CAIE Biology A-level

Topic 19: Genetic technology

Notes

DNA Sequencing

DNA sequencing is defined as the laboratory process used to determine **the exact nucleotide sequence** — the precise order of the four nitrogenous bases (adenine, guanine, cytosine, and thymine) — **within a DNA molecule.**

Knowing this order is fundamental because the sequence of bases acts as a **genetic blueprint**, providing the instructions needed for an organism to develop, function, and reproduce. The rapid advancement of sequencing techniques has increased the speed of sequencing and allowed whole genome sequencing, that is **high-throughput sequencing**.

There are also large databases present which can be used to find out more information about **nucleotide or protein sequences.** Nucleotide sequences can give more information about genes and genomes whereas protein sequences can give more information about proteins and protein structures. These large databases are beneficial as they provide extensive information for many organisms.

Benefits of Nucleotide Sequence Databases (Genes & Genomes)

- **Identification of Genes and Functions:** Researchers can compare an unknown DNA sequence with known sequences in the database to identify specific genes or predict their functions.
- **Determining Evolutionary Relationships:** By comparing the genomes of different species, scientists can calculate the degree of similarity, which indicates how closely related the organisms are and helps in constructing **phylogenetic trees**.
- **Disease Research:** Databases help identify genes responsible for inherited diseases and detect **mutations** associated with specific disorders.
- **Medicine and Drug Discovery:** Identifying the genes involved in a disease allows scientists to find potential targets for drug treatment or develop gene therapies.
- **Model Organisms:** Comparisons can reveal which organisms (e.g., fruit flies) have enough genetic similarity to humans to be used as effective models in medical experiments.

Benefits of Amino Acid Sequence & Protein Structure Databases

- **Predicting Protein Function:** Because the primary structure (amino acid sequence) dictates a protein's 3D shape, databases allow researchers to predict a protein's function and how it might interact with other molecules.
- **3D Modeling and Visualisation:** Databases can provide atomic coordinates that allow scientists to visualise the 3D structure of proteins, which is essential for understanding active sites and binding domains.
- **Rational Drug Design:** Knowing the exact 3D structure of a protein "target" (such as a bacterial enzyme) allows for the design of specific drugs that can fit into and inhibit that protein.

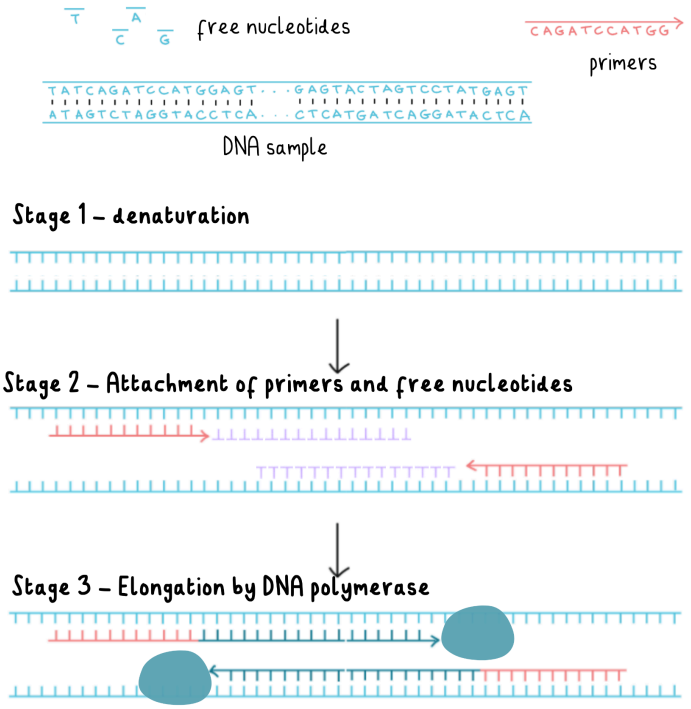
DNA profiling

DNA profiling is a forensic technique used to **identify individuals by characteristics of their DNA**. It can also be used to **determine genetic relationships** between organisms.

