مذكرة الاحياء الجزيئية (Molecular biology)

عملي

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Lab	Name of experiment
Lab 1	**Introduction to molecular biology**
Lab 2	**DNA extraction from fruit**
Lab 3	**DNA extraction from plant (leaves)**
Lab 4	**DNA quantification**
Lab 5	**Gel electrophoresis**
Lab 6	**Proteins isolation from plant tissue by SDS –PAGE**
Lab 7	**PCR and its application**

Molecular genetics :

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

DNA (Deoxyribonucleic Acid):

DNA replication of eukaryotic cells occurs 100,000 times as many of cell replication DNA is highly folded and packaged in a structures called chromosomes in the nucleus. An organism's chromosomes bundle together within the nucleus, but during cell division (mitosis) they become individually distinct (human mitotic chromosomes are X shaped) and can be observed with microscopes.

Structure of DNA:

DNA has two strands.DNA is made up of molecules called **nucleotides.**

Each nucleotide contains (a phosphate group, a sugar group and a nitrogen base).

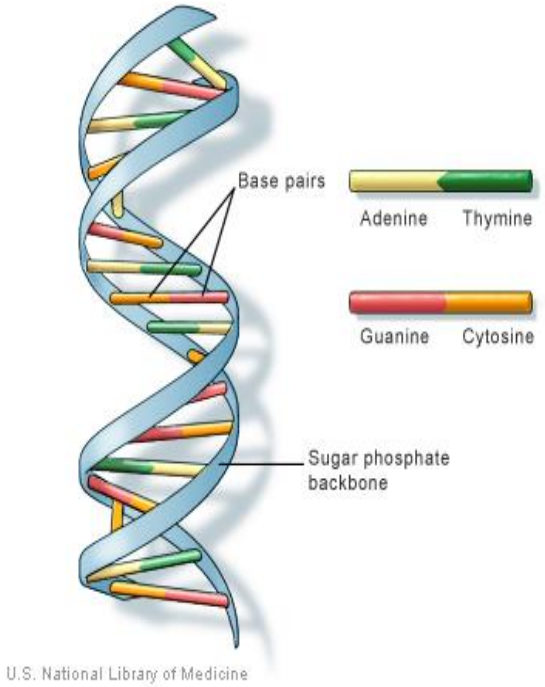
The four types of nitrogen bases:

adenine (A),

thymine (T),

guanine (G)

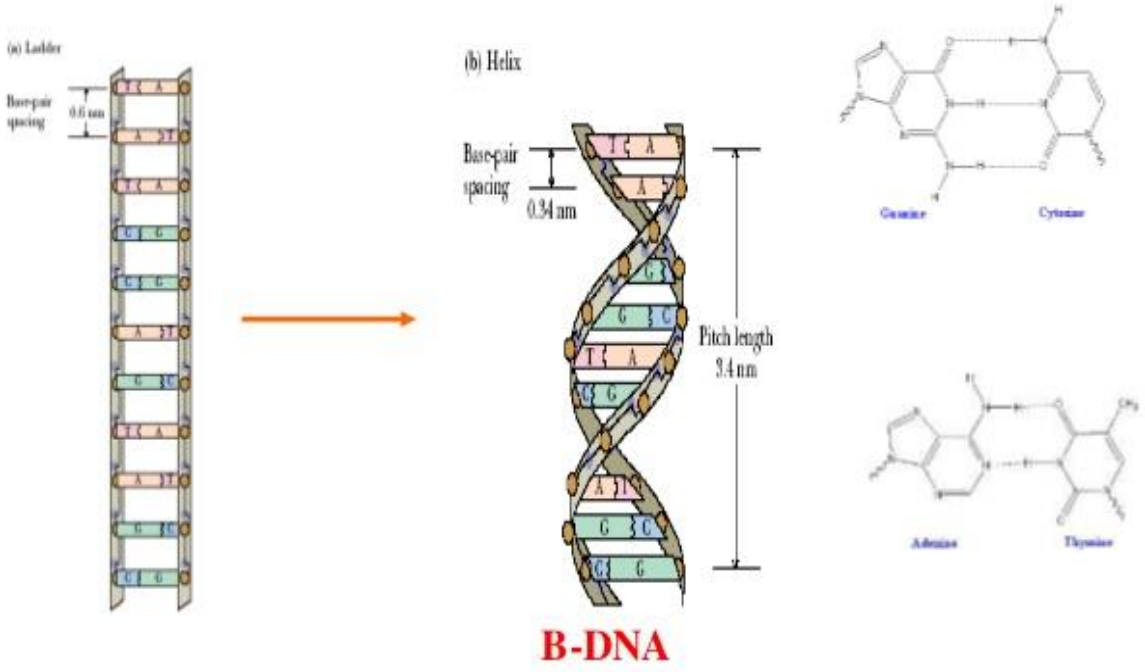
cytosine (C).



DNA bases pair up with each other, A with T and C with G, to form units called base pairs .The two strands are held together by hydrogen bonds between complementary bases. Nitrogenous bases are joined together by covalent bond

DNA Double Helix

DNA has two polynucleotide strands wound together to form a long, slender, helical molecule, the **DNA double helix.**



B-DNA

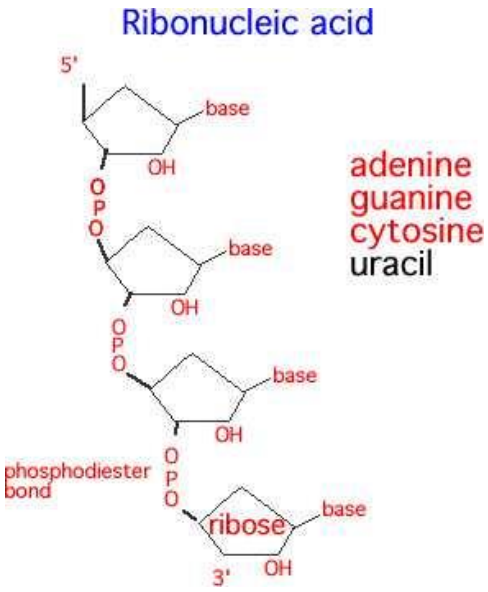
Structure of RNA :

RNA (Ribonucleic acid):

RNA has the basic structure of DNA with two major differences:

1. The pyrimidine base
2. Uracil replace thymine.

Ribose replace deoxyribose. Most cellular RNA molecules are single stranded.

