

DNA IQ™ Method of Extracting DNA from Casework and Reference Samples

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Automated DNA IQ™ Method of Extracting DNA

1 PURPOSE AND SCOPE

This method describes the routine method of DNA extraction using the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms and Promega DNA IQ™ kit. The manual method has been included as a back-up method should it be required.

This method applies to all DNA Analysis staff 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 must be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
Lysates	Samples that have had the Lysis step performed, but have not yet completed the entire extraction process
DNA Extracts	Samples that have had a DNA extraction processes performed
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II PLUS HT EX Platform
DTT	1,4 Dithiothreitol
Pro K	Proteinase K
SDS	Sodium Dodecyl Sulphate
TNE	Tris, NaCl and EDTA Buffer
EDTA	Ethylenediaminetetraacetate
EP-A	Extraction Platform A
EP-B	Extraction Platform B

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer used in the pre-lysis of cellular material contains Proteinase K (Pro K), TNE (Tris, NaCl, EDTA) and SDS. TNE acts as a basic buffer with EDTA chelating ions in solution. SDS is a detergent that lyses open cell membranes. Proteinase K is added to digest protein and cellular material that interferes with the DNA binding capacity of the resin. It is also added to rapidly inactivate enzymatic activity that could potentially degrade DNA (e.g. nucleases).

Proteinase K (also known as 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/next 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.

DNA IQ™ Kit

The DNA IQ™ kit 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. The protocol has been modified to incorporate a number of work practices used in DNA Analysis FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.

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- The increase of Extraction Buffer volume to 500µL for use with the Slicprep™ 96 device.
- The use of tubes and spin-baskets for the off-deck lysis of samples prior to extraction on MPII. Use of a 96-deepwell plate for completion of extraction on MPII.
- The provision of initial incubation with TNE Buffer and retention of a portion thereof for further testing (retained supernatant testing).
- The increase of Lysis Buffer volume to 957µL proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution Buffer volume of 60µL for a final volume of 100µL.
- The use of NUNC Bank-It tubes for storage of final extracts.

Cell lysis is performed with Promega 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, the Lysis Buffer contains 50-75% guanidinium thiocyanate, < 2.5% EDTA, < 2% 3-[(3-Choalamidopropyl)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 Pro K will increase 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 with Lysis Buffer. This wash ensures the DNA is bound to the magnetic bead resin and washes out inhibitors. The next three washing procedures are with a Wash Buffer. This buffer contains an alcohol/aqueous mixture which ensures the DNA is not eluted during washing by keeping the DNA dehydrated, and aqueous phase washes out the inhibitor.

Elution Buffer removes the DNA from the magnetic bead resin. The Elution Buffer changes the salt content. Heating the complex to 65°C 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.

MultiPROBE® II HT EX PLUS with Gripper™ Integration Platform

Within DNA Analysis, routine DNA extractions are performed using either one of two MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms (Extraction Platform A, EP-A) and (Extraction Platform B, EP-B) perform casework or reference samples. Each platform uses a computer – controlled Cartesian X-Y-Z 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 fixed and/or disposable tips (both clear and 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

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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 on all the platforms (except for the Post – PCR MP11) 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, all platforms include a left deck extension.

In this program a platemap is utilised to provide the necessary information for correct volumes and locations for pipetting. It also contains information regarding positions where pipetting must take place at various locations, the number and IDs of samples. Without a platemap the program will fail to work.

4 REAGENTS AND EQUIPMENT

4.1 Reagents

1. DNA IQ™ System Kit – 400 sample Kit
 - Resin
 - Lysis Buffer (LB)
 - 2x Wash Buffer (2xWB)
 - Elution Buffer (EB)
2. Tris/Sodium chloride/EDTA Buffer (TNE)
3. Proteinase K (Pro K) 20mg/mL
4. Dithiothreitol (DTT) 1M
5. 5% TriGene
6. 70% Ethanol
7. 1% Amphyl
8. 0.2% Amphyl
9. Isopropyl alcohol
10. AnalR 100 %Ethanol
11. 20% SDS
12. Decon® 90 solution
13. Nanopure H₂O

Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6120
DTT	Freezer	Room 6120
20% SDS	Shelf	Room 6122
Isopropyl alcohol	Shelf	Room 6122
TNE pH 8 Buffer	Shelf	Room 6122
DNA IQ™ Kit	Shelf	Room 6122
Amphyl (1% and 0.2%)	Shelf	Room 6127
Nanopure Water	Shelf	Room 6127
5% TriGene	Shelf	Room 6127
AnalR 100 %Ethanol	Shelf	Room 6127

Please see Table 2 for the volume of reagents for a full plate or half plate. Refer to “Receipt, Storage and Preparation of Chemicals, Reagents and Kits” (QIS [17165](#)) for

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preparation of the TNE Buffer. All reagents can be made on the bench in Room 6122, except for the Lysis Buffer with DTT which needs to be made in a fume hood. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

Reagent (volume per sample)	Constituent (volume per sample)	Volume req'd for 96 Samples (mL)	Volume req'd for 48 Samples (mL)
Extraction Buffer (500 µL/sample)	TNE Buffer 462.5µL	54	27
	Prot K (20 mg/mL) 25.0 µL	2.9	1.5
	SDS (20 %) 12.5µL	1.5	0.7
Lysis Buffer (with DTT) (1.127mL/sample)	Lysis Buffer (no DTT)	130	66
	DTT (add to Lysis Buffer)	1.3	0.66
Lysis Buffer (with DTT) Reagent Trough	As above	125	63
DNA IQ RESIN Solution (50µL/sample)	Lysis Buffer (with DTT) (from above) 43µL	6	3
	DNA IQ RESIN 7µL	1	0.5
DNA IQ 1X Wash Buffer (300µL/sample)	See Below for preparation	35	18
DNA IQ Elution Buffer (120µL/sample)	Use directly from Kit	14	8

NOTE: For batches not equal to either 96 or 48 samples, refer to Appendix 1 "18.1 Reagents Calculation Tables" Table 7 for batches of <48 samples, and Table 8 for <96 (but >48)

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to Table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots for a 96 sample run. Remove aliquot/s of Proteinase K from the freezer, defrost, vortex and centrifuge before use. Ensure that the 20% (v/v) SDS is completely dissolved (clear) in the stock solution before making the Extraction Buffer. If not dissolved, invert the container a few times and leave longer at room temperature.

Lysis Buffer with DTT

Lysis Buffer is supplied with the kit. Lysis Buffer with DTT is prepared at the start of each run. Remove the DTT from the freezer, defrost, vortex and centrifuge before use. Into a sterilised glass bottle, add 1.3mL of DTT to 130ml of Lysis Buffer for 96 samples. If 48 samples are to be run, use 660µl of DTT to 66ml of Lysis Buffer, again, made up in a sterile glass bottle. Make up the Lysis Buffer with DTT within the Laminar Flow and ensure that full PPE is worn, including face shield. Warning: Lysis Buffer and DTT are toxic.

DNA IQ™ Resin

DNA IQ™ Resin is supplied with the kit. The resin is prepared at the start of each run in a 10mL sterile tube. Ensure the resin is properly mixed by vortexing before pipetting. Look for calculations in Table 2 for the correct volumes of resin and Lysis Buffer (with DTT). In the 10mL tube, mix by inversion before adding to column 4 in the 12-channel reagent plate.

1X Wash Buffer

2X Wash Buffer is supplied with the kit. Once a new kit has been opened, add 35mL of *AnaIR* Ethanol and 35mL of Isopropyl alcohol to the 2X Wash Buffer. Once the reagents have been added, label the lid and side of the bottle with "1X Wash Buffer," initial and date.

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4.2 Equipment

The following equipment (Table 3) and consumables (Table 4) are required for the DNA IQ extraction.

