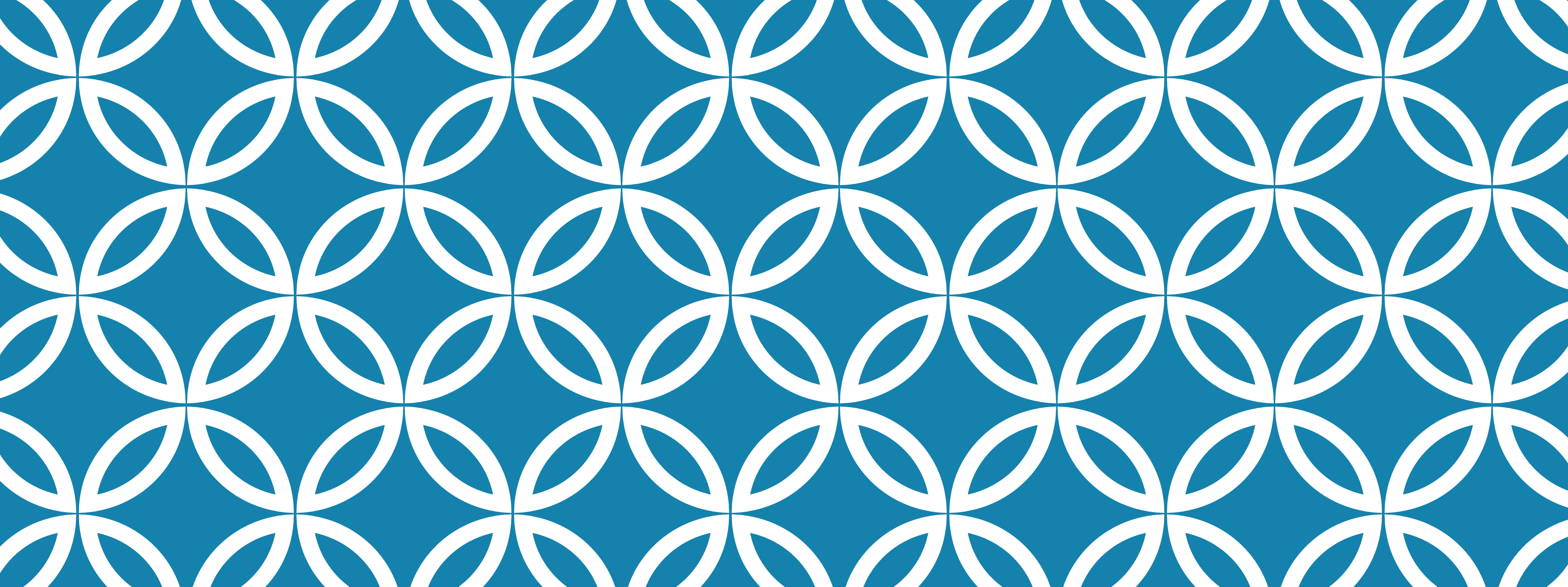


PCR IN BIOCHEMISTRY

Pramod patil
PRN NO. 2018420695
B.N. BANDODKAR COLLEGE

- PCR was developed in 1983 by Kary B. Mullis, an American biochemist who won the Nobel Prize for Chemistry in 1993 for his invention.
- Before the development of PCR, the methods used to amplify, or generate copies of, recombinant DNA fragments were time-consuming and labour-intensive.
- In contrast, a machine designed to carry out PCR reactions can complete many rounds of replication, producing billions of copies of a DNA fragment, in only a few hours.

WHAT IS PCR?

What is PCR?

- The polymerase chain reaction (PCR) was originally developed in 1983 by the American biochemist Kary Mullis. He was awarded the Nobel Prize in Chemistry in 1993 for his pioneering work.
- PCR is used in molecular biology to make many copies of (amplify) small sections of DNA[?] or a gene[?].
- Using PCR it is possible to generate thousands to millions of copies of a particular section of DNA from a very small amount of DNA.
- PCR is a common tool used in medical and biological research labs.

HOW DOES PCR WORK?

The principles behind every PCR, whatever the sample of DNA, are the same.

Five core ‘ingredients’ are required to set up a PCR. We will explain exactly what each of these do as we go along. These are:

- the DNA template to be copied
- primers, short stretches of DNA that initiate the PCR reaction, designed to bind to either side of the section of DNA you want to copy
- DNA nucleotide bases? (also known as dNTPs). DNA bases (A, C, G and T) are the building blocks of DNA and are needed to construct the new strand of DNA
- Taq polymerase enzyme? to add in the new DNA bases
- buffer to ensure the right conditions for the reaction.

PCR involves a process of heating and cooling called thermal cycling which is carried out by machine.

