



DNA replication

Revision notes • IB Biology D1.1: semi-conservative DNA replication, helicase and DNA polymerase, PCR and gel electrophoresis, DNA profiling, and HL replication enzymes.

For IB Diploma Biology, Standard Level and Higher Level (first assessment 2025). **HL only** is additional higher level content, for HL students only.

DNA replication **Spec D1.1**

Exact copies by semi-conservative replication **Spec D1.1.1, D1.1.2**

DNA replication is the production of exact copies of DNA with identical base sequences. It happens before a cell divides, so that each daughter cell receives a full copy of the genome.

- **Reproduction:** DNA must be copied so that it can be passed to offspring, in asexual reproduction and in the production of gametes.
- **Growth:** a multicellular organism grows by mitosis, and every new cell needs a complete copy of the DNA.
- **Tissue replacement:** cells lost through damage or wear (for example skin cells or red blood cell precursors) are replaced by dividing cells, which again need copied DNA.

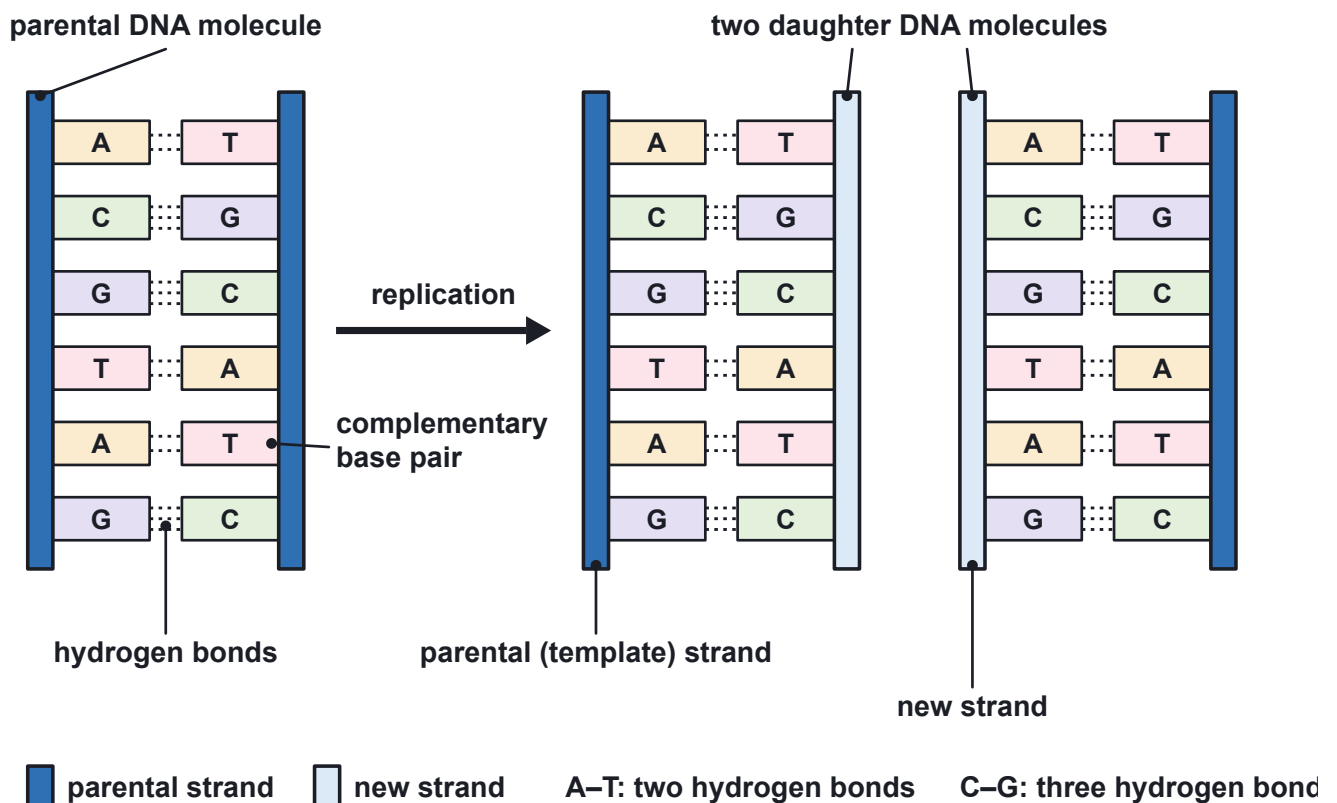
Semi-conservative replication

The two strands of the double helix separate and each one is used as a **template** for a new strand. Each of the two DNA molecules produced therefore has one strand from the original molecule (conserved) and one new strand. This is called **semi-conservative replication**.

