GENETIC TECHNOLOGY

using our knowledge of genetics to improve our lives (health & medicine, agriculture)

1) Genetic Engineering — Recombinant DNA technology / Gene editing
2) PCR
3) Gel electrophoresis
4) Microarrays
5) Bioinformatics

Why?

1) Treating diseases
2) Genetic screening
3) Gene therapy
4) GM plants & animals

Genetic Engineering

→ deliberate manipulation of genetic material to modify specific characteristics of an organism, and may involve transferring a gene into an organism so that the gene is expressed.

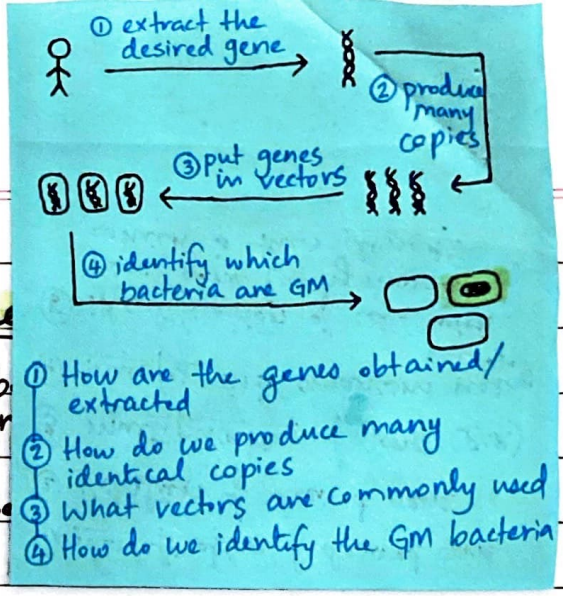
→ changing base sequence of an organism by:
- altering the sequence
- removing a gene
- adding a gene

→ Recombinant DNA Technology

Producing a DNA by artificially joining sections of DNA from different species.

recombinant DNA (rDNA)
artificially created DNA formed by joining nucleotide sequences from diff sources.

Genes to be transferred into an organism may be:
- extracted from the DNA of a donor organism (restriction endonucleases)
- synthesised from the mRNA of a donor organism (reverse transcriptase)
- synthesised chemically from nucleotides



① a) cut the DNA using restriction endonuclease

cuts DNA at specific b
cutting phosphodiester

- highly specific to its substrate (base se
specific restriction sites on DNA.
- separates the 2 strands of DNA by cutting sugar-phosphate backbone.
- may be cut in uneven way to give sticky ends (one strand of DNA fragment longer than the other) OR straight to give blunt ends (nucleotides are added to create sticky ends).

b) synthesise single strand of cDNA from mRNA using reverse transcriptase enzyme

- extract mRNA from organism (that was transcribed for desired gene)

extracted mRNA → (reverse transcriptase) → cDNA → (DNA polymerase) → Double-Stranded DNA

└ no introns

gene with no introns

c) artificially synthesise DNA

- computer used to generate nucleotide sequence to produce gene, by knowing which amino acids are required. no introns
  └ DNA synthesiser machine.

③ Vectors → liposomes (spherical lipids)
→ viruses

↓

Plasmids

- small circular rings of double-stranded DNA.
- used to transfer desired gene into new organism.
- ~~used as they can self-replicate~~

① - same type of restriction endonuclease used to cut plasmid.

② - produces complementary sticky ends to that on DNA fragment (gene).

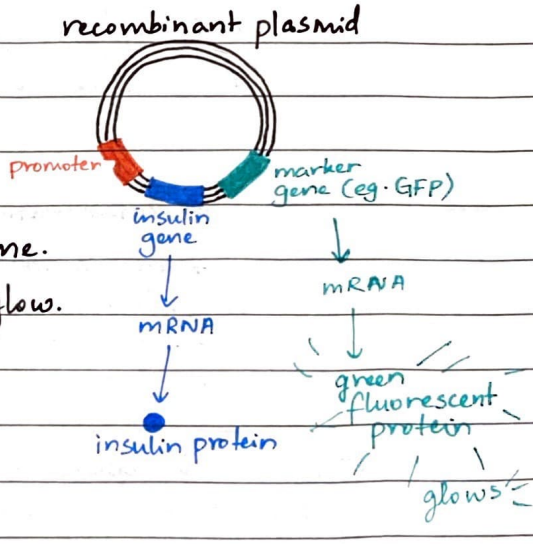
③ - H-bonds form btwn complementary base – pairing of sticky ends.
④ - DNA ligase reforms phosphodiester bonds btwn DNA fragment (gene) and plasmid ⇒ ⑤ recombinant plasmid formed.

