Grinding Protocol:

To start the grinding, here are the things you will need:

- Bucket full of ice
- a plastic grinding tip to fit a microcentrifuge tube
- a power screw driver
- Timer
- Thermometer
- Heating Block
- Kimwipes
- Samples in microcentrifuge tubes
- 2 Blue Aluminum Blocks:
  i. One of which is always kept cold at -20 ^{o}C (Freezer)
  ii. The other one is heated to about 100^{o}C (on heating mantle).

Procedure

Before starting this procedure, the aluminum blocks must be brought to the proper temperatures. The cold block should already be in the freezer. Be sure to put it onto ice right away when removing it. The hot block must be heated to 100 degrees. Place it on a heating block set to the proper temperature. Keep a thermometer in the center hole of the block so you can see when the block is up to temp.

Label all the microcentrifuge tubes with the sample identification information using a Sharpie ultra-fine point marker. Do not use any other brand or type of marker. You will need three tubes for each sample, one for grinding and two for duplicate storage of the clean supernatent.

1. Place the cold Aluminum Block very firmly within the ice, making sure that the block doesn't move while you grind.

   - The surface of the block should fall slightly higher than that of the ice. This way, you will also know that no potential ice bits could go in the sample tubes.

2. Now, place the sample tubes into the spaces in the Aluminum Block. You can place four sample tubes at a time (This will allow you to grind more quickly without having the sample tubes thaw too much).

3. Place a clean Blue tip onto the end of the Black & Decker screwdriver. Insert this into a tube and grind away!

   - The final result of the grinding should resemble a homogenous mixture with **little or no chunks** at all!

   - The blue tip will catch some of the ground mixture, try to keep as much in the tube as possible by tapping the blue tip onto the opening of the sample tube.

4. **RINSE OFF THE BLUE TIP WITH WATER AND THEN DRY IT** before placing it onto the next sample tube. This prevents undesirable cross contamination between the tubes!

Read the entire **step 5**, including the notes before proceeding!

5. Now place the tubes in the 100^{o}C hot Aluminum Block. Let the samples boil for 5 minutes, with the caps **OPEN.** If not open, the pressure build-up will cause the samples to burst open and the extract will spill over.

    - The tubes can experience thermal shock, being that they are taken from a cold to a hot medium. They tend to stick to the aluminum Block. If you wipe the tubes with a kimwipe before placing them in the hot block, you'll prevent this from happening!
    - **Note**: if you are using the heating block for the fist time, make sure the thermometer reads 100^{o}C, before starting the timer.

6. After boiling, place the samples into a centrifuge for 20 minutes at 13 krpm and 4^{o}C.
    - If your centrifuge is not in the immediate area, place the sample tubes back into the cold block to transport them (the cold block should still be on ice when you transport!).

7. DO NOT SHAKE the tubes after centrifugation. Now, using a pipette, transfer the supernatant from each tube into its corresponding new tubes. Start with the pipette set at 10ul. Pipette multiple times, alternating between the two duplicate sample tubes for that sample until all that's left in the original tube is the bottom pellet (Junk)

    - You will use the supernatant when you determine the protein concentration.

    - If you are not determining protein concentration immediately, store the supernatant at -80 ^{o}C (in the ultra-low). Keep in mind that once in the ultra low, the samples should only be taken out when they are going to be immediately used for determining protein concentrations… Thawing and refreezing multiple times will result in BAD samples. You can get away with refreezing once after the original storage, but avoid it if possible or Imene will haunt you in your dreams.

8. Once the supernatant is extracted, the leftover tubes can be thrown away!

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