Green lab protocol for vacuum infiltration transformation of Arabidopsis

Step by step picture demonstration

This protocol is adapted from protocols by Nicole Bechtold (Bechtold et al. 1993), Andrew Bent (Bent et al. 1994) and Takashi Araki. No claims are made that any of the steps are necessary or ideal; these experiments have not been done. However, this protocol gives us very good results, with at least 95% of all infiltrated plants giving rise to transformants, and a transformant rate of 1-4% of seed.

1. Sow seeds of ecotype Columbia in lightweight plastic pots prepared in the following way: mound Arabidopsis soil mixture into pots (We use 3 1/2 inch to 4 inch square pots), saturate soil with Arabidopsis fertilizer, add more soil so that it is rounded about 0.5 to 1 inch above the top, dust with fine vermiculite, cover soil with a square of window screen mesh (Circle Glass Co., Detroit, MI) and secure mesh with a rubber band.

2. Grow plants under conditions of 16 hours light/ 8 hours dark at 20 °C to 22 °C, fertilizing from below with Arabidopsis fertilizer once a week , adding approximately 0.5" to each flat. Thin the plants to one per square inch or fewer per pot. After 4-6 weeks, depending on your conditions, plants will be ready to infiltrate when they are at this stage: the primary inflorescence is 5-15 cm tall and the secondary inflorescences are appearing at the rosette. No clipping of bolts is necessary before infiltration.

3. In the meantime, transform your construct into Agrobacterium tumefaciens strain GV3101 (C58C1 Rifr) pMP90 (Gmr) (Koncz and Schell 1986). When plants are ready to transform, inoculate a 500 ml culture of YEP medium containing 50 mg/l rifampicin, 25 mg/l gentamycin and the appropriate antibiotic for your construct with a 1 ml overnight starter culture. Be sure to water your plants well the day before infiltration so that the stomata will be open that day.

4. Grow culture overnight at 28 °C with shaking, until culture OD_{600} is > 2.0. Spin down the culture and resuspend it in 1 liter of infiltration medium.

   Infiltration medium (l liter)
   2.2 g MS salts
   1X B5 vitamins
   50 g sucrose
   0.5 g MES
   pH to 5.7 with KOH
   0.044 µM benzylaminopurine (be sure the final concentration is micro molar).
   200 µl Silwet L-77 (OSi Specialties request that purchases be
   made at Lehle Seeds, fax # (512) 388-3974

catalog # vis-01)

5. Place resuspended culture in a Rubbermaid container inside a vacuum desiccator. Invert pots containing plants to be infiltrated into the solution so that the entire plant is covered, including rosette, but not too much of the soil is submerged. One good way to do this is to place the corners of the pots on rubber stoppers sitting in the culture. Make sure no large bubbles are trapped under the plant.

6. Draw a vacuum of 400 mm Hg (about 17 inches). Once this level has been obtained, close the suction (i.e., so that the vacuum chamber is still under 17 inches of mercury but the vacuum is not still being directly pulled) and let the plants stay under vacuum for five minutes. Quickly release the vacuum. Briefly drain the pots, place them on their sides in a tray, cover the tray with plastic wrap to maintain humidity, and place the flats back in a growth chamber. The next day, uncover the pots and set them upright. Keep plants infiltrated with different constructs in separate trays from this stage on.

7. Allow plants to grow under the same conditions as before (see step 2). Stake plants individually as the bolts grow. The leaves that were infiltrated will degenerate, but plants continue growing until they finish flowering. Gradually reduce water and then stop watering to let them dry out. Harvest seeds from each plant individually.

8. Prepare large selection plates:
   - 4.3 g/l MS salts
   - 1X B5 vitamins (optional)
   - 1% sucrose
   - 0.5 g/l MES
   - pH to 5.7 with KOH
   - 0.8% phytagar
   - Autoclave.
   - Add antibiotics (30 g/ml works well for kanamycin).
   - Pour into 150 X 15 mm plates.

