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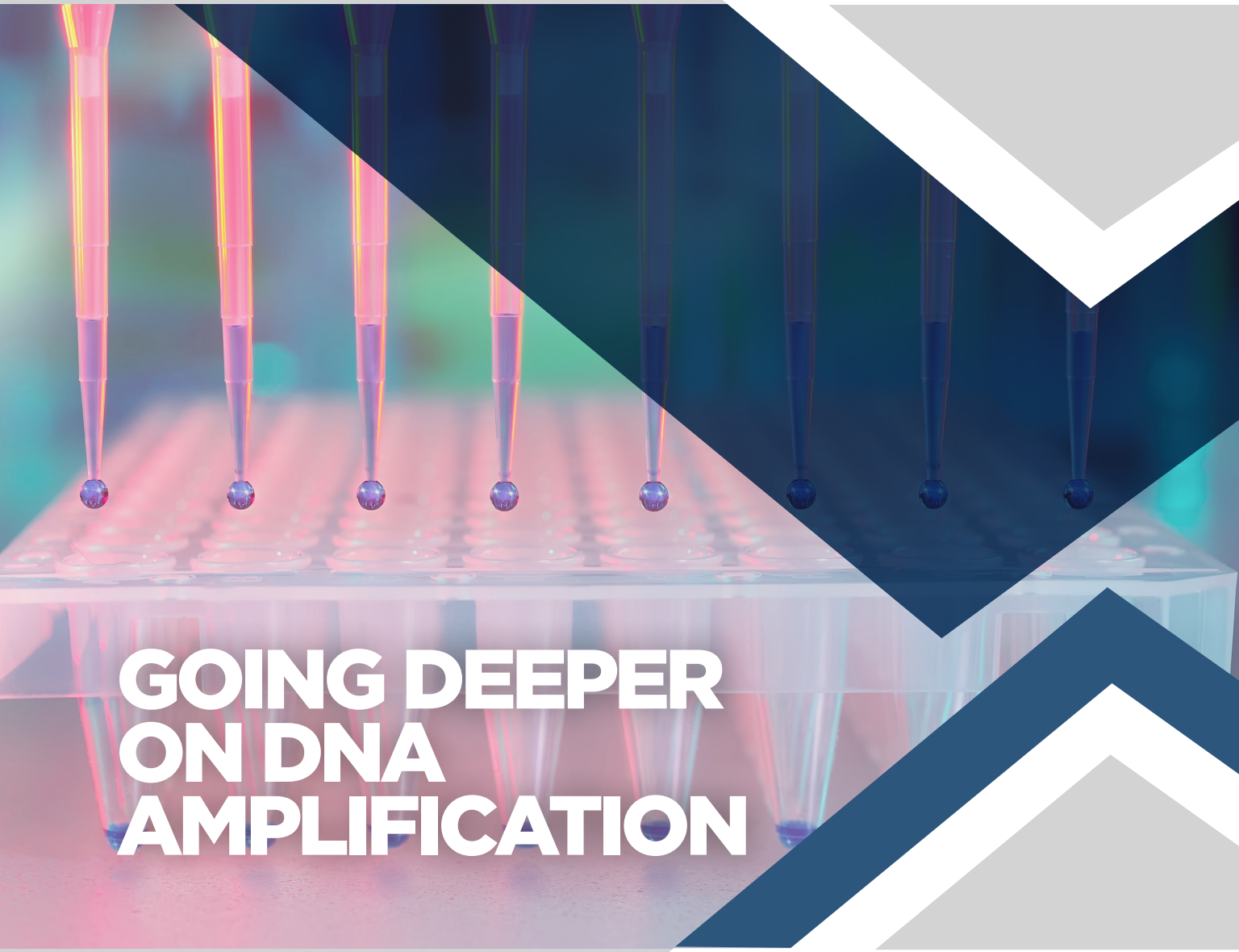

