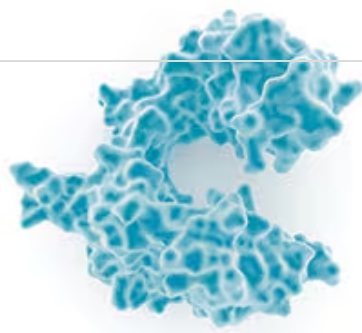




In this article, we discuss the following:

- Tracing the roots: A brief history of PCR
- Hot-start polymerase development
- Pfu: A new phenomenon in PCR
- Phusion DNA Polymerase: Next-Generation PCR technology

Polymerase Chain Reaction (PCR) has revolutionized the field of molecular biology, allowing scientists to amplify DNA sequences for various applications, from genotyping to gene expression analysis. However, the success of PCR was only possible with the evolution of **DNA polymerases**, the enzymes that mediate DNA synthesis during PCR cycles. This article explores the history of modern PCR, from the isolation of the first DNA polymerase to the development of next-generation polymerases such as Thermo Scientific **Phusion DNA polymerases**.

PCR through the ages

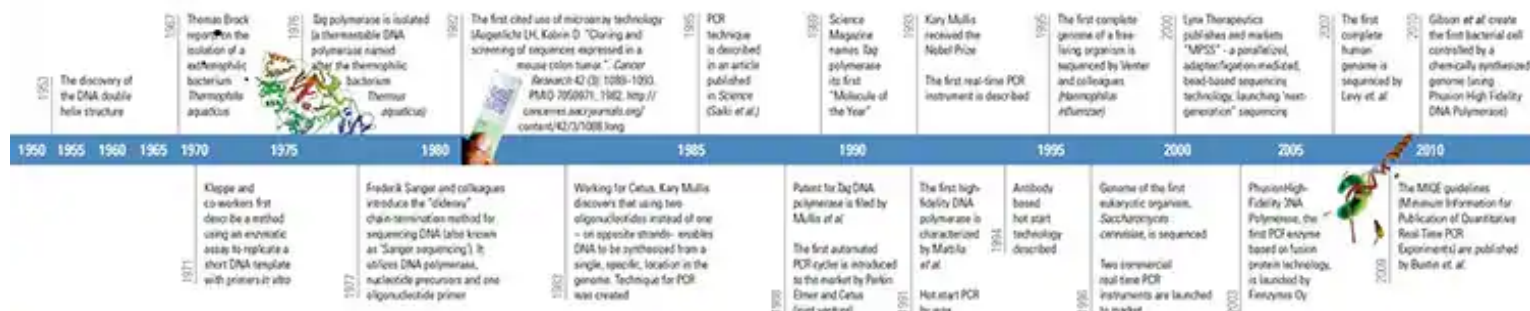


Figure 1. Evolution of PCR (1950–2010). This infographic illustrates the historical development of polymerase chain reaction (PCR) technique over six decades, from its conceptualization in 1950 to its refinement in 2010. The figure highlights the significant contributions of various scientists to the development of PCR and its impact on modern molecular biology.

Tracing the roots: A brief history of PCR

The history of modern PCR begins in 1976 with the isolation of *Taq* DNA polymerase from the thermophilic bacterium *Thermus aquaticus*. Its isolation meant that molecular biologists now had a thermostable enzyme capable of repeat PCR cycling without adding fresh DNA polymerase after each cycle. For those of us who can remember that far back, resetting the PCR reaction as many as 40 times over a 4–5 hour period was not much fun and did not feel like a great use of time, so the *Taq* enzyme made our lives better in so many ways!

Taq DNA polymerase was an instant success; in 1989, Science magazine selected the DNA polymerase molecule as the 'Molecule of the year'. *Taq* enzyme's impact on molecular biology became apparent in 1988 when Kary Mullis and the Cetus Corporation commercialized the enzyme for widespread use. With the introduction of thermal cyclers and commercial *Taq* enzymes, we could finally say goodbye to those laborious afternoons in front of the water bath to amplify DNA.

