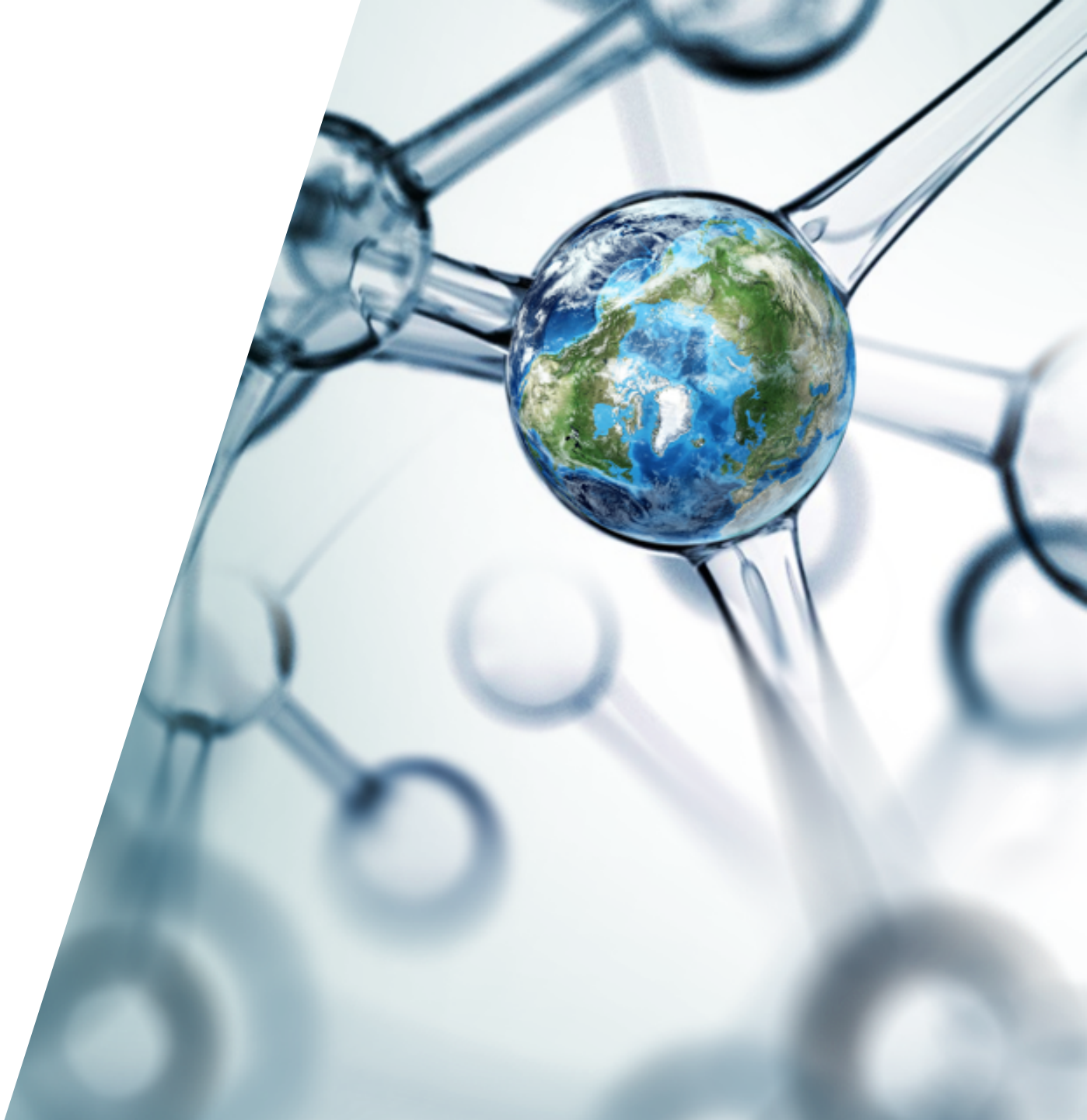
Performance comparison of Phusion Plus and Phusion DNA polymerases



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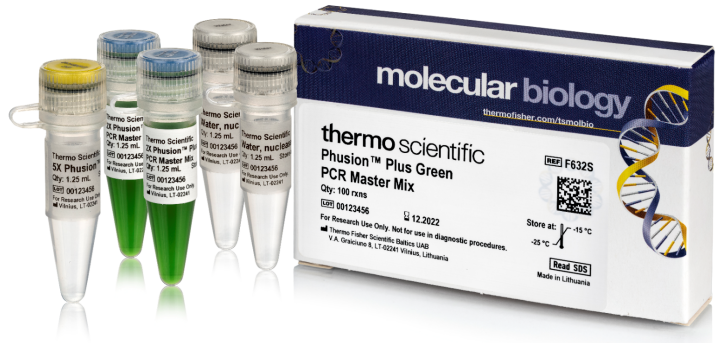
Advantages of Thermo Scientific™ Phusion™ Plus DNA Polymerase over Phusion™ DNA polymerases

Added benefits:

- No more **annealing temperature** (T_m) calculation—uses universal annealing temperature for all primers
- Fewer **PCR runs**—can co-cycle targets of different lengths

Improved benefits:

