Polymerase Chain Reaction (PCR)

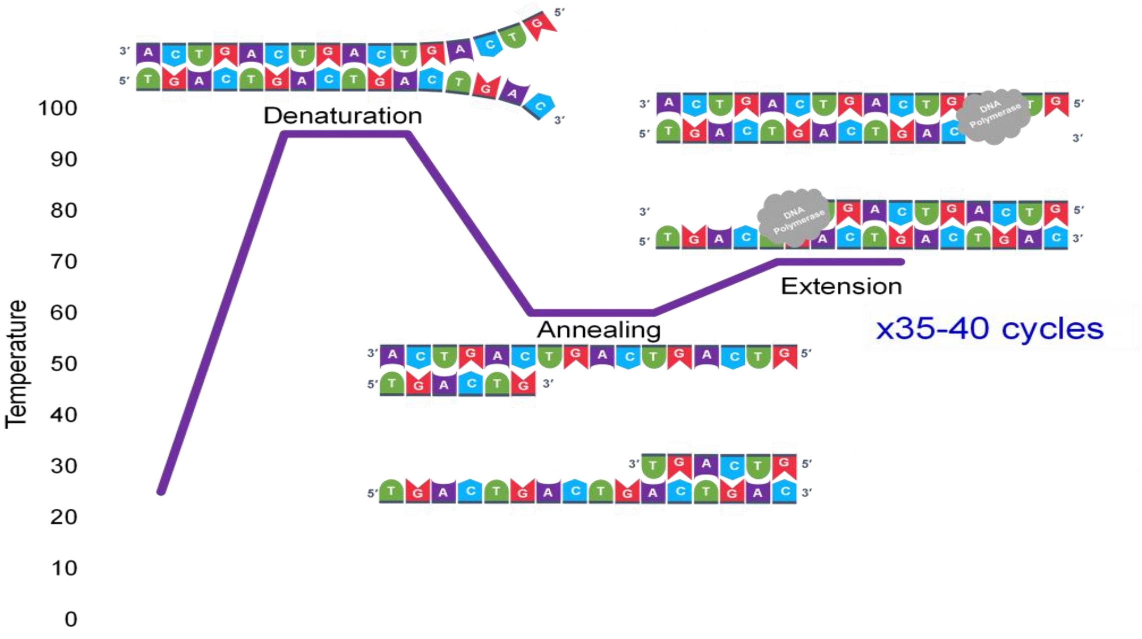
What is PCR?

PCR is a lab technique used to make **millions of copies** of a specific DNA segment. It's essential when only a small amount of DNA is available and more is needed for testing or analysis. It's widely used in research, medicine, and forensic science.

PCR Process – Step-by-Step:

1. **Denaturation (94–98°C):**
   - The DNA is heated so the two strands "melt" apart, breaking the hydrogen bonds between bases.
   - This creates **two single strands** of DNA.
2. **Annealing (50–65°C):**
   - The mixture is cooled so that **primers** (short pieces of DNA that match the start and end of the target sequence) can bind (stick) to each strand.
   - These primers mark the starting point for DNA copying.
3. **Extension (72°C):**
   - The enzyme **Taq polymerase** adds **nucleotides** (DNA building blocks) to the primers.
   - This creates **new DNA strands**, forming double-stranded DNA again.

These three steps make up **one PCR cycle**. It's usually repeated **30–40 times**, leading to exponential DNA amplification—millions of copies!



Lecturer
Dr. Mohammed Salih Hussein

Figure 2. DNA template in the PCR reaction test tube is a double stranded molecule. The targeted sequence of nucleotides for this illustration is shown. The template DNA could be fragments from the chromosome of various lengths. The 5′ and 3′ orientation of both strands is shown. Image by Marjorie Hanneman.

