# Platinum™ II *Taq* Hot-Start DNA Polymerase: PCR simplified with universal annealing

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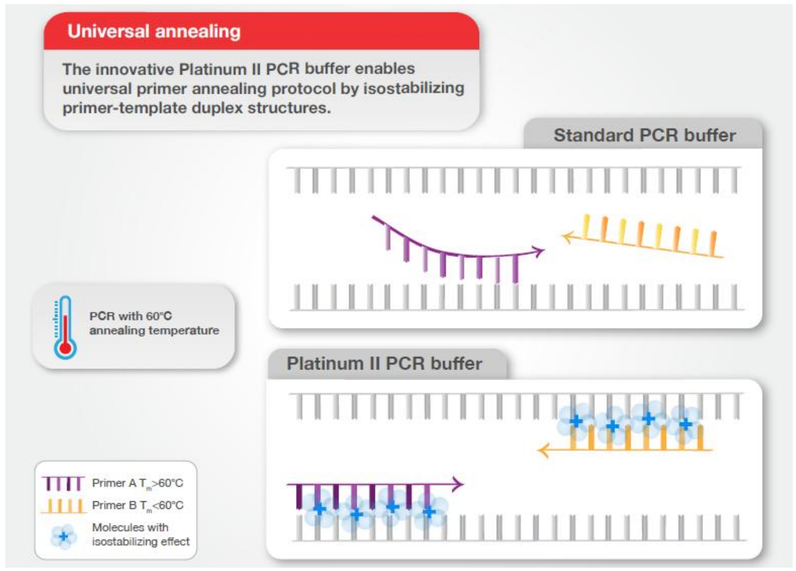
## ABSTRACT

Invitrogen™ Platinum™ *Taq* DNA Polymerase has been trusted by researchers for over two decades and has been used in several thousand publications. Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase is a new generation polymerase designed for fast PCR with minimum optimization. Platinum II *Taq* polymerase combines antibody-based hot-start with two innovative technologies. *Taq* DNA polymerase was engineered by *in vitro* evolution for higher speed and resistance to common PCR inhibitors, while innovative PCR buffer enabled universal annealing temperature. Isostabilizing molecules in the Platinum II PCR buffer increase primer–template duplex stability during the annealing step and contribute to enhanced specificity without the need to optimize annealing temperature for each primer pair. Moreover, Platinum II PCR buffer allows flexibility in the length of the extension step. Therefore different targets of diverse length can be cycled together, increasing the laboratory throughput in PCR. Here we present how Platinum II *Taq* Hot-Start DNA Polymerase provides cycling speed, universal cycling protocol and market leading inhibitor tolerance.

## INTRODUCTION

When there is a need to quickly analyze samples using a number of different PCR assays, it is likely that optimal conditions for each assay will not be the same. First, different assays often will require different annealing temperatures for their primers. In addition, 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.

Platinum II *Taq* Hot-Start DNA Polymerase is designed for fast co-cycling of the PCR assays by two innovative technologies. First, *Taq* enzyme is engineereed by *in vitro* evolution for faster DNA synthesis and inhibitor resistance. Second, Platinum II PCR buffer contains isostabilizing molecules, which increase primer–template duplex stability during the annealing step. This enables optimal and specific binding of primers with different melting temperatures at universal annealing temperature (60ºC). 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.

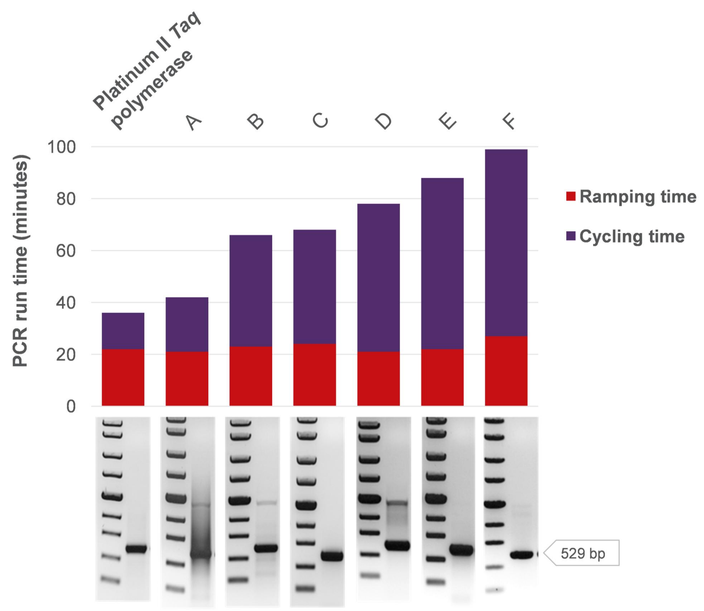



The top panel depicts a situation that often happens in a standard PCR buffer when primers have distinct melting temperatures ($T_m$). At an annealing temperature of 60ºC, chosen as a trade-off between two different Tms, primer A ($T_m$ >60ºC) binds to partially complementary unintended targets. The majority of primer B ($T_m$ <60ºC) 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 60ºC and thus a successful PCR.

Here we show how Platinum II *Taq* polymerase can increase PCR throughput by with fast co-cycling all assays without compromises on PCR yield and specificity.

