Nucleic acid

Nucleic acids are polymers of nucleiotides which function as genetic material. The fundamental unit of nucleic acid is nucleotides.

There are two types of Nucleic acid - DNA and RNA.

Nucleic acid is made up of Nucleioside and nucleiotide.

~~Nucleiside + N~~

Nucleiside is made up of

Pentose sugar and Nitrogenous base.

Pentose sugar + N. base ⟶ Nucleioside.

Example: (i) Deoxyribose + Adenine = Deoxyadenine

(ii) Deoxy ~~guanine~~ ribose + Guanine = Deoxyguanine.

(iii) Deoxyribose + Thymine = Deoxythymine.

(iv) Deoxy ~~cytosine~~ ribose + Cytosine = Deoxycytosine.

Structure of Dioxyribose and Ribose:

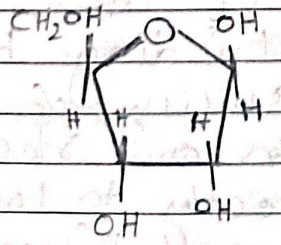


Fig: Ribose

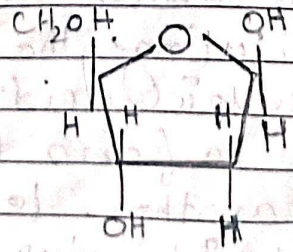


Fig: Deoxyribose.

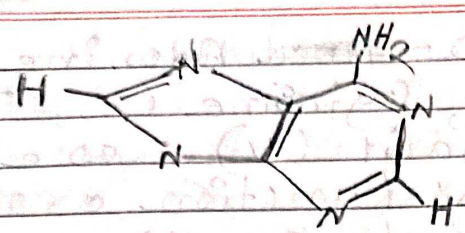
Nucleosides are glycosylamines that can be thought of as nucleotides without a phosphate group. A nucleoside consist simply of a nucleobase (also termed as a nitrogenous base) and a five-carbon sugar whereas a nucleotide is composed of a nucleobase, a five-carbon sugar and one or more phosphate groups. In a nucleoside, the anomeric carbon is linked through a glycosidic bond to the N9 of a purine or the N1 of a pyrimidine. Example of nucleosides include cytidine, Uridine, adenosine, guanosine, thymidine and inosine.

While a nucleoside is nucleobase linked to a sugar, a nucleotide is composed of a nucleoside and one or more phosphate groups. Thus neucleioside can be phosphorylated by specific kinase in the cell on the sugar's primary alcohol group ($-CH_2-OH$) to produce nucleotides.

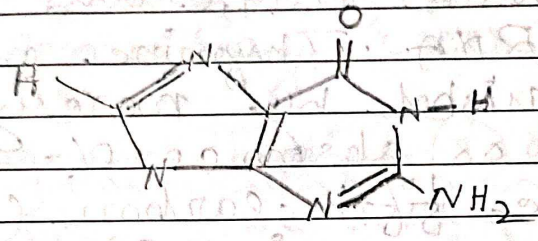
~~Nucleio~~

Neulcobase:—

They are also known as nitrogenous bases or often simply bases, are nitrogen containing biological compounds that form nucleosides, which in turn are components of nucleotides with all of these monomers constituting the basic building blocks of nucleic acid. The ability of nucleobases to form base pairs and stack one upon another leads directly to long chain helical structure such as ribonucleic acid (RNA) and DNA.

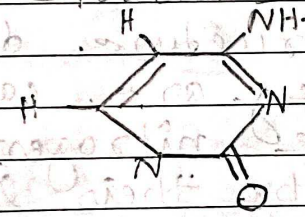


Adenine

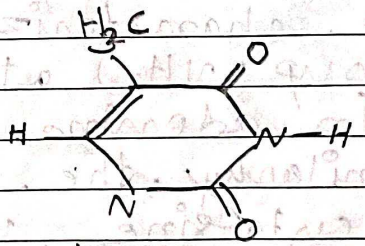


Guanine.

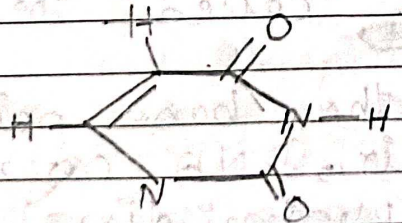
Fig:- Structures of purines.



