

### **Overnight cultures of successful transformants.**

- 1) After determining successful transformants by colony PCR, start overnight cultures of the colonies with inserts.
- 2) Prepare as many 50 ml sterile fisher tubes as you need and add 25 ml of LB broth into each tube. Add 25  $\mu$ l of Ampicillin (200 mg/ml) into each tube and mix.
- 3) Using a sterile inoculating loop carefully pick one colony from the LB-Amp plate and swirl it in the LB-broth. Streak remainder on holding plate if further minipreps are anticipated.
- 4) Cap the tubes, add a piece of scotch tape to ensure cap stays in place, and incubate them at 37°C on an orbital shaker overnight
- 5) Next morning, spin the tubes at 2,700 g for 15 minutes.
- 6) Decant the supernatant and proceed with the Qiagen Miniprep protocol to extract DNA. Alternatively, store the cell pellets at -20 °C.