TECHNICAL MANUAL

Maxwell® FSC DNA IQ™ Casework Kit

Instructions for Use of Products
AS1550 and DC6745

Note: To use the Maxwell® FSC DNA IQ™ Casework Kit, you must have the "DNA IQ Casework" method loaded on the Maxwell® Instrument.

Caution: Handle cartridges with care; seal edges may be sharp.

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TM499

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Maxwell® FSC DNA IQ™ Casework Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: [redacted]

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1. Description

The Maxwell® FSC DNA IQ™ Casework Kit[a] is used with the Maxwell® Instruments specified below and is specifically designed for optimal DNA extraction from forensic casework samples. These samples include blood stains, semen stains, hair, cigarette butts, tissue samples and "touch" DNA samples regularly encountered in forensic DNA analysis.

Extraction from certain sample types (e.g., sperm cells) traditionally requires the use of dithiothreitol (DTT) as part of the extraction method. The Casework Extraction Kit (Cat.# DC6745) includes 1-Thioglycerol, which, unlike DTT, can be stored at 2–10°C, and provides extraction performance comparable to that with DTT. This Technical Manual provides instructions to use 1-Thioglycerol for sample preprocessing.

Supported Instruments

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® FSC	AS4600	TM462
Maxprep™ Liquid Handler	AS9100, AS9101, AS9200, AS9201	TM509

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1. Description (continued)

Maxwell® Instruments are designed for use with predispensed reagent cartridges and elute DNA samples in small volumes. Maxwell® methods for the FSC DNA IQ™ Casework Kit can process from one to the maximum sample number in about 25 minutes.

The Maxwell® FSC DNA IQ™ Casework Kit uses the DNA IQ™ Resin to purify DNA, maximizing DNA yield and purity for use in STR analysis. The Maxwell® Instruments are magnetic-particle-handling instruments that efficiently transport the DNA IQ™ Resin through purification reagents in prefilled cartridges, mixing the resin with the reagents during processing. The paramagnetic particle-based methodology avoids common problems experienced with other automated systems, such as clogged tips or partial reagent transfers, which can lead to suboptimal DNA purification.

Prior to extraction, samples can be preprocessed manually or using the Maxprep™ Liquid Handler. The Maxprep™ Liquid Handler will prepare samples for extraction and can add preprocessed samples to Maxwell® FSC cartridges, transfer plungers to Maxwell® FSC cartridges and dispense elution buffer to elution tubes. Follow the instruction set specific to the preprocessing option used.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® FSC DNA IQ™ Casework Kit	**48 preps**	**AS1550**

Not for Medical Diagnostic Use. Sufficient for 48 automated isolations from forensic casework samples. Includes:

- 48 Maxwell® FSC Cartridges
- 1 Maxwell® FSC Plunger Pack (48 plungers)
- 50 Elution Tubes, 0.5ml
- 20ml Elution Buffer
- 32ml Lysis Buffer

PRODUCT	SIZE	CAT.#
Casework Extraction Kit	**100 reactions**	**DC6745**

Not for Medical Diagnostic Use. Sufficient for 100 extractions of 400µl each. Includes:

- 50ml Casework Extraction Buffer
- 2 × 10mg Proteinase K
- 900µl 1-Thioglycerol
- 1.25ml Nuclease-Free Water

Storage Conditions: Store the Maxwell® FSC DNA IQ™ Casework Kit and Casework Extraction Buffer at 15–30°C. Store 1-Thioglycerol at 2–10°C. Store Proteinase K at –30°C to –10°C. Store Nuclease-Free Water at less than 30°C.

Safety Information: The Maxwell® FSC Cartridges contain flammable substances ethanol and isopropanol, and the harmful substance guanidine thiocyanate. 1-Thioglycerol is a toxic substance. We highly recommend the use of gloves and aerosol-resistant pipette tips.

Maxwell® FSC Cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

3. Sample Preprocessing

The instructions below indicate the manual steps necessary for preprocessing of samples in individual tubes. Perform the preprocessing steps appropriate to the sample type whether using the Maxprep™ Liquid Handler with samples in individual tubes or prior to extraction on the Maxwell® Instrument.

To maximize the amount of DNA purified from forensic casework materials, use the protocol appropriate for your sample type. Protocols include:

1. Samples on a Solid Support (Section 3.A)
2. Liquid Samples (Section 3.B)
3. Differential Extraction Samples (Section 3.C).

All protocols involve a Proteinase K treatment, which is required to maximize recovery and yield from a variety of sample types, including small amounts of sample on a solid matrix, such as a swab or fabric. DNA samples extracted using a Proteinase K treatment generally exhibit better locus-to-locus balance in downstream STR analysis.

Note: These preprocessing protocols use the Casework Extraction Buffer included in the Casework Extraction Kit (Cat.# DC6745) for the Proteinase K digestion. If another proteinase K digestion buffer is used for comparison, the concentration of SDS must be below 0.5% or a precipitate may form when the Lysis Buffer is added.

3.A. Samples on a Solid Support

This preprocessing protocol allows optimal DNA extraction from samples on a solid support, such as swabs or fabric, using a Proteinase K incubation. Two different protocols are listed (Option 1 and 2), depending on which spin baskets and microtubes are selected.

Materials to Be Supplied by the User

- 56°C heat block or water bath
- aerosol-resistant pipette tips
- Casework Extraction Kit (Cat.# DC6745)
- preprocessing spin baskets and tubes (use option 1 or 2 listed below)
    1. DNA IQ™ Spin Baskets (Cat.# V1225) with ClickFit Microtube, 1.5ml (Cat.# V4745)
    2. CW Spin Baskets (Cat.# AS8101) with CW Microfuge Tubes, 1.5ml (Cat.# AS8201)

Preparation of Stock Proteinase K Solution for Sample Preprocessing

Add 556µl of Nuclease-Free Water to one tube of lyophilized Proteinase K, and gently invert to dissolve. The final concentration of Proteinase K will be 18mg/ml. Store Proteinase K Solution at –30°C to –10°C.

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3.A. Samples on a Solid Support (continued)

Option 1: Extraction of Samples on a Solid Support with DNA IQ™ Spin Basket

1. Place solid substrate (e.g., fabric or swab head) at the bottom of a labeled ClickFit Microtube, 1.5ml.
2. Prepare the Extraction Mix by adding the final volume of each reagent listed in Table 1 to a clean tube.

Table 1. Extraction Mix for Solid Support Samples with DNA IQ™ Spin Basket.

Extraction Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Extraction Buffer	386µl	×		=	
Proteinase K (18mg/ml)	10µl	×		=	
1-Thioglycerol	4µl	×		=	
Total Reaction Volume	400µl	×		=	

Note: 1-Thioglycerol is viscous. Pipet slowly.

3. Briefly vortex the Extraction Mix, and dispense 400µl to each ClickFit Microtube containing solid substrate.
4. Close the tube lid, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.
5. Place a DNA IQ™ Spin Basket into a clean labeled ClickFit Microtube, 1.5ml. Transfer the sample to the DNA IQ™ Spin Basket with forceps, being sure to orient the swab or fabric toward the bottom of the spin basket. Transfer the lysate from the incubation tube to the spin basket, and close the tube.
6. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Carefully remove the DNA IQ™ Spin Basket.

   Note: The sample is now ready for Maxprep™ Liquid Handler preprocessing when using the Maxwell® FSC DNA IQ™-Tubes method. Proceed to the instructions in *Maxwell® FSC DNA IQ™ Casework Kit Preprocessing of Sample in Tubes for the Maxprep™ Liquid Handler Technical Manual* #TM592 for use of the Maxprep™ Liquid Handler with samples in tubes.
7. Add 200µl of Lysis Buffer to the tube containing extract.
8. Close the lid of the tube, and vortex the sample for 5–10 seconds.
9. The sample is now ready for automated DNA extraction using the Maxwell® Instrument. Proceed to Section 4 for Maxwell® cartridge preparation and instrument setup.

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

Option 2: Extraction of Samples on a Solid Support with CW Spin Basket

1. Place a CW Spin Basket into a labeled CW Microfuge Tube, 1.5ml.
2. Place solid substrate (e.g., fabric or swab head) at the bottom of a CW Spin Basket.
3. Prepare the Extraction Mix by adding the final volume of each reagent listed in Table 2 to a clean tube.

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Table 2. Extraction Mix for Solid Support Samples with CW Spin Basket.

Extraction Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Extraction Buffer	286µl	×		=	
Proteinase K (18mg/ml)	10µl	×		=	
1-Thioglycerol	4µl	×		=	
Total Reaction Volume	300µl	×		=	

Note: 1-Thioglycerol is viscous. Pipet slowly.

4. Briefly vortex the Extraction Mix, and dispense 300µl to each CW Spin Basket containing solid substrate.
5. Close the tube lid, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.
6. Centrifuge at room temperature for 2 minutes at maximum speed in a microcentrifuge. Carefully remove the CW Spin Basket.

   Note: The sample is now ready for Maxprep™ Liquid Handler preprocessing when using the Maxwell® FSC DNA IQ™-Tubes method. Proceed to the instructions in *Maxwell® FSC DNA IQ™ Casework Kit Preprocessing of Sample in Tubes for the Maxprep™ Liquid Handler Technical Manual* #TM592 for use of the Maxprep™ Liquid Handler with samples in tubes.
7. Add 200µl of Lysis Buffer to the tube containing extract.
8. Close the lid of the tube, and vortex the sample for 5–10 seconds.
9. The sample is now ready for automated DNA extraction using the Maxwell® Instrument. Proceed to Section 4 for Maxwell® cartridge preparation and instrument setup.

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

3.B. Liquid Samples

This preprocessing protocol results in optimal lysis from samples in an aqueous solution using a Proteinase K treatment.

Materials to Be Supplied by the User

- 56°C heat block or water bath
- aerosol-resistant pipette tips
- Casework Extraction Kit (Cat.# DC6745)
- ClickFit Microtubes, 1.5ml (Cat.# V4745) or CW Microfuge Tubes, 1.5ml (Cat.# AS8201)

Note: The ClickFit Microtube or CW Microfuge Tube lid will remain closed during heated incubation.

Preparation of Stock Proteinase K Solution for Sample Preprocessing

Add 556µl of Nuclease-Free Water to one tube of lyophilized Proteinase K, and gently invert to dissolve. The final concentration of Proteinase K will be 18mg/ml. Store Proteinase K Solution at –30°C to –10°C.

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3.B. Liquid Samples (continued)

Extraction of Liquid Samples

1. Pipet liquid sample into the bottom of a labeled ClickFit Microtube or CW Microfuge Tube, 1.5ml.
2. Add 10µl of Proteinase K Solution, 4µl of 1-Thioglycerol and Casework Extraction Buffer to a total volume of 400µl.

   Do not exceed a final volume of 400µl.

   Note: 1-Thioglycerol is viscous. Pipet slowly.
3. Close the tube lid, vortex sample at high speed for 5 seconds, and incubate the sample at 56°C for 30 minutes.

   Note: The sample is now ready for Maxprep™ Liquid Handler preprocessing when using the Maxwell® FSC DNA IQ™-Tubes method. Proceed to the instructions in *Maxwell® FSC DNA IQ™ Casework Kit Preprocessing of Sample in Tubes for the Maxprep™ Liquid Handler Technical Manual* #TM592 for use of the Maxprep™ Liquid Handler with samples in tubes.
4. Add 200µl of Lysis Buffer to each sample.
5. Close the lid of the tube, and vortex the sample for 5–10 seconds.
6. The sample is now ready for automated DNA extraction using the Maxwell® Instrument. Proceed to Section 4 for Maxwell® cartridge preparation and instrument setup.

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

3.C. Differential Extraction Samples

Differential extraction is a method of separating a forensic sample into two fractions: sperm and epithelial. Epithelial cells are lysed using Proteinase K in the absence of a reducing agent (e.g., DTT) to prevent the lysis of spermatozoa. The Differex™ System (Cat.# DC6801, DC6800) can be used to quickly and efficiently separate male and female sample fractions. See the *Differex™ System Technical Manual* #TM331 for a protocol to separate sperm and epithelial fractions, available at: **www.promega.com/protocols**

Materials to Be Supplied by the User

- 56°C heat block or water bath
- aerosol-resistant pipette tips
- Casework Extraction Kit (Cat.# DC6745)
- ClickFit Microtubes, 1.5ml (Cat.# V4745) or CW Microfuge Tubes, 1.5ml (Cat.# AS8201)

Note: The ClickFit Microtube or CW Microfuge Tube lid will remain closed during heated incubation.

Preparation of Stock Proteinase K Solution for Sample Preprocessing

Add 556µl of Nuclease-Free Water to one tube of lyophilized Proteinase K, and gently invert to dissolve. The final concentration of Proteinase K will be 18mg/ml. Store Proteinase K Solution at –30°C to –10°C.

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DNA Extraction from Sperm Fraction

1. Prepare the Extraction Mix by adding the final volume of each reagent listed in Table 4 to a clean tube.

Table 4. Extraction Mix for Sperm Samples.

Extraction Mix Component	Volume Per Extraction	×	Number of Extractions	=	Final Volume
Casework Extraction Buffer	386µl	×		=	
Proteinase K (18mg/ml)	10µl	×		=	
1-Thioglycerol	4µl	×		=	
Total Reaction Volume	400µl	×		=	

Note: 1-Thioglycerol is viscous. Pipet slowly.

2. Briefly vortex the Extraction Mix, and dispense 400µl to the sperm fraction (generally a pellet or liquid volume less than 100µl) into a labeled ClickFit Microtube or CW Microfuge Tube, 1.5ml.
3. Close the lid of the tube, vortex the sample for 5 seconds, and incubate the sample at 56°C for 30 minutes.

   Note: The sample is now ready for Maxprep™ Liquid Handler preprocessing when using the Maxwell® FSC DNA IQ™-Tubes method. Proceed to the instructions in *Maxwell® FSC DNA IQ™ Casework Kit Preprocessing of Sample in Tubes for the Maxprep™ Liquid Handler Technical Manual* #TM592 for use of the Maxprep™ Liquid Handler with samples in tubes.
4. Add 200µl of Lysis Buffer to each sample.
5. Close the lid of the tube, and vortex the sample for 5–10 seconds.
6. The sample is now ready for automated DNA extraction using the Maxwell® Instrument. Proceed to Section 4 for Maxwell® cartridge preparation and instrument setup.

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

DNA Extraction from Epithelial Fraction

1. To an epithelial fraction of up to 400µl, add 200µl of Lysis Buffer.

   Note: Epithelial fraction samples require no further preparation before they are ready for Maxprep™ Liquid Handler preprocessing when using the Maxwell™ FSC DNA IQ™-Tubes method. Proceed to the instructions in *Maxwell® FSC DNA IQ™ Casework Kit Preprocessing of Sample in Tubes for the Maxprep™ Liquid Handler Technical Manual* #TM592 for use of the Maxprep™ Liquid Handler with samples in tubes.
2. Close the lid of the tube, and vortex the sample for 5–10 seconds.
3. The sample is now ready for automated DNA extraction using the Maxwell® Instrument. Proceed to Section 4 for Maxwell® cartridge preparation and instrument setup.

   Note: Store preprocessed sample at room temperature (15–30°C) overnight, if necessary.

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4. Maxwell® FSC DNA IQ™ Casework Cartridge Preparation

1. Change gloves before handling Maxwell® FSC Cartridges, FSC Plungers and Elution Tubes. Place the cartridges to be used in the deck tray(s). Use the Maxwell® Deck Trays appropriate to the Maxwell® Instrument. Place each cartridge in the deck tray with well #1 (the largest well) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.

   Note: Clean sample or reagent spills on any part of the deck tray with a detergent-water solution, followed by 70% ethanol and then water. Do not use bleach on any instrument parts.

2. Transfer each preprocessed sample to well #1 (the largest well) of each cartridge.

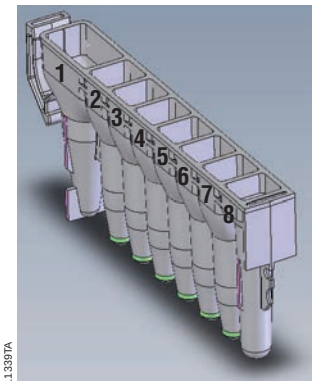
3. Mix the sample in well #1 using the pipette tip to ensure that all sample has been transferred. Change pipette tips between samples.

4. Place one plunger into well #8 of each cartridge.

5. Place an empty elution tube into the elution tube position for each cartridge in the deck tray. Add 50µl of Elution Buffer to the bottom of each elution tube.

   Notes:

   1. Ensure that the Elution Buffer is in the bottom of the tube. If Elution Buffer is on the side of the tube, the elution may be suboptimal.
   2. Use only the Elution Tubes provided with the kit; other tubes may not work with the Maxwell® Instrument.
   3. Use only the Elution Buffer supplied with the Maxwell® FSC DNA IQ™ Casework Kit; other buffers can negatively affect downstream analysis (e.g., poor-quality STR profiles).



User Adds to Wells
1. Preprocessed Sample Lysate
8. FSC Plungers

Figure 1. Maxwell® FSC Cartridge. Preprocessed sample is added to well #1, and a plunger is added to well #8.

Figure 2. Setup and configuration of the deck tray. Elution Buffer is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. Maxprep™ Preprocessing

The Maxprep™ Liquid Handler provides methods for preprocessing of samples prior to extraction using Maxwell® Instruments. Separate, short technical manuals provide instructions for setting up the Maxprep™ Liquid Handler for these methods (available at **www.promega.com/resources/protocols**). Refer to these short technical manuals for information about the methods and how to set up the Maxprep™ Liquid Handler to perform preprocessing of samples prior to extraction using the Maxwell® FSC DNA IQ™ Casework Extraction Kit.

6. Maxwell® Instrument Setup and Run

For detailed information, refer to the technical manual specific to your Maxwell® Instrument.

Instrument	Technical Manual
Maxwell® RSC	TM411
Maxwell® RSC 48	TM510
Maxwell® FSC	TM462

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® software on the Tablet PC. The instrument will proceed through a self-check and home all moving parts.
2. Touch **Start** to begin the process of running a method.
3. Depending on your Maxwell® Instrument model, use one of the following options to select a method:
    a. When running in **Portal** mode, scan the bar code(s) on the deck tray(s). After data has been returned from the Portal software, touch **Continue** to use the sample tracking information for the deck tray(s) or touch **New** to start a run and enter new sample tracking information.
    b. Scan or enter the 2D bar code information on the kit box to automatically select the appropriate method.
    c. Touch the **DNA IQ Casework** method.

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6. Maxwell® Instrument Setup and Run (continued)

4. If applicable to your Maxwell® Instrument model, verify that the DNA IQ Casework method has been selected, and touch the **Proceed** button. If requested by the software, scan or enter kit lot and expiration information as required by the Administrator.

5. On the 'Cartridge Setup' screen, touch the cartridge positions to select/deselect the positions that will be used during this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using 48-position Maxwell® Instruments, touch the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.

6. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

   Inserting the Maxwell® deck tray(s): Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

   Notes:

   1. Check the identifier on 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument.
   2. Make sure elution tubes are open and contain elution buffer before starting a purification run.

7. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

   Warning: Pinch point hazard.

   The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed, and the approximate time remaining in the run.

   Notes:

   1. When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck trays will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen and problem positions will be marked with an exclamation point in a red circle. Resolve all error states, and touch the **Start** button again to repeat deck tray scanning and begin the extraction run.
   2. Pressing the **Abort** button will abandon the run.
   3. If the run is abandoned before completion, you will be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up** when requested. The samples will be lost.

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8. Follow on-screen instructions at the end of the method to open the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the technical manual appropriate to your Maxwell® Instrument (see table above) to perform a **Clean Up** process to attempt to unload the plungers.
9. Remove the deck tray(s) from the instrument. Remove elution tubes containing DNA, and cap the tubes. After the run has been completed, the extraction run report will be displayed. From the report screen, you can print or export this report or both.

Note: Following the automated purification procedure, the deck tray will be warm. It will not be too hot to touch. To remove the deck tray from the instrument platform, hold the sides of the deck tray.

10. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe and then water. All cartridges and tubes should be removed before cleaning. Do not use bleach on any instrument parts.
11. Remove the cartridges and plungers from the deck tray.

Discard the cartridges and plungers as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

Ensure samples are removed before performing any required UV light treatment to avoid damage to the nucleic acid.

7. Storing Eluted DNA

If DNA is not analyzed immediately, store the eluted DNA on ice or at 2–10°C for up to 24 hours. For longer term storage, consult laboratory guidelines. Freezing samples at –30°C to –10°C or below –65°C has been shown to preserve DNA for longer periods of time.

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8. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: [redacted]

Symptoms	Causes and Comments
Low DNA yield	Insufficient sample was processed. Add more starting material for preprocessing to increase yield.
	Too much sample volume was processed. DNA isolation using the Maxwell® FSC Cartridge is most efficient when there is <800µl in well #1. Larger volumes can be processed but may reduce isolation efficiency.
	Insufficient Lysis Buffer. Dispense an equal volume of Lysis Buffer to sample volume during the binding step in well #1. A minimum of 200µl of Lysis Buffer should be used.
Instrument overrun or underrun errors	Verify nothing is physically blocking the movement of the platform, plunger bar or magnetic rod assembly.
	Perform a Self Test from the Settings menu. The instrument will rehome itself. If the error recurs, contact Promega for service.
	The cartridges were not completely seated on the platform. Ensure the cartridges are pressed firmly into place.
	Incorrect elution tube was used. To prevent a Z-axis collision, use only the 0.5ml Elution Tube provided with the Maxwell® FSC DNA IQ™ Casework Kit. Other tubes may have different dimensions.
Resin carryover during elution	The presence of a small amount of resin particles in the Elution Tube will not affect the final DNA concentration or downstream applications. If desired, final elution tubes can be centrifuged to pellet resin and eluates can be transferred to a clean tube or an additional resin capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341).
Instrument unable to pick up plungers	Make sure you are using the plungers in the Maxwell® FSC DNA IQ™ Casework Kit; the plungers for the Maxwell® FSC reagent kits are different than those of other Maxwell® reagent kits.

Symptoms	Causes and Comments
Instrument calibration error	The cartridges were not completely seated in the deck tray. Ensure the cartridges are pressed firmly into place.
	Verify nothing is physically blocking the movement of the platform, plunger bar or magnet bar.
	Confirm that you are using the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550) with FSC plungers.
	Turn the machine off then on to cycle the power. The instrument will rehome itself. If the calibration error occurs again after power cycling, contact Promega for service.
	Turn the machine off then on to cycle the power. After cycling power, run the DNA IQ Casework method without a cartridge in the machine. If another calibration error occurs during the run, contact Promega for service.
	Incorrect elution tube was used. Use only the 0.5ml Elution Tube provided with the Maxwell® FSC DNA IQ™ Casework Kit. Other tubes may have different dimensions.

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9. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxprep™ Liquid Handler, RSC Carriers	1 each	AS9100
Maxprep™ Liquid Handler, RSC Carriers w/ UV Light	1 each	AS9101
Maxprep™ Liquid Handler, RSC 48 Carriers	1 each	AS9200
Maxprep™ Liquid Handler, RSC 48 Carriers w/UV Light	1 each	AS9201
Maxprep™ Carrier, Maxwell® RSC	1 each	AS9402
Maxprep™ Carrier, Maxwell® RSC 48 Front	1 each	AS9403
Maxprep™ Carrier, Maxwell® RSC 48 Back	1 each	AS9404
Nunc™ 2.0ml Deep Well Plates	60/pack	AS9307
2.0ml Deep Well Plates (Non-Sterile)	60/pack	AS9309
Maxprep™ 1000µl Conductive Disposable Tips, Filtered	40 racks of 96 tips/box	AS9303
Maxprep™ 300µl Conductive Disposable Tips, Filtered	60 racks of 96 tips/box	AS9302
Maxprep™ Reagent Reservoir, 50ml	28/pack	AS9304
Maxprep™ Waste Bags, Clear	100/box	AS9305
Maxprep™ Plunger Holder	1 each	AS9408
Maxprep™ 3-Position Reagent Tube Holder	1 each	AS9409
Maxwell® FSC Instrument	1 each	AS4600
Maxwell® FSC Deck Tray	1 each	AS4016
Differex™ System	50 samples	DC6801
	200 samples	DC6800
Proteinase K	100mg	V3021
ClickFit Microtube, 1.5ml	100/pack	V4745
DNA IQ™ Spin Baskets	50/pack	V1225
CW Spin Baskets	50/pack	AS8101
CW Microfuge Tubes, 1.5ml	50/pack	AS8201
Nuclease-Free Water	150ml	P1196
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402

10. Summary of Changes

The following changes were made to the 8/21 revision of this document:

1. Protocol notes in Sections 3.A and 3.B were moved to the correct location.
2. The Maxwell trademark was corrected to ® in two places.
3. Cat.# AS9309 was added to Section 9. Related Products.
4. The TM cover image was updated.

[a]European Pat. No. 1 204 741 and Japanese Pat. No. 4425513.

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All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Proposal#115 – Verification of a new Microcon® DNA Fast Flow Centrifugal Filter membrane

Amy Cheng, Cecilia Iannuzzi, Allan McNevin
DNA Analysis, Forensic and Scientific Services, Queensland Health

1 Abstract

Microcon centrifugal filter devices are used to concentrate (to a factor as high as 100x) and desalt macromolecular solutions of 10-500 µL by centrifugation. Each filter device contains an Ultracel YM membrane, which is sealed around the edge by a medical-grade silicone rubber O-ring. This design allows centrifugation of solutions for set times and speeds without compromising the seal.

The current procedure for the Microcon process is to pipette extracted DNA sample into a Microcon centrifugal device and centrifuge at a speed of 500 x *g* at 1-2 minute intervals for a maximum of 12 minutes until the final volume reaches the desired amount.

The current supplier, Merck Millipore, has announced a discontinuance of a raw material used to manufacture the Microcon® Centrifugal Filters Ultracel® YM-100.

Due to this discontinuance, a replacement membrane has been developed by their own facility, which contains a polyolefin support. Millipore reports that the new Microcon® DNA Fast Flow Centrifugal Filters yield equivalent DNA recovery and shorter spin time when compared to the currently used old membrane.

A verification project was developed to verify the manufacturer's claims and investigate if any modification to current protocols is required. In this project, 10 replicates of an extracted DNA sample were used to carryout a side-by-side comparison between the old and new membranes. Parameters such as concentration / DNA recovery, spin time, quality of profiles and recovery after dryness / over spinning was compared.

Results showed that the newly developed Microcon® DNA Fast Flow Centrifugal Filters did not perform significantly different to the currently used Microcon® Centrifugal Filters Ultracel® YM-100 membranes. Therefore, it is recommended that the newly developed Microcon® Centrifugal Filters DNA Fast Flow kits are deemed suitable for routine use within DNA Analysis Unit.

2 Purpose

The aim of these experiments was to verify the change in Microcon filters from the currently used Microcon® Centrifugal Filters Ultracel® YM-100 (old membrane) to the newly developed product Microcon® Centrifugal Filters DNA Fast Flow (new membrane) provides equivalent quality of DNA profiles after concentration and DNA recovery when compared to each other.

3 Equipment and Materials

- For mock samples
  - 10 x Extracted Positive Controls (FBOT1067)
- 17 x Microcon® Centrifugal Filters Ultracel® YM-100 (Merck Millipore, Germany)
- 17 x Microcon® Centrifugal Filters DNA Fast Flow (Merck Millipore, Germany)
- Nanopure water (Millipore Milli-Q® Advantage A10 system)
- Multitube vortex
- Eppendorf Centrifuge (Eppendorf AG, Germany)
- Miscellaneous consumables (e.g. 1.5ml screw-cap tubes, Nunc tubes, pipettes, pipette tips, septa)
- Quantifiler™ Human DNA Quantification kits (Life Technologies, Foster City, CA, USA) and ABI 7500 Real Time PCR (7500 RT-PCR) System (Life Technologies, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus® PCR Amplification kits including 9947A control DNA (Life Technologies, Foster City, CA, USA)
- MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- GeneAmp 9700 thermalcycler (Life Technologies, Foster City, CA, USA)
- ABI 3130*xl* Genetic Analyzer (Life Technologies, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus® Allelic Ladder (Life Technologies, Foster City, CA, USA)
- GeneScan™ 500 ROX™ Size Standard (Life Technologies, Foster City, CA, USA)
- Hi-Di™ Formamide (Life Technologies, Foster City, CA, USA)
- 3130 POP-4™ Polymer (Life Technologies, Foster City, CA, USA)
- 10x CE Buffer for ABI 3100 (gel company, San Francisco, CA, USA)
- TE^{-4} buffer (10mM Tris HCl, 0.1mM EDTA, pH 8.0)

4 Methods

Mock samples, exhibiting a partial profile were created by initially pooling 10 µL of each of 10 previously extracted Positive Control DNA samples (FBOT1067). Depending on the quantification value, this sample was diluted to make a working bulk solution with a concentration of approximately 0.01 ng/µL. This bulk DNA sample was then quantified, amplified and profiled. This sample was then used for concentration using the Microcon procedure.

4.1 Mock sample creation

4.1.1 Pooling of Positive Control samples

Pooled 10µL of each of the following 10 tubes of extracted positive control samples.

Table1. Positive Control samples used for pooling.

No.	FBOT1067	Quant value
1	511849787	0.915
2	511849919	3.300
3	511849982	0.798
4	543830013	3.230
5	543150656	0.513
6	543150711	2.310
7	543153685	0.295
8	543153696	2.540
9	543154466	1.630
10	543154422	4.180

This pooled DNA sample of 100µL was given barcode #545773655, and was quantified, giving a quantification value of 1.25ng/µL.

4.1.2 Dilution of Positive Control sample

Sample barcode #545773655, which gave a quantification value of 1.25 ng/µL, was diluted to a 1:100 ratio. Therefore 40 µL of Sample barcode #545773655 was diluted in 3960 µL Nanopure H_2O for a final bulk volume of 4000 µL.

This new dilution of 4000 µL was labelled with a new barcode #545774107, and was quantified, amplified & profiled twice.

Quantification results were 0.0133 ng/µL and 0.0125 ng/µL. These results were averaged, and the starting sample for testing was taken to have a quantification value of 0.0129 ng/µL.

4.1.3 Creation of Microcon Batches

Four Microcon batches were created:

Batch 1 was processed using currently available membrane material
- 1 x negative control concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to full

Batch 2 was processed using new membrane material
- 1 negative control concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to full

Batch 3 was processed using Recovery process on currently available membrane material
- 1 x negative control
- 5 x diluted DNA aliquots

Batch 4 was processed using Recovery process on new membrane material

- 1 x negative Control
- 5 x diluted DNA aliquots

4.2 Microcon Processing & Recovery of Diluted DNA Samples

4.2.1 Current Microcon processing for Old and New Microcon membranes

Batches 1 and 2 were concentrated using the current Microcon method for DNA extracts following the Standard Operating Procedure "Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices" (QIS 19544).

These samples were then quantified, amplified and profiled and results were compared.

4.2.2 Current Recovery method for Old and New Microcon membranes

Batches 3 and 4 were spun to membrane dryness. The recovery of the DNA extract was then carried out as per Standard Operating Procedure "Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices" (QIS 19544).

These samples were then quantified, amplified and profiled and results were compared.

4.3 DNA Quantitation

DNA Quantitation results were obtained for all samples, concentrated to 30 µL, according to the standard laboratory procedure outlined in the Standard Operating Procedure "Real Time PCR Quantification of Human DNA using the Quantifiler Human DNA Quantification Kit (Life Technologies)" (QIS 19977). Samples concentrated to full went straight to amplification at maximum volume of 20 µL.

4.4 Amplification

All samples were amplified with the ABI AmpFℓSTR® Profiler Plus® PCR Amplification Kit at the volumes calculated from the quantitation result. The PCR reaction was setup on a PerkinElmer MultiPROBE® II PLUS HT EX Integration Platform according to the Standard Operating Procedure outlined in QIS method "Amplification of Extracted DNA using the AmpFℓSTR Profiler Plus kit or AmpFℓSTR COfiler Kit" (QIS 19976).

4.5 DNA fragment analysis

All samples had fragment analysis performed according to the Standard Operating Procedure "CE Quality Check of Samples from the AB 3130*xl* Genetic Analyzers" (QIS 17130) and "Procedure for the Use and Maintenance of the 3130*xl* Genetic Analyzers" (QIS 15998).

4.6 Genotyping

All sample results were interpreted through the use of Genemapper® *ID-X* Software according to the Standard Operating Procedure "Procedure for the Interpretation & Acceptance of Results using Profiler & COfiler systems" (QIS 17137).

5 Results and Discussion

5.1 Microcon Concentration results

Table 2 Comparison of recovered DNA between old and new membranes after Microcon concentration

No.	Mic'n to	Pre Concentration			Post Concentration - Old Membrane					Post Concentration - New Membrane				
		Volume (µL)	Quant (ng/µL)	DNA Template (ng)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile
(Neg) 1	30µL	100	NA	NA	32	undet	NA	NA	NSD	43	undet	NA	NA	NSD
2		80	0.0129	1.032	32	0.0428	1.3696	133	Full	45	0.0162	0.729	71	Full
3		80	0.0129	1.032	29	0.0410	1.189	115	Full	45	undet	no sample in nunc tube		NSD
4		80	0.0129	1.032	29	0.0529	1.5341	149	Full	46	0.0157	0.7222	70	Full
5		80	0.0129	1.032	30	0.0429	1.287	125	Full	47	0.0178	0.8366	81	Full
6		80	0.0129	1.032	30	0.0480	1.44	140	Full	40	0.0181	0.724	70	Full
7	Full	80	0.0129	1.032	7 (20)	0.0505	1.01	98	Full	14 (20)	0.0422	0.844	82	Full
8		80	0.0129	1.032	7 (20)	0.0618	1.236	120	Full	15 (20)	0.0606	1.212	117	Full
9		80	0.0129	1.032	7 (20)	0.0512	1.024	99	Full	13 (20)	0.0409	0.818	79	Full
10		80	0.0129	1.032	6 (20)	0.0705	1.41	137	Full	17 (20)	0.0581	1.162	113	Full
11		80	0.0129	1.032	7 (20)	0.0756	1.512	147	Full	20	undet	NA	NA	Full
Mean of DNA Template Recovered (%)								**126**					**85**	
T-test (p-value) using DNA Template Recovered					**0.0003**									

Note: Numbers in brackets indicate the total volume of DNA sample after adding TE buffer to enable processing on the Multiprobe II instrument.
Sample number 3 processed using the new membrane filters was not quantified or amplified as the DNA sample from the filter was not transferred into the Nunc tube and was not included in the calculations (in red).
Sample number 11 processed using the new membrane filters gave an "undetermined" quantification value but upon amplification, gave a full DNA profile. This is possibly due to pipetting error of the Multiprobe II instrument during quantification setup (in blue).

Table 2 shows at the pre-concentration stage (in yellow), the starting bulk DNA sample had a quantification value 0.0129 ng/µL. 80 µL of this sample was added to 10 old and 10 new membranes, therefore adding 1.032 ng of DNA template per membrane.

The old membranes (in orange) recovered, on average, 126% of DNA template, whereas the new membranes (in blue) recovered, on average, 85% DNA template.

The results appear to have shown that when using the currently available membrane, more DNA was recovered than what was added at the start of the procedure. This indicated that there was a problem with the quantification reaction with some samples showing a falsely elevated result. Following quantification all samples were amplified, due to the small or lack of volume remaining for each sample, repeat quantification was not feasible, as such this experiment was repeated, refer to section 5.3 below)

Although the "percentage of DNA template recovered" calculations appear unreliable, all the samples that was able to be amplified from the old and new membranes, successfully produced full profiles (with exception of the Negative Control which was NSD as expected).

5.2 Recovery Process results

After centrifugation spinning of the samples for more than the expected time until dryness of the membrane, the samples were recovered by adding 10 µL of TE to the membrane, the membranes were gently agitated, and then inverted into a new tube where the DNA sample was spun into a collecting tube to be quantified and amplified.

Table 3 Comparison of recovered DNA between old and new membranes after Recovery method

No.	Pre Concentration			Final Results - Old Membranes					Final Results - New Membranes				
	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile
(Neg) 1	80	0.0129	1.032	14	undet	NA	NA	NSD	14 (20)	undet	NA	NA	NSD
2	80	0.0129	1.032	5 (20)	0.032	0.630	61	Full	16 (20)	0.027	0.54	52	Full
3	80	0.0129	1.032	5 (20)	0.031	0.620	60	Full	16 (20)	0.030	0.59	57	Full
4	80	0.0129	1.032	5 (20)	0.021	0.426	41	Full	19 (20)	0.058	1.158	112	Full
5	80	0.0129	1.032	5 (20)	0.040	0.794	77	Full	14 (20)	0.043	0.85	82	Full
6	80	0.0129	1.032	5 (20)	0.029	0.588	57	Full	18 (20)	0.045	0.908	88	Full
Mean of DNA Template (%Recovered)							**59**					**78**	
T-test of DNA Template Recovered (p-value)				**0.1582**									

Note: Numbers in brackets indicate the total volume of DNA sample after adding TE buffer to enable processing on the Multiprobe II instrument.

Table 3 shows at the pre-concentration stage (in yellow), the starting bulk DNA sample had a quantification value 0.0129 ng/µL. 80 µL of this sample was added to 5 old and 5 new membranes, therefore adding 1.032 ng of DNA template per membrane.

The old membranes (in orange) recovered, on average, 59% of DNA template, whereas the new membranes (in blue) recovered, on average, 78% DNA template. These results showed that the old membranes were not significantly different to the new membranes (p-value 0.1582).

Although the pre and post DNA samples were quantified the same way as in Section 5.1, where the pre-concentrated DNA sample was quantified on a different batch to the post-concentrated DNA samples, the "percentage of DNA template recovered" was also not deemed a reliable result. However, the Recovery test is performed to primarily assess whether DNA can still be recovered even after the membrane has been spun dry. Therefore, these results can still conclude that the new membrane can recover equivalent and comparable DNA yields to the old membranes.

In addition, all recovered DNA samples successfully produced full profiles (with exception of the negative control which was NSD as expected).

It was also observed that the new membranes appear to lose less liquid in its membrane (approximately 4 µL) as opposed to the old membranes (approximately 8 µL). Also, the new membranes did not fully dry out as fast and retained more liquid above the filter when

compared to the old membranes. This potentially means that it is even less likely that a Recovery process will need to be performed on the new membranes when compared to the old membranes in routine use.

5.3 Repeated Microcon Concentration Experiment

The initial "percentage of DNA template recovered" results (See section 5.1) were deemed unreliable due to the stochastic effects between quantification batches, where the pre-concentrated DNA samples were quantified on a different batch to the post-concentrated samples resulting in a significant difference when comparing the old and new membranes.

Therefore the Microcon concentration process was repeated where the pre and post-concentrated DNA samples was quantified on the same quantification batch. (amplification of the samples was not performed on this round of testing).

Table 4 Comparison of recovered DNA between the old and new membranes after Microcon concentration (repeated process)

No.	Mic'n to	Pre Concentration			Post Concentration - Old Membrane				Post Concentration - New Membrane			
		Volume (µL)	Quant (ng/µL)	DNA Template (ng)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)
(Neg) 1	30µL	100	undet	NA	28	undet	NA	NA	32	undet	NA	NA
2		80	0.025	1.984	30	0.065	1.935	98	32	0.033	1.059	53
3		80	0.025	1.984	30	0.046	1.368	69	25	0.055	1.375	69
4		80	0.025	1.984	30	0.056	1.689	85	28	0.034	0.944	48
5		80	0.025	1.984	32	0.038	1.200	60	30	0.039	1.167	59
6		50	0.025	1.240	32	0.019	0.598	48	29	0.021	0.618	50
7	Full	80	0.025	1.984	8 (20)	0.047	0.938	47	13 (20)	0.036	0.722	36
8		80	0.025	1.984	9 (20)	0.064	1.276	64	12 (20)	0.068	1.352	68
9		80	0.025	1.984	7 (20)	0.059	1.178	59	12 (20)	0.048	0.964	49
10		80	0.025	1.984	7 (20)	0.038	0.754	38	12 (20)	0.054	1.088	55
11		80	0.025	1.984	7 (20)	0.047	0.948	48	12 (20)	0.037	0.734	37
Mean of DNA Template Recovered (%)								**62**				**52**
T-test using DNA Template Recovered (p-value)					**0.187**							

Note: Numbers in brackets indicate the total volume of DNA sample after adding TE buffer to enable processing on the Multiprobe II instrument.

Table 4 shows at the pre-concentration stage (in yellow), the starting bulk DNA sample had a quantification value 0.025 ng/µL. 80uL of this sample was added to 10 old membranes and 10 new membranes, therefore adding 1.984 ng of DNA template per membrane (with exception of sample no. 5 where only 50 uL of bulk sample was remaining, this was taken into account when performing final calculations).

The old membranes (in orange) recovered, on average, 62% of DNA template, whereas the new membranes (in blue) recovered, on average, 52% DNA template. These results did not show a significant difference between the old and new membranes (p-value 0.187).

6 Summary and Recommendations

In this project, the performance of a new Microcon® DNA Fast Flow Centrifugal Filter membrane was compared with the now discontinued Microcon® Centrifugal Filters Ultracel® YM-100 membranes. Analysis of parameters including concentration / DNA recovery, spin time, quality of profiles and recovery after dryness / over spinning demonstrated that the new membranes did not perform significantly different to the currently used old membranes.

Therefore, it is recommended that the newly developed Microcon® DNA Fast Flow Centrifugal Filter kits are deemed suitable replacement for the old membranes for routine use within DNA Analysis Unit.

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Proposal#115 – Verification of a new Microcon® DNA Fast Flow Centrifugal Filter membrane

Amy Cheng, Cecilia Iannuzzi, Allan McNevin
DNA Analysis, Forensic and Scientific Services, Queensland Health

1 Abstract

Microcon [redacted] er devices are used to concentrate (to a factor as high as 100x) and desalt ma [redacted] solutions of 10-500 µL by centrifugation. Each filter device contains an Ultrace [redacted] ane, which is sealed around the edge by a medical-grade silicone rubber O- [redacted] ign allows centrifugation of solutions for set times and speeds without comprom [redacted]

The curre [redacted] for the Microcon process is to pipette extracted DNA sample into a Microcon [redacted] vice and centrifuge at a speed of 500 x *g* at 1-2 minute intervals for a maximum [redacted] s until the final volume reaches the desired amount.

The current supplier, Merck Millipore, has announced a discontinuance of a raw material used to manufacture the Microcon® Centrifugal Filters Ultracel® YM-100. [redacted]

Due to this discontinuance, a replacement membrane has been developed by their own facility, which contains a polyolefin support. Millipore reports that the new Microcon® DNA Fast Flow Centrifugal Filters yield equivalent DNA recovery and shorter spin time when compared to the currently used old membrane. [redacted]

A verification project was developed to verify the manufacturer's claims and investigate if any modification to current protocols is required. In this project, 10 replicates of an extracted DNA sample were used to carryout a side-by-side comparison between the old and new membranes. Parameters such as concentration / DNA recover [redacted] quality of profiles and recovery after dryness / over spinning was compared.

Results showed that the newly developed Microcon® DNA Fast Flow Centrifugal Filters did not perform significantly different to the currently used Microcon® Centrifugal Filters Ultracel® YM-100 membranes. Therefore, it is recommended that the newly developed Microcon® Centrifugal Filters DNA Fast Flow kits are deemed suitable for routine use within DNA Analysis Unit.

2 Purpose

The aim of these experiments was to verify the change in Microcon filters from the currently used Microcon® Centrifugal Filters Ultracel® YM-100 (old membrane) to the newly developed product Microcon® Centrifugal Filters DNA Fast Flow (new membrane) provides equivalent quality of DNA profiles after concentration and DNA recovery when compared to each other.

3 Equipment and Materials

- For mock samples
  - 10 x Extracted Positive Controls (FBOT1067)
- 17 x Microcon® Centrifugal Filters Ultracel® YM-100 (Merck Millipore, Germany)
- 17 x Microcon® Centrifugal Filters DNA Fast Flow (Merck Millipore, Germany)
- Nanopure water (Millipore Milli-Q® Advantage A10 system)
- Multitube vortex
- Eppendorf Centrifuge (Eppendorf AG, Germany)
- [redacted]aneous consumables (e.g. 1.5ml screw-cap tubes, Nunc tubes, [redacted]s, pipette tips, septa)
- [redacted]filer™ Human DNA Quantification kits (Life Technologies, Foster City, [redacted]SA) and ABI 7500 Real Time PCR (7500 RT-PCR) System (Life [redacted]ologies, Foster City, CA, USA)
- [redacted]STR® Profiler Plus® PCR Amplification kits including 9947A control [redacted]Life Technologies, Foster City, CA, USA)
- [redacted]ROBE® II PLUS HT EX with Gripper™ Integration Platform (PerkinElmer, [redacted]rs Grove, IL, USA)
- [redacted]mp 9700 thermalcycler (Life Technologies, Foster City, CA, USA)
- ABI 3130*xl* Genetic Analyzer (Life Technologies, Foster City, CA, USA)
- AmpFℓSTR® Profiler Plus® Allelic Ladder (Life Technologies, Foster City, CA, USA) [redacted]
- GeneScan™ 500 ROX™ Size Standard (Life Technologies, Foster City, CA, USA)
- Hi-Di™ Formamide (Life Technologies, Foster City, CA, USA)
- 3130 POP-4™ Polymer (Life Technologies, Foster City, CA, USA)
- 1[redacted]r for ABI 3100 (gel company, San Francisco, CA, USA)
- TE^{-4} buffer (10mM Tris HCl, 0.1mM EDTA, pH 8.0)

4 Methods [redacted]

Mock samples, exhibiting a partial profile were created by initially pooling 10 µL of each of 10 previously extracted Positive Control DNA samples (FBOT1067). Depending on the quantification value, this sample was diluted to make a working bulk solution with a concentration of approximately 0.01 ng/µL. This bulk DNA sample was then quantified, amplified and profiled. This sample was then used for concentration using the Microcon procedure.

4.1 Mock sample creation

4.1.1 Pooling of Positive Control samples

Pooled 10µL of each of the following 10 tubes of extracted positive control samples.

Table1. Positive Control samples used for pooling.

No.	FBOT1067	Quant value
1	[redacted]	0.915
2		3.300
3		0.798
4		3.230
5		0.513
6		2.310
7		0.295
8		2.540
9		1.630
10		4.180

This pooled DNA sample of 100µL was given barcode [redacted], and was quantified, giving a quantification value of 1.25ng/µL.

4.1.2 Dilution of Positive Control sample

Sample barcode [redacted], which gave a quantification value of 1.25 ng/µL, was diluted to a 1:100 ratio. Therefore 40 µL of Sample barcode #545773655 was diluted in 3960 µL Nanopure H_2O for a final bulk volume of 4000 µL.

This new dilution of 4000 µL was labelled with a new barcode [redacted], and was quantified, amplified & profiled twice.

Quantification results were 0.0133 ng/µL and 0.0125 ng/µL. These results were averaged, and the starting sample for testing was taken to have a quantification value of 0.0129 ng/µL.

4.1.3 Creation of Microcon Batches

Four Microcon batches were created:

Batch 1 was processed using currently available membrane material
- 1 x negative control concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to full

Batch 2 was processed using new membrane material
- 1 negative control concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to 30 µL
- 5 x diluted DNA aliquots concentrated to full

Batch 3 was processed using Recovery process on currently available membrane material
- 1 x negative control
- 5 x diluted DNA aliquots

Batch 4 was processed using Recovery process on new membrane material

- 1 x negative Control
- 5 x diluted DNA aliquots

4.2 Microcon Processing & Recovery of Diluted DNA Samples

4.2.1 C[redacted]con processing for Old and New Microcon membranes

Batches [redacted] concentrated using the current Microcon method for DNA extracts following [redacted] Operating Procedure "Concentration of DNA Extracts using Microcon® Centrifuga[redacted]es" (QIS 19544).

These sa[redacted]en quantified, amplified and profiled and results were compared.

4.2.2 C[redacted]very method for Old and New Microcon membranes

Batches 3 and 4 were spun to membrane dryness. The recovery of the DNA extract was then carried out as per Standard Operating Procedure "[redacted]n of DNA Extracts using Microcon® Centrifugal Filter Devices" (QIS 19544).

These samples were then quantified, amplified and profiled and results were compared.

4.3 DNA Quantitation

DNA Quantitation results were obtained for all samples, concentrated to 30 µL, according to the standard laboratory procedure outlined in the Standard C[redacted]cedure "Real Time PCR Quantification of Human DNA using the Quantifiler Human DNA Quantification Kit (Life Technologies)" (QIS 19977). Samples concentrated to full went straight to amplification at maximum volume of 20 µL.

4.4 Amplification

All samples were amplified with the ABI AmpFℓSTR® Profiler Plus® PCR Amplification Kit at the volumes calculated from the quantitation result. The PCR reaction was setup on a PerkinElmer MultiPROBE® II PLUS HT EX Integration Platform according to the Standard Operating Procedure outlined in QIS method "Amplification of Extracted DNA using the AmpFℓSTR Profiler Plus kit or AmpFℓSTR COfiler Kit" (QIS 19976).

4.5 DNA fragment analysis

All samples had fragment analysis performed according to the Standard Operating Procedure "CE Quality Check of Samples from the AB 3130*xl* Genetic Analyzers" (QIS 17130) and "Procedure for the Use and Maintenance of the 3130*xl* Genetic Analyzers" (QIS 15998).

4.6 Genotyping

All sample results were interpreted through the use of Genemapper® *ID-X* Software according to the Standard Operating Procedure "Procedure for the Interpretation & Acceptance of Results using Profiler & COfiler systems" (QIS 17137).

5 Results and Discussion

5.1 M[redacted] Concentration results

Table 2 Comparison of rec[redacted]etween old and new membranes after Microcon concentration

No.	Mic'n to	Pre Concentration			Post Concentration - Old Membrane					Post Concentration - New Membrane				
		Volume (µL)	Quant (ng/µL)	DNA Template (ng)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile
(Neg) 1	30µL	100	NA	NA	32	undet	NA	NA	NSD	43	undet	NA	NA	NSD
2		80	0.0129	1.032	32	0.0428	1.3696	133	Full	45	0.0162	0.729	71	Full
3		80	0.0129	1.032	29	0.0410	1.189	115	Full	45	undet	no sample in nunc tube		NSD
4		80	0.0129	1.032	29	0.0529	1.5341	149	Full	46	0.0157	0.7222	70	Full
5		80	0.0129	1.032	30	0.0429	1.287	125	Full	47	0.0178	0.8366	81	Full
6		80	0.0129	1.032	30	0.0480	1.44	140	Full	40	0.0181	0.724	70	Full
7	Full	80	0.0129	1.032	7 (20)	0.0505	1.01	98	Full	14 (20)	0.0422	0.844	82	Full
8		80	0.0129	1.032	7 (20)	0.0618	1.236	120	Full	15 (20)	0.0606	1.212	117	Full
9		80	0.0129	1.032	7 (20)	0.0512	1.024	99	Full	13 (20)	0.0409	0.818	79	Full
10		80	0.0129	1.032	6 (20)	0.0705	1.41	137	Full	17 (20)	0.0581	1.162	113	Full
11		80	0.0129	1.032	7 (20)	0.0756	1.512	147	Full	20	undet	NA	NA	Full
Mean of DNA Template Recovered (%)								**126**					**85**	
T-test (p-value) using DNA Template Recovered					**0.0003**									

Note: Numbers in brackets indicate the total volume of DNA sample after adding TE buffer to enable processing on the Multiprobe II instrument.
Sample number 3 processed using the new membrane filters was not quantified or amplified as the DNA sample from the filter was not transferred into the Nunc tube and was not included in the calculations (in red).
Sample number 11 processed using the new membrane filters gave an "undetermined" quantification value but upon amplification, gave a full DNA profile. This is possibly due to pipetting error of the Multiprobe II instrument during quantification setup (in blue).

Table 2 shows at the pre-concentration stage (in yellow), the starting bulk DNA sample had a quantification value 0.0129 ng/µL. 80 µL of this sample was added to 10 old and 10 new membranes, therefore adding 1.032 ng of DNA template per membrane.

The old membranes (in orange) recovered, on average, 126% of DNA template, whereas the new membranes (in blue) recovered, on average, 85% DNA template.

The results appear to have shown that when using the currently available membrane, more DNA was recovered than what was added at the start of the procedure. This indicated that there was a problem with the quantification reaction with some samples showing a falsely elevated result. Following quantification all samples were amplified, due to the small or lack of volume remaining for each sample, repeat quantification was not feasible, as such this experiment was repeated, refer to section 5.3 below)

Although the "percentage of DNA template recovered" calculations appear unreliable, all the samples that was able to be amplified from the old and new membranes, successfully produced full profiles (with exception of the Negative Control which was NSD as expected).

5.2 Recovery Process results

After centrifugation spinning of the samples for more than the expected time until dryness of the membrane, the samples were recovered by adding 10 µL of TE to the membrane, the membran[redacted]y agitated, and then inverted into a new tube where the DNA sample was spun [redacted]ng tube to be quantified and amplified.

Table 3 Comparison o[redacted]NA between old and new membranes after Recovery method

No.	Pre Concentration			Final Results - Old Membranes					Final Results - New Membranes				
	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Profile
(Neg) 1	80	0.0129	1.032	14	undet	NA	NA	NSD	14 (20)	undet	NA	NA	NSD
2	80	0.0129	1.032	5 (20)	0.032	0.630	61	Full	16 (20)	0.027	0.54	52	Full
3	80	0.0129	1.032	5 (20)	0.031	0.620	60	Full	16 (20)	0.030	0.59	57	Full
4	80	0.0129	1.032	5 (20)	0.021	0.426	41	Full	19 (20)	0.058	1.158	112	Full
5	80	0.0129	1.032	5 (20)	0.040	0.794	77	Full	14 (20)	0.043	0.85	82	Full
6	80	0.0129	1.032	5 (20)	0.029	0.588	57	Full	18 (20)	0.045	0.908	88	Full
Mean of DNA Template (%Recovered)				[redacted]			59					78	
T-test of DNA Template Recovered (p-value)				0.1582									

Note: Numbers in brackets indicate the total volume of DNA sample after adding TE buffer to enable processing on the Multiprobe II instrument.

[redacted]

Table 3 shows at the pre-concentration stage (in yellow), the starting bulk DNA sample had a quantification value 0.0129 ng/µL. 80 µL of this sample was added to 5 old and 5 new membranes, therefore adding 1.032 ng of DNA template per membrane.

The old membranes (in orange) recovered, on average, 59% of DNA template, whereas the new membranes (in blue) recovered, on average, 78% DNA template. These results showed that the old membranes were not significantly different to the new membranes (p-value 0.1582).

Although the pre and post DNA samples were quantified the same way as in Section 5.1, where the pre-concentrated DNA sample was quantified on a different batch to the post-concentrated DNA samples, the "percentage of DNA template recovered" was also not deemed a reliable result. However, the Recovery test is performed to primarily assess whether DNA can still be recovered even after the membrane has been spun dry. Therefore, these results can still conclude that the new membrane can recover equivalent and comparable DNA yields to the old membranes.

In addition, all recovered DNA samples successfully produced full profiles (with exception of the negative control which was NSD as expected).

It was also observed that the new membranes appear to lose less liquid in its membrane (approximately 4 µL) as opposed to the old membranes (approximately 8 µL). Also, the new membranes did not fully dry out as fast and retained more liquid above the filter when

compared to the old membranes. This potentially means that it is even less likely that a Recovery process will need to be performed on the new membranes when compared to the old membranes in routine use.

5.3 Repeated Microcon Concentration Experiment

The initial "percentage of DNA template recovered" results (See section 5.1) were deemed unreliable due to the stochastic effects between quantification batches, where the pre-concentra[redacted]ples were quantified on a different batch to the post-concentrated samples r[redacted]significant difference when comparing the old and new membranes.

Therefore[redacted] concentration process was repeated where the pre and post-concentra[redacted]ples was quantified on the same quantification batch. (amplification of the samp[redacted]erformed on this round of testing).

Table 4 Compar[redacted]ered DNA between the old and new membranes after Microcon concent[redacted]ed process)

No.	Mic'n to	Pre Concentration			Post Concentration - Old Membrane				Post Concentration - New Membrane			
		Volume (µL)	Quant (ng/µL)	DNA Template (ng)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)	Volume (µL)	Quant (ng/µL)	DNA Template (ng)	DNA Template Recovered (%)
(Neg) 1	30µL	100	undet	NA	28	undet	NA	NA	32	undet	NA	NA
2		80	0.025	1.984	30	0.065	1.935	98	32	0.033	1.059	53
3		80	0.025	1.984	30	0.046	1.368	69	25	0.055	1.375	69
4		80	0.025	1.984	30	0.056	1.689	85	28	0.034	0.944	48
5		80	0.025	1.984	32	0.038	1.200	60	30	0.039	1.167	59
6		50	0.025	1.240	32	0.019	0.598	48	29	0.021	0.618	50
7	Full	80	0.025	1.984	8 (20)	0.047	0.938	47	13 (20)	0.036	0.722	36
8		80	0.025	1.984	9 (20)	0.064	1.276	64	12 (20)	0.068	1.352	68
9		80	0.025	1.984	7 (20)	0.059	1.178	59	12 (20)	0.048	0.964	49
10		80	0.025	1.984	7 (20)	0.038	0.754	38	12 (20)	0.054	1.088	55
11		80	0.025	1.984	7 (20)	0.047	0.948	48	12 (20)	0.037	0.734	37
Mean of DNA Template Recovered (%)								**62**				**52**
T-test using DNA Template Recovered (p-value)					**0.187**							

Note: Numbers in brackets indicate the total volume of DNA sample after adding TE buffer to enable processing on the Multiprobe II instrument.

Table 4 shows at the pre-concentration stage (in yellow), the starting bulk DNA sample had a quantification value 0.025 ng/µL. 80uL of this sample was added to 10 old membranes and 10 new membranes, therefore adding 1.984 ng of DNA template per membrane (with exception of sample no. 5 where only 50 uL of bulk sample was remaining, this was taken into account when performing final calculations).

The old membranes (in orange) recovered, on average, 62% of DNA template, whereas the new membranes (in blue) recovered, on average, 52% DNA template. These results did not show a significant difference between the old and new membranes (p-value 0.187).

6 Summary and Recommendations

In this project, the performance of a new Microcon® DNA Fast Flow Centrifugal Filter membrane was compared with the now discontinued Microcon® Centrifugal Filters Ultracel® YM-100 membranes. Analysis of parameters including concentration / DNA recovery, spin time, quality of profiles and recovery after dryness / over spinning demonstrated that the new membranes did not perform significantly different to the currently used old membranes.

Therefore, it is recommended that the newly developed Microcon® DNA Fast Flow Centrifugal Filter kits [redacted] suitable replacement for the old membranes for routine use within DNA Analysis [redacted]

Forensic and Scientific Services

Assessment of Low Quantification Value DNA Samples

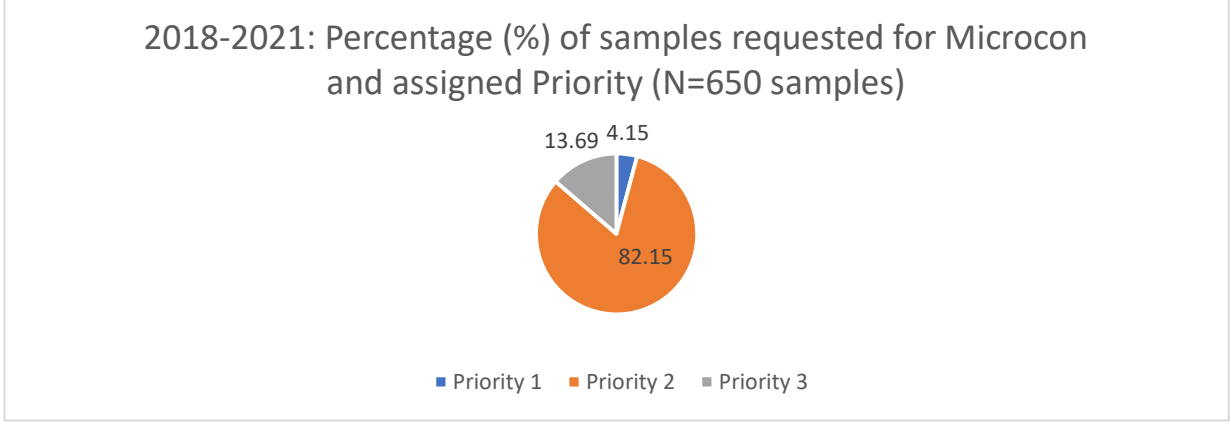
Authors: Cathie Allen, Justin Howes and Paula Brisotto 21 June 2022

Background:

The Queensland Police Service (QPS) implemented a new service model in July 2008, which saw Forensic Officers taking the lead and responsibility for sample selection, examination of some items and case review of forensic results. This change also saw a reduction in case details and case context being supplied to Forensic and Scientific Services (QHFSS). Provision of scientific information to process a sample for DNA profiling remained unchanged. Under this framework, an Options paper was provided to the Superintendent for Forensic Services Group in February 2018 regarding an assessment undertaken to evaluate samples with low quantitation values and subsequent concentration and the DNA profile obtained. The Options paper detailed the assessment of 1449 samples. The QPS selected the option of not DNA profiling samples within a low quantitation range, as a triaging process, and information would be provided electronically on QPRIME (via the Forensic Register) regarding additional work that could be undertaken if requested.

Executive Briefing:

An assessment of all casework DNA samples, with the following criteria was conducted: an initial quantification result of between 0ng/µL and 0.0088ng/µL, underwent a concentration process and reported results issued between 2018 and 2021. This equated to an assessment of 650 DNA samples. The reported DNA result, which may have been completed after one or more amplifications processes, was categorised into two broad categories - 'suitable for comparison purposes' or 'unsuitable for comparison purposes'.



165 DNA samples (25.4%) were categorised as 'suitable for comparison purposes', with most of these samples being major crime samples. 485 DNA samples (74.6%) were categorised as 'unsuitable for comparison purposes' after concentration and amplification processes.

Of the 165 DNA samples categorised as 'suitable for comparison purposes', 41 DNA samples were able to yield a profile suitable for uploading and searching of the National Criminal Investigation DNA Database (NCIDD). This represents 6.3% of total samples selected for processing. This figure is not unexpected as this assessment was based on samples that were actively selected for further processing, determined by either the QPS, QHFSS staff or both organisations using a collaborative approach. This assessment also includes volume crime samples, whereas the previous assessment did not include these samples.

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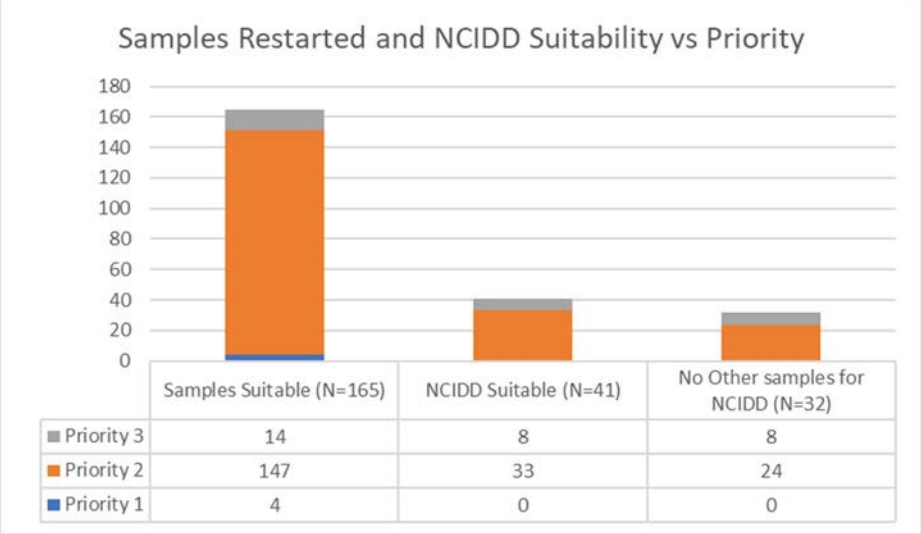
Queen Gover



	Samples Suitable (N=165)	NCIDD Suitable (N=41)	No Other samples for NCIDD (N=32)
Priority 3	14	8	8
Priority 2	147	33	24
Priority 1	4	0	0

Please note the current dataset is different to the previous dataset due to, but not limited to:

- implementation of the statistical interpretation of four-person mixtures (contributes to 5.5% of the total samples deemed 'suitable for comparison purposes in this dataset),
- all DNA samples were selected in this dataset (the previously assessed dataset only included DNA samples assigned to Major Crime cases),
- active selection of samples for processing by either the QPS or Forensic DNA Analysis staff members based on the context of the case or scientific knowledge with respect to the associated parameters from the quantification process,
- if any new instrumentation or consumables were implemented by either the QPS or QHFSS over that period.

Forensic DNA Analysis staff are mindful of consuming all DNA extract when requesting a concentration step. Technologies available in other jurisdictions or future technologies may be applied to DNA extracts, however if all DNA extract has been exhausted through concentration and amplifications processes, no (or very limited) extract will be available for these technologies or for Defence to request external testing. Forensic DNA Analysis staff have limited scope of the case context and other forensic results for the case.

Observations:

Review of quantitation parameters, other than quantitation value, did not identify a discernible trend, however further monitoring of these parameters will continue.

The value of 0.0088ng/µL is based on assessment of the data (and equates to 132 picograms). Validation studies conducted within the laboratory has shown that stochastic effects become apparent from DNA templates below 0.132 ng (132 picograms) making interpretation of the resultant DNA profile more complex.

If a value of 0.0067ng/µL (equating to 100 picograms) is chosen as the threshold for this triage process, DNA samples with a value of between 0.0067ng/µL and 0.0088ng/µL will not be subjected to a concentration step, which may affect the resulting DNA profile.

It was not unexpected that as the quantitation value increases, the ability to yield suitable profiles for interpretation improved.

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Options for Consideration:

1. Continue with the current workflow:
   a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
   b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL (132 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Continue to retain the DNA extract indefinitely, if no request is received.
2. Amend the workflow:
   a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
   b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of 0.001 ng/µL to a newly determined value and process upon request by either the QPS or Forensic DNA Analysis staff members. If requested, the process would include concentration of the DNA sample prior to amplification. Continue to retain the DNA extract indefinitely, if no request is received. DNA samples with a quantitation value of above a newly determined value will be processed as per routine and will not be subject to a concentration process.
   c) The reasoning for a newly determined quantitation value will be agreed upon and documented, including risks.
   d) This amended workflow will require Forensic Register enhancement prior to use.
3. Amend the workflow:
   a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
   b) All priority 2 samples that fall into the quantitation range of either 0.001ng/µL to 0.0088ng/µL or 0.001ng/µL to the newly determined value will be concentrated prior to amplification.
   c) Priority 3 samples that fall into the quantitation range of either 0.001ng/µL to 0.0088 ng/µL or 0.001ng/µL to the newly determined value will be amplified without a concentration step.
   d) This amended workflow will require Forensic Register enhancement prior to use.
4. Amend the workflow:
   a) Priority 1 samples continue to undergo a concentration step prior to amplification if the sample falls into the quantification range of 0.001ng/µL to 0.0088 ng/µL.
   b) Amplify, without concentration, all Priority 2 and 3 samples above 0.001ng/µL value.

Next Steps:

QHFSS will meet with the QPS representatives to discuss the options provided and discuss risks and implications to reach a collaborative decision on the path forward.

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Project 11. Report on the Validation of a manual method for Extracting DNA using the DNA IQ™ System

August 2008

Automation and LIMS Implementation Project Team,

DNA Analysis

Forensic And Scientific Services

Clinical and Scientific Services

Queensland Health

CaSS | Forensic and Scientific Services
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Project 11. Report on the Validation of a Manual Method for Extracting DNA using the DNA IQ™ System

Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C., Ientile, V.
Automation/LIMS Implementation Project, DNA Analysis FSS (August 2008)

1. Abstract

The DNA IQ™ system was found to be the most suitable kit for extracting cell and blood samples that are analysed in DNA Analysis FSS (refer to Project 9). This DNA extraction system, based on magnetic bead technology, was found to generate results that were comparable or better than the current Chelex®-100 protocol. We have validated a manual DNA IQ™ method for extracting DNA from forensic samples, and incorporated studies on sensitivity and consistency, inhibition, substrate type, substrate size, and mixture studies. This manual DNA IQ™ method is suitable for verification on the automated MultiPROBE® II PLUS HT EX extraction platforms.

2. Introduction

A previous evaluation of various DNA extraction systems that were designed specifically for forensic samples was performed in order to select a suitable extraction technology for extracting various sample types that are processed in DNA Analysis FSS. DNA IQ™ was identified as a suitable kit for extracting forensic samples, and was found to outperform both the current Chelex®-100 protocol and also all the other kits evaluated. The results of the evaluation are reported in Project 9 (Gallagher *et al*., 2007a).

DNA purification with silica matrices, either in membrane- or bead-form, commonly uses the affinity of DNA for silica without the need for hazardous organic reagents. However, these systems tend to require extensive washing to remove the guanidium-based lysis buffer. The DNA IQ™ system uses a novel paramagnetic resin for DNA isolation (Promega Corp., 2006). The DNA IQ™ System's basic chemistry is similar to other silica-based DNA isolation technologies, except that the specific nature of the paramagnetic resin, coupled with the formulation of the lysis buffer, is unique. In the DNA IQ™ System, negatively-charged DNA molecules have a high affinity for the positively-charged paramagnetic resin under high salt conditions supplied by the lysis buffer. Once DNA is bound to the magnetic resin, and the resin is immobilised by a magnet, the sample can be washed using an alcohol/aqueous buffer mixture. The high alcohol content of the wash buffer aids to maintain the DNA-resin complex in low-salt conditions, while the aqueous component functions to wash away residual lysis buffer and any inhibitors or non-DNA contaminants such as cellular debris and protein residues. DNA is released from the resin by using a low ionic strength elution buffer, and the purified DNA can be used directly in downstream applications such as PCR.

For samples that are in excess (e.g. reference samples), DNA IQ™ resin will only isolate up to a total of approximately 100ng of DNA due to bead saturation (Huston, 2002).

[redacted] of blood and cell stains on forensic samples using the DNA IQ™ system (Promega Corp., Madison, WI, USA).
[redacted]

4. Equipment and Materials

- DNA IQ™ System (Promega Corp., Madison, WI, USA); 100 samples, Cat.# DC6701), which includes:
    - 0.9mL Resin
    - 40mL Lysis Buffer
    - 30mL 2X Wash Buffer
    - 15mL Elution Buffer
- TNE buffer (10mM Tris, 100mM NaCl, 1mM EDTA, pH 8.0)
- MagneSphere® Magnetic Separation Stand, 12-position (Cat.# Z5342) (Promega Corp., Madison, WI, USA)
- DNA IQ™ Spin Baskets (Cat.# V1221) (Promega Corp., Madison, WI, USA)
- Microtube 1.5mL (Cat.# V1231) (Promega Corp., Madison, WI, USA)
- 95-100% ethanol
- Isopropyl alcohol
- 1M DTT (Sigma-Aldrich, St. Louis, MO, USA)
- Proteinase K (20mg/mL) (Sigma-Aldrich, St. Louis, MO, USA)
- 20% SDS (Biorad, Hercules, CA, USA)
- 0.9% saline solution (Baxter Healthcare, Old Toongabbie, NSW, Australia)
- ThermoMixer Comfort (Eppendorf, Hamburg, Germany)
- Vortex mixer
- Bench top centrifuge
- Cytobrush® Plus Cell Collector (Cooper Surgical, Inc., Trumbull, CT, USA)
- FTA® Classic Cards (Whatman plc, Maidstone, Kent, UK)
- Rayon (155C) and cotton (164C) plain dry swabs (Copan Italia S.p.A., Brescia, Italy)
- Vacuette® K2EDTA blood collection tubes (Greiner Bio-One GmbH, Frickenhausen, Germany)
- Sticky tape (BDF tesa tape Australia Pty Ltd)
- Tannic acid $C_{76}H_{52}O_{46}$ FW1701.25 (Selby's BDH, Lab Reagent >~90%)
- Urea NH_2CONH_2 FW60.06 (BDH, Molecular Biology Grade ~99.5%)
- Indigo carmine $C_{16}H_8N_2Na_2O_8S_2$ FW466.35 PN 131164-100G (Sigma-Aldrich, St. Louis, MO, USA)
- Humic acid sodium salt PN H167520-100G (Sigma-Aldrich, St. Louis, MO, USA)
- Used car motor oil, SW20/SAE50 (Caltex)
- Various clothing materials, including:
    - Best & Less Pacific Cliff, White cotton shirt, XXL
    - Big W Classic Denim, Men's Blue denim jeans, 112
    - Private Encounters, off-white nylon cami, size 14
    - Clan Laird, blue 100% wool kilt
    - Millers Essentials, blue 100% polyester camisole, size 10
    - Unknown, teal green 100% lycra swimwear
    - Leather Belt, brown

5. Methods

5.1 Cell and blood collection

Buccal cells were collected using a modified Cytobrush® protocol (Mulot *et al*., 2005; Satia-Abouta *et al*., 2003). Four donors were chosen. Each donor was asked to brush the inside [redacted] with another Cytobrush®, the other cheek was also [redacted] then resuspended in 2mL of 0.9% saline solution. Multiple collections were taken on different days.

Whole blood was collected from three donors by a phlebotomist as per standard collection procedures in EDTA tubes. Blood samples were refrigerated until spotting onto substrate and cell-counting step.

Table 1 lists the donor sample ID's.

Table 1. List of donor samples used for validating a manual DNA IQ™ method.

Donor ID
Cell samples
D1
D2
D3
D4
Blood samples
D1
D2
D3

5.2 Cell counting

Buccal cell suspensions were diluted using 0.9% saline solution to create a 1/10 dilution of the original sample prior to submitting for cell counting. All counts were performed by the Cytology Department, RBWH (QIS 15393).

Blood cell counting was performed on a 1mL aliquot of the original sample also by the Cytology Department, RBWH (QIS 15393).

5.3 Sensitivity, Reproducibility (Linearity) and Yield

Sensitivity and reproducibility of the DNA IQ™ kit was assessed using dilutions of cell and blood samples.

For cell samples, dilutions were made using a sample from donor 4, diluted in 0.9% saline solution. The dilutions used were:

- Neat
- $^1/_{10}$
- $^1/_{100}$
- $^1/_{1000}$

For blood samples, dilutions were made using a sample from donor 2, diluted in 0.9% saline solution. The dilutions used were:

- Neat
- $^1/_{10}$
- $^1/_{100}$
- $^1/_{1000}$

[redacted] swabs using the above dilutions. The swab heads were removed from the shaft using sterilised scalpel and tweezers. Swab heads were then cut into quarters and each quarter was then added to separate sterile 1.5mL tubes. To each quarter swab, 30µL of each neat sample or dilution was added to

create a total of five replicates. Samples were dried using a ThermoMixer set at 56°C over 2 hours in a Class II biohazard cabinet.

5.4 Inhibition challenge

Quartered cotton swabs in sterile 1.5mL tubes were spotted with 30µL of neat cell suspension and were dried after each addition on a ThermoMixer as described previously. Neat blood samples were also created using the same method.

All the inhibitors except for the motor oil were obtained in powder form. Before making any liquid solution of the powdered inhibitors, research was conducted to determine the likely level of each inhibitor normally encountered in the environment (Hlinka *et al*., 2007). Each solution was made at concentrations based on the information obtained (Table 2).

Table 2. Concentrations of various inhibitors used in the inhibition study.

Inhibitor	Excess/Neat Solution	Mass	Volume H_2O	Final inhibitor concentration
Tannic acid	Excess	600mg	500µL	0.705M
	Neat	200mg	500µL	0.235M
Humic acid	Excess	1g	5mL	20% (w/v)
	Neat	0.1g	5mL	2% (w/v)
Indigo carmine	Excess	0.47g	10mL	100mM
	Neat	0.047g	10mL	10mM
Urea	Excess	0.06g	1mL	1M
	Neat	0.021g	1mL	0.33M

A total of 30µL of each solution containing specified concentrations of various inhibitors was applied to the buccal cell and blood swabs prepared above. The only exception was motor oil, where only 15µL was added to the cell and blood swabs respectively. Each inhibitor sample was replicated in quadruplicate and left to dry overnight in a Class II biohazard cabinet.

To another set of prepared cell and blood swabs, an excess of each inhibitor was applied in quadruplicate for each inhibitor and allowed to dry overnight. This process was achieved by applying another solution of inhibitor exceeding the normal level (Hlinka *et al*., 2007).

5.5 Substrates

Swabs

Four cotton and four rayon swab quarters in sterile 1.5mL tubes were loaded with 30µL of neat cell or blood sample and were extracted once the sample had dried on the swab.

Tapelifts

Two donors were sampled using the tape most commonly used within the laboratory (BDF tesa tape). Strips of tape were firmly applied to the inside of the fore arm and lifted off. This process was then repeated until the tape was no longer adhesive. The tape was wrapped around sticky-side-in, forming a cylinder shape, and placed in a sterile 1.5mL tube. These samples were created in quadruplicate. Tape was not used as a substrate in the blood validation [redacted]

The material types tested included: [redacted]

- White 100% cotton shirt;
- Blue 100% wool kilt;
- Teal green 100% lycra swimwear;
- White 100% nylon camisole;
- Blue 100% polyester camisole; and
- Brown 100% woven leather belt.

All material types except leather were sampled and ten 2.5cm x 2.5cm pieces were cut from each material and washed in 10% bleach following an in-house washing method to remove any contaminating DNA from outside the laboratory (Gallagher *et al*., 2007b). As for the leather, one strand of the leather weave was cut from the belt and washed following the same method. Once dry, the material was then cut into 0.5cm x 0.5cm pieces using sterile techniques, placed in 1.5mL tubes and 30µL of both cell sample and blood was applied to separate pieces. Each substrate sample was created in quadruplicate and dried on a ThermoMixer set at 56°C over 2 hours in a Class II biohazard cabinet.

Gum
Two types of chewing gum were chosen: (1) Wriggley's Extra White (peppermint flavour) and (2) Wriggley's Extra Green (spearmint flavour). The donor was asked to chew the gum for 30 minutes and dispose of the gum into a clip-seal plastic bag. The gum was then air dried in a Falcon tube overnight before it was frozen for roughly an hour before cutting into 3mm x 3mm x 3mm pieces and placed into sterile 1.5mL tubes. Gum substrates were not assessed for blood samples.

Cigarette butts
Two brands of cigarettes were smoked all the way through and then the butts collected. The filter paper of the butt was cut into 0.5mm^2 pieces and placed into sterile 1.5mL tubes. Cigarette butts were not assessed for blood samples.

FTA® Classic Card punches
Eight sterile 1.5mL tubes, each containing four 3.2mm FTA® Classic Card punches, were spotted with 30µL of cells or blood before being dried on a ThermoMixer. Four replicates contained sample from one donor, the other remaining four replicate tubes had a different donor sample added.

5.6 Mixture studies

Buccal cells and whole blood were obtained from a male and female donor. Dilutions were made using 0.9% saline solution to ensure that the cell concentration was equal. Dilutions were then performed on the male sample to obtain the correct ratios.

Mock samples were created using the following ratios of female to male:

- 1:1,
- 1:2,
- 1:10,
- 1:25,
- 1:50 and
- 1:100.

[redacted] was spotted first on to a quarter of a cotton swab [redacted] before adding another 30µL of the male component. Samples were created in quadruplicate for all ratios, for both cell and blood [redacted]

5.7 Substrate size

Various sizes of material were cut from a white cotton shirt:

- 0.5cm x 0.5cm,
- 1cm x 1cm,
- 2cm x 2cm.

Each piece of material was stored in individual, sterile 1.5mL tubes and 30µL of cell sample was added to the material and allowed to dry on a ThermoMixer. The same process was followed for blood samples. Five replicates were made for each sample type.

5.8 Extraction using the DNA IQ™ System (Promega Corp.)

The manual DNA IQ™ method used was based on an automated protocol developed by the Centre of Forensic Sciences (CFS) in Toronto, Ontario (PerkinElmer, 2004). A Proteinase K – SDS Extraction Buffer was made as per the recommended protocol. The 1x Extraction Buffer for one sample consisted of:

277.5µL TNE buffer
15µL Proteinase K (20mg/mL)
7.5µL 20% SDS

The TNE buffer consisted of:

1.211g Tris (10mM Tris)
2mL 0.5M EDTA (1mM EDTA)
5.844g NaCl (100mM NaCl)

The adapted manual DNA IQ™ protocol is described below:

1. Set one ThermoMixer at 37^{o}C and another at 65^{o}C.
2. Ensure that appropriately sized samples are contained in a sterile 1.5mL tube. For every sample, prepare three set of labelled tubes: spin baskets (for every tube except the extraction control), 2mL SSI tubes and Nunc™ tubes.
3. Prepare Extraction Buffer and add 300µL to each tube. Close the lid and vortex before incubating the tubes at 37°C on the ThermoMixer at 1000rpm for 45 minutes.
4. Remove the tubes from the ThermoMixer and transfer the substrate to a DNA IQ™ Spin Basket seated in a labelled 1.5mL Microtube using autoclaved twirling sticks. Then transfer the liquid to a labelled 2mL SSI sterile screw cap tube.
5. Centrifuge the spin basket on a benchtop centrifuge at room temperature for 2 minutes at its maximum speed. Once completed, remove the spin [redacted] remaining solution and pool with the original [redacted] sterile screw cap tube, then vortex.
6. Add 550 µL of Lysis Buffer to each tube. [redacted]

7. Dispense 50µL of DNA IQ™ Resin – Lysis Buffer solution (7µL Resin in 43µL Lysis Buffer) to each tube. Invert the resin tube regularly to keep the beads suspended while dispensing to obtain uniform results.

8. Vortex each tube for 3 seconds at high speed then place in a multitube shaker set at 1200rpm to incubate at room temperature for 5 minutes.

9. Vortex each tube for 2 seconds at high speed before placing the tubes in the magnetic stand. Separation will occur instantly.

   Note: If resin does not form a distinct pellet on the side of the tube, or if the pellet has accidentally mixed with the solution while in the stand, vortex the tube and quickly place back in the stand.

10. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube. If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

11. Remove the tube from the magnetic stand; add 125µL of prepared Lysis Buffer and vortex for 2 seconds at high speed.

12. Return tube to the magnetic stand, allow for separation and then remove and discard the Lysis Buffer.

13. Remove tube from the magnetic stand; add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed.

14. Return tube to the magnetic stand, allow for separation and then remove and discard all Wash Buffer.

15. Repeat Steps 13 to 14 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.

16. In a biohazard cabinet, place the lids of the tubes upside down on a Kimwipe, in their respective order, and the tubes into a plastic rack, and air-dry the resin for 5-15 minutes at room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA. Once dry, screw on the lids.

17. To each samples then add 50µL of Elution Buffer very gently on the top of the magnetic pellet. Do not mix.

18. Close the lid and then incubate the tubes in the ThermoMixer at 65°C for 3 minutes with no shaking and another 3 minutes shaking at 1100 rpm.

19. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand. Tubes must remain hot until placed in the magnetic stand or yield will decrease.

20. Carefully transfer the supernatant containing the DNA to the respective [redacted]

21. Repeat step 17 to 20, transferring the supernatant to the appropriate Nunc™ tube. The final volume after the second elution should be approximately 95µL.

    Note: DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

5.9 DNA quantitation

All DNA extracts were quantified using the Quantifiler™ Human DNA Quantitation kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19977. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

5.10 PCR amplification

DNA extracts were amplified using the AmpFℓSTR® Profiler Plus® kit (Applied Biosystems, Foster City, CA, USA) as per QIS 19976. Reaction setup was performed on the MultiPROBE® II PLUS HT EX (PerkinElmer) pre-PCR platform.

5.11 Capillary electrophoresis and fragment analysis

PCR product was prepared for capillary electrophoresis using the manual 9+1 protocol (refer to Project 15 and QIS 19978). Capillary electrophoresis was performed on an ABI Prism® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) under the following conditions: 3kV injection voltage, 10 sec injection time, 15kV run voltage, 100µA run current, and 45min run time. Data Collection Software version 1.1 was used to collect raw data from the ABI Prism® 3100 Genetic Analyzer. Fragment size analysis was performed using GeneScan 3.7.1. Allele designation was performed using Genotyper 3.7, with thresholds for heterozygous and homozygous peaks at 150 and 300 RFU respectively. The allelic imbalance threshold is 70%.

[redacted]

6. Results and Discussion

6.1 Donor sample cell counts

Aliquots of buccal cell samples were counted at Cytology Department (RBWH) to determine the concentration of viable cells, in order to better estimate the number of cells at any particular dilution. A white cell count was not performed on all the blood samples, and therefore an estimate on the number of nucleated cells could not be determined.

6.2 Sensitivity, consistency and yield

To ensure the reliability and integrity of results for samples containing small amounts of DNA, a sensitivity study was conducted to determine the lowest concentration of DNA that provides reliable results. A consistency study was combined into the sensitivity experiment to determine the maximum acceptable difference between the results obtained. All samples were extracted in identical conditions by the same operator at the same time to minimise variability.

The cell sample used for the experiments was from donor sample 4A, which was counted to be around 3,680 nucleated cells (x 10^6/L). The blood sample used was from donor 6A, which was counted to be around 2,540 nucleated cells (x 10^6/L). The estimated amount of DNA present in each dilution is outlined in Table 3.

Table 3. Amount of DNA in each dilution, as calculated from the cell count.

Sample type	Dilution factor	Number of cells (/µL)	gDNA (ng/µL)	Theoretical total DNA on swab (ng)
Cells	Neat	3680	23.552	706.56000
	1/10	368	2.3552	70.65600
	1/100	36.8	0.23552	7.06560
	1/1000	3.68	0.023552	0.07656
Blood	Neat	2540	16.256	487.68000
	1/10	254	1.6256	48.76800
	1/100	25.4	0.16256	4.87680
	1/1000	2.54	0.016256	0.48768

The DNA yields resulted from extracting the above cell dilutions using the DNA IQ™ System is outlined in Table 4. Blood samples produced higher yields compared to cell samples. On average, blood samples on cotton swabs generated the highest yields. Cell samples on rayon and cotton swabs generated similar yields. All blood dilutions down to 1/1000 produced quantitation results, but cell samples only produced reliable quantitation results down to 1/100 dilution, possibly due to the effects of cell clumping.

[redacted]

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Table 4. DNA quantitation data for diluted cell and blood samples on rayon and cotton substrates.

Sample type	Dilution factor	Theoretical Input DNA (ng)	Rayon swab yield (ng)	Alleles	Cotton swab yield (ng)	Alleles	Rayon average yield (ng)	Rayon Std Dev	Recovery Rayon (%)	Cotton average yield (ng)	Cotton Std Dev	Recovery Cotton (%)
Cells	Neat	706.56000	110.0000	18	117.0000	18	134.5400	41.30	19.04	95.2800	32.69	13.48
			130.0000	18	124.0000	18						
			160.0000	18	46.8000	18						
			83.7000	7	76.6000	18						
			189.0000	17	112.0000	18						
	1/10	70.65600	10.1000	18	12.8000	18	10.4520	1.44	14.79	10.4820	2.52	14.84
			12.7000	18	6.3100	18						
			9.5500	18	11.5000	18						
			9.0100	18	10.1000	18						
			10.9000	18	11.7000	18						
	1/100	7.06560	0.6350	0	0.0000	0	0.9254	0.64	13.10	0.1270	0.18	1.80
			0.4930	0	0.0000	0						
			1.4000	5	0.2770	0						
			1.7900	14	0.3580	0						
			0.3090	0	0.0000	0						
	1/1000	0.7656	0.0000	0	0.3630	0	0.0166	0.04	2.17	0.0726	0.16	9.48
			0.0000	0	0.0000	0						
			0.0000	0	0.0000	0						
			0.0831	0	0.0000	0						
			0.0000	0	0.0000	0						
Blood	Neat	487.68000	216.0000	18	718.0000	18	317.0000	102.36	65.00	447.0000	196.46	91.66
			447.0000	18	297.0000	18						
			215.0000	18	595.0000	18						
			383.0000	7	326.0000	18						
			324.0000	18	299.0000	18						
	1/10	48.76800	113.0000	18	126.0000	18	124.7800	28.10	255.86	97.6600	21.66	200.25
			107.0000	18	91.9000	18						
			145.0000	18	75.4000	18						
			95.9000	18	81.0000	18						
			163.0000	18	114.0000	18						
	1/100	4.87680	14.3000	18	15.9000	18	12.4800	1.62	255.91	16.7600	4.69	343.67
			12.5000	13	12.1000	18						
			13.2000	18	20.8000	18						
			9.9000	18	22.4000	18						
			12.5000	18	12.6000	18						
	1/1000	0.48768	0.7300	18	2.3700	18	0.8894	0.20	182.37	3.0200	0.85	619.26
			0.6990	18	3.1300	18						
			1.1800	18	3.6300	18						
			0.8670	18	1.9700	18						
			0.9710	18	4.0000	18						

The average yield observed within cell and blood samples on either rayon or cotton swabs were comparable (Figure 1). Some inconsistencies were present in cell samples at the lower dilutions of 1/100 and 1/1000 due to unreliable quantitation data at these low dilutions. Blood samples were found to generate higher average yields than cell samples and gave unexpectedly higher recovery values, despite the fact that the input DNA amount was 2-fold higher for cells compared to blood samples (Table 4). This discrepancy may have arisen from inconsistencies in cell suspension uniformity during dilutions of the original cell or blood sample, resulting in inaccurate estimates for average cell concentrations.

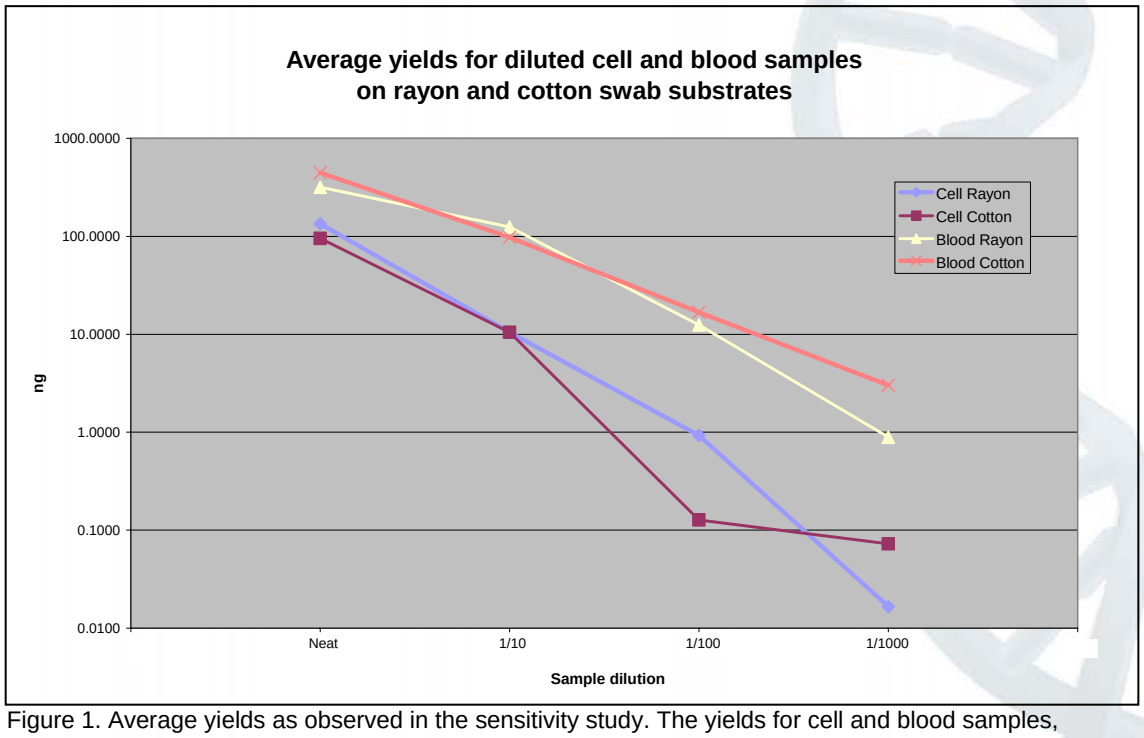


Figure 1. Average yields as observed in the sensitivity study. The yields for cell and blood samples, on two different swab types, were comparable as indicated by overlapping lines on the graph.

The dilution factor was, however, accurately reflected in the average yield for the various dilutions as displayed in Table 4 and Figure 2. An exception to this was the average yields for the neat dilutions (Figure 2). DNA IQ™ isolates a maximum of 100ng DNA as the resin is present in excess, and the system becomes more efficient with samples containing less than 50ng of DNA. Because the amount of DNA was in excess in neat samples, the observed yields varied from sample-to-sample. According to the manufacturer, the DNA IQ™ Database Protocol should be used for samples containing more than 100ng DNA to result in more consistent concentrations between the samples (Huston, 2002).

All five replicates for each neat dilution displayed the highest yields for each dilution series, as expected (Figure 2). For blood samples on rayon and cotton swabs, yields were still [redacted] 00 dilution (Figure 3).

[redacted]

[redacted]

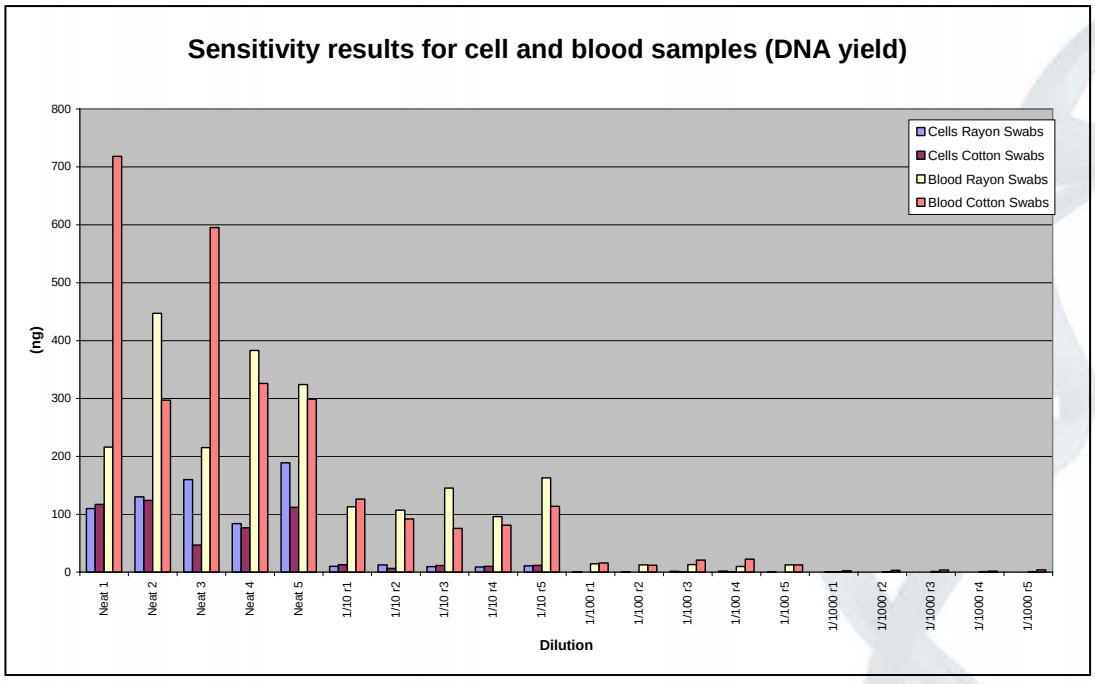
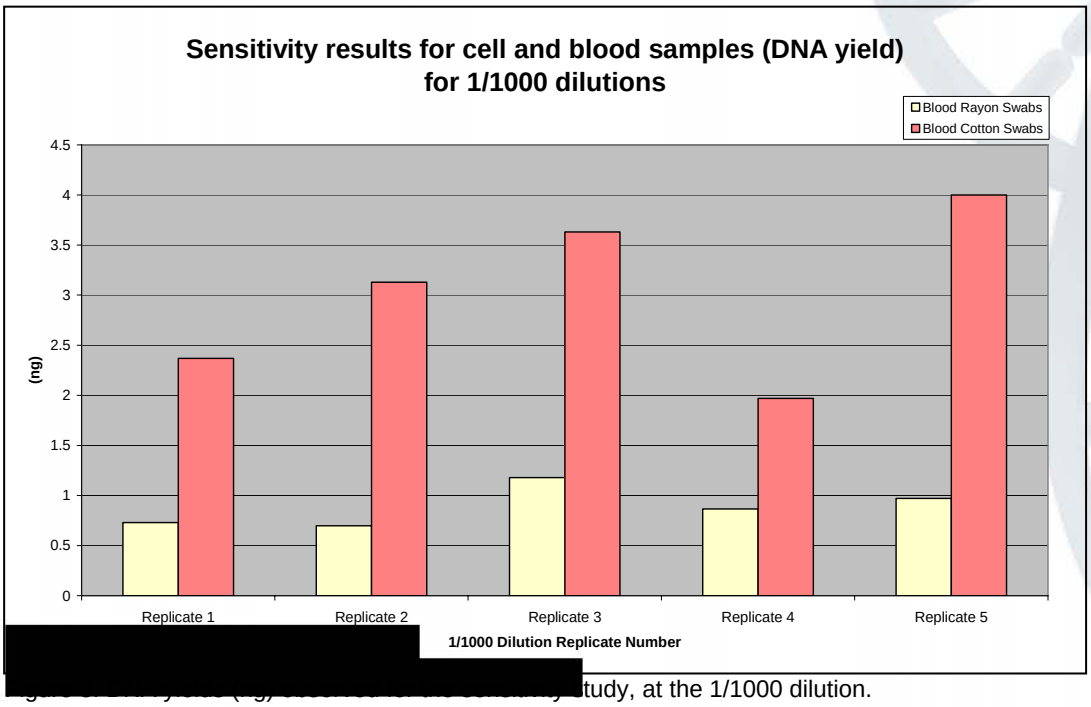


Figure 2. DNA yields (ng) observed for the sensitivity study. As expected, neat samples provided the highest yields. Yields were obtained down to 1/1000 for blood samples and 1/100 for cell samples.



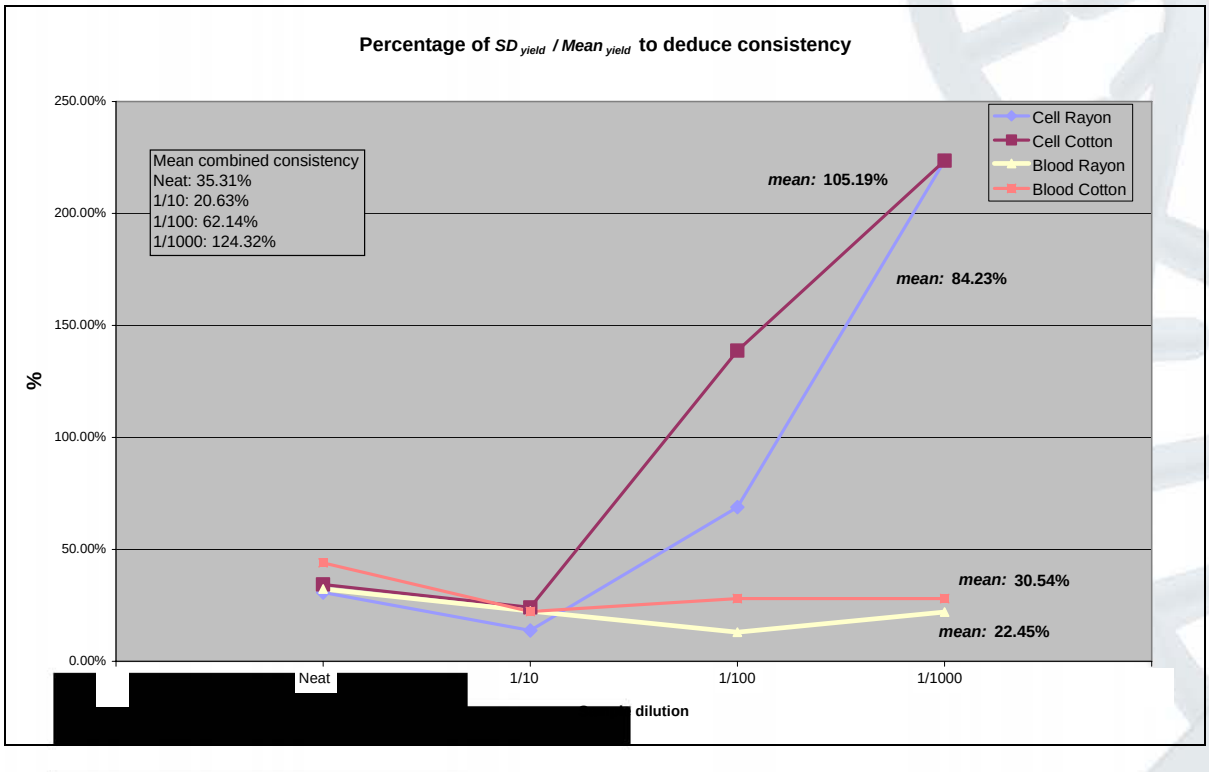
[redacted] study, at the 1/1000 dilution.

[redacted]

When amplified using a 9-locus STR system, all neat samples produced the expected full DNA profile (18/18 alleles), although one outlier was encountered for a cell rayon sample which produced a 7/18 partial profile (Table 4). For cell samples, full profiles could be obtained for samples that were diluted down to 1/10, with partial profiles generated from samples diluted to 1/100. For blood samples, full profiles were generally obtained from all dilutions down to 1/1000. Although two partial profiles were encountered in blood samples on rayon swabs, all blood cotton swabs produced full profiles at all dilutions.

The apparent discrepancy between the results for cell and blood samples can be attributed to inaccurate cell counts or non-uniform sample suspensions when creating the dilutions, as caused by cell clumping or cellular breakdown and precipitation.

For five replicates of each dilution, consistency was observed to vary depending on the dilution (Figure 4). Consistency, as an indication of reproducibility, was calculated as the percentage of the yield standard deviation over five replicates divided by the mean yield of all five replicates (%[SD_{yield} / $mean_{yield}$]). A value closer to 0% indicates minimal sample-to-sample variation and therefore the results are highly consistent. The mean combined reproducibility for all neat, 1/10, 1/100 and 1/1000 dilutions were 35.31%, 20.63%, 62.14% and 124.32% respectively (Figure 4), indicating that there was high reproducibility between the neat and 1/10 dilutions across the four sample types, and reduced reproducibility at the lower 1/100 and 1/1000 dilutions. Overall, the blood samples on rayon and cotton both exhibited high reproducibility across all dilutions at an average of 30.54% and 22.45% respectively (Figure 5). The cell rayon and cotton samples were more variable across all dilutions, producing lower reproducibility at an average of 84.23% and 105.19% respectively (Figure 5). The poor performance of the cell samples can be attributed to inconsistencies in quantitation data observed at the lower 1/100 and 1/1000 dilutions.



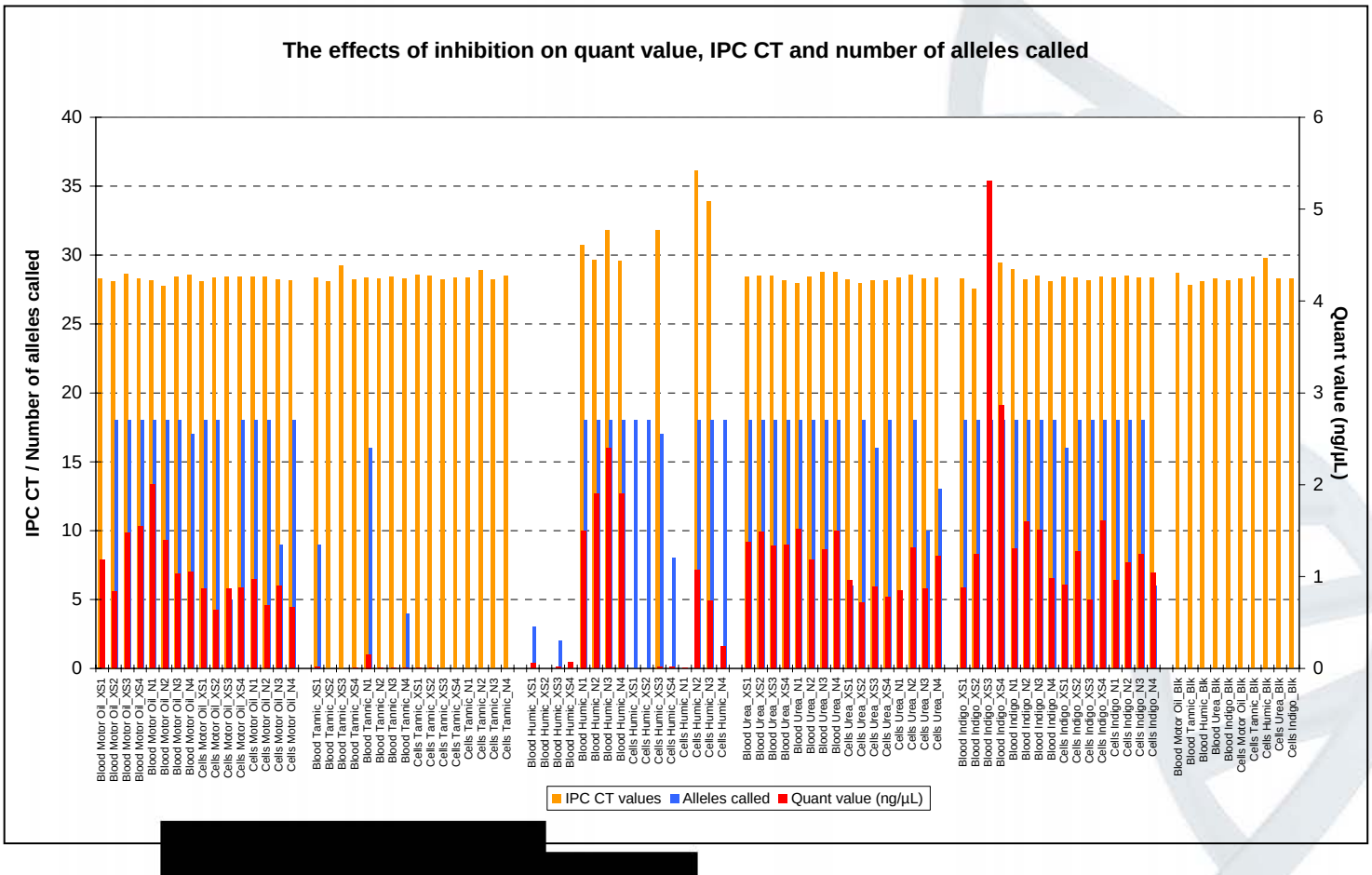
[redacted]cates for cell and blood samples diluted down to 1/1000.

6.3 Inhibition

Forensic samples that are commonly submitted for DNA analysis often contain inhibitors. These inhibitors may inhibit or significantly reduce the efficiency of a DNA extraction system, either by interfering with cell lysis or interfering by nucleic acid degradation or capture, therefore manifesting as extraction inhibitors (Butler, 2005). Inhibitors can also co-extract with the DNA and inhibit downstream PCR amplification processes, therefore acting as PCR inhibitors (Butler, 2005). For example, inhibitors such as hemoglobin and indigo dye likely bind in the active site of the *Taq* DNA polymerase and prevent its proper functioning during PCR amplification.

For the inhibition study, five substances were chosen for their known ability to inhibit PCR and their likelihood of appearing in routine casework samples:

- Indigo carmine: a component of the blue-dye encountered in denim jeans (Shutler, *et al*., 1999).
- Tannic acid: a chemical used in the leather tanning process.
- Urea: a component of urine (Mahony *et al*., 1998).
- Humic acid: a component found in soil and soil products (Tsai and Olson, 1992).
- Motor oil: contains various hydrocarbons and ethanolic compounds that can inhibit PCR.



[redacted]

Figure 3: Effects of various inhibitors on quant value, IPC CT and number of resolved alleles.

[redacted]

The observed effects of these inhibitors at neat and excess concentrations on the ability to extract, quantify and amplify various DNA samples are graphed in Figure 5. Samples were quantified using the Quantifiler™ Human DNA Quantitation Kit (Applied Biosystems) as this kit includes a built-in inhibition detector. Reaction efficiency and the presence of inhibitors can be assessed based on the performance of the internal positive control (IPC), which is known to be detected in this laboratory at around 28 cycles.

