

# Biotechnology & its Applications

- (i) Agriculture - To increased food production, improved quality etc.
- (ii) Medicine / Therapeutics : Use of genetic engineering to make drugs, in treatments (Gene-therapy), diagnosis etc.
- (iii) Bioremediation, waste treatment.  
↳ use of living organism to cope up with soil pollution, water

3 Critical research areas in the field of Biotech -

- (i) Use of improved varieties organism / microbe or enzyme.
- (ii) Providing optimum condition for the catalyst to act.
- (iii) Down stream processing.

## # Biotechnology Application in Agriculture -

To increase agricultural productivity.

- (i) Use of harmful fertilizers, Pesticides etc.
- (ii) Use of Biopesticides, Biofertilizer (use of  $N_2$  fixing bacteria).
- (iii) Use of GMOs (Genetically modified crops).

Note: Green Revolution : Norman E Borlaug : Father of green revolution.

← Inspired from his work MS Swaminathan introduced Green Revolution in India.

## # What was done in Green revolution to ↑ Productivity?

- (i) Use of chemicals like fertilizers & Pesticides.
- (ii) Better management Practices : Better irrigation, crop rotation, advanced machine.
- (iii) improved crop varieties.

\* Green revolution succeeded in trippling the food production but still wasn't enough to meet the demand of growing human population.

\*\* Benefits of using Genetically modified crops -

- (i) Reduce reliance of Pesticides, make the Plant Pest resistant (Bt cotton).
- (ii) Reduced Post Harvest losses (over ripening of tomato can be prevented by silencing a gene responsible for ripening).
- (iii) Can tolerate abiotic stress like change in temperature, pH or other conditions.
- (iv) \* Enhanced nutrition value eg Golden Rice.
- (v) Better mineral usage.

Nutritional Value

Daffodil Plant

↓ has

$\beta$ -carotene gene



cut  $\beta$ -carotene gene



Agrobacterium t.

↓  
(Ti Plasmid)



$\gamma$ -DNA

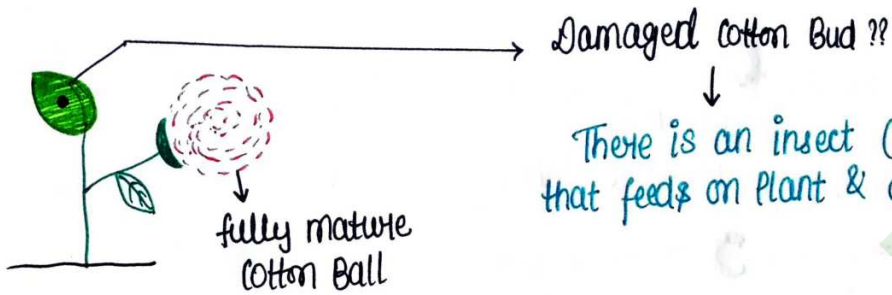
Introduce into normal variety of rice

↓  
 $\beta$ -carotene synthesized in plant

↓  
 $\beta$ -carotene is a precursor of Vit. A,  
so now Vit. A synthesized = Golden Rice  
(Vit A enriched).

## Bt Cotton

→ Pest resistant plant



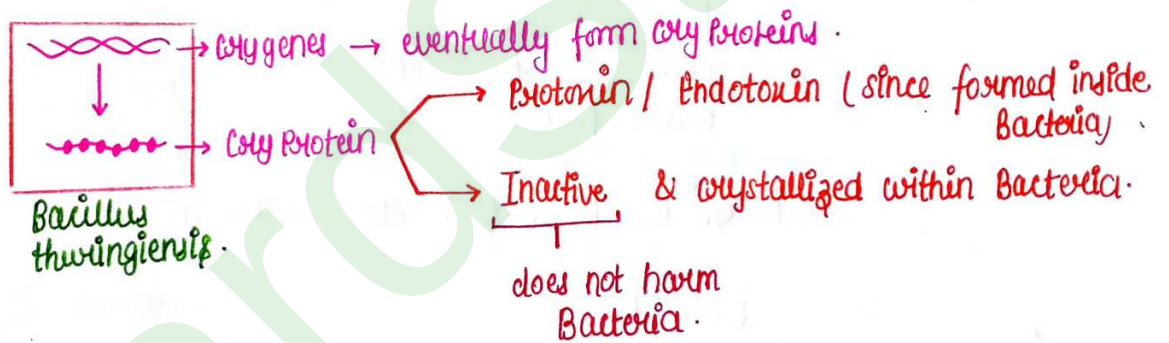
There is an insect (cotton Bollworm) that feeds on Plant & damages the Ball.



