DNA

"Deoxyribonucleic acid"

Fredorich Meischer → "Nucleus" contains an **acidic substance**

(1869)

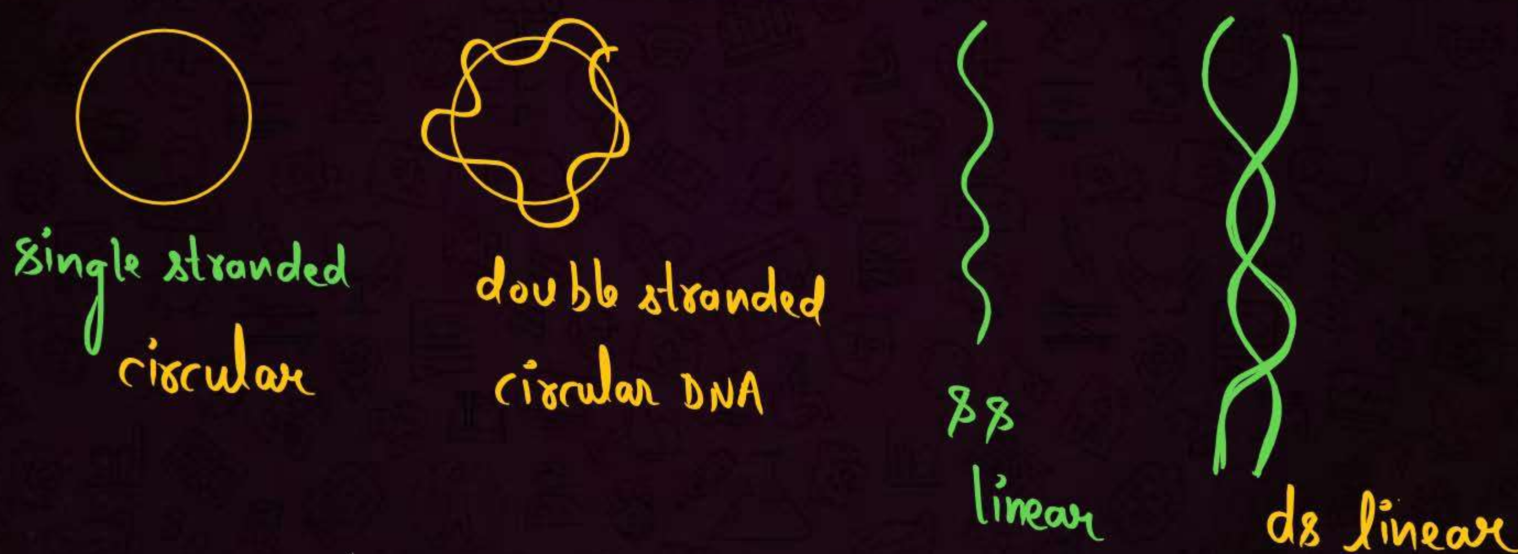
and called it as **NUCLEIN**

↓

later on called as **Nucleic acid**

$\boxed{\text{DNA}} \rightarrow \boxed{\text{POLYMER}}$ (POLYNUCLEOTIDE)

Formed of many $\boxed{\text{Nucleotide}}$



How to measure → $\boxed{\text{length of DNA}}$

In form of

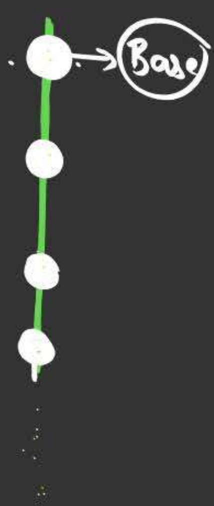
$\boxed{\text{number of nucleotides / Bases}}$

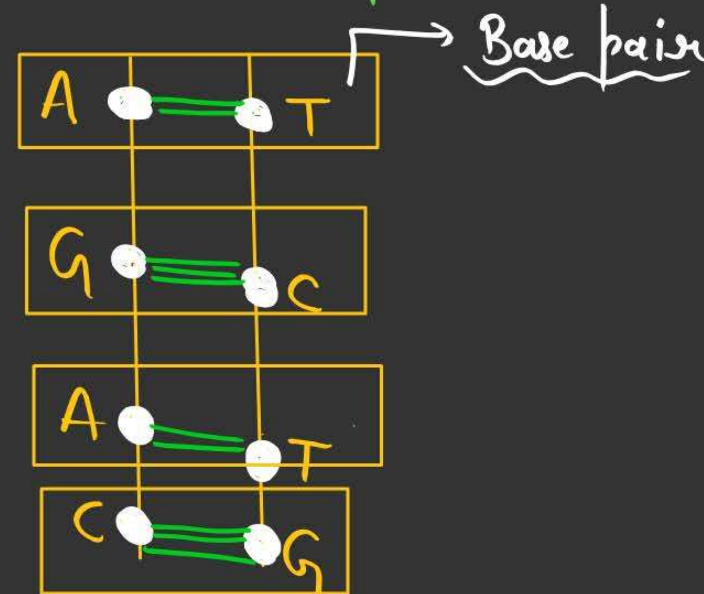
In the form of → ds DNA

$\boxed{\text{Base pairs}}$

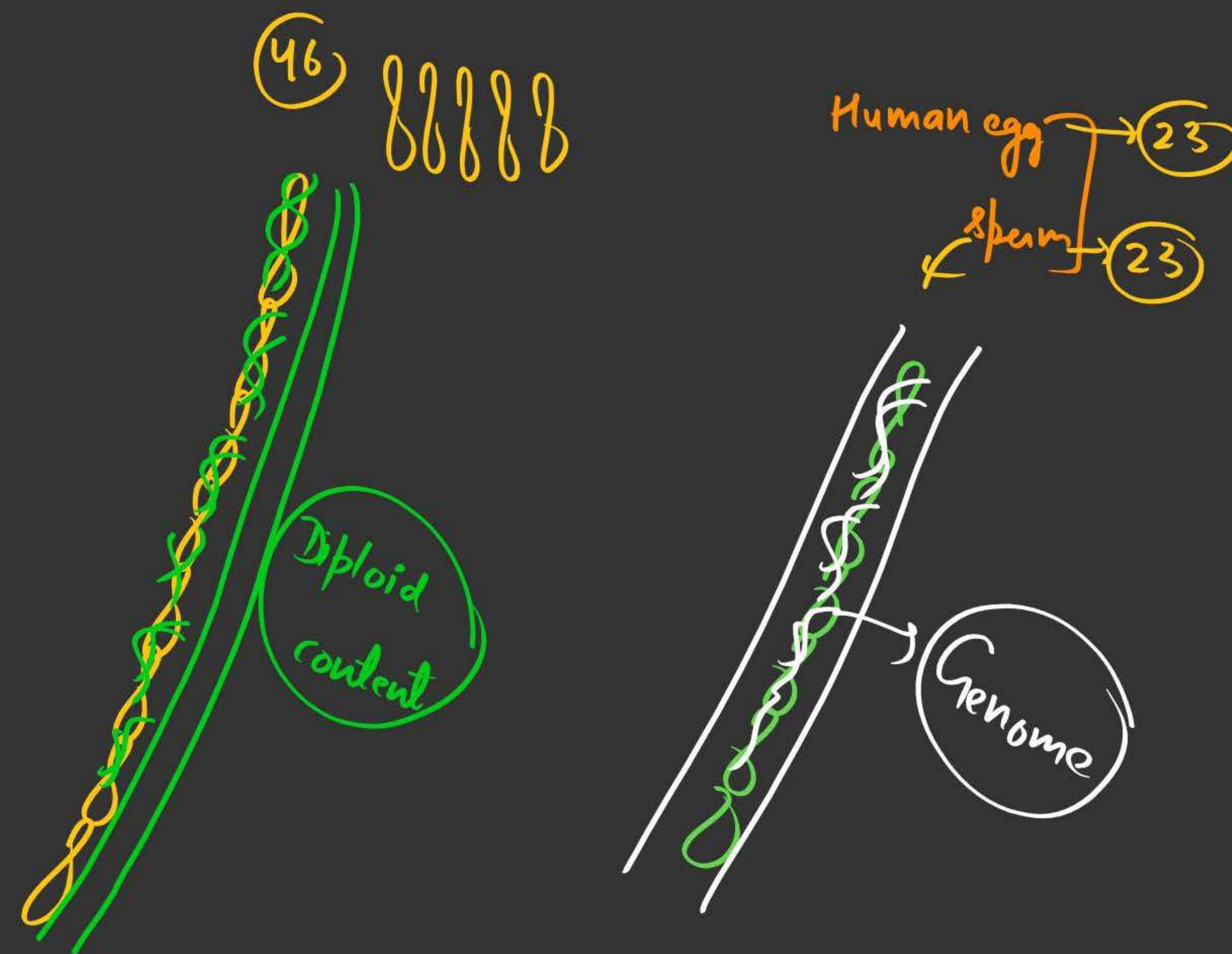
If DNA

is single stranded





Organisms	DNA	(Genome content) DNA length (Bases or Base pairs)
1. ϕ 174 (Haploid) (Virus) (Bacteriophage)	 ss circular	(Haploid content) $\boxed{5386}$ Nucleotides | Bases
2. λ-phage (lambda phage) (Haploid) (Bacteriophage) (Virus)	 ds linear	$\boxed{48,502}$ Base pairs
3. E. coli (Bacteria) (Haploid)	 ds circular	4.6×10^6 base pairs
4. Humans	 ds linear	3.3×10^9 base pairs


46
Diploid content
Human egg
23
Sperm
23
Genome

Q.

Human Skin cell → Diploid cell

DNA content = ?

a) 6.6×10^9 nucleotides

b) 13.2×10^9 nucleotides / Bases

c) 3.3×10^9 bases

d) 3.3×10^9 base pairs

Diploid cell (2n)

↓

6.6×10^9 base pairs

1 base pair = 2 nucleotides / bases

$2 \times 6.6 \times 10^9$ Nucleotides / Bases

13.2×10^9 Nucleotides / Bases

$\boxed{\text{Human Haploid cell}}$

$\downarrow$

Base pairs = 3.3×10^9 bps

Nucleotides | Bases = 6.6×10^9 nucleotides | Bases.

Search for Genetic material

started in 1926

In 1928

Griffith's Experiment

In 1944

Mcleod, McCarty

& Avery

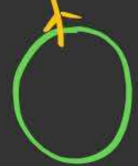
In 1952

Hershey and chase.