Although these developments represented significant progress, *Taq* DNA polymerase could have been better. It was error-prone, unstable at high temperatures, and had difficulty amplifying DNA rich in GC content or with complex secondary structures. These factors played a role in the stunted development of PCR early on, particularly in applications that required high specificity and reliability. It quickly became apparent that the development of DNA polymerases was tied to improvements in PCR technology. To increase the performance of PCR and to expand PCR technology to a broader range of applications, there was a need to engineer more advanced DNA polymerases.

Hot-start polymerase development

 *Taq* DNA polymerase, an enzyme traditionally used in PCR, has intrinsic activity at lower temperatures. This activity can lead to nonspecific amplification, false positive results, and lower target yields. Hot-start techniques were introduced in the late 1980s as a solution to overcome performance issues associated with *Taq* DNA polymerase. Methods of hot-start PCR employ an enzyme modifier such as an antibody, affibody, aptamer, or chemical modification to inhibit DNA polymerase activity at room temperature. After the initial denaturation step of PCR, the hot-start-sensitive modification is released, and the polymerase becomes active.

This controlled activation of the polymerase through hot-start techniques offers several advantages. First, it reduces the likelihood of nonspecific amplification during the initial stages of the PCR reaction, leading to cleaner bands and higher yield of the target DNA. The hot-start mechanism also improves assay sensitivity and amplifies only the target fragment. This is particularly important when working with low-copy number targets. Finally, reactions can be assembled at room temperature using a hot-start DNA polymerase and can be left on the bench for a few hours without compromising PCR results. This feature is especially beneficial for high-throughput laboratories working with many samples at a time and using robotics.

Read more on how hot-start technology is beneficial for your PCR

Hot-start polymerases enhanced PCR performance and are now widely used in various applications, including gene expression analysis, genotyping, cloning, and diagnostic testing.

Pfu: A new phenomenon in PCR

In 1991, the isolation and development of Pfu polymerases derived from *Pyrococcus furiosus*, a hyperthermophilic archaeon found in a hydrothermal vent in the Pacific Ocean, marked a significant breakthrough in molecular biology research. Pfu DNA polymerase has built-in 3' to 5' exonuclease proofreading activity, which could correct nucleotide-incorporation errors, lower error rate, and offer increased specificity. The development and use of both Pfu and *Taq* polymerases continued for some time. PCR played a major role in several groundbreaking studies, such as the sequencing of *Haemophilus influenzae* by Venter and colleagues in 1995. However, while useful for many basic applications, these polymerases could not provide the required accuracy, reliability, and read length to push PCR into new and exciting areas.

Phusion DNA Polymerase: Next-Generation PCR technology

The introduction of **Phusion DNA polymerase** in 2003 was the first step in developing next-generation polymerases and high-fidelity PCR. These specially engineered DNA polymerases could overcome or reduce problems that were limiting PCR development. Created by *Pyrococcus*-like enzyme with a thermostable double-strand DNA-binding domain, the first Phusion High Fidelity DNA polymerases possessed a high proofreading activity, higher affinity to the template, and better performance with long amplicons or difficult templates such as templates with GC-rich regions.