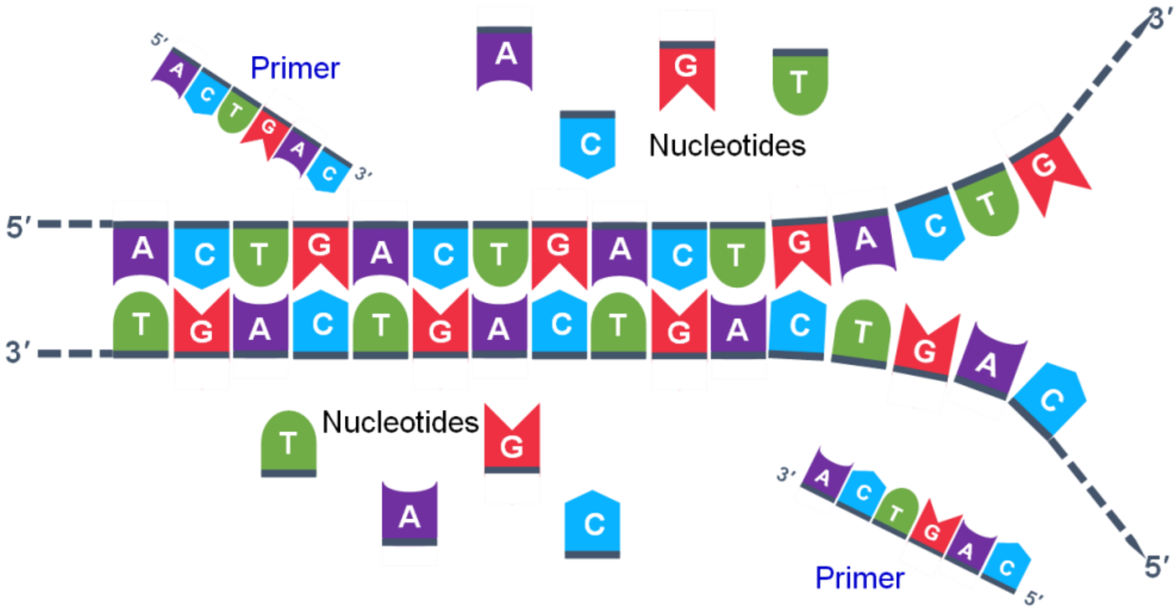


Figure 3. Denaturation: The hydrogen bonds holding the strands of the double stranded template together are broken by heating the test tube. Image by Marjorie Hanneman and Donald Lee.

