

Platinum II *Taq* Hot-Start DNA Polymerase for high-throughput PCR

Abstract

The advances in thermal cycler technology permit a substantial increase in PCR throughput and challenge the capabilities of PCR reagents to enable accelerated PCR workflows without compromising the quality of results. Here we describe Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase, designed to increase the throughput of PCR labs by eliminating optimization steps, supporting faster cycling, and enabling co-cycling of several assays in the same run while retaining superior specificity.

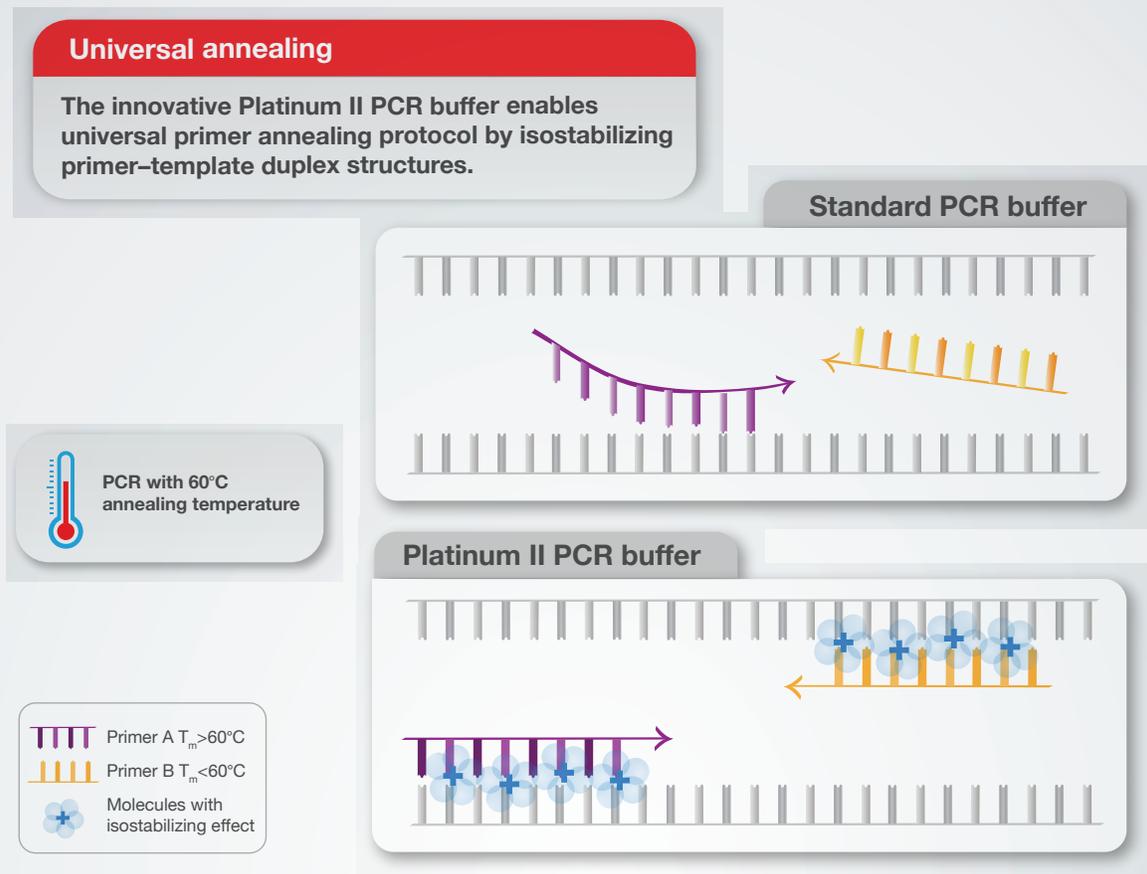
Introduction

When there is a need to quickly analyze a large number of samples using a number of different PCR assays, there are quite a few challenges to overcome. Optimal conditions for each assay have to be determined, and it is likely that they will not be the same. First, different assays often will require different annealing temperatures for their primers. In addition, different amplicons may be designed to be of different lengths and therefore require varying durations of the extension step. With traditional PCR reagents, these complexities might preclude running all of the assays together, due to incompatible cycling protocols. This necessitates sequential runs and causes delay in obtaining results.

