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**Lab 8**

**Polymerase Chain Reaction (PCR)**

What is PCR?

 Is an ingenious technology in molecular biology to amplify a single or

a few copies of a piece of DNA across several orders of magnitude,generating thousands to millions of copies of a particular DNA sequence *in vitro*. This PCR process, invented by **Kary Mullis in 1984** in California, allowed scientists to make millions of copies of a scarce sample of DNA. The technique has revolutionized many aspects of current research, including the diagnosis of genetic defects and the detection of the AIDS virus in human cells. The purpose of a PCR is to make a **huge number of copies of a gene**.

 As a result, it now becomes possible to analyze and characterize DNA fragments found in minute quantities in places like a drop of blood at a crime scene. PCR also affected evolutionary studies because large quantities of DNA can be manufactured from fossils containing but trace

amounts. PCR has been automated for routine use in laboratories worldwide, the development of this technique resulted in an explosion of

new techniques in molecular biology (and a Nobel Prize for Kary Mullins

in 1993) as more and more applications of the method were published.

PCR is now a common and often indispensable technique used in medical and biological research labs for a variety of applications. The invention of the PCR (Polymerase Chain Reaction) technique has resulted in a revolution for evolutionary biologists interested in genetic questions. Suddenly there was a fast, robust and relatively inexpensive technique to

get hold of genetic information from small samples such as skin, blood or

faeces.

An advantage with PCR based molecular studies of DNA is that,

once DNA is extracted and purified, the techniques are very similar

regardless of the taxonomy of the study organisms.The technique was made possible by the discovery of **Taq polymerase**, the DNA polymerase that is used by the bacterium **(*Thermus aquaticus*)** that was discovered from yellow stone National Park hot springs. This DNA polymerase is stable at the high temperatures need to perform the amplification, whereas other DNA polymerases become denatured.Since this technique involves amplification of DNA, the most obvious application of the method is in the detection of minuscule amounts of specific DNAs. This important in the detection of low-level bacterial infections or rapid changes in transcription at the single cell level as well

as the detection of a specific individual's DNA in forensic science. It can also be used in DNA sequencing, screening for genetic disorders,site specific mutation of DNA. The most important consideration in PCR is contamination. If the sample that is being tested has even the smallest contamination with DNA from the target, the reaction could amplify this DNA and report a falsely positive identification. For example, if a technician in a crime lab set up a test reaction (with blood from the crime

scene) after setting up a positive control reaction (with blood from the suspect) cross contamination between the samples could result in an erroneous incrimination, even if the technician changed pipette tips between samples. A few blood cells could volatilize in the pipette, stick to the plastic of the pipette, and then get ejected into the test sample. The

powerful amplification of PCR may be able to detect this cross contamination of samples. Modern labs take account of this fact and

devote tremendous effort to avoiding this problem.

The method relies on thermal cycling, consisting of cycles of repeated

heating and cooling of the reaction for DNA melting and enzymatic

replication of the DNA.

**Requirements of PCR Technology**

1- **The thermal cycler** heats and cools the reaction tubes to achieve the

temperatures required at each step of the reaction.

2- **DNA template** that contains the DNA region (target) to be amplified, the

source of DNA for the PCR amplification. This could be DNA extracted

from blood, skin, feathers, or old PCR products. We use a standard

concentration at 25ng/μl.

3- **Pair of primers:** short artificial DNA fragments containing sequences

complementary to the target region, that are complementary to the 3'

(three prime) ends of each of the sense (forward) and the 5' end of the

anti-sense (reveres) strand of the DNA target, usually the length of 18-30

nt.

4- **Master Mix** which Contains:

• **Taq DNA polymerase** the enzyme that puts the free nucleotides together.

It starts at the 3’end of the primer and uses the complementary DNA strain

as a template.

• **Deoxynucleoside triphosphates** (dNTPs, sometimes called

"deoxynucleotide triphosphates"; nucleotides containing triphosphate

groups), Free nucleotides (G, A, T,C) of which the artificial DNA copies

are made, the building-blocks from which the DNA polymerase synthesizes

a new DNA strand.

• **Buffer solution**, maintains pH and ionic strength of the reaction solution

suitable for the activity of the enzyme.

• **Mg++ ions -** cofactor of the enzyme.

**5- free nuclease water:** must be present for the reaction to work**.**

**Procedure:**

Typically, PCR consists of a series of 25-40 repeated temperature

changes, called cycles, each cycle of PCR includes steps for template

denaturation, primer annealing and primer extension:

• **Initialization step**: This step consists of heating the reaction to a

temperature of 94–96 °C (or 98 °C if extremely thermo stable 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 30sec-1min. It causes DNA

melting of the DNA template by disrupting the hydrogen bonds between

complementary bases, yielding single-stranded DNA molecules.

• **Annealing step**: The reaction temperature is lowered to 50–65 °C for 30

sec-1min 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 formation.

• **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.

• **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

**PCR Steps**：

• Initialization step ：94℃ 10min

• 35 Cycles：

 Denaturation step 94℃ 30s

 Annealing step 55℃ 30s 1 cycle

 Extension step 72℃ 30s

• Final elongation ： 72℃ 10min



 **Applications of PCR:**

1- Medicine: The PCR technique enables early diagnosis of malignant

diseases.

2- Classification of organisms.

3- Mutation detection.

4- Detection of pathogens.

5- Gene therapy.

6- Fingerprint.

7- Forensic science: PCR is very important for the identification of criminal

8- It is also used in diagnosis of retroviral infection, bacterial infections,

cancers, sex determination of embryos.

9- Evolutionary studies: It plays an important role in phylogenetic analysis.

**How do we see our results after running a PCR?**

The most common way of seeing the results of a PCR is by running a gel

electrophoresis. The PCR product is pipetted into a special agar that will

separate the DNA fragments according to their weight by using

electricity.

**Why ““Polymerase”?** It is called “polymerase” because the only enzyme used in this reaction is DNA polymerase.

**Calculating annealing temperature (Tm) of a primer**:

**Tm = 4(G + C) + 2(A + T)**

where G, C, A, T are the number of respective nucleotides. This formula

can be used for primers that are no longer than 25 nt long. Nowadays, some

specialized computer programs can do all calculations and determinations

for proposed primers, for example Gene runner.