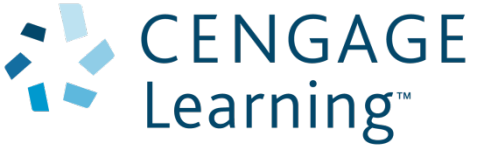
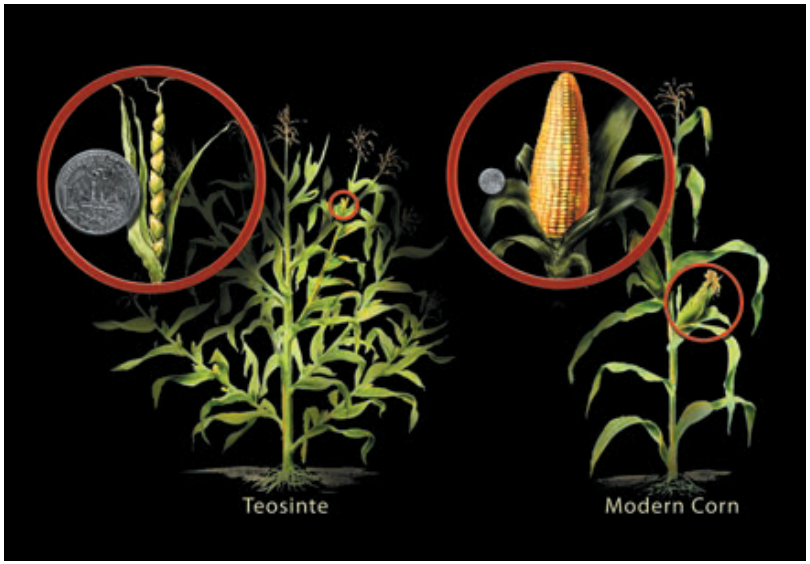
Chapter 6:
Biotechnology – its tools and techniques



Introduction to biotechnology

The term **biotechnology** refers to the artificial tools and techniques used on organisms or the products of organisms to make a product or solve a problem for human benefit.

Biotechnology combines biology and technology to improve our lives and the health of our planet.



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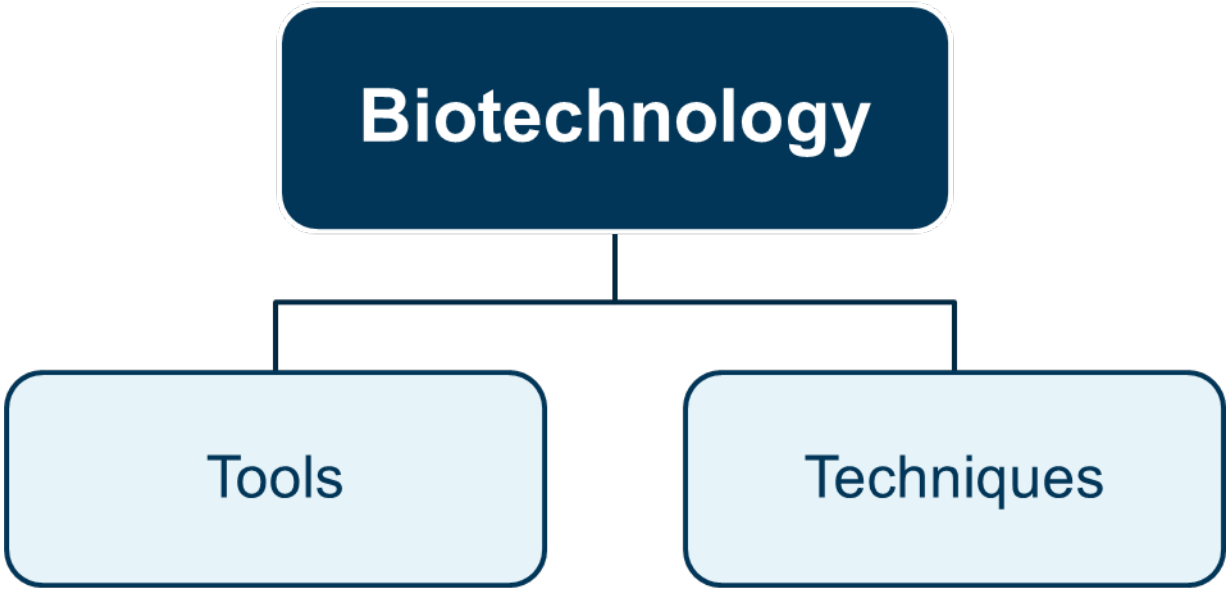
Example:

The corn you eat is the domesticated version of a wild grass called teosinte. Teosinte was selectively bred for thousands of years before the modern corn plant developed.

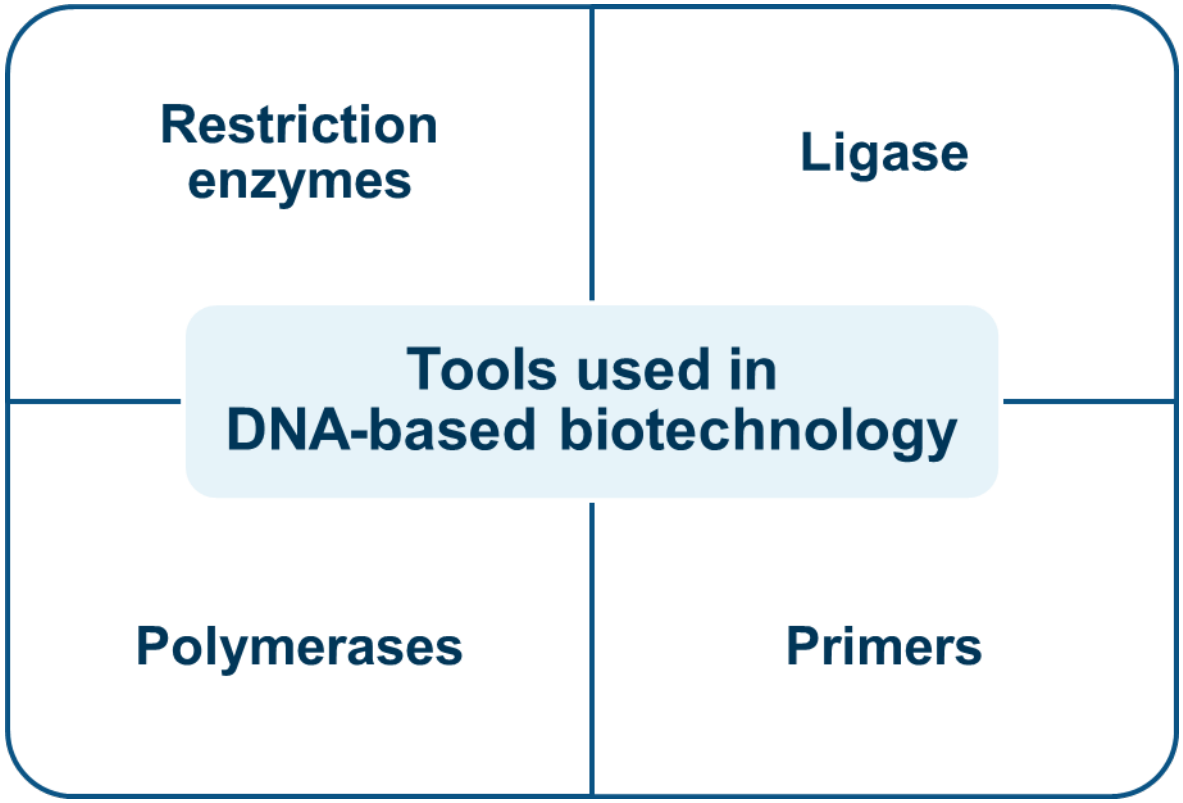
DNA-based biotechnology

Modern biotechnology involves processing and manipulating DNA. Biotechnology has its own set of specialised tools and techniques, which are mostly derived from organisms. These include tools for synthesising, cutting and pasting DNA, along with tools and techniques for viewing and analysing DNA.



