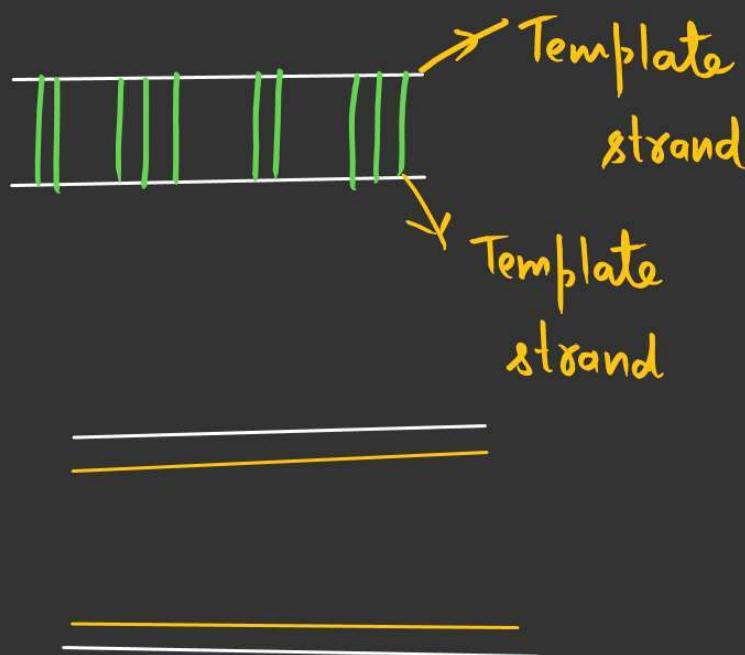
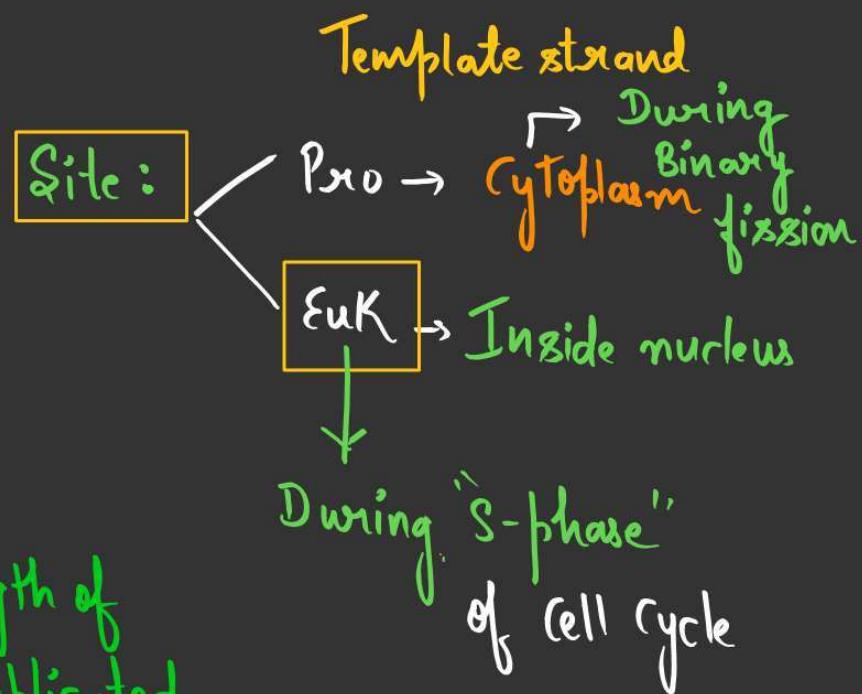


REPLICATION

Duplication of DNA where both strands of DNA act as



NOTE:
Entire length of
DNA is replicated
at the same time.



REPLICOME

(All the proteins & Enzymes together)

↓
Topoisomerase
Releases the tension / supercoiling of DNA.

↓
Helicase / Unwindase

- * Breaks H-bonds between 2 strands of DNA.
- * ATP-dependent Enzyme

↓
DNA polymerase

- * DNA - dependent
- * DNA polymerase (Requires DNA template)

* Polymerizing activity

Arranges nucleotides and forms phosphodiester bond.

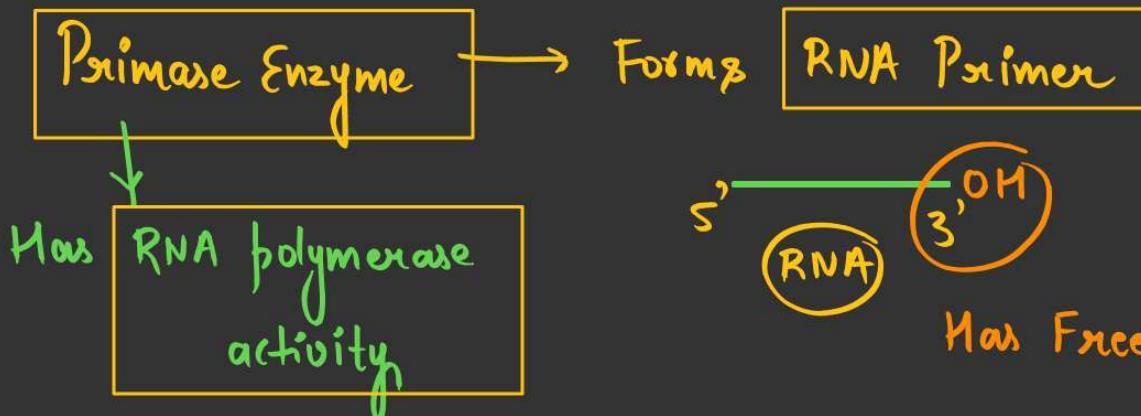
* Polymerizes new DNA strand only in 5' - 3' direction.

* In E. coli
↓
DNA polymerase arranges

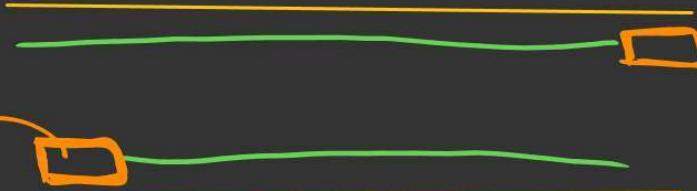
9,000 base pairs
Second

DNA polymerase

Cannot initiate the process.



* Has initiating capacity



RNA primer

DNA ligase

(Molecular glue)

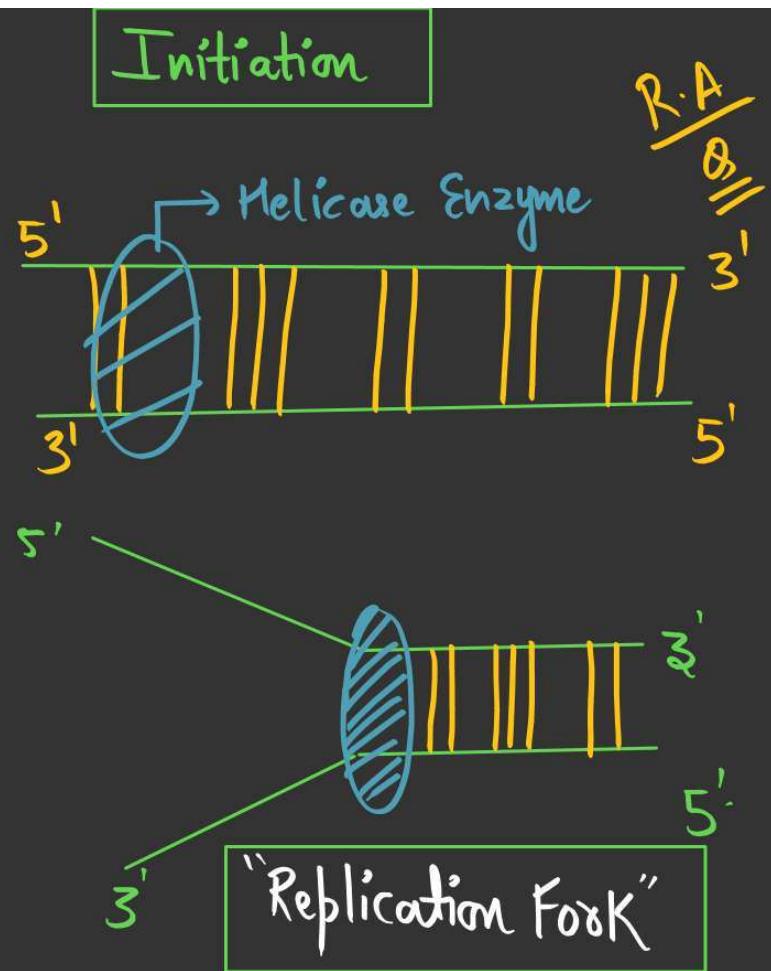
Joins DNA fragments

Process of Replication

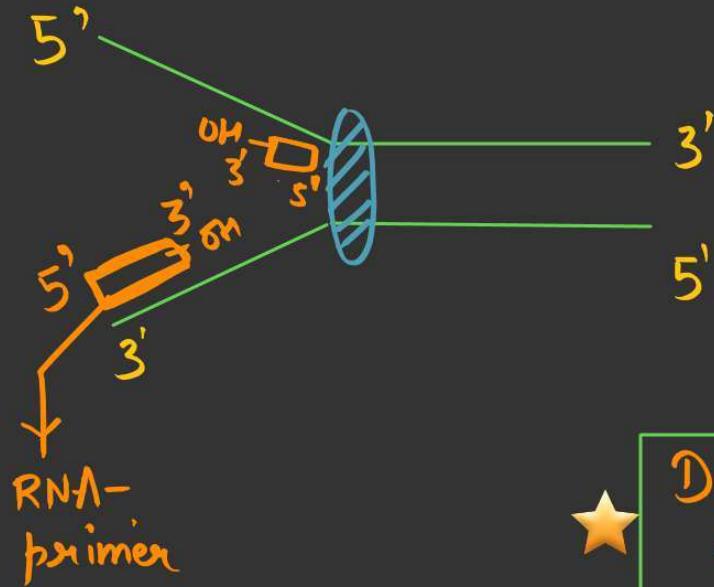


a) Topoisomerase
Releases supercoiling

b) Helicase
Enzyme



Why Replication fork is fork formed?
* Cell cannot provide enough energy to Helicase to open up whole DNA at the same time.



Activation / charging of nucleotides

deoxy nucleoside
monophosphate

deoxy nucleoside
triphosphate

~~P_{YB}~~

Deoxy nucleoside
Triphosphates

(Dual purpose)

Act as
Substrates
for DNA polymerase

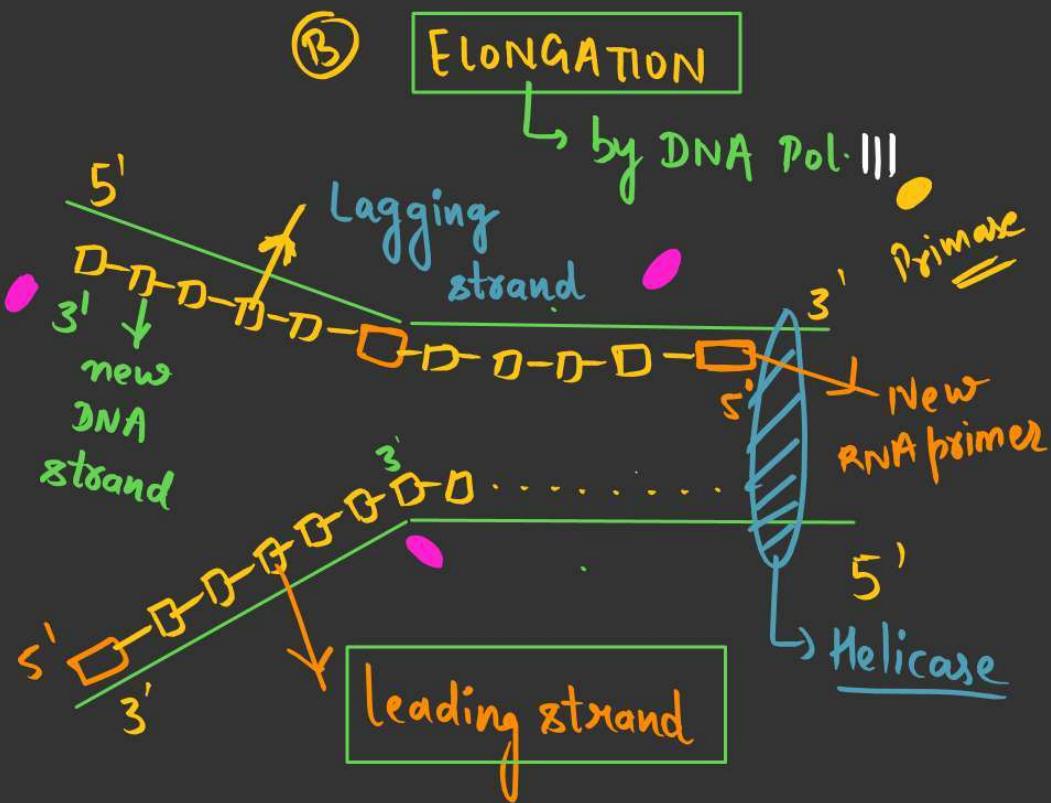
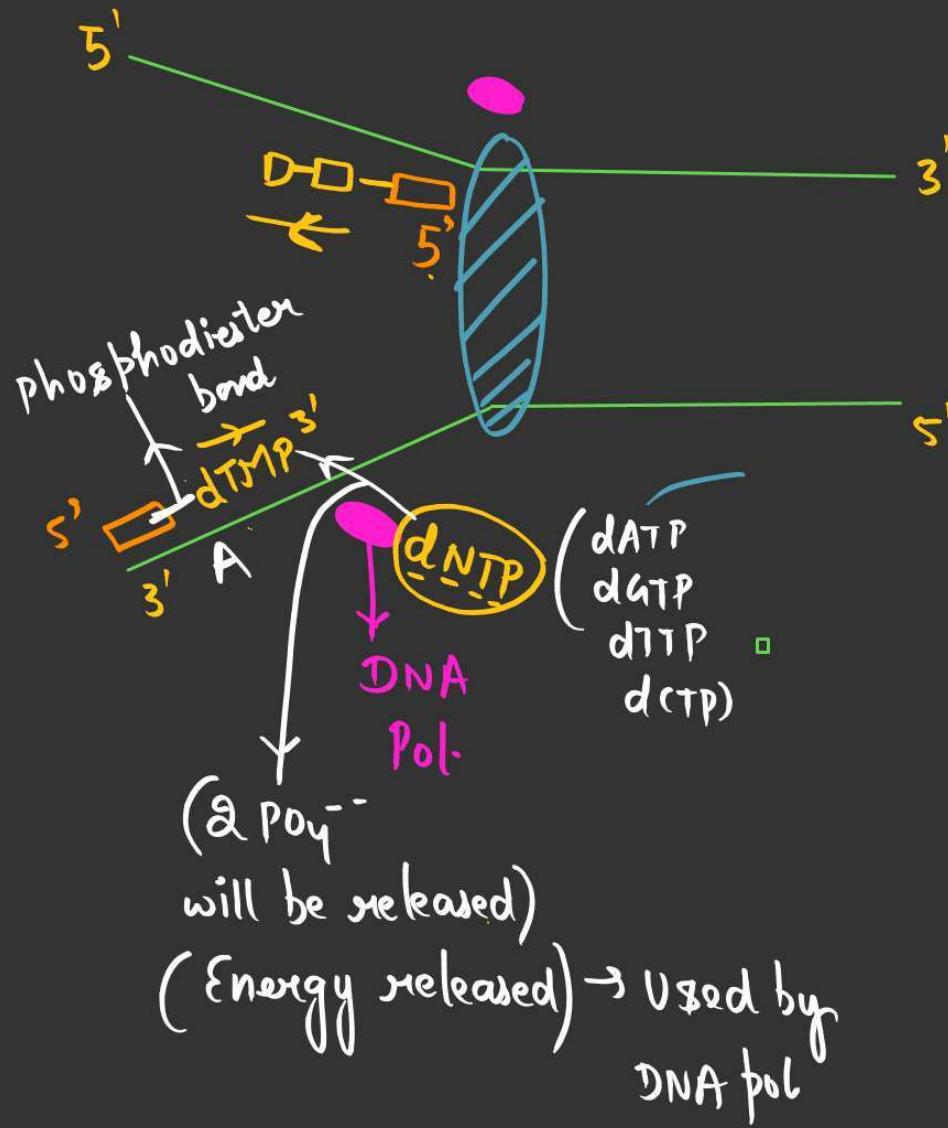
Provide
Energy for
Polymerization.

dAMP → dATP

dCMP → dCTP

dGMP → dGTP

dTMP → dTTP



Answers:

Leading strand

★ Polarity → 5'-3'

★ Polarity of Template

strand → 3'-5'
of leading strand

* Synthesized continuously

* Requires Single RNA Primer

Lagging strand

★ Polarity → 3'-5'

★ Template strand → 5'-3'

* Synthesized discontinuously
in the form of Okazaki fragments
(later on joined by DNA ligase)

* Requires many RNA Primers

* DNA Replication

Semi-conservative

Semi-discontinuous



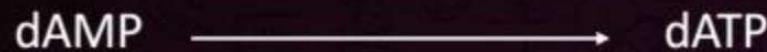
(R·A) \otimes

★ a) DNA pol. polymerizes only in 5'-3' direction ★

★ b) The two template strands of DNA are "Anti-parallel". ★

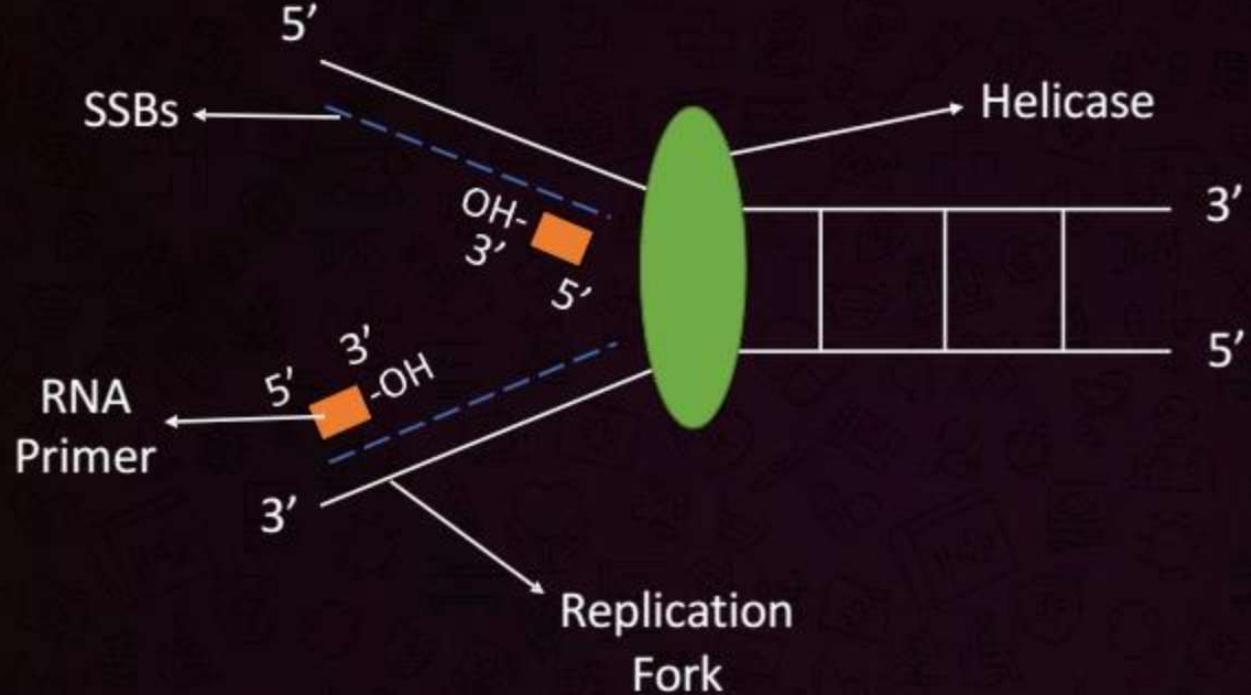
Activation of Nucleotides

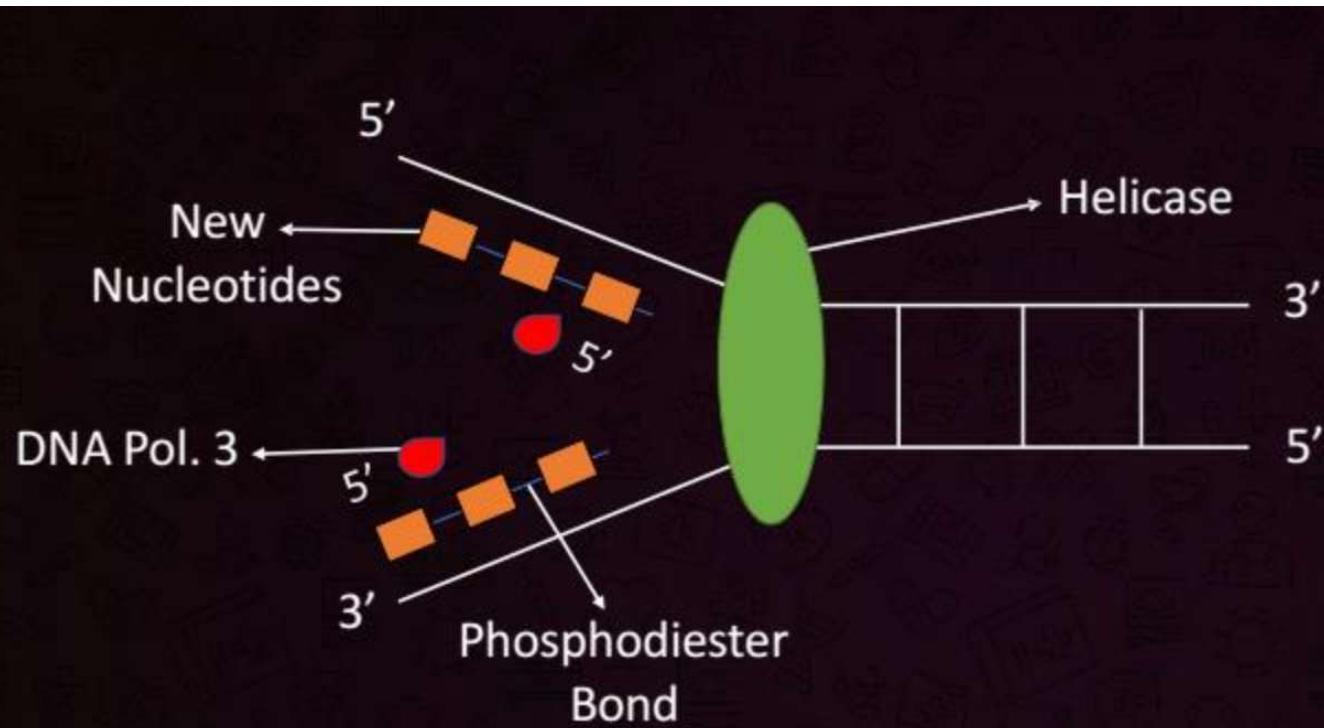
Pyrophosphorylase

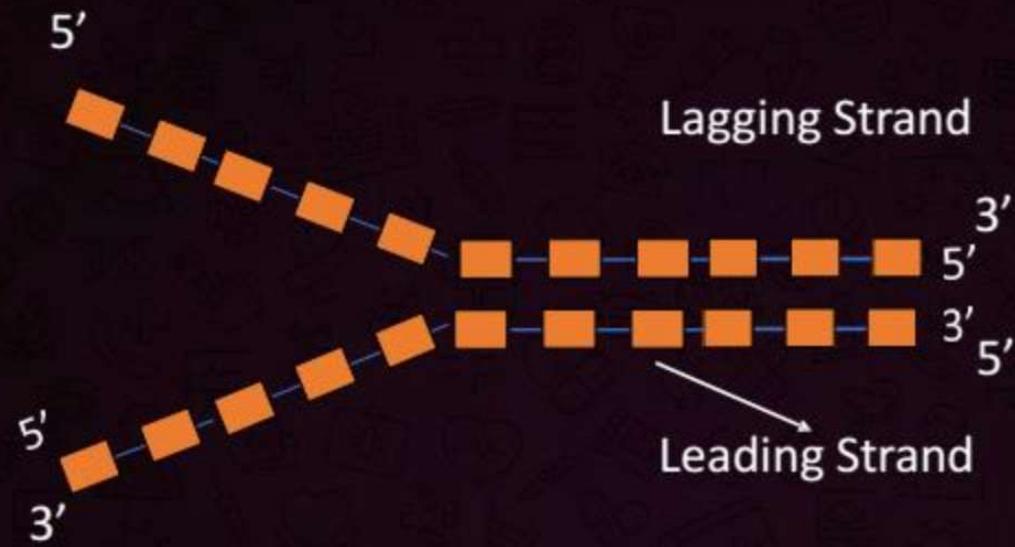


Inactive Nucleotide

Active Nucleotide







DNA Pol.

