Polymerase Chain reaction protocol:

Purpose: Amplify the DNA your isolated from your mini-prep.

- We will be doing 50ul total volume reaction, this will give the opportunity for every student to load the gel three times.
- We are using 2-20 ul micropipettes.

1. In your small PCR tube add these reagents in the following order

- 25ul of OneTaq Master Mix (including Taq DNA polymerase and dNTPs)
- 5ul of DNA
- 2 ul of Forward primer
- 2 ul of reverse primer
- 16 ul of DI water

*Total volume of 50ul

When you are adding the reagents make sure you add to the bottom fo the tube where the solution is, avoid adding to the sides of the tube, you want to be sure that the reagents are being mixed together, so adding to the sides of the tube such small volumes may not allow all of the reagents to mix together well in the right concentrations.

Remember that the DNA polymerase is temperature sensitive, it should always be kept on ice when using it, therefore you should also keep you PCR tube on ice, only take it out when adding reagents and then immediately put it back on ice.

2. Create this program in the Thermocycler
Let it run for 35 cycles

Initial Denaturation	94°C	30 seconds
30 Cycles	94°C 45-68°C 68°C	15-30 seconds 15-60 seconds 1 minute/kb
Final Extension	68°C	5 minutes
Hold	4-10°C	