E-coli Competent Cell

Before proceeding with this experiment make sure that you have the following reagents below!

- CCMB80 buffer
- SOB
- SOC
- Glycerol

CCMB80 Buffer & SOB solution protocol

Materials:

- Detergent-free, sterile glassware and plastic ware (see procedure)
- Table-top OD600nm spectrophotometer
- SOB medium / LB medium
- CCMB80 buffer

CCMB80 buffer:

- 10 mM KOAc pH 7.0 (10 ml of a 1M stock/L)
- 80 mM $CaCl_2.2H_2O$ (11.8 g/L)
- 20 mM $MnCl_2.4H_2O$ (4.0 g/L)
- 10 mM $MgCl_2.6H_2O$ (2.0 g/L)
- 10% glycerol (100 ml/L)
- If pH is above 6.4, adjust pH DOWN to 6.4 with HCl
- Adjusting pH up will precipitate manganese dioxide from Mn containing solutions
- sterile filter and store at 4°C
- slight dark precipitate appears not to affect its function

LB AGAR SOLUTION

Per batch (600 ml):

- 6 g NaCl
- 6 g Bacto-tryptone
- 3 g Yeast extract

Procedure:
Add about 600 ml distilled water
Autoclave 20 minutes
Store in cold room or refrigerator

SOB-medium:

SOB Medium. Used in growing bacteria for preparing chemically competent cells

Materials:

- 0.5% (w/v) yeast extract
- 2% (w/v) tryptone
- 10 mM NaCl
- 2.5 mM KCl
- 20 mM $MgSO_4$

Per liter:

- 5 g yeast extract
- 20 g tryptone
- 0.584 g NaCl
- 0.186 g KCl
- 2.4 g $MgSO_4$

Procedure:

(Note: Some formulations of SOB use 10 mM $MgCl_2$ and 10 mM $MgSO_4$ instead of 20 mM $MgSO_4$.)

Adjust to pH 7.5. This requires approximately 25 ml of 1M NaOH per liter. Autoclave the bottled SOB.

(SOC medium is SOB medium with 20 mM glucose. You can do that by adding 20 ml of 20% glucose into 1 liter of SOB. Make sure everything is sterile though.)

D5α Competent Cell Preparation Procedure

Preparing glassware and media:

Detergent is a major inhibitor of competent cell growth and transformation. Glass and plastic must be detergent free for these protocols. The easiest way to do this is to avoid washing glassware, and simply rinse it out. Autoclaving glassware filled 75% with DI water is an effective way to remove most detergent residue. Media and buffers should be prepared in detergent free glassware and cultures grown up in detergent free glassware.

Pre-chill 250mL centrifuge tubes and screw cap tubes before use.

Preparing seed stocks:

- Streak D5α cells on a plate and grow for single colonies at 37°C room temperature works well
- Pick single colonies into 2 ml of LB medium and shake overnight at 37°C
- Add glycerol to 15%
- Aliquot 1 ml samples to Eppendorf tubes.
- Place in -80°C freezer indefinitely.

Preparing competent cells:

- Inoculate 250 ml of LB medium with 1 ml overnight culture and grow at 37°C to an **OD600nm of 0.3 – 0.4 OD is double the amount every 20-30min so MEASURE OFTEN!**. This takes approximately 2 – 4 hours.
- Room temperature will work. You can adjust the temperature to fit your schedule
- Aim for lower, not higher OD if you can't hit this mark (do not miss exponential phase!)
- Centrifuge at 3000g at 4°C for 10 minutes in 50ml falcon tubes.
- **ALL STEPS BELOW HAVE TO BE PERFORMED ON ICE IN A COLD ROOM!!!**
- Gently resuspend 50ml culture pellet per 20ml **ice cold** CCMB80 buffer (**This can be done in the cold room!**)
- Sometimes, this is less than completely gentle. It still works.
- It is often easier to resuspend pellets by stirring into 1-2 ml of buffer first, *before* adding large amounts of buffer to the desired final volume
- Incubate on ice 20 minutes
- **Centrifuge again at 4°C and resuspend in 10 ml of ice cold CCMB80 buffer.**
- Incubate on ice for 20 minutes
- **Aliquot 50 µl to chilled Eppendorf tubes**
- Store at -80°C indefinitely.
- Flash freezing does not appear to be necessary
- Test competence (see below)
- Thawing and refreezing partially used cell aliquots dramatically reduces transformation efficiency by about 3x the first time, and about 6x in total after several freeze/thaw cycles.

Measurement of competence (only measurement)

- Thaw the cells on ice for 15 minutes
- Transform 75 µl of cells with 2 µl of standard pUC19 plasmid (New England Biolab)
- This was at 50 pg/µl or 10^{-5} µg/µl or 50 µl/ml
- Hold on ice 30 minutes
- Heat shock 60 sec at 42 °C
- Incubate on ice 5 minutes
- Add 500 µl LB
- Incubate at 37 °C for 2 hour in 2 ml centrifuge tubes rotated using 2 ml centrifuge tubes for transformation and regrowth works well because the small volumes flow well when rotated, increasing aeration.
- For our plasmids (pSB1AC3, pSB1AT3) which are chloramphenicol and tetracycline resistant, we find growing for 2 hours yields many more colonies
- Ampicillin and kanamycin appear to do fine with 1 hour growth
- Plate 20 µl on AMP plates using sterile 3.5 mm glass beads
- Good cells should yield around 100 - 400 colonies
- Transformation efficiency is calculated as transformants / µgDNA, this variable can be easily calculated using programs online, e.g. http://www.sciencegateway.org/tools/transform.htm
- The expected transformation efficiency should be between $5x10^{8}$ and $5x10^{9}$ cfu/µgDNA