Transformation protocol when using the pGEM-T and pGEM-T Easy Vector Ligation Reactions

1. Prepare 1 LB/ampicillin//X-Gal (LBA plates are 1ml of 100mM Amp in a liter, 32 ul of 50 ug/ul X-Gal per plate) plates for each ligation reaction. Equilibrate the plates to room temperature (37 C) prior to plating.

2. Centrifuge the tubes containing the ligation reactions to collect contents at the bottom of the tube. Add 2 ul (I recommend all) of each ligation reaction to a sterile 1.5 ml microcentrifuge tube on ice.

3. Remove the tube(s) of frozen JM109 High Efficiency Competent Cells from –80 C storage and place in an ice bath until just thawed (about 5 minutes). Mix the cells by **gently** flicking the tube.

4. **Carefully** transfer 25 ul of cells into each tube prepared in Step 2.

5. **Gently** flick the tubes to mix and place them on ice for 20 minutes.

6. Heat-shock the cells for 45-50 seconds (up to 2 min) in a water bath at exactly 42 C (**Do Not Shake**).

7. Immediately return the tubes to ice for 2 minutes. It is helpful to have a ice/water bath instead of just ice.

8. Add 250 ul room temperature SOC medium to the tubes containing cells transformed with ligation reactions (LB broth may be substituted, but colony number may be lower).

9. Incubate for 1.5 hours at 37 C with shaking (~150 rpm).

10. Plate all of each transformation onto LB/ampicillin/X-Gal plates. For the transformation control, a 1:10 dilution with SOC medium is recommended for plating. If a higher number of colonies is desired, the cells may be pelleted by centrifugation at 1,000 x g for 10 minutes, resuspended in 200 ul of SOC medium, and 100 ul plated on each of 2 plates.

11. Incubate the plates overnight (16-24 hours) at 368C. In our experience, approximately 100 colonies per plate are routinely seen when using competent cells that are 1 x 10^8 cfu/ug DNA, if 100 ul is plated. Longer incubations or storage of plates at 4*C (after 37*C overnight incubation) may be used to facilitate blue color developmnet. White colonies generally contain inserts; however, inserts may also be present in blue colonies.