

Transformation of Arabidopsis with EGAD libraries.

Transformation of EGAD libraries into Agro:

Transform 1 ug of EGAD library DNA into 800 uL of electro-competent GV3101

This can be accomplished by adding DNA to cells on ice, mixing and then transferring the mixture into 4 chilled electroporation chambers (200 ul per .2 cm gap tube). I prefer to do this to keep the ratio of DNA to cells constant for all of the transformations. The idea is to get to an equal representation of each bug transformed on the final plates.

Pulse in biorad gene pulser at 2.5 kV.

Add 1 ml of SOC to cells, and transfer to a 12 ml Falcon tube.

Repeat for remaining electroporation cuvettes, adding the cells to the same Falcon tube.

Fill the falcon tube with SOC to approx. 6 ml final volume

Incubate at 30° C for 1 - 2 hr. with gentle shaking.

Plate the culture onto 10 150 mm petri plates (600 uL per plate).

Grow for 3 - 5 days at 30° C

There should be approximately 5000 - 10000 colonies per plate.

Prepare cells for plant transformation as described below.

Preparation of Agro for Arabidopsis Transformation:

Make 200 mL of infiltration medium per flats to be sprayed.

Or, 2 L per 4 flats by dunking (for each additional flat, make an extra 200 mL).

Scrape Agro off plates using a spatula and transfer to a 50 ml Falcon tube containing 10 ml of infiltration medium (IM) (Transfer cells from 5 plates to one falcon tube).

Resuspend the cells in IM (LB is OK to). This is done most easily by pipetting them up and down with an autopipettor with 10 ml IM. Once the cells are evenly resuspended, fill tube up to 40 ml with IM and then until the cells are evenly resuspended.

Spin for 25 minutes at high speed (3000 rpm) on a clinical desktop centrifuge.

Pour off the supernatant and resuspend the cells in 40 ml IM.

Take the OD₆₀₀ of the resulting solution (dilute 500 fold into IM for taking OD).

I have found that the OD₆₀₀ of the cells at this point is usually between 10 - 50 OD₆₀₀ units.

Add cells to enough of the remaining IM to get a final OD₆₀₀ of 0.5 (this OD₆₀₀ has given me the most consistent + high transformation rates (i.e. 1% and higher). About 1.5 - 2 liters of infiltration solution is enough to transform 4 flats of plants (by the dunking method).

If there are cells remaining, add glycerol to 15% , store at -80. The frozen Agro libraries can be used for subsequent transformations or “booster sprayings”. This is easily accomplished by thawing the cells, plating them out and then prepping them as described above. With the high initial OD of the glycerol stock, efficient growth can occur in 24 hours speeding things up considerably.

Dunking/Spray Transformation of Arabidopsis with Agro:

Plants to be transformed should be grown in pots with mesh coverings to prevent soil loss during dunking.

If you are going to be spraying (instead of dunking), planting the seed in pots or flats without mesh works perfectly fine. Using big pots tends to create big plants and thus increases seed yield, this is valuable if you are attempting to make large numbers of transgenics.

For either method, avoid using plants grown at a high density, as this tends to reduce overall vigor and seed yield. I typically used 8 big pots per flat, with 20 -40 plants per pot. I don't know if high density affects transformation rate, but in my experience it can affect seed yield.

Make sure the plants are well watered prior to dunking to prevent the soil from absorbing an excess of infiltration solution.

Dunking method:

Place 800 ml of infiltration solution into a Pyrex dish large enough to hold about 6 pots (there should be enough solution to cover the leaves when all the pots are added; if not, add more infiltration solution).

Place the plants in the solution (aerial parts submerged). I use plants that have just bolted (4-5 weeks old). Older plants that have been cut back can yield high transformation rates as well. Let the plants sit in the infiltration solution for 2-5 minutes.

Lift the plants out, drain for a few seconds and transfer them back to their flats. Cover with domes to prevent dehydration.

Repeat the transformation process for as many pots as required. I have found that 6 pots will absorb about 150 ml of solution (if they are well watered prior to dunking; they'll absorb all the solution if they are even modestly underwatered). I therefore add about 150-200 ml of infiltration solution to the Pyrex dish before dunking the next set of plants.

Transfer the plants back to their growth space and remove domes after 2 days.

Optional. To achieve higher transformation rates, you can give "booster" sprays weekly with infiltration medium containing Agro.

Spraying Method:

Spray Each flat extensively with 200 mL of Agro/IM.

Cover with plants with domes to prevent dehydration.

Remove domes after 2 days.

Repeat spraying every 4 -5 days for 3 weeks (usually 4 sprayings in total).

When the plants have stopped flowering (4-5 weeks after dunking/first spray), stop watering them and allow them to desiccate. Harvest the seed after the plants have completely desiccated. Allow the seed to dry for about 1 week prior to plating them.

Plate approximately 3000 - 5000 seed per 150 mm petri dish, selecting on glufosinate (or an appropriate antibiotic) at 10 ug/ml.

Infiltration Medium (1 Liter):

Prepare on day of transformation, does not need to be autoclaved.

4.5 g MS salts

1 ml 1000X Gamborgs Vitamins (Sigma)

50 g Sucrose

0.5 g MES, pH'd to 5.7 with KOH (approximately 180 ul of 5 M KOH will pH MES/MS to 5.7)

200 uL Silwet

10 ul 1 mg/ml benzylaminopruine (approx. 0.04 uM final conc.)

qc to 1 L with water.

Notes:

I originally started amplifying the Agro on plates to minimize amplification biases that can result from differential growth rates of bugs in liquid culture. In my experience, using plate grown Agro for transformations doesn't seem to lower transformation efficiency. I have found that growing the Agro on plates is an easy and space efficient way to prep the bugs for transformations, and I have actually started doing this for all of my transformations. Agro grown densely on 10 plates can easily give you about the same amount of OD₆₀₀ units as Agro grown to saturation in 3 liters of lb.

Sean Cutler

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