**Transformation of Chemically Competent E. coli**

Preparation: 42°C water bath; room temperature SOC; 2 plates with appropriate antibiotic warmed to room temperature.

1. Add 1-2 µl of ligation mixture to competent cells thawed on ice. Competent cells are stored in the -70C freezer in L2-130. Keep cells on ice while thawing. Once add the ligation mixture, mix by gently shaking tube (like ringing small bell).

**Note:** in some cases we split the competent cell sample into two by pipeting (gently) 25 µl of cells into a second, sterile screw tip tube. These cells are expensive and if we are ligating multiple samples, we will split the competent cells. If you aren’t sure about when to do this, please ask.

2. Once add DNA, incubate the tube on ice for 5-30 min.

3. Heat shock sample at **42°C for exactly 30 sec**.

4. Add 200 µl room temperature SOC medium to cells. Be sure to use a filter tip and keep the SOC sterile.

**Note:** if you split the competent cells into 2 samples, add 100 µl SOC to each tube.

5. Once mixed, tape tube to the bottom of the 37°C incubator shaker; shake for 1 hr.

6. Spread 100 µl of cell mixture on each of two plates (with correct antibiotic). If we expect the transformation to be very efficient, we will spread only 10-20 µl of mixture on each plate to which additional SOC is added to aid even spreading. Please check before deciding on the amount to spread if you are unsure.

7. To spread add 10-15 sterile glass beads to plate surface and shake plate horizontally to “roll” beads through transformation mix to spread evenly on surface of agarose media.

**Note:** If vector is blue/white selective, coat surface of room temperature plate with 20 µl X-GAL about 10 min prior to spreading cells on plate. X-GAL is stored at -30C in 100µl aliquots.

8. Allow plates to sit for 10 min on counter to allow spread mix to soak into plate. Place in incubator on the shelf with the **bottom facing up (agar on top)** and grow overnight.

9. Next day, parafilm plate and place in refrigerator with the bottom facing up.