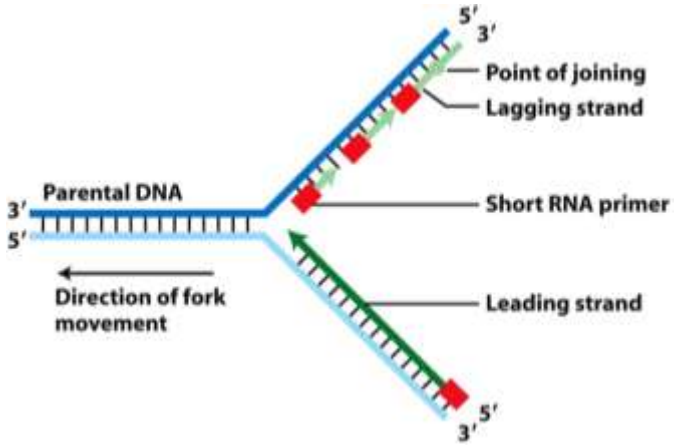
1.2. Replication of DNA

DNA has the capability of **replicating** itself, to make an identical copy of its DNA. This process is performed at the beginning of every cell division so that when the cell divides, each daughter cell will inherit an **identical** copy of the DNA.

There are five requirements for the replication of DNA:

1 – **Original DNA template**	each strand can be used as a template to create a new DNA strand
2 – **Primers**	start process since DNA polymerase can only add nucleotides to an existing strand of DNA
3 – **Enzymes**	**DNA Polymerase** – adds nucleotides to a growing strand of DNA **Ligase** – joins the fragments together
4 – **Free DNA nucleotides**	needed to form the new strands
5 – **ATP**	a chemical which supplies energy for the process to occur

Stages of DNA replication



Stage 1 – The DNA double helix molecule **unwinds** and weak **hydrogen** bonds between base pairs **break**, allowing the two strands to separate ('unzip'). These template strands become stabilised and expose their bases at a Y-shaped **replication fork**.

Stage 2 - DNA polymerase adds complementary free DNA nucleotides (found in the nucleus) to the now exposed bases on both strands (A-T and C-G) in a **5'** to **3'** direction. A **primer** is needed to start replication, since DNA polymerase can only add nucleotides to a pre-existing chain.

1. **Leading strand** is synthesised **continuously**. DNA polymerase adds nucleotides to the deoxyribose (3') ended strand in a 5' to 3' direction.

2. **Lagging strand** is synthesised in **fragments**. Nucleotides cannot be added to the phosphate (5') end because DNA polymerase can only add DNA nucleotides in a 5' to 3' direction. The lagging strand is therefore synthesised in fragments. The fragments are then sealed together by an enzyme called **ligase**.

Stage 3 - The two new strands twist to form a **double helix**. Each is identical to the original strand. Each DNA molecules is known as being **semi-conservative**.

Polymerase chain reaction (PCR)

The Polymerase Chain Reaction (PCR) is a technique used to increase or amplify DNA ***in vitro*** (process happens outside the body of an organism) so that many copies of it can be made from a very small amount.

The PCR process

1. The DNA to be amplified, is heated to **95**°C to break the **hydrogen** bonds between the bases, therefore separating the two strands.
2. The DNA is then cooled to approximately **55**°C to allow the complementary **primers** to bind to its target sequence at the 3' end of the original DNA strands.
3. It is then heated again to **72**°C.
4. Heat-tolerant **DNA polymerase** is added, which adds nucleotides to the primers at the 3'end of the original DNA strands. Two strands are formed.
5. Repeated cycles of heating and cooling are then carried out using the original and the new copies of the DNA to produce millions of copies, within about 3 hours.

Positive and negative controls

It is important to set up control experiments to allow you to verify that you are measuring what you intended.

Positive control	contains a template of DNA with a known sequence to which the primers are complementary. This means that if it is unsuccessful, you know that there is something wrong with the set-up, e.g. problems with the primers or the conditions.
Negative control	would have a template which is not complementary to the primers or might have no DNA template. This means that if there is amplification, there must have been some contamination.

Practical applications of PCR

This technique allows scientists to easily and cheaply turn a single strand of DNA into millions of copies which can then be used for analysis.

The analysis of DNA is used in:

- the Human Genome Project,
- phylogenetics,
- research,
- **paternity** testing,
- prenatal diagnosis of inherited **genetic** disorders
- and the detection of infection.