Protocol

1. Streak *E. coli* onto LB plate* containing antibiotics (if necessary).
2. Pick one colony and grow overnight in 5 ml **LB with antibiotic**.
3. Inoculate 1 ml into 1 L of LB with antibiotic.
4. Grow to OD$_{595}$=0.6; about 3 – 5 hours.
5. Put the cells in ice.
6. Spin cells down at 2,500 g for 5 min at 4°C.
7. Resuspend in 500 ml ice-cold **0.1 M CaCl$_2$**.
8. Incubate on ice for 25 min.
9. Spin cells down 10,000 g for 15 min at 4°C.
10. Resuspend in 10 ml ice-cold 0.1M CaCl$_2$.
11. Add 2 ml of ice-cold **80% glycerol**.
12. Aliquot 250ul into pre-chilled microfuge tubes.
13. Store cells at –80°C.

(Addition by M. Jones - from Promega Protocols and Applications Guide)

Bacterial strains like JM109 and DH5alpha that contain an F’ episome should always be maintained on minimal medium plates (M9) supplemented with thiamine-HCl. This selects for the presence of the F’ episome, which carries a nutritional requirement for growth (proline biosynthesis). This selection will decrease the number of false positives and ensure that competent cells are still capable of alpha-complementation and for use in blue-white colony screening.

**M9 plates (1L)**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>6g</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>3g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.5g</td>
</tr>
<tr>
<td>NH$_4$Cl</td>
<td>1g</td>
</tr>
<tr>
<td>agar</td>
<td>15g</td>
</tr>
</tbody>
</table>

Add deionized water to approximately 1L. Adjust to pH 7.4 with 10N NaOH. Autoclave. Cool to 50°C. Then add:

2.0 ml 1M MgSO$_4$
0.1ml 1M CaCl$_2$
10.0ml 20% glucose
1.0ml 1M thiamine-HCl

Filter the complete medium through a 0.2 µm filter unit.