Hygromycin Selection Protocol for Plants

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Hygromycin B from Bioshop stock = 100mg/ml

Working concentration = 20ug/ml

Therefore, 100ul of stock / 500ml AT media

1) Put seeds out on Hygromycin 20ug/ml plates
2) Keep plates at 4^{o}C wrapped in foil paper for 2-3 days
3) Move plates to continuous light for 6-12hrs at RT (better to do it for 12 hrs)
4) Put plates in the dark again for 2-3 days but this time at RT
    - Sensitive seedlings – short hypocotyls; bleached cotyledons; little or no germination
    - Resistant seedlings – long hypocotyls; green cotyledons

    NB: If no resistant seedlings show up even in positive control population then repeat steps 3 and 4 once again.

5) Keep at bench in low light for a day to let seeds recover
6) Then transfer plates to growth chamber until ready to transplant into soil.