

Transformation *E. coli*: heat shock



Category	Experimental Procedures
Author	Anemoon Drinkwaard
Version	4

Labels:

Introduction

This protocol describes the procedure necessary to transform *E. coli* with your own designed DNA/plasmids.

Materials needed

1. competent cells (*E. coli* DH5 alpha lambda pir) (frozen in -80°C) -> at SSB Chairgroup made by the technician
2. DNA to transform (e.g. ligation mixtures with pSB1C3)
3. LB medium (no agar)
4. Two LB + agar plates with antibiotics corresponding to your DNA/plasmid per DNA
5. Thermo-Shaker

Procedure

1. Thaw competent cells for 20-30 minutes on ice
 2. Add DNA/plasmid solution (e.g. ligation mixture from Golden gate) and let rest for 10 minutes
 3. Perform a heatshock for 1.5 minutes at 42°C (In Thermo-Shaker or water bath)
 4. Put 5 minutes on ice
- NOTE: can do two heat shocks if preferred (step 3 and 4)
5. Add 1 mL LB (without agar)
 6. Shake the tubes at 37°C and 500/600 rpm for 1h (in ThermoShaker)
 7. Plate 100 ul directly
 8. Centrifuge the remaining 900 ul for 5 minutes at 7200 rpm & remove the supernatant (approx 850 uL) & resuspend the pellet with leftover liquid
 9. Plate the resuspension
 10. Incubate the plates at 37°C (in Incubator)

Attachments

No file attachments

*This procedure was originally created by **Anemoon Drinkwaard***

