

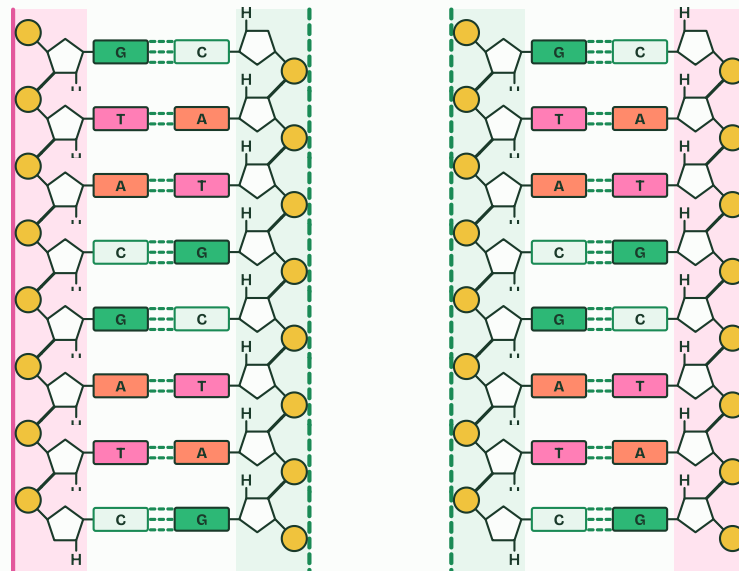
REVISION GUIDE

Copied in *full*, every time a cell divides

DNA replication

Before a cell divides it copies all of its DNA, base by base, and gets almost every one right. Here is how two enzymes unzip and rebuild the helix, how a lab copies a speck of DNA a billion times, and how a profile can name a parent or a suspect.

Two copies



10 chapters · a dry lab

The interactive guide, with every animation:

biologybybradford.com/guides/ib-biology-d1-1-dna-replication



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By the end you can

- ☐ Explain why cells copy their DNA, and why every copy must carry the same base sequence D1.1.1
- ☐ Explain what semi-conservative means, and how complementary base pairing makes copying accurate D1.1.2
- ☐ Describe what helicase and DNA polymerase do at a replication fork D1.1.3
- ☐ Outline how PCR copies a small sample of DNA, with primers, three temperatures and Taq polymerase D1.1.4
- ☐ Explain how gel electrophoresis separates DNA fragments by size, and read sizes against a ladder D1.1.4
- ☐ Use DNA profiles to settle paternity and forensic cases, and explain why more markers mean fewer false matches D1.1.5
- ☐ Explain why DNA polymerases add nucleotides only to the 3' end, so new strands grow 5' to 3' D1.1.6
- ☐ Compare replication on the leading and lagging strands, with Okazaki fragments and RNA primers D1.1.7
- ☐ State what DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase each do D1.1.8
- ☐ Explain how DNA polymerase III proofreads the strand it is building D1.1.9

Tick each one when you can do it without the book.

01

D1.1.1 EXACT COPIES

A copy for every *new cell*

Almost every cell in your body came from another cell dividing. Before a cell divides it has to hand each of the two new cells a full set of instructions, and the instructions are written in its DNA. Making a copy of a DNA molecule is called **DNA replication**.

The copy has to be exact. The message in DNA is the order of its bases, the **base sequence** (A1.2), so a faithful copy has the same bases in the same order. Change the sequence and you may change a protein. A change that slips through is a mutation (D1.3), which is why copying has to be so careful. Chapter 2 shows how the pairing rule keeps it accurate, and chapter 10 how the enzyme checks its own work.

Copying comes before every **cell division**. In a eukaryote it happens in the part of interphase before mitosis, when each chromosome's DNA molecule becomes two identical molecules (D2.1 has the cell cycle). Cells divide for three reasons.

KEY POINTS

- **Reproduction.** A bacterium splits in two, yeast buds and a strawberry plant sends out runners: **asexual reproduction**. Even the cells that become eggs and sperm come from cells that copied their DNA first.
- **Growth.** A fertilised egg becomes a body of about 30 trillion cells, every one made by division. This is **growth**.
- **Tissue replacement.** Skin, the lining of the gut and blood cells are replaced all the time, and a cut heals with new cells: **tissue replacement**.

Copying is how genetic continuity runs from one cell to the next, and from parent to offspring.

KEY TERMS**DNA replication**

Making a copy of a DNA molecule, so that the copy has exactly the same base sequence as the original.

base sequence

The order of the bases along a strand of DNA. It is the message, and every copy must carry the same one.

cell division

One cell splitting into two. The DNA is copied first, so that each new cell gets a full set.

asexual reproduction

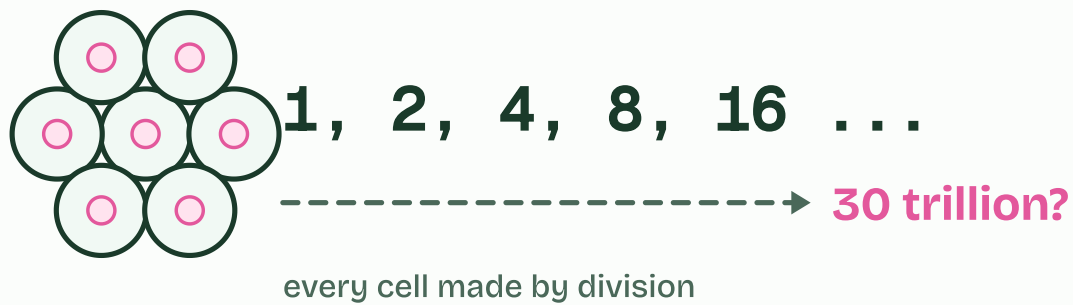
Making offspring from one parent by cell division, so each offspring starts with a copy of the parent's DNA.

growth

An increase in the number of cells in a body, made by division.

tissue replacement

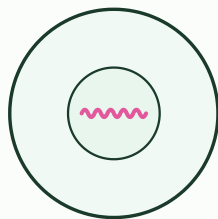
Making new cells by division to replace those that are worn out, lost or damaged.



STEP BY STEP

4 STEPS

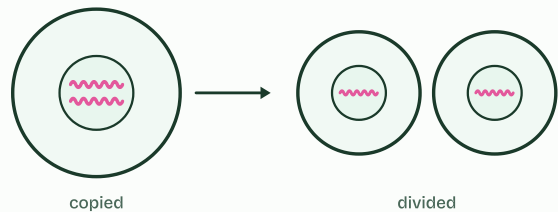
1



one cell, one copy of its DNA

A cell holds one copy of its **DNA**.

2



copied

divided

copy, then divide

Before it divides, it **copies** it, so each new cell has a full set.

3



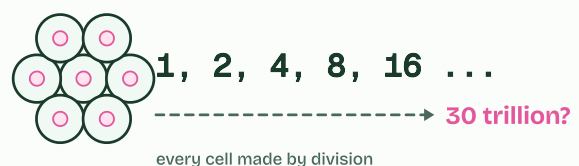
reproduction

growth

tissue replacement

Cells divide to **reproduce**, to **grow** and to **replace** what is lost.

4

Every one of your cells came from one by **division**.

WATCH OUT

Replication is not the same as cell division. Replication copies the DNA; the cell divides afterwards. And the copy is of the whole molecule, not of one gene.

PREDICT

You began as one fertilised egg and now have about 30 trillion cells. If every cell divided in step, about how many rounds of copying and dividing would it take?

- (A) About 30 thousand
- (B) About 45
- (C) About 30 trillion

Decide before you look. Answer on page 41.

02

D1.1.2 SEMI-CONSERVATIVE REPLICATION

One old strand, one *new*

The two strands of a double helix are held together by hydrogen bonds between their bases (A1.2). To copy the molecule, the cell pulls the strands apart. Each old strand then acts as a **template**: free nucleotides line up against its exposed bases, A opposite T and C opposite G, and are joined into a new strand. That is **complementary base pairing**, and nothing else fits.

So each new molecule, a **daughter molecule**, is made of one old strand and one new one. That is what **semi-conservative** means: half of each molecule is conserved from the one before.

Why is the copy so accurate? The pairing rule allows only one base opposite each base of the template. A new strand is therefore the exact complement of its template, and the complement of a complement is the original, so both daughter molecules carry the base sequence of the molecule that went in. Accurate is not the same as perfect: very rarely a wrong base is joined in, and chapter 10 shows how the enzyme catches it.

KEY POINTS

- Each old strand is a template for one new strand.
- Base pairing, A with T and C with G, makes each new strand the complement of its template.
- Each daughter molecule: one whole old strand and one whole new strand.

KEY TERMS

template

A strand of DNA that a new strand is built against. The new strand's bases are set by the template's.

complementary base pairing

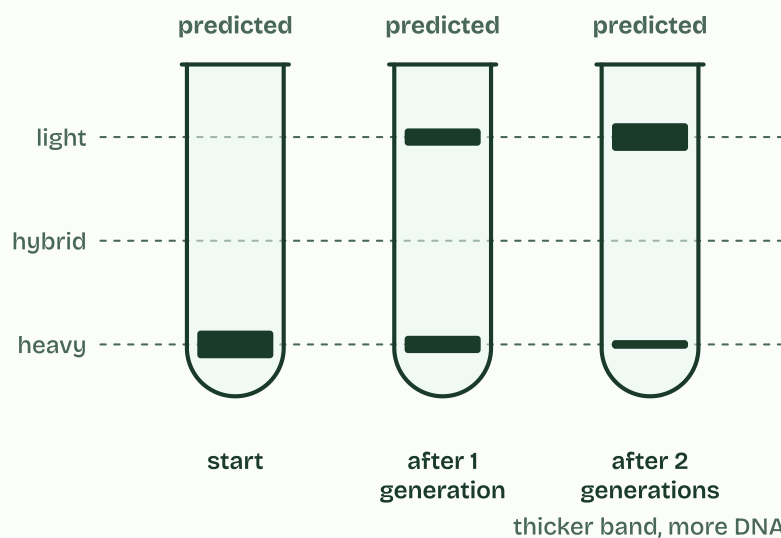
The rule that A pairs only with T and C only with G, held by hydrogen bonds. It fixes which base can sit opposite each base of the template.

daughter molecule

One of the two molecules made when a DNA molecule is copied. Each holds one old strand and one new one.

semi-conservative

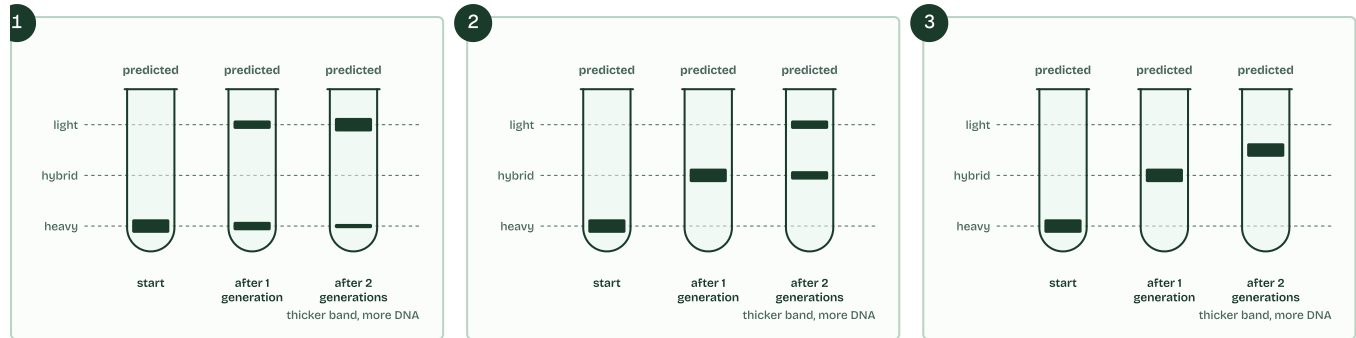
Describes DNA replication in which each daughter molecule keeps one of the two original strands and gains one new strand.



Each tube spins DNA into bands by density. Pick a model, then compare it with what was seen.

