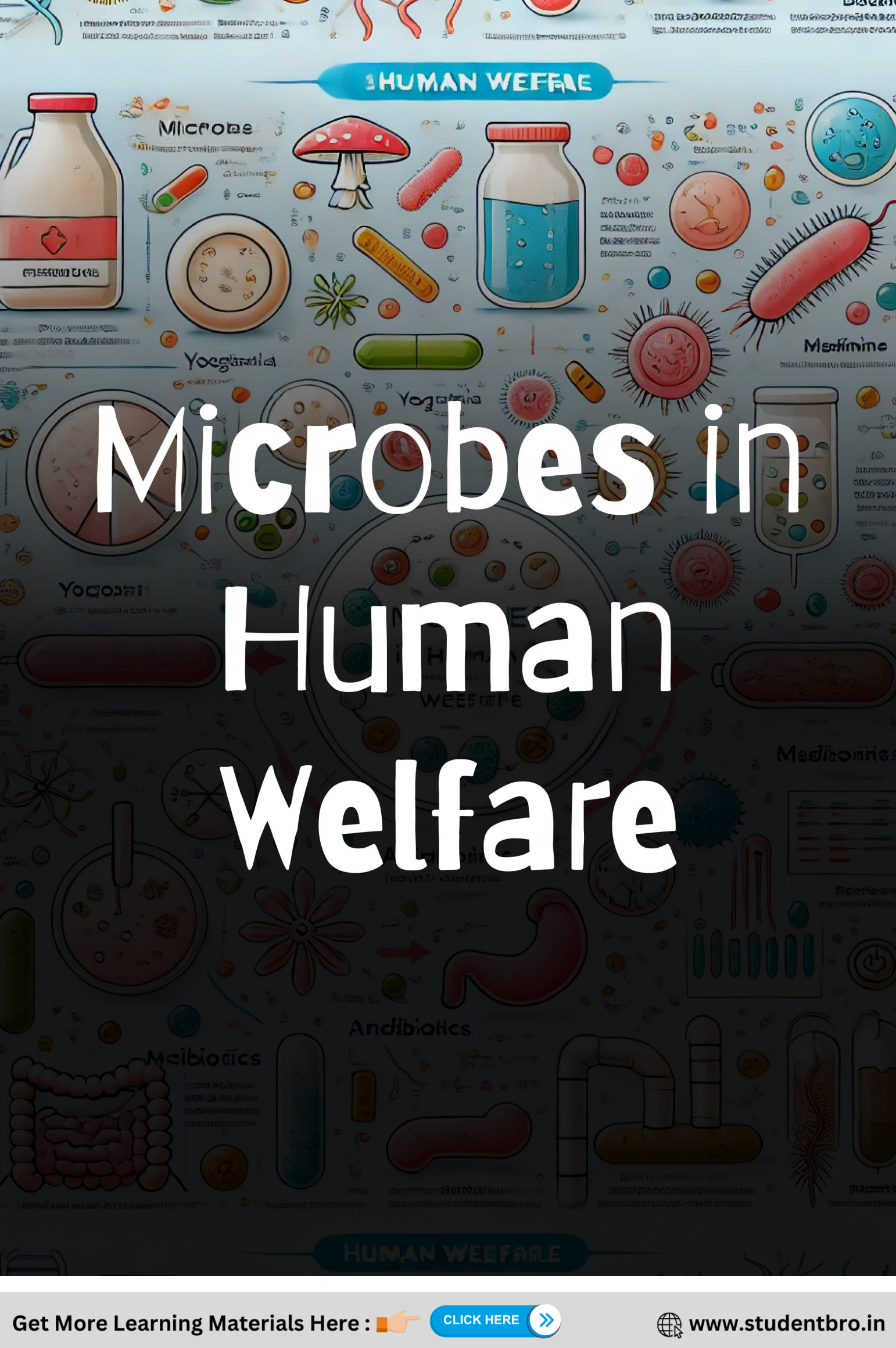


Microbes in Human Welfare

Microbes in human Welfare

Single Cell protein :-

These are high Quantity proteins obtained from uncellular or multicellular Organism.

Sources

- Blue Green Algae : Spiruline
- Bacteria : Methylophilus Methylotropus.
- Candida : candida utilis
- fungi : fuserium Graiemarium ?

Spiruline can be grown over waste water which is coming out from potato processing plant.

Spiruline is a good source of protein, fat, vitamin, carbohydrate, lipid etc.

A 250 kg cow can give 200g protein per day in the same time 250g, methylophilous methylotropus can give 25 tonnes of protein.

Shift from grain to meat diet will results into men consumption of grains beccuse to produce 1kg meat in forming Animal its will use 3 to 10 kg grains.

Biogas

- Biogas is produced from cow dung.
- Burner Efficiency of Biogas is higher in comparision to conventional methods of Burning cow dung.
- The production of Biogas is a three step process.

Step → 1 conversion of complex molecules into simple monomas.

- Lignin remains unaffected all other substances will be converted into monomers.

Step → 2 conversion of monomers into organic acid (Acetic acid).

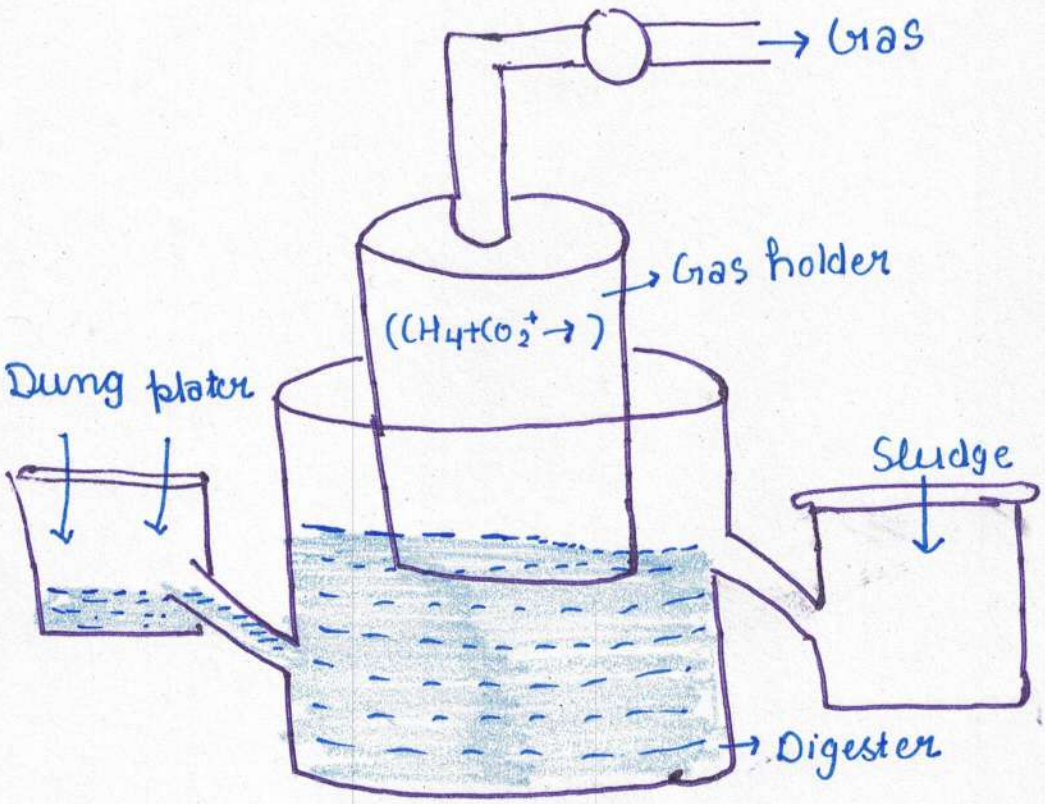
- facultative anaerobic bacteria perform this Action.

Step → 3 conversion of Acetic acid into methane & CO_2.

- **Composition of Biogas:** 60% to 70% methane, 30-40% CO_2, remaining amount (Hydrogen, Hydrogen Sulphide (rotten Egg Smell) Nitrogen.
- degradation of cellulose is very slow process so this step is rate limiting step in Biogas production.
- **Methanobacterium:** are main bacteria which are producing methane gas.

- Calorific Value of Biogas is 23 to 28 MJ/m3.

- The technique for biogas production was given by KVIC (Khadi village Industrial Commission) + IARI (Indian Agricultural Research Institute)



Microbes as Biofertilisers

- Blue-Green Algae, bacteria, fungi they are used as biofertilising Agents.
- fungi-fungi shows symbiotic Association.
- Genus Glomus is used as biofertilising Agents.

Benefits

- Assumption of phosphorus.
- Ectomycorrhiza and Endomycorrhiza are

part of root Ecosystem so it prevents development of root pathogens.

☞ Provide resistance to Abiotic stress (Saline Soil or drought)

☞ They help in overall growth & development of plant.

BLUE GREEN ALGAE

Eg:- Anabenae, Nostoc, Oscillatorion.

useful in paddy fields.

BACTERIA

Rhizobium:- It's a symbiotic bacteria.

fixing of Nitrogen in Organic form

(N2) Nitrogen fixing Zene.

Azatabactor & Azospirillion → They are free Living and perform N_2 fixation.

BIOACTIVE Molecule

cyclosporin :→ It's a Immuno suppressive Agent obtained from Trichoderma Polysporum (fungi).

→ used in organ transplantation.

★ Statin → Obtained from yeast (Monascus Perpevrus) to reduce blood cholestrol level.

★ Statins are given preferably during evening hours because cholestrol synthesis is Maximum at this time.

★ cortisol is preferably given during morning hours.

CURD (Lactic Acid Bacili)

- LAB is responsible for curd.
- It is rich in vitamin B_{12} (only source for Vegetarians)

YOGHURT (श्रीखंड)

- At 40°C streptococcus thermophilous and lactobacillus bulgoris are used.
- The specific taste of Yoghurt is due to presence of acetaldehyde and lactic acid.

CHEESE

- At 38°C streptococcus cremoris or streptococcus lactis are used.
- At 50°C lactobacillus lactis or lactobacillus bulgaris.
  or
  lactobacillus helveticus and streptococcus thermophilus.

Raw cheese (home made cheese)

- Lemon juice is mixed in hot milk.
- Whey: 92% H_2O, 5% lactose.

Swiss Cheese

- cheese with big holes.
- Propinobacterium Sharmanil is used

Blue Cheese

- Penicillium roquefortii is used.

Organic Acids

- Citric Acid → Aspergilus niger (fungus)
- Lactic Acid → Streptococcus lactis, Lactobacillus bulgaris.
- Butyric Acid → clostridium butylicum.
- Acetic Acid → Yeast → Alcoholic fermentation. (Aerobic Oxidation)

Alcohol

- Beer → Barley → 3-6%.
- Wine → Grapes → 10-20%.
- Rum → Molasses → 40%.
- Gin → Rye → 40%.
- Brandy → distillation of wine → 60-70%.

Petroplantation

* These plants secretes latix rasin which has hydrocarbo
  - Euphorbiacege family
  - Apocynacege family
  - Asclepidacege family

Example → Jatropa (plant)

MICROBES AS BIOCONTROL AGENT / BIOPESTICIDES

Example: Lady bird → for controlling Aphids

Dragonfly → Used for Controlling mosquitoes

Bacillus → Thuringiensis can be used → Spray contains Spray and Cry protein.

Baculoviruses → They belong to genus Nucleopoly - hedrovirus.

* They are useful for killing Arthopods and Insects.
* They are specific & Narrow Spectrum.
* They do not attack on non targeted insect.
* They are part of Integrated Pest Management.

Molecular Basis of Inheritance

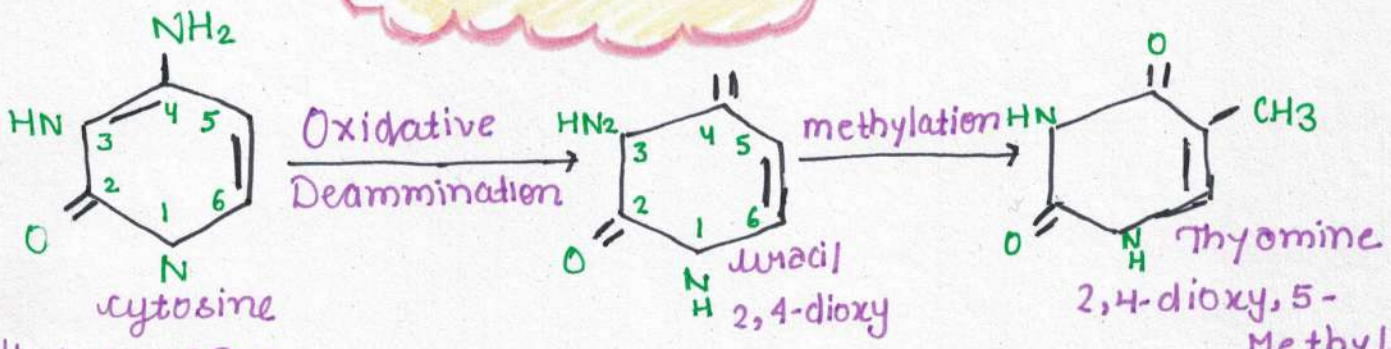
- DNA is a polymer of Nucleotide.
- Nucleotides are made up of Nucleoside + phosphate.
- Nucleotides are made up of Sugar and Nitrogen bases.

Nitrogen base are of 2 types

(i) Purine : Adenine, Guanine.

(ii) Pyrimidine : Cytosine, Uracil and Thiamine.

