Guidelines for a General PCR Protocol

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Materials Required

- Nuclease-Free Water
- Reaction Buffer
- $MgCl_2$, 25mM
- Upstream Oligonucleotide Primer (F)
- Downstream Oligonucleotide Primer (R)
- dNTP Mix (10mM of each dNTP)
- Template DNA
- *Taq* DNA Polymerase

(this protocol is for a 50µl reaction volume but this can easily reduced to 25µl)

Note: To facilitate optimization, troubleshooting and validation of any PCR, include both positive and negative control reactions.

Amplification

1. Combine the components in the order listed in the table below in a thin-walled 0.2ml reaction tube (on ice if necessary).
    1. the exception to this is if you are using a mastermix for several samples in which case the Taq is added to the mastermix which is then aliquoted out and the DNA template is the final addition. See below

2. If the thermocycler you are going to use does not have a heated lid it will be necessary to overlay the reaction with 1–2 drops (20–40µl) of nuclease-free mineral oil to prevent condensation and evaporation.

3. Place the tubes in the heat block and proceed with the thermal cycling profile chosen for your reactions. Generally have

    1. a prolonged initial denaturing step of 94-96^{o}C for 2-5 min

    2. 25-35 cycles of

        - 94-96 ^{o}C for 15-30secs
        - [Tanneal] - 5 ^{o}C for 15-30secs
        - 72 ^{o}C for 15-45secs (this depends on the length of your product

    3. a final extension of 72 ^{o}C for 5-10 min

    4. hold at 10 ^{o}C indefinitely

Note: If working with multiple samples, a master mix consisting of water, $MgCl_2$, 10X Reaction Buffer, individual dNTPs, and *Taq* DNA Polymerase may be assembled. Combine appropriate multiples plus 10% extra (to allow for pipetting error) of the listed reaction components (except template) and add the appropriate volume such that, after template addition, the final volume is 50µl. Initiate the reaction by adding the template. Use individual pipette tips for all additions, being careful not to cross-contaminate the samples.

Store reaction products at -20°C until needed. The reaction products can be further purified using a number of procedures as necessary/appropriate; eg to remove PCR components prior to cloning or to excise a specific band if multiple PCR products are produced and further optimisation of conditions is not possible.

Recommended Volumes of Components for PCR.

Components	Volume	Final Concentration
Nuclease-Free Water	Xµl	(to a final volume of 50µl)
10X Reaction Buffer	5µl	1X
25mM $MgCl_2$	3µl	1.5mM
Downstream Primer	50pmol[1]	1µM
Upstream Primer	50pmol[1]	1µM
dNTP mix (10mM of each dNTP)	1µl	0.2mM each
Template	Yµl[2]	
Taq DNA polymerase (5u/µl)	0.25µl	0.025u/µl

[1]A general formula for calculating the number of nanograms of primer equivalent to 50pmol is: 50pmol = 16.3ng x b; where b is the number of bases in the primer.

[2]If possible, start with $>10^4$ copies of the target sequence to obtain a signal in 25–30 cycles, but keep the final DNA concentration of the reaction at <=10ng/µl. Less than 10 copies of a target can be amplified, but more cycles may be required to detect a signal by gel electrophoresis. Additional cycles may increase nonspecific amplification, evidenced by smeared bands upon gel electrophoresis.

NB many commercial polymerase kits have $MgCl_2$ already in the reaction buffer and further salt is not required – check the product.