

Chapter 1.7 Biotechnology: tools and techniques

Science Understanding

DNA can be extracted from cells.

Modern techniques can be used to analyse even small amounts of DNA.

Segments of DNA can be multiplied using the polymerase chain reaction (PCR); their base sequences can then be identified and analysed.

- Describe PCR, including the roles of
 - heating and cooling
 - primers
 - free nucleotides
 - heat-resistant enzymes.

DNA sequencing enables mapping of species genomes. Electrophoresis is used to sequence DNA. The results may be displayed in an electropherogram.

- Describe electrophoresis.
- Interpret electropherograms that illustrate DNA sequences.

DNA profiling identifies the unique genetic makeup of individuals.

The results of electrophoresis can be used to construct DNA profiles. They may be displayed in an electropherogram or in a table of data.

- Interpret electropherograms and tables of data that illustrate DNA profiles.
- Explain how differences in DNA fragments, identified by DNA profiling, can be used; for example, in forensic science.

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In this chapter students improve their **numeracy** capability by solving problems using calculations and by learning about the impact of ICT on the development of genome sequencing to enrich their **ICT** capability. Students who study the text on genetic testing will have the opportunity to appreciate why respecting and engaging with different cultural views is essential to ensuring the technology is fully inclusive, thus acquiring **intercultural understanding**.

Biotechnology

The use or application of science to provide solutions to problems is called technology. **Biotechnology** refers to forms of technology that are based around biology. It is not new, because humans have been using biological techniques and processes for thousands of years to make bread, wine, cheese and other products. In the last few decades, with the advancement of scientific discoveries, new and continually developing ranges of tools, processes and technologies are emerging that enhance human well-being and potentially help the planet and its ecosystems.

This chapter will present and discuss a range of the most commonly used tools and techniques used in biotechnology and the following chapter (1.8) will examine a range of applications, along with ethical, economic and other issues associated with the technologies.

Extracting DNA from cells

Genetic material can be manipulated. In other words, it can be deliberately altered by scientists e.g. as a result of transferring DNA from one species to the DNA of another species. It is possible to move DNA between species as the genetic code is universal. This means that, for every organism, the same mRNA codon codes for the same amino acid. This makes it possible; for example, to introduce a human gene that can code for insulin to a bacterium which can then make the human protein insulin. As has already been discussed, the human genome has 46 chromosomes and around 21,000 protein-coding genes. Given that the average size of a gene is many thousand bases on the template strand, it is not hard to appreciate how difficult it is to locate or select a particular gene that scientists wish to work with.



Helpful Online RESOURCES about extracting DNA from human cells

To visit a website on this topic use this URL or QRC:

<<http://learn.genetics.utah.edu/content/labs/extraction/>>



Gene probes

Gene probes are single-stranded DNA or mRNA segments that have been constructed in the laboratory. They can be used to select particular genes because they have radioactive or fluorescent markers that enable them to be readily located. The probes work by binding in a complementary manner to the 'gene of interest'. When it binds to the probe, the gene of interest is said to have been **hybridised** with the radioactive probe. Refer to *Figure 171*.

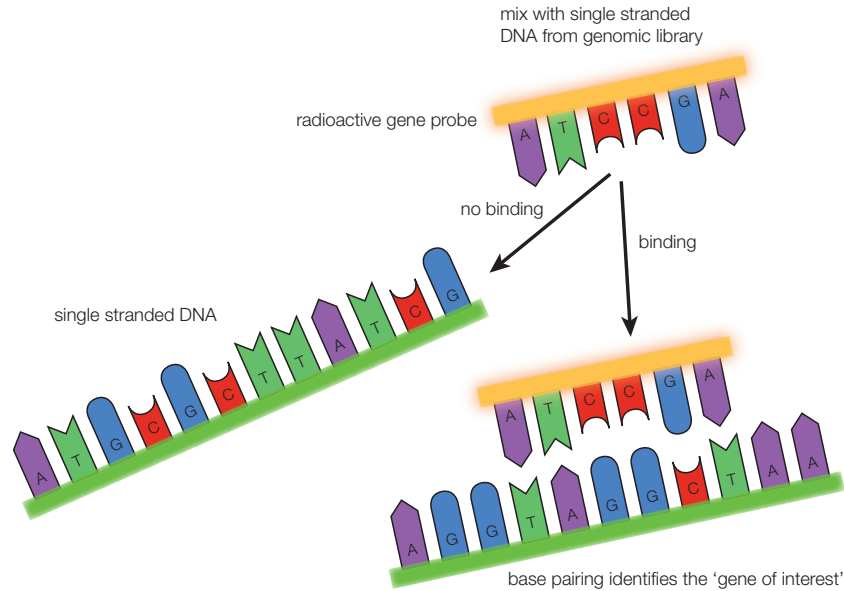


Figure 171 The use of gene probes

Figure 172 reveals a grid of DNA fragments making up the human chromosome 17. Scientists have isolated this to be the site of a defective gene responsible for many cases of inherited breast cancer. The grid represents pieces of DNA which have been spotted onto filter paper and following this, an X-ray plate has been superimposed showing fragments tagged with a radioactive marker. These tagged fragments, some of which correspond to genes, have been hybridised to help researchers map genes on a chromosome.

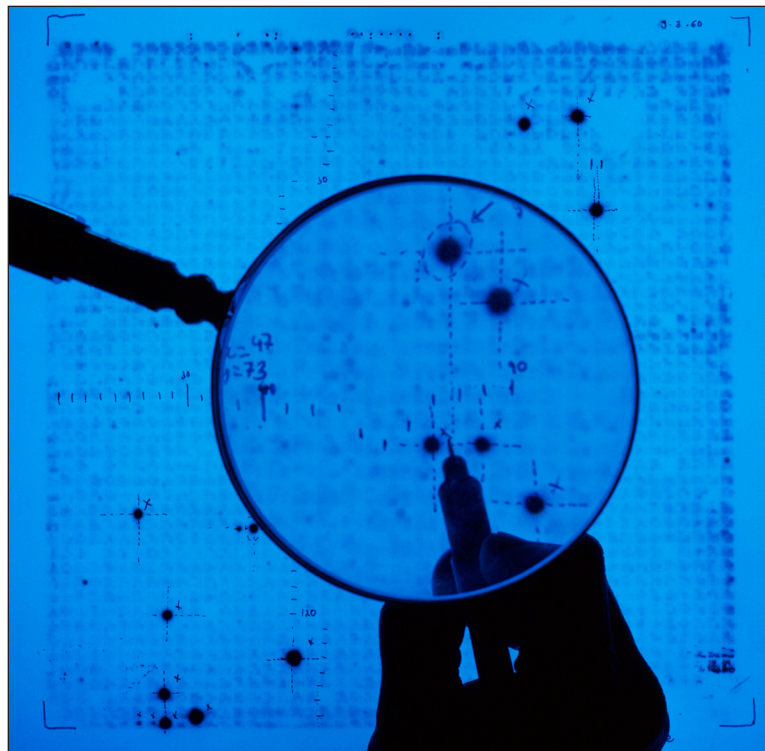


Figure 172 Using hybridisation to locate the gene for breast cancer

Restriction enzymes

Bacteria produce **restriction enzymes** (endonucleases) which cut DNA naturally and thus act as a defence mechanism against viral DNA that may invade the bacteria. These enzymes were discovered in 1970 and not long after they were used in a type of biotechnology called **genetic engineering** to cut DNA at specific sites and thus help remove or isolate genes. Most restriction enzymes recognise short nucleotide sequences; around 4 to 8 and these sites are called restriction sites. They work by making an incision or cut through the sugar phosphate backbone of the DNA. The enzyme named EcoRI was extracted from the bacteria *E Coli* and cuts DNA in a staggered fashion as can be seen in *Figure 173*.