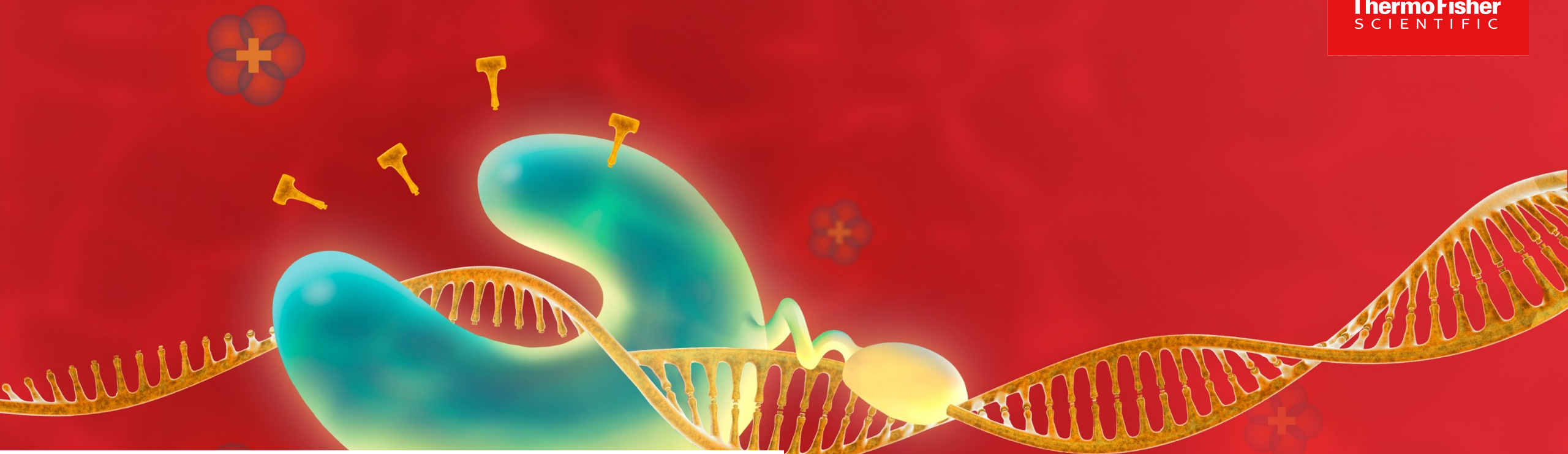
- Improved PCR **sequence accuracy**—enzyme fidelity increases to >100x that of *Taq* enzyme
- Easier detection of **low-abundance targets**—higher PCR sensitivity
- Better results with **GC-rich sequences**—new GC enhancer included
- Higher tolerance to **PCR inhibitors**—reaction buffer reformulated



Not required

T_m calculator no longer needed

thermofisher.com/phusionplus



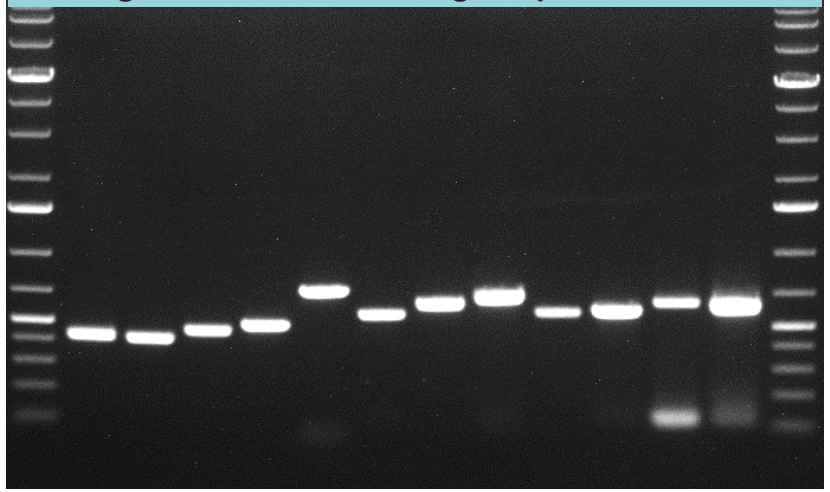
Added benefits

No more T_m calculation

Calculated annealing temperature, °C

59 59 60 60 62 62 64 64 67 67 69 69

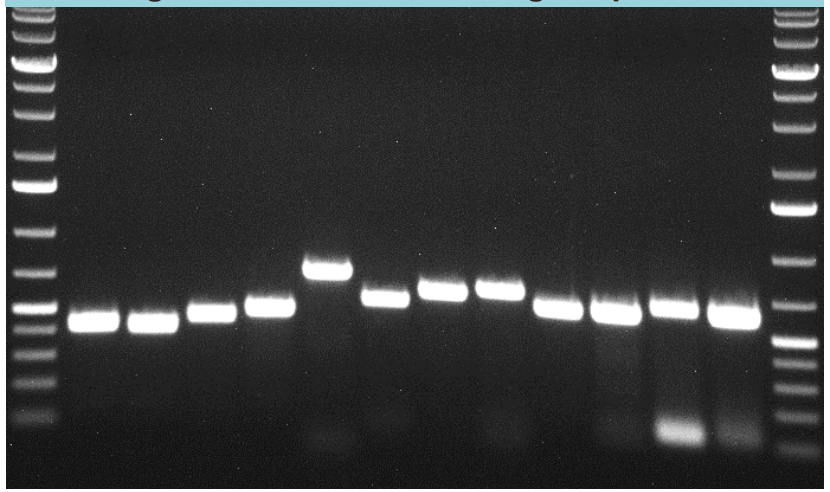
Using a universal annealing temperature of 60°C