# GOING DEEPER ON DNA AMPLIFICATION

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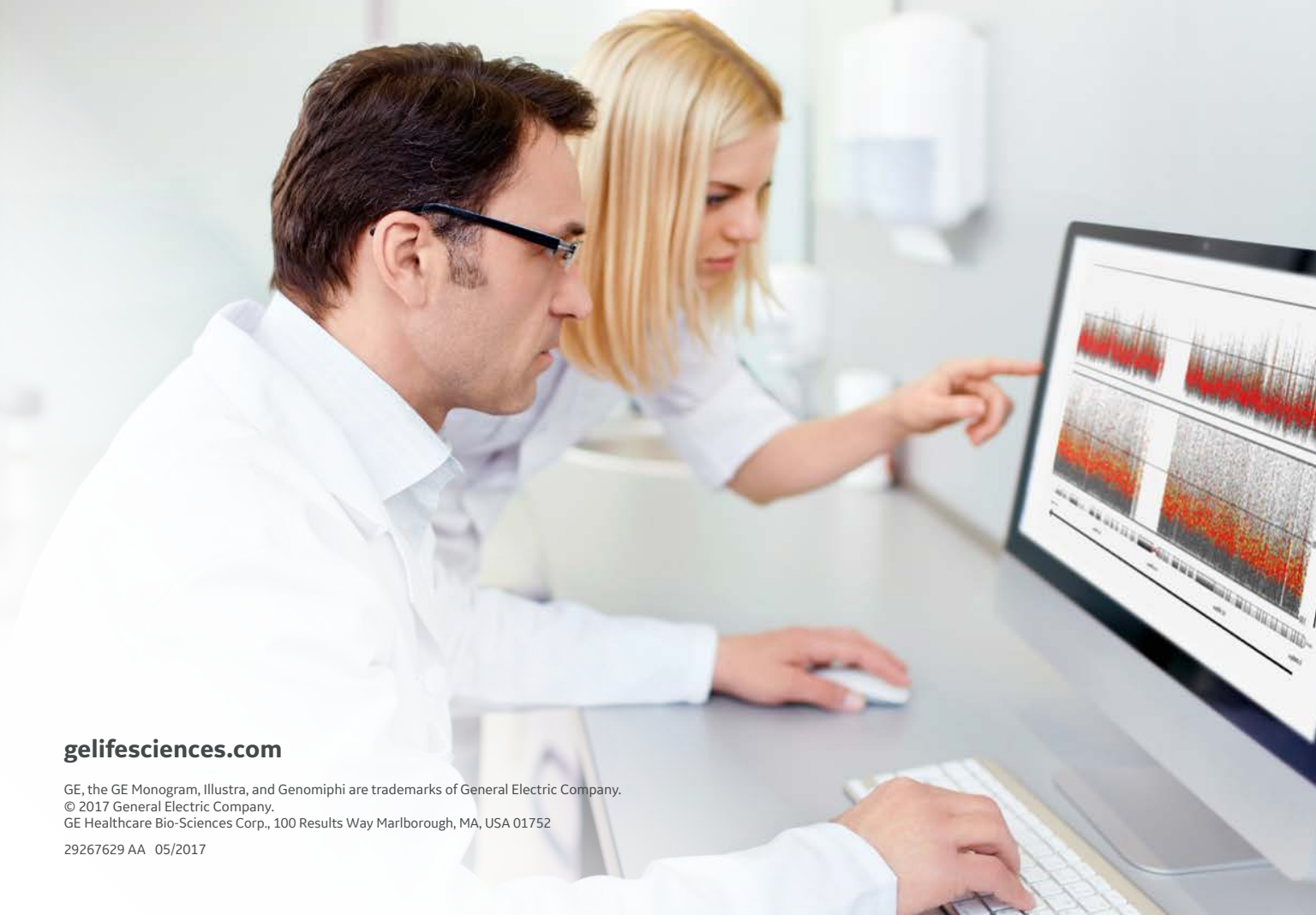

