5. MOLECULAR BASIS OF INHERITANCE

DNA is the genetic material in most of the organisms

Properties of Genetic Material

(i) Be able to **generate its replica**.
(ii) Chemically and structurally be **stable**.
(iii) Potentiality to **generate variation**s → evolution.
(iv) Be able to **express characters** specified by it.

THE SEARCH FOR GENETIC MATERIAL

1. **Griffith's Experiment** (Transforming principle)

- **Frederick Griffith** (1928) performed experiments with *Streptococcus pneumoniae*.

This bacterium has 2 strains-

***S** (smooth) strain*	***R** (Rough) strain*
▪ Has a polysaccharide coat	▪ No coat
▪ Virulent (causes pneumonia)	▪ Non virulent

Experiment:

- S-strain → Inject into mice → Mice die (due to pneumonia)
- R-strain → Inject into mice → Mice live (no ill-effect)
- Heat killed S-strain → Inject into mice → Mice live
- Hk S-strain + R-strain → Inject into mice → Mice die

Conclusion:- Some chemical substances present in the heat killed S-strain **transform** R-strain to S-strain.

2. **Biochemical explanation for Griffith's observation**

Avery, **MacLeod** and **McCarty** (1944) perfected the biochemical substance responsible for transformation in Griffith's experiment.

Experiment:

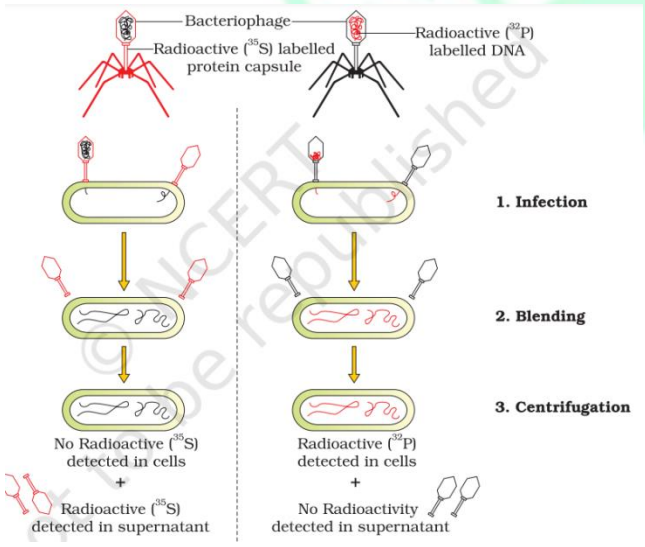
R-cells + Biochemicals (proteins, RNA, DNA etc.) from the heat-killed S cells → S cells

R-cells + " + Proteases → S cells
R-cells + " + RNases → S cells
R-cells + " + DNases → R cells

Conclusion: - Because only DNase inhibits transformation, the transforming substance is DNA and it is the genetic material.

3. **Experiment to confirm DNA as the genetic material**

Experiment of **Hershey** and **Chase** (1952) with *bacteriophages*.



Experiment:-

i. Prepared two cultures of bacteriophage–
In one, **protein coat** were radioactively labelled with ^{35}**S**
In second, **DNA** is radioactively labelled with ^{32}P.
ii. **Radioactive phages** were used separately to infect *E. coli*.
iii. After infection, the *E. coli* was **blended** and **centrifuged**.

Inference:-
Viral DNA gets into bacterium and the protein remains outside.

