POLYMERASE CHAIN REACTION (PCR)

SUBJECT: ANIMAL BIOTECHNOLOGY
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POLYMERASE CHAIN REACTION

- Polymerase chain reaction (PCR) is a common laboratory technique used to amplify or make millions of copies of a particular region of DNA.
- For example, it might be a gene whose function a researcher wants to understand, or a genetic marker used by forensic scientists to match crime scene DNA with suspects.
- It was originally invented by Kary Mullis in 1985 and got the Nobel Prize in 1993.

POLYMERASE CHAIN REACTION

PRINCIPLE:

The basic principle of PCR is that the double stranded DNA molecule, when heated to a high temperature, separate yielding two single-stranded DNA molecules. The single stranded DNA molecules can easily be copied with the help of a DNA polymerase and nucleosides resulting in the duplication of original DNA molecules. By repeating these events, multiple copies of the original DNA molecule can be generated.

REQUIREMENTS:

I. A **thermal cycler** (an instrument having a microprocessor-controlled temperature cycling)

II. **DNA segment** to be amplified

III. **Two primers** [Reverse & Forward] (~10-18 nucleotides bp)

IV. **Taq polymerase** (a DNA polymerase) which is stable at high temperature

V. **MgCl2**

VI. **dNTPs** (dATPs, dTTPs, dGTPs, dCTPs)

- The technique was made possible by the discovery of Taq polymerase, the DNA polymerase that is used by the bacterium (Thermus aquaticus) that was discovered from Yellow Stone National Park hot springs.
- This DNA polymerase is stable at the high temperatures need to perform the amplification, whereas other DNA polymerases become denatured.
- The method relies on thermal cycling instrument(thermal cycler), which provide program consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA.

Requirements of PCR Technology

1. The **thermal cycler** heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction.

2. **DNA template** that contains the DNA region (target) to be amplified. (standard concentration at 25ng/µl).

3. **Pair of primers:** short artificial DNA fragments containing sequences complementary to the target region, 3' ends of each of the sense (forward) and the 5' end of the anti-sense (reveres) (~ 18-30 nt).

Note: DNA purity is very important, because template contaminants (i.e. excesses of phenolic compounds, EDTA) may leads to PCR inhibition and give false-negative results.

4. **Master Mix:** which Contains:

- Taq DNA polymerase the enzyme that puts the free nucleotides together. It starts at the 3'end of the primer, and uses the complementary DNA strain as a template.
- Deoxynucleoside triphosphates (dNTPs, sometimes called "deoxynucleotide triphosphates'), Free nucleotides (G, A, T,C)
- Buffer solution, maintains pH and ionic strength of the reaction solution suitable for the activity of the enzyme.
- Mg++ ions - cofactor of the enzyme.

Procedure:

Typically, PCR consists of a series of 25-40 repeated temperature changes, called cycles, each cycle of PCR includes steps for template: Denaturation, primer annealing and primer extension:

I. **Initialization step:** This step consists of heating the reaction to a temperature of 94–96 °C (or 98 °C if extremely thermostable polymerases are used), which is held for 1–9 minutes. It is only required for DNA polymerases that require heat activation by hot-start PCR.

II. Denaturation step: This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 30sec.-1min. It causes DNA melting of the DNA template by disrupting the hydrogen bonds, yielding single-stranded DNA molecules.

III. Annealing step: The reaction temperature is lowered to 50–65 °C for 30 sec-1min allowing annealing of the primers to the single-stranded DNA template. The polymerase binds to the primer-template hybrid and begins DNA formation.

IV. **Extension/elongation step:** The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified.

V. 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.

PCR Steps :

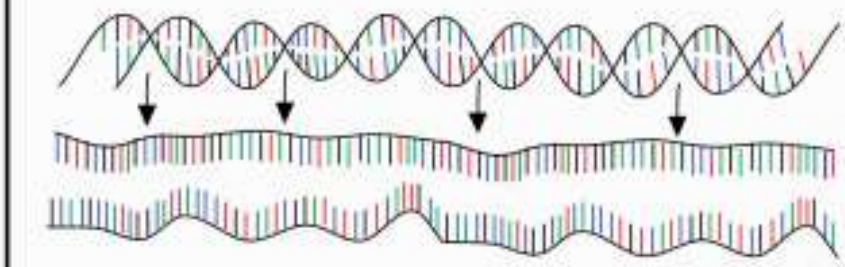
- Initialization step : 94°C 10min
- 35 Cycles :

Denaturation step	94°C 30s	} **cycle**
Annealing step	55°C 30s	
Extension step	72°C 30s	

- Final elongation : 72°C 10min

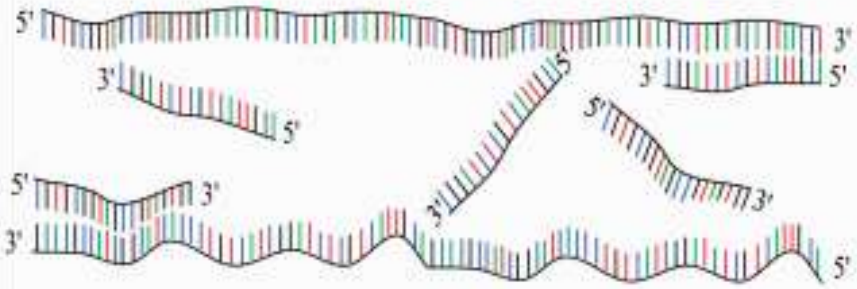
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

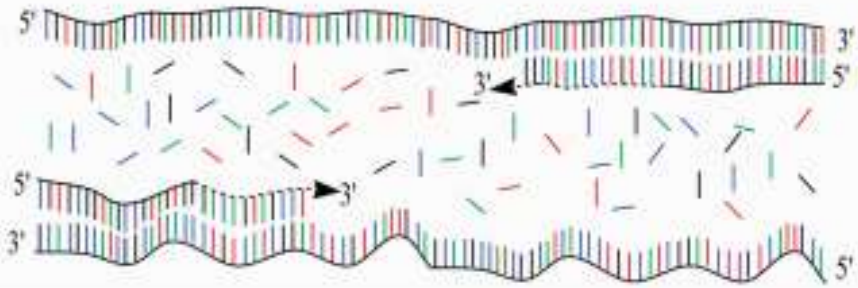
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!



Step 3 : extension

2 minutes 72 °C

only dNTP's

IMPORTANT REMINDERS:

- ALWAYS keep PCR reagents (master mix, primers, DNA template on ICE.
- Gloves are good to use to keep your tubes from getting contaminated.
- Keep your tubes closed and make sure you do not cross contaminate with the tips of the micropipettes.
- VERY IMPORTANT when using the micropipettes be careful and avoid contaminating the micropipettes by slowly releasing the plunger.
- PCR product(amplicon) is separated in agarose and the result is examined via gel electrophoresis


Thank You