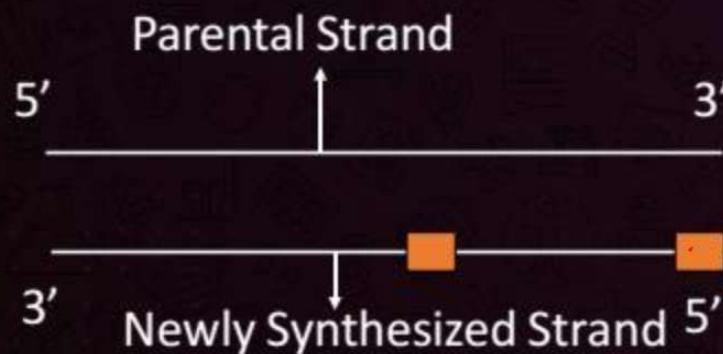
Pol. I

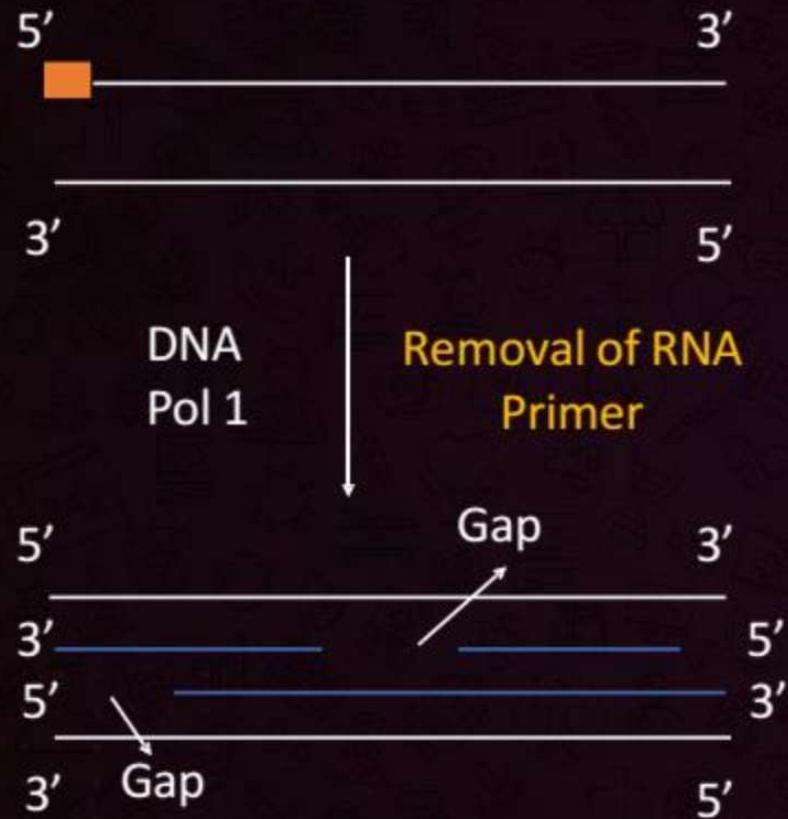
Pol. II

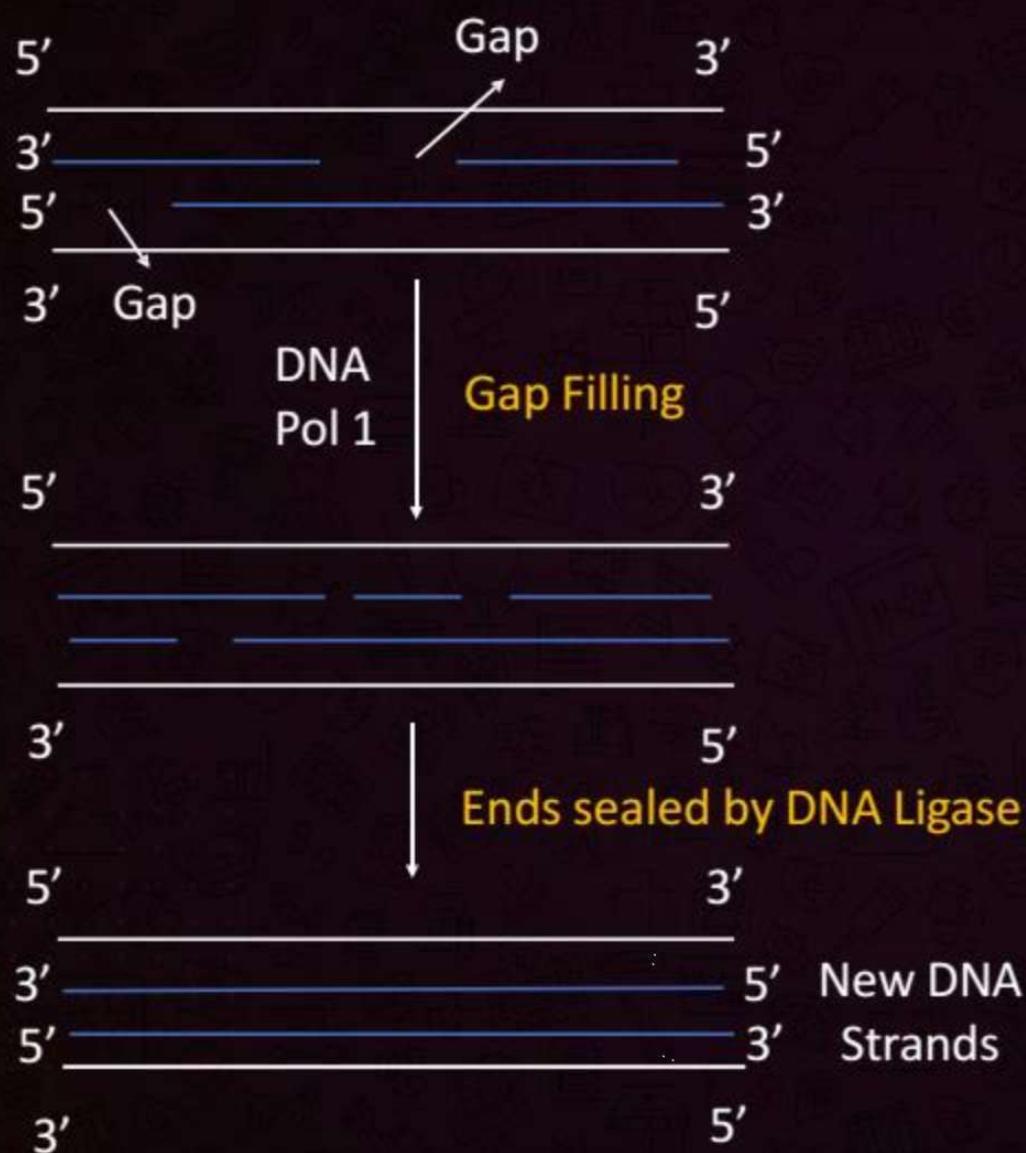
Pol. III

Q After both the new strands are synthesized on template strand.

- Proof Reading is done by DNA Pol.1
- Removal of RNA Primer







Ori^o

- * Origin of Replication
- * A-T rich sequences
- * Replication starts

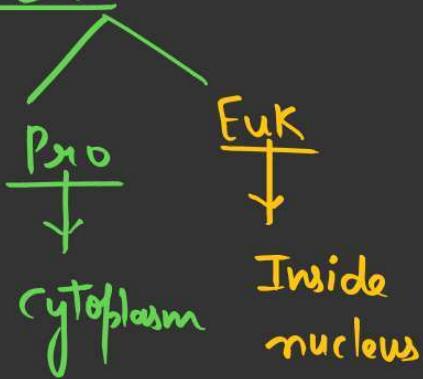
Poly ploidy

After Replication if a cell fails to divide, then poly ploidy occurs.

Transcription

Copying of genetic information from one strand of DNA to m-RNA (RNA)

Site:



Replication

* Both DNA strands → Template

* Entire length of DNA participates

Transcription

* One DNA strand → Template

Polarity → 3'-5'

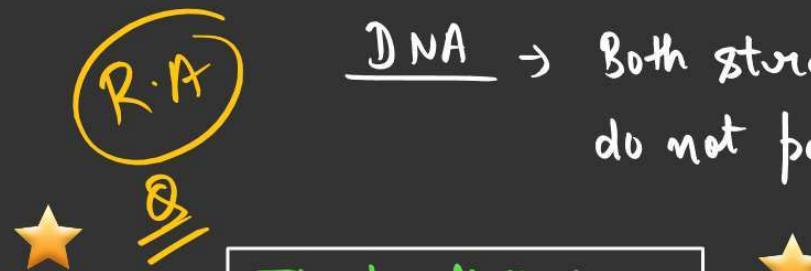
* Only a part of DNA participates (Gene)

Transcription

DNA → mRNA

DNA → t-RNA

DNA → γ -RNA



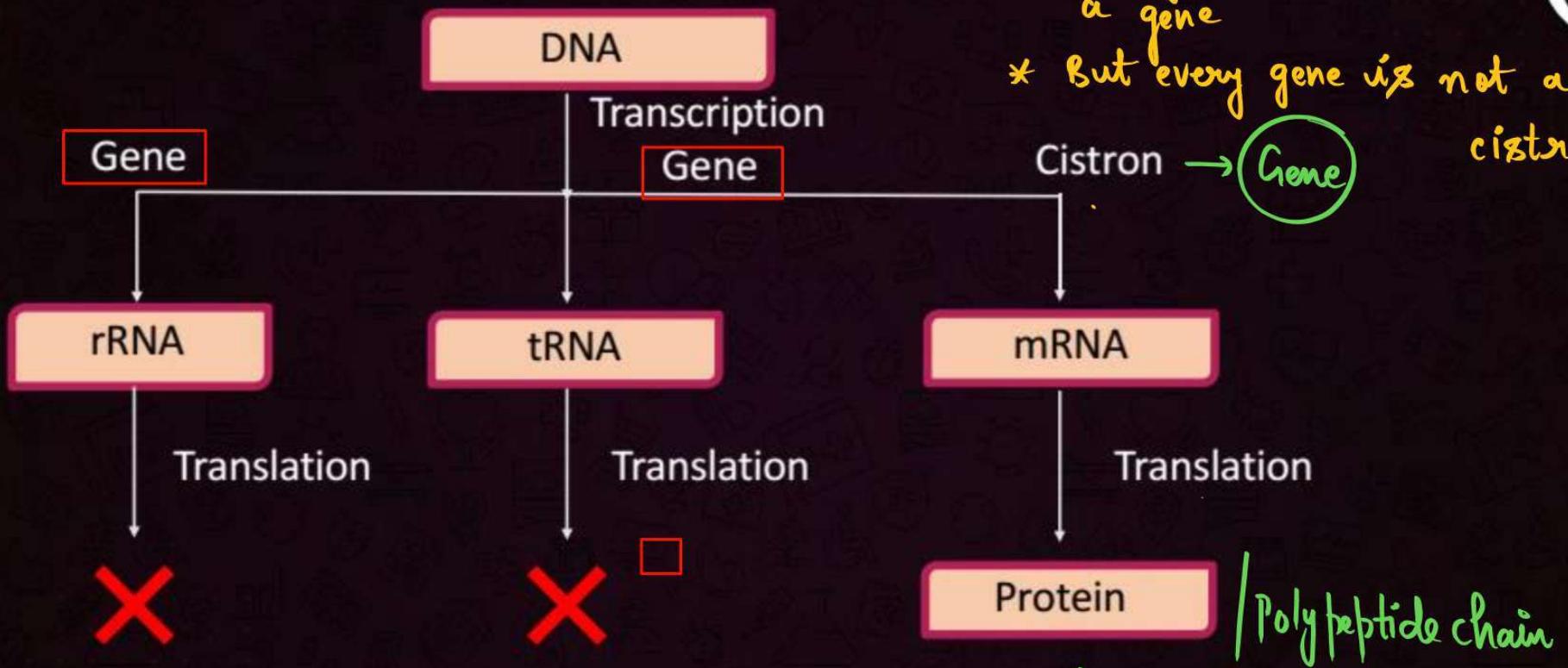
DNA → Both strands
do not participate

If participated

* ds RNA (do not exist)
would have formed

* If two-proteins would have formed then would have interacted

- * Every cistron is a gene
- * But every gene is not a cistron.



RNA Polymerase

- * DNA-dependent RNA polymerase
- * Also synthesizes RNA in 5'-3' direction

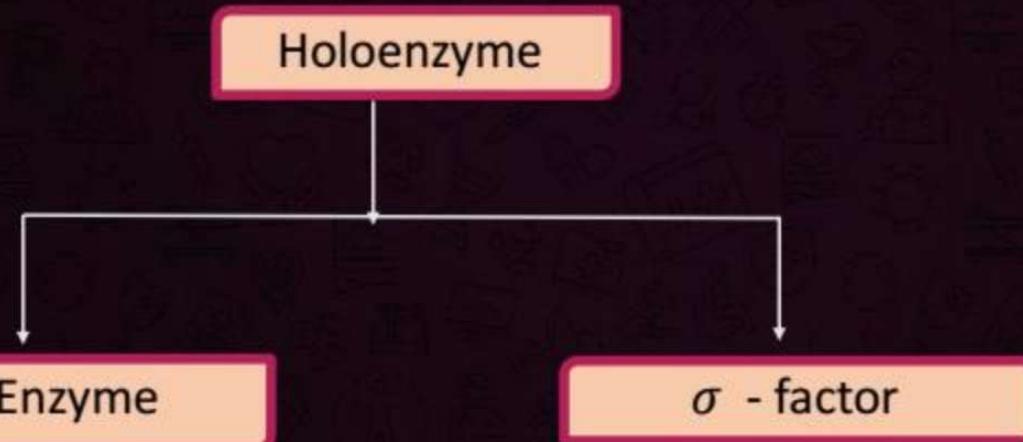
* ★ During Transcription ★
= ↓
RNA polymerase itself Breaks H-bonds

In Prokaryotes

only one type of RNA polymerase

In Prokaryotes

Only one type of RNA polymerase is present.



- It helps in elongation of RNA on the template strand
- It initiates the process

In Eukaryotes

Imp

PYB

P
W

Three types of RNA polymerase is present.

RNA Pol. 1

- 28 srRNA
- 5.8 srRNA
- 18 srRNA

RNA Pol. 2

➤ Hn-RNA
(Heterogenous
nuclear RNA)
↓
(m-RNA)

RNA Pol. 3

- t- RNA
- 5sr RNA
- Sn RNA
- Sc RNA

RNA Polymerase can synthesize only in 5'-3' direction.

Sn RNA
(Small nuclear RNA)

Sc RNA
(Small cytoplasmic RNA)

Transcription Unit

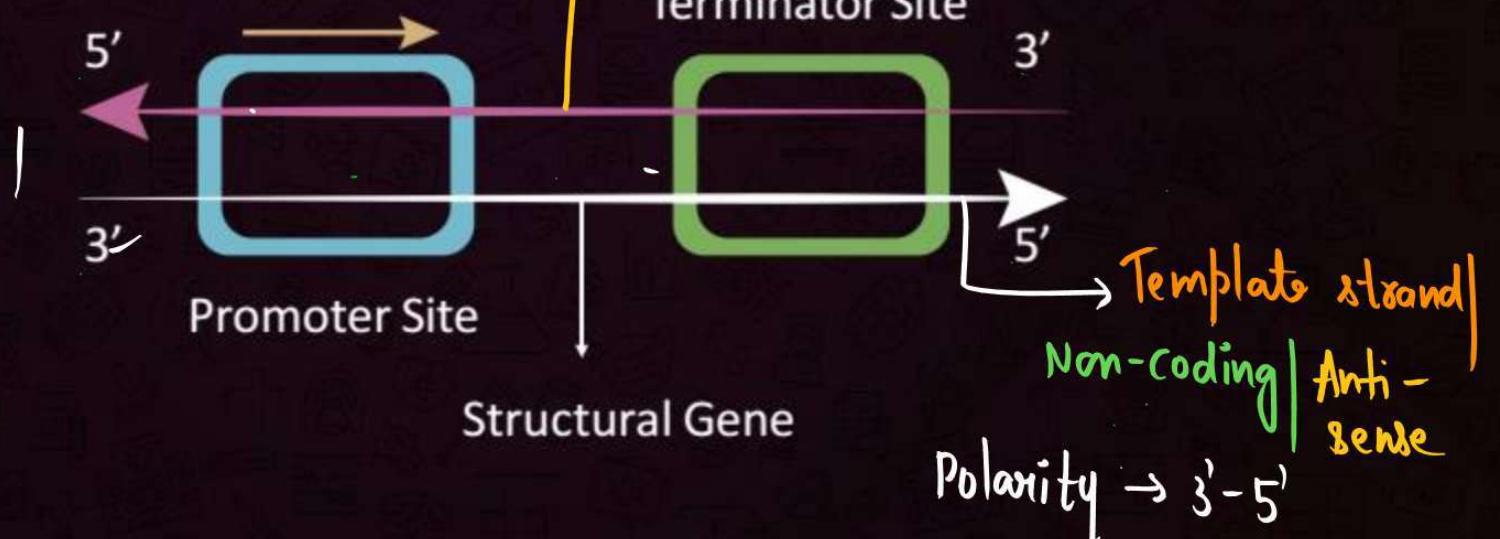
P
W

Promoter Site

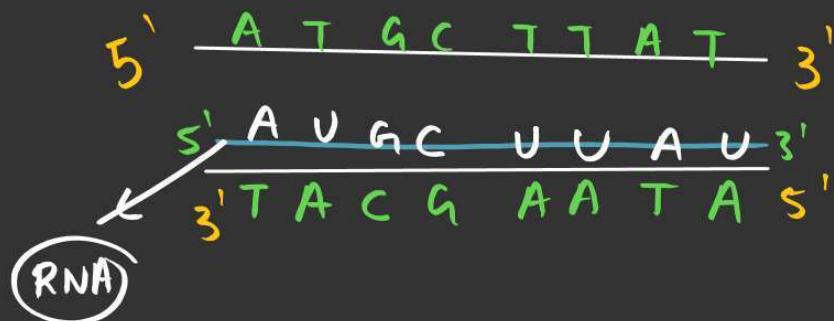
Structural Gene

Terminator Site

Cistron



Coding strand
Frame of reference
Explained Promoter
Terminator



★ The sequence of RNA ★

is similar as of
m-RNA (except thymine)

Promoter

- * Towards 5'-end
- * Upstream sequences
- * RNA-polymerase binding site

Terminator

- Towards 3'-end
- Downstream sequence
- Stops transcription.

Structural gene

↓
Actually codes for.
m-RNA

Cistron



Monocistron



Has information for formation
of single polypeptide chain
(Protein)

* Mostly Eukaryotes.



