PCR

1 The following steps occur during the Polymerase Chain Reaction (PCR).

1. Binding of primer
2. Replication of DNA
3. Heating of sample DNA
4. Separation of DNA strands

In which sequence do these steps occur?

A 1 → 2 → 4 → 3
B 1 → 2 → 3 → 4
C 3 → 4 → 1 → 2
D 3 → 4 → 2 → 1

2 The following are stages in one cycle of the polymerase chain reaction (PCR).

1 Heat tolerant polymerase replicates DNA

2 DNA heated to separate strands

3 Primers bind to DNA

Which of the following is the correct order of the occurrence of these stages in PCR?

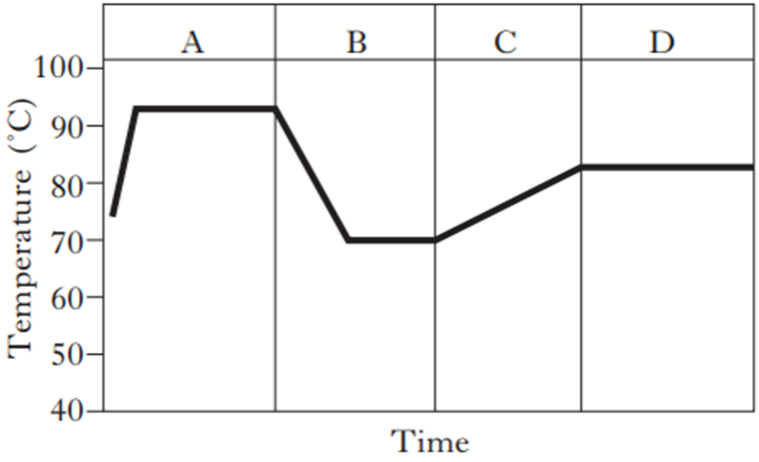
A 2 3 1
B 3 2 1
C 2 1 3
D 3 1 2

3 During the polymerase chain reaction (PCR) samples of DNA are repeatedly heated and cooled.

Why are the samples cooled?

A To denature DNA polymerase
B To slow the reaction down
C To allow primers to bind to target sequences
D To separate the DNA strands

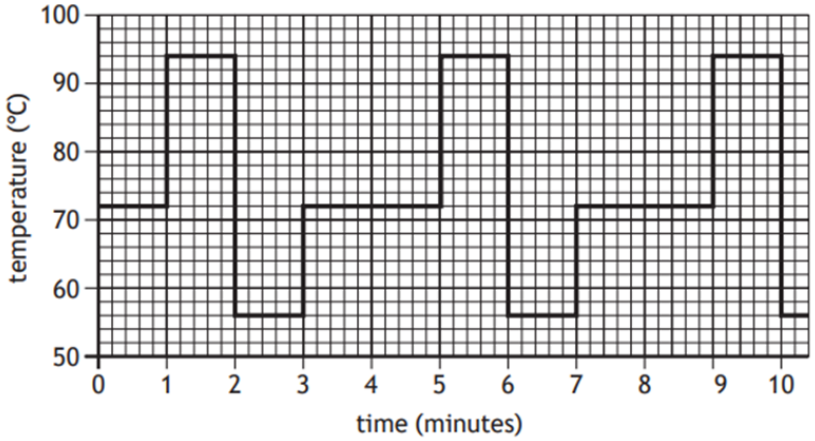
4 The graph below shows the temperature changes involved in one cycle of the polymerase chain reaction (PCR).



Which letter indicates when primers would bind to target sequences of DNA?

PCR

5 The graph shows how temperature changes during repeated cycles of a polymerase chain reaction (PCR).



If there were 500 molecules of DNA at the start, predict how many copies there will be after 20 minutes.

A 16 000
B 8000
C 2500
D 2000

6 PCR was used to amplify a region of DNA. After 5 cycles 32 copies were present.

Calculate the number of **additional copies** present after 4 further cycles.

A 224
B 256
C 480
D 512

7 The polymerase chain reaction (PCR) is used to

A join DNA fragments
B cut open plasmid DNA
C amplify DNA
D extract DNA from cells.

8. Some of the following are steps used in the amplification of DNA by PCR.

1 Decrease temperature to separate the DNA strands
2 Increase temperature to separate the DNA strands
3 Increase temperature for primers to bind to target sequences
4 Decrease temperature for primers to bind to target sequences
5 Increase temperature for DNA polymerase to replicate the DNA
6 Increase temperature for DNA ligase to replicate the DNA

Which line in the table below identifies the correct order of steps involved in a PCR cycle?