DNA-based biotechnology: tools

Tools are required for synthesising, cutting and pasting, viewing and analysing DNA. Many of these tools are enzymes.

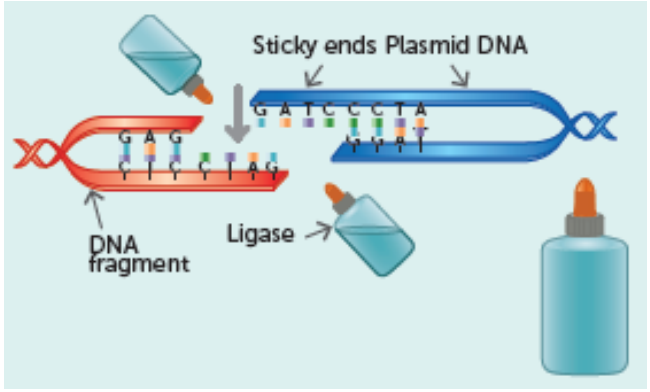


Restriction enzymes

Restriction enzymes are enzymes that cut DNA molecules at **recognition sites** (specific nucleotide sequences), usually 4–8 bases long. The main sources of restriction enzymes are bacteria. Naturally occurring restriction enzymes protect bacteria by cutting foreign DNA and then removing invading organisms.

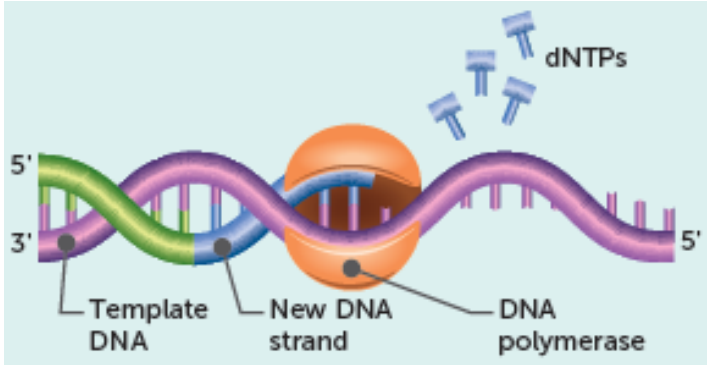
Ligase

Ligase is an enzyme that seals/reassembles DNA fragments in the process of ligation. It sticks the backbone together. (Note: DNA polymerase is the enzyme that joins complementary bases, but the complementary base pairing is temporary. Ligase catalyses a more permanent covalent bond, closing up the sugar–phosphate backbone, forming a phosphodiester bond and sealing the backbone.)



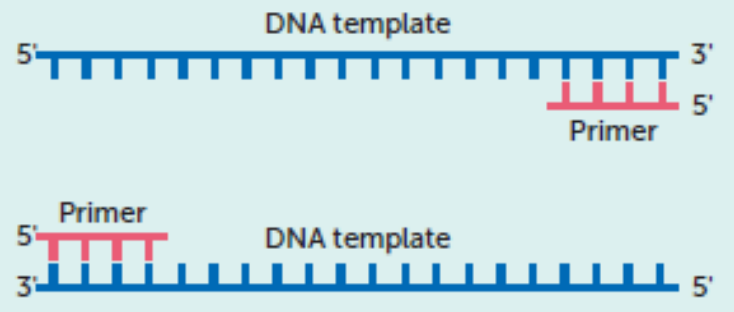
Polymerases

Polymerases are a class of enzymes (found in all living things) that synthesise new strands of DNA/RNA based on a template strand and according to complementary base-pair rules. DNA polymerases are vital tools in biotechnology, enabling efficient and accurate amplification of DNA templates.



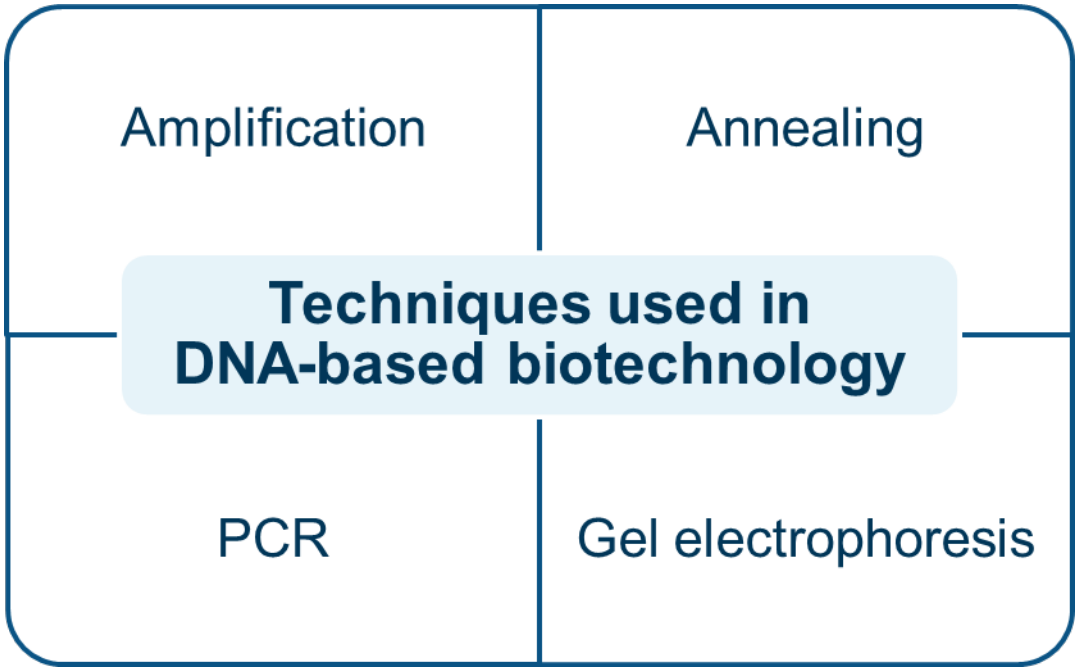
Primers

Primers are short fragments of single-stranded nucleic acid (DNA or RNA). They are made in a laboratory, are about 20 nucleotides in length and are usually labelled with an enzyme, or radioactive or fluorescent dye tag. They are attracted to a target DNA strand by complementary base pairing, and they demarcate on a strand of DNA where elongation/synthesis should start.



