Immunohistochemistry and Molecular Pathology

Sectioning of Frozen Tissue for Nucleic Acid Extraction

a. Tissue collection: Tissue should be collected as soon after surgery as is possible (RNA starts to degrade within minutes). It is customary to freeze the tissue prior to histologic review and sectioning for nucleic acid extraction.

b. Tissue storage (see Cancer Center Tissue Facility): Many different protocols for tissue storage are acceptable. One method which we use routinely is to store the tissue in OCT (Optimal Cutting Temperature compound) in a cryomold cassette at –80°C. In the past we have also used immediate freezing (usually in liquid nitrogen), and then storage in liquid nitrogen or at –80°.

c. Histology: Documenting tissue histology by looking at an H&E stained frozen section is advisable prior to DNA and RNA extraction. If necessary, non-usable tissue (necrotic, non-tumor, etc.) can be removed by trimming of the frozen material prior to nucleic acid extraction. The degree of normal cell contamination of tumor tissue that is acceptable depends on one's needs. For DNA, we require >70% tumor cells, since normal tissue will dilute changes in tumor DNA. For RNA, dilution is also an issue, and a minimum threshold is also used (usually >50-70%).

d. Preparing and sectioning tissue

e. Tissue handling: Note that all fresh tissue should be handled as Biosafety Level 2 materials (wear gloves, lab coat, etc.).

1. Remove samples from –80° freezer, and put on dry ice. Do NOT immediately place them in the cryostat (cryostat is at –20°C).
2. Embed sample in OCT if not already embedded. Place cryomold in cryostat, and pour OCT into cryomold. Wait for crystals to slightly form (this may take a few minutes). Place tissue in center of mold, and make sure OCT completely surrounds tissue.
3. Snap tissue out of cryomold and place on cryostat sectioning chuck (precooled, with OCT on surface). Place metal "heat extractor" on top of tissue to freeze OCT more rapidly.
4. Fasten chuck into holder. After facing block into tissue, cut one thin section (6 microns) as a "before" slide. Label Superfrost Plus slide and place face down on section. Put slide in 100 percent ethanol and then stain with H&E. Use a scalpel to mark orientation of tissue for later trimming.
5. The H&E "before" slide should be reviewed with a collaborating pathologist. This will determine whether tissue is appropriate for use (sufficient % tumor, etc..) Any tissue to be trimmed during microdissection may be directly marked on this slide.
6. Compare slide to sample. Trim tissue and OCT as needed using Feather No. 15 scalpels.
7. Cut multiple 50 micron sections for DNA and RNA extraction. For DNA, place sections into 1.5 or 15 ml screw capped tubes. For RNA, place sections directly into RNA extraction buffer (TRIZOL or similar) for immediate homogenization.

   The number of sections needed varies with the size and type of the tissue. As a very rough estimate, approximately 50 mm3 of cellular tumor tissue generally yields about 100 µg DNA or 50 µg RNA. (Cellular tumor @ 10 mm by 10 mm, x 10 sections [0.050 mm each] = 50 mm3)

   NOTE: DNA generally shows twice the recovery of RNA from the same number of sections.
8. Cut a last 5 µm section as an "after" slide. This is used to verify that tumor remains, and that morphology did not change dramatically during sectioning. This section also represents the next "before" slide for further sectioning of the block.

DNA Extraction from Frozen Tissue Sections

Tissue collection, storage, microdissection, sectioning: See previous section.

Tissue handling: Note that all fresh tissue should be handled as BioSafety Level 2 materials (wear gloves, lab coat, etc.).

1. DNA extraction: The following protocol is based on a standard phenol DNA extraction protocol. Other protocols, and versions of this protocol, are also acceptable.
2. Take pre-cut samples out of the –80°C freezer and thaw.
3. Add 10 ml of PBS (Ca-Mg Free) in hood in BSL2 room to dissolve OCT. Invert tube to make sure all of tissue is in the solution and not stuck on the tube walls.
4. Spin down tissue 1400 rpm (500 x g) for 5 min.
5. Remove supernatant carefully watching the tissue pellet.

   Note: Repeat steps 2-4 (with 5mls PBS) if it looks like there is significant “sticky” OCT left in the tube. If you are repeating the PBS wash step you do not have to get too close to the tissue pellet the first time.
6. Resuspend pellet by vortexing. Add 950 µl digestion buffer (100 µl 10X PCR buffer [100 mM TRIS, 15 mM MgCl2, 500 mM KCl), 5 µl 0.5% tween 20, 845 µl H2O) and 30-50 µl of 20 mg/ml Proteinase K (PK, Sigma P2308).

   Note: this volume should vary depending on the size of the tissue pellet. If the pellet is bigger then add 2 ml total of buffer + PK.
7. Resuspend pellet by vortexing, and pipeting up and down and place in a shaking 50°C water bath overnight. The next day make sure all the tissue has been digested. If necessary, add more PK buffer and allow to digest for a few more hours.
8. Split each tube into two 1.5ml tubes (500 µl per tube).
9. Add 500µl (or equal volume) of room temp Phenol/Chloroform/Isoamyl Alcohol (PCI from Amaresco) into each tube and vortex for 10sec.

   Note: PCI is the lower organic layer.
10. Centrifuge at 14000 rpm (max) for 2 min at room temp.
11. Remove aqueous layer into a new tube and repeat the PCI extraction (steps 8-10).
12. Aliquot the aqueous phase into as few 1.5 ml Epindorf tubes as possible. Maximum volume per tube is 350 µls. Add 1/2 volume of 7.5 M ammonium acetate and mix.
13. Add 2.5 X 100% ethanol, mix by inversion. Leave at RT for 2 hrs, or ON at –20°C.
14. Centrifuge at 14000 rpm for 15 min @ 4°C.
15. Decant supernatant immediately.***Note: watch for the pellet***
16. Wash pellet in 70 ul of 100% ethanol. Make sure you rinse sides, rim of tube. Spin at 14000 rpm for 5 min and dump supernatant.
17. Blot with Kimwipe. Air dry pellet.
18. Add 20-50 ul of TE. The volume will depend on the pellet. (avg ~ 30 µl)
19. Leave at RT for 2 hrs or ON.
20. Place tubes into 65°C for 1 hour (to inactivate DNAse).
21. Combine tubes. Rinse out “empty” tubes with 20 µl TE (the same 20 µl can be used to rinse out all tubes).
22. Measure DNA concentration using a fluorometer with a known standard DNA solution. Very small amounts of DNA can be quantitated by TaqMan analysis with a standard assay.
23. Store DNA at 4°C for short periods, or colder for longer periods. Repeated freezing and thawing may lead to shearing of DNA into smaller pieces.