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)		6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)		6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)		6127
DPC Shaker (Ext A Platform)		6127
DPC Shaker (Ext B Platform)		6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)		6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)		6127
Eppendorf 5804 centrifuge		6127
Vortex		6127
Fridge		6127
Micro centrifuge		6127
Decapper		6127

Table 4. Consumables used for extraction

Consumables	Location
175uL Clear Non-Conductive Filter Tips – Pre-sterilized	6127
MβP Pure 1000uL Tips – Pre-Sterilized	6127
SlicPrep™ 96 device plate	6122
ABgene 96-deepwell plate	6120
Axygen 2mL Deep Well storage plate	6127
1.5ml or 2ml Eppendorf tubes with Spin baskets	6120
12 Channel plate	6127
Nunc tubes	6120
Nunc Caps	6127
Sterile 50mL Falcon tubes	6122
Sterile 10mL tubes	6122
Autoclaved 100mL glass bottles	6122
Autoclaved 250mL glass bottles	6122
Aluminium sealing film	6127
1000uL disposable tips	6120

5 SAFETY

As per the procedures in the QIS document “Operational Practices in the DNA Dedicated Laboratories” (QIS 17120), PPE is to be worn by all staff when performing this procedure.

The automated platforms, labware and associated equipment should be wiped with 5% TriGene™ followed by 70% ethanol before and after use.

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 within the cabinet or on the deck surface.

Warning: Tris base, EDTA, SDS and Lysis Buffer are irritants. DTT is a reducing agent that destroys disulfide bonds. Chaotropic reagents such as guanidinium thiocyanate (GuSCN) are toxic. Do not breathe alcohol fumes. Handle carefully and wear appropriate PPE.

Lysis Buffer contains guanidinium thiocyanate which can be harmful if inhaled, swallowed or comes in contact with skin. Any left over Lysis Buffer with DTT is to be disposed of in a brown Winchester bottle in the fume hood. Never dispose down the sink. If spillage occurs, absorb with liquid-binding material (such as sand, kitty litter, etc) and dispose in a biohazard bin. Again, handle carefully and wear appropriate PPE, including face shield.

6 SAMPLING AND SAMPLE PREPARATION

Samples waiting to be extracted are stored in freezers as described in Table 5.

Table 5. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Low Priority Samples	Walk in Freezer or Freezer in 6117	6109 or 6117-5
Lysates in 1.5ml tubes	Fridge	6120
96 deep well plate containing lysates	Fridge	6127

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed in Table 6.

Table 6. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT277	Negative Extraction control – Empty well
Positive Control	FBOT279	Positive Extraction control – Known Donor dried blood swab

Registration of QC

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 6 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. **Blood** for blood control).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control). Do not assign a priority.
8. Press **[F7] Save** to save the Billing details.
9. Enter **LAB** in the Billing code field and **t** in the date field and **FBQC** in the Loc/Client field.
10. Press **[F4] Save twice** to save the registration details.

Note 1: Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Note 2: for DNA IQ Lysis batches with more than 46 samples (excluding controls) two sets of controls should be registered

Create the DNA IQ Lysis or Retain Supernatant Batch (as required)

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate batch type and press **[F5] Batch Allocation**.
5. Press **[F6] Create batch**.
6. Press **[F8] Print menu**.
7. Press **[F7] Print Sample Label**. (print 3 sets)
8. Press **[F8] Print Worksheet**. (print 2 copies)
9. Press **[SF5] Main menu**.
10. Press **[SF11] Print**.

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11. Press **[SF6] Accept batch**.
12. Press **[Pause/Break]** to exit to the **Main Menu**.
13. Obtain worksheets (**FBLASER3**) and labels (**FBLABEL13-16**) from the Analytical Section printing bench (**Room 6117**).

Locating Samples

To locate samples refer to “Analytical Sample Storage” (QIS [24255](#)).

7 OFF-DECK LYSIS PROCEDURE (NO RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. “18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)”.
2. Separate the batch into two smaller batches of 48 samples, including one set of controls. (If only a single operator the second batch can be started during step 11)

Note: Positions 1-4 will be the two sets of controls, positions 5-50 will be the first 46 samples and 51-96 will be the second set of 46 samples

3. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number within the lysis batch. Retain the original 5mL storage tube for substrate storage.
4. Prepare / assemble spin basket assembly or 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required.

Note: substrates from each sample need to be retained

- a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
- b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws and toothbrush bristles.

5. Label the side of sterile 1.0mL Nunc Bank-It tubes with barcode.
6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.
7. Prepare Extraction Buffer (store at 4°C when not in use).
8. Using a multi-stepper pipette add 500µL of Extraction Buffer and vortex briefly.
9. Incubate in a hotblock at 37°C for 45minutes (note temperature on worksheet).
10. Remove samples from hot block and vortex briefly then return to rack.
11. Increase temperature on hotblock to 65°C (preparation for second incubation step).
12. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).
13. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.
14. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.

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15. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL tube.
16. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10 minutes (note temperature on worksheet)
17. Enter reagent details, temperatures etc. into AUSLAB.
18. Complete batch in AUSLAB.
19. Store lysates at 4°C (fridge in 6120).
20. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.
21. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the lysis batch the samples have progressed from on the worksheets. Stamp as "Urgent" if necessary.

8 OFF-DECK LYSIS PROCEDURE (RETAINED SUPERNATANT)

1. Print or obtain a copy of Appendix 2. "18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)".
2. Remove samples from 5mL storage tubes and label side with barcodes if necessary. If substrates are in a 0.5mL tube, transfer to a labelled 1.5mL tube. Label lid of each sample with position number. Retain 5mL tube for substrate storage.
3. Label the side of 1.5mL tubes with barcodes for retaining supernatant. Also label lid of 1.5mL tube indicating it contains supernatant.
4. Prepare spin basket assembly or a 1.5mL (or 2.0mL) tube and label side with a barcode and lid with position number as required. **Note:**
 - a. Samples requiring a spin basket include swabs, fabric, paper and cigarette butts.
 - b. Samples requiring a 1.5mL include tapelifts, chewing gum, straws and toothbrush bristles.
5. Label the side of sterile 1.0mL Nunc Bank-It tubes with barcode.
6. Have a second operator sequence check all tubes and complete the sequence check in AUSLAB.
7. Using a pipette add 650µL of TNE Buffer and vortex briefly.
8. Incubate at room temperature for 30 minutes.
9. During 30 minute incubation prepare Proteinase K and SDS solutions.
10. Vortex, then centrifuge for 3 minutes at maximum speed (14000rpm).
11. Remove 150µL of supernatant and place into labelled 1.5ml tube (for further testing).
12. Add 25µL of 20ng/µL (mg/mL) Proteinase K and 12.5µL 20% (w/v) SDS to each original sample tube containing TNE Buffer. Vortex briefly.

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13. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).
14. Remove samples from hotblock, vortex briefly and return to rack.
15. Change settings on hotblock to temperature of 65°C (preparation for second incubation step).
16. Transfer substrates to spin baskets if required using twirling sticks (if unable to remove with twirling sticks, use forceps. Forceps must be cleaned between each sample by rinsing in bleach followed by ethanol and flaming).
17. For samples not requiring spin baskets, transfer the lysate to the newly labeled 1.5mL tube. Then store original 1.5mL containing substrate in the original 5mL tube.
18. Centrifuge spin baskets at maximum speed (14000rpm) for 2 minutes.
19. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL.
20. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10minutes (note temperature on worksheet).
21. Enter reagent details, temperatures etc. into AUSLAB.
22. Complete batch in AUSLAB.
23. Store supernatants in Freezer 6117-2 (-20°C).
24. Store lysates at 4°C (Fridge in 6120).
25. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.
26. Print the appropriate worksheets and 3 sets of batch labels for the DNA IQ extraction batch and note the retained supernatant batch the samples have progressed from on the worksheets. Stamp as “Urgent” if necessary.

9 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 6127.

Refer to “*Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform*” (QIS 23939) for instructions on the use and maintenance of the MultiPROBE® II PLUS HT EX platforms.