Main techniques used in DNA profiling are:

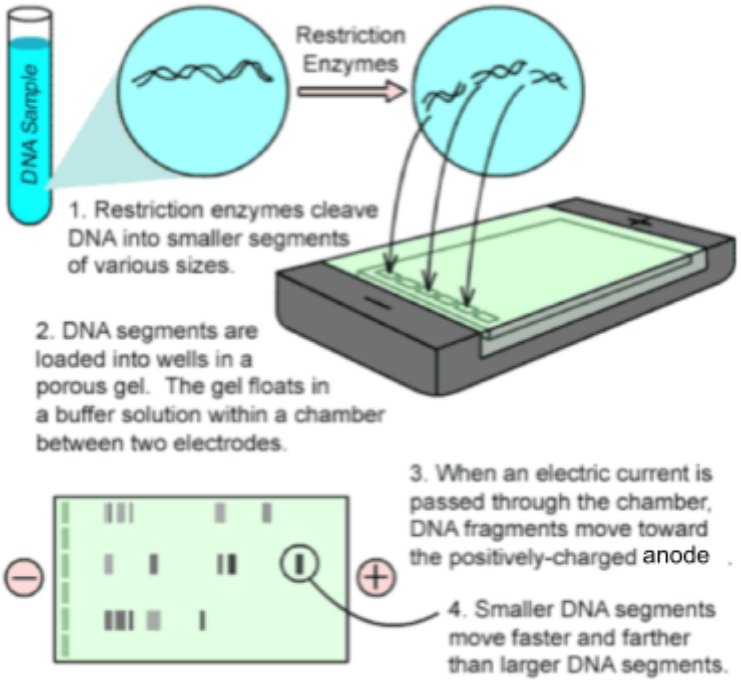
- **Polymerase chain reaction, known as PCR,** which is used to amplify the DNA by making millions of copies of a given DNA sample. This can be used to replicate DNA from crime scenes in forensic investigations. It occurs as follows:

    1. A reaction mixture is set up by mixing the **DNA sample, primers, free nucleotides and DNA polymerase** which is the enzyme involved in creating new DNA strands. The polymerase used is called ***Taq* polymerase**, and comes from organisms living in high temperature environments such as hot springs. This allows the reaction to happen quickly at high temperatures without the DNA polymerase denaturing.
    2. This mixture is then **heated to 95°C** to break the hydrogen bonds and to separate the two strands.
    3. The mixture is **cooled to a temperature between 50-65°C** depending on the type of primers used, so that they can bind to the strands.
    4. Temperature is increased to about **72°C** as this is the temperature *Taq* polymerase works at. ***Taq* polymerase** creates a copy of the sample by **complementary base pairing using the free nucleotides.**
    5. **This cycle is repeated around 30 times** and produces sufficient DNA to generate a DNA profile.



- **Gel electrophoresis** is a process used to **separate the DNA fragments according to their size using an electric current**. It occurs as follows:

1. **Preparation**: DNA is first cut into fragments using **restriction endonucleases.** A loading dye is added to the DNA to increase its **density** (so it sinks into the wells) and to provide a visible **marker** for the progress of the run. DNA fragments are **loaded into wells** in the agarose gel. The gel is submerged in an **electrolyte buffer**.
2. **Charge and Migration:** When the power is turned on, an **electric field** is created. DNA has a net **negative charge** due to the **phosphate** groups in its sugar-phosphate backbone. Therefore, the DNA fragments migrate **away** from the **negative electrode (cathode)** towards the **positive electrode (anode).**
3. **Separation by Size:** As the fragments move through the gel matrix, they encounter **resistance** from the pores.
    a. **Smaller fragments** move more **easily and quickly** through the pores, travelling a **further distance** in a given time.
    b. **Larger fragments** are more impeded by the matrix, move **more slowly**, and stay **closer** to the wells.
4. **Visualisation:** After the run, the DNA is not visible to the naked eye. It must be stained with a **fluorescent dye** and viewed under **UV light**, revealing a **pattern of distinct bands**. Each **band** represents many DNA fragments of the **same length.**
5. **Analysis:** A **DNA ladder** (or marker) containing fragments of known lengths is run in one well simultaneously. By **comparing the position of the sample bands to the ladder,** the approximate **size** of the unknown fragments can be determined.



