Australian Breast Cancer Tissue Bank

STANDARD OPERATING PROCEDURE

Title: DNA Extraction from Whole Blood

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Related SOPs

SOP 5.1.015 Data Entry: Subspecimens from Blood and Tissue
SOP 5.1.018 Caisis User Guide: Basic Specimen Search
SOP 7.1.002 Assessing the Quality of DNA from Whole Blood
SOP 10.1.014 Quantitation of Extracted DNA

Revision History

Version	Issue Date	Reason for amendment	Sections amended	Replaces version	Revised by (initials)
1.0	17/12/2009	Document created	n/a	n/a	LM
1.1	22/03/2010	Formatting only	All	1.0	LM & JEC
1.2	14/02/2011	Equipment change for quantitation step: refer to SOP 10.1.014.	Method step 13.	1.1	LM & JEC
1.3	16/01/2013	Change of label printing and volume of DNA aliquots.	Method steps 3 and 12.	1.2	LM
1.4	15/07/2016	Change of title, additional information added to all sections, reformatted for new template. Volume of reagents changed in 4.4.	All	1.3	JH

Purpose

This SOP details the procedure in use for extraction of DNA from whole blood samples by the ABCTB at the Central Processing Laboratory (CPL).

Scope

DNA samples are routinely extracted by the Tissue Bank Scientist for all tissue bank donors where whole blood specimens are available. After this procedure, all DNA samples are quantitated according to SOP 10.1.014. ABCTB DNA samples also undergo quality assurance measures as covered by SOP 7.1.002.

Materials & Equipment

- Eppendorf epMotion 5075 VAC automated pipetting workstation and epBlue software.

  This equipment is set up on Level 7, Westmead Institute for Medical Research.
- QIAamp 96 DNA Blood Kit
- Qiagen S block 96-well-plates (additional plates are ordered separately to the kit)
- Eppendorf ThermoStat Plus (digital heating block)
- 100% Ethanol
- Injection water
- Cryo-vials and Cryo-labels

Method

Important: Treat all human biological material as potentially biohazardous and follow institutional safety procedures for handling and disposal accordingly.

1. Create a list of samples to be processed and prepare samples for extraction

1.1. A list of 47 blood samples is prepared for a single extraction run. Generally 4 lists are created at the same time, so 188 blood samples need to be pulled out of storage in preparation for extraction. The work plan of running 4 extractions in sequence within one month ensures that the re-constituted working solutions are not stored for too long.

1.2. The default order of processing bloods is in order of freezer box numbers from each ABCTB collection site and bloods in backup ('B') boxes are processed first. The order of process may vary depending on the logistics of transferring bloods between sites, and when particular blood DNA is requested as a priority for research applications.

1.3. Only Whole Blood specimens that have the status 'Available' should be processed.

1.4. To create a list of the next bloods to process, check records of the latest DNA extractions in the folder 'ABCTB DNA' (B:\BreastCancerTissueBank\ABCTB DNA) and continue in order of freezer boxes. A list of whole blood samples contained in each freezer box can then be downloaded using the 'Basic Specimen Search' in Caisis (see SOP 5.1.018). Alternatively, work with the database managers to download the list of specimens as required for extraction.

1.5. If it is necessary to transfer blood boxes from another site, coordinate this with the Tissue Bank Officers.

1.6. Once all bloods for processing are stored at CPL, move all of the Whole Blood samples on your list to new boxes at a temporary -80°C location ready for processing. If any discrepancies are discovered with specimen details (e.g. vial number, location, subtype or tissue bank reference number), then change the status of the sample in question to 'Quarantined', record the discrepancy in the specimen 'Notes' field, and contact the relevant Tissue Bank Officer.

1.7. Finalise your list of Whole Blood samples to process, making any changes necessary after checking the samples in 1.6. Save the list along with the other lists in B:\BreastCancerTissueBank\ABCTB DNA.

1.8. Print a copy of the list to use as a worksheet during the extraction, to make any notes and record storage details before transferring this information to the database.

1.9. A template for the layout of 96 well plate is saved in B:\BreastCancerTissueBank\ABCTB DNA. Print a copy of this layout and map out the position of each whole blood to be extracted and the water control as shown in the layout below:

- Each whole blood will be extracted in duplicate
- The position of the water control should be moved by one position on each run so that every well of the plate is used as a water control over 48 extraction runs. This way any potential cross contamination will be monitored.

