STANDARD OPERATING PROCEDURE	**SOP No. 004** **DNA Extraction from Frozen Tissue**
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1. PURPOSE

To describe the procedure for the extraction of DNA from frozen tissue. NOTE: This SOP does not cover detailed procedures for handling Human Biological Materials or hazardous chemicals and it is recommended that personnel following this SOP refer to institutional safety guidelines.

The following SOP for DNA extraction from frozen tissue is followed by the JHU, NYU, MSKCC and UW Network Sites of the PCBN.

2. RESPONSIBILITIES

Authorized personnel extracting DNA from frozen prostate tissue must ensure that:

- all procedures are followed correctly
- all samples are adequately coded during processing
- all documentation is completed, and accurate records maintained on all samples

3. HEALTH AND SAFETY

Personnel carrying out this procedure must maintain safe working practices and observe all relevant Environmental Health and Safety (EH&S) guidelines. This includes the appropriate use of Personal Protective Equipment (PPE), and the procedures for waste disposal, disinfection & spill clean-up and biosafety.

4. EQUIPMENT AND MATERIALS

Equipment	Materials
Cryostat, chuck, freezing bars Microcentrifuge OCT Thermomixer, shaking water bath, or rocking platform Vortex	Cryovials 100% ethanol Microcentrifuge tubes Qiagen DNeasy Blood and Tissue Kit (Catalog # 69504) Sterile blades for cryostat

5. PROCEDURES

5.1 Sample Preparation / Tissue Lysis (Day 1)

- Collect tissue samples for DNA extraction from -80°C storage and place on dry ice.
- Cut 10 µm sections in a cryostat at -30°C.

  NOTE: The tissue samples may require re-embedding in OCT, before 10 µm sections are cut.

  For each sample, a section is harvested at maximum diameter location at thickness of 5 µm both immediately before and immediately after the tissue sections collected in the tube for H&E staining and stored in the tissue bank.

- Collect sections into 3 ml cryovials (per sample). The starting sample weight should be no more than 25 mg for the DNeasy Kit.

  NOTE: Ensure to use 100% Ethanol to clean knife (or change blade), sample collector and anywhere sample touches before cutting the next sample.

  We have tested the use of differing numbers of tissue sections for DNA extraction and have had success using up to 85, 10 µm sections of tissue, with greater yield from a greater number of sections. The only concern in maximizing the number of sections used is exhaustion of the block.

- Add 180 µl Buffer ATL to tube with sectioned tissue. There is no need to homogenize tissue further.
- Add 20 µl proteinase K. Mix thoroughly by vortexing.
- Incubate at 56°C overnight until the tissue is completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.

5.2 DNA Extraction (Day 2)

- **JHU Network Site Only**: Place sample into an M-tube and tap liquid to the orange lid. Utilize RNA_02.01 pre-set program two times. Transfer liquid into 2mL microcentrifuge tube.

- Add 4 µl RNase A (100 mg/ml, Catalog #19101), mix by vortexing, and incubate for 2 min. at room temperature before continuing.
- Vortex for 15 sec. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing.
- Add 200 µl ethanol (100%), and mix again thoroughly by vortexing.

  NOTE: It is essential that the sample, Buffer AL, and ethanol are mixed immediately and thoroughly by vortexing or pipetting to yield a homogeneous solution.

- Pipet the mixture (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided in kit).
- Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard flow-through and collection tube.

- Place the DNeasy Mini spin column in a new 2 ml collection tube (provided in kit), add 500 µl Buffer AW1

NOTE: Ensure that ethanol has been added to Buffer AW1 before use.

- Centrifuge for 1 min at ≥6000 x g (8000 rpm). Discard flow-through and collection tube.
- Place the DNeasy Mini spin column in a new 2 ml collection tube (provided in kit), add 500 µl Buffer AW2.

  NOTE: Ensure that ethanol has been added to Buffer AW2 before use.

- Centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.
- Place the DNeasy Mini spin column in a clean 1.5 ml microcentrifuge tube (not provided).
- Pipet 100 µl Buffer AE directly onto the DNeasy membrane.
- Incubate at room temperature for 1 min.
- Centrifuge for 1 min at ≥6000 x g (8000 rpm) to elute.

  NOTE: DO NOT discard the eluate.

- Pipet an additional 100 µl Buffer AE directly onto the DNeasy membrane.

  NOTE: There is no need to replace the microcentrifuge tube. The additional DNA elution can be collected into the same tube, up to a total volume of no more than 200 µl.

- Incubate at room temperature for 1 min.
- Centrifuge for 1 min at ≥6000 x g (8000 rpm) to elute, for a total DNA sample volume of 200 µl.

This protocol is adapted from the Qiagen handbook for the DNeasy Blood and Tissue Kit (Catalog # 69504): **Protocol: Purification of Total DNA from Animal Tissues (Spin-Column Protocol).**