SWITCH IT

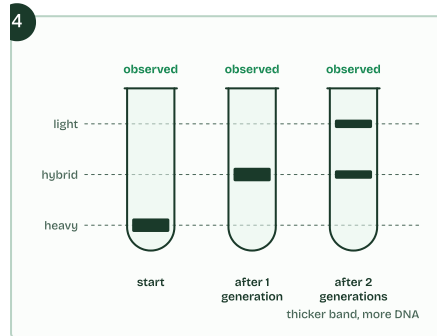
4 SETTINGS



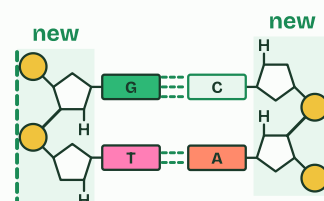
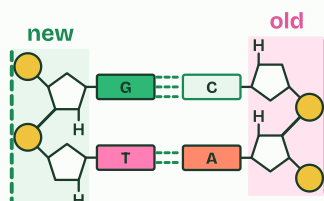
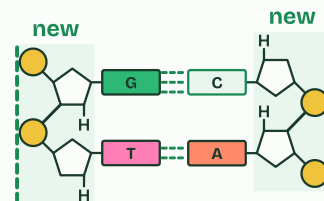
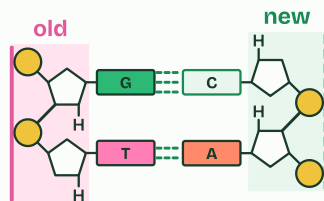
Conservative

Semi-conservative

Dispersive

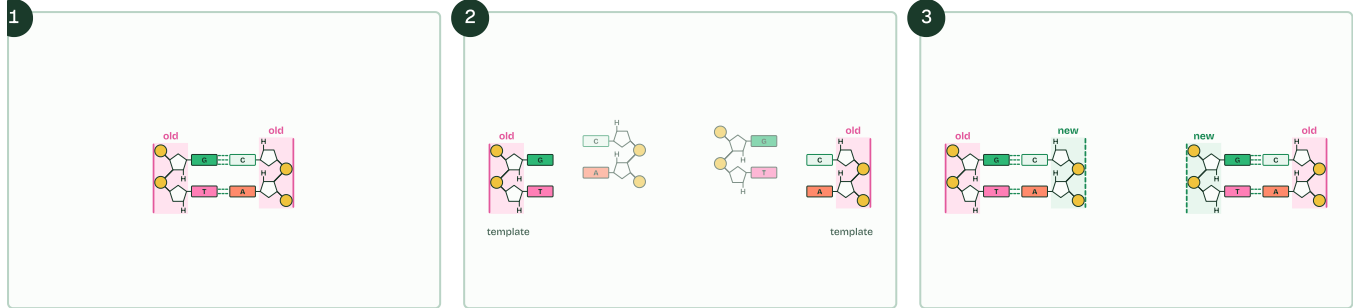
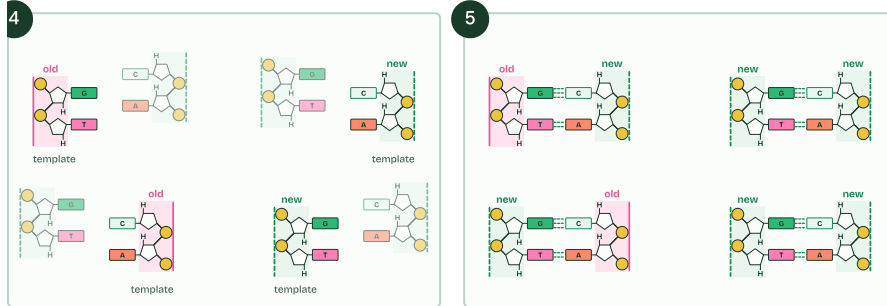


What they saw



STEP BY STEP

5 STEPS

One molecule: two **old** strands.A new partner is built along each strand: its **template**.Each new molecule keeps one old strand: **semi-conservative**.Copied again: **every** strand is a template.

Count the old strands.

PREDICT

A DNA molecule is copied, and then both copies are copied again. Of the four molecules, how many still contain a strand from the original?

- (A) All four: each keeps some of the original
- (B) None: the old strands get broken down after copying
- (C) Two: each old strand sits in one molecule

Decide before you look. Answer on page 41.

WATCH OUT

Semi-conservative does not mean each new strand is half old and half new. Every strand is entirely old or entirely new; it is the molecule that is half and half.

HOW WE KNOW (BEYOND THE IB GUIDE'S STATEMENTS)

People once proposed two other patterns: both old strands staying together in one molecule (conservative), or old and new pieces mixed along every strand (dispersive). An experiment decided between the three.

In 1958 Matthew Meselson and Franklin Stahl grew the bacterium *E. coli* for many generations on food whose nitrogen was the heavy isotope nitrogen-15, so all of its DNA was heavy. They moved the bacteria to food with ordinary nitrogen-14 and took samples after one and after two generations.

Spun very fast in a dense salt solution, DNA settles as a band at the height that matches its density: heavy low, light high, hybrid in between. Each model predicts a different pattern, so the results could rule two of them out. Choose a model to see the tubes it predicts, then what they saw.

03

D1.1.3 HELICASE AND DNA POLYMERASE

Unzip, then *build*

Copying is two jobs, and a different enzyme does each. First the helix has to open, because inside it every base is already paired and tucked away. Then a new strand has to be built against each old one.

Helicase is the unzipper. It moves along the double helix, unwinding it and breaking the hydrogen bonds between the base pairs, so that the two strands part. The point where the helix is opening is the **replication fork**. Only hydrogen bonds break. The covalent bonds along each strand stay whole, so each template keeps its sequence.

DNA polymerase is the builder. **Free nucleotides** drift in the nucleus, or in the cytoplasm of a bacterium. One whose base pairs with an exposed base on the template is joined to the end of the new strand by a covalent bond in the sugar and phosphate backbone. DNA polymerase only joins a nucleotide that pairs correctly, which is why the copy is accurate. It adds whole nucleotides, already made: it does not make bases.

Each template strand has its own DNA polymerase. The strands run in opposite directions, so the two enzymes work in opposite directions, and that makes the two new strands very different: chapter 8 explains how.

KEY POINTS

- Helicase unwinds the helix and breaks the hydrogen bonds between bases.
- DNA polymerase joins free nucleotides into a new strand, matched to the template by base pairing.
- One polymerase works on each template strand.

That answers the first guiding question. New DNA is made by unzipping the old and building a complementary strand on each half. Next, do it yourself: Run the fork.

KEY TERMS

helicase

The enzyme that unwinds the double helix and breaks the hydrogen bonds between its base pairs, so the two strands separate.

replication fork

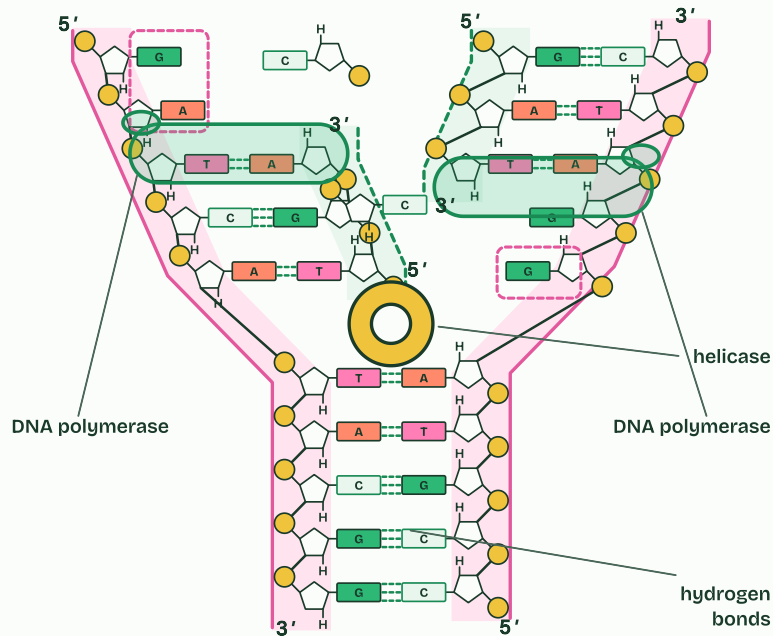
The Y-shaped point where the double helix is being unwound and copied: the unopened helix below, two single strands above.

DNA polymerase

The enzyme that builds new DNA. It joins free nucleotides into a strand, each one matched to the template by base pairing.

free nucleotides

A single nucleotide floating loose in the nucleus (or the cytoplasm of a bacterium), ready to be added to a new strand.



Old strands are on pink bands, new strands on green bands with a dashed edge. The two polymerases face opposite ways because the strands do.

An old strand of the parent molecule. Where the helix has opened, it is a template for a new strand.

Hydrogen bonds hold the base pairs together. They are weak one by one, so they can be broken without harm.

A new strand, complementary to its template.

Exposed bases. Only one kind of nucleotide can pair with each.

A free nucleotide a phosphate, deoxyribose and one of four bases, waiting to pair.

DNA polymerase joins each new nucleotide to the strand it is building, after its base has paired with the template.

An old strand of the parent molecule. Where the helix has opened, it is a template for a new strand.

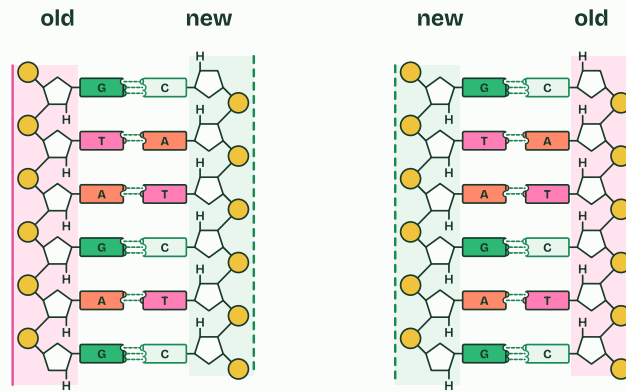
A new strand, complementary to its template.

Exposed bases. Only one kind of nucleotide can pair with each.

A free nucleotide a phosphate, deoxyribose and one of four bases, waiting to pair.

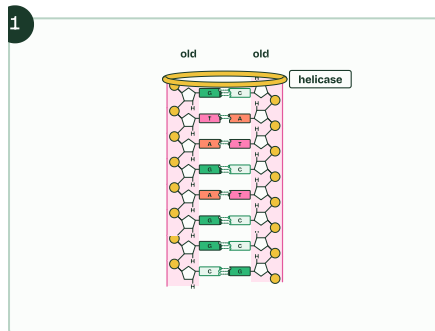
DNA polymerase joins each new nucleotide to the strand it is building, after its base has paired with the template.

Helicase unwinds the helix and pulls the base pairs apart, breaking only the hydrogen bonds that held them. Each strand stays whole.

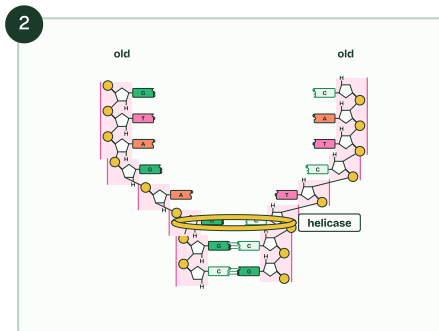


STEP BY STEP

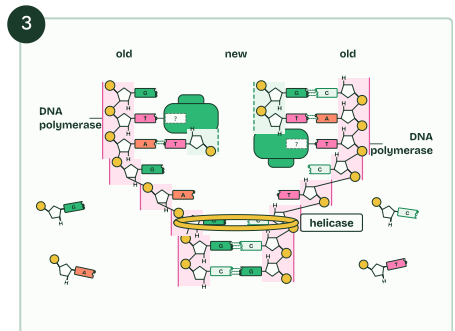
4 STEPS



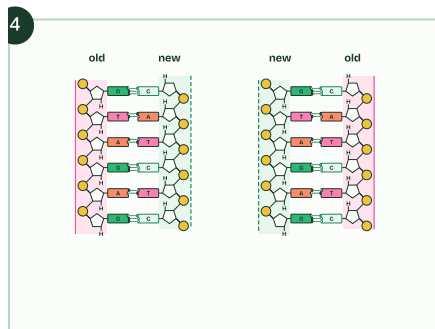
One molecule: two **old** strands.



Helicase unwinds the helix and breaks the hydrogen bonds.