The DNA

Scientist	Contribution
Friedrich Meischer (1869)	Identified DNA as acidic substance in nucleus and named it as **'Nuclein'**
Erwin Chargaff (1950)	Observed that the ratio of A to T and that of G to C is always equal to one
Maurice Wilkins & **Rosalind Franklin** (1953)	Used X-ray diffraction data to study DNA
James Watson & **Francis Crick** (1953)	Proposed **double helix model of DNA**

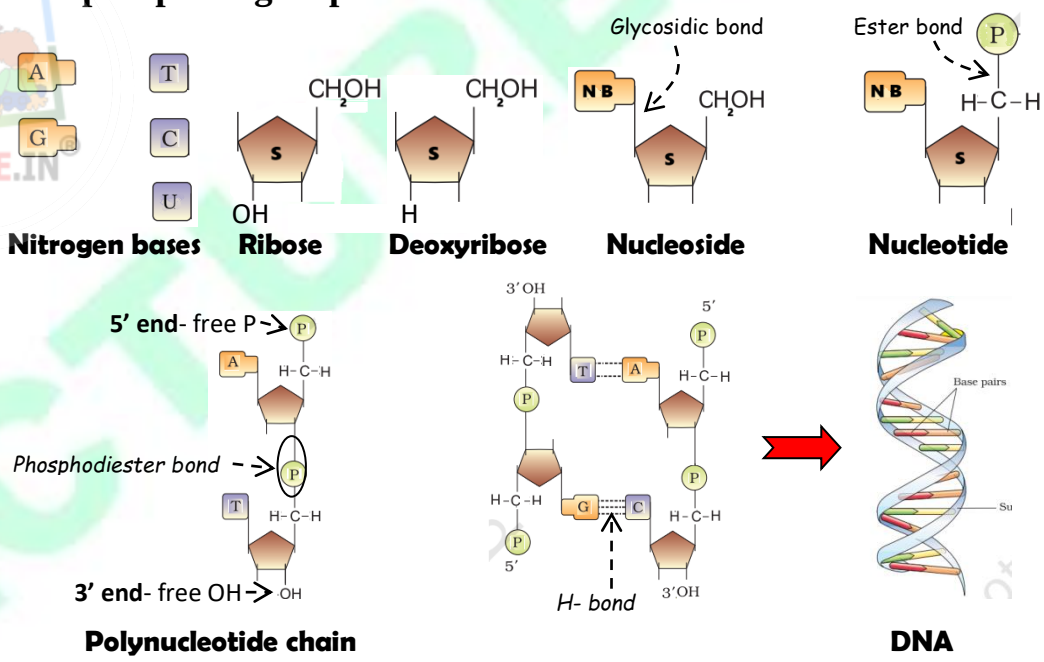
STRUCTURE OF POLYNUCLEOTIDE CHAIN

DNA & RNA are polynucleotides. A nucleotide has 3 components:

1. A nitrogenous base, 2 types -
- **Purines:** It includes **Adenine** and **Guanine.**
- **Pyrimidines:** It includes **Cytosine, Thymine** & **Uracil.**

2. A pentose sugar (ribose in **RNA & deoxyribose** in **DNA)**

3. A phosphate group



Salient features of double helix model of DNA:

(i) Made of 2 polynucleotide chains which are **anti-parallel.**
(ii) Purines and pyramidines in 2 chains are paired through **H-bonds** forming **base pairs** (bp).
A=T (2 H bonds) C≡G (3 H bonds).
(iii) **Length of DNA** = number of bp **x** distance b/w bp (**0.34nm**).

Packaging of DNA Helix

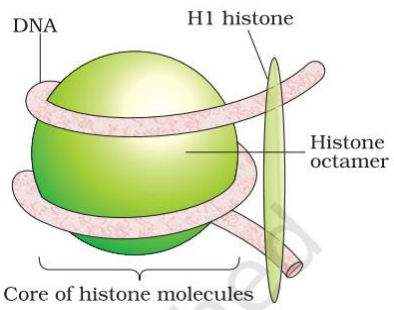
In prokaryotes

♥ DNA$^-$ is held with some proteins$^+$ → **nucleoid** (large **loops).**

In eukaryotes

In eukaryotes, DNA is found in the nucleus.

