Murphy Lab, Emory University, Spring 2000

**Preparation of frozen competent *E. coli Top 10 F’* cells**

IMPORTANT! All chemicals, glassware, bottles etc must be sterile. The bottles and glassware you use to
culture and spin down bacteria may have residual plasmid material. Rinse these with 0.25 M HCl/1.5 M
NaCl, and then with denaturation solution (0.5 M NaOH, 1.5 M NaCl) and then copious amounts of RO
water before autoclaving. Only this rinsing will destroy plasmid DNA, autoclaving will not. It is best to
use only spin bottles reserved for competent cell preps. Follow aseptic technique. Compulsive attention to
detail is essential to make a good batch of cells.

1. Inoculate 20 ml of TMY broth (supplemented with 50 µl tetracycline, 5 mg/ml in 100% ethanol) using
100 ul of E.coli TOP 10 F’ (from frozen stock (e.g. frozen competent cells)) and grow in a 250 ml flask
at 37C at 300 rpm shaking.
2. Grow to midlog phase (OD<sub>600</sub> ~ 0.2 - 0.8).
3. Transfer culture to 2L flask containing 100 ml of TMY (supplemented with 250 µl of tetracycline).
4. Grow with vigorous shaking to OD<sub>600</sub> of 0.5 - 0.9.
5. Dilute culture again by adding to the flask 400 ml of TMY, supplemented with 1 ml tetracycline, and
grow to OD<sub>600</sub> = 0.6.
6. Place flask in ice-water bath and shake gently to ensure rapid cooling.
7. Spin at 4200 rpm at 4C for 10 min using the “competent cell only” centrifuge bottles.
8. Decant supernatant and resuspend pellet in 100 ml of COLD TFBI by gentle pipetting on ice.
9. Spin at 4200 rpm at 4C for 8 min.
10. Decant supernatant and resuspend pellet in 20 ml of COLD TFBI by gentle pipetting on ice.
11. Dispense 600 ul aliquots in pre-chilled microfuge tubes and snap-freeze immediatetl in a dry ice/ETOH
bath (ETOH is cheaper than MeOH and works just as well).
12. Store at −70C.
13. Run a positive control by transforming frozen cell with 5 ng of plasmid, and plate 1/5<sup>th</sup> on LB amp.
Should back calculate to get >10<sup>6</sup> colonies per µg of plasmid.

<table>
<thead>
<tr>
<th><strong>TMY Broth (1 L)</strong></th>
<th><strong>TFBI (200 ml)</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td>20 g bacto-tryptone</td>
<td>0.59 KOAc</td>
<td>0.419 g MOPS</td>
</tr>
<tr>
<td>5 g yeast extract</td>
<td>1.98 g MnCl2</td>
<td>2.2 g CaCl2</td>
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<tr>
<td>5.84 g NaCl</td>
<td>1.50 g KCl</td>
<td>0.15 g KCl</td>
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<tr>
<td>1.2 g MgSO4</td>
<td>0.29 g CaCl2</td>
<td>30 ml glycerol</td>
</tr>
<tr>
<td>Dissolve in QH2O and autoclave</td>
<td>Dissolve all components in QH2O and filter sterilize</td>
<td>Dissolve MOPS in QH2O and adjust pH to 7.0 with IN NaOH. Add remaining components and filter sterilize.</td>
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</tbody>
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