Overview

This protocol is a variant of the Hanahan protocol using CCMB80 buffer for DH10B, TOP10 and Mach 1 strains. It builds on Example 2 of the [Bloom05 patent](http://openwetware.org/images/b/bd/Pat6855494.pdf) as well. This protocol has been tested on TOP10, MachI and [BL21(DE3)](http://openwetware.org/wiki/Talk:TOP10_chemically_competent_cells) cells. See [Bacterial Transformation](http://openwetware.org/wiki/Bacterial_Transformation) for a more general discussion of other techniques. The [Jesse '464 patent](http://openwetware.org/images/0/0c/Pat6960464.pdf) describes using this buffer for DH5α cells. The [Bloom04](http://openwetware.org/images/c/c2/Pat6709852.pdf) 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**](http://openwetware.org/wiki/User:Tk)**and the**[**Registry of Standard Biological Parts**](http://partsregistry.org/) modified by McCarroll MN**.**

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 CaCl2.2H2O (11.8 g/L)
* 20 mM MnCl2.4H2O (4.0 g/L)
* 10 mM MgCl2.6H2O (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
* Note: you can buy pre-made CCMB80 buffer from Teknova

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](http://openwetware.org/wiki/SOB) plate and grow for single colonies at 23°C
  + room temperature works well
* Pick single colonies into 2 ml of SOB medium and shake overnight at 23°C
  + room temperature works well
* Add glycerol to 15%
* Aliquot 1 ml samples to Nunc cryotubes
* Place tubes into a zip lock bag, immerse bag into a dry ice/ethanol bath for 5 minutes
  + This step may not be necessary
* Place in -80°C freezer indefinitely.

**Preparing competent cells**

* Inoculate 250 ml of [SOB](http://openwetware.org/wiki/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
* Centrifuge at 3000rpm at 4°C for 10 minutes in a flat bottom centrifuge bottle.
  + Flat bottom centrifuge tubes make the fragile cells much easier to resuspend
  + It is often easier to resuspend pellets by mixing *before* adding large amounts of buffer
* Discard supernatant by pouring out slowly and pipeting remaining supernatent
* Gently resuspend in 80 ml of ice cold CCMB80 buffer
  + sometimes this is less than completely gentle. It still works.
* Incubate on ice 20 minutes
* Centrifuge again at 4°C and discard supernatant as described above.
* Resuspend in 10 ml of ice cold CCMB80 buffer.
* Test OD of a mixture of 200 μl SOC and 50 μl of the resuspended cells.
* Add chilled CCMB80 to yield a final OD of 1.0-1.5 in this test.
* Aliquot to chilled screw top 2 ml vials or 50 μl into chilled microtiter plates
* 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)
  + 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
* Hold on ice 0.5 hours
* Heat shock 60 sec at 42C
* Add 250 μl [SOC](http://openwetware.org/wiki/SOC)
* Incubate at 37 C for 1 hour in 2 ml centrifuge tubes rotated
  + using 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
* Plate 20 μl on AMP plates using sterile 3.5 mm glass beads
  + Good cells should yield around 100 - 400 colonies
  + Transformation efficiency is (dilution factor=15) x colony count x 105/µgDNA
  + We expect that the transformation efficiency should be between 5x108 and 5x109 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)
* CaCl2 400 mM (200 ml/l of a 2 M solution)
* MnCl2 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 --[Meaganl](http://openwetware.org/wiki/User:Meaganl) 15:50, 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**](http://openwetware.org/wiki/User:Reshma_P._Shetty)**10:49, 11 February 2008 (CST)**: Use of the ligation adjustment buffer is optional.

**SOB Medium**

Summary

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

Ingredients

* 0.5% (w/v) yeast extract
* 2% (w/v) tryptone
* 10 mM NaCl
* 2.5 mM KCl
* 20 mM MgSO4

Per liter:

* 5 g yeast extract
* 20 g tryptone
* 0.584 g NaCl
* 0.186 g KCl
* 2.4 g MgSO4

*Note:* Some formulations of SOB use 10 mM MgCl2 and 10 mM MgSO4 instead of 20 mM MgSO4.

SOB medium is also available dry premixed from Difco, 0443-17.

Adjust to pH 7.5 prior to use. This requires approximately 25 ml of 1M NaOH per liter.

**SOC:**

Follow instructions to make SOB media

After cooling media to less than 50˚C add filter sterilized 2M glucose solution to a final concentration of 20mM. (1mL 2M Glucose to 100mL SOC)

**2M Glucose Stock:**

dissolve 18g Glucose into 50mL (final volume) ddH2O, filter sterilize into 50ml tube

**15/10 medium**

Growth of competent TOP10 cells in Example 2 of the Bloom05 patent is performed in 15/10 broth, which is similar to SOB:

* 1.5% yeast extract
* 1% Bacto-Tryptone
* 10mM NaCl
* 2mM KCl
* 10 mM MgCl2
* 10 mM MgSO4

Extended instructions

* 1 M MgCl2 stock: dissolve 20.33 g MgCl2 6H2O in 100 ml ddH2O (XXX), autoclave on liquid cycle @ XXX°C for 20 min (can be done at the same time as SOC pre-mix below)
* 250 mM KCl stock: dissolve 1.86 KCl in 100 ml ddH2O (XXX)
* combine:

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **for 1 L** | **500 mL** | **100 mL** |
| tryptone | 20 g | 10 g | 2g |
| yeast | 5g | 2.5 g | 0.5 g |
| NaCl | 0.5 g | 0.25 g | 0.05 g |
| 250 nM KCl | 10 mL | 5mL | 1 mL |
| ddH2O | 980 ml | 490 ml | 98 ml |

* adjust pH to 7.0 w/ NaOH
* bring to volume:

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **for 1 L** | **500 mL** | **100 mL** |

* autoclave on liquid cycle @ XXX°C for 20 min
* add autoclaved 1 M MgCl2

|  |  |  |  |
| --- | --- | --- | --- |
| **Reagent** | **for 1 L** | **500 mL** | **100 mL** |
| 1 M MgCl2 | 10 ml | 5 ml | 1 ml |

Source

Adapted From:

F. Ausubel et al., *Short Protocols in Molecular Biology* (John Wiley & Sons, ed. 4, 1999) pg. A1-36