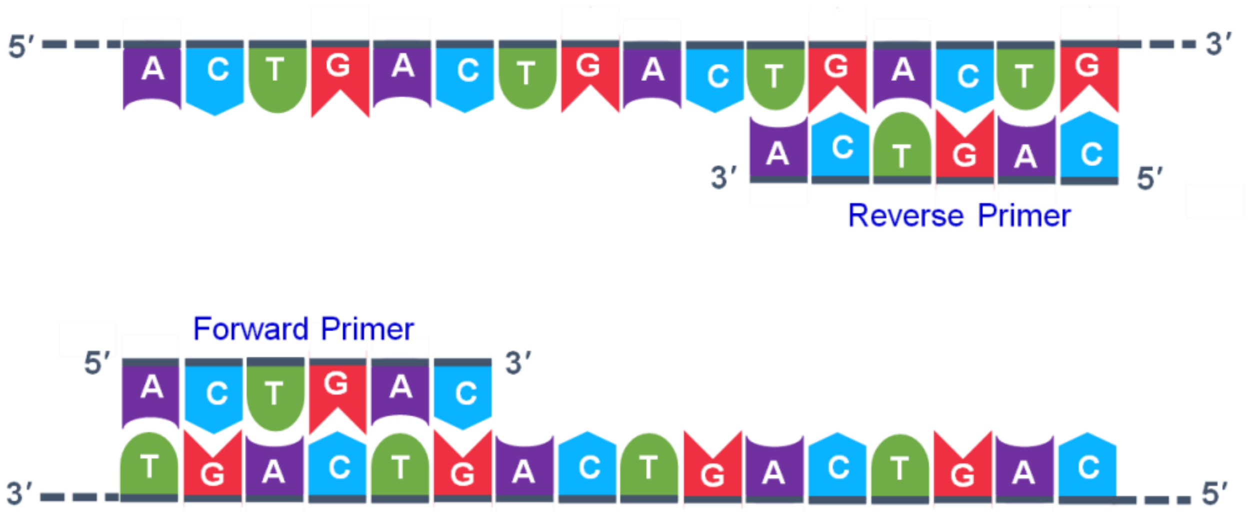


Figure 4. Annealing: The second step of PCR is to cool the test tube which allows the forward and reverse primer to bind to the template. Image by Marjorie Hanneman and Donald Lee.

Lecturer
Dr. Mohammed Salih Hussein

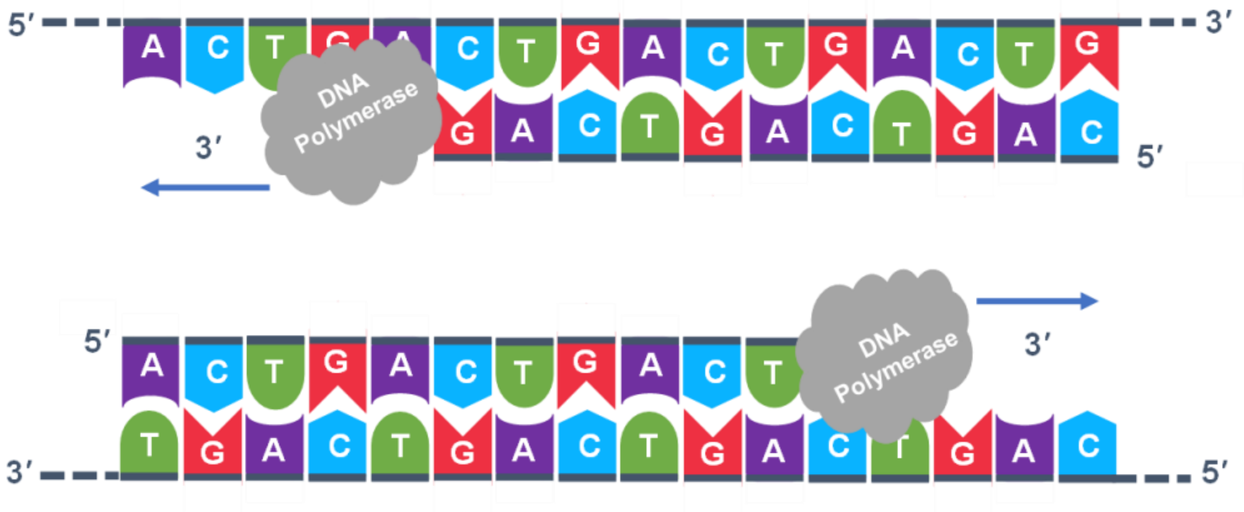


Figure 5. Extension: This step is catalyzed by DNA pol III. The time allowed for this step determines how much of the template DNA pol III can read and replicate. Image by Marjorie Hanneman.

Figure 6a. Cycle 1 is complete; two double stranded DNA molecules are made from the original double stranded template. Image by Marjorie Hanneman and Donald Lee.

