

 **HL IB Biology**

DNA Replication

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Your notes

DNA Replication

Importance of DNA Replication

- The replication of DNA is semi-conservative and depends on complementary base pairing
- Semi-conservative means that **one strand of the 'parent' DNA is kept** in the 'daughter' molecule
 - This is called the **template strand**
- The other half is determined by the code on the template strand and is built up from **free nucleotides** in the nuclear space around the chromosomes
- This takes place in the **nucleus**
 - If an **adenine** is the next exposed base on the original strand, a **thymine** nucleotide is added and *vice versa*
 - If a **cytosine** is the next exposed base on the original strand, a **guanine** nucleotide is added and *vice versa*
 - Nucleotides are added **one by one** to the new strand according to the rules of **complementary base-pairing**
- **Hydrogen bonds** can only form between the template strand and the new strand if the **correct bases** are paired up
- Therefore, the new DNA molecule has **kept half** of the parent DNA and then used this to create a new, daughter strand
- DNA replication is **important in multicellular organisms** for many reasons, such as:
 - **Growth**
 - **Replacement** of old/damaged cells and tissues
 - **Reproduction**

Examiner Tip

Make sure you don't confuse 'parent cell' with 'parent organism'. A **parent cell** is any cell in the body that divides into two cells and the terminology is used to refer to the '**original**' cell that the DNA came from before it was split and replicated semi-conservatively.

Semi-Conservation Replication

- DNA polymerase links nucleotides together to form a new strand, using the pre-existing strand as a template
- **Before** a (parent) cell **divides**, it needs to **copy the DNA** contained within it
 - This is so that the two new (daughter) cells produced will both receive the **full copies of the parental DNA**
- The DNA is copied via a process known as **semi-conservative replication** (half the DNA is kept)
 - The process is called so because **in each new DNA molecule** produced, one of the polynucleotide DNA strands (**half** of the new DNA molecule) is **from the original DNA molecule** being copied
 - The other polynucleotide DNA strand (the other half of the new DNA molecule) has to be **newly created** by the cell

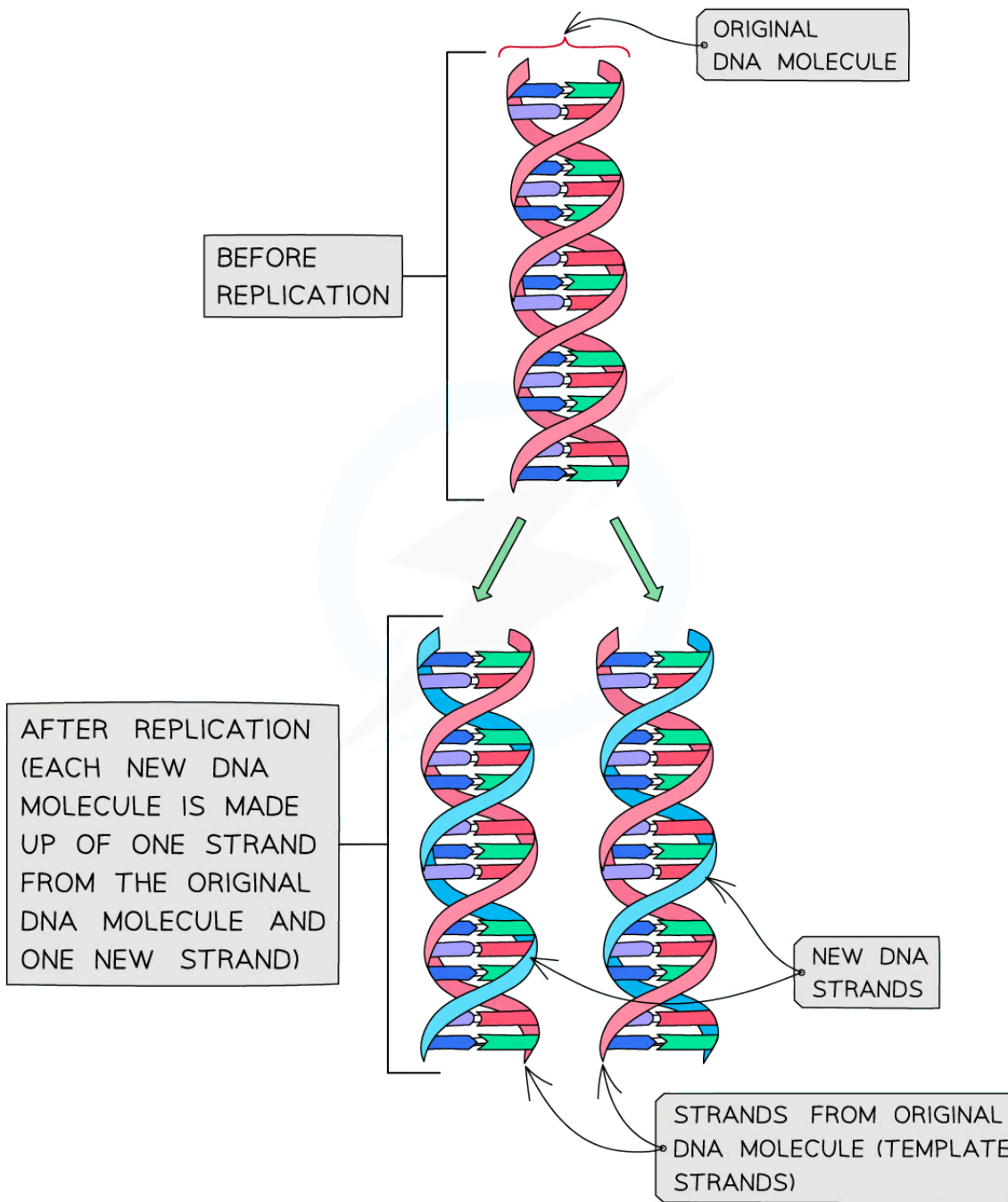
Semi-conservative DNA replication diagram



Your notes



Your notes



Semi-conservative replication of DNA

The importance of keeping one original DNA strand

- It ensures there is **genetic continuity** with a **high degree of accuracy** between generations of cells

- In other words, it ensures that the new cells produced during cell division **inherit all their genes with the correct sequence of DNA bases** from their parent cells

