Kingdom of Saudi Arabia

King Saud University

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PCR

Polymerase Chain Reaction

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ABSTRACT

At the first semester of the Academic Year 2008-2009 we had our graduation project to be supervised by **Mr. Nisar**. He suggests the topic of the project to be "The Coherent Scattered X-Ray". But we chose the topic to be "Polymerase Chain Reaction (PCR)". Which is widely used method in laboratory. The PCR method is using PCR device or Thermocycler device which is the connection between **Biomedical Technology** and **PCR**.

Why PCR?

PCR is a recently developed procedure for the *in vitro* amplification of DNA. PCR has transformed the way that almost all studies requiring the manipulation of DNA fragments may be performed as a result of its simplicity and usefulness. PCR, an acronym for Polymerase Chain Reaction, allowed the production of large quantities of a specific DNA from a complex DNA template in a simple enzymatic reaction.

Contents

1. INTRODUCTION

1.1. What is PCR?

Polymerase Chain Reaction (PCR) is a molecular biological method for amplifying (creating multiple copies of) DNA without using a living organism. PCR is commonly used in medical and biological research labs for a variety of tasks, such as the detection of hereditary diseases, the identification of genetic fingerprints, the diagnosis of infectious diseases, the diagnosis of viruses diseases, the cloning of genes, and paternity testing.

PCR derives its name from one of its key components, which is DNA polymerase. Which is an enzyme catalyzing the DNA replication.

1.2. History

The **history of the PCR** has variously been described as an example of cooperative teamwork between disparate researchers.

At 1971 Kleppe and co-workers first described a method using an enzymatic assay to replicate a short DNA template with primers. However, this early manifestation of the basic PCR principle did not receive much attention, and the invention of the polymerase chain reaction in 1983 is generally return to Kary Mullis.

At the core of the PCR method is the use of a suitable DNA polymerase able to withstand the high temperatures of >90°C (>195°F) required for PCR. The DNA polymerases initially experiments presaging PCR were unable to withstand these high temperatures. So the early procedures for DNA replication were very inefficient, time consuming, and required large amounts of DNA polymerase and continual handling throughout the process.

The discovery in 1976 of **Taq polymerase** (a DNA polymerase purified from the thermophilic bacterium, which naturally occurs in hot environments (50 to 80 °C (120 to 175 °F))) paved the way for dramatic improvements of the PCR method. The DNA polymerase isolated from this bacterium is stable at high temperatures remaining active even after DNA denaturation, thus obviating the

need to add new DNA polymerase after each cycle. This allowed an automated thermocycler-based process for DNA amplification.

When Mullis developed the PCR in 1983, he was working in Emeryville, California for Cetus Corporation, one of the first biotechnology companies. There, he was responsible for synthesizing short chains of DNA. Mullis has written that he conceived of PCR while cruising along the Pacific Coast Highway one night in his car. He was playing in his mind with a new way of analyzing changes (mutations) in DNA when he realized that he had instead invented a method of amplifying any DNA region through repeated cycles of duplication driven by DNA polymerase.

In *Scientific American*, Mullis summarized the procedure: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat. He was awarded the Nobel Prize in Chemistry in 1993 for his invention. Seven years after he and his colleagues at Cetus first put his proposal to practice. However, some controversies have remained about the intellectual and practical contributions of other scientists to Mullis' work, and whether he had been the sole inventor of the PCR principle.

- **Patent wars**

The PCR technique was patented by Cetus Corporation, where Mullis worked when he invented the technique in 1983. The *Taq* polymerase enzyme was also covered by patents. There have been several high-profile lawsuits related to the technique. The pharmaceutical company Hoffmann-La Roche purchased the rights to the patents in 1992 and currently holds those that are still protected.

1.3. Preview about DNA

Before describing PCR it must be know the structure of DNA. DNA is stand for **Deoxyribonucleic acid.** It is a long polymer made from repeating units called nucleotides. The DNA is floating inside the nucleus within DNA polymerase and different DNA solutions. The DNA chain is 22 to 26 ngstr ms wide (2.2 to 2.6 nanometers), and one nucleotide unit is 3.3 (0.33 nm) long. Although each individual repeating unit is very small, DNA polymers can be very large molecules

containing millions of nucleotides. For instance, the largest human chromosome, chromosome number 1, is approximately 220 million base pairs long.