Lecturer
Dr. Mohammed Salih Hussein

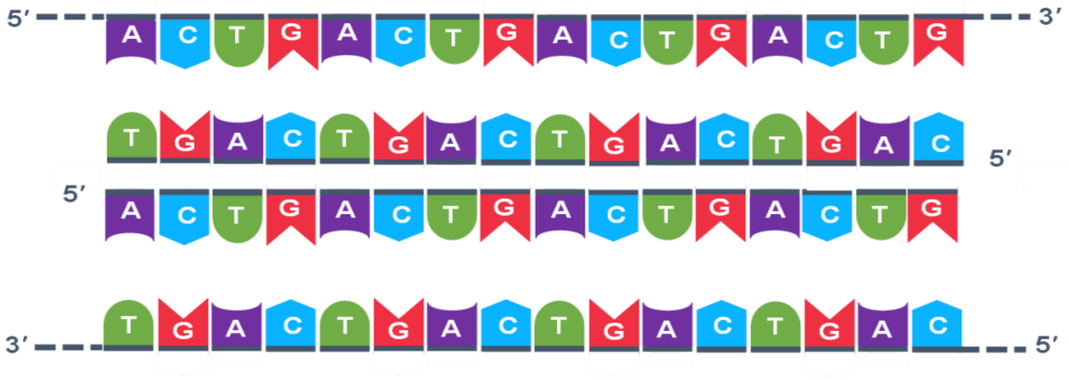


Figure 6b. Denaturation step generates four single strand templates. Image by Marjorie Hanneman

Figure 6c. Annealing and Extension. All templates are primed and four 3′ ends provide a place of DNA pol III extension. Image by Marjorie Hanneman

Figure 7. The thermal cycler is used to carry out PCR reaction. Image by Walter Suza.

Lecturer
Dr. Mohammed Salih Hussein

Gel Electrophoresis

What is it used for?

After PCR, scientists often need to **see and analyze the DNA**. Gel electrophoresis helps do that by **separating DNA fragments** based on their size.

How it works:

1. A gel (usually made of agarose) is prepared with wells (small holes).
2. DNA samples are placed into the wells.
3. An electric current is applied across the gel.
    - DNA is negatively charged, so it moves toward the positive side.
4. **Smaller DNA fragments move faster and travel farther**, while larger fragments move slowly.

After running the gel, the DNA appears as **bands** when viewed under UV light. Each band represents DNA fragments of a certain size.

