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DEPARTMENT OF BIOTECHNOLOGY
Ministry of Science & Technology
Government of India

MOLECULAR BIOLOGY LABORATORY MANUAL

BSc BIOTECHNOLOGY SEMESTER IV

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DEPARTMENT OF BIOTECHNOLOGY

Certificate

Reg. No.

This is to certify that _____ _____

has satisfactorily completed the course of experiments in_____

_____prescribed by the MSRCASC-Autonomous

for B.Sc./M.Sc. _____

program in the Biotechnology laboratory during the year 20____- 20___.

Date: _____

Signature of the faculty in charge	Examiners	Head of the department
1.	1.	
2.	2.	

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DEPARTMENT OF BIOTECHNOLOGY AND GENETICS

COURSE: BSc BIOTECHNOLOGY

COURSE CODE: 24BITP401

MOLECULAR BIOLOGY

LABORATORY MANUAL

ACADEMIC YEAR 2024-25 ONWARDS

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Program: BSc
Semester: IV
Course: Biotechnology Practical

Course Code- 24BITP401: Molecular Biology		
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7.	Estimate the purity of DNA sample and measure the OD ratio for the purity of DNA and protein samples using spectrophotometer	
8.	DNA replication model/ Types of RNA (Model)/ Preparation of models of different forms of DNA	

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Internal Evaluation Report

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			Record/ Performance	Viva	Total	
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Faculty in-charge **HOD**

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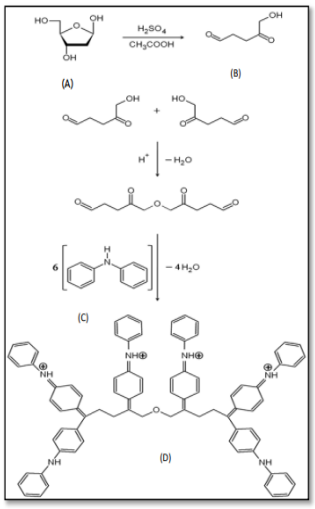
Experiment no. 1: Estimation of DNA by Diphenylamine reaction

Aim: To estimate DNA by diphenylamine (DPA) method

Introduction:

Deoxyribose nucleic acid (DNA) carries the genetic information in a cell and is capable of self-replication and synthesis of RNA. DNA consists of two long chain of nucleotides twisted into a double helix and joined by hydrogen bonds between the complementary bases adenine and thymine or guanine and cytosine. The sequence of nucleotides determines individual hereditary characteristics, are the building blocks of all nucleic acids. Nucleotides have a distinctive structure composed of three components covalently bound together: Nitrogen-containing "base" - either a pyrimidine (one ring) or purine (two rings), five-carbon sugar - ribose or deoxyribose, and a phosphate group. The combination of a base and sugar is called a nucleoside. Nucleotides also exist in activated forms containing two or three phosphates, called nucleotide diphosphates or triphosphates respectively. If the sugar in a nucleotide is deoxyribose, the nucleotide is called a deoxynucleotide; if the sugar is ribose, the term ribonucleotide is used.

Principle: The deoxyribose in DNA in the presence of acid forms β-hydroxylevulinaldehyde which reacts with diphenylamine to give a blue colour with a sharp absorption maximum at 595nm. In DNA, only the deoxyribose of the purine nucleotides reacts, so that the value obtained represents half of the total deoxyribose present.



(A) 2-deoxyribose
(B) β hydroxyl leavulinic aldehyde
(C) Diphenylamine
(D) Blue coloured complex

Reagents required:

- Standard DNA solution (1mg/ml) in 0.1 M NaOH (DNA sample in saline citrate buffer)
- Saline citrate buffer (0.15M NaCl, 0.015M sodium citrate, pH 7.0)
- Glacial acetic Acid
- Concentrated H_2SO_4

DPA reagent: Dissolve 1.5g diphenylamine in 100ml of glacial acetic acid. Add 1.5ml of conc. H_2SO_4. Store the solution in a dark glass bottle. On the day of use, prepare a fresh solution of ethanol

(1ml) in distilled water (50ml). Add 0.5ml of this solution to each 100ml of the diphenylamine solution.

Procedure:

1. Prepare a series of dilutions of standard DNA (0.25mg/ml) in saline citrate buffer to give a concentration of 50-500µg/ml.
2. To 2ml of each dilution of blank, standard and unknown add 4ml of diphenylamine reagent and mix. Tube1 is used as blank and tubes 2 through 7 are used for construction of a standard calibration curve for DNA. Tubes 8-11 are for unknown samples. (Table1)
3. Incubate all the tubes in boiling water for 10 min.
4. Cool the tubes and read the absorbance at 595nm against the blank.
5. Construct a standard curve of absorbance A_{595} vs. quantity of DNA and then calculate the concentration of unknown DNA dissolved in the saline citrate solution.

Calculation: Determine the amount of DNA in the unknown sample by plotting a standard curve of A595 on Y-axis and µg of DNA on X-axis.

Observations and Calculations:

Sl. no.	Vol. of working std. DNA solution (ml)	Vol. of distilled water (ml)	Conc. of DNA (µg/ml)	Vol. of DPA reagent (ml)		O.D at A_{595} nm
1	0.0	1.0	0	2.0	Incubate the Test tubes in boiling water bath for 10 minutes and cool at room temperature	
2	0.2	0.8	200			
3	0.4	0.6	400			
4	0.6	0.4	600			
5	0.8	0.2	800			
6	1.0	0	1000			
7	Unknown (1.0)	1	--			

Result: The amount of DNA present in the unknown sample is_____ µg/ml.

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Paste the graph and photo of the assay here

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Experiment no. 2: Analysis of DNA by Agarose gel electrophoresis

Aim: To analyse the DNA using agarose gel electrophoresis

Introduction:

Agarose gel electrophoresis, a method of gel electrophoresis used in Biochemistry, Molecular Biology and Genetics to separate a mixed population of macromolecules such as DNA, RNA or proteins in a matrix of agarose. Agarose is a natural linear polymer extracted from seaweed that forms a gel matrix by hydrogen-bonding when heated in a buffer and allowed to cool. They are the most popular medium for the separation of moderate and large-sized nucleic acids and have a wide range of separation.

Principle:

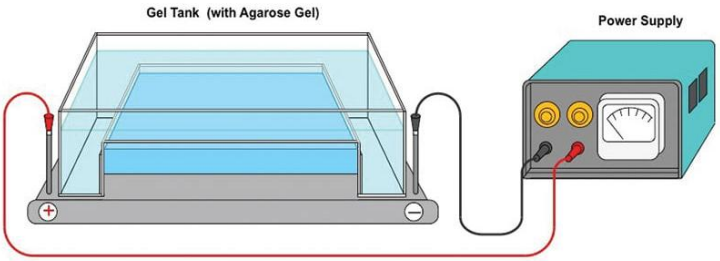
Gel electrophoresis separates DNA fragments by size in a solid support medium such as an agarose gel. Sample (DNA) are pipetted into the sample wells, followed by the application of an electric current which causes the negatively-charged DNA to migrate (electrophorese) towards the anodal, positive (+ve) end. The rate of migration is proportional to size: smaller fragments move more quickly and wind up at the bottom of the gel. DNA is visualized by including in the gel an intercalating dye, ethidium bromide. DNA fragments take up the dye as they migrate through the gel. Illumination with ultraviolet light causes the intercalated dye to fluoresce. The larger fragments fluoresce more intensely. Although each of the fragments of a single class of molecule is present in equimolar proportions, the smaller fragments include less mass of DNA, take up less dye, and therefore fluoresce less intensely. A "ladder" set of DNA fragments of known size can be run simultaneously and used to estimate the sizes of the other unknown fragments.

