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

Outline:

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

- We require some molecular components taken from cells to be able to perform a lot of these manipulations – enzymes.
  - Polymerases
  - Ligases
  - Endonucleases

Discussion: Where do we get these enzymes from?

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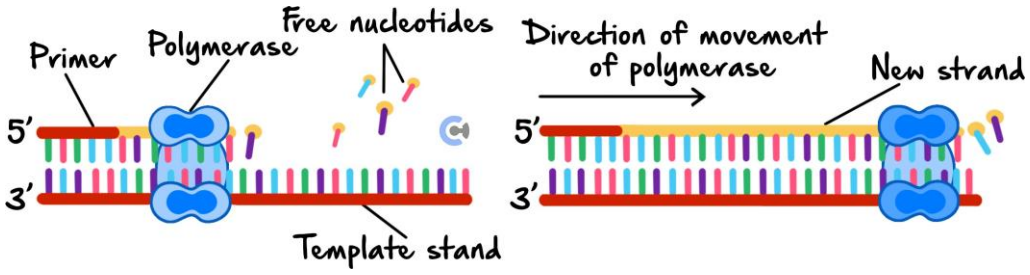
Sub-Section: Polymerases

Do you know any polymerases already?

Polymerases

- These are enzymes that are responsible for copying nucleic acid strands.
- 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 primer 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?

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.

When an endonuclease cuts DNA, the phosphodiester bonds between the sugar and phosphate groups in the DNA backbone are broken.

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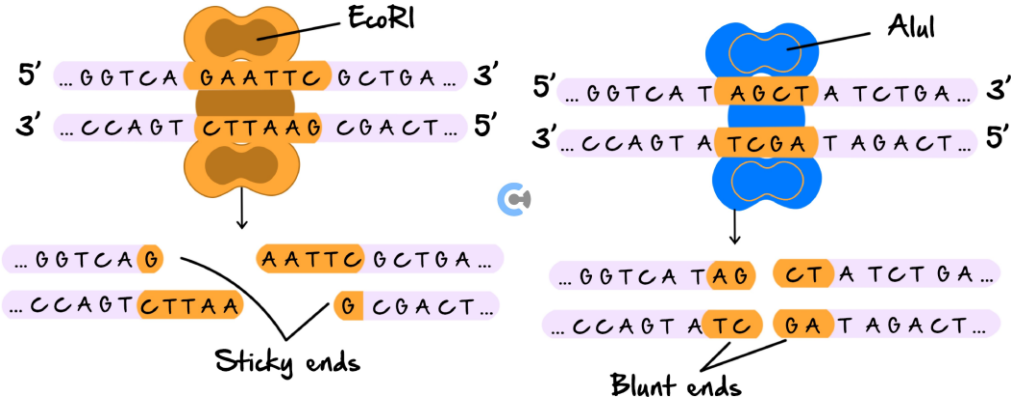


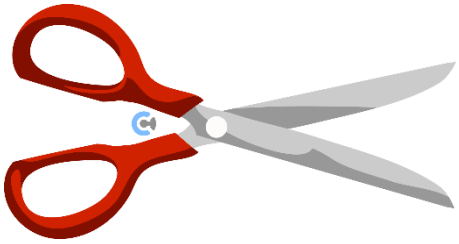
Restriction endonuclease	Recognition sequence (5' to 3' where* is the cut site)
EcorI	5' G* A A T T C 3' 3' C T T A A *G 5'
Hind III	5' A* A G C T T 3' 3' T T C G A *A 5'
AluI	5' A G* C T 3' 3' T C *G A 5'
HaeIII	5' G G* C C 3' 3' C C *G G 5'

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!