Cytosine.



Thymine. (DNA only)



Uracil. (RNA only)

Fig: Structure of pyrimidines.

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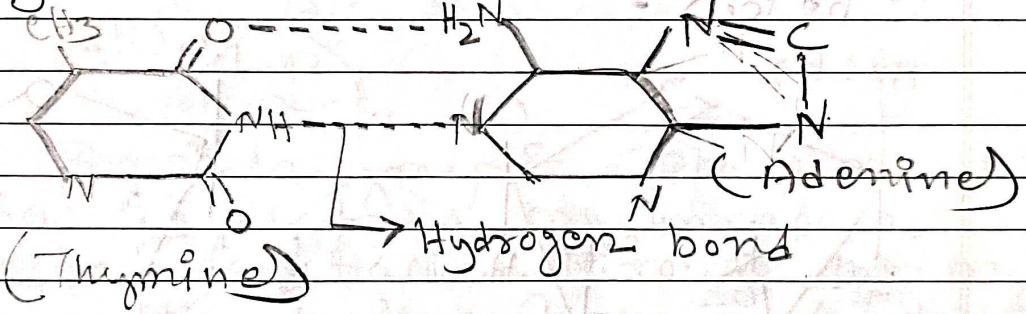
→ Five nucleobases - ~~Five~~ Adenine (A), Guanine (G), Cytosine (C), Thymine (T) and Uracil (U) - are called primary. They function as the fundamental units of genetic code, with the bases A, G, C and T being found in DNA while A, G, C and U are found in RNA. Thymine and Uracil are distinguished by merely the presence or absence of a methyl group on the fifth carbon (C5) of these heterocyclic six-membered rings.

Adenine and guanine have a fused-ring skeletal structure derived of purine, hence they are called purine bases. The purine nitrogenous bases are characterized by their ring amino group (NH_2) at the (C6) carbon site in adenine and C_2 in guanine. Similarly, the simple ring structure of cytosine, uracil and thymine is derived of ~~pyr~~ pyrimidine, so those three bases are called the pyrimidine bases.

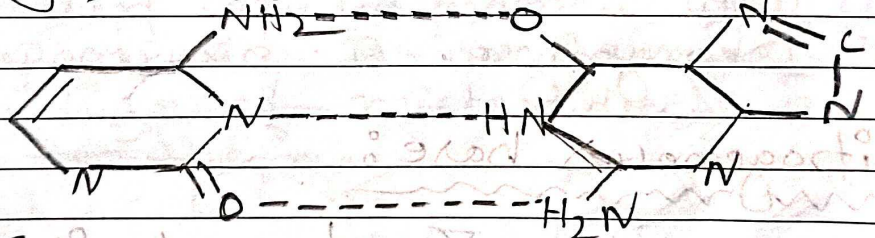
Each of the base pairs in a typical double-helix, DNA comprises a purine and pyrimidine: either an A paired with a T or a C paired with a G. These purin-pyrimidines pairs which are called base compliments connect the two strands of the helix and are often compared to the rungs of a ladder. The pairing of purines and pyrimidines may result

in part from dimensional constraints, as this combination enables a geometry of constant width for the DNA spiral helix. The A-T and G-C pairings functions to form double or triple hydrogen bonds between the amine and carbonyl groups on the complementary bases.

Bonding of Thymine (T) and Adenine (A) (A=T):



Bonding of cytosine (C) and Guanine (G) (C≡G):



Cytosine. Guanine

Structure of nucleotide:-

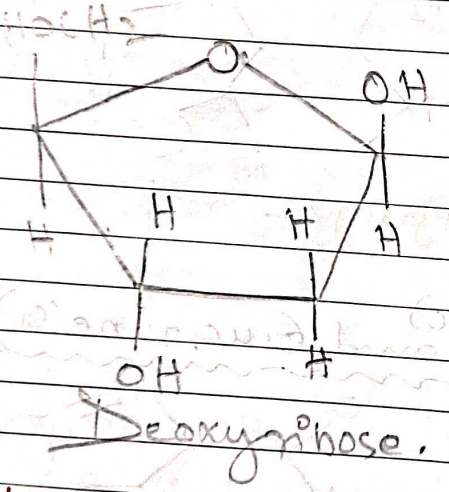
Nucleotides are monomers or building blocks of nucleic acids which are composed of three structural units;

