The big picture

? Guiding question(s)

- How is new DNA produced?
- How has knowledge of DNA replication enabled applications in biotechnology?

Keep the guiding questions in mind as you learn the science in this subtopic. You will be ready to answer them at the end of this subtopic. The guiding questions require you to pull together your knowledge and skills from different sections, to see the bigger picture and to build your conceptual understanding.

Many species around the world are threatened or endangered. Species are going extinct at alarming rates. One of the ways we are working to protect some of these species is the banning of international trade of products of these species. For example, it is illegal to import or export ivory from elephants. How might our

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understanding of DNA (deoxyribonucleic acid) and DNA technologies help us in this fight to protect these species? Can we use DNA in elephant tusks and rhino horns, for example, to track poachers and smugglers (**Figure 1**)?

Figure 1. Confiscated elephant tusks and rhino horns.

Credit: Martin Harvey, Getty Images

Watch **Video 1** to see how DNA can help us to protect endangered species such as the African elephant.

UW biologist Sam Wasser on tracking illegal ivory through DNA

Student view

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Video 1. Tracking illegal ivory trade through DNA profiling.

Creativity, activity, service

Strand: Service
Learning outcome: Demonstrate engagement with issues of global significance

There are endangered species in virtually all parts of the world. Wherever you are there will be local species under threat, either from human activities or from other factors. A possible service project would be to support local, national or international organisations that are working to protect and conserve these species. Some of these organisations may even use DNA evidence as part of their monitoring and conservation efforts. For example, your project could help raise awareness of an endangered species you have an interest in.

Prior learning

Before you study this subtopic make sure that you understand the following:

- the structure of nucleic acids (see subtopic A1.2 (/study/app/bio/sid-422-cid-755105/book/the-big-picture-id-43236/))
- the interdependent components of metabolism (see subtopic C1.1 (/study/app/bio/sid-422-cid-755105/book/the-big-picture-id-43208/)).

D1. Continuity and change: Molecules / D1.1 DNA replication

DNA replication basics

D1.1.1: Production of exact copies of DNA with identical base sequences

D1.1.2: Semi-conservative nature of DNA replication

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

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Learning outcomes

By the end of this section you should be able to:

- Describe DNA replication as the process by which exact copies of DNA are created for use in reproduction, growth and tissue replacement in multicellular organisms.
- Explain the semi-conservative nature of DNA replication and how it allows for a high degree of accuracy when copying base sequences.
- Describe the roles of helicase and DNA polymerase in DNA replication.

Your DNA, contained within the nucleus of your cells, carries your genetic information. From the time you were a single fertilised egg, every cell has had a copy of this information. How did every cell in your body get a copy of this information?

DNA replication

Whenever cell division occurs, whether it is for growth or for repair of tissues through cell replacement, the new cells each need a copy of the organism's DNA. Growth involves the addition of new cells to make the organism larger. Each new cell requires a complete copy of the organism's DNA, which it acquires through DNA replication that must occur prior to cell division. In this way, an organism can grow from a single fertilised egg cell to a large multicellular organism with trillions of cells, most with an exact copy of the DNA from that original fertilised egg cell.

When repairing damaged tissues by replacing cells, the same processes of cell division and DNA replication occur. New cells need to be produced to replace those that were damaged or destroyed. We have all had a cut or scrape. As those wounds heal, new cells are produced to fill in the gap created when the injury

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occurred. Each of these cells will be an exact genetic copy of those around them. They will also be differentiated to the same cell type by the gene expression within those cells.

The DNA of an organism contains the instructions for that organism. The base sequences contained within the DNA need to be copied exactly for the new cells to function properly. The process of DNA replication is able to copy billions of base pairs with amazing accuracy and at incredible speeds. The DNA in one of your cells can be copied completely in about an hour.

Making connections

DNA replication is necessary for cell division. Cell division is covered in subtopic D2.1 (/study/app/bio/sid-422-cid-755105/book/big-picture-id-43548/).

The differentiation of cells into different types with different functions is a result of gene expression. Gene expression is covered in subtopic D2.2 (/study/app/bio/sid-422-cid-755105/book/big-picture-hl-id-43549/).

Semi-conservative nature of DNA replication

The replication of DNA is semi-conservative. As one double strand of DNA is replicated, each new double strand of DNA that is produced contains one strand of the original DNA and one strand of newly synthesised DNA (**Figure 1**). This happens because each strand of the original DNA molecule acts as a template for the new strand to be built from. The complementary base pairing rule of DNA then ensures that the new strands that are built are exact copies of the original. The complementary base pairing rule states that the base adenine (A) always binds with the base thymine (T), and the base cytosine (C) always binds with the base guanine (G).

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Student view

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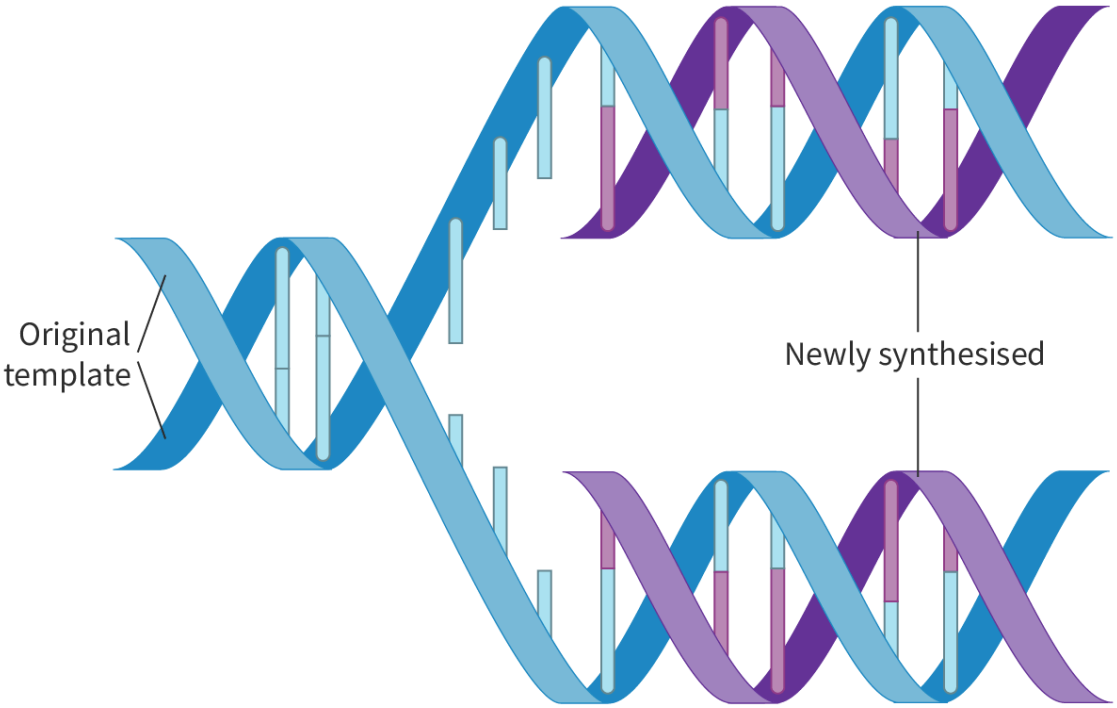

Figure 1. Semi-conservative DNA replication.

More information for figure 1

This diagram illustrates semi-conservative DNA replication. On the left side, there is a blue double helix representing the original DNA strand labeled as "Original template." The diagram shows how each strand of the original DNA is used as a template for creating new DNA strands.

On the right side, there are two purple and blue intertwined strands representing the newly synthesized DNA, labeled "Newly synthesised." The diagram visually demonstrates that each new DNA molecule consists of one original strand and one newly synthesized strand.

The DNA helix is depicted with complementary base pairs between the strands, illustrating key aspects of how DNA strands are copied during the replication process.

[Generated by AI]

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Nature of Science

Aspect: Evidence

Evidence is needed to support theories.

There were three theories for how organisms replicated their DNA — the conservative, semi-conservative and dispersive models. The semi-conservative model was widely believed, but there was no evidence to support it. **Figure 2** illustrates these three theories.

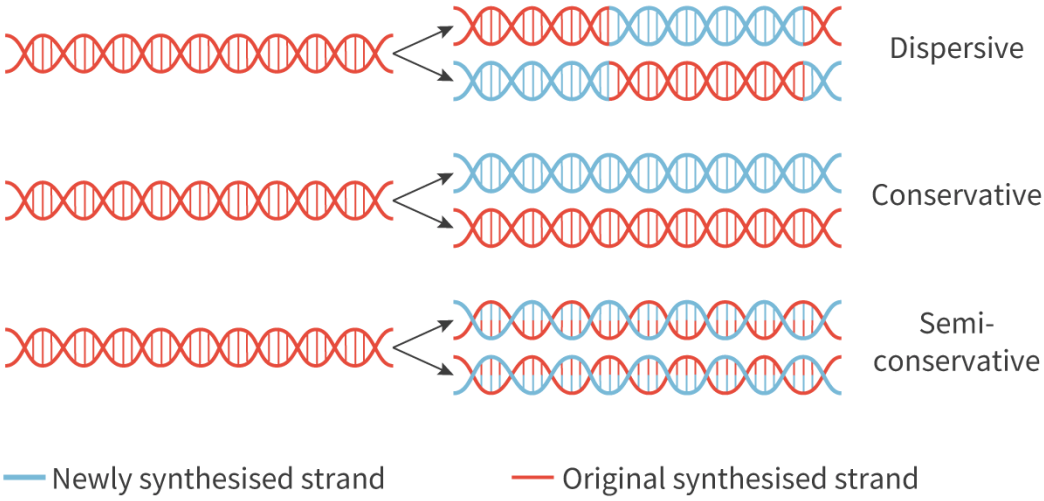

Figure 2. Three theories of DNA replication.

More information for figure 2

The image is a diagram depicting three models of DNA replication. It consists of three horizontal rows representing the dispersive, conservative, and semi-conservative theories. Each row shows two DNA strands represented as double helices.

1. Dispersive Model:
2. The original DNA strands are in red.
3. After replication, the new DNA segments are in blue, interspersed within the original red strands. This creates a mosaic of red and blue within each helix.
4. Conservative Model:
5. The first double helix remains entirely red, indicating the preservation of the original strands.
6. A separate new double helix is formed entirely in blue, representing newly synthesized DNA strands.
7. Semi-conservative Model:

Student view

8. Each resulting double helix contains one original red strand and one newly synthesized blue strand.

Labels indicate 'Newly synthesised strand' in blue and 'Original synthesised strand' in red, enhancing understanding of replication outcomes in each model.

[Generated by AI]

Meselson and Stahl came up with an elegant experiment to show that DNA did replicate semi-conservatively. They realised that they could differentiate between these theories by alternately using 'heavy' (^{15}N) and 'light' (^{14}N) isotopes of nitrogen, which would give DNA of varying masses as replication occurred in successive generations of bacteria. The mass of the DNA in each generation would vary depending upon which of the models of replication was the correct one. The elegance of their experiment is shown by the clever use of several techniques to provide the evidence that demonstrated the semi-conservative model of DNA replication.

Role of helicase and DNA polymerase in DNA replication

DNA replication is an essential process, and it must be carried out with tremendous accuracy to ensure that the new DNA functions exactly as the original. Enzymes play a key role in this process.

The process can be divided into separate steps. Eukaryotic DNA is normally supercoiled by being tightly wound around histones to form nucleosomes. This helps package the DNA and allows it to fit better within the nucleus. Prokaryotic DNA is not associated with these proteins and is referred to as 'naked' DNA. In eukaryotes like us, the first step in DNA replication is to unwind the coils to make the strands accessible to enzymes. The enzyme helicase then unwinds the double helix and separates the two DNA strands by breaking the hydrogen bonds between the bases. This separation of the strands exposes the bases usually protected within the molecule.