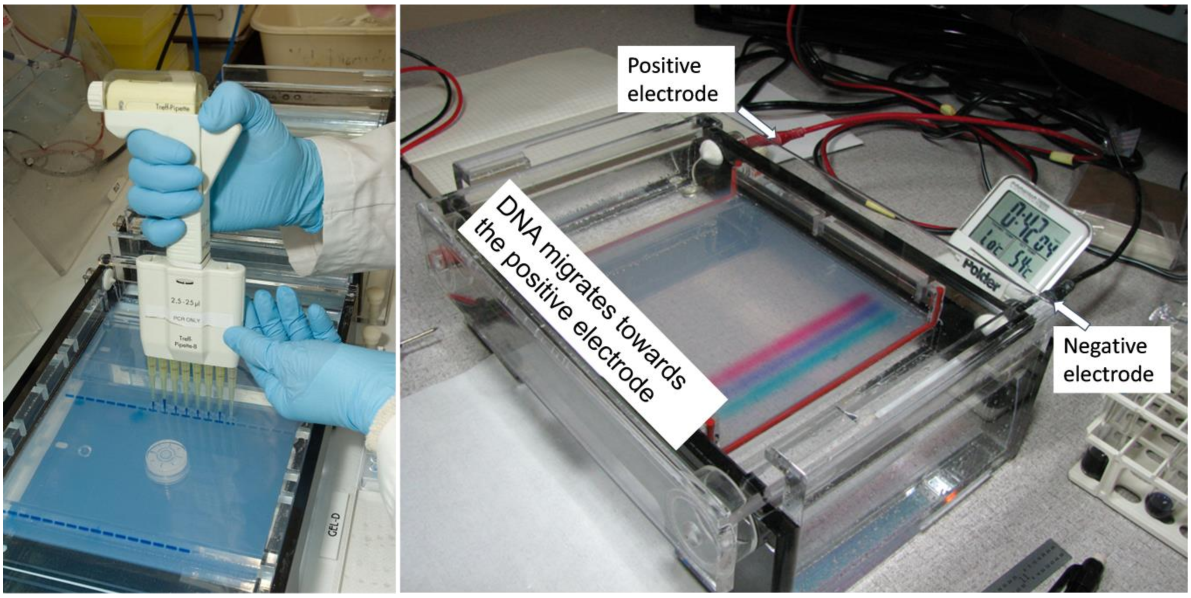


Figure 8. Electrophoresis: A gel electrophoresis set-up with agarose gel with DNA and loading dye on the left and the power supply on the right. Image Source: Michael, CC BY 2.0, via Wikimedia Commons and U. S. Department of Agriculture, CC BY 2.0, via Wikimedia Commons.

Lecturer
Dr. Mohammed Salih Hussein

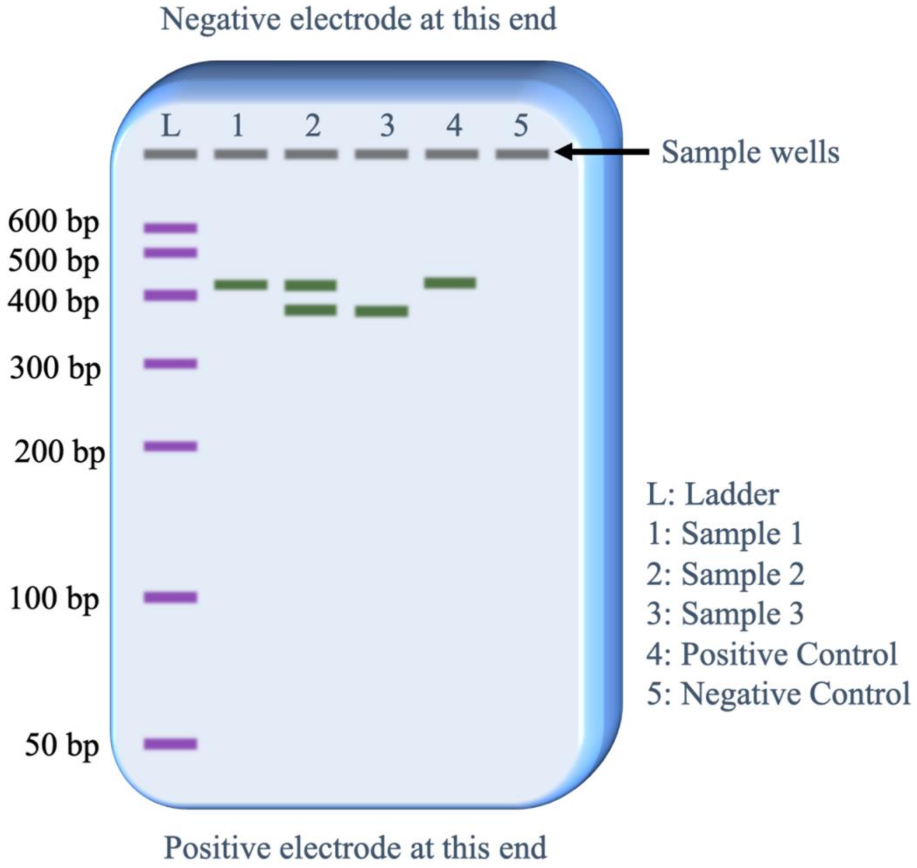


Figure 9. Depiction of an electrophoresis gel with six sample wells that were loaded with either a DNA size ladder (lane L) or a sample from a PCR run (1-5.) The gel was subjected to a DNA staining dye. Image by Marjorie Hanneman.

Why Are These Techniques Important Together?

PCR **amplifies** (copies) a specific DNA segment, and gel electrophoresis lets you **visualize** the result. Together, they're used in:

- **Genetic testing**
- **Disease detection** (like viruses or mutations)
- **Forensic investigations** (DNA fingerprinting)
- **Cloning and gene studies**

Lecturer
Dr. Mohammed Salih Hussein