



Electroporation Transformation of *Agrobacterium*

Materials

- Aliquot of competent cells (50 μ l, from the -70 °C freezer)
- Sterile liquid YEB medium
- Sterile 13 ml tube
- Ice
- Electroporation cuvettes (green cap – 2 mm)
- Biorad Gene Pulser (you can find one in E026)
- YEB plates with the necessary antibiotics
PMP90 C58C1: Rif + Gent + Spec

Preparation

- Pre-cool the electroporation cuvettes on ice
- In the laminar flow: pipet 950 μ l YEB medium in the 13 ml tube
- Optional: For a more efficient transformation: pre-heat the tubes and YEB plates at 28 °C

Protocol

1. Thaw the aliquot of competent cells on ice
2. Add 1 μ l of plasmid DNA (100 ng/ μ l) and mix by pipetting carefully and/or flicking the tube
3. Carefully transfer the mix to the electroporation cuvette by pipetting, make sure there are no air bubbles, otherwise it will arc (you can remove these by tapping the cuvette on the bench)
4. Switch on the Genepulser and select the EC2 program
5. Dry the metal contacts of the cuvette with a tissue and place it in the Genepulser
6. Give a pulse (Check the time constant, it should be around 6 ms. The voltage should be around 2.5 kV)
7. Immediately transfer the pre-heated YEB medium to the cuvette, carefully pipet up and down to mix with the cells, and then transfer back to the 13 ml tube
8. Incubate for 2 hours in a 28 °C shaker at 200 RPM
9. Plate 100 μ l of the mix on a YEB plate with antibiotics
10. Incubate the plate for 2 days at 28 °C
11. Store the left-over at 4 °C in case you need to plate out the rest as well