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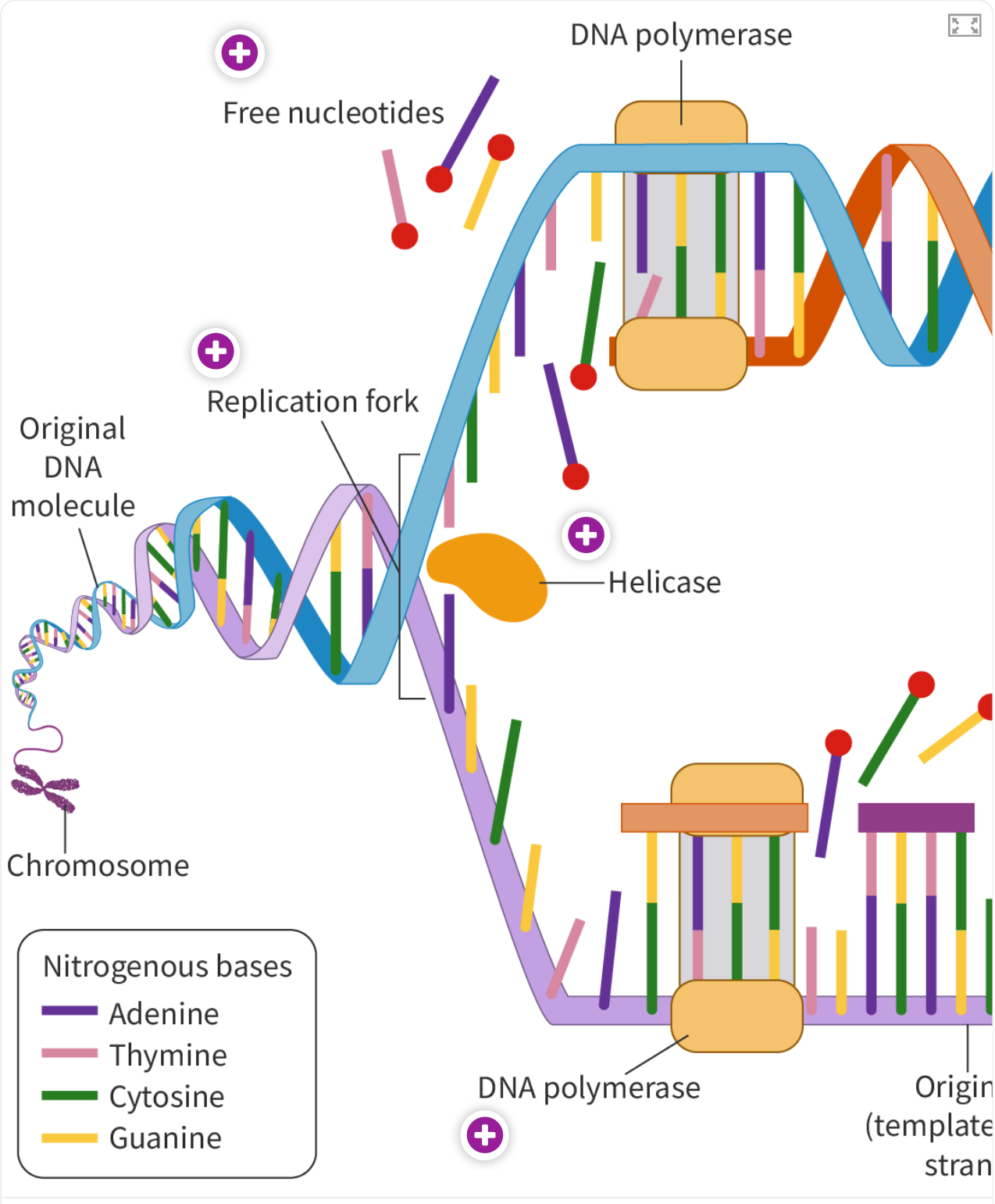
Once the strands are separated and the bases exposed, another enzyme called DNA polymerase can start its job. DNA polymerase will move along the separate DNA strands, using them as templates. It will then begin building a new strand of DNA by placing and attaching free nucleotides in a chain.

Interactive 1 outlines the process of DNA replication.



Interactive 1. DNA Replication.

Student view

More information for interactive 1

An interactive diagram illustrates the molecular mechanism of DNA replication, highlighting the roles of helicase and DNA polymerase in accurately copying genetic material. The illustration begins at the left where the complex "**chromosome**" structure is shown in purple. The structure then unwinds to reveal the basic components of a DNA molecule, which are nitrogenous bases and sugar-phosphate backbone with the label "**original DNA molecule**."
The illustration helps to understand the step-by-step unwinding of original DNA molecules and the synthesis of new DNA strands.
An index at the bottom left shows the color codes representing each nitrogenous base in a box titled "Nitrogenous bases." The color codes are: blue for Adenosine, pink for Thymine, green for Cytosine, and yellow for Guanine.

During the first stage of DNA replication, the helicase enzyme breaks the double-stranded DNA molecule into two separate strands. As the DNA unwinds, a replication fork, a Y-shaped structure where DNA is actively unwound, breaks hydrogen bonds between the nitrogenous bases. The separated DNA strands serve as the templates for new DNA strand synthesis.

Two DNA polymerase enzymes then bind to the two separated DNA strands separately. The polymerase adds free nucleotides to the template strand following the complementary base pairing (A-T, G-C) rule. The new DNA strand is synthesized in the 5' - 3' direction.
Across the illustration, various hotspots explain the elements involved during DNA replication. These hotspots are indicated by plus signs and are numbered. Each of the hotspots when opened read as follows:

Free nucleotides (Hotspot 1): DNA nucleotides that are found in the nucleus. DNA polymerase uses these to build the new strands of DNA.
Replication Fork (Hotspot 2): forms where helicase separates the original DNA strands and moves along the DNA in the same direction as helicase.

Helicase (Hotspot 3): the enzyme that forms the replication
fork by separating the two strands of DNA, breaking the hydrogen bonds between the bases of the two strands.

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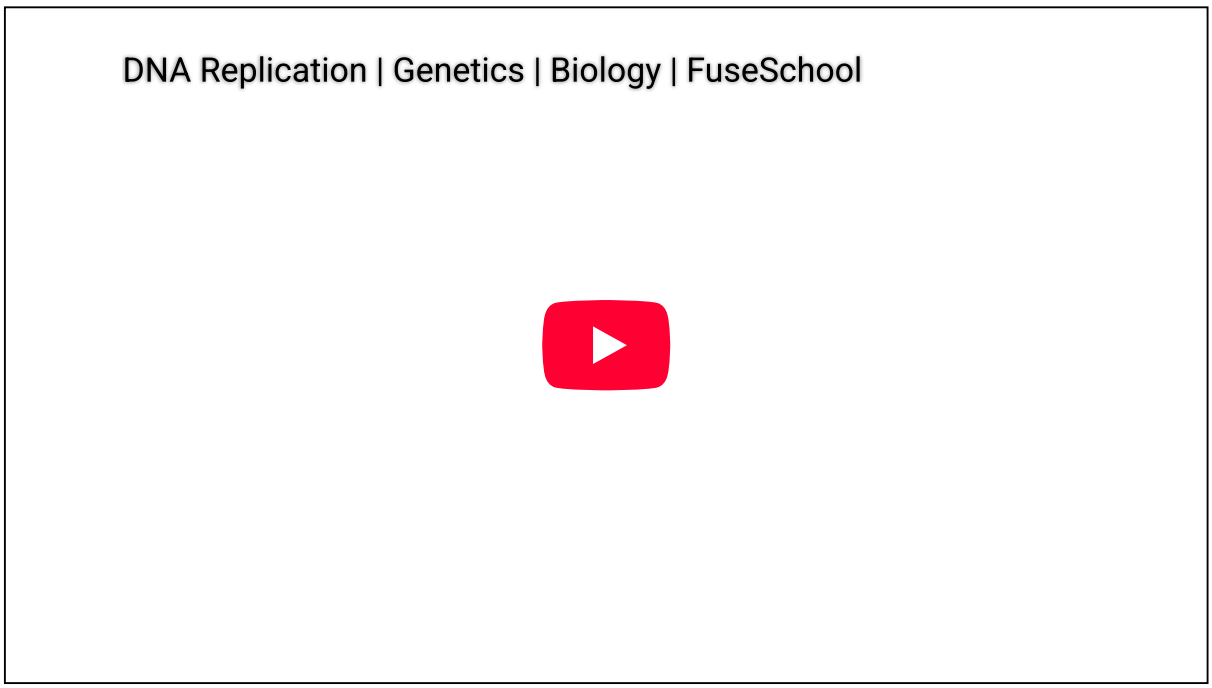
DNA Polymerase (Hotspot 4): assembles the new strands of DNA by adding free nucleotides in the correct sequence based upon the complementary base pairing rule.

Watch **Video 1** for a summary of DNA replication.



Video 1. An overview of DNA replication.

Try this activity to check your understanding of DNA replication.

Activity

- **IB learner profile attribute:** Thinker
- **Approaches to learning:** Thinking skills — Being curious about the natural world, Reflecting on the credibility of results
- **Time required to complete activity:** 15—20 minutes
- **Activity type:** Individual activity

Student view

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The following strand of DNA needs to be replicated. You are to complete this process by adding the correct bases in sequence using the complementary base pairing rule. It is a race, however, so you need to time yourself to see how fast you can do it.

ATCCGTATCAATGGCAATGCCCTAGATGCCGTTAGACCTTTTAGAGCACATAG(

DNA polymerase can build new strands of DNA at a rate of about 1000 bases per second. How long would it take DNA polymerase to do what you just did? Were you faster or slower than DNA polymerase? By how much?

The average human's DNA contains 3.2 billion base pairs. How long would it take DNA polymerase to copy it? How long would it take you?

It takes about an hour for a cell to replicate its DNA. How many DNA polymerases need to work together to replicate the DNA of a cell in an hour?

5 section questions

D1. Continuity and change: Molecules / D1.1 DNA replication

Polymerase chain reaction and gel electrophoresis

D1.1.4: Polymerase chain reaction and gel electrophoresis

D1.1.5: Applications of polymerase chain reaction and gel electrophoresis

Learning outcomes

By the end of this section you should be able to:

- Describe the use of polymerase chain reaction and gel electrophoresis for amplifying and separating DNA.

Student view

- Describe the applications for PCR and gel electrophoresis.

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During the Covid-19 pandemic, testing for Covid-19 often involved a polymerase chain reaction (PCR) test. A sample was collected from your nasal cavity by sticking a swab way up your nose. What happened to that sample? What does a PCR test do?

Polymerase chain reaction and gel electrophoresis

PCR stands for polymerase chain reaction. It is a technique used to amplify small fragments of DNA. The discovery of this technique has revolutionised medical science, forensic science and molecular biology. PCR has enabled scientists to clone genes, to work with minute amounts of DNA found at crime scenes, to identify the dead and, perhaps most extraordinarily, to sequence the DNA of extinct species and other life forms.

Look at **Figure 1** to see how the PCR process works.

