Miniprep PCR
10/17/2013 TO

**Purpose**
PCR (polymerase chain reaction) is a way to amplify a specific strand of DNA. The strand of DNA to be amplified is defined by the forward and reverse primer that you use. PCR is a great way to increase a specific product you want or screen plasmids for your sample.

**Protocol**
1. Obtain a sample to be amplified by miniprep or picking from a colony.
2. When your samples are ready, prepare your PCR Master Mix according to the recipe below and in the same order. Make in 1.5 microcentrifuge tube. MAKE ON ICE! (I like to use the Blue Ice blocks in the freezer. They are easier to use with the small PCR Tubes.)
   a. 33µL ddH2O
   b. 5µL 10x PCR Buffer
   c. 5µL MgCl2
   d. 2µL of 10mM dNTP stock
   e. 1µL of a 20mM stock of your forward primer
   f. 1µL of a 20mM stock of your reverse primer
   g. 1µL of Taq
3. Mix sample well by pipetting to make sure Taq is uniformly distributed.
   a. Add 48µL of your Master Mix to each PCR tube.
4. Add 2µL or your minirepped plasmid into the individual PCR tubes.
5. Transfer to PCR. Run the program “Colony PCR”

   a. 94° for 1 min
   b. 94° for 3 min
   c. 94° for 1 min
   d. 55° for 1 min
   e. 72° for 1 min
   f. Repeat c-e 30X
   g. 72° for 4 min
   h. 4° Pause

6. Run for approx. 30 min. on an Agarose gel
   a. Use small wells on the 1.5 setting
   b. Add 2μL of 6X Loading Dye to 10μL of sample.
      i. This is kept at room temp in the same drawer as SYBR Safe
   c. Use 6μL of 1Kb Plus Ladder