Why plasmids?
① small ∴ easily taken up by bacteria.
② can be copied/ self-replicate.
③ have single target restriction sites. (plasmid only cut at one area)

* Recombinant plasmid has desired gene + promoter
so RNA polymerase can attach, and gene can be expressed.

no promoter = no expression

④ Gene markers
↳ used to identify GMO. eg. GFP gene



- insert recombinant plasmid into bacteria.
- some bacteria express insulin gene & GFP gene.
- they are able to produce insulin & GFP ∴ glow.

→ Gene Editing
form of genetic engineering involving the insertion, deletion or replacement of DNA at specific sites in the genome.
↳ using CRISPR/Cas9 system.

a) Cas9 enzyme
2 active sites to cut the DNA.

b) guide RNA (gRNA)
- can be synthesised in the lab
- guides the Cas9 on where to cut the DNA.

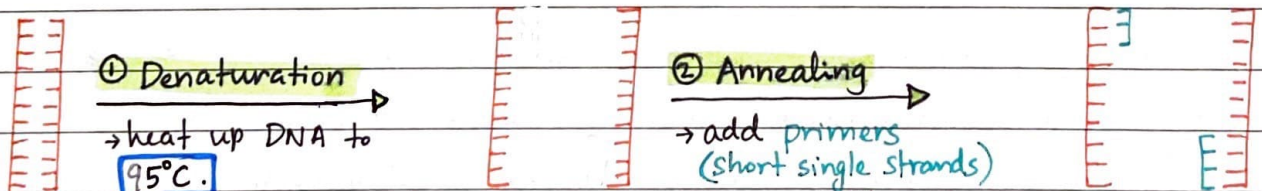
① artificially synthesise gRNA so it is complementary to DNA sequence that needs to be cut.
② Cas9 & gRNA are coupled, and move along chromosome and unwind sections of it, until it reaches complementary section.
③ gRNA binds to target sequence - complementary base pairing.
④ 2 active sites on Cas9 cut the DNA at that section - cuts both strands.
⑤ 2 DNA fragments produced.
↳ deletion / insertion / replacement is then done.

Polymerase Chain Reaction (PCR)

clones & amplifies DNA

amplifies the number of identical DNA molecules; repeated DNA replication.

why?
→ genetic engineering
→ DNA fingerprinting
→ used to detect presence of virus in our body (eg. Covid-19)



① Denaturation
→ heat up DNA to 95°C.
→ this breaks H-bonds
→ strands separated

② Annealing
→ add primers (short single strands)
→ primers will guide the DNA polymerase when forming a new strand.
→ 60°C, so primers can form H-bonds with DNA strands

③ Elongation
→ free DNA nucleotides or dNTPs will form complementary base pairing on template strand.
→ formation of new strands using DNA polymerase
↳ Taq polymerase
→ 72°C, as optimum for Taq polymerase.

* Process is repeated.
* Advantage of Taq polymerase:
  - high optimum temp ∴ can be reused over and over again ⇒ less cost. (doesn't denature when newly formed DNA is heated to 95°C)
  - higher PCR temp ⇒ faster rate

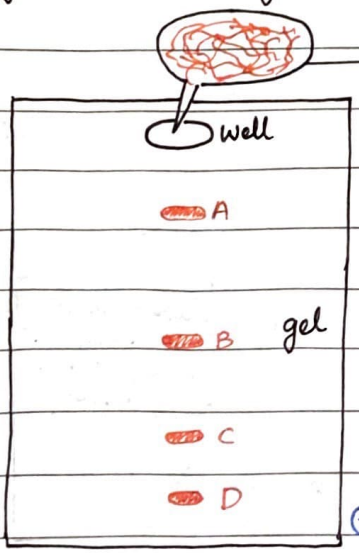
Gel Electrophoresis

separates molecules (proteins / DNA) based on their charge & mass.

method of separating DNA fragments of different lengths, based on charge & mass, to produce banding patterns / DNA fingerprints.

Why?
- → forensics
- → paternity tests
- → genetic screening (to detect specific alleles)

Requirements:
- DNA fragments to be separated
- Polyacrylamide gel
- electric field
  - cathode (-)
  - anode (+)



multiple copies of each fragment (from PCR): So results / bands are clearer/thicker

⊖ cathode

DNA is put on -ve end, as it is -ve charged (due to phosphate groups), and this forces it to move to +ve end.

Gel provides resistance ∴ smaller fragments travel much further than larger ones.