Summary of DNA IQ EXTRACTION Version 2 ODL

1. **Binding of paramagnetic resin to DNA and further Lysis:** add Resin solution (50µL) and Lysis Buffer (957µL). Automated mixing and shaking at room temperature for 5

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minutes. (this occurs at steps 10-15 of the protocol)

2. **Removing lysis reagents:** Slicprep plate is moved to the PKI Magnet to separate beads. Removing of supernatant (1600µL) without disturbing resin, dispense this solution in the storage plate. (this occurs at steps 16-18 of the protocol)
3. **Washing of the resin-DNA complex:** To remove any inhibitors in solution. The first wash is with Lysis Buffer (125µL), shaking at room temperature for 1 minute. The plate is moved to the PKI Magnet and the supernatant is removed into the storage plate. The next three washes are with 1X Wash Buffer (100µL), shaking at room temperature for 1 minute. During each wash cycle, the plate is moved to the PKI Magnet and the supernatant is discarded. (this occurs at steps 21-59 of the protocol)
4. **Removing any excess of 1X Wash Buffer:** air dry at room temperature for 5 minutes. (this occurs at step 60 of the protocol)
5. **Elution of DNA from the Resin-DNA complex:** Add Elution Buffer (60µL) and incubate at 65 °C for 6 minutes (3 minutes no shaking and 3 minutes shaking). The plate is moved to the PKI Magnet. The eluted solution (supernatant) is removed to the Nunc tubes. Elution is repeated twice. (this occurs at steps 63-83 of the protocol)
6. **Flushing of capillaries:** The capillaries are washed with Amphyl and nanopure water.

Preparation of Reagents prior to extraction

1. Refer to table 2 for reagent volumes to make up the required amount of Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.
2. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Sequence Check the Nunc Bank-It™ tubes and Sample Lysates

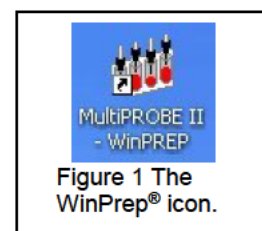
To sequence check storage tubes and transfer DNA lysates to ABgene 96-deep well plates, please refer to method "Procedure for the Use of the STORstar unit for automated sequence checking" (QIS [24256](#)).

ENSURE the Nunc tube rack is labelled with the AUSLAB Batch ID and barcode on the front of the plate.

Setting up the EP-A or EP-B MPIIs

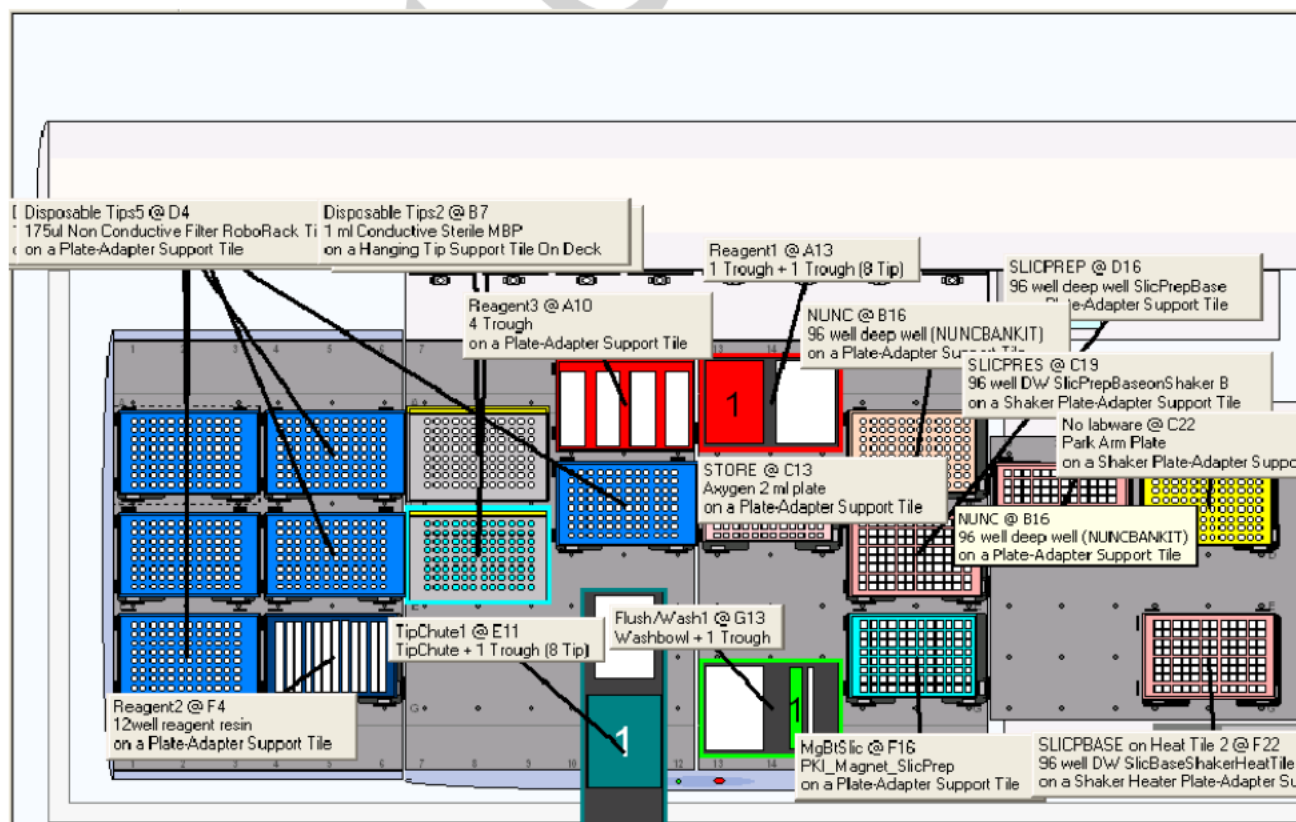
These steps are to be carried out in the Automated extraction Room (Room 6127)

3. Turn on the instrument PC.
4. Log onto the network using the **Robotics** login.
5. Double click the WinPrep® icon on the computer desktop (Figure 1).
6. Log onto the WinPrep® software by entering your username and password, then press "**Enter**".
7. Ensure the **System Liquid Bottle is FULL** before every run and perform a Flush/Wash.



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8. Ensure that the daily/weekly start-up 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.
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 2_ODL.mpt"**
 - Click the **"Open"** button
10. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.
11. Open the required plate map from the network **I:\EXTRACTION**. Check that the plate map is complete. 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, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**
12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).
 - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the ABgene 96-deep well plate must be placed into positions **D16** and **C19**.
 - Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.



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Figure 2. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
For EP-A: Tile 1 at F22 (85°C).
For EP-B: Tile 2 at F22 (85°C).
Note: Press the start/stop button twice at the front of the DPC Shaker to ensure that it displays zero on the screen.
14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.
15. **Nunc tube rack:** Check that is the same Auslab batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Check that the batch label and batch barcode labels are attached to front side of rack. Add B1-Lite generated **"NUNC"** barcode to the right side of the Nunc tube rack. Then place nunc rack into position **B16**.
16. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated **"STORE"** barcode. Then place in position **C13**.
17. Pour the required amount of Lysis Buffer into the labelled 150mL reagent trough. Place Lysis Buffer on the left hand side of the 2 trough holder located in position **A13**.
Note: Ensure that full PPE is worn, including face shield when handling these reagents.
18. **ABgene 96-deep well plate containing lysates:** Centrifuge plate for 2 minutes at 3021rpm before gently removing adhesive seal and place into position **D16** ensuring the plate is oriented such that the long side of the plate with the words "Front" written on at time of STORstar processing is visible from the front. (This should correspond with the cut corner at H1 being visible to the front of the operator)
19. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the **"EXECUTE TEST"** button. While the test is loading, record all run information in the Run Log book.
20. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the ABgene 96-deep well plate containing Lysates located in position **D16**. Once this has been done, click **"Start"**, to continue.
21. Message will appear (Figure 3 below):