DNA polymerase joins nucleotides that pair with each exposed base.



Two molecules, each with one **old** strand and one **new**.

WATCH OUT

Helicase breaks the hydrogen bonds between the bases, not the bonds between sugar and phosphate. And DNA polymerase does not “make bases”: it joins whole nucleotides, already made, into the new strand.

PREDICT

A drug stops helicase but leaves DNA polymerase working. What happens to replication?

- (A) It stops: polymerase needs single strands to read
- (B) It carries on, only more slowly than before
- (C) Polymerase copies the double strand without opening it

Decide before you look. Answer on page 41.

04

D1.1.4 THE POLYMERASE CHAIN REACTION

A *millionfold* copy

A smear of blood, a hair root or a swab holds far too little DNA to study. The **polymerase chain reaction (PCR)** copies one chosen stretch of DNA over and over until there is plenty. Making many copies of a small amount of DNA is to **amplify** it.

The tube holds the sample, plenty of free nucleotides, a buffer, a polymerase and two **primers**: short single strands of DNA, one matching each end of the stretch to be copied. A **thermal cyclor** then changes the temperature again and again. One cycle has three steps.

KEY POINTS

- **Denaturation, about 95 °C.** Heat breaks the hydrogen bonds and the strands separate. It does helicase's job.
- **Annealing, about 55 °C.** The mixture cools and a primer binds, by hydrogen bonds, to its matching sequence at each end of the target.
- **Extension, about 72 °C.** The polymerase adds nucleotides onto each primer and builds a complementary strand.

A cycle takes two or three minutes, and 30 cycles take an hour or two. Each cycle doubles the number of copies, so after n cycles one molecule can give up to 2^n : 30 cycles give 1,073,741,824. In practice the doubling slows near the end, as primers and nucleotides run low.

Why primers? A DNA polymerase cannot start a strand on its own. It can only add to the end of a strand that is already paired with the template. The primers give it that start, and their sequences choose which stretch gets copied; chapter 8 shows that a cell has the same problem.

The polymerase used is **Taq polymerase**, from *Thermus aquaticus*, a bacterium found in hot springs.

PCR mimics replication: strands are separated, nucleotides pair with a template and a polymerase joins them. What differs is that heat does the unzipping instead of helicase, the primers are made by a chemist, and one short stretch is copied instead of a whole chromosome. Chapter 5 shows how the copies are then sorted by size.

KEY TERMS

polymerase chain reaction (PCR)

A method that copies one chosen stretch of DNA again and again in a tube, so that a tiny sample becomes enough to study.

amplify

To make many copies of a small amount of DNA, as PCR does.

primers

A short single strand of DNA, about 20 nucleotides long, that binds to one end of the stretch to be copied and gives the polymerase a place to start.

thermal cyclor

A machine that raises and lowers the temperature of PCR tubes, over and over, on a set cycle.

denaturation

The first step of a PCR cycle, at about 95 °C: heat breaks the hydrogen bonds and the two strands separate.

annealing

The second step of a PCR cycle, at about 55 °C: the mixture cools and a primer binds to its matching sequence at each end of the target.

extension

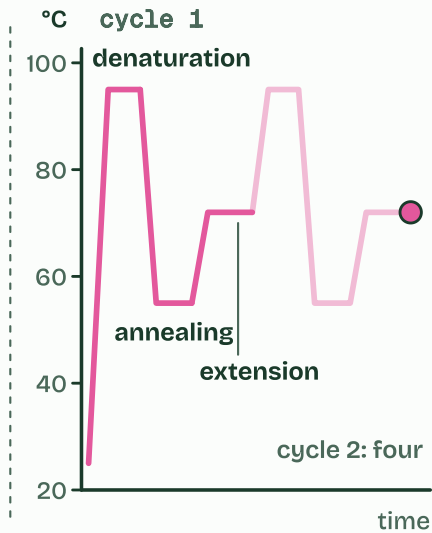
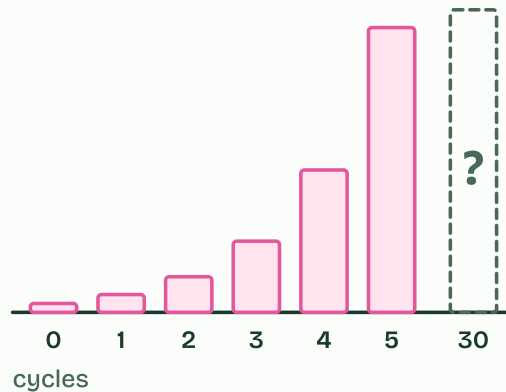
The third step of a PCR cycle, at about 72 °C: the polymerase adds nucleotides to each primer, building a complementary strand.

Taq polymerase

A DNA polymerase from *Thermus aquaticus*, a bacterium that lives in hot springs. It is used in PCR.

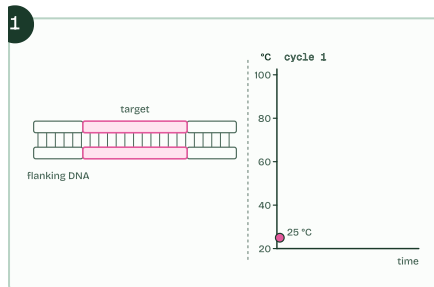
copies of the target

1, 2, 4, 8, 16, 32 ...

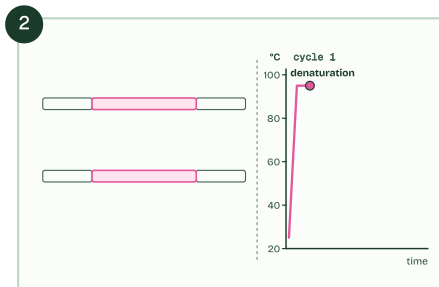
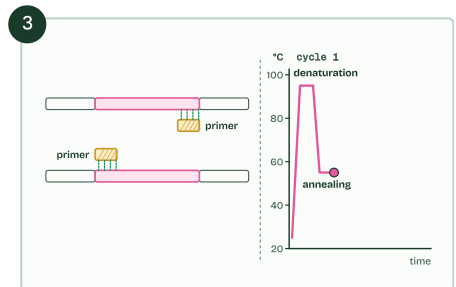
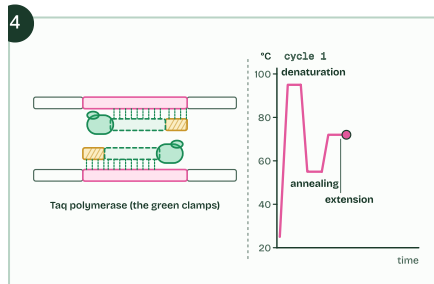
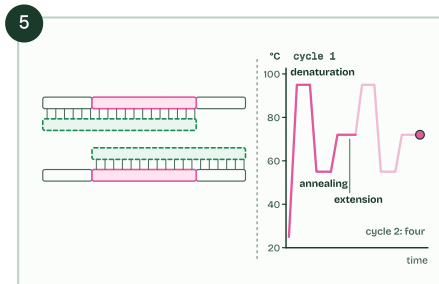
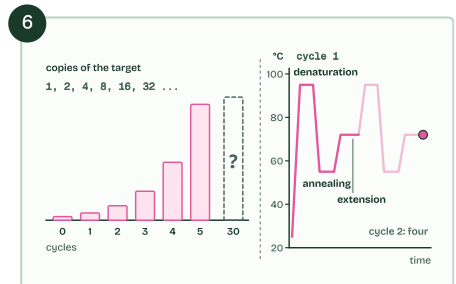


STEP BY STEP

6 STEPS



A sample of DNA, too little to study.

About 95 °C: the strands **separate**.About 55 °C: **primers** bind at each end of the target.About 72 °C: **Taq polymerase** extends each primer.One cycle: the copies **double**.

Thirty cycles later: how many?

DOUBLING

A crime-scene swab holds about 10 copies of the stretch to be tested. After 30 cycles there are $10 \times 2^{30} = 10 \times 1,073,741,824$, about 10.7 billion copies: enough to see as a band on a gel (chapter 5).

REMEMBER IT

One PCR cycle

D Dragons	A Always	E Exhale
Denaturation about 95 °C: the strands separate	Annealing about 55 °C: primers bind	Extension about 72 °C: Taq builds the new strands

WATCH OUT

PCR does not copy a whole genome, and it does not make a new organism. It copies one short stretch chosen by the primers. And denaturing DNA is harmless to it: the strands come apart and pair again, unlike a protein that loses its shape for good.

PREDICT

PCR uses a DNA polymerase from bacteria that live in hot springs. Why not a polymerase from human cells?

- Ⓐ A human polymerase would copy human DNA only
- Ⓑ A human polymerase would be denatured at 95 °C each cycle
- Ⓒ Hot-spring polymerase copies DNA without any primers at all

Decide before you look. Answer on page 41.

05

D1.1.4 GEL ELECTROPHORESIS

Sorted by *size*

PCR gives you a tube of identical copies. To see what is in it, you sort the DNA by size. **Gel electrophoresis** does that: it separates DNA **fragments** by their length, counted in **base pairs (bp)**.

A slab of gel, a jelly made from seaweed, has a row of small pits at one end, the **wells**. The slab lies in a tank of salty buffer between two **electrodes**. Each sample is mixed with a dense loading solution, so it sinks into its well, and a blue dye in the mix shows how far the run has gone. Then the current goes on.

Why does DNA move? Every nucleotide carries a phosphate group with a negative charge, so DNA is pulled towards the positive electrode, and the wells go at the negative end. At the bench: run to red, because the positive lead is usually red.

Why does it separate? The gel is a mesh of fibres. Short fragments slip through quickly and long ones are held back more. Every fragment carries about the same charge for each base pair, so the pull on each base pair is the same, and size decides how far a fragment gets in a set time. The shortest travel furthest.

A **DNA ladder**, a mix of fragments of known sizes, runs in its own lane and gives a scale. To size an unknown band, find the ladder band level with it, or the two it sits between.

DNA is colourless, so afterwards the gel is soaked in a stain that glows under blue or ultraviolet light when it binds DNA. Each set of same-length fragments shows as one band, so the number of bands is the number of different fragment lengths in the sample, and the distances between bands show their relative sizes. A band holds millions of identical fragments, because a single molecule is far too little to see. That is why PCR comes first.

KEY POINTS

- The negative charge of the phosphates pulls DNA towards the positive electrode.
- The mesh holds long fragments back more, so short ones travel furthest.
- The ladder gives the scale for reading sizes.

One more fact for the curious: distance travelled is not in proportion to size. It falls roughly with the logarithm of the size, so doubling a fragment's length does not halve its distance. And real profiling labs run the same separation in a hair-thin tube of gel, a capillary, that can tell apart fragments one base pair different in length. Textbooks, and this guide, draw slab gels.

KEY TERMS

gel electrophoresis

A method that separates DNA fragments by length, by pulling them through a gel with an electric field.

fragments

A piece of DNA of a particular length.

base pairs (bp)

The unit for the length of a DNA fragment: the number of base pairs it contains.

wells

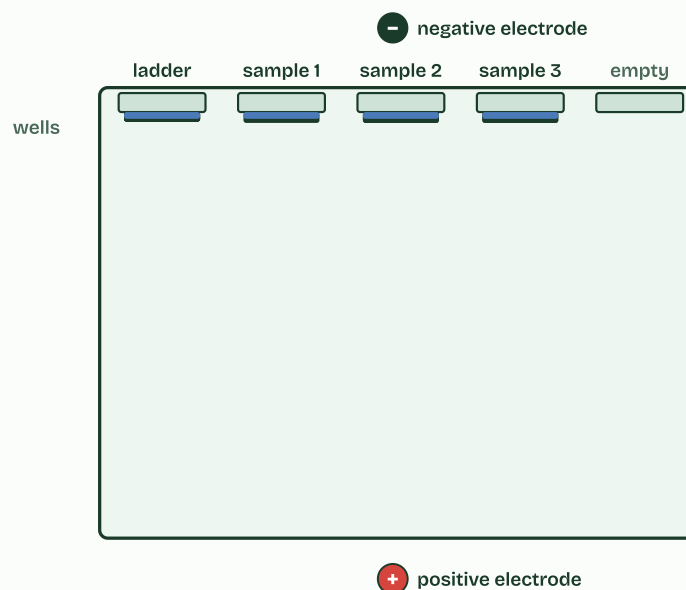
One of the small pits at the end of a gel, where a sample is loaded.

electrodes

A metal lead that carries current into the tank. One is positive and one negative.