Platinum II *Taq* Hot-Start DNA Polymerase is an innovative PCR reagent designed to address these challenges. This polymerase is an engineered *Taq* enzyme capable of faster DNA synthesis. Additionally, isostabilizing molecules in the Platinum II PCR buffer increase primer–template duplex stability during the annealing step and contribute to enhanced specificity. As a result, the need to optimize the annealing temperature for each primer pair is eliminated. Instead, different assays can be run using the same primer annealing temperature and the same elongation time for amplicons of different lengths. In this study, we tested Platinum II *Taq* Hot-Start DNA Polymerase for its ability to increase PCR throughput.



Universal annealing temperature using Platinum II PCR buffer



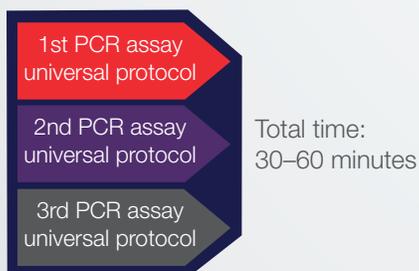
The top panel depicts a situation that often happens in a standard PCR buffer when primers have distinct melting temperatures (T_m). At an annealing temperature of 60°C, chosen as a trade-off between two different T_m s, primer A ($T_m > 60^\circ\text{C}$) binds to partially complementary unintended targets. The majority of primer B ($T_m < 60^\circ\text{C}$) is unbound and cannot participate in PCR. In such a situation, the specificity and yield of amplification is very low. The bottom panel depicts a different situation with the Platinum II PCR buffer. Even though primer A and primer B have different melting temperatures, the stabilizing molecules enable optimal and specific binding of both primers at 60°C and thus a successful PCR.

Time savings enabled by assay co-cycling

PCR assays with conventional DNA polymerase



PCR assays with Platinum II *Taq* polymerase



PCR assays using conventional PCR reagents require specific protocols for amplification of each DNA fragment because of the different primer annealing temperatures and extension steps. Therefore, with traditional PCR reagents, multiple targets often cannot be amplified together in the same PCR run. With Platinum II *Taq* Hot-Start DNA Polymerase, different PCR assays can be cycled together using one protocol with a universal primer annealing temperature and the extension step selected for the longest fragment to be amplified. Moreover, Platinum II *Taq* Hot-Start DNA Polymerase is a fast DNA polymerase, delivering PCR results in as little as 30 minutes.

- Invitrogen™ Platinum™ II Hot-Start PCR Master Mix (2X) (Cat. No. 14000012) or Platinum™ II Hot-Start Green PCR Master Mix (2X) (Cat. No. 14001012)
- Forward and reverse primers
- Invitrogen™ E-Gel™ General Purpose Agarose Gels or equivalent

starting with the guidelines below for reaction setup and thermal cycling (Tables 1–2).

When working with new PCR conditions, we recommend

Table 1. Reaction conditions for PCR.

Component	20 µL reaction	50 µL reaction	Final concentration
Platinum II Hot-Start PCR Master Mix (2X)*	10 µL	25 µL	1X
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Template DNA**	Varies	Varies	≤500 ng/reaction
Platinum GC Enhancer (optional)†	4 µL	10 µL	1X
Water, nuclease-free	To 20 µL	To 50 µL	–

* Provides 1.5 mM MgCl₂ in final reaction.

** 0.5–500 ng genomic DNA, 1 pg–50 ng plasmid or viral DNA, or 1–5 µL of cDNA synthesis reaction per 50 µL PCR reaction.

† Recommended for targets with >65% GC content.

Table 2. Cycling protocol.

PCR cycles	Step	3-step protocol		2-step protocol*	
		Temperature	Time	Temperature	Time
1	Initial denaturation	94°C	2 min	94°C	2 min
25–35	Denaturation	94°C	15 sec	98°C	5 sec
	Annealing**	60°C	15 sec	60°C	15 sec
	Extension†	68°C	15 sec/kb		
Hold		4°C	Indefinitely	4°C	Indefinitely

* The two-step protocol is recommended for simple amplicons up to 1 kb with 45–65% GC content.

** An annealing temperature of 60°C works for most primers. If amplification of a particular primer–template pair does not give satisfactory results, we recommend redesigning the primers.

† The extension step can be prolonged by up to 1 min per kb of amplicon length without a negative effect on specificity, which allows shorter and longer amplicons to be cycled together using the same protocol.

