Polymerase Chain Reaction Protocol

Polymerase chain reaction, or PCR, is the method through which numerous copies of a target region of DNA are made. For the sake of efficiency, many of the ingredients of the reaction are already included in a PCR taq mix. The rest you will have to combine yourself using the protocol. The PCR method described here in detail are intended for producing product for Sanger Sequencing. However, this method can be modified for nearly any PCR application.

Reagents:
Distilled de-ionized H_2O
Bioline *MyTaq* Mix:
- *-10x PCR Buffer*
- *-di-Nucleotide Tri-Phosphates (dNTPs)*
- *-Taq Polymerase*

Two primers (one for forward direction and one for the reverse.)

Equipment and supplies:
PCR tubes
Eppendorf tubes
Micropipettors (1-10 µl and 10-100/200µl)
Thermalcycler

Protocol:
I. Preparing A PCR Master Mix: Each DNA sample requires it own separate PCR reaction and its own set of reagents. Instead of preparing the reagents for each reaction separately, all the reagents will be combined into a master mix. To make the master mix, we need to know how many samples we want to perform a PCR on (N). We will then add one to that number to account for error (N+1). We will use this N+1 number to calculate how much of each reagent goes into our master mix.

1x PCR (25μl) for ribosomal regions (=ITS or 28S, use underlined volumes for single copy protein coding genes.)
ddH_2O = 9 µL / 5 µL
MyTaq mix = 12.5 µL
Primer (forward) = 1.25 µL
Primer (reverse) = 1.25 µL
DNA sample = 1 µL / 5 µL

1) Calculate the number of reactions you will perform (N+1).
2) Calculate the volume of each reagent needed for the master mix.
   a. Multiply N+1 to each volume of the following reagents: ddH_2O, 10x buffer, dNTPs, Primer (f), Primer (r), and Taq.
   b. Do NOT calculate volume of DNA sample. This will be added separately.
3) Combine the above calculated volumes into a single Eppi tube.
4) Prepare and label N number of PCR tubes.
5) Aliquot 24µl of Master Mix to each PCR tube.
6) For each DNA sample, add 1µl to a PCR tube to bring the total volume of that tube to 25µl.
7) RECORD WHICH SAMPLE WENT INTO WHICH PCR TUBE IN YOUR LAB NOTEBOOK!

II. PCR thermalcycler steps for ITS (program "pcr55") and 28S (program "pcr50").

A. Initial Denaturation: DNA melting/making single strands
- 2 minutes @ 95°C

B. Denaturation
- 30 seconds @ 95°C

C. Annealing: Primers bind to single stranded DNA
- 30 seconds @ 50-55°C (depending on protocol)
  ~ 55°C = "pcr55" program on thermalcycler
  ~ 50°C = "pcr50"

D. Extension: Taq polymerase makes the DNA copy
- 2 minutes @ 72°C

E. Repeat steps B-D 32 times.

F. Final Extension
- 5 minutes at 72°C

G. Chill
- Forever at 14°C