PCR AND ITS APPLICATION

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CONTENT

INTRODUCTION

- **Polymerase chain reaction** (**PCR**) is a method widely used in molecular biology to rapidly make millions to billions of copies of a specific DNA sample
- PCR was invented in 1983 by the American biochemist KarryMullis at Cetus corporation . It is fundamental to much of genetic testing including analysis of ancient samples of DNA and identification of infectious agents
- PCR is now a common and often indispensable technique used in medical laboratory and clinical laboratory research for a broad variety of applications including biomedical research and criminal forensics.
- PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp) in length, although some techniques allow for amplification of fragments up to 40 kbp.

A basic PCR set-up

- A *DNA template* that contains the DNA target region to amplify
- A *DNA polymerase* an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process.
- Two DNA *primers* that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target .DNA polymerase can only bind to and elongate from a double-stranded region of DNA.
- a *buffer solution* providing a suitable chemical environment for optimum activity and stability of the DNA polymerase

A thermal cycler for PCR

PRIMER DESIGN

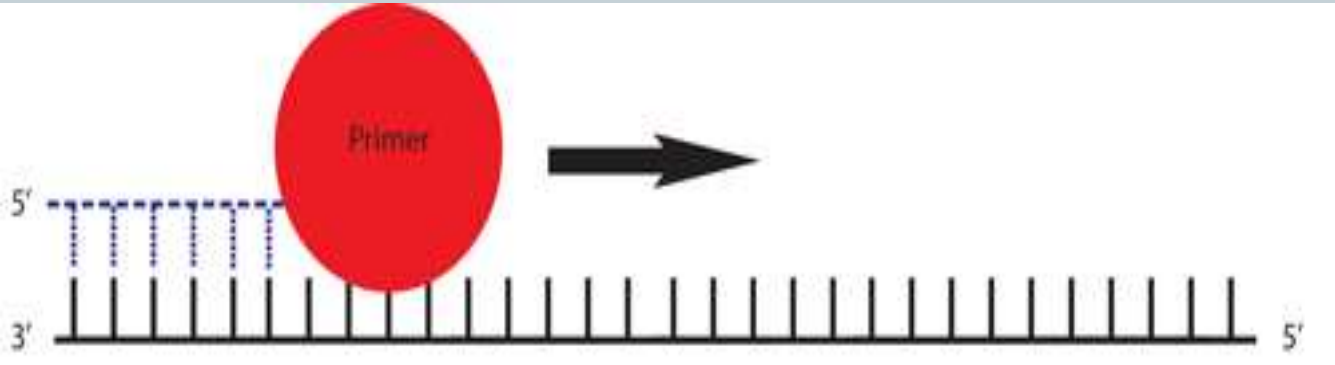
1. Primer Length: It is generally accepted that the optimal length of PCR primers is 18-22 bp. This length is long enough for adequate specificity and short enough for primers to bind easily to the template at the annealing temperature.

2. Primer Melting Temperature: Primer Melting Temperature (T_m) by definition is the temperature at which one half of the DNA duplex will dissociate to become single stranded and indicates the duplex stability. Primers with melting temperatures in the range of 52-58 ^oC generally produce the best results. Primers with melting temperatures above 65^oC have a tendency for secondary annealing.

PRIMER DESIGN

Primers should generally have the following properties:

- Length of 18-24 bases
- 40-60% G/C content
- Start and end with 1-2 G/C pairs
- Melting temperature (Tm) of 50-60°C
- Primer pairs should have a Tm within 5°C of each other
- Primer pairs should not have complementary regions.



DNA POLYMERASES

- DNA polymerase is an essential component for PCR due to its key role in synthesizing new DNA strands.
- Consequently, understanding the characteristics of this enzyme and the subsequent development of advanced DNA polymerases is critical for adapting the power of PCR for a wide range of biological applications.
- The use of *Taq* DNA polymerase in early PCR protocols, significant improvements have been made specifically in the specificity, thermostability, fidelity, and processivity of PCR enzymes.

