

Attachment 1

From: Vanessa Lentile <[REDACTED]>
Sent on: Wed 24/10/2007 11:42:20 AM +10:00
To:
Subject: Fwd: DNA IQ docs
Attachments: DNA IQ FSS Factsheet.pdf (131.91 KB), 24897R0.doc (497.5 KB)

Good morning everyone

A Go Live date for the Extraction automated platforms has been set and we are ramping up to prepare for this.

The Analytical and Automation teams will start using both platforms for casework extractions from Monday the 29th October. Initially as training both in Analytical and other areas is running, the samples will mainly be some of the backlog samples. I would also expect that we will not reach full capacity on these platforms until the new year.

As you would be aware, only Blood and Cell extractions will be run on the new platforms at this stage and this excludes tapelifts.

I understand there are discussions underway about when Major Crime team will commence sampling in the new size format and I would expect this to be in place within the next few weeks.

One of the other areas for us to address is to ensure that all staff have an understanding of the new protocol and the underpinning knowledge of the DNA IQ system. This is especially critical for all reporting scientists.

Attached is the SOP now active in QIS and a Fact Sheet prepared by Iman. Please read both of these and ensure you are familiar with them. A training module has also been developed and is currently being put into the new format. Once this is done it will be activated in QIS.

I expect that at least all reporting scientists will complete the Part B competency and we'll need to set some timeframes about how this will be able to be achieved.

The other action that will be scheduled is a briefing session provided by the Automation team where we will go through in detail the validation report. This will include what was done in the evaluation phase, validation of the manual process, the verification of the automated process and all work done to ensure no cross contamination occurs on the platform.

At this stage, I would like attendance at this workshop to be mandatory for all reporting scientists but of course would like it if as many people as possible attended.

The last thing I wanted to mention was to clarify the use of the 2 platforms designated "A" and "B". At this stage only casework extractions are going live, FTA processing will remain as is for the moment. Both instruments will be utilised for casework.

While casework and reference batches will continue to be extracted separately, I would expect that both platforms at times will be used to do either. This is due to the workload of outstanding casework samples requiring extraction and also in case of any instrument downtime.

The automation team has done an enormous amount of work to get us to this stage and the implementation of these platforms is a major milestone, so I would appreciate everyone getting on board and assisting to make this transition successful. That being said, if anyone has any questions they don't feel are being answered or if there are any suggestions on how to improve the implementation process, I would be more than happy to listen to them and act.

Regards

Vanessa

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DNA IQ™ System from Promega

Introduction

The DNA IQ™ System is a DNA extraction system designed specifically for isolating trace DNA samples as encountered by the forensic community. Although Chelex® extraction is less time consuming, it does a poor job of removing many types of inhibitors (Figure 1). The DNA IQ™ system is designed around a novel magnetic particle capture technique that accommodates DNA binding and removal of inhibitors and impurities in solution. DNA IQ™ can be used to extract DNA from a variety of sample types, including stains and liquid samples (Promega, 2002). The system is designed to increase in efficiency as the sample size decreases, therefore it is most effective with samples containing minimal starting material (Mandrekar *et al.*, 2001). Unlike the Chelex® method, DNA IQ™ is amenable to automation.



Figure 1. A blood sample extracted using DNA IQ™ (left) and Chelex® (right). Not all heme inhibition was removed by the Chelex® method. http://www.promega.com/applications/fnmrid/product_profiles/dnaiq

Basic principle

Cell lysis is performed with **Lysis Buffer** containing DTT and chaotropic salts, promoting binding of DNA to the paramagnetic resin. Applying a magnetic force concentrates the resin to the bottom of the well, allowing removal of inhibitors via washing steps without the loss of sample DNA. **Wash Buffer**, containing an alcohol/aqueous mixture, is used to wash the DNA multiple times and ensures the DNA is not eluted by keeping the DNA dehydrated. After washing, adding **Elution Buffer** and heating the complex to 65°C allows DNA to be released from the paramagnetic resin. The purified DNA can then be used directly in downstream PCR applications. For the detailed in-house protocol, refer to QIS 24897.