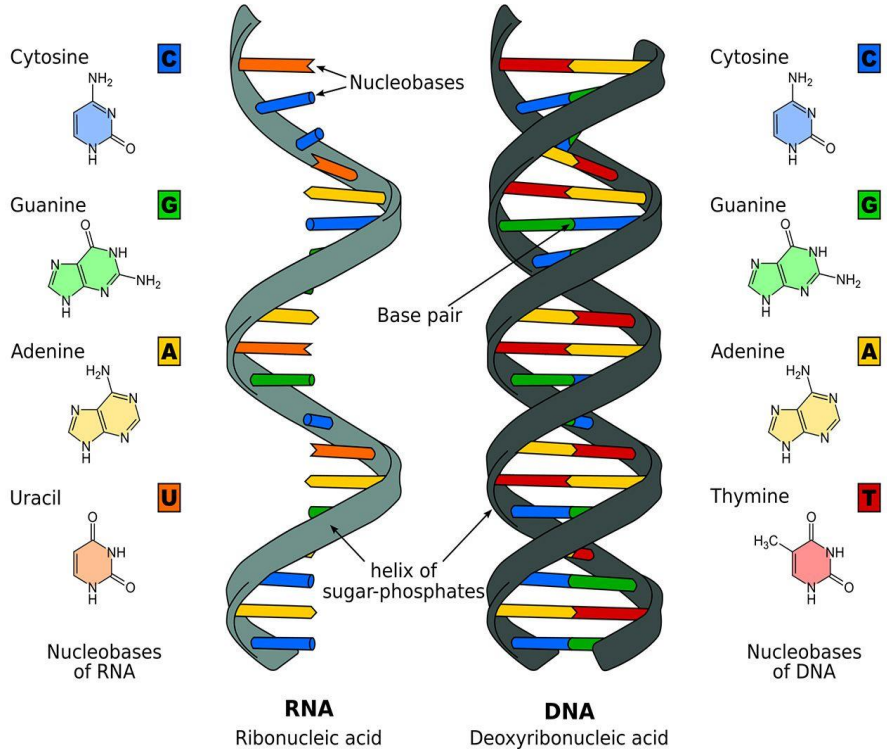


The Differences between DNA and RNA:

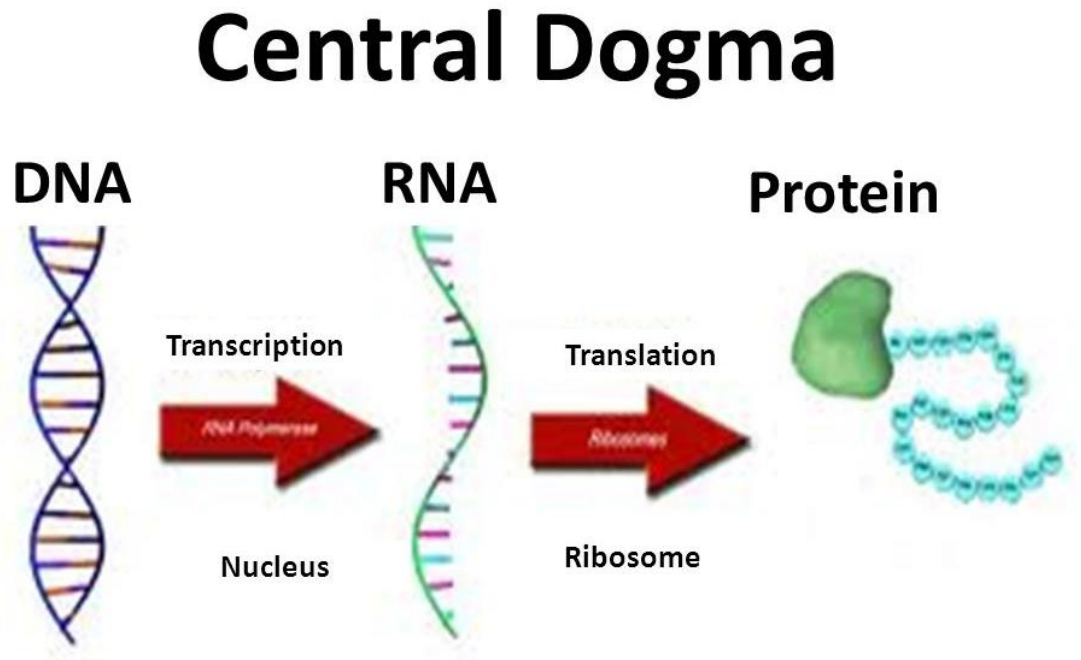
- DNA contains the sugar deoxyribose and RNA contains the sugar ribose.

- DNA contains the bases A,T, G and C and RNA contains the base A, U, G and C.

- DNA is a double helix strand and RNA is single stranded.



The **central dogma** of molecular biology is an explanation of the flow of genetic information within a biological system



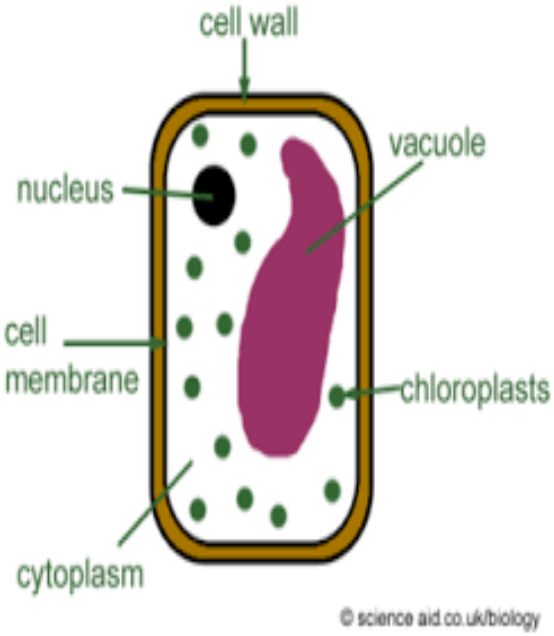
The central dogma states that the pattern of information that occurs most frequently in our cells is:

- From existing DNA to make new DNA (DNA replication).
- From DNA to make new RNA (transcription).
- From RNA to make new proteins (translation).

DNA Extraction from Plant Cells:

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

The process of breaking open a cell is called cell lysis



Cell lysis can be achieved by :

1. Physical actions such as mashing, blending, or crushing the cells cause their cell walls to burst.

2. Chemical actions such as detergent solution cause dissolve the lipids in cell walls.

Importance of DNA Extraction:

1. DNA encodes the instructions for all life processes.

2. Study of heredity and to the treatment of many diseases.

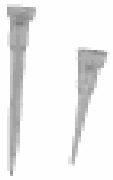
3. Create DNA fingerprints to help diagnose genetic diseases.

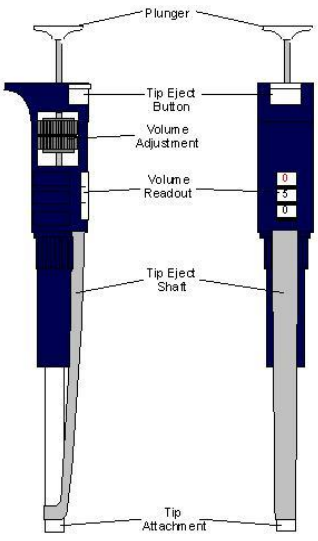
4. Solve criminal cases.

