Polymerase Chain Reaction

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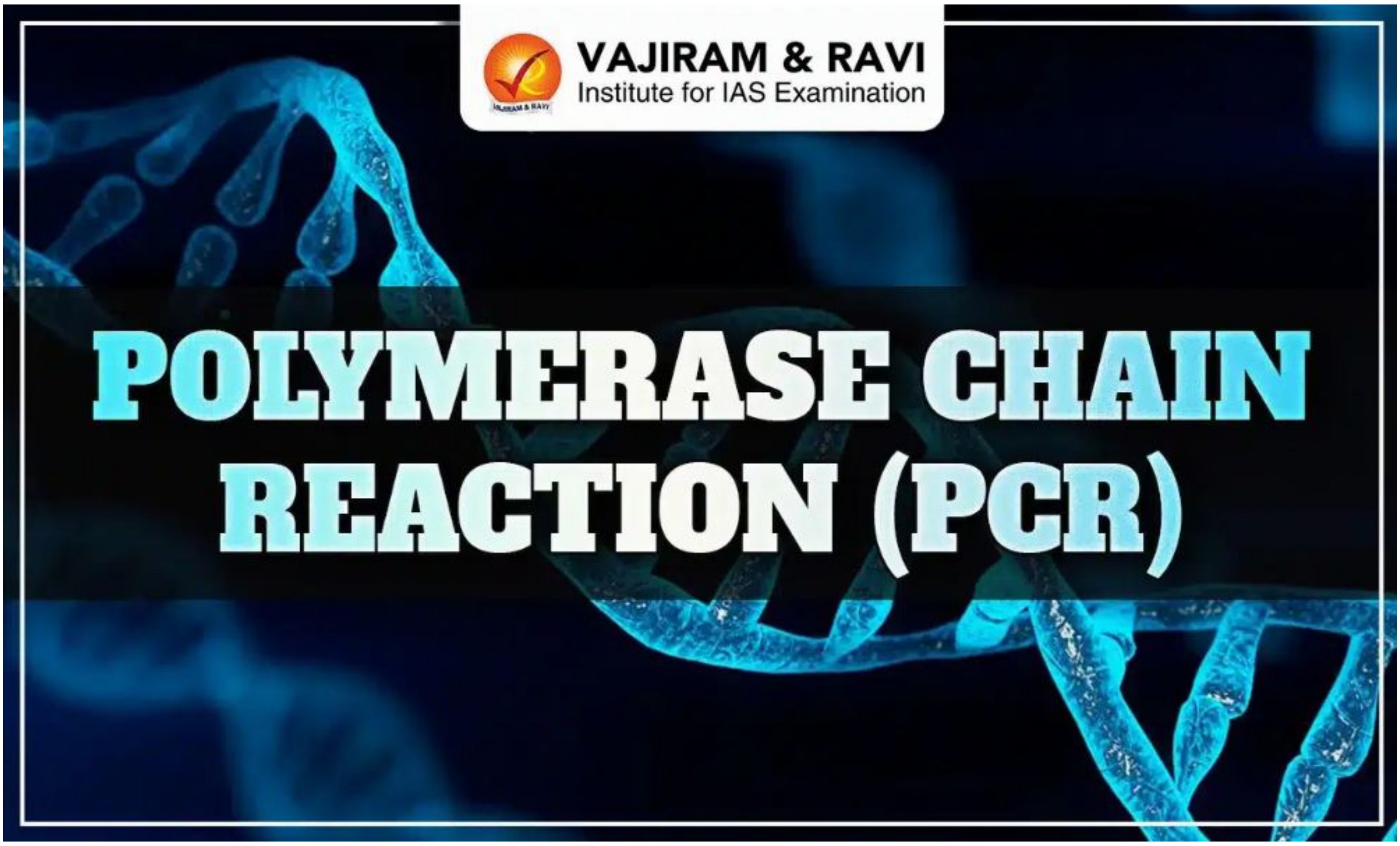


