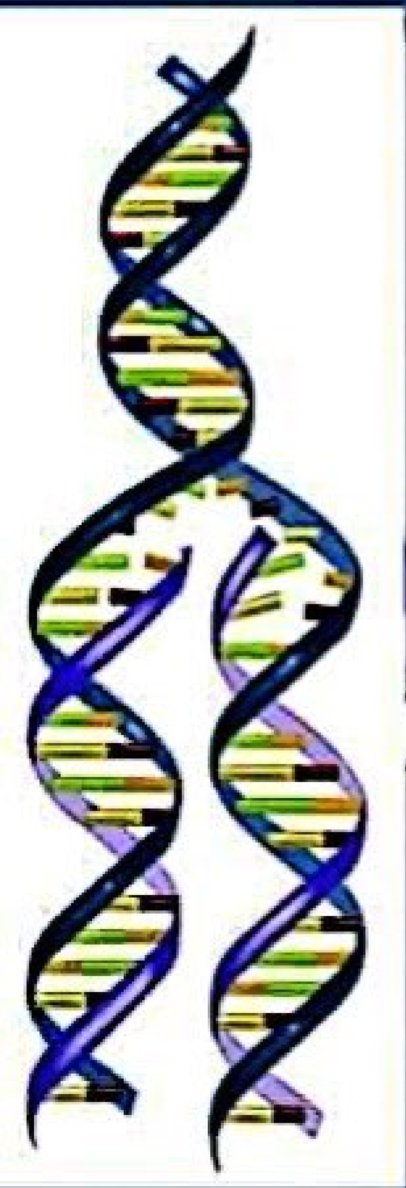

POLYMERASE CHAIN REACTION

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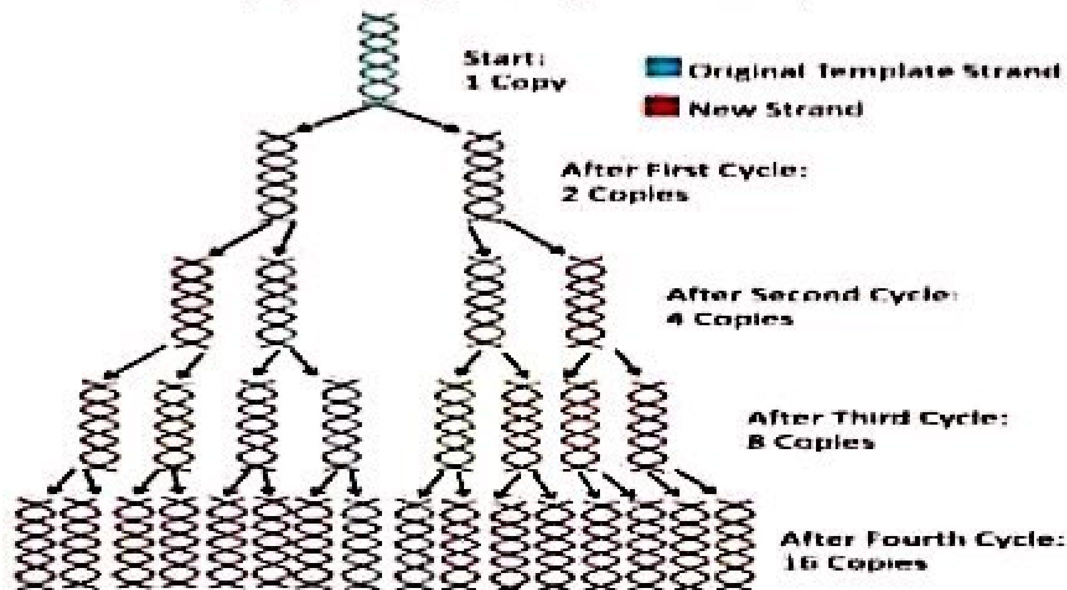
Polymerase chain reaction

Polymerase chain reaction, PCR, is an efficient and cost-effective way to copy or "amplify" small segments of DNA or RNA.

Using PCR, millions of copies of a section of DNA are made in just a few hours, yielding enough DNA required for analysis.



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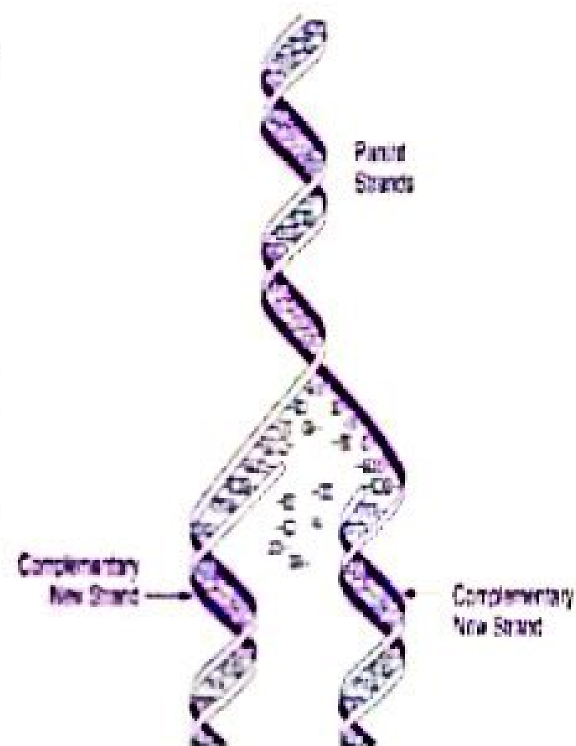
Principle

1. Separation of DNA double-stranded template
2. Primer formation
3. Extension of new DNA strands by a DNA polymerase and deoxynucleotide triphosphates (dNTPs)
4. Other proteins involved



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The Reaction Ingredients

1) Target DNA - contains the sequence to be amplified.

