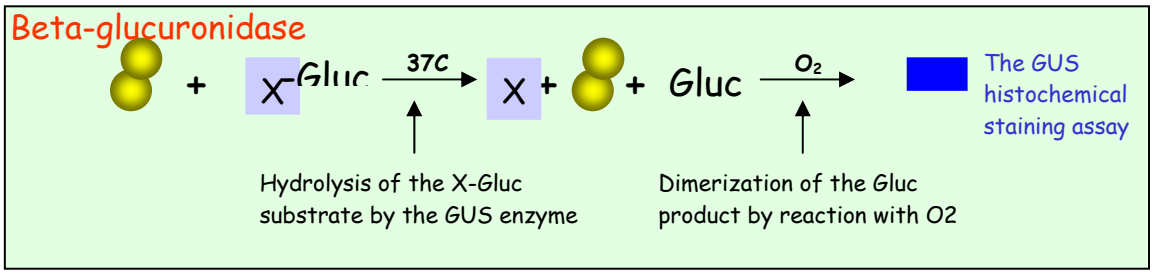
Experiment 6 for the CSS451

Histochemical GUS assay

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The best substrate currently available for histochemical localization of b-glucuronidase activity in tissues and cells is 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc). The substrate works very well, giving a blue precipitate at the site of enzyme activity.



The product of glucuronidase action on X-Gluc is not colored. Instead, the indoxyl derivative produced must undergo an oxidative dimerization to form the insoluble and highly colored indigo dye. This dimerization is stimulated by atmospheric oxygen, and can be enhanced by using an oxidation catalyst such as a K+ ferricyanide/ferrocyanide mixture. Without a catalyst, the results are often very good, but one must be concerned about the possibility that localized peroxidases may enhance the apparent localization of glucuronidase.

Procedure

1. Take fresh leaves directly from WT and transgrenic tobacco plants, respectively.
2. Cut fresh disks into quarters.
3. Transfer sections to 1ml of histochemical reagent (X-GLUC) in 1.5 ml eppendorf
4. Incubate for one hour to overnight at 37C.
5. After staining, rinse sections in 70% ethanol for at least 5 min, and if green tissues clear of chlorophyll for at least 4 hours.

200 mM $NaPO_4$ pH 7.0:
This is made by mixing two stock solutions. Stock A: 0.2 NaH_2PO_4 H_20 (31.2 g/l). Stock B: 0.2 Na_2HP0_4 (28.39 g/l). For pH 7.0, Combine 39 ml A with 61 ml B.

X-GLUC Stain:
Dissolve 5 mg 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) in 1.0 ml dimethyl formamide. Add 50 mM $NaPO_4$ pH 7.0 to 10 ml.

Tissue Fixative if fresh tissues are not used: (0.3% Formaldehyde, 10 mM MES pH 5.6, 0.3 M mannitol)

- Formaldehyde 750.0 ml
- 0.5 M MES 2.0 ml
- Mannitol 5.46 g

Store in fume hood at room temperature for 3 months, or until precipitates appear.

<u>0.5 M MES, pH=5.6:</u>
For 100 ml, dissolve 9.76 g of MES in 80 ml of dH_20. Adjust pH to 5.6 with NaOH and make to volume. Store at room temperature.