Crick and Watson proposed semi-conservative replication

- As part of their discovery of the double-helix structure of DNA, Crick and Watson **made a hypothesis about how DNA copies** during cell growth
- They proposed a **semi-conservative** model, but had **not provided the evidence**
- This was provided by two later scientists, **Meselson** and **Stahl**, in another award-winning piece of research
- Analysis of Meselson and Stahl's results **gave the necessary support for Crick & Watsons' hypothesis** of semi-conservative replication of DNA



Your notes

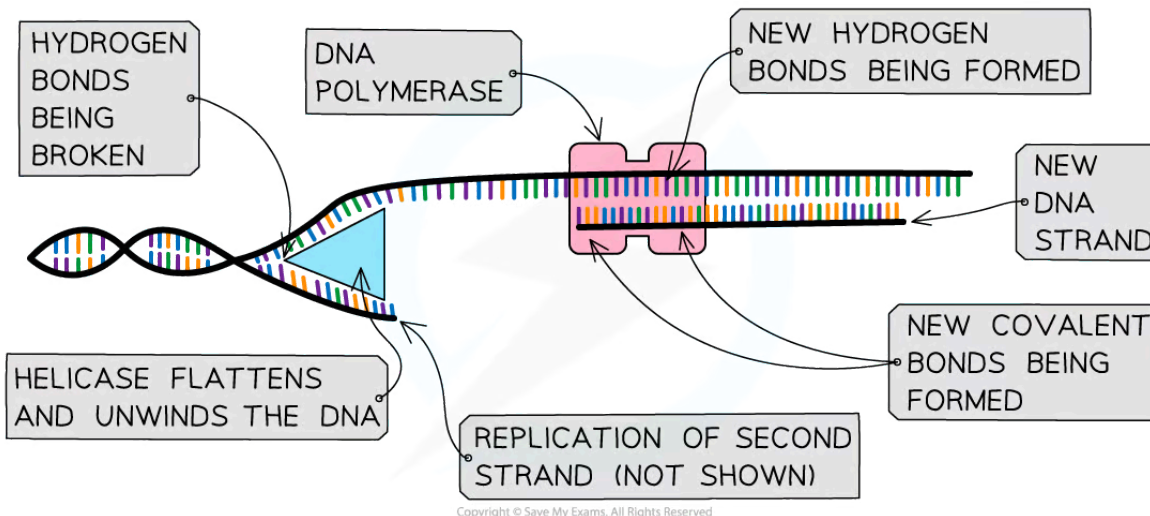


Your notes

Helicase & DNA Polymerase

- DNA replication occurs in preparation for **mitosis**, when DNA must be **doubled** before the parent cell can divide to produce two genetically identical daughter cells
- The enzyme **helicase** first **unwinds the DNA**, by flattening out its helical structure
 - Analogy - think about **untwisting a rope ladder**
- **Helicase** then causes the **hydrogen bonds to break** between pairs of bases, exposing bases on either strand
 - Analogy - **unzipping a zipper**
- Each of these single polynucleotide DNA strands acts as a **template** for the formation of a **new strand** made from free nucleotides that are attracted to the exposed DNA bases by **base pairing**

Function of helicase and DNA polymerase diagram



Helicase and DNA polymerase work together to replicate each strand of DNA

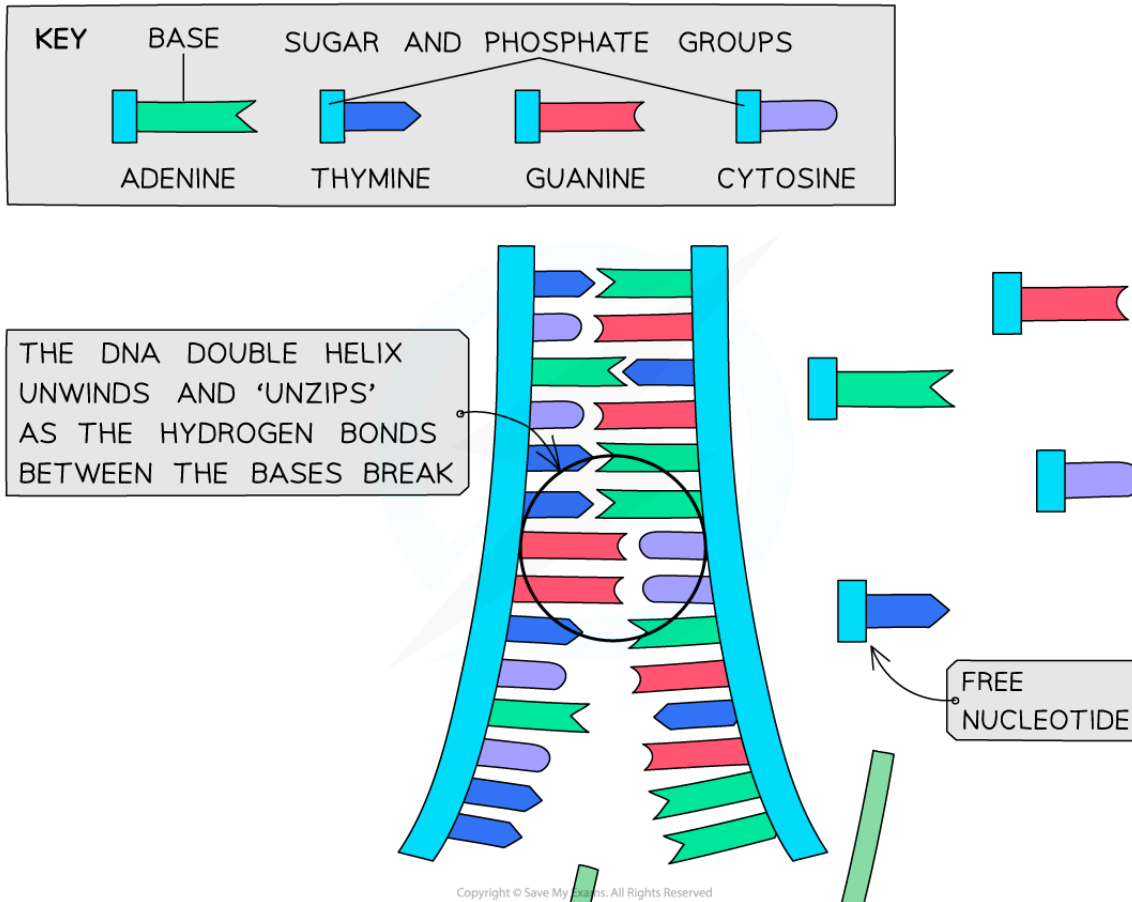
- DNA polymerase links nucleotides together to form a new strand, using the pre-existing strand as a template
 - Following the action of helicase, the template strand is exposed and new nucleotides are **joined together by DNA polymerase**, which catalyses **condensation reactions**, to form a new strand
 - These reactions occur between the **deoxyribose sugar and phosphate groups** of adjacent nucleotides within the new strands, creating the **sugar-phosphate backbone** of the new DNA strands
 - DNA polymerase always works **in the same direction** along a strand of DNA, the 5' to 3' direction
 - This means that it adds the **5' terminal** of the new nucleotide to the **3' terminal** of the strand being built
 - **Hydrogen bonds** then form between the **complementary base pairs** of the template and new DNA strands

