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001595 Rev B

GENERAL GUIDELINES FOR PCR

Primers & Probes: Various free software can be found online to design or analyze primers for PCR and qPCR. Primers/probes are generally 18–30 nucleotides long with a GC content of 40 to 60%. Optimize primer concentrations using a test range of final concentrations of 0.1 µM to 0.5 µM for both primers. The optimal concentration is one that gives the lowest Cq value. Next, optimize probe concentration using a test range of 0.1 µM and 0.5 µM. The optimal probe concentration is the one that gives the lowest Cq value and the highest fluorescent signal.

Additives: Magnesium is a cofactor necessary for Taq polymerase activity. The magnesium ion concentration in the 1X buffer is 3 mM. More magnesium can be added to further optimize the reaction if needed. Amplification of GC rich templates may be improved by using enhancers like DMSO or formamide to stabilize the template for amplification.

PCR consists of three steps: Denaturation, Annealing and Extension.

Denaturation: Heating the DNA to 95 ºC separates the strand and, in hot start mixes, activates the Taq which is inhibited by an aptamer at low temperatures. Initial denaturing of 2 minutes at 95 ºC is sufficient for most templates. For difficult templates, a longer denaturing such as 2-4 minutes at 95 ºC is recommended.

Annealing: The annealing stage allows primers to bind to the DNA. PCR primers copy only a specific sequence of template DNA within a sample. The temperature for annealing depends on the melting temperature (Tm) of the primers. Annealing temperatures can be optimized by performing a gradient PCR starting at 5 ºC below the calculated Tm of the primers. When primers with annealing temperatures above 60 ºC are used, the annealing and extensions steps can be combined, creating a two-step PCR protocol.

Extension: The optimal extension temperature is 68 ºC. At this temperature, the Taq polymerase uses the dNTPs in the Master Mix to make new copies of the target DNA. Extension times are generally 1 minute/kb. Each cycle copies the target DNA available in the sample and effectively doubles it. A final extension time of 5 minutes at 68 ºC is recommended.

Number of Cycles: 30 to 40 cycles generally give sufficient yield for amplification products. 40 cycles may be used for detection of low copy targets.

Refrigeration: Storing the reaction products at 4 ºC after the final extension step can minimize polymerase activity that may happen at higher temperatures. Store at -20 ºC for long-term storage.

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PCR Master Mix 2X

Catalog #: R02100

For research use only

Store at -20 °C
Avoid repeated freeze-thaw cycles

INTRODUCTION

Chai's 2X PCR Master Mix is an optimized ready-to-use solution containing recombinant Taq DNA polymerase, dNTPs, $MgCl_2$, KCl and stabilizers. The Taq polymerase is a thermostable DNA polymerase that possesses a 5′ → 3′ polymerase activity and a 5′ → 3′ exonuclease activity. It can be used for routine PCR applications using pure DNA solutions or cDNA products. The Master Mix can be used for both PCR and qPCR applications, and products between 100 bp and 5 kb can be amplified. Composition of the 2X PCR Master Mix is shown below.

COMPONENT	AMOUNT	FUNCTION
Taq polymerase	50 U/mL	Extends the DNA strand
KCl	100 mM	Stabilizes the DNA strands
$MgCl_2$	6 mM	Co-factor for Taq polymerase
TrisCl pH 8.6	20 mM	Buffer
Glycerol	10%	Enhancer and stabilizer
Trehalose	200 mM	Enhancer
BSA	0.4 mg/mL	Enhancer and stabilizer
Detergents	0.26%	Stabilizer
dNTPs (each)	600 µM	Required for strand elongation

PROTOCOL

Thaw the 2X PCR Master Mix at room temperature. Vortex the Master Mix gently, and spin it briefly in a microcentrifuge to collect the material in the bottom of the tube.

Reaction Set Up: Assemble reaction components on ice and quickly transfer the reactions to a thermocycler preheated to the denaturation temperature (95 °C). The recommended reaction volume is 25 µl. Reaction volumes between 10 and 50 µl may be used; scale up/down the reaction components accordingly. Final concentration of the Master Mix in the reaction should be 1X.

COMPONENT	25 µL REACTION VOLUME	
10 µM Forward Primer	0.5 µL	
10 µM Reverse Primer	0.5 µL	
Template DNA	1 ng - 1 µg	Genomic
	0.5 pg - 5 ng	Plasmid / Viral
	1 ng - 100 ng	cDNA
2X Master Mix	12.5 µL	
Nuclease-Free Water	Bring up to 25 µL	

THERMOCYCLING CONDITIONS

Three-Step PCR

STEP	TEMP.	TIME
Initial denaturation	95 °C	30 s - 2 min
Denature	95 °C	15 - 30 s
Anneal	45 - 68 °C	15 - 30 s
Extend	68 °C	1 min/kb
Final Extension	68 °C	5 min
Hold	4 °C	

● Cycle 30 - 40x

Two-Step PCR

When primer annealing temperatures are above 60 °C, a two-step PCR can be used:

STEP	TEMP.	TIME
Initial denaturation	95 °C	1 - 2 min
Denature	95 °C	15 - 30 s
Anneal/Extension	60 - 68 °C	1 min/kb
Final Extension	68 °C	5 min
Hold	4 °C	

● Cycle 30 - 40x

QUALITY CONTROL ASSAYS

Efficiencies of greater than 95% have been obtained using Chai's 2X PCR Master Mix.

Efficiency and R^2 were determined using a five-point Standard Curve qPCR assay with lambda phage genomic DNA as template and Chai Green dye.

Efficiency and R^2 were determined by using ten-fold serial dilutions of GAPDH/HPRT gene fragments with FAM and HEX labeled probes in both singleplex and duplex reactions.

Absence of endonuclease activity was determined by overnight incubation of *E. coli* amplified DNA with 25 U Taq polymerase, in 1X reaction buffer at 37 °C, and monitored for little or no decrease in original amount of amplicon when resolved by gel electrophoresis.