INTRODUCTION

•PCR is a means to amplify a particular piece of DNA

• Amplify= making numerous copies of a segment of DNA

•PCR can make billions of copies of a target sequence of DNA in a few hours

• PCR was invented by Kary Mullis (Cetus corporation, USA) in the 1983-1984 as a way to make numerous copies of DNA fragments in the laboratory

• Kary Mullis awarded Nobel Prize in Chemistry in 1993.

• 1985, Saiki publishes the first application of PCR (beta-Globin)

• Its applications are vast and PCR is now an integral part of Molecular Biology/RDT/Biotechnology.

• 1966, Thomas Brock discovers Thermus aquaticus, a thermostable bacteria in the hot springs of Yellowstone National Park

• 1985, Cetus Corp. Scientists isolate Thermostable Taq Polymerase (from T. aquaticus), which revolutionized PCR

Component that are needed for PCR

1) DNA(which contains the target sequence)

2)Heat stable DNA polymerase(eg. Taq polymerase)

3)All 4 nucleotide triphosphate

4) Buffers

5)Two short, single stranded DNA molecules that serve as primer

6)Thin walled tubes

7)Thermal cycler(a device that can change temperatures dramatically in a very short period of time)

PCR

The DNA, DNA polymerase, buffer, nucleoside triphosphates, and primers are placed in a thin-walled tube and then these tubes are placed in the PCR thermal cycler PCR

Process of PCR

The basis of PCR is temperature changes and the effect that these temperature changes have on the DNA.

- In a PCR reaction, the following series of steps is repeated 20-40 times

Note: 30 cycles usually takes about 2-3 hours and amplifies the DNA fragment of interest 1,000,000,000 fold

Each PCR cycle consist of 3 steps each requiring only 3 min

Step-1 (denaturing of DNA) – target DNA must be made single stranded and this is done by heating the sample to 92° C.

Step-2 (Priming or Annealing)- The second step involves the specific hybridization of the two primers to the complementary single stranded DNA. The optimal temperature for this process is about 55°c

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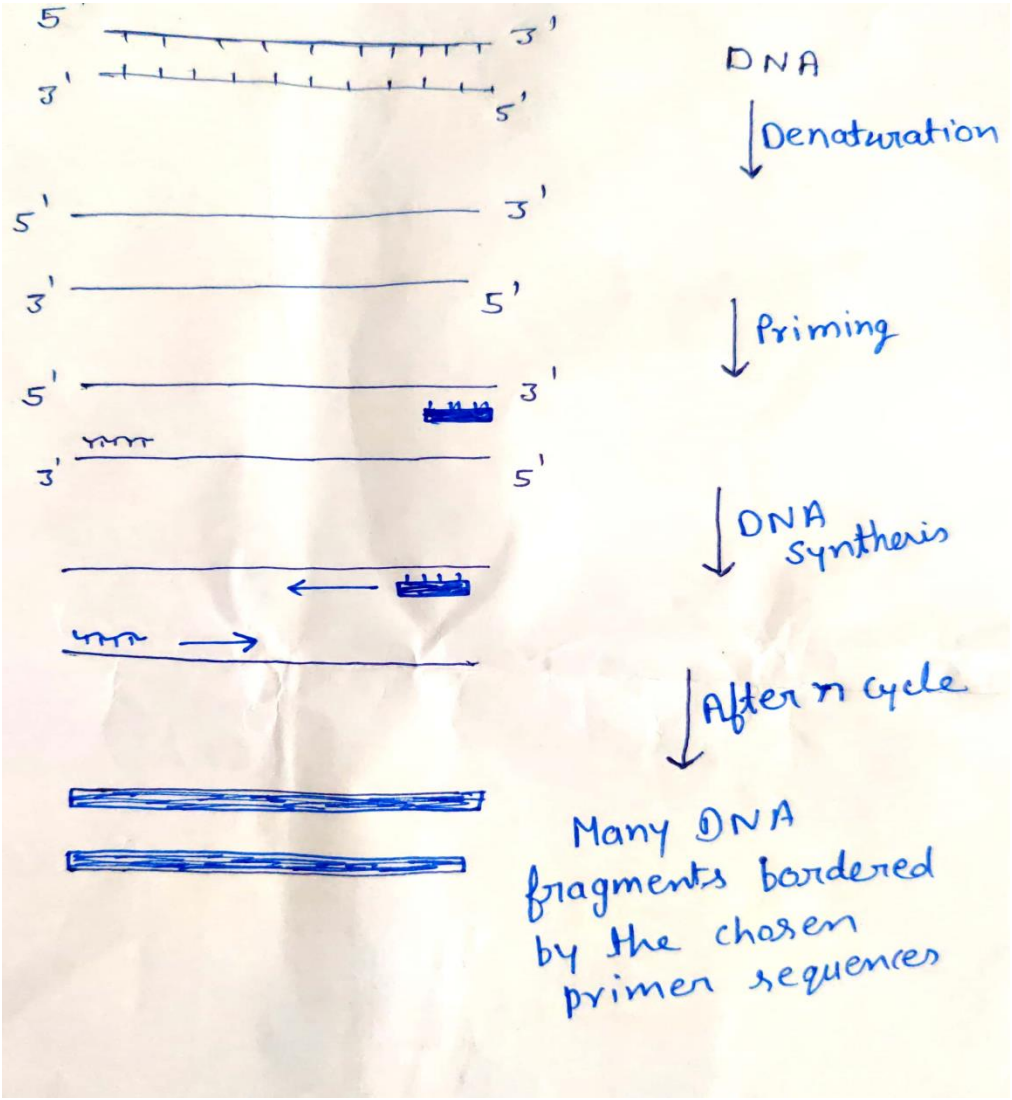
Step-3(synthesis)- DNA polymerase will extend the primer sequence using the single stranded DNA as a template. The optimal extension temperature is about 72 ºC .