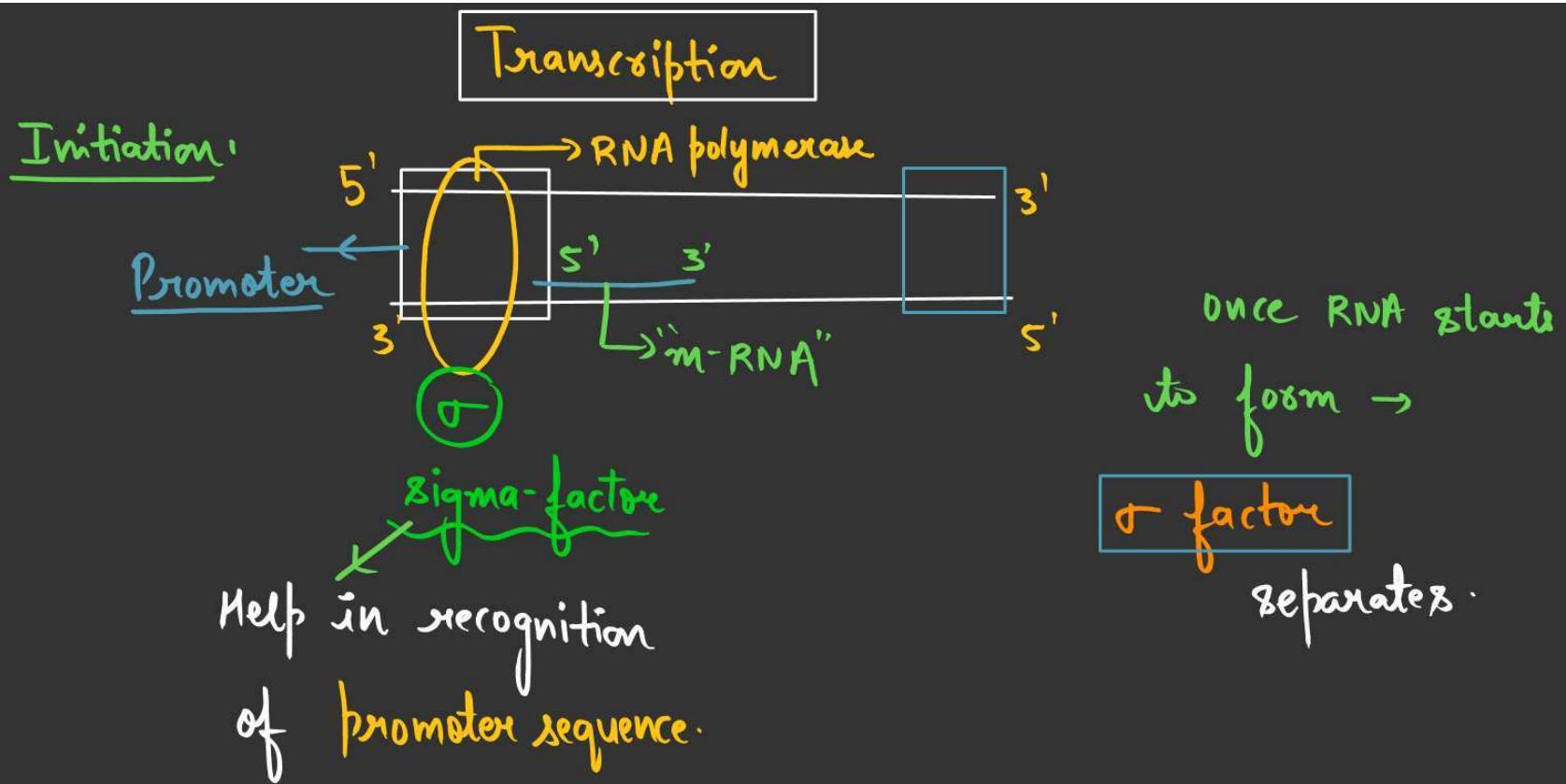
Polycistron

*

Has information for
more than one proteins

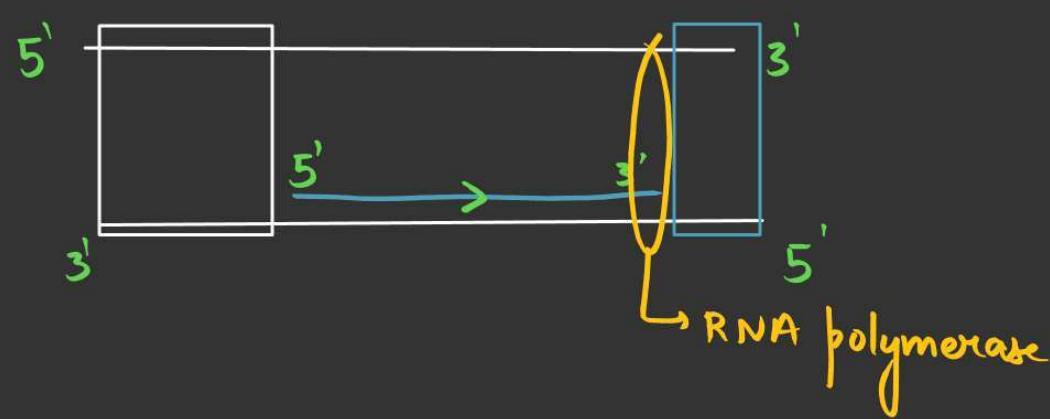
Ex → In prokaryotes (only)

Ex → operon

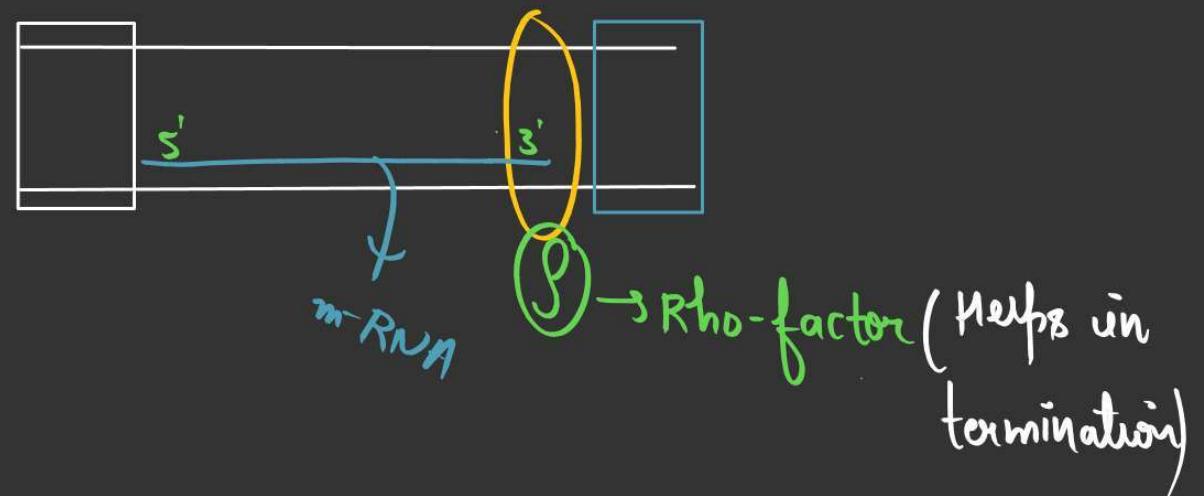


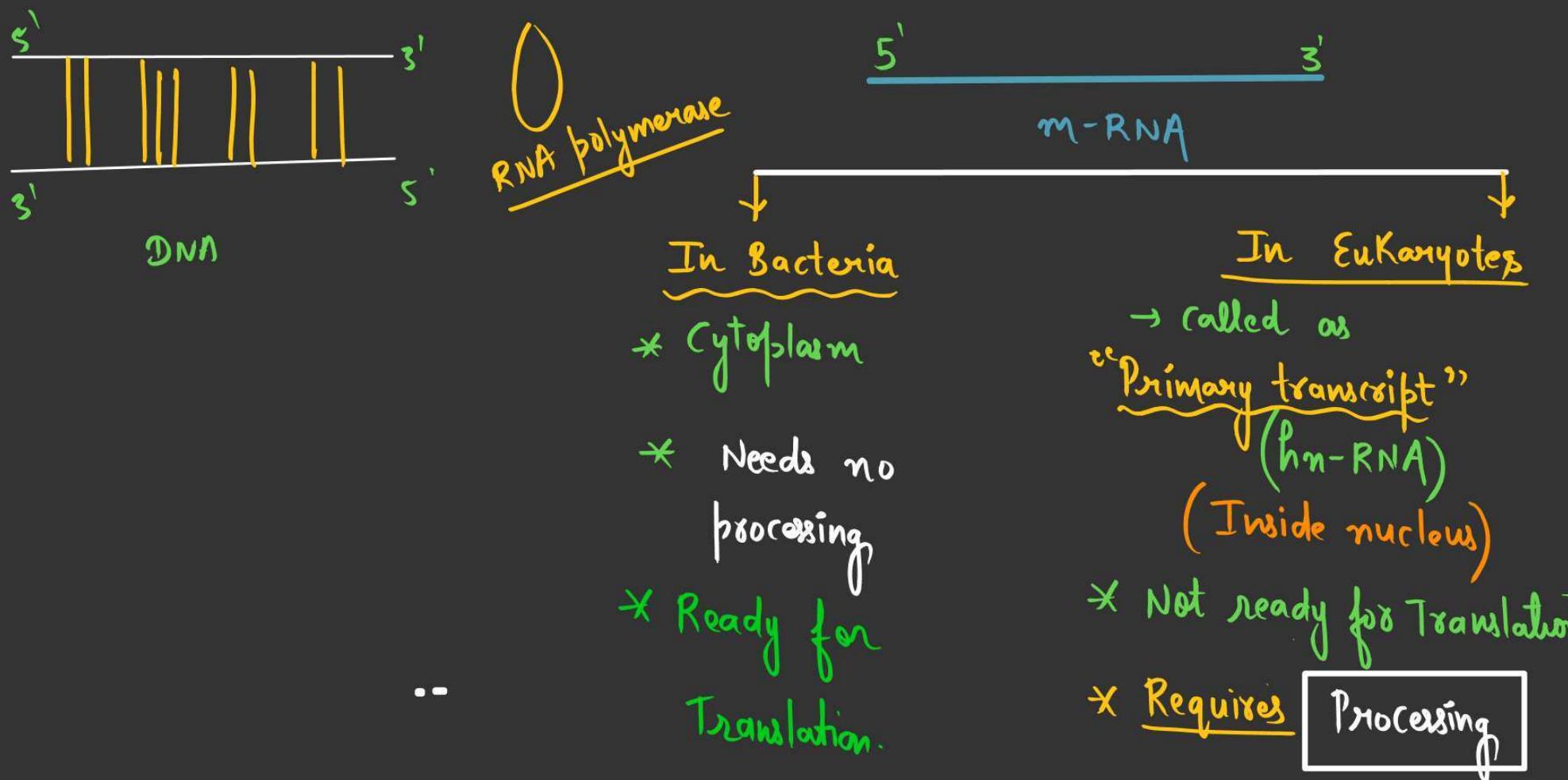
Elongation:

↓
RNA polymerase



Termination:





Types of RNA



m-RNA

r-RNA

t-RNA

Messenger RNA/
Postman RNA/Carrier
RNA /Template RNA

Ribosomal RNA

Transfer RNA/ Soluble
RNA/Adapter
molecule

It constitutes 5% of
total RNA

It constitutes 80% of
total RNA

It constitutes 15% of
total RNA

m-RNA

Longest RNA

Least stable

It carries information from DNA for polypeptide synthesis.

r-RNA

Smaller than mRNA

Most stable

It has structural role & catalytic role

Forms Ribosome
Peptidyl transferase

t-RNA

Smallest of all RNA

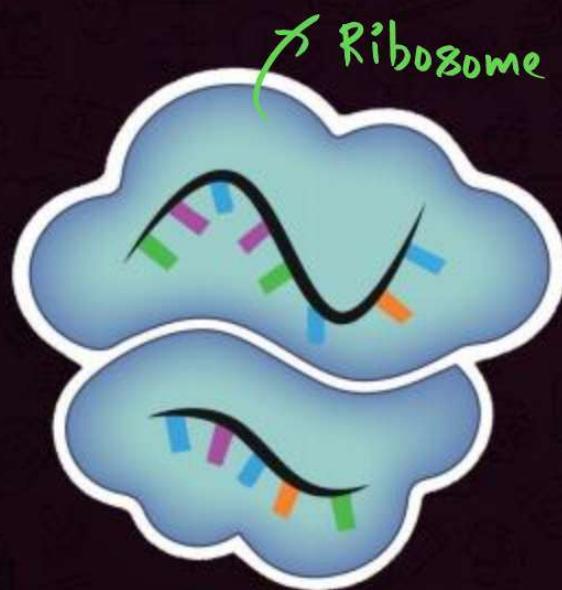
Stable

It carries amino acid from cytoplasm to mRNA during translation.

m-RNA



r-RNA



t-RNA



RNA Processing



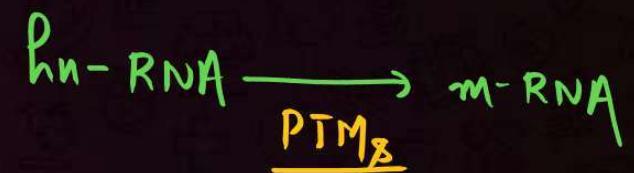
RNA maturation

PTM

(Post Transcriptional
Modification)



Q Occurs in nucleus and only in Eukaryotes.



Capping

Tailing

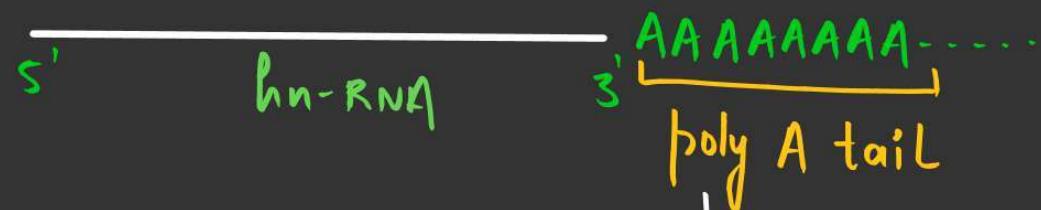
Splicing

① Capping (CAP molecule at 5'-end)



[N -methyl Guanine
Triphosphate]
(GTP)

② Tailing → 200-300 (ATP β)
or
POLYADENYLATION
Adenylate / Adenylic residues
are added at 3'-end

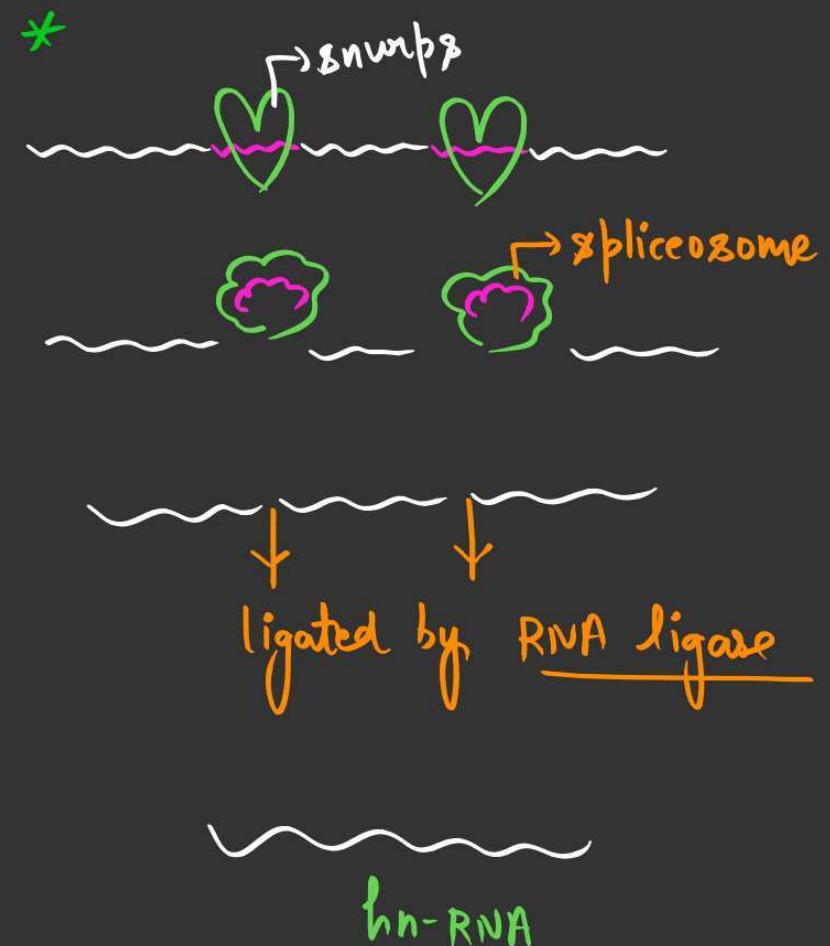
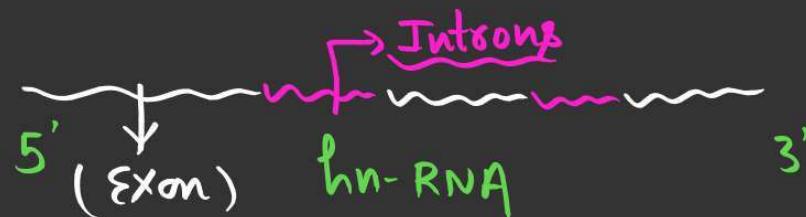


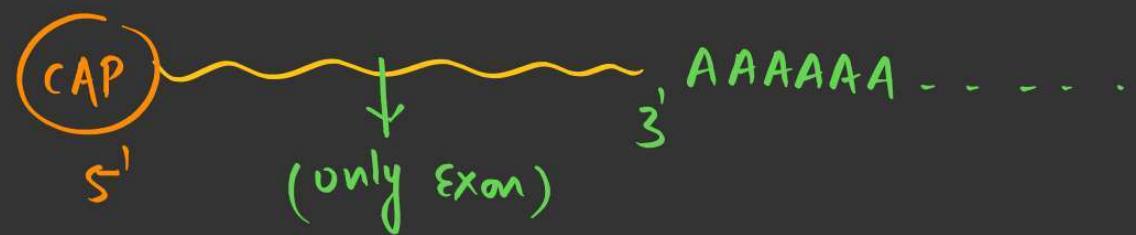
Done by
Poly A polymerase | Adenylate
polymerase
Template independent
Enzyme

③

Splicing

Removal of Introns from hn-RNA





Now it is called as

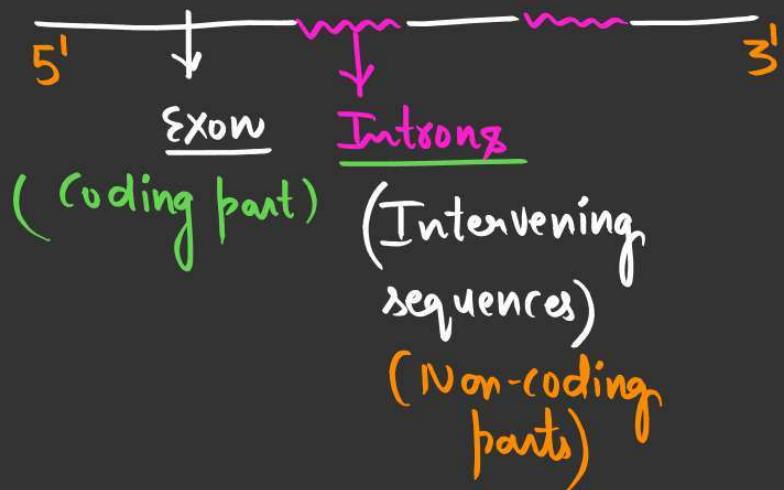
m-RNA

Ready to be transported
to cytoplasm for Translation.

"split-Gene"

Split-gene Arrangement

→ only in
Eukaryotes



Introns

DNA
m-RNA

only in Eukaryotes

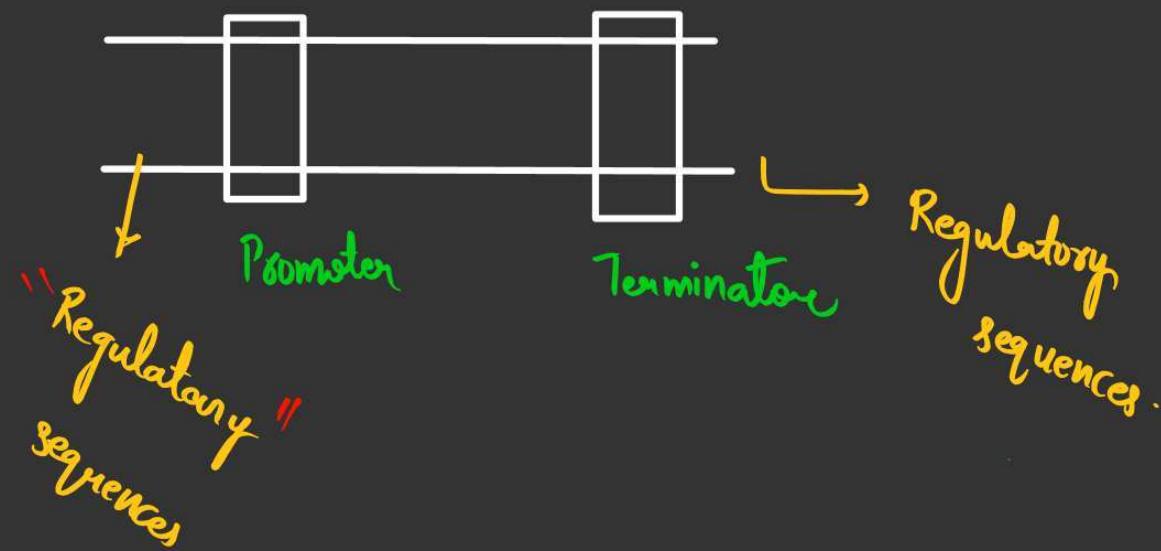
Introns are Absent in

Prokaryotes

Important 3-lines

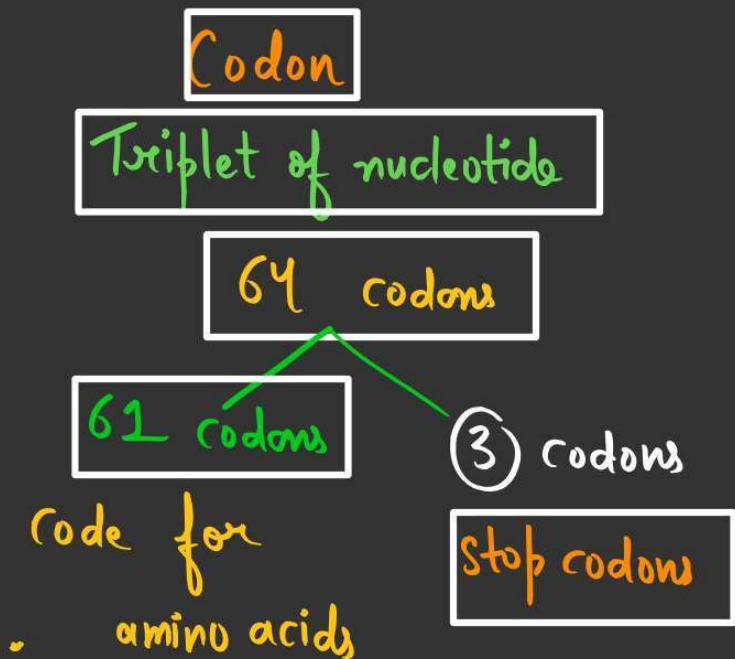
1. split-gene arrangement is the ancient feature of genome.
2. Presence of introns viz the Reminiscent of Antiquity
3. Splicing represents Dominance of RNA world.