   We also add vancomycin at 500mg/l to control bacterial growth.

9. Dry plates well in the sterile hood before plating. Twenty minutes to half an hour with the lids open is usually sufficient.

10. For each plant sterilize up to 100 µl of seeds (approximately 2500 seeds) and plate out individually. Sterilize seeds (7 minutes rocking in 50% bleach/0.02% Triton X-100, 3 rinses in sterile distilled water). Resuspend seeds in approximately 8 ml sterile 0.1% agarose and pour onto large selection plates as if plating phage. Tilt plate so seeds are evenly distributed,

and let sit 10-15 minutes. After a while the liquid should soak into the medium; if evaporation is too slow, open the plate in the hood and let dry until the excess liquid is gone. Seal plates with Parafilm or paper surgical tape and place in a growth room.

11. After 5 to 7 days, transformants will be visible as green plants. Transfer these onto "hard selection" plates (100 x 20 mm plates with same recipe as selection plates but with 1.5% phytagar) this allows roots to elongate and eliminates any false positives. Place in growth room.

12. After 6-10 days, plants will have at least one set of true leaves. Transfer normal conditions. Keep covered for several days. Note: We usually move just one transformant to soil from any one plant that was infiltrated, to ensure independent transformants.

References:

- Bechtold N, Ellis J, Pelletier G (1993) C. R. Acad. Sci. Paris 316:1194-1199
- Bent A, Kunkel BN, Dahlbeck D, Brown KL, Schmidt R, Giraudat J, Leung J,
- Staskawicz BJ (1994) Science 265:1856-1860
- Koncz C, Schell J (1986) Mol. Gen. Genet. 204:383-396

Solutions

YEP medium (1 liter)

10 g Bacto peptone
10 g yeast extract
5 g NaCl

1000X B5 vitamins (10 ml)

1000 mg myo-inositol
100 mg thiamine-HCl
10 mg nicotinic acid
10 mg pyridoxine-HCl
Dissolve in ddH2O and store at -20oC.

Arabidopsis fertilizer (10 liters)

50 ml 1M KNO3
25 ml 1M KPO4 (pH 5.5)
20 ml 1M MgSO4
20 ml 1M Ca(NO3)2
5 ml 0.1M Fe.EDTA

10 ml micronutrients (see below)
Dissolve in ddH2O and store at room temperature

Arabidopsis micronutrients (500 ml)

70 ml 0.5M boric acid
14 ml 0.5M MnCl2
2.5 ml 1M CuSO4
1 ml 0.5M ZnSO4
1 ml 0.1M NaMoO4
1 ml 5M NaCl
0.05 ml 0.1M CoCl2
Dissolve in ddH2O and store at room temperature

Commonly asked questions about this vacuum infiltration method:

Q. Why add vancomycin to the seed selection medium?

A. We find it decreases Agrobacterium growth that may occur as the seedlings germinate. Although the seed coat is surface sterilized, in our experience some seeds may contain Agrobacterium inside the seed coat.
We have found an inexpensive source of vancomycin at our university pharmacy. You may want to check at your pharmacy, indicating it is for research purposes only, and ask for it in injectable vials.

Q. Why thin plants to just a few per pot?

A. We found that it increased the transformation rate of primary plants to at least 95%. It also increases the number of seeds generated per plant and it facilitates harvesting individual plants. We harvest plants individually and choose one transformant per primary plate to assure that transformants are independent.

Q. If the leaves die shortly after infiltration, is something wrong?

A. No, we always see rapid degeneration of leaves after infiltration. Keep watering and growing plants until they finish flowering.

Q. What method does the Green lab use to transform Agrobacterium tumefaciens ?

A. We prefer electroporation. Refer to the manual of your electroporation unit for method.

Any other questions or comments about the Green lab protocol for vacuum infiltration of Arabidopsis can be sent to Linda Danhof.