# ISOLATION OF NUCLEI.

**(Crude prep for chromatin not run-offs))**

1. Grind 10 g leaves in liquid nitrogen

2. Resuspend in 25 ml **Isolation Buffer &** stir until temperature reaches –10°C.

3. Filter through 60 μM nylon mesh. To make a filter, cut a 50 ml Falcon tube in half and cut out the centre of the cap. Place the mesh in the cap and screw onto the tube. Pour the leaf bits into the tube.

4. Pellet cells by centrifugation at 10,000 g for 15' at 0°C.

5. Decant supernate, and resuspend pellet in 15 ml Isolation Buffer using a paintbrush.

6. Pellet cells by centrifugation at 10,000 g for 15' at 0°C.

7. Resuspend pellet in 10 ml **Isolation Buffer** using a paintbrush.

8. Pellet cells by centrifugation at 6,000 g for 15' at 0°C.

9. Resuspend pellet in 10 ml **Isolation Buffer** using a paintbrush.

10. Pellet as in 8 and resuspend in 2 ml **Isolation Buffer**.

11. Dispense into 100μl aliquots, freeze in liquid nitrogen and store at –70°C. (each aliquot is equivalent to 10 mg DNA)

**Isolation Buffer** (100ml)

| | |
|---|---|
| 20mM Tris pH7.8 | 1ml 2M |
| 250mM sucrose | 8.5g |
| 5mM $MgCl_2$ | 0.5ml 1M |
| 5mM KCl | 0.5ml 1M |
| 40% glycerol | 40ml |
| 0.25% triton X-100 | 0.25ml |

| | |
|---|---|
| 0.1% B-mercaptoethanol | 0.1ml |