Basic Steps and Guidelines for Polymerase Chain Reaction (PCR)

Set-up:

1. Handle everything with latex gloves and be conscious of breathing/loose hairs/dust. Use bleach to clean benchtop. A clean lab bench is an important component of success.
2. Get sterile or autoclaved tubes (small PCR tubes/strip tubes equal to your sample number and larger tubes for preparing a master mix), a PCR tube rack, and a source of PCR-grade water (ddH_2O). Label PCR tubes.
3. Thaw tubes of PCR mastermix, $MgCl_2$, primer 1 and 2, 1X BSA, and your DNA samples. If your procedure will take a long time, its recommended to keep these reagents in a cold plate.
4. Turn up PCR machine (use code 637 when prompted) and identify appropriate protocol. Use edit button to check protocol if necessary.
5. When pipetting, check for accurate uptake and dispensation of reagents. Mix reagents occasionally by pipetting up and down.
6. Prepare a PCR solution (sometimes with extra added, ie 10%) by starting with reagents that are least likely to cause cross contamination (i.e. water first, then mastermix, then $MgCL_2$, then BSA, and last primers). Use filter tips, and change tips when possible contamination can occur (tip touches a foreign object, tip touches a reagent that will contaminate another reagent, etc).
7. Thoroughly mix the master mix (finger-flicking, or vortexing). Aliquot master mix to each PCR tube.
8. Add DNA sample to the PCR tubes.
9. Spin down PCR tube if necessary. Load thermocycler and start PCR reaction.

Generalized Insect COI-COII mtDNA Recipe

Reagents	**1X Reaction**
*ddH_2O	7.5ul
PCR mastermix (MM)	12.5ul
*25mM $MgCl_2$	1.0ul
1X BSA	1.0ul
Forward Primer 10uM	1.0ul
Reverse Primer 10uM	1.0ul
*DNA sample	1.0ul
TOTAL VOLUME	**25ul**

NOTES:

1. Sometimes new dilutions of BSA or primers need to be made. BSA stock comes in 100X concentration, so it should be diluted as 1ul in 99 ul of ddH_2O. Primer stocks are made as 100 uM concentration, so it should be diluted as 1 ul in 9 ul of ddH_2O.
2. Typically, modifications (*) of this recipe involve altering $MgCl_2$ concentration (& readjusting dd H_2O) or the DNA sample volume.

Generalized Touchdown Thermocycler Protocol: Arthropod mtDNA

Step	Cycle #	Temperature °C	Duration
*Denature	1	94	2min
Step-down PCR	25X	94	30s
		55 (-0.4/cycle)	30s
		72	90s
Low Hold PCR	15X	94	30s
		45	30s
		72	90s (+3s/cycle)
Extension	1	72	7min
Cold Hold	1	10	Infinity

1. The first denaturing step is usually 2 minutes, unless you are using a different PCR mastermix.
2. The touchdown protocol is a solution to not knowing the optimal annealing temperature for your gene and species. You can alter this protocol by adjusting the starting annealing temperature (raising the temperature above 55 °C will increase the specificity of primer-DNA annealing, lowering the temperature will reduce the specificity- but the specificity depends on your primer length and degree of mismatch to target DNA). You can also adjust the "Low Hold" temperature, but typically you don't want to lower it below 43 °C (raising it above 45 °C will lead to more specific annealing). Finally, you can adjust the number of cycles. 40 cycles, as in this protocol, is a lot- typically between 30 and 40 cycles is recommended. Adding more cycles will eventually result in reagent degradation or reagent exhaustion.

"Universal" Arthropod mtDNA Thermocycler Protocol

Step	Cycle #	Temperature °C	Duration
*Denature	1	94	2min
Universal PCR	*35-40X	94	30s
		45	30s
		72	*60-90s
Extension	1	72	7min
Cold Hold	1	10	Infinity

1. This protocol's low annealing temp (45°) can produce multiple bands. Optimizing this temperature is recommended when many PCRs are planned.
2. Adjust the cycle number depending on the DNA concentration (very small arthropods and dilute extractions should be run for 40 cycles).
3. You can adjust your extension time for the length of the region you are working on. If it is larger than 1000 bp, then it is probably a good idea to have a 90s extension time.