PCR

Fast endpoint PCR protocol using Phusion Flash High-Fidelity PCR Master Mix

Introduction

The polymerase chain reaction (PCR) amplifies DNA from minimal samples, facilitating its detection, analysis, and manipulation. This technique is crucial for genotyping, cloning, pathogen detection, gene expression studies, and DNA sequencing. However, PCR faces challenges such as extended cycling times and errors introduced by Taq polymerase, that lead to reduced fidelity.

Thermo Scientific™ Phusion™ Flash High-Fidelity PCR Master Mix overcomes these issues by delivering results more than twice as rapidly as Taq polymerases while maintaining high fidelity and yield across various target lengths. This study highlights the effectiveness of Phusion Flash High-Fidelity PCR Master Mix in fast PCR applications.

Important notes:

- Phusion Flash DNA Polymerase has fidelity 25 times that of *Taq* polymerases, maintaining sequence accuracy during amplification.
- To calculate the correct primer annealing temperature, use the T_m calculator at **thermofisher.com/tmcalculator**.
- The thermal cycler ramp rate can be adjusted for fast cycling protocols to minimize ramp up times.
- Higher percentage Invitrogen™ E-Gel™ agarose gels are recommended for small fragment analysis.
- Samples must be diluted prior to loading on E-Gel agarose gels.

Materials and methods

DNA amplification

- Thermo Scientific™ Phusion™ Flash High-Fidelity PCR Master Mix (Cat. No. F548S))
- Invitrogen™ Nuclease-Free Water (not DEPC-Treated) (Cat. No. AM9938)
- Forward and reverse PCR primers, constructed following **PCR primer design guidelines**.
- Template DNA: pBR322 plasmid DNA, *E. coli* genomic DNA, and human genomic DNA
- Applied Biosystems™ ProFlex™ PCR System, 3 x 32-well (Cat. No. 4484073), with the ramp rate set at 6°C/sec

Amplicon analysis

- Invitrogen™ E-Gel™ Sample Loading Buffer, 1X (Cat. No. 10482055)
- Invitrogen™ E-Gel™ 1 Kb Plus Express DNA Ladder (Cat. No. 10488091)
- Invitrogen™ E-Gel™ EX Agarose Gels, 2% (Cat. No. G401002) for shorter amplicons (up to 0.5 kb)
- Invitrogen™ E-Gel™ EX Agarose Gels, 1% (Cat. No. G401001) for amplicons from 1 kb to 4 kb
- Invitrogen™ E-Gel™ Power Snap Plus Electrophoresis System (Cat. No. G9101)

Table 1. Composition of final PCR mixture.

Component	Amount	Final concentration
2X Phusion Flash High-Fidelity PCR Master Mix	10 µL	1X
10 µM forward primer	1 µL	0.5 µM
10 µM reverse primer	1 µL	0.5 µM
Template DNA*	Varies	Varies
Nuclease-free water	Add to a final volume of 20 µL	–

* 50 ng human genomic DNA, 5 ng *E. coli* genomic DNA, or 500 pg plasmid DNA per 20 µL PCR reaction.

Table 2. Fast cycling protocol using Phusion Flash High-Fidelity PCR Master Mix.

Step	Temperature	Time	Cycles
Initial denaturation	98°C	10 sec	1
Denaturation	98°C	1 sec	30
Annealing*	X°C	5 sec	
Extension	72°C	4 sec/kb for amplicons <1 kb; 8 sec/kb for amplicons ≥1 kb	
Final extension	72°C	1 min	1
Hold	4°C	–	–

* The optimal annealing temperature for Phusion DNA polymerases may differ significantly from that of *Taq*-based polymerases. For optimal results, calculate primer T_m with the T_m calculator at **thermofisher.com/tmcalculator**.