Calculated annealing temperature, °C

59 59 60 60 62 62 64 64 67 67 69 69

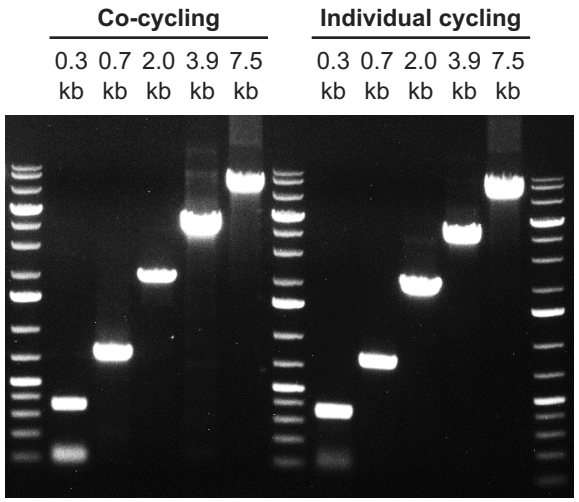
Using the calculated annealing temperatures



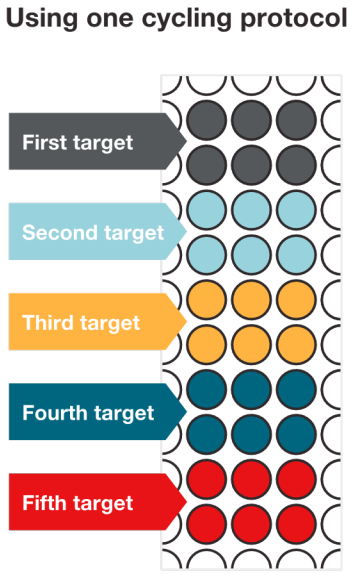
PCR cycling under two annealing conditions. 12 targets with varying calculated annealing temperatures (indicated above each lane) were amplified from 50 ng of human genomic DNA (gDNA), following a universal annealing temperature of 60°C (left), or the annealing temperatures calculated with the T_m calculator (right). The molecular weight marker is Thermo Scientific™ ZipRuler™ Express DNA Ladder 2.

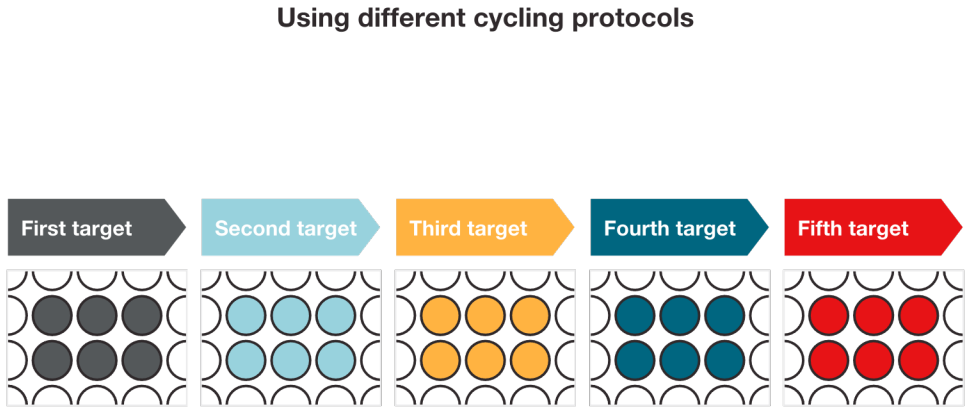
Phusion Plus DNA Polymerase provides convenience and flexibility in PCR preparation

Fewer PCR runs



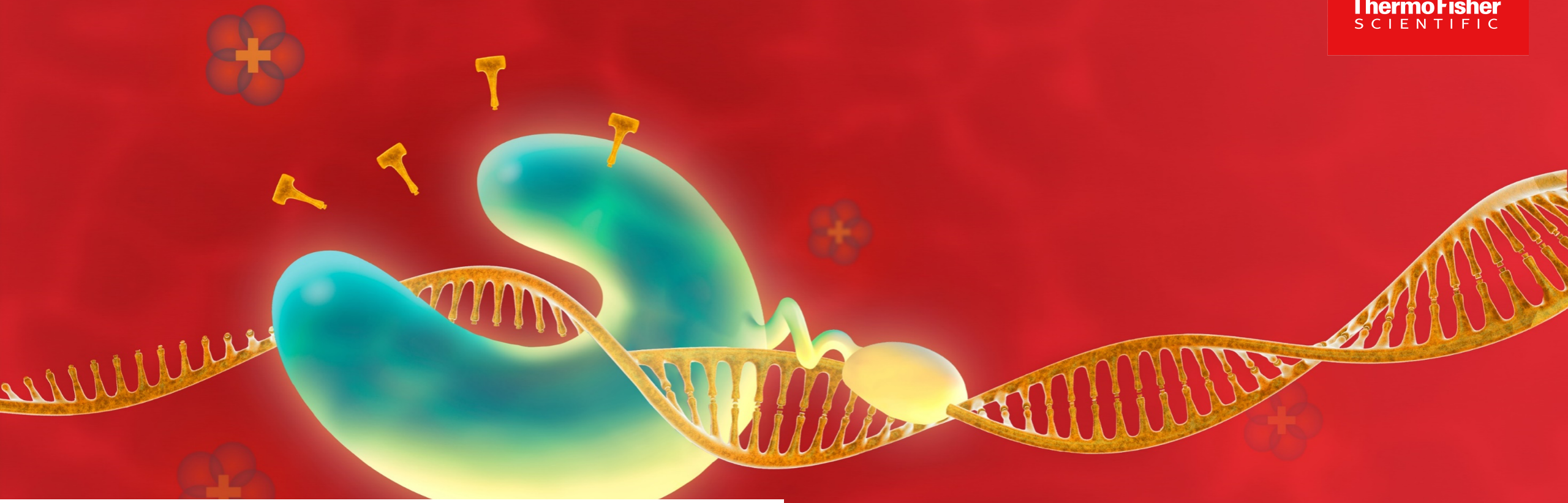
PCR co-cycling. Five targets of different lengths were amplified from human gDNA using a universal cycling protocol for all targets (up to 7.5 kb), with the extension time of the longest amplicon (3 min 45 sec for 7.5 kb) (left) or following separate cycling protocols with a different extension time calculated for each target (9 sec for 0.3 kb, 21 sec for 0.7 kb, 60 sec for 2 kb, 2 min for 3.9 kb, 3 min 45 sec for 7.5 kb) (right). The molecular weight marker is ZipRuler Express DNA Ladder 2.