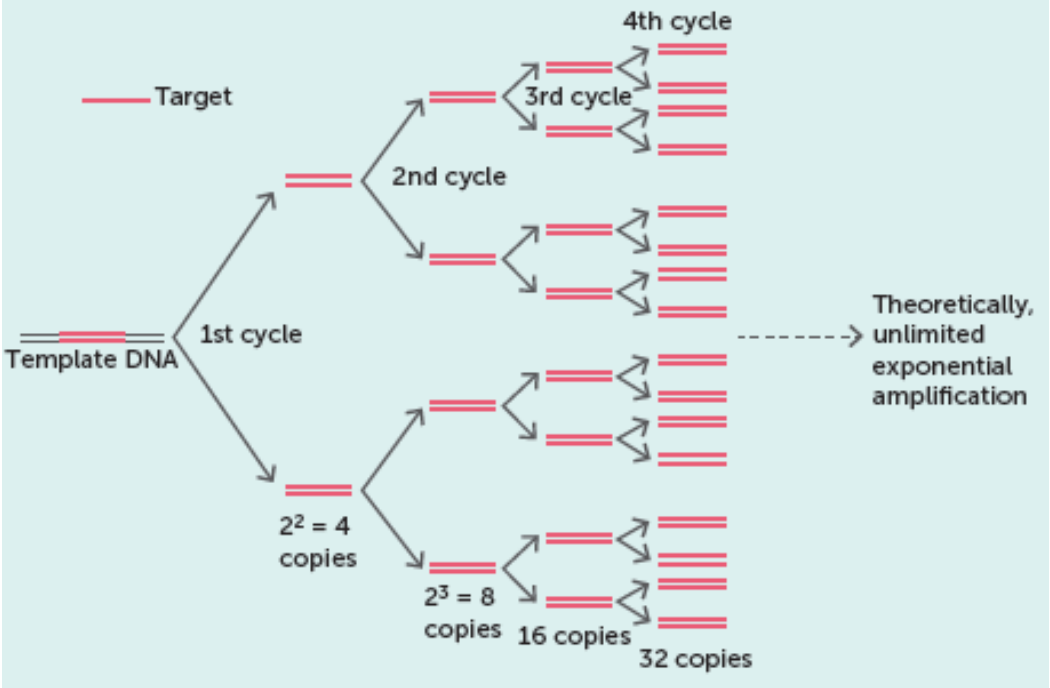
DNA-based biotechnology: techniques

DNA tools are used in conjunction with DNA techniques to help make products or solve biological problems. Techniques such as PCR and gel electrophoresis are used in multiple biotechnological processes such as DNA sequencing, mapping, profiling and recombinant DNA methods.



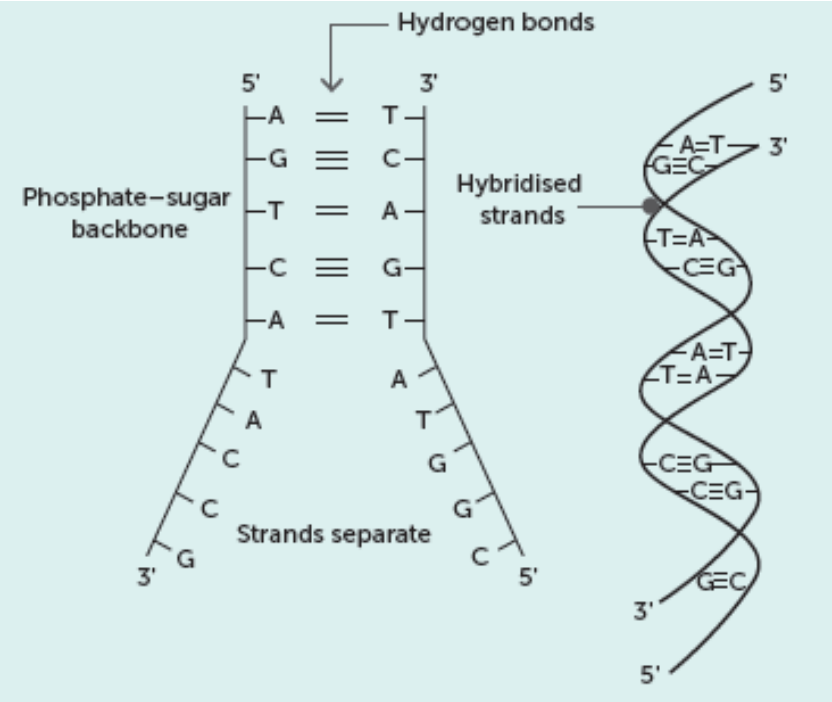
Amplification

Amplification is used to greatly increase the number of copies of a DNA sequence for further laboratory use. This can be achieved either in vivo, by inserting the sequence into a cloning vector that replicates within a host cell, or in vitro, by polymerase chain reaction (PCR).



Annealing

Annealing is the process of joining two pieces of DNA by complementary base pairing (joining of overhanging sticky ends). The two pieces are joined by weak hydrogen bonds only, and therefore only temporarily.



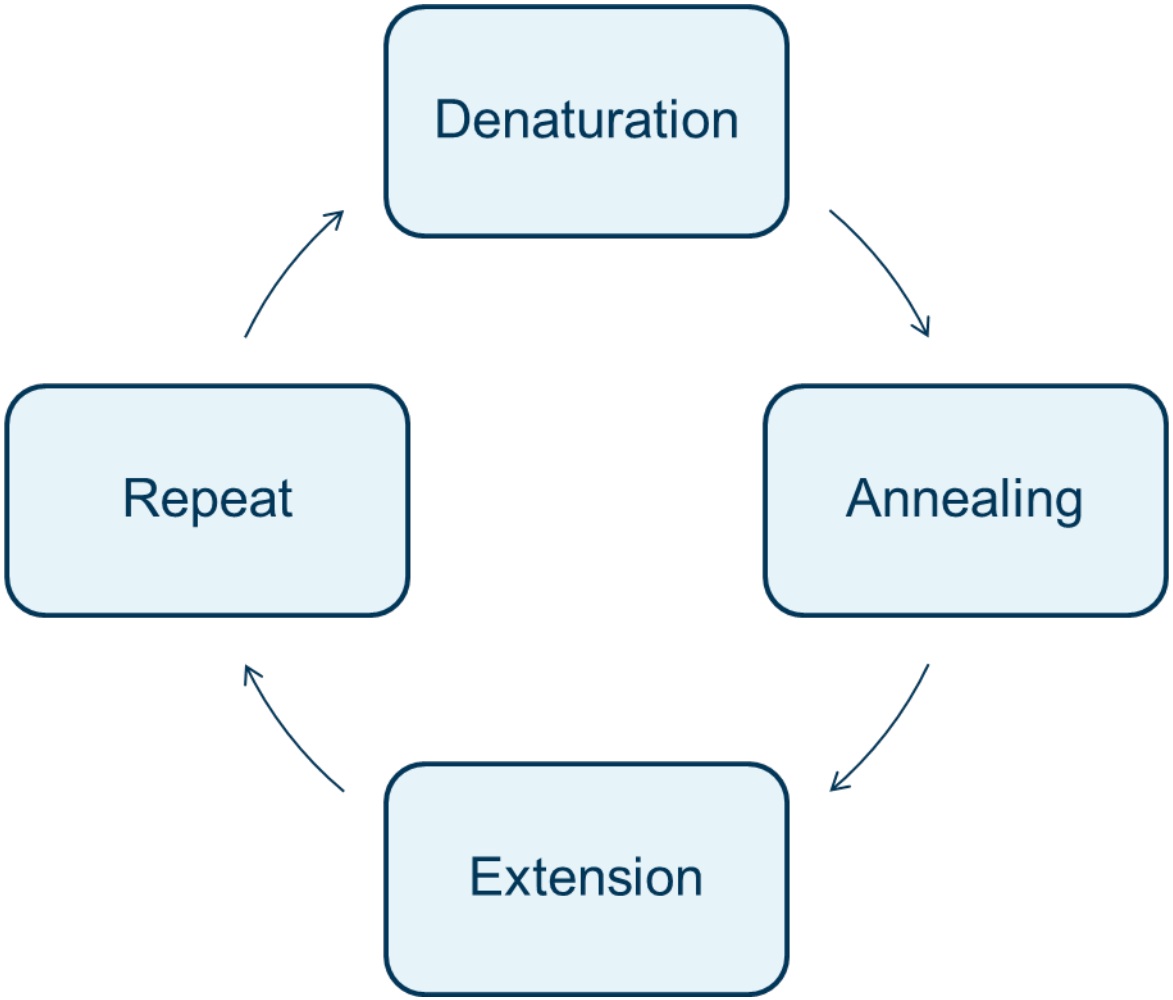
Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is cyclic method used to rapidly amplify (replicate many times) relatively small numbers of particular sequences of DNA into extremely large numbers of copies. The DNA is then suitable for further laboratory uses such as gel electrophoresis and DNA profiling.