The observations that were made include:

- Samples that were spiked with motor oil, urea and indigo carmine dye did not show inhibition as determined by the IPC, and resulted in quantifiable DNA templates after extraction using DNA IQ™. The average DNA concentration observed for all samples was around 1ng/µL. The majority of samples yielded full DNA profiles, with the exception of several cell samples that were treated with urea (both at excess and neat concentrations).
- Blood and cell samples that were spiked with tannic acid did not show inhibition in Quantifiler™, as the IPC performed as expected. However, almost no amplifiable template DNA could be quantified and the majority of samples did not produce DNA profiles. This suggests that the original template DNA was degraded by application of tannic acid to the sample. It should be mentioned at this point that the tannic acid used was in the form of a yellow-brown paste substance that was applied directly to the sample swabs. The tannic acid paste, even at the neat concentration, may have been strong enough to severely fragment DNA to result in non-amplifiable templates. It was observed that three blood samples (1 with tannic acid in excess and 2 with tannic acid at neat concentration) yielded partial profiles (between 4-16 reportable alleles), and none of the cell samples produced reportable alleles. This may be caused by: (1) the concentration of viable cells in the buccal cell samples was lower than blood samples; (2) the drying of the blood stain on the substrate may have created a better barrier to protect the blood components from the degradative effects of the tannic acid.
- Blood and cell samples that were treated with humic acid in excess appeared to retain inhibition after extraction using DNA IQ™. However, at neat concentration, the effect of the humic acid inhibitor was overcome and amplifiable DNA template was purified as demonstrated by high DNA concentration yields. Residual inhibition was still present at neat concentration, as evidenced by higher CT values for the IPC (closer to 30), but full profiles were still produced. For some cell samples with humic acid in excess, the Quantifiler™ data suggested full inhibition (undetermined IPC CT and quantitation results), but two samples resulted in full DNA profiles.
- All reagent blanks were undetermined, indicating the absence of contamination in the results.

The results show that the DNA IQ™ system could be used to extract blood or cell samples that were spiked with motor oil, urea and indigo carmine at both excess and neat concentrations. Blood samples that contained humic acid in excess did not yield amplifiable template DNA, but 2 out of 4 cell samples with humic acid in excess appeared to produce full profiles. Samples that were exposed to tannic acid, at both neat and excess concentrations, resulted in non-amplifiable DNA, but the inhibitor was effectively washed out of the extract by DNA IQ™ as evidenced by the amplification of the IPC at the expected CT. Based on these results, we conclude that the DNA IQ™ system effectively removes inhibitors that are present in the original sample, resulting in a DNA extract that is of sufficient quality and is suitable for PCR amplification.

[redacted]

[redacted]

6.4 Substrates

The substrate types examined included: swabs (cotton and rayon), tapelifts, fabric (denim, cotton, wool, lycra, nylon, polyester, leather), gum, cigarette butts, and FTA® paper. Cell and blood materials were spotted on to the substrates and extracted using DNA IQ™. The results for the two different sample types are presented in Figures 6 and 7 below.

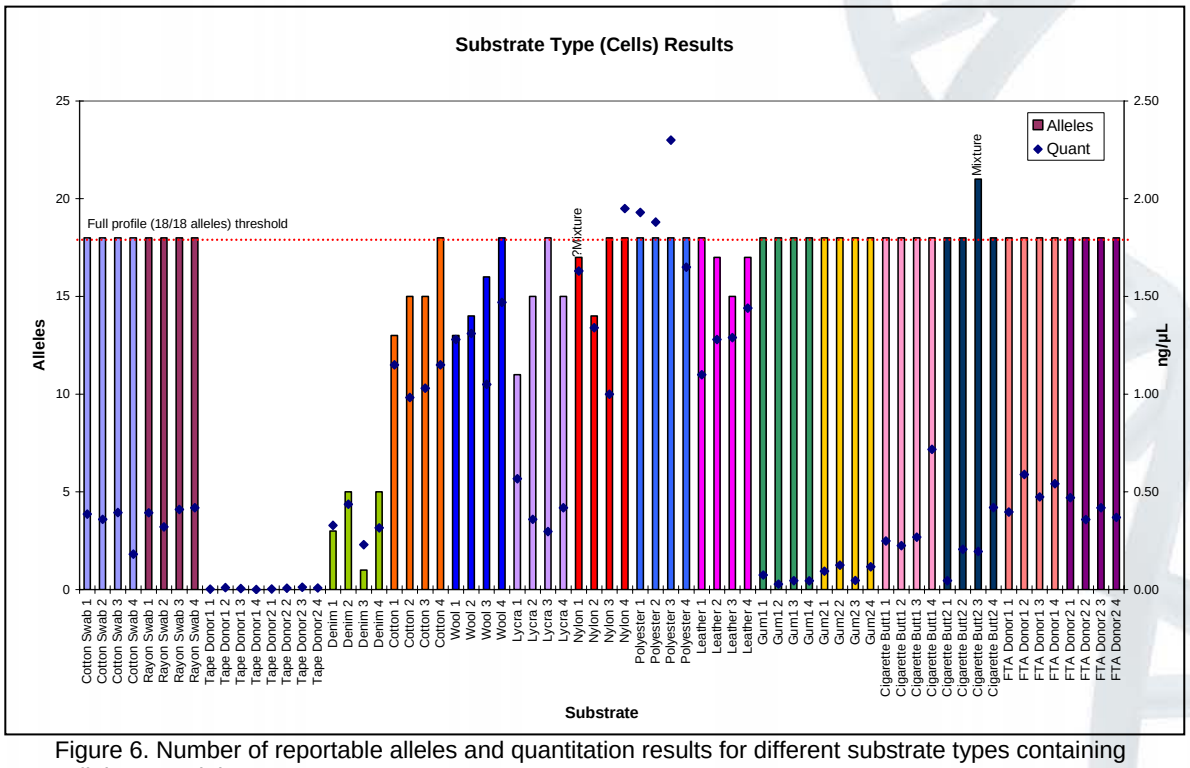


Figure 6. Number of reportable alleles and quantitation results for different substrate types containing cellular material.

[redacted]

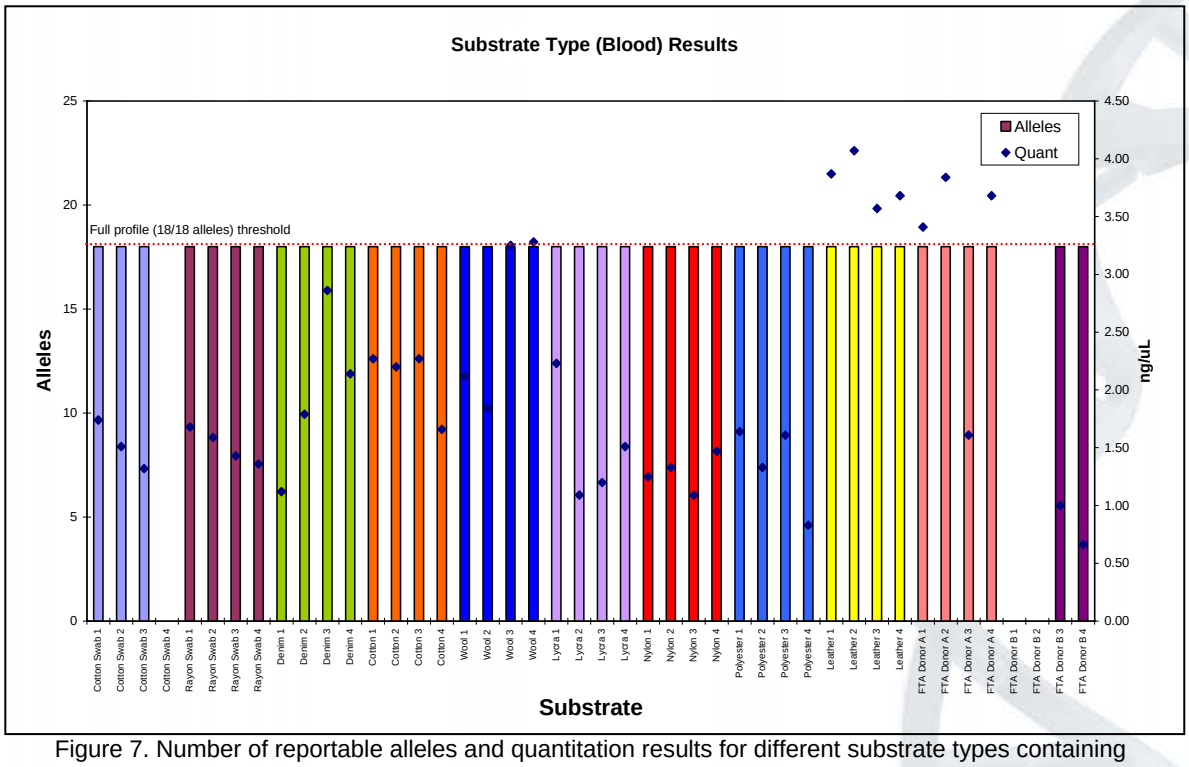


Figure 7. Number of reportable alleles and quantitation results for different substrate types containing blood material.

For cell samples:

- Full DNA profiles (18/18 alleles) were obtained from samples on cotton and rayon swabs, gum, cigarette butts and FTA® paper.
- The quantitation results for most of these samples were less than 0.5ng/µL. For gum samples, the average quantitation result was 0.072ng/µL, and therefore a PCR amplification at maximum volume (20µL) resulted in a total input DNA amount of 1.44ng which is sufficient to result in a full DNA profile.
- Tapelift samples gave an average quantitation result of 0.006ng/µL (just 0.002ng/µL higher than the observed background), and yielded no reportable alleles at all.
- The performance of clothing substrates was variable.
    - Cells on denim yielded quantitation results less than 0.5ng/µL but only partial profiles (maximum 5 reportable alleles), although Quantifiler™ results did not indicate any inhibition of the IPC. The poor performance of these samples may have been a result of sample preparation due to cell clumping.
    - Cells on cotton, wool and nylon resulted in higher quantitation values than lycra, but all substrates generated a similar number of reportable alleles (mean = 14 alleles). Only 25% of samples generated full DNA profiles.
    - [redacted]

For blood samples:

- All substrate types generated full DNA profiles.
- On average, the DNA quantitation results for all blood samples was greater than those resulted from cell samples. This is as per expected and was observed previously (see Project 9 report), because the concentration of nucleated cells in the blood samples were hypothesised to be higher than the concentration of buccal cell samples.
- Because of processing error, data was not available for the following samples: Cotton Swab 4, FTA Donor B 1 and FTA Donor B 2.

The results above are initial amplification results that do not take into account any reworking options.

We found that samples on tapelift substrates performed the worst; however this was probably due to the sampling method devised for this experiment, which did not adequately sample a sufficient number of cells.

6.5 Mixture studies

A mixture study was performed as part of the validation, however the results are not presented in this document because the mixture ratio was found to be inaccurate because cell counts were not performed on the saliva samples. Therefore, little information could be deduced from these results.

6.6 Substrate size

Blood on cotton swabs produced full DNA profiles for all sample sizes, ranging from 0.5 x 0.5cm to 2.0 x 2.0cm (Figure 8). Cells on cotton swabs did not perform as well (Figure 8), possibly due to the nature of the cells and difficulties in obtaining full DNA profiles from cell samples as observed in previous experiments.

Although the same starting amount of sample was used, it was observed that the 0.5 x 0.5cm samples generated higher quantitation results (therefore, also higher yields) compared to the 2.0 x 2.0cm samples (Figure 8). It appears that extraction efficiency decreases as the substrate surface area increases. This may be due to insufficient mixing and distribution of the lysis buffer over a larger substrate surface area, causing insufficient lysis of cellular material. This observation is in line with other reports that the DNA IQ™ system works more efficiently with smaller samples (Promega, 2006). The resulting IPC CT fell within the narrow range of 27.91 – 28.43 (mean = 28.10), indicating that both small and larger samples resulted in DNA extracts of similar quality, but the overall yield was lower for larger substrates (Figure 8 & 9).

[redacted]

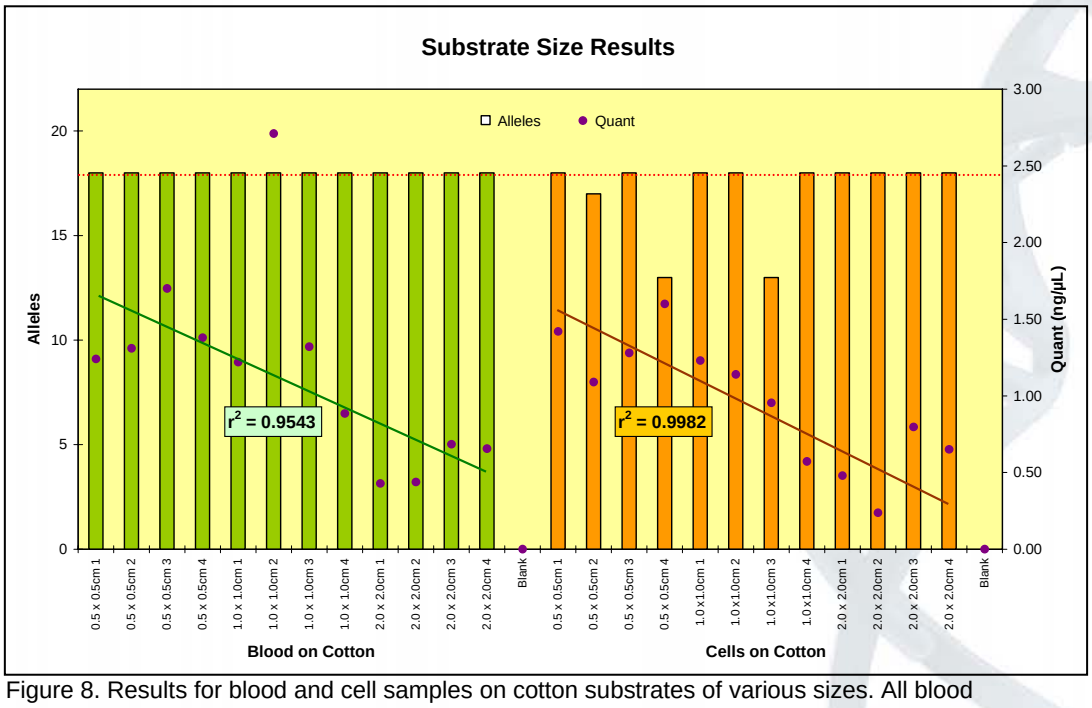


Figure 8. Results for blood and cell samples on cotton substrates of various sizes. All blood samples generated full profiles, but cell samples were more variable. The quantitation results for 0.5 x 0.5cm samples were higher than those for 2.0 x 2.0cm samples (blood r^2 = 0.9543*; cell r^2 = 0.9982; *Note: an outlier was removed from the calculation).

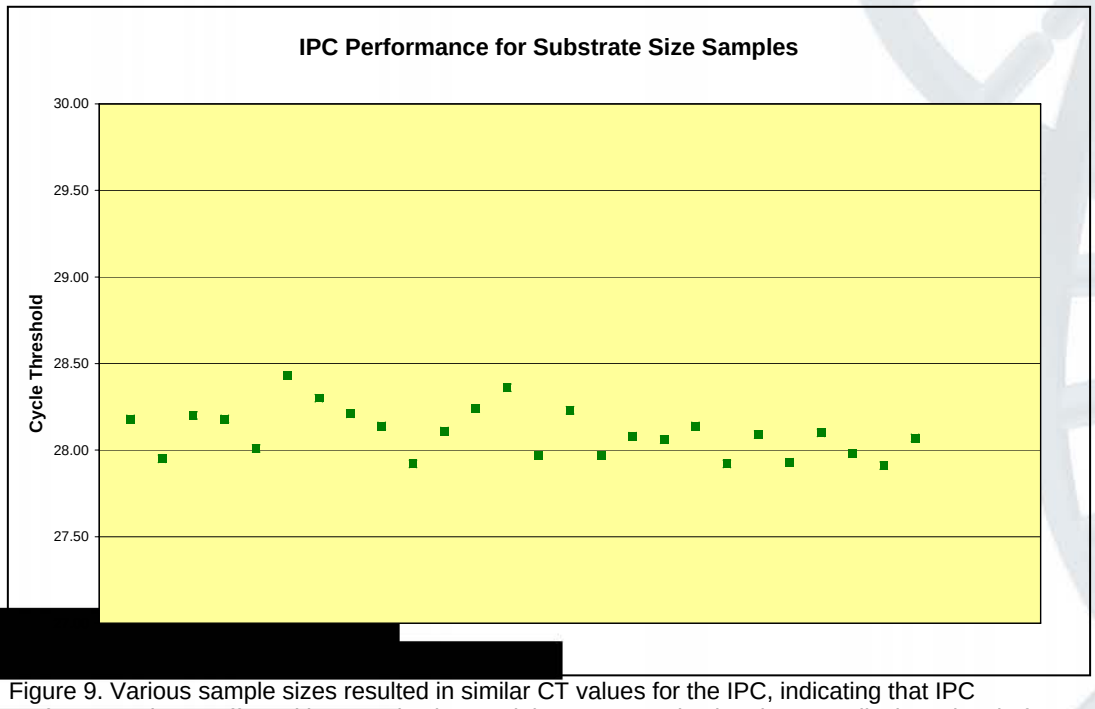


Figure 9. Various sample sizes resulted in similar CT values for the IPC, indicating that IPC [redacted] e size, and that one sample size does not display a level of inhibition that is different to another sample size.

7. Summary and Recommendations

Based on the findings of this validation report, we recommend:

1. To enable processing of cell and blood samples using the validated manual DNA IQ™ protocol, except for samples on tapelift substrates.
2. To design and verify an automated protocol of the validated DNA IQ™ method for use on the MultiPROBE® II PLUS HT EX platforms, for processing blood and cell samples.

8. Acknowledgements

We wish to thank the Cytology Department at the Royal Brisbane and Women's Hospital for assistance with the cell-counting protocols.

9. References

Butler JM (2005). Forensic DNA Typing: Biology, Technology, and Genetics of STR Markers, 2nd Edition. Elsevier: Burlington, MA, USA.

Gallagher B, Hlinka V, Iannuzzi C, Lundie G, Muharam I, Nurthen T, Ientile V (2007a). Project 9: Report on the Evaluation of Commercial DNA Extraction Chemistries [Laboratory Report]. DNA Analysis, FSS: Brisbane, QLD, Australia.

Gallagher B, Hlinka V, Muharam I, Iannuzzi C, Lundie G, Nurthen T, Ientile V (2007b). Mock sample creation for cell and blood samples [Laboratory Report]. DNA Analysis FSS: Brisbane, QLD, Australia.

Hlinka V, Gallagher B, Iannuzzi C, Lundie G, Muharam I, Nurthen T, Ientile V (2007). Evaluation of the effect of inhibitors as assessed by a silica-based DNA extraction method and real-time PCR [in preparation].

Huston K (2002). DNA IQ™ System "Frequently Asked Questions". *Profiles in DNA* 5(1):11-12.

Mahony J, Chong S, Jang D, Luinstra K, Faught M, Dalby D, Sellors J and Chernesky M (1998). Urine specimens from pregnant and nonpregnant women inhibitory to amplification of *Chlamydia trachomatis* nucleic acid by PCR, ligase chain reaction, and transcription-mediated amplification: identification of urinary substances associated with inhibition and removal of inhibitory activity. *Journal of Clinical Microbiology* 36:3122-3126.

Muharam I, McNevin A, Iannuzzi C, Nurthen T, Ientile V (2007). Project 15: Report on the verification of automated capillary electrophoresis setup using the MultiPROBE® II PLUS HT EX platform [Laboratory Report]. DNA Analysis, FSS: Brisbane, QLD, Australia.

Mulot C, Stücker I, Clavel J, Beaune P, Loriot M-A (2005). Collection of human genomic DNA from buccal cells for genetic studies: comparison between Cytobrush, mouthwash and treated card. *Journal of Biomedicine and Biotechnology* 2005(3):291-6.

PerkinElmer (2004). MultiPROBE II Liquid Handling Forensic Workstation Application Guide: Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation [PN 8842157]. PerkinElmer Life and Analytical Sciences: Downers Grove, IL, USA.

Promega Corporation (2006). DNA IQ™ System – Small Sample Casework Protocol [PN TB296, Rev. 4/06]. Promega Corporation: Madison, WI.

[redacted]ronchoalveolar lavage (BAL) cell count and differential [redacted]. Anatomical Pathology, RBWH: Brisbane, Australia.

[redacted]

QIS 19976 R1 (2007). Automated amplification of extracted DNA using the AmpFℓSTR® Profiler Plus® or AmpFℓSTR® COfiler® kit [Standard Operating Procedure]. DNA Analysis FSS: Coopers Plains, Brisbane, Australia.

QIS 19977 R1 (2007). Automated quantification of extracted DNA using the Quantifiler™ Human DNA Quantification Kit [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

QIS 19978 R0 (2008). Capillary electrophoresis setup [Standard Operating Procedure]. DNA Analysis, FSS: Coopers Plains, Brisbane, Australia.

Satia-Abouta, King IB, Abouta JS, Thornquist MD, Bigler J, Patterson RE, Kristal AR, Shattuck AL, Potter JD, White E (2002). Buccal cell DNA yield, quality and collection costs: comparison of methods for large-scale studies. *Cancer, Epidemiology, Biomarkers & Prevention* 11:1130-3.

Shutler GG, Gagnon P, Verret G, Kalyn H, Korkosh S, Johnston E and Halverson J (1999). Removal of a PCR inhibitor and resolution of DNA STR types in mixed human-canine stains from a five year old case. *Journal of Forensic Sciences* 44(3):623-626.

Tsai YL and Olson BH (1992). Rapid method for separation of bacterial DNA from humic substances in sediments for polymerase chain reaction. *Applied and Environmental Microbiology* 58(7):2292-2295.

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Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices

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1 Purpose and Scope

This method describes the use of Microcon® Centrifugal Filter Devices to concentrate extracted DNA. It is used for samples where the yield of DNA is not sufficient to obtain a DNA profile using the PowerPlex® 21 system.

Concentrating the DNA extract using Microcon® devices typically reduces the volume from approximately 100 µL to ≤35 µL for samples processed using PowerPlex® 21.

2 Definitions

FR	Forensic Register
PP21	PowerPlex® 21

3 Principle

Microcon® Centrifugal Filter Devices are centrifugal filter devices that desalt and concentrate macromolecular solutions such as DNA-containing solutions. They employ Amicon's low binding, anisotropic, hydrophilic regenerated cellulose membrane.

The low-adsorption characteristics of the Ultracel® YM-100 membrane and the device's component parts, together with an inverted recovery spin, combine to yield high recovery rates (typically >95 % of the sample, with concentration factors as high as 100x).

4 Reagents, Equipment and Consumables

4.1 Reagents

Table 1 outlines all the reagents and their storage locations for concentration of DNA extracts using the Microcon® Centrifugal Filter Device.

Table 1: Reagent storage room and location

Reagent	Room	Location
Amplification grade water	3188	In-use tray or shelf
70% v/v ethanol	3188, 3189, 3191	Sink or bench
0.5% v/v Bleach	3188, 3189, 3191	Sink or bench
5% v/v Trigene Advance	3191, 3188	Sink

4.2 Equipment

Table 2 outlines the equipment and their locations required for concentration of DNA extracts using the Microcon® Centrifugal Filter Device.

Table 2: Location of required equipment

Equipment	Location
Fridge	3189
Freezers	3189 / 3190
Biological safety cabinet class II x 4	3188 x 1, 3189 x 4
96 well tube racks	3189
Vortex x 4	3189
Centrifuge x 4	3189 / 3194
Finnpipettes 100 – 1000 µL	3189
Finnpipettes 20 – 200 µL	3189

4.3 Consumables

Table 3 outlines the consumables and their locations required for concentration of DNA extracts using the Microcon® Centrifugal Filter Device.

Table 3: Location of required consumables

Consumables	Location
Racks	3189
Nunc™ tubes	3189
Nunc™ caps	3189 / 3194
Microcon® Centrifugal Filter Devices	3189
Sharps bins	3189
20 µL pipette tips	3189
300 µL pipette tips	3188 / 3189
1000 µL pipette tips	3188 / 3189

Additional consumables can be found in the store room (3184).

4.4 Entering Reagents, Equipment, Consumables and Locations and into FR

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).
2. Click the **Edit/Update Batch** icon.
3. Using the relevant dropdown menu select the correct consumables and reagents.
4. Scan the equipment and location barcodes into the required fields (Figure 1).
5. Click the **Save Batch** icon.

Note: Fields should be filled out contemporaneously while processing the batch. These steps can be performed at any stage prior to batch completion, and entries can be modified after saving.

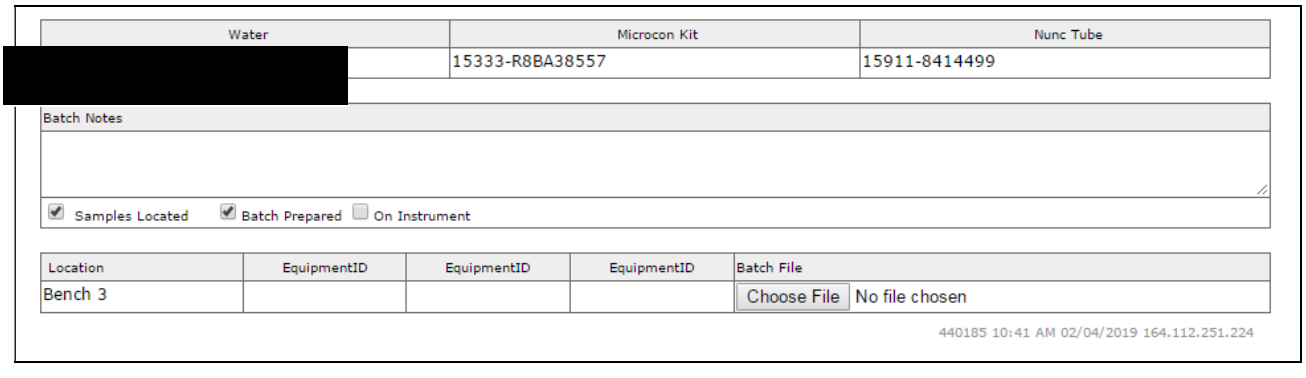

Figure 1: Entering batch details

5 Safety

As per the Anti-contamination procedure (22857), PPE is to be worn by all staff when performing this procedure.

6 Sample location and Batch Preparation

6.1 Batch Creation

Create extraction batches according to the Forensic DNA Analysis Workflow Procedure (34034).

6.2 QC Samples

One negative control will be registered automatically by FR when creating Microcon® extraction batches according to the Forensic DNA Analysis Workflow Procedure (34034). The negative extraction control consists of 100 µL of amplification grade water.

6.3 Sample Location and Locating Samples

Samples waiting to be extracted are stored in freezers as described in Table 4.

Table 4: Sample storage locations

Sample type	Storage Device	Storage Location
DNA extracts	Freezer	3194

Locate samples according to Forensic DNA Analysis Workflow Procedure (34034).

6.4 Analytical Notes

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).

2. On the batch **Exhibit Analysis** page in FR hover the cursor over the samples to check the analytical notes for specific processing comments (e.g. Microcon to Full) (Figure 2).

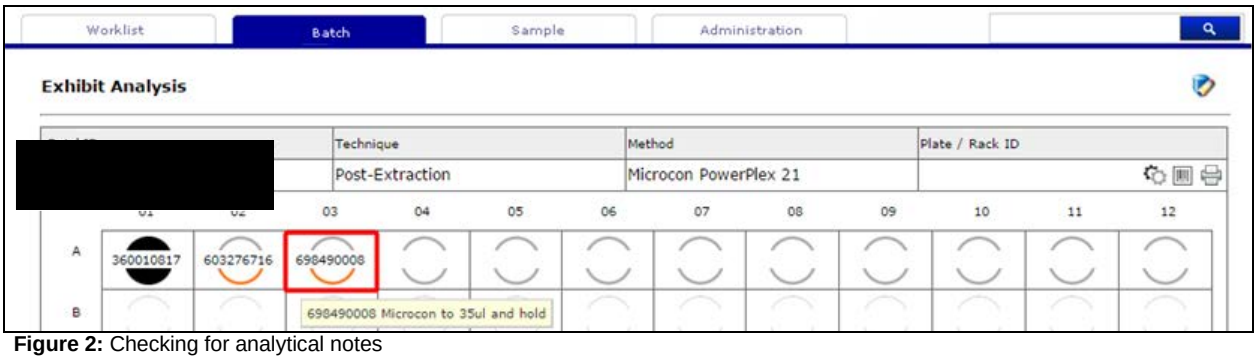

Figure 2: Checking for analytical notes

6.5 Creating and Printing Sample Labels

1. Access the batch according to the Forensic DNA Analysis Workflow Procedure (34034).

2. Click the **Batch Labels** icon to display the sample labels. Print the labels to the designated label printer.

6.6 Sequence Checking

1. Perform a sequence check of all tubes for the batch by clicking the **Sequence Check & Lock** icon.

2. Scan all tubes in the order they are positioned in the rack corresponding with the FR platemap. The parent barcode must be the first barcode scanned.

Note: If a barcode is scanned incorrectly during the sequence check an error message will appear above the virtual rack. Check the position the error message indicates and rescan with the correct barcode.

3. To complete the sequence check, check the **Confirm Sequence Check** box. (Figure 3). The **Confirm Sequence Check** box should not be checked if there are any errors showing on the sequence check screen.

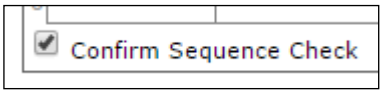

Figure 3: Confirm Sequence Check box

4. Click the **Lock Batch** icon.

6.7 Printing Sample Transition Page

1. Click **Sample Transition** icon.
2. Print **Sample Transition** page.
3. **Do not** save **Sample Transition** page at this stage. Press the back button to return to the batch **Exhibit Analysis** page.

7 Procedure

7.1 Microcon® to Full

1. All manual extractions are to be performed within a biological safety cabinet in Room 3189.
2. Check for Analytical notes as per Section 6.4 and action as required.
3. Print labels required as per Section 6.5.
4. For each sample, prepare and label:
   - Microcon® collection tube and Microcon® sample reservoir (Microcon® assembly) (**parent** barcode)
   - Separate Microcon® collection tube (without Microcon® sample reservoir) (**parent** barcode)
   - Nunc™ tube (**parent** barcode)

Note: Prior to use, check the membrane of the Microcon® sample reservoir is intact and check during each step of the procedure it remains intact (Figure 4)

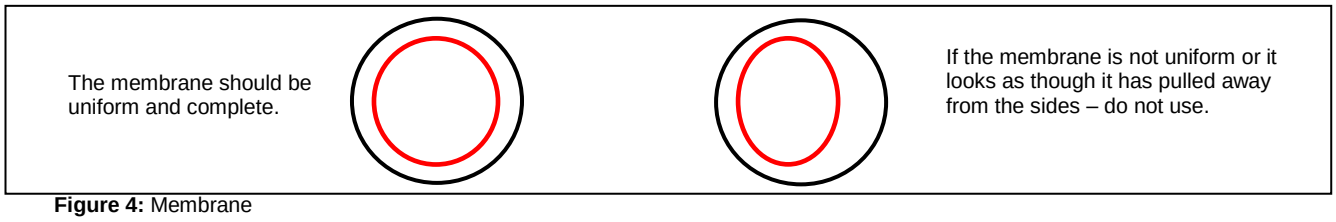

Figure 4: Membrane

5. Ensure a sequence check is performed as per Section 6.6 prior to continuing.
6. Print **Sample Transition** page as per Section 6.7.

7. Once thawed, vortex and pulse spin the samples at 1000 rcf for ~5 seconds.

8. Pipette 100 µL of amplification grade water into the Microcon® sample reservoir of the Microcon® assembly for the negative extraction control.

9. Transfer the extract into Microcon® sample reservoir without touching the membrane. Ensure no magnetic beads are pipetted into the column. Seal with the attached cap.

10. Place the Microcon® assembly into the centrifuge and spin at 500 rcf (2 minute intervals are optimal, however can be spun for less time at the operator's discretion). The Microcon® assembly can only be spun for a total of 12 minutes.

11. Remove the Microcon® assembly from the centrifuge and check the Microcon® sample reservoir for remaining liquid. The amount of liquid remaining above the membrane should be observed as per figure 5. The membrane should be damp but never completely dry. **The final volume to aim for is between 5-15 µL for samples and 35 µL (PP21) for the negative extraction control**

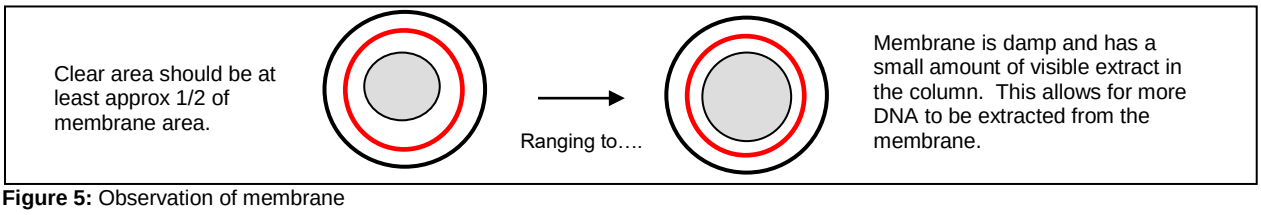

Figure 5: Observation of membrane

12. Once the membrane resembles that in Figure 5, remove the Microcon® assembly from the centrifuge and separate the Microcon® sample reservoir from the Microcon® collection tube.

13. Place the Microcon® sample reservoir upside down into the separate labelled Microcon® collection tube. With the tube cap open carefully place the assembly into the centrifuge. Spin for 3 minutes at 1,000 rcf.

14. Carefully remove the Microcon® assembly from the centrifuge. Separate the Microcon® sample reservoir from the Microcon® collection tube and place back into the original flow through Microcon® collection tube.

15. Measure the final volume and transfer to a labelled Nunc™ tube. Note the final volume.

16. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer as per Storage Guidelines for Forensic DNA Analysis (23959).

17. Discard Microcon® sample reservoir and Microcon® collection tube containing flow through.

7.2 Microcon® to Specific Volumes

1. All manual extractions are to be performed within a biological safety cabinet in Room 3189.

2. Check for Analytical notes as per Section 6.4 and action as required.

3. Print labels required as per Section 6.5.

4. For each sample, prepare and label the following:
    - Microcon® collection tube and Microcon® sample reservoir (Microcon® assembly) (**parent** barcode)
    - Separate Microcon® collection tube (without Microcon® sample reservoir) (**parent** barcode)
    - Nunc™ tube (**parent** barcode)

Note: Prior to use, check the membrane of the Microcon® sample reservoir is intact and check during each step of the procedure it remains intact (Figure 4).

5. Ensure a sequence check is performed as per Section 6.6 prior to continuing.

6. Print **Sample Transition** page as per Section 6.7.

7. Once thawed, vortex and pulse spin the samples at 1000 rcf for ~5 seconds.

8. Pipette 100 µL of amplification grade water into the Microcon® sample reservoir of the Microcon® assembly for the negative extraction control.

9. Using a pipette measure the volume of the extract being transferred into the Microcon® sample reservoir without touching the membrane. Ensure no magnetic beads are pipetted into the column. Note the volume.

10. Based on the final volume requested in the analytical note calculate the amount of flow through required.

Note: Allow for approximately 5 µL of unrecoverable volume trapped beneath the membrane.
Casework PP21 samples and negative controls aim for final volumes of 35 µL (unless a specific final volume has been requested in the analytical note).
Reference PP21 samples and negative controls aim for final volumes of 23 µL

11. Place the Microcon® assembly into the centrifuge and spin at 500 rcf (2 minute intervals are optimal, however can be spun for less time at the operator's discretion). The Microcon® assembly can only be spun for a total of 12 minutes.

12. Remove the Microcon® assembly from the centrifuge and check the volume of flow through. Repeat this after each spin until the required flow through volume has been reached or the Microcon® assembly has been spun for 12 minutes.

13. Once the calculated flow through volume has been reached separate the Microcon® sample reservoir from the Microcon® collection tube.

14. Place the Microcon® sample reservoir upside down into the separate labelled Microcon® collection tube. With the tube cap open carefully place the assembly into the centrifuge. Spin for 3 minutes at 1,000 rcf.

15. Carefully remove Microcon® assembly from the centrifuge. Separate the Microcon® sample reservoir from the Microcon® collection tube and place back into the original flow through Microcon® collection tube.

16. Measure the final volume and transfer to a labelled Nunc™ tube. Note the final volume.

17. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer as per Storage Guidelines for Forensic DNA Analysis (23959).

18. Discard Microcon® sample reservoir and Microcon® collection tube containing flow through.

8 Batch Finalisation

1. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4.

2. Click **Sample Transition** icon.

3. Enter the final volumes for each sample in the **µL field.**

4. Select the appropriate technique and method from the dropdown menus (Figure 6). If samples have an **Extract & Hold** or **NWQPS** analytical note, the **Technique** and **Method** fields need to be left blank. This also applies for samples with a final volume <23 µL. For these samples a Manual Amp needs to be ordered.

NOTE: Samples processed through Microcon® to full need to progress to a manual amp. **Technique** and **Method** should be blank. See **Figure 6**. To order a Manual Amp refer to Appendix 14.2 Ordering a Manual Amp.

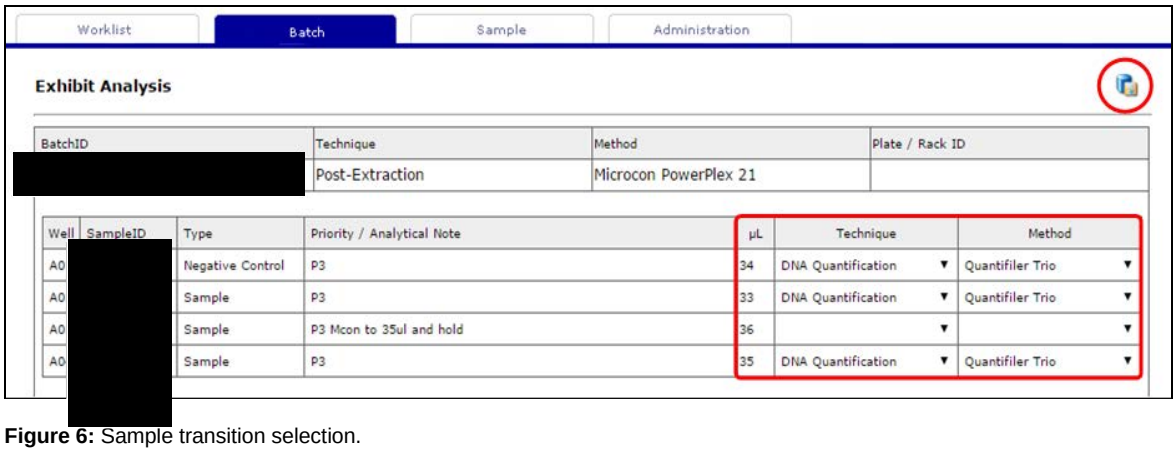

Figure 6: Sample transition selection.

5. Ensure check box Batch Prepared has been ticked prior to completing the batch.

6. Click the **Complete Batch** icon.

7. Click the **Edit/Update Batch** icon.

8. Complete the **Run Date & Run Time** fields (Figure 7) (manually enter and date and time, or select the current date from the calender which autofills the time field upon batch completion).

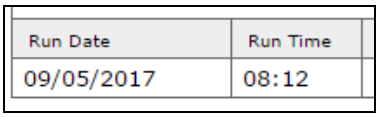

Figure 7: Run date and Run Time field

9. Click the **Complete Batch** icon to complete the batch.

9 Validation

Cheng, A., Iannuzzi, C., and McNevin, A. "Proposal#115 - Verification of a new Microcon® DNA Fast Flow Centrifugal Filter membrane". December 2012

10 Quality Assurance/Acceptance Criteria

One negative control is included in each extraction batch and is processed as a normal sample through to completion.
If any results are obtained from the negative control, either at quantification or during the CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible. This is covered in Capillary Electrophoresis (CEQ) Quality Check (34131) and Miscellaneous Analytical Section Tasks (34064).

11 Associated Documents

QIS 22857 Anti-contamination Procedure
QIS 23959 Storage Guidelines for DNA Analysis
QIS 34034 Forensic DNA Analysis Workflow Procedure
QIS 34064 Miscellaneous Analytical Section Tasks
QIS 34131 Capillary Electrophoresis (CEQ) Quality Check

12 Amendment History

Version	Date	Author/s	Amendment
1	01/12/2016	Adam Kaity	Changeover of FR from AUSLAB to Forensic Register – update FR related sections.
2	May 2017	Megan Mathieson, Lisa Farrelly	Added a table of contents page. Updated Section numbers and headings. Included consumables to reagents and equipment section. Added in sections 6.1, 6.5 to 6.8. Separated into two sections Microcon to full and Microcon to specific volumes. Changed terminology of reservoir to Microcon sample reservoir and microcon tube to microcon collection tube. Removed adding water and TE to microcon to full samples. Standardised final volumes for all samples types to 23µL. Created section 8 batch finalisation. Updated quality assurance and acceptance criteria for negative controls. Moved troubleshooting to an Appendix.

Version	Date	Author/s	Amendment
3	February 2019	Tara Prowse	Updated all Hyperlinks. Removed all reference to P+ (Profiler Plus) processing. Section 4.4 Added a picture showing the Batch prepared check boxes. Removed Section 6.1 Electronic Workflow Diary. Section 6.4.2 updated E.g. to Micrcon to Full. In Section 7.2, point 10. Note: Added comment about specific volumes requested in Analytical Notes. In Section 7.2, removed point 17 referring to making up to 23uL with Amplification water for Reference samples. Section 8 Added a step before step 5 Ensure check box batch prepared has been ticked before completion. Updated Section 8.4 to include that if the volume is <23uL the dropdown menu needs to left blank and a Manual Amp needs to be ordered. Removed Section 11 References. Added Appendix 13.2 Ordering a Manual Amp.
4	January 2021	Melissa Cipollone	Standard Operating Procedure reviewed. Table 5 updated to reflect the new STR Amplification method for casework (PowerPlex21 3500xL) Removed some hyperlinks and updated 'Refer to' Steps in Section 7 as they were incorrect

13 Appendices

13.1 Troubleshooting
13.2 Ordering a Manual Amp

13.1 Troubleshooting

Note: Ensure that detailed **Sample Notations** outlining that nature of the issue are added for affected samples from the **Exhibit Testing** page as outlined in the Miscellaneous Analytical Section Tasks (34064).

13.1.1 Volumes >500 µL

If volumes greater than 500 µL are encountered then pipette in the same manner but split the respective sample over more Microcon® centrifugal filter devices (pool after completion of subsequent steps).

13.1.2 Initial Spin sees ALL liquid pass through the membrane

If all liquid has passed through the membrane after the first spin, a membrane failure may have occurred. The following should be performed:

1. Add 10 µL of amplification grade water (PP21 samples) to the faulty sample reservoir.
2. Agitate gently for 30 seconds.
3. Invert the sample reservoir into the **original** collection tube and spin at 1,000 rcf for 3 min to collect and pool.
4. Transfer into a new Microcon assembly and re-start the process.

13.1.3 Recovery (required when over spinning/sample dryness has occurred)

1. Add 10 µL of Amplification grade H_2O (for PP21 samples) to the sample reservoir.
2. Agitate gently for 30 seconds.
3. Re-invert the sample reservoir into a **new** collection tube.
4. Centrifuge at 1,000 rcf for 3 min to collect.
5. Add an appropriate volume of Amplification grade H_2O (for PP21 samples) so that the final volume is 23 µL.

13.1.4 No Fluid appears to be passing through the filter

In the case of no fluid passing through filter, the filter is to be inverted into a new collection tube and spun at 1,000 rcf for 3 minutes. The sample is then to be transferred to a new Microcon assembly and the process re-started.

13.1.5 Excess Samples remaining after Microcon® Procedure

Should there be excess sample left after a Microcon® to Full, enter a **Sample Notation** for the case scientist indicating that there is sample remaining. Refer to Miscellaneous Analytical Section Tasks (34064).

13.2 Ordering a Manual Amp

1. Access the **Exhibit Detail** page by entering the sample barcode into the FR search field.

2. Click the **Add Exhibit Test** icon, to the right of the Exhibit Testing table.

3. On the **Exhibit Testing** page choose a **Technique** of STR Amplification from the drop down menu.

4. Select the appropriate **Method** from the drop down box, refer to Table 5.

Table 5: STR Amplification Methods

Technique	Method
STR Amplification (Casework)	PowerPlex21 3500xL Manual
STR Amplification (Reference)	PowerPlex21 3500xL Manual

5. Change the SV1 Volume to match the Final volume of the sample after Microcon. Change the TV1 Volume so that the sum of SV1 and TV1 is equal to 15 µL for Casework and 7.5 µL for Reference samples.

6. Click the **Save** icon.

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Procedure for Case Management

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Not Current

1 Purpose

The purpose of this procedure is to describe the components of a case record, processes involved in compiling and completing a case record and tracking of case records.

2 Scope

This procedure shall apply to all Forensic DNA Analysis staff that case manage any component of a case record.

3 Definitions

Case record	All information relating to a particular case. This can include all case histories, receipts, communication with clients, examination notes, Analytical data, internal communications, results and reports.
DAD/DADI	The DNA Analysis Database Interface (DADI) is used to maintain the data stored within the DNA Analysis Database (DAD) and to provide results management functions.
GSI	Generic system interface (AUSLAB interface to QPS)
Examining scientist	The scientist/s who has/have examined exhibits for a case.
EXH	Exhibit report (post 8/10/2008)
EXR	Exhibit report (pre 8/10/2008)
Case managing scientist	The scientist(s) that has (or have) been involved in the assessment of results and compilation of the case file in preparation for statement writing or peer review.
In tube	An item that has been sub-sampled by the QPS and submitted to the laboratory in a tube ready for analysis.
Paperless	A type of case that does not involve a traditional paper case file. Work is performed almost entirely in AUSLAB
Paper	A type of case that involves a traditional paper case file.
STRmix™	A statistical program used during case management to interpret certain types of DNA profiles.
DRMU	DNA Results Management Unit – A QPS Unit that transfers the EXR/EXH and LKR results from the Forensic Register to QPRIME. They also perform quality checks on the validity of the information/results received.
Reporting Scientist	The scientist who is responsible for writing a Statement of Witness outlining the results of a case and for presenting evidence in a court of law.
CRISP	Crime Recording Information System for Police (Pre 2008)
QPRIME	Queensland Police Records and Information Management Exchange (Post 2008)
AmpFℓSTR® Profiler Plus®	The amplification kit made by Life Technologies – This is used for low priority cases (Volume crime cases).
AmpFℓSTR® COfiler®	The amplification kit made by Life Technologies – this was used for additional loci in paternity cases. This is no longer used.

Not Current

PowerPlex® 21 system kit	The amplification kit made by Promega that is currently used for all reference samples and high priority case work samples.
SSLU	Scientific Services Liaison Unit
Modify 8	Code utilised by AUSLAB to perform automated actions with AUSLAB
P+	AmpF*l*STR® Profiler Plus®
PP21	PowerPlex® 21 system kit
ERT	Evidence Recovery team
FRIT	Forensic Reporting and Intelligence Team
VAR	Variant allele
OLA	Off ladder allele
ULP	Unlabelled allele
XOVER	Cross over allele, allele migrates into an adjacent marker bin.
NCIDD	National Criminal Investigation DNA Database
QFLAG	Quality checking procedure to investigate potential staff and elimination database matches
STATSWG	Statistics Working Group
EPG	Electropherogram
GMIDX	GeneMapper ID-X, software used for allele designation after capillary electrophoresis

4 Case file overview

Each case file has a unique case number (Occurrence/QPRIME number, CRISP number or SSF/COR/CA number).
CRISP numbers were used by QPS for cases prior to 2007; the format used is XX/XXXXXX. Occurrence numbers (QPRIME) are generated by QPS and are recorded in AUSLAB with a prefix e.g. QP1400000000. SSF/COR/CA numbers are generated in AUSLAB and usually indicate a coronial matter.

Table 1 - UR prefix summary

UR prefix	Description	Use	Date range
F	F numbers Biol	Historic	1965-2004
QPS	QPS CRISP	Historic	2004 - Mid 2007
QT	QPRIME Temp	Historic	Mid 2007 - 2008
QP	QPRIME	Current cases	Mid 2007 - Present
FBQA	Forensic Biology Q.A	External proficiency	2003 - present
FBOT	FBiology Robotics Validation	Internal Controls	2005 - present
FBE	Forensic Biol Environmental	Environmental monitoring	2005 - present
SSF	SS-Forensic	Generic forensic	2002 - present
COR	QWIC (QLD Magistrates)	Coronial cases	2003 - 2009
CA	Coronial Autopsy	Coronial cases	2009 - present
LKR	Link reports	Link reports	2003 - present

Information relating to a case, such as offence type, is recorded in AUSLAB. See QIS 17116 *Processing DNA Exhibits/Samples in the Forensic Sciences Property Point* and QIS 16004 *AUSLAB Users Manual – DNA Analysis*.

If a case contains items or documentation citing more than one case number, enquiries can be made to the QPS Sample Management Unit (SMU) to establish whether the cases are linked and can be reported together. Details of these checks must be included in the UR notes

Not Current

All cases registered in AUSLAB will have a CS test panel (case management screen) requested (except for F numbers). The CS is requested on the first laboratory number registered for the case. There should be only one CS test panel for each case. The CS test panel provides general details on the case. The tests displayed on the CS are:

- **Status** (CASEST)–the case status is used to track the workflow in the milestones page. Options for this field are:
  - RECEIVED – case has been received but not allocated.
  - ALLOCATED – case has been allocated
  - AWAITING ADVICE – more information has been requested. Case is on hold until further notice. This status is not tracked in the milestones.
  - STARTED – Examination has commenced
  - SENT TO PEER REVIEW – case has been completed and has been sent for Administrative and/or Technical review.
  - RETURNED FROM PEER REVIEW – case has been returned to Case Scientist for amendments during the review process.
  - REPORT ISSUED – case has been reviewed and statement has been sent. Entered by the reviewer following tech/admin review.
  - ANALYSED - REPORT NOT REQUIRED – case has been reviewed and no statement has been requested. Entered by the reviewer following administrative review.
  - NO TESTING REQUIRED – case has been written off, no work was required or no longer required.
  - REACTIVATED – case has been reopened for more work or statement preparation.
  - ON HOLD SAMPLED AND STORED – Used for low priority cases for samples that have not been sent for processing
- **People involved in the case (Surname, First name, DOB, Class)** – (CSN1-CSN10) names, dates of birth and classes of people involved in the case, e.g. complainants, suspects, defendants, deceased, elimination.
- **Case Scientist** (CSCI) – scientist who has been allocated the case, generally the reporter.
- **Primary Case Scientist** (CSCIP) – allocated case manager or reviewer.
- **Case Type** (CASET) – e.g. Armed Robbery, Assault, Murder, Property, Proficiency Testing, Paternity, Sexual Assault, Coronial/DVI. This is supplied by QPS.
- **Crime Class Code** (CCCODE) – information provided by QPS, subdivisions of the case type categories.
- **Operation** (CASEO) – used to record police operation name if applicable.
- **Investigating Officer** (INVOFF) – Contact officer for the case.
- **Due Date** (FTDC) – date when statements or results are required, used mainly for Coronial/DVI cases or major investigations.
- **Court Date** – date of court proceedings. May be committal or trial. Details are recorded in UR notes.
- **Biol/Police Priority** (FORCAT) – used to assist in case prioritisation.
- **Date Completed** (FBCDAT) – date case review is completed.
- **Exhibits** (EXRTR) – indicates status of exhibits.

4.1 When is a case file required?

Since the 1st of September 2009, low priority Volume Crime cases have been treated as 'paperless' and therefore do not have case files. In April 2010, paperless case management and review was expanded to also include all cases of both high and low priority (Volume and Major Crime) and some Sexual Assault cases (Yellow Team cases) except for cases

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involving excessive numbers of crime scene/reference samples or complex profiles. In April 2015 all cases are initially managed as paperless cases.

Case files are generally created

- At the time of case management (for complex cases) or
- When a statement is requested or
- When a case manager/reporter deems it necessary for efficient case management.

A case that has been initially managed paperlessly may be converted to a paper file if further exhibits are received at a later date. Case and examination notes are stored in 'Paperless' folders stored in Evidence Recovery, Reporting and Admin areas.

If a case has been converted from paperless to paper, it is not necessary to annotate all of the epgs with the interpretations unless a statement has been requested. The reporter of the statement will update the epgs with interpretations as they are documented in AUSLAB.

4.2 How to create a case file

Additional information is provided in QIS 24126 *DNA Analysis Unit Administrative Officer Case Management*. It is advisable for efficiency purposes that casefiles are created by the Forensic DNA Analysis Administration Team.
To request a casefile to be created, email [redacted] with instructions. Alternatively, instructions are given below:

1. Check AUSLAB to see if a case file already exists. If a file does exist, a new one does not need to be prepared and the file should be located.
2. If a file does not exist and it has been determined that one is required, it will need to be prepared.
3. Place a barcode for the first received receipt (if multiple receipts are registered) on the case file folder in the box in the top right hand corner labelled 'case barcode'. This first receipt barcode should also be the barcode that the CS screen is registered under in AUSLAB. See Appendix 1 for details on the CS screen.
4. Write the case number at the top of the folder in the 'Case Number' field.
5. If applicable, write the initials of the reporting scientist in the 'Reporting Scientist' field. If no statement is to be issued, this field should have 'N/A' entered in it.
6. Write the complainant's and suspect's/defendant's names (if known) on the lines next to the 'NAMES' fields (one name per line). Surnames should be written in capitals and given names in lower case. Write '(Comp/Def't)' or '(Sus)' after each name as appropriate. If the complainant is a company, the company name can be written on the case file, but for reporting purposes the complainant in these cases is 'Regina'.
7. Turn the folder to the side and fill out the details along the edge as indicated: case number; case type - found on the CS screen for the case in AUSLAB, e.g. assault (N/S); names - divide the line in half with a diagonal line and write the surname of the complainant on the left side and the surname of the suspect(s)/defendant(s) (one on top of the other if necessary) on the right side.
8. Place the receipt barcode on the Case File Particulars page (QIS 17038) in the box in the top left hand side of the page labelled 'Case File Barcode'. If no barcodes are available, print a new barcode from AUSLAB.
9. On the Case File Particulars page write the case number in the 'Case #' field.
10. Place the Case File Particulars page and receipt page(s) (scanned signed copies of the receipt) inside the case file and track the file in AUSLAB to the appropriate storage location. Since 26th September 2011, the QP127 (if provided) will be scanned into AUSLAB by Property Point staff and the original handed back to the QPS Officer with the exception of SAIKs where the QP127 will be scanned into

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AUSLAB and attached to the GMO notes, tracked and transferred to Forensic DNA Analysis.

4.3 Additional Elements of a case file

Upon completion, a case file may also contain:

1. Examination notes
2. Diagrams, photographs and/or photocopies
3. Statistical calculations
4. Copies of results (Genotyper or GeneMapper ID-X printouts)
5. Interpretations of results
6. Copy of statement or intelligence report
7. Records of any internal or external communication relating to the case, e.g. UR notes or emails.
8. STRmix™ output files

4.4 Handwritten results and corrections within a case file

As is required by NATA ISO 17025 - as case notes etc. are subject to subpoenas; no pencil is to be used in the case file (unless used in diagrams or pictorial representations).

Any calculations, interpretations or changes to notes or results must be initialled and dated by the person performing the action.

4.5 Case file storage and movement

Case files are required to be kept indefinitely as per accreditation requirements.

1. No exhibits are to be stored in the case file. This includes external proficiency samples. Original QPS property tags or reference sample envelopes are also NOT to be stored in the case file.
2. Case file movement is to be recorded in AUSLAB.
3. Active case files are stored with the case analyst or in a designated storage location for the work area.
4. Upon completion, scientists should transfer cases to either FBCFF1 (Case File Finish) or FBPR1. Administration assistance slips are available to attach to the front of the case file to direct the storage of the file or to outline any further administrative tasks that need to be performed prior to storage.
5. FBCFF1 is the location from which administrative staff will track case files (sequentially) into the compactus or another designated storage location. No further administrative tasks will be carried out on these cases.
6. FBPR1 is the location which administrative staff will collect cases from in order to perform further administrative tasks such as page numbering and administrative reviews (left hand side only).

5 Workflows

5.1 Priorities

Table 2 details the DNA priorities that are used in Forensic DNA Analysis. These are not to be confused with case priorities. Case priorities are received from the QPS via the Forensic Register interface.

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Table 2 - DNA Priorities in Forensic DNA Analysis

Priority	Description	CW Use	Ref Use
1~SS	Urgent	Urgent	Priority/investigation
2~SS	High Pri	Major crime	High priority
3~SS	Med Pri	Volume	Normal
4~SS	Low Pri	No longer used	N/A
5~SS	Very low	No longer used	N/A
6~SS	Cease	Cease work	Cease work

Urgent (5 day TAT) cases are specifically allocated to a case scientist and/or reporting scientist as they arrive into the department. The Management Team will be notified of the arrival of an urgent case by email and appropriate UR notes will be entered. A supervising scientist will allocate to an appropriate case manager. This does not mean that the case managing scientist will necessarily become the reporting scientist should a statement be required.

Priority 1 samples will be list inserted to the GREECM so they can be monitored by the Intelligence Team. The Supervising scientist or the case manager will list insert the case to their communication list. They must be managed as soon as results become available and reviewed as soon as results are interpreted. To ensure there is no delay in QPS being informed of 5 day TAT results as soon as they are available, a workflow has been created for samples that are expected to be completed on a Friday. See QIS 17119 *Procedure for the Release of Results*. Reworks on priority 1 samples are to be ordered and case managed by the original allocated scientist.

Priorities 4 & 5 are no longer used; if a sample has one of these priorities and needs to be case managed the priority must be changed to 3.

5.2 PowerPlex®21 system kit vs AmpFℓSTR® Profiler Plus® case management

Cases that have been started in one STR system will generally be completed in that system. The exception to this single system testing is reactivation and receipt of further exhibits for a high priority case that has been tested with AmpFℓSTR® Profiler Plus® (P+). These samples can be processed in PowerPlex®21 system kit (PP21) if the previous results were all reported as 'No DNA detected'. Approval from a Team Leader or the Managing Scientist is required in this scenario. Other exceptions include specific requests from QPS Insp for DNA Results Management Section/ Senior Sergeant delegate to a Forensic DNA Analysis Team Leader.

All major crime cases that have been allocated a case priority of 1 or 2 are processed in PP21 with a DNA priority of 1 or 2. All other cases are processed in P+ (Volume priority 3).

New evidence samples received for a case which has been profiled using P+ will be profiled using PP21. Only the 9 common loci between kits will be used for comparison purposes. For cases where a statement is required, a paragraph explaining the comparison of P+ casework samples and PP21 reference samples must be entered in the preambles section. See also QIS 17119.

5.3 STRmix versions

Any cases with a priority 1 or 2 received from Jan 1 2015 are processed with STRmix™ version 2.0.6 Refer to QIS 31523 *Use of STRmix™ Software*. All likelihood ratios (LRs) are

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generated using STRmix™ v2.0.6 regardless of the version of the deconvolution. See Table 3 below.

Table 3 – STRmix version use

Date case received	Decon	LR (at time of receival)	LR (New comparison)
19 Dec 2012 – 30 June 2014	v1.05	v1.05	v2.0.6
1 July 2014 – Jan 2015	v2.0.1	v2.0.1	v2.0.6
30 Jan 2015 – present	v2.0.6	v2.0.6	v2.0.6

5.4 Case management workflows

See Appendix 1 for a high level overview of case management lists/batches used.

Allocation of cases to a particular scientist usually only happens if a statement is required, the case is large or has been assigned an Operation by QPS. These cases will otherwise be routinely case managed by the Reporting and Intelligence teams. However, to reduce the amount of double handling by case managers, individual samples initially case managed by a particular person will be completed by the same person. This includes reworking and STRmix deconvolutions.

Unallocated paper case file may be stored in the FRITCM filing cabinets stored in the far end of the reporting area in Block 3.

Internal controls, external and internal proficiency, internal and external environmental monitoring samples case managed by the Analytical, Evidence recovery and Quality teams. The presence of these teams in the BTEAMS field triggers the Modify 8 equation to list insert to the 1AS (Analytical), ERT (Evidence recovery) or BQUAL (Quality) lists.

At the time the results of the DNA profiling are imported into AUSLAB; pre configured autovalidation rules (contained within the results import autovalidation mask) add a test (BID1, BID2 or BID3) determined by the presence of a core comment. The Modify 8 equation is triggered to list insert these samples onto workflow lists if certain requirements are met.

1. When the Sample Status (**XPRES**) or DNA Profile Result (**9PLEXS**) is “PDF ready” the system checks to see if the sample is ready for case management. This is defined in the system as incomplete or complete sample statuses and the plate reading comment matching the BID test ordered.
    i. If the sample statuses are incomplete the Modify 8 equation list inserts the sample to the appropriate hold list
    ii. If the sample statuses are complete the Modify 8 equation lists inserts the sample to the appropriate case management list.
2. Following case management of PP21 samples, the samples can be added to the STRMX deconvolution list if HP2 assistance is required by changing the Sample Status to ‘STRmix decon’. This triggers the Modify 8 equation to list insert to the sample to the STRMIX list and list removes from the current case management list.
    i. The comments field of the XPLEX page should have clear instructions for the STRmix operator, as opposed to an interpretation note for case managers.
    ii. Suggested instruction format for STRmix: ‘STRmix: v2, 3p/LR’. Suggested format for interpretation: ‘CM: 3p drop D21 tri’. If STRmix and interpretation notes are wanting to be added to the comments field together, the suggested format is: ‘STRmix: v2, 3p/LR | CM: 3p drop D21 tri’.

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3. On completion of this task the HP2 will change the Sample status of the sample to 'STRmix complete'. This triggers the Modify 8 equation to auto list insert to the STRMXCM (STRmix CM) list and list remove from the STRMX list.
4. Samples are manually list inserted to the appropriate review list, adding the Sample status (**XPRES**) of 'For Review' triggers auto-list removal from the current list.

Table 4 - Criteria to direct samples to appropriate lists

Test	GMIDX CORE COMMENT	BID TESTCODE	ON HOLD LIST	CM LIST
XPLEX	NSD	BID1	SSHOLD	SSCM
XPLEX	SS	BID1	SSHOLD	SSCM
XPLEX	MIX	BID2	MIXHOLD	MIXCM
XPLEX	COMPLEX	BID3	COMPHOLD	COMPCM
XPLEX	(BLANK/no Core Comment)	(NONE)	(NONE)	BLCM
9PLEX	NSD	BID1	(NONE)	VOLLOW
9PLEX	SS	BID1	(NONE)	VOLLOW
9PLEX	MIX	BID2	(NONE)	VOLLOW
9PLEX	COMPLEX	BID3	(NONE)	VOLLOW
9PLEX	(BLANK/no Core Comment)	(NONE)	(NONE)	BLCM

The hold lists are SSHOLD (SS On Hold), MIXHOLD (MIX On Hold), COMPHOLD (Complex On Hold) and VOLHOLD (Volume On Hold).

The case management lists are – SSCM (Single source case management), MIXCM (Mixture Case Management), COMPCM (Complex Case Management) and VOLLOW (Volume Low priority).

A further list BLCM (No team Case management) is utilised for samples that do not contain a core plate reading comment. This is managed by the FRIT Team Leader.

The 'Hold' and 'Case management' lists are sorted by the Case Manager field, then the request date. See Figure 1 below.

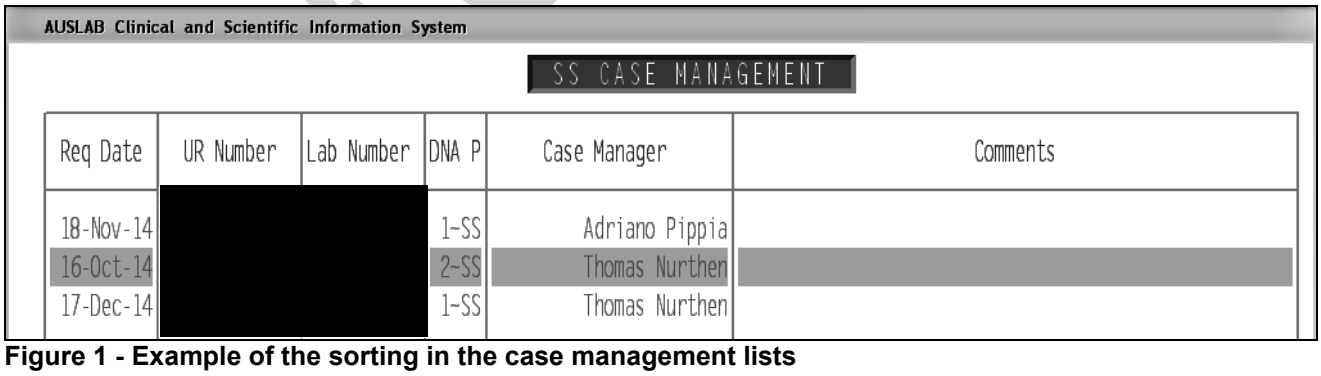

Figure 1 - Example of the sorting in the case management lists

Review of results is managed through the following AUSLAB worklists: SSREV (single source review), SSUPREV (Single Source Upload Rev), MIXREV (Mixture Review), MIXUPREV (Mixture Upload Review), COMPREV (Complex Review), VCREV (Vol Review) and VCMIX (Volume Paperless Mixtures) or for paper case files, MIXACT or the reviewer's storage location.

Various tools may be employed to assist in meeting timeframes and to cover absence such as scheduling Outlook appointments or tasks.

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Rework tests ordered on samples trigger the Modify 8 Equation to remove the sample from the current list.

6 Case management

The purpose of case management is to collate and report any DNA results that have been obtained and to prepare the case file for a statement (if required) or for peer review. To achieve this, the case managing scientist may be required to:

1. Assess DNA results to determine whether reworking is required to improve or confirm results.
2. Assess reworked and initial results to determine which is the best profile (for P+ cases only).
3. Enter final EXR/EXH results into AUSLAB.
4. Compile case file and finalise AUSLAB pages.

The steps for case management are listed below and a checklist provided in Appendix 2.

6.1 Check quality

Samples should not be progressed or reported until the various quality checks that are in place have been completed. These checks are designed to identify potential issues with samples before they are reported to the QPS.

6.1.1 Batch statuses

Check that the statuses of the processing batches are fully completed (XPLEX samples will have up to five test panels that list batch statuses (PLEXB,PLEXC,PLEXD,MCONC and NSPNC panels - See Figure 2). Please note that 9PLEX batches only have a final batch ID for each rework and no status fields. 9PLEX samples that require further consideration will have a ‘See batch audit’ specimen note.

Samples under investigation will have a batch status of ‘Investigation’. These samples will not have an end date and will populate the appropriate ‘hold’ list until the investigation is complete.

Absent batch statuses may indicate a batch has not undergone final quality checks. Do not case manage samples unless there is a batch status or you have checked the status of the batch from the batch audit itself. If this is the case, then add a specimen note to indicate what has been checked and why.

If the batch has been passed without any issues the status will be ‘Pass’. This status does not require any checking of the batch audit.

It is optional for the case manager to check the batch audit if the batch status is ‘EXTN < 3pks’.

If there has been an issue noted during processing of a sample, the Analytical staff member/delegate will enter a status of ‘See batch’. The case manager **MUST** check the batch audit and add a specimen note to indicate what the issue is and that it has been assessed and they have deemed the sample OK to report.

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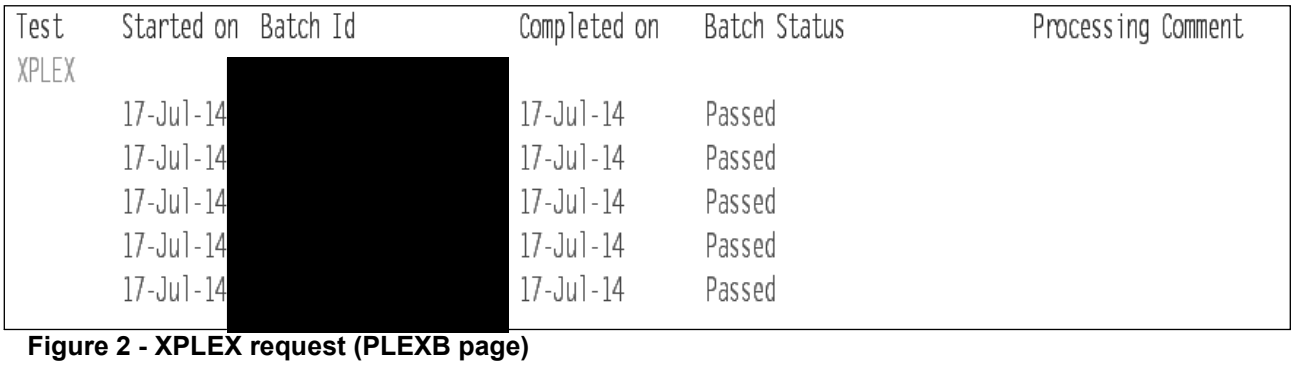

Test	Started on	Batch Id	Completed on	Batch Status	Processing Comment
XPLEX					
	17-Jul-14		17-Jul-14	Passed	
	17-Jul-14		17-Jul-14	Passed	
	17-Jul-14		17-Jul-14	Passed	
	17-Jul-14		17-Jul-14	Passed	
	17-Jul-14		17-Jul-14	Passed	

Figure 2 - XPLEX request (PLEXB page)

6.1.2 UR notes

Check UR notes for relevant information. UR notes are the repository for information that relates to a whole case. Information such as allocation to an individual case manager/reporter, SAIK strategies, court timeframes, communication with DRMU etc are recorded in the UR notes.

6.1.3 Specimen notes

Check for relevant information in the specimen notes. Specimen notes are used when information is specific to that laboratory number. Specimen notes are used for communication regarding processing. .e.g. that an issue raised in a batch audit has been assessed by the case manager.

6.1.4 Digital images

Check the digital images attached to the record in AUSLAB. These may be photo(s) of the exhibit(s), exhibit packaging or labelling.
To check the digital images, access the digital imaging component of AUSLAB

- From the sample barcode press [**CTRL + INSERT**]
- A list of all of the images for that barcode will appear
- Select an entry and press [**Enter**]

If the samples are 'In tube', the details on the envelope should match those registered in AUSLAB. If they do not and no action has already been taken refer to Section 4 of QIS 26071 *Examination of In-tube samples*.

For whole items, check the image and barcodes in the image match descriptions in AUSLAB.

For additional information on digital imaging refer to QIS 20080 *Digital Imaging in DNA Analysis*.

6.2 Check case information

Case information may be relevant to only particular samples or the whole case. This information may be used to guide the case manager's choice of processing and reporting.

6.2.1 Check for reference samples associated to the case

The presence or absence of reference samples may affect the workflow path a sample takes. If reference samples have been received for a case, these will be compared against

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representative single source DNA profiles, all interpretable mixed DNA profiles to generate a LR (XPLEX only) or to attribute contributions e.g. major, minor DNA profiles (9PLEX) and reported back to QPS via EXH/EXRs.

1. From any test panel in the case, press [**SF9**]
2. Page through the list and look for the specimen type FTA Evidence or FTA Specimen -Elimination (Intel purposes).

Alternatively the association of a reference sample to a case is documented by SSLU/Laboratory staff in the UR notes.

FTA specimens- Elimination (Intel Purposes) are associated to the case file but are not used in statements. The result of the comparison is reported back via an EXH the same as Suspect checks.
FTA specimens may **only** be used for comparisons if the QPS have requested a suspect check.

6.2.2 Check for case allocation

It is necessary to check if a case has been allocated to a particular case manager or reporter before case managing a sample. Check the CSCI field on the CS test panel or the UR notes to see if a case has been allocated.

6.2.3 Check for paper file/case notes.

Check the AUSLAB storage locations to see if a paper file has been created.

1. Press [**SF9**]
2. Press [**SF5**] **Specimen Search**, check for specimen type 'Case File' and take note of the storage location and description

This method can result in known display issues if the rack descriptions are too long.

Another method is listed below.

1. Press [**SF9**]
2. Select the entry with the CS request and press [**Enter**]
3. Press [**SF8**] Audit
4. Press [**SF8**] **Storage Audit**
5. Take note of the storage rack mnemonic.

If you are unsure whether the mnemonic refers to a paperless location or an actual case file check in the AUSLAB storage

- **Main Menu**
- Select **2. Sample Processing**
- Select **6. Sample Storage**
- Enter the mnemonic in the Rack Name field and press [**Enter**]

The screen will update and the Rack name and Location will be displayed.
If a paper case file exists; results and interpretations must be printed and filed in the case file. This includes the epg and STRmix deconvolutions. Reviews of the results in a paper case file are managed through the MIXACT or individual intray location as per Section 8 below.

6.2.4 Check ownership of item

Ownership of an item may be required before interpretation of a DNA profile or an exhibit is sampled. The ***ELF*** (***Item Ownership***) AUSLAB list is for requesting ownership details of items. This information can be requested by the Evidence Recovery team (ERT) before or

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at the time of registration/sampling or by a case manager. Add the details required to the AUSLAB specimen notes and list insert to the ELF list.

SSLU give the QPS three days to respond to the request before they contact them for a follow up.

6.2.5 Check case status

The case status in AUSLAB is sent back to the QPS via the GSI interface. As the status is changed the details are recorded in the FBMILE (Milestone page). The order in which the case status must be changed to update the Milestone page in AUSLAB correctly is *Received→Allocated→Started→Sent to Peer*.

Information may be received that indicates that a case is no longer required. This may be in the form of an email from the QPS,a UR note, an 'N' appearing in the Forensic Relationship field for a case/sample in AUSLAB, or insertion on the FBNLR work list by SSLU. Staff are rostered to FBNLR list for a week at a time.
For cases dated prior to September 2009: for items no longer required, SSLU will add NWQPS into the EXH, case manager will finalise case.

For cases dated September 2009 onwards: SSLU will add the relevant samples to FBNLR list, case manager will add appropriate EXH (NWQPS or NWQPSR) and will finalise case.

If notification is received that work is no longer required, yet the sample is at profile interpretation review stage, there is no need to complete the interpretation review. The EXH line 'NWQPSR' (No further work required as per QPS – results available) should be used to inform QPS that there are results, but no further work has been conducted for now based on advice. Any associated AUSLAB pages (eg. COMIX) can be removed from AUSLAB, or completed in such a way that the allele designations do not inform any other case management actions eg. NCIDD loading.

If the EXH 'NWQPSR' is reported back for a sample, and QPS decide that results are required, SMU will advise SSLU via email. This will then be communicated by SSLU via the CSRC AUSLAB work list so that case can be reactivated and the relevant sample(s) reported. The priority will be consistent with the case type.

If work is to be re-started on a sample at a later date due to a priority upgrade or advice from QPS, order a re-work test code as normal and change the priority as appropriate. If the sample had not been extracted prior to the CWORK being ordered, a second barcode is required in the same way that re-extracts are ordered. An appropriate EXH line should also be entered for samples that have been re-started, for example, 'Testing restarted on advice from QPS' (refer to QIS 23008 *Explanations of EXR/EXH Results* for further detail).

6.2.5.1 Finalising samples no longer required

1. Open the ***FBNLR*** list in AUSLAB.
2. Check the ***Forensic relationship*** field and UR notes/scanned emails(some sample may still require testing)
3. Check the testing status of the samples not required.
   a. If the samples have not been allocated to amplification, capillary electrophoresis or GeneMapper batches, order a cease work (**CWORK**) rework test.
      i. Change the DNA priority of the sample to '6'
      ii. Complete the fields on the cease work screen and press [***F6***] ***Validate***
      iii. Enter the final EXH result 'NWQPS' – into the next available EXH line

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b. If the samples have been allocated to an amplification, capillary electrophoresis or GeneMapper batch
   i. Enter the final EXH result 'NWQPSR' – into the next available EXH line

4. Enter 'Rev' and Press [**F6**] ***Validate***
5. Press **[F6]** ***Validate*** on the FBEXAM screen if not already validated.
6. If the ***Completed*** date (***9PLCD*** for the 9PLEX panel or ***XPCD*** for the XPLEX panel) is empty, add the current date (t)
7. Add your name to the case manager field (***XPCM*** for XPLEX, ***CSCI*** for 9PLEX)
8. Enter 'N' in the **CM NCIDD** field
9. Enter 'N' in the **Rev NCIDD** field
10. Enter 'N/A' in the ***Review*** field (***XPRV*** for XPLEX or ***9PLREV*** for 9PLEX)
11. Enter 'Finished' (for 9PLEX): or 'Completed' (for XPLEX) into the ***Sample Status***
12. Press **[F6]** ***Validate*** for each of the batch status pages if they are not already validated.
13. Remove the sample from the appropriate 'on hold' or case management list.
14. Remove from the FBNLR list
15. If the whole case is not required
    a. Change the **Status** (CASET) to 'NOTRQ'
    b. Complete the **Exhibits** (EXRTR) field. Most of the time this will be 'DESTR', 'RTORSR' or 'N/A' (e.g. paternity cases)
    c. Add the current date to the **Date Completed** (FBCDAT) field
    d. Delete the Administrative review test panel FBAR
    e. Validate all test panels
16. If a paper case file exists
    a. Add a comment to the case file particulars – 'No longer required'
    b. Track the case file to FBPR1

If

6.3 Assess results

All samples have alleles designated as per QIS 17137 *Procedure for STR fragment analysis using GeneMapper ID-X software* or QIS 31389 *STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software*.

When results become available for a sample, an assessment needs to be made as to whether reworks are required or whether sufficient information has already been obtained. This can be performed as each result becomes available. Not all results need to be available at the same time for these assessments to take place.

Samples that have been processed in Profiler Plus have their epgs saved to AUSLAB via digital imaging.

To aid in the assessment of results, several macros have been created. The PDF finder macro locates the epgs (both zooms and standard) for a particular laboratory number or a whole case.

1. Open the macro (I:\Macros\PDF Finder.xls)
2. Click the Find all .pdf files button
3. Enter the required laboratory number or case number
4. Select the years to search by ticking the year required
5. Press the Search button or 'Enter' key
6. A list of .pdf files will be displayed
7. Select the .pdf required and double click or press the open .pdf button.

Not Current

The Stutter check macro is a tool used to check the stutter and potential pull up for a whole plate or a single sample. The macro uses the allele sizes, allele peak heights, and validated stutter thresholds. This macro is only used for the assessment of PowerPlex®21 samples as stutter is left on and modelled by STRmix™.

1. Open the macro (I:\Macros\Macros to update\Stutter Check.xls)
2. Click the Check for Stutters (1 sample) button
3. Enter the laboratory number in the Sample ID field
4. Enter the GeneMapper plate ID in the Plate field
5. Press Ok button.

6.3.1 Assess the number of contributors to the DNA profile

The number of contributors to a DNA profile is required to perform interpretation. Counting the number of alleles at each locus is the first step in assessing the number of contributors. However, counting called peaks alone may not be suitable in determining the number of contributors due to the presence of PCR artefacts such as stutter. Allelic imbalance (AI) also known as heterozygote balance (Hb) can also be used as an indication of the number of contributors. Forensic DNA Analysis does not have a threshold for AI for casework DNA profiles because STRmix is designed to model the heterozygote balance as a continuous system. Although internal validation studies indicate that the calculated AI threshold varies depending on the DNA input, the values detailed in the study can be used as a guide.

The document '*Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis_version 2*' is used as a guideline for determining the number contributors within a PowerPlex 21 sample.

The validated stutter thresholds (as published in QIS 31389 for PowerPlex 21 samples and QIS 17137 for Profiler Plus samples) are used as a guide to aid in the determination of contributors to a DNA profile.

Additional information (for Profiler Plus samples only) is detailed in the document '*A guideline for the assessment of complex DNA profiles*'.

6.3.2 Assess the overall quality of the DNA profile

The quality of the DNA profile in conjunction with the number of contributors will determine if a DNA profile is suitable for interpretation.
The following factors should be considered

1. Whether a reasonable assumption of the number of contributors can be made.
2. The degradation slope (the tendency for higher molecular weight loci to have lower peak heights compared with smaller molecular weight loci).
3. The total amount of DNA input used in the amplification.
4. Adverse events affecting the sample.

6.3.3 Check VAR/OLA/ULP/XOVER calculations

If a variant and/or off ladder allele or stutter has been observed on a GeneMapper ID-X (GMIDX) profile it is not necessary to re-amplify to confirm its presence.

For mixed DNA profiles with variant and/or off ladder alleles, the repeat of these samples is at the case manager/reporter's discretion. Things to consider include whether the profile with variant and/or off ladder alleles has already had this questioned allele confirmed, matches a deconvoluted contribution, or if the sample description suggests the mixed DNA

Not Current

profile could be conditioned on the reference DNA profile (with variant and/or off ladder alleles).

The case manager must independently perform the calculation for allele designation including if the calculated allele falls in the stutter position. Refer to QIS 17137 or QIS 31389 for details. The case manager must also agree with the calculation performed by the plate reading scientist (enter a specimen note stating this). In the absence of an extract, a re-run is acceptable.

Variant/OLA/ULP/crossover calculations do not require checking if the DNA profile has been assessed as unsuitable for interpretation.

6.3.4 NAD samples

If a sample is flagged as No Analysed Data (NAD) at CE quality checking stage, the sample will be re-prepared by Analytical staff. To confirm the DNA profile is correct and has not been taken from a different well the sample needs to be re-amplified to confirm.

6.3.5 Edit DNA profiles

As interpretation of PP21 samples uses a continuous method of interpretation (STRmix™) rather than a binary method of interpretation, the homozygote and stutter thresholds are used only to guide the scientist in assessing the number of contributors to a DNA profile.

At the plate reading stage of PP21 casework plates, readers do not apply any homozygous threshold. Similarly, putative stutter peaks are not removed from the DNA profile as this information is required by the STRmix™ program for modelling. Following plate reading, PowerPlex®21 casework plates are processed by a macro (QIS 26045 *GeneMapper ID-X File Conversion*) to call a single peak at a locus (for example‘15’, rather than ‘15,-‘ or ’15,NR’, regardless of peak height.) for AUSLAB upload , to check against the staff database, and create a STRmix upload file.

For single source samples that require NCIDD upload , After processing in STRmix™, the case manager will then change the allele designation in AUSLAB (via the [**SF7**] ***Results History***) to either ’15,15’ or ’15,NR’ (based on the STRmix™ deconvolution). The amended profile will then need to be re-saved or edited on the XPLEX page.

Peaks that have been assessed by the case manager to be removed or re-added are list inserted to the 1JMM list for a profile edit. In the specimen notes add the locus name, locus designation to be removed or added, the test name, GeneMapper IDX batch ID and reason. The person editing the DNA profiles from the 1JMM list will liaise with the case manager if the edit is disputed.

The procedure for editing a DNA profile is outlined in section 8.8 Changing Completed GeneMapper Profiles within the QIS 31389. This is a weekly rostered task performed by a competent plate reader.

The DNA profile should be amended in AUSLAB (via the [**SF7**] ***Results History***) by the case manager and if there is a paper case file, reprint the corrected epg.

6.3.6 Rework DNA extract if necessary.

QPS in conjunction with Forensic DNA Analysis have decided that if reworking is required, that additional testing will only be conducted on Major Crime samples and selected Volume Crime samples (high priority cases - DNA priorities 1 & 2) via any means available.

Not Current

For PP21:
Internal validation studies have shown that samples with low template DNA (~132 pg) that are amplified with PP21 may exhibit significant stochastic effects such as large allelic imbalance and allele drop-out. These effects can complicate the interpretation of both single source and mixed DNA profiles. Reworking may improve the quality of the DNA profile.

For P+:
Low priority Volume Crime (DNA priority 3) cases are only to be reworked via re-amplification, re-running (previously known as re-Genescanning) or re-reading in GMIDX until 12 alleles are obtained (National Criminal Investigation DNA Database-NCIDD uploading threshold). No NucleoSpin cleanups or Microcon concentrations are to be ordered on low priority samples. Other valid reasons for reworking these samples include investigations of adverse events or other quality issues are suspected.

If allelic imbalance occurs (<70% in a Genotyper profile or <50% in a GMIDX profile), the alleles can be reported as single source if the case managing scientist is confident that both alleles at the locus are paired from a single contributor. The profile should be examined carefully for evidence of a mixture; if there is no evidence of a mixture both alleles can be reported. The case managing scientist wanting to accept the alleles as true should record this on the epg. For paperless cases this is recorded in the specimen notes for the sample. By validating the 9PLEX page for such a sample, the reviewing scientist is agreeing that they approve of this interpretation.

If a partial profile or NSD profile is obtained for a sample, an assessment should be made as to whether reworking that sample will be beneficial or if there are other profiles within the case that satisfy reporting requirements.

Rework strategies:
If it is determined that a better profile is required, the following should be considered when determining the best rework strategy:

1. **The type of sample**
   e.g. blood versus cells. Due to the generally high number of nucleated white cells in whole blood, a DNA profile is usually obtained from such samples. On the other hand, shed skin cells contain few or no nuclei and therefore it is more difficult to obtain a profile of DNA that has been deposited on an item through casual contact.

2. **The quantitation value**
   The quantitation value is displayed in the 'FBQUAN' or 'QUANT' field in AUSLAB. The quantitation value is an estimate and should be assessed in conjunction with other factors. Sample workflows based on the quantitation value are listed below:
   See also Appendix 3 – Quantitation

   1. P+ samples with an undetermined quantitation value will not be further processed and will be reported in their associated EXH as 'No DNA detected', regardless of priority.
   2. P+ samples with any detectable quantitation value will be amplified.
   3. P+ samples in cases that were previously High Priority (P2) that require a microcon rework (MICFCW), need to have instructions added to the Processing comments for the type of microcon eg. Mic to full, mic to half, mic to 30uL.

Not Current

4. PP21 samples with a quantitation value <0.001 ng/µL will not be further processed and will be reported in their associated EXH as 'No DNA detected', regardless of priority.
5. PP21 QPS environmental samples with a quantitation value of >0.001 ng/µL but <0.0088 ng/µL as per QPS –Forensic DNA Analysis agreement will not be routinely amplified or sent for Microcon processing. This result will be communicated using the 'DNA insufficient for further processing' EXH line.
6. PP21 samples with an initial quantitation value between 0.001 ng/µL and 0.0088 ng/µL will automatically be sent for a Microcon to 35 µL and be re-quantitated then amplified.
7. PP21 samples with an initial quantitation value of > 0.0088 ng/µL are amplified.

A partial or NSD profile from a sample with a high quantitation value may indicate inhibition. It should be noted that while quantitation values can be used as an indicator for the presence of inhibitory compounds in an extracted sample, lack of inhibition in a quantitation amplification (as indicated by the IPCCT and possibly the CT as well) does not necessarily mean there will be no inhibition in an STR amplification. This is because different primers, target DNA and amplification conditions are used in each reaction and this could result in inhibition to one reaction and not the other. Also, 2 µL of extracted sample is added to a quantitation amplification, whereas in an STR amplification the sample may be diluted before being added (which would decrease the concentration of any inhibitory substances in the amplification reaction), or up to 20 µL may be added to the reaction for a P+ sample, 15 µL for a PP21 full volume amplification (which would change the relative concentration of inhibitory substances in the amplification reaction). Further information on DNA quantification is found in QIS 33407 *Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit*.

There may be additional quantitation information available for a sample (depending on whether it was processed pre/post AUSLAB batch functionality)

3. The number of alleles obtained

A full DNA profile is the aim of any DNA amplification but a partial DNA profile does not necessarily need to be reworked.

The minimum number of alleles required to upload to NCIDD is 12 alleles. Samples below this stringency, but above 6 alleles, may be loaded to NCIDD under special circumstances and searched against the database (refer to QIS 23890 *Uploading and Actioning Samples on NCIDD*.

If an assumption of a single contributor has been determined, Partial DNA profiles do not have to be reworked to obtain a full DNA profile.

4. **Examination notes**

Certain substances are known to be inhibitory to the PCR process. This includes a variety of commonly encountered substances, such as dyes used in clothing (particularly denim dyes) and some biological material (in particular, the haem in blood). Semen samples have also been observed to return an NSD profile after initial extraction with no indication of inhibition. Performing a NucleoSpin clean up may result in improved or full profiles for these samples.

Not Current

5. **Offence Details (if available)**
   The information from the QP127, item packaging or from case conferences may assist in determining the evidential value of a particular item. If the case has a paper file, it will already have been printed and added to the file, if it hasn't or for paperless cases the QP127 will have been scanned into AUSLAB usually on the case barcode.

6. **Results already obtained**
   If multiple samples have been submitted for an item and one or more full profiles or mixtures have already been obtained there may be no need to continue reworking other samples from that same item. A partial 'matching' profile is often sufficient if other better profiles already exist for the same item. This must be considered carefully and in the context of the case. If it is a possibility that there may be a different profile present, such as in the case of multiple offenders, then reworks should be considered.

AmpFℓSTR® COfiler®

Prior to samples routinely being processed using PP21, additional work using the AmpFℓSTR® COfiler® (COfiler) kit could be ordered if required. Samples processed using the P+ kit could be amplified with the COfiler kit in order to amplify an additional 4 loci.

Reworks on AUSLAB batch functionality samples

When requesting reworks ensure that the case scientist field (**XPCM**) is completed; this will reduce double handling as the same case manager will take ownership of that particular sample.

All reworks for current samples are carried out through the [**SF7**] ***Results History*** screen of the sample as per QIS 24469 *Batch Functionality in AUSLAB*.
Specific tests codes are available for each rework. Refer to Table 5 below and QIS 24486 *Explanations of Analytical Test Codes and Batch Types* for the reworks available in AUSLAB and the test codes to be used.

Table { SEQ Table * ARABIC } - Case work test panel and rework tests

Description	XPLEX Panel	Processing Comment	9PLEX Panel	Processing Comment
Primary test panel	XPLEX	XPC1	9PLEX	9PLEXP
Re-Amplification	XAMP1	XPC2	AMP1CW	N/A
Re-Amplification	XAMP2	XPC3	AMP2CW	N/A
Re-Amplification	XAMP3	XPC4	AMP3CW	N/A
Re-Quantification	XREQC	XPC7	REQC	N/A
Microcon procedure	MCONC1	XPC8	MICFCW	MICFCP
Microcon procedure	MCONC2	XPC9	MICCW2	MICF2P
Re-Run	XRRC	XPC5	RRCW	RRCWP
Re-Run	XRRC2	XPC6	RRCW2	RRCW2P
NucleoSpin clean up	NSPNC1	XPC10	NCLCW	NCLCWP
NucleoSpin clean up	NSPNC2	XPC11	NCLCW2	NCLC2P
Re-Read	N/A	N/A	GMCW	

The total PCR volume used for a PP21 amplification is 25 µL and P+ amplification is 50 µL and the corresponding total allowable sample volume (SV1) is 15 µL and 20 µL respectively.

SV1 and TV1 must add up to 15 µL (for XPLEX) and 20 µL (for 9PLEX).

Not Current

SV2 and TV2 are used when a dilution is required.SV2 and TV2 combined cannot exceed 20 µL. If a dilution is not required these must be filled in as '0'. To enter the volumes required for a dilution see QIS 24012 *Miscellaneous Analytical Section Tasks*.

The MultiPROBE® II PLUS HT EX Forensic Workstations are unable to pipette volumes of less than 1 µL, concentrated samples should be diluted so that the minimum volume is 1 µL or greater. The MultiPROBE® II PLUS HT EX Forensic Workstations are unable to pipette from dead volumes of less than 20 µL, these samples are manually prepared from amplification.

If a re-run rework test is requested (XRRC, XRRC2, RRCW and RRCW2), the amplification batch and sample position must be specified in the Processing comments (Refer to Table 2 above for the corresponding test results). The information is obtained from the Audit Trail (if the sample has been previously amplified multiple times, be careful to choose the correct batch). The information entered needs to be checked by a second case managing scientist and a specimen note entered indicating that the information is correct.

When ordering these reworks the user is prompted to add a processing comment, Enter this information when prompted or it can be added later on the secondary test panels 9PLEX2, XPLEX 2, XPLEX 3, Microcon Casework, NucleoSpin Casework pages in the re-processing comments field.

Amplification products are not kept indefinitely. The availability of a PCR product should be checked prior to ordering a re-run. For more recent batches, the Analytical Section enters audit notes against the amplification batch when the PCR product has been discarded.

If the rework is for a GMIDX re-read, the original Genotyper ID and folder number must be specified in the processing comments. Enter this information when prompted after you enter in the rework code or it can be added on the 9PLEX(2) page in the Microcon processing comment field.

Extra barcodes required for processing of a sample

There is a limit to the number of test codes available for reworks on a single barcode. For 9PLEX or XPLEX case work samples

Extra barcodes are required when:

1. A sample requires further reworking but the available test codes have been used.
2. Re-extracting from a spin basket; this requires an additional barcode as there can only ever be one extract in existence for each barcode.
3. Between 29 October 2007 (when the first automated DNA IQ casework batch was created) and 19 March 2008 (when routine Off Deck Lysis procedures began) the extraction process required some samples to be sub-sampled in Analytical prior to extraction. If this was the case for a particular sample, a specimen note will indicate that the sample was too large and was sub-sampled by Analytical scientists. If satisfactory results were not obtained from the extraction of the portion sub-sampled by Analytical scientists, the remainder of the sample can be requested to be processed under another barcode.
4. Pooling of DNA extracts. Refer to procedure below and QIS 17142 *Examination of Items* for pooling post-batch functionality samples.
5. Dilution of high quantity DNA extracts or inhibitors -Samples that require a dilution greater than 1 in 20 (for 9PLEX) or 1 in 15 (for XPLEX) or have exhausted the available re-amplification test codes will also require an additional barcode to be requested.

Not Current

If a sample has been completed in DNAMaster/DAD and subsequent reworks are required they are requested in AUSLAB.

To request a connected barcode and connected barcode reworks:

1. View the XPLEX,9PLEX, 9PLEXX or FBOLD page of the sample
2. Press [**SF10**] ***Registration***
3. Press [**SF5**] ***Copy Entry***
4. Scan in the new barcode
5. Change the ***Received*** date to the current date and time
6. Change the ***Collected*** date to the current date and time
7. Change the ***Specimen***
    i. to '***Trans***' specimen type
    ii. to '***Pooled***' specimen type if pooling pre batch samples
    iii. to '***DNAD***' specimen type for diluting samples.
8. Enter the appropriate DNA priority for the sample
9. Enter details in the ***Sample Info 1*** field
    i. If the sample is being transferred from a **XPLEX** or **9PLEX** sample add the source barcode to the ***Sample Info 1*** field.
    ii. If the sample is a pre batch functionality sample then the ***Sample Info 1*** field must contain the DNA# of the sample.
10. Request a 9PLEX or XPLEX test code depending on the request from QPS.
    i. If pooling samples - When prompted to enter a processing comment identify the type of rework required, e.g. Microcon to full
    ii. If diluting, specify the dilution required e.g. 1:100
11. Press [**F4**] Save to complete and save the registration
12. If XPLEX is requested on a pre batch functionality sample, add to the DNA number to the DNAN (**DNA#**) field.
13. If 9PLEX is requested on a pre batch functionality sample, the DNA# must then be entered into the '***Connected barcode***' field.
14. Fill out the connected barcode field (BARCON for 9PLEX), Comment field for XPLEX with the source barcode and from the source barcode identify the connected barcode in the same fields.
15. For pooled samples, Request a **POOLED** test code for each barcode being pooled.
    i. In the 'This lab number has been pooled with Lab Number' field put in the other samples that the sample has been pooled with (**including** the barcode and **DNA#**).
    ii. In the 'Processed Using Lab Number' field enter the extra barcode given for the processing of the pooled samples.
    iii. The 'Reported Under Lab Number' field will be filled in when final results are available

6.4 Manage samples

Sample management refers to the management of an individual sample. It incorporates the necessary steps to be completed in AUSLAB and if a paper case file exists, the information required to be transcribed onto the case file records.

Cases are not usually allocated to an individual case manager/reporter. The exception to this rule may be some urgent or Blue cases, QPS operations, linked cases or sensitive matters. Samples are case managed by staff from the case management lists. However, if at the time of statement allocation there are outstanding results, a reporter can choose to rework or case manage the outstanding results themselves.

Not Current

Cases with paper files require the epg to be annotated with the results and interpretations. As a minimum, the type of DNA profile. e.g. single source matching UKM1 is required. These annotations need to be signed and dated by the case manager. The historical 'Reported profile' annotation is not required for PP21 DNA profiles. If a re-run (re-CE/ReGS) has been performed the identification of which run used in STRmix is necessary.

6.4.1 Interpret

6.4.1.1 Paired Kinship/Paternity Trios

Any samples for Paternity trios etc. are interpreted as detailed in QIS 25303 *Statistical Analysis for Paired Kinship and Paternity Trio / Missing Child Scenarios*.

6.4.1.2 PP21 interpretation

Statistics for PP21 results are generated by the STRmix™ program as outlined in QIS 31523 *Use of STRmix™ Software*.

If a sample has replicate amplifications they must all be included in the STRmix™ deconvolution unless they have a particular processing issue such as different amplification volumes (half volume vs full volume PCR amplifications), excess peak heights and pull up, a Re-run has been performed or the runs are not consistent with each other (at the discretion of the case manager). A Re-run and the source amplification results cannot be included in the same deconvolution as they come from the same amplification, a choice as to the best or most appropriate run must be made by the case manager, and replaces the less informative result. At minimum, a specimen note (for paperless cases) and an annotation on epg (for paper casefiles) must be added to explain why particular amplifications were not included.

In the instance that a case has had half volume PCR amplifications performed, it is not necessary to include these in the case file as they won't be reported.

All reference samples received for a particular case are to be compared against all interpretable mixtures (to generate a Likelihood Ratio - LR) and single source samples within a case.

The number of contributors will have been determined as per section 6.3.1 above.

Samples processed with STRmix™ V2.0.6 use three populations (Australian Caucasian, Aboriginal and Asian) for the calculation of LRs, the figure that is quoted is the most conservative of the three with a factor of N and 99% one tailed HPD. The case manager is to ensure that this is the figure that is used to determine which EXH line will be used.

Table { SEQ Table * ARABIC } – Quick reference when to use STRmix™

Scenario	Decon	LR
SS <32 & matches assumed known contributor	No	No
SS <32 & matches a reference sample	Yes	Yes
SS <32 & new Unk & NCIDD	Yes	N/A
SS <32 & matches an Unk	No	N/A
First SS >32 DNA profile & matches a reference sample & NCIDD	Yes	No
First SS >32 DNA profile & matches a reference sample no NCIDD	No	No
SS >32 DNA profile & new Unk & NCIDD	Yes	No
Subsequent SS >32 DNA profile and matches a reference sample/Unk	No	No
2P or 3P & no reference samples & not likely to resolve for NCIDD	No	N/A
2P or 3P cond & no other reference samples & not likely to resolve for NCIDD	No	N/A
2P or 3P & reference samples	Yes	Yes

Not Current

Single source DNA profiles

Deconvolution with STRmix™ is required:

1. If the sample is the first single source DNA profile that matches a reference sample and needs to be loaded to NCIDD or
2. The sample requires loading to NCIDD (e.g. UNK). And/or
3. This DNA profile has a less than 32 alleles.

LR generation with STRmix™ is not required for single source DNA profiles:

1. If a reference sample does not match the single source sample.
2. If a matching reference sample has previously had an LR generated (and the new interpretation would not be more probative).
3. If the single source DNA profile has 32 or more alleles, the sample can be reported with the appropriate EXH line (As per QIS 23008) and doesn't require deconvolution and an LR generated as per the recommendations in the document 'The determination of the threshold number of alleles, above which single source DNA profiles can confidently be ascribed a likelihood ratio in excess of 100 billion.' [Parry et al 2014].

If a single source DNA profile has one peak at a locus and another peak is visible sub threshold, STRmix™ may designate the locus as a homozygote (with a ≥99 % weighting), the case manager should order a rework in an attempt to amplify the second peak.

Homozygote alleles for single source samples that will not be loaded to NCIDD do not require editing in AUSLAB. Homozygote alleles designated by STRmix™ need to be updated in AUSLAB as follows.

1. From the XPLEX screen of the sample
2. Press [**SF7**] ***Results History***
3. Select the appropriate/representative test panel
4. Move the cursor to the allele
5. Press [**F2**] to edit the allele
6. When prompted 'Are you sure (y/n) ?' type 'y'
7. Add the second allele in the format x,x
8. Press [**Enter**]
9. Repeat for the other homozygote alleles
10. Press [**F7**] **Select Preferred**
11. Press [**SF6**] **Save Preferred Profile**

A mixed DNA profile would be reported as a single source profile with sub-threshold peaks using the appropriate EXH line (as per QIS 23008) in the following circumstances:

1. If the only indication of a mixture is a labelled Y peak at Amelogenin or
2. If the only indication of a mixture is a labelled Y peak at Amelogenin and sub-thresholdpeaks that do not affect the called alleles.

This is done because STRmix cannot 'see' Amelogenin or sub-threshold peaks and the low level contribution does not affect the interpretation of the 'single source' profile.

Two and three person mixtures

Deconvolution with STRmix™ is not required:

1. If the case does not have any reference samples and the profile is not likely to be deconvoluted by STRmix™ into contributions for NCIDD or

Not Current

2. If the case does not have any reference samples and if the DNA profile is likely to be deconvoluted into a contribution that matches an already reported unknown in the case. These are reported with the appropriate EXH.

If reference samples are later received they will be compared against the mixture and the LRs reported back via EXH. This is covered in sections 6.5 and 13.1 below.

Deconvolution with STRmix™ is required for all other two and three person mixtures. All three person mixtures have the number of iterations increased to 100 000 burn in and 500 000 accepts.

Two and three person mixtures may run for extended periods of time, additional support is provided by HP2 staff to run deconvolutions on dedicated STRmix™ computers. This releases Reporting Scientists' computers for other tasks.
To have a HP2 staff member run a deconvolution:

1. Add the STRmix™ parameters required as per Table 7 below to the XPLEX comments field.
2. Add your name to the Case Scientist (**XPCM**) field
3. Change the Sample status (**XPRES**) to 'STRmix decon' – the system will list insert the sample to the 'STRMIX' (STRmix decon) list
4. When the STRmix™ deconvolution (and any required reference sample comparisons) have been completed by the HP2 staff member they will change the Sample Status (**XPRES**) to '*STRmix complete'* and the system will list insert the sample to the STRmix CM list
5. The **STRMIXCM** list is sorted by Case Manager then Request Date (in ascending order)

Table { SEQ Table * ARABIC }- Comment for STRMIX list

Decon Interp	**Decon Instruction Truncated**
STRmix™ v1.05	v1
STRmix™ v2.0.6	v2.6
1 contributor	1p
2 contributors	2p
3 contributors	3p
Add c for condition and specify REF name	c SMITH
Add LR for any ref comparisons (add / if after conditioned REF name)	LR (eg. c SMITH/LR)
Decon all runs	No comment added
Decon specific runs	List runs by testcode only eg. XPLEX XAMP1 MCONC1

Conditioning mixtures
It may be possible to condition mixtures from intimate swabs and items (said to have come from a person). The decision to condition is at the discretion of the case manager (and reviewer). Additional information regarding ownership may be required – as per section 6.2.4 above.

Other mixtures
Mixtures that have been assessed as having four or more contributors are considered unsuitable and are not interpreted with STRmix™.

STRmix™ results output

Not Current

After the STRmix™ deconvolution and/or reference comparison has been completed and processed with the QIS 32139 *STRmix™ Report macro*, the following quality checks must be performed on each result produced by STRmix™.

1. STRmix™ version
2. Casework sample number is correct
3. Reference sample number (if any) is correct
4. Number of contributors assumed to be present is correct
5. Casework DNA profile (correct allelic designations entered and correct run(s) have been included)
6. Individual locus LRs appear have an intuitive fit
7. Check all loci had successfully deconvoluted (component interpretation complete)
8. Settings values (especially check full vs. half variances)
9. Reference DNA profile (correct allelic designations entered)
10. The overall LR is reasonable given the reference and casework DNA profiles

It is important when a STRmix™ analysis is carried out, that the results are interpreted by examining the weightings of various genotypes and the DNA profile(s) observed. There are instances when the results obtained do not intuitively seem correct. Sometimes (particularly if the model must account for drop-in) the failure of the Markov chain to properly converge means that some parameters will not have optimised properly. Examples of this are:

1. Large LRs are obtained for each locus, except one where the LR is low or 0
2. The mixture proportions do not reflect what is observed
3. The degradation does not reflect what is observed
4. Genotype combinations do not reflect all likely allele sets (especially likely if sub-threshold peaks are present at a locus)
5. The probability of dropout at a particular locus has been given a low value but sub-threshold peaks are clearly visible in the DNA profile.

Effectively, a zero LR means that the genotype of the POI has not been accepted by the MCMC at any time through the course of the analysis. Common causes for making a genotype an unlikely contributor are large required dropouts, drop-ins or imbalances, or when the peak heights at a locus exceed the general degradation slope (and are therefore penalised). The standard values for an increased iteration analysis is 100K/500K for burn-in and MCMC respectively. If further iterations are chosen, then the MCMC will have more opportunity to accept the less supported genotypes, however a reference sample with a poor fit to the DNA profile will still have a low LR for a particular locus or loci. It is best practice to attempt to resolve the mixture biologically first, that is through rework, prior to resorting to increased iterations.

If it is noted that the epg has a plate reading error, such as a stutter peak that has been inappropriately removed or an artefact that has been left in, then the sample must be reread and the corrected GMIDX file exported. See section 6.3.4 above.

Paper cases have the STRmix™ report printed and added to the case file, Paper and paperless cases have the results stored on a network drive I:/STRmix results/ .

Repeated Analysis

Each time a DNA profile is analysed using STRmix™ the results will vary slightly. This is a natural consequence of the random nature of the Monte Carlo property. To be as unbiased as possible, each analysis should only ever be run once and the result reported. If a STRmix™ result has been generated for a DNA profile at case management stage, then that same result should be the one used for statement writing. If additional reference

Not Current

samples are received in the case, the reference sample(s) should be run against all original deconvolutions for all samples in the case where mixtures are present. The exception to this is when an analysis has produced a result that requires further investigation and hence further analysis or if the underlying assumptions made about the profile have changed (eg. a two-person mix is reassessed as being a three-person mix).

Consequently, if at review or at a subsequent stage in reporting it is decided that a different number of contributors better fits the DNA profile, the deconvolution for that sample can be rerun using the new assumption. Case-managers/Reporters should discuss any decision to change a reviewed result with the original reviewer.

If multiple analyses have been conducted, then only the STRmix™ results from the most appropriate analysis should be included in the casefile (e.g. the higher number of acceptances or the more appropriate number of contributors). All relevant STRmix™ results will need to be included in the casefile and previous results removed from the casefile.

The electronic STRmix™ results from the multiple analyses that are not used must be moved into a sub-folder labelled “Do not use” in the case folder in the STRmix results folder.

Use of Ignore Loci function
In certain circumstances a particular locus or loci may be dropped from the interpretation. These include where a Tri-allele pattern has been observed in a reference profile and inconsistent sizing of an allele is observed. There is an option in STRmix™ version 2.0.6 to exclude these loci from the calculations. The Ignore Loci option in the STRmix™ options menu is used. See QIS 31523 Section 8.

Use of the database Search
If there is a large number of reference samples associated to a case, the database (DB) search function in STRmix™ is used. This is the preferred method in this scenario as the number of LR results to be checked and reviewed will be kept at a minimum. The database search function will generate a LR for each reference sample in the file.

Reference DNA profiles that contain partial loci information (e.g. 15, NR) cannot be used in STRmix™ v1.05 or 2.0.6 Database searches. Reference DNA profiles that are partial (whole locus/loci missing) can only be used in STRmix™ v2.0.6 database searches.

A database of reference samples for a particular case is created as per Database search section in QIS 31523.

The created database must be checked by a second case manager/reporter before the database is used in STRmix™. After checking the alleles and sample IDs are correct the checker must replace the case ID on each line with their AUSLAB login mnemonic.

The STRmix DataBase macros incorporates the Factor of N. When the macro is run on STRmix™ V2.0.6 database results, it will ask whether the mixture is 2 or 3 people so that the correct factor can be applied. The reviewer must check that the correct Factor of N has been applied on the printout. It should be 2 for two person mixtures and 6 for three person mixtures.

The results of the DB search are reported back via an EXH (see section 6.5.4). Reference DNA profiles that generate LRs supporting low or non-contributions can be excluded if there are sub-threshold peaks that support the exclusion.

Not Current

As the DB search does not incorporate the HPD (Highest Posterior Density) or θ (Theta/FST) correction, when a statement is requested, reference samples that give an LR > 0 (except if an exclusion can be made on sub-threshold peaks) need to be compared directly against the original deconvolution to generate an LR incorporating HPD & θ. This ensures that the most appropriate LR is reported in the statement.

