Protocol

1. Introduction

Taq DNA polymerase is a heat-stable enzyme and synthesizes DNA under suitable conditions from single-stranded templates in the presence of specific primers and dNTPs. Our product Taq DNA Polymerase is a recombinant enzyme with the molecular weight of approximately 94kd.

Taq DNA Polymerase possesses the capability of 5'→3' DNA polymerase and weak 5'→3' exonuclease, yet, no potential of 3'→5' exonuclease which means there will appear a dA overhang at the 3' end. Using the characteristic, it can be utilized in adding dA to 3' end of blunt-end for TA cloning. In PCR reaction, elongation rate of Taq DNA Polymerase is generally 1 kb/min for most templates, for gene with high complexity it may need 1-2kb/min.

This Master Mix contains dye which means it can be directly electrophoresed after the amplification without the need to add a loading buffer.

If the templates possess more secondary structure, higher GC content or **amplicons longer than 5 kb**, you may require more optimization, or you can turn to our product **hyPerFUsion™ high-fidelity DNA polymerase** (**Cat. K1032**). If needed to subject the PCR product to agarose gel electrophoresis, our product **SYBR Safe DNA Gel Stain (Cat: A8743)** is available.

2. Setup PCR Reaction

Prepare your PCR experiment as the following table, or employ your own parameters.

Thaw completely, mix and briefly centrifuge each component before use. We recommend assembling all reaction components on ice.

Component	20-µL Reaction	50-µL Reaction	Final Concentration
ddH2O	Add to 20 µL	Add to 50 µL	/
10 µM forward primer	0.8 µL	2 µL	0.4 µM
10 µM reverse primer	0.8 µL	2µL	0.4 µM
Template DNA	varies	varies	1–500 ng
2X Taq PCR Master Mix(with dye)	10 µL	25 µL	1X

Note:

a. The template can be cDNA, gDNA or λDNA.

b. The final concentration of Taq DNA polymerase is 1 U/50 µl reaction system.

Mix and briefly centrifuge the reaction tube. Quickly transfer to the thermocycler after the reactions set.

3. Cycling Conditions

Incubate reactions in a thermal cycler.

	Temperature	Time	Cycles
Initial denaturation	94℃	3min	1
Denaturation	94℃	30 sec	25-35 (variable) cycles
Annealing	~55℃(Depending on primer Tm)	30 sec	
Extension	72℃	1 min/kb	
Final extension	72℃	5min	1
Hold	4℃	+∞	1

Note:

a. You can take an annealing temperature subtracting 3-5 degrees from the calculated Tm(The lower one of two Tm).

When the primer is shorter than 20bp, Tm (℃)= 4 (G + C)+ 2 (A + T).

When the primer is longer than 20bp, Tm (℃)= 81.5+0.41*(% of GC) − (675/length)

You could utilize a temperature gradient to determine the annealing temperature alternatively.

For some highly complex genomic DNA or cDNA templates, extension time can be increased to 90 seconds per kb.

4. Electrophoresis

This Master Mix contains dye which can be directly electrophoresed after the end of PCR without the need to add a loading buffer. If you need to subject the PCR product to agarose gel electrophoresis, our product **SYBR Safe DNA Gel Stain (Cat: A8743)** is available. You can use the PCR product immediately for down-stream applications, or store it at -20°C.

5. Note

5.1. PCR reactions should be assembled in a DNA-free environment. Use of "clean" dedicated automatic pipettors and aerosol resistant barrier tips are recommended. Always keep the positive/negative control DNA and other templates to be amplified isolated from the other components. Microcentrifuge tubes used should be nuclease-free.

5.2. Oligonucleotide primers utilized are generally 20–40 nucleotides in length and ideally have a GC content about 40–60%. The final concentration of each primer in a reaction may be 0.05–1 µM, typically 0.1–0.5 µM.

5.3. Amplification of some difficult targets, like GC-rich sequences, may be improved with some additives, such as DMSO or formamide.

5.4. For difficult templates such as GC-rich sequences, a longer initial denaturation such as 5 min at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minute denaturation at 95°C is recommended.

5.5. The annealing time could range between 15–60 seconds. Annealing temperature is based on the Tm of the primer pair and is typically 45–72°C. Annealing temperatures can be optimized by doing a temperature gradient PCR, for example, starting 5°C below the calculated Tm.

5.6. The recommended extension temperature is 72°C. Extension times are generally 1 minute per kb for most template.

5.7. Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets. Be careful that too much cycles will lead to nonspecific bands.

5.8. When primers are with annealing temperatures above 72°C, a 2-step protocol is recommended.

	Temperature	**Time**	**Cycles**
Initial denaturation	94℃	3min	1
Denaturation	94℃	30 s	variable
Extension	72℃	1 min/kb	
Final extension	72℃	5min	1
Hold	4℃	+∞	1

5.9. The PCR products generated by Taq DNA Polymerase contain dA overhangs at the 3´end; therefore the PCR products can be ligated to dT/dU-overhang vectors.