DAPI Nuclei Prep from Solid Tissue Samples

Date:_____

[Sample(s) Info:

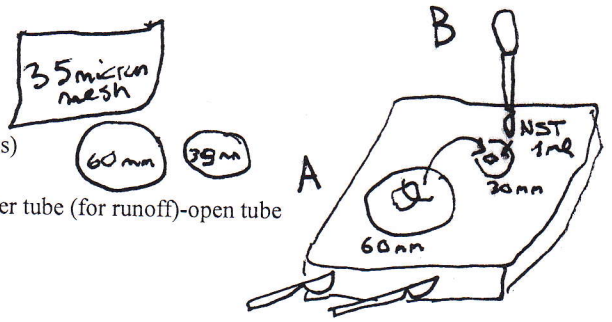
1. **Take out:** (*consider all tissues to be pathogenic*)
   - a. 500ml beaker with ~300ml of bleach
   - b. Clean bench pad
   - c. Box of scalpels
   - d. 35 and 60mm nunclon dishes
   - e. 5ml culture tubes (polystyrene)
   - f. Small round styrofoam for dry ice/sample
   - g. Ice pack for under tissue in dish

   TURN OFF LIGHTS IN HOOD *DAPI is light sensitive

   - h. Forceps for handling mesh squares
   - i. P1000(set to ~1ml) and P10(set to 5µl)
   - j. Ice bucket with wet ice
   - k. Multi-side tube rack
   - l. RNase from -20°C and DAPI/ NST Buffer from L.R.'s 4°C 200ug/ml in 1.5ml tube
   - m. **TISSUE SAMPLES on dry ice**

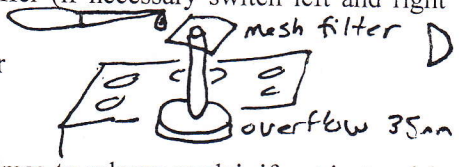
Filter 35µm
Small Parts Inc
CHN-35-D
monofilament cloth
Nylon Mesh
35µm 12x24"

2. **Set up hood as follows:**
   - a. Bench pad down
   - b. Ice pack (with small kimwipe on top) in front
   - c. Bleach beaker to the upper center
   - d. 60mm dish on top of icepack (use lid for deflecting any flying tissue particles)
   - e. 2 scalpels resting on open 60mm dish
   - f. Label 5ml tube prior to starting, put in tube rack with lidless 35mm dish under tube (for runoff)-open tube
   - g. Keep tissue in dry ice (upper left)—**DO NOT LET THAW!!!**



3. **Preparing tissue for sorting:**
   - a. Carefully open stock tube of tissue and dump/tap out tissue into 60mm dish on ice pack - block half the dish with lid
   - b. Using 2 scalpels: use left to stab tissue and hold in place and use right to cut small piece (~2mm square) [use tip of scalpel to snap frozen piece off]
   - c. Put remainder back in original tube, close tube up and **place tissue immediately back on dry ice**
   - d. **Take tissue in dish off of ice pack and put at a tilt on "yellow folded rubber pad"**
   - e. Take up **~~1.0-1.5~~ml of DAPI/NST Buffer** (depending on tissue) and add buffer on top of tissue in 60mm dish(ex. 1ml or "normal control" and ~1.5ml for "sample")
   - f. Take small pieces off of tissue and move to edge of buffer, while holding smaller piece steady in dish with left scalpel, use right scalpel to cut/pull apart tissue into tiny pieces mixing with the buffer (if necessary switch left and right scalpels to keep right scalpel sharp, or take a new one)
   - g. Repeat cutting up smaller pieces until all of larger piece is cut up into the buffer
   - h. Put both scalpels into the bleach
   - i. **Place a small ~37µm mesh square over the open 5ml tube using forceps**
   - j. Use 1ml tip to pick up buffer/sample→pipet up and down if possible a few times to release nuclei, if not just swish over pieces with buffer
   - k. Pick up buffer/sample, leaving behind as many of the big pieces of tissue remaining (but collecting droplets around pieces attached to plate)
   - l. Very carefully, steadily and slowly add droplets of buffer/sample above the mesh (do not touch tip to mesh)→start releasing droplets slightly towards one edge and let drips start to flow through
   - m. Once started add buffer/sample slowly making sure it drips down the inside of the tube - also if it begins to collect on top of the mesh or run to one side→carefully lift mesh up by corner and drain liquid into tube→replace with new mesh and continue filtering sample (if some of the liquid drips outside of the tube into 35mm dish, after filtering through mesh, then when finished carefully pipet the liquid off the bottom of the tube and from dish back into tube) (make sure there is no bubble at the top of the tube after removing a mesh square, if so pop it with the corner of the mesh)
   - n. After filtering ~<1.0-1.5ml of sample, add **5µl of RNase** [200µg/µl], mix by tapping and place tube on wet ice
   - o. REPEAT FOR EACH TISSUE TO PREPARE WITH NEW SETUP (ex. Dish, scalpels and tubes)
   - p. Bring samples to "FAC Sorting facility" (bring folder for reports) **(IF STOREING FOR LSR ANALYSIS, THEN ADD 8-10% DMSO TO SAMPLE THEN FREEZE AT -80°C)**

1-2ml depending on size



4. **Cleaning up:**
   - a. Make sure all items that touched tissue have been bleached
   - b. After all plates (remaining pieces and mesh) have been sitting with bleach for while, poor off into beaker
   - c. Poor off bleach into new empty beaker→poor bleach down the sink with running water
   - d. Remove all scalpels from bleach beaker to sharps container
   - e. Remove any other items from bleach beaker to the bench pad
   - f. Wrap all tubes, dishes and etc. in a package of the bench pad and tape and dispose in biohazard box
   - g. Clean hood really well