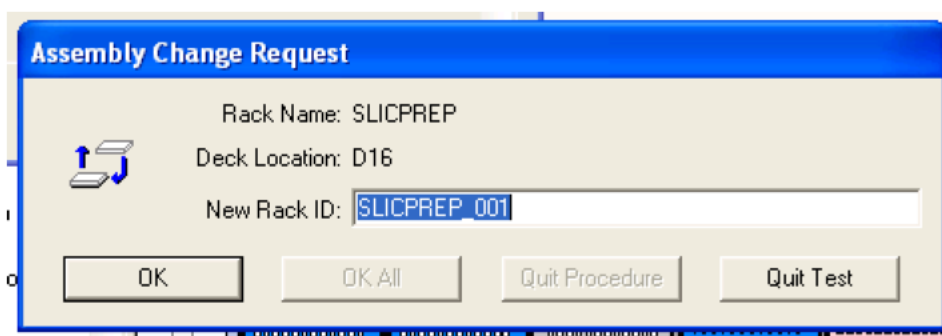


Figure 3. Scan batch ID request

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Into "New Rack ID:" Scan barcode of ABgene 96-deep well plate (matches batch ID) and press "OK"

22. 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, followed by clicking "**Next**"
23. After the barcodes have been read, a user prompt will appear as a reminder to:

"Ensure

 - 1. Shaker and heat box are on.**
 - 2. Deck has been populated correctly.**
 - 3. The Lysis Buffer is on the left side at A13.**

Click "**OK**" to continue.
24. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the ABgene 96-deep well plate containing Lysates.
25. Place the 12 channel plate into position **F4** then add the Elution Buffer to the plate by splitting the amount of Elution Buffer in half between channels 11 and 12.
26. Place the Wash Buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10**)
27. The next User prompt will appear with the following directions:

"Ensure Wash Buffer has been added. Manually add 50uL of Resin and place the ABgene plate in position D16. Ensure Elution Buffer has been added." Press "**OK**" when steps 24-26 have been performed.
28. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85^oC (real temp 65^oC). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^oC.
29. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:

"Push down the plate on the PKI Magnet, Check Nunc tubes are uncapped at position B16, then press OK."

Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.
30. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet
31. Once the program is completed, a final User Message prompt appears asking to:

"Remove plates and cover them with aluminium sealing film. Remove Nunc rack and recap Nunc tubes."

Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click "**OK**" to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

Automated DNA IQ™ Method of Extracting DNA

32. Remove the resin-Lysis-DTT solution from the 12 channel plate in the glass Lysis-DTT bottle used. Discard the plate in the biohazard waste bin.
33. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.
34. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H₂O upon completion of the run.
35. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
36. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.
37. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the “**Completed Extractions**” folder.

Recording Reagent Details and other information in AUSLAB

38. To record reagent lot numbers, log into the **AUSLAB Main Menu**.
39. Select **5.Workflow Management**.
40. Select **2. DNA Batch Details**.
41. Scan in the Extraction Batch ID.
42. Press **[F6] Reagents**.
43. Press **[SF8] Audit**.
44. Press **[F5] Insert Audit Entry**, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

45. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
46. 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)”
47. 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**”. (refer to Figure 4. below)

Automated DNA IQ™ Method of Extracting DNA

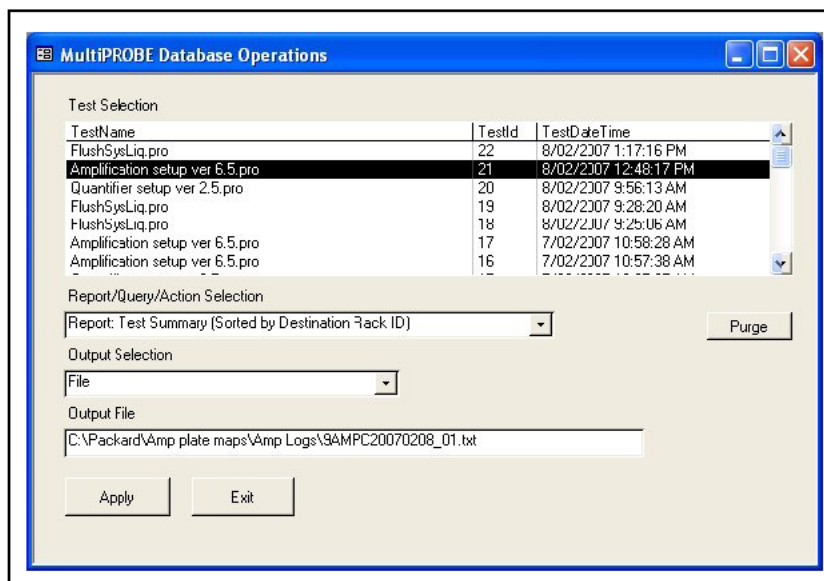


Figure 4. The MultiPROBE log database for collecting MP II run information

48. 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.
49. Copy the log file to I:\EXTRACTION\EXT A MPII\LOGS or I:\EXTRACTION\EXT B MPII\LOGS for uploading to AUSLAB.
50. Log into the **AUSLAB Main Menu**.
51. Select **5.Workflow Management**.
52. Select **2. DNA Batch Details**.
53. Scan in the Extraction Batch ID barcode.
54. Press **[SF6] Files**.
55. Press **[SF6] Import Files**.
56. AUSLAB prompts "**Enter filename**"; enter the filename and extension and press **[Enter]**. (e.g. I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv)
57. AUSLAB prompts "**Is this a result file Y/N?**" enter **N** and press **[Enter]**.
58. Press **[Esc]**.

Importing Extraction "Results" into AUSLAB

59. Log into the **AUSLAB Main Menu**.
60. Select **5. Workflow Management**.
61. Select **2. DNA Batch Details**.
62. Scan the Extraction batch ID barcode located on the worksheet.
63. Press **[SF6] Files**.
64. Press **[SF6] Import Files**.
65. AUSLAB prompts "**Enter filename**"; enter batch name and extension and press **[Enter]**.(e.g. CWIQEXT20071115_01.txt)
66. AUSLAB prompts "**Is this a results file y/n?**" enter "**y**" and press **[Enter]**.
67. The file will be imported into AUSLAB and appear in the DNA file table.
68. Highlight entry and press **[Enter]**, for access to the DNA results table.
69. Page down through the table and check that all sample results have been imported.
70. Press **[SF8] Table Sort Order**, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
71. For all samples that have failed check the **Processing Comments**, by entering into the sample.
72. a) If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. Microcon, NucleoSpin and pooling

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- b) Press **[Esc]** to exit back to the DNA results table.
 - c) Do not toggle accept.
73. a) If processing comment does not state next step for sample the sample will be processed as normal.
- b) Press **[Esc]** to exit back to the DNA results table.
 - c) Highlight any entries to be changed and press **[SF7] Toggle Accept**
74. 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.
75. File the Extraction worksheet into the relevant folder in Room 6117.

10 SAMPLE STORAGE

Please refer to “*Analytical Sample Storage*” (QIS [24255](#)) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, ABgene 96-deep well and Axygen store plates.