DNA ladder

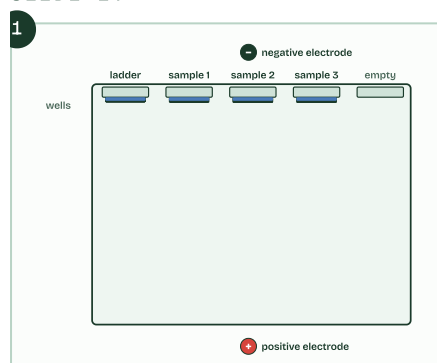
A mixture of DNA fragments of known sizes, run in its own lane to give a scale for reading the sizes of the other bands.



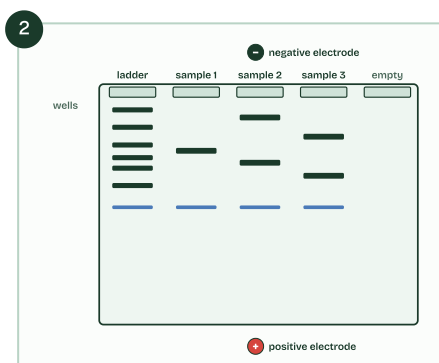
Seen from above. The DNA is stained, so every band shows. The sizes are in base pairs.

SLIDE IT

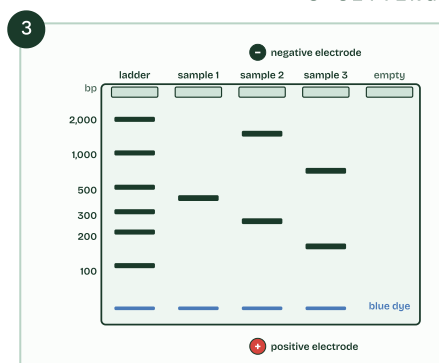
3 SETTINGS



Run time (min) 0



Run time (min) 30



Run time (min) 58

WATCH OUT

Bigger fragments do not travel further because they are “heavier” or “more charged”: they travel less far, because the gel holds them back. And DNA moves to the positive electrode, because it is negative.

PREDICT

Fragments of 300, 1,000 and 5,000 base pairs are run on the same gel. Which ends up nearest the wells?

- (A) 300 base pairs: small fragments stick to the gel
- (B) 1,000 base pairs: middle sizes are held back most
- (C) 5,000 base pairs: long fragments move slowest

Decide before you look. Answer on page 41.

06

D1.1.5 APPLICATIONS OF PCR AND GEL ELECTROPHORESIS

Profiles, parents and *crime scenes*

PCR and gel electrophoresis are used far beyond the classroom. A few of the jobs they do:

KEY POINTS

- Forensic investigations and paternity tests, by DNA profiling.
- Diagnosing infections. The COVID-19 test copies a stretch of the virus's genes (the virus's RNA is first copied into DNA).
- Testing for inherited conditions.
- Identifying species: a fish sold as cod that is not, the source of a meat sample, wildlife trading.
- Copying a gene so that it can be studied or moved.
- Studying ancient DNA from bones.

Most of our DNA is the same from one person to the next, but some places hold a short sequence repeated over and over, end to end: a **short tandem repeat (STR)**, such as GATA GATA GATA. People differ in how many repeats they have. Such a place is a **marker**, each version (a number of repeats) is an **allele**, and each person has two alleles at each marker, one from each parent.

DNA profiling starts with a sample: blood, saliva, a hair root, or skin cells left on a handle. PCR copies the markers, using primers that bind either side of each repeat. Electrophoresis then separates the copies by length: more repeats, longer copy, shorter distance. The pattern of bands at several markers is the person's profile.

In a **forensic** investigation, the profile from the scene is compared with those of the suspects and the victim. A match at every marker is strong evidence that the sample came from that person, and a single clear mismatch excludes them. Profiling has cleared innocent people as well as convicting guilty ones.

In a **paternity** test, a child has at each marker one allele from the mother and one from the father. A band in the child that the mother does not have must come from the father, so a man without it is excluded. Labs look for exclusions at two or more markers, because a rare new mutation can change one.

So how many markers are enough? One marker proves little, because many people share any one pair of alleles. Each extra independent marker is another test that a person must pass by luck, so the chance of a **false match** is multiplied by another small number and falls very fast. Reliability rises with the number of measurements. Real profiles use about 15 to 20 markers: 16 in the UK and 20 in the USA.

Take care all the same. Identical twins share a profile, close relatives share more than strangers, and a sample can be contaminated. The evidence is strong, but not proof on its own.

KEY TERMS

short tandem repeat (STR)

A short sequence of bases, such as GATA, repeated over and over end to end. The number of repeats differs from person to person.

marker

A place in the DNA, such as an STR, that is compared between people in DNA profiling.

allele

One version of a gene or a marker. For an STR it is a particular number of repeats. Each person has two at each marker, one from each parent.

DNA profiling

Comparing the alleles of a few markers, found by PCR and gel electrophoresis, between a sample and the people it might have come from.

forensic

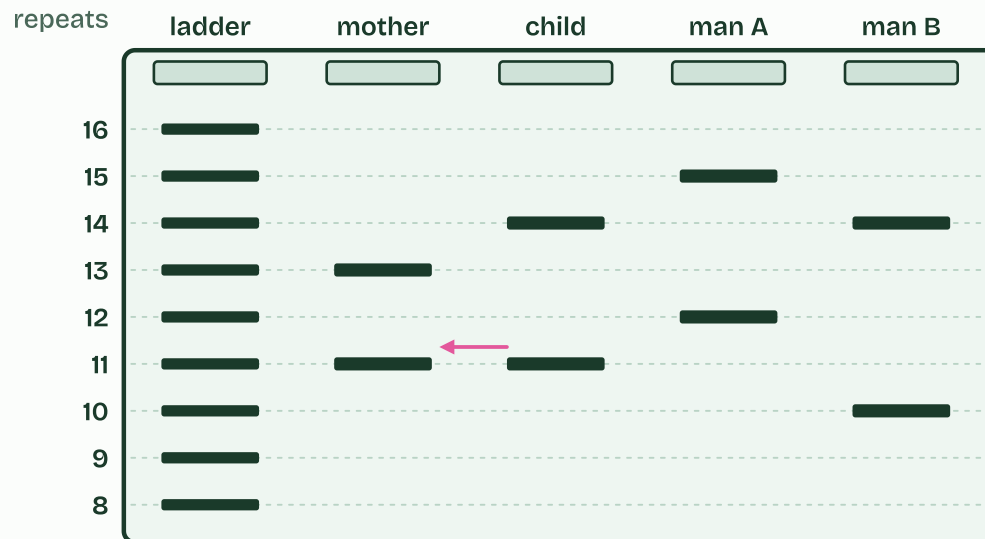
To do with finding evidence for a court of law.

paternity

To do with who a child's father is.

false match

Two different people having the same profile by chance.

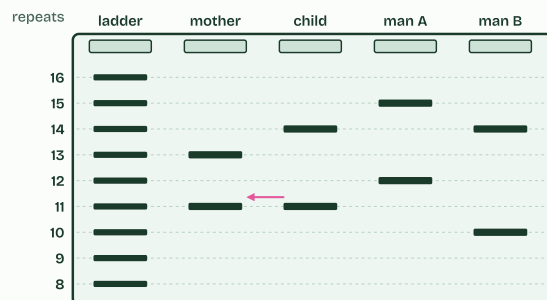


The ladder has a band for every allele, numbered by its repeats. More repeats make a longer copy, which travels less far.

SWITCH IT

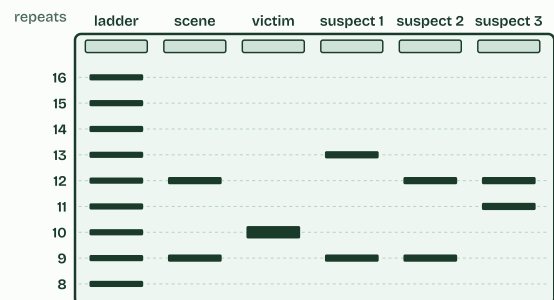
2 SETTINGS

1



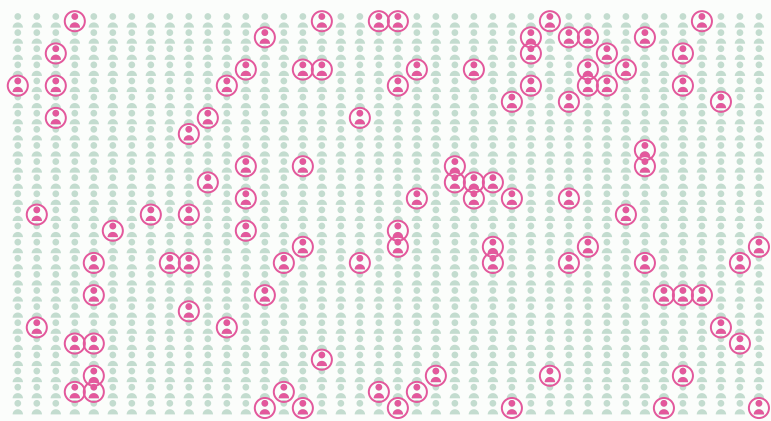
Paternity

2



Crime scene

a city of a million people matches by chance



1 marker: about 1 person in 10 matches by chance.
In this city of a million, that is about 100,000 people.
each dot is 1,000 people

Each marker is taken as shared by about 1 person in 10, so each extra marker divides the chance of a false match by 10. Real markers vary, and many are rarer.

SWITCH IT

4 SETTINGS

1

a city of a million people matches by chance

1 marker: about 1 person in 10 matches by chance.
In this city of a million, that is about 100,000 people.
each dot is 1,000 people

2

a city of a million people matches by chance

3 markers: about 1 person in 1,000 matches by chance.
About 1,000 people in this city: just one dot.
each dot is 1,000 people

3

a city of a million people matches by chance

10 markers: about 1 person in 10 billion.
Probably nobody in the city.
each dot is 1,000 people

1 marker

3

10

4

a city of a million people matches by chance

20 markers: about 1 in 10²⁰.
Far fewer than one person in everyone who has ever lived.
each dot is 1,000 people

20

WATCH OUT

A child does not share half of each parent's bands in some fixed way. At each marker the child has exactly one allele from each parent, and those may happen to be the same size as the other parent's. And a profile is not the person's whole genome: it is a handful of repeat counts.

PREDICT

At one marker, 1 person in 10 shares the crime-scene pattern by chance. With ten independent markers, each 1 in 10, how many people in a city of 10 million would you expect to match by chance?

- Ⓐ Almost certainly none of them
- Ⓑ About 1 million: one person in every ten
- Ⓒ About 100, as the chances add up each time

Decide before you look. Answer on page 41.

07

D1.1.6 HL DIRECTIONALITY OF DNA POLYMERASES

Only ever at the *3' end*

From A1.2: the carbons of the sugar in a nucleotide are numbered 1' to 5', and in a strand each phosphate links the 5' carbon of one nucleotide to the 3' carbon of the one before. So the two ends of a strand are different. One end has a free phosphate on a 5' carbon, the **5' terminal**. The other has a free hydroxyl group (OH) on a 3' carbon, the **3' terminal**.

DNA polymerases follow one rule. They join the 5' phosphate of an incoming nucleotide to the 3' OH at the end of the strand they are building, by a covalent bond in the sugar and phosphate backbone. So nucleotides are only ever added at the 3' end, and a new strand grows 5' to 3'. This one-way working is the **directionality** of DNA polymerases.

The template is read the other way. As the new strand grows 5' to 3', the polymerase moves along its template 3' to 5', because the two strands are antiparallel. At a fork the two templates run in opposite directions, so the two new strands have to grow in opposite directions. Chapter 8 follows what that means.

A polymerase also cannot start a strand from nothing. It needs a 3' end to add to, which is what the primers of chapter 8 are for.

KEY POINTS

- Nucleotides are added only at the 3' end of the new strand.
- So a new strand grows 5' to 3', and its template is read 3' to 5'.
- Antiparallel templates mean the two new strands at a fork grow in opposite directions.

