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VCE Biology ¾
Introduction to DNA Manipulation Techniques [1.5]
Workbook

Outline:

Enzymes and DNA Manipulation Pg 3-12
- Introduction to DNA Manipulation
- Polymerases
- Endonucleases
- Ligases

Polymerase Chain Reaction Pg 13-17
- Process of PCR

Gel Electrophoresis Pg 18-24
- The Process

DNA Profiling Pg 25-32
- DNA Fingerprinting/Profiling
- Genetic Testing

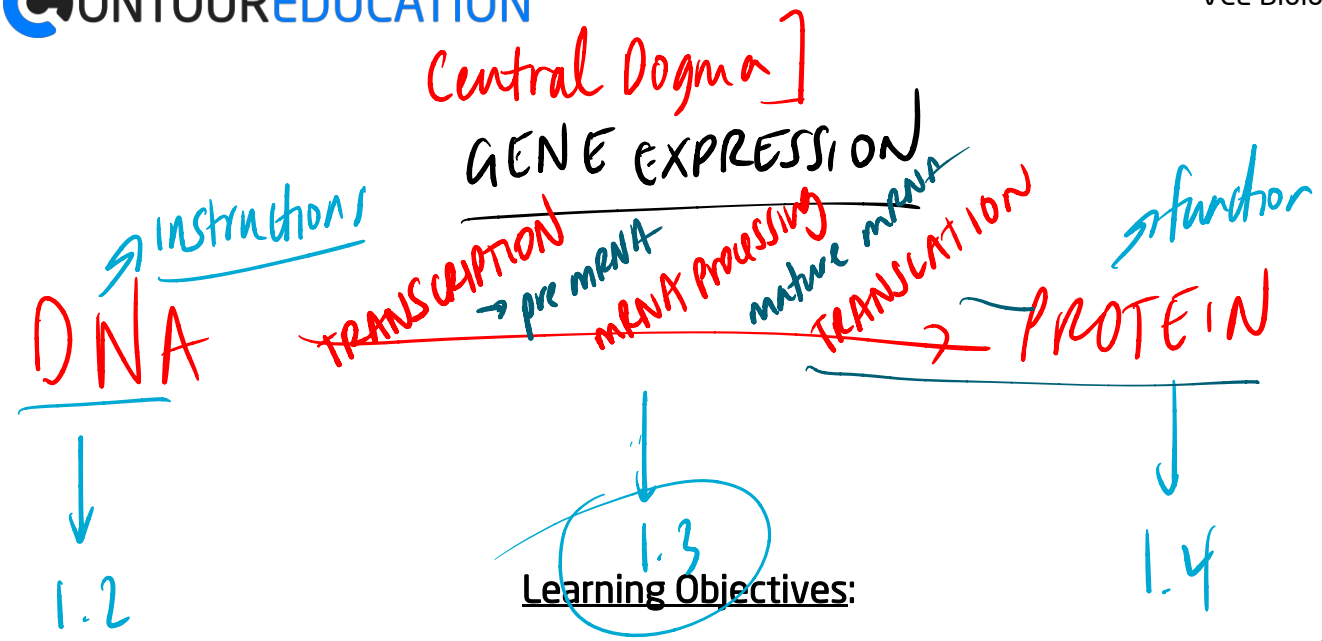
Study Design Key Knowledge:

Study Design: DNA manipulation techniques and applications

The use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA, and endonucleases to cut DNA.

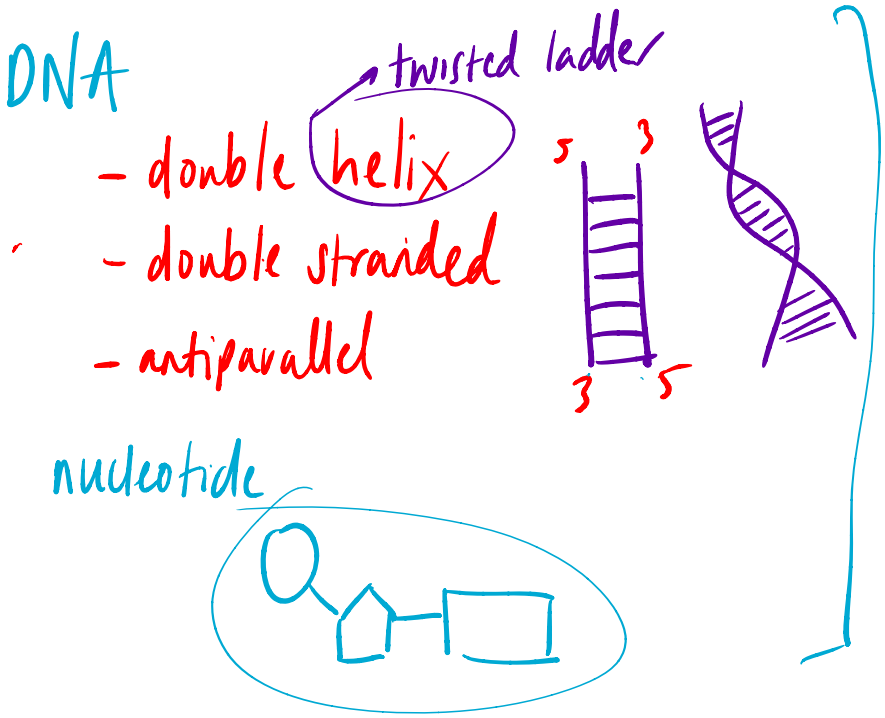
Amplification of DNA using polymerase chain reaction and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling.

https://www.vcaa.vic.edu.au/Documents/vce/biology/2022BiologySD.docx



Learning Objectives:

- **BI34 [1.5.1]** - Identify and describe the function of polymerases, endonucleases, and ligases in DNA manipulation.
- **BI34 [1.5. 2]** - Identify the ingredients required, describe the process, and recall key applications of PCR.
- **BI34 [1.5.3]** - Describe the process of gel electrophoresis, and describe how it may be used to differentiate DNA samples or to obtain a "DNA profile".
- **BI34 [1.5.4]** - Explain the factors that affect the movement of fragments in gel electrophoresis.
- **BI34 [1.5.5]** - Define satellite DNA and STRs, and explain their use in identifying people through DNA profiling for crimes and paternity testing.



Section A: Enzymes and DNA Manipulation

Sub-Section: Introduction to DNA Manipulation

What does it mean to "manipulate" DNA?

Discussion: What are some examples of where we can "manipulate" DNA?

- There have been many advances in this technology over the course of the past century, from DNA fingerprinting to producing synthetic proteins to editing and changing organisms' DNA!

DNA Manipulation

Definition: speed up the rate of chemical reaction

- We require some molecular components taken from cells to be able to perform a lot of these manipulations - enzymes.
  - POLYMERASE
  - ENDONUCLEASES
  - LIGASES

Discussion: Where do we get these enzymes from?

BACTERIA?

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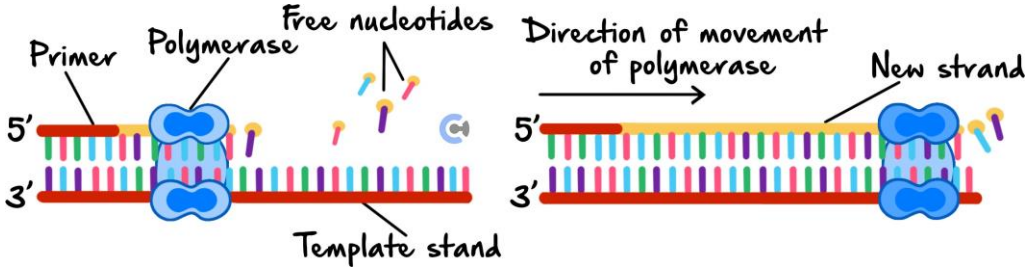
Sub-Section: Polymerases

Do you know any polymerases already?

