Sri Satya Sai College for Women

Session-2021-22

CCE-1

Class: B.Sc 2nd year

Subject: MicroBiology

Submitted to: Dr. Shikha Nandlal

Submitted by:

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Date:

C.C.E-2

Questions:

Ques 1) Describe Messelson and Stahl Experiment.

Messelson and Stahl conducted an experiment to demonstrate the semi-conservative nature of DNA replication. They used two cultures of E. coli, one growing in medium with N^{14} isotope while other growing in medium with N^{15} isotopes. They were allowed to grow separately for several generations. When these were subjected to density gradient centrifugation the cells growing on N^{14} medium contained both the strand of N^{14} while the cells of N^{15} medium contained both the strands of N^{15}, which settled down during centrifugation due to higher density.

In the second experiment, the N^{15} culture was transferred to N^{14} media. The cells were to density gradient centrifugation after on

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generation. It was found that the daughter cells produced bands of DNA which was located at intermediate position between N^{14} and N^{15} which lead to the conclusion that the resultant daughter DNA contained two strands one of N^{14} and the other is of N^{15} which act as an evidence for the semi-conservative nature of DNA replication.

The aim of Meselson and Stahl's experiment was to prove the mode of DNA replication is semi-conservative.

Matthew Meselson and Franklin Stahl in 1958 performed experiments on E. coli to prove that DNA replication is semi-conservative.

They grew E.coli in a medium containing ^{15}N (in which ^{15}N is the heavy isotope of Nitrogen) for many generations.

As a result, ^{15}N got incorporated into newly synthesised DNA.

This heavy DNA can be differentiated from normal DNA by centrifugation in (CsCl) density gradient

DNA composition

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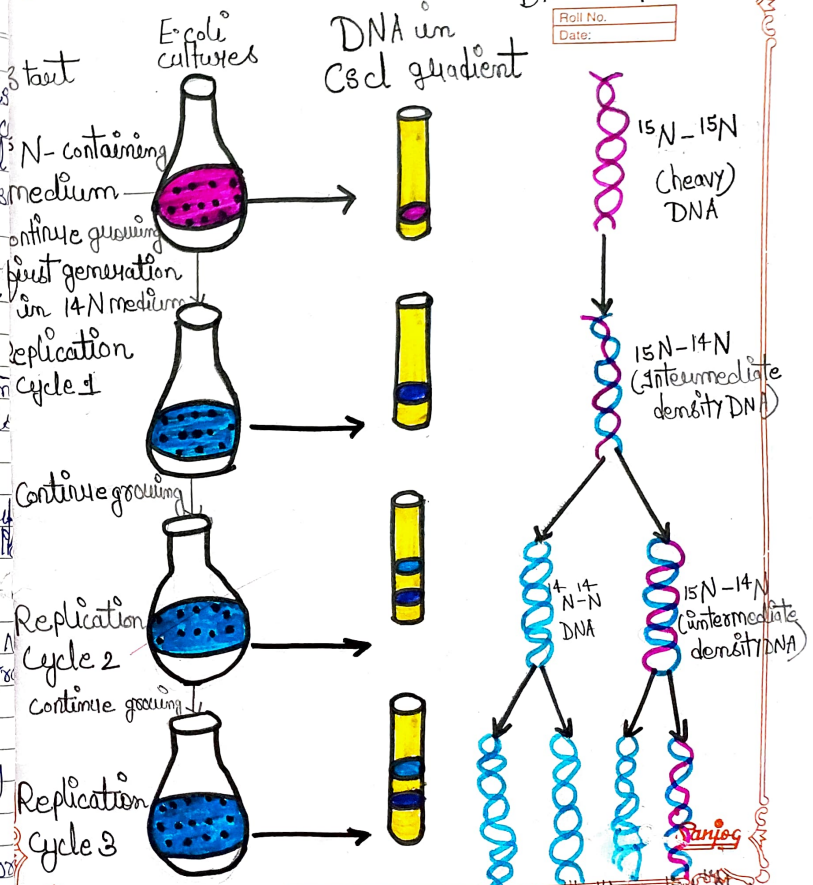

Meselson-Stahl Experiment

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Then they transferred the cells into a medium with normal $^{14}NH_4Cl$ and took the samples at various definite intervals as the cells multiplied.

The extracted DNAs were centrifuged and measured to get their densities.

The DNA extracted from the culture after one generation of transfer from the ^{15}N medium to ^{14}N medium (i.e, after 20 minutes). E. coli divides every 20 minutes) showed an intermediate hybrid density.

