### Biotechnology

The **genome** is all the genetic material contained within an organism or cell.

**Gel electrophoresis** is used to separate DNA samples based on their size.

* To carry out gel electrophoresis agarose gel is required, along with a buffer solution, an electric current, and DNA samples.
* DNA is negatively charged, so they move from the positive terminal towards the negative terminal of the electric current through the agarose gel.
* Agarose gel is porous. Smaller DNA samples move through the gel at a faster rate than large samples.
* A comparison ladder with a known number of base pairs may sometimes be used for comparison purposes.
* At the end of the trial the DNA fragments are spread out according to their size (i.e. number of base pairs.) A sample of interest then may be isolated so it can be copied using PCR or recombinant DNA.

**PCR** stands for **polymerase chain reaction**. It amplifies a small DNA sample to make many copies.

* PCR requires the ‘ingredients’ *Taq* polymerase, free nucleotides, a section of DNA containing the gene of interest, 2 different DNA primers (to bracket the gene of interest), a buffer solution, and a thermocycler machine.
* The first step is **denaturing**. The temperature is set to 92°C. The hydrogen bonds of the DNA section are broken, leaving two single strands.
* The second step is **annealing**. The temperature is set to 65°C. This allows the two primers to anneal to the ends of the DNA strands according to complimentary base pairing rules.
* The third step is **extension**. The *Taq* polymerase extends the DNA strand by adding free nucleotides, according to complimentary base pairing.
* These steps can be repeated to produce a large amount of copies of a single section of DNA within a relatively short time.
* Making many copies of a DNA sample is also called **amplifying**.

**DNA sequencing** is highly similar to PCR.

* DNA sequencing uses forms of nucleotides called **dideoxynucleotides** (**ddNTPs**.) Once a dideoxynucleotide has been added to a chain, no more nucleotides can be added. They also contain a fluorescent marker.
* A number of different lengths of DNA are created as a result of the addition of these nucleotides.
* The fragments are then run through gel electrophoresis. A laser reads the fluorescent marker and sequences the DNA.
  + Originally, four different ddNTPs were used in four different solutions.
  + The fragments were then run through gel electrophoresis and the bases had to be manually sequenced.
  + This technique is known as **Sanger sequencing** and it is still used for smaller sequencing projects.
* DNA sequencing has applications in **comparative genomics** where the entire genomes of different species are sequenced and compared.

**DNA transformation** uses **bacterial** **plasmids** in order to create many copies of a gene of interest.

* First a **gene of interest** must be identified. **Restriction enzymes** (also called **restriction endonucleases**) are used to cut up or isolate the gene of interest. Restriction enzymes are named after the bacteria they are derived from.
* The DNA fragment may have either **sticky** or **blunt** ends. Sticky ends have an overhang of nucleotides; so complimentary base pairing helps ensure the DNA is re-joined in the correct places. Blunt-ended fragments are less specific.
* A **bacterial plasmid** is then removed from a bacterium and cut using the same **restriction enzyme** that was used to isolate the gene of interest.
* The DNA fragment then anneals to the plasmid according to complimentary base pairing rules. **Ligase** may be used to seal the fragments together.
* The product is a **transgenic organism** possessing DNA from more than one species.
* The plasmid is now called a **recombinant plasmid** and is reintroduced to the bacterial culture. Heat or an electric current may be used to shock the bacteria in order to reintroduce the plasmid. The gene of interest, if taken up by the culture, will be expressed and produce protein.
* This process can also be referred to as **gene cloning**.

**DNA ligation** can be improved by using restriction enzymes that create a sticky end. Ligation is indiscriminate and will join DNA even from two different species.

**DNA profiling** is done to compare the base pair sequence of two or more individuals.

* **Short tandem repeats** (**STRs**) and **variable nucleotide tandem repeats** (**VNTRs**) are present in all members of a population.
* Each individual has a different number of repeats.
* As each individual has their own pattern of repeats there is a basis for identification of individuals.
* The process requires use of both PCR and gel electrophoresis.
* The reliability of the results is related to the number of restriction enzymes used in the sample.

In biotechnology a **vector** is a tool that can be used to transfer DNA between organisms. They can be effective where use of bacteria is not wanted or needed. There are three main types.

* **Plasmids** can be directly donated to the recipient organism. Though this process is efficient the plasmids are typically not very stable in the recipient’s body.
* **Viruses** can be useful vectors as they exist to inject their genes into foreign cells. However, the human immune system attacks viruses. Viral DNA insertion can also disrupt normal gene regulation and lead to the development of cancer.
* **Liposomes** are small spheres surrounded by a phospholipid bilayer. They can be artificially created. The bilayer fuses with the membranes of the cells to deliver its contents.

**Gene transformation** is the basis of gene therapy. It involves inserting functional copies of a gene into a target to prevent or remedy genetic disease. Gene delivery is usually achieved using a vector.

