Bot358
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Molecular Biology Of Plants

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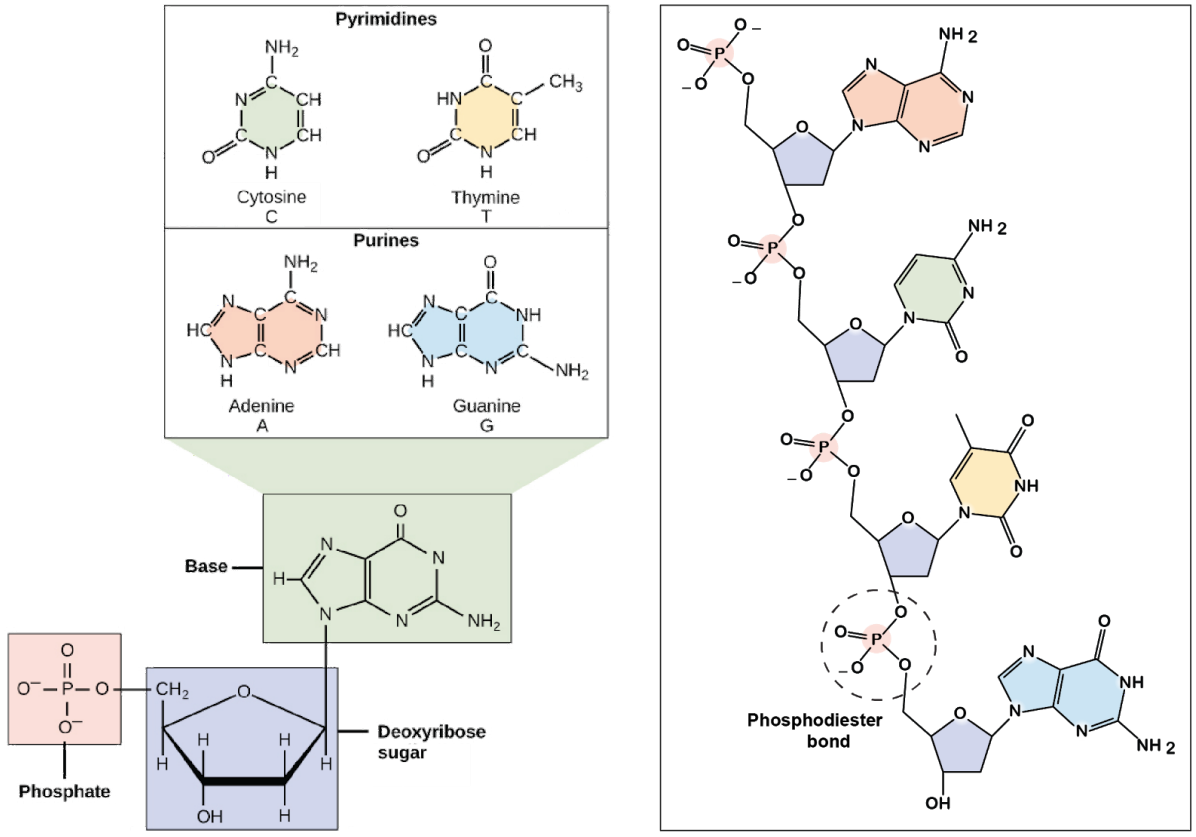
Introduction To Molecular Biology:

Molecular genetics :is the study of structure and function of genes at a molecular level. The field studies how the genes are transferred from generation to generation.
Molecular genetics employs the methods of genetics and molecular biology components of DNA

The components of DNA

From the work of biochemist Phoebus Levene and others, scientists in Watson and Crick's time knew that DNA was composed of subunits called **nucleotides**. A nucleotide is made up of a sugar (deoxyribose), a phosphate group, and one of four nitrogenous bases: adenine (A), thymine (T), guanine (G) or cytosine (C).
C and T bases, which have just one ring, are called **pyrimidines**, while A and G bases, which have two rings, are called **purines**.



