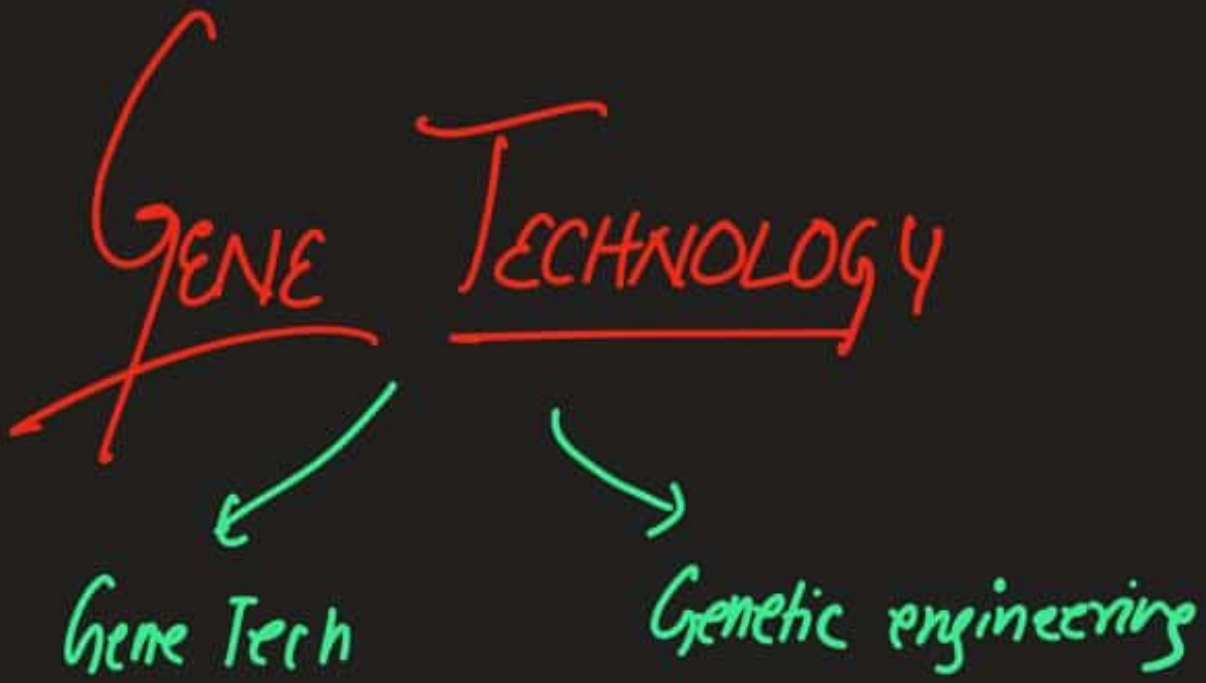

Gene Technology
Gene Tech
Genetic engineering

GENE TECHNOLOGY

* Gene Technology refers to alteration in an organism's genotype which alters protein synthesis during translation.

* Genetic Engineering refers to transfer of genetic material from one organism to another to produce transformed organisms.

Proteins Produced via Genetic Engineering

* Examples of a few proteins produced industrially via genetic engineering include:

i) Insulin

ii) Growth Hormone

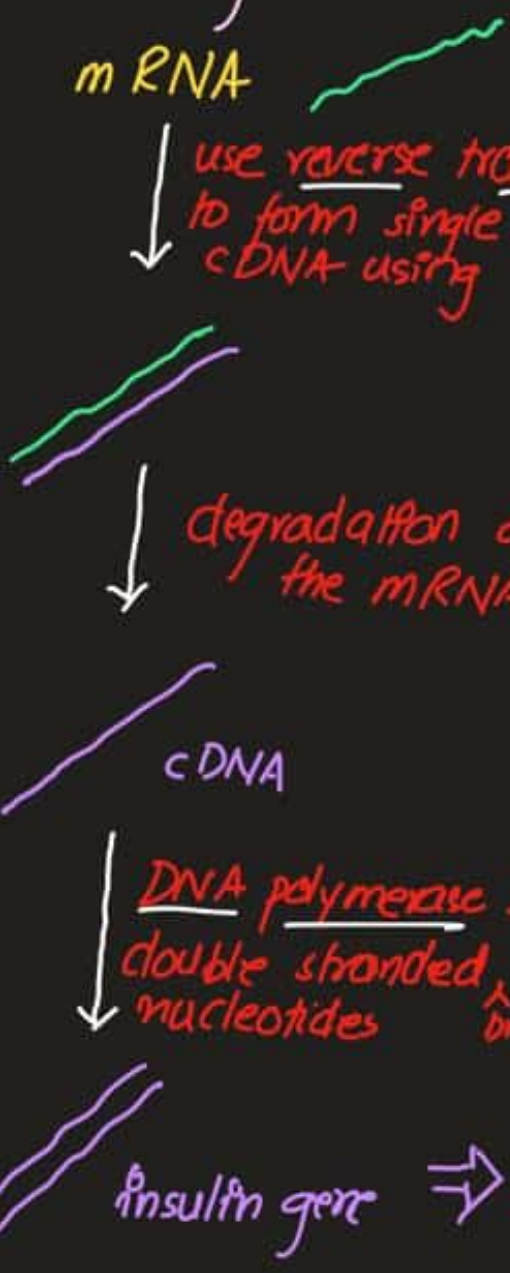
iii) Factor VIII.

Production of Human Insulin

* Before insulin from genetically modified bacteria became available, people with diabetes mellitus were treated with insulin extracted from the pancreas of pigs or cattle.

I. Extraction of human insulin gene

(a) Extraction of mRNA from the β-cells of the pancreas



⇒ synthesise numerous copies of the gene via PCR.

(b) Isolation of the insulin gene from the β-cells

(c) Artificial synthesis of the gene

I. Extraction of human insulin gene

* The gene can be extracted or synthesized in different ways which are summarised below:

a. Extraction of mRNA from B-cells of the Pancreas

b. Isolating gene from the DNA

c. Artificial synthesis of the gene

@ Extraction of mRNA from B-cells of the Pancreas

1

mRNA extracted from tissue from the pancreas

human pancreas – here insulin is synthesised in β cells of islets of Langerhans – so **mRNA for insulin production** may be extracted from this tissue

purified mRNA coding for insulin

A U G G A A C A C U G G C A C C G U U G C U G U

reverse transcriptase enzyme added – this synthesises a complementary strand of DNA (using a pool of nucleotides) by base pairing with the sequence of bases of the mRNA

mRNA strand is then discarded

mRNA being used as a template for DNA synthesis

A U G G A A C A C U G G C A C C G U U G C U G U

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

cDNA strand
– DNA complementary to base sequence of mRNA

cDNA being used as a template for DNA synthesis

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

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

DNA polymerase
enzyme added –
this synthesises a second DNA strand, complementary to the base sequence of cDNA

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

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

the two DNA strands are the **gene for insulin**

polymerase synthesises complementary DNA strand

forming

copy of human gene for insulin

(a) Extraction of mRNA from β-cells of the Pancreas

* Isolating mRNA is easier than extracting the human insulin gene from DNA.
* The mRNA is devoid of introns and there are multiple copies of mRNA present within the cytoplasm.

* This mRNA is then converted into single-stranded complementary DNA (cDNA) using the enzyme reverse transcriptase.

* The single-stranded DNA is converted into double-stranded complementary DNA using the enzyme DNA Polymerase.

* Multiple copies of this gene can thereafter be formed using the Polymerase Chain Reaction (PCR).

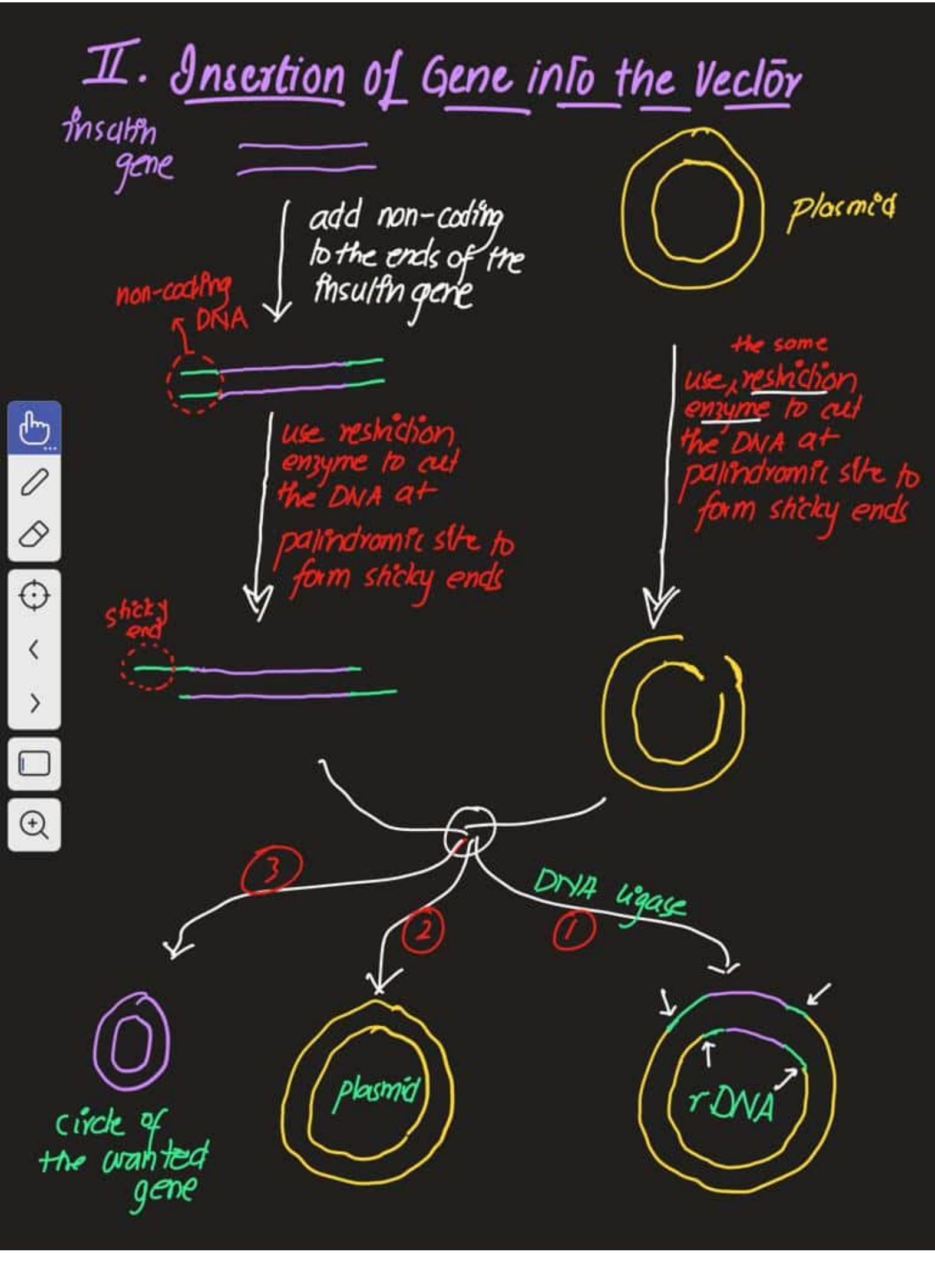
(b) Isolating Gene from the DNA

* The insulin gene can be extracted from the DNA within the B-cells of the pancreas.
* Introns are removed from the gene before multiple copies of the gene are produced.

(c) Artificial Synthesis of the Gene

* Since the amino acid sequence of the insulin protein is known, an artificial gene can be synthesised. Multiple copies of the gene can be produced using PCR.

II. Insertion of Gene into the Vector



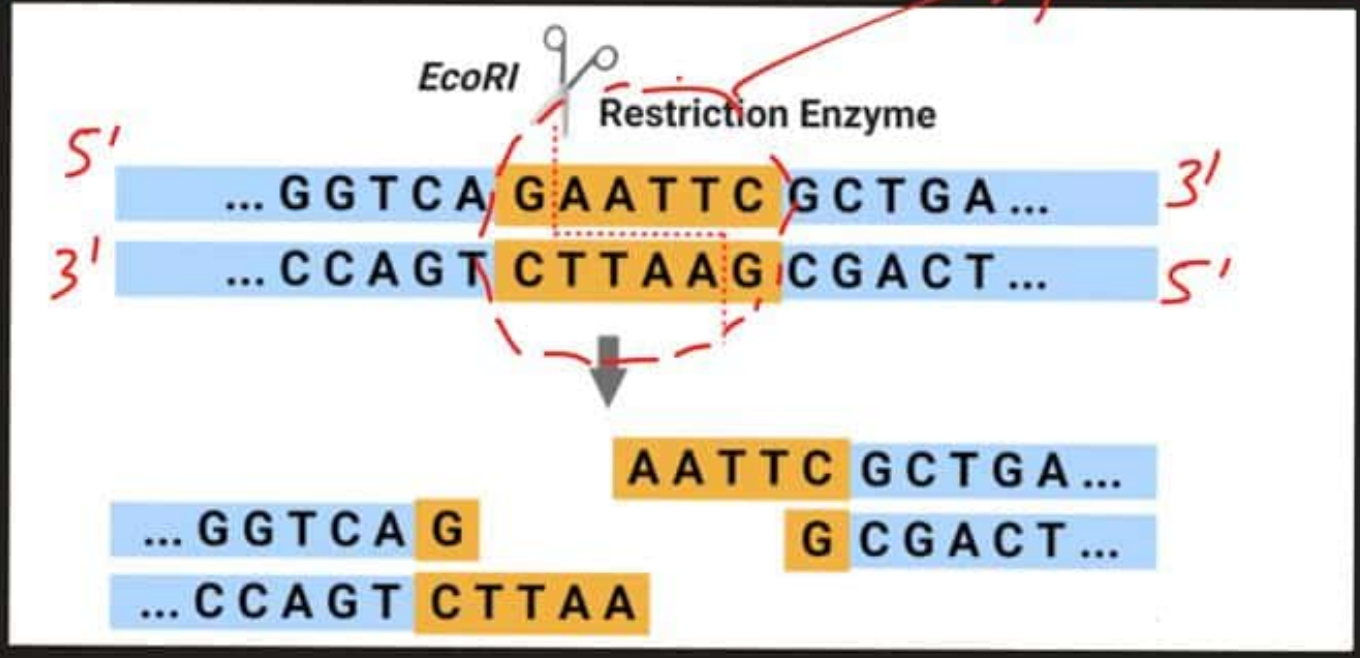
II. Insertion of Gene into the Vector

* The human insulin gene is now modified by adding non-coding DNA segments which will be cut by specific restriction enzymes To produce sticky-ends.

This same restriction enzyme will serve To cut the vector plasmid at the same palindromic site.

* These plasmids are extracted from the bacteria E. coli, by exposing them To certain enzymes which digests their cell wall.

Palindromic site of EcoRI

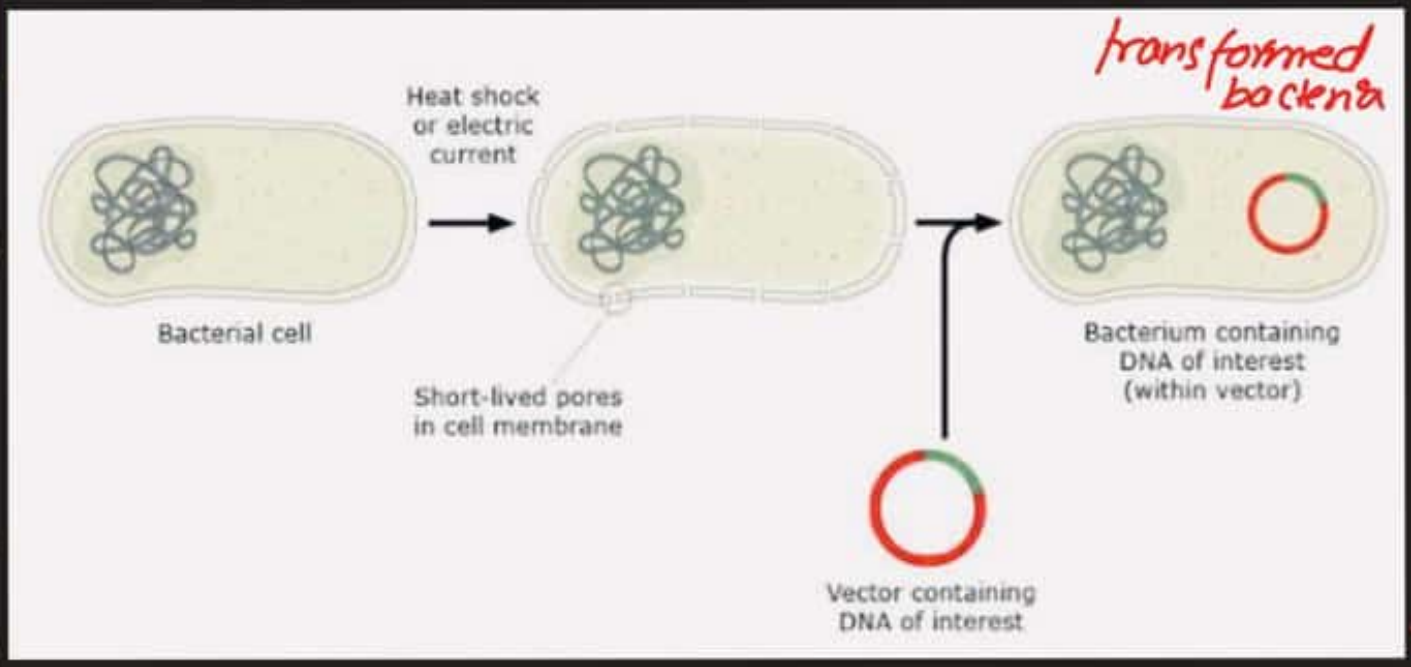


* The plasmids are then mixed with the insulin gene to allow complementary base pairing to occur b/w their sticky-ends.

The enzyme DNA ligase forms phosphodiester bonds b/w the ends of DNA fragments.

The resultant plasmid formed is known as the Recombinant DNA (rDNA).

III. Insertion of the vector into the bacterium


transformed bacteria
Heat shock or electric current
Bacterial cell
Short-lived pores in cell membrane
Bacterium containing DNA of interest (within vector)
Vector containing DNA of interest

Insertion of the vector into the Bacterium (E·coli)

* The genetic material (plasmids) is inserted into the bacteria by mixing them with E·coli in a medium containing a high conc. of Ca^{2+} at 0°C.

* Ca^{2+} increase the permeability of the cell membranes of bacteria to allow uptake of plasmid in the presence of a heat shock.

* The temperature is rapidly raised to 40°C during the heat shock.

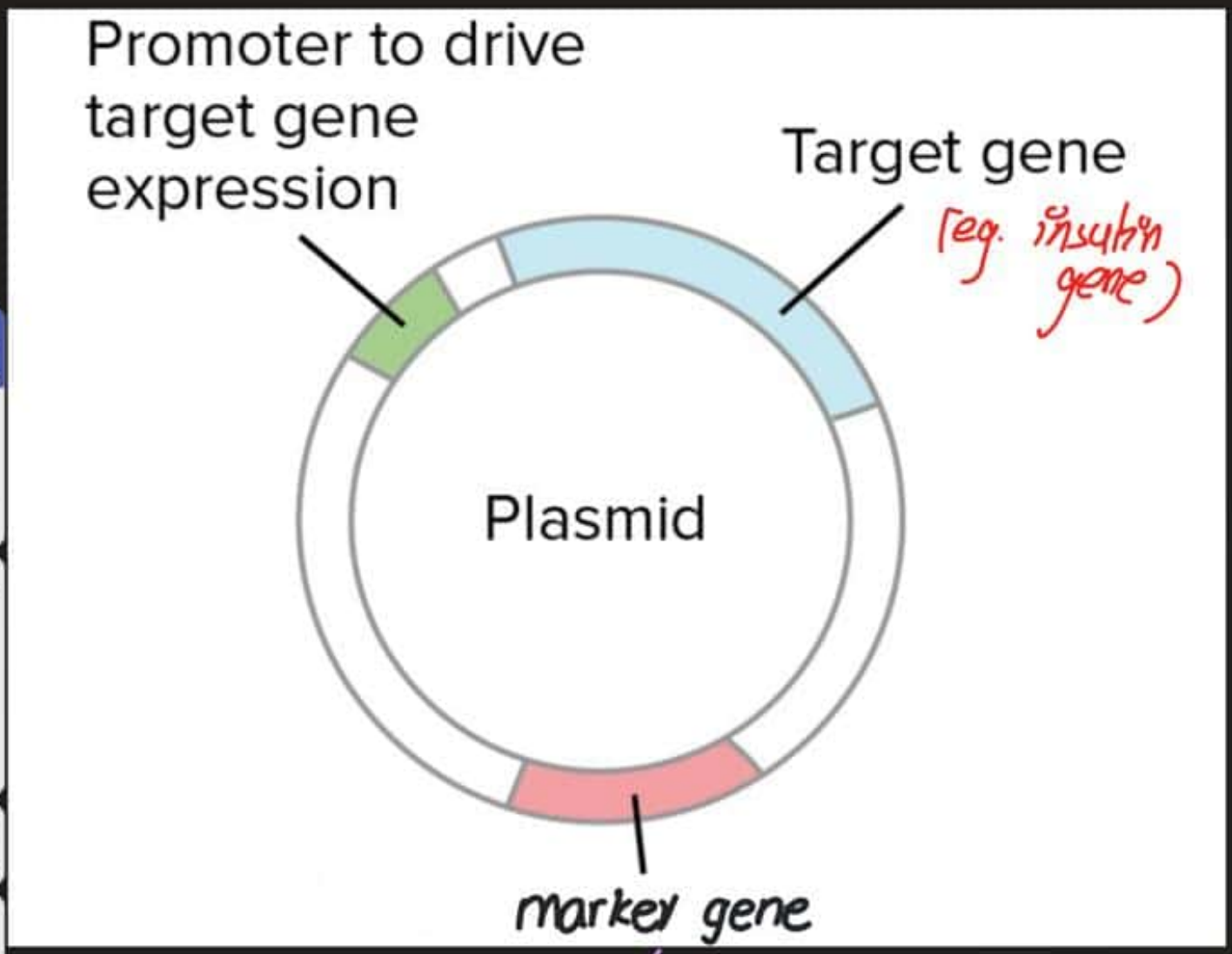
* Bacteria that take up the recombinant DNA (rDNA) are termed as transformed bacteria.

* Less than 1% of the bacteria actually undergo transformation.

* Most either fail to take up plasmids or;
  1) take up non-recombinant plasmids
  2) take up circles of the wanted gene.

* Thus, it's essential to select for transformed bacteria.

II. Selection of transformed bacteria



Selectable markers → antibiotic resistance genes

Screening markers → GFP gene

Selection of Transformed Bacteria

* Selection of transformed bacteria is achieved via genes termed as marker genes.

These genes are either already present in the vector (plasmid) or inserted into the plasmid along with the desired gene (e.g. insulin gene).

* Marker genes are of two types:

1. Selectable Markers (genes)
2. Screening Markers (genes)

* Selectable markers are antibiotic resistance genes. These genes enable identification of transformed bacteria by monitoring their survival in the presence of an antibiotic. E.gs ampicillin resistance gene/Tetracycline resistance gene. These genes may be apart of the vector (plasmid) used for genetic engineering.

* Screening markers are genes which enable identification of transformed bacteria by changing their visual appearance. E.gs. include GFP gene (Green fluorescent protein), X-gene (recall the lac operon), etc.

We will discuss HOW these marker genes enable identification of transformed bacteria later.

V. Cloning

* Insulin is a quaternary protein made up of two polypeptides (A & B).

* Bacteria CANNOT produce quaternary proteins => it's devoid of rER/Golgi.

* It only produces polypeptides A and B in large amounts (genes for these polypeptides are usually inserted on different plasmids).

CLONING

* Transformed bacteria are allowed to grow in large fermenters in a suitable growth medium.

* Bacteria multiply rapidly to produce a large population of transformed bacteria.

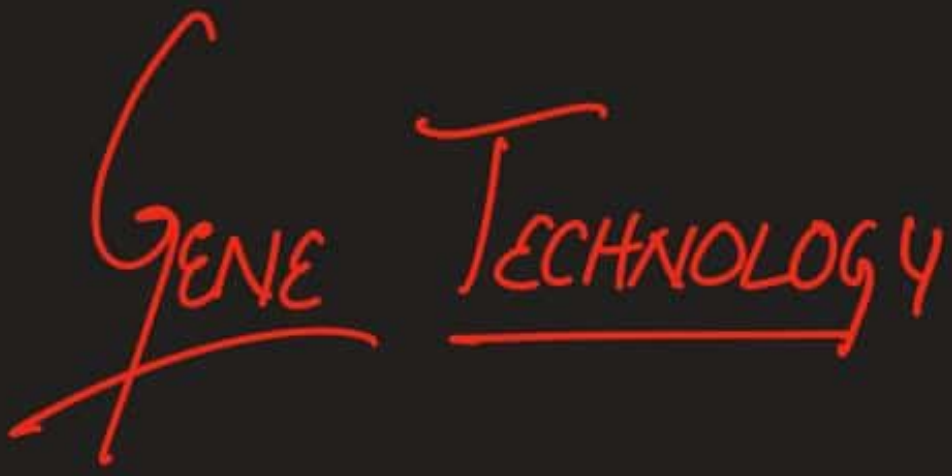
* These bacteria replicate their plasmids (and hence the insulin gene) rapidly which eventually leads to production of large amounts of insulin.

VI. Isolation, modification & purification of insulin

Isolation, Modification & Purification of Insulin

* The two polypeptides are isolated and chemically modified to produce human insulin.

The human insulin is then purified before being packaged for human consumption.


Gene Technology

Previously,

* Gene technology & genetic engineering
* Production of genetically engineered human insulin

Production of human insulin via genetic engineering

* Steps to produce human insulin:

1. Extraction of the Human Insulin Gene
2. Insertion of the gene into the vector (plasmid)
3. Insertion of the modified vector (r-DNA) into the bacteria.
4. Selection of transformed bacteria
5. Cloning
6. Isolation, Modification and Purification of Insulin

Q1: Describe how the gene for human insulin is obtained and inserted into the vector?

Ans: The mRNA of the human insulin gene is extracted from the cytoplasm of the β-cells of the pancreas. The enzyme reverse transcriptase is used to make single-stranded complementary DNA using the mRNA. DNA Polymerase thereafter converts the single-stranded DNA into double-stranded DNA. PCR (Polymerase Chain Reaction) may be

used to amplify this DNA. Plasmids are obtained from bacteria such as E. coli. These plasmids are treated with restriction enzymes to cut the plasmid at palindromic sites to form sticky-ends. The same restriction enzyme creates sticky ends at the ends of the insulin gene. The insulin gene is thereafter mixed with the plasmid in the presence of the enzyme DNA Ligase which seals the nicks in the sugar-phosphate backbone.

Q2: Describe the structure of a plasmid?

Ans: A plasmid is an extra chromosomal circular form of double-stranded DNA. It has a low molecular mass. It contains palindromic sites cut by restriction enzymes. It may contain antibiotic resistance genes.

Q3: Describe the benefits of using plasmids in genetic engineering?

Ans: 1. Plasmids can be easily extracted & reinserted after modification into the bacteria.