11 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc tube rack barcode, if the Gripper is not picking up or placing the Nunc tube rack properly on the deck, just manually place the rack properly on the plate adapter support tile.
3. When reading the Store plate barcode, if the Gripper is not picking up or placing the Store plate properly on the deck, just manually place the plate properly on the plate adapter support tile.
4. When reading the Slicprep plate barcode if the Gripper is not picking up the plate properly :
 - a. if the plate was not properly placed on the plate adapter support tile with the Wallac Isoplate, just manually place the plate properly.
 - b. if the plate was properly placed on the plate adapter support tile with the Wallac Isoplate on it: it means that the gripper needs to be initialised. Abort the run, Initialise the instrument and restart the run. If problem persists, shutdown the MPII and PC, restart and then initialise the whole instrument. Otherwise, contact your line manager.
 - c. Calibrate relevant labware using the SlicPrep Calibration plate. This has preset standardised positions that need to be the same on all labware where the Slicprep plate is being moved. The same plate is used on both extraction platforms A and B.
 - d. Check the calibrations against the run program DNAIQGripperTest.pro. This program moves the Slicprep across all the labware the gripper moves across. Start with the Slicprep at D16.
5. In steps 18 or 26, if a message is stating that the instrument is having a motor problem when picking up 1 mL tips and the only option is to Abort, abort, initialise and open program version **1.3a** (if the problem is in step 18) or version **1.3b** (if the problem is in step 26).

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Figure 5. Example of DLL error

As the program will start the gripper will pick up the plates, it is not necessary that the Nunc tube rack is in position (B16), only ensure that it is reading the correct barcode. It is **important not** to place the Slicprep in the original position (E13) as the Slicprep plate has the Spin basket part removed (ie keep at D16), ensure it will scan the correct batch barcode. The Store plate remains in the original position. If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.

6. If the program has already started step 18 and the previous message is appearing, you need to abort. Initialise the instrument and open program version 1.3a.
Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 for all the samples that the Lysis Buffer have been dispensed (Column 6), ensure that the number of samples where the Lysis Buffer was added is the same as the ones where the volume needs to be changed.
Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.
7. If the program has already started step 26 and the previous message is appearing, you need to abort. Initialise the instrument and open program version 1.3b.
Go to the plate map in C:\PACKARD\ EXT PLATE MAPS and change volume to 0 in all the samples that the Lysis Buffer and Extraction Buffer have been removed (Column 9), ensure that the number of samples where the solution was removed is the same that the ones the volume need to be changed.
Save the changes and use this plate map for the restart of the program. Please read troubleshooting 5 for barcode reading of plates.
If the problem persists even after restarting, replace the rack of disposable 1 mL conductive sterile MBP tips for a new one.
8. If a disposable tip gets stuck on the 8 tip arm during disposal of tips a user message will appear. Remove and press retry and then continue.
9. If the message Figure 6 below has appeared:

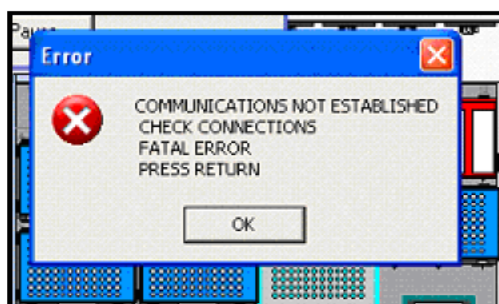
Automated DNA IQ™ Method of Extracting DNA

Figure 6. Example of error

press OK and the program will be aborted automatically. Check that all the connections to the instrument (shaker, heater and computer) are properly plugged in. If everything is OK, you need to close WinPrep, shut down the instrument, shaker, heater and PC. After 2 min restart everything. Once Winprep has been opened, reinitialise the instrument and start the program (check version number according to which step the message has came up). Please read troubleshooting 5 for barcode reading of plates.

12 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

13 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

- A negative control (also known as the reagent blank) is included with each batch of extractions. This reagent blank is processed as a normal sample through to completion. If any results are obtained from this sample, either at the quantitation step or the Genescan analysis step, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.
- Positive and negative controls are included in each extraction batch as per table 4.

14 REFERENCES

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8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
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15 STORAGE OF DOCUMENTS

All worksheets are stored in the Analytical area (Room 6117).

16 ASSOCIATED DOCUMENTS

- QIS 17120 Operational Practices in the DNA Dedicated Laboratories
 QIS [17171](#) Method for Chelex Extraction
 QIS 17165 Receipt, Storage and Preparation of Chemicals, Reagents and Test Kits
 QIS [23939](#) Operation and Maintenance of the MultiPROBE® II PLUS HT EX and MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform
 QIS 24255 Analytical Sample Storage
 QIS [24256](#) Sequence Checking with the STORstar Instrument
 QIS 24469 Batch functionality in AUSLAB

17 AMENDMENT HISTORY

Revision	Date	Author/s	Amendments
0	23 Oct 2007	B. Gallagher, T. Nurthen, C. Iannuzzi, V. Hlinka,	First Issue

Automated DNA IQ™ Method of Extracting DNA

		G. Lundie, I Muharam.	
1	12 Dec 2007	M Harvey, C Iannuzzi, A McNevin	Reviewed and updated after initial training
2	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

Not Current

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18 APPENDIX

18.1 Reagents Calculation Tables

Table 7. Less than 48 samples (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	$(N \times 1.35) + 0.75$	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	$N \times 0.56$	
Prot K (20 mg/L)	$N \times 0.03$	
SDS (20 %)	$N \times 0.015$	
DNA IQ RESIN Solution		
LYSIS Buffer	$0.054 \times (N + 8)$	
DNA IQ RESIN	$0.009 \times (N + 8)$	
DNA IQ 1X Wash Buffer	$N \times 0.36$	
DNA IQ Elution Buffer	$N \times 0.144$	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

Table 8. Greater than 48 samples (note difference is in DNA IQ RESIN Solution)

Lysis-DTT Buffer		Volume (in mL)
Lysis Buffer	$(N \times 1.35) + 0.75$	
DTT (1M)	Lysis Buffer/100	
Extraction Buffer		
TNE Buffer	$N \times 0.56$	
Prot K (20 mg/L)	$N \times 0.03$	
SDS (20 %)	$N \times 0.015$	
DNA IQ RESIN Solution		
LYSIS Buffer	$0.054 \times (N + 16)$	
DNA IQ RESIN	$0.009 \times (N + 16)$	
DNA IQ 1X Wash Buffer	$N \times 0.36$	
DNA IQ Elution Buffer	$N \times 0.144$	

N = number of samples on batch including positive and negative controls, round up to nearest multiple of 8)

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18.2 Reagent & Batch details recording tables (DNA IQ™ Lysis Batch & Extraction Batch)

Off Deck Lysis Batch ID:	DNA IQ Extraction Batch ID:

Lysis batch:

Samples located by:	
For samples 1-48	For samples 49-96
Operator:	Operator:
Sequence check performed by:	Sequence check performed by:
Transfer tubes sequence checked:	Transfer tubes sequence checked:

Extraction Buffer made by:	TNE Buffer Lot#:
20% SDS Lot#:	Proteinase K Lot#:
Comments:	

Extraction batch:

Plate Lot#:	Lysate/STORstar Operators:
Nunc tube/STORstar Operators:	Lysate Logfile uploaded:
Nunc Logfile uploaded:	
Comments:	

MultiPROBE Platform:	Operator:
Date and Start time:	

Kit Lot#:	1xWash Buffer Lot#:
Lysis Buffer Lot#:	DTT Lot#:
Resin Lot#:	Elution Buffer Lot#:
MP II Logfile uploaded:	Results file uploaded:
Comments:	

18.3 Fully automated method for extraction using DNA IQ™

18.3.1 Sampling and Sample Preparation

FTA® Samples waiting for extraction will have been punched into a Slicprep™ 96 device according to “FTA® Processing” SOP (QIS document 24823) and stored in the Fridge located in room 6127.

18.3.2 Procedure

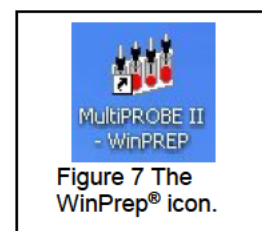
Preparation of Reagents prior to extraction

1. Defrost Prot K and DTT
2. Refer to table 2 for reagent volumes to make up the required amount of Extraction Buffer, Lysis Buffer (with DTT) and Resin solution. Also measure the required amount of 1X Wash Buffer.
3. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.