Griffith → In 1928

Bacteria: Streptococcus / Pneumonococcus

Cell wall



R-II strain

→ Capsule Absent

→ Rough strain

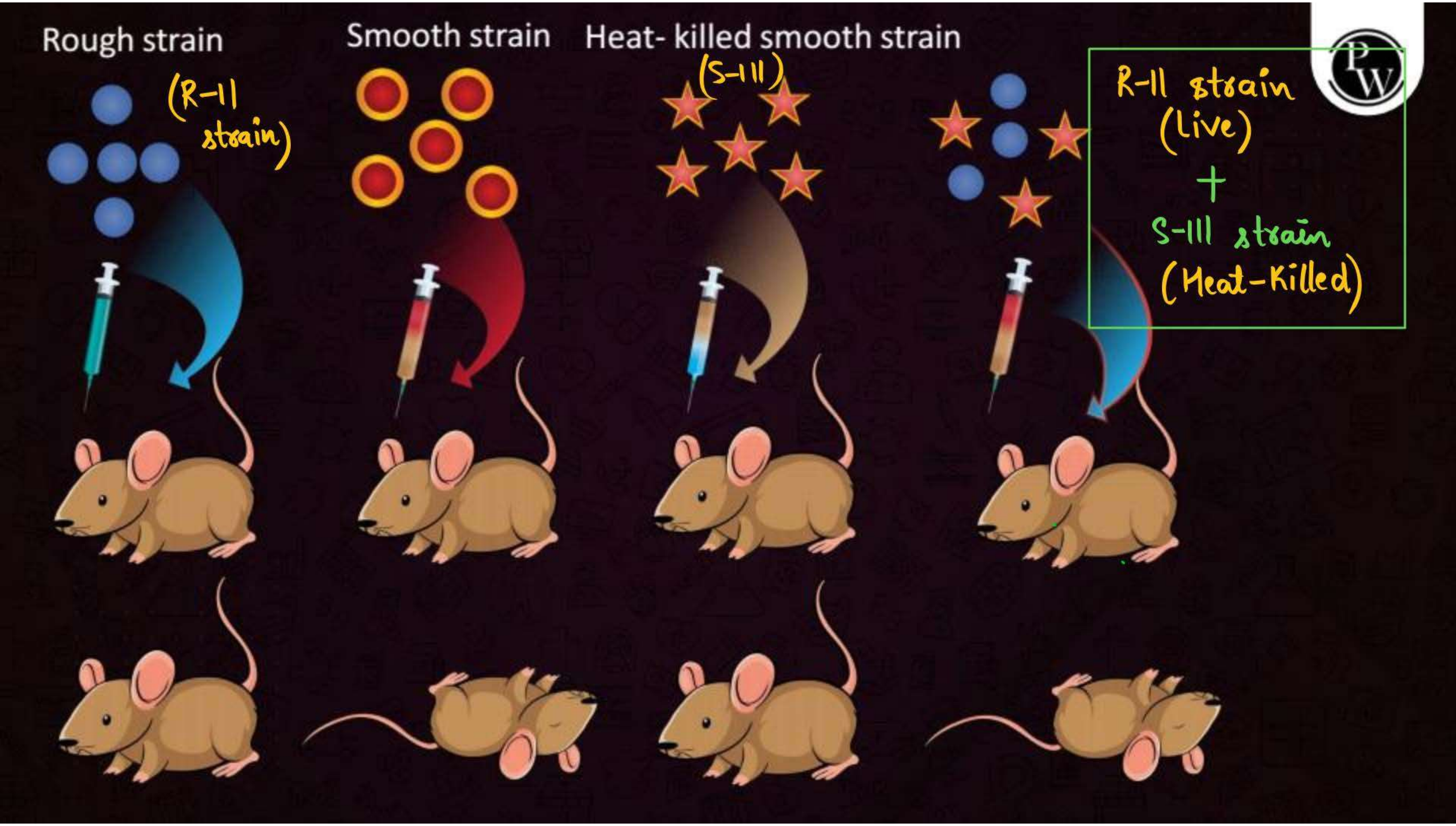
→ Non-virulent | Non-pathogenic

S-III strain

→ Capsule present

→ Smooth strain

→ Virulent | Pathogenic



Rough strain
(R-II strain)
Smooth strain
Heat- killed smooth strain
(S-III)
R-II strain (Live)
+
S-III strain (Heat-Killed)

Conclusion: There is some $\boxed{\text{Transforming Principle}}$ which is released by S-III strain & accepted by R-II strain. Due to $\boxed{\text{R-II strain}}$ transformed to S-III strain.

* He did not know the $\boxed{\text{Biochemical Nature}}$ of $\boxed{\text{Transforming Principle}}$

From 1933–1944 → Protein is the Genetic material.

Mcleod, McCarty and Avery → In 1944

↓

Biochemical Nature of Transforming Principle is DNA

* First suggested that "DNA is the genetic material".

Heat-Killed S-III strain

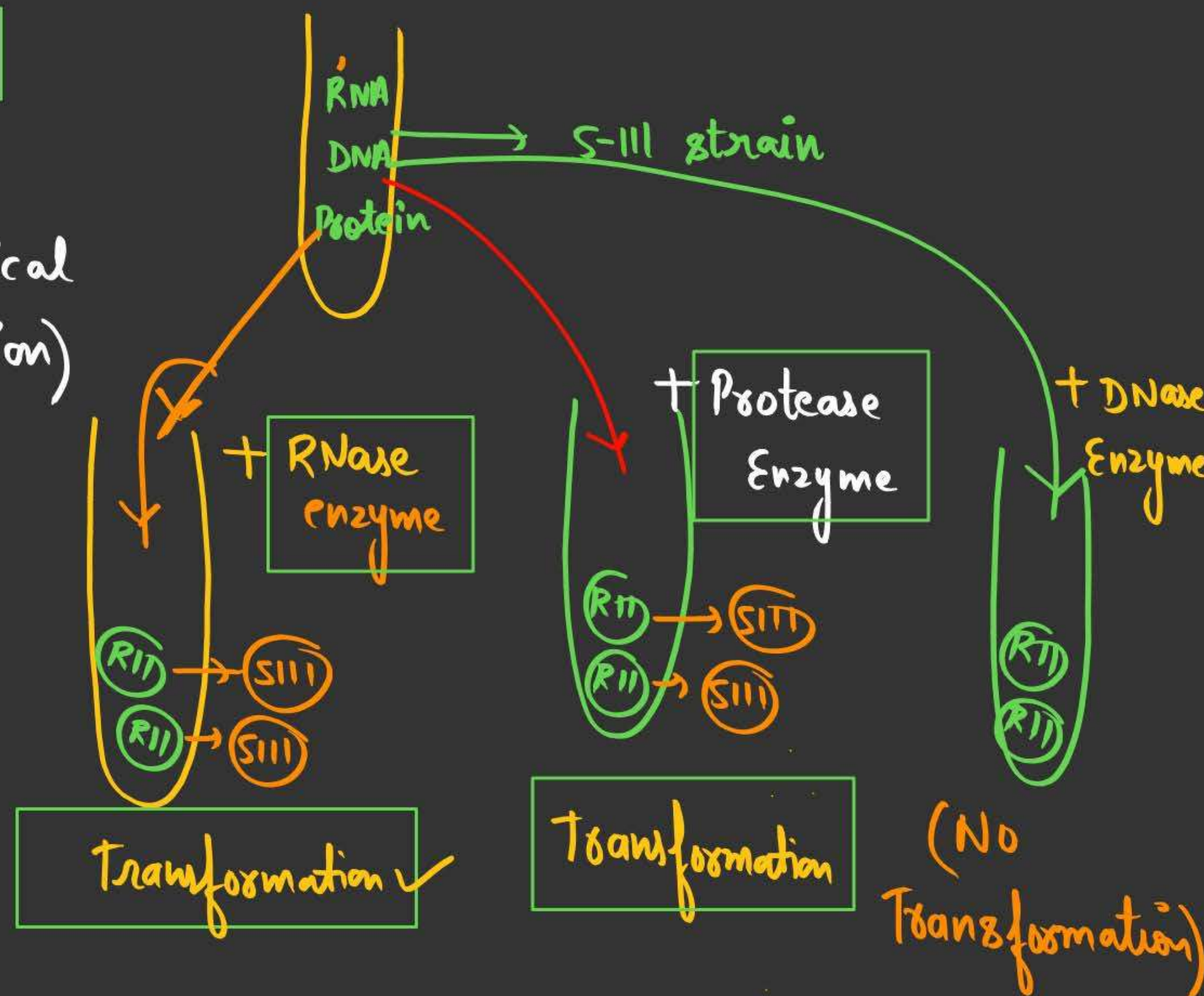
↓

Extracted (Biochemical extraction)

→ RNA

→ DNA

→ Protein

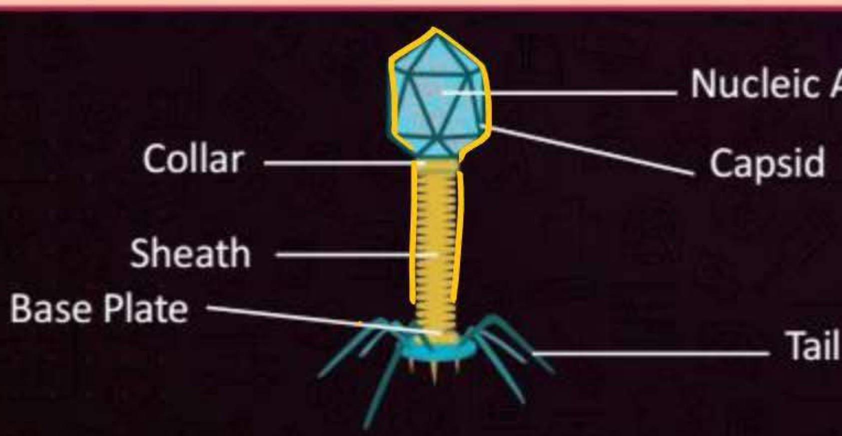


Hershey & Chase Experiment

→ In 1952

Provided Unequivocal Proof that DNA is the genetic material.

Experiment
↓
Virus
(T_2 - Bacteriophage)
↓
Infects E.coli



(DNA) ds linear

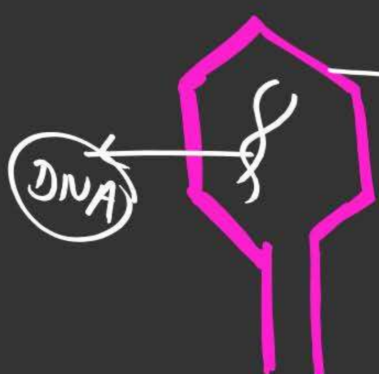
Radioactively labelled the virus

Virus with $\boxed{\text{Radioactive Protein coat}}$

Protein

$\boxed{\text{Sulphur}}$

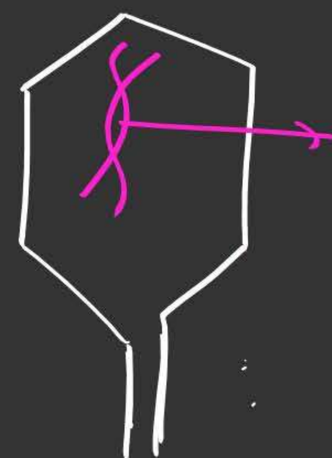
→ Grew virus on Radioactive sulphur medium ($^{35}SO_4$)



Protein coat (S^{35})