Complementary base pairing and accuracy

Free DNA nucleotides are added to the new strand according to **complementary base pairing**: adenine pairs only with thymine (two hydrogen bonds) and cytosine pairs only with guanine (three hydrogen bonds). Because the base on the template strand decides which base is added, the new strand has exactly the sequence complementary to the template, and the new double helix is identical to the original.

A base that does not pair correctly does not fit or form the right hydrogen bonds, so mismatches are rare. This is why replication copies base sequences with a very high degree of accuracy.



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Semi-conservative replication: each daughter molecule keeps one parental strand and gains one new strand.

Common mistake: Semi-conservative does not mean half of each strand is new. Each daughter molecule has one complete old strand and one complete new strand.

Roles of helicase and DNA polymerase Spec D1.1.3

Two enzymes are central to replication.

Helicase	Unwinds the double helix and separates the two strands by breaking the hydrogen bonds between complementary bases. Each separated strand can then act as a template.
DNA polymerase	Assembles a new strand: it brings free DNA nucleotides into position opposite their complementary bases on the template strand and links them together with covalent (phosphodiester) bonds to form the sugar-phosphate backbone.

1. Helicase moves along the DNA, unwinding it and breaking hydrogen bonds between base pairs, so the strands separate.
2. Free DNA nucleotides pair with exposed bases on each template strand (A-T, C-G).
3. DNA polymerase links the paired nucleotides into a continuous new strand.
4. Two DNA molecules result, each with one old and one new strand.

Exam tip: Say helicase breaks hydrogen bonds between bases, not covalent bonds. DNA polymerase forms covalent bonds between adjacent nucleotides of the new strand.

PCR and gel electrophoresis Spec D11.4

Polymerase chain reaction (PCR)

The **polymerase chain reaction (PCR)** makes millions of copies of a chosen DNA sequence from a tiny sample. The reaction mixture contains the sample DNA, free DNA nucleotides, two **primers** and **Taq polymerase**, in a buffer. A thermal cycler changes the temperature in repeated cycles.

One cycle of PCR

Stage	Typical temperature	What happens
Denaturation	about 95 °C	Hydrogen bonds between the strands break, so the double helix separates into single strands.
Annealing	about 50–65 °C (often about 55 °C)	Primers bind by complementary base pairing to the ends of the target sequence on each single strand.
Extension	about 72 °C	Taq polymerase adds nucleotides to the primers, building a new complementary strand.

- **Primers** are short single-stranded sequences of DNA. Two different primers are used, one for each strand, and they mark the two ends of the sequence to be copied. DNA polymerase can only add nucleotides to an existing strand, so the primer gives it a starting point.
- **Taq polymerase** is a DNA polymerase from *Thermus aquaticus*, a bacterium of hot springs. It is not denatured at 95 °C and works best at about 72 °C, so it survives every cycle and does not need to be replaced.
- **Temperature changes** drive the cycle: high temperature separates strands, a lower temperature allows primers to bind, and an intermediate temperature suits Taq polymerase.

Each cycle doubles the number of copies of the target sequence, so the amount of DNA increases exponentially. Thirty cycles typically take one to two hours.

$$\text{number of copies} = \text{starting number of copies} \times 2^n \text{ (n = number of cycles)}$$

Maths skill: copies after PCR

A sample contains 50 copies of a target DNA sequence. How many copies are present after 20 cycles of PCR, assuming each cycle doubles the DNA?