5. Identify victims of disaster and war.

6. Scientists can genetically engineer changes in DNA to create robust, disease-resistant genetically modified plants and animals.

Micropeipetties :

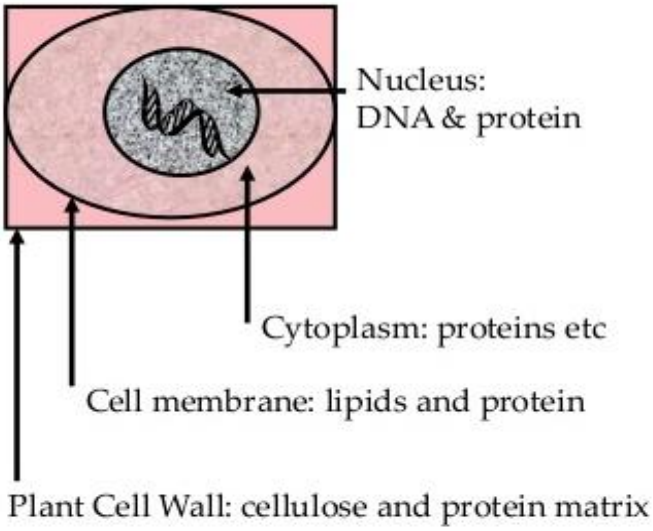
Size	Range	Top view and Color	Example Setting	Tip size and color	Tip sample
P-10	0.5-10 µl	0.5 ~ 10 white	0 6 5 0 → 6.5 µl	micro white	
P-20	2-20 µl	2 ~ 20 yellow	1 7 8 → 17.8 µl	medium white or yellow	
P-200	20-200 µl	20 ~ 200 yellow	1 5 0 → 150 µl		
P-1000	200-1000 µl	200 ~ 1000 blue	0 6 7 0 → 670 µl or 0.67 ml	large white or blue	

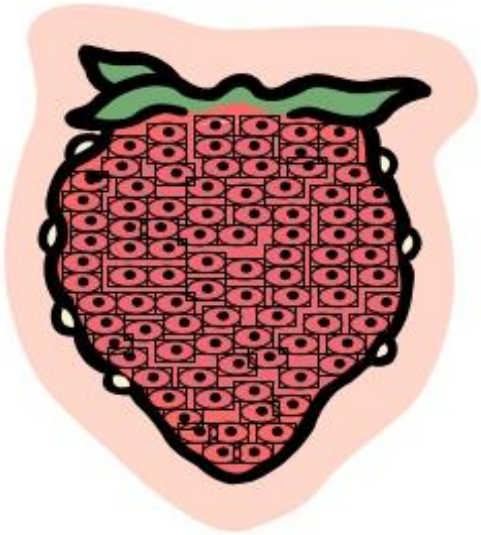


Strawberry DNA Extraction

Lab.2

How do you get DNA out of a strawberry?





To extract DNA, you must remove:

- Cell membrane
- Cytoplasm
- Nuclear membrane
- Proteins

Materials:

- 50-mL tubes with lids and bases
- 15-mL tubes with lids
- Plastic bags
- Wooden sticks
- Funnels
- Cheesecloth
- Pipets
- Ethanol 95%(100 mL)
- Liquid detergent
- 10g salt

Procedure:

1- Obtain one fresh or one frozen and thawed strawberry. If you are using a fresh strawberry, remove the green sepals (tops) from the berry.

2- Place the strawberry in a resealable plastic bag.

3- Close the bag slowly, pushing all of the air out of the bag as you seal it.

4- Being careful not to break the bag, thoroughly mash the strawberry with your hands for two minutes.

5- Pour the 10-mL aliquot of extraction buffer into the bag with the mashed strawberry. Reseal the bag.

6- Mash the strawberry for one additional minute.

aliquot of extraction buffer :

(Liquid detergent + salt)

The soapy solution helps removes proteins and phospholipids.

7- Place a funnel into a 50-mL centrifuge tube. Fold the cheesecloth in half along the longer side and place it in the funnel to create a filter. The cheesecloth will overlap the edge of the funnel.

8- Pour the strawberry mixture into the funnel, filtering the contents through the cheesecloth and into the 50-mL centrifuge tube.

9- Carefully pour 2 mL of the filtered contents from the 50-mL tube into a clean 15-mL tube. Use the lines on the side of the 15-mL tube to help measure the amount added.

10- Hold the 15-mL tube at an angle. Using a transfer pipet, carefully add 5 mL of cold 95% ethanol by running it down the inside of the tube. Add the 95% ethanol until the total volume is 7 mL (use the lines on the side of the tube to help you measure). You should have two distinct layers.

11- Caution: Do not mix the strawberry extract and the ethanol!

12- Watch closely as translucent strands of DNA begin to clump together where the ethanol layer meets the strawberry extract layer. Tiny bubbles in the ethanol layer will appear where the DNA precipitates.