RNA Polymerase

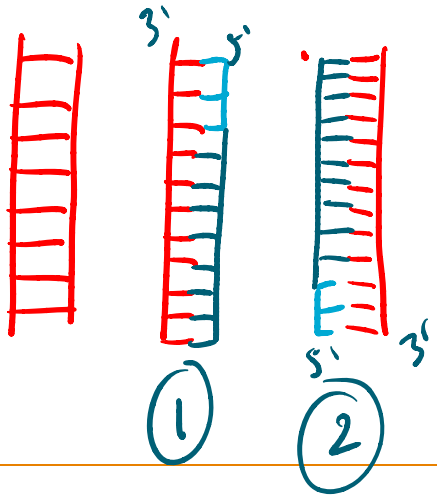
Polymerases

- These are enzymes that are responsible for copying nucleic acid molecules.
- DNA polymerase is used to synthesise DNA strands from existing ones.
  - Initially the DNA must be unzipped; in an actual cell, this is achieved by a helicase enzyme.
  - The polymerase requires a short primers sequence to be able to bind to the DNA strand and then moves along synthesising a complementary strand from the template.



Exploration: DNA replication is referred to as semi-conservative. What does this mean?

- As we replicate DNA, how do we make sure that the message is the same each time?



↳ new DNA molecule is old strand from which a new strand is composed from

NOTE: Polymerase is commonly used to refer to DNA polymerase, whereas RNA is usually specified.

Analogy: Photocopier

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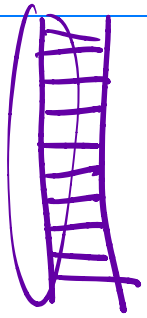
Sub-Section: Endonucleases

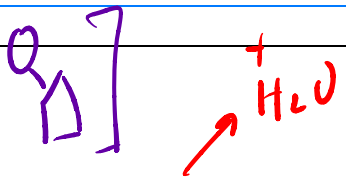
Endonucleases

- Enzymes responsible for cutting DNA strands.
  - They cut DNA at specific sequences called recognition sites.
  - They can form blunt end cuts or sticky end cuts.



Question 1

Explain what bonds are being broken when an endonuclease cuts DNA.



Phosphodiester Bond → hydrolysis

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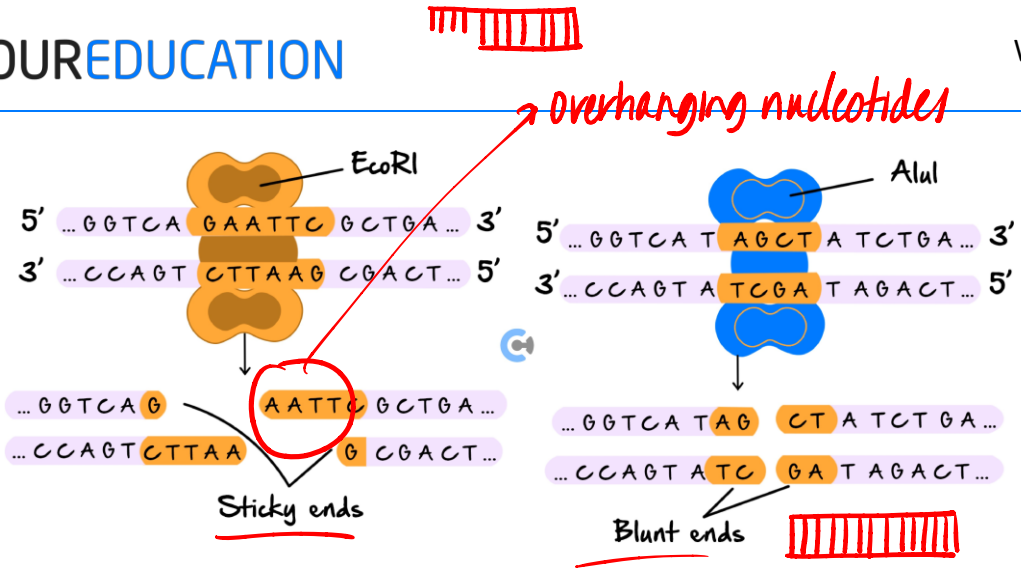


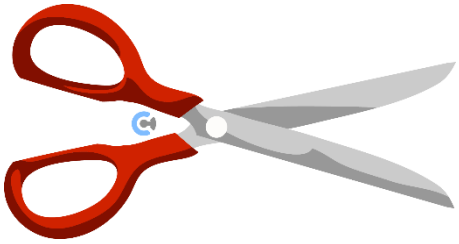
Restriction endonuclease	Recognition sequence (5' to 3' where* is the cut site)
EcorI S	5' G* A A T T C 3' 3' C T T A A *G 5'
Hind III S	5' A* A G C T T 3' 3' T T C G A *A 5'
AluI B	5' A G* C T 3' 3' T C *G A 5'
HaeIII B	5' G G* C C 3' 3' C C *G G 5'

Which ones above are sticky ends and which ones are blunt ends?

NOTE: They can also be referred to as restriction enzymes, and cutting as digestion!

ALSO NOTE: Their names come from the bacteria that they are sourced from!

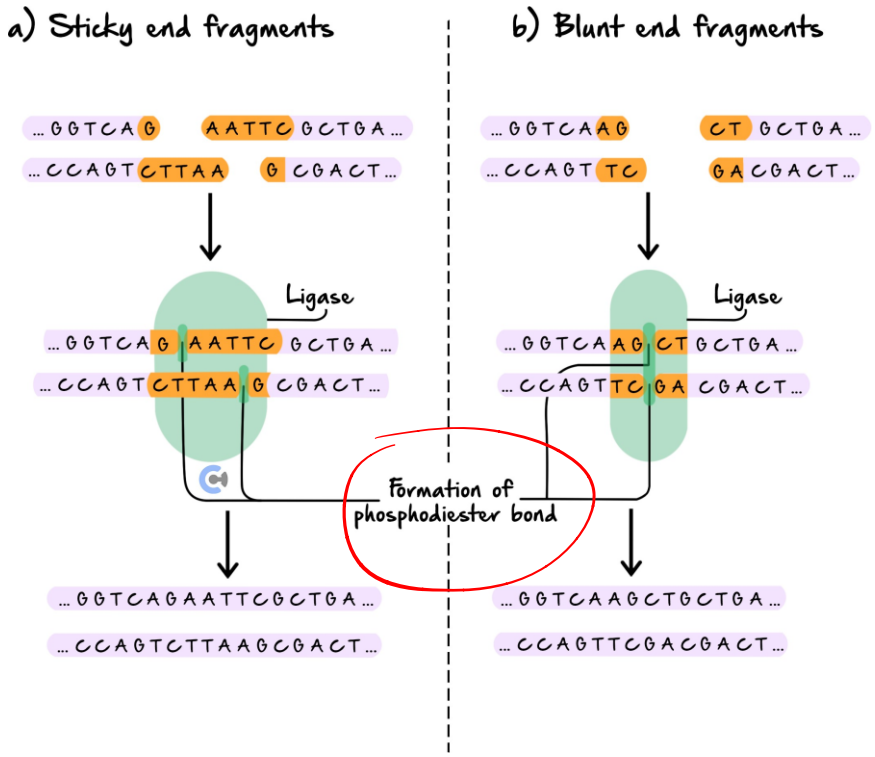
Analogy: Scissors



Sub-Section: Ligases

Ligases

- Enzymes responsible for joining fragments of DNA together.



Analogy: Glue

What bonds are being reformed here? What reaction is taking place?

Exploration: Understanding the uses of sticky ends and blunt ends

- Are sticky ends better, or blunt ends? Explain.

Sticky end fragments are better as they have greater specificity when joining fragments together. This is because the overhanging nucleotides of each fragment MUST be complementary to each other.

Summary

Enzyme	Action	Diagram
Endonuclease	CUT	
Ligase	JOIN	
Polymerase	COPY	

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Question 2 (1 mark)

EcoR1 is a tool used in the 'cutting' manipulation of DNA.