Donors of reference samples may be intuitively excluded, in which case the DB search output can be overridden by case managers making this decision (see Appendix 3 – Intuitive Exclusion Guide).

6.4.1.3 P+ interpretation

Samples that are processed with P+ are not interpreted using STRmix™ as this system has not been validated for use with P+ data. Interpretation of P+ samples is outlined in QIS 17168 *Procedure for Single Source DNA Profile Statistics*, QIS 25302 *Interpretation of Mixed DNA (STR) Profiles using Profiler Plus*.

Single source
Single source P+ samples are matched directly against reference profiles and if required for a statement, have a statistic performed as per QIS 17168.

Mixtures
Mixtures are interpreted as detailed in QIS 25302.
P+ mixtures may be transcribed into AUSLAB on the MIXT/MIXC test panels.

Reference samples are compared to the contributions and CODIS Popstats may be used to generate a LR for mixtures that cannot be separated into a major and minor DNA profiles.

Complex mixtures may not have all of the alleles exported from GeneMapper IDx because only 6 alleles per loci are exported and therefore they may not have been identified in the QFLAG checking.

If your profile is able to be interpreted (complex cannot exclude/ complex unable to load, or a major/minor), and at any loci has 7 or more alleles, then an assessment needs to be made to suitability for QFLAG checking. If the major or “large” peaks are captured within the first 6 alleles for each loci, then those peaks have undergone quality flag checking. If any peaks within the major or ‘large’ peaks fall outside of the first 6 alleles at more than one loci (as the staff match macro allows for 1 mismatch) then a quality flag check can be undertaken for the major/large peaks by the Quality and Projects Senior Scientist. This sample can be inserted onto the BQUAL list with a specimen note “for Quality flag checking”. The Quality team or delegate will then perform the quality search on the larger peaks within the profile and add a specimen note, inserting back onto the case managers comms list.

6.4.2 Complete LIMS

AUSLAB is pre-programmed with a number of auto-validation rules to speed up case management. These rules rely on certain conditions being met. At the time that a DNA profile is imported into AUSLAB, auto-validation occurs to allocate the sample to an appropriate hold or case management list. The core comments used by plate readers QIS 31389, direct the sample via test panels that are ordered by the GIDXXPLX or GMIDX auto-validation mask. The system will ‘Save prefer’ the DNA profile triggering an additional AUSLAB equation to add the completed date and change the sample status to ‘Completed’ At the end of case management and review of a sample all test panels are required to be fully validated.

Not Current

For PowerPlex 21 samples (XPLEX request):
Refer to Figure 3 below:
Case managers as a minimum must complete the following fields:

a. XPCM (Case scientist) with their mnemonic
b. XPNR (CM NCIDD) with either “Y” or “N”

Case Managers must also ensure that the

a. XPCD (Completed Date) is populated
b. XPRES (Sample Status) with “For Review”

The essential fields (fields that must be completed for full validation of the request) for XPLEX are:

a. XPCD (Completed Date)
b. XPRES (Sample Status)
c. XPCM (Case scientist)
d. XPNR (CM NCIDD)
e. XPRV (Reviewed By)
f. XPRN (Rev NCIDD)
g. XPDAT (Reviewed Date)

If the Case Manager name and Reviewer name are the same, AUSLAB is programmed to delete the Reviewer.

For COMIX
Case managers as a minimum must complete the following fields:

a. CMXDAT (Date completed)
b. CPROS1 (Profile)
c. CP1N (CM A NCIDD)
d. CPROS2 (Profile) – only if second NCIDD load
e. CP2N (CM B NCIDD)-only if second NCIDD load
f. CMXA01 – CMXA21 (Locus fields)
g. CMIX1-CMIX21 (Allele fields)

The essential fields (fields that must be completed for full validation of the request) for COMIX are:

a. CMXDAT (Date completed)
b. CMREV (Reviewed By)
c. CXMRDT (Reviewed)

For Profiler Plus samples (9PLEX request):
Refer to Figure 4 below:
Case managers as a minimum must complete the following fields:

a. CSCI (Case scientist) with their mnemonic
b. 9PLEXS (DNA Profile Result) with “For Review”

The essential fields (fields that must be completed for full validation of the request) for 9PLEX are:

a. 9PLCD (Completed Date)
b. 9PLEXS (DNA Profile Result)
c. 9PLREV(Reviewed By)

Not Current

d. 9PLDAT (Reviewed Date)

Mixed DNA profiles that can be interpreted or deconvoluted are displayed in AUSLAB using one of three test panels – MIXT,MIXC (9PLEX samples) or COMIX (XPLEX samples).

For MIXT/MIXC samples:
Refer to Figures 6 & 7 below.
Case managers as a minimum must complete the following fields

a. MIXCD (Completed Date)
b. MIN101 – MIN110 (Known Contributor/Major)
c. MIN201- MIN210 (Remaining Profile/Minor)
d. MIN301-MIN310 (Ratio)

The essential fields (fields that must be completed for full validation of the request) for MIXT/MIXC are:

a. MIXREV (Reviewed By)
b. MIXDAT (Review Date)
c. MIXCD (Completed Date)

Connected barcodes
Once reworks are finalised on a connected barcode, the 'Accepted Barcode' field needs to be filled out. If the original DNAmaster profile is the best profile, enter the DNA# into this field. If the AUSLAB profile is the best profile, add the barcode to this field.

If a NCIDD load is to occur from the original barcode, the DNA profile needs to be manually added to the XPLEX or 9PLEX page. The alleles designations must be checked by the reviewer to ensure transcription errors are avoided

Hold samples
Samples that have been extracted and held (such as epithelial fractions) need to have the XPLEX page completed in AUSLAB. This ensures that laboratory numbers clear outstanding lists and are shown as fully validated in the SF9 summary screen.

1. Enter your name in the XPCM (Case scientist) field
2. Enter 'No' in the XPNR (CM NCIDD) field
3. Enter 'No' in the XPRN (Rev NCIDD) field
4. Enter 'N/A' in the XPRV (Reviewed By) field
5. Validate all test panels for the request

Last sample interpreted in a case
If all of the samples within the case have been interpreted; on the CS test panel change

1. Case status (CASET) to PRSENT – Sent to Peer Review
2. Exhibit (EXRTR) to
    i. Ready to return (no submissions on whole item) or
    ii. Ready to return subsample retained (where one or more submissions from whole items) or
    iii. Destroyed subsample retained (in-tube case)
    iv. N/A (for Paternity cases with FTAs only)

Not Current

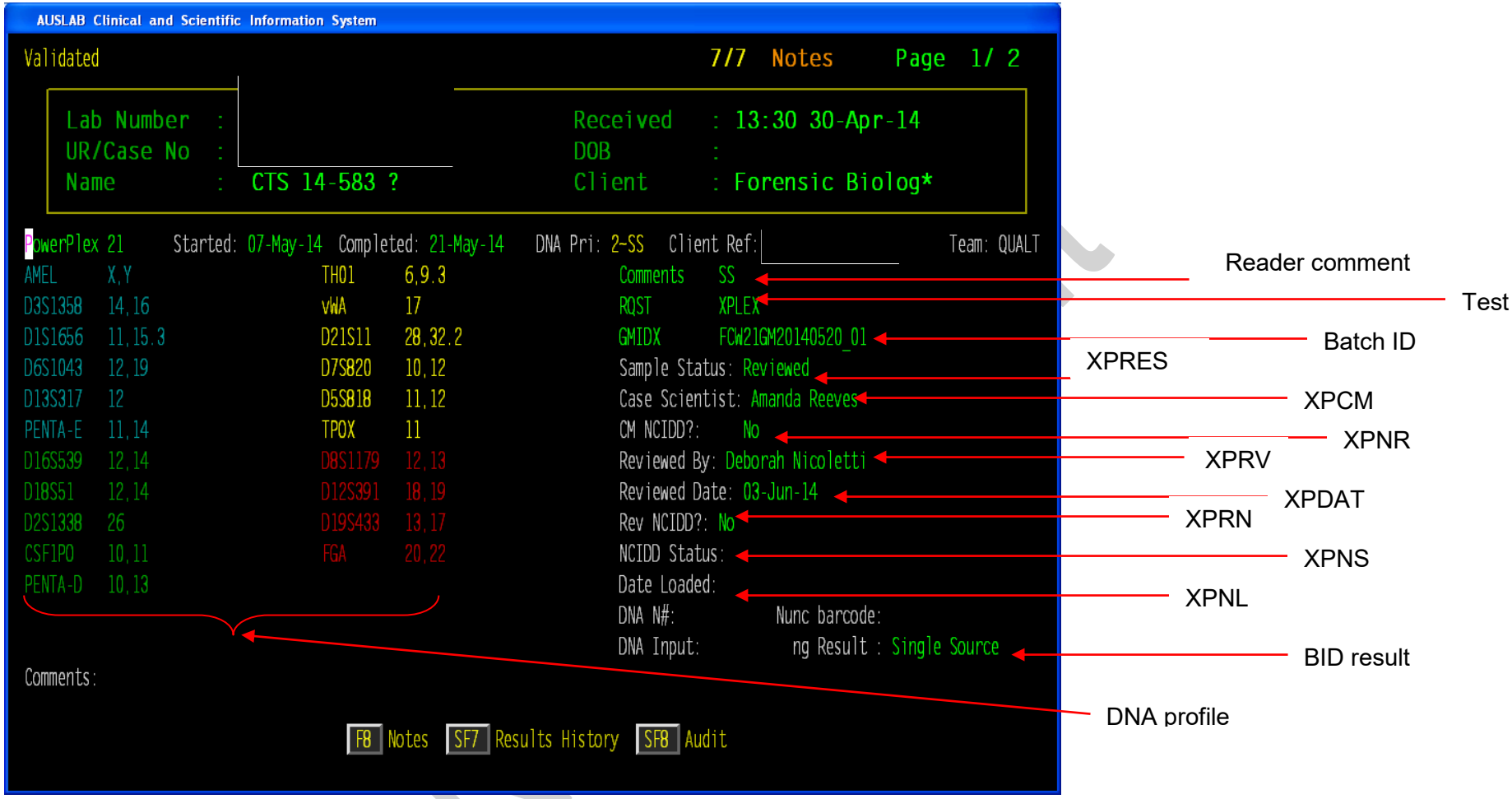

Figure { SEQ Figure * ARABIC } - XPLEX request showing AUSLAB result mnemonics

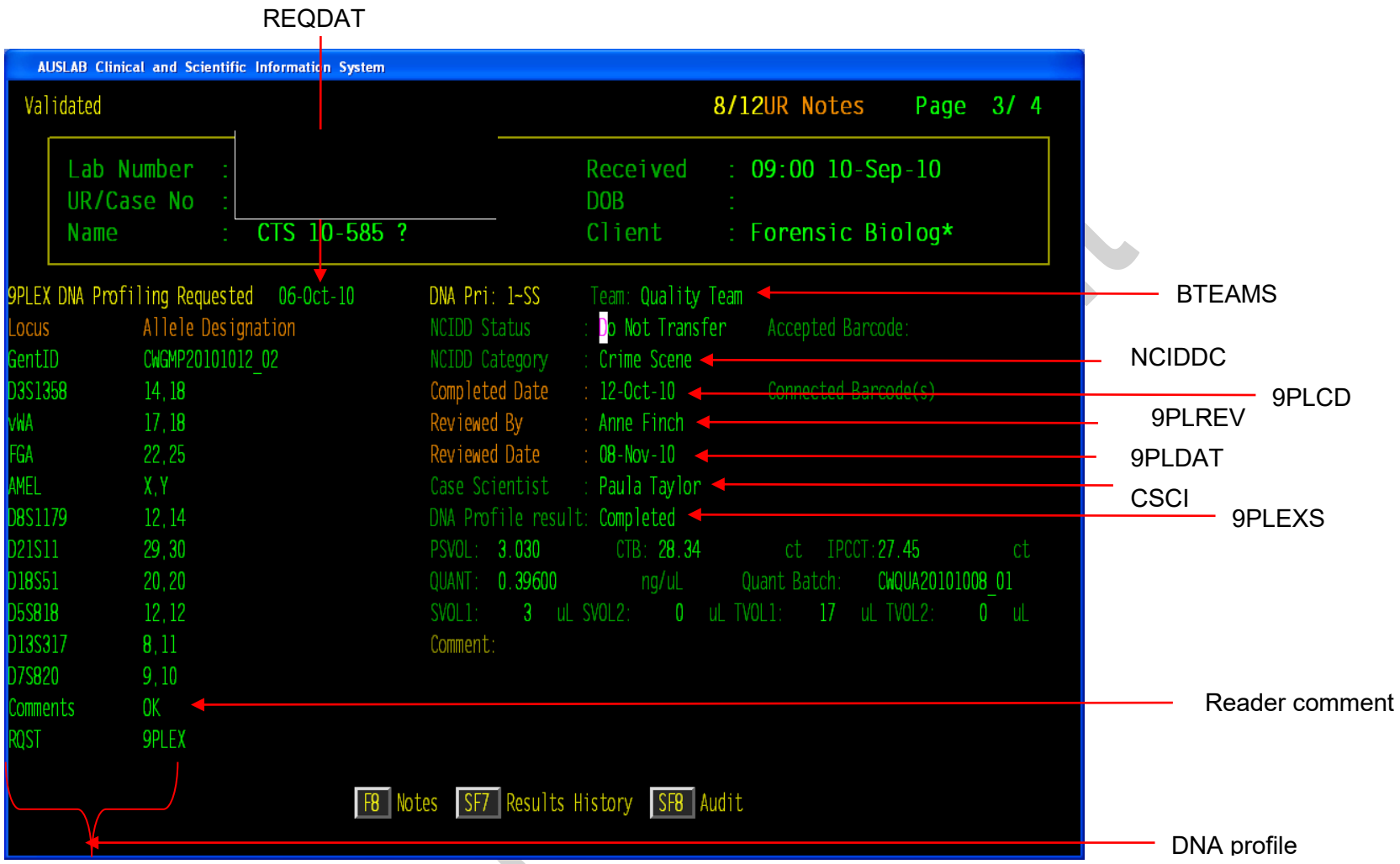

Figure { SEQ Figure * ARABIC } - 9PLEX request showing AUSLAB result mnemonics

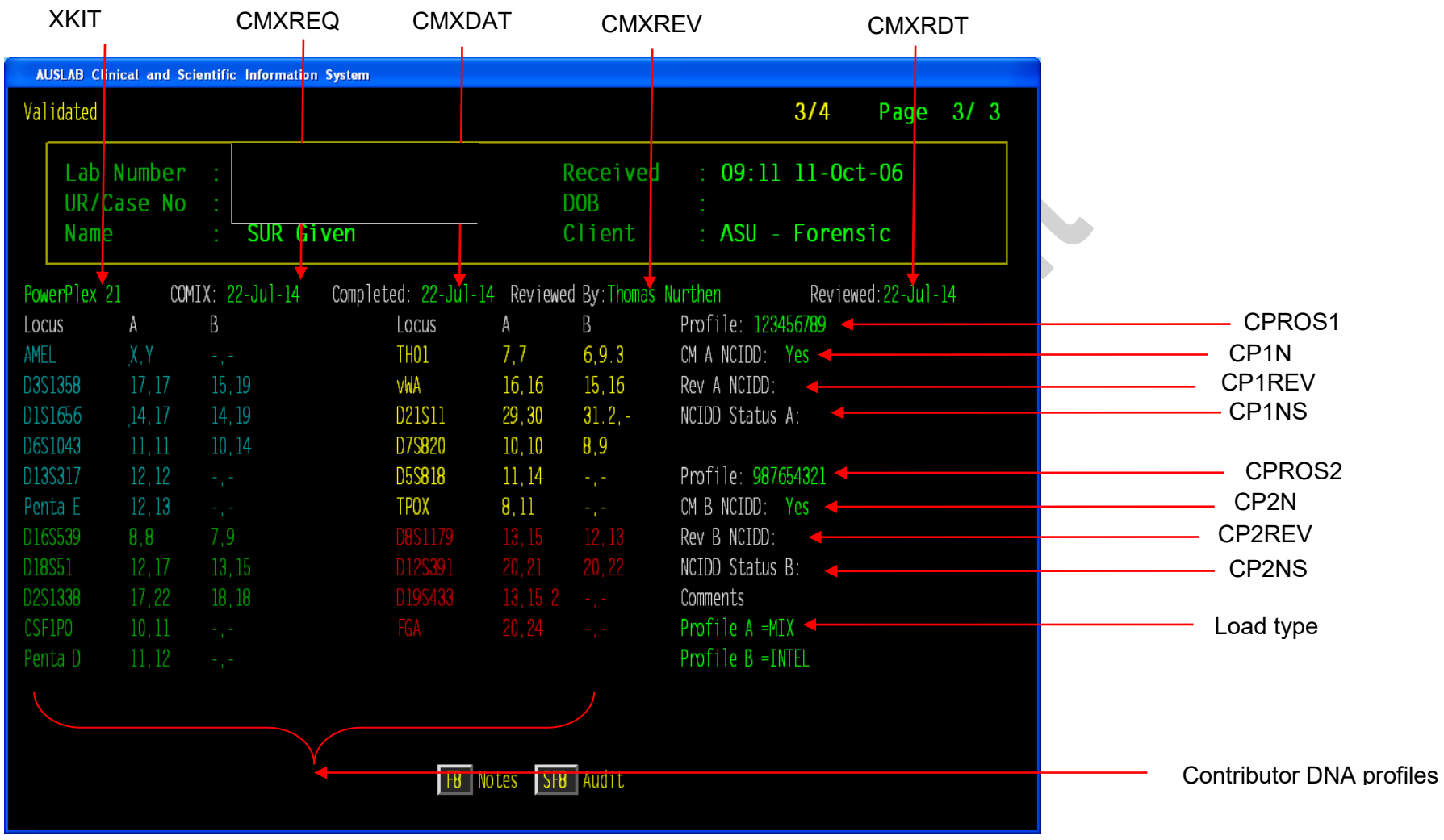

Figure { SEQ Figure * ARABIC } - COMIX request showing AUSLAB result mnemonics

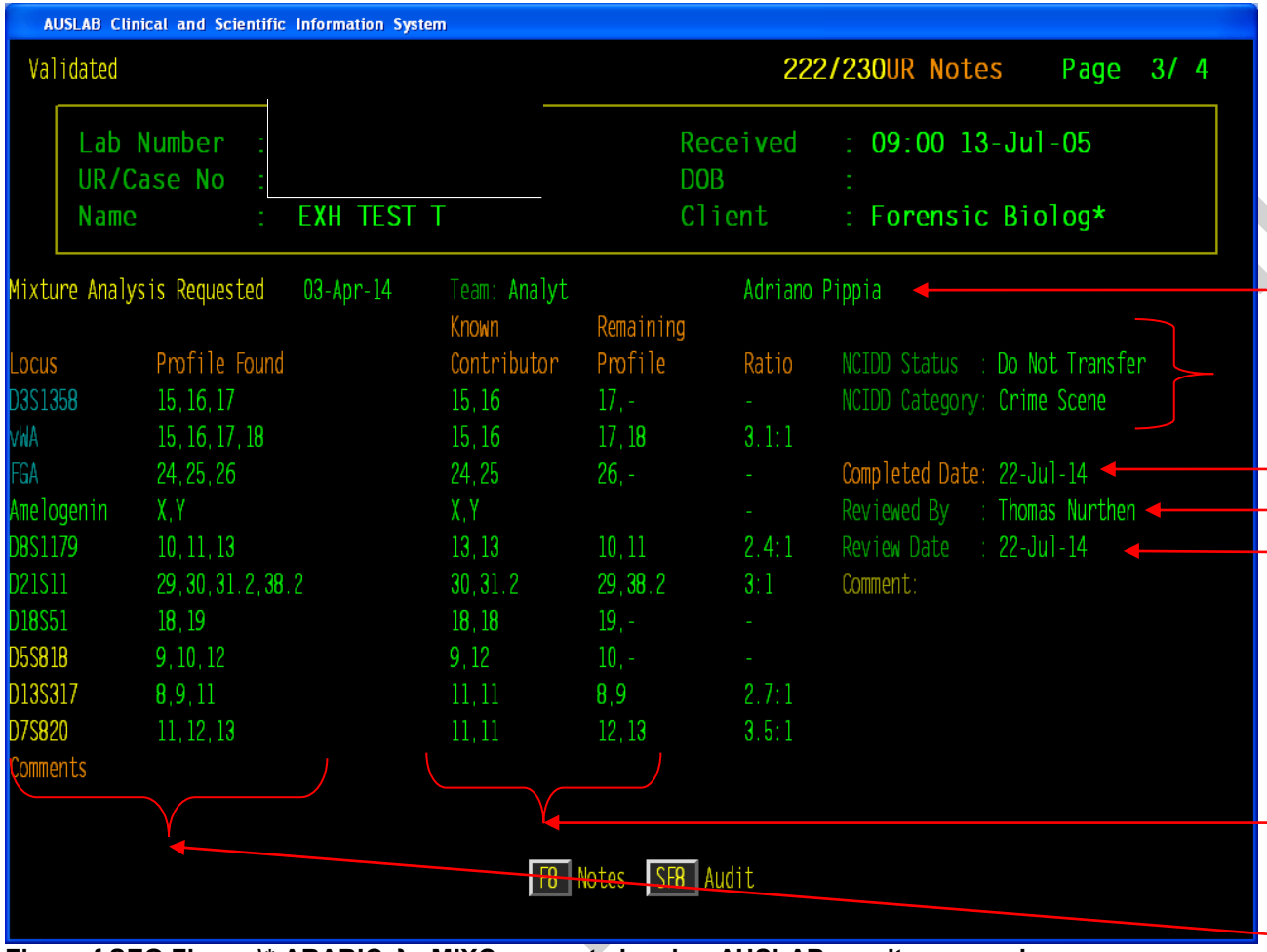

Figure { SEQ Figure * ARABIC } - MIXC request showing AUSLAB result mnemonics

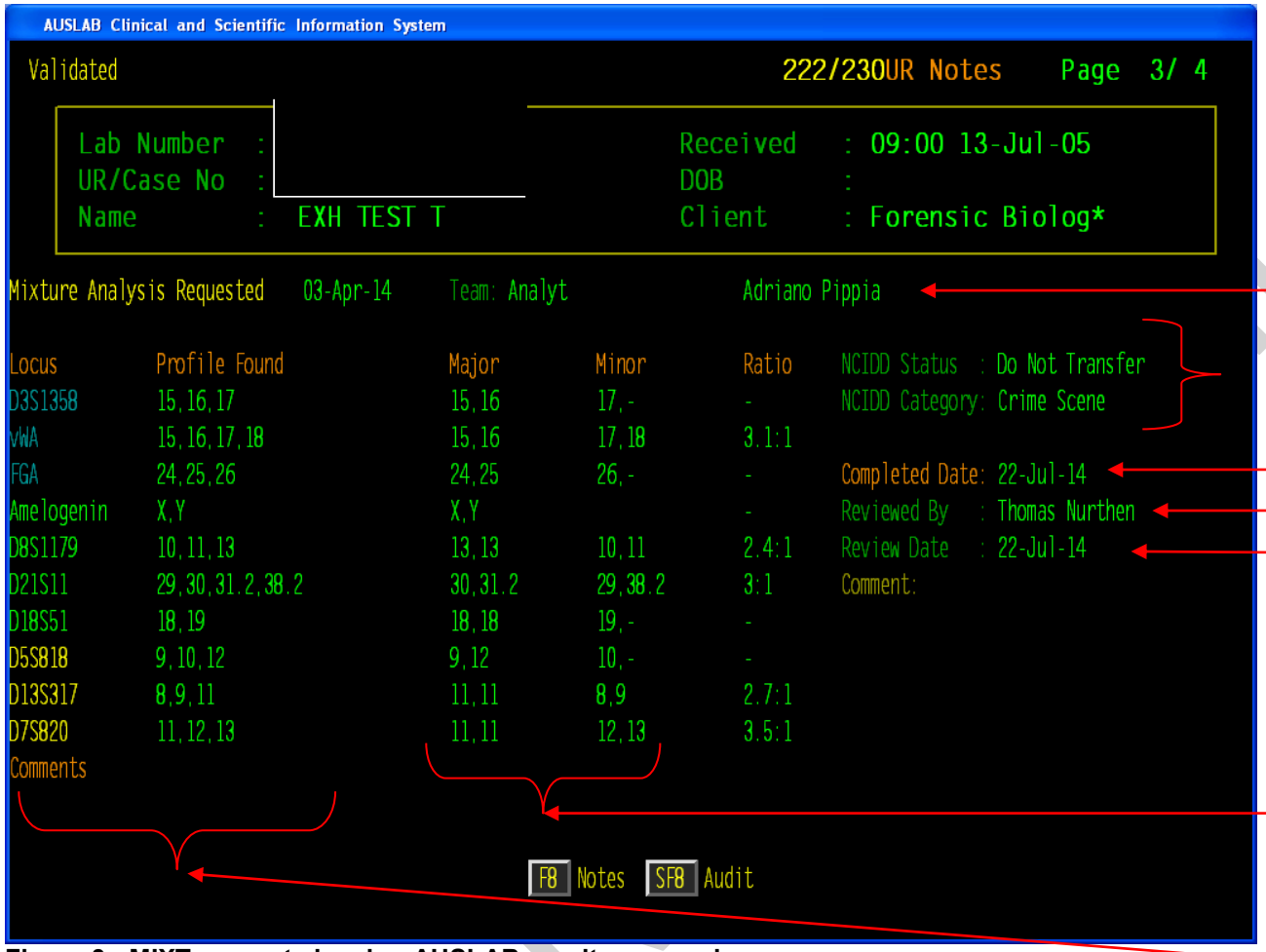

Figure 3 - MIXT request showing AUSLAB result mnemonics

6.5 Report Results

All results are to be communicated as outlined in QIS 23968 *Forensic DNA Analysis Communications Procedure* and QIS 24015 *Procedure for Intelligence Reports and Interstate/Interpol Requests*.
Statements and intelligence reports are to be prepared according to QIS 17119 *Procedure for the Release of Results*.

EXR and EXH pages vary slightly in their appearance however the information entered is the same (for 9PLEX samples). If a case has started processing with EXR lines, additional information must be added to EXR lines NOT EXH lines.

EXH pages for XPLEX samples are the same as for 9PLEX pages, however due to interpretational changes in DNA profiles processed through PP21 most of the EXH lines used will be different. The full list of available EXH lines and the full expansions as seen by QPS is detailed in QIS 23008 *Explanations of EXR/EXH Results*.

NOTE: If a sample cannot be explained by one of the EXH results available, an intelligence letter should be sent to QPS to outline the interpretation. See QIS 24015 .

EXR/EXHs are entered into AUSLAB with the following hierarchy:
If multiple similar results are available for an exhibit with sub samples, the first barcode (e.g. SAIK presumptive has been reported) must always be reported back, if there are subsequent more informative results they are reported from the same EXH

1. Presumptive EXH lines
2. Interim EXH lines (only applicable to DNA Prioritiy1)
3. Final EXH lines
   a. DNA profile type
      i. Complex, Single source,2 person,3 person or Similar
   b. Conditioning information (if applicable)
   c. NCIDD load information (if applicable)
   d. Reference sample comparison interpretations (e.g. STRmix LRs) (if applicable)
4. EXH updates/Incorrects (if applicable)

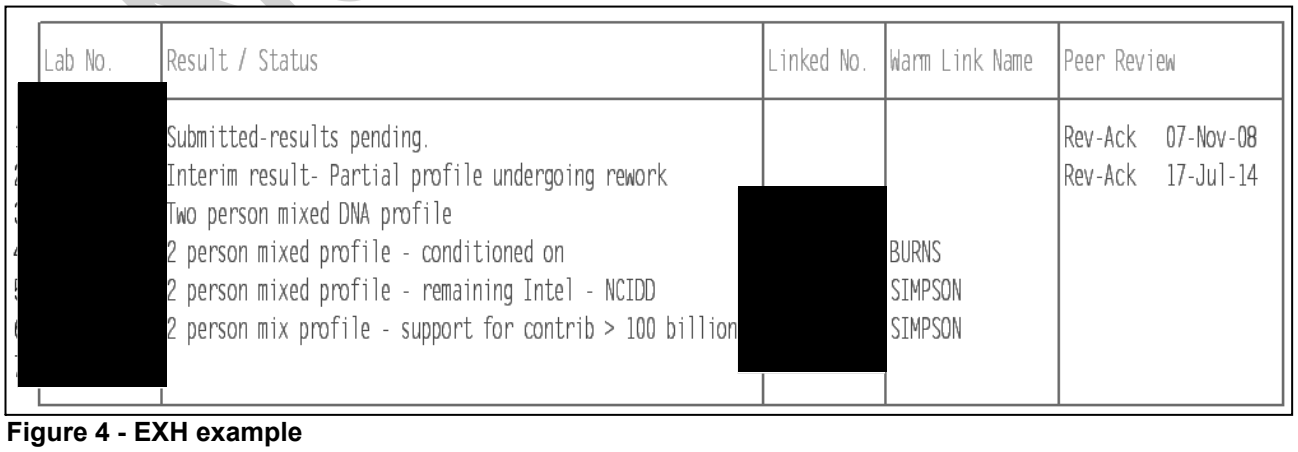

Figure 4 - EXH example

6.5.1 Interim EXH lines

Once a rework is ordered, an interim EXH must be entered only for urgent/Priority 1 cases. If the interim EXH that has been selected contains no interpretation, such as 'interim result

Not Current

– sample undergoing rework', the result can be validated by the person who entered it. If an interpretation is included in the selected interim EXH, such as 'Interim result - Mixed profile obtained - rework reqd', the result must be validated by someone other than the person who entered it (and must be competent to do so).

6.5.2 Final EXH/EXR lines

If the barcode of the final preferred profile is the same as the original barcode used to report the presumptive results, the final result can be reported via EXR/EXH under that barcode.
If the barcode of the final preferred profile is different to the original barcode used to report the presumptive results (but refers to the same sample), an EXR/EXH line explaining why must be entered. The final result is then reported under the barcode that it was processed under.

If a profile obtained from a casework sample does not match any reference profiles within a case (or if the case has no reference profiles) 'UKM1' (for a male profile), 'UKF1' (for a female profile) or 'UKP1' (if gender is undetermined) is entered into the Linked No. field. Each subsequent unknown is given a consecutive denomination, e.g. UKM2, UKM3 etc. This applies to single source samples profiled through PP21. It also applies to single source P+ samples, Major/Minor mixtures and 'Remaining' profiles. See also QIS 31523 .

Example 1 (for 9PLEX/XPLEX samples):
A sample with barcode 111111111 was submitted to Analytical. The sample was diluted in Analytical due to an excessive quantitation value. This diluted sample was assigned the barcode 222222222, and continued through the amplification process. The profile for 222222222 was assessed to be male, full and unique within the case (with no reference profiles). The final EXH below is for a 9PLEX sample –See Figure 9 below. The EXH would be different for a XPLEX sample i.e. 'NCIDD upload single source DNA profile' would be the 'Result/Status' field.

	Lab No.	Result / Status	Linked No.	Warm Link Name	Peer Review
1	111111111	Submitted-results pending.			
2	111111111	Sample processed and final results under	222222222		
3	222222222	9 loci DNA profile. Uploaded to NCIDD	UKM1		

Figure 5 - Example 1 EXH

Example 2 (for 9PLEX/XPLEX samples):
Wet and dry swabs of the handle of a knife were taken and submitted separately to the Analytical section. Exhibit barcode (knife) – 111111111; Wet swab – 222222222; Dry swab – 333333333. The presumptive EXR/EXH was entered under the barcode for the wet swab. Both the wet and dry swabs yielded partial profiles with sub-threshold peaks, and it was decided to pool the samples. Samples were pooled under barcode 444444444 and processed further. A partial profile (13 alleles) matching that of the suspect (John Smith), barcode: 555555555, was obtained. The EXH would be different for a XPLEX sample ie. 'SS DNA profile < 9 loci LR 1 million - 1 billion' would be the 'Result/Status' field. See Figure 10 below.

Current

	Lab No.	Result / Status	Linked No.	Warm Link Name	Peer Review
1	222222222	Submitted-results pending.			
2	222222222	Sample pooled and processed under	444444444		
3	444444444	Partial DNA profile. Uploaded to NCIDD	555555555	SMITH, J.	

Figure { SEQ Figure * ARABIC } - Example 2 EXH

6.5.3 EXH/EXR line updates

EXH/EXR lines may require updating after additional information is available or additional testing has been completed. Commonly, EXH/EXRs are updated after a reference sample for case has been received and new information needs to be sent back to QPS.
If an EXH/EXR has been reported incorrectly (or further information such as ownership becomes available) the line(s) must be incorrected by FRIT Senior Scientists or Team Leader. See Figure 11 below.

	Lab No.	Result / Status	Linked No.	Warm Link Name	Peer Review
1	[redacted]	Submitted for cells. Presumptive saliva test pending.			11-Aug-14
2		Single source DNA profile	UKM1		Incorr. 23-Sep-14
3		Single source DNA profile	UKM1		Reviewed 23-Sep-14

Figure { SEQ Figure * ARABIC } - Example of EXH update

The new correct interpretation is added to the next available line by the case manager and the incorrect line (IRCBx) is changed to 'INR' (Incorrect) by the peer reviewer. The corrected laboratory number must be communicated to QPS DRMU via email (Minor Change register has the most up to date email addresses for DRMU).

If there is a change to the interpretation after the EXH has been reviewed (eg. at statement writing stage), a note in the Specimen notes detailing the reason for the change is a preferable course of action. If there is a significant effect on the result (eg. a NCIDD load and Link are affected and requiring a change), this should be communicated to a Supervising Scientist or Team Leader to inform the QPS DRMU Senior Sergeant prior to sending the email notification.

6.5.4 Suspect checks

If a suspect check has been requested by QPS for a reference sample profiled in P+ and the sample is not intuitively excluded from the mixture, the reference sample needs to be reworked in PP21 to increase the amount of data available to NCIDD and .

Instructions for reworking reference samples are documented in section 9.1 below.

Suspect checks have reserved EXH lines for reporting; refer to QIS 23008 for the appropriate EXH line.

6.5.5 STRmix™ Database searches

For paper based cases – when a STRmix™ DB search is performed, the date of the DB search and the number of reference samples used are included in the Warm link field see Figure 12 below:

	Lab No.	Result / Status	Linked No.	Warm Link Name	Peer Review
1	[redacted]	ubmitted-results pending.			Rev-Ack 03-Nov-14
2		hree person mixed DNA profile			
3		ixture-low support for contrib or supports non contrib		13ref 1uk 4.12	
4		CIDD upload - mixed DNA profile	UKF2		

F orting DB search

If for the reference samples changes then:

- Add 'This sample has undergone further processing' line
- Add all relevant lines to the interp just like it is being done for the first time. This means, the slate was wiped clean with SUFP so re-add '3MX' etc. etc..
- Do not incorrect any lines
- Do not send an email (DRMU understand the situation may occur)

For paperless cases the date of the DB search and the surnames of the reference samples used are added to a specimen note by the case manager.

6.5.6 Samples with undetermined quantitation values or insufficient DNA

This applies to 9PLEX samples that have an undetermined quantitation value, and for XPLEX samples that have a quantitation value less than the Quantification limit of detection (LOD) which is 0.001 ng/uL.

Competent Analytical Section staff members will case manage these samples. See also QIS *Automated Quantification of Extracted DNA using the Quantifiler Human DNA Quantification Kit* and QIS 24012.

1. Access the **NDNACM** outstanding batches (No DNA Case Management)
2. Create a batch
   a. For 9PLEX samples check that each sample has an IPC <30, the Quant is Undetermined, the Team is Volume & the priority is 3.
   b. For XPLEX samples check that each sample has an IPC <30, the Quant is less than the Quant LOD (0.001 ng/uL)
3. On the 9PLEX/XPLEX page press [**SF7**] ***Results History*** to access the results.
4. Save the profile (which will be blank) by pressing [**F7**] ***Select Preferred*** then [**SF6**] ***Save Preferred Profile***. This will transfer the quantitation information to the 9PLEX/XPLEX page.
5. Add sample management details to AUSLAB
   a. For 9PLEX samples
      a. Add your name to case scientist field - **CSCI** field
      b. Add 'NDNAD' -No DNA detected to the DNA profile result field – **9PLEXS**,
      c. Add today's date to the completed date field on the 9PLEX
      d. On the 9PLEX(2) page, enter the Quant batch ID to complete the batch, and press [**F6**] ***Validate*** the page
6. For XPLEX samples
      a. Add your name to case scientist field - **XPCM** field

Not Current

b. Add 'N' to **XPNR** field
c. Add 'NDNAD' -No DNA detected to the Sample status field - **XPRES**
d. Add today's date to the completed date – **XPCD** field

7. On the EXH page enter the barcode into the Lab no field of the next blank EXH line and enter 'NDNAD' (no DNA detected) into the result/status field.

   NOTE: if the sample is a sub-sample, the EXH will be entered into the parent barcode (see Client Reference barcode to find parent barcode).
8. Use [**SF9**] to access the results summary page.
9. If the sample is the only sample in the case, or all other samples in the case are also undetermined or completed, then the case can be written off. Enter into the CS test panel and change the case status (CASET) to '*Sent to Peer Review*'

6.5.7 Paternity Samples

For paternity cases, an EXH will have to be registered under a new barcode so that results can be communicated back to QPS via AUSLAB. A follow up phone or email should be made to DRMU to explain the new barcode and EXH. The format for the results to be entered into the new EXH is: child barcode in Lab No. field, profile result in Result/Status field, alleged father/mother in Linked No. field, name in Warm Link Name (of alleged father/mother).

- If a Guthrie card is used and an EXH is registered on that barcode, a subsample barcode will usually be registered for the sample submission. The initial line in the EXH will be: Guthrie card barcode in Lab No. field, profile result in Result/Status field.
- If the putative father sample is an intelligence sample, the relevant EXH would be 'Intel report required for further Interpretation'.
- Consult the Senior Scientist or Team Leader to liaise with DRMU if required to determine the appropriate EXH line to use to best suit their method of reporting.

6.5.8 Using Coronial samples as Reference Samples in EXHs

- If a sample has been processed with casework conditions is to be used as a reference sample, it needs to be deconvoluted in STRmix because there is no homozygote threshold. This deconvoluted DNA profile is used as the reference in all comparisons.
- An EXH is to be requested on the parent barcode of the item (e.g. bone). A subsample for the bone/muscle is to be reported back matching itself, and therefore appear as a reference sample match in AUSLAB.
- This allows the coronial sample to become the reference sample for that continuation of the case.
- Barcode details and person details (Date of Birth, name and type of exhibit) are to be provided to QPS DRMU before the information in the EXH is sent.

7 NCIDD

Case managers are responsible for choosing a representative profile for each unique profile seen within a case for upload to NCIDD. These profiles must have at least 12 alleles for NCIDD matching.

To upload an allele to NCIDD for PP21 samples, a 99% deconvolution is required at a locus as per the Statistics Working Group (STATSWG) recommendations.
e.g.

Not Current

- [9,11] 99% = 9,11 uploaded
- [9,11] 98% [9,9] 2% = 9,NR uploaded
- [9,11] 98.8% [9,9] 0.6% [11,11] 0.6% = 9,11 uploaded
- [9,11] 98.8% [9,10] 0.6% [10,11] 0.6% = NR,NR uploaded
- If four alleles exist in a deconvolution [9,11] 98.1%[9,10] 0.4% [9,12] 0.5% = 9,NR uploaded
- If in doubt as to the combination to load e.g. [9,10] 98.5% [9,NR] 0.5% [10,NR] 0.5% = NR,NR uploaded
- If in doubt as to the combination to load and the allele count is borderline for NCIDD load (11 alleles) refer to a senior for direction.

≥99% deconvolution at all PP21 loci is known as a 'full' NCIDD load
≥99% deconvolution at ≥ 12 PP21 loci is known as an 'Intel' NCIDD load.

Profiles of less than 12 alleles may be loaded to NCIDD in special circumstances. Please consult with Senior Scientist in the Intelligence Team prior to uploading. All matches generated will be reported through an EXR/EXH. In certain circumstances, a profile with less than 12 alleles (including sub-threshold information) can be loaded to NCIDD, and any matches will be reported back to QPS via an Intelligence report written by the case scientist or Intelligence Team member. This is an intel/upload process and is not for court purposes. Intel/NCIDD work does not get heard in court unless special authorisation is given by the Judge/Justice due to potential to prejudice court.

Only one representative DNA profile is loaded to NCIDD for a person in a case. Profiles that match known deceased persons or complainants in sexual assault cases are not to be uploaded to NCIDD. By the same rationale, unknown DNA profiles previously loaded to NCIDD that match known deceased and sexual assault victims are also removed from NCIDD. Refer to QIS 23890 *Uploading and Actioning Samples on NCIDD* for Forensic DNA Analysis procedure on which case work samples can be uploaded to NCIDD.

7.1 Single Source NCIDD load

7.1.1 9PLEX samples

The steps are listed below to request a load to NCIDD for a single source 9PLEX sample.
The case manager:

- Add the appropriate EXH line as per QIS 23008
- List insert to **NCIDD worklist**

The Intelligence Team:

- Monitor the NCIDD work list
- Request the SNCIDD test panel
- Add 'Load' to the **9PLEXP** field

7.1.2 XPLEX samples

The steps are listed below to request a load to NCIDD for a single source XPLEX sample.
The case manager:

- Adds their name to the **XPCM** field
- Adds 'Y' to **XPNR** field
- Add the appropriate EXH line as per QIS 23008

The Reviewer:

- Adds their name to the **XPRV** field
- Adds 'Y' to the **XPRN**

Not Current

- Review the EXH line

The Modify 8 equation has been programmed to list insert a sample to the NCIDD list:

1. If **XPNR** is 'Yes'
2. If **XPNR** is 'No' and **XPRN** is 'Yes'

If **XPNR** and **XPRN** are both 'Yes" the sample is removed from the NCIDD list and a SNCIDD test ordered by the system.

7.2 Mixed DNA samples NCIDD load

7.2.1 9PLEX samples

If a mixture is present in a profile and it is possible to separate the mixture into major and minor contributors, or if the mixture can be conditioned against a known profile, a mixture page should be registered and completed in AUSLAB.

The MIXT or MIXC panel is used for 9PLEX samples only. Unlike the COMIX panel, both the mixture and the contributions are displayed on the same panel. The mixed DNA profile will copy from the 9PLEX page (If one exists on the same barcode).

- Add the MIXT/MIXC panel
- Manually add the allele designations of the major/known contributor and the minor/remaining profile
- Add the corresponding ratios (or N/A if not applicable)
- The completed date (**MIXCD**) must also be filled out at the time of the interpretation
- To print the mixture interpretation page for inclusion in the case file press [**SF11**], then [**F7**] and direct to a printer.
- Add an entry to the **Comment** field on mixture page e.g. 'major – intel load'
- List insert to NCIDD worklist
- Review EXH line, 9PLEX and mixture pages, and the sample is on the NCIDD list.
- List insert to the appropriate review list.

Within high priority cases and low priority cases in exceptional circumstances, mixed DNA profiles can be interpreted for Intelligence purposes and uploaded to NCIDD.
The case manager is required to:

- Add the appropriate EXH line as per QIS 23008
- If necessary, write an Intelligence report

The Intelligence Team:

- Monitor the NCIDD work list
- Request the SNCIDD test panel
- Upload the profile to NCIDD with the suffix '-Intel'.

If an Intelligence report is required, the report will be stored within the Intelligence Team area of Block 6 after it is emailed to QPS DRMU.

7.2.2 XPLEX samples

If a mixture is present in a profile which is assessed by the case manager as suitable for STRmix™ deconvolution and subsequent NCIDD upload, a COMIX test panel is required to be completed and reviewed. Up to two contributors from a mixed DNA profile can be recorded in a COMIX page. If there is a third DNA profile that requires an NCIDD load use the QIS 31512 *Manual NCIDD Upload form PowerPlex®21* form. The EXH reviewer will

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then deliver the paperwork to the Intelligence team for upload to NCIDD. Following upload, the paperwork will be scanned into AUSLAB.

- Complete the XPLEX page, Add 'N' to **XPNR** field.
- Request a COMIX test panel
- To add the loci names to the COMIX page, see Appendix 2 in QIS 32019 *Procedure for using the Generic Instrument Interface in Forensic DNA Analysis*.
- Manually add the allele designations from the STRmix™ deconvolution consistently using either a '-' or 'NR' for partial loci. e.g. '15,- ' or '15,NR'
- Add the current date to the **CMXDAT** (***Completed Date***) field.
- Add 'Yes' to the **CP1N** (***CM A NCIDD***) field
- In the **Comments** field add the type of NCIDD load e.g. Intel or MIX

NCIDD loads from COMIX are requested on a different barcode to the source. Each NCIDD upload requires a new barcode for NCIDD upload.

- Press [**SF10**] ***Registration***
- If the **Cli. Ref** is empty add the sample barcode (this allows the source barcode and the NCIDD load barcode to sort together)
- Press [**SF5**] ***Copy Entry***
- Scan a new barcode
- Change the **Received Date** to the current time
- Change the **Collected Date** to the current time
- Change the **Specimen** type to 'Misc'
- If the **Cli. Ref** field is empty then add the source barcode
- Add a designation to the **Sample Info 1** field (e.g. UKM1 or if matching reference Surname.
- Add the NIDD1 request
- Press [**F4**] **Save**
- Add the new barcode to the **CPROS1** field on the source **COMIX** page.
- List insert to **MIXUPREV** list

Conditioned DNA profiles loading to NCIDD

After a mixed DNA profile has been conditioned in STRmix™, the deconvolution will list that each conditioned allele has been deconvoluted to 100%, a conditioned component of a mixed DNA profile can be loaded to NCIDD provided that :

- The upload alleles are able to be visually separated (i.e. major or minor)
- Upload matching alleles in an even mixture where there is a strong representation

Do not upload contributions from low level mixed minors where we may be confident enough to condition but not load to NCIDD.

7.3 Printing an NCIDD upload form from DADI

Samples that are selected for upload cannot be list inserted on the NCIDD work list and an upload form must be printed and delivered to the Intelligence Team.
To obtain an upload form for a sample on DNAmaster/DAD, open DADI and search for the sample. Click on the Print Upload Form button. Refer to QIS 25583 Use of DNA Analysis Database Interface (DADI).

A barcode may be printed from AUSLAB and attached to the form in the appropriate location for these samples to allow for easier scanning into AUSLAB.

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7.4 NCIDD removal

There may be times when a DNA profile that has been loaded to NCIDD needs to be removed or updated with a better DNA profile.

To have a DNA profile removed from NCIDD:
The Case manager is required to:

- Add the barcode of the DNA profile to be removed to the next available row (**EXRL1-21**)
- Add the Result 'DNA profile removed from NCIDD' (PRNCID) to corresponding Result/Status field (**DUM1-21**).

The Reviewer is required to:

- Add the 'Review' to corresponding Peer Review field (**IRCB1-21**)

The Modify 8 equation has been programmed to list insert the EXH barcode to the NCIDD removal list.

The Intelligence Team:

- Monitor the NCIDD Removal list
- Remove the DNA profile from NCIDD
- Update the NCIDD status to 'Removed from NCIDD'

Not Current

8 Send to peer review

All results must be peer reviewed prior to release to the QPS. Peer review can be at a sample level or case level, Technical or Administrative. QIS 17113 *Technical Review* and QIS 17123 *Administrative Review* outline the procedures involved in review within Forensic DNA Analysis.
Different lists/storage locations are used in AUSLAB for the review of different results. Table 8 below is a summary of the AUSLAB review lists and storage locations.

Table { SEQ Table * ARABIC } - Review lists/storage locations used

Type of result	Test panel	AUSLAB review list
Complex	XPLEX, 9PLEX	COMPREV
Single Source NCIDD	XPLEX,9PLEX	SSUPREV
Single Source	XPLEX	SSREV
Single Source	9PLEX	VCREV
Mix NCIDD	XPLEX,9PLEX	MIXUPREV
Mix	XPLEX	MIXREV
Mix	9PLEX	VCMIX
All unallocated paper	XPLEX	MIXACT
All allocated paper	XPLEX,9PLEX	Individual intray locations

Peer review of 'No DNA detected' and 'DNA insufficient for further processing' is performed by a competent Analytical Section staff member.

1. Access the VOLUND list and check the information completed by the case manager is correct.
2. For 9PLEX samples
    i. Enter your name into the Reviewed by (**9PLREV**) field and
    ii. Press [**F6**] ***Validate*** on each page 9PLEX, 9PLEX(2)
3. For XPLEX samples

i. Enter your name in the Reviewed by (**XPRV**) field
ii. Enter 'N' in Reviewer NCIDD? (**XPRN**) field

4. Review the information entered into the EXH page. If correct, enter 'rev' or 'r' into the corresponding peer review field and press [**F6**] ***Validate***

5. Use SF9 to access the results summary page. If the sample is the only sample in the case, or all other samples in the case are also undetermined or completed, then the case can be written off.
6. Enter into the CS page and change the case status (***CASEST***) to 'Analysed Report not Required' (ARNR),
7. In the date completed field the current date (t) and the exhibits field to 'destroyed, subsample retained' (DESTSR)
8. Press **[F6]** ***Validate*** to validate the page
9. Navigate to the administrative review page and enter your name into both Reviewed by fields, today's date in both Date of review fields, 'N/A' in all other fields except for 'Yes' in the All results checked and validated field
10. Press [**F6**] ***Validate*** the page and remove the sample from the VOLUND list

9 Reference sample management

9.1 Reworking evidence reference samples

Detailed reference processing information is presented in QIS 31389.

GMIDX plate reading comments are the trigger for AUSLAB (via the GIDXR21 autovalidation mask) to automatically rework reference samples. All Evidence reference samples are reworked to yield a full PP21 DNA profile (1 x locus with allelic imbalance and/or incomplete Amelogenin is acceptable). Table 9 below summarises the rework tests available for PP21 reference testing and their equivalent P+ historical tests.

If a buccal FTA sample after the first two direct amplification procedures (1x 1.2 mm spots) fails to yield an acceptable DNA profile; the sample is punched (4x 3.2mm spots), extracted and amplified.
Blood FTA samples due to the potential high concentration of DNA are extracted without any direct amplification.

Table 5 - Reference sample reworks

Description	REF21 Panel	PowerPlex 21 Amp conditions	9FTAR Panel	Profiler Plus Amp conditions
Direct re-amp low conc	R21RUN	1x 1.2 mm spot in 12.5 µL @ 27 cycles	FTARUN	3x 1.2 mm spots in 25 µL@ 25 cycles
Direct repeat analysis issue	R21RPT	1x 1.2 mm spot in 12.5 µL @ 26 cycles	FTARPT	2x 1.2 mm spots in 25 µL@ 25 cycles
Direct re-amp high conc	R21OSD	1x 1.2 mm spot in 12.5 µL @ 25 cycles	FTAOSD	1x 1.2 mm spot in 25 µL@ 25 cycles
Extraction	EREF	N/A	EFTA	N/A
Re-Amplification	R21AM1	Normalised DNA extract to 0.5 ng in 12.5 µL @ 30 cycles	AMP1RE	Normalised DNA extract to 1.2 ng in 50 µL @ 28 cycles
Re-Amplification	R21AM2		AMP2RE	
Re-Amplification	R21AM3		AMP3RE	
Re-Quantification	21REQR		REQR	
Microcon procedure	MCONR		MCREF	
Re-Run	21RRRF	N/A	RRREF	N/A
ReRun	21RRF2	N/A		

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If the reworked sample still has analysis issues, the plate reader enters the comment EVDRW and list inserts the sample onto the EVDCM worklist. This worklist is monitored by the Senior Scientist of the Intelligence Team on a weekly basis. The aim is to get a full DNA profile from the evidence samples.

- The rework history is assessed to determine if there is a need for a full PP21 profile
    - Evidence samples that have all of the P+ alleles present and are associated to P+ cases only, can be accepted as complete.
    - Evidence samples that have a replicate sample (e.g. coronial samples may be submitted with a FTA cell sample and a blood sample) that already has a full DNA profile can be accepted within the same case.
    - Evidence samples that are not associated to a case or are partial and don't have matching single source crime scene samples or interpretable mixtures, can be accepted. If however, these conditions change, reworking will be required to obtain a full DNA profile.

Reworking strategies:
After an evidence sample has been reworked via direct amplification, the evidence sample will be sub-sampled, the DNA extracted, quantified and amplified at the optimum DNA input where possible. If an evidence sample fails to generate a usable DNA profile after this process or it has not undergone any direct amplification processing (as in the case of blood FTA samples, evidence swabs and reference hair) additional reworks from the DNA extract may be required.
.
It may be necessary for the case manager to order reworks on evidence reference samples if it has not yet been actioned from the EVDCM work list.

- XS samples – Evidence samples (already extracted) that are excess DNA are reworked by decreasing the amount of DNA template added to the PCR. The 'PP21 half volume SV1 calculation' macro (I:\Macros\PP21 half volume SV1 calculation.xls) can be used to re-calculate the template. The sample is reworked with a Re-amplification test code (As per Table 9). Dependant on how excess these samples are may require several reworks to obtain a full DNA profile.
- NSD samples – Samples that consistently yield NSD or low partial profiles and have low quantitation values are generally reported to QPS as Failed to Profile (FTP). FTP samples are actioned by QPS SMU to submit a further evidence sample, if an additional sample cannot be obtained, and a request is made to be reprocessed and costed, a connected barcode can be used to try to obtain a usable DNA profile.
- Extra peaks – Evidence samples that exhibit extra peaks or other analysis issues require consideration of the previous results obtained. This run may be altered or a previous run that has been confirmed may be accepted. DNA profile edits are processed as per section 6.3.4 above.

It is critical that the correct rework test code is ordered for reference samples via the [**SF7**] ***Results History*** screen for the correct test panel and except for FTAR,FTAREF,FTAR and REF21 must not be ordered via the [**SF10**] ***Registration*** screen. Reference sample reworks must not be ordered with case work test codes.

Once a sample has been processed under an EREF (or for historical samples EFTA/EBLD), an extract exists. If the above reworking techniques have not yielded a full profile, a further extraction can be ordered but must be under a new barcode. There can only be one extract in existence for each barcode.

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Connected barcodes
To request a connected barcode and Connected barcode reworks:

1. View the FTAR,9FTAR, REF21 or FTAREF page of the sample
2. Press [**SF10**] ***Registration***
3. Press [**SF5**] ***Copy Entry***
4. Scan in the new barcode
5. Change the ***Received*** date to the current date and time
6. Change the ***Collected*** date to the current date and time
7. Enter the appropriate ***DNA priority*** for the sample
8. Enter details in the ***Sample Info 1*** field ,Enter 'From xxxxxxxxx ' where xxxxxxxxx is the source barcode
9. Request a **REF21** and if necessary a **FTAREF** (to re-bill the request if this process has been requested by QPS on FTA intelligence samples).
10. Press [**F4**] ***Save*** to complete and save the registration
11. Enter the details of the connected barcode in the Comments of the source barcode.

Requesting a REF21 will add the barcode to an outstanding batch allocation dictated by the specimen type. If the connected barcode does not require additional direct amplification (e.g. a microcon) a rework testcode must be ordered (via the [**SF7**] ***Results History***) and the REF21 removed from the batch allocation list as per QIS 24469 (Batch Functionality in AUSLAB) .

9.2 Reporting Reference Samples

Reference samples are collected by QPS under the Police Powers and Responsibilities Act 2000. QPS send registration information to Forensic DNA Analysis via the POLARIS GSI interface (from QPRIME). The NCIDD category and specimen type determine if a reference sample will be loaded to NCIDD.

The same interface sends QPS notification of successful/unsuccessful DNA profiling via the QPSGSI request.

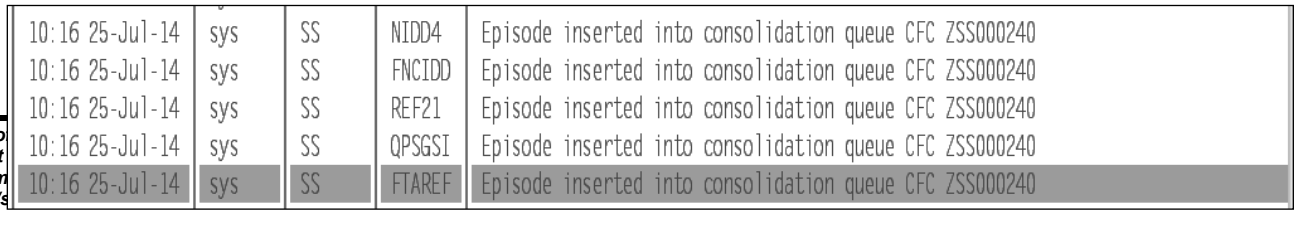
Autovalidation rules have been configured in AUSLAB to automatically request the required test panel (FNCIDD) for loading to NCIDD. For the autovalidation rules to be triggered and result in an FNCIDD being requested the following must occur:

- REF21 must be ordered and
- R21RES (Sample Status) = 'FBPOS' and
- R21NS (NCIDD Status) = 'Load'

If a reference sample has been profiled under a connected barcode, the original source barcode must have the DNA profile and result information transcribed from the connected barcode. This will ensure that the reference sample is able to be validated in AUSLAB and send a result back to QPS. A macro (MOD batch file fix) is used by the Intelligence team to replace the connected barcode with the source barcode in the NCIDD upload file.

9.3 Billing reference samples

New reference samples and reference samples that QPS have specifically requested to be reworked (eg. Upgrade a Profiler Plus profile to PowerPlex 21) are able to be billed via the FTAREF request. For billing to be successful the entire laboratory number must have all of the requests validated.
To check if billing will occur check the specimen audit for the entry as in Figure 13 below.



10:16 25-Jul-14	sys	SS	NIDD4	Episode inserted into consolidation queue CFC ZSS000240
10:16 25-Jul-14	sys	SS	FNCIDD	Episode inserted into consolidation queue CFC ZSS000240
10:16 25-Jul-14	sys	SS	REF21	Episode inserted into consolidation queue CFC ZSS000240
10:16 25-Jul-14	sys	SS	QPSGSI	Episode inserted into consolidation queue CFC ZSS000240
10:16 25-Jul-14	sys	SS	FTAREF	Episode inserted into consolidation queue CFC ZSS000240

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Figure 7 - Specimen Audit billing consolidation audit

10 Court Appearances

Notices to attend and give evidence should be recorded in AUSLAB refer to Figure 14 below. Any court appearances should be recorded in AUSLAB. A **CCD** code should be requested and the two pages filled out appropriately.

- The **Date** field (***CSD1-3***) refers to the date of the court appearance
- The **Attended by** field (***CSAB1-3***) refers to the scientist that gave evidence at court
- The **Time Box** (***CSTM1-3***) refers to amount of time giving evidence
- The **Time Out** (***CSTM8-11***) refers to the amount of time the scientist was absent from general duties
- The **Court** (***CSCT1 -3***) refers to the type of court (e.g. Supreme)
- The **Location** field (***CSLOC1-3***) refers to the court location
- The **Trial Type** (***CSTTP1-3***) refers to the type of trial (e.g. Trial)
- The **Evidence** by (***CSEV1-3***) refers to the type of evidence given (e.g. In person)
- The second page of the CCD relates to the content of the court evidence.
- Ensure that the pages are validated by pressing [**F6**] ***Validate***

Court Details

Date	Attended By	Time Box	Time Out	Court	Location	Trial Type	Evidence by	Reviewed by	Pass
29-Jul-14	tenf2	00:30	00:30	Supreme	Rockhampton	Committal	Phone		
30-Jul-14	aapf1	01:00	04:00	Supreme	Rockhampton	Trial	In Person		

Figure { SEQ Figure * ARABIC } - CCD request court appearances

11 Case conferences

The CCD request also provides a table for recording details of case conferences, refer to Figure 15 below.

- The **Date** (***CSD4-7***)field refers to the Date of the case conference
- The **Attended by** (***CSAB4-7***) field refers to the scientist (s) and QPS/DPP staff that attended the case conference
- The **Purpose** (***CSPD1-4***) field refers to the purpose of the case conference.
- The **Time Box** (***CSTM4 -7***) refers to how long the case conference went for
- The **Time out** (***CSTM11 -14***) refers to the amount of time the scientist was absent from general duties
- The details of the case conference can be added to the second page of the CCD or scanned into AUSLAB and the appropriate UR notes added.
- Ensure that the pages are validated by pressing [**F6**] ***Validate***

	Date	Attended By	Purpose	Time Box	Time Out
Conference Details	27-Jul-14	aapf1	Discuss SAIK results	01:00	01:00

Figure { SEQ Figure * ARABIC } - CCD request case conference

12 File compilation

12.1 Suggested order of pages (from top to bottom)

1. Case file particulars page
2. Copy of final statement (if written)
3. Most recent printout of UR notes, emails and other communications*
4. DNA results table (if deemed necessary)
5. Reference samples – receipt page then profile
6. QP127 (if available) and receipt page
7. Examination notes:

a. Description of item
b. Diagrams

8. Photos/photocopies/AUSLAB images*
9. Profiles
10. Mixture interpretation sheets (if applicable)
11. Statistical calculations (if applicable)
12. Subsequent QP127 (if available), receipt page and examination notes etc…

* these items are not required to be printed if the case is not going to court

12.2 Page numbering

Only cases that are going to court (Statements of Witness or Evidentiary Certificates) need to be page numbered. Assistance is available from the Administrative Team for page numbering.

1. The Case File Particulars page is always Page 1 (except upon reactivation when the additional Case File Particulars page will be numbered page 1 and the original Case File Particulars page will be renumbered as the next consecutive number in the case file).
2. Case Files are numbered from the back of the case file to the front.
3. Number and initial each page, including the reverse of the page if both sides have been used.
4. Ensure the Case number is recorded on each page.
5. Write the total number of pages on the front of the case file and initial and date as indicated.

For those cases that aren't going to court, the total number of pages simply needs to be counted and noted on the front of the case file, that is, each individual page does not need to be numbered.

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12.3 Statement compilation

Refer to QIS 17119 for the correct format for statements or reports issued by Forensic DNA Analysis.

12.4 AUSLAB finalisation

EXR/EXH page – ensure each sample has the appropriate interpretation entered and validated.
9PLEX/XPLEX page – ensure each sample has a saved preferred profile and validated.
FBEXAM – ensure this page is validated. This page will not validate if required fields do not have entries, e.g. Examination Trolley. Enter N/A or refer to the examination notes for details.
CS – change the status to "Sent to Peer" and validate.
Forensic Receipt page – ensure this page is validated
Return Destruction and Report Release Details – Validate these pages.
Tech Review Page (FBTR) – order an FBTR from the registration page if required, fill in and validate.
FBCALC – order an FBCALC (for 9PLEX samples only) from the registration page if Kinship has been used. Complete and validate

12.5 Preparing a case file for peer review

Prior to submitting a case file for final review or prior to a statement being issued, the following is required:

- Ensure that all items/exhibits have been examined or prioritised appropriately.
- Ensure that appropriate reworks have been performed.
- Establish whether further testing needs to be performed
- Ensure that all samples are finalised and a preferred profile saved on the 9PLEX/XPLEX page.
- Enter the final results in to the EXR/EXH.
- Samples that have been reported as 'No DNA detected' or 'DNA insufficient for further processing' need to be documented in the case file. This can be done by either printing the EXH, annotation of the receipt or annotation of the packaging image.
- All profiles have been printed and included in the case file. Each profile should have a brief description of the sample written on it. An interpretation of the profile must also be written on the epg and signed and dated by the person making that interpretation.
- Ensure that appropriate profiles have been selected for upload to NCIDD. Only one example of each profile is to be loaded to the database.
- Ensure that the reference sample receipt is printed for each evidence sample. To print the reference sample receipt page press SF11, then F7 and direct to a printer.
- If there are multiple epgs for a particular reference sample, only the reported profile need be printed and annotated as the final profile.
- Ensure that all evidence samples associated with the case are present in the final table (if one exists).
- STRmix™ printouts for all paper cases.
- For 9PLEX cases: if a statement has been requested, ensure that profiles requiring a genotype frequency have had the statistical calculation performed through the Kinship program and that the results are printed and included in the file.
- For XPLEX cases: if a statement has been requested, ensure that profiles requiring a STRmix™ interpretation have had the statistical calculation performed through the STRmix™ program and that the results are printed and included in the file.
- For 9PLEX cases: ensure that any mixed profiles have been interpreted using the MIXT or MIXC pages in AUSLAB or the determination of genotypes (if required). A copy of the worksheet or a print-out of the page from AUSLAB should be included in the file.

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- For XPLEX cases: ensure that any relevant profiles have been interpreted using the STRmix™ program.
- For 9PLEX cases: if applicable, ensure that any non major/minor or conditioned mixtures are interpreted with appropriate 'Popstats' hypotheses and/or calculations.

13 Reactivated cases and case requiring updated interpretations

13.1 Reactivated cases

On occasion, some cases require further work after they have been finalised and reviewed. When a case file is reactivated the case status must be changed to "Reactivated" and the case completed date should be deleted from the CS page.

A UR note should be entered detailing the reason for the re-activation, e.g. Reactivated for further examination of exhibits, or Reactivated for statement purposes. This is helpful for Property Point when deciding whether or not exhibits can be returned to QPS.

An assessment of previously reported and uploaded profiles should be undertaken. In July 2007, it was decided (in conjunction with QPS) that all crime scene profiles (except Known Deceased and complainants in sexual assault cases) would be uploaded. Prior to this any crime scene sample that matched a complainant profile for any case type was not uploaded to NCIDD. Since the introduction of GMIDX software and lower thresholds, it may also be advantageous to rework samples in an attempt to obtain more information from a profile than may have previously been reported as insufficient for interpretation (if time restrictions permit).

A new case file particulars page is required to easily distinguish between the original section of the file that has been reviewed and the newly added pages.

Any additional Administrative and Technical Review needs to be requested on a new barcode.
Technical reviews are only required on cases with a statement / addendum statement.
If the case was processed using P+, any samples on future receipts for that case will also be processed with P+.

1. If there is a statement request for a low priority (priority 3) volume crime case, and one or more samples have been reported as 'No DNA detected' in the EXH, testing does not recommence. This result will be reported in the statement as 'DNA was not detected in this sample during the initial steps of DNA analysis and as a result no further testing was conducted' (wording is at the discretion of the reporting scientist).
2. If there is a statement request for a low priority (priority 3) volume crime case, and particular sample(s) may benefit from a rework such as a Microcon, the sample(s) can be submitted by the reporter.
3. If a volume crime case (low -3 to high-2 or urgent -1, or sample has its priority changed from low (3) to high (2), and the sample has been previously reported as 'No DNA detected' in the EXH, testing does not recommence. The sample will remain as 'No DNA detected'.
4. If there are samples that have been reported as 'No DNA detected', and written communication is received from QPS that testing is to recommence, these samples will be processed but will not be reworked unless the case priority is changed from low to high.
5. If a high priority case has been tested with P+ and all results were undetermined, additional new items received can be tested with PP21.

Not Current

13.2 Case requiring updated interpretations – EVDREV

Evidence samples are added to the 1EVD list by Operational Officers when received through list insertion from the case CS test panel. When a reference sample is completed it will be case managed from the 1EVD list.

1. Check Case status
   If there is a paper case and
   a. If the case status is START (Started), print the reference sample epgs and and case manage as a paper file.
   b. If the case status is ARNR (Analysed - Report Not Required) or PRSENT (Sent to Peer Review), the subsequent comparison to reference samples can be performed paperlessly. A note should be made in the UR notes.
2. Change **Case status** to '**REA**' (***Reactivated***)
3. The evidence sample(s) should then be compared to all relevant profiles.
4. Update sample management details
   a. Case manager to delete original case manager and reviewer name, add their name as the case manager.
5. Update the EXRs/EXHs/mixture if required.
   a. If the ref comparison has been conducted and there are no changes to any interpretations, a UR note to say 'ref XX compared to case awaiting review' or similar note is required.
6. Manually list insert case or EXH to EVDREV for review. if there is a new upload for NCIDD, list insert the EXH to SSUPREV or MIXUPREV to enable quick review
7. UR note to be added for 9PLEX/XPLEX cases to say 'comparison complete-awaiting review', change status to 'sent to peer', manually delete sample from 1EVD list.

Cases that have been received and processed using P+ will have any new receipts of casework samples profiled using the P+ system.

New evidence samples received for a case which has been profiled using P+ will be profiled using PP21. Only the 9 common loci between kits will be used for comparison purposes. The EXR/EXH lines used for update purposes will need to be selected appropriate to the kit used for initial processing (P+ / PP21). See also 23008 (Explanations of EXR/EXH Results).

Any interstate person samples submitted for analysis by the DNA Management Section (QPS) that have been obtained from people located interstate are to be treated as Evidence samples (as per advice from the QPS).

Not Current

14 Case file management off site

When case files are required for court appearances they should be tracked in AUSLAB to the reporting scientist's intray and then the Borrow function should be used.

14.1 Borrowing case files

1. Main Menu
2. Select **2. Sample Processing**
3. Select **7. Sample Storage Search**
4. Enter the barcode and press [**Enter**]
5. Press [**F7**] ***Borrow Sample***
6. Enter a comment when prompted "Please enter borrow comment:'
7. Press [**Enter**]

A comment should be recorded when prompted documenting why the case file is to be borrowed. The person taking the file off site is solely responsible for maintaining chain of custody and confidentiality at all times.

If the case file is requested by court officials a photocopy of the file is to be offered. The case file is the original copy and this must be retained by Forensic DNA Analysis.

There are some rare circumstances where an original case file may be required off-site and not be in the custody of a Forensic DNA Analysis staff member (e.g. Freedom of Information Requests). Where this is the case the Team Leader must be notified in advance and the entire case file must be photocopied and kept within Forensic DNA Analysis. UR notes should be completed explaining the circumstances, where the file is going, provide a contact name if possible and the anticipated return date. When the original case file is returned the copy should be destroyed.

14.2 Returning case files

1. Main Menu
2. Select **2. Sample Processing**
3. Select **7. Sample Storage Search**
4. Enter the barcode and press [**Enter**]
5. Press [**F8**] ***Return Sample***
6. Enter a comment when prompted “Please enter return comment:’
7. Press [**Enter**]

15 Records

1. Case file records – the location of paper case files is recorded in AUSLAB.
2. Paperless case examination notes (FBP1 – FBPX) – all but the current folder is stored in Block 3 Reporting.
3. Batch paper records - Filing Storage area (room 6112) or the Exhibit Room (room 6106)
4. DAD-Prior to AUSLAB Batch Functionality, all results obtained were loaded into an Excel spreadsheet known as DNAmaster. In 2008 these results were transferred to the DNA Analysis Database (DAD).
5. AUSLAB
6. Electropherogram pdf/jpeg files for samples:
   - Genotyper profiles are located in J:\User3100\Results Finalised\PRE-LIMS and I:\User3100\AAARESULTS FINALISED\POST-LIMS
   - As of the 16th February 2009, results have been analysed using GeneMapper ID-X. GeneMapper ID-X profiles are located in P:\Profile PDFs and only accessible from computers with GeneMapper ID-X installed (contains all DNA profile results from 16th February 2009 until June 2012).
   - As of July 2012, all DNA profile results are located in O:\Profile PDFs (accessible from all network PCs).
7. STRmix™ result files are stored on a network drive - I:\STRmix Results\

16 Associated Documentation

QIS: 16004 – AUSLAB Users Manual - DNA Analysis

QIS: 17038 – Case File Particulars

Not Current

QIS: 17113 – Technical Review

QIS: 17116 – Processing DNA Exhibits/Samples in the Forensic Sciences Property Point

QIS: 17119 – Procedure for the Release of Results

QIS: 17123 – Administrative Review

QIS: 17137 – Procedure for STR fragment analysis using GeneMapper ID-X software

QIS: 17142 – Examination of Items

QIS: 17168 – Procedure for Single Source DNA Profile Statistics

QIS: 19544 – Concentration of DNA Extracts using Microcon Centrifugal Filter Devices

QIS: 33407 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit.

QIS: 20967 – NucleoSpin® method for DNA extraction and clean-up of DNA extract

QIS: 23008 – Explanations of EXR/EXH Results

QIS: 23959 – Storage Guidelines for Forensic DNA Analysis

QIS: 23968 – Forensic DNA Analysis Communications Procedure

QIS: 24012 – Miscellaneous Analytical section Tasks

QIS: 24015 – Procedure for Intelligence Reports and Interstate/Interpol Requests

QIS: 24126 – Forensic DNA Analysis Administrative Officer Case Management

QIS: 24469 – Batch Functionality in AUSLAB

QIS: 24486 – Explanations of Analytical test codes and Batch types

QIS: 25302 – Interpretation of Mixed DNA (STR) Profiles using Profiler Plus

QIS: 25303 – Statistical Analysis for Paired Kinship and Paternity Trio / Missing Child Scenarios

QIS: 25368 – Kinship Software – Genotype Frequency Module

QIS: 25583 – Use of the DNA Analysis Database Interface (DADI)

QIS: 25581 – Kinship Software - Paired Kinship and Paternity Trio/Missing Child Modules

QIS: 31389 – STR fragment analysis of PowerPlex® 21 profiles using GeneMapper® ID-X software

QIS: 31512 – Manual NCIDD Upload form PowerPlex 21

QIS: 31523 – Use of STRmix™ Software.

QIS: 32019 – Procedure for using the Generic Instrument Interface in Forensic DNA Analysis

QIS: 32172 – GII filemaker

17 References

National Association of Testing Authorities (NATA). Forensic Science ISO/IEC 17025 Application Document, July 2015. Refer to NATA website: http://www.nata.com.au

Nurthen T., Mathieson M. & Allen C., (2013) Amplification of Extracted DNA validation v2.0

Not Current

Nurthen T., Mathieson M., Scott K. & Allen C., (2012) PowerPlex® 21-Direct Amplification of Reference FTA® samples validation.

Parry, R., Caunt., E & Allen C., (2012) Verification of the DNA profile module of STRmix™ using the Promega PowerPlex® 21 system.

Parry, R., Caunt., E & Allen C., (2013) Verification of the DNA profile module of STRmix™ for Full Volume Amplifications using the Promega PowerPlex® 21 system.

Parry,R., Howes, J., & Allen, C. (2014) The determination of the threshold number of alleles, above which single source DNA profiles can confidently be ascribed a likelihood ratio in excess of 100 billion.

Police Powers and Responsibilities Act 2000, Current as of 22 September 2014

Police Powers and Responsibilities Regulation 2012, Current as of 22 September 2014

Howes, J., (2010) A guideline for the assessment of complex DNA profiles.
Caunt, E., Morgan, R., Gardam, T.,Howes, J. & Allen, C. (2014) Verification and implementation of STRmix™ V2.0.1.

Morgan, R., & Caunt E. (2015) Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis.

Caunt E., Morgan, R., Howes, J & Allen, C. (2015) Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis_version 2

18 Amendment History

Revision	Date	Updated By	Amendments
1	11 Nov 1998	V Ientile	
2	28 Mar 2001	V Ientile	
3	11 Jun 2001	V Ientile	
4	18 Jul 2001	V Ientile	
5	08 Jan 2002	V Ientile	9(3) – Completed case codes for FACTS
6	21 Nov 2002	V Ientile	Changes to section 9, completing a case
7	19 Nov 2003	V Ientile L Freney	Refer to AUSLAB. Remove FACTS in many places
8	07 Jun 2005	M Gardam	Included requirements for paperwork in case file ie No loose pages
9	03 Aug 2006	M Gardam	List of reference articles added
10	25 Sep 2006	M Gardam	Off site case file management, compilation of case file, case management.
11	13 Feb	L Weston	Update with processes for AUSLAB

Not Current

	2007		
12	Apr 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
Version	**Date**	**Updated by**	**Amendments**
12	10 Apr 2008	J Connell	Transferred section on preparing case file for presumptive EXR/EXH validation to Examination of Items SOP
13	12 Feb 2009	K Lee	Major rewrite; Inserted subheadings and table of contents; changed order of information to reflect current processes; expanded on reworking information and other processes undertaken as part of case management; added information regarding dilutions and requesting processing of samples sub-sampled in analytical; summarised finalisation requirements for samples with extra barcodes; added examples for entering final EXR lines. Hyperlinked associated documents.
14	28 Oct 2009	K Lee	Updated with reference to GeneMapper *ID-X* software; changed "Pre/Post LIMS" references to "Pre/Post AUSLAB Batch Functionality"; removed unnecessary flow charts; updated hyperlinks and associated documents; introduced paperless case management; re-arranged for better flow and grammatical correctness; Introduced more definitions; included instruction on locating profiles for printing.
15	27 Jan 2012	K Pippia	Introduced new worklists; added section on reworking evidence samples; added VOLUND process; addressed changes in processes since last update; removed references to re-Genescanning and introduced references to re-reads; updated hyperlinks; addressed comments raised against last revision; updated FBNLR process
16	12 Nov 2012	Alicia Quartermain, Emma Caunt, Justin Howes	Updated all processes to include implementation of PowerPlex®21 and STRmix™
17	Jan 2015	Thomas Nurthen	Incorporation of updated workflows, major rewrite , New template
18	August	Thomas Nurthen	Fixed typos, referenced new

Not Current

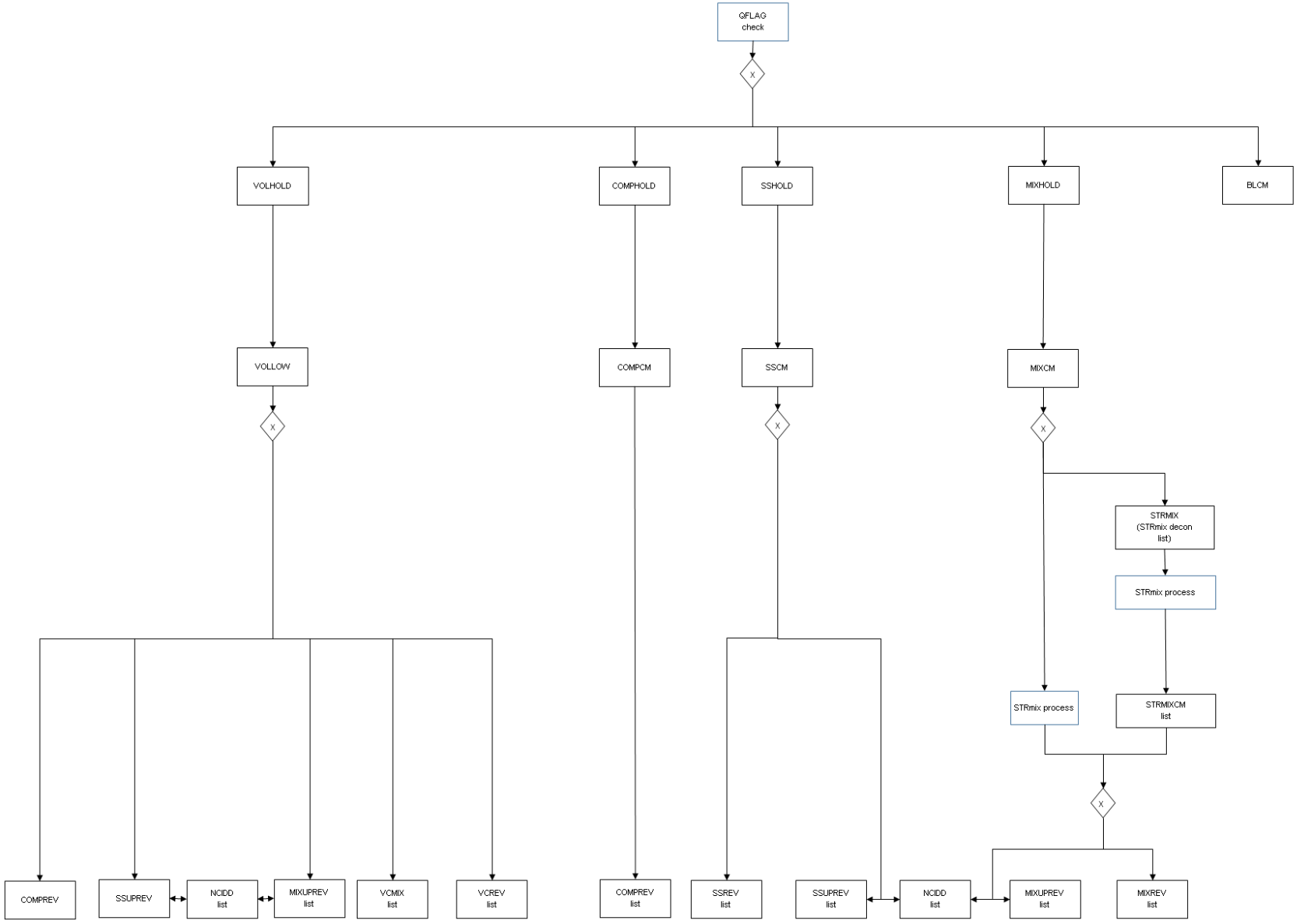
	2015		document for number of contributors, additional steps for FBNLR process, added NCIDD removal process, updated STRmix versions, NCIDD load requirements
19	07 April 2017	Justin Howes	Changed example on p41 to [9, NR]; added information to 5.4 regarding strmix instructions; added eg P+ to PP21 to 9.3; section 6.3.6 – added info on P+ and microcon instructions; changed LOD Quant from 0.00214ng/uL to 0.001ng/uL; added information to 6.5.3 re incorrects; added first line to Table 6;added information to 6.2.5 on no further work process; added Appendix 3 – Intuitive Exclusion Guide and details to 6.4.1.2; changed 19977 to 33407; fixed title of 24126 and hyperlinking throughout; edited amendment history versions/revisions to align with QIS.

19 Appendices

1 Appendix 1 Case management overview
2 Appendix 2 Case management checklist
3 Appendix 3 Intuitive Exclusion Guide
4 Appendix 4 Quantification workflow

Not Current

19.1 Case Management overview



19.2 Case Management Check list

Check Quality

- ☐ Batch statuses are fully completed
- ☐ Check specimen notes
- ☐ Check UR notes
- ☐ Check the photo of the crime scene envelope.

Check case

- ☐ Check for Reference samples.
- ☐ Check for Allocation
- ☐ Check for paper file.
- ☐ Ownership of item
- ☐ Case status

Assess results

- ☐ View results already reported
- ☐ Assess the number of contributors to the DNA profile.
- ☐ Assess the quality of the DNA profile
- ☐ Rework DNA extract if necessary/possible
- ☐ Variant/OLA /ULP calculations
- ☐ DNA profile edits

Manage Samples

- ☐ Interpret - STRmix™, POPSTATS etc
- ☐ Reference comparison
- ☐ LIMS completion
- ☐ Paper case files

Report results

- ☐ DNA profile type line
- ☐ Conditioning information line if necessary
- ☐ NCIDD load lines if necessary

Send to peer review

- ☐ List insert to peer review list or track casefile to location

Not Current

19.3 Intuitive Exclusion Guide

Guide for performing intuitive exclusions

Once the scientist has made an assessment of the number of contributors to a DNA profile, the next stage is to compare DNA profiles obtained from reference samples associated to the case to determine whether these people could be potential contributors. For a single source DNA profile, this is a simple process since the reference sample must match all alleles present within the crime scene profile in order for it to be considered a potential contributor. This process is more complex for mixed DNA profiles as there are more allelic peaks, and combinations of these peaks, to be considered.

As an example, for a two person mixture where there are four alleles at a locus, the reference sample must match two of those alleles to be considered as a potential contributor. If this condition is not met then, under the assumption of two contributors, the reference profile is excluded.

As the number of contributors increase and/or the contributor proportions change, the decision process for excluding a reference profile also changes.

The table below provides a guide for when a reference sample can be excluded as a potential contributor to a DNA profile when comparing the reference sample to an individual locus. It describes the following:

- the mixture type that may be encountered;
- the component of the mixture to which the reference sample is being compared (for an even mixture this would be the entire mixture, for the minor component of a major/minor mixture this would be only the minor alleles);
- the type of match to the reference sample (one or two alleles matching and whether the reference sample is homozygous or heterozygous at that locus).

Where there is an 'S', this means that the reference sample may not be able to be excluded intuitively and that a LR should be calculated using STRmix™. Where there is an 'x', this means that the reference sample can be excluded without the need to calculate a LR in STRmix™.

The number of alleles being compared does not include peaks that could be -2, -1 or +1 repeat stutter. These alleles can be either above or below the LOR but must be above the LOD.

The 'component' of the mixture being compared refers to whether the scientist is comparing to the whole mixture as in an even mixture, or to part of the mixture e.g. major, minor, remaining.

Mixture Type	Number of alleles at locus being examined in component of mixture being compared	**Reference sample**			
		Doesn't match any alleles	Homozygous matching 1 allele	Heterozygous matches 1 allele	Matches 2 alleles
2 person even mixture - drop-out is unlikely to have occurred	1	x	S	x	n/a
	2	x	S	x	S
	3	x	S	x	S

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	4	x	x	x	S
2 person even mixture - drop-out is <u>likely</u> to have occurred	1	S	S	S	n/a
	2	S	S	S	S
	3	x	S	S	S
	4	x	x	x	S
2 person major/minor mixture **- comparison to minor** (major is considered single source)	1	x	S	S	n/a
	2	x	x	x	S
2 person conditioned mixture **- comparison to remaining**	1	x	S	S	n/a
	2	x	x	x	S
3 person even mixture - drop-out is <u>unlikely</u> to have occurred	1	x	S	x	n/a
	2	x	S	x	S
	3	x	S	x	S
	4	x	x	x	S
	5	x	x	x	S
	6	x	x	x	S
3 person even mixture - drop-out is <u>likely</u> to have occurred	1	S	S	S	n/a
	2	S	S	S	S
	3	S	S	S	S
	4	S	S	S	S
	5	x	S	S	S
	6	x	x	x	S
3 person major/minor mixture - 2 people in major - drop-out of major is unlikely to have occurred **- comparison to major**	1	x	S	x	n/a
	2	x	S	x	S
	3	x	S	x	S
	4	x	x	x	S
3 person major/minor mixture - 2 people in major **- comparison to minor** (minor single source)	1	x	S	S	n/a
	2	x	x	x	S
3 person major/minor mixture - 2 people in minor **- comparison to minor** (major is considered single source)	1	S	S	S	n/a
	2	S	S	S	S
	3	x	S	S	S
	4	x	x	x	S
3 person conditioned mixture - 2 people in remaining **- comparison to remaining**	1	S	S	S	n/a
	2	S	S	S	S
	3	x	S	S	S
	4	x	x	x	S
3 person conditioned mixture - remaining is 2 person major/minor **- comparison to remaining minor** (single source) (major is considered single source)	1	x	S	S	n/a
	2	x	x	x	S

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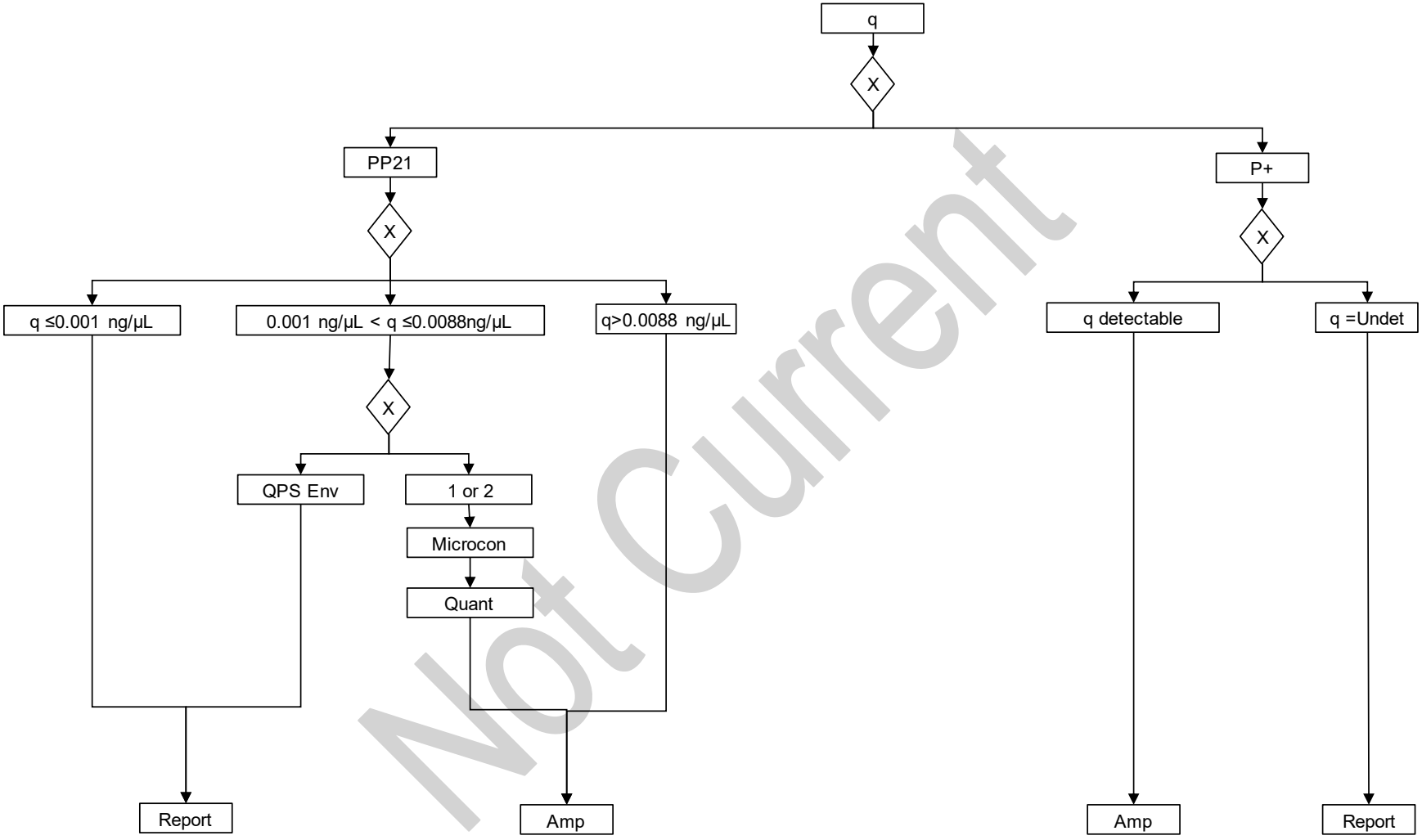
19.4 Quantification workflow



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HealthSupport Queensland

Forensic and Scientific Services

Procedure for Case Management

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1 Purpose

The purpose of this procedure is to describe the components of a case record, processes involved in compiling and completing a case record and tracking of case records.

2 Scope

This procedure shall apply to all Forensic DNA Analysis staff that case manage any component of a case record.

3 Definitions

AUSLAB	Laboratory Information System (routinely used prior to the FR)
Case managing scientist	The scientist(s) that has (or have) been involved in the assessment of results and compilation of the case file in preparation for statement writing or peer review.
Case record	All information relating to a particular case. This can include all case histories, receipts, communication with clients, examination notes, Analytical data, internal communications, results and reports.
CE	Capillary Electrophoresis
DAD	DNA Analysis Database
DNA Master	Repository of DNA profiling information prior to FR
DNA Mgt	DNA Management Unit – A QPS Unit that transfers the exhibit results and link results from the Forensic Register to QPRIME. They also perform quality checks on the validity of the information/results received.
EPG	Electropherogram
Examining scientist	The scientist/s who has/have examined exhibits for a case.
FR	Forensic Register – Laboratory Information Management System since July 2017.
GMIDX	GeneMapper ID-X, software used for allele designation after capillary electrophoresis
In tube	An item that has been sub-sampled by the QPS and submitted to the laboratory in a tube ready for analysis.
LR	Likelihood Ratio
NCIDD	National Criminal Investigation DNA Database
OLA	Off ladder allele
PDA	Profile Data Analysis – page in the FR to record the DNA profile interpretation and actions
Profiler Plus	AmpF*l*STR® Profiler Plus®: The amplification kit made by Life Technologies
PP21	PowerPlex® 21 system kit
Paperless	A type of case that does not involve a traditional paper case file.
PowerPlex® 21 system kit	The amplification kit made by Promega that is currently used for all samples.
QFLAG	Quality checking procedure to investigate potential staff and elimination database matches

QPRIME	Queensland Police Records and Information Management Exchange (Post 2008)
Reporting Scientist	The scientist who is responsible for writing a Statement of Witness outlining the results of a case and for presenting evidence in a court of law.
RFU	Relative Flourescent unit (a measure of peak heights in electropherograms)
SCI	QPS Scientific Officer
SOCO	QPS Scenes of Crimes Officer
SSLU	Scientific Services Liaison Unit
StatsPWG	Statistics Project Working Group
STRmix™	A statistical program used during case management to interpret certain types of DNA profiles.
UKN	Unknown DNA profile
ULP	Unlabelled allele
VAR	Variant allele
XOVER	Cross over allele, allele migrates into an adjacent marker bin.

4 Case file overview

Since the 1st of September 2009, low priority Volume Crime cases have been treated as 'paperless' and therefore do not have case files. In April 2010, paperless case management and review was expanded to also include all cases of both high and low priority (Volume and Major Crime) and some Sexual Assault cases except for cases involving excessive numbers of crime scene/reference samples or complex profiles. In April 2015 all cases are initially managed as paperless cases.

Case files are generally created

- At the time of case management (for complex cases) or
- When a statement is requested or
- When a case manager/reporter deems it necessary for efficient case management.

For cases previously managed paperlessly that become reactivated upon receipt of further items, they may be considered for conversion to a paper file. Case and examination notes (when the case was managed paperlessly) are stored in 'Paperless' folders stored in Evidence Recovery, Reporting and Admin areas.

If a case has been converted from paperless to paper, it is not necessary to annotate all of the EPGs with the item description or interpretations unless a statement has been requested. At such a time, the reporting scientist may continue with EPGs not being annotated as long as the casefile also includes a printout of the relevant PDA page from the FR.

4.1 How to create a case file

To request a casefile to be created, email FSS.FDNA.Admin@health.qld.gov.au with instructions. Admin edit the Statement Request/Task that a casefile is being created, assign a barcode for the casefile and create a storage location (see QIS 33773 and 34248).

4.2 Additional Elements of a case file

Upon completion, a case file may also contain:

1. Examination notes

2. Diagrams, photographs and/or photocopies
3. Statistical calculations.
4. Copies of results (GeneMapper ID-X printouts).
    a. As a minimum, reference samples require the final/reported profile. Casework samples should have all EPGs printed.
5. Interpretations of results
6. Copy of statement or intelligence report
7. Records of any internal or external communication relating to the case, e.g. Casefile Notations, Requests/Tasks or emails.
8. STRmix™ output files/report. STRmix™ v2.7 it is not recommended to include the STRmix™ report, rather a printout of the PDA page with the EPG is sufficient.

4.3 Handwritten results and corrections within a case file

As is required by NATA ISO 17025 - as case notes etc. are subject to subpoenas; no pencil is to be used in the case file (unless used in diagrams or pictorial representations).

Any calculations, interpretations or changes to notes or results must be initialled and dated by the person performing the action.

4.4 Case file storage and movement

Case files are required to be kept indefinitely as per accreditation requirements.

Exhibits are not to be stored in the case file. This includes external proficiency samples. Original QPS property tags or reference sample envelopes are also NOT to be stored in the case file.

Case file movements are to be recorded in the FR. If a case previously managed within AUSLAB is reactivated, remove the tracking from AUSLAB, create a casefile in the FR (using the same barcode) and track in the FR.

Active case files are stored with the case analyst or in a designated storage location for the work area.

Upon completion, scientists should transfer cases to Admin via the FR. Administration assistance slips are available to attach to the front of the case file to direct the storage of the file or to outline any further administrative tasks that need to be performed prior to storage. Admin In-Tray – Casefile Finish is the location from which administrative staff will track case files (sequentially) into the compactus or another designated storage location. No further administrative tasks will be carried out on these cases.

If a casefile in the custody of the case scientist is taken out of the laboratory for court, or for court preparation, movement of the casefile should be recorded as a casefile notation in the FR.

5 Workflows

5.1 Priorities

Table 2 details the DNA priorities that are used in Forensic DNA Analysis. These are not to be confused with case priorities eg. one sample may be processed as Priority 1, but the case as a whole is Priority 2 (Major Crime).

Table { SEQ Table * ARABIC } - DNA Priorities in Forensic DNA Analysis

Priority	Description	CW Use	Ref Use
1	Urgent	Urgent	Priority/investigation
2	High Pri	Major crime	High priority
3	Low Pri	Volume	Normal

Urgent (5-day Turnaround (TAT)) cases are specifically allocated to a case scientist and/or reporting scientist as they arrive into the department. The Managing Scientist and Team Leaders will be notified of the arrival of an urgent case by email and appropriate notes will be entered. A supervising scientist will allocate to an appropriate case manager. This does not mean that the case managing scientist will necessarily become the reporting scientist should a statement be required, however this is preferred to maintain consistency in reporting.

P1 samples must be managed as soon as results become available and reviewed as soon as results are interpreted. To ensure there is no delay in QPS being informed of 5-day TAT results as soon as they are available, a workflow has been created for samples that are expected to be completed on a Friday (see QIS 23968, 33773 and 34006).

5.2 PowerPlex®21 system kit vs AmpFℓSTR® Profiler Plus® case management

Since the end of testing with AmpFℓSTR® Profiler Plus® (Profiler Plus) in January 2018, all samples are received and processed with PowerPlex®21 system kit (PP21).

This does not mean the reporting method for Profiler Plus samples is invalid; therefore, in consultation with a senior scientist, samples may be re-processed with PP21 for case consistency or only newly received items will be processed and reported with PP21 and STRmix™.

5.3 STRmix™ versions

The date of first installation and processing of cases with various versions of STRmix™ are listed in Table 3 below.

Table 1 – STRmix™ version use

Date case received	Decon	LR (at time of receival)	LR (New comparison)
19 Dec 2012	v1.05	v1.05	v2.0.6
1 July 2014	v2.0.1	v2.0.1	v2.7.0
30 Jan 2015	v2.0.6	v2.0.6	v2.7.0
16 Jan 2019	v2.6.0	v2.6.0	v2.7.0
24 June 2019	v2.6.2	v2.6.2	v2.7.0
10 Feb 2020	v2.7.0	v2.7.0	v2.7.0

If new samples are received for cases that had other samples in the case previously analysed with earlier STRmix™ versions, they are to be analysed with the current version of STRmix™. Discussion with a Senior Scientist on whether to migrate previously reported samples to the current version should be held.

5.4 Case management workflows

For the process to allocate samples and/or cases, see QIS 33773.

For worklists and information on how these are populated, refer to QIS 33773.

Allocation of cases to a particular scientist usually only happens if a statement is required, the case is large or has been assigned an Operation by QPS. These cases will otherwise be routinely case managed by the competent case managers. However, to reduce the amount of double handling by case managers, individual samples initially case managed by a particular person will be completed by the same person. This includes reworking and STRmix™ deconvolutions.

Unallocated paper case files may be stored in the filing cabinets stored in the far end of the reporting area in Block 3.

Internal controls, external and internal proficiency (where applicable), internal and external environmental monitoring samples are case managed by the Analytical, Evidence recovery and Quality teams.

Various tools may be employed to assist in meeting timeframes and to cover absence such as scheduling Outlook appointments or tasks.

6 Case management

The purpose of case management is to collate and report any DNA results that have been obtained and to prepare the case file for a statement (if required) or for peer review. To achieve this, the case managing scientist may be required to:

1. Assess DNA results to determine whether reworking is required to improve or confirm results.
2. Enter final Exhibit reports via the Profile Data Analysis (PDA) page in the FR.
3. Compile case file.

6.1 Check quality

Samples should not be progressed or reported until the various quality checks that are in place have been completed. These checks are designed to identify potential issues with samples before they are reported to the QPS.

6.1.1 Batch statuses

Check that the statuses of the processing batches are fully completed (see QIS 33773).

If there has been an issue noted during processing of a sample, the Analytical staff member/delegate will enter a status of ‘See batch’. The case managers (PDA operator and reviewer) **MUST** check the batch audit and add a Sample Note to detail that they have deemed the sample OK to report.
It is acceptable that the note is added by the PDA operator or reviewer. If there is a critical element to a Batch that could affect the sample processing or interpretation strategy, and there is no note added by the PDA operator, then a discussion between the PDA operator and reviewer should occur.

Results can be released prior to the batches being formally 'passed'. In these instances, the PDA operator and reviewer will need to check the relevant batches and added a comment or sample notation to describe this.

6.1.2 Casefile Notations

Check Case Management tab in the FR for Casefile Notations and Request/Tasks (and UR notes for cases processed with AUSLAB) for relevant information related to the case. This may include information such as allocation to an individual case manager/reporter, court timeframes, communication with DNA Management etc.

6.1.3 Notations

Check for relevant information in the Exhibit Testing tables for notations and Analytical Notes (see QIS 33773), and Specimen Notes for cases processed with AUSLAB.

6.2 Check case information

Case information may be relevant to only particular samples or the whole case. This information may be used to guide the case manager's choice of processing and reporting.

6.2.1 Check for reference samples associated to the case

The presence or absence of reference samples may affect the workflow path a sample takes. If reference samples have been received for a case, these will be compared against all single source DNA profiles, and all interpretable mixed DNA profiles to generate a LR.

See QIS 33773 and 34006.

6.2.2 Check for case allocation

It is necessary to check if a case has been allocated to a particular case manager or reporter before case managing a sample.
Check the Case Management tab in the FR for details or on the PDA page, it can be viewed in the 'Case Scientist' field. See QIS 33773.
In AUSLAB (if some or all of the case was processed with AUSLAB (pre July 2017), it may be recorded in the UR notes and/or the CS page.

6.2.3 Check for paper file/case notes.

Check the Exhibit Register for a barcode created for a casefile to enable storage and tracking (see QIS 33773).

6.2.4 Check ownership of item

Ownership of an item may be required before interpretation of a DNA profile or an exhibit is sampled. If unknown, send a Request/Task to the SOCO or SCI in the first instance to obtain this information. If a response is not received in a timely manner, send a Request/Task to QPS DNA Management for the information.

6.2.5 Finalising samples no longer required

See QIS 34006.

6.3 Assess results

All samples have alleles designated as per QIS 34112.

When results become available for a sample, an assessment needs to be made as to whether reworks are required or whether sufficient information has already been obtained. This can be performed as each result becomes available. Not all results need to be available at the same time for these assessments to take place.

If viewing a case via AUSLAB and with samples processed with Profiler Plus, the EPGs were saved to AUSLAB as jpegs, or if they were samples from major crime cases, they had their EPGs saved to the P drive.

If the case was processed before implementation of the FR, the EPG PDF will be stored on the network.

To assess the stutter percentages, a worksheet or macro may be used to perform the calculation checks (see QIS 35008 or QIS 35406). The former requires manual addition of the alleles and peak heights to calculate the stutters, and the latter spreadsheet uses a macro to calculate the stutters after importation of the STRmix™ text file generated by the FR.

6.3.1 Assess the number of contributors to the DNA profile

The number of contributors to a DNA profile is required to perform interpretation. Counting the number of alleles at each locus (above and below Limit of Reporting threshold, above Limit of Detection) is the first step in assessing the number of contributors.

However, counting called alleles alone may not be suitable in determining the number of contributors due to the presence of PCR artefacts such as stutter. Allelic imbalance (AI) also known as heterozygote balance (Hb) can also be used as an indication of the number of contributors. Forensic DNA Analysis does not have a threshold for AI for casework DNA profiles because STRmix™ is designed to model the heterozygote balance as a continuous system. Although internal validation studies (Nurthen et al 2013) indicate that the calculated AI threshold varies depending on the DNA input, the values detailed in the study can be used as a guide.

See Appendix 1 for a workflow designed within the internal Change Management project #149 to assist in deciding on a reasonable number of contributors to the DNA profile. Note that the stochastic range in RFU values will be different depending on the CE instrument. The workflow is a guide only.

The validated stutter thresholds (as published in QIS 34112) are used as a guide to aid in the determination of number of contributors to a DNA profile.

6.3.2 Assess the overall quality of the DNA profile

The quality of the DNA profile in conjunction with the number of contributors will determine if a DNA profile is suitable for interpretation.
The following factors should be considered

1. Whether a reasonable assumption of the number of contributors can be made.
2. The degradation slope (the tendency for higher molecular weight loci to have lower peak heights compared with smaller molecular weight loci).
3. The total amount of DNA input used in the amplification
4. Adverse events affecting the sample.

6.3.3 Check VAR/OLA/ULP/XOVER calculations

If a variant and/or off ladder allele or stutter has been observed on a GeneMapper ID-X (GMIDX) profile it is not necessary to re-amplify to confirm its presence.

For mixed DNA profiles with variant and/or off ladder alleles, the repeat of these samples is at the case manager/reporter's discretion. Things to consider include whether the profile with variant and/or off ladder alleles has already had this questioned allele confirmed, matches a deconvoluted contribution, or if the sample description suggests the mixed DNA profile could be conditioned on the reference DNA profile (with variant and/or off ladder alleles).

A case manager must independently perform the calculation for allele designation including if the calculated allele falls in the stutter position. Refer to QIS 33773.

Variant/OLA/ULP/crossover calculations do not require checking if the DNA profile has been assessed as unsuitable for interpretation.

If there are broad peaks observed in the EPG and the sample has not been Re-CE'd, the case manager may order a Re-CE. This is especially important if the DNA profile is to be assessed by STRmix™, or if the case manager determines that the broad peak could be masking other peaks such that it may affect the number of contributors assessment.

6.3.4 NAD samples

If a sample is flagged as No Analysed Data (NAD) at CE quality checking stage, the sample will be re-prepared by Analytical staff.

6.3.5 Edit DNA profiles

See QIS 33773 and 34006.

6.3.6 Rework DNA extract if necessary.

For processes relating to ordering reworks, see 33773.

See Appendix 2 for information on reworking strategies and considerations when assessing sample information and profiles.

If a sample was completed in DNAMaster/DAD and AUSLAB, any subsequent reworks that are required are requested in the FR.

As of 30 June, 2019, any rework on a previously reported Major Crime (Priority 2) result is not to be ordered without Managing Scientist or Executive Director authorisation. A MS Form can be used to provide information to the Managing Scientist of Executive Director to assess the reasons for the rework, and the potential risks associated with proceeding (or not proceeding) with a requested rework. This form can be accessed via Office 365, then selecting MS Forms. The operator fills out the details in the DNA Rework Authorisation form. After submission, the form then goes to the Team Leader for consideration and endorsement prior to the Managing Scientist (or Executive Director) for final consideration.

Internal validation studies (Nurthen et al 2013) have shown that samples with low template DNA (~132 pg) that are amplified with PP21 may exhibit significant stochastic effects such as large allelic imbalance and allele drop-out. These effects can complicate the interpretation of both single source and mixed DNA profiles. Reworking may improve the quality of the DNA profile. It is standard for P2 samples with less than 132pg (Quant of 0.0088ng/µL) to not be processed initially and a result line of 'DNA Insufficient for further processing' be released. DNA Mgt may request these samples to be reactivated for processing by sending a Request/Task in the FR to the Supervising Scientist of the Analytical Section.

In 2008, QPS in conjunction with Forensic DNA Analysis decided that for Low priority Volume Crime (Priority 3) cases, samples are only to be reworked via re-amplification, or Re-CE'ing until 12 alleles are obtained (National Criminal Investigation DNA Database-NCIDD uploading threshold). NucleoSpin cleanups or Microcon concentrations are not to be ordered on low priority samples, unless in exceptional circumstances. Other valid reasons for reworking these samples include investigations of adverse events or if other quality issues are suspected.

If a partial profile or NSD profile is obtained for a sample, an assessment should be made as to whether reworking that sample will be beneficial or if there are other profiles within the case that satisfy reporting requirements.

Amplification products are not kept indefinitely. The availability of a PCR product should be checked prior to ordering a Re-CE. For more recent batches, the Analytical Section enters audit notes against the amplification batch when the PCR product has been discarded.

Rework strategies:
If it is determined that a better profile is required, the following should be considered when determining the best rework strategy:

1. **The type of sample**
   e.g. blood versus cells. Due to the generally high number of nucleated white cells in whole blood, a DNA profile is usually obtained from such samples. If a DNA profile is not obtained, this may be due to insufficient nucleated cells in the sample, or could indicate an issue with the efficacy of the processing, or it could be that the sample is inhibited. Reworks may assist in obtaining an interpretable profile.