The DNA extracted from culture after two generations (i.e, after 40 minutes) showed equal amounts of light DNA and hybrid DNA.

Observation:

Because E. coli divides every 20 minutes, the DNA extracted after 20 minutes in the experiment had a hybrid density. DNA extracted after 40 minutes had equal amounts of hybrid and light densities.

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Conclusion:

This implies that the newly synthesised DNA c one of its strands from the parent.

Messelson and Stahl reasoned that these experiments showed that DNA replication was semi-conservative: the DNA strands se and each makes a copy of itself, so that e daughter molecule comprises one "old" a one "new" strand. Thus, replication was semi-conservative.

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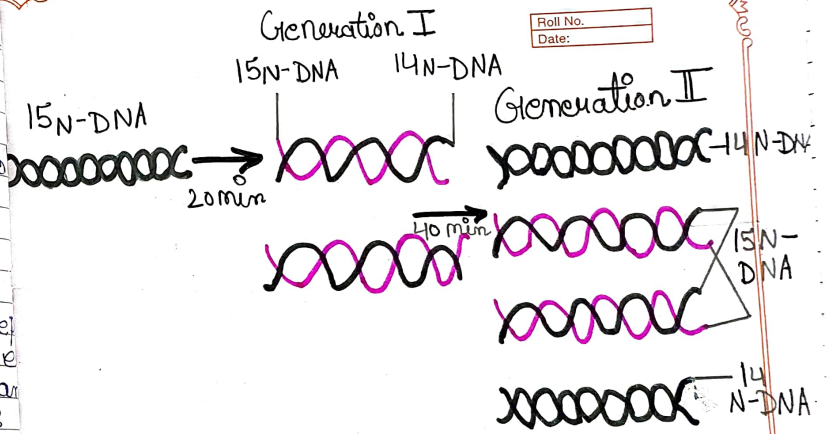

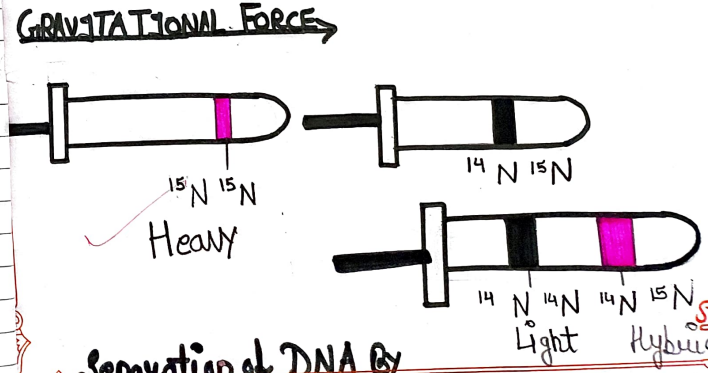

Separation of DNA By CENTRIFUGATION METHOD

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Write short notes on:

(a) DNA topology:-

DNA is essentially an extremely long- double-stranded rope in which the two strands are wound about one another. As a result topological properties of the genetic material including DNA underwinding and overwinding, knotting, and tangling, profoundly influence virtually every major nucleic acid process.

During this catalytic event, topoisomerases maintain genomic stability by forming covalent phosphotyrosyl bonds b/w active site residues and the newly generated DNA termini.

Topoisomerases are essential for cell survival. However, because they cleave the genetic material these enzymes also have the potential to fragment the genome.

The normal biological functioning of DNA occurs only if it is in the proper topological state. In such basic topological processes as RNA transcription and DNA replication, the recognition of a base sequence requires the

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local separation of complementary polynucleoti strands. The negative supercoiling of natura occurring DNAs results in a torsional that promotes such separations since it ten unwinds the duplex helix (an increase in T be accompanied by a decrease in W). If DNA lacks the proper superhelical tensio the above vital processes (which themselv supercoil DNA; sections occur quite slo if at all.

There are two classes of topoisomerases:

1. Type I topoisomerases act by creating transien single strand breaks in DNA. Type I en are further classified into type I A and type II B topoisomerases on the basis o their amino acid sequences and reaction mechanisms.

2. Type II topoisomerases act by making transie double strand breaks in DNA. A large number of type II topoisomerases genes f both prokaryotic and eukaryotic cornes sou have been identified and sequenced.