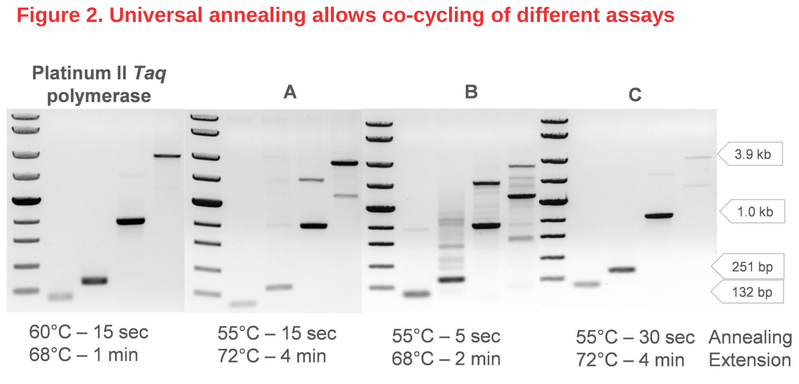
## RESULTS

**Figure 1. Fast cycling reduces PCR run time while retaining high specificity**



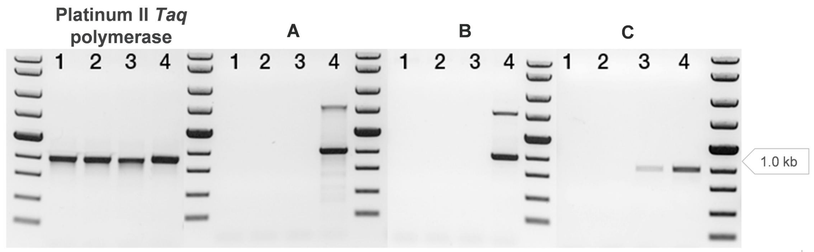

Amplification of a 529 bp fragment from 50 ng of human genomic DNA in 50 µL reactions for 35 cycles was carried out using Platinum II *Taq* Hot-Start DNA Polymerase and hot-start DNA polymerases from other suppliers: (A) Sigma-Aldrich KAPA2G™ Fast HotStart PCR Kit, (B) NEB OneTaq Hot Start DNA Polymerase, (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 (6ºC/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.

**Figure 2. Universal annealing allows co-cycling of different assays**




Four targets of different length were amplified from 50 ng of human genomic DNA in 50 µL reactions using Platinum II *Taq* and other hot-start DNA polymerases: (A) NEB OneTaq Hot Start DNA Polymerase, (B) Qiagen Fast Cycling PCR Kit, and (C) Roche FastStart Taq DNA Polymerase. The same protocol was used for all targets with the annealing and extension settings indicated.

**Figure 3. Successful amplification with samples of suboptimal purity**




Amplification of a 1 kb fragment from 50 ng of human genomic DNA in 50 µL reactions using Platinum II *Taq* Hot-Start DNA Polymerase or competitor DNA polymerases: (A) - KAPA 2G Robust Hot Start, (B) – NEB OneTaq Hot Start, and (C) – Promega GoTaq G2. Reaction mixtures contained: 1— humic acid (up to final concentration of 1.3 µg/mL), 2— hemin (up to final concentration of 6 µM), 3— xylan (up to final concentration of 0.26 mg/mL), or 4—no inhibitor control. The molecular weight marker is ZipRuler Express DNA Ladder 2.

**Table 1. Platinum II *Taq* polymerase features**

| | |
|---|---|
| Universal annealing protocol | **Yes** |
| Speed | **15 sec/kb** |
| Flexible extension step* | **Yes** |
| Inhibitor resistance | **Yes** |
| Controlled low level of human and bacterial DNA | **Yes** |
| Target length | Up to 5 kb |
| GC-rich templates | Yes |
| Hot-start modification | Antibody |
| Initial activation | 2 min |
| Fidelity versus Taq DNA Polymerase | 1x |
| Blunt or 3'A end | 3'A |
| Benchtop stability of assembled reactions | 24 h |

*The extension step can be extended up to 60 sec/kb without the effect on specificity

**Table 3. Application notes: Why Platinum II *Taq* polymerase?**

| Mouse genotyping | Direct PCR with blood | PCR from bacterial 16S rRNA genes | High-throughput PCR |
|---|---|---|---|
|  |  |  |  |
| **Tolerance to common PCR inhibitors and high specificity allow amplification from whole blood and other samples of suboptimal purity** | **Controlled low level of bacterial DNA during manufacturing allows detection of bacterial DNA in various samples** | | **Universal protocol, fast cycling, and benchtop stability are ideal for high-throughput applications** |

## CONCLUSIONS

Platinum II *Taq* Hot-Start DNA Polymerase provides an unique combination of trusted antibody hot-start technology with an innovative buffer and engineered *Taq* DNA polymerase. This unique combination enables fast cycling, co-cycling of all assays, and successful amplification with samples of suboptimal purity. Platinum II *Taq* polymerase shows exceptional PCR results, even in the toughest applications.


The new product is available in the following formats:

- Platinum™ II Master Mix (2X)
- Platinum™ II Green PCR BufferTaq Hot-Start DNA Polymerase
- Platinum™ II Hot-Start PCR Master Mix (2X)
- Platinum™ II Hot-Start Green PCR

More information on thermofisher.com/platinumiitaq

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## TRADEMARKS/LICENSING



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