DNA POLYMERASES

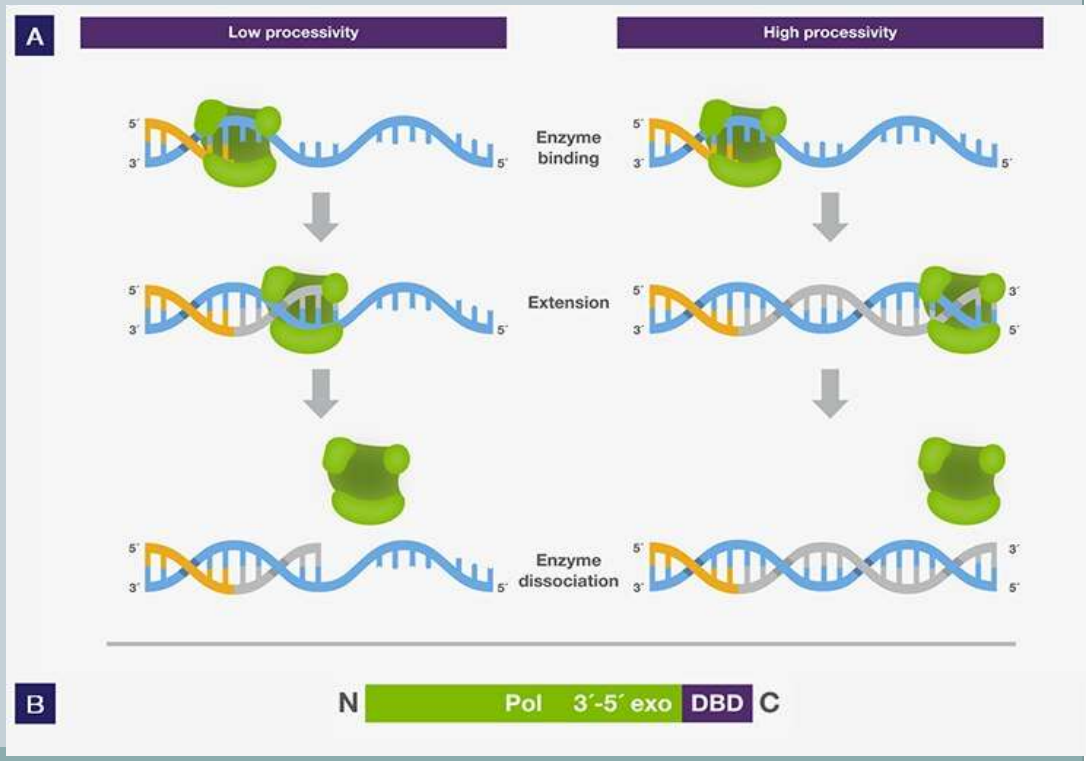
- DNA polymerases have been modulated in combination to enhance PCR as:

*Specificity

*Thermostability

*Fidelity

*Processivity



TYPES OF PCR

Some common types of PCR are,

*MULTIPLEX

* NESTED

* REVERSE TRANSCRIPTASE

* REAL TIME

* TOUCHDOWN

* HOT START

* COLONY

TYPES OF PCR

MULTIPLEX PCR: Multiplex PCR is a variant of **PCR method** in which more than one target sequence is amplified using multiple sets of primers within a single PCR mixture. This enables amplification of several gene segments at the same time, instead of specific test runs for each. This technology was first used by Chamberlain et al. for the diagnosis of Duchenne muscular dystrophy (1988).

NESTED PCR: Nested PCR is a modification of **PCR** designed to increase the sensitivity and specificity of the assay reaction. It involves the use of two primer sets directed against the same target and two successive PCR reactions.

TYPES OF PCR

REVERSE TRANSCRIPTASE:**RT-PCR**, also known as **Reverse Transcriptase PCR**, is a variation of the **polymerase chain reaction** that typically measures RNA expression levels. In **RT-PCR**, complementary DNA (cDNA) is made by **reverse** transcribing of the RNA templates with the enzyme **reverse** transciptase.