EcoR1 is a:

A. Polymerase enzyme from a bacterium.

B. Ligase enzyme from a bacterium.

C. Restriction enzyme from a bacterium.

D. Reverse transcriptase enzyme from a bacterium.

Another name for endonuclease is restriction enzyme

Question 3 (1 mark)

endonuclease

If PSTI (the molecule that was used in the example in the diagram above to carry out the process) was used for another sample and three fragments were formed, a total of:

A. Six recognition sites were recognised.

B. One recognition site was recognised.

C. Three recognition sites were recognised.

D. Two recognition sites were recognised.

Question 4 (1 mark)

Biotechnologists use a variety of enzymes for their work. These include ligase, polymerase, and endonuclease.

Their functions are:

	Ligase	Polymerase	Endonuclease
A.	pasting	replicating	cutting
B.	replicating	pasting	cutting
C.	cutting	replicating	pasting
D.	pasting	cutting	replicating

Key Takeaways

DNA Manipulation

- **Molecular components (enzymes)** are used for DNA manipulation.
  - Types of enzymes:
    - **Polymerases** (e.g., DNA polymerase, RNA polymerase)
    - **Endonucleases** (e.g., restriction enzymes)
    - **Ligases**

Polymerases

- Polymerases are enzymes responsible for copying nucleic acid strands.
- DNA polymerase synthesises DNA strands using an existing template:
  - DNA must first be unzipped by helicase.
  - The enzyme requires a **primer** to bind and begin synthesising a complementary strand.
- DNA replication is referred to as **semi-conservative** because each new DNA molecule contains one original strand and one newly synthesised strand. This process ensures the genetic message remains accurate and minimises errors.
- **Analogy**: Acts like a photocopier, duplicating genetic material.

Endonucleases

- Endonucleases are enzymes responsible for cutting DNA strands at specific sequences called recognition sites:
  - They can produce **sticky ends** (overhanging sequences) or **blunt ends** (straight cuts).
- Sticky ends are often preferred because their overhanging sequences can form complementary base pairings, making it easier to join fragments accurately.
- These enzymes break **phosphodiester bonds** in the DNA backbone.

- **Examples of restriction enzymes**:
  - EcoRI: Creates sticky ends.
  - AluI and HaeIII: Create blunt ends.
- **Analogy**: Works like scissors, cutting DNA at precise locations.

Ligases

- Ligases are enzymes responsible for joining DNA fragments together:
  - They reform **phosphodiester bonds** to create a continuous DNA strand.
  - Can join both sticky and blunt ends, although sticky ends are easier to work with due to complementary overhangs.
- During this process, ligases facilitate the formation of new bonds, effectively sealing the DNA fragments.
- **Analogy**: Functions like glue, sealing DNA fragments into a continuous strand.

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Section B: Polymerase Chain Reaction

Overview

- Polymerase Chain Reaction (PCR) is used to "amplify" samples of DNA.
  - What do we mean by "amplifying" DNA?
    - ↳ making more copies of DNA → increasing size of DNA sample
- Involves repetitive cycles, which can increase the amount of DNA in a very short amount of time.

Sub-Section: Process of PCR

What are the materials required for PCR?

PCR component	Purpose
DNA sample	the template from which multiple copies of DNA are replicated
Primers	short sequence that binds to 3' end of template strand of DNA. Allows polymerase to bind and therefore replicate the DNA molecule.
DNA polymerase / Taq polymerase	HEAT RESISTANT POLYMERASE
Free nucleotides (dNTPs – deoxyribonucleotide triphosphate)	used to synthesise the DNA strands during replication

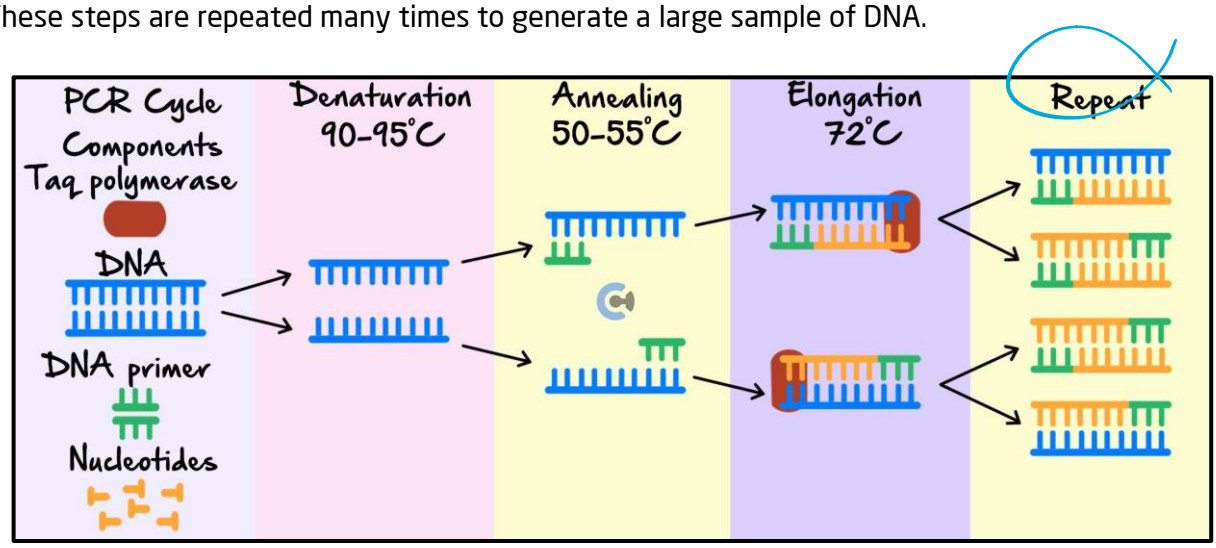
Mix buffer	maintain stability
PCR tube	

What are the steps of PCR?

The Process

- Essentially this requires cycling the DNA alongside a buffer solution, moving it along different temperatures.
- Denaturing - 90-95°C
  - This is to break the two strands apart - performing the role that helicase would be in a natural setting. Hydrogen bonds between the complementary bases will break apart, separating the strands.
- Annealing - 50-55°C
  - Lowering the temperature keeps the strands separate whilst allowing primers to anneal to the DNA strands. They bind via the 3' end, and will allow the polymerase to begin replication.
- Elongation - 72°C
  - Raised temperature allows a heat-resistant polymerase (*TAQ* polymerase) to function optimally and replicate the DNA, extending the primers until the end of the sample or a termination sequence. 72°C is the optimal temperature for *TAQ* polymerase.

- These steps are repeated many times to generate a large sample of DNA.



TIP: Follow the same framework for answering questions!

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Sample Response:

- Denaturing - 90-95°C

This is to break the two strands apart - performing the role that helicase would be in a natural setting. Hydrogen bonds between the complementary bases will break apart, separating the strands.

- Annealing - 50-55°C

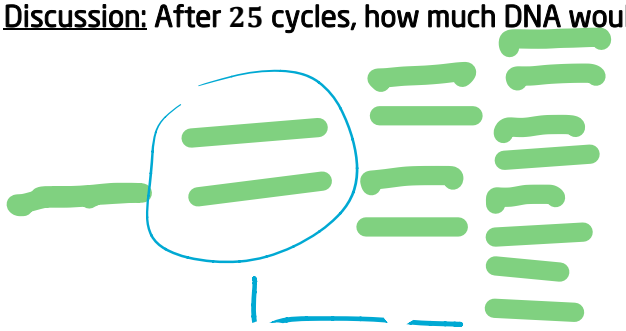
Lowering the temperature keeps the strands separate whilst allowing primers to anneal to the DNA strands. They bind via the 3' end and will allow the polymerase to begin replication.

- Elongation - 72°C

Raised temperature allows a heat-resistant polymerase (*TAQ* polymerase) to function optimally and replicate the DNA, extending the primers until the end of the sample or a termination sequence. 72°C is the optimal temperature for *TAQ* polymerase.