1-Left panel: structure of a DNA nucleotide. The deoxyribose sugar is attached to a phosphate group and to a nitrogenous base. The base may be any one of four possible options: cytosine (C), thymine (T), adenine (A), and guanine (G). The four bases have differences in their structure and functional groups. Cytosine and thymine are **pyrimidines** and have just one ring in their chemical structures. Adenine and guanine are **purines** and have two rings in their structures.
Right panel: a strand of linked DNA nucleotides. The sugars are connected by phosphodiester bonds. The DNA strand consists of alternating phosphate groups

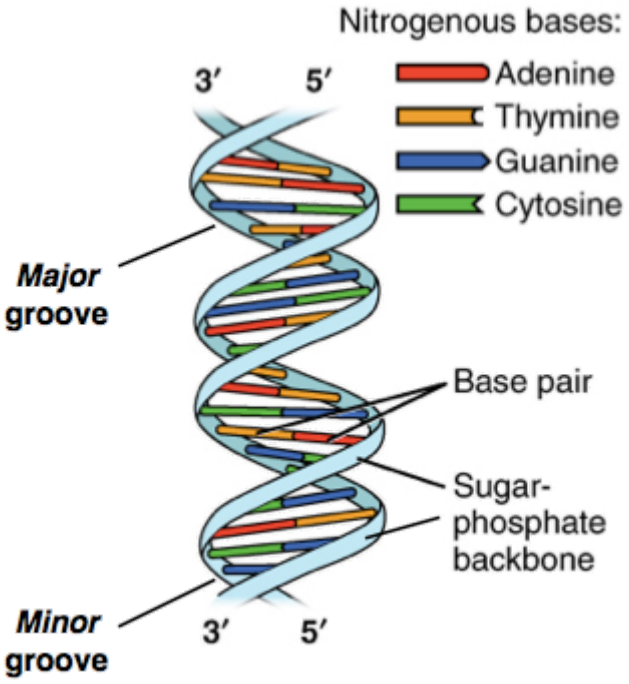
and deoxyribose sugars (sugar-phosphate backbone), with the nitrogenous bases sticking out from the deoxyribose sugars.
Image credits: **2-left panel**,{ image modified from "Nucleic acids: Figure 1," by OpenStax College, Biology (CC BY 3.0). Right panel, image modified from "DNA chemical structure," by Madeleine Price Ball (CC0/public domain).}
DNA nucleotides assemble in chains linked by **covalent bonds**, which form between the deoxyribose sugar of one nucleotide and the phosphate group of the next. This arrangement makes an alternating chain of deoxyribose sugar and phosphate groups in the DNA polymer, a structure known as the **sugar-phosphate backbone**
.

Watson and Crick's model of DNA

The structure of DNA, as represented in Watson and Crick's model, is a double-stranded, antiparallel, right-handed helix. The **sugar-phosphate backbones** of the DNA strands make up the outside of the helix, while the **nitrogenous bases** are found on the inside and form hydrogen-bonded pairs that hold the DNA strands together.
In the model below, the orange and red atoms mark the phosphates of the sugar-phosphate backbones, while the blue atoms on the interior of the helix belong to the nitrogenous bases.

Antiparallel orientation

Double-stranded DNA is an **antiparallel** molecule, meaning that it's composed of two strands that run alongside each other but point in opposite directions. In a double-stranded DNA molecule, the 5' end (phosphate-bearing end) of one strand aligns with the 3' end (hydroxyl-bearing end) of its partner, and vice versa.
[What is the purpose of the prime marks in 3' and 5'?]
The carbon atoms of the deoxyribose sugar in DNA nucleotides are labeled with numbers accompanied by prime marks. Prime marks look similar to apostrophes (e.g., 3').



Watson and Crick model of DNA

Base pairing

In Watson and Crick's model, the two strands of the DNA double helix are held together by hydrogen bonds between nitrogenous bases on opposite strands. Each pair of bases lies flat, forming a "rung" on the ladder of the DNA molecule.

Base pairs aren't made up of just any combination of bases. Instead, if there is an A found on one strand, it must be paired with a T on the other (and vice versa). Similarly, an G found on one strand must always have a C for a partner on the opposite strand. These A-T and G-C associations are known as **complementary base pairs**.

