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

Homework Outline:

Compulsory Questions	Pg 2 – Pg 21

Section A: Compulsory Questions (46 Marks)

Sub-Section [1.5.1]: Identify and Describe the Function of Polymerases, Endonucleases, and Ligases in DNA Manipulation

Question 1

Definitions:

a. Endonuclease

An enzyme that breaks the phosphodiester bond between two nucleotides in a polynucleotide chain.

b. Recognition site

A specific target sequence of DNA where endonucleases will cut.

c. Sticky end

Where an endonuclease cuts each strand of DNA at a different point, leaving overhanging exposed nucleotides.

d. Blunt end

Where an endonuclease cuts each strand of DNA at the same point, leaving no overhanging exposed nucleotides.

e. Polymerase

An enzyme that synthesises a polymer by joining monomers together, including nucleic acids.

f. Ligase

An enzyme that joins fragments of DNA together by connecting the sugar-phosphate backbone together by creating a phosphodiester bond.

Question 2 (1 mark)

A scientist is attempting to combine a gene of interest into a plasmid. Which pair of enzymes would they most likely use?

A. Polymerase and ligase.

B. Helicase and polymerase.

C. Endonuclease and ligase.

D. Endonuclease and polymerase.

Question 3 (1 mark)

During a DNA repair process, which enzyme is likely involved in sealing the gap between adjacent nucleotides?

A. Endonuclease

B. Polymerase

C. Ligase

D. Restriction enzyme

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Question 4 (1 mark)

A student observes that after cutting a DNA sample, the fragments have "sticky ends." Which enzyme likely produced these fragments?

A. Ligase

B. DNA Polymerase

C. Endonuclease

D. Helicase

Question 5 (1 mark)

In a PCR experiment, which enzyme replicates the DNA template by adding nucleotides?

A. Restriction enzyme

B. Taq Polymerase

C. DNA Ligase

D. Topoisomerase

Question 6 (1 mark)

If an endonuclease cuts DNA into fragments and a ligase enzyme fails to function, what would be the consequence?

A. DNA will replicate incorrectly.

B. DNA fragments will remain unattached.

C. The DNA will form sticky ends.

D. The fragments will rejoin naturally.

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Question 7 (1 mark)

Which enzyme would be most useful for amplifying a specific DNA sequence from a small sample?

A. DNA Ligase

B. DNA Polymerase

C. Endonuclease

D. Helicase

Question 8 (2 marks)

Explain why sticky ends are preferred over blunt ends when cutting DNA from manipulation using an endonuclease.

They form complementary overhangs, which allow for specificity when joining the fragments together, via hydrogen bonding.

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Sub-Section [1.5.2]: Identify the Ingredients Required, Describe the Process, and Recall Key Applications of PCR

Question 9

Definitions:

a. Primer

A short single strand of nucleic acids that acts as a starting point for polymerase enzymes to attach.

b. PCR

Polymerase chain reaction, a laboratory technique used to produce many copies of DNA from a small initial sample.

c. Denaturation

In reference to PCR, the disruption of the double stranded DNA structure by heat.

d. Elongation

Phase of PCR where the polymerase is functioning to synthesise a strand.

e. Annealing

Phase of PCR, where primers join to the complementary DNA strands before being elongated by the polymerase.

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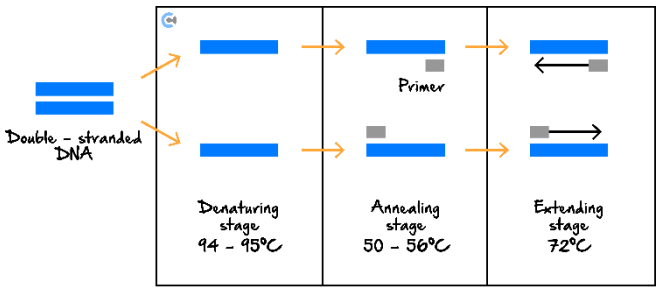
Question 10 (1 mark)

The diagram below represents a method of DNA manipulation.