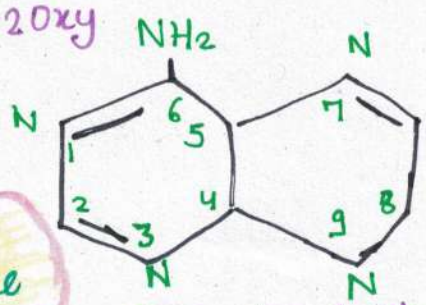
Pyrimidine



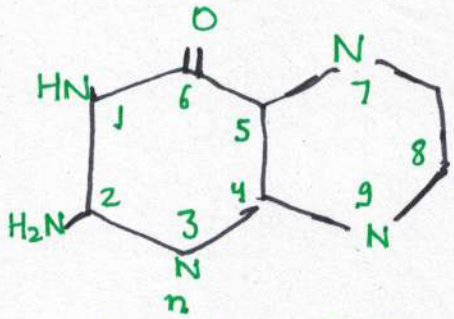
Cytosine
4-Amino 2Oxy

Uracil
2,4-dioxy

Purine



6-Amino purine
Adenine

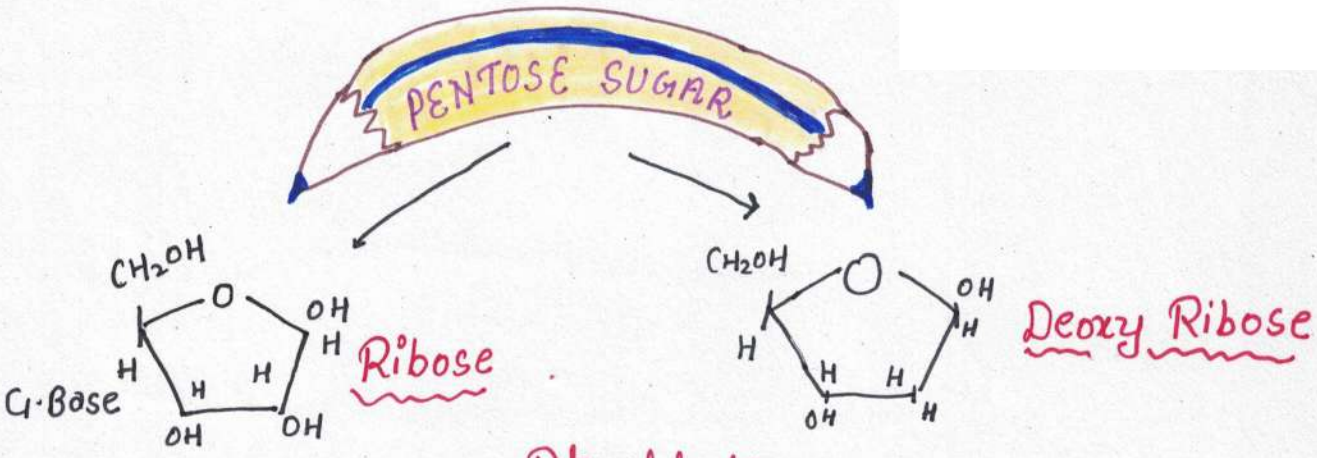


2-Amino-6-oxy purine
Guanine.

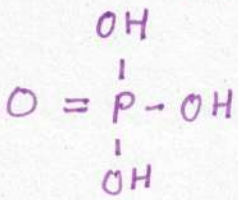
Pyrimidine ring : 4C+2N

Imidazole ring : 3C+2N

N = 1, 3, 7, 9



Phosphate



☞ Virus have four type of Nucleotide because they have Either DNA or RNA.

☞ In remaining organism all Eight type of Nucleotides are present.

☞ Nucleotides perform dual role :-

They Act as substrate.

They are Energy source during replication process.

NUCLEOSIDES

Nitrogen base + Sugar = Nucleoside.

- Adenine + Ribose = Adenosine
- Adenine + Deoxy Ribose = Deoxy adenosine
- Guanine + Ribose = Guanosine
- Guanine + Dioxy Ribose = Deoxy guanosine
- Cytosine + Ribose = cytidine.
- Cytosine + Deoxy Ribose = Deoxy cytidine.
- Uracil + Ribose = uridine.
- Thymine + Deoxy Ribose = Deoxy thymidine.

Nucleotides

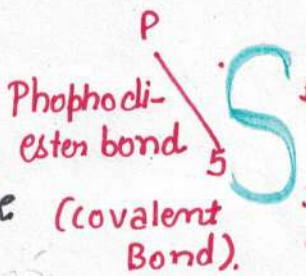
Nucleoside + Phosphate = Nucleotide.

- Adenasine + P = Adenylic acid (AMP)
- Deoxy Adenasine + P = Deoxy adenylic acid (dAMP)
- Guanosine + P = Guanylic Acid (GMP)
- Deoxy guanosine + P = Deoxy guanylic Acid (dGMP).
- cytidine + P = cytidylic Acid (CMP).
- Deoxy cytidine + P = Deoxy-cytidylic Acid (dCMP)
- uridine + P = Uridylic Acid (UMP).
- Deoxy thymidine + P = Deoxy thymidylic Acid (dTMP)

C_1 - Base

C_3 - OH

C_5 - phosphate

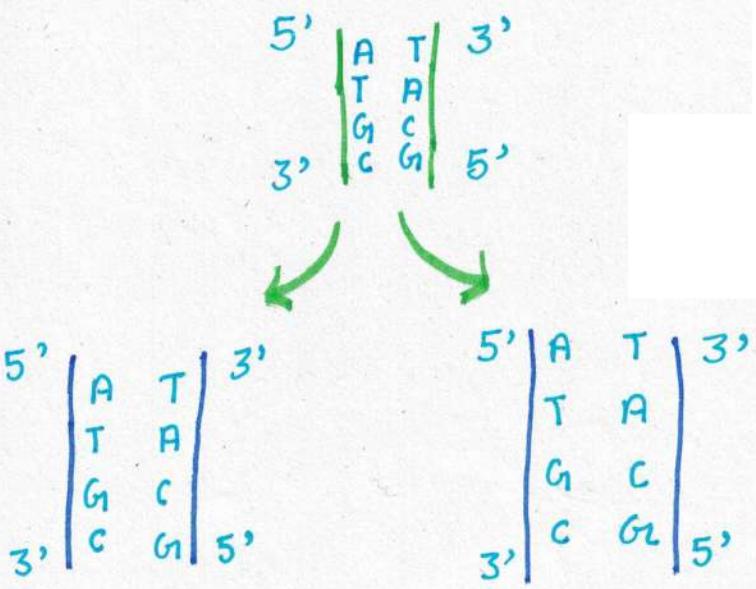


DNA

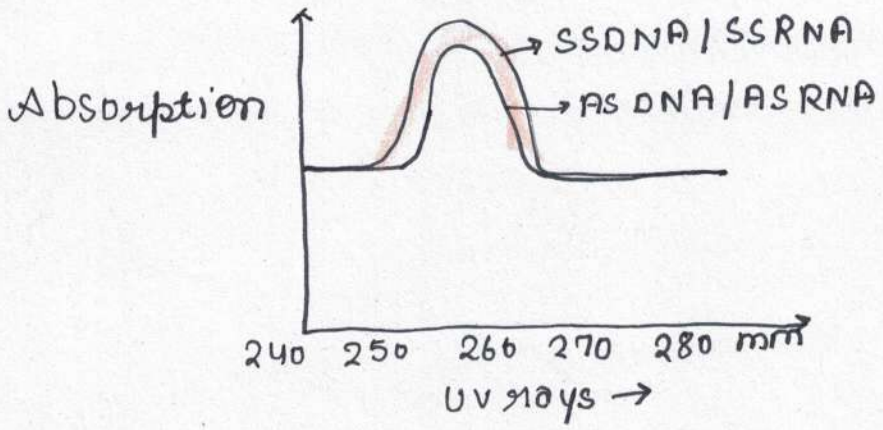
- Fredwrick Meischer discovered DNA from pus cells and called it nuclein.
- Altmen gave views about acidic nature of DNA.
- Zacharis called it DNA
- Wilkins and Franckin studied DNA structure with the help of x-Ray crystallography.
- Wateson and crick on the basis of @ x-Ray crystillography data.
  (b) chargaff's Rule gave double helix model of DNA.

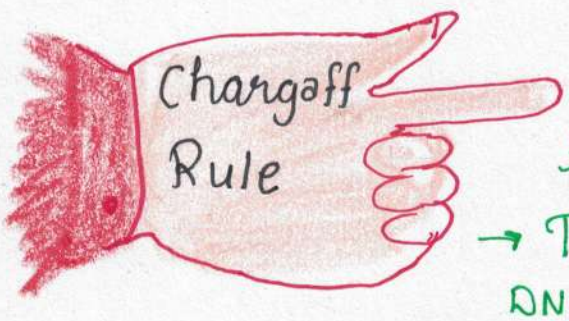
Sailent feature of DNA ....

- DNA is double Stranded.
- Both strands are complementry to Each other but they run in Anti-parallel direction.



- DNA has Acidic nature and negatively charged due to phosphate.
- charge on any fragment of DNA is same so their movement in Electric field / Electrophoresis depends on molecular weight.
- DNA has optical activity due to presence of Sugar.
- DNA can absorb UV rays due to presence of Nitrogen base.





Amount of purine is Equal to Amount of pyrimidin.

→ This rule was given by for ds DNA having complementary base sequence.

(i) $A = T$ $\quad G = C$

(ii) $A + G = T + C$ $\quad$ (3) $\frac{A+G}{T+C} = 1$

★ $\frac{A+G}{T+C} = 1 \rightarrow$ ds DNA ★ $\frac{A+G}{U+C} = 1 \rightarrow$ ds RNA

★ $\frac{A+G}{T+C} \neq 1 \rightarrow$ SS DNA ★ $\frac{A+G}{U+C} \neq 1 \rightarrow$ SS RNA.

In a double stranded DNA what will be the amount of C if A is 30%

Answer:-

A + T = 30 | G = C = ?

A + T ≠ 60 | G + C = 40

G = C = 20

If %age of Guanine is 15 in human DNA, what is the %age of T.

ANSWER:-

G = C = 15%

Total = 30%

T = A = 1/2 x 70

T = A = 35

Base Ratio

- It is specific for a species.
- It is calculated by $\frac{A+T}{G+C}$
- On the basis of base ratio, We can Identify the source of DNA.

EUKARYOTIC DNA	PROKARYOTIC DNA
$\frac{A+T}{G+C} > 1$	$\frac{A+T}{G+C} < 1$
AT type.	GC Type.
Low Tm.	High TM.
Eg:- Nucleous DNA	Eg:- Prokaryotic DNA and cytoplasmic Eukaryotic DNA.

NUCLEAR DNA

Inside the Nucleous

Replicate in S-phase

AT type DNA

Low temp.

Biparental Inheritance.

CYTOPLASMIC DNA

Mitochondria and plastid.

can replicate in any phase.

GC Type

High temp.

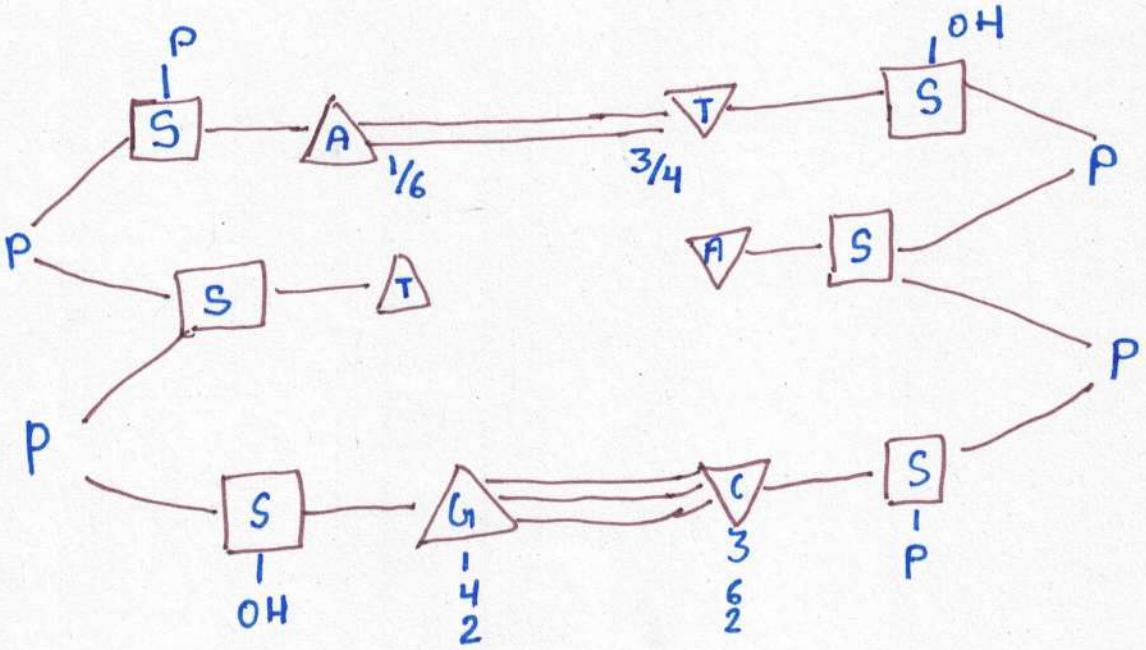
uniparental Inheritance.
(ममी बीमार....).

Human Base Ratio : 1.55

E-coli base ratio : 0.98-0.95.

Question Base ratio in one strand of DNA is .6, what is the base ratio of complementory strand?

Question In a ds DNA ratio of $\frac{A+G}{T+C}$ is .6 in complementary strand of the data DNA what will be the ratio of $\frac{A+G}{T+C}$.



P-P → 20 A°

S-S → 11.3 A°

B-B = 2.8 to 3.0 A°

Solution $\frac{A+G}{T+C} = 6/10 = .6 = x$

A	T
T	A
C	G
G	C
A	T
G	C
G	C
A	T
T	A
C	A
C	A
C	G
T	G
	A

$\rightarrow 10/6 = \frac{A+G}{T+C} = 1/x$

$1/0.6 = 1.66.$

- Due to Arrangement of sugar in both stand of DNA they are Anti parallel to Each other.
- Diameter of DNA is 20 A° and it remain same throughout because one purine is Arranged with one pyrimidine vice-versa.
- **No. of phosphodiester bond :-**

(i) SS DNA = (n-1) n = no. of Nucleotides.

(ii) ds DNA = (n-2) n = total Nucleotide in both stand.

(iii) In circular DNA = n

- No. of phosphodiester bond = 2 (at 5' end on both strand).

In a ds DNA total 700 H-bond are present, no. of GC base paird is 100. what is the total base pair Number.

Solution G·C = 100 ⇒ 300 H-Bond.

1 · G·C = 3

$400/_2$ = 200 AT 1 AT = 2 H-bond

400 H·bond = 200 AT.

% age of Adenine :-

100 GC → 100 G
→ 100 C

200 AT → 200 A
→ 200 T

$\frac{\text{Adenine}}{\text{Total}} \times 100 = \frac{200}{600} \times 100$

= 33.33 %.

Right Handed DNA :- Clockwise twisting.

DNA	Helix length	No. of base points	Distance between two pairs	Diameter.
'A'	28 A°	11 pairs	2.56 A°	23 A°
'B'	34 A°	10 pairs	3.4 A°	20 A°
'C'	31 A°	9.33 pairs	3.32 A°	19 A°
'D'	24.24 A°	8 points	3.03 A°	19 A°

LEFT HANDED DNA :- Anticlockwise twisting.

'Z'	45.6 A°	12 (6 dimers)	3.75 A°	18.4 A°

Packaging of DNA

- Total length of Human DNA in one cell is 22m.
- It is highly coiled inside the cell in prokaryote and Eucaryotes both.