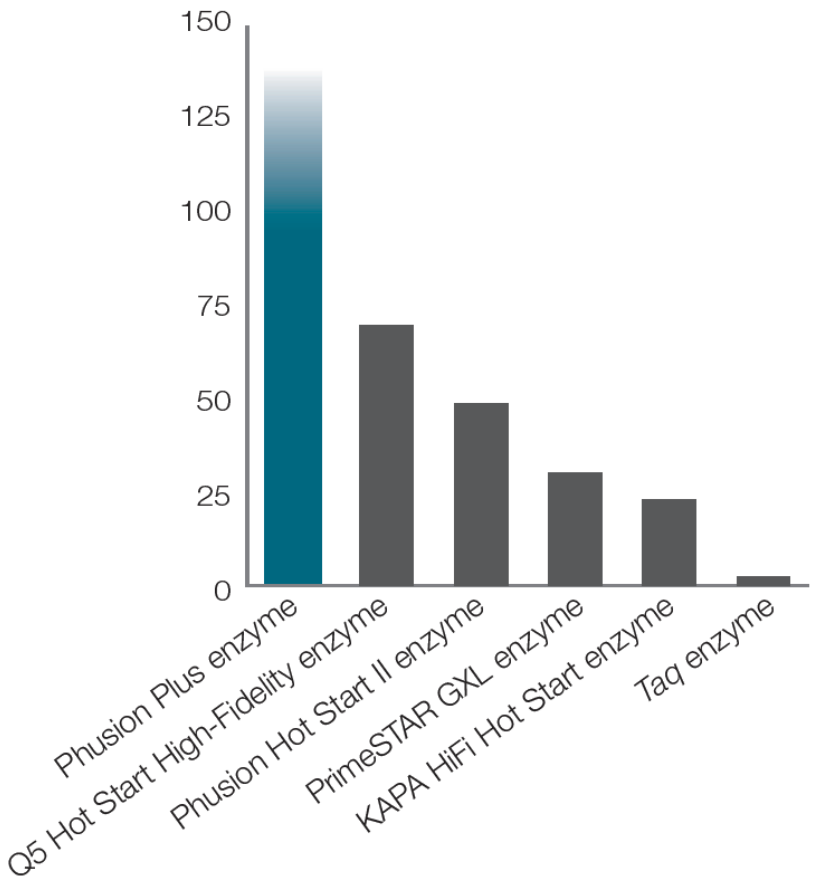
The universal annealing feature of Phusion Plus DNA Polymerase also allows a **universal cycling protocol**, helping you to circumvent multiple PCR runs and save time. One annealing temperature (60°C) and one extension time based on the longest amplicon can be used for targets of different lengths—i.e., **co-cycling different targets** on the same block—without compromising PCR yields and specificity.

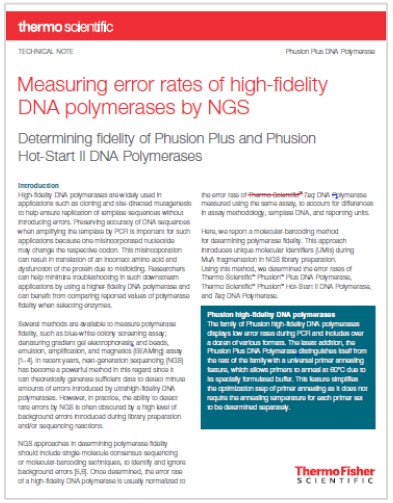
Different PCR targets can be co-cycled using Phusion Plus DNA Polymerase



Improved benefits

Improved PCR sequence accuracy

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TECHNICAL NOTE

Phusion Plus DNA Polymerase

Measuring error rates of high-fidelity DNA polymerases by NGS

Determining fidelity of Phusion Plus and Phusion Hot-Start II DNA Polymerases

Introduction

ThermoFisher SCIENTIFIC

Tech note: Learn more about enzyme fidelity measurement using NGS with molecular barcodes.

