

Modeling the Organism: The Cell in Development

Laboratory 4: Exploring the plant body

Introduction

The model plant *Arabidopsis thaliana* has many advantages for biological study: small size, short generation time, simple culture requirements, and well-developed biochemical, genetic and molecular tools for studying the complexities of plant development.

One approach to studying the molecular bases of cell differentiation and tissue organization has been to transform plants with reporter gene constructs. Sequences encoding fluorescent GFP (Green Fluorescent Protein) or the colorimetric marker Beta-glucuronidase under the control of a weak promoter are introduced randomly into the plant genome via *Agrobacterium*-mediated transformation, where their expression is strongly influenced by local endogenous gene enhancer sequences. Thus, if a reporter gene is located near a strong enhancer driving in the expression of a nuclear-encoded gene involved in photosynthesis, the reporter will be strongly co-expressed in the greening cotyledons (seed leaves) but not in the roots. This approach, called enhancer trapping, has revealed much about the organization and molecular identities of different cells and tissues in the plant body. A nice review of enhancer trapping and related technologies [[Patricia Springer \(2000\) Gene Traps: Tools for Plant Development and Genomics. The Plant Cell 12:1007-1020](#)] (including examples of staining patterns that we may observe) is available if you wish to learn more about this important technique.

In this week's lab, we will sow *Arabidopsis* seeds on Petri dishes of a gelled agar medium. This medium provides a source of the inorganic salts and trace nutrients normally obtained from soil by the plant. You should obtain three plates. Gently invert the plates (the agar is very soft, so do not drop or jar them) and, using a Sharpie permanent marker, draw a single line across the diameter of the bottom of each plate. Add your LAB DAY, INITIALS, AND either WT, LINE 1, OR LINE 2. Then flip the plates right-side-up again. The plates are your molecular fields, the line the furrow for your planting, and WT or LINE # is the variety you are going to grow!

Next, select tubes of wild type (WT) and two of the enhancer trap mutants available at your bench. The tubes are small and the seeds quite precious, so please take care not to misplace or drop the tubes or spill the seed.

Working under the dissecting microscope with a single tube at a time, and a pair of tweezers, carefully plant 5-6 wild-type seeds along the agar along the middle half of the "furrow" you drew on the bottom of the plate. Then repeat for two of the varietal tubes, recording their numbers in your notebook.

Once the dried seeds have been placed on the agar surface, they will absorb water (this process is termed imbibition). The plates should then be placed in the cold room on the tray labeled with your lab's day. After 48 hours of cold treatment (termed vernalization) we will

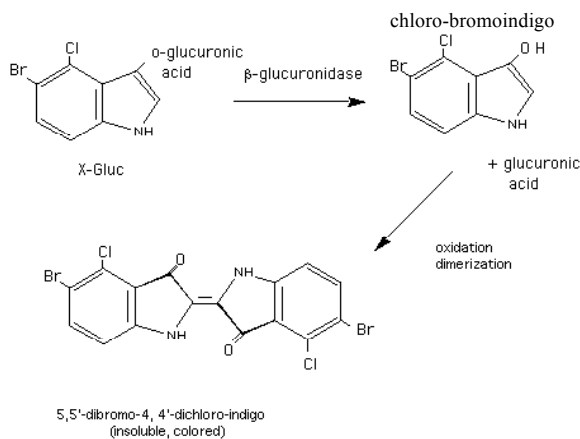


A week-old *Arabidopsis* seedling

(source: <http://www.oeb.harvard.edu/faculty/holbrook/projects/seedling.jpg>)

transfer the plates into the lab, where they will be placed under fluorescent lights. The seeds will germinate within a day or two of transfer to room temperature and then rapidly grow into seedlings like the one pictured above.

In next week's lab (week 2), recover your plates and examine your "crop". Record the appearance of your seedlings in your notebook, carefully noting any differences between as well as within each group. Then using a pair of tweezers carefully transfer several healthy seedlings from each plate into a marked microfuge tube containing distilled water (dig a bit at the agar to keep as much of the root system intact as possible). Then use a pipette to change the water for 0.5 ml of GUS-staining solution. Transfer the microfuge tubes to the rack labeled for your lab day (on the central bench). We will incubate the tubes at 37°C for 48+ hours to permit color development.



Source: <http://www.biology.purdue.edu/people/faculty/karcher/blue2000/blue.html>

The GUS substrate is a soluble, colorless chemical (called X-Gluc, for 5-bromo-4-chloro-3-indolyl-beta-D-glucuronic acid) that will be converted by the GUS enzyme into a pink/purple colored product that precipitates in place. Thus, cell/tissue specific patterns of GUS activity will result in colored sectors that match the patterns gene expression in the plant.

After the 48 hour incubation we will transfer the racks to the Bio 200 cold room. Before your next lab section, please come to lab and exchange the liquid your seedlings with 70% Ethanol

(one wash is sufficient – use a pipette and please transfer the spent staining solution to the labeled waste collection container). This will reduce any non-specific staining and will extract the chlorophyll from the green parts of your seedlings to reveal GUS staining in those organs. When transferring solutions, use a pipette to carefully remove the liquid from around the fragile seedlings without crushing or bruising the tissue; then add the new solution and recap the tube securely. Waste solutions should be discarded in the appropriate waste containers in the cold room.

In lab during week 3, transfer your stained seedlings from the microfuge tube to the lid of a Petri dish and carefully observe under the compound microscope. Record your staining observations in your notebook. Did the wild-type seedlings stain for GUS activity? Why or why not? What patterns of staining did you observe in the enhancer lines? Travel around the lab and observe several other groups' staining patterns, recording your observations and the varietal number in your notebook.

Lab report:

Each student will be responsible for submitting a one page lab "brief" describing your results from one of the following lab modules: (1) this *Arabidopsis* experimental module; (2) the concurrent *Planaria* regeneration experiment (which we will start in week 2) or (3) the *Drosophila* patterning investigation (starting in week 3 of this module). Your briefs should include some hand drawings / images (which we will collect on using a digital microscope camera in the lab) of your results, and will be due at the start of lab in the last week of the semester.