Fall 2013 Biology 322

Arabidopsis thaliana: **Phenotypic and genotypic analysis of F2 segregants from *aha-3/+* heterozygotes.**

Part 1 Planting seeds and assessing phenotypes of seedings

Part 2 Direct detection of seedling genotype using PCR

Background information on Arabidopsis aha-3 gene and T-DNA mutations:
http://fire.biol.wwu.edu/trent/trent/aha3intro.pdf
Background information on PCR:
http://fire.biol.wwu.edu/trent/trent/13.05.20lecture.pdf

Phenotypic Assessment of Seedlings: Looking and Seeing

Both the worm and the plant lab focus on assessing phenotypes -- but in different contexts. In the worm lab you know what phenotypes you should see and you will hone your skills in assessing individual animals and placing them in a phenotypic category. In contrast, in the plant lab you don't know what the phenotype of the *aha3-1* mutants should look like or even whether you will see a phenotype at all. So you will need to assess phenotypic variation in a less prejudiced way.

Inferring Genotypes of worms and plants

In both the worm and the plants labs you will infer the genotypes of specific individuals. But, you will do this *indirectly* in the worm lab by assessing the self-progeny of wild-type hermaphrodites of unknown genotype; in contrast, you will assess genotype *directly* in the plant lab using a PCR based assay.

Seed Stocks:

- F2 seeds segregating from an *aha3-1*/*aha*+ heterozygote
- Wild-type controls: *aha*+/*aha*+ (that is, homozygous for a wild-type *aha* allele)

Tues Oct 1

- See pages 2 & 3: Sterilize and plant seeds collected from a genotypically wild-type plant and a plant heterozygous for a *aha-3* T-DNA insertion**.** Wrap plates in foil and place at 4^{o} C for 2 days.

Thurs Oct 3

- Place plates under lights (100 mmol m^{-2} s^{-1}) at room temperature

Tues Oct 8

- See pg 4. Assess germination and initial examination of phenotypes of seedlings

Mon Oct 14 Lecture

- Introduction to *Arabidopsis*, the *aha* gene family and T-DNA induced mutations

Tues Oct 15

- See pg 4. Assess & categorize seedling phenotypes
- Extract genomic DNA from seedlings (wild-type controls and F2 segregants)
- Subject each prep of DNA to two PCR reactions each: one to detect wild-type template and one to detect the presence of the T-DNA insertion

Thurs Oct 17

- Gel electrophoresis of PCR reaction & analysis of results
- ***Lab Report Due on or around ***** assuming we remain on schedule for data collection***

SEED STERILIZATION

Seed Stocks:

- F2 seeds segregating from an *aha3-1*/*aha*+ heterozygote
- Wild-type controls: *aha*+/*aha*+ (that is, homozygous for a wild-type *aha* allele)

Other Reagents

70% ETOH/0.1% Triton X
95% ETOH
Whatman filter paper, 6 cm diameter circles
Sterile plastic petri dishes (label bottom of the dish), round

1. Place a piece of filter paper in a petri dish and sterilize by wetting with 95% ETOH. (Set up one petri dish for each tube of seeds that you will be handling.)

2. Obtain microcentrifuge tubes containing seeds and add 1ml 70% ETOH/0.1% Triton solution to each tube. Shake so that all seeds are suspended. After 5 minutes (shaking tube once or twice during this time), allow seeds to sink and then pour out as much solution as possible.

3. Rinse seeds with 1ml 95% ETOH, allow seeds to sink, pour off ETOH.

4. Add 0.5ml 95% ETOH, shake tube to suspend seeds

5. Using a p1000 tip*, pipette as many seeds as possible and expel seeds and ETOH onto the Whatman paper. Repeat step if necessary to remove the majority of seeds from the tube.
   * Cut tip off with scissors or razor blade.

6. Allow ETOH to evaporate with the petri lid slightly ajar. *If planting seeds immediately, go to instructions below*.

7. When seeds are dry, remove the Whatman paper with sterilized tweezers by lightly shaking to remove seeds. If not planting the seds immediately seal the petri dish with 3m surgical tape or Parafilm.

Individual SEED PLANTING on AGAR

Each student should set up:

- One plate of F2s from an *aha-3*/+ heterozygote: 20 seeds per plate.
- One plate of wildtype (*aha+)* control seeds: 20 seeds per plate

1. Place a p200 pipette tip onto the end of utensil of some sort (the paint brush in your drawer will work great, use the non-bristle end).

2. Close the tip of the pipette by briefly holding it in the flame of an alcohol lamp…just a second or so to melt the tip closed, and to prevent fumes.

3. Once the tip has cooled, dip it into a microcentrifuge tube that has 95% ETOH, then allow to air dry.

4. Select a Murishige and Skoog plate (MS plate) and label the side of the bottom with your name, the date, and the experiment.

5. Dip the tip of your sterile seed planter in the agar (at the edge of the plate) in order to wet and "stickify" the tip.

6. Touch a single sterile seed, then place the seed ***on the surface*** of the MS plate. Do not push the seed into the agar. Space seeds in rows, widely and evenly.

7. Wrap the perimeter of each plates with a strip of sterile gauze; be sure that the plates are sealed.

8. Place plates in light-tight box wrapped in aluminum foil. Place at 4° C (refrigerator temp) for 2 days.

Phenotypic Assessment of Seedlings: Looking and Seeing

***Each student will have one plate of segregants from an aha3-1/aha+* heterozygote and *one plate of aha+/aha+* seedlings.**

Examine the agar plates carefully. You may want to use your stereomicroscope or a hand lens to examine each seedling.

Germination Basics: http://herbarium.desu.edu/pfk/page1/page2/page2.html

- What fraction of plated seeds germinated?
- Are all of the seedlings approximately the same size with the same number of leaves?
- How extensive is the root system for each plant?
- If we are seeing sufficient phenotypic variation, as a class we will develop phenotypic categories and then you will determine the number of your plants in each category.

Each student should select F2 plants and wild-type plants for DNA extraction.

- To consider: if the seedlings vary with respect to phenotypic category, how should you select plants for further analysis?
- Give each plant an ID and keep track of its phenotypic category

(A) Stage 0.1, imbibition.
(B) Stage 0.5, radicle emergence.
(C) Stage 0.7, hypocotyl and cotyledons emerged from seed coat.
(D) Stage 1.0, cotyledons opened fully.
(E) Stage 1.02, two rosette leaves >1 mm in length.
(F) Stage 1.04, four rosette leaves >1 mm in length.
(G) Stage 1.10, ten rosette leaves >1 mm in length.
(H) Stage 5.10, first flower buds visible (indicated by arrow in inset).
(I)
Stage 6.00, first flower open.

Numbers indicate principle growth stages:
0 = Seed germination
1 = Leaf development
5 = Inflorescence emergence
6 = Flower production