1. $2^{20} = 1\,048\,576$

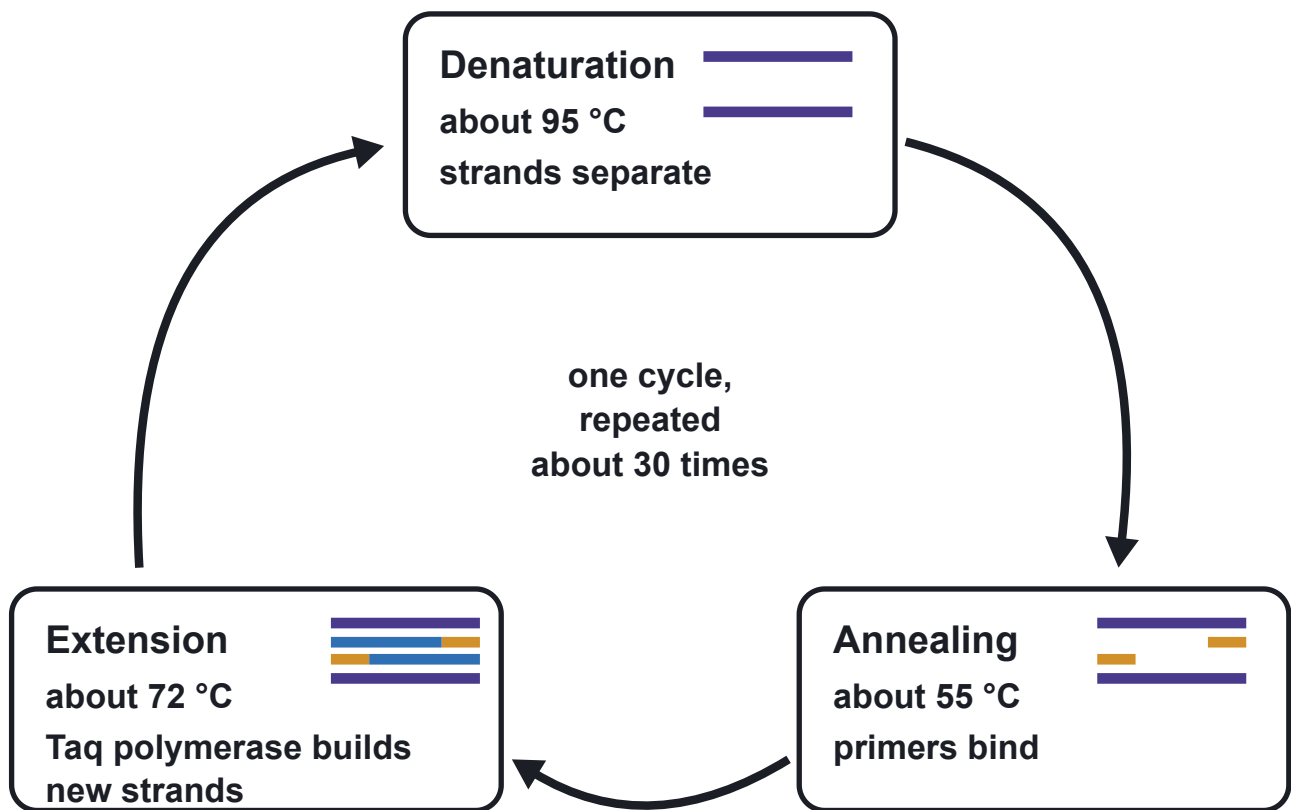
2. $50 \times 1\,048\,576 = 52\,428\,800$

Answer: About 5.2×10^7 copies.