Automating DNA IQ™

The automated Forensic Biology DNA IQ™ protocol differs slightly from the conventional manual method:

- ✚ Use of an Extraction Buffer containing Pro K that allows increased yields.
- ✚ Lower lysis incubation temperature to cope with heat-labile fabrics.
- ✚ Use of the SlicPrep™ 96 Device for simultaneous extraction of 96 samples.

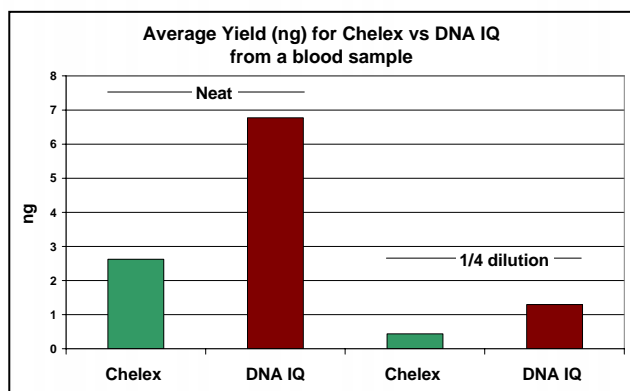


Figure 2. Average yields were higher for the in-house DNA IQ™ protocol compared to the current Chelex® method.

The in-house DNA IQ™ protocol generates higher yields compared to the current Chelex® protocol (Figure 2).

The crude Chelex® method does not gain any benefits from automation as the DNA is not immobilised and is free in solution. Because of this, the DNA solution cannot be “washed” and hence inhibitors are retained in the final extract.

The DNA IQ™ method is superior compared to Chelex® because of its ability to yield better quality DNA. Other advantages of DNA IQ™ over Chelex® are highlighted in Table 1 below.

Table 1. Characteristics of the Chelex® and DNA IQ™ protocols.

	Chelex®	DNA IQ™
Able to wash and remove PCR inhibitors	x	✓
Minimal sample transfers	✓	✓
Resulting extract is purified DNA	x	✓
Consistent yield	x	✓
Isolates all DNA present in a sample	✓	✓
Isolates mitochondrial DNA	✓	✓
DNA can be used directly in PCR	✓	✓
Residual resin in extract inhibits PCR	✓	x
Use system as clean-up procedure	x	✓
Substrate material can be easily stored	x	✓
Amenable to automation	x	✓

DNA IQ™ compared to other kits

In addition to DNA IQ™, Forensic Biology performed an evaluation of other DNA extraction kits, including QIAamp® Micro (Qiagen), ChargeSwitch® (Invitrogen), NucleoSpin® 8 Trace (Macherey-Nagel), and forensicGEM™ (Zygem). The criteria for which the different kits were assessed on included:

- ✚ Ability to remove inhibitors;
- ✚ Validated MultiPROBE® II automated protocols;
- ✚ Total DNA yield;
- ✚ Quality of the resulting DNA profiles.

Although some kits satisfied several of these criteria, the DNA IQ™ system satisfied all of them and also resulted in the most number of full profiles using Profiler Plus®.

What is changing in the laboratory?

With implementation of the automated DNA IQ™ system in Forensic Biology, several minor changes are required to our workflow processes:

- ✚ Size of sample required for extraction has been reduced (refer to Sample Size Chart);
- ✚ All tapelifts should have the specimen type “Nucleospin Cells”, not “Cells”;
- ✚ Continue to register samples with specimen type “Blood” or “Cells” and “REF Blood” or “REF cells”;
- ✚ New batch types for DNA IQ™ extractions:
 - **CW IQEXT** (CW DNA IQ extraction)
 - **RF IQEXT** (Reference DNA IQ EXT)
- All samples extracted with Chelex® will only have an audit entry referring to **BLDEXT**, **CELEXT**, **RBEXT**, **RCEXT**;
- ✚ Batch sizes increased from either 24 or 36 samples to a total of 96 samples;
- ✚ STORstar® is being used to sort and sequence check samples into the SlicPrep™ 96 Device;
- ✚ All DNA IQ™ substrates, lysates and paramagnetic resin will be stored for a short period of time following the extraction procedure.