	First stage	*Second stage*	*Third stage*
A	3	1	6
B	3	1	5
C	2	4	5
D	2	4	6

PCR

1. The polymerase chain reaction (PCR) is a technique carried out to amplify target sequences of DNA. It involves repeated cycles of heating and coolin

Two different primers are used in each PCR procedure.

(i) Give a reason why two different primers are used.

_____

_____

(ii) State a temperature at which primers bind to the target sequence o DNA.

_____ °C

b) One complete cycle of a PCR took 3 minutes.

Calculate how many copies of the DNA there would be after 9 minutes from an original sample of 30 DNA molecules. **1**

Space for calculation

_____ copies

(c) Explain the importance of using heat-tolerant DNA polymerases in PCR. **1**

_____

_____

d) (i) The base sequence of a primer used in the PCR procedure is shown below.

A T G A C A A A T C G

Give the base sequence of a DNA fragment to which this primer would bind. **1**

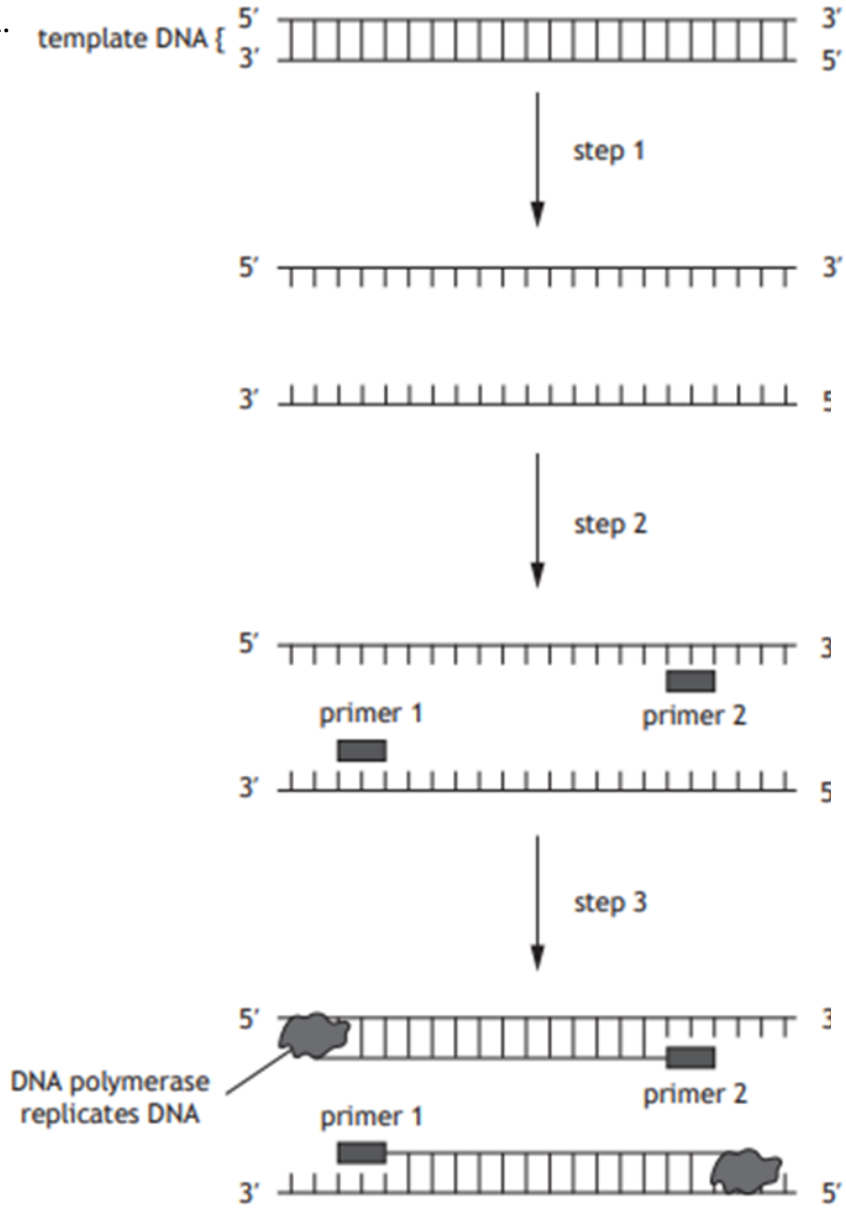
_____

(ii) Complete the table below to show the temperatures used in two stages of the PCR procedure and the reasons for using these temperatures. **2**

Temperature (°C)	*Reason*
94	
	Allows primer to bind to target sequence

PCR

2.



(i) Give a temperature for step 1 to occur. **1**

_____ °C

(ii) Describe one role of the primers in PCR. **1**

_____

_____

(b) (i) The diagram shows the role of DNA polymerase in PCR.

