Chemical Transformation

1. Remove tube of frozen competent cells from -70°C and place on ice. Allow cells to thaw. Note: Keep cells chilled on ice to ensure high transformation efficiency.

2. Mix cells by flicking the tube gently, then remove 100μl per transformation into a sterile pre-chilled (on ice) Falcon 2059 tube.

3. Add 1-50ng of DNA (in a volume no greater than 10μl) per 100μl cells. Quickly flick the tube several times to ensure the even distribution of DNA. To determine the transformation efficiency, add 1-10ul (0.1-1ng, depending on how competent the cells are) of the pGEM-3Z control plasmid (or alternate control).

4. Immediately place tubes on ice for at least 10 minutes.

5. Heat shock the cells for 45-50 seconds in a water bath at exactly 42°C. Do not shake.

6. Immediately place tubes on ice for 2 minutes.

7. Add 900μl of room temp (or 37°C) SOC or LB medium and incubate for 1 hour at 37°C with shaking at ~225 rpm.

8. Plate 100-200μl of the transformation mix or an appropriate dilution onto antibiotic plates. You may have to plate all of the transformation mix if you know your cells or DNA produce low transformation efficiencies (common with plasmid mini-preps); the cells may be pelleted by centrifugation at 1000 x g for 1 minute, then the cells can be resuspended in 50-200μl of SOC or LB medium and plated. (The maximum amount of solution that may be spread on a plate is ~200μl).

For the positive control DNA, a 1:100 to 1:1000 dilution is recommended for plating on LB plates.

9. Place plates in the 37°C incubator and grow overnight 14-18 hrs depending on the cell growth rate (XL1-Blues usually grow slower than BL21s; it's best to keep an eye on the growth of the cells the next day).

To determine the transformation efficiency (colony forming units; cfu):

Transformation efficiency (cfu/μg DNA) = [(cfu on control plate)/(ng of pGEM DNA used)] x (1x10^3 ng/μg) x (dilution plated)

Solutions
**SOC medium**

2.0 g Bacto-Tryptone  
0.5 g Bacto-Yeast extract  
1 ml 1M NaCl  
0.25 ml 1M KCl  
1 ml 2M Mg stock (1M MgCl2-6H2O, 1M MgSO4-7H2O), filter sterilize  
1 ml 2M Glucose, filter sterilize  
Up to 100 ml ddH2O

Add Bacto-Tryptone, Bacto-Yeast extract, NaCl and KCl to 97ml ddH2O. Stir to dissolve. Autoclave and cool to room temp. Add 2M Mg stock and 2M Glucose, each to a final concentration 20mM. Filter the complete medium through a 0.2um filter. The pH should be 7.0.

**LB plates (per liter)**

10 g Bacto-Tryptone  
5 g Bacto-Yeast extract  
5 g NaCl  
15 g agar

Adjust the pH to 7.0 with NaOH (~200ul 5M NaOH). Autoclave and allow to cool to 50 C before adding antibiotics. Autoclaved media can be stored at room temp if top is tightened and then be used by microwaving (w/ top loosened completely to allow steam to vent) at 70% for ~15 minutes (keep an eye on the bottle because to top can pop off; it helps to mix the solution a few times during the 15 minutes of microwaving). For liquid media leave out the agar.