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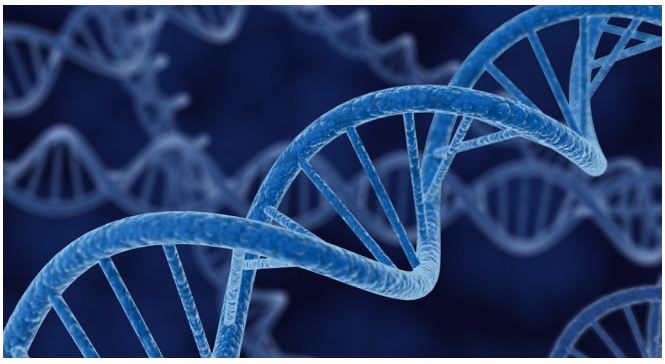
# DNA Amplification: Replicate and Repeat



A common tool of the molecular biologist, the polymerase chain reaction (PCR), uses repeated rounds of thermal cycling to amplify a specified region of DNA for downstream use or analysis. PCR relies on the harmonious cooperation of a variety of molecules to affect efficient amplification. The original strand of DNA serves as the template for strands being built. The amplification machinery, DNA polymerase, is a large protein that binds to single-stranded DNA and adds complementary nucleotides one by one, processively adding bases until it falls off of the strand. The polymerase knows where to bind to the DNA thanks to small (~18–22 nt.) fragments of complementary DNA that signal the start of where the new strands should begin. This amplification process copies each parental DNA strand resulting in daughter strands that contain one newly synthesized strand and one parental strand.

PCR is an iterative process that leads to the exponential amplification of the original target sequence. It proceeds as follows:

**Step 1:** Denature: This first step disrupts the hydrogen bonds between the nucleobases by heating (94–96 °C) the DNA template mixture, resulting in single-stranded DNA strands.

**Step 2:** Anneal: Lowering the reaction temperature (50–67 °C), allows the site-directed primers to bind to the single-stranded DNA template. The annealing temperature depends on the melting temperature of the primers and may need to be optimized.

**Step 3:** Extend: The reaction temperature is raised slightly (typically 72 °C) and free nucleotides in the solution are added to the annealed primers by the enzyme polymerase.

**Step 4:** Repeat: Steps 1 through 3 are repeated, typically 25–35 times, resulting in the exponential amplification of the target DNA segment.

The PCR process allows a small amount of DNA to be simply and accurately amplified in a relatively short amount of time.

