Tissue Fractionation via Differential Centrifugation

Fractionation Buffer (FRAC):

	Molecular Weight	mM Concentration	For 50mL Stock
HEPES	260.27	20	260.27 milligrams
Sucrose	342.3	250	4.28 grams
EDTA	292.23	0.1	1.46 milligrams

HEPES (260.27mg) ➔ pH to 7.4 ➔ Sucrose (4.28mg) ➔ EDTA (1.46mg)

A 50mL stock solution should be made without inhibitors. A working solution should be made fresh the day of harvest with 2 protease tablets and 200uL phosphatase 2&3 per 10mL FRAC buffer.

Fractionation Procedure:

1. Remove tissue under anesthesia and weigh.
2. Mince sample with razor blade on parafilm into ~1mm3 pieces and place sample in a 5mL polyethylene tube on ice.
3. Add 15 volumes FRAC buffer to weight (1.5mL for a 100mg muscle) to tube.
4. Homogenize the sample with a polytron homogenizer at 40% power for 15seconds in an ice-water bath.
5. Immediately transfer homogenate to microcentrifuge tube on ice labeled P_0.
6. Centrifuge sample at 800g for 10 minutes.
7. Transfer the supernatant, S_0, into a microcentrifuge tube on ice labeled S_1.
8. Resuspend the original pellet, P_0, in 5 volumes FRAC buffer and transfer to a 5mL glass homogenizer in an ice bath. Homogenize resuspended pellet with 10 passes and transfer resulting homogenate through taught cheese cloth into a microcentrifuge tube on ice labeled P_1.
9. Centrifuge P_1 at 800g for 10 minutes and discard resulting supernatant. **Nuclear Fraction**
10. Turn attention to original supernatant, S_1, from step 7. Centrifuge S_1 at 9000g for 10 minutes.
11. Pipette the resulting supernatant into a separate tube on ice labeled S_2. **Cytosolic Fraction**
12. Resuspend the resulting pellet, P_2, in 2 volumes FRAC buffer and centrifuge at 11,000g for 10 minutes. Discard supernatant and keep pellet. **Mito Fraction**

Fractions into Sample Buffer:

For each sample, use best judgment on volume of 2x Laemmli sample buffer with phosphatase and protease inhibitors to obtain a suitable protein concentration.

For 100mg GA muscle the following ratios work well: 200uL 2xSB for nuclear fraction, 30uL 2xSB for mitochondrial fraction, 100uL 4xSB in 200uL cytosolic fraction. Heat fractions for 5 minutes at 95°C and store at -80°C.