Setting up the EP-A or EP-B MPIIs

These steps are to be carried out in the Automated extraction Room (Room 6127)

4. Turn on the instrument PC.
5. Log onto the network using the **Robotics** login.
6. Double click the WinPrep® icon on the computer desktop (Figure 7).
7. Log onto the WinPrep® software by entering your username and password, then press “**Enter**”.
8. Ensure the **System Liquid Bottle is FULL** before every run and perform a Flush/Wash.
9. Ensure that the daily/weekly start-up 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](#).
10. 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_Ver1.3.mpt.**”
 - Click the “**Open**” button
11. Check the whole program there are no bold fonts (e.g. plates may lose gripability and the program will not run). See the line manager.
12. Decontaminate the required labware by wiping with 5% TriGene followed by 70% ethanol before placing the labware on to the designated grid positions as outlined in the virtual deck view within WinPrep® (Figure 2).
 - The white WALLAC Isoplates (catalogue #1450-514) that are used to support the SlicPrep™ 96 device plate must be placed into positions **E13**, **D16** and **C19**.



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- Ensure that the PKI Magnet at **F16** is stable in its insert. This is critical so that beads and DNA are not lost during pipetting.

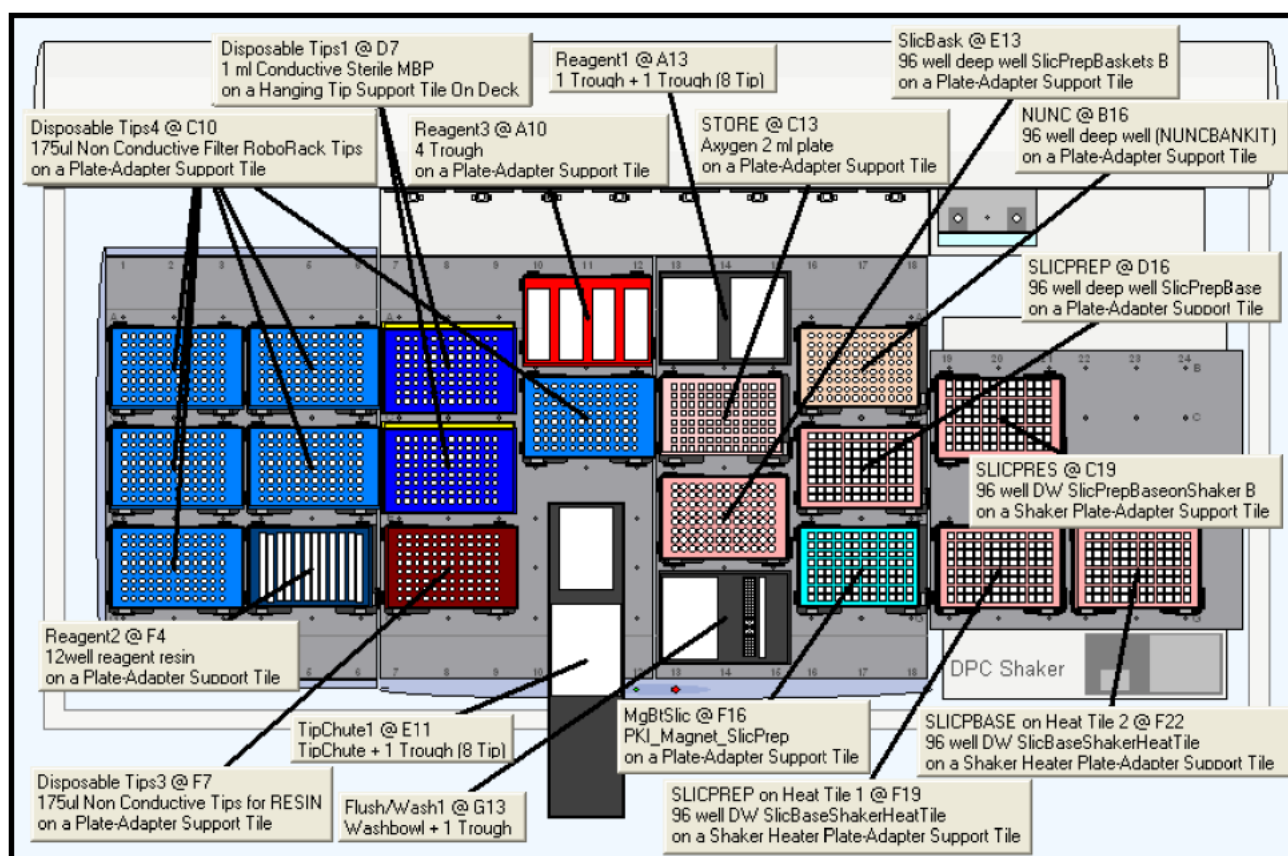


Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC Shaker and Heater Controller Box are switched on.
For EP-A: Tile 3 should be at F19 (50°C), Tile 1 at F22 (85°C).
For EP-B: Tile 1 should be at F19 (50°C), Tile 2 at F22 (85°C).
Note: Press the start/stop button twice at the front of the DPC Shaker to ensure that it displays zero on the screen.
14. To the Amphyl wash station at **A10**, add fresh 1% Amphyl to the trough on the far left hand side, 0.2% diluted Amphyl to the middle left and place an empty reagent tough in the middle right position of the station. The nanopure water goes to position **G13** into a 160mL trough in the Flush-Wash station.
15. Pour the required amounts of Extraction Buffer and Lysis Buffer into the labelled 150mL reagent troughs. Place Lysis Buffer on the left hand side and the Extraction Buffer on the right hand side of the 2 trough holder located in position **A13**.
Note: Ensure that full PPE is worn, including face shield when handling these reagents
16. **Nunc tube rack:** Check that is the same Auslab batch number as the platemap to be run. Ensure that the barcode of the first (A1) and last tube (H12) are the respective ones. Attach batch label and batch barcode label to front side of rack. Add B1-Lite generated “**NUNC**” barcode to the right side of the nunc tube rack. Then place nunc rack into position **B16**

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17. On an Axygen 2ml deep well Storage plate, label the left side of the plate with both the Batch ID and barcode. Label the right side of the plate with a B1-Lite generated "STORE" barcode. Then place in position **C13**.
18. **Slicprep™ 96 device**: Gently remove septa mat from Slicprep™ 96 device and check that substrates are at the bottom of the Spin baskets, if not push them down with a sterile disposable tip and place the Slicprep™ 96 device into position **E13**.
19. In I drive from Extraction folder open the required plate map. Check that the plate map is complete. 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, then save the changes. Copy the plate map to the following file path: **C:\PACKARD\EXT PLATE MAPS**
20. After ensuring that all the necessary labware has been positioned in the correct grid position as displayed within WinPrep®, click the "EXECUTE TEST" button. While the test is loading, record all run information in the Run Log book.
21. 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, followed by clicking "Next"
22. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the platemap selected matches the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16**. Once this has been done, click "Start", to continue.
23. After the barcodes have been read, a user prompt will appear as a reminder to:
 - "Ensure
 - 1. Shaker and heat box are on.**
 - 2. Deck has been populated correctly.**
 - 3. The Lysis Buffer is on the left side and Extraction Buffer is on the right at A13.**
 Click "OK" to continue.
24. Once the Extraction Buffer has been added to the plate, a message will appear waiting for the heating tile to reach 50°C (real temp 37°C). When current temperature reaches 50°C click "Continue".
25. The next prompt that appears will request the following:
 - "Cover Slicprep with the Aluminium sealing film, then place in position **F19**.
 - Press "OK."
26. After shaking, a User Prompt will appear with the following directions:
 - "Remove plate, add white plastic collar and centrifuge 5mins at 3021rpm, then in the cabinet, remove the spin basket part and place it in the empty 1 ml tip container."
 Place the Slicprep™ 96 device into the plate centrifuge and ensure the correct balance plate is used. Once the plate has been centrifuged, carry the plate to the hood and remove the basket of the Slicprep™ 96 device, storing the basket in an empty 1mL tip box, discard the Collar. Complete the step by clicking "OK".
27. Add the Resin to the 12 Channel plate. Pipette mix thoroughly. Then dispense 50uL of resin into each well of the Slicprep™ 96 device.
28. Place the 12 channel plate into position **F4** then add the Elution Buffer to the plate by splitting the amount of Elution Buffer in half between channels 11 and 12.