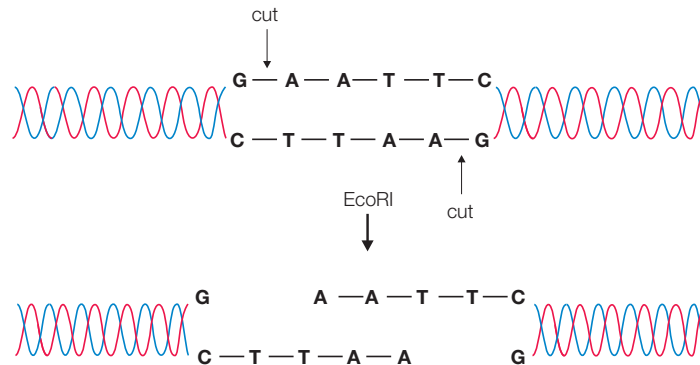


Figure 173 The action of a restriction enzyme 'EcoRI'

As seen in *Figure 173*, the enzyme cuts between the G and A bases and the effect is that the DNA is cut leaving overlapping ends. When preparing to transfer DNA, the same restriction enzyme is used for the host and the donor so that the cuts are made in the same way. When this occurs, the overlapping sections are referred to as 'sticky ends' and the sticky ends enable complementary binding between host DNA and donor DNA. An enzyme, **DNA ligase**, is used to join the host and donor DNA. The incorporation of DNA into bacteria like this is called bacterial transformation.

Refer to *Figure 174* which shows the joining of bacterial plasmid DNA (host) and human DNA (donor) using a restriction enzyme to cut the DNA and a ligase enzyme to join the two pieces together; the resulting DNA is termed **recombinant DNA**. Techniques that permit or produce recombinant DNA are called recombinant DNA technology.

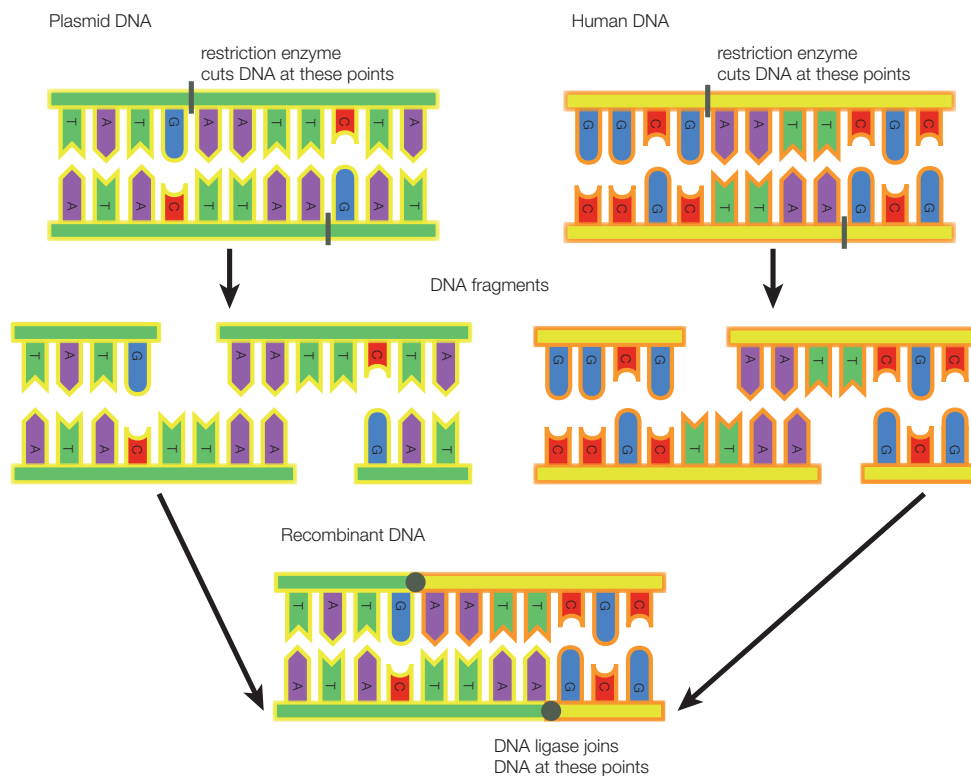


Figure 174 The production of recombinant DNA



Helpful Online RESOURCES about restriction enzymes

To visit a website on this topic use this URL or QRC:

<<https://www.youtube.com/watch?v=pDHCHa1C85Y>>



Polymerase Chain Reaction (PCR)


 Often the sample of DNA that scientists have to work with is from the nucleus of cells (nuclear DNA) and there isn't a lot of it. To solve this problem a process called the **polymerase chain reaction (PCR)** was developed that can be used to create many more copies of the same DNA until there is sufficient for analysis or other application. DNA that has been put through the PCR process is said to have been multiplied, or amplified. PCR is carried out in a thermal cycler shown in *Figure 175*.



Figure 175 A thermal cycler

To carry out this process, the following ingredients are required:

- the DNA template that needs to be copied
- **DNA primers** which are short sections of DNA that bind to either side of the template (target) DNA
- free DNA nucleotides which are required to assemble the new DNA copies
- a Taq **DNA polymerase** enzyme from bacteria that can tolerate higher temperatures than human DNA polymerase
- buffer solutions to provide a suitable chemical environment for the process.

Three stages can be identified in the process:

1. Denaturing

The template DNA is heated to about 95°C so that the hydrogen bonds holding the double helix together are broken and this exposes the bases.

2. Annealing

The mixture is cooled to around 60°C and this enables the primers to bind to the complementary bases that are exposed on the template DNA.

3. Extension

The temperature is raised to approximately 72°C to enable the nucleotides to bind with the exposed DNA bases and the Taq DNA polymerase will then join them into a DNA strand.

This way each of the original strands has formed a new complementary strand. The DNA formed can be heated and separated and the above process repeated. New DNA is made into many copies in an exponential fashion. Refer to *Figure 176* which represents three cycles of PCR. Note that sometimes mitochondrial DNA (mtDNA) is collected and analysed. This may decrease the need for the PCR process because each cell has many mitochondria, so many copies of the mtDNA already exist.



ECV - All about the polymerase chain reaction (PCR)

To hear an *Essentials Concept Video* on this topic use:

<<http://essentialseducation.com.au/resources/sace2/biology/ecv-pcr/>>



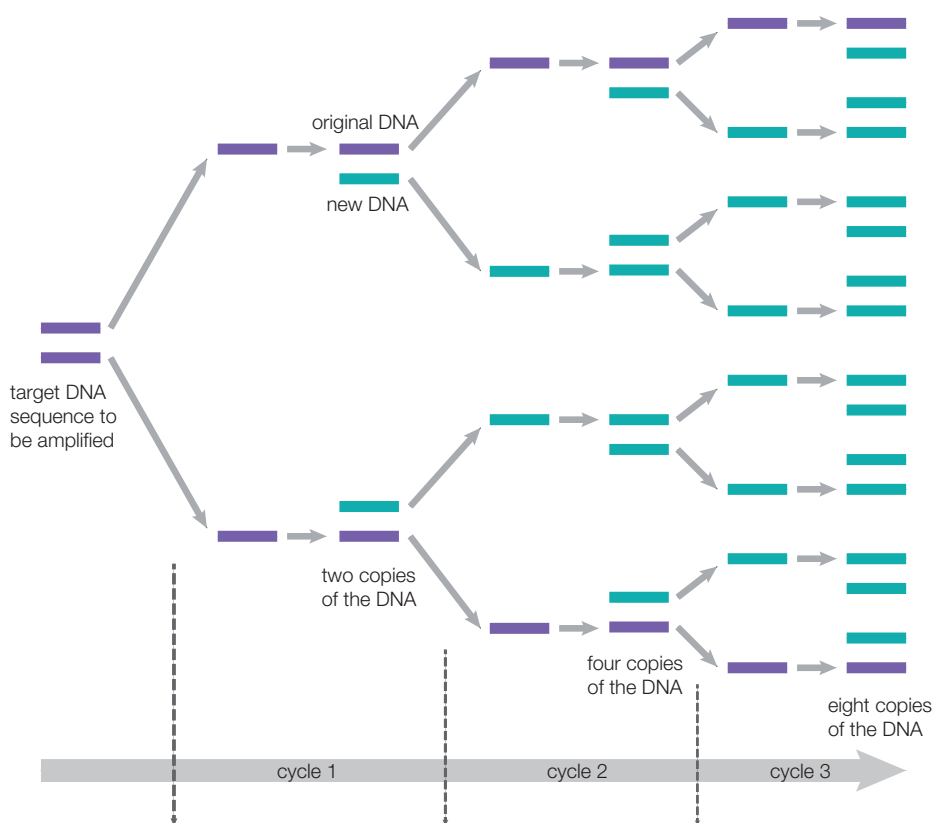


Figure 176 The process of PCR

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C Intercultural understanding: Inclusive genetic testing