Gel electrophoresis

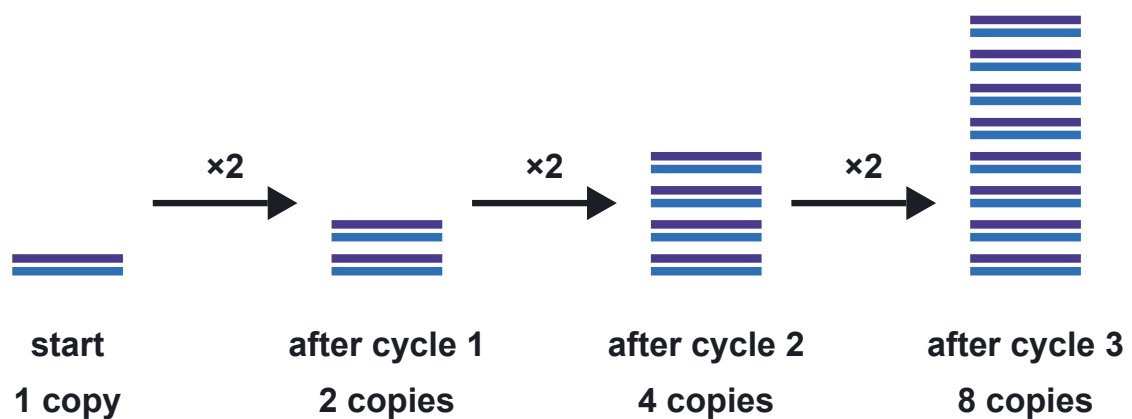
Gel electrophoresis separates DNA fragments according to their length. Samples are placed in wells at one end of an agarose gel and a voltage is applied. DNA has a **negative charge** (from its phosphate groups), so fragments move through the gel towards the positive electrode. The gel acts like a mesh: **smaller fragments move faster and travel further** than larger ones in the same time.

A DNA ladder (a mixture of fragments of known lengths) is run in one lane so that fragment sizes can be estimated. The DNA is stained so that the bands can be seen.



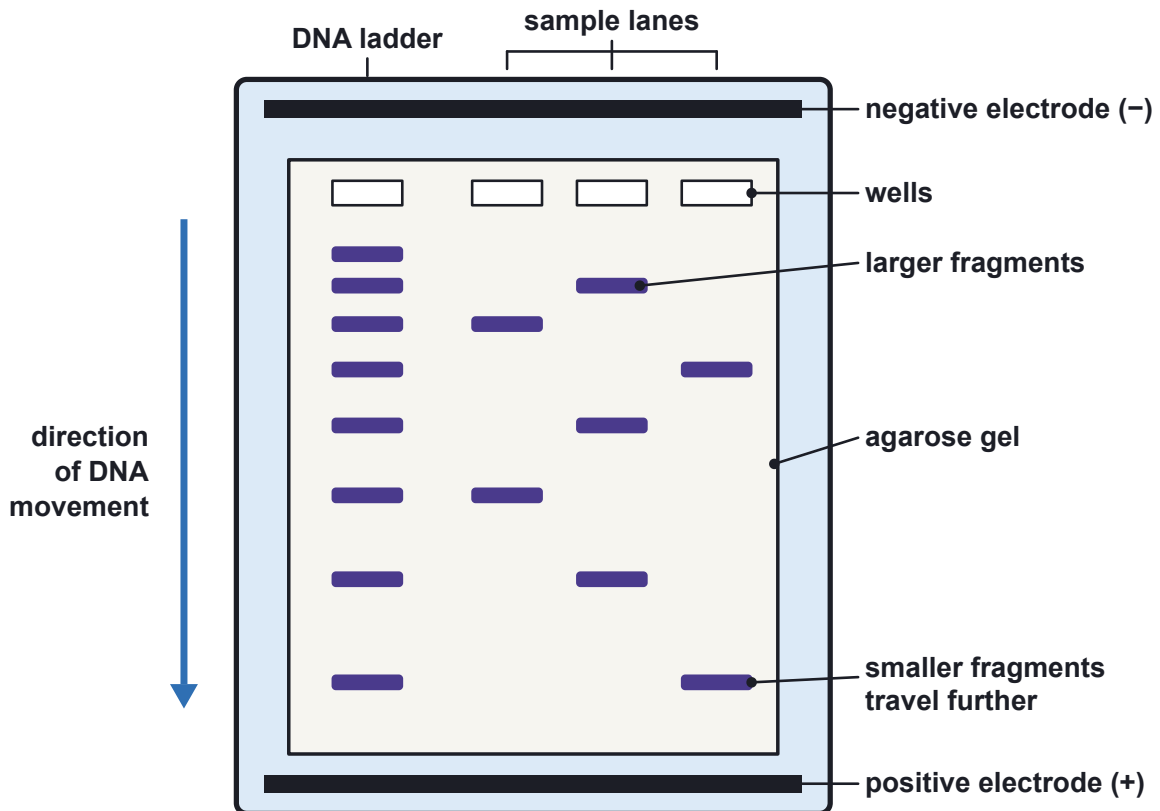
— template DNA
 — primer
 — new strand

copies of the target sequence double every cycle



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One cycle of **PCR**. Each cycle doubles the number of copies of the target sequence.



