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 to have 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 aguaticus* (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 fragrnent 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.

Seeds collected from a plant heterozygous for the *aha*3-1 insertion were aseptically planted 4/28, 4^o C till 4/30, then placed in the light (100 mmol m^{-2} s^{-1}.)

To Do: (after germination, see *Planting Arabidopsis* Handout)

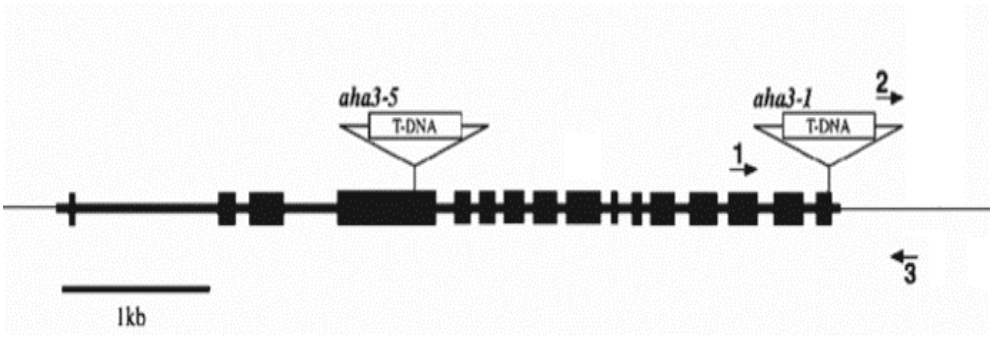
5/5…

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

5/7…

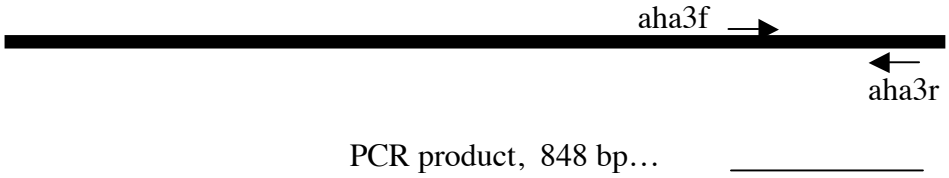
- Genotype,
  - Run 10 ml of the PCR reactions on an ethidium stained gel,
  - photograph, analyze results.

PCR Genotyping: *aha*3-1 Segregating Populations



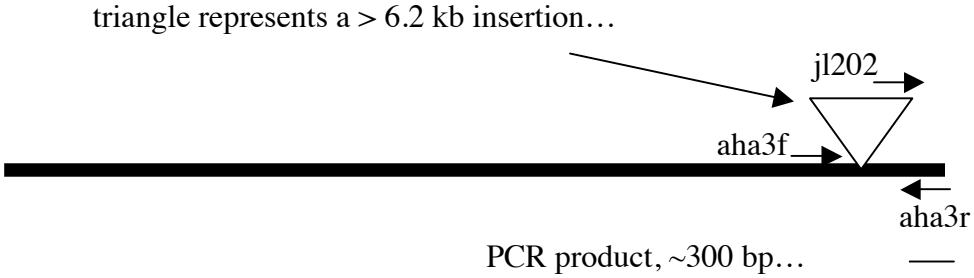
AHA3 Genomic DNA (AT1g17250): Maps to chromosome V. *AHA*3 codes for a plasma membrane H^+-ATPase protein. H^+-ATPases create chemiosmotic potential that in turn drives solute transport across the plasma membrane. The*AHA*3 gene is expressed mainly in the phloem and developing pollen.

- **aha3f (1)** and **aha3r (3)** are PCR primers that amplify wild type DNA templates,



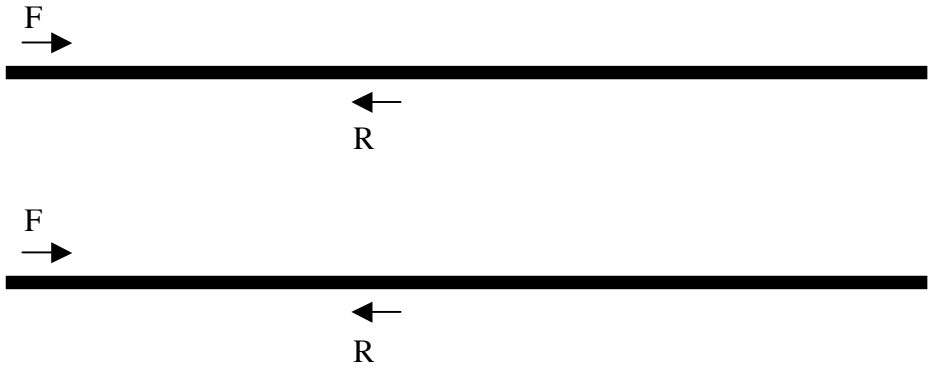
T-DNA Location; T-DNA is a plasmid from a soil bacterium. Upon infection, the T-DNA is passed from the bacteria to the host plant, where it inserts "randomly" into the plant's genome. When inserted into a gene, the gene's function is disrupted.

