PREPARATION OF PROTEIN SAMPLES

1. Grind tissue in liquid N$_2$ and transfer to appropriate volume of homogenization buffer in a 30 ml tube. Use 3 ml buffer per g tissue.

2. Filter samples through cheesecloth into microfuge tubes (two tubes per 1 g sample) and spin 10’ in a microfuge.

3. For soluble proteins, add 0.6 x volumes 2x Stop Dye to the supernate. i.e 500 µl supernate plus 300 µl dye.

4. For insoluble proteins, extract the pellet with 0.1 x volume of original homogenate with Pellet Buffer. i.e. for 1 g of tissue, add 300 µl of buffer to each tube, mix and add 180 µl 2 x stop dye.

5. Boil both samples for 10’ and then place on ice before loading on a gel. Store at -70°C.

Protein concentrations should be approximately 2 mg ml$^{-1}$ from young leaves but do a Bradford assay if concentration is critical.

**Homogenization Buffer** (50 ml) (make fresh)
- 18% sucrose: 15 ml 60%
- 10 mM MgCl$_2$: 0.5 ml 1 M
- 100 mM Tris-HCl, pH 8.0: 2.5 ml 2 M
- 40 mM β-mercaptoethanol: 140 µl

**Pellet Buffer** (10 ml)
- 2% SDS: 2 ml 10%
- 6% sucrose: 1 ml 60%
- 40 mM β-mercaptoethanol: 28 µl

**2 X Stop Dye** (20 ml)
- 1% SDS: 2 ml 10%
- 0.1% bromophenol blue: 2 ml 1%
- 10 mM EDTA: 400 µl 0.5 M
- 20% Ficoll: 4 g