

Alternative protocol for Preparing competent *Agrobacterium tumefaciens* for electroporation

protocol according to J.M. Kwak *in* UCSD by personal communication

What you need:

1. *Agrobacterium tumefaciens* cells. Stock is in a box in the – 70 °C freezer.
2. selective plates (e.g. YEP + rifarm for strain GV3101)
3. appropriate antibiotic for O/N culture (e.g., streptomycin for GV3101)
4. sterile test tubes
5. YEP medium
6. sterile cold sucrose-glycerol buffer (272mM Sucrose, 15% Glycerol)
 - about 25ml for 1L starting cell culture
7. sterile cold water – 1.5L for 1L starting cell culture

What you do:

1. Inoculate 1L of YEP broth with 1/100 volume of a fresh overnight culture.
2. Grow cells at 28°C with vigorous shaking to an OD600 of 0.8 to 1.0 (about 12 hrs)
3. To harvest, chill the flask on ice for 15 to 30 min and centrifuge in a cold rotor at 4000xg for 15min. (alternatively 6000rpm 7min)
4. Remove as much of the supernatant as possible. Resuspend in a total of 1L of cold water. centrifuge as in step 3.
5. Remove as much of the supernatant as possible. Resuspend in a total of 0.5L of cold water. centrifuge as in step 3.
6. Resuspend in 20ml pf cold 272mM sucrose, 15% glycerol. centrifuge as in step 3.
7. Resuspend to a final 4~5ml of cold 272mM sucrose, 15% glycerol.
8. This suspension should be frozen in aliquots (200µl) on LN, and stored at -70°C.

Clean-up:

1. Every tool that came in contact with the bacteria has to be sterilized (bleach or autoclave)!
Agrobacterium tumefaciens is a plant pathogen and must not be released to the environment!
2. electroporation cuvettes can be cleaned the following way: (a) soak in 1% bleach for < 5 min, (b) rinse several times with sterile water, (c) rinse twice with isopropanol, (d) invert on kimwipe to dry, (e) replace cap and store until next use.

Transformation of *Agrobacterium tumefaciens* with binary plasmids

electroporation protocol according to C.H.Shaw *in*: Methods in Molecular Biology, vol. 49: Plant Gene Transfer and Expression Protocols. H.Jones (ed.) Humana Press, Totowa, NJ.

note: Condition for electroporator has been changed by Eunsook Park.

What you need:

1. competent *Agrobacterium tumefaciens* cells.
They are already pre-made in a box in the -70°C freezer.
2. minipreps of binary plasmids. (Check them first to make sure they contain the right insert.)
3. electroporation cuvettes with 1 mm gap distance (may be able to borrow from Von Arnim lab)
4. sterile test tubes and pasteur pipettes
5. YEP medium
6. selective plates (e.g. YEP + streptomycin + kanamycin)

What you do:

1. make sure you can use the electroporator in the von Arnim lab.
2. get competent *Agrobacterium* (strain LBA 4404) from -70° freezer and **thaw on ice**.
3. set up electroporator to use $25\ \mu\text{F}$, $2.5\ \text{kV}$, $200\ \Omega$. Main switch is on power supply (don't change any settings on it).
4. set toggle switch to "charge" while machine is "disarmed" (meter will go up to ~ 15)
5. use $2\ \mu\text{l}$ of miniprep DNA for $40\ \mu\text{l}$ of bacteria, mix briefly, transfer to pre-chilled cuvette.
6. wipe down outsides of cuvette with kimwipe to remove moisture
7. place cuvette in electroporator, switch to "armed", flip toggle switch to pulse
8. disarm electroporator, set to charge again, remove cuvette
9. add 1 ml of pre-chilled YEP medium and transfer bacteria to test tube
10. incubate bacteria at 28°C for 3 to 4 hours on roller drum
11. plate bacteria on selective medium (typically YEP plates with streptomycin and kanamycin).