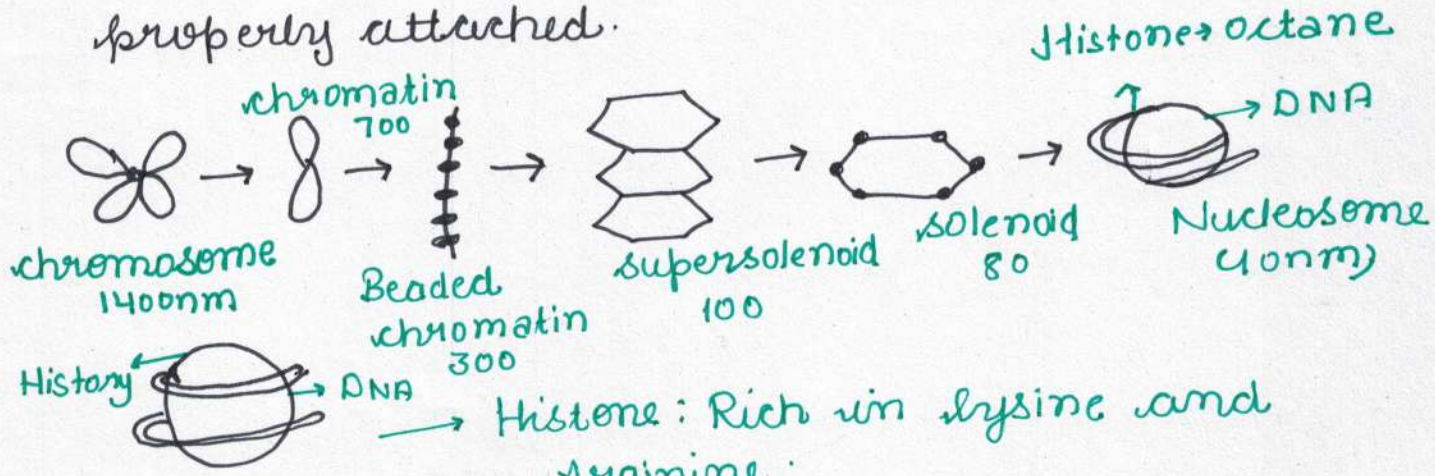
Prokaryote

- In prokaryote DNA is supercoiled and circular it is known as Nucleoid / Genophone.
- In prokaryotes polyamines perform the same function like histone protein in human.

Eukaryote

In Eukaryote nucleosome model of DNA packaging.

- Histone is a basic protein, In Nucleosome positive charge is outside, so DNA (Negatively charged) is properly attached.



→ Histone : Rich in lysine and Arginine.

Seating protein → H1, H2A, H2B, H3, H4 x2 · octamer

(2nm) → ≃ 60 bp - linker DNA.
→ ≃ 146 bp - take 1.75 turn
- 1 3/4 turn.

Non-histone chromosomal Protein
- Structural → Scaffolding protein.
- Functional → DNA P, RNA P.
- Regulatory → HMG Protein. (high-Mobility group) protein.

B-DNA

(i) clockwise twisting
(ii) Right handed.
(iii) Smallest unit: Mononucleotide.
(iv) One turn length = 34A°
(v) Diameter : 20 A°

Z-DNA

(i) Anticlockwise-twisting.
(ii) Left handed.
(iii) Smallest unit : Dinucleotide
(iv) One turn length 36.6 A°
(v) Diameter = 18.4 A°

Search for Genetic Material

- Mendal gaves detail about unit factor (controlling a character).
- Sutton and Boveli Established parallel behaviour between gene and chromosome.
- Morgon Established presence of gene over chromosome.

Character of a genetic Material.

- It must have replication ability.
- It must be stable.
- It must express genetic Information.
- It must resist mutation.

Initially, proteins were thought to be genetic Materia because DNA has four Nucleotide and proteins have twenty Amino Acids.

Griffith transformation Experiment

Griffith Effect :- When genetic material is transformed from one bacterian to another without any help of other organism and it result into chax in nature of organism. (R-II $\xrightarrow{\text{transformation}}$ S-II)

Biochemical nature of transforming substance was Explained by Avery, Mcleod, Mc carty.

Ex:- Steptococcus pneumoni/ Pneumococcus pneumoni/ Diplococcus pneumoni.

Smooth Strain (S)	Rough Strain (R).
★ S-III	★ R-II
★ Mucoid Substance	★ Non-Mucoid
★ Smooth colonies	★ Rough colonies.
★ Pathagenic	★ Non pathagenic.

Experiment

	S-III	R-II	Heat killed SIII (HKS-III)	HKS-III + R-II
Injected in:	Mice	Mice	Mice	Mice
Result:	death	Alive	Alive	death
Recovery:	S-III strain	R-II	HKS-III	S-III strain.

Biochemical Nature conformation :-

	+R-II	HKS-III		
Injected in:	Mice	Mice	Mice	Mice
+	+	+	+	+
Enzyme :	Protease	carbohydra-tase	RNase	DNase.
	↓	↓	↓	↓
	Death	Death	Death	Alive
	R-II → S-III		R-II ⟶ S-III	

Even after this Experiment many scientists were not convinced about DNA as genetic Material.

Hershy-Chase transduction Experiment

This Experiment gave unequivacal proof (undoubtedly) for DNA as genetic Material.

P^{32} and S^{35} were used.

Phosphorus = part of DNA

Sulphur = part of protein.

Bacteriophages can never take radioactive material directly from the medium because they never take Nutrition directly from the medium.

So, E.coli growth was prepared and these substances were introduced in them. Now, Bacteriophages can take these radioactive Material from E.Coli.

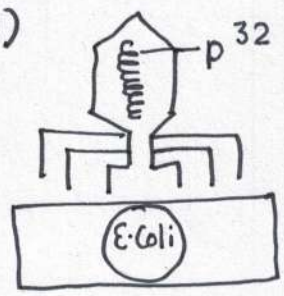
Transduction :-

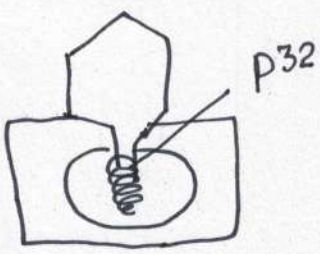
Transfer of genetic material from one bacteria to another with help of Viruses (Bacteriophage).

Step 1

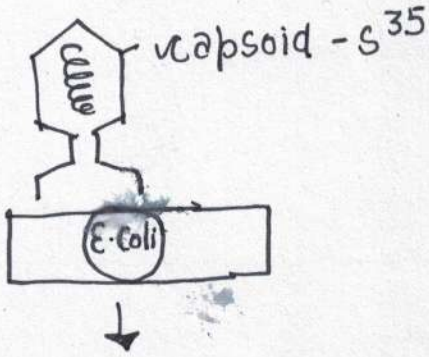
Infection: Association of Bacteriophage. (radio Active with E.Coli).

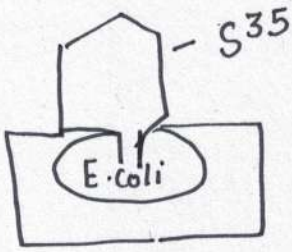
(A)





(B).

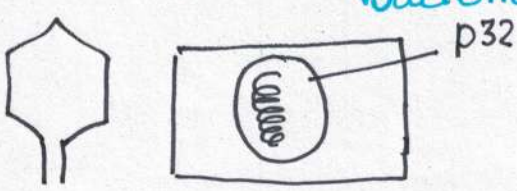


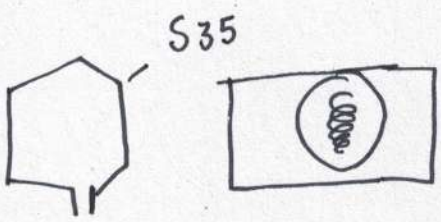


Step 2

Blending :- To dissociate bacteriophage from Bacteria.

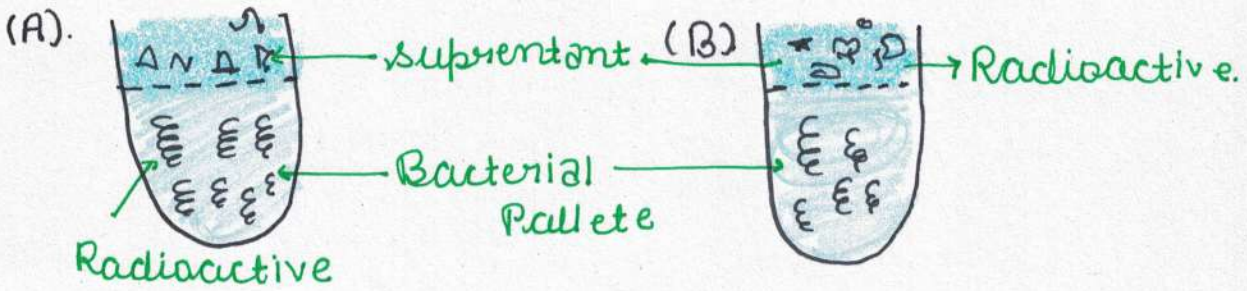
Purpose :-





Step 3

centrifugation:- To segregate bacteriophage and bacterial pallate in a different layer so radioactivity presence can be Establish (Supernatant or bacterial pallate).



DNA REPLICATION

- DNA replication takes place in S-phase
- DNA is elongated in this phase.
- Replication of Cytoplasmic DNA can take place in any phase.

features

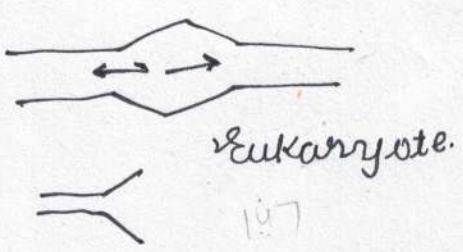
- It is Auto catalytic → for Energy DNA is not dependent on Mitochondria.

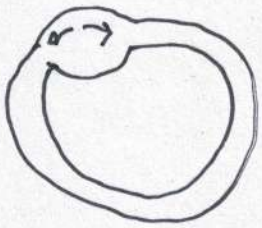
dGMP $\xrightarrow{PPi}$ dGTP (Nucleoplasm; Activation) $\longrightarrow$ dGMP + PPi (for DNA Replication)

PPi $\xrightarrow{\text{Pyrophosphatase}}$ Pi + Pi + Energy

Energy → to attach New Nucleotide

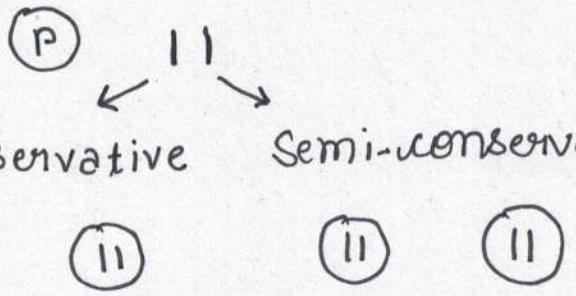
- DNA replication is bidirectional in prokaryote and Eukaryote both.
- unidirectional DNA replication is seen in mitochondria, coliphase P_2.
- Due to bidirectional replication the fork Appears like .Y- shape.





Prokaryote.

(3) DNA replication is semi-conservative.



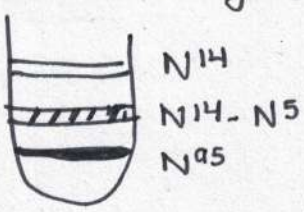
Taylor-et-all :-

the performed his experiment vicia faba he used tritiated thymidine.

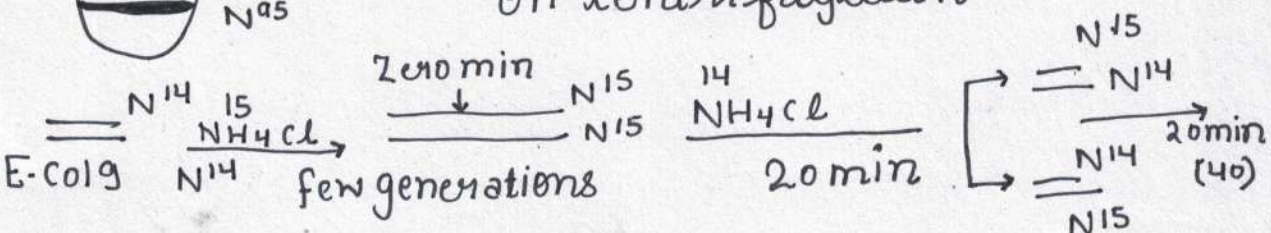
He proved semi-conservative method at chromosomal level.

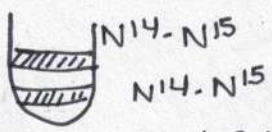
Meselson and Stahl :-

N^{15} → Heavy Isotope.

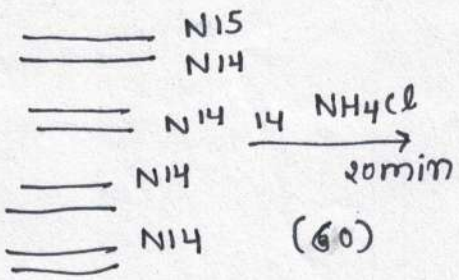


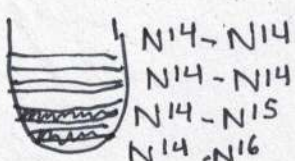
Cscl density based on centrifugation.



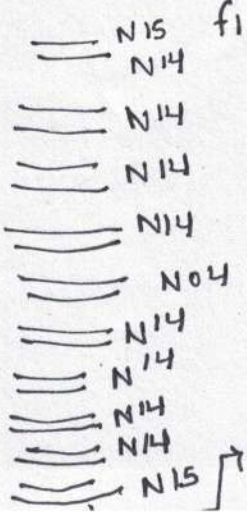


100K Hybrid DNA

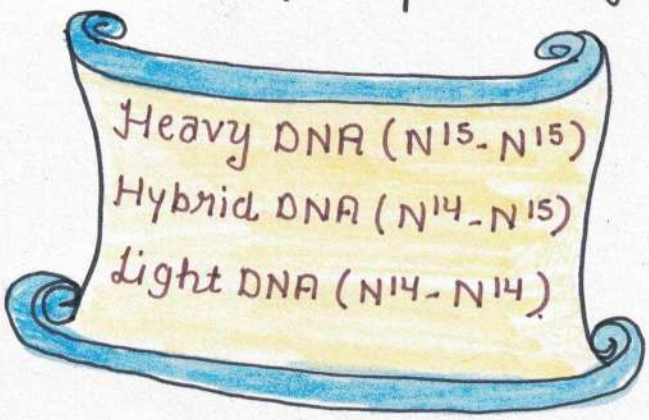




50% of Hybrid f2



E·coli was initially grown in medium containing heavy Nitrogen - N^{15} for few generations then E·Coli containing N^{15}-N^{15} in DNA was isolated and trasnferred to medium containing N^{14} Nitrogen. and Now in Every generation content of hybrid. DNA (N^{14}- N^{15}) keeps on getting half.



content of hybrid DNA in a generation is $2/2^n \times 100$.

? A heavy DNA was grown in N^{14} medium after 60 min, what will be (a) content of heavy DNA (b) No. of hybrid DNA (c) % Age of light DNA.