Source: Genome Research Limited, in Your Genome, <www.yourgenome.org>

The method represented is:

A. Gel electrophoresis.

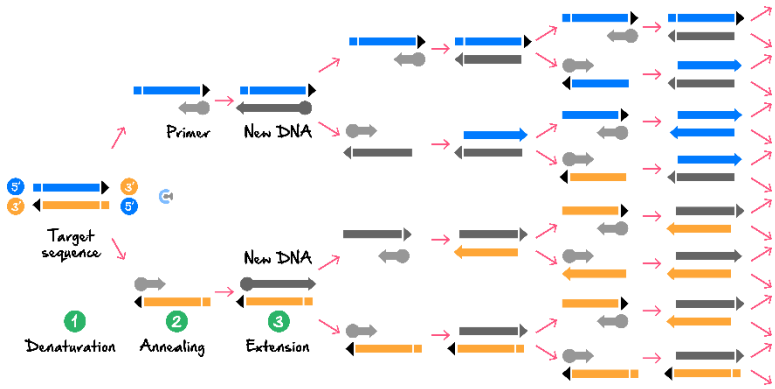
B. DNA transformation.

C. Bacterial transformation.

D. Polymerase chain reaction.

The following information applies to the two questions that follow.

Question 11 (1 mark)



During the extension stage, the most ideal temperature is typically:

A. 72 degrees.

B. 50 degrees.

C. 82 degrees.

D. 95 degrees.

Amplification of DNA using the polymerase chain reaction	Question 34 During the extension stage, the most ideal temperature is typically A. 72 degrees B. 50 degrees C. 82 degrees D. 95 degrees	*A*	*In polymerase chain reaction, the extension phase is typically carried out at 72 degrees.*

Amplification of DNA using the polymerase chain reaction

Question 35
The purpose of a primer in this reaction is
A. to act as a short sequence of nucleotides that provides a starting point for DNA synthesis.
B. to move along the original DNA strand and add complementary nucleotides.
C. to act as the 'glue' to join complementary nucleotides together.
D. to separate the DNA and prime it, ready for a copy to be made.

A *Primers are required for the enzyme to commence reading the DNA.*

Question 12 (1 mark)

The purpose of a primer in this reaction is

A. to act as a short sequence of nucleotides that provides a starting point for DNA synthesis.

B. to move along the original DNA strand and add complementary nucleotides.

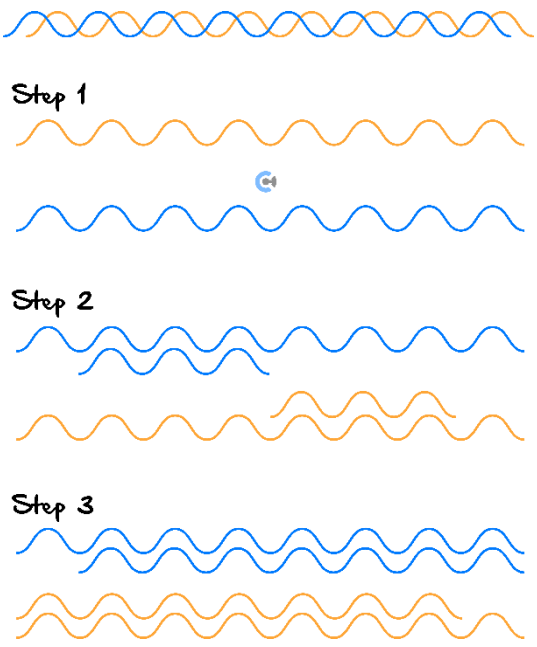
C. to act as the 'glue' to join complementary nucleotides together.

D. to separate the DNA and prime it, ready for a copy to be made.

The following information applies to the two questions that follow.

Polymerase chain reaction (PCR) has been used to primarily amplify specific sections of DNA from small samples. It has uses in DNA sequencing, forensic analysis and genetic testing for diseases.

The diagram below illustrates the steps involved in each replication cycle.



Question 32 B
A PCR cycle is repeated many times to generate copies of target sections of DNA. A temperature of about 90°C is involved in step 1 which breaks the hydrogen bonds holding the original DNA strand together. The sample is then cooled to about 50°C where primers are then able to anneal (stick) onto complementary sections of each DNA strand, as shown in step 2. Finally, in step 3, the sample is warmed to about 70°C and the *taq* polymerase enzyme works to replicate each strand using the primer as an anchor for the enzyme. This completes one cycle and it then begins again.

Question 13 (1 mark)

A temperature of 50°C is needed for step:

A. 1 only.

B. 2 only.

C. 3 only.

D. 2 and 3 only.

Question 14 (1 mark)

At the beginning of the first PCR cycle there was 1 DNA strand.