In living organisms, DNA does not usually exist as a single molecule, but instead as a tightly-associated pair of molecules. These two long strands entwine like vines, in the shape of a double helix. The nucleotide repeats contain both the segment of the backbone of the molecule, which holds the chain together.

The DNA double helix is stabilized by hydrogen bonds between the bases attached to the two strands. The four bases found in DNA are Adenine (abbreviated A), Cytosine (C), Guanine (G) and Thymine (T).

Adenine combines by hydrogen bond only with Thymine. And Cytosine combines only with Guanine. With this property the code of DNA is formed as showing in the figure.

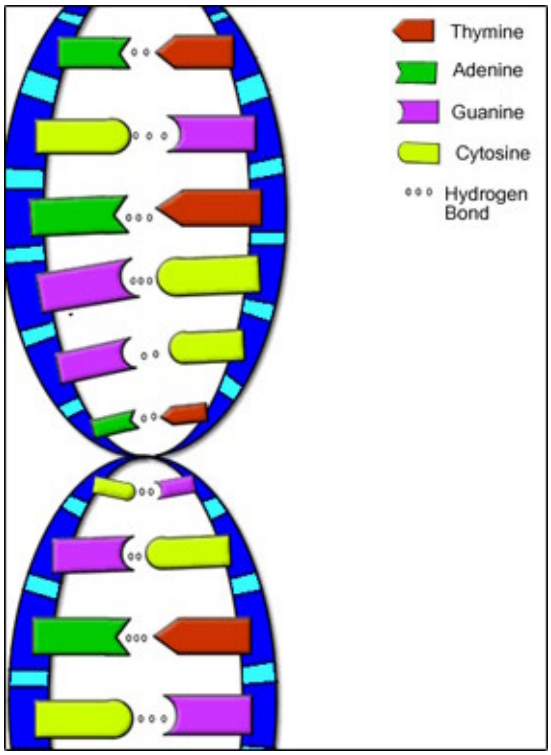

Fig 1.1 DNA structure

2. PRINCEPLE OF PCR

2.1. PCR Equipment (Thermocycler)

- PCR main device is the *Thermocycler* (most called PCR device) and it is mainly consist of:
    a. *Heater* for raise the temperature to 60°, 72° and 95°C
    b. *Fan* for cooling the samples from high temperature (95°C) to a lower temperature (72° and 60°C). The fan also to distribute the heat.
    c. *Thermo sensor* to measure the temperature of sample.
    d. *Thermo chamber* making the heat homogenously distributed on all samples.
    e. And all these components are connected to a *microcomputer or microcontroller* to control the process of raising and dropdown the temperature.
    f. *Carousel* or *Sample Block* to hold the samples.

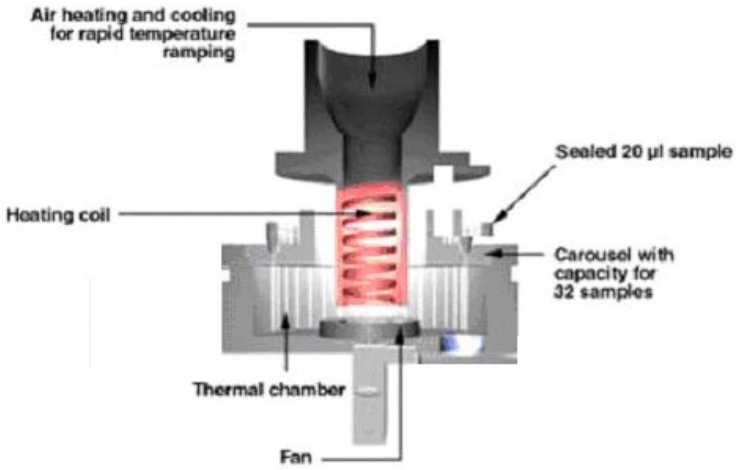

Fig 2.1 Diagram explaining the components of *thermocycler*

Fig 2.2 One of the most popular models of *Thermocycler* (GeneAmp® PCR System)