Requirements/ Instrumentation

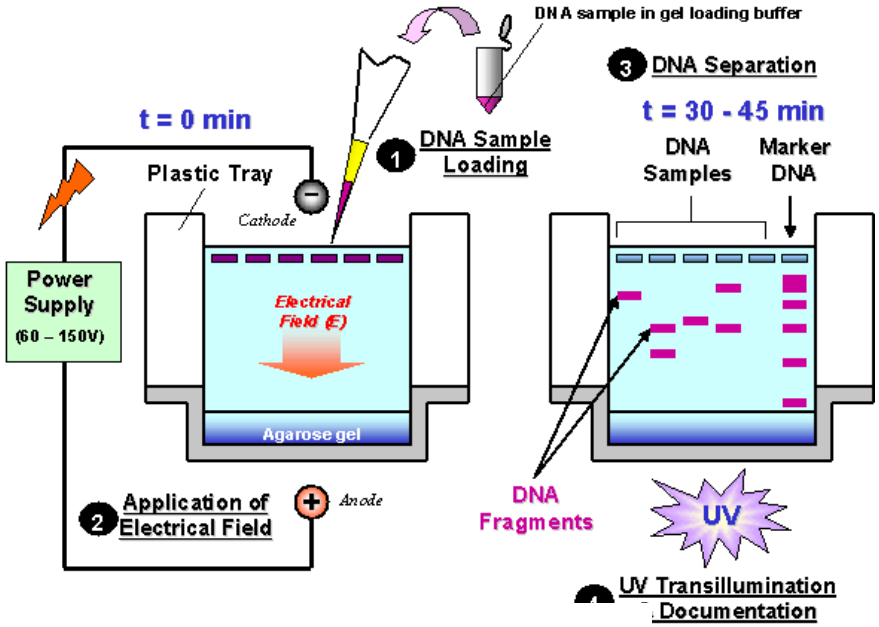
The equipment and supplies necessary for conducting agarose gel electrophoresis are relatively simple and include:

1. An **electrophoresis chamber** and **power supply**
2. **Gel casting trays**- which are available in a variety of sizes and composed of UV transparent plastic. The open ends of the trays are closed with tape while the gel is being cast, then removed prior to electrophoresis.
3. **Sample combs**- Fix the combs around which molten medium is poured to form sample wells in the gel.
4. **Electrophoresis buffer**- Tris-acetate-EDTA (TAE) or Tris-borate-EDTA (TBE).

5. **Loading buffer**- which contains something dense (e.g. glycerol) to allow the sample to "fall" into the sample wells, and one or two tracking dyes, which migrate in the gel and allow visual monitoring or how far the electrophoresis has proceeded.
6. **Staining**: DNA molecules are easily visualized under an ultraviolet lamp when electrophoresed in the presence of the extrinsic fluor ethidium bromide. Alternatively, nucleic acids can be stained after electrophoretic separation by soaking the gel in a solution of ethidium bromide. When intercalated into double stranded DNA, fluorescence of this molecule increases greatly. It is also possible to detect DNA with the extrinsic fluor 1-anilino 8-naphthalene sulphonate.
7. **Transilluminator** (an ultraviolet light box), which is used to visualize stained DNA in gels.



Procedure:



1. To prepare gel, agarose powder is mixed with electrophoresis buffer to the desired concentration, and heated in a microwave oven to melt it.

Agarose Gel concentration preparation

- The percentage of agarose used depends on the size of fragments to be resolved.
- The concentration of agarose is referred to as a percentage of agarose to volume of buffer (w/v), and agarose gels are normally in the range of 0.2% to 3%.

- The lower the concentration of agarose, the faster the DNA fragments migrate.
- In general, if the aim is to separate large DNA fragments, a low concentration of agarose should be used, and if the aim is to separate small DNA fragments, a high concentration of agarose is recommended.

2. Ethidium bromide is added to the gel (final concentration 0.5 ug/ml) to facilitate visualization of DNA after electrophoresis.
3. After cooling the solution to about 60°C, it is poured into a casting tray containing a sample comb and allowed to solidify at room temperature.
4. After the gel has solidified, the comb is removed, taking care not to rip the bottom of the wells.
5. The gel, still in plastic tray, is inserted horizontally into the electrophoresis chamber and is covered with buffer.
6. Samples containing DNA mixed with loading buffer are then pipetted into the sample wells, the lid and power leads are placed on the apparatus, and a current is applied.
7. The current flow can be confirmed by observing bubbles coming off the electrodes.
8. DNA will migrate towards the positive electrode, which is usually colored red, in view of its negative charge.
9. The distance DNA has migrated in the gel can be judged by visually monitoring migration of the tracking dyes like bromophenol blue and xylene cyanol dyes.

The conformation of a piece of DNA can affect its migration through an agarose gel, and accordingly, an estimation of its molecular weight. For example, plasmid DNA can exist in multiple conformations: Supercoiled, relaxed circular, and linear. Supercoiled plasmid DNA is the native DNA conformation found *in vivo* and occurs when extra twists are introduced into the double helix strand. Following isolation, spontaneous nicks can accumulate in the plasmid. Here, a break in one of the strands results in a piece of DNA that remains circular, but is in a relaxed conformation because the supercoils have been released. Linearized DNA results when the DNA helix is cut in both strands at the same place.

The migration of DNA molecules of the same sequence but different conformations is affected by the compactness of each conformation as they move through the gel pores. Supercoiled DNA is the most compact, and therefore migrates the fastest, followed by flexible linear and, lastly, relaxed circular molecules. The relatively small, compact conformation of Supercoiled DNA means that it sustains less resistance against the agarose matrix than a larger, floppy, open conformation of relaxed circular DNA. Therefore, for the same DNA molecule in different conformations, the supercoiled form runs faster than the relaxed, circular form. Linear DNA sustains less resistance through an agarose gel than relaxed circular DNA, but more than Supercoiled, causing it to run at an intermediate rate.

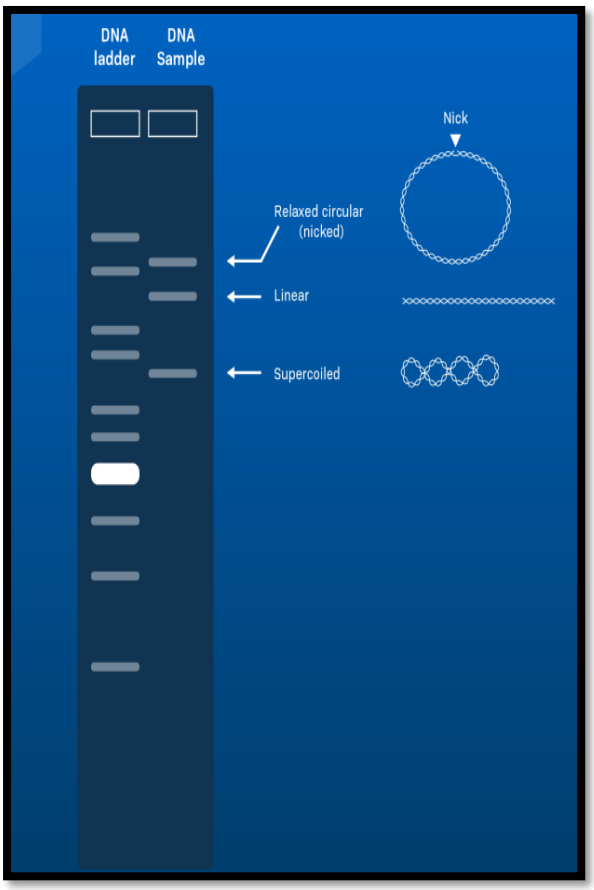


Figure: The same DNA in different conformations will migrate differently through an agarose gel.