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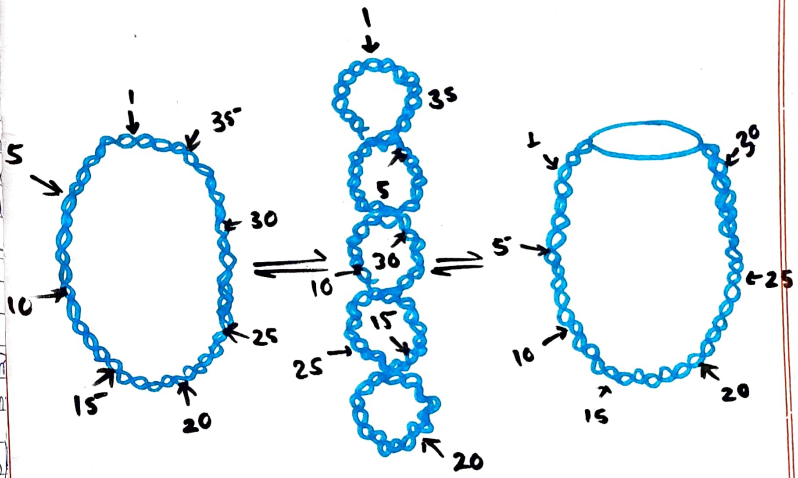

DNA TOPOLOGY

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DNA topology is the focus of an interdiscipline between molecular biology and mathematics and as a term refers to DNA supercoiling, knotting and catenation. It studies the shape and path of the DNA helix in three dimensional space. It refers to the relationship between the two strands of the double helix and includes the concept of supercoiling.

It should be an integral component of biochemistry and molecular biology curricula for a no. of reasons, including:

- Topology affects virtually every nucleic acid process that requires the double helix to be opened or moved within the cell.
- Topoisomerases, the enzymes that regulate the topological structure of DNA, play critical roles in fundamental cellular processes including replication, transcription, recombination, and mitosis.
- They are the targets for some of the most important anticancer and antibacterial drugs currently in clinical use.

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b) Super coiling of DNA.

DNA supercoiling is a special property of double-stranded DNA that is topological. It confers new structural and energetic It refers to the over- or under - winding of a DNA strand, and is an expression of the strain on that strand. Supercoi important in a number of biological proc such as compacting DNA, and by regulating to the genetic code, DNA supercoiling affects DNA metabolism and possibly gene ex

DNA supercoiling describes a higher-order structure. The double-helical structure of entails the interwinding of two complementa strands around one another and aroun a common helical axis. When the ends of linear DNA molecule are ligated to pro a covalently closed circle, the two become intertwined like the links of chain, and will remain so unless one the strands is broken. The no. of one strand is linked with the other described by a fundamental property DNA supercoiling, the linking no (Lk).

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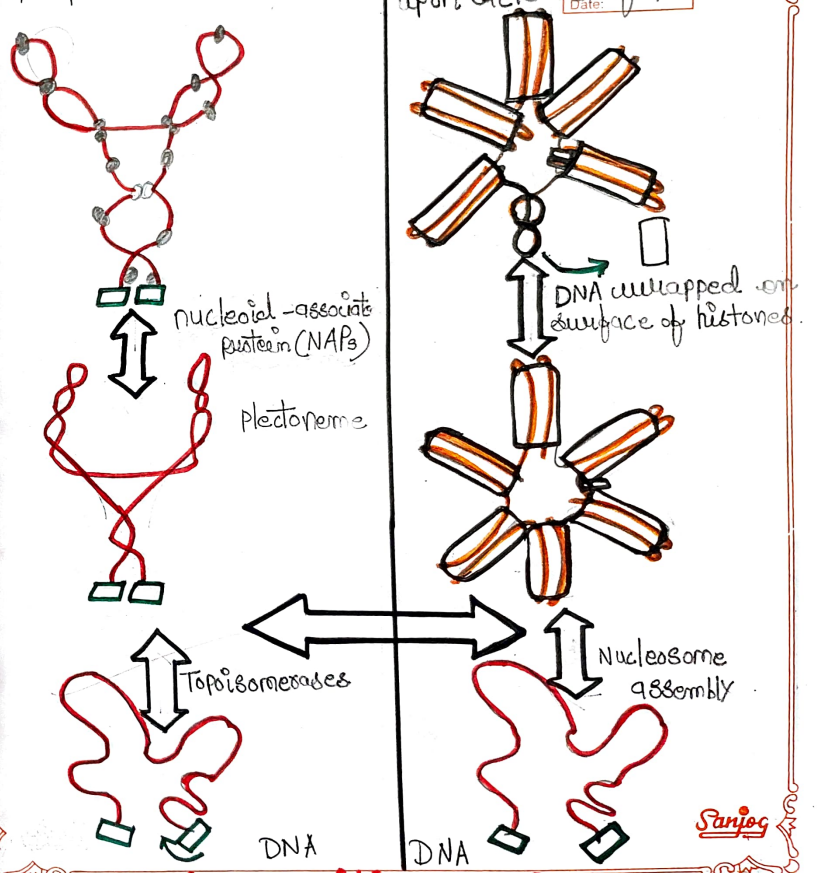
50% supercoils constrained by NAPs