2. **The Quantitation value**
   The quantitation value is displayed in the FR. The quantitation value is an estimate and should be assessed in conjunction with other factors. Sample workflows based on the quantitation value are listed below:

1. PP21 samples with a quantitation value <0.001 ng/µL will not be further processed and will be reported post-quant with the result line 'No DNA detected', regardless of priority.
2. PP21 samples with an initial quantitation value between 0.001 ng/µL and 0.0088 ng/µL will be reported post-quant with the result line of 'DNA insufficient for further processing'. Priority 1 samples will proceed to a microcon concentration step prior to re-quant and amplification as per QPS –Forensic DNA Analysis agreement.
3. Samples reported as 'No DNA detected' or 'DNA insufficient for further processing' can be requested by QPS for further processing via the Request/Task system to the senior scientist of the Analytical section.
4. PP21 samples with an initial quantitation value of > 0.0088 ng/µL are amplified.

A partial or NSD profile from a sample with a high quantitation value may indicate inhibition or may be due to degradation. The Degradation Index is available within the Quantification data and provides an indication that degraded DNA may be present. It should be noted that while quantitation values can be used as an indicator for the presence of inhibitory compounds in an extracted sample, lack of inhibition in a quantitation amplification (as indicated by the IPCCT and possibly the CT as well) does not necessarily mean there will be no inhibition in an STR amplification. This is because different primers, target DNA and amplification conditions are used in each reaction and this could result in inhibition to one reaction and not the other. Also, 2 µL of extracted sample is added to a quantitation amplification, whereas in an STR amplification the sample may be diluted before being added (which would decrease the concentration of any inhibitory substances in the amplification reaction). Up to 15 µL of DNA extract can be used for a PP21 amplification (which would change the relative concentration of inhibitory substances in the amplification reaction). Further information on DNA quantification is found in QIS 34045.

3. The number of alleles obtained

A full DNA profile is the aim of any DNA amplification but a partial DNA profile does not necessarily need to be reworked.

The minimum number of alleles required to upload to NCIDD is 12 alleles. Samples below this stringency, but above 6 alleles, may be loaded to NCIDD under special circumstances and searched against the database (refer to QIS 34246 and 33773).

If an assumption of a single contributor has been determined, partial DNA profiles do not have to be reworked to obtain a full DNA profile.

4. Examination notes

Certain substances are known to be inhibitory to the PCR process. This includes a variety of commonly encountered substances, such as dyes used in clothing (particularly denim dyes) and some biological material (in particular, the haem in blood). If managing a case where semen samples were extracted with Chelex – for example, if the case is reactivated for further processing - these samples were sometimes observed to return an NSD profile after initial extraction with no indication of inhibition. Performing a NucleoSpin clean up was noted to improve the chances of obtaining an interpretable DNA profile for these samples.

5. **Offence Details (if available)**
   Information from the QPS entered into the FR, present on item packaging, or from case conferences may assist in determining the evidential value of a particular item.

6. **Results already obtained**
   If multiple samples have been submitted for an item and one or more full profiles or mixtures have already been obtained there may be no need to continue reworking other samples from that same item. A partial 'matching' profile is often sufficient if other better profiles already exist for the same item. This must be considered carefully and in the context of the case. If it is a possibility that there may be a different profile present, such as in the case of multiple offenders, then reworks should be considered.

6.4 Manage samples

The sample management tab in the FR contains the worklists relevant to PDA entry and review (see 33773 and 33744).

Cases are not usually allocated to an individual case manager/reporter. The exception to this rule may be some urgent cases, QPS operations, linked cases or sensitive matters. Samples are case managed by staff from the worklists in the FR.

Cases with paper files may have EPGs annotated with the results and interpretations, although if the PDA page is also printed, this may be not required (see 33773). If annotated, as a minimum, the type of DNA profile. e.g. single source matching UKM1 is required. These annotations need to be signed and dated by the case manager.

6.4.1 Interpret

6.4.1.1 Paired Kinship/Paternity Trios

Any samples for Paternity trios etc. are interpreted as detailed in QIS 25303.

Reporting of the analysis outcomes is detailed in QIS 34006 and QIS 34308.

6.4.1.2 PP21 interpretation

Statistics for PP21 results are generated by the STRmix™ program as outlined in QIS 35007.

If a sample has replicate amplifications they must all be included in the STRmix™ deconvolution unless they have a particular processing issue such as excess peak heights and pull up, a Re-CE has been performed, or the runs are not consistent with each other (at the discretion of the case manager). A Re-CE and the source amplification results cannot be included in the same deconvolution as they come from the same amplification, a choice as to the best or most appropriate run must be made by the case manager and replaces the less informative result. At a minimum, a Sample Note should be added to explain why particular amplifications were not included.

All reference samples received for a particular case are to be compared against all interpretable mixtures (to generate a Likelihood Ratio - LR) and single source samples within a case.

The number of contributors will have been determined as per section 6.3.1 above.

STRmix™ V2.7 uses a stratified approach to reporting the Likelihood Ratio where the relative proportions of the population are factored into the final LR.

Single source DNA profiles

Deconvolution with STRmix™ is required if:

1. The sample is the first single source DNA profile that matches a reference sample and needs to be loaded to NCIDD, or
2. The sample requires loading to NCIDD (e.g. UNK), and/or
3. This DNA profile has less than 32 allelic peaks. The count of peaks is such that homozygous loci are counted as one peak. It is only through STRmix that single-peak loci are determined to be homozygous.

LR generation with STRmix™ is not required for single source DNA profiles:

1. If a reference sample does not match the single source sample.
2. If a matching reference sample has previously had an LR generated (and the new interpretation would not be more probative).
3. If the single source DNA profile has 32 or more allelic peaks, the sample can be reported with the appropriate result line (as per QIS 34229) and doesn't require deconvolution and an LR generated as per the recommendations in the document 'The determination of the threshold number of alleles, above which single source DNA profiles can confidently be ascribed a likelihood ratio in excess of 100 billion.' [Parry et al 2014] and further Risk Assessment after moving to STRmix™ V2.7.0.

If a single source DNA profile has one peak at a locus and another peak is visible sub threshold, STRmix™ may designate the locus as a homozygote (with a ≥99 % weighting), the case manager should consider ordering a rework in an attempt to amplify the second peak.

Homozygote alleles for single source samples that will not be loaded to NCIDD do not require editing in the FR PDA page.

A mixed DNA profile would be reported as a single source profile with sub-threshold peaks using the appropriate exhibit result line in the following circumstances:

1. If the only indication of a mixture is a labelled Y peak at Amelogenin or
2. If the only indication of a mixture is a labelled Y peak at Amelogenin and sub-threshold peaks that do not affect the called alleles.

This is done because STRmix™ cannot 'see' Amelogenin or sub-threshold peaks and the low-level contribution does not affect the interpretation of the 'single source' profile.

Mixed DNA profiles (two, three, four person mixtures)

Deconvolution with STRmix™ is not required if:

1. The case does not have any reference samples and the profile is not likely to be deconvoluted by STRmix™ into contributions for NCIDD, or
2. The case does not have any reference samples and if the DNA profile is likely to be deconvoluted into a contribution that matches an already reported unknown in the case.

If reference samples are later received then the deconvolution will be run and these reference sample profiles will be compared against the mixture and the LRs reported back via exhibit result lines.

Deconvolution with STRmix™ is required for all other two, three and four person mixtures.

Deconvolutions of mixed DNA profiles may run for extended periods of time. Additional support is provided by other staff in Forensic DNA Analysis (mostly Forensic Technicians) to run deconvolutions on dedicated STRmix™ computers. This releases Reporting Scientists' computers for other tasks.

To have another staff member run a deconvolution, see QIS 33773.

Conditioning mixtures

It may be possible to condition mixtures from intimate swabs and items (said to have come from a person). The decision to condition is at the discretion of the case manager (and reviewer). Additional information regarding ownership may be required.

Table 2 – Quick reference when to use STRmix™

Scenario	Decon	LR
SS <32 & matches assumed known contributor	No	No
SS <32 & matches a reference sample	Yes	Yes
SS <32 & new Unknown profile & NCIDD	Yes	N/A
SS <32 & matches an Unknown profile	No	N/A
First SS >32 DNA profile & matches a reference sample & NCIDD	Yes	No*
First SS >32 DNA profile & matches a reference sample no NCIDD	No	No*
SS >32 DNA profile & new Unknown profile & NCIDD	Yes	No
Subsequent SS >32 DNA profile and matches a reference sample/Unknown profile	No	No*
2P to 4P & no reference samples & not likely to resolve for NCIDD	No	N/A
2P to 4P cond & no other reference samples & not likely to resolve for NCIDD	No	N/A
2P to 4P & reference samples	Yes	Yes

*Where matching a reference samples, a Likelihood Ratio is not calculated in these instances, but they are reported in the FR as >100 billion favouring contribution.

STRmix™ results output

After the STRmix™ deconvolution and/or reference comparison has been completed and processed, the following quality checks must be performed on each result produced by STRmix™.

1. STRmix™ version
2. Casework sample number is correct
3. Reference sample number (if any) is correct
4. Number of contributors assumed to be present is correct
5. Casework DNA profile (correct allelic designations entered and correct run(s) have been included)
6. Individual locus LRs appear have an intuitive fit
7. Check all loci had successfully deconvoluted (component interpretation complete)
8. Check that the Diagnostic tools are all performing to expectation
9. Settings values (especially check full vs. half variances)
10. Reference DNA profile (correct allelic designations entered)

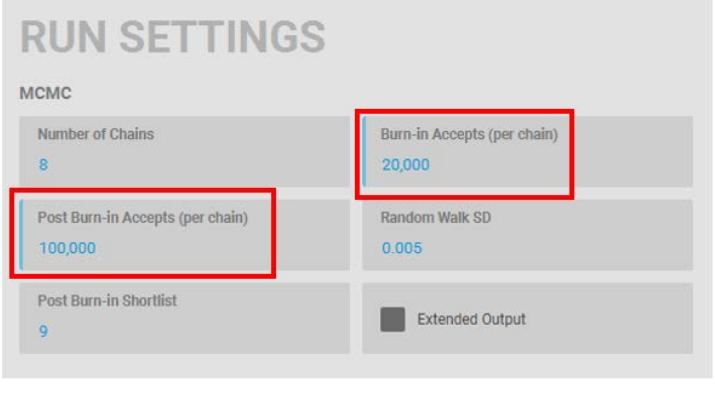
11. The overall LR is reasonable given the reference and casework DNA profiles

It is important when a STRmix™ analysis is carried out, that the results are interpreted by examining the weightings of various genotypes and the DNA profile(s) observed. There are instances when the results obtained do not intuitively seem correct. Sometimes (particularly if the model must account for drop-in) the failure of the Markov chain to properly converge means that some parameters will not have optimised properly. Examples of this are:

1. Large LRs are obtained for each locus, except one where the LR is low or 0
2. The mixture proportions do not reflect what is observed
3. The degradation does not reflect what is observed
4. Genotype combinations do not reflect all likely allele sets (especially likely if sub-threshold peaks are present at a locus)
5. The probability of dropout at a particular locus has been given a low value but sub-threshold peaks are clearly visible in the DNA profile.

Effectively, a zero LR means that the genotype of the POI has not been accepted by the MCMC at any time through the course of the analysis. Common causes for making a genotype an unlikely contributor are large dropouts, drop-ins or imbalances, or when the peak heights at a locus exceed the general degradation slope (and are therefore penalised). If further iterations are chosen, then the MCMC will have more opportunity to accept the less supported genotypes, however a reference sample with a poor fit to the DNA profile will still have a low LR for a particular locus or loci. It is best practice to attempt to resolve the mixture biologically first, that is through rework, prior to resorting to increased iterations.

It is possible that the deconvolution does not fit with the intuitive assessment of the DNA profile, e.g. there is a clear major profile but the deconvolution has not resolved C1 (Contributor 1) to ≥99%. There are a number of reasons why this may occur including there being insufficient accepts to enable STRmix to converge on the best probability space. In this instance, the user can increase the number of burnin accepts and post-burnin accepts by a factor of 2 (to 20,000 and 100,000 respectively) in the run settings when setting up the deconvolution.



If it is noted that the EPG has a plate reading error, such as a stutter peak that has been inappropriately removed or an artefact that has been left in, then the sample can be edited in the FR and EPGs manually edited as per QIS 33773.

It is not necessary for STRmix™ v2.6 (and beyond) cases to have the STRmix™ report printed and included in the casefiles. A printout of the PDA page and EPG is sufficient. All cases have the pdfs imported and retained in the FR (see QIS 33773).

Repeated Analysis

Each time a DNA profile is analysed using STRmix™ the results will vary slightly. This is a natural consequence of the random nature of the Monte Carlo property. To be as unbiased as possible, each analysis should only ever be run once and the result reported. If a STRmix™ result has been generated for a DNA profile at case management stage, then that same result should be the one used for statement writing. If additional reference samples are received in the case, the reference sample(s) should be run against all original deconvolutions for all samples in the case where mixtures are present. The exception to this is when an analysis has produced a result that requires further investigation and hence further analysis or if the underlying assumptions made about the profile have changed (eg. a two-person mix is reassessed as being a three-person mix).

Consequently, if at review or at a subsequent stage in reporting it is decided that a different number of contributors better fits the DNA profile, the deconvolution for that sample can be rerun using the new assumption. Case-managers/Reporters should discuss any decision to change a reviewed result with the original operator/s. For High Priority samples, if a rework after a result has been released, this will need Managing Scientist or Executive Director approval (see 6.3.6).

If multiple analyses have been conducted, then only the STRmix™ results from the most appropriate analysis should be reported (e.g. the higher number of acceptances or the more appropriate number of contributors). If there are printouts of the STRmix™ results in the casefile, the previous results will need to be removed.

The electronic STRmix™ results from the multiple analyses that are not used must be moved into a sub-folder labelled “Do not use” in the case folder in the STRmix™ results folder.

Use of Ignore Loci function

In certain circumstances a particular locus or loci may be dropped from the interpretation. These include where a Tri-allele pattern has been observed in a reference profile and inconsistent sizing of an allele is observed. See QIS 35007.

If a case has a reference sample with a mutation, all scene profiles within the case (except single-source profiles that do not match the reference sample in question) should have the loci removed from the interpretation. If the reference sample is received after the initial deconvolution was performed, the deconvolutions should be repeated with the relevant locus/loci ignored.

Amended Results

If an amended result is required to be released, this should be accompanied by an Intelligence Report (in most circumstances as per QIS 33773) and cleared by the Managing Scientist or Executive Director prior to release.

6.4.1.3 Profiler Plus interpretation

Since January 2018, Profiler Plus DNA profiles were no longer produced by Forensic DNA Analysis. Samples may still be added to statements (if requested) and reported in a binary fashion. This difference should be explained in the statement of witness.

Samples that are processed with Profiler Plus are not interpreted using STRmix™ as this system has not been validated for use with Profiler Plus data. Interpretation of Profiler Plus samples is outlined in QIS 17168 and 25302.

See QIS 33773 for the use of the FR in reporting Profiler Plus DNA profile interpretation results.

6.5 Report Results

All results are to be communicated as outlined in QIS 23968 and 34308.

Statements and intelligence reports are to be prepared according to QIS 34006 and 34308.

For cases processed and previously reported via AUSLAB, all new items received and/or updated interpretations should be reported via the FR.

If a sample cannot be explained by one of the result lines available, an intelligence letter should be sent to QPS to outline the interpretation. See QIS 34308.

When reporting 4p mixture interpretations where the LR is in the in the range 2-1million favouring contribution, a result is acceptable to be reported via Request/Task in the FR by using the following process:

- PDA Reviewer to ask for the Request/Task when reviewing the sample,
- Using a template (below), case manager/reporter to direct a Task to the reviewer with the information,
- PDA Reviewer directs to Sgt DNA Results Management Unit at same time as reviewing.

- Template to use:
- *Sample barcode: XXXXXXXXX*

- *Result reported: Mixed DNA profile*
- *LR reported: Mix – Support for contribution 2 to 1 million: Person barcode YYYYYYYYY*
- *Actual LR: [number]: Person barcode YYYYYYYYY*

6.5.1 Exhibit Result lines

See QIS 33773 and 34006 for details on how to report result lines in the FR.

For urgent/Priority 1 samples only, an interim exhibit report may be entered.

6.5.2 Exhibit Result line updates and amendments

Exhibit result lines may require updating after additional information is available or additional testing has been completed. Commonly, these lines are updated after a reference sample for the case has been received and new information needs to be sent back to QPS eg. the profile is now to be ‘conditioned’.

If the DNA profile has undergone further work and the result line 'SUFP: sample undergone further processing' has been used, the final interpretation result lines need to be added to the FR at the same time and supersede the previous result lines. This means all lines need to be added that are relevant to the updated DNA profile interpretation.

If an incorrect result is needing to be reported, the result line must be marked as incorrect by Senior Scientists or Team Leaders. See QIS 33773 and 34006.

The correct result should be added and reviewed at the same time as marking the previous result as 'incorrect', (see QIS 34006).

If an Intelligence Report is required to be sent to the QPS Inspector of DNA Management Unit to explain and incorrect or amended result, this report needs to be initially sent to the Managing Scientist for awareness. See 34308 for a template for this report.

6.5.3 Suspect checks

If a suspect check has been requested by QPS for a reference sample profiled in Profiler Plus and the sample is not intuitively excluded from the mixture, the reference sample needs to be reworked in PP21 to increase the amount of data available for comparison.

Instructions for reworking reference samples are documented in QIS 34245.

Suspect checks have reserved Exhibit result lines for reporting; refer to QIS 34229.

LR reports from STRmix™ for Suspect Checks need to be retained in the FR. These can be attached as a sample notations for the crime scene sample, or attached to the Result line pertaining to the LR outcome for the comparison.

6.5.4 Samples with undetermined quantitation values or insufficient DNA

It is understood by QPS that samples reported post-quant as 'No DNA Detected' or 'DNA Insufficient for further processing' can be requested for processing at any time.

This request for further processing is made by the QPS sending a Request/Task to the Senior Scientist of the Analytical section to reactivate the sample for processing.

Similarly, case managers may at their discretion order a rework in cases where the only results are low quant samples.

6.5.5 Paternity Samples

For paternity cases, results are reported via the barcode for the child (see QIS 33773).

If the putative father sample is an intelligence sample, the relevant result line would be 'Intel report required for further Interpretation'. The Intel Report is issued as per QIS 34308.

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6.5.6 Using Coronial samples as Reference Samples in Exhibit results.

If a sample has been processed with casework conditions is to be used as a reference sample, it needs to be deconvoluted in STRmix™ because there is no homozygote threshold. This deconvoluted DNA profile is used as the reference in all comparisons.

6.5.7 Using Covert samples to compare to DNA profiles

Covert samples are ones that have been identified by the QPS as being taken in lieu of a official reference sample. Covert samples are treated as crime scene samples and can present to the laboratory as items such as straw swabs, swabs of drink containers and cigarette butts, among others.

The DNA profiles obtained from these covert samples may be requested to be compared to specific, or all crime scene samples. The results of these comparisons should be entered in an Intelligence Report and issued to QPS DNA Management Section, unless specifically informed otherwise.

See QIS 34308, 33773 and 34006.

7 NCIDD

Case managers are responsible for choosing a representative profile for each unique profile seen within a case for upload to NCIDD. These profiles must have at least 12 alleles for NCIDD matching.

To upload an allele to NCIDD for PP21 samples, a 99% deconvolution is required at a locus as per the Statistics Project Working Group (StatsPWG) recommendations.

- ≥99% deconvolution at all PP21 loci is known as a ‘full’ NCIDD load
- ≥99% deconvolution at ≥ 12 PP21 loci is known as an ‘Intel’ NCIDD load.

In certain circumstances, a profile with less than 12 alleles (including sub-threshold information) can be loaded to NCIDD, and any matches will be reported back to QPS via an Intelligence report written by the case scientist or Intelligence Team member. This is an intel/upload process and is not for court purposes. Intel/NCIDD work does not get heard in court unless special authorisation is given by the Judge/Justice due to potential to prejudice court.

Only one representative DNA profile is loaded to NCIDD for a person in a case. Profiles that match known deceased persons or complainants in sexual assault cases are not to be uploaded to NCIDD. By the same rationale, unknown DNA profiles previously loaded to NCIDD that match known deceased and sexual assault victims are also removed from NCIDD. Refer to QIS 34246 and 33773.

7.1 Conditioned DNA profiles loading to NCIDD

After a mixed DNA profile has been conditioned in STRmix™, the deconvolution will list that each conditioned allele has been deconvoluted to 100%, a conditioned component of a mixed DNA profile can be loaded to NCIDD provided that :

- The upload alleles are able to be visually separated (i.e. major or minor)
- Upload matching alleles in an even mixture where there is a strong representation

Do not upload contributions from low level mixed minors where we may be confident enough to condition but not load to NCIDD.

8 Peer review

All results must be peer reviewed prior to release to the QPS. Peer review can be at a sample level or case level, Technical or Administrative (see QIS 34322 and 34006).

Peer review of 'No DNA detected' and 'DNA insufficient for further processing' is usually performed by a competent Analytical Section staff member.

9 Reference sample management

Refer to QIS 34245.

10 Case Managing a file with a 'Just in Case' SAIK

'Just in Case' (JIC) kits are sexual assault investigation kits that are distributed to Pathology Queensland (PQ) Laboratories and are used in instances where a patient has disclosed a sexual assault but are not ready to involve police. A forensic examination can be requested "Just in Case" a police complaint may be made at a later date.

The JIC kits include swabs in a tamper evident bag (similar to standard SAIKs), pathology request form, JIC consent form and chain of custody form.

The JIC kits are registered in AUSLAB (Pathology) by Pathology Queensland and received at Forensic Property Point (FPP), FSS within AUSLAB (Pathology) and electronically tracked.

FSS will hold the JIC kits for 12 months, at which time they will be destroyed if the complaint has not progressed.

If the complaint progresses, the JIC kits will be registered in the Forensic Register (FR) by the Queensland Police Service using a barcode allocated by FPP. This may be different to the Pathology Queensland allocated barcode, as FR cannot currently accept the series 2 ten digit barcodes. The AUSLAB audit trail and notation in the FR will link these barcodes. FPP will enter the delivery officer details as per the initial AUSLAB (Pathology) entry, with appropriate notes regarding the date and time the samples were originally received in the FR. The AUSLAB (Pathology) audit trail will be scanned to the FR. NB. the test code "TRAIL" in AUSLAB will output the entire audit trail for the case into a report.

Testing will proceed through standard examination and analysis within Forensic DNA Analysis.

The consent form, pathology request form and Chain of Custody form will be scanned into the FR.

Refer to https://qheps.health.qld.gov.au/hsq/forensics/response-to-sexual-assault for more information.

11 File compilation

11.1 Suggested order of pages (from top to bottom)

1. Case file particulars page (QIS 34307)
2. Copy of final statement (if written)
3. Most recent printout of casefile notations, emails*
4. Exhibit Register list
5. Reference samples – receipt page then profile
6. QP127 (if available)
7. Examination notes:
    i. Description of item
    ii. Diagrams
8. Photos/photocopies/packaging/envelope images*
9. DNA profiles (EPGs)
10. Statistical calculations (if applicable)#

* these items are not required to be printed if the case is not going to court
# STRmix™ v2.6.0 (and beyond) deconvolution and likelihood Ratio reports are not necessary for casefiles. The PDA page may be substituted as it displays the LRs.

11.2 Page numbering

Only cases that are going to court (Statements of Witness or Evidentiary Certificates) need to be page numbered. Assistance is available from the Administrative Team for page numbering.

1. The Case File Particulars page is always Page 1 (except upon reactivation when the additional Case File Particulars page will be numbered page 1 and the original Case File Particulars page will be renumbered as the next consecutive number in the case file).
2. Case Files are numbered from the back of the case file to the front.
3. Number and initial each page, including the reverse of the page if both sides have been used.
4. Ensure the Case number is recorded on each page.
5. Write the total number of pages on the front of the case file and initial and date as indicated.

For those cases that aren't going to court, the total number of pages simply needs to be counted and noted on the front of the case file, that is, each individual page does not need to be numbered.

11.3 Statement compilation

Refer to QIS 34006 for the correct format for statements or reports issued by Forensic DNA Analysis.

11.4 Preparing a case file for peer review

Prior to submitting a case file for final review or prior to a statement being issued, the following is required:

- Ensure that all items/exhibits have been examined or prioritised appropriately.
- Ensure that appropriate reworks have been performed.
- Establish whether further testing needs to be performed
- Ensure that all samples are finalised
- Samples that have been reported as 'No DNA detected' or 'DNA insufficient for further processing' need to be documented in the case file. This can be done by either printing the PDA page, annotation of the receipt or annotation of the packaging image.
- All profiles have been printed and included in the case file. It is not necessary for EPGs within a casefile to be labelled, instead a copy of the PDA page can be printed to accompany the EPG(s). The PDA page contains all of the sample and interpretation information and can be associated with the EPG via its barcode.
- Ensure that appropriate profiles have been selected for upload to NCIDD. Only one example of each profile is to be loaded to the database.
- Ensure that the reference sample receipt is printed for each evidence sample (AUSLAB only).
- If there are multiple EPGs for a particular reference sample, only the reported profile need be printed and annotated as the final profile.
- Ensure that all evidence samples associated with the case are present.
- STRmix™ printouts for all cases that used this program for statistical calculations. It is not necessary to print the report for STRmix™ v2.6.0 (or beyond) as it contains a large number of pages; a printout of the PDA page and EPG is sufficient.
- For Profiler Plus cases: if a statement has been requested, ensure that profiles requiring a genotype frequency have had the statistical calculation performed through the Kinship program (see QIS 25368) and that the results are printed and included in the file. Any mixture interpretation pages, including Popstats where appropriate, must be included in the casefile.

12 Working Remotely

See QIS 34006 for writing and reviewing statements from a location other than at work (eg. working from home).

In these situations, printed casefiles with all contents may not be necessary unless a court requirement eventuates. Casefiles will be needed to be created to contain, at the very least, the hard-copy of the Statement of Witness to enable tracking to occur in the FR.

At times where actions are performed (or not performed) that differ to the standard approach to casefile compilation, these actions should be recorded as casefile notations in the FR.

13 Case file management off-site

When case files are required for court appearances they should be tracked to the Reporting Scientist in the FR.

If a file is taken off-site (in exceptional circumstances eg. flight for court evidence outside Brisbane), then a casefile notation should be added to the FR to detail this occurrence.

14 Reactivated cases and case requiring updated interpretations and testing in other labs

14.1 Reactivated and Cold Case Management

On occasion, some cases require further work after they have been finalised and reviewed. In compiling cases that were previously managed with AUSLAB, it is recommended to print UR notes and any associated communications for the reactivated case, and commence tracking within the FR (QIS 33773).

An assessment of previously reported and uploaded profiles should be undertaken. In July 2007, it was decided (in conjunction with QPS) that all crime scene profiles (except Known Deceased and complainants in sexual assault cases) would be uploaded. Prior to this any crime scene sample that matched a complainant profile for any case type was uploaded to NCIDD.

New evidence samples received for a case which has been profiled using Profiler Plus will be profiled using PP21. It should be discussed with a Senior Scientist or Team Leader and in consultation with DNA Management as to whether the case is transitioned to PP21 profiling.

Any interstate person samples submitted for analysis by the DNA Management Section (QPS) that have been obtained from people located interstate are to be treated as Evidence samples (as per advice from the QPS).

If a case is reactivated for attention, a Request/Task is usually sent to the Team Leader. The case may already have been allocated to an existing staff member or can be considered for allocation to a new case manager.

The reactivation may be for a number of reasons including, but not limited to:

- Check into property holdings at FSS;
- Check into any remnants of testing still held at FSS (ie. spin baskets, extracts);
- Check into what volumes of extracts may remain for consideration of profiling at FSS, or at an external facility;
- Seeking advice on potential for external testing (extract volume and reference sample dependent);
- Request for a copy of the casefile as held at FSS (QIS 34248).

If samples were quantified prior to 04 November, 2015, they would not have been processed with Quant-Trio. These samples would benefit from a re-Quant with Quant-Trio so that the indicators of Degradation and Y-Quant are obtained.

If new samples are received for these Cold Cases, these are usually accompanied by a request for ‘Quant and Hold’ (see QIS 33773 and 34006).

In some instances, it may be possible upon consultation with QPS Homicide Cold Case Investigation Team Forensic Co-Ordinator to pool samples from the same parent item. Consideration of whether to pool prior to profiling, or after profiling can be discussed. DNA profiling of the sample/s may be before, or after a microcon post-extraction step. Pooling samples may hinder the ability to obtain a usable DNA profile if one sample is complex, or has raised a Quality Flag.

14.2 Testing in other laboratories

Consideration of further profiling interstate or overseas can be made:

- Highly sensitive DNA profiling, using Minifiler and LCN technology, may assist degraded or low-level DNA profiles. The Institute of Environmental Science and Research (ESR) in New Zealand offers this testing.

- Y-STR profiling is performed in most other Australian jurisdictions, and in New Zealand. This technology may be useful if there are male reference DNA profiles, and the DNA profile has a quant value associated to the Y-Quant from Quant-Trio.

- Mitochondrial DNA profiling may be useful if the sample is likely to be single-sourced. This technology is useful for samples that are highly degraded or aged eg. recovered skeletal remains. Currently, Victorian Institute of Forensic Medicine (VIFM) offer this profiling service. This technology may be useful if there are males or females from the same maternal lineage.

If testing for certain samples has been approved to be conducted in other jurisdictions, the appropriate discussions and authorisations with QPS DNA Management should be retained in the FR.

Approvals and packaging process is outlined in QIS 30917.

If a casework sample is processed in another jurisdiction, it should be reported in a statement by that testing laboratory. Reference sample data (including EPG) may be requested by this reporting jurisdiction, which can be sent via DNA Management Unit.

If a casework sample is processed in QLD and Reference sample data is received from another jurisdiction, this should be reported to DNA Management Unit via Intelligence Report.

15 Records

1. Case file records – the location of paper case files is recorded in the FR, or for pre-FR cases, this is recorded in AUSLAB.
2. Paperless case examination notes - all but the current folder is stored in Block 3 Reporting.
3. Batch paper records - Filing Storage area (room 6112) or the Exhibit Room (room 6106)
4. DAD-Prior to AUSLAB Batch Functionality, all results obtained were loaded into an Excel spreadsheet known as DNAmaster. In 2008 these results were transferred to the DNA Analysis Database (DAD).
5. AUSLAB
6. Electropherogram pdf/jpeg files for samples:
    - Genotyper profiles are located in J:\User3100\Results Finalised\PRE-LIMS and I:\User3100\AAARESULTS FINALISED\POST-LIMS
    - As of the 16th February 2009, results have been analysed using GeneMapper ID-X. GeneMapper ID-X profiles are located in P:\Profile PDFs and only accessible from computers with GeneMapper ID-X installed (contains all DNA profile results from 16th February 2009 until June 2012).

- As of July 2012, all DNA profile results are located in O:\Profile PDFs (accessible from all network PCs).

7. STRmix™ result files are stored on a network drive - I:\STRmix Results\

16 Associated Documentation

QIS: 17168 – Procedure for Single Source DNA Profile Statistics

QIS: 23968 – Forensic DNA Analysis Communications Procedure

QIS: 25302 – Interpretation of Mixed DNA (STR) Profiles using Profiler Plus

QIS: 25368 – Kinship Software – Genotype Frequency Module

QIS: 25581 – Kinship Software - Paired Kinship and Paternity Trio/Missing Child Modules

QIS 30917 – Forensic DNA Analysis – Procedure for external transfer of samples and subsamples

QIS: 32139 - STRmix™ Report macro

QIS: 33744 – Forensic Register Training Manual

QIS: 33773 – Procedure for Profile Data Analysis using the Forensic Register

QIS: 34006 – Procedure for Release of Results using the Forensic Register

QIS: 34045 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit.

QIS 34307 – Forensic DNA Analysis - Case File Particulars

QIS: 34112 – STR Fragment Analysis of PowerPlex 21 profiles using GeneMapper ID-X software – FR

QIS: 34229 - Explanations of Exhibit Results for FR

QIS: 34245 – Reference Sample Result Management

QIS: 34246 – Uploading and Actioning on NCIDD - FR

QIS: 34248 - Administrative Team - Case File related duties using the Forensic Register

QIS 34308 – Procedure for Intelligence Reports and Interstate/Interpol Requests in the Forensic Register.

QIS 34322 – Technical and Administrative Review of Records Created in the Forensic Register

QIS 35007 – Use of STRmix v2.7.0 software

QIS 35008 – Allele specific stutter threshold worksheet

QIS 35406 – STRmix Stutter Calculator

17 References

Brisotto P, Ryan L, & Scott K. (2020). Observed Reduction in Volume Post-PCR May 2020.

Caunt E, Morgan R, Gardam T, Howes J & Allen C. (2014) Verification and implementation of STRmix™ V2.0.1

Caunt E, Morgan, R, Howes, J & Allen, C. (2015) Assessment of the Number of Contributors for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis_version 2

Caunt E, McNevin A, Howes J & Allen, C. (2018) Interpretation of four person mixtures using STRmix v2.0.6

Caunt E, McNevin A, Howes J & Allen, C. (2018) Validation of STRmix™ V2.6.0.

Caunt E, Pattison H, McNevin A, Howes J & Allen, C. (2019) Validation of STRmix™ V2.7.0.

McNevin A, Caunt E & Allen C. (2019) Verification of STRmix™ V2.6.2.

Morgan, R., & Caunt E. (2015) Development of Guidelines for Mixed PowerPlex® 21 DNA Profiles within Forensic DNA Analysis_version 2 (Change Management #149).

National Association of Testing Authorities (NATA). Refer to NATA website: http://www.nata.com.au

Nurthen T, Mathieson M & Allen C. (2013) Amplification of Extracted DNA validation v2.0

Nurthen T., Mathieson M., Scott K. & Allen C. (2012) PowerPlex® 21-Direct Amplification of Reference FTA® samples validation.

Parry R, Caunt, E & Allen C. (2012) Verification of the DNA profile module of STRmix™ using the Promega PowerPlex® 21 system.

Parry R, Caunt, E & Allen C. (2013) Verification of the DNA profile module of STRmix™ for Full Volume Amplifications using the Promega PowerPlex® 21 system.

Parry R, Howes J, & Allen C. (2014) The determination of the threshold number of alleles, above which single source DNA profiles can confidently be ascribed a likelihood ratio in excess of 100 billion.

Parry R, Caunt E, & Lloyd A. (2020) 4p Mixture Discussion Paper

Police Powers and Responsibilities Act 2000, Current as of 22 September 2014

Police Powers and Responsibilities Regulation 2012, Current as of 22 September 2014

18 Amendment History

Revision	Date	Updated By	Amendments
1	11 Nov 1998	V Ientile	
2	28 Mar 2001	V Ientile	
3	11 Jun 2001	V Ientile	
4	18 Jul 2001	V Ientile	
5	08 Jan 2002	V Ientile	9(3) – Completed case codes for FACTS
6	21 Nov 2002	V Ientile	Changes to section 9, completing a case
7	19 Nov 2003	V Ientile L Freney	Refer to AUSLAB. Remove FACTS in many places
8	07 Jun 2005	M Gardam	Included requirements for paperwork in case file ie No loose pages
9	03 Aug 2006	M Gardam	List of reference articles added
10	25 Sep 2006	M Gardam	Off site case file management, compilation of case file, case management.
11	13 Feb 2007	L Weston	Update with processes for AUSLAB
12	Apr 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
Version	**Date**	**Updated by**	**Amendments**
12	10 Apr 2008	J Connell	Transferred section on preparing case file for presumptive EXR/EXH validation to Examination of Items SOP
13	12 Feb 2009	K Lee	Major rewrite; Inserted subheadings and table of contents; changed order of information to reflect current processes; expanded on reworking information and other processes undertaken as part of case management; added information regarding dilutions and requesting processing of samples sub-sampled in analytical; summarised finalisation requirements for samples with extra barcodes; added examples for entering final EXR lines. Hyperlinked associated documents.
14	28 Oct 2009	K Lee	Updated with reference to GeneMapper *ID-X* software; changed “Pre/Post LIMS” references

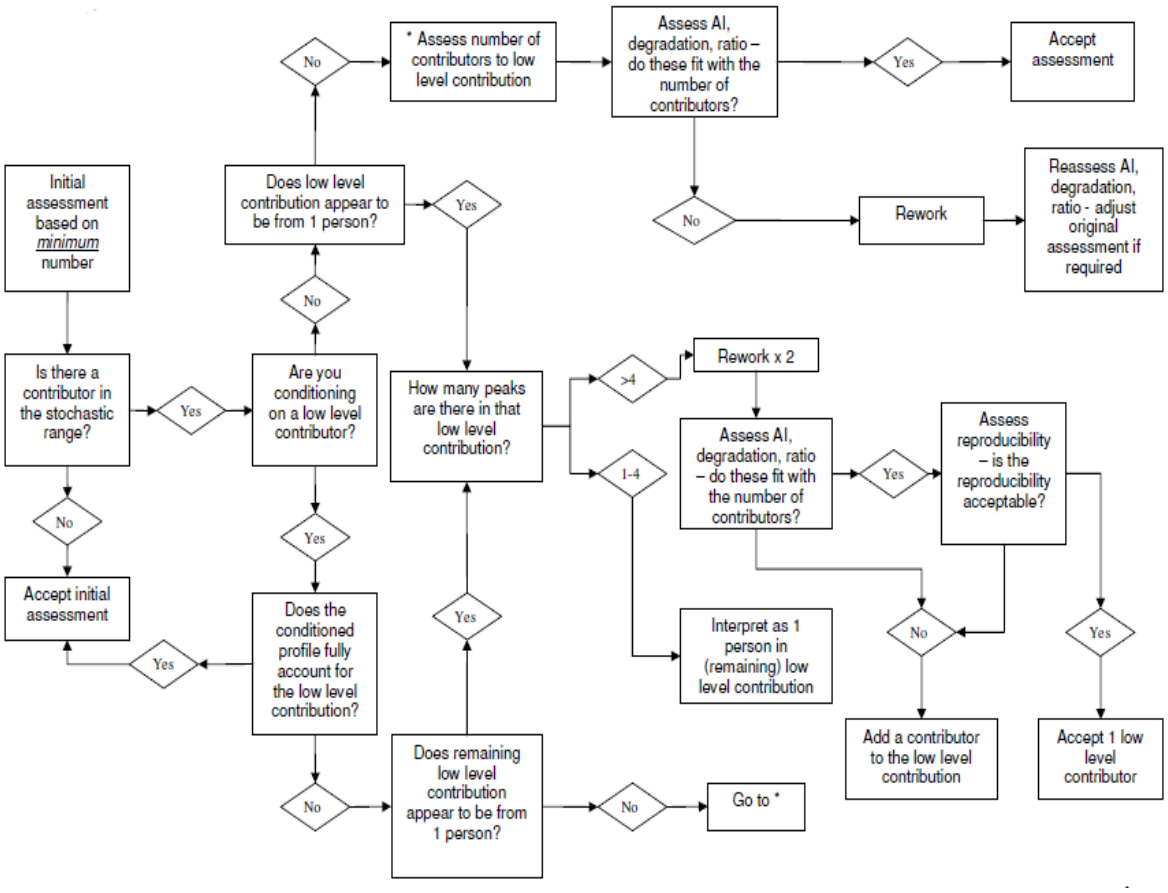
			to “Pre/Post AUSLAB Batch Functionality”; removed unnecessary flow charts; updated hyperlinks and associated documents; introduced paperless case management; re-arranged for better flow and grammatical correctness; Introduced more definitions; included instruction on locating profiles for printing.
15	27 Jan 2012	K Pippia	Introduced new worklists; added section on reworking evidence samples; added VOLUND process; addressed changes in processes since last update; removed references to re-Genescanning and introduced references to re-reads; updated hyperlinks; addressed comments raised against last revision; updated FBNLR process
16	12 Nov 2012	Alicia Quartermain, Emma Caunt, Justin Howes	Updated all processes to include implementation of PowerPlex®21 and STRmix™
17	Jan 2015	Thomas Nurthen	Incorporation of updated workflows, major rewrite , New template
18	August 2015	Thomas Nurthen	Fixed typos, referenced new document for number of contributors, additional steps for FBNLR process, added NCIDD removal process, updated STRmix versions, NCIDD load requirements
19	07 April 2017	Justin Howes	Changed example on p41 to [9, NR]; added information to 5.4 regarding strmix instructions; added eg Profiler Plus to PP21 to 9.3; section 6.3.6 – added info on Profiler Plus and microcon instructions; changed LOD Quant from 0.00214ng/uL to 0.001ng/uL; added information to 6.5.3 re incorrects; added first line to Table 6;added information to 6.2.5 on no further work process; added Appendix 3 – Intuitive Exclusion Guide and details to 6.4.1.2; changed 19977 to 33407; fixed title of 24126 and hyperlinking throughout; edited amendment history versions/revisions to align with QIS.
20	24 December 2018	Justin Howes	Major revision due to implementation of FR and other new SOPs (for the FR).
21	17 February	Justin Howes	Updated definition list; changed

	2021		EXH to result; changed statswg to statsPWG; added 35406 and 35008 to associated docs and details to 6.3; updated title of no. contributors guidelines document; added details to 6.3.1; 6.3.4 edited to remove the requirement for reamps;added authorisations to 6.3.6; removed App 17.2 (intuitive exclusion guide); replaced 're-run' with re-CE'; added 35007 and 30917 to assoc docs, removed 31523; removed details on no. iterations for STRmix in 6.4.1.2; edited the title of mixed profiles to include four-person mixtures; added Sections on remote, cold cases and off-site; added info on broad peaks to 6.3.3; 6.5.2 added info on further processing; added information on increasing iterations; removed 17038 and replaced with 34307; added reference to Intel Report template for amended results in 6.5; updated formatting, added information to section 4.4 and removed numbers; edited 11.1 to remove AUSLAB references; removed checklist (was App 19.1); added contributors workflow to appendix; added reworking strategies to appendix; add information to 6.3.6 and 6.1.1, updated reference list, updated workding in 6.4.1.2; added section 6.5.7, edited wording in section 12 (remote working),6.1.1 and 6.5.3.

19 Appendices

1 Determination of Number of Contributors workflow
2 Considerations in assessing samples for reworks

19.1 Determination of Number of Contributors workflow



19.2 Considerations in assessing samples for reworks

Reworks are required for case work samples for several reasons including optimisation of profiles, confirming information and assessing the impact of quality issues.

Below is a brief set of options to consider when deciding to rework a sample and choosing an appropriate rework strategy. This set of options will not cover every scenario and each sample should be considered on its own merit and within its own case. Samples may exhibit more than one issue that might warrant a rework. In this case select the one that will overcome the majority of issues in one go for maximum efficiency.

Problem/Profile Type	Rework Strategy/Considerations
Quality Issue noted in Batch Notes - Reduced Volume Post PCR - Other batch issue affecting the sample	Refer to the Report on Observed Reduction in Volume Post-PCR (Brisotto et al 2020). The wells commonly affected are A01, A012, H01 and H012. A reduced remaining volume may impact on the rework able to be ordered. If a suboptimal amplification (amp) is obtained due to reduced amp volumes, consider a re-quantification (quant) or re-amp as an appropriate strategy. Only rework if necessary in order to confirm a profile after a quality issue has been found to impact the sample. The best rework strategy will be dependent on the issue affecting the batch and the possible implications of the batch issue itself. Consider that re-extracting the spin basket may be best option. If the profile is considered unsuitable for interpretation, a rework or re-extraction may not assist. Consult a Senior Scientist if in doubt.
Quantification - Quant issue	If the profile seems inconsistent with the quant value or if the quant value is unexpected given other results or testing (such as numerous spermatozoa present), consider a re-quant as the best option. A profile with an inaccurate quant might be able to be identified in a sample with a strong quant with low degradation however with a poor quality or low level profile. Check the quant batch to assess the IPCCt value. A particularly low value (< 27) can be a contributing factor as this does not flag (as it does if it is a high IPCCt). If IPCCt value is low and degradation high, a re-quant should be ordered. If the IPCCt value appears to be low, a Nucleospin clean-up is still an available option for reworking.

	Note that Quantification of samples is only an estimation of the amount of DNA present within a sample and the true value can vary. A re-quant will use less extract and is more likely to obtain an accurate profile. Microconning a sample with an incorrect quant value can consume the entire extract and potentially obtain an uninformative profile that is unsuitable for interpretation.
- Low quant	A profile displaying limited information due to the low level of DNA present might benefit from a re-amp at maximum volume. If the sample has already been amplified at the maximum volume, consider concentrating the sample via microcon to 35ul (a microcon to full can be a helpful option for low level single source profiles). When considering a microcon, bear in mind that the optimal amplification DNA input is approximately 500pg or 0.033ng/ul quant value. A sample with a quant value less than 0.03 is more likely to benefit from a microcon. The presence of multiple peaks at loci in a low quant profile does not in itself mean that the microconned profile will be complex, it could lead to a clean mixed profile that might be interpreted. This should be considered within the case context.
CE issues	
- Poor Baseline and/or Pull Up	A profile with an unclean baseline can create difficulty in interpretation particularly if pull-up is interfering with true alleles and causing uncertainty as to the number of contributors to the profile. A re-CE is the best first option. A re-amp might be useful if the re-CE doesn't fix the issue.
- Artefacts such as ULPs or VARs etc.	It is no longer policy within DNA Analysis to confirm unlabelled peaks or variant alleles unless there are questions raised as to their accuracy. A re-CE can confirm whether they are truly present however a re-amp will confirm the allele designations.
- Broad Peaks	Broad peaks are peaks considered to be wider than standard. Broad peaks can interfere with STRmix™ deconvolutions of mixed profiles. A mixed DNA profile with labelled broad peaks will require a re-CE before being processed through STRmix™. A re-CE is preferable due to reduced costs and faster turn arounds however a re-amp is a second alternative. If the profile is considered complex or unsuitable for

	interpretation, a rework is not necessary. Note that a single source profile displaying broad peaks that also requires STRmix™ deconvolution does not necessarily require a rework. This is because STRmix™ will assign the broad peaks correctly to the one contributor without much penalty. If the sample has broad peaks and is not being reworked, add a sample note on the PDA page that broad peaks have been observed however are not affecting the overall interpretation.
Degradation	Degradation of a sample can vary from nil to extreme. The greater the degradation, the less the certainty of the interpretation or number of contributors to the profile. Degradation can be identified by taking the quant value into account along with the severity of the slopes of peaks from left to right of the profile. Provided inhibition has not been detected (low/high IPCCt value), re-amplifying using above optimal volume input (but below what might saturate the amplification) may assist. If the Degradation Index is significant, consider if the IPCCt value is appearing satisfactory. A re-quant may be necessary.
Amplification Issues - Preferential Amplification	Preferential amplification is noted by the ski slope effect from left to right across the profile in conjunction with an indication of degradation as per the Degradation Index. Whilst this is relatively rare within casework samples, it can be negated by re-amplifying at slightly lower volumes than previous.
- Poor Amplification	Poor amplifications might occur for a number of reasons including bad injections or pipetting issues. They can generally be identified after a good quality profile followed by a poor quality profile after a re-amp. First consider a re-CE or else re-amp at the same volume. A poor amp can be used for information but may not be particularly useful as part of a STRmix™ deconvolution.

Determination of Number of Contributors	
- Single Source Profiles	Consider that single source profiles only require 12 alleles and preferably as many P+ alleles as possible to be loaded to NCIDD. Therefore a partial single source may not require reworking depending on the sample and case. If the profile is low level and falls within the stochastic range, a re-amp might be beneficial to confirm any high stutters or potentially interfering sub threshold information.
- Two Contributor Profiles	Refer to the Number of Contributor Guidelines (Morgan R and Caunt E, 2015 – Change Management #149) for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility. However if both contributors are clearly present across all loci, there may be no need to rework unless the profile is within stochastic range or STRmix™ might have a better chance at deconvolution with extra runs.
- Three Contributor Profiles	Refer to the Number of Contributor Guidelines for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility. If a profile is assessed as 3 contributors, a re-amp might help to assess if drop out has occurred.
- Four Contributor Profiles	Refer to the Number of Contributor Guidelines for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility
- Uncertain Contributor Profiles	Refer to the Number of Contributor Guidelines for reworking to determine the number of contributors to a profile. In general terms, re-amps are the most appropriate rework for reproducibility. Two additional re-amps (if necessary) are considered appropriate.
- Complex profiles	Complex profiles should not be reworked unless it is considered that the profile is complex due to other amplification or quantification issues.
- General Mixed profiles	There is NO NEED to rework a profile unless there is good reason to do so. Consider the risks of doing so. Does the number of contributors assessed correlate with the appearance of the profile, rather than just counting the number of peaks? If not, consider a rework to see if an extra contributor might be involved or to allow STRmix™ more certainty. Remember that

	the assumption of the number of contributors to a mixed profile is the minimum number of contributors to reasonably explain the DNA profile. Note that the Number of Contributor Guidelines are GUIDELINES ONLY and interpretation can occur without added reworks.

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DNA Extraction and Quantification of Samples Using the QIAsymphony® SP and AS Modules

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1 Purpose and Scope

This document outlines the automated procedures for extracting DNA from reference and casework samples using the QIAsymphony® DNA Investigator® Kit (QIAGEN Group, 40724 Hilden, DE). The automated procedure utilises the QIAsymphony® SP module. The QIAsymphony® SP instrument in combination with the QIAsymphony® DNA Investigator® Kit allows extraction from a range of cell types and substrates including: blood, cells, tissue, fingernails, hair, FTA cards, tapelifts, cigarette butts, chewing gum, fabric, swabs, paper, plastic, wood, bone, teeth, etc.

In the event of an instrument error or when a run is cancelled, a 'clean-up' procedure is performed. This is where samples are manually recovered, and the extraction process finalised using the manual DNA IQ method.

An 'Integrated Run' is the automated extraction procedure that is followed by a quantification assay set-up.

This procedure applies to all staff members who are required to lyse and extract DNA from samples using the automated QIAsymphony® SP module, and quantify samples using the QIAsymphony® AS module.

2 Definitions

Adaptors	Support tiles specifically designed for the QIAsymphony® instrument to insert reagents and consumables that are placed on the worktable
ARTEL	Is the Dual–Dye Ratiometric Photometry method utilising colourimetric analysis to assess pipetting performance of the QIAsymphony® AS module.
AS	Abbreviation for 'Assay Set-up' and refers to the AS module of the QIAsymphony® instrument
ATE	Buffer included in the QIAsymphony® DNA Investigator Kit used during the QIAsymphony run (contained within the Carrier RNA and within the reagent cartridge)
ATL	Buffer included in the QIAsymphony® DNA Investigator Kit used during the pre-treatment of samples
AVE	Buffer included in the QIAsymphony® DNA Investigator Kit that is **not required** and discarded appropriately.
Carrier RNA	Reagent included in QIAsymphony® DNA Investigator Kit used during the QIAsymphony run (contained within the enzyme racks)
Channels	Are the piston-driven pipetting mechanism used for liquid transfer during a procedure. Channels are numbered 1-4 from the back of the instrument to the front
Data files	Information of a batch run which includes system log files, report files, loading information files and rack files
DTT	1,4 Dithiothreitol
Extracts	Samples that have undergone DNA extraction
FR	Forensic Register
Lysates	Samples that have undergone pre-lysis but not DNA extraction
Magnetic particles	Particles included in the QIAsymphony® DNA Investigator Kit which bind and unbind DNA through the application of magnetic forces with the assistance buffers
PCR	Polymerase chain reaction
Pro K	Proteinase K
Protocol	An automated method created by QIAGEN® used to perform a run. A Protocol includes all information regarding a run including steps, movement, reagents, consumables, as well as reagent volumes to be added

QIAsymphony®	The QIAsymphony® Instrument which includes both SP and AS modules
QIAsymphony® AS	The QIAsymphony® Instrument 'Assay Set up' module that performs the quantification assay set-up of extracted samples
QIAsymphony® DNA Investigator Kit	The kit used in conjunction with the QIAsymphony® SP for the extraction and purification of casework and reference samples
QIAsymphony® SP	The QIAsymphony® Instrument 'Sample Preparation' module that performs the final stages of extraction and purification of lysed samples
QS	Abbreviation for 'QIAsymphony®'
Quantifiler® Trio Kit	The DNA Quantification reagents utilised for the assay set-up on the AS module
Samples	Sample substrates in tubes awaiting a DNA extraction process
SP	Abbreviation for 'Sample Preparation' and refers to the SP module of the QIAsymphony® instrument
Worktable	The platform on the instrument where disposable tips, reagent cartridges, tubes and other labware are positioned

3 Principle

3.1 QIAsymphony® SP Module

DNA extractions are performed on casework and reference samples using the QIAsymphony® SP module located in Room 3191.

The QIAsymphony® SP module is a fully automated modular system which enables the automated purification of DNA of up to 96 forensic casework or reference samples on a single run. It uses pre-programmed protocols with the QIAsymphony® DNA Investigator Kit – utilising cartridge-based magnetic-particle technology. The instrument's integrated components include a barcode reader, lysis station, a 4-channel pipetting system, robotic gripper and an array of magnetic rods.

The instrument is operated and controlled via software through an integrated touchscreen located at the front of the QIAsymphony® SP module. As an alternative, a PC with QIAsymphony® Management Console V4.0 software installed may be connected to the QIAsymphony® instrument to extend its capabilities.

All necessary information required for a run, including consumable and reagent movements, pipetting volumes and locations are all hard coded into custom-designed protocols created by QIAGEN.

3.2 QIAsymphony® AS Module

Samples that have been extracted using the QIAsymphony® SP can progress through to an assay set up using the QIAsymphony® AS module located in Room 3191.

The QIAsymphony® AS module is a fully automated modular system which enables the quantification assay set up of extracted samples using the Quantifiler® Trio DNA Quantification Kit (QIS 34045).

An 'Integrated Run' on the AS module enables the quantification of samples immediately after extraction on the SP module. An 'Integrated Run' uses a pre-programmed protocol, combining the sample preparation protocol (extraction) and the assay set-up (quantification).

3.3 QIAsymphony® DNA Investigator Kit

The QIAsymphony® DNA Investigator Kit (QIAGEN Group, 40724 Hilden, DE) is designed for automated purification of total DNA from varying substrates. The magnetic particle-based technology provides high-quality DNA, which is suitable for direct use in downstream applications, such as quantitative PCR and STR analysis.

The kit must be stored at room temperature (15-25 °C) and when stored appropriately, the kit is stable and viable until the registered expiration date on the kit.

The kit also contains a ready-to-use Proteinase K solution that can also be stored at room temperature. However, for extended use, storage at 2-8 °C is recommended.

Reagents for purification of DNA are contained in the QIAsymphony® DNA Investigator Reagent Cartridge. Each trough of the reagent cartridge contains a particular reagent, such as magnetic particles, lysis buffer, wash buffer, or elution buffer. Opened and partially used reagent cartridges can be stored for a maximum of two weeks at room temperature when re-sealed with the supplied re-usable seal strips - enabling cost effective use of reagents.

The QIAsymphony® DNA Investigator Kit includes the following components:

- 2 x 50 mL bottles of Buffer ATL
- 3 x vials of Proteinase K solution (20 mg/mL)
- 1 x lyophilized carrier RNA
- 2 x enzyme racks
- 1 x 20 mL bottle of Buffer ATE
- 2 x reagent cartridges
- 1 x 20 mL bottle of Buffer AVE (not required for the extraction protocol)

QIAsymphony® technology combines the speed and efficiency of silica-based DNA purification with the convenient handling of magnetic particles. The purification procedure is designed to ensure safe and reproducible handling of crime scene forensic samples. This extraction method comprises of 4 stages: lysing (during the pre-lysis process), binding, washing and eluting.

A modified version of the QIAGEN sample pre-lysis and automated extraction protocol was utilised and validated in-house.

The lysing stage is performed using a pre-lysis procedure validated using the Buffer ATL, Proteinase K and 1,4 Dithiothreitol (prepared in-house). The Buffer ATL contains chaotropic salts, required for DNA binding to the magnetic beads.

The Proteinase K solution possesses highly specific activity that remains stable over a wide range of temperatures and pH ranges that has substantially increased activity at higher temperatures. Proteinase K is a recombinant protein expressed in *Pichia pastoris* and is particularly suitable for short digestion times. Proteinase K solution is added to the extraction buffer to aid in the digestion of cellular material to release DNA from within cells. It also digests proteins that interfere with the DNA binding capacity of the resin and rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

1,4 Dithiothreitol is a reducing agent also used in extractions to break disulfide bonds of proteins.

The kit is supplied with lyophilized carrier RNA to be used in the QIAsymphony® DNA Investigator Casework protocols. 1 x vial of Carrier RNA and 2 x enzyme racks are supplied

with each QIAsymphony® DNA Investigator kit. Carrier RNA enhances the binding of DNA to the magnetic beads, which is essential for samples containing very few target molecules. The carrier RNA is dissolved using the Buffer ATE included in the kit. Dissolved carrier RNA is stable for 4 weeks when stored at 2-8 °C and for longer periods when stored at -20 °C.

The magnetic particles are the foundation of the QIAsymphony® DNA Investigator Kit which contains novel paramagnetic particles covered with silica. The magnetic particles have a negative charge at basic and near neutral pH. The addition of the lysate, containing Buffer ATL, alters the pH and salt concentration which subsequently alters the magnetic beads from a negative to a positive charge, enabling free DNA in solution to bind to the magnetic beads. The addition of the QSL3, QSW2, the magnetic particles, the carrier RNA and the samples to the sample prep cartridges constitutes the binding stage of the extraction process.

Inhibitors and cellular debris are removed directly after the binding stage and during the washing stages of the extraction process which includes the addition of QSW1 and QSW2. The first washing step is performed using the QSW2 wash buffer (binding step), which ensures the DNA is bound to the magnetic bead resin and to wash out inhibitors. The next three washing steps involve using QSW1 and QSW2 wash buffer.

The Buffer ATE changes the pH and salt concentration of the solution. Combined with heating – set in the extraction protocol - the DNA is released from the magnetic particles. This constitutes the eluting stage of the extraction process.

4 Reagents, Equipment and Consumables

4.1 Reagents

Table 1 outlines all the reagents and their storage locations required for the pre-lysis, SP extraction and integrated procedures.

Table 1: Reagents with storage room and location

Reagent	Room	Location
70 % v/v ethanol	3188 / 3189 / 3191	Sink/ Reagent bottles container/ Bench
0.5 % v/v CleanTech Bleach	3188 / 3189 / 3191	Sink/ Reagent bottles container/ Bench
5 % v/v Trigene Advance	3188 / 3189 / 3191	Sink/ Reagent bottles container/ Bench
DNA Investigator Kit Buffer ATE	3188	In-use tray or shelf
DNA Investigator Kit Buffer ATL	3188	In-use tray or shelf
DNA Investigator Kit Carrier RNA	3188	In fridge or freezer in 3188
DNA Investigator Kit Enzyme Rack	3188, 3191	In-use shelf / fridge in 3191
DNA Investigator Kit Proteinase K	3188	Proteinase K reagent box in fridge
DNA Investigator Kit Reagent Cartridge	3188 / 3191	Bench or in-use shelf / bench
*QIAGEN Proteinase K 10mL	3188	Fridge
*QIAGEN Carrier RNA 310mg	3188	Shelf
DTT (1M)	3188	Freezer
Quantifiler® Trio reagents	3188	Fridge/Freezer
Quantifiler® Trio standards	3194	Fridge in Room 3194
Amp grade water aliquot	3194	Bench in Room 3194

***Note:** QIAGEN Proteinase K and Carrier RNA are purchased individually if required. If an additional Carrier RNA tube is required, notify the Analytical HP5.

4.1.1 Pre-lysis Buffer

Prepare pre-lysis buffer in the biosafety cabinet in room 3188 prior to commencing the pre-lysis procedure.

1. Refer to Table 2 to determine the required reagent volumes for the pre-lysis buffer.

Table 2: Reagent volumes for pre-lysis procedure

Extraction Buffer	**Volume per sample (µL)**	**Volume 24 samples (µL)***
Buffer ATL	450	11700
QIAGEN Proteinase K	25	650
DTT	25	650

***Note: Extra reagent has been factored into calculation (extra 2 samples).**
****Note: If QIAGEN Pro K is not available the validated in-house Pro K may be used.**

2. Gently mix the Buffer ATL by swirling **(do not shake)**. Check for precipitation by lifting the bottle up against the light. If precipitation is present, sit the bottle (closed) in lukewarm water until completely dissolved.

3. Remove aliquot of DTT from the freezer and thaw. Vortex and centrifuge before use.

4. Remove the QIAGEN Proteinase K solution from the fridge, vortex mix and briefly centrifuge before use.

5. Aliquot 11700uL of Buffer ATL into a 50 mL falcon tube.

6. Add 650uL of QIAGEN Proteinase K and 650uL DTT to the Buffer ATL and invert gently to mix.

7. Label with **"Pre-lysis Buffer"**, initial and date.

4.1.2 Carrier RNA

Note: Prepared Carrier RNA is stored in the fridge in Room 3188, check if Carrier RNA is required to be prepared.

Carrier RNA tubes should be prepared in the biological safety cabinet in room 3188.

1. Gently mix the Buffer ATE by swirling **(do not shake)**. Check for precipitation by lifting the bottle up against the light. If precipitation is present, sit the bottle (closed) in lukewarm water until completely dissolved.

2. Add 1.6 mL of Buffer ATE to the Carrier RNA tube. Vortex until dissolved and briefly centrifuge.

Note: Final volume of Carrier RNA must be exactly 1.6 mL.

3. Affix its corresponding pre-printed label showing lot details on the tube, and initial and date the tube if required to store remaining contents.

Note: One prepared tube of Carrier RNA is sufficient for the two reagent cartridges contained in the kit. The dissolved Carrier RNA is stable for 4 weeks when stored between 2-8 °C. For longer periods, store the vial of carrier RNA at -20 °C.

4.1.3 Enzyme Rack

Note: Check if an enzyme rack is required to be prepared. Check by looking for prepared enzyme racks (from previous extractions) in Room 3191 or in the QIAsymphony electronic diary.

Enzyme racks should be prepared in the biological safety cabinet in room 3188 prior to commencing the extraction protocol on the QIAsymphony® SP.

1. Gently mix the Buffer ATE by swirling (**do not shake)**. Check for precipitation by lifting the bottle up against the light. If precipitation is present, sit the bottle (closed) in lukewarm water until completely dissolved.

2. Uncap the two tubes in the enzyme rack and add 400 µL of the prepared Carrier RNA into each tube.

Note: **If the enzyme rack is from a new kit, the Carrier RNA will need to be prepared, refer to Section 4.1.2.**

3. Add 1.2 mL of Buffer ATE to each tube of the enzyme rack and gently pipette mix several times.

4. Affix its corresponding pre-printed labels showing lot details for the Enzyme rack, Carrier RNA and ATE. Initial and date the enzyme rack.

5. Store any remaining Carrier RNA in the fridge.

4.1.4 Reagent Cartridge

Note: Check if a reagent cartridge is required to be prepared. Check by looking for prepared reagent cartridges (from previous extractions) in Room 3191 or in the QIAsymphony electronic diary. New reagent cartridges are stored in Room 3188.

In use reagent cartridges are stored in Room 3191.

1. Remove the reagent cartridge from the packaging. Ensure that its corresponding pre-printed label showing lot details has been affixed to the Reagent Cartridge.

2. Check for any visible precipitation in the reagent troughs of the cartridge by lifting it up against the light. If precipitation is present, partly submerge reagent cartridge in luke-warm water for approxiamtely 30 minutes.

3. Place the reagent cartridge into the reagent cartridge holder (Figure 1).

Figure 1: Reagent Cartridge being insterted into the reagent cartridge holder

4. Check that the 2D barcodes on reagent cartridge(s) and enzyme racks(s) ae present and intact. If required, use a Kimwipe and wipe the barcodes.

5. Remove the trough of magnetic particles and check that the sealed correctly (Figure 2).

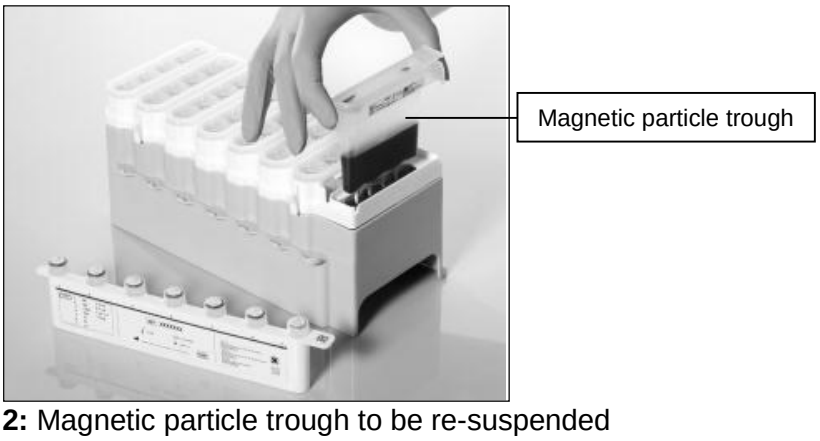

Figure 2: Magnetic particle trough to be re-suspended

Warning: The magnetic particles may cause an allergic skin reaction. Handle with care and wear appropriate PPE.

6. Vigorously vortex the magnetic particle trough, then shake using the Mixmate for 3 minutes at 1000 rpm.

7. Place back into the reagent cartridge frame and carefully remove the seal.

8. Uncap the two Carrier RNA tubes of the corresponding enzyme rack and insert onto the reagent cartridge (Figure 3).

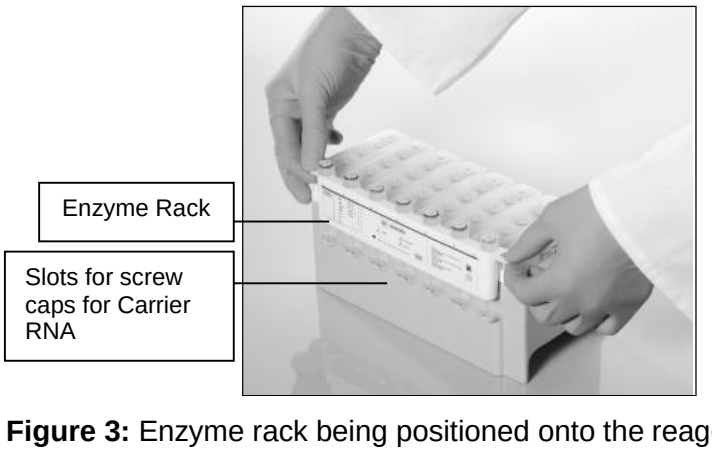

Figure 3: Enzyme rack being positioned onto the reagent cartridge holder

9. If using a new reagent cartridge, place a piercing lid on top of the reagent cartridge in the correct orientation (Figure 4). Carefully push from both ends to ensure it has clicked into place.

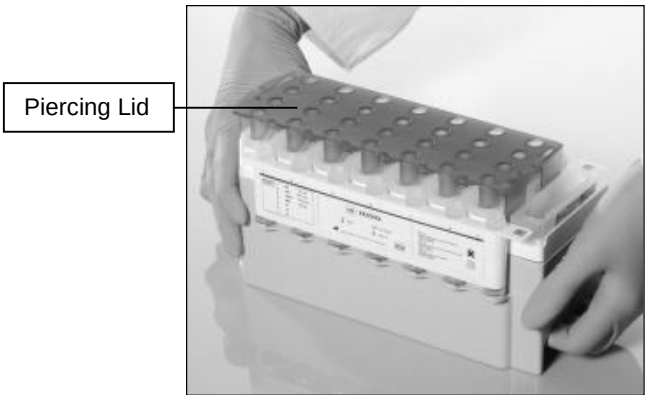

Figure 4: Piercing lid being positioned onto the reagent cartridge holder

Note: To avoid evaporation, the reagent cartridge should only be open for a maximum of 15 hours (including run times) at a maximum environmental temperature of 30°C.

4.1.5 Quantifiler® Trio Reagents

The QIAsymphony® AS module requests a minimum amount of Quantifiler® Trio master mix. This amount (1688 µL) can be found by selecting on the Eluate and Reagent drawer of the AS module and selecting the master mix tube.

Prepare the master mix using number of samples equalling 94 (or 93) as per Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit (QIS 34045).

Prepared Quant Trio standards are stored in the Pre-PCR fridge (Room 3194). When required, obtain the Quant Trio standards and prepare as per Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit (QIS 34045).

4.2 Equipment

Table 3 outlines the equipment and its location for the pre-lysis, SP extraction and integrated procedures.

Table 3: Location of required equipment

Equipment	Location	Procedure
QIAsymphony® SP module (A & B)	3191	SP Extraction, Integrated
QIAsymphony® AS module (A & B)	3191	Integrated
STORstar (A)	3190	SP Extraction, Integrated
STORstar (B)	3194	SP Extraction, Integrated
LabElite® Integrated I.D. Capper™	3191	SP Extraction, Integrated
Labogene Scanspeed 1248	3191	Integrated
Fridge	3189, 3191	Pre-lysis, SP Extraction, Integrated
Freezers	3189 / 3190	Pre-lysis
Biological safety cabinets class II	3189	Pre-lysis
96 well tube racks	3189	SP Extraction, Integrated
Vortex	3189 / 3191	Pre-lysis, SP Extraction, Integrated
Centrifuge	3189 / 3191	Pre-lysis, SP Extraction, Integrated
Mini/Micro centrifuges	3189	Pre-lysis
Pipettes 100 – 1000 µL	3189	Pre-lysis
Thermomixers	3189	Pre-lysis
Magnetic rack	3189	Sample recovery method
Mixmate	3191	SP Extraction, Integrated

4.3 Consumables

Table 4 outlines the consumables and their locations for the pre-lysis, SP extraction and integrated procedures.

Table 4: Consumables and locations for each procedure

Consumables	Location	Procedure
1500 µL QIAGEN Filter tips (32 per rack)	3191	SP Extraction, Integrated
200 µL QIAGEN Filter tips (32 per rack)	3191	SP Extraction, Integrated
50 µL QIAGEN Filter tips (32 per rack)	3191	Integrated
2 mL SSI sterile tubes	3188 / 3189	Pre-lysis
2 mL QIAGEN tubes	3191	Integrated
Spin basket assemblies	3189	Pre-lysis
50 mL sterile Falcon tubes	3188 / 3189	Pre-lysis
200 µL & 1000 µL filtered pipette tips	3188 / 3189	Pre-lysis
Sample Prep Cartridges (28 per box)	3191	SP Extraction, Integrated
8-Rod Covers (12 per box)	3191	SP Extraction, Integrated
Inoculation sticks	3189	Pre-lysis
Nunc™ tubes	3189	SP Extraction
Sharps bin	3188 / 3189	Pre-lysis
96-well optical plate	3191 / 3194	Integrated

Additional consumables can be found in the Store Rooms 3184 and 6105.

4.4 Entering Reagents, Equipment, Consumables and Locations into FR

1. Access the batch as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).
2. Select the **Edit/Update Batch** icon.
3. Using the relevant dropdown menus, select the correct consumables and reagents.
4. Scan the equipment and location barcodes into the required fields (Figure 5).
5. Select the **Save** Batch icon.

Note: Fields should be filled out contemporaneously while processing the batch. These steps can be performed at any stage prior to batch completion and entries can be modified after saving.

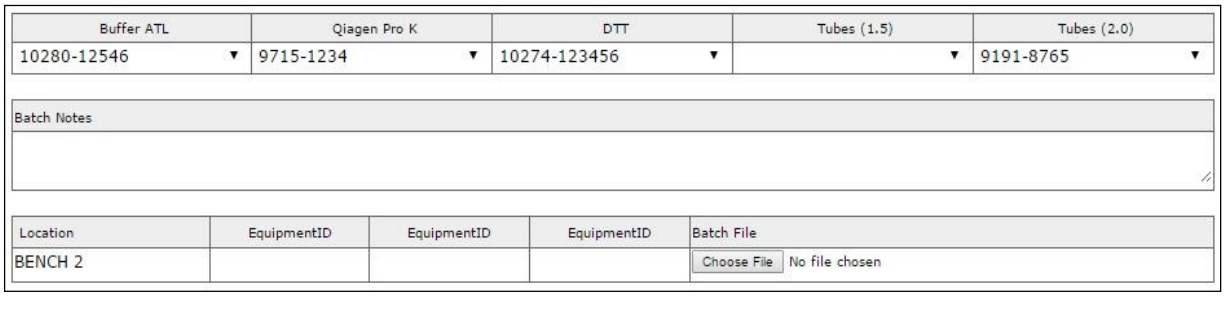

Figure 5: Entering batch details

5 Safety

As the Anti-Contamination Procedure (QIS 22857), PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

Labware and biosafety cabinets are wiped with 5 % v/v Trigene **only**, followed by 70 % v/v ethanol.

Centrifuges are cleaned/rinsed weekly with 5 % v/v Decon, followed by nanopore water and then followed by final rinse with 70 % v/v ethanol.

Work benches and non-metallic equipment should be wiped with 0.5 % v/v bleach, followed by 70 % v/v ethanol.

Warning: Reagents contained in the Buffer ATL are irritants and hazardous. DTT is a reducing agent that destroys disulfide bonds. Wash Buffers are flammable and hazardous. Handle carefully and wear appropriate PPE. Wash Buffer used in the Manual DNA IQ method is not recommended to be prepared / handled by pregnant women.

The contents of liquid waste bottles are to be disposed of in a Waste collection container / bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If a spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin.

Do not wipe areas of the waste container with bleach as the reagents may produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when cleaning up spills. If any reagents or contents of the waste container are spilt onto PPE (eg gowns), remove and discard in a biohazard bin.

6 Sample Location and Batch Preparation

6.1 FR Workflow Diary and Electronic Diary

Batches that require processing can be found in the FR Workflow diary tab (refer to the Forensic DNA Analysis Workflow Procedure (QIS 34034).

The electronic QIAsymphony instrument diary (I:\AAA Electronic Workflow Diary\AAA QIAsymphony Diary) is used to record daily use of the instruments. Maintenance, batches processed, and any issues are recorded in the diary.

6.2 Batch Creation

Create extraction batches as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

Note: QIAsymphony® SP extraction batches ≤4 pre-lysis batches
QIAsymphony® integrated batches 3 pre-lysis batches
QIAsymphony® SP extraction URGENT batches with 1 pre-lysis batch for those batches that contains sample/s that have been upgraded to Priority 1.

6.3 QC Samples

One negative control and one positive control will be automatically registered by the FR when creating pre-lysis batches as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

Duplicates of the Quantifiler® standards and a reagent blank will be automatically allocated by the FR when creating the quantification batch for the QIAsymphony® integrated procedure according to the Forensic DNA Analysis Workflow Procedure (QIS 34034).

6.4 Sample Location and Locating Samples

Samples required for the pre-lysis procedure are found in the extraction sorting room (3190) freezers and in the fridge in Room 3189 once the pre-lysis batches are prepared. Samples ready for the extraction on the QIAsymphony® instrument are found in the fridge in the auto extraction room 3191 (Table 5).

Table 5: Sample storage locations

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples required for pre-lysis batches	Freezer	3190 / Block 6 walk-in freezer*
Samples required for extraction batches	Fridge / Freezer	3191 / 3190

***** Some medium and low priority storage boxes are located in the Exhibit Room (6106)

Locate samples as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

6.5 Analytical Notes

1. Access the batch in FR as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

2. On the batch **Exhibit Analysis** page, check samples for analytical notes (orange semi-circle). For each analytical note, hover the cursor over sample to check the analytical note (e.g. Extract and hold, NWQPS) (Figure 6).

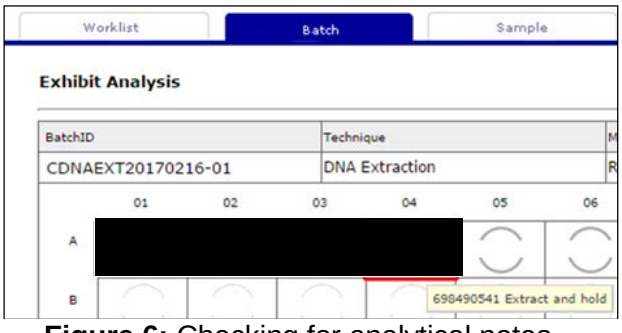

Figure 6: Checking for analytical notes

6.6 Creating Subsamples and Printing Labels

For Pre-Lysis Batches:

1. In FR, enter into the Pre-lysis batch.

2. On the batch **Exhibit Analysis** page in FR, select the **Subsamples** icon to create additional barcodes for samples requiring a spin basket label (substrates that need to be stored after exrtaction).

Note: Substrates that require a spin basket include absorbant substrates such as swabs and fabric. Substrates that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles. Small absorbant substrates such as paper and cigarette butt papers can be spun in spin baskets at the operators discretion.

3. For samples that do not require substrate storage (eg. negative extraction controls or hair), uncheck the corresponding **SPIN** box (Figure 7).

4. Select **Create Subsamples** icon (Figure 7).

Note: Once subsamples have been created and saved, this option is no longer available. If you require to create a spin basket label or substrate after saving, this will need to be done following Miscellaneous Tasks SOP QIS 34064.

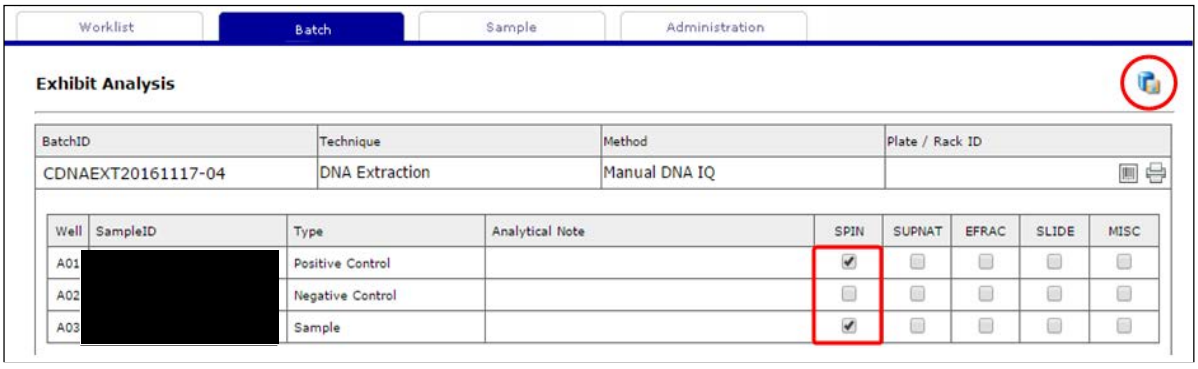

Figure 7: Creating substrate storage barcodes

5. Select the **Sample Labels** icon to display the sample labels. Print the labels to the designated label printer.

Note: When labelling 2.0 mL tubes for pre-lysis, ensure to adhere the label so the first number is closest to the tube cap, the label is applied squarely and on the smooth surface close to the top of the tube. This will ensure effective scanning in later processes on the QIAsymphony®.

For QIAsymphony® Integrated Batches:

1. In FR, enter into the created quantification batch.

2. Select the **BatchID Label** icon to display the batch label. Print the label to the designated printer. This label will be required for labelling the 96 well optical plate.

6.7 Sequence Checking

6.7.1 Pre-lysis Batches

1. To perform a sequence check of all tubes for the batch, select the **Sequence Check & Lock** icon.

2. Scan all tubes in the order they are positioned in the rack corresponding with the FR plate map. The parent barcode must be the first barcode scanned.

Note: If a barcode is scanned incorrectly during the sequence check an error message will appear above the virtual rack. Check the position that the error message indicates and rescan with the correct barcode.

3. Complete the sequence check by clicking on the **Confirm Sequence Check** box at the bottom right hand side. The **Confirm Sequence Check** box should not be checked if any errors shown on the sequence check screen.

4. Select the **Lock Batch** icon.

6.7.2 QIAsymphony® Extraction Batch Lysate Tubes

1. Allow lysates to come to room temperature to ensure there is no precipitation.

2. Vortex and centrifuge all lysates at 10000rpm for 30 seconds.

3. Load the 2.0 mL lysates tubes into the correct colour-coded and numbered sample tube carriers as per Step 29 in Section 7.3 SP Extraction Procedure.

4. Access the batch in FR and select the **Sequence Check and Lock** icon.

5. Scan all tubes in the order they are positioned in the tube carriers corresponding with the plate map. If an error message appears, clear the error and rescan the highlighted box with the correct barcode.

Note: If more than one sample tube carrier is to be loaded onto a QIAsymphony® SP platform for the same run, it is critical that each carrier of samples is scanned into the sequence check and immediately loaded onto the platform before the subsequent sample tube carrier is scanned into the sequence check. This ensures the carriers are loaded in the correct order on the instrument.

6. Once all sample tube carriers have been scanned and loaded, complete the sequence check by clicking on the **Confirm Sequence Check** box at the bottom right hand side. The **Confirm Sequence Check** box should not be checked if any errors shown on the sequence check screen.

7. Select the **Lock Batch** icon.

6.7.3 QIAsymphony® Extraction Batch Nunc™ Tubes

Follow instructions as per Procedure for the use of the STORstar Unit for Automated Sequence Checking using Forensic Register (QIS 34042) **with the exception of Sequence checking and locking the batch through FR** (as the Sequence check and locking of the batch through FR is performed by the QIAsymphony operator).

1. Ensure that they output log has been uploaded to FR.

2. Check the first and last Nunc™ tube sample number against the batch in FR and ensure they are in the correct orientation.

6.7.4 Quantification Batch from QIAsymphony® Integrated Run

A quantification batch that is created from a QIAsymphony® Integrated batch is sequence checked during the loading the AS module.

1. Using the QIAsymphony touchscreen, select the blue Slot 2 [2. Samples] icon.

2. Select **[List view]** on the righthand side to view a list of the loaded samples IDs.

8. Access the quantification batch in FR.

9. Compare the labelled Optical PCR plate with the Quantification Batch ID in FR and ensure that they match.

10. Compare the sample IDs loaded onto the QIAsymphony to the quantification batch in FR and ensure that they match.

11. To enter the sequence check in FR, select the **Sequence Check & Lock** icon.

12. Complete the sequence check by clicking on the **Confirm Sequence Check** box (bottom left hand side), followed by the **Lock Batch** icon.

7 Procedure

7.1 Pre-lysis Procedure

Note: This procedure is used for all sample types excluding bone/teeth.

All pre-lysis procedures are performed within a biological safety cabinet in Room 3189.

1. In the Clean Reagent Room (3188) prepare Pre-lysis Buffer as per Section 4.1.1.
2. Turn on a thermomixer and set temperature to 70°C.
3. Check for Analytical notes as per Section 6.5 and action as required.
4. Create subsamples and print labels as required as per Section 6.6.
5. For each sample label:
   - Original sample tube (use **spin** barcode)
   - Spin basket collection tube assembly as required (**parent** barcode)
   - 2.0 mL SSI tube (**parent** barcode)
   - 1.0 mL Nunc™ tube (**parent** barcode)

Note: If the substrate is to be stored in the original tube post extraction, ensure that the 'SPIN' label is applied over the barcode of the original tube making sure the original tube label number is still visible.

6. Ensure a sequence check is performed as per Section 6.7.1.
7. Add 500 µL of Pre-lysis Buffer to each sample and ensure that substrates are submerged in the buffer.
8. Vortex each sample for 10 seconds.
9. Incubate samples on a thermomixer at 70°C for 45 minutes, shaking at 1400 rpm.
10. Remove samples from the thermomixer. Vortex all samples briefly and pulse spin to remove condensation from the lids.
11. If required, transfer substrates to spin basket or transfer the lysate to an appropriately labelled SSI 2.0 mL tube. Retain the original tubes containing the substrate.

Note: Do not transfer any solid material as this may clog the tips during automated DNA purification.

12. Centrifuge spin baskets at 15800 rcf for 2 minutes.

Note: Final lysate MUST be retained in a 2.0 mL SSI tube. If the original lysis tube is not a 2.0 mL SSI tube (i.e. QPS tube), then pulse spin the original tube and transfer

the lysate to the 2.0 mL SSI tube. The substrate will then be retained in the original lysis tube and lysate pooled into the 2.0 mL SSI tube.

13. Transfer the substrate from the spin basket to its corresponding 2 mL tube and retain. Transfer the flow through back to original lysis tube.

14. **Check all 2 mL lysate tubes to ensure that all contain lysate (no substrates) and that none are labelled with a 'SPIN' barcode.**

15. Store the lysates in temporary storage boxes in the fridge in the automated extraction room (Room 3191) as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

Note: Lysates can be stored for up to 192 hours before being extracted.

16. Store tubes containing substrates in the spin basket or discard boxes located in the Pre-PCR sorting room (3194) freezer as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

17. Store labelled Nunc™ tubes to a temporary Sarstedt box in order and write batch ID as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

18. Proceed to Section 8.1 for batch finalisation.

7.2 Bone Pre-lysis Procedure

Note: This procedure is used <u>ONLY</u> for bone/teeth samples.

All pre-lysis procedures are performed within a biological safety cabinet in Room 3189.

1. In the Clean Reagent Room (3188) prepare Pre-lysis Buffer as per Section 4.1.1.

2. Turn on a thermomixer and set temperature to 56°C.

3. Check for Analytical notes as per Section 6.5 and action as required.

4. Create subsamples and print labels as required as per Section 6.6.

5. For each sample label:
   - Original sample tube (use **spin** barcode)
   - Spin basket collection tube assembly as required (**parent** barcode)
   - 2.0 mL SSI tube (**parent** barcode)
   - 1.0 mL Nunc™ tube (**parent** barcode)

Note: If the substrate is to be stored in the original tube post extraction, ensure that the 'SPIN' label is applied over the barcode of the original tube making sure the original tube label number is still visible.

6. Ensure a sequence check is performed as per Section 6.7.1.

7. Add 500 µL of Pre-lysis Buffer to each sample and ensure that substrates are submerged in the buffer.

8. Vortex each sample for 10 seconds.

9. Incubate samples on a thermomixer at 56°C for overnight (at least 12 hours), shaking at 1400 rpm.

Note: A shorter incubation protocol of 56°C for 5 hours, shaking at 1400 rpm may be used where:

- **there is a large number of samples and/or where time critical processing is requred (i.e for DVIs), or**
- **samples are expected to provide good DNA yields and there is sufficient material for retesting if required.**

Please seek permission from HP5 before using this alternate incubation protocol.

10. Remove samples from the thermomixer. Vortex all samples briefly and pulse spin to remove condensation from the lids.

11. If required, transfer substrates to spin basket or transfer the lysate to an appropriately labelled SSI 2.0 mL tube. Retain the original tubes containing the substrate.

12. Centrifuge spin baskets at 15800 rcf for 2 minutes.

Note: Final lysate MUST be retained in a 2.0 mL SSI tube. If the original lysis tube is not a 2.0 mL SSI tube (i.e. QPS tube), then pulse spin the original tube and transfer the lysate to the 2.0 mL SSI tube. The substrate will then be retained in the original lysis tube and lysate pooled into the 2.0 mL SSI tube.

13. Transfer the substrate from the spin basket to its corresponding 2 mL tube and retain. Transfer the flow through back to original lysis tube.

14. Centrifuge all samples (that contain bone powder) for 3 minutes at the highest speed.

15. Using a 200uL pipette, transfer lysate (top layer only) to an appropriately labelled SSI 2.0 mL tube. Be careful not to disturb the powdered pellet.

Note: Do not transfer any solid material as this may clog the tips during automated DNA purification. A 200uL pipette is used to avoid transfer of any particles.

16. **Check all 2 mL lysate tubes to ensure that all contain lysate (no substrates) and that none are labelled with a 'SPIN' barcode**.

17. Store the lysates in temporary storage boxes in the fridge in the automated extraction room (Room 3191) as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

Note: Bone lysates are to be extracted on the QIAsymphony instrument immediately after the Bone pre-lysis procedure.

18. Store tubes containing substrates in the spin basket or discard boxes located in the Pre-PCR sorting room (3194) freezer as per Storage Guidelines for Forensic DNA Analysis (QIS 23959). Bone powder discarded.

19. Store labelled Nunc™ tubes to a temporary Sarstedt box in order and write batch ID as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

20. Proceed to Section 8.1 for batch finalisation.

7.3 SP Extraction Procedure

1. In the Clean Reagent Room (3188) prepare the Carrier RNA and enzyme rack(s) as per Sections 4.1.2 and 4.1.3.

2. The automated QIAsymphony® SP extraction procedure is performed using the QIAsymphony® SP modules located in Room 3191.

3. Remove the 2 mL lysates tubes from the fridge and allow to come to room temperature prior to commencing the extraction procedure.

Note: Once at room temperature, check lysates tubes to ensure that substrates are not present as it will cause an instrument error/crash.

4. In FR access the **Extraction batch** to be run and enter in the equipment and reagents to be used as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

5. If required, turn on the QIAsymphony® SP. Initialisation will take a few minutes.

6. Log into the QIAsymphony® SP module using your operator username and password.

7. Check that the daily maintenance has been performed.

8. Select **Sample Preparation**.

9. Open the 'Waste' drawer, check that it has been cleaned and full waste unit boxes replaced.

10. Close the 'Waste' drawer and select **Scan** to start the inventory scan.

11. Once scanning is complete, a prompt will appear, select **OK**.

12. Ensure that the Nunc™ extract tube rack has the correct **batch ID** with rack**.** Additionally, check the barcodes of the first and last samples in the rack to match against the batch in FR.

Note: An automated sequence check of the Nunc™ extract tubes using the STORstar instrument must be performed prior to the run as per Section 6.7.3.

13. Decap the Nunc™ extract tubes using the LabElite® Integrated I.D. Capper™.

14. Place the rack of tubes in the correct orientation on the appropriate adaptor including the transport frame, again cross-checking the first and last samples on the rack to FR.

15. Open the 'Eluate' drawer and load the plate adaptor holding the Nunc™ tube rack onto slot 1 with the barcode on the plate adaptor facing the front.

Note: The transport frame is required for Integrated batches, but is also used for the SP extraction procedure. Ensure that the transport frame hook is at the back and the plate adaptor barcode is at the front.

16. Select **Elution Slot 1** on the touchscreen, this position will highlight **blue**. The **Racks** icon will also be highlighted indicating that a rack type is required to be selected.

17. From the **Available rack types** panel, select the **Deep well** option.

18. Select **NU#374078 *Racked tubes 96** from the drop down menu. The **Elution Slot 1** will highlight in **orange** and the Nunc™ tube type will appear in this slot.

19. Select **Rack ID** on the right side and scan or manually enter the extraction batch ID.

20. Select **OK**.This will direct back to the **Eluate Drawer** screen, showing the entered Rack ID with the **Elution Slot 1** highlighted **blue**.

21. Close the 'Eluate' drawer and select **OK** to start the inventory scan.

22. Open the 'Reagents and Consumables' drawer.

23. Add the required amount of tips, sample preparation cartridges and rod covers. Refer to Table 6 for quantities of consumables required.

Table 6: Consumables required for SP Extraction Procedure

Batch Size	24 samples (1 x 24)	72 samples (3 x 24)	96 samples (4 x 24)
200 µL disposable filter-tips	28 (1 x rack)	84 (3 x racks)	122 (4 x racks)
1500 µL disposable filter-tips	52 (3 x racks)	156 (7 x racks)	208 (9 x racks)
Sample Prep Cartridges	15 (1 x unit box)	45 (2 x unit boxes)	60 (3 x unit boxes)
8-Rod Covers	3 (1 x unit box)	9 (1 x unit box)	12 (1 x unit box)

Note: When using a used reagent cartridge the instrument requires an additional two 200 µL and seven 1500 µL tips per reagent cartridge to measure the reagents. If using used reagent cartridge(s), add an additional rack of tips per tip type.

24. Prepare the required reagent cartridge/s as per Section 4.1.4.

25. Ensure that there is enough reagents remaining in the cartridges. For previously opened cartridges, the number of samples remaining should be noted on the cartridge. If not, scan the reagent cartridge. The instrument will measure the reagent cartridge and a sample calculation can then be performed.

26. Place the reagent cartridge/s onto the worktable.

Note: Place the used (or lowest number of samples remaining) reagent cartridge into position 1 (closest to the back).

27. Close the 'Reagent and Consumables' drawer and start the inventory scan. When the inventory scanning prompt appears on the screen:
    Select **yes** for:
    - Tips Racks
    - Unit Boxes
    - Reagents

    Select **No** for:
    - Buffer Bottle
    - Accessory trough

28. Select **Scan**. This may take some time.

29. Vortex mix and centrifuge the samples (lysates) at 10000rpm for 30 seconds in the batch and place them into the correct 'numbered' and 'colour coded' sample tube carrier in the same order as per the FR plate map. **Check that substrates are not present in the lysates and ensure all tubes are 2.0 mL SSI tubes.**

Note: The sample tube carriers must contain the 3B inserts specific for the 2.0 mL tubes.

30. Sequence check the samples in the first sample tube carrier as per Section 6.7.2.

Note: If more than one sample tube carrier is to be loaded onto a QIAsymphony® SP platform for the same run, it is critical that each carrier of samples is scanned into the sequence check and immediately loaded onto the platform before the subsequent sample tube carrier is scanned into the sequence check. This ensures the carriers are loaded in the correct order on the instrument as they appear in FR.

31. Remove lysate tube lids from the sequence checked carrier and place on a clean KimWipe.

32. To load the first tube carrier containing lysates, place the sample tube carrier onto the start line of the chosen slot ensuring that the number and colour between the slots and the sample tube carriers match.

33. Wait for the **green** light to flash, then slide the carrier into the slot smoothly until it is all the way in. A **SP Batch** icon highlighted in blue will appear in the Sample Preparation screen as the first batch.

Note: An error will appear if the carrier has been loaded too quickly or not smoothly. In this case, remove the rack and re-insert. If all positions are red, logging back in into the instrument may be required.

34. Select the **SP Batch** icon to go to the **SP|Batch 1|Define samples** screen.

35. Check that the sample tube carrier has scanned successfully.

Note: Samples highlighted in blue indicate that the tube carrier inserts and sample barcodes have both scanned successfully. Samples highlighted in orange indicate that barcodes have scanned unsuccessfully. Samples highlighted in dark orange indicate that both the tube carrier insert and/or sample barcode have scanned unsuccessfully.

36. If samples have scanned unsuccessfully, either remove the sample tube carrier and re-insert or enter the sample information manually. There are two options available:
    a. To manually enter the sample barcodes:
        - Select **ID/Type** button on the right side of the screen.
        - Select the sample that requires changes and select **Sample ID**.
        - Manually edit the sample ID. Select **Next.**
        - Select **Tubes**, the default tube type will appear **SSI_FIX_#2341-SO T2.0 ScrewSkirt**

    b. Alternatively, the QIAsymphony can generate a unique sample ID:
        - Select to highlight all samples that have scanned unsuccessfully.
        - Select **Generate ID**, a prompt will appear, select **OK**.
        - Select **Tubes**, the default tube type will appear

Note: A hand symbol will appear next to the sample ID when manually edited.

37. Check that the positions of the sample barcodes loaded and visible on the QIAsymphony screen, corresponds to the same positions and sample barcodes on the FR plate map. Additionally, check the barcodes of the first and last samples in the rack

to match against the batch in FR. If the instrument fails to scan these 2 barcodes, cross check another 2 barcodes from this carrier.

38. Check that the default tube type (**SSI_FIX_#2341-SO T2.0 ScrewSkirt**) is correct and select **Next**.

39. If the correct tube type has not been automatically assigned, it can be selcted by:
    - Select **Select All** on the right side to highlight all samples.
    - From the **Inserts/tube type** drop down menu, under the **Tube Insert 3B** menu, select **SSI_FIX_#2341-SO T2.0 ScrewSkirt**.

40. On the **Assign assay** screen, select **Select All** to highlight all samples.

41. From the **Applications/ACS** panel, select **Customs Protocol** followed by **CW500ADV CR21344 ID1647 V5** from the drop down menu.

42. Select **Next**.

43. On the **Sample Preparation | Elution slot and volume** screen. All batches that have been inserted with their allocated Batch IDs will be displayed.

44. Select the Batch that have just been inserted to highlight it **dark blue**.

45. Ensure that the eluate volume is set a 100 and select **Queue**.

46. On the **Sample Preparation** screen, the batch will have changed colour to **green** to indicate it has been queued.

47. Repeat Steps 31-46 to load subsequent sample tube carriers required one at time.

48. Select **Run** to start the extraction batch.

Note: If there are insufficient consumables/reagents, it will flag at this point. Open the 'Reagents and Consumable' drawer and add the required consumables. Close the drawer and Scan when prompted.

7.4 Completing a SP Extraction Procedure

1. Once the run is complete, the blue lights on the instrument will flash and the 'Eluate' drawer will also flash yellow.

2. Open the 'Eluate' drawer and remove the eluate rack, adaptor and the Nunc™ tubes containing the extracts.

3. Re-cap the Nunc™ tube extracts using the LabElite® Integrated I.D. Capper™ and store in the upright freezer in the Pre-PCR sorting room (Room 3194 A).

4. Clean the 'Eluate' drawer and adaptors with 70 % Ethanol.

5. Close the drawer, select **slot 1** on the eluate screen and select **Remove** (from the **Configure** tab).

6. A prompt will appear asking if the batch is to be removed. Select **Yes**.

7. Select **OK** to scan the eluate drawer.

8. Select the **Reagents and Consumables** icon on the bottom of the screen.

9. Select **Sample Calc** on the right hand side of the screen.

10. From the **Applications/ACD** drop-down menu, select **Custom Protocol**.

11. Select the protocol **CW500ADV CR21344 ID1647 V5**. An automated calculation of the consumables and reagents remaining on the deck will be performed.

12. Take note of the number of samples remaining for the reagent cartridge and select **OK**.

13. Open the 'Reagents and Consumables' drawer and remove the reagent cartridge/s.

14. Remove the enzyme rack/s from the reagent cartridge/s and re-cap the enzyme tubes.

15. Tightly seal all troughs of the reagent cartridge with the 'Reuse Seal set' (Figure 8).

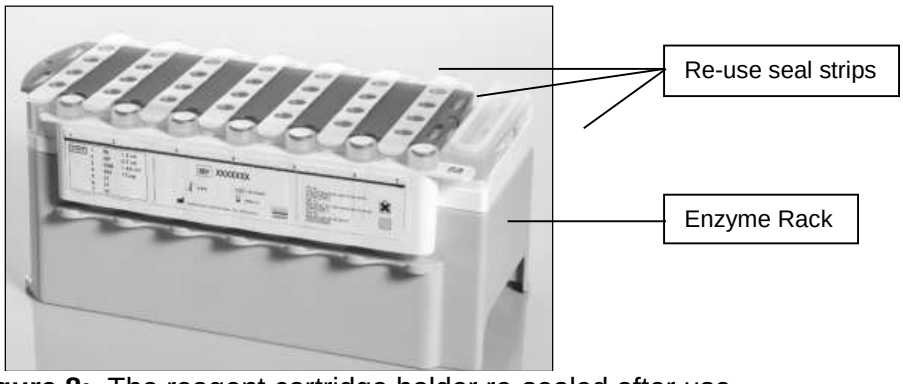

Figure 8: The reagent cartridge holder re-sealed after use

16. Remove the reagent cartridge from the reagent cartridge holder and place it in the white reagent cartridge base.

17. On the front of both the enzyme rack and the reagent cartridge, write the number of samples remaining for that cartridge (from Step 11), initial and date.

18. If the enzyme rack has samples remaining, store in the fridge (Room 3191). If no samples are remaining, discard in a biohazard bin.

19. If the reagent cartridge has samples remaining, store at room temperature (bench in Room 3191). If no samples are remaining, discard in a biohazard bin.

20. Wipe the reagent cartridge holder/s with 70 % Ethanol and store in the cupboard.

21. Remove the partially used unit boxes. Re-cap unit boxes and write the number of consumables remaining on the box.

22. Discard empty tip racks. Replace partially used tip racks into the tip boxes.

23. Wipe the 'Reagents and Consumables' drawer and worktable with 70 % Ethanol.

24. Close the drawer. The scanning inventory window will appear, select **No, nothing changed**.

25. Remove the sample tube carrier 1 from the 'Sample' drawer. Removing these will automatically clear the run from the instrument.

26. Check sample numbers 1 and 24 against the FR extraction batch screen.

27. Repeat Steps 25 and 26 for the subsequent sample tube carriers.

28. Enter a batch entry under the extraction batch in FR stating that sample numbers have been checked against those displayed in FR.

Note: If sample tube carriers are removed during processing of the batch (prior to completion), select the SP Batch icon and then select Remove to clear the run from the instrument.

29. Check that all lysate tubes are empty before discarding into a biohazard waste bin.

30. If any lysate remains in the 2.0 mL tube, recap the tube for it to be stored. To store the reamining a lysate a new barcode needs to be registered in FR, refer to Miscellaneous Analytical Procedures and Tasks (QIS 34064).

    a. In FR, access the **Exhibit Testing Table** page of the sample and click the **Add** button on the **Exhibit Testing / Examinations** table.

    b. Click on the **Process** drop down menu and select **Subsample**.

    c. Click on the **SubType** drop down menu and select **Misc**.

    d. Enter a **new barcode** under **SubID**.

    e. In the **Notes** text box, add a descriptive note and click the **Save** icon.

    f. Select the newly created exhibit testing record entry and then select to display and print the new barcode.

    g. Label the lysate tube with the new barcode and store in the freezer (Room 3194) as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

31. Wipe the 'Sample' drawer with 70 % Ethanol and close the drawer.

32. Wipe the sample tube carriers with 70 % Ethanol and store in the cupboard.

33. Open the 'Waste' drawer and remove any full waste unit boxes. Re-cap boxes and seal with parafilm prior to discarding into biohazard bin.

34. Thoroughly clean the 'Waste' drawer with 70 % Ethanol as items are removed and replaced.

35. Move any partial waste unit boxes into positions 1 and 2 and load empty unit boxes into the empty slots. Ensure that box in **slot 4 is completely empty**.

36. Remove the liquid waste container, transfer the contents into a waste collection container located in the ventilated cupboard in room 3191.

37. Wipe the bottom of the tip chute with 70% v/v ethanol. Remove tip chute and rubber drop catcher from the drawer.

Note: DO NOT USE ethanol on the rubber drop catcher.

38. Remove Tip Park Station from the 'Waste' drawer and soak in nanopure water with 5% v/v TriGene™ followed by rinsing with 70% v/v ethanol. Set aside to air dry.

39. Re-install spare clean liquid waste bottle, tip chute, rubber drop catcher and Tip Park Station into the 'Waste' drawer.

40. Close the 'Waste' drawer, a prompt to scan will appear select **Scan**, to scan the drawer.

41. Remove the Tip guards and soak in nanopure water with 5% v/v TriGene™ (soak the tip guards separately to the Tip Park Station). After soaking, wipe them with 70% v/v ethanol.

42. Re-install spare clean Tips guards.

43. Start the UV light protocol as per Section 5.3 of Operation and Maintenance of the QIAsymphony® SP/AS (QIS 33756).

44. Rinse and then soak the liquid waste container, tip chute and rubber drop catcher using copious amounts of tap water. Soak in tap water with 5% v/v TriGene™, rinse and leave to air dry.

45. The QIAsymphony® is left on during the week and shutdown at the end of the week.

7.5 Integrated Procedure

1. In the Clean Reagent Room (3188) prepare the carrier RNA and enzyme rack(s) as per Sections 4.1.2 and 4.1.3.

2. The QIAsymphony® integrated procedure is performed using the QIAsymphony® SP and AS modules located in Room 3191.

3. If required, turn on the QIAsymphony® SP. Initialisation will take a few minutes.

4. In FR access the **Extraction batch** and enter in the equipment and reagents to be used as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

Note: In FR check that the extraction batch has been created using the Integrated method. If the batch has not been created using the Integrated method, the Quantification batch will not create correctly. Process the batch on the QIAsymphony® using the SP extraction protocol.

5. Create the **Quantification batch** in FR as per in the Forensic DNA Analysis Workflow Procedure (QIS 34034) with the following exceptions as per Figure 9:

    - Create the batch from the **DNA Quantification Batch** section.
    - Use the **Batch / SBox / File** sample source option.
    - Use the batch ID of the QIAsymphony Integrated batch as the **Batch Source / Storage Box**.

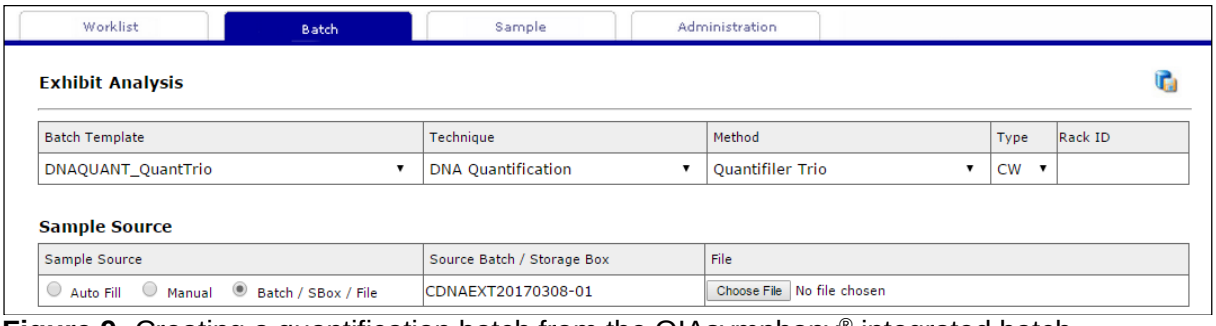

Figure 9: Creating a quantification batch from the QIAsymphony® integrated batch

6. Print the Quantification Batch ID label as per Section 6.6.

7. Log into the QIAsymphony® SP module using your operator username and password.

8. Select the **Integrated Run** Tab.

9. Check that the daily maintenance has been performed.

10. Open the 'Waste' drawer, check that it has been cleaned and full waste unit boxes replaced.

11. Ensure that the Nunc™ extract tube rack has the correct **batch ID** with rack**.** Additionally, check the barcodes of the first and last samples in the rack to match against the batch in FR.

Note: An automated sequence check of the Nunc™ extract tubes using the STORstar instrument must be performed prior to the run as per Section 6.7.3.

12. Decap the Nunc™ extract tubes using the LabElite® Integrated I.D. Capper™.

13. Place the rack of tubes in the correct orientation on the appropriate adaptor including the transport frame, again cross-checking the first and last samples on the rack to FR.

14. Open the 'Eluate' drawer and load the plate adaptor holding the Nunc™ tube rack onto slot 1 with the barcode on the plate adaptor facing the front.

15. **Note: The transport frame is required for Integrated batches. Ensure that the transport frame hook is at the back and the plate adaptor barcode is at the front.**

16. Select **Elution Slot 1** on the touchscreen, this position will highlight **blue**. The **Racks** icon will also be highlighted indicating that a rack type is required to be selected.

17. From the **Available rack types** panel, select the **Deep well** option.

18. Select **NU#374078 *Racked tubes 96** from the drop down menu. The **Elution Slot 1** will highlight in **orange** and the Nunc™ tube type will appear in this slot.

19. Select **Rack ID** on the right side and scan or manually enter the extraction batch ID.

20. Select **OK**.This will direct back to the **Eluate Drawer** screen, showing the entered Rack ID with the **Elution Slot 1** highlighted **blue**.

21. Close the 'Eluate' drawer and select **OK** to start the inventory scan.

22. Open the 'Waste' drawer, check that it has been cleaned and full waste unit boxes replaced.
23. Close the 'Waste' drawer and select **Scan** to start the inventory scan.

24. Open the 'Reagents and Consumables' drawer.

25. Add the required amount of tips, sample preparation cartridges and rod covers. Refer to Table 7 for quantities of consumables required.

Table 7: Consumables required for SP Extraction Procedure

Batch Size	24 samples (1 x 24)	72 samples (3 x 24)	96 samples (4 x 24)
200 µL disposable filter-tips	28 (1 x rack)	84 (3 x racks)	122 (4 x racks)
1500 µL disposable filter-tips	52 (3 x racks)	156 (7 x racks)	208 (9 x racks)
Sample Prep Cartridges	15 (1 x unit box)	45 (2 x unit boxes)	60 (3 x unit boxes)
8-Rod Covers	3 (1 x unit box)	9 (1 x unit box)	12 (1 x unit box)

Note: When using a used reagent cartridge the instrument requires an additional two 200 µL and seven 1500 µL tips per reagent cartridge to measure the reagents. If using used reagent cartridge(s), add an additional rack of tips per tip type.

26. Prepare the required reagent cartridge/s as per Section 4.1.4.

27. Ensure that there is enough reagents remaining in the cartridges. For used cartridges, the number of samples remaining should be noted on the cartridge. If not, scan the reagent cartridge. The instrument will measure the reagent cartridge and a sample calculation can then be performed.