An illustration of a DNA sample containing 3 conformations of the same piece of plasmid DNA (relaxed circular, linear, and Supercoiled; right lane) is presented. Each conformation is depicted to the right of the gel. Relaxed circular plasmid DNA is nicked, and therefore assumes an open circular conformation. This conformation takes up the most volume, causing it to migrate the most slowly through the gel. Linearized plasmid DNAs move through the gel at a slightly higher rate. Intact, Supercoiled plasmid DNA is the most compact, and therefore migrates the fastest through the gel.

Applications of Agarose Gel Electrophoresis

Agarose gel electrophoresis is a routinely used method for separating proteins, DNA or RNA.

- Estimation of the size of DNA molecules
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
- Separation of restricted genomic DNA prior to Southern analysis, or of RNA prior to Northern analysis.
- The agarose gel electrophoresis is widely employed to estimate the size of DNA fragments after digesting with restriction enzymes, e.g. in restriction mapping of cloned DNA.
- Agarose gel electrophoresis is commonly used to resolve circular DNA with different supercoiling topology, and to resolve fragments that differ due to DNA synthesis.
- In addition to providing an excellent medium for fragment size analyses, agarose gels allow purification of DNA fragments. Since purification of DNA fragments size separated in an agarose gel is necessary for a number molecular techniques such as cloning, it is vital to be able to purify fragments of interest from the gel.

Advantages of Agarose Gel Electrophoresis

- For most applications, only a single-component agarose is needed and no polymerization catalysts are required. Therefore, agarose gels are simple and rapid to prepare.
- The gel is easily poured, does not denature the samples.
- The samples can also be recovered

Disadvantages of Agarose Gel Electrophoresis

- Gels can melt during electrophoresis.
- The buffer can become exhausted.
- Different forms of genetic material may run in unpredictable forms.

Experiment no. 3: Estimation of RNA by Orcinol method

Aim: To estimate the concentration of RNA by orcinol reaction.

Introduction:

The colorimetric method suitable for pentose determination have been utilize for measuring RNA and involve reaction with orcinol, aniline, phloroglucinol etc. One of the widely used reaction is those of RNA with orcinol commonly used for estimation purpose.

Principle:

The orcinol method is a colorimetric technique for quantifying RNA based on the reaction between ribose, the sugar in RNA, and orcinol. In the presence of a strong acid (like HCl) and a catalyst ($FeCl_3$), RNA is hydrolyzed to release ribose, which on heated with conc. HCl then dehydrates to form furfural. This furfural reacts with orcinol to produce a green-colored product with a maximum absorbance at 665 nm, allowing for the RNA concentration to be determined by measuring the color intensity.

Requirements:

1. Standard RNA solution- 200µg/ml in 1 N perchloric acid/buffered saline.
2. Orcinol Reagent- Dissolve 0.1g of ferric chloride in 100 ml of concentrated HCl and add 3.5 ml of 6% w/v orcinol in alcohol.
3. Buffered Saline- 0.5 mol/litre NaCl; 0.015 mol/litre sodium citrate, pH 7.

Procedure:

1. Take 7 test tubes; label them as B, 1, 2, 3, 4, 5 and U.
2. Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard in to the series of labelled test tubes (1, 2, 3, 4, and 5).
3. A tube with 1 ml of distilled water serves as the blank (B).
2. Pipette out 1 ml of the given unknown sample into another test tube (U).
3. Make up the volume to 1 ml with distilled water in all the test tubes.
4. Now add 2 ml of orcinol reagent to all the test tubes including the test tubes labelled 'blank' and 'unknown'.
5. Mix the contents of the tubes by vortexing / shaking the tubes and heat on a boiling water bath for 20 min.
6. Then cool the contents and record the absorbance at 665 nm against blank.
7. Then plot the standard curve by taking concentration of RNA along X-axis and absorbance at 665 nm along Y-axis.
8. Then from this standard curve calculate the concentration of RNA in the given sample.

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Observations and Calculations:

Sl. no.	Vol. of working std. RNA solution (ml)	Vol. of distilled water (ml)	Conc. of RNA (µg/ml)	Vol. of Orcinol reagent (ml)		O.D at A_{665} nm
1	0.0	1.0	0	2.0	Incubate the Test tubes in boiling water bath for 10 minutes and cool at room temperature	
2	0.2	0.8	200			
3	0.4	0.6	400			
4	0.6	0.4	600			
5	0.8	0.2	800			
6	1.0	0	1000			
7	Unknown (1.0)	1	--			

Result: The given unknown sample contains _____ µg RNA/ml.

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Paste the graph and photo of the assay here

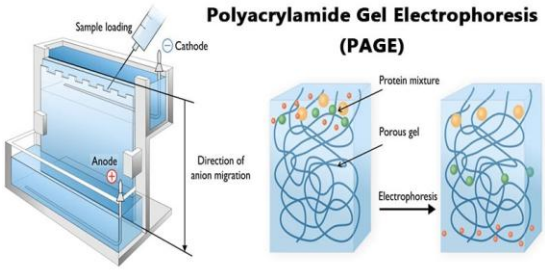
Experiment no. 4: Demonstration of protein separation by SDS-Polyacrylamide Gel Electrophoresis (PAGE)

Aim: To separate proteins based on their molecular weight by SDS PAGE.

Introduction:

Polyacrylamide Gel Electrophoresis (PAGE)

- Electrophoresis through agarose or polyacrylamide gels is a standard method used to separate, identify and purify biopolymers, since both these gels are porous in nature.
- Polyacrylamide gels are chemically cross-linked gels formed by the polymerization of acrylamide with a cross-linking agent, usually N,N'-methylenebisacrylamide.
- The reaction is a free radical polymerization, usually carried out with ammonium persulfate as the initiator and N,N,N',N'-tetramethylethylendiamine (TEMED) as the catalyst.
- Polyacrylamide gel electrophoresis (PAGE) is a technique widely used in biochemistry, forensic chemistry, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility.
- The most commonly used form of polyacrylamide gel electrophoresis is the Sodium dodecyl suplhate Polyacrylamide gel electrophoresis (SDS- PAGE) used mostly for the separation of proteins.