*Bacillus thuringiensis* (Bacteria that is seen in some Plants)

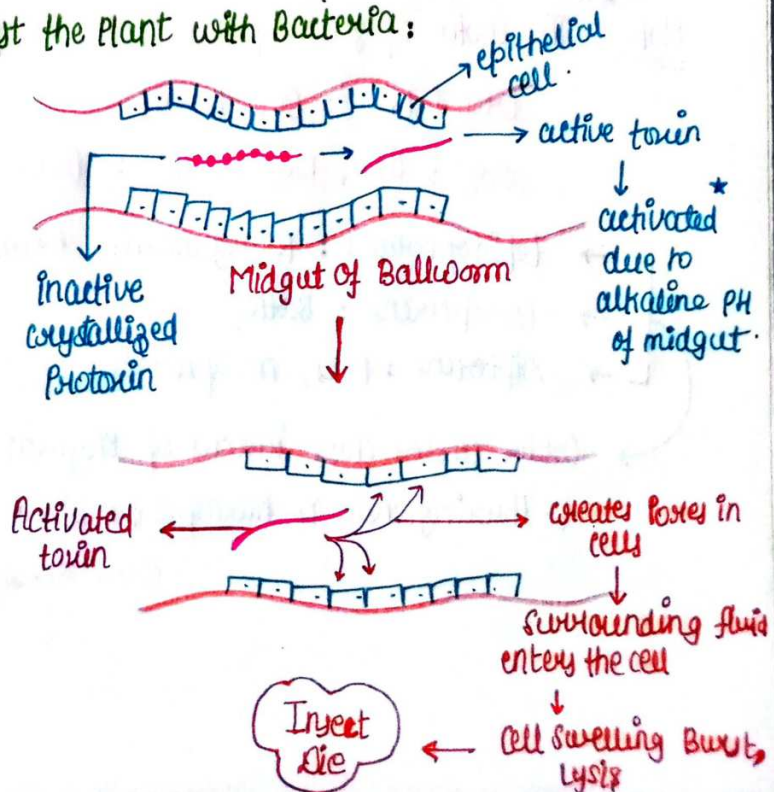
→ There is an observation that when insect feed such Plant the insect die eventually.