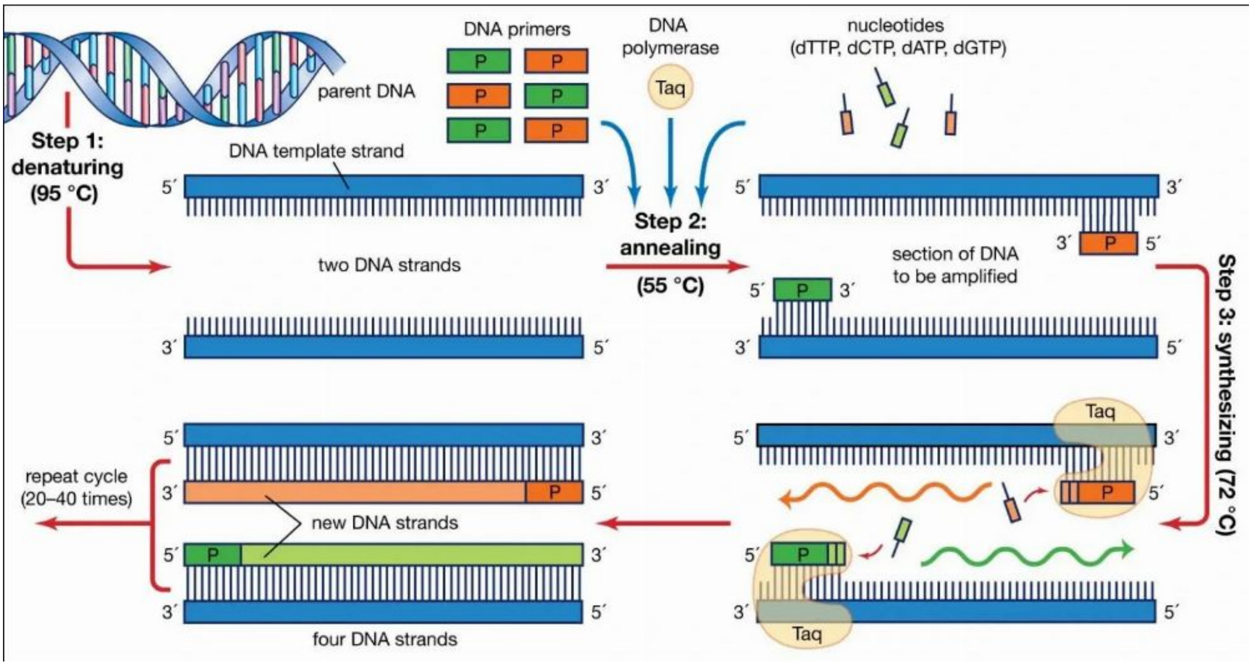
Polymerase Chain Reaction (PCR) has been an indispensable tool in the field of genetic engineering enabling amplification of DNA sequences in vitro. Invented by **Kary Mullis in 1983**, PCR allows exponential copying of minute quantities of DNA through **thermocycling and enzyme-mediated replication.** By generating billions of copies from just a few molecules, Polymerase Chain Reaction catalyzed genetic engineering by facilitating gene isolation, cloning, sequencing and targeted mutagenesis.

Refinements like real-time **PCR, RT-PCR** and assembly PCR have expanded their applications in **recombinant DNA technology, synthetic biology** and **genome editing**. By enabling amplification and manipulation of DNA in vitro, Polymerase Chain Reaction has been indispensable for the rapid growth of genetic engineering across medicine, biotechnology and life.

What is Polymerase Chain Reaction?

A standard Polymerase Chain Reaction (PCR) is an **in-vitro** method that allows a single, short region of a DNA molecule (single gene perhaps) to be copied multiple times by **Taq Polymerase.** Key aspects of the Polymerase Chain Reaction (PCR):

- **Discovery of PCR:** In **1983, Kary Mullis** conceptualized and later demonstrated PCR while working at the Cetus Corporation. This breakthrough discovery earned him the **Nobel Prize in 1993.**
- **Concept of PCR:** It involves **repetitive cycles** of controlled heating and cooling of the reaction mixture to alternately melt, anneal and extend short **oligonucleotide primers** that flank the target DNA region of interest.
- **Mechanism of PCR:** It relies on thermal cycling, exploitation of a **DNA polymerase enzyme** and primers that initiate/limit DNA synthesis.
- **Key Reagents in PCR:** Template DNA, primers, DNA polymerase enzyme, nucleotides (dNTPs), buffer and magnesium ions.
- **Polymerase Chain Reaction Equipment:** Thermal cycler machine with controllable heating, cooling and holding; tubes/plates to hold reagents.
- **PCR Cycling Steps:** Initialisation (hot start), denaturation, annealing, extension and final elongation. Each cycle doubles the number of DNA copies.
- **Exponential Amplification:** Even a single copy of the template can be amplified to **~108** or more copies **after 30-40 cycles,** enabling detection and analysis.

