SEED STERILIZATION

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

1. Place seeds ** in an eppendorf microtube (does not need to be sterile). Add 1ml 70% ETOH/0.1% Triton solution. 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.

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

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

    - Pipette *** as many seeds as possible and expel seeds and ETOH onto the Whatmans paper. Repeat step if necessary to remove the majority of seeds.

5. Allow ETOH to evaporate with the petri lid slightly ajar,

    - When seeds are dry, remove the Whatmans paper with sterilized tweezers by lightly shaking to remove seeds. Petri dishes may be sealed with Parafilm for storage. Label the **bottom** of plate.

* Sterilize by wetting with 95% ETOH after placing in the petri plate.

** This procedure can be done with up to 0.5ml of seeds. Multiple seed lines can be done at once.

*** Cut the tip off to allow seeds to pass, sterilize briefly with 95% ETOH.

Save your seeds, you'll use them again. And, if your plate becomes contaminated, you'll need to <u>replant</u>.

Individual SEED PLANTING on AGAR
aha3-1 F2 and controls

1. Place a "yellow" 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 a Bunsen Burner…just a second or so to melt the tip closed, and to prevent fumes.

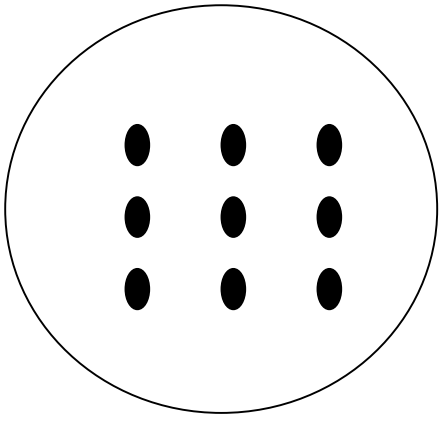
3. Once the tip has cooled, dip it into an eppendorph tube that has 95% ETOH, then allow to air

dry.

4. Select a Murishige and Skoog plate (MS plate) and label the bottom with your name, the date, and the experiment. Two plates each, 9 seeds per plate.

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 plant on the MS plate in the following pattern…



…space seeds widely and evenly.

7. Wrap the plates with sterile gauze, be sure that the plates are sealed.

8. Wrap in aluminum foil and place at 4° C for 2-4 days.

Bulk SEED PLANTING on AGAR
EMS seeds

1. Once sterilized seeds and filter paper are completely dry, carefully shake the seeds from the filter paper into the petri dish (same one used used to sterilize the seed coats).

2. Slowly and carefully tap the seeds out onto the ACC/MS plates (4 plates per student). Your goal should be to spread the seeds widely and evenly, so that when they germinate, you will be able to score individual plants.

3. Wrap the plates with sterile gauze, be sure that the plates are sealed.

4. Wrap in aluminum foil and place at 4° C for 2-4 days.

Planting Schedule
4/30/08

1. Surface sterilize *aha*3-1 seeds. Plant two plates each, 16 seeds each plate on MS plates. Seal with surgical tape. Label plates, wrap in foil, label foil using lab tape.

2. Surface sterilize EMS treated seeds. Sprinkle all seeds evenly over 4 ACC/MS plates. Seal with surgical tape. Label plates, wrap in foil, label foil using lab tape.

- **note**: drug and chemical treatment of plants in culture often requires organic solvents to suspend the compound in the media. Controls, with the solvent only, are run to determine the effect of the solvent on plant growth. In this experiment, we made a 10mM ACC solution, suspended in 10% ETOH. We used this to make a 50 μM ACC based media. How much ETOH was present, and what would you expect the result to be on hypocotyl growth, given the following growth response curve?