2) Pair of Primers - oligonucleotides that define the sequence to be amplified.

3) dNTPs – deoxynucleotide triphosphates: DNA building blocks.

4) Thermostable DNA Polymerase - enzyme that catalyzes the reaction

5) Mg^{++} ions - cofactor of the enzyme

6) Buffer solution – maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme

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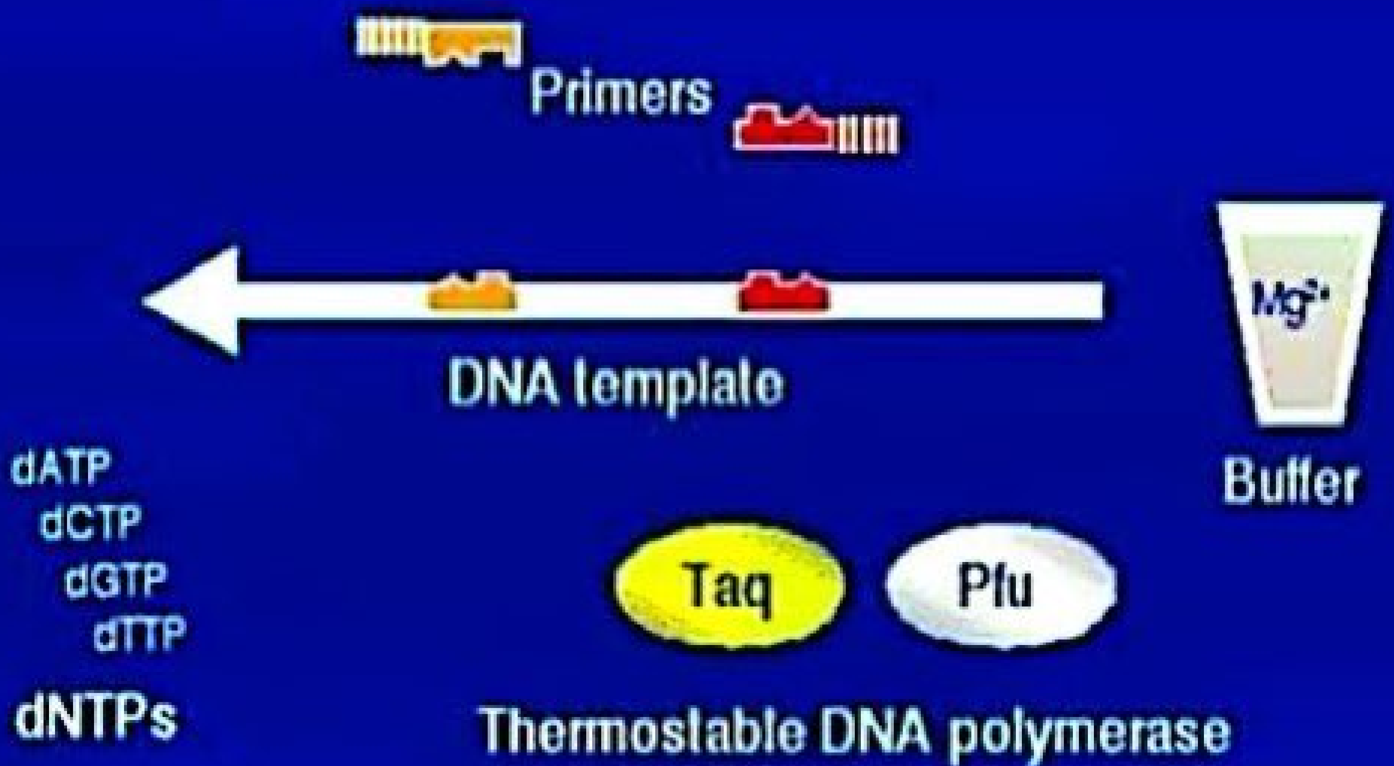

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PCR process

1. Separating the Target DNA - Denaturation

The tube containing the sample DNA is heated to more than 90 degrees Celsius which separates the double-stranded DNA into two separate strands.

2. Binding Primers to the DNA Sequence - Annealing

Two primers are used in step two - one for each of the newly separated single DNA strands. The primers bind to the beginning of the sequence that will be copied, marking off the sequence for step three. During step two, the tube is cooled and primer binding occurs between 40 and 60 degrees Celsius

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PCR process(Cont......)

3. Making a Copy - Extension

The temperature is increased to approximately 72 degrees Celsius. Beginning at the regions marked by the primers, nucleotides in the solution are added to the annealed primers by the DNA polymerase.

After completing the extension, two identical copies of the original DNA have been made.

4. Final elongation

This single step is occasionally performed at a temperature of 70–74 °C for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully extended.