After 5 cycles, there would be:

A. 5 strands.

B. 16 strands.

C. 32 strands.

D. 64 strands.

Question 33 C

There was initially 1 strand and after the first cycle there were 2 strands. After 2 cycles there were 4 strands. After 3 cycles, there were 8 strands. After four cycles, there were 16 strands. After 5 cycles there were 32 strands.

Question 15 (6 marks)

Scientists investigating a transgenic strain of the *Arabidopsis* plant called kojak carried out a gel electrophoresis to find the root hair gene that had been transferred into the kojak strain from a species of barley.

The root hair gene was removed along with some other DNA using restriction enzymes, and underwent PCR prior to the gel electrophoresis being run.

a. Why was PCR performed on the DNA sample prior to the gel electrophoresis being carried out? (1 mark)

Worked solution

PCR is performed to amplify the DNA segment so that there is enough to be visible in the gel electrophoresis.

Mark allocation: 1 mark

- 1 mark for recognising that PCR is performed to increase the amount of DNA in the sample

b. Outline the three major steps in PCR. (3 marks)

Worked solution

1. Heat the DNA to 90 °C
2. Cool to attach primers
3. Taq polymerase copies DNA strands

Mark allocation: 3 marks

- 1 mark for identifying that the DNA must be heated to 90 °C
- 1 mark for stating 'cool to attach primers' or equivalent
- 1 mark for stating 'Taq polymerase copies DNA strands' or equivalent

c. Describe the role of primers in PCR. (2 marks)

- Primers will bind to the 3'end of each DNA strand allowing TAQ polymerase to bind and replicate the DNA strands.
- There are two different primers which will bind to each strand, allowing for a specific sequence to be transcribed.

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Sub-Section [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"

Question 16

Definitions:

a. Gel Electrophoresis

A technique that separates DNA fragments based on their molecular size.

b. Well

Indent in the agarose gel in which the samples are loaded.

c. Standard Ladder

A mixture of DNA fragments of a known length, which are used to be able to infer the sizes of the unknown sample.

d. Buffer

lonic solution that allows for the conduction of charge through the gel.

e. Electrode

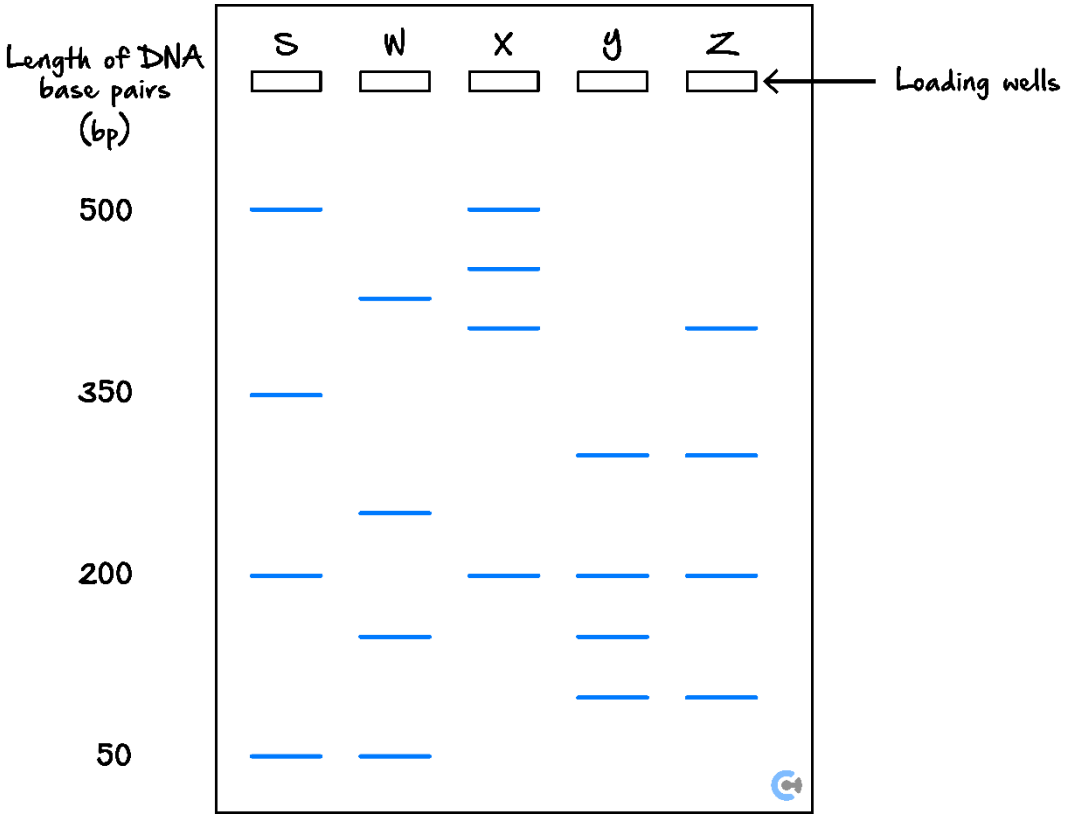
Conductors of electricity that allow a current to be passed through the gel.