2. Plasmid has a low molecular mass.

3. Plasmids can be easily cut by restriction enzymes due to presence of multiple palindromic sites.

4. It's easy to insert genes into the plasmid.

Q4: Describe the role of the following enzymes;

a) Reverse Transcriptase (2)

b) DNA Polymerase (2)

c) DNA ligase (2)

Ans: a) Reverse Transcriptase is responsible for producing single stranded cDNA from mRNA.

b) DNA Polymerase is responsible for producing the second strand of cDNA using activated nucleotides. A pairs with T and G pairs with C. The product is a double-

stranded DNA.

c) DNA Ligase seals nicks* in the sugar-phosphate backbone to form recombinant DNA (rDNA).

* a nick is a break in the sugar-phosphate backbone of DNA.

Qs: Explain the meaning of the term restriction enzymes and describe its role in genetic engineering? (4)

Ans: Restriction enzymes are chiefly produced by bacteria as a defense mechanism to protect itself from bacteriophages (viruses that infect bacteria). These enzymes cut the viral DNA.

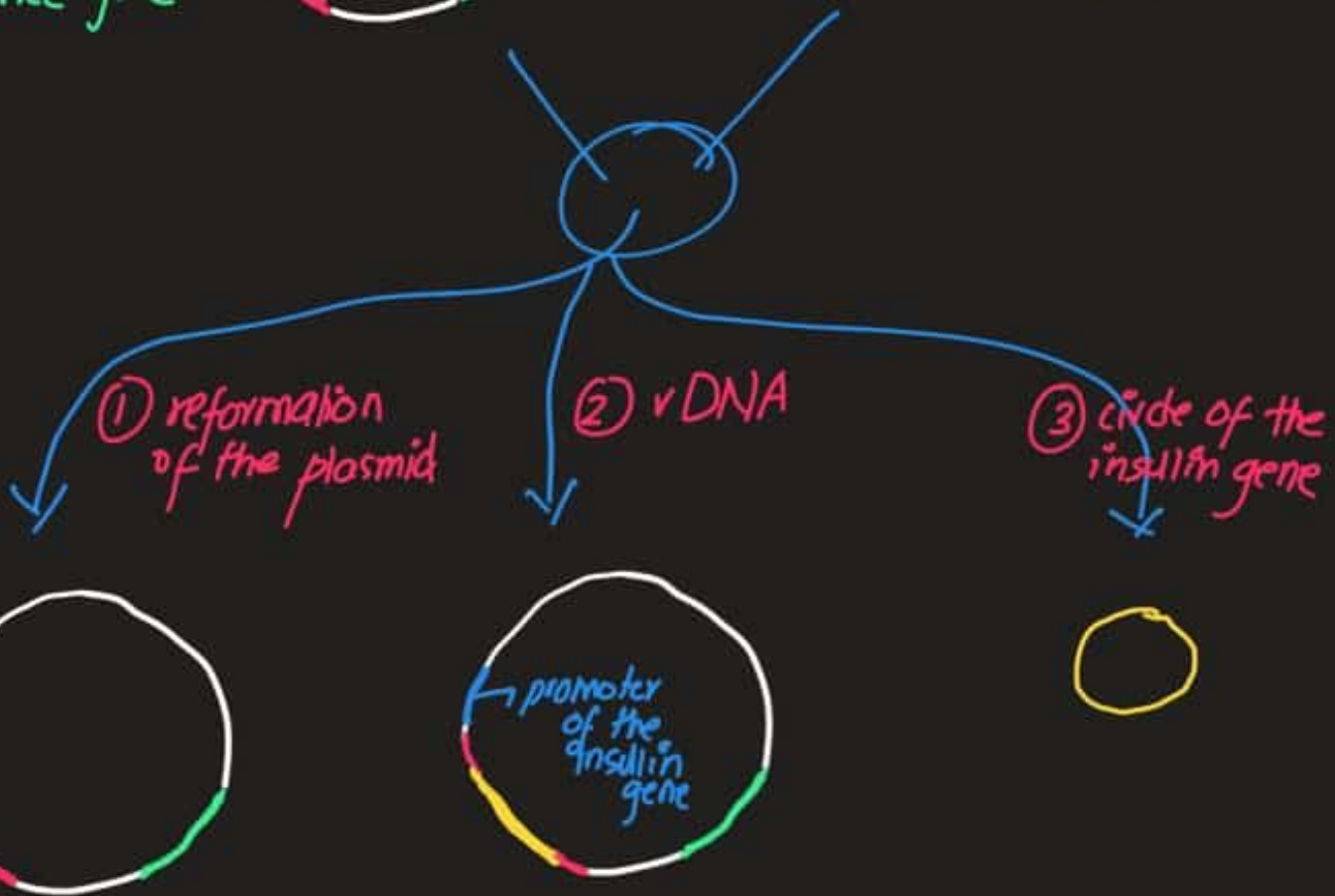
In genetic engineering, restriction enzymes are used to cut DNA at palindromic sites to produce sticky-ends.

Selection of transformed organisms using selectable / screening markers

A. Using selectable markers (antibiotic resistance genes)

- ampicillin resistance gene
- tetracycline resistance gene

+ insulin gene



the bacteria that take up this plasmid will be resistant to BOTH tetracycline & ampicillin

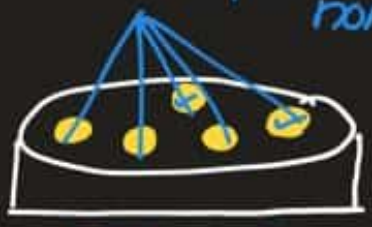
Bacteria that take up this plasmid are transformed bacteria. They are resistant to tetracycline but sensitive to ampicillin

bacteria that take up the insulin gene are sensitive to BOTH ampicillin and tetracycline

REPLICA PLATING

nutrient rich agar + tetracycline

incubate it for 24 hrs



transformed and/or non transformed bacteria

MASTER PLATE

velvet cloth

REPLICA PLATE

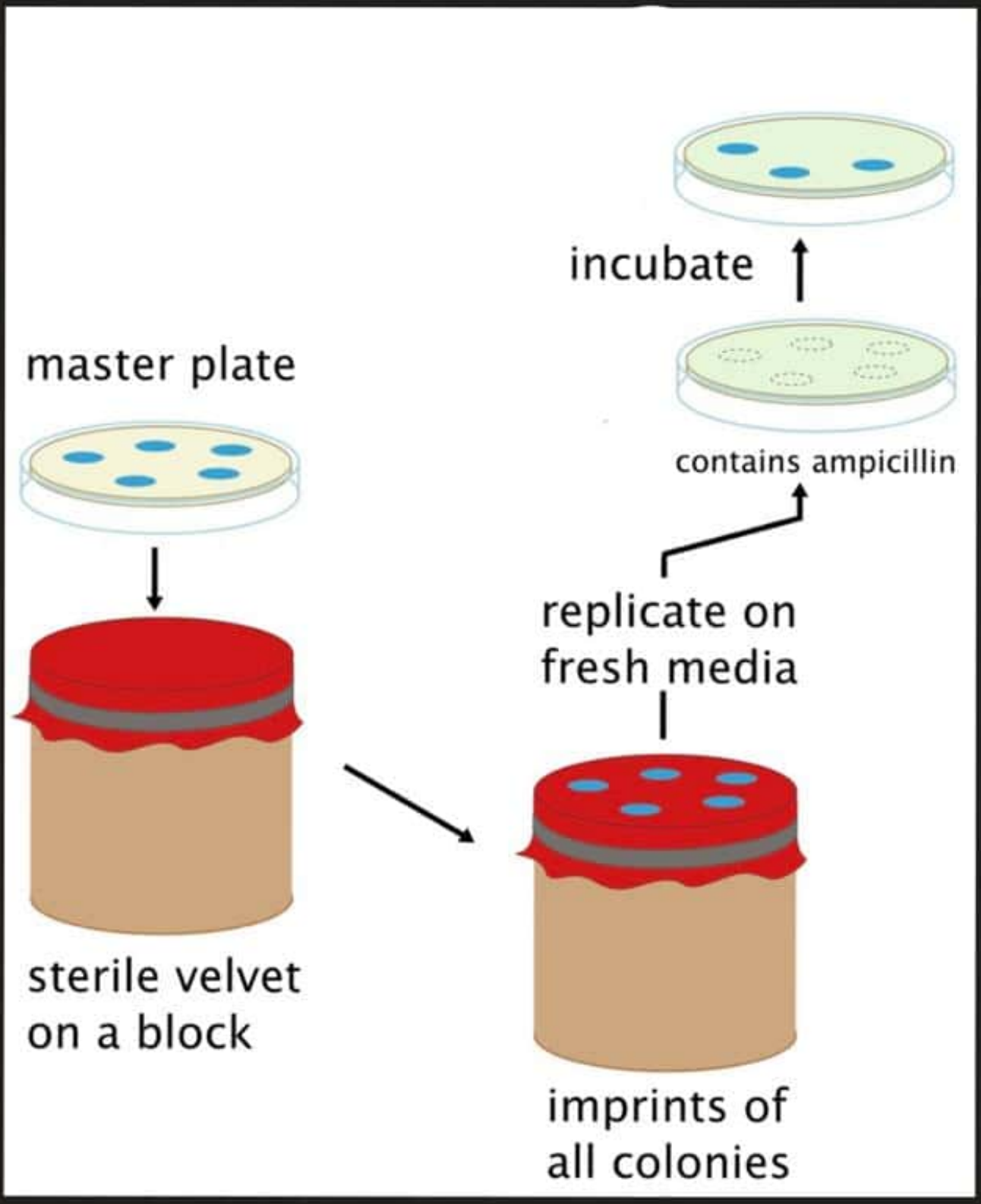
nutrient rich agar + ampicillin

incubate for 24 hrs

REPLICA PLATE

nutrient rich agar + ampicillin

missing colonies of bacteria on the replica plate ⇒ these colonies are the transformed bacteria


incubate
master plate
contains ampicillin
replicate on
fresh media
sterile velvet
on a block
imprints of
all colonies

Identifying Transformed Organisms Using Selectable Marker Genes (via Replica Plating)

Replica Plating:

* method used to identify transformed bacteria using antibiotic resistance genes.

* We generally use plasmids with two different antibiotic resistance genes.

- ampicillin resistance gene
- tetracycline resistance gene.

* Insulin gene is inserted into the plasmid by cutting through the ampicillin resistance gene.

– insulin gene

recombinant DNA (modified plasmid)

* The genetic material is mixed with E.coli to allow uptake of the recombinant DNA. (rDNA).

* Less than 1% of the bacteria actually take up the rDNA.

* Most either take up the unmodified plasmid OR the circle of insulin gene OR nothing at all.

* Selecting transformed bacteria is therefore an important step.

* Bacteria from the mixture are allowed to grow in an agar plate rich in nutrients and tetracycline. This is known as the Master Plate.

1. incubate the agar with transformed & non-transformed bacteria

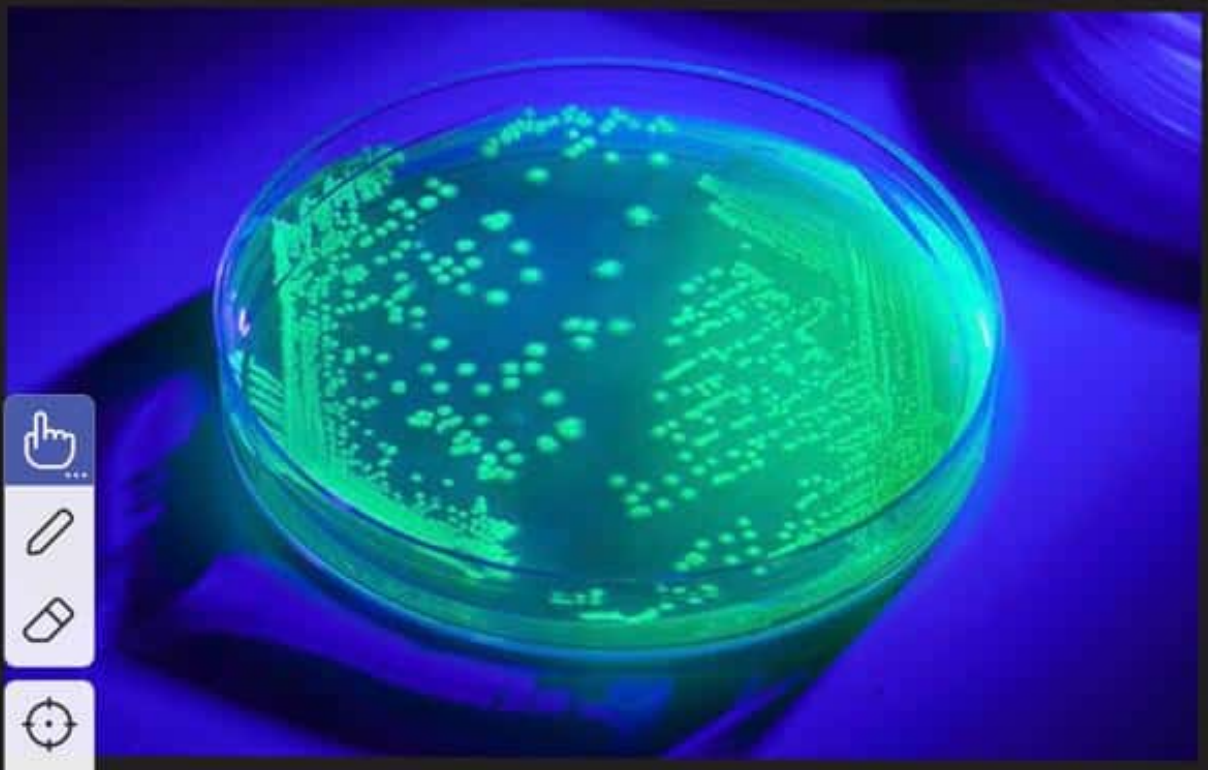
2. Observe bacterial colonies → label this agar plate as the master plate.

3. Press a velvet cloth against the master plate and replicate bacterial colonies onto another agar plate which contains ampicillin. This is the Replica Plate. Retain the master plate.

4) Observe the growth of the bacterial colonies on the replica plate.

Missing colonies on the replica plate are the colonies that contain transformed bacteria. Samples of these bacteria can be taken from the master plate.

B. Using screening markers



Identifying Transformed Organisms Using Screening Marker Genes

* Screening markers are genes that enable identification of transformed organisms based on the changes in their visible appearance.

* Commonly used screening markers include:

- GFP gene (Green Fluorescent Protein Gene)

the GFP gene is extracted from the Jellyfish. The protein produced by this gene fluoresces green under UV light.

- **B-Gus gene (coding for B-Glucuronidase Enzyme)**

B-Gus gene which codes for the enzyme B-Glucuronidase enables identification of transformed organisms by breaking down non-blue glucuronides to produce a toxic blue product.

The Blue White Method

which involves use of a marker gene known as the Z-gene. Z-gene codes for the enzyme B-galactosidase, which splits a colourless galactoside to produce harmless blue product.

* The marker gene for GFP codes for a non-enzymatic protein, whereas, the other two screening markers code for enzymes. These enzymes can produce a large number of product molecules since they can be re-used.

Benefits of genetically engineered human insulin

Benefits of Producing Genetically Engineered Human Insulin

* Less likely to cause allergic reactions.
* Production of genetically engineered human insulin is relatively cheaper if insulin is produced on a large scale.
* is acceptable to religious groups who are against the usage of insulin derived from pigs or cattle.

* Likely to be more effective in causing reduction in the plasma glucose because of its better binding to the insulin receptor.

Human insulin has a more rapid onset of action and is less likely to cause Tolerance.

Q:Explain how bacteria can be genetically modified and then identified using antibiotic resistance genes?

Ans: Transformed bacteria are produced by mixing plasmids with bacteria in a solution containing a high concentration of calcium ions at 0° C . Heat shock (temperature raised to 40°C) allows uptake of these plasmids into the bacteria.
Replica plating is a technique used to select transformed bacteria using antibiotic resistance genes. Non-recombinant plasmids contain an intact ampicillin resistance gene and a tetracycline resistance gene. Recombinant plasmids contain an intact tetracycline resistance gene but inactive ampicillin resistance gene as the new gene (e.g, insulin) is inserted within the ampicillin resistance gene. Bacteria that take up the plasmids (recombinant or non-recombinant) grow in an agar containing the antibiotic tetracycline. Bacterial colonies are replicated onto the replica agar plate containing the antibiotic ampicillin. A velvet pad is used to transfer the bacterial colonies . The bacterial colonies on the master plate which DO NOT grow on the replica plate are the transformed bacteria.

Q: The pink bollworm moth, Pectinophora gossypiella, is a pest of cotton crops. The size of its population can be reduced by releasing large numbers of sterile male moths into cotton fields. The sterile male moths mate with wild females from the cotton fields, but no offsprings are produced.

Over a period of 3 years, 20 million genetically modified (GM) sterile male moths were released in the USA. Each insect contained a gene coding for a red fluorescent protein (DsRed) taken from a species of reef coral. The added DNA also included a promoter.

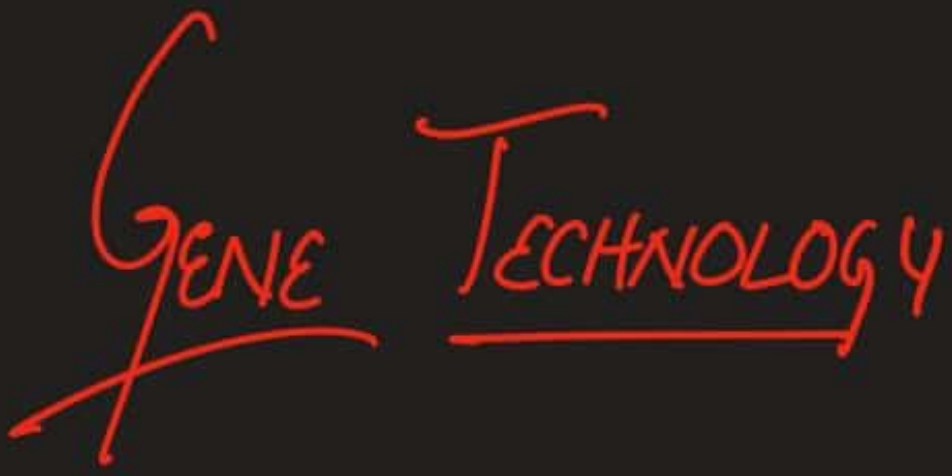
a) Explain why, in gene technology:

Genes for fluorescent proteins such as DsRed are now more commonly used as markers than are genes for antibiotic resistance.[2]

Ansa): It's easier and faster to identify transformed organisms based on changes in their visible appearance. Antibiotic resistance genes may be transferred to other bacteria spreading antibiotic resistance. This reduces the effectiveness of antibiotics.

b)Explain why, in gene technology:
A promoter needs to be included when transferring a gene from a coral reef into an insect. [3]

Ansb: Promoter is a control sequence necessary for gene expression. It serves as the binding site of the enzyme RNA polymerase (and the transcription factors) to allow transcription to occur. Promoter also helps identify the template strand in a double-stranded DNA.

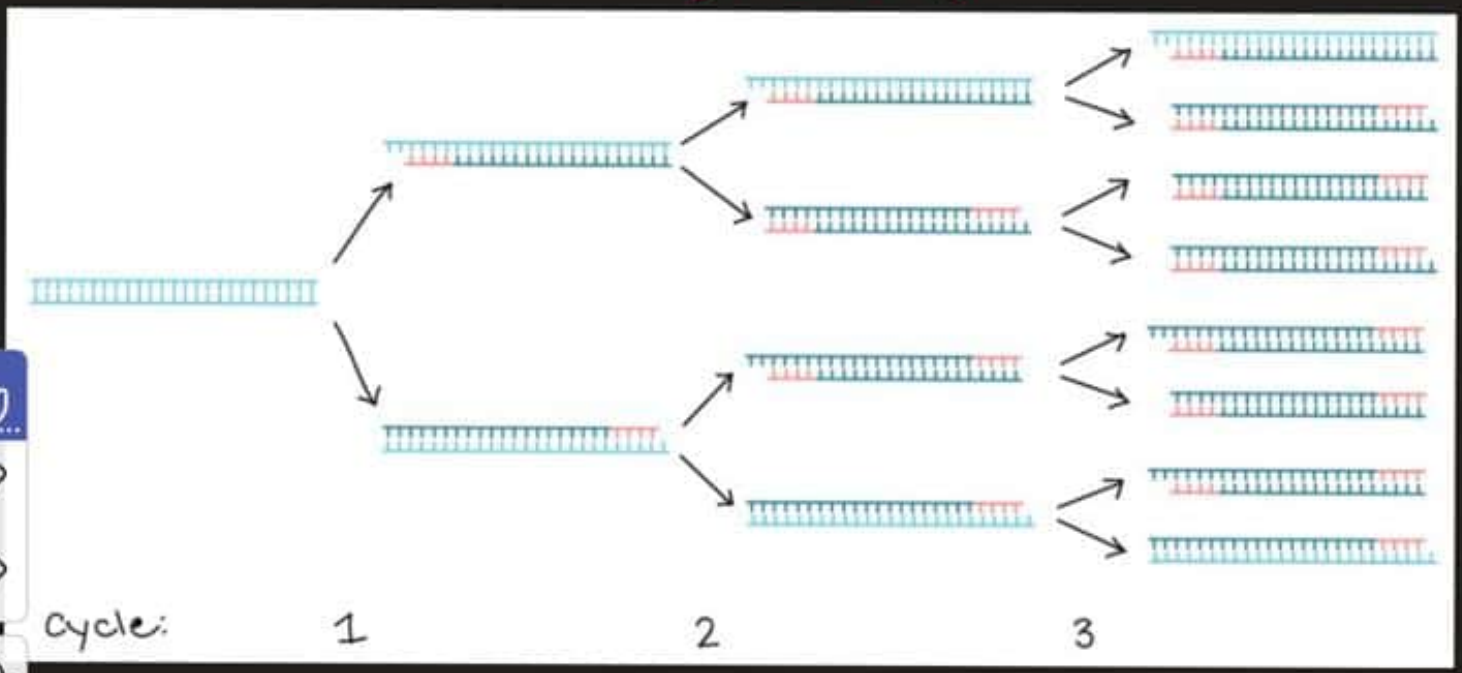

Gene Technology

Previously,

* Gene technology & genetic engineering
* Production of genetically engineered human insulin
* Marker genes for selection of transformed organisms

Polymerase Chain Reaction (PCR)

In each cycle
- a. denaturation (95°C)
- b. annealing (65°C)
- c. elongation (72°C)



cycles (n)	no. of DNA copies (2^n)
1	2
2	4
3	8
4	16
⋮	⋮

* Polymerase used in PCR ⇒ Taq polymerase
  ↙ derived from
  Thermophilus aquaticus

POLYMERASE CHAIN REACTION (PCR)

* It is a technique used to amplify a gene or a DNA segment, thereby, producing numerous copies of the genetic material.

* PCR is highly sensitive and can make use of a single copy of DNA to produce numerous copies.

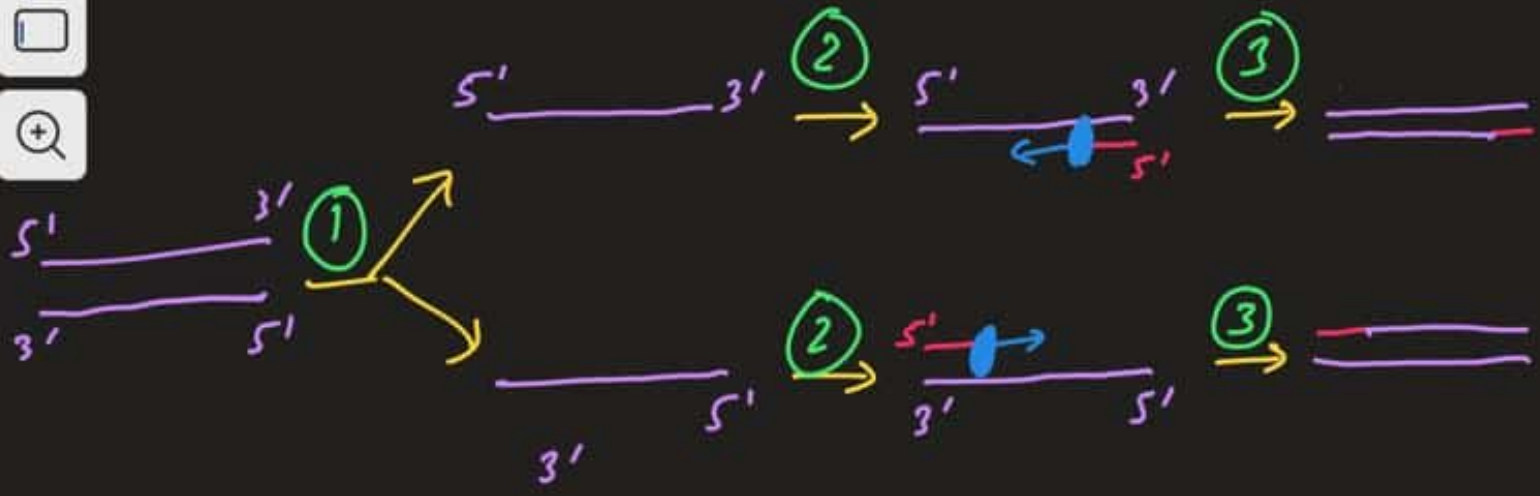
* PCR makes use of a thermally stable DNA Polymerase known as Taq Polymerase.

* This enzyme has a high optimum temp. & is resistant to denaturation at high temps.

* Taq polymerase is extracted from the bacterium Thermophilus aquaticus.

* Each cycle of PCR involves the following 3 stages:

1. DENATURATION (at 95°C)
2. ANNEALING (at 65°C)
3. ELONGATION/POLYMERISATION (at 72°C)



Substrate ⇒ dNTPs → dATP, dTTP, dCTP, dGTP

PCR MAKES USE OF THE FOLLOWING:

1) DNA Primers - short lengths of single-stranded DNA molecules that are approximately 20 bases long.

2) Taq Polymerase - a thermally stable enzyme extracted from the bacterium Thermophilus Aquaticus. Taq polymerase has the following advantages:

i) it is resistant to denaturation at high temps due to presence of disulfide bridges. (95°C)

ii) it has a relatively higher optimum temp.

3) dNTPs (deoxyribo-Nucleotide Tri Phosphates)

dNTPs serve as a :

i) substrate for formation of new DNA molecules.

ii) source of energy due to extra phosphates attached to them.

4) Buffer Solution - To maintain the pH of the reaction medium. It also contains Mg^{2+}. (cofactors of Taq polymerase)

5) Required gene or DNA that needs to be amplified.

DENATURATION

* Denaturation involves melting of DNA to produce single-stranded DNA molecules.
* The energy required for melting is provided by a higher temperature (95 °C) within the PCR thermal cycler.
* Melting occurs when the hydrogen bonds b/w the two complementary strands break.

ANNEALING:

* Annealing involves pairing up of the DNA primers To the 3'-end of the single-stranded DNA molecules.

* This process requires a low temperature of 65°C.

