Protein Homogenization & Extraction Protocol

**Equipment/Materials:**

* Sample size: 20-30mg
* Lysis buffer: ~~2% SDS, 10 mM EDTA, 6 mM Tris-HCl, pH 7.4 with~~ protease inhibitors (25 μg/mL aprotinin and 10 μg/mL leupeptin)
  + NOTE: when practicing, do not include protease inhibitors
* FastPrep-24 5G Tissue and Cell Homogenizer (MP Biomedicals; catalog #116004500)
* Lysing Matrix-D tubes (MP Biomedicals; catalog #116913050)
* Fisherbrand™ Mini-Centrifuge 100-240V, 50/60Hz Universal Plug, Grey
* Sorvall Legend Micro 21 Centrifuge with 24-sample rotor
* Ultrafree-MC Centrifugal Filter Units (0.5mL filter tubes with 5.0µm microporous membrane and 2mL collection tube) (EMD Millipore Corp.; catalog #UFC30SV00)

**Procedure:**

* Sample collection:
  + Collect 20-30mg of frozen heart tissue from the apex
* Homogenization: 24 sample maximum
  + Place each frozen sample into a Lysing Matrix-D tubes.
  + Add in cold lysis buffer (1mL buffer:50mg tissue) to suspend sample in tube
  + Load samples into FastPrep-24 5G Homogenizer
  + Homogenize at velocity of 6.0m/s for 40s at 15,000x*g* (1 cycle)
  + Remove Lysing Matrix-D tubes from homogenizer and place samples on ice
  + Spin down Lysing Matrix-D tubes in mini-centrifuge for 1-2mins to condense foam and increase yield of liquid homogenate
* **Filtration:**
  + Transfer homogenate from Lysing Matrix-D tubes into centrifugal filter units.
    - Simply dump the entire contents of the Lysing Matrix-D tubes into the Centrifugal Filter Units, beads and all.
  + Load Centrifugal Filter Units into Sorvall Legend Micro 21 Centrifuge rotor
  + Centrifuge samples 15,000x*g* for 12min.
  + Once centrifuged, remove the top filter unit and place the 2mL centrifuge tube on ice.
* **Protein Preparation & Quantification:**
  + Need a method to quantify protein and standardize so we are running the same amount every time.