Competent *E. coli* cells

We take DH5α and XL1-blue *E. coli* strains.

Overview

This protocol is a variant of the Hanahan protocol [1] using CCMB80 buffer for DH10B, TOP10 and MachI strains. It builds on Example 2 of the Bloom05 patent as well. This protocol has been tested on NEB10, TOP10, MachI and BL21(DE3) cells. See OWW Bacterial Transformation page for a more general discussion of other techniques. The Jesse '464 patent describes using this buffer for DH5α cells. The Bloom04 patent describes the use of essentially the same protocol for the Invitrogen Mach 1 cells.

This is the chemical transformation protocol used by Tom Knight and the Registry of Standard Biological Parts.

Materials

- **Detergent-free, sterile glassware and plasticware (see procedure)**
- Table-top OD600nm spectrophotometer
- SOB

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)
- adjust pH DOWN to 6.4 with 0.1N HCl if necessary
    - 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

Procedure

Preparing glassware and media

Eliminating detergent

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 3/4 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.**

Prechill plasticware and glassware

Prechill 250mL centrifuge tubes and screw cap tubes before use.

Preparing seed stocks

- Streak TOP10 cells on an SOB/LB plate and grow for single colonies at 23° (RT works well)
- Pick single colonies into 2 ml of SOB medium and shake overnight+day at 23°C (RT works well)

- [Add glycerol to 15% (300 µl 50% glycerole (sterile) + 700 µl culture), Aliquot 1 ml samples to Nunc cryotubes, Place in -80°C freezer indefinitely.]

Preparing competent cells

- Ethanol treat all working areas for sterility.
- **Inoculate 250 ml of SOB medium with 1 ml vial of seed stock and grow at 20°C to an OD600nm of 0.3.**
  - This takes approximately 16 hours.
  - Controlling the temperature makes this a more reproducible process, but is not essential.
  - Room temperature will work. You can adjust this temperature somewhat to fit your schedule
  - Aim for lower, not higher OD if you can't hit this mark
- Fill an ice bucket halfway with ice. Use the ice to pre-chill as many flat bottom centrifuge bottles as needed.
- Transfer the culture to the flat bottom centrifuge tubes. Weigh and balance the tubes using a scale
  - Try to get the weights as close as possible, within 1 gram.
- **Centrifuge at 3000g at 4°C for 10 minutes in a flat bottom centrifuge bottle**.
  - Flat bottom centrifuge tubes make the fragile cells much easier to resuspend
- Decant supernatant into waste receptacle, bleach before pouring down the drain.
- **Gently resuspend in 80 ml of ice cold CCMB80 buffer**
  - Pro tip: add 40ml first to resuspend the cells. When cells are in suspension, add another 40ml CCMB80 buffer for a total of 80ml
  - Pipet buffer against the wall of the centrifuge bottle to resuspend cells. Do not pipet directly into cell pellet!
  - After pipetting, there will still be some residual cells stuck to the bottom. Swirl the bottles gently to resuspend these remaining cells
- **Incubate on ice for 20 minutes**
- **Centrifuge again at 3000g at 4°C**. Decant supernatant into waste receptacle, and bleach before pouring down the drain.
- **Resuspend cell pellet in 10 ml of ice cold CCMB80 buffer.**
  - If using multiple flat bottom centrifuge bottles, combine the cells post-resuspension
- Use Nanodrop to measure OD of a mixture of 200 µl SOC and 50 µl of the resuspended cells
  - Use a mixture of 200 µl SOC and 50 µl CCMB80 buffer as the blank
- Add chilled CCMB80 to yield a final OD of 1.0-1.5 in this test.
- **Incubate on ice for 20 minutes. Prepare for aliquoting**
  - Make labels for aliquots (strain code see fridge)+ name the boxes with strain+date. Use these to label storage microcentrifuge tubes/microtiter plates
  - Prepare liquid nitrogene in a Dewar-bucket. **Pre-chill tubes**/plates at 4°C.
- Aliquot (cut off tip of a yellow tip to lower shearing forces) into chilled 1.5 ml microcentrifuge tubes or 50 µl into chilled microtiter plates
- Freeze in liquid nitrogene, 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 total after several freeze/thaw cycles.

