

Highly competent *E. coli* cells

Based on Inoue, H. et al. (1990) Gene 96 23-28

Preparation:

To get good results it is very important to start with fresh cells. The strain to be made competent should be plated out on LB (or minimal medium) plates with the appropriate antibiotics, the day before starting the liquid culture for the competent cells. Use the plate straight from 37°C to start the cultures, storing the plates at 4°C will lead to less competent cells.

The paper warns against growing liquid pre-cultures for inoculation but we have done that and do not see a big difference.

After the cells have been spun down they should not leave the cold room until they are ready and frozen. Use liquid N₂ in a thermos flask or a tight styrofoam box to freeze the cells. Prepare the tubes into which the cells will be aliquoted before starting the manipulations and place them in cold room.

Buffers and media:

SOB (1l)

| | | |
|-----|---|---------------------|
| 20 | g | Bacto tryptone |
| 5 | g | Bacto yeast extract |
| 0.5 | g | NaCl |

- Dissolve in ~ 950 ml dH₂O
- Add: 10 ml 250 mM KCl
- Bring to pH 7.0 with NaOH
- Adjust volume to 1 L
- Aliquot, autoclave and cool to ~60°C
- Add: 5 ml Sterile 2 M MgCl₂ solution

SOC (100 ml)

Add 2 ml sterile 1 M Glucose solution to 100 ml SOB.

HTB

| | | |
|-----|----------------------|---------|
| 10 | mM HEPES * | 0.477 g |
| 15 | mM CaCl ₂ | 0.441 g |
| 250 | mM KCl 3.728 | g |

- Dissolve in ~150 ml dH₂O
- Adjust pH to 6.7 with KOH (a few drops of 4M solution)
- Then add MnCl₂ and dissolve
55mM MnCl₂ (1.384g)

- Adjust volume to 200 ml
- Filtersterilize with a 0.45 μ filter
- Store at 4°C

* 10 mM PIPES or BES buffers are even better than HEPES according to the original paper, and MOPS can be used as well. We have compared PIPES and HEPES and see no real difference.

Procedure:

The procedure describes the production of ~17 ml of competent cells, but can of course be scaled up or down as one wishes.

1. Use about ten large colonies from a freshly grown plate to start a 200 ml culture in SOB medium. Grow the cells at 18°C (room temperature ~22°C works as well) with vigorous shaking. Use large flasks for good aeration. Catching the culture at the correct density is very important and growth is slow at this temperature, so start the cultures the day before the cells are to be made competent. It's advisable to make a growth curve for your strain to estimate doubling times so you don't have to harvest in the middle of the night.
2. The cells should be grown to an OD₆₀₀ of 0.45 - 0.60. 0.45 is the theoretical optimum but 0.60 gives more cells. The density must be monitored carefully. If you overshoot - start again. When the correct density has been reached the culture should be split into sterile centrifuge tubes (4 x 50 ml Falcon tubes) and placed on ice for 10 min
3. Spin for 15 min at 2500 g (~3500 rpm) and carefully discard all of the supernatant
4. Resuspend in 64 ml HTB (4 x 16 ml) and place on ice for 10 min
5. Spin again as before and discard supernatant
6. Resuspend in 16 ml HTB (each pellet in 4 ml and then pool)
7. Slowly add 1.2 ml filtersterilized DMSO while gently swirling the cell suspension
8. Aliquot immediately into convenient aliquots (88 x 200 µl in snap cap tubes) and quick freeze by throwing into liquid N₂. Store at -80. Avoid all temperature fluctuations

Expected competency 1×10^9 transformants/µg pBR322