Heavy DNA i.e. N^{15}-N^{15}, after 60 min only hybria and light DNA's are there so content of Heavy DNA = 0.

(b) No. of heavy DNA = 2

(c) % Age of light DNA = 75%.

? A light DNA (N^{14}-N^{14}) was grown in growth medium containing N^{15}. In 40 min. what will be the content of hybric DNA.

content of hybrid DNA = 2 i.e. 50%.

Enzymes of DNA Replication

(1) Helicase

- ATP dependent Enzymes.
- Requires Magnesium (Mg^{+2}) as co-factor.
- Need Alkaline pH to perform well.

function :→

- To break hydrogen bond.
- It breaks H-bond A-T rich Area / only two H-bond.
- These sites are known as ORI Site.

ORI Site : Origin of Replication Initiation Rate.

- In Eukaryotes many ORI sites are present.
- In Prokaryotes only one ORI site is present.

(2.) TOP Isomerase :-

Due to action of helicase supercoilling is introduced infront of its Action sites. So to release the tension top isomerase will cut the DNA strand it will relief the supercoiling. Top Isomerase 1 can cut only one strand of DNA but top Isomerase 2 will cut both strand of DNA and After release of tension it will rejoin these strand.

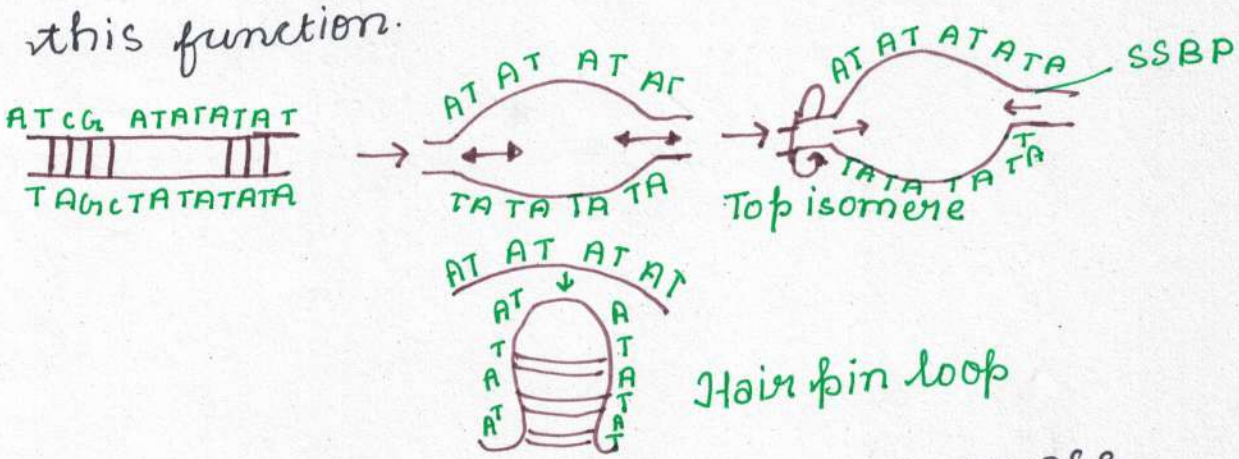
In Eukaryote top isomerase 2 is used, in prokaryote DNA gyrase is used.

Single Stranded Binding protein/ Helix Stabilizing Protein
SSBP/HSP

SS.S
It has tetramer structure and it will prevent formation of hair pin loop in DNA segment where A-Bonds are broken

Primase

- This responsible for formation of RNA primer.
- RNA primer will provide free OH group at 3' with a this DNA replication can not be started.
- In Eukaryote DNA polymerase Alpha performs this function.



- Primase is DNA dependent RNA polymerase.
- RNA primer is 8 to 12 Nucleotide long.

DNA Polymerase

SSS
In case of prokaryote DNA polymerase is of three type:-

	5'-3' polymerase	3'-5' Exonuclease (Proof reading) ✓	5'-3'-Exonuc (Removal of Primer) ✓
DNA-P-I	1000 Nucleotide per Second.		

DNA-P-II ✓ 50 ✓ X
Least

DNA-P-III ✓ 2000 ✓ X
Main

S.S

In Eukaryte DNA polymere is of 5 types.

- α → lagging strand.
- β → Repair.
- γ → cytoplasmic DNA replication.
- δ → leading strand
- ε → Proof reading.

DNA Lygose

this is responsible for joining of two strand of DNA or two nuclei by phosphodiester bond.

In the phosphodiester band formation 3'-OH and 5' phosphase are used.

Mechanism of DNA Replication

Initiation

Nucleotides are recruited from nucleoplasm they will serve dual function as a substrate and as Energy source.

Helicase Enzyme will break H-Bond at ORI Site (one replication in prokaryote, Many replications in Eukaryote)

Top. Isomerase II / Gyrase will release supercoiling in the DNA.

SSBP will prevent hair pin loop formation.

- Primase Enzyme / DNA dependent RNA polymerase will form oligonucleotide RNA primer.
- New stand is always formed in 5'3 direction.

Elongation

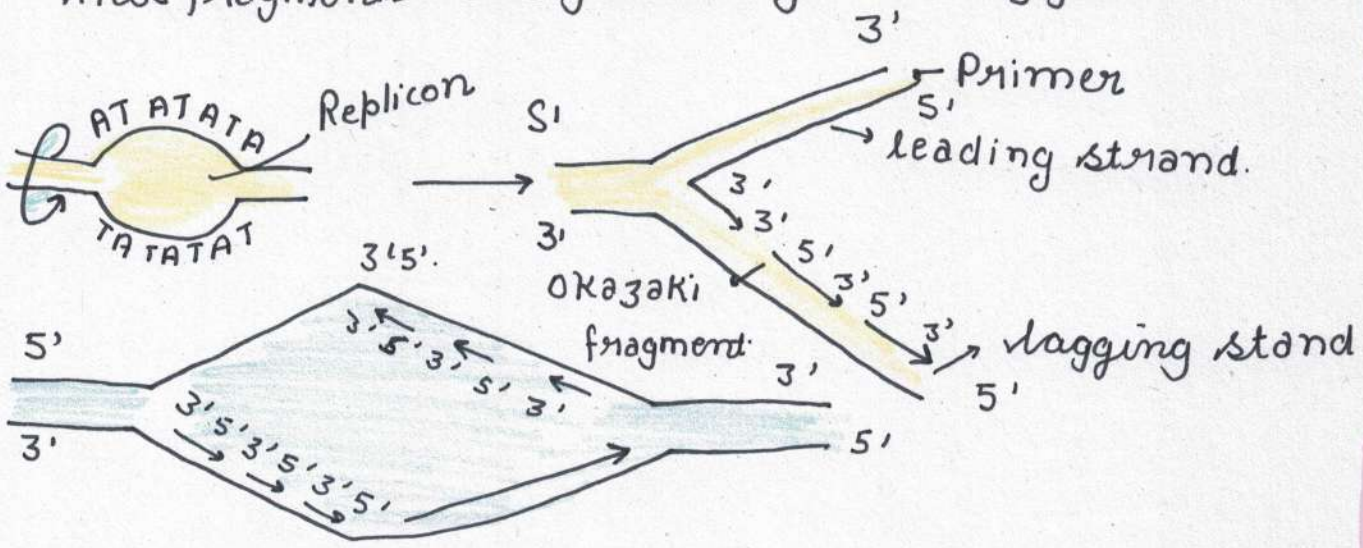
New Nucleotide will get attach to three prime (3) OH group by forming phosphodister bond.

- One stand will be formed in continuous manner in 5'-3' direction It is known as leading stroam or continuous Bond.
- Loading strand require only one primer.
- Replication of another strand is discontinuous and this strand is known as lagging strand.
- Logging strand is require many primer.
- RNA primer is removed by DNP-P-1.
- DNA polymerase-1 will fill these gaps.

PROOF READING

- 3 'to 5' Exonucleous Activity is responsible for proof reading.
- one in 10,000 bp can get abnormal so it need to be connected by proof reading.
- All fragments of logging stand are known as Okazaki fragment

These fragments are joined by DNA ligyase.



R.N.A.

- RNA was first genetic Material.
- RNA is less stable form in comparision to DNA because
  - (a.) At 2'OH group is present in Ribac Sugar (more react)
  - (b). In place of thymine, uracil is present.

RNA is of two types :-

(i) Genomic RNA :- RNA is Acting as genetic Material
Ex :- Tobacco, Masaic Virus, QB bacteriophage, Rheo Virus.

(ii) Non-Genomic RNA :- It is of three types :-
(a). M-RNA (b) tRNA (c) γRNA.

γ-RNA (ribosomal RNA)

- 72 to 80%.
- It is most stable form.
- This RNA has structural and Enzymatic Activity

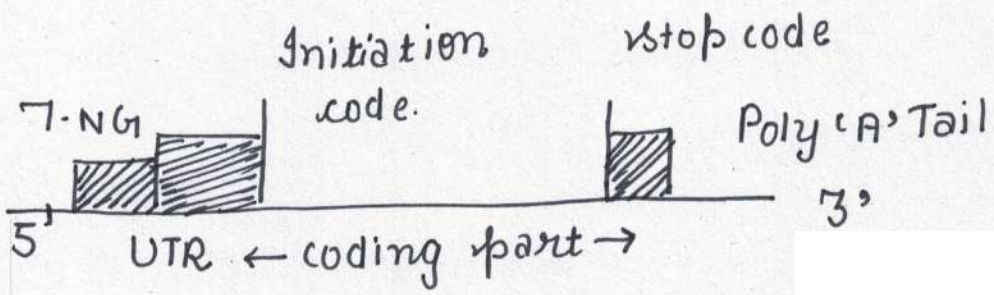
In Eukaryotes 80S		In prokaryotes 70S
60S (large subunit)	40S (Small Subunit)	50S
-28S	-18S	-23S
-58S		-5S

- 5S RNA → for attachment of tRNA to large subunit.
- 58S RNA → translocase action.
- 16S and 18S → for attachment of mRNA.
- 23S and 28S → peptidal transferase Activity.

m-RNA (Messanger RNA)

- formation of mRNA over DNA transcription process.
- 1 to 5%
- Least stable.

Prokaryotic mRNA	Eukaryotic mRNA
• Very short life cycle (few seconds to few Minutes)	• Short life cycle (few minutes to few hour).
• No split Gene	• Split gene present.
• Transcription is coupled with translation	• Transcription is followed by translation.
• Polycistronic m-RNA	• Monocistronic m-RNA.
• At 5' end Shine-Delgamo seaquence is present.	• At 5' End 7 methyl Guanosine cap is present
• one cistrone - 1 polypeptide.	• At 3' end poly Adenylate tail is attached (200 to 300 Adenine) and both side of coding part UTR is present.



RNA (transfer RNA)

- It is 10 to 15%.
- It is smallest RNA
- t-RNA is known as adaptor RNA
- t-RNA is known as soluble RNA
- With the help of t-RNA genetic information of m-RNA will be converted in polypeptide chain.
- t-RNA has abnormal bases due to which loops are present in t-RNA.
  - Eg:- Inosine (I), pseudouricil t (Ψ), Dihydrouridine (DHC) Thymine-T.
- t-RNA has partial double standed Appearance.
- Holley → 2D → clover leaf.
- Kim → 3D → Inverted 'L'
- t-RNA has three loops and four arms.

i) Pseudouracil loop (TψC):-

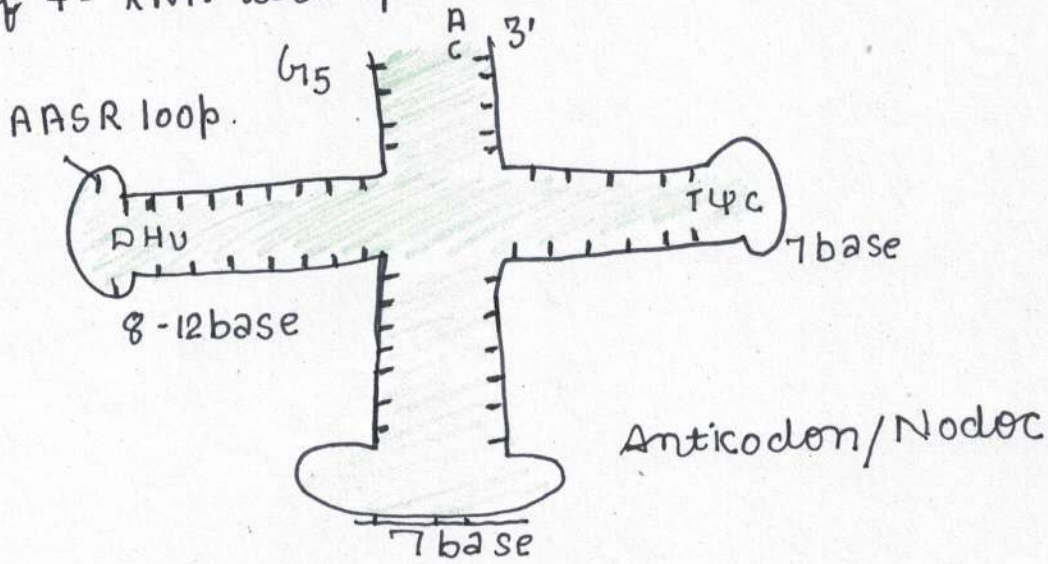
- It is attached to ribosome large subunit through 5S.

(ii) Anticodon loop / Nodoc loop:-

- This part has three nucleotide sequence which are complementory to mRNA codon.

(iii) DHU loop:-

- This loop is Amino Acyl synthetase recognition loop (AAS)
- Amino Acid will be attached to 3OH group of t-RNA through Ester bond charging of t-RNA
- for 20 Amino Acids twenty Amino Acyl synthetase are present and for twenty amino Acid total 64 codon are present.
- Three codon are non sense codon for which there is no anti-codon. So more than 60 type of t-RNA are present.



Transcription :-

DNA $\xrightarrow{\text{Transcription}}$ MRNA $\xrightarrow{\text{Translation}}$ Polypeptide.

Synthesis of m-RNA over DNA template strand is transcription.

Prokaryotic transcription

- Only one type of RNA polymerase is present
- Holo Enzyme
  - σ-factor
  - core Enzyme → main RNA-P
- σ-factor : Identifies initiation point
- RHo(S) factor : Identifies termination point
- Recognition → TATAAT
  10 N_2 bp upstream
  "TATA box"
  "Pribnow box"
- Attachment
  TTGACA
  -35 bp stream.

Eukaryotic transcription

- Three types of RNA polymerase is present
- RNA Polymerase
  - I → 28S, 18S, 5.8S
  - II → Hn-RNA → m-RNA
  - III → t-RNA, 5S, Sc RNA, 5o RNA
- RNA-P perform both function
- Recognisition - TATATAT or TATAAAA
  20 N_2 bp upstream
  "TATA box"
  "HOGNESS box"
- Attachment
  CAAT box - 70 bp upstream.
  GC box - 50-100 bp upstream.