f. Band

Line seen in the gel after staining the DNA with the ethidium bromide at the conclusion of the gel.

The following information applies to the two questions that follow.

Four samples of DNA were loaded into four different wells in lanes W, X, Y and Z. A standard ladder was loaded into the well in lane S. The results of gel electrophoresis are shown below.



Question 17 (1 mark)

Which lane represents a sample that was loaded with DNA fragments of four different lengths: 100 bp, 150 bp, 200 bp and 300 bp?

A. W

B. X

C. Y

D. Z

Question 18 (1 mark)

Which lane contains the band that is closest to the negative electrode?

A. *W*

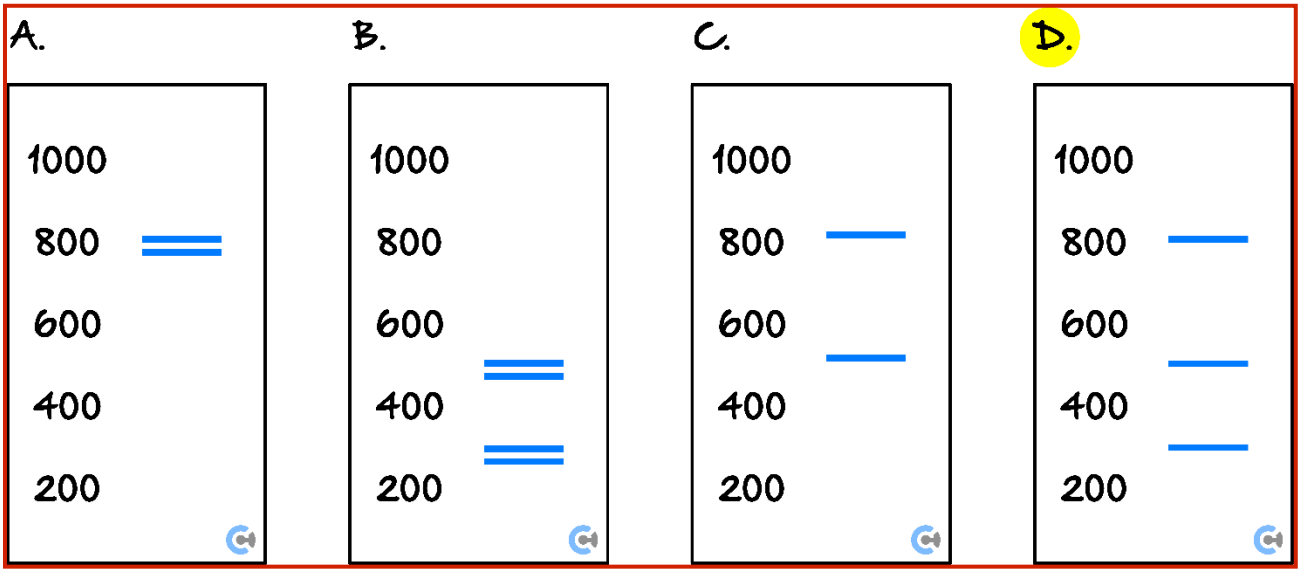
B. *X*

C. *Y*

D. *Z*

Question 19 (1 mark)

Beta thalassemia is an autosomal recessive genetic disorder. Genetic screening is now available that can test for one common genetic mutation that causes this disorder. A restriction enzyme is used which cuts the defective gene into two strands of length 280 base pairs and 520 base pairs respectively. Which of the following results would indicate the person tested was a carrier of beta thalassemia?



