

Plant Molecular Genetics Workshop

Outreach Project for Grade 12 Students

Genotyping and Gene Expression Analysis

Presented in collaboration with the
McMaster University Department of Biology

Description of Activity

This workshop offers students a “hands-on” experience performing molecular genetic experiments with the model plant *Arabidopsis thaliana*. During the workshop students genotype plants by amplifying DNA from transgenic and wild-type plants using PCR (polymerase chain reaction) and they monitor *GUSA* reporter gene activity through the detection of GUS (beta-glucuronidase) activity. The students are assisted by a number of undergraduate, graduate, and post-graduate personnel who volunteer their time to participate as mentors for the workshop. The workshop takes approximately five hours to complete (including a one-hour break for lunch) and teachers can get more details by contacting Dr. Elizabeth Weretilnyk at weretil@mcmaster.ca.

Genotyping: The transgenic plants we use are distinguished from wild-type plants by the presence of the *GUSA* reporter-transgene. After setting up and performing PCR, students use agarose gel electrophoresis to identify the presence of a DNA fragment arising from the transgene.

Reporter gene use in molecular studies: Reporter genes are commonly placed next to the promoter of a gene of interest. In this way, each time the gene of interest is expressed the reporter gene is expressed as well. By monitoring the expression and activity of the reporter gene we can study how the promoter of the gene of interest is controlled. For example, imagine creating a reporter gene system composed of a light-responsive gene promoter and the *GUSA* reporter gene. If you were to screen plants for GUS activity, you would expect that plants in the light would have GUS activity and if it does, a blue precipitate would form when the substrate for GUS, X-gluc, is present. In contrast, when plants are kept in the dark there should be no GUS activity and so plants will not have the enzyme needed to make a blue precipitate.



Arabidopsis plants containing a light-responsive *GUSA* reporter gene. The plant has been in the light and in the light the GUS enzyme is present and active and cleaves X-GLU to form the blue precipitate.

Glossary:

Genotype: The genetic composition of an organism

Transgene: A foreign or manipulated native gene that has been integrated into a host's genome. The resulting organism is classified as being "transgenic" or "genetically modified".

Gene Promoter: Segment of DNA upstream (in front of) a gene that controls the expression (turning on or off) of that gene.

Reporter Gene: A gene whose product is very easily detected. Reporter genes are either placed next to a promoter or fused to a gene of interest, such that every time the gene of interest is expressed the reporter gene is expressed as well. We can then infer the expression of the gene of interest by monitoring the expression of the reporter gene.

PCR: A molecular biology technique used to amplify specific segments of DNA for detection of the products on an agarose gel following electrophoresis.

Gel electrophoresis: A technique that uses an electric charge to separate DNA segments based on size.

GUSA: gene that codes for the enzyme beta-glucuronidase (GUS) whose activity cleaves the chromogenic (color generating) substrate X-gluc (5-bromo-4-chloro-3-indolyl β -D-glucuronic acid), to produce an insoluble blue precipitate, Cl-Br-indigo dimer.