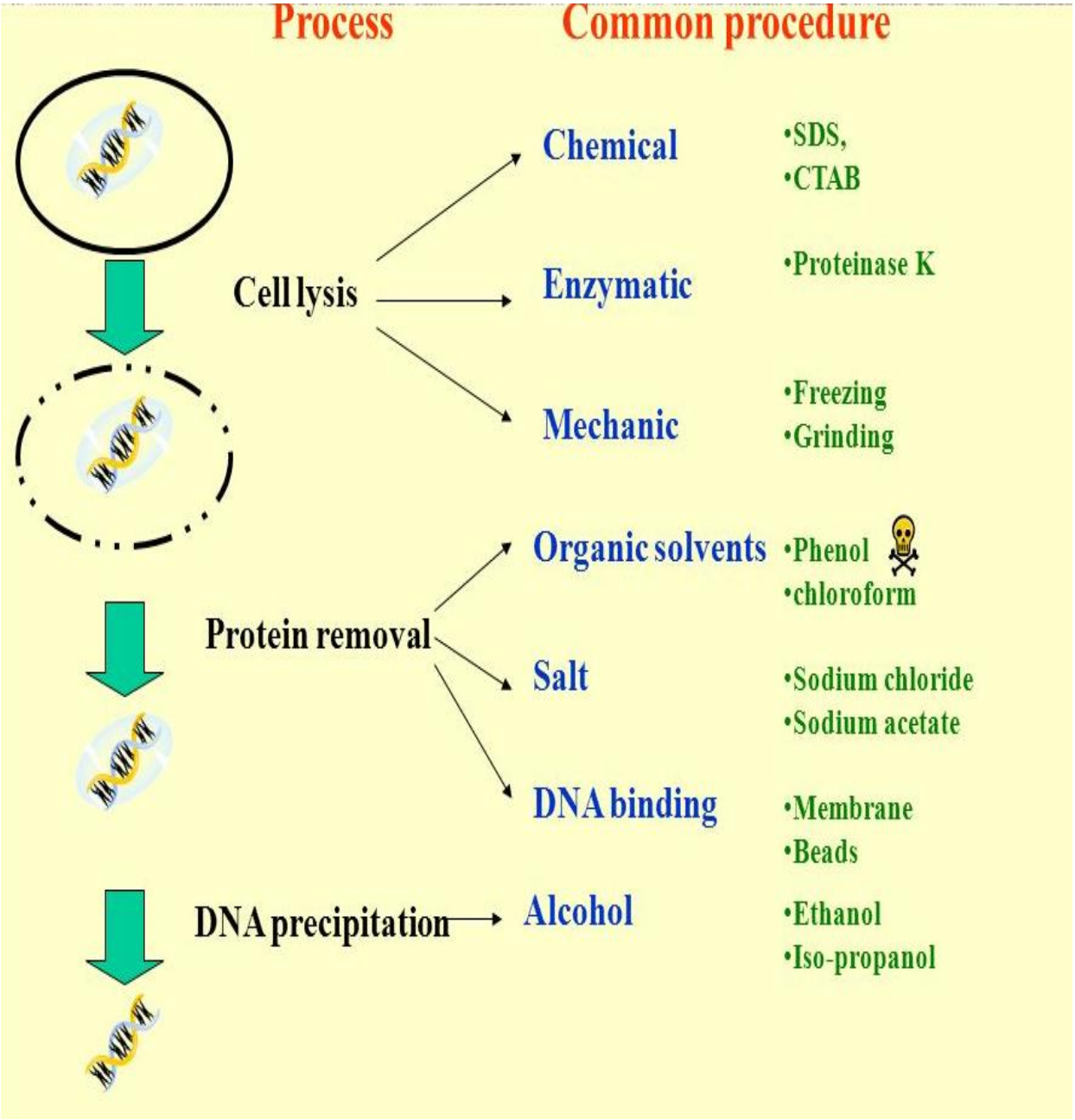
DNA normally stays dissolved in water, but when salty DNA comes in contact with alcohol it becomes undissolved. This is called precipitation. The physical force of the DNA clumping together as it precipitates pulls more strands along with it as it rises into the alcohol.

13- Slowly and carefully rotate the wooden stick in the ethanol directly above the extract layer to wind (or "spool") the DNA. Remove the wooden stick from the tube and observe the DNA **.**

DNA isolation from plant materials

Lab3

DNA extraction-the basic concept:



Protocol :

1. Collecting plant materials

2. Phase Separation

3. DNA Precipitation

4. DNA Wash

5. Re-dissolving DNA

Collecting plant materials :

1. The plant most be clean and free of soil, insect and microscopic fungi.

2. Keep the specimen inside clean container or zip lock bag.

3. Label information should be placed inside the zip lock bag with specimen which include: Taxon name, collection name, Date of collection.

4. If some time must elapse before shipping, refrigerate but do not freeze the plant.

5. Get a 2 sample from every specimen one for molecular work other to keep it in the university herbarium.

6. For extraction of DNA you will need a small piece of plant.

7. Plant tissues may be efficiently powdered by first freezing in liquid nitrogen or dry ice/ethanol before DNA extraction.

Materials:

- CTAB buffer (Hexadecyl trimethy l-ammonium bromide)
- Microfuge tubes, Mortar and Pestle, Microfuge
- Absolute Ethanol (ice cold)
- 70 % Ethanol (ice cold)
- 7.5 M Ammonium Acetate
- 55 ^{o}C water bath
- Chloroform: Iso Amyl Alcohol (24:1)
- Water (sterile)
- Agarose
- 6x Loading Buffer
- 1x TBE solution (Tris base, boric acid, EDTA (pH 8.0)).
- Agarose gel electrophoresis system Ethidium Bromide solution

Procedure :

I. Phase Separation:

1. Grind 0.02 mg of plant tissue to a fine paste in approximately 500 µl of CTAB buffer.

2. Transfer CTAB/plant extract mixture to a microfuge tube.

3. Incubate the CTAB/plant extract mixture for about 15 min at 55 ^{o}C in a recirculating water bath.

II. DNA Precipitation :

4. After incubation, transfer the microfuge tube to centrifuge at 12000 rpm for 5 min.

5. Add 250 µl of Chloroform : Iso Amyl Alcohol (24:1) and mix the solution by vortex . After mixing, spin the tubes at 13000 rpm for 1 min.

(In this step the DNA will be separated in the upper aqueous).

6. Transfer the upper aqueous phase only (contains the DNA) to a clean Eppendorf.

7. Add 50 µl of 7.5 M Ammonium Acetate followed by 500 µl of ice cold absolute ethanol.

8. Invert the tubes slowly to precipitate the DNA.

Generally the DNA can be seen to precipitate out of solution. Alternatively the tubes can be placed for 1 hr at -20 o C after the addition of ethanol to precipitate the DNA.

III. DNA Wash:

9. After precipitation, the DNA can be pipetted off by slowly rotating a tip in the cold solution. The precipitated DNA sticks to the pipette and is visible as a clear thick precipitate.

To wash the DNA, transfer the precipitate into a microfuge tube containing 500 µl of ice cold 70 % ethanol and slowly invert the tube.

10. transfer the tube to centrifuge at 13000 rpm for 1 min.

11.Remove the supernatant and wash the DNA pellet by adding two changes of ice cold 70 % ethanol of 500 µl)).

12. Allow the DNA pellet to dry (approximately 15 min).

13**. Do not allow the DNA to over dry or it will be hard to re-dissolve.**

IV. Re-dissolving the DNA:

14. ADD 500 µl distilled water and after that add 4 µl RNAse to remove RNA in the preparation.

15. After resuspension, the DNA is incubated at 65° C for 20 min to destroy any DNases that may be present and store at 4 C.

16. Agarose gel electrophoresis of the DNA will show the integrity of the DNA, while spectrophotometry will give an indication of the concentration and cleanliness

Using the Nano Drop to Quantify DNA

Lab4

OBJECTIVE:

To quantify the amount of DNA in a phage or genomic DNA sample.

DNA yield can be assessed using various methods including :

Absorbance (optical density), agarose gel electrophoresis, or use of fluorescent DNA-binding dyes, all three methods are convenient, but have varying requirements in terms of equipment needed, ease of use, and calculations to consider.

Nucleic acids absorb light at a wavelength of 260nm. If a 260 nm light source shines on a sample, the amount of light that passes through the sample can be measured, and the amount of light absorbed by the sample can be inferred. For double stranded DNA, an Optical Density (OD) of 1 at 260 nm correlates to a DNA concentration of 50 ng/µl, so DNA concentration can be easily calculated from OD measurements.