Possible plectonemic struct upon release of histone octam



Supercoiling of DNA

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This is related to two geometrical properties of the molecule, the twist (Tw, rotation of the strands about the helical axis) and the writhe (Ws, which measures the path of the helix axis in space). These three properties are related by:

$$Lk = Tw + Ws$$

A relaxed, closed circular DNA molecule has a linking number (Lk°) given by:

$$Lk^\circ = N/h.$$

where N is the no. of base pairs, and h is the helical repeat under the experimental conditions. If a linear DNA molecule with an exact no. of turns under the prevailing conditions were ligated into a planar circle in the absence of torsional force, it would have a linking number that equaled the no. of turns in the original linear molecule.

A negatively supercoiled molecule has a linking deficit (Lk) relative to the relaxed species, i.e.,

$$\Delta Lk = Lk - Lk^\circ < 0.$$

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It is often convient to express the level of supercoiling in the form of a density effectively independent of the size of molecule considered.

Superhelix density (σ) is given by:

$$\sigma = \Delta Lk / Lk^{\circ}.$$

Many natural bacterial DNA species are in and negatively supercoiled. A plasmid ex from E. coli in mid-exponential growth typically supercoiled to the extent of although inside the cell, the unconstrain supercoiling in general takes about half value. By contrast, the DNA of some thermophiles is positively supercoiled.

In the absence of strand breakage Lk is and therefore the sum of twist and changes is constant in any structura change that maintains strand integrity. Thus, the linking deficit is partitioned b geometric alternatives in the molecule: torsional and flexural character:

$$\Delta Lk = \Delta Tw + \Delta Wr$$

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These changes in the shape and geometry of the supercoiled molecule lead to different physical properties, such as sedimentation and frictional properties.

The writhing of the helical axis in space defines the DNA Superhelical structure (DNA tertiary structure). For a circular DNA or a linear DNA with its ends anchored to create a loop, there is a tight topological coupling b/w the DNA superhelical structure and the double-helical structure (DNA 2nd structure). Hence, DNA superhelicity can influence the DNA winding/unwinding, thereby affecting the biological functions of DNA. In nature, there exists a ubiquitous class of enzymes, DNA topoisomerases which can mediate the topological transformation in DNA molecules.

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C) Linking number.

Linking number is a topological property of DN
Linking number is a sum of twists and wri
The numbers of time one strand of DNA
around another strand is called a twist
while inter-coiling of the double helix is
termed as writhe. In short, writhe
no. of a time DNA double helix is crossed
coiled over each other or the no. of time
strand wrap around another strand.

The linking number, Lk, is the defining pro
of DNA elementary topological domains.
Lk is a measure of the total number
complete revolutions that either strand
makes about the other. As long as the
strands remain intact, Lk is a conserved qua
Two otherwise identical closed duplex DNAs
that differ only in Lk are termed topoison
Even though they contain exactly the same
nucleotide sequence and covalent connecti
the property of the members of a family
topoisomers depend strongly on Lk. The lin
no. of DNA can be described formally in
several different ways, all of which ma
readily generalized to any type closed

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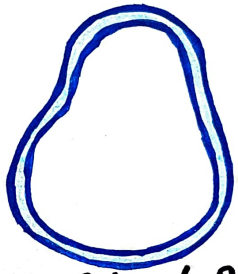

Relaxed DNA
Lk = 200

ΔLk = -2

ΔLk = +2

Negative Supercoils
Lk = 198

Positive Supercoils
Lk = 202

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Linking Number

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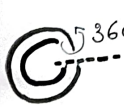

Twist = -1, Writhe = 0.

Twist = 0, Writhe = -1.

Twist = -2, Writhe = 0.

Twist = 0, Writhe = -2.

Twist = +1, Writhe = 0

Twist = 0, Writhe = +1.

Twist = 0, Writhe = -4.

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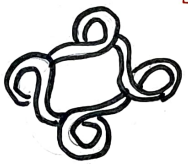
Plectonemic



Toroidal

Twist = 0
Writhe =

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in space.