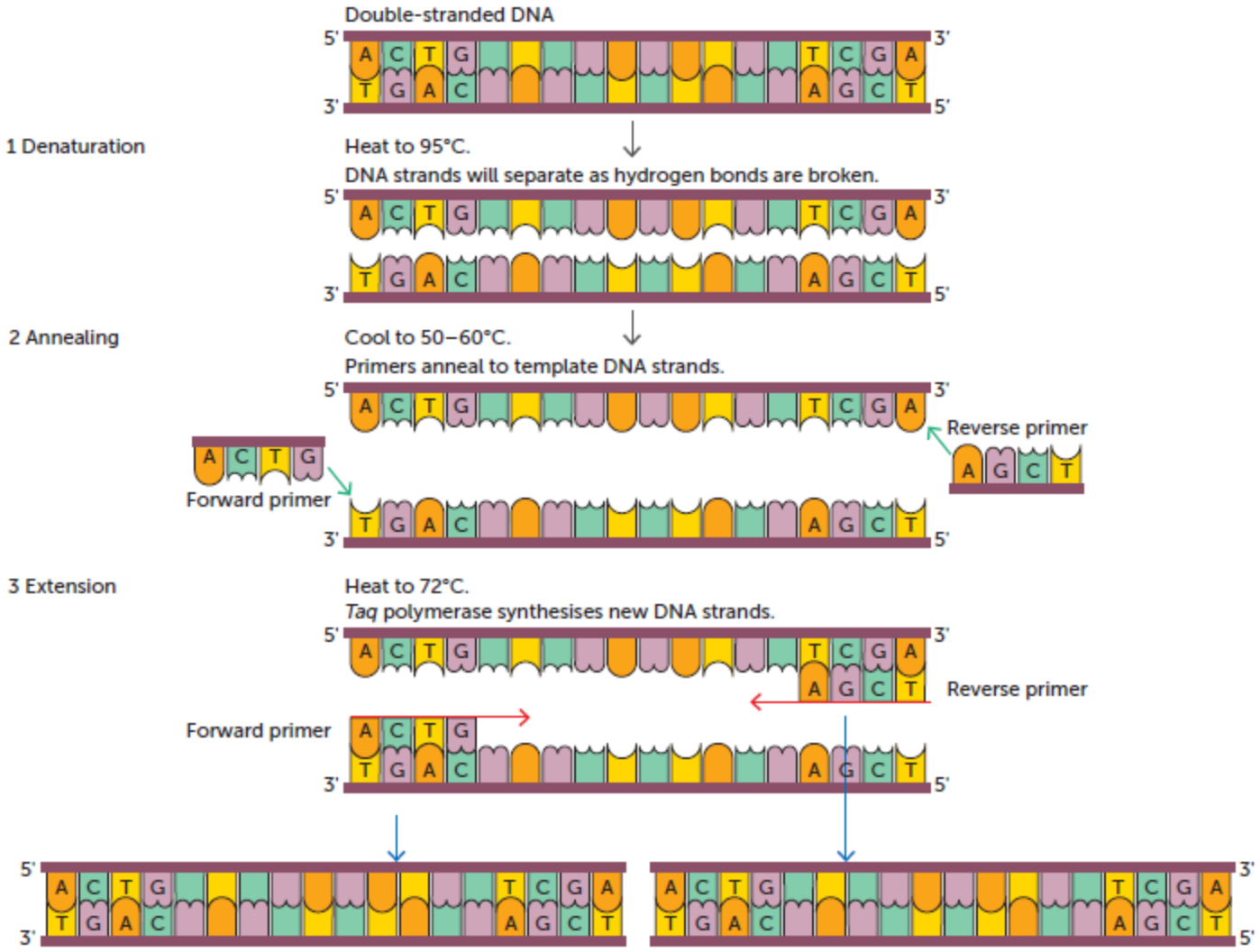
Requirements for PCR:

- Template DNA
- A special DNA polymerase (taq polymerase)
- Buffer solution to maintain pH
- A supply of the four nucleotides (i.e. A, T, C, G)
- Two sets of single-stranded DNA primers.
- A thermocycler that is able to rotate through the necessary temperatures

Steps in PCR: a cycle



Steps in PCR: a cycle



Steps in PCR: a cycle

Name of step	Temperature of mixture	Description
Denaturation	95°C	Double-stranded DNA heated to around 95°C, breaking the weak hydrogen bonds between complementary bases, causing the two template strands to denature (separate).
Annealing	50–60°C	Temperature is reduced to 50–60°C, allowing the single-stranded DNA primers to anneal (join via hydrogen bonds) to complementary sequences on opposite ends of each strand. Primers attach according to complementary base-pairing rules to the 3' end of each template strand.

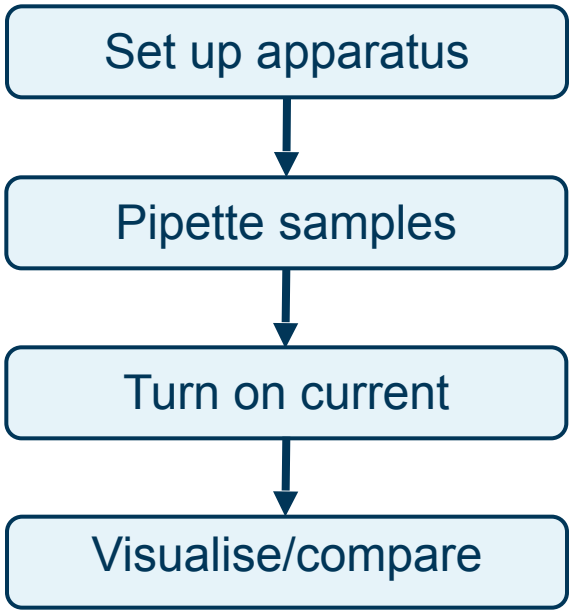
Steps in PCR: a cycle

Name of step	Temperature of mixture	Description
Extension	72°C	DNA taq polymerase extends the new strand, starting from primers. New DNA strands are synthesised using taq polymerase and available nucleotides. At the end of this phase, there are two copies of each original strand of the double-stranded DNA. The DNA has doubled at the end of this step.
Repeat cycle	The process is repeated many times.	Each new strand can act as template strand; therefore, DNA is amplified exponentially.

Gel electrophoresis

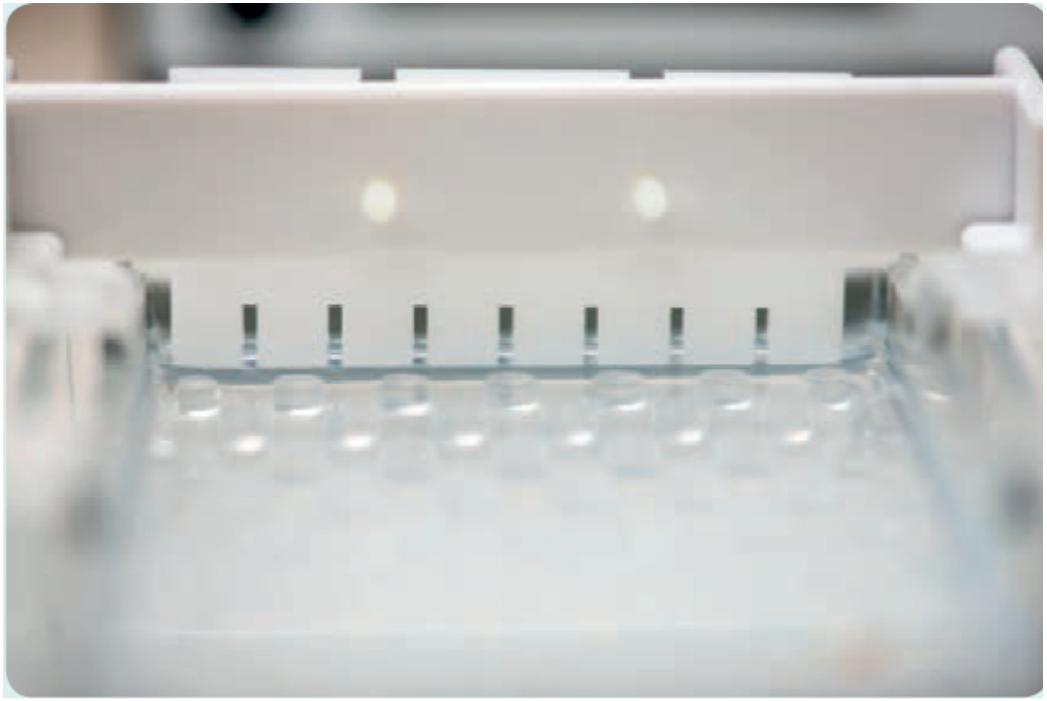
Gel electrophoresis is a technique that can separate large charged molecules (such as dyed fragments of DNA or proteins, according to size and charge, so that they can be visualised and identified by comparison with a standard.