Student view

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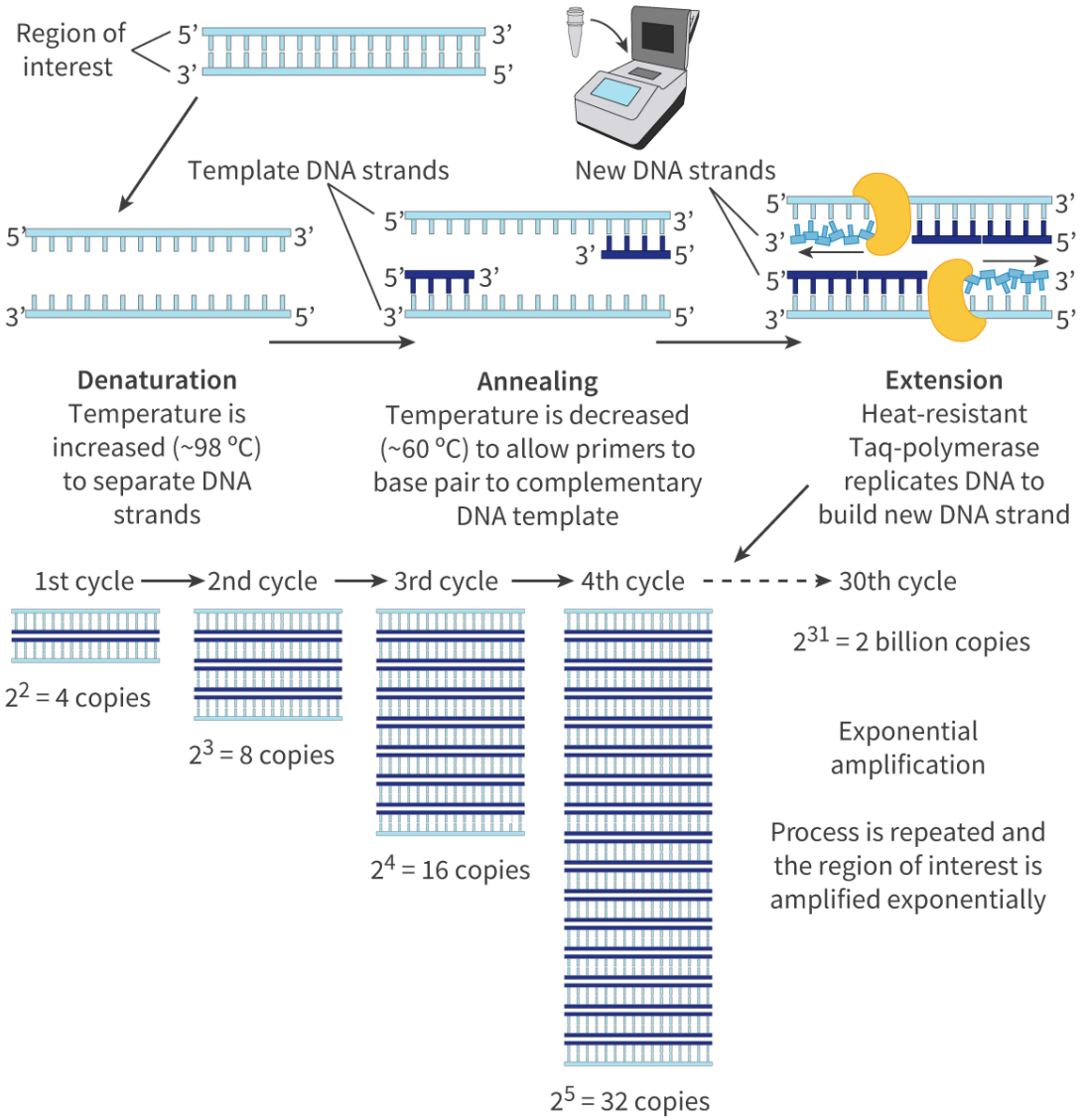

Figure 1. The PCR technique and numbers of copies of the target sequence generated.

More information for figure 1

The image illustrates the Polymerase Chain Reaction (PCR) process. The top row shows the initial DNA template strands labeled as 'region of interest' with sequences in 5' to 3' orientation. Nearby, a machine represents the PCR equipment.

Below, three main stages are depicted:

1. **Denaturation:** Temperature is increased (~98°C) to separate DNA strands into single strands.
2. **Annealing:** Temperature is reduced (~60°C) to allow primers to attach to complementary bases on the DNA template.
3. **Extension:** Heat-resistant Taq-polymerase extends the primers, synthesizing new DNA strands.

Student view

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The process is cyclic, represented visually by DNA strand illustrations indicating exponential amplification: - **1st cycle:** 2^2 = 4 copies - **2nd cycle:** 2^3 = 8 copies - **3rd cycle:** 2^4 = 16 copies - **4th cycle:** 2^5 = 32 copies - **30th cycle:** 2^31 = 2 billion copies

An annotation mentions "Exponential amplification," describing how the initially targeted DNA region is exponentially amplified after 30 cycles.

[Generated by AI]

The desired section of DNA is placed in a reaction chamber that contains:

- many free nucleoside triphosphates
- primers that will allow replication to occur from the desired point, and
- a special heat-stable version of DNA polymerase called Taq polymerase.

Taq polymerase was originally found in bacteria that live in hot springs. It is used because it does not denature at the high temperatures used in PCR and can therefore continue to function in repeated cycles.

First, the DNA is heated enough to break the hydrogen bonds that hold the two strands of the double helix together. This is called the denaturation phase. This occurs at a temperature of around 98 °C. Then, as the sample is allowed to cool to around 60 °C, the short primer sequences will bond to complementary sequences in the DNA sample. This is known as the annealing phase.

The final phase is the extension phase, which happens at about 72 °C. The bonding of primers allows *Taq* polymerase to replicate DNA using the primer as a starting point. (DNA polymerases are not able to add the first nucleotide of a DNA strand; they are only able to extend existing strands.) Once the DNA has been replicated, the DNA strands are heated to the point of separation and the process begins again.

Student view

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Each time a cycle occurs, the amount of DNA doubles, resulting in exponential growth. Within a few hours, enough cycles of PCR have occurred to create billions of copies of the DNA sequence. This provides ample copies for gel electrophoresis and other tests.

Watch **Video 1** for an overview of PCR.

Video 1. How PCR works.

Sometimes scientists need to know more about an individual's DNA, but determining the sequence of the entire genome would be excessive. In these cases, a technique called gel electrophoresis can be used to identify some key features of the DNA. Gel electrophoresis uses an electrical current to move molecules through a semisolid medium or gel. The DNA molecules are separated by their size and amount of charge.

DNA molecules have a negative electrical charge and will move towards the positive electrode in an electric field. DNA molecules are often millions of base pairs long; too long to be separated by electrophoresis.

Student view

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To get fragments of appropriate size, usually 250–30 000 base pairs (bp) in length, DNA is digested with special enzymes called restriction endonucleases. These enzymes cut the backbone of the DNA double helix at highly specific sequences, producing shorter DNA segments and distinctive fragment patterns (**Figure 2**). These patterns can be used to produce DNA profiles or DNA fingerprints, combinations of DNA sequences that are unique to each individual. This allows anyone (with the exception of identical twins) to be identified by their DNA.

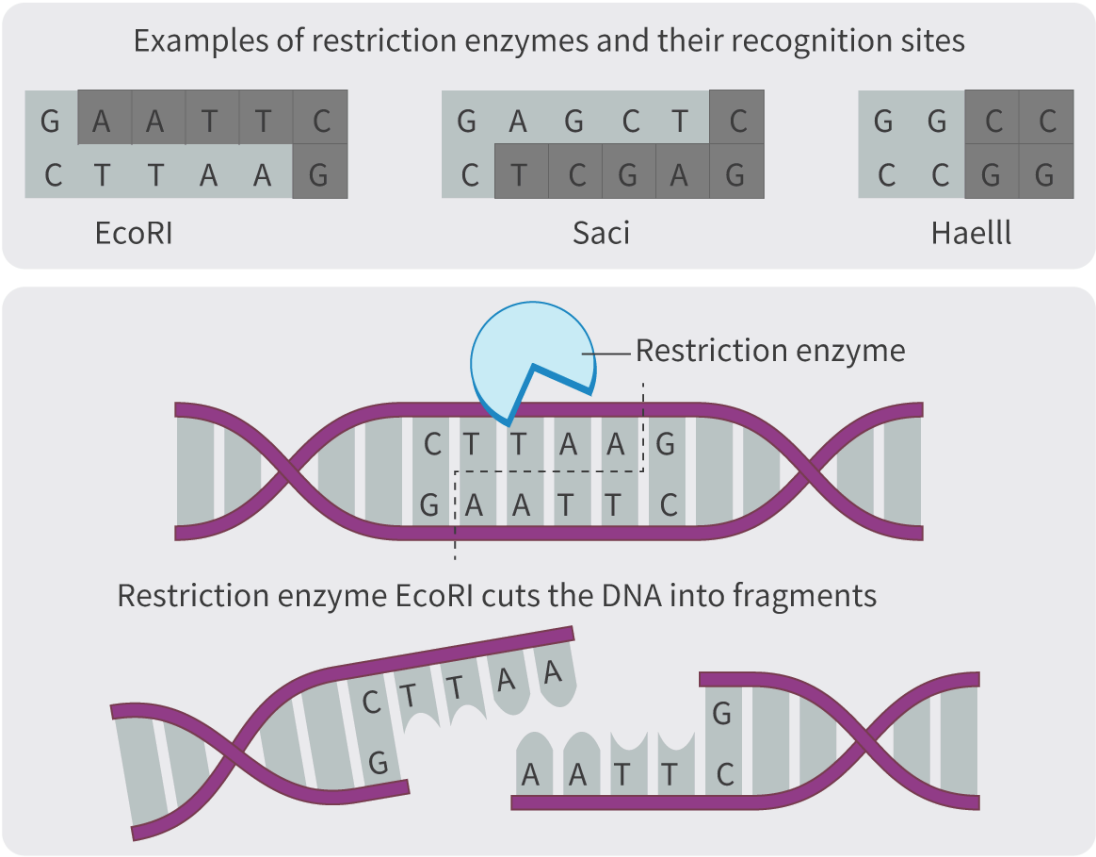

Figure 2. Restriction enzymes cut DNA into fragments for gel electrophoresis.

More information for figure 2

The diagram consists of two main sections: the top section displays examples of restriction enzymes (EcoRI, Saci, and HaeIII) and their recognition sites on DNA. Each enzyme is associated with a specific DNA sequence that it recognizes and cuts, shown in shaded boxes. "G A A T T C" / "C T T A A G" for EcoRI, "G A G C T C" / "C T C G A G" for Saci, and "G G C C" / "C C G G" for HaeIII.

The bottom section illustrates how the restriction enzyme EcoRI cuts DNA. The DNA double helix is depicted, with the specific recognition site shaded and labeled. A representation of the restriction enzyme is shown as a blue shape interacting with the DNA at the recognition site "G A A T T C" / "C T T

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A A G." The DNA strand is cut by the enzyme, resulting in two separate segments, depicted with jagged edges indicating the cut locations.

[Generated by AI]

Samples with fragments of DNA are loaded into small depressions, called wells, on one end of the gel (a jelly-like polymer). The gel is submerged in a buffer solution, and an electric current is run through the gel. The DNA samples must begin near the negative pole, so that they can spread out as they are drawn toward the positive pole (**Figure 3**).