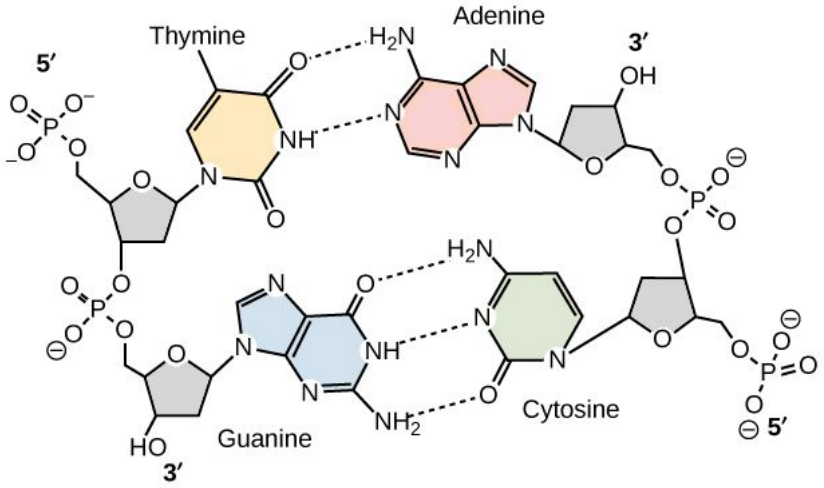


Diagram illustrating base pairing between A-T and G-C bases. **A and T** are found opposite to each other on the two strands of the helix, and their functional groups form two hydrogen bonds that hold the strands together. Similarly, **G and C** are

found opposite to each other on the two strands, and their functional groups form three hydrogen bonds that hold the strands together.

Strawberry DNA Extraction

Objectives

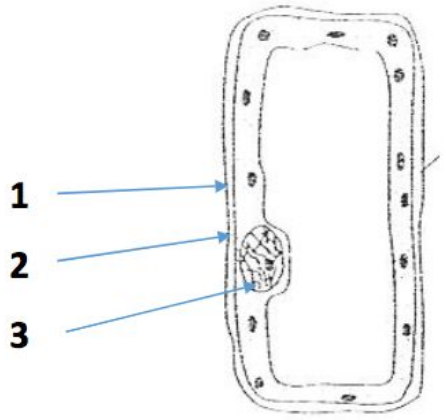
Understand how cell barriers are broken and how to extract DNA from strawberry cells.

DNA Extraction From Plant Cells:

The DNA of a plant cell is located within the **cell's nucleus**. The nucleus is surrounded by a nuclear membrane and the entire cell is encased in both a **cell membrane and a cell wall.**

These barriers protect and separate the cell and its organelles from the surrounding environment.

In the plant cell the DNA surrounded by 3 briers:



p!ant cell

The function of each step:

Step	Function
Crushing the strawberries	Open many of the strawberry cells, releasing the nuclei where the DNA is.
The soap in the detergent or shampoo in the extraction buffer	Breaks down the fatty membranes of the cells, breaks open the nuclear membrane and releases the DNA into solution
The salt	Makes the DNA molecules stick together, and separate from the proteins that are also released from the cells.
Alcohol	DNA is insoluble in alcohol, so it precipitates. What you see is the precipitation of strawberry DNA - long, thread-like DNA molecules at the interface of the alcohol and DNA solution.
The gauze	The gauze retains strawberry cell debris. The strawberry DNA is dissolved in the DNA extraction buffer, which will pass through the gauze into the test tube.

DNA isolation from plant materials

Protocol

1.Collecting plant materials
2.Phase Separation
3.DNA Precipitation
4.DNA Wash
5.Redissolving the DNA

Phases with main step:

Phase	Main Step
Phase Separation	Grind 0.02 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer
DNA Precipitation	Using Chloroform : Iso Amyl Alcohol (24:1) and 7.5 M Ammonium Acetate
DNA Wash	Using ice cold 70 % ethanol
Redissolving the DNA	Resuspend the DNA in sterile DNase free water