28. Place the reagent cartridge/s onto the worktable.

Note: Place the used (or lowest number of samples remaining) reagent cartridge into position 1 (closest to the back).

29. Close the 'Reagent and Consumables' drawer and start the inventory scan. When the inventory scanning prompt appears on the screen:

Select **yes** for:

- Tips Racks
- Unit Boxes
- Reagents

Select **No** for:

- Buffer Bottle
- Accessory trough

30. Select **Scan**. This may take some time.

31. Vortex mix and pulse spin the samples (lysates) in the batch, 24 samples at a time and place them in the correct 'numbered' and 'colour coded' sample tube carrier, in the same order into as per the FR plate map. **Check that substrates are not present in the lysates and ensure all tubes are 2.0 mL SSI tubes.**

Note: The sample tube carriers must contain the 3B inserts specific for the 2.0 mL tubes.

32. Sequence check the samples in the first sample tube carrier as per Section 6.7.2.

Note: If more than one sample tube carrier is to be loaded onto a QIAsymphony® SP platform for the same run, it is critical that each carrier of samples is scanned into the sequence check and immediately loaded onto the platform before the subsequent sample tube carrier is scanned into the sequence check. This ensures the carriers are loaded in the correct order on the instrument as they appear in FR.

33. Load the sample tube carrier containing lysates by placing the sample tube carrier onto the start line of the chosen slot ensuring that the number and colour between the slots and the sample tube carriers match.

34. Wait for the **green** light to flash, then slide the carrier into the slot smoothly until it is all the way in. A **READY TO DEFINE** icon will appear underneath the associated **SP Carrier** (1, 2, and 3).

Note: An error will appear if the carrier has been loaded too quickly or not smoothly. In this case, remove the rack and re-insert. If all positions are red, logging back in into the instrument may be required.

Note: An error message will appear if a sample tube carrier is loaded when the instrument is scanning the 'Reagent and Consumables' drawer. Select OK on the error message, remove sample tube carrier and wait until scanning is completed prior to loading the sample tube carrier.

35. Select **Define Run**. This will direct to the overview screen for an **Integrated Setup**.

36. Select the required (first) **SP Batch** icon to highlight **grey**.

37. Select **Edit Samples** to check that the sample tube carrier has scanned successfully.

Note: Samples highlighted in blue indicate that the tube carrier inserts and sample barcodes have both scanned successfully. Samples highlighted in orange indicate that barcodes have scanned unsuccessfully. Samples highlighted in dark orange indicate that both the tube carrier insert and/or sample barcode have scanned unsuccessfully.

38. If samples have scanned unsuccessfully, either remove the sample tube carrier and re-insert or enter the sample information manually. There are two options available:
    a. To manually enter the sample barcodes:
        - Select **ID/Type** button on the right side of the screen.
        - Select the sample that requires changes and select **Sample ID**.
        - Manually edit the sample ID. Select **Next.**
        - Select **Tubes**, the default tube type will appear **SSI_FIX_#2341-SO T2.0 ScrewSkirt**
    b. Alternatively, the QIAsymphony can generate a unique sample ID:
        - Select to highlight all samples that have scanned unsuccessfully.
        - Select **Generate ID**, a prompt will appear, select **OK**.
        - Select **Tubes**, the default tube type will appear

Note: A hand symbol will appear next to the sample ID when manually edited.

39. Check that the positions of the sample barcodes loaded and visible on the QIAsymphony screen, corresponds to the same positions and sample barcodes on the FR plate map. Additionally, check the barcodes of the first and last samples in the rack to match against the batch in FR. If the instrument fails to scan these 2 barcodes, cross check another 2 barcodes from this carrier.

40. Check that the default tube type (**SSI_FIX_#2341-SO T2.0 ScrewSkirt**) is correct and select **Next**.

41. If the correct tube type has not been automatically assigned, it can be selcted by:
    - Select **Select All** on the right side to highlight all samples.
    - From the **Inserts/tube type** drop down menu, under the **Tube Insert 3B** menu, select **SSI_FIX_#2341-SO T2.0 ScrewSkirt**.

42. Select **OK**. This will direct to the **Integrated Setup** screen.

43. Select the first **SP Batch** icon again to highlight in **grey** and then select **Define Assays**.

44. On the **Assign Assay** screen, select **Select All** to highlight all samples.

45. From the **Assays** panel, select **Customs Protocol** followed by **IR Quantifiler Trio (4ºC)** from the drop down menu.

46. This will highlight all samples in **green**, and a '**1**' will appear in each sample.

47. Select **OK**. This will direct back to the **Integrated Setup** screen.

48. Repeat Steps 32 – 47 to set up the other SP batches.

49. Select all **SP batches** to highlight in **grey** and select **Create AS batch**. A **1** will appear in the **AS Batch** column against each SP batch.

50. Select **OK**. The AS will now appear as **Queued**.

51. Select **RUN** to start the extraction. A prompt indicating the required time to set up the AS module will appear, select **OK**.

Note: The Integrated run can start (using the the SP) without setting up the AS module. *This delay is recommended to prevent condensation in the Quant Standard tubes, Mastermix tube and optical plate.* A message will appear upon commencement of the run, stating the time at which the AS module is due to commence and when it is required to be set up by.

Note: Setting up the the AS module with reagents and consumables within the appropriate time will prevent the integrated run to pause.

52. Set up the AS module when the SP run is processing the third pre-lysis set.

53. In the Clean Reagent Room (3188) prepare the Quantifiler® Trio reagents as per Section 4.1.5.

54. Prepare the Quantifiler® Trio standards as per Section 4.1.5 and obtain an aliquot of amp grade water.

55. In FR access the **Quantification batch** and enter in the equipment and reagents to be used as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

56. Label an Optical PCR plate with the Quantification Batch ID and place it onto the adaptor (**PCR Plate 96 QS QIAGEN 9020732**).

57. Open both the 'Eluate and Regents' and 'Assay' drawers, this will direct to the **Assay Setup** screen.

Note: Slot 2 will be hightlighted in blue, indicating that no actions are required. This is the position where the eluates will be transferred to after the extraction from the SP module.

58. Perform a sequence check of the quantification batch as per Section 6.7.4. Save the appropriate platemap into I:/ABI Quantifilier.

59. Place the adaptor containing the labelled Optical PCR quantification plate onto Slot 5.

60. Slot 5 **Assay** is highlighted **orange**, indicating that an action is required. Select **Slot 5** to access the **Assay Setup** screen.

61. Select **Rack ID** on the right and then scan or manually enter the Quant batch ID.

62. Select **Load**. Slot 5 has now changed colour to **blue** on the **Assay Setup** screen.

63. Into the 'Eluate and Reagents' drawer, load the required amount of tips that is shown on the screen. (Table 8 outlines the tip types and amounts required).

Table 8: Consumables and reagents required for an integrated run

	3 pre-lysis batches (83 samples total)
50 µL disposable filter-tips	83 (3 x racks)
200 µL disposable filter-tips	8 (1 x rack)
Master mix volume	1688 µL

64. Slot 3 **Reagent** is highlighted **orange** which indicates that an action is required. Select **Slot 3** to move to the **Assay Setup** screen.

65. Position the master mix, amplification water and standards to the adaptor (**Reagent Holder 1 QS QIAGEN 9018090**) as displayed on the screen and uncap all reagent tubes.

Note: Selecting the adaptor positions on the screen will display the required reagent and the minimum volume required in the QIAGEN 2.0 mL tube.

66. Place the adaptor containing all reagents into **Slot 3**.

67. Select **Load**. Slot 3 has now changed colour to **blue** on the **Assay Setup** screen.

68. Close both the 'Eluate and Regents' and 'Assay' drawers.

69. Select **Scan** and then select the consumables that require scanning.

70. Select **Scan** to perform an inventory scan.

Note: If there are insufficient consumables/reagents, it will flag at this point. Open the 'Eluate and Reagents' and/or 'Assay' drawer and add the required consumables. Close the drawer(s) and Scan when prompted.

71. On the **Integrated Run** screen, the batch will display on the AS as **Queued**.

72. Select **Run** to start the batch.

7.6 Completing an Integrated Procedure

1. When the run has completed, the **Assay** drawer icon will be green.

2. Select the green Assay icon, a prompt that the run has completed will appear. Select **OK**.

3. Select the **Integrated Run tab** and then select the **Integrated Batch**. The following prompt will appear: "Shall the complete Integrated batch be removed", select **Yes.**

4. Alternatively, select the **Remove** icon. A prompt will appear advising that the cooling will stop, select **Yes**.

5. Open the 'Assay' drawer and remove the quantification plate.

6. Clean the 'Assay' drawer and adaptor with 70 % Ethanol and then close the drawer.

7. Check that the wells of the quantification plate contain the required volume. Seal the plate with an optical adhesive seal.

8. Centrifuge the quantification plate for 1 minute at 2000 rpm and ensure that bubbles are not present. Place the plate in the pass-through hatch to the CE Room (3194).

Note: Bubbles in the wells may affect the accuracy of the quantification results.

9. Refer to Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit (QIS 34045) for performing the quantifiler run on the QS5 instrument and results analysis and finalisation.

10. Open the 'Eluate and Reagents' drawer, remove the adaptor containing the reagents. Re-cap the quantification standards and return the standards to the Pre-PCR fridge, discard other tubes.

11. Clean the 'Eluate and Reagents' drawer and adaptor with 70 % Ethanol.

12. Close the 'Eluate and Regents' drawer and select **Scan**.

13. Follow steps 2 – 42 of Section 7.4 Completing a SP Extraction Procedure.

7.7 AS module Procedure

A standalone AS procedure is used for ARTEL calibrations (QIS 26628) and for AS module contamination (Soccerball) check batches (refer to Environmental Monitoring procedure QIS 34280). The following procedure is used for the setup of an AS Module contamination check batch.

1. Prepare your 'Sample' tubes as per Environmental Monitoring SOP (QIS 34280).

2. In the Clean Reagent Room (3188) prepare the Quantifiler® Trio reagents as per Section 4.1.5 (use n=106).

3. Prepare the Quantifiler® Trio standards as per Section 4.1.5 and obtain an aliquot of amp grade water.

4. In FR access the **Quantification batch** and enter in the equipment and reagents to be used as per the Forensic DNA Analysis Workflow Procedure (QIS 34034).

5. Ensure that the daily maintenance on the QIAsymphony® has been performed.

6. Select the **Assay Setup** tab at the top of the touch screen.

7. Select **New**. This will direct to the 'Sample rack(s)' screen displaying slot 2 (2 Sample) in **orange**.

8. Select on **Rack ID** on the righthand side of the screen.

9. Enter the Quantification batch ID to name the run (e.g. VDNAQUA20190829-01).

10. Select **Ok**. The entered rack ID will now appear under Slot 2.

11. From the **Available rack types** panel, select the **Deep well** option.

12. From the drop down menu, select **NU#374078 *Racked tubes 96**. This will now appear under the sample position in Slot 2.

13. Once the rack ID and tube type has been assigned, the '2 Sample' position in Slot 2 will be highlighted blue. Select **Next**.

14. The 'Sample Rack Layout' screen will appear. Select positions D2 to H12 to highlight these positions. Select **Sample** to assign these positions as samples. An 'S' will appear in these positions once assigned.

15. Select **Select all** to highlight all samples for the batch.

16. Select **volume** and enter **70uL**.

17. Select **Ok**. The volume assigned will now appear in each sample position.

18. Select **Next**. The assay selection screen will now appear.

19. From the 'available assays' panel on the left hand side of the screen, select **Custom Protocols** and highlight the protocol **Quant Trio (4C)**.

20. Select the **arrow** to transfer the protocol across to the 'selected assays' panel.

21. Select **Next**. This will direct to the 'Assay assignment' screen.

22. Select **Select All** to select all the positions.

23. Select **Assign** to assign the selected protocol previously selected (**Quant Trio (4C)**) to all positions. The positions will now appear in green with a '**1**'.

24. Select **Queue**. This will direct to the 'Loading information' screen displaying the worktable layout for the reagents.

Note: If any of the slots are highlighted orange, information is required for those positions. It will also display the tip types and quantities required for the protocol.

25. Open both the 'Eluate and Regents' and 'Assay' drawers, this will direct to the **Assay Setup** screen.

26. Ensure that the Nunc™ extract tube rack is labelled with the correct **batch ID** on the **front** of the rack**.** Additionally, check the barcodes of the first and last samples on the rack to match against the batch in FR.

27. Decap the Nunc™ extract tubes using the LabElite® Integrated I.D. Capper™.

28. Place the rack of tubes in the correct orientation onto the appropriate adaptor. Ensure that the batch ID of the rack is facing the barcode on the adaptor.

29. Load the plate adaptor holding the Nunc™ tube rack onto slot 2 of the 'Eluate and Regents' drawer with the barcode on the plate adaptor facing the front.

30. On the 'Loading information' screen, select the **[2 Sample]** position.

31. Select **[Load].** The position will now appear blue.

32. Label an Optical PCR plate with the Quantification Batch ID and place it onto the adaptor (**PCR Plate 96 QS QIAGEN 9020732**).

33. Perform a sequence check of the quantification batch as per Section 6.7.4. Save the appropriate platemap into I:/ABI Quantifilier.

34. Place the adaptor containing the labelled Optical PCR quantification plate onto Slot 5.

35. Slot 5 **Assay** is highlighted **orange**, indicating that an action is required. Select **Slot 5** to access the **Assay Setup** screen.

36. Select **Rack ID** on the right and then scan or manually enter the Quant batch ID.

37. Select **Load**. Slot 5 has now changed colour to **blue** on the **Assay Setup** screen.

38. Into the 'Eluate and Reagents' drawer, load the required amount of tips that is shown on the screen. (Refer to Table 8).

39. Slot 3 **Reagent** is highlighted **orange** which indicates that an action is required. Select **Slot 3** to move to the **Assay Setup** screen.

40. Position the master mix, amplification water and standards to the adaptor (**Reagent Holder 1 QS QIAGEN 9018090**) as displayed on the screen and uncap all reagent tubes.

Note: Selecting the adaptor positions on the screen will display the required reagent and the minimum volume required in the QIAGEN 2.0 mL tube.

41. Place the adaptor containing all reagents into **Slot 3**.

42. Select **Load**. Slot 3 has now changed colour to **blue** on the **Assay Setup** screen.

43. Close both the 'Eluate and Regents' and 'Assay' drawers.

44. Select **Scan** and then select the consumables that require scanning.

45. Select **Scan** to perform an inventory scan.

46. Select **Run** to start the batch.

8 Batch Finalisation

8.1 Pre-lysis Procedure

Note: It is not possible to halt processing of a sample once it has been sequence checked (locked) on a pre-lysis batch. The sample must progress to a QIAsymphony extraction procedure.

1. Ensure all reagents, equipment, consumables and locations are selected against the batch as per Section 4.4

2. Select the **Edit/Update Batch** icon and click on the **Run Date & Run Time** field, select the current date from the calender which autofills the time field upon batch completion.

3. Type '**EXT Ready**' in the Comment field (Figure 10).

Run Date	Run Time	Batch Controls / Status	Comment
19/05/2017	14:13	PASS FAIL INV N/R CEQ	EXT ready

Figure 10: Run date and Run Time field and 'EXT ready' in Comment field

4. Select the **Complete Batch** icon to complete the batch.

8.2 SP Extraction Procedure

Note: <u>Do not</u> halt processing a sample once it is on an extraction batch. A sample can only be placed on hold at the sample transition step at the end of the extraction procedure.

1. Ensure all reagents, equipment and consumables are selected against the batch as per Section 4.4.

2. Select **Sample Transition** icon and select the appropriate technique and method from the dropdown menus (Figure 11). For samples that have an **Extract & Hold** or **NWQPS** analytical note, the **Technique** and **Method** fields are changed to be blank.

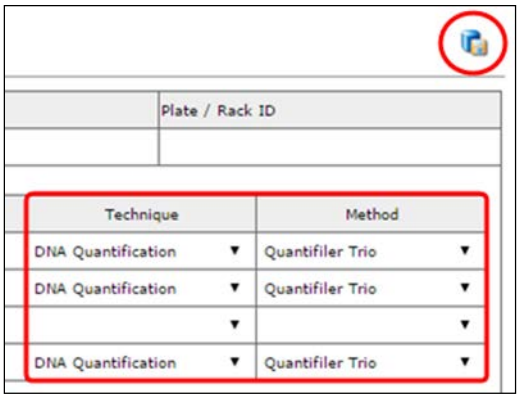

Figure 11: Checking for processing comments and sample transition

3. Select the **Save Batch** icon.

4. Select the **Edit/Update Batch** icon and click on the **Run Date & Run Time** field, select the current date from the calender which autofills the time field upon batch completion.

5. Select **PASS** in the Batch Controls/Status field (Figure 12).

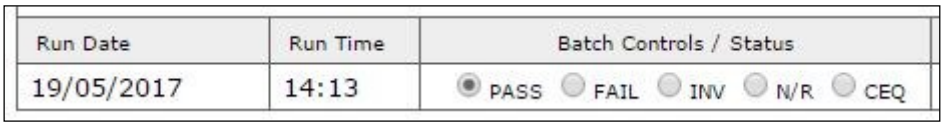

Figure 12: Run date & Time field and changing batch status to “PASS”

6. Remove the ‘**QIA**’ from the Comment field for each of the pre-lysis batches included in the SP Extraction batch.

7. Select the **Complete Batch** icon to complete the batch.

8.3 Integrated Procedure

Note: <u>Do not</u> halt processing a sample once it is on an extraction batch. A sample can only be placed on hold at the Quant Results page at the end of the quantification procedure.

1. Ensure all reagents, equipment and consumables are selected against the batch as per Section 4.4.

2. Select the **Edit/Update Batch** icon and click on the **Run Date & Run Time** field, select the current date from the calender which autofills the time field upon batch completion.

3. Select **PASS** in the Batch Controls/Status field (Figure 13).
4. Remove the ‘**QIA**’ in the Comment field for each of the pre-lysis batches included in the Extraction batch.

5. Select the **Complete Batch** icon to complete the batch.

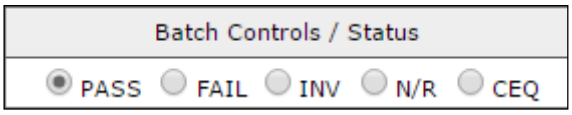

Figure 13: Changing QIAsymphony – integrated batch status

Note: DO NOT select and save the Sample Transition page. This will cause samples to populate to the quantification worklist, when the samples have already been quantified.

6. The Quantification batch will be finalised by the operators rostered in Pre-PCR as per Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit (QIS 34045).

9 Troubleshooting

9.1 General Troubleshooting

Issue	Actions
A prompt appears advising that the scan of reagents was not possible	Check and wipe the barcode of each reagent trough of the Reagent Cartridge with 70 % Ethanol, dry with a kimwipe and re-scan.
The barcodes on the sample tube carriers do not scan consistently	Wipe the barcodes with 70 % Ethanol, dry with a kimwipe and try again.
The reagent cartridge does not appear to contain the amounts stated on the cartridge.	Check the reagent reservoirs/trophs in the reagent cartridge for bubbles.Ensure that the Enzyme Rack has also been added to the cartridge for scanning.
The hood is opened during the first stages of the extraction protocol	The run will not be able to continue. The samples at this stage have not been added to the sample prep cartridges. Thus, the run is required to be restarted.
The run has been paused or cancelled and cannot be continued	Note the command number on the QIAsymphony® Instrument. A recovery of the samples via the clean-up procedure is required for that 24 sample batch set. Refer to Section 14.2.1 and Section 14.2.2 to perform these procedures.
QIAsymphony® instrument issues are preventing the extraction of the lysates	The Maxwell extraction procedure can be utilised to complete the extraction (refer to Section 9.2).
Residue is noticed on the outside of the Tip guards after/during a procedure	After run has completed, remove the Tip guard and transfer the residue (using a swab) into a 2mL tube. Register and process the sample as an environmental sample as per QIS 34280. Add a “See Batch” to the comment field, select “INV” and enter appropriate batch notes. After residue is quantified, additional notes are added to enter the quantification value.

9.2 Extraction of Lysates using Maxwell Instrument

Samples that have undergone the pre-lysis procedure can be extracted using the Maxwell® instrument. This method was validated due to the limited storage time of samples lysates and the possibility of QIAsymphony® instrument issues and/or extended instrument downtime.

Refer to DNA IQ™ Extraction using the Maxwell® 16(QIS 34044) or Maxwell® FSC(QIS 35605).

1. Split one QIAsymphony® Pre-Lysis batch into two Maxwell batches:

   a. Samples in positions 1-16 of the Pre-Lysis batch will go onto the first Maxwell batch.

   b. Samples in positions 17-24 of the Pre-Lysis batch will go onto the second Maxwell batch. This Maxwell batch will require a Positive and Negative control added to the batch (as per a routine Maxwell batch).

2. Add Batch notes to both Maxwell batches stating that the samples are QIAsymphony® lysates that are having the extraction completed using the Maxwell instrument.

3. Prepare the required amount of Maxwell reagents for the two batches as per QIS: 34044 or 35605). However, Maxwell extraction buffer is only required for the Positive and Negative control of the second Maxwell batch.

4. Set up required tubes: Elution buffer tubes and Nunc tubes (Nunc tube will already be labelled from the Pre-Lysis batch). The second Maxwell batch will also require a spin basket tube and 1.5/2.0mL tube for the Positive control and a 1.5/2.0mL tube for the Negative control.

5. For each Maxwell batch, print the batch sample labels and label the required tubes. Then perform sequence check of the batches.

   Note: SPIN label is only required for the Positive control of the second Maxwell batch, uncheck ALL other SPIN boxes.

6. For the first Maxwell batch, add 200 µL of Lysis Buffer to each sample and process as per:
   - QIS 34044 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction) from steps 11 – 19, or
   - QIS 35605, Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction) from steps 11 – 19.

7. For the second Maxwell batch:

   a. Add the Maxwell Extraction buffer to the Positive and Negative control and process as per:
      - QIS 34044 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction) from steps 8 – 16.;or
      - QIS 35605 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction) from steps 8 – 16

   b. For the remaining samples (QIAsymphony® Pre-Lysis lysates) add 200 µL of Lysis Buffer to each sample and process as per:
      - QIS 34044 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction), from step 11; or

- QIS 35605., Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction), from step 11.

c. The controls and lysates are now at the same stage, complete the Maxwell process as per:
   - QIS 34044 section 7.1 (Pre-Lysis Procedure for Maxwell Extraction), from steps 17 – 19, or
   - QIS 35605 section 7.1 (Pre-Lysis Procedure for Maxwell Extraction), from steps 17 – 19.

9.3 Recovery & Clean up Procedure

If the QIAsymphony® Instrument run has errored or has been interrupted and cannot continue, inform the Analytical HP5 and/or HP4 and proceed with the recovery of the samples via the clean-up procedure. If required, ask for assistance from at QIASymphony® Trainer.

The recovery and clean up procedure comprise of 4 steps and should be performed in the following order:

1. Error analysis (Section 14.3.1) – identification of the error and the step in the protocol in which it occurred.
2. Sample recovery (Section 14.3.2) – recovery of the unprocessed and/or semi-processed samples from the instrument (e.g. removal of samples from the instrument). This is procedure is performed via the clean-up procedure, where “yes” to the use of a CW protocol is selected.
3. Clean up procedure (Section 14.3.3) –re-sets the instrument before starting a new run. This ensure that no consumables are left on the instrument that may cause errors/crashes. This procedure is performed via the clean-up procedure, where “no” to the use of a CW protocol is selected.
4. Manual processing (Section 14.3.4) – identification of sample positions in the sample prep cartridges and the completion of the extraction of the recovered samples using DNA IQ™ manual method.

Note: Ensure batch notes/sample notations are entered for affected batches/samples. See Miscellaneous Analytical Procedures and Tasks (QIS 34064) for instructions on how to enter sample notations.

9.3.1 Error Analysis

Identify the step in which the protocol was interrupted by performing the following:

1. Remove the elution rack (Nunc™ extract tube rack) to generate the result file.
2. If the **Sample** drawer icon is yellow, press it to obtain the error message box.
3. If an error message box appears (Figure 14), record the error message including the error message ID and the command number in brackets.

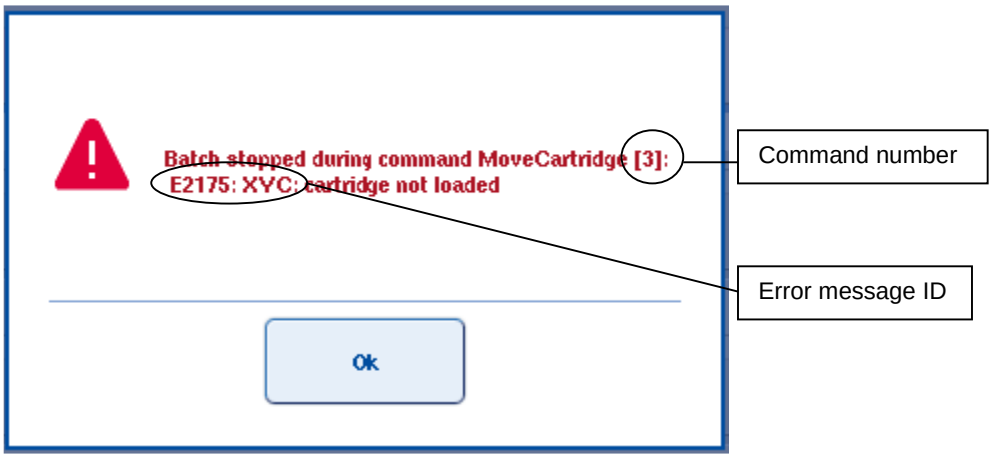

Figure 14: An example of an error message box

4. If an error message does not appear, proceed to step 5.

5. Copy the result file and log file (including the error.log file) from the QIAsymphony® instrument to the PC as per Section 6.3 of the Operation and Maintenance of the QIAsymphony® (QIS 33756).

6. Open the result file of the erroneous run and locate the error message and command number under the **Messages** section.

7. Record the error message and the command number in brackets.

9.3.2 Sample Recovery

To recover unprocessed and/or semi-processed samples from the instrument by performing the following:

1. Samples (lysates) that are in the original 2.0mL tubes in the carrier racks, are to be re-capped and set aside. These samples will be extracted either using the QIAsymphony® once the error/issue has been resolved or extracted using the Maxwell®16 instrument as per Section 9.2.

2. Using the *QIAsymphony® SP Recovery Procedure for DNA Investigator® Applications (software version 4.0 or higher)* manual located in Room 3191, refer to Table 1 (Command numbers and associated sample recovery instructions) on pages 4-13.

3. Using the obtained Command Number, navigate to the associated instructions in Table 1. Follow and perform these steps only up to obtaining the "**Recovery Forensic**" protocol (1, 2, or 3). Record the required recovery protocol.

Note: Ensure the hood is closed before starting a Forensic Recovery Protocol.

4. Select the **Tools** tab.

5. Open the **Maintenance SP** menu and select **Cleanup**.

6. Follow the instructions on the touchscreen until a prompt appears asking if a casework protocol is being used. Select **YES** to confirm the message.

7. On the '**Choose protocol**' screen select the appropriate protocol that was obtained (in step 4).

8. Follow the instructions on the screen until the sample recovery procedure is complete.

9. When removing the sample prep cartridges, it is important to keep the correct orientation and positioning of the sample prep cartridges. Write “front” on sample prep cartridges and label with 1, 2, or 3.

IMPORTANT: If possible, remove the Magnetic Head Cover (if not instructed in Step 5) to ensure that no consumables are left on the instrument.

The clean-up procedure MUST be performed after a Forensic Recovery Protocol is performed and/or before starting a new run. This is to ensure that no consumables (eg. rod covers) are left behind that will cause instrument crashes.

9.3.3 Clean-up Procedure

Re-set the instrument after sample recovery by performing the following:

1. Select the **Tools** tab and open the **Maintenance SP** menu and select **Cleanup**.

2. Follow the instructions on the touchscreen until a prompt appears asking if a casework protocol is being used. Select **No** to start the clean-up procedure.

3. The **Input | State of the QIAsymphony®** screen will appear. Select **Other** and then select **OK**.

4. Follow the instructions on the screen until the clean-up procedure is complete.

5. When the QIAsymphony® SP Clean-up procedure is finished, the instrument is ready to start a new run.

9.3.4 Manual Processing Part A: <u>Transfer of Samples</u>

This section outlines the procedure for transferring the samples (lysates) from the sample prep cartridges to 2.0mL tubes for manual extraction of recovered samples.

Figure 15 shows the positions of samples within the tube carrier, sample prep cartridges and the elution racks during an extraction procedure.

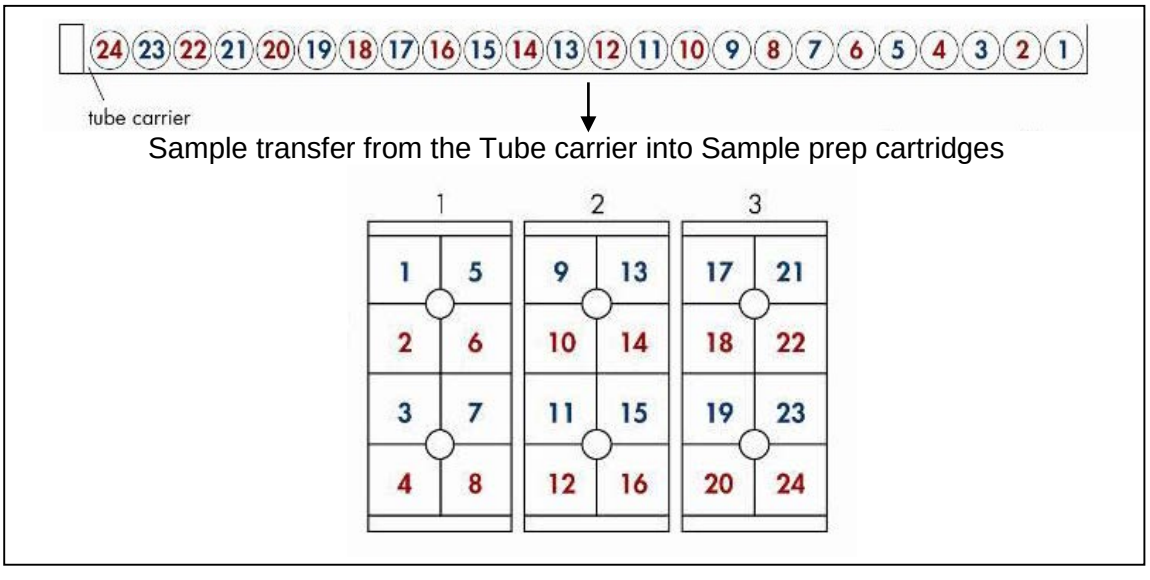

Figure 15: Sample positioning of 24 samples from the tube carrier to sample prep cartridges

1. Print a set of sample labels as per Section 6.6 for the (Pre-lysis) batch required for transfer from the sample prep cartridges.

2. Label a 2.0 mL tube for each sample and arrange the corresponding Nunc™ tube (from the **Eluate** drawer) into a rack.

3. A sequence check of the labelled tubes is required by a second operator and a batch note (under the affected Pre-Lysis batch) detailing the sequence check added in FR.

4. If the sample prep cartridge has been sealed, carefully unseal the cartridge. Only unseal one sample prep cartridge at a time.

5. Pipette mix the contents of the sample prep cartridge well and carefully transfer the contents including the magnetic particles into the prepared 2.0 mL tube.

NOTE: The transfer of sample from the sample prep cartridge to the 2.0 mL tube is to be performed under supervision (by the 'sequence check' person).

6. Repeat for all samples.

9.3.5 Manual Processing Part B: Manual DNA IQ extraction

This section outlines the procedure for the extraction of the recovered samples (lysates) using the manual DNA IQ method. This is performed after the samples have been transferred from the sample prep cartridges to (2.0mL) tubes.

1. Turn on Thermomixer and set the temperature to 65°C (for use in the elution steps).

2. Prepare the required reagents as per Appendix 15.

3. If the QIAsymphony® has errored during the elution steps, continue from step 12 (addition of elution buffer), otherwise continue below.

4. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

5. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

6. Carefully remove and discard the liquid, ensuring that the resin is not disturbed. Remove from the magnetic stand.

Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

7. Add 125 µL of Lysis Buffer-DTT solution. Vortex and place into the magnetic stand.

8. Once separation has occurred, remove and discard the liquid. Remove the tube from the magnetic stand.

9. Add 100 µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the liquid. Remove the tube from the magnetic stand.

10. Repeat step 9 twice for a total of three Wash Buffer washes. Ensure that all the liquid has been removed after the last wash. Remove the tube from the magnetic stand.

11. Uncap the tubes and place the lids down onto a clean rediwipe within the biohazard cabinet. Allow the resin to air-dry in the hood for 15 minutes and then recap the tubes.

Note: Do not allow the resin to dry for > 20 minutes as this may inhibit DNA elution.

12. Add 50 µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

13. Incubate using the thermomixer at 65 °C for 3 minutes, followed by a further 3 minutes at 65 °C shaking at 1100 rpm. If no thermomixer is available, incubate samples within a hot block and vortex mix at the start and end of the second 3 minute incubation. Remove samples.

14. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

15. Carefully transfer the DNA extract to the corresponding labelled Nunc™ tube.

16. Remove from the magnetic stand and repeat steps 12-13 once. The final volume after the second elution is approximately 95 µL of DNA extract.

17. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room 3194-A in the upright freezer as per Storage Guidelines for Forensic DNA Analysis (QIS 23959).

Ensure that adequate batch notes are entered in the QIAsymphony® extraction batch explaining the issues and steps taken.

9.4 Dropped Substrate during Pre-Lysis Procedure

If Substrate has been dropped **during transfer**

1. Unaffected lysate continues through processing as per standard procedure through to quantification.

2. The volume of the unaffected substrate is measured and noted in FR as a **Sample Notation.**

3. The dropped substrate is placed in a clean 2 mL tube and labelled with the **"SPIN"** barcode

4. The **"SPIN"** barcode is made into an exhibit barcode (refer to Section 9.5 for how to make a subsample an exhibit). Add the Analytical Note to the Spin barcode exhibit "Hold after extraction".

5. Create a new Maxwell extraction batch according to the Forensic DNA Analysis Workflow Procedure (34034) using the Manual fill option.

6. Manually add the dropped **"SPIN"** barcode to position 3 of the Maxwell batch.

8. Add Batch notes to the Maxwell batch stating that the samples are QIAsymphony® substrates that are having the extraction completed using the Maxwell instrument.

9. Prepare the required amount of Maxwell reagents as per QIS 34044 or 35605. Note, Maxwell extraction buffer is only required for the Positive and Negative controls.

10. Set up, label and sequence check required tubes as per QIS 34044 or 35605.

11. Process the Maxwell batch as follows:

    a. Add the Maxwell Extraction buffer to the Positive and Negative control and process as per:
        - QIS 34044 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction) from steps 8 – 10.;or
        - QIS 35605 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction) from steps 8 – 10

    b. The controls and QIAsymphony® Pre-Lysis substrate/s are now at the same stage, complete the Maxwell process from the addition of 200 µL of Lysis Buffer and process as per:
        - QIS 34044 Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction), from step 11; or
        - QIS 35605., Section 7.1 (Pre-Lysis Procedure for Maxwell Extraction), from step 11.

7. At the completion of the Maxwell extraction procedure the Positive and Negative control are transitioned as standard procedure. The dropped substrate is held after extraction.

8. A **Sample Notation** is added under the original barcode and the new subsample barcode in FR.

9. Add an entry in the Forensic DNA Analysis Adverse Events Log.

If Substrate has been dropped **after** being spun in spin basket

1. Continue to extract unaffected lysate as per standard procedure through to quantification.

2. Store substrate in spin basket storage as per standard procedure.

3. Add **Sample Notation** for the sample in FR stating that the substrate was dropped post spinning.

4. Add an entry in the Forensic DNA Analysis Adverse Events Log.

9.5 Creating an Exhibit from a Subsample

1. Open the parent barcode in the Case Management side of FR.

2. Click the **Exhibit Register** tab and then click the 'add an exhibit' icon

3. Enter the **subsample** barcode in the 'Exhibit Barcode' field.

Note: Once the subsample barcode has been entered the following warning will appear.
Warning: This Barcode has previously been allocated to a subsample.

4. Choose a 'Category' from the drop-down box. This should be the same as the parent (e.g. swab/trace DNA).

5. Fill in the 'Description' field with a description of what the subsample was from (e.g. Subsample of 123456789/re-extract of 123456789).

6. Enter FSS in the 'Located / Owner' field.

7. **IMPORTANT.** Add the **parent** barcode in the 'Parent Barcode' field.

Note: If the parent barcode is not entered, the subsample will no longer be associated with the parent barcode. This step is essential for continuity.

8. Check *Admission / Intel* in the 'Ownership / Relationship / Prioritisation' field.

9. Check the "*Sample has been collected in strict compliance with CSE101 Biological Evidence [Required]*" box in the 'Forensic Biology Analytical Advice' field.

10. Enter FR user number in the Delivery Officer Rego field and tab across to Surname to populate that field.

11. Change Station field to "Queensland Health Scientific".

12. Click the 'Save' icon

10 Validation

- M. Aguilera, B. Micic, Pierre Acedo, L. Ryan, C. Allen. Proposal # 168: Validation of the QIAsymphony® SP/AS Modules, November 2016.
- M. Aguilera, M. Mathieson, L. Ryan, C. Allen. Internal Project: Replacement of the Extraction Multiprobe with the QIAsymphony® SP/AS, July 2015.
- M. Cipollone, L. Ryan, M. Mathieson, C. Allen. Proposal # 192: Validation of QIAsymphony SP for Bone Extraction, April 2018.
- M. Cipollone, L. Ryan, M. Mathieson, C. Allen. Proposal # 192: Validation of QIAsymphony SP for Bone Extraction- Supplementary Repeatability and Reproducibility, March 2020.

11 Quality Assurance/Acceptance Criteria

- Casework and reference samples are extracted and quantified on separate batches. If casework and reference batches are to be extracted on the QIAsymphony® SP on the same day, the instrument and all associated labware must be decontaminated between operations.
- Positive and negative controls are included in each extraction batch and are processed in the same manner as samples through to completion.
- If any results are obtained from the negative control at either quantification or CEQ check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if required. Refer to Capillary Electrophoresis (CEQ) Quality Check (QIS 34131) and Miscellaneous Analytical Procedures and Tasks (QIS 34064).

12 References

- QIAsymphony® DNA Investigator® Handbook 10/2008
- QIAsymphony® SP Protocol Sheet: Casework_500_ADV_V7 06/2012
- QIAsymphony® SP Recovery Procedure for DNA Investigator® Applications Handbook 03/2014 (Electronic copy located I:\AAA Analytical\Communications from Vendors\Qiagen communications)
- Quantifiler® HP and Trio DNA Quantification Kits User Guide, Applied Biosystems by Life Technologies; Publication Number: 4485354, Rev. B
- Promega, FAQs –DNA IQ™ System.
- Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
- Promega, DNA IQ™ System –Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
- Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
- Promega, Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
- Promega Corporation 2006 Material Safety Data Sheet. Lysis Buffer. Article number: A826.

13 Associated Documents

34103 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
22857 Anti-Contamination procedure
33756 Operation and Maintenance of the QIAsymphony®
34045 Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit
33759 DNA Extraction and quantification of casework and reference samples using QIAsymphony® Instrument Training Module
23959 Storage Guidelines for Forensic DNA Analysis
34042 Procedure for the use of the STORstar unit for automated sequence checking
34034 Forensic DNA Analysis Workflow Procedure
34064 Miscellaneous Analytical Procedures and Tasks
34044 DNA IQ™ Extraction using the Maxwell® 16
35605 DNA IQ™ Extraction using the Maxwell® FSC
34131 Capillary Electrophoresis (CEQ) Quality Check

14 Amendment History

Version	Date	Author/s	Amendments
1	March 2017	M Aguilera, P Acedo, A Kaity	First Issue for LIMS changeover from AUSLAB to FR. Replaces 33758.
2	June 2017	B Micic, M Mathieson, L Farrelly	Included sections 6.1, 6.5 to 6.7. Removed duplication steps from sections 7.4 & 7.5. Included section 8 batch finalisation.
3	Dec 2017	P Acedo, B Micic	Updated the Integrated Run steps and the Sample Recovery/Clean Up steps, added Manual IQ procedure and reagents. Minor wording/formatting changes.
4	Feb 2019	P Acedo	Updated SP and AS protocols and fixed/updated hyperlinked documents.
5	Mar 2020	B Micic	Minor changes throughout. Added: additional cRNA; steps to scan waste drawer after run; quant batch sequence check, AS setup section; sampling of tip guard residue; check of

Version	Date	Author/s	Amendments
			lysate tubes. Changed General troubleshooting section to a table, expanded Integrated section. Added Bone Pre-lysis section, updated Section 10.
6	Mar 2021	M Aguilera A Darmanin	Made procedural changes to SOP in accordance to OQI# 54156. Corrected hyperlinks throughout SOP. Included labelling of Enzyme rack to also include cRNA and ATE lots used in its preparation. Minor changes throughout. Made amendments as per reviewer comments. Added dropped substrate troubleshooting methods in Sections 9.4 & 9.5.

15 Appendix

15.1 Manual DNA IQ™ Reagents, Equipment and Consumables

Table 9 outlines the reagents and the storage locations required for the Manual DNA IQ™ extraction of lysates recovered from the QIAsymphony®.

Table 9: Reagents with storage room and location

Reagent	Room	Location
DNA IQ™ Elution Buffer	3188	In-use tray or shelf
DNA IQ™ Lysis Buffer	3188	In-use tray or shelf
DNA IQ™ 2x Wash Buffer	3188	In-use tray or shelf
1,4 Dithiothreitol (DTT) 1M	3188	Freezer
AnalR 100 % v/v Ethanol	3188	Shelf
2- Propanol	3188	Shelf

15.1.1 Lysis Buffer with DTT Solution

Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the Manual DNA IQ™ procedure.

1. Determine reagent volumes required by referring to Appendix 15 Table 12.

2. Remove the appropriate aliquot/s of DTT from the freezer and thaw. Vortex mix and centrifuge briefly before use.

3. In the biohazard cabinet, add the required volume of Lysis Buffer to a 10 mL tube.

4. Add the required volume of DTT to the Lysis Buffer and gently swirl to mix.

5. Label with **"Lysis Buffer + DTT"**, initials and date.

15.1.2 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. A 1x Wash Buffer is required for the procedure.

1. In a biohazard cabinet, add 35 mL of *AnalR* 100% Ethanol to the 2x Wash Buffer bottle.

2. Add 35 mL of 2- Propanol to the 2x Wash Buffer bottle.

3. Cross out the 2x on the bottle and replace with 1x and initial and date the amendment. Refer to Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits (QIS 34103). Update the 1x Wash Buffer reagent log in FR by:

   - At the FR main menu click the **Reference** tab and the **Supply** button.
   - Type **DNA IQ** into the **Category** field followed by the enter key.
   - Click the **Received Date** of the wash buffer that is being prepared.
   - Click the **Edit** icon at the top right of the screen.
   - Click in the **Comments** box and specify the 1x Wash Buffer aliquot (e.g. A1) and the lot numbers of the ethanol and isopropyl alcohol used.
   - Click the **Save** icon at the top right of the screen.

Note: 1x Wash Buffer can be prepared and stored at room temperature prior to use.

4. Determine reagent volumes required by referring to Appendix 15 Table 12.

5. In the biological safety cabinet, aliquot the required amount of 1x Wash Buffer into a 10 mL tube.

6. Label with **"Wash Buffer"**, initials and date.

15.1.3 Elution Buffer

Elution Buffer is supplied with the DNA IQ™ kit.

1. Determine reagent volumes required by referring to Appendix 15 Table 12.

2. In the biohazard cabinet, aliquot the required amount of Elution Buffer into a 5 mL tube.

3. Label with **"Elution Buffer"**, initials and date.

15.1.4 Equipment

Table 10 outlines the equipment and the locations required for Manual DNA IQ™ extraction.

Table 10: Location of required equipment

Equipment	**Location**
Fridge	3189
Freezers	3189 / 3190
Biological safety cabinet class II x 4	3188 x 1, 3189 x 4
96 well tube racks	3189
Vortex x 4	3189
Hot block x 4	3189 / 3191 / 3194
Centrifuge x 4	3189 / 3194
Mini/Micro centrifuges x 4	3189
Finnpipettes 100 – 1000 µL	3189
Finnpipette 20 – 200 µL	3189
Thermomixer x 4	3189
Magnetic rack	3189
Multitube Shaker	3191

15.1.5 Consumables

Table 11 outlines the consumables and the locations required for Manual DNA IQ™ extraction.

Table 11: Location of required consumables

Consumables	**Location**
Racks	3189
2 mL tubes	3188 / 3189
Nunc™ tubes	3189
5 mL, 10 mL sterile tubes	3188
Sharps bins	3189
20 µL pipette tips	3189
300 µL pipette tips	3188 / 3189
1000 µL pipette tips	3188 / 3189

Additional consumables can be found in the Store Room (3184).

15.2 Reagent Volumes for Manual DNA IQ™ Extraction

Table 12: Reagent volumes required for manual DNA IQ™ extraction

	Reagent	Volume for 24 samples (µL)
Lysis Buffer-DTT	DNA IQ™ Lysis Buffer	3300
	DTT	33
Wash Buffer	DNA IQ™ 1x Wash Buffer	7800
Elution Buffer	DNA IQ™ Elution Buffer	2600

Note: The volumes entered for 24 samples have been calculated for an 'extra' 2 samples (i.e. calculated for 28 samples)

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Forensic and Scientific Services

Procedure for Profile Data Analysis using the Forensic Register

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1 Purpose

The purpose of this document is to describe the procedure for managing DNA profile results using the Forensic Register (FR).

2 Scope

This procedure shall apply to all Forensic DNA Analysis staff who interpret DNA profile results.

This procedure does not include the guidelines for the scientific interpretation of DNA profiling results. This information can be found in QIS 17117 *Procedure for Case Management*.

Use of the Profiler Plus amplification kit within Forensic DNA Analysis was ceased in January 2018 (Appendix 13 – Profiler Plus). From this date all samples were amplified using the PowerPlex 21 amplification kit.

3 Definitions

To date, the interpretation and management of DNA profile results in Forensic DNA Analysis has been referred to as “Case Management”. Within the Forensic Register, the term “Case Management” refers to the management of a case as a whole rather than the individual exhibit results. It is therefore necessary for terminology within Forensic DNA Analysis to be brought in line with the FR. The interpretation and management of DNA profile results will now be referred to as “Profile Data Analysis” and a person who performs Profile Data Analysis will be referred to as a “Profile Analyst”.

Forensic Register (FR)	Forensic DNA Analysis LIMS
PDA	Profile Data Analysis
PP21	PowerPlex 21
P+	Profiler Plus
NCIDD	National Criminal Investigation DNA Database
CX	Complex unsuitable
NP	No profile
PU	Partial unsuitable
ST	Sub-threshold peaks
PRT	Profile Record Table
CPT	Case Profiles Table
GM	GeneMapper IDX
WL	Worklist
DNAIntel	Person sample profiles with the descriptor “DNA Person Sample Intel”
AC	Assumed contributor
UK	Unknown (a profile that does not match to a reference or DNAIntel sample)
Epg	Electropherogram

4 Worklists

There are a number of worklists to direct the workflow of samples that require interpretation, review or STRmix analysis. These lists are summarised in Table 1 below. Also refer to Appendix 2 – Profile Data Analysis (PDA) Worklists (Crime Scene).

To access these lists, the profile analyst / reviewer needs to navigate to the sample management pages of the FR. This can be done by entering 'sm' into the global search field of the primary case management page.

If a sample has populated a crime scene PDA list but one or more batches are under investigation then the 'GMIDx' column will display 'INV' and the sample will be greyed-out at the bottom of the list. Once all batches are completed the sample will appear as normal to the appropriate place on the list.

To action exhibits that are no longer required, refer to QIS 34006 – see Appendix 7. Whilst to action samples with no DNA detected or DNA insufficient for further processing results, refer to QIS 34064.

Table 1 – Worklists

Worklist	Sub-list	Purpose	Population triggers	Removal triggers	Default filter	Additional optional filters
Profile Data Analysis	Reference	Reference sample analysis	CE batch passed; QFLAG passed; profile PDF uploaded and a reader comment that doesn't trigger an auto-rework	Profile copied down or rework ordered	Date & time of receipt	ALL OK EVDRW FTP LINK UK INV
	Case Work PP21	PP21 profile data analysis (Priority 1 and 2 samples)	CE batch passed; QFLAG passed; profile PDF uploaded	Rework ordered; 'STRMix' technique (worklist) ordered; 'Profile Review' test ordered	1. User is PDA analyst 2. User is Reporter 3. Priority 1 then 2 4. Date & time of receipt 5. Samples with batch at investigation (INV)	ALL SS MIX COMPLEX UNDEFINED INV ENV
	Case Work PP21 (P3)	PP21 profile data analysis (P3 samples)		Rework ordered; 'STRMix' technique (WL) ordered; 'Profile Review' test ordered		
	Case Work UK CE	Profile data analysis of samples with unknown chemistry (dilutions and cut-over samples)		Rework ordered; 'STRMix' technique (WL) ordered; 'Profile Review' test ordered		
	Case Work + Ref	Profile data analysis of completed samples where new reference samples have been received	Casework sample adds to list if: 1. Ref newly associated to case 2. Ref comment is 'OK' and PDF uploaded 3. PDA results for casework sample are validated	'Profile Review' test ordered	1.User is PDA analyst 2. User is Reporter 3. Priority 4. Date & time of receipt	

			4. Casework sample is 1, 2, 3 or 4 contributors			
Profile Review	Case Work PP21	PP21 profile review (P2 samples)	'Profile Review' test ordered	'Profile Review' test incorrected, validated or 'Click to Rework' selected	1. User is Reviewer 2. Priority 3. Date & time of receipt	ALL SS SSNCIDD MIX MIXNCIDD COMPLEX PATERNITY ENV (except Case Work + Ref list)
	Case Work PP21 (P3)	PP21 profile review (P3 samples)				
	Case Work Unknown	Review of profiles with unknown chemistry (dilutions and cut-over samples)				
	Case Work +Ref	Profile data analysis of completed samples where new reference samples have been received				
STRmix		STRmix analysis request	'STRMix' technique (WL) ordered	'STRMix' test ordered	Date & time of receipt	None
Awaiting Review	STRmix	STRmix analysis allocation and completion	'STRMix' test ordered	'STRMix' test validated	Date & time of receipt	None
Interim DNA Results		Profile data analysis or review of interim results	'-interim' suffix in Profile Record Table	Same profile copied down into Profile Record Table with a suffix other than '-interim'	1.User is PDA analyst 2. Priority 3. Date & time of receipt	None

For additional optional filters, it is possible to request more through the FR provider if a specific workflow requirement is identified. For example, an OQI filter for samples affected by a specific adverse quality event.

5 Basic Functions of the PDA Page

The PDA page is split into five main sections:

- Exhibit Detail – provides relevant information about the sample
- Profile Analysis – provides batch information including volumes. Where GeneMapper IDx information and STRmix import files can be obtained
- Profile Interpretation – used to record basic interpretation information and STRmix deconvolution. Where auto-add result lines can be generated
- Profile Record – used to record single source profiles and contributors resolved from a mixture. Also used to record profiles for upload to NCIDD

- Case Profiles – used to record all associated reference samples, reference sample comparisons and unknowns for a case

Other functions on the page are as follows:

This icon is found in the top right hand corner of the page. From here STRmix report files (in PDF format) can be added. Clicking this icon will open the 'STRmix File Import' page. The scientist can drag the required files into the box (or browse by clicking 'Add Files') and click 'Start Upload'. Once the upload is complete, click the save icon ( ) and the STRmix files will be filed in the Profile Interpretation section of the PDA page. Also refer to Section 12. The STRmix PDF must have the following naming convention:

- For a deconvolution 'QPxxxxxxxxxx_yyyyyyyyy' where 'xxxxxxxxx' is the QP number and 'yyyyyyyyy' is the crime scene sample barcode
- For a LR 'QPxxxxxxxxxx_yyyyyyyyy_LRPrev_1_zzzzzzzzz' where 'xxxxxxxxx' is the QP number, 'yyyyyyyyy' is the crime scene sample barcode and 'zzzzzzzzz' is the reference sample barcode

This icon is found in two places on the page:
In the top right hand corner – clicking this icon **will change between the PDA page and the** Exhibit Testing / Movement page
Above the Case Profiles table – clicking this icon will produce a profile table for all reference samples and unknowns in the case. This table will default to the kit type used for the processing of the sample. This can be changed between P+ and PP21 by clicking on [P+] and [PP21] underneath the table. Clicking [P+ All] or [PP21 All] will display all profiles in the case that have been entered into the Profile Record table

This icon is found in the top right hand corner of the page. Clicking on this icon will open a new window containing the GeneMapper® record for all CE runs for the sample. The information in this record is the same as that detailed in Section 6.2 however it does not contain the height and size columns. All epgs can be opened from this window either individually by clicking on the relevant PDFs or all at once by clicking on 'Open All'

This icon is found in the top right hand corner of both the PDA page and Exhibit Testing / Movement page. Clicking on this icon utilises a built in 'PDA Robot' designed to check all results lines entered for a sample against the completed information in the PDA page. A new window opens and any discrepancies are highlighted with a red cross showing anticipated result lines versus entered result lines. These potentially incorrect results should be investigated prior to PDA review (Section 18).

This icon is found above the Profile Interpretation table. Clicking on this 'Add Results' icon generates the exhibit result lines for a sample (only three lines at a time) based on the completed information in the Profile Interpretation, Profile Record and Case Profiles sections of the PDA page (Section 18).

This icon is found above the Profile Analysis table. Clicking this icon will allocate the sample to the scientist clicking the icon and their name will populate to the left of the icon. If a second scientist were to subsequently click this icon, then the sample will be allocated to this scientist in place of the first scientist. Once a sample is allocated it cannot be unallocated, it can only be reallocated to another scientist.

This icon is found above the Profile Analysis table and is used to enable the page to be edited.

This icon replaces the edit icon when enabled and is used to save changes made to the page.

The Sample Notes section can be used to record auditable notes against a sample.

6 Profile Analysis Table

Figure { SEQ Figure * ARABIC } – Profile Analysis Table

1. Volume post extraction – applies to microcon batches only
2. Quantification value (short fragment)
3. Amplification volumes
4. CE batch position – click here to access GeneMapper Record (Section 6.2)
5. Check box to show that this run is to be included in STRmix analysis
6. Click to export STRmix input file for this run

Hovering over the quant value will display a pop-up box with all of the Quant Trio results (Figure { SEQ Figure * ARABIC }).

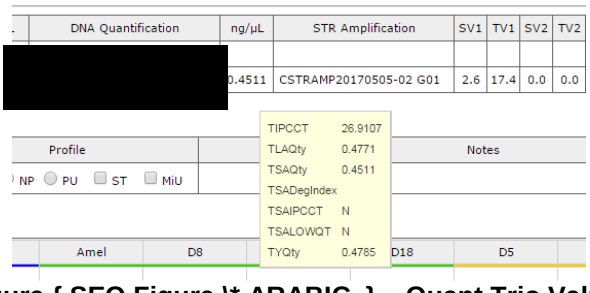


Figure { SEQ Figure * ARABIC } – Quant Trio Values

6.1 Quality Control

When entering into a sample to interpret the results, the first action by the scientist is to check the quality markers of the batches associated with the sample.

The 'Profile Analysis' table (Figure 1) details all of the batches that a sample has been processed on. Each batch will have a quality marker in the form of a coloured square. The colour of the square indicates the status of the batch as follows:

Red = batch failed
Orange = batch in progress or under investigation
Green = batch passed

If there is a ⚠ symbol, this indicates there is a batch comment. Hovering over this symbol or the batch id will show all or part of the comment and the progress of the batch. If the comment is '<3', this means there are less than 3 peaks in a negative control and the comment does not need to be actioned further. If the comment is 'see batch', this means the scientist needs to enter into the batch and read the associated notes to ensure the result for the relevant sample is reportable. The batch notes should be acknowledged as 'noted' in the 'Sample Notes' section of the PDA page.

Quality flags can be acknowledged by the reviewing scientist, provided both entering and reviewing scientists are aware of any critical quality issues.

A batch can be entered into by clicking on the batch id in the Profile Analysis table.

Results should not be reported until all batches have passed and display a green square. However, there are exceptions to this stipulation. For urgent results, the scientist can check all batch notes and any associated control profiles for issues. If no quality issues are identified, the urgent results can be interpreted with notes added to the Sample Notes section accepting all batches. For no DNA detected or DNA insufficient for further processing results, the scientist can interpret these samples once the quantification batches have passed.

6.2 GeneMapper® Record

Clicking on the position number adjacent to the CE batch id will open a new window containing the GeneMapper® record for that CE run (Figure { SEQ Figure * ARABIC }). The 'Alleles (GeneMapper)' column (highlighted in green) displays all of the designations as exported from GeneMapper at the time of plate reading; the information in this column cannot be changed. The 'Alleles' column displays all of the designations as exported from GeneMapper at the time of plate reading and the information in this column can be changed. The 'Height' and 'Size' columns display the peak heights and sizes of the alleles in their respective order.

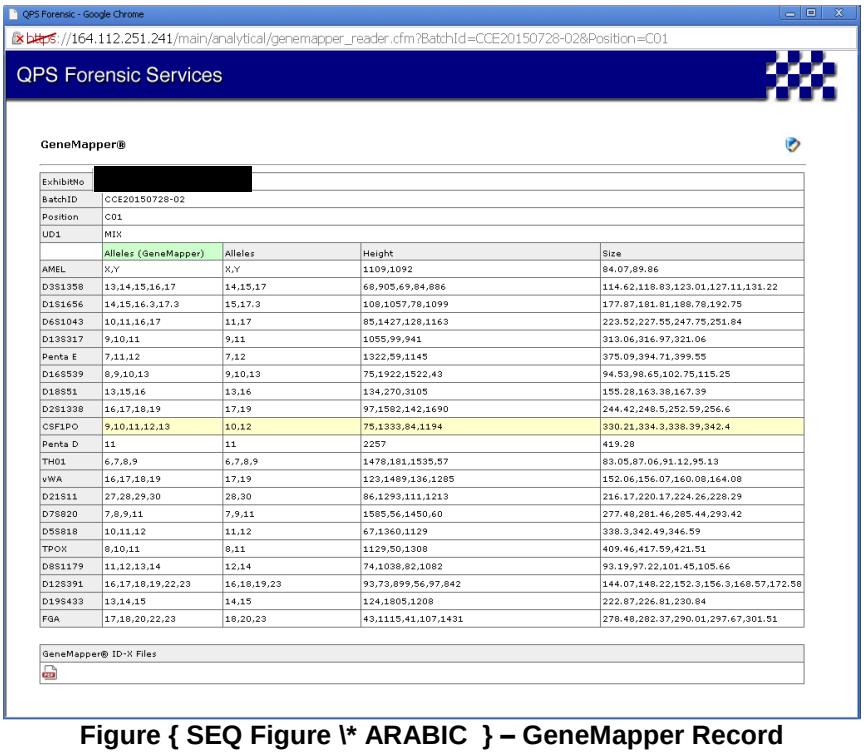


Figure { SEQ Figure * ARABIC } – GeneMapper Record

6.2.1 PowerPlex 21

The following functions apply to this page:

1. In the default view, the 'Alleles' column shows the allele designations only, the stutter peaks are hidden from view. Clicking the edit button ( ) displays the stutters as well

as the alleles. Clicking the save button ( ) reverts to the default view (without stutters). The heights and sizes relating to all peaks (stutters and alleles) are displayed in the 'Height' and 'Size' columns at all times

2. The 'Alleles', 'Height' and 'Size' columns are used to generate the input file for STRmix. These fields can be edited
3. When the record is edited, the line will highlight yellow to show that the record has been changed. The reason for any manual changes should be recorded in the Sample Notes
4. PDFs of the epg can be accessed from the 'GeneMapper ID-X Files' box at the bottom of the window

6.2.1.1 Removing Peaks from PP21 Profiles

On occasion a peak may be left on the profile by the plate reader in error, such as a -2 repeat stutter peak or an artefact peak (QIS 34112). This peak can be removed from the GeneMapper Record by the following process:

a. Click the edit button ( )
b. Delete the allele in question along with the respective height and size
c. Click the save button( )

It is important to ensure that the correct height and size is removed. Following the removal of the peak, only the non-zoomed epg needs to be updated / annotated and uploaded into the FR. This can be done as described in Appendix 3 – Amending PDFs, alternatively the peak can be removed in GeneMapper and the epg re-PDF'd. Add a sample note detailing the amendment.

6.2.1.2 Adding Peaks Back on to PP21 Profiles

On occasion a peak may be removed from the profile by the plate reader in error. In order to add the information back into the GeneMapper Record it is necessary to obtain the correct allele designation and its associated height and size from GeneMapper. The Profile Analyst must add the peak onto the profile using GeneMapper and re-PDF the epg ensuring that the alleles are labelled with the allele designation, the height and the size of the peak. This is required so that the reviewer is able to easily check this information from the epg. This peak can be added to the GeneMapper Record by the following process:

a. Click the edit button ( )
b. Enter the allele into the alleles column ensuring the alleles are in number order and are separated by a comma
c. Enter the height into the height column ensuring that it is in the same position as the allele, e.g. if the allele is the second entry in the 'alleles' column, the height must be the second entry in the 'height' column
d. Enter the size into the size column ensuring that it is in the same position as the allele and height
e. Click the save button( )

The new non-zoomed epg must be uploaded into the FR as described in Appendix 3 – Amending PDFs. Add a sample note detailing the amendment.

7 Profile Interpretation Table

The Profile Interpretation table is where the number of contributors, STRmix deconvolution including resolved alleles and simple interpretations for a DNA profile can be recorded. The notes section within this table is for reminders only since the entries within this section are not recorded in the audit trail. An example of when this notes section might be used is to remind the scientist to look at a particular locus when the rework is completed. Since it is expected that the majority of profile data analysis will be paperless, this section is analogous to using a sticky note in a case file.

7.1 PowerPlex 21

Figure 1 – PP21 Profile Interpretation Table

1. This section is used to record the number of contributors to a profile
2. With the exception of ST, this section is used to record interpretations that don't require any further action:
   a. CX is to record a profile that is a complex mixture and is unsuitable for further interpretation
   b. NP is used to record a no profile result
   c. PU is used to record a profile that is partial and unsuitable for further interpretation
   d. ST can be used in conjunction with the number of contributors or NP to record that there are also sub-threshold peaks within the profile
3. When a STRmix deconvolution PDF file is uploaded (Appendix 6 – STRmix Workflow), a PDF icon will be displayed. Clicking on this icon will download the PDF
4. Notes section (not audited)

When a STRmix deconvolution PDF is uploaded into the FR the individual contributions will be displayed at the bottom of the Profile Interpretation Table (Figure { SEQ Figure * ARABIC }).

Profile Interpretation

Contributors	Profile	STRmix™	Notes
1 2 3 4 5	CX NP PU ST	PDF	

	D3	D1	D6	D13	PentE	D16	D18	D2	CSF	PentD	TH01	vWA	D21	D7	D5	TPOX	D8	D12	D19	FGA
1	14,15	14,16.3	13,19	10,12	10,16	10,13	15,18	20,21	10,11	9,11	6,7	14,16	28,28	8,13	11,12	8,12	13,16	16,17	12,12.2	18,22
2	18,0	0,0	0,0	0,0	0,0	0,0	13,0	0,0	0,0	0,0	9.3,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

Figure { SEQ Figure * ARABIC } – Profile Interpretation Table with Mixture Contributions

8 Profile Record Table

The FR contains a database of profiles from various sources including casework and reference profiles, staff profiles and elimination database profiles and person sample profiles with the descriptor "DNA Person Sample Intel" (DNAIntel samples). A crime scene profile (single source or resolved component of a mixture) is added to this database when it is entered into the Profile Record table and the record saved. A profile can be added to the table manually as well as by a process called 'copy down' from the GeneMapper Record, a STRmix results file or a combination of 'copy down' followed by manual edit (Section 8.3.2 and Appendix 4 – Profile Data Analysis of a Single Source PP21 Profile for NCIDD).

When entering a profile each locus must have two alleles separated by a comma and missing alleles must be represented as a zero '0'. 'NR' is not recognised in this table and should not be used. Amelogenin must be represented as 'X,X'; 'X,Y'; 'X,0' or by leaving the field blank. 'X' on its own must not be used as this is not recognised by NCIDD.

The Profile Record table can be cleared whilst in edit mode by clicking the icon. This will delete all records in the table that have not been validated. Clicking the 'CLR' button above the Profile Record table will clear the entry that has been made in the same edit.

The '+NCIDD' box (see 4 in Figure 10) enables the scientist to nominate the profile for upload to NCIDD.

A suffix should be selected for all profiles copied down into the Profile Record table to describe the profile type (see 3 in Figure { SEQ Figure * ARABIC }). If the profile is nominated for NCIDD, this is the suffix that should be used for NCIDD.

Refer to Section 8.3.1 and Section 9 for a description of the '+CPT' box.

The following profiles MUST be recorded in the Profile Record table:

- All single source profiles with ≥ 12 alleles;
- All resolved components of mixed profiles with ≥ 12 alleles;
- Single source profiles with <12 alleles where the profile is able to be matched / designated (one sample per case);
- Resolved components of mixed profiles with <12 alleles where the component is going to be matched / designated (one sample per case), e.g. PP21 two person conditioned mixture where the remaining consists of 7 alleles and is being designated as UKM1
- All conditioned components of mixed profiles

Unresolved mixed profiles should not be recorded in the profile record table.

The Profile Record table should be completed for a single contribution in one action as once the record is saved it can only be edited by deleting the whole record (by clicking the button whilst in edit mode).

8.1 FR Database Matching

If a profile entered into the Profile Record table consists of ≥12 alleles, clicking the icon (5Figure 10) will cause it to be searched against the FR database for possible matches in the following order:

1. Staff elimination database (Forensic DNA Analysis staff, QPS staff and FBUNKs)
2. Reference samples within the case
3. DNAIntel (reference) samples external to the case
4. Unknown profiles within the case

If a match is obtained it will be displayed in the match cell below the profile (5). The matching rules allow one mismatch with the number of matching and mismatching alleles being displayed in brackets in the match cell; the first number is the number of matches, the second number is the number of mismatches.

If a match to the staff elimination database occurs, refer to QIS 34281 for required action.

If the scientist agrees with the match nominated by the database then the designation can remain, if the scientist does not agree with the match the scientist can overwrite the match information.

If a partial profile is copied down and a match to a reference sample is displayed, the scientist may have additional information (such as sub-threshold peaks) that excludes that person as being a match. In this instance the scientist may decide that the profile is unknown and overwrite the match information with an unknown designation in the format described below.

If a partial profile is copied down and no match to existing profiles is displayed, the scientist needs to be aware that if there are less than 11 matching alleles, it is possible for a match to an existing partial profile to be missed by the FR (Figure 6). In this instance the scientist can manually add the match information to the Profile Record table (i.e. remove the FR generated designation and replace with the designation of the matching profile).

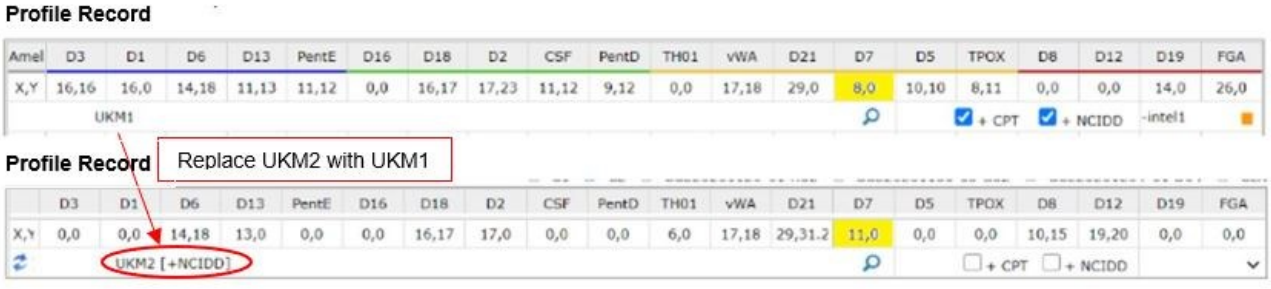


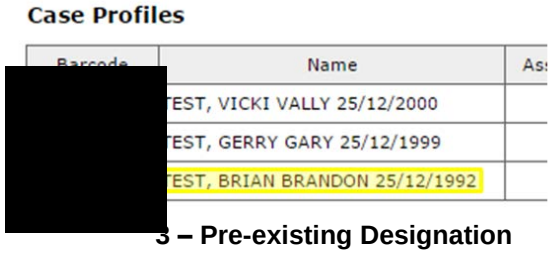
Figure 2 – Partial Profile Matching in the Profile Record Table

If no match is obtained, the FR will suggest the next unknown designation available for use in the case. This means that if a previous profile in the case has been designated as unknown male 1 (UKM1), the FR will suggest the next unknown male profile be designated UKM2.

Although this matching function is of assistance to the scientist, this does not override the requirement for the scientist to check the match through the comparison of epgs.

Profiles consisting of <12 alleles will not be searched and will populate the match cell with its own exhibit barcode. The designation for this profile should be entered manually into the match cell (❺) in the following format:

- If the designation is new the format is xxxxxxxxx UKyz (where 'xxxxxxxxx' is the barcode of the sample being interpreted; 'y' is 'M', 'F' or 'P' representing male, female or person respectively; 'z' is the number of the unknown designation). There should be only one space between the barcode and the designation
- If the designation is pre-existing (either an unknown, a named individual or DNAIntel) then the barcode and name / designation should be copy and pasted from the CPT with a space in between the barcode and the name / designation (Figure 7).



3 – Pre-existing Designation

8.2 Nomination for Upload to NCIDD

When a search against the FR database has been performed (≥12 alleles), the FR will highlight whether a profile / component should be considered for upload to NCIDD as follows:

1. If the profile / component matches to a reference sample in the case and a representative profile has not been nominated for upload to NCIDD, then '[+NCIDD]' will appear in the match cell (Figure 8);
2. If the profile / component is a new unknown for the case or a representative profile of the unknown has not been nominated for upload to NCIDD, then '[+NCIDD]' will appear in the match cell;
3. If a representative profile has previously been nominated for upload to NCIDD and the current profile / component would be a better upload then '[+NCIDD [Replace xxxxxxxxx-yy]]' will appear in the match cell (Figure { SEQ Figure * ARABIC }) where 'xxxxxxxxx' is the barcode of the sample requiring replacement and 'yy' is it's associated suffix. Refer to Section 9 for guidance on replacing a NCIDD upload with a better profile.

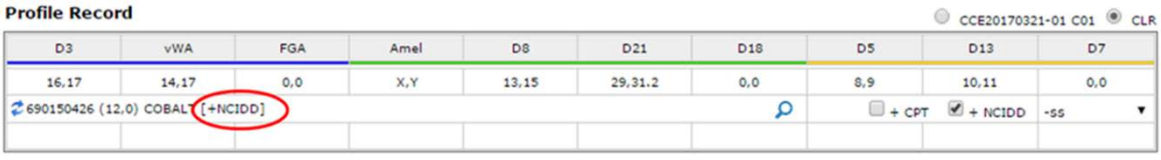


Figure 4 – NCIDD Indicator

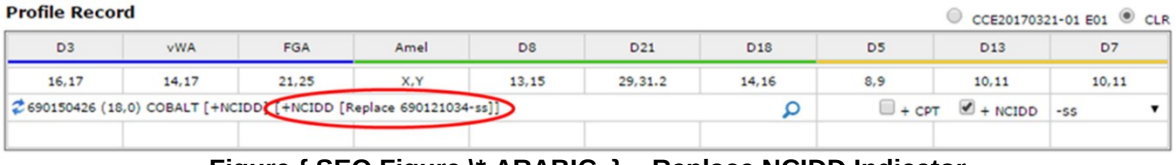


Figure { SEQ Figure * ARABIC } – Replace NCIDD Indicator

8.3 PowerPlex 21

When a DNA profile is obtained that is either single source or one or more contributions are able to be resolved then the Profile Record table is to be completed as per Section 8. Upon review these resolved profiles will be added to the FR database as a record of profiles that have been obtained for this sample. For a PP21 profile, an allele or genotype is considered to be resolved if it has a weighting of ≥ 99% from the STRmix deconvolution.

Clicking the edit icon on the PDA page will enable the Profile Record table to be edited. The profile can then either be entered manually or 'copied down' from the resolved contributions in the Profile Interpretation table or the GeneMapper file (❶ Figure { SEQ Figure * ARABIC }) by clicking on the appropriate circle. If required, the CLR button (❷) will clear the profile entered. If there is more than one GeneMapper file for the sample then there will be the option to choose which result to copy down (distinguished by the CE batch id).

The suffix list (❸) records the type of profile for identification of individual contributions in NCIDD (Table 2). A suffix should be selected for every profile / contribution that is copied down into the Profile Record table. If there are multiple resolved contributions from a mixture, the FR will automatically number this suffix.

If a profile requires upload to NCIDD then a NCIDD process is automatically ordered for each nominated upload (Section 11.1.1).

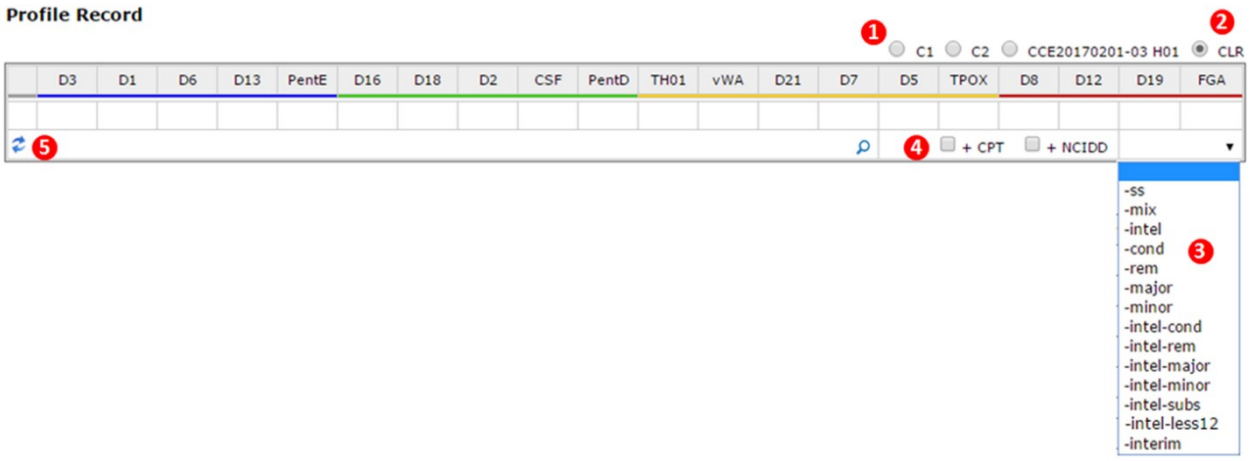


Figure { SEQ Figure * ARABIC } – PP21 Profile Record Table

8.3.1 Single Source Profiles

For important procedural information, refer to Appendix 4 – Profile Data Analysis of a Single Source PP21 Profile for NCIDD.

Single source profiles can be copied down in the following ways:

- By selecting 'C1' (❶ Figure { SEQ Figure * ARABIC }) if a STRmix deconvolution has been uploaded. In this instance the scientist will need to complete the 'Amel' box manually with 'X,X', 'X,Y', 'X,0' or leaving the field blank
- By selecting the appropriate CE batch (❶) if a STRmix deconvolution has not been uploaded. In this instance, any loci that have only one allele will be assigned the values "allele,0" to represent the inability to designate a locus as homozygous without STRmix. Stutter peaks may need to be removed.
- By entering the profile manually

Before saving the record, the scientist will click the ⇄ icon (❺) to commence searching.

If the profile is ≥ 12 alleles then the FR will suggest a designation / match for the profile (refer to Section 8.1 for further details). If the scientist does not agree with the match / designation proposed by the FR database then the scientist must replace it with the correct match / designation in the format described in Section 8.1.

If the profile is <12 alleles then no designation / match will be suggested by the FR and the match cell (❺) will populate with the barcode of the sample. The scientist must enter a designation / match manually in the format described in Section 8.1.

If the profile is required to be loaded to NCIDD then the '+NCIDD' box (❹) should be checked and the '-ss' suffix selected from the drop-down list (❸). If the profile consists of <12 alleles then the '-intel-less12' suffix is to be used. Profiles consisting of <12 alleles are only to be loaded to NCIDD in exceptional circumstances and this should be discussed with a Supervising Scientist or above before doing so. In the case that a profile with <12 alleles is to be loaded to NCIDD the Intelligence Team should be notified as the profile will need to be searched in NCIDD manually and added to a search list.

If the profile is not being uploaded to NCIDD then the '-ss' suffix should be selected from the drop-down list (❸).

If the profile is unknown and is not listed in the 'Case Profiles' table then the '+CPT' box must be checked to add the profile to the table.

If a reference sample matches the casework profile and they are an assumed known contributor then the appropriate 'AC' box must be checked in the Case Profiles table (Section 9).

Table { SEQ Table * ARABIC } – Suffix Meanings

Suffix	Purpose	Kit
-ss	Single source component	PP21
-mix	Fully deconvoluted mixture component	PP21
-intel	Partially deconvoluted mixture component	PP21
-cond	Conditioned component	PP21
-rem	Remaining component	PP21
-intel-cond	Conditioned component where the profile is conditioned for intelligence purposes only	PP21
-intel-rem	Remaining component where the profile is conditioned for intelligence purposes only	PP21
-intel-subs	Single source component where sub-threshold peaks are used for intelligence purposes only	PP21
-intel-less12	Single source component with less than 12 alleles	PP21
-interim	Any component that is loaded as an interim measure pending rework results	P1 cases only

8.3.2 Resolved (Fully or Partially) Mixed Profiles

When a profile can be resolved (fully or partially) into its individual contributions, and the STRmix PDF has been uploaded, then each contribution should be recorded in the Profile Record table as per Section 8. This is done as follows:

a. Click the edit icon
b. Copy down the profile by selecting the appropriate contribution
c. Complete the 'Amel' box manually with 'X,X', 'X,Y' or 'X,0'
d. Click the icon (❺ Figure { SEQ Figure * ARABIC }) to commence searching
e. Check / enter the match / designation into the match cell (❺) in the format as described in Section 8.1
f. Check the '+NCIDD' box (❹) if this contribution is required to be loaded to NCIDD (ticking this box triggers the ordering of an 'NCIDD' process when the record is saved)
g. Select the appropriate suffix. If appropriate, FR will add numbering (Table { SEQ Table * ARABIC })
h. Check the '+CPT' box (❹) if this contribution is a new designation for the case
i. Save the record by clicking the save icon
j. If appropriate, repeat the process for the other resolved contributions of the profile (≥ 12 alleles)

When accepting a mixed profile with a labelled stutter peak (below the laboratory stutter threshold) that is presenting in the Profile Record table as an allele because it is above the STRmix stutter threshold, add explanatory notes to the 'Notes' field including any amendments necessary due to uploading to NCIDD.

8.3.3 Complex Profiles

If a profile is considered unsuitable for interpretation, it is not necessary to copy down the result. In this instance only the CX or PU box is checked in the Profile Interpretation table.

The PDA analyst may add notes to the Sample Notes section explaining why the profile has been assessed as complex, particularly if the reasons for this determination are not obvious (e.g. unable to determine the number of contributors or some unusual processing / analysis issue).

8.3.4 Tri Alleles

The Profile Record table should only contain two allele designations. If a tri-allele is obtained, drop the locus and add a sample note stating that a tri-allele is present and all three designations. If the profile is required for upload to NCIDD then the details of the tri allele should be added to the 'NCIDD User Comment' field in the NCIDD process (Section 11.1). This will assist with the investigation of any potential mis-matches on NCIDD.

9 Case Profiles Table (CPT)

This table lists all of the reference samples (including their NCIDD category) associated with a case and can be expanded to include unknown profiles and profiles matching to DNAIntel samples.

All evidence reference samples that are associated to the case, including reference samples that are registered by Forensic DNA Analysis, will populate this table by default.

Unknowns populate the table at the request of the scientist by checking the '+CPT' box in the Profile Record table when an unknown is designated. The barcode in the table relating to the unknown is the barcode of the exhibit from which the unknown originated. If a better profile of the unknown is obtained from another exhibit in the case, the table should be updated by checking the '+CPT' box relating to the better profile and replacing the matching barcode in the match cell with the barcode of the exhibit from which the better profile has been obtained. This will ensure that the barcode of the better unknown profile populates the Case Profiles table.

For example, the exhibit in Figure { SEQ Figure * ARABIC } has the barcode 690153578. You can see that it has matched to UKM1 which has come from exhibit 690153595 (displayed in the match cell of the Profile Record table). Exhibit 690153578 has a better profile for UKM1 than its originating exhibit (690153595). In order to update the profile for UKM1, the barcode in the match cell (inside the red circle) needs to be manually edited and replaced with barcode 690153578. This will enable both the profile and originating barcode for UKM1 to be updated.

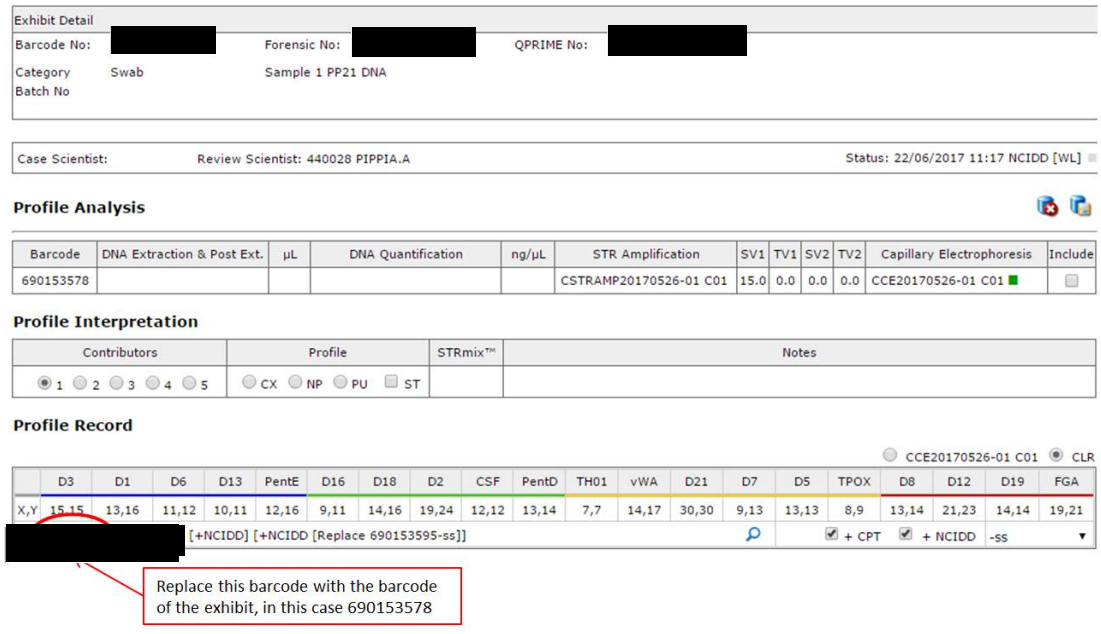


Figure { SEQ Figure * ARABIC } – Updating Unknown Profile in CPT

Any unknown profiles should be added to the Case Profiles table to alert scientists working on other samples in the case that the unknown has been identified.

Profiles matching to DNAIntel samples populate the table at the request of the scientist by checking the '+CPT' box in the Profile Record table when a DNAIntel match is identified.

ALL newly identified unknown profiles and profiles matching to DNAIntel samples should be added to the Case Profiles table.

Clicking on the barcode associated with the reference sample, DNAIntel sample or unknown will open the PDA page for that sample.

Clicking the icon above the Case Profiles table will produce a table in a new window containing the profiles of all of the samples in the Case Profiles table (Figure 12).

Once the table has been produced, the information can be displayed in a number of ways. Clicking [P+] will display the only the Profiler Plus loci, clicking [PP21] will display the PP21 loci. Clicking [P+ All] or [PP21 All] will display all profiles copied down into the Profile Record table for all exhibits in the case in the appropriate format.

A reference sample profile will not populate this table until the reference sample PDA page has been validated. Refer to QIS 34245 for information regarding the profile data analysis of reference samples.

Figure { SEQ Figure * ARABIC } – Profile Table

There are a number of indicators in the category column of the Case Profiles Table (Table 3).

Table 2 – Indicators in CPT

Profile Type	Indicator	Colour	Meaning
Reference sample	Square	Orange	Reference sample is being processed
		Green	Reference sample has been validated
	Triangle	Orange	Crime scene profile matching to reference sample has been nominated for upload to NCIDD
		Green	Crime scene profile matching to reference sample has been nominated for upload to NCIDD and this nomination has been validated
Unknown	Square	Orange	Unknown crime scene profile has been identified
		Green	Unknown crime scene profile has been identified and validated
	Triangle	Orange	Unknown crime scene profile has been nominated for upload to NCIDD
		Green	Unknown crime scene profile has been nominated for upload to NCIDD and this nomination has been validated

If a profile previously designated as an unknown subsequently matches to an associated reference sample or an identified DNAIntel sample, this is documented in the 'Association' column. Whilst in edit mode, the barcode of the matching reference sample can be entered into the association field that accompanies the unknown. In turn the association field that accompanies the matching reference sample will update with the unknown designation. Only the association fields that accompany an unknown or DNAIntel sample are able to be edited. Also refer to Section 14.2.

9.1 PowerPlex 21

The Case Profiles table is used to record the results of reference sample comparisons to all crime scene profiles and to store STRmix PDFs relating to LR calculations (Figure { SEQ Figure * ARABIC }).

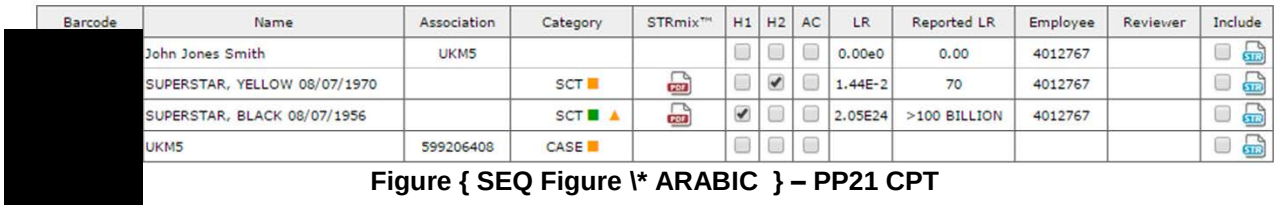


Figure { SEQ Figure * ARABIC } – PP21 CPT

If a STRmix PDF for a LR is loaded into the FR then the LR relating to the comparison of the reference sample will populate the 'LR' box for that reference sample. At the same time the appropriate 'H1' and 'H2' boxes will be checked and the figure will transform into a usable format in the 'Reported LR' box. Alternatively the scientist may enter the LR manually into the 'LR' box in the format described below and the remaining boxes will fill automatically.

A description of the columns used to record the interpretation is as follows:

'H1' – this box is checked if the LR favours contribution
'H2' – this box is checked if the LR favours non-contribution
'AC' – this box is checked if the reference sample is an assumed contributor either for single source profiles or conditioned mixtures (this is a manual function only)
'LR' – the LR in its original form is entered here, this must be in scientific number format to two decimal places, e.g. 1.54e4, 2.63e-7

'Reported LR' – the LR entered into the 'LR' column is transformed by the FR into the format that is required for statement purposes

If the reference sample is an assumed contributor then the scientist should check the 'AC' box. For all other interpretations the analyst is only required to enter the LR in its original form into the 'LR' column. The FR will then check the appropriate 'H1' and / or 'H2' boxes and transform the original number into numbers greater than one as described in the following examples:

LR of 1.10e-4 is entered	→	FR will check 'H2' box to indicate LR favours non-contribution and will populate the 'Reported LR' column with the reciprocal of 1.10e-4
LR of 5.71e17 entered	→	FR will check 'H1' box to indicate LR favours contribution and will populate the 'Reported LR' column with ">100 BILLION"
LR of 1.00e12 entered	→	FR will check 'H1' box to indicate LR favours contribution and will populate the 'Reported LR' column with ">100 BILLION" (single source profile matching to an associated reference sample profile)
LR of 0.00e0 entered	→	FR will not check any boxes but will populate the 'Reported LR' column with "0.00" (exclusion)
LR of 1.00e0 entered	→	FR will check both the 'H1' and 'H2' boxes and populate the 'Reported LR' column with "1.00" (inconclusive)

The LR can be manually edited after a STRmix PDF for an LR has been uploaded. For example, if an LR has been calculated for a reference sample but the analyst subsequently decides the reference sample is intuitively excluded. Entering 0 into the LR column will override the LR and exclude the relevant reference sample.

Unknown profiles are not compared to casework profiles for the purposes of calculating a LR routinely; these calculations are to be performed for P1 samples and at the request of the QPS only. If a LR is calculated for an unknown, the STRmix PDF file is added to a notation and the LR is entered by the analyst.

10 DNAIntel Matching

Person sample profiles with the descriptor "DNA Person Sample Intel" are termed 'DNAIntel samples' and are held in the FR database. When a profile is copied down into the Profile Record table and the search button clicked, this profile will be searched against the FR database as per Section 8.1.

If a profile in the Profile Record table matches to a single DNAIntel sample then this match will be displayed in the match cell (Figure { SEQ Figure * ARABIC }).

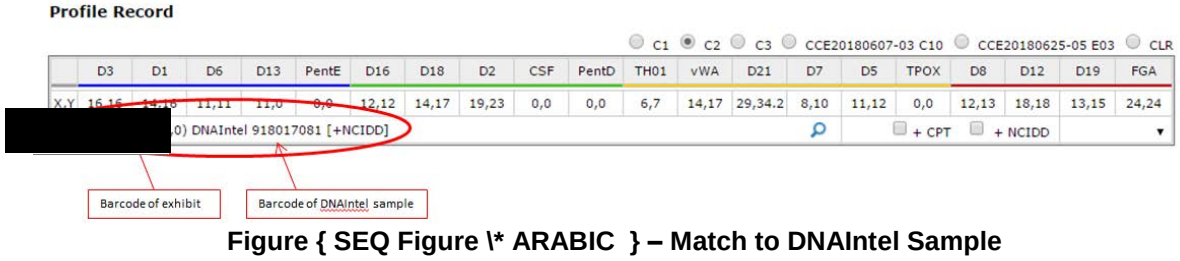


Figure { SEQ Figure * ARABIC } – Match to DNAIntel Sample

If a profile in the Profile Record table matches to multiple DNAIntel samples then the match cell will default to the next unknown designation. Any DNAIntel sample matches can be seen

in a separate table by clicking the button to the right hand side of the match cell (Figure 15).

Figure 5 – Match to Multiple DNAIntel Samples

If a profile matches to multiple DNAIntel samples this will not be seen unless the button is clicked as the search will default to the next unknown designation. These DNAIntel matches do not require actioning and the unknown designation can be reported.

If a profile matches to a single DNAIntel sample then this match will be seen in the match cell and the DNAIntel sample profile can be accessed by clicking the button. The profile analyst should compare the DNAIntel sample to the copied down profile of the crime scene sample and the epgs to ensure that there are no exclusions either above or below threshold. It is not necessary to locate the epg of the DNAIntel sample to perform this comparison, it is sufficient to use the allele designations within the table produced by the FR.

If the DNAIntel sample is excluded then the profile analyst should overwrite the information in the match cell with the appropriate match / designation in the format described in Section 8.1.

If the DNAIntel sample is not excluded then the DNAIntel profile should be added to the Case Profiles table (as per Section 9) if it is not there already. This match should then be reported to the QPS via an Exhibit Result line in the same way as an unknown profile, i.e. by using the appropriate line(s) for the interpretation and placing the barcode of the DNAIntel sample in the 'Linked No.' field.

Since DNAIntel samples are reported in the same way as unknown profiles, it is only necessary to report the result to the QPS if the profile is single source or if a component is being uploaded to NCIDD. LR calculations are not required.

In some instances, a DNAIntel match may be obtained part way through the case, i.e. what has previously been reported as UKM1 now matches to a DNAIntel sample. If this occurs, the following process should be followed:

1. Do not update any previously reviewed results
2. For the new sample with the DNAIntel match, in the Profile Record table, tick the '+CPT' box but do not tick the NCIDD box. Click save
3. In the Case Profile table, associate the new sample with the DNAIntel match to the previously reviewed result with UKM1. Add a sample note 'UKM1 = DNAIntel XXX'
4. All samples analysed from this point forward should refer to the DNAIntel match where required

11 Exhibit Testing Table

The Exhibit Testing table is on the Exhibit Testing / Movement page (Figure 16) which can be accessed from the PDA page by clicking the icon in the top right corner of the page.

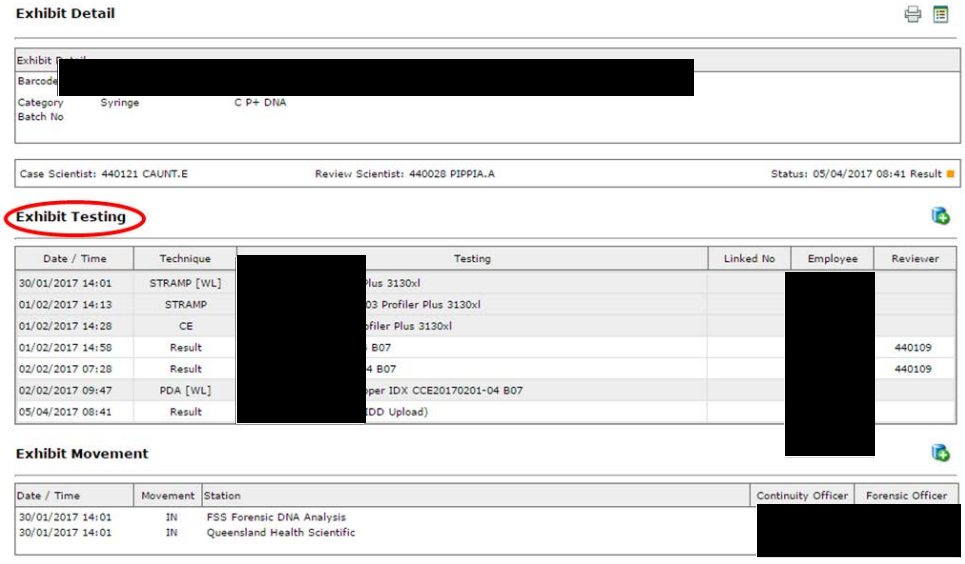


Figure 6 – Exhibit Testing / Movement Page

The Exhibit Testing table records all testing and analyses that have been performed on a sample, all techniques (via worklists) that have been ordered for a sample and all results that have been reported for a sample. The information within the table includes the date and time that the testing was ordered, the type of test and the person that performed / reviewed the test. A test can be added by clicking the add button ( ) which opens the page shown in (Figure 17).

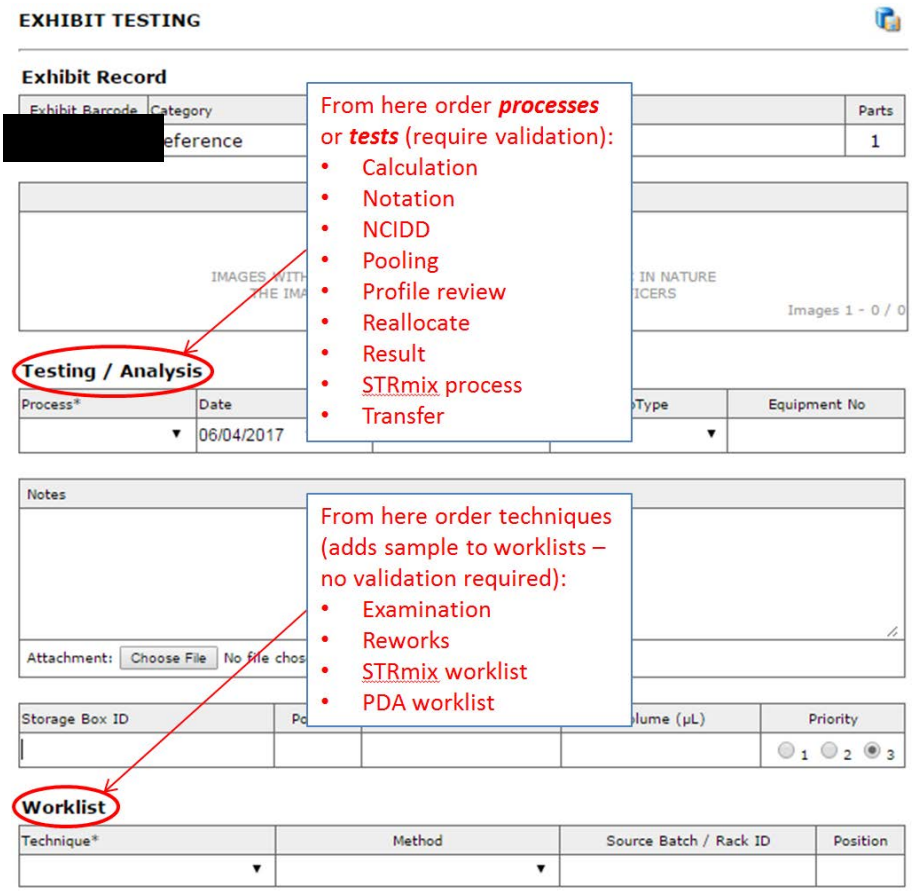


Figure 7 – Page to Order Processes and Techniques

All tests require validation however some tests auto-validate. A test that is awaiting review has an orange box beside it in the exhibit testing table; this box turns green when the review is complete.

Entries in the exhibit testing table that have a grey background represent techniques or worklist / batch list entries. Where applicable the coloured boxes for these entries represent the status of the batch (Section 6.1). Entries in the exhibit testing table that have a white background represent tests that require validation.

If the scientist requires a rework to be performed on a sample, it will be ordered from this page.

11.1 NCIDD

11.1.1 NCIDD Upload

For important procedural information, refer to Appendix 4 – Profile Data Analysis of a Single Source PP21 Profile for NCIDD.

Each crime scene profile / component that is to be loaded to NCIDD must have an NCIDD process ordered. An ‘NCIDD’ process is ordered automatically when the ‘NCIDD’ box on the PDA page is ticked and the record is saved (Section 8.3.2 and Figure 4 – NCIDD Indicator).

If necessary, an NCIDD process may be ordered manually as follows:

1. Select ‘NCIDD’ from the ‘Process’ drop-down menu (Figure 18)

2. Select ‘Upload’ from the ‘NCIDD Method’ drop-down menu
3. Enter the suffix of the profile / component to be uploaded in the exact same format as it is in the profile record table after the barcode number that is already present in this field
4. If required, enter any tri-allele information
5. Click the save icon to save the record
6. Upon validation by the reviewer, this request will automatically populate the NCIDD worklist
7. Repeat this process for all profiles / components to be uploaded for the sample

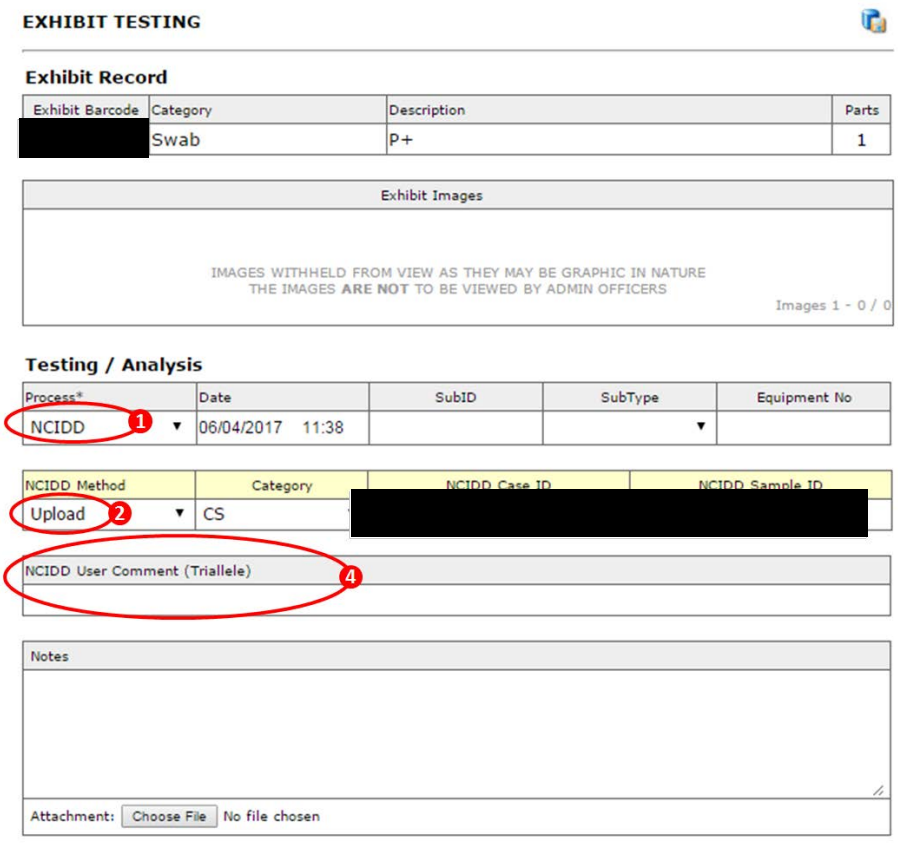


Figure 8 – NCIDD Upload

11.1.2 Modify Profile on NCIDD

If a better profile has been obtained from a different sample within a case and this profile is to be uploaded to NCIDD or if a better profile has been obtained from the same sample but the suffix has changed (e.g. –intel has changed to –mix) then the modify process is not used. In this case, the sample that is being replaced is to be removed from NCIDD and the better profile is to be uploaded to NCIDD as a new upload.

If a better profile has been obtained from the same sample, for example through rework, AND the suffix has not changed then the modify process will be used as follows:

1. On the PDA page, copy down the new profile and select the suffix that is the same as the suffix used for the profile being modified (Figure 19)
2. Select ‘NCIDD’ from the ‘Process’ drop-down menu (Figure 8)
3. Select ‘Modify’ from the ‘NCIDD Method’ drop-down menu
4. Enter the suffix of the profile / component being modified ‘-yyyy’ after the barcode number that is already present in the NCIDD Sample ID field

5. Enter the reason why the profile / component is being modified in the Notes section
6. Click the save icon to save the record
7. Upon validation by the reviewer, this request will automatically populate the NCIDD worklist

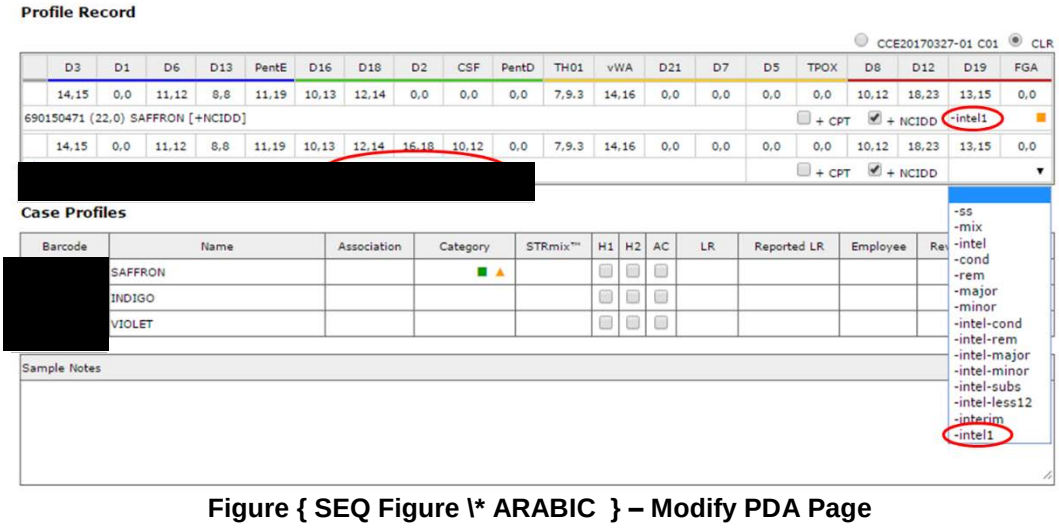


Figure { SEQ Figure * ARABIC } – Modify PDA Page

A profile that has been modified will remain in the Profile Record table but will be greyed out (Figure 20).

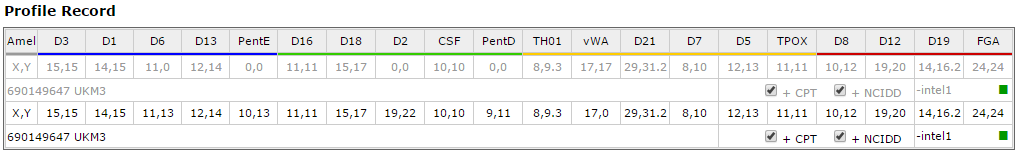


Figure 9 – Modified Profile

11.1.3 Remove Profile from NCIDD

A profile / component can be removed from NCIDD as follows:

1. Select ‘NCIDD’ from the ‘Process’ drop-down menu (Figure 18)
2. Select ‘Delete’ from the ‘NCIDD Method’ drop-down menu
3. Enter the suffix of the profile / component to be removed in the format ‘-yyyy’ after the barcode number that is already present in the NCIDD Sample ID field
4. Enter the reason why the profile / component is being removed in the Notes section
5. Click the save icon to save the record
6. Upon validation by the reviewer, this request will automatically populate the NCIDD worklist

The Exhibit result line associated with removing a profile from NCIDD should not have anything in the Linked No. field (Section 18).

11.2 Ordering Reworks

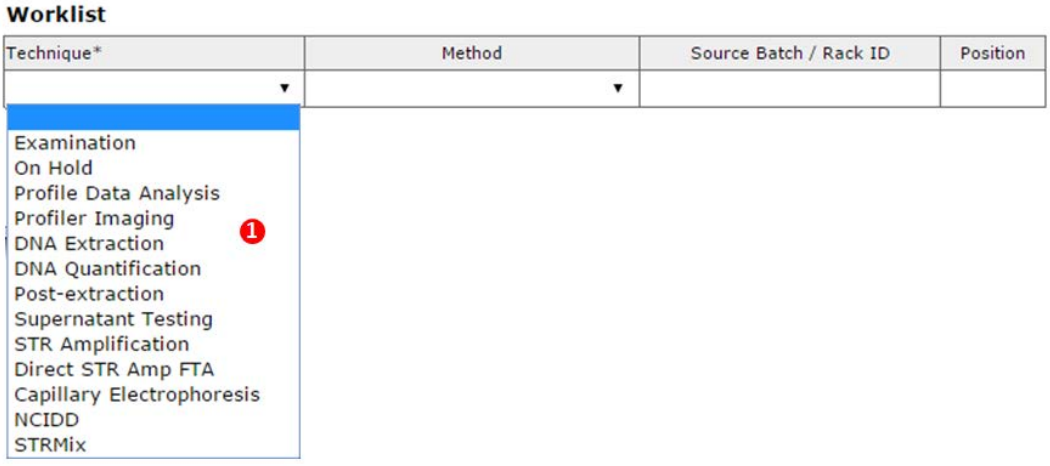


Figure { SEQ Figure * ARABIC } – Technique / Worklist Ordering

11.2.1 Re-amplification

1. If the sample is a dilution with a child / subsample barcode, add the dilution barcode to the 'SubID' field
2. Select 'STR Amplification' from the dropdown list (1Figure 21)
3. An amplification method can be chosen from the drop down menu under 'Method' (Figure { SEQ Figure * ARABIC })

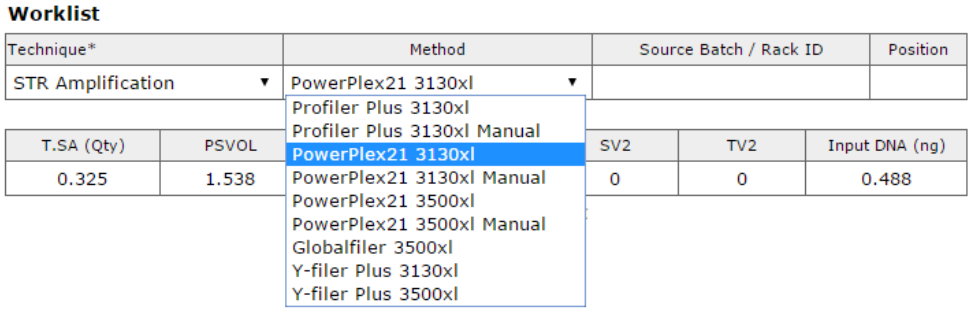


Figure { SEQ Figure * ARABIC } – Amplification Methods

4. An additional box will appear that shows the quant value for the sample and volumes required to amplify at the optimal amount (Figure 23). These volumes can be edited to enable the scientist to change the amount of DNA amplified

T.SA (Qty)	PSVOL	SV1 (µL)	TV1	SV2	TV2	Input DNA (ng)
0.325	1.538	1.5	13.5	0	0	0.488

Figure 10 – Amplification Volumes

5. Click the save icon to save the record (pressing enter will cause the record to save; use the 'tab' key or the mouse to move between fields)
6. This request will automatically populate the appropriate analytical worklist

For PP21, change the default method of 'PowerPlex21 3130xl' in the method box to 'PowerPlex21 3500xl'.