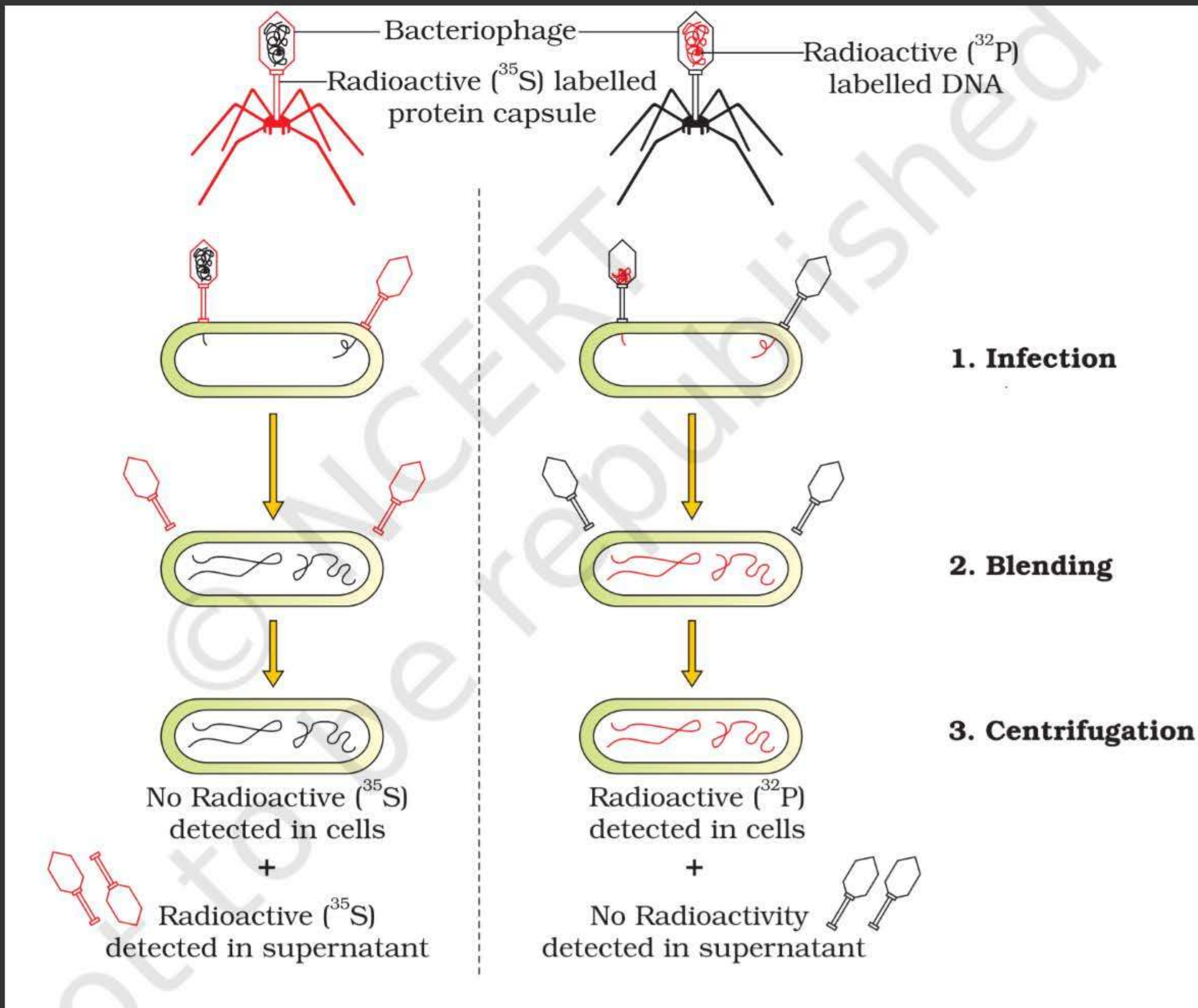
Virus with $\boxed{\text{Radioactive DNA}}$

→ Medium → $P^{32}O_4$



DNA

(P^{32})



supernatant

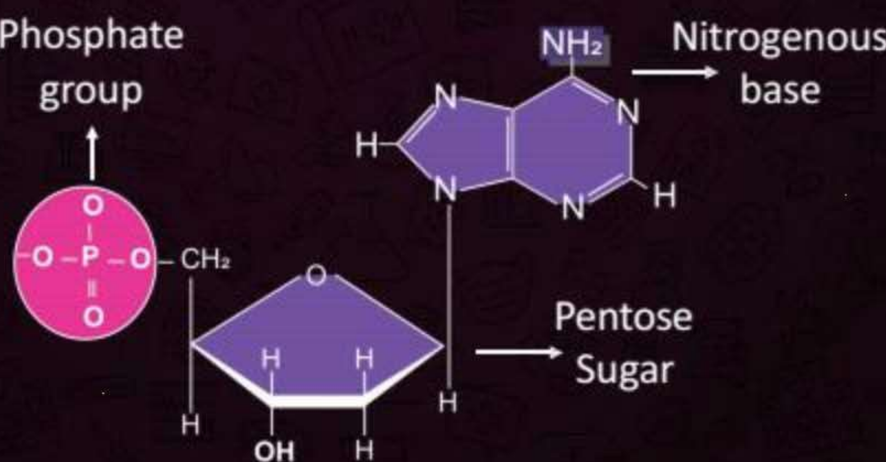
(Part of virus not injected in E.coli

E.coli + part of virus

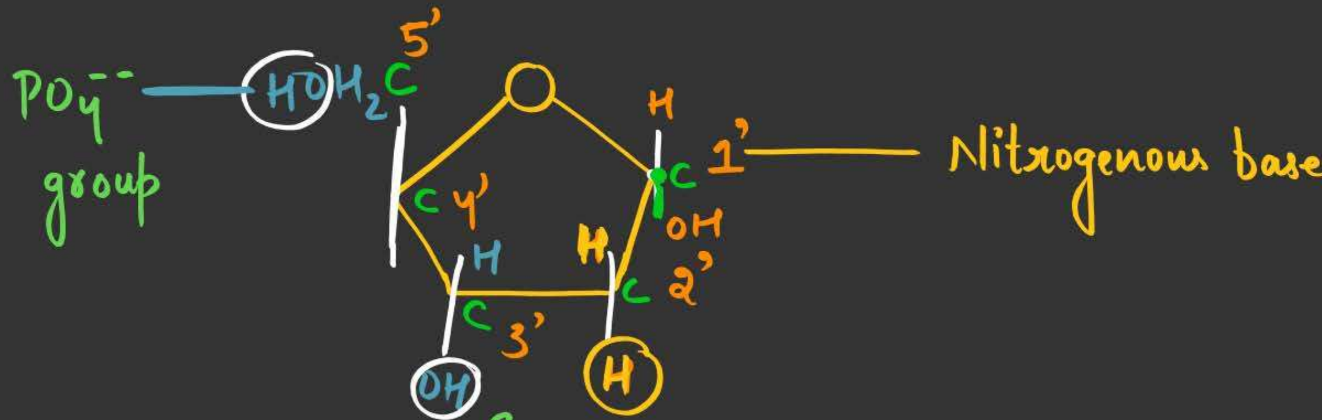
Structure of Polynucleotide chain

The basic unit of DNA is a nucleotide which has three components

- Nitrogenous base
- Pentose sugar (deoxyribose)
- Phosphate group



Nucleotide



Sugar

(Pentose Sugar)

(Deoxyribose sugar)

(2'-C there is no OH group)

Nucleotide

Sugar + PO_4^{--} + Nitrogenous base

Nucleoside

Sugar + Nitrogenous base

$\boxed{\text{Nucleotide} = \text{Nucleoside} + PO_4^{--} \text{ group}}$

Nucleotide

Monophosphate	Diphosphate	Triphosphate form
NMP	NDP	NTP
(Nucleoside monophosphate)	(Nucleoside diphosphate)	(Nucleoside triphosphate)

Nucleoside

AMP → Adenosine Monophosphate

CMP → Cytidine Monophosphate

GMP → Guanosine ,,

TMP → Thymidine ,,

UMP → Uridine ,,

RNA

ADP → Adenosine diphosphate

GDP

TDP

CDP

UDP

ATP → Adenosine triphosphate

GTP

TTP

CTP

UTP

DNA

Nucleotides

dAMP
(deoxyadenosine monophosphate)

dGMP

dTMP

dCMP

RNA

AMP ✓
(Adenosine monophosphate)

GMP ✓

CMP ✓

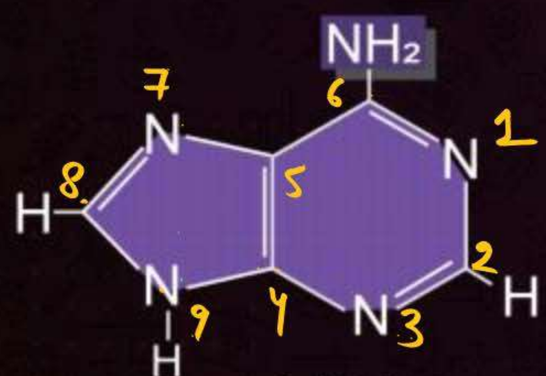
UMP ✓

Nitrogenous base

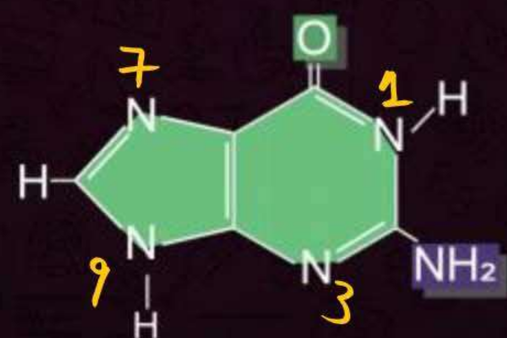
Purines — Adenine, Guanine

Pyrimidines — Uracil → RNA, Thymine, cytosine

Purines - Heterocyclic 9-membered double-ring structure with N at position 1, 3, 7 and 9.
Eg: Adenine (A) and Guanine (G).



Adenine



Guanine

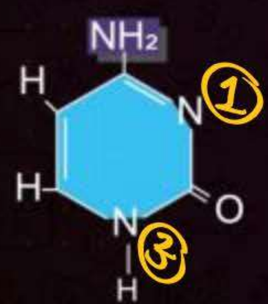
Nitrogenous base

Imp

Thymine
↓
5-methyl Uracil

Pyrimidines- Heterocyclic, 6-membered single-ring structure with N at 1 and 3 position,
Eg., Cytosine (C), Thymine and Uracil. Cytosine is common in both DNA and RNA; thymine is present in DNA and uracil is present in RNA at the place of thymine.

Cytosine



Thymine

Uracil

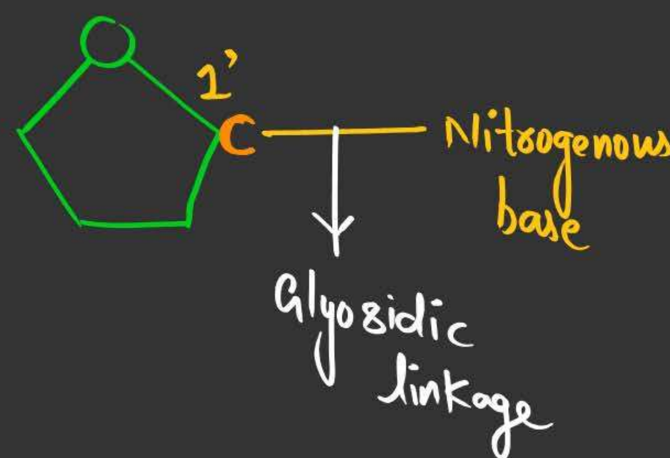
✗ Rest are (Covalent Bonds)

Bonds / linkages in a Single Nucleotide

N - Glycosidic Linkage

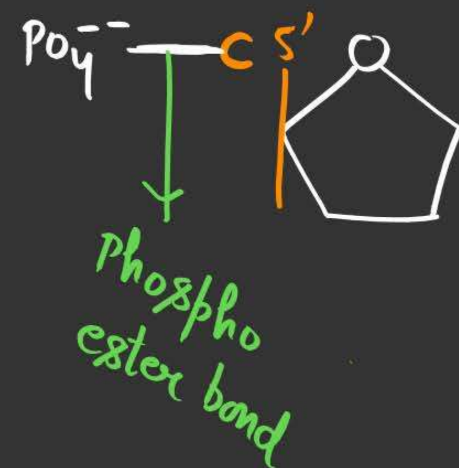
Between

1'C of sugar and Nitrogenous base



Phosphoester linkage

Bond b/w 5'-C of sugar & PO_4^{--} group



Linkage

(Glycosidic bond) =

Purine

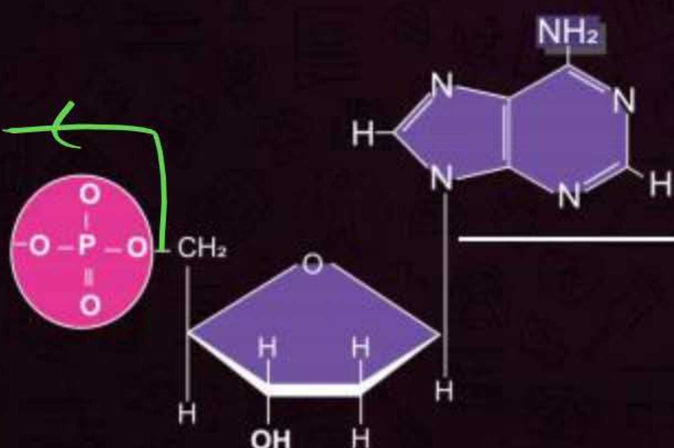
1'C of sugar — 9th N of purine

N-glycosidic linkage: A nitrogenous base is linked to the pentose sugar through a N-glycosidic linkage to form a nucleoside Purine nucleosides have 1-9 glycosidic linkage. Pyrimidine nucleosides have 1-1 linkage.

