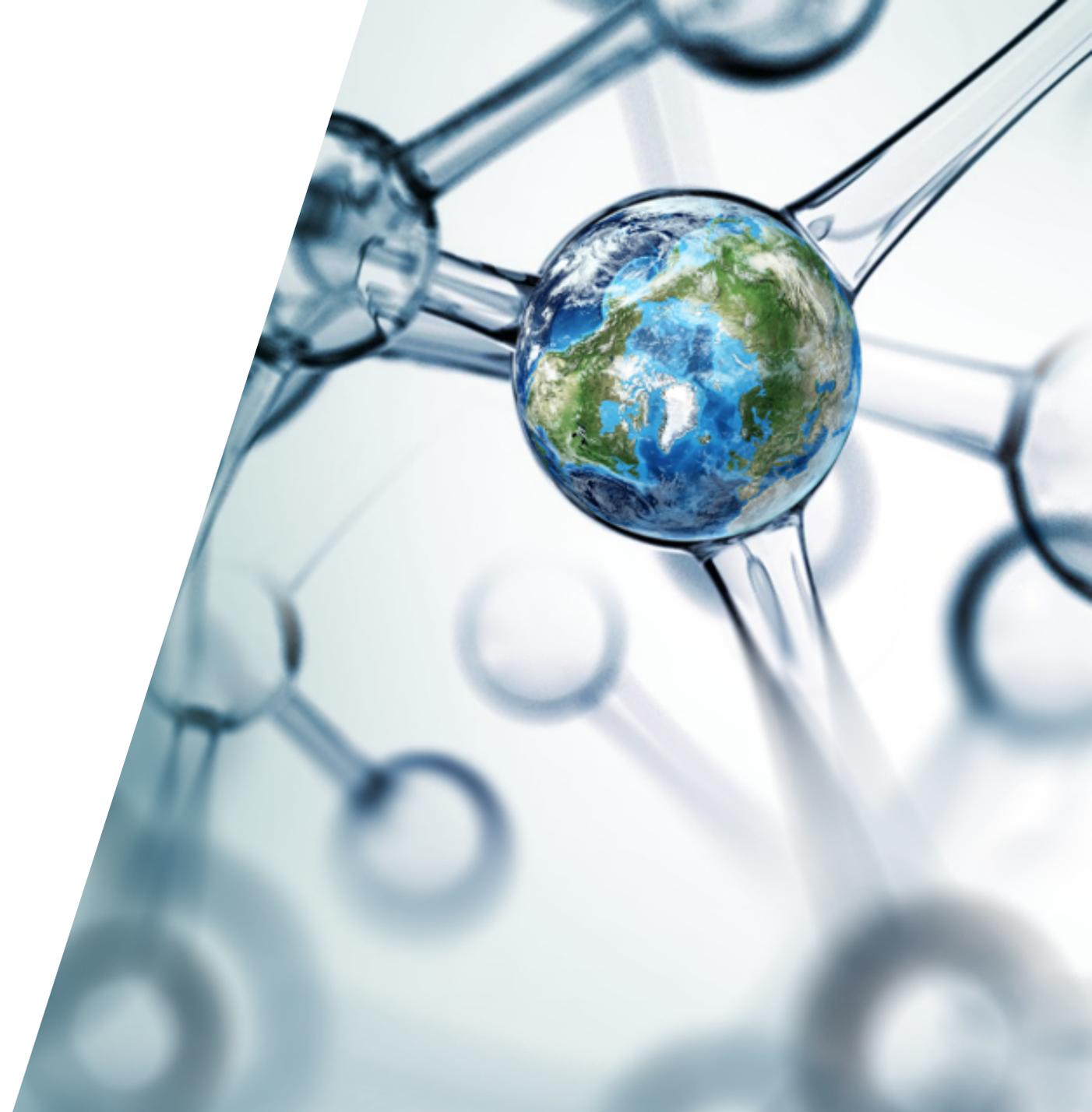


# Performance comparison of Phusion Plus and Phusion DNA polymerases

 The world leader in serving science



# 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:

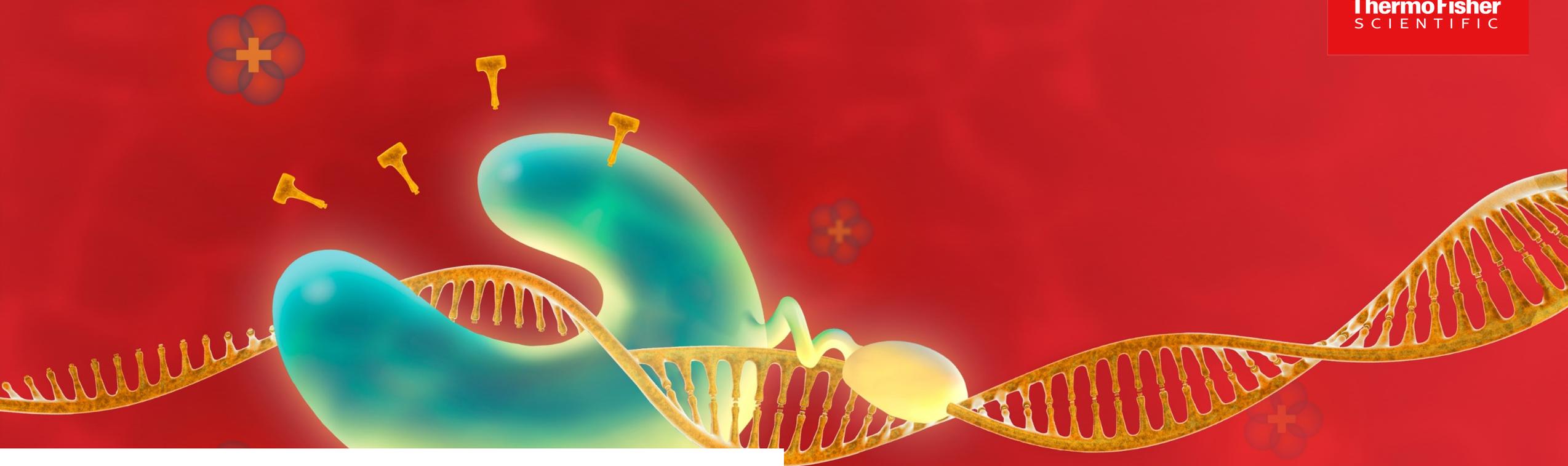
- Improved PCR **sequence accuracy**—enzyme fidelity increases to >100x that of *Taq* enzyme
- Easier detection of **low-abundance targets**—higher PCR sensitivity
- Better results with **GC-rich sequences**—new GC enhancer included
- Higher tolerance to **PCR inhibitors**—reaction buffer reformulated