It is now possible to test for hundreds of human genetic disorders. In many cases this involves using PCR with primers that bind to the genes associated with them. The presence or absence of the disease-causing mutation in the amplified DNA can then be determined by DNA sequencing. Common genetic tests include those for sickle-cell anaemia, cystic fibrosis and the blood clotting disorder called haemophilia. The use of such tests permits the identification of the disease before the onset of symptoms. Many can also be used to identify carriers of faulty-disease causing genes who do not have the disease but have the potential to have children with the disease. In some cases, identification of a genetic disease can even be confirmed before birth. Many genetic tests are in development and new ones are introduced every year; for example, a more accurate test for bowel cancer called *Colvera* that became available in Australia for the first time in 2017.

Until recently, genetic testing required the patient to go to a clinic or hospital, have the test there, and then have a follow-up with a doctor or genetic counsellor to accurately interpret the results. This helps people begin to come to terms with what can be confronting information and the fact that genetic tests do not always provide 'clear-cut' results. Furthermore, the implications of confirmation of a serious genetic disease go well beyond the self – they have implications for family members, employment and decisions about how lifestyle should or can be adjusted. It is possible to advise and counsel patients like this in a way that is culturally inclusive as well. This is important because people of different cultural origin may hold different views and customs that influence how they receive, process and react to information like genetic test results and related advice that may be about having blood tests, decisions about family planning, or the termination of a pregnancy.

Direct-to-consumer (DCT) genetic testing provides a more patient-independent way to get genetic information about the risks of a genetic disease developing at some point in the future. On paying for the test, a person sends a sample (usually saliva or an inner cheek swab) which is analysed by a laboratory overseas who then send the results directly back to the person who sent the sample. There is no involvement of a health or genetic professional which promotes inaccurate processing of the information and incorrect decisions about how it should be used. While DCT genetic testing may appeal to some whose cultural views forbid for instance blood tests (a blood test is not required), transmission of genetic information like this is very unlikely to be respectful of or engage with broader cultural views thereby undermining its inclusiveness.

Helpful Online RESOURCES about PCR

To visit a website on this topic use this URL or QRC:
 <<https://www.youtube.com/watch?v=laKXv1JqBtY>>



EVA - The polymerase chain reaction (PCR)

To view an *Essentials Video Animation* on this topic use:
 <<http://essentialseducation.com.au/resources/sace2/biology/eva-polymerase-chain-reaction>>



Gel electrophoresis

? **Gel electrophoresis** is a technique used to separate proteins or fragments of DNA according to their size and charge. A gel is prepared in a thin layer and placed in a container as shown in *Figures 177(a) and (b)*.

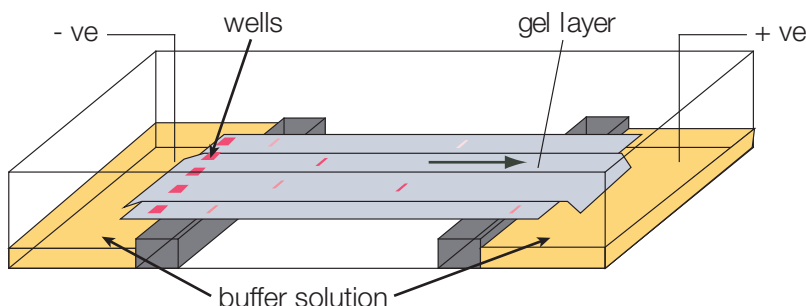


Figure 177 (a) The principle of gel electrophoresis

If samples of DNA fragments are loaded into the wells and the electrophoresis tank is connected to a power supply to generate an electric field, the DNA fragments (-ve charge) will be repelled away from the wells (-ve charge) - the smaller the DNA fragments (less DNA base pairs) the further the fragment moves (refer to SIS1.7). An **electropherogram** is a plot of results from separation done by electrophoresis.

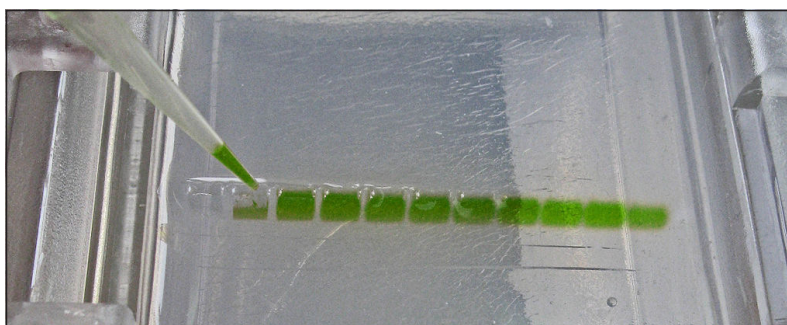


Figure 177 (b) An actual gel electrophoresis tray

Figure 178 illustrates a ladder of known sizes of DNA fragments, (in base pairs) that can be used to identify unknown fragments. Sometimes chemicals can be added to make these fragments easier to observe e.g. fluorescent dyes.

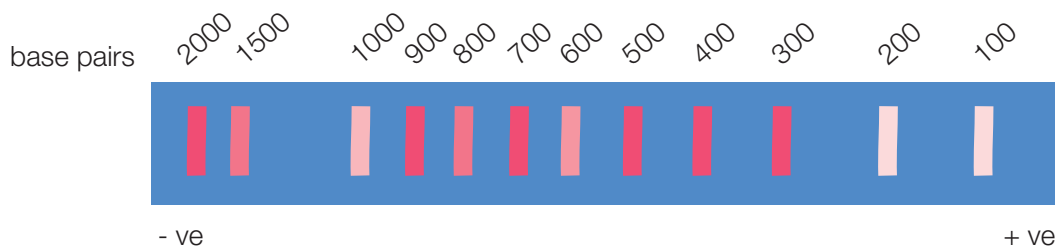


Figure 178 A sample ladder of DNA fragments

Helpful Online RESOURCES for (virtual) gel electrophoresis

To visit a website on this topic use this URL or QRC:

<<http://learn.genetics.utah.edu/content/labs/gel/>>



DNA profiling

DNA profiling (or DNA fingerprinting as it was once called) is a technique that identifies the unique genetic make-up of individuals or compares DNA from different sources without mapping the entire genome. An application is in forensic science where DNA bands from a suspect match those found from samples (e.g. blood, semen, hair) at a crime scene; refer to *Figure 179*.

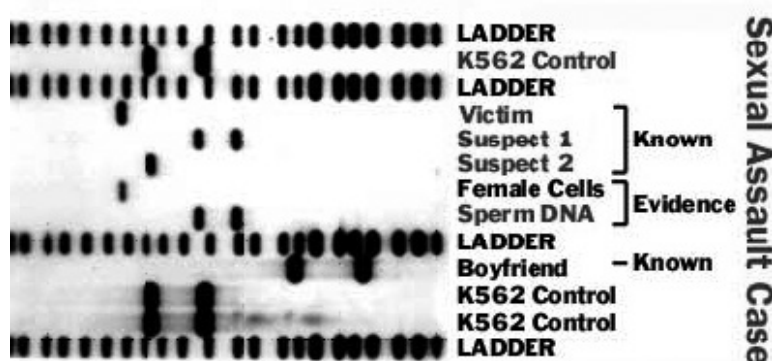


Figure 179 An example of the use of DNA profiling for forensic purposes

Helpful Online RESOURCES for DNA profiling (or fingerprinting)

To visit a website on this topic use this URL or QRC:

<<https://www.youtube.com/watch?v=AkBUriMK9u8>>



ECV - All about genetic fingerprinting and profiling

To hear an *Essentials Concept Video* on this topic use:

<<http://essentialseducation.com.au/resources/sace2/biology/ecv-fingerprinting-profiling/>>



Another use here includes paternity testing whether the DNA of a man can be compared with a potential offspring's DNA.

