Course: M.Sc. Biotechnology

Paper: BIOT4009: Genetic Engineering and Gene Therapy

1

UNIT – III
POLYMERASE CHAIN REACTION

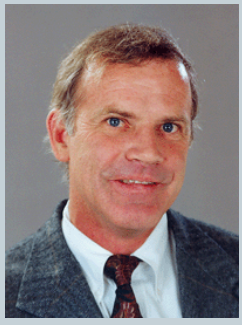
BRIJESH PANDEY
DEPARTMENT OF BIOTECHNOLOGY
MAHATMA GANDHI CENTRAL
UNIVERSITY, BIHAR

Polymerase Chain Reaction:PCR

History

2

1. Method was invented by Kary B Mullis (Cetus Corporation, USA), published in 1985
2. Awarded with Nobel Prize in 1993 which he shared with Michael Smith (Site Directed Mutagenesis) for Chemistry



Kary Banks Mullis

Polymerase Chain Reaction: PCR

3

History contd.

3. **It is an *in vitro* method used for production of multiple copies of a DNA**
4. **PCR was amalgamation of informations derived from experiments related to**
   - **Repair replication (Khorana et al)**
   - **Thermal denaturation and renaturation of DNA (Julies Marmur and Paul Doty)**
   - **Synthesis of primers**
   - **Use of DNA dependent DNA Polymerases**

Polymerase Chain Reaction: PCR

4

Facts and events

- Is results into exponential amplification of a selected region of a DNA molecule.
- Also called a **Molecular Photocopier**: it produces identical copies of DNA at the end.
- DNA dependent DNA polymerase is used to synthesize strand of DNA.
- In this chain reaction, product of first reaction become substrates for the following one, and so on)

Polymerase Chain Reaction: PCR

5

Components of reaction mixture

- **Template DNA (1-1000 ng)**
- **Primers, i.e oligonucleotides with 3'OH (5-100 p mol each)**
- **20-200 µM each dNTP (dATP, dCTP, dGTP, dTTP)**
- **DNA polymerase (preferably thermostable *Taq* DNA polymerase)**
- **Tris-HCl (10-50 mM) , pH 8.3**
- **KCl (Up to 50 mM)**
- **1.5 mM or higher $MgCl_2$**
- **Additionally, ammonium sulphate, BSA and DTT etc. are also used**

Polymerase Chain Reaction: PCR

6

Thermal Profile

Thermal stages governing status of DNA and activity of enzyme

Polymerase Chain Reaction: PCR

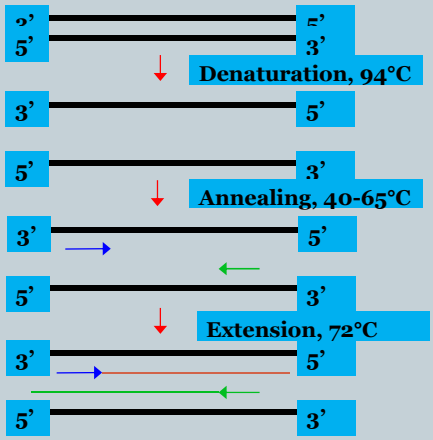
7

Thermal Profile contd.

Initial denaturation 94°C for separation of DNA strands

20-35 cycles of PCR thermal stages (as shown in right panel)

Final extension (polymerization) for 2-5 minutes at 72°C

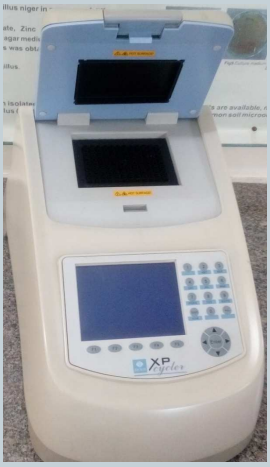


Cyclic thermal stages

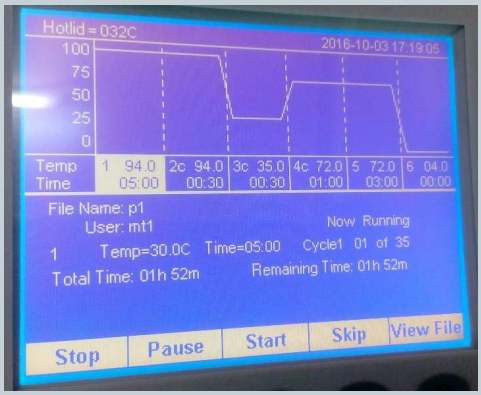
Polymerase Chain Reaction: PCR

8

PCR machine



The machine



Display panel with thermal profile

Polymerase Chain Reaction: PCR

- Denaturation: leads to strand separation
- Annealing: allows binding of primers on single strand DNA
- Extension/ polymerization stage- primer is extended in template dependent synthesis of DNA
  - Mediated by polymerase
  - Building blocks are dNTPs
  - Required supporting components are Mg^{++} ions, buffer, KCl etc.

Polymerase Chain Reaction: PCR

10

Cyclic progress in copies of DNA

$$Nc = No\ (1+Y)^{n-1}$$

Nc: the no. of DNA templates after a given cycle number,

No: the no. of target molecule at the beginning (0 cycle),

Y: efficiency,

n: number of cycles

Polymerase Chain Reaction: PCR

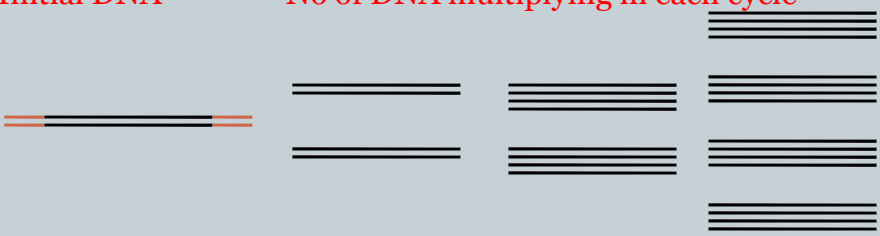
11

Exponential increase in copy no.

Target gene

Initial DNA

No of DNA multiplying in each cycle



Polymerase Chain Reaction: PCR

12

The critical parameters to optimize

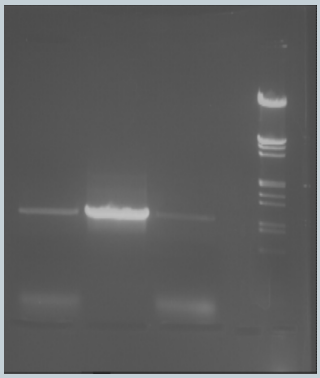
- Concentration of
  - DNA template
  - Nucleotides
  - Divalent cations (Mg^{2+})
- Primer sequence and annealing temperature
- Polymerase and their error rate (*Taq, Vent, Pfu*)

Polymerase Chain Reaction: PCR

13

The visualization of results

- Amplified DNA is visualized by many methods.
- Agarose gel elctrophoresis in 0.7-2 % agarose gel containing ethidium bromide is most widely used method.
- UV transillumination visualizes the DNA as fluorescent bands



Thanks

TO BE CONTINUED