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29. Place the Wash Buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10**)
30. The next User prompt will appear with the following directions:
**“Ensure Wash Buffer has been added to trough 4 at A10.
 Manually add 50uL resin to each well of the SlicPrep plate
 Place the plate in position D16.
 Add the Elution Buffer to the 12 channel plate.
 THEN
 Press OK when ready.”** Press **“OK”** when steps 27-29 have been performed.
31. Once the lysis and wash steps have been completed, a message will appear waiting for the heating tile to reach 85⁰C (real temp 65⁰C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85⁰C.
32. After the first elution step when the plate is moved to the PKI Magnet, a User message will appear requesting:
**“Check Nunc tubes are uncapped at position B16
 Push down the Slicprep on the PKI Magnet then press OK.”**
Note: Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet.
33. After the second elution step, the above prompt will appear again. **Note:** Allow the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet
34. Once the program is completed, a final User Message prompt appears asking to:
**“Remove all the plates starting with the Slicprep plate, place the Spin Basket into the Slicprep plate.
 Cover the Storage plate with the aluminium sealing film.”**
Recap the NUNC tubes
 Once all plates are removed from the deck and sealed, place into small clipseal plastic bags. Click **“OK”** to proceed to the Amphyl wash step to decontaminate the system tubing.

Finalising the MP II run

35. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
36. Remove Lysis Buffer with DTT (wear face shield) and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and dispose in the brown Winchester bottle located in the fume hood.
37. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H₂O upon completion of the run.
38. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
39. Remove the tip chute and funnel, rinse with warm tap water to help loosen and remove any residue inside the chute before cleaning with 5% TriGene and 70% ethanol. Hang the chute to dry on the rack beside the sink. If running another extraction straight after, replace the tip chute with another spare, clean chute.
40. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the **“Completed Extractions”** folder.

Recording Reagent Details and other information in AUSLAB

41. To record reagent lot numbers, log into the **AUSLAB Main Menu**.
42. Select **5.Workflow Management**.
43. Select **2. DNA Batch Details**.
44. Scan in the Extraction Batch ID.
45. Press **[F6] Reagents**.
46. Press **[SF8] Audit**.
47. Press **[F5] Insert Audit Entry**, enter the lot number details, operator name and Extraction platform the batch was run on and press **[Enter]**.

Importing the MP II log file into AUSLAB

48. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
49. 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)"
50. 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**". (refer to Figure 9. below)

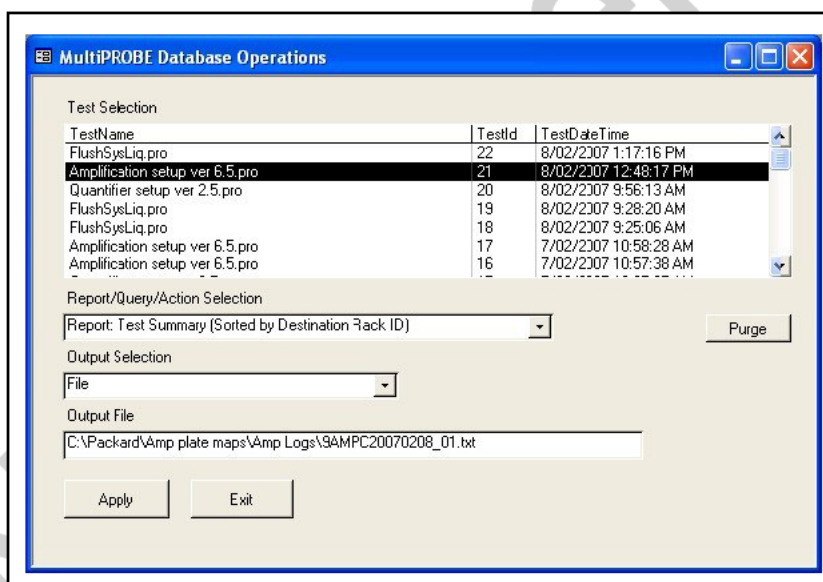


Figure 9. The MultiPROBE log database for collecting MP II run information

51. 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.
52. Copy the log file to **I:\EXTRACTION\EXT A MPII\Logs** or **I:\EXTRACTION\EXT B MPII\Logs** for uploading to AUSLAB.
53. Log into the **AUSLAB Main Menu**.
54. Select **5.Workflow Management**.
55. Select **2. DNA Batch Details**.
56. Scan in the Extraction Batch ID barcode.
57. Press **[SF6] Files**.
58. Press **[SF6] Import Files**.
59. AUSLAB prompts "**Enter filename**"; enter the filename and extension and press **[Enter]**. (e.g. **I:\EXTRACTION\EXT A MPII\Logs\CWIQEXT20071115_01.csv**)
60. AUSLAB prompts "**Is this a result file Y/N?**" enter **N** and press **[Enter]**.
61. Press **[Esc]**.

Importing Extraction “Results” into AUSLAB

62. Log into the **AUSLAB Main Menu**.
63. Select **5. Workflow Management**.
64. Select **2. DNA Batch Details**.
65. Scan the Extraction batch ID barcode located on the worksheet.
66. Press **[SF6] Files**.
67. Press **[SF6] Import Files**.
68. AUSLAB prompts “**Enter filename**”; enter batch name and extension and press **[Enter]**. (e.g. CWIQEXT20071115_01.txt)
69. AUSLAB prompts “**Is this a results file y/n?**” enter “**y**” and press **[Enter]**.
70. The file will be imported into AUSLAB and appear in the DNA file table.
71. Highlight entry and press **[Enter]**, for access to the DNA results table.
72. Page down through the table and check that all sample results have been imported.
73. Press **[SF8] Table Sort Order**, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
74. For all samples that have failed check the **Processing Comments**, by entering into the sample.
75. **a)** If processing comments state sample is to be sent to another batch type **other** than quant. Return the sample to the correct next batch type – e.g. microcon, nucleospin and pooling
 - b)** Press **[Esc]** to exit back to the DNA results table.
 - c)** Do not toggle accept.
76. **a)** If processing comment does not state next step for sample the sample will be processed as normal.
 - b)** Press **[Esc]** to exit back to the DNA results table.
 - c)** Highlight any entries to be changed and press **[SF7] Toggle Accept**
77. 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.
78. File the Extraction worksheet into the relevant folder in Room 6117.

18.3.3 Sample Storage

Please refer to “Analytical Sample Storage” (QIS 24255) for how to store the old original 5 mL sample tubes, the DNA extract NUNC tubes, Slicprep with Basket and Axygen store plates.

18.4 Manual method for extraction using DNA IQ™

18.4.1 Sampling and Sample Preparation

Samples waiting to be extracted are stored in freezers as described in Table 9.

Table 9. Sample storage locations.

Sample type	Storage Device	Storage Location
Urgent/High/Medium Priority Samples	Freezer	6117-2
Medium Priority Samples	Walk in Freezer	6109
Low Priority Samples	N/A	

QC samples

All extraction batches require two controls to be registered. These controls, UR numbers and positions are listed below in Table 10.

Table 10. Extraction Quality Controls

QC	UR Number	Extraction types
Neg Control		All
QC swab (blood)		Blood

1. Log into the **AUSLAB Main Menu**.
2. Select **1. Request Registration**.
3. Select **2. Full Reception Entry**.
4. Scan in barcode of control.
5. Enter the UR number as per Table 4 and press **[Enter]**.
6. Enter the appropriate Specimen type (e.g. Blood for blood extraction).
7. Request a **9PLEX** test, when prompted to enter the processing comment, enter **EXTP** (Positive extraction control) or **EXTN** (Negative extraction control).
8. Press **[F7]** Enter **LAB** in the Billing code field.
9. Press **[F4]** **Save** to save the Billing details.
10. Press **[F4]** **Save** to save the registration details.