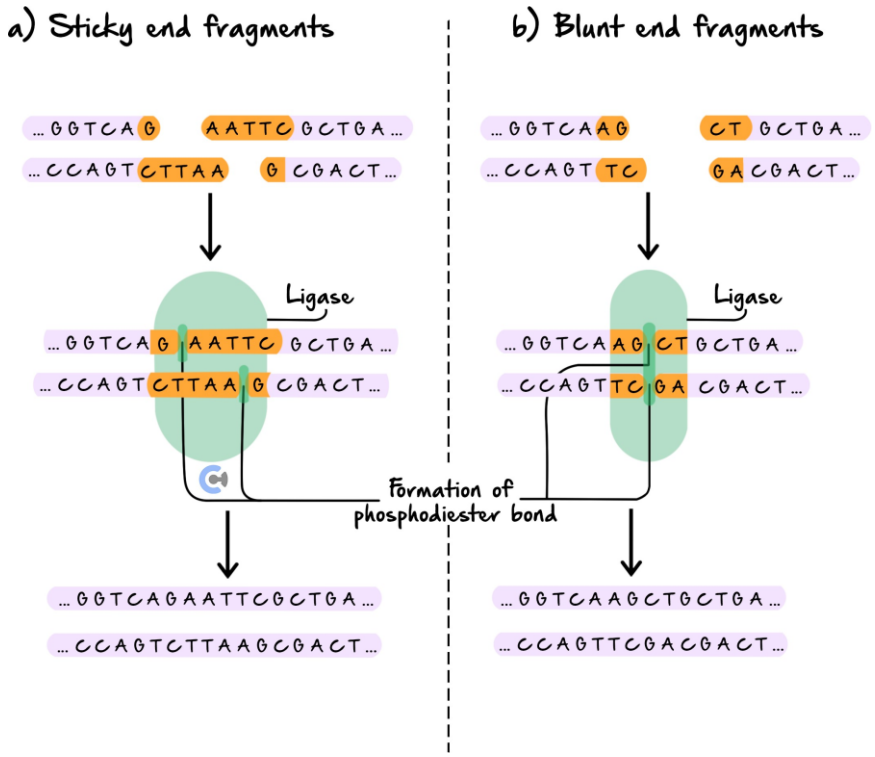
Analogy: Scissors



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.

_____

_____

_____

Summary

Enzyme	Action	Diagram
Endonuclease		
Ligase		
Polymerase		

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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.

Explanation: EcoRI from *E. coli* is used in cutting desired lengths of DNA and is a restriction enzyme. A polymerase enzyme is used in the polymerase chain reaction (PCR),and ligase enzymes are used in joining pieces of DNA. Reverse transcriptase is used in making single-strand lengths of DNA.

Question 3 (1 mark)

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.

Explanation: One recognition site is required for producing two strands of DNA. If three fragments are formed, then two recognition sites were recognised by PSTI.

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	
D.	pasting	cutting	

With DNA technology, restriction enzymes (also called endonucleases) are used to cut DNA in specific recognition sites. Occasionally DNA needs to be reconnected (pasted), and ligase enzymes are used to form a covalent bond between nucleotides. Polymerase connects free nucleotides onto a single-stranded DNA template.

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?
- 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	To provide a template to produce copies of in PCR.
Primers	To bind to the single-stranded DNA and to provide a point in which DNA synthesis can be initiated and designate the sequence to be copied.
Taq polymerase	To make multiple copies of the DNA strand by adding nucleotides.
Free nucleotides (dNTPs – deoxyribonucleotide triphosphate)	To be added by *Taq* polymerase to produce the new DNA strand.

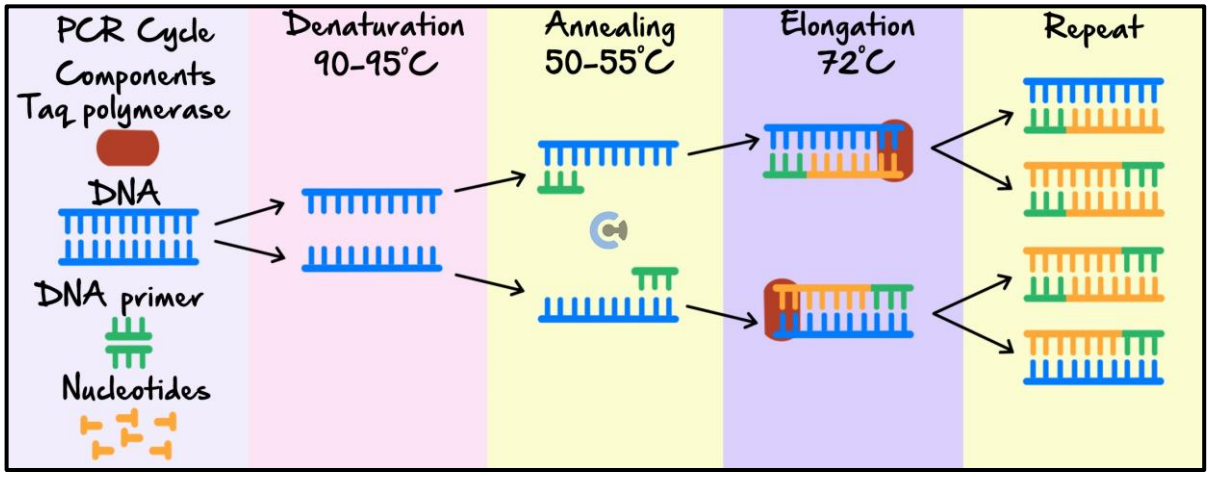
Mix buffer	To provide a suitable chemical environment for the activity of *Taq* polymerase by maintaining the appropriate pH and providing any required salts
PCR tube	To provide a vessel for the PCR reaction to occur. The tube will contain the DNA sample, polymerase, primers, nucleotides, and buffer.