**Not required**

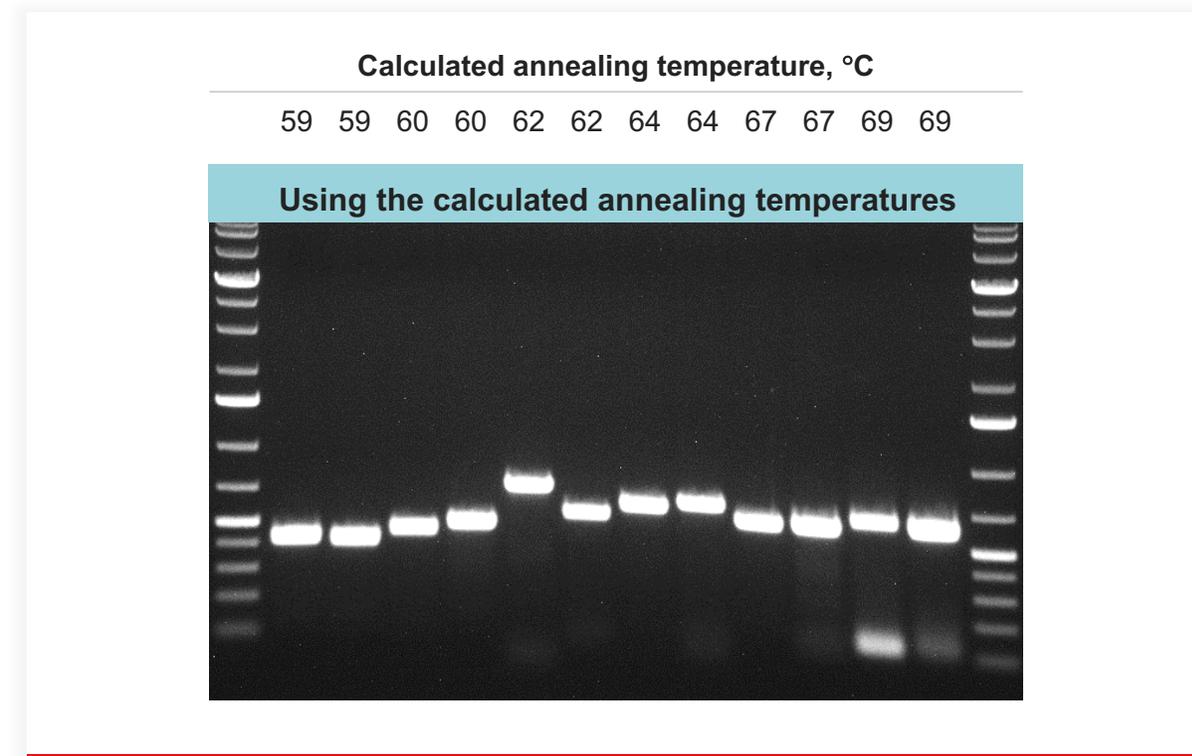
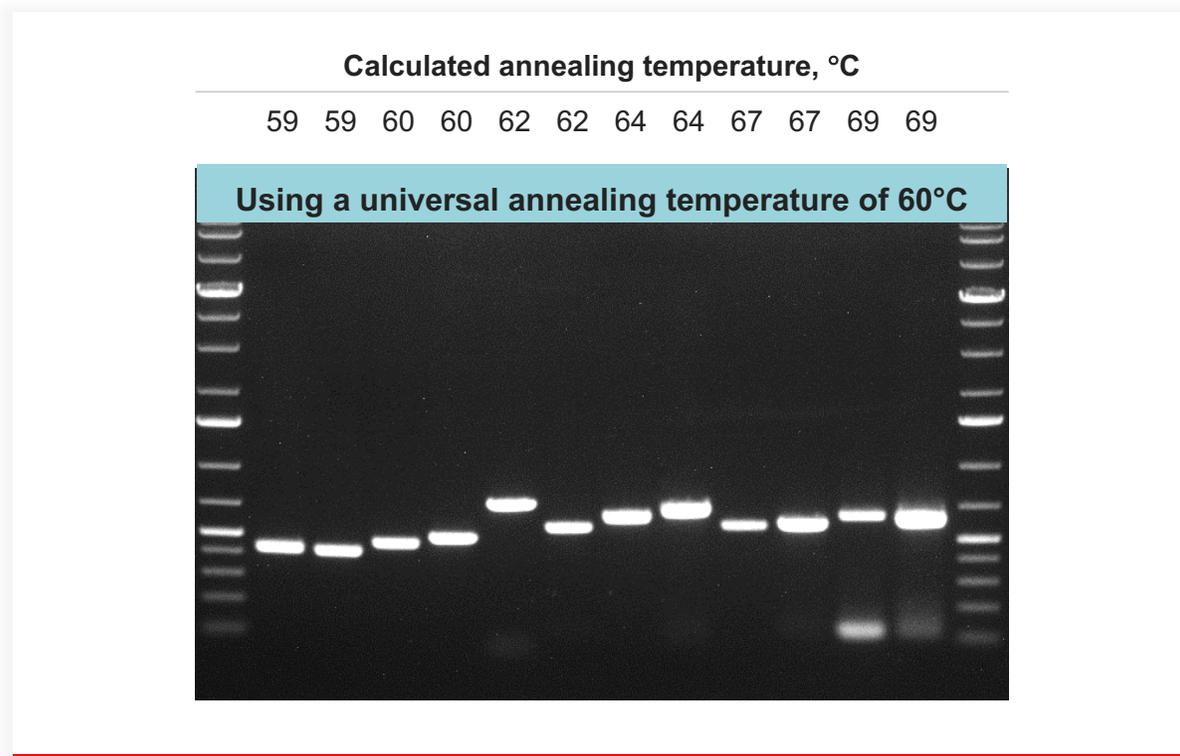
$T_m$  calculator no longer needed

