Medical Instrumentation Laboratory

Molecular Biology equipment

PCR Machine

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1 st Class

(What is Polymerase Chain Reaction(PCR)?

PCR is a technique that takes a specific sequence of DNA of a small amount and amplifies it to be used for

.further testing in-vitro technique

Dr. Kary Mullis developed PCR **:**1983

Purpose

To amplify a lot of double-stranded DNA molecules

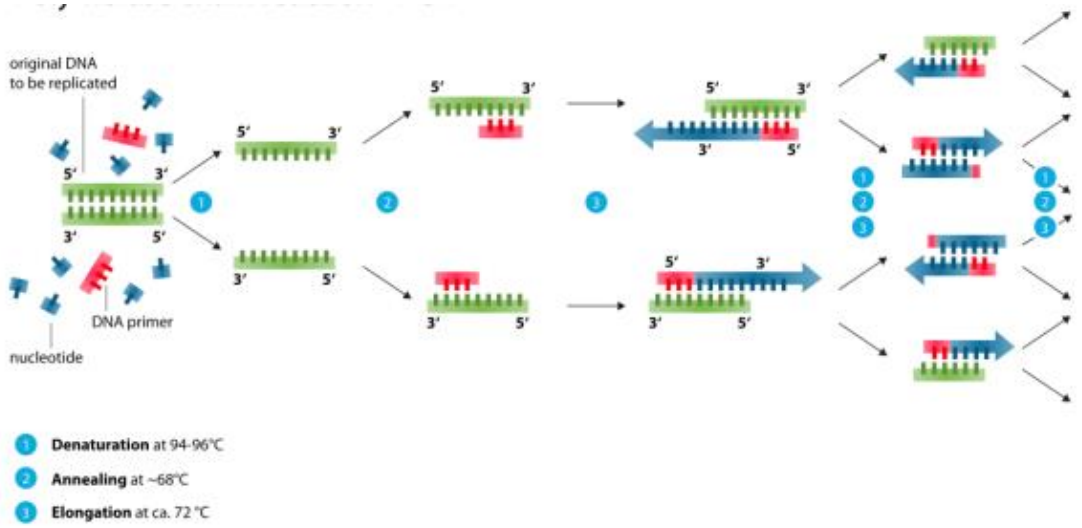
fragments) with the same (identical) size and)

sequence by enzymatic method and cycling condition

Condition

1. Denaturation of double strands (ds) DNA template 2. Annealing of primers 3. Extension of ds DNA molecules

The polymerase chain reaction (PCR) is a molecular biology technique to amplify a single or a few copies of a piece of a particular DNA sequence. PCR is used to reproduce (amplify) selected sections of DNA or RNA for analysis.



The PCR reaction requires the following components

- **DNA template**: DNA template is DNA target sequence. High temperature is applied to separate both the DNA strands from each other.

- DNA polymerase: DNA polymerase sequentially adds nucleotides complimentary to template strand at 3'-OH of the bound primers and synthesizes new strands of DNA complementary to the target sequence.

- Primers: Primers are synthetic DNA strands of about 18 to 25 nucleotides.

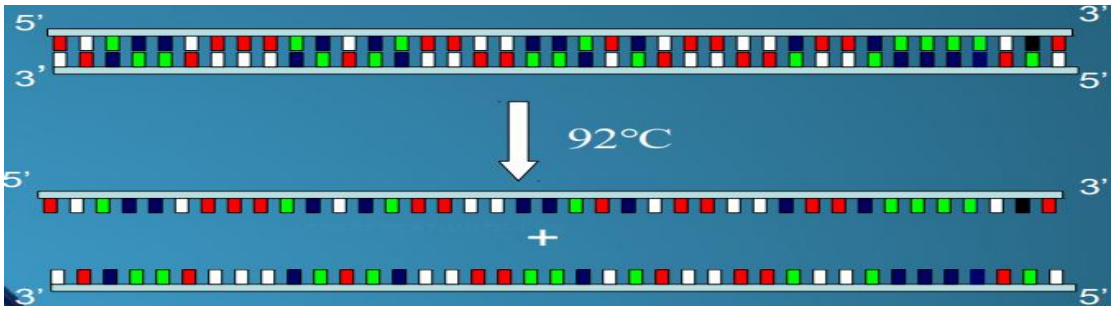
complementary to 3' end of the template strand. DNA polymerase starts synthesizing new DNA from the 3' end of the primer. Two primers must be designed for PCR; the forward primer and the reverse primer. The forward primer is complimentary to the 3' end of antisense strand (3'-5') and the reverse primer is complimentary to the 3' end of sense strand (5'-3').