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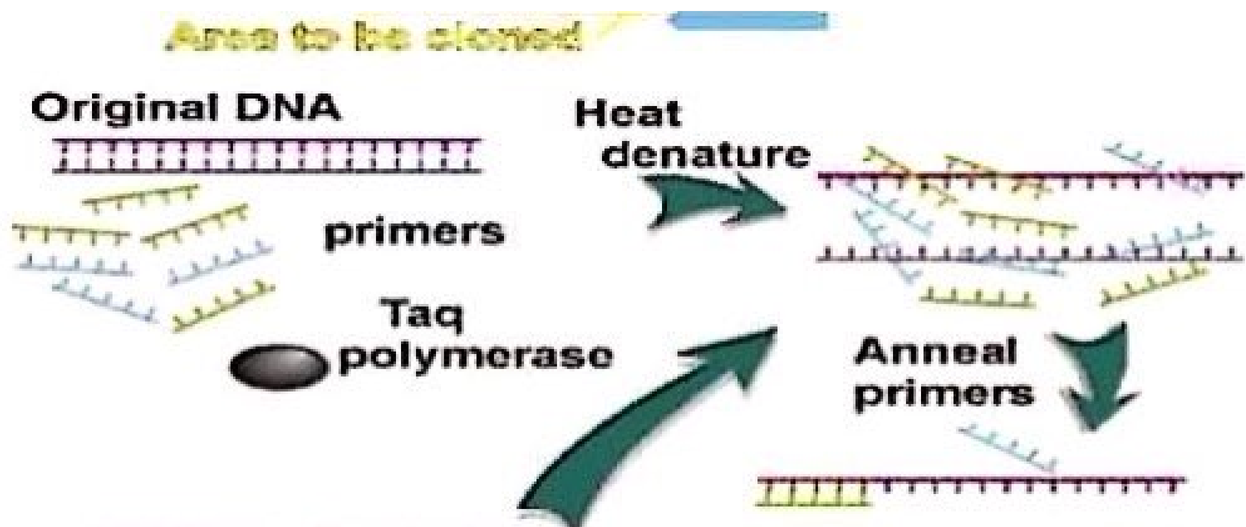

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Applications of PCR

Basic Research

- Mutation screening
- Drug discovery
- Classification of organisms
- Genotyping
- Molecular archaeology
- Molecular epidemiology
- Molecular ecology
- Bioinformatics
- Genomic cloning
- Site-directed mutagenesis
- Gene expression studies

Applied Research

- Genetic matching
- Detection of pathogens
- Pre-natal diagnosis
- DNA fingerprinting
- Gene therapy

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Types of PCR

1. Real time
2. Quantitative
3. Reverse transcriptase
4. Multiplex
5. Hot-start
6. Asymmetric
7. Methylation specific
8. Allele specific
9. RAPD
10. Nested
11. Touch down
12. In Situ
13. Assemble

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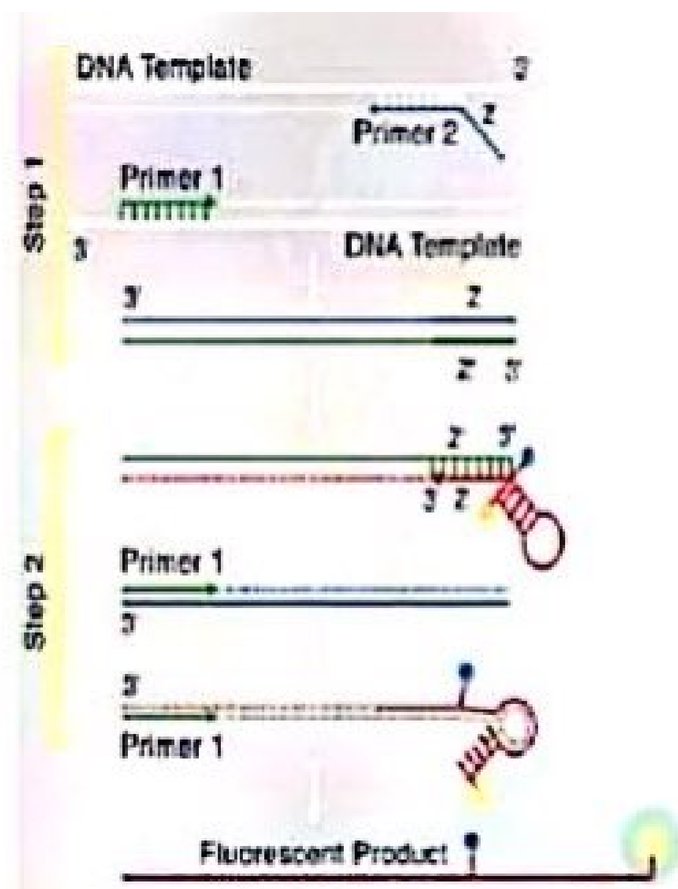
A real-time polymerase chain reaction monitors the amplification of a targeted DNA molecule during the PCR, i.e. in real-time, and not at its end, as in conventional PCR. Real-time PCR can be used quantitatively, semi-quantitatively, i.e. above/below a certain amount of DNA molecules or qualitatively.