Inheritance of a character is also affected by promoter and regulatory sequences of a structural gene. Hence, sometime the regulatory sequences are loosely defined as regulatory genes, even though these sequences do not code for any RNA or protein.



Genetic Code → Term → George Gamow

Inter-relationship between nucleotide sequences on DNA / m-RNA
and amino-acid sequences on protein chain.



Decipher | Crack → Genetic Code

Nirenberg & Matthaei

Used only Homopolymers

Har-Gobind Singh Khurana

Used both Homopolymers &

(Heteropolymers | Co-polymers)



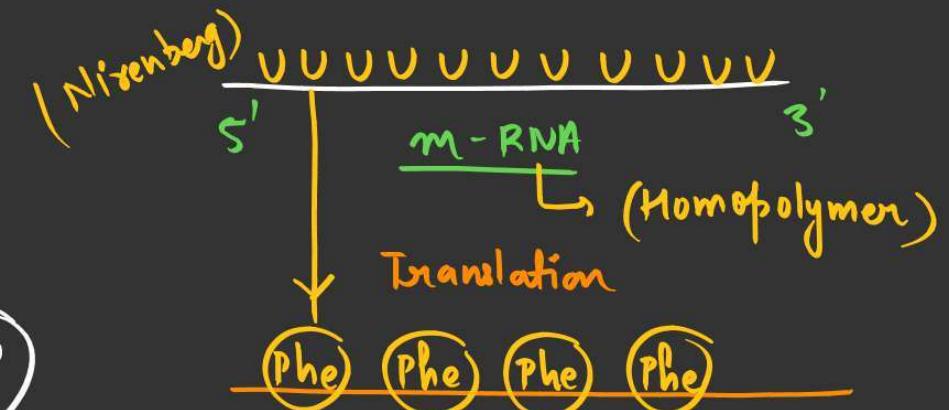
Nirenberg & Har Gobind Singh Khurana

} Nobel Prize

* Synthetic m-RNA
was formed.

By
Severo - ochoa Enzyme/
Polynucleotide phosphorylase

* works in
DNA-template
independent manner



UUU
codon
→ Phenylalanine
(amino-acid)

Khurana

UCUCUCUCUC
Co-polymer

Characteristics of Genetic Code

Imp

Triplet nature

Suggested by Gamow

$$4^1 = 4$$



$$4^2 = 16$$



$$4^3 = 64$$



Codons → AUG

UUU

Proved by Nirenberg & Mathael

Universal ✓

All the codons code for same amino acids in all organisms.

Exception – In mitochondrial DNA of mammals & Yeast, "UGA" codes for "Tryptophan" (otherwise it is stop codon). "AGG & AGA" – Stop codon (otherwise they code for Arginine)

UAA ✓
UAG ✓
AGG ✓
AGA ✓

Only in mitochondrial DNA of mammals & Yeast

AUG - methionine

In all organisms.

PW

Stop codons

Termination Codons

Non-sense Codons

Does not code for amino acids

Also called termination codon.

UAA = Ochre

UAG = Amber

UGA = Opal

8
=

Initiation codons

(Dual function)

Bifunctional

Behave as
Initiation codon

Code for
"methionine"

Non- ambiguous

(clear) (specific)

Each codon codes for a specific amino-acid.



Exception

GUG is
ambiguous

Generally it codes
for "Valine"

When behaves
as initiation
codon(in
prokaryotes) it
codes for
"methionine"

Commaless/Continuous

Punctuationless

• A U G U U U A A A A G X

• AUG U U U A A A A G ✓

Non-overlapping

• A U G U U U A A A A G



[U] [A] [A] [A] [A] [G]



• A U G U U U A A A A G



[U] [A] [G] [U] [A] [A] [A] [G]



Degenerate

Q One amino acid is coded by more than one codon.

Arginine → Coded by
Amino-acid) ⑥ codons

Serine → ⑥ codons

Non-degenerate

Exception:

Methionine

AUG

Tryptophan

UGG



Structure of tRNA



Clover Leaf Model

Plant (*Trifolium*)

2-D Model

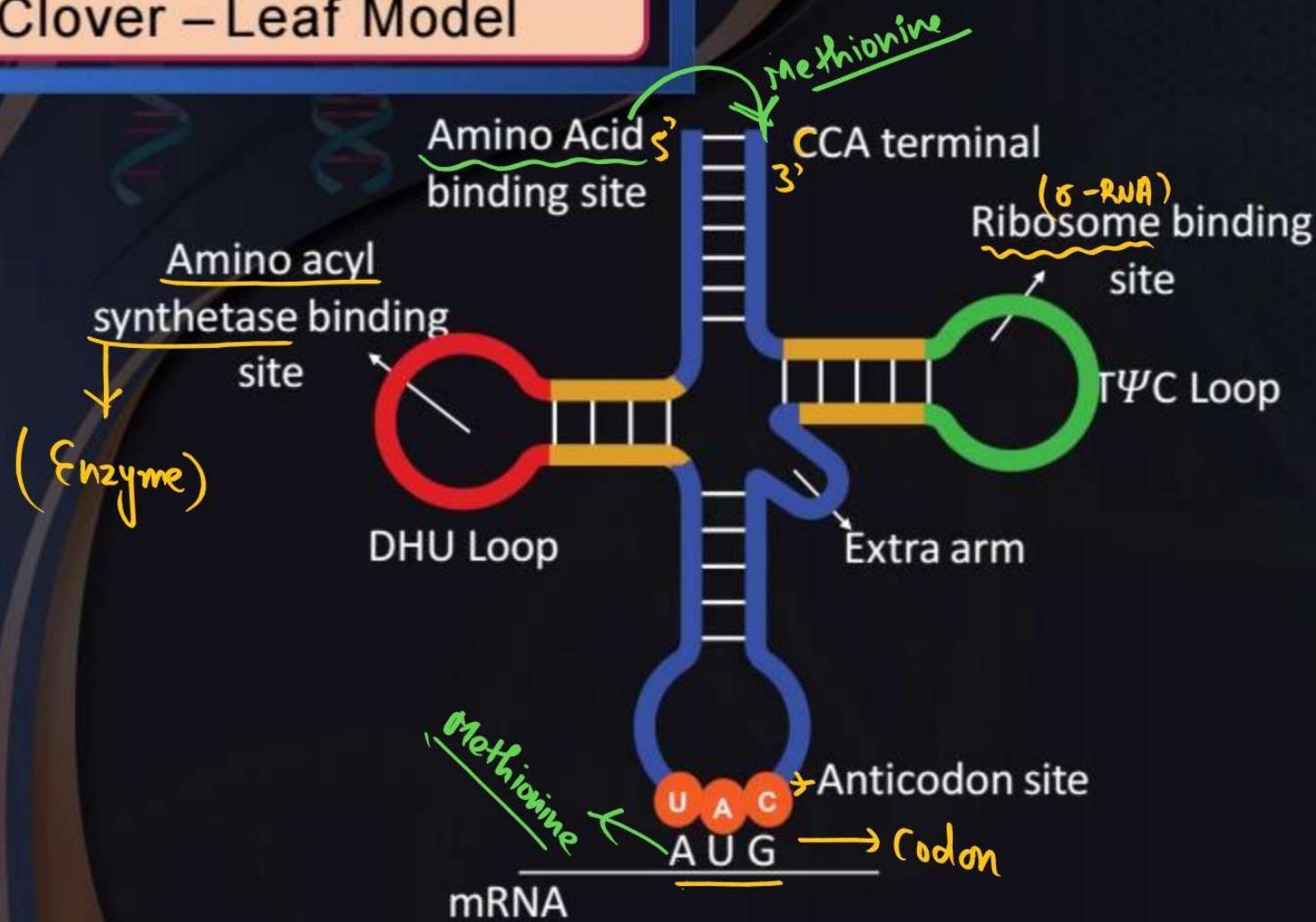
Given by "Halley"

L shaped Model

3-D Model

Given by "Kim & Klugs"

Clover – Leaf Model



t-RNA → Initially called as S-RNA
(Soluble RNA)

Later on:

Francis Crick
told that $\xrightarrow{\quad}$ t-RNA (Adapter molecule)
Understands Genetic Code ^{on} one hand
and can carry amino-acids on other hand.

Translation

* Formation of protein chain on m-RNA

* Site < Pro → Cytoplasm
 Euk → Cytoplasm

Initiation

Activation/Activation of amino acid

Amino acid

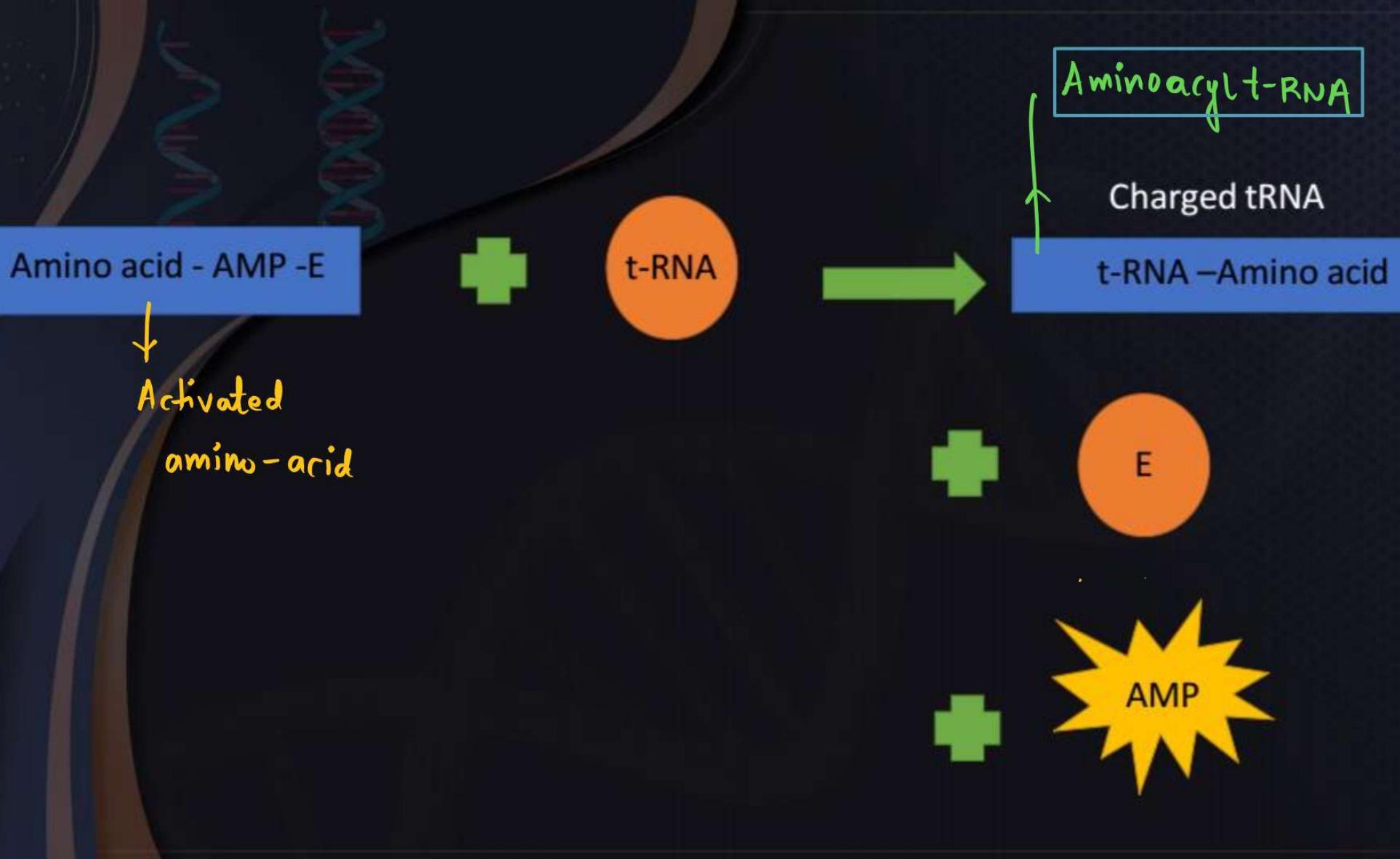
ATP

E

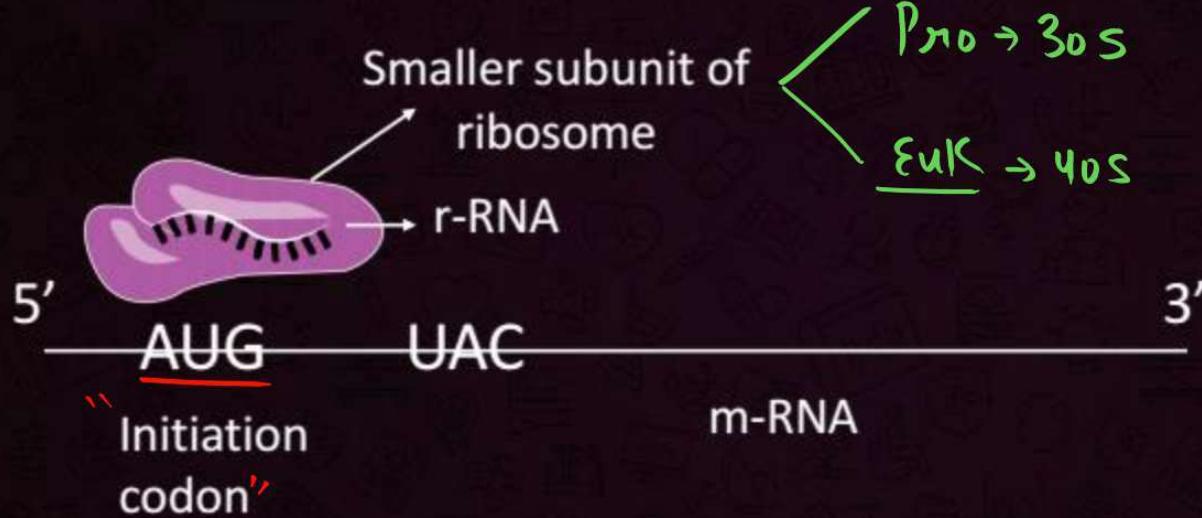
Amino acid + AMP - E

Amino acyl
synthetase Enzyme

P_i



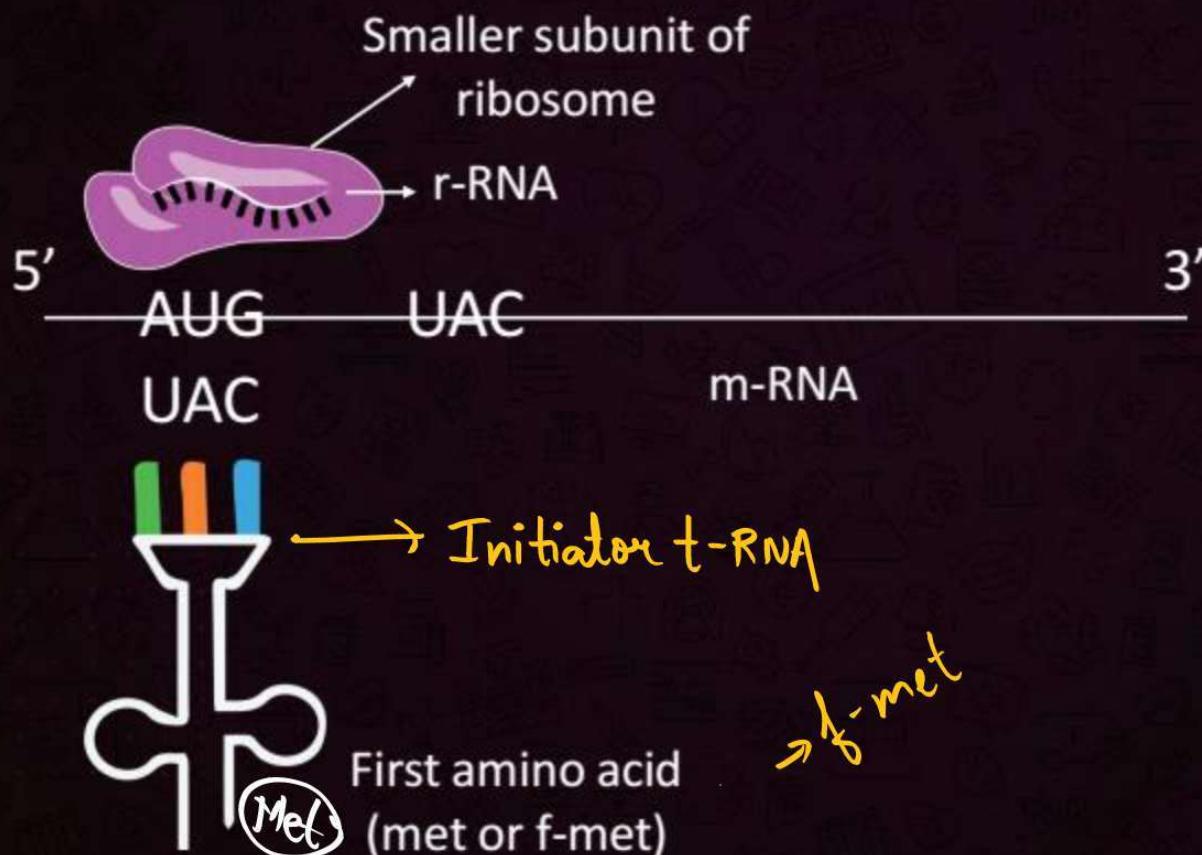
Binding of smaller subunit of ribosome to m -RNA



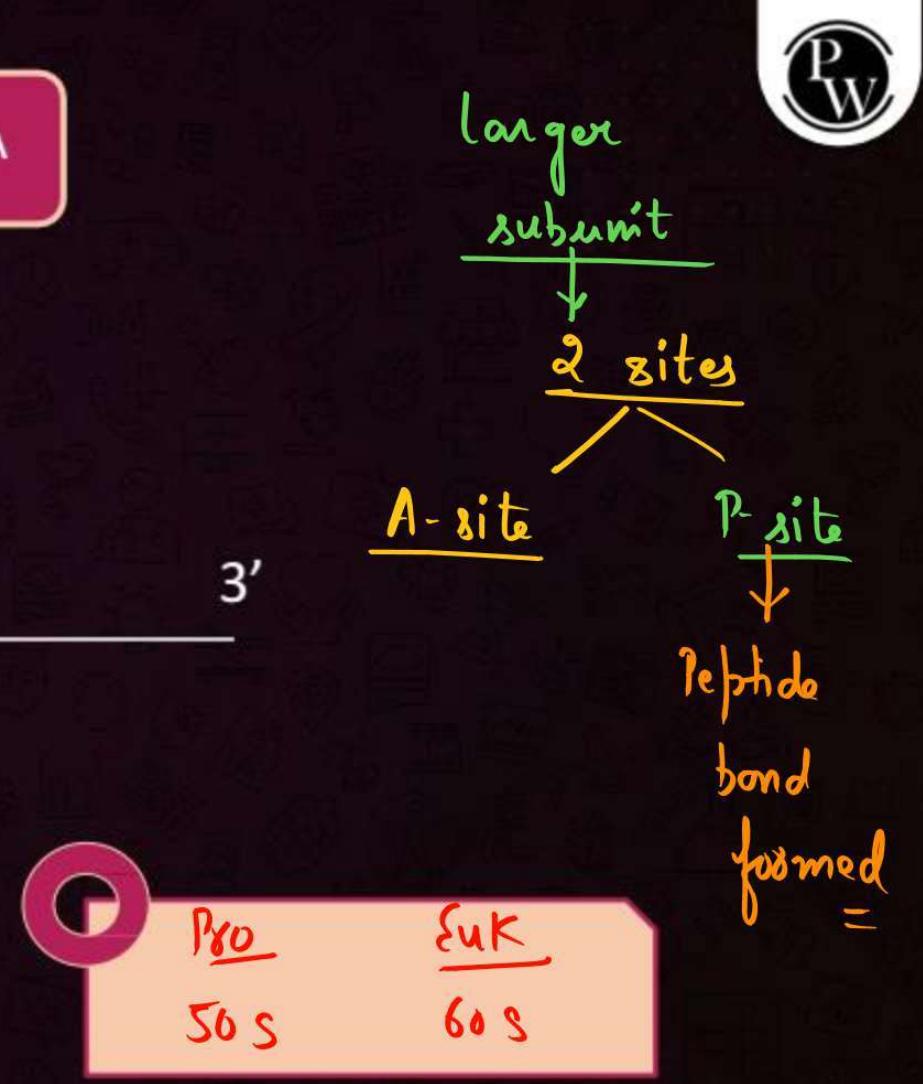
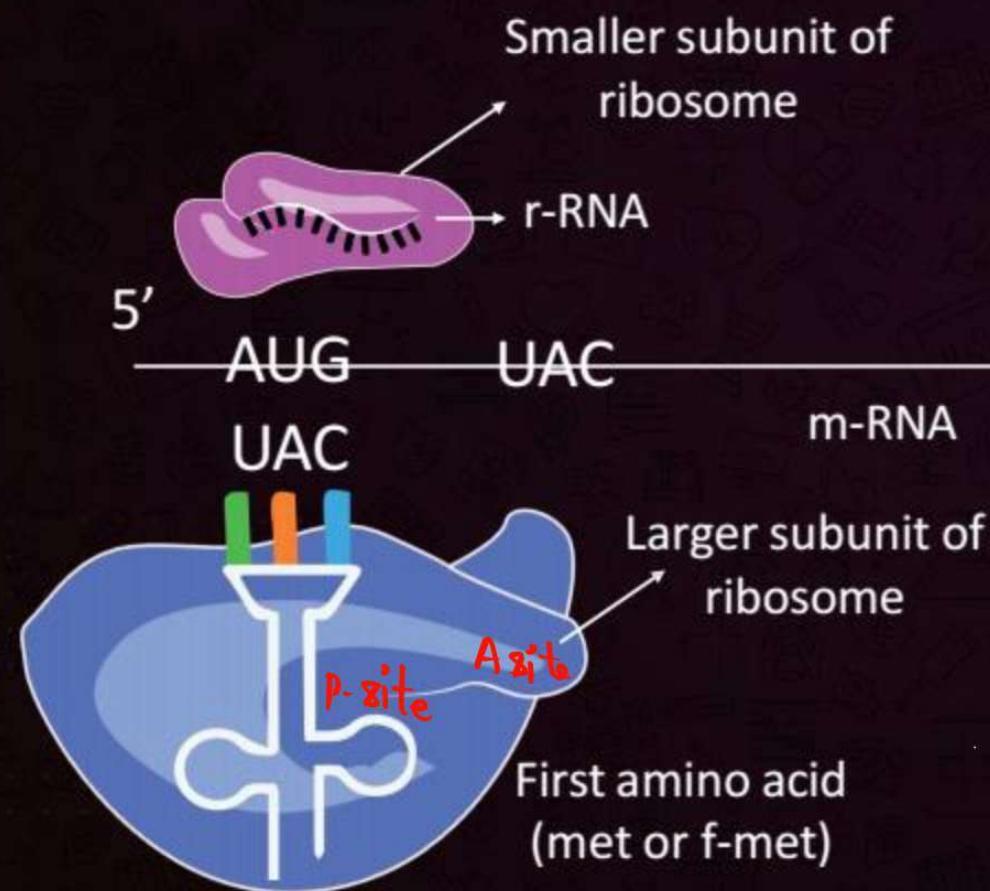
Ribosome



