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 dH₂O; bring the pH to 7.0 with NaOH. Bring the volume up to 1 liter with dH₂O. For plates add 15 g of agar per liter.

**Addition of antibiotics:**

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

Stock solutions of antibiotics dissolved in dH₂O 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 CaCl₂ · 2H₂O (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
ddH₂O to 100 ml.

**Note:** It is best to store CaCl₂ containing solutions in plastic rather than glass.

### SOB media

To 950 ml of ddH₂O 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 ddH₂O. Sterilize by autoclaving. Allow the solution to cool to 60°C or less and add 5 mls of 2M MgCl₂ (19g MgCl₂ per 100ml ddH₂O).

Taken from: Sambrook et.al Molecular Cloning, A Laboratory Manual; second edition. Look here for more detailed information.
entered by TAA 2/4/91
updated by DMP 7/24/95

### 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 ddH₂O).

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