Protein Extraction Protocol
Version: 3-24-08
Modified by Jessie after receiving recipes from Ung

I. Recipes

2X Sample Buffer (50ml)

i. 6mL 1M Tris (pH=8)
    1. 121.1 g Tris
    2. 750 mL DD water into graduated cylinder
    3. 40 mL HCl (add slowly)
    4. Adjust to pH 8.0 by slowly adding HCl
    5. Adjust final volume to 1L
ii. 10mL 20% SDS
iii. 15g Sucrose
iv. 0.06g E-Aminocaproic Acid
v. 0.018g Benzamidine
vi. fill to 50mL with ddwater.

Immediately before using

vii. dilute to 1X working buffer
viii. add DTT at 0.5g per 50mL of buffer (for 65mM final conc.)

II. Extraction Procedure

- Time: Day 1 - 2 Hours
- Equipment and Supplies:
    - Homogenizers (pestles)
    - Centrifuge
    - Micro-centrifuge Tubes
    - Heater (w/heater block)
    - Cooler of dry ice
    - Cooler of ice
- Steps:
    1. Set up equipment:
        i. Turn on heater with block for microcentrifuge tubes.
        ii. Set to 95C.
        iii. Set-up and label ALL microcentrifuge tubes necessary for process.
    2. Prepare sample buffer for use including dilution to 1X working buffer (see steps vii and viii in recipes above)
    3. Add 100uL of buffer to each tube
    4. Move frozen tissue samples to be used to a cooler with dry ice. Keep these frozen!
    5. For each individual tube:
        - place tube with buffer on the scale, then tare.
        - Working quickly, remove a piece of frozen tissue (10-15mg) from the sample tube and submerge it in the buffer.
        - Weigh the leaf tissue and record the weight.
        - Using a pestle, grind the leaf in the buffer. Make sure tissue is completely ground.
        - Place the tube on regular wet ice
    6. Boil for 5mins @ 95^{o}C using heater with block drilled for microcentrifuge tubes. After the first two minutes are up, open the caps and close them up again.

    i. Do this for all the tubes.
    ii. This step, you will notice, is just to relieve the pressure buildup that occurs when heating.
7. Centrifuge for 20 minutes @ 13,000 rpm
8. Move tubes back to regular wet ice and keep them on ice for remaining steps, until they are frozen.
9. Transfer (with a pipette set at 10ul) supernatant to new tubes. (Discard old tube w/ pellet at bottom).
10. For storage, split into two or three aliquots so that you can thaw one at a time.
11. Store in Ultra Low (-80C) and DO NOT thaw and re-freeze multiple times!