[thermofisher.com/phusionplus](https://thermofisher.com/phusionplus)



**Added benefits**

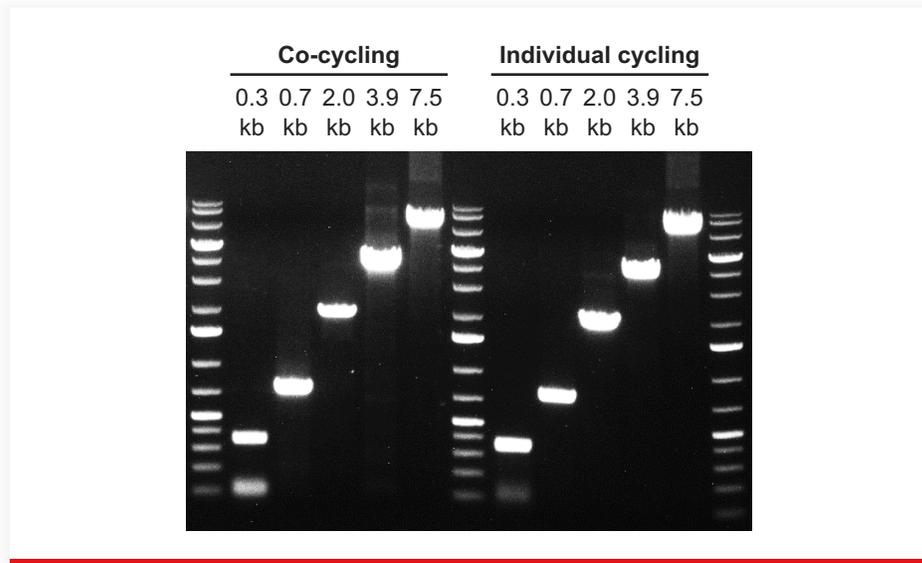
# No more $T_m$ calculation



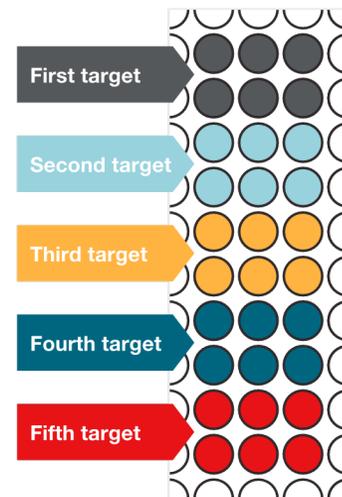
**PCR cycling under two annealing conditions.** 12 targets with varying calculated annealing temperatures (indicated above each lane) were amplified from 50 ng of human genomic DNA (gDNA), following a universal annealing temperature of 60°C (left), or the annealing temperatures calculated with the  $T_m$  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**