Directionality rules other copying too. RNA is built 5' to 3' in transcription, and a ribosome reads messenger RNA 5' to 3' in translation (D1.2).

KEY TERMS

5' terminal

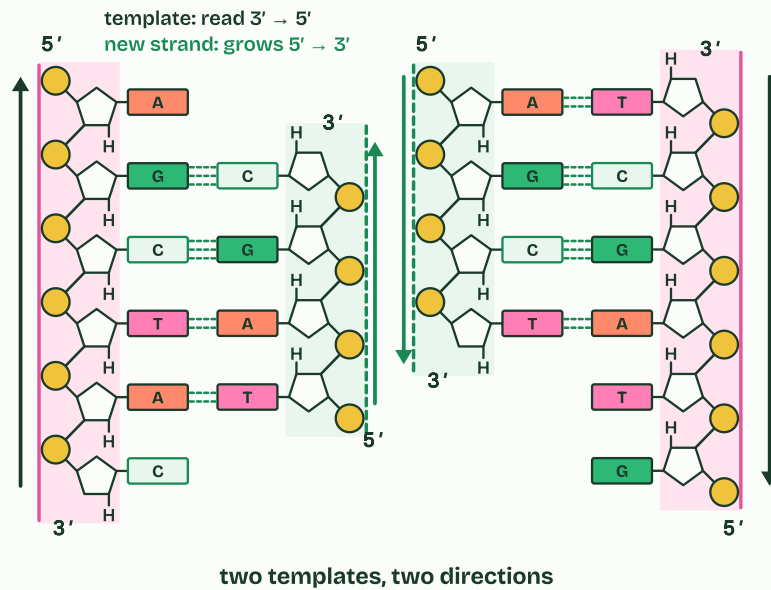
The end of a strand of nucleotides that has a free phosphate group on a 5' carbon.

3' terminal

The end of a strand of nucleotides that has a free hydroxyl group on a 3' carbon. It is where new nucleotides are added.

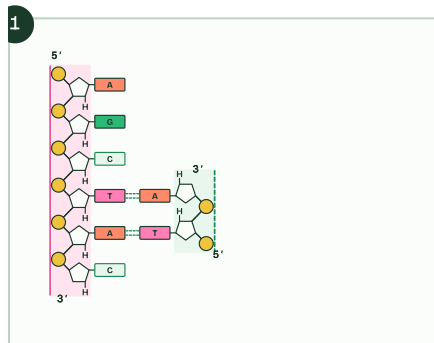
directionality

Working in one direction only. DNA polymerases add nucleotides at the 3' end of a strand, so a new strand grows 5' to 3'.

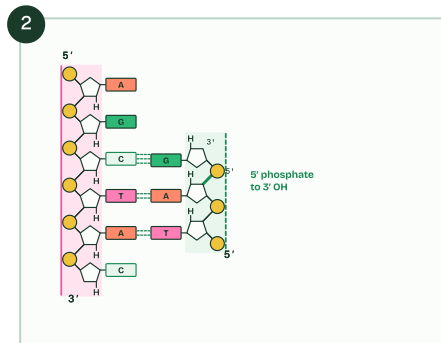


STEP BY STEP

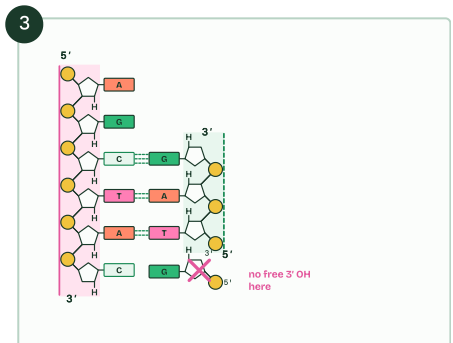
5 STEPS



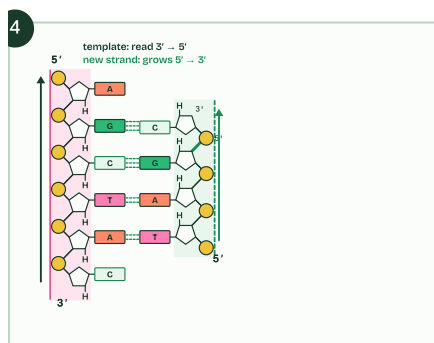
Every strand has a **5'** end and a **3'** end.



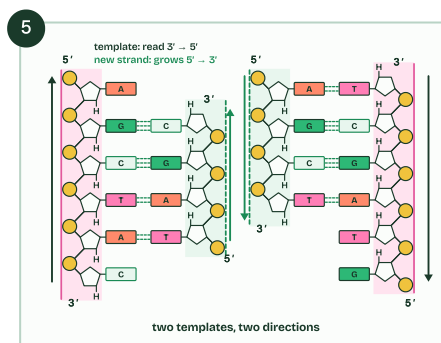
DNA polymerase joins a nucleotide's **5'** phosphate to the **3'** end.



There is nothing to join to at the **5' end**.



So new strands grow **5' to 3'**.



The templates are antiparallel, so the new strands grow in **opposite** directions.

WHERE THE ENERGY COMES FROM (NOT NEEDED FOR THE EXAM)

Free nucleotides arrive carrying three phosphates. DNA polymerase cuts off two, and the energy released makes the new bond.

WATCH OUT

"5' to 3'" describes the new strand. The template is read 3' to 5'. And 5' and 3' name carbons of the sugar, not ends of a gene.

PREDICT

A free nucleotide arrives at the 5' end of a growing new strand, and its base pairs with the template. What happens?

- ☐ (A) It is joined, and the strand grows at both ends
- ☐ (B) It is not joined: there is no free 3' OH there
- ☐ (C) It is joined if its base pairs with the template

Decide before you look. Answer on page 41.

08

D1.1.7 HL LEADING AND LAGGING STRANDS

One strand smooth, one *in pieces*

The fork moves along the DNA as helicase unwinds it. One template is the right way round for its new strand, growing 5' to 3', to grow in the same direction as the fork moves. DNA polymerase follows right behind helicase and builds without stopping. This is the **leading strand**, made **continuously**.

On the other template the new strand must also grow 5' to 3', and for this one that is away from the fork. As the fork opens a fresh stretch of template, a polymerase starts near the fork and builds back towards the piece before it. Then the fork opens a little more and the polymerase starts again. This is the **lagging strand**, made **discontinuously** in short pieces called **Okazaki fragments**.

A polymerase cannot start a strand from nothing, as chapter 7 said. So every new piece of DNA begins with a short **RNA primer**, made by an enzyme you meet in chapter 9, which gives the polymerase a 3' end to add to. The leading strand needs a primer only once, at its start. The lagging strand needs one for every Okazaki fragment.

Why "lagging"? Its pieces are finished a little behind the fork. Its polymerase is not lazier: both strands are made at the same fork at the same time. The pace differs, though. The leading strand grows steadily, at the speed the fork opens; the lagging strand grows in stop-start bursts, one fragment at a time, and keeps up only on average.

KEY POINTS

- Leading strand: built continuously towards the fork, from one primer.
- Lagging strand: built discontinuously, away from the fork, as Okazaki fragments, each from its own primer.
- Both are made at the same fork at the same time.

You will run both strands yourself in Run the fork's higher level rounds, after chapter 10.

KEY TERMS

leading strand

The new strand that is built towards the fork, in the same direction as the fork moves. It needs only one primer.

continuously

Built in one piece, without stopping, as the leading strand is.

lagging strand

The new strand that is built away from the fork, in pieces, each started again as the fork opens more template.

discontinuously

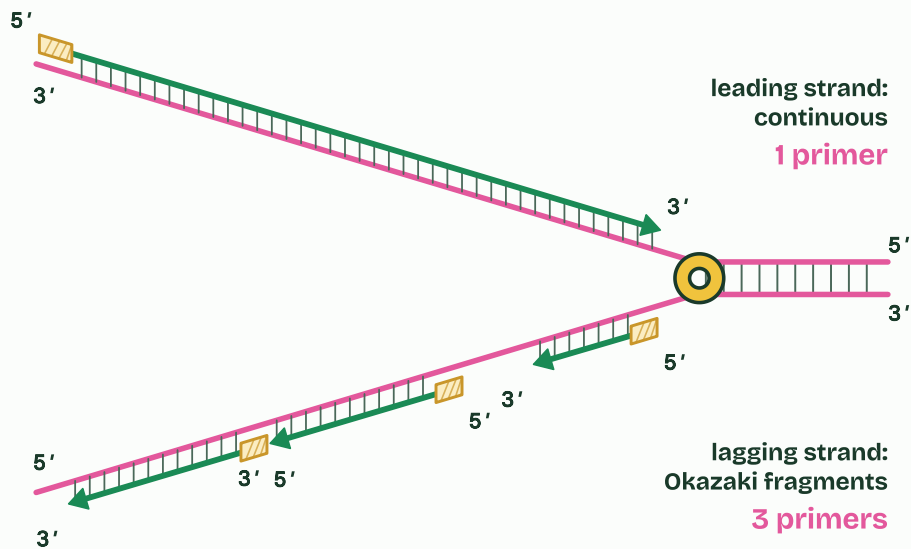
Built in separate pieces, each started again, as the lagging strand is.

Okazaki fragments

One of the short pieces of the lagging strand, named after Reiji and Tsuneko Okazaki, who found them in the 1960s.

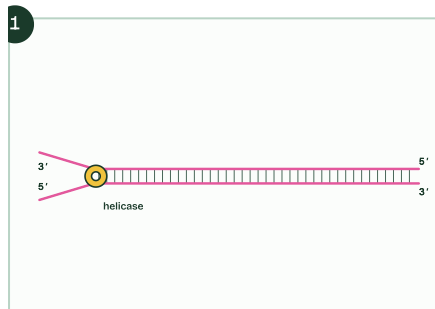
RNA primer

A short strand of RNA, made by an enzyme, that is paired with the template and gives DNA polymerase a 3' end to add to.

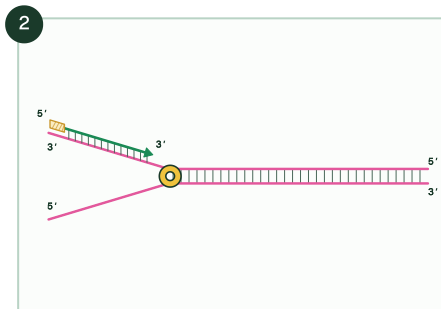
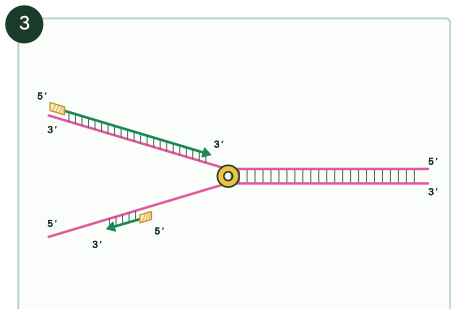
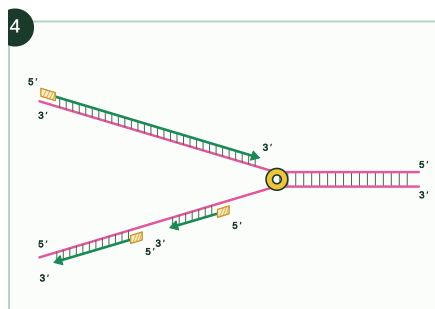
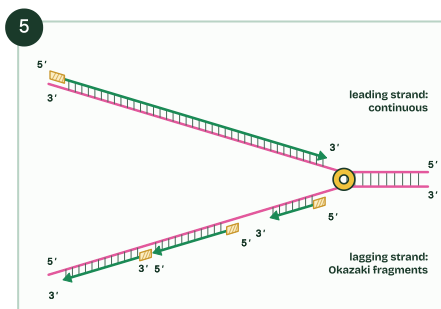
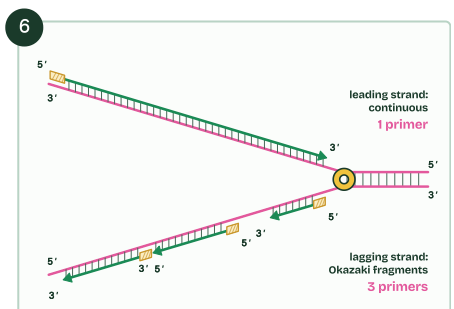


STEP BY STEP

6 STEPS



Helicase opens the fork and moves on.