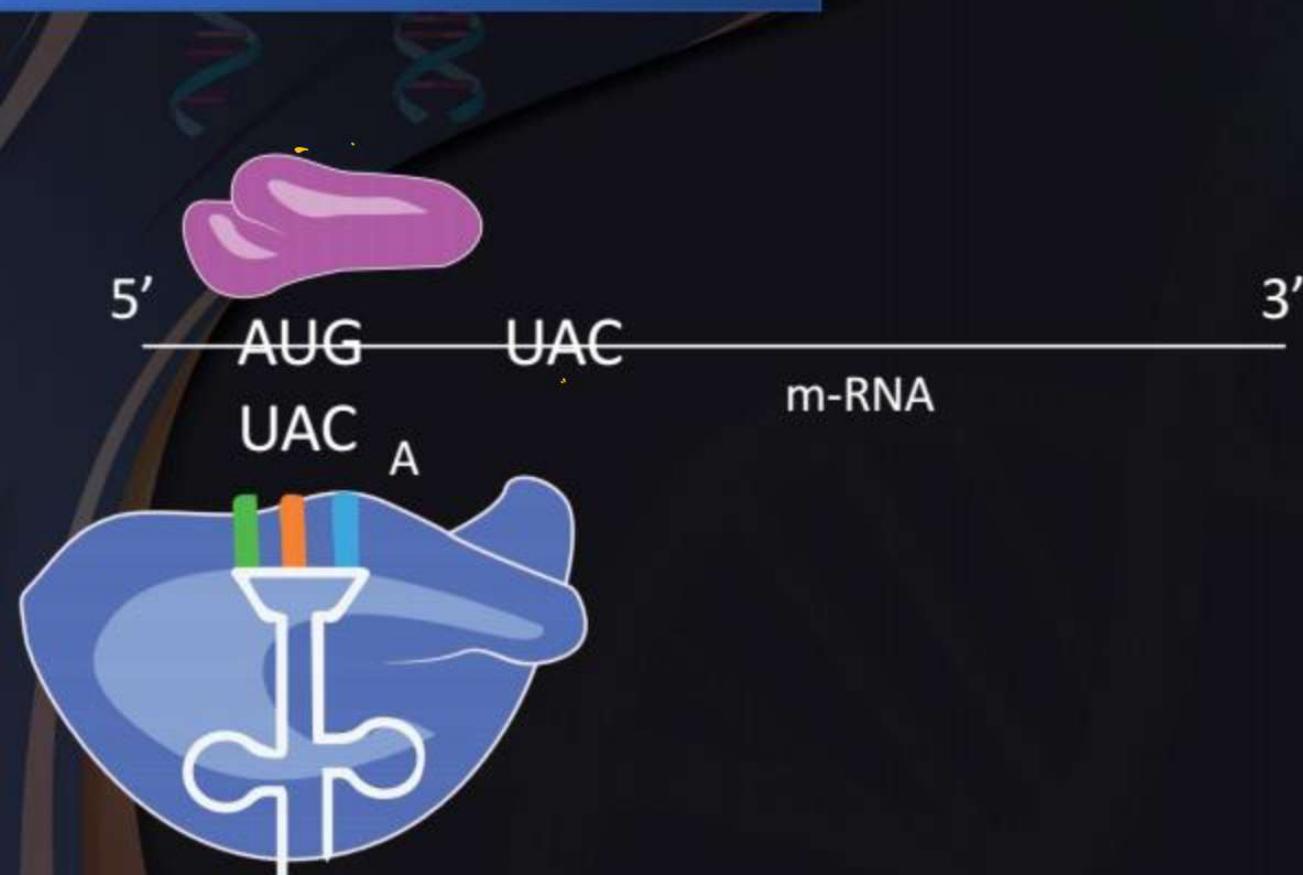
Binding of charged t-RNA to m -RNA



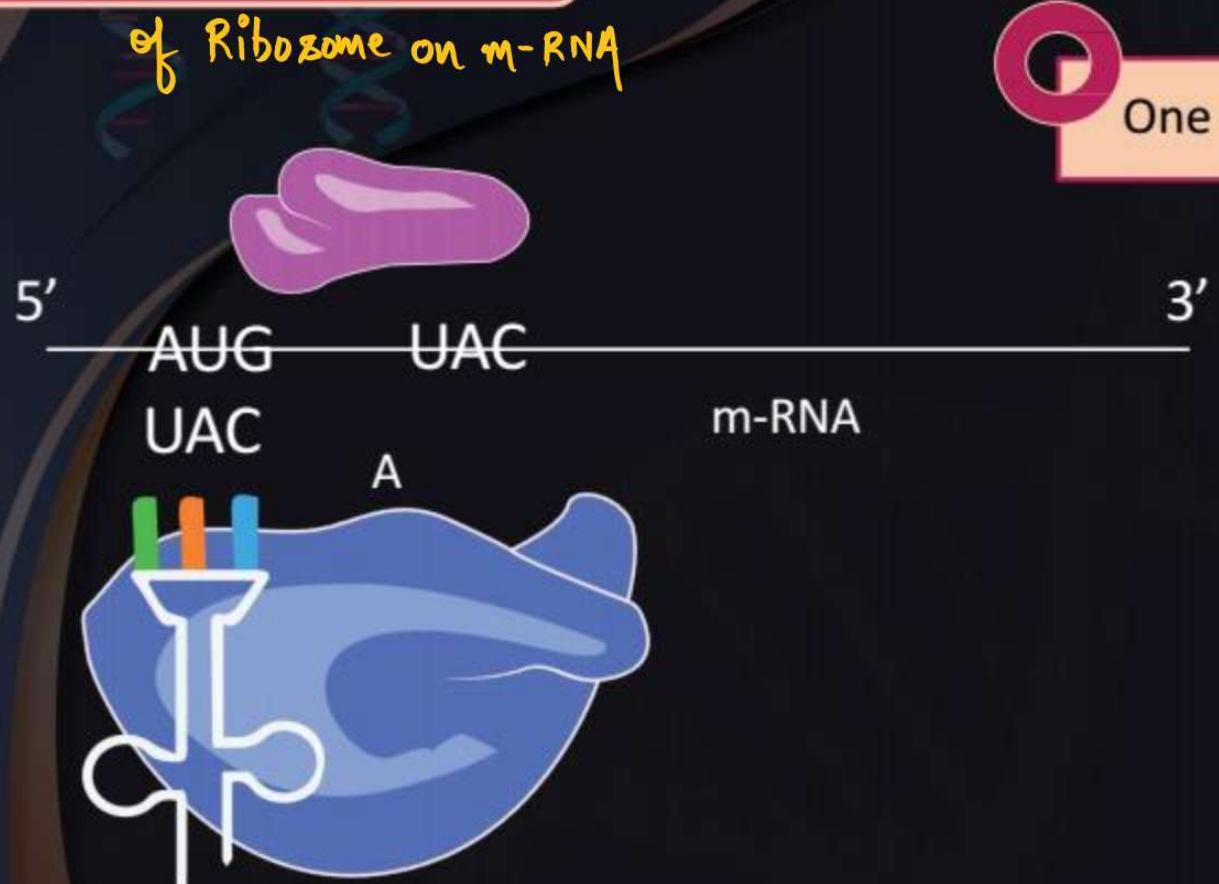
Binding of larger subunit of ribosome to m-RNA



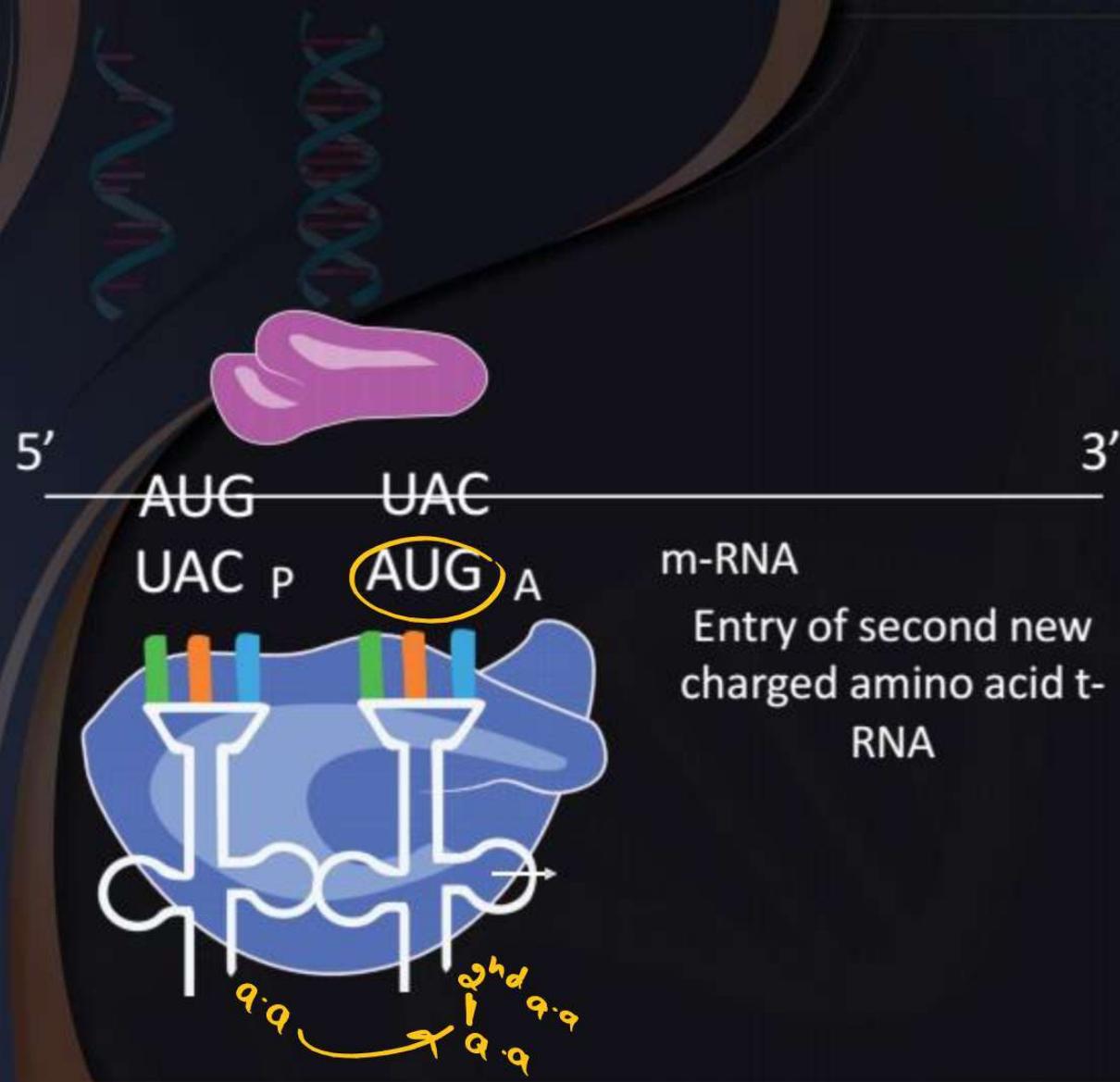
Elongation

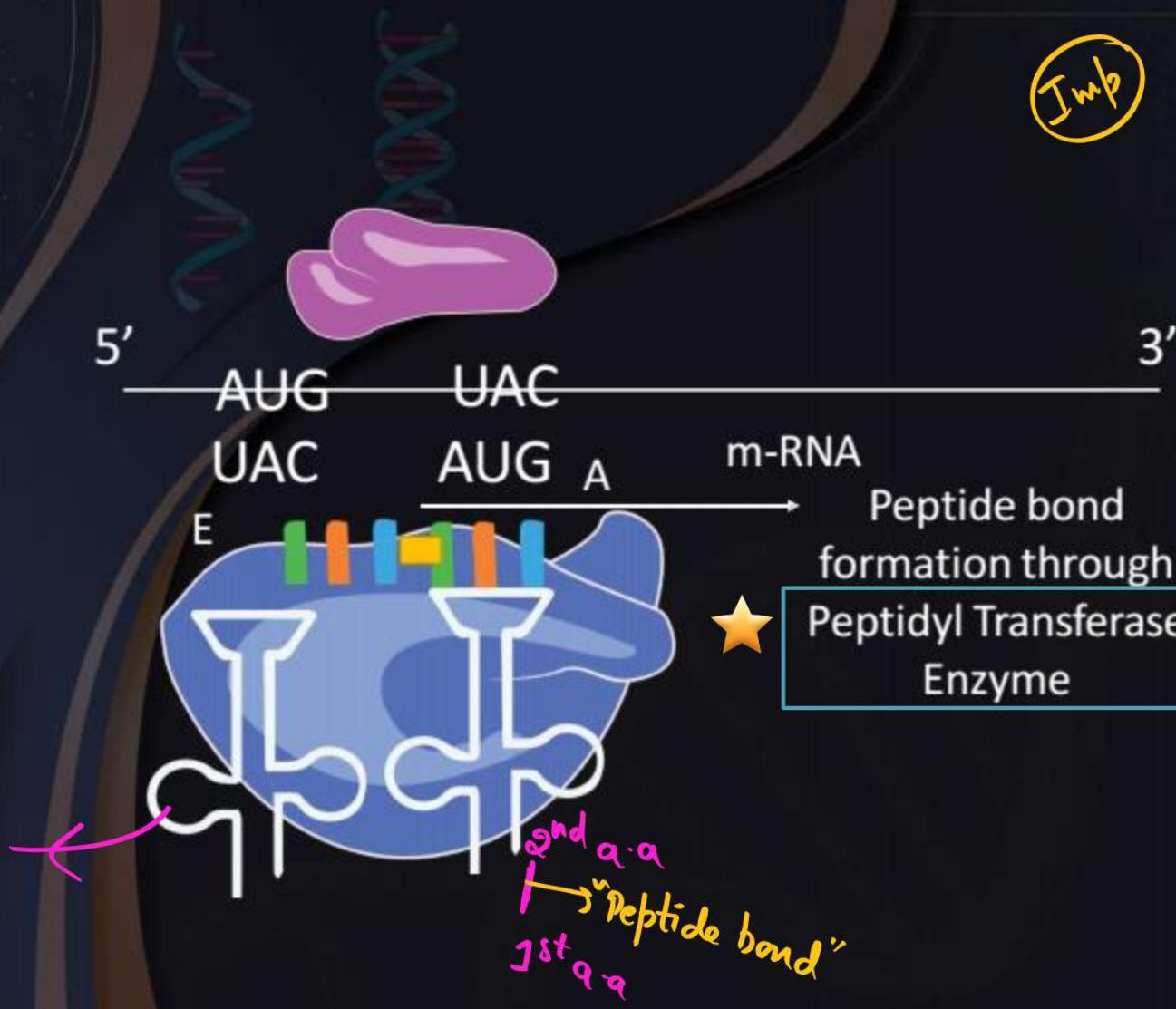


Translocation



One GTP molecule is consumed





Imp

Prokaryotes

23 s σ -RNA in
larger subunit

(Peptidyl transferase)

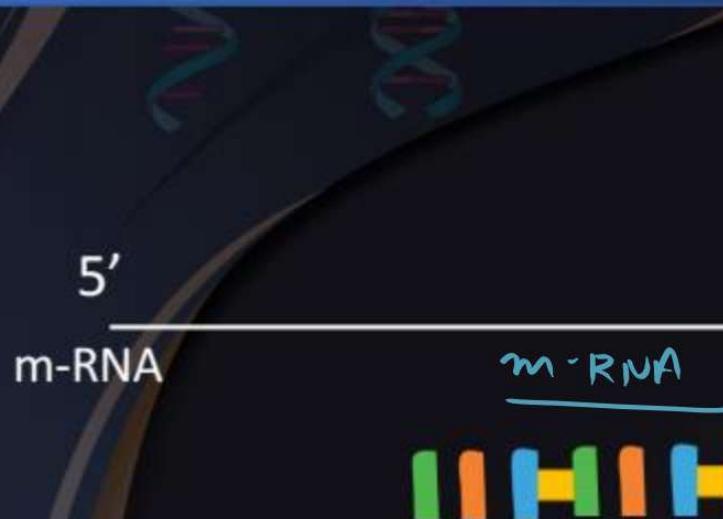
Euk

large subunit

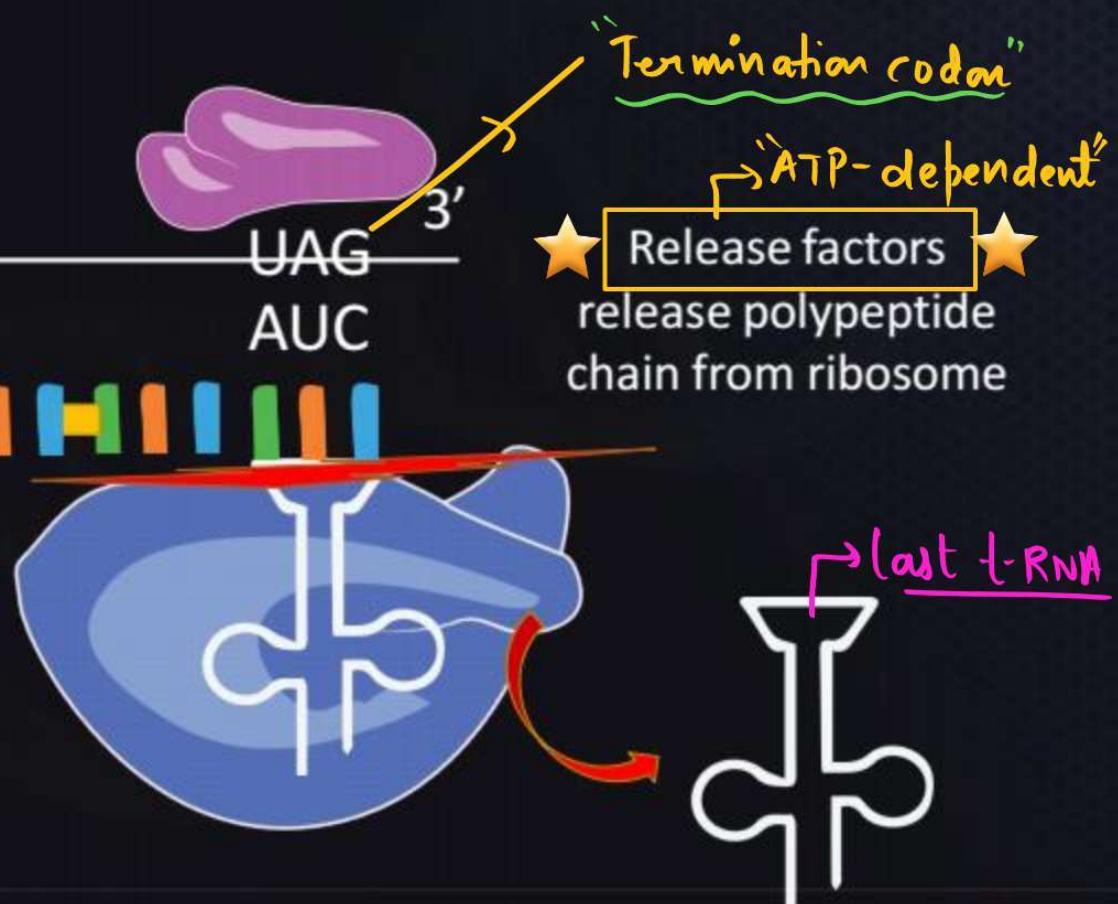
28 s σ RNA

(Peptidyl transferase)

Termination



There are no t-RNAs for
stop-codon



Protein chain
released

Termination

5'
m-RNA



Polypeptide
chain



Dissociation
Factors
dissociate the
two subunits
of ribosomes



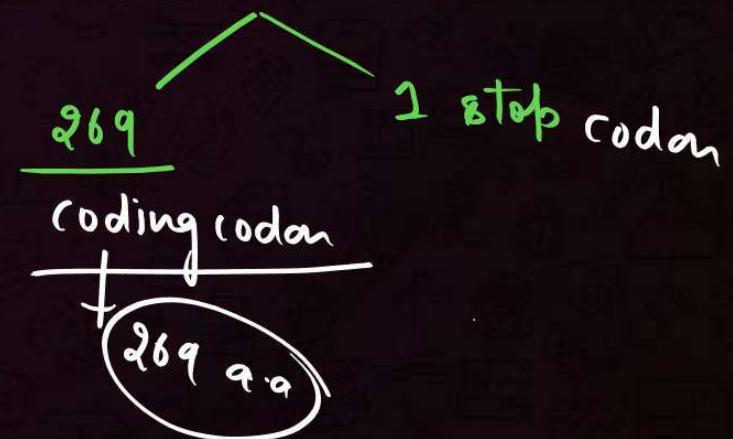


If an m-RNA molecule has 810 nucleotides then calculate the number of amino acids by this m-RNA.

