* Made JIT Statement re changes from Manufacturer that were made.

FSS.0001.0080.6677

21/05/2008

? what system did I use for my PhD ?

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

- 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-Choalamidopropryl)dimethylammonio] propanesulfonic and < 2% polyethylene glycol tert-octylphenyl ether.

The basis of the DNA IQ™ kit is a magnetic bead resin which contains novel paramagnetic particles covered with silica. The magnetic bead resin usually has a DNA binding capacity of 100ng but the addition of 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

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
	Sarcosyl (40 %) 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	5.536	3
	DNA IQ RESIN 7µL	0.901	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 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 40% (w/v) Sarcosyl 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 ***AnalR*** 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.

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 no 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***.

14. Transfer flow through from spin baskets back to original lysis tube, retain spin basket in 5mL tube.

15. Vortex Lysates briefly, then incubate in hotblock at 65°C for 10 minutes (note temperature on worksheet)

16. Enter reagent details, temperatures etc. into AUSLAB.

17. Complete batch in AUSLAB.

18. Store lysates at 4°C (fridge in 6120).

19. Enter into completed batch in AUSLAB and determine the DNA IQ extraction batch ID the DNA lysates have progressed to.

20. 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, fingernail clippings 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. Vortex, then centrifuge for 3 minutes at maximum speed (14000rpm).

10. Remove 150µL of supernatant and place into labelled 1.5ml tube (for further testing).

11. Add 25µL of 20ng/µL (mg/mL) Proteinase K and 12.5µL 40% (w/v) Sarcosyl to each original sample tube containing TNE Buffer. Vortex briefly.

12. Incubate in hotblock at 37°C for 45 minutes (note temperature on worksheet).

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™ Bank-It™ 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.

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™Bank-It™ tube rack is labelled with the AUSLAB Batch ID and barcode on the right hand side of the plate.

Preparation of Reagents & Lysates 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 worksheet (printed from appendix 2 and in the AUSLAB batch audit entry.

3. Remove the deep well plate containing Lysates from either storage (either freezer or fridge as case may be) to allow to come to room temperature before starting extraction procedure.

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 1).

Figure 1 The WinPrep® icon.

7. Log onto the WinPrep® software by entering your username and password, then press **"Enter"**.

8. Ensure the **System Liquid reservoir is FULL** and tubing is fully submerged in the system liquid before every run and perform a Flush/Wash.

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

14. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 1 at F22 (85^{0}C).
    For EP-B: Tile 2 at F22 (85^{0}C).
    Note: Press the start/stop button twice at the front of the DPC shaker to ensure that it displays zero on the screen.

15. Ensure the heat transfer tile is clicked into the plate adapter tile properly. This is critical to ensure correct incubation temperatures.

16. 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.

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. **Nunc™ Bank-It™ 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™ Bank-It™ tube rack. Then place the rack into position **B16**.

19. 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**.

20. **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)

21. 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.

22. Message will appear (Figure 3 below):

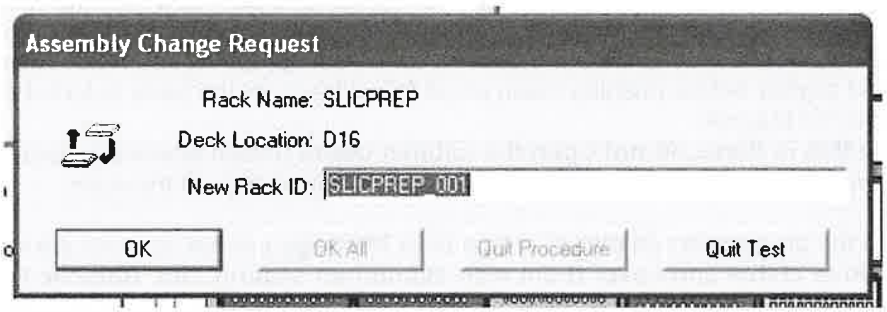

Figure 3. Scan batch ID request

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

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. **Note:** review the supernatant storage plate for the transfer of beads.

Finalising the MP II run

34. 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.

35. 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.

36. Empty the contents of the Amphyl wash station into the sink and rinse the troughs with nanopure H_2O upon completion of the run.

37. Remove all labware from the deck and clean with 5% TriGene™ followed by 70% ethanol, and setup for the next run if necessary. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach and 70% Ethanol.

38. 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.

39. Move the platemap to **C:\PACKARD\EXT PLATE MAPS** to the "**Completed Extractions**" folder.

Recording Reagent Details and other information in AUSLAB

40. To record reagent lot numbers, log into the **AUSLAB Main Menu.**
41. Select **5.Workflow Management.**
42. Select **2. DNA Batch Details.**
43. Scan in the Extraction Batch ID.
44. Press **[F6]** ***Reagents***.
45. Press **[SF8]** ***Audit***.
46. 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

47. To extract the MP II log file, click on the Microsoft Access icon in the WinPrep® main menu to open the MultiPROBE log database.
48. 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)"
49. 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)

the SF8 request rework functions (e.g. samples requiring Microcon, NucleoSpin and pooling)

b) Press **[Esc]** to exit back to the DNA results table.

c) Do not toggle accept.

d) add the extraction batch ID into the 9PLEX or 9FTAR completed date fields in AUSLAB

75. **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
76. 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.
77. 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™ Bank-It™ tubes, ABgene 96-deep well and Axygen store plates.

