Polymerase Chain Reaction

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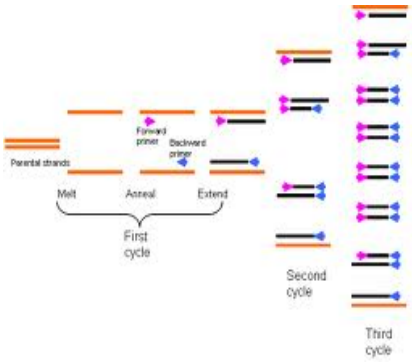
PCR

Thepolymerase chain reaction (PCR) is invented by Karry Mullis in 1983. It is used to amplify the specific DNA sequence by using a pair of oligo nucleotide primers. It requires to generate DNA fragment for cloning experiments.



Some uses of PCR

- Amplification of chosen genomic DNA sequences with or without subsequent cloning
- DNA sequencing in the absence of clone
- For diagnostic using DNA fingerprinting
- To mutate the DNA molecules
- To analyze the previous generation's DNAs

Karry Mullis was awarded the Nobel prize for this great discovery.

Requirements for PCR

- DNA Template
- Primers
- Enzymes

DNA Template:

Any source that contains one or more target DNA molecules to be amplified can be taken as template.

Primers:

Primers are designed to anneal on opposite strands of target sequence. So that they will be extended towards each other by addition to their 3’ ends. A pair of oligonucletides of about 18-30 nucleotides with similar G+C contents act as a primer.

Enzymes:

Taq polymerase is widely used in PCR. It is isolated fromThermus aquaticus which is a thermo-stable bacterium. It can survive at 95 degree C for few minutes. It will have a half life of 2 hours if continuously kept in this high temperature.

Limitations of PCR

- Requires two primers
- Amplifies sequences between two primers only.
- In vitro DNA replication is 100 to 10,000 fold more inaccurate than in vivo DNA replication.

Application of PCR

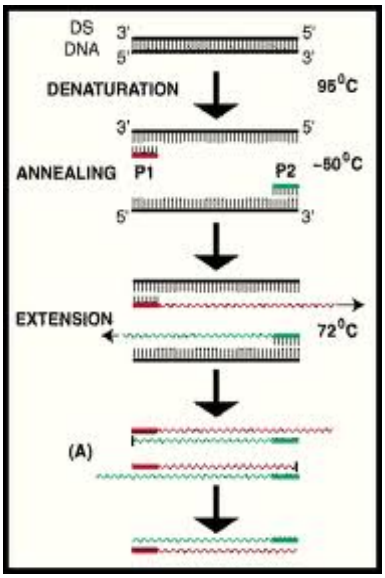
After polymerase chain reaction’s discovery, modern biology has been revolutionized in each and every aspect. Many areas are benefited by PCR application. In that the main areas are

- Diagnosis of pathogens
- Prenatal diagnosis
- DNA fingerprinting
- Palaentology
- Diagnosis of specific mutation
- Research

Working Mechanism of PCR

The mechanism involves many cycles. One amplification has three steps.

1. Denaturation (melting)
2. Annealing
3. Polymerisation (Extension)



Denaturation:

The two strands of DNA are separated if we apply high temperature. This separated strand acts as a template

.

Annealing:

The Nucelotide sequence is complementary to 3’ end of single stranded template. The primers hybridizes

each template. That is done at low temperature. But if we apply very low temperature the chances of mispairing will be high.

Extension:

It is the final step ofamplification cycle.

Want to know more about PCR mechanisms?Click here to schedule live online session with e Tutor!

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Reference Links:

- http://en.wikipedia.org/wiki/Polymerase_chain_reaction
- http://www.sumanasinc.com/webcontent/animations/content/pcr.html
- http://www.dnalc.org/resources/animations/pcr.html
- http://www.ncbi.nlm.nih.gov/projects/genome/probe/doc/TechPCR.shtml

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