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DNA is negatively charged, so it moves towards the **positive electrode**; **smaller fragments travel further**.

Practical skill: When reading a gel, compare each band with the ladder lane. Bands at the same distance from the wells in different lanes are fragments of the same length.

Applications of PCR and gel electrophoresis Spec D1.1.5

Because PCR can amplify a chosen sequence from a tiny sample and gel electrophoresis can separate fragments by length, the two techniques have a broad range of applications.

- **DNA profiling** for paternity and forensic investigations.
- **Detecting pathogens:** PCR detects DNA (or RNA copied into DNA) of a virus or bacterium in a patient sample, as in tests for viral infections.
- **Genetic screening:** testing for alleles that cause genetic conditions.
- **Research:** amplifying genes for sequencing or for comparing species.

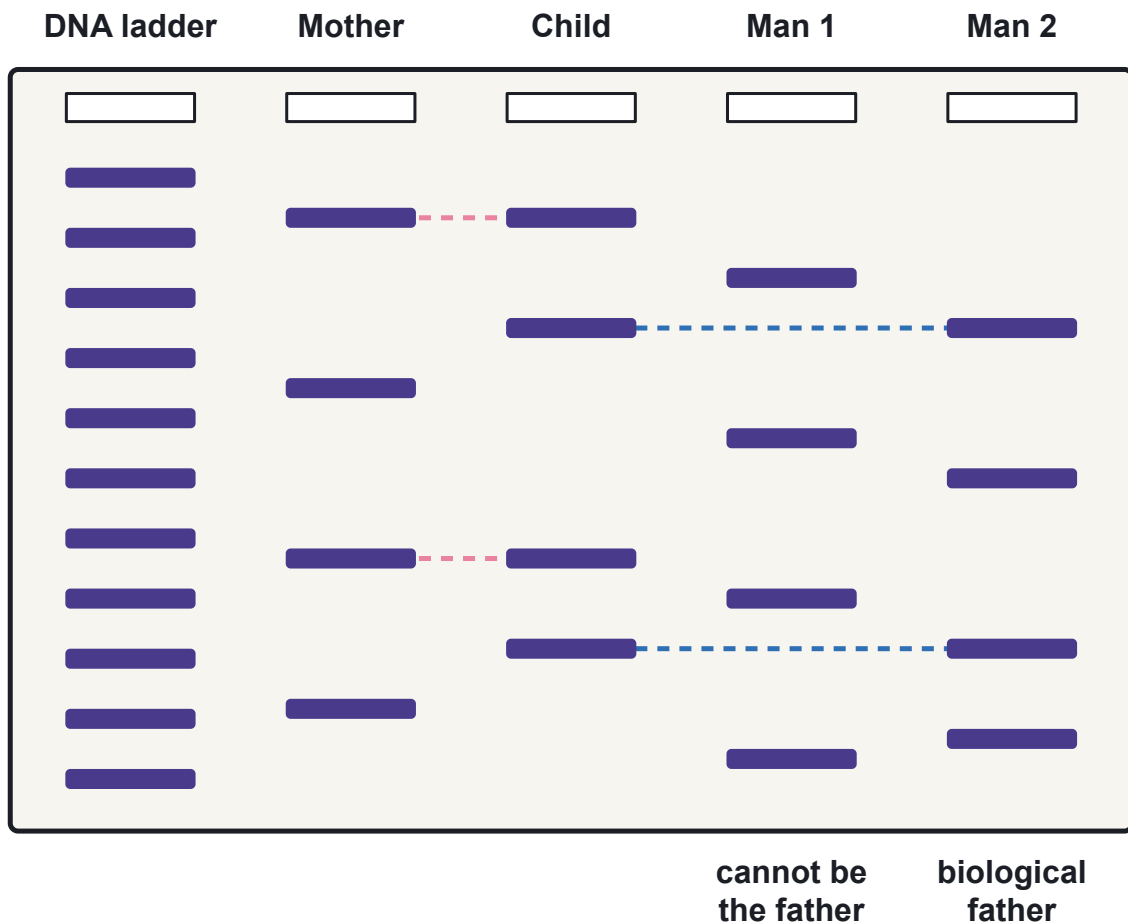
DNA profiling

Some regions of the genome contain **short tandem repeats (STRs)**: a short base sequence repeated many times. The number of repeats at each region varies between individuals, so the length of the region varies. In **DNA profiling**, several of these regions are amplified by PCR and the fragments are separated by gel electrophoresis, giving a pattern of bands (a DNA profile) that is almost unique to each person.