Pyrimidine

1'c of sugar – 1st N of pyrimidine

Phospho-ester linkage



N-glycosidic Linkage

Phosphodiester Bond

Present between $\boxed{2 \text{ Nucleotides}}$

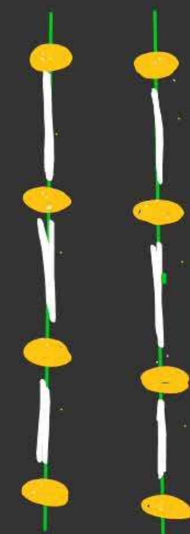
ds DNA = 4 base pairs
↓
8 nucleotides

ss DNA

→ Phosphodiester bond (P·B)

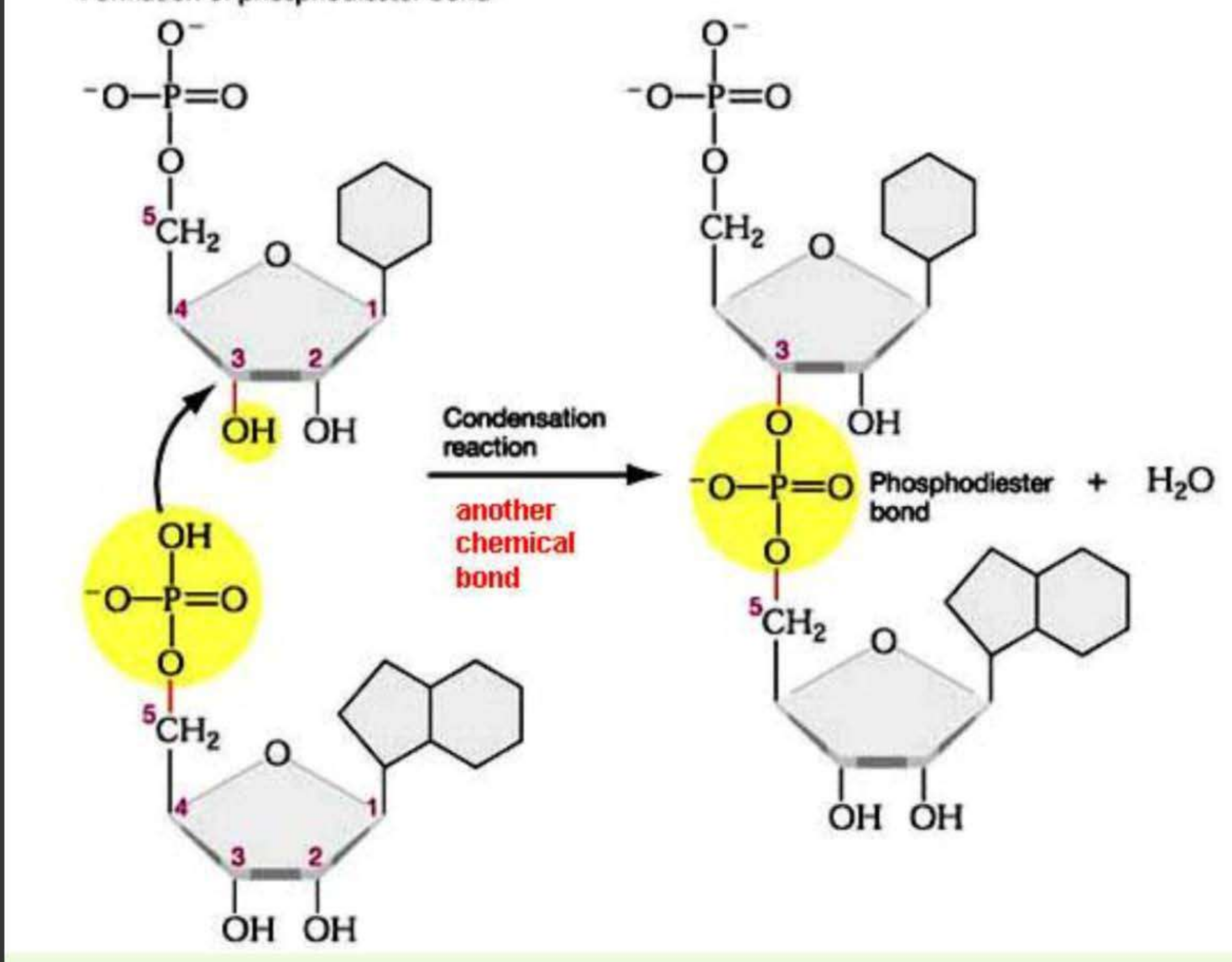
$$\boxed{P\cdot B = \text{No. of nucleotides} - 1}$$

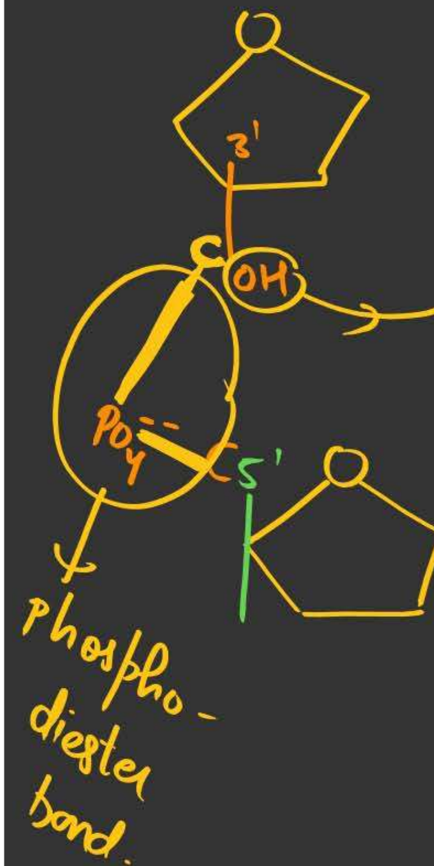
ds DNA

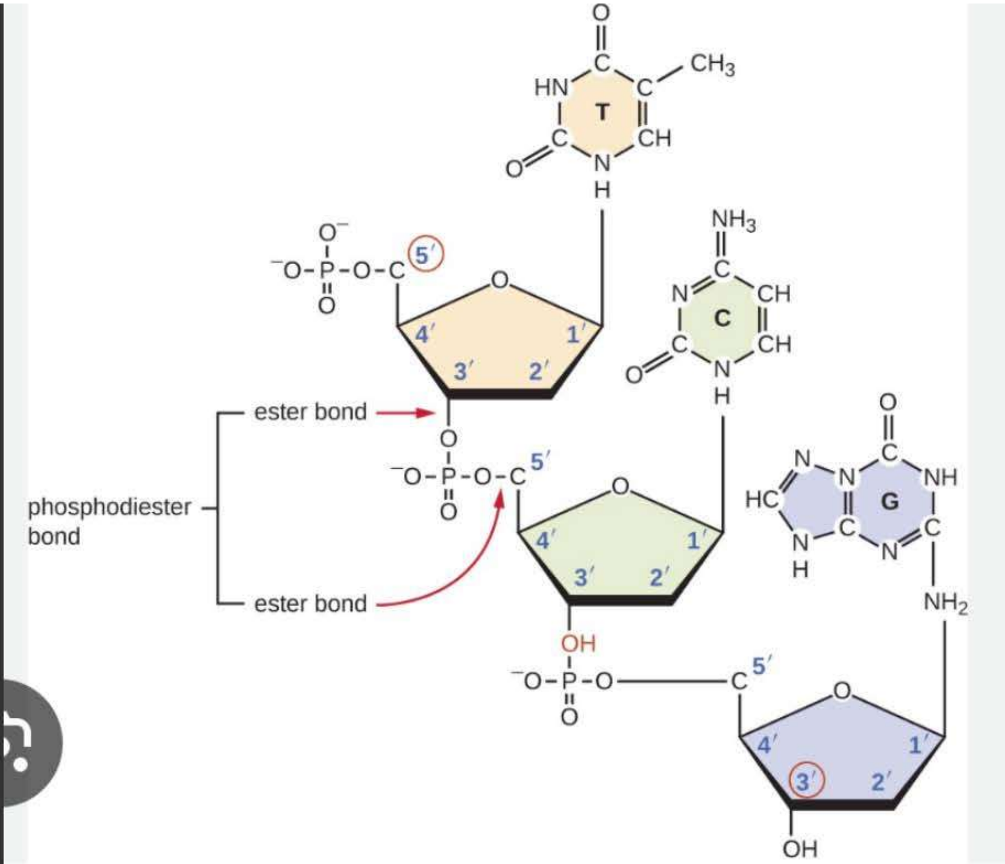


$$\boxed{P\cdot B = \text{No. of nucleotides} - 2}$$

Formation of phosphodiester bond







Phosphodiester bond

⭐ Polarity → $\boxed{3' - 5'}$ ⭐

Q) $\underline{\text{DNA}}$ = $\boxed{\text{1,000 base pairs}}$ = $\boxed{2,000}$ nucleotides/Bases
(ds DNA)

a) No. of glycosidic linkages = 2,000

b) No. of sugar molecules = 2,000

c) No. of phosphate groups = 2,000

d) No. of phosphodiester Bonds = P.B = No. of nucleotides − 2
2,000 − 2
= 1998

Derivation of DNA Structure

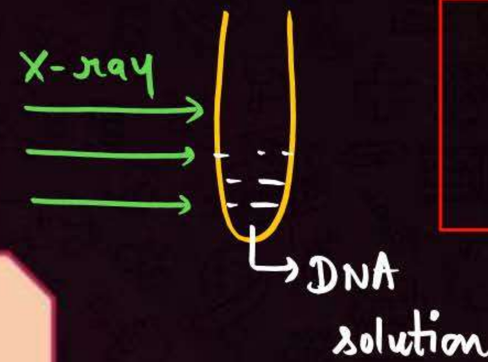
Two lines of investigations helped in derivation of DNA structure

X-ray Crystallography → Wilkins & Franklin

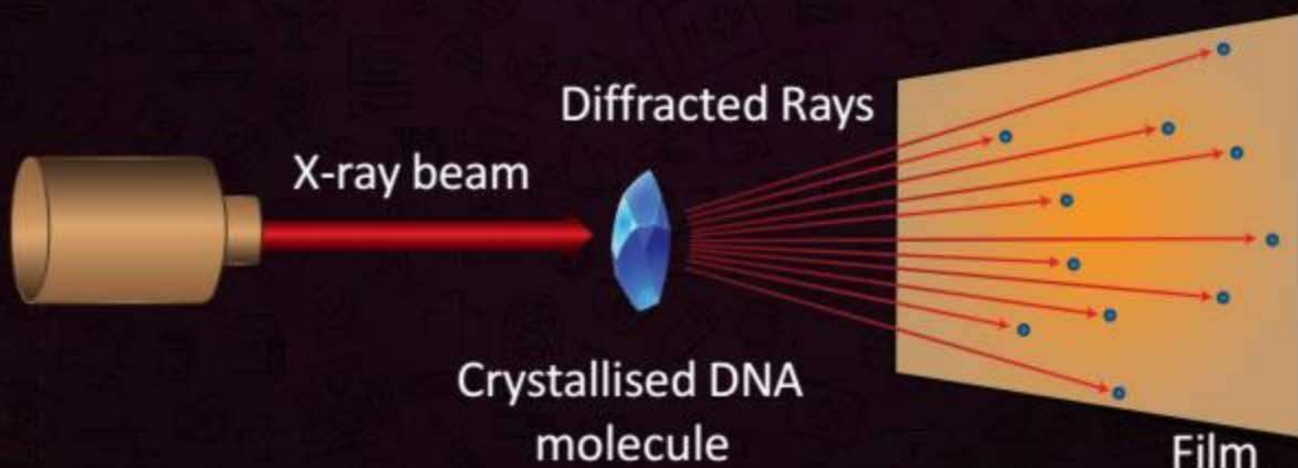
Chargaff's rule → Erwin Chargaff

X-ray Crystallography

→ Diffaraction study



Maurice Wilkins and Rosalind Franklin obtained very fine X-ray diffraction pictures of DNA.