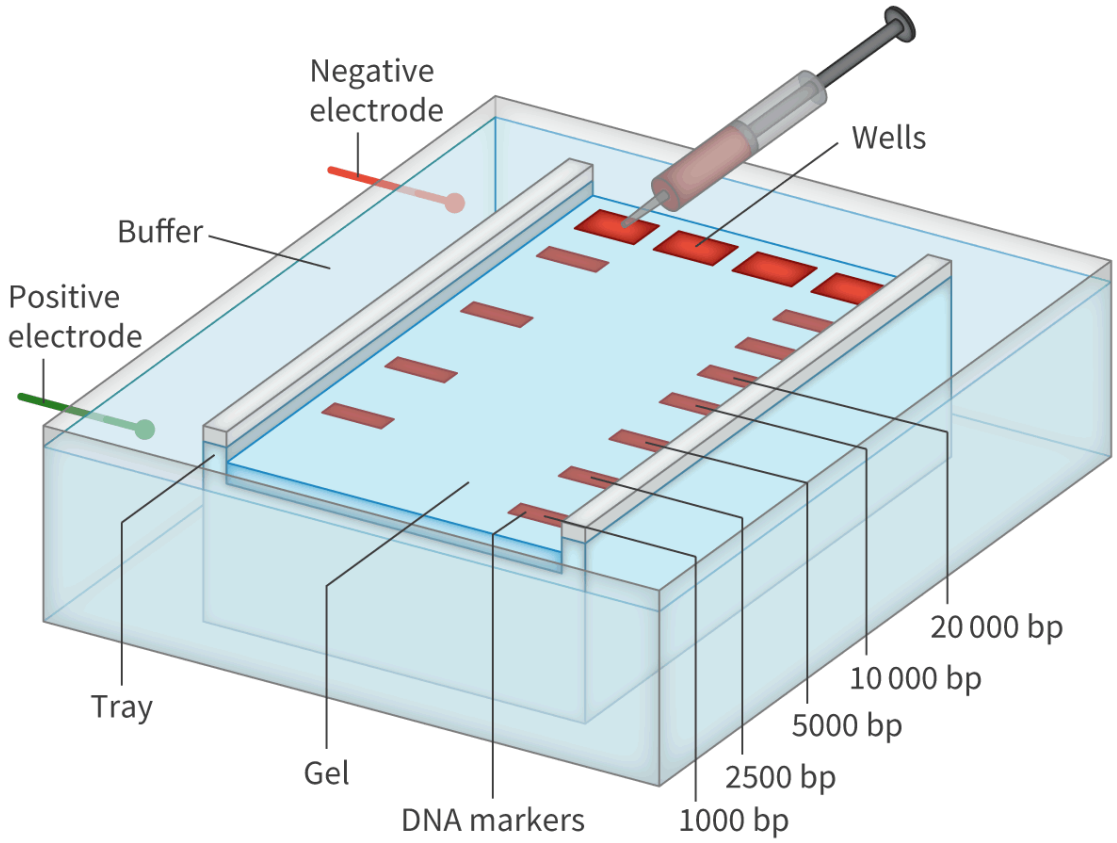

Figure 3. Gel electrophoresis apparatus.

More information for figure 3

This is a labeled diagram of a gel electrophoresis apparatus. The apparatus is shown in perspective view. At the center is a rectangular gel slab, where DNA samples are placed. The gel has wells at one end where samples are loaded with a pipette. There are two electrodes indicated: a negative electrode

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shown in red and a positive electrode in green, placed at opposite ends of the gel, creating an electric field across the gel. The gel is immersed in a buffer solution, which facilitates the movement of DNA. The DNA fragments travel from the wells towards the positive electrode due to the electric field.

Positioned along the gel are DNA markers with lengths labeled as 1000 bp, 2500 bp, 5000 bp, 10,000 bp, and 20,000 bp, showing the migration pattern of DNA fragments. The entire setup is placed on a tray, with the buffer surrounding the gel. The diagram includes labels for all these components, highlighting the structure and function of the gel electrophoresis process.

[Generated by AI]

The gel is porous, and the DNA must travel through the spaces within the gel. Smaller pieces can slip through the spaces more easily, allowing them to travel further along the gel in a given amount of time. Usually, one or more of the wells is filled with a ‘DNA ladder’, which contains DNA fragments with a range of known lengths. By using the DNA ladder, the length of sample fragments can be determined.

Figure 4 shows a photograph of DNA fragments that have been separated within a gel. Because the DNA fragments themselves do not have a colour, the gel must be dyed. Ethidium bromide is commonly used because it binds to DNA and then fluoresces in ultraviolet light. Ethidium bromide binds strongly to DNA, so it produces a clear signal, but is mutagenic, so sometimes safer alternatives are used.

Student view

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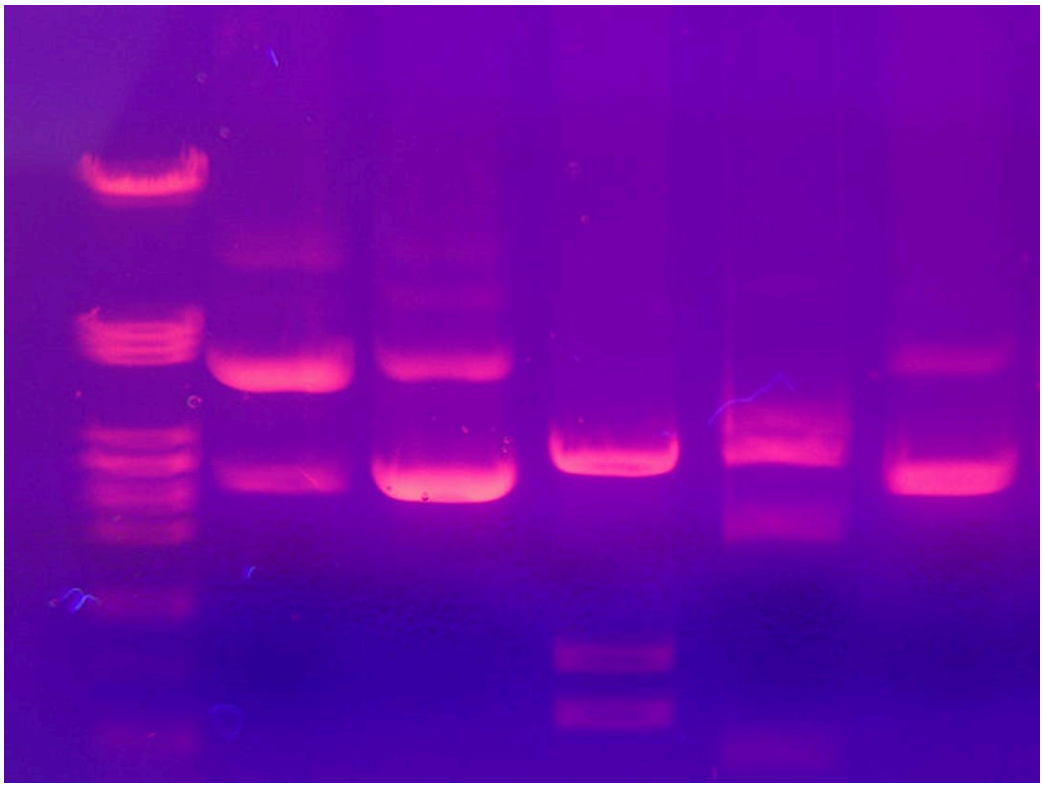

Figure 4. DNA fragments separated by gel electrophoresis.

Source: "Gel electrophoresis 2 (https://commons.wikimedia.org/wiki/File:Gel_electrophoresis_2.jpg)" by Mnolf is licensed under CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0/deed.en)

More information for figure 4

The image is a photograph of gel electrophoresis used to separate DNA fragments. The gel is illuminated under ultraviolet (UV) light, causing the DNA fragments, stained with ethidium bromide, to fluoresce as visible bright bands. This creates distinct lines or bands across the gel. These bands represent DNA fragments of various sizes, allowing for analysis of DNA based on fragment length. The gel appears predominantly purple due to the UV light, with the bands glowing in shades of pink or red, showing a progression from left to right of bands varying in intensity and size.

[Generated by AI]

Watch **Video 2** for an overview of gel electrophoresis.

Student view

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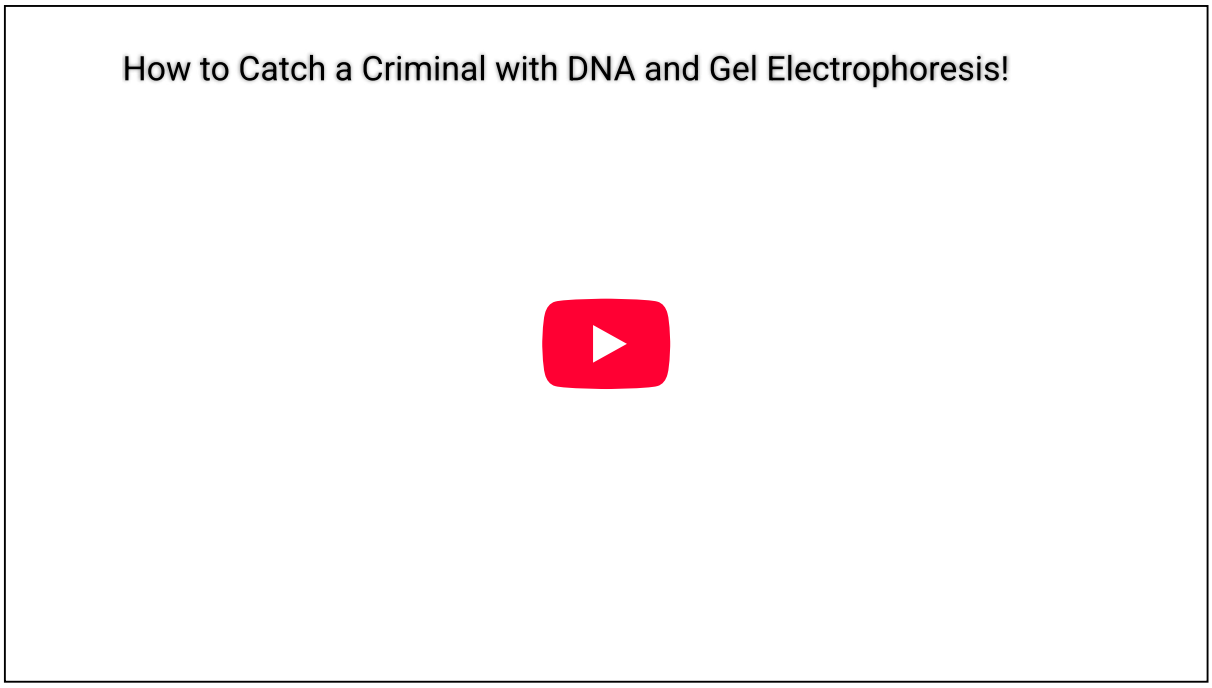

Video 2. Catching criminals – an application of gel electrophoresis.

Applications of PCR and gel electrophoresis

Both PCR and gel electrophoresis have many applications. One example is DNA profiling, which can be used for paternity testing and in forensic investigations. DNA profiling is a technique that examines variable portions of DNA to create a profile or 'fingerprint' that is unique to the individual. Forensics is the use of science in criminal and legal cases, and DNA profiling is a powerful forensic tool. Each of our cells contains our entire genome, and we shed cells continually, leaving them in the environment around us. By analysing residue on a doorknob, a drinking glass, a piece of clothing and so on, we can determine if an individual's DNA is present. For example, a blood stain on the suspect's jacket that contains the victim's DNA could be very powerful evidence.

Theory of Knowledge

How do the tools that we use shape the knowledge that we produce?

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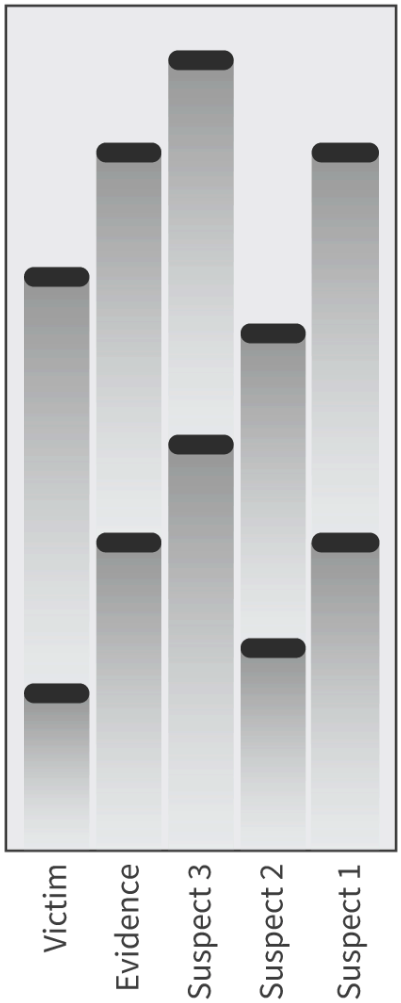
The development of tools such as PCR and gel electrophoresis allow new types of knowledge to be produced, including big data in the form of genetic databases with extensive applications in treatment of disease and forensic investigations.