Using DNA profiles

Use	How profiles are compared
Paternity	A child inherits one allele of each region from each parent. Every band in the child's profile must match a band in the mother's or in the father's profile. A man whose profile lacks the non-maternal bands cannot be the father.
Forensic investigation	DNA from blood, hair or skin cells at a crime scene is amplified and profiled, then compared with profiles from suspects. A match links a person to the scene; a mismatch excludes them.

Exam tip: Nature of science: reliability is enhanced by increasing the number of measurements. In DNA profiling, using more markers (more STR regions) reduces the probability of a false match between two different people.



--- child's band matches a band from the mother

--- child's band matches a band from Man 2

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Paternity test: each band in the child comes from one parent, so Man 2 is the father and Man 1 is excluded.

Directionality: leading and lagging strands Spec D1.1.6, D1.1.7 HL only

5' and 3' ends

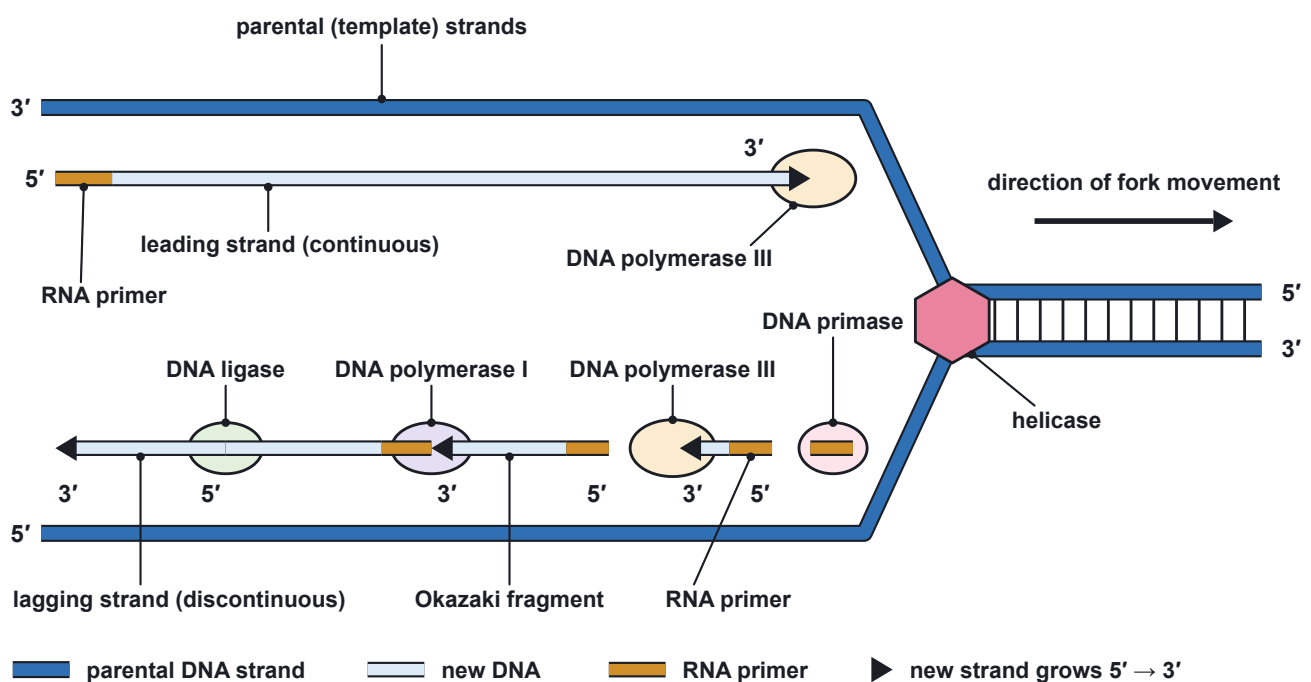
Each nucleotide in a strand is linked to the next through its deoxyribose. The **5' terminal** of a strand has a phosphate group attached to carbon 5' of the sugar; the **3' terminal** has a free -OH group on carbon 3'. The two strands of the double helix are antiparallel: they run in opposite directions.