Principle:

SDS-PAGE (Polyacrylamide Gel Electrophoresis), is an analytical method used to separate components of a protein mixture based on their size. The technique is based upon the principle that a charged molecule will migrate in an electric field towards an electrode with opposite sign. The general electrophoresis techniques cannot be used to determine the molecular weight of biological molecules because the mobility of a substance in the gel depends on both charge and size. To overcome this, the biological samples needs to be treated so that they acquire uniform charge, then the electrophoretic mobility depends primarily on size. For this different protein molecules with different shapes and sizes, needs to be denatured (done with the aid of SDS) so that the proteins lose their secondary, tertiary or quaternary structure. The proteins being covered by SDS are negatively charged and when loaded onto a gel and placed in an electric field, it will migrate towards the anode

(positively charged electrode) are separated by a molecular sieving effect based on size. After the visualization by a staining (protein-specific) technique, the size of a protein can be calculated by comparing its migration distance with that of a known molecular weight ladder (marker).

Requirements:

- Acrylamide solutions (for resolving & stacking gels)
- Isopropanol / distilled water
- Gel loading buffer
- Running buffer
- Staining, destaining solution
- Protein samples
- Molecular weight markers

The equipment and supplies necessary for conducting SDS-PAGE includes:

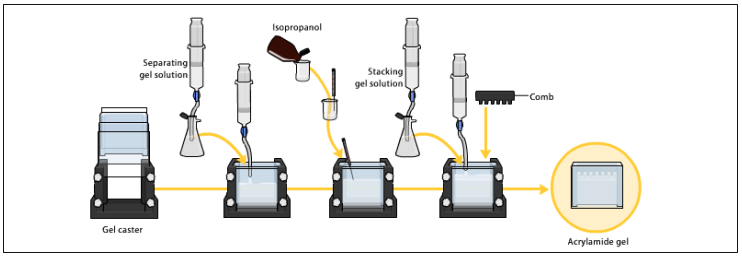
- An electrophoresis chamber and power supply.
- Glass plates (a short and a top plate).
- Casting frame
- Casting stand
- Combs

Procedure

1. **Sample preparation**

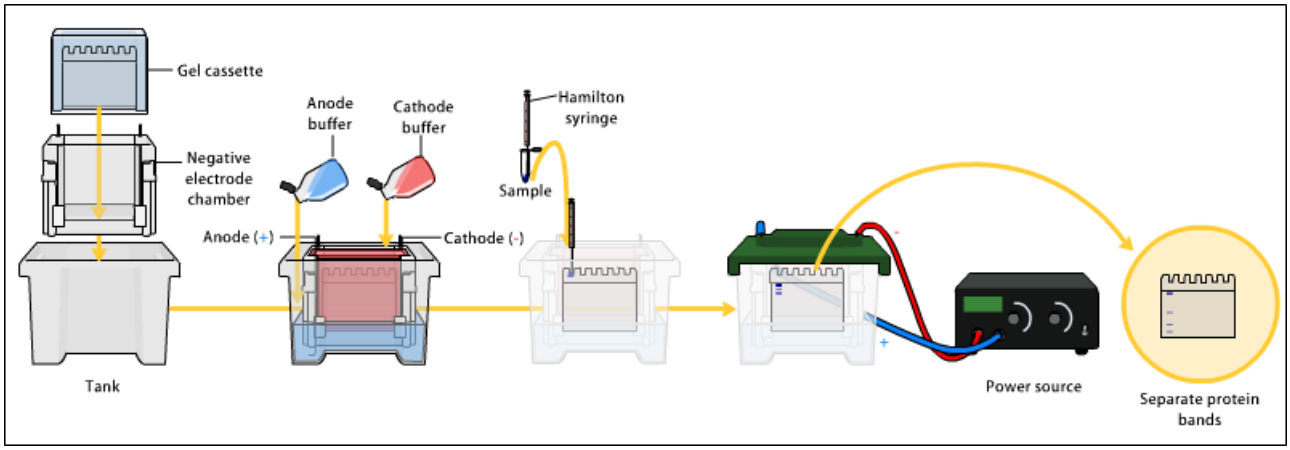
- Samples may be any material containing proteins or nucleic acids.
- The sample to analyze is optionally mixed with a chemical denaturant if so desired, usually SDS for proteins or urea for nucleic acids.
- SDS is an anionic detergent that denatures secondary and non–disulfide–linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass. Urea breaks the hydrogen bonds between the base pairs of the nucleic acid, causing the constituent strands to anneal. Heating the samples to at least 60 °C further promotes denaturation.
- A tracking dye may be added to the solution. This typically has a higher electrophoretic mobility than the analytes to allow the experimenter to track the progress of the solution through the gel during the electrophoretic run.

2. **Preparation of polyacrylamide gel**



- The gels typically consist of acrylamide, bisacrylamide, the optional denaturant (SDS or urea), and a buffer with an adjusted pH.
- The ratio of bisacrylamide to acrylamide can be varied for special purposes, but is generally about 1 part in 35. The acrylamide concentration of the gel can also be varied, generally in the range from 5% to 25%.
- Lower percentage gels are better for resolving very high molecular weight molecules, while much higher percentages of acrylamide are needed to resolve smaller proteins,
- Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells.
- After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis.

3. **Electrophoresis**



- Various buffer systems are used in PAGE depending on the nature of the sample and the experimental objective.
- The buffers used at the anode and cathode may be the same or different.
- An electric field is applied across the gel, causing the negatively charged proteins or nucleic acids to migrate across the gel away from the negative and towards the positive electrode (the anode).
- Depending on their size, each biomolecule moves differently through the gel matrix: small molecules more easily fit through the pores in the gel, while larger ones have more difficulty.
- The gel is run usually for a few hours, though this depends on the voltage applied across the gel.
- After the set amount of time, the biomolecules will have migrated different distances based on their size.
- Smaller biomolecules travel farther down the gel, while larger ones remain closer to the point of origin.

- Biomolecules may therefore be separated roughly according to size, which depends mainly on molecular weight under denaturing conditions, but also depends on higher-order conformation under native conditions.

4. **Detection**

- Following electrophoresis, the gel may be stained (for proteins, most commonly with Coomassie Brilliant Blue or autoradiography; for nucleic acids, ethidium bromide; or for either, silver stain), allowing visualization of the separated proteins, or processed further (e.g. Western blot).
- After staining, different species biomolecules appear as distinct bands within the gel.
- It is common to run molecular weight size marker sof known molecular weight in a separate lane in the gel to calibrate the gel and determine the approximate molecular mass of unknown biomolecules by comparing the distance traveled relative to the marker.

Applications

- Measuring molecular weight.
- Peptide mapping.
- Estimation of protein size.
- Determination of protein subunits or aggregation structures.
- Estimation of protein purity.
- Protein quantitation.
- Monitoring protein integrity.
- Comparison of the polypeptide composition of different samples.
- Analysis of the number and size of polypeptide subunits.
- Post-electrophoresis applications, such as Western blotting.
- Staining of Proteins in Gels with Coomassie G-250 without Organic Solvent and Acetic Acid.
- Pouring and Running a Protein Gel by reusing Commercial Cassettes.
- Selective Labelling of Cell-surface Proteins using CyDye DIGE Fluor Minimal Dyes.
- Detection of Protein Ubiquitination.

