



Heat-Shock Transformation of Agrobacterium

Materials

- Aliquot with competent cells (from the -70 °C freezer)
- Sterile liquid YEB medium
- Ice
- Liquid nitrogen
- Heat block for 1.5 mL tubes
- YEB plates (YP when transforming EHA105) with the necessary antibiotics
PMP90 C58C1: Rif + Gent + Spec
EHA105: Rif + Spec

Before you begin

- Set the heat block to 37 °C
- Optional: For a more efficient transformation: pre-heat the YEB medium and the YEB plates at 28 °C

Protocol

1. Thaw a 200 µl aliquot of competent cells on ice
2. Add 1 µg of plasmid DNA and mix carefully by pipetting or flicking the tube
3. Incubate the tube on ice for 5 minutes
4. Move the tube to liquid nitrogen for 5 minutes
5. Let the cells thaw on the bench, and then incubate for 5 minutes at 37 °C
6. In the laminar flow: add 800 µl of liquid YEB medium
7. Incubate for 2 - 4 hours in a 28 °C shaker at 200 RPM
8. Plate 200 µl of the mix on a YEB or YP plate with antibiotics
9. Incubate the plate for 2 days at 28 °C
10. Store the leftover at 4 °C in case you need to plate out the rest as well