Mechanism of transcription

Transcription results into formation of m-RNA over DNA template strand.

Template strand/ Anti Sense strand/ Non coding strand :-

template stand has. promotor and terminator sequences on both side (flanked) of structural Gene.

coding strand/ sense strand :-

This strand has similar Nucleotide sequence. Except thymine is present instead of uracil

Initiation

for transcription, transcription unit Required.

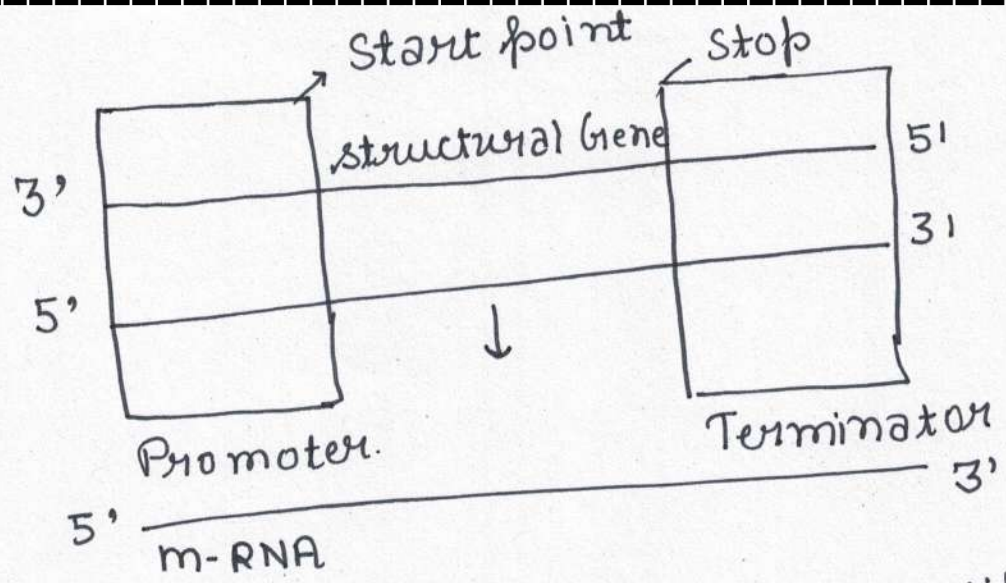
transcription unit has three parts

Promoter | Structural gene | Terminator

Promoter :→ This part has TATA box and ~~After~~ other sequence so RNA polymerase will recognise and attach to these sides.

Structural Gene :→ from this part m-RNA will be transcribed.

Terminator :→ This part is responsible for termination of transcription.



- RNA polymerase with σ-factor identifies initiation point and σ-factor will leave the core Enzyme (RNA polymare).
- RNA polymerase will form Nascent RNA which is 4 to 9 Nucleotide long sequence.

Elongation

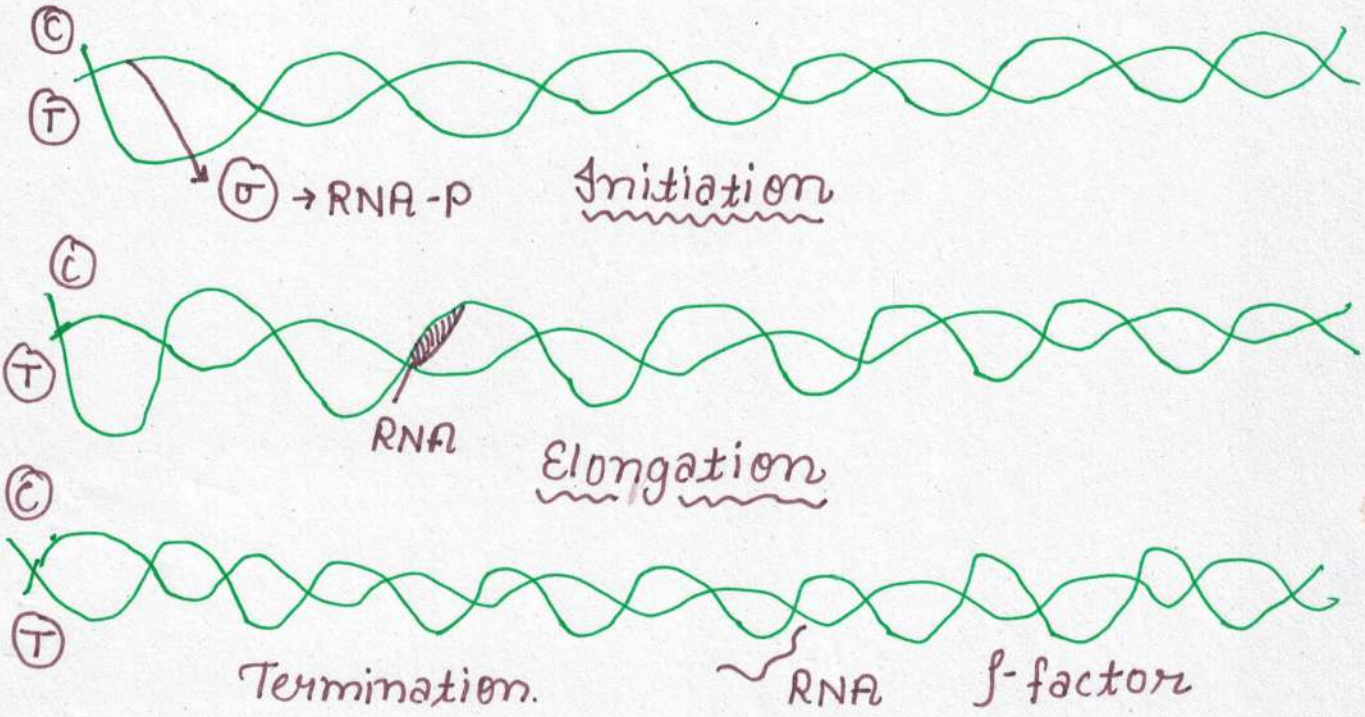
- ATP, GTP, CTP, UTP nucleotides are used for elongation process and Mg^{++} is also required.
- New nucleotide is attached to previous nucleotide by phosphodiester bond between 3'OH and 5' phosp.

TERMINATION

P-factor identifies the termination point and approximately 70 bp upstream (prior) to termination point it attaches to RNA polymerase so at termination point it will break H-bonds Between RNA or DNA template strand.

- This mechanism is ρ-factor dependent termination and ρ-factor has helicase Activity.

- When AT rich or GC rich area is present termination can take place without ρ-factor due to loop-formation It is ρ Independent termination.



Transcription of both strand of DNA cannot take place at same time only template strand undergoes transcription because (NCERT)

- If template and coding both strand undergoes transcription then two complementory RNA will be formed out it will result into formation of double stranded RNA which will not be of any use.

(b). If both these RNA undergoes translation then two polypeptide chain will be formed out of which only one chain will be useful other chain will not be of any use.

Replication	Transcription
• Both strand participate in Replication.	• only template strand participate in transcription.
• Whole DNA undergoes replication process.	• only structural gene part will undergo transcription.
• Take place during S-phase.	• During anytime.

- In case of prokaryote transcription is coupled with translation so more than one Ribosome Attaches themselves to mRNA is only why many polypeptide chain copies are formed.
- In Eukaryote m-RNA undergoes post transcriptional modification prior to its release.

POST TRANSCRIPTIONAL MODIFICATION

Capping :- At 5' end with the help of Guanyl transfer Enzyme 7-Methyl guanasine cap is attached.

Tailing :- Poly A-polymerase Enzyme forms poly-A-tail at 3' · 200-300 adenylate.

Splicing :- In Eukaryote split are gene present.

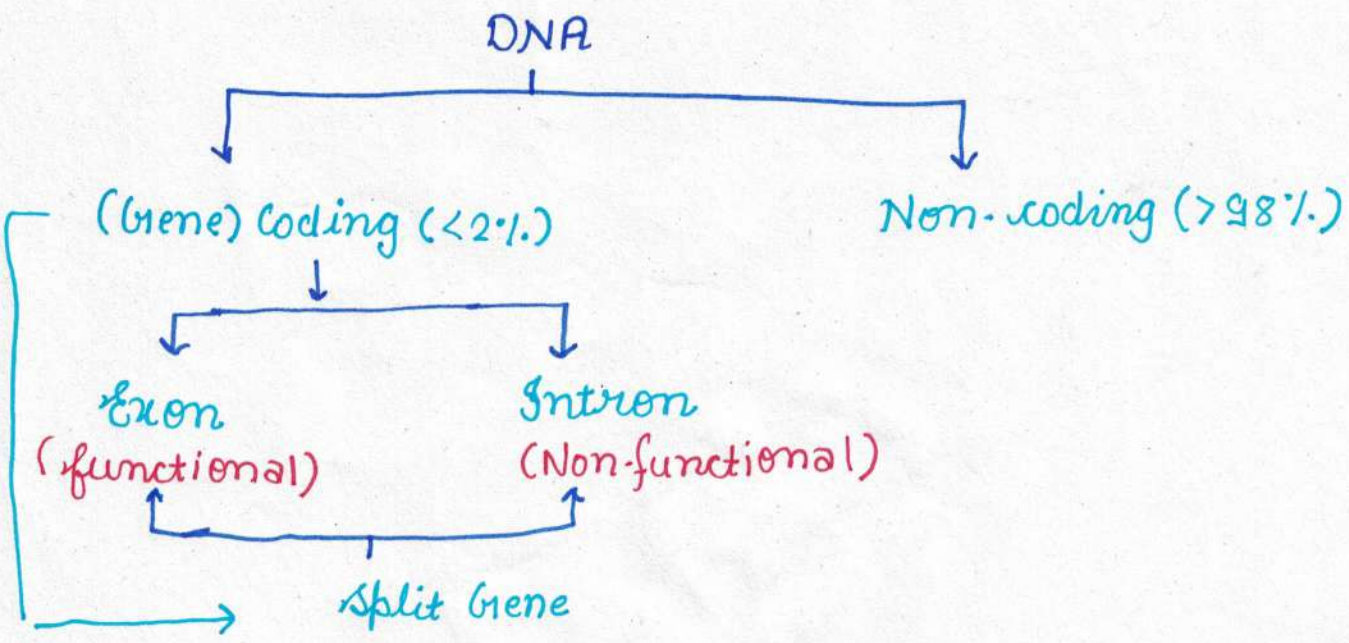
★ Robert and sharp discovered it split gene.

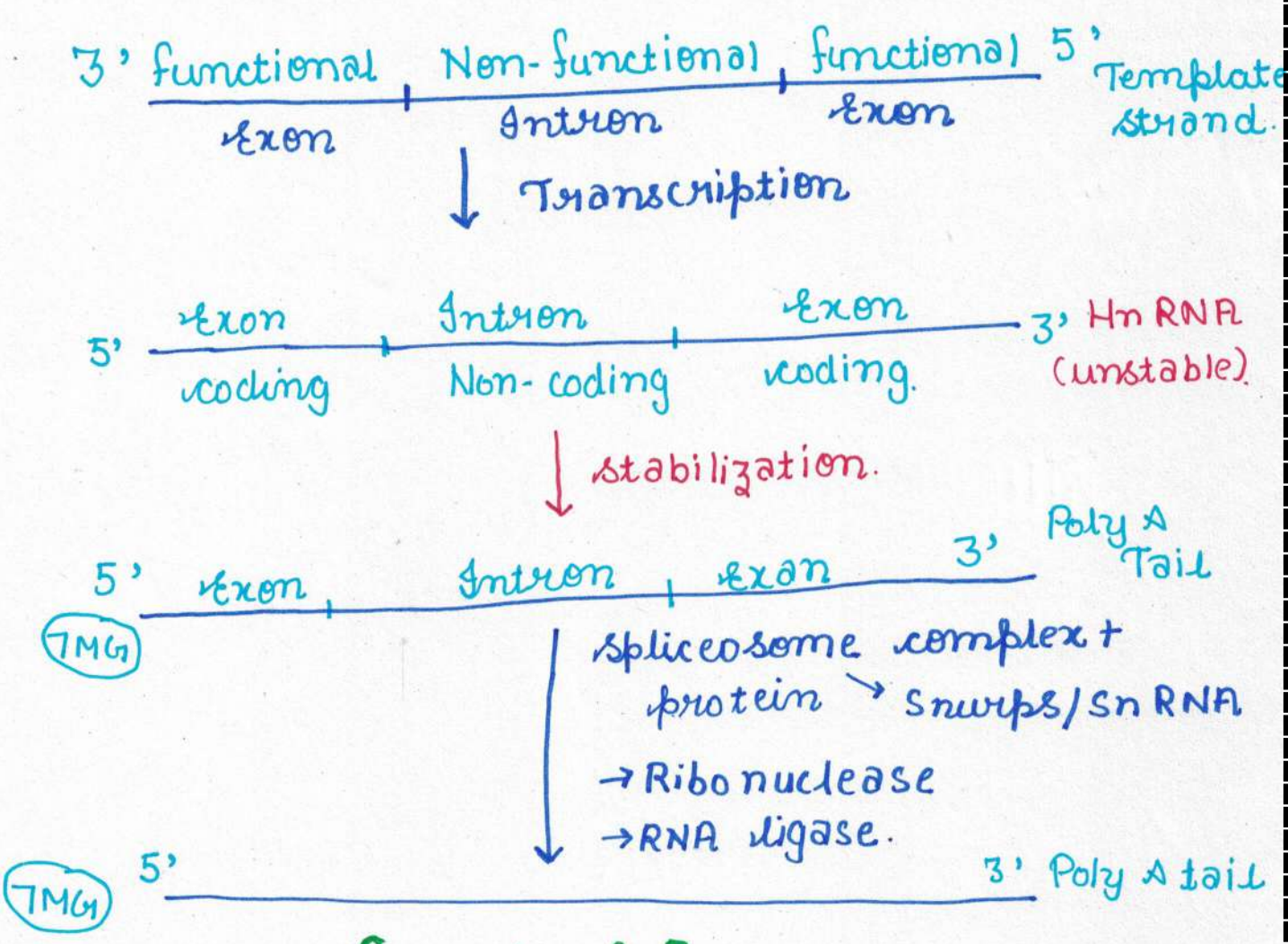
★ In Eukaryote all genes are split genes (Exon + Intron)

Except :→ Histone and Interferon (only Exon).

- In prokaryote no Intron are present so no need of splicing.
- Thymidiate synthatase Enzyme gene in prokaryote is split gene.

Splicing → Removal of Introns.





Genetic Code

The term was given by George Gamow and was described by Nirenberg, Mathai & Khorana.

Definition:- These are nucleotide sequences present on mRNA/DNA which will be translated into poly peptide chain sequence.

