

TRANSFORMATION OF E.COLI

E.coli transformation (CaCl₂ method)

1. For up to ten transformations. Set up 40ml culture of 1:100 dilution of o/n E.coli culture in 2xTY in a 250ml flask i.e. 0.4ml in 40ml.
2. Grow 37°C for 1-2 hours until A₆₀₀=0.6. Transfer to 50ml Falcon tube and chill on ice 15 min. Spin 3K 15 min 4°C. Use precooled pipettes and try to keep the bugs as cold as possible.
3. Resuspend pellet in 20ml cold 50mM CaCl₂. Leave on ice for as long as possible, at least > 15 min.
4. Spin 3K 10 min 4°C.
5. Gently resuspend in the required amount of cold 50mM CaCl₂ (i.e. no. tf'ns * 0.2, max. 2ml). Leave on ice 15 min.
6. Add 0.2ml of cells to DNA in 0.1ml 0.1M Tris 7.2 in precooled glass tubes. Leave on ice 20 min.
7. Heat shock 2 min 42°C.
8. Add 1ml 2xTY*. Incubate 37°C 20 min.
9. Pour onto plates warmed to 37°C and spread with beads. Grow o/n 37°C.

notes

can use LB instead of 2xTY

can use SOC instead of 2xTY at this stage