♥ 200 bp DNA$^-$ is wrapped around 8 **histone** proteins$^+$ → **nucleosome**



♥ Nucleosomes —repeat→ **Chromatin**

♥ Chromatin → **chromatin fibers** —condense at metaphase→ **Chromosomes**

❖ Chromatins include -

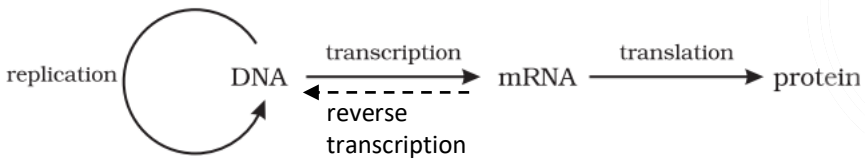
Euchromatin	**Heterochromatin**
1. **Loosely-packed** region	1. **Densely-packed** region
2. **Lightly-stained** regions	2. **Darkly-stained** regions
3. Transcriptionally **active**	3. Transcriptionally **inactive**

DNA $^{V}/_{s}$ RNA

DNA	RNA
Acts as the genetic material in most of the organisms	- Acts as the genetic material in some viruses (eg: TMV, QB bacteriophage) - functions as messenger (mRNA). - Act as enzyme (***ribozyme***).
DNA was derived from RNA	RNA is the first to evolve
DNA is a better genetic material ∵ Chemically less reactive and structurally more stable due to • Being double stranded • Presence of thymine (5-methyl Uracil). • Absence of 2′-OH in deoxyribose sugar	**RNA is better for the transmission of genetic information** ∵ easily degradable due to • Being single stranded • Presence of uracil (less stable compared to thymine). • Presence of 2′-OH in ribose sugar (a reactive group)
Resist mutation by repair mechanism	Mutate at a faster rate
DNA is dependent on RNA for synthesis of proteins DNA → RNA → Protein	Can directly code for the synthesis of proteins

CENTRAL DOGMA OF MOLECULAR BIOLOGY

Proposed by **Francis Crick** (1953)



DNA Replication

➔ **DNA replication:** *Parental DNA → 2 DNA*

Watson & **Crick** (1953) proposed **Semi-conservative model** of replication.

Experimental proofs for semi conservative DNA replication

1. **Messelson & Stahl** (1958) experiment on *E. coli* (prokaryote)

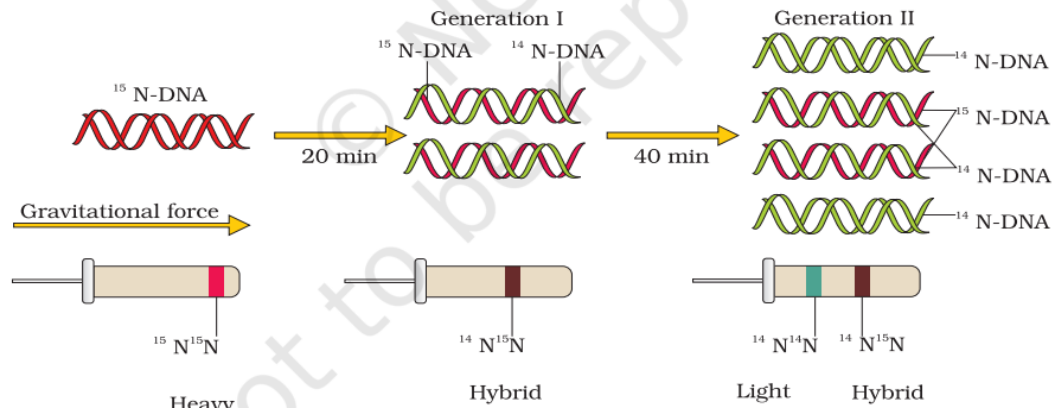
Steps:-

(i) Grew *E. coli* in a medium containing heavy ^{15}**N** for many generations→ DNA and become **heavier**.

(ii) The cells were transferred into a medium with normal ^{14}**N**

(iii) Took **samples** at various definite time intervals to extract DNA.

