PCR – basic mtDNA and nDNA

1. 12.8 µl ddH_2O
2. 1.8 µl 10mM $MgCl_2$
3. 2.0 µl 10mM dNTPs
4. 2.5 µl 10x PCR Buffer (with 15 mM $MgCl_2$)
5. 2.5 µl 2µM primer1
6. 2.5 µl 2µM primer2

Mix and add 24 µl of master mix to each reaction. Add 1 µl DNA (5-100 ng/µl).

1. 68°C for 60 sec. (prewarm)
2. 92°C for 35 sec. (denature)
3. 48-55°C for 35 sec. (anneal)
4. 68°C for 90 sec. (extend)
5. 68°C for 7 min. (final extend)

Repeat the parameters 2 – 4 35 times. I have found that a 2 or 5 minute "hot start" at 94°C does not work that well. The last 68°C extension can also be left out.

This is the standard mix for PCR reactions which works well and is usually strong enough so that the DNA can be purified directly. If the bands are not strong enough, I will run them out on NuSieve Agarose, then cut them out with a Blue Tip, resuspend them in 50 µl of ddH_2O, and then use this resuspended DNA instead of the genomic DNA. I use the same procedure if I get multiple bands in the initial amplification, although some of those bands can also be eliminated by reducing the amount of $MgCl_2$ added to the mix. When I reamplify, I also raise the annealing temperature to 55°C (depending on the annealing temperature of the primers, but remember this time the match between the priming sites and the primer is perfect). An alternative to multiple bands is to use internal sequencing primers.