⊕ anode

Microarrays

(chk last few pages)

Bioinformatics

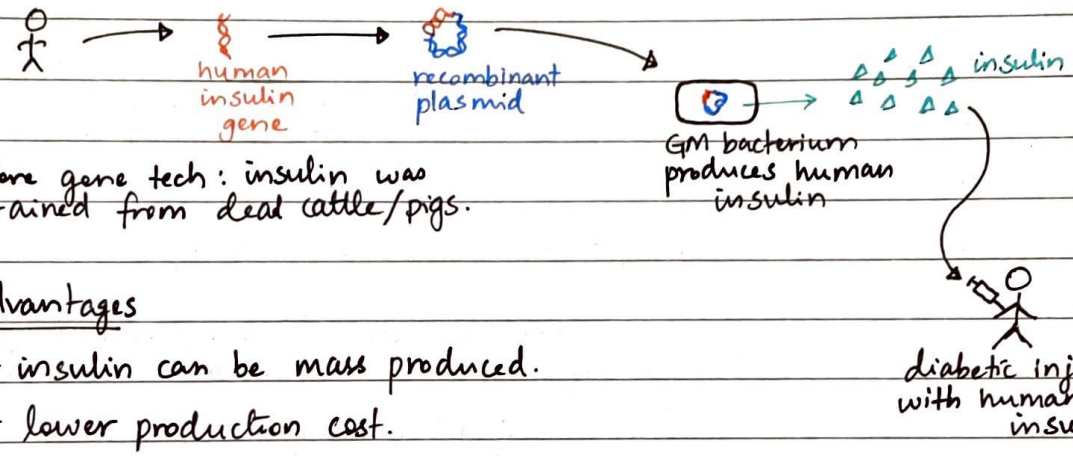
(learn from papers)

- → Bioinformatics is a store/database of:
  - nucleotide sequences of genes & genomes.
  - amino acid sequences of proteins & protein structures.
- → these sequence data are pooled from all over the world.
- → it used to compare base/amino acid sequence data with known sequences in database.
- → it uses search/analysis programmes/ softwares/ algorithms.
- → can be used for modelling 3D tertiary protein structure OR identifying role of protein

Genetic Technology in Medicine

① **Recombinant DNA Technology** – Recombinant human proteins to treat disease.

a) Treatment of Diabetes Mellitus

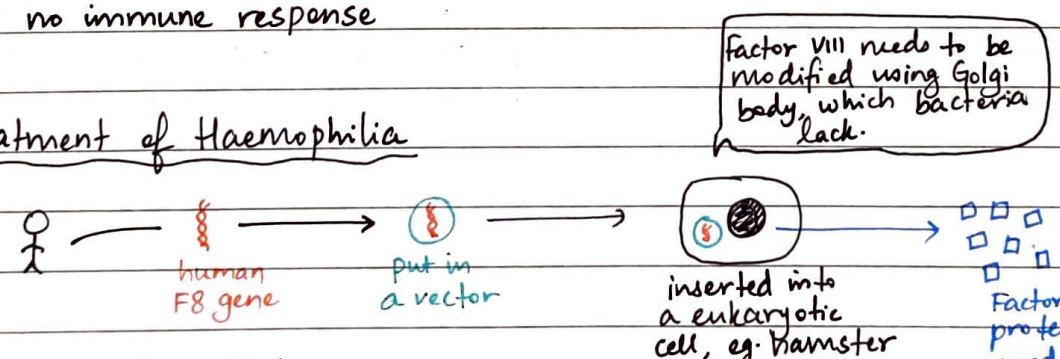


* Before gene tech: insulin was obtained from dead cattle/pigs.

Advantages

- insulin can be mass produced.
- lower production cost.
- fewer ethical & religious issues.
- no immune response

b) Treatment of Haemophilia



* Before gene tech: Factor VIII was donated to Haemophiliacs through blood transfusions.

Advantages

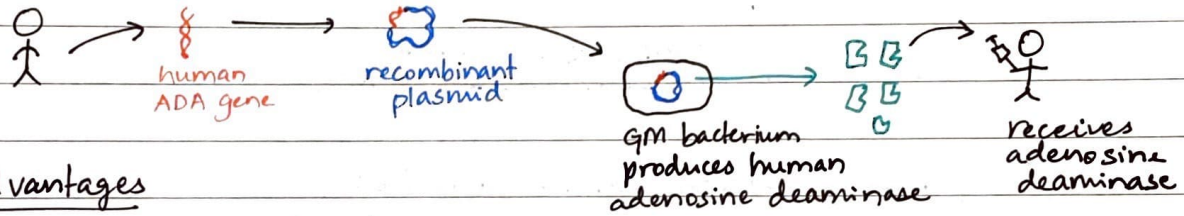
- can be mass produced.
- lower production cost.
- no need to worry about incompatible blood types (no immune response).
- no risk of pathogen transmission.

c) Treatment of SCID

* mutation of ADA gene → unable to produce adenosine deaminase

SCID!! ← destruction of T- lymphocytes

* before gene tech: adenosine deaminase was obtained from dead cattle.



Advantages

- can be mass produced.
- lower production cost.
- fewer ethical and religious issues.
- no immune response.

② Genetic Screening

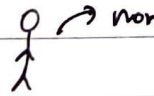
testing an embryo/fetus/adult for the presence of specific alleles. (determining the genotype of a person).