(iv) Then it is undergone for **centrifugation** in ***CsCl*** and measured to get their densities.



Inference:-

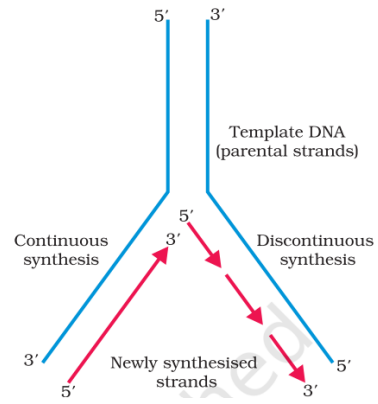
The newly synthesised DNA obtains one of its strands from the parent.

2. **Taylor** (1958) on *Vicia faba* (eukaryote).

Using **radioactive thymidine** to detect distribution of newly synthesized DNA in the chromosomes.

MECHANISM OF DNA REPLICATION

1. The replication of DNA takes place at S-phase of the cell-cycle.
2. DNA replicates in 5′➔3′
3. DNA replication starts at a point called **origin of replication *(ori)***.
4. The replication occurs within a small opening of the DNA helix, referred to as **replication fork**.
5. **DNA polymerase** uses DNA template to catalyse the polymerisation of deoxynucleotides.
6. **Deoxyribonucleoside triphosphates** serve dual purposes-
   - act as **substrates**
   - **provide energy** for polymerisation.



7. 2 new strands are synthesised along the replication fork-

Template with polarity 3′➔ 5’- <u>continuous replication</u>

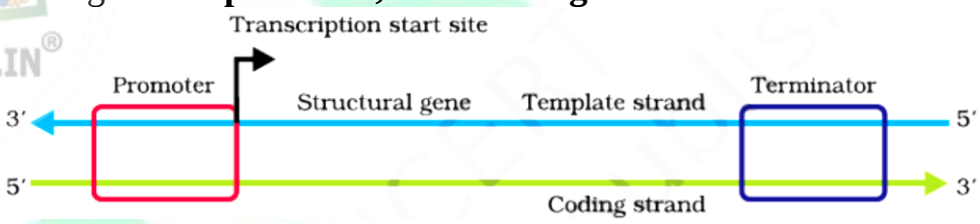
Template with polarity 5′➔ 3’- <u>discontinuous</u> (**DNA ligase** joins it)

Transcription (RNA Synthesis)

➔ **Transcription**: DNA → RNA.

Transcription Unit

- *It is the segment of DNA which takes part in transcription*. It consists of 3 regions: **A promoter, Structural gene and a terminator**.



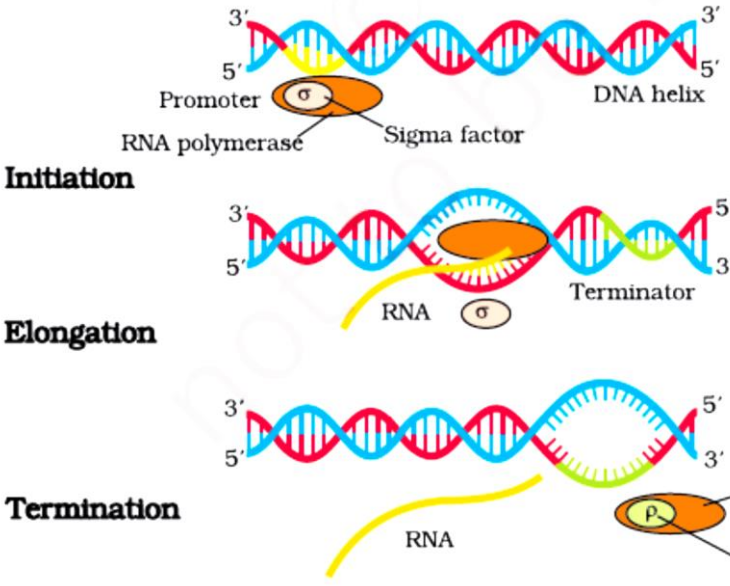
- RNA polymerase

Use strand with polarity 3′→5′ as template strand

New strand is formed in 5′→3′ direction

Steps of transcription

- **Initiation:** ***RNA polymerase*** + ***σ- factor*** binds to promoter.
- **Elongation:** Polymerising **ribonucleoside triphosphates**.
- **Termination:** ***RNA polymerase*** reaches at terminator region. + ***ρ – factor.*** Transcription stops.