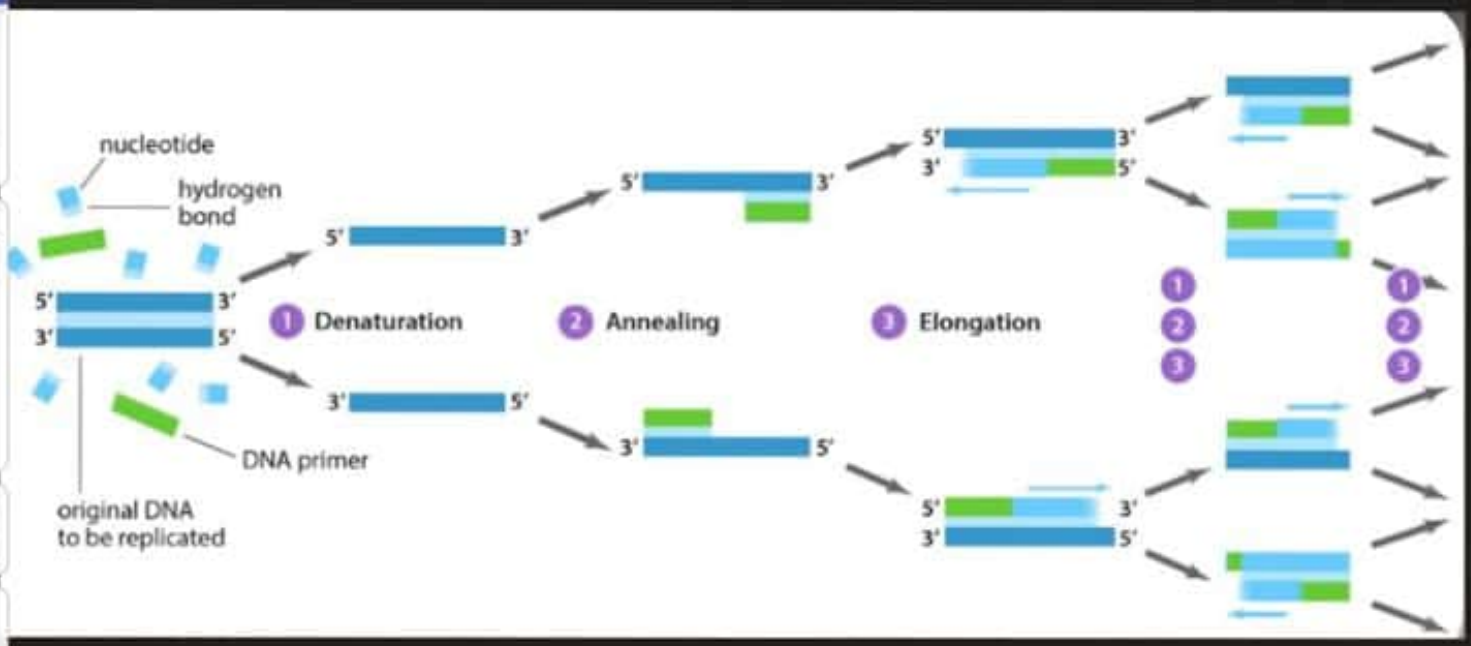
* These DNA primers guide the Taq Polymerase To elongate the nucleotide chain.

ELONGATION / POLYMERISATION

* Elongation is the process in which the Taq Polymerase elongates the nucleotide strand in the 5'-3' direction by forming phospho-diester bonds b/w the neighbouring dNTPs.

* These dNTPs pair up with the complementary bases in accordance with the rules of base pairing : A pairs up with T, G pairs up with C.

* A PCR thermal cycler can produce 2^n copies of DNA where n refers To the no. of cycles, for example, 25 cycles of PCR can produce 2^{25} copies of DNA.


nucleotide
hydrogen bond
5'
3'
3'
5'
original DNA to be replicated
DNA primer
1 Denaturation
2 Annealing
3 Elongation
1
2
3


Questions

Q.

3 The polymerase chain reaction (PCR) is used to produce large amounts of DNA from a very small original sample. The main stages of a PCR are shown in Fig. 3.1.

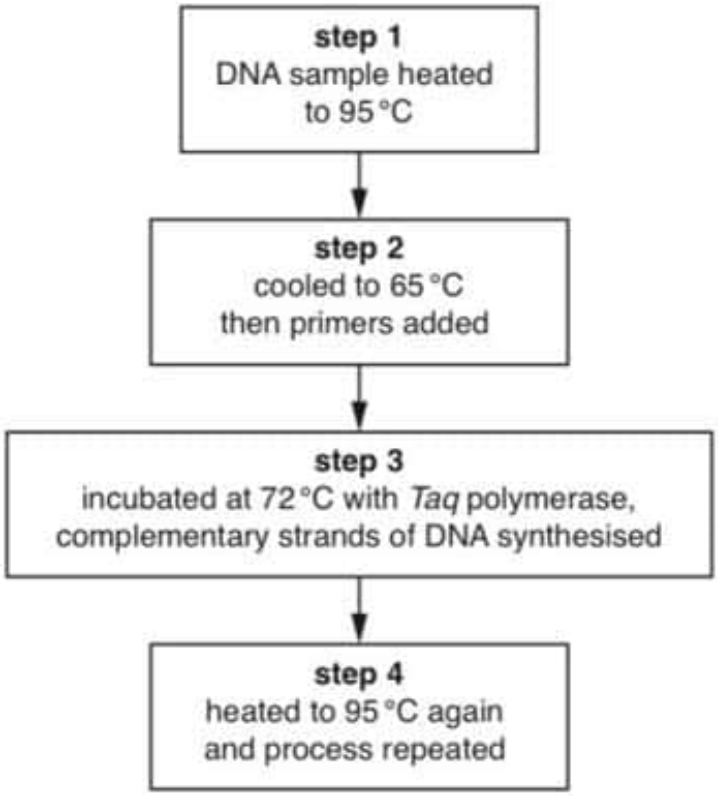


Fig. 3.1

(a) (i) Explain why the DNA sample is heated to 95 °C in **step 1**.

* to break the hydrogen bonds between the two strands
* these strands serve as a template to form new strands

[2]

(ii) Explain why primers are added in **step 2**.

* primers attach to (anneal) the 3'-end of single stranded DNA b/c
* Taq polymerase cannot attach to single stranded DNA

[2]

(iii) Explain why the enzyme *Taq* polymerase is used in **step 3**.

* Taq polymerase has a high optimum temperature, and
* is resistant to denaturation at high temperatures

[2]

3 Human insulin can be synthesised in a laboratory strain of *Escherichia coli* using recombinant DNA (rDNA) technology.

The starting point for the process is mRNA coding for insulin, isolated from human pancreas cells.

Four enzymes are needed:
- reverse transcriptase
- DNA polymerase
- restriction enzyme
- DNA ligase.

(a) (i) State the role of each of these enzymes in producing rDNA carrying the gene for human insulin.

reverse transcriptase

produces single stranded cDNA using mRNA

DNA polymerase

produces double stranded DNA using cDNA

restriction enzyme

cut the DNA (plasmid and the gene) at palindromic sites to produce sticky ends

DNA ligase

joins the plasmid with the gene by forming phosphodiester bonds [4]

(ii) Outline the role of insulin in a healthy human.

* insulin regulates blood glucose concentration
* binds to its receptors on liver/muscle cells and promote uptake of glucose by increasing membrane permeability
* stimulates glycolysis → resp. of glucose
* stimulates glycogenesis → conv. of glucose to glycogen [3]

(iii) Describe and explain **one** advantage of treating diabetics with human insulin produced by rDNA technology.

* more rapid response
* it's cheaper with more reliable supply as compared to animal insulin b/c it's produced in large volumes [2]

(b) It is possible to use rDNA technology to produce insulin with a slightly different structure from that of human insulin. The effect of the changed structure can then be investigated.

The activities of equal quantities of two insulins, both produced by *E. coli*, were compared in healthy, non-diabetic subjects:

- human insulin
- insulin **X**, in which the positions of two amino acids, lysine and proline, were exchanged. Lysine has a hydrophilic R group and proline has a hydrophobic R group.

The results of the investigation are shown in Fig. 3.1.

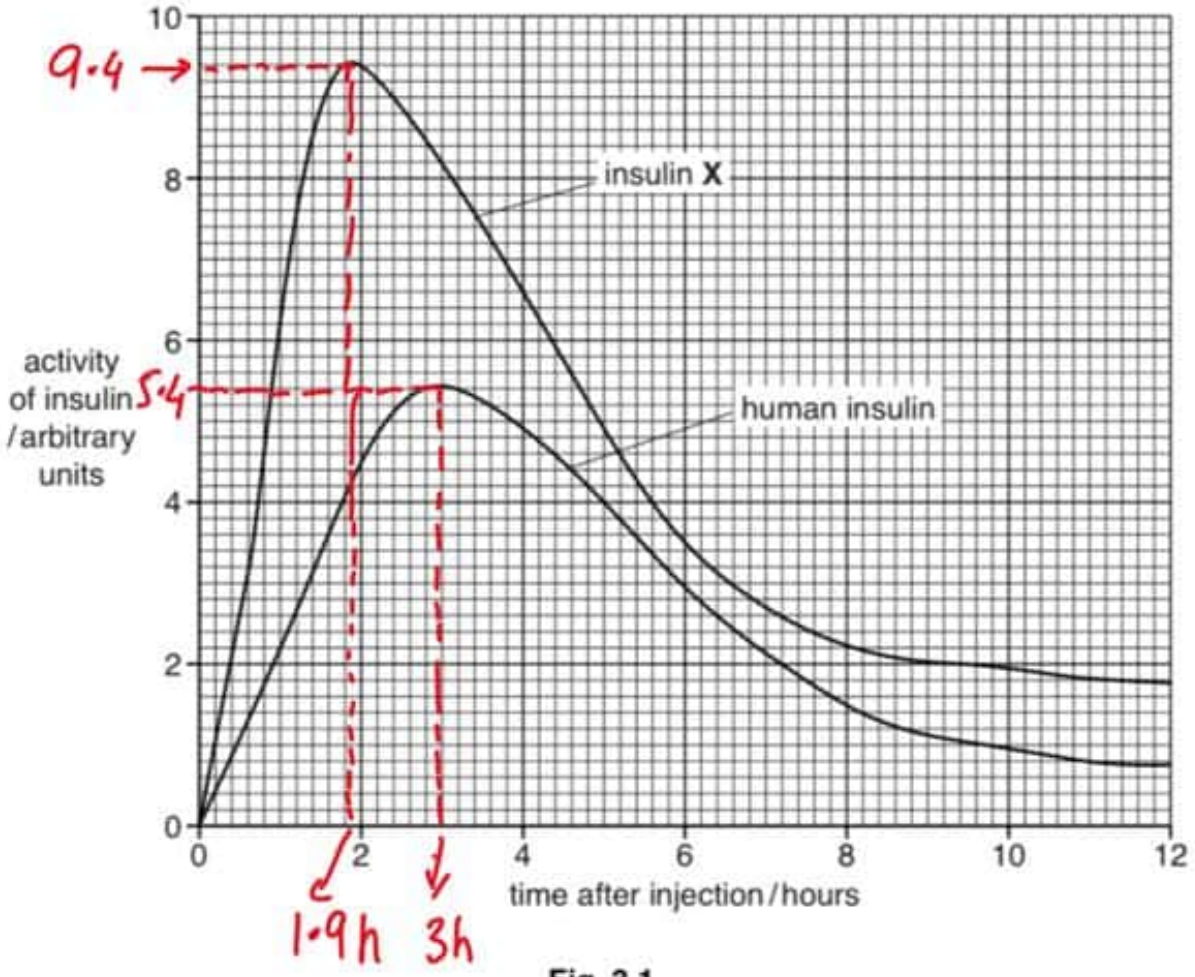


Fig. 3.1

(i) With reference to Fig. 3.1 describe the differences in activity between human insulin and insulin **X**.

* insulin X showed a steeper rise in activity at the start after injection
* the peak activity with insulin X was at 9.4 compared to 5.4 with human insulin
* Insulin X reached peak activity 1-9 hours after injection compared to 3 hrs with human insulin
* Insulin X had a greater activity than human insulin over 12hrs after injection

[4]

(ii) Suggest how exchanging the position of two amino acids in the insulin molecule can result in differences in activity.

* insulin X may bind with a better fit with the insulin receptor
* which may affect the second messengers within the target cells

[2]

* change in tertiary structure may also make the insulin X more soluble in the blood plasma

Q.

4 Enzymes are globular proteins that catalyse specific reactions.

(a) Explain how enzymes catalyse specific reactions.

* enzymes have an active site complementary to the shape of the substrate
* substrate binds with the enzyme to form the E-S complex
* substrate is held within the active site via hydrogen bonds

[3]

(b) Restriction enzymes cut DNA into fragments. They cut at specific sites determined by the sequence of bases. Fig. 4.1 shows the base sequences cut by three restriction enzymes and a section of DNA cut by one of these enzymes.

restriction enzyme	DNA sequence
HindIII	A AGCTT
EcoRI	G AATTC
BamHI	G GATCC

section of DNA

TACGAATTCGTAA
ATGCTTAAGCATT

TACG
ATGCTTAA

AATTCGTAA
GCATT

Fig. 4.1

(i) Identify the restriction enzyme that has cut the section of DNA shown in Fig. 4.1.

EcoRI [1]

(ii) State the name given to the unpaired base sequences that remain after DNA has been cut by the three restriction enzymes shown in Fig. 4.1.

sticky ends [1]

(c) Human genes may be cloned by inserting lengths of DNA into bacteria. This is carried out by inserting the DNA into a plasmid.

Explain how lengths of DNA, cut by restriction enzymes, are inserted into plasmids.

* the plasmid and the gene are mixed together
* in the presence of the enzyme DNA ligase
* the plasmid and the gene attach via hydrogen bonds bw sticky ends
* DNA ligase makes phosphodiester bonds between the gene and the plasmid

[3]

Q.

5 Traditional techniques for genetically modifying organisms use three enzymes:

- restriction endonuclease
- reverse transcriptase
- DNA ligase.

For example, these enzymes have been used to produce genetically modified (transgenic) pigs containing the *GFP* gene coding for green fluorescent protein, originally sourced from jellyfish.

(a) Outline how these three enzymes could be used in genetically engineering a transgenic pig containing the *GFP* gene.

.....

.....

.....

.....

.....

.....

..... [3]

A new technique that aims to cause a **deletion** in a gene uses an enzyme called Cas9 nuclease. It is injected into zygotes along with an RNA sequence (the guide RNA) that is complementary to a target gene. The Cas9 nuclease causes a deletion in the target gene in the zygotes, preventing the expression of that gene.

The toxicity and efficiency of the new technique was tested on four groups of pig zygotes. These pig zygotes were produced by IVF using:

- ova from a female non-transgenic pig.
- sperm from a male transgenic pig whose somatic (body) cells contained one copy of the *GFP* gene per cell.

The pig zygotes in three groups were injected with different concentrations of Cas9 nuclease and guide RNA **targeted at the *GFP* gene**.

The fourth group of pig zygotes (control group) was **not** injected with Cas9 nuclease and guide RNA.

(b) Explain why the *GFP* gene was chosen for testing the new technique.

* marker gene
* deletion of GFP gene will lead to no fluorescence

..... [2]

Some of the zygotes in each group survived and after six days each had developed into a group of cells called a blastocyst.

The blastocysts were counted using a light microscope. A filter was then added to the microscope, so that only blastocysts expressing the green fluorescent protein showed up. These were counted and the results are summarised in Table 5.1.

Table 5.1

concentration of Cas9 nuclease and guide RNA / ng mm^{-3}	number of blastocysts seen under white light	number of blastocysts seen under filter
0 (control)	68	46
10	40	0
20	24	0
50	15	0

Aa = Aa aa

(c) (i) Calculate the percentage of zygotes in the control group that were transgenic.

Show your working. % = 46/68 × 100 = 67.6

Aa Aa aa aa

67.6 ..... % [1]

(ii) Explain whether the percentage you calculated for **(i)** is higher or lower than expected.

* higher than the expected 50% .....

..... [1]

(iii) Name a statistical test that would allow you to test the significance of the difference between the percentage you calculated in **(i)** and the expected percentage.

* X^2 test (chi-squared test) ..... [1]

(iv) State the best concentration of Cas9 nuclease and guide RNA to use to cause a deletion in the *GFP* gene **and** give reasons for your choice.

* 10 ng mm^{-3}

* B/c of the highest no. of blastocysts and lesser toxicity

..... [3]

ELECTROPHORESIS

* simple technique used to separate a mixture of charged molecules
* separation is on the basis of charge to mass ratio
* the process is generally used to separate;

a) mixture of polypeptides/amino acids
b) mixture of DNA fragments

ELECTROPHORESIS

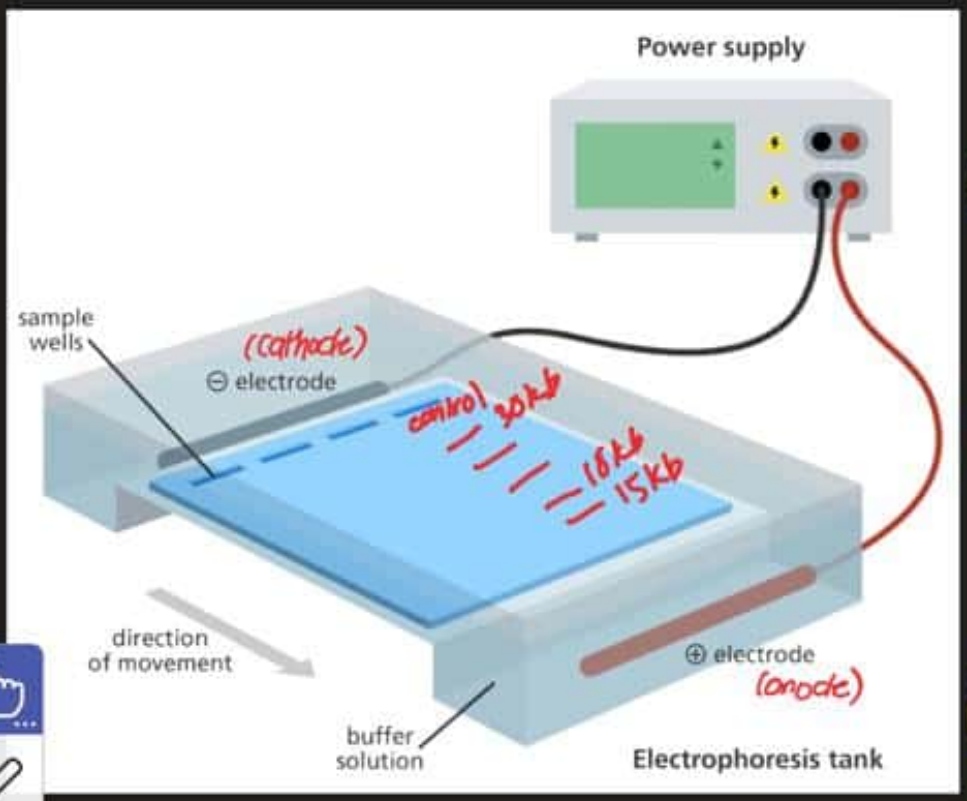
* Electrophoresis is a simple technique used for the separation and analysis of charged molecules, such as proteins & nucleic acids, based on their size & charge.

* Electrophoresis is commonly used to separate:

a) Mixture of polypeptides / amino acids (polyacrylamide gel) PAGE

b) Mixture of DNA fragments (agarose gel) AGE

Separating DNA fragments via electrophoresis



① Extract the DNA from the sample

② Amplify the DNA using PCR

③ Cut the DNA into fragments using restriction enzymes

④ Load the wells with the DNA fragments

⑤ Apply potential difference to allow separation of the fragments using the charge : mass

⑥ Visualise the DNA fragments using ;

a. labelled DNA probes → radiolabelled / fluorescent stain labelled

b. Staining techniques

* This process of separating DNA fragments via electrophoresis involves the following steps:

1- Extraction of DNA

2- Amplification of the extracted DNA using PCR.

3- The DNA is then cut into fragments using restriction endonuclease.

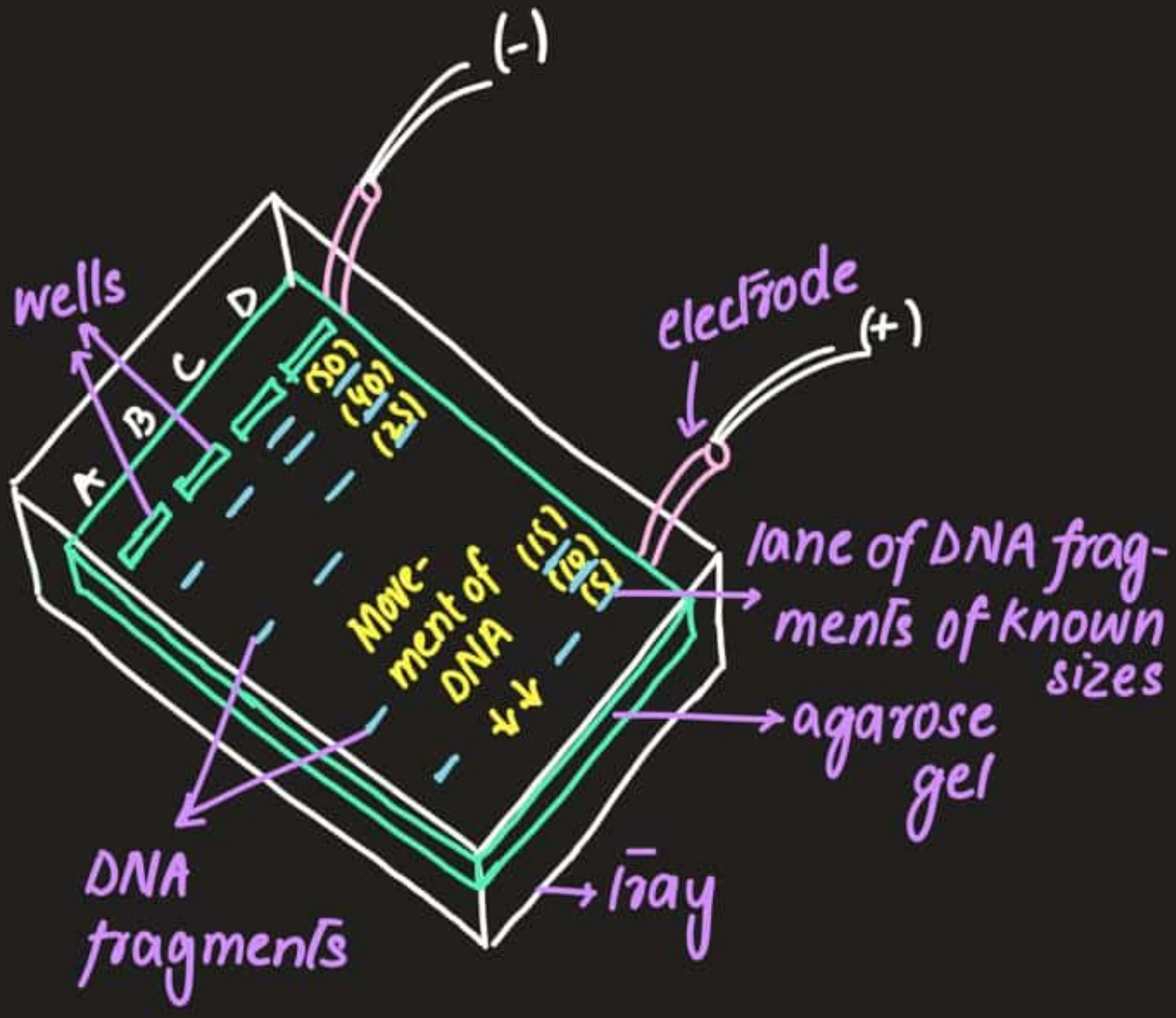
4- The DNA fragments are poured into wells cut in the agarose gel. These wells are placed close to the cathode.

5- The agarose gel is placed inside a buffer solution in an electrophoresis tray.

6- A potential difference is applied across the agarose gel which allows negatively charged DNA fragments to move towards the anode. These negative charges on DNA are due to the presence of the phosphate groups within the sugar-phosphate backbone.

* These DNA fragments are separated based on their molecular size and the charge. The rate of movement of the DNA fragments is therefore determined by:

1) The molecular mass
2) The charge on the fragments
3) The molecular spacing within the gel.
4) Temperature
5) The potential difference applied across the gel.

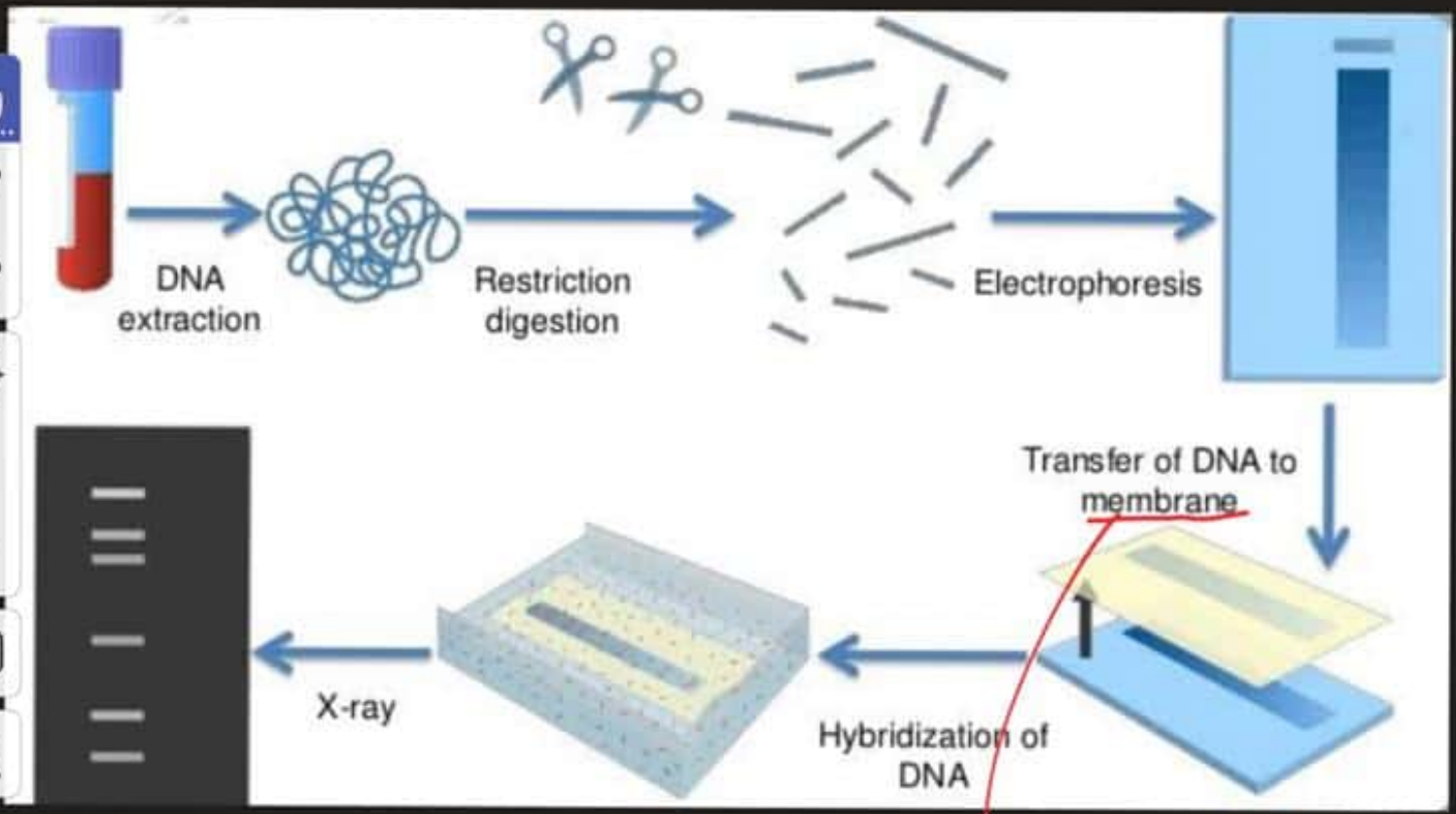

(-)
wells
A
B
C
D
(50)
(40)
(25)
electrode
(+)
Move-
ment of
DNA
(15)
(10)
(5)
lane of DNA frag-
ments of known
sizes
agarose
gel
DNA
fragments
tray

Visualisation of the DNA fragments may be achieved by one of the following two ways:

i) using labelled DNA probes

ii) Staining Techniques using dyes.

