2x PaCeR™ HP™ Master Mix

Catalog # PCR002

GeneBio Systems, Inc.™

Introduction

PaCeR™ HP™ DNA Polymerase is a superior performance, highly flexible enzyme for robust PCR with extreme fidelity, featuring 53x error rate lower than *Taq* polymerase and extension rate as fast as 1 sec / kb*. PaCeR™ HP™ DNA Polymerase possesses 5´→ 3´polymerase activity, 3´→5´exonuclease activity and will generate blunt-ended products. This product is also capable of amplifying long fragments such as 20 kb genomic DNA and 40 kb λDNA. Our suggested applications include essentially all your PCR applications, including cloning, sequencing, genomic DNA amplification and high throughput PCR, etc. It is ideal for use as all-round, versatile, one-for-all enzyme for a busy and productive PCR lab. In a convenient 2 x Master Mix format, this reagent makes it easy to set up PCR reactions.

** The extension rate varies with individual application.*

Advantages

Robust: High probability of PCR success with minimal optimization.

Hot Start: The enzyme has an inherent hot start capability

Extreme Fidelity: 53× error rate lower than that of *Taq*, 6 x lower than that of *Pfu*

High Yield: Increased product yield compared to those using regular PCR reagents.

Versatile: Recommended for routine PCR, or those with long, and/or difficult templates or direct PCR

Package Information

Components	PCR-002	PCR-002	PCR-002
2× PaCeR™ HP™ Master Mix	1 ml	5 ml	15 ml

All materials should be stored at -20 ℃.

Protocol

Recommended reactions setup:

Components	25µL Reaction	50µLReaction
2xPaCeR™ HP™ Master Mix	12.5 µL	25 µL
DNA template (100 ng / µl)	Variable	Variable
Upstream primer (10 µM)	1 µL	2 µL
Downstream primer (10 µM))	1 µL	2 µL
Distilled water (dH_2O)	To 25 µL	To 50 µL

** Do not use dNTP mix or primers that contain dUTP or dITP.*

1. Add the components into nuclease-free PCR tubes, mix thoroughly and gently.
2. Place PCR tubes to a PCR thermal cycler.

3. Suggested cycling parameters for using PaCeR™ HP™ DNA Polymerase are provided below. Perform PCR reaction using optimized cycling conditions. Analyze PCR amplification products on a 0.7–1.0% (w/ v) agarose gel. Real-time PCR can also be performed by adding a fluorescent dye such as SYBR Green.

Cycling parameters

Segment	Number of cycles	3-step protocol		2-step protocol	
		Temperature ℃	Duration	Temperature ℃	Duration
Initial	1	95	3 min (30 sec)[a]	95	3 min (30 sec)[a]
PCR	25-35[b]	95	15 sec	95	15 sec
		55-65 [c]	15 sec	72	15-30 sec/ kb
		72	15-30 sec/ kb		
Final	1	72	5 min	72	5 min
Hold	1	4	∞	4	∞

Notes:

a. This mix contains a hot-start DNA polymerase, thus the pre-denaturation activation condition should be set to 95 ℃ for 3 minutes (for genomic DNA and cDNA) or 30sec (for plasmid DNA and virus DNA) to thoroughly activate the enzyme.

b. Optimized cycling parameters mat be adapted according to your thermal cyclers, reaction volumes and reaction tubes/plates. Consult the instrument manufacturer's recommendations if further optimization of cycling parameters is required.

c. The annealing temperature should be chosen based on the Tm values of the primers.

d. For primers with annealing temperatures ≥ 72℃, a 2-step protocol is recommended.

Trouble Shooting

No product at all or low yield

1. High quality or purified DNA templates are preferred to enhance the success of PCR.
2. Use fresh high purity dNTPs that have not undergone several freeze-thaw cycles.
3. Template DNA may be damaged. Use carefully purified template and make sure template is not fragmented.
4. Make reaction conditions more conducive to amplification: a) Use more templates; b) Increase extension time; c) Increase the number of cycles; d) Lower annealing temperature, e) Optimize enzyme concentration; f) Optimize the denaturation time;

   Please note: If you are getting a low yield or even no amplification with PaCeR, please consider lowering the annealing temperature by 3-5 °C and/or increasing the annealing temperature (doubling). Talk to our Tech Support as you need.;
5. Check primer design to ensure tendency to primer dimer formation is minimized. Ensure sufficient purity and concentration of the primers.

Non-specific products - High molecular weight smears

Consider making reaction conditions more stringent by experimenting with one or more of the recommendations:

a). ease enzyme concentration; b) decrease extension time; c) reduce the total number of cycles, d) increase annealing temperature or try 2-step protocol; e) increase denaturation temperature; or f) decrease primer concentration.

Non-specific products - Low molecular weight discrete bands

Consider making reaction conditions more stringent by experimenting with one or more of the recommendations:

a). decrease enzyme concentration; b) decrease extension time; c) reduce the total number of cycles, d) increase annealing temperature or try 2-step protocol; e) increase denaturation temperature; or f) decrease primer concentration.