Making Heat-Shock Competent Cells

(Modified from: http://openwetware.org/wiki/Preparing_chemically_competent_cells)

1. Grow a 5ml overnight culture of cells in LB media. In the morning, dilute this culture back into 25-50ml of fresh LB media in a 200ml conical flask. You should aim to dilute the overnight culture by at least 1/100.
2. Grow the diluted culture to an OD_{600} of 0.2 - 0.5. (You will get a very small pellet if you grow 25ml to OD_{600} 0.2)
3. Put eppendorf tubes on ice now so that they are cold when cells are aliquoted into them later. If your culture is X ml, you will need X tubes. At this point you should also make sure that your TSS is being chilled (it should be stored at 4°C but if you have just made it fresh then put it in an ice bath).
4. Split the culture into two 50ml falcon tubes and incubate on ice for 10 min.

All subsequent steps should be carried out at 4°C and the cells should be kept on ice wherever possible

1. Centrifuge for 10 minutes at 3000 rpm and 4°C.
2. Remove supernatant. The cell pellets should be sufficiently solid that you can just pour off the supernatant if you are careful. Pipette out any remaining media.
3. Resuspend in chilled TSS buffer. The volume of TSS to use is 10% of the culture volume that you spun down.
4. Add 100 µl aliquots to your chilled eppendorfs, freeze with liquid nitrogen and store at − 80°C.

Transformation of Heat-Shock Competent Cells

(Modified from http://openwetware.org/wiki/Transforming_chemically_competent_cells)

1. Thaw competent cells on ice
2. Pipet 50 ul of cells and 2 ul of DNA into an eppendorf tube, mix gently
3. Incubate tubes on ice for 30 minutes
4. Incubate tubes in 42 °C for 1 minute
5. Incubate tubes on ice for 5 minutes
6. Add 200 ul of SOC
7. Incubate cells at 37 °C for 2 hours
8. Plate 125 ul of cell suspension onto an appropriate antibiotic LB-agar plate
9. Incubate overnight at 37 °C

Making Electrocompetent Cells

(modified from http://openwetware.org/wiki/Knight:Preparing_electrocompetent_cells)

- All steps should be carried out at 4 °C and the cells should be kept on ice whenever possible!

1. Make a 300 ml liquid culture of cells. When OD600 = 0,5 - 0,6 (-1,0), chill flask on ice.
2. Pour 35 ml cell culture into 8 falcon tubes. Centrifuge cells 3000 rcf 10 minutes at 4 °C. Discard supernatant, resuspend cells gently (do not vortex!) in 30 ml of ice cold water.
3. Centrifuge cells 3000 rcf 10 minutes at 4 °C. Discard supernatant and resuspend cells gently in 15 ml of ice cold water. Combine two tubes into one so that you'll have 4 tubes.
4. Repeat previous step and combine four tubes into two.
5. Centrifuge cells 3000 rcf 10 minutes at 4 °C. Discard supernatant and resuspend cells gently in 10% glycerol (as small amount as possible).
6. Pipet 100 ul of cell suspension into 0,5 ml eppendorf tubes
7. Shock freeze tubes with liquid nitrogen
8. Store at -80 °C
9. Before use, thaw tubes on ice

Transformation of Electrocompetent Cells

(modified from http://openwetware.org/wiki/Knight:Electroporation)

1. Chill electroporation cuvettes, DNA samples and eppendorf tubes on ice
2. Place appropriate antibiotic LB-agar plates in 37°C incubator to warm
3. Remove electrocompetent cells from -80°C freezer and thaw on ice
4. Pipet 60 ul of electrocompetent cells into pre-chilled tubes
5. Pipet 0,7µL of DNA ligation mix and add to electrocompetent cells. Swirl tip around gently in cells to mix DNA and cells
6. Place cells back on ice to ensure they remain cold
7. Turn on electroporator and set voltage to 2.5 kV (2mm cuvettes)
8. Transfer cell-DNA mixture to cuvettes
9. Tap the cuvette on the counter gently so that cells are at the bottom and to remove any air bubbles
10. Wipe off excess moisture from outside of cuvette
11. Place in chamber of electroporator
12. Slide the chamber in so that the cuvette sits snugly between electrodes
13. Pulse the cells with a shock by pressing button on electroporator

14. Remove cuvette from the chamber and immediately add 940 ul of SOC (room temperature). This step should be done as quickly as possible to prevent cells from dying off.
15. Transfer SOC-cell mixture to chilled eppendorf tube
16. Chill sample on ice for 2 mins to permit the cells to recover
17. Incubate tubes for 1 hour at 37 °C
18. Plate 150 ul (and the rest on another plate if needed) of transformation onto pre-warmed plate supplemented with appropriate antibiotic
19. Incubate plate overnight at 37°C.