The linking number has several simple and highly useful attributes.

(1) Lk is an integer for superhelical DNA - (but not necessarily for protein - sealed DNA loops) - this follows from the requirements that both strands be closed curves;

(2) In general, Lk is constant as long as the topological domain may be broken by a single- or double-stranded DNA chain scission or, if appropriate, by the disruption of links to the protein sealing the domain;

(3) the linking no. is a topological quantity, and its value is independent of DNA geometry - that is, Lk does not vary with deformation of the trajectory of either strand nor with changes in the characteristic duplex geometric quantities (pitch, roll, twist, tilt, propeller twist, etc);

(4) The linking no. is independent of the ordering of the two curves. Thus, for two DNA strands C_1 and C_2, $Lk(C_1, C_2) = Lk(C_2, C_1)$ -

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(d) Melting curve of DNA and Tm value deter

Melting curve analysis is an assessm
the dissociation characteristics of
stranded DNA during heating. The
at which 50% of DNA is denatur
is known as the melting temperature
As the temperature is raised, the do
begins to disassociate leading to a
the absorbance intensity, hyperchromici

This further can be used to infer the
and identity of single-nucleotide
(SNP). This is because G-C base pa
3 hydrogen bonds between them while A-
pairs have only 2 DNA with a higher
G-C content whether because of
(G-C contents: E. coli 0.50 M, Lutetis 0.
& (AT) 0.00) or, as previously mentione
of SNPs, will have a higher melting
than DNA with a higher A-T content.

The information also gives vital clues to
molecule's mode of interaction with
Molecules such as intercalators sli
base pairs and interact through pi-
This has a stabilizing effect on DNAs

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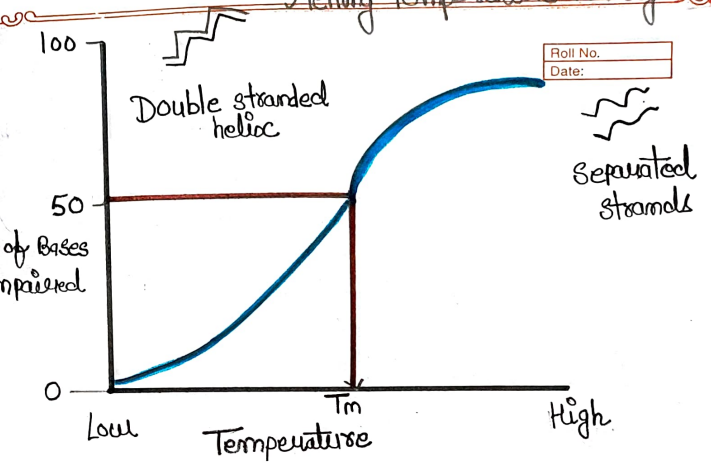

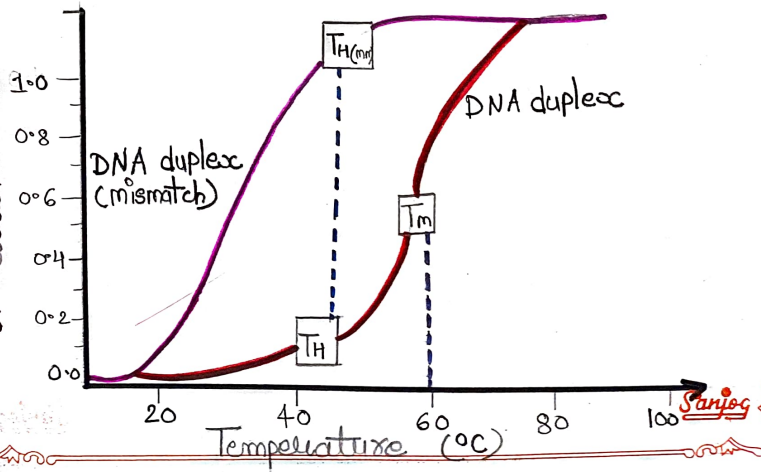

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which leads to a raise in its melting temperature. Likewise, increasing salt concentrations helps diffuse negative repulsions between the phosphates in the DNA's backbone. This also leads to a rise in the DNA's melting temperature.

Conversely, pH can have a negative effect on DNAs stability which may lead to a lowering of its melting temperature. It monitors the melting temperature of a PCR product amplified from bisulfate-treated DNA in real time using a thermocycler coupled to a fluorometer.

