PCR

Amplify DNA targets up to 4 kb in under 40 minutes with Platinum II *Taq* Hot-Start DNA Polymerase

Introduction

The polymerase chain reaction (PCR), a foundational molecular biology tool, is used for the amplification of DNA. PCR enables many molecular biology applications, including genotyping, pathogen detection, gene expression studies, and sequencing. Many standard PCR runs can take over an hour to complete. With the protocol and enzyme described here, amplification can be completed in 21 minutes for 100 bp and in 38 minutes for 4,000 bp.

In addition to producing results more than two times faster than by using other DNA polymerases, Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase has several exceptional features. Its innovative buffer allows for a universal annealing temperature for almost all primer pairs, which facilitates co-cycling of multiple PCR reactions in the same run. The premium hot-start technology allows for room temperature reaction setup and greater sensitivity than the standard technology. This study demonstrates how Platinum II *Taq* Hot-Start DNA Polymerase can be effectively used for fast PCR applications.

Important notes:

- Due to the composition of the Platinum II PCR buffer, the annealing temperature is 60°C for any primer pair constructed following general design rules.
- Due to the universal annealing temperature, multiple fragments can be co-cycled to save time.
- The thermal cycler ramp rate can be adjusted for fast cycling protocols to minimize ramp-up times.
- Higher percent 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

- Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase (Cat. No. 14966001)
- Invitrogen™ dNTP Mix (10 mM each) (Cat. No. 18427013)
- 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	20 µL reaction	Master mix for 10 samples + 1*	Final concentration
5X Platinum II PCR Buffer**	4 µL	44 µL	1X
10 mM dNTP mix	0.4 µL	4.4 µL	200 µM each
10 µM forward primer	0.4 µL	4.4 µL	0.2 µM
10 µM reverse primer	0.4 µL	4.4 µL	0.2 µM
Template DNA†	Varies	–	Varies
Platinum II *Taq* Hot-Start DNA Polymerase	0.16 µL	1.76 µL	0.04 U/µL
Nuclease-free water	Add to a final volume of 20 µL	Amount needed x 11 samples	-

* For easier pipetting, prepare the master mix with all components except the sample. Add enough reagent for one additional sample to allow for pipetting error.

** Provides 1.5 mM $MgCl_2$ in final reaction concentration.

† 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 Platinum II *Taq* Hot-Start DNA Polymerase.

Step	Temperature	Time	Cycles
Initial denaturation	94°C	2 min	1
Denaturation	94°C	5 sec	30
Annealing*	60°C	5 sec	
Extension	68°C	4 sec/kb for amplicons ≤1 kb; 8 sec/kb for amplicons >1 kb	
Hold	4°C	–	–

* 60°C was used for all primer pairs due to universal annealing temperature feature of Platinum II *Taq* Hot-Start DNA Polymerase.

Results

Platinum II *Taq* Hot-Start DNA Polymerase was used to generate amplicons of varying lengths from three different DNA samples. Target lengths were 100 bp, 250 bp, 500 bp, 1 kb, 2 kb, and 4 kb. Amplification was performed using a fast cycling protocol in which denaturation, annealing, and extension times were reduced compared to the standard cycling protocol. Analysis of the PCR products on agarose gels 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).