References

- Mandrekar PV, Krenke BE, Tereba A (2001). DNA IQ™: The Intelligent Way to Purify DNA. *Profiles in DNA* July 2001. Madison, WI: Promega Corp.
- Promega (2002). DNA IQ™ System – Small Sample Casework Protocol, PN TB296. Madison, WI: Promega Corp.
- QIS 24897. Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates.

Automated DNA IQ™ Method of Extracting DNA from Blood and Cell Substrates

1 PURPOSE AND SCOPE

This method describes the automated DNA extraction of cell and blood samples on the PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms using the Promega DNA IQ™ system.

This method applies to all Forensic Biology staff that is required to extract cell and blood 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 should be decontaminated between operations.

2 DEFINITIONS

Samples	Samples awaiting DNA extraction
DNA Extracts	Samples that had DNA extraction processes performed
EB	Extraction Buffer Solution that lyses cells and breaks down proteins
LB	Promega DNA IQ™ Lysis Buffer Solution
WB	Promega DNA IQ™ Wash Buffer
DNA IQ™ Resin	Magnetic Resin Beads used to bind DNA
MP II	MultiPROBE® II 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 – back wall platform
EP-B	Extraction Platform B – side wall platform

3 PRINCIPLE

Sample Pre-lysis

The Extraction Buffer (EB) 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 Forensic Biology FSS. These are:

- The use of the Slicprep™ 96 device (Promega) for removing substrate from lysate.
- The increase of extraction buffer volume to 500 L for use with the Slicprep™ 96 device.
- The increase of Lysis Buffer volume to 957 L proportional to the increase of Extraction Buffer volume, according to the manufacturer's instructions.
- Double Elution step, with an Elution buffer volume of 60 L for a final volume of 100 L.
- 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 silica bead resin which contains novel paramagnetic particles. The silica 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 silica beads 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 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 resin and washes out inhibitors. The next three washing procedures are with a 1xWash 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 beads. 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 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 Forensic Biology, blood and cell extractions are performed using 2 MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms – one primarily for Reference samples (Extraction Platform A, EP-A) and the other mainly for Casework samples (Extraction Platform B, EP-B).

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 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. Decon® 90 solution
11. Nanopure H₂O



Table 1. Reagent storage locations.

Reagent	Device	Storage Location
Pro K	Freezer	Room 6122
DTT	Freezer	Room 6122
20% SDS	Shelf	Room 6127 Shelf 7
Isopropyl alcohol	Shelf	Room 6127 Shelf 7
TNE pH 8 Buffer	Shelf	Room 6127 Shelf 7
DNA IQ™ Kit	Shelf	Room 6127 Shelf 5
Amphyl (1% and 0.2%)	Shelf	Room 6127 Shelf 7
Nanopure Water	Shelf	Room 6127 Shelf 7
5% TriGene	Shelf	Room 6127 Shelf 7
70% ethanol	Shelf	Room 6127 Shelf 7

Please see Table 2 for the volume of reagents for a full plate or half plate. See QIS 17165 (Receipt, Storage and Preparation of Chemicals, Reagents and Kits) for preparation of the TNE buffer. All reagents, except for the Lysis Buffer with DTT, can be made on the bench in Room 6122. DNA IQ reagents are prepared by staff performing the DNA IQ method.

Table 2. Table of reagent volumes.

		Volume for	
		96 samples	48 samples
Extraction Buffer (500 µL/sample)	TNE buffer 462.5µL	54mL	27mL
	Prot K (20 mg/mL) 25.0 µL	2.9mL	1.5mL
	SDS (20 %) 12.5µL	1.5mL	0.7mL
Lysis buffer (with DTT) (1.127mL/sample)	Lysis buffer (no DTT)	130mL	66mL
	DTT (add to Lysis buffer)	1.3mL	660µL
Lysis Buffer (with DTT) Reagent Trough	From above	125mL	63mL
DNA IQ RESIN Sol (50µL/sample)	Lysis buffer (with DTT) (from above) 43µL	6mL	3mL
	DNA IQ RESIN 7µL	1mL	0.5mL
DNA IQ 1X Wash B (300µl/sample)		35mL	18mL
DNA IQ Elution B (120µl/sample)		14mL	8mL

Extraction Buffer

Prepare the buffer just before extraction in a 100mL sterile glass bottle according to the table 2. Use one aliquot of 20mg/mL Proteinase K (1.5mL) for a 48 sample run and two aliquots of Proteinase K 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 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.

