Tissue Derivatives – Extraction of DNA

Category:	**Materials Handling and Documentation**		
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1.0 PURPOSE

The purpose of this SOP is to establish standardized procedures for the extraction of DNA from tissue derivatives at Nourah's Tissue Biobank using Qiagen Tissue Extraction Kits. The procedures outlined cover both manual and automated methods to ensure the integrity, purity, and quality of the extracted DNA for downstream applications in research.

2.0 SCOPE

This SOP applies to all personnel involved in the extraction of DNA from tissue samples within Nourah's Tissue Biobank, using Qiagen Tissue Extraction Kits. It covers both manual and automated processes using Qiagen equipment, as well as documentation using the LabVantage LIMS system.

3.0 ROLES AND RESPONSIBILITIES

This SOP applies to all personnel of Nourah's Tissue Biobank members

Biobank Personnel	Responsibility
Laboratory Technicians	Responsible for performing DNA extraction according to this SOP, ensuring the proper handling, processing, and storage of DNA.
Biobank Manager	Responsible for overseeing DNA extraction activities, ensuring compliance with this SOP, and addressing any issues that arise.
Quality Assurance (QA) Officer	Responsible for auditing the DNA extraction process, ensuring adherence to protocols and managing documentation.
All Personnel	Responsible for following the procedures outlined in this SOP to maintain the integrity and quality of DNA samples.

4.0 MATERIALS, EQUIPMENT, AND FORMS

Listing of the materials, equipment, and forms being used to achieve the goals of the SOP, this list will mainly contain necessary materials and, or recommendations that may be substituted by alternative or equivalent materials more suitable at the time of testing.

Material to be used	Site
Qiagen DNA extraction kit (e.g., QIAamp DNA Mini Kit, QIAmp DNA FFPE Tissue Kit)	
Qiagen automated system (e.g., QIAcube, QIAcube Connect)	
Centrifuge	
Pipettes and sterile pipette tips	
RNase-free water and tubes	
Ethanol (96-100%)	
Vortex mixer	
Disposable gloves, lab coat, face shield (PPE)	
Biohazard waste containers	
Ice or cold block for DNA stabilization	
DNA storage tubes (e.g., RNase-free cryovials)	

5.0 POTENTIAL HAZARDS

In this part of the SOP, we explain the potential hazards from chemicals and methodologies used in this procedure. It will also contain information on how to handle these chemicals and the level of biosafety

Material	Safety and handling

6.0 PROCEDURES

This section outlines the steps involved in the manual and automated extraction of DNA from tissue derivatives at Nourah's Tissue Biobank. These procedures ensure the integrity and purity of the extracted DNA for subsequent research applications.

6.1 PREPARATION OF FRESH TISSUE

1. Obtain tissue samples that have been properly stored and logged in the LabVantage LIMS system.
2. Thaw any frozen tissue samples on ice or in a cold block to preserve DNA integrity.
3. Weigh and cut tissue samples into smaller pieces (typically 25-50 mg) suitable for extraction.
4. Assemble all components of the Qiagen Tissue Extraction Kit as per the manufacturer's instructions.
5. For automated extraction, ensure that the Qiagen automated system (e.g., QIAcube) is operational and the required protocols are programmed.
6. Prepare any necessary reagents (e.g., ethanol) as indicated in the Qiagen kit protocol.

6.2 MANUAL DNA EXTRACTION USING FRESH TISSUE

1. Place the tissue sample into a clean, RNase-free tube.
2. Add the appropriate volume of lysis buffer and Proteinase K from the Qiagen kit to the sample.
3. Vortex briefly and incubate the mixture at 56°C until the tissue is completely lysed (typically 1-3 hours).
4. Add ethanol to the lysate according to the Qiagen kit protocol.
5. Transfer the mixture to the DNA binding column provided in the kit.
6. Centrifuge the column at the recommended speed to allow DNA binding to the membrane.
7. Wash the column with the buffers provided in the kit (e.g., Buffer AW1 and Buffer AW2) according to the Qiagen protocol.
8. Ensure that all traces of wash buffer are removed by centrifugation.
9. Elute the DNA from the column using RNase-free water or the elution buffer provided in the Qiagen kit.
10. Centrifuge the column to collect the purified DNA in a clean, RNase-free tube.