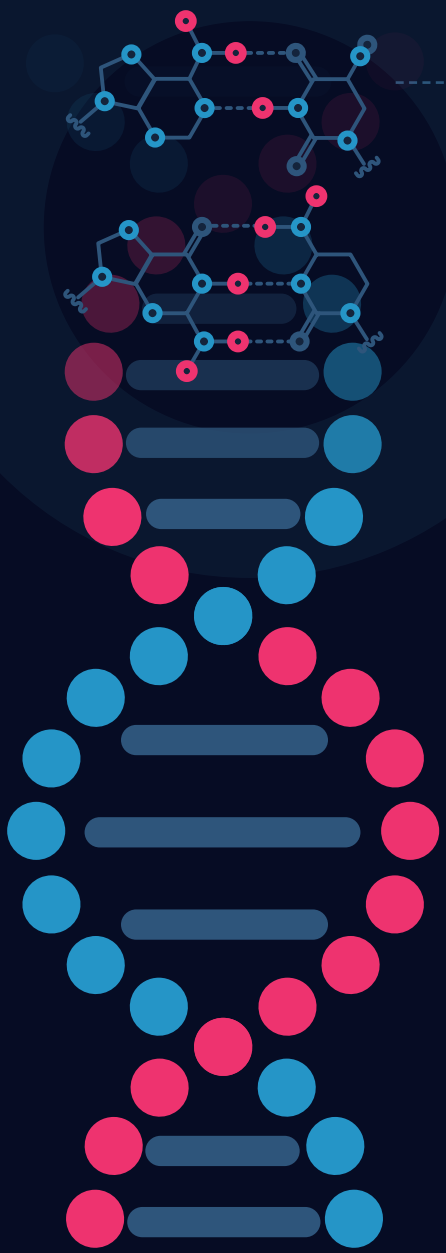
## Prudence & Problems

The introduction of PCR in the '80s revolutionized molecular biology and genetic studies, due to its low cost, sensitivity, and specificity. Despite its widespread acceptance to reliably and efficiently amplify DNA for genotyping, sequencing, cloning, forensics, and diagnostics, interpretation of results should always consider the limitations of the technique.

Firstly, the target sequence must be known in order to enable the design of primers for initiating the amplification step. Technique modifications may be necessary where the starting sequence is unknown. Secondly, primers must be carefully designed for specificity to the target region and do not cross react with another region of DNA within the original sample to ensure only the desired sequence is amplified. Thirdly, polymerases used to amplify DNA have an inherent error rate and may introduce mutations during the PCR process. High fidelity polymerases may be necessary for amplifications when it is necessary to minimize mutation rates or where long-range regions are being amplified. Finally, due to the exponential amplification process, even seemingly trivial amounts of contaminating DNA can result in inaccurate results. Maintaining a clean workflow and the appropriate controls are key components to ensuring reliable amplification results.

# A, T, G, C: THE LANGUAGE OF LIFE

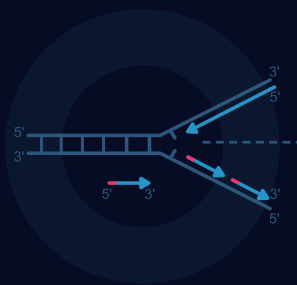
On the surface, learning the language of life seems fairly straightforward; after all, it's composed of just four letters. Its simplicity belies its central importance to the biological sciences. Getting to know DNA, from the bases up, may inspire you to look at the twisty, trusty-old double helix with fresh eyes.



**Adenine (A)**, **Thymine (T)**, **Guanine (G)**, and **Cytosine (C)** are nitrogenous bases that, together with sugar and phosphate residues, constitute nucleotides, the building blocks of deoxyribonucleic acid (DNA). When the nucleotides of one strand of DNA come together with another strand, they form hydrogen bonds that hold them together.

**IMPORTANT!** The two strands of a double helix are built from opposite ends, making them antiparallel. DNA is also right-handed, which refers to the directionality of the helix.

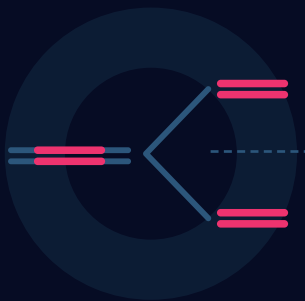

## DNA AMPLIFICATION: FROM ONE, MANY



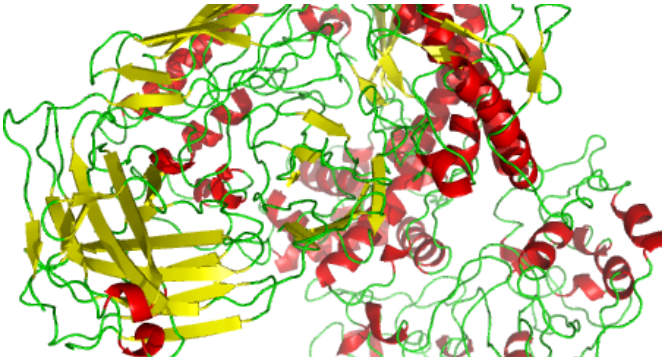

DNA cannot self-replicate. With the help of several enzymes, DNA is unwound, copied, and resealed each and every time a strand of DNA must be generated, which happens every time a cell divides.