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?

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

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

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?

DNA moves through the gel because it has a negative charge due to its phosphate backbone, which is attracted to the positive electrode.

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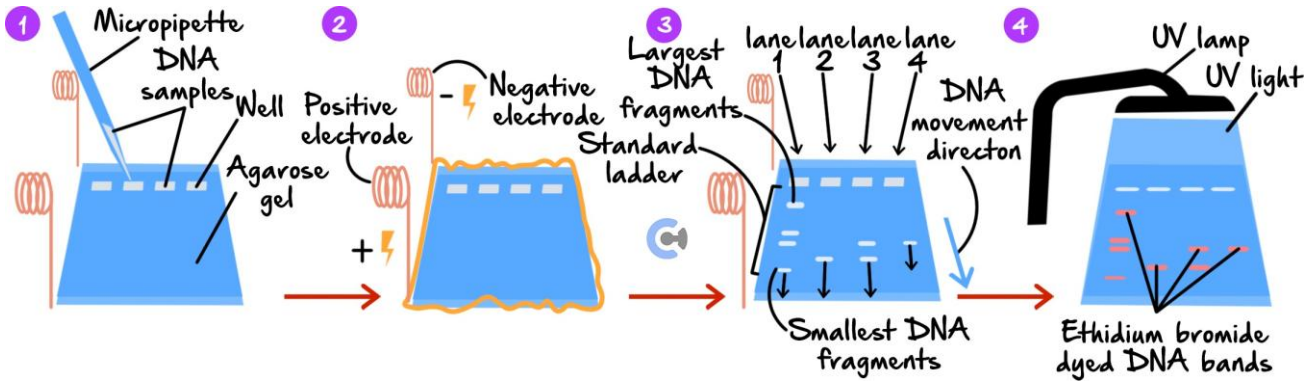
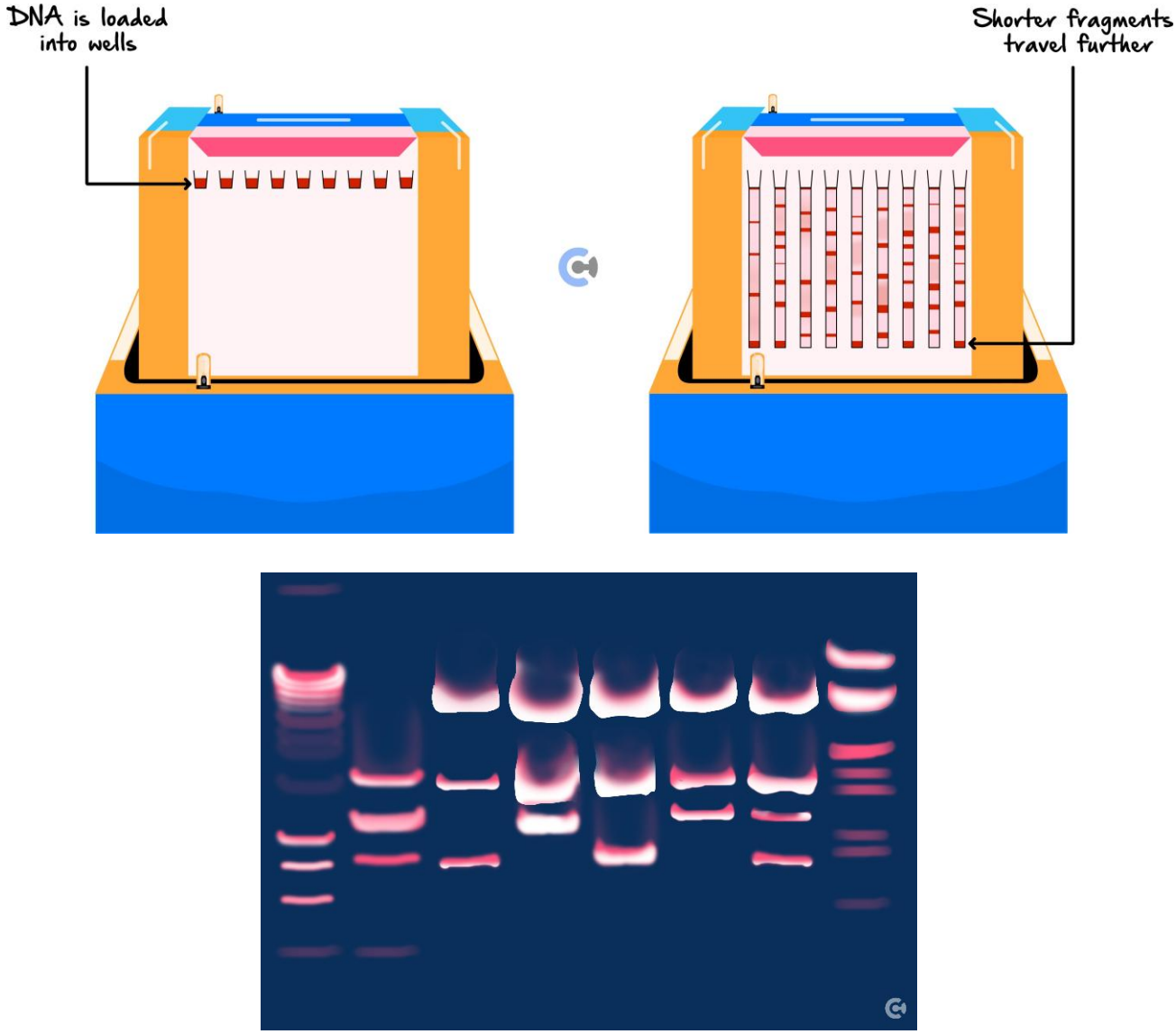
Sub-Section: The Process