The technique has proven to be successful in comparing what are termed **short tandem repeats (STR)** from one individual to another. STR's are short sections of the DNA, usually around 2 to 6 base pairs, that are repeated a huge number of times and the pattern has been found to be highly variable between individuals and thus a good technique for identification. An example of a profile using gel electrophoresis showing the fluorescent markers is seen in *Figure 1710*.

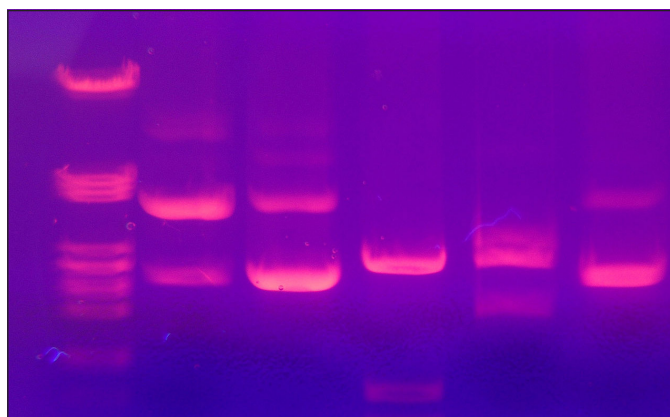


Figure 1710 Gel electrophoresis using fluorescent markers

Electrophoresis and electropherograms

Capillary electrophoresis has mostly replaced the use of gel separation techniques but the general principles of electrophoresis still apply:

- DNA fragments move from the negative electrode to the positive electrode.
- Shorter DNA fragments move faster and further through the tube than longer ones.

Frederick Sanger, a British biochemist developed a technique called the chain termination method to sequence the DNA bases in segments of DNA. Currently the process of sequencing DNA is highly automated and carried out by DNA sequencing machines.

In sequencing DNA the strands are tagged at the last nucleotide by a specifically coloured molecule. It is the colour of the tag, detected using a laser that indicates the nucleotide at the end of the DNA fragment. Refer to *Figure 1711*.

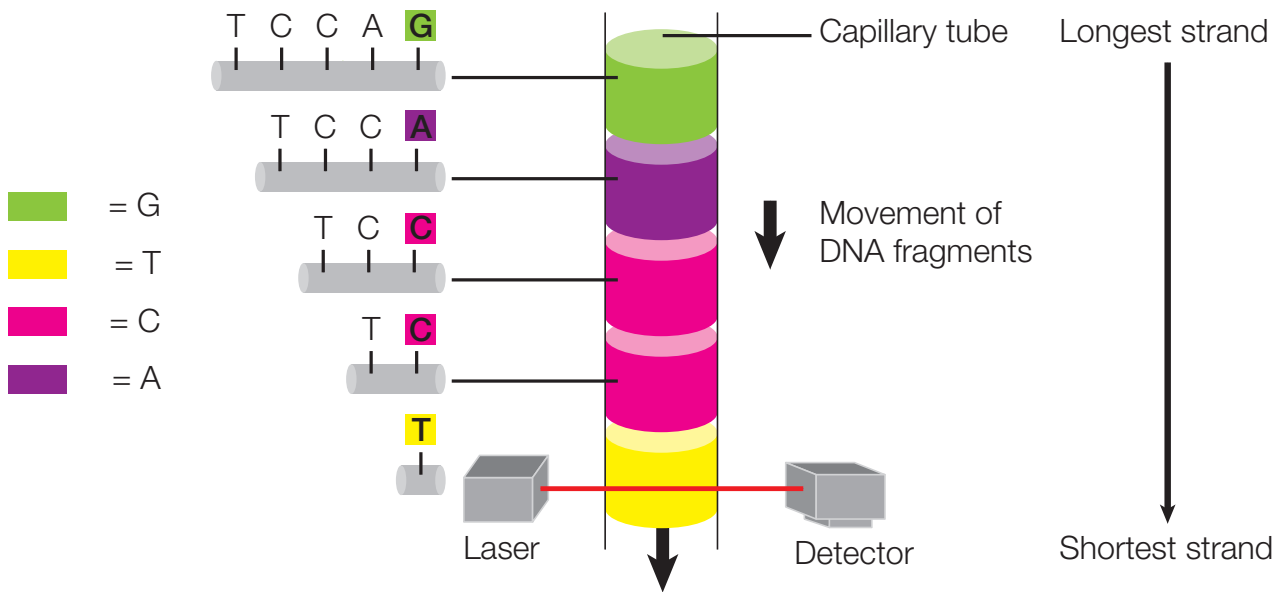


Figure 1711 The principle of electrophoresis

The graph produced is called an electropherogram where each peak signifies a specific DNA nucleotide in the DNA sequence. Refer to an example shown in *Figure 1712*.

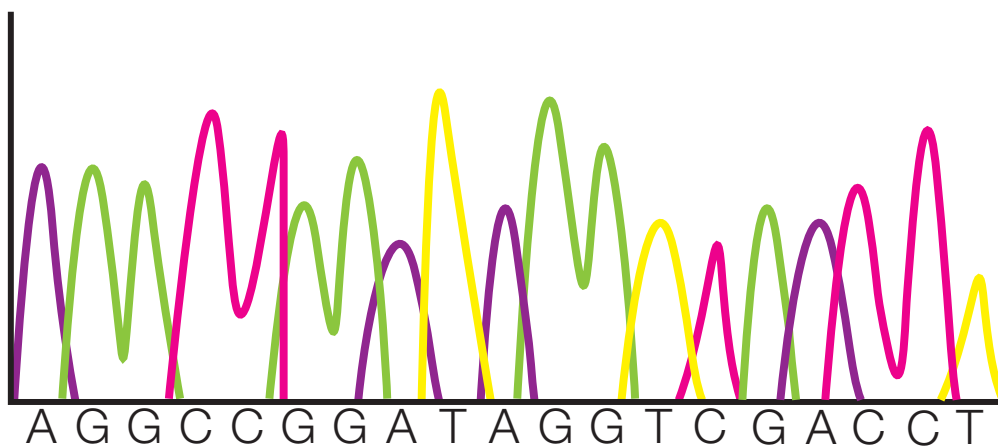


Figure 1712 An example of an electropherogram

DNA profiles and electropherograms

Humans have about 99.9% of their DNA in common, which means they are very similar in the order, number and type of DNA bases. At several locations, however, these base pairs are different and it is these differences that enable scientists to distinguish between the DNA of different people. The variable regions that are used are called short tandem repeats (STR's) and these are known as markers that are found in specific locations known as loci – they are usually around 2-5 base pairs long – eg GATA.

The STR's are highly variable between people and this enables forensic scientists to establish which DNA belongs to which person. The length of an STR varies between individuals and are signified by a number. For example '11' implies that the allele has 11 repeating units whereas '13' implies 13 of the repeating units. Each person inherits one of the alleles from each parent.

Again using electrophoresis DNA fragments are separated by size and the information is shown by a graph called an electropherogram. See *Figure 1713*.

