E. coli Heat Shock Transformation

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Abstract

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Protocol

Step 1.
Thaw competent cells on ice (20-30min).

Step 2.

Combine 1-5µl of DNA (10pg - 100ng) into 50µL of competent cells in a microcentrifuge tube. Flick the bottom of the tube with your finger a couple of times to mix.

Step 3.
Incubate cell/DNA mixture on ice for 20-30min.

Step 4.
Place transformation tube into a 42°C water bath for 42 seconds (30-60 sec).

Step 5.
Return tube to ice for 2 min.

Step 6.
Add 500µl LB medium, grow in 37°C shaking incubator for 1 h.

Step 7.
Plate transformation on LB agar plates containing the appropriate antibiotic. Often e.g. 50 uL on one plate and 200 uL on another gives a good chance of single colonies. The rest of the transformation can be left on the benchtop overnight and plated the next day if needed.

Step 8.
Incubate plates at 37°C overnight.