Freezing Competent Cells

1. Streak out glycerol stock of cells onto LB plate(s).

2. Choose one large colony and inoculate 1 ml of LB. Incubate at 37°C with shaking for 2.5 -3 hours.

3. Inoculate 100ml from the 1 ml and allow the 100 mls to grow to log phase (O.D. = 0.3 - 0.4) .

4. Spin down cells 15 minutes at 2.0 - 2.5 K rpm.

5. Remove the media and resuspend pellet in 1/3 volume of freezing buffer, and chill on ice 10 - 60 minutes.

6. Spin down cells 15 minutes at 1.5K RPM.

7. Decant supernatant and resuspend pellet in 1/12 volume of freezing buffer.

8. Aliquot and flash freeze.

9. Thaw at room temperature.

10. Place cells on ice for 10 minutes mixing every 2 minutes. **Pre-chill all tubes and solutions to be used in transformation.**

11. Add 10-20 µl of DNA ligation mixture, or less than 5% volume of DNA. One nanogram of supercoiled plasmid will saturate a 50µl aliquot of cells.

12. Swirl, put on ice for 30 minutes.

13. Heat shock cell at 42°C for 1-2 minutes. (Note: Optimal heat shock time may vary among batches of competent cells and does vary with different types of tubes. Optimal conditions will be included on a competent cell lab stock sheet found in front the glycerol stocks book.)

14. Place cells on ice for 2 minutes.

15. Add 1 ml of 42°C SOC media and shake at 37 °C for 1 hour (NOTE: Ken adds: at least 1 hour for Amp, at least 30 minutes for tetracycline.)

16. Plate cells with the appropriate selection. May want to plate a volume of cells with 100µl of SOB to help with spreading.

SOLUTIONS

LB Media

5 g of yeast extract

10 g of NaCl

10 g of tryptone

Add 800 ml of dH_2O; bring the pH to 7.0 with NaOH. Bring the volume up to 1 liter with dH_2O. For plates add 15 g of agar per liter.

Addition of antibiotics:

	Stock Solution(-200C) concentration	Working concentration stringent plasmid	Working concentration relaxed plasmid
Ampicillin	25-50 mg/ml	20µg/ml	60-100µg/ml
Carbenicillin	50 mg/ml	20µg/ml	60µg/ml
Chloramphenicol	34 mg/ml in EtOH	25µg/ml	170µg/ml
Kanamycin	10mg/ml	10µg/ml	50µg/ml
Streptomycin	10mg/ml	10µg/ml	50µg/ml
Tetracycline	5mg/ml in EtOH	10µg/ml	50µg/ml

Stock solutions of antibiotics dissolved in dH_2O should be filter sterilized through a 0.22 micron filter. Antibiotics in EtOH need not be sterilized. Store solutions in light-tight containers. Magnesium ions are antagonists of tetracycline. Use media without magnesium salts (e.g. LB media) for selection of bacteria resistant to tetracycline.

NOTE: Add filter-sterilized antibiotics to LB liquid before use. Add sterile antibiotics to cooled LB agar after autoclaving before plates are poured. These concentrations are those suggested by Sambrook et.al. Different laboratories/experiments may call for concentrations differing from those listed above.

Freezing buffer

(per 100 ml)
100 mM KCL (10 ml of 1 M)
50 mM $CaCl_2 \cdot 2H_2O$ (0.7351g)

10% w/v glycerol (10 ml of 100%)
10 mM Potassium acetate (334µl of 3 M)
pH to 6.2 - 6.4 with 0.1N HCl
ddH_2O to 100 ml.
Note: It is best to store $CaCl_2$ containing solutions in plastic rather than glass.

SOB media

To 950 ml of ddH_2O add:
20 g bacto-tryptone
5g yeast extract
0.5g NaCl

Allow solids to dissolve and add 10ml of 250mM KCL. Adjust the pH to 7.0 with 5M NaOH (~ 2.0ml) . Adjust the volume to 1 liter with ddH_2O . Sterilize by autoclaving. Allow the solution to cool to 60°C or less and add 5 mls of 2M $MgCl_2$
(19g $MgCl_2$ per 100ml ddH_2O).

Taken from: Sambrook et.al Molecular Cloning, A Laboratory Manual; second edition. Look here for more detailed information.
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SOC media

per liter:

Follow protocol to make SOB media and at the end add 20ml of filter sterilized 1M glucose (18g glucose per 100ml of ddH_2O).

Taken from: Sambrook et.al Molecular Cloning , A Laboratory Manual; second edition.
Entered TAA 3/91
DMP