- This method of replicating DNA is known as **semi-conservative replication** because **half of the original** DNA molecule is kept (**conserved**) in each of the two new DNA molecules



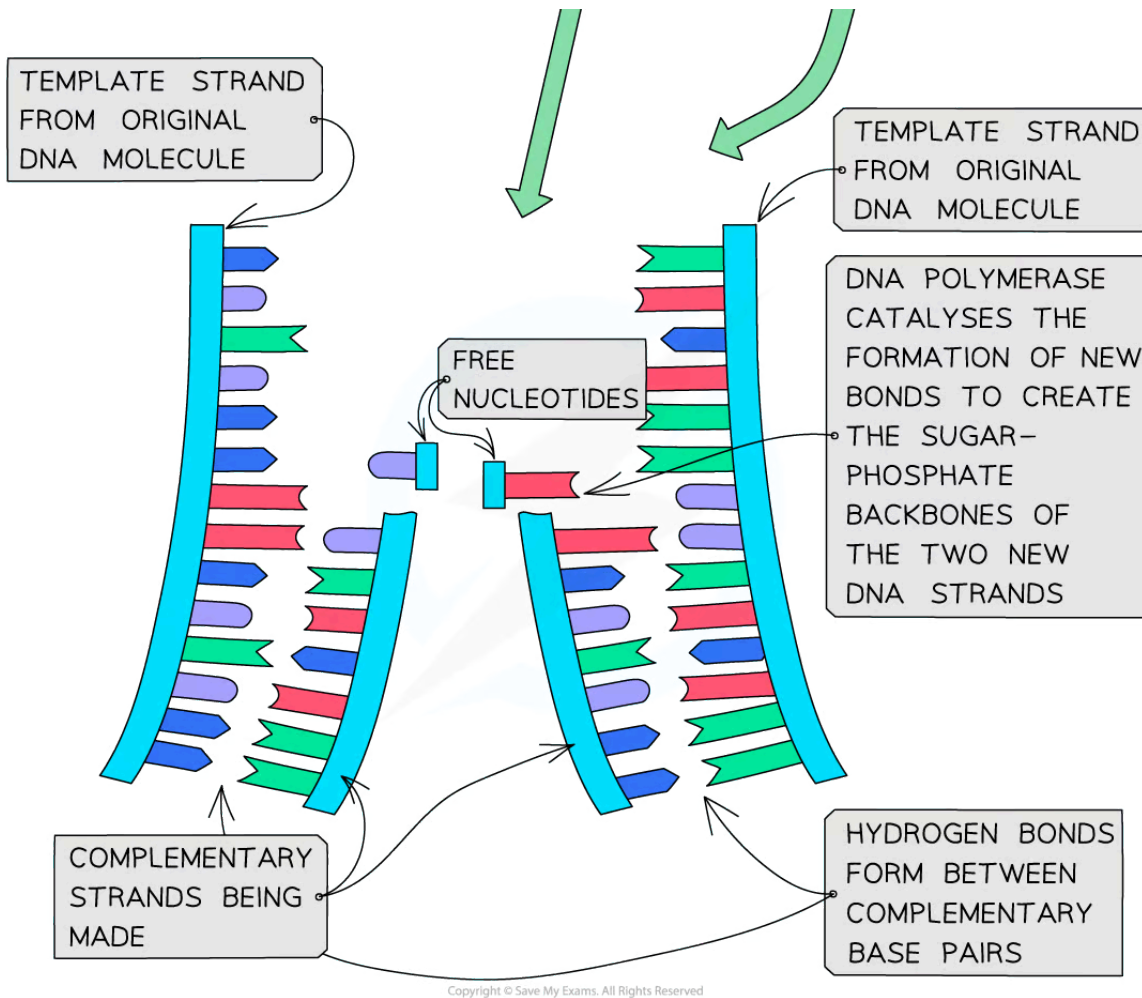
Your notes

Overview of DNA replication diagram





Your notes



The combined actions of helicase and DNA polymerase create new complementary DNA strands



Your notes

Electrophoresis & PCR

Electrophoresis & PCR

Gel electrophoresis

- **Gel electrophoresis** is a technique used widely in the analysis of DNA, RNA, and proteins
- During electrophoresis, the **molecules** are **separated with an electric current** according to their **size or mass** and their **net (overall) charge**
- This separation occurs because of:
 - The **electrical charge** molecules carry:
 - Positively charged molecules will move towards the cathode (negative pole), whereas negatively charged molecules will move towards the anode (positive pole), e.g. **DNA is negatively charged** due to the **phosphate** groups and thus, when placed in an electric current, the molecules **move towards the anode**
 - The **different sizes** of the molecules:
 - Different sized molecules move through the gel (agarose for DNA and polyacrylamide for proteins) at different rates. The tiny pores in the gel result in **smaller molecules** moving **quickly**, whereas **larger molecules** move **slowly**
 - The **type of gel**:
 - Different gels have different sized pores that affect the speed at which the molecules can move through the gel

DNA separation

- DNA can be collected from almost anywhere on the body, e.g. the root of a hair or saliva from a cup. After collection, DNA must be prepared for gel electrophoresis so that the **DNA** can be **sequenced** or analysed for genetic profiling (fingerprinting)
- To prepare the fragments, scientists must first increase (amplify) the number of DNA molecules by the **Polymerase Chain Reaction (PCR)**
- Then restriction (DNA-cutting) enzymes are used to chop the DNA into fragments

