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Working

Arabidopsis flower dip transformation

Forked from Arabidopsis flower dip transformation

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ABSTRACT

A standard protocol how to transform Arabidopsis plants using a flower-dip method.

For higher rates of transformation, plants may be dipped two times at 5-7 day intervals. We suggest one dip two days after clipping, and a second dip one week later.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

SAFETY WARNINGS

1 Grow healthy Arabidopsis plants until they are flowering.

2 Prepare Agrobacterium tumefaciens strain carrying gene of interest on a binary vector. Grow a large liquid culture @ 28C in LB with antibiotics to select for the binary plasmid, or grow in other media.

3 Spin down Agrobacterium, resuspend to OD600 = 0.8-1.0 (can be higher or lower) in LB with 5% Sucrose. You will need 250 ml for each four small pots to be dipped.

4 Before dipping, add Silwet L-77 to a concentration of 0.05% (125 ul/250mL) and mix well.

5 Dip above-ground parts of plant in Agrobacterium solution for 10-30 seconds, with gentle agitation. You should then see a film of liquid coating plant.

6 Place dipped plants under a cover in a dark O/N to maintain high humidity (plants should be laid on their side).

7 Water and grow plants normally. Stop watering as seeds become mature.

8 Harvest dry seed. Transformants are usually all independent, but are guaranteed to be independent if they come off of separate plants.

9 Select for transformants using antibiotic or herbicide selectable marker.

10 Transplant transformants to soil.