DNA concentration (µg /µl) = OD at 260 nm × dilution times × standard value .If OD260 is 1.00, it is equivalent to 50 µg DNA per ml (standard). We are taking 50 µl of DNA in 1 ml TE buffer, which means it is diluted 20 times.

For example: if the reading is 0.112 (say), then the concentration of DNA is calculated as, DNA concentration (µg/µl) = 0.112 × 20 × 50= 112 µg/ml.

A pure DNA solution has an OD260:OD280 ratio of **1.8 ± 1**

DNA purity and the amount of contaminants through: A260 / A280 The higher the ratio or the greater the 1.8 the better the mean that the concentration of DNA relative to the concentration of protein and other pollutants. Pure RNA has an A260/A280 ratio of 2.0 this could suggest RNA contamination. **RNA can easily be removed by adding RNase A during purification.**

The purity of the DNA and the amount of salts through: A260 / A230 The higher the ratio or the greater 1.5, the better and mean that the amount of salts in the DNA sample is few.

Most DNA extracts are not reasonably pure and therefore estimates of concentration using spectrophotometric measurements of UV absorption may be misleading because of the interference by RNA or non-nucleic acids contaminants.

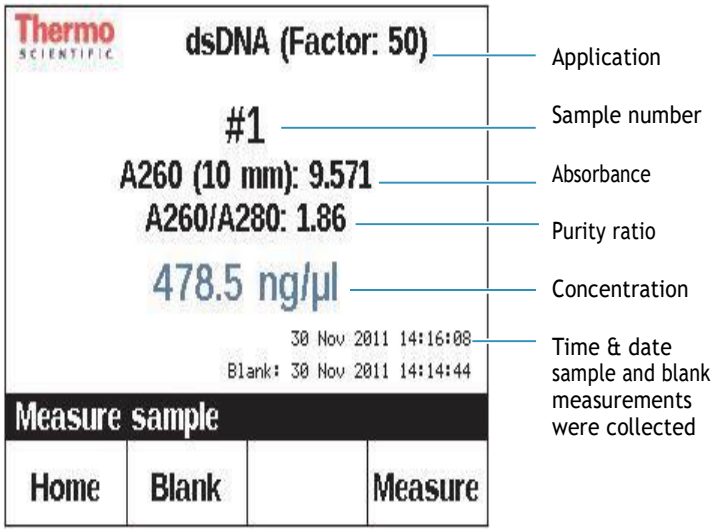
MATERIALS :

- Sample to be measured.
- Nano Drop.
- P2 or P10 micropipettor with tips.
- Lint – free lab wips.
- Purified water.
- Blanking solution (H2O , TE, EB, Tris, or other depending on your sample).

Procedure :

1. Select the appropriate application from the **Home** screen (**DNA** or **RNA**). For DNA measurements, select either the **dsDNA** or **ssDNA** assay.
2. Following the on-screen instructions, establish a blank by pipetting 1-2 µl of the blanking buffer onto the bottom pedestal, lower arm and press **Blank**.
3. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.
4. Confirm Blank by pipetting a fresh aliquot of blanking buffer onto the bottom pedestal, lower the arm and press **Blank**.
5. When measurement is complete, raise the arm and wipe the buffer from both the upper and lower pedestals using a dry laboratory wipe.
6. Measure sample by pipetting 1-2 µl of sample onto the bottom pedestal, lower arm and press **Measure**.
7. Wipe the upper and lower pedestals using a dry laboratory wipe and the instrument is ready to measure the next sample.

Sample measurement screen:

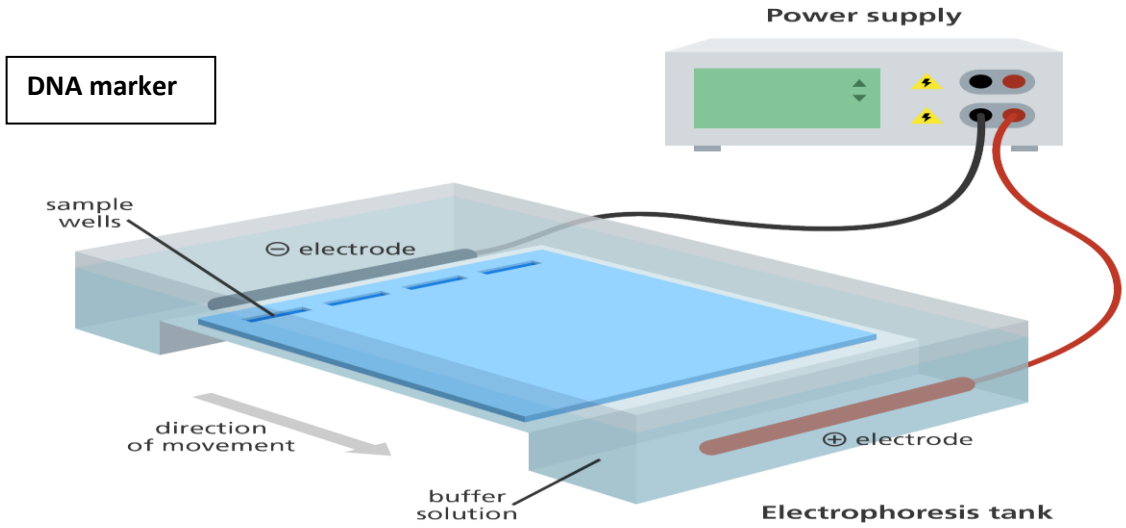


Gel electrophoresis

Lab5

Gel electrophoresis is a method used to separate nanoparticles by charge or size. It used to :

1. Separate a mixed population of DNA and RNA fragments by length.
2. To estimate the size of DNA and RNA fragments.



Gel electrophoresis :

Gel electrophoresis is a technique commonly used in laboratories to separate charged molecules like DNA , RNA and proteins according to their size. Charged molecules move through a gel when an electric current is passed across it. An electric current is applied across the gel so that one end of the gel has a positive charge and the other end has a negative charge. The movement of charged molecules is called migration. Molecules migrate towards the opposite charge. A molecule with a negative charge will therefore be pulled towards the positive end. The gel consists of a permeable matrix, a bit like a sieve, through which molecules can travel when an electric current is passed across it.

Smaller molecules migrate through the gel more quickly and therefore travel further than larger fragments that migrate more slowly and therefore will travel a shorter distance. As a result the molecules are separated by size.

Gel electrophoresis and DNA:

- DNA is negatively charged, therefore, when an electric current is applied to the gel, DNA will migrate towards the positively charged electrode.
- Shorter strands of DNA move more quickly through the gel than longer strands resulting in the fragments being arranged in order of size.
- A DNA marker with fragments of known lengths is usually run through the gel at the same time as the samples.

How is gel electrophoresis carried out ?

