PCR Protocol

Polymerase chain reaction (PCR) is a simple method to amplify specific sequences of target DNA using 1) two, flanking DNA primers, 2) template DNA, 3) a special DNA polymerase called Taq, 4) free nucleotides (dNTPs), and other additives, including MgCl2 and other salts. All of these components are added together and subjected to repetitive thermal cycling in order to achieve amplification. Performing PCR is generally straightforward, but variables such as the concentration and identity of primers and target DNA, annealing temperature, cycle number, and other factors, can determine the overall success. Thus, it is important to always consider the **essential controls** when performing PCR, including a 'NO Template' negative control. Identifying all of the control and test samples will aid in the PCR setup. A simple protocol for performing most PCR reactions is detailed below.

Reminders:

- Keep the reagents, master mix tubes, and sample tubes on ice at all times.
- Make sure to briefly spin each of the individual reagent tubes in a mini centrifuge before you begin because some of the liquid may be stuck at the top of the tube.
- Label all reaction tubes with a black Sharpie marker so they can be identified after the reaction; if the labels on any reagent or stock tubes are becoming faded, re-label them with a black Sharpie marker.
- After all reagents are added to reaction tubes, they should be quickly vortexed (2-3 seconds) and spun down in a mini centrifuge before putting them in the PCR thermal cycler.

PCR Setup

We generally use a HotStart 2xTaq Pol Master Mix that contains all of the necessary reagents except the primers and the template DNA. You will generate a Master Mix solution that contains all of the common ingredients. You will distribute the Master Mix into the appropriate number of sample tubes corresponding to your experiment. After distributing the Master Mix, you will then add the DNA templates or water control to each tube. Set up the PCR reaction based on info in the table below.

Reagent	[Stock]	[Final]	Volume of Stock per Sample	# Samples	Total Volume of Stock Added to Master Mix
HotStart 2x Taq MM	2X	1X	10 uL		
Forward primer	10 uM	0.3 uM	0.6 uL		
Reverse primer	10 uM	0.3 uM	0.6 uL		
Nuclease-free H20	***Up to 19 uL*		8.8 uL		
DNA template	DNA or Control		1 uL		
Total Reaction Volume			**20 uL**		

1. Determine the total number of samples you will be amplifying, i.e. each of the different template DNA samples, along with the appropriate controls (including a 'NO Template' control).
2. Create a Master Mix **for each primer set** that contains all of the common reagents (everything **except** the genomic DNA). Calculate the amount of each reagent that will be added to the Master Mix by multiplying the amount per sample by the total number of samples.
3. Get a 1 mL centrifuge tube and label it 'Master Mix'. Begin adding the appropriate amounts of each reagent, including the HotStart 2x Taq MM, each of the primers, and water.
4. Briefly vortex the Master Mix, spin it in the mini centrifuge, and put it aside.
5. Label the appropriate number of small PCR reaction tubes with an identifier for the primer set and the DNA template added, i.e. 1A = primer set 1, worm 1; 2B = primer set 2, worm 2.
6. Add 19 uL of the Master Mix to each of the PCR tubes corresponding to those primers. Add 1 uL of the corresponding DNA to each of the tubes.
7. Briefly vortex each of the PCR tubes and spin in a mini centrifuge.
8. Keep the PCR tubes on ice until you add them to the PCR machine for thermal cycling.
9. Clean up your lab bench and return all reagents to the appropriate boxes in the -20C freezer.

Thermal Cycling

The reactions you assembled will be put into a machine that will adjust the temperature conditions to allow PCR to occur. Some reactions may require specific modifications to the annealing temperature, extension time, or number of cycles; but a standard protocol described below will serve as a good starting point.

1. You will turn on the PCR machine, navigate to the menu, and select or modify the protocol that corresponds to your specific reaction.
    a. If you are running a new PCR reaction, you should name a new protocol with the primer name and save it on the machine before you set up the reactions.
    b. If you are running an existing PCR reaction, select it and ensure the parameters are correct.
2. Put your samples in the thermal cycler, close the lid, and select ‘Start’.
3. Ensure that the procedure starts and note the time.

Standard PCR Protocol for HotStart Taq Polymerase

1. Initial Denaturation: 95°C for 10 minutes.
2. Cycle Denaturation: 95°C for 20 seconds.
3. Cycle Annealing: 55°C for 20 seconds.
4. Cycle Extension: 72°C for 30 seconds (per 1kb of target)
5. Repeat Steps 2 - 4 for 30 cycles.
6. Final Extension: 72°C for 5 min.
7. Hold: 4°C forever.