Codon :- These are nucleotide sequences triplet in nature which codes for amino Acids.

Characteristics of Genetic Code

- Genetic codes are triplet in nature → Neirenberg
- Mathai and Khorana they stablished triplet Nature of genetic code.
- Four types of Nucleotide are present → A, U, G, C

(Types of Nucleotide)^n No. of Amino Acids.

n = Nature of codon.

(i) If n=1 → Singlet → $(4)^1 = 4$ AA. X

(ii) If n=2 → doublet → $(4)^2 = 16$ AA. X

(iii) If n=3 → triplet → $(4)^3 = 64$ AA. ✓

Human have 20 Amino Acids so codon are triplet in nature.

Question:- In an organism 6 types of nucleotides are present and 38 Amino Acids are present Nature of codon will be...

Type of Nucleotide = 6

No. of Amino Acids = 36 n=?

$(6)^n = 36 \rightarrow (6)^n (6)^2$

n = 2 → doublet.

Question:- If 40 Amino Acids in previous question what is the nature.

Triplet.

Genetic codes are non-overlapping in nature. In a triplet codon one nucleotide will participate in only one codon.

AUGCGU → no. of nucleotide / 3 = no. of Amino Acids.

AUGCGU → no. of nucleotide − 2 = no. of a.a.

1st 2nd 3rd 4th

Total Nucleotide in MRNA are 150 then what will be no. of Amino Acids if

(i) Overlapping is not present.

(ii) Overlapping is presence.

Solution

Total Nucleotide = 150 no. of a.a = ?

(i) Overlapping (−)nt, then no. of nucleotide / 3 = no. of a.a

= 150/3 = no. of a.a.

⇒ 50 = no. of a.a.

(ii) Overlapping (+)nt then, no. of nucleotide − 2

= no. of a.a.

= 150 − 2 = no. of a.a.

= 148 = no. of a.a.

S.S.S

Genetic codes comma less there is no punctuation in Genetic code because genetic codes are commaless so, shifting of one nucleotide will shift the whole frame

a b c d

AUG GUA CUA CCA

a c d

AUG GUA CUA CCA

a

AUG GGU ACU ACCA

e f g

SSS

Genetic codes are non-Ambiguous.

one triplet codon → code for only one aa.

Exception:- GUG → It codes for methionine and Valine but according to its position.

Initiation Codon And Termination Codon

Initiation :- In every organism initiation codon AUG.

AUG → P → N-formyl - Methionine.
AUG → E → Methionine.

GUG → At Initiation → Methionine.
GUG → other than Initiation - Valine.

TERMINATION CODON / STOP CODON / Non Sense Codon

- UAA → Ochar
- UAG → Ambar
- UGA → opal

for this codon there is no Anti-codon tRNA.

Genetic Codes are Universal.

Triplet codon code for same aa in bacteria, Virus unicellular organism, Multicellular organism.

Exception: Mitochondria
- UGA → tryptophan
- AGA, AGG → Stop codon.

Protozoa → Paramaecium
– UAA, UGA → Glutamine.

Degeneracy of Genetic Code

- For 20 amino acids 61 triplet codon are present so for many amino acids more than one triplet codon are possible.
- Third Nucleotide base on mRNA is known as wobbly base, It is non specific sometime.

Leucin → CUA, CUU, CUC, CUG
(6) UUA, UUG

Serine → UCA, UCU, UCC, UCG
(6) AGU, AGC

Arginine → CGA, CGU, CGC, CGG
(6) AGA, AGG.

Methionine → AUG
Tryptophan → UGG.

Question:- On mRNA 150 nucleotides are present then how many amino acids will be formed.

1.) 39 2.) 50 3.) 150 4.) 148 (stop codon not considered)

or

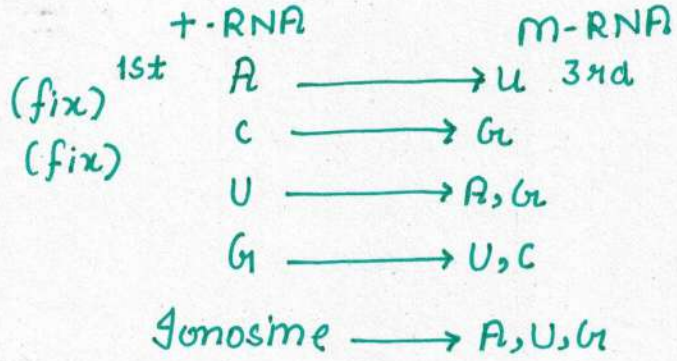
a.) 49 b.) 50 c.) 150 d.) 148 (stop codon is considered).

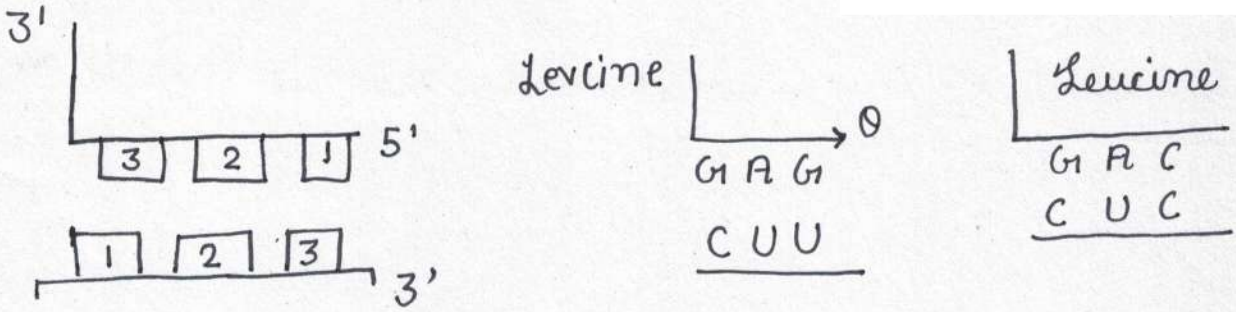
Wobble Hypothesis

For 20 Amino Acids 61 are present but for 61 codons all 61 anticodons are not present.

For 61 codon (mRNA) less than 61 Anti codon (tRNA)

First Nucleotide of +RNA and third Nucleotide of RNA they show wobble hypothesis.





Translation

Translation is a process in which genetic information stored in mRNA is converted into peptide polypeptide chain. Prior to translation following two steps take place.

Activation of Amino Acid :- for twenty type of Amino Acids twenty Amino Acyl synthatase (AAS) enzyme are present.

$$A\cdot A\cdot + \underset{\underset{E}{|}}{A\cdot A\cdot S} + ATP \longrightarrow AA-AMP-E + 2Pi$$

(2.) **charging of tRNA →** With the help of DHU loop +RNA can attach to particular Aminoacyl synthatase so amino acid will be transferred to +RNA charging of t Rna also called Aminoacylation of +Rna

$$AA-AMP-E + + RNA \longrightarrow \underset{\underset{COOH}{|}}{AA} - \underset{\underset{3OH}{|}}{tRNA} + E + AMP$$

Ester bond.

Translation is a three step process:-

Initiation Elongation Termination

- **Initiation :-**

Both subunit of ribosome are dissociated prior to translation.

In case of prokaryote:-

(a) $30S + mRNA \xrightarrow{IF_3, Mg^{+2}} \underset{\underset{SD}{|}}{\underset{\underset{ASD}{|}}{30S-mRNA}}$

(b). $30S-mRNA + tRNA \xrightarrow[GTP]{IF_3, Mg^{+2}} 30S-mRNA-tRNA$

(c). $30S-mRNA-tRNA + 50S \xrightarrow{IF_1\ Mg^{+2}}$

IFs 1, 2, 3, sequence of functioning → 3, 2, 1

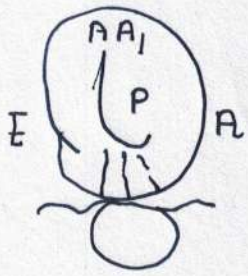
★ In case of Eukaryotes :-

(a). $40S + mRNA \xrightarrow{eIF_2, Mg^{+2}} 40S-mRNA$

(b.) $40S-mRNA + tRNA \xrightarrow[GTP]{eIF_3, Mg^{+2}} 40S-mRNA-tRNA$

(c). 40S-mRNA - tRNA + 60S $\xrightarrow{eIF_1, Mg^{+2}}$

eIf = 1,2,3 , 2, 3, 1 ÷ sequence of functioning



2.) Elongation

Larger subunit of ribosome has three sites:

(i) E-Site ⇒ Exit site (tRNA without Amino Acid)

(ii) P-Site ⇒ peptidal site (tRNA with Amino Acid)

(iii). A-Site ⇒ Amino Acyl-site (tRNA with amino Acid).

Elongation factors:-

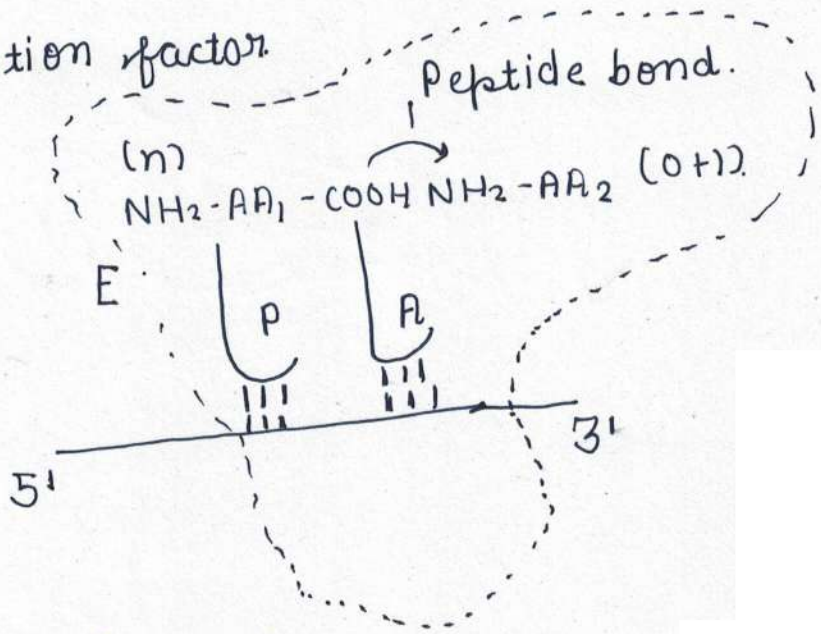
Eukaryote		Prokaryote.	
eEF → 1α	⟶	EF → Tu	] peptidal transferance Activity.
eEF → 1β	⟶	EF → Ts	
eEF → 2	⟶	EF → G →	Translocation.

EF-Tu-GDP $\xrightarrow{EP-Ts/GTP}$ EF-TU-GTP.

New t-RNA will come to A-site with Amino-Acid one it will form peptide bond between COOH group of Amino Acid at P-Site and NH_2 group of Amino-Acid at A-Site.

Hydrolysis take place at P-Site tRNA.

Peptidal transferase (23S and 28S) is reasponsible for formation of peptide bond with the help of Elongation factor.



Translocation

Ribosome will move over mRNA so,

Old site		New site
E	→	outside.
P	→	E
A	→	P

Translocase (5-BS) with the help of EFG will perform this function.

Termination

When stop codons comens at A-site there is no t-RNA ~~interaction~~ anticodon for stop codon so peptide chain will be release because A-Site will be unoccupied.

stop codon		t-RNA anticodon	
UAA	Present	AUU	Absent.
UAG		AUC	
UGA		ACU	

In prokaryote releasing factor 1,2,3

RF_1 → UAA, UAG
RF_2 → UAA, UGA
RF_3 → Activation RF_1 & RF_2.

In Eukaryote Eukaryotic Releasing factor 1 (e RF-1) perform all these functions.

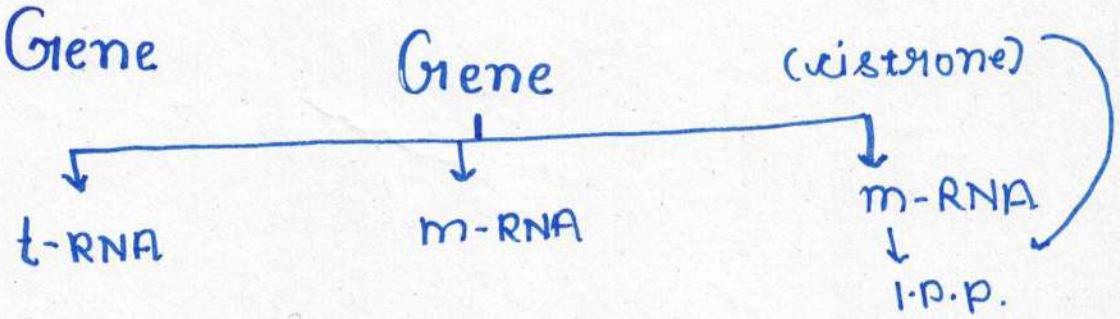
To release last Amino Acid from P-site one GTP is required.

What will be no. of ATP and GTP to incorporate 25 Amino acid in a poly peptide chain ?

To Incorporate 1aa → 1ATP + 2GTP is required
so for 25 aa = 25 X (1ATP + 2 GTP)
= 25 ATP + 50 GTP.

What will be no. of ATP and GTP required to Incorporate and release 25aa long polypeptide chain.

To Incorporate 25aa 25ATP + 50GTP is required (-See previous Question)
and to release aa from Psite one GTP is required. So total
25 ATP and 51 GTP is required.

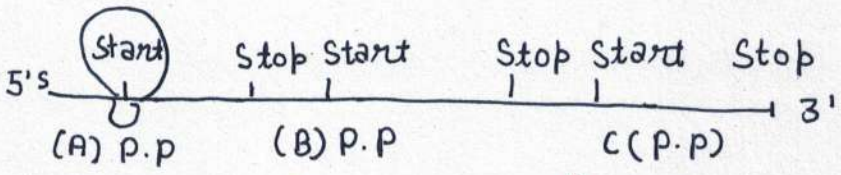


Gene

Eukaryote

Prokaryote

Monocistronic



Polycistronic

CRacking of Genetic code / Deciphering of Genetic Code

⇝ Neirenberg, Mathai and Khorana gave detail About Nature of genetic code.

⇝ They studied homopolymer and co-polymer method.

Homopolymer Method :-

✱ Neirenberg and Mathai studied this Method.

Eg :→ UUU UUU UUU UUU or ABC ABC ABC ABC

Phenyl alanine.

Heteropolymer / Co-polymer Method :-

Eg :→ UG UG UG UG CG

cystein Valine.

✱ Severo-Ochoa-Enzyme is a RNA polymerase. This Enzyme do not require template.

✱ In Eukaryote gene introns are present in split gene (Archaebacteria also have introns). so presence of Intron is primitive feature (Reminance of Antiquity).

Central dogma

Crick explained flow of genetic information is in one direction.