- Agarose gels[?] are typically used to visualise fragments of DNA. The concentration of agarose used to make the gel depends on the size of the DNA fragments you are working with.
- The higher the agarose concentration, the denser the matrix. Smaller fragments of DNA are separated on higher concentrations of agarose whilst larger molecules require a lower concentration of agarose.

- **Prepare 1X TAB + 1% Argarose :**

1. Add 250 ml of 1X TAB solution in 250 ml flask
2. Add 2.5 g of Agarose
3. Boil in a microwave.

(To make a gel, agarose powder is mixed with an electrophoresis buffer and heated to a high temperature until all of the agarose powder has melted).

- The molten gel is then poured into a gel casting tray and a "comb" is placed at one end to make wells for the sample to be pipetted into.

- Once the gel has cooled and solidified (it will now be opaque rather than clear) the comb is removed.

- The gel is then placed into an electrophoresis tank and electrophoresis **buffer** is poured into the tank until the surface of the gel is covered.

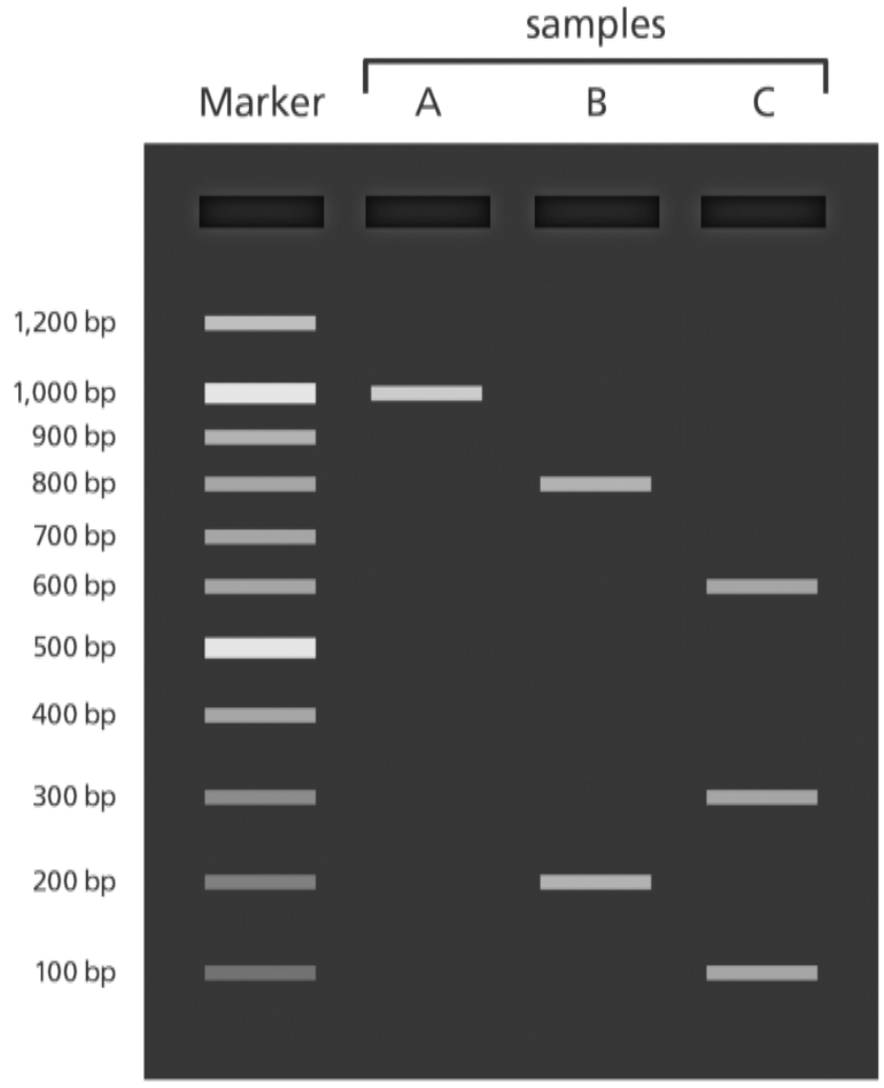
- **Preparing the DNA for electrophoresis**:

- A dye is added to the sample of DNA prior to electrophoresis to increase the viscosity of the sample which will prevent it from floating out of the wells and so that the migration of the sample through the gel can be seen.

- A DNA marker (also known as a size standard or a DNA ladder) is loaded into the first well of the gel. The fragments in the marker are of a known length so can be used to help approximate the size of the fragments in the samples.

- **Visualising the results:**

To visualise the DNA, the gel is stained with a fluorescent dye that binds to the DNA, and is placed on an ultraviolet transilluminator which will show up the stained DNA as bright bands. It is then possible to judge the size of the DNA in your sample by imagining a horizontal line running across from the bands of the DNA marker. You can then estimate the size of the DNA in the sample by matching them against the closest band in the marker.



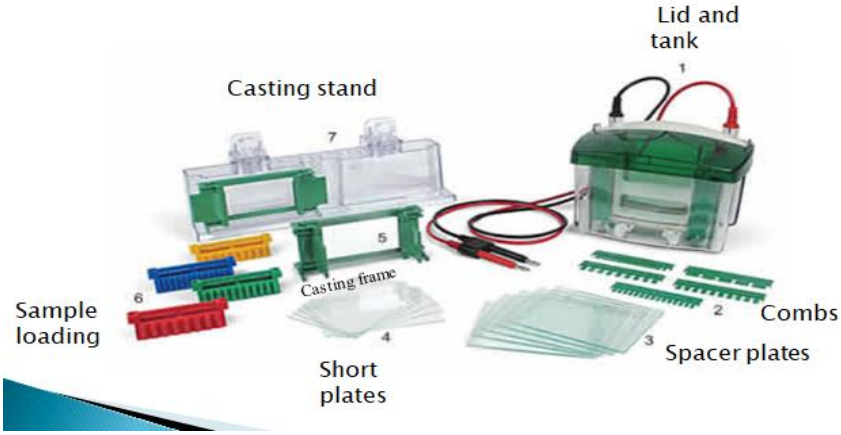
Protein isolation from plant tissue (SDS – PAGE)

Lab6

Protein Extraction from Plant

Proteins are extracted from plant tissues for a wide range of reasons, including to assay an enzyme in a crude extract for physiological studies, to purify a protein in order to identify the gene that encodes it, and to resolve plant proteins by SDS-PAGE. SDS-PAGE (sodium dodecyl sulphate -polyacrylamide gel electrophoresis) is commonly used in the lab for the separation of proteins based on their molecular weight. SDS-PAGE separates proteins according to their molecular weight, based on their differential rates of migration through a sieving matrix (a gel) under the influence of an applied electrical field

Polyacrylamide gel electrophoresis apparatus



Resolving Gel

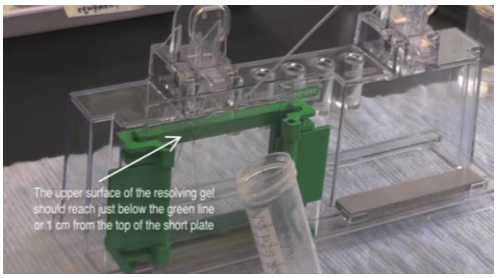
- Acrylamide **(Warning :Acrylamide is a neurotoxin. Use gloves, do not ingest)**
- 1.5M Tris, PH 8,8
- SDS
- ddH2O (Distilled water)
- Amonium persulphate (APS)
- TEMED

Prepar the 12% resolving Gel solution in 50mL falcon tube. Mix 3.3 mL of dd water 4.0 mL of 30% acrylamide solution (**should be handled with caution)** and add 100 microliters of 10% SDS together. Right before you are to pour the gel , add 10 & mL of 10% APS and 4mL of TEMED.