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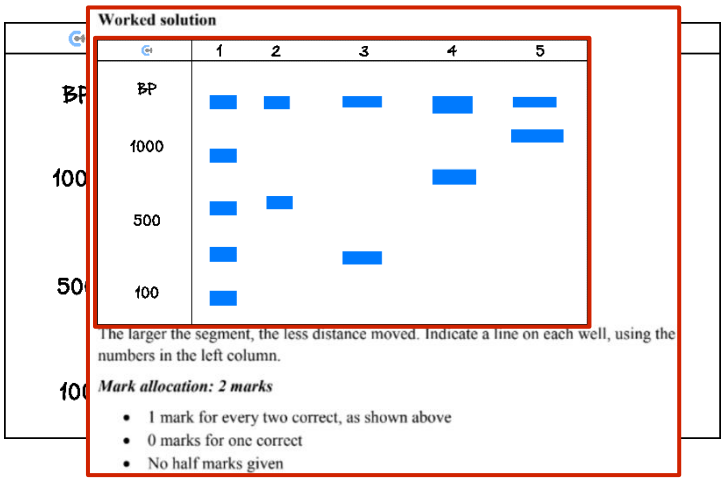
Question 20 (4 marks)

The table below gives the sizes of the various DNA fragments (genes) used in the genetic engineering of the *Arabidopsis* plant.

Gene	Size (base pairs)	Well (gel electrophoresis)
Normal *Arabidopsis* root hair gene	520	2
Mutant kojak *Arabidopsis* gene	450	3
Barley root hair gene	600	4
Recombined mutant *Arabidopsis* + barley genes	1050	5

a. On the diagram below, indicate where each of the DNA fragments would be positioned after the gel electrophoresis has been run. (2 marks)



Worked solution

The larger the segment, the less distance moved. Indicate a line on each well, using the numbers in the left column.

Mark allocation: 2 marks

- 1 mark for every two correct, as shown above
- 0 marks for one correct
- No half marks given

b. What is placed in well 1? (1 mark)

Worked solution

Standard DNA fragments of known lengths

Mark allocation: 1 mark

- 1 mark for identifying a standard DNA fragments of known lengths

c. What is the purpose of the standard ladders? (1 mark)

To provide a point of reference by which unknown fragments can be compared with known ones.

Sub-Section [1.5.4]: Explain the Factors That Affect the Movement of Fragments in Gel Electrophoresis

Question 21 (1 mark)

If a gel electrophoresis was left to run for 24 hours longer than normal, what would be expected to happen to the DNA fragments?

A. They would get darker.

B. They would all collect at the positive terminal.

C. They would all collect at the negative terminal.

D. There would be no apparent difference.

Question 22 (1 mark)

A particular DNA marker used in DNA profiling has four possible variations of length, namely 8, 10, 16 and 21. If this marker was used as part of a DNA profile, which of the following results is least likely?

A. 8, 14

B. 10, 10

C. 8, 16, 21

D. 21, 10

Question 23 (4 marks)

List and explain the factors that affect the movement of fragments in a gel electrophoresis experiment.

Suitable responses for factors affecting the migration of DNA fragments through the agarose gel during gel electrophoresis included three of the following:

- the size of the molecules, as the larger molecule will move more slowly
- the charge of the molecule, as the negative charge means that DNA moves towards the positive electrode
- the length of time the voltage is applied, as there may not be enough time for the DNA to migrate through the gel
- the concentration of the agarose, as denser agarose results in the molecules moving more slowly.

Sub-Section [1.5.5]: Define Satellite DNA and STRs, and Explain Their Use in Identifying People Through DNA Profiling for Crimes and Paternity Testing

Question 24

Definitions:

a. DNA Profiling

The process of identifying an individual based on their genetic information.

b. Genetic testing

Screening an individual's DNA for anomalies that may make them susceptible to particular disease or disorder.

c. STRs

Short tandem repeats, 4-5 nucleotides long repeating themselves over in non-coding region of genes.