Advantages

- Stable chemically cross-linked gel
- Greater resolving power (Sharp bands)
- Can accommodate larger quantities of DNA without significant loss in resolution
- The DNA recovered from polyacrylamide gels is extremely pure
- The pore size of the polyacrylamide gels can be altered in an easy and controllable fashion by changing the concentrations of the two monomers.
- Good for separation of low molecular weight fragments

Disadvantages

- Generally, more difficult to prepare and handle, involving a longer time for preparation than agarose gels.
- Toxic monomers
- Gels are tedious to prepare and often leak
- Need new gel for each experiment Stable chemically cross-linked gel

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Experiment no. 5: Extraction and partial purification of protein from animal source by organic solvents

Aim: To extract and partial purify the proteins from animal source using organic solvents.

Introduction: Proteins are precipitated using different percentage of organic solvents solution. It is one of the widely used methods to remove interfering substances and precipitate the proteins. In the present experiment, acetone dissolves most of the lipids in animal tissue and proteins are precipitated by chilled acetone at different concentrations. Lower temperature aids the protein conformation to stay in its nature form. The amount of extracted protein is estimated by Biuret test. This is the most commonly employed technique for estimation (Quantitative to confirm the presence & qualitatively presence of proteins). In this method, the peptide bonds of proteins interact with Cu^{2+} ion, in alkaline medium to give violet solution, absorbance recorded at 540nm against blank.

Principle: Extraction and partial purification of protein from animal sources using organic solvents involves disrupting the sample, adding a solvent like cold acetone or a solvent mixture (e.g., t-butanol), centrifuging to precipitate the proteins, and then washing and redissolving the protein pellet. The organic solvent removes non-polar components and impurities, while the cold temperature helps prevent denaturation. This process concentrates the protein by separating it from many other cellular components.

Reagents required:

1. Chilled acetone – 30% and 50%
2. 0.1M Tris HCL Buffer (pH-8.2) – 1.2gms of Tris HCL is dissolved in 80ml of distilled water, adjust the pH to 8.2 with 0.1N HCL / 0.1M NaOH. Make up the volume to 100ml with distilled water.
3. Standard Stock solution: 100mg of BSA is dissolved in 100ml of distilled water to get 1mg/ml of concentration.
4. Biuret Reagent: 0.3gms of copper sulphate and 0.9gms of sodium potassium Tartarate in 50ml of 0.2N NaOH. Add 5g of Potassium iodide and make up the volume to 100ml with 0.2N NaOH.

Procedure:

Preparation of Standard graph

1. Pipette out various volume of protein working standard stock solution into a series of test tubes.
2. Pipette out 2ml of distilled water which is considered blank.
3. Make the volume of all test tubes to 2ml with distilled water
4. Add 3ml of Biuret's reagent to all the test tubes.
5. Mix the contents of test tubes; incubate all the test tubes for 10minutes at 37^0C.
6. Cool the test tubes to room temperature, record the absorbance at 540nm against the BLANK.

7. Plot the standard graph curve by taking amount of protein along with x axis and absorbance at 540nm along y axis.

Preparation of animal extract for partial purification protein

1. Cut the provided liver tissue into smaller pieces, wash with distilled water.
2. Weigh 5g of liver tissue into mortar and pestle, homogenise with 10ml of cold phosphate buffer.
3. Filter the homogenate with two layered muslin cloth.
4. Centrifuge the homogenate at 5000rpm for 5 minutes.
5. Collect the supernatant in test tubes and divide into two parts. Part 1 + 2ml of 30% acetone and Part 2 + 2ml of 50% acetone.
6. Centrifuge the homogenate at 5000rpm for 5 minutes.
7. Collect the precipitate and re-dissolve in 3ml Tris HCL Buffer.
8. Pipette out 1ml extract from each sample into separate test tube and make up the volume upto 2ml.
9. Add 3ml of Biuret reagent to above test tubes and incubate at 37^0C for 10 minutes.
10. Cool the test tubes at room temperature and record the absorbance at 540nm.
11. Determine the amount of protein in the liver tissue using standard graph.

Observations and Calculations:

Sl no.	Vol. of BSA (ml)	Vol. of distilled water (ml)	Amount of Protein	Vol. of Biuret's reagent (ml)		O.D at A_{540} nm
1	0.0	2.0	0	3.0	Incubate the test tubes at room temperature for 10 minutes	
2	0.2	1.8	2000			
3	0.4	1.6	4000			
4	0.6	1.4	6000			
5	0.8	1.2	8000			
6	1.0 Acetone liver Tissue Extract **(30%)**	1	?			
7	1.0 Acetone liver Tissue Extract **(50%)**	1	?			

Result: The amount of protein present in 30% Acetone is _____ and 50% Acetone is _____.

Paste the graph and photo of the assay

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Experiment no. 6: Determination of absorption spectra of DNA and protein using UV-Visible spectrophotometer

Aim: To determine the absorption maxima of DNA and protein using UV-Vis spectrophotometer.

Introduction:

UV/Vis spectroscopy is an analytical technique that measures how much ultraviolet or visible light a sample absorbs. The amount of light a protein, DNA or RNA sample absorbs is directly proportional to the concentration. Proteins absorb most at 280 nm while DNA and RNA absorption peaks at 260 nm. With Beer's law, the sample concentration is determined from the absorbance at 280 nm or 260 nm (called A_{280} or A_{260}), the known path length and the sample's extinction coefficient or concentration factor. UV/Vis spectrophotometers perform these measurements and calculations automatically and without any additional reagents. The best spectrometers can measure at high-throughput and with low sample volumes. Absorption spectrum is a measure of absorbance as a function of wavelength (λ). Absorption spectrum of a molecule is obtained by measuring its absorbance in a certain wavelength range where the molecule absorbs light. The wavelength where the maximum absorbance takes place is known as the absorption maxima (λ max) of that molecule. Absorption spectroscopy is often used for DNA and RNA analysis. DNA is double stranded and is composed of four bases, namely, Adenine (A), Guanine (G), Thymine (T) and Cytosine (C) along with phosphate and deoxyribose pentose sugar. While RNA differs from DNA in three aspects, it is single stranded, rather than thymine (T), it has uracil (U) and it contains ribose rather than deoxyribose sugar.

Principle:

Absorption maxima (λ max) is the wavelength at which a substance absorbs the most light in a UV-Vis spectrum. It represents the highest point on the spectrum's curve and is a unique identifier for a compound, similar to a fingerprint. This wavelength is used in UV spectroscopy to both identify and quantify the concentration of a substance in a solution, using the Beer-Lambert law. Aromatic amino acids and nitrogenous bases [A, G (purines), and C, U, T (pyrimidines)] are intrinsic chromophores that form part of the structure of proteins and DNA respectively. All of them absorb light in UV range. Thus detection of peptides, proteins and DNA can be done at 210, 280 and 260nm respectively. This property is useful in quantitative and qualitative analysis of proteins and nucleic acids. This analysis is usually done using double beam UV-visible spectrophotometer. Nitrogenous bases (A, T, G, C, U) absorb ultraviolet light around 260 nm. By virtue of these bases, nucleic acids absorb strongly at 260 nm. Thus, choose the spectra range of 200-400 nm i.e. the UV range. Absorption spectrum is then plotted which is a measure of absorbance as a function of wavelength. The plot tells at what wavelength nucleic acids absorb maximally.

Reagents required:

Stock solutions

2 mg/ml DNA: Weigh 10 mg of DNA and to it add 5 ml 10mM Tris pH 8.0.