**Key Enzymes of DNA Amplification:**

- DNA Helicase: Unwinds the double helix
- DNA Polymerase: Adds nucleotides to the 3′ end of the new strand
- DNA Ligase: Seals the nicks left in the DNA



In the laboratory, isolated DNA is frequently amplified to increase the amount of a particular region of DNA. This most commonly involves the polymerase chain reaction (PCR), a series of temperature gradients designed to break the hydrogen bonds of the double helix, enable binding of specific primers, and allow for amplification to proceed. It's an iterative process that yields an exponential increase in DNA.



# Pick Your Polymerase

The humble polymerase, responsible for synthesizing long chains of nucleic acids, is the lifeblood of DNA amplification. For many, choosing a polymerase for their reaction is as simple as picking a tube of *Taq* polymerase out of the freezer, but fully understanding the pros and cons of different DNA polymerases can improve your amplification process.

## Mr. Popular: *Taq*

*Taq* polymerase, originally isolated from the thermophilic bacteria *Thermus aquaticus*, is the most widely-used polymerase for DNA amplification. When first discovered, its thermostability heralded a breakthrough for PCR, as it meant that fresh polymerase no longer had to be introduced after every denaturation cycle. *Taq* polymerase operates optimally around 75 °C, which means the entire PCR amplification process has to be run at high temperatures, typically above 50 °C. This augments primer specificity and helps limit non-specific primer dimerization, which can occur at lower temperatures.

*Taq* polymerase is widely available, stable, and efficient, but it has one major drawback: it lacks proofreading activity. It has been found to have an error rate of $3 \times 10^{-5}$ per base.[1] This is not a major concern for most applications, as standard PCR amplicons do not exceed a few hundred base pairs in size, and any transcription errors may not affect the production or detection of the end-product.[2]

## High-fidelity DNA polymerases

However, DNA replication fidelity is critical for certain applications, such as cloning for protein production, single-nucleotide pair (SNP) mutation analysis, or DNA sequencing applications. For these amplicons and assays, a high-fidelity DNA polymerase should be used.

High-fidelity DNA polymerases innately possess "proofreading" $3' \rightarrow 5'$ exonuclease activity. When the polymerase detects a misincorporated base, it transfers the strand to the active site of the exonuclease domain for removal. The new strand then returns to the polymerase domain and resumes replication.

High-fidelity DNA polymerases also offer accuracy-augmenting structural and kinetic properties that facilitate a 99.6% incorporation rate for matches and a 99.9% release rate for mismatches,[3] resulting in an error rate between $1 \times 10^{-6}$ and $4 \times 10^{-7}$.[1] Since high-fidelity polymerases such as *Pfu* polymerase (from the thermophile bacteria *Pyrococcus furiosus*) possess the same or better thermostability as *Taq* polymerase, they are excellently positioned for thermocycler-based amplifications requiring high transcriptional accuracy.

## Isothermal DNA polymerases

Isothermal amplification offers advantages in amplification magnitude and speed. They do not require thermocyclers and are well suited for ambient-temperature applications. As such, they present a low-cost and low-energy footprint alternative for situations where advanced infrastructure is not present.

Isothermal amplification techniques mostly use strand-displacing DNA polymerases with helicase activity to expose single strands for replication. As such, specific primers are not required. One notable isothermal polymerase is Phi29, which is especially suitable for multiple displacement amplification (MDA), a technique that generates significantly more end-product than traditional PCR methods[4] and it is capable of producing large fragments. Phi29 possesses an extremely fast DNA synthesis rate due to a strong affinity for single-stranded DNA, a high processivity without an accessory protein, and contains a proofreading domain resulting in an error rate of $1 \times 10^{-6}$ to $1 \times 10^{-7}$ per base.[5,6]

## The Right Tool for the Right Amplification

Ultimately, each DNA polymerase presents its own strengths and weaknesses. Make sure you have the right one for your research needs.