- **jl202 (2)** and **aha3r** are PCR primers that amplify DNA from insertion templates.

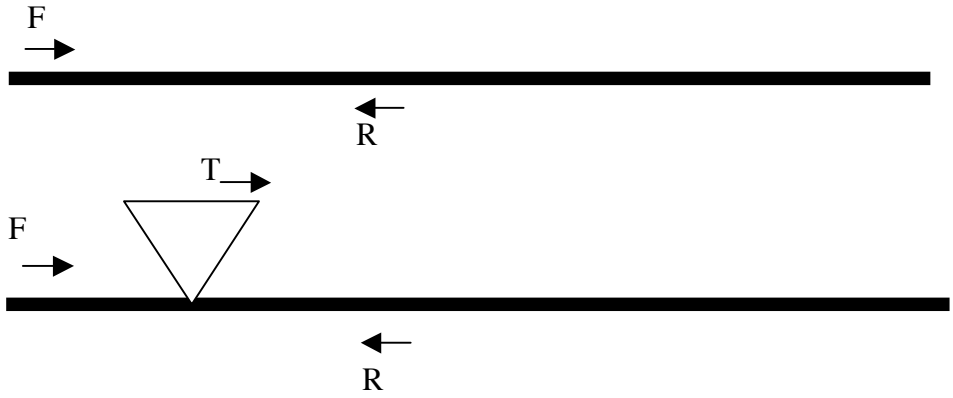


Note: No wt product will be formed from tagged template. The PCR reaction we will be using fails on templates over 3 kb.

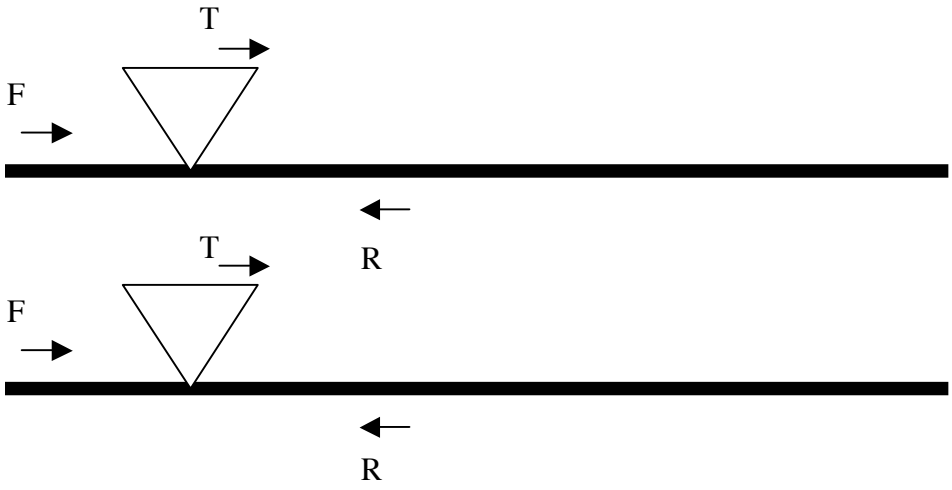
Homozygous wt plant.



Heterozygous plant.



Homozygous mutant plant.



DNA Extraction
(quick prep)

one small seedling
10-20 seeds
~20 mm^2, etc.

- **Place tissue in 500 µl of 'Extraction Buffer' in a labeled (initials) microtube (1.7 ml),**
    - **use a micropestle to grind tissue, this breaks cell walls and membranes, allowing nuclear DNA to go into solution.**
    - **use a different pestle for each DNA prep, avoid contamination between tubes.**
- **Cap tubes, spin 3 minutes (highest speed). This pellets the tissue,**
    - **be sure to balance the centrifuge rotor.**
- **Move 200 µl of the supernatant to a clean tube (initialed) that contains 200 µl of isopropyl alcohol, be sure to exclude plant tissue (re-spin if necessary),**
    - **be sure to change pipette tips for each tube.**
    - **vortex for 10 seconds,**
    - **spin 5 minutes (highest speed),**

4. **<u>As soon as spin is over</u>, pour off the liquid, save the tube w/ pellet.**

5. **To rinse off excess salts, add 500 µl 70% ETOH (ethyl alcohol), spin at high speed for 2 minutes, pour off the liquid, save the tube w/ pellet.**

- **be sure to balance the centrifuge rotor.**

6. **To rinse and aid drying, add 500 µl 95% ETOH (ethyl alcohol), spin at high speed for 2 minutes, pour off liquid,**

- **be sure to balance the centrifuge rotor.**

5. **Allow pellet to dry.**

7. **Suspend DNA pellet in 400 µl H_2O. Use 2 µl for the PCR reaction.**

<u>Extraction Buffer</u> (100 ml)

0.2 M Tris-HCL, pH 9.0	…pH Buffer, inhibits nucleases.
0.4 mM LiCl	…nucleic acids precipitate in solutions with salt and alcohol.
25 mM EDTA	…chelator, inhibits nucleases.
1% SDS	…aids in disrupting membranes.