The Process

- Requires a few components – agarose gel, buffer solution, stain, DNA sample.
- 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?

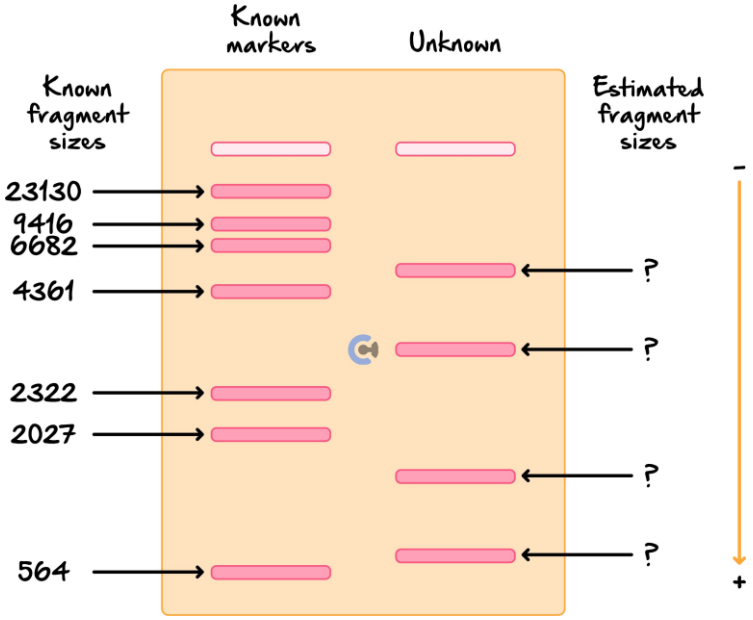
- They contain known fragment sizes, so we can compare the unknown fragments by looking at the relative positions to the bands.
- They are specific to a certain set of conditions – in one gel the same fragment may move $5cm$ whereas in another, they may move $6\ cm$.

- What factors impact the distance travelled in a gel?

- **Voltage**: The stronger the electric force generated by the electrodes, the further DNA travels towards the positive electrode.
- **Gel composition**: Gels with a greater density and agarose concentration increase the difficulty for larger fragments to move through.
- **Buffer concentration**: The greater the concentration of the ions in the buffer, the more the electric current is conducted through the gel, which causes DNA to move further down the lane.
- **Time**: The longer the electric current is applied, the further the DNA will travel.

  Note: If too much time passes, the DNA may move out of the gel.

- Looking at the results of a gel experiment!