Genetic engineering

Genetic engineering is the process by which genetic material is deliberately **manipulated** in order to modify an organism's specific characteristics. In order for the gene to be expressed, it needs to be transferred into the organism.

The gene that needs to be transferred into the organism can be obtained from the following methods:

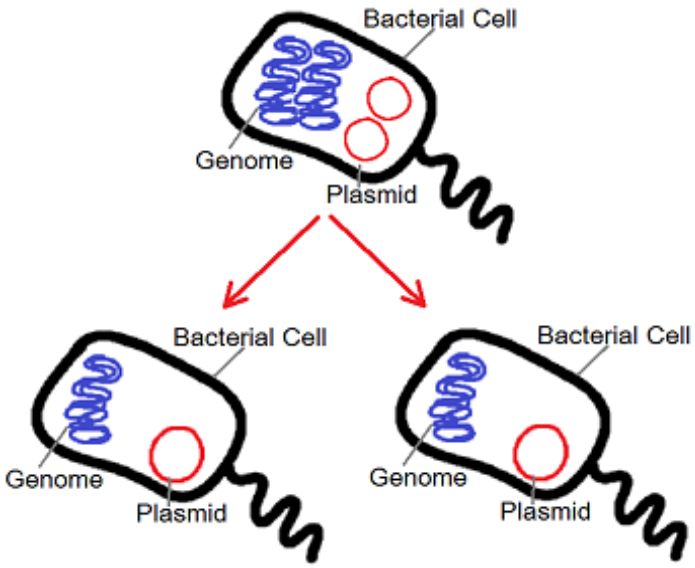
- It can be **extracted** from the donor's DNA.
- It can be **synthesised** from the donor organism's mRNA.
- It can be **chemically synthesised** from nucleotides.

Isolated DNA fragments can be placed in plasmids in the following way:

1. **Plasmid and gene** are cut with the same restriction enzymes **(restriction endonucleases)** to create complementary ends. If sticky ends are missing, they can be added. Plasmids are used as **vectors** because they exist naturally and are small and easy to use.

2. The fragments are incubated with the plasmids. If a plasmid takes up the insert, base pairing takes place between the complementary ends which are then sealed with the use of **DNA ligase** which forms **phosphodiester linkages.**

3. A **recombinant DNA** molecule is created. Recombinant DNA is a combination of DNA from **two different organisms**.



Bacteria which have successfully taken up a plasmid are identified using marker genes. For instance, if a plasmid contains an antibiotic resistance gene, the bacteria will be resistant to the antibiotic, and if grown on the medium, only bacteria that have been successfully transformed will survive. Other types of vectors include bacteriophages, liposomes and yeast artificial chromosomes.

Recombinant DNA can be used to produce human proteins such as:

- **Insulin** – bacteria can be used to produce high-quality insulin. This is advantageous as bacteria replicate faster.
- **Factor 8** for treatment of **haemophilia** – can be produced by genetically modified hamster cells, lower risk of infection as it does not come from donated blood which could be a means of HIV transmission.
- **Adenosine deaminase (ADA)** for treating **severe combined immunodeficiency (SCID)** – ADA is a recombinant human protein that can be given to SCID individuals which lacks ADA enzyme in purine metabolism.

Another form of genetic engineering is **gene editing.** This is the **deletion, insertion**, or the **replacement** of DNA at specific sites of the genome of a living organism.

Genetic technology applied to medicine

Bioinformatics is the science of collecting and analysing biological data using computer software. For instance, it can be used to build a database of gene sequences as well as complete genomes. Bioinformatics can be used to determine the extent of relatedness between organisms and to identify human gene counterparts in other species. It can also be used to study the genomes of parasites such as *Plasmodium* to develop new means of controlling them.

Genetic technology enables screening for genetic conditions such as breast cancer caused by faulty alleles of BRCA1 and BRCA2 genes. If a carrier is identified, they may undergo increased monitoring or preventative treatments, such as prophylactic surgery or drug therapy, to reduce cancer risk. Genetic screening can also be used **for preimplantation genetic diagnosis and prenatal testing** such as **chorionic villus sampling and amniocentesis.**

Genetic technology also allows screening for **cystic fibrosis** and **Huntington's disease.** It enables us to find out if a person is a carrier of the **CFTR gene** which leads to development of cystic fibrosis.