REAL TIME: **Real-time polymerase chain reaction** (**real-time PCR**) is commonly used to measure gene expression . It is more sensitive than microarrays in detecting small changes in expression but requires more input RNA and is less adaptable to high-throughput studies (1). It is best suited for studies of small subsets of genes.

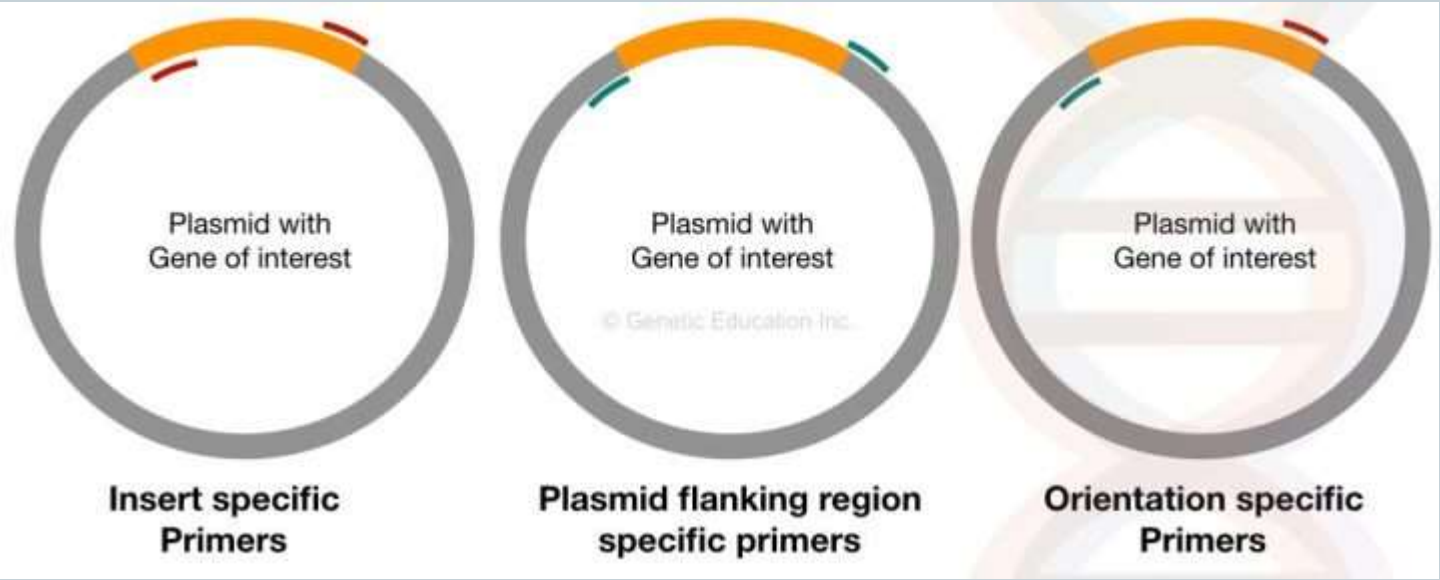
TYPES OF PCR

TOUCH DOWN:TD-**PCR** is a modification of **PCR** in which the initial annealing temperature is higher than the optimal Tm of the primers and is gradually reduced over subsequent cycles until the Tm temperature or "**touchdown** temperature" is reached, much like the **touchdown** of an airplane**.**

HOTSTART: **Hot Start PCR** is a technique that inhibits **Hot Start** Taq polymerase activity or the incorporation of modified dNTPs during reaction set up until a heat activation step occurs. **Hot Start PCR** allows for reaction set up at room temperature without non-specific amplification and primer dimer formation

TYPES OF PCR

COLONY: **Colony PCR** is a rapid, high throughput **PCR** method to determine the presence or absence of the inserted DNA into plasmid directly from the bacterial **colonies**. Molecular cloning is one of the most popular methods for DNA transformation since long.