If the sample is likely to have less than 20 µL of extract remaining (if it has previously undergone microcon concentration) then a manual amplification method must be ordered as there will be insufficient volume to be pipetted on the automated platform. Also, if the sample is likely to have less than the requested amplification volume remaining, add an analytical note when the technique is ordered, requesting that all of the remaining extract is amplified.

11.2.2 Microcon / Nucleospin / Dilution

1. Select 'Post-extraction' from the dropdown list (❶Figure 21)
2. The default entry is Microcon PowerPlex 21 however an alternative post-extraction method can be chosen from the drop down menu under 'Method' (Figure { SEQ Figure * ARABIC })

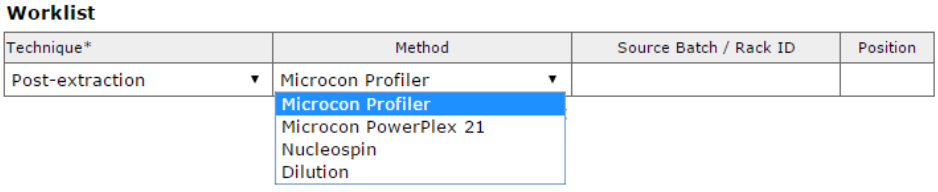


Figure { SEQ Figure * ARABIC } – Post Extraction Methods

3. An analytical note can be added at the same time as requesting the rework to request microcon volumes and dilutions (Section 11.2.6)
4. Click the save icon to save the record (pressing enter will cause the record to save; use the 'tab' key or the mouse to move between fields)
5. This request will automatically populate the appropriate analytical worklist

The default final volume for a PP21 microcon is 35 µL; an analytical note is only required for any exceptions outside of this, e.g. M'con to full.

11.2.3 Re-CE

1. Select 'Capillary Electrophoresis' from the dropdown list (❶Figure { SEQ Figure * ARABIC })
2. For PP21, change the default method of 'PowerPlex21 3130xl' in the method box to 'PowerPlex21 3500xl' (Figure 25)

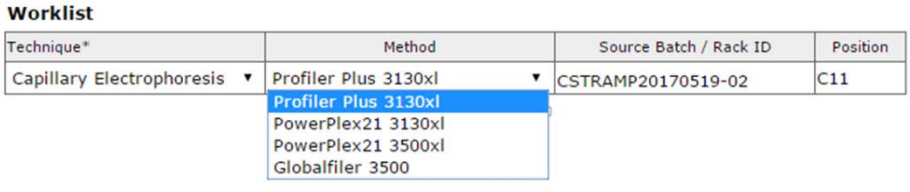


Figure 11 – CE Methods

3. Enter the batch ID and position number of the amplification batch containing the amplification product requiring re-CE by copy and pasting from the PDA page
4. Click the save icon to save the record (pressing enter will cause the record to save; use the 'tab' key or the mouse to move between fields)
5. This request will automatically populate the appropriate analytical worklist

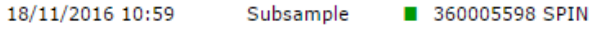
With the implementation of the STARlet in CE, if a DNA profile is obtained from a sample that was previously NAD, the second run can be accepted without further confirmation for both casework and reference samples.

11.2.4 Re-quantification

1. Select 'DNA Quantification' from the dropdown list (❶Figure 21)
2. Quantifiler Trio is the only Method currently available
3. An analytical note can be added at the same time as requesting the rework to request a 'quant and hold' (Section 11.2.6)
4. Click the save icon to save the record (pressing enter will cause the record to save; use the 'tab' key or the mouse to move between fields)
5. This request will automatically populate the appropriate analytical worklist

11.2.5 Re-extraction of Spin Baskets

1. Find the barcode of the spin basket for your sample from the exhibit testing table



2. Register the subsample as an exhibit as described in Appendix 9 – Registering a Subsample as an Exhibit
3. Navigate to the exhibit testing table
4. Open up a test page by clicking the add button ( )
5. Select 'DNA Extraction' as the technique and select the required extraction method (Figure { SEQ Figure * ARABIC })

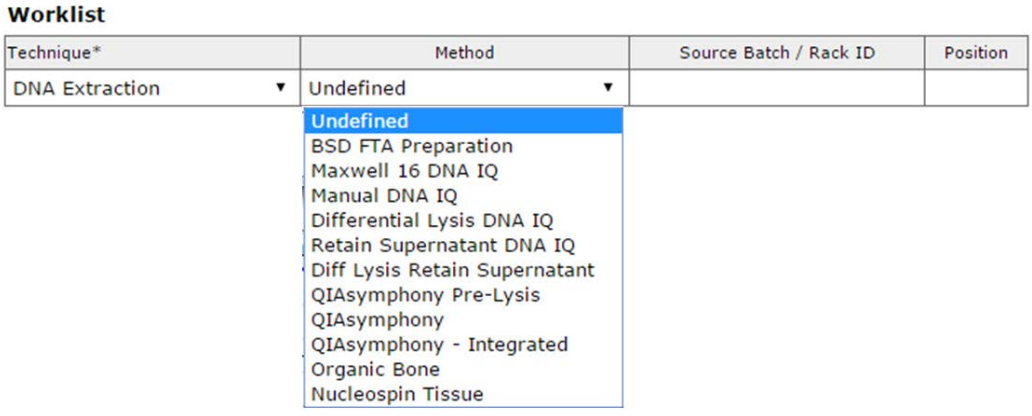


Figure { SEQ Figure * ARABIC } – Re-extraction

6. Click the save icon to save the record (pressing enter will cause the record to save; use the 'tab' key or the mouse to move between fields)
7. This request will automatically populate the appropriate analytical worklist

11.2.6 Analytical Notes

1. To enter an analytical note for a sample (e.g. M'con to full), select 'Analytical Note' from the dropdown list under the 'Testing / Analysis' heading (❶Figure 27).
2. Enter the details of the analytical note into the 'Notes' section (❷)
3. Click the save icon to save the record (pressing enter will cause the record to save; use the 'tab' key or the mouse to move between fields)
4. This request will automatically highlight to analytical scientists that an analytical note exists for that sample
5. An analytical note can be added at the same time any relevant technique is requested

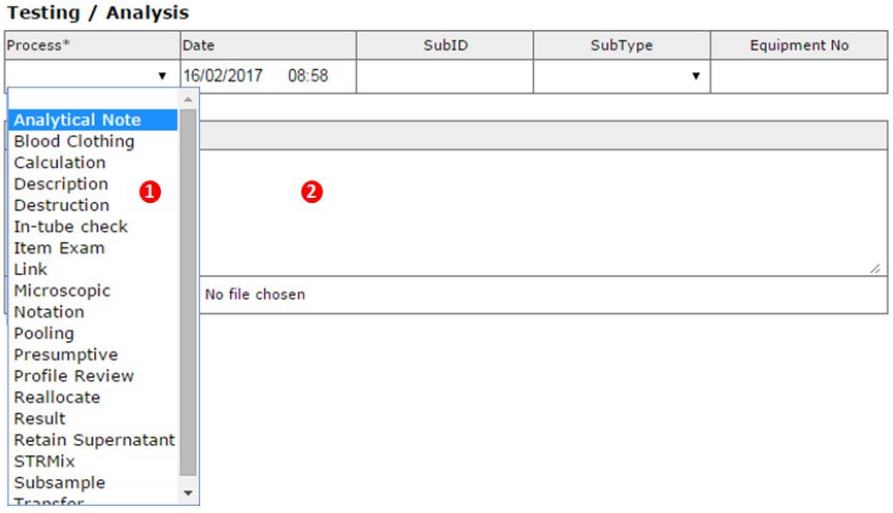


Figure 12 – Analytical Notes

11.2.7 Reviewing

If the PDA Analyst / Reviewer considers further processing / reworking is necessary during the review process, the PDA Analyst will select '[CLICK TO REWORK]' from the profile review page and rework as per Section 11.2 (QIS 34006 – Section 4.4.7).

11.3 Pooling

Before pooling, each sample / subsample to be pooled must be registered as an exhibit (Appendix 9 – Registering a Subsample as an Exhibit). Once registered correctly, refer to Appendix 11 – Process for Pooling Samples.

11.4 Transfer

Samples processed pre-batch functionality were assigned a DNA number. On occasion it is necessary to rework these samples with new technology. Refer to Appendix 10 – Processing of DNA Number Exhibits for the workflow required to register this sample in the FR and order a 'Transfer' process.

11.5 Changing Priority

The priority of samples is set by the QPS, with major crime samples being Priority 1 or 2 and volume crime samples being Priority 3.

It may be necessary for the scientist to change the priority of a sample, for example to meet a court date. This can be done as follows:

1. Select 'Notation' from the 'Process' drop-down menu (Figure { SEQ Figure * ARABIC })
2. Enter appropriate notes in the 'Notes' section
3. Change the priority
4. Click the save icon to save the record
5. This request will automatically update the priority of the sample in all worklists

The priority of a sample can only be changed if there is a process selected.

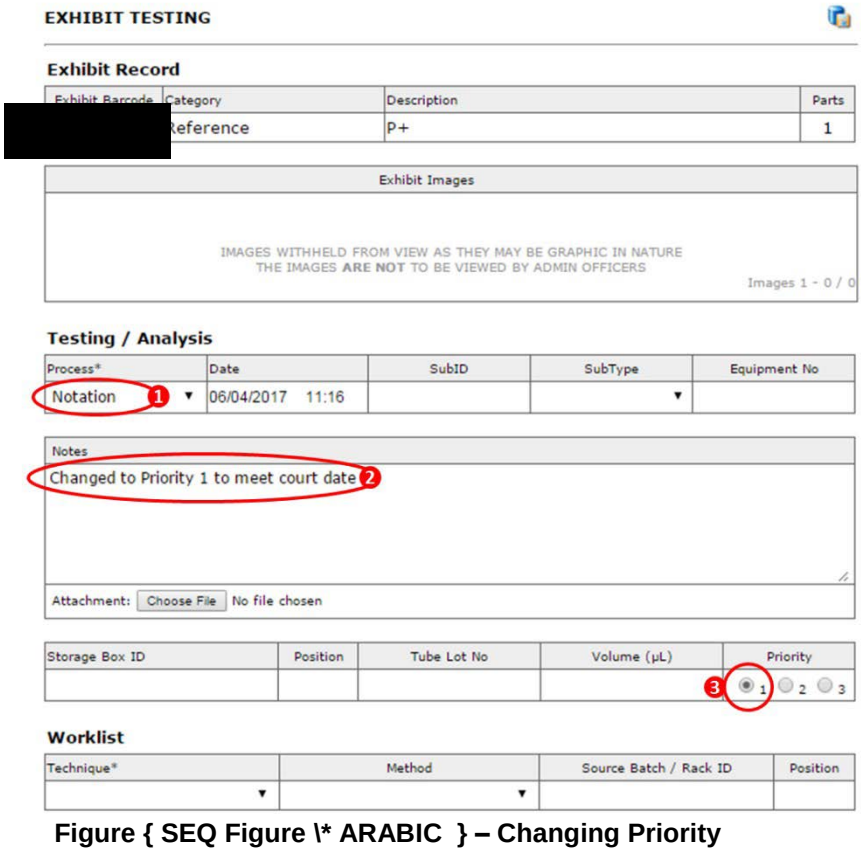


Figure { SEQ Figure * ARABIC } – Changing Priority

11.6 Requesting Additional Examinations

11.6.1 Re-examination

1. Select ‘Examination’ from the ‘Technique’ drop-down menu (Figure 29)
2. Select ‘Re-examination’ from the ‘Method’ drop-down menu
3. Select ‘Notation’ from the ‘Process’ drop-down menu
4. Enter appropriate notes in the ‘Notes’ section
5. Click the save icon to save the record
6. This request will automatically populate the Examination worklist

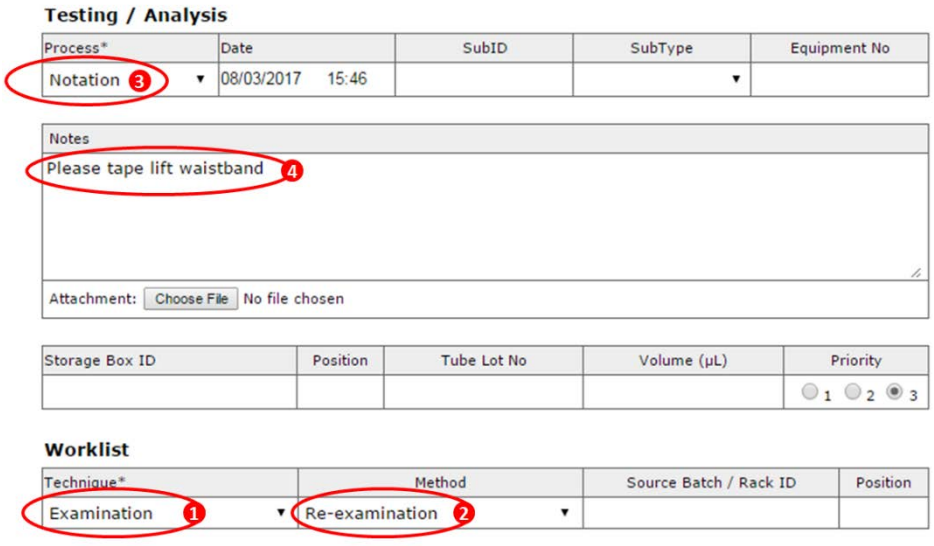


Figure 13 – Re-examination Ordering

11.6.2 Diff Slide Examination

1. Select ‘Examination’ from the ‘Technique’ drop-down menu (Figure 30)
2. Select ‘Slide microscopy’ from the ‘Method’ drop-down menu
3. Enter the subsample barcode of the diff slide in the ‘SubID’ box (the SubID of the slide can be found in the exhibit testing table)
4. Click the save icon to save the record
5. This request will automatically populate the Examination worklist

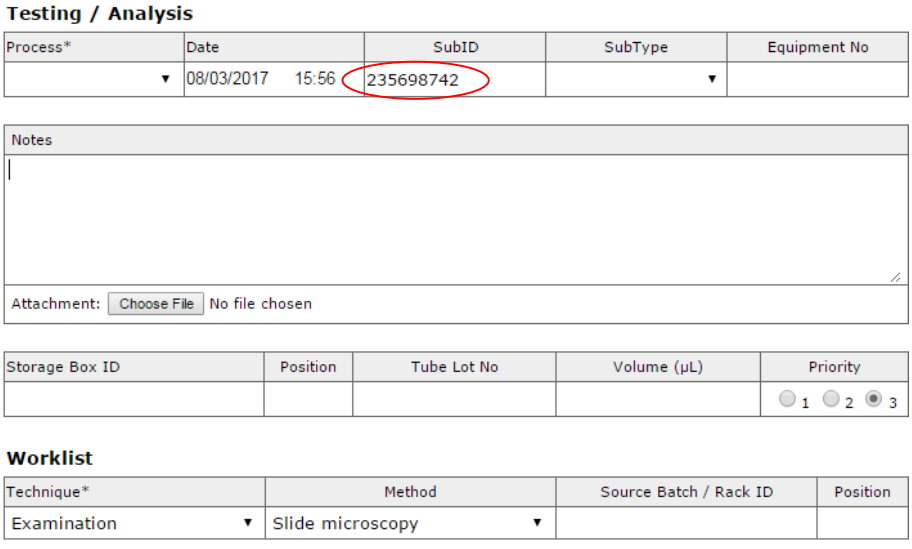


Figure 14 – Diff Slide Examination Request

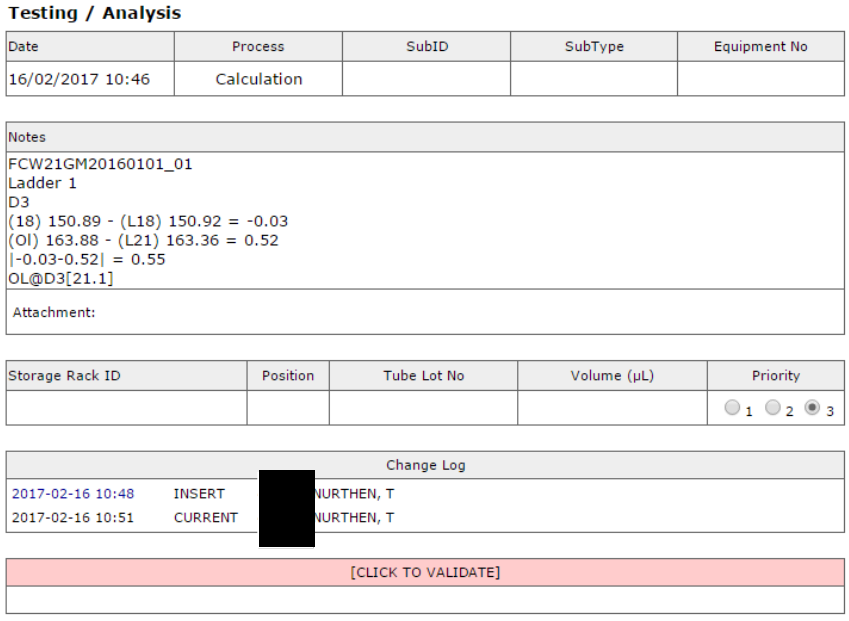
11.6.3 Examination of Prioritised Exhibits

Select ‘Examination’ from the ‘Technique’ drop-down menu (Figure 30)
Select ‘Item Exam’ from the ‘Method’ drop-down menu
Click the save icon to save the record
This request will automatically populate the Examination worklist

11.7 Calculations

If a VAR / OLA / ULP / XOVER calculation is required at plate reading the plate reader will order a 'Calculation' process and record the details of the calculation in the 'Notes' section (Figure { SEQ Figure * ARABIC }). The profile analyst will enter into the calculation record and check the calculation. If the calculation is correct then the profile analyst will validate the record by clicking the red 'CLICK TO VALIDATE' bar. If there is an error in the calculation the profile analyst may correct the error however they will be unable to validate the record. In this instance the reviewer will validate the calculation. Alternatively, the plate reader may be contacted to amend the calculation.

For complex profiles not suitable for interpretation, calculations do not need to be checked provided the calculation is not critical to the interpretation. The profile analyst should add 'Calculation not checked' to the top of the 'Notes' section in the calculation record for the reviewer to validate.



Testing / Analysis

Date	Process	SubID	SubType	Equipment No
16/02/2017 10:46	Calculation			

Notes
FCW21GM20160101_01 Ladder 1 D3 (18) 150.89 - (L18) 150.92 = -0.03 (OI) 163.88 - (L21) 163.36 = 0.52 |-0.03-0.52| = 0.55 OL@D3[21.1]
Attachment:

Storage Rack ID	Position	Tube Lot No	Volume (µL)	Priority
				1 2 3

Change Log		
2017-02-16 10:48	INSERT	[redacted]NURTHEN, T
2017-02-16 10:51	CURRENT	[redacted]NURTHEN, T

[CLICK TO VALIDATE]

Figure { SEQ Figure * ARABIC } – Calculations

11.8 Notations

A 'Notation' can be used to record notes against a sample, however it is preferable to use the 'Sample Notes' on the PDA page as this is more easily seen and likely to be included in a case file.

See points 3-6 in Section 11.6.1 for the process for ordering a notation.

Notations self-validate upon saving.

If the sample is on the 'On Hold' worklist, adding a notation will remove it from this list. If this occurs and the sample needs to be on the 'On Hold' list, it will need to be re-added manually.

11.9 Reallocate

The ‘Reallocate’ process is used to remove a sample from a worklist where the most recent entry in the exhibit testing table displays [WL]. For example, if a re-amp is ordered in error, the sample can be removed from the amplification worklist using the ‘Reallocate’ process as follows.

Order a ‘Reallocate’ process from the drop-down menu (Figure { SEQ Figure * ARABIC }) and add a note stating the reason for the reallocate.

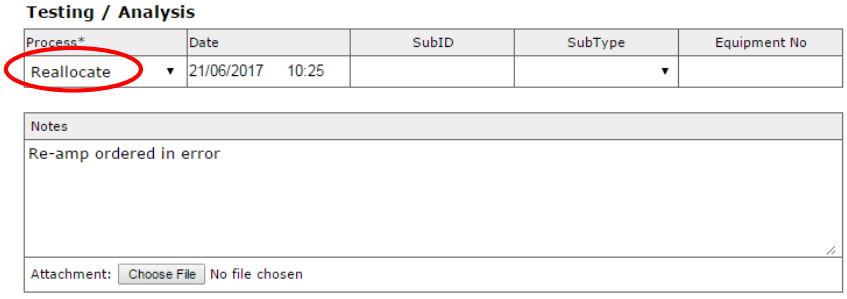


Figure { SEQ Figure * ARABIC } – Reallocate Process

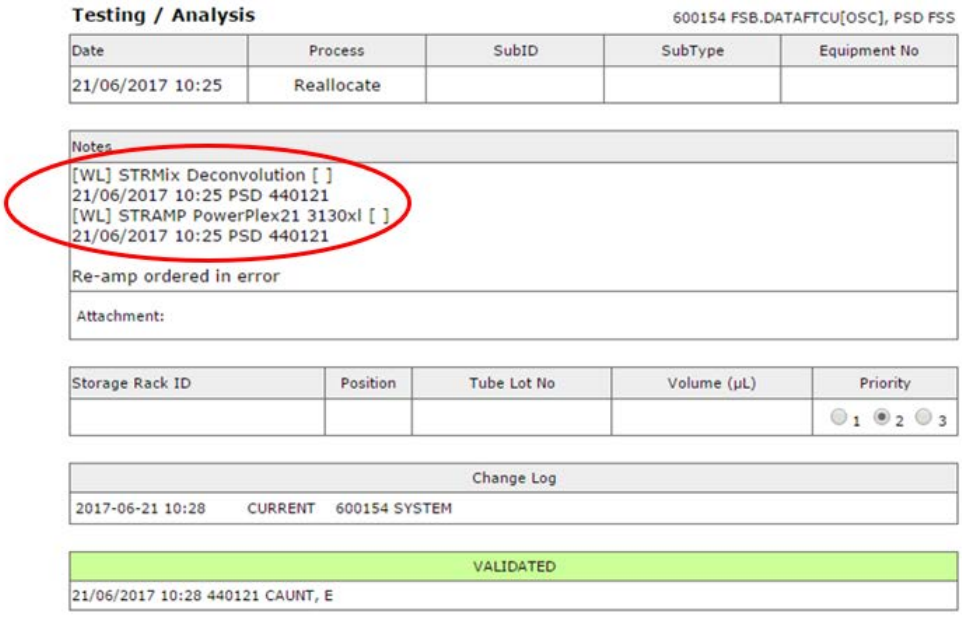
By ordering a ‘Reallocate’ the sample will be removed from ALL worklists that it is currently sitting on. The scientist will need to add the sample back onto the worklists that it should remain on.

The ‘Reallocate’ process and the worklist the sample has been removed from will be visible in the Exhibit Testing table (Figure 33).

Figure 15 – Reallocate Display

Clicking on the date and time relating to the reallocate will open the record. This record shows all of the worklists that the sample has been removed from if multiple worklists are involved. From this, the scientist can see what worklists that sample needs to be added to.

The sample below (**Error! Reference source not found.**) has been removed from the STRmix and STRAMP worklists. Since the sample was added to the amplification worklist in error, it only needs to be added back to the STRmix worklist.



Testing / Analysis 600154 FSB.DATAFTCU[OSC], PSD FSS

Date	Process	SubID	SubType	Equipment No
21/06/2017 10:25	Reallocate			

Notes

[WL] STRMix Deconvolution [ ]
21/06/2017 10:25 PSD 440121
[WL] STRAMP PowerPlex21 3130xl [ ]
21/06/2017 10:25 PSD 440121

Re-amp ordered in error

Attachment:

Storage Rack ID	Position	Tube Lot No	Volume (µL)	Priority
				1 2 3

Change Log

2017-06-21 10:28 CURRENT 600154 SYSTEM

VALIDATED

21/06/2017 10:28 440121 CAUNT, E

Figure 16 – Reallocate Example

Reallocate processes self-validate upon saving.

If a sample is only added to a worklist when a process is ordered, e.g. ‘Profile Review’ (no ‘[WL]’ associated with the entry in the exhibit testing table), this sample can be removed from the worklist by selecting ‘[CLICK TO REWORK]’ from the profile review page or by making the process incorrect (Section 13).

11.10 Staff Matches

If a match is obtained to the staff elimination database (Section 8.1) then this needs to be investigated by the Quality team. The sample should be placed on the ‘On Hold – Quality Review’ worklist as follows:

1. Select ‘On Hold’ from the ‘Technique’ drop-down menu (Figure 35)
2. Select ‘Quality Review’ from the ‘Method’ drop-down menu
3. Select ‘Result’ from the ‘Process’ drop-down menu
4. Enter a note for the Quality team
5. Click the save icon to save the record
6. This request will automatically populate the ‘On Hold’ worklist

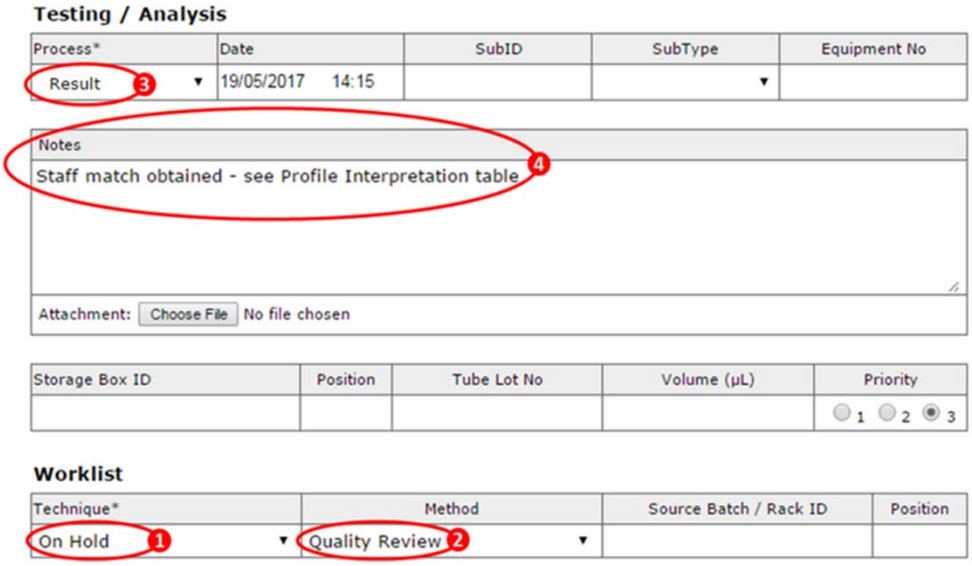


Figure 17 – Staff Match

If the sample is on the 'On Hold' worklist then adding a notation will remove it from this list (this is why a 'Result' is added). If this occurs and the sample needs to be on the 'On Hold' list, it will need to be added manually.

12 STRmix Analysis

STRmix input files for casework samples and reference samples that have been associated to a case are created by the FR. These can be downloaded by clicking the icons located in the Profile Analysis and Case Profiles tables. It is suggested that the downloaded input files be saved in a location convenient for access from STRmix.

Instructions for uploading STRmix PDFs to the PDA page can be found in Section 5 and Appendix 6 – STRmix Workflow.

Scientists may enlist the assistance of HP2 staff to run STRmix analyses. This is managed through the STRmix worklists.

For details on the STRmix workflow, refer to Appendix 6 – STRmix Workflow.

13 Incorrect Results

When a process is ordered it populates the Exhibit Testing table, however if this process is ordered in error it is not possible to remove it from the Exhibit Testing table.

Processes are able to be made incorrect by anybody prior to validation. Once made incorrect, the process will have a strike through in the Exhibit Testing table and will not be able to be accessed.

To incorrect a process, enter into it by clicking on the associated date and time stamp in the Exhibit Testing table (Figure { SEQ Figure * ARABIC }})

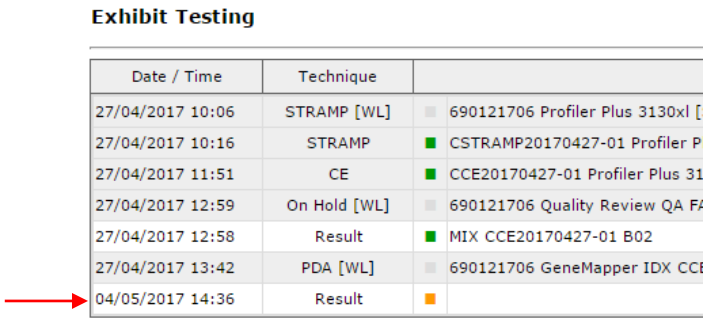
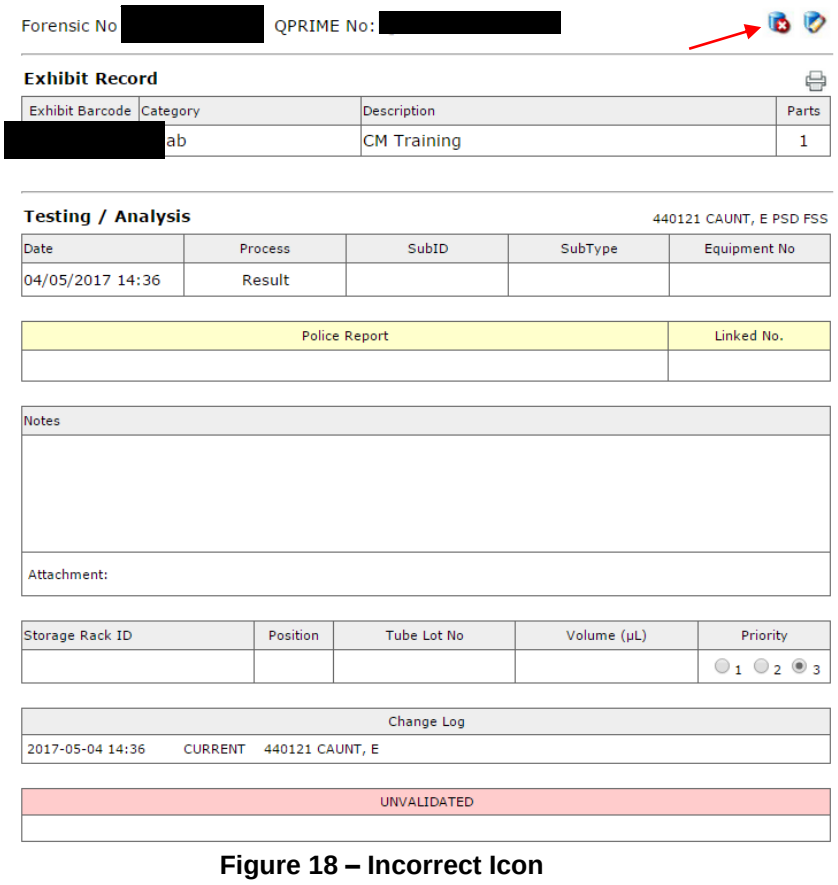


Exhibit Testing

Date / Time	Technique	
27/04/2017 10:06	STRAMP [WL]	690121706 Profiler Plus 3130xl [
27/04/2017 10:16	STRAMP	CSTRAMP20170427-01 Profiler P
27/04/2017 11:51	CE	CCE20170427-01 Profiler Plus 31
27/04/2017 12:59	On Hold [WL]	690121706 Quality Review QA FA
27/04/2017 12:58	Result	MIX CCE20170427-01 B02
27/04/2017 13:42	PDA [WL]	690121706 GeneMapper IDX CCE
04/05/2017 14:36	Result	

Figure { SEQ Figure * ARABIC } – Incorrect Entry

Click the icon in the top right of the page (Figure 37)



Forensic No [redacted] QPRIME No: [redacted]

Exhibit Record

Exhibit Barcode	Category	Description	Parts
[redacted]	ab	CM Training	1

Testing / Analysis 440121 CAUNT, E PSD FSS

Date	Process	SubID	SubType	Equipment No
04/05/2017 14:36	Result			

Police Report	Linked No.

Notes
Attachment:

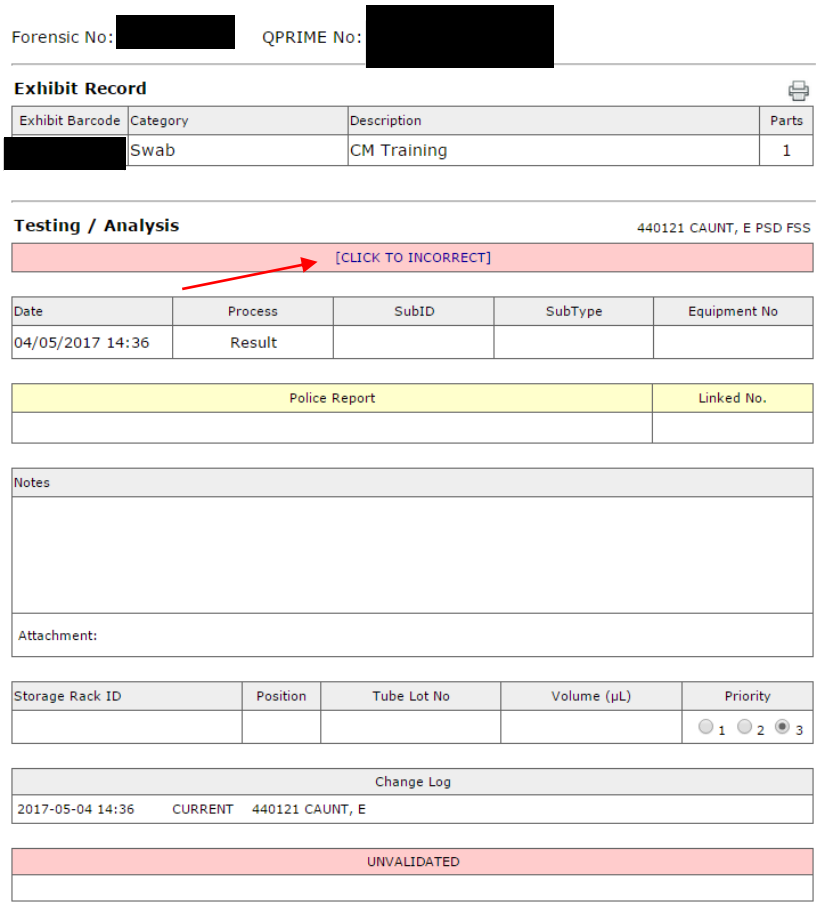
Storage Rack ID	Position	Tube Lot No	Volume (µL)	Priority
				1 2 3

Change Log
2017-05-04 14:36 CURRENT 440121 CAUNT, E

UNVALIDATED

Figure 18 – Incorrect Icon

Click the [CLICK TO INCORRECT] bar at the top of the page (Figure 38)



Forensic No: [redacted] QPRIME No: [redacted]

Exhibit Record

Exhibit Barcode	Category	Description	Parts
[redacted]	Swab	CM Training	1

Testing / Analysis 440121 CAUNT, E PSD FSS

[CLICK TO INCORRECT]

Date	Process	SubID	SubType	Equipment No
04/05/2017 14:36	Result			

Police Report	Linked No.

Notes
Attachment:

Storage Rack ID	Position	Tube Lot No	Volume (µL)	Priority
				1 2 3

Change Log
2017-05-04 14:36 CURRENT 440121 CAUNT, E

UNVALIDATED

Figure 19 – Incorrect Bar

The process entry in the Exhibit Testing table will now have a strike through (Figure 39)

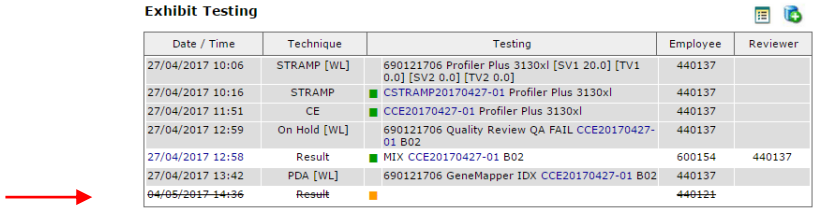
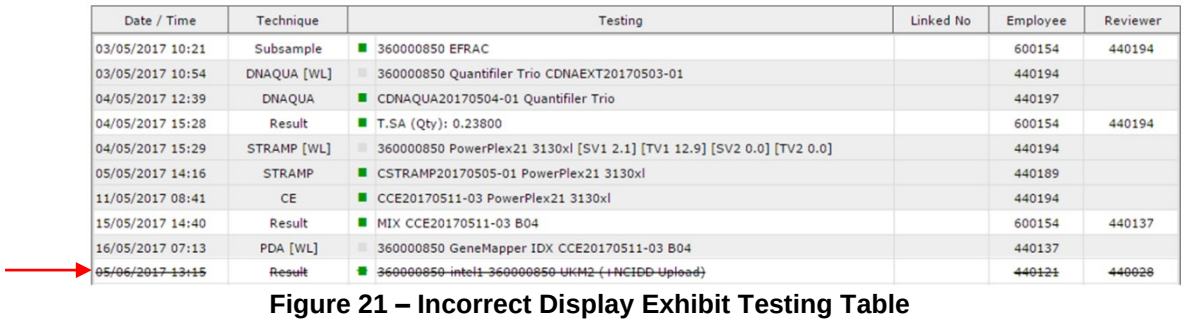


Exhibit Testing

Date / Time	Technique	Testing	Employee	Reviewer
27/04/2017 10:06	STRAMP [WL]	690121706 Profiler Plus 3130xl [SV1 20.0] [TV1 0.0] [SV2 0.0] [TV2 0.0]	440137	
27/04/2017 10:16	STRAMP	CSTRAMP20170427-01 Profiler Plus 3130xl	440137	
27/04/2017 11:51	CE	CCE20170427-01 Profiler Plus 3130xl	440137	
27/04/2017 12:59	On Hold [WL]	690121706 Quality Review QA FAIL CCE20170427-01 B02	440137	
27/04/2017 12:58	Result	MIX CCE20170427-01 B02	600154	440137
27/04/2017 13:42	PDA [WL]	690121706 GeneMapper IDX CCE20170427-01 B02	440137	
~~04/05/2017 14:36~~	~~Result~~		~~440121~~	

Figure 20 – Initial Incorrect Display Exhibit Testing Table

Once a copied down profile has been reviewed, this profile cannot be removed from the Profile Record table. If this profile is incorrect then the result in the Exhibit Testing table relating to that copied down profile should be made incorrect (Figure 40)



Date / Time	Technique	Testing	Linked No	Employee	Reviewer
03/05/2017 10:21	Subsample	360000850 EFRAC		600154	440194
03/05/2017 10:54	DNAQUA [WL]	360000850 Quantifiler Trio CDNAEXT20170503-01		440194	
04/05/2017 12:39	DNAQUA	CDNAQUA20170504-01 Quantifiler Trio		440197	
04/05/2017 15:28	Result	T.SA (Qty): 0.23800		600154	440194
04/05/2017 15:29	STRAMP [WL]	360000850 PowerPlex21 3130xl [SV1 2.1] [TV1 12.9] [SV2 0.0] [TV2 0.0]		440194	
05/05/2017 14:16	STRAMP	CSTRAMP20170505-01 PowerPlex21 3130xl		440189	
11/05/2017 08:41	CE	CCE20170511-03 PowerPlex21 3130xl		440194	
15/05/2017 14:40	Result	MIX CCE20170511-03 B04		600154	440137
16/05/2017 07:13	PDA [WL]	360000850 GeneMapper IDX CCE20170511-03 B04		440137	
~~05/06/2017 13:15~~	~~Result~~	~~360000850 intel1 360000850 UKM2 (+NCIDD Upload)~~		~~440121~~	~~440028~~

Figure 21 – Incorrect Display Exhibit Testing Table

In turn, this will strike through the profile in the Profile Record table on the PDA page (Figure 41)

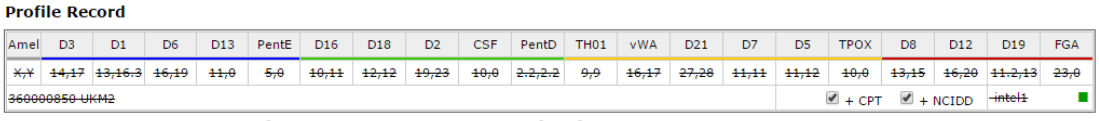


Figure 22 – Incorrect Display PDA Page

Validated results are incorrected in the same way; however these can only be performed by a Supervising Scientist or above.

When a validated result is identified as incorrect, the following should occur:

1. PDA page amended and correct result lines added to the relevant sample by the PDA analyst
2. Note added to the Sample Notes section explaining why the original results are incorrect or Notation added with Intelligence Report (QIS 34308). Requirements for amended results are outlined in Table 4
3. If an Intelligence Report is required, e-mail this report to the QPS DNA Management Unit
4. Ask a Supervising Scientist or above to incorrect the results at the same time the correct results are validated

Table 3 – Requirements for Amended Results

Type of Final Result Change	Intelligence Report Requirement	Managing Scientist Notification
Change in number of contributors only (no LR changes)	No	Yes
Change in LR: Support for contribution to support for contribution (same or different range)	No	No
Change in LR: Support for non-contribution to support for non-contribution	No	No
Change in LR: Support for contribution to support for non-contribution (and vice versa)	Yes	Yes
Change in LR: Support for contribution to exclusion (and vice versa)	Yes	Yes
Change in LR: Support for non-contribution to exclusion (and vice versa)	No	Yes
Any ‘suitable for interpretation’ to ‘unsuitable for interpretation’ (and vice versa)	Yes	Yes
Addition or removal of possible sub-threshold information	No	No

14 Additional Workflows

14.1 Suspect Checks

Requests for suspect checks will be received via SSLU or the QPS in a CM request.

Suspect check reference samples are not associated to cases in the FR and therefore these samples will not appear in the CPT.

If the crime scene sample against which the suspect check reference sample is to be compared has been profiled in PP21 then STRmix analysis may be required. As the suspect check reference sample does not populate the CPT, the STRmix input file cannot be obtained from the PDA page of the FR.

If the suspect check reference sample results are located in AUSLAB then the STRmix input file for the suspect check reference sample can be located in I:\STRmix Profiles.

If the suspect check reference sample results are located in the FR then the STRmix input file will need to be generated using the 'Build GMIDX reference' macro located in I:\Macros. Alternatively, this file can be generated from the Reference PDA page of the FR, however it may need to be amended.

The resultant STRmix PDF file should be added to a 'Notation' against the sample.

Exhibit results lines will be used to communicate results back to the QPS for all suspect checks (P+ and PP21) as per Section 18.

Once a suspect check has been completed, where appropriate, it will be necessary to complete the suspect check request.

14.2 Receipt of New Reference Samples

Often reference samples will be received for a case after the profile data analysis has been completed. The reference sample will require comparison to all interpretable DNA profiles in the case. The crime scene profiles with validated results that require comparison will add to the 'Profile Data Analysis – Case Work + Ref' worklist.

If the newly associated reference sample matches to the crime scene profile, the Case Profiles table will need to be updated and new result line(s) added.

In the example below (Figure { SEQ Figure * ARABIC }), consider that the new reference sample matches UKM1. The profile analyst should enter the barcode of the new reference sample into the 'Association' field for UKM1. This will update the association field for the reference sample.

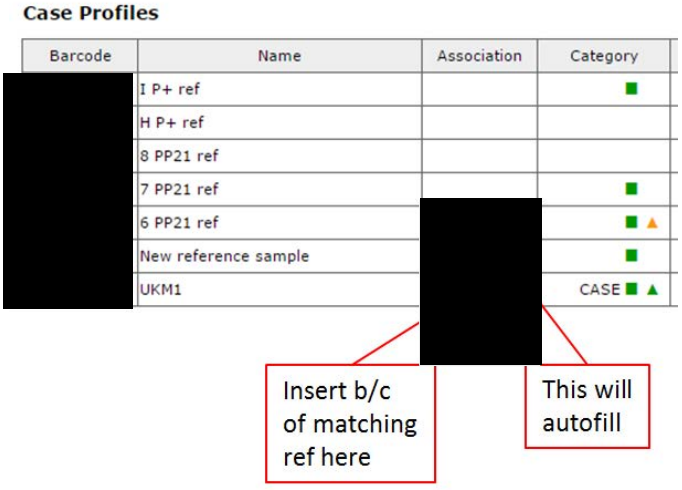


Figure { SEQ Figure * ARABIC } – Association of Unknowns

For new reference samples matching a DNAIntel sample, the profile analyst should update the DNAIntel 'Association' field in the CPT with the new reference sample barcode.

14.2.1 New Reference Comparisons

Newly associated reference samples can be identified by the absence of LR information in the Case Profiles table.

The comparison of the reference sample should be recorded as follows:

1. If required, complete a LR calculation in STRmix and import the PDF
2. If required, manually update the LR information in the Case Profiles table (e.g. intuitive exclusion)
3. If the reference sample matches an unknown or DNAIntel sample for the case, update the 'Association' fields (Figure 42)
4. Re-copy down the profile into the Profile Record table, click the icon to perform a search and select the original suffix, provided there is no change to the interpretation. Do not re-tick either the +CPT or +NCIDD boxes
5. Click 'Add Results' from the PDA page to update the result lines for the reference sample comparison. Alternatively, order a 'Result' process for the Exhibit Testing / Examinations table and manually enter the appropriate result lines for the reference sample comparison (Section 18)
6. If no additional results lines are required i.e. the results have not changed, then a Sample Note to this effect should be added (e.g. duplicate reference sample)
7. Order a 'Profile Review' process (a 'Profile Review' process should be ordered regardless of whether a result line is entered)

14.2.2 New Reference Comparisons that Require a Change in Interpretation

If a reference sample is received that requires the original interpretation of the profile to be changed due to the profile now being able to be conditioned, then the following process should be followed (otherwise follow the incorrect process as per Section 13):

1. Upload the new STRmix PDFs to the PDA page (these will overwrite the original PDFs)

2. Click 'Edit' and update the Profile Record table by copying down the appropriate profiles, searching the database, checking the +NCIDD box if necessary and selecting suffixes as per Section 8
3. Incorrect all 'Result' entries in the exhibit testing table that relate to the profiles that were copied down from the original interpretation. Although the original interpretation was correct, this will cross out the profiles originally copied down in the Profile Record table and remove them from the FR database
4. Enter appropriate result lines as per Section 18 (may need 'Sample undergone further work – conditioned' line)
5. Order a 'Profile Review'

14.3 Paternity / Paired Kinship Cases

To enable paperless PDA of paternity / kinship cases, Kinship reports can be printed to PDF and attached to a 'Notation' against the sample. The Kinship report and manual entry audit both have areas for the scientist to sign and date the report. Since NATA require the sign and date fields to be filled, the scientist can edit the PDF and enter 'N/A' in the sign and date fields (Appendix 3 – Amending PDFs for instructions on editing PDFs). Alternatively, these reports can be digitally signed.

14.3.1 Product of Conception (POC) Received

When a paternity / paired kinship analysis is performed on a POC the FR records should be completed as follows:

1. Copy down the profile / resolved component of profile into the Profile Record table and perform a search
2. Check the '+CPT' box and save the record
3. Order a 'Notation' and upload the Kinship report (two 'Notations' will be required if there is an audit report)
4. Order a 'Result', select the appropriate Exhibit Result line and enter the barcode of the alleged parent in the 'Linked No.' field (where subsamples exist for the reference sample of the alleged parent, the parent barcode should be entered into the 'Linked No.' field)
5. Order a 'Profile Review'

14.3.2 Reference Sample for Alleged Child Received

When a paternity / paired kinship analysis is performed on a reference sample then the results are reported to the QPS via an Exhibit Result on the reference sample of the alleged child. The FR records should be completed as follows:

1. Order a 'Notation' on the reference sample of the child and upload the Kinship report (two 'Notations' will be required if there is an audit report)
2. Order a 'Result' on the reference sample of the child, select the appropriate Exhibit Result line and enter the barcode of the alleged parent in the 'Linked No.' field (where subsamples exist for the reference sample of the alleged parent, the parent barcode should be entered into the 'Linked No.' field)
3. Order a 'Profile Review'
4. The reference sample of the child will populate the 'Profile Review' worklist.

14.4 Coronial Cases

If required, Exhibit Results can be reported back to the QPS on a bone sample using the parent barcode in the same way as paternity / paired kinship cases (Section 14.3.2).

14.5 Reactivated Cases

The QPS may request a volume check or 'Quant & Hold' for samples within a particular case type such as 'Cold' Cases.

If a volume check is requested:

1. Send a Request / Task to the Senior Scientist of the Analytical Section
2. Add details to the 'Comments' field
3. Save the record
4. Click the 'Add Exhibits' icon to add samples registered in the FR (refer to Appendix 23.14 for samples not appearing in the FR)

For a Quant and Hold request, refer to QIS 34006 Section 4.4.17 for detailed steps on actioning these samples.

14.6 Covert Samples

The QPS may submit covert samples for DNA analysis. Covert samples may be identified through information entered by the QPS in the 'Exhibit Notes & FSS Advice' field of the Exhibit Record page. Alternatively, the QPS may alert a HP5 or above of these samples. If not clearly identified, a notation should be added to the 'Exhibit Testing / Examinations' table of the relevant samples.

For the PDA page of a covert sample, do not complete the Profile Record or Case Profiles tables. All results for these samples including any reference sample comparisons should be reported back to the QPS in an Intelligence Report for the relevant sample (QIS 34308) and not through the standard exhibit result line process.

15 Sample Status

The status of a sample can be easily seen in the 'Status' field of the PDA page (Figure 43).

Figure 23 – Sample Status

The information displayed in this field is taken from the last entry in the Exhibit Testing table along with the date and time of that entry.

To access the status of all samples for a case, click the sample barcode '690149606' at the top left corner of the Exhibit Detail table. Click the Exhibit Register tab twice, select 'DNA Analysis' then 'All Exhibits' (Figure 44). This can be further filtered by profile type once DNA profiling has been completed:

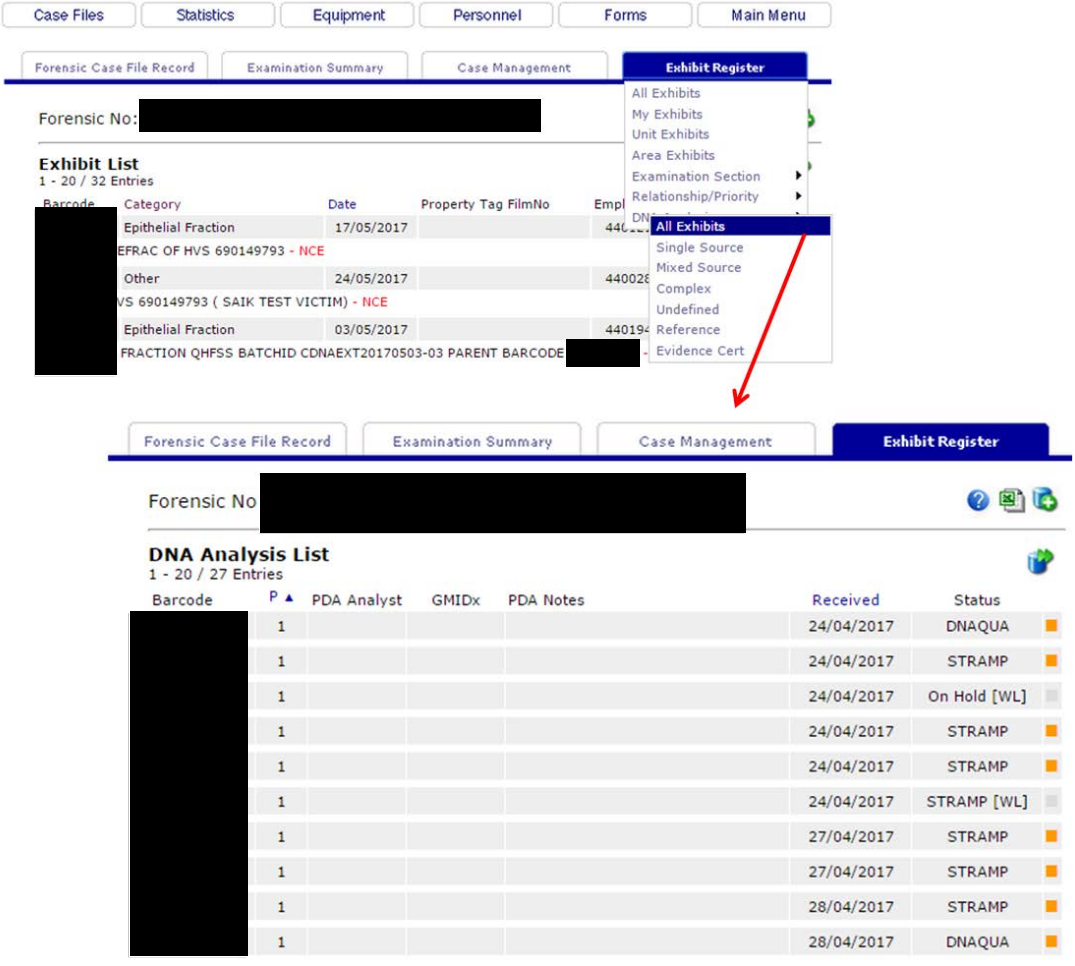


Figure 24 – Status of All Samples in a Case

This sample status summary can also be accessed from the Integrated Case Management System (ICS) page that presents after log-in. Click the 'Case Files' button at the top of the page, enter the case number, click enter and then access the 'Exhibit Register' tab as detailed above.

16 SAIKs and Multiple Items from One Exhibit

Often exhibits are received that contain multiple samples for DNA analysis. For example, SAIKs that contain multiple swabs and exhibits containing multiple cigarette butts.

Once received in Evidence Recovery for examination, these exhibits will be split into their individual components and new barcodes assigned. Consider a SAIK that contains three swabs. Evidence Recovery will register each of the three swabs as individual exhibits; these swabs are 'children' of the 'parent' exhibit (SAIK). As the 'children' are registered as exhibits, they will each have a PDA page and a result for each of these 'children' should be reported back to the QPS.

Since each of the 'child' exhibits from the SAIK will undergo DNA analysis, they will have subsample barcodes associated with them in order to store the individual parts created during the DNA analysis process (Figure 45 and Appendix 12 – Forensic Register Storage Architecture).

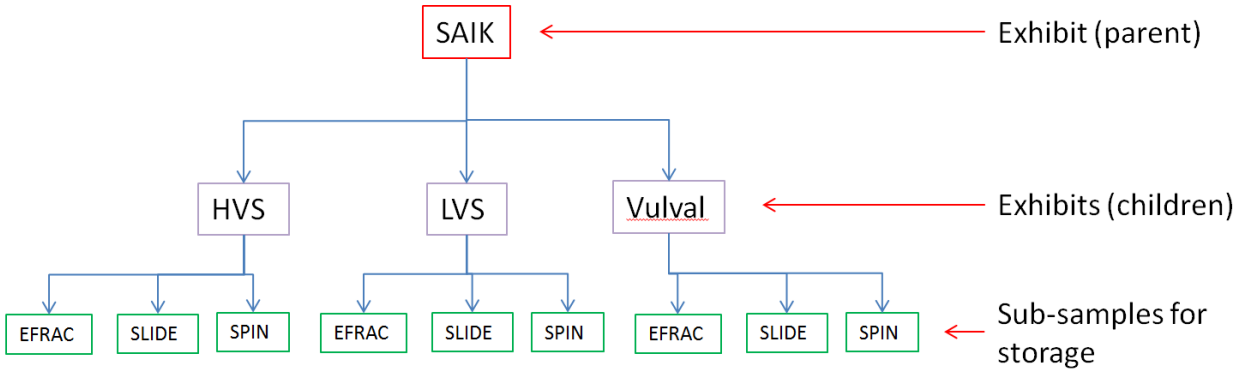


Figure { SEQ Figure * ARABIC } – SAIK Hierarchy

If the spin basket from the HVS requires re-extraction, this will need to be made into an exhibit to enable subsample barcodes to be created for storage of its individual parts (Figure 46).

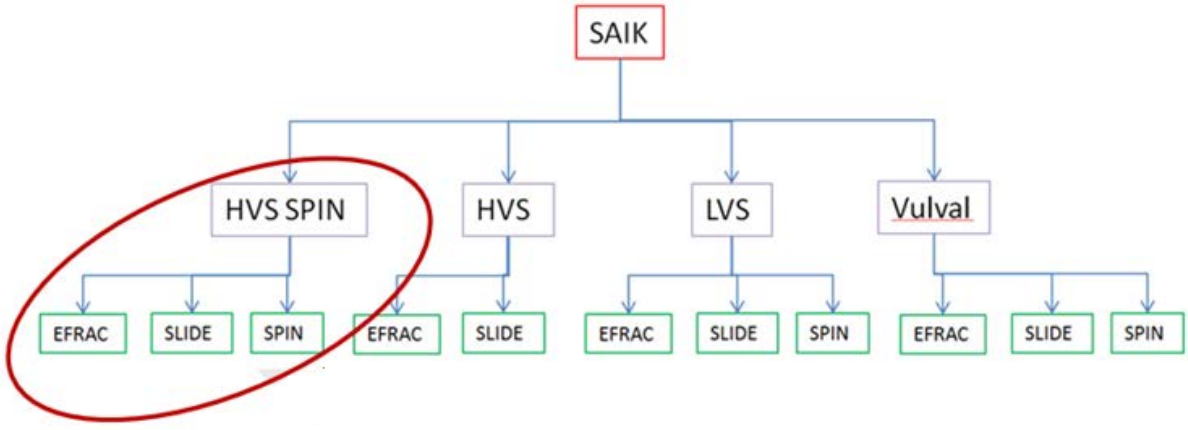
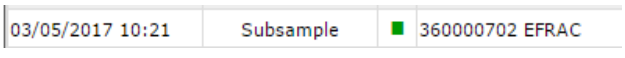


Figure 25 – Re-extract of Spin Basket

The following process should be followed to order the processing of an EFRAC that has been held at extraction:

1. Find the barcode of the EFRAC for your sample from the exhibit testing table



2. Register the EFRAC as an exhibit as described in Appendix 9 – Registering a Subsample as an Exhibit
3. Navigate to the exhibit testing table
4. Open up a test page by clicking the add button ( )
5. Select ‘DNA Quantification’ as the technique and ‘Quantifiler Trio’ as the method
6. Click the save icon to save the record

17 Subsamples and Subsample Processing

Subsamples are not exhibits and do not have records in their own right. Any process performed on a subsample is recorded within the parent exhibit from which the subsample originates.

Some exhibits require multiple samples to be processed with only one final result being reported back to the QPS. This is most likely to happen with bone samples when there are multiple aliquots.

In the example below (Figure { SEQ Figure * ARABIC }) the bone exhibit has barcode 695362707; the bone has two aliquots with barcodes 695362647 and 695362638 which are subsamples of the bone. The profile information for both subsamples is displayed in the Profile Analysis table on the PDA page of the bone. This enables the results from both aliquots to be combined into a consensus profile for reporting.

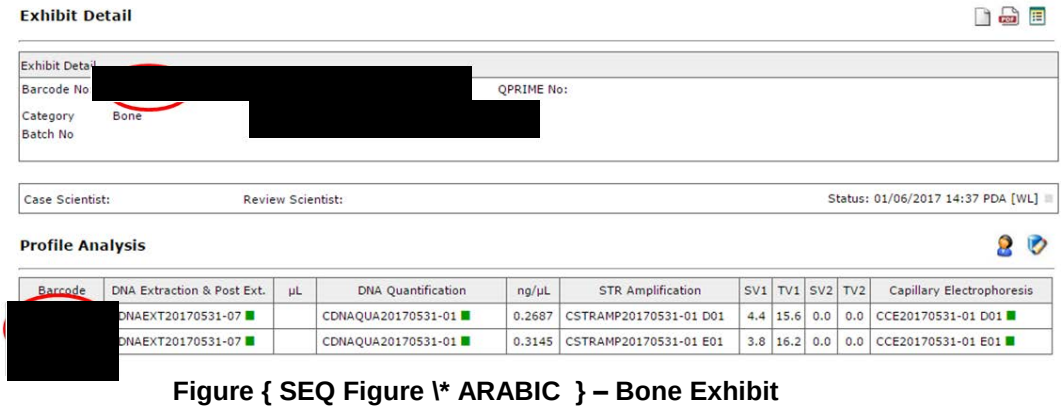


Figure { SEQ Figure * ARABIC } – Bone Exhibit

Another example of when a subsample will be encountered is if a sample has been diluted. Below (Figure 48) exhibit 603315267 has been processed but later needed a dilution which has barcode 360001753. The Profile Analysis table shows the batches that related to the processing of the original extract under the exhibit barcode followed by the processing of the dilution. In this instance the sample has been processed under the dilution barcode but the result will be reported back under the exhibit barcode.

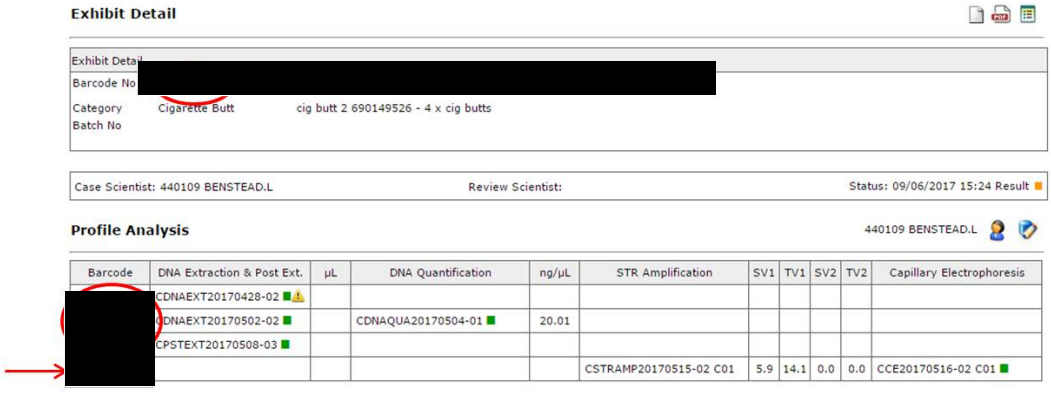


Figure 26 – Dilution

When processing of subsamples is complete they will populate the PDA lists under the subsample barcode; this enables visibility of the progress of each of the individual subsamples. Since the results of the Profile Data Analysis are reported back on the exhibit, it is the exhibit and not the subsamples that will populate the Profile Review lists. Subsamples only remove from the PDA lists when a Profile Review is ordered with the subsample barcode in the 'SubID' field. Alternatively, 'Reallocate' from the parent barcode with the subsample barcode will remove subsamples from the PDA lists.

If a subsample requires a rework, the exhibit test is ordered on the exhibit and the barcode of the subsample should be entered into the SubID field of the exhibit testing page. In the

example below (Figure 49) the subsample with barcode 695362647 is being submitted for re-amplification.

For any action that is required to be performed on the subsample, the barcode of the subsample must be entered into the SubID field.

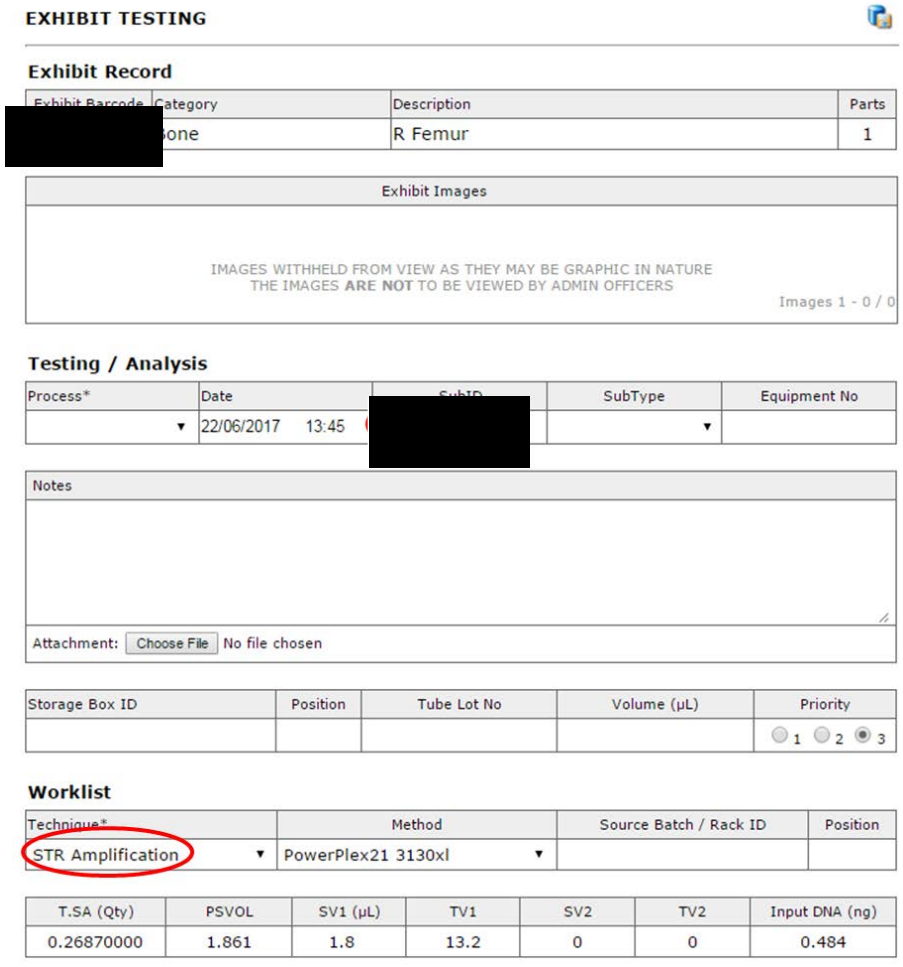


Figure 27 – Processing of SubID

Subsamples may be registered as exhibits if a result needs to be reported back on that subsample in isolation of any other subsamples or if pooling is required (Section 11.3).

In the example of pooling, subsamples are made into exhibits to enable the pooling process to occur however the final result will be reported on the pooled barcode. In this instance the samples that have been pooled require peer review at the same time as the results on the pooled barcode. Each of the exhibits that have been pooled should have a profile review ordered on them to remove them from the PDA list and enable the reviewer to record the review of these samples.

18 Exhibit Results

Exhibit result lines are created to communicate results to the QPS electronically (QIS 34229 for Explanations of Exhibit Results for FR). Exhibit results relating to DNA profile interpretation are entered into the FR by selecting appropriate lines:

1. Select the ‘Add Results’ icon from the PDA page

2. Check all automated lines
3. Click the save icon to save the record
4. Repeat until all relevant exhibit result lines have been entered (‘Add Results’ allows for the addition of three lines at a time)

On occasion, it may be necessary to select the appropriate result lines manually:

1. Select the ‘Add Results’ icon from the Exhibit Testing / Examinations table, followed by ‘Result’ from the ‘Process’ drop-down menu (Figure 50)
2. Select the appropriate exhibit result line from the drop-down menu. The mnemonic can be entered into the box for additional filtering
3. Enter the associated reference sample barcode, unknown designation or DNAIntel barcode into the ‘Linked No.’ field
4. Click the save icon to save the record
5. Repeat until all relevant exhibit result lines have been entered

Provided they are unvalidated, result lines can be edited (e.g. feedback, re-working or finalisation of a critical reference sample). If the selection of result lines from the drop-down menu are limited, edit and save a result line as a blank record and then re-enter this record with the intent to edit. The full complement of result lines should now be available for use.

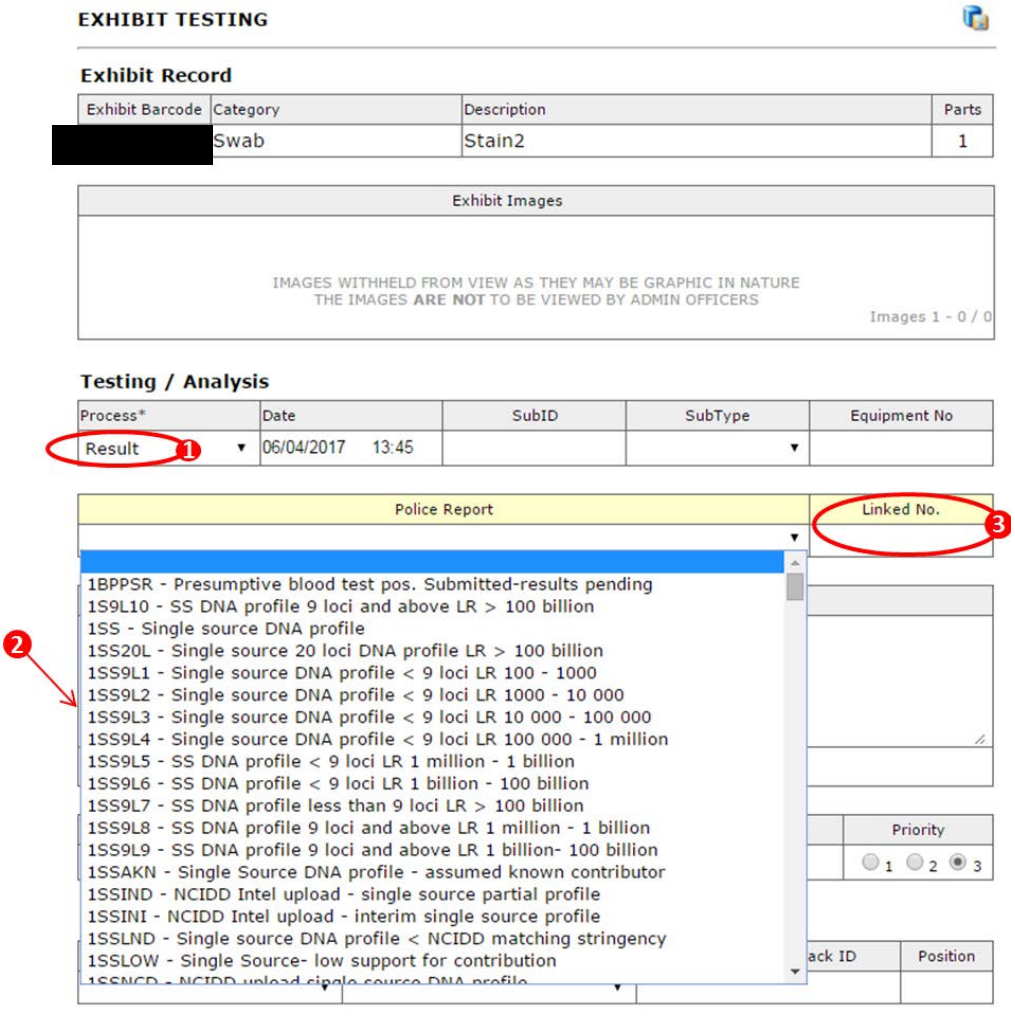


Figure 28 – Exhibit Result

Once all exhibit result lines have been entered a 'Profile Review' process should be ordered (Figure 51) which removes the sample from the PDA list and adds it to the appropriate review list.

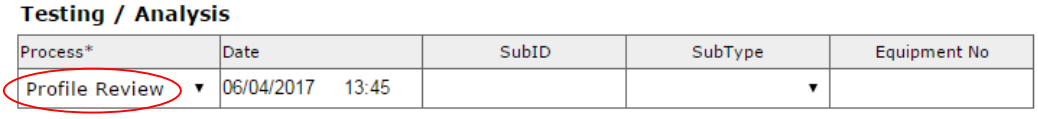


Figure 29 – Profile Review

These result lines can be checked by clicking on the 'PDA Robot' icon . Any discrepancies will be highlighted by a red cross and should be investigated prior to PDA review.

An additional process is required for four person mixtures with an LR in the range 'Mix – Support for contribution 2 to 1 million':

1. Await request from the reviewer for a new task
2. Create a Request / Task from the Case Management tab
3. Allocate this task to the reviewer
4. Select 'CM' as the Request Type
5. In the 'Comments' field add
    a. Sample barcode: XXX
    b. Result reported: Mixed DNA profile
    c. LR reported: Mix – Support for contribution 2 to 1 million; Person barcode XXX
    d. Actual LR: (Value); Person barcode XXX
6. Click the save icon

The exhibit result line associated with removing a profile from NCIDD should not have anything in the Linked No. field (Section 11.1.3).

If a sample undergoes further processing after review, a complete set of new exhibit result lines should be entered (supersedes any previously reported result lines).

19 Case Files

Generally speaking, cases without a statement are managed paperlessly. A paper case file can be created at any stage if it is necessary for efficient case management or if a statement is required (Appendix 8 – Creating and Tracking a Case File).

For cases with paper case files tracked in non-FR LIMS requiring further work or tracking:

1. 'Remove' the case file from the non-FR LIMS storage and add an audit entry 'Removed to track in FR'
2. Create and track this case file in FR as per Appendix 8 – Creating and Tracking a Case File using the existing case file barcode
3. Add a note to the 'Exhibit Notes & FSS Advice' field stating the case file was previously tracked in non-FR LIMS

It is not necessary for epgs within a case file to be labelled; instead a copy of the PDA page can be printed to accompany the epg(s). The PDA page contains all of the sample and interpretation information and can be associated with the epg via its barcode.

Case notes (e.g. GMO notes) are stored in 'Paperless' folders stored in Evidence Recovery, Reporting and Admin areas.

19.1 Case File Storage and Movement

Case files should be tracked at all times. FR storage locations are represented by a nine or ten digit barcode and a description. Each scientist has their own FR storage location, with additional storage locations assigned for general case file storage.

General FR case file storage locations are detailed in Table { **SEQ Table * ARABIC** }.

Table { SEQ Table * ARABIC } – General Case File Storage Locations

Location barcode	Location description	Physical location	Purpose	AUSLAB location
[redacted]	FRIT In-tray - PDA (Paper file)	Block 3	Ongoing case files for PDA	FRITCM
	FRIT In-tray - PDA Review (Paper file)	Block 3	Paper case file requires PDA review	MIXACT
	FRIT In-tray - Case Files for Reporting	Block 3	Case files created by admin for statement	FRITCM
	FRIT In-tray - Statement Review	Block 3	Statements for review	STAREV
	Admin In-tray - No Further Testing Review (Admin)	Block 6	Paper case file, no statement, new pages added, requires admin review	FBPR1
	Admin In-tray - Case File Finish	Block 6	Case file requires filing	FBCFF1
	Admin In-tray – Statements	Block 6	Admin in-tray for statement finalisation	FBSI24
	Admin In-tray – Scanning	Block 6	Scanning in-tray	
	ER In-tray - Incoming Paperwork	Block 6	ER in-tray	CFLIT

20 Records

Nil

21 Associated Documentation

QIS: 17117 – Procedure for Case Management
QIS: 34245 – Reference Sample Result Management
QIS: 34229 – Explanations of Exhibit Results for FR
QIS: 34006 – Forensic Register Procedure for the Release of Results
QIS: 33744 – Forensic Register Training Manual
QIS: 34064 – Miscellaneous Analytical Procedures and Tasks
QIS: 34281 – Procedure for the Use and Maintenance of the Forensic DNA Analysis Elimination Databases

QIS: 34308 – Procedure for Intelligence Reports and Interstate / Interpol Requests in the Forensic Register
QIS: 34112 – STR fragment analysis of PowerPlex® 21 profiles using GeneMapper™ ID-X software

22 Amendment History

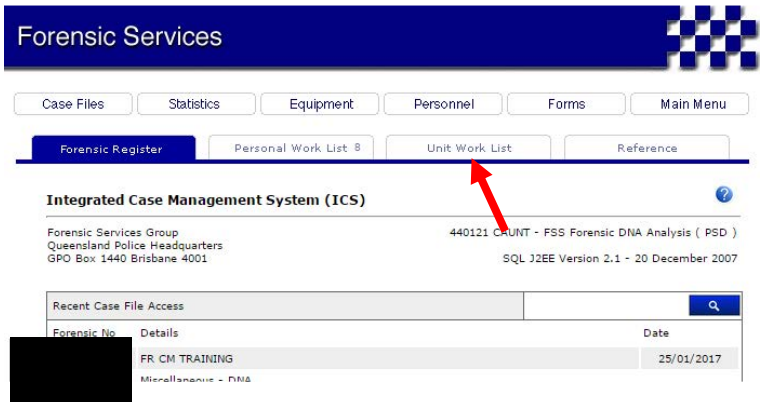
Version	Date	Updated By	Amendments
1	May 2017	E. Caunt	First issue
2	October 2019	A. Keller and J. Entwistle	Incorporated comments including audit recommendations; Incorporated FR enhancements including automatic ordering of NCIDD process; Added appendices; Moved Profiler Plus processes to appendix; General edit of document
3	January 2022	A. Keller and J. Entwistle	Incorporated comments; Upgraded template; Removed Appendix 5; Added Flowchart to Appendix 14

23 Appendices

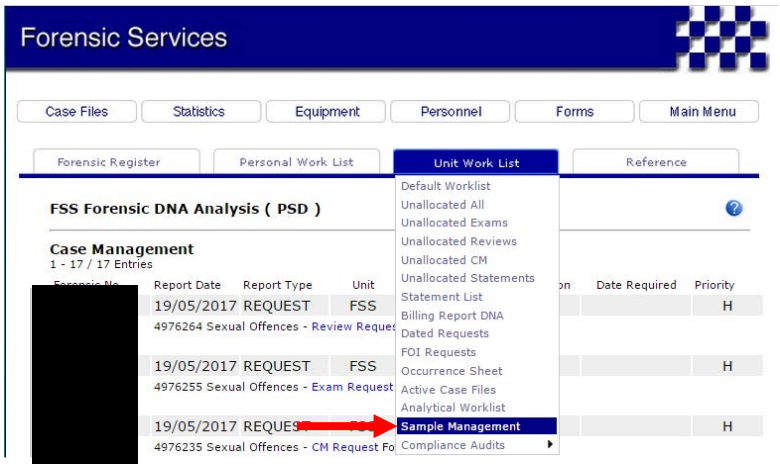
23.1 Appendix 1 – Navigation for Profile Data Analysis

Following log-in, click on 'Forensic Register' button

Click 'Unit Work List' tab

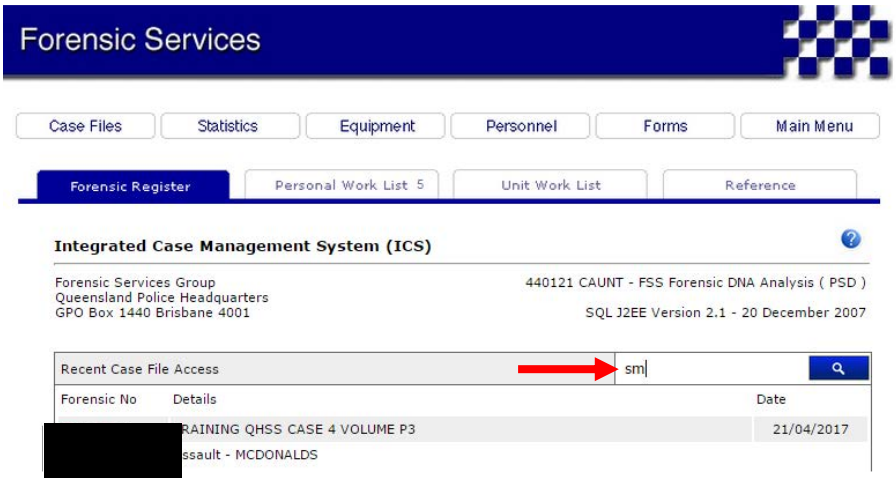


Click 'Sample ment'

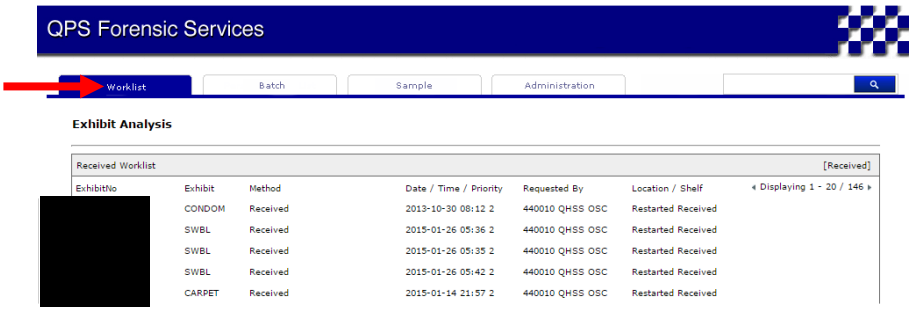


This will open a new tab in Chrome

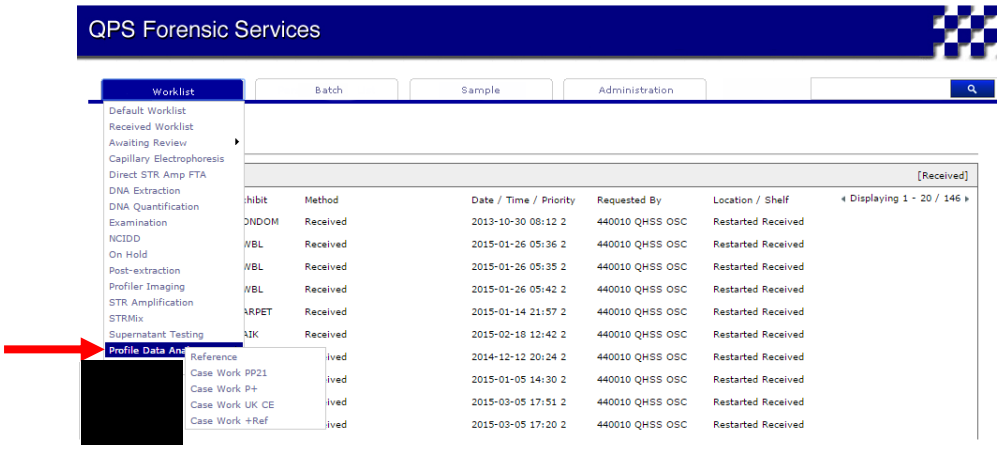
Alternatively, entering ‘sm’ into the search field will take you to the same place



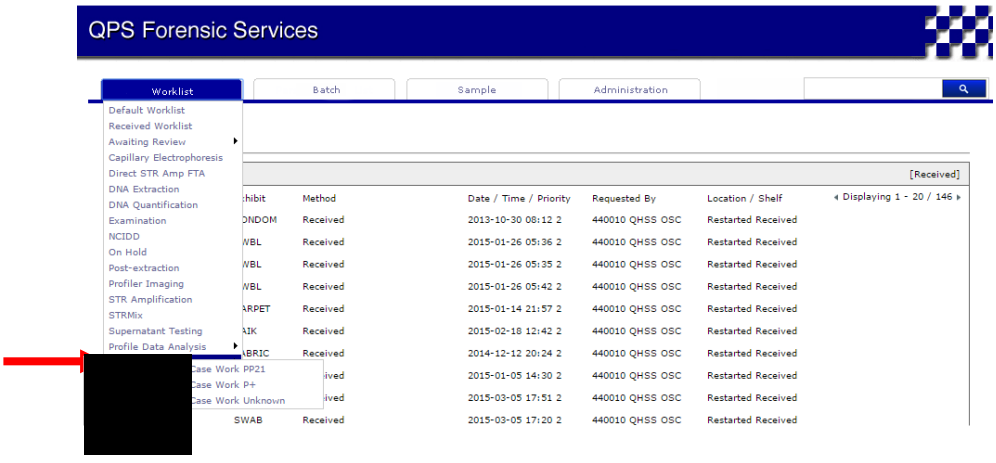
Click the ‘Worklist’ tab



Hover over ‘Profile Data Analysis’ and this will bring up the menu of PDA worklists. Clicking on the appropriate list title will open the list

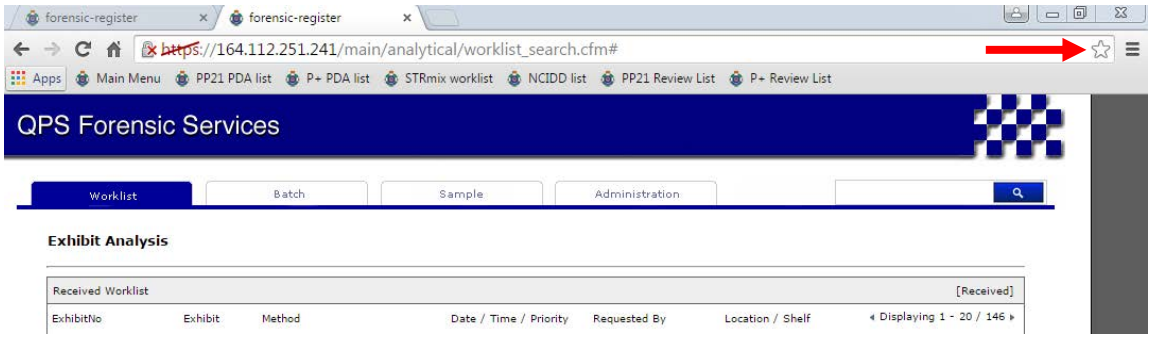


Hover over ‘Profile Review’ and this will bring up the menu of review worklists. Clicking on the appropriate list title will open the list

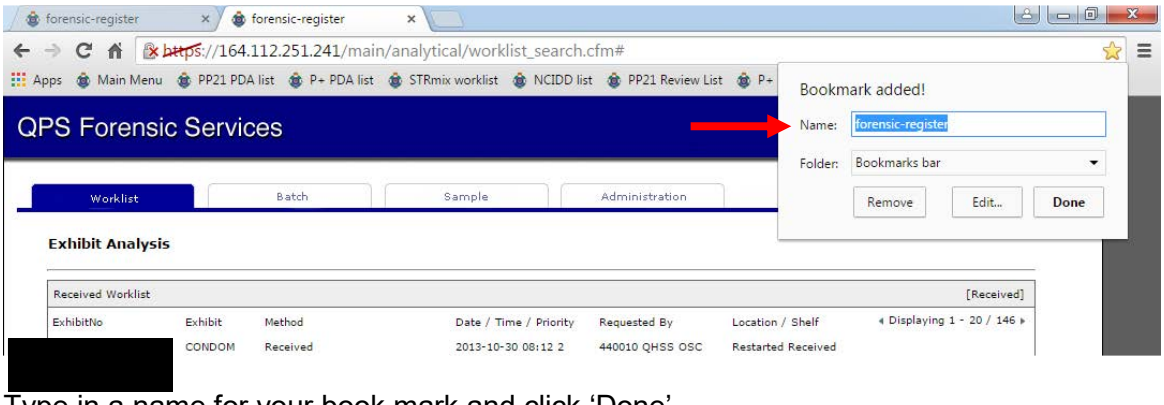


You can bookmark any page in the FR to your tool bar as follows

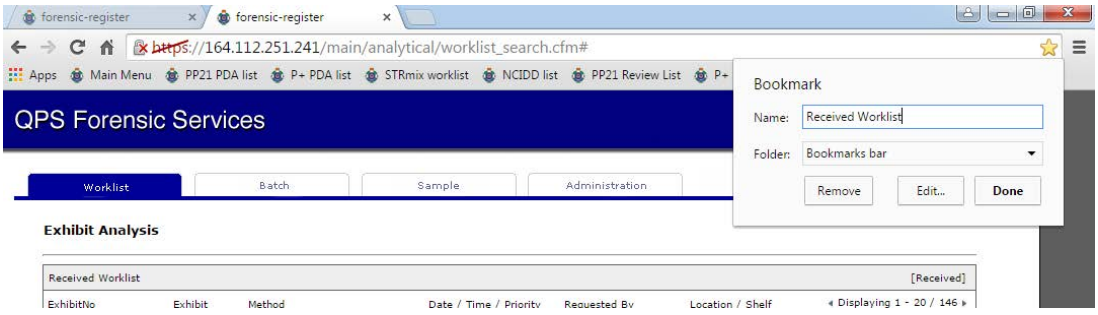
When on the page that you would like to bookmark, click the star in the toolbar



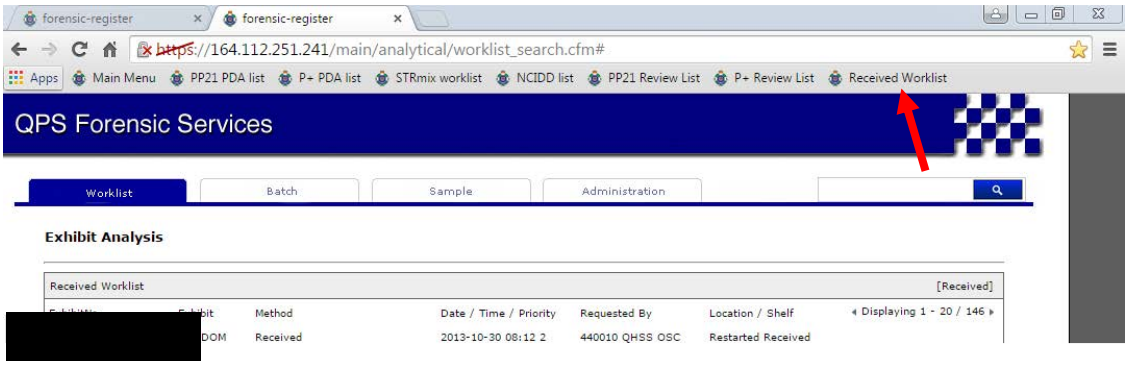
This will bring up a window for you to name your book mark



Type in a name for your book mark and click 'Done'



Your book mark will appear on your tool bar



Also refer to QIS 34006 Section 4.1.1 for Navigation to PDA Worklists

23.2 Appendix 2 – Profile Data Analysis (PDA) Worklists (Crime Scene)

The PDA worklists for casework are detailed in Table 1. These worklists populate and sort in the same way.

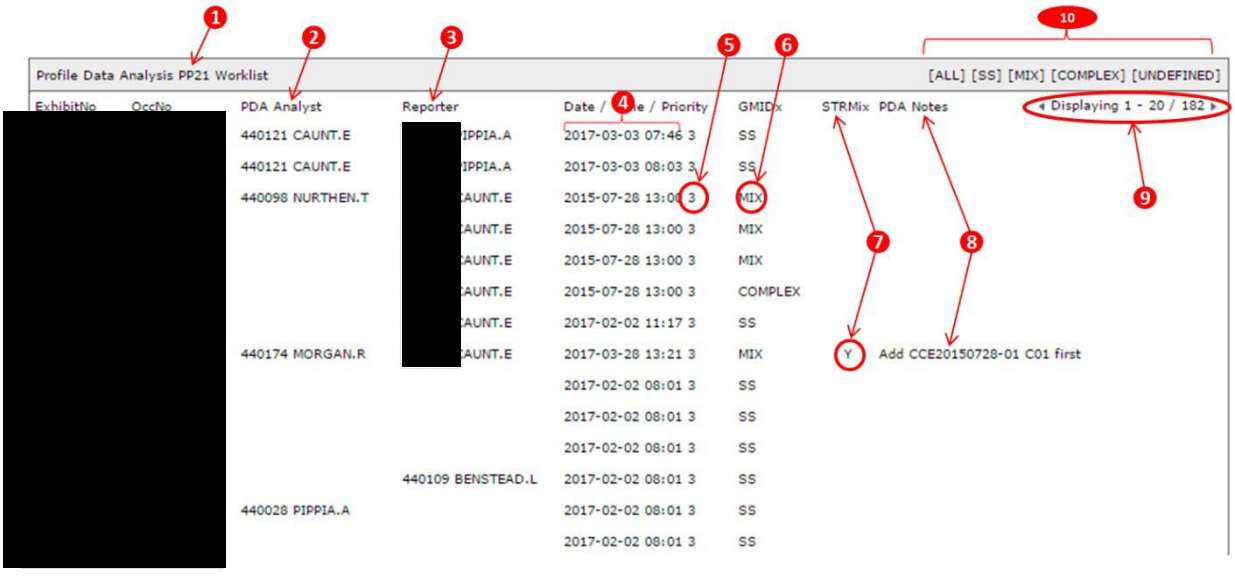
A sample populates the relevant PDA list under the following circumstances:

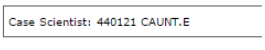
- The GeneMapper file has been uploaded and a profile PDF has been added, unless a QFLAG is raised in which case the sample will populate the 'On Hold' list
- Upon completion of the STRmix process which requires validation of the STRmix page
- The sample is manually inserted onto the PDA list

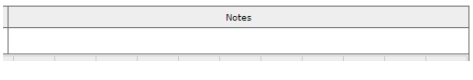
Samples processed through PP21 will populate onto lists based on the amp batch type and priority. If there is no amp batch present, the sample will populate the 'UK CE' worklist.

For the 'PP21 + Ref' worklist, all validated mixture and single source samples for a particular case populate this worklist when any associated reference samples are finalised.

The PDA lists are structured as shown below:



1. Name of worklist
2. Name of PDA Analyst for sample (assigned by clicking icon on the PDA page)
3. Name of PDA Analyst / Reporter for entire case. Displayed as 

on the PDA page (Appendix 7 – Allocating a Case to a Reporter / Profile Analyst)
4. Date and time that the sample was received
5. Priority of the sample
6. Reflection of plate readers UD1 comment in GeneMapper
    a. If the UD1 comment contains 'SS', 'MIX' or 'COMPLEX' then the sample will appear on the list as 'SS', 'MIX' or 'COMPLEX' respectively;
    b. If the UD1 comment does not contain 'SS', 'MIX' or 'COMPLEX' then the sample will appear on the list as 'UNDEFINED'
7. If the sample has populated the PDA list following STRmix analysis, a 'Y' will populate the STRMix column
8. Notes from the 'Notes' field on the PDA page 

9. Number of samples on the list. You can navigate between pages by clicking on the arrows
10. Filters for filtering the list based on profile type (also refer to QIS 34006 Section for Filtering Samples on Worklists – Section 4.3.1). You can search for a particular barcode by entering it on the Worklist tab page

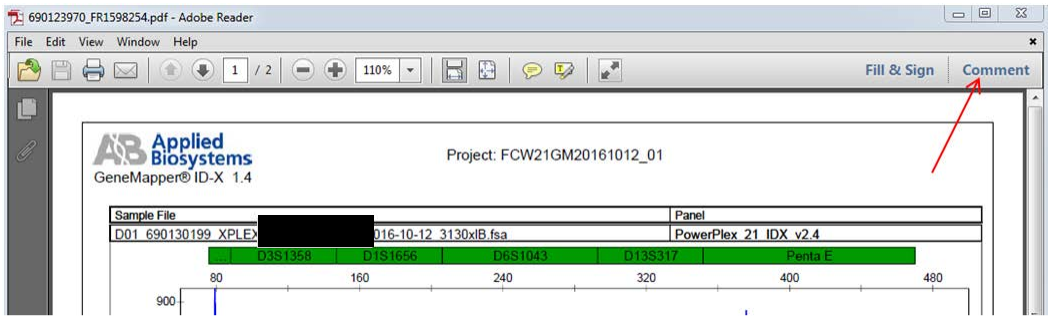
Samples are removed from the list under the following circumstances:

- The sample is added to the STRmix worklist
- A rework is ordered
- A 'Profile Review' process is ordered (with subsample barcode if applicable)
- A 'Reallocate' process is ordered

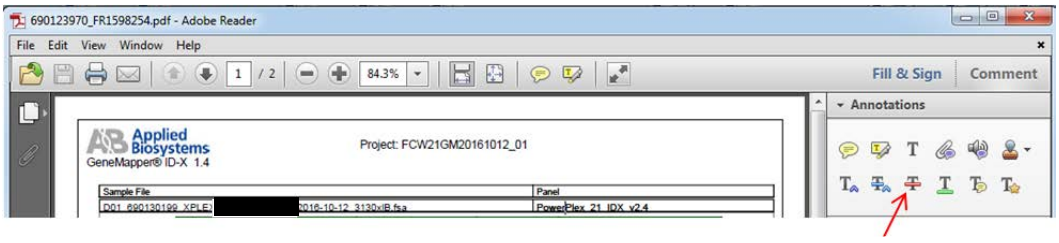
23.3 Appendix 3 – Amending PDFs

Open epg as PDF

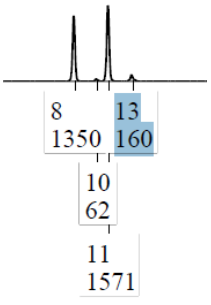
On PDF click ‘Comment’



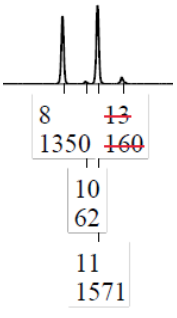
Under ‘Annotations’ click the strikethrough icon



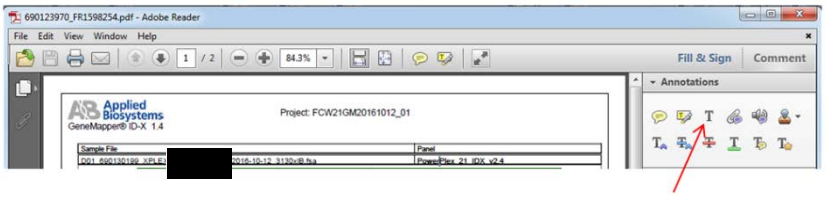
Highlight the text to be crossed out



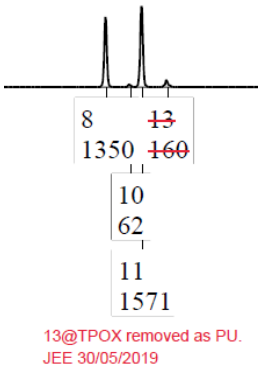
The text will strikethrough



Click the text icon and place the cursor where you need to add text



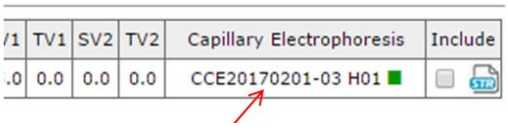
Add a note explaining the reason for the edit and add your initials and date



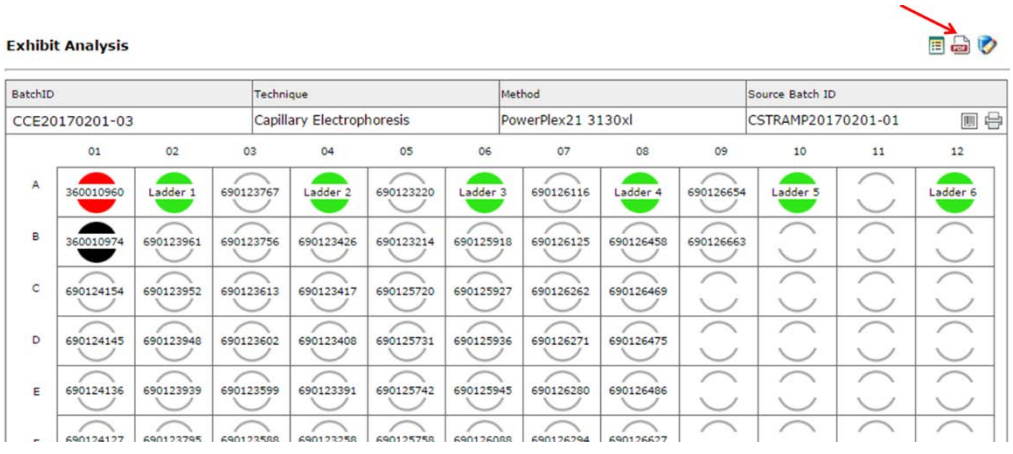
Save the edited PDF to a convenient location with the file name in the format 'CE position number-sample barcode-QP number-suffix' eg H01-690123970-[redacted]-EJC

The file name requires a suffix to ensure that any previously uploaded PDFs are not overwritten

Open the CE batch for the PDF that has been edited



Click the icon in the top right corner of the batch page



Drag the amended PDF into the box or select the file from its location, click 'Start Upload', click the icon to save. The PDF should now be accessible from the PDA page of the sample

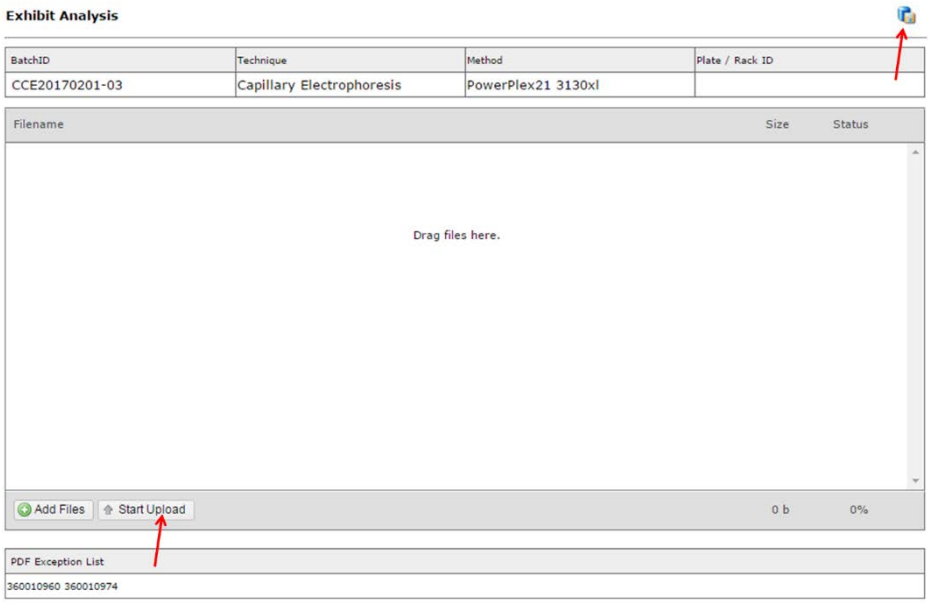
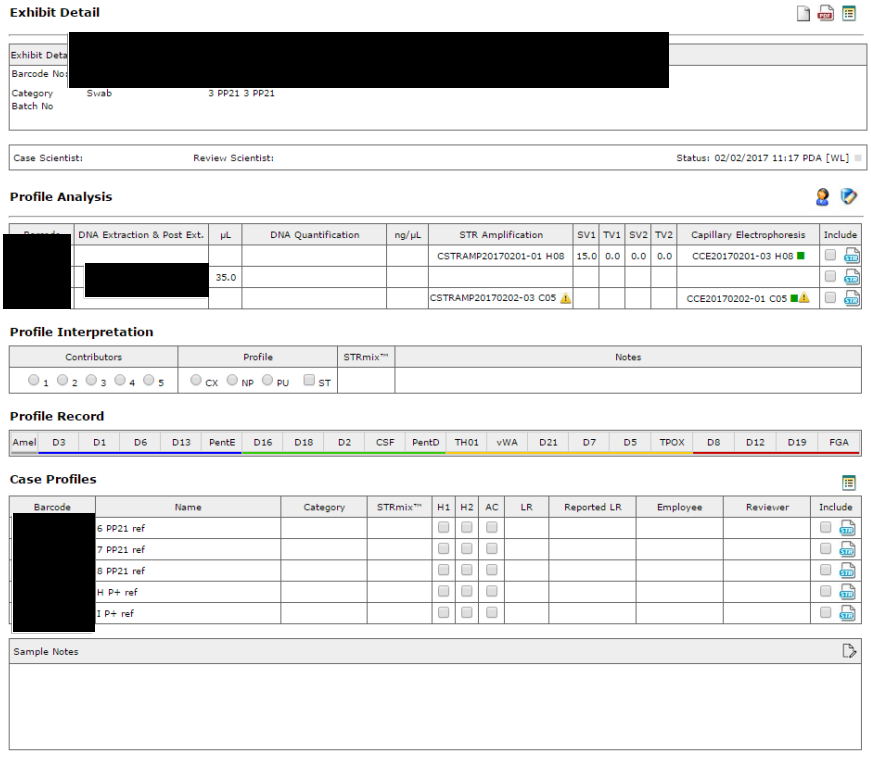


Exhibit Analysis
BatchID
Technique
Method
Plate / Rack ID
CCE20170201-03
Capillary Electrophoresis
PowerPlex21 3130xl
Filename
Size
Status
Drag files here.
Add Files
Start Upload
0 b
0%
PDF Exception List
360010960 360010974

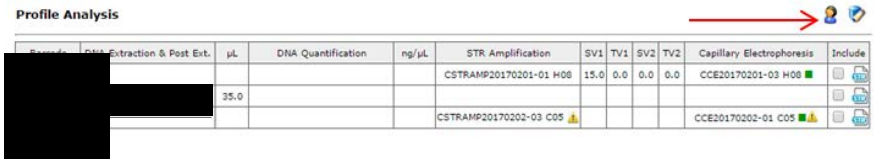
23.4 Appendix 4 – Profile Data Analysis of a Single Source PP21 Profile for NCIDD

A request for further information may be necessary before proceeding with the PDA of a sample. For example, establishing the ownership of an exhibit taken from a piece of intimate clothing such as underwear, to enable the conditioning of a sample. Refer to QIS 34006 Section 4.4.15 as well as QIS 34006 Sections 4.4.4 and 4.4.5 for detailed steps on accessing further information from cases and associated samples.

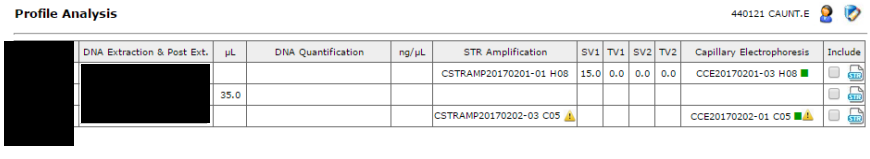
The PDA page for a sample yet to be interpreted will look like this



The first action is to allocate the sample to yourself by clicking on the icon



Your name will appear next to the icon



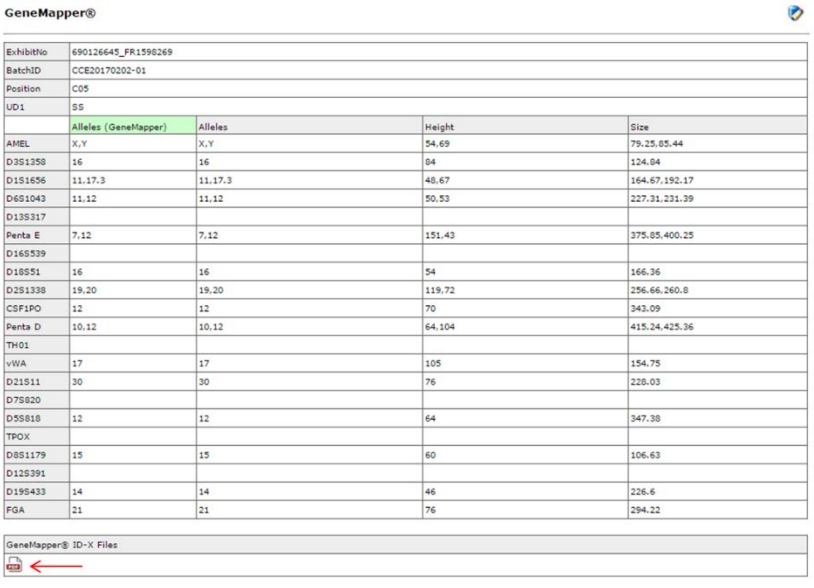
Batch records can be accessed by clicking on the batch id

The GeneMapper record and profile PDFs can be found by clicking on the position next to the appropriate CE batch id

Profile Analysis 440121 CAUNT.E

Barcode	DNA Extraction & Post Ext.	µL	DNA Quantification	ng/µL	STR Amplification	SV1	TV1	SV2	TV2	Capillary Electrophoresis	Include
[redacted]					CSTRAMP20170201-01 H08	15.0	0.0	0.0	0.0	CCE20170201-03 H08	
	T20170202-03	35.0									
					CSTRAMP20170202-03 C05					CCE20170202-01 C05	

This will open a new window. The profile PDFs can be opened by clicking on the icon.