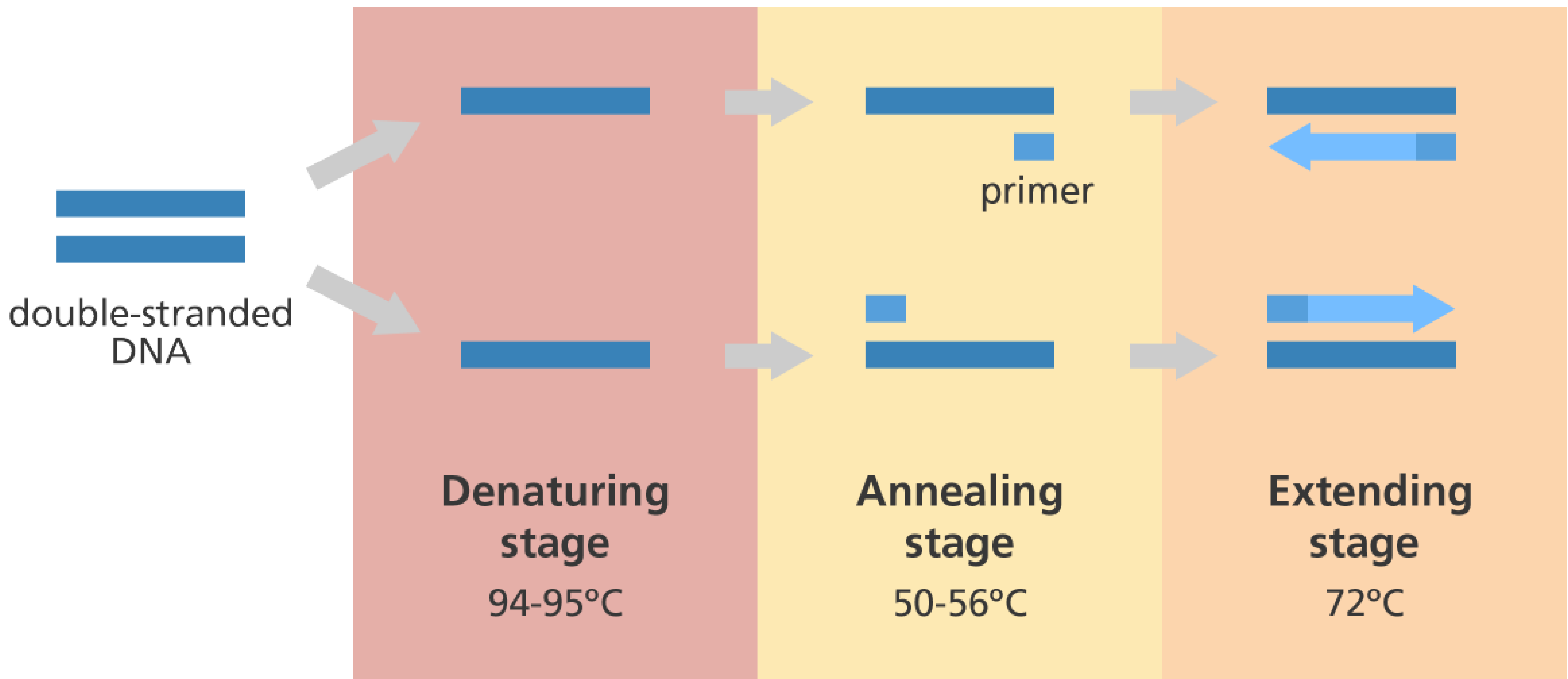
There are three main stages:

- **Denaturing –** when the double-stranded template DNA is heated to separate it into two single strands.
- **Annealing –** when the temperature is lowered to enable the DNA primers to attach to the template DNA.
- **Extending –** when the temperature is raised and the new strand of DNA is made by the Taq polymerase enzyme.

These three stages are repeated 20-40 times, doubling the number of DNA copies each time.

A complete PCR reaction can be performed in a few hours, or even less than an hour with certain high-speed machines.

After PCR has been completed, a method called electrophoresis can be used to check the quantity and size of the DNA fragments produced


double-stranded
DNA
primer
Denaturing
stage
94-95°C
Annealing
stage
50-56°C
Extending
stage
72°C

Annealing stage

- During this stage the reaction is cooled to 50-65^{0}C. This enables the primers to attach to a specific location on the single-stranded template DNA by way of hydrogen bonding (the exact temperature depends on the melting temperature of the primers you are using).
- Primers are single strands of DNA or RNA[?] sequence that are around 20 to 30 bases in length.
- The primers are designed to be complementary[?] in sequence to short sections of DNA on each end of the sequence to be copied.
- Primers serve as the starting point for DNA synthesis. The polymerase enzyme can only add DNA bases to a double strand of DNA. Only once the primer has bound can the polymerase enzyme attach and start making the new complementary strand of DNA from the loose DNA bases.
- The two separated strands of DNA are complementary and run in opposite directions (from one end - the 5' end – to the other - the 3' end); as a result, there are two primers – a forward primer and a reverse primer.
- This step usually takes about 10-30 seconds

Denaturing stage

•During this stage the cocktail containing the template DNA and all the other core ingredients is heated to 94-95^{0}C.

•The high temperature causes the hydrogen bonds? between the bases in two strands of template DNA to break and the two strands to separate.

•This results in two single strands of DNA, which will act as templates for the production of the new strands of DNA.

•It is important that the temperature is maintained at this stage for long enough to ensure that the DNA strands have separated completely.

•This usually takes between 15-30 seconds.

Extending stage

- During this final step, the heat is increased to 72^0C to enable the new DNA to be made by a special Taq DNA polymerase enzyme which adds DNA bases.
- Taq DNA polymerase is an enzyme taken from the heat-loving bacteria? *Thermus aquaticus*.
    - This bacteria normally lives in hot springs so can tolerate temperatures above 80^0C.
    - The bacteria's DNA polymerase is very stable at high temperatures, which means it can withstand the temperatures needed to break the strands of DNA apart in the denaturing stage of PCR.
    - DNA polymerase from most other organisms would not be able to withstand these high temperatures, for example, human polymerase works ideally at 37˚C (body temperature).
- 72^0C is the optimum temperature for the Taq polymerase to build the complementary strand. It attaches to the primer and then adds DNA bases to the single strand one-by-one in the 5' to 3' direction.
- The result is a brand new strand of DNA and a double-stranded molecule of DNA.
- The duration of this step depends on the length of DNA sequence being amplified but usually takes around one minute to copy 1,000 DNA bases (1Kb).

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YOU