

Vapor-Phase Sterilization of Arabidopsis Seed

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The following protocol contains a convenient method for surface-sterilization of plant seeds. In particular, the method does not require all of the soaking and rinsing of liquid-phase bleach-sterilization methods. While this protocol substantially reduces hands-on manipulations, it does require some incubation time (a few hours to overnight).

We often use this protocol in conjunction with selection of Arabidopsis transformants on MS medium + antibiotics. See the related "[Simplified Arabidopsis Transformation Protocol](#)." This vapor-phase sterilization protocol was adapted from a version supplied by Maud Hinchee and colleagues at Monsanto.

- 1. Obtain a vessel for seed sterilization, typically a dessicator jar. Place in fume hood.
- 2. Place seed that is to be sterilized into appropriate resealable containers (for example, microcentrifuge tubes).
- 3. Place open containers of seed into a rack or stand inside the dessicator jar.
- 4. Place a 250 ml beaker containing 100 ml bleach into the dessicator jar.
- 5. Immediately prior to sealing the jar, carefully add 3 ml concentrated HCl to the bleach.
- 6. Seal jar and allow sterilization by chlorine fumes to proceed for a period of between three and sixteen hours. The time needed will vary based on the configuration of seed and the extent to which seed is contaminated. Three to four hours is often sufficient for reasonably clean seed. Overnight is usually acceptable although some seed killing may occur, especially if seed is not fully mature and dry.
- 7. Depending on the application, open container in fume hood or in sterile laminar flow hood, seal microcentrifuge tubes or other seed containers, and remove surface-sterilized seed for use.

Notes:

-Some lab members suspect that sterilization in 15 ml orange-cap tubes is more likely to result in seed mortality.

-Obtain proper approval for transformation work from institutional authorities. Autoclave and properly dispose of all materials.

Link to protocol online: <http://www.arabidopsis.org/portals/education/vapor.jsp>