(1) Pentose sugar, (2) Nitrogenous base

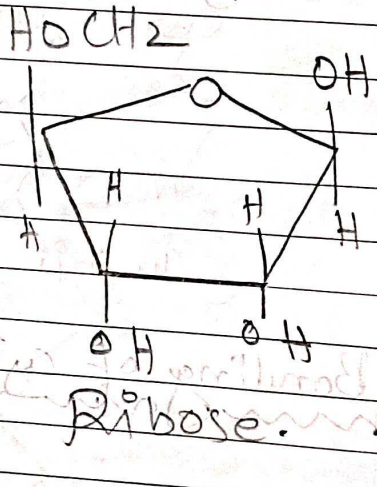
(3) phosphate group.

Pentose Sugar:

Two types of pentose sugars are present in nucleic acids. They are ribose and deoxyribose. If the pentose is ribose, the nucleic acid is called ribonucleic acid (RNA) and if the pentose sugar is deoxyribose, the nucleic acid is called deoxyribonucleic acid (DNA). Structure of these two sugar are given below.



Deoxyribose.



Ribose.

Nitrogenous base:

Two types of nitrogenous bases are found in nucleic acids: Purine and pyrimidine. Adenine, Guanine are purine bases, whereas Cytosine and Thymine and Uracil are pyrimidine bases. Pentose sugar and nitrogenous base (without the phosphate group) are collectively known as nucleosides.

Nucleoside + phosphate group = Nucleotide.

Example:-

(i) Deoxyadenosine + PO_4 = Deoxyadenosine monophosphate. (AMP)

(ii) Deoxyguanosine + PO_4 = Deoxyguanosine monophosphate (GMP)

(iii) Deoxythymine + PO_4 = Deoxythymine monophosphate (TMP)

(iv) Deoxycytosine + PO_4 = Deoxycytosine monophosphate (CMP)

Phosphate group:

Phosphate group is a phosphorus atom surrounded by four oxygen atoms. Phosphate is attached through the oxygen atom of the hydroxyl group of the 5' carbon of the pentose sugar. Thus a nucleotide can be named as nucleoside monophosphate. As an example, the structure of nucleotide with a ~~g~~ purine base ~~is shown~~.

(The carbon residues in the pentose sugar are numbered 1' to 5'. The N-base is attached to the 1' position of the sugar and the phosphate is attached to the 5' position. When a polynucleotide chain is formed, the 5' phosphate of one nucleotide is attached to the 3' hydroxyl group of the end of the growing chain. The N-based are important components of nucleotides. They contain carbon and Nitrogen. They are bases because they contain an amino group which has the potentiality

to binding an extra hydrogen. And therefore decreases the H^+ conc^n making it more basic.