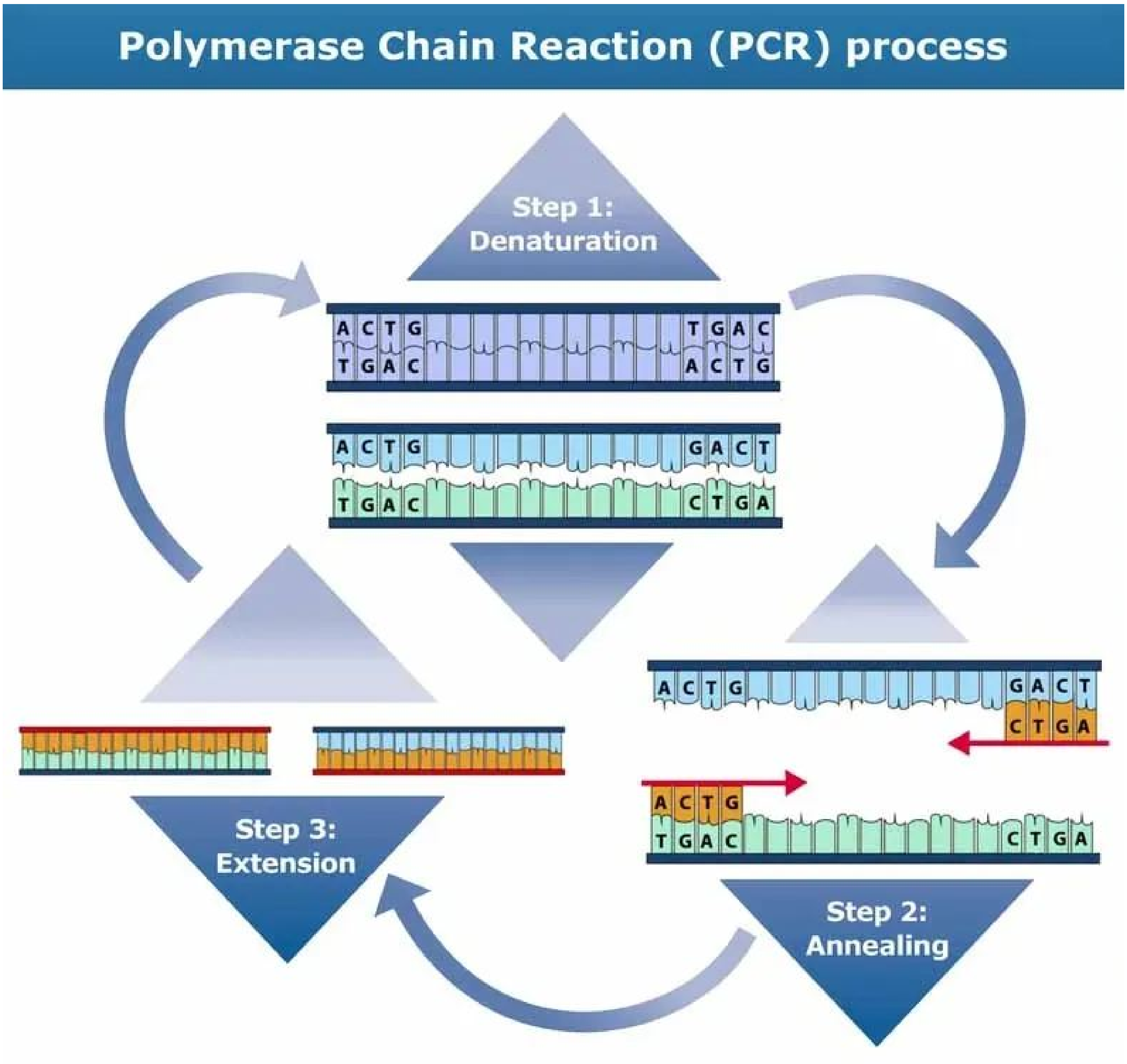


Steps in Polymerase Chain Reaction

While Polymerase Chain Reaction is conceptually simple, careful experimental design and optimization are vital for success. Key steps include:

- **Denaturation:**
  - This initial heating step **(90-96°C)** separates the **double-stranded DNA template into single strands**, a prerequisite for primers to anneal in the next step.
  - Higher initial denaturation temperatures (~98°C) ensure complete strand separation, especially for **GC-rich templates**. Initial denaturation times range from 30 seconds to 3 minutes depending on sample type, template complexity, and reaction volume.
- **Annealing:**