4.2 Equipment

Table 3. Equipment used and location.

Equipment	Asset No.	Location
STORstar (B system)	10238493	6122
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext A Platform)	10076438	6127
MultiPROBE® II PLUS HT EX with Gripper™ Integration Platform (Ext B Platform)	10076437	6127
DPC Shaker (Ext A Platform)	N/A	6127
DPC Shaker (Ext B Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext A Platform)	N/A	6127
Automated Temperature Controller, Heat Block tiles and heat block adapters (Ext B Platform)	N/A	6127
Eppendorf 5804 centrifuge	10238416	6127
Vortex	30087015	6127
Fridge	30433424	6127
Micro centrifuge	None	6127

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 disulphide 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. 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 4.

Table 4. 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 5.

Table 5. Extraction Quality Controls

QC Name	UR Number	Description
Negative Control	FBOT33	Negative Extraction control – Empty well
Positive Control	FBOT35	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 4 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).
8. 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

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow management**.
3. Select **1. DNA workflow table**.
4. Highlight the appropriate Extraction batch type and press **[F5] Batch Allocation**.
5. Press **[F6] Create batch**.
6. Press **[F8] Print menu**.
7. Press **[F6] Print Batch label**.
8. Press **[F7] Print Plate Label**. (print 3 sets)
9. Press **[F8] Print Worksheet**.
10. Press **[SF5] Main menu**.
11. Press **[SF11] Print**.
12. Press **[SF6] Accept batch**.
13. Press **[Pause/Break]** to exit to the **Main Menu**.
14. Obtain worksheets (FBLASER3) and labels (FBLABEL13-16) from the Analytical Section printing bench (Room 6117).
15. Collect 1 NUNC and 1 STORE labware label from the roll of labels already printed in 6127.

Locating Samples

To locate samples refer to “*Analytical Sample Storage*” (QIS 24255).

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

To sequence check sample substrates and storage tubes please refer to method “*Procedure for the Use of the STORstar unit for automated sequence checking*” (QIS 24256).

7 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.



Competent Analytical Section staff members perform all the following steps.

