

Arabidopsis PCR Genotyping

Review PCR in your Genetics text book if required.

PCR Principle: The polymerase chain reaction (PCR) is a method for the enzymatic amplification of a specific DNA sequence of interest. This technique is capable of recognizing and amplifying a target sequence from nanogram amounts of template DNA within a large background of irrelevant sequences. A prerequisite for amplifying a sequence using PCR is the knowledge of unique sequences flanking the segment of DNA to be amplified, so that specific oligonucleotides (primers) can be obtained. It is not necessary to know anything about the intervening sequence between the primers. The PCR product is amplified from the DNA template using a heat-stable DNA polymerase from *Thermus aquaticus* (Taq DNA polymerase) and using an automated thermal cycler. The thermocycler puts the reaction through 30 or more cycles of denaturing, annealing of primers, and polymerization. After amplification by PCR, the products are separated by gel electrophoresis and are directly visualized after staining with ethidium bromide.

PCR Genotyping: PCR applications are myriad, and PCR genotyping can take any of a number of approaches towards identifying the genetic make-up of an individual. In this experiment, we will be using PCR to identify the genotype of plants that are segregating an 'insertional mutagen'. To determine the function of a specific gene, in this case a H⁺-ATPase primary transport gene, a foreign 6.2 kb DNA fragment has been inserted into the coding sequence. We will use PCR to identify the presence of this insert and identify whether the plant assayed is homozygous wild-type, heterozygous or homozygous mutant.

To Do:

Seeds collected from a plant heterozygous for a T-DNA insertion will be planted "aseptically", placed at 4° C for 2-4 d, then placed in the light 100 mmol m⁻² s⁻¹. DNA extraction can proceed after 7 days in the light.

Day 0..

- Sterilize and plant seedlings on media agar. Wrap in foil, place at 4° C for 2-4 days

Day 1...

- Place plate in the light.

Day 7 (or later)...

- Extract DNA (two seedlings each).
- Subject DNA to two PCR reactions each,
 - One for wild-type template, one for the mutagenic insert template.

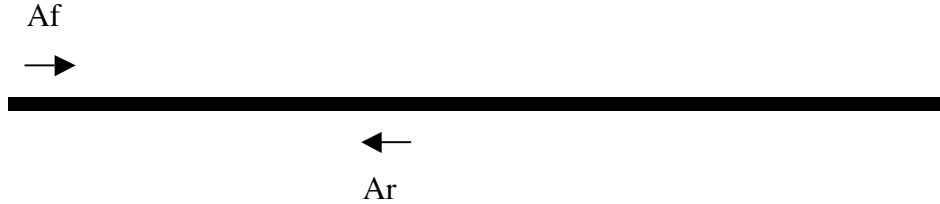
Next Class ...

- Genotype,
 - Run 10 µl of the PCR reactions on an ethidium stained gel, photograph, analyze results.

PCR Genotyping: *T-DNA* Segregating Populations

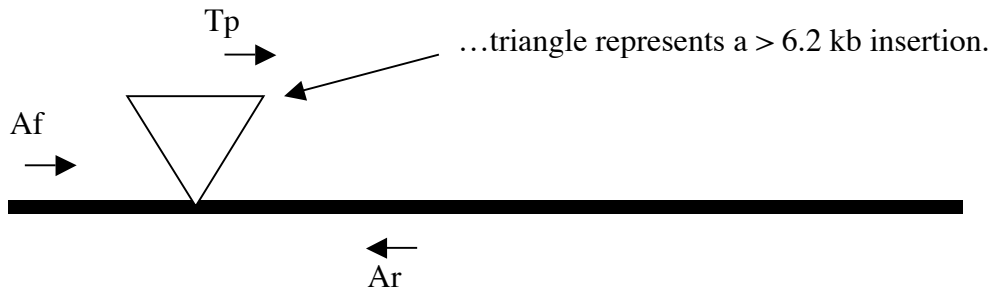
Arabidopsis Locus (At5g57350): Maps to chromosome 5.

- **Af** and **Ar** are PCR primers that amplify wild type DNA templates,

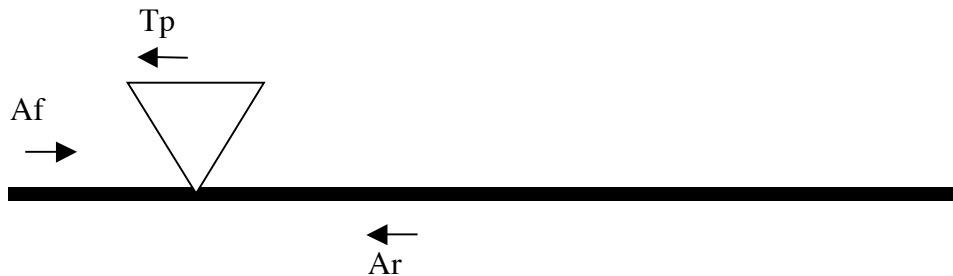


T-DNA Location; A plant line has been identified that has an T-DNA insertion in this locus. T-DNA is coded on a plasmid from the soil bacterium *Agrobacterium*. Upon infection, the T-DNA is passed from the bacteria to the host plant, where it inserts "randomly" into the plant's genome. When inserted into a gene, the gene's function is disrupted.