$\frac{0}{=}$

3 nucleotides \rightarrow ① codon

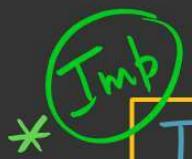
$$810 \text{ " } \rightarrow \frac{810}{3} = 270 \text{ codons}$$



Regulation of Gene Expression

(Control)

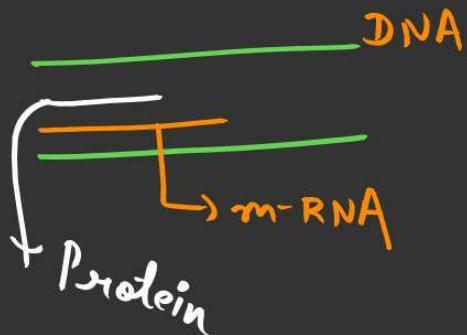
Simple in Prokaryotes



Initiation of Transcription

via the Predominant site

for Regulation.



Transcription & Translation

are Coupled in
Bacteria
in same compartment (cytoplasm)

Complicated

Regulation of Gene Expression in Eukaryotes

4 steps:

- ① Transcriptional level: Formation of **primary transcript** (hn-RNA) (Nucleus)
- ② Processing: Splicing of hn-RNA
- ③ Successful transfer of m-RNA to cytoplasm.
- ④ Translation (cytoplasm)
Ex → **Embryo-development**

Jacob & Monod

OPERON

only in Prokaryotes

DNA

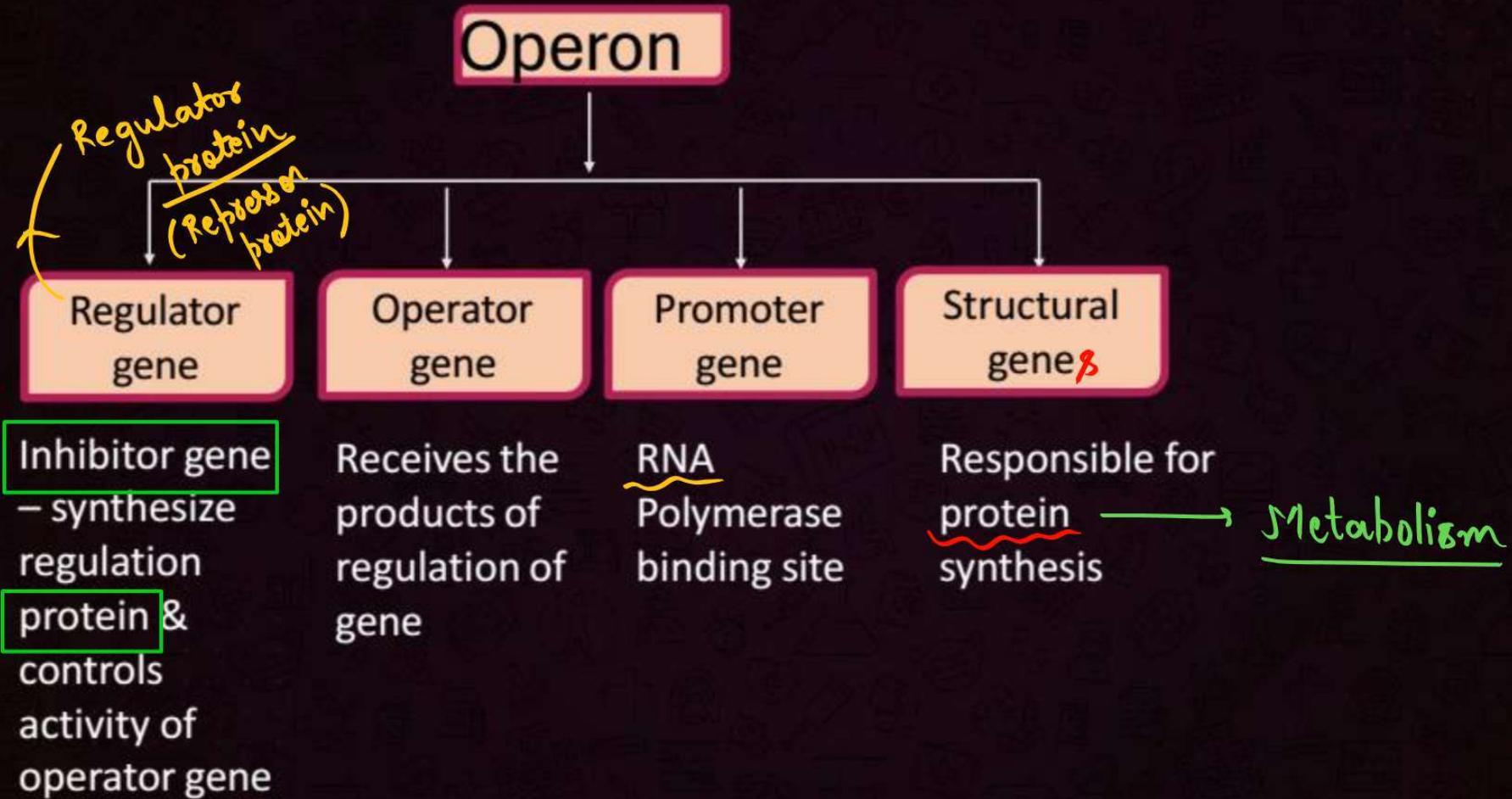
Segment of DNA → Has clusters of genes

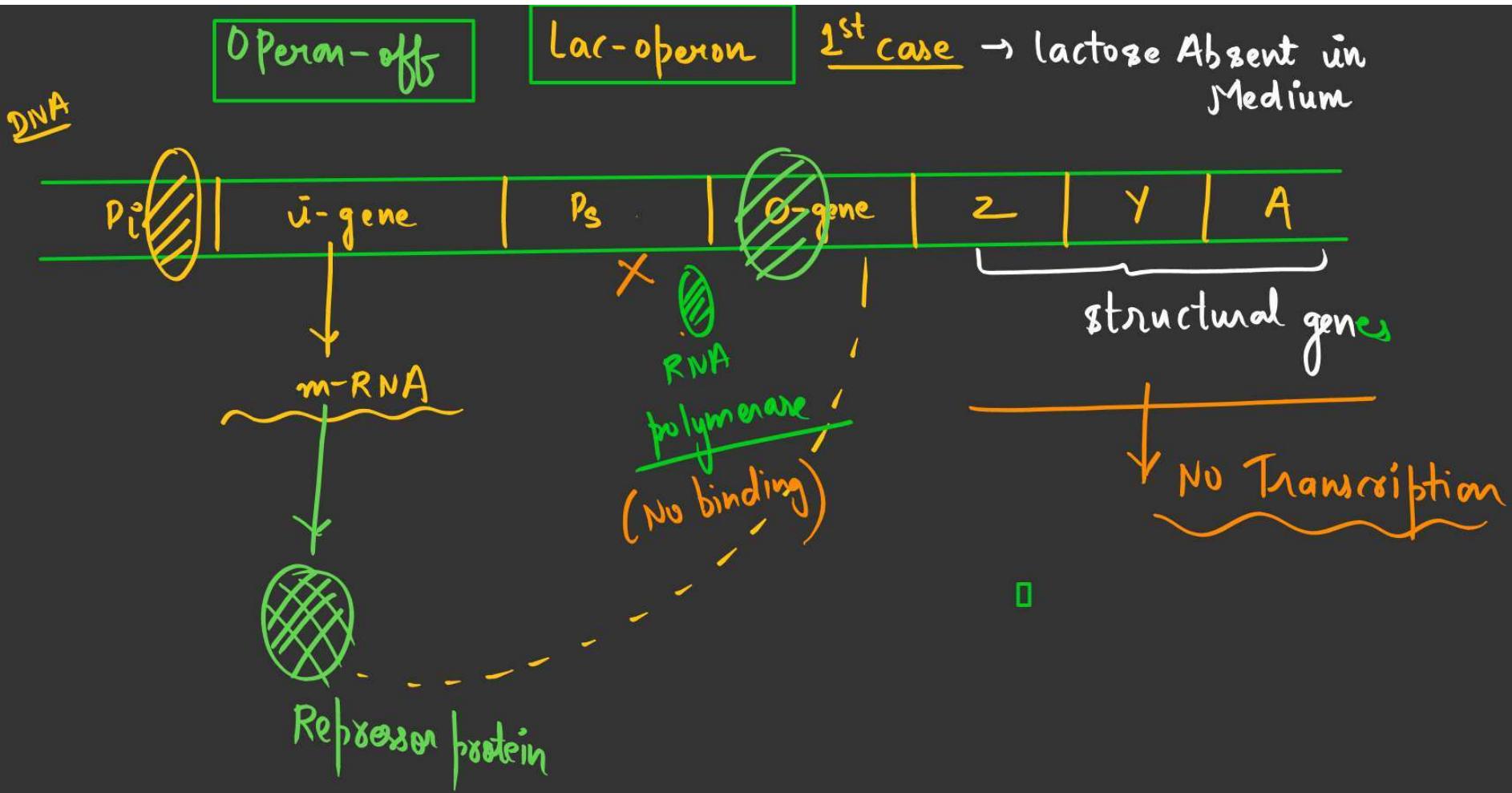
* lac-operon

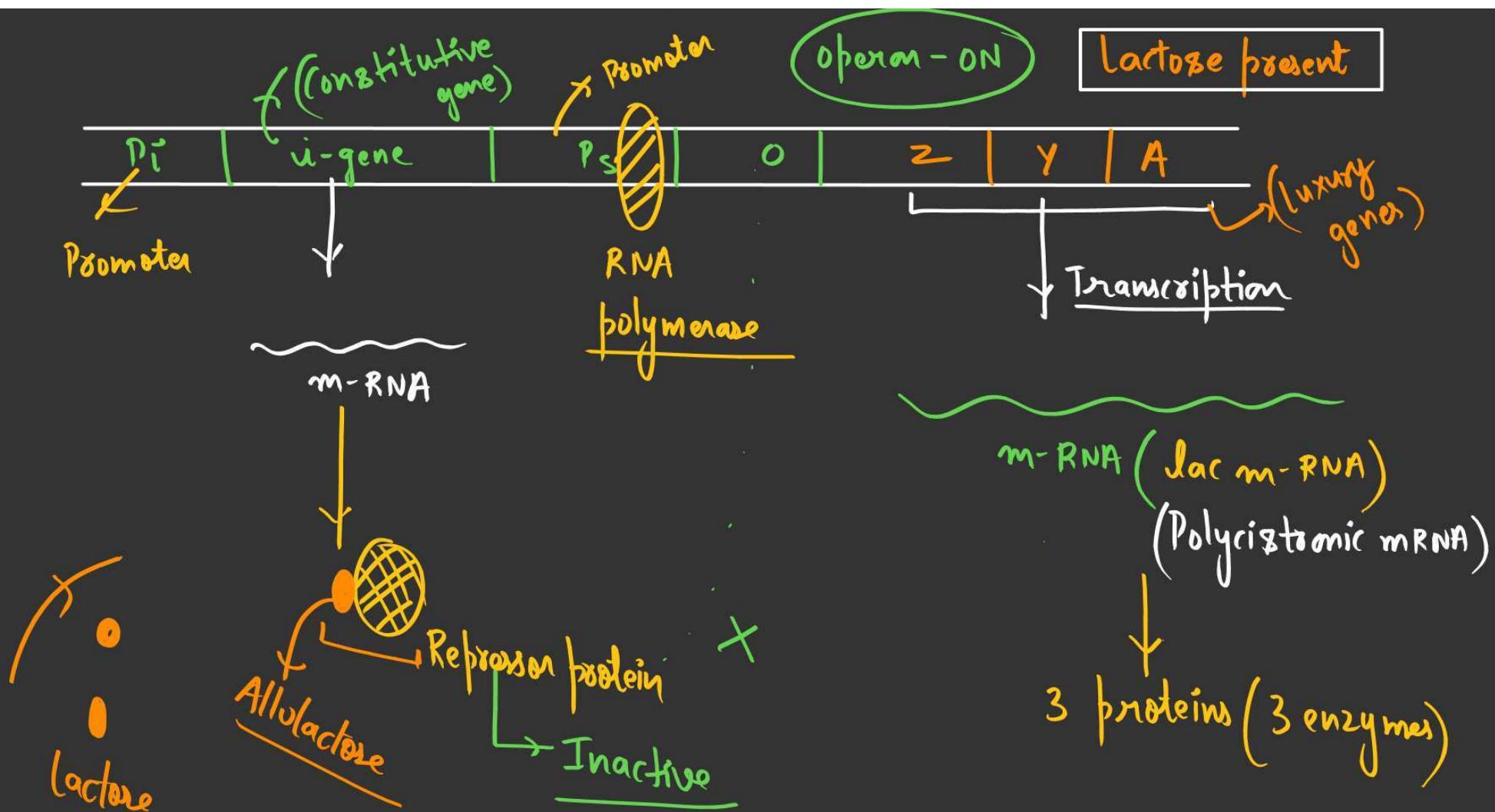
* trp-operon

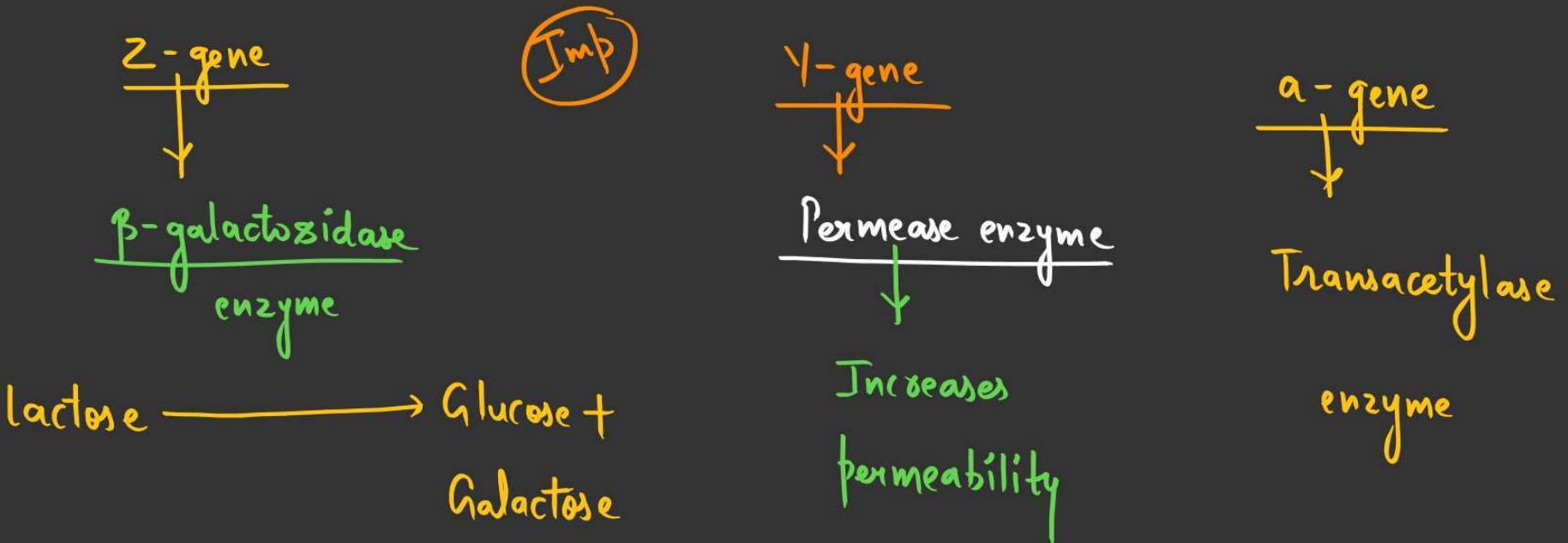
* his-operon

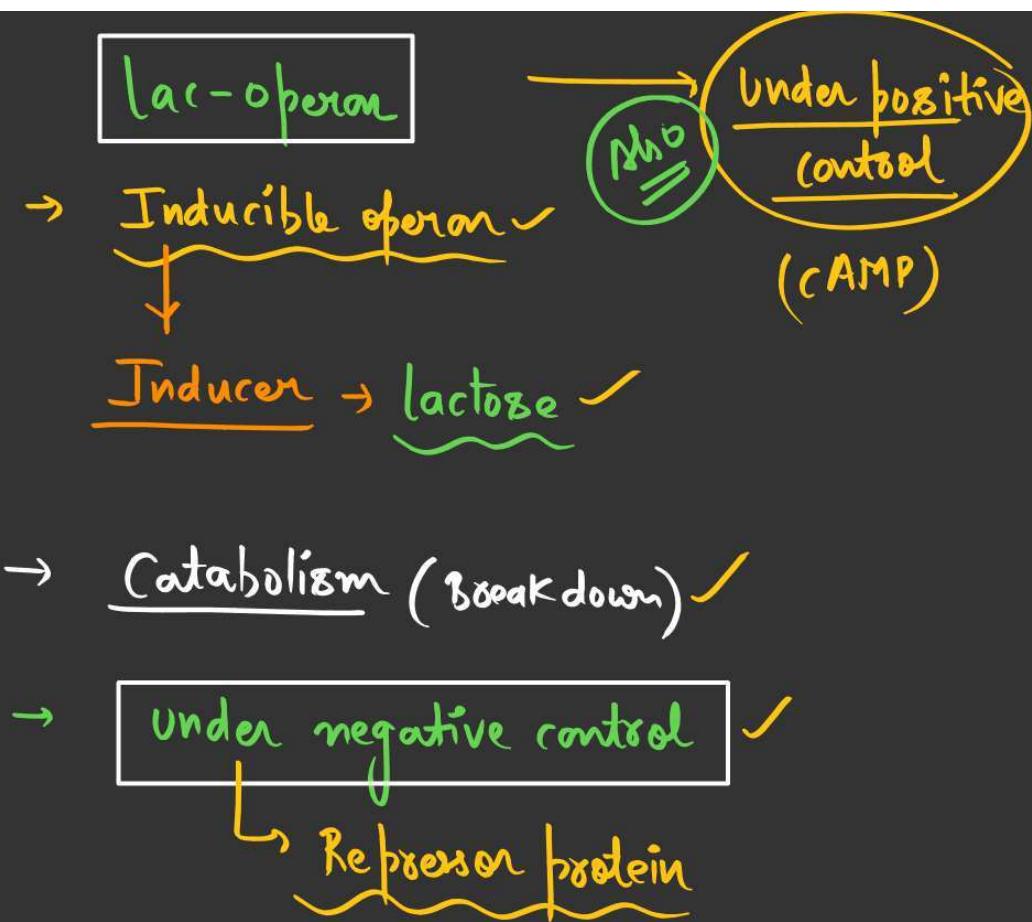
→ Responsible for
metabolism.







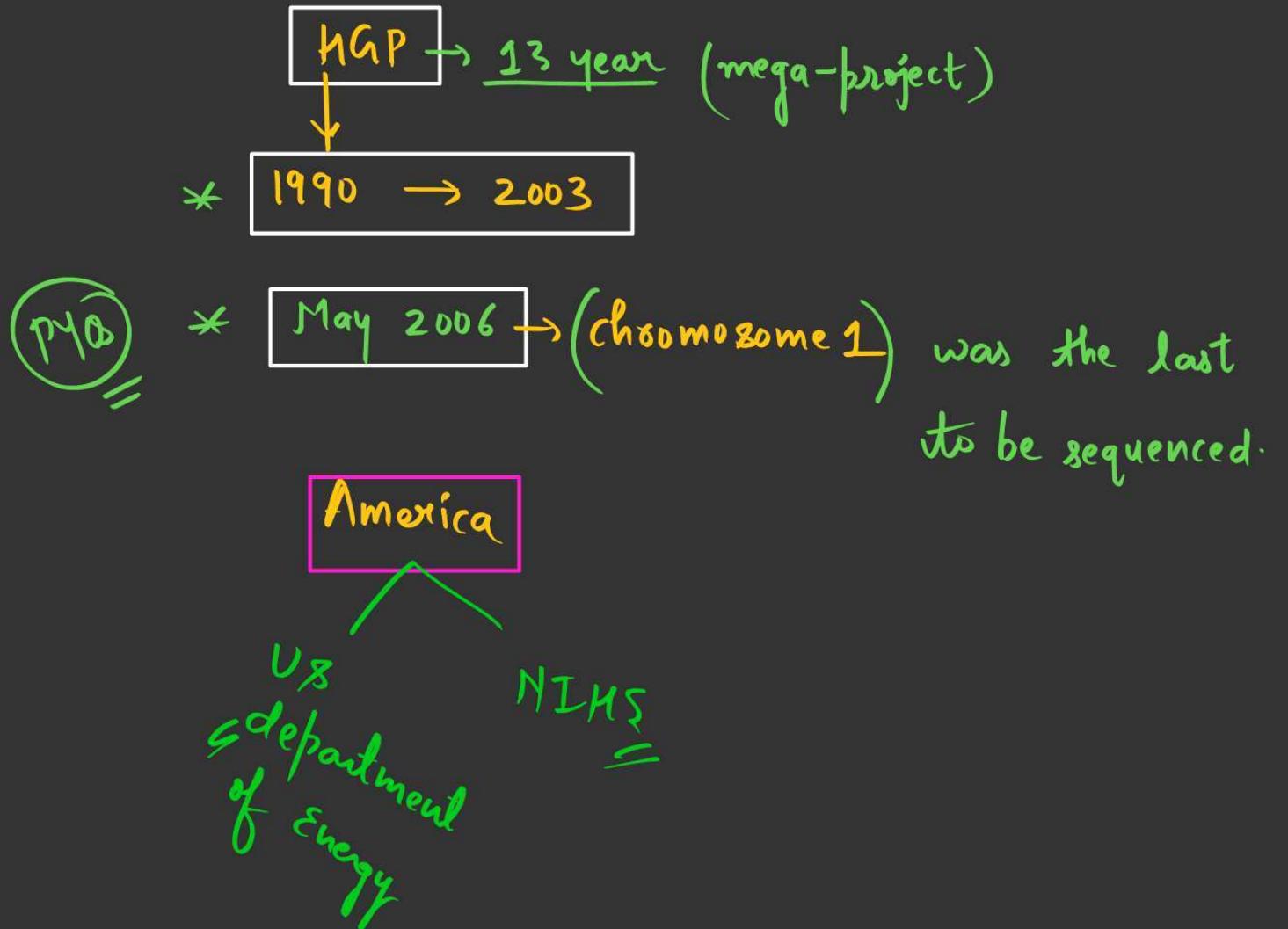




*

little amount
expression of operon
is always there.