**Microarrays** are used to test thousands of sections of DNA at one time. They were developed through tumour research.

* Small sections of DNA are placed on a glass plate. The DNA sections are single-stranded and their gene/chromosome of origin is known.
* The DNA responsible for the tumour will create mRNA and then protein which will be expressed as a tumour in the same way other proteins are copied and expressed.
* mRNA is focused on over DNA as this allows only the genes that are being expressed to be investigated.
* mRNA will then be isolated from the tumour. **Reverse transcriptase** (an enzyme used by viruses) the creates single-stranded **cDNA** (**copy DNA**.) A red fluorescent marker is placed on the cDNA.
* The same process is carried out for a normal (non-tumour causing) version of the gene, with a green fluorescent marker placed on it.
* The DNA chip is washed over with the red-marked cDNA which anneals to some sites.
* The chip is also washed with the green-marked cDNA and some will anneal.
* The microarray chip is then read with a laser. The computer investigates the difference in gene expression between the normal and non-normal sections of DNA.
* If the red probe only is attached to the DNA it is active in producing the tumour. If it is green, it’s active in producing the normal version of the gene.
* If neither or both colours have annealed, the gene is not relevant or does not have an effect on the tumour.
* Microarrays do not identify or diagnose the problem, but they do identify the location of the problem. The differences between the marked genes can then be investigated.

**Gene probes** are single strands of DNA, complimentary to a gene of interest. In biotechnology they can be used to identify, locate, or isolate a gene on a chromosome.

Biotechnology has many applications in human health.

* **Microarrays** can identify the genes associated with human diseases and illnesses.
* **Electrophoresis** has many applications in the identification of individuals and forensic science, as does **DNA profiling**.
* **DNA profiling** is also used to find family relationships between individuals and to carry out paternity / maternity tests.
* When carrying out any procedure it is essential to follow guidelines to ensure the cross-contamination of samples does not occur, especially as samples are typically very small.

### Applications of Biotechnology

* **Recombinant DNA** has been found to have many applications in agriculture.
  + It is used to produce crops with faster growth rates, higher yield, and disease resistance.
  + **Golden rice** has been engineered that has a high amount of beta-carotene (a precursor of vitamin A.) It is hoped that this genetically-engineered crop will be able to combat nutritional deficiency in third-world countries.
  + The bacterium ***Bacillus thuringiensis***has been successfully introduced to cotton plants through recombinant DNA technology. The *Bt.* cotton produced is a natural pesticide for the *Heliothis* caterpillar.
  + **Roundup ready** canola is produced by the company Monsanto. The canola is resistant to the herbicide **Roundup** (also produced by Monsanto) allowing farmers to safely use the herbicide to kill weeds. The active constituent in Roundup is glyphosate.
* **Recombinant DNA** has also been used for other purposes.
  + Following oil spills, recombinant bacteria have been introduced with the ability to degrade some of the components of oil. This helps lessen the devastating impact of oil spills and aids the removal of the oil from the natural environment.
  + Recombinant viruses have also been introduced into feral rabbit populations. Following the emergence of feral populations resistant to viruses such as *Myxomatosis* this technology has been applied and produced a new virus that induces infertility in the rabbits, hindering the growth and/or sustenance of wild populations.
* **DNA identification technology** (electrophoresis, profiling, sequencing) has found many applications in conservation.
  + Identification techniques can be used to monitor or research individuals of endangered species in order to track or learn more about them.
  + Identification techniques can also be used to assess gene pools and preserve genetic diversity in breeding programs, especially those for critically endangered species or those with an otherwise smaller gene pool.
* **DNA identification technology** can also be used in agriculture.
  + It assists in selective breeding by allowing genetic markers to identify desirable traits.
  + It can also be used for quarantine purposes, helping to identify pest or invasive species.
* **Transgenic organisms** (those with DNA from more than one individual) have also been engineered through biotechnology.
  + “Enviropigs” have been produced which have a lower level of phosphorus in their waste.
  + Cows have also been bred that produce milk similar to human breast milk.

### Effects of Biotechnology

Especially in regard to biotechnology, the **three Rs** must be addressed when considering research involving animals.

* **Replacement:** the substitution of conscious, living animals for insentient material. Especially important where the same result can be achieved without harming animals.
* **Reduction:** the number of animals used to obtain a certain level of precision and amount of information is important to consider. The minimum number of animals to be used must be statistically determined, however this number must not come at higher expense to individuals. A valid conclusion must also be reached from the research, otherwise it is deemed unethical.
* **Refinement:** decreasing the severity of inhumane procedures that have to be used. The impact of any procedure on an animal’s wellbeing must assessed. Strategies must then be implemented to eliminate and/or minimise the impact of any procedures carried out.

**Agricultural biotechnology** has many advantages and disadvantages. Typically, the benefits outweigh the harm.

* The benefits of agricultural biotechnology are mostly related to the crops produced.
  + Hardier crops give a higher yield
    - Crops can be bred to be resistant to salt and drought
    - Can also be bred to be resistant to herbicides, meaning less weeds impeding on crop growth
* Disadvantages also include financial elements.
  + Market dominated by few companies (e.g. Monsanto)
  + Reduction in variety of alleles
    - Biodiversity reduced
    - Loss of genes
  + Possible link drawn to increase in allergies
  + Unknown long-term effects

**Transgenic organisms** also carry the potential to have negative effects. It is possible for an organism to spread an adaptation meant to defend against a pest to the pest organism, rendering the biotechnology useless. By breeding many organisms with the same gene or genes, genetic biodiversity is also compromised.

The practical applications of **medicinal biotechnology** are staggering. However, so are the number of ethical considerations and possible problems.

* Genetic tests could be used to determine the risk of insuring people. There are a number of potential confidentiality implications associated with this use of technology.