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Real-time PCR In Diagnosis of Disease

✓Quantitatively measurment of Human Immunodeficiency Virus

✓Detection of Thalassemia, hemophilia, Sickle cell anemia

✓Cystic fibrosis

✓Phenyl ketonuria

✓Use in forensic medicine

✓Noninvasive prenatal diagnosis by analysis of fetal DNA in maternal plasma

✓Detection of circulating Plasmodium falciparum DNA

✓Effect of antimicrobial peptides on host cells

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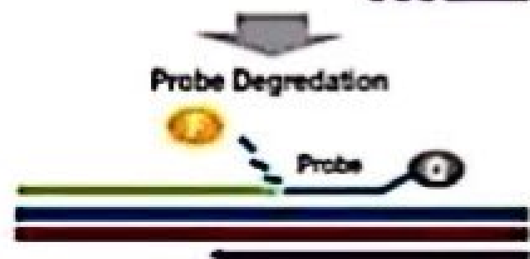
Quantitative PCR (qPCR)

Quantitative *PCR* is used to measure the quantity of a target sequence (commonly in real-time).

Step 1:
Primers and probe bind to target DNA.

Step 2:
PCR occurs, primers are extended on forward and reverse DNA strands.



Step 3:
Probe is degraded as a result of polymerization and flourescent signal is generated.

Step 4:
Taregt DNA is amplified and flourescent signal can be measured

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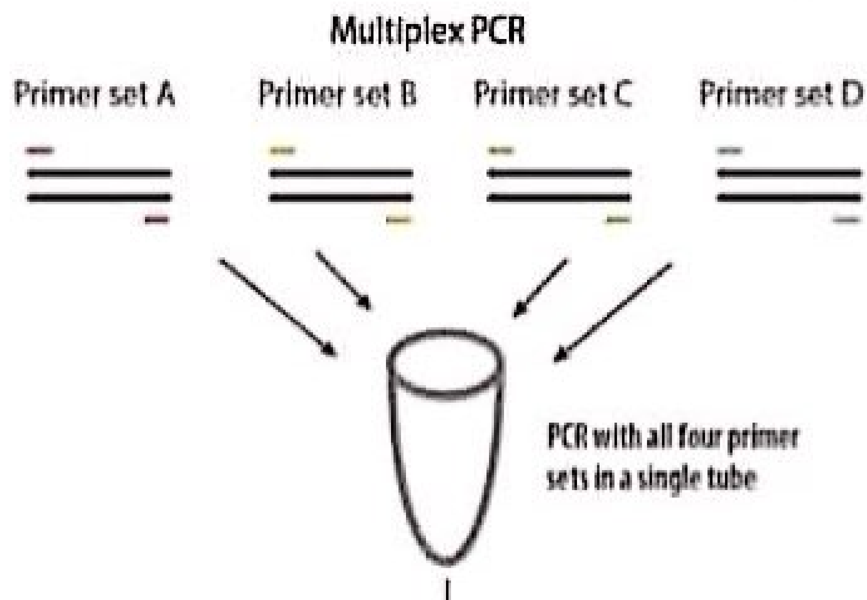
Multiplex PCR

Multiplex PCR is used to amplify several different DNA sequences simultaneously (as if performing many separate PCR reactions all together in one reaction).



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Applications of multiplex PCR include:

1. Pathogen identification
2. High throughput SNP genotyping
3. Mutation analysis
4. Gene deletion analysis
5. Template quantification
6. Linkage analysis
7. RNA detection
8. Forensic studies
9. Diet analysis

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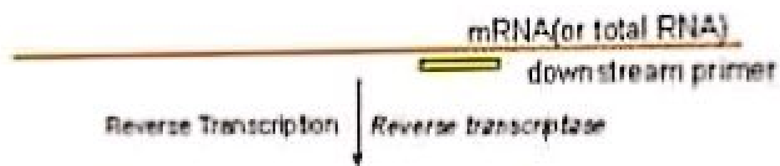
Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)



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Application of RT PCR

- Detection of infectious agents
- Diagnosis of genetic mutations
- Gene expression in a tissue

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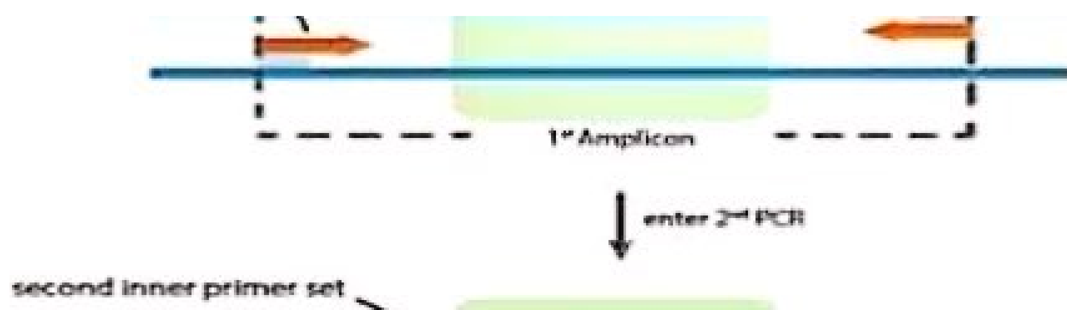

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Allele-specific PCR

Allele-specific PCR is a diagnostic or cloning technique based on single-nucleotide variations. It requires prior knowledge of a DNA sequence, including differences between alleles, and uses primers whose 3' ends encompass the SNV.

