Plant Morphology - SPURRS Propylene Oxide

Procedure for fixing and embedding plant tissue for morphological study

**Protective Equipment**; Safety glasses, Lab coat, gloves and Chemical Fume hood

Remember to **label all vials** with; sample name, fixative type, your name & date

1°Fixation:

2- 4% Paraformaldehyde/ 2% - 3% Glutaraldehyde Overnight, 4° C

 in 0.05 M Phosphate

Rinse: with 0.05 M Phosphate Buffer 3x 5 – 10 min ea

Secondary fixation(optional):

Osmium tetroxide (1-2%) 2% -1h room temp (r.t.),

 Or 1% - overnight 4° C

Rinse: With ddH2O after OsO4 3x 5 -10 mins each

Dehydration:

 30% Ethanol 10 mins

 50% Ethanol 10 mins

 60% Ethanol 10 mins

 70% Ethanol 10 mins

 80% Ethanol 10 mins

 95% Ethanol 15 mins

 100% Ethanol 2x, 10 mins each

 100% Propylene Oxide 1 - 2x, 10 mins

Infiltration: :

 SPURRS : Propylene Oxide

 1 : 2 2 hours to overnight (in hood), the denser the

 1 : 1 tissue the longer the incubation time

 2 : 1 (optional, depends on density of samples)

 100% SPURRS **overnight**, in hood **3x**

**Warning**:Don’t leave samples in 100% SPURRS for more than one night at room temperature as polymerization may occur.

Embedding: Change resin just before embedding in molds. Place paper label in mold, then sample

Curing/Polymerization: Overnight in oven in hood.