2 mg/ml RNA: Weigh 10 mg of RNA and to it add 5 ml 10mM Tris pH 8.0.

1 mg/ml protein solution- Weigh 100mg/ 100ml PBS (pH 7.0)

Working solutions

50 µg/ml of DNA in 10mM Tris pH 8.0: Take 0.25 ml of 2 mg/ml DNA stock
and to it add 9.75 ml of 10 mM Tris pH 8.0

40 µg/ml of RNA in 10mM Tris pH 8.0: Take 0.2 ml of 2 mg/ml RNA stock
and to it add 9.8 ml of 10 mM Tris pH 8.0

1. Take 1 ml of 10 mM Tris pH 8 in each of the two cuvettes. Place them in reference and sample holder in dual beam spectrophotometer.
2. Discard the contents of sample cuvette and instead add 1 ml of 50 µg/ml of DNA in sample cuvette.
3. Take absorbance in the range of 200 nm to 400 nm.
4. Repeat steps (i) to (iii) taking 1 ml of 40 RNA µg/ml sample instead of DNA.
5. Plot a graph by taking wavelength at x axis against absorbance reading on y-axis. Calculate the lambda max from the graph.
6. Repeat the steps for protein samples also.

Wavelength	Absorbance of DNA sample	Wavelength	Absorbance of RNA sample	Wavelength	Absorbance of protein sample
200					
210					
220					
230					
240					
250					
260					
270					
280					
290					
300					

310					
320					
330					
340					
350					
360					
370					
380					
390					
400					

Results: The absorption maxima obtained for DNA and protein were --------.

Paste the graph here

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Experiment no. 7: Estimate the purity of DNA sample and measure the OD ratio for the purity of DNA and protein samples using spectrophotometer

Aim: To estimate the purity of DNA and protein samples using spectrophotometer

Introduction: To evaluate DNA purity, measure absorbance from 230nm to 320nm to detect other possible contaminants. The most common purity calculation is the ratio of the absorbance at 260nm divided by the reading at 280nm. Good-quality DNA will have an A_{260}/A_{280} ratio of 1.7–2.0. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

Principle:

DNA purity:

Nucleic acids (DNA and RNA) absorb ultraviolet (UV) light most strongly at 260 nm. Proteins, especially aromatic amino acids like tyrosine and tryptophan, absorb strongly around 280 nm. By comparing the absorbance at these two wavelengths, you can determine the ratio of nucleic acid to protein in a sample.

Protein purity:

A pure protein sample would show very little absorbance at 260 nm and 230 nm, and therefore, this ratio is not used for protein purity. Instead, the A260/A280 ratio can be used as an indicator of DNA or RNA contamination in a protein sample, where a low ratio would indicate the presence of nucleic acids. *A*260/*A*230 ratio, can also be used to assess for contaminants like phenol or guanidine, which absorb at 230 nm. A pure protein sample would show very little absorbance at 260 nm and 230 nm, and therefore, this ratio is not used for protein purity. Instead, the ratio can be used as an indicator of DNA or RNA contamination in a protein sample, where a low ratio would indicate the presence of nucleic acids. A common contamination found in whole cell lysates is DNA. The 260/280 ratio can be used to gauge the purity of an isolated protein when evaluating purified proteins. For typical proteins, a 260/280 ratio of 0.6 is appropriate. Higher ratios can be a sign that DNA has contaminated isolated proteins.

Procedure:

Set up the spectrophotometer: Calibrate the instrument with a blank solution (e.g., nuclease-free water) to zero the reading. For a more accurate reading, ensure the path length is correctly set, especially if using a micro-volume spectrophotometer

1. **Take the reading at 260 nm:** Pipette the sample into the spectrophotometer's cuvette or onto its analysis surface. Measure the absorbance at 260 nm (A_{260}).
2. **Take the reading at 280 nm:** Without changing the cuvette or sample, measure the absorbance at 280 nm ((A_{280})
3. **Calculate the ratio:** Divide the A_{260} reading by the A_{280} reading to get the ratio. For a pure DNA sample, the ratio should be between 1.8 and 2.0. For a pure DNA sample, the ratio should be between 1.8 and 2.0.
4. **Interpret the ratio:**
   - **Ratio ≈is approximately equal to**

     ≈1.8–2.0: The sample is pure DNA.

   - **Ratio < 1.7:** The sample may be contaminated with protein or acidic phenol.
   - **Ratio > 2.0:** The sample may be contaminated with basic substances.
5. **Consider the A_{260}/A_{230} ratio:** If you suspect other contaminants like guanidine salts or phenol, take an additional reading at 230 nm and calculate the A_{260}/A_{230} ratio. A pure DNA sample will have an A_{260}/A_{230} ratio of approximately 1.8–2.2. A ratio that is too low may indicate the presence of these contaminants.
6. **Set up the spectrophotometer:** Calibrate the instrument with a blank solution (e.g., nuclease-free water) to zero the reading. For a more accurate reading, ensure the path length is correctly set, especially if using a micro-volume spectrophotometer.
7. **Take the reading at 260 nm:** Pipette the sample into the spectrophotometer's cuvette or onto its analysis surface. Measure the absorbance at 260 nm (A_{260})
8. **Take the reading at 280 nm:** Without changing the cuvette or sample, measure the absorbance at 280 nm (A_{280})
9. **Calculate the ratio:**

   For a pure DNA sample, the ratio should be between 1.8 and 2.0.

10. **Interpret the ratio:**
    - **Ratio ≈is approximately equal to ≈1.8–2.0:** The sample is pure DNA.
    - **Ratio < 1.7:** The sample may be contaminated with protein or acidic phenol.
    - **Ratio > 2.0:** The sample may be contaminated with basic substances.
11. **Consider the A_{260}/A_{230} ratio:** If you suspect other contaminants like guanidine salts or phenol, take an additional reading at 230 nm and calculate the A_{260}/A_{230}
12. *A*260/*A*230ratio: A pure DNA sample will have an *A*260/*A*230 ratio of approximately 1.8–2.2. A ratio that is too low may indicate the presence of these contaminants.

Procedure:

Steps involved in measuring DNA concentration using 500 µl quartz cuvettes and a 10mM Tris-Cl, pH 7.0 buffer.

1. Turn on the spectrophotometer and set the wavelength to 260 nm.

2. Transfer 495 µl of 10mM Tris-Cl, pH 7.0 to two 500 µl quartz cuvettes.

3. Insert the first cuvette into the spectrophotometer and zero the instrument. Note: This cuvette acts as the blank

4. Insert the second cuvette into the spectrophotometer and record the reading.

Note: This is called matching the cuvettes.

5.The value recorded is the correction factor that will be added or subtracted (depending if it is negative or positive) from the reading recorded for the DNA sample.

6. Remove the second cuvette and add 5 µl of the DNA sample.

7. Mix by placing parafilm over the cuvette and inverting several times.

Protocol for measuring protein purity

Prepare the sample: Dilute your purified protein sample in a suitable buffer. Make sure the buffer components do not absorb strongly at 260nm or 280nm, as this can skew the results.

1. **Calibrate the spectrophotometer:** Blank the spectrophotometer with the same buffer used for the sample. This ensures that any readings are specific to the protein and contaminants, not the buffer itself.

2. **Take absorbance readings:** Measure the absorbance of the diluted sample at 260nm and 280nm.