Genetic diseases that are screened:

→ Huntington's Disease

→ Potential breast cancer

- due to mutations of BRCA-1 & BRCA-2 genes



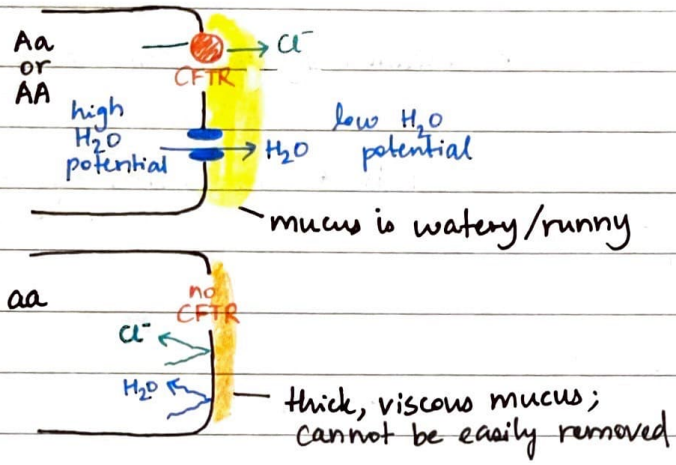
↗ normal BRCA-1 gene: causes cell death if DNA damage not repaired

↳ normal BRCA-2 gene: prevents cell from dividing uncontrollably

→ Cystic Fibrosis

CFTR gene ↗ A: normal CFTR protein

↳ a: no CFTR protein



Genetic screening can be done by:

① gel electrophoresis
② microarrays

} easily done in adults

Prenatal diagnosis: genetic screening of fetus before birth.

↳ how to obtain their cells?

① Amniocentesis (15-16 weeks of pregnancy)
- collect amniotic fluid surrounding fetus, which contains fetus cells.
- lower miscarriage risk.

② Chorionic villus sampling (10-13 weeks of pregnancy)
- collect placenta tissue of fetus
- higher miscarriage risk.

③ Collect the mother's blood
- may contain fetus cells.
- no miscarriage risk.
- less accurate than the other 2.

③ Gene Therapy

treating genetic diseases by:

a) inserting a genetically corrected cell
b) inserting functional allele ~~directed~~ directly into the cells

How to insert an allele into a cell:

Vectors

* plasmids not used as they are not found in human eukaryotic cells ∴ cannot be inserted into them.

a) Liposomes

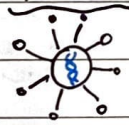
- spherical lipid membrane

(§) allele is put into it.

☺ - it fuses with cell surface membrane, and delivers the allele into cell.

☹ - doesn't deliver allele directly into nucleus ∴ might get destroyed, and cannot be expressed.

b) Viruses



allele is inserted into virus, and its own genetic material is modified to make it less harmless.

Retrovirus

☺ - delivers the allele into the genome.

☹ - does it randomly, which may cause DNA damage / mutation.

☹ - immune response due to antigens on surface: virus destroyed before it can deliver + physical reactions - fever, etc.

Adeno-associated virus (AAV)

☺ - delivers the allele into the nucleus.

☺ - does not randomly insert it into the genome / doesn't damage or cause mutation of host cell chromosome.

☹ - immune response

* SCID = severe combined immunodeficiency

Using Gene Therapy to treat SCID

↳ so child doesn't need to be constantly injected with the adenosine deaminase (pg. 7)

① extract 1 blood stem cell (cell has the mutated alleles only.) from bone marrow of infant with SCID.
② AAV inserts the normal ADA allele into the child's nucleus.
③ genetically corrected cell is produced, that can produce adenosine deaminase.
④ re-insert this cell into bone marrow.
⑤ this blood stem cell becomes a lymphocyte with functional ADA, producing adenosine deaminase.

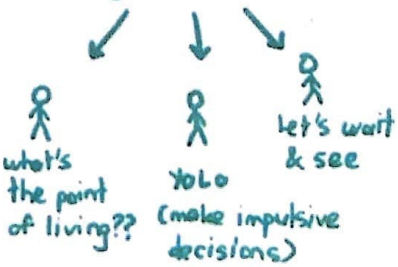
Using Gene Therapy to treat Leber Congenital Amaurosis (LCA)

RPE 65 gene → D: allows for pigment regeneration in retina.
→ d: prevents pigment regeneration ⇒ cell death ⇒ blindness.

① virus containing dominant allele.
② injected directly into the retina.
③ virus delivers the dominant allele.
④ allows pigment regeneration & prevents cell death.

* chance of immune response from virus is low, as eye doesn't have many WBCs.

