PCR

Fast endpoint PCR amplification protocol using Phire Hot-Start II DNA Polymerase

Introduction

The polymerase chain reaction (PCR) is one of the most widely used technologies in molecular biology and has become essential for a variety of applications, including genotyping, cloning, and detection of pathogens. Its reliability and simplicity make it an indispensable tool.

To reduce turnaround time and improve workflows, faster PCR protocols are required. Thermo Scientific™ Phire™ Hot Start II DNA Polymerase addresses this need by producing results more than twice as fast as other *Taq* polymerases. Phire Hot Start II DNA Polymerase also features Affibody™-mediated hot start to increase specificity. This study demonstrates how Phire Hot Start II DNA Polymerase can be used effectively for fast PCR applications.

Important notes:

- 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™ Phire™ Hot Start II DNA Polymerase (Cat. No. F122S)
- 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
- Invitrogen™ dNTP Mix (10 mM each) (Cat. No. 18427088)

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 reaction mixture.

Component	Final concentration
5X Phire Reaction Buffer	1X
10 mM dNTP mix	200 µM each
10 µM forward primer	0.5 µM
10 µM reverse primer	0.5 µM
Template DNA*	Varies
Phire Hot Start II DNA Polymerase	0.4 µL
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.

Step	Temperature	Time	Cycles
Initial denaturation	98°C	30 sec	1
Denaturation	98°C	5 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 Phire Hot-Start II DNA Polymerase may differ significantly from that for *Taq*-based polymerases. For optimal results, calculate primer T_m with the T_m calculator at **thermofisher.com/tmcalculator**

Results

Phire Hot Start II DNA Polymerase 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 were analyzed on E-Gel EX Agarose Gels, which revealed that all six target lengths were successfully amplified (Figure 1). The yield achieved with the fast-cycling protocol was comparable to that of the standard protocol (not shown).

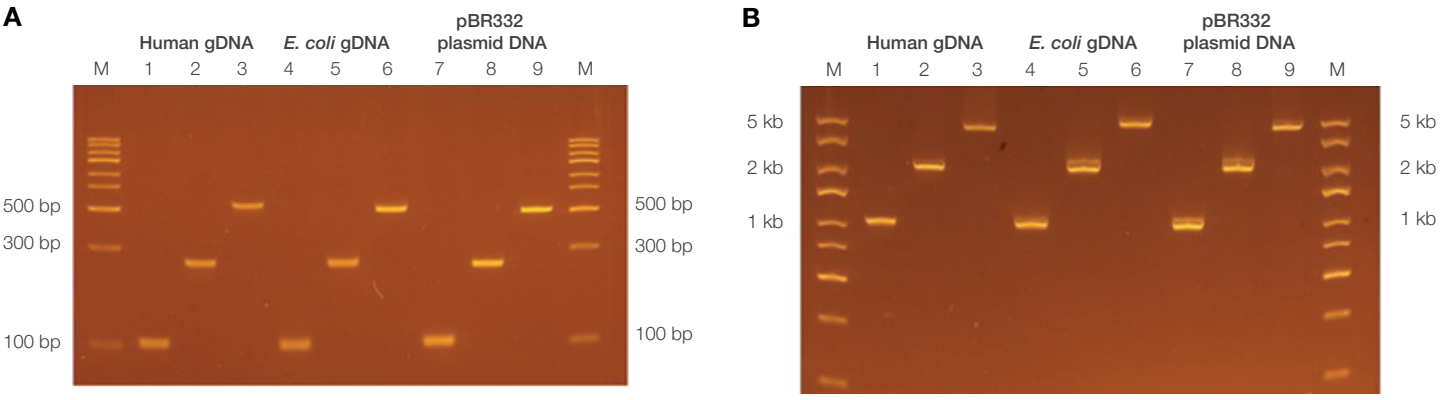

Figure 1. PCR products generated using fast PCR cycling conditions and Phire Hot Start II DNA Polymerase. All fragments were amplified from human genomic DNA, *E. coli* genomic DNA, or pBR322 plasmid DNA using fast PCR cycling conditions. **(A)** Short amplicons (100 bp, 250 bp, and 500 bp) were analyzed 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 Phire Hot Start II DNA Polymerase 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)		
Phire Hot Start II DNA Polymerase (Colorless)	200 reactions	F122S
Phire Hot Start II DNA Polymerase (Green)	200 reactions	F124S
dNTP Mix (10 mM each)	1 mL	18427088
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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