Applications of Melting curve of DNA

(i) Researchers often use melt curve analysis to assess whether their intercalating dye PCR/qPCR assays have produced single, specific products. It is used as a diagnostic tool for assessing qPCR amplicon length with intercalating dye qPCR assays.

(ii) DNA melt-curve analysis- applying temperature to melt and characterize the resulting curve profiles of double-stranded DNA samples has proven useful for scanning for sequence variations, primarily to confirm the specificity of primers by ensuring no primer-dimers.

are present in quantitative PCR assays.

(iii) Therefore, an understanding of melting temper (Tm) provides information on when and the DNA or RNA strands are going hybridize and defines the rules for hybridiz tion. It is very important to unders this process so that you can better de and optimize the oligos for experiments

(iv) Many research and clinical examples exis the literature that show the use of me curve analysis to obviate or complement Sequencing efforts, and thus reduce

(v) It is less expensive and simpler in design to develop probeless melting curve, as

(vi) The probe based technique is sensitive, en to detect single-nucleotide polymorphisms and can distinguish between homozygous wildtype, heterozygous and homozygous alleles by virtue of the dissassociation p produced.

The melting temperature (Tm) by definition is the temperatur at which one half of the oligo (primer) duplex will dissociate to become single stranded and indicates the duplex stability.

For multi-peak DMCs, Tm value determined in this way is the closest to the thermodynamic melting temperature.

Basic Melting Temperature (Tm) calculations:

For sequences less than 14 nucleotides the formula is $Tm = (wA + xT) * 2 + (yG + zC) * 4$. where w, x, y, z are the number of the bases, A, T, G, C in the sequence, respectively.

For sequences longer than 13 nucleotides, the equation used is $Tm = 64.9 + 41 * (yG + zC - 16.4) / (wA + xT + yG + zC)$.

Low Tm will mean lower hybridization temperature needed.
Low specify results.
Low Tm will mean lower annealing temperatures needed.
Low specificity and lower efficiency in strand generation.

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e) Buoyant Density of DNA and its relations mole (G+C) content in DNA.

Definition: A measure of the tendency of a substan float in some other substance, in which molecules are distinguished by their differ buoyant densities in some standard flu Buoyant density of majority of DNA is which is equal to density of 6M CsCl

Buoyant Density Centrifugation

One of the most common methods of fractio DNA is to exploit differences in density different types of DNA in concentrated solutions. Caesium chloride is the most comm used. The method involves prolonged high Centrifugation of purified DNA in concent CsCl. The centrifugation produces a g of salt concentration in the centri tube and the DNA migrates to occupy a p in the gradient which corresponds to own buoyant density.

A no. of metals factors affect the buoyant d such as the nature of the caesium sa the presence of heavy metals or DNA-b

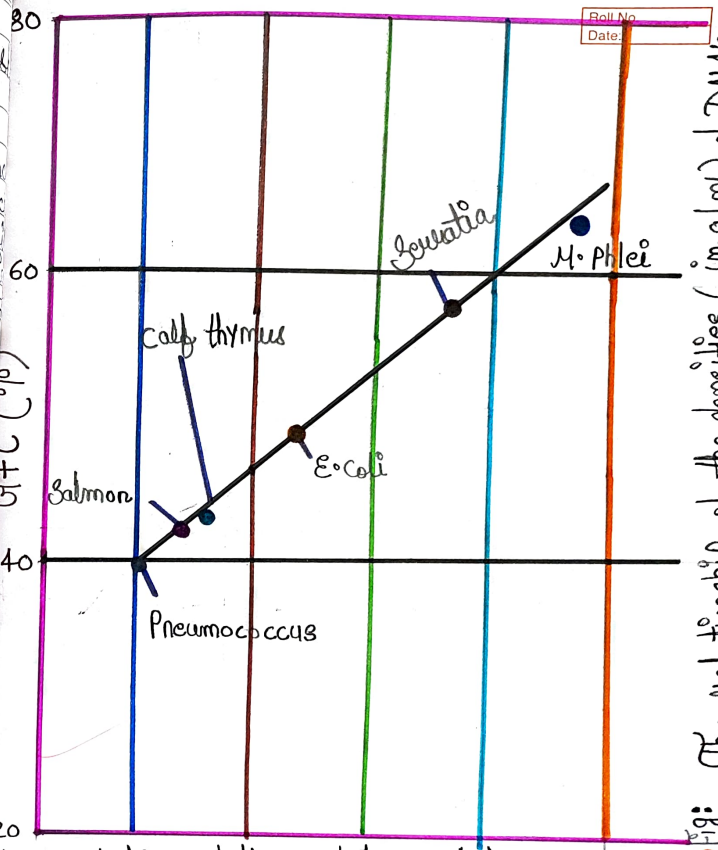

Fig: The relationship of the densities (in g/mL) of DNAs from various sources and their (G+C) content