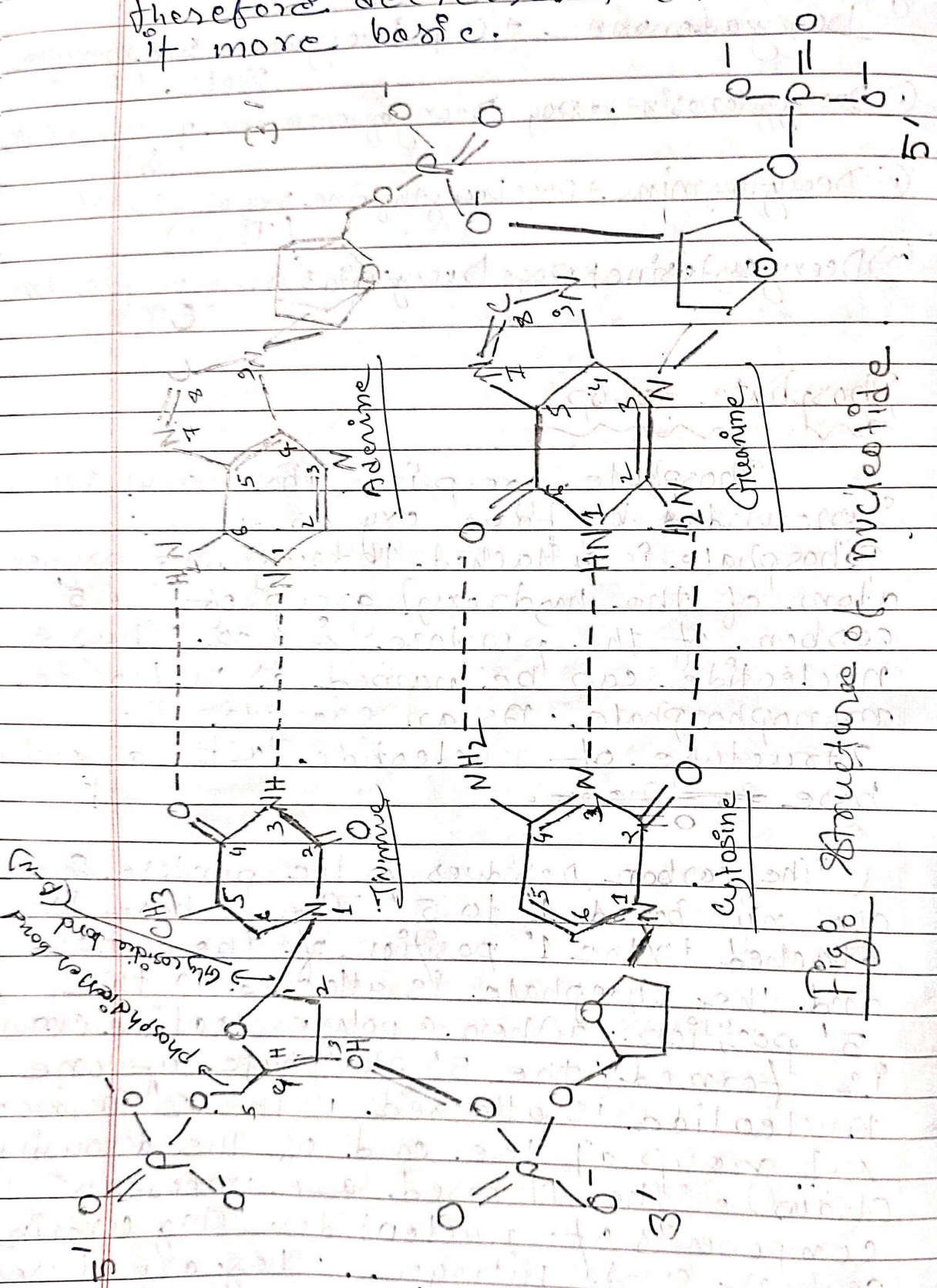


Fig 2 Structure of Nucleotide

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DNA denaturation :

① DNA denaturation is the process of breaking down the DNA molecule generally for the purpose ~~is called~~ of comparison of seqvence.

② When DNA is heated up in water, the energy of the heat pull the two strands of DNA a-part. This process is called denaturation. After denaturation the two strands still have the same nucleotide sequences, therefore they are still complimentary.

③ If the DNA is subjective to cool after the denaturation it reforming the double strands. This process is called DNA renaturation or annealing or by bridization.

④ Double helical native DNA are highly viscous at pH 7 and room temperature (25°C).

⑤ When the DNA solution is subjected to extream pH or temperature above 80°C, its viscosity significantly decreases, which indicates the DNA has undergone physical changes.

⑥ Distruption of the hydrogen bond between base pairs causes unwinding of the double helix to form two single strands, completely separate from each other along entire lenght of the molecule. However, no-covalent bonds in the DNA are broken during this process.

Factor affecting DNA denaturation:

DNA denaturation can be occur by three main method:—

(i) Temperature (ii) Salt (iii) pH.

Temperature affecting DNA denaturation:

When a DNA solution is heated up to approximately (≈) 90°C or above, there will be enough kinatic energy produced causing denaturation of the DNA completely forming two single strands.

The temperature at fluids 50% of double stranded DNA (dsDNA) is converted to single stranded DNA is called melting temperature. (Tm)

The higher Tm, the greater the G≡C content.

