SOP Name: Competent *E. coli* cells for heat-shock
Date: 24/05/17
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Source(s): N/A
Time Required: Up to 5 hours

Notes: Protocol for 200ml of cell culture (10-15 aliquots of 100 µl Ca^{2+}-competent cells).

Materials:

- 200 ml sterile LB media
- 150 ml pre-chilled sterile 100Mm $CaCl_2$
- 200 µl of 50% glycerol
- Preculture
- 8 large 50ml falcon vials
- Sterile microfuge tubes

Preculture:

1. Incubate plate overnight
2. Pick a single colony and inoculate 5 ml LB
3. Incubate overnight at 37°C

Procedure:

1. Inoculate 200/400 ml LB with 1/2 ml of preculture
2. Incubate culture at 37°C on a shaker (180-200 rpm) for 2 hours
3. Start measuring OD_{600} till $0.1<OD_{600}<0.2$
4. Switch on the centrifuge to cool to 4°C
- All further steps to be carried out on ice
5. Transfer into one large centrifugation vial (8 large 50ml falcons)
6. Centrifuge cells 20 minutes at 4°C at 4000 rpm
7. Carefully discard the supernatant
8. Resuspend each pellet in 10 ml pre-chilled 100Mm $CaCl_2$
9. Keep cells on ice for 40 minutes EXACTLY!!
10. Centrifuge cells 20 minutes at 4°C at 4000 rpm
11. Carefully discard the supernatant
12. Resuspend and combine 4 pellets in 1ml pre-chilled 100Mm $CaCl_2$.

13. Add 200 µl of 50% glycerol to each pellet to give a final concentration of 10%
14. Vortex
15. Aliquot 100 µl mix into sterile microfuge tubes
16. Shock-freeze cells in liquid nitrogen
17. Store samples at -80°C