Cool growth competent *E. coli*

This protocol is very effective and simple. The fact that the cells are grown at low temperature greatly increase the OD range during which the cells will prep up at high efficiency (Inoue et al., 1990). Cell prepared this way standardly have a competency over $10^8$ pfu/ug for a 10 kb plasmid.

**Day 1:** Streak bacteria onto fresh LB plate from frozen stock. Incubate at 37°C overnight.

**Day 2:** Pick a single colony from streaked plate and inoculate 3ml overnight pilot culture.

**Day 3:** Inoculate 250 ml of SOB media (2 liter flask) with 0.5 ml overnight culture. Shake 250 ml culture in sterile 2L Erlenmeyer flask at RT (20°C in our case). Grow bacteria to A600 ~0.6 (~28 hrs). Approximate doubling time is ~200 min for DH5α cells at 20 °C. Make sure SOB, TB, and tubes for aliquots are prepared and sterile. Since RT varies, one can either use a 20 °C (Room/incubate/bath) or grow at RT. Note that growth will be significantly faster at 23 or 24. Keeping notes on the doubling time in your media at your standard RT simplify timing the growth.

**Day 4:** An OD of 0.6 is ideal for collection. While anywhere from 0.2 to 1.0 will work, for the best competency attempt to collect cells between 0.5 and 0.75.

**Chill the following:** >100 ml ice-cold TB (4°C), centrifuge rotor (GSA rotor, 4°C), 500 ml centrifuge bottles, and sterile tubes (microcentrifuge tubes with caps that seal well at -80°C).

**Once OD reaches 0.6 or collection OD:**

For best results the remainder of this protocol should be done as quickly as possible and in a cold room (4°C).

- Place on ice 10 minutes.
- Transfer to iced centrifuge bottle; spin 2500 x g, 10 min, 4°C.
- Gently re-suspend pellet in 80 ml ice-cold TB, pipetting and swirling on ice as necessary.
- Place on ice for 10 min.
- Spin again 2500 x g, 10 min, 4°C.
- Gently re-suspend in 20 ml ice-cold TB by swirling on ice.
-Add DMSO, while swirling on ice, to final 7% (~1.4 ml). DMSO must be at RT since it goes solid at 4°C.
-Place on ice, 10 min.
-Dispense into freezing tubes (~0.2-1 ml ea).
-Freeze in liquid N2; transfer to -80°C.

Transformation
1. Thaw an aliquot of cells on ice.
2. Distribute in 50-100 ul aliquot in ependorf tubes precooled on ice.
3. Add DNA and vortex briefly to mix. This does not harm cells.
4. Incubate 20-40 minutes (each 10 minutes below 40 min will reduce efficiency 2 fold.)
5. Heat shock 40 second at 42°C.
6. Return to ice for 2-10 minutes. (Longer the better and longer may even work better).
7. Add > 5 volumes of SOC (SOB + 20 mM glucose).
8. Incubate at 37 for 1 hr with shaking (each reduction of 15 minute will reduce efficiency 2 fold).
9. Plate on drug selection plates. One can plate 100 ul or spin down cells and resuspend in SOC and plate.

Notes
1. Cells can be refrozen at least once after being thawed on ice by placing back in liquid nitrogen and will continue to have high competency.
2. These cells will remain very competent for >6 months in the -80, but they will keep even longer in liquid nitrogen, so if your lab uses cell only occasionally, consider only keeping only a working aliquot at -80.

Troubleshooting
1) The main problem we have observed is low competency we believe is due to detergent film in dishware. We have a dedicated set of two liter flasks for making competent cells that we just rinse out with distilled water after use.
2) Low quality DMSO can reduce competency greatly according to Hannahan (1983).

3) Adding magnesium to the SOB is critical.

4) Filter sterilizing units sometime contain detergent. We pre-rinse the sterilizing units with 100 ml of distilled water before filter sterilizing the TB.

**Media:**

**1L SOB:**
20 g bactotryptone  
5 g bacto-yeast extract  
0.5g NaCl  
Dissolve all in 950 ml ddH2O; add 10 ml 250 mM KCl; pH to 7.0 with NaOH. Adjust volume to 1 liter and autoclave.  
Just before use, add 5 ml sterile 2M MgCl2.

**1L TB:**
3.35 g PIPES Na salt, final concentration: 10 mM  
2.2 g CaCl2 (Fisher), final concentration: 15 mM  
18.64g KCl (Aldrich), final concentration: 250 mM  
10.9 g MnCl2 (Aldrich), final concentration: 55 mM  
Combine PIPES, CaCl2 and KCl; pH to 6.7; add MnCl2; filter sterilize.

**REFERENCES**


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