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Preparation and Transformation of Competent E. coli cells (CCMB80 Method)

Tobias von der Haar[1]

[1]University of Kent at Canterbury

1 Works for me dx.doi.org/10.17504/protocols.io.hayb2fw

Tobias von der Haar

ABSTRACT

This method is based on that originally described by Hanahan et al (1991), with the difference that we grow cells faster at a higher temperature (this works fine for us).

Preparation of Bacterial Seed Stocks

1 Pick single colonies from the stock plate into 5 ml of SOB or LB medium and shake overnight at 30°C.
Add 600 µl of glycerol (15% final concentration) to the culture
Aliquot 1 ml samples into cryotubes and place in a -80ºC freezer for long-term storage.

Preparation of Glass Ware

2 **Note:** this step is designed to remove any residual detergent from glassware. In may cases it will improve transformation efficiency as competent cells are sensitive to residual detergent.

1. Fill a 1 litre conical flask and a 1 litre Duran bottle with deionised water and autoclave.
2. Discard the water and use the empty flask and bottle for preparation of the growth medium and of the CCMB80 buffer.

Preparation of Buffer and medium

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1. In the 1 litre conical flask, prepare 250 ml of SOB medium (1 .25 g yeast extract, 5 g tryptone, 0.15 g NaCl, 0.05 g KCl, 0.6 g $MgSO_4$ in 250 ml water). Close the conical flask with a foam bung and autoclave.
2. In the 1 litre Duran bottle, prepare 1 litre of CCMB80 buffer by adding 0.98 g KOAc (10 mM), 11.8 g $CaCl_2$ *$2H_2O$ (80 mM), 4.0 g $MnCl_2$ *$4H_2O$ (20 mM), 2.0 g $MgCl_2$ *$6H_2O$ (10 mM), 100 g glycerol (10%), and water to a final volume of 1 litre. Measure pH, if it is above 6.4 adjust down to 6.4 with dilute HCl. Do not adjust if pH is below 6.4. Sterile filter the buffer and store at 4ºC. The buffer has a shelf live of several months. A slight dark precipitate does not affect its function.

Preparation of Competent Cells

4
1. Inoculate the SOB medium with one vial of seed stock and grow at 30°C to an oD_{600} of 0.3. While the cells grow, place CCMB80 buffer on ice.
2. Harvest cells by centrifugation at 3000 rpm and 4°C for 10 minutes.
3. Slowly pour off the supernatant. Remove any residual liquid from the cells with a pipette.
4. Gently resuspend the cells in 80 ml of ice cold CCMB80 buffer (swirl the cells in the buffer rather than shaking or pipetting vigorously). Incubate the resuspended cells on ice for 20 minutes.
5. Centrifuge as before and discard supernatant.
6. Resuspend cells in 10 ml of ice cold CCMB80 buffer.
7. Test the oD_{600} of a mixture of 200 µl SOB and 50 µl of the resuspended cells. Add chilled CCMB80 to the cell slurry to yield a final OD of 1 .0-1 .5 in this test.
8. Aliquot into microcentrifuge tubes and freeze at -80ºC.

Transformation of Competent Cells

5 1. Pipet DNA (1 µl of miniprep-grade plasmid DNA, or up to 9 µl of ligation reaction) into an Eppendorf tube and place this on ice. Place an aliquot of competent cells on ice and leave until thawed (~10-20 minutes).
2. Add 100 µl of competent cells to the DNA, and incubate for 20-30 minutes on ice.
3. Heat shock in a water bath or metal block at 42ºC. The ideal time is strain dependent, between 30 and 60 seconds (if unsure, use 60 seconds). Immediately place on ice.
4. Add 1 ml of LB or SOB medium and shake at 37ºC for 30-60 minutes. If you transform miniprep DNA, plate out 100 µl of the cells onto a suitable selective plate. If you transform ligation reactions, spin the cells at 3000 rpm for 5 minutes. Pour off most of the medium, leaving 100-200 µl in the tube. Resuspend the cells in this remaining medium and plate everything onto suitable selective plates.