Reference   Meyerowitz Lab
(http://iprotocol.mit.edu/protocol/119.htm)

X-GLUC Protocol:

Material   X-Gluc. Stock Solution:

Add 1.92 ml N-N Dimethylformamide to 100 mg of X-GLUC to make a 100 mM solution. Store in the freezer (it will remain a solution at -20) and limit the solutions exposure to light.   The solution will last several months, and, if it has gone bad it will turn a bright red color.

Reagents Needed:

1. 0.5 M Na_2HPO_4

2. 100 mM $K_4Fe(CN)_6$

3. 90% Acetone

Working Solutions

Rinse solution:

50 mM $NaPO_4$ pH 7.2 (68.4 parts of Na_2HPO_4 with 31.6 parts of NaH_2PO_4)

0.5 mM $K_3Fe(CN)_6$

0.5 mM $K_4Fe(CN)_6$

Staining Solution:

50 mM $NaPO_4$ pH 7.2

0.5 mM $K_3Fe(CN)_6$

0.5 mM $K_4Fe(CN)_6$

2 mM X-Gluc

Make enough staining solution that will be used in a couple of days store in at 4 ^{o}C.

Procedure

a. Fix tissue: immerse in 90% cold acetone (10% H_2O), incubate on ice for 15 - 20 minutes.

b. remove acetone, rinse tissue with the rinse solution.

c. Remove rinse solution then replace with X-gluc staining solution. Totally immerse your tissue, but use no more than that amount.

d. vaccuum infiltrate 1 or more times. You do not want any air bubbles clinging onto the tissue, and you want the tissue to be in the solution, not floating at the surface.

e. Incubate o/n at 37 ºC.

Remove chlorophyll by doing an ethanol series, and do additional fixation if desired:

EtOH series: 15%, 30%, 50% (or instead of the 50%, use FAA fixative, which is 50% ethanol (recipe below), 70%, 85%, 95%, 100%, 100%. Note: Fixing the tissue with acetone prior to staining aids in the chlorophyll removal. At a convenient point, move tissue from microfuge tubes to scintillation vials.

Fixative Mix:

Ethanol 50.0% 50 ml
Acetic Acid 5.0% 5 ml
Formaldehyde 3.7% 10 ml of 37%
Water 41.3% 35 ml

Total: 100 ml

Xylene: Minimize exposure to xylene as the blue product of the staining reaction is slowly solubilized by xylene. If you leave the sample in xylene for several days, all the blue will eventually disappear.

25% xylene/75% ethanol (leave for 1 hour per step)

50% xylene/50% ethanol

75% xylene/25% ethanol

100% xylene, 100% xylene (note: if whole mounts are desired, skip the embedding and boat pouring steps)

Embedding:

100% xylene, add paraplast chips, inc at 42, keep adding chips until the vial is full, replace the xylene/wax mixture with 100% molten paraplast wax. Try to proceed from the first step of the xylene series to 100% wax within one day.

Pour boats in the usual manner

Sectioning: do 10 u sections, cook on the hot plate for at least 1 hour. Use xylene to remove the wax, again minimize the time the sample is exposed to xylene. Stain tissue if desired (not methylene blue, other non-blue stains may be ok). Mount with permount, visualization with dark field is the most sensitive.

Whole Mount:

From xylene, move tissue into a pool of permount on your slide, position as desired, put coverslip on, and use weighted vials to mash the tissue. Let dry.

Reference Meyerowitz Lab
(http://iprotocol.mit.edu/protocol/119.htm)

Overview This protocol is adapted from Jefferson, R.A., et al., 1987. EMBO 6:3901-3907. and from
Rodrigues-Pousada et al. 1993. The Plant Cell 5:897-911.