2.2 Components of PCR reaction

- *DNA template* that contains the DNA region (target) to be amplified.
- *Primers*, which are complementary to the DNA (Designed chemically and each DNA has special primer).
- *Taq polymerase* or another DNA polymerase with a temperature optimum at around 70°C.
- *Deoxynucleoside triphosphates* (dNTPs; also very commonly and erroneously called deoxynucleotide triphosphates), the building blocks from which the DNA polymerases synthesizes a new DNA strand.
- *Buffer solution*, providing a suitable chemical environment for optimum activity and stability of the DNA polymerase.
- *Divalent cations*, magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be utilized for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis
- *Monovalent cation* potassium ions.

2.3 PCR process

- *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.

- *Denaturation step*: This step is the first regular cycling event and consists of heating the reaction to 94-98°C for 20-30 seconds. It causes melting of DNA

template and primers by disrupting the hydrogen bonds between complementary bases of the DNA strands, yielding single strands of DNA.

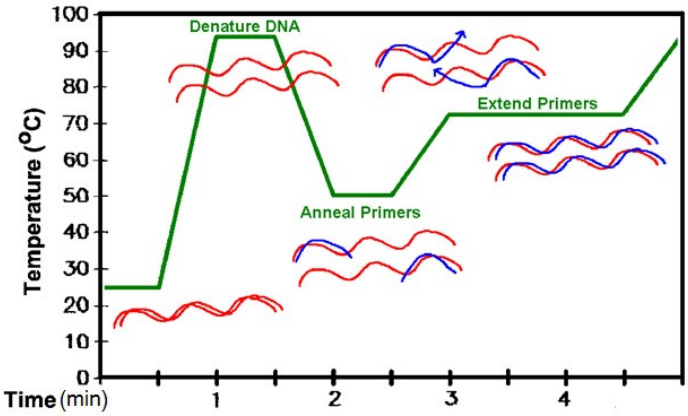

Fig 2.3 The temperature change at different steps (one cycle)

- *Annealing step*: The reaction temperature is lowered to 50-65°C for 20-40 seconds allowing annealing of the primers to the single-stranded DNA template. Typically the annealing temperature is about 3-5 degrees Celsius below the Tm of the primers used. Stable DNA-DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence. The polymerase binds to the primer-template hybrid and begins DNA synthesis.

- *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, and commonly a temperature of 72°C is used with this enzyme. At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5' to 3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxyl group at the end of the nascent (extending) DNA strand. The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to be amplified. As a rule-of-thumb, at its optimum temperature, the DNA polymerase will polymerize a thousand bases per minute. Under optimum conditions, i.e., if there are no limitations due to limiting substrates or reagents, at each extension step, the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA fragment.