**Leading strand:** one primer, then built continuously, towards the fork.**Lagging strand:** built away from the fork, so it must start again.Each new stretch gets its own **primer**.The lagging strand comes in **Okazaki fragments**.

One primer on the leading strand; one per fragment on the lagging strand.

IN E. COLI

Okazaki fragments are about 1,000 to 2,000 nucleotides long, and the fork moves about 1,000 base pairs a second. Copying begins at one place on the circular chromosome and two forks travel away from it in opposite directions, meeting on the far side about 40 minutes later, for 4.6 million base pairs. Each fork has its own leading and lagging strand.

WATCH OUT

The lagging strand is not copied later, after the leading strand is finished: both are made at the same fork at the same time. And "continuous" and "discontinuous" describe how each new strand is built, not the template.

PREDICT

A fork opens 10,000 bases of a bacterial chromosome. Okazaki fragments there are about 1,000 to 2,000 nucleotides long. How many RNA primers does each new strand need?

- ☐ (A) Leading about 5 to 10, lagging just 1
- ☐ (B) Both need about 5 to 10, one for each stretch
- ☐ (C) Leading just 1, lagging about 5 to 10

Decide before you look. Answer on page 42.

09

D1.1.8 HL PRIMASE, POLYMERASES I AND III, LIGASE

Four enzymes at the *fork*

Helicase has opened the fork. Four more enzymes finish the job. This is the system of a bacterium such as *E. coli*, which is the one the IB asks for, and the order they act in is the order to learn.

KEY POINTS

- **DNA primase** makes a short RNA primer (about ten nucleotides in *E. coli*) on the template, complementary to it: once at the start of the leading strand, and once for each Okazaki fragment.
- **DNA polymerase III** extends each primer from its 3' end, one nucleotide at a time, each paired with the template, so it builds almost all of each new strand, 5' to 3'. It also proofreads (chapter 10).
- **DNA polymerase I** removes the RNA primers and replaces their nucleotides with DNA nucleotides. Like every DNA polymerase it needs a 3' end to add to, so it works from the 3' end of the newer fragment that has grown up to the primer, taking out RNA ahead of it and filling in DNA behind. A primer is only replaced once the next fragment has reached it.
- **DNA ligase** joins the fragments. It makes the last covalent bond in the sugar and phosphate backbone, where one fragment's 3' end meets the next fragment's 5' end. The unjoined gap is a **nick**.

Why RNA primers, and then their removal? The finished strand must be DNA all through, so every primer's RNA has to be swapped out. That is also why ligase works many times on the lagging strand, once at every join, and once on the leading strand, where its single primer was replaced. It also answers why neighbouring Okazaki fragments are not joined at first: between them lies an RNA primer at first, then, once polymerase I has swapped it for DNA, a nick, and only then can ligase seal it.

Helicase, from chapter 3, is the fifth enzyme at the fork: named again here, not taught again.

KEY TERMS

DNA primase

The enzyme that makes a short RNA primer on a template strand, to give DNA polymerase III a 3' end to start from.

DNA polymerase III

The main DNA polymerase of a bacterium. It extends each primer from its 3' end, builds almost all of each new strand, and proofreads.

DNA polymerase I

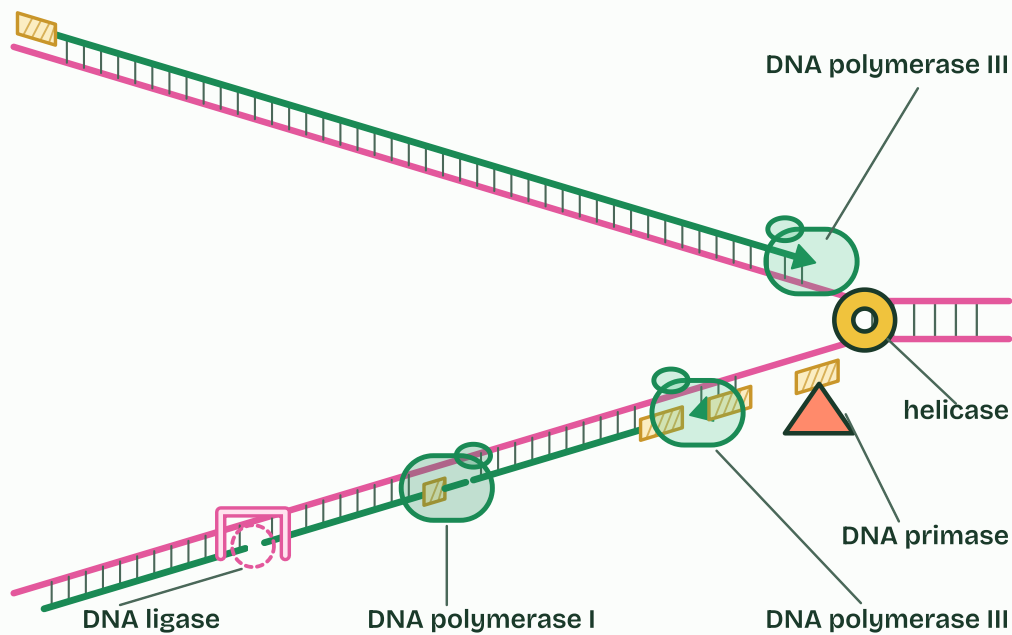
The DNA polymerase that removes the RNA primers and replaces their nucleotides with DNA nucleotides.

DNA ligase

The enzyme that joins two fragments of DNA by making the last covalent bond in the sugar and phosphate backbone.

nick

A gap in the sugar and phosphate backbone of DNA, between two fragments that sit side by side with one bond missing.



The fork of the last figure at a later moment. Old strands are pink, new DNA is green and primers are hatched yellow.

The leading strand is built continuously towards the fork, from a single primer.

An Okazaki fragment one short piece of the lagging strand. In *E. coli* it is 1,000 to 2,000 nucleotides long; here it is drawn much shorter.

An RNA primer a few RNA nucleotides that give DNA polymerase III a 3' end to start from.

DNA ligase joins fragments: it makes the last covalent bond in the sugar and phosphate backbone where one fragment's 3' end meets the next fragment's 5' end.

DNA polymerase III extends each primer from its 3' end, one nucleotide at a time, each paired with the template. It builds almost all of each new strand, 5' to 3', and checks its work (chapter 10).

DNA primase makes a short RNA primer on the template, complementary to it: once at the start of the leading strand, and once for each Okazaki fragment.

An RNA primer a few RNA nucleotides that give DNA polymerase III a 3' end to start from.

An RNA primer a few RNA nucleotides that give DNA polymerase III a 3' end to start from.

A nick two DNA fragments side by side, with one bond missing in the backbone.

DNA polymerase I removes the RNA primers and fills the gap with DNA nucleotides. It works from the 3' end of the newer fragment, taking out RNA ahead of it and adding DNA behind.

DNA polymerase III extends each primer from its 3' end, one nucleotide at a time, each paired with the template. It builds almost all of each new strand, 5' to 3', and checks its work (chapter 10).

Helicase opens the fork ahead of the other enzymes, unwinding the helix and breaking the hydrogen bonds between the base pairs.

REMEMBER IT

The enzymes at the fork, in order

H Hungry	P Pandas	P Prefer	P Plain	L Lettuce
Helicase unwinds and breaks hydrogen bonds	DNA primase lays an RNA primer	DNA polymerase III builds the new strand 5' to 3', and proofreads	DNA polymerase I swaps RNA primers for DNA	DNA ligase seals the nicks

WATCH OUT

DNA polymerase III does not remove primers: polymerase I does. Ligase does not add nucleotides: it makes one bond where two pieces meet. And primers are RNA, which is why they must be replaced.

PREDICT

A bacterium makes a faulty DNA ligase. What would you find in its newly copied DNA?

- Ⓐ Lagging strands left as short fragments
- Ⓑ RNA primers left in both new strands
- Ⓒ No new strands at all, as copying cannot start

Decide before you look. Answer on page 42.

10

D1.1.9 HL DNA PROOFREADING

Checking as it *goes*

Base pairing makes the right base far likelier than a wrong one, but now and then DNA polymerase III joins a nucleotide whose base does not pair with the template. That is a **mismatch**.

Proofreading deals with it. DNA polymerase III checks the nucleotide it has just added. If its base is a mismatch, the enzyme backs up, removes that nucleotide from the 3' terminal of the new strand, adds a correctly matched one in its place, and carries on. It is the last nucleotide that gets checked because a mismatched base at the 3' end does not pair properly, and the polymerase cannot extend well from it. That is how the mistake is caught before it is buried.

A mismatch that is left in becomes a **mutation** in one of the two daughter molecules after the next copying (D1.3). Accuracy matters because every cell copies billions of bases.

KEY POINTS

- A mismatch is a new nucleotide whose base does not pair with the template.
- DNA polymerase III backs up, removes it from the 3' end and adds the correct one.
- It checks as it builds, not afterwards.

Accurate copying plus proofreading is how genetic continuity is kept between generations, and the rare errors that remain are where new alleles come from.

Now put the whole fork together in Run the fork, below.

KEY TERMS

mismatch

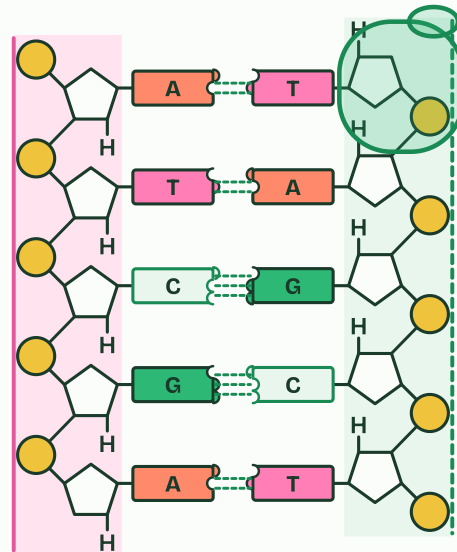
A nucleotide in a new strand whose base does not pair with the template's base opposite it.

proofreading

DNA polymerase III checking the nucleotide it has just added. If the base is a mismatch it removes the nucleotide from the 3' terminal and adds a correct one.

mutation

A change in the base sequence of DNA.

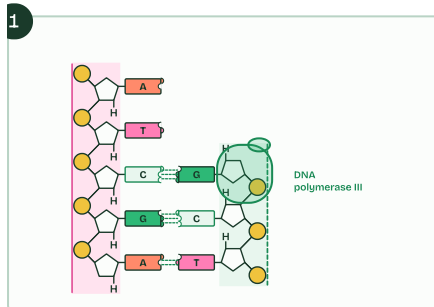


checked: every base

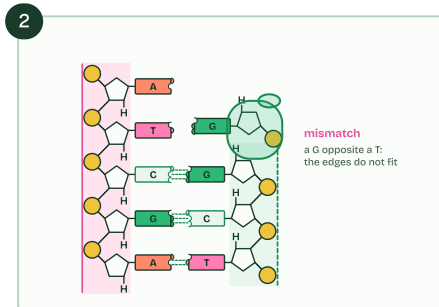
The knobs and notches stand for the atoms that form hydrogen bonds (A1.2). A knob fits a notch, and a blank fits a blank.

STEP BY STEP

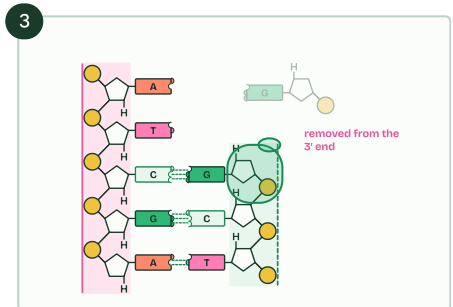
5 STEPS



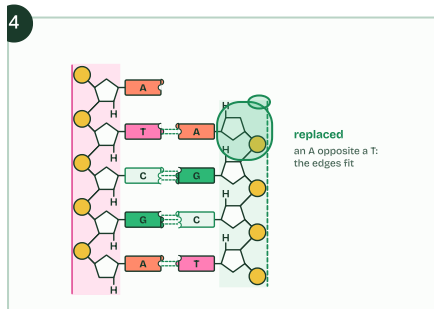
DNA polymerase III adds nucleotides at the 3' end.