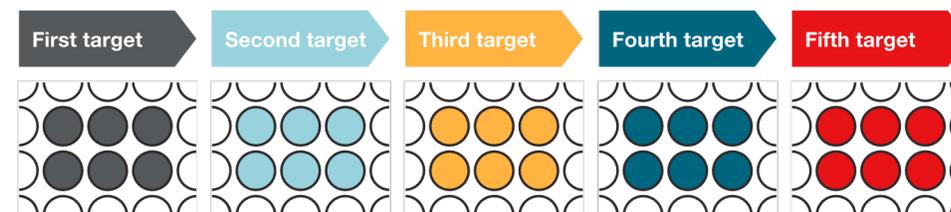
# Fewer PCR runs



Using one cycling protocol



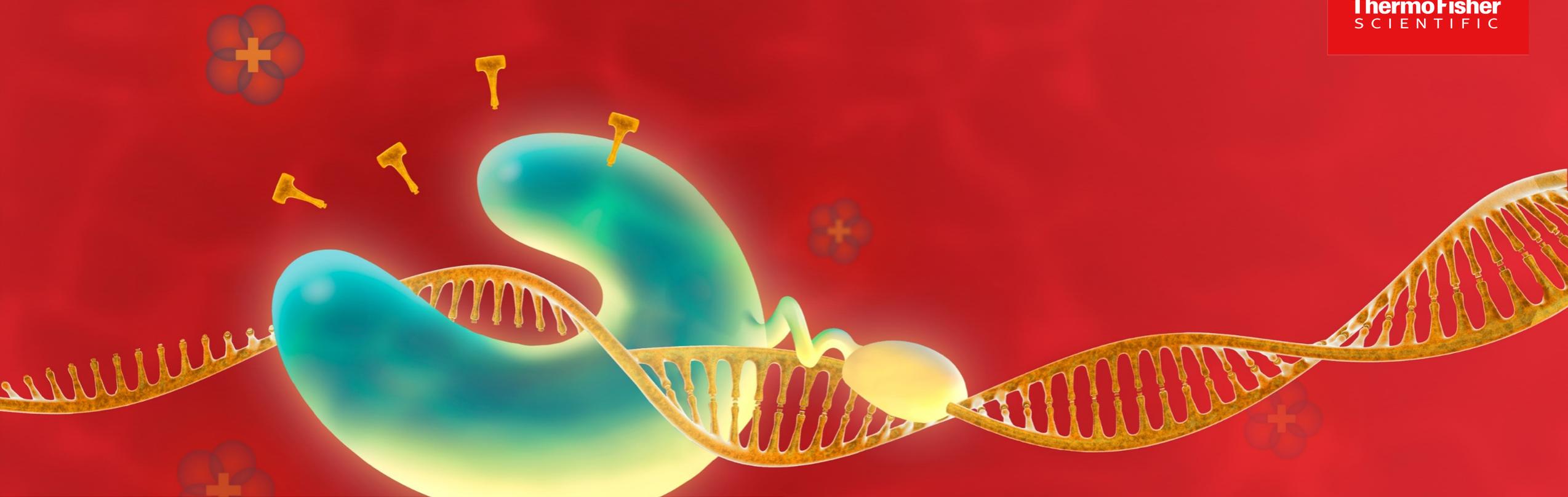
Using different cycling protocols



**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](#).

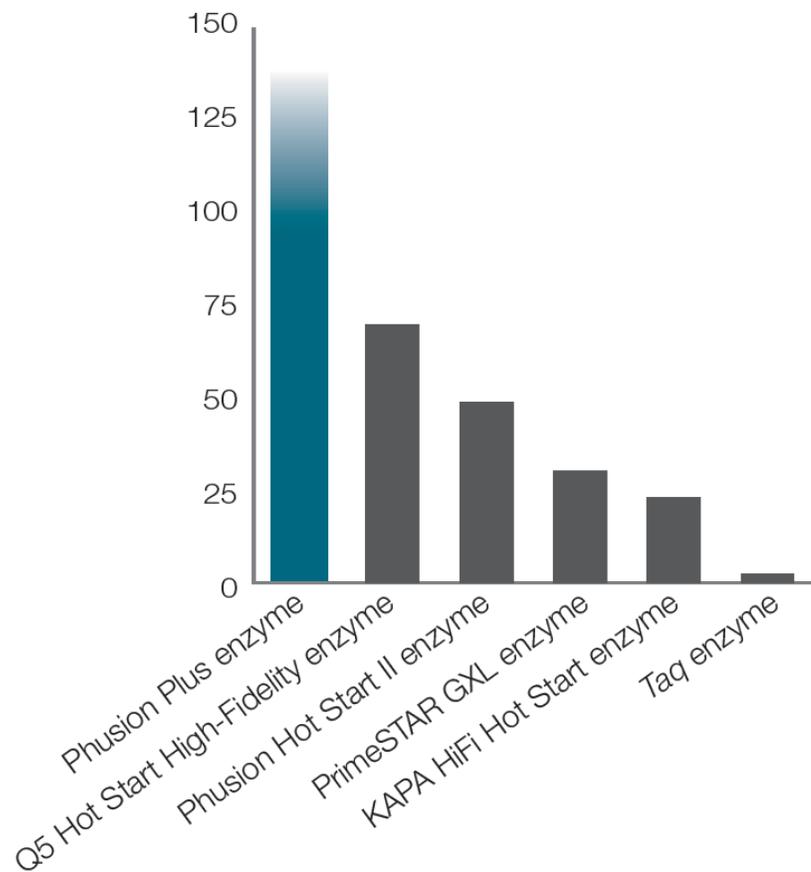
The universal annealing feature of Phusion Plus DNA Polymerase also allows a **universal cycling protocol**, helping you to circumvent multiple PCR runs and save time. One annealing temperature (60°C) and one extension time based on the longest amplicon can be used for targets of different lengths—i.e., **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



thermo scientific
Phusion Plus DNA Polymerase

TECHNICAL NOTE

### Measuring error rates of high-fidelity DNA polymerases by NGS

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

**Introduction**

High fidelity DNA polymerases are widely used in applications such as cloning and site-directed mutagenesis to help ensure replication of template sequences without introducing errors. Preserving accuracy of DNA sequences when amplifying the template by PCR is important for such applications because one misincorporated nucleotide may change the respective codon. This misincorporation can result in translation of an incorrect amino acid and dysfunction of the protein due to misfolding. Researchers can help minimize misincorporation in such downstream applications by using a high fidelity DNA polymerase and can benefit from comparing reported values of polymerase fidelity when selecting enzymes.

Several methods are available to measure polymerase fidelity, such as site-directed mutagenesis, sequencing gradient gel electrophoresis, and beads, splitters, amplification, and magnetite (BAMING) assay [1-4]. In recent years, next-generation sequencing (NGS) has become a powerful method in this regard since it can increasingly generate sufficient data to detect minor amounts of errors introduced by ultrahigh-fidelity DNA polymerases. However, in practice, the ability to detect rare errors by NGS is often obscured by a high level of background errors introduced during library preparation and/or sequencing reactions.

NGS approaches to determining polymerase fidelity should include single molecule consensus sequencing or molecular barcoding techniques, to identify and ignore background errors [5]. Once determined, the error rate of a high-fidelity DNA polymerase is usually normalized to

the error rate of ThermoFisher® Taq DNA Polymerase measured using the same assay, to account for differences in assay methodology, template DNA, and reporting units.