Application

1. Detection of Single Nucleotide Polymorphism
2. Used to identify single base differences in DNA

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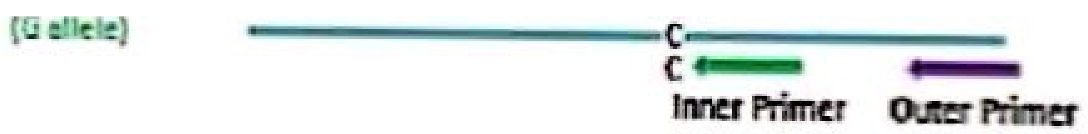

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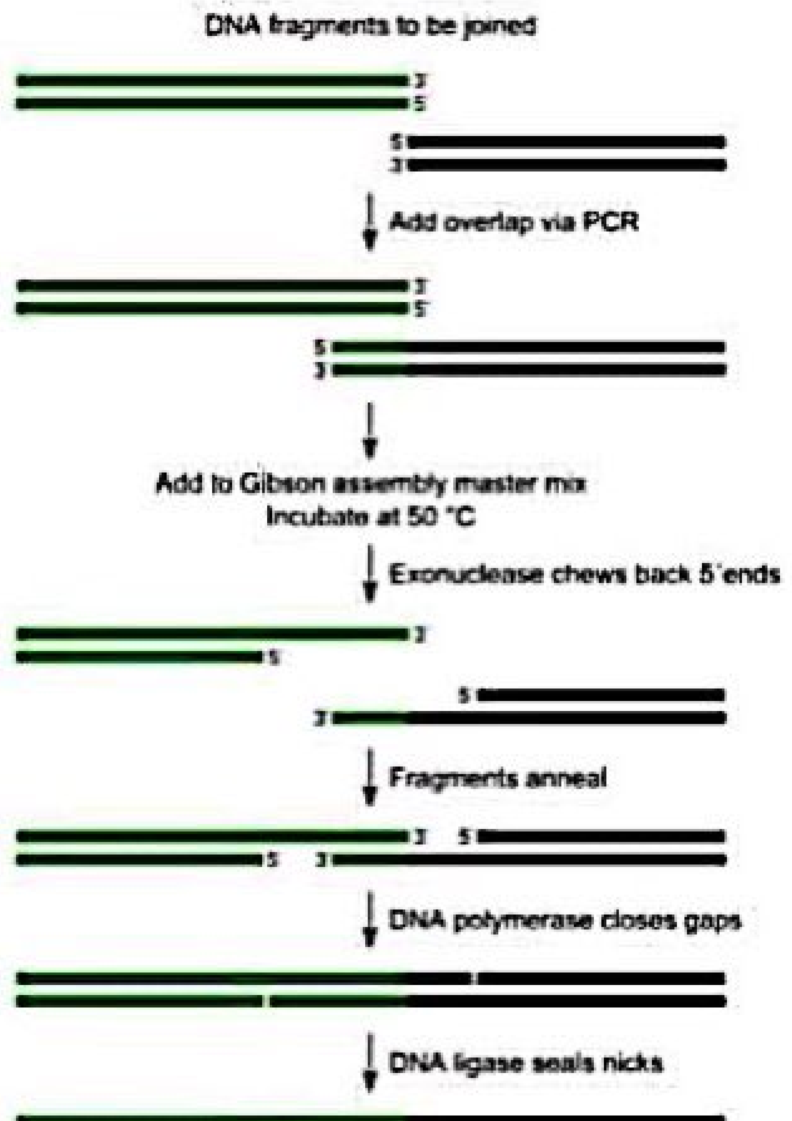
Assemble PCR

It's an artificial synthesis of long DNA sequences by performing PCR on a pool of long oligonucleotides with short overlapping segments.

Assembly PCR can be used to assemble two gene-sized pieces of DNA into one piece for easier cloning of fusion genes/parts



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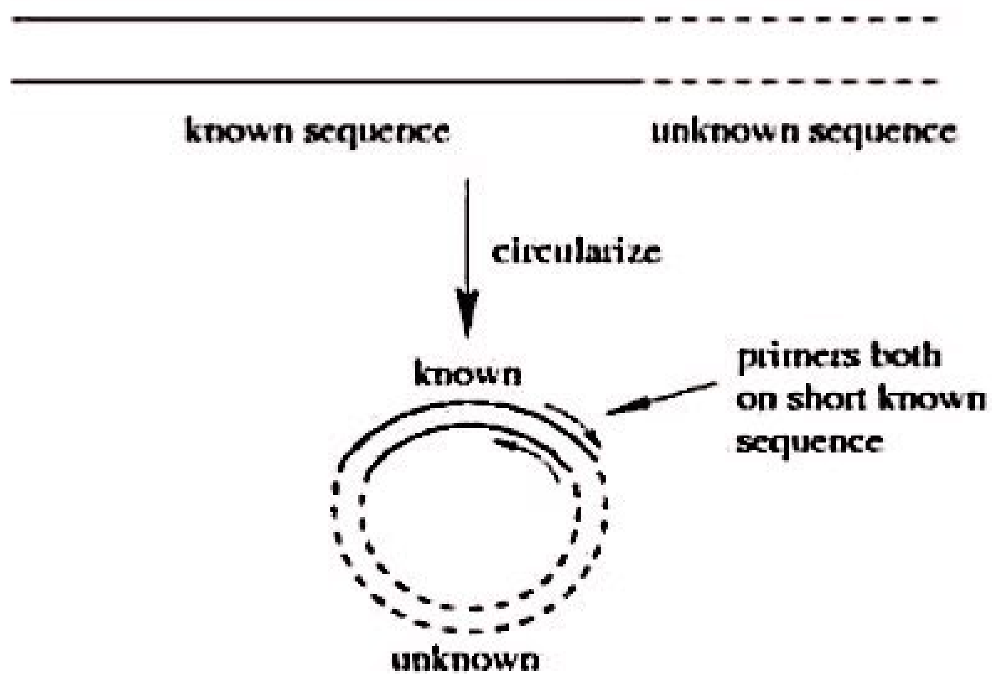
Inverse PCR

It involves a series of DNA digestions and self ligation, resulting in known sequences at either end of the unknown sequence.