- **Tp** is a primer that amplifies DNA from insertion templates when paired with a gene specific primer.



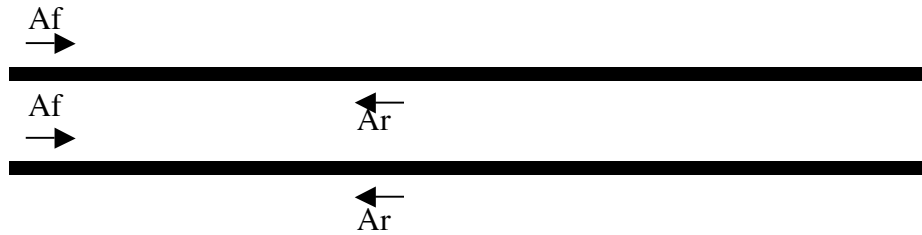
- or -



No wt product formed from tagged template. The PCR reaction we will be using fails on templates over 3 kb.

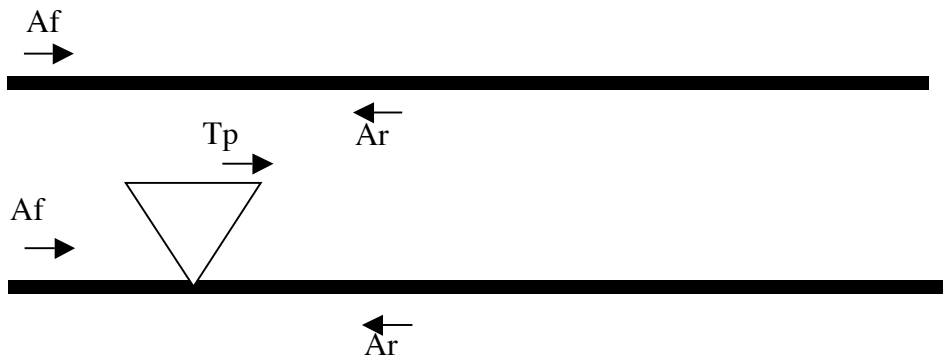
Genotyping Worksheet

Homozygous wt plant.



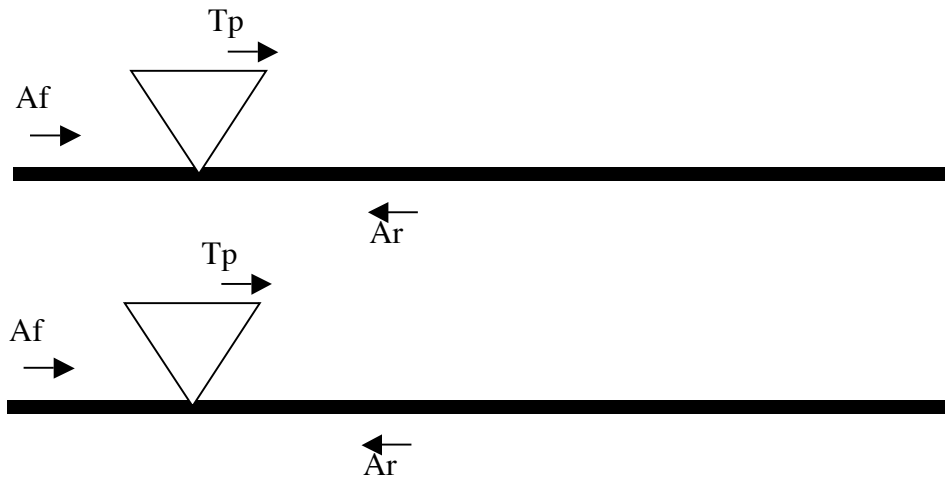
Will the Af and Ar primer combination produce PCR products? _____
Will the TP and Ar primer combination produce PCR products? _____

Heterozygous plant.



Will the Af and Ar primer combination produce PCR products? _____
Will the TP and Ar primer combination produce PCR products? _____

Homozygous mutant plant.



Will the Af and Ar primer combination produce PCR products? _____
Will the TP and Ar primer combination produce PCR products? _____