Name another enzyme necessary for DNA replication in cells.

Suggest why this enzyme is **not** required for step 3 in PCR. **2**

Name _____

Suggestion _____

_____

_____

_____

(ii) Explain why DNA polymerase extracted from human cells would not replicate DNA in PCR. **1**

_____

_____

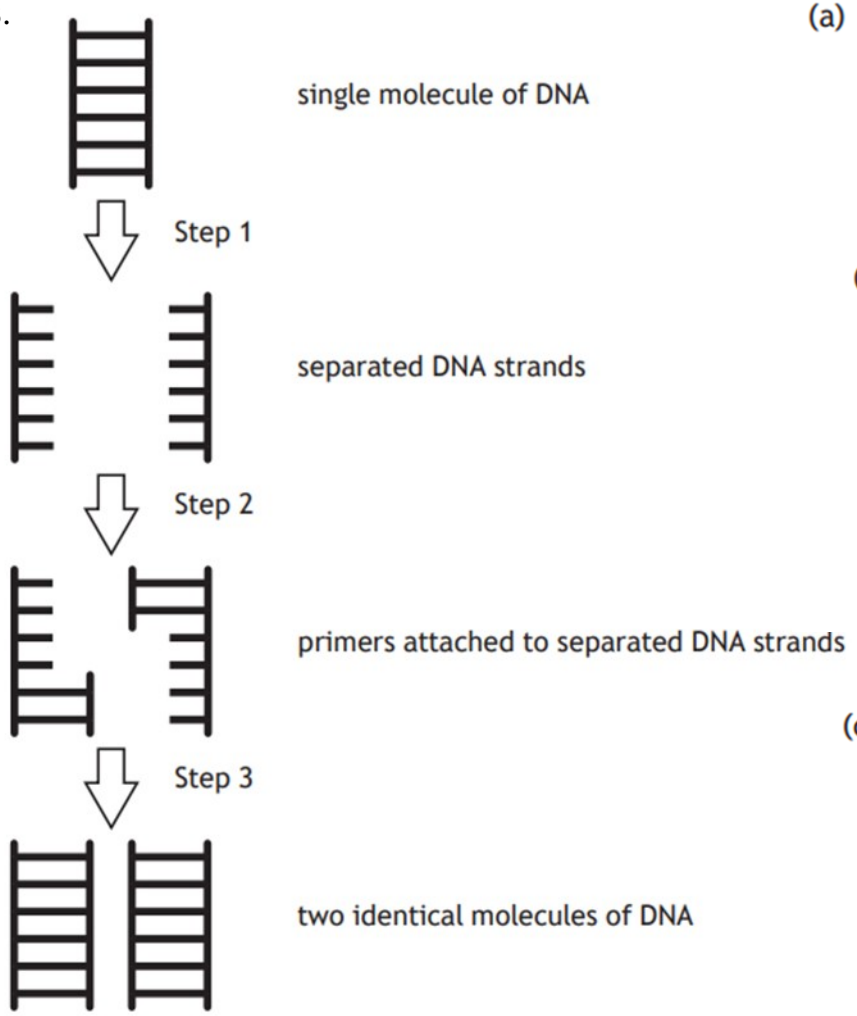
(c) State one practical application of PCR. **1**

_____

_____

PCR

3.



(a) Give values to describe the change in the temperature that occurs in step 2. **1**

_____

_____

(b) (i) Name the enzyme used in step 3. **1**

_____

(ii) Suggest an advantage of using a heat tolerant form of this enzyme during PCR. **1**

_____

_____

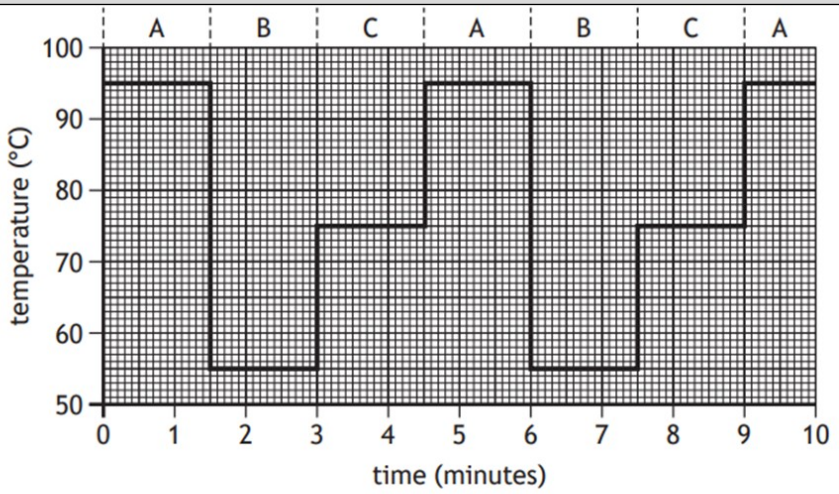
(c) Calculate the number of DNA molecules produced from a single molecule of DNA after 10 cycles of PCR. **1**

Space for calculation

_____DNA molecules

PCR

4a)



(i) Describe the events that occur during stage A and stage B. **2**

Stage A _____

_____

Stage B _____

_____

(ii) An original sample of DNA contained 100 copies of the target sequence.