Method

- **To separate the DNA fragments** in gel electrophoresis:
 1. Create an **agarose gel** plate in a tank. **Wells** (a series of small rectangular holes) are cut into the gel at one end
 2. Submerge the gel in an **electrolyte** solution (a salt solution that conducts electricity) in the tank
 3. Load (insert) the DNA fragments into the wells using a **micropipette**
 4. Apply an **electrical current** to the tank. The negative electrode must be connected to the end of the plate with the wells as the DNA fragments will then move towards the anode (positive pole) due to the attraction between the negatively charged phosphates of DNA and the anode
 5. DNA fragments with a smaller mass (i.e. shorter DNA fragments) will move faster and further from the wells than the larger fragments



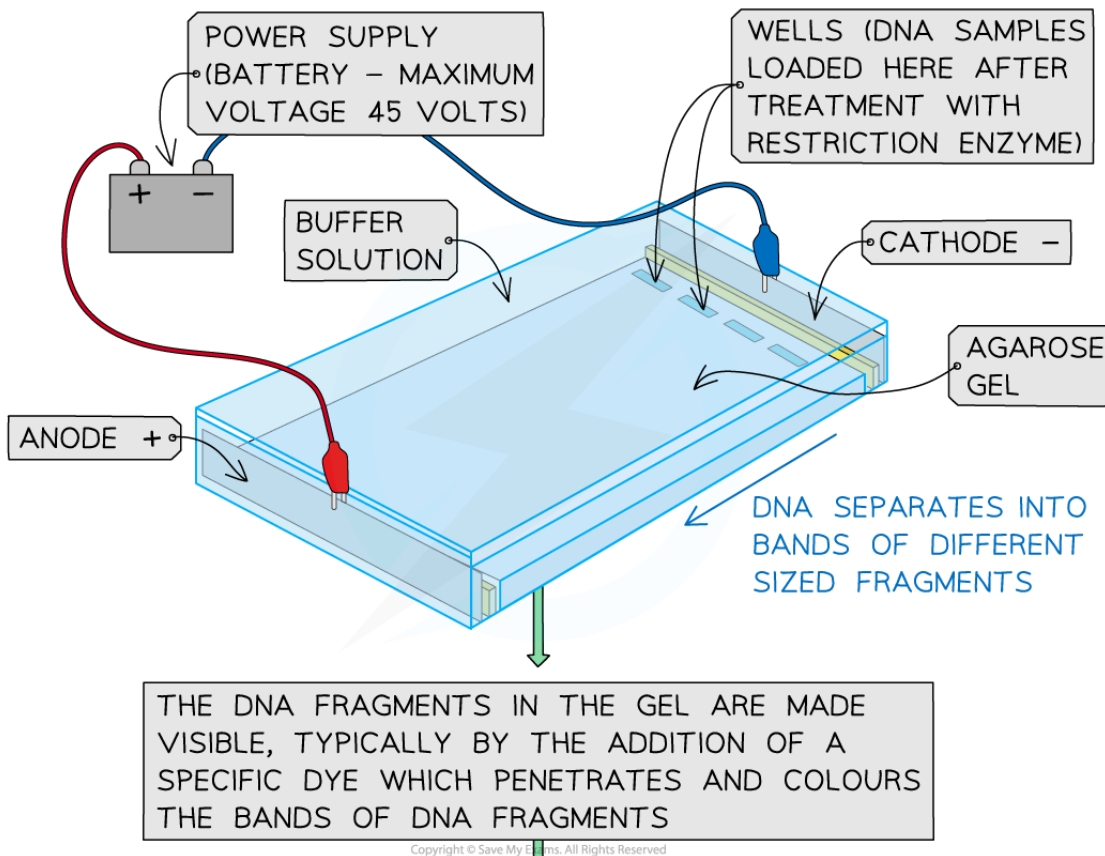
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6. **The fragments are not visible** so must be transferred onto absorbent paper or nitrocellulose which is then heated to separate the two DNA strands

7. **Probes** are then added to develop a visual output, either:

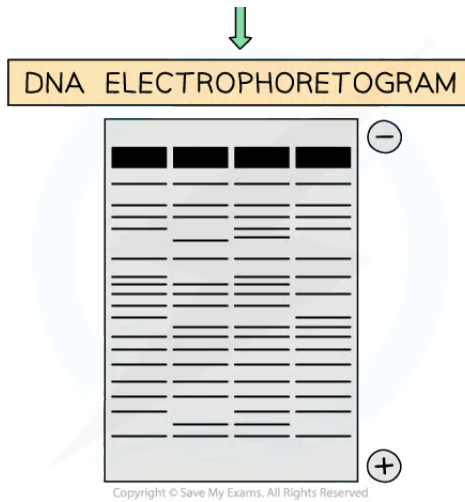
- A **radioactive label** (eg. a phosphorus isotope), which causes the probes to emit radiation that makes the X-ray film go dark, creating a pattern of dark bands
- A **fluorescent stain or dye** (eg. ethidium bromide), which fluoresces (shines) when exposed to ultraviolet (UV) light, creating a pattern of coloured bands

Gel electrophoresis diagram





Your notes



The process of electrophoresis

Examiner Tip

Remember gel electrophoresis is the separation of molecules according to their size and charge (negatively charged DNA molecules move to the positive pole). Examiners like to ask questions about gel electrophoresis, so make sure you understand each of the different steps in the process.

Polymerase Chain Reaction (PCR)

- **Polymerase Chain Reaction (PCR)** is a common molecular biology technique used in most applications of gene technology
 - For example, it is used in DNA profiling (e.g. identification of criminals and determining paternity) or genetic engineering
 - In the **COVID-19 pandemic**, PCR has been used in **routine diagnostic testing** to amplify small amounts of viral RNA
- It can be described as the ***in vitro* method of DNA amplification**
- It is used to produce **large quantities** of specific fragments of DNA or RNA from very small quantities (even just one molecule of DNA or RNA)
 - Using PCR, scientists can produce **billions of identical copies** of the DNA or RNA samples within a few hours, these can then be used for **analysis**

The requirements of PCR

- Each PCR reaction requires:
 - The **target DNA** or **RNA** that is being amplified
 - It's important that **the whole genome is not required** to be copied - only specific sections that vary from one individual to another
 - These sections are identified by adding a primer sequence that binds to them



Your notes

- **DNA polymerase** - the enzyme used to build the new DNA or RNA strand. The most commonly used polymerase is **Taq polymerase** as it comes from a thermophilic bacterium *Thermus aquaticus*
 - This means it **does not denature** at the **high temperature** involved during the first stage of the PCR reaction
- **Free nucleotides** - used in the construction of the DNA or RNA strands
- **Buffer solution** - to provide the optimum pH for the reactions to occur in

