

**Biology 423L**

**Dec. 1/2**

### **Mapping a Mutation in Arabidopsis.**

**Readings:** **Readings:** Hartwell Chapter 10 pp 354-365.. 514-515, 518-521.  
Lukowitz et al 2000 Positional cloning in Arabidopsis. Plant Physiol. 123; 795-805 (see web site for PDF)  
Szymanski et al 2000, Progress in the molecular genetic analysis of trichome initiation and morphogenesis in Arabidopsis. Trends in Plant Science 5: 214-219 (see web site for PDF)

#### **Objectives:**

In this exercise we will try to locate a gene by its cosegregation with DNA markers. We will use a procedure called interval mapping. We start with an F2 population of plants generated from a cross of a homozygous mutant with a homozygous wild type. The mutant had a different genetic background (ecotype) from the wild type. This allows us to locate the mutation by its cosegregation with DNA markers that reveal polymorphisms between the genetic background of the mutant and the wild type. We will do an initial mapping step in order to determine which chromosome carries the mutation.

After this step is complete, a researcher would normally use DNA markers from that chromosome to more finely locate the gene. Because very many polymorphic markers have been identified in Arabidopsis, it is possible to map the gene to a DNA interval between two markers that contains only a few gene sequences. Then matching segments of DNA from a wild type genome can be used to transform mutant plants. If the transformed plants have a wild type phenotype, the mutation is complemented by the cloned gene(s). Complementation means we have correctly identified the gene responsible for the mutation.

#### **Introduction:**

There are several ecotypes used for mapping in Arabidopsis. Hundreds of DNA markers have been identified which reveal polymorphisms between these ecotypes and their location on the chromosomes has been determined by genetic and physical mapping. In our cross, we used plants of the Columbia-0 (Col-0) genetic background that were homozygous for a recessive mutation in a gene required for leaf hair development called *GL1* (*Glabrous 1*). Because it is recessive, the mutant allele is written as *gl1*. The homozygous mutants were crossed to wild type plants from the Landsberg-erecta (La-er) ecotype. The F1 plants from this cross were heterozygous at every locus, hundreds of which can be identified using defined PCR primers. In the F2 generation, the alleles will segregate randomly. We can detect the type of alleles in each F2 plant using PCR markers similar to the single gene markers we used in the forensics lab.

In order to map the location of the *GL1* gene, we will collect only the F2 plants that have the recessive allele at the *GL1* locus by their phenotype of having smooth leaves. These will all have received 2 copies of the *gl1* allele from the mutant parent. It was of the Columbia ecotype. Since all the plants we will choose have the Columbia allele at the *GL1* locus, they will be very likely to have the Columbia allele at any closely linked genes. In contrast, loci that are far away or on different chromosomes will segregate randomly (i.e. 1 Col-0/Col-0: 2 Col0/La-er: 1 La-er/La-er). We can identify the closest mapped markers because they will most frequently cosegregate with the Col-0 *gl1* allele.

We will use a very simplified DNA prep to make DNA from the chosen F2 plants for PCR reactions. The next step would be to amplify the DNA using primers directed to loci that represent major sections of the five Arabidopsis chromosomes. Generally a set of about 20 markers, well distributed across the chromosomes, is used. In the next lecture period, we will analyze gel images showing PCR products from DNA pooled from about 25 chosen F2 plants with the mutant phenotype. If the marker locus we test is close to the *gl1* locus, the PCR products will mainly be of the Col-0 allele.

In the lecture period, we will discuss the steps used to identify a small genome interval that must contain the *GL1* gene defined by recombination events in the chromosomes of F2 plants with the *gl1/gl1* phenotype like the ones we have used for DNA preps. Then we will examine the annotated genome sequence in our interval using the Arabidopsis Information Resource (TAIR) database. We will discuss the final steps to proving that a candidate gene in the defined interval is responsible for the mutant phenotype we chose to analyze.

## Protocol

### DNA preparation

#### Materials for DNA preps:

Pots of F2 seedlings 3 weeks after planting in small pots

1 pot of *gl1* mutants (Col-0 ecotype)

1 pot of La-er ecotype (parental type).

Tweezers

Paper towels

Dissecting microscopes

Boiling water bath with room for 50 samples

Sucrose Solution 5 ml per group

Recipe: 50 mM Tris-HCl pH 7.5,

300 mM NaCl

300 mM sucrose

Autoclave and aliquot into sterile tubes.

Microfuge  
Gloves  
Yellow tips  
Blue tips  
1.5 ml eppendorf tubes (10 per group Each group of 2 will do 5 DNA preps)  
Blue pestles for grinding with eppendorf tubes  
Boiling water bath and supports for eppendorf tubes

### **Procedure:**

#### **1. Pick leaves for DNA prep.**

Label 2 sets of 5 1.5 ml eppendorf tubes with your initials and 1-5. Choose F2 plants that have the glabrous (hairless) phenotype. You may pull the plants out of their pots and examine their leaves under the dissecting microscopes. Only the leaves are affected by the mutations, not the roots.

Each group should make DNA from 3 different F2 plants with the glabrous phenotype. In addition make one prep from each parental type ie. glabrous mutant in Col-0 background and wild type in La-er background.

Use the numbering system below.

Tube 1 is the Col-0 parent

Tube 2 is the La-er parent

Tubes 3-5 will each contain leaf material from a different individual glabrous F2 plant.

#### **2. DNA prep. Wear Gloves**

- For each sample, add 400µl sucrose solution to an appropriately labeled 1.5 ml tube.
- Remove 2 small leaves from plant and place in labeled tube.
- Grind by hand using a blue pestle until no large chunks of tissue remain.
- Boil samples for ten minutes in the boiling water bath using a flotation device for the tubes.
- Cool tubes on ice until ready to centrifuge.
- Spin for 10 seconds in the microfuge on max speed to bring debris to bottom of tube. Transfer the supernatant to a new labeled tube and keep on ice.

1 ul of supernatant is enough for each subsequent PCR analysis.

Next Monday we will discuss the next steps for identification of the *GL1* gene.

**There will not be a written lab report for this lab.**