Q. What is in *Bacillus thuringiensis* that leads to death of Insects (cotton Ball Worm)?

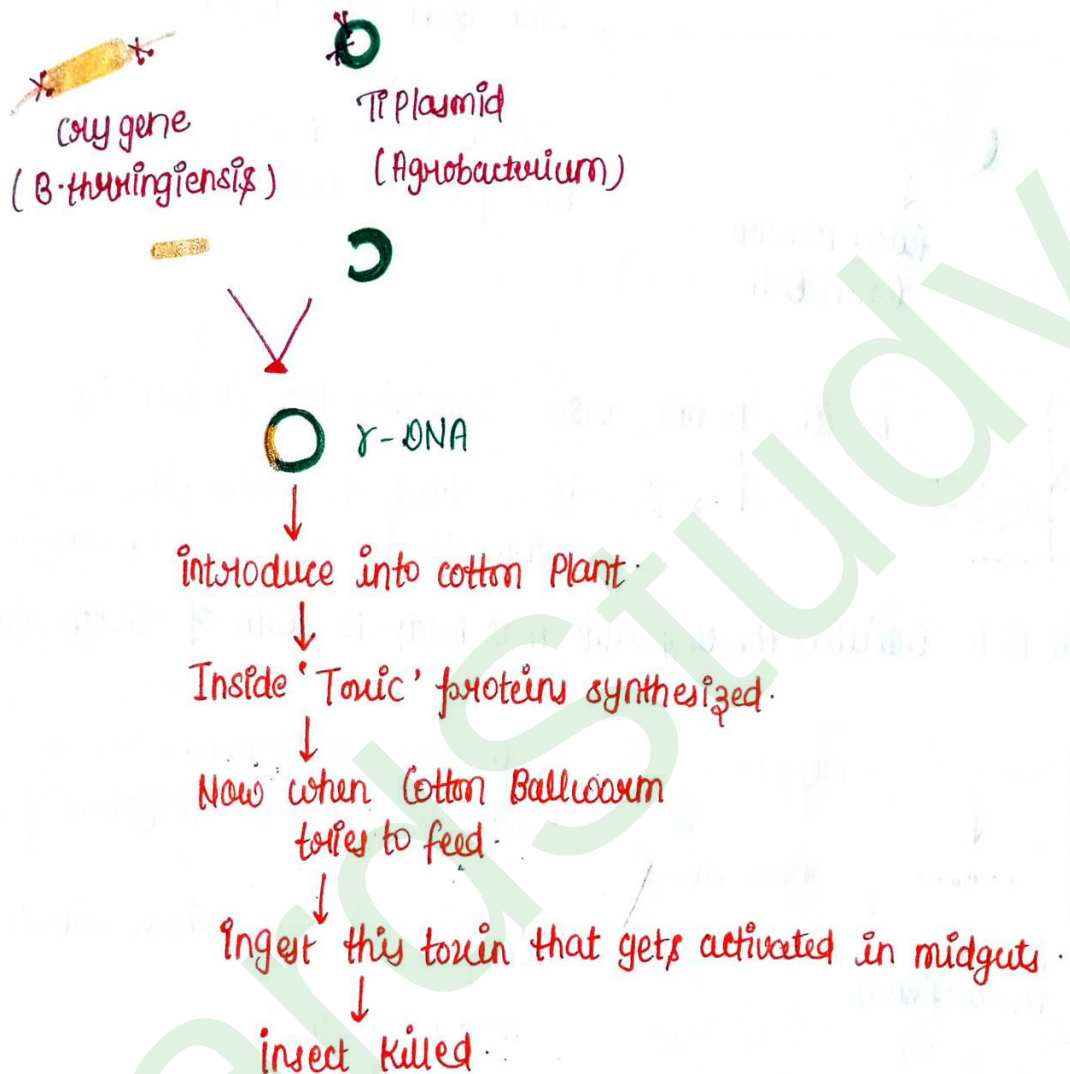


→ Once cotton Ball worm ingest the Plant with Bacteria:

In the midgut of insect:



② How did we make 'Bt - cotton' ?  
*Bacillus thuringiensis*.



Note \*\*\* The choice of gene depends on the crop and the pest.

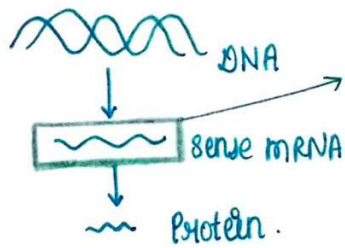
'cry I Ab' : Corn Borer.

cry I Ac, cry II Ab : Cotton Bollworm.

- Lepidopterans : Army worm, tobacco bud worm, corn borer, Cotton Bollworm
  - Coleopterans : Beetles.
  - Dipterans : Flies, mosquitoes.
- Order under class Insecta of Phylum Arthropoda.  
*B. thuringiensis* is harmful for above.

## RNAi / RNA interference / mRNA silencing / Gene silencing-

### Central Dogma



if we stop translation of this (silencing), the specific protein will not be formed.

- mRNA  $\rightarrow$  single stranded : Protein.
- mRNA  $\rightarrow$  If dsRNA : No Protein.

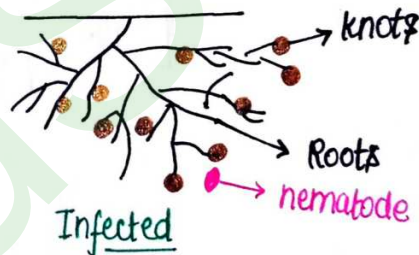
$\rightarrow$  \* A nematode *Meloidogyne incognita* (Aschelminthes)

Infects the Roots of Tobacco plants.

Root KNOT - Disease



Normal Plant



Infected

What's in Nematode?