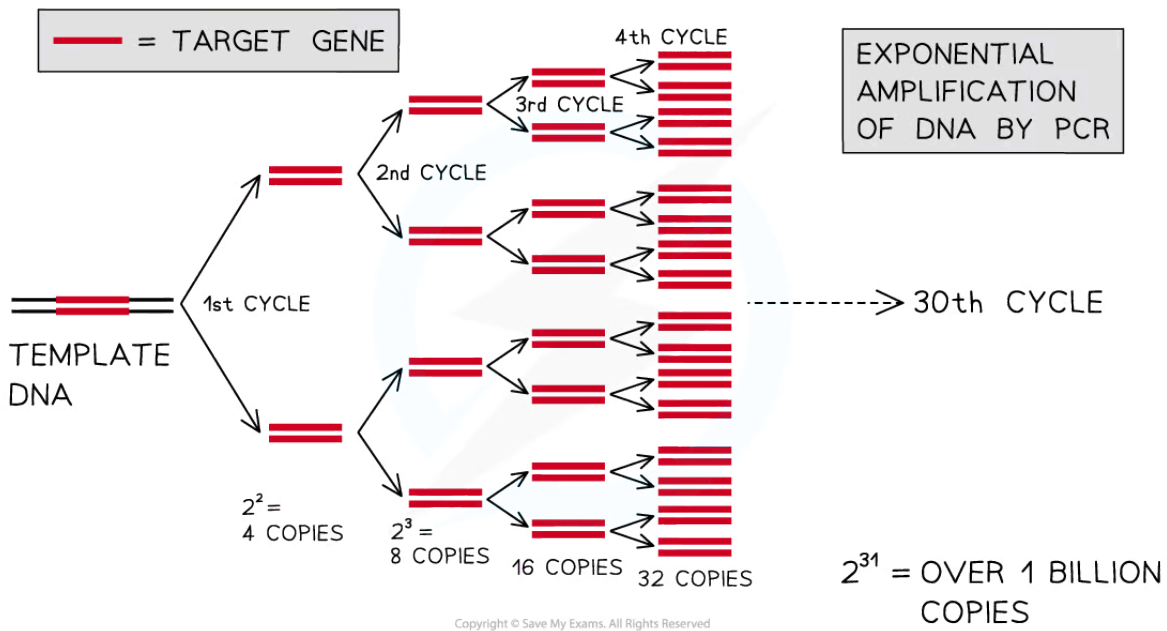
The key stages of PCR

- The PCR process involves **three key stages** per cycle
- In each cycle the DNA is doubled (so in a standard run of 20 cycles a million DNA molecules are produced)
- The PCR process occurs in a piece of specialist equipment called a **thermal cycler**, which automatically provides the **optimal temperature** for each stage and controls the **length of time** spent at each stage
- The three stages are:
 1. **Denaturation** - the double-stranded DNA is heated to 95°C which breaks the hydrogen bonds that bond the two DNA strands together
 2. **Annealing** - the temperature is decreased to between 50 - 60°C so that primers can anneal to the ends of the single strands of DNA
 3. **Elongation / Extension** - the temperature is increased to 72°C for at least a minute, as this is the optimum temperature for *Taq* polymerase to build the complementary strands of DNA to produce the new identical double-stranded DNA molecules
- **Each whole cycle takes a few minutes**, so 30 cycles can take just a few hours and can generate 2^{31} (over 1 billion) copies of a gene from a single DNA molecule, by **exponential amplification**

DNA amplification during PCR diagram



Your notes



Target DNA sequences can be copied exponentially by PCR to generate billions of copies in a short time



Your notes

Electrophoresis & PCR: Applications

Electrophoresis & PCR: Applications

DNA profiling

- **DNA profiling** (genetic fingerprinting) enables scientists to **identify suspects of a crime** and **identify corpses**
- Every person (apart from identical twins) has repeating, short, non-coding regions of DNA (20 to 50 bases long) that are unique to them
- These regions of DNA are called **VNTRs** (Variable Number Tandem Repeats)
- DNA profiling involves using **gel electrophoresis** to separate VNTR fragments according to length to create a pattern of bands that is unique to every individual, sometimes called the **genetic fingerprint**
- To **create a DNA profile** from the DNA being tested scientists complete the following in sequence:
 1. **Obtain the DNA**, which can be extracted from the root of a hair, a spot of blood, semen or saliva
 2. Increase the quantity of DNA by using **PCR** to produce **large quantities** of the required fragment of DNA from very small samples (even just one molecule of DNA or RNA).
 3. Use **restriction endonucleases** to cut the amplified DNA molecules into fragments
 4. Separate the fragments using **gel electrophoresis**
 5. Add **radioactive or fluorescent probes** that are complementary and therefore bind to specific DNA sequences
 6. X-ray images are produced or UV light is used to produce images of the fluorescent labels glowing
 7. These images contain **patterns of bars** (the DNA profile) which are then **analysed and compared**

Use of DNA profiling in Paternity Investigations

- Every person inherits their DNA VNTRs from **both** their mother and their father
- A man may sometimes **deny being the father** of a child to evade parenting responsibilities
- A woman may not know for sure which of her recent sexual partners is the father of a child
- A child may wish to know definitively who his/her father is to **be aware of possible inherited illnesses** that might affect him/her in future
- DNA profiles of the mother and child are compared, along with the profile of the alleged father (**all three** are needed)
- **Patterns of bands are compared** on all three genetic profiles
 - Any band that appears in the child's profile **must show in either the mother's or father's profiles**; if not, the alleged true father is a different man