Main steps of electrophoresis



Steps in electrophoresis: Set up the apparatus

Set an agarose gel and make wells with a comb. Place the gel into the apparatus and pour a buffer solution over to regulate pH. Cut the DNA fragments with restriction enzymes. Dye them with a binding chemical, such as ethidium bromide, that fluoresces under UV light.



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Steps in electrophoresis: Pipette samples

Pipette the samples and DNA ladder (standard) into the wells of the agar. Make sure the negatively charged samples are at the end of the apparatus where the negative electrode is situated. Discard used micropipette tips after each use.

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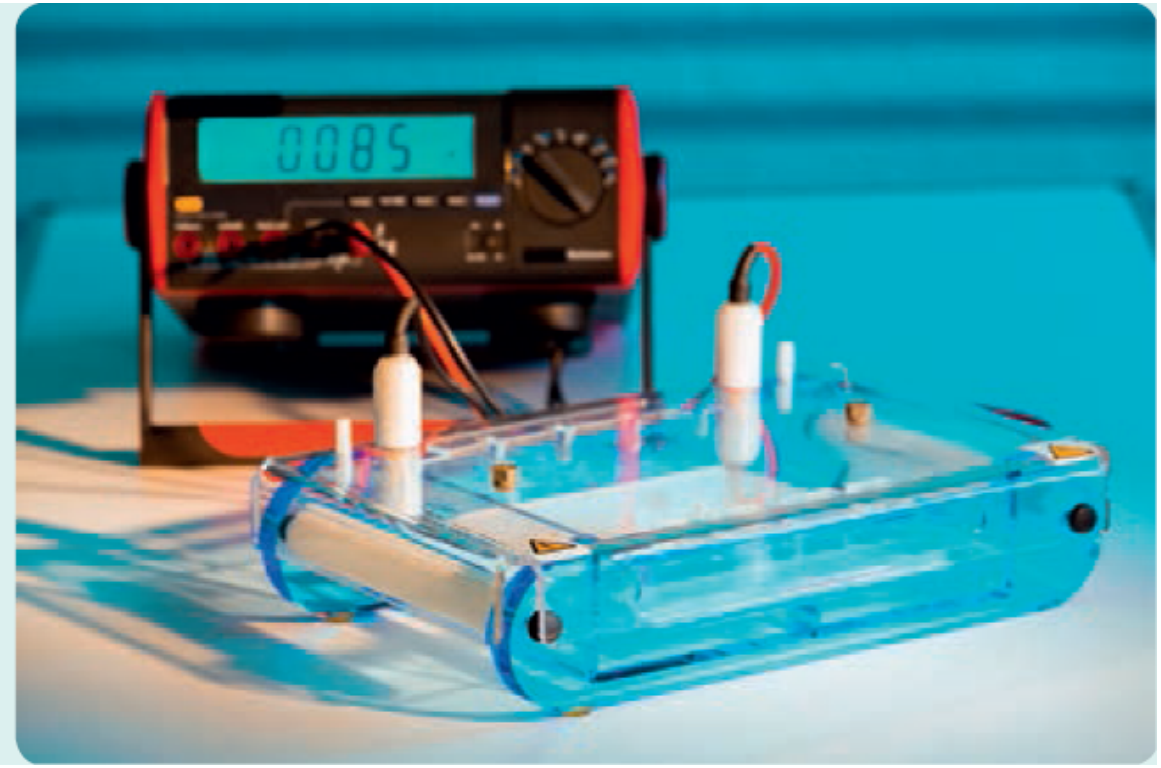
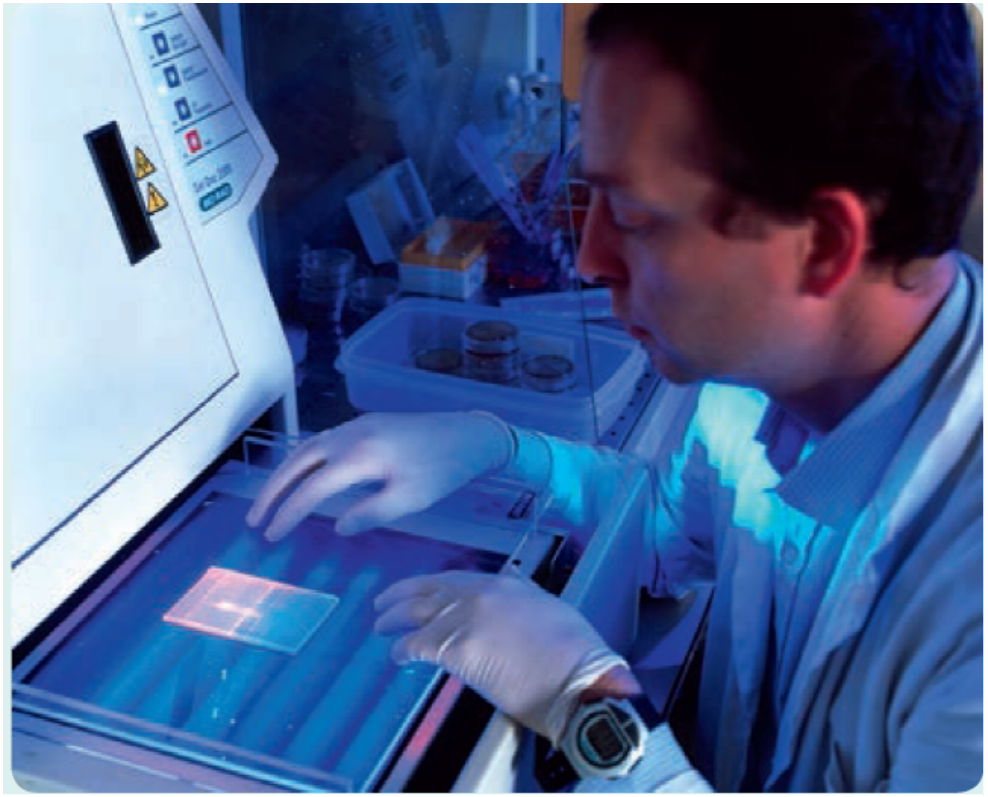
Steps in electrophoresis: Turn on the current

Turn on the current. The negative molecules are repelled by the negative electrode and travel (migrate) towards the positive electrode, which they are attracted to. The smaller fragments move faster and further, and the larger molecules move more slowly and not so far.



Steps in electrophoresis: Visualise and compare

Visualise the fragments by shining a UV light on the apparatus and photographing the results. The bands in each lane can be compared with the ladder (standard) to determine the length of the sample in number of base pairs (bp).