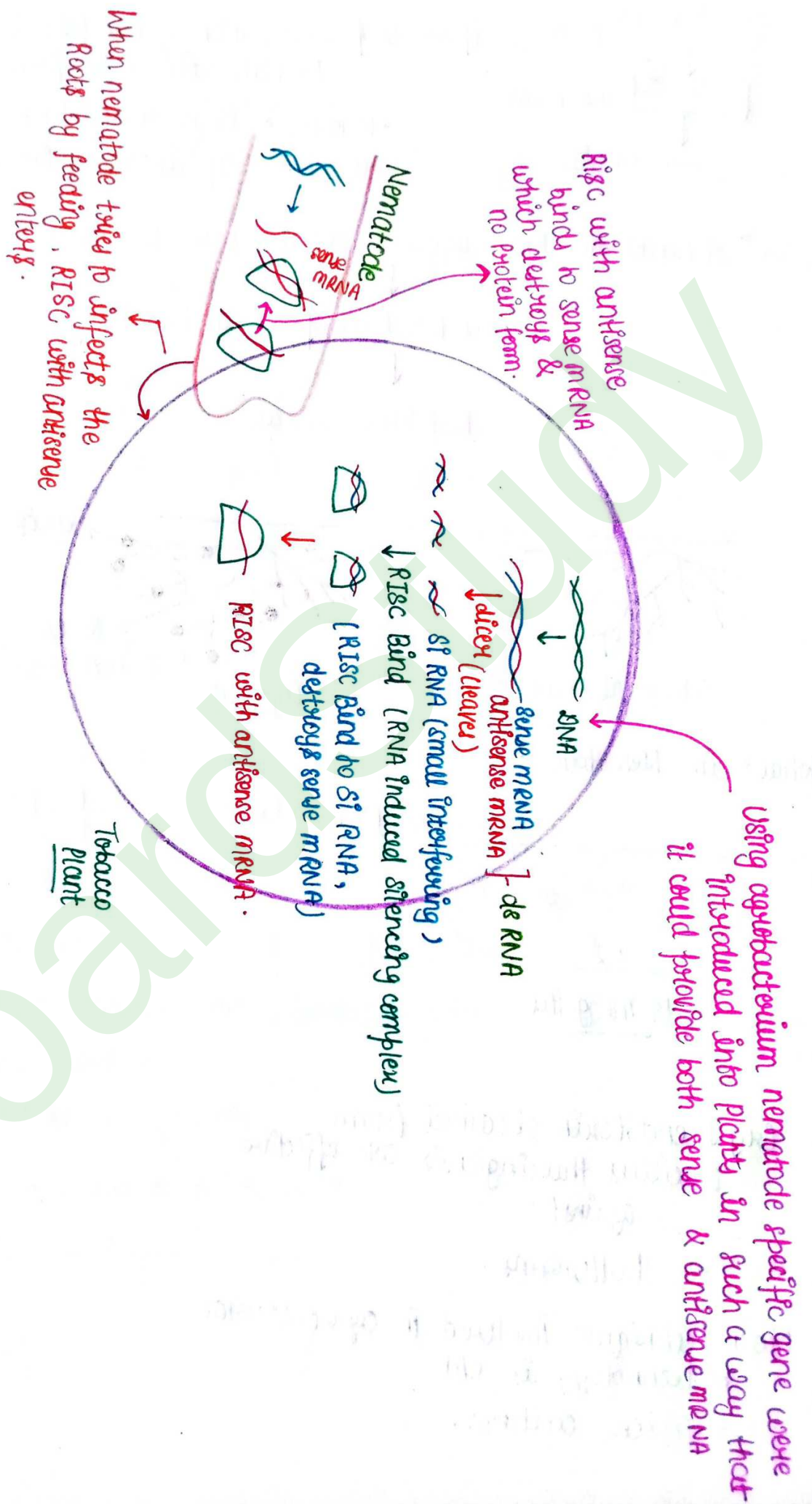
This protein helps in growth, feeding & survival of nematode.

\* Cry I endotoxin obtained from *Bacillus thuringiensis* are effective against.

$\rightarrow$  Bollworms.

\* Main technique involved in agricultural biotechnology is cl.

$\rightarrow$  Tissue culture.



## Some Important Points

(i) RNAi initiated due to formation of a dsRNA.

(ii) Nematode : *Meloidogyne incognita*.

