5x PCR Master Mix

PCR*opsis* 5x PCR Master Mix contains all necessary components for PCR (polymerase chain reaction).

Storage:
4°C Short-Term (months)
-80°C Long-Term (years)

Applications

- Polymerase Chain Reaction (PCR)
- DNA fingerprinting (VNTR, STR, and RAPD)
- Mouse genotyping
- RT-PCR

Stability and Storage

The undiluted 5x PCR Master Mix can be stored at 4°C for up to 12 months or frozen at -80°C for years. Repeated freezing and thawing should be avoided. 5x PCR Master Mix ships at ambient temperature overnight.

Thermostable Taq polymerase in 5x PCR Master Mix:

- Prolonged exposure to room temperature does not affect activity (storage at room temperature is not recommended)
- No loss of activity detectable after 12 months at 4°C
- No loss of activity detectable after 10 freeze / thaw cycles at -80°C

Product Composition

The Master Mix is provided as a 5x concentrate. The concentrations given below are for the final 1x concentration as needed for running a PCR reaction.

Components	Final (1x) Concentration
Tris-HCl pH 8.6	10 mM
KCl	50 mM
MgCl2	1.5 mM
Taq Polymerase	30 U / mL
dNTPs	1 mM
Stabilizers	6.85%

Required Resources

- Template DNA, gene-specific PCR primer pair
- Water, PCR grade
- Thermal block cycler

- PCR tubes
- Sterile reaction tubes for preparing master mixes and dilutions

Guidelines

We recommend assembling all reaction components on ice and promptly transferring the reactions to a thermocycler pre-heated to the denaturation temperature (95°C). Common template concentrations range between 10 ng - 250 ng of human genomic DNA and 0.1 ng - 15 ng plasmid DNA. It is possible to scale the reaction down to 10 ~ 25 µl total volume by proportionately using small volumes of each reagent. Scaling down is not recommended for realtime PCR, where sensitivity is a direct function of the amount of fluorophore present per volume unit.

Preparing a PCR Reaction

- Thaw 5x PCR Master Mix (if frozen), primers and template DNA. Keep them on ice after complete thawing, and mix each thoroughly before use.
- Prepare reaction mix accord to the table below and thoroughly mix by gently pipetting up and down a few times.
- Dispense appropriate volumes into PCR tubes or the wells of a PCR plate.
- Program the thermal cycler according to the manufacturer's instructions.

For each PCR reaction:

Components	50 µL Reaction
Nuclease-free water	38 µL
5x PCR Master Mix	10 µL
Forward Primer (10 mM)	0.5 µL
Reverse Primer (10 mM)	0.5 µL
DNA Template	1 µL

PCR Reaction Conditions

Optimal conditions for the reaction vary depending on the sequence being amplified. The following program is recommended as a starting point. If you do not achieve satisfactory results, please consult the section on PCR Troubleshooting and Optimization.

Thermal Cycling Program

Step	Temperature	Time
Initial denaturation	95°C	
Amplification (30 cycles)	95°C	15~30 seconds
	45~68°C	15~60 seconds
	72°C	60 seconds / kb
Final Extension	72°C	1~5 minutes
Hold	4°C	

Product Quality Control Testing for Each Lot

Each lot of PCR*opsis* 5x PCR Master Mix is tested for the ability to amplify a DNA fragment of GAPDH when 1 pg of human placenta DNA in added to the PCR reaction mixture. In addition, we verify that no amplification is detected when human placenta DNA is excluded from the PCR reaction mixture. This second control verifies that extraneous genomic DNA is not present in the 5x PCR Master Mix. Only lots that show appropriate amplification with template DNA and no amplification without template DNA pass quality control testing and are available for commercial use.

Troubleshooting and Optimization for PCR

Each PCR reaction is different. In rare cases, success can depend on variables outside of the user's control, such as template-specific DNA secondary structures, or other poorly understood sequence-specific idiosyncrasies. If a reaction is not working as expected, the best possible solution is to select a different primer pair, or a different amplification strategy to accomplish the same goal. If that is not possible, there are some specific troubleshooting tips one can follow, in order to get a specific primer/template pair to work:

Fuzzy or smeared bands

- Try using shorter primer or a primer with a reduced G/C content (45-55%).
- Decrease the amount of primers and/or Master Mix relative to the reaction.
- Add up to 2 µl of DMSO per 50 µl reaction.
- Vary the amount of template used. Either too much or too little template can cause this problem.
- Possible contamination with nuclease or PCR inhibitors – purify the template better.

Absence of bands or weak bands

- Reduce the annealing temperature.
- Increase the extension time (should be 1000 bp / minute).
- Increase the amount of primers and/or Master Mix relative to the reaction.
- Add up to an extra 5 mM of magnesium chloride to the reaction.
- Vary the amount of template used. Either too much or too little template can cause this problem.
- Possible contamination with nuclease or PCR inhibitors – purify the template better.
- Choose a longer / different primer pair, if you can.

Well-defined, but false bands

- Specific binding of the primers to the wrong sequences can cause false bands. Using homology searching (e.g. NCBI's BLAST algorithm) of the primers against the template can catch this problem in advance.
- A very short false band can be the result of the primers annealing to each other (a "primer dimer"). Eliminate any homologies between your primers, especially at the ends. As little as 2 bp homology at the ends can cause this problem.
- Look out for possible overlaps between the two primers and within in each primer (pairing with molecules of its own kind). If you cannot change the primer sequences, follow the troubleshooting tips for "Fuzzy or smeared bands".

Overhangs

- If PCR is used to add non-template sequences to the template (e.g. restriction enzyme overhangs), the single most important thing to do is to have the annealing temperature follow the behavior of the annealing sequences over time: At first, only part of the primer will pair to the template (excluding the overhang). But after the first few cycles, enough reaction product containing the overhang will have accumulated to crowd the original template out. Now the entire primer hybridized with these products (including the overhang) and therefore the optimal annealing temperature rises. The PCR program should reflect this, stepping the annealing temperature up to the fully hybridized length (primer+overhang) after the first few cycles. This is sometimes called touch-up PCR.
- Taq polymerase usually adds a single 3' T overhang to the PCR product. This is useful for A/T based cloning schemes, but can get in the way of other cloning schemes. It's important to keep in mind that it does that and take it into account when designing a cloning strategy.