CLONING OF PCR

- PCR cloning differs from traditional cloning in that the DNA fragment of interest, and even the vector, can be amplified by the Polymerase Chain Reaction (PCR) and ligated together, without the use of restriction enzymes.
- PCR cloning is a rapid method for cloning genes, and is often used for projects that require higher throughput than traditional cloning methods can accommodate. It allows for the cloning of DNA fragments that are not available in large amounts.

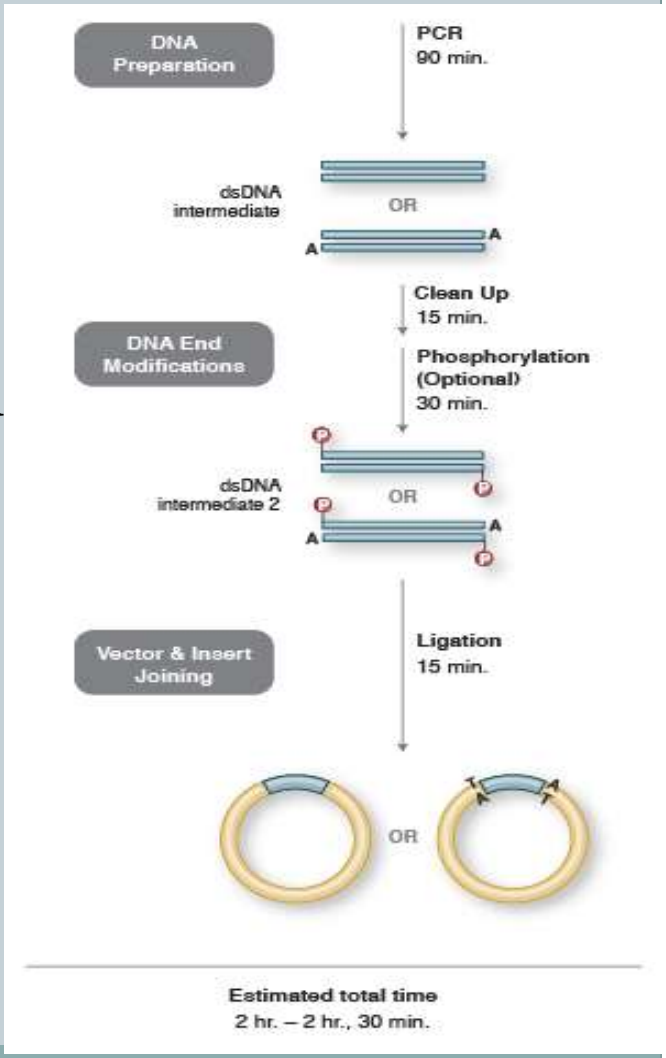
Advantages: *High efficiency, with dedicated vectors

*Amenable to high throughput

CLONING OF PCR

Disadvantages:

