

Histostaining for Tissue Expression Pattern of Promoter-driven GUS Activity in *Arabidopsis*

Xiyan Li*

Department of Genetics, Stanford University, Stanford, USA

*For correspondence: lixiyan@stanford.edu

[Abstract] Promoter-driven beta-glucuronidase (GUS) activity is the most commonly used technique for tissue-specific expression patterns in *Arabidopsis*. In this procedure, the GUS enzyme converts 5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) to a blue product. The staining is very sensitive. Processed samples can be examined under dissecting microscope or Differential Interference Contrast (Nomaski) microscope for bright blue color over cleared transparent background. Note that this assay does not provide accurate information on subcellular levels.

Materials and Reagents

1. Transgenic plants that contain genomic integration of a promoter: GUS expression cassette
2. Potassium Ferrocyanide
3. Potassium Ferricyanide
4. Triton X-100
5. 50 mM NaHPO₄ buffer (pH 7.2)
6. Dimethylformamide (DMF)
7. Acetone
8. NaHPO₄ buffer
9. 5-bromo-4-chloro-3-indolyl beta-D-glucuronide cyclohexamine salt (X-Gluc)
10. 200 proof ethanol (once opened, 200 proof becomes essentially 190 proof)
11. Staining buffer (see Recipes)
12. Stock solutions (see Recipes)

Equipment

1. Eppendorf tubes
2. Vacuum
3. Dissecting or light microscope

4. Differential Interference Contrast (Nomaski) microscope

Procedure

1. Harvest tissue and place in cold 90% Acetone on ice. This should stay on ice until all samples are harvested. For sample containers, Eppendorf tubes and glass scintillation vials work well.
2. When all samples are harvested, place at room temperature (RT) for 20 min.
3. Remove acetone from the samples, and add staining buffer on ice.
4. Add X- Gluc to the staining buffer to a final concentration of 2 mM from a 100 mM stock solution of X-Gluc in DMF- this must be kept in the dark at -20 °C.
5. Remove staining buffer from samples and add staining buffer with X-Gluc on ice.

Note: Do not infiltrate when make LR embedding, instead infiltrate in the fixatives or 10% ethanol.

Infiltrate the samples under vacuum, on ice, for 15 to 20 min. Release the vacuum slowly and verify that all the samples sink. If they don't, infiltrate again until they all sink to the bottom when the vacuum is released.

6. Incubate at 37 °C (I usually do it for 2 h for strong promotors and up to overnight for weak promotors. It is not advisable from my experience to go too long (over two days) as the tissue seems to begin deteriorating during long incubations.
7. Remove samples from incubator and remove staining buffer.
8. Go through ethanol series from 10%, 30%, 50%, 70% (you may heat the sample to 60 °C to get rid of chloroplasts), to 95% (avoid light); 30 min each step and then finally 100%. You may store at 4 °C for up to a month, seal well.
9. Go to embedding procedure, or observe directly under dissecting or light microscope. To mount, simply apply a few drops of water to the samples.

Recipes

1. Staining buffer (final conc.) (fresh)
0.2% Triton X-100 (may be reduced to 0.05%)
50 mM NaHPO₄ buffer (pH 7.2)
2 mM potassium Ferrocyanide
2 mM potassium Ferricyanide
Water to volume

Note: Higher Ferricyanide and ferrocyanide concentrations give lower overall staining level, but more specificity. 2 mM works well for most applications, but the concentrations may need to be adjusted for certain needs.

2. Stock solutions (4 °C)
 - 10% Triton X-100
 - 0.5 M NaHPO₄ buffer (pH 7.2)
 - 100 mM potassium Ferrocyanide (store in the dark at 4 °C)
 - 100 mM potassium Ferricyanide (store in the dark at 4 °C)
 - 100 mM X-Gluc in DMF

References

1. Li, X., Chanroj, S., Wu, Z., Romanowsky, S. M., Harper, J. F. and Sze, H. (2008). [A distinct endosomal Ca²⁺/Mn²⁺ pump affects root growth through the secretory process.](#) *Plant Physiol* 147(4): 1675-1689.
2. Padmanaban, S., Chanroj, S., Kwak, J. M., Li, X., Ward, J. M. and Sze, H. (2007). [Participation of endomembrane cation/H⁺ exchanger AtCHX20 in osmoregulation of guard cells.](#) *Plant Physiol* 144(1): 82-93.