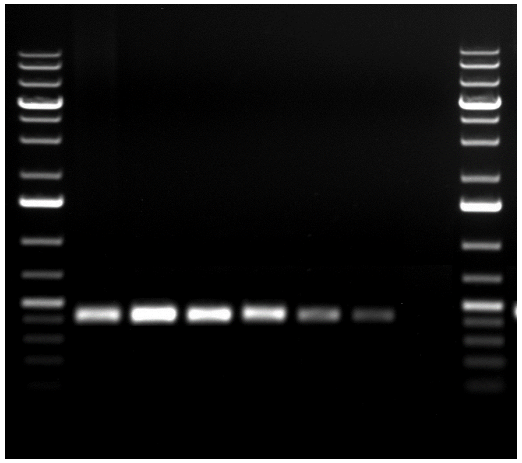
Fidelity of high-fidelity polymerases measured relative to *Taq* DNA polymerase. Error rates were determined by next-generation sequencing (NGS) using molecular barcodes, then normalized to that of *Taq* DNA polymerase.

Phusion Plus DNA Polymerase's fidelity is >100x that of *Taq* DNA polymerase, giving you more confidence in PCR sequence accuracy

Easier detection of low-abundance targets

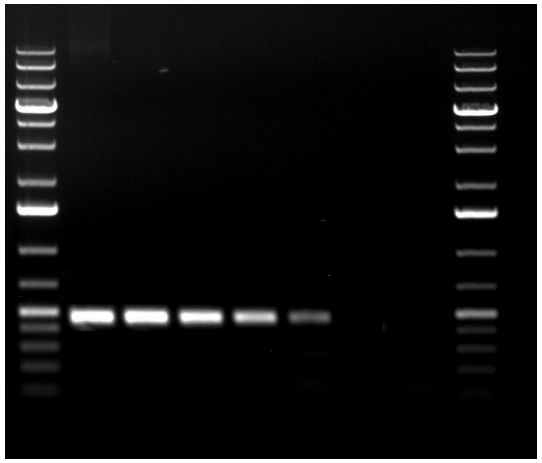
Phusion Plus DNA Polymerase

250 ng | 50 ng | 10 ng | 2.0 ng | 0.4 ng | 0.08 ng | 0 ng



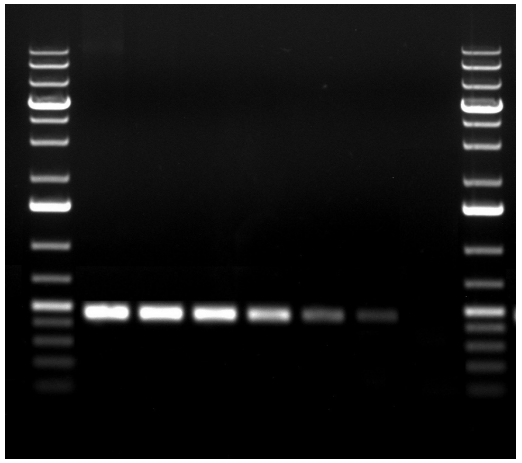
Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerase

250 ng | 50 ng | 10 ng | 2.0 ng | 0.4 ng | 0.08 ng | 0 ng



Thermo Scientific™ Phusion™ Hot Start II DNA Polymerase

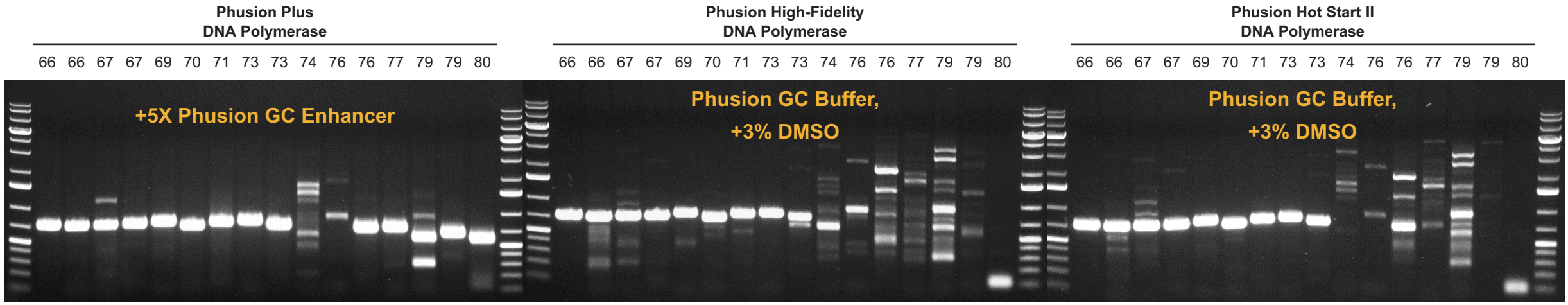
250 ng | 50 ng | 10 ng | 2.0 ng | 0.4 ng | 0.08 ng | 0 ng



High sensitivity of Phusion Plus DNA Polymerase. A 0.5 kb target was amplified from different amounts of human genomic (gDNA). The molecular weight marker is the Thermo Scientific™ GeneRuler™ 1 kb Plus DNA Ladder.