Primer recommendations

- Design 18- to 30-mers with 40–60% GC content.
- Aim for primer melting temperatures (T_m) between 55°C and 65°C.
- Avoid primer pairs with a difference of >10°C in melting temperature (T_m).
- Avoid self-complementary primers, and primer pairs that are complementary at their 3' ends.
- If possible, design the primers with one or two G or C bases at the 3' end.
- Verify primer complementarity to a single template region using programs for sequence alignment.

Results and discussion

Co-cycling of different assays with Platinum II *Taq* Hot-Start DNA Polymerase

Before defining protocol settings for a standard PCR, the annealing temperature for each primer pair has to be determined. Various calculation algorithms can be employed, though sometimes they provide conflicting values. In complicated cases, different annealing temperatures are tested experimentally or gradient PCR is performed. Each target also needs a specific extension time, as too-long extension of short targets can lead to nonspecific amplification. We sought to test if Platinum II *Taq* polymerase, with extension times longer than 15 sec/kb, would maintain faithful amplification of specific fragments in PCR. Four pairs of primers with annealing temperatures in the range of 52–59°C were used to amplify

four amplicons of different lengths. Reactions were cycled together in the same thermal cycler block, using the extension time required for the longest target. Platinum II *Taq* Hot-Start DNA Polymerase successfully amplified fragments from 132 bp to 3.9 kb, while suboptimal annealing temperatures and/or extension times resulted in nontarget amplicons when several other hot-start DNA polymerases were used (Figure 1). Strong and specific amplification results show that the isostabilizing molecules in Platinum II PCR buffer allow use of the same annealing temperature (60°C) as well as flexibility in the duration of the extension step. Therefore, in a PCR mix with Platinum II *Taq* Hot-Start DNA Polymerase, different targets of diverse lengths can be cycled together, increasing the throughput of the entire lab.

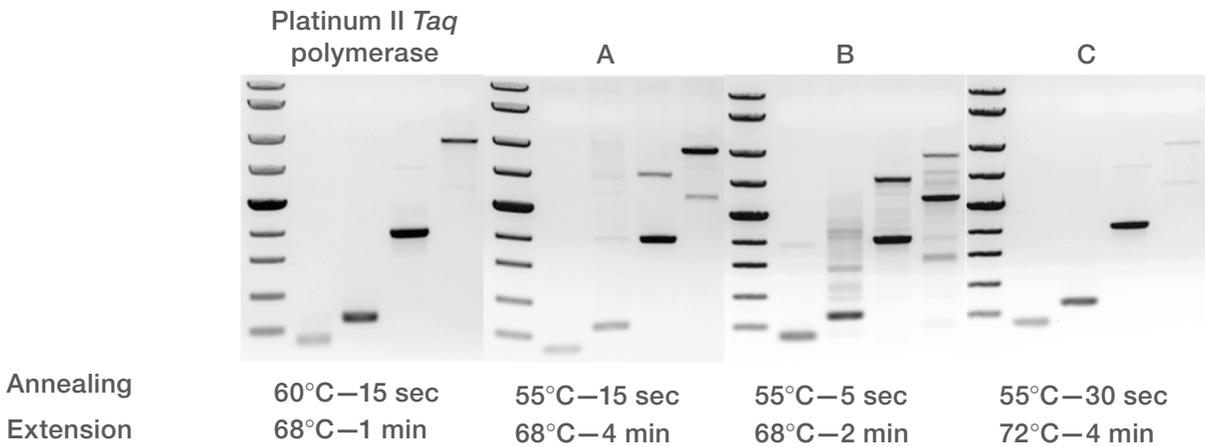


Figure 1. Platinum II *Taq* Hot-Start DNA Polymerase enables cycling of shorter and longer amplicons together. 132 bp, 251 bp, 1,005 bp, and 3.9 kb fragments were amplified from 50 ng of human genomic DNA in 50 μ L reactions using Platinum II *Taq* Hot-Start DNA Polymerase or other hot-start DNA polymerases: (A) NEB OneTaq™ Hot Start DNA Polymerase, (B) Qiagen Fast Cycling PCR Kit, (C) Roche FastStart™ *Taq* DNA Polymerase. The same protocol was used for all four targets with the annealing and extension settings indicated. The size marker is Thermo Scientific™ ZipRuler™ Express DNA Ladder 2.