Wilkins & Franklin

Conclusion: * DNA has double-helical structure

* DNA has periodicity of 3.4 Å

→ Distance between 2 base pairs = 3.4 Å

or

0.34 nm

Erwin Chargaff's Rules

Performed $\boxed{\text{Base composition studies on DNA}}$ → Applied only to $\boxed{\text{dsDNA}}$

- Purines and pyrimidines occur in equal amounts. A+G=T+C

$$\boxed{\frac{A+G}{T+C} = 1}$$

- Sugar deoxyribose and phosphate residues occur in equal number.

- Purine adenine is equimolar with pyrimidine thymine

$$A = T \quad \frac{A}{T} = 1$$

- Purine guanine is equimolar with pyrimidine cytosine.

$$C = G \quad \frac{C}{G} = 1$$

Erwin Chargaff's Rules

This value is constant for all species

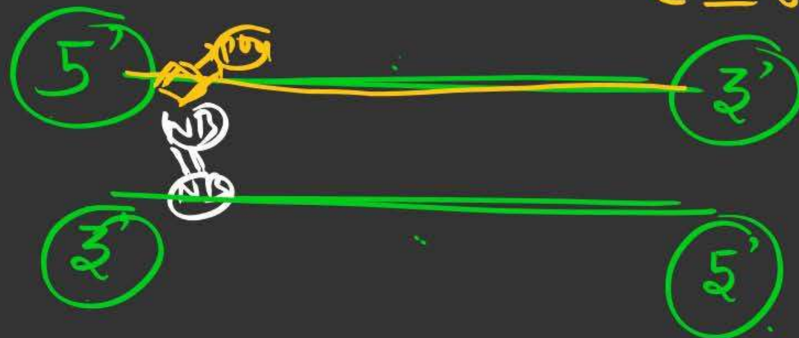
$$\frac{A+G}{T+C} = 1$$

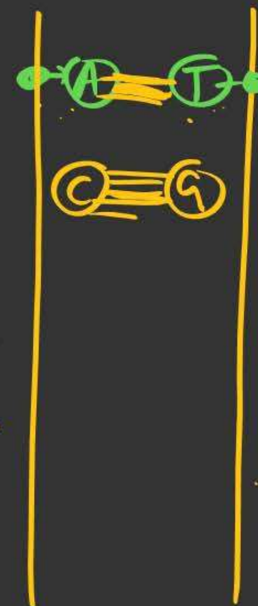
Base ratio is specific for a species.
It is used to identify a species.

$$\frac{A+T}{G+C}$$

$A = T$

$C \equiv G$





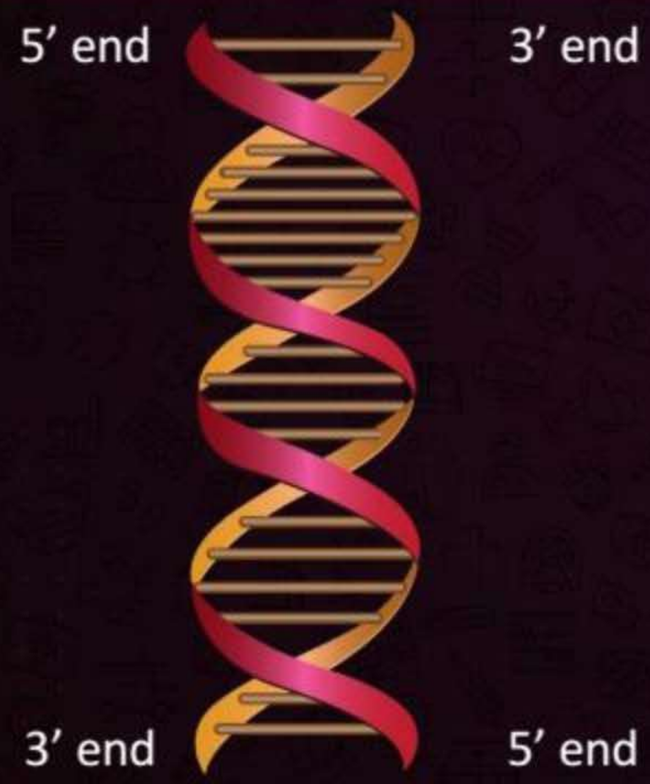
Salient features of DNA → Watson & Crick Model → In 1953 ⇒ B-DNA

(Right-handed coiling)

James Watson and Francis Crick on the basis of previous information proposed a very simple but famous double helix model for the structure of DNA.

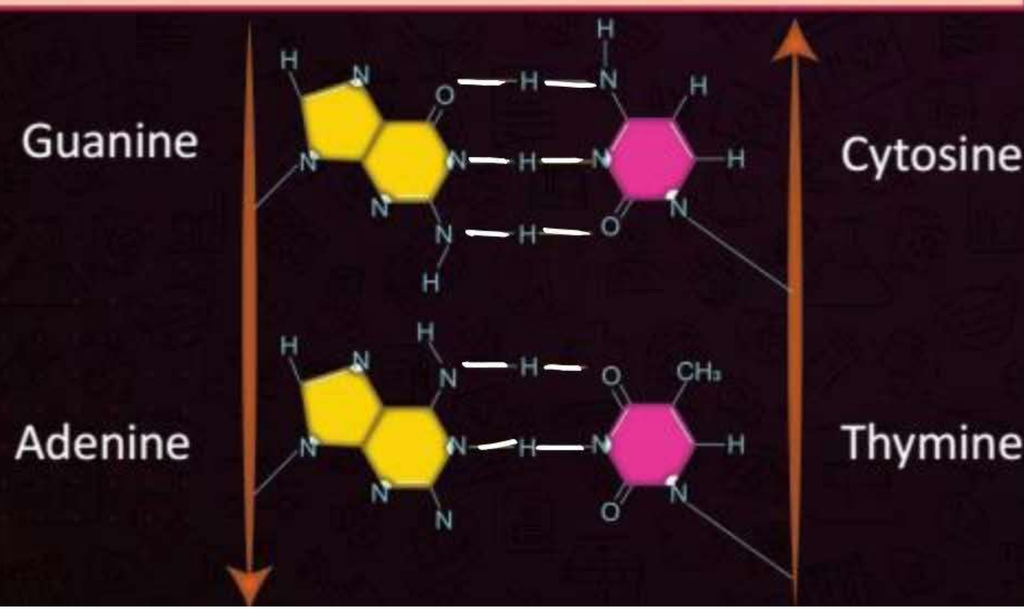
DNA consists of two polynucleotide chains. The backbone is constituted by sugar-phosphate and the bases project inside

The two chains of DNA run in anti-parallel fashion with 5'→3' polarity in one and 3'→5' polarity in other chain.



The bases in two strands are paired through hydrogen bonds forming base pairs (bp).

Adenine forms 2 H-bonds with thymine from opposite strand and vice versa. Similarly, guanine is bonded with cytosine with three H-bonds.

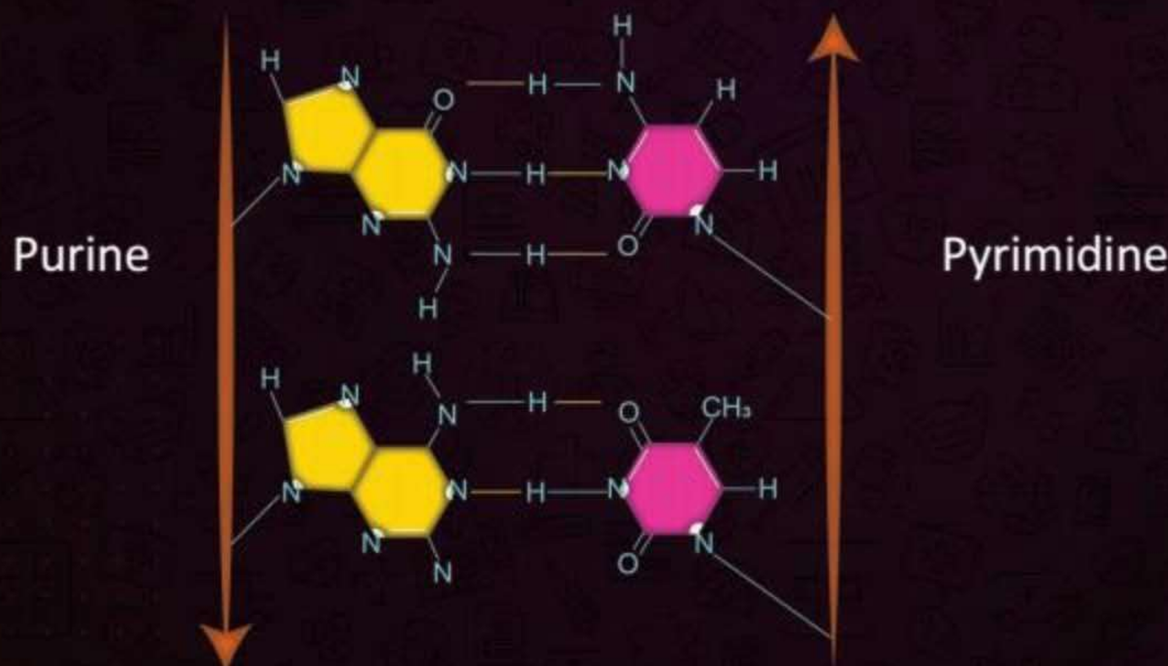


* $\boxed{\text{Hallmark}}$ of watson & crick Model is $\boxed{\text{Complementary Base-pairing}}$

A = T (Based on Erwin Chargaff's work).

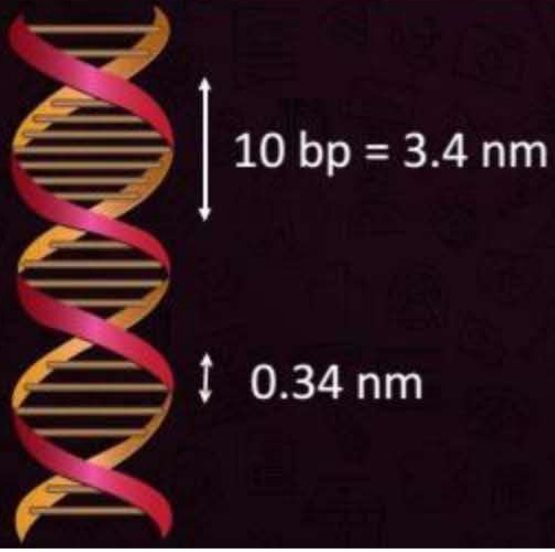
C ≡ G

As a result, always a purine comes opposite to a pyrimidine. This generates approximately uniform distance between the two strands of helix.