Phusion Plus DNA Polymerase can detect targets from as little as 0.08 ng of human gDNA

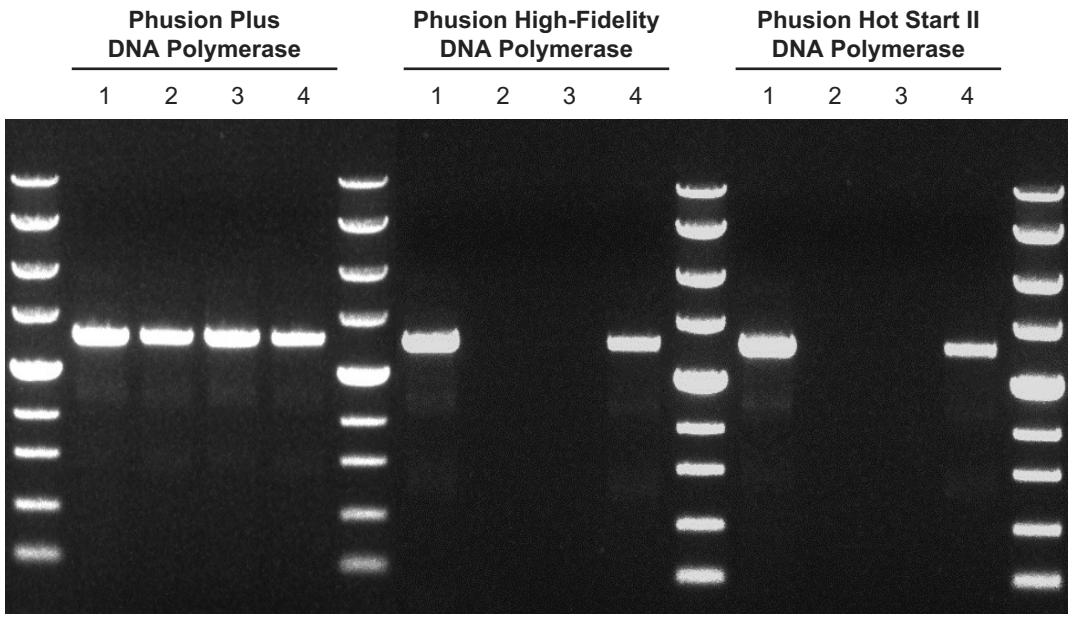
Better results with GC-rich sequences



Efficient GC-rich amplification of Phusion Plus DNA Polymerase. 16 targets with high GC content (their percentages indicated) were amplified from 50 ng of human gDNA. The molecular weight marker is Thermo Scientific™ ZipRuler™ Express DNA Ladder 2.

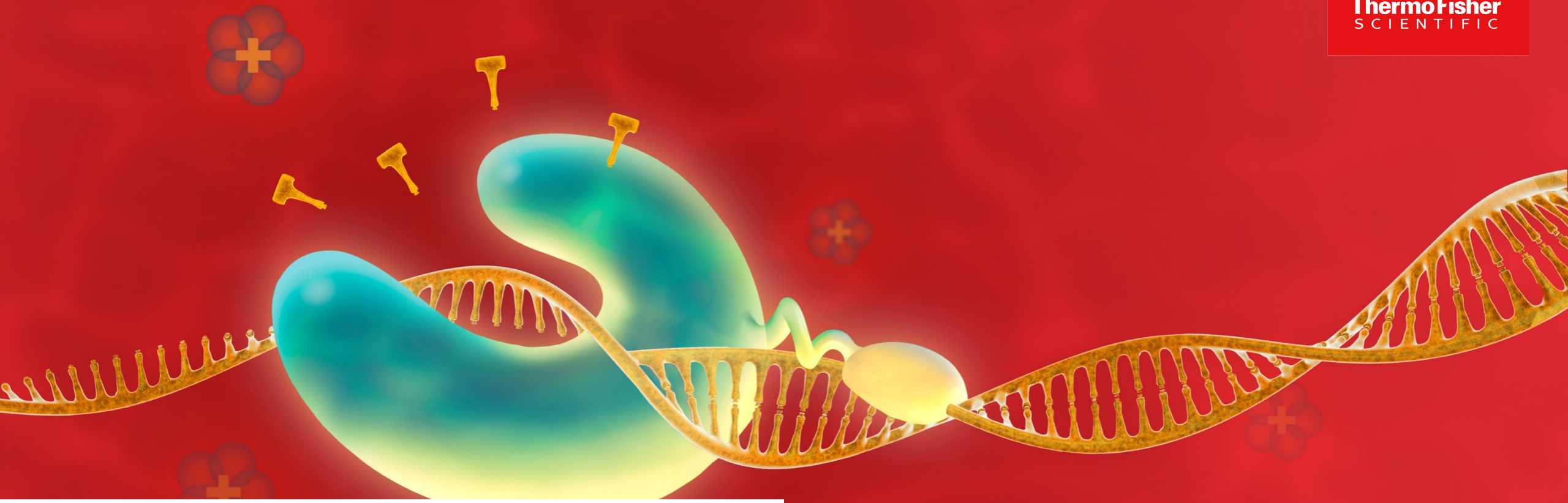
Phusion Plus DNA Polymerase includes the new Phusion™ GC Enhancer in the package for more efficient amplification of sequences with >65% GC content

