Polymerase Chain Reaction

31

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Abstract

Polymerase chain reaction or PCR is a method of multiplying DNA copies and was invented in 1983. This process depends on denaturation of DNA at high temperatures, annealing of primers to the DNA, and elongation and synthesis of new DNA strands by the heat-resistant *Thermophilus aquaticus* polymerase. It utilizes nucleotides and magnesium chloride as a cofactor. PCR can generate more than 30 billion copies of DNA in a couple of hours. Its advanced method, real-time PCR (RT-PCR), can quantify simultaneously while multiplying the DNA and is, hence, also known as quantitative PCR (qPCR). It uses fluorescence-emitting dyes such as SYBR green and TaqMan to quantify DNA multiplication. PCR is used in forensics to multiply DNA evidence, in diagnosing genetic diseases and mutations from body fluids like saliva and blood, and in genetic engineering.

Keywords

Polymerase chain reaction (PCR) · Real-time PCR · qPCR · SYBR green · TaqMan

31.1 Introduction

Polymerase chain reaction (PCR) or the process of multiplying DNA copies was invented by Dr. Kary Mullis in 1983. The DNA of interest may come from any source available such as blood, saliva, fossil, or a crime scene.

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31.2 Utility of PCR

- To generate a high quantity of the desired DNA which can be used for forensic testing.
- To detect mutations in the amplified DNA copies by further downstream processes like electrophoresis.
- To diagnose diseases such as sickle cell anemia.
- To utilize the DNA in gene cloning or genetic engineering.

31.3 Materials Required for a PCR Reaction

- Thermocycler: A thermal device which rapidly changes temperature as per the settings.
- *Thermophilus aquaticus* (Taq) polymerase.
- Water.
- Primers are oligonucleotides which bind to the complementary sequence of single-stranded DNA and act as starting points.
- Nucleotides which are the building blocks of DNA (dATP, dGTP, dCTP, dTTP).
- Magnesium chloride acts as a cofactor and catalyzes the PCR.
- The number of copies generated can be calculated by the formula $2^n - 2n$. For example, if the PCR runs 35 times in a couple of hours, it generates $2^{35} - 70$ copies of the desired gene, i.e. 34,359,738,368 – 70 = 34,359,738,298 copies (Table 31.1).

31.4 Real-Time PCR

- Real-time PCR (RT-PCR) was developed in 1992 by Higuchi et al. Real-time PCR, as the name suggests, amplifies as well as analyzes the DNA simultaneously, i.e., in real time.
- In conventional PCR, to analyze the product, a separate process has to be run such as gel electrophoresis. Electrophoresis separates the products as per their size by making the DNA run toward a positive pole inside a gel, DNA being inherently negatively charged.

Table 31.1 Steps of polymerase chain reaction

Sequence	Step name	Temperature	Process
1	Denaturation	96 °C	DNA strands separate
2	Annealing	56 °C	Primers bind to the corresponding site on both strands of DNA
3	Elongation	72 °C	Taq polymerase utilizes nucleotides to synthesize new DNA
1, 2, 3	Repeat for around 35 cycles		

Taq *Thermophilus aquaticus*-derived DNA polymerase which is heat resistant

31.4.1 Principles of RT-PCR

- Dye such as SYBR green is included in the PCR mixture. This dye binds to the amplified DNA.
- Fluorescence emitted by the dye is measured, and its intensity lets us know the quantity (if any). In case the desired DNA has not been amplified, no fluorescence is detected.
- Therefore, the amount of product synthesized is proportional to the fluorescence emitted. For this reason, RT-PCR is also known as *quantitative PCR (qPCR)*.
- RT-PCR estimates the quantity of DNA produced as well, whereas conventional PCR-electrophoresis only estimates quality, i.e., tells us if desired DNA is present or not.

31.5 Labels Used to Quantify DNA Multiplication

- **SYBR green**
  - Fluorophores with intrinsically strong fluorescence such as SYBR green, which emit fluorescence on binding to the minor groove of DNA. The fluorescence is 1000 times more upon binding than in the resting phase. There are chances of less sensitivity due to binding additionally to primer-dimer products. SYBR green is cost-effective.
- **Hydrolysis-based labels such as TaqMan**
  - These are oligonucleotides and have a quencher at one end and detector at the other end. The detector emits fluorescence even at baseline, but this is absorbed by the quencher.
  - When the Taq polymerase elongates sequences in each cycle of PCR, Taqman probe utilizes the endonuclease activity of Taq polymerase. When the oligonucleotide breaks, the quencher and detector separate, and now the fluorescence is no longer "quenched" but detected in proportion to the amount of DNA produced.

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