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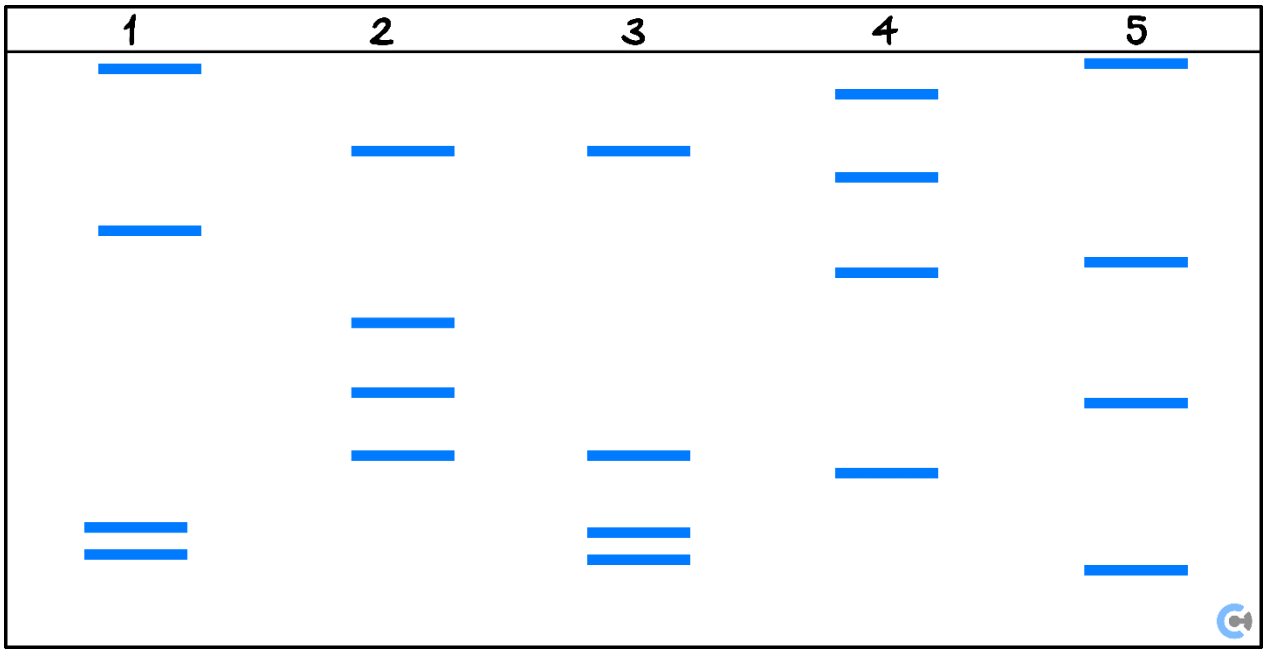
Question 25 (1 mark)

A woman with a child marries a man. The couple then have a child of their own, after which they adopt a third child. Genetic fingerprinting was carried out and the results are shown below. Lane 1 contains the woman's DNA; lane 2 contains the man's DNA; and lanes 3, 4 and 5 contain the children's DNA.



Use the information provided to identify each of the children as being the woman's child, the couple's child or the adopted child.

	Lane 3	Lane 4	Lane 5
A.	Woman's child	Couple's child	Adopted child
B.	Adopted child	Couple's child	Woman's child
C.	Couple's child	Woman's child	Adopted child
D.	Couple's child	Adopted child	Woman's child

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Answer: D

Explanatory notes

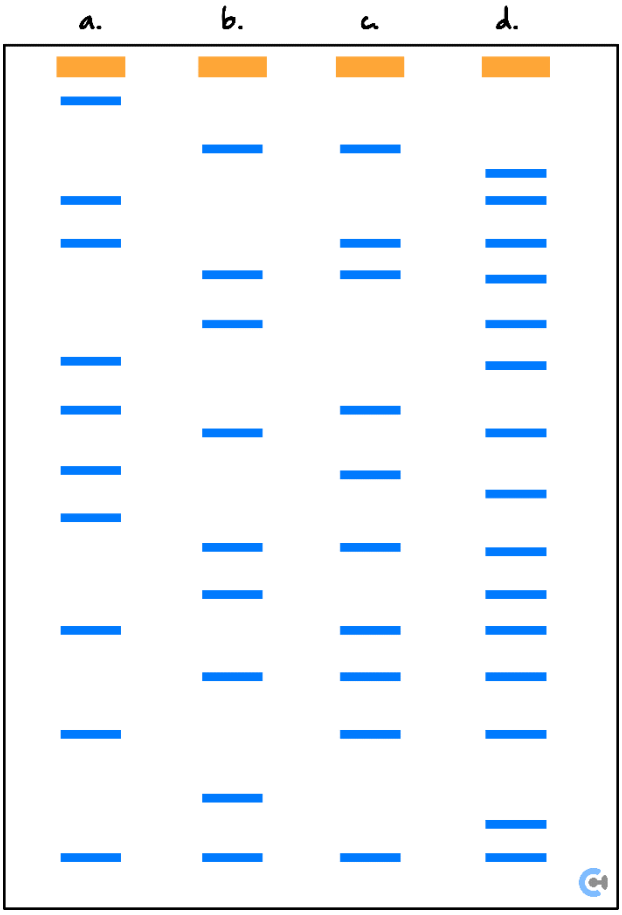
Lane 3 contains the DNA sample from the couple's child. The first two bands in this fingerprint match the man and the last two bands match the woman.