Higher tolerance to PCR inhibitors



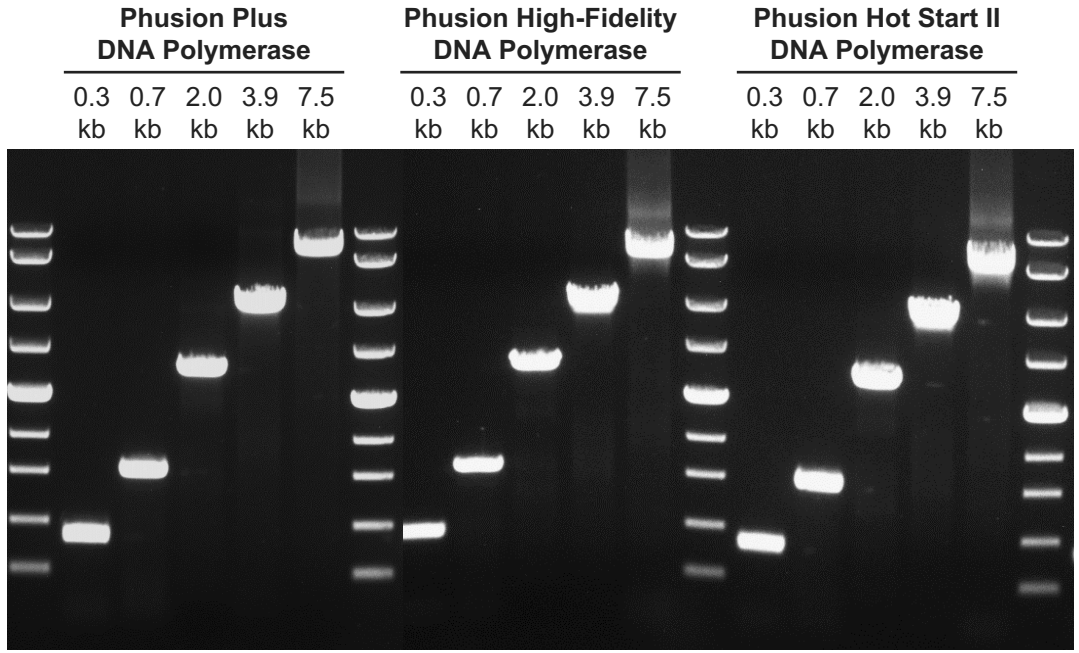
High inhibitor tolerance of Phusion Plus DNA Polymerase. A 2 kb target was amplified from 50 ng of human gDNA. The reaction mixtures contained at a final concentration of: 1—no inhibitor, 2—humic acid (0.5 µg/mL), 3—hemin (2.5 µM), or 4—xylan (250 µg/mL). The molecular weight marker is the GeneRuler 1 kb Plus DNA Ladder.

Phusion Plus DNA Polymerase better tolerates inhibitors from plants (e.g., xylan), soil (e.g., humic acid), and blood (e.g., hemin) during PCR



