SEED STERILIZATION

A. In liquid bleach:

-- Gets rid of mold and bacteria on the surface of seeds

-- Solutions:
  sterile water
  sterile 0.1% agarose
  50% bleach solution with a drop of Tween-20

-- All in a sterile hood, using sterile technique:

  1. Put seeds in a 1.7 ml tube.
  2. Add 1 ml 50% bleach solution with Tween. Vortex, and let stand no more than 5 minutes. Overbleaching can kill seeds; not enough will not sterilize sufficiently.
  3. Wash with 1 ml sterile water 3 times.
  4. Add 0.5 ml sterile agarose and resuspend seed. Drop onto plates with a pipetman and sterile pipet tip.
  5. Dry the plate for a few minutes with the lid off; then add the lid and wrap with filter tape.
  6. Incubate the plates in the fridge 2 days, then put in the incubator to grow.

B. With bleach vapor:

1. Put 100 to 500 ml seed into a labeled eppendorf tube. (A VWR marker is best).

2. Place the tube in a rack inside a sealable plastic container in the fume hood.

3. Wearing appropriate safety gear, add 4 ml concentrated HCl to 100 ml bleach in a 250 ml glass beaker set inside the container.

4. Quickly seal the container and let it sit in the hood for 5-6 hours.

5. Vent in the fume hood and close the tubes. Now they are ready to place on plates in the laminar flow hood.