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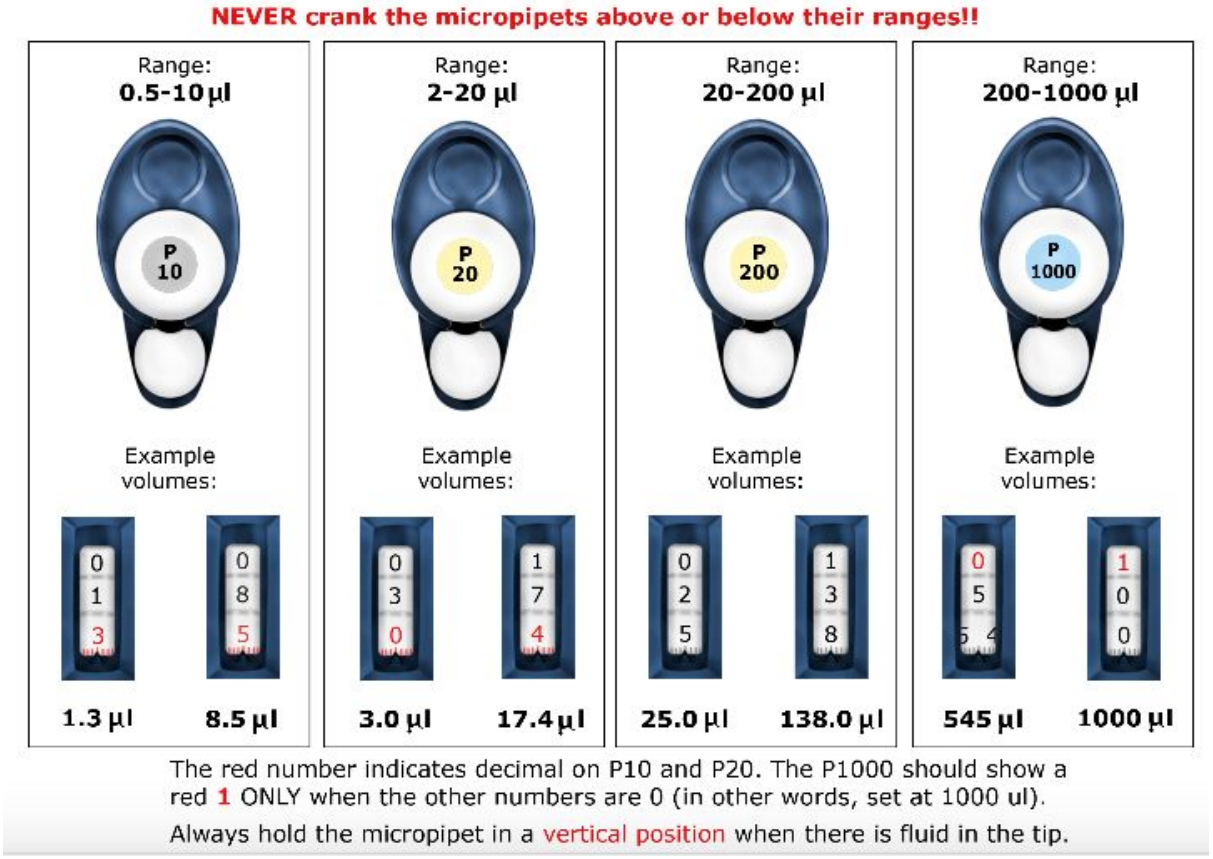
How to use micropipette



The red number indicates decimal on P10 and P20. The P1000 should show a red **1** ONLY when the other numbers are 0 (in other words, set at 1000 ul).

Always hold the micropipet in a vertical position when there is fluid in the tip.