The ethics of genetic screening

Good	Bad (maybe...)	Grey Area
① Screening for BRCA-1 & BRCA-2 mutations can help detect cancers earlier & take precautions.	① Genetic screening is expensive (not available to everyone)	① Being aware of some mutations may cause anxiety & stress
② Parents may terminate pregnancies where the fetus has severe genetic diseases	② Potential abuse	eg: A person who is told they have the allele that causes Huntington disease
→ future parents do not go through the stress of caring for the child	→ Parents may terminate pregnancies if the fetus has a 'mild' genetic disease, or even if the desired alleles are absent!	
→ less harmful allele frequency in the population!	or sex selection!	

The ethics of gene therapy

Good	Bad	Grey Area
① Successful treatment of LCA & SCID ↓ restoration of eyesight ↓ intact immune system ↳ better quality of life for those with genetic diseases...	① usage of viruses as vectors may cause immune responses (due to antigens) ② Limitations a) not 100% effective b) unable to treat diseases caused by dominant alleles!! ③ expensive (not available for everyone)	① Some argue that it can be a form of 'eugenics' (deliberately choosing desirable characteristics in humans) → instead of treating genetic diseases, it may be used to 'improve' humans.... whatever that may be...

Genetic Technology in Agriculture (Food Production)

Problems:
① crop plants have to compete with weeds for water, minerals, and space.
② crop plants are damaged by pests.
③ Salmon grows very slowly to reach adulthood (3 yrs)
↳ only produces growth hormones intermittently (spring & summer).

① **Herbicide resistance crop plants – soybean**
- herbicide resistance gene inserted into plant.
- spray herbicides
- crop plant ~~or~~ unaffected by herbicides.

Advantages	Disadvantages → GM seeds expensive
→ increased crop production.	→ overuse of herbicides may result in evolution of weeds to become herbicide-resistant
→ more food.	→ GM plant could become a weed in another farm.
→ better profits for farmers.	→ GM plant may interbreed with wild relative and produce hybrid weeds that are herbicide resistant.

② **Insect resistance in crop plants - cotton**

- insert Bt toxin gene into embryo of plant.
- crop plant produces Bt toxin.
- pests that ingest Bt toxin will die.

Advantages	Disadvantages
→ (same as herbicide)	→ Bt toxin can act as selection pressure, to allow pests to evolve and become toxin resistant.
→ less usage of pesticides: - less cost for farmers. - non-pest species not killed.	→ (same as herbicide)

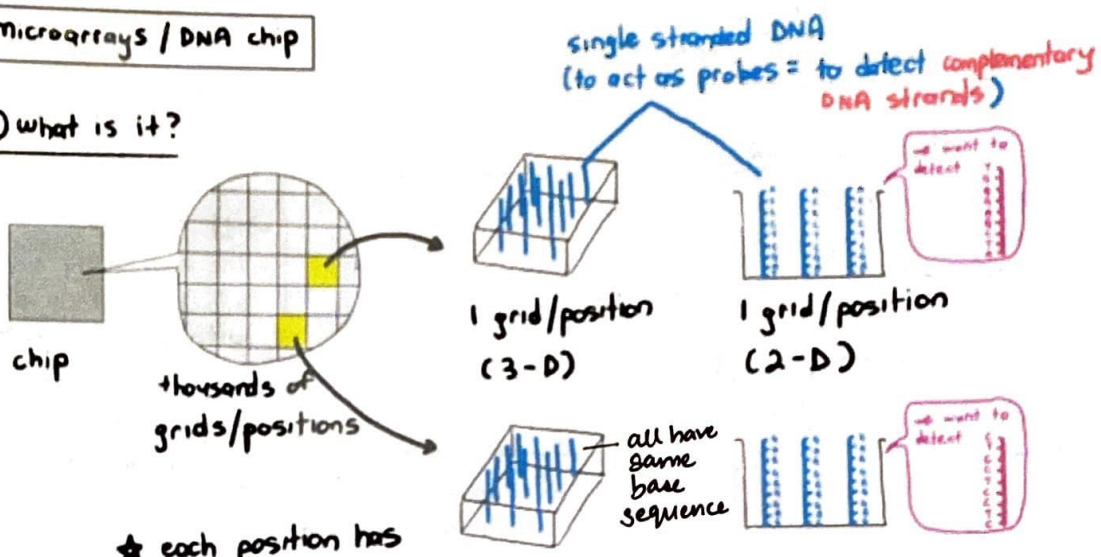
③ **GM animals - GM salmon**

- growth hormone regulating gene + promoter inserted into embryo.
- GM salmon produced, that produces growth hormone constantly.
- reaches adult size in 18 months (1.5 yrs)

Advantages	Disadvantages
→ faster food production for humans.	→ GM salmon may outcompete wild salmon.
	→ ethical considerations when genetically modifying animals.
	→ consumption of GM animals may have harmful side effects.