Plants : Tobacco

Vector : *Agrobacterium* (Nematode specific gene into Plants)

(iii) Disease : Root KNOT Disease.

(iv) \* RNAi takes place in all eukaryotes as a mechanism of self defence.

The source of complementary RNA could be from infection of Virus (RNA genome) or transposons / Jumping genes that replicates via RNA intermediate.

## Biotechnology Applications in Medicine

→ Recombinant therapeutic drugs are very effective & do not generate unwanted immune system responses.

→ At Present 30 recombinant therapeutics used worldwide & 12 currently marketed in India.

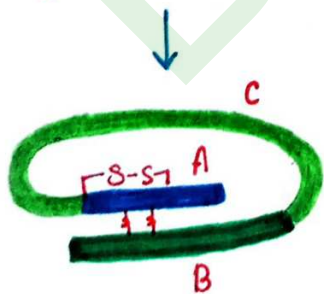
③

→ Genetically Modified Insulin :

⇒ Insulin : Diabetes mellitus treatment.

\* Earlier insulin was extracted from Non human resources like Pigs & Cattles (slaughtered) but when used in human caused Allergy.

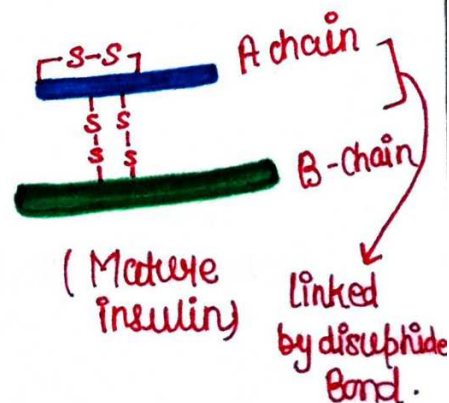
How insulin is synthesized in human body ?



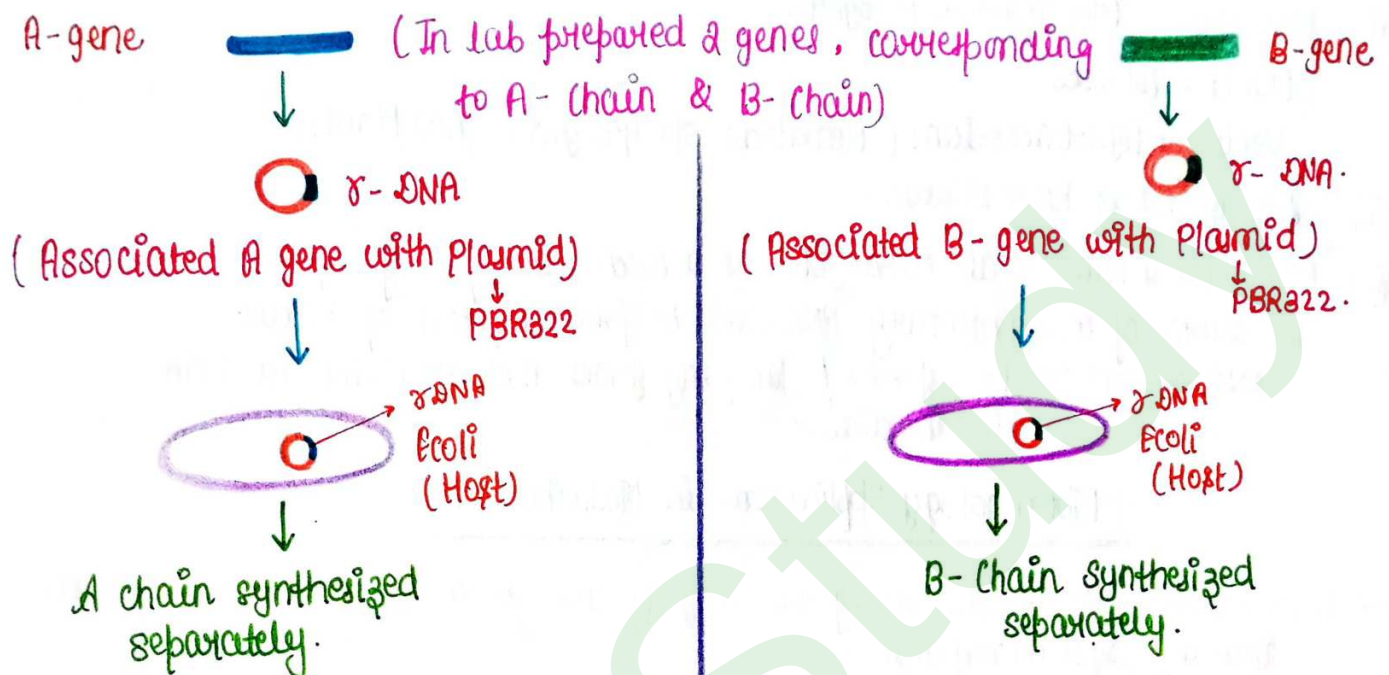
(Pro-insulin)  
(immature)