Pre-implantation genetic diagnosis – embryos created through IVF are tested for genetic disorders before they are implanted into the woman's uterus.

Chorionic villus sampling – this test is carried out at **8 to 12 weeks** of pregnancy. A sample of **embryonic tissue** is taken from the placenta and the DNA is then analysed. This test is quicker than amniocentesis.

Amniocentesis is carried out at **14-16 weeks** and a sample of **amniotic fluid** is obtained using a needle which contains **fetal cells**. The DNA is then analysed. Results are available after 2-3 weeks as fetal cells need to be grown in culture first.

There are many social and ethical issues surrounding genetic testing. Some of the viewpoints are:

- There is a **risk of harm to the foetus** as it can lead to miscarriage.
- The outcome of testing might lead to an **abortion.**
- The foetus has a **right to life.**
- **The cost** of bringing up a baby with a genetic disorder.
- **Emotional and mental issues** surrounding the birth of a baby with a disorder.

Gene therapy

Gene therapy is the insertion of a normal allele into target cells to replace a faulty allele, such as the allele which causes cystic fibrosis. Diseases such as severe combined immunodeficiency (SCID) and some eye diseases can also be treated with gene therapy.

There are two types of gene therapy:

- **Somatic gene therapy** where the allele is introduced to the target cells only.
    - Somatic gene therapy is a short-term solution only and needs to be repeated as it does not affect the sperm and egg cells
    - The effects do not last very long.

- **Germ line gene therapy** where the allele is introduced to **embryonic cells**, so the allele may be passed on to future generations (this is not permitted in humans).
    - This is a permanent solution which will be passed down to the offspring.

Ethical and social implications of gene therapy:

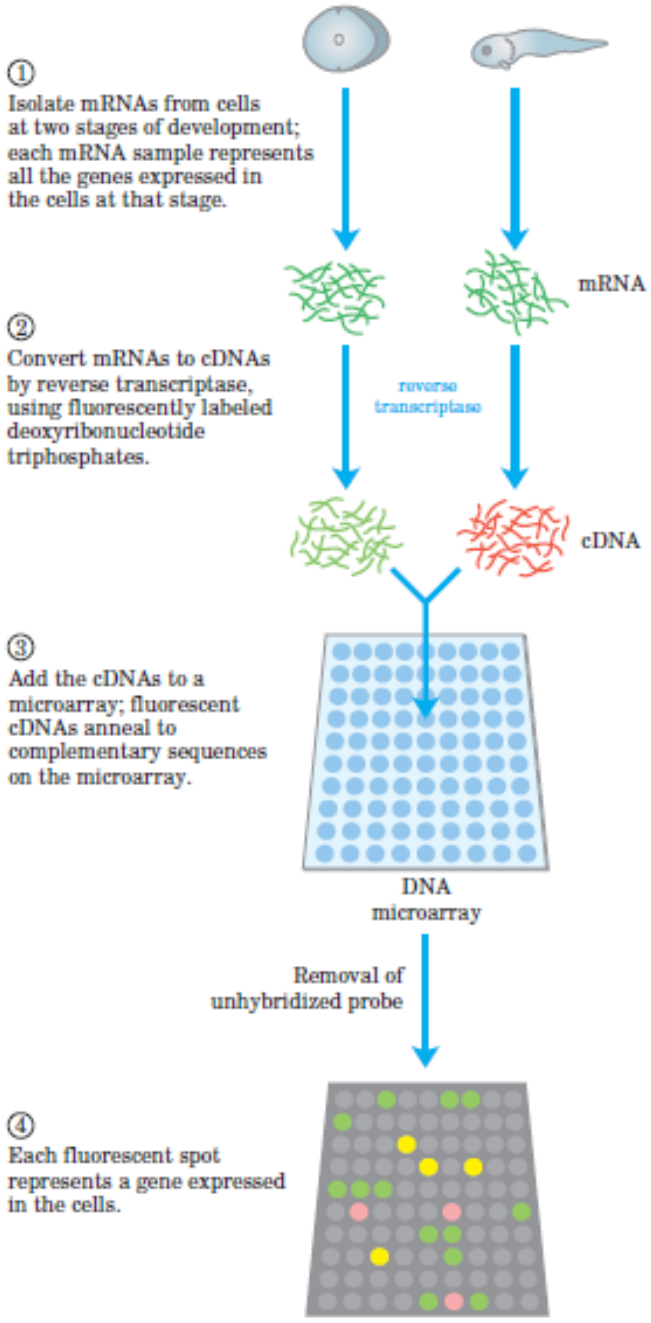
- Some people believe you are violating the unborn individual's human rights.
- We also do not know the impact that intervention will have on the germ cell.
- Our current knowledge on gene therapy does not guarantee that the faulty allele is replaced in the right place. This could lead to other problems in the genetics of the organism.
- Side effects could be very severe causing more pain than the original faulty allele did.