(i) Using labelled DNA probes



(absorbent paper)

VISUALISATION OF DNA FRAGMENTS USING DNA PROBES

* DNA probes are single-stranded short segments of DNA that are either tagged with a fluorescent stain or radioactively labelled phosphorus isotope.

* An absorbant paper is placed on the electrophoresis gel which causes the DNA fragments to stick on the paper.

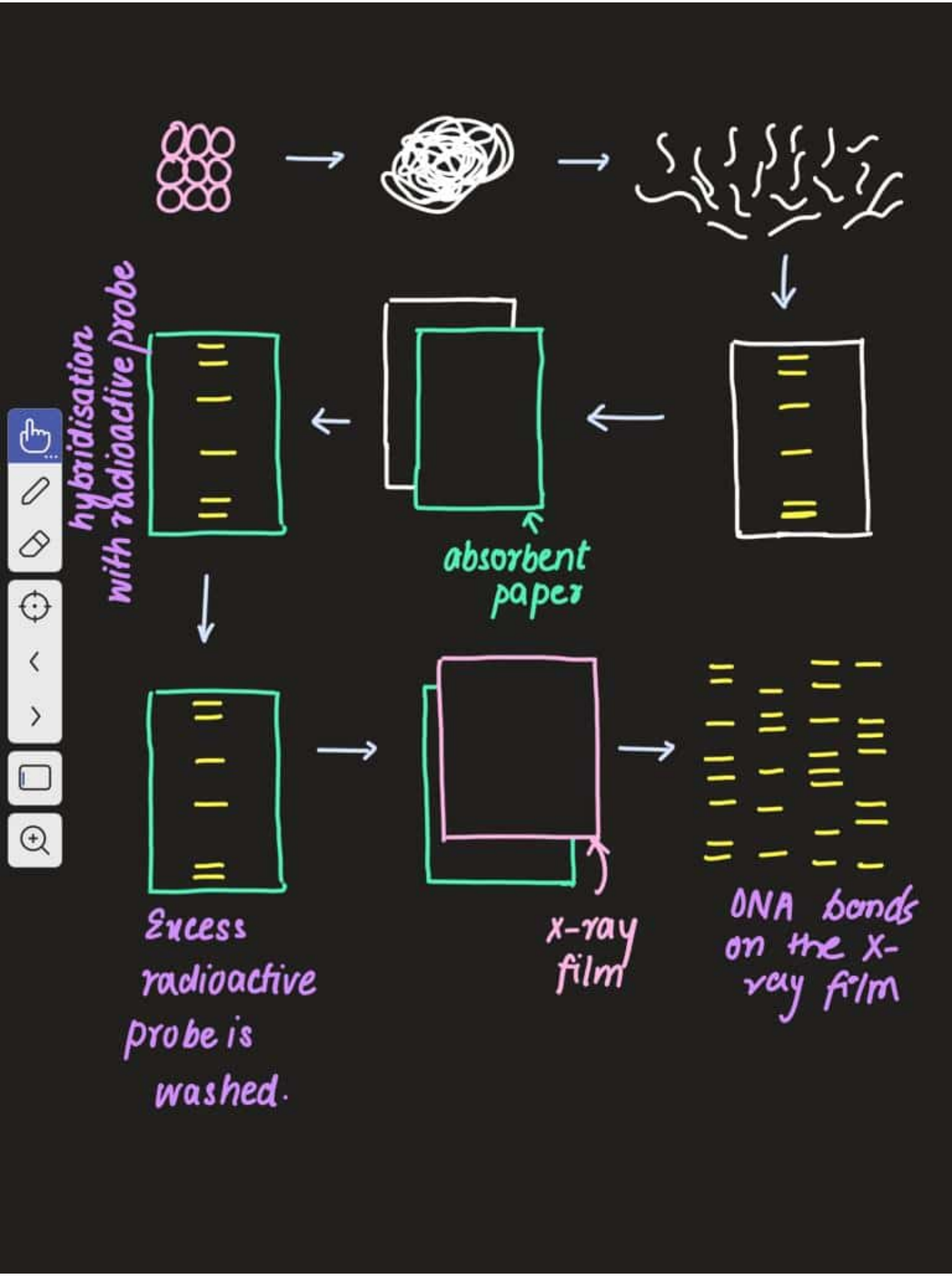
* The absorbant paper is gently heated to melt the DNA, thereby producing ssDNA fragments.

* Radioactively labelled DNA probes are then added to the absorbent paper to allow them to bind to the ss DNA fragments (hybridisation).

* Excess DNA probes are washed off.

* The absorbent paper is then exposed on an x-ray film which produces a pattern of dark bands due to the radio emissions by the radioactive phosphorus isotope.

* Alternatively these DNA probes may be tagged with a fluorescent stain.


hybridisation with radioactive probe
absorbent paper
Excess radioactive probe is washed.
X-ray film
DNA bands on the X-ray film

(ii) Using staining techniques

VISUALISATION OF DNA FRAGMENTS USING STAINING TECHNIQUES

* The following dyes can be used To stain the DNA fragments :

a) Ethidium Bromide which binds To the DNA fragments and fluoresces under U.V light.

b) Methylene Blue Stain which binds To the DNA fragments and stains them blue as they move along the gel. Methylene blue also stains the buffer solution and the electrophoresis gel.

c) Nile Blue Stain which binds To the DNA fragments and stains them blue without staining the gel or the buffer solution.

Applications of electrophoresis

A. Separating proteins

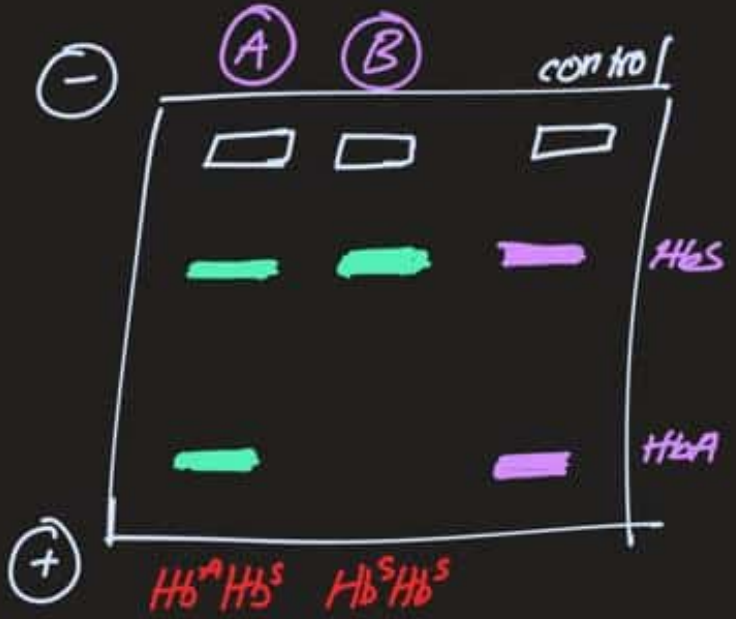
Hb electrophoresis to diagnose sickle cell anaemia or sickle cell trait

Hb A

(+) glutamine

Hb S

(+) valine



SEPARATING PROTEINS (E.G. HAEMOGLOBIN) VIA ELECTROPHORESIS

* Haemoglobin is a tetramer of 2 alpha & 2 beta globin chains.

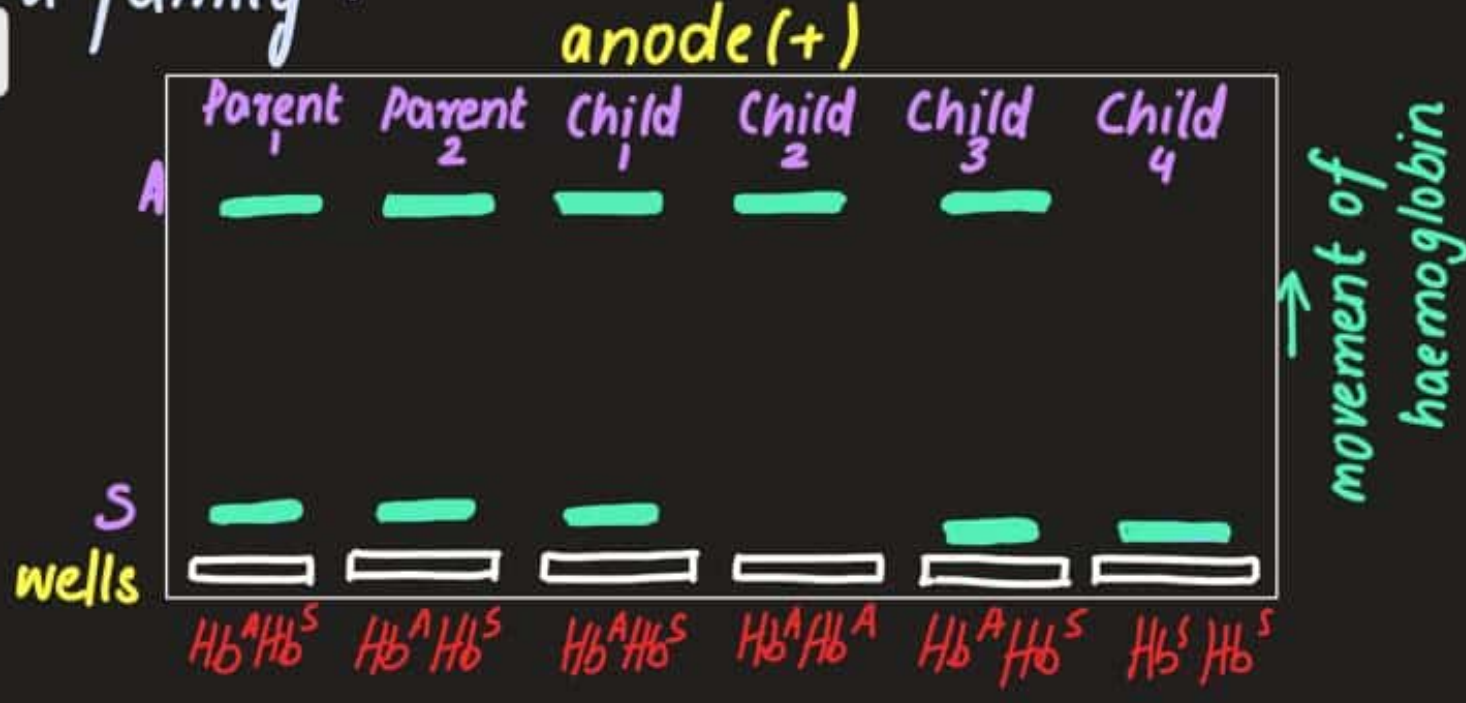
* In sickle cell disease alteration of one nitrogenous base in the beta globin gene results in a change in the amino acid at the sixth position.

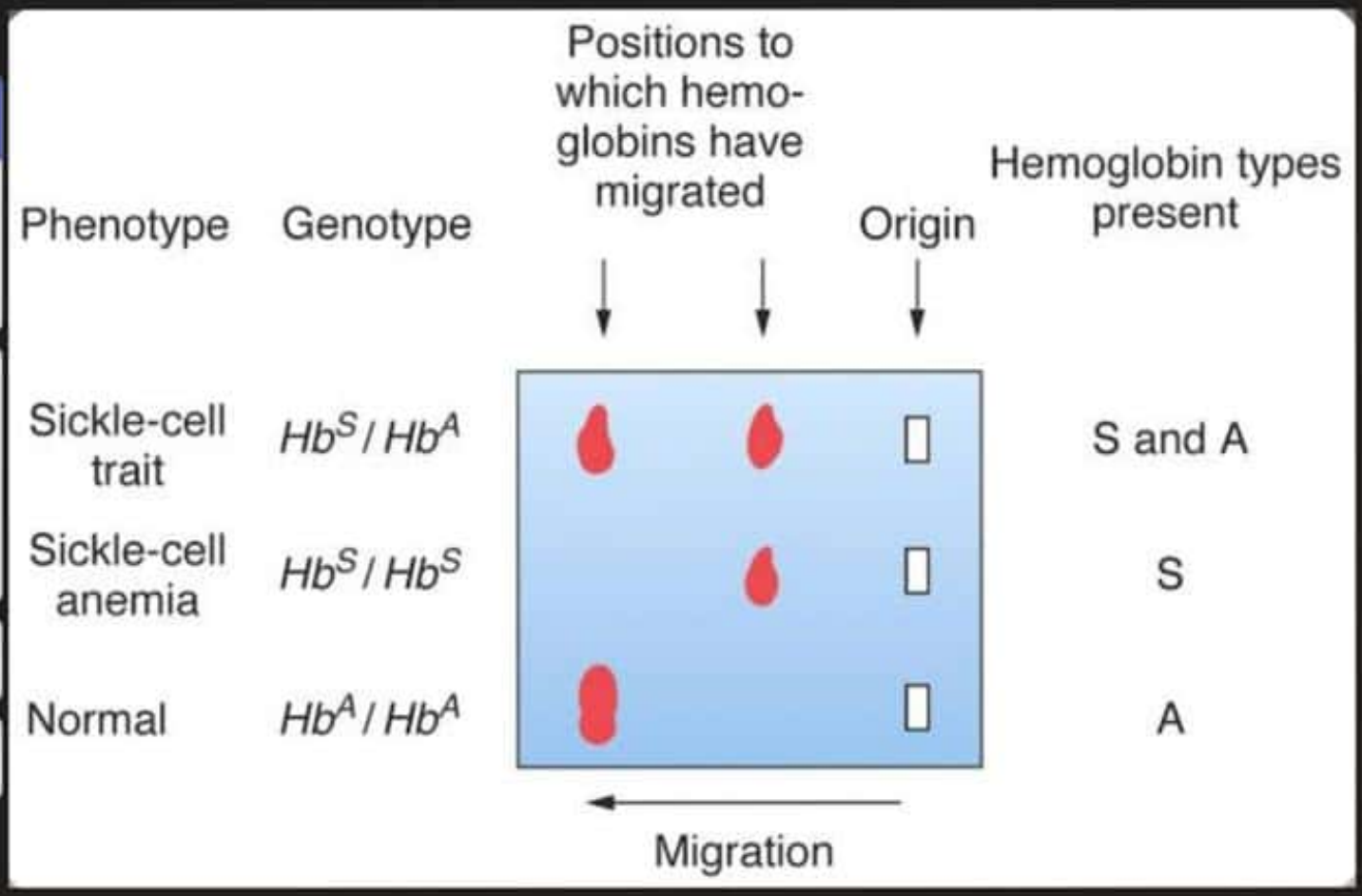
* The mutated gene codes for an amino acid that has a non-polar R group.

* This decreases the overall negative charge on the haemoglobin molecule.

* The mutated haemoglobin does not travel as far in the gel when compared to normal haemoglobin.

* The diagram below shows haemoglobin electrophoresis to determine the genotype of a family:




Positions to which hemo-globins have migrated
Hemoglobin types present
Phenotype
Genotype
Origin
Sickle-cell trait
Hb^S / Hb^A
S and A
Sickle-cell anemia
Hb^S / Hb^S
S
Normal
Hb^A / Hb^A
A
Migration

B. Genetic profiling (DNA)

↓

the genome of each individual has numerous non-coding repetitive sequences of bases termed as VNTRs

↓

VNTR ≡ variable number of tandem repeats

↓

the length of the set of VNTRs in a particular individual is unique to that individual

↓

these VNTRs can be separated using electrophoresis

GENETIC PROFILING (Genetic fingerprinting)

* DNA profiling (or genetic fingerprinting) is an analytical technique used to detect the specific characteristics of an individual's DNA.

It is now routinely used in forensic medicine, paternity testing, crime scene investigations, genealogy (study of family history and lineage) and medical research, besides other uses.

* DNA profiling involves the collection, processing and analysis of repetitive non-coding nucleotide sequences within the genome of an individual which are unique to it.

* These repititive non-coding sequences vary in length amongst individuals at different gene loci. These are known as the variable Number of Tandem Repeats or the VNTRs. Two individuals (other than identical twins) will never have identical VNTRs.

* DNA can be extracted from an individual's tissue or bodily fluids, such as, hair, saliva or blood.

* The quantity of DNA can be increased using PCR.

* The DNA is then broken into fragments by using restriction endonucleases that cleave the DNA close to the VNTR regions. (UNTRs)

* DNA fragments are separated using electrophoresis, smaller molecules travel faster.

* The fragments are not visible yet.

* Absorbent paper is placed over the gel To transfer the fragments onto it.

*The paper is heated to separate the DNA strands.

* DNA probes are introduced.

* They have base sequences complementary to the VNTR regions and contain a radioactive phosphorus isotope.

* When placed on an x-ray film the radiation causes the film To produce dark bands.

Crime-scene investigation

Victim

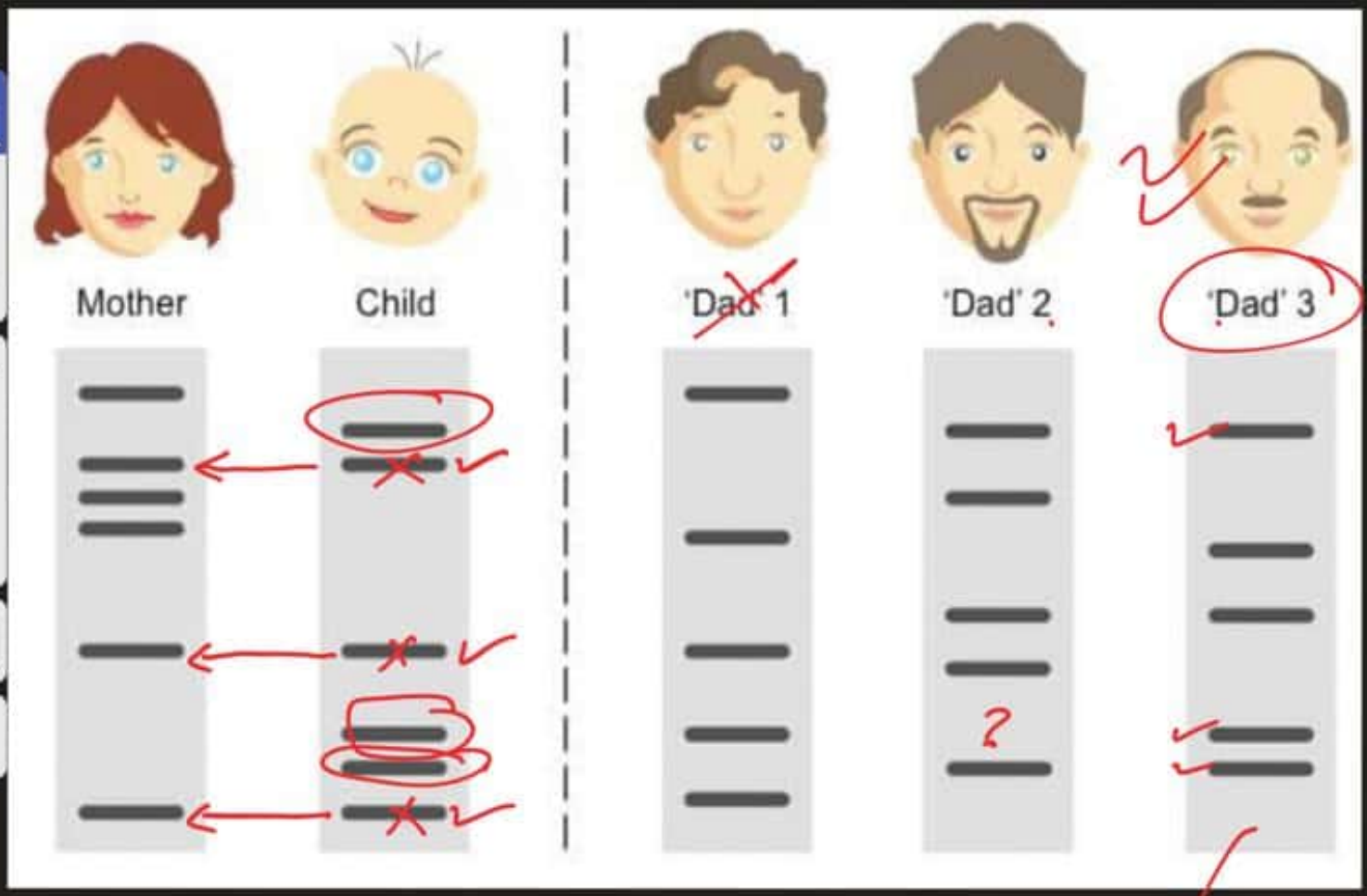
Crime Scene

Suspect 1

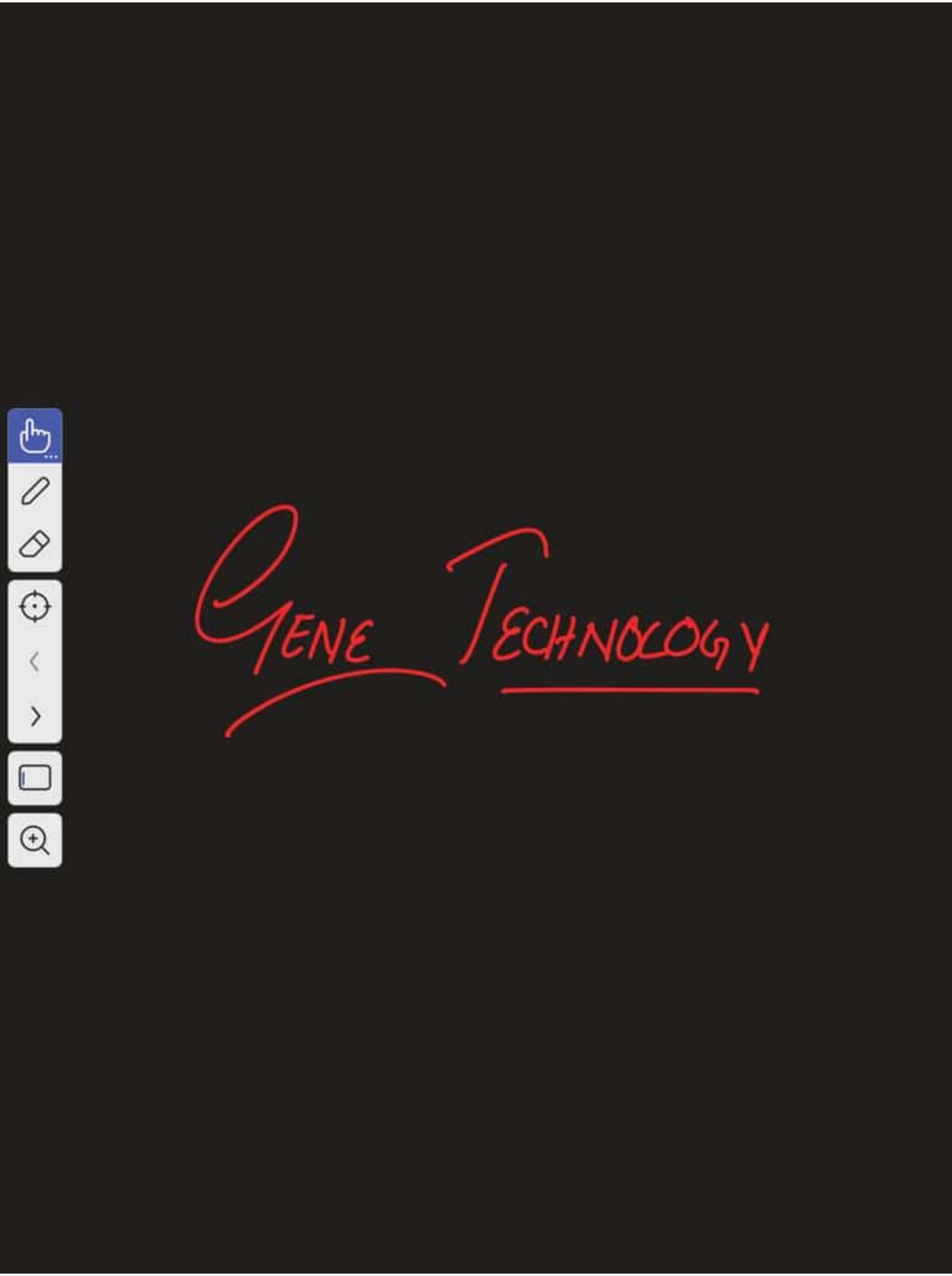
Suspect 2

Suspect 3

Paternity testing



genetic father


Gene Technology

Previously,

* gene technology &; genetic engineering
* production of genetically engineered human insulin
* Marker genes to select transformed bacteria
* PCR


Questions

4 There are a number of mutations affecting the production of fetal haemoglobin, HbF, and normal adult haemoglobin, HbA.

- The Hb^A allele codes for the normal β-globin polypeptide of haemoglobin.
- The Hb^S allele, caused by a base substitution mutation, codes for an abnormal β-globin polypeptide.
- The base substitution results in the amino acid glutamine, which has a polar R group, to be replaced by valine, which has a non-polar R group, in the polypeptide.

The abnormal haemoglobin molecules (HbS) form fibres in low partial pressures of oxygen (pO_2). The fibres cause red blood cells to become sickle shaped and the cells can block blood capillaries.

Individuals with adult haemoglobin molecules that are all abnormal (HbS) have sickle cell anaemia. This is a painful chronic condition that can be life-threatening.

(a) Explain why this mutation causes the HbS to form fibres.

* the polar amino acid glutamine is changed by non polar valine which changes the 3° structure
* this causes the HbS molecules to stick together

[2]

(b) Fetal haemoglobin, HbF, is produced by the fetus until just before birth, when adult haemoglobin begins to be made.

By the age of six months, adult haemoglobin has replaced most of the HbF. This change occurs when the genes coding for HbF are switched off and the genes coding for adult haemoglobin are switched on.

- A base substitution, British-198, causes fetal haemoglobin to continue to be produced.
- Normally by the age of six months, the concentration of HbF reduces to less than 1% of total haemoglobin.
- With the British-198 mutation, the concentration of HbF may be as high as 20% of total haemoglobin in an adult.
- HbF has a higher affinity for oxygen at low pO_2 than adult haemoglobin.

Individuals who have both sickle cell anaemia and British-198 mutation have reduced symptoms of sickle cell anaemia.

(i) Suggest why having the British-198 mutation reduces the symptoms of sickle cell anaemia.

* both HbF and HbS are present
* reduced formation of HbS fibres which leads fewer RBCs having sickle shape.

[2]

(ii) In adults with the British-198 mutation, the gene coding for a fetal haemoglobin polypeptide remains switched on. This is due to the presence of a protein that controls gene expression.

State the term that is used to describe a protein that controls gene expression.

transcription factors [1]

(c) Gel electrophoresis can be carried out to test individuals for the different versions of haemoglobin: HbA, HbS and HbF.