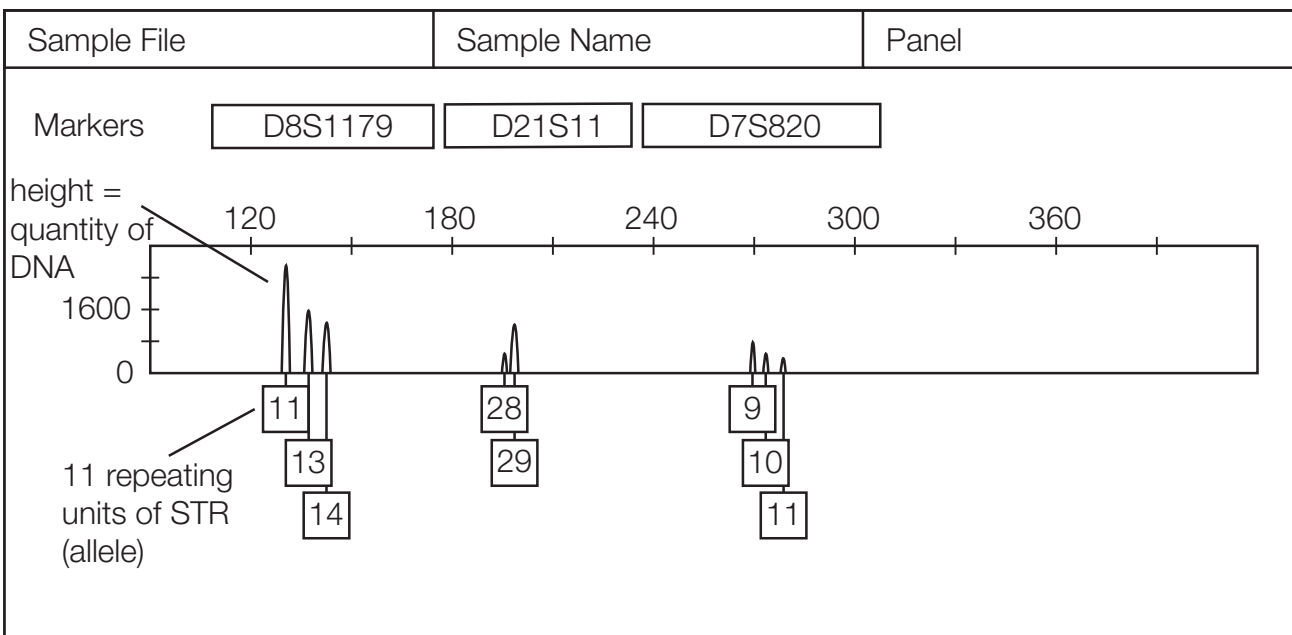


Figure 1713 Another example of an electropherogram

This type of data can also be displayed in a data table; an example is shown below.

In the example below there are 3 sets of data shown at 3 markers. One set of data is from evidence taken at the scene of a crime, the next DNA is from the suspect and the last is from the victim.

The numbers in the table represent the numbers of the repeating STR found at the allele position. Typically forensic scientists examine 13- 15 specific markers in determining someone's genetic profile.

	DNA evidence at crime scene				Suspects DNA				Victims DNA			
	Allele				Allele				Allele			
Marker	1	2	3	4	1	2	3	4	1	2	3	4
D8S1179	11	13	14	-	11	14	-	-	11	13	-	-
D21S11	28	29	-	-	29	-	-	-	28	29	-	-
D7S820	9	10	11	-	9	-	-	-	10	11	-	-

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DNA sequencing

DNA sequencing involves techniques that are used to determine the actual sequence of DNA bases (A, T, G, C) in the gene segment of DNA and the entire genome of an organism (in which case it is often called genome sequencing). The process was first developed with small, simple organisms such as bacteria and also viral particles and culminated in the first sequence published of the entire human genome through the Human Genome Project (HGP) that was completed in 2003.

This work continues in laboratories around the globe as scientists continue to map the genomes of different species. Collating and analysing this information continues to advance our understanding of genetics, gene control and evolutionary relationships between organisms. It also enhances our ability to use biotechnology and genetic techniques to enhance the well-being of humans and other species.

DNA sequencing has advanced from its first appearance in 1977 to the latest computer generated large-scale automated analyses. Refer to *Figure 1714* of a circular genome map showing shared genetic material between humans (outer ring) and (from inner ring outwards) chimpanzee, mouse, rat, dog, chicken and zebrafish chromosomes. The colours within each ring form a 'heat map', the pattern of which represents hot spots of shared genetic material. This map was created using 'Circos' software which visualises complex genetic comparisons between different species. *Note: This diagram is provided for interest only and specific details are not required knowledge in this course.*

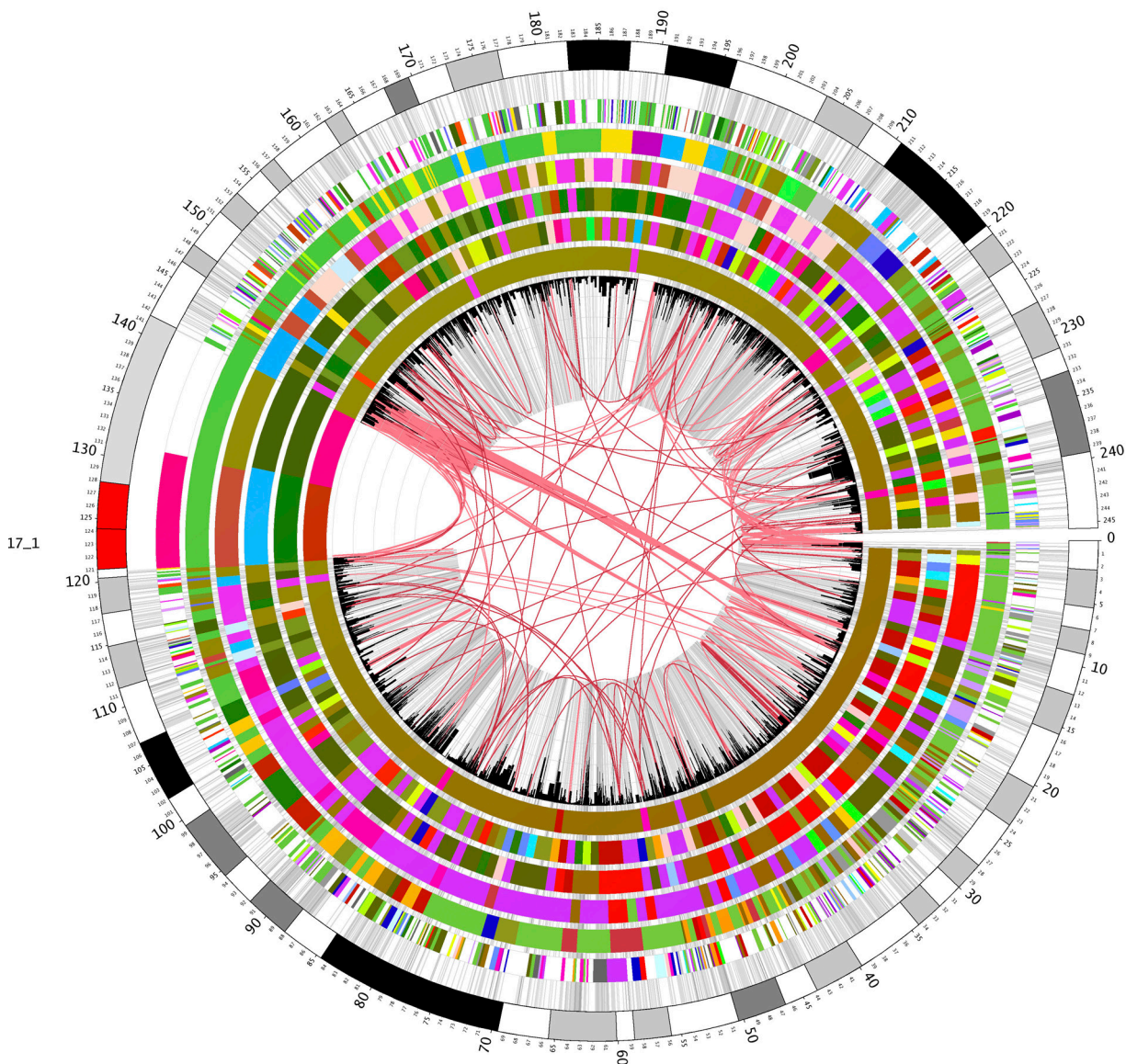


Figure 1714 A map comparing genomes of different species.

