

## **Cloning Protocol**

### **Material Locations:**

- 1.) X-Gal – upright -20 freezer door, upper shelf
- 2.) IPTG – large fridge door, upper shelf
- 3.) SOC – large fridge door, upper shelf
- 4.) Plates – either buy new ones, or poured plates are in the bottom shelf of fridge
- 5.) E. coli – upright -80
- 6.) Vector kit – horizontal -20

### **Preparation:**

- 1.) Stick plates in incubator at 37° for ~30 min to dry.
- 2.) Spread X-Gal (20ul) + IPTG (100ul) on plates and stick back in the incubator to warm up
- 3.) Put SOC on bench top to warm up to room temp.

### **Ligation:**

PCR product	2ul
Salt solution	1ul
dd H <sub>2</sub> O	2ul
Vector	<u>1ul</u>
	6ul

Incubate at room temp. for 5 min.

### **Transformation:**

1. Thaw E. coli on ice
2. Add 2ul of ligation mixture
  - Mix GENTLY
3. Incubate on ice for ~ 15 min
4. Heat shock @ 42° for 30 seconds
5. Add 250 ul of Room Temp. SOC
  - Screw cap on tight (we don't want E.coli spilling out)
  - Go to Begun lab and tape tubes to shaker in the 37° incubator.
  - Shake for 1 hour
6. Spread 50 ul of E. coli into prewarmed plates
7. Incubate overnight at 37°

### **Harvesting Colonies and Sequencing:**

- 1.) Pick a single colony from a plate using a sterilized 10 ul pipet tip. Pick ones that are white and not touching neighbors (NOTE: blue colonies mean failed transformations)
- 2.) Drop pipet tip with the colony on the tip into a sterile 15 ml tube filled with 3-6 ml of LB medium.
  - NOTE: for higher yields use more LB medium

- See sequencing lab website for minimum concentrations depending on plasmid size

3.) Tape tubes onto the shaker in the Begun lab incubator overnight.

4.) Clean up with Quiaquick Spin Miniprep kit sold at Vet med.