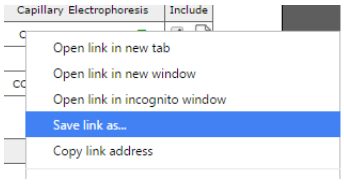
GeneMapper®

ExhibitNo	690126645_FR1598269			
BatchID	CCE20170202-01			
Position	C05			
UD1	SS			
	Alleles (GeneMapper)	Alleles	Height	Size
AMEL	X,Y	X,Y	54,69	79.25,85.44
D3S1358	16	16	84	124.84
D1S1656	11,17.3	11,17.3	48.67	164.67,192.17
D6S1043	11,12	11,12	50,53	227.31,231.39
D13S317				
Penta E	7,12	7,12	151,43	375.85,400.25
D16S539				
D18S51	16	16	54	166.36
D2S1338	19,20	19,20	119,72	256.66,260.8
CSF1PO	12	12	70	343.09
Penta D	10,12	10,12	64,104	415.24,425.36
TH01				
vWA	17	17	105	154.75
D21S11	30	30	76	228.03
D7S820				
D5S818	12	12	64	347.38
TPOX				
D8S1179	15	15	60	106.63
D12S391				
D19S433	14	14	46	226.6
FGA	21	21	76	294.22

GeneMapper® ID-X Files

All available profile PDFs can be accessed from the PDA page by clicking on the icon, followed by 'Open All' or individual icons.

The STRmix input files are accessed directly from the FR. Clicking the icon will download the STRmix input file for the associated casework run. This input file can be saved to the desktop or other convenient location for access from STRmix. Alternatively, right clicking on the icon will allow the file to be saved directly.



When setting up the STRmix analysis **only the QP number is to be entered into the 'Case Number' field and only the barcode of the casework sample is to be entered into the 'Sample ID' field**. This is also the case when running LRs. If additional information is to be added, such as the barcode of the reference sample, then this can be added to the 'Case Notes' field.

Once the STRmix analysis is complete, the STRmix macro should be run and the PDFs uploaded into the FR as follows:

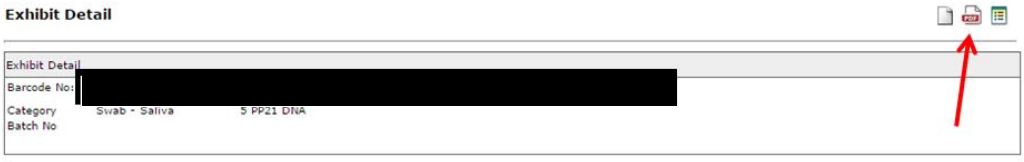
- Run the STRmix macro QIS: 35009 – MoveSTRmixPDF Macro

- The STRmix files and PDF will be stored to I:\STRmix Results and a copy of the PDF will be stored in the FR upload folder (shortcut on desktop)
- The FR upload folder should be emptied at regular intervals as this is only a transient storage location. A copy of the PDF will always be stored in I:\STRmix Results

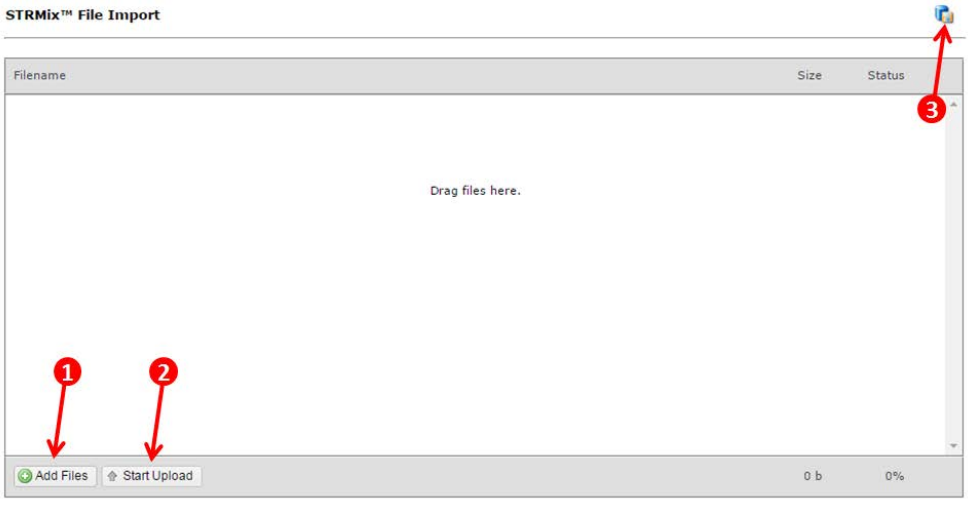
The macro will name the PDF such that the FR will file the PDF in the correct place. This naming convention is based on the 'Case Number' and 'Sample ID' fields in STRmix, therefore if additional information is added into these fields the FR cannot file the PDFs appropriately.

Upload STRmix PDF into the FR as follows:

1. From the PDA page of the crime scene sample, click the icon in the top right corner



2. Drag and drop all of the STRmix PDFs pertaining to that sample into the window or click on 'Add Files' (1) and browse. Once all files are listed in the window, click 'Start Upload' (2) and then click the save icon (3)

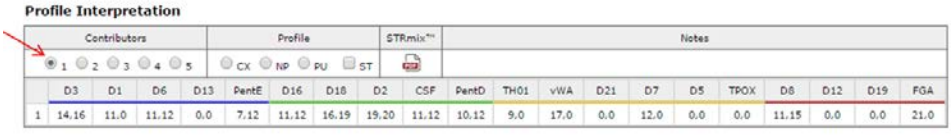


All of the STRmix PDFs will file in the appropriate places. If you have STRmix PDF files for multiple samples they can all be dragged into the window at the same time regardless of which sample the window was opened from and they will all file appropriately.

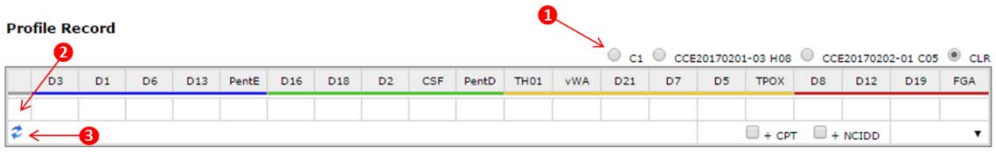
Now you can complete the PDA page

Click the edit icon

Check the '1' circle in the Profile Interpretation table

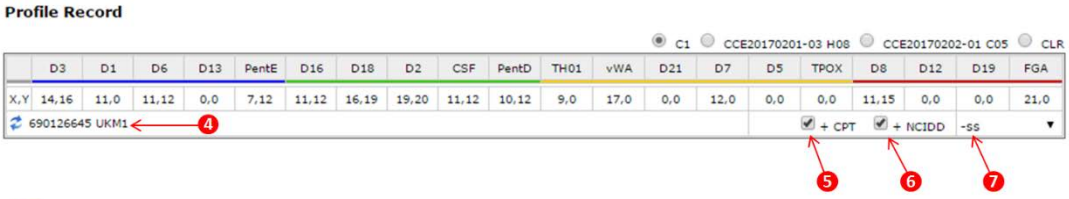


Copy down the profile into the profile record table by checking the 'C1' circle (1); enter AMEL (2) and click the search button (3)

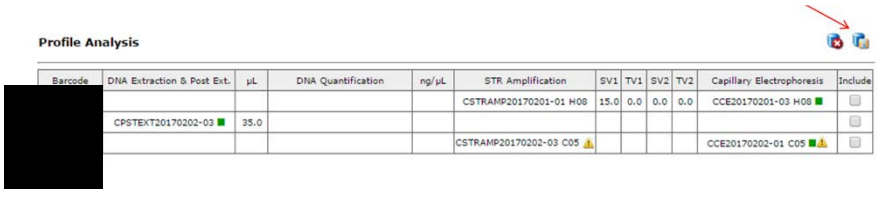


New Unknown

Check that you agree with the FR designation of UKM1 (4) for this sample. If UKM1 does not match any reference samples and is a new unknown for the case, check the '+CPT' box (5); check the '+NCIDD' box (6) and select the '–ss' suffix from the drop-down menu (7)



Click



Option 1

Whilst still on the PDA page, click the icon above the Profile Interpretation table. This will open a 'Result' page with automatically populated result lines generated from the completed fields of the PDA page (up to three result lines at a time, repeat this process if more are required). Check that you agree with these result lines. Click

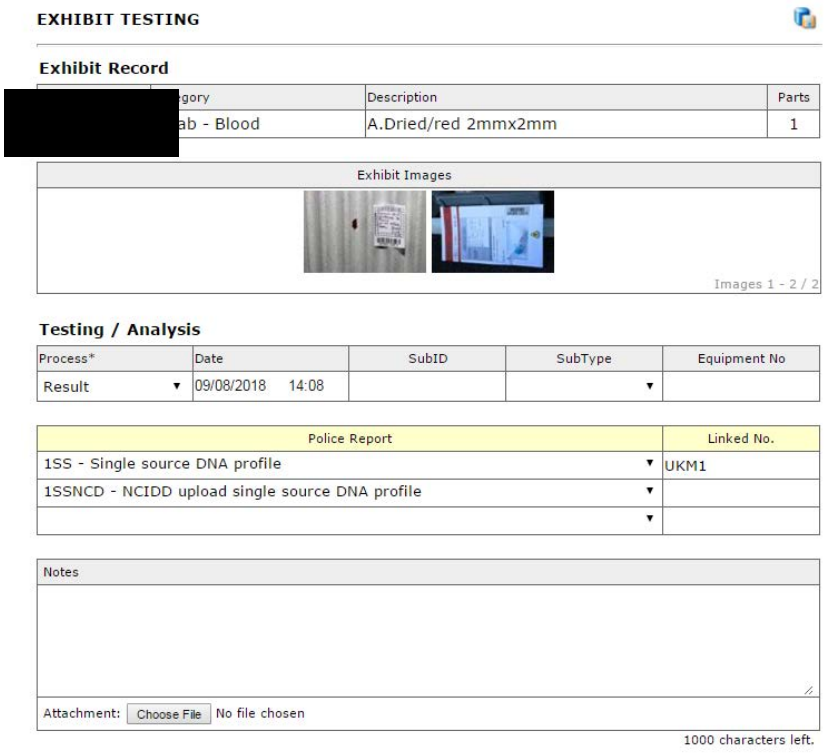


EXHIBIT TESTING

Exhibit Record

[redacted]	Description	Parts
ab - Blood	A.Dried/red 2mmx2mm	1

Exhibit Images

Images 1 - 2 / 2

Testing / Analysis

Process*	Date	SubID	SubType	Equipment No
Result	09/08/2018 14:08			

Police Report	Linked No.
1SS - Single source DNA profile	UKM1
1SSNCD - NCIDD upload single source DNA profile	

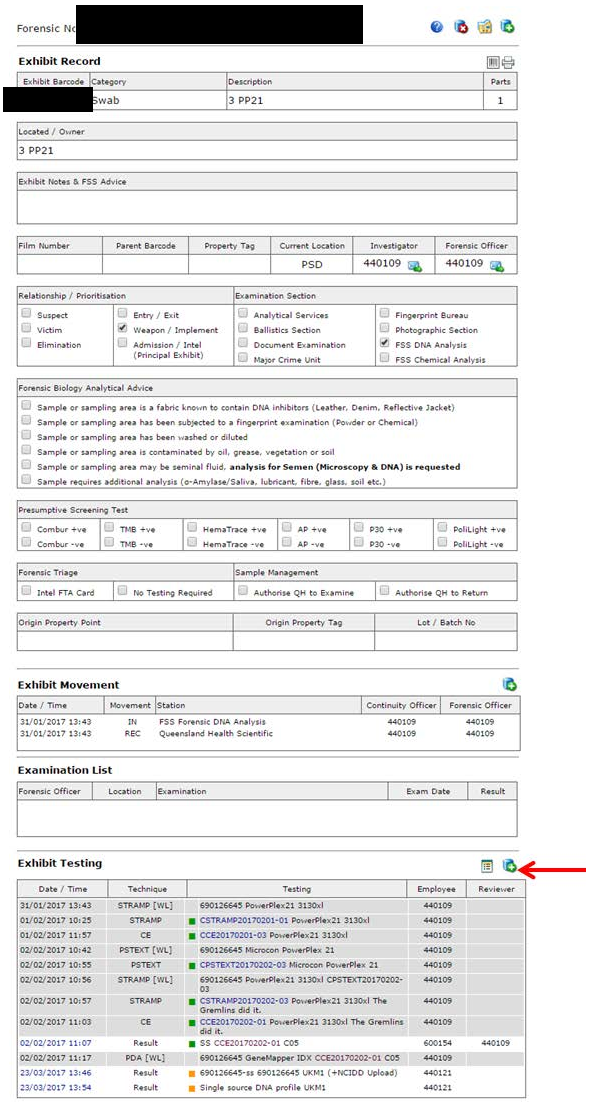
Notes

Attachment: Choose File No file chosen

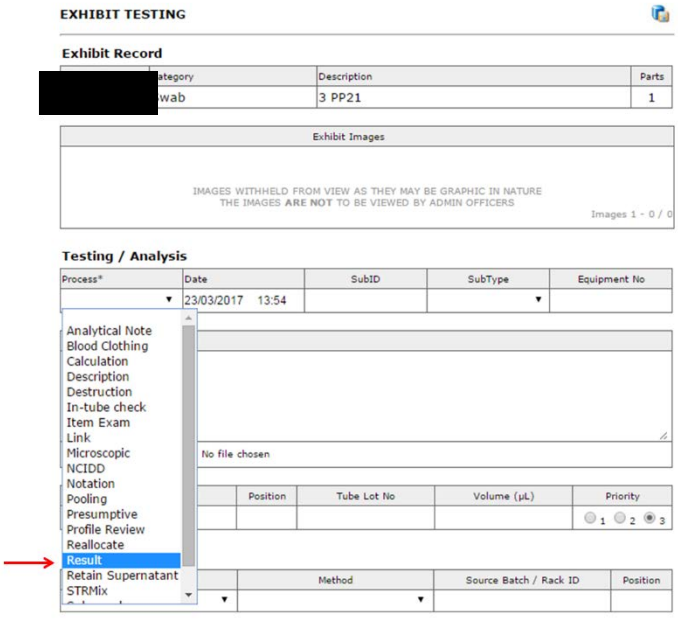
1000 characters left.

Option 2

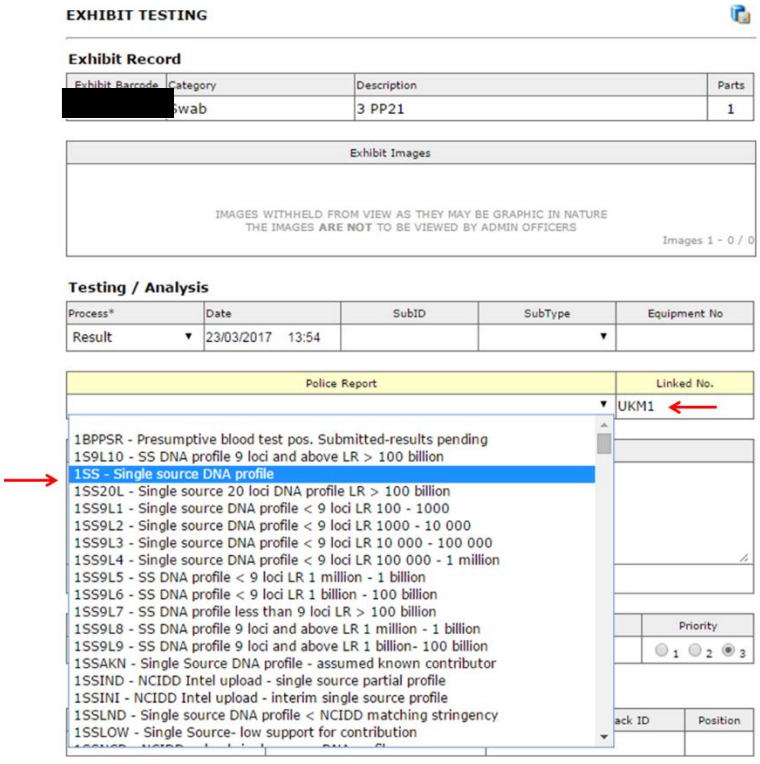
From the PDA page, either click to access the Exhibit Testing / Examinations table on the Exhibit Testing / Movement page OR click the sample barcode to access the Exhibit Testing table as shown below. In both cases, then click the icon



Select the process of ‘Result’ from the drop-down list

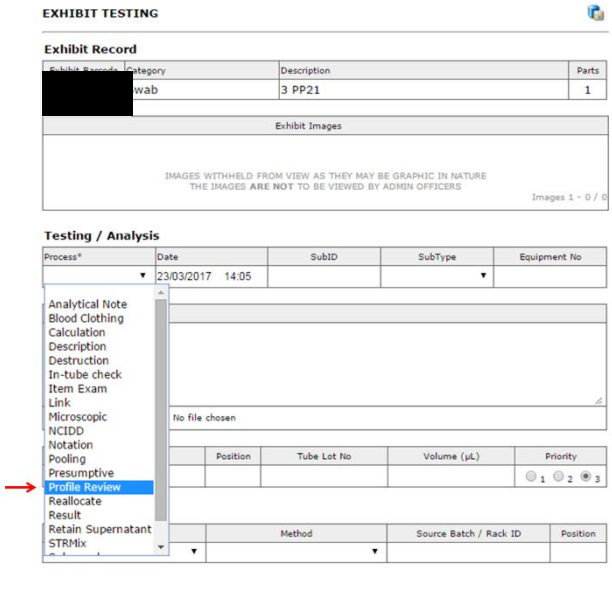


Select the result lines from the drop-down menu and enter the designation in the 'Linked No.' field



Click

Once all exhibit report lines have been entered, order a process of 'Profile Review'. Click



This will remove the sample from the PDA list and add it to the Profile Review list

The Exhibit Testing table should look something like this

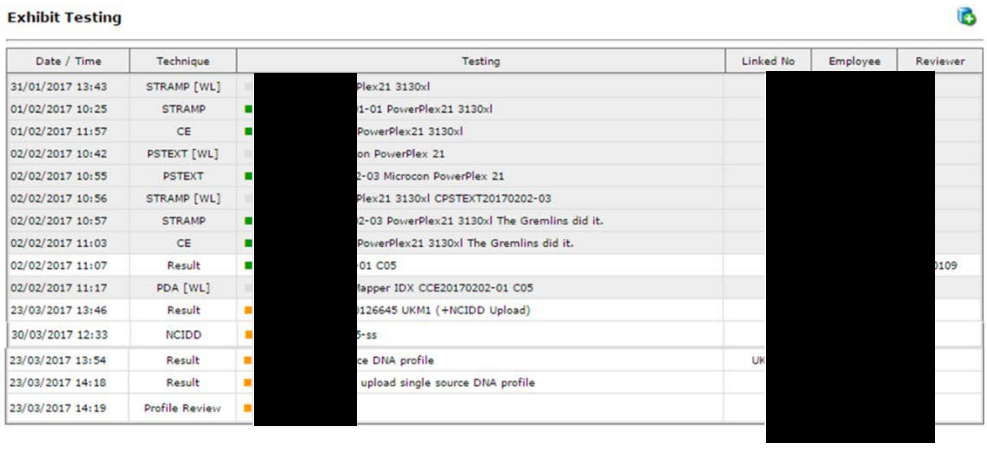


Exhibit Testing

Date / Time	Technique	Testing	Linked No	Employee	Reviewer
31/01/2017 13:43	STRAMP [WL]	[redacted]Plex21 3130xl		[redacted]	
01/02/2017 10:25	STRAMP	[redacted]1-01 PowerPlex21 3130xl			
01/02/2017 11:57	CE	[redacted]PowerPlex21 3130xl			
02/02/2017 10:42	PSTEXT [WL]	[redacted]on PowerPlex 21			
02/02/2017 10:55	PSTEXT	[redacted]2-03 Microcon PowerPlex 21			
02/02/2017 10:56	STRAMP [WL]	[redacted]Plex21 3130xl CPSTEXT20170202-03			
02/02/2017 10:57	STRAMP	[redacted]2-03 PowerPlex21 3130xl The Gremlins did it.			
02/02/2017 11:03	CE	[redacted]PowerPlex21 3130xl The Gremlins did it.			
02/02/2017 11:07	Result	[redacted]01 C05			0109
02/02/2017 11:17	PDA [WL]	[redacted]lapper IDX CCE20170202-01 C05			
23/03/2017 13:46	Result	[redacted]126645 UKM1 (+NCIDD Upload)			
30/03/2017 12:33	NCIDD	[redacted]5-ss			
23/03/2017 13:54	Result	[redacted]ce DNA profile	UK		
23/03/2017 14:18	Result	[redacted] upload single source DNA profile			
23/03/2017 14:19	Profile Review	[redacted]			

Check an NCIDD process has been added to send the profile to the NCIDD worklist (this process should be ordered automatically when the 'NCIDD' box on the PDA page is ticked and the record is saved). If not, click on the icon in the top right corner of the PDA page

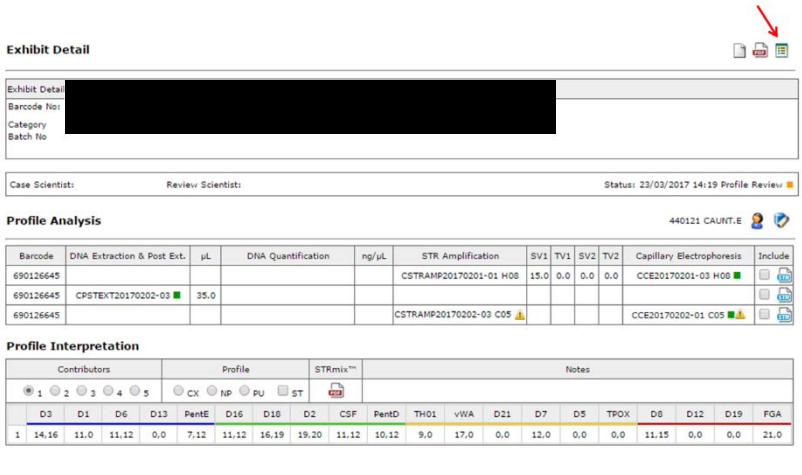


Exhibit Detail

Exhibit Detail: [redacted]
Barcode No:
Category
Batch No

Case Scientist: Review Scientist: Status: 23/03/2017 14:19 Profile Review

Profile Analysis

440121 CAUNT.E

Barcode	DNA Extraction & Post Ext.	µL	DNA Quantification	ng/µL	STR Amplification	SV1	TV1	SV2	TV2	Capillary Electrophoresis	Include
690126645					CSTRAMP20170201-01 H08	15.0	0.0	0.0	0.0	CCE20170201-03 H08	
690126645	CPSTEXT20170202-03	35.0									
690126645					CSTRAMP20170202-03 C05					CCE20170202-01 C05	

Profile Interpretation

Contributors: 1 2 3 4 5 | Profile: CX NP PU ST | STRmix™ | Notes

	D3	D1	D6	D13	PentE	D16	D18	D2	CSF	PentD	TH01	vWA	D21	D7	D5	TPOX	D8	D12	D19	FGA
1	14,16	11,0	11,12	0,0	7,12	11,12	16,19	19,20	11,12	10,12	9,0	17,0	0,0	12,0	0,0	0,0	11,15	0,0	0,0	21,0

This will take you to the exhibit testing table. Click the icon

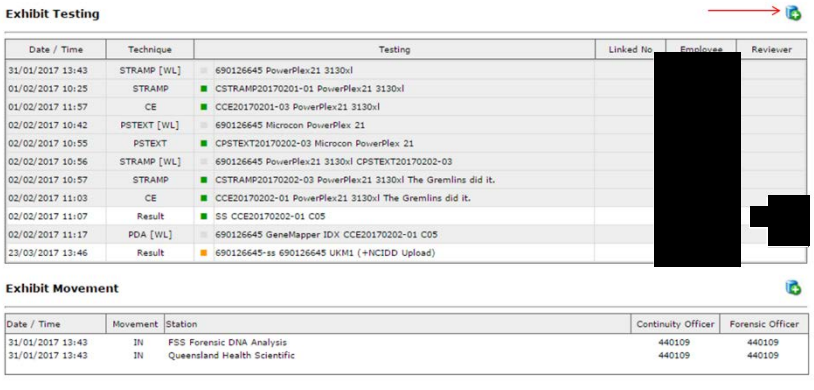


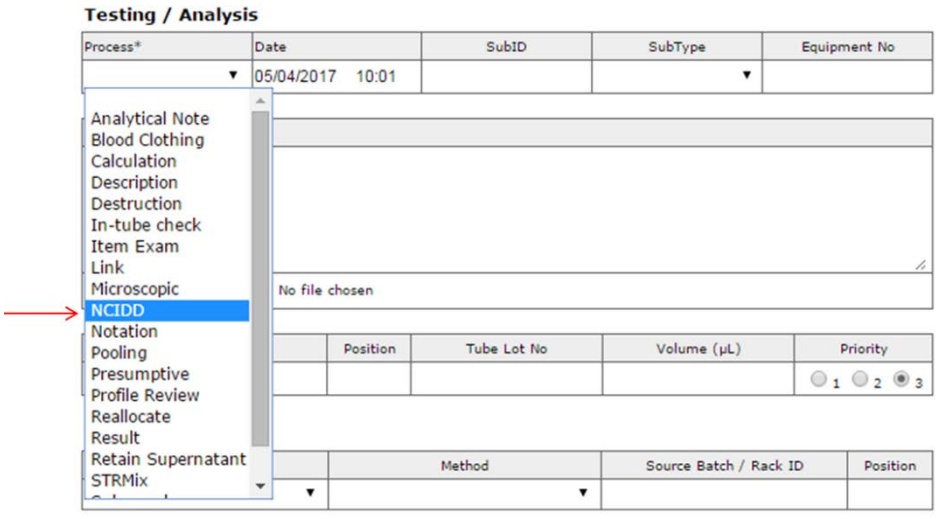
Exhibit Testing

Date / Time	Technique	Testing	Linked No	Employee	Reviewer
31/01/2017 13:43	STRAMP [WL]	690126645 PowerPlex21 3130xl		[redacted]	
01/02/2017 10:25	STRAMP	CSTRAMP20170201-01 PowerPlex21 3130xl			
01/02/2017 11:57	CE	CCE20170201-03 PowerPlex21 3130xl			
02/02/2017 10:42	PSTEXT [WL]	690126645 Microcon PowerPlex 21			
02/02/2017 10:55	PSTEXT	CPSTEXT20170202-03 Microcon PowerPlex 21			
02/02/2017 10:56	STRAMP [WL]	690126645 PowerPlex21 3130xl CPSTEXT20170202-03			
02/02/2017 10:57	STRAMP	CSTRAMP20170202-03 PowerPlex21 3130xl The Gremlins did it.			
02/02/2017 11:03	CE	CCE20170202-01 PowerPlex21 3130xl The Gremlins did it.			
02/02/2017 11:07	Result	SS CCE20170202-01 C05			
02/02/2017 11:17	PDA [WL]	690126645 GeneMapper IDX CCE20170202-01 C05			
23/03/2017 13:46	Result	690126645-ss 690126645 UKM1 (+NCIDD Upload)			

Exhibit Movement

Date / Time	Movement	Station	Continuity Officer	Forensic Officer
31/01/2017 13:43	IN	FSS Forensic DNA Analysis	440109	440109
31/01/2017 13:43	IN	Queensland Health Scientific	440109	440109

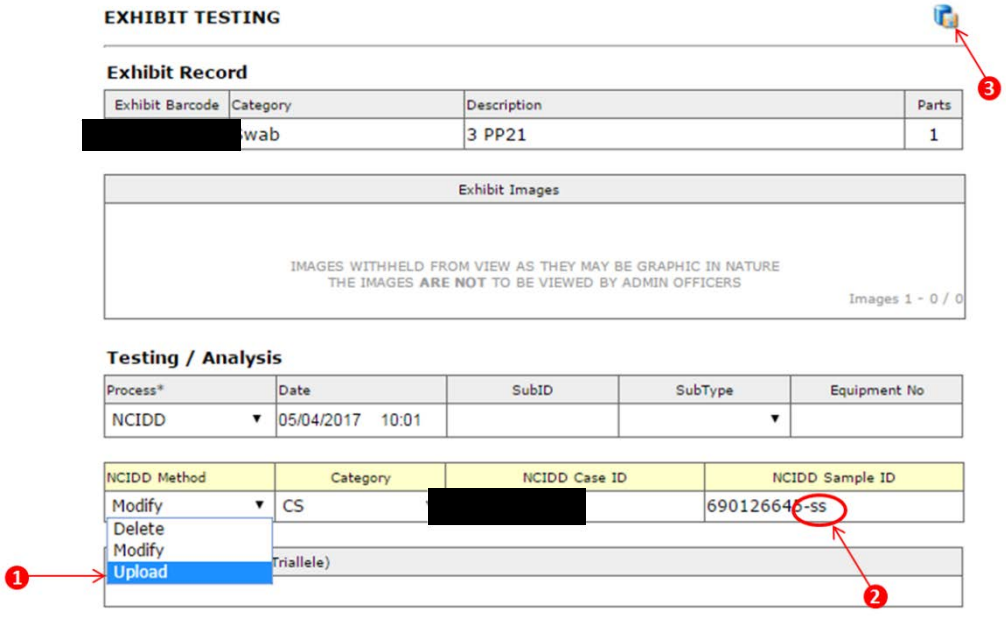
Select the process of 'NCIDD' from the drop-down list



Select the NCIDD Method of ‘Upload’ from the drop-down box (1)

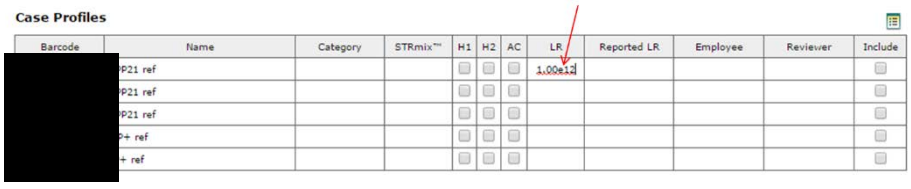
Enter the suffix of ‘-ss’ into the NCIDD Sample ID (2)

Click save (3)



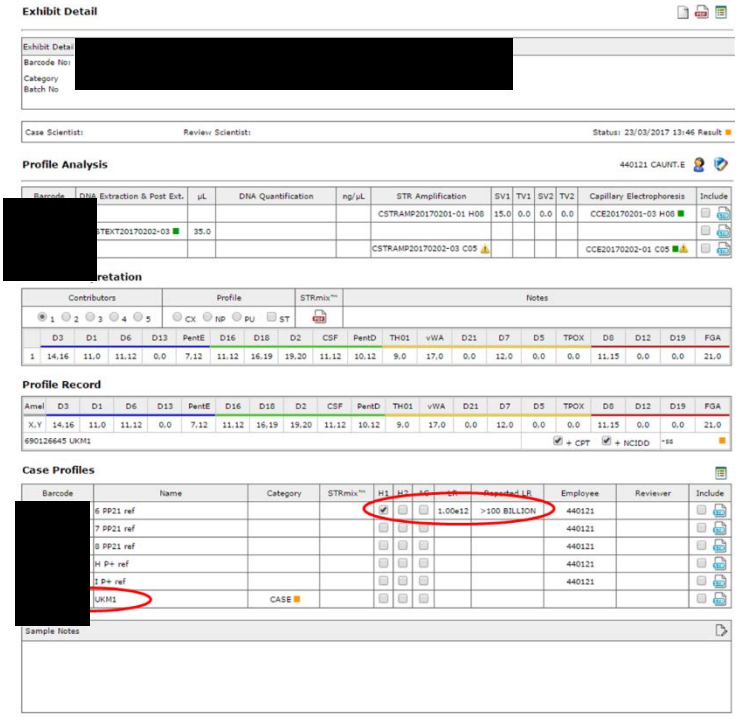
New Reference Comparison

If a previously designed unknown from a crime scene sample now matches a new reference sample for the case, then the Case Profiles table on the PDA page needs updating. Click and type “1.00e12” into the ‘LR’ box associated to the matching reference sample

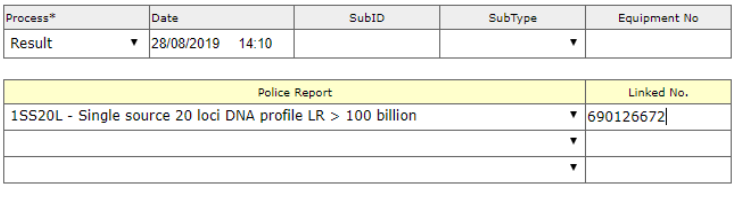


Click

The PDA page will now look like this



Associate the matching reference sample to the unknown by adding the reference sample barcode to the 'Association' column of the unknown. Click the icon above the Profile Interpretation table to update the associated result line



Click

Once the result line has been updated, order a process of 'Profile Review'. Click

23.5 Appendix 5 – PowerPlex 21 Priority 3 Workflow

Profiles assessed as complex:

- Check 'CX' box on PDA page
- Enter result 'CMPU' (complex unsuitable)
- Order 'Profile review'

Single source profiles:

If sub-threshold peaks are present but they do not affect the interpretation, report as per below with the extra result line 'PSTI' (possible sub-threshold information). If at any point the number of contributors is ambiguous, an assessment of suitability needs to be made (is the profile complex). Also, consider the possibility that the profile is a two contributor mixture.

Single source profile, <6 alleles

- Check '1' box on PDA page
- No STRmix required
- Enter result '1SSUND' (Single source DNA profile- unsuitable for NCIDD searching)
- Order 'Profile review'

Single source profile, ≥ 6 & <12 PP21 alleles

- Check '1' box on PDA page
- No STRmix required
- Enter result '1SSLND' (Single source DNA profile < NCIDD matching stringency)
- Order 'Profile review'

Single source profile, ≥ 12 PP21 alleles

- Check '1' box on PDA page
- Deconvolution in STRmix either by PDA analyst or HP2
- STRmix file imported
- Unknown person assigned
- Uploaded to NCIDD if appropriate
- Enter result
- Order 'Profile review'

For detailed steps, refer to Appendix 4 – Profile Data Analysis of a Single Source PP21 Profile for NCIDD.

2 to 4 contributor mixtures:

Even 2 to 3 contributor mixtures, no reference sample

- Check '2' or '3' box on PDA page
- Deconvolution in STRmix not required
- Enter result '2MX' or '3MX' and NSIP (no statistical interpretation performed)
- Order 'Profile review'

Major / minor mixed profile, expected to resolve, no reference sample

2 contributor mixture

- Check '2' box on PDA page
- Deconvolution in STRmix as 2p either by PDA analyst or HP2
- STRmix file imported

- Unknown persons assigned
- Major uploaded to NCIDD if appropriate
- Enter result of '2MX'
- Check NCIDD upload line entered if appropriate (intel or mix)
- Order 'Profile review'

3 contributor mixture with 2 contributors in the minor / 4 contributor mixture with 3 contributors in the minor (rework recommended)

- Check '3' or '4' box on PDA page
- Deconvolution in STRmix as 3 or 4p either by PDA analyst or HP2
- STRmix file imported
- Unknown person assigned
- Major uploaded to NCIDD if appropriate
- Enter result '3MX' or '4MX'
- Check NCIDD upload line entered if appropriate (intel or mix)
- Order 'Profile review'

Major / minor mixed profile, expected to resolve, reference samples

2 contributor mixture, reference sample matches major (check for the possiblity of conditioning through ownership – QIS 34006; Section 4.4.15)

- Check '2' box on PDA page
- Deconvolution and LR in STRmix as 2p either by PDA analyst or HP2
- STRmix file imported
- Unknown person assigned if any
- Major uploaded to NCIDD if appropriate
- Enter result '2MX'
- Enter reference comparison result
- Check NCIDD upload line entered if appropriate (intel or mix)
- Order 'Profile review'

3 contributor mixture with 2 contributors in the minor, reference sample matches major (check ownership – QIS 34006; Section 4.4.15) / 4 contributor mixture with 3 contributors in the minor, reference sample matches major (rework recommended, check ownership – QIS 34006; Section 4.4.15)

- Check '3' or '4' box on PDA page
- Deconvolution and LR in STRmix as 3 or 4p either by PDA analyst or HP2
- STRmix file imported
- Unknown person assigned if any
- Major uploaded to NCIDD if appropriate
- Enter result '3MX' or '4MX'
- Enter reference comparison result
- Check NCIDD upload line entered if appropriate (intel or mix)
- Order 'Profile review'

Reference samples received after initial PDA finalised (CW+Ref list):

See as per above where there is an associated reference sample. Reworks must be considered at this stage if there is any doubt as to the number of contributors.

23.6 Appendix 6 – STRmix Workflow

For STRmix troubleshooting, refer to QIS 17117.

The Profile Analyst will use the 'Contributors' section in the 'Profile Interpretation' table to inform the HP2 of the number of contributors for the STRmix analysis.

The Profile Analyst will use the 'Include' check boxes on the PDA page to inform the HP2 of the parameters for the STRmix analysis.

The 'Include' boxes in the 'Profile Analysis' table show which runs are to be used in the deconvolution; the 'Include' boxes in the 'Case Profiles' table show which reference samples are to be used in the LR. The 'AC' box in the 'Case Profiles' table is used to show that the associated reference sample is to be conditioned.

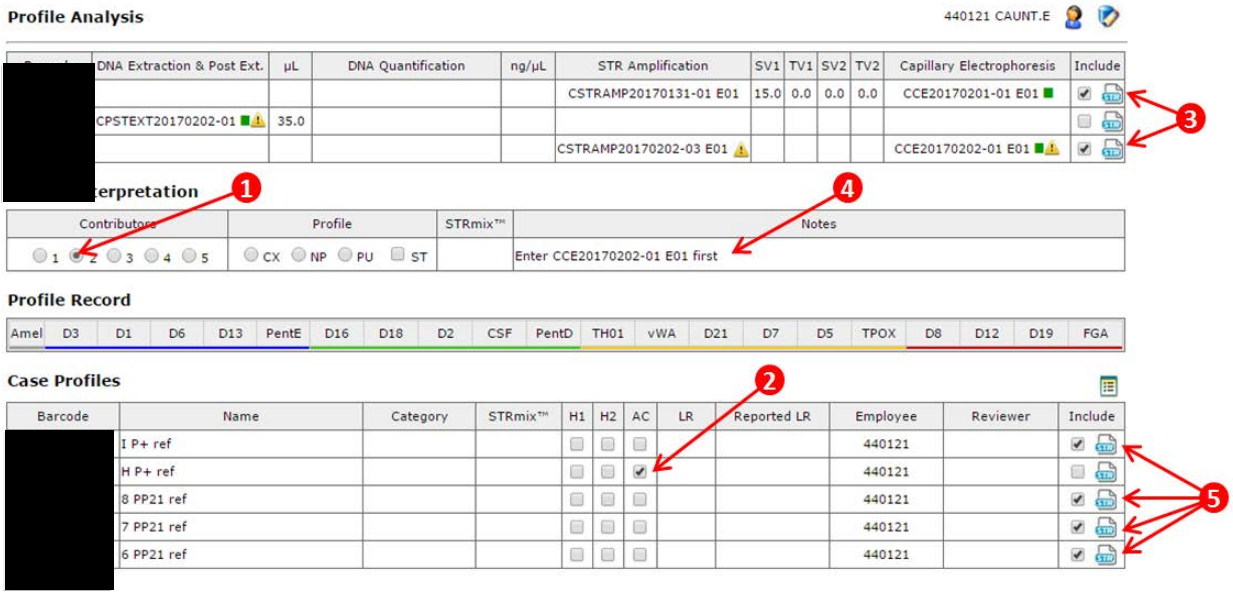
Any further instructions will be entered into the 'Notes' box such as:

- STRmix version
- Extra processing (e.g. re-deconvolution or increased iterations)
- CE instrument
- Dropped loci (e.g. vWA)

In instances where a profile has more than 12 alleles at a locus, the STRmix file may need to be modified prior to analysis.

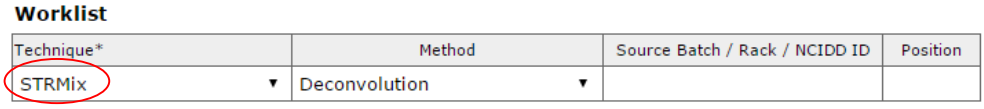
The example below shows that the STRmix analysis needs to be performed as follows:

1. Two contributors
2. Condition on "H P+ ref"
3. Include two CE runs
4. Any further instructions
5. Calculate LRs for the remaining four reference samples



If a deconvolution already exists and to request that LRs only are performed, add a note 'LRs only' and check the include boxes for the reference samples that require LRs.

The sample now needs to be added to the 'STRMix' worklist by requesting a STRmix deconvolution from the Exhibit Testing / Examinations table. This is done by adding the 'Technique' of 'STRMix'. The method of 'Deconvolution' adds by default and does not preclude the addition of LRs to the list. For samples with dilutions, the Profile Analyst should add the dilution barcode to the SubID field on the STRmix request page. To highlight that the STRmix process needs to be ordered on the dilution barcode, add 'STRmix: DILN' to the 'Notes' box on the PDA page.



Worklist

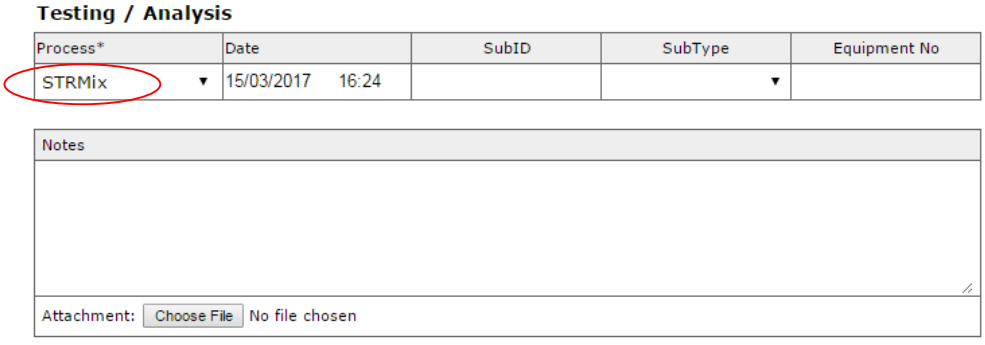
Technique*	Method	Source Batch / Rack / NCIDD ID	Position
STRMix ▾	Deconvolution ▾		

The sample will now be on the STRMix worklist.

STRMix Worklist

ExhibitNo	Exhibit	Method	Date / Time / Priority	Requested By
[redacted]	SWAB	Deconvolution	2017-03-01 15:16 2	440121 PSD
	SWAB	Deconvolution	2017-03-15 16:18 3	440121 PSD

When the HP2 takes a sample to run the STRmix analysis the first action is to order a STRmix process. If the sample has the PDA note 'STRmix: DILN', check the sample has a dilution and then order the STRmix process using the dilution barcode in the SubID field.



Testing / Analysis

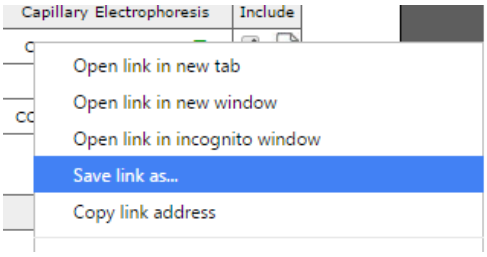
Process*	Date	SubID	SubType	Equipment No
STRMix ▾	15/03/2017 16:24		▾	

Notes

Attachment: Choose File No file chosen

This will remove the sample from the STRmix worklist, thereby preventing anybody else from running the same analysis, and simultaneously add the sample to the STRmix Review worklist. The HP2 will then run the STRmix analyses. If a sample with a dilution needs to be removed from the STRmix worklist, order a reallocate with the dilution barcode in the SubID field. Check the sample has only been removed from the STRmix worklist.

The HP2 will access the STRmix input files directly from the FR. Clicking the icon will download the STRmix input file for the associated casework run or reference sample. This input file can be saved to the desktop or other convenient location for access from STRmix. Alternatively, right clicking on the icon will allow the file to be saved directly.



When setting up the STRmix analysis only the QP number (or FR number if there is no QP number) is to be entered into the 'Case Number' field and only the barcode of the casework sample is to be entered into the 'Sample ID' field. This is also the case when running LRs. If additional information is to be added then this can be added to the 'Notes' field.

Once the STRmix analysis is complete, close the PDF generated by STRmix. The STRmix macro may be run and the PDFs uploaded into the FR as follows:

1. Run the STRmix macro

2. If not already present, the macro will create a folder called 'FR upload' and add a shortcut to your desktop

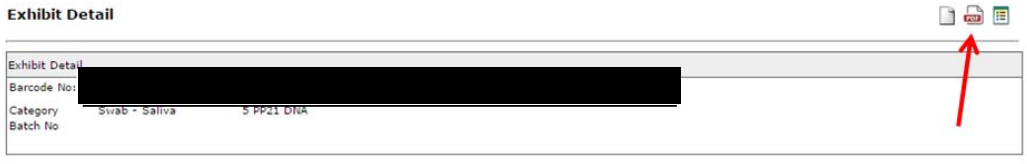
3. The STRmix files and PDF will be stored to I:\STRmix Results and a copy of the PDF will be stored in the FR upload folder

4. The FR upload folder should be emptied at regular intervals as this is only a transient storage location. A copy of the PDF will always be stored in I:\STRmix Results

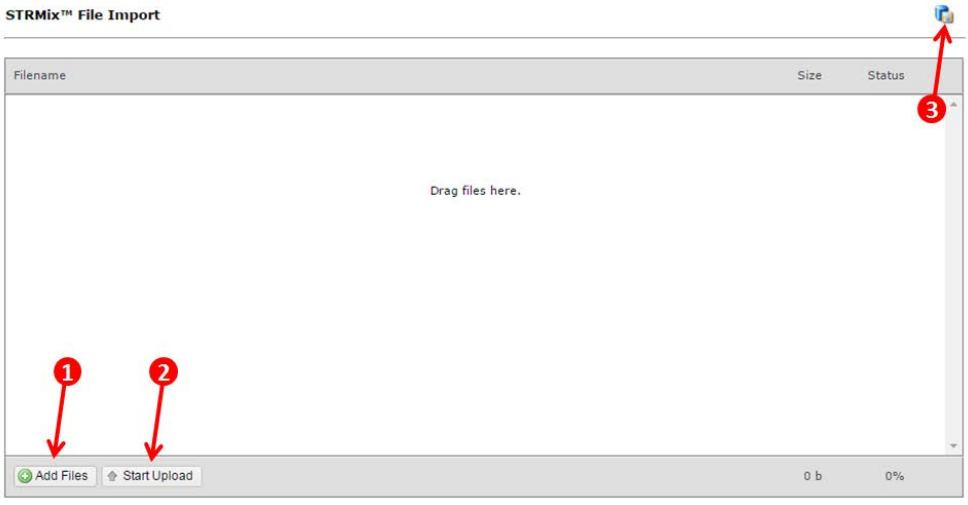
The PDF is named such that the FR will file the PDF in the correct place. This naming convention is based on the 'Case Number' and 'Sample ID' fields in STRmix, therefore if additional information is added into these fields the FR cannot file the PDFs appropriately.

The STRmix PDFs are uploaded into the FR as follows:

1. From the PDA page of the crime scene sample, click the icon in the top right corner



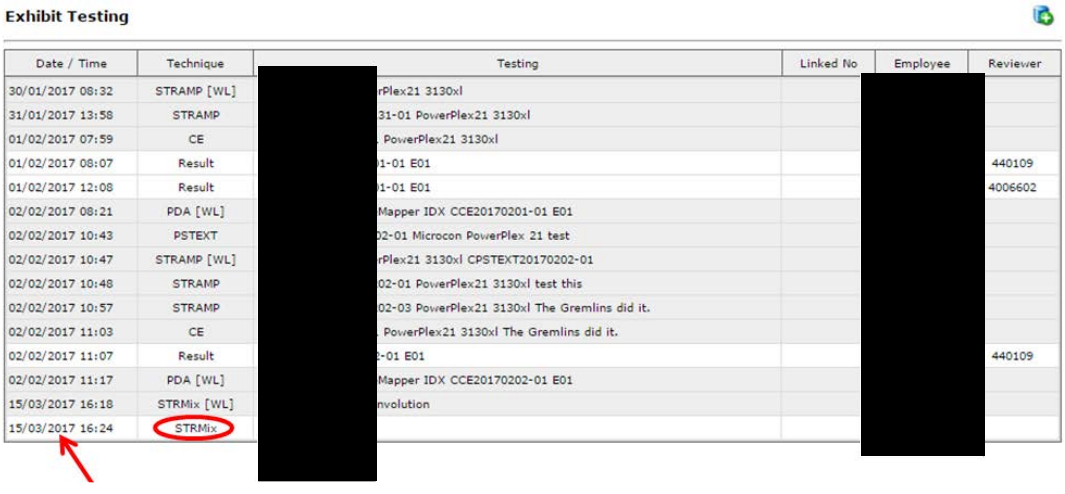
2. Drag and drop all of the STRmix PDFs pertaining to that sample into the window or click on 'Add Files' (1) and browse. Once all files are listed in the window, click 'Start Upload' (2) and then click the save icon (3)



All of the STRmix PDFs will file in the appropriate places. If you have STRmix PDF files for multiple samples they can all be dragged into the window at the same time regardless of which sample the window was opened from and they will all file appropriately.

If an LR has been performed against an unknown profile the STRmix PDF should not be uploaded to the FR using this process, instead the PDF should be attached to a 'Notation' against the sample.

Once the STRmix PDFs have been uploaded, the STRmix process should be closed. Enter into the STRmix process record from the Exhibit Testing table by clicking on the associated date and time.



Click the red '[CLICK TO VALIDATE]' button.

This will remove the sample from the STRmix Review worklist and simultaneously add it to the PDA worklist.

Once a STRmix PDF has been uploaded, it cannot be removed by Forensic DNA Analysis. A second STRmix PDF can be uploaded and it will overwrite the first PDF if the file names are the same.

If a STRmix PDF needs to be completely removed, a Forensic Register Change Request will need to be submitted as follows:

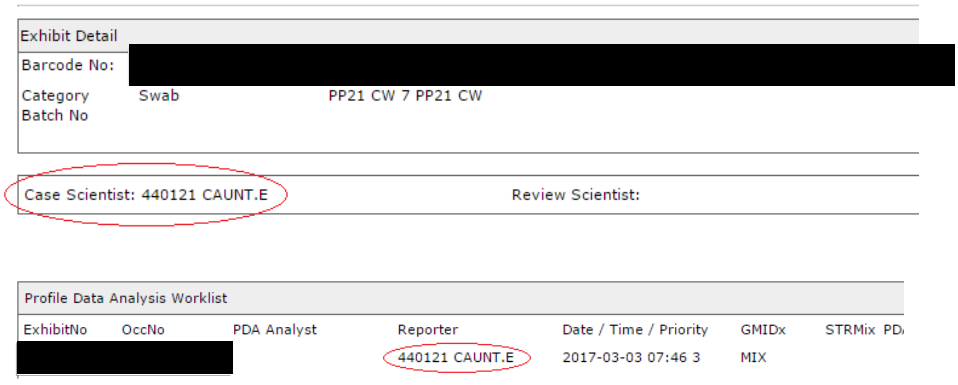
1. Enter into the Exhibit Record for the sample on the Case Management side of the FR
2. Click the icon in the top right of the screen
3. Complete the change request with the appropriate information

Any STRmix analysis no longer required should not be deleted but moved into a ‘Do not use’ folder within the relevant case number folder containing all of the STRmix results.

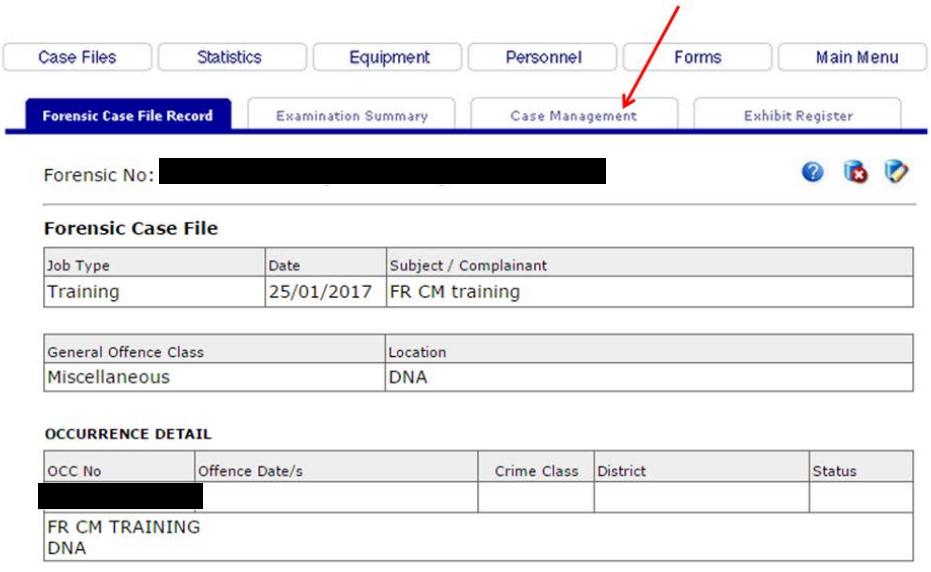
23.7 Appendix 7 – Allocating a Case to a Reporter / Profile Analyst

A whole case may be allocated to a scientist for the purposes of reporting but also during the early stages of a cases progression through the system to assist with consistency and efficiency (e.g. P1, operation, anticipated to be large or unique aspects).

Allocating a case to a scientist for PDA entry adds the name of the nominated scientist to the PDA page for every crime scene sample in the case, including new samples that are received at a later date. The name of the allocated scientist will appear against samples on the PDA and profile review lists.

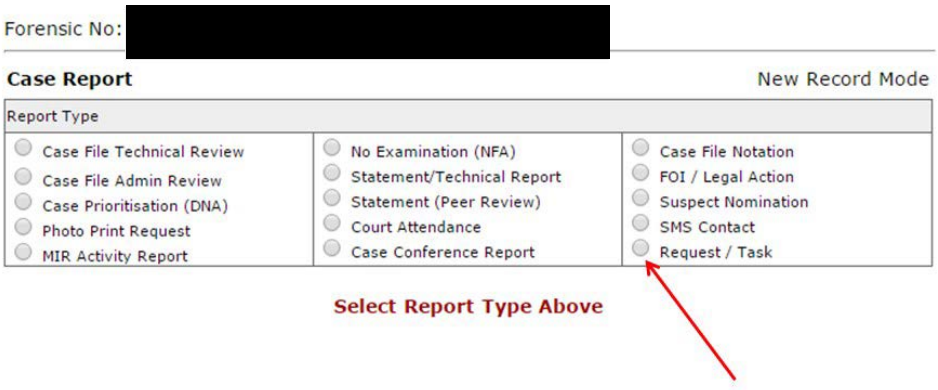


To allocate a case to a scientist enter into the Forensic Case File Record for the case



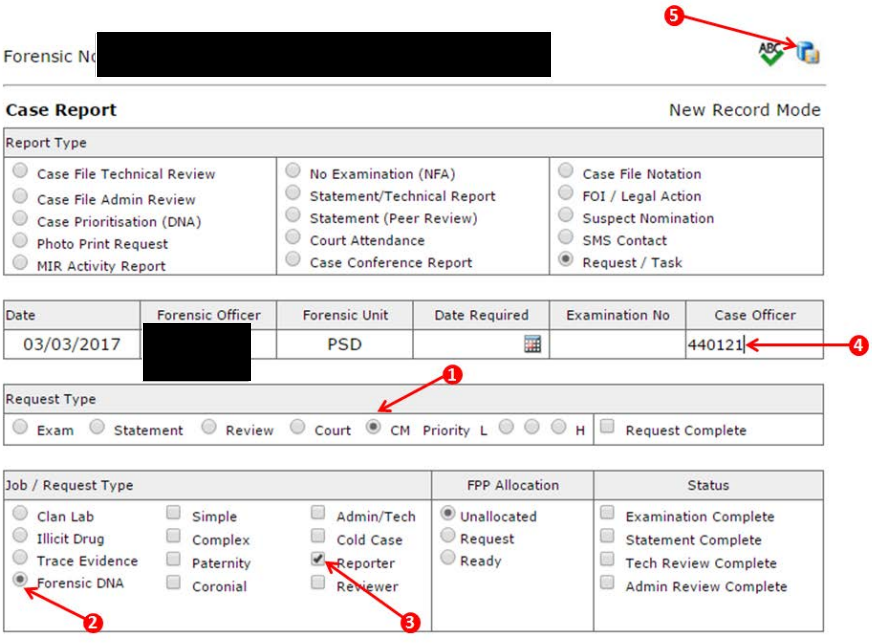
Click the Case Management tab and click the add button

Click Request / Task



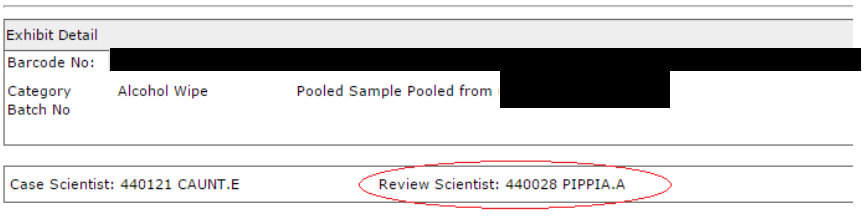
Select the following:

1. CM
2. Forensic DNA
3. Reporter
4. Enter the scientist (QPS user ID) that the case is being allocated to
5. Click save

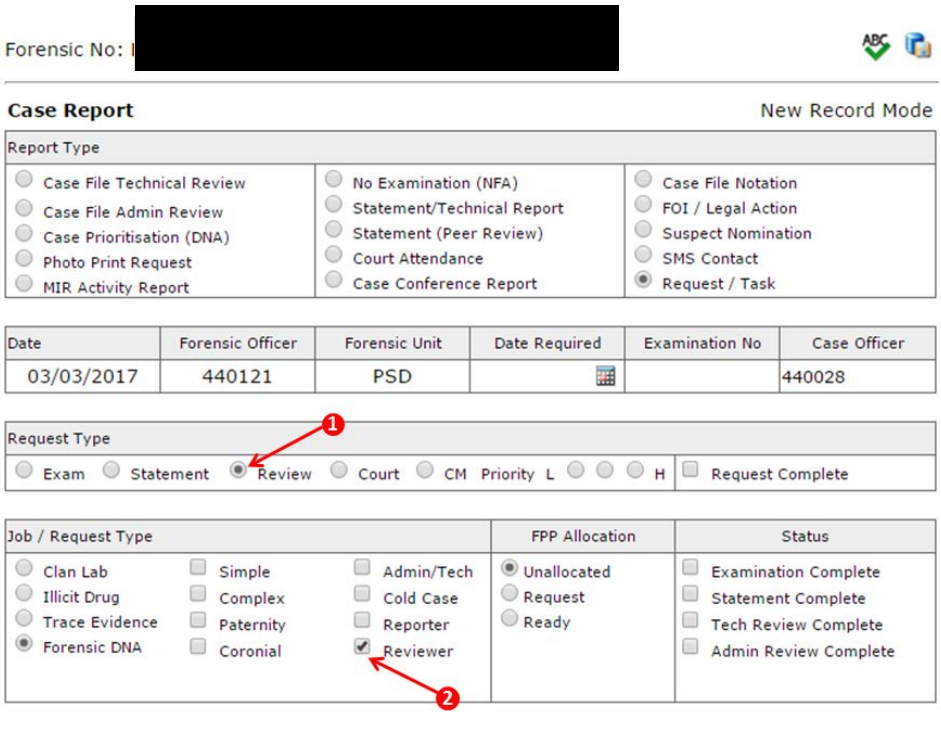


Allocating a Case to a Reviewer

A whole case may be allocated to a scientist for review to assist with reporting, consistency and efficiency. Allocating a case to a scientist for review adds the name of the nominated scientist to the Review Scientist field of the PDA page for every crime scene sample in the case. The name of the allocated review scientist will appear against samples on the profile review lists.

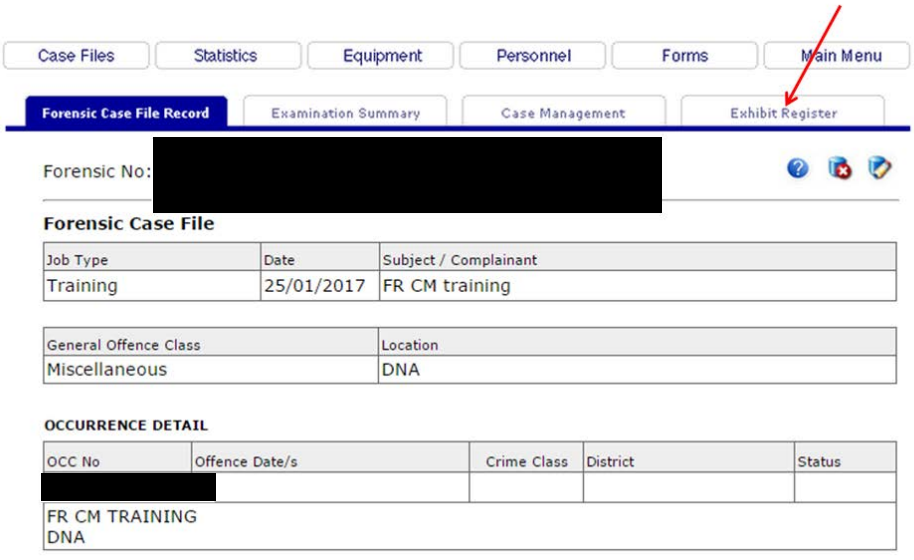


A case is allocated to a reviewer in the same way as allocating the case scientist; however 'Review' and 'Reviewer' are selected in place of 'CM' and 'Reporter' (see 1 and 2 below).



23.8 Appendix 8 – Creating and Tracking a Case File

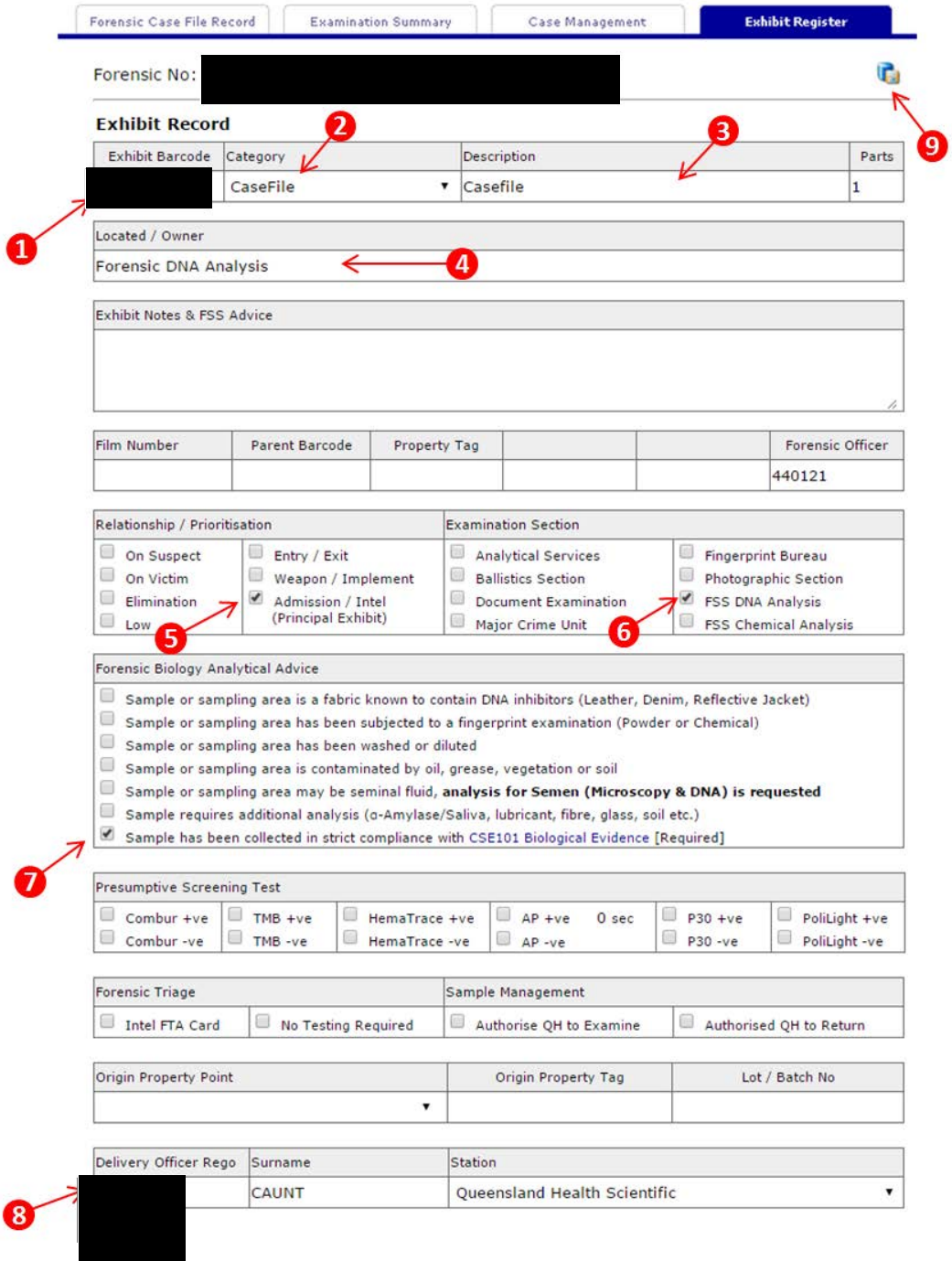
Enter into Forensic Case File Record. Click 'Exhibit Register' tab



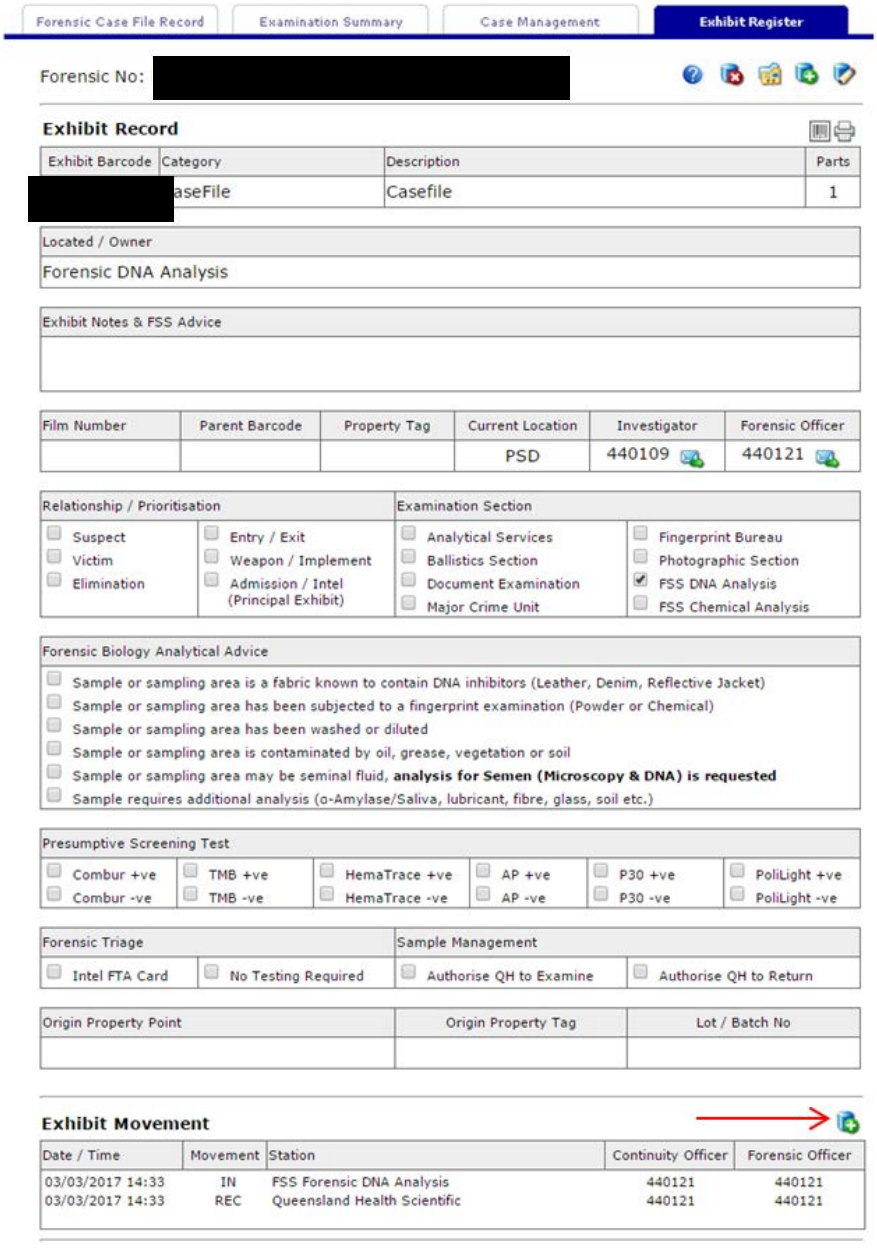
Click the add button

Enter the following information:

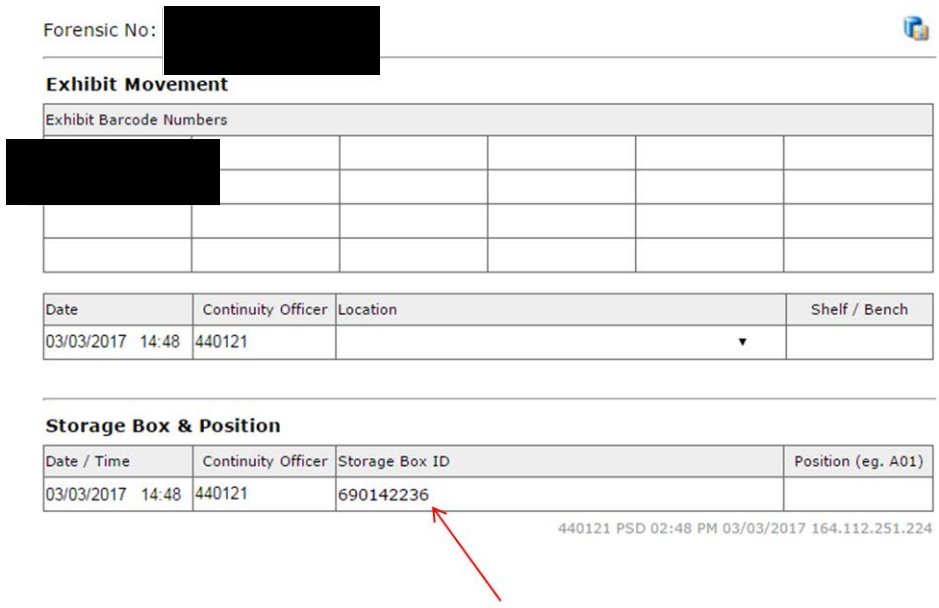
1. Barcode of case file (new nine or ten digit number or from existing case file previously tracked in AUSLAB)
2. Select 'CaseFile' from the drop-down list
3. Enter 'Case file' into the description
4. Enter 'Forensic DNA Analysis' into the 'Located / Owner' box
5. Check 'Admission / Intel'
6. Check 'Sample has been collected in strict compliance……'
7. Enter your QPS user ID, check that your surname is correct and select 'Queensland Health Scientific' from the drop-down Station menu
8. Click save



To track the case file, click the add button above the ‘Exhibit Movement’ table

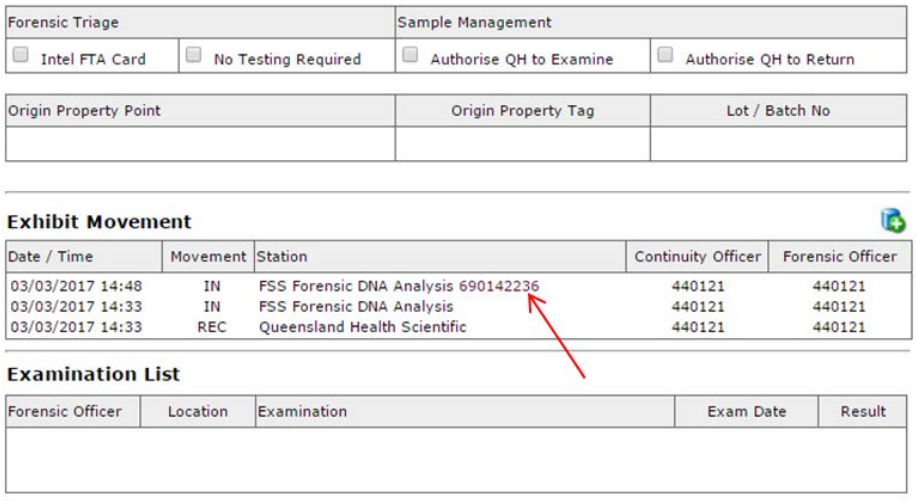


Enter the number of the storage box where the case file is to be stored in the 'Storage Box ID' field or start typing the name of the storage location and the relevant option will appear for selection (Section 19 and Appendix 12 – Forensic Register Storage Architecture)

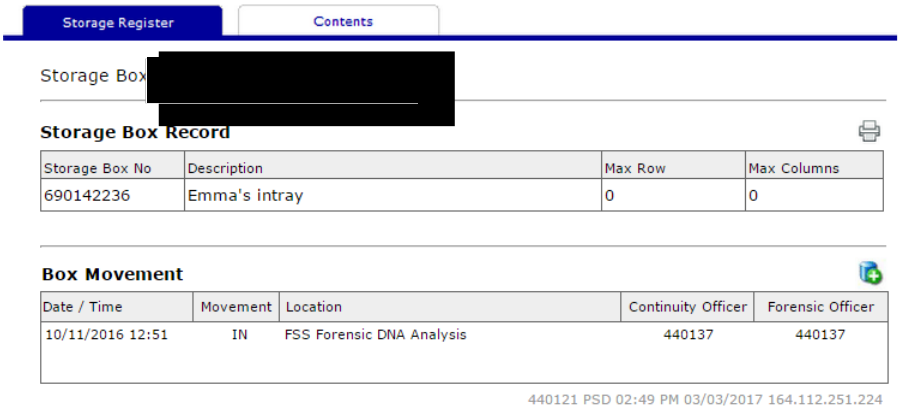


Click save

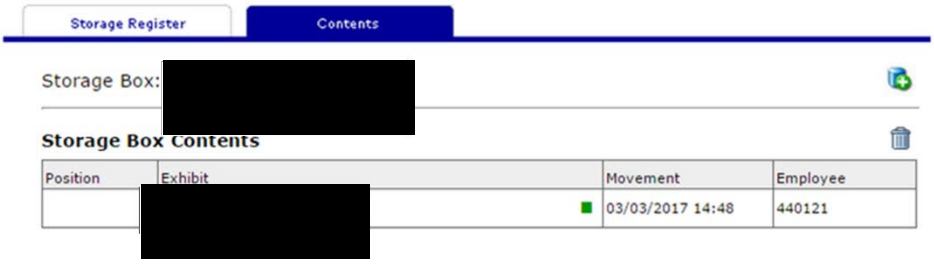
The location of the file will be displayed in the Exhibit Movement table



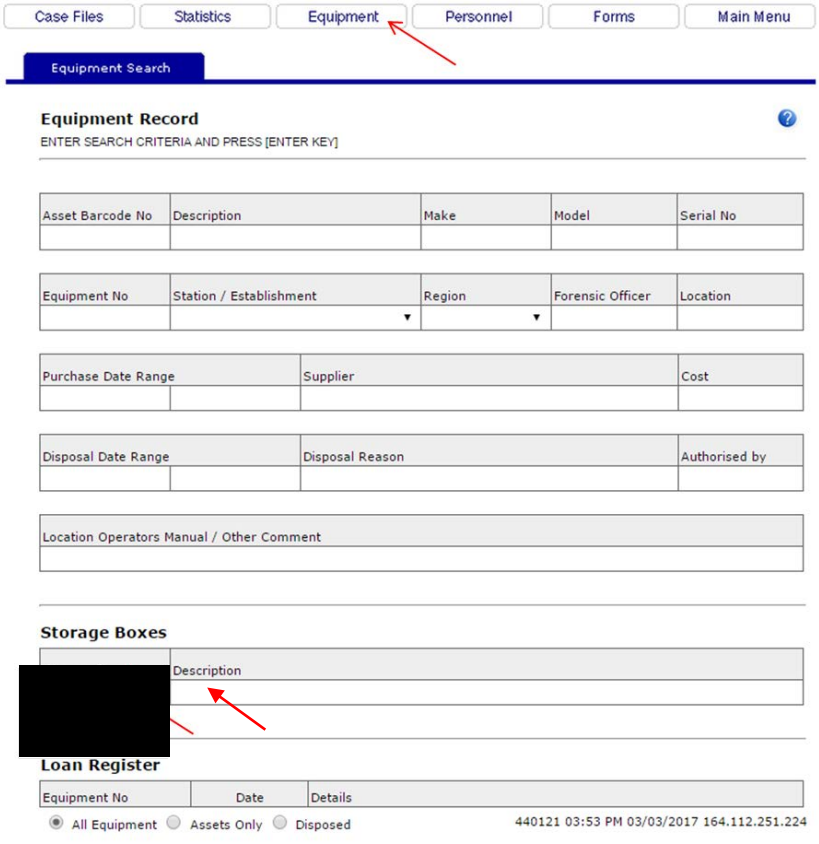
Clicking on the storage location will open the storage box record



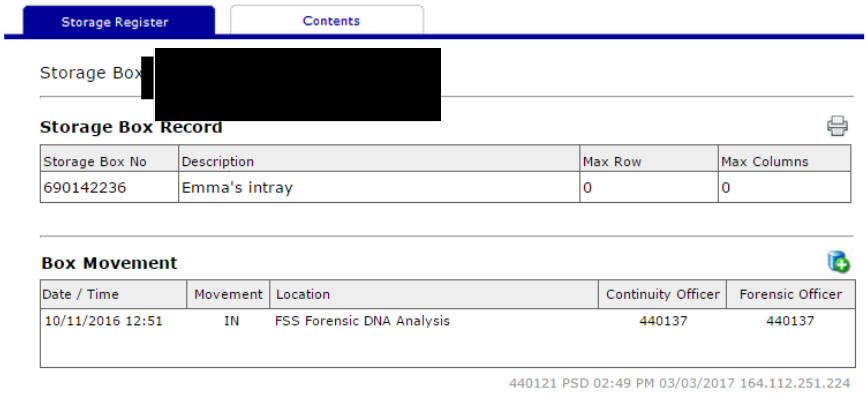
Clicking on the ‘Contents’ tab will display the contents of the storage box



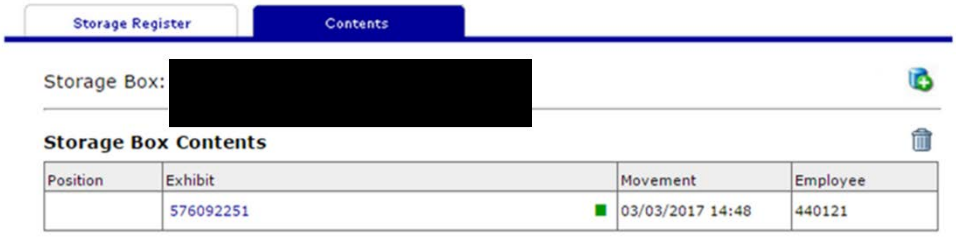
Storage boxes can also be found by clicking on the ‘Equipment’ tab and searching on the storage box number or the storage box description



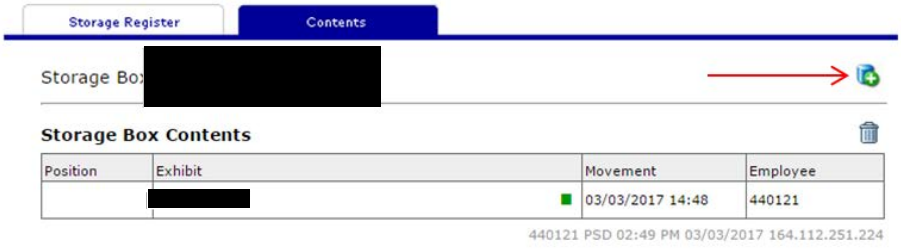
Press ‘Enter’



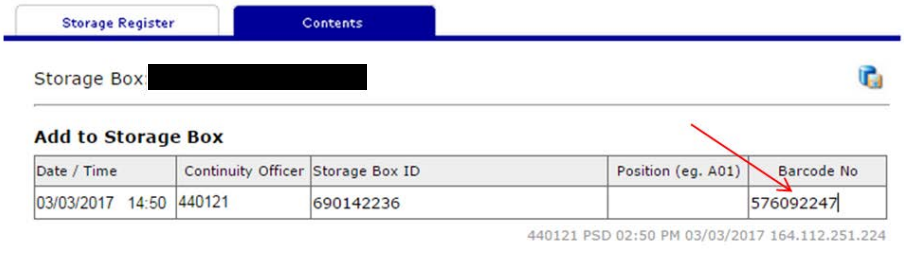
Clicking on the 'Contents' tab will display the contents of the storage box

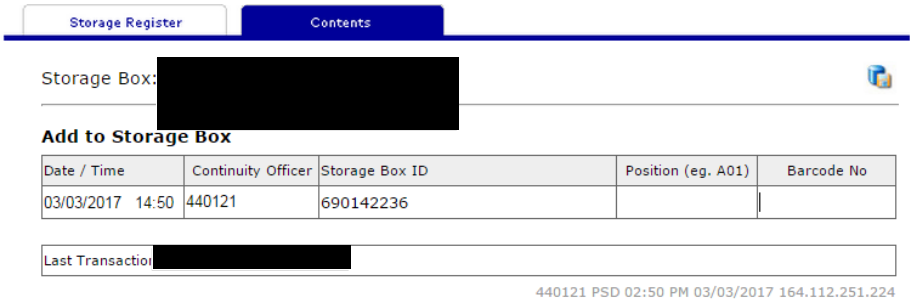


To add an item to a storage box (e.g. case file to intray), click the add button

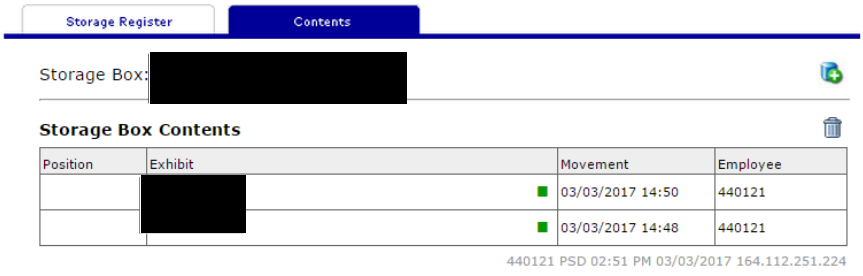


Enter the barcode of the item requiring storage in the 'Barcode No' field and click save

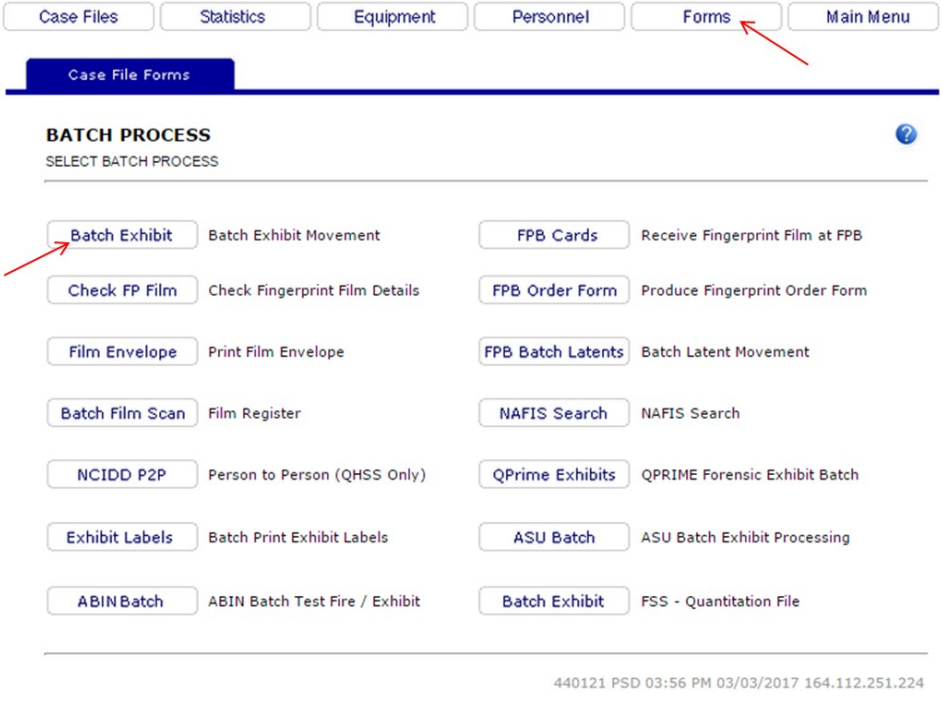




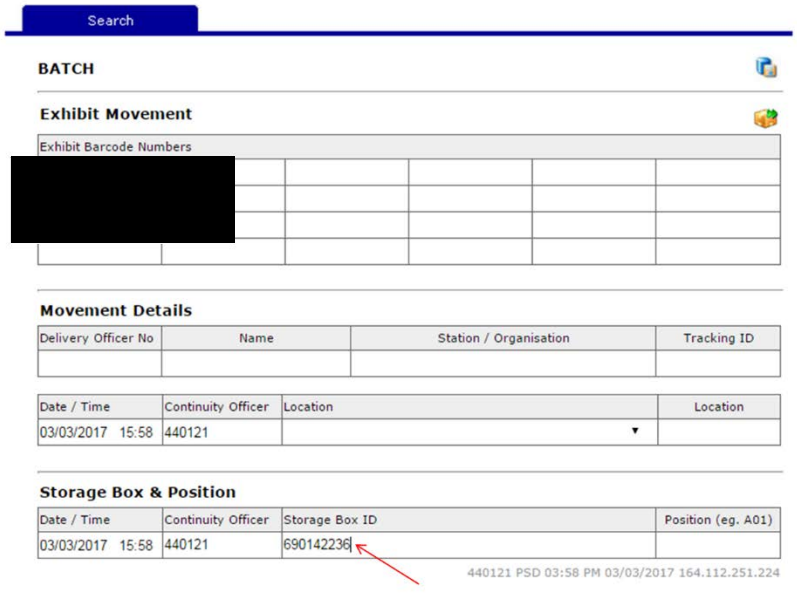
Clicking on the 'Contents' tab again will show the contents of the storage box



Multiple items can be transferred at once by clicking on the 'Forms' tab followed by the 'Batch Exhibit' button



Enter the items to be stored and the storage box ID and click save



Search

BATCH

Exhibit Movement

Exhibit Barcode Numbers					
[redacted]					

Movement Details

Delivery Officer No	Name	Station / Organisation	Tracking ID

Date / Time	Continuity Officer	Location	Location
03/03/2017 15:58	440121		

Storage Box & Position

Date / Time	Continuity Officer	Storage Box ID	Position (eg. A01)
03/03/2017 15:58	440121	690142236	

440121 PSD 03:58 PM 03/03/2017 164.112.251.224

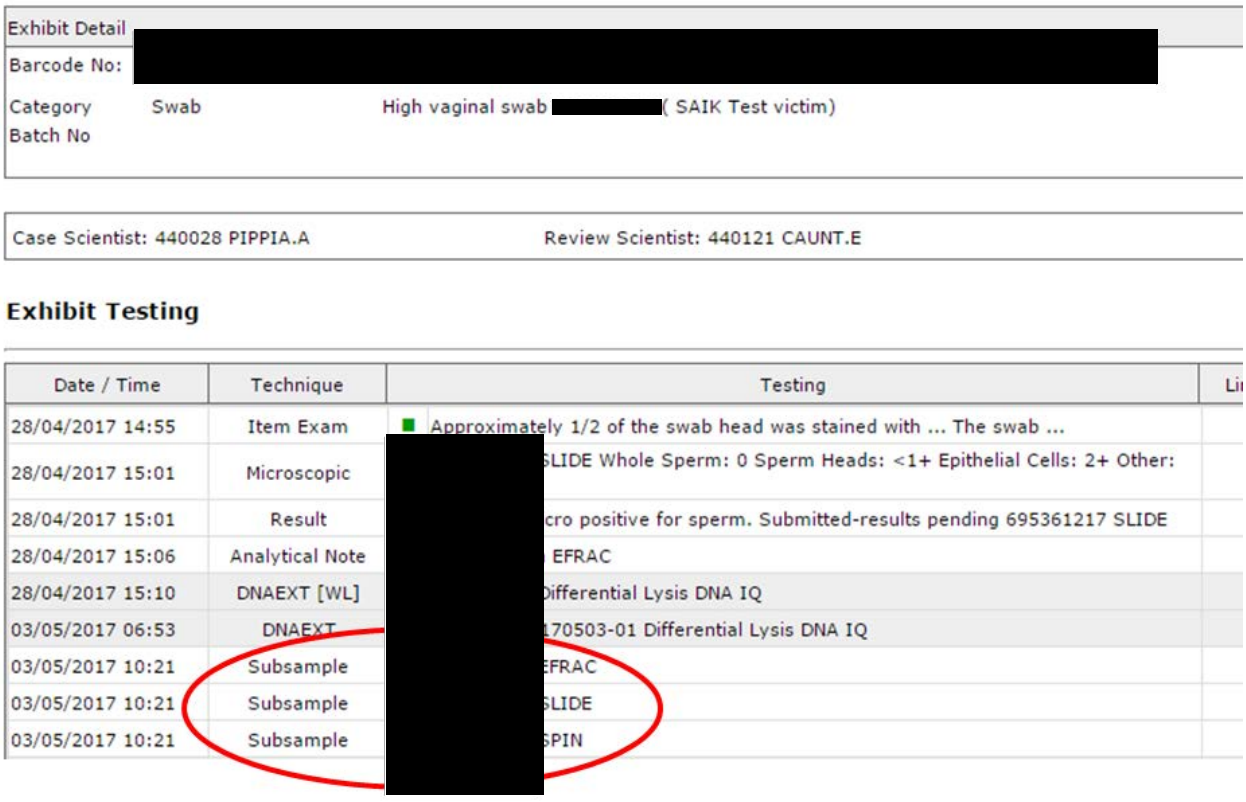
23.9 Appendix 9 – Registering a Subsample as an Exhibit

Registering a subsample as an exhibit may be required for samples such as epithelial fractions from high vaginal swabs, re-extracts of spin baskets (Section 11.2.5) and dilutions.

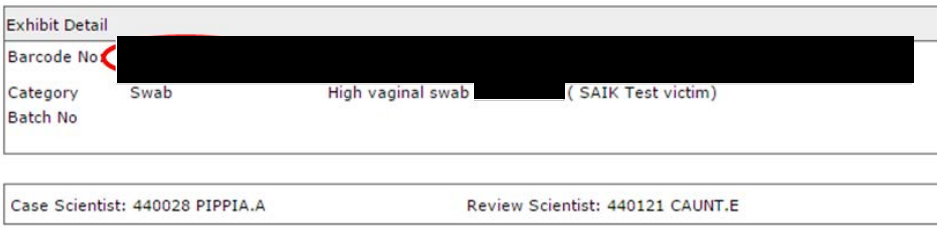
Consider a high vaginal swab that is submitted for DNA analysis. At the extraction stage a number of subsamples will be created to enable different parts of the high vaginal swab to be stored. These will include a spin basket, slide and EFRAC.

In the first instance the EFRAC from the HVS will not usually be processed, however the profile analyst may require the EFRAC to be profiled. In this instance it will be necessary to register the subsample as an exhibit to enable further processing.

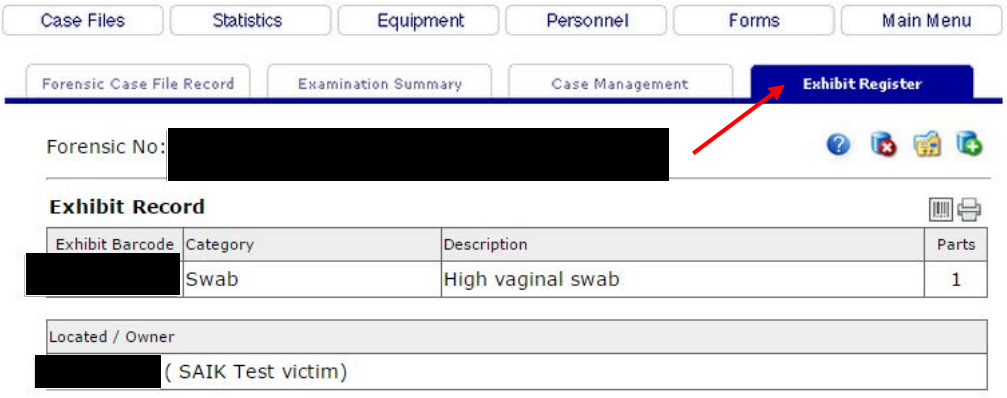
The exhibit testing table for the HVS will show the subsamples that have been created and their barcodes.



