HealthSupport Queensland

Forensic and Scientific Services

Procedure for Case Management

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, emai[redacted]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)

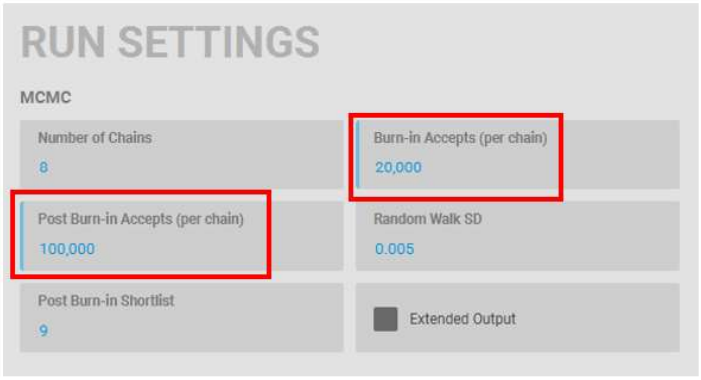
11. The overall LR is reasonable given the reference and casework DNA profiles

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.

.

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

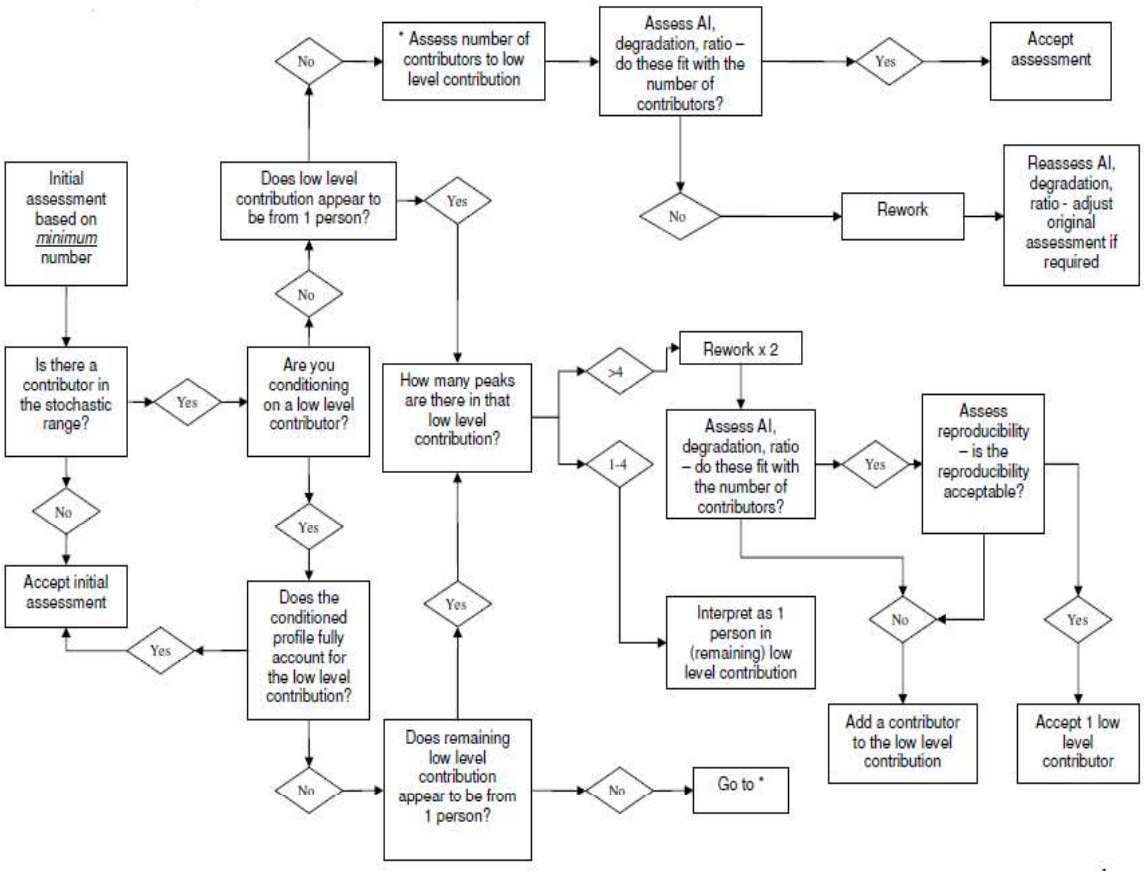
			to "Pre/Post AUSLAB Batch Functionality"; removed unnecessary flow charts; updated hyperlinks and associated documents; introduced paperless case management; re-arranged for better flow and grammatical correctness; Introduced more definitions; included instruction on locating profiles for printing.
15	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.