DNA Quantification

Methods for Quantitating Nucleic Acid

There are several common laboratory techniques used to estimate nucleic acid sample concentration. A common misconception, however, is that all these methods have similar accuracy or even measure the same thing: how much DNA or RNA is in the sample. In fact, every method measures something different. Depending on what method you choose, contamination or or other issues can lead to an inconsistent estimation of concentration

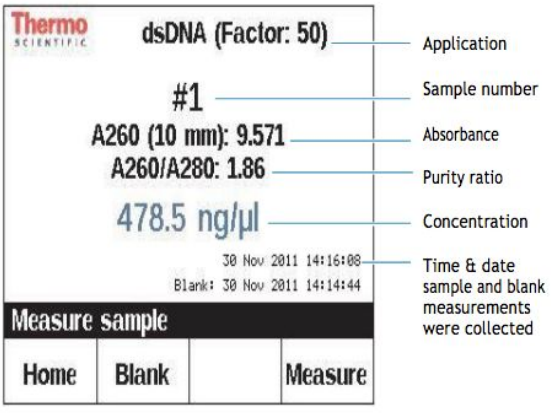
Spectrophotometry

The Beer-Lambert Law relates the absorption of light to the properties of the material through which the light travels. This law states that there is a logarithmic dependence between the transmission of light through a substance and the product of the absorption coefficient of the substance and the path length (Figure 1). For DNA and RNA, the heterocyclic rings of nucleotides (adenine, guanine, cytosine and thymine/uracil) result in nucleic acid molecules absorbing ultraviolet (UV) light maximally at 260nm (λmax = 260nm).

$$A = \varepsilon \cdot b \cdot c$$

A = absorbance at a particular wavelength
ε = extinction coefficient
b = path length of the spectrophotometer
c = concentration of sample

Sample measurement screen:

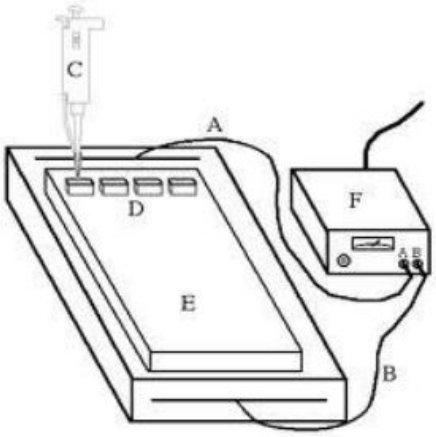


Gel ELectrophoresis

is a method used to separate nanoparticles by charge or size. It is used to:
1.Separate a mixed population of DNA and RNA fragments by length.
2.To estimate the size of DNA and RNA fragments or to separate proteins by charge.

Gel electrophoresis apparatus
An agarose gel is placed in this buffer-filled box and electrical field is applied via the power supply to the rear.
negatively-charged of DNA to migrate across the gel towards the positive (+) electrode (anode)

There are 2 types of gel was commonly used in electrophoresis apparatus: Agarose gel and Polyacrylamide Gel.



C	DNA sample
F	Power source
A	Negative electrode(Cathode)
B	Positive electrode(Anode)
E	Gel
D	Pore

Electrophoresis apparatus

Agarose gel	Polyacrylamide Gel
used for the separation of DNA fragments	gel electrophoresis (PAGE) is used for separating proteins

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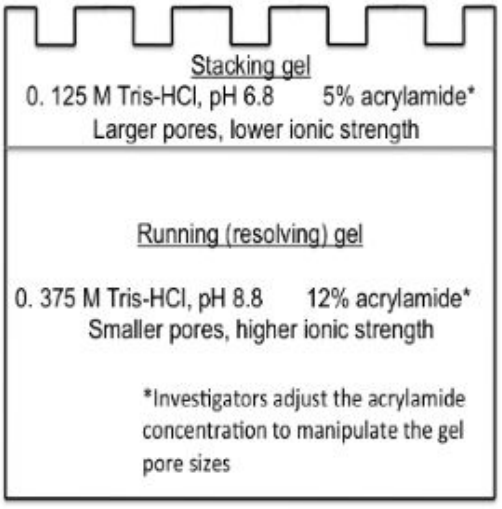
SDS-PAGE technique

