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POLYMERASE CHAIN REACTION

INTRODUCTION

PCR is a technique that results in exponential amplification of a selected region of a DNA molecule.

Polymerase chain reaction is a method used widely in molecular biology to make millions to billions of copies of a specific DNA sample rapidly.

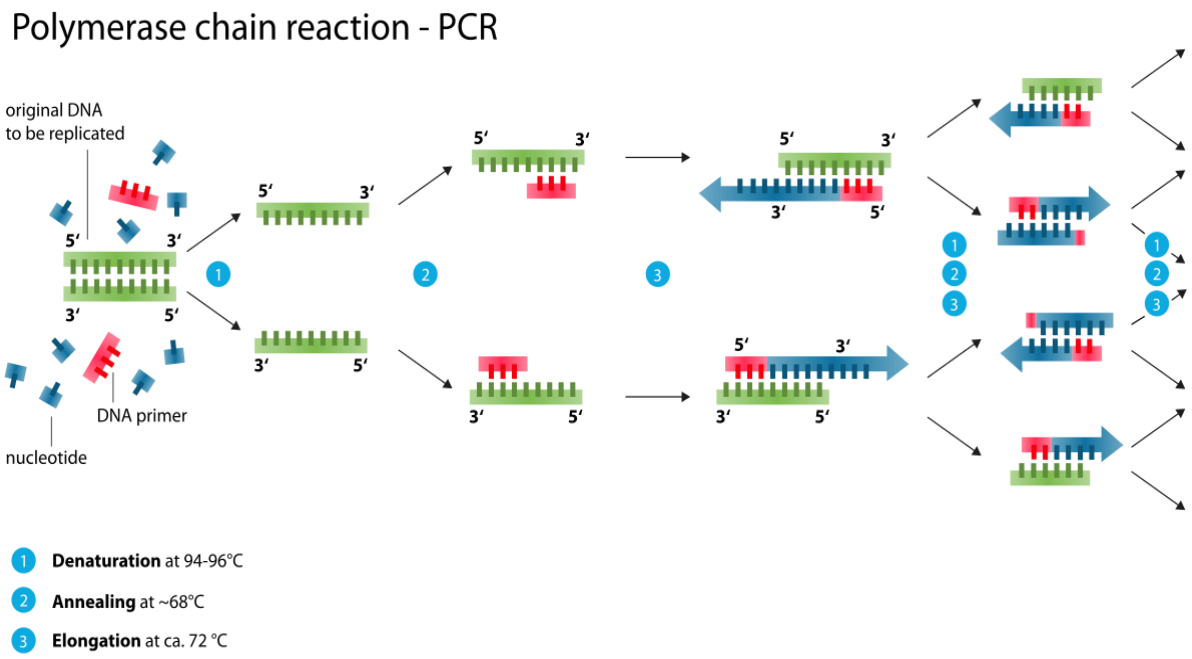
PCR was discovered by Kary Mullis in 1985. For this work Mullis received the Nobel Prize in Chemistry jointly with Michael Smith in 1993. Taq DNA Polymerase the enzyme used in PCR was chosen as the molecule of the year in 1989.

PCR is fundamental for much of genetic testing including analysis of ancient samples of DNA and identification of infectious agents.

STEPS IN PCR REACTION

To perform PCR following steps are followed

1. **Denaturation** of Double Stranded DNA (ds DNA)
   The template strands that are bound together cannot be replicated, so the first step is to separate them by heating up the sample.
2. **Annealing** of Primers to Single Stranded DNA template
   The sample is cooled just enough to allow the primers to bind to the ends of each of the two template strands.
3. **Extension** of primer or Synthesis of ds DNA
   DNA Polymerase attaches to the primers and makes a copy which is complementary to each template strand.



The method consists of repeated heating and cooling which causes melting (separation of the two strands) and replication of the original DNA, also called a template. Short DNA fragments consisting of DNA sequences complimentary to the ends of the template, called primers and a DNA polymerase are key materials for selective and repetitive steps.

After the first cycle, there are four DNA strands. The process repeats with the four strands which will go on to make eight strands, then repeat itself again to make sixteen strands. In this way PCR doubles the amount of DNA in a sample after each cycle, making it possible to obtain millions of copies of a DNA strand overnight.