DNA polymerases add the 5' of a DNA nucleotide to the 3' end of a strand of nucleotides. The phosphate on carbon 5' of the incoming nucleotide is bonded to the 3' -OH of the strand. Free nucleotides arrive as deoxynucleoside triphosphates, and the release of two phosphates provides the energy for the bond. New DNA therefore always grows in the 5' → 3' direction.

Leading and lagging strands

Replication at a replication fork		
Feature	Leading strand	Lagging strand
Direction of synthesis relative to the fork	Same direction as the fork moves	Opposite direction to the fork
Type of replication	Continuous	Discontinuous, in short sections called Okazaki fragments
RNA primers	Needed only once, at the start	Needed repeatedly, one for each Okazaki fragment
Joining	Little joining: the single primer is replaced by DNA and the one nick sealed by DNA ligase	Fragments joined by DNA ligase

Because the template strands are antiparallel and synthesis can only go 5' → 3', one new strand can follow the helicase as the fork opens, while the other must be made in short pieces backwards from the fork, each started afresh as more template is exposed.



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At a replication fork, new DNA always grows 5' → 3': the **leading strand** is continuous and the **lagging strand** is made in Okazaki fragments.

Exam tip: Linking question: what biological mechanisms rely on directionality? Replication, transcription and translation all proceed 5' to 3', which follows from the structure of the sugar-phosphate backbone.

Replication enzymes and DNA proofreading Spec D1.1.8, D1.1.9 HL only

The functions below are for the **prokaryotic system**, as the IB requires.

Enzymes of prokaryotic DNA replication

Enzyme	Function
DNA primase	Synthesises a short RNA primer complementary to the template. This provides the 3' end that DNA polymerase III needs to start adding nucleotides. Once on the leading strand; at the start of every Okazaki fragment on the lagging strand.
DNA polymerase III	Adds DNA nucleotides to the 3' end of the primer and then of the growing strand, 5' → 3', following complementary base pairing. Makes most of the new DNA. Also proofreads.
DNA polymerase I	Removes the RNA primers nucleotide by nucleotide and replaces them with DNA nucleotides.
DNA ligase	Seals the nicks between adjacent sections of DNA (for example between Okazaki fragments) by forming a covalent bond in the sugar-phosphate backbone.

DNA proofreading

Occasionally DNA polymerase III adds a nucleotide with a base that does not pair with the template. A mismatched base pair distorts the double helix at the 3' end of the new strand. DNA polymerase III detects this, **removes the nucleotide with the mismatched base from the 3' terminal** and then adds a correctly matched nucleotide in its place before continuing. **Proofreading** greatly reduces the error rate of replication.

Common mistake: In IB answers, proofreading of the new strand is credited to DNA polymerase III. The role to give for DNA polymerase I is removing RNA primers and replacing them with DNA (it can also correct errors, but this is not required).

Quick check

1. Why is DNA replication described as semi-conservative?
2. What is the role of helicase?
3. Why is Taq polymerase used in PCR?
4. Why do smaller DNA fragments travel further in gel electrophoresis?
5. **HL only** Why are many RNA primers needed on the lagging strand?

Quick check answers

DNA replication

1. Each new DNA molecule contains one strand from the original molecule and one newly synthesised strand.
2. It unwinds the double helix and breaks hydrogen bonds between bases so the strands separate.
3. It comes from a hot-spring bacterium and is not denatured at 95 °C, so it survives the denaturation step of every cycle.
4. They pass more easily through the mesh of the gel, so they move faster towards the positive electrode.
5. It is made discontinuously as Okazaki fragments, and each fragment must start from its own primer.