Procedure

There are three major steps in a PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

1. Denaturation : During the heating step (denaturation), the reaction mixture is heated to92- 94°C for 1 min, which causes separation of DNA double stranded. Now, each strand acts as template for synthesis of complimentary strand.

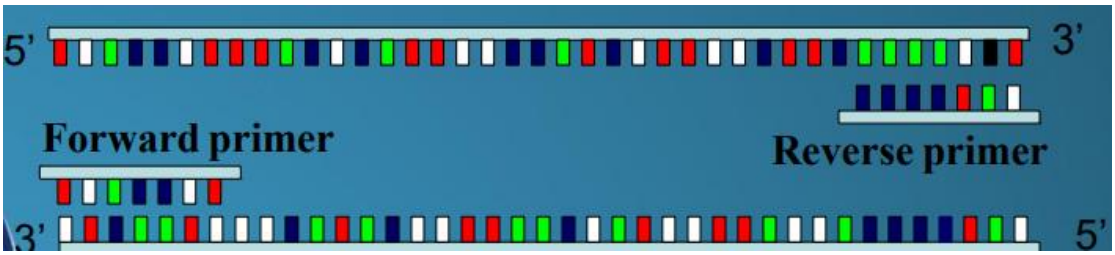
Primer is a short nucleic acid sequence of DNA used for DNA synthesis.



2. Annealing

Temperature50-70℃ : (dependant on the melting temperature of the expected duplex) Primers bind to their complementary sequences.

Melting Temperature (MT): is the temperature that one-half of the DNA duplex will dissociate to become single-stranded.