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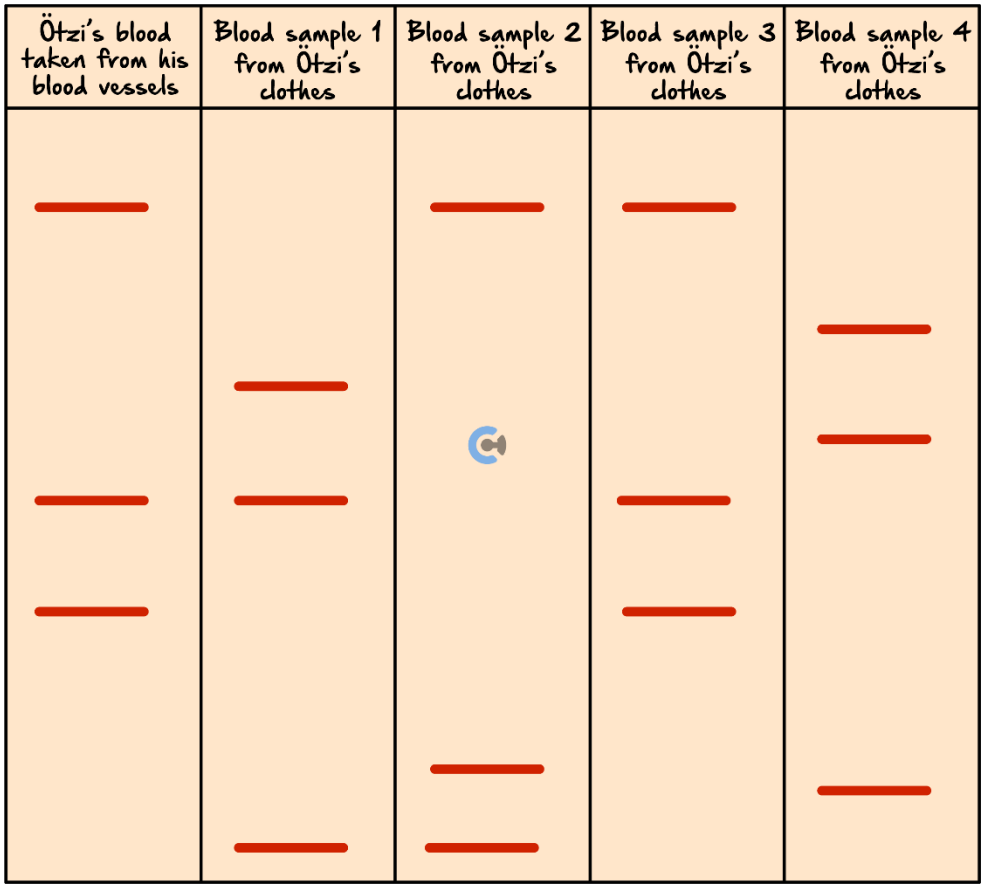
Question 6 (5 marks)

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)

A Good Response included the following steps:
- Heat DNA to approximately 90°C.
- Cool to attach primers.
- Taq polymerase copies strands.
- Process is repeated many times.

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)

Sample 3

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

Blood samples came from other people or from animals.

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.

Space for Personal Notes

Section D: DNA Profiling

Overview

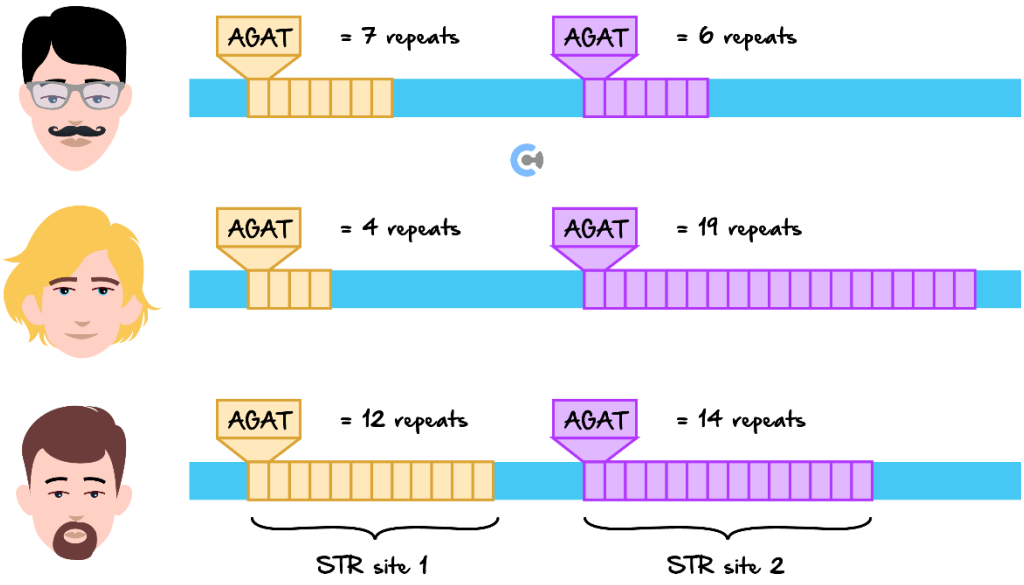
- We can combine these 2 technologies to apply them to DNA profiling – using DNA to identify people. This technique is widely used in forensic science and genetic testing.
- Let's work through how a crime is "solved" to cover this topic.