Most genomes, including that of humans, have short, repeated DNA sequences called tandem repeats. The number of repeats can vary greatly between individuals in a population, meaning there may be dozens of versions. Restriction enzymes are used to chop the DNA into fragments that vary in length depending on the number of repeats. After amplifying with PCR, the resulting mix of DNA fragments is separated using gel electrophoresis.

To match the DNA in a piece of evidence to the DNA of an individual, there must be exactly the same number and length of DNA fragments, since a person's DNA sequence is the same in all cells. In **Figure 5**, there is a match between the evidence and suspect 1.



Student view

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Figure 5. DNA profile of evidence from a crime scene and suspects.

More information for figure 5

The image is a chart depicting DNA profiles in the form of vertical columns. Each column represents the DNA fragments for different subjects: Victim, Evidence, Suspects 3, 2, and 1. The columns are of varying heights, signifying different lengths of DNA fragments. The column labeled 'Evidence' matches perfectly in height with 'Suspect 1', indicating a DNA match. The other columns ('Victim', 'Suspect 3', and 'Suspect 2') do not match in height with the 'Evidence' column, which clearly differentiates which suspect's DNA aligns with the evidence provided.

[Generated by AI]

A match for one particular site, as shown in **Figure 5**, can completely eliminate suspects 2 and 3 as the source of DNA in the evidence. Although suspect 1 is a match, it is possible that there are other individuals with the same combination of alleles. In forensics, several dozen sites may be analysed. The chance of several dozen matches occurring randomly is a tiny fraction of a percent.

Although DNA profiling is most commonly used in forensics and determining family relationships, it has other uses as well, and can be used with any species. For example, it can be used to study biodiversity in wild populations or to determine which strains of bacteria cause more serious illness.

Nature of Science

Aspect: Measurement

Reliability is enhanced by increasing the number of measurements in an experiment or test. In DNA profiling, increasing the number of markers used reduces the probability of a false match. Markers are the short tandem repeat sequences found in DNA that are used in DNA profiling. There are many in our DNA and usually 18—20 are used. The more short

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tandem repeats that are analysed and compared, the more reliable the results of the DNA profiling (see section 1.5.4 (/study/app/bio/sid-422-cid-755105/book/data-analysis-id-46700/) for data processing).

International Mindedness

DNA profiling is used and applied slightly differently in different countries around the world. For example, countries may use different amounts of markers as their standard. In North America a set of 20 markers is used, while the United Kingdom uses 17 and Australia uses 18.

Try this activity to test your understanding of applications of PCR and gel electrophoresis in paternity testing.

Activity

- **IB learner profile attribute:** Inquirer
- **Approaches to learning:** Thinking skills — Providing a reasoned argument to support conclusions; Applying key ideas and facts in new contexts
- **Time required to complete activity:** 15—20 minutes
- **Activity type:** Individual/pair activity

DNA profiles are also used to determine family relationships, most commonly to determine paternity. Individuals inherit fragment length from their parents, just as they do other alleles.

In the **Figure 6**, three gels show the DNA profiles for three different paternity tests. The profile of the child should contain DNA fragments from the mother and the father, and each of the child's DNA fragments must be able to be mapped to one of the parents.

Try to determine which of the three alleged fathers is the biological father of the child. Half of the bands on the child's gel can be mapped to the mother. Now see which of the fathers has bands which can be

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mapped to the child so that there are no bands left without a match on the child's gel.

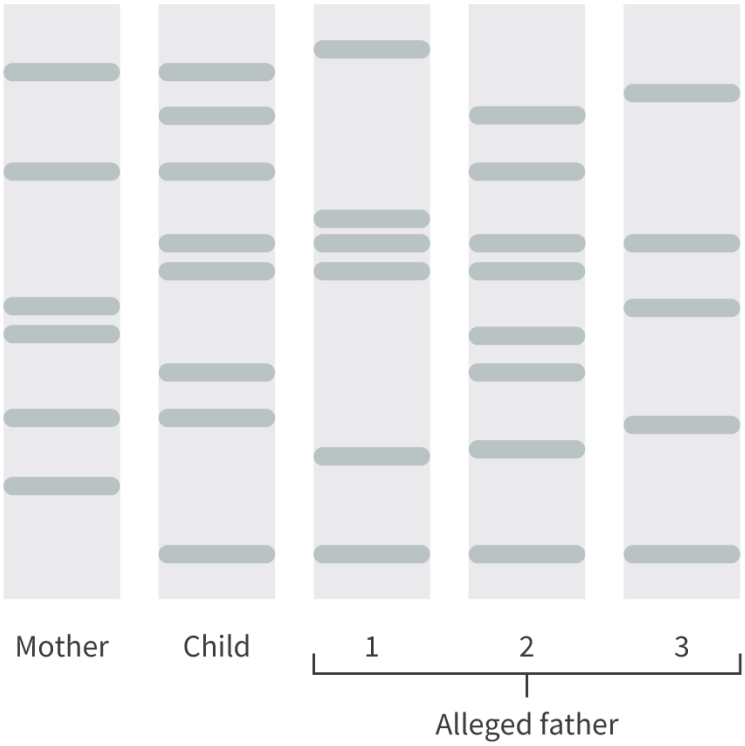

Figure 6. DNA profiles for a paternity case.

More information for figure 6

The image displays five vertical columns representing DNA profiles labeled as Mother, Child, and three alleged fathers numbered 1, 2, and 3. Each profile is composed of several horizontal bands, varying in position and intensity. To determine paternity, match half of the child's bands with those of the mother. Then, compare the remaining child's bands with each alleged father's bands to find a father whose profile matches all the child's unaccounted bands. This will help identify any father whose bands share a complete match, thus indicating biological relation.

[Generated by AI]

Next, consider the DNA profiles shown in **Figure 7**. A child that was adopted at birth is searching for their birth parents. The DNA profile shows the child compared to four sets of possible birth parents.

Determine which set of parents are the most likely parents of the adopted child. Explain your reasoning.

Student view

Overview
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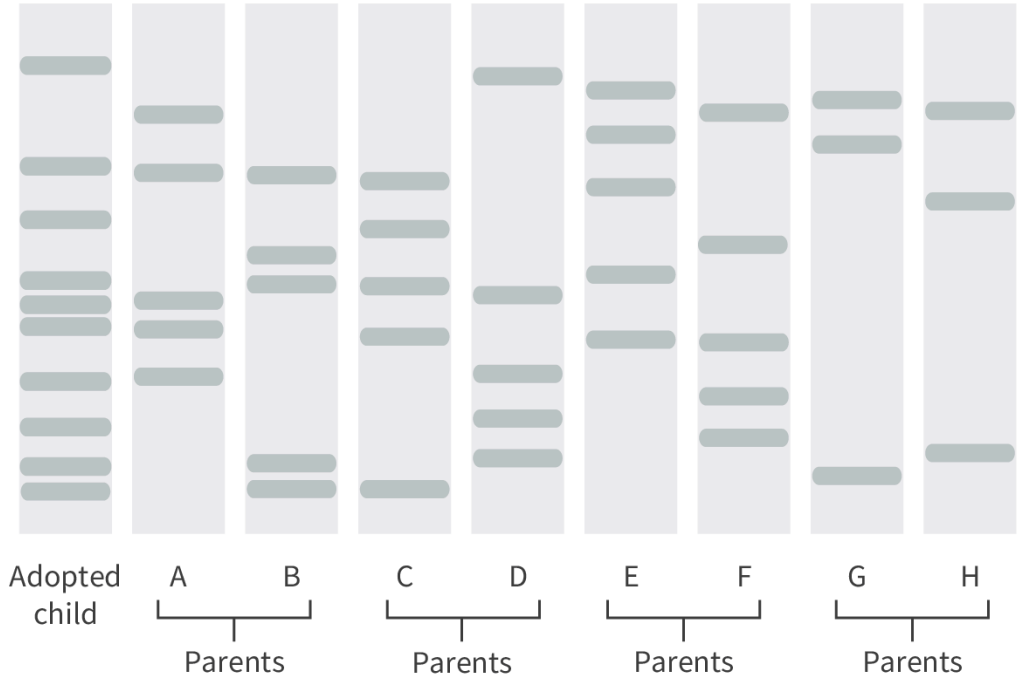

Figure 7. DNA profiles for adopted child.

More information for figure 7

The image shows a set of DNA profiles arranged vertically like barcode patterns, which are used to compare an adopted child with several sets of potential parents. Each profile consists of several horizontal bands. The profiles are grouped and labeled from left to right. The first column is labeled 'Adopted child,' followed by groups labeled 'Parents' with corresponding letters: A, B, C, D, E, F, G, and H. Each group has two columns representing a pair for each parent group. The profiles contain bands at various heights, suggesting the alleles present in each individual. The task is to identify which set of parents' DNA profiles match the adopted child's profile by analyzing the matching bands.

[Generated by AI]

5 section questions

D1. Continuity and change: Molecules / D1.1 DNA replication

Directionality of DNA replication (HL)

Student view

D1.1.6: Directionality of DNA polymerases (HL) D1.1.7: Replication on the leading strand and the lagging strand (HL)

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Higher level (HL)

Learning outcomes

By the end of this section you should be able to:

- Describe the directionality of DNA polymerases based on the difference between the 5′ and 3′ terminals of strands of nucleotides.
- Describe replication on both the leading and lagging strands and how these differ.

If DNA is a code, does it matter in which direction it is read? Does it matter in which direction you write the letters in a word or the words in a sentence? Would the words or sentences make sense if they were written in reverse?

Directionality of DNA polymerases

To understand the directionality of DNA polymerases, we need to review our understanding of the structure of DNA. DNA is a polynucleotide, meaning it is made of many nucleotides (**Figure 1**).

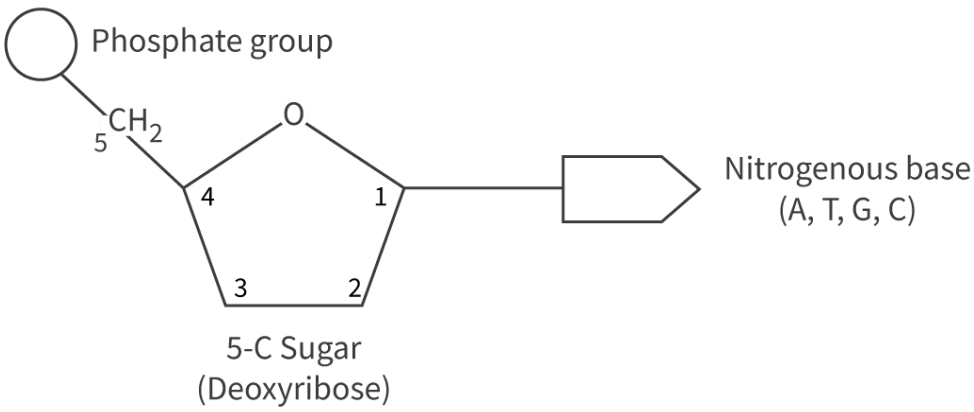

Figure 1. A DNA nucleotide.

More information for figure 1

Student view

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The image is a diagram depicting the structure of a DNA nucleotide. It consists of a pentagon-shaped 5-carbon sugar (deoxyribose) in the center. Attached to the 5-carbon position of this sugar is a 'Phosphate group,' which is depicted as a circle connected by a single line labeled with '5'. This indicates the beginning of the polynucleotide chain. On the opposite end of the sugar, an 'Nitrogenous base' is connected, which is represented as a rectangle with a pointed end. The nitrogenous bases are given as letters (A, T, G, C) indicating adenine, thymine, guanine, and cytosine. This diagram helps in understanding the basic composition and connectivity of a nucleotide in DNA, which is foundational for its directionality and function in forming the DNA polymer.