Lane 4 contains the DNA sample from the adopted child. None of the bands in this fingerprint match the fingerprints of the adults.

Lane 5 contains the DNA sample from the woman's child. The first and last bars in this fingerprint match the woman's fingerprint and the remaining bars do not match the man's fingerprint.

Question 26 (1 mark)

The image below depicts a gel electrophoresis run from a husband (a) and wife (b).



From the information above, which of the following statements is correct?

A. Neither lane *c* or *d* is likely to be a child of the husband or wife.

B. Lane *d* is likely the child of the husband and wife.

C. Lane *c* is likely the child of the husband and wife.

D. Lane *c* and *d* are identical twins.

The use of gel electrophoresis in sorting DNA fragments, including interpretation of gel runs

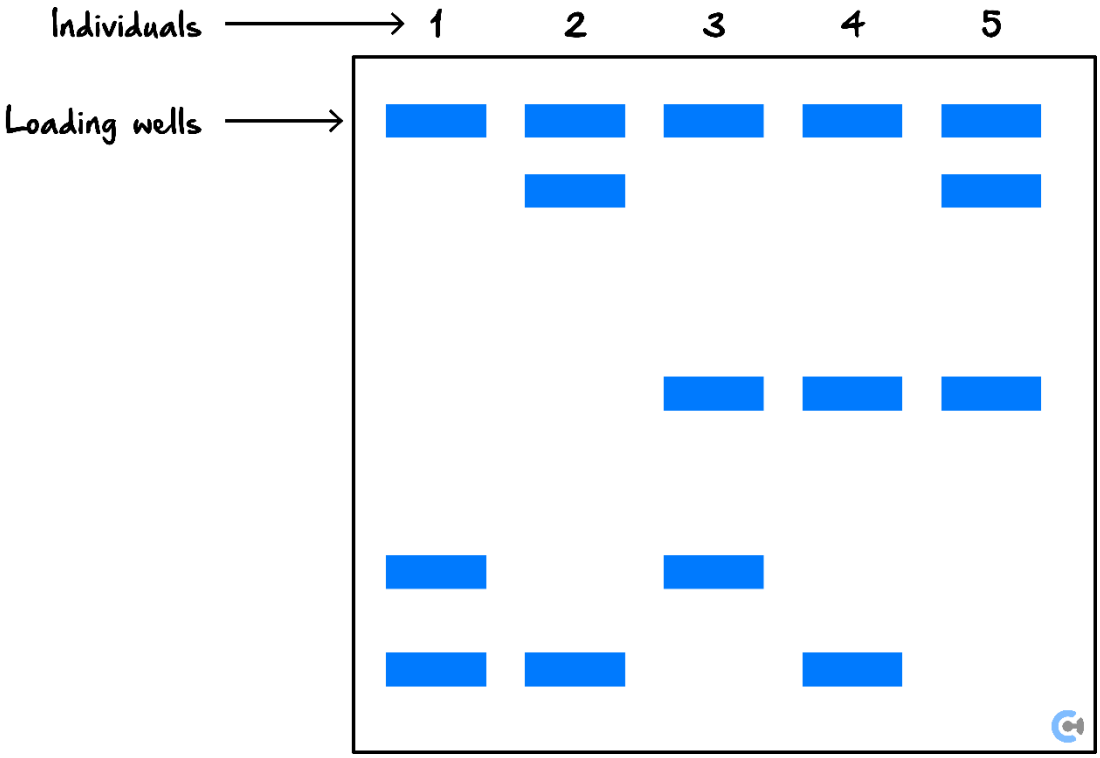
Question 33
From the information above, which of the following statements is correct?
A. neither lane c or d is likely to be a child of the husband or wife
B. lane d is likely the child of the husband and wife
C. lane c is likely the child of the husband and wife
D. lane c and d are identical twins

C *All of the banding patterns for lane c match either the husband or wife's banding patterns.*

Space for Person

The following information applies to the two questions that follow.

