CW, 1-07
Revise MR 110530
Rev. TM 11.8.2011

Preparation of competent E. coli cells

Preparation

Day 1 morning: Prepare fresh streak of cells on LB or LB/resistance plate. In the evening, pick single colony and inoculate 10 ml medium (in 100 ml flask). Shake at 250 rpm at 37°C.

Day 2 morning: Inoculate 100 ml with 1 ml of O/N culture. Shake at 250 rpm at 37°C. Grow to OD <<0.3 (550 nm). Measure every 30 min. (it works very well if the OD is between 0.15 and 0.2!)

Label Eppendorf tubes and pre-cool at –80°C.

Spin down in Falcon tubes for 10 min. at 2.400 rpm, 4 °C. Quickly discard supernatant and stand tubes briefly upside down on clean paper towel to drain off medium.

Resuspend cells in 30 ml TFB I, vortex vigorously. Combine all pellets in 1 50 ml tube.

Store cells on **ice-water** for 8 min.

Spin 10 min as above. From now on, cells are very fragile. Quickly discard supernatant.

Add 4 ml TFB II and resuspend cells by very gentle swirling in ice-water bath (it takes > 10-20 min). Keep it in ice-water during that time.

Distribute 50 (1 transformation), 100 (2 trsf.) or 200 µl (4 trsf.) aliquots in Eppendorf tubes pre-cooled in EtOH/dry ice (use blunted blue Gilson tip). Store at –80°C.

Solutions

Prepare all solutions with cell culture grade water and chemicals of highest purity. Rinse bottle and culture flasks with sterile water before use.

Medium: LB, adjusted to pH 7.5. (**For LB Medium, use cell culture grade water from commercial supplier)**

Prepare buffers fresh or one evening before and cool down at 4°C!

TFBI: prepare always fresh from salts! Amounts are for 100 ml.

1. 30 mM KAc, (Mr 98.15) 0.29445 g (Applichem A279)
2. Adjust to pH 5.8 with 0.2 M acetic acid (it is necessary to adjust the pH first on this step)
3. 50 mM $MgCl_2$, x 6 H_20 (Mr 203.3) 1.0165 g (Applichem A4425)
4. 100 mM KCl, (Mr 74.56) 0.7456 g (Applichem A2939)
5. 15 % (vol./vol.) ultrapure glycerol (Applichem A2926).

Add water to 95 ml, control final pH and adjust to pH 5.8, if necessary, with 0.2 M acetic acid. Add cell culture grade water up to 100ml. **Filter sterile**!

TFB II: prepare always fresh from salts! Amounts are for 100 ml.

1. 10 mM MOPS, (Mr 209.27) 0.2093 g (Applichem A2947)
2. adjust pH to 7 with NaOH (it is necessary to adjust the pH first on this step)
3. 10 mM KCl, (Mr 74.56) 0.07456 or 1 ml of 1 M sol. (Appl. A2939)
4. 75 mM $CaCl_2$,x 2 H_20 (Mr 147.02) 1.103 (Applichem A4689)
5. 15 % (vol./vol.) ultrapure glycerol.

Add water to 95 ml, control final pH and adjust to pH 7 with 1 M NaOH.
Filter sterile!

Comments

- Always test bacterial stock for contamination by streaking out on LB/Amp and LB/Kana plates in addition to LB plates. If contamination is found, discard plate and go back to stab culture. Restreak from that.
- Before putting new lot into use, test for competence with standard pUC18 plasmid prep (see below). Any stock that is frozen must be better than 2 x 10^6 per µg of the above DNA. If not, throw away.
- Note in lot description the size of aliquots.

Transformation

Thaw cells on ice.

Use 50 µl cells and up to 5 µl ligation mixture per transformation.

For **re-transformation** of purified plasmids, 5 µl cells and 1-5 ng of DNA are sufficient.

Mix gently and incubate on ice for 30 min.

Heat-shock for 90 sec. at 42°C.

Return to ice instantly for 2 min.

Add 450 µl LB medium and incubate at 37°C for 1h.

Plate 50 µl and rest on appropriate resistance plates.

Test every new batch of competent E. coli cells as follows:

Standard DNA: pUC18 at 10 ng/ ul. Dilute an aliquot 1:100 immediateoly before transformation.

Thaw 50 µl of cells and add 1µl of freshly diluted pUC18 (=0.1 ng), mix gently and incubate on ice for 30 min.

Heat-shock for 90 sec. at 42°C.

Return to ice instantly for 2min.

Add 450 µl LB medium and incubate at 37°C for 1h.

Plate 50 µl and rest on appropriate resistance plates (amp)

Next day count all the colonies per 0.1 ng and calculate the competence of new batch as measured in colony number per 1µg of pUC18.

Typically, the protocol produces competence of ~ 6×10^6 to 1×10^7 colonies /1µg plasmid