Extra C-peptides / chain removed.

Later immature insulin converts into mature insulin.



→ Eli Lilly, 1983, an American company constructed the first recombinant insulin.



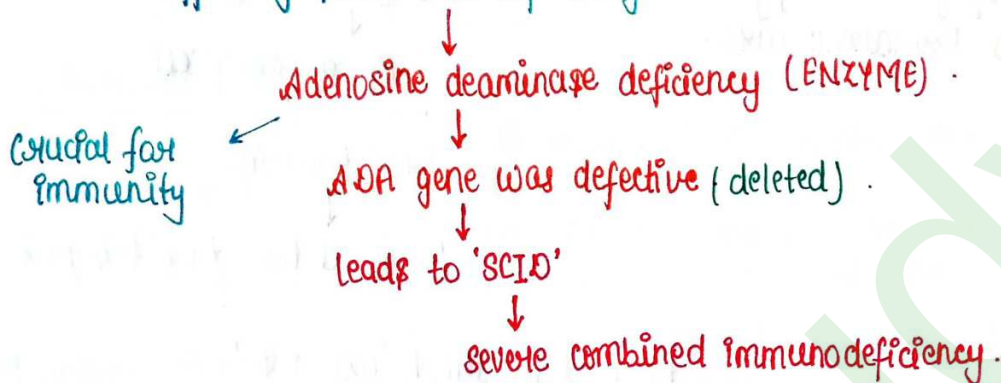
Note: Both chains prepared separately were extracted & linked with disulphide bonds in lab.

→ Major Challenge faced by Eli Lilly was the assembly of insulin into mature form but was resolved by the above method.

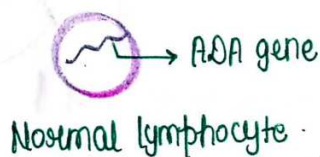
## Gene therapy

Replacement of a defective gene with correct set of Gene.

→ first clinical gene therapy was performed in 1990, to a 4 year old girl child suffering from 'ADA deficiency'.



\* ADA enzyme : Proliferation of 'Lymphocytes' : IMMUNITY.



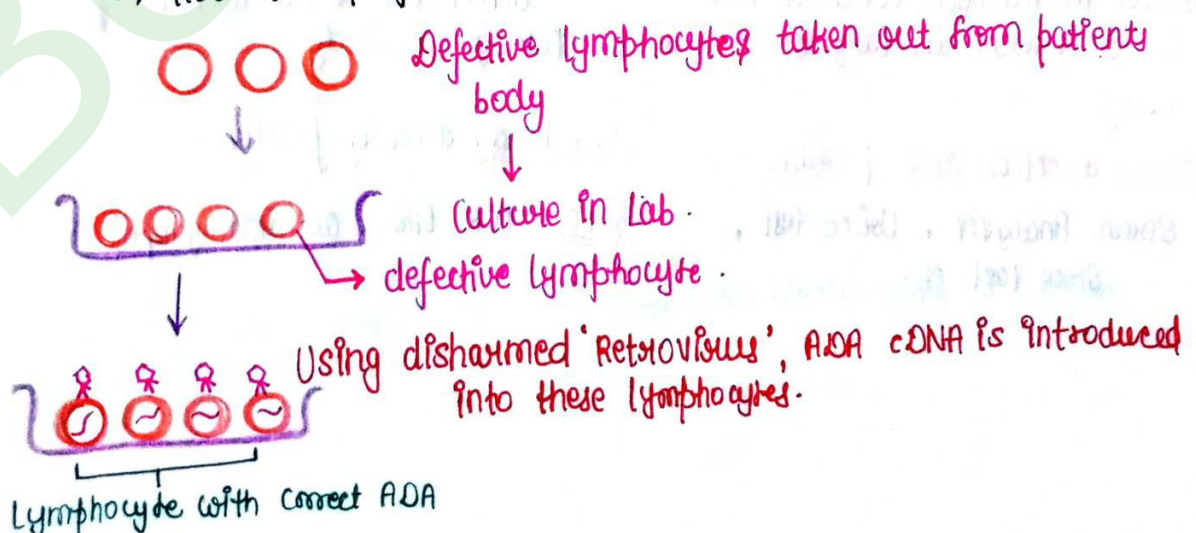
'ADA' deletion / SCID, Abnormal Lymphocyte.