11 TROUBLESHOOTING

1. If the barcode reader is not reading the barcodes of the Nunc™ Bank-It™ tube rack, or the Slicprep Plate or the Store plate, manually scan the appropriate barcodes.
2. When reading the Nunc™ Bank-It™ tube rack barcode, if the Gripper is not picking up or placing the Nunc™ Bank-It™ 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).

everything is OK, you need to close WinPrep, shut down the instrument, DPC 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

1. Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., & van der Noordaa, J., Rapid and Simple Method for Purification of Nucleic Acids. J. Clin. Microbiol., 1990. 28: p. 495-503.
2. Chen., C.W.T.J., C.A., Recovery of DNA Segments from Agarose Gels. Anal Biochem., 1980. 101: p. 339-341.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.
7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.

17 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
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
3	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland

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:	
Sample set 1	**Sample set 2**
Operator:	Operator:
Sequence check performed by:	Sequence check performed by:
Transfer tubes sequence checked:	Transfer tubes sequence checked:
37°C Incubation temp:	37°C Incubation temp:
65°C Incubation temp:	65°C Incubation temp:

Extraction Buffer made by:	TNE Buffer Lot#:
40% Sarcosyl 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:	

- 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.

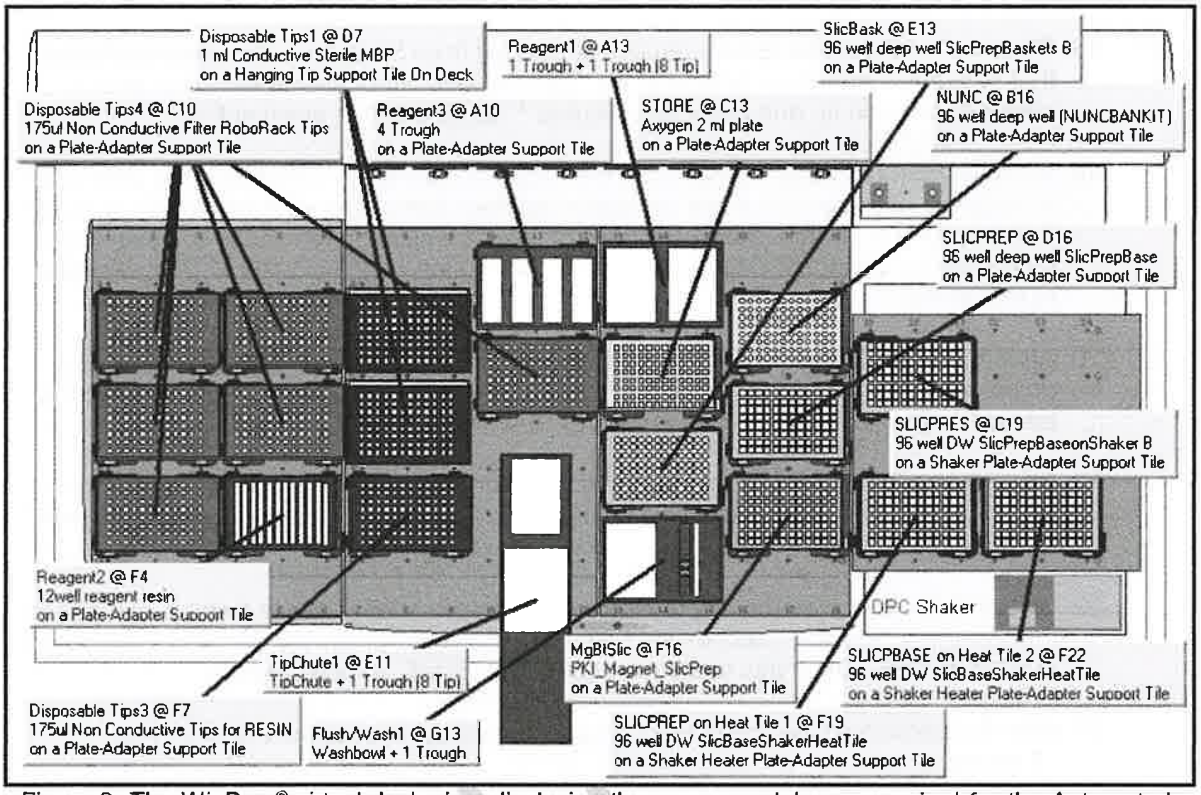

Figure 8. The WinPrep® virtual deck view displaying the necessary labware required for the Automated DNA IQ™ Method of Extraction on Extraction Platform A.

13. Ensure that the DPC shaker and Heater Controller Box are switched on.
    For EP-A: Tile 3 should be at F19 (50^{0}C), Tile 1 at F22 (85^{0}C).
    For EP-B: Tile 1 should be at F19 (50^{0}C), Tile 2 at F22 (85^{0}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™ Bank-It™ 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™ Bank-It™ tube rack. Then place the rack into position **B16**

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 85^{0}C (real temp 65^{0}C). **DO NOT PRESS CONTINUE** it will continue automatically when temperature has reached 85^{0}C.

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 and dispose of left over reagent into the glass bottle used previously. Bring this bottle to the room 6122 and transfer into 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 H_2O 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. In addition, clean the work area around the MPII instrument with 10% (v/v) Bleach and 70% Ethanol.
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.

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™ Bank-It™ tubes, Slicprep with Basket and Axygen store plates.

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 regents 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™ Bank-It™ 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.

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 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™ Bank-It™ 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**.