6.3 AUTOMATED DNA EXTRACTION FROM FRESH TISSUE USING QIAGEN QIACUBE

1. Transfer the tissue sample into the specified sample tubes or cartridges compatible with the QIAcube.
2. Load the tubes into the QIAcube system, ensuring they are securely placed.
3. Select the appropriate DNA extraction protocol on the QIAcube, ensuring all required reagents and consumables are in place.
4. The system will perform tissue lysis, DNA binding, washing, and elution automatically.
5. Once the automated run is complete, carefully remove the DNA-containing tubes from the system.
6. Inspect the eluted DNA for volume and quality as indicated by the system's output.

6.4 PREPARATION OF FFPE TISSUE

1. Obtain tissue samples that have been properly stored and logged in the LabVantage LIMS system.
2. Section the tissue samples using a microtome producing 4-8 sections with 10 µm thickness
3. Assemble all components of the Qiagen FFPE Tissue Extraction Kit as per the manufacturer's instructions.
4. For automated extraction, ensure that the Qiagen automated system (e.g., QIAcube) is operational and the required protocols are programmed.
5. Prepare any necessary reagents (e.g., ethanol) as indicated in the Qiagen kit protocol.

6.5 MANUAL DNA EXTRACTION FROM FFPE

1. Place the sections of tissue sample into a clean, RNase-free tube.
2. Fill the sample tube with Xylene to dissolve Paraffin Wax
3. Add the appropriate volume of lysis buffer and Proteinase K from the Qiagen kit to the sample.
4. Vortex briefly and incubate the mixture at 90°C until the formalin crosslinking are reversed
5. Transfer the mixture to the DNA binding column provided in the kit.
6. Centrifuge the column at the recommended speed to allow DNA binding to the membrane.
7. Wash the column with the buffers provided in the kit (e.g., Buffer AW1 and Buffer AW2) according to the Qiagen protocol.
8. Ensure that all traces of wash buffer are removed by centrifugation.
9. Elute the DNA from the column using RNase-free water or the elution buffer provided in the Qiagen kit.
10. Centrifuge the column to collect the purified DNA in a clean, RNase-free tube.

6.6 AUTOMATED DNA EXTRACTION FROM FPPE USING QIAGEN QIACUBE

1. Transfer the tissue sample into the specified sample tubes or cartridges compatible with the QIAcube.
2. Load the tubes into the QIAcube system, ensuring they are securely placed.
3. Select the appropriate DNA extraction protocol on the QIAcube, ensuring all required reagents and consumables are in place.
4. The system will perform tissue lysis, DNA binding, washing, and elution automatically.
5. Once the automated run is complete, carefully remove the DNA-containing tubes from the system.
6. Inspect the eluted DNA for volume and quality as indicated by the system's output.

6.7 QUALITY CONTROL AND STORAGE

Measure the concentration and purity of the DNA using a spectrophotometer (e.g., NanoDrop) or fluorometer.

Assess the A260/A280 ratio to ensure DNA purity (ratios between 1.8 and 2.0 are typically acceptable).

Aliquot the DNA into DNase-free tubes and store at -20°C for short-term use or -80°C for long-term storage.

Log the DNA samples into the LabVantage LIMS system, recording the concentration, volume, and storage location.

6.8 INCIDENT MANAGEMENT

1. In case of any deviations from the SOP (e.g., temperature excursions, delays), document the incident and notify the Biobank Manager immediately.
2. Implement corrective actions as necessary to mitigate any impact on sample integrity.
3. Complete an incident report detailing the deviation, corrective actions taken, and any follow-up measures required.
4. Submit the report to the QA Officer for review and documentation.

7.0 REFERENCES

1. CTRnet SOPs "08.03.008 Tissue Derivatives - Extraction of DNA"
2. QIAmp DNA extraction Kit manual
3. QIAmp DNA FFPE Tissue Kit manual
4. Declaration of Helsinki.
   http://www.wma.net/en/30publications/10policies/b3/index.html
5. Human Tissue and Biological Samples for use in Research. Operational and Ethical Guidelines. Medical Research Council Ethics
   http://www.mrc.ac.uk/Utilities/Documentrecord/index.htm?d=MRC002420
6. 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/Search/search.asp?zoom_query=best+practices+for+repositories
7. National Bioethics Advisory Commission: Research involving human biological materials: Ethical issues and policy guidance, Vol. I: Report and recommendations of the National Bioethics Advisory Committee. August 1999.
   http://bioethics.georgetown.edu/nbac/hbm.pdf
8. US National Biospecimen Network Blueprint
   http://biospecimens.cancer.gov/resources/publications/reports/nbn.asp

8.0 REVISION HISTORY

SOP No.	Date Revised	Author	Summary

9.0 APPENDICES