1	1	9	9	17	17	H_2O	H_2O	32	32	40	40
2	2	10	10	18	18	25	25	33	33	41	41
3	3	11	11	19	19	26	26	34	34	42	42
4	4	12	12	20	20	27	27	35	35	43	43
5	5	13	13	21	21	28	28	36	36	44	44
6	6	14	14	22	22	29	29	37	37	45	45
7	7	15	15	23	23	30	30	38	38	46	46
8	8	16	16	24	24	31	31	39	39	47	47

Layout of sample aliquots on 96-well-plate

2. Print cryo-labels for DNA samples

2.1. Open the following three files from the 'ABCTB DNA' folder (B:\BreastCancerTissueBank\ABCTB DNA):

- The list of Whole Bloods to process that was saved in step 1.7.
- The Excel worksheet 'Template_create blood DNA labels'.
- The Word document 'Template_print blood DNA labels'

2.2. Four DNA aliquots will be stored from each whole blood extracted, so you will need to print four labels for each specimen reference number. The suffixes 'D1' to 'D4' are added to the whole blood reference number to create unique identifiers for each DNA aliquot. See how a formula is set up to create each aliquot label in the spreadsheet 'Template_create blood DNA labels'.

Copy all of the specimen reference numbers and tissue bank reference numbers from your list to their respective columns on label template spreadsheet. Paste the list four times to get the D1 to D4 labels (each time copying over the top of the previous list in the label template). The DNA labels should be generated automatically by the formula in the last column H (if not, copy down the formula).

2.3. Copy all the labels from 'Template_create blood DNA labels' into the Word file 'Template_print blood DNA labels'. Copy 13 labels at a time to fill each column and check that each label appears in three rows like the one below (adjust the spacing if necessary):

04-14-046
400008623D4
10.12.2015

Note: the font should be Arial black size 8. Also note that the "0" at the beginning of the vial number will be missing due to space restrictions on the label. Therefore, you will need to add a "0" at the beginning of the vial number when searching for the sample within the database.

2.4. Print the labels with a laser printer. Set the print setting to use the manual feed tray and Letter size.

2.5. Label the cryovials in advance of the extraction.

3. Make up reagents

Note: Follow the instructions within the QIAamp DNA Blood Blood Handbook, June 2012. The quantities here are for four extraction runs (188 Whole Blood samples) as per the work plan in 1.1 above.

3.1. It is best to make up the reagents the day before the first run and use the made up solutions for the subsequent three runs.

3.2. The reconstituted protease is stable for 2 months when stored at 2-8°C. Alternatively, it is recommended to aliquot the reconstituted protease and store at -20°C for later use. Storage at -20°C will prolong its life but repeated freezing and thawing should be avoided. Keeping the protease stock solution at room temperature for prolonged periods of time should also be avoided.

3.3. QIAGEN protease: Reconstitute the lyophilized QIAGEN protease by adding 5.5mL of Protease Solvent then gently mix well.

3.4. Buffer AW1: add 130mL of 100% ethanol to obtain 228mL of Buffer AW1. Mix thoroughly and always keep the bottle firmly closed.

3.5. Buffer AW2: add 160mL of 100% ethanol to obtain 226mL of Buffer AW2. Mix thoroughly and always keep the bottle firmly closed.

4. Preparation on the day of extraction

4.1. Take out the box of Whole Blood samples and leave it at room temperature for at least one hour for the blood to thaw.

4.2. Set up the software ready to run:

4.2.1. Turn on the computer beside the epMotion and open the 'Eppendorf epBlue' program.

4.2.2. Login with the account name 'demo' and password 'demo'.

4.2.3. Click on 'open/run applications' and choose the file 'demo/DNA extraction/BCTB_QIAamp_96x.dws'.

4.2.4. Click on 'work table' to view a picture of the layout of the work bench.

4.3. Set up your work table according to the diagram on the epBlue program which shows the location of the following items:

- Tips
- Metal reagents rack with six plastic tubs from position 1 to 6. (See detail in table below (4.4).
- Lysis plate (S block)
- Heated vac lid
- Vac lid holder
- Elute collection plate with adaptor
- Waste tray with plastic bag inside
- Waste tub, adaptor plate, Vac lid 2 and filter plate.