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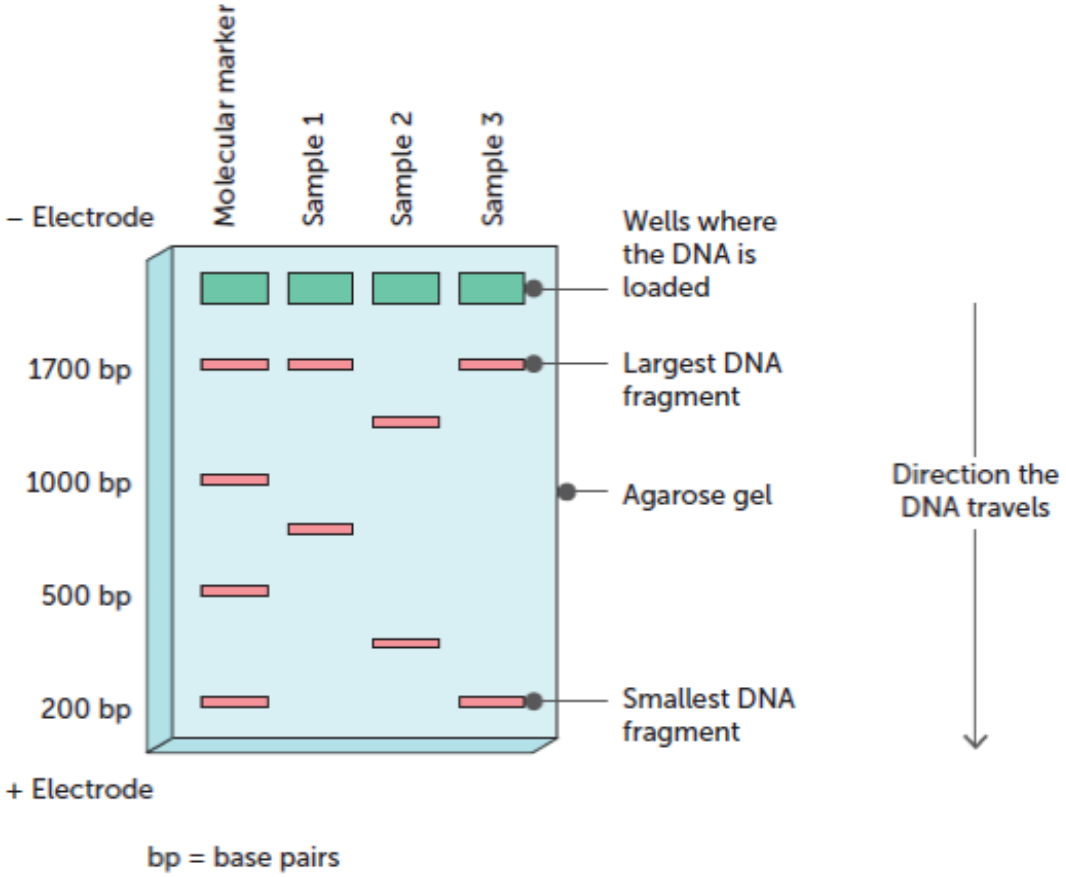
Steps in electrophoresis: Result

Molecular markers of known size are run alongside samples and allow estimation of the size of the DNA fragments migrating through the gel.



DNA sequencing

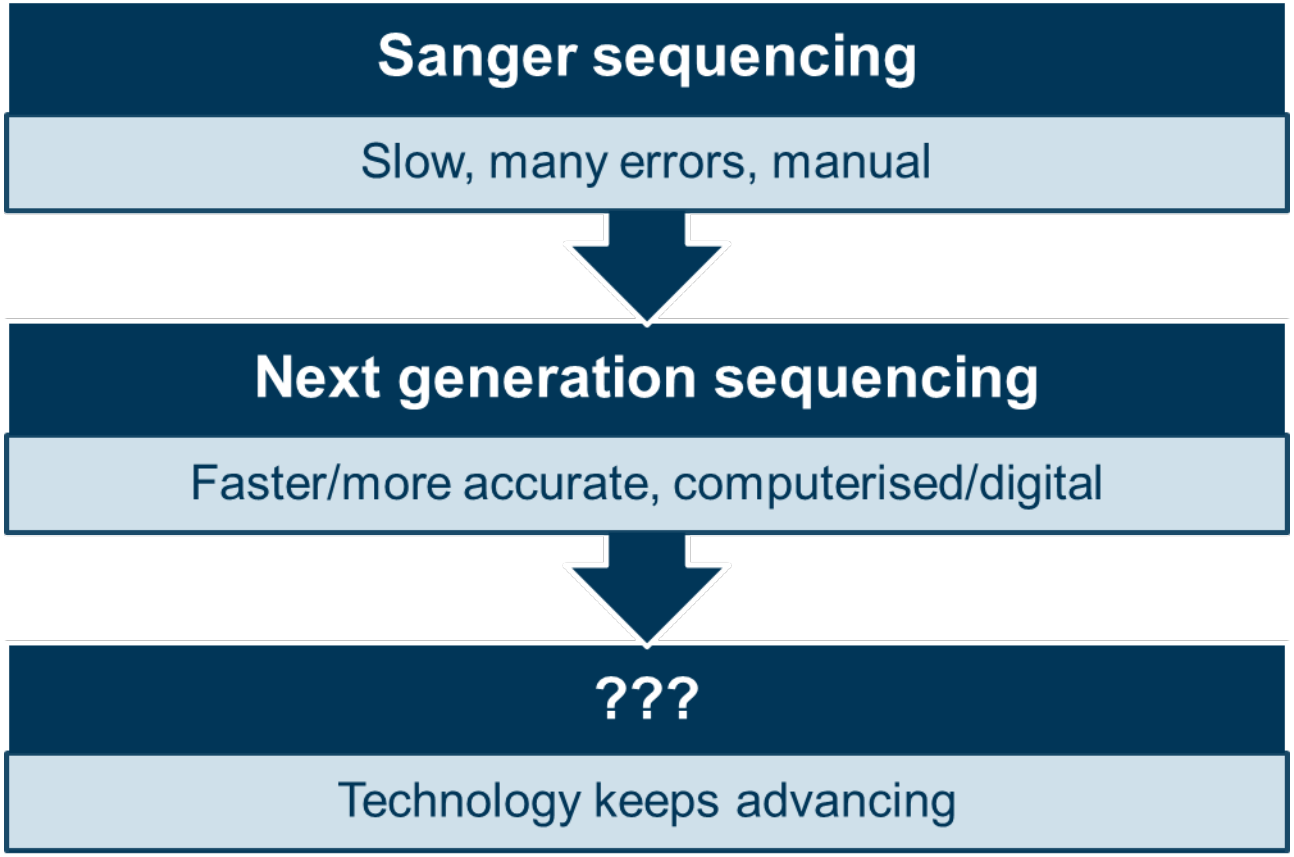
DNA sequencing refers to the methods and technologies used to determine the orders of the nucleotide bases in a DNA molecule: adenine (A), guanine (G), cytosine (C) and thymine (T). Scientists cut DNA into fragments to sequence one section at a time. The entire set can then be put together to create a whole genome

The genomes of thousands of species have been sequenced, allowing genomes and genes to be compared.

Knowing the sequences can help scientists determine the genetic code for particular phenotypes. There may be survival benefits in identifying, for example, genes that increase drought resistance or salt tolerance.

