

Tissue Grinding in Liquid Nitrogen

Assemble the following:

- 1) Large square Styrofoam box with 4 aluminum block tube holders (in two stacks of two with holes up).
- 2) Two med-sized ceramic mortars with matching pestles.
- 3) Cotton liners and a pair of large vinyl gloves for your hands.
- 4) Paper towels sufficient to provide insulation for your hands while working the pestle and stabilizing the mortar during grinding.
- 5) Notebook and pen to record tube numbers and information written on the frozen tissue packets that you will start with.
- 6) Delrin rack, a small bag of 2-ml eppendorf tubes, 18 gauge needle, sharpie pen for numbering the tubes, small spatula for transferring tissue powders from the ceramic mortar into the 2-ml tubes, forceps.

Set-up:

Pour liquid nitrogen (N) into the Styrofoam box to a depth sufficient to just cover the stacked aluminum blocks.

Place the two ceramic mortars and pestles into the liquid N.

There will be some vigorous boiling as the metal and ceramic pieces are cooled.

After the boiling settles down, add liquid N to bring the level in the box to within 1 inch of the top surface of the stacked aluminum blocks.

Once this is all set up, it will last for a couple of hours worth of work before you may need to add more liquid N.

Label the tops of the tubes as instructed by the owner of the tissues you are grinding and aliquoting. Record the numbers in a column in your notebook where you will later enter matching information that is marked on the foil packets containing the whole tissues. *Be very careful with your book-keeping. Do not 'streamline' or 'automate' in such a way that there is any possibility for mix-ups. The grinding process comes after someone has spent months planning, running, and sampling an experiment. Work carefully and conservatively.*

CRITICALLY IMPORTANT SAFETY STEP: Use the syringe needle to punch a single hole through the center of the eppendorf tube cap. **Do not forget this step.** If you

forget to do this, there is a good chance that you, or someone accessing the tubes later will explode a tube with sufficient force to put out an eye, not to mention, lose the sample.

Keep the labeled tubes in the Delrin rack at room temperature until it is time to chill the tubes that are 'next in line' for aliquoting the a frozen powder.

Proceed to the tissue grinding:

General objective: *ENSURE THAT THE SAMPLE IS KEPT AT LIQUID N TEMPERATURE (OR VERY NEAR THAT TEMPERATURE UNTIL THE SAMPLE IS FULLY CRUSHED TO A VERY FINE POWDER AND PLACED INTO THE -80°C FREEZER FOR LONG-TERM STORAGE.*

Segregate the first foil-wrapped sample you are going to grind from the other foil packets. The system for how you do this will vary with each researcher. KEEP THE PACKET IN THE LIQUID NITROGEN AT ALL TIMES. NEVER PLACE A PACKET OR BOX OF PACKETS ONTO A WARM SURFACE, **NOT EVEN FOR A FEW SECONDS.**

- 1) Record packet information in your notebook in a column next to the tube number that will be assigned to however many identically numbered tubes you will need for the powder from that sample. For example, if you need 'n' tubes for sample 1432, enter 1432 just once in your record along with (n) for however many tubes received that number. Next to that, enter the packet information.
- 2) Place the designated tubes for the first packet into one of the aluminum blocks in the Styrofoam box.
- 3) Remove one of the mortar and pestle sets from the Styrofoam box, empty nearly all the liquid N back into the Styrofoam box and place the two items together on a ½ inch stack of paper towels.

The Grinding Process:

Immediately transfer the tissue to be powdered into the mortar for grinding. THE OBJECT OF GRINDING IS TO END UP WITH A UNIFORM, LIGHT GREEN OR TAN POWDER WITH NO OBVIOUS 'PIECES'. UNIFORM VERY VERY SMALL PARTICLE SIZE IS THE OPERATIVE HERE.

For about 30 seconds, use firm vertical pressure with a rocking motion to break harder tissue pieces into smaller pieces. Your free hand should be used to prevent brittle tissue pieces from flying out of the mortar during this step.

For about 3 minutes, continue to break up and grind the tissue.

- 1) Keep the tissue pieces/powder as low in the mortar as possible.
- 2) Do **not** add more liquid N after the initial ¼ inch or so evaporates off.
- 3) Periodically take the liquid N-chilled small spatula out of the aluminum block and use it to brush tissue pieces from the barrel of the pestle back into the mortar.
- 4) Use moderate force on the pestle (no need to apply anything that remotely resembles maximum brute force that causes you to tire after a few samples) to move the powder around and around the bottom of the mortar as it is crushed.
- 5) Vary your motion, or increase force if you see after about a minute of grinding that you still have a non-uniform chunky mixture of tissue pieces.
- 6) If you do not have a fine powder after 3 minutes, you really are being overly light handed, or you are too restricted in the range of your motion. The point is to apply enough force to do the job, while remembering that rhythmic, constant, **lateral/circular motion** as well as **vertical force** are what break down the tissue.

Aliquoting the Frozen Powder:

- 1) Use a liquid-N chilled spatula to gather the powder from the sides and bottom of the mortar toward the pouring spout.
- 2) Place a paper towel over the mortar to prevent ice condensation on the powder, and transfer the aluminum block with the designated tubes from the Styrofoam box to the paper towels that your mortar should be sitting on.
- 3) Dip the end of the spatula into the liquid N for a few seconds to chill it thoroughly, remove the paper towel that is covering the mortar, and begin to push the powder from the mortar into the designated tubes with minimum spillage. It is quite easy to do this without spillage, but it takes careful, deliberate motion. Remember; **slow** and **deliberate**, not fast and sloppy!
- 4) Close the lid of the tube after using your gloved fingers to warm the hinge sufficiently so that it does not snap apart. This 'hinge-warming' step should be applied as the tube sits in its hole in the aluminum block.
- 5) Place the aluminum block w/ closed tubes back into the Styrofoam box.
- 6) Empty loose residual powder by tapping the mortar upside down over paper towels, and then use a single laboratory tissue to wipe clinging residual powder

from the mortar into the trash. Place the wiped mortar into a corner of the Styrofoam box.

- 7) This mortar will take a few minutes to truly re-equilibrate with the liquid N. For that reason, we alternate between the two mortars as we go through the samples.

Move on to the next sample.