- A buffer with alkaline pH is used to make all haemoglobin molecules negatively charged.
- HbS molecules have an additional positive charge compared to HbA.

(i) Describe **and** explain how gel electrophoresis is used to diagnose sickle cell anaemia.

* apply potential difference across the gel
* the Hb molecules move towards the anode
* HbS travels a lesser distance due to less negative charge
* compare the positions of the bands with reference bands

[4]

(ii) Four individuals had their haemoglobin analysed by gel electrophoresis. One of the individuals was heterozygous for the Hb^A and Hb^S alleles and had a condition known as sickle cell trait (SCT).

Some of the results are shown in Fig. 4.1. In Fig. 4.1, lane 1 and lane 5 are complete.

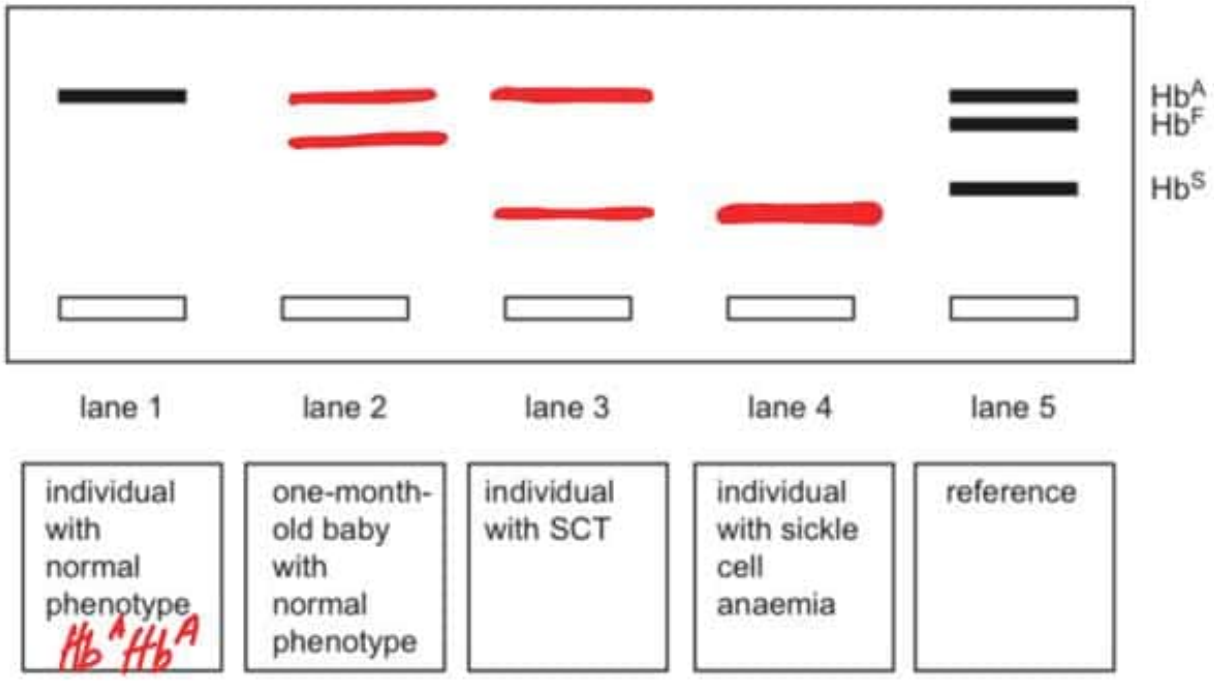
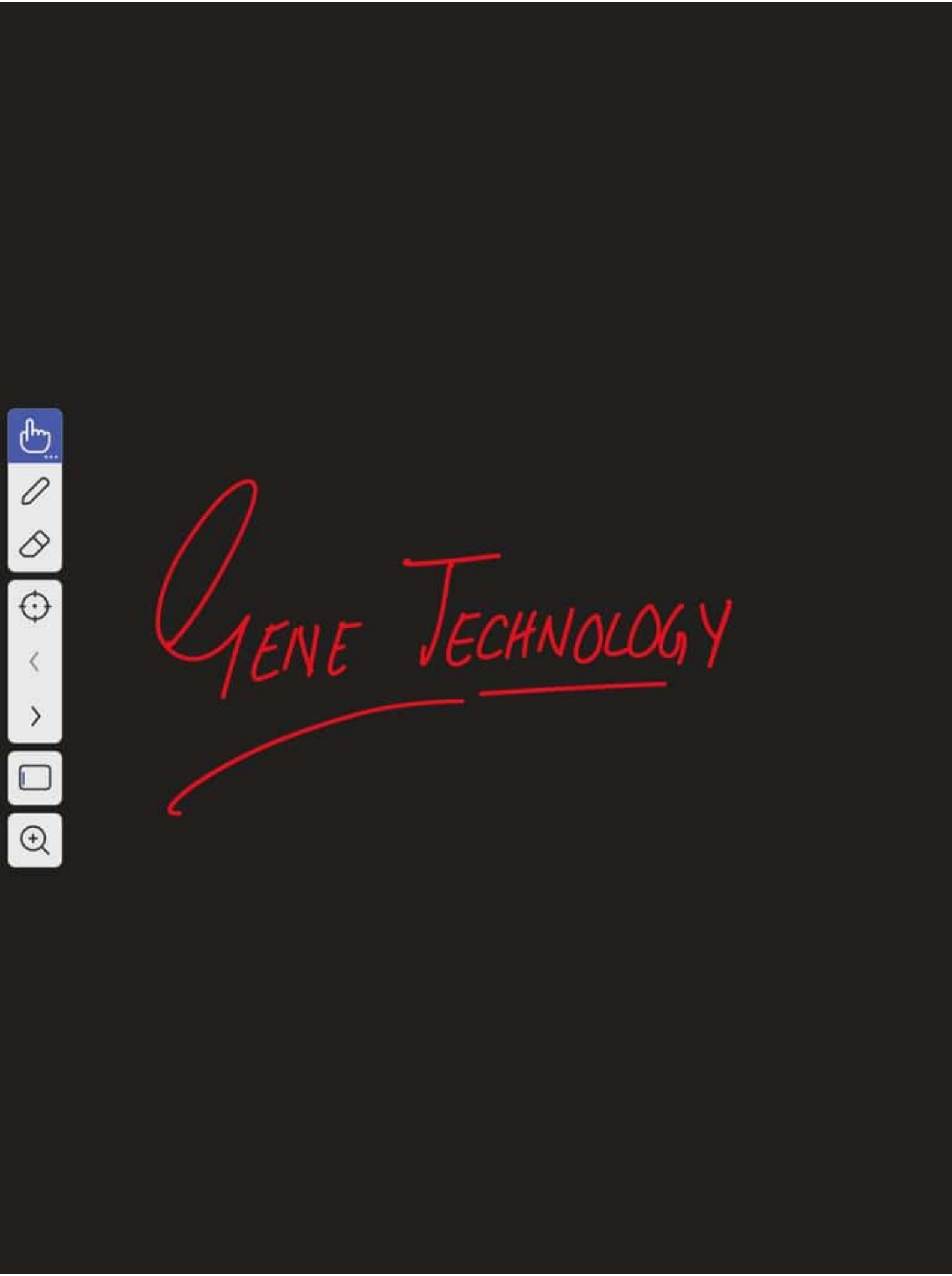


Fig. 4.1

Predict the results for the individuals analysed, by adding bands to lanes 2, 3 and 4 on Fig. 4.1. [2]


Gene Technology

Previously,

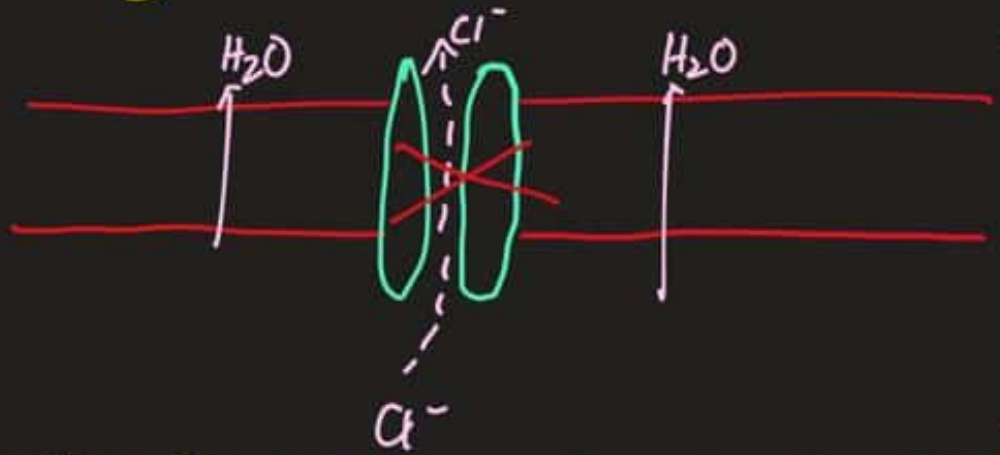
* gene technology & genetic engineering
* production of genetically engineered human insulin
* Marker genes to select transformed bacteria
* PCR
* Electrophoresis & it's application in,
  a. Hb electrophoresis
  b. DNA profiling

Cystic Fibrosis

* autosomal recessive
* homozygous recessive
* mutation of in the CFTR gene
* CFTR gene codes for CFTR protein

CFTR ≡ Cystic Fibrosis Transmembrane Regulator

* the gene mutation in CFTR gene can be → triplet deletion (508th) → phenylalanine
  → base substitution {nonsense mutation} → premature stop codon
* CFTR protein is a Cl^- channel → responsible for allowing Cl^- to leave the cell



* Mutation in CFTR gene prevents the CFTR protein from functioning normally → Cl^- fail to leave the cells → thickens the mucus lining
* CFTR protein is found in → resp. epithelium, pancreatic duct, vas deferens

Cystic Fibrosis

* It is an autosomal recessive disease.

* The gene that undergoes mutation is the CFTR gene.

* CFTR stands for Cystic Fibrosis Transmembrane Regulator.

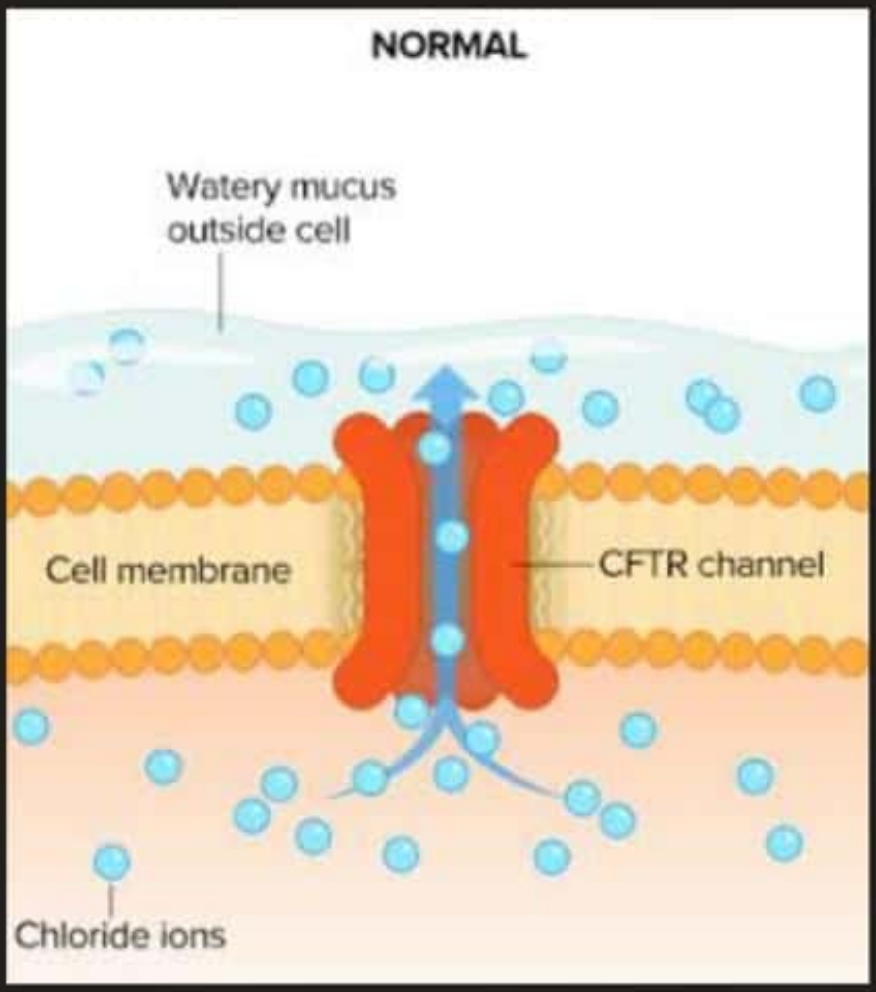
* The CFTR gene codes for the CFTR protein.

* CFTR is a chloride ion channel which controls the movement of chloride ions across the cell surface membrane.

* The CFTR gene is expressed in the cells lining tubular structures, such as;

a) cells lining the tracheal lumen
b) cells lining the pancreatic duct.
c) cells lining the vas deferens.

CFTR protein & it's role



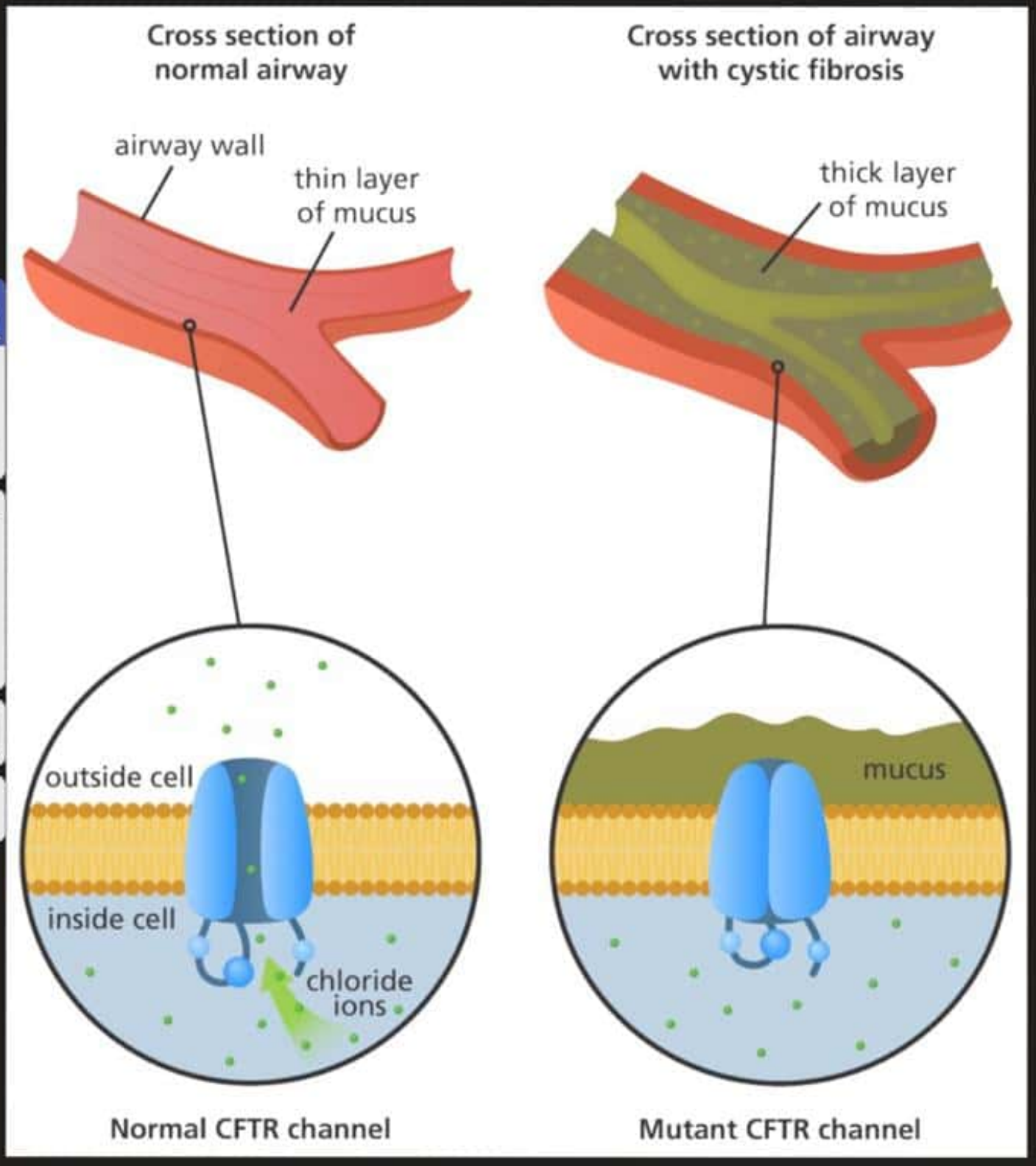
MUTATIONS IN CFTR GENE

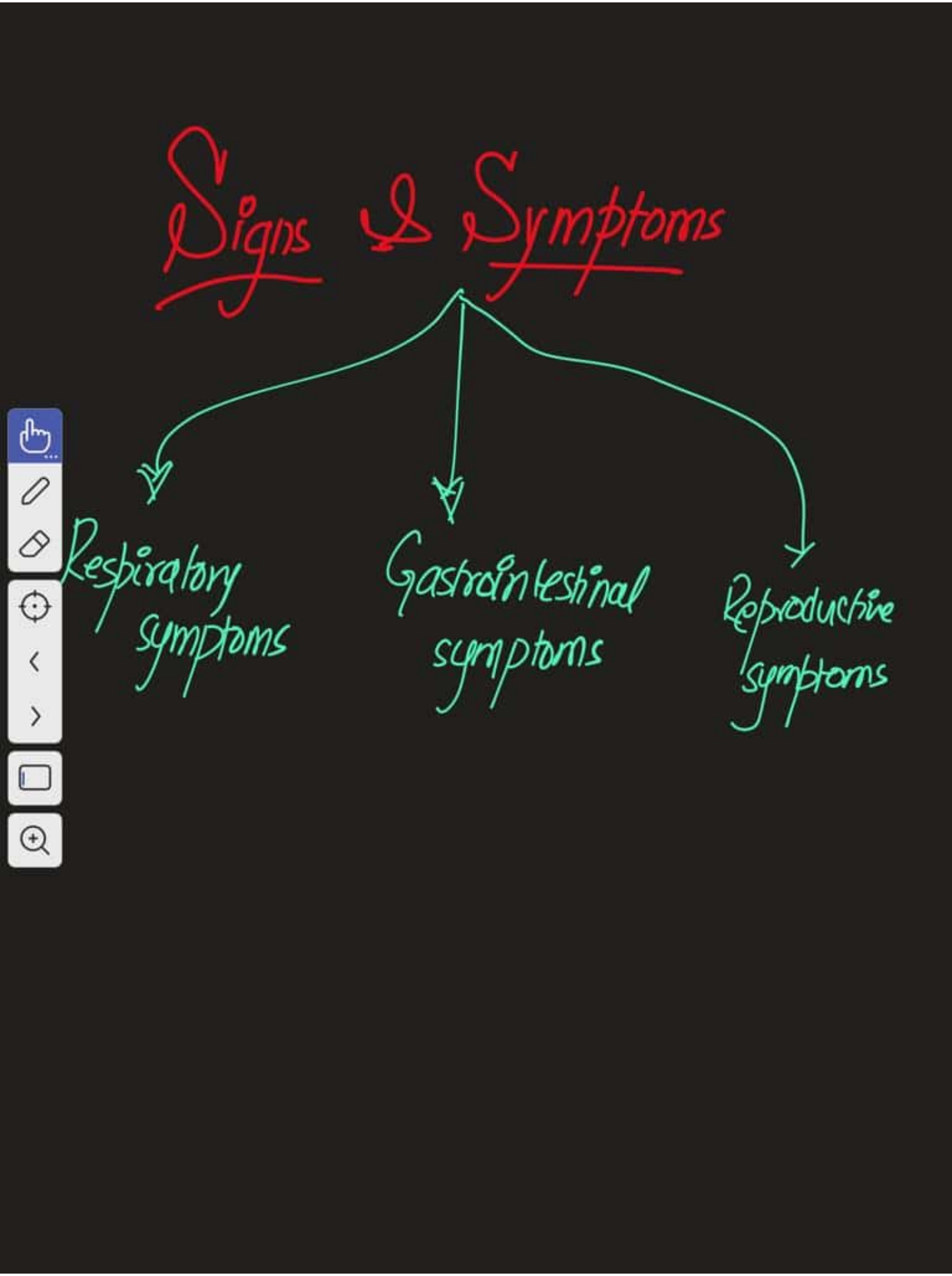
* The most common mutation causing cystic fibrosis is a triplet deletion at the 508th position which codes for the amino acid phenylalanine. This deletion is commonly referred to as the ΔF508.

Mutations other than triplet deletion may also cause cystic fibrosis, such as, a single base substitution which introduces a stop codon along the nucleotide sequence of the gene. Such a base substitution is referred to as non-sense mutation.

* The CFTR protein usually allows the chloride ions to leave the cell causing water to follow via osmosis. This causes the mucus lining the tubular structures to become runnier.

* Mutation in the CFTR gene reduces the transport of chloride ions through the ion channel, thereby causing the mucus to become more viscous.


Cross section of normal airway
airway wall
thin layer of mucus
outside cell
inside cell
chloride ions
Normal CFTR channel
Cross section of airway with cystic fibrosis
thick layer of mucus
mucus
Mutant CFTR channel


Signs & Symptoms
Respiratory symptoms
Gastrointestinal symptoms
Reproductive symptoms

* The signs & symptoms of cystic fibrosis can be subdivided into 3 main categories:

- Respiratory Symptoms
- GastroIntestinal Symptoms
- Reproductive Symptoms.

Respiratory Symptoms

* Which result due to thickening of the mucus lining the respiratory passages.
* Cilia fail to swap this mucus upwards leading to pooling of the mucus into the lower respiratory passages.
* This mucus contains entrapped dust & bacteria.
* Bacteria thrive on nutrition provided by the mucus and multiply in numbers to cause respiratory infections, such as, pneumonias.
* Pneumonia is the most common cause of death in patients with cystic fibrosis.

Gastrointestinal Symptoms

* Blockage of the pancreatic duct, thereby preventing the pancreatic enzymes from reaching the small intestine.
* These enzymes thereafter damage the pancreatic tissue, leading to inflammation of the pancreas, termed as pancreatitis.
* Absence of pancreatic enzymes within the small intestine leads to maldigestion and malabsorption leading to stunted growth & development.

* Lipid digestion is affected the most since pancreatic lipase initiates the lipid digestion in the gastrointestinal tract.

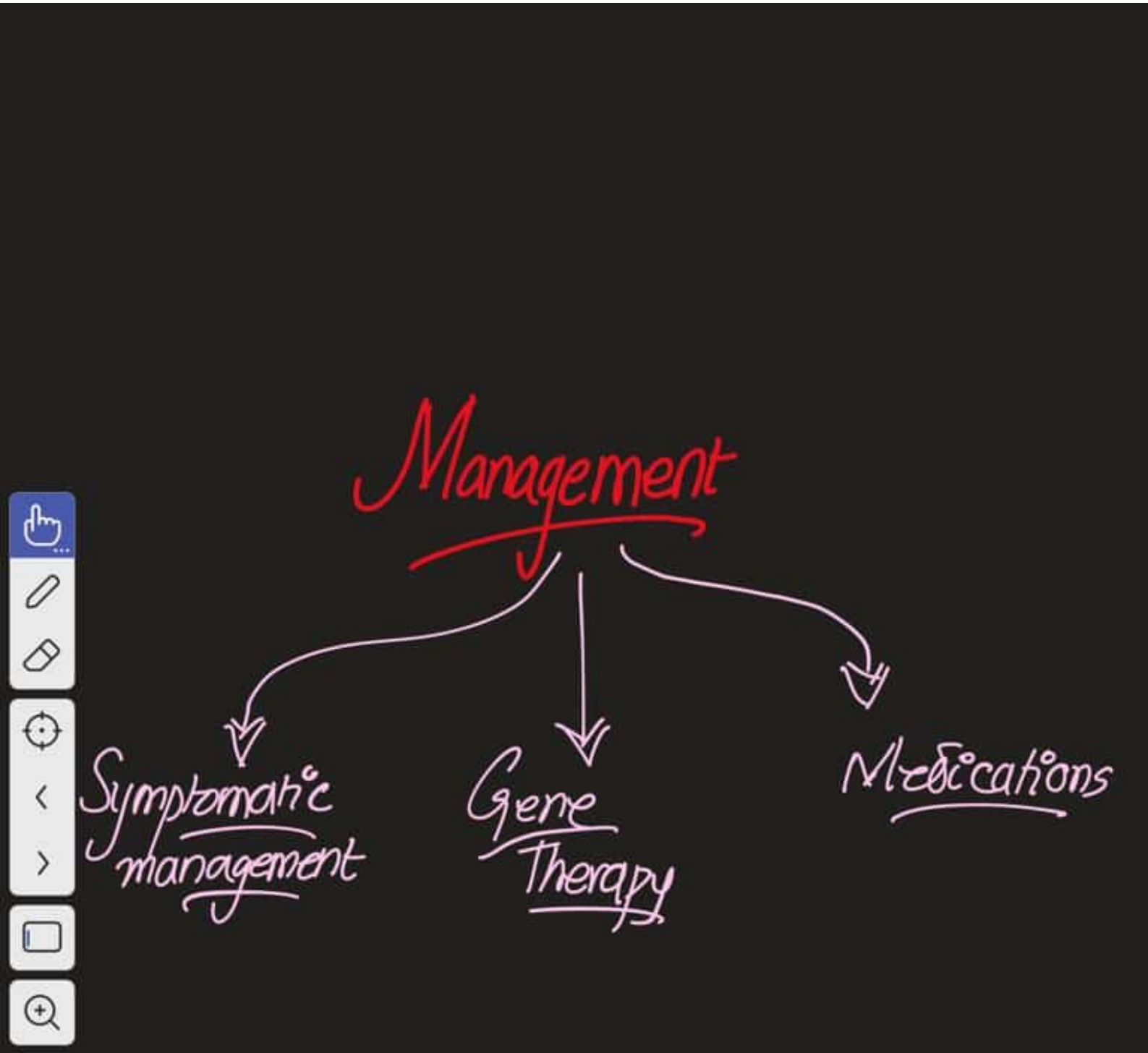
* Individuals with cystic fibrosis, therefore, pass out faeces with high lipid content.

Reproductive Symptoms

* Blockage of the vas deferens in males leading to sterility in males.

Q1:Explain how the faulty CFTR proteins in the cell surface membrane lead to symptoms of cystic fibrosis?

Ans: CFTR protein is a Cl^- channel which usually allows Cl to leave the cell. Water follows via osmosis . Faulty CFTR protein does NOT allow Cl ions to leave the cell. Less water thereafter leaves the cell via osmosis which causes mucus lining the passages to become thick. In lungs, the thickened mucus cannot be cleared by cilia and pools in lower respiratory passages narrowing the airways. This reduces the exchange of gases across the alveoli causing breathing problems. Accumulation of mucus also allows entrapped bacteria to thrive and cause respiratory infections (such as pneumonia). Blockage of the pancreatic duct causes maldigestion and malabsorption . Pancreatic enzymes cannot enter the intestine causing maldigestion. This leads to stunted growth and development. Blockage of the vas deferens leads to infertility in males.