Heat-stable DNA Polymerase

- PCR involves very high temperatures, it is imperative that a heat-stable DNA polymerase to be used in the reaction.
- Most DNA polymerases would denature (and thus not function properly) at the high temperatures of PCR.
- *Taq DNA polymerase was purified from the hot springs* bacterium *Thermus aquaticus in 1976*
- *Taq has maximal enzymatic activity at 75 °C to 80 °C, and* substantially reduced activities at lower temperatures.

Process of PCR



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- Size of the DNA Fragment Produced in PCR is Dependent on the Primers
- The PCR reaction will amplify the DNA section between the two primers.
- If the DNA sequence is known, primers can be developed to amplify any piece of an organism's DNA.

APPLICATION

- Primers can be created that will only bind and amplify certain alleles of genes or mutations of genes
- This is the basis of genetic counseling and PCR is used as part of the diagnostic tests for genetic diseases.
- Some diseases that can be diagnosed with the help of PCR:
- Huntington's disease
- Cystic fibrosis
- Human immunodeficiency virus

Huntington's Disease (HD)

- HD is a genetic disorder characterized by abnormal body movements and reduced mental abilities
- HD is caused by a mutation in the Huntingtin (*HD) gene*
- In individuals with HD, the *HD gene is "expanded"*– In non-HD individuals, the *HD gene has a pattern called* trinucleotide repeats with "CAG" occurring in repetition *less than 30 times.*
- – IN HD individuals, the "CAG" trinucleotide repeat occurs more that 36 times in the *HD gene*
- PCR can be performed on an individual's DNA to determine whether the individual has HD.

– The DNA is amplified via PCR and *sequenced (a technique by* which the exact nucleotide sequence is determined) and the number of trinucleotide repeats is then counted.

Cystic Fibrosis (CF)

- CF is a genetic disease characterized by severe breathing difficulties and a predisposition to infections.
- CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CTFR) gene.
- In non-CF individuals, the CTFR gene codes for a protein that is a chloride ion channel and is involved in the production of sweat, digestive juices and mucus.
- In CF individuals, mutations in the CTFR gene lead to thick mucous secretions in the lungs and subsequent persistent bacterial infections.
- The presence of CTFR mutations in a individual can be detected by performing PCR and sequencing on that individual's DNA.

Human Immunodeficiency Virus (HIV)

- HIV is a retrovirus that attacks the immune system.
- HIV tests rely on PCR with primers that will only amplify a section of the viral DNA found in an infected individual's bodily fluids.
- Therefore if there is a PCR product, the person is likely to be HIV positive.
- If there is no PCR product the person is likely to be HIV negative.
- Protein detection based tests are available as well but blood is tested usually by PCR.

COVID-19

RT –PCR technique is used for the diagnosis of corona virus disease.

Note - Reverse transcription polymerase chain reaction (RT-PCR) is a laboratory technique combining reverse transcription of RNA into DNA (in this context called complementary DNA or cDNA) and amplification of specific DNA targets using polymerase chain reaction (PCR).

INVERSE PCR

- The method allows the amplification of those DNA sequences which are away from the primer and not of those which are flanked by the primer. consider the case , if the border sequences of the DNA segment are not known and those of the vector are known, then the sequences to be amplified may be cloned in a vector and border sequence of the vector may be used as a primer in such a way that the polymerization proceeds in reverse direction ,*i.e*. away from the vector sequence flanked by the primer and towards the sequence of the inserted segment.

Anchored PCR

- In this techniques only one primer may be used instead of the two. Only one strand may be copied first, after which a poly G tail is attached at the end of the newly synthesized stand.
- This newly synthesized strand with the poly G tail at its 3’ end will serve as the template for the daughter strands synthesis utilizing an anchor primer with which a poly C sequence to complement to Poly G of the template is linked. In the next cycle both the original primers and the anchored primer are used.