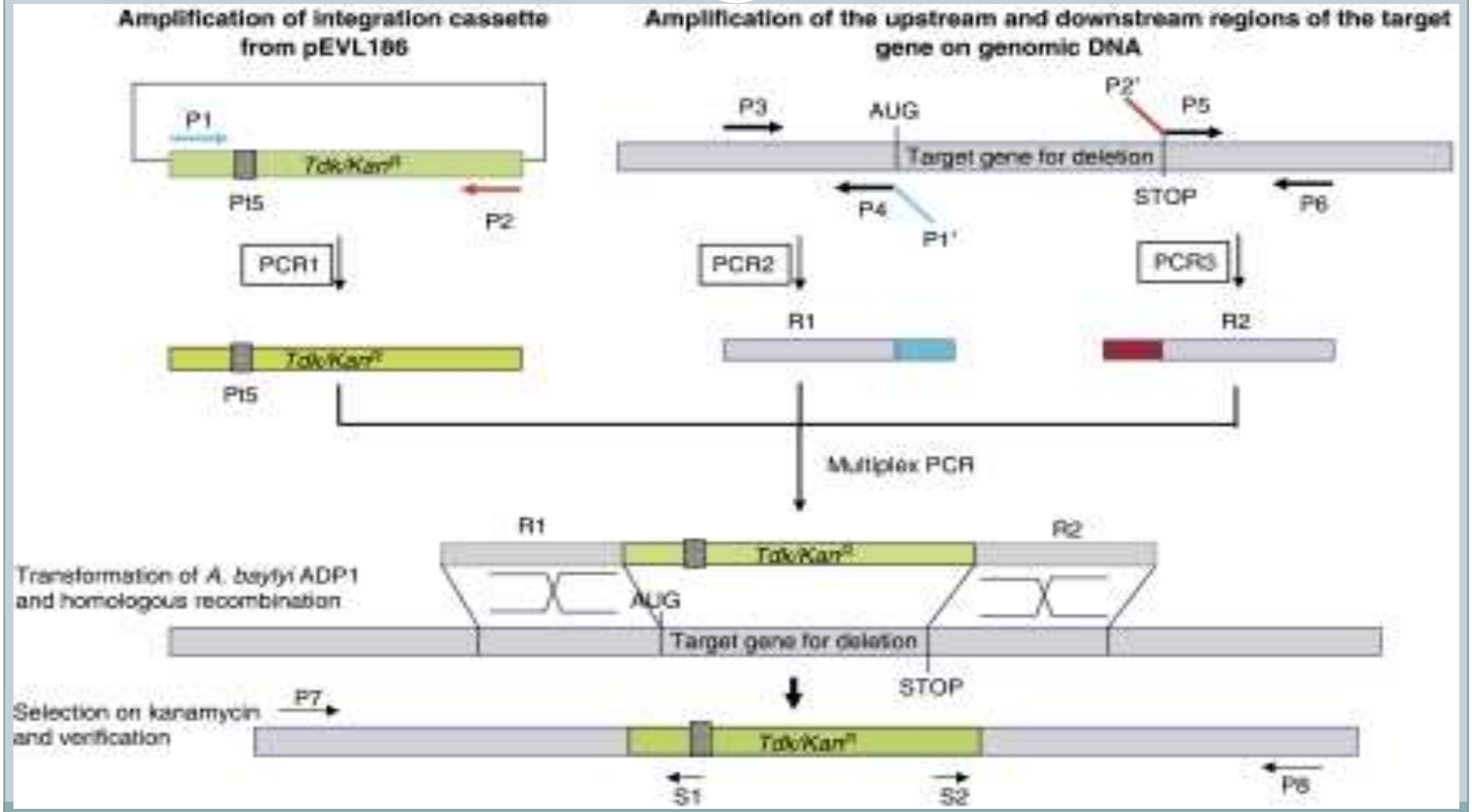
- Limited vector choices
- Higher cost
- Lack of sequence control at junction
- Multi-fragment cloning is not straight forward
- Directional cloning is difficult.



PCR IN GENE RECOMBINATION

- Mutagenesis by the overlap extension PCR has become a standard method of creating mutations including substitutions, insertions, and deletions. Nonetheless, the established overlap PCR mutagenesis is limited in many respects. In particular, it has been difficult to make an insertion larger than 30 nt, since all sequence alterations must be embedded within the primer
- Mutagenesis is usually employed to understand the regulatory regions of genes and the relationship between the protein structure and its function .
- To ensure an accurate amplification by PCR, versions of the high-fidelity DNA polymerases are usually available for site mutagenesis. The common trait of this kind of polymerases is their low error rate.

PCR IN GENE RECOMBINATION



PCR BASED MUTAGENESIS

- **PCR mutagenesis**. **PCR mutagenesis** is a method for generating **site-directed mutagenesis**. This method can generate mutations (base substitutions, insertions, and deletions) from double-stranded plasmid without the need for subcloning into M13-

  based bacteriophage vectors and for ssDNA rescue**.**