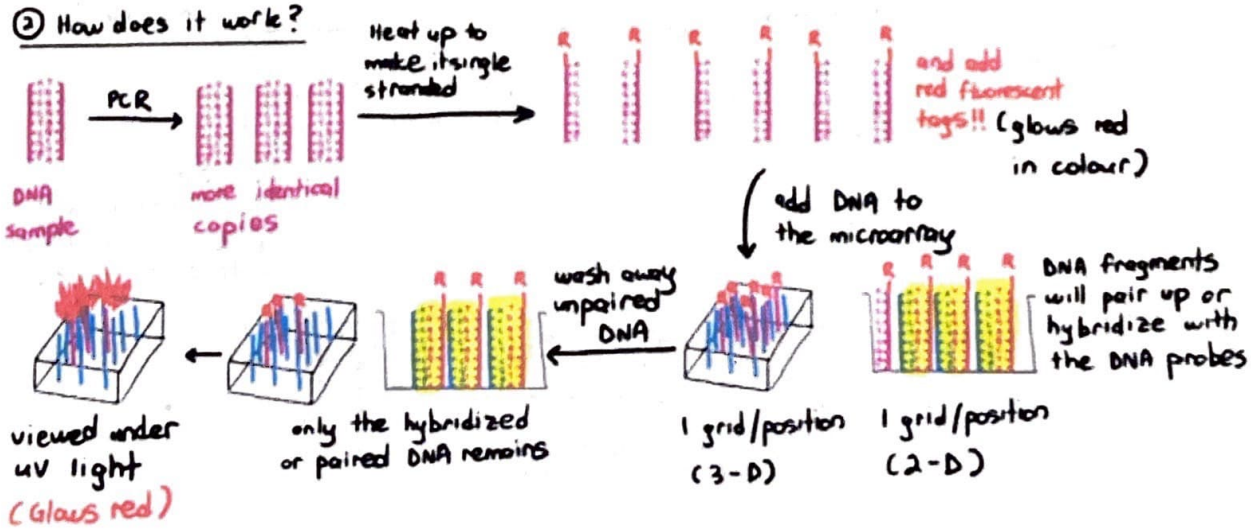
Microarrays / DNA chip

① What is it?



★ each position has 1 type of DNA probe
∴ each position has 1 DNA base sequence

② How does it work?



③ using microarrays to detect presence of genes

what gene is present in this species?

DNA sample → PCR → more identical copies

a) Heat up to make it single stranded

b) and add red fluorescent tags!! (glows red in colour)

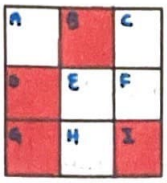
c) add DNA to the microarray and allow it to hybridize with DNA probes

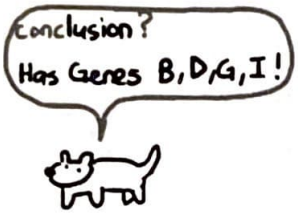
microarray

each alphabets represent a type of Gene.

d) wash away unpaired DNA

f) viewed under uv light





Explanation

DNA sample → PCR → more identical copies

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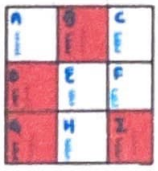
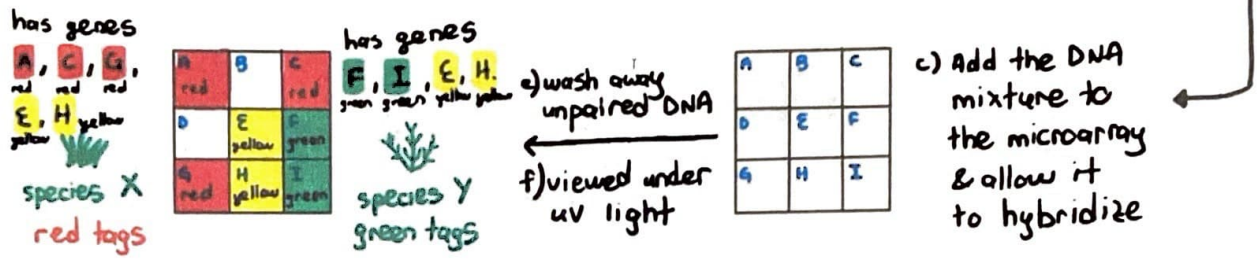


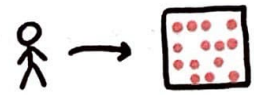
③ using microarrays to detect presence of genes

species X → DNA sample → PCR → more identical copies → a) Heat up to make it single stranded → b) Add red tags to the DNA

species Y → DNA sample → PCR → more identical copies → a) Heat up to make it single stranded → b) Add green tags to the DNA