- These steps are repeated many times to generate a large sample of DNA.

Discussion: After 25 cycles, how much DNA would we have?

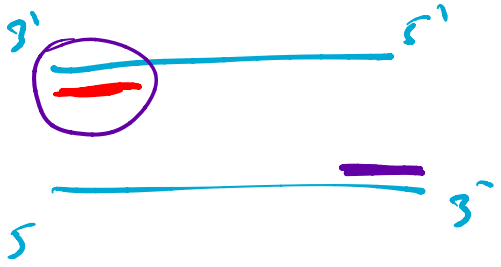


2^n = number of cycle

33mil

Discussion: Will the primers for each strand be the same?

- If yes, why? If not, why not? Use a diagram to help you!



2 types
- forwards
- reverse

the 3' end of each strand is different

Key Takeaways

- PCR is used to **amplify** samples of DNA, meaning to create multiple copies of a specific DNA segment. It involves repetitive cycles that significantly increase the amount of DNA in a very short amount of time.
- The materials required for PCR include:
  - **DNA sample**: Provides the template to produce copies.
  - **Primers**:
    - **The forward primer** binds to the start of the DNA segment.
    - **The reverse primer** binds to the complementary strand at the opposite end. These primers designate the sequence to be copied and provide a starting point for DNA synthesis.
  - **Taq polymerase**: A heat-resistant enzyme that makes multiple copies of the DNA strand by adding nucleotides.
  - **Free nucleotides (dNTPs)**: Added by Taq polymerase to produce the new DNA strand.
  - **Mix buffer**: Maintains the appropriate pH and provides the required salts for Taq polymerase activity.
  - **PCR tube**: Serves as the vessel for the reaction, containing all the components.
- The process of PCR involves the following steps:
  1. **Denaturing (90–95°C)**: The two DNA strands are broken apart, performing the role that helicase would in a natural setting. Hydrogen bonds between the complementary bases are broken, separating the strands.
  2. **Annealing (50–55°C)**: Lowering the temperature keeps the strands separate while allowing primers to anneal to the DNA strands. The primers bind at the 3' end, allowing the polymerase to begin replication.
  3. **Elongation (72°C)**: The raised temperature allows a heat-resistant polymerase (Taq polymerase) to function optimally. It extends the primers, replicating the DNA until the end of the sample or a termination sequence. 72°C is the optimal temperature for Taq polymerase.
- These steps are repeated many times (typically 20– 40 cycles) to generate a large sample of DNA.

Section C: Gel Electrophoresis

How can we analyse DNA samples?

Overview

- Gel electrophoresis is a method by which DNA fragments can be sorted and separated based on size, as DNA moves through a gel after a current has been applied.
- Used typically after a sample has been cut up by a restriction enzyme.

Discussion: What could be the purpose of gel electrophoresis?

Question 5 (1 mark)

Why does DNA move through the gel when a charge is applied?

negatively charged → attracted to positive when current applied

→ PHOSPHATE

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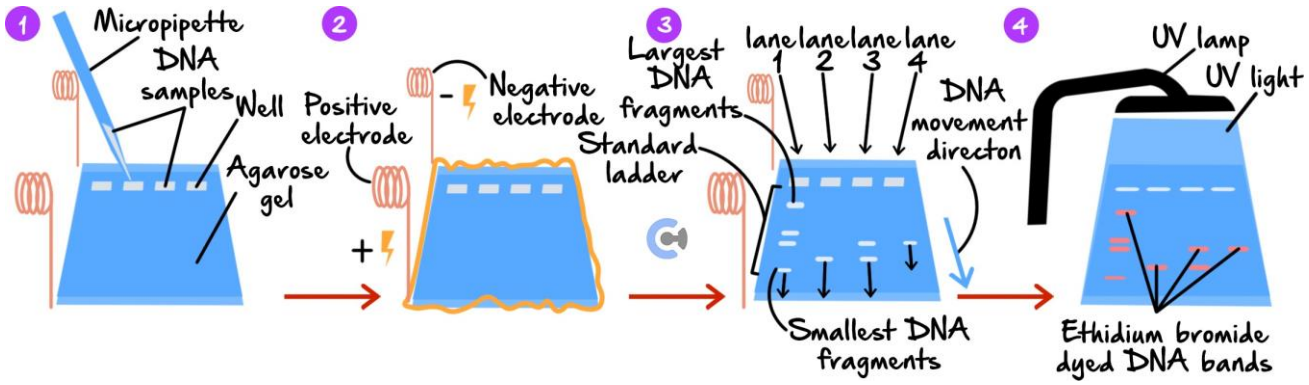
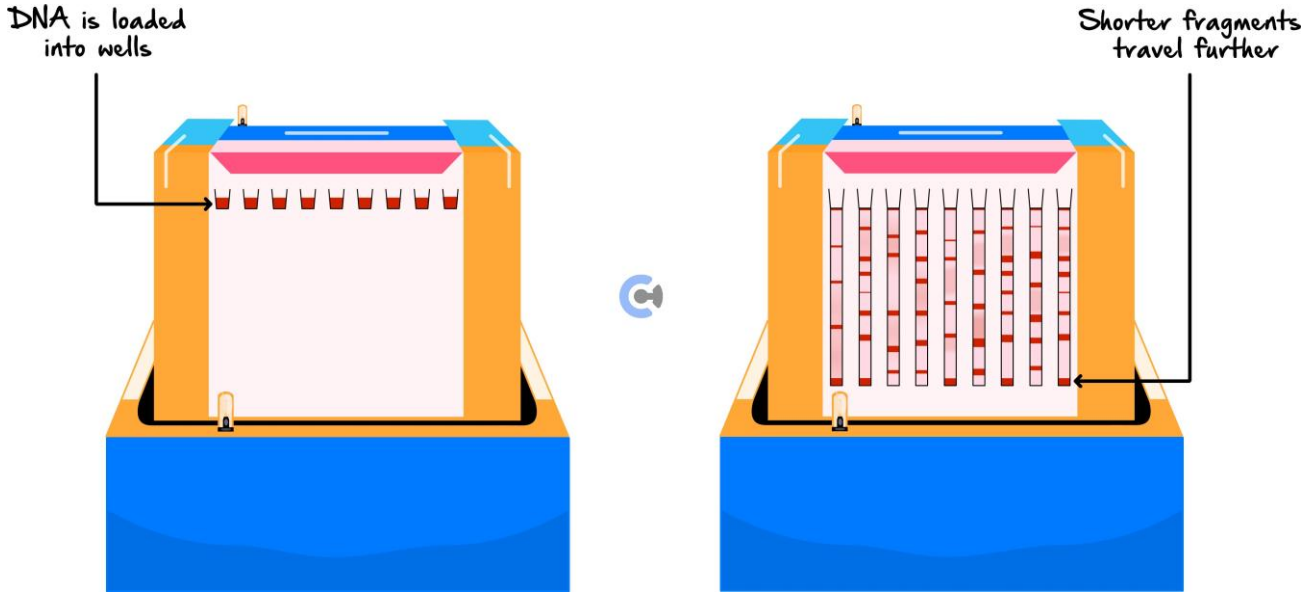
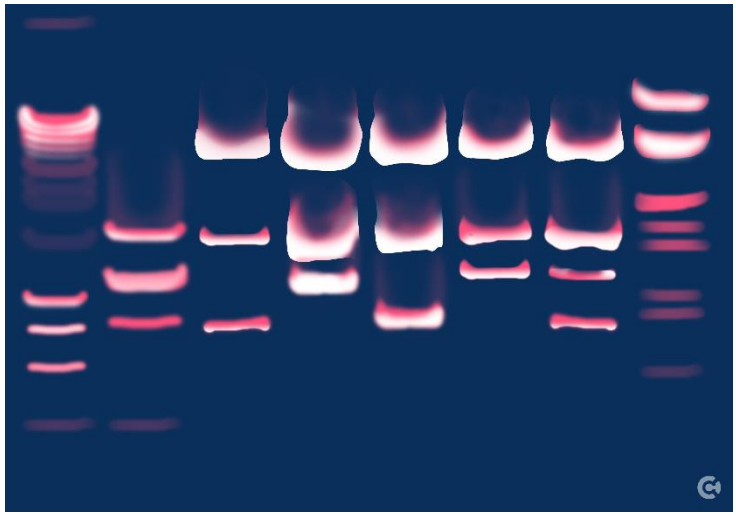
Sub-Section: The Process