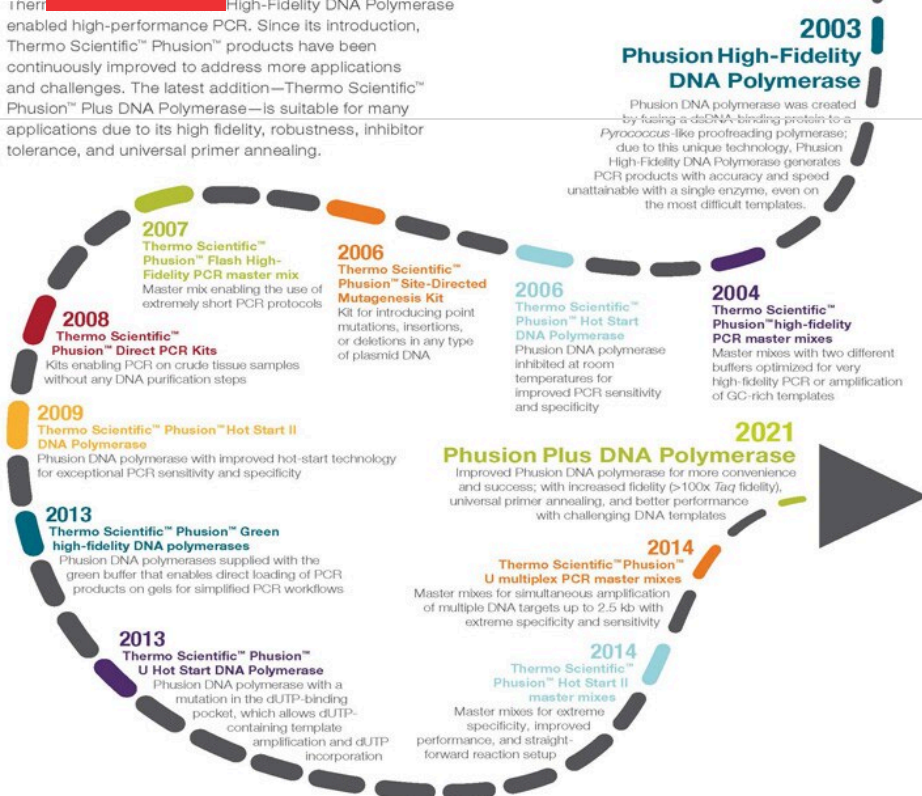
Over the years, Thermo Fisher Scientific has continued to develop Phusion DNA polymerase stand-alone enzymes, master mixes, and kits optimized for different applications, such as site-directed mutagenesis and fast PCR. Recently, **Thermo Scientific Phusion Plus DNA Polymerase** was introduced—an improved version of Phusion DNA Polymerase with increased fidelity (>100X *Taq* polymerase's fidelity), universal primer annealing, and better performance with challenging DNA templates—making it more convenient and easier to use.

Figure 2. A timeline of the development of Phusion high-fidelity DNA polymerases. The first Phusion DNA polymerase was created in 2003 by fusing a dsDNA-binding protein to a *Pyrococcus*-like proofreading polymerase. The result was a high-fidelity enzyme with higher processivity and inhibitor tolerance. Since then, Phusion products have been continuously expanded, such as introducing the Phusion Flash High-Fidelity PCR master mix in 2007, Phusion Direct PCR Kits in 2008, and Phusion Hot Start II DNA Polymerase in 2009, among others. The latest addition is the Phusion Plus DNA polymerase, which provides even higher fidelity, universal primer annealing, and better performance with challenging DNA templates.

Phusion polymerase the power of innovation

ThermoFisher
SCIENTIFIC

Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerase enabled high-performance PCR. Since its introduction, Thermo Scientific™ Phusion™ products have been continuously improved to address more applications and challenges. The latest addition—Thermo Scientific™ Phusion™ Plus DNA Polymerase—is suitable for many applications due to its high fidelity, robustness, inhibitor tolerance, and universal primer annealing.



In summary, the evolution of DNA polymerase has played a crucial role in advancing PCR technology. Advances in engineering DNA polymerases allowed for faster, more reliable, and more accurate PCR amplification opening new possibilities in genetics, medicine, and biotechnology. Phusion DNA polymerase is a prime example of this progress, making it a valuable tool for molecular biologists. The combination of next-generation polymerases and **real-time and digital PCR technologies** has contributed to significant advancements in scientific research and healthcare. With further advancements in DNA polymerase enzymes and PCR methodologies, we can anticipate greater breakthroughs in scientific research that can help positively impact the world.

Resources

Molecular Biology Resource Library

Access valuable support for standard molecular biology techniques from our library of webinars, videos, articles, and more.

Molecular Biology Web Tools

Utilize free online tools to help with primer analysis, reaction setup, biochemical conversions, and calculators.

Product selection guides

[OEM Enzyme Selection](#)

[FastDigest Restriction Enzyme](#)

[PCR Enzymes](#)

[qPCR and PCR Plastics](#)

[TOPO PCR Cloning](#)

Molecular Biology Handbook

Optimize your experiments. Our latest edition is improved with more technical tips, educational tools, and guidance to help you select the right molecular biology products.

Molecular Biology Product Discontinuations

Find an alternate to a discontinued molecular biology product.

Services and Support

Email or call our Technical Application Scientists for additional questions regarding molecular biology products.