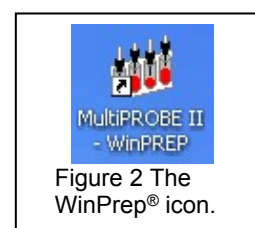
Test Outline	
Initial User Query (x 1)	51. Flush/Wash_2 (x 1)
1. BarcodeSetup (x 1)	52. ShakerOnWash2 (x 1)
2. ReadBarcode (x 1)	53. Shake 1 minute Wash2 (x 1)
3. User Message - Hardware setup (x 1)	54. StopShakerWash2 (x 1)
4. Initial Flush/Wash_1 (x 1)	55. Flush/WashWash2 (x 1)
5. OpenComm to Shaker (x 1)	56. Move Plate SlicPrep to PKI MagnetWash2 (x 1)
6. Set Heater Temperature at 37 C (x 1)	57. Bind 1 minute_Wash2 (x 1)
7. Set Heater Temperature at 65C (x 1)	58. Remove wash buffer 2 (x File: Records)
8. Add 500 ul Extraction Buffer to SlicBask (x File: Records)	59. Move SlicPrep from PKI Magnet to Shaker 4 (x 1)
9. Wait for 37 Temperature (x 1)	60. Add wash buffer 3 (x File: Records)
10. Seal plate (x 1)	61. Flush/Wash_3 (x 1)
11. ShakerOn_1 (x 1)	62. ShakerOnWash3 (x 1)
12. Incubate 45 min on heater/shaker_1 (x 1)	63. Shake 1 minute Wash3 (x 1)
13. StopShaker_1 (x 1)	64. StopShakerWash3 (x 1)
14. Centrifuge (x 1)	65. Flush/WashWash3 (x 1)
15. Place SlicPrep D16 (x 1)	66. Move Plate SlicPrep to PKI MagnetWash3 (x 1)
16. Flush/Wash_1 (x 1)	67. Bind 1 minute_Wash3 (x 1)
17. Add Resin 50ul (x File: Records)	68. Remove wash buffer 3 (x File: Records)
18. Flush/Wash_3 (x 1)	69. Dry 5 minutes (x 1)
19. Add DNA IQ Lysis Buffer (957 ul) to SlicPrep at D16 (x File: Records)	70. Flush/Wash_4 (x 1)
20. Flush/Wash_1 (x 1)	71. Wait for 65 Temperature_1 (x 1)
21. Move Plate_1 (x 1)	72. Add Elution Buffer (60ul) Elut1 (x File: Records)
22. ShakerOn_2 (x 1)	73. Move SlicPrep from PKI Magnet to Tile2 on Shaker_1 (x 1)
23. Time 5 min_1 (x 1)	74. 3 minutes Timer_1 (x 1)
24. StopShaker_2 (x 1)	75. ShakerOnElut1 (x 1)
25. Move SlicPrep to PKI Magnet (x 1)	76. Shake 3 minute Elu1 (x 1)
26. Time 1 min - Wait to Bind Resin_1 (x 1)	77. StopShakerElu1 (x 1)
27. Remove 1600ul to AxSuper (x File: Records)	78. Move SlicPrep from Tile2 to PKI Magnet_1 (x 1)
28. Flush/Wash_3 (x 1)	79. Push Down SlicPrep Elut1 (x 1)
29. Move SlicPrep to shaker (x 1)	80. Bind 1 minute Elut1 (x 1)
30. Dispense Lysis Buffer (125 ul) (x File: Records)	81. Transfer Eluted DNA_Elut1 (x File: Records)
31. Flush/Wash_4 (x 1)	82. Flush/Wash_Elut1 (x 1)
32. ShakerOn_3 (x 1)	83. Add Elution Buffer (60ul) Elut2 (x File: Records)
33. Timer_1 (x 1)	84. Move SlicPrep from PKI Magnet to Tile2 on Shaker_2 (x 1)
34. StopShaker_3 (x 1)	85. 3 minutes Timer_2 (x 1)
35. Flush/Wash_1 (x 1)	86. ShakerOnElut2 (x 1)
36. Move SlicPres to PKI Magnet (x 1)	87. Shake 3 minute Elut2 (x 1)
37. Time 1 minute (x 1)	88. StopShakerElu2 (x 1)
38. Remove Lysis Buffer (125 ul) to STORE (x File: Records)	89. Move SlicPrep from Tile2 to PKI Magnet_2 (x 1)
39. Move SlicPrep from PKI Magnet to Shaker 1 (x 1)	90. Push Down SlicPrep Elut2 (x 1)
40. Add wash buffer 1 (x File: Records)	91. Bind 1 minute Elut2 (x 1)
41. Flush/Wash_1 (x 1)	92. Transfer Eluted DNA_Elut2 (x File: Records)
42. ShakerOnWash1 (x 1)	93. Flush/Wash_6 (x 1)
43. Shake 1 minute Wash1 (x 1)	94. Close Heater Comm (x 1)
44. StopShakerWash1 (x 1)	95. Close Shaker Comm (x 1)
45. Flush/WashWash1 (x 1)	96. Remove Nunc tubes (x 1)
46. Move Plate SlicPrep to PKIMagnetWash1 (x 1)	97. AmphyI_concentrate (x 8)
47. Bind 1 minute_Wash1 (x 1)	98. AmphyI_dilute (x 8)
48. Remove wash buffer 1 (x File: Records)	99. Water wash (x 8)
49. Move SlicPrep from PKI Magnet to Shaker 2 (x 1)	100. Flush/Wash_5 (x 2)
50. Add wash buffer 2 (x File: Records)	End of Test

Figure 1. The Test Online of the program DNA IQ Extraction_Ver1.1.

Setting up the EP-A or EP-B MPIIs

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

1. Turn on the instrument PC.
2. Log onto the network using the **Robotics** login
3. Double click the WinPrep® icon on the computer desktop (Figure 1).



4. Log onto the WinPrep® software by entering your username and password, then press **[Enter]**.
5. 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.
6. Ensure the System Liquid Bottle is full before every run and perform a Flush/Wash.
7. 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_Ver1.1.mpt.”**
 Click the **“Open”** button
8. 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**.

