



Harvesting and GUS Histological Assay (ISSA)

This information sheet describes the harvesting procedure and GUS assay used following the *in vivo* stem and bud transformation methods. There are some differences but these relate to the way in which the samples are harvested and dissected only. We have modified this protocol from the one described by Hawkins *et al.* (2002).

1. Dissect stem segments containing cambial windows from stem (*in vivo* stem) or new shoots from dormant lateral buds (*in vivo* bud) after appropriate growth period.
2. For cambial windows derived from *in vivo* stem technique, trim off most of the surrounding tissue and further dissect into small pieces (0.5-1.5 mm thick) to allow for penetration of GUS reagent. The plane of dissection is dependent on the trait under investigation. For shoots derived from the *in vivo* bud technique, similar dissection is undertaken but in this case only the lowest 5-10 cm of the stem requires sampling. Place dissected stem samples in appropriate sized tubes. For this we use round bottom 10-12 ml Falcon tubes.
3. Wash dissected stem pieces in ice cold 90% acetone on a rotary shaker (to allow for mixing) for one hour in cold room at 4°C. This removes surface tension and allows for increased penetration of GUS reagent. **This step is optional.** We no longer use this step.
4. Rinse dissected stem pieces twice in 0.1 M phosphate buffer pH 7 to equilibrate samples to right pH and to wash off acetone if used.

NOTE: Important to make sure that pH 7 is maintained as we have found that a lower pH, e.g. pH 6 or lower, can lead to endogenous GUS activity which can mask the identification of GUS stained sectors as well as lead to the identification of false positive sectors.

5. Remove excess phosphate buffer from samples and fill with GUS reagent until sample is fully covered (see below for recipe). We have found around 5-7 ml of reagent is sufficient to cover samples.
6. Incubate samples for 10 min at 55°C in the dark. This step has been shown by others to be useful in removing endogenous GUS activity (Muhitch 1998; Hansch *et al.* 1995; Hodal *et al.* 1992).
7. Remove tubes from heat source and incubate overnight at 37°C on a shaker/incubator in the dark. It is important that the samples are shaken to stop any localized pH increases which could lead to endogenous GUS activity.
8. On the following day remove tubes from incubator and confirm pH 7 has been maintained.
9. Remove GUS reagent and store in 70% ethanol (or other appropriate storing solution) in cold room.



Reagents

0.1 M (sodium) phosphate buffer pH 7

- 57.7 ml 1M Na₂HPO₄
- 42.3 ml 1M NaH₂PO₄

Mix and make up to 1 L with distilled H₂O

Check pH and adjust if required.

GUS reagent

Stock – can be made several days in advance and stored in cool room for several months

For 100 ml of reagent

In 90 ml 0.1 M phosphate buffer pH 7 add

- 0.5 ml Triton X-100 (0.5% v/v final concentration)
- 372 mg EDTA (10 mM final concentration)

Mix and make up to 100 ml with 0.1 M phosphate buffer

GUS reagent – on day of assaying

For 100 ml of reagent

In 90 ml of GUS reagent stock add

- 29.09 mg X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid) (0.5 mM final concentration)
- 16.4 mg Potassium Ferricyanide (III) (0.5 mM final concentration)
- 21.1 mg Potassium Ferrocyanide (II) (0.5 mM final concentration)

Mix and make up to 100 ml GUS reagent stock and use.



References

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