# Protocol



**Creation Date: 5/18/2016**
**Revision Date: 9/11/2018**

## Histochemical Staining of GUS Reporter Activity

### Adapted from D. Preuss (1996): *Arabidopsis* Genetics Course

## Introduction

A useful method for studying pollen gene expression is to analyze the levels of a reporter enzyme such as β-glucuronidase (GUS) under the control of a promoter of interest. One promoter that can be studied using this GUS gene fusion system is the LAT52 promoter. The LAT52 gene is found in tomato and shares some homology with sequences in tobacco. Studies have found the LAT52 promoter is pollen-specific and is active in many plant species, including *Arabidopsis*. Because transcription under LAT52 regulation occurs only after meiosis, plants heterozygous for a LAT52-GUS transgene will produce both GUS-positive and GUS-negative pollen grains in a 1:1 ratio. Inviable pollen grains or pollen that fail to develop properly will form little GUS protein and will stain poorly. Furthermore, some strains may have low pollen viability because they are backcrossed only once following transformation. Here, we describe the use of GUS to study pollen gene expression using transgenic plants containing a fusion of the LAT52 promoter to β-glucoronidase.

## Materials

- Genetically modified plants
- Microcentrifuge tube
- 90% Acetone
- Vortexer
- Pipet
- 96-well plate
- Centrifuge
- X-Gluc Stock Solution 20 mg/ml**.** Dissolve 30 mg X-Gluc (GoldBio Catalog # G1281) in 1.5 ml DMF. Cover with foil and store at -20°C.
- X-Gluc Stain Solution using X-Gluc (GoldBio Catalog # G1281). Cover with foil and store at 4°C. See Table 1.

**Note: 4ml of stain solution are necessary to stain one 96-well plate.**

**Table 1.** Reagents needed for the X-Gluc Stain Solution.

| Reagents | Working Volume (4 ml) |
|---|---|
| 50mM ferrocyanide | 400 µl |
| 50mM ferricyanide | 400 µl |

**Gold Biotechnology**
St. Louis, MO
**Ph**: (800) 248-7609
**Web:** www.goldbio.com
**Email:** contactgoldbio86@goldbio.com

| | |
|---|---|
| 500mM $NaPO_4$ at pH 7 | 400 µl |
| 20 mg/ml X-Gluc (in DMF) | 100 µl |
| $dH_2O$ | 2700 µl |
| **Total** | **4000 µl** |

## Storage and Handling

- Store X-Gluc desiccated at -20°C and protect from light. This product is soluble in DMF.
- This product may be shipped in blue ice and should be stored immediately upon arrival at -20°C.
- Store the stock solution at -20°C and protect from light.
- Store the Stain Solution containing X-Gluc at 4°C and protect from light.

## Method

1. Place flowers from plants to be tested in a microcentrifuge tube.
2. Add ~200 µl of 90% acetone (to cover flowers) and vortex briefly to dislodge the pollen.
3. Pipet 20-40 µl of pollen suspension into a flat-bottom 96-well plate (polystyrene).
4. Centrifuge the plate at 3000 g for 3-5 minutes and carefully remove the acetone.
5. Air dry at room temperature until the acetone is completely evaporated.
6. Add 40 µl of X-Gluc Stain Solution to each well and incubate for a minimum of 1 hour, to overnight if desired, at 37°C.
7. Assess pollen staining in the light microscope.

## Associated Products

- X-Gluc (GoldBio Catalog # G1281)

## References

Preuss, D. (1996). Genetics and Mutant Screen, Cold Spring Harbor Arabidopsis Genetics Course. University of Chicago.

Preuss, D., Lemieux, B., Yen, G., and Davis, R. W. (1993). A conditional sterile mutation eliminates surface components from Arabidopsis pollen and disrupts cell signaling during fertilization. *Genes & development*, 7(6), 974-985.

Preuss, D., Rhee, S. Y., and Davis, R. W. (1994). Tetrad analysis possible in Arabidopsis with mutation of the QUARTET (QRT) genes. *Science*, 264(5164), 1458-1460.

Twell, D., Wing, R., Yamaguchi, J., and McCormick, S. (1989). Isolation and expression of an anther-specific gene from tomato. *Molecular and General Genetics MGG*, 217(2-3), 240-245.

Twell, D., Yamaguchi, J., and McCormick, S. (1990). Pollen-specific gene expression in transgenic plants: coordinate regulation of two different tomato gene promoters during microsporogenesis. *Development*, 109(3), 705-713.