Management
Symptomatic management
Gene Therapy
Medications

Management of Cystic Fibrosis

* Management of cystic fibrosis involves :

a) Symptomatic Management

b) Gene Therapy

c) Medication.

Symptomatic Management

Respiratory Symptoms

1. Prophylactic antibiotics
2. Suction along with percussion therapy
3. Bronchodilators
4. O_2 therapy
5. Mucolytic agents

Gastrointestinal Symptoms

1. Oral enzyme supplements
2. IV nutrition

Reproductive Symptoms

1. ARTs
   ↓
   eg IVF
   ICSI

a) Symptomatic Management :

* Symptomatic management is based on relieving signs and symptoms associated with cystic fibrosis :

i) Respiratory Symptoms :

→ prophylactic antibiotics are given to prevent the recurrance of lung infections.

→ Suction along with precussion therapy is advised to clear the airways of excessive mucus.

→ Bronchodilators are advised to dilate the airways.

→ Oxygen may be administered to ensure oxygenation of the tissues.

→ Mucolytic agents may be advised to break down excessive mucus in the airways.

ii) Gastrointestinal Symptoms:

→ Blockage of the pancreatic duct due to excessive mucus prevents the pancreatic enzymes from reaching the small intestine which thereafter leads to maldigestion & malabsorption.

& Oral enzyme supplements are therefore given to promote digestion within the small intestine.

→ Intravenous nutrition (parenteral) is advised if the patient cannot take oral enzyme supplements.

iii) Reproductive Symptoms

Male sterility is very common in patients with cystic fibrosis. These patients may be advised assisted reproductive techniques, such as, IVF (In Vitro Fertilisation) or ICSI (Intra Cytoplasmic Sperm Injection)

GENE THERAPY

* alteration of an organism's genotype to treat a genetic disorder
* only for recessive disorders
* two types: ① Germline gene therapy
② Somatic cell therapy

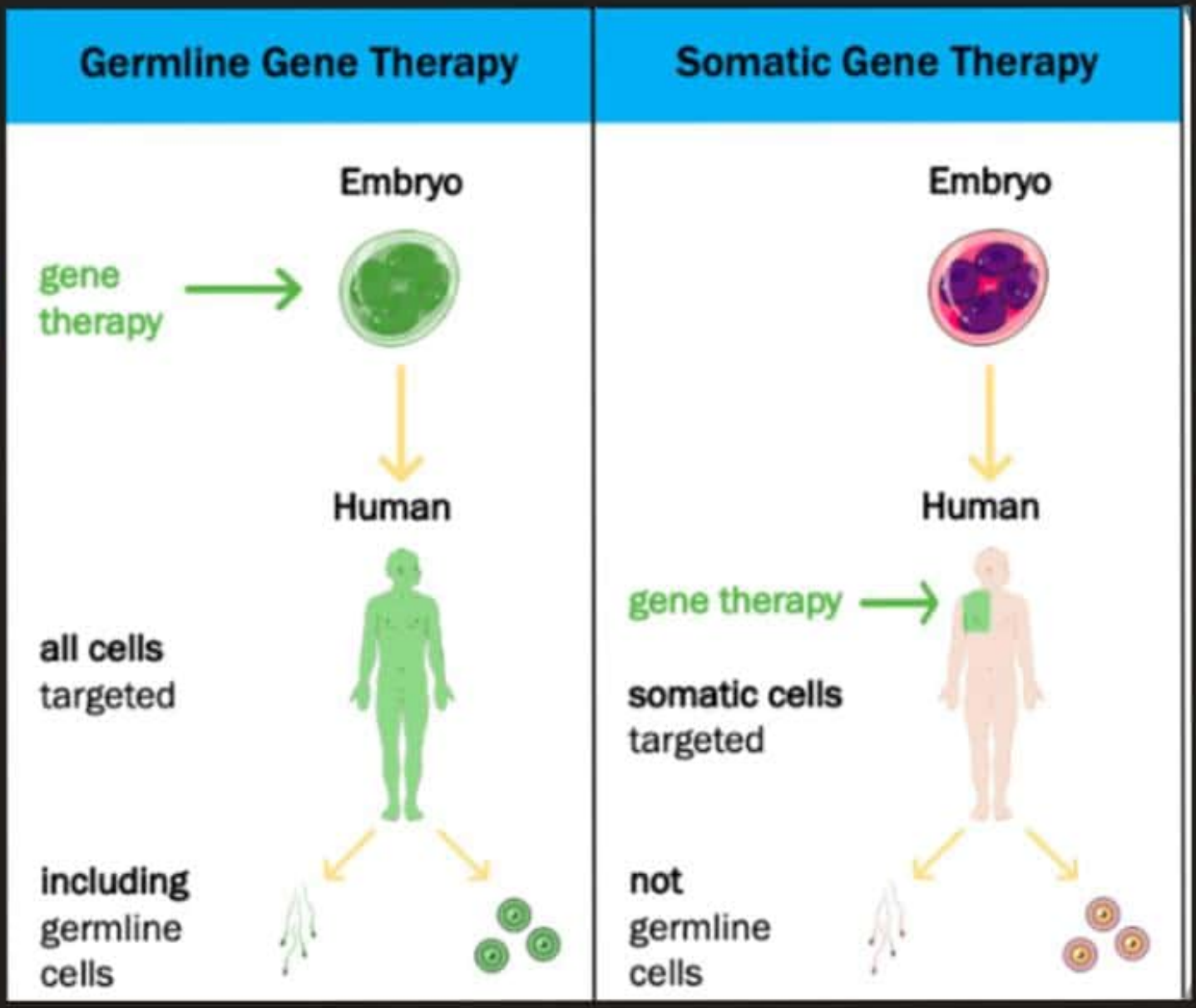
GENE THERAPY

* Gene therapy refers to alteration in the genotype of an individual to treat a genetic disorder.

* Gene therapy exists in two different forms:

i) Somatic Cell Therapy

ii) Germ Cell Therapy


Germline Gene Therapy
Embryo
gene therapy
Human
all cells targeted
including germline cells
Somatic Gene Therapy
Embryo
Human
gene therapy
somatic cells targeted
not germline cells

Somatic and Germ Cell Therapy

- Somatic cell therapy involves alteration of the genotype of a somatic cell that cannot divide by meiosis to form gametes.
- Germ cell therapy involves the introduction of the desired allele into an oocyte or a sperm cell which can then be fertilised in vitro to produce a zygote that contains the desired allele. This allele will then be transferred to all the cells which result due to mitotic divisions of the zygote.

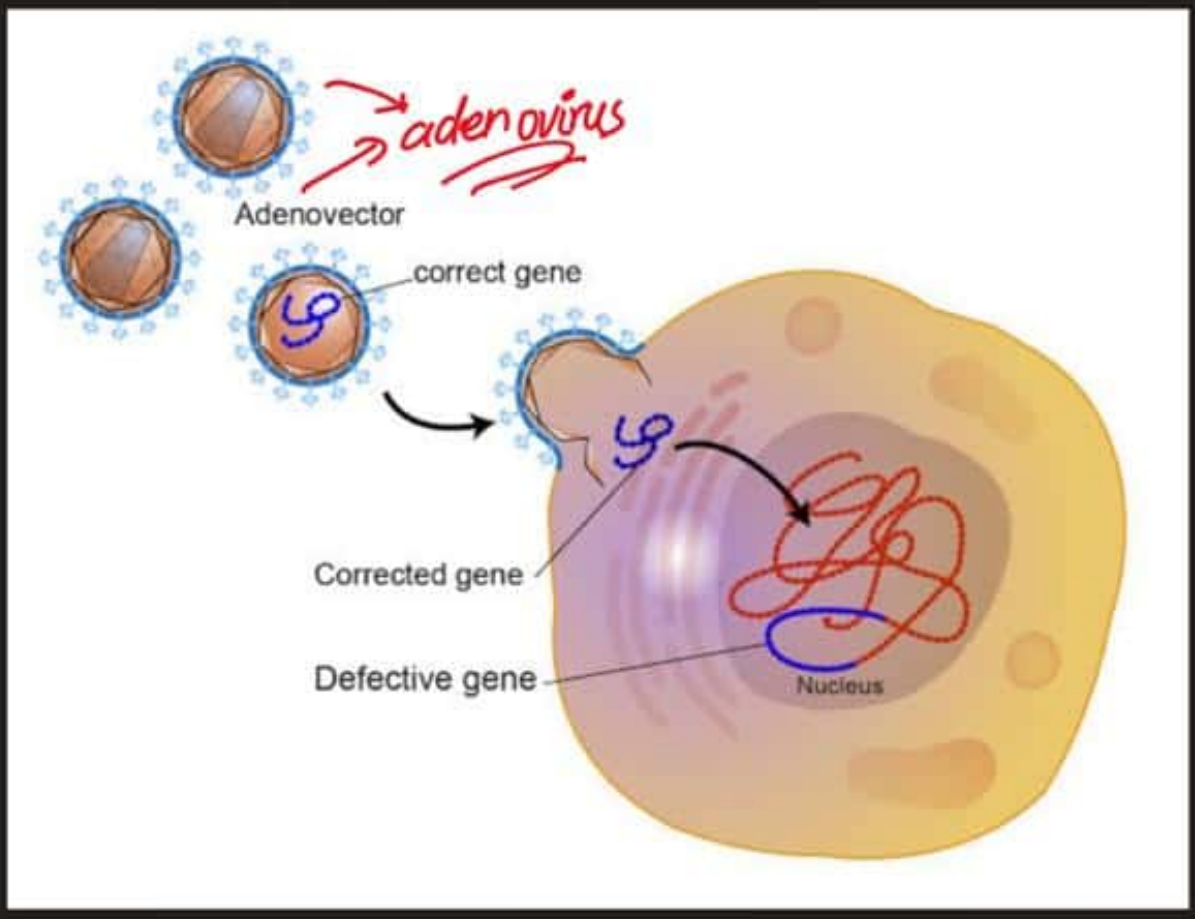
• Germ cell therapy is legally banned in many parts of the world due to ethical issues which may result due to introduction of desirable alleles into the 'germ line'.

Gene therapy for cystic fibrosis

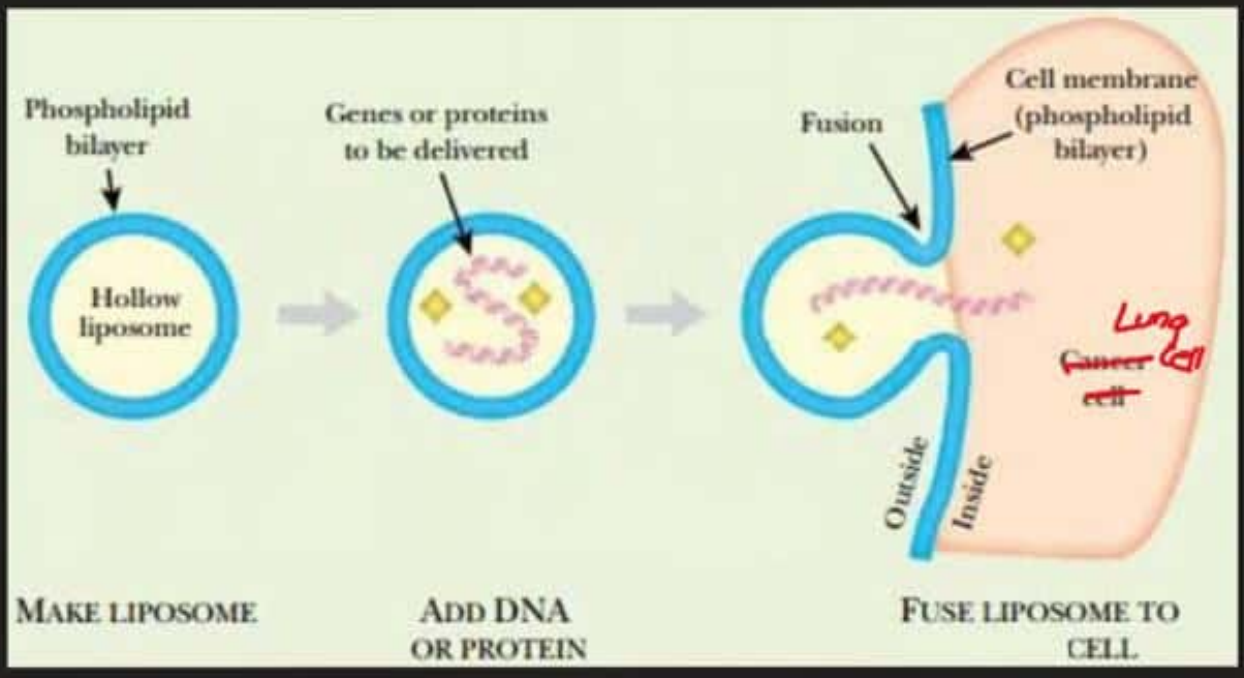
* Gene therapy to treat cystic fibrosis involves the use of viral or non-viral vectors to deliver the normal CFTR allele to the affected cells.

* Cystic fibrosis being a recessive disorder can be managed by delivering the dominant allele (normal) which will render the individual heterozygous.

Viral Vector



Non Viral Vector



i) Viral Delivery Systems

Viruses such as adeno viruses which normally infect the lung cells may be used as vectors to deliver the normal CFTR gene to the lung cells. These viruses may be inhaled in the form of aerosol droplets. The viruses enter the lung cells and integrate the dominant CFTR allele into the host cell genome.

Viral vectors are, however, not very effective because:

a) The individual may develop an immune response or an allergic response to the virus.

b) The effect of integrating the dominant allele is short-lived since the lung cells are replaced continuously

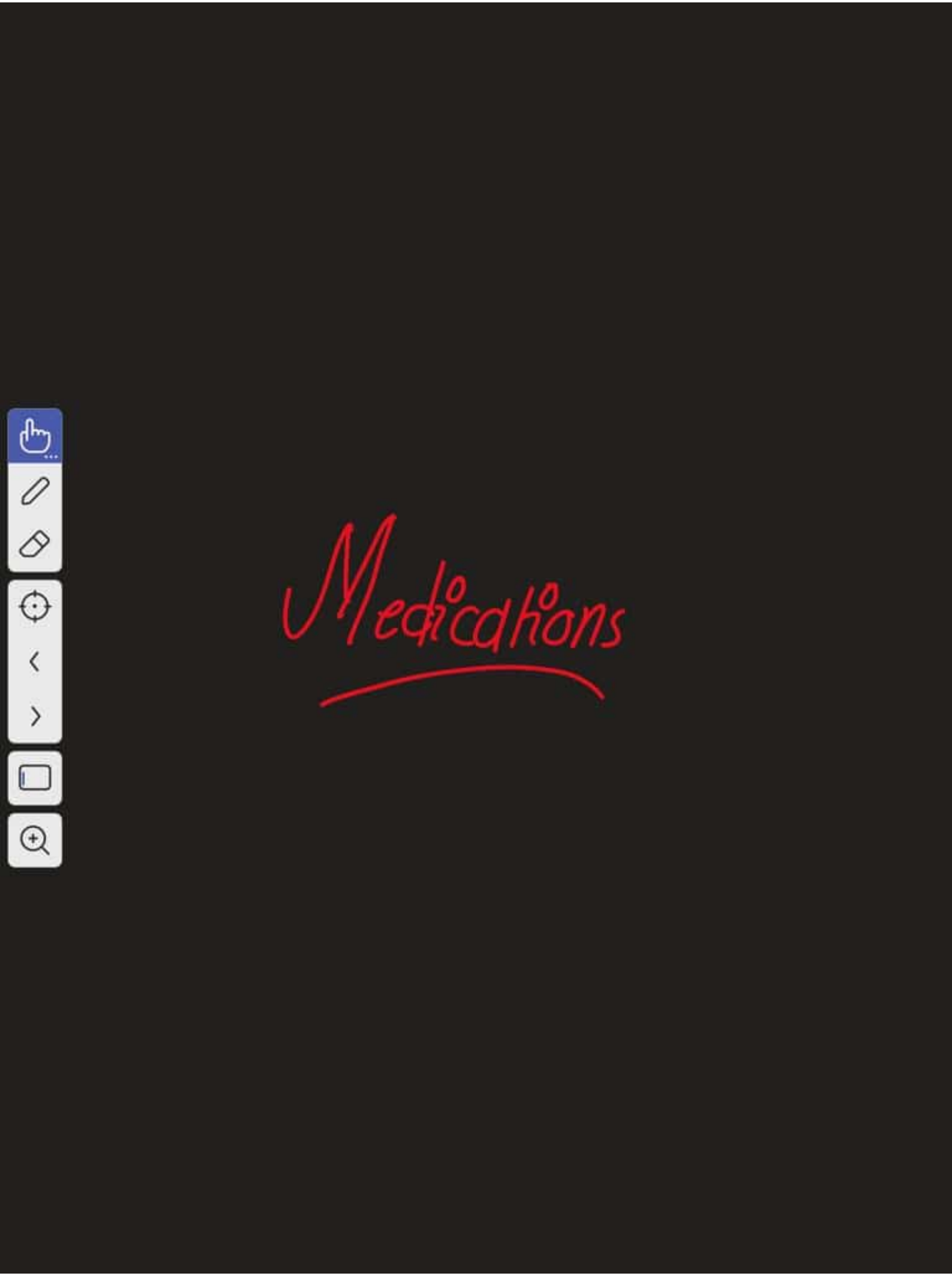
ii) Non-viral Delivery Systems:

The non-viral delivery systems include:

a) Lipid spheres known as liposomes - which are inhaled in the form of droplets.

b) Naked DNA compressed in a small volume.

None of non-viral delivery systems have been 100% effective in treating cystic fibrosis


Medications

c) Medication

* A form of cystic fibrosis results due to a base substitution which leads to formation of a stop codon, thereby, producing a non-functional polypeptide.

* The ribosomes are not able to complete translation due to introduction of this stop codon along the mRNA.

* Ataluren (PTC124) is a drug which is given as a pill a day to help patients with this form of cystic fibrosis.

* The drug works by enabling the ribosomes to skip the stop codon.

Gene Technology

Previously,

* gene technology & genetic engineering
* production of genetically engineered human insulin
* Marker genes to select transformed bacteria
* PCR
* Electrophoresis & it's application in,
  a. Hb electrophoresis
  b. DNA profiling
* Cystic Fibrosis → gene therapy

analysis of a person's DNA to detect a particular allele

GENETIC SCREENING

Screening individuals or couples for the carrier state ⇒ CARRIER Screening

PRENATAL SCREENING

NEWBORN SCREENING

GENETIC SCREENING

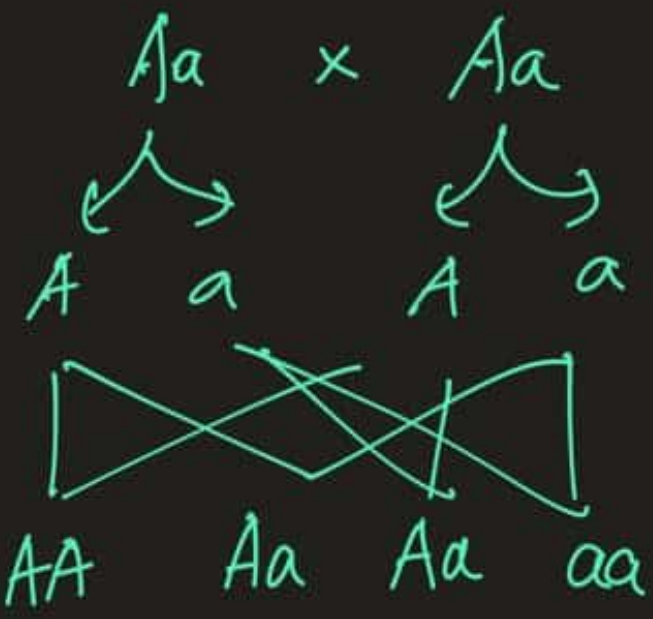
* Genetic screening refers to the analysis of a person's DNA to detect a particular allele.
* Genetic screening is of different types which can be summarised as below:

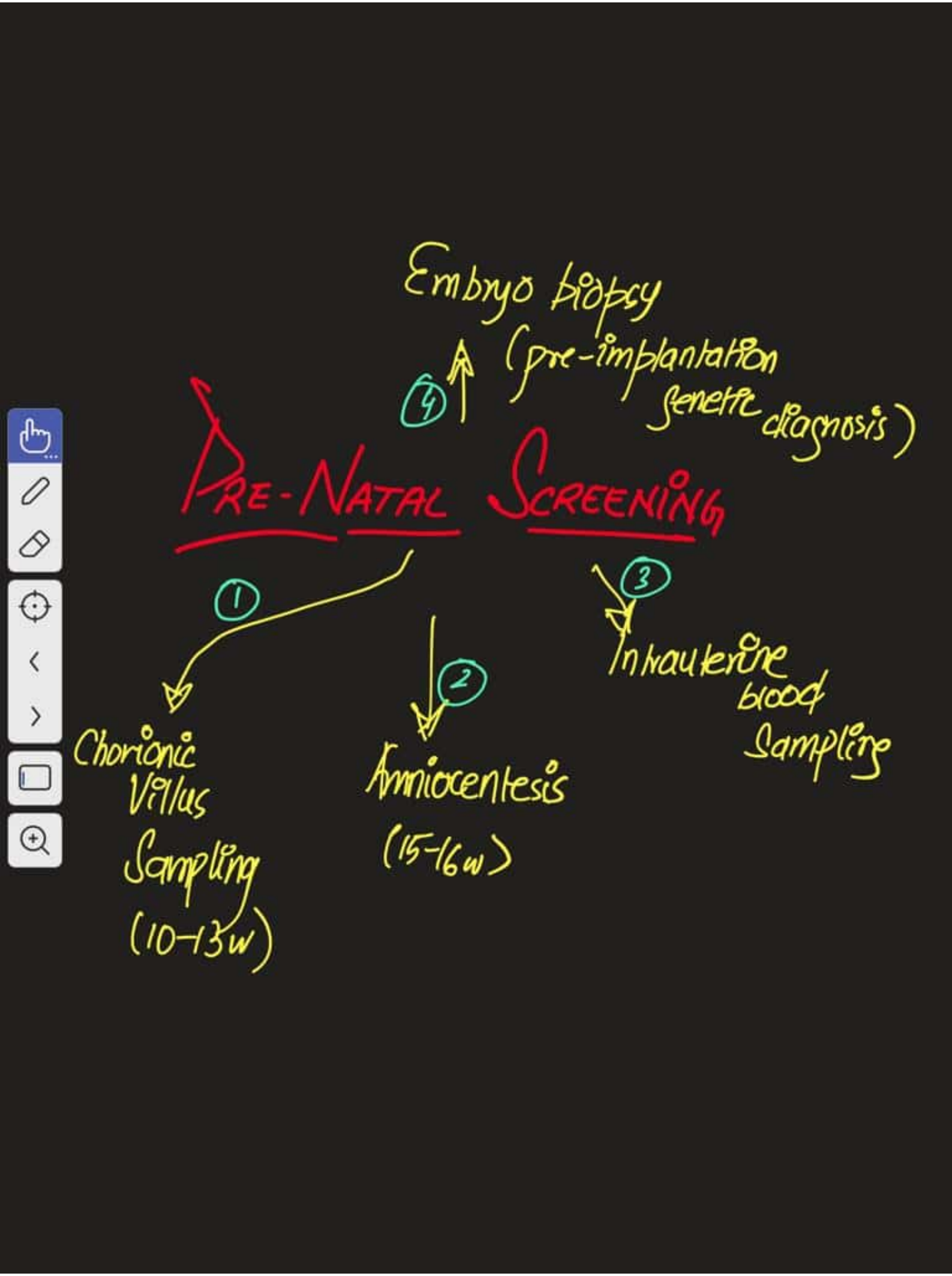
i) Carrier Screening for Individuals & Couples
ii) Pre-natal Screening
iii) New-Born Screening

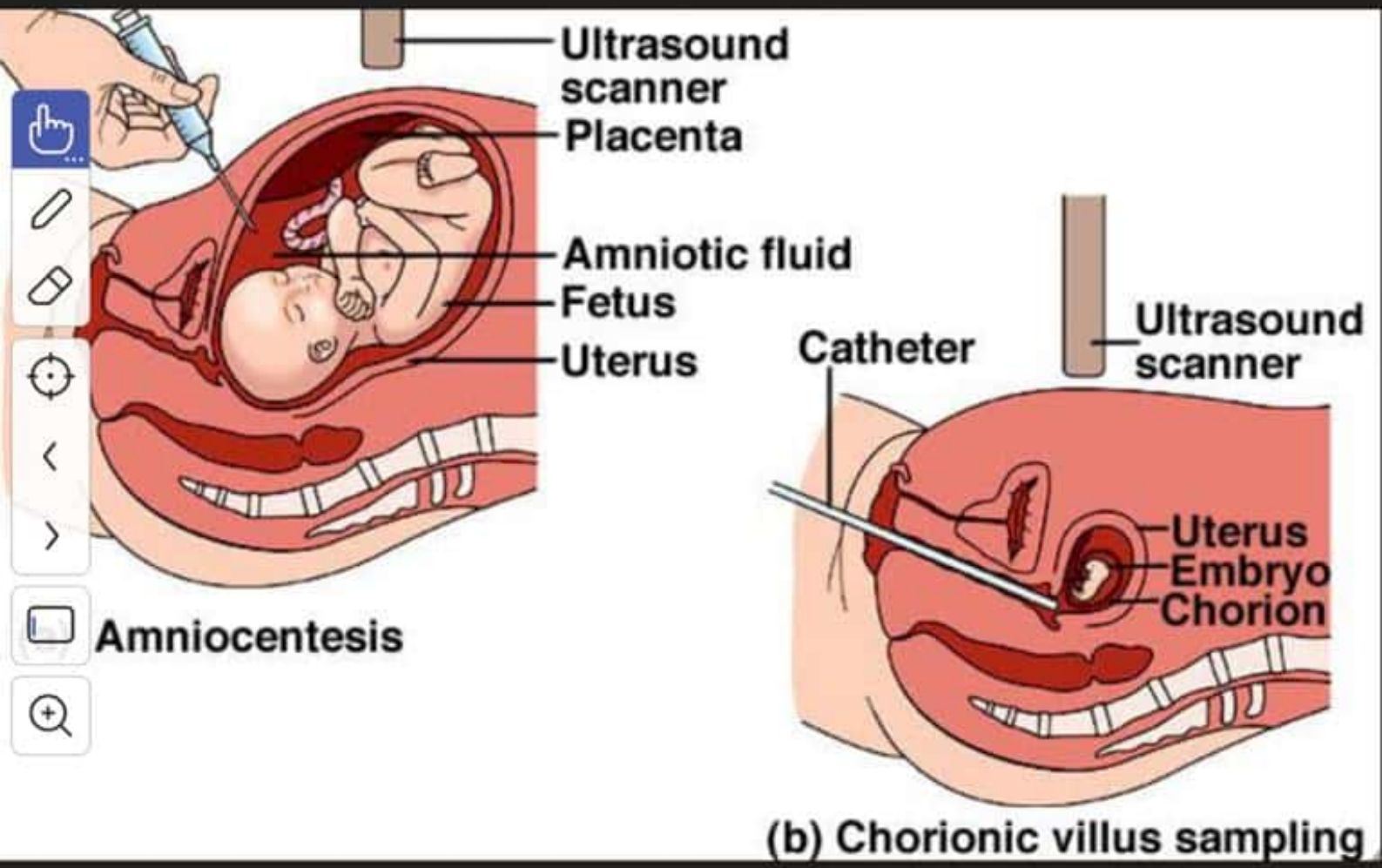
i) CARRIER SCREENING

* It involves screening for carrier state.
* Individuals who have a family history of genetic disorder, such as breast cancer or thalassemia are candidates for carrier screening, for example, screening for BRCA 1 and BRCA 2 genes in individuals who have a family history of breast cancer is important to ensure prompt management. If an individual is positive for these genes, such an individual may be advised elective (scheduled) mastectomy.

