Genetic Engineering

- Genetic engineering aims to remove a gene or genes from one organism and transfers it to another organism which can then express the genes transfered. The new DNA which now contains genes from two different organisms is called recombinant DNA.

- The organism which expresses the new combined DNA is a transgenic organism and is called Genetically Modified Organism (GMO).

* Overview of Gene Transfer

1. The gene that is required is identified. It may be cut from a chromosome, made from mRNA by reverse transcription or synthesized from nucleotides.

2. Multiple copies of the gene are made using a technique known as polymerase chain reaction. (PCR)

3. The gene is inserted into a vector which delivers the gene to the cells of the organism. Examples are plasmids, lysosomes and viruses

4. The vector takes the gene into the cells. The cells that have the new gene are identified & cloned.

Enzymes (restriction endonucleases, ligase and reverse transcriptase)

- Restriction endonucleases are produced by bacteria and are able to break down the DNA of invading viruses. These enzymes cut the sugar phosphate backbone of DNA at specific places with specific sequences of bases. Some restriction enzymes cut straight across DNA to give blunt ends, while others cut in a staggered fashion to give sticky ends.

→ Usually produced by bacteria and break down DNA of enemy viruses.

Restriction enzyme	Site of cutting across DNA
EcoR1	-G|AATTC- -CTTAA|G-
BamH1	-G|GATCC- -CCTAG|G-
Hind III	A|AGCTT TTCGA|A

- Vectors

→ Vectors are used to get a new gene into a recipient cell. Ex: Plasmid

→ Plasmids are naturally occuring circular DNA in bacteria which can be transferred from one bacteria to another of the same or

different species.

→ To extract plasmids, bacteria containing them are treated with cell wall - breaking enzymes. The plasmid is cut using restriction enzymes to produce sticky ends and same are used on the gene to produce complementary sticky ends.

→ The cut plasmids and new gene are mixed together and DNA ligase is used to link the sugar phosphate of DNA with the plasmid.

★ Properties of plasmids for gene cloning

- Low molecular mass
- An origin of replication that allows them to be easily copied.
- Several single target sites for different restriction enzymes.
- One or more marker genes that allow identification of cells that have taken up the plasmid.

★ Genes coding for easily identifiable substances that can be used as markers

- In order to ensure successful transmission of genes, it is important to use markers. Ex: enzymes that produce fluorescent

markers.

Inserting key regions

→ It is important to remember that some genes have promoters. Hence, when they are being copied and transferred, the promoter regions must be copied and transferred too.

Gel Electrophoresis

- Gel electrophoresis is a technique used to separate different molecules. It involves placing a mixture of molecules into wells cut into an agarose gel and applying an electric field.

→ Note: so DNA is cut using restriction enzymes into fragments and is stained with a dye so that DNA is visible. Then we can load the DNA into wells. It is then connected to an electric source. The molecules move in response to electric field by:

- Net overall charge: Negatively charged molecules move towards the anode (+) while positively charge molecules move toward the cathode (-). Highly charged molecules also move faster than those with an overall lesser charge

- Molecular size: smaller molecules move faster than larger ones.

Gel composition: Polyacrylamide is used for proteins while agarose is used for DNA. The size of the pores within the gel will determine the speed with which they will move.

- Allele separation: different alleles of the same gene can be separated using gel electrophoresis

→ used for genetic profiling

Procedure

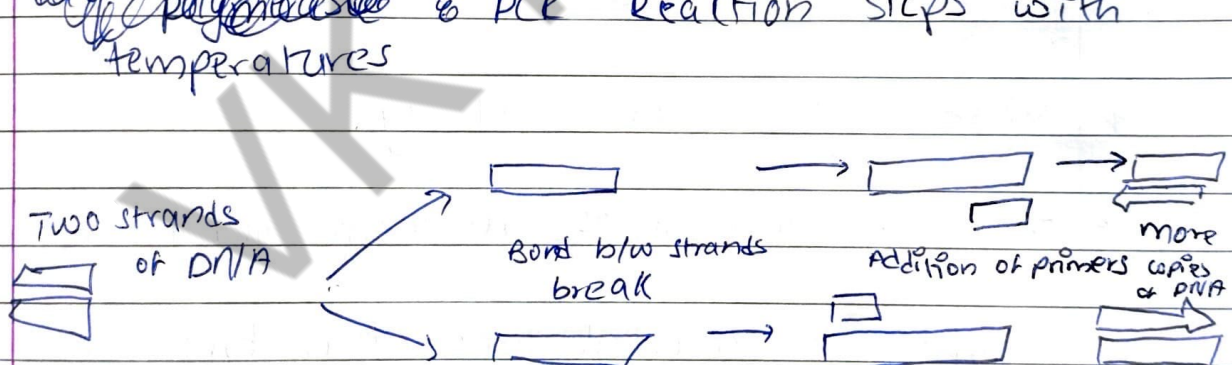
1. The gel is prepared by dissolving powder in hot water. A comb is placed at one end until gel solidifies and this leaves the wells where samples are loaded.
2. Buffer solution is poured in gel giving a constant pH.
3. A micropipette is used to transfer DNA to the wells and DNA is stained to identify how far the material in samples have travelled.
4. A reference sample with DNA fragments is also placed used to determine lengths of fragments of DNA in samples.
5. An electrode is connected with the negative electrode at the same end as the wells containing DNA.
6. Once the dye has moved across most of the gel, the battery is disconnected
7. The buffer solution is poured away and a suitable stain is added to the gel. The stain is rinsed away to reveal bands across the gel showing

the positions of the DNA fragments. Alternatively the probes are labelled with a fluorescent stain and viewed under UV light. The length of DNA fragments can be determined by comparing them with the DNA 'ladder' at the side of the gel.

☆ Polymerase chain reaction (PCR)

- PCR is a technique that is used for the rapid production of a large number of copies of a particular DNA fragment. Even from a small molecule of DNA, multiple copies can be made

Taq polymerase & PCR reaction steps with temperatures



Taq polymerase: This was the first heat-stable DNA polymerase. It was isolated from thermophilic bacterium. It is valuable as: It is not destroyed during denaturation so there is no need to

replace it after every denaturation cycle. It has a high optimum temperature so there is no need to reduce the temperature of elongation below that of annealing.

Microarrays

- Microarrays are used to find the genes present in an organism's genome and to find out which genes are expressed within cells at any particular time
- Microarrays are used to compare DNA from two different species.
- Microarrays are used to compare which genes are active by identifying the genes that are transcribed into mRNA.

Microarrays & Gene activity

- To identify which genes are active you track mRNA.

1. mRNA is collected from two types of cells and converted to cDNA using reverse transcriptase. The cDNA may need to be increased using PCR.
2. cDNA is labelled with fluorescent tags and denatured to give single-stranded DNA
3. The DNA is allowed to hybridize with probes on the microarray

4. Fluorescent spots will indicate the genes that were being transcribed in the cell.

5. The intensity of light emitted by each spot indicates the level of activity of each gene.

6. A high intensity means many mRNA molecules were present in the sample & vice versa.