

Electroporation of *Agrobacterium tumefaciens*

1. Incubate *A. tumefaciens* in 30 ml of YEB overnight at 28C with shaking.
2. Chill the bacteria on ice and centrifuge at 3000 rpm for 10 min. at 4C
3. Xxx
4. Take the bacteria up in 1 ml ddH₂O at 0C.
5. Place an electroporation cuvette on ice to chill. Pipette in 100 ul bacteria. Add 200 to 500 ng of plasmid in water or TE. Used 1 ul of a 1:10 dilution of plasmid and gently mixed with the pipette tip.
6. Electroporate at 200 ohms, 25 uF, 2.5 kV at room temperature (takes only a few seconds). For 0.2 cm cuvettes, use 2.5 kV. For 0.1 cm cuvettes, use 1.8 kV. These specifications are for the BioRad electroporator for *E. coli*, cat. no. 165-2098.
7. Add immediately 400 ul of YEB + 1% mannitol.
8. Incubate bacteria in a eppendorf tube or the cuvette for 3 hours at 28C.
9. Plate bacteria on YEB (+1% mannitol?) with selective antibiotic and incubate for two to three days at 28C.

Prepare ahead of time:

YEB:

- 5 g/l beef extract (Tryptone)
- 1 g/l yeast extract
- 5 g/l peptone
- 5 g/l saccrose
- 1.8% agar
- Adjust pH to 7.2
- After cooling, add 2 ml/l of 1 M MgSO₄
- Rif (rifampicyna) added at 50 mg/l

100 ml of double distilled water at 0C