Al-Mustaqbal University
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Department

جامـــــعة المـــــستـقبـل
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كلية العـــــــلوم
قـســـــــم علوم الأنظمة الطبية الذكية

LECTURE: (5)

Subject: Molecular Biology
Level: Second
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Polymerase Chain Reaction(PCR)

Introduction

Advanced molecular technology has become a crucial tool for identifying new genes with importance in medicine, agriculture, animal production, health, environment, industry other related areas. Among the applications of molecular techniques is important to highlight the use of the Polymerase Chain Reaction (PCR) in the identification and characterization of viral, bacterial, parasitic and fungal agents. PCR is a process used in molecular biology to amplify a single copyor a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. Mechanisms involved in this methodology are similar to those occurring in vivo during DNA replication

Definition of PCR It is a genetic technique that occurs in vitro which allows the enzymatic synthesis of large quantities (amplification) of a targeted region of DNA in exponential manner. DNA is synthesized in the same manner as that seen in vivo (in the cells) using a DNA polymerase (enzymes that cells use to replicate their DNA) .

Types of PCR

• Conventional (Qualitative)PCR. • Multiplex PCR. • Nested PCR. • RT-PCR and qRT-PCR. • Hot-start PCR. • Touchdown PCR. • Assembly PCR. • Colony PCR.

•LAMP assay . •Methylation-specific PCR

Uses of PCR

1- Medicine: detecting infectious organisms, discovering variations and mutations in genes.
2- Genome Projects: DNA sequencing 3- The law: Genetic fingerprinting
4- Evolutionary biology: taxonomic classification 5- Zoology: research on animal behavior
6- Ecology: studies on seed dispersal, reducing illegal trade in endangered species, monitoring release of GMOs
7-Archaeology and paleontology: ancient DNA, analyzing geneticvariations in animals and plants .

Advantages of PCR

1. Simple technique .
2. Sensitivity .
3. Specificity
4. Fast technique .
5. Versatility

Applications of PCR

1. Detecting pathogens using genome-specific primer pairs in clinical samples.
2. Detection of viral pathogens and other microorganisms which persist in low levels in infected cells and are difficult to be identified by routine methods such as HIV or HPV
3. Diagnosis of genetic disorders such as hemophilia, sickle cell anemia and thalassemia.
4. Identification of genetic mutations like deletions, insertions and point mutations.
5. Screening specific genes for unknown mutations.
6. Identification and analysis of mutations in eukaryotic DNA.
7. Gene polymorphisms.
8.Gene expression
9. Forensic Odontology

Basic Protocol for Polymerase Chain Reaction

A/ Components and reagents A basic PCR set up requires the following essential components and reagents

1. Template DNA containing the DNA region (target) to be amplified.

2. 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).

3. Taq polymerase or other thermo stable, high fidelity DNA polymerase (Pfupolymerase isolated from Pyrococcus furiosus).

4. Deoxyribonucleotides triphosphates (dNTPs), which are the building-blocks for a newly synthesized DNA strand.

5. Buffer solutions to provide a suitable chemical condition for optimum activity and stability of the DNA polymerases.

6. Divalent cations (eg. magnesium or manganese ions). They act as a co-factor for Taq polymerase which increases its polymerase activity. Generally Mg2+ is used, but Mn2+ can be applied to achieve PCR-mediated DNA mutagenesis. This is because higher Mn2+ concentration leads to higher error rate during DNA synthesis.

B/ Procedure Typically PCR is designed of 20-40 repeated thermal cycles, with each cycle consisting of 3 discrete temperature steps: denaturation, annealing and extension. The thermal cycles are often proceeded by a temperature at a high range

(>90°C), and followed by final product extension or brief storage at 4 degree celsius. 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. The various steps involved are:-

a) Initial Denaturation

b) Denaturation
c) Annealing
d) Extension
e) Final extension

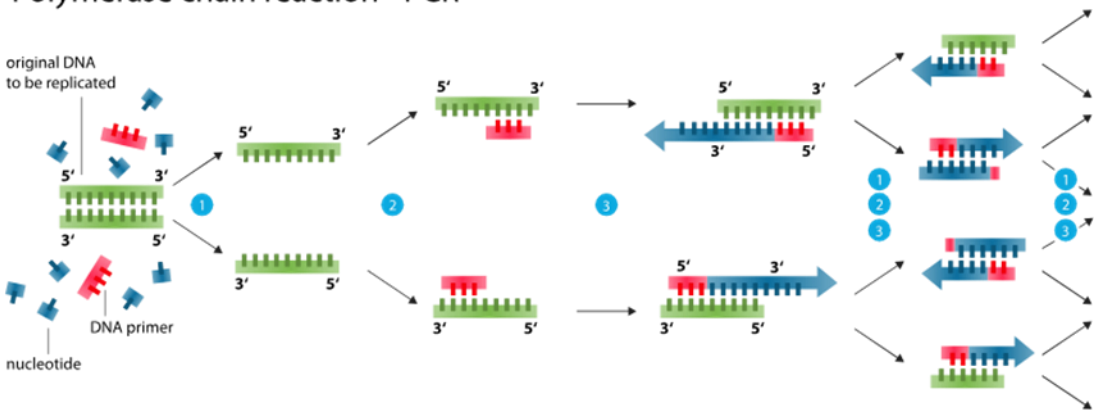
Initial denaturation involves heating of the reaction to a temperature of 94–96 °C for 7- 10 minutes (or 98 °C if extremely thermostable polymerases are used). For specifically engineered DNA polymerases (Hot start Taq polymerases) activity requires higher range of temperature. The initial heating for such a long duration also helps in gradual and proper unfolding of the genomic DNA and subsequent denaturation, and thus exposing target DNA sequence to the corresponding primers.

Denaturation requires heating the reaction mixture to 94–98 °C for 20–30 seconds. It results in melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules. Annealing Following the separation of the two strands of DNA 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 Tm of the primers. Stable complimentary binding are only formed between the primer sequence and the template when there is a high sequence complementarity between them. The polymerase enzymes initiate the replication from 3’ end of the primer towards the 5’end of it.

Extension/elongation step includes 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.

Final elongation & Hold Final elongation 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 maybe done for short-term storage of the reaction mixture. After around 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) , agarose gel electrophoresis is employed for separation of the PCR products based on size. The determination ofsize(s) of PCR products is performed by comparing with a DNA ladder, which contains DNA fragments of known size, run on the gel alongside the PCR products.

Polymerase chain reaction - PCR



1. **Denaturation** at 94-96°C
2. **Annealing** at ~68°C
3. **Elongation** at ca. 72 °C