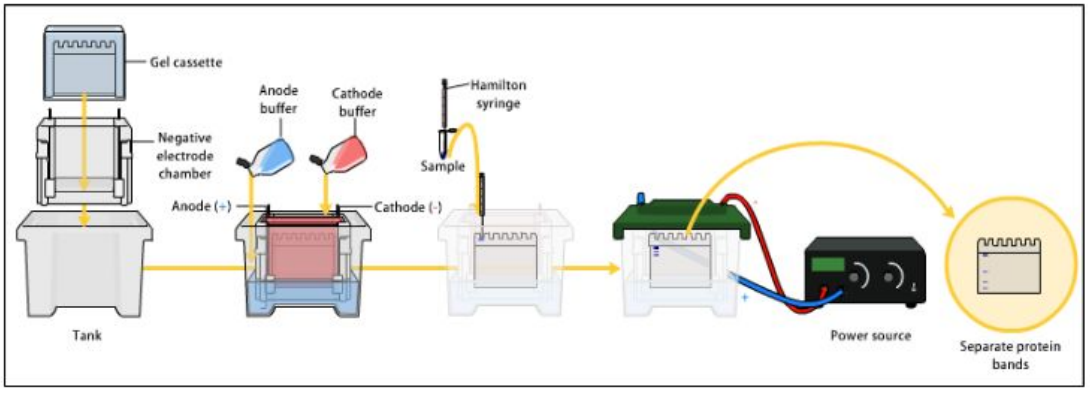
Summary of SDS-PAGE procedure:

Phase	Summary
Sample preparation	Samples may be any material containing proteins
Mixing with SDS	The sample is mixed with SDS, anionic detergent. To denatures secondary and non–disulfide–linked tertiary structures. To apply a negative charge to each protein
Heating	The samples are heated at 60°C. To promote protein denaturation, helping SDS to bind
Tracking dye	A tracking dye may be added to the protein solution. As it has a higher electrophoretic mobility which allow the experimenter to track the progress of the protein solution through the gel.
Preparing acrylamide gels	Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the wells.

	these gels between the two glass plates have two layers which difference in their structure the Top one called(Stacking gel) and the bottom one called (Lacking gel)
Electrophoresis	Various buffer systems are used in SDS-PAGE depending on the nature of the sample and the experimental objective. An electric field is applied across the gel, causing the negatively -charged proteins to migrate across the gel towards the positive (+) electrode (anode)



Stacking and Running gels



Summary of SDS PAGE technique

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The polymerase chain reaction(PCR)

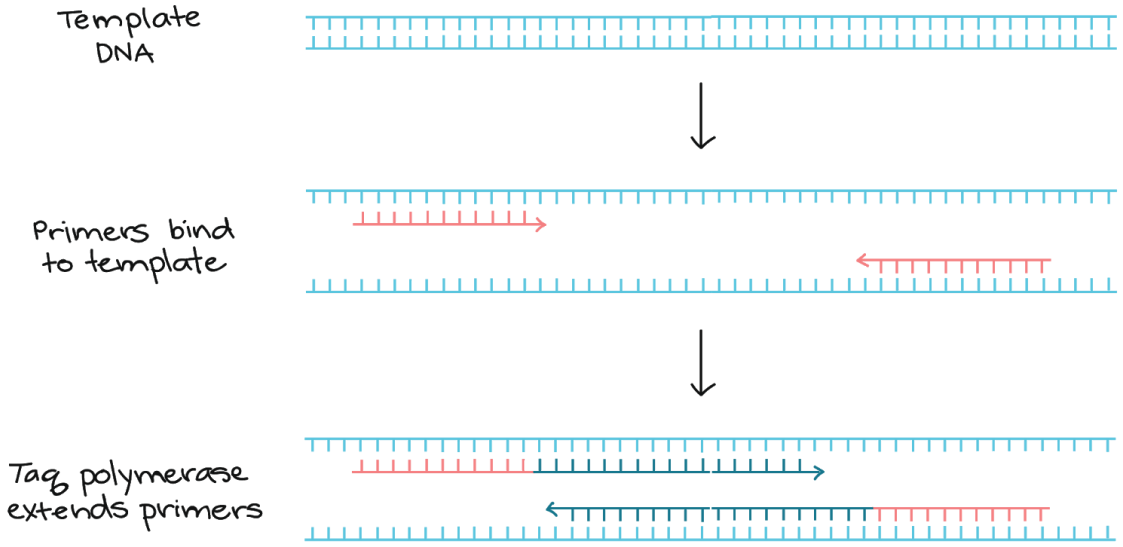
- The polymerase chain reaction (PCR) :is a scientific technique in,molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

- Developed in 1983 by Kary Mullis.