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Methylation-specific PCR

Methylation-specific PCR (MSP) is a method for analysis of DNA methylation patterns in CpG islands. For performing MSP, DNA is modified by and PCR performed with two primer pairs, which are detectable methylated and unmethylated DNA, respectively. MSP is a rapid measure for assessment of the methylation status in CpG island.

Used to identify patterns of DNA methylation at cytosine-guanine islands in genomic DNA

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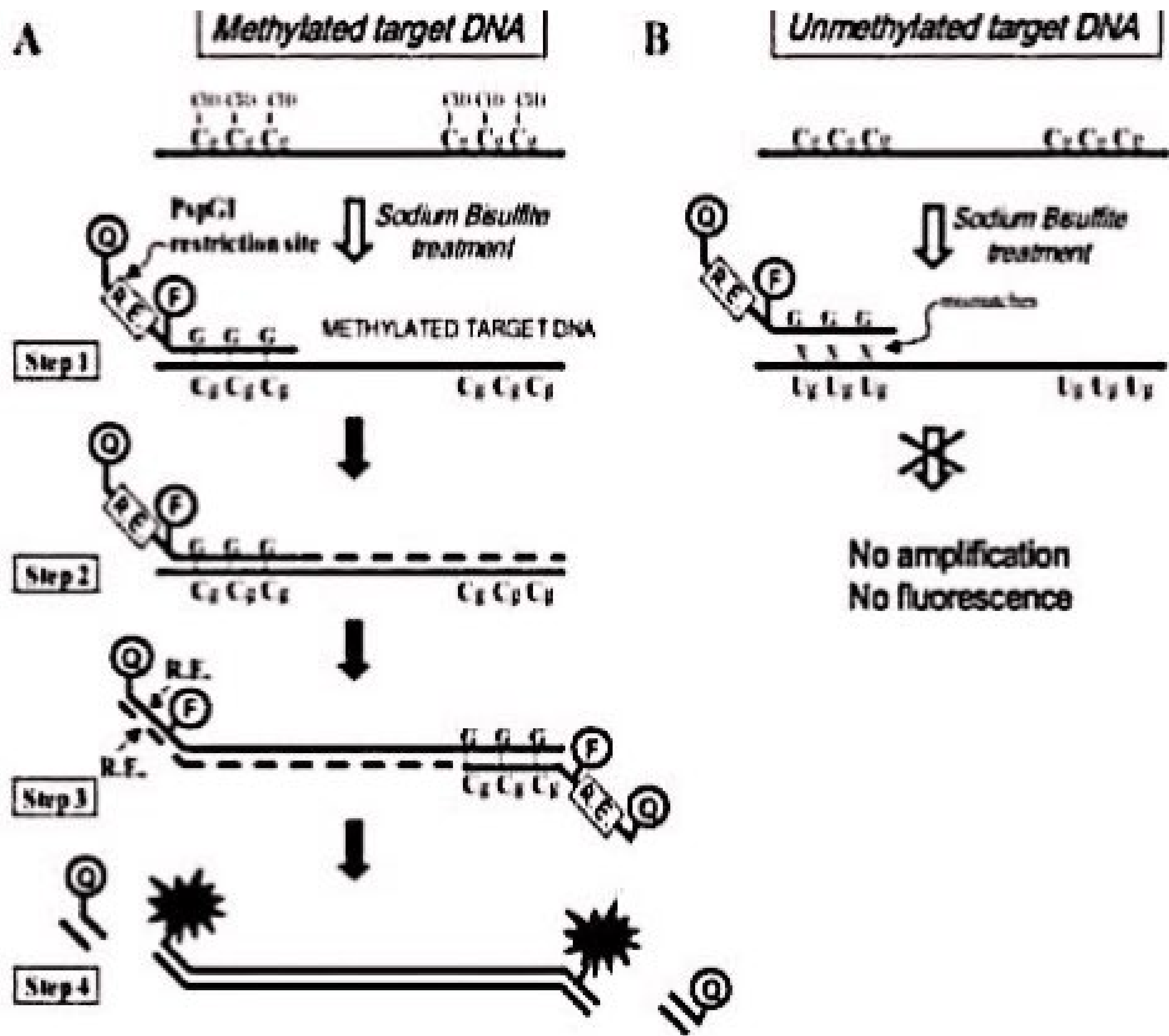

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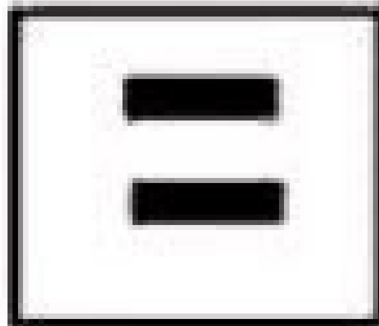
DNA template