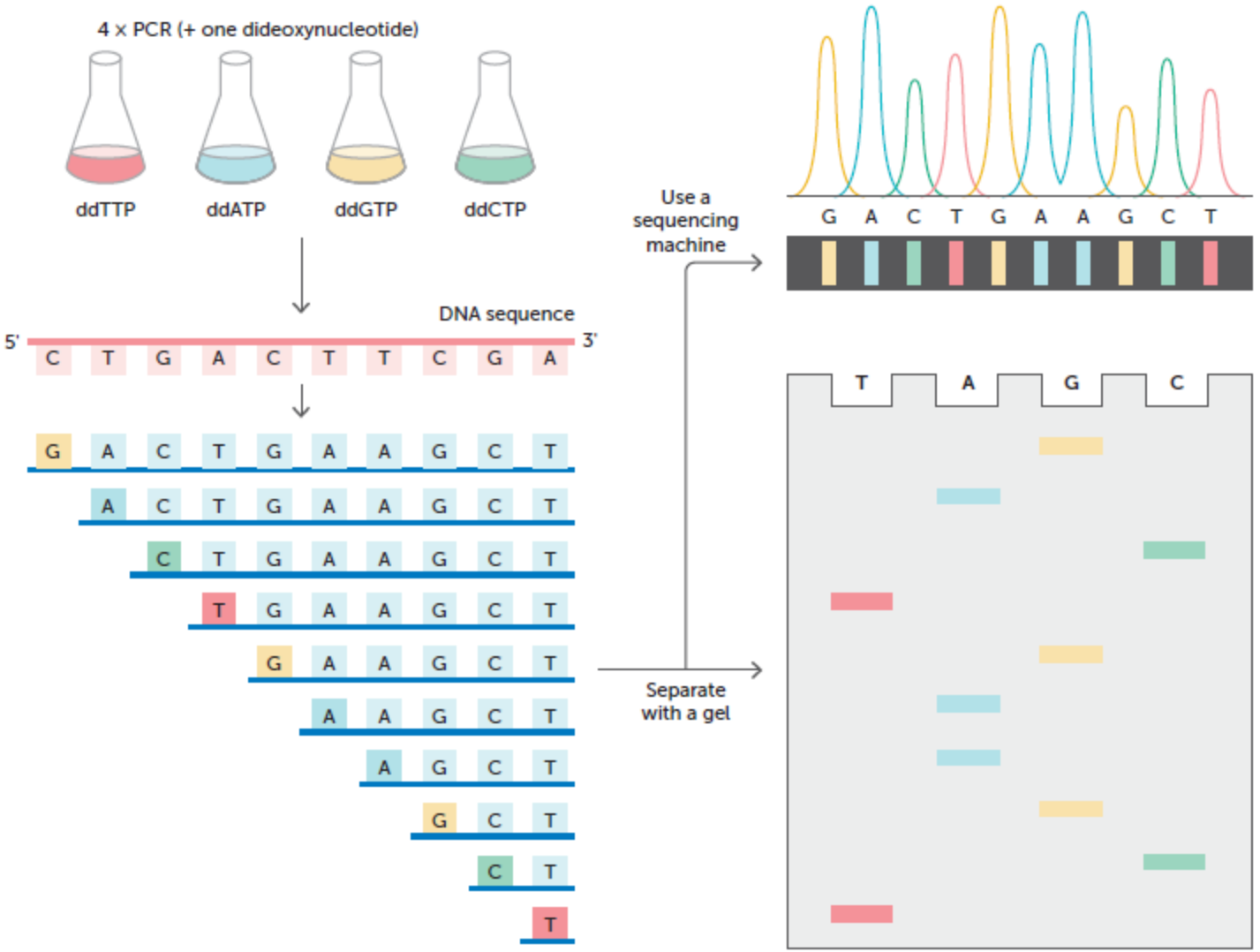
Sequencing genes of different species has assisted scientists in determining genetic relatedness and evolutionary links.

DNA sequencing methods



DNA sequencing

The Sanger sequencing method



The terminated strands line up from smallest to largest. The various colours enable identification of the nucleotide in each position.

CENGAGE Learning

DNA sequencing enables mapping of species' genomes

The genome includes genes and non-coding sequences. DNA sequencing has led to mapping of species genomes.

Gene mapping involves identifying and recording the positions (or relative positions, the 'loci') of genes and the distances between genes on a chromosome.

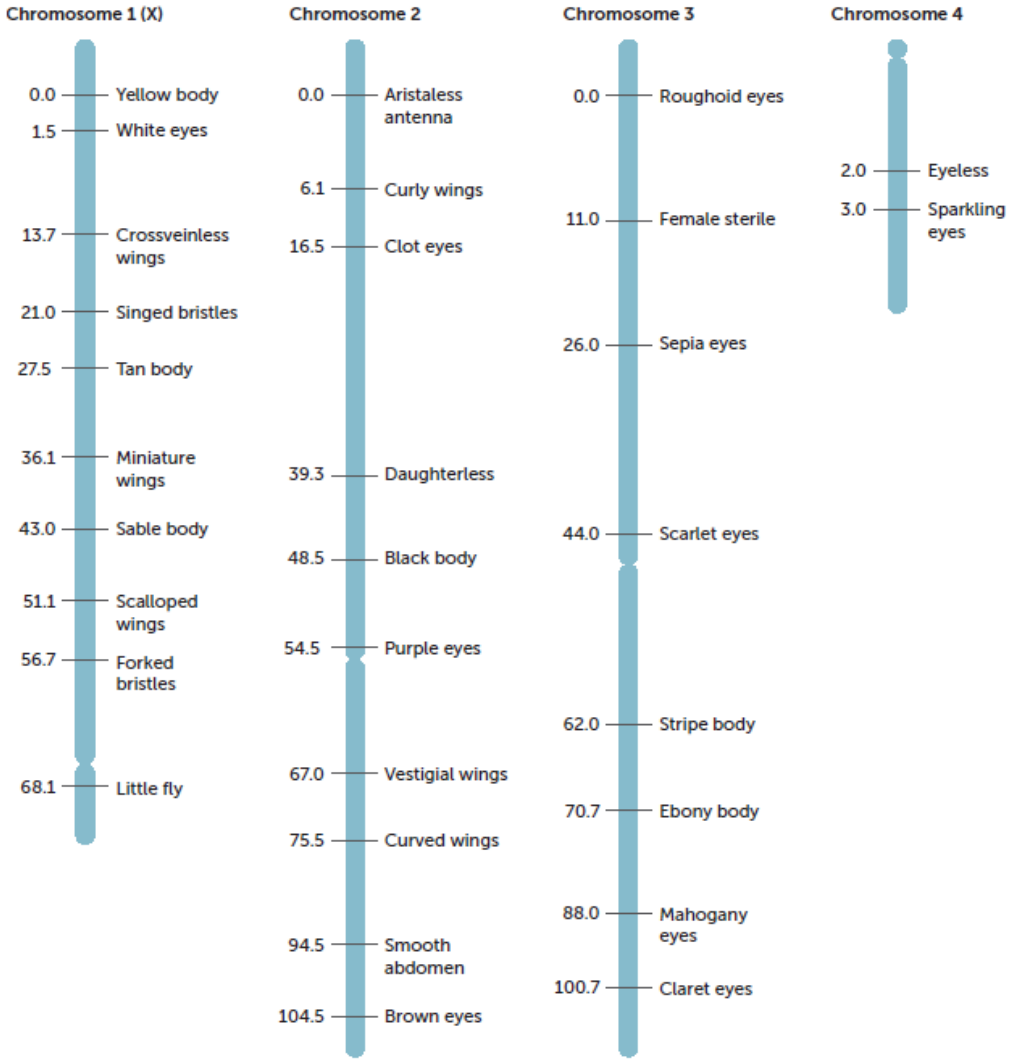
When a species' genome is mapped, all chromosomes in a somatic cell are mapped. Once the position of a gene is known, it can be shown on a diagram.

DNA sequencing

DNA sequencing enables mapping of species' genomes

Landmarks (genetic markers) are used on a genome map (e.g. fruit fly, at right).

