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on deck lysis

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).

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 MPII) 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 H_2O

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

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

Figure 2 The WinPrep® icon.

4. Log onto the WinPrep® software by entering your username and password, then press **[Enter]**.

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.

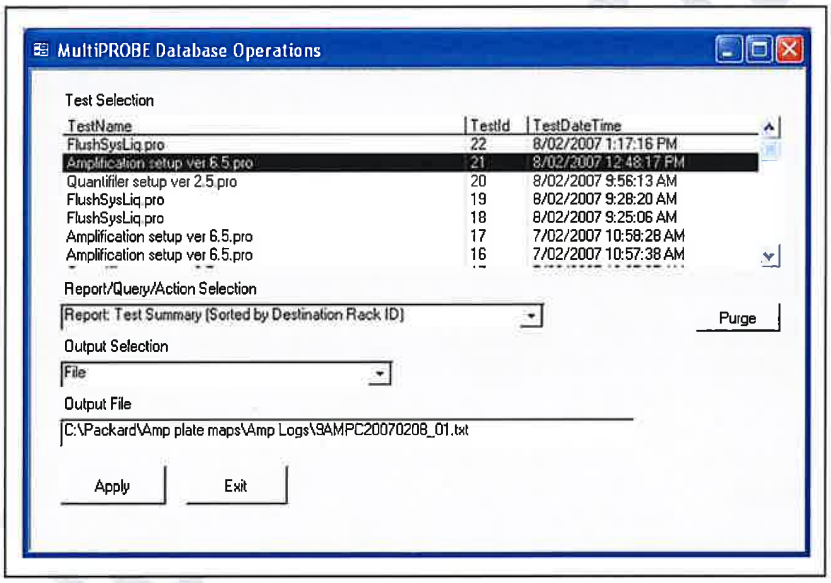
21. Once the extraction buffer has been added to the plate, a user prompt will appear requesting the following:

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 H_2O 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

11 REFERENCES

1. Boom, R., Sol, C.J.A., Salimans, M.M.M., Jansen, C.L., Wertheim-van Dillen, P.M.E., & van der Noordaa, J., Rapid and Simple Method for Purification of Nucleic Acids. J. Clin. Microbiol., 1990. 28: p. 495-503.
2. Chen., C.W.T.J., C.A., Recovery of DNA Segments from Agarose Gels. Anal Biochem., 1980. 101: p. 339-341.
3. Cowan, C., The DNA IQ™ System on the Tecan Freedom EVO® 100 Profiles in DNA. Profiles in DNA, 2006: p. 8-10.
4. Eminovic, I., Karamehić, J., Gavrankapetanović, F. & Helijić, B., A Simple method of DNA Extraction in Solving Difficult Criminal Cases. MEDARH, 2005. 59(1): p. 57-58.
5. Greenspoon, S.B., J., Robotic Extraction of mock sexual assult samples using the Biomek® 2000 and the DNA IQ™ System. Profiles in DNA, 2002. 5: p. 3-5.
6. Komonski, D.I., Marignani, A., Richard, M.L., Frappier, J.R.H., & Newman, J.C., Validation of the DNA IQ™ System for use in the DNA extraction of high volume forensic casework. Can.Soc.Forensic Sci.J., 2004. 37(2): p. 103-109.
7. Mandrekar, P., V., Flanagan, L., & Tereba, A., Forensic Extraction and Isolation of DNA Form Hair, Tissue and Bone. Profiles in DNA, 2002: p. 11.
8. Mandrekar, P.V., Kreneke, B. E., & Tereba, A., DNA IQ™: The Intelligent Way to Purify DNA. Profiles in DNA, 2001: p. 16.
9. Marko, M.A., Chipperfield, R., & Birnboim, H.C., A Procedure for the Large Scale Isolation of Highly purified Plasmid DNA using alkaline extraction and binding to glass powder. Anal. Biochem., 1982. 121: p. 382-387.
10. Melzak, K.A., Sherwood, C.S., Turner, R.F.B. & Haynest, C.A., Driving forces for DNA Adsorption to Silica in Percholorate Solutions. J. Colloid. Interface Sci., 1996. 181: p. 635-644.
11. PerkinElmer, Automated DNA IQ™ System for Mixed Casework Sample DNA Isolation. MultiPROBE II Liquid Handling - Forensic Workstation Application Guide, 2004: p. 1-25.
12. Promega, FAQs –DNA IQ™ System.
13. Promega, Protocols & Applications Guide. Chapter 9. rev. 7/06.
14. Promega, DNA IQ™ System -Small Casework Protocol. Promega Technical Bulletin #TB296 2006. Rev 4/06: p. 1-14.
15. Promega, DNA IQ™ System-Database Protocol. Promega Technical Bulletin #TB297, 2006. Rev 4/06: p. 1-14.
16. Promega, Tissue and hair Extraction Kit (for use with DNA IQ™) Protocol. Promega Technical Bulletin #TB307, 2006. Rev 5/06: p. 1-11.
17. Schiffner, L.A., Bajda, E. J., Prinz, M., Sebestyen, J., Shaler, R. & Caragine, T.A., Optimisation of a Simple, Automatable Extraction Method to Recover Sufficient DNA from Low Copy Number DNA Samples for Generation of Short Tandem Repeat Profiles. Croat Med J, 2005. 46(4): p. 578 -586.
18. Vandenberg, N., van Oorchot., R. A. H., & Mitchell, R. J., An evaluation of selected DNA extraction strategies for short tandem repeat typing. Electrophoresis, 1997. 18: p. 1624-1626.

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