Fast protocol for minimum cycling time

A standard *Taq* DNA polymerase requires 60 seconds to synthesize 1 kb of DNA, so a PCR run can take several hours to complete. Platinum II *Taq* Hot-Start DNA Polymerase is an engineered *Taq* polymerase with a higher synthesis rate of 15 sec/kb in the extension step. To compare PCR cycling times, we amplified a 529 bp fragment on the Applied Biosystems™ ProFlex™ PCR System with different DNA polymerases, and compared the total time to delivery of results. A PCR run with Platinum II *Taq* polymerase was completed in 36 minutes, while the reactions with most of the other DNA polymerases

required more than 1 hour to complete. Gel analysis revealed that Platinum II *Taq* polymerase produced a clean single band, while some other fast PCR enzymes had apparent nonspecific amplification (Figure 2). These results demonstrate that Platinum II *Taq* Hot-Start DNA Polymerase can amplify DNA fragments in a shorter time without sacrificing specificity.

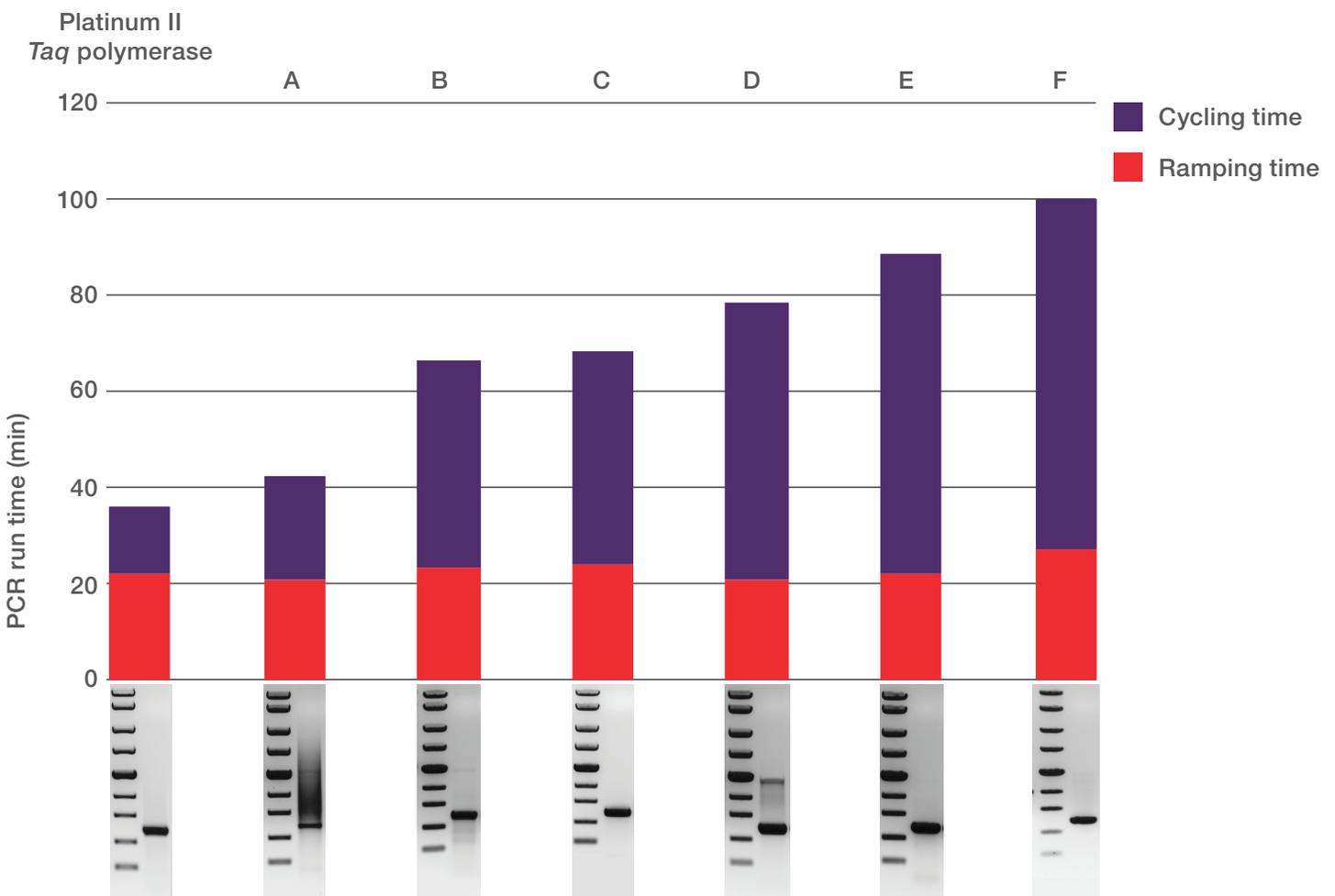


Figure 2. Fast cycling reduces PCR run time. Amplification of a 529 bp fragment from 50 ng of human genomic DNA in 50 μ L reactions for 35 cycles was carried out using Platinum II *Taq* Hot-Start DNA Polymerase and hot-start DNA polymerases from other suppliers: (A) Sigma-Aldrich KAPA2G™ Fast HotStart PCR Kit, (B) NEB OneTaq Hot Start DNA Polymerase, (C) Promega GoTaq™ G2 DNA Polymerase, (D) Toyobo Quick *Taq*™ HS DyeMix, (E) Roche FastStart *Taq* DNA Polymerase, and (F) Sigma-Aldrich JumpStart™ *Taq* DNA Polymerase. Cycling times for each polymerase are shown in purple, while ramping times on the ProFlex PCR System (6°C/sec peak block ramp rate) are shown in red. PCR product analysis in 1% TAE agarose gels is presented below the graph. The size marker is the ZipRuler Express DNA Ladder 2.

Reaction stability for high-throughput applications

