DNA REPLICATION

Content Statements:

D1.1.1 DNA replication as production of exact copies of DNA with identical base sequences

D1.1.2 Semi-conservative nature of DNA replication and role of complementary base pairing

D1.1.3 Role of helicase and DNA polymerase in DNA replication

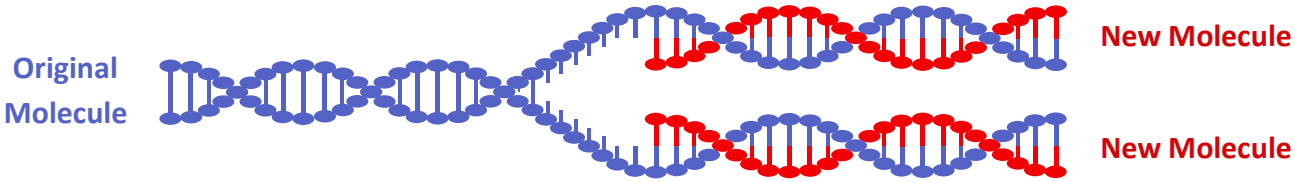
D1.1.4 Polymerase chain reaction and gel electrophoresis as tools for amplifying and separating DNA

D1.1.5 Applications of polymerase chain reaction and gel electrophoresis

SEMI-CONSERVATIVE

DNA replication is the process by which the genetic information in the cell prior to the production of new cells. It is required for both **reproduction** (asexual or sexual) and **organism growth** or **tissue replacement** in multicellular organisms. DNA replication is semi-conservative, meaning that whenever the DNA is copied:

- One strand will be from the original template molecule (it is 'conserved' from the original source)
- One strand will be newly synthesised (it is *not* conserved and is composed of new nucleotides)



The DNA is copied with a high degree of accuracy because each nitrogenous base can only pair with its complementary base partner. Adenine and thymine pair together via two hydrogen bonds, while guanine and cytosine pair together with three hydrogen bonds. This system of pairing ensures new DNA molecules will have an **identical base sequence** to the original molecule, meaning genetic instructions remain intact.

REPLICATION ENZYMES

The process of DNA replication is controlled and coordinated by many enzymes, however there are two main enzymes that are primarily involved in the separation and copying of the double-stranded molecule:

Helicase	DNA Polymerase
Unwinds and separates the two DNA strands by breaking the hydrogen bonds between base pairs	Covalently joins the free nucleotides together to form a complementary strand (semi-conservative)
Once the strands are separated, free nucleotides will align opposite their complementary partner	Catalyses the formation of a phosphodiester bond between a sugar and phosphate (condensation)

Because DNA molecules can be extremely long, replication is initiated at multiple points simultaneously. New strands are synthesised bi-directionally from these **origins of replication**, eventually fusing together to form a complete sequence. This functions to limit the time required for the process of replication to occur.

Fun Fact: An average chromosome is 150 million bp in length and DNA polymerase copies at ~50 bp/sec.

POLYMERASE CHAIN REACTION

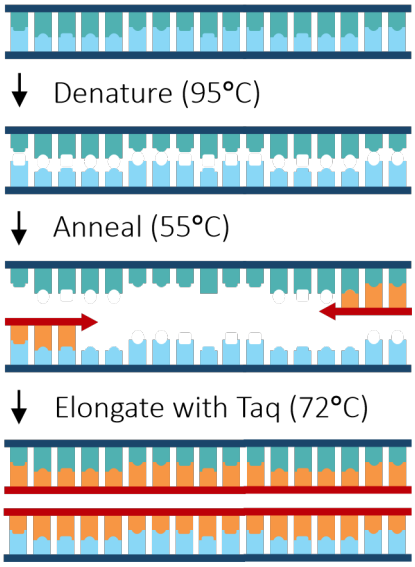
The polymerase chain reaction is an artificial method of DNA copying that uses a thermal cycler to rapidly amplify a specific DNA sequence

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:

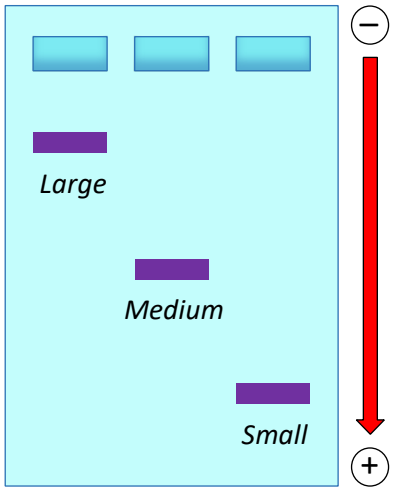
- **Denaturation (90°C):** DNA is heated to separate the two strands
- **Annealing (55°C):** Primers bind to strands (designates copy region)
- **Extension (75°C):** Taq polymerase copies the two DNA strands

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



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 paternity tests, every band present in the DNA profile of the infant must come from one of the parents