CTRNet Standard Operating Procedure DNA Extraction from Blood			
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Subject:	DNA Extraction from Blood	Category	Material Handling and Documentation

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REVISION HISTORY

SOP Number	Date Issued	Author (Initials)	Summary of Revisions
LP 001.001	2005	JdSH	CTRNet Generic SOP for Blood Collection and Processing
8.2.004	2008	JdSH	Revised to cover only extraction of DNA from blood cells

1.0 PURPOSE

Tissue samples are collected from patients that have been through the informed consent process and agreed to participate in the tumour repository program. Genomic studies often utilize nucleic acids (DNA and RNA) derived from these samples. When extracting and storing DNA from blood samples all efforts should be made to avoid contamination, prevent degradation and preserve molecular integrity. The purpose of this document is to outline standardized procedures for CTRNet repositories to follow when extracting DNA from blood samples.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how DNA should be extracted from blood samples. The SOP does not cover detailed safety procedures for handling Human Biological Materials (HBMs) or hazardous chemicals and it is recommended that personnel follow institutional safety guidelines.

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3.0 REFERENCE TO OTHER POLICIES AND SOPS

1. CTRNet Policy: POL 005.001 Records and Documentation
2. CTRNet Policy: POL 002.001 Ethics
3. CTRNet Policy: POL 004.001 Privacy and Security
4. CTRNet Policy: POL 007.001 Material and Information Handling Policy
5. CTRNet SOP: 8.2.001 CTRNet Generic SOP for Blood Collection and Processing
6. CTRNet SOP: 8.1.002 Biohazardous Material Waste Management

4.0 ROLES AND RESPONSIBILITY

The policy applies to all personnel from CTRNet member repositories who are responsible for extracting DNA from blood.

Tumour Bank Personnel	**Responsibility/Role**	**Site Specific Personnel and Contact Information**
Lab Technician	Responsible for labelling tubes and extracting DNA from blood samples.	

5.0 MATERIALS,REAGENTS EQUIPMENT AND FORMS

The materials, equipment and forms listed in the following list are recommendations only and may be substituted by alternative/equivalent products more suitable for the site-specific task or procedure.

Materials and Equipment	**Materials and Equipment (Site- Specific)**
Markers, ink and pens	
Appropriate labels for tubes and vials	
Tube of previously isolated Buffy Coat from blood sample	
2 ml Microfuge tubes	
1.5 ml centrifuge tubes	
2 ml cryotubes	
Racks for Microfuge tubes	
Tube Racks for water bath	
Vortex Mixer	
Microcentrifuge	
Pipettes	
Sterile pipette tips with aerosol barrier	
Micropipettors	
Transfer pipettes	
Isopropanol	

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DNA Extraction Kit such as Qiagen Flexgene	
A shaking Heat Block like the Eppendorf Thermomixer or normal heating block or water bath.	
-80º C and -20º freezer	
Storage Boxes	
Disposable Gloves	
Hot Water Bath (set at 55° C)	
Tube Racks for water bath	
TRIS Saturated Phenol	
Rolling Rack (nutator mixer)	
Glass pipettes for transferring phenols and chloroforms (do not use polystyrene)	
Pipettors for large glass pipettes	
95% Ethanol	
70% Ethanol	
Buffer A*	
Proteinase K*	
20% SDS*	
Tris Buffer*	
TRIS EDTA (TE) Buffer*	
Phenol Chloroform/Isoamyl Alcohol*	
Chloroform /Isoamyl Alcohol*	
Refrigerator at 4° C	

* See Appendix A

6.0 DEFINITIONS

DNA: Deoxyribonucleic acid. A long molecule that is found in all living organisms. DNA is present in the nucleus of eukaryotic cells. DNA is found in the form of a double helix, and is composed of four basic elements that define the genetic code.

Buffy Coat: A thin grayish white layer of white blood cells (leukocytes and platelets) found covering the top of packed erythrocytes (red blood cells) of a hematocrit.

7.0 PROCEDURES

This procedure is intended to ensure that DNA is extracted from blood samples in a safe and consistent manner while eliminating the risks of contamination and loss of molecular and structural integrity. Consistency in procedure is important for obtaining comparable and reliable test results.

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7.1 Extraction of DNA from blood samples using a kit such as Qiagen Flexgene

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the sample. This protocol is scaled to 100-500 µl of isolated Buffy Coat.

1. Treat all blood as potentially infectious.
2. DNA Extraction is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Have materials and equipment ready. Have as many tubes and cryovials as needed labelled and ready.
4. Thaw the previously frozen Buffy Coat by gentle agitation in a 37º C water bath.
5. Keep the thawed tube on ice until starting the extraction procedure.
6. Use a DNA Extraction kit such as the Qiagen Flexigene Kit and follow the protocol outlined for the product: http://www.ebiotrade.com/buyf/productsf/qiagen/1019478HBQAFlexiGene_0102WW.pdf
7. Genomic DNA can be stored at 4ºC.
8. DNA is a weak acid and at 4ºC may be subject to acid hydrolysis. For longer term storage keep DNA at -80º C. Avoid subjecting the DNA to freeze/thaw cycles to prevent fragmentation of the genomic DNA).
9. Place DNA in storage boxes and record storage location.

