Arabidopsis Seed Sterilization Protocol for Screening – Rahul Patharkar

1. Get a 15 mL tube and label with genotype of seeds
2. Fill with desired seeds
3. Add 2 ml 15% bleach with 0.1% Triton X-100 to the tube (per 200-250ul of seeds).
4. Shake by hand to make sure every part of the tube is touched with bleach (there should be no seed clumps-this ensures that every individual seed is sterilized)
5. Rock the tube for about 5-7 minutes (the total time the seeds are exposed to 15% bleach should be limited to 10 minutes including handling and centrifuge time).
6. Spin down for 12 seconds in in a swinging bucket centrifuge (spinning for 12 sec will reach approximately 1500xg in a Heraeus Multifuge X1R with rotor 75003629).
7. Carefully pour off the bleach solution.
8. Fill the tube to 14 ml with sterile water. Shake vigorously until all seeds are dispersed.
9. Spin down for 12 sec.
10. In the hood carefully pour off the bleach and water into a beaker.
11. Fill tube with sterile water to about 14 mL and shake upside down until all seeds are dispersed.
12. Spin down for 12 sec.
13. In the hood, pour off water slowly.
14. Repeat steps 11-13 two more times. There should not be foaming for the last wash indicating that you have removed all of the detergent and bleach.
15. Optionally, add 1 µl 300 mg/mL timentin per mL of wet seeds. Mix well. Timentin inhibits agrobacterium growth that survived the sterilization under the seed coat.
16. If BASTA selection is to be performed on plates, add 1 µl 20 mg/mL glufosinate ammonium per mL of wet seeds.
17. Cold treat at 4°C for 1-3 days.
18. Plate seeds on appropriate plates with appropriate protocol for the selectable marker.

Notes:

1. 200 µL dry seeds = 5400 seeds = 0.135 g

See video protocol here:
https://rahulpatharkar.000webhostapp.com/2018/11/sterilizing-seeds-and-selecting-for-hygromycin-resistance