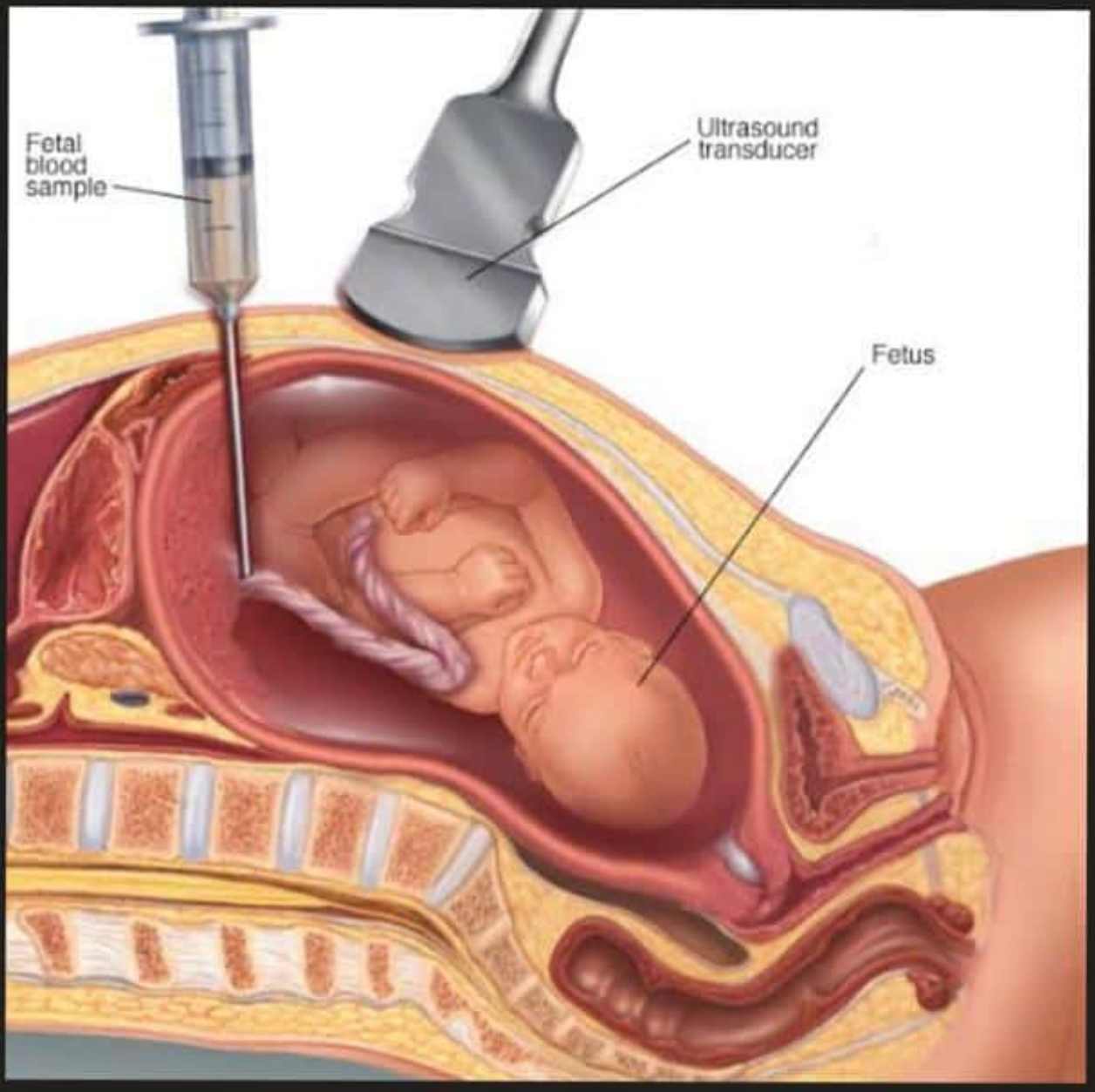
* Screening for carrier state in couples may be advised to determine the probability of transmitting the disease to their offspring. If both the parents are carriers for a particular disease, such as, sickle cell anaemia then there is a 25% chance that the child born will have sickle cell anaemia.




Embryo biopsy
(pre-implantation genetic diagnosis)
4
PRE-NATAL SCREENING
1
2
3
Chorionic Villus Sampling (10-13w)
Amniocentesis (15-16w)
Intrauterine blood Sampling


Ultrasound scanner
Placenta
Amniotic fluid
Fetus
Uterus
Amniocentesis
Catheter
Ultrasound scanner
Uterus
Embryo
Chorion
(b) Chorionic villus sampling

Intra-uterine blood sampling



ii) PRE-NATAL SCREENING

* Screening for genetic disorders prior To birth.

* Ways in which pre-natal screening can be carried out include:

a) Chorionic Villus sampling → which is carried out b/w 10-13 weeks of gestation (pregnancy). In this procedure, a part of the placental tissue known as the chorion is sampled & screened for genetic disorders.

b) Amniocentesis → which is usually carried out b/w 15-16 weeks of gestation. In this procedure, an ultrasound guided needle is used to sample out a small volume of the amniotic fluid. This fluid contains fetal cells which can be used to screen for genetic disorders.

) Intra-Uterine Blood Sampling → in which the fetal blood is sampled from umbilical vessels. The WBCs present within the blood sample are used to screen for genetic disorders.

d) Pre-Implantation Genetic Diagnosis (PIGD)

OR Embryo Biopsy → is a new technique which usually accompanies IVF. At the 8 cell stage a cell is removed from multiple embryos and screened for genetic disorders. Embryos with genetic disorders are discarded while others are transferred To the female uterus.

→ Pre-natal screening is usually carried out for the following genetic disorders:

a) Gene disorders, such as, sickle cell anaemia, albinism & Huntington's disease.

b) Chromosomal disorders, such as, Down's Syndrome & Turner's Syndrome

c) Neural tube defects, such as, the spina bifida and anencephaly (no brain present)

Newborn Screening

* Hypothyroidism
* Phenylketonuria (PKU)
  (phenylalanine hydroxylase)

iii) New-Born Screening

* It is usually carried out for the following genetic disorders:
    a) Phenyl kelonurla (PKU)
    b) Hypothyroidism
* Both PKU and hypothyroidism can lead to mental retardation if not managed properly
* PKU is managed by consuming a diet free of phenylalanine.
* Hypothyroidism is corrected by offering the hormone thyroxine.

Advantages of genetic screening

ADVANTAGES OF GENETIC SCREENING

1. Provides information about the increased risk of people having genetic conditions.
2. It identifies carriers of any condition.
3. It allows couples who are both carriers of a genetic condition to make decisions about starting a family.
4. It allows parents to prepare for the birth of a child who will need treatment for a considerable time after birth or even lifetime.
5. It allows people to prepare for late onset genetic conditions, such as Huntington's chorea.

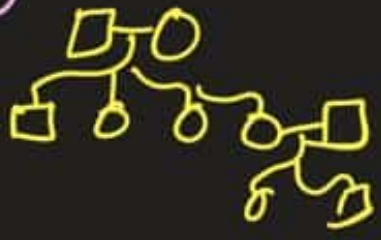
6- The genetic tests can identify whether the embryos produced by IVF or the ones that develop in the uterus are going to develop a genetic condition.

Genetic Counseling

* medical service offered to discuss
* the nature of a genetic disorder
* and the probability of transmitting it to future generations

* people who offer genetic counselling are termed as genetic counselors.

- may advise genetic screening
- construct pedigrees
- discuss the results of screening



GENETIC COUNSELLING

* It is a medical service to determine the nature of the genetic disease and its probability of transmission to future generations.
* Individuals who offer genetic counselling are termed as genetic counsellors.
* Genetic counsellors carry out pedigree analysis.
* They may advise genetic screening based on the family history of an individual or a couple.

* Genetic counsellors explain the results of the genetic screening tests and offer advise based on the risk of transmission of the disease.

* In cases where the genetic screening of the embryo indicates that there is a genetic disorder, a genetic counsellor may advise on termination of pregnancy as well as the ethical and financial implications of having an affected child.

* If a couple's test is positive for a particular allele that causes a genetic disease, the genetic counsellor explains how the disease is transmitted and may advise IVF to check for the genetic disorders in the embryo prior to implantation.

Screening & Counselling for specific cases:

A. THALASSEMIA

* Thalassemia is a blood disorder characterised by production of abnormal haemoglobin.
* It is an autosomal recessive disorder.
* Thalassemia are of two types: alpha- and beta- depending upon which globin chains are primarily affected.
* Unlike sickle cell anaemia, thalassemia is not specific to beta-globin chains.
* Genetic counselling has decreased the incidence of thalassemia over the years.

* Couples identified as carriers of the mutant allele are counseled.

* Couples are advised on the possibility of terminating pregnancy if the felus has thalassemia. This is known as therapeutic abortion.

B. Huntington's Disease

* Huntington's chorea is an autosomal dominant disorder.

* It usually develops in middle age.

* Genetic screening for huntington allele poses ethical issues:

① Nothing can be done to manage the disorder if the person tests positive for the Huntington's allele.

② The person may not develop the disorder throughout his life despite the abnormal allele.

Genetically Modified Organisms (GMOs)

transformed organisms

transgenic organisms

GMOs

GM crop plants

* ① Vitamin A enriched rice (Golden rice)
* ② Herbicid resistant oil seed rape plant + tobacco plant
* ③ Bt modified maize and cotton
④ Flavr Savr tomatoes

GM animals

* ① GM salmon
② GM mosquitoes
③ GM glow in the dark animals

Genetically Modified Organisms (GMOs)

* GMOs are organisms whose genotype & hence protein expression has been altered via genetic engineering techniques.

GMOs include both transformed organisms & transgenic organisms. An organism is said to be "transformed" if it takes up the foreign gene via genetic engineering. An organism is said to be "transgenic" if it expresses the gene (& hence the phenotype) following transformation.

* Thus, all transgenic organisms are transformed but all transformed organisms may NOT be transgenic.

* GMOs are now commercially produced on a large scale for various reasons.

* While GMOs have their own importance, their synthesis have social and ethical implications. Let's discuss a few examples of GMOs and their importance before discussing these social and ethical implications.

Examples of GMOs can be classified into :

1- Genetically modified crop plants

a) Vitamin A enriched rice (Golden Rice)

b) Herbicide Resistant oil seed rape plants & tobacco plants .

c) Bt modified cotton plant & maize plants

d) Flavr Savr tomatoes.

2. Genetically Modified Animals

a) GM salmon (Aqu advantage)

b) GM insects

c) GM glow in the dark animals

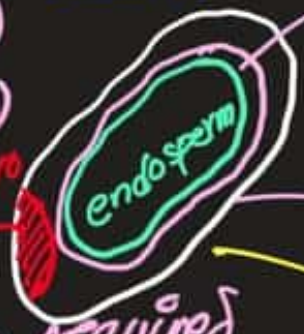
d) GM chickens

e) GM mosquitoes

Vitamin A enriched rice (GOLDEN RICE)

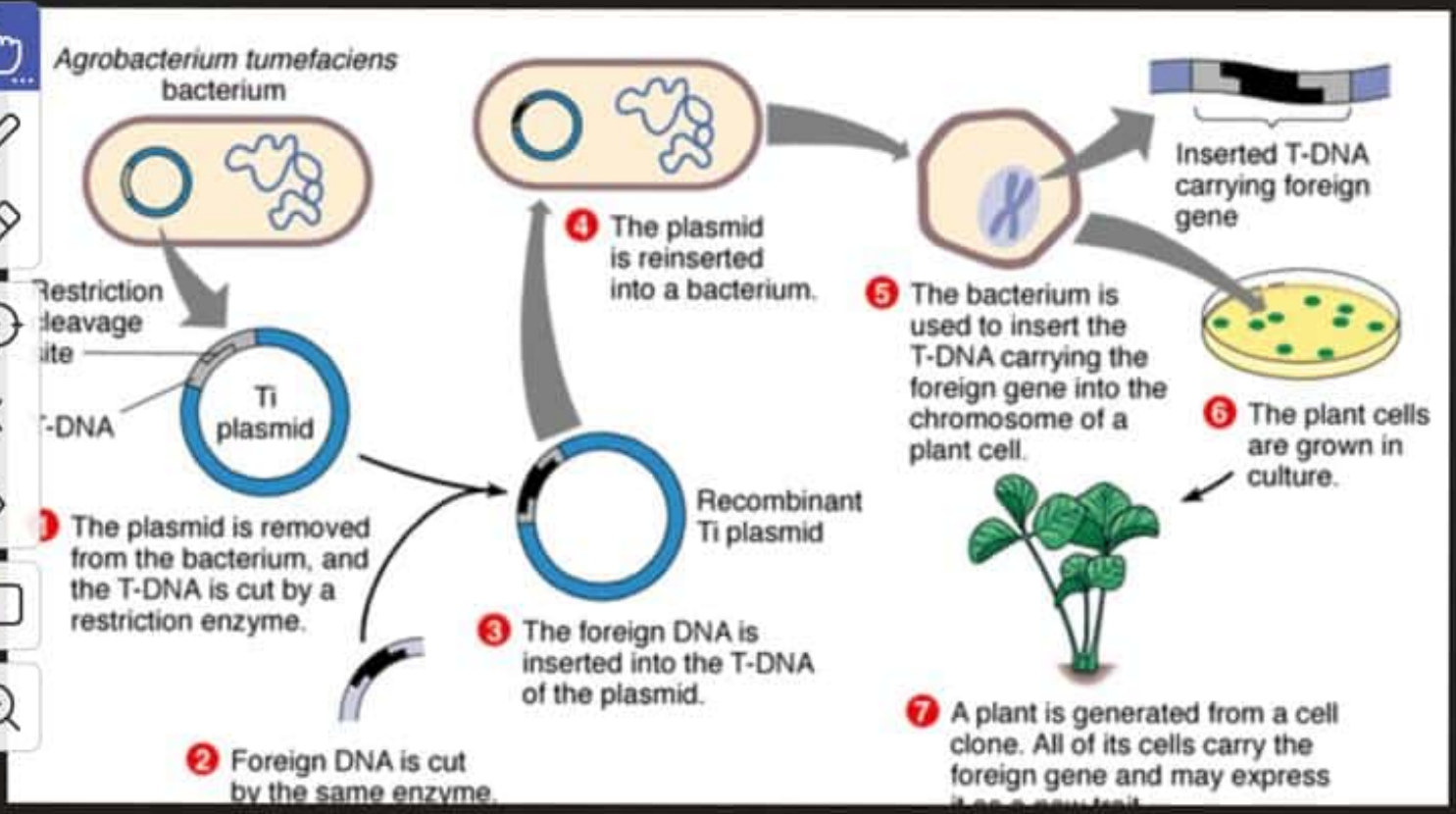
these genes were inserted into the plasmids of *A. tumefaciens* ↑ Ti plasmid

① psy gene (Daffodil)
② crt I gene (bacterium)
↑



two relevant genes required for synthesis of β carotene were identified and extracted
↑
GM of white rice aimed at producing β-carotene in the endosperm
↑
but can be stored for a longer period of time
↑
which is a poor source of β-carotene
↑
this produces WHITE RICE

⇒ BROWN RICE has the aleurone layer which contains β-carotene
↓
β-carotene can be converted into vit A in our body which is essential for normal vision and immunity
↓
Brown rice does NOT have a long shelf life
↓
a problem which is averted by removing the aleurone layer through POLISHING


Agrobacterium tumefaciens bacterium
Restriction cleavage site
T-DNA
Ti plasmid
1 The plasmid is removed from the bacterium, and the T-DNA is cut by a restriction enzyme.
2 Foreign DNA is cut by the same enzyme.
3 The foreign DNA is inserted into the T-DNA of the plasmid.
Recombinant Ti plasmid
4 The plasmid is reinserted into a bacterium.
5 The bacterium is used to insert the T-DNA carrying the foreign gene into the chromosome of a plant cell.
Inserted T-DNA carrying foreign gene
6 The plant cells are grown in culture.
7 A plant is generated from a cell clone. All of its cells carry the foreign gene and may express it as a new trait.

Vitamin A ENHANCED GOLDEN RICE

* Rice contains aleurone layer which is rich in proteins and certain fats.
* These fats provide a medium for storing β-carotene which is a precursor for vitamin A.

* Brown rice contains aleurone layer & is therefore rich in β-carolēne.

* Brown rice cannot be slōred for a long period of time due To The presence of aleurone layer which is thereafter removed by polishing brown rice To produce whiTe rice.

White rice can be stored for a long period of time but serves as a poor source of β-carotene. White rice can therefore be genetically modified to enhance the β-carotene content within their endosperm.

*The main aim is To provide vitamin A To third world, developing countries where malnutrition and vitamin A deficiency is common.

* The genes required for biosynthesis of β-carolene is extracted from a Daffodil (psy gene) & a bacterium (crtI gene)

* Once extracted, these genes are introduced into the Ti plasmid (Tumor Inducing plasmids) of the bacterium Agrobacterium Tumefaceins along with their promoters

* These bacteria naturally infect the embryo of the rice plants.

* Mixing rice plant embryos with Agrobacter.
ium Jumefaceins will enable the uptake of
genes by the rice plant cells.
* The bacterium and Ti plasmid serve as
the vectors in the process.

* When these embryos grow into a plant, they produce seeds that have an endosperm rich in β-carotene.

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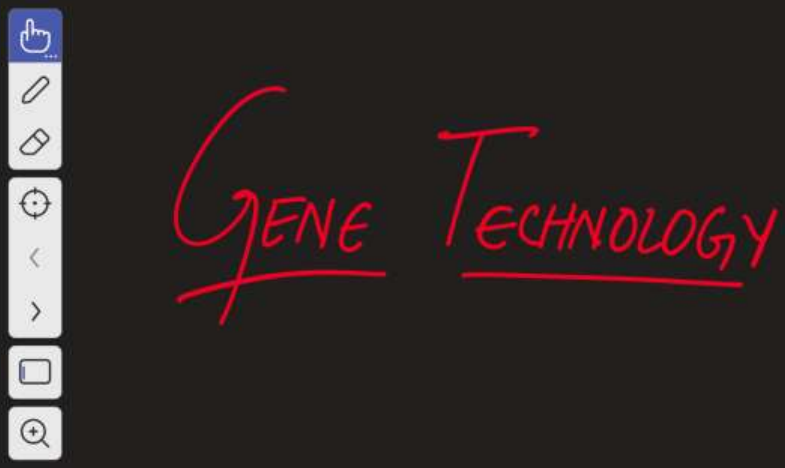
Gene Technology



With
Mohammad Hussham Arshad, MD

HEUSER

ADVANCED LEVEL BIOLOGY 9700

Learning Objectives:

- Genetically Modified Organisms (GMOs) II

Video Lecture 8 Slides

Mohammad Hussham Arshad, MD
Biology Department

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Gene Technology

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Previously,

* gene technology & genetic engineering
* production of genetically engineered human insulin
* Marker genes to select transformed bacteria
* PCR
* Electrophoresis & it's application in,
  a. Hb electrophoresis
  b. DNA profiling
* Cystic Fibrosis → gene therapy
* Genetically modified organisms (GMOs)

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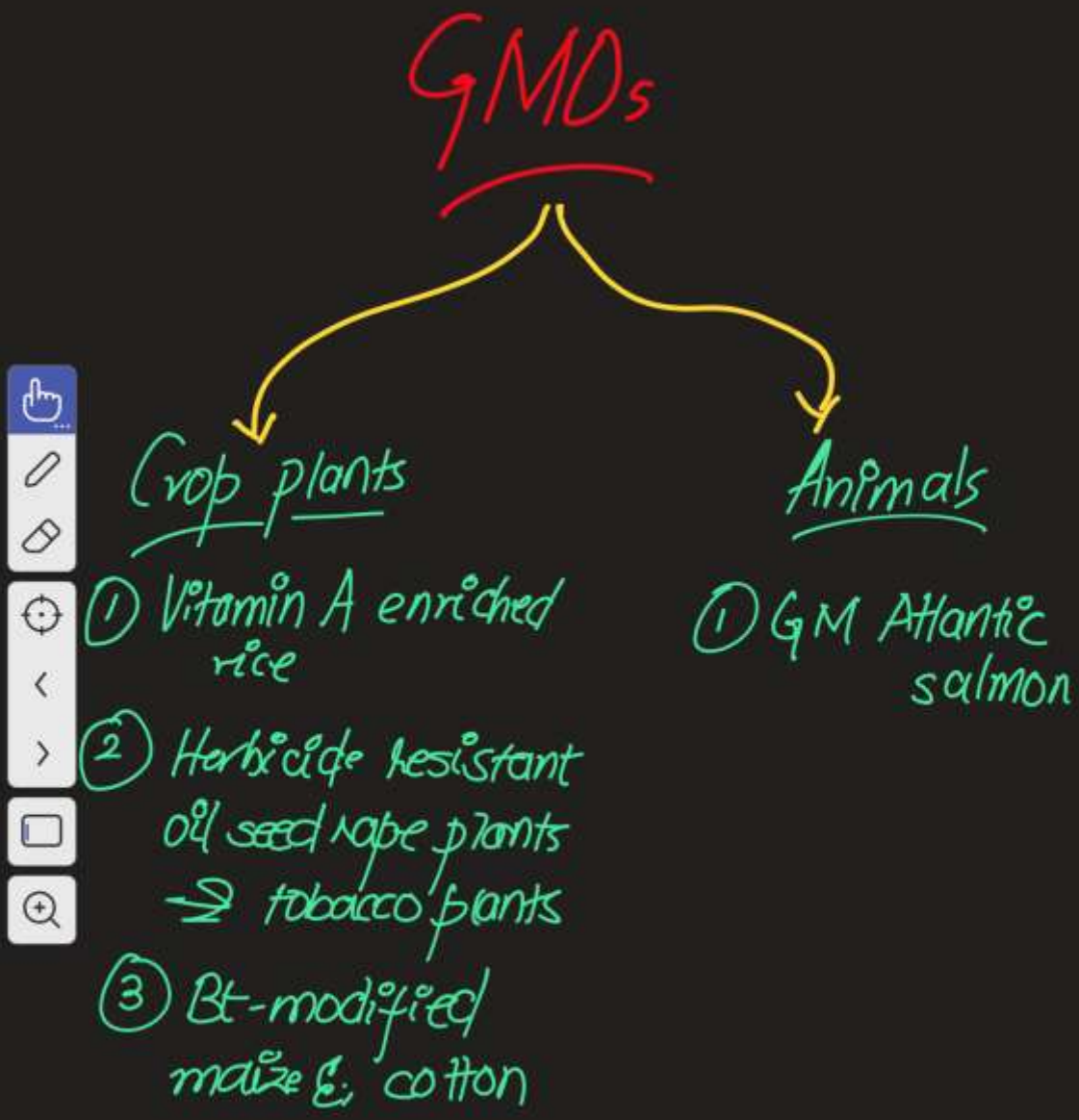
GMOs
Crop plants
1) Vitamin A enriched rice
2) Herbicide resistant oil seed rape plants → tobacco plants
3) Bt-modified maize & cotton
Animals
1) GM Atlantic salmon

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Vitamin A enriched rice (GOLDEN RICE)

these genes were inserted into the plasmids of *A. tumefaciens*
↳ Ti plasmid

rice grain

① psy gene (Daffodil)
② crt I gene (bacterium)

two relevant genes required for synthesis of β carotene were identified and extracted

⇒ BROWN RICE has the aleurone layer which contains β-carotene

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but can be stored for a longer period of time

which is a poor source of β-carotene

this produces WHITE RICE

β-carotene can be converted into vit A in our body which is essential for normal vision and immunity

Brown rice does NOT have a long shelf life

a problem which is averted by removing the aleurone layer through POLISHING

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ADVANTAGES & DISADVANTAGES OF G·M· RICE

1. The production of β-carolēne in endosperm can happen due to genes added via genetic engineering.
2. β-carolēne is needed to make vitamin A, which helps with eyesight.
3. can be used in areas where vitamin A deficiency is common so it helps to prevent blindness

1. The amount of golden rice needed for sufficient vitamin A intake may be too great.
2. spreading, 'escaping' or crossing of genes from genetically modified crops. This could create unwanted plants (weeds).
3. food from GM plants may harm people.
4. seed for GM plants can b expensive.

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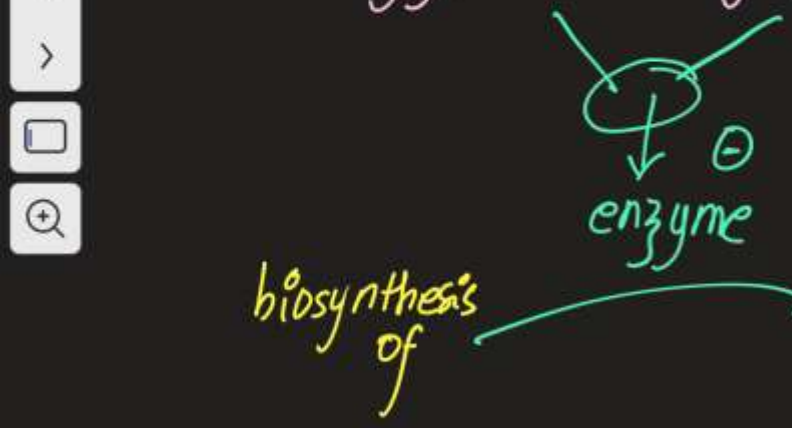
Herbicide Resistant Oil Seed Rape Plant & Tobacco Plant

* Weeds grow along oil seed rape plants and compete for soil resources thereby
* making the use of herbicides necessary
* herbicide sprays contain; glyphosate & glufosinate



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HERBICIDE RESISTANT OIL SEED RAPE PLANT

* Oil seed rape plants are grown very commonly in many parts of the world.
* Weeds grow along with these plants and compete for soil resources such as soil water, oxygen, mineral ions and nutrients. This reduces the yield of oil seed rape plants.
* Herbicide sprays are therefore used to kill these weeds which unfortunately affect the oil seed rape plant.

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* Production of herbicide resistant oil seed rape plant via genetic modification can therefore tackle the problem by killing the weeds without affecting the oil seed rape plant in the presence of herbicide sprays.
* The oil produced from the seeds of these plants is used for the following purposes:
  - as a biofuel
  - as a lubricant
  - household for human consumption.

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* The main component of herbicide sprays are the chemicals glyphosate & Glufosinate which competitively inhibit an enzyme involved in the biosynthesis of the amino acids phenylalanine, tyrosine & Tryptophan.
* These amino acids are essential for the growth of plants.
* Certain microorganisms produce an enzyme that is not affected by the herbicide glyphosphate and glufosinate.
* One such microorganism is the bacterium Agrobacterium Tumefaceins.

* The gene for this enzyme is therefore extracted from the bacterium and generally engineered into the seedlings of oil seed rape plants.