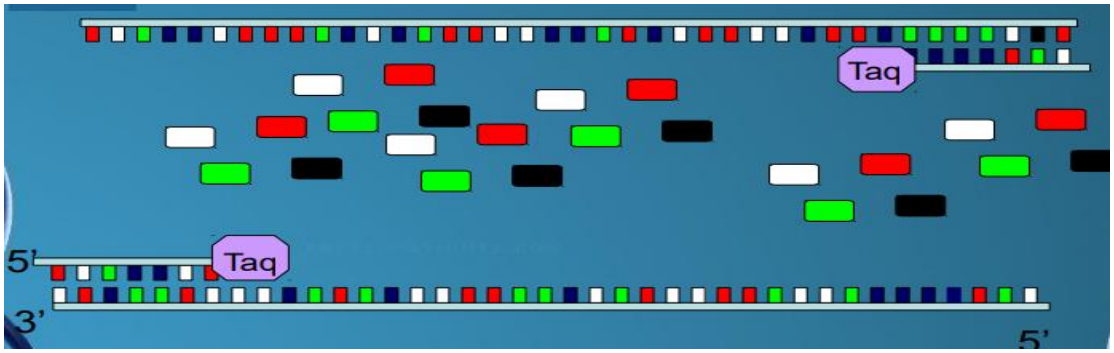
3. Extension

Temperature: ~72 ℃

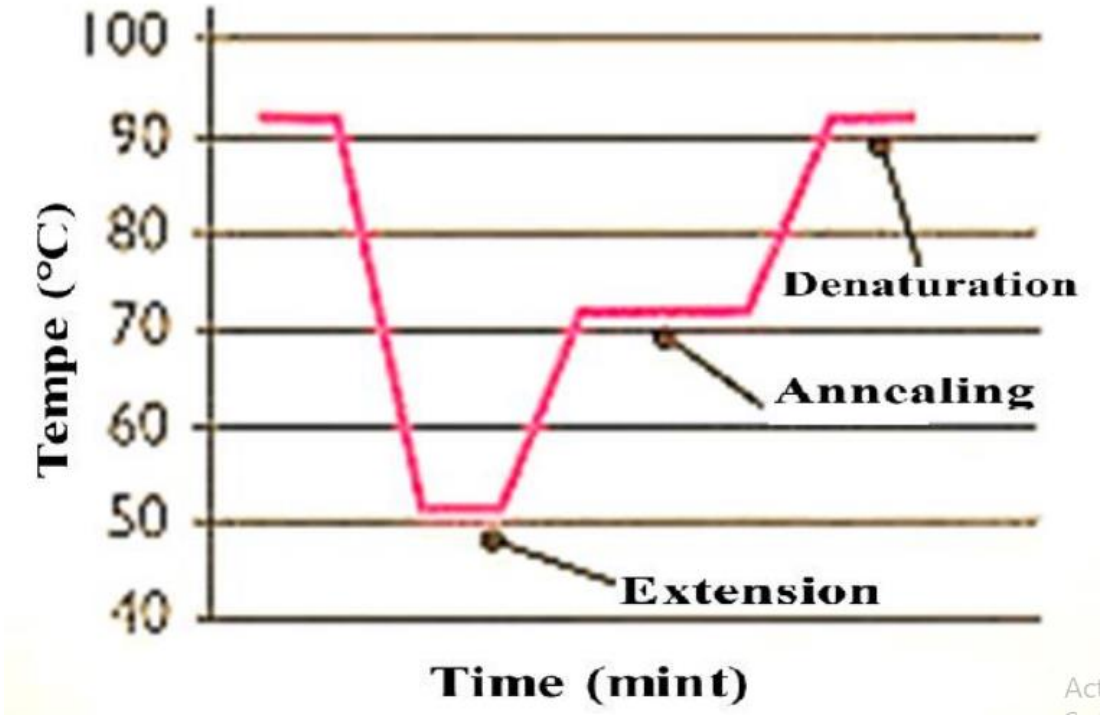
• Time: 0.5-3min

DNA polymerase binds to the annealed primers and extends DNA at the 3' end of the chain

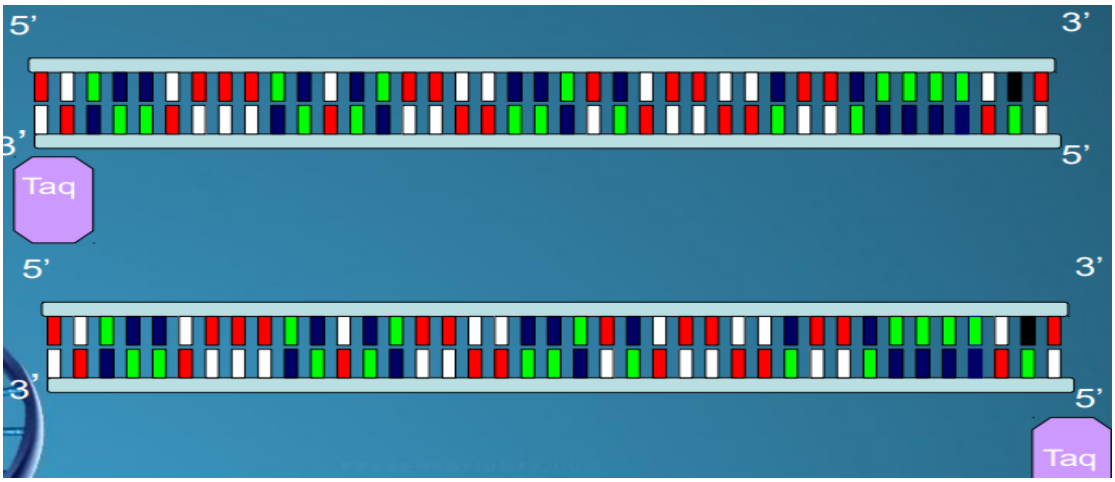
The reaction mixture is heated to 72°C which is the ideal working temperature for the Taq polymerase. The polymerase adds nucleotide (dNTP's) complimentary to template on 3' –OH of primers thereby extending the new strand.


Taq
5'
Taq
3'
5'

Cycling

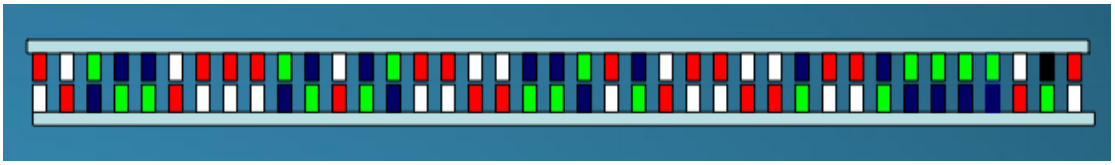

100
90
80
70
60
50
40
Tempe (°C)
Denaturation
Anncaling
Extension
Time (mint)