DNA $\xrightarrow{\text{Transcription}}$ RNA $\xrightarrow{\text{Translation}}$ Enzyme

Temin and Baltimor studied Rous Sarcoma Virus and Explained reverse transcriptase activity.

DNA $\underset{\text{Reverse transcriptase}}{\overset{\text{Transcription}}{\rightleftarrows}}$ RNA $\xrightarrow{\text{Translation}}$ Enzyme.

Types of Genes

(i) Jumping Gene / Transposons / Retroposones / Mobile Element :→

Barbra Mclintek (Lady Mendel) discovered jumping Area. in maize as chromosome – no. 3

(Ac and Ds)
Activator Dissociation.

Drosophila → P and I Element.
→ Copia like Element.

Human → "Alu"

This genes can switch their position from one chromose to another.

2.) Constitutive Gene :→ ★ These genes are always switch on Active.

★ They are known as house-keeping gene.

★ Eg → Genes for respiratory Enzyme.

Nor2.) Constitutive Gene

These genes are always luxury genes they can be switch on according to need.

Two types

Inducible	Repressible
Generally, switch off need to be switch on by Inducer	Generally they are switch on need to be switch off by repressor.
Switch off → switch on (Inducer.)	Switch on → Switch off (Repressor)
catabolic in nature	Anabolic in Nature.
LAC operon	Tryptohon operon.

Operon

They are segment of DNA consisting of more than one type of gene.

(i) Regulator Gene (ii) Promotor gene.
(iii). operator Gene (iv) Structural Gene (>1)

Two types of operon system:-

(i). Inducible → eg lac operon.
(ii) Repressible → Eg. Tryptophan operan.

Lac Operan (In E. Coli) E.coli को Glucose पसंद है।

- E. coli utilises glucose Mainly.
- It's a Inducible operon.
- It's a catabolic

Generally, remain switch off need to be switch on.

Parts of Lac operon

(i). **Regulator Gene:-** It is constitutive gene.

- Regulator gene forms repressor protein.
- It has two surfaces of Attachment:-
  - for operator Gene.
  - for Inducive (lactose).
- But at one time repressor attaches from one side.
- Switch off → Repressor gene + operator gene (Active).
- Switch on → Repressor gene + Inducer. (Inactive)

(ii) **Promoter Gene:-** It provides attachment sites for RNA polymerase.

(iii) **Operator Gene:-** It gives passage to RNA polymerase so it can reach to structural Gene.

(iv) **Structural Gene:-** These gene has three points z, y and a.

- Synthesis of polycistronic m RNA from structural Gene.

Z-Part

Synthesis of β-galactosidase Enzyme.

Lactose $\xrightarrow{\beta\text{galactosidase}}$ Glucose + Galactose.

β-galactosides.

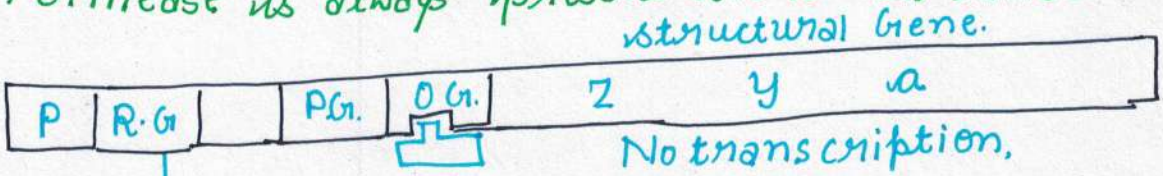
Y-Part

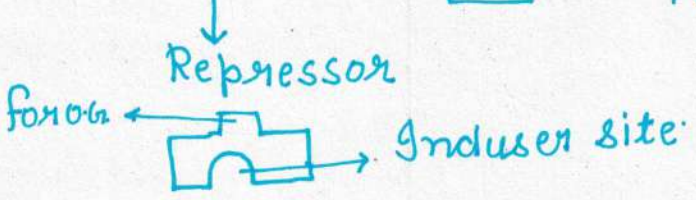
- Synthesis of permease enzyme.
- Permease Enzyme is needed for increasing the permiability for β-galactosides (lactose)

a-part

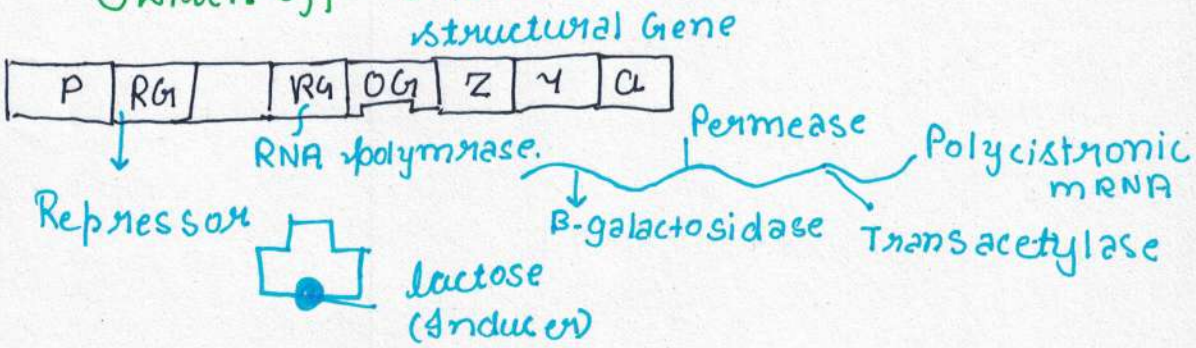
- Synthesis of transacetylase Enzyme.
- Transacetylase (to restore acetyl group at β-galactosides)

Permease is always present in small Amount.





"Switch off" (lactose Absent).



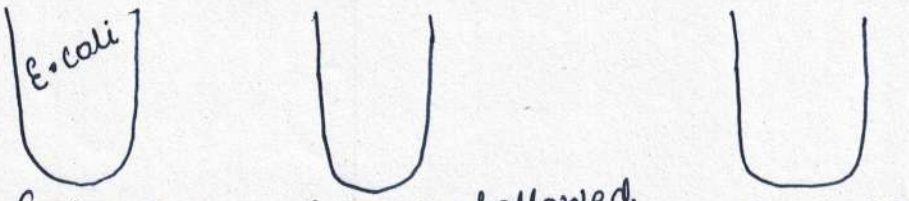
'Switch on' (lactose present).

Recon: unit of recombination (crossing over)

Muton: unit of Mutation.

cistron > Recon > Muton.

Lac operon: 'OFF' OFF → ON 'ON'



Glucose Glucose followed by Lactose Lactose

Tryptophan operon

- It's a anabolic operon.
- It's a responsible operon.
- Generally remain switch ON need to be switch OFF.

Parts of Tryptaphan Operan :-

1. **Regulator Gene** → Production of Apo-repressor protein.

- Apo-represser alone cannot switch off, the operon
- Tryptophan which is synthesized by this operan is continuously utilised so collection of tryptophan is not so regularly seen.
- If tryptophan is collected it will act as co-represent

Switch off → Apo-repressor + Co-Repressor + operator Gene.

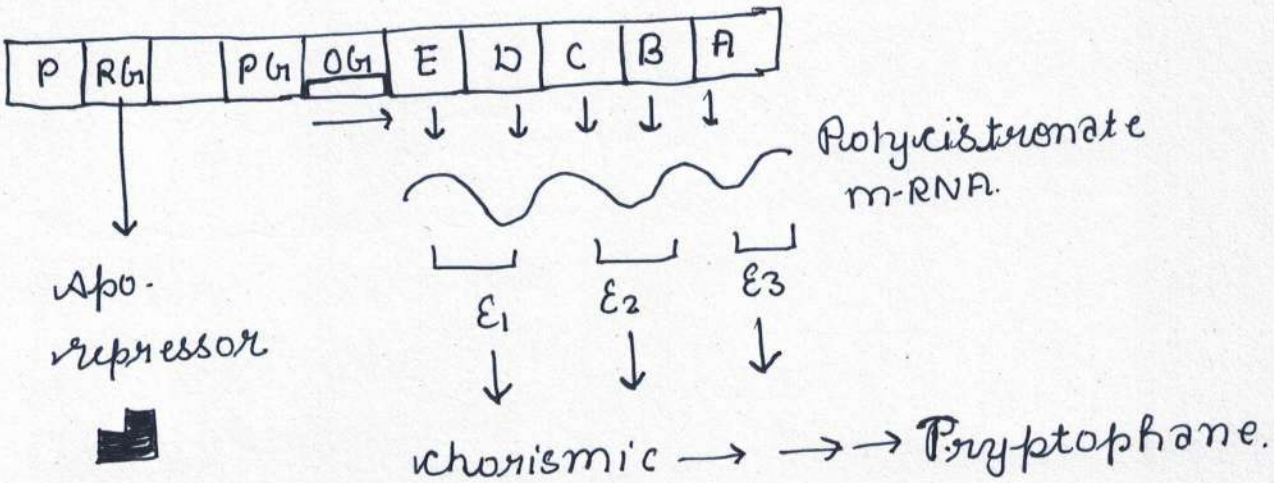
2. **Promoter Gene** → Attachment of RNA polymerase.

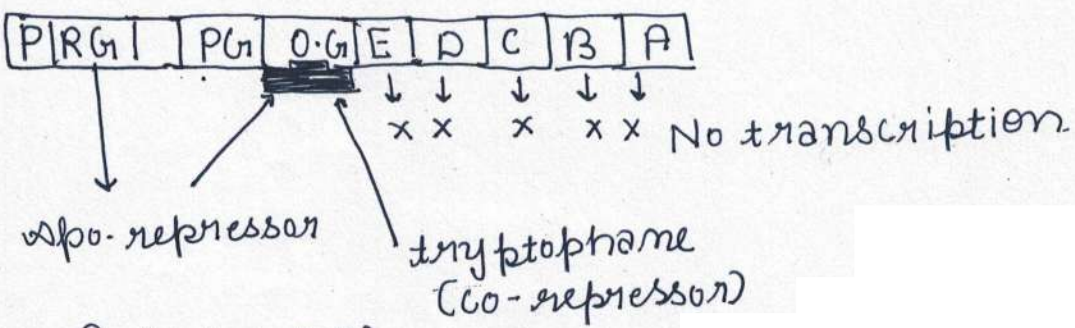
3. **Operator Gene** → Provide passage to RNA polymerase.

4. **Structural Gene** → It has five parts :-

- Synthesis three Enzymes :-

Polycistronic mRNA is formed.





Human Genome Project (HGP)

- It was Mega project.
- Started in 1990 and completed in 2008.
- Six countries participated and twenty research centres were Established.
- Expenditure 3 U.S. $ per base pair (Total base pair 3.3×10^9).
- from U.S.A: Department of Energy and National Institute of health.
- from UK (England): Welkane Trust

Bioinformatics:-

If all the data of human Genome Project is converted in hard copies then 3300 books will be needed, Every book having only 1000 pages, Every page having 1000 letters. So to deal with this biology was combined with Information technology and bioinformatics started.

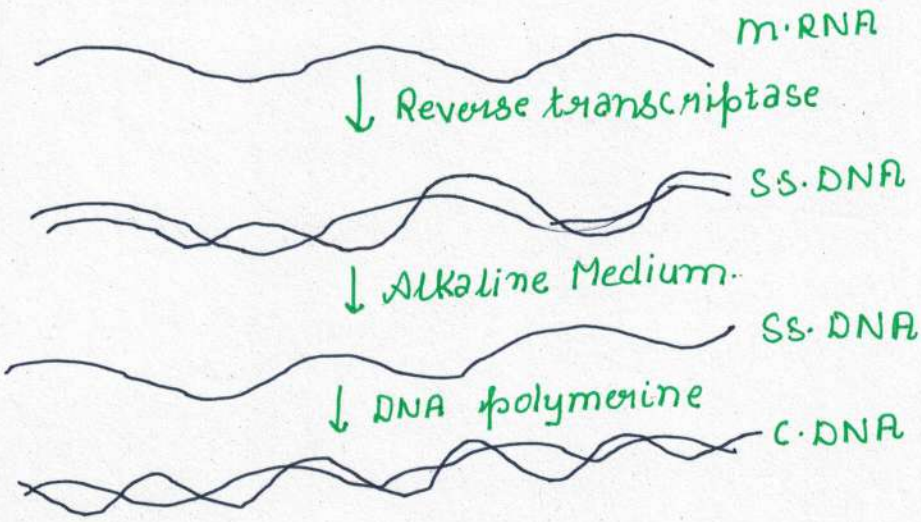
Goals of HGP:-

- To Identify all the genes in human genome.
- To determine sequencing of base pair in human gene.
- To store the data.
- To Improve the data tools for future Analysis.
- Transfer of technology to the Industry.
- To deal with E.L.S.I (Ethical legal social Issues).

Methadology of HGP

(i). Expressed Sequence Tags (EST)

Only coding part of DNA were studied because they have Expenssive Sequences.



(ii). Sequence Annatation (S·A).

It's a blind Approach because studied DNA is not known. It is coding or non-coding.

Host Bacteria → Vector Bacterial Artificial chromosome (BAC) → Passanger DNA Desired Gene.

Yeast → Yeast Artificial chromosome (YAC)

☞ Friedrich discovered automated DNA sequencer which was computerised and it works on overlapping region principle to study Human Genetic Project.

☞ Di-deoxynucleotide chain termination method studied (22+x+y). was also used.

☞ Total 24 chromosome Sequence were studied (22+x+y)

Salient features of HGP :→

- 3×10^9 nucleotide base pair are present in human genome.
- Total number of genes in human genome and around 20,000 to 30,000 (previously it was thought that 80,000 to 1 lakh genes).
- chromosome no.1 has highest number of genes. (i.e. 2968)
- Minimum genes are present on Y chromosome (i.e 231)
- Average gene size is 3000 base pairs
- Shortest or smallest gene TDF → 14 bp.
- Longest gene DMD (Duchenen Muscular Dystrophy). → 24 million base pair.

- 50% of the discoved gene's function is unknown.
- Mutation in non-coding region is responsible for DNA polymorphism it gives information about statics, dynamics and Evolution.

