STANDARD OPERATING PROCEDURE	**SOP No. 006** **RNA Extraction from Frozen Tissue**
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1. PURPOSE

To describe the procedure for the extraction of RNA 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 RNA extraction from frozen tissue is followed by the JHU, NYU and MSKCC Network Sites of the PCBN.

2. RESPONSIBILITIES

Authorized personnel extracting RNA 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 Centrifuge gentleMACS Dissociator (JHU and NYU) OCT Qiagen TissueLyser (MSKCC) Refrigerated Microcentrifuge Vortex	Chloroform Cryovials 75% Ethanol in RNase free H_2O gentleMACS M Tubes Isopropyl alcohol Microcentrifuge tubes RNAse Away RNas-free water Sterile blades for cryostat TRIzol® Reagent (Invitrogen Catalog # 15596-18)

5. PROCEDURES

5.1 Sample Preparation

- Collect tissue samples for RNA 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 biorepository.

- Collect sections into 3 ml cryovials (per sample). The starting sample weight should be no more than 50-100 mg for the TRIzol® Reagent.

  NOTE: Use clean practice techniques for RNA handling (use of RNAse Away or similar product on all surfaces, use RNase-free equipment, etc.). Change blade before cutting the next sample.

  We have tested the use of differing numbers of tissue sections for RNA 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.

5.2 RNA Extraction

- Add 1 ml of TRIzol® Reagent to tube with sectioned tissue. **NYU Networking site only:** no further homogenization step required.

- **MSKCC Networking site only:** Use the Qiagen TissueLyser to homogenize tissue.

- Incubate the homogenized sample for 5 min at 15-30°C to permit the complete dissociation of nucleoprotein complexes.

- **JHU Networking site only:** Transfer homogenous solution into a gentleMACS M Tube. Utilize the gentleMACS Dissociator on the RNA 02.01 pre-set program 3 times per sample to ensure complete homogenization of the tissue. Centrifuge M Tubes and transfer liquid into a fresh, sterile 1.5 ml microcentrifuge tube.

- Add 0.2 ml of chloroform per 1 ml of TRIzol® Reagent.
- Cap sample tubes securely. Shake tubes vigorously by hand for 15 sec and incubate at 15-30°C for 2-3 min.
- Centrifuge the samples at no more than 12,000 × g for 15 min at 2-8°C.

  Following centrifugation, the mixture separates into a lower red, phenol-chloroform phase, an

interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The volume of the aqueous phase is about 60% of the volume of TRIzol® Reagent used for homogenization.

- Transfer the aqueous phase to a fresh, sterile 1.5 ml microcentrifuge tube.
- Precipitate the RNA from the aqueous phase by mixing with isopropyl alcohol. Use 0.5 ml of isopropyl alcohol per 1 ml of TRIzol® Reagent used for the initial homogenization.
- Incubate samples at 15-30°C for 10 min.
- Centrifuge at no more than 12,000 × *g* for 10 min. at 2-8°C.

The RNA precipitate, often invisible before centrifugation, forms a gel-like pellet on the side and bottom of the tube.

- Remove the supernatant and discard, leaving the pellet untouched.
- Wash the RNA pellet once with 75% ethanol, adding at least 1 ml of 75% ethanol per 1 ml of TRIzol® Reagent used for the initial homogenization.
- Mix the sample by vortexing and centrifuge at no more than 7,500 × *g* for 5 min at 2-8°C.
- Remove the supernatant and discard, leaving the pellet untouched.
- Briefly dry the RNA pellet (air-dry or vacuum-dry for 5-10 min).

NOTE: Do not dry the RNA by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility.

- Dissolve RNA in 200 µl (**JHU and NYU**) or 120 µL (**MSKCC**)RNase-free water by passing the solution a few times through a pipette tip, and incubating for 10 min at 55-60°C.

This protocol is adapted from the Invitrogen handbook for TRIzol® Reagent (Catalog # 15596-018).