Measurement of competence

- Transform 50 µl of cells with 1 µl of standard pUC19 plasmid (Invitrogen) [we use pSB1A3 at the same concentration]
  - This is at 10 pg/µl or 10^{-5} µg/µl
  - This can be made by diluting 1 µl of NEB pUC19 plasmid (1 µg/µl, NEB part number N3401S) into 100 ml of TE
- Incubate on ice 0.5 hours. Pre-heat water bath now.
- Heat shock 60 sec at 42°C
- Add 250 µl SOC (or LB)
- Incubate at 37 C for 1 hour in 2 ml centrifuge tubes, using a mini-rotator
  - Using flat-bottomed 2ml 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 (Ampicillin as little as 5 min)
- Add 4-5 sterile 3.5mm glass beads to each agar plate, then add 50 µl of transformation
  - After adding transformation, gently move plates from side to side to re-distribute beads. When most of transformation has been absorbed, shake plate harder
  - Use 3 plates per vial tested
- Incubate plates agar-side up at 37 C for 12-16 hours
- Count colonies on light field the next day
  - Good cells should yield around 250 - 1000 colonies
  - Transformation efficiency is (dilution factor=6) x colony count x 10^5/µgDNA
  - We expect that the transformation efficiency should be between $1.5x10^8$ and $6x10^8$ cfu/µgDNA

5x Ligation Adjustment Buffer

- Intended to be mixed with ligation reactions to adjust buffer composition to be near the CCMB80 buffer
- KOAc 40 mM (40 ml/liter of 1 M KOAc solution, pH 7.0)
- $CaCl_2$ 400 mM (200 ml/l of a 2 M solution)
- $MnCl_2$ 100 mM (100 ml/l of a 1 M solution)
- Glycerol 46.8% (468 ml/liter)
- pH adjustment with 2.3% of a 10% acetic acid solution (12.8ml/liter)
  - Previous protocol indicated amount of acetic acid added should be 23 ml/liter but that amount was found to be 2X too much per tests on 1.23.07 --Meagan15:50, 25 January 2007 (EST)
- water to 1 liter

- autoclave or sterile filter
- Test pH adjustment by mixing 4 parts ligation buffer + 1 part 5x ligation adjustment buffer and checking pH to be 6.3 - 6.5

- **Reshma 10:49, 11 February 2008 (CST)**: Use of the ligation adjustment buffer is optional.

References

1. Hanahan D, Jessee J, and Bloom FR. *Plasmid transformation of Escherichia coli and other bacteria.* Methods Enzymol 1991; 204 63-113. pmid:1943786.PubMed HubMed [Hanahan91]

2. Reusch RN, Hiske TW, and Sadoff HL. *Poly-beta-hydroxybutyrate membrane structure and its relationship to genetic transformability in Escherichia coli.* J Bacteriol 1986 Nov; 168(2) 553-62. pmid:3536850. PubMed HubMed [Reusch86]

3. Addison CJ, Chu SH, and Reusch RN. *Polyhydroxybutyrate-enhanced transformation of log-phase Escherichia coli.* Biotechniques 2004 Sep; 37(3) 376-8, 380, 382. pmid:15470891. PubMed HubMed [Addison04]

4. US Patent 6,709,852 pat6709852.pdf

   [Bloom04]

5. US Patent 6,855,494 pat6855494.pdf

   [Bloom05]

6. US Patent 6,960,464 pat6960464.pdf

   [Jesse05]

All Medline abstracts: PubMed HubMed

Protocol generously provided by the lab
Prof. Thorsten Mascher
Großhadernerstr. 2-4
82152 Planegg-Martinsried
www.syntheticmicrobe.bio.lmu.de