

## PCR from Bacterial Colonies

1. Each student will set up one PCR reaction. Label your 0.2 ml PCR tube with your initials and group number on the top and side.
2. Using a toothpick or a sterile pipet tip, gently scrape some bacterial cells from a single colony on your Petri dish from last week, then rub the toothpick on the bottom of your PCR tube to transfer some of the cells. You just need a small amount of cells in the tube, not a big glob.
3. Add all components for your PCR reaction, in the order shown below, to a 0.5 ml tube containing 0.5  $\mu$ l Taq Polymerase. The mix should be centrifuged briefly (3-5 seconds) to bring all components to the bottom of the tube. Next, pipette 50  $\mu$ l of the reaction mix to the PCR tube (0.2 ml) containing your bacterial cells.

<u>Reaction Mix</u>	<u>Amount to add for one reactions</u>
10X PCR Buffer	5 $\mu$ l
dH <sub>2</sub> O	39.5 $\mu$ l
Primer mix	1.0 $\mu$ l
2.5 mM dNTPs	4.0 $\mu$ l
<u>Taq polymerase (already in tube)</u>	<u>0.5 <math>\mu</math>l</u>
Total in tube:	50 $\mu$ l

4. Your PCR samples should be placed on ice until everyone has finished assembling their reactions.
5. The reactions will be cycled using a MJ Research DNA Engine Peltier Thermal Cycler. The reaction conditions are as follows:

94°C for 30 seconds	} 25 cycles
94°C for 30 seconds	
55°C for 30 seconds	
72°C for 60 seconds	

6. Prepare a sample from the PCR reaction for electrophoresis. Obtain your PCR reactions from the TA. The samples should be centrifuged briefly (5-10 seconds). 10  $\mu$ l of each sample should be added to new tubes. 2  $\mu$ l of loading dye is then added. The reactions should be thoroughly mixed, centrifuged briefly, and loaded onto a prepared agarose gel as directed by the instructors.
7. Photograph the gel and calculate the size of the band, if any.

### **Materials**

The primer mix contains both PCR primers at concentrations of 10 pmoles/ $\mu$ l each. The 10X PCR buffer will give a final reaction concentration of 50 mM Tris-HCL pH 9.1, 16 mM ammonium sulfate, 3.5 mM MgCl<sub>2</sub> and 150  $\mu$ g/ml BSA.

Primer M13F

5'-GTAAAACGACGGCCAGTG -3'

T<sub>m</sub>= 55°C

Primer Lec1R

5'-GGCCTCATGCAACACAAAGC -3'

T<sub>m</sub>= 59°C