CTRNet Standard Operating Procedure
Tisssue Derivatives: Extraction of DNA

SOP Number:	8.3.008	Version	e1.0
Supersedes:	SR 001.001	Effective Date	09 Jan 08
Subject:	Tisssue Derivatives: Extraction of DNA	Category	Material Handling and Documentation

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REVISION HISTORY

SOP Number	Date Issued	Summary of Revisions
LP 002.001	2005	CTRNet Generic SOP for Collection and Processing of Tumour Tissue
8.3.008	08-01-2008	Revised to cover extraction of DNA only

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 tissue specimens 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 tissue samples.

2.0 SCOPE

The Standard Operating Procedure (SOP) describes how DNA should be extracted from tissues. 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 Generic Procedure: FS 002.001 CTRNet Generic SOP for Collection and Processing of Tumour Tissue
6. CTRNet SOP: 8.3.003 Preservation of tissue: Snap Freezing
7. CTRNet SOP: 8.3.004 Preservation of tissue: Freezing in OCT
8. 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 tissue.

Tumour Bank Personnel	Responsibility/Role	Site Specific Personnel and Contact Information
Lab Technician	Responsible for labelling tubes and extracting DNA from tissue and storing 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 vials	
Microfuge tubes	
Racks for Microguge tubes	
Hot Water Bath (set at 55°C)	
Tube Racks for water bath	
TRIS Saturated Phenol	
Rolling Rack (nutator mixer)	
Microcentrifuge	
Pipettors for large glass pipettes	
Glass pipettes for transferring phenols and chloroforms (do not use polystyrene)	

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Micropipettors	
Sterile pipette tips	
Buffer A*	
Proteinase K*	
20% SDS*	
Tris Buffer*	
TRIS EDTA (TE) Buffer*	
Phenol Chloroform/Isoamyl Alcohol*	
Chloroform /Isoamyl Alcohol*	
95% Ethanol	
-80º C or -20º C freezer	

*Consult Appendix 1 for the preparation of DNA Extraction Solutions and Buffers. For additional details on preparing buffers see reference #10 (Section 8)

6.0 DEFINITIONS

DNA: Deoxyribonucleic acid

Preservation: Use of chemical agents, alterations in environmental conditions or other means during processing to prevent or retard biological or physical deterioration of a specimen.

RNA: Ribonucleic acid

7.0 PROCEDURES

This procedure is intended to ensure that DNA is extracted from tissue 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. The following steps are based on procedures followed at The Cancer Network of the Fonds de la recherche en santé du Québec (FRSQ).

7.1 Extraction of DNA from tissue

NOTE: Volumes indicated are recommendations only and should be scaled according to the size of the tissue sample.

1. Treat all tissue 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.

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4. Suspend frozen tissue in 500 µl of Buffer A Cut tissue (mince) into small pieces with a sterile scissors or scalpel blade. Alternatively, frozen tissue can be wrapped in Aluminum foil and fragmented with a hammer.
5. Add 20 µl of 20% SDS and 20 µl of Proteinase K (10 mg/ml).
6. Incubate 3 hours or overnight in 55° C water bath (with agitator).
7. Add 500 µl of Tris Saturated phenol.
8. Mix for 10 minutes at room temperature on a nutator.
9. Centrifuge at 180000 x g for 10 min at room temperature.
10. Transfer supernatant to a clean microfuge tube.
11. Add 500 µl of Phenol/Chloroform/Isoamyl Alcohol.
12. Mix by inverting tubes.
13. Repeat step #'s 9 and 10.
14. Add 500uL of chlorophorm/Isoamyl Alcohol
15. Mix by inverting tubes.
16. Repeat steps 9 and 10.
17. Add 1 ml cold 95% Ethanol to the supernatant.
18. Incubate at -80º C for 30 minutes to 2 hours or at -20º C overnight.
19. Precipitated DNA will resemble a white gelatinous fibre, scoop this with a clean pipette tip into 500µl of 70% Ethanol in a microfuge tube.
20. Centrifuge at 180000 x g for 5 minutes at room temperature.
21. Remove the supernatant and let the DNA dry for 10 minutes at room temperature (or until alcohol evaporates).
22. Resuspend the DNA pellet in TE buffer to get an appropriate concentration of DNA in solution.
23. Incubate the tube in a 55° C water bath (with agitation) for one hour to dissolve the pellet.
24. DNA can be stored at 4ºC.
25. For longer term store DNA at -20º C or lower. Avoid subjecting the DNA to freeze/thaw to prevent DNA fragmentation).

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

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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

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

6. Jewell, S. et al. 2002, Analysis of the Molecular Quality of Human Tissues, an experience from the Cooperative Human Tissue Network. Am. J. Clin. Pathol. 118:733-741.

7. Guideline – Fresh Tissue Working Group of BIG and NCI breast cancer Cooperative Groups http://ctep.cancer.gov/forms/guidelines_fresh_tissue.pdf

8. Snell L. and P. H. Watson. 2006, Breast Tissue Banking: Collection, Handling, Storage and Release of Tissue for Breast Cancer Research. Methods Mol Med. 120:3-24.

9. DNA Extraction procedure from Fonds de la recherche en santé du Québec (FRSQ).

10. Sambrook, Fitsch, Maniatis. Molecular Cloning – A Laboratory Manual, 2nd Edition, Preparation of Reagents and Buffers used in molecular cloning. Appendix B.

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APPENDIX 1.

PREPARATION OF BUFFERS AND REAGENTS REQUIRED FOR DNA EXTRACTION:

NOTE: Suppliers and Brands are as used by Fonds de la recherche en santé du Québec and 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)

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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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