The electrophoresis gel shown below shows the results of a restriction enzyme digest of five individuals, a mother and father and their three children.



Question 27 (1 mark)

The pattern shown by the fragments on the gel is a result of the:

A. Heaviest fragments moving the furthest towards the negative pole of the gel.

B. Lightest fragments moving the furthest towards the negative pole of the gel.

C. Lightest fragments moving the furthest towards the positive pole of the gel.

D. Heaviest fragments moving the furthest towards the positive pole of the gel.

Question 28 (1 mark)

From the results, it can be stated that:

A. Individuals 2 and 3 are the parents and 1,4 and 5 are the children.

B. Individuals 4 and 5 are the parents and 1,2 and 3 are the children.

C. Individuals 1 and 5 are the parents and 2,3 and 4 are the children.

D. Individuals 2 and 4 are the parents and 1,3 and 5 are the children.

Question 29 (10 marks)

Beginning in 1996 the FBI launched a national DNA database known as CODIS. The database stores information relating to 13 specific loci which have variable numbers of short tandem repeat sequences (STRs). One of these is the CSF1PO locus which has between six and fifteen repeats of the AGAT tetranucleotide.

a. Prior to analysis, genetic samples are amplified using PCR. Briefly identify the three stages of PCR and explain what occurs during each stage. (3 marks)

Denaturing. The PCR mix is exposed to a temperature of approximately 92°C in order to separate the two template strands of DNA.

1 mark

AND

Annealing. The PCR mix is cooled to about 55°C which enables the primers to bond to the ends of the DNA template.

1 mark

AND

Extension or elongation: The PCR mix is heated to approximately 72°C. This enables taq polymerase to bind to the primers, read the template strands and assemble the complementary strands.

1 mark

b. Why is PCR carried out prior to analysing a DNA sample? (1 mark)

Often the DNA sample will be very small, so many copies are made prior to analysis.

c. Identify an enzyme which carries out a function in DNA replication, but is not used in a PCR mix. Explain why this enzyme is not used during PCR. (2 marks)

DNA helicase is involved in DNA replication by unwinding the double helix to separate the two strands. However, it is not used in PCR because the denaturation step in PCR, which separates the DNA strands, is achieved by heating the reaction mixture to $90 - 95°C$. This high temperature causes the DNA to separate without the need for helicase.

d. Explain how the size of a DNA fragment affects its movement through an electrophoresis gel and why DNA moves towards the positive terminal. (2 marks)

The negative charge of DNA causes it to move through the gel towards the positive terminal.

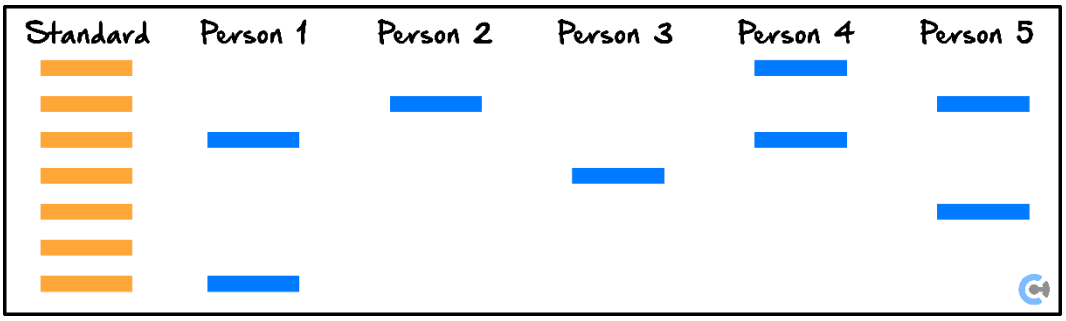
1 mark

AND

The size of the fragment determines the extent to which the fragment will move through the gel.

1 mark

The following diagram shows an example of an electrophoresis gel containing samples from five different people. The DNA of each person would have been inserted into a well at the top of the diagram.



e. Which individual has the variation with the most STR regions? Provide a reason to support your answer. (2 marks)

Person 4.

1 mark

AND

Increasing the number of STR regions will increase the size of the DNA fragment. Large fragments of DNA stay close to the origin. Person 4 has the band that has stayed closest to the origin.

1 mark

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