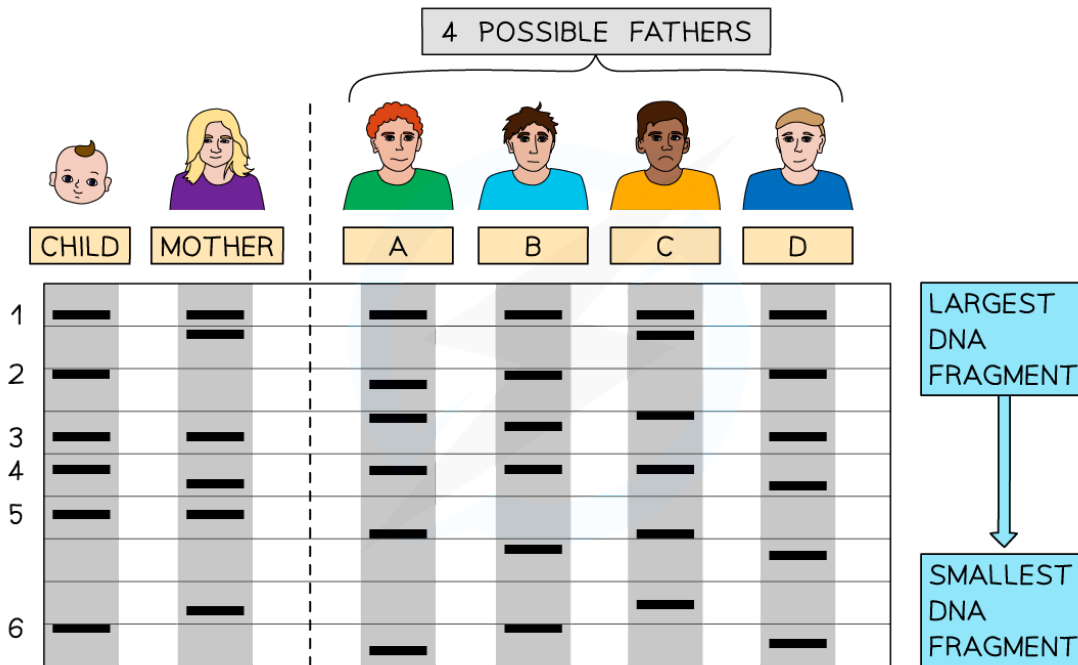


Your notes

Worked example

Who's the Father? – Use the DNA profiles of all 6 people shown to work out who the child's father is

DNA profiles of a child, a mother and 4 possible fathers



Remember, any band showing in the child's profile must be present in the mother **OR** father's profile, **OR** both. If not, that man is not the child's father.

Answer:

Step 1: Look at the child's first DNA band (labelled 1)

The mother possesses this same band, so the child could have inherited that DNA from its mother. It is therefore needless to look at whether any of the men possess that band

Step 2: Look at the child's second DNA band (labelled 2)

The mother does not possess this band, so the child must have inherited it from its father. Only men B and D possess this band, so men A and C are eliminated

Step 3: Look at the child's third DNA band (labelled 3)

As with band 1, the mother possesses this same band, so the child could have inherited that DNA from its mother. It is therefore needless to look at whether any of the men possess that band

Step 4: Look at the child's fourth DNA band (labelled 4)

The mother does not possess this band, so the child must have inherited it from its father. Only men A, B and C possess this band, but A and C have already been eliminated

Step 5: Conclude that B is the father

Step 6: Look for supporting evidence from band 6

The mother does not possess this band, and the only man who possesses it is B. **This reinforces the conclusion that Man B is the child's father**



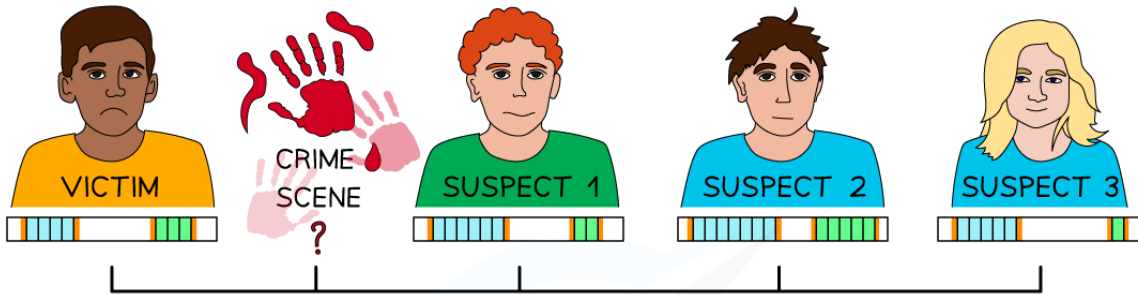
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Use of DNA profiling in Forensic Investigations

- DNA profiling has been used by forensic scientists to **identify suspects** of crimes
 - Samples of body cells or fluids (e.g. blood, saliva, hair, semen) are taken from the crime scene or victims body (e.g. rape victims)
 - DNA is **removed** and **profiled**
 - The profile is **compared to samples from the suspect** (or criminal DNA database), victim and people with no connection to the crime (control samples)
 - Care must be taken to **avoid contamination** of the samples
- DNA profiling can also be used in forensics to **identify bodies** or body parts that are unidentifiable (e.g. too badly decomposed or parts remaining after a severe fire)
- DNA profiling from a crime scene can also **eliminate innocent people** whose DNA may happen to appear there



Your notes



KNOWN LENGTHS	Control DNA	Victim	Crime scene	Suspects		
				1	2	3
—	—	—	—	—	—	—
—					—	
—				—		
—			—		—	—
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Using DNA profiling in criminal investigations. Suspect 3 has the most fragments in common with the crime scene DNA so it is likely that Suspect 3 is the culprit.

Examiner Tip

In the exam, you will be expected to interpret the results of gel electrophoresis experiments used to separate DNA fragments. For example, you will be given a few different genetic fingerprints and will have to match the victim to the crime or determine the parents of children. In these questions, you need to look for the most bands in common or a combination of parents' fingerprints that covers all the child's bands.

NOS: Reliability is enhanced by increasing the number of measurements in an experiment or test

- Increasing the **reliability** of scientific conclusions is a very important aspect of all experimentation, but this is particularly important when evidence is being collected for use in a court of law
- It is **almost impossible** to be 100% certain that any evidence provided is absolutely correct
- In DNA profiling, reliability can be increased by **increasing the number of VNTR markers** being observed and compared