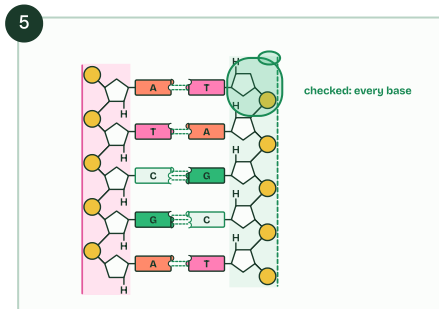
Rarely, a **mismatch** is joined in.



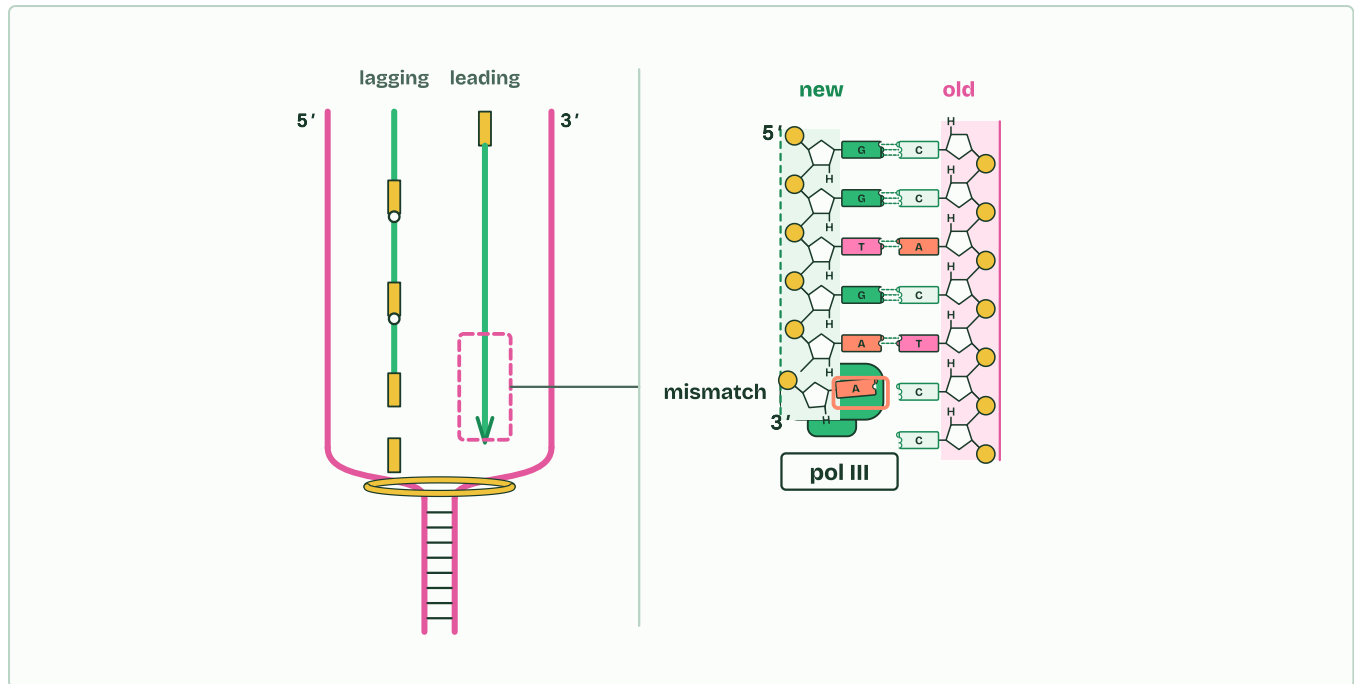
It backs up and **removes** the mismatched nucleotide.



It adds the **correct** one.

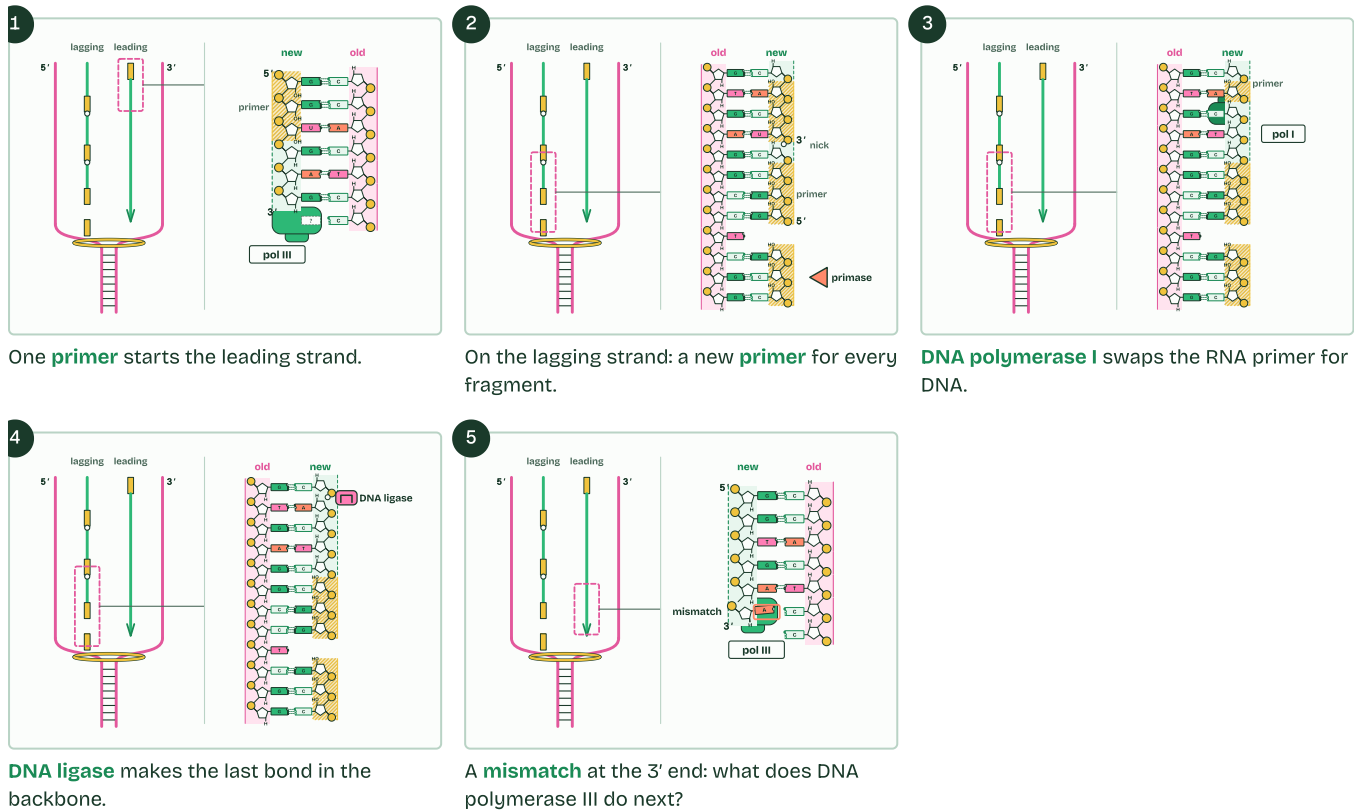


Then it carries on: about 1 mistake in 10 million survives.



STEP BY STEP

5 STEPS



HOW GOOD IS IT?

Without proofreading, DNA polymerase III gets about 1 base in 100,000 wrong. Proofreading catches about 99 in 100 of those, so about 1 in 10 million slips through. (Cells have further checks after copying, which take it lower still, but they are beyond the IB guide.)

WATCH OUT

Proofreading does not check the whole strand afterwards: it removes the nucleotide just added at the 3' end, while the strand is being built. And it is DNA polymerase III that proofreads (in the IB's account), not polymerase I or ligase.

PREDICT

Without proofreading, DNA polymerase III gets about 1 base in 100,000 wrong. With it, about 1 in 10 million. Copying a bacterial chromosome means adding about 9 million new bases. About how many mistakes would each give?

- ☐ (A) About 45 without, and none at all with it
- ☐ (B) About 90 without, about 1 with it
- ☐ (C) About 1 without, about 90 with it

Decide before you look. Answer on page 42.

Key terms

Every term this guide defines, A to Z, with the chapter it is in. Cover the right-hand side and test yourself.

3' terminal 07

The end of a strand of nucleotides that has a free hydroxyl group on a 3' carbon. It is where new nucleotides are added.

5' terminal 07

The end of a strand of nucleotides that has a free phosphate group on a 5' carbon.

allele 06

One version of a gene or a marker. For an STR it is a particular number of repeats. Each person has two at each marker, one from each parent.

amplify 04

To make many copies of a small amount of DNA, as PCR does.

annealing 04

The second step of a PCR cycle, at about 55 °C: the mixture cools and a primer binds to its matching sequence at each end of the target.

asexual reproduction 01

Making offspring from one parent by cell division, so each offspring starts with a copy of the parent's DNA.

base pairs (bp) 05

The unit for the length of a DNA fragment: the number of base pairs it contains.

base sequence 01

The order of the bases along a strand of DNA. It is the message, and every copy must carry the same one.

cell division 01

One cell splitting into two. The DNA is copied first, so that each new cell gets a full set.

complementary base pairing 02

The rule that A pairs only with T and C only with G, held by hydrogen bonds. It fixes which base can sit opposite each base of the template.

continuously 08

Built in one piece, without stopping, as the leading strand is.

daughter molecule 02

One of the two molecules made when a DNA molecule is copied. Each holds one old strand and one new one.

denaturation 04

The first step of a PCR cycle, at about 95 °C: heat breaks the hydrogen bonds and the two strands separate.

directionality 07

Working in one direction only. DNA polymerases add nucleotides at the 3' end of a strand, so a new strand grows 5' to 3'.

discontinuously 08

Built in separate pieces, each started again, as the lagging strand is.

DNA ladder 05

A mixture of DNA fragments of known sizes, run in its own lane to give a scale for reading the sizes of the other bands.

DNA ligase 09

The enzyme that joins two fragments of DNA by making the last covalent bond in the sugar and phosphate backbone.

DNA polymerase 03

The enzyme that builds new DNA. It joins free nucleotides into a strand, each one matched to the template by base pairing.

DNA polymerase I 09

The DNA polymerase that removes the RNA primers and replaces their nucleotides with DNA nucleotides.

DNA polymerase III 09

The main DNA polymerase of a bacterium. It extends each primer from its 3' end, builds almost all of each new strand, and proofreads.

DNA primase 09

The enzyme that makes a short RNA primer on a template strand, to give DNA polymerase III a 3' end to start from.

DNA profiling 06

Comparing the alleles of a few markers, found by PCR and gel electrophoresis, between a sample and the people it might have come from.

DNA replication 01

Making a copy of a DNA molecule, so that the copy has exactly the same base sequence as the original.

electrodes 05

A metal lead that carries current into the tank. One is positive and one negative.

extension 04

The third step of a PCR cycle, at about 72 °C: the polymerase adds nucleotides to each primer, building a complementary strand.

false match 06

Two different people having the same profile by chance.

forensic 06

To do with finding evidence for a court of law.

fragments 05

A piece of DNA of a particular length.

free nucleotides 03

A single nucleotide floating loose in the nucleus (or the cytoplasm of a bacterium), ready to be added to a new strand.

gel electrophoresis 05

A method that separates DNA fragments by length, by pulling them through a gel with an electric field.

growth 01

An increase in the number of cells in a body, made by division.

helicase 03

The enzyme that unwinds the double helix and breaks the hydrogen bonds between its base pairs, so the two strands separate.

lagging strand 08

The new strand that is built away from the fork, in pieces, each started again as the fork opens more template.

leading strand 08

The new strand that is built towards the fork, in the same direction as the fork moves. It needs only one primer.

marker 06

A place in the DNA, such as an STR, that is compared between people in DNA profiling.

mismatch 10

A nucleotide in a new strand whose base does not pair with the template's base opposite it.

mutation 10

A change in the base sequence of DNA.

nick 09

A gap in the sugar and phosphate backbone of DNA, between two fragments that sit side by side with one bond missing.

