**Competent Cells & Transformation - Inoue Method**

(Sambrook & Russell, Molecular Cloning: A Laboratory Manual, 3rd ed., 2001)

This protocol produces "ultra-competent" cells with transformation efficiencies of >1x108 colonies/µg DNA. The major difference from other protocols is that the bacterial culture for the competent cells is grown at 18-23°C. The slow cell growth is best done overnight.

**Competent Cells**

1) Prepare Inoue transformation buffer (~100 mL is needed for the following protocol):

 55 mM MnCl2

 15 mM CaCl2

 250 mM KCl

10 mM PIPES (add from sterile 0.5 M, pH 6.7 stock made with free acid and

pH'ed with KOH)

 Sterilize with a 0.2 µm filter.

2) Pick a colony from a fresh O/N plate and inoculate 25 mL LB. Incubate for 6-8 hrs at 37°C.

3) At about 6pm, inoculate three 1 L flasks containing 250 mL SOB with 10, 4 and 2 mL of the above starter culture. Incubate O/N with moderate shaking at **18-22°C**.

4) The next morning, monitor the OD600 of the three cultures until one reaches 0.55. Transfer this culture to an ice-water bath for 10 min and discard the other two cultures.

5) Pellet cells at 2500g for 10 min at 4°C.

6) Decant supernatant and remove last drops of media with vacuum aspirator. Resuspend cells in 80 mL ice-cold Inoue transformation buffer.

7) Pellet cells at 2500g for 10 min at 4°C.

8) Decant supernatant and remove last drops of media with vacuum aspirator. Resuspend cells in 20 mL ice-cold Inoue transformation buffer.

9) Add 1.5 mL DMSO and store on ice for 10 min.

10) Working quickly, dispense 200 µL aliquots into chilled, sterile microcentrifuge tubes. Immediately snap-freeze in liquid nitrogen. Store at -80°C.

**Transformation**

1) Remove competent cells from -80°C freezer. Thaw with finger warmth and then immediately store in an ice bath for 10 min.

2) Transfer to sterile Falcon 2057 tubes prechilled in ice bath (50 µL aliquots should be sufficient).

3) Add transforming DNA (up to 25 ng per 50 µL rxn - do not exceed 5% of volume) and swirl gently. Store on ice for 30 min.

4) Incubate in a preheated 42°C water bath for exactly 90 seconds. Do not shake.

5) Immediately transfer to an ice bath and cool 1-2 min.

6) Add 800 µL SOC media to each tube and incubate at 37°C for 45 min.

7) Plate onto SOB media containing 20 mM MgSO4 with the appropriate antibiotic (≤200 µL should be sufficient for plating - e.g. from miniprep DNA - but entire culture can be centrifuged and resuspended in 200 µL SOC if necessary - e.g. for ligations).

8) Incubate O/N at 37°C.

**Competent Cells & Transformation - Calcium Chloride Method**

(Sambrook & Russell, Molecular Cloning: A Laboratory Manual, 3rd ed., 2001)

This procedure is relatively simple and rapid. It yields competent bacteria that transform with efficiencies of >5x106 colonies/µg DNA. It is useful for routine transformation with intact plasmids, though it can also be successful for transforming ligation reactions.

1) Pick a colony from a fresh O/N plate and inoculate 100 mL LB. Harvest when OD600 reaches 0.35. It is *essential* that the OD600 does not exceed ~0.4.

2) Transfer to centrifuge tube and cool on ice bath for 10 min. Centrifuge at 2700*g* for 10 min at 4°C.

3) Decant supernatant and remove last drops of media with vacuum aspirator. Resuspend cells in 30 mL ice-cold MgCl2-CaCl2 solution (80 mM MgCl2, 20 mM CaCl2).

4) Centrifuge cells at 2700*g* for 10 min at 4°C.

5) Decant supernatant and remove last drops of media with vacuum aspirator. Resuspend cells in 4 mL ice-cold MgCl2-CaCl2 solution.

6) Add 140 µL DMSO, mix and store on ice for 15 min. Add another 140 µL DMSO, swirl and return to ice.

7) Working quickly, dispense 200 µL aliquots into chilled, sterile microcentrifuge tubes. Immediately snap-freeze in liquid nitrogen. Store at -80°C.

**Transformation**

1) Remove competent cells from -80°C freezer. Thaw with finger warmth and then immediately store in an ice bath for 10 min.

2) Transfer 200µL aliquot to sterile Falcon 2057 tubes prechilled in ice bath.

3) Add transforming DNA (no more than 50 ng in 10 µL volume) and swirl gently. Store on ice for 30 min.

4) Incubate in a preheated 42°C water bath for exactly 90 seconds. Do not shake.

5) Immediately transfer to an ice bath and cool 1-2 min.

6) Add 800 µL SOC media to each tube and incubate at 37°C for 45 min.

7) Plate onto SOB media containing 20 mM MgSO4 with the appropriate antibiotic (≤200 µL should be sufficient for plating - e.g. from miniprep DNA - but entire culture can be centrifuged and resuspended in 200 µL SOC if necessary - e.g. for ligations).

8) Incubate O/N at 37°C.