Sub-Section: DNA Fingerprinting/Profiling

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

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 repeats ___ (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.

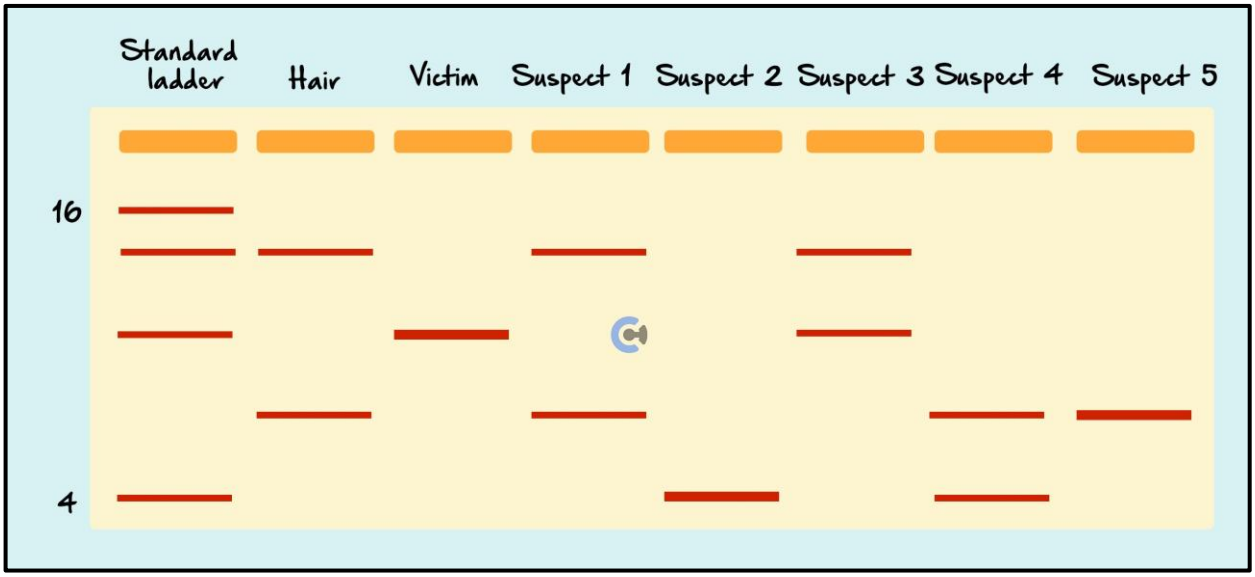


Exploration: How can we actually compare the STRs of a sample with another person?

- 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

- Definition of STRs: STRs are regions in the DNA where a short sequence of nucleotides (typically 2-6 bases in length) is repeated multiple times in a row. These repeats are highly polymorphic, meaning they vary greatly among individuals.
- **Location and Variation**: STRs are scattered throughout the human genome. The number of repeats in a specific STR region varies between individuals, making them useful for identification purposes.
- **Extraction and Amplification**: To analyse STRs, DNA is first extracted from biological samples like blood, saliva, or hair. Then, the regions containing STRs are then amplified using a technique called Polymerase Chain Reaction (PCR), making them easier to analyse.
- **Multiplexing**: In DNA profiling, multiple STR loci are examined simultaneously. This is called multiplexing. Forensic scientists typically analyse 13-20 different STR loci for a comprehensive profile.
- **Detection and Analysis**: After PCR, the amplified STRs are separated using electrophoresis, based on their size (number of repeats). The resulting pattern of STR sizes is unique to each individual (except identical twins).
- **Applications in Forensic Science**: STR profiles are used to match biological samples found at a crime scene with suspects or to identify victims. They are also used in paternity testing and in identifying remains in disaster victim identification.
- **Advantages of STRs in Profiling**
  - **High Discrimination Power**: Due to the high variability in STR regions, the probability of two unrelated individuals having the same STR profile is extremely low.
  - **Small Sample Size**: STR analysis can be performed with very small DNA samples, which is often the case in forensic investigations.
  - **Robustness**: STR analysis is relatively robust against DNA degradation, a common issue with samples from crime scenes.
- **Limitations**:
  - **Not Informative for Related Individuals**: STRs are less discriminating among closely related individuals.
  - **Mixed DNA Samples**: Analysing STRs in mixed DNA samples (from multiple individuals) can be complex and challenging.
- **Ethical and Privacy Considerations**: The use of STRs in DNA databases raises ethical and privacy issues, including concerns about consent, data security, and potential misuse of genetic information.

