**Titin Sample preparation: Solubilizing (~2-15 mg) Tissue Samples in 8M Urea Buffer**

**Materials:**

* 2 mL Kontes Dounce style homogenizer with close fit pestle (stored at -20°C)
* [8M Urea buffer](#Urea_Buffer) (stored at -20°C)
* [50% Glycerol](#Glycerol) with 4X Leupeptin, E-64 and PMSF inhibitors (stored at -20°C)
* Tabletop test tube incubator set to 73°C)
* Tube of Ice and Liquid nitrogen

**Methods:**

1. Cut the samples in liquid nitrogen and place them in the appropriate pre-labeled and pre-chilled tubes.
2. Obtain weights (mg) of each sample by weighing on a small ice block cooled with liquid Nitrogen. Make sure the tissue is not thawed and is on liquid nitrogen all along. After weighing place the tissue into the respective tube. This process can be done on day 1 if the numbers of samples are many. \*Aim to obtain as close to 10 mg of sample\*
3. Allow 8M urea buffer to thaw and place 50% Glycerol on ice. You will be add the 8M urea buffer at room temp and adding the 50% Glycerol cold.
4. Cool Dounce style homogenizer in liquid Nitrogen.
5. Quickly, add pre-weighed out tissue to the Dounce style homogenizer and grind the tissue sample by placing the Dounce style homogenizer on the table and pushing down/twisting until the tissue becomes completely ground and extremely smooth. (10 seconds in liquid Nitrogen and, 10 seconds out, for a maximum of 5 minutes or ground fine, whichever is earlier.
   * Do not take out the pestle from mortar after grinding. Keep the unit intact until it is further processed.
6. Temper sample in -20°C for a minimum of 10 minutes (max is 60 min) on ice.
7. After 10 minutes, bring out the first sample. **Add 40 times amount of 8M Urea buffer** to the sample by running down tube.
   * **(weight of sample in mg \* 40 = amount of 8M Urea buffer in uL)**
8. Repeat by **adding equal amount of 50% glycerol + protease inhibitors buffer to the sample**. Mix the sample with the buffer slowly 10 times up and down. NO BUBBLES!!!
   * **(weight of sample in mg \* 40 = amount 50% glycerol in uL)**
9. Transfer sample into a pre labels 1.5 mL centrifuge tube. VORTEX.
10. Incubate the sample @ 73°C in tabletop heater. Incubate for at least 10 minutes, no more than 15 minutes. Vortex every 5 minutes for 10 at least seconds.
11. After incubation, plunge the sample with a syringe and a 21 G or 23 G needle. Plunge at least 5 times.
    * This shears any large stands of DNA
12. Centrifuge sample at maximum speed of tabletop centrifuge (roughly 14.9 k) for 5 minutes.
13. Pour supernatant into a pre labeled cryogenic tube and flash freeze in liquid Nitrogen and store at designated location in -80°C.
14. Keep a record of sample location along with above pertinent information.
    * Wash all glassware in 0.2% SDS, rinse with distilled water and further with nanopure water.