The Process

- Requires a few components – agarose gel, buffer solution (ionic), electrodes, stain
- Can be described through 4 steps:
  - DNA samples are placed at 1 end of the sample in small holes called wells in the agarose gel. One of the wells includes fragments of known sizes (standard ladder).
  - Gel is immersed in a buffer solution that contains ions allowing for the conductance of charge, and an electric current is passed using 2 electrodes, and the negative one at the end with the wells. When the current is applied, the DNA will move from the wells to the positive electrode.
  - Smaller fragments will move faster, whilst larger ones will move slower, so when the current is switched off, they will have moved different distances and therefore will have separated.
  - A dye is applied to visualise the results – commonly ethidium bromide. The DNA fragments and sample appear as fluorescent bands under UV light.







NOTE: You will rarely have to ever describe the full process, but you need to relate the theory of what happens to the information that we gain from it in clinical applications!

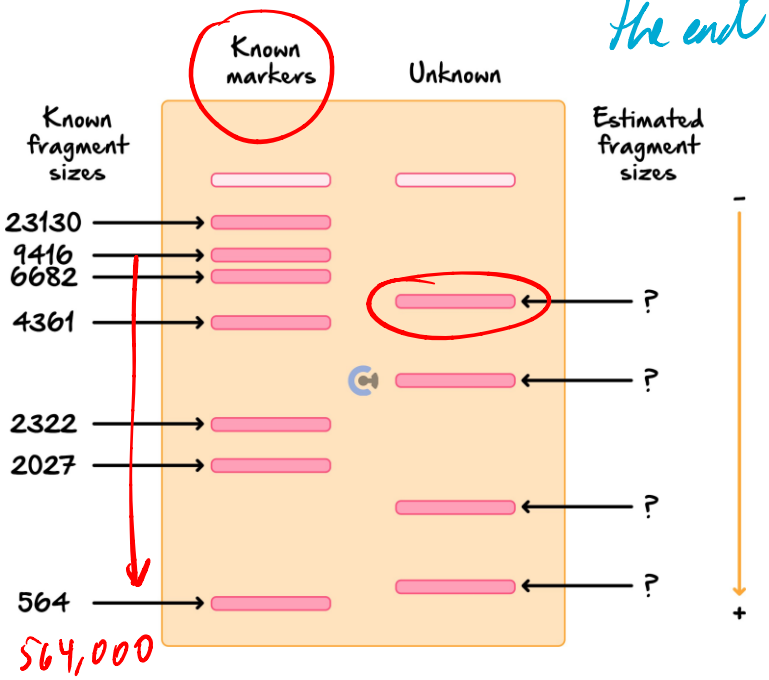
Exploration: Why are standard ladders important?

↳ a sample of DNA with known fragment size which can be used to help estimate unknown fragment size!

- What factors impact the distance travelled in a gel?

SIZE → larger fragments more slower
→ smaller fragment are faster
CONCENTRATION OF SOLUTION → higher conc = faster
DENSITY OF THE GEL – more dense = slower
CURRENT/VOLTAGE = more current = faster
TIME – more time longer → if you leave it too long all fragments reach the end

- Looking at the results of a gel experiment!



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Question 6 (5 marks)

In 1991, the body of a man was found frozen beneath a glacier in Italy. Researchers named him Ötzi. It was determined that Ötzi died 5300 years ago and that his body is the oldest mummified human body ever found. Scientists have successfully extracted DNA from the nucleus of his frozen cells.

a. Describe the process scientists would use on a small sample of Ötzi's DNA to obtain larger quantities of identical DNA. (3 marks)

Polymerase Chain Reaction (PCR)

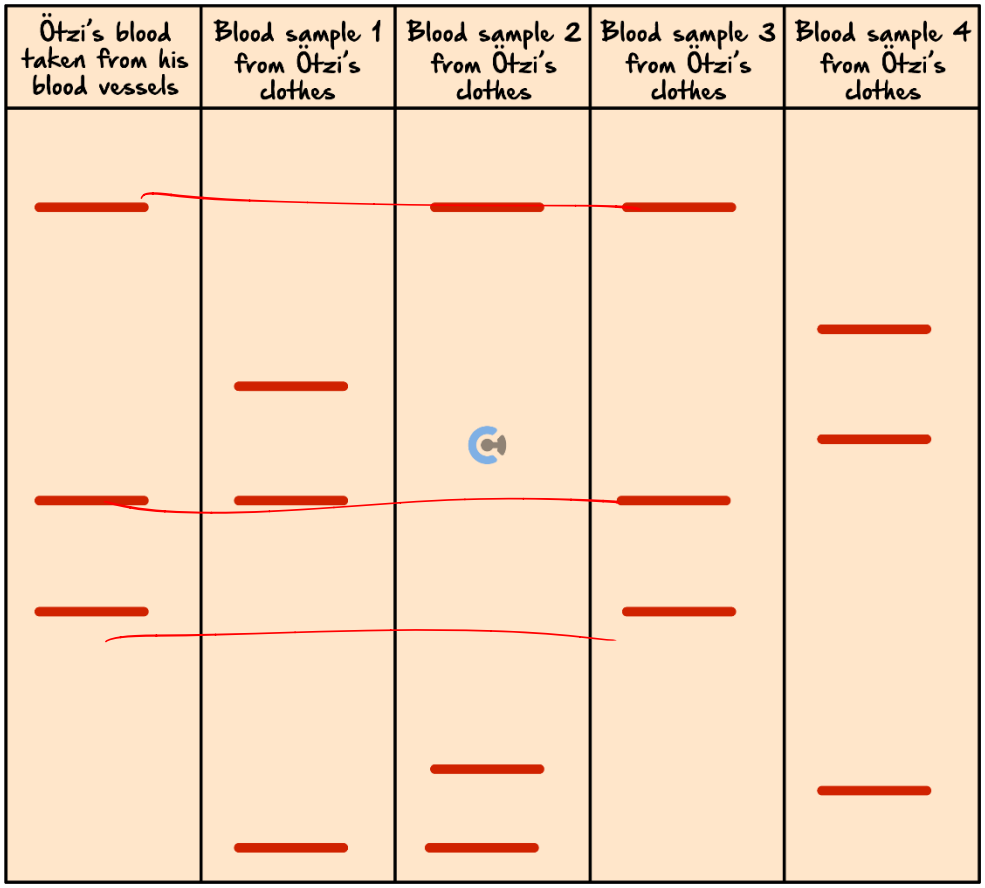
D 90-95°C

A 50-55°C

E 72°C

Repeat!

Using gel electrophoresis, scientists discovered that there were four different types of blood on Ötzi's clothes. Their results were as follows:



b.

i. Which blood sample on Ötzi's clothes belongs to Ötzi? (1 mark)

3

ii. Propose **one** hypothesis that would explain the presence of the other blood samples on Ötzi's clothes. (1 mark)

Hunting
Fight

Key Takeaways

Purpose

- ☑ Gel electrophoresis is used to analyse DNA samples by sorting and separating DNA fragments based on size.
- ☑ Typically performed after DNA has been cut with a restriction enzyme.
- ☑ Allows for size comparison of DNA fragments, visualisation using fluorescent dyes, and separation of DNA based on fragment size.

Why DNA Moves Through the Gel

- ☑ DNA is negatively charged due to its phosphate backbone.
- ☑ When an electric current is applied, DNA moves toward the positive electrode.