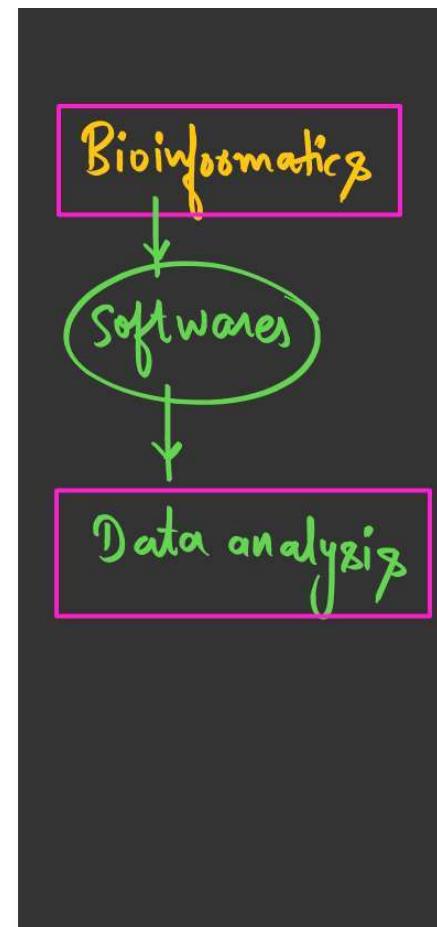
(little amount of
Permease enzyme
is always present).

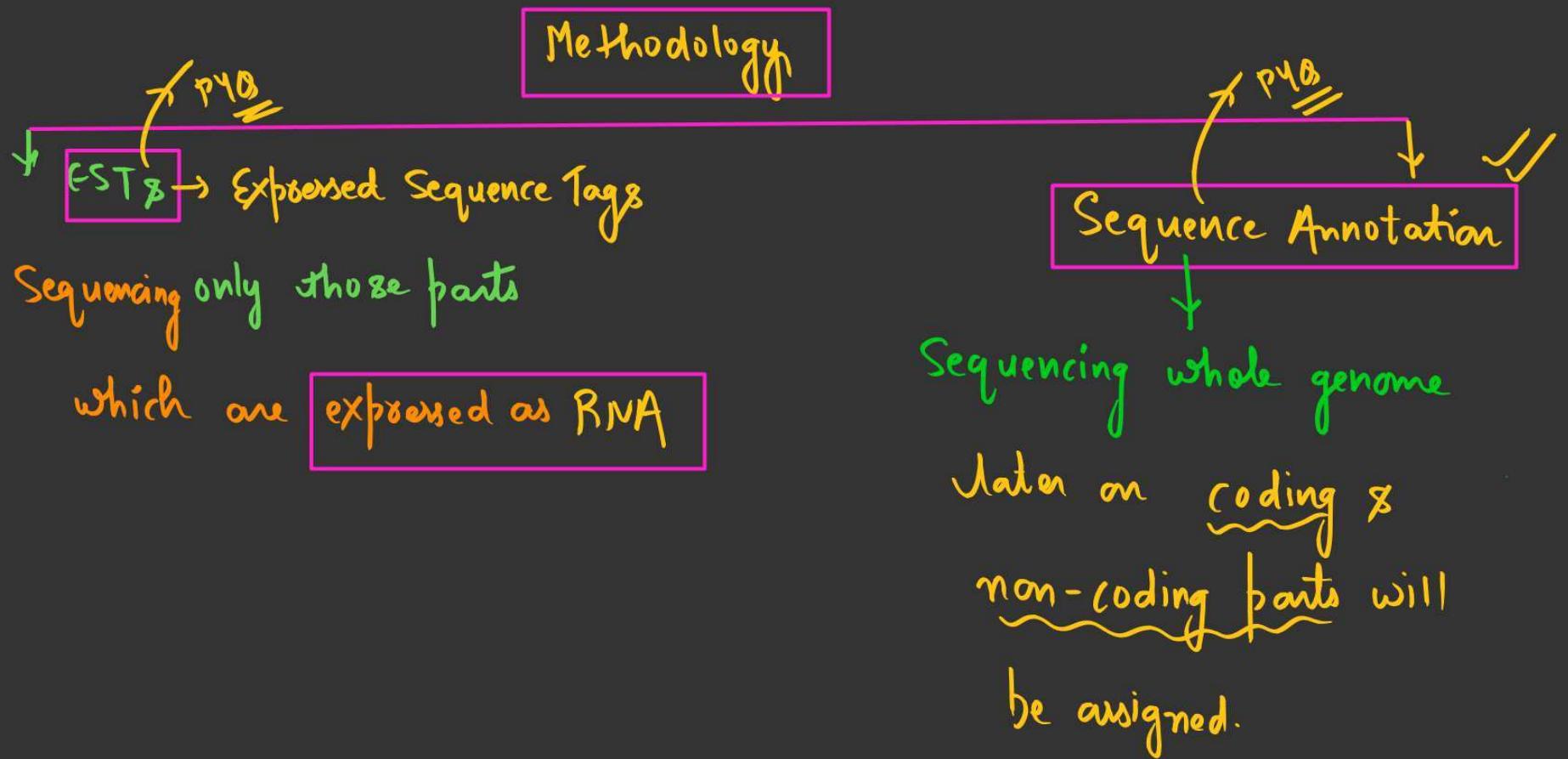


Goals of HGP

Some of the important goals of HGP were as follows:

- (i) Identify all the approximately 20,000-25,000 genes in human DNA;
- (ii) Determine the sequences of the 3 billion chemical base pairs that make up human DNA;
3 × 10⁹ bp's
- (iii) Store this information in databases;
- (iv) Improve tools for data analysis;
- (v) Transfer related technologies to other sectors, such as industries;
- (vi) Address the ethical, legal, and social issues (ELSI) that may arise from the project.





Non-human organism

Genome Sequence

- a) Bacteria
- b) Yeast
- c) *Caenorhabditis* (worm)
- d) *Drosophila* (Fruitfly)
- e) Rice (plant)
- f) *Arabidopsis* (plant)

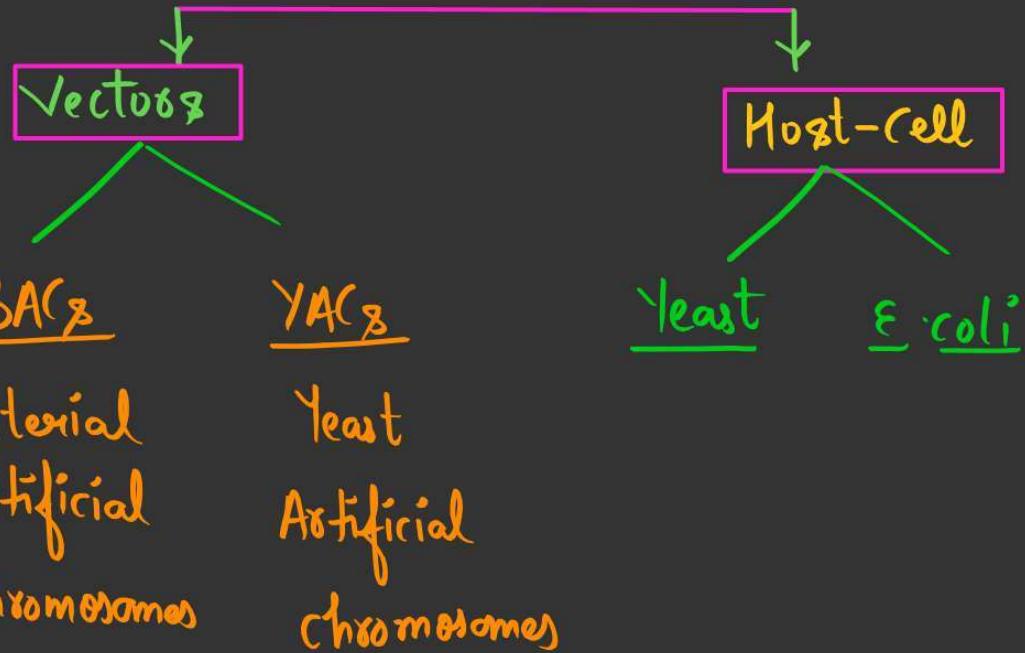
① DNA isolation

② Fragments of DNA
(Restriction Endonuclease)

③ Cloning
(Millions of copies of each DNA fragment)

Process

Cloning



⑤

Sequencing



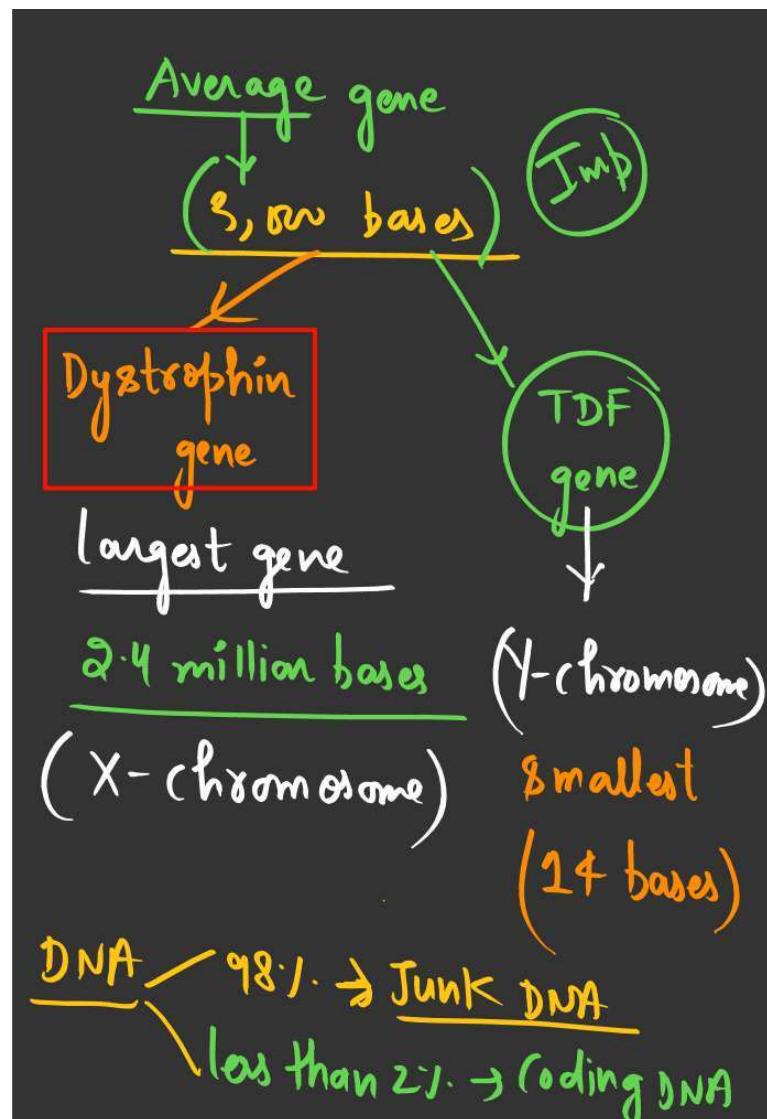
By Sanger's technique

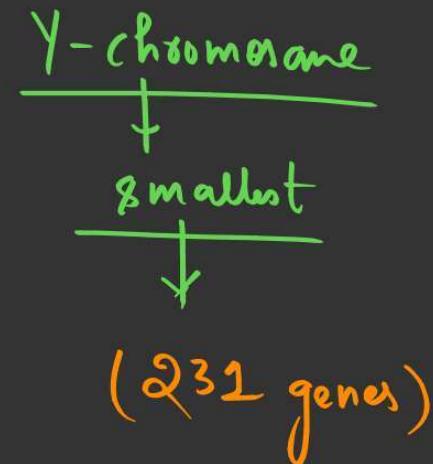
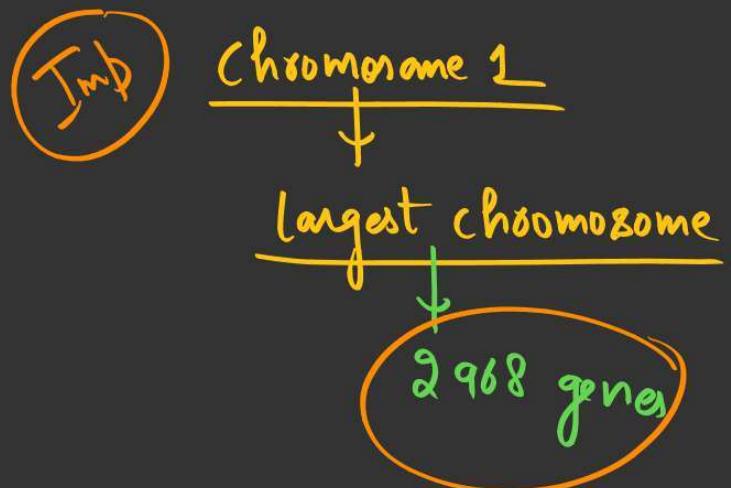
(Automated
sequencing)

5.9.1 Salient features of Human Genome

Some of the salient observations drawn from human genome project are as follows:

- (i) The human genome contains **3164.7 million bp.**
- (ii) The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.
- (iii) The total number of genes is estimated at 30,000—much lower than previous estimates of **80,000 to 1,40,000** genes. Almost all **(99.9 per cent)** nucleotide bases are exactly the same in all people.
- (iv) The **functions are unknown** for over **50 per cent** of the discovered genes.
- (v) Less than 2 per cent of the genome codes for proteins.
- (vi) **"Repeated sequences"** make up very large portion of the human genome.
- (vii) Repetitive sequences are stretches of DNA sequences that are repeated many times, sometimes hundred to thousand times. They are thought to have **no direct coding functions**, but they shed light on chromosome structure, dynamics and evolution.
- (viii) Chromosome 1 has most genes (2968), and the Y has the fewest (231).
- (ix) Scientists have identified about 1.4 million locations where single-base DNA differences (**SNPs – single nucleotide polymorphism**, pronounced as 'snips') occur in humans. This information promises to revolutionise the processes of finding chromosomal locations for disease-associated sequences and tracing human history.

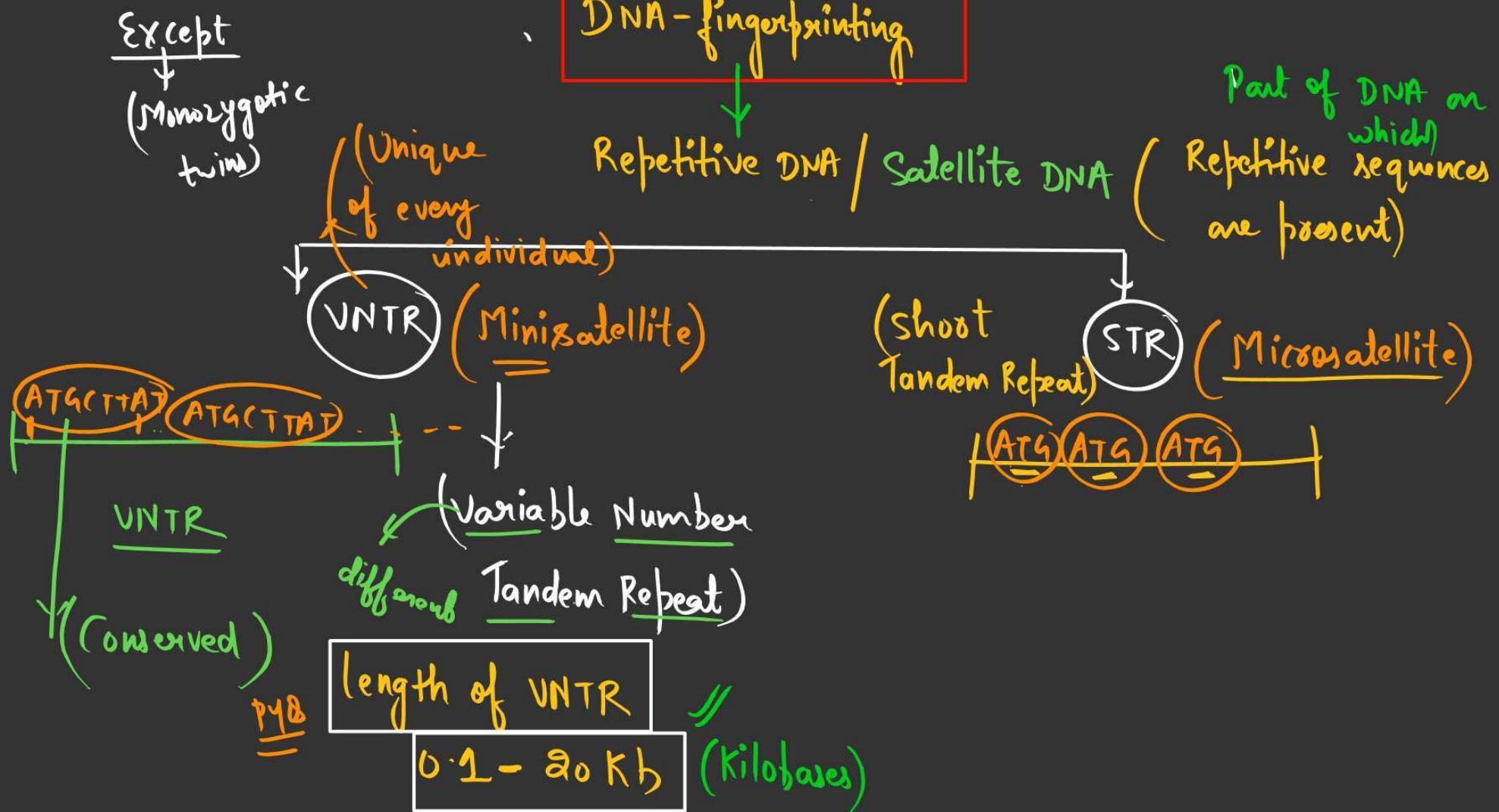




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Snips | SNPs → "Single Nucleotide Polymorphism"
 "1.4 million locations"

DNA-fingerprinting

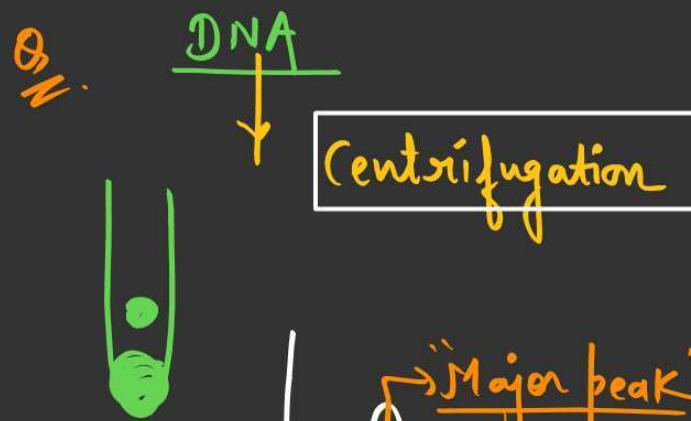
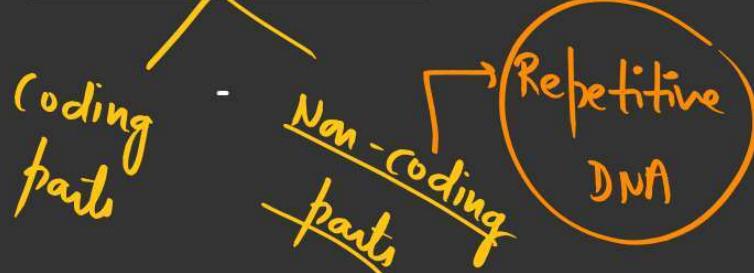


All Humans
99.9% → Similar

0.1% → Different

0.1% of 3×10^9 bps

3×10^6 bps



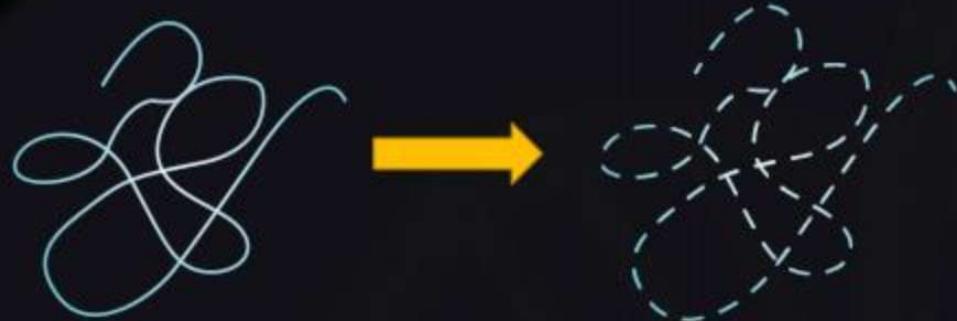
1st step → Isolation of DNA

↓

(By enzymes)

Digestion of DNA

Fragments of DNA



Process

DNA is cut by
restriction
endonuclease

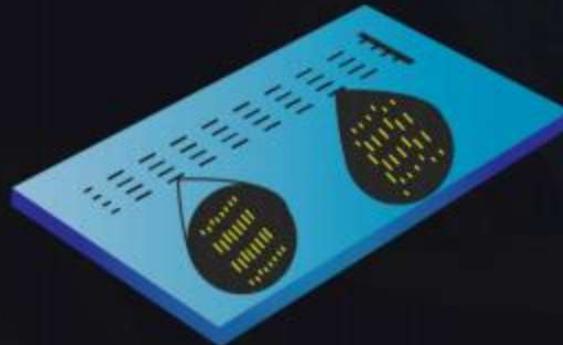
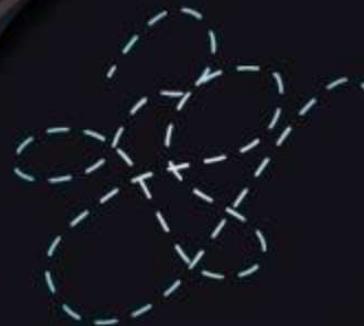