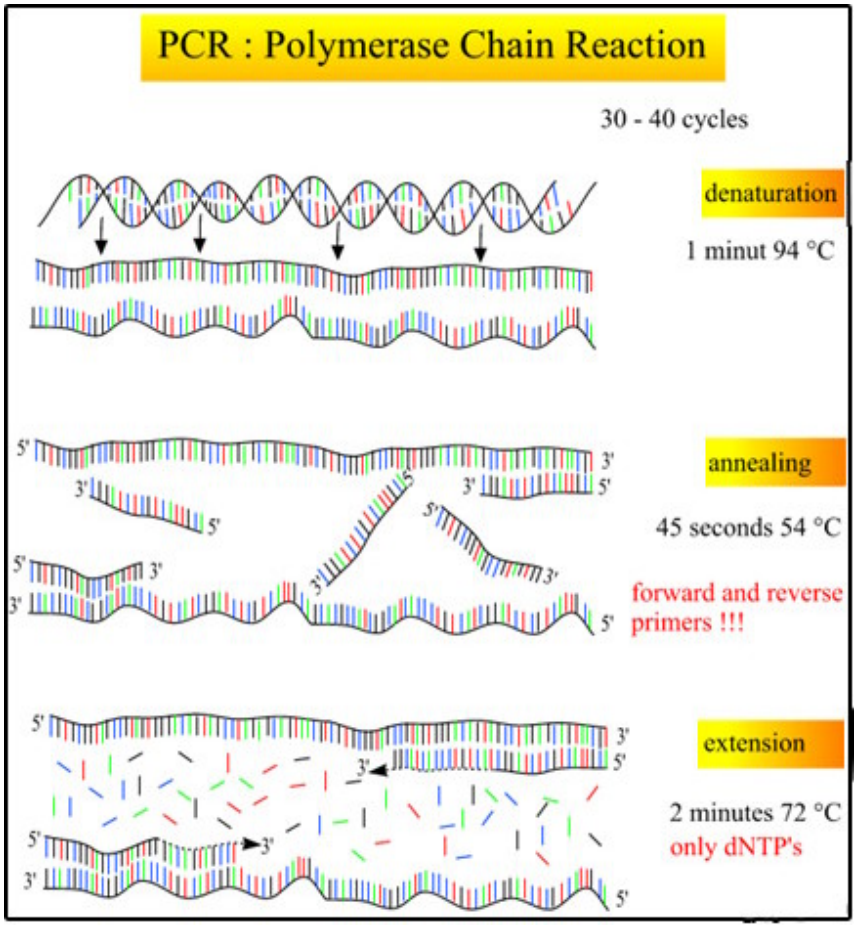

Fig 2.4 Diagram showing PCR process

- *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.

- *Final hold*: This step at 4-15°C for an indefinite time may be employed for short-term storage of the reaction.

  The result of each cycle is doubled DNA as (Fig 2.5)