3. **Calculate the ratio:** Divide the absorbance reading at 260nm by the absorbance reading at 280nm to get the *A*260/*A*280 ratio.
4. **Interpret the results:**

   Pure protein: A ratio between 0.6 and 0.8 is considered pure.

   Nucleic acid contamination: A ratio above 0.8 indicates the presence of DNA or RNA, which absorb strongly at 260 nm.

   Other contamination: A ratio below 0.5 can indicate contamination with compounds like phenolic compounds.
5. **Consider alternatives for contaminated samples:** If you have a contaminated sample or are using a buffer with high UV absorbance, it is best to use a colorimetric assay like the Bradford or BCA assay instead, as they are not affected by buffer components.

Calculations:

Result: The purity of DNA and protein are --------- respectively.

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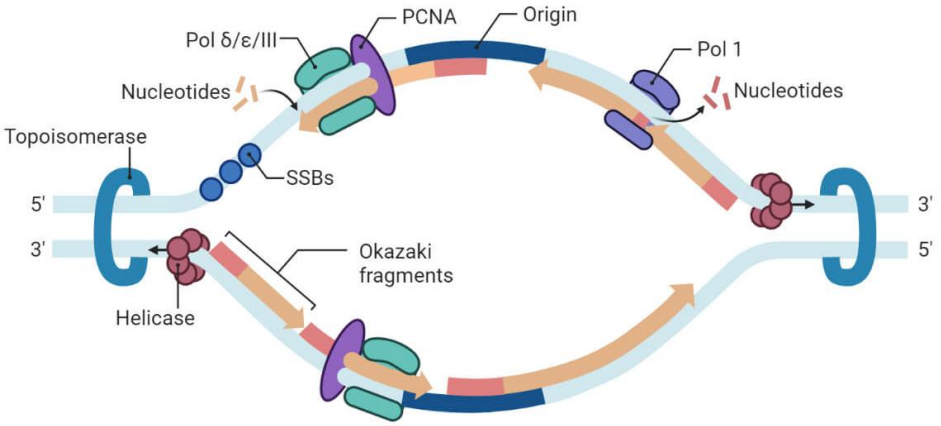
Experiment no. 8: DNA replication model/ Types of RNA (Model)/ Preparation of models of different forms of DNA

DNA replication models – a) Semiconservative replication b) Rolling circle model c) Theta model

Semiconservative replication



Semiconservative model of replication

In the semiconservative model of DNA replication, two copies of the original DNA molecule are produced, each copy containing one original strand and one newly synthesized strand.

Semiconservative DNA Replication

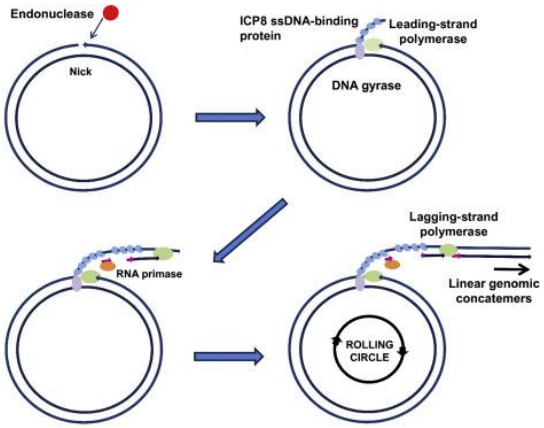
- Watson and Crick's double helix model of the DNA molecule features an integrated template system for self-replication or autocatalysis.
- Because of the specificity of base pairing, Adenine to Thymine and Guanine to Cytosine, the base sequence along one chain automatically determines the base sequence along the other.
- This leads to each double helix chain serving as the template for synthesizing the other.
- For the replication of DNA molecules, Watson and Crick proposed that replication involved the disruption of hydrogen bonds followed by a rotation and separation of the two polynucleotide strands.
- Each purine and pyrimidine base of each polynucleotide strand attracts a complementary free nucleotide available for polymerization in the cell, and to hold it in place means of the specific hydrogen bonds.

- Once held in place on the parent template chain, the free nucleotides are linked together by forming the phosphodiester bonds that linked adjacent deoxyribose residues, forming a new polynucleotide molecule of a predetermined base sequence.

- The result of replication is two double-stranded DNA molecules with sequences identical to the original one.
- The original left strand is present in one of these daughter molecules, while the original right strand is in the other.
- Since each progeny retains half of the parent DNA molecule, this replication pattern is considered semi-conservative.
- The DNA replication in prokaryotes and eukaryotes is similar but not the same. The process is more complicated in eukaryotes than in prokaryotes.

In addition to the semiconservative mode, the next two models of DNA replication are equally possible.

Rolling circle model



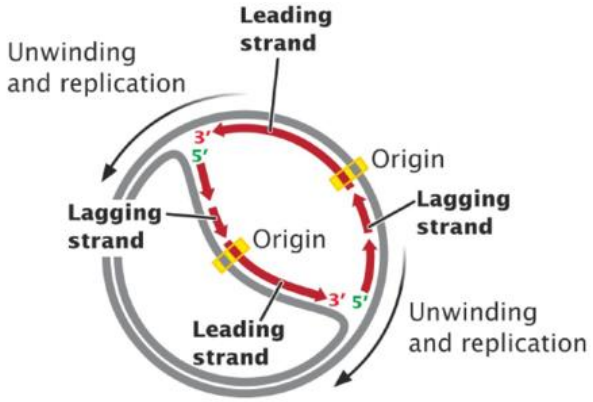
Rolling circle model of DNA replication

Steps in rolling circle replication

1. **Initiation**: An initiator protein nicks one strand of the double-stranded circular DNA at the origin of replication. The protein remains bound to the 5' end of the nicked strand.
2. **Elongation**: The exposed 3' end of the nicked strand serves as a primer for a DNA polymerase. DNA is synthesized continuously along the intact circular strand, which acts as the template.
3. **Displacement**: As the new DNA strand is synthesized, the original, nicked strand is peeled away and displaced, often coated with single-stranded DNA-binding proteins.

4. **Lagging strand synthesis**: The displaced, single-stranded DNA acts as a template for the lagging strand, which is replicated in fragments (Okazaki fragments) by a DNA polymerase.
5. **Termination**: The process can result in multiple copies of the original circular DNA. The 5' end of the displaced strand is eventually rejoined by ligase to form a new circular molecule.

Theta model of DNA replication



Theta model of DNA replication

The theta model is a mechanism for **circular DNA replication** where two replication forks move in opposite directions from an origin of replication, creating a **theta-shaped structure** in the intermediate stage. This process begins at the origin of replication, proceeds through initiation, elongation (bidirectional synthesis), and termination, and is common in prokaryotes like bacteria, as well as in organelles like mitochondria and chloroplasts**.**

Initiation: Replication starts at a specific sequence called the origin of replication (ori). Initiator proteins bind to the ori and unwind the DNA helix, which recruits other proteins to form a replication complex.

Elongation: DNA polymerase enzymes begin synthesizing new DNA strands in both directions from the origin.

Bidirectional replication: Two replication forks are created and move away from the origin in opposite directions, moving around the circular chromosome.

Theta structure: As the two forks move, the structure of the replicating DNA resembles the Greek letter theta (θ), hence the name.

