PfuUltra II Fusion HS DNA Polymerase

Catalog #600670, 600672, and 600674

For Research Use Only. Not for use in diagnostic procedures.

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MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #600670	**Catalog #600672**	**Catalog #600674**
PfuUltra II Fusion HS DNA Polymerase	40 reactions	200 reactions	400 reactions
10× *PfuUltra* II Reaction Buffer	1 ml	2 × 1 ml	4 × 1 ml

Storage: Store at –20°C upon receipt.

INTRODUCTION

In Agilent's *PfuUltra* II fusion HS DNA polymerase, we couple the fusion polymerase technology with our engineered *PfuUltra* DNA polymerase, hotstart antibody, and proprietary ArchaeMaxx PCR enhancing factor to achieve extreme accuracy, high specificity, and long target-length capability while dramatically reducing overall PCR extension times.

OPTIMIZATION PARAMETERS (50 µL REACTION VOLUME)

Parameter	Targets: ≤10 kb (vector or genomic DNA)	Targets: >10 kb (vector or genomic DNA)	cDNA Targets
Extension time	15 seconds for targets ≤1 kb; 15 seconds per kb for targets >1 kb	30 seconds per kb	30 seconds for targets ≤1 kb; 30 seconds per kb for targets >1 kb
PfuUltra II fusion HS DNA polymerase	1 µl	1 µl	1 µl
Input template	100 ng genomic DNA; 5–30 ng vector DNA	200–250 ng genomic DNA; 5–30 ng vector DNA	1–2 µl cDNA from RT-PCR reaction (50–500 ng starting total RNA template)
Primers (each)	0.2 µM each primer	0.4 µM each primer	0.2 µM each primer
dNTP concentration	250 µM each dNTP (1 mM total)	500 µM each dNTP (2 mM total)	250 µM each dNTP (1 mM total)
Final reaction buffer conc	1.0×	1.0×	1.0×
Denaturing temperature	95°C	92°C	95°C
Extension temperature	72°C	68°C	72°C

PCR PROTOCOL

The reaction conditions given here are for amplification of a typical single-copy chromosomal target of ≤10 kb. See the Optimization Parameters section for guidelines on amplifying longer targets. The reaction conditions are for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 50 µl. Add the components in order into sterile thin-walled PCR tubes while mixing gently.

Reaction Mixture for a Typical Single-Copy Chromosomal Locus PCR Amplification (≤10 kb)

Component	Amount per reaction
Distilled water (dH_2O)	40.5 µl
10× *PfuUltra* II reaction buffer[a]	5.0 µl
dNTP mix (25 mM each dNTP)	0.5 µl
DNA template (100 ng/µl)	1.0 µl[b]
Primer #1 (10 µM)	1.0 µl
Primer #2 (10 µM)	1.0 µl
PfuUltra II fusion HS DNA polymerase	1.0 µl
Total reaction volume	50 µl

[a] The 10× buffer provides a final 1× Mg^{2+} concentration of 2 mM.

[b] The amount of DNA template required varies depending on the type of DNA being amplified. Generally 100 ng of genomic DNA template is recommended. Less DNA template should be used for amplification of lambda or vector (5–30 ng) PCR targets.

Perform PCR using optimized cycling conditions. Suggested cycling parameters are indicated in the table below. The PCR cycling parameters have been tested on the following: the MJ Research DNA Engine PTC-200, the Applied Biosystems GeneAmp PCR system 9700, the Applied Biosystems GeneAmp PCR system 9600, and the Agilent Mx3000P QPCR system. Optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers. Analyze the PCR amplification products on a 0.7-1.0% (w/v) agarose gel.

PCR Cycling Parameters for *PfuUltra* II fusion HS DNA Polymerase[a]

Targets ≤10 kb (vector DNA, genomic DNA, and cDNA)

Segment	Number of cycles	Temperature	Duration (vector or genomic DNA)	Duration (cDNA)
1	1	95°C[b]	2 minutes	1 minute
2	30 cycles for vector or genomic DNA; 40 cycles for cDNA	95°C	20 seconds	20 seconds
		Primer T_m – 5°C[c]	20 seconds	20 seconds
		72°C	15 seconds for targets ≤1 kb 15 seconds per kb for targets >1 kb	30 seconds for targets ≤1 kb 30 seconds per kb for targets >1 kb
3	1	72°C	3 minutes	3 minutes

Targets >10 kb (vector or genomic DNA)

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	30	92°C	10 seconds
		Primer T_m – 5°C[c]	20 seconds
		68°C	30 seconds per kb
3	1	68°C	5 minutes

[a] Thin-wall PCR tubes are highly recommended. These PCR tubes are optimized to ensure more efficient heat transfer and to maximize thermal-cycling performance.

[b] Denaturing temperatures above 95°C are recommended only for GC-rich templates.

[c] The annealing temperature may require optimization. Typically annealing temperatures will range between 55° and 72°C.

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