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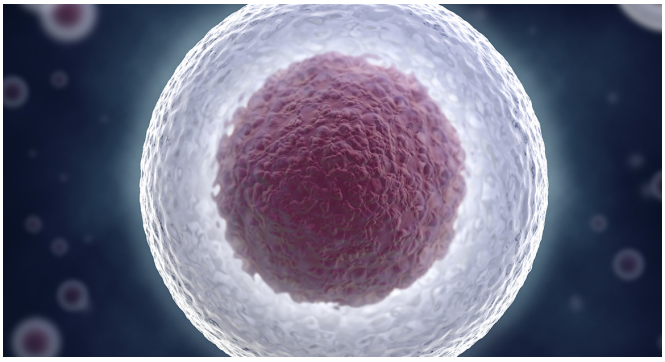

# One Cell at a Time: Whole-Genome Amplification

Whole-genome amplification (WGA) is a process designed to amplify an entire genome in order to facilitate experimentation and analysis. WGA starts with nanogram-levels of DNA and yields microgram quantities, making it vital to providing sufficient material for effective single-cell analysis studies.

## Some like it hot: thermostable polymerase methods

WGA can be performed using thermostable PCR-based techniques and isothermal polymerases. The most popular PCR-based techniques are degenerate oligonucleotide-primed PCR (DOP-PCR) and primer-extension-preamplification (PEP)-PCR. In DOP-PCR, primers bind randomly throughout the genome. These primers contain degenerate bases—bases that can bind multiple nucleic acids—in order to increase the number of potential binding sites. This interaction is promoted by low-temperature cycles at the start of the PCR. Following initial annealing, high-temperature cycles then restrict amplification specificity only to areas where annealing has occurred. In addition, the higher temperatures favor increased polymerase activity.[1]

In PEP-PCR, primers consisting entirely of degenerate bases are used to greatly increase the number of potential priming sites. Here, the annealing step for each PCR cycle begins at a low temperature to promote primer binding and then ramps to higher temperatures to increase amplification specificity. Since PEP-PCR uses completely random primers, there are not only more binding sites along the template, but their distribution is more uniform, leading to less amplification bias than DOP-PCR.[2]

## Others play it cool: isothermal amplification

In contrast to PCR-based methods, isothermal protocols, such as multiple displacement amplification (MDA), use high-fidelity polymerases with innate proofreading capabilities to quickly and efficiently amplify DNA templates at ambient temperatures. MDA begins with random hexamer primers to anneal to template DNA. Transcription then begins at those annealing sites. When the polymerase reaches another annealing site, it debranches the newly synthesized strand and continues along the original template strand. Primers then anneal to the debranched strand, facilitating additional transcription and amplification. Finally, nucleases cleave branches at displacement sites, generating DNA fragments for sequencing.[3,4]

PCR-based methods present an inherent drawback in their use of thermostable polymerases. *Taq*, the most common thermostable polymerase, does not possess proofreading capabilities, increasing the transcriptional error rate. Efforts have been made to improve these techniques by introducing a proofreading polymerase alongside *Taq*.[2] In contrast, isothermal protocols result in lower error rates, greater yields, and are faster than thermocycler-based protocols because no temperature cycling is required. In addition, isothermal polymerases such as Phi29 do not require extremely high temperatures for inactivation, resulting in less DNA damage.[4]

## Tricks of the trade: the potential of WGA

When utilizing either isothermal or thermostable WGA procedures, there are various biases that can be introduced due to template degradation, polymerase artifacts, DNA chimera formation, and non-uniform primer annealing. Researchers need to carefully examine their finished product to make sure that their amplified product matches the original template.[5]

Nonetheless, WGA offers a solution for critical situations where starting sample is scarce or where resources are limited. Amplified DNA can be used in many downstream applications, including next-generation sequencing, mutation/single-nucleotide polymorphism (SNP) analysis, multi-locus investigations, and comparative genomic hybridization.

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# When consistency in your DNA amplification matters...

Illustra™ PuReTaq Ready-To-Go™ PCR Beads are pre-mixed, pre-dispensed, single-dose reactions optimized for performing standard PCR amplifications.

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