[Generated by AI]

The nucleotides are held together by phosphodiester bonds. A phosphodiester bond occurs when two of the hydroxyl groups in phosphoric acid react with hydroxyl groups on other molecules to form two ester bonds. Phosphodiester bonds are central to all life on Earth, as they make up the backbone of the strands of nucleic acid and are the reason for its directionality. In DNA, the phosphodiester bonds occur between the phosphate group attached to the 5′ carbon of the deoxyribose of one nucleotide and the hydroxyl group on the 3′ carbon of deoxyribose on the next nucleotide. This arrangement allows us to describe the directionality in terms of 5′ and 3′.

When assembling a new strand of DNA, DNA polymerase III adds the 5′ end of a DNA nucleotide to the 3′ end of the previously added nucleotide. Like other enzymes, DNA polymerase III has an active site that is complementary to only a very specific shape. This is why it is only able to build new DNA strands in a 5′ to 3′ direction. If it worked in the other direction, the shape would be different.

Making connections

Go to section A1.2.11 (/study/app/bio/sid-422-cid-755105/book/dna-base-sequences-hl-id-46211/) to learn more about the directionality of ribonucleic acid (RNA) and DNA, and to section D1.2.12—14a (/study/app/bio/sid-422-cid-

Student view

Overview (/study/ap 422-cid-755105/o

755105/book/controlling-transcription-and-translation-hl-id-46505/) to learn how non-coding sequences in DNA do not code for polypeptides.

The leading strand and the lagging strand

As new strands of DNA can only be assembled by DNA polymerase III in a 5′ to 3′ direction, only one strand can be replicated in the same direction as the helicase is unwinding and unzipping the original strand. As the helicase moves along the DNA, it forms the replication fork where replication can occur. The strand that can be replicated in the same direction as the helicase moves is called the leading strand. This strand is orientated from 3′ to 5′ and can be replicated continuously.

The other strand, as it is orientated in the opposite direction, does not allow DNA polymerase III to move in the same direction as helicase. It must work in the opposite direction. This strand is known as the lagging strand and the replication along this strand is discontinuous. DNA polymerase III replicates the new strand in sections, having to repeatedly move further along the strand to continue replicating it. These sections of newly formed but disconnected DNA are known as Okazaki fragments.

Watch **Video 1** for an overview of DNA replication.

Student view

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Video 1. A detailed overview of DNA replication.

Try this group activity to enhance your understanding of DNA replication.

Activity

- **IB learner profile attribute:** Thinker
- **Approaches to learning:** Thinking skills — Being curious about the natural world; Applying key ideas and facts in new contexts
- **Time required to complete activity:** 15 minutes
- **Activity type:** Group activity

In a group of three or four, discuss the directionality of DNA replication and other examples of directionality. Consider the following questions in your discussion.

- How would DNA replication potentially differ if there was no directionality?
- Would it be beneficial to an organism to evolve a DNA polymerase that could build new DNA in both directions? What if the directionality was reversed?
- Can you think of any other examples of directionality? What about written language? Does directionality affect

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how we understand written language?

The expected outcome of this activity is that you are all given an opportunity to think about and express your ideas on directionality and its significance to DNA replication. In your group, make sure all members are given the opportunity to speak. To aid your group in this, select one member to be the facilitator. Their role is to manage the discussion, decide who speaks and make sure only one person is sharing at a time.

5 section questions

D1. Continuity and change: Molecules / D1.1 DNA replication

The enzymes of DNA replication (HL)

D1.1.8: DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase (HL) D1.1.9: DNA proofreading (HL)

Higher level (HL)

Learning outcomes

By the end of this section you should be able to:

- Describe the functions of DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase in replication of prokaryotic DNA.
- Explain DNA polymerase III's role as a proofreader of replicated DNA.

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Functions of enzymes in DNA replication

Several enzymes are involved in the process of DNA replication. These include DNA helicase, DNA polymerase (actually DNA polymerase III), DNA primase, DNA polymerase I and DNA ligase. As it is easier to understand, we will limit our understanding of these enzymes and how they function to the DNA replication within prokaryotic cells. The following outlines the functions of these enzymes.

Helicase – unwinds and unzips the DNA molecule by breaking the hydrogen bonds holding the complementary bases together. This forms the replication fork. The strands are then kept from coming back together by single strand binding proteins. These proteins attach to the single strands of DNA and prevent them from re-forming the hydrogen bonds between their complementary bases until replication can be carried out.

Gyrase – moves ahead of helicase, relieving the tension created by the unwinding and unzipping of the DNA double helix.

DNA primase – attaches small RNA primers, made of several RNA nucleotides, to the template strand. This allows DNA polymerase III to attach and begin assembling the free nucleotides into a new strand of DNA. Without the RNA primers, DNA polymerase III is unable to attach to the DNA strand properly. As replication is continuous on the leading strand, only a single primer is required. However, on the lagging strand, primers need to be placed at regular intervals to allow DNA polymerase III to attach at the multiple points necessary for the discontinuous replication on this strand.

DNA polymerase III – assembles the new strands of DNA by placing free nucleotides in the correct sequence according to the base sequence of the template strand and the complementary base pairing rule. It is only able to build new strands in the 5′ to 3′ direction. It can replicate continuously on the leading strand but must replicate the lagging strand discontinuously. This means it must replicate the strand in short sections called Okazaki fragments.

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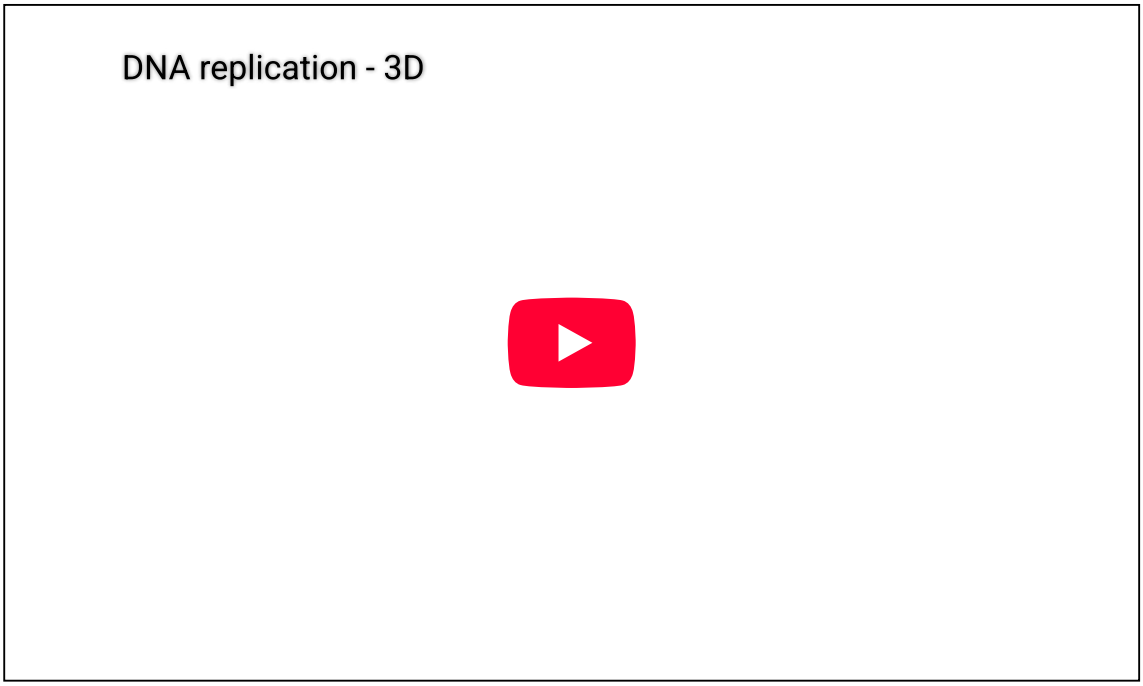
DNA polymerase I – removes the RNA nucleotides of the primers and replaces them with the correct DNA nucleotides.

DNA Ligase – catalyses the formation of the phosphodiester bonds between the Okazaki fragments. This makes the replicated strand built using the lagging strand into a single strand that can function normally.

Watch **Video 1** for a summary of DNA replication, including the roles of the key enzymes.



Video 1. DNA replication. (Note: the exonuclease described in the video is DNA polymerase I.)

Making connections

Review section A1.2.8 (/study/app/bio/sid-422-cid-755105/book/complementary-base-pairing-id-43801/) to learn about the role of complementary base pairing in allowing genetic information to be replicated and expressed. Section A1.2.6 (/study/app/bio/sid-422-cid-755105/book/rna-and-dna-polymers-id-45990/) describes the detailed structure of DNA.

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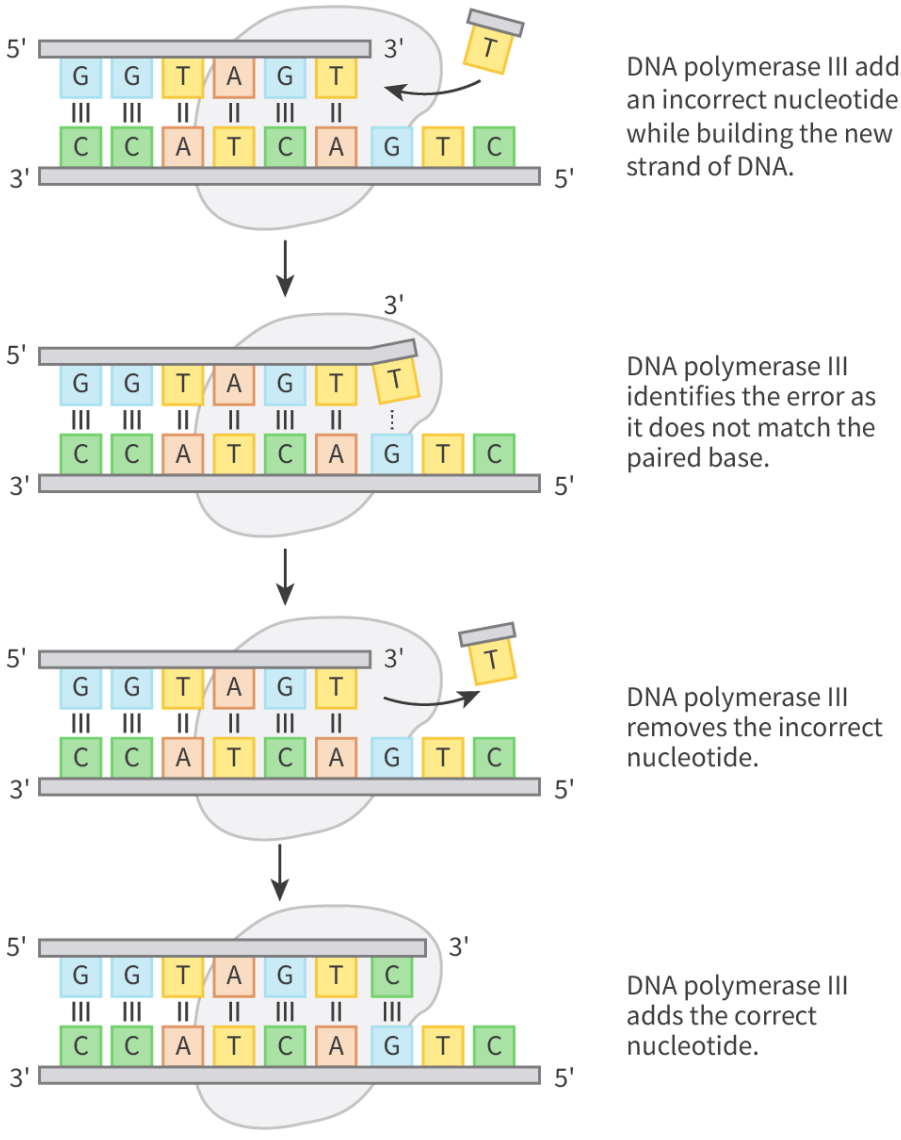
Read through section C1.1 (/study/app/bio/sid-422-cid-755105/book/enzymes-as-catalysts-id-46232/) to learn about the role of enzymes as globular proteins with an active site for catalysis.