→ In this case we can perform :-

- (i) Enzyme replacement therapy : ADA can be directly injected into the patient's body  
→ Not a permanent sol<sup>n</sup>.
- (ii) Bone marrow transplantation : From a suitable donor, Bone marrow cells can be transplanted but still not a permanent solution.
- (iii) Gene therapy : Correct ADA gene directly introduce into patient's defective lymphocyte.

⇒ How it is performed ?



Returned to patient's body but still not a permanent sol<sup>n</sup> as lymphocytes are not immortal.

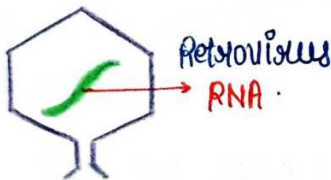
④ Note → If gene therapy is done in the embryonic stage it can be a permanent cure.

↓  
Totipotent stem cell

↓  
Total potential

↓  
Every cell will have this gene.

→ 'ADA cDNA'

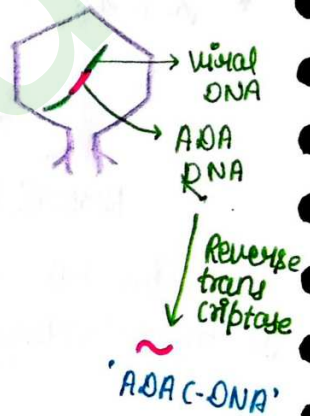


→ We need to attach 'ADA-RNA' with viral RNA

→ ADA DNA

↓ forms  
ADA RNA

↓ attach with  
Viral DNA



Molecular Diagnosis

→ for treatment of any disease (effective)   
 ↳ Diagnosis (detection)   
 ↳ Pathophysiology (Symptoms produced by the disease).

Old techniques

→ Conventional techniques that can only detect the pathogen when it has multiplies or present in large amount in a body.

→ Early detection not possible.

eg. Serum Analysis, Urine test, Stool test etc.

Modern technique

→ Uses r-DNA technology & can detect even small amount of pathogen in body.

→ Early detection possible.

eg. PCR, ELISA, autoradiography.

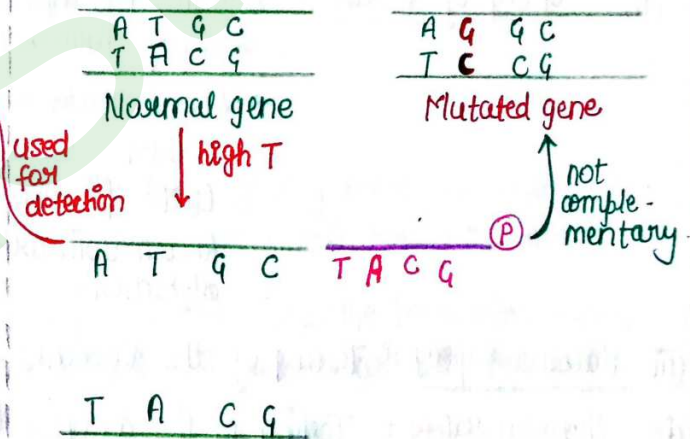
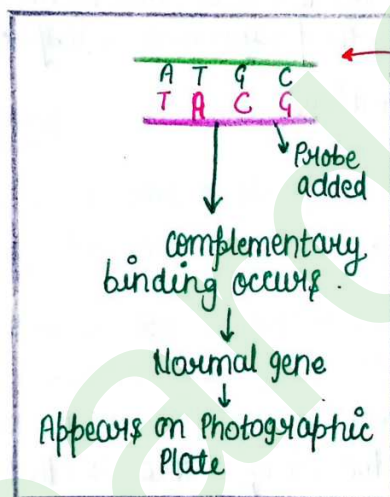
(i) PCR : Polymerase chain Reaction.