C ICT: Genome sequencing

The development of the technique of genome sequencing provides a good example of the impact of ICT on the development of biology. The first genome sequencing methods took a long time and were very expensive. The determination and then publication of the human genome sequence in 2003 was a major achievement but it took over 14 years and cost about \$3 billion. By 2013, the development of advanced high speed ICT-driven automated DNA sequencers was yielding human genome sequences in only three days for as little as \$1000. In December 2016, the UK-based company Oxford Nanopore Technologies announced genome-sequencing-in-a-day capability using an easy to operate portable DNA sequencer called a MinION.

The applications for super-fast, cheap genome sequencing are numerous and very wide-ranging. It is already possible for biotechnology companies to sequence thousands of genomes a year. This greatly increases the ability of them to contribute to major research projects; for example, those looking into understanding and developing new treatments for cancers or genetic disorders. The technologies also make possible more personalised medicines that, informed by an individual's own genome, respond more effectively to drug treatments with less side-effects. Even more ground-breaking is the potential ability to sequence genomes from a huge number of people so that in the not too distant future analysis of them may usher in what might be called prognostic genomics – an individual's genome will be used to discover vulnerability to disease and to inform the steps to be taken before it develops, or to assist treatment choices if it has developed.

Transferring genes between species

Gene transfer is a technique in which genetic material is transferred from one organism to another. Where this is done by scientists it is for the purpose of modifying the genetic information of the organism receiving the genes so that it may acquire a new, improved characteristic or produce a different protein of benefit. The process is called **transgenesis** (and often GM or genetic modification) and organisms formed in this manner are called transgenic organisms. Gene transfer can be achieved chemically, physically or by using a virus or plasmids from a bacterium. Viruses and plasmids used in this way are called vectors. These methods are explained in more detail in the sections below.

Microinjection

Microinjection is a physical method of gene transfer where DNA from a donor is injected directly inside the cell and into the cell nucleus through a very thin micropipette. This technique is highly flexible but is slow and requires highly skilled technicians. This method is also used in vitro fertilisation to improve the chance of fertilisation of an ovum with a sperm (the technique is called intra-cytoplasmic sperm injection).

Refer to *Figure 1715* which illustrates the technique of microinjection.



Figure 1715 Microinjection

Bacterial plasmids

It has already been stated that plasmids are small, circular DNA structures capable of independent replication and found in prokaryotes such as bacteria. These plasmids are able to transfer DNA between bacteria and this characteristic has been utilised by scientists so that they can be used as a vector (**plasmid vectors**) in transmitting genes to other species. The desirable gene for transfer is incorporated into the plasmid for further incorporation into a recipient or host cell. Bacterial plasmids can also be used to mass produce copies of genes in a process called gene cloning (refer to EVA)

The use of a virus

Viruses naturally infect plant and animal cells and in the process transfer their DNA into the DNA in the nucleus of the cells they are invading. If the viral DNA is modified to contain a 'desired' gene this can also be transferred into a host or target cell nucleus. These are called **viral vectors**.

The aim of these and other gene transfer technologies is to integrate selected genes into host DNA so that the host cell can express the gene and make a desirable gene product, or this leads to production of an organism that does this, or one that has an altered phenotype that is desirable.

Figure 1716 represents a brief summary of three gene transfer techniques:

- On the left (a) microinjection; note that the red strand represents a gene of interest and the two sets of scissors the restriction enzyme used to remove it from the surrounding DNA.
- In the middle (b) using a plasmid vector; this (recombinant) plasmid may be introduced into plant cells directly or indirectly by placing it in the cytoplasm of bacteria that naturally infect the plant cells.
- On the right (c) another technique using metallic particles where DNA is fired into the nucleus of plant cells by a gene gun.

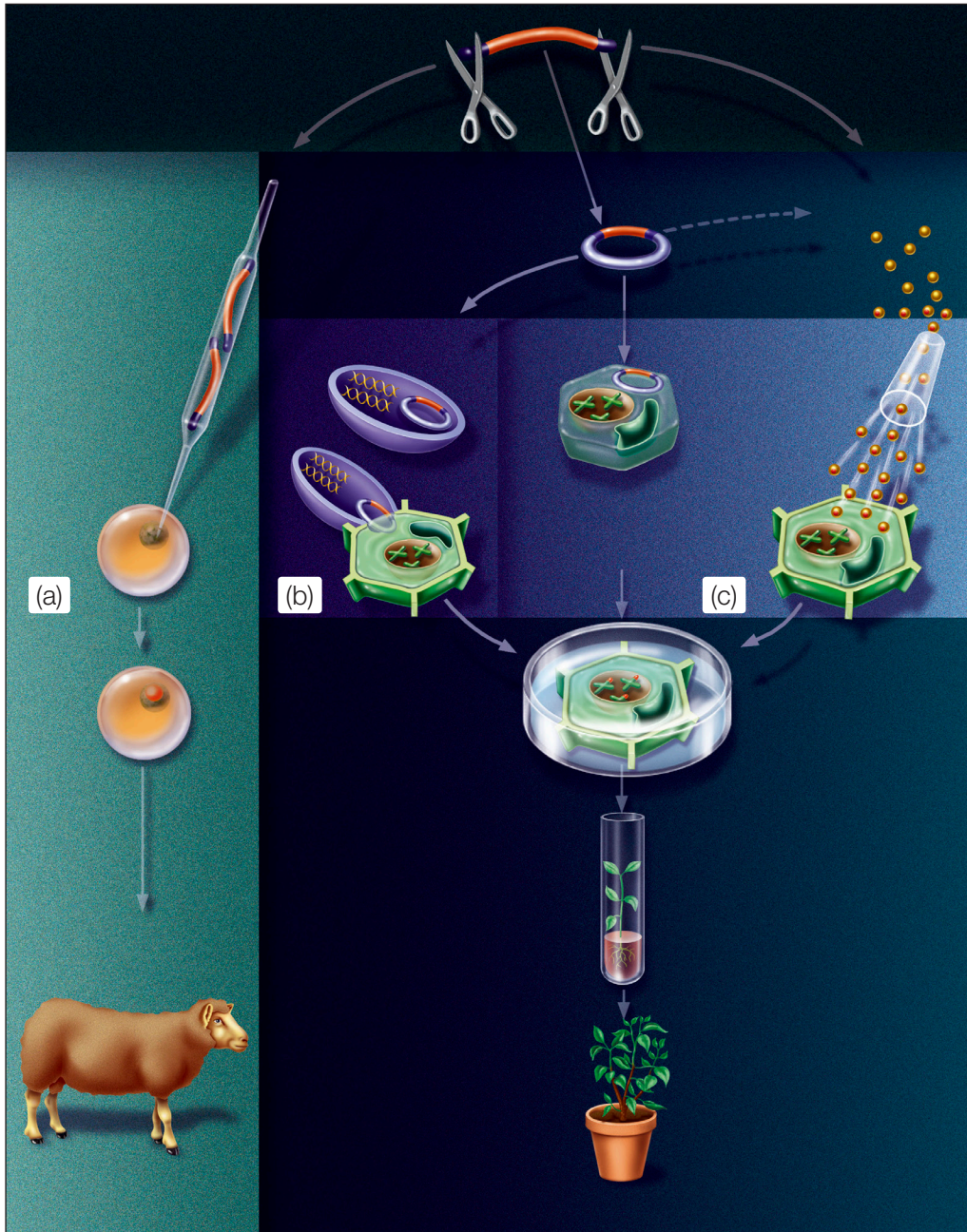


Figure 1716 (a), (b) and (c) Gene transfer techniques

▶ EVA - The cloning of genes

To view an *Essentials Video Animation* on this topic use:

<<http://essentialseducation.com.au/resources/sace2/biology/eva-cloning-genes/>>



CRISPR

'Clustered regularly interspaced short palindromic repeat' (or **CRISPR**, pronounced 'crisper') technology is a genomics editing tool that has the capacity to edit parts of the gene by removing, adding or altering sections of DNA.