Process

1. DNA samples are placed in wells in an **agarose gel**.
   - One well contains a **standard ladder** with fragments of known sizes.
2. The gel is placed in a **buffer solution** that conducts electricity.
   - A negative electrode is at the well end, and a positive electrode is at the opposite end.

3. An electric current is applied, and DNA fragments move through the gel:
   - Smaller fragments move faster.
   - Larger fragments move slower.
4. A dye (commonly **ethidium bromide**) is added to visualise the results under UV light.
   - DNA fragments appear as fluorescent bands.

Importance of Standard Ladders

- ☑ Contain DNA fragments of known sizes.
- ☑ Used to compare and estimate the size of unknown DNA fragments.
- ☑ Specific to experimental conditions for accurate results.

Factors Affecting DNA Movement

- ☑ **Voltage**: Higher voltage increases movement but may distort separation.
- ☑ **Gel Composition**: Denser gels slow larger fragments.
- ☑ **Buffer Concentration**: Affects electrical conductivity and DNA movement.
- ☑ **Time**: Longer runs allow DNA to travel further, but excessive time may cause DNA to move out of the gel.

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Section D: DNA Profiling

Overview

- We can combine these 2 technologies to apply them to DNA profiling – using DNA to identify people. This technique is widely used in forensic science and genetic testing.
- Let's work through how a crime is "solved" to cover this topic.

Sub-Section: DNA Fingerprinting/Profiling

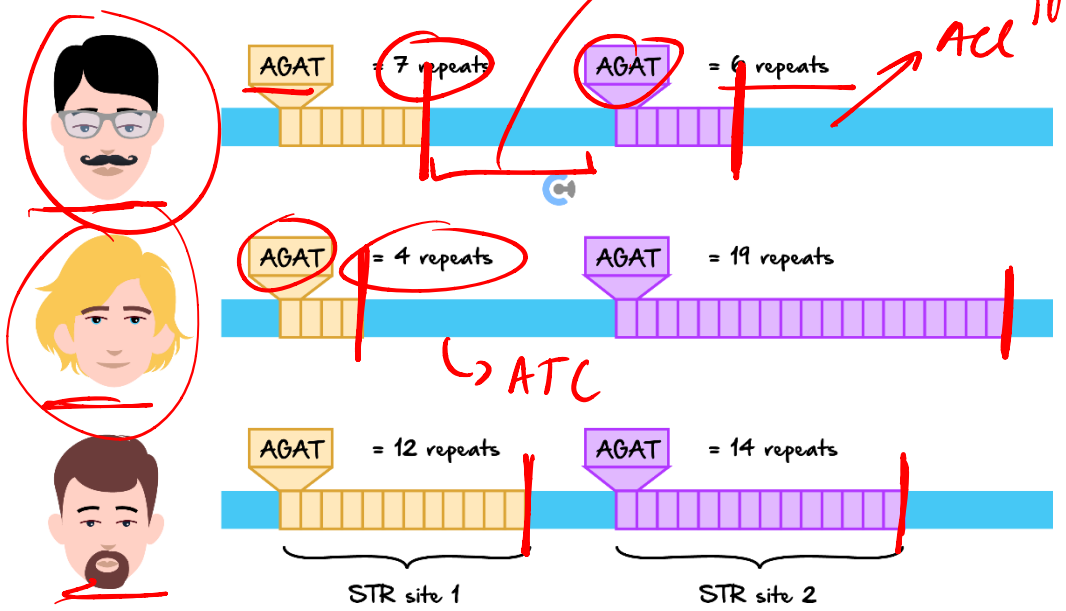
Exploration: What is the first step in every crime scene investigation? What do they find?

1. FIND DNA SAMPLE
2. PCR → amplify the DNA

ATCGACG
ATCGAGT

The Case Continues....

- Everyone's DNA is different, so theoretically, we could compare the entire genome of the sample to our suspects to see if there is a match.
  - Why don't we actually do that? Why don't we compare every letter to see if the DNA matches or is different?
- Instead, what we do is actually focus on the short tandem repeat (STRs) in the DNA- these are small sections of repeated DNA that are found in non-coding regions of chromosomes.
- These STRs are found in satellite DNA which is non-coding.
  - People will have different STRs on different chromosomes. It is very unlikely that all 6-12 chromosomes tested will be a match for two different people.

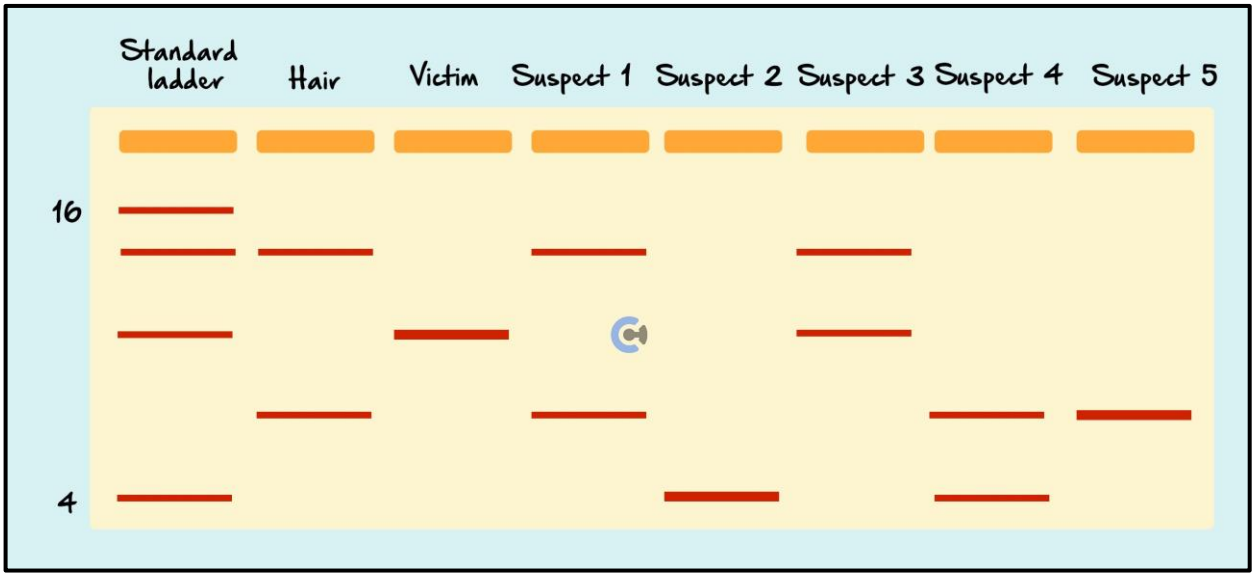


Exploration: How can we actually compare the STRs of a sample with another person?

- Gel Electrophoresis!

- Owing to each person's different STRs, if cut with the same restriction endonuclease, each sample will create fragments of differing lengths.

- Our Results! What do they show?



