Preparing competent *Agrobacterium tumefaciens* for 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. *Agrobacterium tumefaciens* cells. Stock is in a box in the – 70 °C freezer.
2. selective plates (e.g. LB + streptomycin for strain LBA4404)
3. appropriate antibiotic for O/N culture (e.g., streptomycin for LBA4404)
4. sterile test tubes
5. LB medium
6. sterile HEPES buffer (10 mM, pH 7) with 10 glycerol

What you do:

1. grow overnight culture of *Agrobacterium* (typically 4 tubes of 3 ml)
2. spin down cells
3. resuspend in xx ml **ice-cold** HEPES + glycerol buffer
4. repeat steps 2 and 3 twice (total of 3 washes)
5. aliquot bacteria into prechilled microfuge tubes, 40 μl each
6. freeze in liquid nitrogen
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!

updated on 03.01.09 by Andreas