There are two major molecules involved in the CRISPR process:

- an enzyme Cas 9 which acts as a pair of molecular scissors to cut DNA at specific sites
- guide RNA which ensures that the Cas 9 cuts at the right spots on the DNA.

This technology is currently the fastest and best system for editing genes. It can also be applied to the DNA of virtually any species. Chapter 1.8 will examine some further aspects of CRISPR technology. *More information about this technique is given in the following chapter.*



Helpful Online RESOURCES about how CRISPR works

To visit a website on this topic use this URL or QRC:

<<https://www.youtube.com/watch?v=0Mkie4R7haA>>



Key Concepts

1. Using biotechnology involves an impressive array of ever developing tools and techniques to manipulate DNA.
2. Gene probes are short pieces of DNA or RNA that bind to, and thus help scientists to select particular genes (or locate the position of genes on chromosomes).
3. Restriction enzymes were discovered in bacteria and provide a very useful DNA (cutting tool) that bind to specific sites on DNA and cut it, allowing particular genes to be removed from chromosomes.
4. The polymerase chain reaction (PCR) enables multiple copies of DNA to be made from a small, original sample that can then be used in laboratory work and forensic science.
5. DNA profiling identifies the unique genetic make-up of individuals. This enables DNA from different sources to be compared e.g. in forensic science and paternity testing.
6. Gel electrophoresis can separate fragments of DNA based on their charge and size. It has many applications from DNA profiling, DNA sequencing and evolutionary comparisons of DNA.
7. DNA sequencing determines the actual base sequence in segments of DNA and nowadays is carried out by high-speed automated DNA sequencing machines.
8. There are a range of techniques used to transfer genes between species. They include using vectors e.g. plasmids or viruses or a physical method such as microinjection.
9. CRISPR is a new technology that can be used to edit and/or transfer genes. It is potentially a very powerful process, the development of which is only in its infancy.

What have you learned?

Key Terms

- biotechnology.....
- genetic engineering
- gene probe
- hybridisation
- restriction enzyme
- DNA ligase
- recombinant DNA
- polymerase chain reaction (PCR)
- DNA primer
- DNA polymerase
- gel electrophoresis
- electropherogram
- DNA profiling
- short tandem repeats (STR)
- gene transfer
- transgenesis
- microinjection
- plasmid vector
- viral vector
- CRISPR

Knowledge and Understanding

1. Briefly describe how it is possible for a bacterium to produce a human protein. *(No details of processes are required.)*

2. State two characteristics of molecules that are able to act as gene probes.
 - a)
 - b)
3. Often, during PCR, the DNA, enzyme and nucleotide mixture is heated to temperatures well in excess of 60-70°C.
 - a) State why it is necessary to heat the DNA in this manner.

 - b) Explain why this heating would prevent the use of human DNA polymerase to catalyse a reaction.

4. Describe the benefit in genetic engineering when the same restriction enzyme is used to cut the DNA of both the host and the donor.

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5. In DNA profiling, bands or segments of DNA appear at different spots in the electrophoretic gel. Explain why segments appear at different spots after electrophoresis.

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6. Microinjection is not always successful in altering the genetic information of a host. State why, giving a likely reason.

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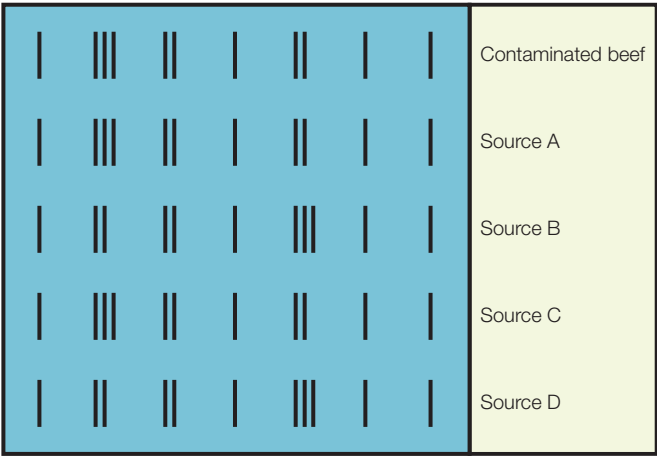
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7. PCR is carried out ‘in cycles’ in a thermal cycler machine. As a result of each cycle the amount of DNA is doubled; in other words, after one cycle, there are 2 copies of the sample of DNA to be amplified, after two cycles there are 4 copies, and so on. How many copies of the original sample of DNA will have been produced after 10 PCR cycles? Show your working.

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8. A cattle industry carried out a study using DNA profiling to determine if beef from various sources was contaminated. Refer to the DNA profile below from the study.



- a) How many sources of beef were contaminated?.....
- b) What percentage of the total number of sources used in the DNA profile above were contaminated? Show your working.

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Application, Analysis and Evaluation

9. State and briefly give reasons regarding which technique would most likely be used in the following situations:

a) Multiplying or amplifying the DNA of a suspected criminal's hair follicle cells found at a crime scene.

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b) Comparing human DNA with chimpanzee DNA, looking for differences in coding for a particular protein.

.....

c) Analysing DNA in an embryo, looking for susceptibility to a particular genetic abnormality.

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10. *In order to answer this question, you will need to refer back to Chapter 1.2 and Figures 128(a) and 128(b) indicating which codons code for particular amino acids.*

One of the techniques used to locate genes of interest is to use the protein product to trace the gene.

If the beginning of an amino acid sequence was: **serine - glycine - proline - methionine**

a) Give a sample DNA sequence that could have coded for this.

.....

b) If a complementary gene probe was made to help locate the gene, what would its DNA sequence be for the given section?

.....

11. In a hypothetical experiment, a gene for silk produced by a species of spider is incorporated into the DNA of female sheep. It is discovered that these sheep then go on to produce 5.0g of the spider silk per litre of milk per day.

a) The sheep above makes 8 litres of milk per day. How much spider silk would this contain? **Show your working.**

.....

b) Clones of the sheep are produced. How many of these sheep would be needed to make 2 kg of the spider silk per day? **Show your working.**

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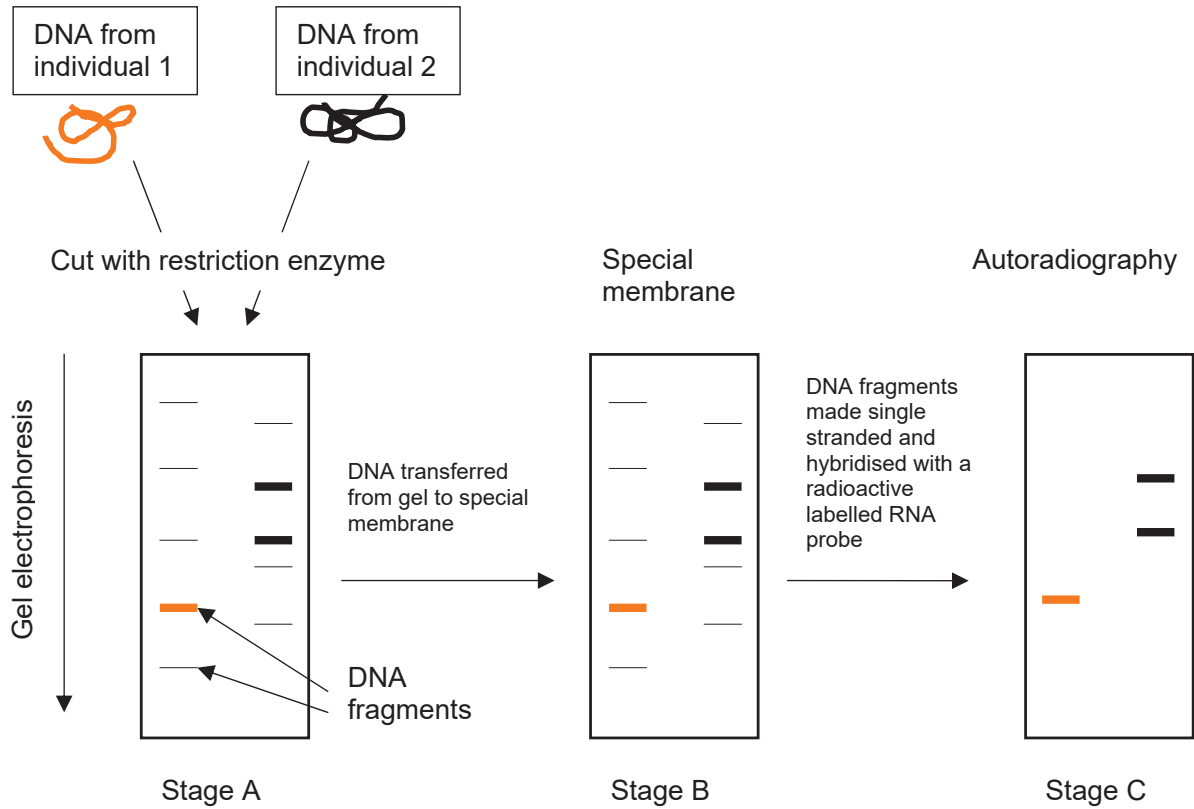
12. When a restriction enzyme cuts a DNA molecule, the cuts are not even and leave single-stranded ends. Suggest why this is an advantage in recombinant DNA technology.