7.2 Extraction of DNA from blood samples using the Phenol/Chloroform Method.

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the sample. It is possible to keep the supernatant at 4º between each step. For buffer recipes see Appendix A.

1. Treat all blood as potentially infectious.
2. DNA Extraction is performed by the laboratory technician or trained personnel designated by the tumour repository.
3. Resuspend the blood (buffy coat) cell pellet in 500 µl of Buffer A, 20 µl of 20% SDS and 20 µl of Proteninase K (10 mg/ml).
4. Mix with a pipette tip. To avoid shearing the genomic DNA **do not** vortex to mix.
5. Incubate 3 hours to overnight in a water bath at 55º C.
6. Add 500 µl of Tris saturated Phenol.
7. Mix 10 min. on nutator (rolling rack) at room temperature.

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8. Centrifuge at 180000 x g for 10 min. in a microfuge at room temperature.
9. Transfer upper phase to an microfuge tube.
10. Add 500 µl of Phenol/Chloroform/Isoamyl Alcohol.
11. Mix by inversion.
12. Centrifuge at 180000 x g for 10 min. in a microfuge at room temperature.
13. Transfer upper phase to a clean microfuge tube.
14. Add 500 µl of Chloroform/Isoamyl Alcohol.
15. Mix by inversion.
16. Centrifuge at 180000 x g for 10 min. in a microfuge at room temperature.
17. Transfer upper phase to a clean microfuge tube.
18. Add 1 ml of cold 95% Ethanol (stock kept at -20º C.
19. Incubate at -80º C for 2 hours or at - 20º C overnight.
20. Harvest DNA with a pipette tip and transfer it to 500 µl of 70% Ethanol to wash.
21. Centrifuge at 180000 x g for 5 min. in a microfuge at room temperature.
22. Discard supernatant and allow DNA to dry for 10 min.
23. Resuspend DNA pellet in TE buffer
24. Incubate at in a 55º C water bath to dissolve DNA if needed.
25. Store DNA at -80º C.

8.0 APPLICABLE REFERENCES, REGULATONS AND GUIDELINES

1. Declaration of Helsinki. http://ohsr.od.nih.gov/helsinki.php3 http://www.wma.net/e/policy/b3.htm
2. Tri-Council Policy Statement; Ethical Conduct for Research Involving Humans; Medical Research Council of Canada; Natural Sciences and Engineering Council of Canada; Social Sciences and Humanities Research Council of Canada, August 1998. http://www.pre.ethics.gc.ca/english/policystatement/policystatement.cfm
3. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics Series. http://www.mrc.ac.uk/pdf-tissue_guide_fin.pdf
4. Best Practices for Repositories I. Collection, Storage and Retrieval of Human Biological Materials for Research. International Society for Biological and Environmental Repositories (ISBER). http://www.isber.org

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5. US National Biospecimen Network Blueprint
http://www.ndoc.org/about_ndc/reports/NBN_comment.asp

6. SOP #: BIO-SOP-BLD-PRO-DNA. Blood Sample Processing November 20, 2006 Procure, Quebec Prostate Cancer Biobank

7. Qiagen Flexigene DNA Kit Handbook

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Appendix A. Preparation of Buffers and Reagents required for DNA EXtraction:

NOTE: Suppliers and Brands can be substituted with other appropriate brands.

Buffer A (for 500 mL) : 10mM TRIS pH 7,9
2 mM EDTA pH 8
40mM NaCl

Proteinase K : 20 units/mg (Invitrogen # 25530-015)
Resuspend in 10mL of storage solution
Dilution = 10mg/mL

Storage solution for proteinase K (for 50mL) :
10 mM TRIS pH7,5
20 mM $CaCl_2$
50% Glycerol (Life Technologies cat# 25530-015)

TRIS saturated phenol :

- Put at 55°C to liquefy the crystal phenol.
- Add 0,1% of 8-hydroxyquinolin ⇒ RNAse inhibitor
- Mix
- Add an equal volume of TRIS 1M pH8
- Mix 30 minutes
- Let stand until the phases separate (3 hours to overnight, at 4°C)
- Take out the supernatant
- Add 500 mL TRIS 0,1M pH8
- Repeat until the phenolic phase pH is > 7.6
- Store at 4°C in a dark bottle

Phenol/Chloroform/Iso : Keep at 4°C in a dark bottle
Mix in following ratio- 25:24:1)
For 200 ml use
Phenol – 100 ml
Chloroform – 96 ml
Isoamyl Alcohol – 4 ml

Chloroform/Iso : Keep at Room Temperature in a dark bottle
Ratio (24:1)
For 200 ml solution use 192 ml Chloroform and 8 ml Isoamyl Alcohol.

TE buffer : 10mM TRIS pH 7,6
1mM EDTA pH 8

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