Single Nucleotide Polymorphism:

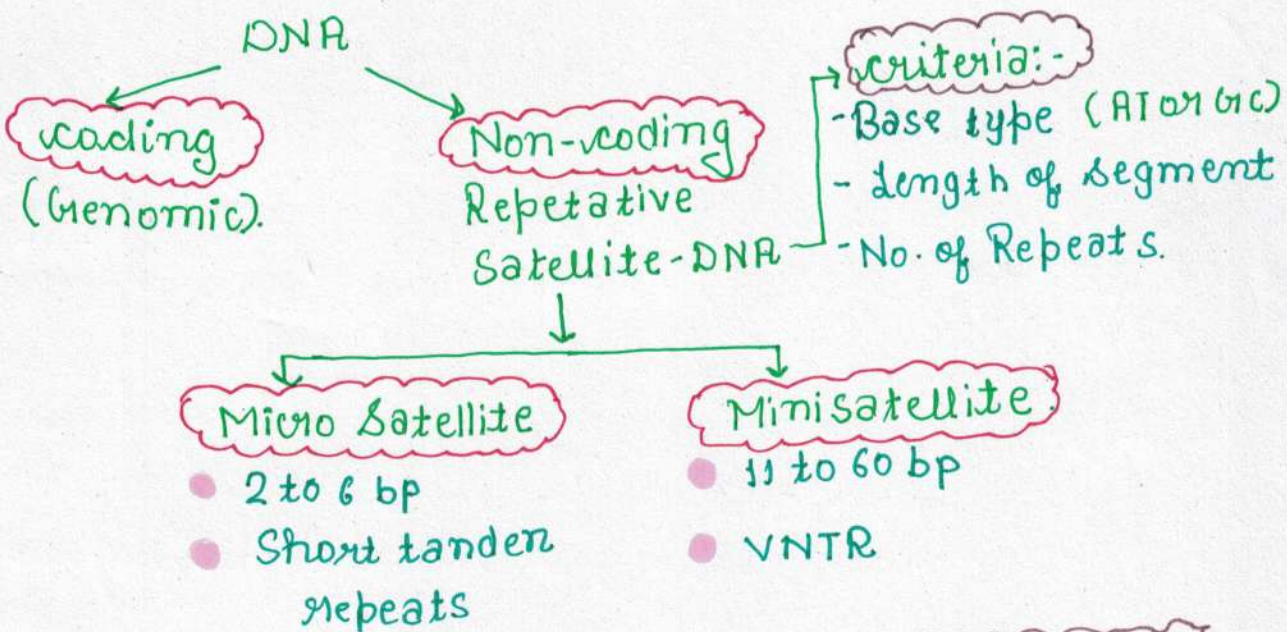
- At 1.4 million locations it is found that DNA shows polymorphism in single nucleotide (SNPs).
- These are important for identifies of location of disease and tracing human history.

First genome to be sequence in :-

- First prokaryote :- Haemophilus influenzea.
- First Eukaryote :- Saccharomyces cerviciae.
- First plant :- Arabidopsis thaliana (Small Mustard plant)
- First Animal :- caenorhabitidis Elegance.

DNA fingerprinting :-

- Alec. Jeffery is known as Father of DNA fingerprinting.
- In India Lalji Singh and V.K. Kashyap is known as father of DNA fingerprinting.



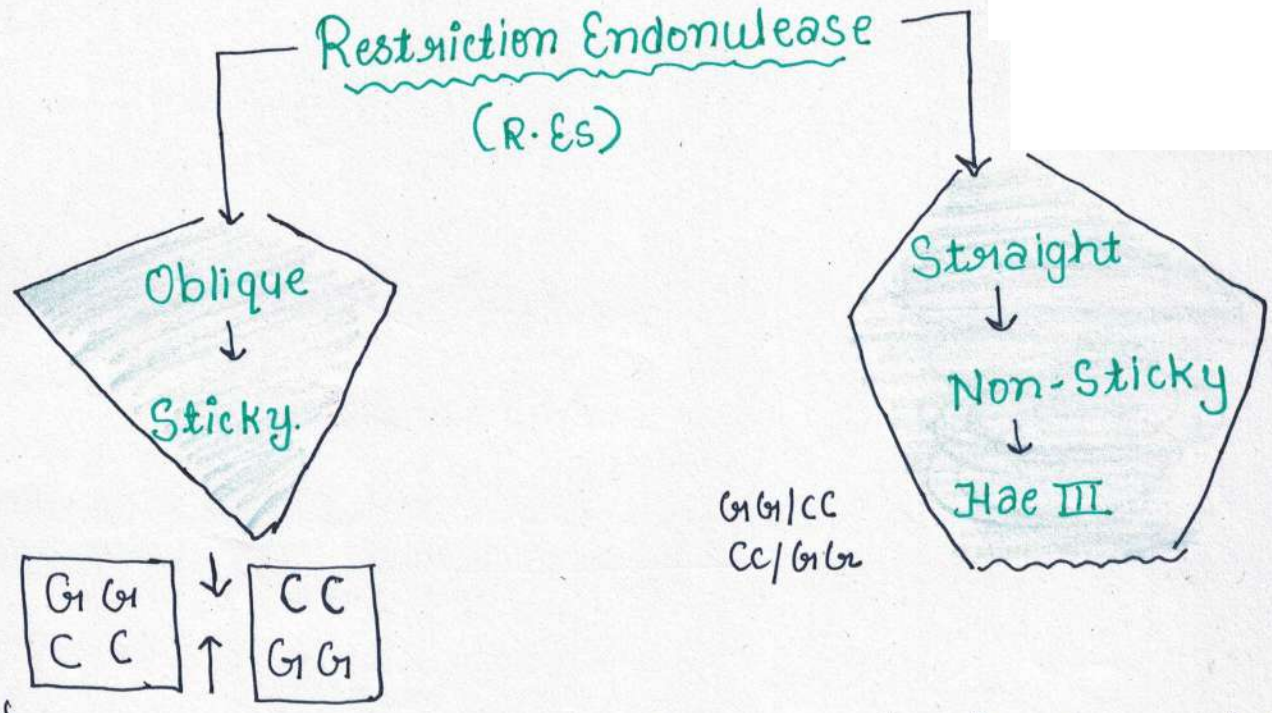
VNTR (Variable Number Tandem Repeats)

- It's a mini satellite.
- 11 to 60 bp sequence are repeated.
- Number of repeats may vary upto 100 (sometimes Even more)

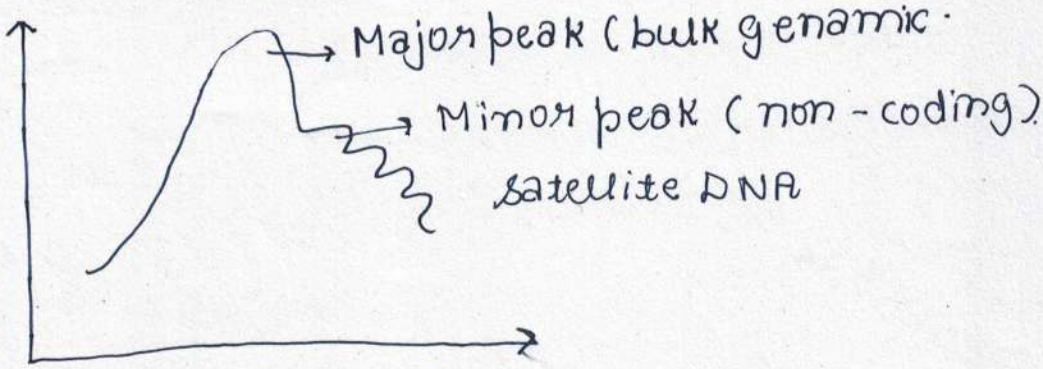
In VNTR due to mutation very high degree of polymorphism is seen in repeat sequence and Number of repeats.

Even in one homologous pair of chromosome. (one from father and another from mother) VNTR sequence show polymorphism.

Size of VNTR → 0.1 Kb to 20 K.b.
(K.b. Kilobase).

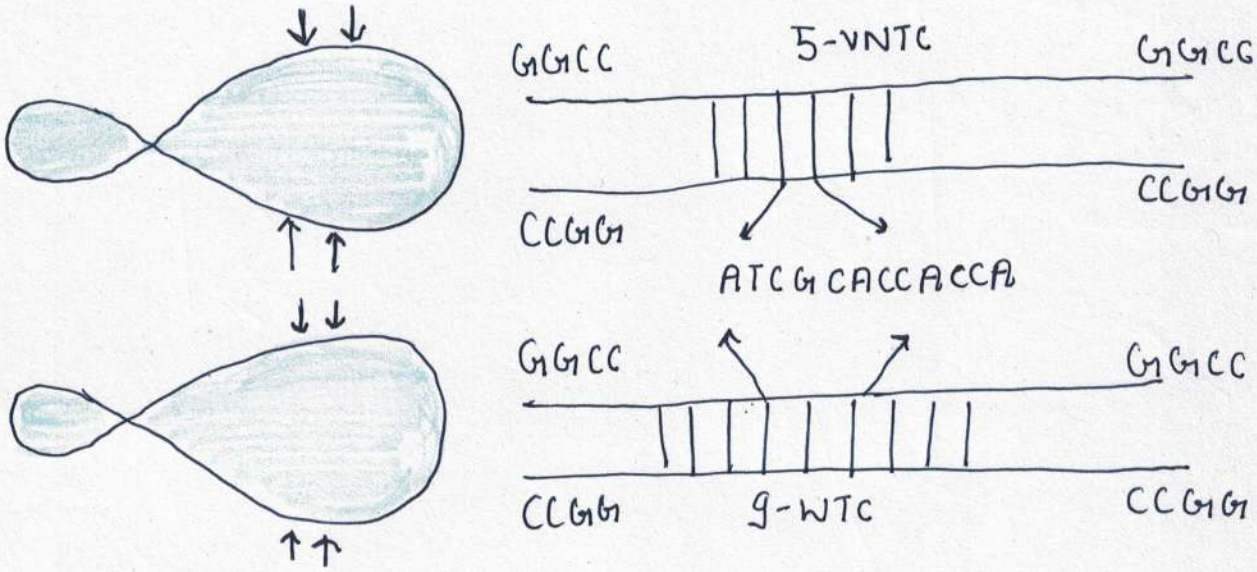


Density gradient centrifugation is performed to differentiate coding (bulk genomic part) from non-coding part.



RFLP (Restriction fragment length polymorphism)

Due to action of restriction Endonuclease (Hae III) restricted fragment length are obtained. These fragments show polymorphism in length. so they are known as restriction fragment length polymorphic segment.



RFLP → fragment.

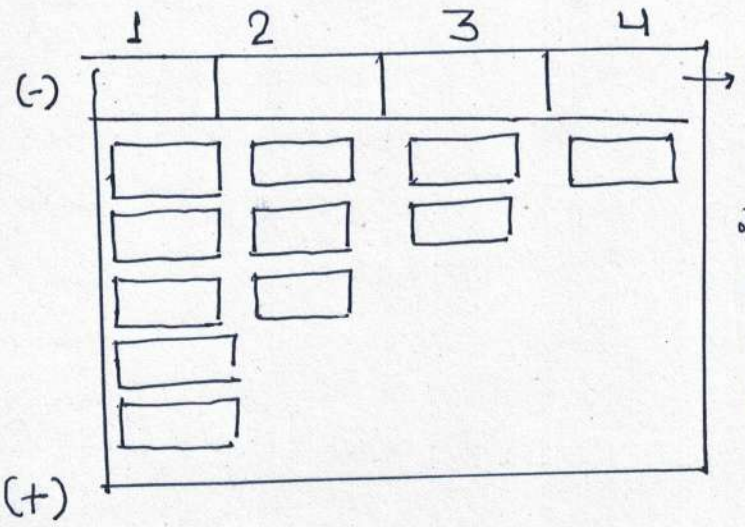
Mechanism of DNA fingerprinting

- **collection of Sample:-** samples can be collected from Mucusa cells. blood cells, Hair follicles, Semen bone marrow etc.
  (All cells containing DNA can be taken as sample.)
- **Isolation of DNA:-** Cell lysis is performed with the help of lipose and proteases.
- **Exposure to restriction Endonuclease:-and density gradient centrifugation:-** Hae III are used and mini satellite are obtained.

4.) Gel Electrophoresis :- It's a colloidal gel in solid form.

→ Positive and Negative both Electrodes are applied

→ DNA fragments will move according to charge them and their molecular weight.



Length same है
सबकी width इसलिए
करा हो रही है ताकि ये
show हो पाए कि
molecular wt कम
हो रहा है।

→ Gel Electrophoresis is used to separate fragments on the basis of their molecular weight.

Example :-

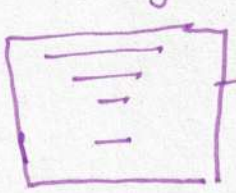
- DNA fingerprinting.
- To differentiate protein fragments

5.) Southern Blotting :- souther blotting was given by Edward Southern.

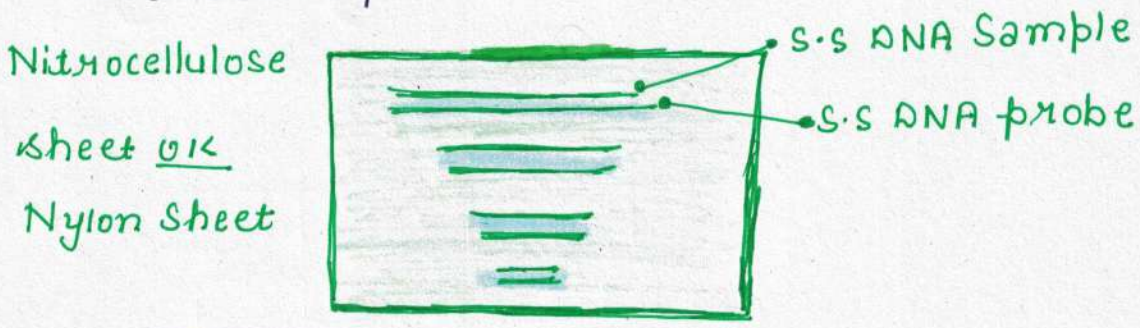
Southern blotting → DNA finger printing.
Northen blotting → RNA.
Western blotting → for proteins.

Gel Electrophoresis (ds DNA) $\xrightarrow[\text{Alkaline Medium}]{<90^\circ C}$ SS DNA $\xrightarrow[\text{or Nylon sheet}]{\text{Nitrocellulose sheet}}$

Blotting → चिपका कर आना।



6. Hybridisation :→ Single standed Radio Active (phosphate) Nucleotide sequences are prepared which are complementary to VNTR, they are known as radioactive probe.



Alkalyting Agents

These agents couses depurination and at their places another purine (transition) or replaced by pyrimidine. (transversion).

Example :- (i) Ethyl Methane Sulphonate.
(ii). Methyl Methane Sulphernate.

Acridine and Proflouins

- Acrine and proflavins can insert or delete a nucleotides.
- They will cause frame shift mutation.

b.) Physical Mutagens (Radiations) :→

Radiations are of two types:
(i). Ionizing (ii). Non-Ionizing.

(i) Ionizing Radiations :-

- X-Rays → They causes chromosomal structural changes.
- α, β, γ - Rays → They causes substitution.

(ii). Non-Ionizing Radiations:-

- UV rays → They are absorbed superficially. They causes thymine dimer formation so it leads to xeroderma pigmentasa.
- U.V Rays can cause mutation in Micro-Organism.
- chemical-Mutagens are more harmful → they have generalised Effect.
- Protection from chemicals is not possible (not so Easy).