RAPD - PCR

Gel electrophoresis of amplified fragments



Detection of the size of the amplified fragments and comparison

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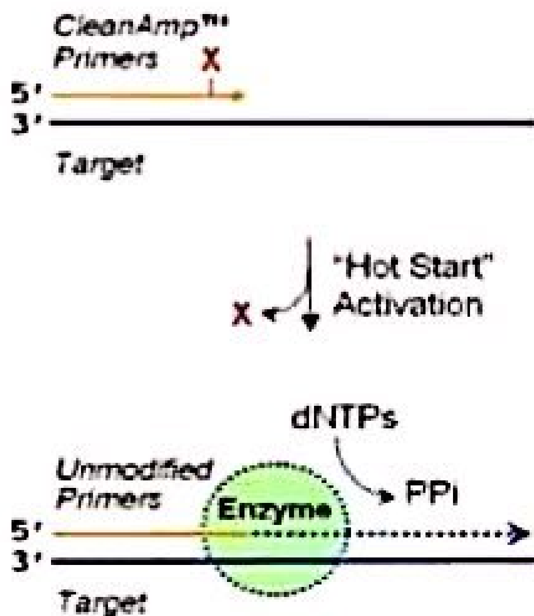
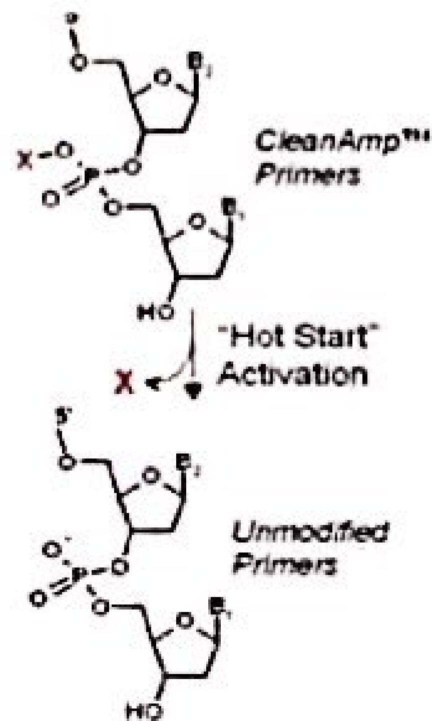
Hot-start PCR

Hot-start PCR is a technique that reduces non-specific amplification during the initial set up stages of the PCR. It may be performed manually by heating the reaction components to the denaturation temperature (e.g., 95 °C) before adding the polymerase.





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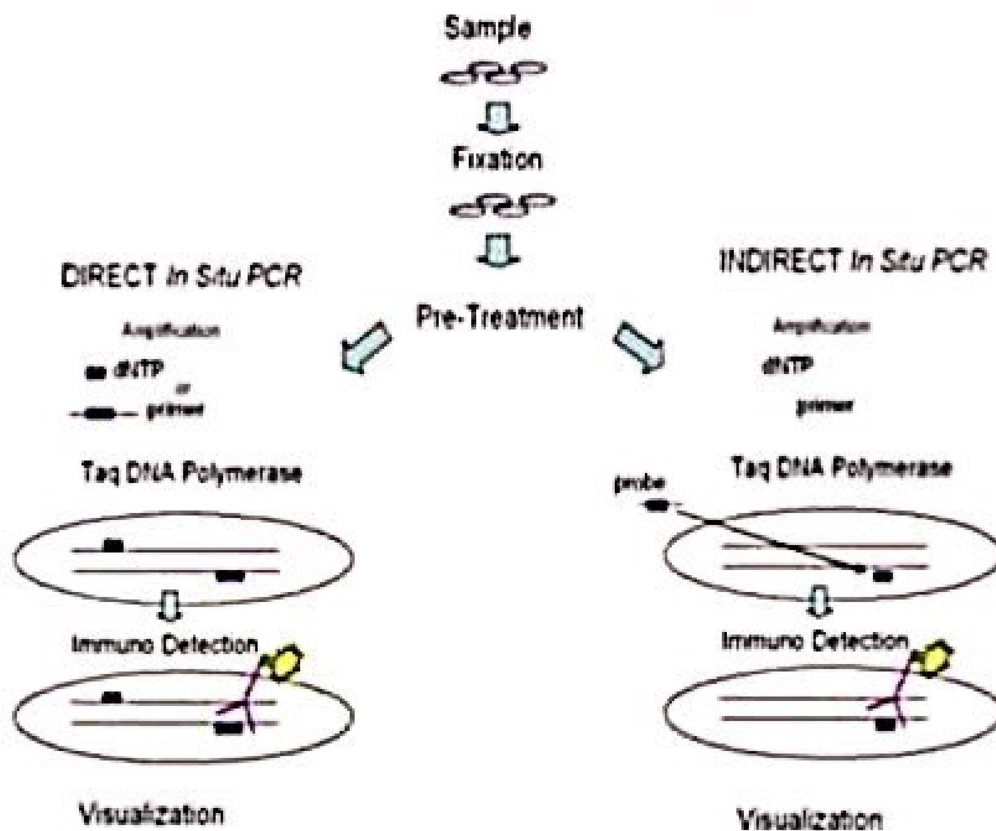
In Situ PCR

In situ PCR is a polymerase chain reaction that actually takes place inside the cell on a slide. In situ PCR amplification can be performed on fixed tissue or cell. It applies the methodology of hybridization of the nucleic acids.