Here, we report a molecular barcoding method for determining polymerase fidelity. This approach introduces unique molecular barcodes (UMI) during MPA fragmentation in NGS library preparation. Using this method, we determined the error rates of ThermoFisher® Phusion® Plus DNA Polymerase, ThermoFisher® Phusion® Hot Start II DNA Polymerase, and Taq DNA Polymerase.

**Phusion High-Fidelity DNA polymerases**

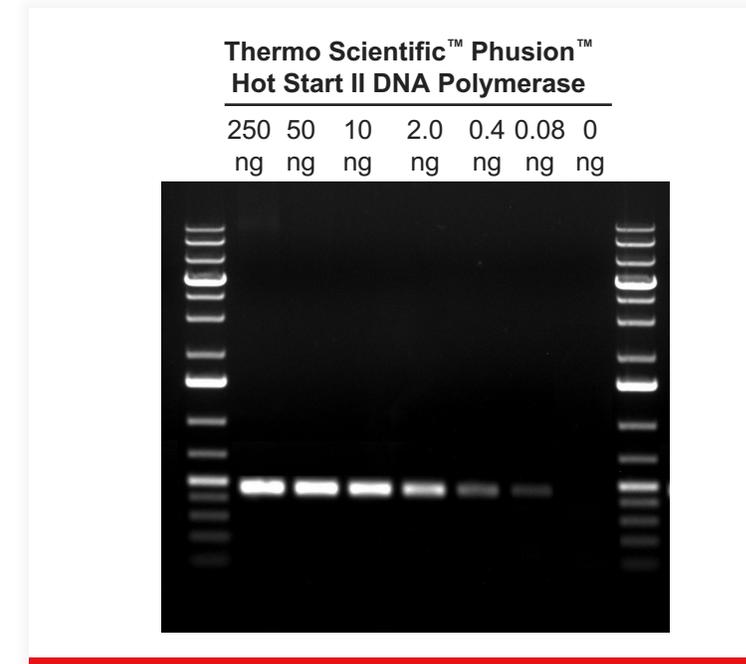
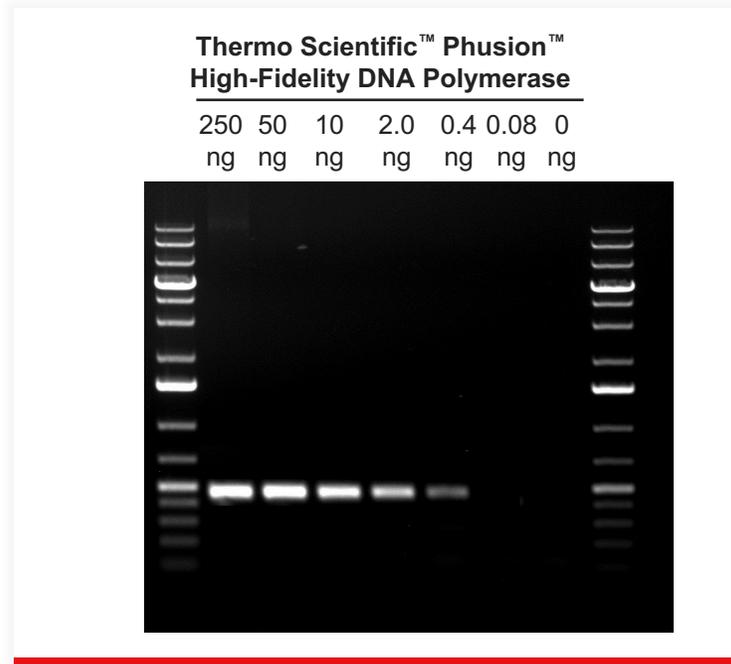
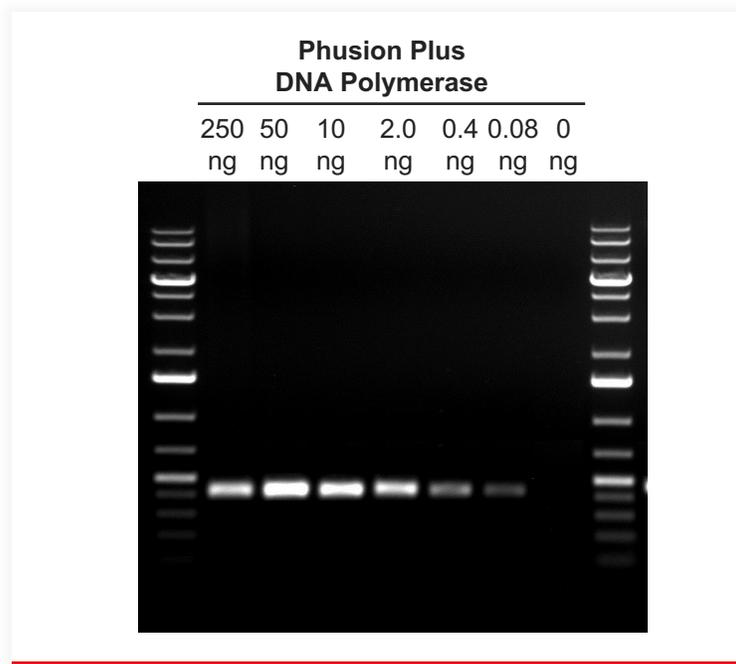
The family of Phusion High-Fidelity DNA polymerases is designed for error-reducing PCR and includes over a dozen of various formats. The base polymer, the Phusion Plus DNA Polymerase, distinguishes itself from the rest of the family in a universal primer annealing feature, which allows primers to anneal at 60°C due to its specially formulated buffer. This feature simplifies the optimization step of primer annealing as it does not require the routinely temperature for each primer set to be determined separately.

