APPLICATION OF POLYMERASE CHAIN REACTION (PCR) IN MEDICAL PRACTICE

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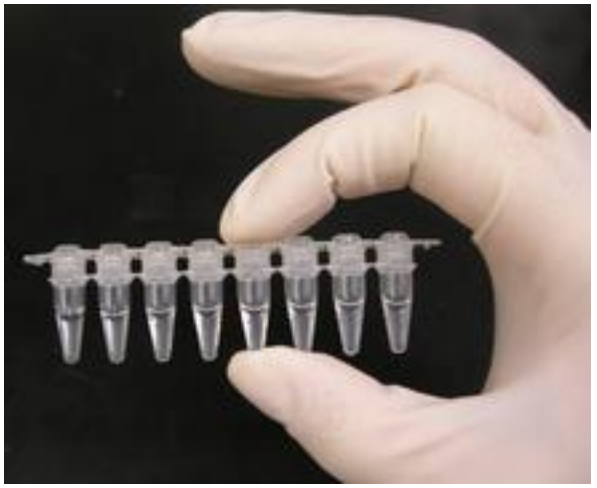
SUMMARY

PCR is an in vitro technique for generating large quantities of a specific DNA sequence. In simple word it is automated version of DNA replication. The vast majority of PCR methods rely on thermal cycling. Thermal cycling exposes reactants to repeated cycles of heating and cooling to permit different temperature-dependent reactions—specifically, DNA melting and enzyme-driven DNA replication. PCR employs two main reagents - primers (which are short single strand DNA fragments known as oligonucleotides that are a complementary sequence to the target DNA region) and a DNA polymerase. In the first step of PCR, the two strands of the DNA double helix are physically separated at a high temperature in a process called DNA melting. In the second step, the temperature is lowered and the primers bind to the complementary sequences of DNA. The two DNA strands then become templates for DNA polymerase to enzymatically assemble a new DNA strand from free nucleotides, the building blocks of DNA. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the original DNA template is exponentially amplified. Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis, functional analysis of genes; diagnosis and monitoring of hereditary diseases; amplification of ancient DNA; analysis of genetic fingerprints for DNA profiling and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

APPLICATION OF POLYMERASE CHAIN REACTION IN MEDICAL PRACTICE

Polymerase chain reaction (PCR) is a method widely used in molecular biology to make many copies of a specific DNA segment. Using PCR, copies of DNA sequences are exponentially amplified to generate thousands to millions of more copies of that particular DNA segment. PCR is now a common and often indispensable technique used in medical laboratory and clinical laboratory research for a broad variety of applications including biomedical research and criminal forensics. PCR was developed by Kary Mullis in 1983 while he was an employee of the Cetus Corporation. He was awarded the Nobel Prize in Chemistry in 1993 (along with Michael Smith) for his work in developing the method.



PRINCIPLES

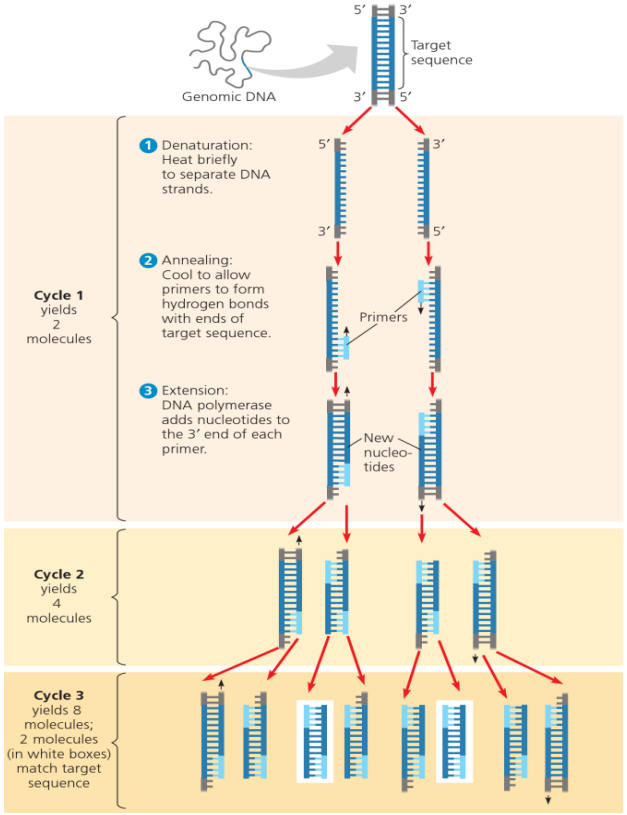
A basic PCR set-up requires several components and reagents, including:

1. DNA template that contains the DNA target region to amplify.
2. DNA primers that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target.
3. DNA polymerase; an enzyme that polymerizes new DNA strands; heat-resistant Taq polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process.
4. Deoxynucleoside triphosphates, or dNTPs (sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand.
5. Buffer Solution providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
6. Bivalent cations, typically magnesium (Mg) or manganese (Mn) ions and monovalent cations, typically potassium (K) ions.

The reaction is commonly carried out in a volume of 10–200 µL in small reaction tubes (0.2–0.5 mL volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction

PROCEDURE

Typically, PCR consists of a series of 20–40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of two or three discrete temperature steps. The individual steps common to most PCR methods are as follows:



1. **Denaturation:** This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.
2. **Annealing:** In the next step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region.
3. **Extension/elongation:** In this step, the temperature is raised at 72^{C} [162^{F}]. The DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free DNTPS from the reaction mixture that are complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the DNTPS with the 3'-hydroxy group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify.

APPLICATION OF PCR IN MEDICAL PRACTICE

1. PCR can be used to create copies of DNA

2. PCR can detect infectious disease like HIV, TB before standard serological laboratory test, so allowing treatment to start much earlier.

3. PCR can also be used to detect bacterial and viral DNA in the environment for example looking at pathogens in water supplies.

4. PCR can be used in analysis of gene expression for example looking at level of expression and when gene are switched on and off in physiological processes including in health and diseases.

5. PCR is especially useful for infections that are difficult to culture in the laboratory, such as tuberculosis.

6. Genetic testing where a sample of DNA is analyzed for the presence of genetic disease mutation.

7. PCR is used in personalized medicine to select patients for certain treatments, e.g. in cancer when patients have a genetic change that makes a patent more or less likely to respond to a certain treatment.

8. PCR is used to monitor and track the spread of infectious disease within an animal or human population.

9. PCR is also useful for screening donated blood for infections.

10. PCR permits early diagnosis of malignant diseases such as leukemia and lymphomas Infectious disease applications.

11. PCR allows for rapid and highly specific diagnosis of infectious diseases, including those caused by bacteria or viruses.

12. PCR also permits identification of non-cultivatable or slow-growing microorganisms such as *mycobacteria*, anaerobic bacteria, or viruses from tissue culture assays and animal models.

ADVANTAGES OF PCR

1. PCR is specific/ sensitive
2. PCR is simple to use, rapid, relative/ inexpensive
3. Amplifies from low quantities
4. Works on damaged DNA

LIMITATIONS OF PCR

1. Contamination risk
2. Primer complexities
3. Primer – binding site complexities
4. Amplifies rare species
5. Detection methods

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