

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.

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. LB medium
6. selective plates (e.g. LB + 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 $50\ \mu\text{F}$, 1.8 kV, $150\ \Omega$. 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 SOC medium and transfer bacteria to test tube
10. incubate bacteria at 28°C for 1 to 2 hours on roller drum
11. plate bacteria on selective medium (typically YEP plates with streptomycin and kanamycin).

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.