N.B Quality controls should not have a DNA priority allocated at time of registration to ensure they are included in the top positions of a batch

Create the Extraction Batch

14. Log into the **AUSLAB Main Menu**.
15. Select **5. Workflow management**.
16. Select **1. DNA workflow table**.
17. Highlight the appropriate Extraction batch type and press **[F5]** **Batch Allocation**.
18. Press **[F6]** **Create batch**.
19. Press **[F8]** **Print menu**.
20. Press **[F6]** **Print Batch label**. (for the deep well plate)
21. Press **[F7]** **Print Sample labels**. (print four sets of labels for all extractions)
22. Press **[F8]** **Print Worksheet**.
23. Press **[SF5]** **Main menu**.
24. Press **[SF11]** **Print**.
25. Press **[SF6]** **Accept batch**.
26. Press **[Pause/Break]** to exit to the **Main Menu**.
27. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table/Batch details table print out. The columns **Rack** and **Pos** respectively identify the rack and the grid location where the sample has been stored. Remove the samples from the storage rack and place in an orange rack (12x8).

When all samples have been located:

1. Log into the **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **7. Search Sample storage**.
4. Scan in the sample barcode that is affixed to the sample tube.
5. Press **[F6] Remove Sample**.
6. AUSLAB prompts "**Are you sure you want to remove XXXX-XXXX? (Y/N)**", Enter **Y** and press **[Enter]**.
7. AUSLAB prompts "**Please enter remove comment**", No comment is required. Press **[Enter]**.
8. Press **[Scroll lock]** to clear.
9. Repeat steps 5 - 8 until all of the samples have been removed from their rack.

Sequence Check the tubes

1. Thaw samples at room temperature and label 1.5mL sample tubes.
2. Sequence check the tubes.
3. Add the sequence check details into AUSLAB.
4. Log into **AUSLAB Main Menu**.
5. Select **5. Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the appropriate extraction batch ID barcode.
8. Press **[F5] Sequence Check**.
9. Scan in the appropriate extraction batch ID barcode.
10. Press **[Pause/Break]** to exit to **Main Menu**.

18.4.2 Procedure

1. Enter the number of samples to be extracted (including controls) into the "No of Samples" column of the DNA IQ Reagent Calculations Table to calculate the volumes of each reagent to be measured out for the extraction. Aliquot reagents into either 5ml tubes or 50ml Falcon tubes. **Note:** The volume of Lysis Buffer calculated includes the volume used in the resin-lysis solution
2. Turn on the Eppendorf Thermo mixer and set the temperature to 37°C.
3. Remove 1.5ml tube and retain the 5mL tube. Prepare the Spin baskets by placing a DNA IQ™ Spin basket into a 1.5mL Microtube. Label the spin baskets (for every tube except Ext. control), 2mL SSI tubes and Nunc storage tubes (for every sample) with the sample barcodes. Have a second operator perform a sequence check of all tubes. This person must Press **[F5] Sequence Check** against the batch in AUSLAB
4. Using the Reagents table, prepare Extraction Buffer, Lysis Buffer with DTT, & Resin Solution. Reagents need to be prepared fresh before each run.
5. Add 300 µL of Extraction Buffer to each tube. Vortex each tube before incubating the tubes at 37°C on the Thermomixer at 1000 rpm for 45 minutes.

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6. Remove the tubes from the Thermo mixer and place into a rack, increase the temperature on the Thermomixer to 65°C (for use in the Elution steps).
7. Transfer the substrate from the original tube to a DNA IQ™ Spin Basket using autoclaved twirling sticks. Centrifuge the Spin basket for 2 minutes at room temperature at its maximum speed. Once completed, remove the spin basket & retain in the original 5ml tube. Ensuring minimal contamination and transfer the extract to a labelled 2mL SSI sterile screw tube.
8. Transfer the remaining extract from the original tube to the corresponding 2mL tube. Vortex the tube gently.
9. Add 500 µL of Lysis Buffer to each tube.
10. Into a separate, clean 2mL SSI tube, aliquot the required amount of Lysis Buffer for the Resin solution. Ensure that the DNA IQ™ Resin solution has been thoroughly mixed by vortexing the resin bottle before adding the required resin volume to the Lysis Buffer. Pipette mix the solution to clear the tip of any lingering resin beads. Mix the solution by inverting the tube to prevent bubbles forming.
11. Add 50µL of DNA IQ™ Resin-Lysis solution into each tube. Invert the resin-lysis tube at regular intervals to keep the resin suspended within the solution to ensure uniform results.
12. Vortex each tube for 3 seconds at high speed before placing the tubes in the Multitubeshaker set at 1200 rpm to incubate at room temperature for 5 minutes.
13. Remove from the Multitubeshaker and vortex the tubes 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.

14. Carefully remove all of the solution in the tube into the original 1.5mL tube (i.e. the tube which originally contained the substrate), ensuring that the resin is not disturbed from its place on the side of the tube.

Note: If some resin is drawn up in tip, gently expel resin back into tube to allow re-separation.

15. Add 125µL of prepared Lysis Buffer solution and vortex for 2 seconds at high speed. Return the tubes to the magnetic stand and allow for separation to occur. Once separation has occurred again remove the Lysis Buffer into the original 1.5mL tube (i.e. the tube which originally contained the substrate).
16. Add 100µL of prepared 1X Wash Buffer and vortex for 2 seconds at high speed. Return tube to the magnetic stand and once separation has occurred remove and discard all Wash Buffer.
17. Repeat Step 16 another two times for a total of three washes. Ensure that all of the solution has been removed after the last wash.
18. Wipe down a Biohazard hood with bleach followed by ethanol. Uncap the tubes, placing the lids inside down onto a clean rediwipe in consecutive order and place the tubes in the same order into a clean plastic rack. Air-dry the resin in the hood for 15

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minutes at Room temperature. Do not dry for more than 20 minutes, as this may inhibit removal of DNA during the elution phase.

19. Once the resin is dry, replace the lids on the corresponding tubes and remove from the hood. Add 50µl of Elution Buffer to each of the samples by carefully pipetting the liquid to the side of the tube, above the pellet. Do not mix.
20. With the lids on, incubate the tubes in the Thermomixer at 65°C for 3 minutes. After the three minutes are up, continue to incubate for a further 3 minutes shaking at 1100 rpm.
21. Remove the tubes and vortex for 2 seconds at high speed. Immediately place the tube in the magnetic stand while hot to ensure maximum DNA yield during elution.
22. Carefully transfer the DNA containing supernatant to the corresponding labelled Nunc tubes.
23. Remove tubes from the magnetic stand and add carefully another 50 µL of Elution Buffer above the magnetic pellet.
24. Repeat step 20 to 22. The final volume after this elution should be approximately of 95 µL of DNA solution.
25. DNA can be stored at 4°C for short-term storage or at -20 or -70°C for long-term storage.

18.4.3 Sample storage

1. Log into **AUSLAB Main Menu**.
2. Select **2. Sample Processing**.
3. Select **6. Sample Storage**.
4. Scan in Rack barcode.
5. Press **[SF5] Fill Rack**.
6. Scan in sample barcode and place in rack in scanned position.
7. Repeat for all samples.
8. Press **[Esc]**.
9. Press **[Pause/Break]** to return to the **Main Menu**.
10. Select **3. Patient Enquiry**.
11. Scan in Rack barcode.
12. Tab down to the next blank **DNA Batch No** field and press **[F2] Edit**.
13. Scan in the Batch ID of the samples stored.
14. Press **[Pause/Break]** to return to the **Main Menu**.