Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Davison Life Sciences Building, Lab B310

Chemical(s)	MS (Murashige and Skoog) Basal Salt, Vitamin solution, Gellan Gum, Triton X-100, Kanamycin, Timentin, Bleach, Ethanol.
Process	**Arabidopsis Seed Sterilization and Plating**
Specific Hazards *referred to MSDSs for more detailed information*	Bleach: Corrosive. Ethanol: Flammable.
Personal protective equipment	☒ 3-5 mil nitrile gloves □ double gloves (w/ concentrated stock) ☒ lab coat (except when used in microcentrifuge tubes) □ chemical safety goggles (when splash potential exists)
Engineering/ventilation controls	Emergency shower and eyewash accessible.
Special handling procedures and storage requirements	MS salt, Vitamin: @ 4°C. Kanamycin, Timemtin: @ -20°C. Bleach: stored under the sink.
Spill and accident procedures	Skin exposure: Wash skin with water for 15-20 minutes. If irritation develops, call a physician. Eye exposure: Hold eye open and rinse with water for 15-20 minutes. Remove contact lenses, after first 5 minutes. Continue rinsing eye. Call a physician.
for hazardous chemicals only	Spill: Containerize liquid and use absorbents on residual liquid; dispose appropriately. Wash area and let dry. For spills of multiple products, responders should evaluate the MSDS's of the products for incompatibility with sodium hypochlorite. Breathing protection should be worn in enclosed, and/or poorly ventilated areas until hazard assessment is complete.
Waste disposal	Bleach < 10% can be drain into sink with large quantity of water.
Special approval	No special authorization needed after SOP training & reading MSDSs.
Prepared by	Name/date: Kate Tay and C-J Tsai, 8/11/09, revised 03/22/10
Reviewed by	

Arabidopsis Seed Sterilization and Plating

General Information: 100 µl dry seed volume ≈ 2500 seeds

Petri Dish	Size (mm)	1L media make:	Each plate holds:	Per 100 µl dry seed:
Normal Petri dish	85 x 13	40 dishes	~1,250 seeds	2 plates
Large Petri dish	140 x 13	20 dishes	~2,500 seeds	1 plate

(A) Media preparation for Arabidopsis seed germination:

Refer to the "Transformation of *Populus tremula* x *P. alba* INRA 717-1B4" SOP for detailed instructions on preparation of media

	500 ml	1 L
½ MS salt **(no sucrose, no vitamin)**	1 g	2 g
Adjust pH to 5.7		
Gellan Gum	1.5 g	3 g
Autoclave for 25 min/500 ml or 40 min/1 L & cool to ~60°C		
Optional: 50 mg/L Kan [stock = 100 mg/ml], or 5 mg/L Hyg [stock = 100 mg/ml], or other selecting agent	250 µl 25 µl	500 µl 50 µl
Optional: 300 mg/L Timentin [stock = 200 mg/ml]	750 µl	1.5 ml
Pour to plates & allow to solidify		

Also autoclave 0.1% gellan gum in 5-10 ml ddH_2O for use in Step B-8 below.

(B) Arabidopsis Seed Sterilization and plating:

For each transformation, screen > 2,500 seeds (1% transformation efficiency = 25 seeds)

1. Aliquot 100 µl of seeds into a 1.5 ml tube
2. Add 1 ml 70% EtOH, shake or vortex for 5 min (making sure seeds are dispersing freely – not in clumps)
3. Spin briefly to pellet the seeds, and remove supernatant
4. Add 1 ml 50% Bleach (with 0.1% or 1 µl of Triton X-100), shake for 30 min
5. Spin briefly and remove supernatant in a laminar flow
6. Wash with 1 ml sterilized water 3 times, for 5 min each, by shaking or vortexing.
7. Spin briefly to pellet the seeds, and remove supernatant
8. Add 1 ml autoclaved 0.1% gellan gum (in ddH_2O) to resuspend the seeds.
9. Use 1 ml pipet tip to dispense the seed/agar slurry drop-by-drop onto the plate.
10. If necessary, add sterile water to "splash" seed clumps in order to more evenly distribute the seeds. Remove excess water with a pipet, dry the plate for ~10min.
11. Store @ 4°C for 2days
12. Place the plates in the tissue culture room under 16h/8h light

Notes:

* For T2 seeds, 70% EtOH for 5 min and 50% bleach for 10 min usually work well.
* Seeds will germinate in one day @ RT, with an 85-90% germinate rate.
* It may take 2 weeks or longer to kill non-transformed seeds that germinated.
* You may need to adjust the plating density of seeds for specific experiments
* If plating seed one-by-one, 1 small petri dish can plate ~ 60 seeds.