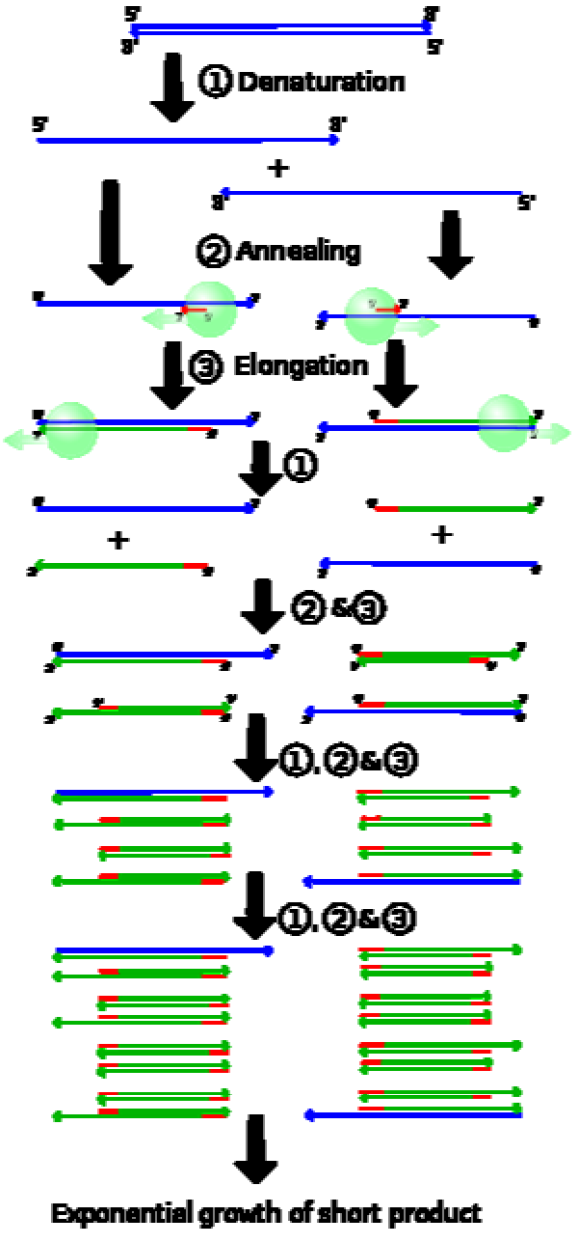

Fig 2.5 Doubling DNA each cycle

3. APPLECATIONS OF PCR IN MEDICINE

3.1 Diagnosis of infectious diseases

PCR is applied in detecting DNA or RNA of infecting organisms as bacteria and viruses. In this respect, PCR has the advantage of detecting the occurrence of infection even in the early stages, in which only a small amount of nucleic acid is available in blood. In addition, real time-PCR (RT-PCR), which is a special modification of PCR technique, allows the quantitative determination of viral infection. This developed technique facilitates the decision of proper and adequate medication and the follow up of patients' condition and their response to treatment.

Detection of infectious diseases by PCR is nowadays recommended to be performed as a routine for blood of donors in blood transfusion. This can help to limit risk of transmitting infected blood with viruses (as hepatitis B, HIV, etc) from carriers who may show negative results by ordinary serological methods which depend on antibodies detection in serum. Appearance of antibodies in serum may take a long time to develop.

Examples of infectious organisms detected by PCR

- *Bacterial infections*: Tuberculosis (TB).
- *Viral infections*: hepatitis B and C virus.
- *Human Immunodeficiency Virus* (HIV) which causes AIDS.

3.2 Detection of mutations in single genes

Some single gene mutations may cause inborn errors of metabolism. Accurate and early diagnosis even before appearance of clinical signs of such disorders may help to avoid serious consequences as it permits treatment to be applied in proper time.

A set of primers is designed to amplify the single gene of interest. Amplified gene can be further studied by sequencing in order to identify the presence of mutations of any type. Sometimes, it is required to design many sets of primers for different locations on the same gene. This technique is called multiplex-PCR.

Examples of application of PCR in single gene mutation

▪ Cystic fibrosis

It is an autosomal recessive genetic disease. The clinical disorder results from mutations in the Cystic Fibrosis Transmembrane Regulator (CFTR) gene. The most common mutation is three-base deletion that results in the loss of a phenylalanine residue from the CFTR protein.

PCR helps to verify the CFTR gene for the presence of mutation. As the mutant allele is three bases shorter than the normal allele, it is possible to distinguish them from each other by the size the PCR products obtained by amplifying the portion of the DNA.

▪ Duchene muscular dystrophy (DMD)

DMD is among the most common human genetic diseases. This gene is greater than 2 million base pairs in size and contains at least 70 exons separated by an average intron size of 35 Kb. Intragenic deletion mutations account for up to 60% of all cases of DMD. In order to detect deletions in this large gene by PCR, at least nine separate regions of the dystrophin gene are amplified at the same time using a single PCR procedure called multiplex PCR. To fulfill technique requirements, several sets of PCR primers are designed and applied in equimolar concentrations to permit specific and simultaneous amplification of suspected loci on the gene.

3.3 Forensic applications of PCR

DNA isolated from a single human hair, a tiny spot of blood or a semen sample is sufficient to check whet4er the sample belongs to a specific individual or not. Accordingly, evidences from crime scenes could be analyzed in order to verify whether a suspect (if available) is guilty or not guilty.

3.4 Molecular Genetics and Molecular Biology research applications of PCR

The study of segments of DNA may require their amplification by PCR. Amplified DNA can be analyzed by sequencing. In this respect, PCR technique as a relatively rapid technique of DNA amplification, facilities the human genome project which achieved to sequence all DNA in human cells.

In addition, scientists try to understand the physiological function of each

domain of various proteins as enzymes. This could be easily performed by exploiting the cDNA coding these proteins. cDNA of a protein of interest may be amplified by PCR. Then, amplified cDNA is cloned in expression vectors. Cloned vectors are introduced to expression prokaryotic or eukaryotic host cells in order to synthesize the protein of interest.

Many studies of genes may require inducing mutagenesis in a specific single or multiple loci on the DNA coding the protein by using PCR. Then, the mutated gene is further amplified by PCR and cloned in expression vectors. Expressed mutated protein is studied for the biological importance of the knocked out region of DNA.

4. ACKNOWLADGMENTS

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Finally we thank all who read this report.

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