Various types of vectors can be used for gene therapy including viruses and liposomes.

Microarrays

Microarrays are used to analyse **genomes** and to detect **mRNA levels** in studies of **gene expression** by allowing many DNA sequences to be analysed simultaneously. A microarray consists of a solid surface onto which thousands of **short, single-stranded DNA probes** are fixed in an organised grid. Each probe corresponds to a known gene or DNA sequence.

In **genome analysis**, DNA from an organism is extracted and fragmented. The DNA fragments are labelled with a **fluorescent marker** and allowed to hybridise with complementary probes on the microarray. If a fragment contains a sequence complementary to a probe, it will bind. The microarray is then scanned, and fluorescent signals indicate which genes or sequences are present in the genome being analysed. This allows identification of specific genes, gene variants, or comparisons between genomes.



In studies of **gene expression**, microarrays are used to detect and compare **mRNA levels** in different cells or under different conditions. mRNA is extracted from cells and converted into **complementary DNA (cDNA)** using reverse transcriptase. The cDNA is labelled with a fluorescent marker and applied to the microarray. Genes that are being expressed produce mRNA, so their corresponding cDNA will bind to probes on the microarray. The **intensity of fluorescence** at each probe indicates the **level of gene expression**, allowing comparisons between samples.

Microarrays enable the simultaneous analysis of thousands of genes, making them a powerful tool for studying **gene activity**, identifying genes involved in disease, and comparing patterns of gene expression between different tissues or environmental conditions.

Genetically modified organisms in agriculture

The production of crops such as **maize, cotton, tobacco and oilseed rape,** which is the source of vegetable oil and biodiesel fuel, may be increased by using varieties that are genetically modified for **herbicide resistance**.

Crop yield is increased when the fields are sprayed with herbicide. Weeds which **compete** with the plant for resources are killed, thus making it easier for the plant to grow. **Herbicide-resistant crops** can also be created to **increase yield. Soybeans** have been genetically modified to be resistant to the **herbicide glyphosate** (commonly known as Roundup). Farmers can spray entire fields with glyphosate to kill weeds **without harming the resistant soybean crops**, leading to increased productivity.

Insect-resistant crops can also be created to **increase yield**. For instance, **cotton** has been engineered to be resistant to pests like **bollworms** by using genes from the bacterium ***Bacillus thuringiensis (Bt).*** Bollworms are insect larvae that feed on cotton bolls, causing massive yield losses and requiring heavy pesticide use. The Bt bacterium produces a protein called **Cry toxin,** which is toxic to certain insects when ingested. This reduces the need for external chemical insecticide sprays and increases the overall cotton yield.

However, genetically modified seeds need to be purchased each season and are expensive, therefore they are not available to all farmers.

Another example of a GMO used in food production is the **GM salmon**: a gene which regulates a growth hormone is injected into fertilised salmon fish eggs, which causes the fish to reach the desired size in half the time. It also allows them to grow all year instead of seasonally. This leads to a higher yield of larger fish.

Ethical and social implications of using genetically modified organisms (GMOs) in food production:

- Herbicides and pesticides can be **toxic** and are linked to cancer, thyroid problems and other diseases such as Parkinson's in humans.
- GMOs can produce **allergies.**
- Herbicides and pesticides can **damage the ecosystem** by killing plants and animals and damaging the food chain. For example, there are concerns that Bt corn is harming the Monarch butterfly population.
- There is **little research into the long-term effects** of GMOs.