(TEMED catalyzes the polymerization of acrylamide)

Add pipette 200mL of isopropanol on the resolving gel.

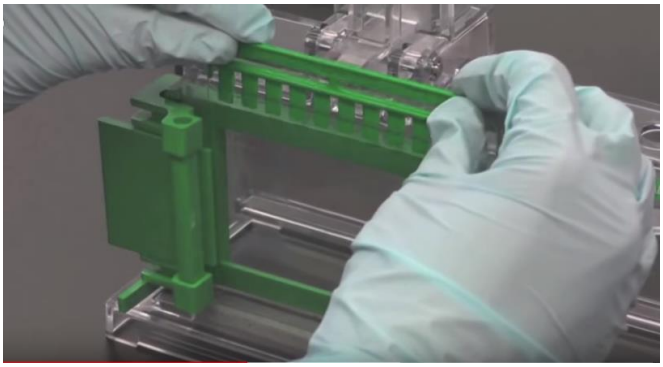
(you should observe a thin layer on the gel)



Staking Gel

- 1.35 mL dd water (Distilled water)
- 0.335 mL Acrylamide solution.
- 0.25 mL upper stock .
- 20µL 10% SDS.

Mix 1.35mL dd water , 0.335 mL acrylamide stock and 20µL 10% SDS. Once the resolving gel has formed which takes roughly 30 min drain the isopropanol. Right before you are ready to pour the stacking gel.add20mL of 10%APS and 2.0mL of TEMED into the stacking gel solution , mix well. Immediately pipette about 1 mL of the stacking gel solution on top of the resolving gel in between the plates. Fill to the top, then insert the comb into the solution

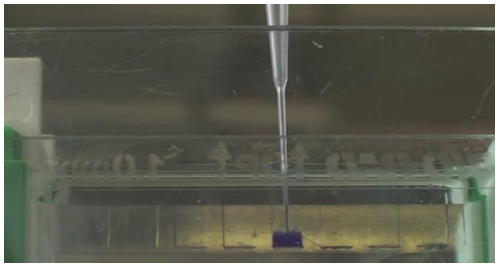


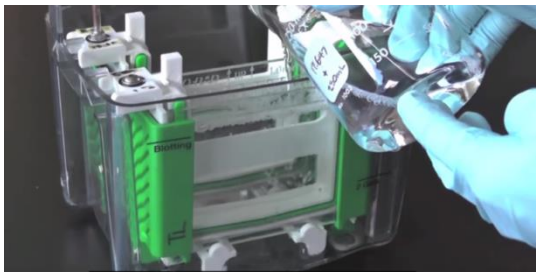
After the gel is formed , unclip the casting frame with the gel form the casting stand. Then carefully remove the glass plates from the casting frame.

Sample preparation

- Pipette 40mL of the protein sample into the microfuge tube and add 40 mL of sample buffer.
- Heat the tubes at 100̊C for 3minutes. Then pulse centrifuge for 5 seconds.

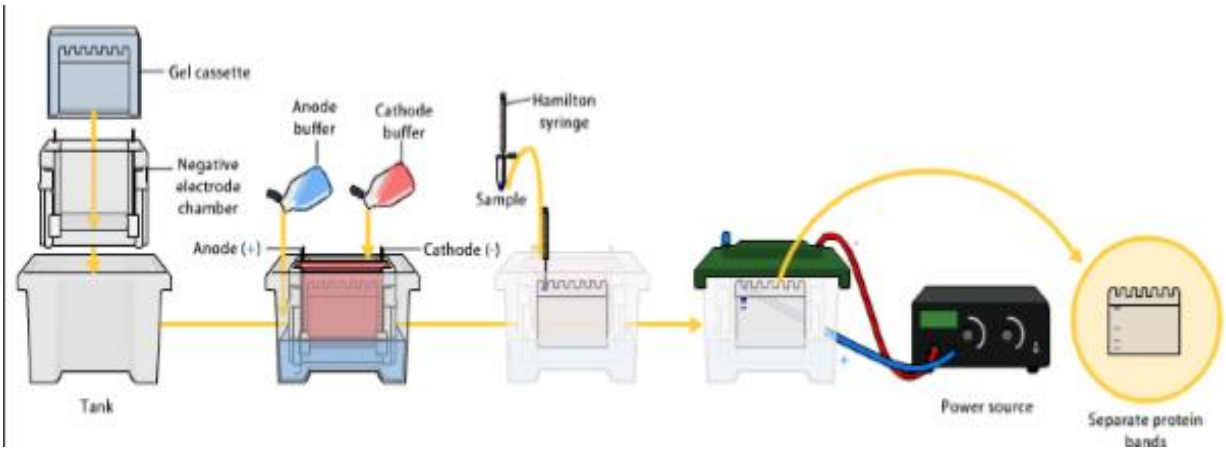
Insert the running module into the tank .Pour the 10x diluted running buffer solution into the running module between the two plates. You can install the loading on the gel



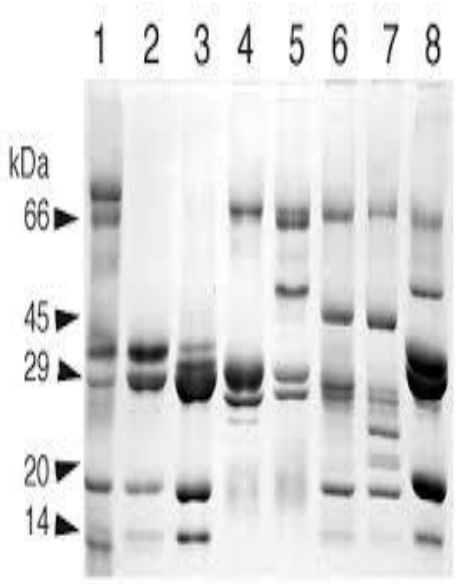


When all the sample are loaded, close the lid on the tank. Turn on the supply power .Observe the sample

The negatively charge protein to migrate across the gel towards the positive electrode.