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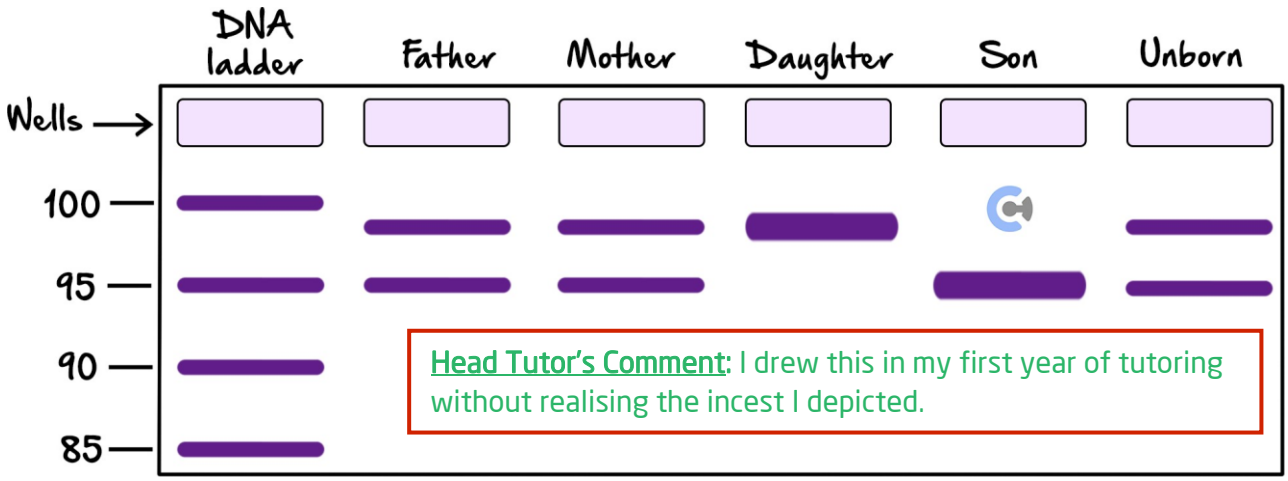
Use this to guide the discussion- ultimately, students should be able to understand what STRs are, understand that they originate from non-coding satellite DNA, and the basic probabilities behind how they can be used to discriminate individuals based on DNA profiles.