- The more VNTRs used, the **lower the chance of a false match**



Your notes



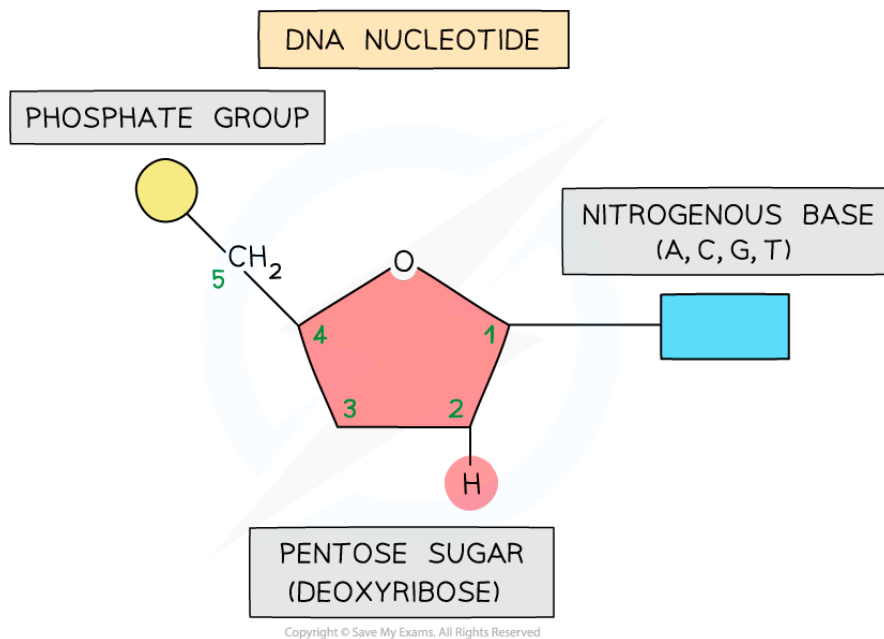
Your notes

Mechanism of DNA Replication (HL)

Directionality of DNA Polymerase

- Similar to transcription and translation, DNA replication must occur in the **5' to 3' direction**
- DNA polymerase only works in a 5' to 3' direction, **adding nucleotides to the 3' end** of a strand of nucleotides
- DNA nucleotides have a **phosphate** bonded to the **5' carbon** of the deoxyribose sugar
- When DNA polymerase adds a new nucleotide to extend the DNA strand, the 5' phosphate group of the **incoming DNA nucleotide** bonds to the free 3' -OH group on the **growing strand**

DNA nucleotide structure diagram

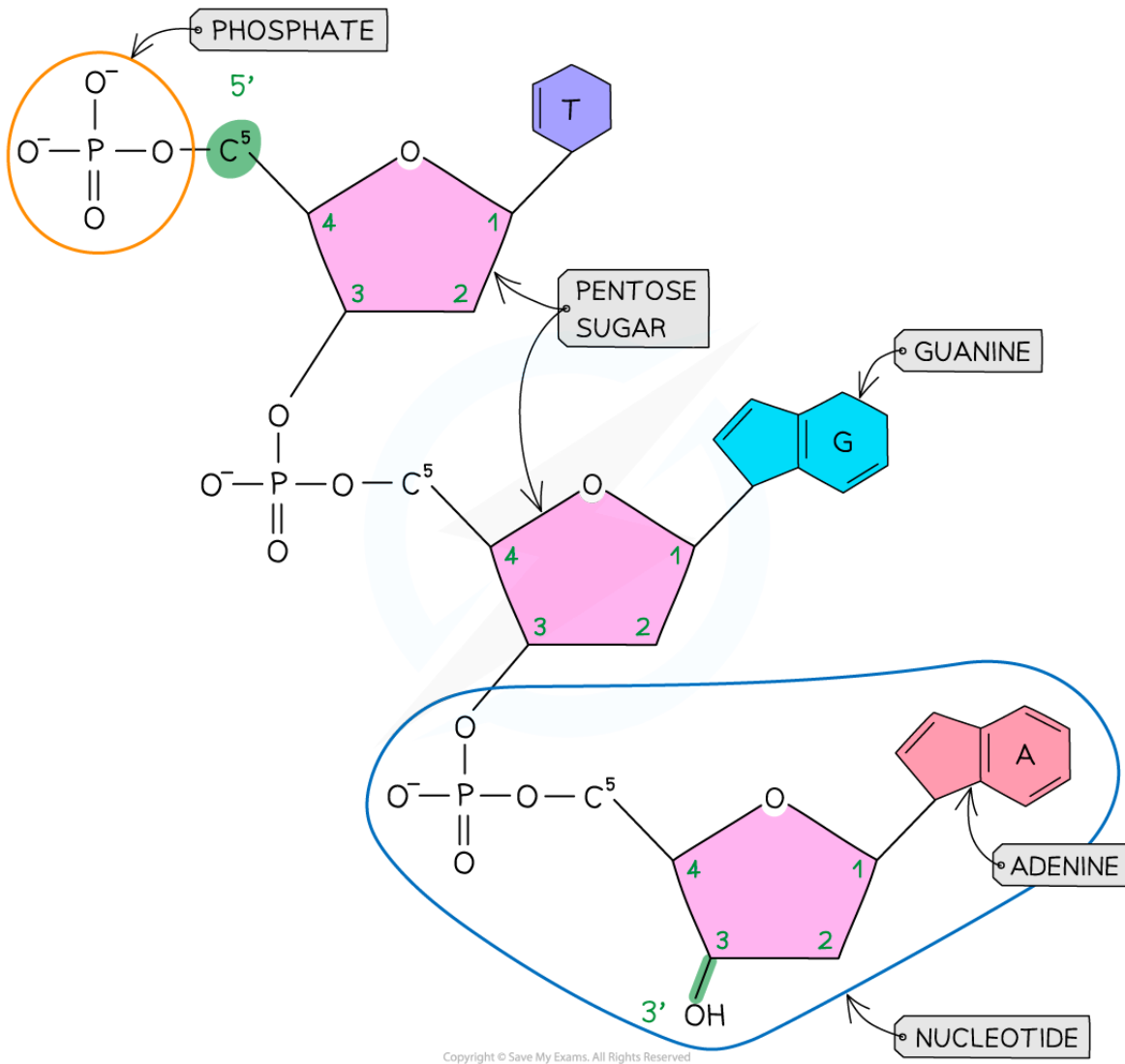


DNA nucleotides have a phosphate bonded to the 5' carbon of the pentose sugar

5' and 3' ends of a DNA strand diagram



Your notes



When DNA polymerase adds a new nucleotide, the 5' phosphate group of the incoming nucleotide bonds to the free 3' -OH group on the growing strand