Separation protein bands:



PCR (the polymerase chain reaction)

Lab7

- PCR is a common tool used in medical and biological research labs. It is used in the early stages of processing DNA for sequencing, for detecting the presence or absence of a gene to help identify pathogens during infection, and when generating forensic DNA profiles from tiny samples of DNA

- 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.

How does PCR work?

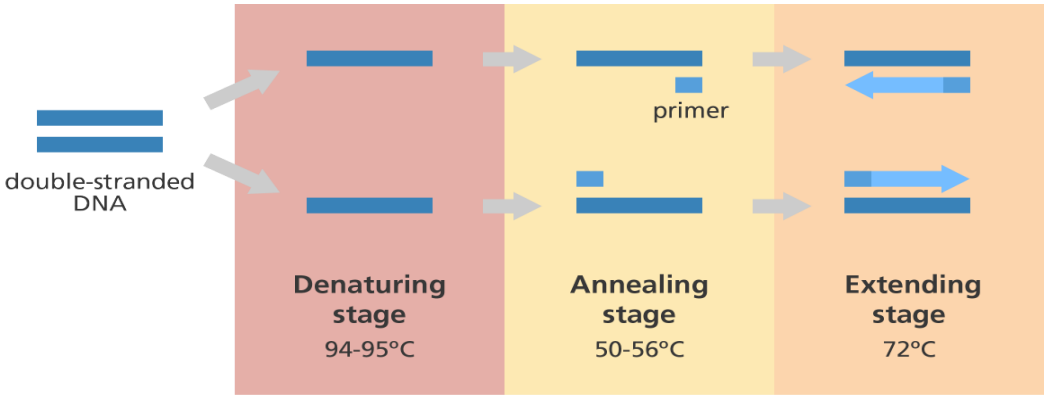
Five core 'ingredients' are required to set up a PCR. These are:

1. the DNA template to be copied
2. 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
3. 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
4. Taq polymerase enzyme to add in the new DNA bases
5. 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 in the polymerase chain reaction (PCR):

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

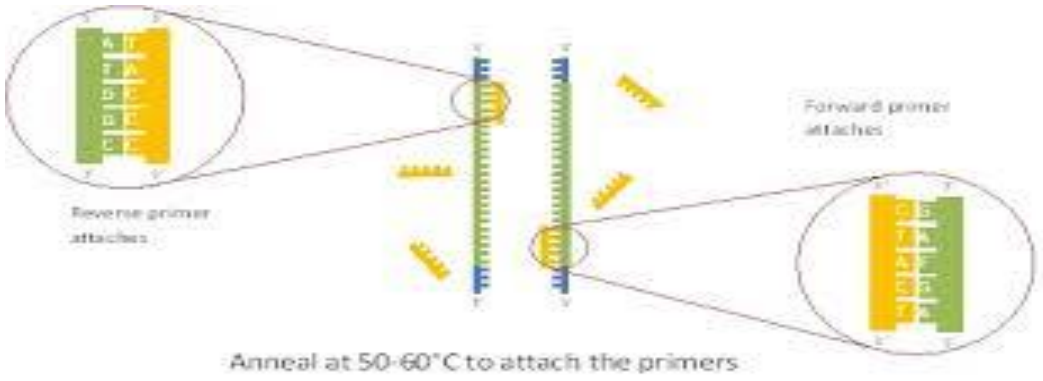


Denaturing stage:

- During this stage the template DNA and all the other core ingredients is heated to 94-95ºC.
- 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.
- This usually takes between 15-30 seconds.

Annealing:

- During this stage the reaction is cooled to 50-65ºC. This enables the primers to attach to a specific location on the single-stranded template DNA by way of hydrogen bonding.
- 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.



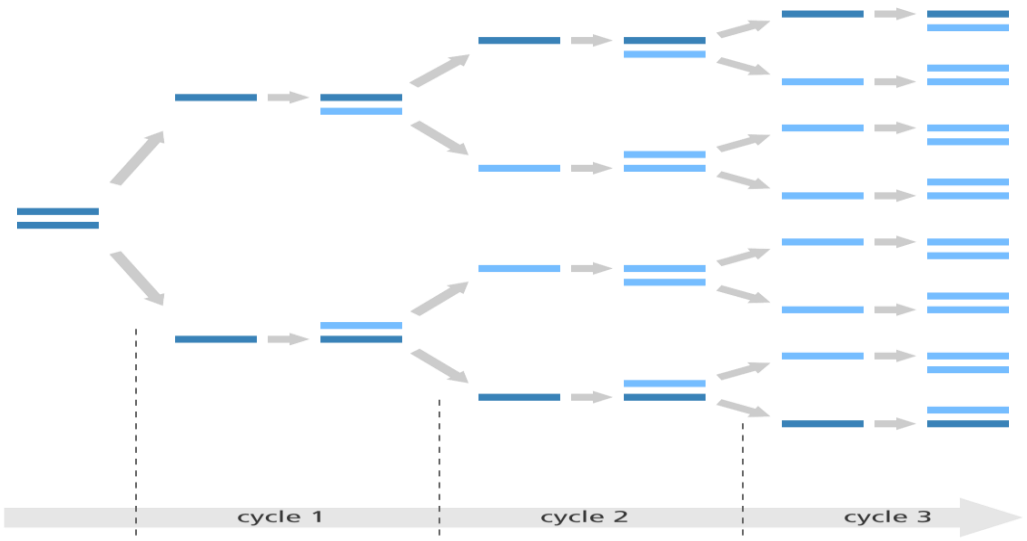
- This step usually takes about 10-30 seconds.

Extending stage:

- During this final step, the heat is increased to 72ºC to enable the new DNA to be made by a special Taq DNA polymerase enzyme which adds DNA bases.

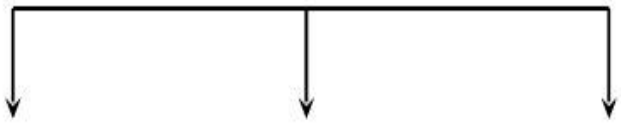
- 72ºC is the optimum temperature for the Taq polymerase to build the complementary strand. It attaches to the primer and then adds DNA bases to the single strand one-by-one in the 5’ to 3’ direction.

- The result is a brand new strand of DNA and a double-stranded molecule of DNA.



how the polymerase chain reaction (PCR) produces lots of copies of DNA.

Applications of PCR



Molecular Identification

- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- DNA fingerprinting
- Classification of organisms
- Genotyping
- Pre-natal diagnosis
- Mutation screening
- Drug discovery
- Genetic matching
- Detection of pathogens

Sequencing

- Bioinformatics
- Genomic cloning
- Human Genome Project

Genetic Engineering

- Site-directed mutagenesis
- Gene expression studies