The DNA denaturation can be accelarated by using certain chemical reagents maintain urea and Formamide. The chemicall enhances the aqeas solubility of purines and pyrimidines. This separation of double helix DNA is called melting as it occurs abruptly at the certain characteristic temperature called Tm. Or denaturation temperature.

The melting of DNA can be followed ~~Spectro~~ spectrophotometrically by monitoring the absorbance of DNA at 260nm.

The melting temperature (Tm) is analogus to the melting point of DNA crystal. The Tm value depends on the nature of the DNA. When several samples of DNA are melted, it is founded the Tm is highest for those DNA which have G≡C content. Thus the value is used to estimate the percentage of G≡C in a DNA sample. The Tm depends on both the length of the DNA and the specific nucleotide sequence composition of that DNA molecule.

The G≡C pair is bounded by three hydrogen bonds, while A=T pairs is bounded by two hydrogen bonds.

DNA with high G≡C content is more stable than DNA with low G≡C content. Higher G≡C content indicates higher melting temperature.

Denaturation involves the following changes of DNA property:-

(a) Increases in absorption of UV light:- When denaturation is followed Spectrophotometrically by monitoring the absorbance of light at 260 nm. It is observed that the absorbance at 260nm increases when DNA become denature. This phenomenon is called Hyperchromatic effect or hyperchromicity. This increasing absorbance is due to the un-stacking of base pairs.

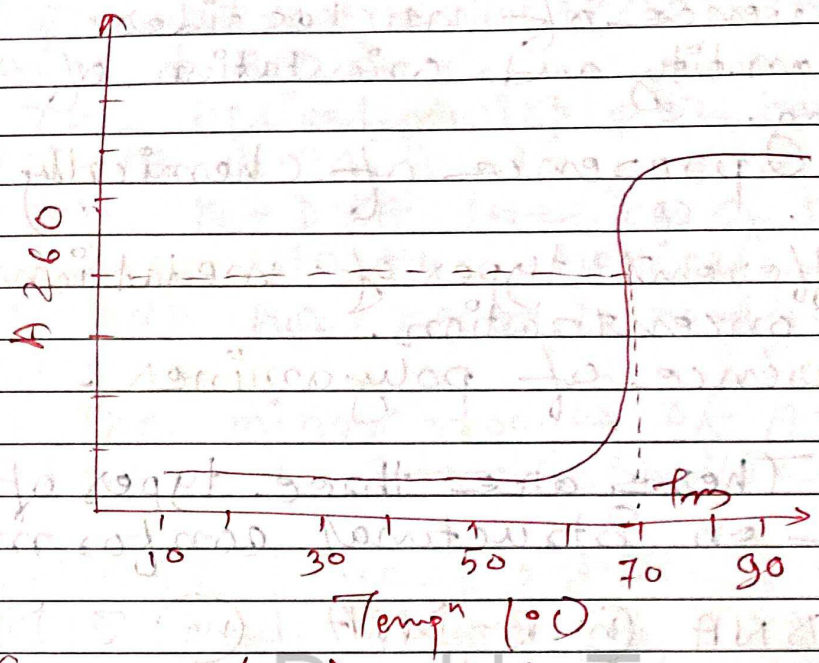
(ii) Decrease in Specific optical rotation:
Double stranded DNA (dsDNA) shows a strong positive rotation which highly decreases with denaturation. This changes is analogous to the change in rotation observed when the proteins are denatured.

(iii) Decrease in viscosity:
The solutions of native DNA exhibit high viscosity, because of the relatively rigid double helical, long and rod like structure of DNA molecule Denaturation causes significant decrease in viscosity.

Effect of ph on the denaturation of DNA:

Denaturation occurs at acidic and alkaline solution when ionic changes of purines and pyrimidins bases occur. In acidic solution of (pH = 2-3) the amino group binds with protons and the DNA double helix distrupted.

Similarly alkaline solutions (pH = 12) the enolic hydroxyl (OH) group ionize, thus preventing the keto-amino hydrogen bonding.



