**Mitochondrial Isolation from Liver**

* Place the following on ice:
  + Isolation Buffer
  + PBS
  + Glass dounce homogenizer
  + 6 sorval tubes
    - 2 labeled WT, 2 labelled Het and 2 labeled KO
* Turn on Sorval, insert the Ss-34 rotor and cool to 4oC
* Remove livers from -80oC and immediately add 10mL of ice cold PBS to the tissue
  + Record any animal information / notes:
    - WT =
    - Het =
    - KO =
* Dump off PBS and place livers in weigh boat
  + Record weights
    - WT =
    - Het =
    - KO =
  + While tissue is in weigh boat, use curved scissors to mince the tissue (~11 cuts)
* Add 5mL of isolation buffer to the minced tissue, swirl and dump into the dounce homogenizer
  + Homogenize on ice (~7 pumps)
    - Dump homogenate into properly labeled Sorval tube
      * Wash dounce with an additional 5mL of isolation buffer and add to Sorval tube as well (total volume = 10mL)
        + Repeat with each liver sample
* Spin samples for 7’ @ 500g
* Following the spin, dump off the supernatant into the next appropriately labeled, pre-cooled Sorval tube and spin samples for 10’ @ 9400rpm
* Following this spin, dump off the supernatant into a waste container and slowly wash the pellet (do not re-suspend) by rinsing with 10mL of fresh isolation buffer.
* Spin the washed pellets again for 10’ @ 9400rpm
  + After spin, dump off the supernatant (wash) and re-suspend the pellet in TNEB (+ fresh protease inhibitors) based on the size of the pellet
    - Size of a dime = 500uL
      * Record volume used for re-suspension:
        + WT =
        + Het =
        + KO =
      * Back pipette and record the total volume of the pellet following re-suspension:
        + WT =
        + Het =
        + KO =
* Prepare for protein assay using the following dilutions:
  + 1:25
  + 1:50
  + 1:100
    - From protein assay - we need to calculate the volume of sample needed for 2mg/mL total protein (as many tubes as we can possible make...)

**Isolation Buffer:**

100mL of 10mM EGTA (pH = 8.0) [Final] = 2mM

5mL of 1M Tris-HCl (pH = 7.5) [Final] = 10mM

42.79g of sucrose [Final] = 250mM

Bring up to 400mL

pH to 7.4

Bring up to 500mL final

Store at 4oC