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.

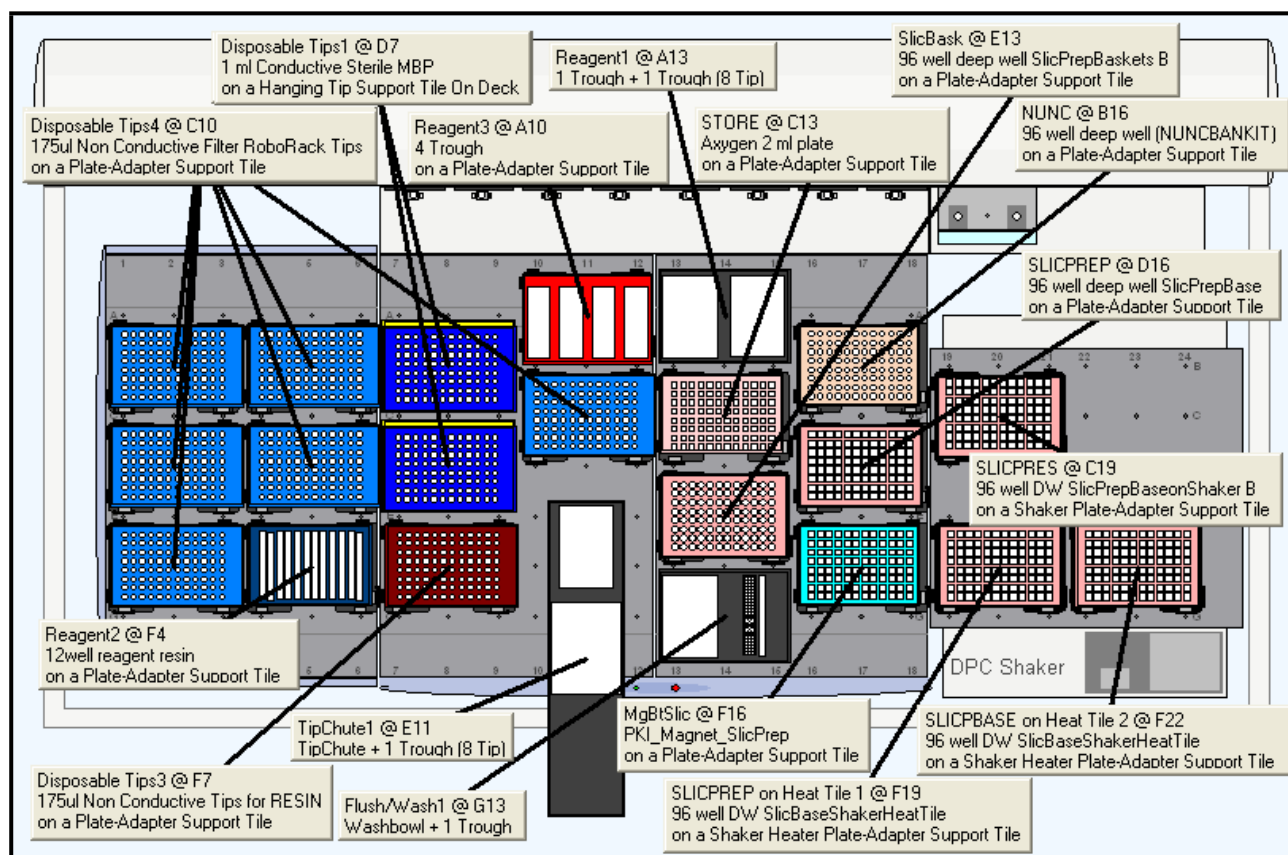


Figure 3. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

9. 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).
10. Referring to the table of reagent volumes (table 2), use the volumes of TNE, SDS and Prot K to make up the required amount of Extraction Buffer. Pour the required amounts of Extraction Buffer and Lysis Buffer (with DTT) into the labelled 150mL reagent troughs, using the reagent volume table as a guide to the volumes. Ensure that full PPE is worn, including face shield when handling these reagents.
 11. Place Lysis Buffer on the left hand side of the 2 trough holder (**A13**) and the Extraction buffer on the right hand side next to the Lysis buffer (**A13** also).
 12. Using the left over Lysis Buffer, make up the Resin Solution according to table 2. Add the resin solution to the fourth channel and split the amount of elution in half between channels 11 and 12 of the 12 channel reagent plate (**F4**). Ensure that the face shield is worn while making up and dispensing the resin.
 13. Record the Lot numbers of all the reagents used onto the AUSLAB worksheet.
 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 trough in the middle right position of the station. The nanopure water needed to complete the Amphyl wash goes to position **G13** into a 160ml trough in the Flush-Wash station. Only fill to the designated level markers.
 15. If not already done, label the Slicprep™ 96 device, with the AUSLAB Batch ID label with the Batch ID label on the front and the barcode on the right hand side of the plate. Place the Slicprep™ 96 device containing the substrates in the support tile position assigned in the program with the Batch label facing forward. For the Nunc plate and Axygen 2ml deep well Storage plate, label the front of the plate with the Batch ID. On the right hand side of the plates, label with corresponding Labware barcode - either the "NUNC" barcode or the "STORE" barcode, depending upon the type of plate. De-cap the Nunc tubes before placing in the support tile on the deck.
 16. Transfer the batch's platemap from the Extraction folder within I:\ drive to the following file path:
C:\PACKARD\EXT PLATE MAPS
 17. 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.
 18. 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**"
 19. Select the correct platemap by browsing to **C:\PACKARD\EXT PLATE MAPS**. Ensure that the plate maps selected match the batch ID affixed to the 96-well Slicprep™ 96 device in position **D16**. Once this has been done, click "**Start**", to continue.