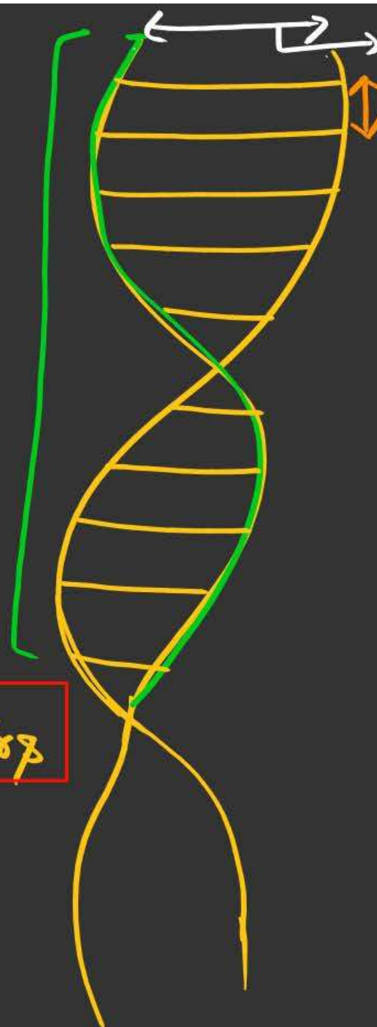
The double chain of DNA is helically twisted in a right-handed fashion.

Each turn of double helix or the pitch of the helix is 3.4 nm. It has roughly 10 base pairs in each turn. The distance between two adjacent base pair is approximately equal to 0.34 nm.



1 Helix | 1 Pitch

(1 complete turn)



Helical diameter

Distance b/w 2 pairs = $\boxed{0.34\ nm}$ / $3.4\ \mathring{A}$

$\boxed{\text{length of one Helix / Pitch}}$

= $\boxed{3.4\ nm}$ / $34\ \mathring{A}$

1 Helix

Has $\boxed{\text{10 Base pairs}}$

Helical diameter | Distance between 2 strands

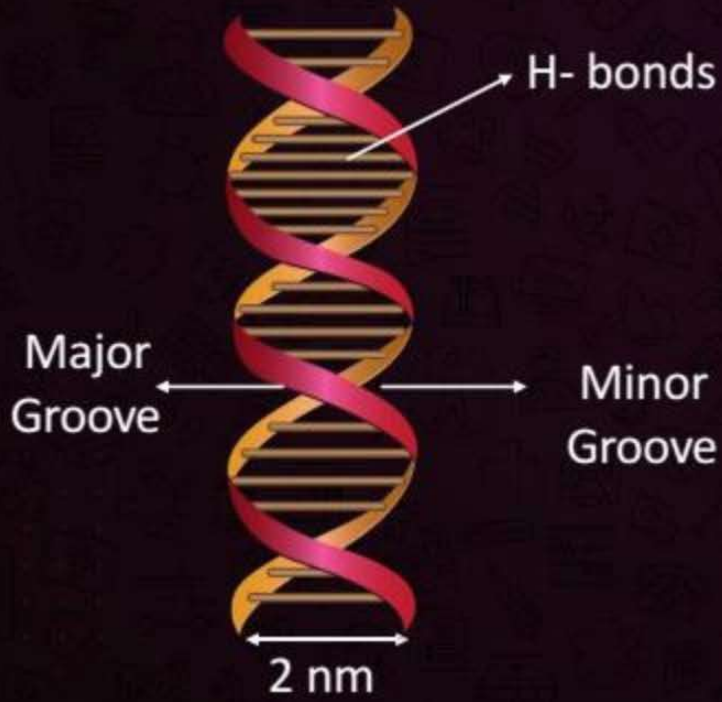
= $\boxed{2\ nm\ \text{or}\ 20\ \mathring{A}}$

The plane of one base pair stacks over the other in double helix. This, in addition to H-bonds, contribute stability to the helical structure.

Stability to Helical structure

1) Hydrogen - Bonding

2) Plane of one base pair stacks over the plane of other base pair.



If a DNA molecule has 2000 bp then, calculate the

(a) Number of sugar and phosphate molecules

(b) Number of N-glycosidic linkage

DNA was extracted from Streptococcus bacterium. The proportion of Adenine was found to be 28%, then calculate the amount of cytosine

$A = T$

$A = 28\%$

$T = 28\%$

56%

$C = G$

100
56
44%

$C = 22\%$

$G = 22\%$

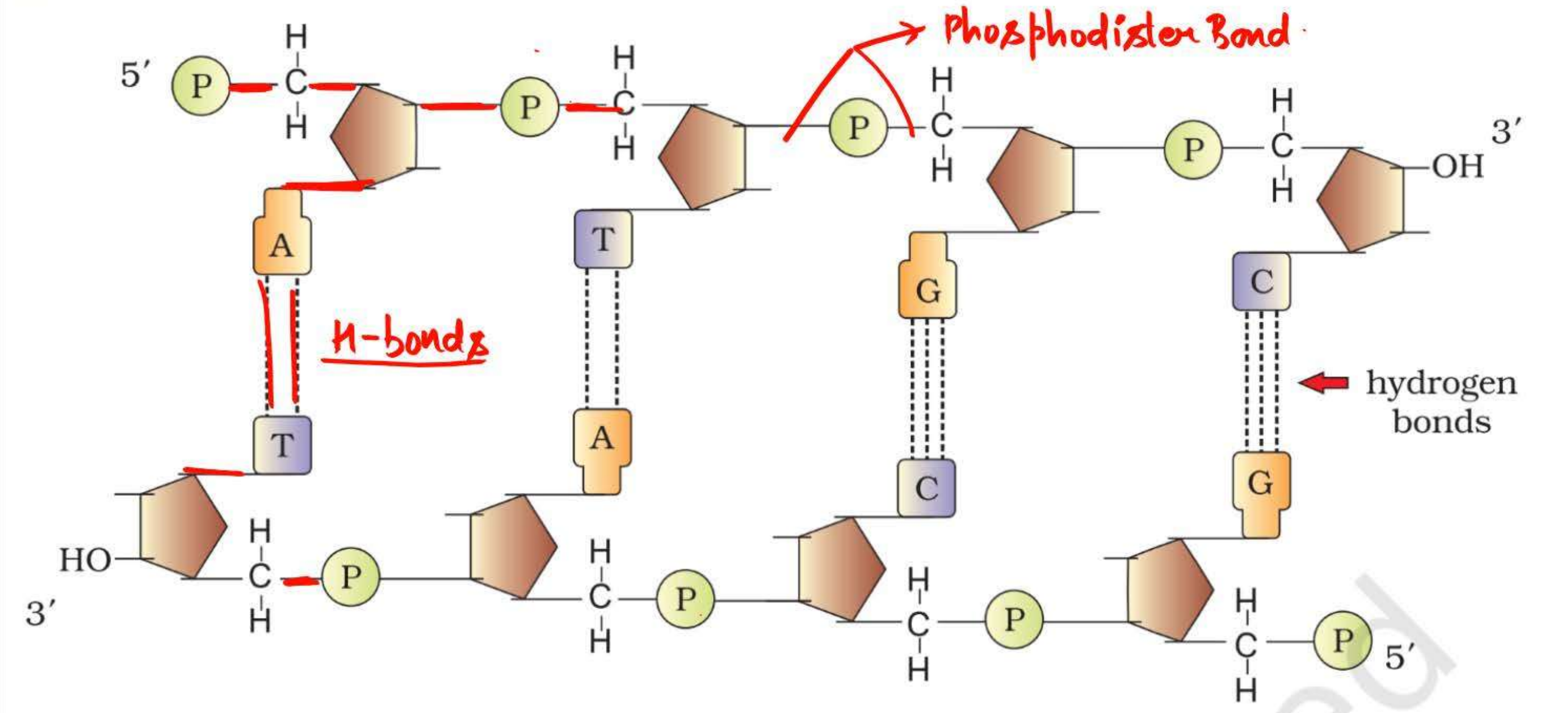


Figure 5.2 Double stranded polynucleotide chain

If the sequence of one strand of DNA is written as follows:

5- TG CA GC TA GC TA GC AT CG-3’

Write down the sequence of complementary strand in ~~5'→3'~~ direction.

3'-5'

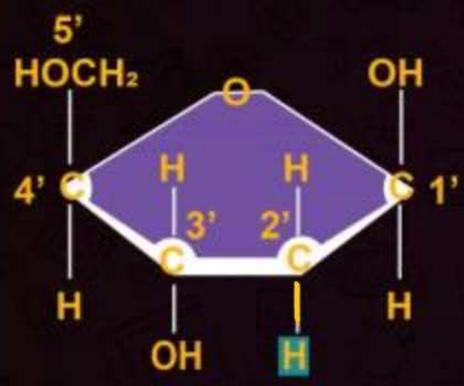
HW

DNA VS RNA

DNA	RNA
➢ ds	➢ ss

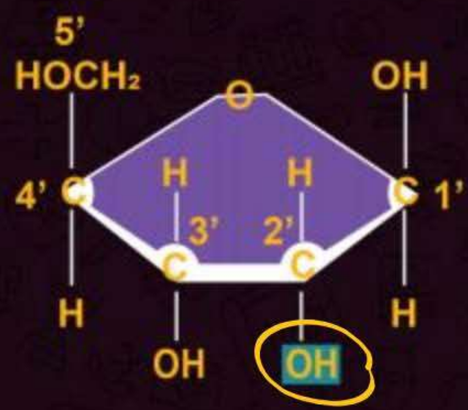
DNA has repairing mechanism

DNA



- Contains Deoxyribose Sugar
- Act as a genetic material

RNA



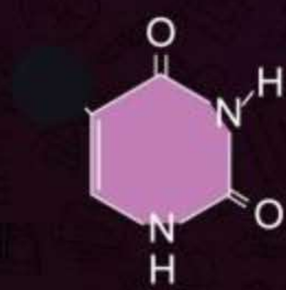
- Contains Ribose Sugar
- Used to act as a genetic material
- Act as Catalyst
- Has structural role as well

DNA

RNA

- Have Thymine

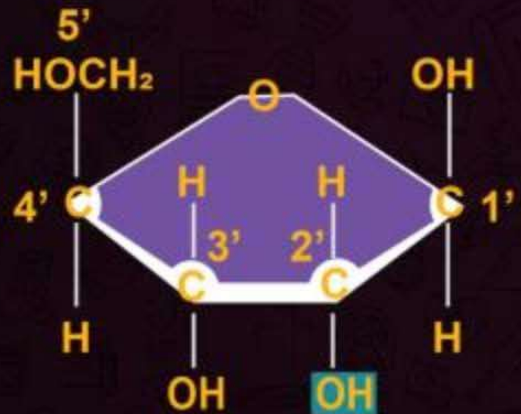
- Have Uracil



- Has evolved from RNA
- Was the first genetic material

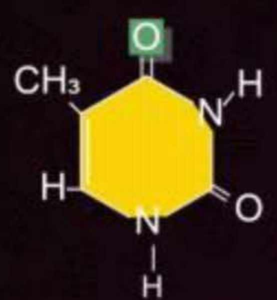
Properties of RNA

Free 2'OH of RNA makes it more labile and easily degradable. Therefore DNA in comparison is stable.



Presence of thymine (5-Methyl uracil) at the place of uracil also confers additional stability to DNA.

DNA has Thymine



RNA has Uracil

RNA being unstable, mutates at a faster rate. Consequently, viruses having RNA genome can din code for the synthesis of proteins, hence can easily express the characters.

RNA World

RNA was the first genetic material.

There are evidences to suggest that essential life processes, such metabolism, translation, splicing etc. evolved around RNA.

But, RNA being a catalyst was reactive and hence unstable. Therefore DNA has evolved from RNA with chemical modifications that make it more stable.

RNA

- Act as Catalyst
  - To act as Enzyme (Ribozyme)
    - Peptidyl transferase
    - Snurps
- Structural Role
  - Form structure of Ribosome (r-RNA)

DNA being double strand and having complementary strand further resists changes by evolving a process of repair.

RNA also acts as a structural molecule.

Thus, it can be said DNA being stable is preferred for storage of genetic material and for the transmission of genetic information RNA is better material.