- Small amount of pathogens can be detected by amplification using PCR
- for eg: → A patient suspected of HIV infection can be diagnosed.
  - Mutations caused in cancer can be detected.
  - Genetic disorder can be detected.

(ii) Autoradiography - Gene mutations can be detected.

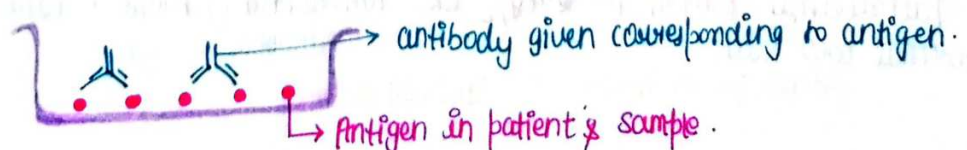
→ A radiolabelled PROBE (ss DNA / RNA that is radiolabelled) is used.

- Suppose, a gene is suspected to be mutated, we use this probe to form complementary binding with suspected gene.
- If gene is normal, Binding will occur & it will appear on the photographic film, if mutated, it will not appear.



(iii) ELISA (Enzyme linked immunosorbent assay)

- It is used to detect both presence of Antigen as well as antibody in patient.
- Based on antigen - antibody interaction.



## Transgenic animals -

- Animals whose gene has been manipulated to express a foreign gene.
- Many transgenic animals have been created : SHEEP, cow, Pig, monkey, mice, fish etc.
- 95% of transgenic animals are MICE.

- chromosomal similarity to humans.
- short life cycle.
- easy to maintain.

### Why transgenic animals?

#### (i) Normal Physiology & Development :

If a foreign gene is expressed in a transgenic animal we can study its role in the normal growth & development.

#### (ii) Study of disease : Several transgenic animals are a disease model for study of various disease, its progression, impact.

→ Disease model exist for disease :

Cancer

Cystic fibrosis

Rheumatoid arthritis.

Alzheimer's.

#### (iii) Vaccine Safety : Testing of the vaccines.

#### (iv) Chemical Safety : Testing of the drugs. Transgenic animals are made more sensitive towards the drugs tested, so that minor problems can also be detected.

#### (v) Biological Products : ① $\alpha$ -1 antitrypsin is made in transgenic sheep for treatment of emphysema.

→ Models exist for treatment of cystic fibrosis, PKU (Phenylketonuria).

② First transgenic cow ROSIE was created in 1997, where a gene Human  $\alpha$ -Lactalbumin was introduced to produce human  $\alpha$ -Lactalbumin protein in 2.4g/L was nutritionally more balanced than normal cow milk.

## Ethical issues

- Ethics in biotechnology refers to the moral principles that guide the use & development of biotechnological technologies.
- It involves questions about what is right or wrong when manipulating living organism, genes & and ecosystems and considers the impact on human health, animal welfare, the environment and society.
- Key concerns include safety, fairness, the potential for harm, and respect for living beings.

GEAC : 'Genetic engineering approval committee'.

↓  
Organisation by the Indian government  
that decides.

↓  
Validity of GM research and safety to introduce  
them for public services.

## Patent

Patent is a legal right granted to an inventor, giving them the exclusive ability to make, use, or sell their invention for a certain period of time.

In exchange, the inventor must publically disclose how the invention works. This prevents other from copying or using the invention without permission.

Biopatent : The patent given for Biological product.

Biopiracy : Biopiracy refers to the unethical practice of using or exploiting biological resources (such as plants, animals or genetic materials) from indigenous or local communities without proper permission, compensation or acknowledgement.

- There are 2 lakh varieties of Rice in India, 27 documented varieties of Basmati which is known for its unique aroma & flavour.
- \* In 1997, an American company got patent on one of the Basmati by US government.

Indian Basmati X Semi dwarf variety

↓  
New Variety.

Note Several attempts have been made to patent  
Turmeric & Neem.