 20. 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.

21. Once the extraction buffer has been added to the plate, a user prompt will appear requesting the following:
“Cover Slicprep with the Aluminium sealing film, then place in position F19. Press OK.”
Once the Slicprep™ 96 device has been covered with an aluminium seal and been placed onto the deck at the correct position, click OK on the user message.
22. 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.”
Following the above steps 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.
23. Once OK has been clicked, another User message (step 15) will appear requesting:
“Place the Slicprep in position D16. Ensure wash buffer has been added. Press OK when ready.”
Place the Wash buffer in the far right hand side of the 4 well reagent trough holder (Amphyl wash station at **A10**).
24. After the first elute where the plate has been heated to 65°C and moved to the PKI Magnet, a User message (step 79) will appear requesting:
“Push down the Slicprep on the PKI Magnet then press OK.”
Allow to the plate to cool slightly before pushing down on all four corners of the plate to lock it into place on the PKI Magnet. Once it is firmly in place, click OK to continue. After the second elute, the prompt will appear again. Repeat the steps.
25. Once the program is completed, a final User Message prompt appears asking to:
“Remove all the plates starting with the NUNC tubes (recap). Place the Spin Basket into the original base. Cover the other plate with the aluminium sealing film.”
Remove and seal the Nunc Bank-It tubes first by recapping the tubes. Seal the 2mL storage plate with aluminium foil seal. Remove the Slicprep™ 96 device from the deck and replace the basket on it, make sure the basket part is fitted in the right position. Click **“OK”** to proceed. The platform will perform an Amphyl wash to decontaminate the system tubing.
26. Once the program has finished, remove the tip chute and 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. While wearing the face shield, remove Lysis buffer with DTT and dispose of left over reagent into a brown Winchester bottle.

Recording Reagent Details in AUSLAB

1. To record reagent lot numbers, log into the **AUSLAB Main Menu**.
2. Select **5.Workflow Management**.
3. Select **2. DNA Batch Details**.
4. Scan in the Extraction Batch ID.
5. Press **[F6] Reagents**.