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13. Refer to the diagram below showing a procedure that can be used to compare DNA and answer the questions below.



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- a) Explain why this procedure could be used to identify a suspect at the scene of the crime.

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- b) In Stage C the DNA of the gel has been hybridized with a radioactive probe. State the purpose of this step in the procedure.

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14. Short tandem repeats (STR's), found in segments of DNA, have been found to be very useful in discriminating between individuals in the process of DNA profiling. The segments have very low mutation rates. Explain why these low mutation rates are also an advantage in DNA profiling.

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15. Compare the techniques and applications of DNA sequencing and profiling.

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16. Cystic fibrosis is caused by a single faulty gene. Gene therapy trials have been undertaken trying to deliver the functional gene into the cells of the respiratory tract using viruses.

a) Explain how such a technique might work.

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b) This technique has experienced quite a high failure rate and also comes with risks to the patient. Suggest possible reasons for this.

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17. CRISPR is a recent advance in gene editing and modification.

a) State the function of the protein 'Cas 9' in the process.

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b) Explain how 'guide RNA' works in ensuring that the action of 'Cas 9' occurs in the right position.

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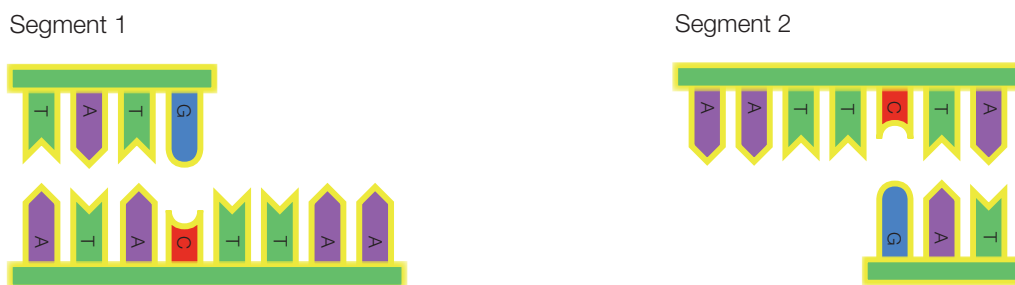
c) Predict how the 'guide RNA' might end up in the wrong position.

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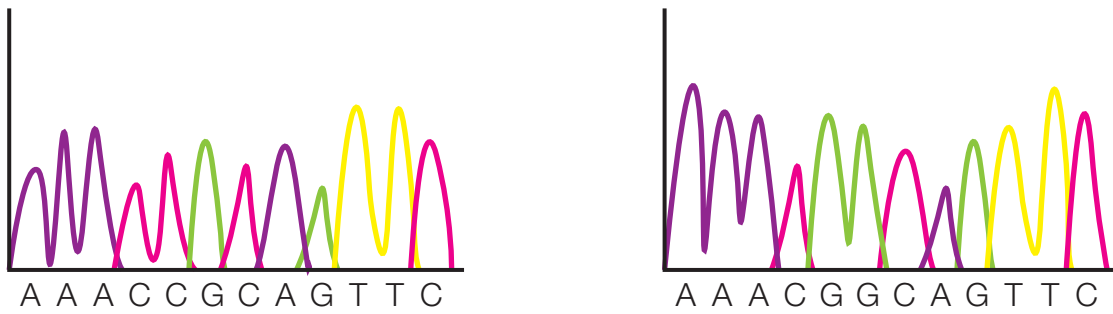
18. These two segments of DNA were produced by the action of a specific restriction enzyme.



a) On the left side, draw a diagram of the single DNA segment prior to the action of the enzyme.

b) Indicate on the diagram, or below, the specific recognition site for this enzyme and where the cuts occur.

19. Using capillary electrophoresis, DNA fragments can be separated by their molecular weight (size). A DNA sequence can be worked out using this technique and a laser to detect bases marked with fluorescent dyes.



a) State 2 factors that may affect the rate at which molecules move through the capillary.

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b) Compare the DNA sequences shown below in the 2 electropherograms.

(i) State the change which has occurred from sequence 1 (left) compared with sequence 2 (right).

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(ii) Using your knowledge from earlier in this Topic, suggest what possible impact this might have on the polypeptide produced when this sequence is transcribed and translated.

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20. The results of electropherograms are used to construct DNA profiles and thus are key in forensic science. Refer to *Figure 1713* and the table to answer the questions that follow.

a) State the importance of STR's in the production of a DNA profile of an individual.

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b) Explain why each individual has 2 alleles for each marker shown.

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c) Explain, giving reasons which marker does not provide strong support as to whether the suspect might be guilty.

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d) Explain how the other 2 markers do provide evidence to support the fact that the suspect may have been at the scene of the crime.

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Science as a Human Endeavour 1.7 - PCR and gene cloning

Development

New technologies improve...procedures, data collection and analysis

The polymerase chain reaction (PCR) is a straightforward way to produce a huge number of copies of DNA from even the tiniest of samples. Billions of copies of specific fragments of DNA can be copied in only a few hours. The PCR technique was first developed in California in the United States in 1985 by a DNA chemist called *Kary Mullis*. It quickly had an enormous impact on biological research and in particular the science of genetic engineering. Using data collection and analysis made possible by PCR, scientists have become able to match suspects to crime scenes (or prove them to be innocent), confirm family relationships, identify victims of catastrophic natural disasters, and help to diagnose or monitor diseases. Mullis was awarded the Nobel Prize for Chemistry in 1993 and subsequent refinements of PCR have cemented its claim to be one of the key biological advances of the 20th century.

You may need to refer to the websites below to answer the exercises that follow:

1. The PCR technique developed by *Kary Mullis* was an improvement on DNA-copying procedures that existed at the time in part because of its simplicity. Describe the equipment and materials the procedure he invented requires and what they are needed for.

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2. Although the original PCR technique has been refined many times, errors during the replication of DNA fragments limit the number of 'good copies' that can be produced. One solution to this problem has been the development of a technique called gene cloning. Briefly explain how the use of gene cloning makes possible the production of many more 'error-free' copies of genes, improving the efficiency of the gene copying process.

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Helpful Online RESOURCES for more about PCR

To visit a website on this topic use this URL or QRC:
 <<http://learn.genetics.utah.edu/content/labs/pcr/>>



Helpful Online RESOURCES for more about gene cloning

To visit a website on this topic use this URL or QRC:
 <<https://www.youtube.com/watch?v=b-gtadSdHS0>>