Types of DNA

DNA molecule can't be accomodated perfectly in the nucleus or cell as lines - conformation. Almost all the cells from simple bacteria to complex eukaryotes the DNA must be compacted by more than 1000 folds in oder to fit inside the cell or nucleus. Based on - X-Ray crystallography of short pices of synthetic DNA, it has shown that there is considerable variance of the helical structure of DNA, based on the sequence.

DNA can adopt different types of structural conformations. The various types of conformations that DNA can adopt depends on various factors. Such as -

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① Level of hydration.
② Salt concentration.
③ Sequence of nucleotides.
④ Quantity and orientation of super coiling.
⑤ presence of chemically modified bases.
⑥ Different types of metal ions and its concentration.
⑦ presence of polyamines.

There are three types of DNA based on Structural conformation:-

① A-DNA ② B-DNA ③ Z-DNA.

① A-DNA: It is a rare type of Structural conformations of DNA under dehydrating conditions. A-DNA is ds DNA (helical structure) with a shorter and more compact Structural organization compare to B-DNA.

Structural features of A-DNA:-

① A-DNA is formed from B-DNA under dehydrating condition.
② A-DNA is much wider than B-DNA.
③ It is a right handed helix. Therefore called right handed DNA.
④ The Diameter of A-DNA is 26 Å.
⑤ The pitch of the helix of A-DNA is 28.6 Å.
⑥ A-DNA is 20-25% shorter than B-DNA

(7) A-DNA containing 11-6 base pairs per turn.
(8) The distance betn adjacent base pairs is 2.9 Å.
(9) The helical twist per base pair in A-DNA is 31°
(10) In A-DNA the base pair are inclined to the helical axis.
(11) A-DNA has narrow and deep major grooves.
(12) The minor grooves of A-DNA are wide and ~~short~~ shallow.

B-DNA: The B-DNA is most common and abundant pre-dominant types of structural conformation of DNA in the cells. B-DNA was described by James Watson and Crick in 1953.

Structural features of B-DNA :-

(1) Majority of the DNA found in a cell is in B-DNA conformation.
(2) In B-DNA the N-base occupied at the core. Whereas the sugar-phosphate back bone occurs at the periferi of the helix.
(3) B-DNA is right handed helix.
(4) Each base pair in B-DNA has the same width.
(5) The helical diameter of B-DNA is 20 Å.
(6) Each turn in B-DNA consist of 10 base pairs (BP).
(7) The distance betn in adjacent base pair 3.4 Å.

(8) Each base pair in B-DNA have a helical twist of 36°.
(9) The plane of inter-strand hydrogen bonds are perpendicular to the helical axis.
(10) The major groove of B-DNA is wide and deep.
(11) Minor groove of B-DNA is narrow and deep
(12) The glycosidic bond conformation in B-DNA is anti form.

Structural features of Z-DNA:

(1) Z-DNA has a left handed double helical conformation where the double helix winds to the left in a zig-zag pattern.
(2) The DNA strand has a complimentary nucleotides with alternating purine and pyrimidines, thus forming Z-DNA conformation at high salt conc.
(3) The helical diameter of Z-DNA is 18 Å.
(9) The Z-DNA was discovered by Andres Wang and Alexander Rich. It is the one of the biologically active forms of DNA found in vivo.

Cot curve analysis:

It is a technique used to measuring the complexity of DNA or genome. This technique was discovered by Roy Britten and Eric Davidson in 1960. This technique is based on the principle of DNA renaturation

DNA Renaturation kinatics = The rate at which heat-denaturate DNA will re-naturates which depends on DNA concentration, cation conc^n and viscosity.

The principle of cot curve analysis:

The rate at which a particular sequence of a DNA re-associates or Reanilling is proportional to the number of times of the sequence found in the DNA or genome. Given enough time all the DNA that is denatured will re-associate in a given DNA sample.

The more the repetative sequences, the less will be the time taken for renaturation.

Procedure of cot curve analysis:

This technique involved denaturation of DNA by heating and allowed to renaturation by coiling. The re-naturation of DNA is assed by ~~Stor~~ Stereoscopically. The large DNA molecule takes longar time to re-naturation.

cot value:-

The Renaturation of DNA depends on the following factors:

(i) DNA concentration.
(ii) Renaturation temperature
(iii) Cation concentration.
(iv) Viscosity.