Okazaki fragments 08

One of the short pieces of the lagging strand, named after Reiji and Tsuneko Okazaki, who found them in the 1960s.

paternity 06

To do with who a child's father is.

polymerase chain reaction (PCR) 04

A method that copies one chosen stretch of DNA again and again in a tube, so that a tiny sample becomes enough to study.

primers 04

A short single strand of DNA, about 20 nucleotides long, that binds to one end of the stretch to be copied and gives the polymerase a place to start.

proofreading 10

DNA polymerase III checking the nucleotide it has just added. If the base is a mismatch it removes the nucleotide from the 3' terminal and adds a correct one.

replication fork 03

The Y-shaped point where the double helix is being unwound and copied: the unopened helix below, two single strands above.

RNA primer 08

A short strand of RNA, made by an enzyme, that is paired with the template and gives DNA polymerase a 3' end to add to.

semi-conservative 02

Describes DNA replication in which each daughter molecule keeps one of the two original strands and gains one new strand.

short tandem repeat (STR) 06

A short sequence of bases, such as GATA, repeated over and over end to end. The number of repeats differs from person to person.

Taq polymerase 04

A DNA polymerase from *Thermus aquaticus*, a bacterium that lives in hot springs. It is used in PCR.

template 02

A strand of DNA that a new strand is built against. The new strand's bases are set by the template's.

thermal cycler 04

A machine that raises and lowers the temperature of PCR tubes, over and over, on a set cycle.

tissue replacement 01

Making new cells by division to replace those that are worn out, lost or damaged.

wells 05

One of the small pits at the end of a gel, where a sample is loaded.

Dry lab: *PCR and gel: whose DNA is it?*

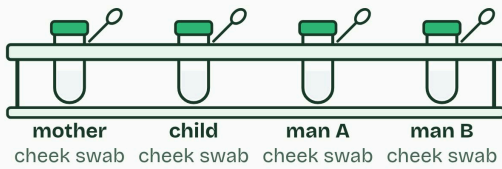
Copy a trace of DNA in a thermal cycler, run it on a gel beside a ladder, and read the bands to settle a case. Then add markers and watch the chance of a false match fall.

Who's the father?

A made-up case.

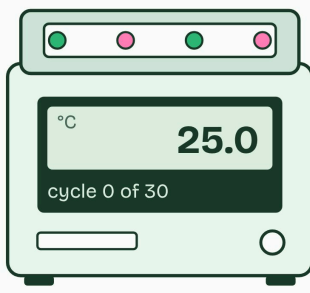
A mother has asked whether either of two men is her child's father. The mother, the child and both men each gave a cheek swab.

Who could be the father?

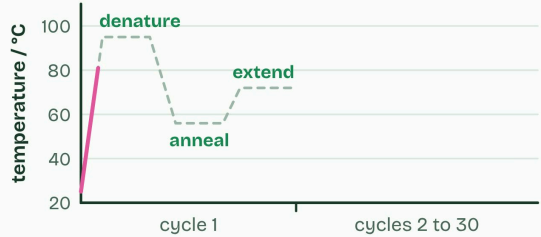


mother child man A man B
cheek swab cheek swab cheek swab cheek swab





thermal cycler



temperature / °C

cycle 1 cycles 2 to 30

Run it online: change the variables, run the trials, and the table and graph fill in as you go.
biologybybradford.com/guides/ib-biology-d1-1-dna-replication#lab

Your question

Do the profiles show who the father is, or whose blood was left at the scene, and how many markers do you need before you can be confident?

What you can change

The case; how much DNA you start with; the number of PCR cycles; the three temperatures; the polymerase; the primers; the gel; how long the gel runs; how many markers you run.

What to record

For each person and each marker: the sizes of the bands, read against the ladder, and the alleles they stand for. Then your verdict, and the chance that an unrelated person would match by luck.

Find out

- **Who's the father?** Run three markers. Can you decide yet? If not, use **Run more markers on this case** until you can. How many did it take, and what was the chance of a false match then?
- **Whose blood?** Decide, then check the chance that an unrelated person would match at the markers you ran. Play a few cases with different numbers of markers and watch the graph under the bench.
- **What makes PCR work?** With the father case, change one PCR setting at a time and run the gel each time: 15, 20, 25 and 30 cycles; denaturation at 85 and 95 °C; annealing at 45, 56 and 68 °C; the other polymerase. Record which settings give bands.
- **Read the ladder.** Switch to the size ladder. Use the standard curve: plot log size against distance, fit a line and read a band's size from it. Work out its number of repeats (the markers' sizes are under **What the model is**). How close is it to the allelic ladder's answer?
- **COVID test.** Call each patient. What are the two control tubes for?
- **Harder cases.** Tick the box and play each case again. What goes wrong, and how would a real lab spot it?

Record

- Each person's alleles at each marker, in the profile table, read against the ladder.
- Your verdict, and what the bands support, marker by marker.
- How many markers you ran, and the chance of a false match for those markers.
- For the PCR settings, a table of the setting you changed and whether bands appeared. **Download CSV** keeps every reading and verdict.

QUESTIONS ON THE LAB

QUESTION 1

With the polymerase from *E. coli*, the copies line never rises and the gel shows only the ladder. Why?

- (A) It cannot copy human DNA, only the DNA of bacteria
- (B) It is denatured in the first heating to 95 °C
- (C) It needs more primers than Taq polymerase does

Answer on page 42.

QUESTION 2

In a COVID run, the no-DNA tube (water, no swab) shows a faint viral band. What should the lab do?

- (A) Call every patient who has a viral band positive
- (B) Ignore it: the band is fainter than the patients' bands
- (C) Treat the whole run as void, and repeat it

Answer on page 42.

QUESTION 3

With one marker, both men could be the child's father. With six, one of them is excluded. What does this show?

- (A) Each extra marker makes a chance match much less likely
- (B) The first marker was read wrongly, so it must be ignored
- (C) Fathers share more bands with a child at some markers only

Answer on page 42.



Check yourself

The check for this guide is online: a fresh paper of past-paper style questions from the exam bank on D1.1 each time, marked as you go, and saved to your Exam practice progress when you are signed in. Scan the code, or type the link.

[biologybybradford.com/exam-maker/sit?
topics=D1.1&level=HL&paper=quiz&marks=15&src=ep](https://biologybybradford.com/exam-maker/sit?topics=D1.1&level=HL&paper=quiz&marks=15&src=ep)

Answers

PREDICTIONS IN THE CHAPTERS

- 01** You began as one fertilised egg and now have about 30 trillion cells. If every cell divided in step, about how many rounds of copying and dividing would it take?

B About 45

About 45. Each round doubles the number of cells, so 45 rounds give 2^{45} , about 35 trillion. Real cells do not divide in step, and many die and are replaced, so your DNA has been copied far more times than that in all, and every one of those copies had to be exact. Doubling comes back in chapter 4, where it is the secret of PCR.

- 02** A DNA molecule is copied, and then both copies are copied again. Of the four molecules, how many still contain a strand from the original?

C Two: each old strand sits in one molecule

Two. The original's two strands are never broken up or thrown away: each stays whole and ends up in one molecule, paired with a new partner. The other two molecules are made entirely of new strands. Every round after that, the two old strands are still in just two molecules, however many copies there are.

- 03** A drug stops helicase but leaves DNA polymerase working. What happens to replication?

A It stops: polymerase needs single strands to read

It stops. DNA polymerase can only pair new nucleotides with bases it can reach, and in the double helix every base is already paired and tucked inside. No unzipping, no templates, no copying. (Some antibiotics and anti-cancer drugs work by jamming the copying enzymes of fast-dividing cells.)

- 04** PCR uses a DNA polymerase from bacteria that live in hot springs. Why not a polymerase from human cells?

B A human polymerase would be denatured at 95 °C each cycle

Every cycle heats the tube to about 95 °C to separate the strands. A human polymerase, made to work at 37 °C, would lose its shape in the first heating and never work again. Taq polymerase, from a bacterium that lives in hot springs, keeps its shape through 30 heatings, so it can be added once at the start. (It still needs primers, like every DNA polymerase.)

- 05** Fragments of 300, 1,000 and 5,000 base pairs are run on the same gel. Which ends up nearest the wells?

C 5,000 base pairs: long fragments move slowest

The longest, 5,000 base pairs. All three carry the same charge per base pair, so the pull per base pair is the same. What differs is how easily each threads through the mesh of the gel: long fragments snag more and move slowest, so they stay nearest the wells. Nothing sticks to the gel.

- 06** At one marker, 1 person in 10 shares the crime-scene pattern by chance. With ten independent markers, each 1 in 10, how many people in a city of 10 million would you expect to match by chance?

A Almost certainly none of them

Almost certainly none. The chances multiply, not add: 1 in 10, ten times over, is 1 in 10 billion. In a city of 10 million that is an expected 0.001 people, so a chance match is very unlikely. That is why a profile uses many markers: each one makes a false match far rarer.

- 07** A free nucleotide arrives at the 5' end of a growing new strand, and its base pairs with the template. What happens?

B It is not joined: there is no free 3' OH there

It is not joined. DNA polymerase only makes the bond between a nucleotide's 5' phosphate and the 3' OH of the strand. At the 5' end there is a phosphate, not an OH, so there is nothing to join to, however well the base pairs. Every DNA polymerase works this way, so every new strand grows 5' to 3'.

- 08** A fork opens 10,000 bases of a bacterial chromosome. Okazaki fragments there are about 1,000 to 2,000 nucleotides long. How many RNA primers does each new strand need?

C Leading just 1, lagging about 5 to 10

The leading strand is primed once and then built continuously as the fork moves, so it needs one primer. The lagging strand is built in fragments, each started from its own primer: 10,000 bases in pieces of 1,000 to 2,000 is about 5 to 10 fragments, so about 5 to 10 primers.

- 09** A bacterium makes a faulty DNA ligase. What would you find in its newly copied DNA?

A Lagging strands left as short fragments

Without ligase, nothing seals the last bond between one fragment and the next, so the lagging strand stays in pieces (and the leading strand keeps one nick where its primer was). Primase and polymerase III still start and build the strands, and polymerase I still swaps the primers for DNA. Only the joining fails.

- 10** Without proofreading, DNA polymerase III gets about 1 base in 100,000 wrong. With it, about 1 in 10 million. Copying a bacterial chromosome means adding about 9 million new bases. About how many mistakes would each give?

B About 90 without, about 1 with it

9 million ÷ 100,000 is 90 mistakes a copy without proofreading, and 9 million ÷ 10 million is about 1 with it: proofreading makes copying about a hundred times more accurate. Not zero, though: the odd mismatch still gets through, which is one source of mutations.

QUESTIONS ON THE DRY LAB

- Q1** With the polymerase from *E. coli*, the copies line never rises and the gel shows only the ladder. Why?

B It is denatured in the first heating to 95 °C

An enzyme from a bacterium that lives at 37 °C loses its shape at 95 °C, and every run starts with that heating, so it never copies anything. Taq polymerase comes from *Thermus aquaticus*, a bacterium of hot springs, and keeps working through 30 heatings, so it can be added once at the start.

- Q2** In a COVID run, the no-DNA tube (water, no swab) shows a faint viral band. What should the lab do?

C Treat the whole run as void, and repeat it

Water has no virus in it, so a band there means something got into the tubes: contamination. PCR is so sensitive that a few stray copies become billions, so any patient's band could be contamination too, and the run is repeated. The controls are there to catch exactly this; the positive control shows the PCR itself worked.

- Q3** With one marker, both men could be the child's father. With six, one of them is excluded. What does this show?

A Each extra marker makes a chance match much less likely

At one marker, many men carry the child's paternal allele by chance. Each independent marker is another test a man must pass, and the chance of passing them all by luck multiplies down fast. More measurements give more reliable conclusions, which is why real profiles use about 15 to 20 markers.

How this was made. The course structure, the activities and the teaching choices come from my own classroom, my presentations and my years of teaching IB and AP Biology. I use Claude, an AI, to help write and build the interactive guides, lesson plans, slides and practice questions, and to draw the diagrams and code the interactives. I direct the work and review it against the IB and AP specifications before it goes live.

Written and drawn for Biology by Bradford, biologybybradford.com. Free to print and share with a class.