**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**

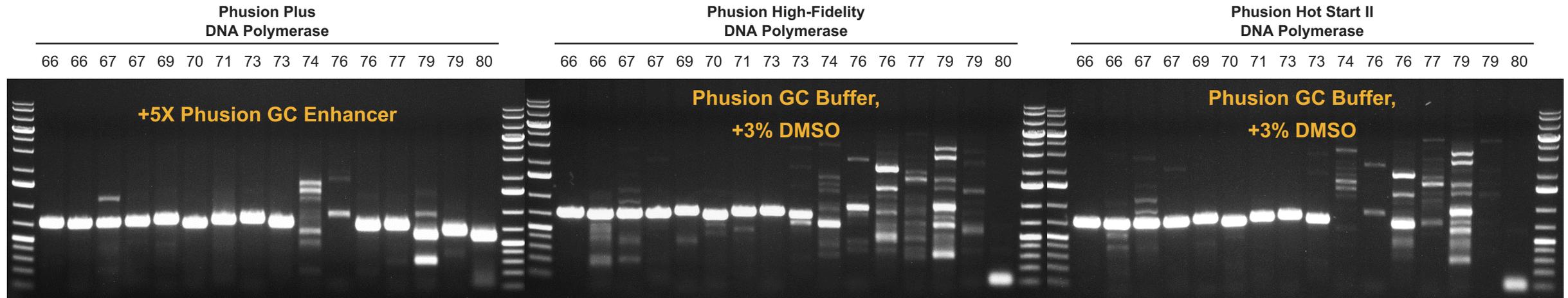
# Easier detection of low-abundance targets



**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

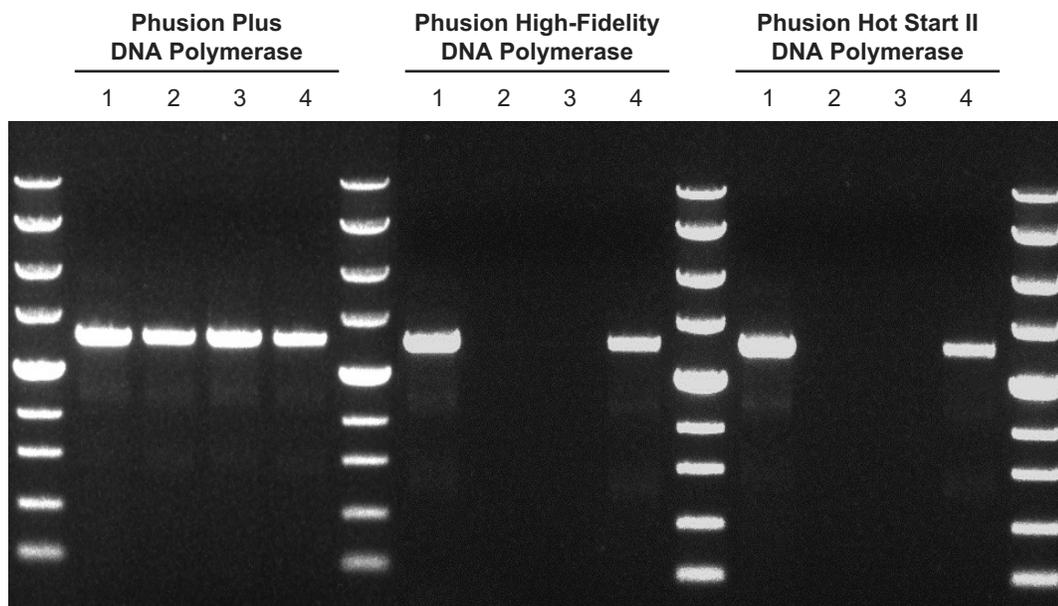


**Efficient GC-rich amplification of Phusion Plus DNA Polymerase.** 16 targets with high GC content (their percentages indicated) were amplified from 50 ng of human gDNA. The molecular weight marker is [Thermo Scientific™ ZipRuler™ Express DNA Ladder 2](#).

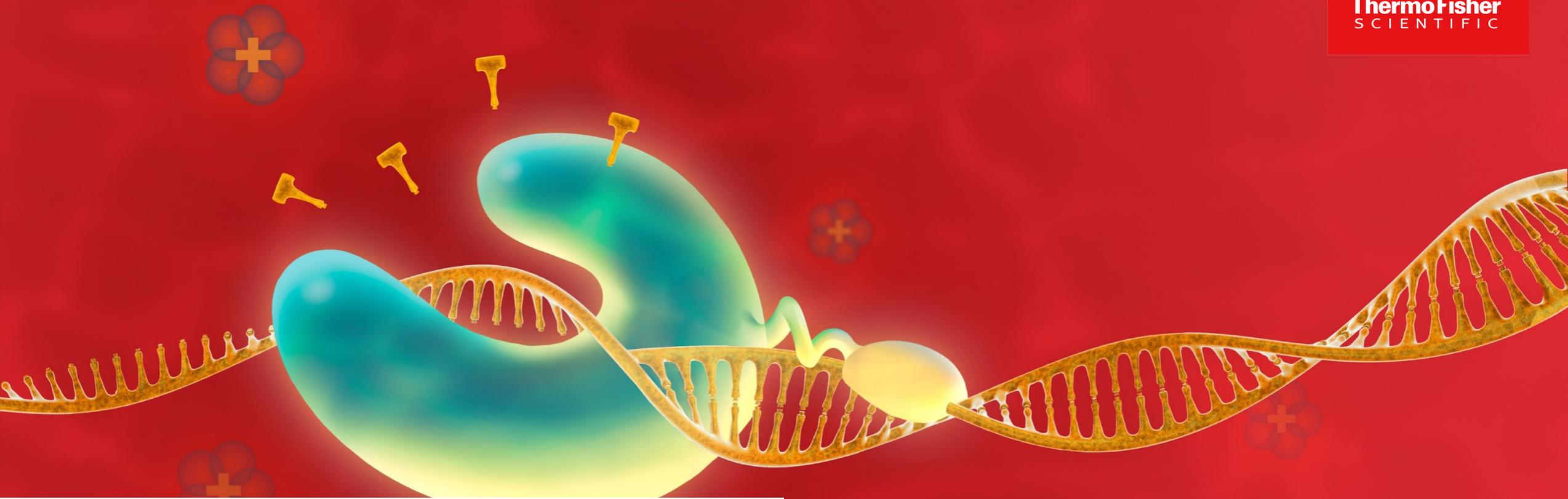
**Phusion Plus DNA Polymerase includes the new Phusion™ GC Enhancer in the package for more efficient amplification of sequences with >65% GC content**

