Procedure for grinding and quenching tissue samples for a single extraction.

This procedure is developed for preparing tissue slices (samples smaller than 50 mg wet weight). The MeOH rinse can also be used to quench a subsample of a larger tissue sample after the bulk of the ground tissue has been transferred to a bar code tube (see SPEX grind procedure for Bulk tissue and replace RNA later rinse with MeOH rinse).

Material:

1. Prepare 3x polystyrene boxes for the following usage:
    a. Extra LN_2 to fill the grinder and keep the other containers full. With a plastic beaker for dipping out the LN_2.
    b. Pre-cool cell assembly and extra sample storage
    c. Sample tube storage with a sample box (cardboard with a grid, preferably one with holes in the bottom).
2. One flat tub of LN_2 for grinder cell assembly and sample handling. With a cardboard grid (to hold the sample tubes), a white, 24-place microfuge tube rack (for holding the weigh boat), and a 9-place 50ml tube rack (for holding the grinding tube).
3. Grinding cell End-Plug puller, weigh boats, grinding cell assembly parts, large tweezers, and tray of warm detergent in water.
4. 50:50 MeOH:H_2O (1 ml per sample), 100% Acetonitrile, Tris master mix prepared as 0.51:0.2 H_2O:0.2 mM Tris (.71 ml per sample), 1ml pipette and tips.
5. Ice tray for the quench solutions and quenched samples.
6. 15 ml Sardsted tubes labeled for the quenched tissue samples.

Preparation:

Night before (or enough time for the methanol to completely evaporate):

Wash all parts of the cell with methanol and let dry between paper towels and Kim Wipes. Prepare 10% more cells than you anticipate needing, in case of breakage or some other mistake.

On the day:

Fill SPEX grinder with LN_2 until filling line and let it cool down. You will need to top up again before experiment because the chamber is at room temp before hand. Always keep the grinder partly closed so that the part that holds the sample is submerged in LN_2. Never allow the level of LN2 in the grinder to fall below the sensor, or it will need to be fully warmed and dried before it will work again.
Transfer tissues from -80 to LN_2 in the polystyrene box you prepared.

For each sample:
Insert bottom plug to the cell body and place, with top plug and impactor separately, in the polystyrene box you prepared with LN_2 to precool. **Note:** don't put more than 2 sets in LN_2 at a time, so they don't bang into each other. Make sure the bottom plug is inserted before placing in LN_2. When bubbles stop forming around the cell, it is cooled.

Check the level of LN_2 in the grinder and add if needed.

Empty the LN_2 from the cooled cell and place it in the tube rack in the flat tub you prepared. Transfer the tissue sample from the snap cap tube to the grinding cell. Remove the grinding cell from the holder and support it at an angle in the corner of the tub, with the bottom (where the sample is) submerged. Carefully add impactor, followed by top plug (make sure there is no LN_2 in side the cell before top plug assembly). The assembled cell can be submerged horizontally in the LN_2 until time to insert it in the grinder.

Carefully lift the assembled cell, supporting the bottom so it cannot fall apart. Insert cell in to the SPEX grinder and press run button on screen. (Make sure LN_2 is filled up in the chamber. If you need to fill it, put the cell with the sample back in the tub, submerged in LN_2).

Set a weigh boat on the tube rack in the tub. Add 1.79 ml 100% Acetonitrile to the 15 ml quench tube labeled for this sample.

When grinding is finished, take out cell with both hands, tap the cell to make sure all of the tissues are at the bottom and transfer it vertically in to the rack in the LN_2.

Remove top plug with Tool (black) or your gloved hand (make sure you do not lift the grinder cell up), and place top plug in the weigh boat.

Add 1ml of 50% MeOH to the cold grinding cell and seal with top plug. Take the cell and the weigh boat over to the ice tray you have prepared.

Hold the cell horizontally and gently massage the cell between two palms, while 50% MeOH is melting, tilt the cell on both sides to collect all residual tissue. You can roll the cell so that the impactor rolls over all parts of the inside of the tube.

As soon as the solution is thawed, remove the grinding cell top plug and the impactor and place in the weigh boat. Transfer the 50% MeOH to the 15 ml quench tube that has been pre-loaded with the 1.79 ml 100% Acetonitrile on ice. It is critical to get the tissue into the Acetonitrile as soon as possible.

Replace the impactor back into the cell and add 0.71 ml of your Tris master mix. Reinsert the top plug and roll the cell so that the solution rinses all parts of the cell and impactor. Remove the top plug and, using the same pipet tip you used for the first transfer, transfer this solution to the 15 ml quench tube. You should have a total of 3.5 ml of quench solution that is 51% Acetonitrile and 14% MeOH (remainder water with Tris).

Detergent wash all grinding cell parts and air dry. Freeze quenched tissue samples upright in -80 freezer until extraction (within a week) or proceed directly to extraction.