BYS 340
Laboratory Exercise
By Lynn Boyd
at University of Alabama in Huntsville

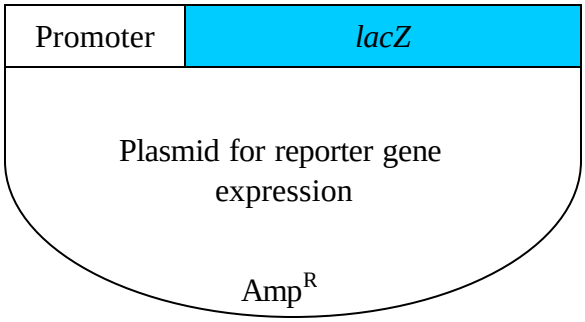
b-galactosidase staining in transgenic strains of *Caenorhabditis elegans*

Introduction:

The nematode, *Caenorhabditis elegans,* is a free-living, non-parasitic worm that normally lives in the soil. Researchers have been using this species as a model for development and neurobiology for several decades. The nematode is easy to grow in the laboratory and has a short generation time. Nematodes are grown on agar plates that have been seeded with a small dollop of *E. coli* bacteria. The nematodes eat the bacteria and reproduce hermaphroditically.

It is fairly easy to generate transgenic strains of *C. elegans.* A transgenic strain is one that contains foreign DNA. In *C. elegans,* DNA can be injected into the gonad of an adult worm and this DNA is taken up by the developing oocytes. If the DNA is taken up by the oocyte nuclei, it will ultimately be passed on to future generations. These transgenic strains can be used to study various gene functions. For example, if you are studying a particular gene and you want to know where it is expressed in the worm, you can use a reporter gene and generate a transgenic strain. First, you would create a plasmid that contains the transcriptional control region for the gene (i.e. promoter, etc.) connected to a reporter gene. Then, you would inject that plasmid into worms and assess the expression of the reporter gene.

One of the most popular reporter genes is the *lacZ* gene which codes for the enzyme, β-galactosidase. This enzyme makes an excellent reporter because its presence can be detected by staining with a substrate that turns blue in the presence of the enzyme. The substrate that we will use is called X-gal. Any blue color in the worm indicates that the reporter gene is expressed in that cell.



The plasmid that has been injected into the worms contains the promoter region of a worm gene connected to the coding region for the bacterial *lacZ* gene.

The goal of this investigation is to determine the expression patterns of β-galactosidase in two different transgenic strains of *C. elegans.* The PD55 strain contains a plasmid with the promoter from the *unc-54* gene fused to *lacZ*. The JW29 strain has a plasmid with the promoter of the *mec-7* gene fused to *lacZ*.

You will need to stain the worms for β-galactosidase expression using the procedure below. Then, describe the expression pattern using the template on the next page. Describe the type of tissues these genes might be expressed in. Hypothesize on the types of functions that these genes may have.

PROTOCOL

1. Remove worms from the petri dishes by washing the plates with 2 ml water. Place worms into 1.5 ml microcentrifuge tubes.
2. Allow the worms to settle under gravity for a few minutes. Remove the liquid from the top of the tube. Add 1.5 ml H_2O. Invert.
3. Allow the worms to settle under gravity for a few minutes. Remove the liquid from the top of the tube. Leave a little bit of liquid with the worms.
4. Pipette 20 µl of worms onto a coated microscope slide. Cover with a coverslip.
5. **Immediately,** use forceps to immerse the slide in liquid nitrogen.
   WEAR GOGGLES.
   BE CAREFUL. DO NOT SPLASH OR SPILL NITROGEN.
6. Remove the slide from the liquid nitrogen with forceps. Flip off the coverslip with a razor blade. **Immediately** place the slide in a Coplin jar filled with methanol (on ice). Leave in methanol for 5 minutes.
7. Move the slide to a Coplin jar filled with Acetone (ice). Leave for 5 minutes.
8. Remove slide and allow to air dry.
9. Add 25 µl of staining solution to slide.

10. Carefully place a coverslip over the solution.
11. Seal edges of the coverslip with nail polish.
12. Place slide at 37º until blue color appears.