4.4. Load the reagents into the tubs in the quantities shown in the table below:

Tub position	Tub size (mL)	Reagent	Volume (mL)
1	30	Protease	2.6
2	30	Lysis buffer	20
3	100	100% Ethanol	55
4	100	Buffer AW1	53
5	100	Buffer AW2	53
6	30	Buffer AE	20

Reagents for a 96-well-plate

5. Perform the extraction

5.1. In the epBlue program, tick off ' level' and tick on 'tip' and 'location'.

5.2. Click "start".

5.3. After the protease is dispensed into each well, the program will pause for you to add the blood samples manually. Take out the S block and add duplicates of 200µL of blood from each sample into the plate according to the layout planned in step 1.9 above. Pipette blood directly from the cryovials. For any samples where the volume of blood is less than 0.4 mL, make up the volume with Injection Water.

5.4. Return the plate back to the epMotion and continue the program.

5.5. After the lysis buffer has been mixed with blood, take the plate out of the epMotion and incubate in the Eppendorf ThermoStat Plus at 40°C for 15 minutes.

5.6. Return the plate back to the epMotion and continue the program.

5.7. When the first vacuum step is complete, the program will pause again. Inspect the filter plate to ensure the lysate in every well has gone through the membrane. If there are any blockages, manually pipette the lysate up and down.

5.8. Return the plate back to the epMotion and continue the program. As it is running, inspect the filter plate again for any wells where the lysate has failed to go through the membrane. If there are any failed wells, pause the program to take out the plate and empty them. Mark any such wells in the working list as failed.

5.9. When the extraction has finished, manually pipette to combine the duplicates of DNA from each case into the 'D1' vial.

5.10. Refer to SOP 10.1.014 to measure and record the concentration of each DNA with 2µL of DNA from the 'D1' vials.

5.11. Run the 'demo/DNA extraction/Aliquot purified DNA.dws' program on the epMotion to automatically aliquot 55µL of DNA from vial 'D1' into vials 'D2', 'D3' and 'D4'. The volume that will remain in 'D1' will be approximately 70µL.

6. Storage and data entry

Note: DNA is stored at -80°C. As a precaution in case of equipment failure, the four DNA aliquots from each case are split into four different storage boxes to make it easy for later transfer to another ABCTB collection site for backup storage. See SOP 5.1.016 for creating new freezer boxes in the database.

6.1. Check the previous worksheet or the database to find the next storage locations for DNA. Record the boxes and positions on the worksheet for all the DNA aliquots.

6.2. Transfer the DNA aliquots on wet ice to their allocated locations.

6.3. Enter all of the details for DNA specimens and from your worksheet into the database following SOP 5.1.015.

Safety

Institutional safety requirements must be adhered to at all times.

Personal protective equipment such as gowns and gloves must be worn at all times when in the laboratory.

All human sample material must be handled in a Class II Biohazard Cabinet.

All local Chemical and Sharps policies must be adhered to.

Ordering Information

Item	**Supplier**	**Catalogue number**
QIAamp 96 DNA Blood kit (12)	Qiagen Pty. Ltd.	51162
S Blocks (24)	Qiagen Pty. Ltd.	19585
epT.I.P.S.® Motion pipette tips, with filter, PCR clean, 1000 µL, 960 tips (10 racks × 96 tips)	Eppendorf	0030014499
epT.I.P.S.® Motion pipette tips, with filter, PCR clean, 300 µL, 960 tips (10 racks × 96 tips)	Eppendorf	0030014456
ep*Motion*® reservoir 100 mL, 10 × 5 reservoirs in the bag, PCR clean, polypropylene	Eppendorf	0030126513
ep*Motion*® reservoir 30 mL, 10 × 5 reservoirs in the bag, PCR clean, polypropylene	Eppendorf	0030126505
SSI, sterile screw tubes with standard caps, 0.5ml, natural	Interpath Services	2141-S0
Cryo-label laser tags, -196oC to 121oC, pack 1664, 24x19.5 mm	Molecular solutions	LCL-32W
Ethanol Absolute 100%	POCD (Point of Care Diagnostics Australia Pty Ltd)	ETHABS2.5

References

1. QIAamp DNA Blood Blood Handbook, June 2012