CAIE Biology A-level

Topic 19: Genetic technology

Notes

DNA Sequencing

DNA sequencing begins with the process of **mapping** where the existing information about the genome is used to identify the locus of a particular gene within the genome. The gene is fragmented with the use of **restriction enzymes** and the fragments produced are inserted into **bacterial artificial chromosomes**. This step results in the formation of a **genomic DNA library**. The fragments obtained from the bacterial cultures are again broken down into smaller fragments with the use of restriction enzymes and sequenced with the use of the **chain-termination**. This technique was developed by Sanger and is based on selective incorporation of **chain terminating nucleotides** into a growing chain by **DNA polymerase during replication.**

It occurs as following:

- The DNA sample is divided into **four separate sequencing reactions** which contain all four standard **nucleotides, DNA polymerase, primers required for replication and modified nucleotide**s which have been **fluorescently labelled** for ease of identification.
- When a modified nucleotide is incorporated into a growing chain, replication is terminated
- DNA fragments of different lengths are formed across the reaction vessels
- **High resolution electrophoresis** is used to separate the fragments by size – single base differences can be seen
- The fragments are visualised under **UV light**, thus enabling the base sequence to be read from the bottom of the gel upwards

The rapid advancement of techniques used in sequencing increased the speed of sequencing and allowed whole genome sequencing, that is. **high-throughput sequencing**.

Gene sequencing allows for genome-**wide comparisons** between individuals and between species. Comparing genomes between species is significant as it allows **evolutionary relationships** between species to be determined, and it is also beneficial to **medical research.** Comparing genomes of individuals enables differences to be identified which can then be used for development of personalised medicine tailored to a particular genome, as well as in studies of human diseases.

Apart from allowing genome-wide comparisons to be made, gene sequencing has allowed for the sequences of amino acids in polypeptides to be predicted and has allowed for the development of **synthetic biology.**

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 such a broad spectrum of information for a broad amount of organisms.

DNA profiling

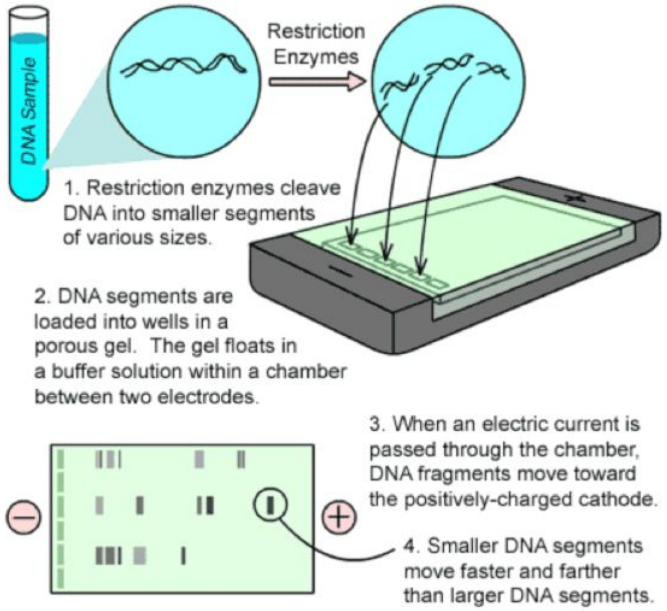
DNA profiling is a forensic technique used to **identify individuals by characteristics of their DNA**. It can also be used to **determine genetic relationships** between organisms. Main techniques used in DNA profiling are:

- **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 following:

    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 environment 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 **70°C** as this is the temperature DNA polymerase works at. **DNA polymerase** creates a copy of the sample by **complementary base pairing using the free nucleotides.**
    5. **This cycle is repeated around 30 times** and gives rise to an amount of DNA sufficient to create a DNA profile.

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

Figure S-2: Gel Electrophoresis



Genetic engineering

Figure SEQ Figure * ARABIC 1
Living Environment Course

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 organisms mRNA
- Could **chemically** synthesise it from nucleotides

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

1. **Plasmid and gene** are cut with the same restriction enzymes called **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 2 different organisms.

