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POLYMERASE CHAIN REACTION IN AGRICULTURAL TECHNOLOGY

Popular Article

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ABSTRACT

Polymerase chain reaction (PCR) is the quick, easy method for generating unlimited copies of any fragment of DNA. This method relies on the exponential amplification of specific DNA fragments, resulting in millions of copies that can serve as templates for different kinds of analyses. The polymerase chain reaction has been elaborated in many ways since its introduction and is now commonly used for a wide variety of applications including genotyping, cloning, mutation detection, sequencing, microarrays, forensics, and paternity testing.

Introduction

Polymerase chain reaction (PCR) is a technique used to exponentially amplify a specific target DNA sequence, allowing for the isolation, sequencing, or cloning of a single sequence among many. PCR was developed in 1983 by Kary Mullis, who received a Nobel Prize in chemistry in 1993 for his invention. Typically, PCR is designed of 20-40 repeated thermal cycles, with each cycle consisting of 3 discrete temperature steps. The thermal cycles are often proceeded by a temperature at a high range (>90°C), and followed by final product extension and brief storage at 4°C. In PCR cycles, the temperatures and the duration of each cycle is determined based on various parameters like the type of DNA polymerase used, the melting temperature (Tm) of the primers, concentration of divalent ions and dNTPs in the reaction etc.

Basic steps in Polymerase Chain Reaction: following essential Components and reagents:

- Template DNA containing the target DNA region, to be amplified.
- Primers that are complementary to the 5' ends of each of the sense (Forward primer) and anti-sense strand of the DNA target (Reverse primer).
- *Taq* polymerase an enzyme which apmplifies the target DNA.
- Deoxyribonucleotide triphosphates (dNTPs), which are the building-blocks for a newly synthesizing DNA strand.
- Buffer solutions to provide a suitable chemical conditions for optimum activity and stability of the DNA polymerases.
- Divalent cations (eg. magnesium or manganese ions). They act as a co-factor for Taq polymerase which increases its polymerase activity. Generally Mg^{2+} is used, but Mn^{2+} can be applied to achieve PCR-mediated DNA mutagenesis. This is because higher Mn^{2+} concentration leads to higher error rate during DNA synthesis.

PCR involes various steps namely: 1) Initial Denaturation. 2) Denaturation. 3) Annealing. 4) Extension. 5) Final extension.

Initial denaturation: Involves heating up of reaction to a temperature of 94–96°C for 7-10 minutes (or 98°C if extremely thermostable polymerases are used). Initial heating for such a long duration also helps in gradual and proper unfolding of the genomic DNA and subsequent denaturation. Thus exposing target DNA sequence to the corresponding primers.

Denaturation: Requires heating the reaction mixture upto 94–98°C for 20–30 seconds. It helps in melting of the DNA template by disrupting the hydrogen bonds

between complementary bases, by yielding single-stranded DNA molecules.

Annealing: Following the separation of two DNA strands during denaturation, the temperature of the reaction mix is lowered to 50–65°C for 20–50 seconds to allow annealing of the primers to the single-stranded DNA templates. Typically the annealing temperature should be about 3-5°C below the T_m of the primers. Stable complimentary binding are only formed between the primer sequence and the template when there is a high sequence complimentarity between them. The polymerase enzymes initiate the replication from 3' end of the primer towards the 5'end of it.

Extension /elongation: This step addition of dNTPs to the 3' end of primer with the help of DNA polymerase enzyme. The type of DNA polymerase applied in the reaction determines the optimum extension temperature at this step. DNA polymerase synthesizes a new DNA strand complementary to its template strand by addition of dNTPs, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. Conventionally, at its optimum temperature, DNA polymerase can add up to a thousand bases per minute. The amount of DNA target is exponentially amplified under the optimum condition of elongation step. The drawback of Taq polymerase is its relatively low replication fidelity. It lacks a 3' to 5' exonuclease proofreading activity, and has an error rate measured at about 1 in 9,000 nucleotides.

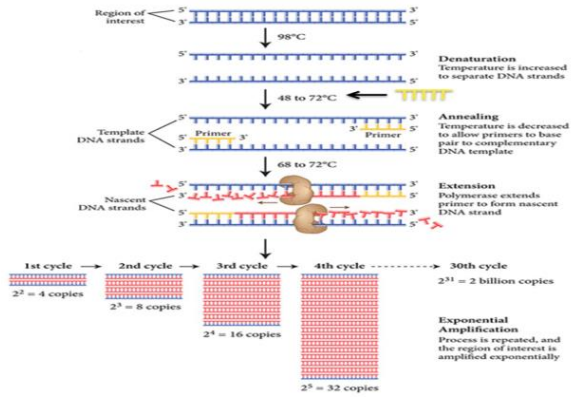

Fig 1. The sequential steps of PCR

Final elongation & Hold: This step is occasionally performed for 5–15 minutes at a temperature of 70–74°C after the last PCR cycle to ensure amplification of any remaining single-stranded DNA.

Final hold step at 4°C may be done for short-term storage of the reaction mixture.

After around 25-30 cycles of Denaturation, Annealing and Extension, there will be over a billion fragments that contain only your target sequence. This will yield a solution of nearly pure target sequence. To check the desired PCR amplification of the target DNA fragment (also sometimes referred to as the amplicon or amplimer), agarose gel electrophoresis is employed for separation of the PCR products based on their size. The determination of size(s) of PCR products is performed by comparing with a DNA ladder, which contains DNA fragments of known size.

Conclusion

The discovery of Polymerase Chain Reaction (PCR) introduced a technological advancement and has become an essential research and diagnostic tool for improving knowledge regarding identification, characterization, detection and diagnosis by increasing the sensitivity, precision and accuracy of the diagnosis. PCR technology allows scientists to take a specimen of genetic material, even from just one cell, copy its genetic sequence over and over, and generate a test sample sufficient to detect the presence or absence of a specific virus, bacterium or any particular sequence of genetic material. Therefore, it is hard to exaggerate the impact of the polymerase chain reaction.

PCR, the quick, easy method for generating unlimited copies of any fragment of DNA, is one of those scientific developments that actually deserve timeworn superlatives like "revolutionary" and "breakthrough".

Medical research and clinical medicine are profiting from PCR mainly in two areas: detection of infectious disease organisms and detection of variations and mutations in genes, especially human genes and it can also be more accurate than standard tests.

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