Or

DNA → $\boxed{\text{Storage of genetic information}}$

$\boxed{\text{RNA → Transmission of genetic information}}$

Properties of Genetic Material

1. Chemically stable

2. Structurally stable

3. Transmit itself in form of Mendelian laws.

4. Mutation: → At slow rate

5. Should replicate itself.

Central Dogma of Molecular Biology

Proposed by Francis Crick, it explains one way or unidirectional flow of information from master copy DNA to working copy RNA and from RNA to building molecule.

"Replication"

DNA $\xrightarrow{\text{"Transcription"}}$ mRNA $\xrightarrow{\text{Translation}}$ Protein

Responsible for Expression.

Reverse Central Dogma or Teminism

* In some Retroviruses (HIV)

Has $\boxed{\text{Reverse Transcriptase Enzyme}}$

RNA $\longrightarrow$ DNA

Replication

$$\text{DNA} \underset{\text{"Reverse Transcription"}}{\overset{\text{Transcription}}{\rightleftarrows}} \text{mRNA} \xrightarrow{\text{Translation}} \text{Protein}$$

Packaging of DNA

In Prokaryotes (Bacteria)

(1 - 2 μm)

Bacteria

E. coli = $\boxed{4.6 \times 10^6 \text{ base pairs}}$

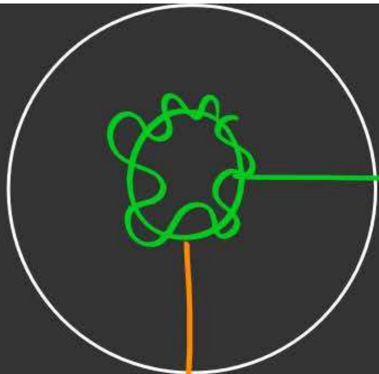
DNA length ~ $\boxed{1.36 \text{ mm}}$

(10^{-3} m)

DNA
base pairs
distance

[= 0.34 nm]

* $\boxed{\text{DNA is packed | supercoiled}}$



NUCLEOID

Region where packed DNA is present

DNA is not scattered

* DNA packaging

↓

Proteins → POLYAMINES

→ Basic proteins

→ Positively charged

DNA

↓

Negatively charged

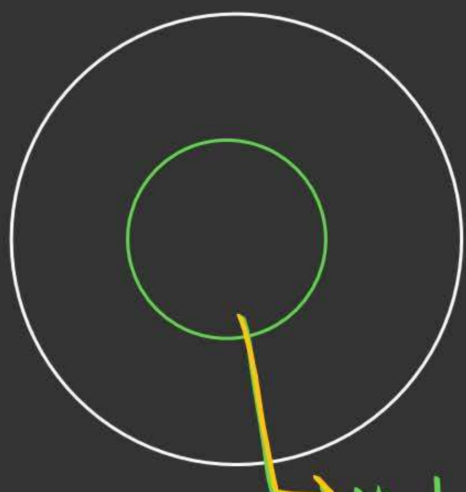
Eukaryotes

Human Diploid cell = $\boxed{6.6 \times 10^{9} \text{ base pairs}}$

Distance b/w ② bps = 0.34 nm $(0.34 \times 10^{-9}$ m$)$

DNA length $= 6.6 \times 10^{9} \times 0.34 \times 10^{-9}$

$= \boxed{2.2 \text{ metres}}$



Nucleus $(10^{-6}$ m$)$

Histone Proteins

→ Basic proteins

→ Rich in Lysine & Arginine

→ Positively charged

Histone Proteins ↓ 5 - types:

- H_1
- H_2A
- H_2B
- H_3
- H_4

H_2A, H_2B, H_3, H_4 ↓ Participates in formation of **Histone octamer**

$\boxed{\text{Histone octamer}}$ / Nu-body

↓

8 molecules of Histone-proteins

Histone octamer

2 H_2A

2 H_2B

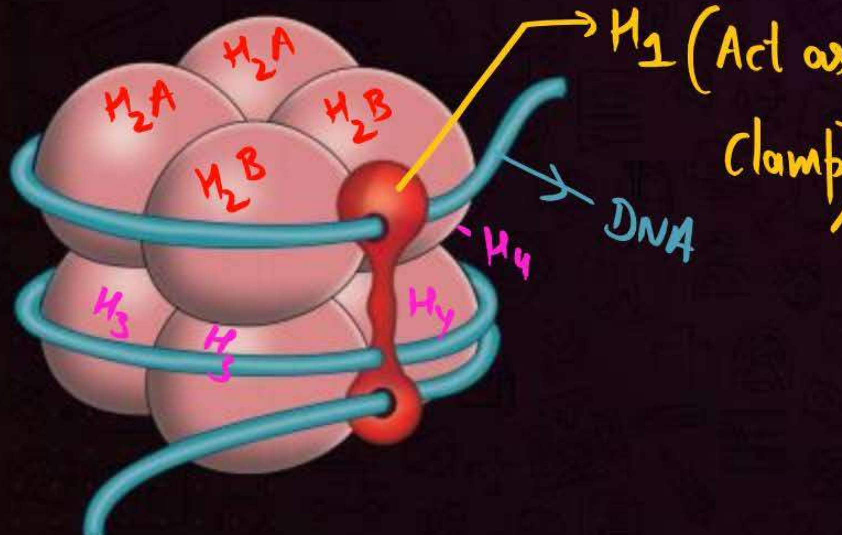
2 H_3

2 H_4

$\boxed{\text{Nucleosome}}$ = Histone octamere + DNA

$\boxed{\text{Single Nucleosome}}$ has $\boxed{\text{200 base pairs}}$ of DNA.

Nucleosome



DNA takes 1.75 / 1 3/4 turns on octamer.

* Nucleosome appears as $\boxed{\text{Beads on string}}$ in electron microscope

The nucleosome constitute repeating unit of a structure in nucleus called chromatin. The nucleosomes in chromatin gives a "Beads on string" appearance under electron microscope.

Q How many nucleosomes in Human diploid cell?

Ans

$$200 \text{ bps} \rightarrow 1 \text{ nucleosome}$$

$$1 \text{ bps} = \frac{1}{200} \text{ nucleosome}$$

$$6.6 \times 10^9 \text{ bps} = \frac{1}{200} \times 6.6 \times 10^9$$

$$= \boxed{3.3 \times 10^7 \text{ Nucleosomes}}$$

*

$\boxed{\text{Higher level of packaging}}$

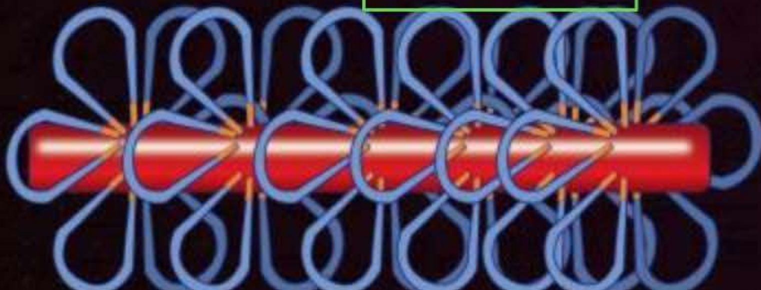
NHCs → Non-Histone chromosomal Proteins

⇒ "Acidic in nature"

____Chromatin____ are further coiled and condensed at metaphase stage of cell division to form chromosomes.

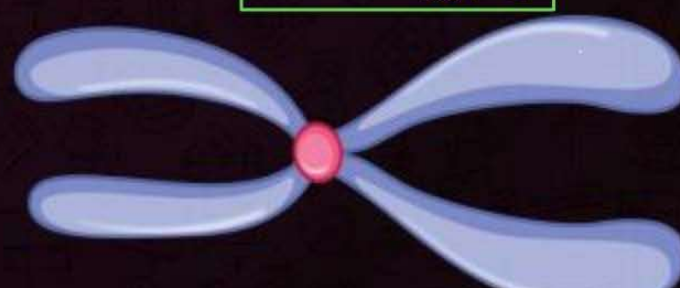
The packaging of chromatin at higher level requires additional set of proteins that collectively are referred as ___NHCs___

