PaCeR™ HP™ DNA Polymerase

Catalog # PCR001

GeneBio Systems, Inc.™

Introduction

PaCeR™ High Performance (HP)™ DNA Polymerase is a new generation engineered enzyme derived from Pfu DNA Polymerase for robust PCR with extreme fidelity. High amplification efficiency and template adaptability makes PaCeR™ HP™ suitable for almost all PCR reactions. Ideal for use as an one-for-all, all-round versatile polymerase for a busy PCR lab.

The unique extension factor, specificity-promoting factors and plateau-inhibiting factor in PaCeR™ HP™ greatly improve its long-fragment amplification ability, specificity and yield. PaCeR™ HP™ is capable of amplifying long fragments up to 40 kb λ DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of PaCeR™ HP™ is 53-fold lower than that of Taq and 6-fold lower than that of Pfu. In addition, PaCeR™ HP™ has a good resistance to PCR inhibitors and can be used for direct PCR amplifications with bacteria, fungi, plants, animal tissues, and even whole blood samples. PaCeR™ HP™ contains two monoclonal antibodies inhibiting the 5′→3′ polymerase activity and 3′→5′ exonuclease activity at room temperature. Super fidelity and supreme amplification efficiency make PaCeR™ HP™ the best choice for high fidelity PCR. Amplification will generate blunt-ended products, which are compatible with all our Blunt end Cloning Kits (See the Blunt End Cloning category).

Package Information

Components	PCR-001 100 rxn (50 µl/rxn)	PCR-001 500 rxn (50 µl/rxn)	PCR-001 1,000 rxn (50 µl/rxn)
PaCeR™ HP™ DNA Polymerase (1 U/µl)	100 µl	PCR-001 × 5	PCR-001 × 10
2× PaCeR™ HP™ Buffer	2 × 1.25 ml		
dNTP Mix (10 mM each)	100 µl		

Please store all components at -20℃.

Please note: Sample Packs do not contain dNTP or Loading Buffer

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble products in 30 minutes at 74℃. with activated salmon sperm DNA as the template / primer.

Recommended PCR System

2× PaCeR™ HP™Buffer[a]	12.5 µl	25 µl
dNTP Mix (10 mM each)[b]	0.5 µl	1 µl
Template DNA [c]	x µl	x µl
Primer 1 (10 µM)	1 µl	2 µl
Primer 2 (10 µM)	1 µl	2 µl
PaCeR™ HP™ DNA Polymerase (1 U/µl)[d] ddH_2O	0.5 µl To 25 µl total reaction	1 µl To 50 µl total reaction

a. 2× PaCeR™ HP™ Buffer contains Mg^{2+}. The final concentration of Mg^{2+} is 2 mM.

b. DO NOT use dUTP. DO NOT use any primers or templates that contains Uracil.

c. Optimal reaction concentration varies from templates and is usually as follows (per 50 µl): genomic DNA, 50 - 400 ng; plasmid or virus DNA, 10 pg - 30 ng; cDNA, 1 - 5 µl.

d. The recommended final concentration of PaCeR™ HP™ is 1 U/50 µl, which can be optimized between 0.5 U/50 µl and 2 U/50 µl for each target, if necessary. The PaCeR™ HP™ DNA Polymerase has strong proofreading activity. For TA cloning, the PCR products must be A-tailed.purified before dA-Tailing.

Recommended PCR Programs

Steps	Temperature	Time	Cycles
Pre-denaturation [a]	95℃	30 sec / 3 min	1
Denaturation	95℃	15 sec	25 - 35
Annealing [b]	56℃ - 72℃	15 sec	
Extension [c]	72℃	30 - 60 sec / kb	
Final Extension	72℃	5 min	1

a. For pre-denaturation, the recommended temperature is 95℃, and the recommended time is 30 sec for plasmid / virus DNA and 3 min for genomic DNA / cDNA.

b. For annealing, the recommended temperature is the T_m of the primers. If the T_m of the primers is higher than 72℃, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized in a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature in 3℃ increment is helpful to improve poor amplification specificity.

c. Longer extension time is helpful to increase the amplification yield.

**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. **

For Long-fragment PCR

* Use long primers whenever possible; use high-quality templates and improve the template usage;

* When the recommended PCR program does not work, try the Touch Down Two-step PCR as follows:

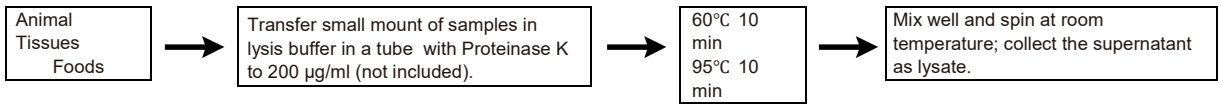
Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	1
Denaturation	95℃	15 sec	5
Extension	74℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	72℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	70℃	60 sec / kb	
Denaturation	95℃	15 sec	25
Extension	68℃	60 sec / kb	
Final Extension	68℃	5 min	1

For PCR using Crude Material as Template

PaCeR™ HP™ has a good resistance to PCR inhibitors and can be used for direct PCR amplifications with bacteria, fungi, plants, animal tissues, and even whole blood samples. Crude materials that have been successfully amplified using PaCeR™ HP™ are as follows:

Sample Type	Amplification Method	Template Recommendation (50 µl PCR)
Whole Blood	Direct PCR	1-5 µl
Filter Paper Dry Blood	Direct PCR	1-2 mm^2 filter paper
Cultured Cells	Direct PCR	Scrap by a pipette tip or 1 µl suspension
Yeast	Direct PCR	Colony touched by a pipette tip or 1 µl suspension
Bacteria	Direct PCR	Colony touched by a pipette tip or 1 µl suspension
Fungi	Direct PCR	Scrap by a pipette tip or 1 µl suspension
Sperm	Direct PCR	Scrap by a pipette tip or 1 µl suspension
Plankton	Direct PCR	Scrap by a pipette tip or 1 µl suspension
Plant Tissue	Direct PCR	1-2 mm^2 tissue
Mouse Tail	PCR with lysate	1-5 µl lysate*
Food	PCR with lysate	1-5 µl lysate*

* Recommended Lysate Preparation protocol:



Note: Lysis Buffer composition is: 20 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 0.1% SDS, pH 8.0 (not included).

Primers Designing Notes

1. Whenever possible, choose C or G as the last base of the 3'-end of the primer;

2. Avoid more than 1 mismatch at the last 8 bases of the 3'-end of the primer;

3. Avoid hairpin structure at the 3'-end of the primer;

4. T_m of the primers should be within the range of 55℃ - 65℃;

5. Additional tag sequence non-homologous to target should not be included when calculating Tm of the primers;

6. GC content of the primers should be within the range of 40% - 60%;

7. T_m and GC content of forward and reverse primers should be as similar to each other as possible.