A genetic marker is a gene or sequence on a chromosome with a known location that is associated with a specific trait.



DNA profiling identifies unique genetic makeup

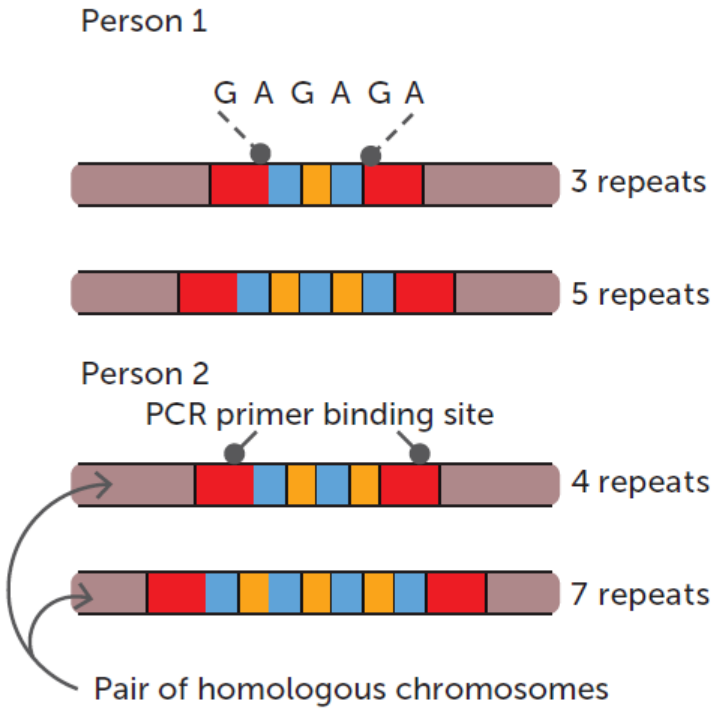
DNA profiling (also known as DNA fingerprinting) is a technique by which individuals can be identified by comparing a DNA sample with their respective DNA profiles.

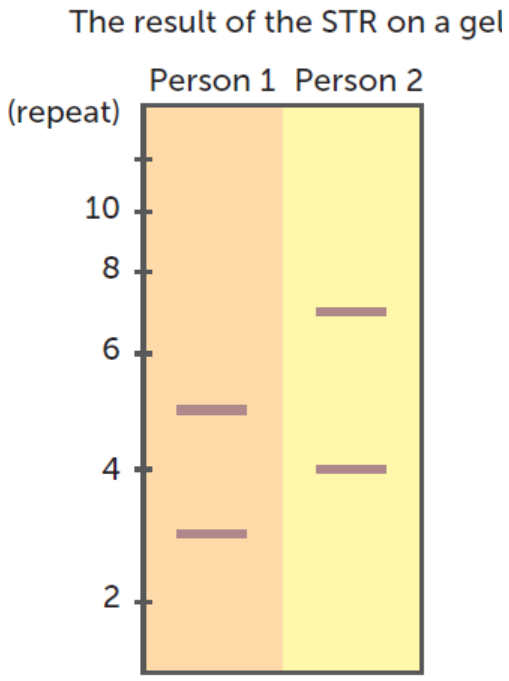
Within the non-coding regions of an individual's genome, there exists satellite DNA – long stretches of DNA made up of repeating elements called short tandem repeats (STRs).

As individuals will likely have different numbers of repeats at a given satellite DNA locus, they will generate unique DNA profiles.

DNA profiling identifies unique genetic makeup

DNA profiles can be visualised using gel electrophoresis. Different individuals of the same species will generate unique banding patterns, indicative of their unique genetic makeup.





Recombinant DNA technology and transgenic organisms

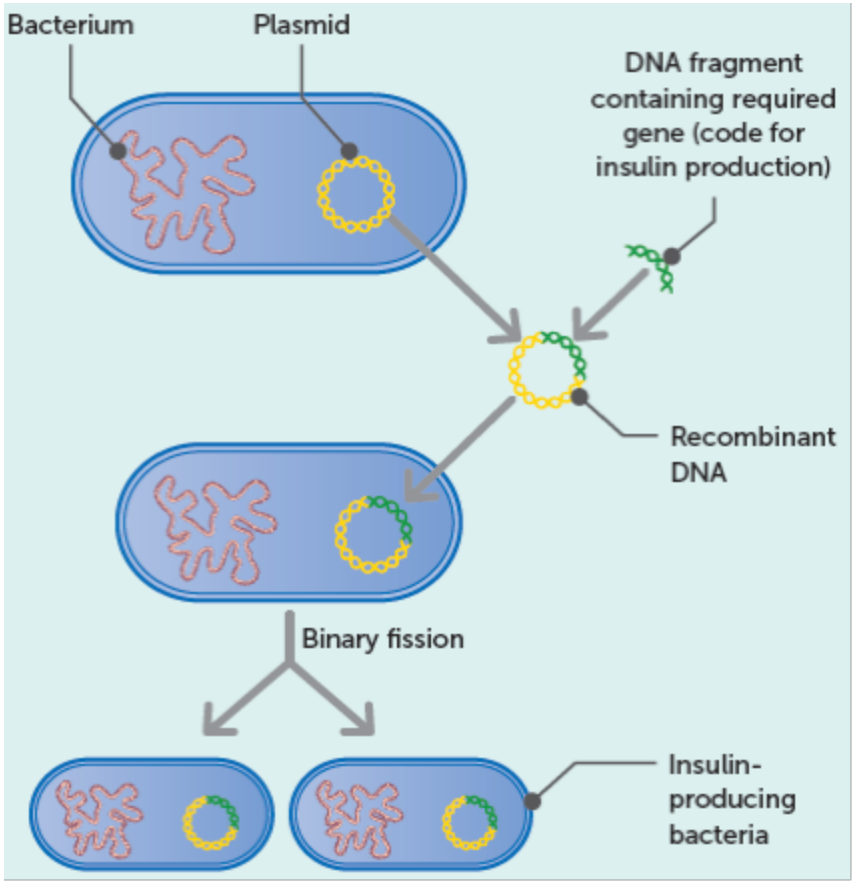
Recombinant DNA is DNA that is composed of one or more genes from two different organisms, usually two different species.

A **transgenic organism** has ‘foreign’ DNA transferred into its genome, usually from a different species, and is then expressed.

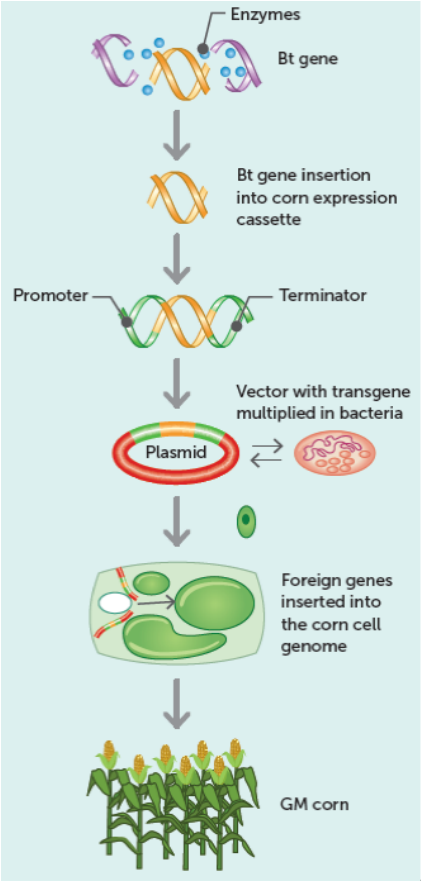
Transgenic organisms are also known as genetically modified organisms.

Recombinant DNA technology

Technique used to create a transgenic organism



Transformation and expression of bacteria



Bacterial vectors deliver target gene to host