The presence of **Taq DNA polymerase** and all the four essential nucleotide triphosphates allow synthesis of complementary strands in usual manner. In a thermal cycle the process is automatically repeated 20-30 times, so that a millions and billions of copy can be synthesized. **Thermostable** enzyme **Taq DNA polymerase** has been isolated from **Thermus *aquaticus*** (Archaea growing in hot springs). This enzyme acts at 72degrees, and even at 90 degrese its enzymatic activity doesn't destroys. Other thermostable polymerases are

Pflu DNA polymerase isolated from **Pyrococcus *furiosus***
Vent polymerase isolated from **Thermococcus *litoralis***

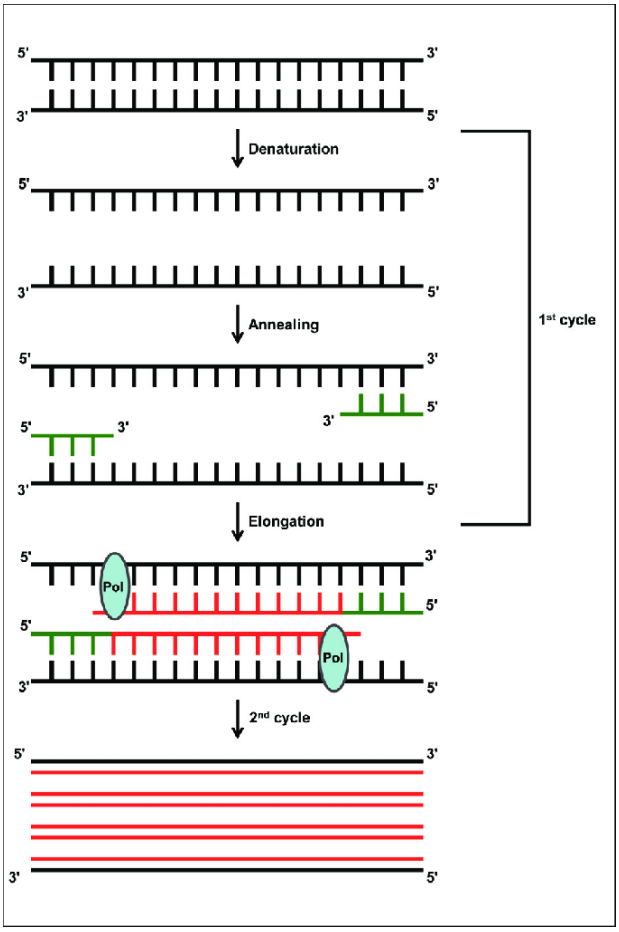
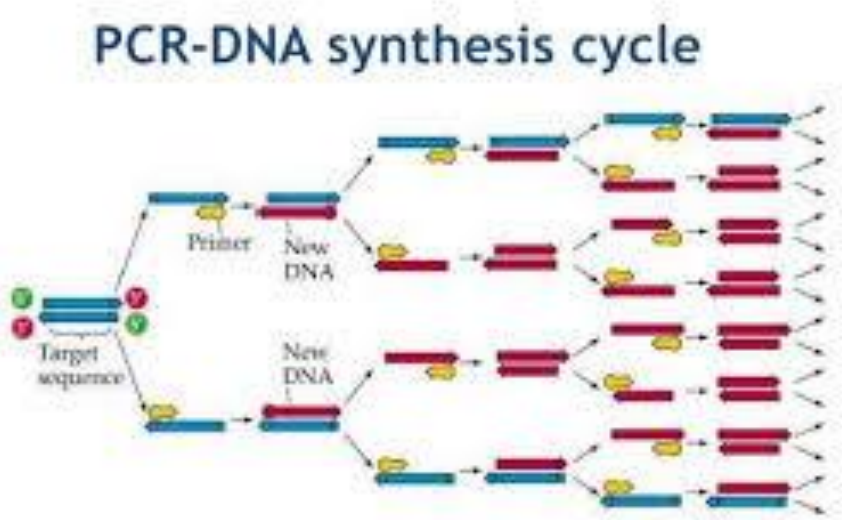


Fig: STEPS OF PCR



APPLICATIONS

1. PCR may be compared with gene cloning as both are used for increasing number of DNA fragments. Improvement in PCR method has led to develop an automatic PCR machine which has increased its application many fold. It has replaced unnecessary gene cloning which is usually done with specialized person's efforts but are error prone, costly and take 2-4 days to complete the whole process. Very small amount of DNA was unable to be amplified by gene cloning. PCR can multiply smallest fragments of DNA (nanograms) automatically with less effort and investment. Manipulation is faithful without error.
2. It is being used for Parental diagnosis
3. DNA fingerprinting
4. As confirmation test for gene tagging
5. Electronic PCR (e-PCR)
6. Frequently used for Archaeological & Palaenological (fossil) studies.
7. Amplification of gene for various industrial applications, where it enhances the end product.

Fig: Commercially used automated PCR machines

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