Exploration: A bit more of an exploration into STRs and Satellite DNA

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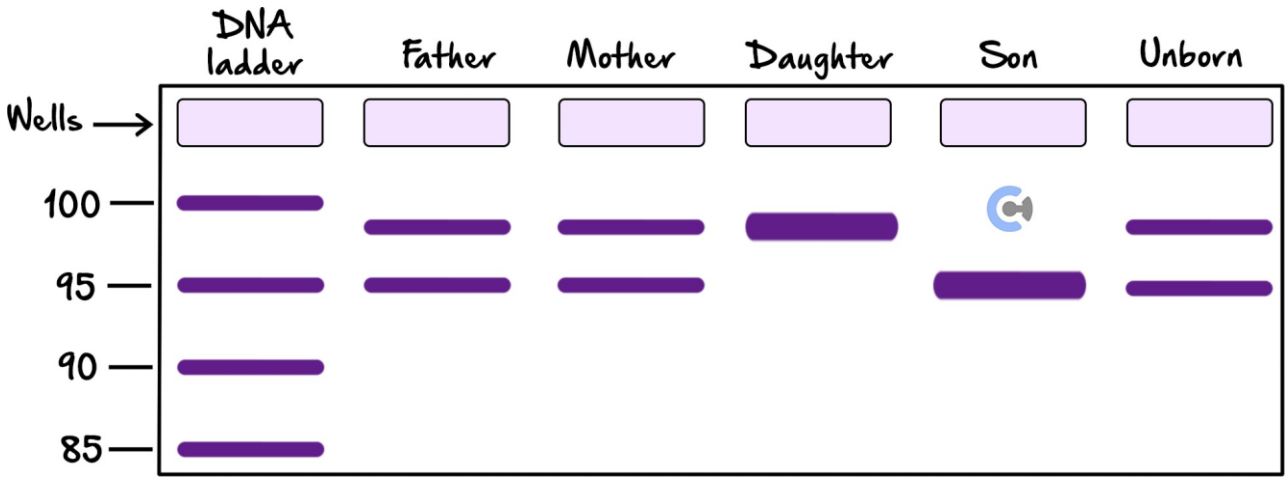
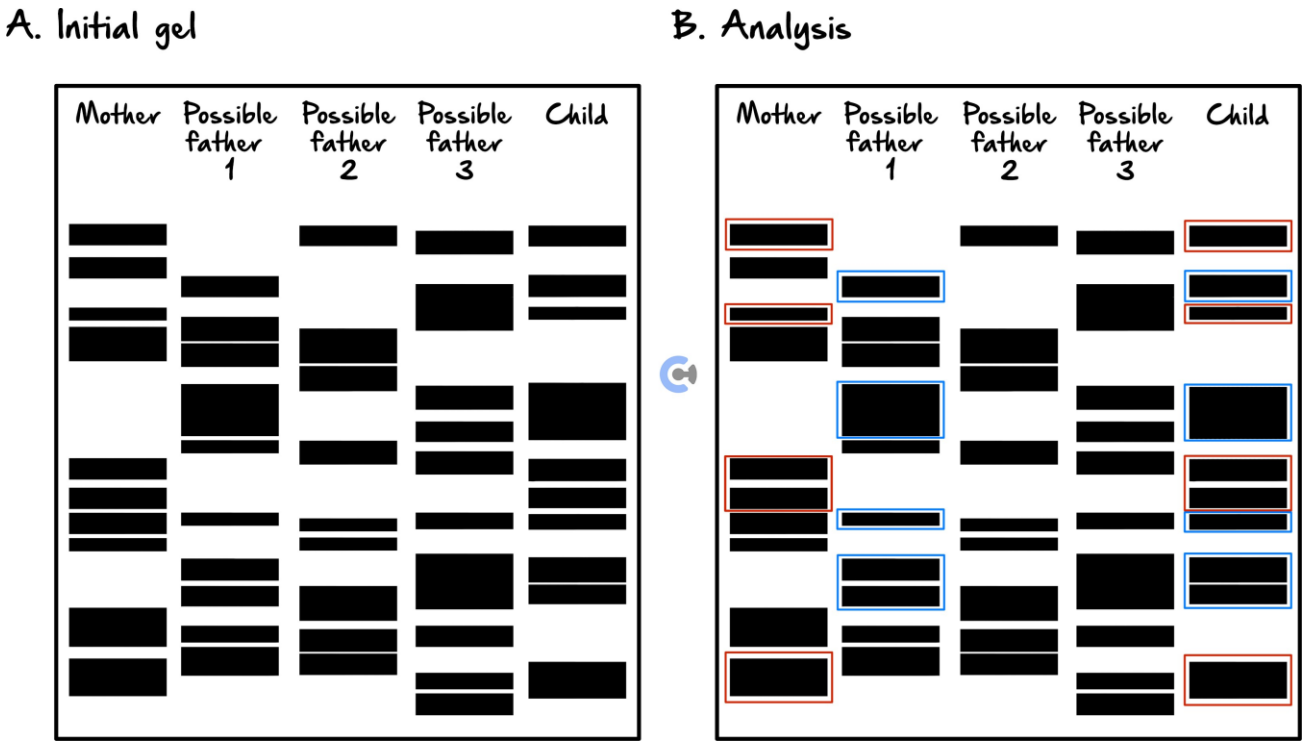
Sub-Section: Genetic Testing

Genetic Testing

- As children get half their chromosomes from each parent, their fragments should be a mix of their parents, i.e., they inherit the fragments of the chromosome they get from their parents.



Ayo, there's something wrong here.....

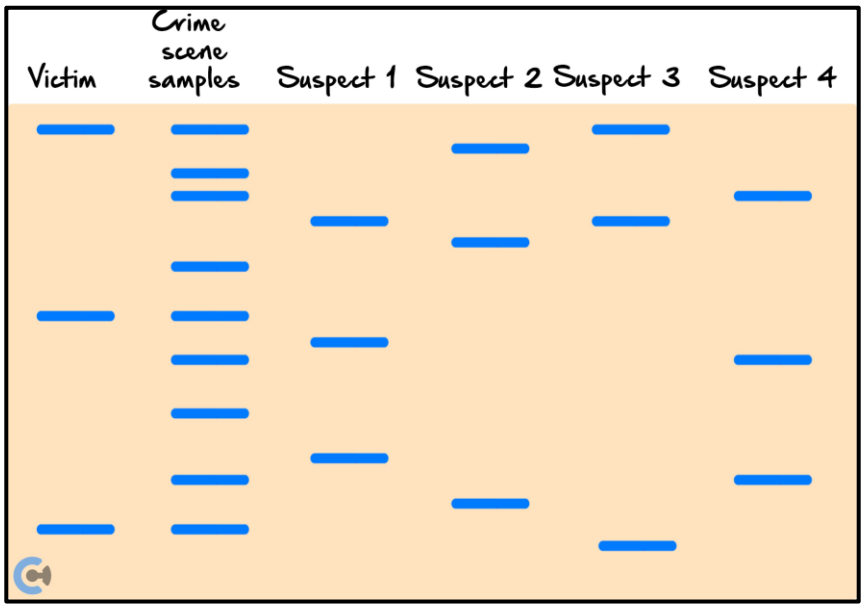


Okay, now that's a little better...

Summary of DNA Profiling

- Involves the combination of 2 techniques, PCR, and Gel Electrophoresis.
  - PCR: To amplify the sample – we can use primers to focus on the STRs.
  - Gel Electrophoresis: To be able to tell the DNA samples apart- different samples cut with the same enzyme, will have different sizes and thus create a different gel.
  - Then we can compare the sample that has been collected, to the sample that has been received from a suspect.

Question 7 (1 mark)



The person most likely to have been at the crime scene is:

A. Suspect 1

B. Suspect 2

C. Suspect 3

D. Suspect 4

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Question 8 (1 mark)

In a disputed paternity case, 3 gene loci were tested and run through a gel. The results of the test are shown in the diagram below:

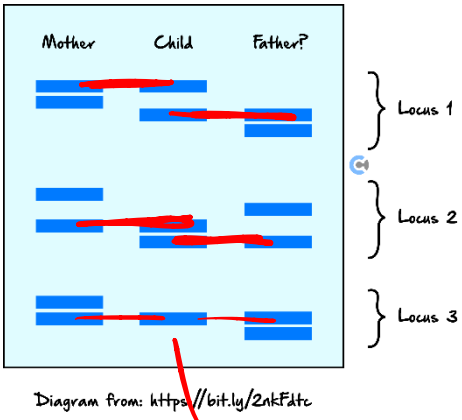

Diagram from: https://bit.ly/2nkFdte

This fragment should be thicker

What conclusion can be made about the paternity case?

A. The male tested is not the father.

B. The male tested is the father.

C. The male tested could be the father, but more gene loci would need to be tested.

D. The child only has 1 copy of gene locus 3.

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Key Takeaways

Overview:

- ☑ DNA profiling combines technologies like **PCR** and **Gel Electrophoresis** to identify individuals using DNA.
- ☑ Widely used in forensic science to solve crimes and in genetic testing to establish relationships.

