**LEE LAB RESEARCH LABORATORY**

**GENERATION OF LYSATE INOCULATION MATERIALS**

**Soluble/Insoluble lysate preparation from harvested brainstem/spinal cord tissue:**

1. Tissue acquired from 4-month-old asymptomatic TgA53T (Line G2-3) and end-stage tissue harvested and stored at -80C prior to use.
2. Tissue weighed and suspended in 0.9% sterile saline (1:10 w/vol)
3. Homogenize and centrifuge for five minutes at 3000xg at 4C.
   1. Supernatant (S3000)
4. Centrifuge S3000 for 45 minutes at 150,000xg at 4C
   1. Supernatant (S150) – Highly soluble fraction
5. Wash pellet and resuspend in sterile saline (half of original volume) by sonication (3x10s pulses)
   1. Resuspended pellet (P150) – Insoluble fraction

**Endoplasmic Reticulum (ER)-enriched fractionation:**

1. Homogenize freshly harvested tissues (1:10 w/vol) in lysis buffer (250 mM sucrose, 20mM HEPES, 10mM KCl, 1.5mM MgCl2, 2 mM EDTA, protease-inhibitor cocktail)
2. Centrifuge at 1000xg and collect supernatant
3. Centrifuge at 10,000xg
   1. Pellet = mitochondria
4. Centrifuge supernatant at 100,000xg
   1. Supernatant = cytosol
   2. Pellet = ER