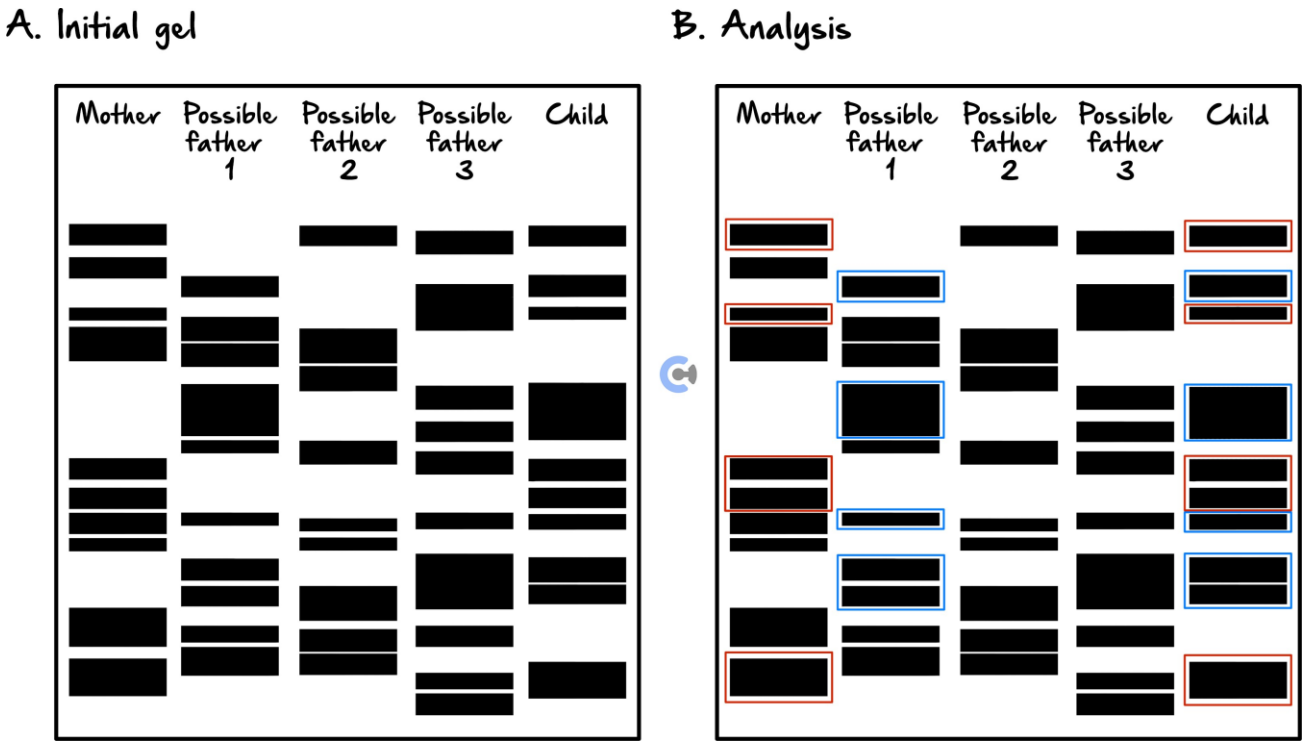
Sub-Section: Genetic Testing

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.....

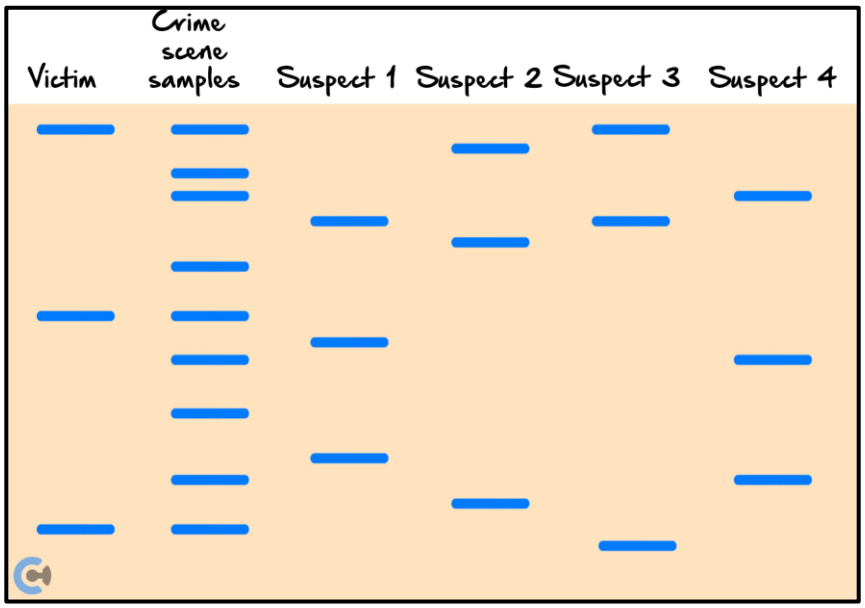


Okay, now that's a little better...

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:

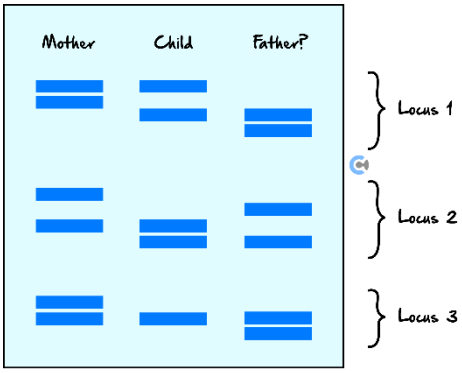

Diagram from: https://bit.ly/2nkFdtc

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

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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In these type of cases, there is 1 allele of the 3 gene loci passed from each parent to the child. Locus 1 and 2 have provided 1 allele from each of the parents, and based on this information, the child could be biologically related to both, the mother and father. Locus 3 has only 1 band, so each parent could pass the same-sized fragment to the child. It would be expected that this common band would be "thicker," illustrating more DNA. D is incorrect, as each person has 2 alleles. It cannot be concluded if the man is or is not the biological father, so the only way to sort out this dispute would be to test more gene loci.

Study Design Reference:

Techniques that apply DNA knowledge (specifically gene cloning, genetic screening, and DNA profiling), including social and ethical implications and issues.

Web Link:

http://genetics.thetech.org/ask/ask20

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.

Space for Personal Notes

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** (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 the ____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.____.

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**:
    - _____Forward primer_____ binds to the start of the DNA segment.
    - _____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 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.

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 _____ phosphate backbone _____.
  - When an electric current is applied, DNA moves towards 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 one end of the well, 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 (**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.

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:**
  - Voltage: Higher voltage increases movement, but may distort separation.
  - Gel Composition: Denser gels slow down 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.

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 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.
  3. **Digestion with Restriction Enzyme**:
     - DNA is cut with the same restriction enzyme 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 varying 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 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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