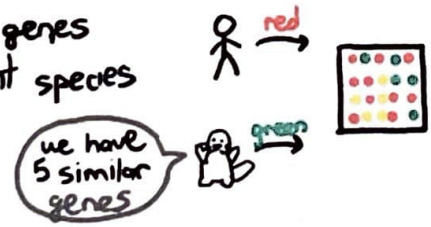


a) we can detect the presence of thousands of genes in 1 species



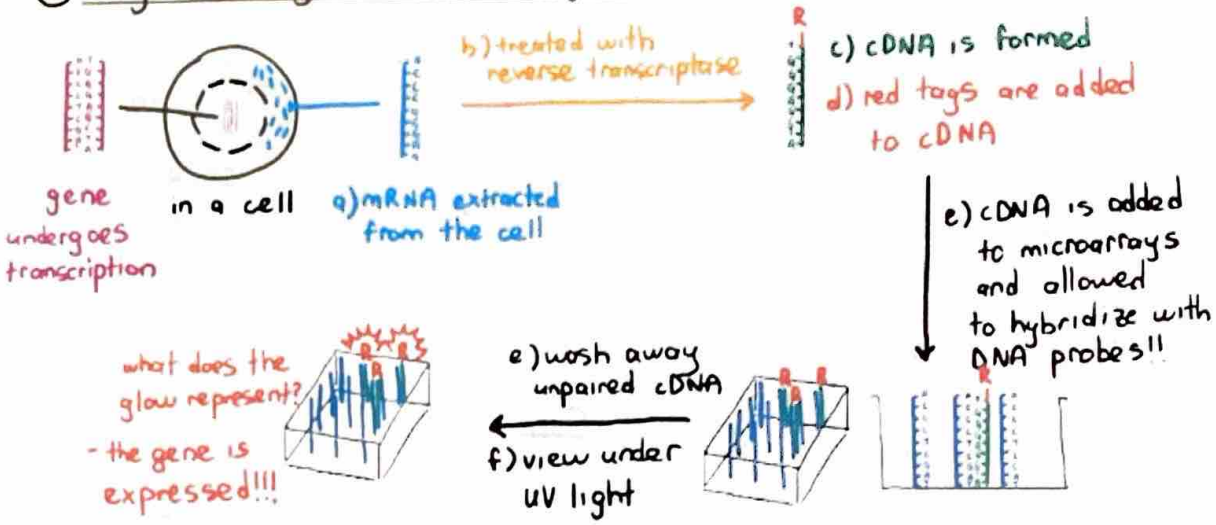
fluorescence proves presence of genes in our DNA

b) we can compare genes between different species

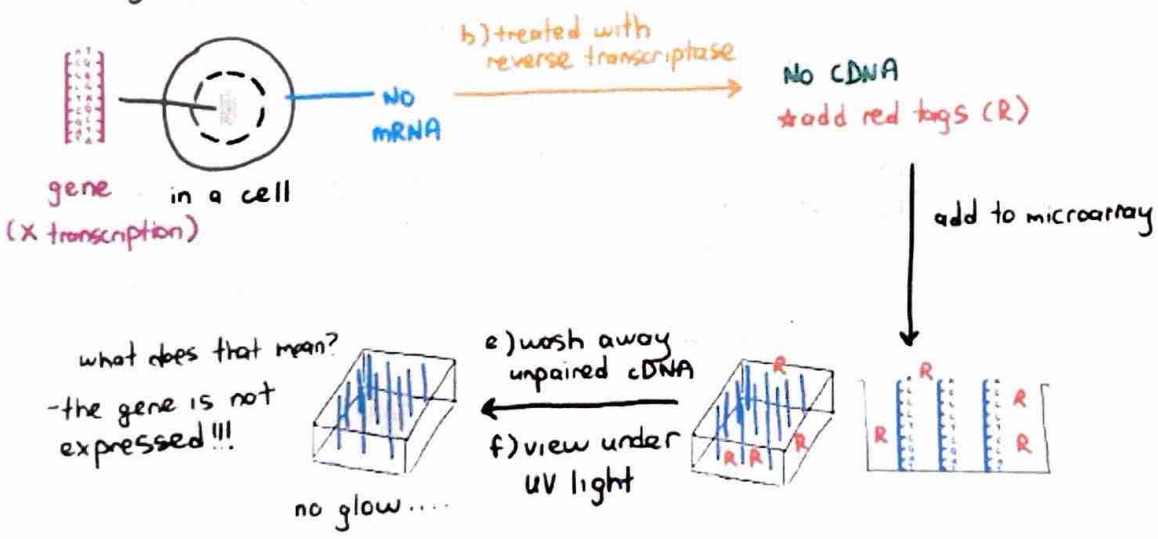


we can quickly compare genetic similarities between 2 different species!!