In prokaryotes	In eukaryotes
Single ***RNA polymerase*** catalyses all transcription	**RNA poly I:** rRNAs (28S, 18S, 5.8S). **RNA poly II:** hnRNA. **RNA poly III:** tRNA, 5S rRNA, snRNAs
mRNA not required any processing	Need processing: **1. Capping-** add methyl GTP at 5′ end 2. **Splicing-** remove introns and join exons 3. **Tailing-** add adenylate residues at 3’-end
Transcription and translation can be coupled	Transcription and translation occurs separately.

TYPES OF RNA

Type	Role
mRNA (messenger RNA)	Provide template for translation
rRNA (ribosomal RNA**)**	Play structural & catalytic role during translation
tRNA (transfer RNA / soluble RNA**)**	Brings amino acids for protein synthesis and reads the genetic code

Genetic Code

➔ *It is the nucleotide sequence in mRNA that specifies the amino acid sequence of protein.*

Scientists involved in revealing Genetic Code

Scientist	Contribution
George Gamow	Suggested codon is made up of 3 nucleotides (4 types of nucleotides codes 20 amino acids).
Har Gobind Khorana	Synthesised RNA with defined combinations of bases.
Marshall Nirenberg	Developed **cell-free system** for protein synthesis.
Severo Ochoa	***Polynucleotide phosphorylase*** is used to make RNA in a template independent manner.
Frederick Sanger	Developed method for determination of amino acid sequences in proteins

Salient features of Genetic Code

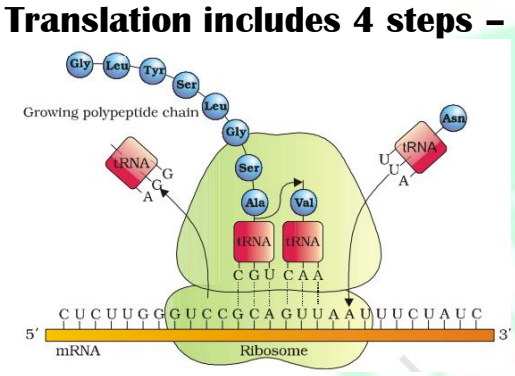
- **Codon** is a set of 3 nucleotides (triplet).
  - 61 codons code for 20 amino acids.
  - 3 codons (**UAA, UAG** & **UGA)** act as **stop codons.**
- ***Universal***: The same code applies to all organisms with exceptions.
- ***Comma less:*** The codon is read in mRNA in a contiguous fashion.
- ***Degeneracy:*** Some amino acids are coded by more than one codon.

Translation (Protein Synthesis)

➔ **Translation**: RNA → Protein.
It takes place in ribosomes, in cytoplasm.

- A **translational unit** in mRNA is the sequence of RNA that codes for a polypeptide, flanked by the start codon (AUG) and the stop codon and.

Translation includes 4 steps –



1. **Charging of tRNA / Aminoacylation of tRNA**
   - **ATP + amino acids** linked to specific tRNA.
2. **Initiation**
   - The ribosome binds at start codon (AUG) of mRNA at 5'-end → **initiator tRNA** pair with AUG.
3. **Elongation**
   - The ribosome moves from codon to codon along the mRNA. Amino acids are added one by one, translated into protein.
4. **Termination**
   - At the end, a **release factor** binds to the stop codon, terminate translation → release the protein.

