TOPIC: BIOTECHNOLOGIES

Key Knowledge:

- The use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA and endonucleases to cut DNA
- Amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling

DNA MANIPULATION

The manipulation of genetic sequences requires the use of a number of different types of specific enzymes:

- **DNA Polymerases:** Synthesise new nucleotide strands from a complementary template strand of DNA
- **Endonucleases:** Fragments DNA by cleaving the sugar-phosphate backbone at specific recognition sites
- **Ligases:** Joins together the sugar-phosphate backbones of two DNA fragments (with covalent bonding)
- **Reverse transcriptase:** Converts mRNA sequences into cDNA sequences (will not contain any introns)

POLYMERASE CHAIN REACTION

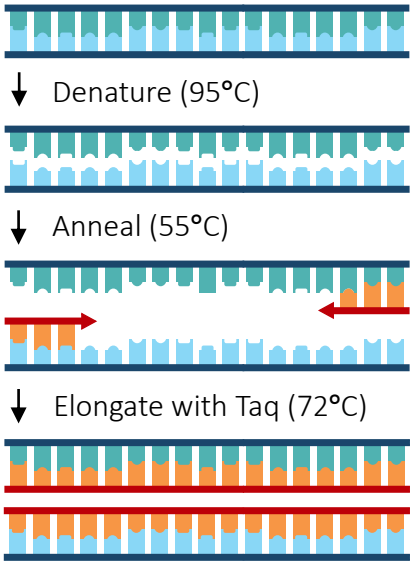
The polymerase chain reaction is an artificial method of DNA copying that uses a thermal cycler to rapidly amplify a specific DNA sequence

Taq polymerase is used in this reaction as it is a heat-tolerant enzyme isolated from the thermophilic bacterium ***Thermus aquaticus*** (Taq)

PCR involves a three-step process that utilises temperature variations:

- **Denaturation (90°C):** DNA is heated to separate the two strands
- **Annealing (55°C):** Primers bind to strands (designates copy region)
- **Extension (75°C):** Taq polymerase copies the two DNA strands

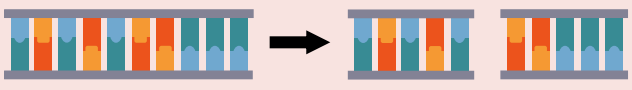
Each cycle of the polymerase chain reaction doubles the DNA amount, so a reaction of 30 cycles would produce more than 1 billion sequences



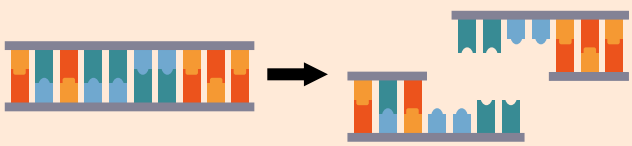
DIGESTION

Digestion describes the cutting of DNA molecules with restriction enzymes (endonucleases). The enzymes target specific sequences called recognition sites and cleave the DNA to generate either **blunt ends** or **sticky ends** (with complementary overhangs). Digestion may be used to either fragment DNA molecules for identification purposes (via **gel electrophoresis**) or insert genes of interest into deliver vehicles (**vectors**). Fragments with sticky ends can only be ligated to fragments with complementary sticky ends (i.e. cut with the same restriction endonuclease), while any two blunt ends can be ligated together to form a molecule.

BLUNT ENDS (NO OVERHANGS)

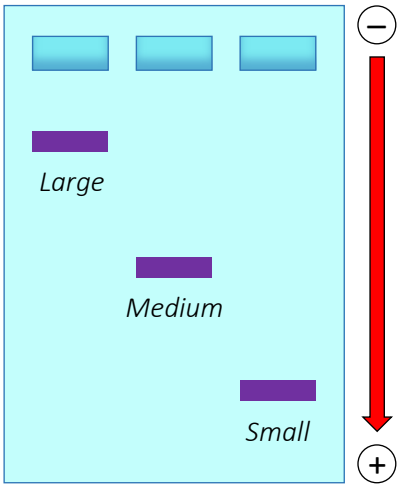


STICKY ENDS (OVERHANGS)



GEL ELECTROPHORESIS

Gel electrophoresis is a laboratory technique used to separate DNA or proteins based on size. Samples are placed in a block of gel and then an electric current is applied to cause the sample to move (DNA will move towards the **positive anode** as it is negatively charged). Smaller samples are less impeded by the gel matrix and will move both faster and further through the gel. This causes samples of different sizes to separate out as they travel at different speeds. While proteins can also be separated via the same basic process, they must be treated with an anionic detergent (SDS) in order to linearise the protein and impart a net negative charge. DNA samples are loaded onto an **agarose gel**, while protein samples are placed into a polyacrylamide gel. The concentration of the gel and the voltage applied will influence the degree of separation of the molecules. DNA samples may be fragmented with specific restriction enzymes and compared against a **DNA ladder** composed of fragments of known size.



*Gel electrophoresis will separate based on **size***

DNA PROFILING

DNA profiling is a technique by which individuals can be identified and compared via their respective DNA profiles. Within the non-coding regions of an individual's genome are loci comprised of repeating elements called **short tandem repeats** (STR). As individuals likely have different numbers of repeats at any STR locus, they generate unique DNA profiles. In larger populations more STR loci are needed to form unique profiles.

DNA profiling is used in **criminal investigations** and in **paternity disputes**. The same procedure is used:

- A DNA sample is collected (e.g. from blood, semen, saliva, etc.) and then amplified using PCR
- The STR loci are cut out with specific restriction enzymes to generate fragments of variable length
- The fragments are then separated out using gel electrophoresis and the resulting profiles are compared

In forensic investigations (below, left), suspects should be a complete match with the DNA sample taken from the crime scene. In paternity tests (below, right), the child should possess a combination of paternal and maternal fragments (all fragments not inherited from the mother **must** have come from the father).