# 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  $\mu\text{g}/\text{mL}$ ), 3—hemin (2.5  $\mu\text{M}$ ), or 4—xylan (250  $\mu\text{g}/\text{mL}$ ). The molecular weight marker is the [GeneRuler 1 kb Plus DNA Ladder](#).

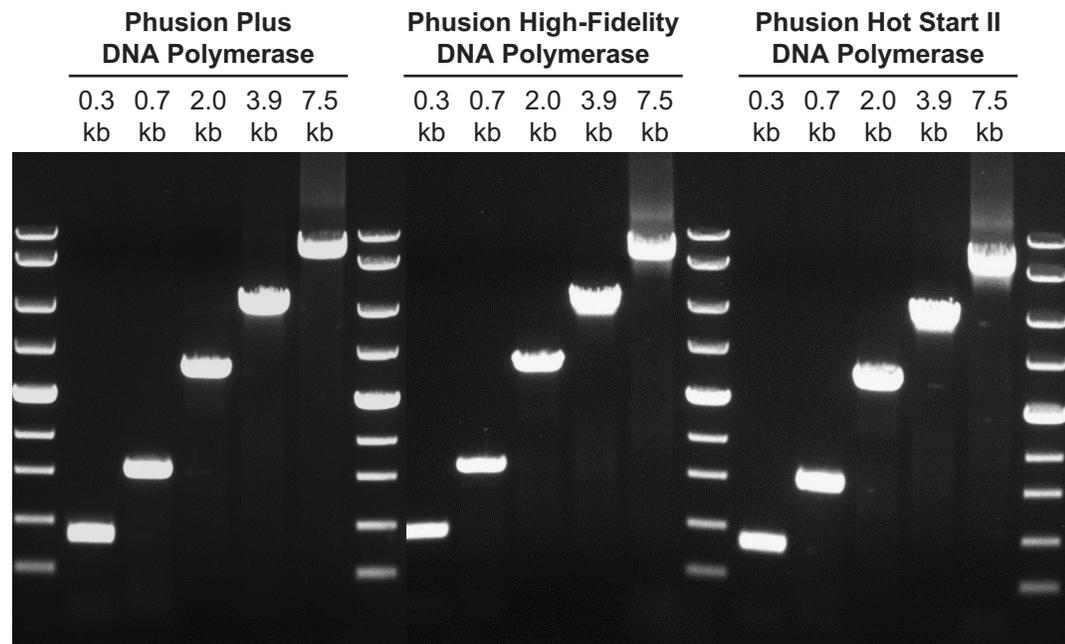


**Phusion Plus DNA Polymerase better tolerates inhibitors from plants (e.g., xylan), soil (e.g., humic acid), and blood (e.g., hemin) during PCR**