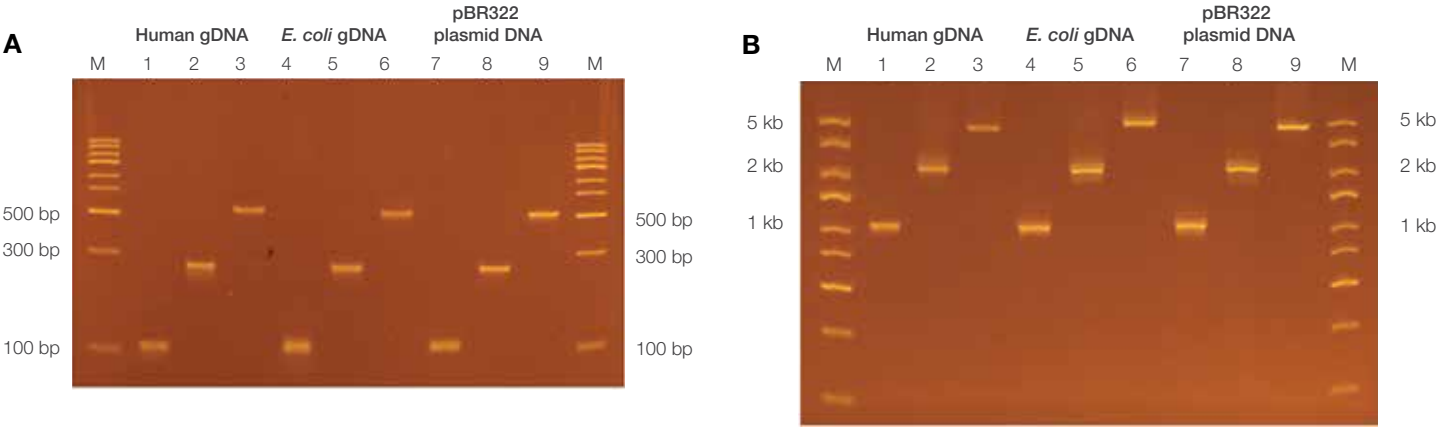

Figure 1. PCR products generated using fast PCR cycling conditions. All fragments were amplified from human genomic DNA, *E. coli* genomic DNA, or pBR322 plasmid DNA using fast PCR cycling conditions. **(A)** Short amplicons (100 bp, 250 bp, and 500 bp) were analyzed on a 2% E-Gel EX Agarose Gel. **(B)** 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 Platinum II *Taq* Hot-Start DNA Polymerase is highly effective for rapid endpoint PCR amplification. By implementing a new fast cycling protocol, the total PCR time can be significantly reduced, helping cut reaction time by more than one-third compared to standard cycling conditions (Figure 2).

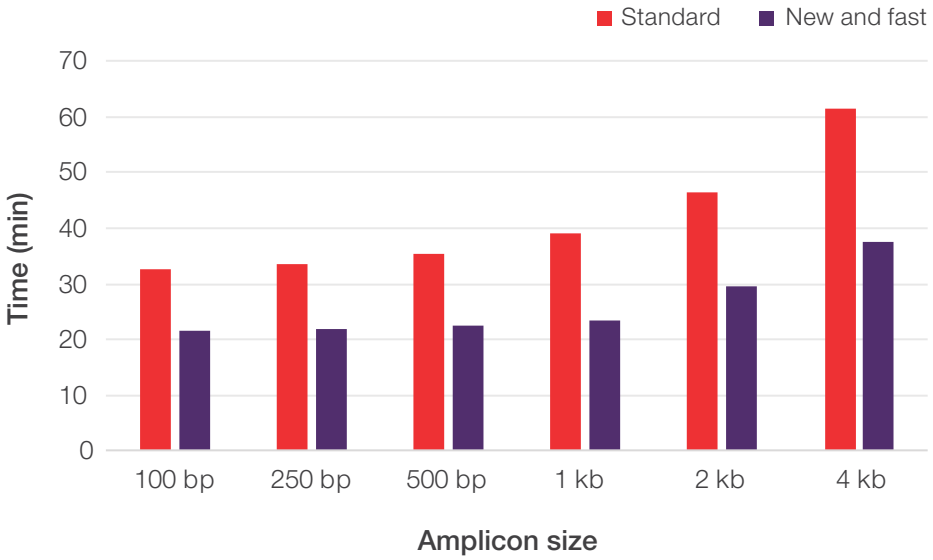

Figure 2. Cycling time comparison of standard protocol and the new fast protocol. Red bars: standard protocol time. Purple bars: fast protocol time. **Note:** Out of the total time, instrument ramping time (time needed for PCR cycler to change temperature between each step) takes 14.5 min for 30 cycles of PCR.

Ordering information

Description	Quantity	Cat. No.
DNA amplification (PCR)		
Platinum II *Taq* Hot-Start DNA Polymerase	100 reactions	14966001
Platinum II Hot-Start PCR Master Mix (2X) (colorless)	50 reactions	14000012
Platinum II Hot-Start Green PCR Master Mix (2X)	50 reactions	14001012
dNTP Mix (10 mM ea)	1 mL	18427088
Water, nuclease-free	100 mL	AM9938
ProFlex PCR System, 3 x 32-well	1 unit	4484073
Agarose gel electrophoresis		
E-Gel Sample Loading Buffer	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	1 unit	G9301

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