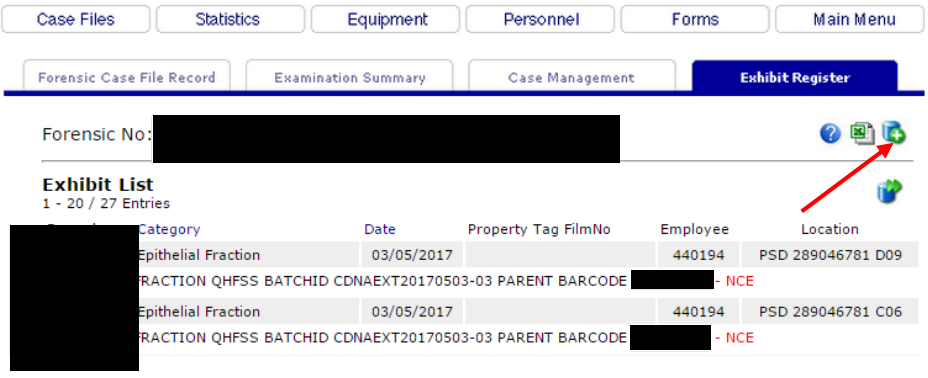
To register the EFRAC as an exhibit, click on the barcode of the HVS



Click the 'Exhibit Register' tab

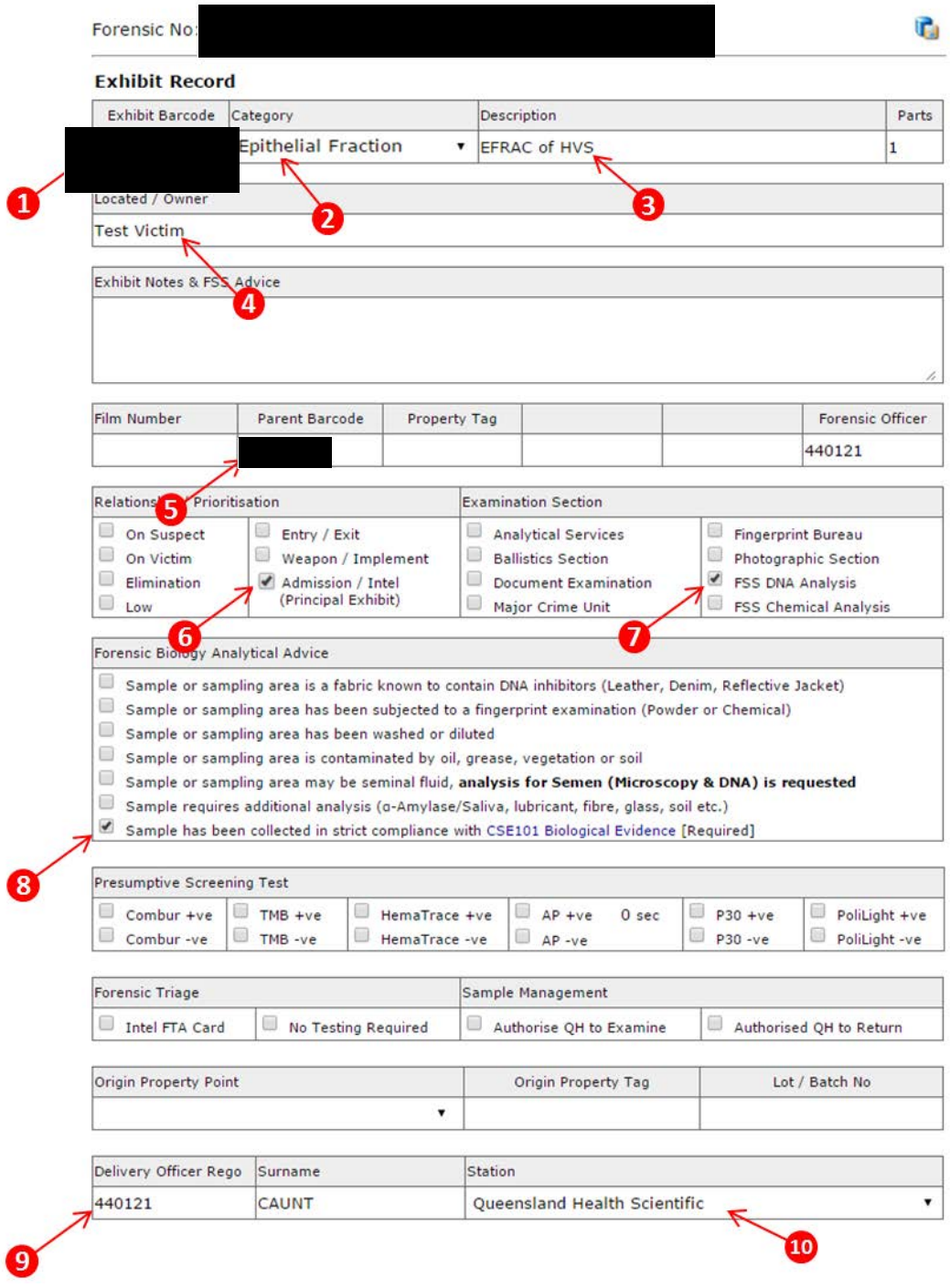


Click the button



Enter the following information:

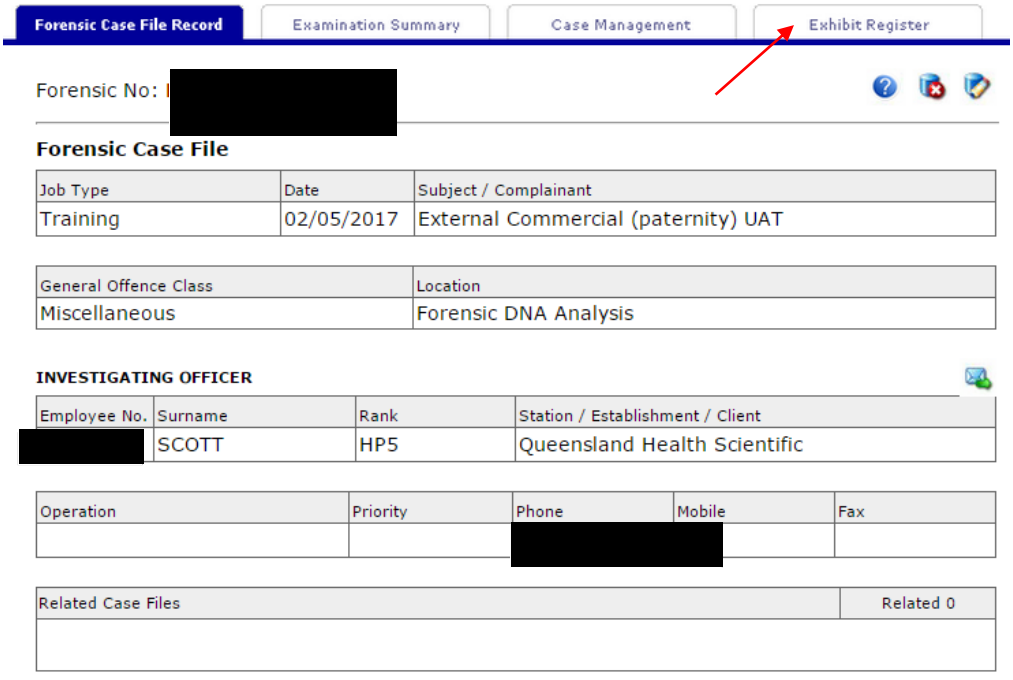
1. Barcode of EFRAC subsample
2. Select ‘Epithelial Fraction’ from the drop-down list (or other appropriate category)
3. Enter a description (for re-extraction of spin baskets also add the parent barcode and description ‘Spin basket – Parent barcode; Description’)
4. Enter information into the ‘Located / Owner’ box
5. Enter the barcode of the HVS into the ‘Parent Barcode’ box
6. Check ‘Admission / Intel’
7. Check ‘FSS DNA Analysis’ has been ticked
8. Check ‘Sample has been collected in strict compliance……’
9. Enter your QPS user ID
10. Select ‘Queensland Health Scientific’ from the drop-down list
11. Click save


Forensic No: [redacted]
Exhibit Record
Exhibit Barcode
Category
Description
Parts
[redacted]
Epithelial Fraction
EFRAC of HVS
1
Located / Owner
Test Victim
Exhibit Notes & FSS Advice
Film Number
Parent Barcode
Property Tag
Forensic Officer
[redacted]
440121
Relationship Prioritisation
On Suspect
On Victim
Elimination
Low
Entry / Exit
Weapon / Implement
Admission / Intel (Principal Exhibit)
Examination Section
Analytical Services
Ballistics Section
Document Examination
Major Crime Unit
Fingerprint Bureau
Photographic Section
FSS DNA Analysis
FSS Chemical Analysis
Forensic Biology Analytical Advice
Sample or sampling area is a fabric known to contain DNA inhibitors (Leather, Denim, Reflective Jacket)
Sample or sampling area has been subjected to a fingerprint examination (Powder or Chemical)
Sample or sampling area has been washed or diluted
Sample or sampling area is contaminated by oil, grease, vegetation or soil
Sample or sampling area may be seminal fluid, analysis for Semen (Microscopy & DNA) is requested
Sample requires additional analysis (α-Amylase/Saliva, lubricant, fibre, glass, soil etc.)
Sample has been collected in strict compliance with CSE101 Biological Evidence [Required]
Presumptive Screening Test
Combur +ve
Combur -ve
TMB +ve
TMB -ve
HemaTrace +ve
HemaTrace -ve
AP +ve
0 sec
AP -ve
P30 +ve
P30 -ve
PoliLight +ve
PoliLight -ve
Forensic Triage
Intel FTA Card
No Testing Required
Sample Management
Authorise QH to Examine
Authorised QH to Return
Origin Property Point
Origin Property Tag
Lot / Batch No
Delivery Officer Rego
Surname
Station
440121
CAUNT
Queensland Health Scientific
1
2
3
4
5
6
7
8
9
10

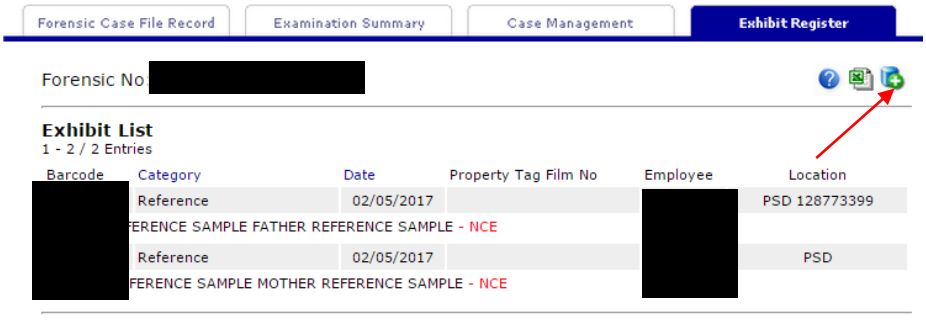
23.10 Appendix 10 – Processing of DNA Number Exhibits

Register a new barcode for the sample to be transferred as follows (e.g. DNA# / Transfer samples):

From the 'Forensic Case File Record' for the case, click the 'Exhibit Register' tab

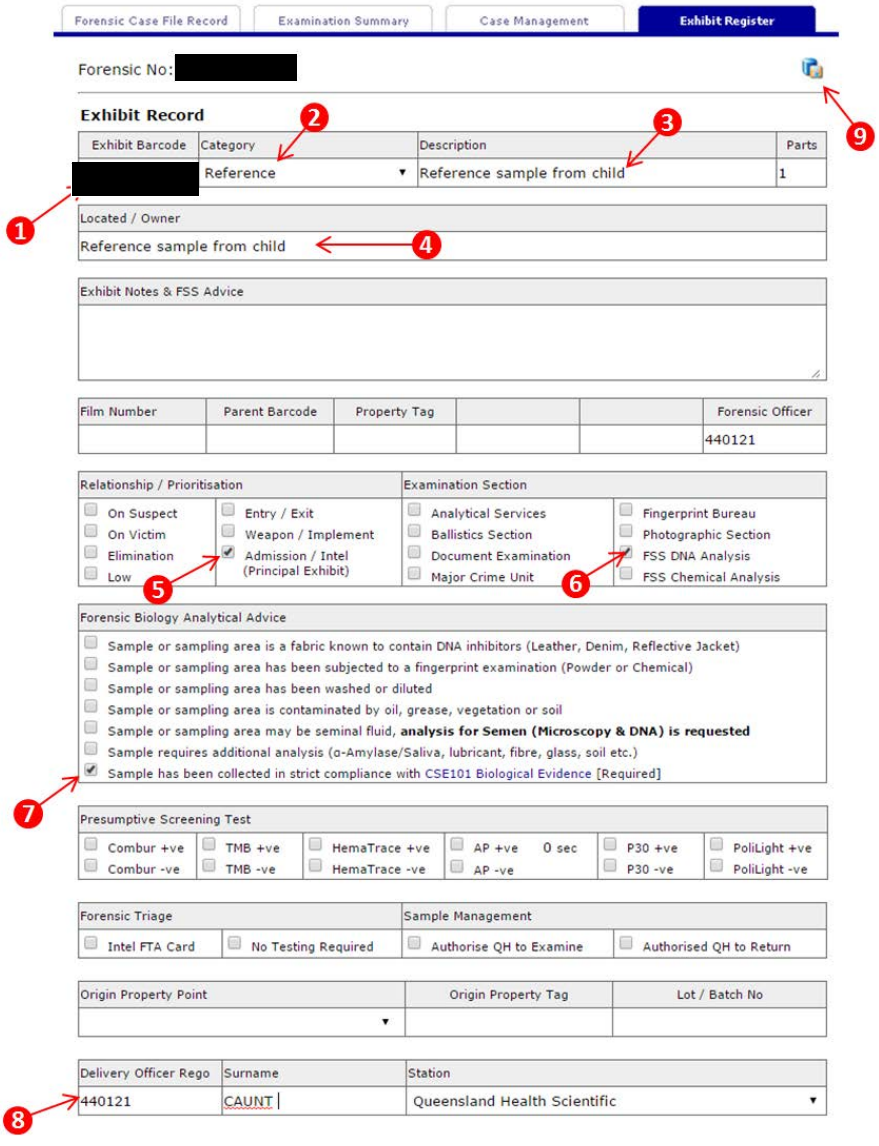


Click the icon to add a new exhibit to the case

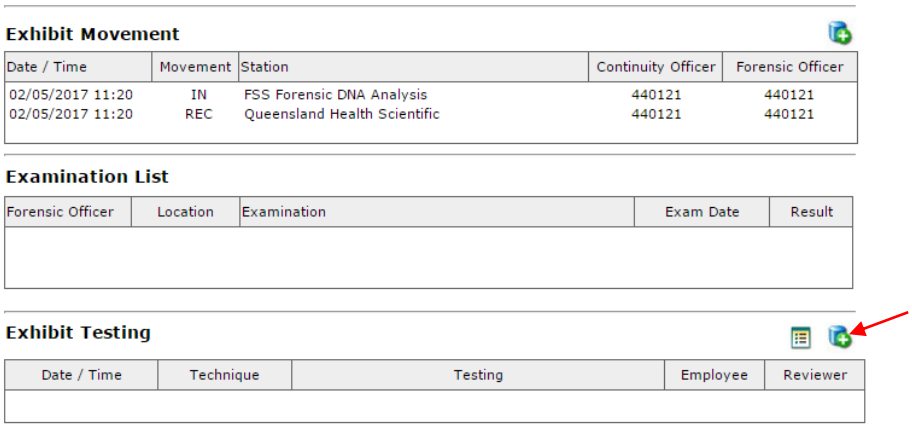


Complete the appropriate fields as below:

1. Enter new barcode for the exhibit
2. Select appropriate category from the drop-down list
3. Enter description
4. Enter owner / location (including DNA#)
5. Check the 'Admission / Intel' box
6. Check 'FSS DNA Analysis' has been ticked
7. Check the 'Sample has been collected in strict compliance with CSE101…' box
8. Enter your QPS user ID and press the tab key to fill your name
9. Click save

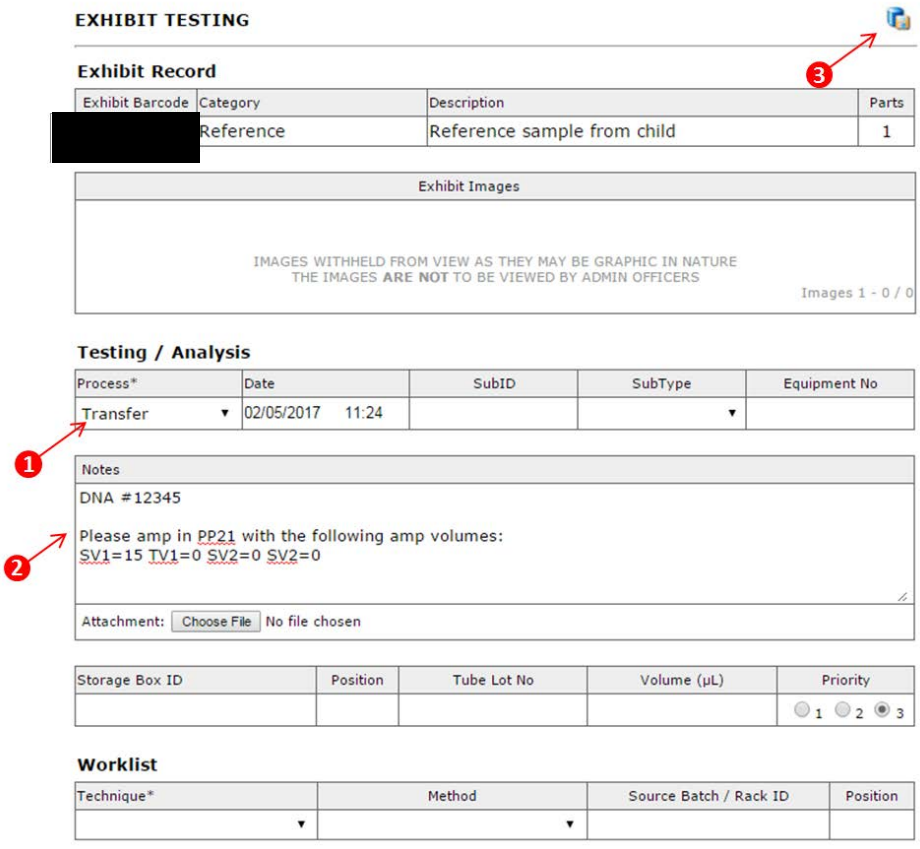


Once the record has been saved, a 'Transfer' process needs to be ordered. Click the icon above the 'Exhibit Testing' table:



Complete the Exhibit Testing page as follows:

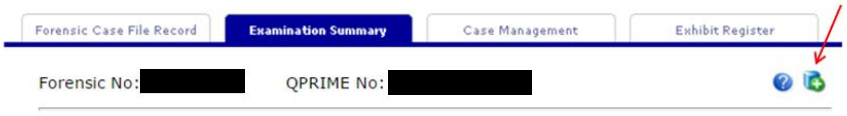
1. Select the process of ‘Transfer’ from the drop-down list
2. In the notes section, enter the DNA number and the details of the processing required
3. Click save



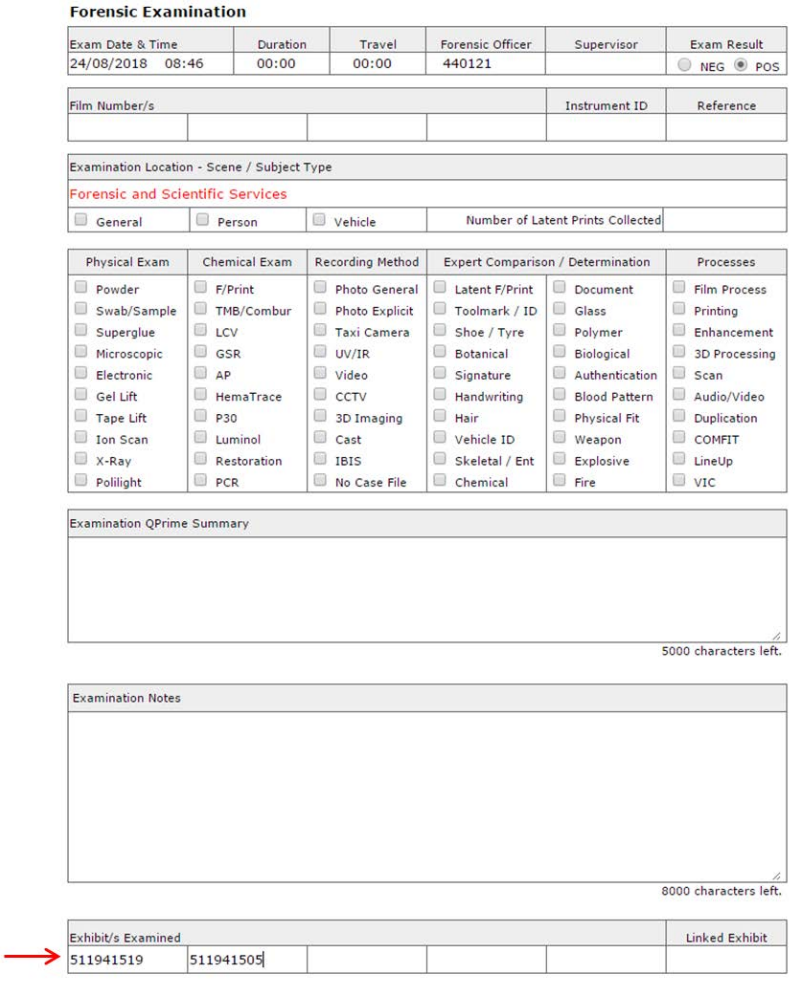
23.11 Appendix 11 – Process for Pooling Samples

1. On the Case Management side of the FR enter into a sample in the case where the pooling is to occur
2. Click the 'Examination Summary' tab

3. Click add

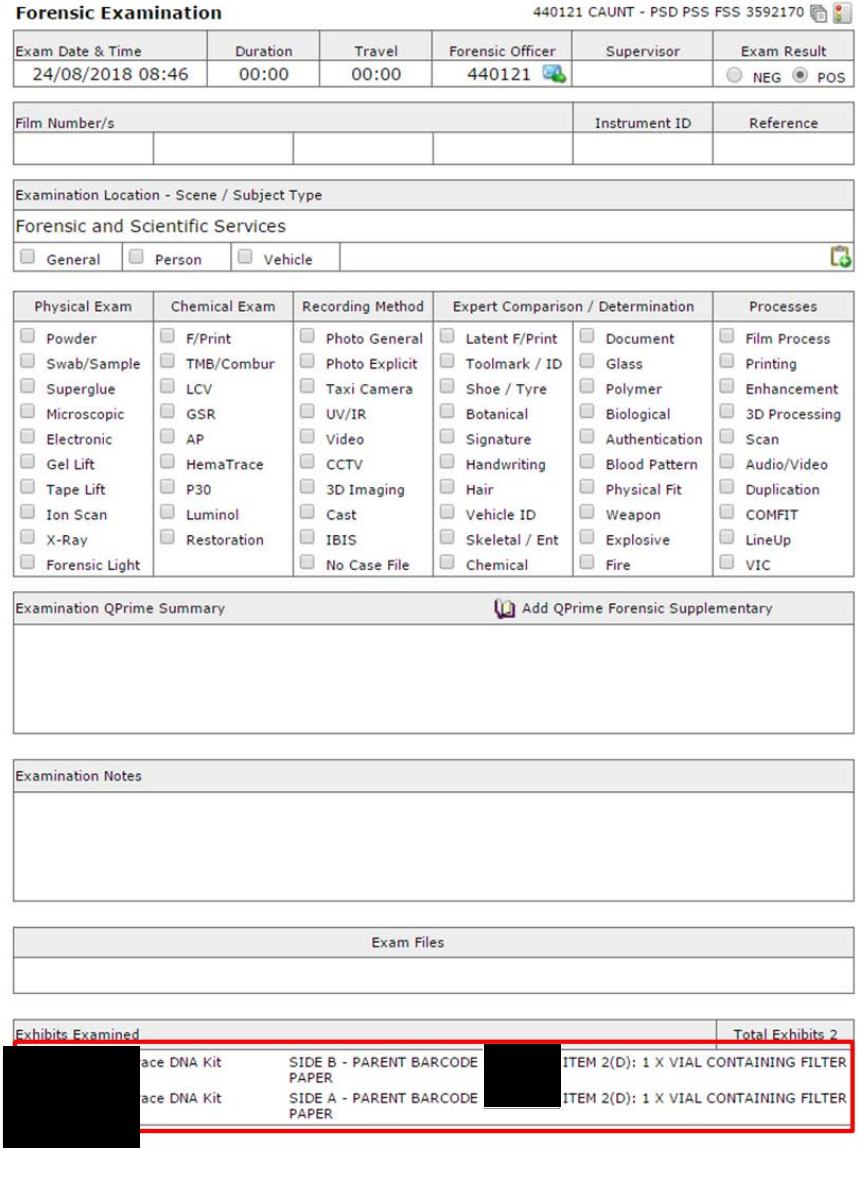


4. Enter the barcodes of the samples that require pooling in the 'Exhibit/s Examined' section. **Ensure that these samples are registered as exhibits before pooling**

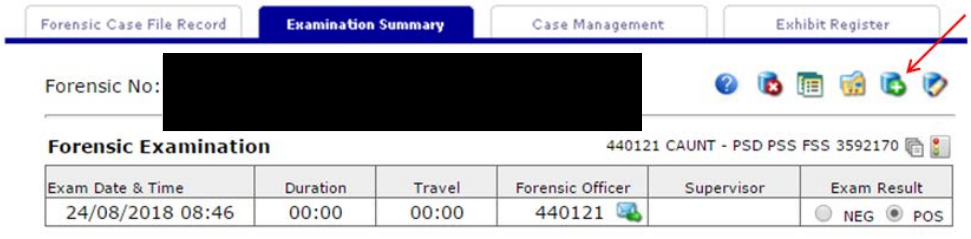


5. Click save

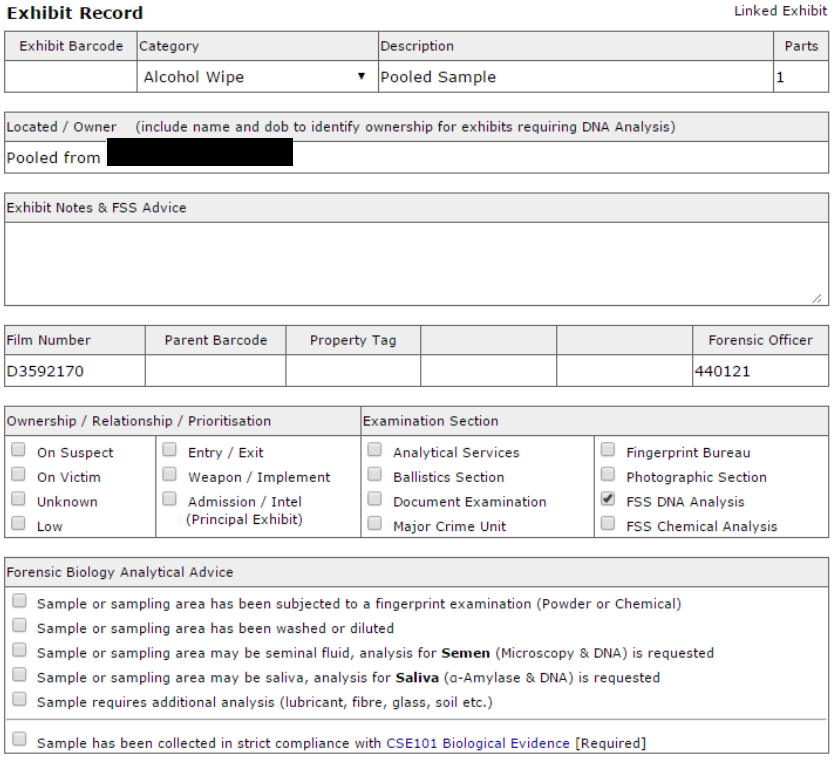
The samples to be pooled will appear on the page as below



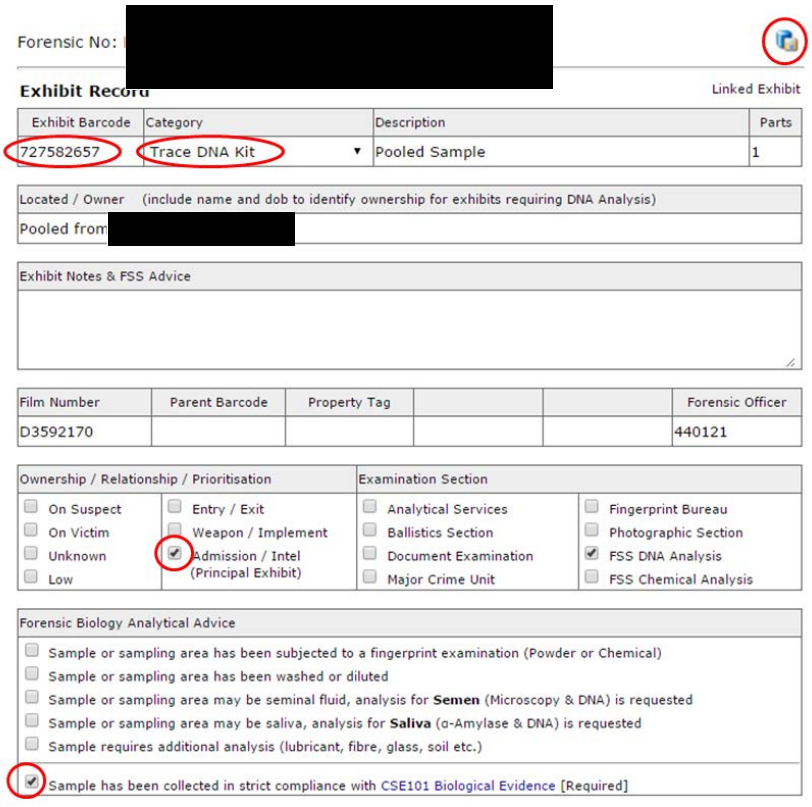
6. Add a related exhibit to the examination summary by clicking add



7. The following page will appear which will allow you to register a new barcode for the final pooled sample



8. Enter a new barcode into the ‘Exhibit Barcode’ field
    a. Change the ‘Category’ to the same as that for the parent item
    b. Tick the ‘Admission / Intel’ box
    c. Tick the ‘Sample has been collected…..’ box
    d. Click save



Forensic No: [redacted]

Exhibit Record Linked Exhibit

Exhibit Barcode	Category	Description	Parts
727582657	Trace DNA Kit	Pooled Sample	1

Located / Owner (include name and dob to identify ownership for exhibits requiring DNA Analysis)

Pooled from [redacted]

Exhibit Notes & FSS Advice

Film Number	Parent Barcode	Property Tag			Forensic Officer
D3592170					440121

Ownership / Relationship / Prioritisation		Examination Section	
☐ On Suspect	☐ Entry / Exit	☐ Analytical Services	☐ Fingerprint Bureau
☐ On Victim	☐ Weapon / Implement	☐ Ballistics Section	☐ Photographic Section
☐ Unknown	☑ Admission / Intel (Principal Exhibit)	☐ Document Examination	☑ FSS DNA Analysis
☐ Low		☐ Major Crime Unit	☐ FSS Chemical Analysis

Forensic Biology Analytical Advice

- ☐ Sample or sampling area has been subjected to a fingerprint examination (Powder or Chemical)
- ☐ Sample or sampling area has been washed or diluted
- ☐ Sample or sampling area may be seminal fluid, analysis for **Semen** (Microscopy & DNA) is requested
- ☐ Sample or sampling area may be saliva, analysis for **Saliva** (α-Amylase & DNA) is requested
- ☐ Sample requires additional analysis (lubricant, fibre, glass, soil etc.)
- ☑ Sample has been collected in strict compliance with CSE101 Biological Evidence [Required]

9. The sample will now appear on the 'Pooling' review list for the Analytical section to action
10. Add an 'Analytical Note' to advise what testing is required, eg 'Pool; Quant and hold'
11. The pooled samples will have the 'Sample pooled and processed under' line automatically added and validated. They will also have an examination record. This examination record should be validated as per QIS 34298

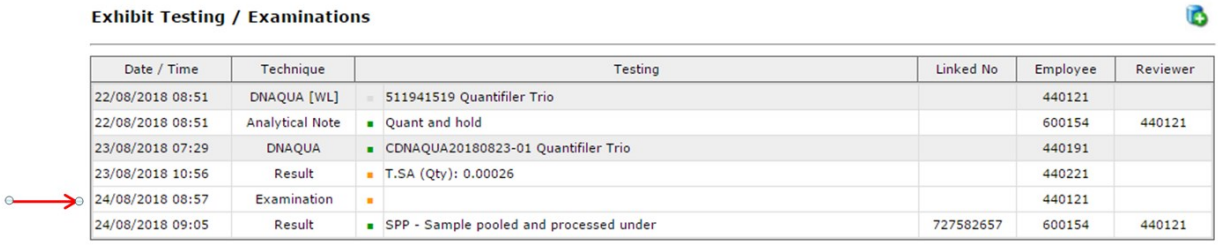
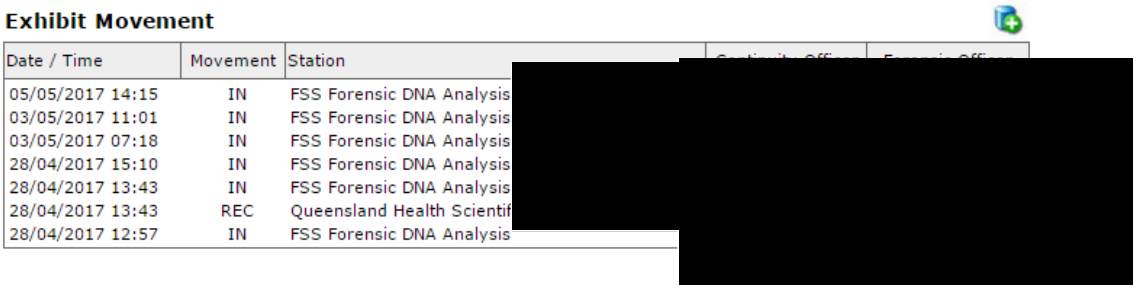


Exhibit Testing / Examinations

Date / Time	Technique	Testing	Linked No	Employee	Reviewer
22/08/2018 08:51	DNAQUA [WL]	511941519 Quantifiler Trio		440121	
22/08/2018 08:51	Analytical Note	Quant and hold		600154	440121
23/08/2018 07:29	DNAQUA	CDNAQUA20180823-01 Quantifiler Trio		440191	
23/08/2018 10:56	Result	T.SA (Qty): 0.00026		440221	
24/08/2018 08:57	Examination			440121	
24/08/2018 09:05	Result	SPP - Sample pooled and processed under	727582657	600154	440121

23.12 Appendix 12 – Forensic Register Storage Architecture

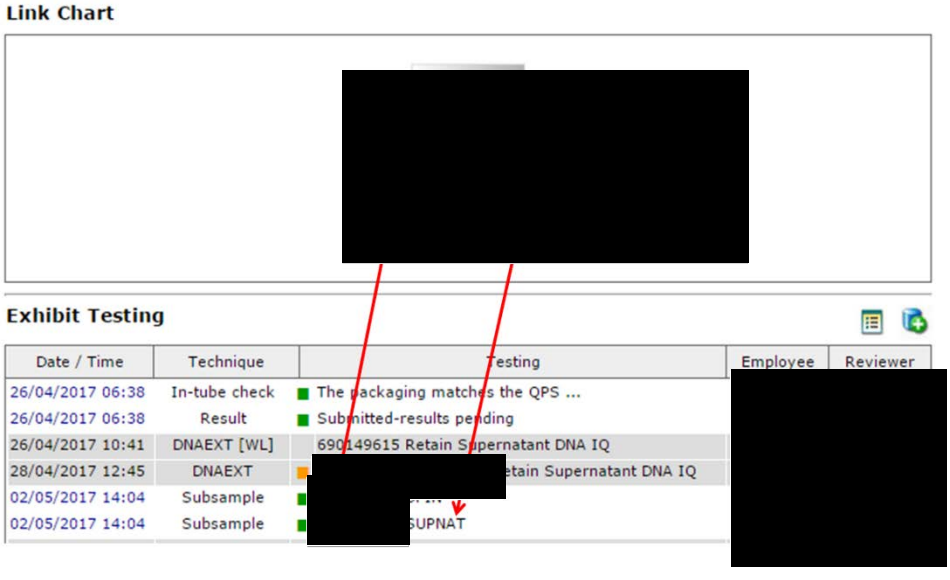
Exhibit movement in the FR works by updating the location of the exhibit. The most recent location of an exhibit is displayed at the top of the list in the Exhibit Movement table.



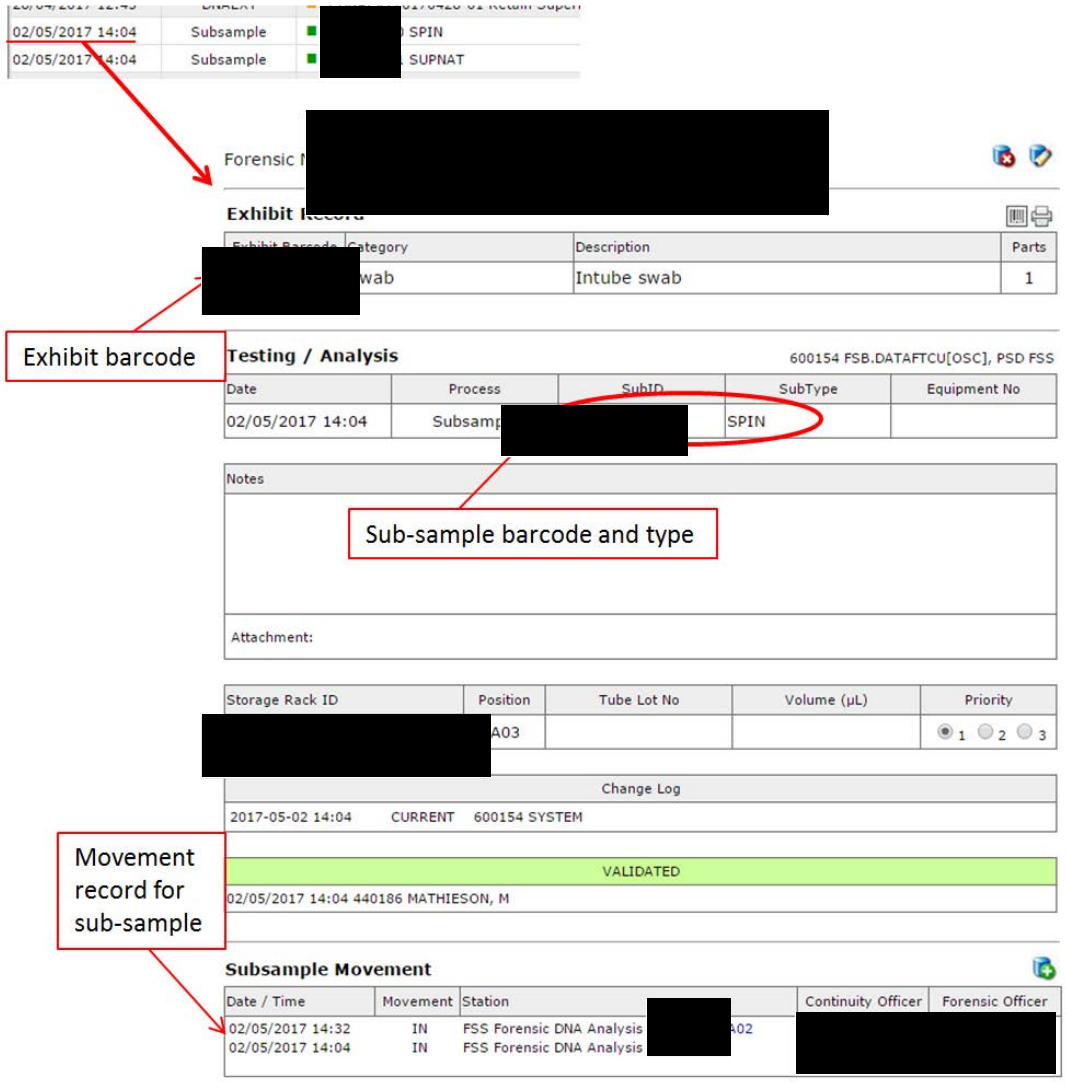
By adding an exhibit to a location, it is automatically removed from its previous location. This means that an exhibit can only be in one location at any one time. An exhibit often comprises of multiple parts that require individual storage. The storage of these multiple parts is managed using subsample barcodes.

When a sample undergoes DNA analysis, it splits into multiple parts. In its most simple form a sample will branch into an extract and a spin basket. The extract will retain the exhibit barcode for storage purposes and a new barcode will be created for the spin basket storage. If the sample requires testing of the supernatant then a new barcode will also be created for the supernatant storage.

In the example below we can see that exhibit [redacted] has a spin basket with barcode [redacted] and a supernatant with barcode [redacted] (additional sample information becomes available by hovering over the individual barcodes on the 'Link Chart').



The storage record for a subsample can be accessed by clicking on the Date / Time associated with that subsample.



For analytical processes, most subsample barcodes that may be required are generated automatically at the extraction stage. The subsample types are:

- Spin baskets (SPIN)
- Microscope slides (SLIDE)
- EFRACs (EFRAC)
- Supernatants (SUPNAT)
- Miscellaneous (MISC)

If a spin basket or EFRAC requires further analytical processing then it will be necessary to change the subsample into an exhibit to allow the parts created from these additional processes to be stored and a PDA page to be created.

Storage boxes are identified by a nine or ten digit barcode and a description. The barcode is displayed in the Exhibit Movement table and is hyperlinked to the storage box record. Hovering over the storage box barcode will show the fixed location where the box is stored.

Exhibit Movement

Date / Time	Movement	Station	[redacted]	Continuity Officer	Forensic Officer
05/05/2017 14:16	IN	FSS Forensic DNA Analysis		[redacted]	
02/05/2017 14:29	IN	FSS Forensic DNA Analysis			
28/04/2017 13:06	IN	FSS Forensic DNA Analysis			
26/04/2017 06:39	IN	FSS Forensic DNA Analysis			

Fixed storage locations are identified by a 12 character code e.g. FDNA-EXFZ-0001. The first four letters represent the department e.g. FDNA = Forensic DNA Analysis. The second four characters represent the type of storage e.g. shelf, freezer, fridge. The last four characters represent the shelf number.

The codes for the second four characters for storage locations within Forensic DNA Analysis are as follows:

CMP	Compactus
RT	Returns
FZ	Freezer
FR	Fridge
SH	Shelf
CG	Cage
DR	Drawer
EX	Exhibit room
ER	Evidence recovery
ES	Extraction sorting
ADMN	Admin
INTL	Intel
EVRT	Evidence recovery
LDNA	Low DNA room
AE	Analytical extraction
QA	Pre-PCR
CE	CE
BONE	Bone room
FRXX	Walk in fridge
FZXX	Walk in freezer
AD	Admin
SHLF	Walk in fridge/freezer direct shelf storage
CHST	Chest freezer
QUAL	Quality
ANLT	Analytical
FRIT	FRIT
FILE	File store

Storage Locations

Fixed locations:

- Exhibit room shelves, Fridge shelves, Freezer shelves, Rooms

Storage box (open):

- FTA boxes, Freezer storage tubs, Staff in-trays
- Storage boxes can “move” e.g. Kirsten's in-tray can be stored to the Admin fixed location
- Locally configured, with unlimited storage positions

Storage box (grid format):

- Perm DNA, Temp DNA, ERT-AS boxes
- Storage boxes can “move” e.g. from ERT lab to Freezer in extraction sorting
- Locally configured, with defined and limited positions in the format A01, A02, A03, B01, B02 etc.

General Storage Functionality

Five basic methods / functionalities are available for storage. Use of method is determined in part by storage type e.g. if the storage box is “open” or “grid” format.

1. **Batch exhibit movement (Main Menu – Forms – Batch Exhibit):**
    a. Used to move up to 24 barcodes to a fixed location or open storage box
    b. Not suitable for movements to grid-format boxes
    c. Likely used by property point, storage of FTA cards, storage of case files

2. **Fill box function (Main Menu – Equipment – Search storage box ID):**
    a. Add samples one at a time to a storage box (grid or open format)
    b. Not applicable for fixed locations
    c. Likely used in extraction sorting/Pre-PCR where large number of items stored to grid boxes

3. **Exhibit/Sub-ID movement (Exhibit Movement Table):**
    a. Standard movement to any location
    b. All storage formats: fixed, open box and grid box
    c. Used for single movements of item. Subsamples will have their own movement history

4. **Movement while adding a test:**
    a. Add samples one at a time to a storage box (grid or open format)

5. **Storage box movement:**
    a. Movement of a box (open or grid) to a fixed location. Available from the storage box screen

23.13 Appendix 13 – Profiler Plus Amplification Kit

GeneMapper® Record Page:

When the GeneMapper file is uploaded to the FR, loci with only one allele will be transformed into the format “allele,allele” or “allele,0” in the ‘Alleles’ column according to the homozygous threshold. Loci with no alleles will be transformed into the format “0,0”. The row will highlight yellow to reflect the change.

The ‘Alleles’ column contains the allele designations to be used on the PDA page.

If a peak requires removing, refer to Section 6.2.1.1.

Profile Interpretation Table:

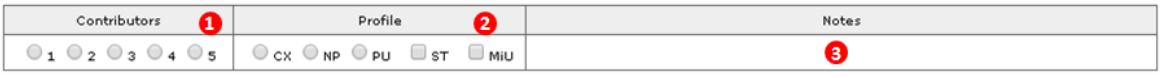


Figure 30 – P+ Profile Interpretation Table

1 This section is used to record the number of contributors to a profile. If the interpretation is ‘at least two’ contributors then the ‘2’ is to be checked; likewise ‘at least three’ will see the ‘3’ checked

2 This section is used to record interpretations or parts of interpretations that don’t require any further action:

a. CX is to record a profile that is a complex mixture and is unsuitable for further interpretation
b. NP is used to record a no profile result
c. PU is used to record a profile that is partial and unsuitable for further interpretation
d. can be used in conjunction with the number of contributors or NP to record that there are also sub-threshold peaks within the profile
e. MiU is not used at this stage (for automated Exhibit Results)

3 Notes section (not audited)

Profile Record Table:

When a DNA profile is obtained that is either single source or one or more contributions are able to be resolved the Profile Record table is to be completed for each resolved contribution.

Clicking the edit icon on the PDA page will enable the Profile Record table to be edited. The profile can then either be entered manually or ‘copied down’ from the GeneMapper file (1 Figure 52) by clicking on the appropriate button. If required, the CLR button (2) will clear the profile entered. If there is more than one GeneMapper file for the sample then there will be the option to choose which result to copy down (distinguished by the CE batch id); this should be the ‘Reported profile’ result or the profile with the most informative information (statistics for P+ use the ‘Reported profile’ only).

The suffix list (3) records the type of profile that has been entered. A suffix must be selected for every profile entered into the table.

If a profile requires upload to NCIDD then a NCIDD process is automatically ordered for each nominated upload (Section 11.1.1).

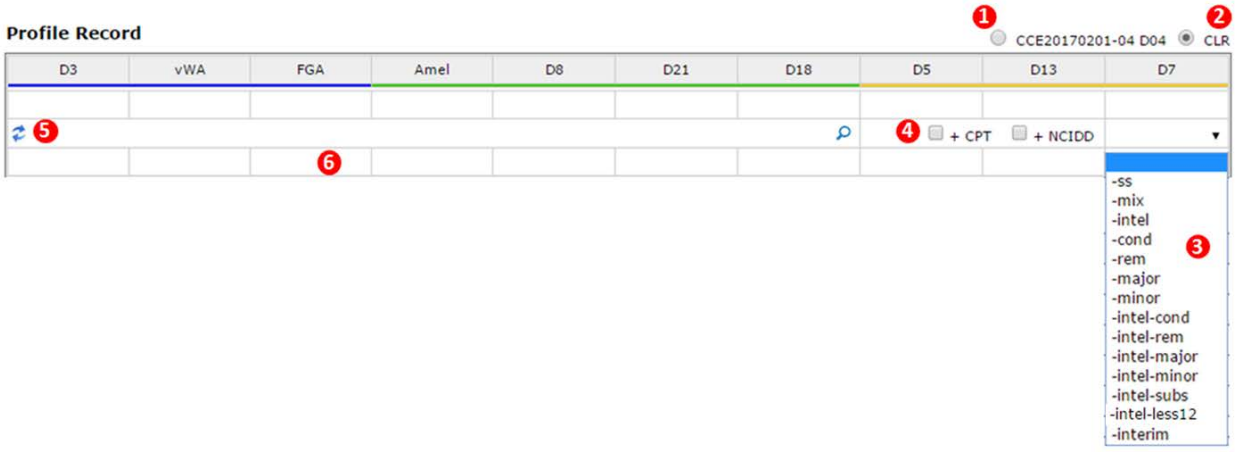


Figure { SEQ Figure * ARABIC } – P+ Profile Record Table

1. Single Source Profiles
   When a single source profile is obtained the scientist will copy down the profile by selecting the appropriate CE batch or entering manually. The scientist will then click the ⇄ icon (❺Figure 53) to commence searching.

   If the profile is ≥ 12 alleles then the FR will suggest a designation / match for the profile (refer to Section 8.1 for further details). If the scientist does not agree with the match / designation proposed by the FR database then it must be replaced with the appropriate match / designation in the format described in Section 8.1.

   If the profile is <12 alleles then no designation / match will be suggested by the FR and the match cell (❺) will populate with the barcode of the sample. The designation for this profile should be entered manually into the match cell (❺) in the format described in Section 8.1.

   If the profile is required to be loaded to NCIDD then the '+NCIDD' box (❹) should be checked. The '-ss' suffix should be selected from the drop-down list (❸) (Table 6).

   If the profile is unknown and is not listed in the 'Case Profiles' table then the '+CPT' box (❹) must be checked to add the profile to the Case Profiles table.

Table 4 – Suffix Meanings

Suffix	Purpose	Kit
-ss	Single source component	P+
-cond	Conditioned component	P+
-rem	Remaining component	P+
-major	Major component	P+
-minor	Minor component	P+
-intel-cond	Conditioned component where the profile is conditioned for intelligence purposes only	P+
-intel-rem	Remaining component where the profile is conditioned for intelligence purposes only	P+
-intel-major	Major component where the term 'major' has been assigned for intelligence purposes only	P+
-intel-minor	Minor component where the term 'minor' has been assigned for intelligence purposes only	P+
-intel-subs	Single source component where sub-threshold peaks are used for intelligence purposes only	P+

-intel-less12	Single source component with less than 12 alleles	P+
-interim	Any component that is loaded as an interim measure pending rework results	P1 cases only

2. Fully Resolved Mixed Profiles
   When a profile can be fully resolved into its individual contributions then each contribution should be recorded in the Profile Record table as follows:

   a. Click the edit icon
   b. Copy down the profile by selecting the appropriate CE batch and delete the alleles that do not apply to the contribution being entered, or enter the profile manually
   c. Click the icon to commence searching
   d. Check the match / designation in the match cell (❺Figure 53) and replace as necessary as per Section 8.1
   e. Check the '+NCIDD' box (❹) if this contribution is required to be loaded to NCIDD (ticking this box triggers the ordering of an 'NCIDD' process when the record is saved)
   f. Select the appropriate suffix for this contribution from the list (❸)
   g. Check the '+CPT' box if this contribution is a new designation for the case
   h. Save the record by clicking the save icon
   i. Repeat the process for the other resolved contributions of the profile entering the mixture ratios into the boxes below each locus (❻).

3. Partially Resolved Mixed Profiles
   When a profile can only be partially resolved, for example a major profile with a complex minor profile, then only the resolved contribution is recorded in the Profile Record table by following steps a.-i. as per above paragraph.

4. Unresolved Mixed Profiles
   When a profile is unable to be resolved then no information will be recorded in the Profile Record table. In this instance only the ○2 or ○3 box is checked in the Profile Interpretation table.

5. Complex Profiles
   If the profile is not suitable for interpretation because it is partial or consists of an unknown number of contributors or ≥ 4 contributors, it is not necessary to copy down a profile. In this instance only the ○CX or ○PU box is checked in the Profile Interpretation table.

6. Tri Alleles
   The Profile Record table should only contain two allele designations. If a tri-allele is obtained, drop the locus and add a sample note stating that a tri-allele is present and all three designations. If the profile is required for upload to NCIDD then the details of the tri allele should be added to the 'NCIDD User Comment' field in the NCIDD process (Section 11.1).

Case Profiles Table:

The Case Profiles table is used to identify reference and unknown profiles that are available for comparison in the case and to associate them with one another (Figure { SEQ Figure * ARABIC }). It is also used to show when a reference comparison has been completed.

Barcode	Name	Association	Category	CE	NM	II	Employee	Reviewer
690126672	6 PP21 ref			☐	☐	☐		
690126681	7 PP21 ref			☐	☐	☐		
690126690	8 PP21 ref			☐	☐	☐		

Figure { SEQ Figure * ARABIC } – P+ CPT

When a reference sample has been compared with the crime scene profile then the associated 'CE' box should be checked. This enables any new reference samples to be easily identified.

The 'NM' and 'II' boxes are not used currently.

New Reference Comparisons:

Newly associated reference samples can be identified by an unchecked box in the 'CE' field of the Case Profiles table.

The comparison of the reference sample should be recorded as follows:

a. Once the comparison has been performed, check the associated 'CE' box
b. If the reference sample matches an unknown in the case, update the 'Association' fields (Figure 42)
c. If required, order a 'Result' process and enter the appropriate Exhibit Result line for the reference sample (Section 18)
d. If no additional Exhibit Results lines are required i.e. the results have not changed, then a Sample Note to this effect should be added
e. Order a 'Profile Review' process (Note: a 'Profile Review' process should be ordered regardless of whether an Exhibit Result line is entered)

23.14 Appendix 14 – Managing Cases Across Different LIMS (Pre-AUSLAB, AUSLAB and FR)

Cases from 2017 or earlier, can have samples across a number of different LIMS systems. Flowchart 1 provides a number of different pathways and the SOPs required to manage such cases / samples.

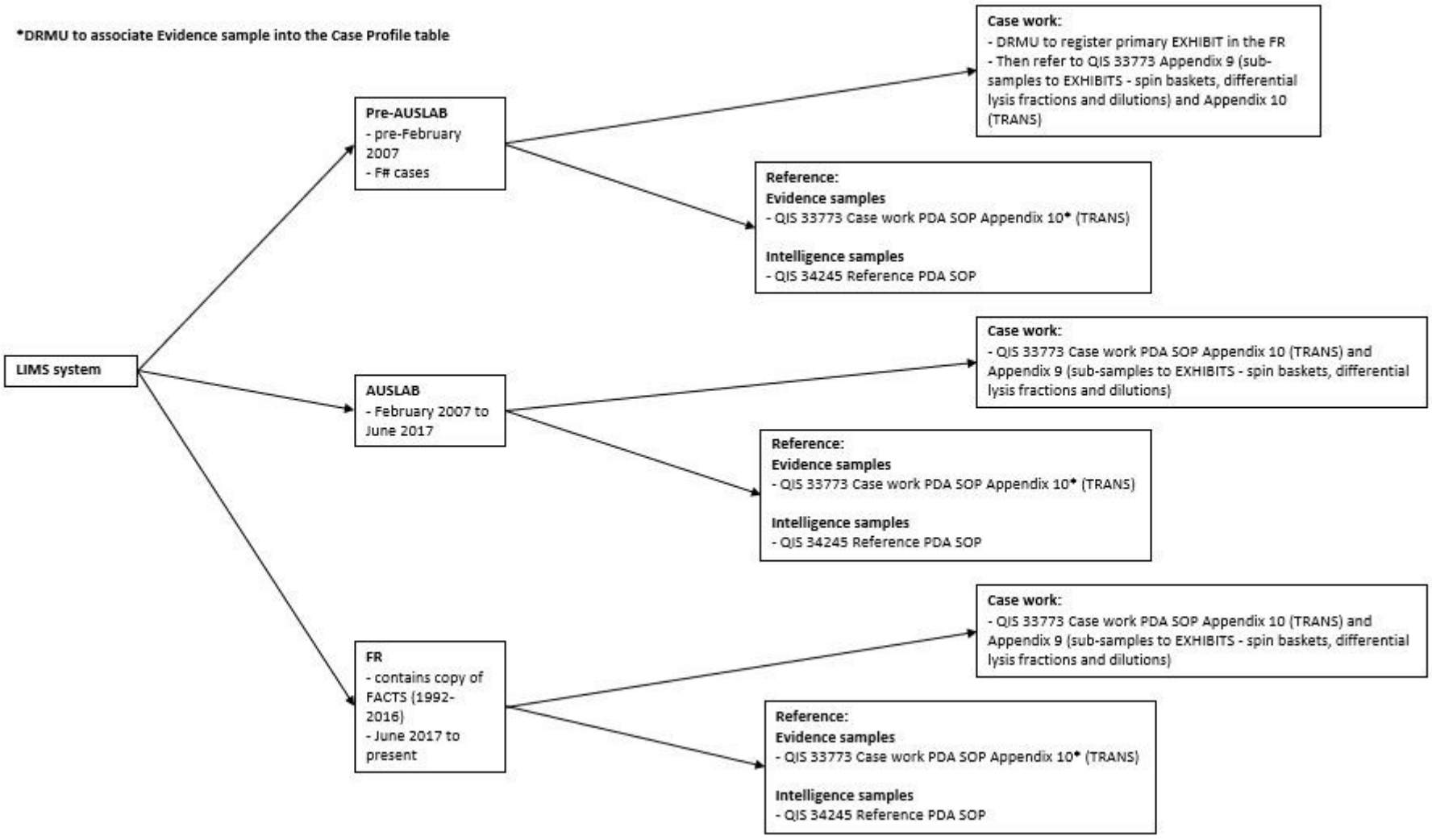
Tracking AUSLAB Case in FR

For cases with paper case files tracked in AUSLAB requiring tracking (e.g. court):

1. 'Remove' the case file from the AUSLAB storage and add an audit entry 'Removed to track in FR'
2. Create and track this case file in FR as per Appendix 8 – Creating and Tracking a Case File using the existing case file barcode
3. Add a note to the 'Exhibit Notes & FSS Advice' field stating the case file was previously tracked in AUSLAB
4. Proceed as normal

Cases with AUSLAB and FR Unknowns

If a case has an unknown in AUSLAB followed by unknowns in the FR, the subsequent FR unknowns will appear in the CP table without accounting for the previously designated AUSLAB unknowns (i.e. they do not appear correctly in the CP table on the PDA page). To resolve this situation, register the sample in the FR, manually enter the DNA profile and designation, and then tick the +CPT box so it appears in the CP table of the FR. Alternatively, contact QPS DNA Management (DRMU) to request registration of the AUSLAB unknown(s).


*DRMU to associate Evidence sample into the Case Profile table
LIMS system
Pre-AUSLAB
- pre-February 2007
- F# cases
AUSLAB
- February 2007 to June 2017
FR
- contains copy of FACTS (1992-2016)
- June 2017 to present
Case work:
- DRMU to register primary EXHIBIT in the FR
- Then refer to QIS 33773 Appendix 9 (sub-samples to EXHIBITS - spin baskets, differential lysis fractions and dilutions) and Appendix 10 (TRANS)
Reference:
Evidence samples
- QIS 33773 Case work PDA SOP Appendix 10* (TRANS)
Intelligence samples
- QIS 34245 Reference PDA SOP
Case work:
- QIS 33773 Case work PDA SOP Appendix 10 (TRANS) and Appendix 9 (sub-samples to EXHIBITS - spin baskets, differential lysis fractions and dilutions)
Reference:
Evidence samples
- QIS 33773 Case work PDA SOP Appendix 10* (TRANS)
Intelligence samples
- QIS 34245 Reference PDA SOP
Case work:
- QIS 33773 Case work PDA SOP Appendix 10 (TRANS) and Appendix 9 (sub-samples to EXHIBITS - spin baskets, differential lysis fractions and dilutions)
Reference:
Evidence samples
- QIS 33773 Case work PDA SOP Appendix 10* (TRANS)
Intelligence samples
- QIS 34245 Reference PDA SOP

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DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

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1 PURPOSE AND SCOPE

This method describes the routine method for the extraction of DNA using the DNA IQ™ kit (Promega Corp., Madison, WI, USA). The automated method is the preferred procedure, utilising the MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (PerkinElmer BioDiscovery, Downers Grove, IL, USA). The manual method has also been included. This method applies to all DNA Analysis staff members that are required to extract DNA from samples.

Reference samples and casework samples must be extracted separately. If casework and reference samples are to be extracted on the same instrument, the instrument (including all required labware) must be decontaminated between operations.

2 DEFINITIONS

DNA IQ™ Resin	Magnetic resin beads used to bind DNA
DTT	1,4 Dithiothreitol
EDTA	Ethylenediaminetetraacetatic acid
EP-A	Extraction Platform A
EP-B	Extraction Platform B
Extracts	Samples that have had a DNA extraction processes performed
Lysates	Samples that have had the off-deck lysis step performed, but have not yet completed the entire extraction process
MPII	MultiPROBE® II PLUS HT EX Platform
Paramagnetic	To become magnetic with the application of a magnetic force
Pro K	Proteinase K
Samples	Sample substrates (in tubes) awaiting DNA extraction
Sarcosyl	N-Lauroylsarcosine sodium
TNE	Tris, NaCl and EDTA buffer (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0)

3 PRINCIPLE

3.1 OFF-DECK LYSIS

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE buffer (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0) and Sarcosyl. TNE acts as a basic buffer with EDTA chelating ions in solution. Sarcosyl is a detergent that lyses open cell membranes. Proteinase K is added to digest cellular material, helping the DNA to be released. It also digests proteins that interfere with the DNA binding capacity of the resin. In addition, Proteinase K rapidly inactivates enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (or Endoproteinase K) is a fungal enzyme from Engyodontium album (formerly Tritirachium album). It is a stable S8 family serine alkaline protease that has broad specificity and is inactivated by diisopropylfluorophosphate, phenyl-methane sulfonyl fluoride and Hg2+ ions. As a highly active endopeptidase, it cleaves peptide bonds adjacent to the carboxyl group of N-substituted (blocked alpha amino) hydrophobic, aliphatic and aromatic amino acids. Therefore, it actively hydrolyses proteins, glycoproteins, peptides and esters of amino acids. The smallest peptide that can be hydrolysed with Proteinase K is a tetrapeptide.

3.2 MANUAL DNA IQ™ KIT

The DNA IQ™ kit (Promega Corp., Madison, WI, USA) is designed to purify DNA from a number of different substrates and has been optimised for trace DNA samples. An in-house validation was performed using a modified version of the PerkinElmer automated protocol.

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The in-house protocol includes:

- Off-deck lysis steps with the option to retain a portion of the supernatant for further testing;
- The use of 300µL Extraction Buffer containing TNE (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0) and Proteinase K to lyse cellular material prior to performing the DNA IQ process;
- The use of tubes and spin baskets for off-deck lysis of samples prior to extraction on the MPII platform. At the conclusion of off-deck lysis, lysates are transferred to individual Nunc Bank-It™ tubes;
- Nunc Bank-It™ tubes (arranged in sequence using STORstar) containing lysates are presented to the MPII platform for automated transfer of lysates into a 96-deep well plate;
- DNA IQ™ Resin is added using the MPII platform, followed by addition of two volumes of DNA IQ™ Lysis Buffer;
- The 96-deep well plate containing DNA IQ™ Resin and Lysis Buffer is sealed using a heat sealed piercing film and is placed on a MixMate to mix the contents of each well. The plate is centrifuged and the film is then pierced using a 96 well half skirt PCR microplate and the plate is returned to the MPII platform;
- A double elution step is performed using two dispenses of DNA IQ™ Elution Buffer at 60µL, resulting in a final DNA extract volume of 100µL;
- DNA extracts are automatically transferred into Nunc Bank-It™ tubes for storage.

Cell lysis is performed using DNA IQ™ Lysis Buffer containing Dithiothreitol (DTT). 1,4 Dithiothreitol is a reducing agent used in extractions to break disulfide bonds of proteins. The Lysis Buffer used is a proprietary buffer containing chaotropic salts required for binding of DNA to the magnetic beads. According to the MSDS (Promega Corporation 2006), the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of Proteinase K increases the binding capacity. Samples with small amounts of DNA are more efficient than samples with large amounts of DNA, and as a result a small sample size is critical to ensure efficient recovery of DNA.

The magnetic beads with silica have a negative charge at basic and near neutral Ph. The Lysis Buffer changes the Ph and salt concentration of the solution and the silica on the magnetic beads becomes positively charged, which is then able to bind the DNA.

Several washing steps are employed in the protocol to remove inhibitors. The first washing step is performed using Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures incorporate the use of DNA IQ™ Wash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and the aqueous phase washes out the inhibitor.

The DNA IQ™ Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65ºC allows the DNA to be released from the magnetic beads. The Elution Buffer is a low ionic strength buffer that reduces the affinity of the DNA for the silica that covers the magnetic beads, by re-hydration of the phosphate backbone.

The DNA IQ™ kit isolates DNA greater than 80bp, smaller DNA is removed selectively to prevent PCR inhibition.

Not Current

3.3 MULTIPROBE® II HT EX PLUS WITH GRIPPER™ INTEGRATION PLATFORM

Within DNA Analysis, routine DNA extractions are performed on casework or reference samples using two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (EP-A or EP-B) located in Room 3191.

Each platform uses a computer-controlled Cartesian XYZ liquid handling system designed for the efficient automation of sample preparation. Liquid transfer is performed by an 8-tip system with VersaTip® and VariSpan™ options. The VersaTip® option allows the use of both fixed and disposable tips (conductive and non-conductive). The VariSpan™ option permits variable probe spacing between each of the individual probes so that a wide range of labware such as micro plates, tubes, vials and reagent troughs can be accessed. Each sample probe is capable of independent motion in the Z direction due to independent Z drives.

The 8-tip system is also capable of multichannel liquid-level sensing by utilising Accusense™ technology. This technology works by each probe detecting a change in capacitance within the liquid. This capacitive mode of detection is also possible when using conductive disposable tips. Accusense™ also permits the detection of low or non-ionic polar solutions and solvents. Pipetting on the platforms is driven by 8 individually controlled syringes via the positive displacement of the system liquid (nanopure water) when transferring liquid.

The Gripper™ Integration allows for automated identification of labware via the scanning of barcodes and also allows for automated transfer of plates from one position on the deck to another. To increase deck space and capacity, the platforms include a left deck extension.

For automated DNA extraction using the DNA IQ™ kit, a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding batch and plate identification, sample positions, lab numbers of samples, and lists all the samples in the batch, providing the total number of samples. The program will fail to work without a platemap.

4 REAGENTS AND EQUIPMENT

4.1 REAGENTS

- DNA IQ™ System Kit (400 sample kit)
  - DNA IQ™ Resin
  - Lysis Buffer (LB)
  - 2x Wash Buffer (2Xwb)
  - Elution Buffer (EB)
- TNE (10Mm Tris, 100Mm NaCl, 1Mm EDTA, Ph 8.0)
- Proteinase K (Pro K) 20mg/Ml
- Dithiothreitol (DTT) 1M
- 5% TriGene
- 70% Ethanol
- 10% Bleach 7x Solution
- 1% Amphyl
- 0.2% Amphyl
- Isopropyl Alcohol
- AnalR 100% Ethanol
- 40% Sarcosyl
- Nanopure Water

These reagents are stored in locations as per Table 1.

Not Current

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 3188
DTT	Freezer	Room 3188
40% Sarcosyl	Shelf	Room 3188
Isopropyl Alcohol	Shelf	Room 3188
AnalR 100 %Ethanol	Shelf	Room 3188
TNE Ph 8 Buffer	Shelf	Room 3188
DNA IQ™ Kit	Shelf	Room 3188
Amphyl (1% and 0.2%)	Shelf	Room 3191
Nanopure Water	Shelf	Room 3188
5% TriGene	Shelf	Room 3191
10% Bleach 7x Solution	Shelf	Room 3191

4.1.1 Extraction Buffer

Note: Prepare Extraction Buffer just prior to commencing the off-deck lysis and manual DNA IQ procedures

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Remove the required amount of 20mg/mL Proteinase K from the freezer and thaw. Vortex and centrifuge before use.
3. Ensure that the 40% (w/v) Sarcosyl is completely dissolved and appears clear in the stock solution. If not dissolved, invert the container a few times and leave at room temperature.
4. Aliquot out the appropriate amount of TNE buffer required. It is best to not remove the buffer directly from the stock solution.
5. Add the appropriate volumes of 20mg/mL Proteinase K and 40% (w/v) Sarcosyl to the falcon tube containing TNE buffer, and invert gently to mix.
6. Label the tube with **"Extraction Buffer"**, your initials and the date.

4.1.2 Lysis Buffer with DTT Solution

Note: Lysis Buffer is supplied with the DNA IQ™ kit. The Lysis Buffer with DTT solution is prepared just prior to commencing the extraction procedure.

Warning: Lysis Buffer and DTT are toxic, use a fume hood to prepare these reagents and wear appropriate PPE including safety glasses when handling Lysis Buffer.

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Remove the required amount of DTT from the freezer and thaw. Vortex and centrifuge before use.
3. In the biohazard cabinet, add the required volume of Lysis Buffer to a sterilised glass Schott bottle or 50mL falcon tube and then add the required volume of DTT.
4. Label the glass Schott bottle with **"Lysis Buffer + DTT"**, your initials and the date.

4.1.3 DNA IQ™ Resin

Note: DNA IQ™ Resin is supplied with the DNA IQ™ kit. The resin is prepared at the start of each run. Ensure the resin is properly mixed by *vortexing* prior to use.

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Into a 10mL (or 5mL or 2mL) sterile tube, add the required volume of Lysis Buffer with DTT solution (from 4.1.2) followed by the required volume of DNA IQ™ Resin.
3. Mix by gentle inversion.
4. Label the tube with **"Resin"**, your initials and the date.

Not Current

4.1.4 1x Wash Buffer

Note: 2x Wash Buffer is supplied with the DNA IQ™ kit. To prepare 1x Wash Buffer, add 35mL of *AnalR* Ethanol and 35mL of Isopropyl Alcohol to the 2x Wash Buffer bottle. The freshly made 1 x wash buffer reagent must be entered into Auslab:

- Log into Auslab
- 2 – Sample Processing
- 8 – Materials Processing
- 2 – Consumable Inventory
- Find the Wash Buffer in the list and enter
- Esc
- Highlight the lot number that is applicable
- Shift F8
- Add audit entry to state that the additional reagents were added.

1. Determine the required volumes of reagents by using the appropriate appendix.
2. Into a 50mL (or 10mL) tube, add the required volume of 1x Wash Buffer.
3. Label the tube with **"Wash Buffer"**, your initials and the date.

4.1.5 Elution Buffer

Note: Elution Buffer is supplied with the DNA IQ™ kit. The Elution Buffer can be used directly from the kit, which is stored in the clean room (3188), however it must be aliquoted into a new tube rather than using directly from the stock solution.

4.2 EQUIPMENT

4.2.1 Equipment and consumables required for Off Deck Lysis processes

Table 2. Equipment used and their locations

Equipment	Asset No.	Location
Vortex x 4	30435255 30435256 002123941 806021325	3189
Fridge	30433424	3189
Centrifuge x 4	30433323 30433324 10233209 30433322	3189
Hot Block x 4	30435115 30435113 30435114 30435112	3189
Mini Centrifuges x 4	30434993 30087075 30087057 041129	3189
Finnpipettes 100 µL – 1000 µL	N/A	3189

Table 3. Consumables used and their locations.

Consumables	Location
Racks	3189/3184
Spin baskets	3189/3184
1.5mL or 2mL tubes	3189/3184
Nunc Bank-it™ tubes	3189/3184
Nunc Bank-it™ caps	3189/3184
Sterile 50mL Falcon tubes for reagents	3189/3184
Sharps Bin	3189/3184
300µL ART tips	3189/3184
1000µL ART tips	3189/3184
Twirling Sticks	3189/3184

Not Current

4.2.2 Equipment and consumables required for Manual DNA IQ™

Table 4. Equipment used and their locations

Equipment	Asset No.	Location
Vortex x 4	30435255 30435256 002123941 806021325	3189
Fridge	30433424	3189
Centrifuge x 4	30433323 30433324 10233209 30433322	3189
Hot Block x 4	30435115 30435113 30435114 30435112	3189
Mini Centrifuges x 4	30434993 30087075 30087057 041129	3189
Finnpipettes 100 µL – 1000 µL	N/A	3189

Table 5. Consumables used and their locations

Consumables	Location
Racks	3189
Spin baskets	3189
Nunc Bank-it™ tubes	3189
1.5mL or 2mL tubes	3189
Nunc Bank-it™ caps	3189
Sterile 50mL Falcon tubes for reagents	3188
10mL Sterile tubes	3188
5mL Sterile tubes	3188
Sharps Bin	3189
300µL ART tips	3189
1000µL ART tips	3189
Twirling Sticks	3189
Magnetic Stands	3189

Further consumables can be found in the Store Room (3184)

4.2.3 Equipment and consumables required for Automated DNA IQ™

Table 6. Equipment used and their locations.

Equipment	Asset No.	Location
STORstar (B system)	10238493	3190
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-A)	10076438	3191
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (EP-B)	10076437	3191
DPC shaker (EP-A)	N/A	3191
DPC shaker (EP-B)	N/A	3191
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-A)	N/A	3191
Automated Temperature Controller, Heat Block tiles and heat block adapters (EP-B)	N/A	3191
Eppendorf 5804 centrifuge	10238416	3191
Vortex	30087015	3191
Fridge	30433424	3191
MixMate	30512822	3191
Decapper	None	3191
4titude 4seal Sealer	30512847	3191

Not Current

Table 7. Consumables used and their locations

Consumables	Location
175µL Clear Non-Conductive Filter RoboRack tips – Pre-Sterilised	3191
MβP Pure 1000µL Tips – Pre-Sterilised	3191
Abgene 96-deep well plate	3191
Axygen 2mL deep well storage plate	3191
96 well Half Skirt PCR Microplate	3191
4titude Piercing Film	3191
12 Channel plate	3191
Nunc Bank-it™ tubes	3191
Nunc Bank-it™ caps	3191
Sterile 50mL Falcon tubes	3188
Sterile 10mL or 5mL tubes	3188
Autoclaved 100mL glass bottles	3191
Autoclaved 250mL glass bottles	3191
Aluminium sealing film	3191
300uL ART tips	3188
1000µL ART tips	3191

Further consumables can be found in the Store Room (3184)

5 SAFETY

As per the procedures in the QIS document "*Anti-Contamination procedure*" (QIS 22857), PPE is to be worn by all staff when performing this procedure. This includes the use of safety glasses.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% Ethanol before and after use. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

While the MPII is running a procedure, refrain from placing any part of the body inside the cabinet. Either pause the program or use the emergency STOP button located at the front of the instrument before touching anything on the deck surface. Pressing the emergency STOP button may cause the program to pause or abort.

Warning: Tris base, EDTA, Sarcosyl and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate (GuSCN) which is toxic and can be harmful if inhaled, swallowed or comes in contact with skin. Left over Lysis Buffer-DTT is disposed of in a brown Winchester bottle in the ventilated cupboard in Room 3191. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand or kitty litter) and dispose in a biohazard bin. Do not combine bleach with Lysis Buffer and do not wipe areas of Lysis Buffer spillage with bleach, as the two reagents produce cyanide gas when combined. Handle carefully and wear appropriate PPE, including safety glasses, when handling Lysis Buffer. If Lysis Buffer is spilt onto PPE (eg. Gloves, gowns), discard the PPE and obtain new PPE.

6 SAMPLING AND SAMPLE PREPARATION

6.1 SAMPLE LOCATIONS

Samples waiting to be extracted are stored in freezers as described in Table 8.

Table 8. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	3190 or 6106*
Lysates in 1.0mL Nunc Bank-it™ tubes	Freezer	3190
Extracts in 1.0mL Nunc Bank-It™ tubes	Freezer	3194 A

*** Note:** Some Medium and Low Priority storage boxes are located in Block 6, within the Exhibit Room (6106).

Not Current

6.2 QC SAMPLES

For all off-deck lysis batches one negative control and one positive control are required to be registered. In addition, 5 blanks are also to be registered. For manual DNA IQ and retain supernatant batches, one negative and one positive control are required to be registered.

Table 9. Extraction Quality Controls

QC Name	Batch Type	Description
Negative Control	Off-Deck Lysis,	Negative Extraction control
	Manual DNA IQ	
	Retain Supernatant	
Positive Control	Off-Deck Lysis	Positive Extraction control – dried blood swab from a known donor
	Manual DNA IQ	
	Retain Supernatant	
Blank Control x 5	Off-Deck Lysis	Negative Extraction control x 5

6.3 REGISTRATION OF QC SAMPLES

The registration of control samples is covered in QIS24919 DNA Analysis workflow procedure.

6.3.1 Create the DNA IQ™ Lysis, Manual DNA IQ™, Retain Supernatant batch or Automated Extraction

Creation of Lysis and retain supernatant batches is covered in QIS 24919 DNA Analysis Workflow Procedure.

6.3.2 Locating Samples

To locate samples refer to QIS 23959 Storage Guidelines for DNA Analysis.

6.4 ELECTRONIC WORKFLOW DIARY

An electronic workflow diary (I:\AAA Electronic Workflow Diary) is used for the recording of batches that are to be created and for scientists to delegate the work amongst themselves. Once batches are listed and created within the electronic workflow diary (by the operational officers), scientists are required to type in their initials next to the batch that they are to complete. This can also be used to record which scientist has nominated themselves for a particular task on each day and assists the analytical senior scientist with the recording of key performance indicators.

7 OFF-DECK LYSIS PROCEDURE

7.1 OFF-DECK LYSIS (NO RETAIN SUPERNATANT)

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
   - Original sample tube
   - Spin basket as required
   - 1.0mL Nunc Bank-It™ tube
   - 1.5mL or 2.0mL tube

Not Current

Note:
Samples that require a spin basket are: swabs, fabric, paper and cigarette butts.
Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.

5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

6. Add 300µL of Extraction Buffer and vortex briefly. Ensure that substrates are immersed in the Extraction Buffer.

7. Incubate for 45 minutes at 37 degrees on a hotblock. It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.

8. Remove from the hotblock. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL or 2mL tube. Retain original tube containing the substrate if no spin basket is used.

9. Centrifuge spin baskets at 15800 g for 2 minutes.

10. Transfer the substrate in the spin basket to a new and appropriately labelled 1.5mL or 2mL tube and retain.Transfer the flow through back to original lysis tube.

11. Vortex lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

12. Centrifuge at 15800 g for 1 minute.

13. Transfer 300µL of lysate to the corresponding Nunc Bank-It™ tube.

Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

14. In AUSLAB, enter reagent and hotblock temperature details and complete the batch.

15. Store lysates in temporary storage boxes located within the extraction sorting room in the orford Freezer with equipment number 102384430 (Room 3190).

16. Store tubes containing substrates or extra lysate in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

7.2 OFF-DECK LYSIS (RETAIN SUPERNATANT)

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

2. All manual extractions are to be performed within a biohazard cabinet in Room 3189

3. For each sample label:
   - Original sample tube
   - Spin basket as required
   - 1.5mL tube (also labelled with "**sup**" to indicate supernatant)

Not Current

- 1.0mL Nunc Bank-It™ tube
- 1.5mL or 2.0mL tube

Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require spin baskets are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. If substrate is in a 0.5mL tube, transfer to a labelled 1.5mL or 2.0mL tube.

5. Have a second operator sequence check the tubes and enter the sequence check in AUSLAB.

6. Add 450µL of TNE buffer and vortex.

7. Incubate at room temperature for 30 minutes.

8. Vortex, then centrifuge at 15800 g for 3 minutes.

9. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with "**sup**" (for further testing).

10. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

11. Incubate for 45 minutes at 37 degrees on the hotblock. If the batch is ≤24 samples, then the thermomixer can be used (incubate 45min at 37 degrees and 1000rpm). It is best practice to vortex the samples intermittently throughout the duration of the incubation period. Record temperature on worksheet.

12. Remove from the hotblock/thermomixer. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 1.5mL tube. Retain original tube containing the substrate in if no spin basket used.

13. Centrifuge spin basket at 15800 g for 2 minutes.

14. Transfer the substrate in the spin basket to a new and appropriately labelled 2mL tube to be retained. Transfer the flow through back to original lysis tube.

15. Vortex Lysate, then incubate on hotblock at 65°C for 10 minutes. Record temperature on worksheet.

16. Centrifuge at 15800 g for 1 minute.

17. Transfer 300 µL of lysate to the corresponding Nunc Bank-It™ tube.

    Note: If more than 300µL of lysate is present, retain the remaining lysate in the 1.5mL tube. In AUSLAB, write a specimen note for that sample (eg. extra lysate retained from sample XXXXXXXXX.). Store the retained 1.5mL lysate tube in appropriate box in freezer.

18. In AUSLAB, enter reagent and hotblock/Thermomixer temperature details and complete the batch.

19. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

Not Current

20. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

21. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

22. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List'**:

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

8 AUTOMATED EXTRACTION OF LYSED SAMPLES

8.1 BATCH CREATION

Creation of extraction batch is covered in QIS 24919 DNA Analysis Workflow Procedure.

8.2 SAMPLE LOCATION

To locate samples refer to QIS 23959 Storage Guidelines for DNA Analysis.

8.3 SEQUENCE CHECKING THE NUNC BANK-IT™ TUBES

The procedure for the automated checking of sample tubes is covered in QIS 24256 Procedure for the use of the STORstar unit for automated sequence checking.

8.4 MPII EXTRACTION PROCEDURE

Automated setup of DNA IQ extractions in 96-deep well format is performed using the MultiPROBE® II PLUS HT EX EP-A and EP-B platforms located in Room 3191.

Refer to QIS 23939 *Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform* for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

8.5 PREPARATION OF REAGENTS FOR THE AUTOMATED EXTRACTION PROCESS

All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

8.6 SUMMARY OF DNA IQ™ EXTRACTION VERSION 6.6_ODL (FOLLOWING OFF-DECK LYSIS PROCESS)

8.6.1 Transfer of lysates from Nunc Bank-It™ tubes into the Abgene 96-deep well plate

Lysates from the off-deck lysis protocol, contained in individual Nunc Bank-It™ tubes, are transferred automatically into an Abgene 96-deep well plate prior to commencing automated sample processing. Instead of a 96-deep well plate, the use of individual Nunc Bank-It™ tubes for storage of lysates removes the need for an adhesive film and therefore reduces the risk of well-to-well contamination from the action of peeling back an adhesive plate cover.

Not Current

8.6.2 Automated addition of DNA IQ™ Resin and Lysis Buffer

DNA IQ™ Resin is added automatically into the Abgene 96-deep well plate on the platform. The program uses a waste mode dispensing step to add 53µL DNA IQ™ Resin-Lysis Buffer solution to each well in either half the plate or the whole plate. Because of this, batch sizes are restricted to either 48 or 96 samples in order to maintain efficiency and economy. Two volumes of Lysis Buffer are then added to promote binding of DNA to the paramagnetic resin.

8.6.3 Mixing using a MixMate to bind DNA to resin

Manual intervention is required to seal the Abgene 96-deep well plate with a 4titude Pierce Seal film using the 4titude sealer pre-heated at 175 °C. The plate is then transferred onto a MixMate instrument for mixing at 1100rpm for 5 minutes, followed by centrifugation on an Eppendorf 5804 centrifuge at 3000rpm for 2 minutes. The seal is then carefully pierced with a 96 well PCR microplate and the Abgene plate is returned to the Applied Biosystems magnet on the MPII platform.

8.6.4 Removing lysis reagents for storage

At this point, most of the DNA is bound to the paramagnetic resin. With the positioning of the Abgene plate on the ABI magnet, DNA IQ™ Resin becomes immobile at the bottom of the plate. The lysis reagents from each well are transferred automatically to a storage plate on the MPII platform without disturbing the DNA IQ™ Resin.

8.6.5 Washing of the resin-DNA complex

Washing steps are performed to remove any inhibitors in solution. The first wash uses 125µL Lysis Buffer with shaking at room temperature for 1 minute on the DPC shaker to ensure that the DNA is bound to the paramagnetic resin. The plate is moved to the ABI magnet and the supernatant is transferred into the storage plate. The next three washes incorporate 100µL of 1x Wash Buffer with shaking at room temperature for 1 minute on the DPC shaker. During each wash cycle, the Abgene 96-deep well plate is moved to the ABI magnet and the supernatant is discarded into the tip chute.

8.6.6 Removing any excess of 1x Wash Buffer

The samples are allowed to air dry at room temperature for 5 minutes in order to remove all traces of Ethanol from the Wash Buffer. The presence of Ethanol may potentially inhibit both the elution process and also downstream PCR.

8.6.7 Elution of DNA from the resin-DNA complex

A double elution method is employed in this procedure. At each elution step, 60µL of Elution Buffer is added to each sample, followed by incubation at 65°C for 6 minutes (3 minutes without shaking followed by 3 minutes shaking on the DPC shaker). The Abgene plate is moved to the ABI magnet and the eluted solution (supernatant containing eluted DNA) is transferred to fresh Nunc™ Bank-It™ tubes.

8.6.8 Flushing of liquid pathway

As a decontamination measure, the liquid pathway is washed with Amphyl and Nanopure water at the conclusion of the automated extraction process.

8.7 SETTING UP THE MPII PLATFORMS FOR AUTOMATED DNA IQ™ PROCESSING

1. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

The following steps are carried out in the automated extraction room (Room 3191).

Not Current

2. Remove the Nunc Bank-It™ tubes containing lysates from the fridge to allow to come to room temperature before commencing the extraction procedure.

   Note: If the lysates are frozen, remove them from the freezer and thaw in Room 3191.

3. Restart or turn on the instrument PC.

4. Log onto the network using the **Robotics** login.

5. Open WinPrep® by double clicking icon on the computer desktop (Figure 1).

Figure 1.
The WinPrep® icon.

6. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

7. Ensure that the daily/weekly start-up and maintenance has been performed before running any program. If WinPrep® has been closed or been idle for a long period of time initialise the MP II platform as described in QIS 23939.

8. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash. If visible air bubbles have appeared in tubing or in syringes between setting up the deck and executing the test, another flush wash will need to be performed before starting the MPII extraction run.

9. Open the Extraction setup MP II test file in WinPrep® by selecting:

   - **File**
   - **Open**, navigate to **C:\PACKARD\MULTIPROBE\BIN\QHSS PROTOCOLS**
   - Select "**DNA IQ Extraction_Ver 6.6_ODL.mpt**"
   - Click the "**Open**" button

10. Check the tree pane of the whole program for any bold fonts. See the Analytical Senior Scientist if bold fonts are present.

11. Copy the required plate map from the network folder **I:\EXTRACTION** into the local folder **C:\PACKARD\EXT PLATE MAPS.** Open the plate map using Excel and check that the plate map is complete, and make changes if necessary. If there are blanks (samples that have been removed), make all volumes to 0. If it is not a full batch, delete the rows of blank samples at the end of the platemap. Save all changes made to the platemap (as a .txt file).

12. Decontaminate the required labware with 5% TriGene followed by 70% Ethanol and place onto the instrument deck in the designated grid positions (as shown in the WinPrep® software). Additionally, ensure the DPC shaker is positioned properly.

13. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^0C).
    For EP-B: Tile 2 at F22 (85^0C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

14. Ensure the heat transfer tile is clicked into the plate adapter tile properly.
    Note: This is critical to ensure correct incubation temperatures.

15. To the Amphyl wash station in position **A10**, add 1% Amphyl to the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty (waste) reagent trough in the middle right position of the station.

Not Current

16. Add Wash Buffer to the far right hand side trough of the Amphyl wash station in position **A10**.

17. Add Lysis Buffer to the labelled 150mL reagent trough on the left hand side of the 2 trough holder in position **A13**.

18. Place the 12 channel plate into position **A16**. Add Elution Buffer to the plate by dividing the Elution Buffer between channels 11 and 12.

19. Add Resin to channel 1 of the 12 channel plate in position **A16**. It is important to add the resin in a uniform fashion to ensure equal distribution of resin along the channel.

20. **Nunc Bank-It™ lysate tubes:** The lysates should now be at room temperature. Ensure that the rack is labelled with the correct **AUSLAB batch ID** and that the label matches the batch ID on the worksheet and platemap. Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed.

21. Place the rack onto the MixMate to shake for 1 minute at 1000rpm.

22. Centrifuge the rack at 3000rpm for 2 minutes in the Eppendorf 5804 centrifuge and then place into position **C13**.

22. **Abgene 96-deep well plate:** With the plate in its correctt orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word "**FRONT-lysate"** on the front of the plate. Place the plate in its correct orientation in position **E13**.

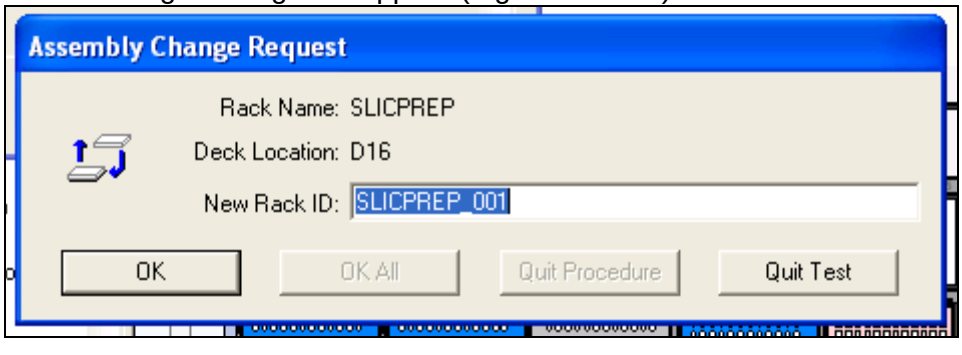
23. **2mL 96-deep well storage plate:** With the plate in its correct orientation label the **left hand side** of the plate with both the correct **AUSLAB batch ID** and **batch ID barcode**. With a marker, print the word **"FRONT-store"** on the front of the plate. Place the plate in its correct orientation in position **E16**.

24. **Nunc Bank-It™ extract tubes:** Ensure that the rack is labelled with the correct **AUSLAB batch ID** on the **front** of the Nunc Bank-It™ tube rack**.** Additionally, randomly check some visible barcodes on the side of lysate tubes against the positions on the worksheet to ensure the correct batch of samples is to be processed. Place the rack in its correct orientation in position **G16**.

25. Add Nanopure water to the 160mL trough in the Flush/Wash station in position **G13**.

26. Ensure that all necessary labware have been positioned correctly as displayed within WinPrep®, then click "**EXECUTE TEST"**. Record run information in the Run Log book.

27. The following message will appear (Figure 2 below):



Not Current

Figure 2. Scan batch ID request

Into **"New Rack ID:"** scan barcode off the worksheet. It is important this corresponds to the labelling of labware on the deck and the plate maps used.

28. Click "**Reset Tip Boxes**" and ensure that the number of tips displayed in this window match those as available on the deck platform. Fill new tips if necessary. Click "**Close**" to accept the tip count, and then click "**Next**".

29. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected corresponds to the labelling of labware on the deck, the paperwork used and the **"New Rack ID"** entered.

30. For a full batch of 76 samples, ensure that all nodes are checked. Click "**Next**" to check all other nodes.

31. For a batch of 48 samples or less, uncheck the node: **"Add resin to second half of plate"**. Click "**Next**" to check all other nodes.

32. Click "**Start**" to continue.

    - The MPII instrument will begin and user prompts will appear as a reminder to confirm the deck setup. **Always decap tubes from positions H1 to A1, H2 to A2 etc.**
    - Ensure all steps on the first prompt have been complete, Click **OK** to continue**.**
    - Ensure all steps on the second prompt have been complete, Click **OK** to continue**.**

33. The program will progress to transfer the lysates followed by automated addition of the Lysis-DTT buffer and the DNA IQ™ Resin solution. The next user prompt will appear. Follow the steps as outlined in the user prompt. Then click **OK** when ready.

    Note: Ensure that the plate is heated and sealed properly with the Pierce Seal on the 4titude Heat Sealer. Mix the plate on the mixmate for 5min at 1100rpm and centrifuge. Pierce the plate with a PCR Microplate and then discard. (A new plate is to be used each time). Ensure the pierced plate is returned to the magnet.

34. Once lysis steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

    Note 1: The Lysate Nunc tubes (including the rack) should be placed into a clipseal bag, sealed and discarded into a biohazard waste bin. The Store plate can also be removed, heat sealed and discarded into a biohazard waste bin.

    Note 2: When the supernatant storage plate is sealed, check the plate for the transfer of beads. To do this, place the plate onto the ABI magnet (if beads are present they will settle to the bottom of the plate) and then inspect the bottom of the plate. If no beads are present, discard the store plate. If, however, beads are present, refer to the Section 15, Troubleshooting.

35. Once the wash steps have been completed, a user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

36. **Note:** The Nunc extract tubes can be decapped on the bench in the MPII cabinet while the samples are drying and the heating tile is stabilising. At this point, the operator has approximately **12 minutes** to decap the tubes. If the operator has been interrupted and requires more time to decap the tubes, the program may be paused at this step.

Not Current

37. A message will appear waiting for the heating tile to reach 85°C (for incubation at 65°C). **DO NOT PRESS CONTINUE** as the program will continue automatically when the temperature has been reached with sufficient stability.

38. A user prompt will appear. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

39. Once the elution is completed, a user prompt appears. Follow the directions as outlined in the user prompt. Then click **OK** when ready.

40. The Lysate Plate is heat sealed and kept in temporary storage for one month.

41. Once all plates are removed from the deck and sealed. Click "**OK**" to proceed to the Amphyl wash step.

42. A final message will advise that the run has completed. Click "**OK**".

8.8 FINALISING THE MPII RUN

1. Transfer left over Resin solution from the 12 channel plate and the Lysis Buffer-DTT (wearing safety glasses) into the glass Lysis-DTT bottle previously used. Discard the 12 channel plate in the biohazard waste bin. Transfer the contents of glass bottle into the brown Winchester bottle located in the safety cupboard in room 3191.

2. Discard the contents of the Amphyl wash station into the sink and rinse the troughs with Nanopure water.

3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% Ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach 7x and 70% Ethanol.

4. Remove the tip chute and funnel, rinse with warm tap water to remove any residue inside the chute before cleaning with 5% TriGene and 70% Ethanol.

5. Import the platemap into Auslab and then delete it.

8.9 IMPORTING MPII LOG FILE INTO AUSLAB

1. Click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.

2. Click **"Open"** when prompted.

3. Click on the relevant run Test ID in the Test Selection box. In the Report/Query/Action Selection dropdown menu, select **"Report: Test Summary (Sorted by Destination Rack ID)"**
4. In the Output Selection dropdown menu, select **"File"**. Save the output file as *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click "**Apply**".

5. Open the log file and check for any errors that may have arisen during the extraction process. Compare the listed errors to any that were encountered during the run. Report any critical errors to the line manager.

6. Import the log file, entering the path, filename and extension (e.g. C:\Packard/ext plate maps/ext logs….) and press **[Enter]**. Delete the log file after importing.

Not Current

7. For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

8.10 IMPORTING EXTRACTION "RESULTS" INTO AUSLAB

1. Import the results file, entering the filename and .txt extension (e.g. CWIQEXT20100917_06.txt). For more details on importing files into AUSLAB refer to Batch functionality in AUSLAB SOP (QIS 24469).

2. The file will be imported into AUSLAB and appear in the Results Import Table.

3. Highlight entry and press **[Enter]**, for access to the Results Table.

4. Page down through the table and check that all sample results have been imported.

5. Press **[SF8]** ***Table Sort Order***, this sorts the table, sorting samples that have failed Autovalidation to the top. Samples that have failed are those that have processing comments present.

6. For samples that have failed, check the **Processing Comments,** by entering into the sample.

7. If processing comments state sample is to be sent to another batch type **other** than quant. Proceed with the following steps:
   a. Request the appropriate rework test code via the **[SF7]** results history table and the **[SF8]** request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling).
   b. Press **[Esc]** to exit back to the DNA results table. Do not toggle accept.
   c. Add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB.

8. If processing comments do not state next step the sample will be processed as normal:
   a. Press **[Esc]** to exit back to the DNA results table.
   b. Highlight any entries to be changed and press **[SF7]** ***Toggle Accept*****.**

9. Press **[F7]** ***Complete Batch***, all of the samples with a ***Yes*** in the Accept column will be transferred to the outstanding Quant Casework or Quant Reference lists.

10. File the extraction worksheet into the relevant intray for scanning in room 3194A (Pre PCR-Sorting).

8.11 SAMPLE STORAGE

Refer to QIS 23959 Storage Guidelines for DNA Analysis for how to store the DNA extract Nunc™ Bank-It™ tubes and Abgene 96-deep well.

8.12 TROUBLESHOOTING WITH THE MPII

1. If the resin is not pipette mixing correctly (eg. Resin is not being drawn up into the pipette tip), pause the Winprep program and check that the support tile is clicked onto the MPII deck correctly. Alternatively, pipette and mix resin manually one more time from the corresponding column in the 12 channel plate.

2. If the Gripper is not placing the rack/plate properly on the deck, pause the program and manually adjust the rack/plate properly on the adapter support tile.

3. If the Gripper appears to be not gripping a plate correctly, or the pipetting alignment of a labware appears to be incorrect, the deck and / or labware can be re-calibrated. This is

Not Current

covered in QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform

9 MANUAL METHOD FOR EXTRACTION USING DNA IQ™

9.1 SAMPLING AND SAMPLE PREPARATION

Refer to section 8.0 above.

9.2 QC SAMPLES

All extraction batches require two controls to be registered. The registration of control samples is covered in QIS 24919 DNA Analysis Workflow Procedure.

9.3 CREATING THE EXTRACTION BATCH AND LOCATING SAMPLES

Refer to QIS 24919 DNA Analysis Workflow Procedure.

9.4 PROCEDURE FOR MANUAL DNA IQ™ (NO RETAIN SUPERNTANT)

1. All manual extractions are to be performed within a biohazard cabinet in Room 3189

2. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

Note: Lysis Buffer-DTT solution and Resin solution need to be prepared in the biohazard cabinet.

3. Turn on the Eppendorf Thermomixer and set the temperature to 37˚C.

Label for each sample:

- Original sample tube
- Spin basket if required
- 2mL tube and
- Nunc™ Bank-It™ storage tube.

Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

4. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

5. Add 300µL of Extraction Buffer. Ensure that large substrates including tape lifts are fully submerged.

6. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. Tapelifts MUST go onto a thermomixer.

7. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

8. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate if no spin basket used.

9. Centrifuge spin basket at 15800 g for 2 minutes.

Not Current

10. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained.Transfer the flow through (lysate) back into the original sample tube.

    Note: If original sample tube is not a 2mL tube, transfer the lysate from spin basket and the supernatant from the original tube into a 2mL tube.

11. Add 550µL of Lysis-DTT Buffer solution.

12. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

13. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

14. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

15. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

16. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

17. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

18. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

19. Repeat the Wash Buffer step (step 17) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

20. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes.

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

21. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

22. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

23. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

24. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

Not Current

25. Remove from the magnetic stand and repeat the Elution Buffer steps (step 19-22). The final volume after the double elution is approximately 95µL of DNA extract.

26. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

27. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

9.5 PROCEDURE FOR MANUAL DNA IQ™ (RETAIN SUPERNTANT)

1. All manual extractions are to be performed within a biohazard cabinet in Room 3189

2. All reagents are to be made fresh within the clean room (3188) prior to commencing each process. For reagent volumes, please refer to the appropriate Appendix.

3. Turn on the Eppendorf Thermomixer and set the temperature to 37ºC. Alternatively an appropriately calibrated hot block may be used.

4. Remove DTT 1M (small aliquot) and Prot K 20mg/mL (small aliquot) from freezer to thaw.

5. Label for each sample:

   - Original sample tube
   - 1.5mL tube (for supernatant) these tubes should not be in contact with the substrate
   - Spin basket if required
   - 2mL tube
   - Nunc™ Bank-It™ storage tube.

Note: Samples that require a spin basket are: swabs, fabric, paper and cigarette butts. Samples that do not require a spin basket are: tapelifts, chewing gum, straws, fingernail clippings/scrapings and toothbrush bristles.

6. Have a second operator perform a sequence check of all tubes. This person must enter the sequence check in AUSLAB.

7. Add 450µL of TNE buffer and vortex.

8. Incubate at room temperature for 30 minutes.

9. Vortex, then centrifuge at 15800 g for 3 minutes.

10. Remove 150µL of supernatant and place into the respective 1.5mL tube labelled with **"sup"** (for further testing).

11. Add 14µL of 20mg/mL Proteinase K and 7µL 40% (w/v) Sarcosyl to the original sample tube containing substrate and TNE buffer, then vortex.

12. Vortex, then incubate at 37°C on the Thermomixer at 1400 rpm for 45 minutes. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of the incubation and at least one during the incubation.

13. Remove from the Thermomixer and increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).

Not Current

14. Transfer substrate to spin basket if required or transfer the lysate to an appropriately labelled 2.0mL tube. Retain original tube containing the substrate in if no spin basket used.

15. Centrifuge spin basket at 15800 g for 2 minutes.

16. Transfer the substrate in the spin basket into a new and labelled 2mL tube to be retained. Transfer the flow through (lysate) back into the original sample tube.

17. Add 550µL of Lysis-DTT Buffer solution.

18. Add 50µL of DNA IQ™ Resin-Lysis solution. Pipette mix the Resin-Lysis solution between each addition to keep the resin suspended in solution.

19. Vortex and place on the Multitube shaker set at 1200 rpm for 5 minutes.

20. Remove from the Multitube shaker, vortex and immediately place into the magnetic stand. Separation will occur instantly.

    Note: If resin does not form a distinct pellet on the side of the tube, or if the magnetic beads have re-suspended while in the stand, vortex the tube and quickly place back in the stand.

21. Carefully remove and discard the solution, ensuring that the resin is not disturbed. Remove from the magnetic stand.

    Note: If some resin is drawn up in the pipette tip, gently expel resin back into tube to allow re-separation.

22. Add 125µL of Lysis-DTT Buffer solution. Vortex and place into the magnetic stand.

23. Once separation has occurred, remove and discard the Lysis-DTT Buffer. Remove from the magnetic stand.

24. Add 100µL of 1X Wash Buffer, vortex and place into the magnetic stand. Once separation has occurred remove and discard the Wash Buffer. Remove from the magnetic stand.

25. Repeat the Wash Buffer step (step 23) two times for a total of three washes. Ensure that all of the solution has been removed after the last wash. Remove from the magnetic stand.

26. Uncap the tubes and place the lids down onto a clean rediwipe. Air-dry the resin in the hood for 15 minutes and then recap tubes

    Note: Do not dry for more than 20 minutes, as this may inhibit the elution of DNA.

27. Add 50µl of Elution Buffer by carefully pipetting the solution to the side of the tube, above the pellet. **Do not mix**.

28. Incubate in the Thermomixer at 65°C for 3 minutes and then continue to incubate for a further 3 minutes shaking at 1100 rpm. If no thermomixer available, incubate samples within a hot block, vortex mix at the start and end of 2nd 3 minute incubation. Remove samples.

29. Vortex and immediately place into the magnetic stand while hot to ensure maximum DNA yield during elution.

Not Current

30. Carefully transfer the DNA extract to the corresponding labelled Nunc™ Bank-It™ tube.

31. Remove from the magnetic stand and repeat the Elution Buffer steps (step 26-29). The final volume after the double elution is approximately 95µL of DNA extract.

31. Store the DNA extracts in temporary storage located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

32. Store tubes containing substrates in Spin Basket boxes located within the Pre-PCR sorting room in the upright freezer with equipment number 30512883 (Room 3194 A).

33. Store supernatants in the S/N Retention boxes located within the Extraction sorting room in the Orford Freezer 30512883 (Room 3190).

34. Ensure that the negative extraction control from the batch is also inserted onto the **'Saliva List':**

    - Log into Auslab
    - 3 for Patient Enquiry
    - Type/Scan the lab number for the negative extraction control
    - From the 9Plex Page press Shift F12
    - A prompt will appear 'Enter List Name'
    - Type Saliva or use the F1 lookup list.
    - Press Enter

9.6 SAMPLE STORAGE

Refer to QIS 24919 DNA Analysis Workflow Procedure

10 VALIDATION

- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 11: Report on the Validation of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 13: Report on the Verification of the Automated Extraction Chemistry Kit using the MultiPROBE® II PLUS HT EX with Gripper™ Integration Casework Platform." 2007.
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 21: A Modified DNA IQ™ Method Consisting of Off-Deck Lysis to Allow Supernatant Retention for Presumptive Identification of α-Amylase" 2008
- Muharam I., Hlinka V., Gallagher, B., Lundie, G., Iannuzzi, C., Nurthen T., McNevin A., Ientile V. "Project 22: A Modified DNA IQ™ Method for Off-Deck Lysis Prior to Performing Automated DNA Extraction" 2008

11 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- Positive and negative (reagent blank) controls are included in each extraction batch. However, for off deck-lysis batches, an additional five negative (reagent blank) controls are included. These controls are processed as normal samples through to completion.
- If any results are obtained from the reagent blank, either at quantification or during the CE Q check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

Not Current

12 REFERENCES

1. Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., & van der Noordaa, J., Rapid and Simple Method for Purification of Nucleic Acids. J. Clin. Microbiol., 1990. 28: p. 495-503.
2. Chen., C.W.T.J., C.A., Recovery of DNA Segments from Agarose Gels. Anal Biochem., 1980. 101: p. 339-341.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.
7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
9. Marko, M.A., Chipperfield, R., & Birnboim, H.C., A Procedure for the Large Scale Isolation of Highly purified Plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem., 1982. 121: p. 382-387.
10. Melzak, K.A., Sherwood, C.S., Turner, R.F.B. & Haynest, C.A., Driving forces for DNA Adsorption to Silica in Percholorate Solutions. J. Colloid. Interface Sci., 1996. 181: p. 635-644.
11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling – Forensic Workstation Application Guide, 2004: p. 1-25.
12. Promega, FAQs –DNA IQ™ System.
13. Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
14. Promega, DNA IQ™ System –Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
16. Promega, Tissue and Hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
17. Promega Corporation 2006 Material Safety Data Sheet. Lysis Buffer. Article number: A826.
18. Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.
19. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

13 STORAGE OF DOCUMENTS

The off-deck lysis worksheets are to be stored within the extraction sorting room (3190) in the nominated intray. It is essential that ALL off-deck lysis worksheets go within this intray so the operational officers are aware that batches are ready and waiting to be combined for extraction on the automated robots.

All worksheets, after auto extraction has been completed, are stored within the Pre-PCR sorting room (3194-A) in an allocated in-tray for scanning by the operational officers.

Not Current

14 ASSOCIATED DOCUMENTS

QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
QIS 17171 Method for Chelex Extraction
QIS 22857 Anti-Contamination procedure
QIS 23939 Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
QIS 23959 Storage Guidelines for DNA Analysis
QIS 24256 Sequence Checking with the STORstar Instrument
QIS 24469 Batch functionality in AUSLAB
QIS 24919 DNA Analysis Workflow Procedure

15 AMENDMENT HISTORY

Version	Date	Author/s	Amendments
R0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka, G. Lundie, I Muharam.	First Issue
R1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
R2	19 March 2008	M Harvey, B Andersen, C Iannuzzi, A McNevin	Addition of Off-deck Lysis procedure, Retention of fully automated method as Appendix, addition of reagent record tables into Appendix
R3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
4	13 March 2009	QIS2 migration	Version incremented by one on migration to QIS2
5	03 June 2009	M Aguilera, B Micic, C Iannuzzi, A. Cheng, V. Hlinka, I. Muharam, G. Lundie, C. Weber	Major changes to reflect new procedure. Updated to reflect changes in procedure as an outcome of internal and external audits. Created ver.6.4 ODL in MPII Platforms. Minor changes in procedures using 4titude 4seal heat sealer to seal plates.
6	29 June 2009	A McNevin, K Lancaster	Removed references to retaining lysate and beads, fixed minor formatting errors. Created ver6.5 ODL in MPII Platforms. Substrates now to be retained in 2mL tube
7	30 September 2010	M.Cipollone, M.Mathieson	Major changes made RE: room numbers to reflect the move to Block 3. Re-formatted entire SOP. New Appendices added which outline all reagent volumes. All equipment and associated asset numbers have been included. Use of the electronic diary now included as an additional section.

Not Current

			Preparation of reagents within the clean room (3188) now to be done prior to starting each process. Storage of worksheets updated. New software version 6.6 on automated extraction robots. S/N Retention Boxes now stored in Manual Ext Room. Associated Documents and hyperlinks updated. Consumables and Equipment table added for Manual DNA IQ.

Not Current

16 APPENDICES

16.1 REAGENT VOLUMES FOR OFF DECK LYSIS PROCESSES (NO RETAIN SUPERNATANT)

Table 10 - Table of reagent volumes.

Reagent	Volume for 48 samples (mL)	Volume for 38 Samples (mL)
Extraction Buffer		
TNE buffer	16	12
Proteinase K (20mg/mL)	0.864	0.648
Sarcosyl (40%)	0.432	0.324
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin		

solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

16.2 REAGENT VOLUMES FOR OFF DECK LYSIS PROCESSES (RETAIN SUPERNATANT)

Table 11 - Table of reagent volumes.

Reagent	Volume for 48 samples (mL)	Volume for 38 Samples (mL)
TNE buffer	22	17
Proteinase K (20mg/mL)	0.672	0.504
Sarcosyl (40%)	0.336	0.252
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	N/A	N/A
DTT (1M)	N/A	N/A
DNA IQ™ Resin solution		
Lysis-DTT Buffer	N/A	N/A
DNA IQ™ Resin	N/A	N/A
DNA IQ™ 1x Wash Buffer	N/A	N/A
DNA IQ™ Elution Buffer	N/A	N/A

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

No Current

16.3 REAGENT VOLUMES FOR AUTOMATED DNA IQ™

Table 12 - Table of reagent volumes.

Reagent	Volume for 96 samples (mL)	Volume for 76 samples (mL)	Volume for 48 samples (mL)
Extraction Buffer (300µL/sample)			
TNE buffer	N/A	N/A	N/A
Proteinase K (20mg/mL)	N/A	N/A	N/A
Sarcosyl (40%)	N/A	N/A	N/A
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	90	70	50
DTT (1M)	0.9	0.700	0.5
DNA IQ™ Resin solution			
Lysis-DTT Buffer	6.0	6.0	3
DNA IQ™ Resin	1.0	1.0	0.5

DNA IQ™ 1x Wash Buffer	35.0	30.0	18
DNA IQ™ Elution Buffer	14.0	12.0	8

Note: Do not directly pipette from reagent stock bottles. Stock reagents should be decanted into working containers where appropriate.

16.4 REAGENT VOLUMES FOR MANUAL DNA IQ™ (NO RETAIN SUPERNATANT)

Table 13 - Table of reagent volumes

Reagent	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	4.0	8.0
Proteinase K (20mg/mL)	0.216	0.432
Sarcosyl (40%)	0.108	0.216
Lysis-DTT Buffer		
DNA IQ™ Lysis Buffer	10	20
DTT (1M)	0.1	0.2
DNA IQ™ Resin solution		
Lysis-DTT Buffer	0.645	1.29
DNA IQ™ Resin	0.105	0.210
DNA IQ™ 1x Wash Buffer	4.0	8.0
DNA IQ™ Elution Buffer	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

Not Current

16.5 APPENDIX FIVE REAGENT VOLUMES FOR MANUAL DNA IQ™ (RETAIN SUPERNATANT)

Table 14 - Table of reagent volumes

Reagent	Volume per sample (uL)	Volume for 12 samples (mL)	Volume for 24 Samples (mL)
TNE buffer	450	5.4	10.8
Proteinase K (20mg/mL)	14	0.168	0.336
Sarcosyl (40%)	7	0.084	0.168
Lysis-DTT Buffer			
DNA IQ™ Lysis Buffer	660	10	20
DTT (1M)	6.6	0.1	0.2
DNA IQ™ Resin solution			
Lysis-DTT Buffer	43	0.645	1.29
DNA IQ™ Resin	7	0.105	0.210
DNA IQ™ 1x Wash Buffer	300	4.0	8.0
DNA IQ™ Elution Buffer	100	1.4	2.8

Note: The volume of Lysis-DTT Buffer calculated includes the volume used in the Resin-Lysis solution preparation.

Not Current

Cathie Allen

From: Cathie Allen
Sent: Friday, 3 June 2022 3:58 PM
To: Lara Keller; Alison Slade
Subject: Data and costs

Hi Lara & Alison

Option 1 – Preferred:

Revert to pre 2018 workflow – which is where all samples above a quant value of 0 are processed through to DNA profiling. Samples that identify as being beneficial for concentration can be based on DNA profile achieved, item criticality and case context.

Option 2 – Not the preferred:

Discontinue 2018 workflow and concentrate samples with a quant value between 0 and 0.0088ng/uL and then process through to DNA profiling stage. This has a risk of there being no DNA sample available for testing by other technologies not undertaken in Queensland, future technologies or testing requested by Defence. In previous discussions, the QPS did not support an automatic concentration process, as the sample hadn't been assessed in the context of the case and may leave no sample remaining for future testing.

Costs:
Approx 4,400 samples were marked as DNA Insufficient during 2021 (calendar year).

Therefore 2,200 samples would be processed in a 6 month period. Additional costs of reagents would be: Profiling Kits: $40,000 and Concentration Kits (if option 2 chosen) $15,000.

Risks: additional Labour required to process – could result in manual injury to staff (WH&S), fatigue and increase in lab errors, additional cost in overtime to maintain throughput; Increase in TAT for results to the QPS (essentially adding 1 months work to a 6 month period – ie 7 months work to process in 6 months) – which may equate to an increase in 1 week TAT - increase from 2 weeks to 3 weeks. This may create a backlog situation and require additional resources to clear the backlog, however training needs to be considered. It takes 12 months to train a staff member to report results and provide a Statement of Witness and give court evidence. There is a decrease in throughput during training as competent staff members are producing less work due to the training burden.

Cheers
Cathie

Cathie Allen **BSc, MSc (Forensic Science) (She/Her*)**
Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the
Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

[redacted]

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here

Helen Gregg

From: Helen Gregg
Sent: Wednesday, 20 July 2022 12:36 PM
To: 'Pobar.DarrenJ[OSC]'
Subject: RE: Further clarification previous email: Assessment of low quant DNA samples report
Attachments: FSS advice regarding DNA reporting

Hi Darren,

I have reached out to my colleagues to assist me with this response:

In 2018, an Options Paper was provided to the QPS with options regarding processing. The QPS reviewed the options and approved for the implementation of the Option where samples with a quant value between 0.0001 and 0.0088ng/ul would be advised as 'DNA Insufficient for processing' and QPS officers could request testing of these samples, which would involve a concentration step prior to amplification.

A Follow-up paper was provided to the QPS last month or so ago, regarding samples that had been concentrated prior to amplification and the outcome of those samples.

Prior to the announcement of the commission of inquiry, the DG requested options for processing that did not include the 'DNA insufficient' process. Options were provided and the Premier announced that Cabinet had decided the DNA insufficient process was no longer being used, and all samples were being processed. From this, we take it that the Premier and Cabinet did not appear to choose the option that included concentration of samples within a particular range, given potential workplace health and safety issues.

Lara advised Supt McNab of the decision and process in the attached email, given the announcement by the Premier of the Cabinet's decision.

Samples are processing through DNA profiling and upon review of the profile obtained, staff will assess if concentration of the sample would be of benefit, within the context of the case. The option of concentration is available, as it has always been since it's implementation in the late 1990's.

From a Forensic DNA Analysis perspective, the most conservative option has been chosen – in that all samples are being profiled, concentration can be done once an appropriate evaluation of the resulting profile has been reviewed, and allows the work unit to gather data on the effectiveness of the concentration step when applied to samples with low quantitation values.

Regards
Helen

From: Pobar.DarrenJ[OSC] <[redacted]
Sent: Wednesday, 20 July 2022 9:51 AM
To: Helen Gregg <[redacted]
Subject: Further clarification previous email: Assessment of low quant DNA samples report

This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.

Good morning Helen

Further to the below query, I am seeking further clarification of the current testing process by QHFSS announced by the Minister. With the 0.0088ng/ul threshold removed, are some samples now being processed without any microconcentration step in place. Ie those between .001 and .0088 which would potentially benefit from concentration.

Regards
Darren

Darren Pobar | Acting Superintendent
Forensic Services Group
Operations Support Command
Queensland Police Service
[redacted]

From: Pobar.DarrenJ[OSC]
Sent: Friday, 15 July 2022 12:00
To: [redacted]
Subject: Assessment of low quant DNA samples report

Good morning Helen

I am currently relieving for a short term in Superintendent Bruce Mcnab's role in Forensic Services Group.

I refer to attached report provided by Acting Executive Director Lara Keller to Supt Mcnab on 24 June 2022 regarding a review assessment of low quant DNA samples and I thank QHFSS for compiling and providing this new report. I note that the success rate in this new review of the micro-concentration process is approximately 25%. This is considerably higher than predicted in the 2018 Options Paper that recommended the removal of the process as a matter of routine. We are still considering the material provided and hope to discuss the options with QHFSS in the near future.

I understand the Health Minister announced on 30 May 2022 the .0088ng/uL processing threshold has been removed and that all samples are now processed as a matter of routine. I am seeking clarification on the current process on testing low quant value samples. If correct that all samples from priority 1 to 3 are being processed despite low quant values, the QPS has concerns how this change will impact anticipated backlogs and turn around times of results. Should this present as a risk, could you also please advise what strategies are in place to mitigate this issue.

Thank you again for providing the report and I look forward to receiving your advice on these queries.

Regards

Darren Pobar | Acting Superintendent
Forensic Services Group
Operations Support Command
Queensland Police Service

[redacted]

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From: Helen Gregg
Sent: Thursday, 25 August 2022 1:54 PM
To: Justin Howes; Paula Brisotto
Subject: FW: new workflow implemented by the DG - potential issues

From: Kylie Rika <[redacted]
Sent: Thursday, 25 August 2022 1:41 PM
To: Helen Gregg <[redacted]
Subject: new workflow implemented by the DG - potential issues

Hi Helen

I know you are coming over to talk with us soon. I just wanted to give you the heads up on a few potential issues a few of us see with the new process:

The workflow does not provide the scientist the ability to assess everything in relation to the sample to get the best result. It may be that the sample could be pooled (or combined) with another sample in the case to maximise the amount of DNA, or if the sample was at the higher end of the quant range, the scientist might want to try amping first rather than microconning particularly if conserving sample is a requirement.

Places undue restrictions on the scientist to get the best result as permission is required from the QPS to perform a second amp. The QPS may not necessarily be in the position to determine whether a second amp might make a profile interpretable.

The workflow does not enable the scientist to assess which rework strategy would be the best based on their scientific knowledge and the circumstances to the case.

The workflow places emphasis on conserving sample for future testing, however in doing so reduces the ability of the scientist to get the best result for the case now. Perhaps the better option would be for the QPS to let us know if any particular sample requires conserving before testing commences.

One process for all samples is not appropriate. Each sample should be assessed on its own merits

Under the new process, all P3 samples are not being microconned – they are still being amped at 15uL. Why aren't P3 samples being treated the same as P2 samples, especially since conservation of sample is less of an issue with P3 samples?

Cold cases in this range are held after quant to enable the scientist to make a decision on further processing – why is this not the case for all samples

With the process that was implemented post 6 June 2022 where samples went straight for a 15uL amp we were able to subsequently microcon to full. If a sample has a 15uL amp and then has a microcon to full then there is a 4.9 times concentration.
With this new process of the sample going straight to microcon at 35uL there is only a 2.5 times concentration.
This means that the new process is much worse than that implemented 6 June 2022.

Thanks
Kylie

Kylie Rika
Senior Scientist, Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health

[redacted]

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Kylie Rika

From: Paula Brisotto
Sent: Friday, 24 June 2022 3:08 PM
To: Kylie Rika
Subject: RE:

Thanks Kylie.

While I think there are pros and cons to either strategy, the decision on the process is not ours at this time.

I think this does provide an opportunity to assess the effectiveness of amplifying the samples, and then subsequently microconning (which is data we had limited information on previously and cannot obtain from straight to microcon samples). This will be valuable information, and I am looking forward to seeing this data.

In relation to the s/sheet, as it is not a full set of data I am not sure how it can be used. Whether you continue using it is up to you and the FRIT managers.

Happy to chat about anything further if you want.

Thanks,
Paula

From: Kylie Rika <[redacted]
Sent: Friday, 24 June 2022 2:05 PM
To: Paula Brisotto <Paula.[redacted].gov.au>
Subject: RE:

Hi Paula

As per the mgmt. meeting minutes from Nov 2021, I asked Ingrid to add it to the s/sheet at G:\ForBiol\AAA Forensic Reporting & Intel\DIFP 2021 examples. I haven't had anyone ask me to stop adding to this s/sheet so hope this is OK.

This sample is really, just an example of Q. 0.004 and good profile obtained loaded to NCIDD.

Agree that the current process worked, but I still worry about the situation where we amp those under 0.0088 at 15ul straight up without the chance to consider m'con first (15ul of our extract is gone – which means less extract to concentrate). In this sample [redacted], we have also added an extra step in the processing – increasing the chance of more sample waste and extra step to possibly expose to contamination.

As Cathie mentioned at our mgmt. meeting, this new process is a government decision that was made after she presented them the options if we remove the DIFP. So I know there is probably not much we can do about it – I just wanted to raise my concern regardless.

Thanks
Kylie

From: Paula Brisotto [redacted]
Sent: Friday, 24 June 2022 1:46 PM
To: Kylie Rika [redacted]
Subject: RE:

Hi Kylie,

Thanks! This seems to be a great example of the process working and the case manager evaluating an appropriate rework strategy based on the initial profile obtained.

Is this being recorded somewhere, or just for noting? (sorry, not sure if I'm supposed to do anything further with this)

Thanks,
Paula

From: Kylie Rika <[redacted]
Sent: Friday, 24 June 2022 12:41 PM
To: Paula Brisotto <[redacted]
Subject: FW:

Hi Paula

In Justin's absence, please note this example.

Thanks
Kylie

From: Ingrid Moeller <[redacted]
Sent: Friday, 24 June 2022 12:30 PM
To: Kylie Rika <Kylie.[redacted]
Subject:

Hi Kyles,

R[redacted] - one of the DIFP samples originally amped at 15uls – sort of crappy profile, then I microconed to full and there will be a new upload.

Ingrid Moeller
Scientist

Forensic & Scientific Services
Prevention Division, Queensland Health

e [redacted] w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Kylie Rika

From: Paula Brisotto
Sent: Monday, 16 May 2022 9:38 AM
To: Kylie Rika
Subject: RE: Minutes Planning and Development meeting 12/05/2022

Hi Kylie,

I am thinking of adding these comments to the minutes:

Media/DNA Review –

Discussed Team response and feeling regarding DNA Review announcement and Terms of Reference. Team feeling okay – confident in processes in place and standards of work.

Data grab from FR performed by bdna. No progress on data interrogation at this time as per ED advice – pending assessment as per scope of review and recommendations provided.

I will run these by Wendy, as she took the minutes for the meeting.

I think this covers the general discussions. While there is mention in the DNA review of DIFP, there are a number of data grabs requested as discussed in the meeting as well – Nucleospin, sexual assault samples, DIFP and NDNAD. The data grabs from FR is something we want to build in to regular reports from FR so we can do trend analysis, post-implementations reviews etc as a part of BAU, as general success rates is something we had ready access to in AUSLAB.

Thanks for raising this.

Paula

From: Kylie Rika <[redacted]
Sent: Monday, 16 May 2022 8:44 AM
To: Paula Brisotto <[redacted]
Subject: Edits required: Minutes Planning and Development meeting 12/05/2022

Hi Paula

I think we need to include a summary of the conversation about the data dump we got from bdna – albeit analysis not progressing at this time, because it shows that the mgmt. team are keen to do more data analysis on a variety of things possibly including DIFP (continuous improvements) – especially since it was raised by Adrian and I back in Nov 2021.

Thanks
Kylie