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Application of In Situ PCR

- ➢Detection and diagnosis of viruses and other infectious agents in specific cell types within tissues
- ➢Detection and characterization of tumor cells within tissue
- ➢Detection and diagnosis of genetic mutations in inherited diseases
- ➢Detection of gene and gene expression in a tissue
- ➢Any assay in which identification of cells expressing a target gene is required

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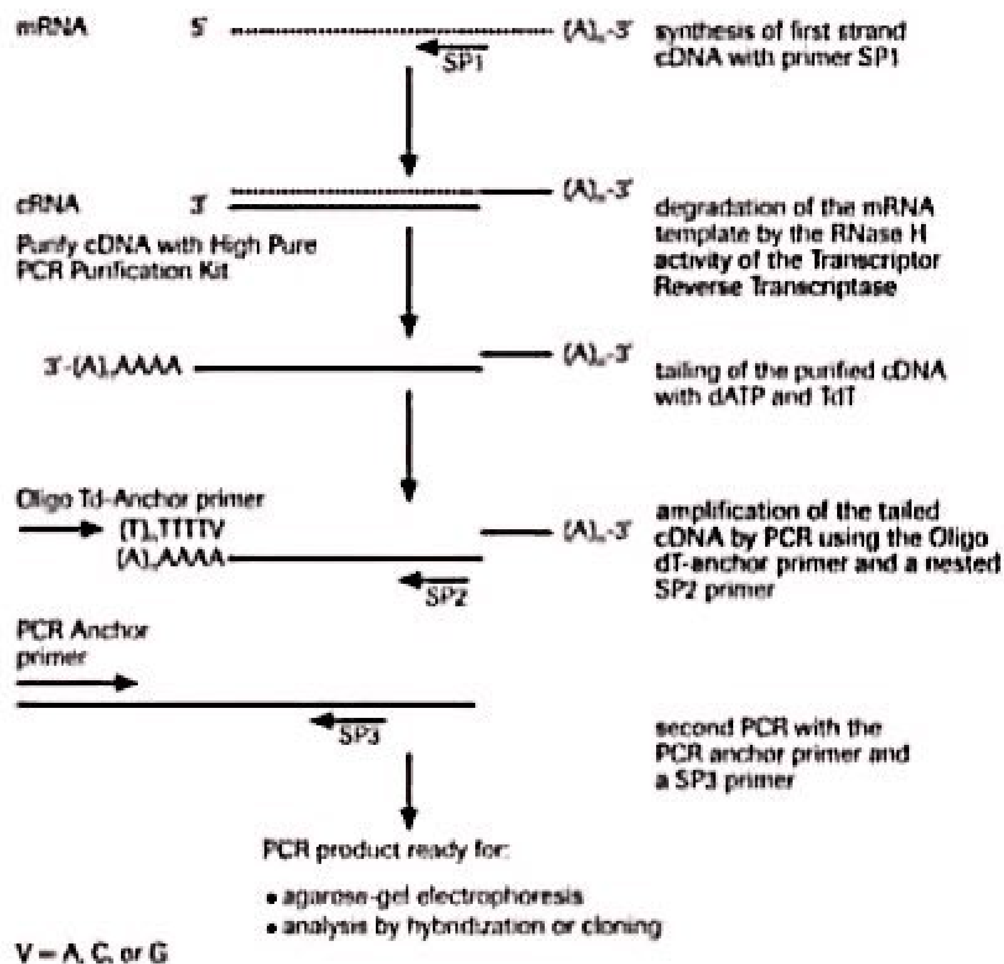
RACE PCR

Rapid amplification of cDNA ends (RACE) is a technique used in molecular biology to obtain the full length sequence of an RNA transcript found within a cell.



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Asymmetric PCR

Asymmetric PCR preferentially amplifies one DNA strand in a double-stranded DNA template. It is used in sequencing and hybridization probing where amplification of only one of the two complementary strands is required.

Digital PCR (dPCR)

Digital PCR (dPCR)is used to measure the quantity of a target DNA sequence in a DNA sample. The DNA sample is highly diluted so that after running many PCRs in parallel, some of them do not receive a single molecule of the target DNA. The target DNA concentration is calculated using the proportion of negative outcomes.

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Touchdown PCR

Touchdown PCR aims to reduce nonspecific binding by gradually lowering the annealing temperature as PCR cycling progresses. The annealing temperature at the initial cycles is usually a few degrees (3–5 °C) above the T_m of the primers used, while at the later cycles, it is a few degrees (3–5 °C) below the primer T_m. The higher temperatures give greater specificity for primer binding, and the lower temperatures permit more efficient amplification from the specific products formed during the initial cycles.

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Ligation-mediated PCR

Uses small DNA oligonucleotide 'linkers' that are first ligated to fragments of the target DNA. PCR primers that anneal to the linker sequences are then used to amplify the target fragments.

Principle

- Ligation with excess of primers
- Polymerase chain reaction of individual fragments

This method is applied for

- DNA sequencing
- Genome walking
- DNA footprinting
- To map DNA damage
- Reveal DNA–protein interactions inside living cells

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Suicide PCR

Suicide PCR is typically used in paleogenetics or other studies where avoiding false positives and ensuring the specificity of the amplified fragment is the highest priority.

Degenerate PCR

- Mixed PCR primers for a given sequence.
- Powerful tool to find 'new' gene or gene families. By aligning the sequences from a number of related proteins, the conserved and variable part can be determined.

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