RFLP

Restriction Fragment
length Polymorphism

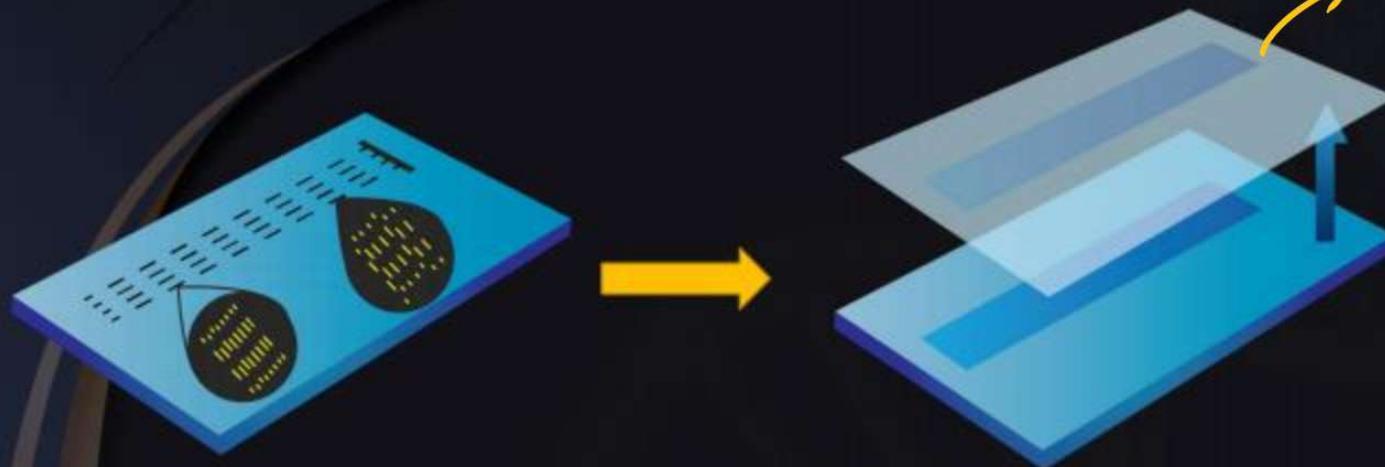
Separation of DNA by Gel Electrophoresis

fragment



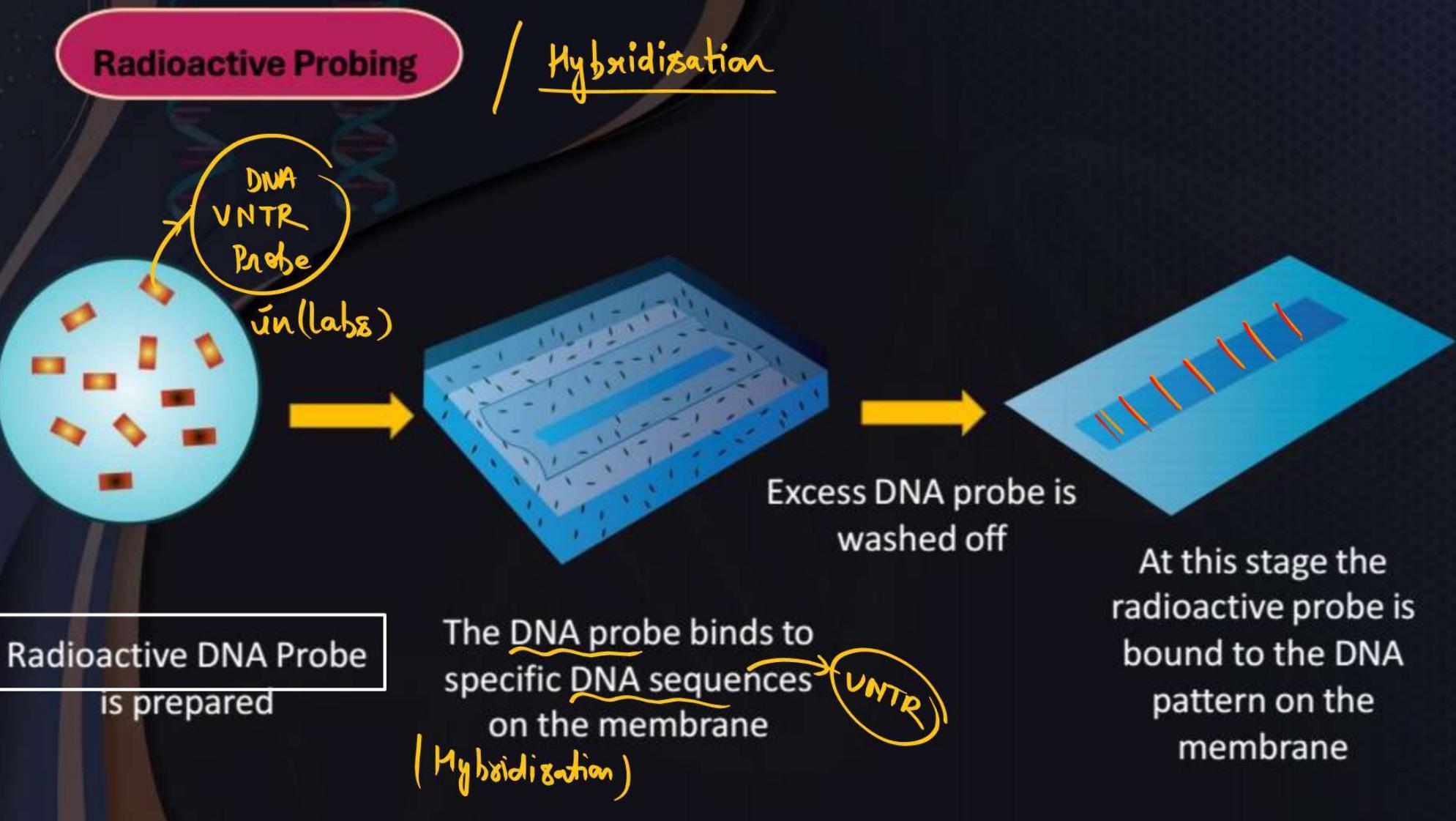
The DNA fragments are separated into bands during electrophoresis in an agarose gel

Southern Blotting

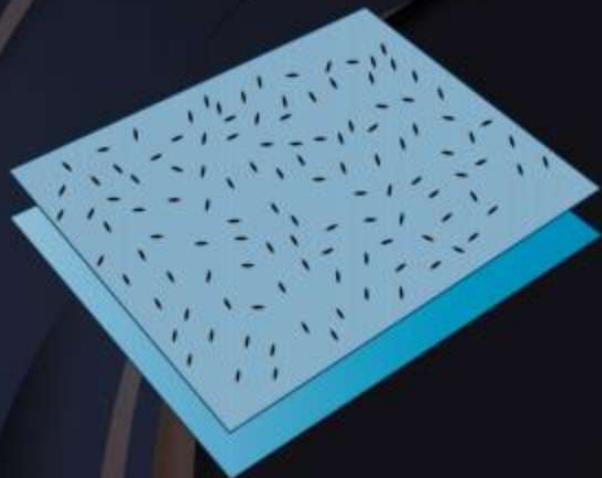


Nylon membrane/
Nitrocellulose
membrane

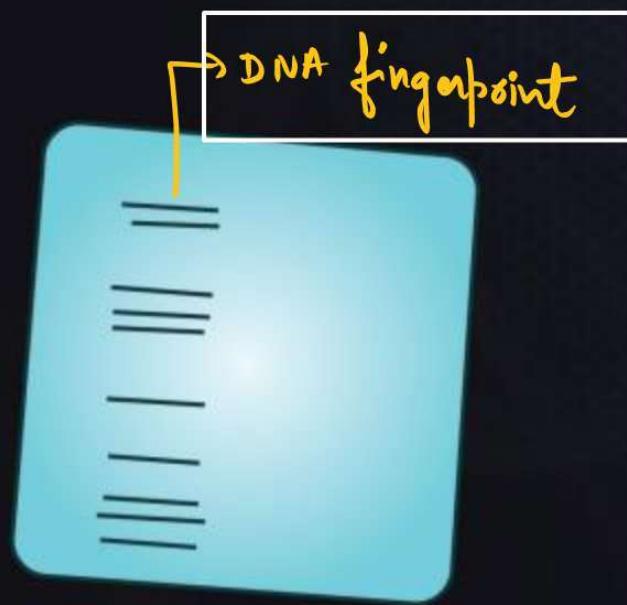
The DNA band pattern in
the gel is transferred to a
nylon membrane by a
technique known as
southern blotting



Detection by autoradiography



X-ray film is placed next to the membrane to detect the radioactive pattern



The X-ray film is developed to make visible the pattern of bands which is known as a DNA fingerprinting



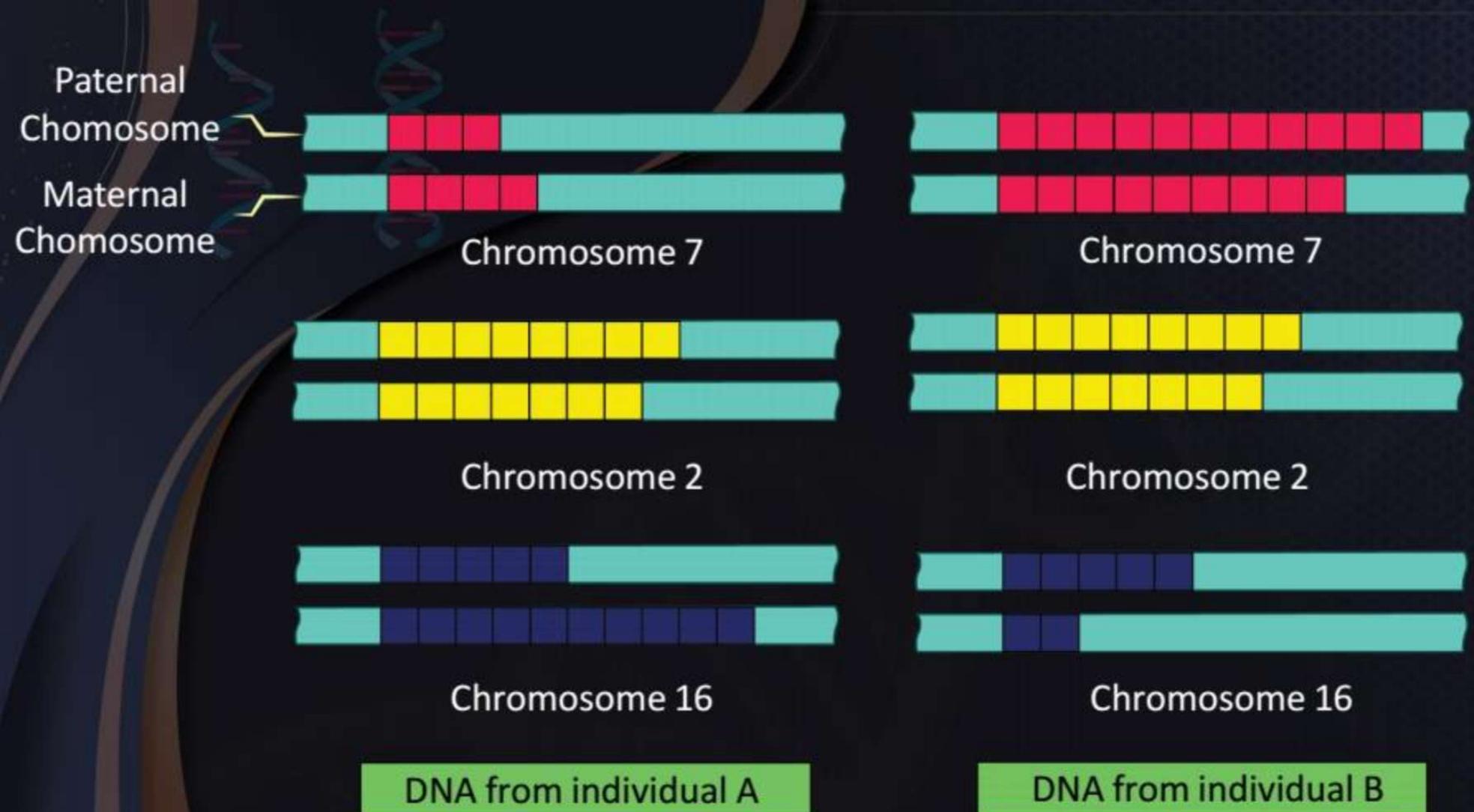
Practical Applications

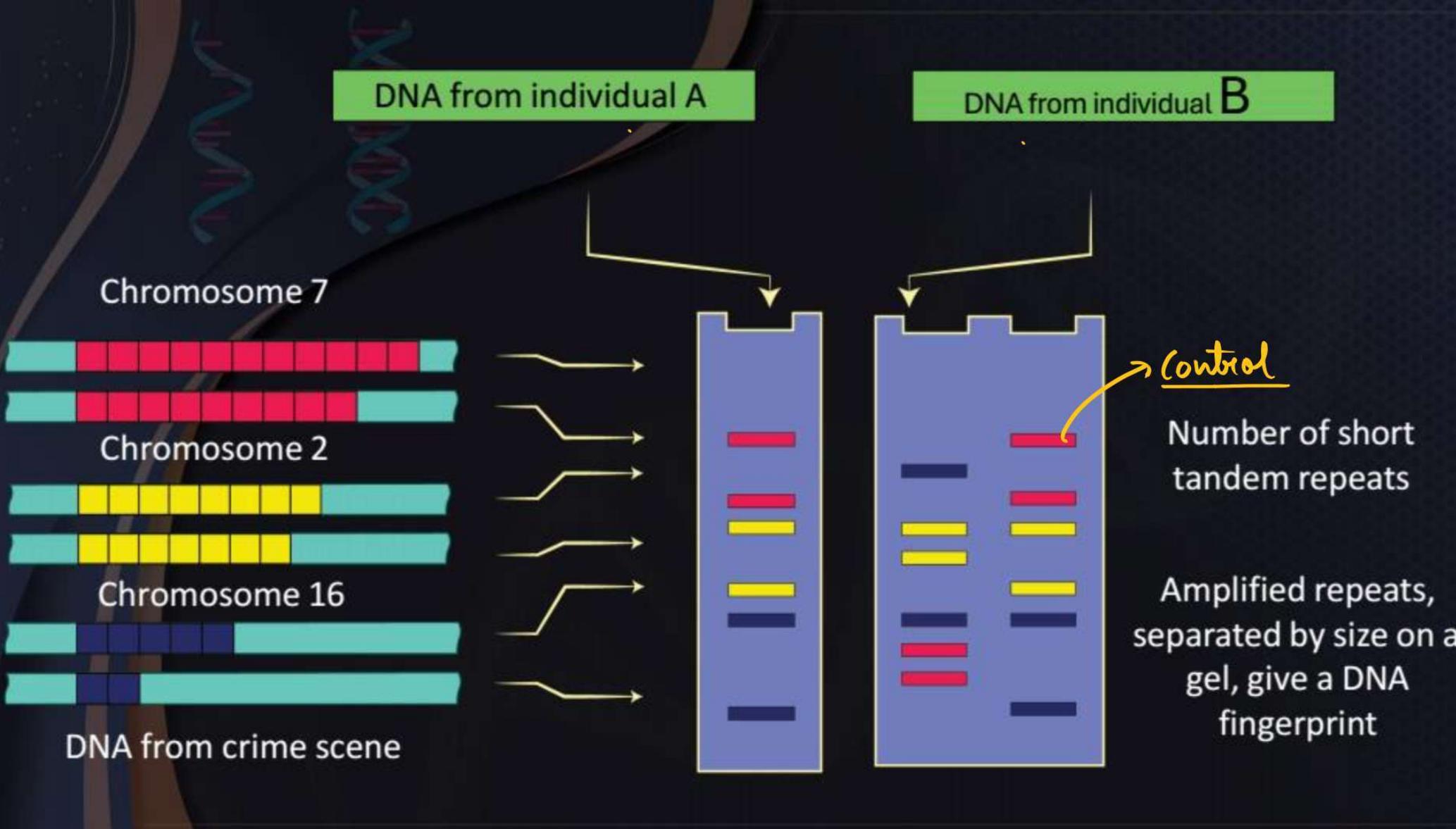
Paternity-Maternity disputes

Criminal identification and forensics

Personal Identification

Close relations of an intending immigrant

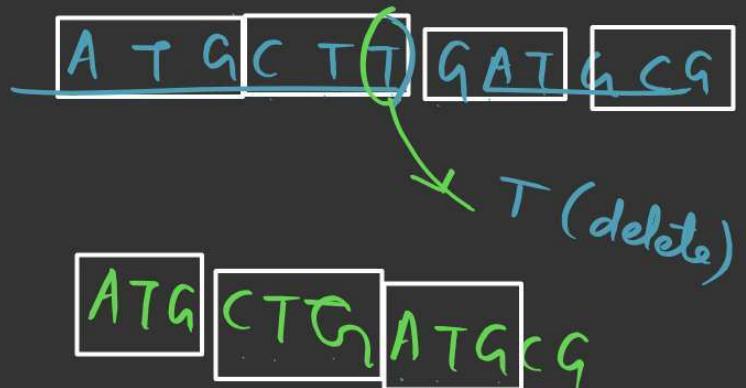




Mutation

Frame-shift Mutation

Addition
Deletion



NOTE : If whole codon is added or deleted
then mutation is there but not frameshift mutation

Questions for Homework

For **answers & Solutions**

See

Uthaan Series

on **Competititonwallah**
channel



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



QUESTION

Which of the following acts as substrate as well as provide energy for DNA polymerisation?

- 1** Ribonucleoside
- 2** Deoxyribonucleoside
- 3** Ribonucleotide
- 4** Deoxyribonucleoside triphosphate



QUESTION

The mRNA of prokaryotes is

- 1** Polycistronic
- 2** Monocistronic
- 3** Formed by splicing of hnRNA
- 4** Carries genetic message to DNA



QUESTION

Capping in hnRNA is catalysed by

- 1** Poly A polymerase
- 2** SnRNA
- 3** Guanyl transferase
- 4** Catalytic RNA

QUESTION



Which of the following type of ribosomal RNA is not present in eukaryotic cytoplasm?

1 18 S

2 28 S

3 5.8 S

4 16 S



QUESTION

Which of the following codons is known as ochre?

1 UAG

2 UGA

3 UAA

4 UUU



QUESTION

Which of the following is an ambiguous codon?

1 AUG

2 GUG

3 UAG

4 GAG



QUESTION

Activation of amino acids during translation is done by

1 Peptidyl transferase

2 Aminoacyl-tRNA synthetase

3 Methionine

4 Initiation factors

QUESTION



How many structural genes are present in lac-operon of *E. coli*?

1 4

2 3

3 2

4 1



QUESTION

Select incorrectly matched pair

1 Lac z – Constitutive gene

2 Operator gene – Smallest gene of lac operon

3 Lac a – Transacetylase

4 Promotor gene – RNA polymerase

QUESTION

Sequencing the whole set of genome that contained all the coding and non-coding sequences and later assigning different regions in the sequence with functions is known as

- 1** Sequence annotation
- 2** PCR
- 3** Northern blot
- 4** Microarray



QUESTION

The last step of DNA fingerprinting is

- 1 Blotting
- 2 Autoradiography
- 3 Hybridisation
- 4 Isolation of desired DNA



QUESTION

In humans, the largest gene is present on

- 1** Chromosome-1
- 2** Y-chromosome
- 3** X-chromosome
- 4** Chromosome-7



QUESTION

TDF gene is the smallest gene in humans with

- 1** 231 bp
- 2** 14 bp
- 3** 2968 bp
- 4** 3000 bp



QUESTION

SNPs stands for

- 1** Single nucleoside polymorphism
- 2** Simple nucleotide polymorphism
- 3** Single nucleotide polymorphism
- 4** Simple nucleoside polymorphism



QUESTION

Find out the incorrect match.

- 1** UUU - Phenylalanine
- 2** UAG - Sense codon
- 3** GUG - Valine
- 4** UGG - Tryptophan



QUESTION

Mark the incorrect option w.r.t. lac operon

- 1** Is under positive as well as negative control
- 2** Controls catabolic pathway
- 3** Shows feed back repression
- 4** Discovered by Jacob and Monod



QUESTION

In *lac* operon, the *lac* mRNA

- 1 Has several initiation and termination codons
- 2 Forms four different enzymes
- 3 Is not transcribed in the presence of lactose
- 4 Is involved in an anabolic reaction



QUESTION

What is correct for bacterial transcription?

- 1** mRNA requires processing to become active
- 2** Translation can begin when mRNA is fully transcribed
- 3** Transcription and translation takes place in the same compartment
- 4** Rho factor initiates the process

QUESTION

In eukaryotes, RNA polymerase III catalyses the synthesis of

- 1** 5 S rRNA, tRNA & SnRNA
- 2** mRNA, HnRNA & SnRNA lo ajaeritaya
- 3** 28 S rRNA, 18 S rRNA & 5 S rRNA
- 4** All types of rRNA & tRNA



QUESTION

How many amino acids will be coded by the mRNA sequence - 5'CCCUCAUAGUCAUAC3' if a adenosine residue is inserted after 12th nucleotide?

- 1** Five amino acids
- 2** Six amino acids
- 3** Two amino acids
- 4** Three amino acids

QUESTION

The DNA strand showing replication using Okazaki fragments also shows

- 1** Continuous growth in 5' → 3' direction
- 2** Discontinuous growth on 5' → 3' parental strand
- 3** Discontinuous growth on 3' → 5' parental strand
- 4** Involvement of one primer only



QUESTION

Assertion: Sigma factor of RNA polymerase recognizes the start signal region in prokaryotes.

Reason: Promotor region lies at 5' of template strand.

- 1** Both Assertion & Reason are true and the Reason is a correct explanation of the Assertion.
- 2** Both Assertion & Reason are true but Reason is not a correct explanation of the Assertion.
- 3** Assertion is true but Reason is false.
- 4** Assertion is false but the Reason is true.



QUESTION

Assertion: Peptidyl transfer site is contributed by larger sub-unit of ribosome.

Reason: The enzyme peptidyl transferase is contributed by both 23S and 16S ribosomal sub-units.

- 1** Both Assertion & Reason are true and the Reason is a correct explanation of the Assertion.
- 2** Both Assertion & Reason are true but Reason is not a correct explanation of the Assertion.
- 3** Assertion is true but Reason is false.
- 4** Assertion is false but the Reason is true.