Embedding for light microscopy of GUS-stained tissues

Use this protocol for best structural preservation

1. Immediately after X-Glc stain, proceed as for standard LRW embedding, starting with 3-5 rinses in 25 mM phosphate buffer, pH 7.2, 15 min each rinse.
2. Fix in 3% glutaraldehyde in 25 mM phosphate buffer, pH 7.2, at room temperature for 90 min or longer. If necessary (should not be necessary after X-Glc stain), vacuum infiltrate first for a few min until material sinks.
3. Rinse in 25 mM phosphate buffer 4 times over approx 60 min.
4. Dehydrate in ethanol series:

Concentration of ethanol	Dehydration times
25%	1-2 h
50%	1-2 h
70%	overnight at 4°C
95%	2 h
100%	1-2 h, repeat at least 3 times

5. Infiltrate in LR White resin (medium grade):

Concentration of resin	Infiltration times
2:1 ethanol : LRW	8 h to overnight
1:2 ethanol : LRW	8 h to overnight
100% LRW	At least overnight, then repeat 3 times, at least 8 h each

5. Embed in rubber moulds or gelatine capsules. In each case, a thin layer can be polymerized first to facilitate positioning of tissue in mould. Alternatively, for large tissues, flat embed in foil planchettes. Polymerise a thin layer of resin on the bottom of the planchettes before embedding (at 70°C under nitrogen gas).
6. Polymerise in vacuum oven at 70°C for about 90 minutes under nitrogen gas (polymerisation is complete when resin is hard), adjust flow rate to give 250-300 kPa on the nitrogen tank gauge.

Waste LRW and LRW-solvent mixtures go into glass jar in fume hood.

Waste LRW-contaminated pipettes go into plastic container in fume hood.

Embedding for light microscopy of GUS-stained tissues

Use this protocol if tissue has already been rinsed and cleared in 70% ethanol

1. Proceed as for standard LRW embedding:

   Dehydrate in ethanol series:

Concentration of ethanol	Dehydration times
70%	overnight at 4°C
95%	2 h
100%	1-2 h, repeat at least 3 times

2. Infiltrate in LR White resin (medium grade):

Concentration of resin	Infiltration times
2:1 ethanol : LRW	8 h to overnight
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Waste LRW and LRW-solvent mixtures go into glass jar in fume hood.

Waste LRW-contaminated pipettes go into plastic container in fume hood.

Notes:

i. Larger and denser tissues need longer in every stage. For example, cereal grains can be fixed overnight or longer, need long vacuum infiltration of fixative, need at least 8 hours in each dehydration step (preferably longer), and need slow infiltration with resin – smaller increases in resin concentration and longer at each stage – at least a day (24 h) in each. Very hard/dense tissues may need several days to several weeks for resin infiltration.

ii. Larger tissues (as above) may need to be vacuum infiltrated in resin to remove any remaining solvent.

iii. Impermeable tissues need slower and longer infiltration or the tissues will shrink and be deformed – impermeable walls allow solvent (EtOH) to diffuse out much more rapidly than the resin monomers can diffuse in, hence cell collapse. For example, *Arabidopsis* roots show cell collapse if the resin concentration is increased too rapidly – commonly seen in GUS-stained tissues if not careful.

iv. Hard tissues such as dry grains may also need to be embedded in harder resin – hard grade rather than medium grade.