    - The reaction temperature is lowered to **50-60°C** to enable **primer annealing** to complementary sequences flanking the target DNA segment.
    - The annealing temperature is optimised based on **primer length** and **composition** - usually around **2-5°C below** the primer melting temperature (Tm).
    - Insufficient annealing hampers **primer binding, reducing yield**, while excessive annealing promotes nonspecific binding and artefacts.
- **Extension:**
    - Raising the temperature to **~72°C enables Taq polymerase** to synthesise new strands by adding dNTPs complementary to the template.
    - The extension time depends on the **amplicon length** and **polymerase** used. Taq polymerase typically adds nucleotides at **~1000 bases/minute.**
    - Longer extensions (2-3 minutes) are used for longer amplicons or with slower polymerases.
- **Repeated Cycling:**
    - Repeating **~25-40 cycles** exponentially amplify the target segment framed by the primers.
    - The cycle number is optimised based on the starting **template amount** and **amplicon length**.
    - **Excessive cycling** should be avoided to minimise non-specific products and artefacts.
- **Final extension:**
    - After the repeated cycles, a **final 5-15-minute** extension step at **72°C** ensures all **single-stranded products** are fully extended. This maximizes the final yield.
- **Optimisation:**
    - While Polymerase Chain Reaction is straightforward in principle, optimal conditions (annealing temperature, cycle number, extension time etc.) must be empirically determined for **each target** and **primer pair**.



Types of Polymerase Chain Reaction and their Applications

Various PCR modifications and alternative techniques have emerged over the years for specialised applications:

PCR Type	Key Features	Applications
Standard PCR	The original PCR method. Used for routine DNA amplification.	General lab applications like **cloning, sequencing** etc.
Real-time Polymerase Chain Reaction	- Allows real-time monitoring of DNA amplification through fluorescence. - Can quantify the starting template.	**Gene expression studies,pathogen detection** and **diagnostics**.

Reverse Transcription-PCR (RT-PCR)	- Converts **RNA to cDNA** before normal PCR amplification. - Used to study gene expression.	**Viral detection**, **cancer biomarker discovery** etc.
Multiplex PCR	- Amplifies multiple DNA regions simultaneously using primer multiplets. - Allows high-throughput DNA analysis.	**for Genetic disease testing**, agricultural **GMO detection** etc.
Nested PCR	Uses two sets of primers in two successive runs for enhanced specificity.	Forensic **DNA fingerprinting,paleo genomics** etc.
Assembly PCR	Assembles long DNA constructs from oligo building blocks.	**Synthetic biology** and **metabolic engineering.**

Significance of Polymerase Chain Reaction

Polymerase chain reaction (PCR) is a technique that enables the detection of small amounts of DNA. It has significantly impacted various fields, including genomics, forensics, diagnostics, archaeology, and palaeontology. The following are some of the key points that highlight the importance of PCR:

- Polymerase chain reaction has played an essential role in various fields of molecular biology and genomics, including **molecular cloning, DNA sequencing, and gene expression analysis.**
- It has revolutionised **forensic investigations**, allowing the identification of criminals from tiny traces of DNA evidence left at crime scenes.
- PCR has also transformed **clinical microbiology** and infectious disease management by enabling the diagnosis of infectious diseases through the detection of pathogen DNA signatures.
- It has facilitated **paternity testing, genetic fingerprinting,** and DNA databasing for forensic identification.
- Polymerase chain reaction has enabled the **DNA barcoding of species,** catalysing metagenomics studies of environmental microbial diversity.
- PCR has democratised access to genomic information by enabling the generation of vast DNA data from minute samples.

Applications of Polymerase Chain Reaction

PCR has a wide range of specialised applications and is used by scientists in all fields of biology. It has become an indispensable tool in molecular biology. Here are some of the key applications of Polymerase Chain Reaction:

- **Medical diagnostics:** PCR is used to detect trace amounts of viral or bacterial DNA/RNA in patient samples,
    - Enabling the diagnosis of infectious diseases like **COVID-19, HIV, and hepatitis.**

- **Clinical research:** PCR is used to analyze gene sequences and mutations associated with genetic disorders.
    - Like **cystic fibrosis and thalassemia,** which is crucial for developing diagnostics and treatments.
- **Forensics:** It is used to amplify tiny samples of DNA collected from crime scenes to identify suspects through DNA fingerprinting and establish forensic evidence.
- **Agriculture:** Polymerase Chain Reaction is used to detect the presence of **GMOs, plant pathogens, antibiotic resistance markers**, and more in crops and soil, which is important for biosecurity.
- **Paleogenomics:** It is used to amplify ancient DNA from fossils and artefacts to study evolution, migrations, and relationships between extinct species.
- **Metagenomics:** It is used to sequence microbial communities in environmental samples to catalogue biodiversity.
- **Synthetic biology:** PCR is used to assemble DNA fragments and create artificial genes and engineered biological systems.
- **Food safety:** It is used to **screen food items** for contaminating microorganisms or allergens.

Thus, Polymerase Chain Reaction has become an indispensable technology across diverse domains including medicine, forensic science, biological research, agriculture, and biotechnology. Its sensitivity and specificity to amplify trace amounts of DNA has wide-ranging applications.