Products of Extension

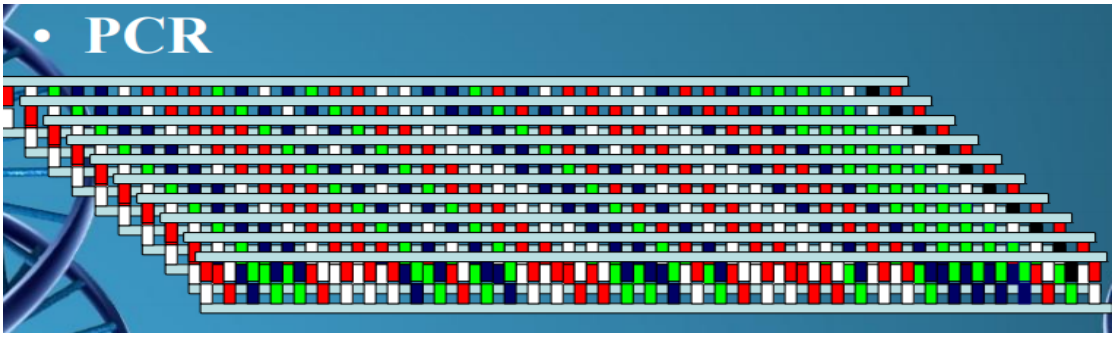


Overall Principle of PCR

- **DNA – 1 copy**



The Sequence of interest was a Known sequence



Chemical Components

Magnesium chloride: 5-2.5mM •

Buffer: pH 8.3-8.8 •

dNTPs (ATP, GTP, CTP, TTP): 20-200µM •

-Primers (Forward and Reverse primer): 0.1 -0.5 Mm

DNA Polymerase isolated from bacteria •

Thermus aquaticus (Taq.): 1-2.5 units

Target DNA (since strand): 1 µg •

Basic requirements for PCR reaction

• **1) DNA sequence** (DNA Templet) of the target region must be known.

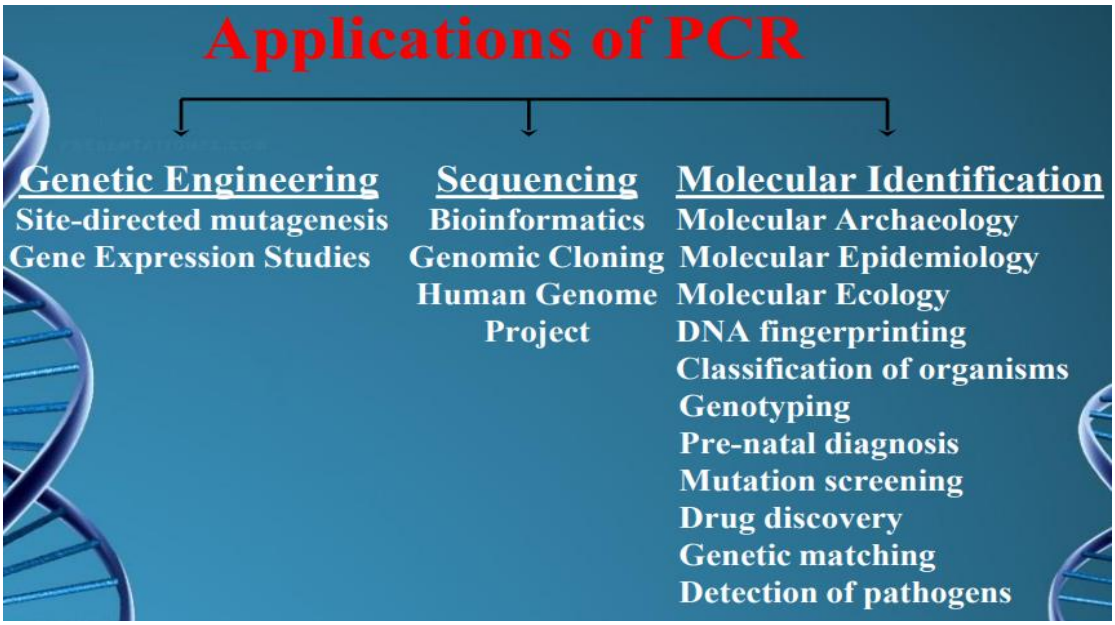
• 2) Primers - typically 20-30 bases in size. These can be readily produced by commercial companies. Can also be prepared using a DNA synthesizer.

• 3) Thermo-stable DNA polymerase eg. Taq polymerase which is not inactivated by heating to 95 ℃.

• 4) DNA thermal cycler - a machine that can be programmed to carry out heating and cooling of samples over a number of cycles.

Advantages of PCR

- Small amount of DNA is required per test
- Result obtained more quickly - usually within 1 day for PCR
- Usually not necessary to use radioactive material (32P) for PCR.
- PCR is much more precise in determining the sizes of alleles - essential for some disorders.
- PCR can be used to detect point mutations.



DNA hybridization generally refers to a molecular biology technique that measures the degree of genetic similarity between pools of DNA sequences. It is usually used to determine the genetic distance between two organisms. This has been used extensively in phylogeny and taxonomy.

DNA hybridization is the process of joining two single strands of DNA thus forming dsDNA. Joining of the two strands is facilitated by the formation of hydrogen bonds between the nucleotide bases. DNA hybridization may be used to assess the genetic similarity between two populations of DNA or to detect a specific sequence of DNA in a population of DNA molecules. This is achieved by using a nucleic acid probe

A probe is a piece of single-stranded DNA (ssDNA) or RNA with a marker attached for detection after hybridization.

DNA sequencer is a scientific instrument used to automate the DNA sequencing process. Given a sample of DNA, a DNA sequencer is used to determine the order of the four bases: G (guanine), C (cytosine), A (adenine) and T (thymine). This is then reported as a text string, called a read.