The steps of PCR

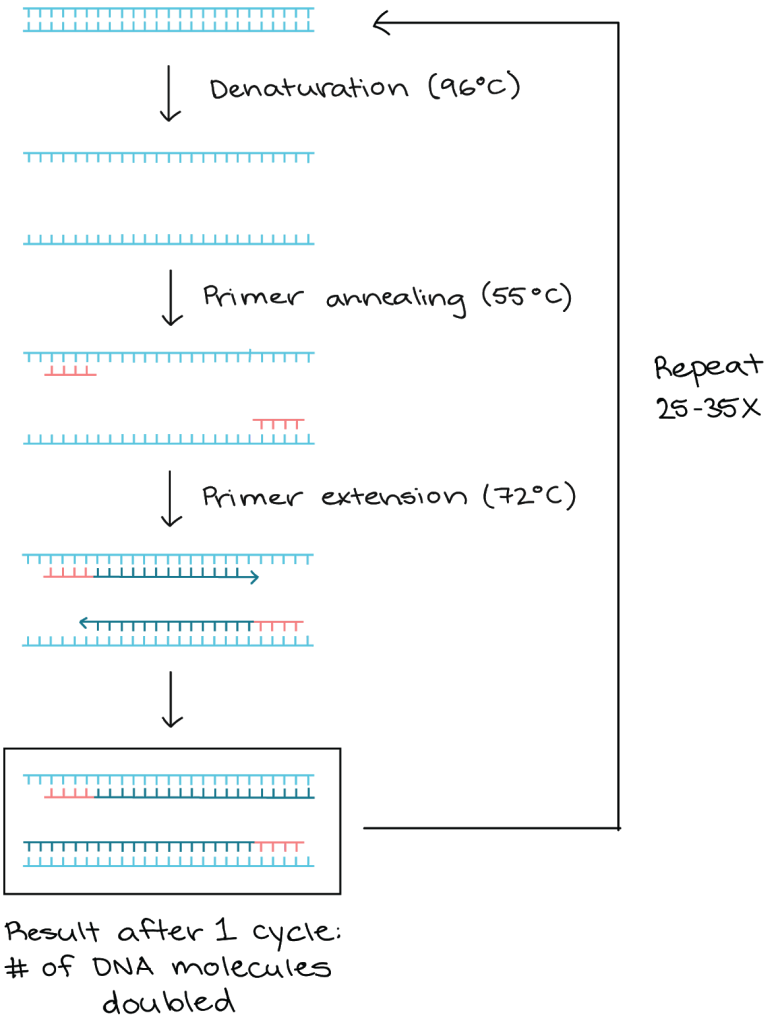
The key **ingredients** of a PCR reaction are:
1- *Taq* polymerase.
2-primers.
3-template DNA.
4-nucleotides (DNA building blocks).

The ingredients are assembled in a tube and are put through repeated cycles of heating and cooling that allow DNA to be synthesized.



The basic steps are:

1. **Denaturation** (96°C): Heat the reaction strongly to separate, or denature, the DNA strands. This provides single-stranded template for the next step.
2. **Annealing** (55-65°C): Cool the reaction so the primers can bind to their complementary sequences on the single-stranded template DNA.
3. **Extension** (72°C): Raise the reaction temperatures so *Taq* polymerase extends the primers, synthesizing new strands of DNA.



Applications of PCR

Using PCR, a DNA sequence can be amplified millions or billions of times, producing enough DNA copies to be analyzed using other techniques. For instance, the DNA may be visualized by gel electrophoresis, sent for sequencing, or digested with restriction enzymes and cloned into a plasmid.

PCR is used in many research labs, and it also has practical applications in forensics, genetic testing, and diagnostics. For instance, PCR is used to amplify genes associated with genetic disorders from the DNA of patients (or from fetal DNA, in the case of prenatal testing). PCR can also be used to test for a bacterium or DNA virus in a patient's body: if the pathogen is present, it may be possible to amplify regions of its DNA from a blood or tissue sample.