* These seedlings are grown in tissue culture to produce genetically engineered oil seed rape plants

Additional info .....

* The oil produced contains toxic chemicals, such as, Erucic Acid. A genetically modified train of oil seed rape plants produced in Canada is low in its acid content & is commonly sold as the 'CANola oil'.

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Environmental consequences....

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ENVIRONMENTAL CONSEQUENCES OF PRODUCING HERBICIDE RESISTANT OIL SEED RAPE PLANTS

- Evolution of herbicide resistant weeds.
- Transfer of pollen grains containing herbicide resistant genes to the wild relatives of the oil seed rape plants. This problem can be tackled by planting these crops at a safe distance from its wild relatives (200 m)
- The transformation of herbicide resistant oil seed rape plants into agricultural weeds.
- It may lead to reduction in genetic biodiversity over a long period of time.

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GM Tobacco Plants

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PRODUCING GENETICALLY MODIFIED HERBICIDE RESISTANT TOBACCO PLANTS.

* Genetically modified tobacco plants contain genes which confer resistance to herbicide containing sulfonylureas.

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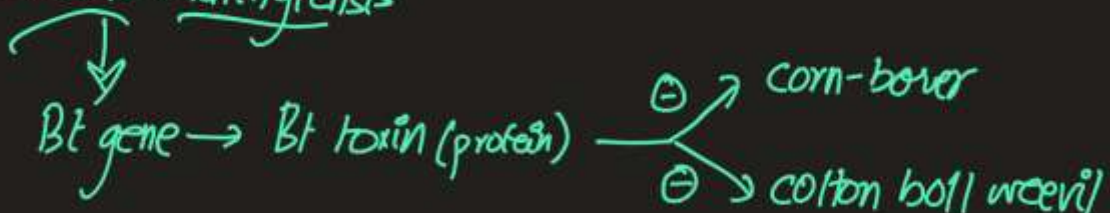
Bt-modified maize & cotton

* corn-borers and cotton boll weevils are insects that feed on these crop plants thereby reducing their yield
* This makes the use of insecticides necessary which
* kills the non-target insect species as well thereby reducing biodiversity

Bacillus thuringiensis



Genetically Modified Maize & Cotton Plants

* The yields of maize and cotton plants are significantly affected by pests, such as, corn borer (maize) & cotton boll weevil (cotton).
* These insects lower the yield by feeding on these crop plants, thereby, making it necessary to use insecticides.
* Use of insecticides has environmental consequences, such as reduction in biodiversity due to their effect on non-target insect species.

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* Genetic modification of maize & cotton plant to make them resistant to insects can therefore help cut down the use of insecticides.
- A particular toxin known as the Bt Toxin is potentially lethal for these insects.
- Maize & cotton has been genetically modified to produce this Bt toxin by inserting the gene for this toxin into these plants.

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* The gene for Bt toxin is extracted from the bacterium **Bacillus Thuringiensis**.
* This toxin is lethal to insects but harmless to humans
* The toxin requires an **alkaline pH** for activation and gets inactivated by the acidic pH in the human stomach.
* Genetic modification ensures that only the insects that feed on these plants are affected while other non-target species stay un-affected.

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Environmental Impact of GM Maize & Cotton

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* The adverse environmental consequences of producing Bt modified crop plants include:

- Evolution of Bt resistant insect stains.
- Transfer of pollen grains containing the gene for Bt Toxin To other wild plant species, for example, planting Bt-modified maize in Mexico has a potential risk of transferring the Bt Toxin gene To a wild relative of a maize plant, Teosinte.
- Food chain may be affected due To reduction in the no. of corn borers & cotton boll weevils.
- Reduction in biodiversity.

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* Environmental advantages of producing Bt modified crops include:

- Decrease in the use of insecticides.
- Non-target species are not affected because only those insects which feed on these crop plants die due to the toxin.

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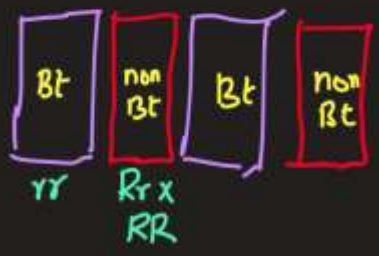
Strategies to counteract environmental consequences of Bt modified crops

evolution of Bt resistant insects can be minimised via HDR (high-dose refuge) strategy

- resistant insects (rr)
- non resistant (Rr or RR)



Safe Planting distance to avoid the transfer of pollen grains to wild relatives

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Stralegies to counleract environmental conseq uences of Bt modified crops

HIGH DOSE REFUGE (HDR) STRATEGY:

* This involves cultivating non-Bt maize crops along with Bt maize crops.
* Most of the adult corn borers in the Bt maize crops would have developed resistance against the Bt loxin.
* A recessive allele (r) is responsible for conferring resistance against the Bt loxin.

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* Most of the adult corn borers in Bt- maize fields will be homozygous recessive (rr), whereas, the adult corn borers in non-Bt maize fields will either be homozygous dominant (RR) or heterozygous. (Rr)

* HDR strategy aims to allow crossing b/w Bt maize and non-Bt maize adult corn borers so that most of the offsprings are heterozygous and therefore not resistant to Bt toxin.

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SAFE PLANTING DISTANCE:

* For Bt maize plants grow in Mexico, there is potential risk of transferring the gene for Bt Toxin to its wild plant relative, Teosinte
* Pollen grains from Bt maize plants can travel in wind for 2 hours.
* Therefore, planting Bt maize crops at a safe distance, such that its wild relatives are not pollinated. These pollen grains can reduce the risk of forming wild Teosinte plants with the gene for Bt-toxin.

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Genetically modified salmon

(Aqu Advantage)

* Atlantic salmon takes 3 years (12 seasons) to grow to it's full mature size
* Atlantic salmon requires 6 seasons to grow to it's full size but it only grows in sumers and spring.
* It lacks a gene required for growth in other seasons.
* We extract the gene (growth hormone regulating gene) and it's promoter and insert it into the embryo of the altantic salmon.
* The GM salmon grows to it's full size in 1.5 years

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Genetically Modified Salmon

* Production of GM animals is not as common as the production of GM crops.
* Very few animals have been genetically modified so far and even fewer have been approved for human consumption.

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* An example of the GM animals is the Salmon fish which is usually available by the name of Aqu Advantage.

* GM Atlantic Salmon is produced by inserting the growth hormone regulating gene along with its promoter into the fertilised egg of the salmon.

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* The growth hormone regulating gene is extracted from the Pacific salmon. whereas, the promoter is extracted from other fish species, known as the ocean pout.

* The non-genetically modified Atlantic salmon takes 3 years to reach its full mature size because it only grows during spring & summer each year.

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* Genetic modification of this Atlantic Salmon enables it To grow throughout the year which is why it reaches its mature size in only 18 months.

* The GM Atlantic Salmon is not known To disturb the ecological environment in its natural habitat, therefore, the production of GM Atlantic Salmon has been approved by regulating authorities, such as FDA (Food & Drug Administration).

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Gene Technology

With
Mohammad Hussham Arshad, MD

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ADVANCED LEVEL BIOLOGY 9700

Learning Objectives:

- Bioinformatics
- Microarrays

Video Lecture 9 Slides

Mohammad Hussham Arshad, MD
Biology Department

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Gene Technology

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Previously,

* gene technology & genetic engineering
* production of genetically engineered human insulin
* Marker genes to select transformed bacteria
* PCR
* Electrophoresis & it's application in,
  a. Hb electrophoresis
  b. DNA profiling
* Cystic Fibrosis → gene therapy
* Genetically modified organisms (GMOs)

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Bioinformatics

* Collection, processing and analysis of biological information using computer databases.

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BIOINFORMATICS

* It is the collection, processing & analysis of biological information & data using computer databases.

These are databases holding freely available, continually updated information such as the;

- nucleotide sequences of genes (gene sequences)
- amino acid sequences of proteins
- whole genome sequences
- mutations
- protein structures, and
- phenotypic data.

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Genetic Databases

- **Ensembl**

which holds data on the genomes of eukaryotic organisms.

- **BLAST**

(basic local alignment search tool) is a computer software which compares nucleotide or protein sequences.

- **GenBank** (DNA sequence)
- **UniProt**

(Universal Protein resource) which holds info on the primary sequence of proteins & their functions.

- **PDB**

(Protein Data Bank) is a database for the three-dimensional structural data for large biological molecules, such as proteins & nucleic acids.

- **COSMIC**

(Catalogue of Somatic Mutations in Cancer) an online database of somatically acquired mutations found in human cancer.

- **The Human Genome Project.**

(HGP) is an international research effort to determine the sequence of the human genome & identify the genes that it contains.

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BIOINFORMATICS HAVE A ROLE TO PLAY IN....

→ targetting drug design;

→ investigating evolutionary links by comparing gene & protein sequence data;

→ searching for the functions of genes;

→ identifying mutations

→ identifying genetic risk factors;

→ gene therapy;

→ The entire genome of Plasmodium species can now be accessed via databases. This is useful in developing vaccines against malaria to control the transmission of the malarial parasite.

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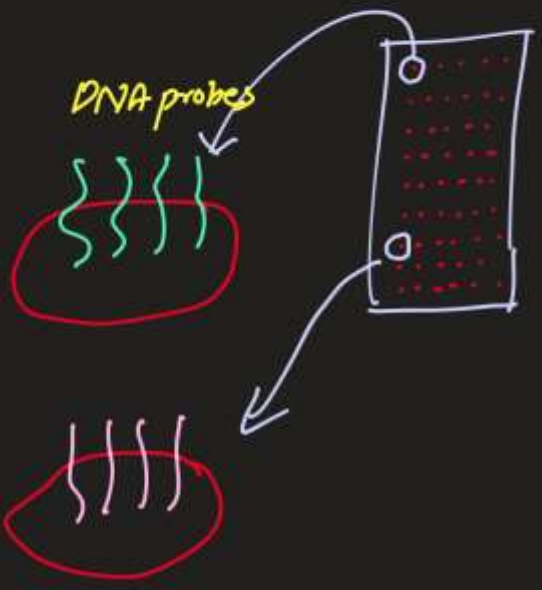
Micro-arrays (DNA chip technology)

- Compare the genome of two species (A and B)
- determine gene expression in normal vs diseased cells



20,000 microscopic DNA spots (wells)

↓

detect for 20,000 different genes

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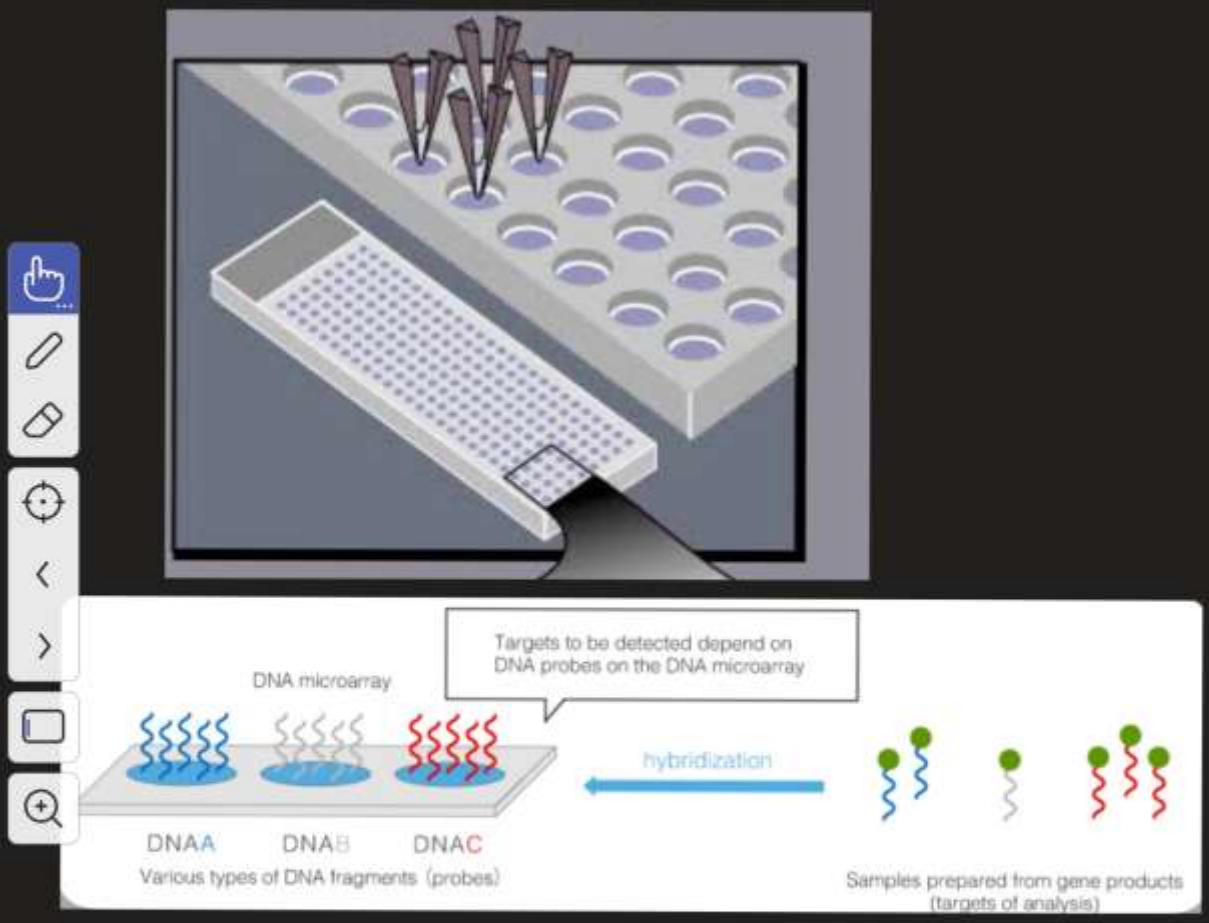
Targets to be detected depend on
DNA probes on the DNA microarray
DNA microarray
hybridization
DNAA
DNAB
DNAC
Various types of DNA fragments (probes)
Samples prepared from gene products
(targets of analysis)

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MICROARRAYS

*A microarray is made of glass or plastic with an area of $2cm^2$ containing numerous microscopic DNA spots (or wells).

*There are approximately 10000 or more different wells per cm^2.

*Numerous copies of a particular DNA probe are present in each well.

*The probes are from known locations across the chromosomes of the organisms involved.

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* Microarrays can be used To compare the genes present in two different species.
* DNA from each species (species A and B) is cut up into fragments and denatured to give lengths of single-stranded DNA.
* The DNA is labelled with fluorescent tags, red (A) and green (B) for the different species.
→ The DNA from both the species is mixed together and allowed To hybridise with the probes on the microarray.
→ The unbound DNA is washed off & the microarray examined under fluorescent light

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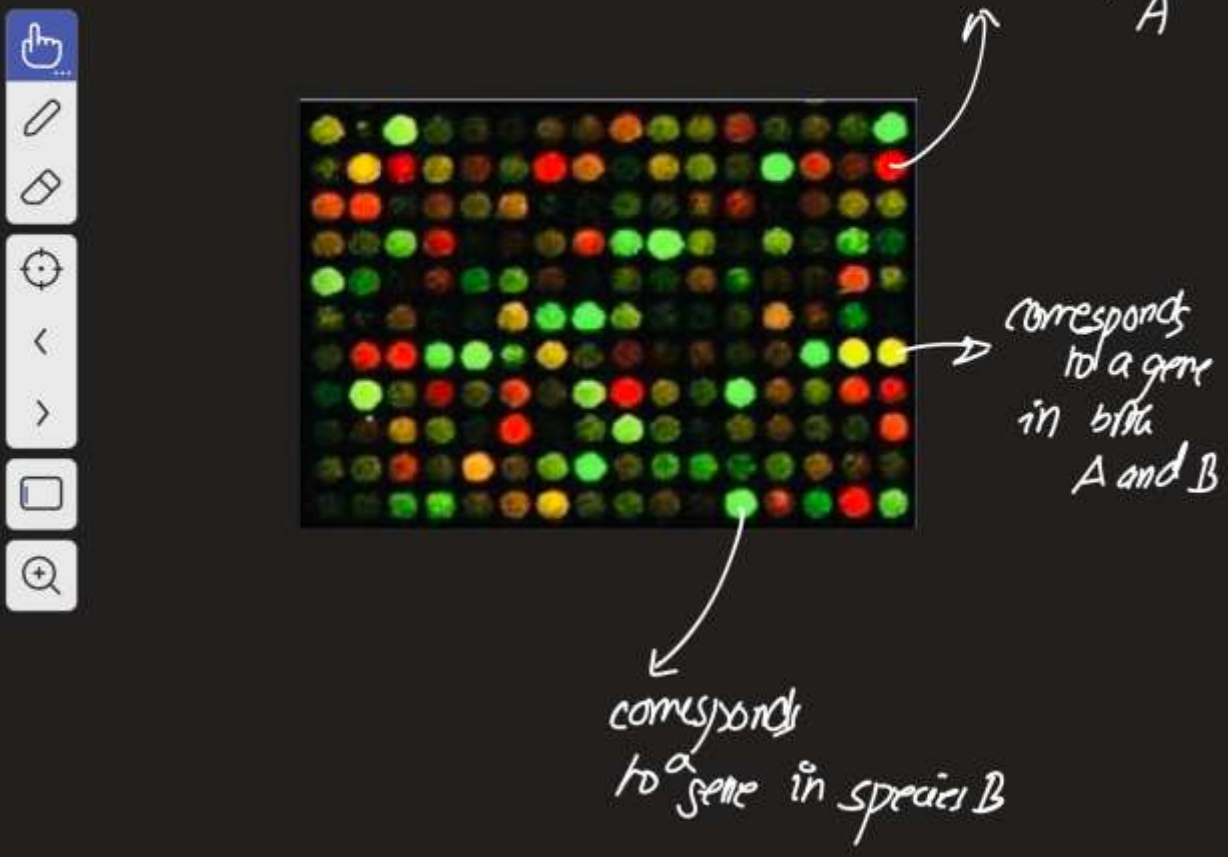
* Places where hybridisation has taken place are visible under the fluorescent light.

* Where DNA from only one species hybridized, it fluoresces its respective colour.

* Where DNA from both the species hybridizes, yellow colour is found.

* The microarray is scanned & the data obtained stored in a computer.


Couesponds to
a seme in species
A
corresponds to a gene in both A and B
corresponds to a seme in species B

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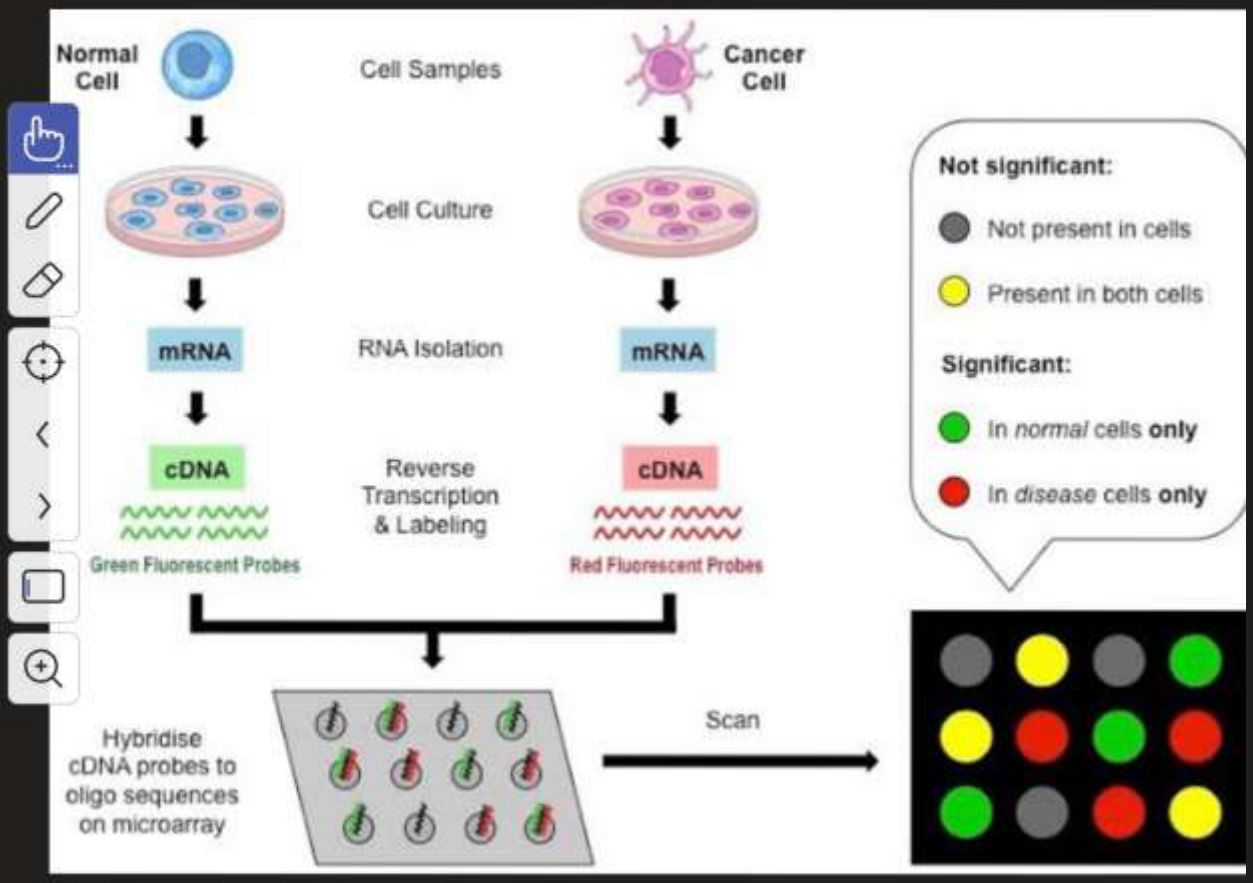
It can also be used to detect the expression of genes, for example in normal vs. cancerous cells.


Normal Cell
Cell Samples
Cancer Cell
Cell Culture
mRNA
RNA Isolation
mRNA
cDNA
Reverse Transcription & Labeling
cDNA
Green Fluorescent Probes
Red Fluorescent Probes
Not significant:
Not present in cells
Present in both cells
Significant:
In normal cells only
In disease cells only
Hybridise cDNA probes to oligo sequences on microarray
Scan

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Questions

Q.

4 Gene therapy can be used to treat some genetic disorders. An appropriate vector is chosen to carry the normal allele into the target cell. Three types of vectors commonly chosen are naked DNA, viruses and liposomes.

(a) A trial of gene therapy to treat cystic fibrosis used a viral vector. The viral vector caused a primary immune response with the production of memory cells.

Explain why the production of memory cells prevents the gene therapy from working in long-term chronic conditions such as cystic fibrosis.

* repeat treatment stimulates a more rapid and bigger secondary immune response
* so the target cells expressing the protein will be shortlived
* which will reduce the expression of the normal allele

[3]

(b) With reference to the three types of vectors that are commonly used, discuss the challenges in choosing appropriate vectors for use in gene therapy.

Do **not** include problems associated with an immune response in your answer.

* viral vector → integration of the allele into the host cell genome
* liposome vector → lowered ability of the the allele being delivered to the target cells
* naked DNA → * rapidly broken down
  * reduced uptake of the DNA by target cells

[4]

(c) A trial was carried out to find a new vector for use in gene therapy.

The new vector was made from red blood cells taken from the person with the genetic disorder. The cells had most of their cytoplasmic content removed and were then broken up to make small spherical vectors.

Most of these vectors lacked the ability to bind to receptors on the target cells.

To solve this problem, genetically engineered stem cells taken from the person were used to form red blood cells. These red blood cells had membrane proteins that were complementary to the target cell receptors. The vectors that were produced were well-tolerated by the immune system.

(i) Explain why the vectors were well-tolerated by the immune system.

* red blood cells had self antigens which
* were not recognised as foreign by the body

[2]

(ii) Suggest why it is not possible to produce genetically engineered red blood cells, except by using genetically engineered stem cells.

* red blood cells are devoid of a nucleus → hence cannot synthesize proteins

[1]

[Total: 10]

Q.

4 Lung epithelial cells have a thin layer of watery mucus on their surface.

The normal allele of the *CFTR* gene codes for a transport protein that transports chloride ions out of epithelial cells.

Fig. 4.1 is a diagram of part of the cell surface membrane and the mucus layer of an epithelial cell with normal CFTR proteins.

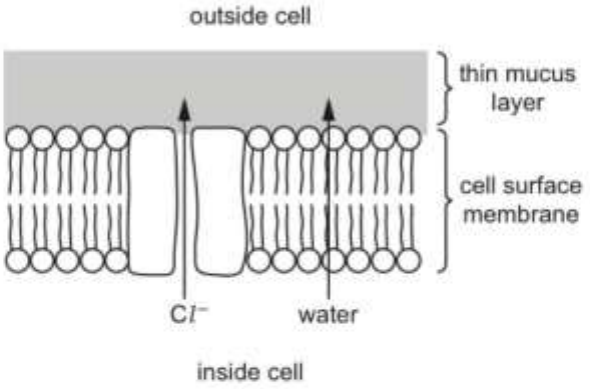

Fig. 4.1

Cystic fibrosis (CF) is a genetic disorder caused by having two recessive alleles of *CFTR*. In severe cases of CF, the transport proteins are not added to the cell surface membrane. This causes the mucus layer to be thick and sticky.

(a) Explain why the absence of CFTR proteins will cause the mucus layer to be thick and sticky.

* Cl^- can't leave the epithelial cell
* which prevent water from following via osmosis
* thereby making the mucus viscous

..... [2]

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(b) The probability of a baby having CF when both parents are heterozygous carriers for CF is 25%.

It is possible to carry out prenatal screening to check for CF by using one of these tests:

- amniocentesis, using cells from the amniotic fluid
- chorionic villus sampling, using cells from the placenta.

Both tests slightly increase the probability of the pregnancy failing (miscarriage).

Outline the advantages of carrying out prenatal screening for CF.

* If the screening is negative → reduces parental stress and anxiety
* if the screeny is positive →
  ① parents can opt for therapeutic abortion
  ② make arrangements for early treatment of CF in the newborn [3]

(c) Embryos produced by IVF may be screened for genetic abnormalities:
- to test for a specific genetic disease, such as cystic fibrosis
- to check whether there is an abnormal number of chromosomes present.

To improve the success of implantation and pregnancy, only embryos without any form of genetic abnormality are transferred to the woman's uterus.

A new double screening method was trialled where a single embryo biopsy was taken and used to test for a specific genetic disease **and** to check the number of chromosomes.
In the trial, 1122 embryos were tested using this double screening method.

In the trial, of the 1122 embryos tested:
- 50.6% did not have a genetic disease
- 27.5% did not have a genetic disease **and** did not have an abnormal number of chromosomes (normal embryos).

Only normal embryos were transferred into the women. The percentage of embryo transfers that resulted in pregnancy was calculated.

The results of the trial using double screening of a single biopsy were compared to the results of IVF procedures that used standard screening methods, as shown in Table 4.1.

Table 4.1

IVF method	percentage of embryo transfers that resulted in pregnancy
IVF with standard screening	32
IVF with double screening	49

Using the data in Table 4.1, discuss the social **and** ethical considerations of double screening for cystic fibrosis and chromosomal abnormalities in a single biopsy.

* cheaper b/c of a higher success rate
* there is greater probability of a successful pregnancy
* it is safer

..... [3]

[Total: 8]