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dyes, the pH and the temperature. Under constant conditions (usually 25°C in CsCl at neutral pH) the buoyant density of DNA is related to the GC content:

DNAs with different base compositions can therefore be separated by this method. Most nuclear DNAs from higher plants have buoyant densities within the range 1·69–1·71 g cm^{-3}. However, the presence of 5-methyl-cytosine serves to reduce the density slightly thereby giving rise to an under estimate of the GC content. In general, 1% methylation decreases the buoyant density by 1 mg cm^{-3}. Certain sequences of bases may also distort the relationship between base composition and buoyant density. Furthermore, single-stranded DNA is denser than double-stranded DNA of similar base composition by approximately 0·015 g cm^{-3} and under alkaline conditions the density is increased by 0·06 g cm^{-3}. This is due partly to the fact that DNA becomes single-stranded under these conditions and also partly because the deprotonated adenine and thymine residues are neutralized by binding caesium ions.

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Method:

The most common methods for determination plus cytosine (G+C) content of DNA (%G+C, mole percent) are buoyant density centrifugat thermal denaturation, and high - performance liquid chromatography (HPLC) of DNA. Densit gradient centrifugation is a common meth separating macromolecules, particularly nu acids, in solution. A cell extract with a solution of CsCl to a final densit about 1.7 g/cm³ and centrifuged at high speed (40,000 rpm, giving relative c forces of about 200000 g). The biological molecules in the extract will move to eq positions in the CsCl gradient that reflect their buoyant densities. A number of fact affect the buoyant density, such as

- the nature of the cacsium salt,
- the presence of heavy metals or DNA-bi dyes,
- the pH and
- the temperature.

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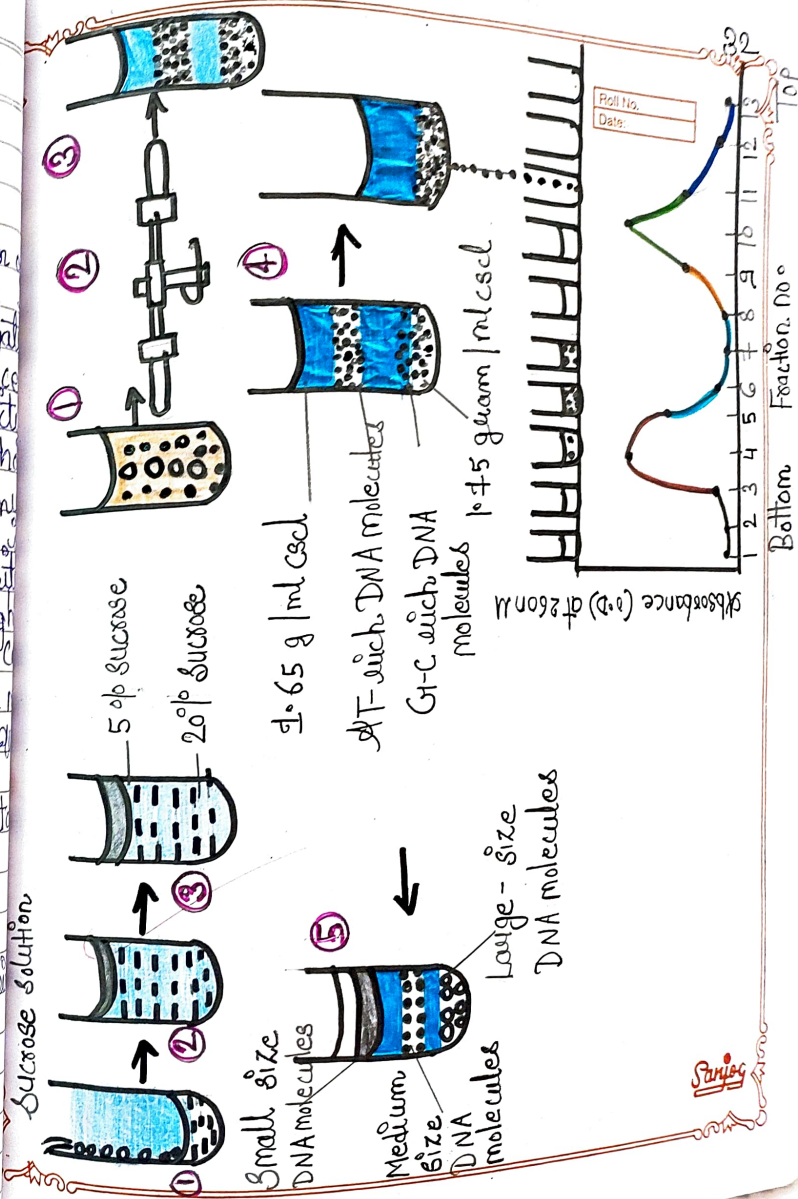