In the formation of transgenic microorganisms, **electroporation** is used to stimulate bacterial cells to take up plasmids. Electroporation facilitates the process by **increasing the fpermeability of bacterial membranes,** thus increasing the chance of success. This is achieved via the use of calcium salts and rapid temperature increase from 0 to 40°C. Bacteria which have successfully taken up a plasmid with the help of 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 media, only the bacteria which 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, advantageous as it acts faster
- **Factor VII** 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 transmission of HIV

- **Adenosine deaminase (ADA)** for treating **severe combined immunodeficiency (SCID)** - produced by genetically modified insect larvae, this type of treatment is used when gene therapy is not available

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. This can be used to determine the extent of relatedness of organisms, as well as for identification of human gene counterparts in other organisms. Studying the genome of parasites such as Plasmodium can be used 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. In a case where a positive carrier is identified, they can undergo vasectomy to reduce their risk of breast cancer. 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 **Huntignton'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 and a sample of **embryonic tissue** is taken from the placenta and the DNA is then analysed, this form of testing 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's **a risk of harm to foetus** of miscarriage
- The outcome of testing might lead to an **abortion**
- **Right to life**
- **The cost** of bringing up a baby with a genetic disorder

- **Emotional and mental issues** surrounding the birth 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 a 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 doesn't affect the sperm and egg cell
    - The effects doesn't last very long

- **Germ line gene therapy** where the allele is introduced to **embryonic cells**, thus meaning every cell contains the normal allele.
    - 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 individuals human rights.
- We also don't know the impact that intervention will have on the germ cell.
- As well as this 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.
- Furthermore 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.

Cystic fibrosis

Cystic fibrosis is a genetic disorder caused by a mutation of a single gene which is the gene coding for the **CTFR protein**. CTFR is a channel protein **which transports chloride ions out and into the mucus**, this channel protein makes the mucus watery as it causes water to move into mucus by osmosis. Therefore a **mutation in this gene makes the mucus very thick** as a mutant CTFR protein is less efficient at transporting chloride ions. Sticky and thick mucus causes many problems in **gas exchange, reproduction and digestion.**

Respiratory system:

- Build-up of mucus in the **lungs traps bacteria thus increasing the risk of infection**

- Build-up of mucus in the airways decreases the number of **alveoli** involved exposed to fresh air therefore **reducing the surface area for gas exchange**

Reproductive system:

- Cervical mucus **prevents the sperm from reaching the egg**
- In men, the **sperm duct is blocked** with mucus meaning that **sperm produced cannot leave the testes**

Digestive system:

- The **pancreatic duct** which connect pancreas to the small intestine can become blocked with mucus so the **digestive enzymes do not reach the small intestine**, as a result of that food is not properly digested so **fewer nutrients are absorbed**
- The **mucus lining in the duodenum is very thick** thus reducing the absorption of nutrients.
- Mucus can cause **cysts** to form in the pancreas and damage the insulin producing cells thus leading to **diabetes.**

Genetically modified organisms in agriculture

The production of crops such as **maize, cotton, tobacco and oil seed 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 increases as 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.

However, there is a chance that the **pollen** transfers the gene for herbicide and insect resistance to wild relatives, thus producing **hybrid offspring** which are herbicide resistant weeds.

Insect-resistant crops can also be created to **increase yield**, for instance genetically modified **Bt maize** are able to produce their own insecticide in the form of **Bt toxin**. This makes Bt maize resistant to **corn borers.**

The increasing demand for food in the world can be solved with the use of genetic engineering. Examples include **golden rice** containing **beta-carotene** producing genes taken from daffodils and ***Pantoea ananatis*** which can be used to treat **vitamin A deficiency** as beta-carotene produced by the plants can be converted to vitamin A in **human cells**.

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

Other examples of GMOs used in food production is the **Atlantic 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