DNA proofreading

DNA polymerase III has an additional, but essential, function in this process. It proofreads the newly formed DNA strand as it is being built (**Figure 1**). If a nucleotide is placed with a mismatched base, the incorrect nucleotide is removed and replaced with the correctly matching one. For example, if A is matched to C, the A would be recognised as being incorrect and it would be removed and replaced with G that is complementary to C. This is just one of several ways that cells are able to avoid the vast majority of errors that would lead to potential mutations.

Section 1 of 10 Feedback Print (/study/app/bio/sid-422-cid-755105/book/directionality-of-dna-replication-hl-id-46377/print/) Assign



Student view

Overview (/study/ap 422-cid-755105/o

Figure 1. DNA polymerase III proofreading.

More information for figure 1

This diagram illustrates the proofreading function of DNA polymerase III during DNA replication. It consists of four stages:

1. The first stage shows the DNA polymerase adding an incorrect nucleotide (T) to the new DNA strand opposite a C on the template strand. This incorrect addition is highlighted as it doesn't match the template base.
2. The second stage indicates the recognition of this mismatched base by DNA polymerase III. The enzyme identifies the error because the new nucleotide (T) does not properly pair with the existing base on the template strand (C).
3. The third stage depicts DNA polymerase III removing the incorrect nucleotide (T) from the newly synthesized strand, correcting the error.
4. The fourth and final stage shows DNA polymerase III replacing the incorrect nucleotide with the correct one (G) that complements the template strand's C.

Accompanying each stage in the diagram, there are written explanations that align with the visual steps explaining the proofreading mechanism.

[Generated by AI]

Try the interactive exercises in this activity to help you understand the enzymes involved in DNA replication.

Activity

- **IB learner profile attribute:** Thinker
- **Approaches to learning:** Thinking skills — Being curious about the natural world
- **Time required to complete activity:** 15 minutes
- **Activity type:** Individual activity

Complete the following three interactive activities.

Student view

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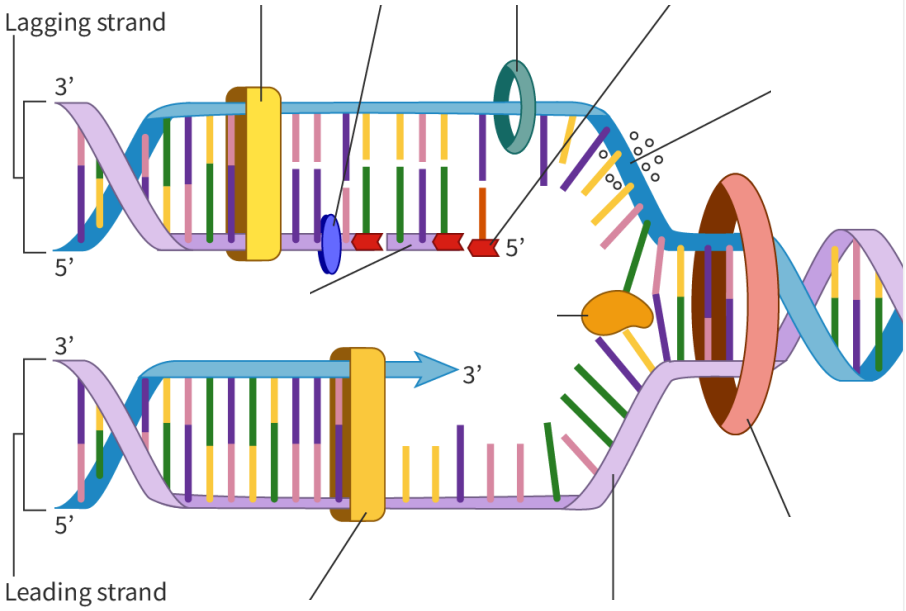
In **Interactive 1**, correctly label the diagram showing DNA replication.



Gyrase | DNA polymerase I | Single strand binding proteins | DNA primase | Okaza fragme

Helicase | DNA polymerase III | RNA primer | Replication fork | DNA lig

Check

Interactive 1. Key Proteins in DNA Replication.

In **Interactive 2**, correctly drag the statements into the correct order to summarise the stages of DNA replication.

Student view

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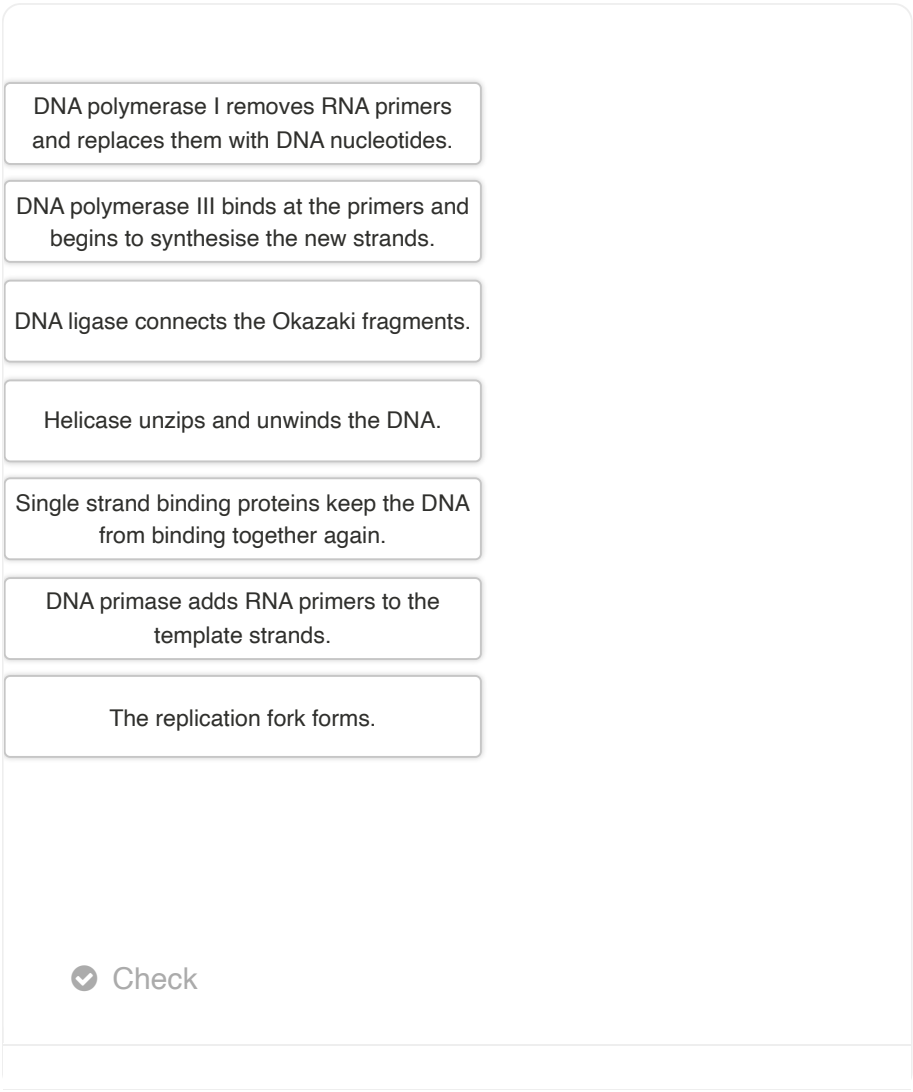

Interactive 2. Enzymes Involved in DNA Replication.

In **Interactive 3**, drag the correct term to the correct description.

Student view

Relieves the tension in the DNA as it is unwound.

Unwinds and unzips the DNA by breaking hydrogen bonds between bases.

Builds the new strands of DNA using the original strands as templates.

Places RNA primers on the template strands.

Replaces RNA primers with DNA nucleotides.

Connects the Okazaki fragments by forming phosphodiester bonds.

Keep the two strands of DNA separated and stable during replication.

DNA polymerase I | DNA polymerase III | DNA primase | Gyrase | Single strand binding pro | Helicase | DNA ligase

Check

Interactive 3. Key Enzymes in DNA Replication.

5 section questions

D1. Continuity and change: Molecules / D1.1 DNA replication

Summary and key terms

- DNA replication occurs any time a new cell is formed, whether for growth or for replacement of cells in damaged tissues. DNA replication is able to produce exact copies of the entire sequence of bases of a cell's DNA.

Overview (/study/ap 422-cid-755105/o

- DNA replication is semi-conservative, which means each new DNA double helix is composed of one strand from the original parent DNA and one newly synthesised strand.
- The enzyme helicase is used in DNA replication to unwind and unzip the DNA double helix by breaking the hydrogen bonds between the complementary bases of the two strands. DNA polymerase then builds the new strands using the original strands as a template, using the complementary base pairing rule to place nucleotides in the correct sequence.
- PCR is a technique used to amplify small samples or target sequences of DNA that can then be used for other types of analysis. Thermal cycling is used to repeatedly denature, anneal and extend the DNA strands. This leads to the exponential growth in the numbers of target strands, giving you millions or even billions of individual sections of DNA within several hours.
- Gel electrophoresis is a technique used to separate DNA strands based on their size. Samples taken from different sources can be compared and used for forensic analysis.

Higher level (HL)

- The structure of DNA nucleotides means that they have a different shape at opposite ends of the molecule. In a single strand of DNA, the 5′ end of a nucleotide that ends with a phosphate group is bonded to the 3′ end of the adjacent nucleotide that ends in a hydroxide group. This makes directionality in DNA-related processes very important. Enzymes such as DNA polymerase III are only able to add nucleotides to a strand in the 5′ to 3′ direction.
- Due to the directionality of DNA replication, the two strands of a DNA molecule are replicated differently. One strand can be replicated continuously in the direction of movement of the replication fork and helicase. This is the leading strand. The other needs to be replicated discontinuously as it runs in the opposite direction. This forms Okazaki fragments which must then be later joined together. This is the lagging strand.
- There are a number of important enzymes involved in DNA replication. Helicase unwinds and unzips the DNA molecule. DNA polymerase III builds the new strand by placing free nucleotides in the correct position complementary to the template strand. DNA primase places RNA primers on the template strand to allow DNA polymerase III to attach and carry

Student view

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out its function. DNA polymerase I replaces the RNA nucleotides in the primers with DNA nucleotides. DNA ligase bonds adjacent Okazaki fragments together to form a continuous strand.

- While carrying out its main role of assembling the new strands of DNA, DNA polymerase III also does another important job. It proofreads the new strand for errors. If it detects an incorrect nucleotide that is not complementary to the template, it is removed by DNA polymerase III and replaced with the correct nucleotide.

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Key terms

Student view

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Review these key terms. Do you know them all? Fill in as many gaps as you can using the terms in this list.

1. Whenever new cells are needed for growth or repair of tissues, DNA needs to occur. This process is , meaning each new DNA molecule is made of one original parent strand and one newly synthesised strand.
2. is the enzyme responsible for unwinding and unzipping the DNA molecule. Then can assemble , forming the new strands using the original strand as a template.
3. is used to amplify small samples of DNA. is able to separate fragments of DNA based on size, creating DNA profiles that can be used in forensics and other investigations.
4. [HL] The of DNA replication means it can only occur in a 5′ to 3′ direction. On the , replication can occur continuously. However, on the , replication is discontinuous. This forms .
5. [HL] DNA replication in prokaryotes involves the following: , which attaches RNA primers to the template strands of DNA. , which assembles the new strands of DNA and them as it goes. , which removes the RNA primers and replaces them with DNA nucleotides. , which connects the Okazaki fragments.