Monoclonal antibodies built into Platinum II *Taq* Hot-Start DNA Polymerase prevent the extension of misprimed oligos at room temperature by reversibly inactivating the polymerase. The stringency of DNA polymerase inactivation and the stability of other components of the PCR mix determine if PCR reactions can be assembled and held at room temperature before PCR cycling. To test the benchtop stability of reaction mixes with Platinum II *Taq* polymerase, we compared the amplification of a 735 bp fragment from various amounts of human genomic DNA

in PCR reactions that were set up at room temperature and cycled either immediately or after 24 hours of storage on the bench. Results indicate that PCR reactions with Platinum II *Taq* Hot-Start DNA Polymerase can be held for 24 hours at room temperature prior to cycling, without any effect on PCR specificity and sensitivity (Figure 3).

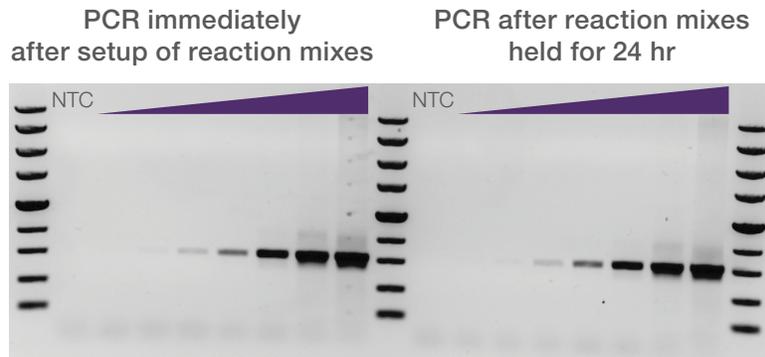


Figure 3. Stability of PCR reactions at room temperature. A 735 bp fragment was amplified from 0, 0.016, 0.08, 0.4, 2, 10, 50, and 250 ng of human genomic DNA using Platinum II *Taq* Hot-Start DNA Polymerase. PCR was performed either immediately after reaction setup or after 24 hr of holding the reaction mixes at room temperature.

Conclusion

Our findings demonstrate that Platinum II *Taq* polymerase is especially suitable for high-throughput PCR. Primers with different melting temperatures can specifically amplify targets of different lengths with the same PCR cycling protocol—more than 2 times faster than using conventional PCR enzymes. Furthermore, PCR assays can be set up

and held at room temperature for up to 24 hours. In summary, Platinum II *Taq* Hot-Start DNA Polymerase can help to increase PCR lab throughput by fast co-cycling of several assays in the same run.



Helpful tip

Are you looking to automate your high-throughput PCR?

Whether you've implemented an automated liquid handling system or may do so in the future, the Applied Biosystems™ Automated Thermal Cycler (ATC) is the right choice for automated PCR results. The Automated Thermal Cycler provides the reliability and performance you've come to expect from Applied Biosystems™ instruments, now in a small, easy-to-integrate, and compatible format.

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