Making SOC Broth

100ml
0,5 g Yeast extract
2 g Tryptone
0,05 g NaCl
g KCl
$MgSO_4 \cdot 6 H_2O$
H_2O

- autoclave

c=n/V, n=m/M, m=0,01L*221,322g/mol=2,213g

1M $MgCl_2$: 2,213g $MgCl_2 \cdot 7 H_2O$ in 10ml of H_2O
1M glucose: 18,02 g glucose in 100ml of H_2O

- autoclave separately

Add in the broth after autoclavation

- 1 ml 1M $MgCl_2$
- 2 ml 1M glucose

Restriction Digestion

50 ul

…… ul DNA (amount needed)
…… ul H_2O (to fill to tot vol. 50ul)
5 ul 10X NEBuffer 2
0,5 ul BSA (100X)
1 ul enzyme 1 **Add enzymes last!**
1 ul enzyme 2 **Add enzymes last!**

Program in thermocycler:

“37804” (incubation time can be prolonged if complete digestion needs to be assured.)

1. 30-60 min in 37 °C incubation
2. 20 min in 80 °C inactivation
3. 4 °C

Ligation

Remember to make a control without the insert! (just add 8 ul of H2O instead)**!**

20 ul
5 ul H2O
4 ul first Biobrick (digested)
4 ul second Biobrick (digested)
4 ul plasmid backbone (digested)
2 ul 10x T4 DNA Ligase Buffer (vortex and spin down before use!)
1 ul T4 DNA Ligase **Add enzyme last!**

If gel purified parts are used, calculate the amounts to match 250 ng vector and 3:1 ratio of insert:vector. Add H2O to fill up to 20 ul.

Program in the thermocycler:

“16804” (incubation time can be prolonged if difficult ligation)

1. 1,5h 16 °C:ssa incubation
2. 20 min 80 °C:ssa inactivation
3. 4 °C

PCR

200 ul
148 ul H2O
40 ul 5x buffer
4 ul Kapa (Hotstart) polymerase
6 ul 10mM dNTP
1 ul primer 1
1 ul primer 2
+ template (1 pg - 10 ng, too much can inhibit the process!)

Programs in thermocycler:

“BackBAmp” for backbone amplification

1. 30 s 98 °C
2. 10 s 98 °C

“ColPCR” for colony PCR

1. 3 min 95 °C
2. 10 s 95 °C

3. 20 s 55 °C
4. 2 min 72 °C
5. go to 2. 29 more times
6. 7 min 72 °C
7. 4 °C

3. 20 s 55 °C
4. 2 min 72 °C
5. go to 2. 29 more times
6. 7 min 72 °C
7. 4 C°

Agarose Gel Electrophoresis

(modified from: http://openwetware.org/wiki/Knight:Agarose_gel_electrophoresis)

1. Add 50 ml 1X TAE to a conical flask.
2. Measure out sufficient agarose to cast either a 0,7% (0,35 g), 1,0% (0,50 g) or 1,2% (0,60 g) gel.
3. Add the agarose to the TAE buffer in the conical flask.
4. Swirl to mix.
5. Microwave the flask on high until the gel starts to bubble and is transparent.
6. Let cool by either sitting on bench top.
7. While gel is cooling, assemble casting trays and gel combs and verify that the trays are level.
8. Once gel is cooled so that it can be touched comfortably with your gloved hand, add one drop of ethidium bromide (NOTE: Ethidium bromide is an acute toxin and a strong mutagen, be cautious and dispose gloves immediately after handling it!)
9. Pour gel into casting trays.
10. Let gel sit until they are solidified.
11. Remove comb.
12. Place your gel in gel box.
13. Add 1X TAE buffer to gel box such that buffer just covers the top of the gel.
14. Load 10 µL of prepared ladder.
15. Mix 2 µL of loading dye with 10 µL of sample.
16. Place gel box cover on gel box such that your samples will run towards the positive, red electrode.
17. Run your gel at ~70-80 volts for 45-60 min (NOTE: running voltage and time depend on the agarose percentage and length of the DNA samples).
18. Visualize the gel with a gel imager.