Results

Phusion Flash High-Fidelity PCR Master Mix was used to generate amplicons of varying lengths (100 bp, 250 bp, 500 bp, 1 kb, 2 kb, and 4 kb) from different DNA samples. Amplification was performed using a fast cycling protocol in which extension time was reduced compared to the standard cycling protocol. PCR products analyzed on

E-Gel EX Agarose Gels revealed that all six target lengths were successfully amplified (Figure 1). The yields achieved with the fast cycling protocol were comparable to those of the standard protocol (not shown).

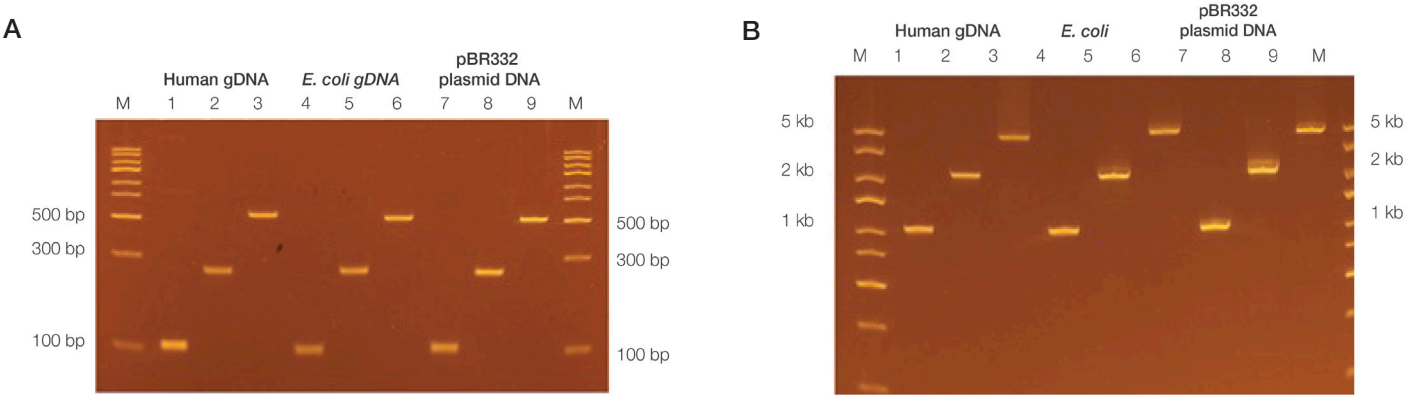

Figure 1. PCR products generated using fast PCR cycling conditions and Phusion Flash High-Fidelity PCR Master Mix. All fragments were amplified from human genomic DNA, *E. coli* genomic DNA, or pBR322 plasmid DNA using fast PCR cycling conditions. **(A)** Short amplicons (100 bp, 250 bp, and 500 bp) were analyzed on a 2% E-Gel EX Agarose Gel. **(B)** Longer amplicons (1 kb, 2 kb, and 4 kb) were analyzed on a 1% E-Gel EX Agarose Gel. Lane M: E-Gel 1 Kb Plus Express DNA Ladder.

Summary

This study illustrates that Phusion Flash High-Fidelity PCR Master Mix is highly effective for rapid endpoint PCR amplification. Results can be obtained in as little as 21 min depending on amplicon size.

Ordering information

Description	Quantity	Cat. No.
DNA amplification (PCR)		
Phusion Flash High-Fidelity PCR Master Mix	100 reactions	F548S
Nuclease-Free Water (not DEPC-Treated)	100 mL	AM9938
ProFlex PCR System, 3 x 32-well	Each	4484073
Agarose gel electrophoresis		
E-Gel Sample Loading Buffer, 1X	4 x 1.25 mL	10482055
E-Gel 1 Kb Plus Express DNA Ladder	2 x 1.25 mL	10488091
E-Gel EX Agarose Gels, 2%	10 gels/pk	G401002
E-Gel EX Agarose Gels, 1%	10 gels/pk	G401001
E-Gel Power Snap Plus Electrophoresis System	Each	G9301

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