Continuous and discontinuous synthesis: Just like in linear replication, the leading strand is synthesized continuously, while the lagging strand is synthesized discontinuously in short segments called Okazaki fragments.

Termination: The two replication forks continue until they meet at a termination site on the opposite side of the chromosome. At this point, the two new circular DNA molecules separate, completing the replication process

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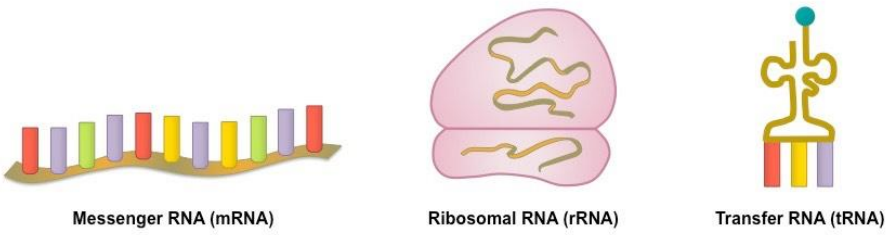
Types of RNA (Model)



Types of RNA (Model)

The main types of RNA models are **messenger RNA (mRNA)**, **transfer RNA (tRNA)**, and **ribosomal RNA (rRNA)**, which are all involved in protein synthesis. Other RNA types exist, including noncoding RNAs like **microRNA (miRNA)**, **small interfering RNA (siRNA)**, and **small nuclear RNA (snRNA)**.

RNA types involved in protein synthesis

- **Messenger RNA (mRNA):** Carries the genetic code copied from DNA to the ribosome, where it serves as a template for protein synthesis.
- **Transfer RNA (tRNA):** Brings the correct amino acid to the ribosome based on the mRNA sequence.
- **Ribosomal RNA (rRNA):** Forms the structural and catalytic core of ribosomes, the sites where proteins are made.

Other types of RNA

- **Noncoding RNAs (ncRNAs):** A large group that does not code for proteins but performs other functions.
  - **Regulatory ncRNAs:** Control gene expression. Examples include:
  - **Long non-coding RNA (lncRNA):** Longer RNA molecules with regulatory roles.
  - **Small ncRNAs:** Shorter molecules, further divided into several categories:

- **microRNA (miRNA):** Involved in gene regulation.
- **Small interfering RNA (siRNA):** Also involved in gene silencing.
- **Small nuclear RNA (snRNA):** Found in the nucleus and involved in RNA splicing.
- **PIWI-interacting RNA (piRNA):** Protects germline cells from transposable elements.

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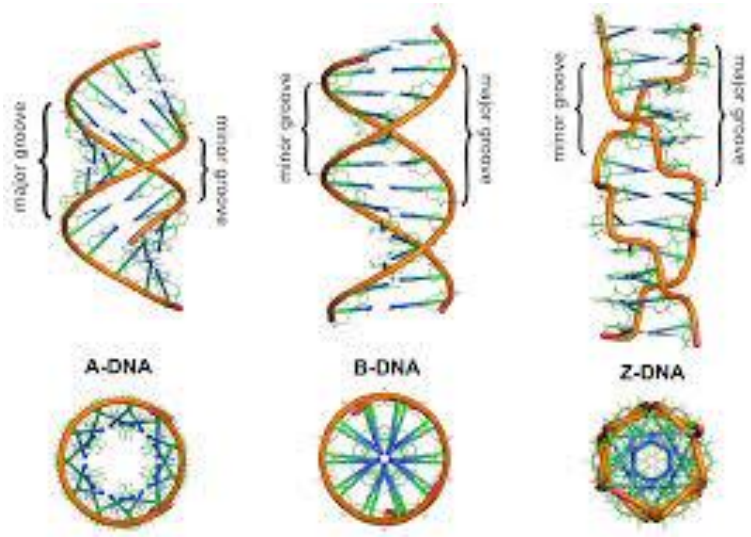

Different forms of DNA

The main types of DNA are A-DNA, B-DNA, and Z-DNA, which are all double helix structures with different helical properties and are stable under different environmental conditions. B-DNA is the most common form, with a right-handed helix, while A-DNA is also right-handed and forms under low hydration conditions, and Z-DNA is a left-handed, zigzag-shaped helix that can form under high salt concentrations or during transcription.

A-DNA

- **Structure:** A right-handed double helix.
- **Characteristics:** Wider and shorter than B-DNA, with 11 base pairs per turn.
- **Formation:** Typically forms under low-hydration conditions.

B-DNA

- **Structure:** The most common and stable form, a right-handed double helix.
- **Characteristics:** Has 10.5 base pairs per turn.
- **Formation:** The standard form found under normal physiological conditions.

Z-DNA

- **Structure:** A left-handed double helix with a zigzag pattern.
- **Characteristics:** Narrower than B-DNA, with 12 base pairs per turn.
- **Formation:** Can form in regions with alternating purine-pyrimidine sequences, especially in high-salt conditions or during processes like transcription.

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REFERENCES

1. Glick, B. R., Pasternak, J. J. (2022). Principles and applications of recombinant DNA. ASM, Washington DC, 683. ISBN-13: 9781683673644 (Print), 9781683673668 (eTextbook)
2. Howe, C. (2007). Gene cloning and manipulation. Cambridge University Press. Pages: 278, ISBN-13: 9780521521055
3. Keller, E. F. (2000). The century of the gene. Harvard University Press.
4. Nicholl, D. S. (2023). An introduction to genetic engineering. Cambridge University Press.
5. Green, M. R., Sambrook, J. (2021). Cloning and transformation with plasmid vectors. Cold Spring Harbor Protocols, 2021(11), pdb-top101170.
6. Dubey, R. C. (2022). A textbook of Biotechnology. S. Chand Publishing.
7. Das, H. K. (2007). Textbook of biotechnology. John Wiley & Sons.
8. Bhatia, S. C. (2005). Textbook of Biotechnology. Atlantic Publishers

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ABOUT THE DEPARTMENT

Department of Biotechnology and Genetics in the M S Ramaiah College of Arts, Science and Commerce, was established in the year 2000 offering both UG and PG programs. The main objective of the program is to provide conducive learning environment for the students and to mitigate the shortage of biotechnologists in the field of food, agriculture, medicine and environmental management. Highly qualified and experienced faculty members deliver the lectures and conduct the practical in various subjects as per the curriculum developed by the Bangalore City University. The department focuses mainly on teaching the basics, applications and hands-on training in a state-of-the-art classroom and laboratory environment. It also facilitates students to broaden their knowledge for multitasking.

ABOUT THE MANUAL

Molecular Biology laboratory manual outlines the discovery and understanding of biological processes, experimental procedures, safety rules, report writing formats, and detailed protocols for key experiments in these fields. The manual serves as a comprehensive guide to the students to learn the experimental techniques, principles of analysis, data recording, and scientific communication in Molecular Biology. Molecular Biology techniques are the prerequisite to analyse nucleic acids for several applications, genetic testing, diagnosis of infectious diseases and development of new drugs. By learning the concepts in the Molecular Biology practical class of Molecular Biology, students are nurtured with laboratory skills and preparedness for the advanced higher studies. This manual is authored by Dr. Radha Dayanidhi, Assistant Professor, Department of Biotechnology and Genetics, MSRCASC, Bengaluru.

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