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Density of DNA is dependent on relative G:C content. G:C rich DNA has a significantly higher density than A:T rich DNA.
Furthermore, a linear relationship exists b/w the buoyant densities of DNA from different sources and their G:C content.
For every 10% increase in GC content, the density rises by 0.12 units.

On the basis of their buoyant density the differentiation between the small size, medium and large DNA molecule can be done via this method, more than that the variation in the DNA composition that is AT and GC content can be found out with this method, as AT rich DNA would be having DNA density band near the 1.65 gram/ml CsCl and GC rich DNA would be having density band near the 1.75 g/ml CsCl.

Under constant conditions (usually 25°C in CsCl at neutral pH) the buoyant density of DNA is related to the GC content:

$$\%\ G+C\ content = \frac{\text{buoyant density } (g cm^{-3}) - 1.66}{0.098} \times 100$$

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The enzymes involved in DNA replication are:

(i) DNA polymerase.

- DNA polymerases are enzymes used for the d of DNA by adding nucleotide one b are to the growing DNA chain. The enzy incorporates complementary amino acids to template strand.
- DNA polymerase is found in both prokaryot and Eukaryotic cells. They both contain different DNA polymerases responsible different functions in DNA replication and DNA repair mechanisms.

(ii) DNA Helicase enzyme

- This is the enzyme that is involved in unw the double-helical structure of DNA allow DNA replication to commence.
- It uses energy that is released during AT hydrolysis, to break the H-bond betw the DNA bases and separate the strands
- It forms two replication forks on each

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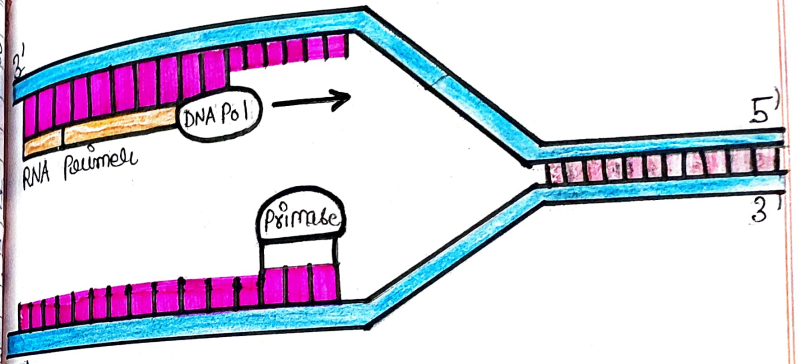

DNA Primase Enzyme

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separated strand opening up in opposite directions.

- At each replication fork, the parental DNA strand must unwind exposing short sections of single-stranded templates.
- The helicase enzyme accurately unwinds the strands while maintaining the topography on the DNA molecule.

(iii) DNA primase Enzyme –

- This is a type of RNA polymerase enzyme that is used to synthesize or generate RNA primers, which are short RNA molecules that act as templates for the initiation of DNA replication.

(iv) DNA ligase enzyme –

- DNA ligase is a specific type of enzyme, a ligase, that facilitates the joining of DNA strands together by catalyzing the formation of a phosphodiester bond.
- DNA ligase is used in both DNA repair and DNA replication.
- The mechanism of DNA ligase is to form this covalent phosphodiester bonds b/w 3' hydroxyl ends

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of one nucleotide ("acceptor") with the 5' phospho end of another ("donor").

- ATP is required for the ligase reaction, which p in three steps.

(v) Exonuclease

- These are a group of enzymes that remove nucleotid bases from the end of a DNA chain.

(vi) Topoisomerase

- This is the enzyme that solves the problem of the topol stress caused during unwinding.

They one or both strands of the DNA allowing the strand to move around each other to relieve tension before it rejoins the ends.

And therefore, the enzymes catalyste the reversible breakage it causes by joining the broken strandes

Topoisomerase is also known as DNA gyrase in E. coli.

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