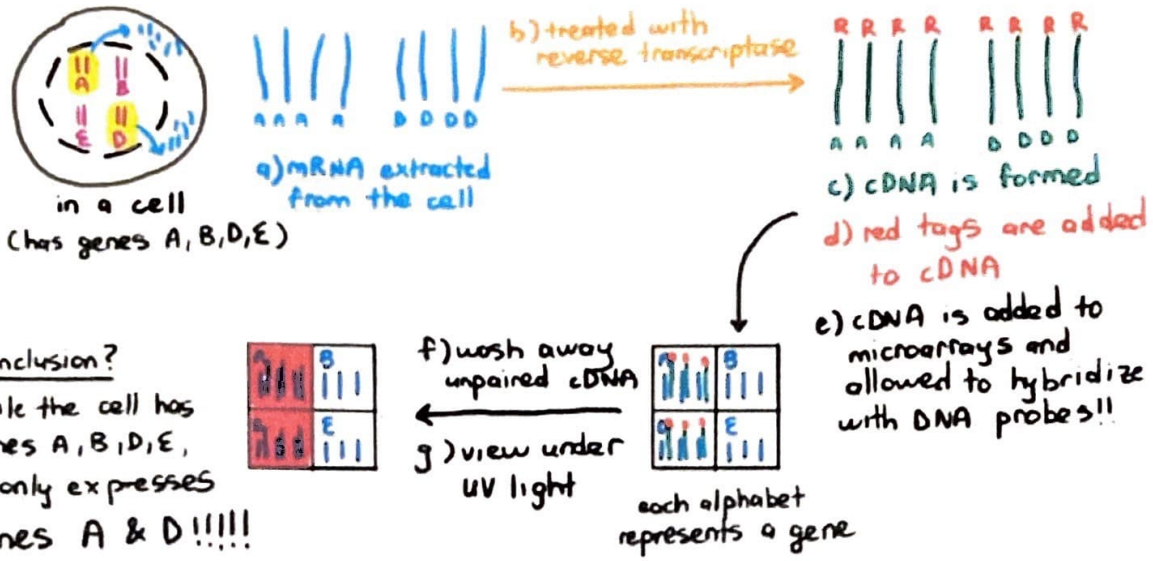
④ using microarrays to determine gene expression



④ using microarrays to determine gene expression



④ using microarrays to determine gene expression



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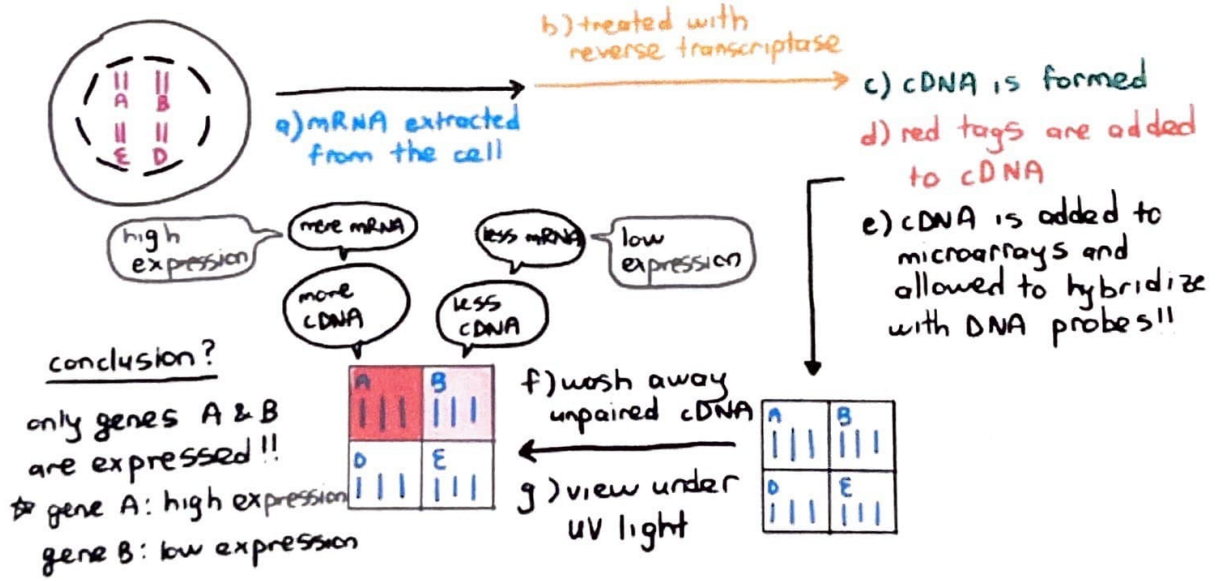
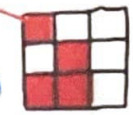


Summary

a) To detect presence of genes in an organism

extract DNA → PCR (optional) → Denature DNA (make it single stranded) → add red tags → add to microarray and allow it to hybridize → wash away unpaired DNA and view under UV light

glow indicates gene is present!!!



b) To detect gene expression

extract mRNA → treat with reverse transcriptase → forms cDNA → add red tags → add to microarray and allow it to hybridize → wash away unpaired DNA and view under UV light

glow indicates the gene is expressed!