



BLEACH STERILIZATION OF ARABIDOPSIS SEEDS

References

Blomme, Jonas, et al. "The heat is on: a simple method to increase genome editing efficiency in plants." *BMC Plant Biology* 22.1 (2022): 142.

Materials

- Miracloth filter
- 500 mL glass bottle
- Ethanol 70% v/v
- Sodium hypochlorite (NaOCl) 4.8% v/v (prepare fresh)
- Sterile distilled water
- Stapler

Protocol

1. Cut out 10 × 10 cm pieces of Miracloth filter. Label one piece per construct with pencil in the middle.
2. Pack the seeds in miracloth packages.
3. Transfer selected seeds to the middle of the piece of Miracloth. Fold Miracloth into a small package. Close using a staple.
4. Put all packages into a 500 mL glass bottle.
5. Work in a laminar flow cabinet. Add 500 ml of 70% ethanol and incubate 2 min at room temperature. Shake the bottle gently to allow contact with all packages.
6. Pour off 70% ethanol into a glass beaker. Discard in the appropriate waste stream.
7. Add 500 ml of 4.8% sodium hypochlorite (NaOCl; bleach). Incubate 13 min at room temperature. Shake the bottle gently every minute to allow contact with all packages.
8. Pour off the 4.8% sodium hypochlorite into a glass beaker. Discard in the appropriate waste stream.
9. Add sterile distilled water. Incubate for 5 min, shaking the bottle gently to allow contact with all packages.
10. Pour off the distilled water into a glass beaker. Discard in the appropriate waste stream.
11. Repeat the wash step with fresh sterile distilled water at least four times to remove all traces of bleach. The seeds are ready to be sown.