chromatin



Further condensation of chromatin

chromosome



Duplicated chromosome

Chromatin

→ DNA

→ Histone proteins

→ NHCs

→ Traces of RNA

→ Ca^{+2}, Mg^{+2}

Chromosome

↓

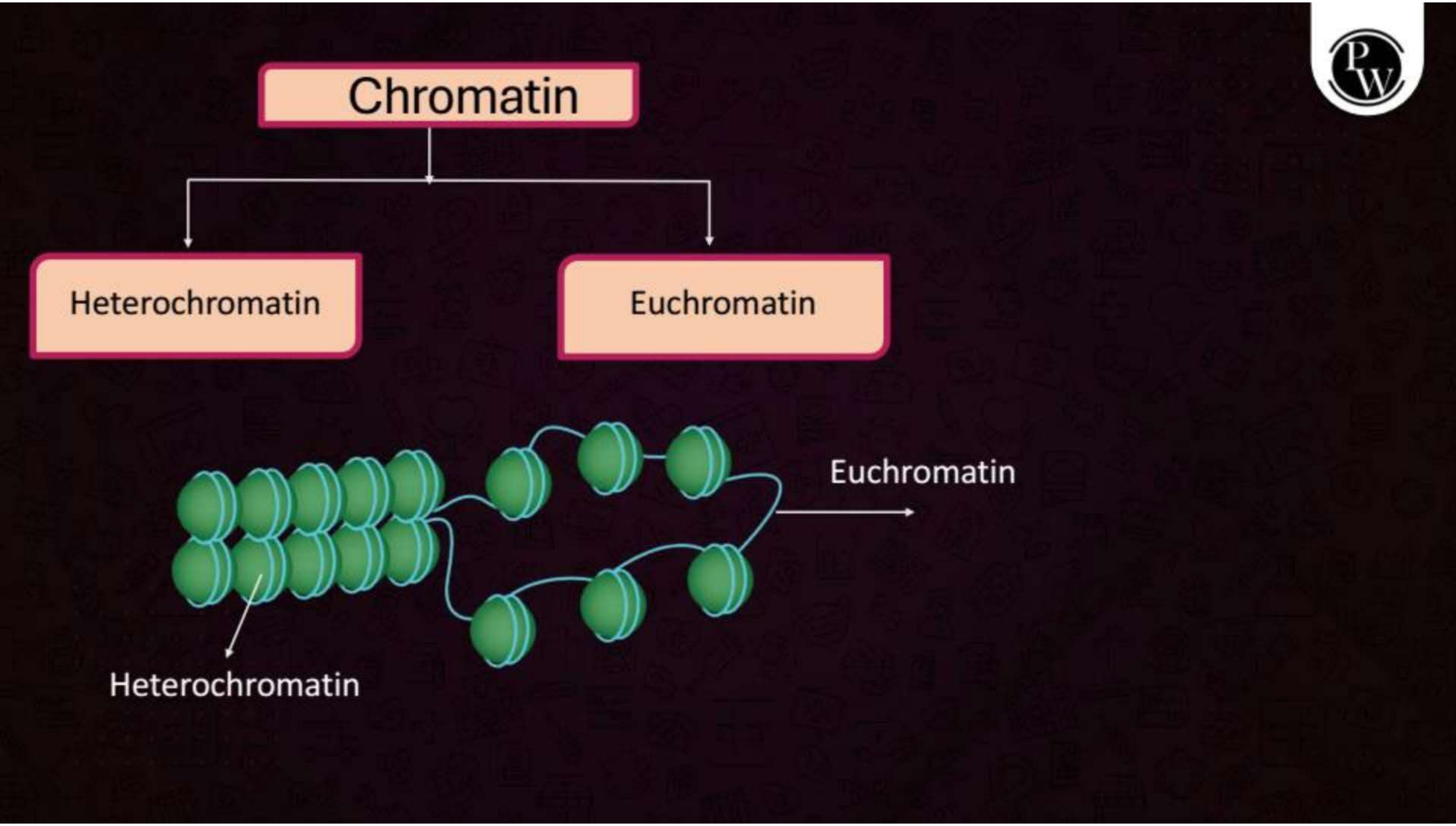
Appears in Dividing cell

↓

At Metaphase

chromosome

- Chromatin
  - Heterochromatin
  - Euchromatin


Chromatin
Heterochromatin
Euchromatin
Euchromatin
Heterochromatin

Heterochromatin

→ Densely packed

→ Darkly stained

→ $\boxed{\text{Transcriptionally Inactive}}$

↓ Are not expressed

↓ RNA not formed

Euchromatin

→ loosely packed

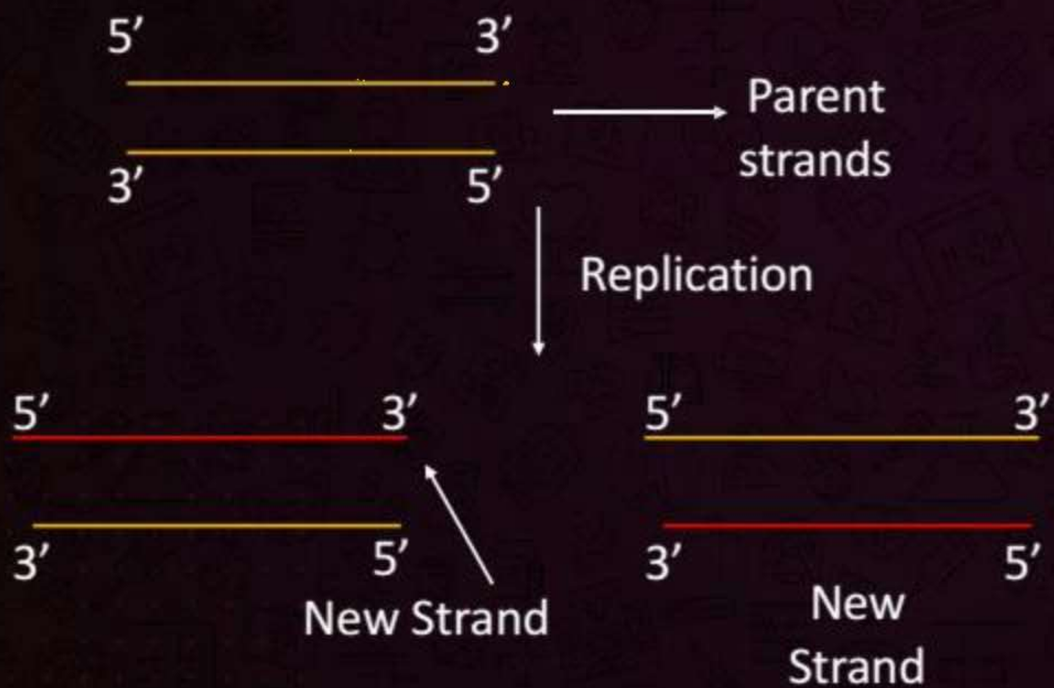
→ Lightly stained

→ $\boxed{\text{Transcriptionally Active}}$

↓ (Expressed parts)

→ Majority of parts

Semi-Conservative Nature of DNA Replication



$\boxed{\text{Watson \& Crick}}$

↓

Suggested that Replication is

⭐ $\boxed{\text{Semi-Conservative in Nature}}$ ⭐

Semi-Conservative Nature of DNA Replication

Proved in Prokaryotes

- By Messelson and Stahl
- In *E.coli*

Proved in Eukaryotes

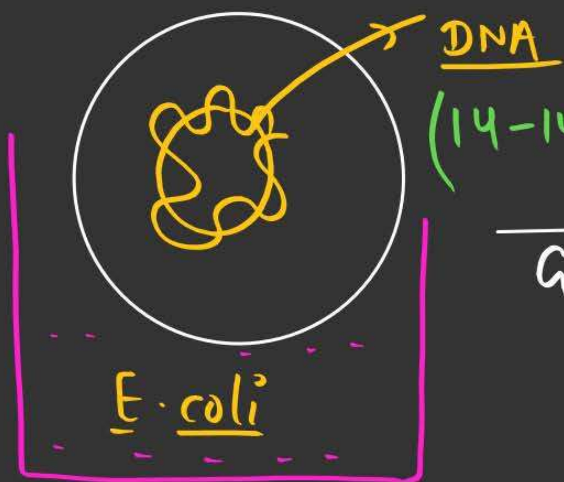
- Proved by Taylor et al
- In *Vicia faba* (Plant) beans (Flat)

Messelson and Stahl Experiment

(1958)

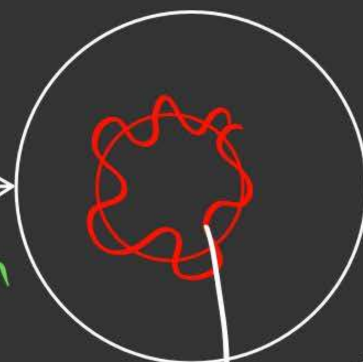
N-14

N-15 → Heavy isotope of Nitrogen



Grown in medium $^{15}NH_4Cl$

"for several generations"

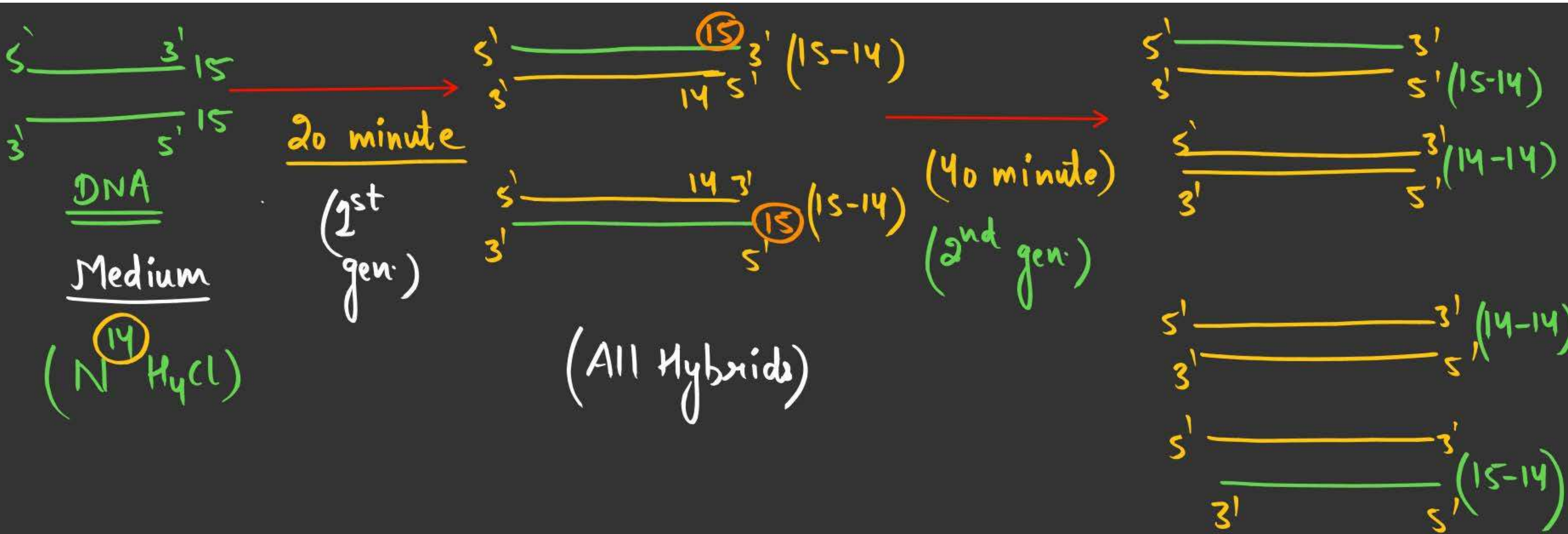


→ (15-15) DNA

14-14 → Pure light DNA

15-14 → Hybrid

15-15 → Pure Heavy



50% → Hybrids

50% → Pure-light

DNA $(15-15)$

Medium (N^{14})

20 min (1st gen.)

100 % Hybrids

$(15-14)$

40 min (2nd gen.)

50 % Hybrids

50 % Pure light

60 min

(3rd gen.)

25 % Hybrids

75 % Pure light

80 min

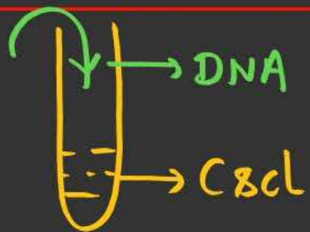
(4th gen.)

(12.5 % Hybrids)

87.5 %

(Pure light)

Density-Based Centrifugation → In CsCl (Caesium chloride solution)



E. coli

(15-15)

↓ CsCl

1st gen.

(20 min)

(15-14)

↓ CsCl

2nd gen.

(40 min)

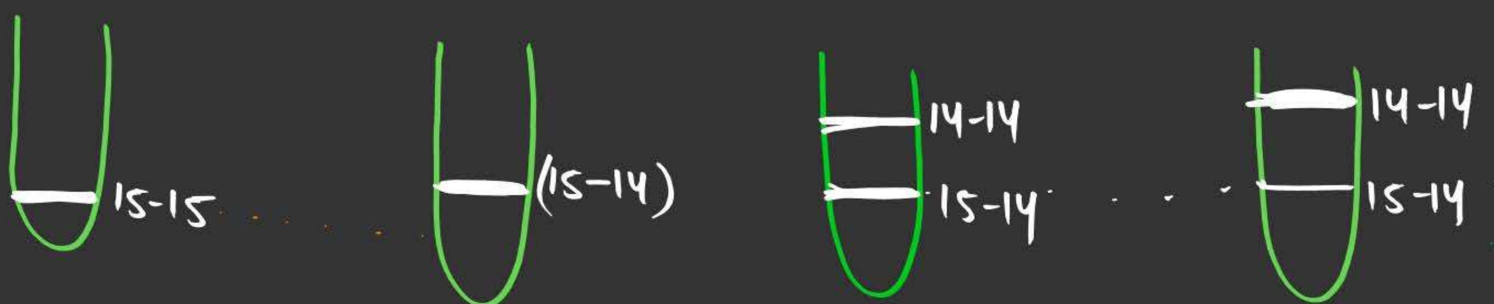
50% → 15-14

50% → 14-14

3rd gen.

25% → 15-14

75% → 14-14



A 14-14 E.coli cell is grown in $N^{15}H_4Cl$ medium. Calculate the proportion of hybrids & pure E.coli cells after 3 generations.

PYQ

Opposite of Experiment

3rd gen.

Hybrid → 25%

Pure heavy → 75%

Pure light → 0

QUESTION

Hallmark of the Watson and Crick three dimensional DNA model was based upon the findings of

1. Wilkins and Franklin
2. Erwin Chargaff
3. Hershey and Chase
4. Meselson and Stahl

QUESTION

Heterochromatin

1. Is transcriptionally active ✗
2. Is densely packed ✓
3. Replicated during early S-phase ✗
4. Stains lightly ✗

QUESTION

The number of glycosidic bonds associated with DNA of diploid human cell are

1. 6.6×10^9
2. $2 \times 6.6 \times 10^9$
3. 3.3×10^9
4. $3.3 \times 10^9 - 2$

QUESTION

Which of the following does not confer stability to the helical structure of DNA?

1. Phosphodiester bond ✗
2. H-bond ✓
3. N-glycosidic linkage ✗
4. More than one option is correct

QUESTION

The biochemical nature of transforming principle was defined by

1. Griffith
2. Avery, Macleod, McCarty
3. Watson and Crick
4. Taylor