Process:

- ☑ **DNA Collection**: DNA is collected from a crime scene (e.g., blood, hair, or saliva) and compared to the samples from suspects or victims.

PCR Amplification:

- ☑ The collected DNA is amplified using **PCR** to produce multiple copies of specific DNA regions, particularly **Short Tandem Repeats (STRs)**.
- ☑ STRs are short, repeated DNA sequences found in **non-coding regions (Satellite DNA)**, which vary significantly between individuals.

Digestion with Restriction Enzyme:

- ☑ DNA is cut with the **same restriction enzyme** to ensure consistent fragment sizes across all samples.

Gel Electrophoresis:

- ☑ The cut DNA fragments are separated based on size using gel electrophoresis.
- ☑ STRs create fragments of varying lengths unique to each individual.
- ☑ Fragments are visualised as fluorescent bands under UV light using dyes like ethidium bromide.

Why STRs Are Used:

- ☑ STRs are highly variable between individuals, even within non-coding regions.
- ☑ Testing multiple STR loci ensures reliable identification and reduces the chance of matching unrelated individuals.

Interpreting Results:

- Samples with identical banding patterns indicate a match.
  - For forensic cases:
    - Matching the crime scene sample to a suspect's STR profile can link them to the scene.
  - For genetic testing:
    - Banding patterns confirm familial relationships (e.g., paternity tests).
    - Children inherit STR fragments from both parents, which can be observed in shared patterns.

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Contour Check

Learning Objective: [1.5.1] Identify and describe the function of polymerases, endonucleases, and ligases in DNA manipulation.

Study Design:

The use of enzymes to manipulate DNA, including polymerase to synthesise DNA, ligase to join DNA, and endonucleases to cut DNA.

Key Takeaways:

- **Molecular components (enzymes)** are used for DNA manipulation.
  - Types of enzymes:
    - _____
    - _____
    - _____

Polymerases:

- Polymerases are enzymes responsible for _____.
- DNA polymerase synthesises DNA strands using an existing template:
  - DNA must first be unzipped by _____.
  - The enzyme requires a _____ to bind and begin synthesising a complementary strand.
- DNA replication is referred to as **semi-conservative** because _____

  _____

  _____

  _____
- **Analogy**: _____.

Endonucleases:

- Endonucleases are enzymes responsible for cutting DNA strands at specific sequences called _____:
  - They can produce **sticky ends** (_____) or **blunt ends** (_____).
- Sticky ends are often preferred because

  _____

  _____
- These enzymes break the _____ in the DNA backbone.
- **Examples of restriction enzymes**:
  - EcoRI: Creates sticky ends.
  - AluI and HaeIII: Create blunt ends.
- **Analogy**: _____.

Ligases:

- Ligases are enzymes responsible for joining DNA fragments together.
  - They reform _____ to create a continuous DNA strand.
  - Can join both _____ and _____, although sticky ends are easier to work with due to complementary overhangs.
- During this process, ligases facilitate the formation of new bonds, effectively sealing the DNA fragments.
- **Analogy**: _____.

Learning Objective: [1.5.2] Identify the ingredients required, describe the process, and recall key applications of PCR.

Study Design:
Amplification of DNA using polymerase chain reaction, and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling.

Key Takeaways:

- PCR is used to **amplify** samples of DNA, meaning to create multiple copies of a specific DNA segment. It involves repetitive cycles that significantly increase the amount of DNA in a very short amount of time.
- The materials required for PCR include:
  - **DNA sample**: Provides the template to produce copies.
  - **Primers**:
    - _____ binds to the start of the DNA segment.
    - _____ binds to the complementary strand at the opposite end. These primers designate the sequence to be copied and provide a starting point for DNA synthesis.
  - _____ A heat-resistant enzyme that makes multiple copies of the DNA strand by adding nucleotides.
  - _____ Added by Taq polymerase to produce the new DNA strand.
  - _____: Maintains the appropriate pH and provides the required salts for Taq polymerase activity.
  - _____: Serves as the vessel for the reaction, containing all the components.

- The process of PCR involves the following steps:

1. **Denaturing (90-95°C)**: The two DNA strands are broken apart, performing the role that helicase would in a natural setting.

   _____

   _____

2. **Annealing (_____)**: Lowering the temperature keeps the strands _____, while allowing primers to anneal to the DNA strands. The primers bind at the _____, allowing polymerase to begin replication.

3. **Elongation (72°C)**:

   _____

   _____

   _____

   _____

- These steps are

  _____

Learning Objective: [1.5.3] Describe the process of gel electrophoresis, and describe how it may be used to differentiate DNA samples or to obtain a "DNA profile".

Study Design:
Amplification of DNA using polymerase chain reaction, and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling.

Key Takeaways:

- **Purpose**:
  - Gel electrophoresis is used to analyse DNA samples by sorting and separating DNA fragments based on size.
  - Typically performed after DNA has been cut with a restriction enzyme.
  - Allows for size comparison of DNA fragments, visualisation using fluorescent dyes, and separation of DNA based on fragment size.
- **Why DNA Moves Through the Gel**:
  - DNA is negatively charged, due to its _____.
  - When an electric current is applied, DNA moves towards the _____ electrode.
- **Process**:
  1. DNA samples are placed in wells in an _____.
     - One well contains a **standard ladder** with fragments of _____.
  2. The gel is placed in a _____ that conducts electricity.
     - A _____ electrode is at one end of the well, and a _____ electrode is at the opposite end.

3. An electric current is applied, and DNA fragments move through the gel:
   - ☐ Smaller fragments move _____.
   - ☐ Larger fragments move _____.
4. A dye (**ethidium bromide**) is added to visualise the results under UV light.
   - ☐ DNA fragments appear as _____ bands.

☐ **Importance of Standard Ladders**:

- _____
- _____
- _____

Learning Objective: [1.5.4] Explain the factors that affect the movement of fragments in gel electrophoresis.

Study Design:
Amplification of DNA using polymerase chain reaction, and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling.

Key Takeaways:

☐ **Factors Affecting DNA Movement:**

- _____
- _____
- _____
- _____

Learning Objective: [1.5.5] Define satellite DNA and STRs, and explain their use in identifying people through DNA profiling for crimes and paternity testing.

Study Design:
Amplification of DNA using polymerase chain reaction, and the use of gel electrophoresis in sorting DNA fragments, including the interpretation of gel runs for DNA profiling.

Key Takeaways:

- **Overview**:
  - DNA profiling combines technologies like **PCR** and **Gel Electrophoresis** to identify individuals using DNA.
  - Widely used in forensic science to solve crimes and in genetic testing to establish relationships.
- **Process**:
  1. **DNA Collection**: DNA is collected from a crime scene (e.g., blood, hair, or saliva) and compared to samples from the suspects or victims.
  2. **PCR Amplification**:
     - The collected DNA is amplified using _____ to produce multiple copies of specific DNA regions, particularly **Short Tandem Repeats (STRs)**.
     - STRs are short, repeated DNA sequences found in _____, which vary significantly between individuals.
  3. **Digestion with Restriction Enzyme**:
     - DNA is cut with the _____ to ensure consistent fragment sizes across all samples.

4. **Gel Electrophoresis**:
   - The cut DNA fragments are separated based on size using gel electrophoresis.
   - STRs create fragments of _____ lengths unique to each individual.
   - Fragments are visualized as fluorescent bands under UV light using dyes like ethidium bromide.

- **Why STRs Are Used**:
  - STRs are highly variable between individuals, even within non-coding regions.
  - Testing multiple STR loci ensures reliable identification, and reduces the chance of matching unrelated individuals.
- **Interpreting Results**:
  - Samples with _____ indicate a match.
  - For forensic cases:
    - Matching the crime scene sample to a suspect's STR profile can link them to the scene.
  - For genetic testing:
    - Banding patterns confirm familial relationships (e.g., paternity tests).
    - Children inherit STR fragments _____, which can be observed in shared patterns.