**Comparable performance**

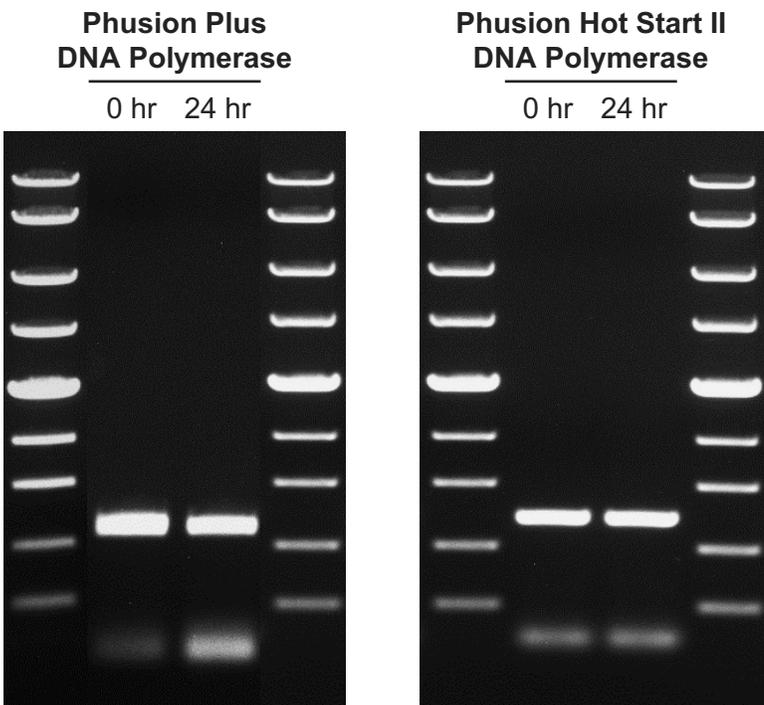
# High yields and specificity



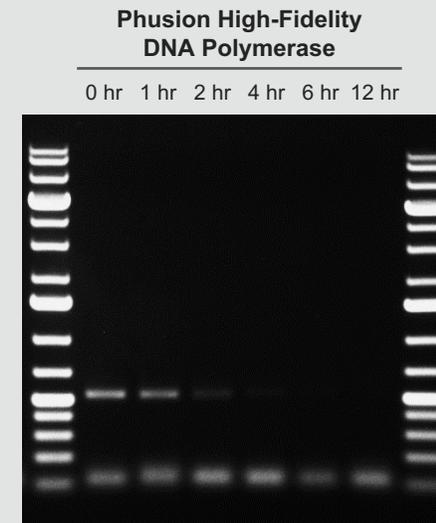
**High yields and specificity using Phusion Plus DNA Polymerase.** 0.3–7.5 kb DNA targets were amplified from 100 ng of human gDNA. The molecular weight marker is the [GeneRuler 1 kb Plus DNA Ladder](#).

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

# Benchtop stability of assembled reactions

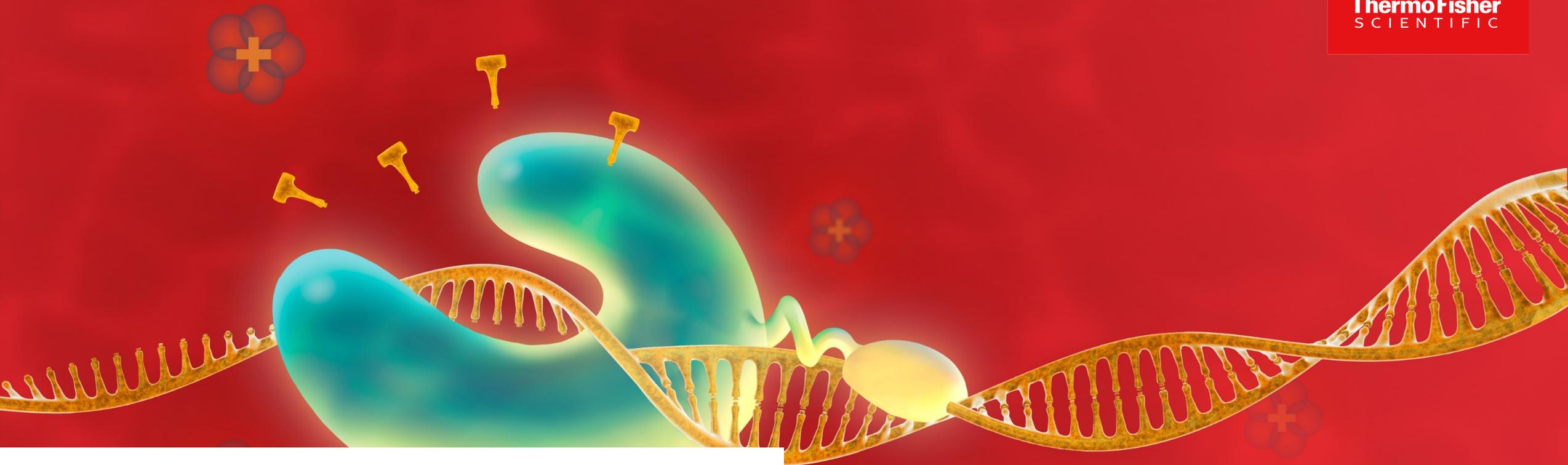


Non-hot-start DNA polymerases are not recommended for lab automation setup because assembled reactions are not stable at room temperature for an extended period.



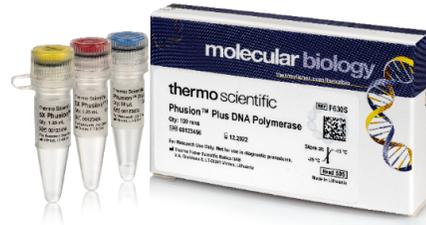
**Benchtop stability of Phusion Plus DNA Polymerase.** A 0.5 kb target was amplified from 50 ng of human gDNA. Assembled PCR reactions were loaded immediately onto a thermal cycler (0 hr) or set at room temperature for 24 hr before cycling (24 hr). The molecular weight marker is the [GeneRuler 1 kb Plus 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 |
|--|-----------------------------|--------------------------------------|-------------------------------------|
| <b>Fidelity</b> (vs. <i>Taq</i> DNA polymerase)                            | >100x                       | 50x                                  | 50x                                 |
| <b>Hot-start modification</b> (Affibody molecule-mediated)                 | Yes                         | No                                   | Yes                                 |
| <b>Universal annealing temperature</b> (no $T_m$ calculator needed)        | Yes                         | No                                   | No                                  |
| <b>Universal cycling protocol</b> (co-cycling targets of different length) | Yes                         | No                                   | No                                  |
| <b>PCR sensitivity</b>   | +++                         | ++                                   | ++                                  |
| <b>GC-rich amplification</b>   | +++<br>(New GC enhancer)    | ++                                   | ++                                  |
| <b>Inhibitor tolerance</b>   | +++                         | ++                                   | ++                                  |
| <b>PCR yields and specificity</b>  | +++                         | +++                                  | +++                                 |
| <b>Benchtop stability</b> (of assembled reactions)                         | Up to 24 hr                 | N/A                                  | Up to 24 hr                         |
| <b>Stand-alone and master mix formats</b>                                  | Yes                         | Yes                                  | Yes                                 |

Find your upgrade at [thermofisher.com/phusionplus](https://www.thermofisher.com/phusionplus)

# Thank you

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