Helicase | free nucleotides | lagging strand | DNA polymerase I | DNA polymerase III | proofreads | Gel electrophoresis | DNA polymerase | leading strand | Okazaki fragments | DNA primase | semi-conservative | Polymerase chain reaction | directionality | DNA ligase | replication

Student view

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Interactive 1. DNA Replication Process and Key Steps.

Checklist

Section Student... (0/0) Feedback Print (/study/app/bio/sid-422-cid-755105/book/checklist-id-46503/print/) Assign

What you should know

After studying this subtopic you should be able to:

- Describe DNA replication as the process by which exact copies of DNA are created for use in reproduction, growth and tissue replacement in multicellular organisms.
- Explain the semi-conservative nature of DNA replication and how it allows for a high degree of accuracy when copying base sequences.
- Describe the roles of helicase and DNA polymerase in DNA replication.
- Describe the use of polymerase chain reaction and gel electrophoresis for amplifying and separating DNA.
- Describe the applications for PCR and gel electrophoresis.

Section Student... (0/0) Feedback Print (/study/app/bio/sid-422-cid-755105/book/summary-and-key-terms-id-46502/print/) Assign

Higher level (HL)

- Describe the directionality of DNA polymerases based on the difference between the 5′ and 3′ terminals of strands of nucleotides.
- Describe replication on both the leading and lagging strands and how these differ.
- Describe the functions of DNA primase, DNA polymerase I, DNA polymerase III and DNA ligase in replication of prokaryotic DNA.

Section Student... (0/0) Feedback Print (/study/app/bio/sid-422-cid-755105/book/the-enzymes-of-dna-replication-hl-id-46501/print/) Assign

Student view

- Explain DNA polymerase III's role as a proofreader of replicated DNA.

Investigation

- **IB learner profile attribute:** Thinkers
- **Approaches to learning:** Thinking skills – Providing a reasoned argument to support conclusions; Applying key ideas and facts in new contexts
- **Time required to complete activity:** 30–40 minutes
- **Activity type:** Individual activity

Your task

Restriction enzymes are used to cut DNA at specific locations. There are different restriction enzymes that are specific to different DNA sequences. For example:

The *EcoRI* enzyme cuts DNA at the following site:

Figure 1. *EcoRI* enzyme.

More information for figure 1

Overview (/study/ap 422-cid-755105/o

The diagram illustrates the DNA cutting site for the EcoRI enzyme. It shows the DNA sequence GAATTC targeted by EcoRI, with the enzyme creating a staggered cut. The cut occurs between the G and A nucleotides on the upper strand (GAATTC) and between A and G on the complementary lower strand (CTTAAG), resulting in staggered ends. The sequence is typically recognized by EcoRI, making it a specific site for cutting DNA in molecular biology applications.

[Generated by AI]

The *SmaI* enzyme cuts at a different site.

Figure 2. *SmaI* enzyme.

More information for figure 2

The image depicts a DNA sequence involving a restriction enzyme cut site. The sequence shown is vertical and contains two strands. The upper strand reads CCCGGG, and the complementary lower strand reads GGGCCC. A vertical red line is positioned between the C and G on both strands, indicating where the enzyme cuts. This is a depiction of a palindromic sequence typical of restriction enzyme recognition sites.

[Generated by AI]

Student view

When different restriction enzymes are used to cut up a DNA sample, different numbers of fragments will be created and these will be of different sizes. It is the number and sizes of these fragments that are used to identify individuals and to

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make comparisons between a DNA sample and a suspect or person of interest in a forensic investigation.

Try the following exercise as an example.

- How many fragments would the following sequence of DNA bases be separated into using *EcoRI*?
- How many fragments would the following sequence of DNA bases be separated into using *SmaI*?

ATCATCCCGGGAATTCTAGCCCGGGAAATAGGCCCGGGATGAATTCT

TAGTAGGGCCCTTAAGATCGGGCCCTTTATCCGGGCCCTACTTAAGA

Figure 3. A sequence of DNA bases.

More information for figure 3

The image shows a sequence of DNA bases composed of letters representing nucleotides. It consists of two rows of letters, which include A, T, C, and G. These letters are arranged in a specific order to represent the genetic sequence. The top row reads: ATCATCCCGGGAATTCTAGCCCGGGAAATAGGCCCGGGGATGAATTCT. The bottom row reads: TAGTAGGGCCCTTAAGATCGGGCCCTTTATCCGGGGCCTACTTAAGA. This representation indicates a section of DNA, which is crucial in DNA profiling and examining genetic material for various purposes such as investigating poaching and trade of endangered species.

[Generated by AI]

As you saw in the big picture, DNA profiling can be used to investigate the illegal poaching and trading of endangered animals. In the following activity, you will be analysing DNA profile evidence to investigate the illegal poaching and international trade of black rhinoceros (*Diceros bicornis*). Black rhino populations were decimated by hunters and poachers in the twentieth century, decreasing

Student view

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their population by approximately 98%. From their low of about 2500 individuals, intensive conservation efforts have helped them to recover to about 6000 animals today (**Figure 4**).

Figure 4. Black rhinoceros (Diceros bicornis).

Credit: Munib Chaudry, Getty Images

One of the conservation efforts in place is the use of DNA evidence taken directly from rhino horns confiscated from their illegal trade and from the carcasses of illegally killed animals (**Figure 5**). The evidence is then used in criminal cases brought against suspected poachers and smugglers.

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Figure 5. Confiscated rhino horns.

Credit: Stockbyte, Getty Images

First let's use the following quick simulation to review how a DNA profile of a DNA fingerprint is created.

https://www.pbs.org/wgbh/nova/interactive/create-dna-fingerprint (https://www.pbs.org/wgbh/nova/interactive/create-dna-fingerprint)/

The DNA profiles shown in **Figure 6** were obtained from four horns seized by police and customs officials from a shipping container leaving a port in Tanzania. There are also the profiles from four rhinos found killed with their horns removed by poachers. The restriction enzyme *EcoRI* was used.

Rhino 1 was found in Tanzania, Rhinos 2 and 4 were found in South Africa and Rhino 3 was found in Namibia.

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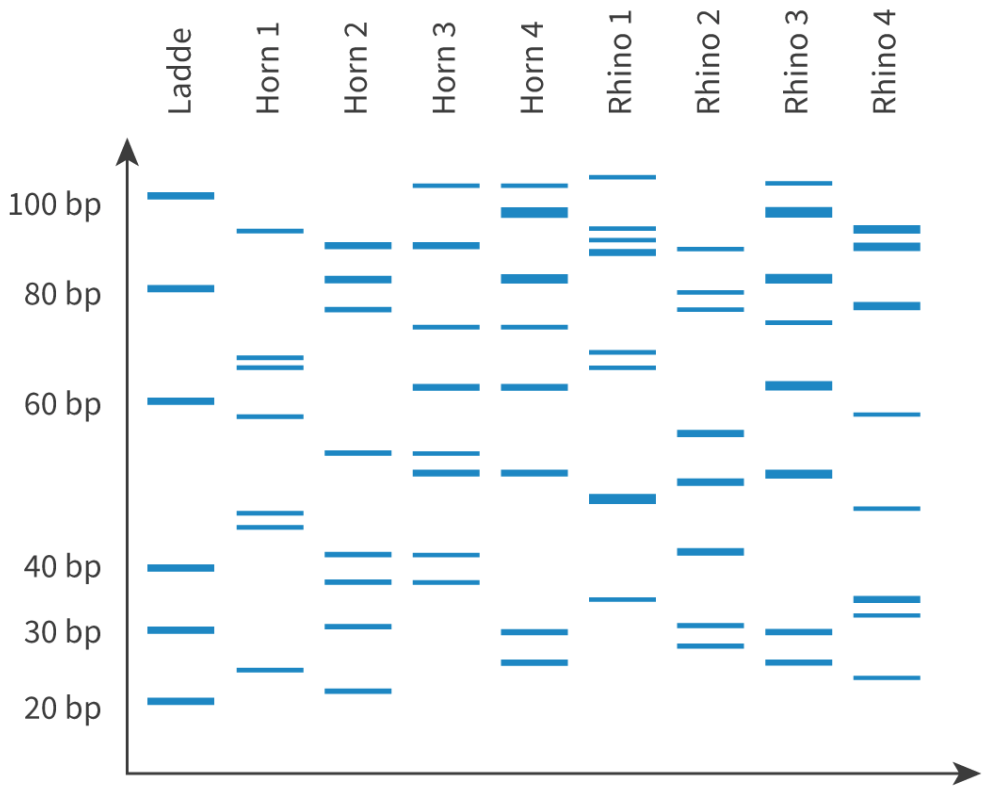

Figure 6. DNA profiles of confiscated rhino horn DNA and DNA found from rhinos killed by poachers.

More information for figure 6

The image displays a diagram representing DNA profiles of rhino horn samples and rhino samples. The horizontal axis lists samples under 'Ladder,' 'Horn 1,' 'Horn 2,' 'Horn 3,' 'Horn 4,' 'Rhino 1,' 'Rhino 2,' 'Rhino 3,' and 'Rhino 4.' The vertical axis is labeled with base pair (bp) values: 20 bp, 30 bp, 40 bp, 60 bp, 80 bp, and 100 bp.

Each sample column contains several blue bands aligned horizontally at various bp levels, indicating the DNA presence at those points. 'Ladder' serves as a reference, displaying bands at consistent intervals. For instance, 'Horn 1' shows bands around 100 bp, 80 bp, and 40 bp, while 'Rhino 1' exhibits a different pattern, sharing some bands with 'Horn 1' but also with unique bands.

The pattern and number of bands vary across samples, providing a visual representation of DNA similarities or differences among the horns and rhinos, useful for identification and comparison purposes.

[Generated by AI]

Student view

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- What conclusions can you draw from the DNA profiles?
- Can you match any horns to any individuals?
- Can you prove that any horns have been transported across international borders?
- Are there any relationships between individual rhinos?
- How would these profiles be different if another restriction enzyme such as *SmaI* was used? Would your conclusions be different?

D1. Continuity and change: Molecules / D1.1 DNA replication

Reflection

Section Student... (0/0) Feedback Print (/study/app/bio/sid-422-cid-755105/book/reflection-id-46890/print/) Assign

Teacher instructions

The goal of this section is to encourage students to reflect on their learning and conceptual understanding of the subject at the end of this subtopic. It asks them to go back to the guiding questions posed at the start of the subtopic and assess how confident they now are in answering them. What have they learned, and what outstanding questions do they have? Are they able to see the bigger picture and the connections between the different topics?

Students can submit their reflections to you by clicking on 'Submit'. You will then see their answers in the 'Insights' part of the Kognity platform.

Reflection

Now that you've completed this subtopic, let's come back to the guiding question introduced in The big picture (/study/app/bio/sid-422-cid-755105/book/big-picture-id-43546/).

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- How is new DNA produced?
- How has knowledge of DNA replication enabled applications in biotechnology?

With these questions in mind, take a moment to reflect on your learning so far and type your reflections into the space provided.

You can use the following questions to guide you:

- What main points have you learned from this subtopic?
- Is anything unclear? What questions do you still have?
- How confident do you feel in answering the guiding questions?
- What connections do you see between this subtopic and other parts of the course?

⚠ Once you submit your response, you won't be able to edit it.

0/2000

Submit

Rate subtopic D1.1 DNA replication

Help us improve the content and user experience.

☆ ☆ ☆ ☆ ☆

Student view