6. Press **[SF8] Audit**.
7. Press **[F5] Insert Audit Entry**, enter the lot number details and press **[Enter]**.

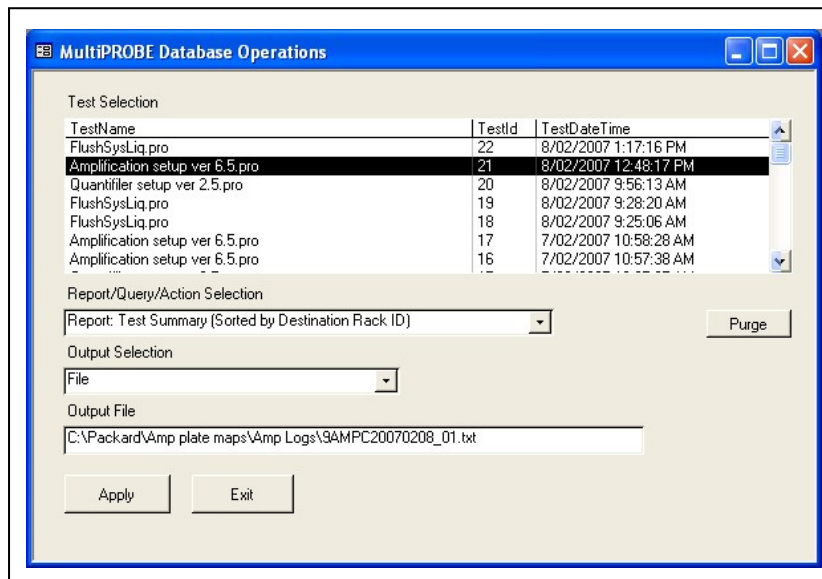
Finalising the MP II run

1. Discard the 12-channel plate used for Resin and Elution Buffer in the biohazard waste bin.
2. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H₂O upon completion of the run.
3. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary.
4. Move the platemap in **C:\PACKARD\EXT PLATE MAPS** to the “Completed Extns” folder.

Importing the MP II log file into AUSLAB

1. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database. 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)” and in the Output Selection dropdown menu, select “File”. Save the output file in *.csv format to **C:\PACKARD\EXT PLATE MAPS\EXT LOGS** with the same name as the AUSLAB batch ID and click **“Apply”**.

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



2. 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.
3. Copy the log file to **I:\EXTRACTION\EXT A MPII\LOGS** or **I:\EXTRACTION\EXT B MPII\LOGS** for uploading to AUSLAB.
4. Log into the **AUSLAB Main Menu**.
5. Select **5.Workflow Management**.
6. Select **2. DNA Batch Details**.
7. Scan in the Extraction Batch ID barcode.
8. Press **[SF6] Files**.
9. Press **[SF6] Import Files**.
10. AUSLAB prompts **“Enter filename”**; enter the filename and extension and press **[Enter]**.
11. AUSLAB prompts **“Is this a result file Y/N?”** enter **N** and press **[Enter]**.
12. Press **[Esc]**.

Importing Extraction “Results” into AUSLAB

1. Log into the **AUSLAB Main Menu**.
2. Select **5. Workflow Management**.
3. Select **2. DNA Batch Details**.
4. Scan the Extraction batch ID barcode located on the worksheet.
5. Press **[SF6] Files**.
6. Press **[SF6] Import Files**.
7. AUSLAB prompts “**Enter filename**”; enter file name and extension and press **[Enter]**.
8. AUSLAB prompts “**Is this a results file y/n?**”; enter “**y**” and press **[Enter]**.
9. The file will be imported into AUSLAB and appear in the DNA file table.
10. Highlight entry and press **[Enter]**, for access to the DNA results table.
11. Page down through the table and check that all sample results have been imported.
12. Press **[SF8] Table Sort Order**, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table.
13. Highlight the first entry that has failed and press **[Enter]**.
14. Confirm the reason for the failure by checking the **Processing Comment**, and return the sample to the correct next batch type dependant upon the type of Processing Comment – e.g. Processing comment of Microcon should see the sample returned to the Microcon outstanding allocations list.
15. Press **[Esc]** to exit back to the DNA results table.
16. Repeat steps **13-15** until all entries that failed Autovalidation have been checked.
17. Highlight any entries to be changed and press **[SF7] Toggle Accept**
18. 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.
19. File the Extraction worksheet into the relevant folder in Room 6117.

8 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.

9 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.

10 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.

11 REFERENCES

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12 STORAGE OF DOCUMENTS

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



13 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 [24469](#) Batch functionality in AUSLAB
 QIS [24256](#) Sequence Checking with the STORstar Instrument
 QIS [24255](#) Analytical Sample Storage

14 AMENDMENT HISTORY

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