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04 Transformation

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MATERIALS

NAME	CATALOG #	VENDOR
Shaker incubator		
Ice		
Centrifuge		
Competent Cells		
LB medium	/	

BEFORE STARTING

Preparation competent cells.

1 **Preparation of competent cells**

1.Streak out the E.coli strain on an LBM plate (no ampicillin!) to isolate colonies and incubate at 37 degrees C overnight (16-20 hours).
2. Use a sterile inoculating loop to collect cells from a single colony and inoculate 50 ml sterile 1X LBM Grow at 37 degrees C overnight (16-20 hours) in a shaker incubator.
3. Add 25 ml of the overnight culture to each 250 ml LBM flask.
4. Grow the cultures to OD600 = 0.5
5.Decant supernatant and resuspend the cells in 1/4 original volume (87.5 ml) ice cold 100 mM MgCl2. Hold on ice for 5 minutes. Transfer the cells to pre-chilled sterile large centrifuge bottles. Spin in the for 10 minutes using the rotor 4000 rpm at 4 degrees C.
6.Decant the supernatant and resuspend the cells in 1/20 original volume (17.5 ml) of ice cold 100 mM CaCl2. Hold on ice for 20 minutes. Pellet as above 4000 rpm for 10 minutes.
7.Decant the supernatant and resuspend the cell pellet in 1/100 original volume (3.5 ml) of a solution that is 85% v/v 100 mM CaCl2 and 15% v/v glycerol (100%). For each culture processed chill approximately 15 labeled eppendorf tubes in a dry ice-EtOH bath. Pipet 300 ul cells into each tube and place immediately into the dry ice-EtOH bath.
8.Transfer the frozen competent cell aliquots to -80 degrees C.

2 Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins).

-80 °C

3 Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to room temperature and then (optional) incubate in 37°C incubator.

37 °C

4 Mix 1µl of DNA (usually 10 pg - 100 ng) into competent cells. Gently mix by flicking the bottom of the tube with your finger a few times. Incubate the competent cell/DNA mixture on ice for 20-30 mins.

10 ng ~ 100 ng

5 Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 90 secs.

00:01:30

42 °C

6 Put the tubes back on ice for 2 min.

00:02:00

7 Add 600µl LB media (without antibiotic) to the bacteria and grow in 37°C shaking incubator for 45 min.

600 µl

00:45:00

37 °C

8 The bacterial liquid was centrifuged at 3500rpm for 3 minutes, 400 microliters of supernatant was discarded, and the bacterial liquid was suspended again.

00:03:00

9 Plate the transformation onto a LB agar plate containing the appropriate antibiotic.

10 Incubate plates at 37°C overnight.

37 °C

12:00:00