Regulation of Gene Expression

In eukaryotes:-

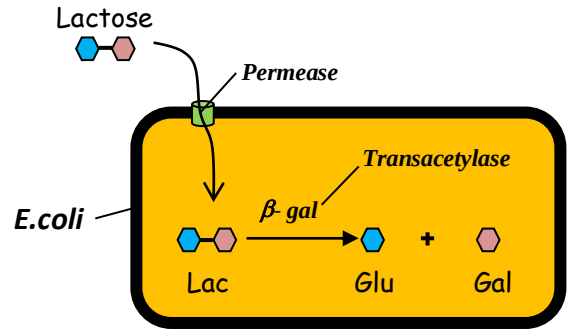
The regulation includes the following levels:-
1. Transcriptional level (formation of primary transcript)
2. Processing level (regulation of splicing)
3. Transport of mRNA from nucleus to the cytoplasm
4. Translational level.

In prokaryotes:-

The regulation is mainly at transcriptional level.

- *A set of genes regulating a metabolic pathway is called an* ***Operon.***

Lac operon in *E. coli*:



The operon controlling lactose metabolism. It was first elucidated by **Francois Jacob** and **Jacque Monod** (1961).

It consists of –

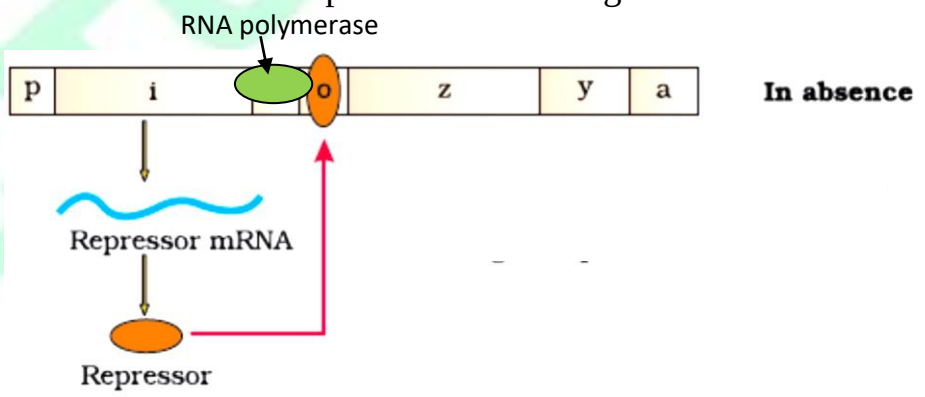
a) **3 structural genes:**
   - **z gene:** Codes for ***β- galactosidase*** (lactose $\xrightarrow{\beta\text{- gal}}$ gal + glu).
   - **y gene:** Codes for ***permease*** (increase lactose permeability).
   - **a gene:** Codes for a ***transacetylase*** (unknown function)**.**

b) **Promoter gene (P)**.
c) **Operator gene (O)**.
d) **A regulatory or inhibitor (*i*) gene:** *Codes for the* ***repressor***.
e) **Inducer** (here, lactose).

Functioning of Lac operon:-

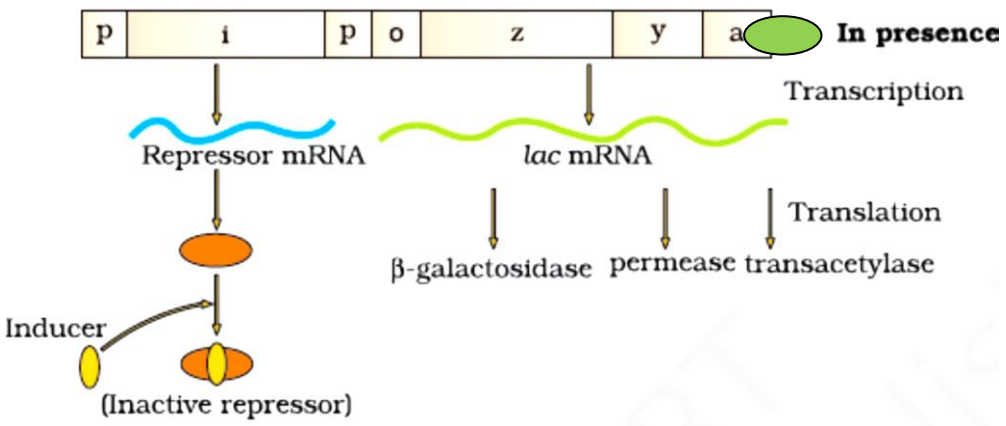
When lactose (inducer) is absent:

Step 1. The *i*-gene produces **repressor**
Step 2. Repressor binds to the **operator** and blocks RNA polymerase.
Step 3. Prevents the transcription of structural gene.



When lactose is present:

Step 1. Lactose binds to the **repressor** making it *inactive*.
Step 2. The RNA polymerase binds with promoter gene and transcribe structural genes



HUMAN GENOME PROJECT

Goals of HGP

(i) Identify all the 20,000-25,000 genes in human DNA.
(ii) Sequences the 3 billion bp
(iii) Store this information in databases **(Bioinformatics)**.
(iv) Improve tools for data analysis.
(v) Transfer related technologies to other sectors
(vi) Address the **ethical, legal, and social issues (ELSI)** that may arise from the project.

Methodologies:

1. **Expressed Sequence Tags** (ESTs)- Identifying all the genes that are expressed as RNA.

2. **Sequence Annotation** -Sequencing the whole set of genome containing all the coding & non-coding sequence.

   Procedure: -

   i. DNA is cut into fragments of smaller sizes
   ii. Introduced into ***Bacterial Artificial Chromosome*** (BAC-vector) in bacteria (host) or in ***Yeast Artificial Chromosome*** in yeast for amplification.
   iii. Sequenced using *Frederick Sanger* method.
   iv. These sequences were then arranged based on some overlapping regions using computer programs.
   v. These sequences were annotated and were assigned to each chromosome.

Salient Features of Human Genome

i. Human genome contains **3164.7 million** nucleotide bases.
ii. The total number of genes = 30,000.
    The **largest human gene** is **dystrophin**.
iii. **99.9 %** nucleotide bases are exactly the same in all people.
iv. Functions of **50 %** of genes are unknown.
v. Less than **2%** codes for proteins.
vi. **Repeated sequences** make up large portion of the human genome.
vii. About **1.4 million** locations where single-base DNA differences (**SNPs – single nucleotide polymorphism**) occur in humans.

➔ Many non-human model organisms also been sequenced.
e.g: bacteria, yeast, *Caenorhabditis elegans*, *Drosophila*, rice, *Arabidopsis* etc.

DNA Fingerprinting

- **DNA fingerprinting** *is the technique to identify the variation in individuals at genetic level.*
- Developed by **Alec Jeffreys**

Applications

∵ It is **forensic tool** to solve paternity disputes, rape case, murder etc.
∵ To study genetic diversities and evolutionary biology

Basis of DNA fingerprinting

♥ **Repetitive DNA:** DNA carries some non-coding repeated sequences**.**
Number of repeats is specific from person to person.

♥ **Satellite DNA:** These are highly-repeated short sequences in the repetitive DNA.

Important types of Satellite DNA

A. Micro-satellites- 5-8 bp long
B. **Mini-satellites-** 11-60 bp long e.g.: **VNTR** (Variable Number of Tandem Repeats).

Steps of DNA fingerprinting

(i) **Isolation of DNA**
(ii) Digestion of DNA by **restriction endonucleases**
(iii) Separation of DNA fragments by **electrophoresis**
(iv) Transferring (southern **blotting**) of separated DNA fragments to **nitrocellulose** or **nylon**
(v) **Hybridisation** using labelled VNTR probe
(vi) Detection of hybridised DNA fragments by **autoradiography**.