Comparable performance

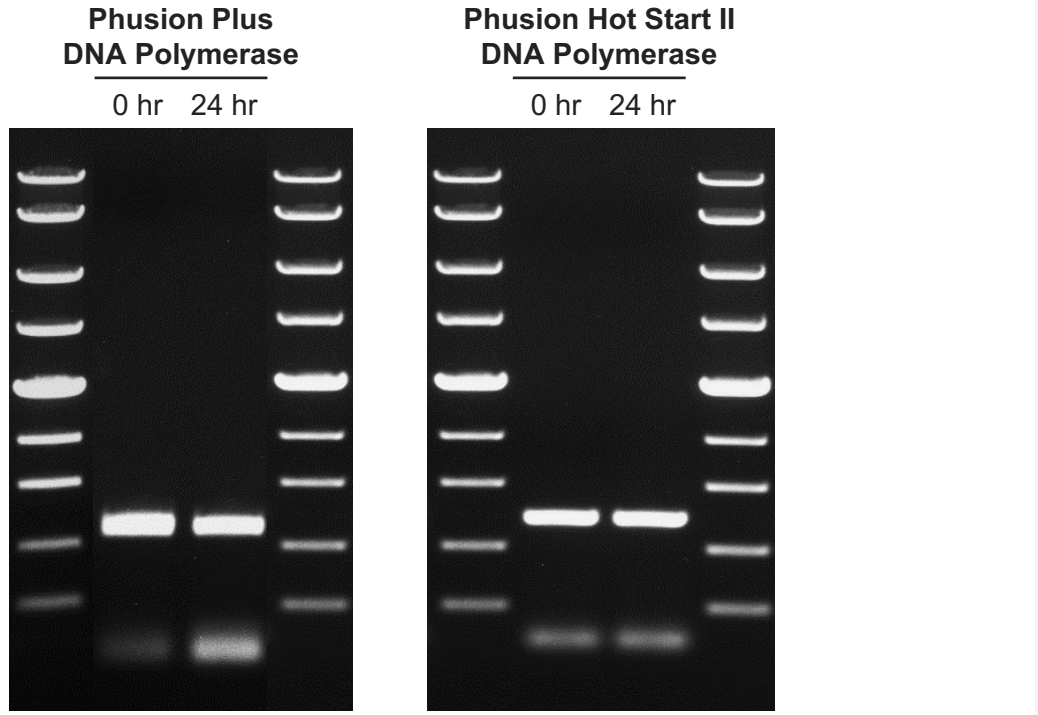
High yields and specificity



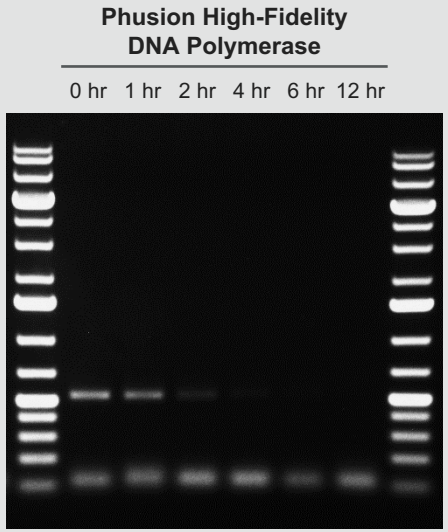
High yields and specificity using Phusion Plus DNA Polymerase. 0.3–7.5 kb DNA targets were amplified from 100 ng of human gDNA. The molecular weight marker is the GeneRuler 1 kb Plus DNA Ladder.

Similar PCR specificity and yields can be achieved with Phusion Plus DNA Polymerase

Benchtop stability of assembled reactions

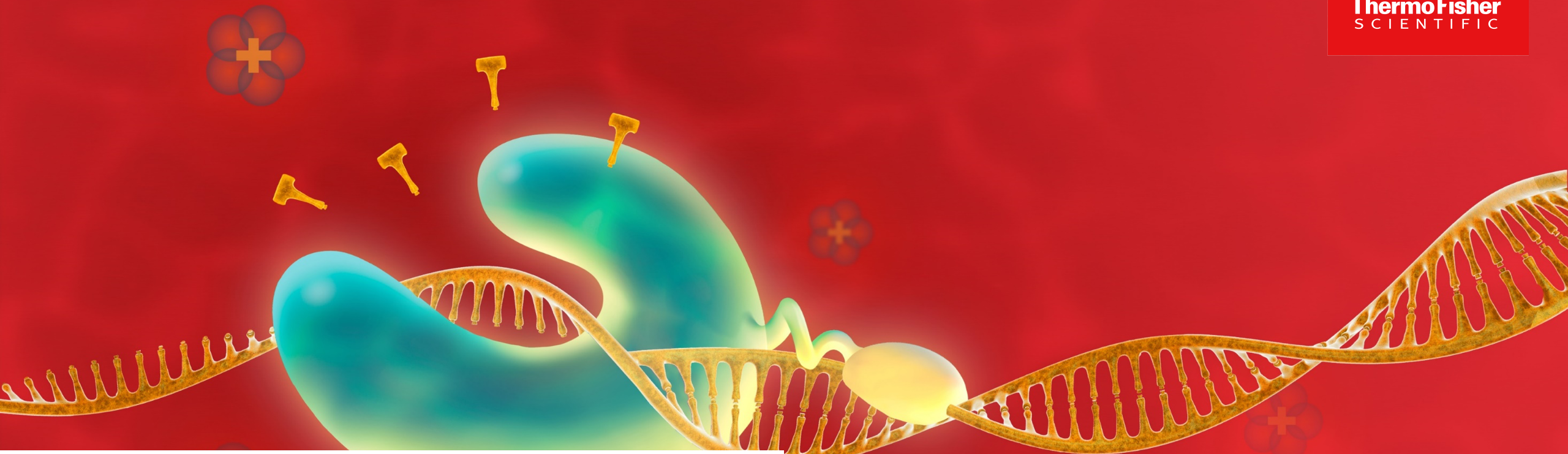


Non–hot-start DNA polymerases are not recommended for lab automation setup because assembled reactions are not stable at room temperature for an extended period.



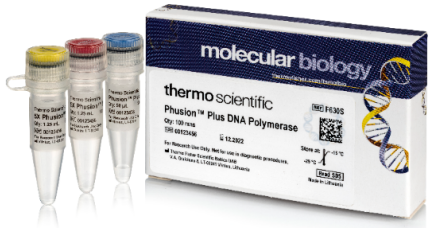
Benchtop stability of Phusion Plus DNA Polymerase. A 0.5 kb target was amplified from 50 ng of human gDNA. Assembled PCR reactions were loaded immediately onto a thermal cycler (0 hr) or set at room temperature for 24 hr before cycling (24 hr). The molecular weight marker is the GeneRuler 1 kb Plus DNA Ladder.

PCR reactions assembled with Phusion Plus DNA Polymerase are stable at room temperature for up to 24 hr, enabling lab automation setup



Summary

Summary: comparison of Phusion Plus, Phusion High-Fidelity, and Phusion Hot Start II DNA polymerases



	Phusion Plus DNA Polymerase	Phusion High-Fidelity DNA Polymerase	Phusion Hot Start II DNA Polymerase
Fidelity (vs. *Taq* DNA polymerase)	>100x	50x	50x
Hot-start modification (Affibody molecule–mediated)	Yes	No	Yes
Universal annealing temperature (no T_m calculator needed)	Yes	No	No
Universal cycling protocol (co-cycling targets of different length)	Yes	No	No
PCR sensitivity	+++	++	++
GC-rich amplification	+++ (New GC enhancer)	++	++
Inhibitor tolerance	+++	++	++
PCR yields and specificity	+++	+++	+++
Benchtop stability (of assembled reactions)	Up to 24 hr	N/A	Up to 24 hr
Stand-alone and master mix formats	Yes	Yes	Yes

Find your upgrade at thermofisher.com/phusionplus

Thank you