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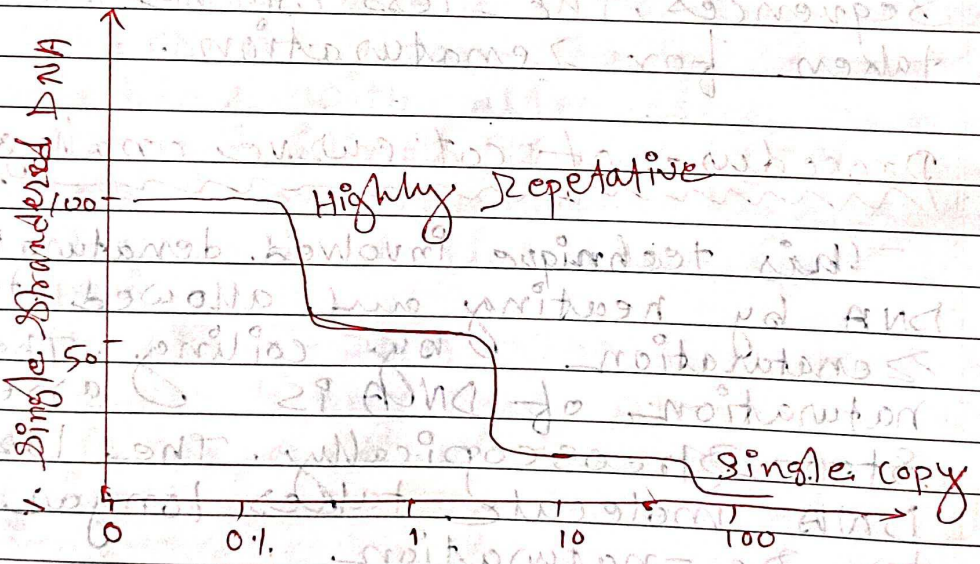
Cot = DNA conc^n (mole/L) × Renaturation time (in second) × Buffer factor (Account for the effect of cations on the speed of Renaturation)

C_0 = Conc^n of DNA before denaturation.
t = time taken for renaturation.

Low cot value indicate more number of repetative sequence.

High cot value indicate less number of repetative sequence or more number of unique sequence.



Example of cot value:

Nucleotide concentration (C_0) = 0.05m
Renaturation time (t) = 344 sec
Buffer factor (SPB) @ (0.5m) = 5.820
Cot value.
= Cot × t × buffer factor
= 0.05 × 344 × 5.820
= 100.00

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Application of Cot analysis:

(i) Determination of genome size and complexity.

(ii) Determination of complexity of sequences in DNA or genome.

(iii) Determination of the relative proportion of single copy and repetative sequence.

Types of RNA:-

There are different types of RNA describe below:-

(1) Coding RNA (messenger RNA - mRNA):

Messenger RNA: carries the genetic code from DNA in a form that can be recognized to make proteins.

(2) Non-coding RNA (ncRNA):

Ribosomal RNA is the catalytic component of the ribosomes. In the cytoplasm, rRNA and protein components combine to form a nucleoprotein complex called the ribosomes which binds mRNA and synthesizes protein.

(3) Small nuclear RNAs (snRNA): (Cont)

Small nuclear RNAs are always associated with a group of specific proteins to form the complexes referred to as "small nuclear ribonucleoproteins" (snRNA) in the nucleus.

Their primary function is to process the precursor mRNA.

④ Small nucleolar RNAs (SnoRNA; 60-300 nt)

Small nucleolar RNAs are components of small nucleolar ribonucleoproteins (SnoRNP), which are complexes that are responsible for sequence-specific nucleotide modification.

⑤ Piwi-interacting RNAs (piRNA; 24-30 nt):

Piwi-interacting RNAs bind the PIWI subfamily proteins that are involved in maintaining genome stability in germline cells.

⑥ MicroRNAs (miRNA; 21-22 nt):-

MicroRNA are small ncRNAs of 22 nucleotides (nt) and the most widly studied class of ncRNAs.

⑦ Long noncoding RNAs (lncRNA):

Long noncoding RNAs are a heterogeneous group of non-coading transcripts larger than 200 nt in size.