The Leading & Lagging Strand

- Double-stranded DNA consists of **two antiparallel strands**
 - This means that one strand runs from 5' to 3', while the other strand runs from 3' to 5'
- During DNA replication, the two strands are 'unzipped' and DNA polymerase moves along each template strand linking nucleotides together to form a new strand
 - Crucially, **DNA polymerase can only add new nucleotides in a 5' to 3' direction**
 - As the template strands are antiparallel, replication needs to proceed in opposite directions
- As the replication fork opens up in one direction only, each new strand is synthesised differently
 - The **leading strand** is made **continuously**, following the fork as it opens
 - The **lagging strand** is made **discontinuously**, in short fragments, away from the fork
 - These fragments are called **Okazaki fragments**
- As more template strand is exposed, new fragments are created
 - Okazaki fragments are later joined together by DNA ligase to form a continuous complementary DNA strand
- Before new DNA nucleotides can be added to the new DNA strand, first an **RNA primer** must be added to create a binding point for **DNA polymerase III**
 - The RNA primer only has to be **added once on the leading strand** but **several are needed on the lagging strand** to initiate each fragment

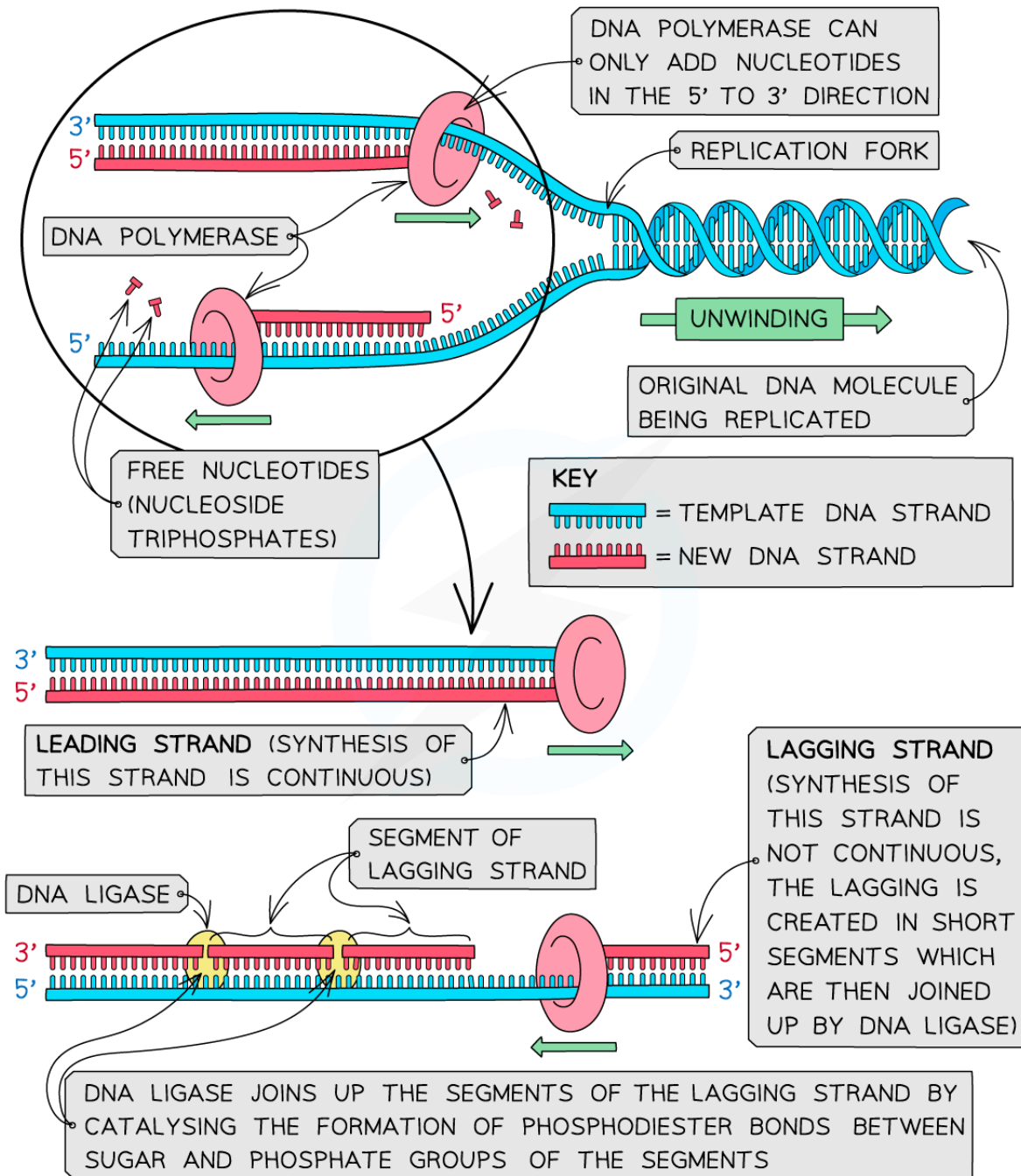
Difference between replication on the lagging and leading strands of DNA diagram



Your notes



Your notes



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During DNA replication, synthesis of the leading strand is continuous but synthesis of the the lagging strand is discontinuous in small fragments (not all the enzymes involved are shown)



Your notes

Enzymes in DNA Replication

- DNA replication is carried out by a **complex system of enzymes** working as a team
- **Helicase** unwinds the DNA double helix at the replication fork
 - Helicase then causes the hydrogen bonds between the two strands to break so that they can separate
- **Single-stranded binding proteins** keep the separated strands apart whilst the template strand is copied
- **DNA primase** generates a short RNA primer on the template strands
 - Providing an initiation point for DNA polymerase III to add new nucleotides
- A **number of polymerases** are involved in DNA replication, each with different functions
 - Two of these polymerases are
 - **DNA polymerase III**, which starts replication next to the RNA primer linking nucleotides in a 5' to 3' direction to form a new strand
 - **DNA polymerase I**, which removes the RNA primers on the leading and lagging strands and replaces it with DNA
- **DNA ligase** joins up the Okazaki fragments by catalysing the formation of sugar-phosphate bonds

Proofreading Replicated DNA

- Each time a human cell replicates it requires **3 billion new base pairs** to be synthesised in order to fully replicate the genome
- The copying process is not 100% perfect and mistakes do occur, these are called mutations
 - Mutations can be harmful to the functioning of the new cell and lead to diseases such as cancer
- In prokaryotes, in order to reduce mistakes during replication the enzyme **DNA polymerase III** acts as a **proof-reader** of the new daughter strand of DNA
 - It can **recognise incorrect DNA nucleotides** in the daughter strand
 - It **reverses direction** in order to **remove** the incorrect nucleotide from the **3' end of this strand**
 - The **correct nucleotide is then inserted** and the polymerase III enzyme continues replication