Calculate how long it would take to produce at least 25 000 copies of this sequence. **1**

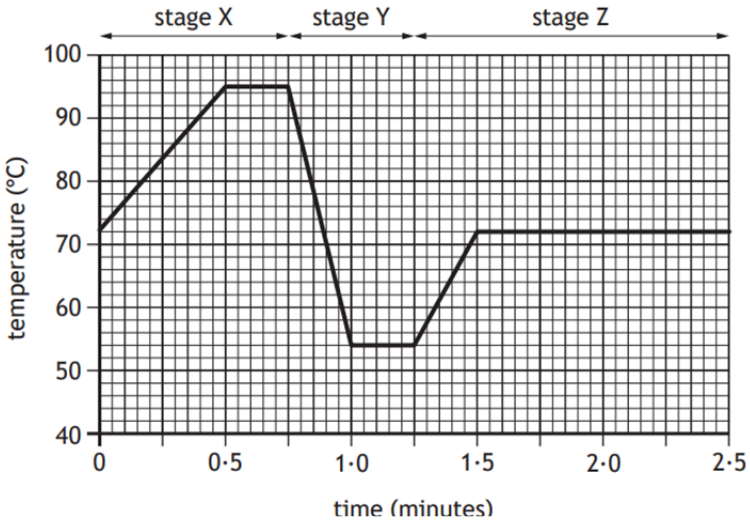
Space for calculation

_____ minutes

b) State **one** practical application of PCR. **1**

_____

5.



(i) Before the reaction began there were 1000 copies of a DNA fragment in the reaction tube.

Calculate the time it would take until there were at least one million copies of this DNA fragment present. **1**

Space for calculation

(ii) Explain why the reaction tube is heated in stage X. **1**

_____

_____

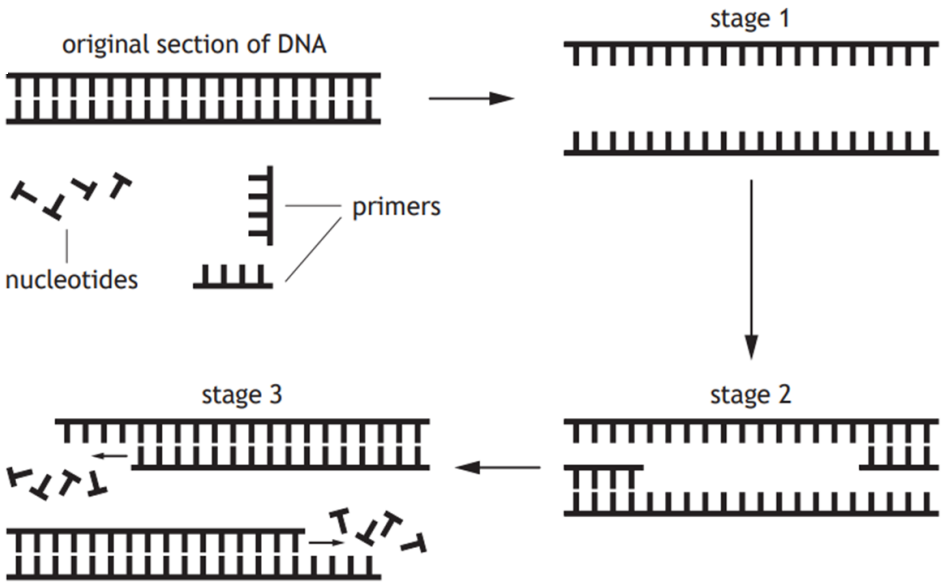
(iii) Explain why the reaction tube is cooled in stage Y. **1**

_____

_____

PCR

6.



(a) Each stage of PCR is temperature dependent.

Complete the table for stages 1 and 3.

Stage	Temperature (°C)	Reason
1	95	
3		To allow replication of DNA

(b) Each PCR cycle produces two copies of a section of DNA.

This PCR cycle takes 3 seconds.

Calculate how long it would take for at least 2000 copies of the original section to be produced. **1**

Space for calculation

(c) Describe the role of primers in stage 2 and stage 3. **2**

Stage 2 _____

_____

Stage 3 _____

_____

(d) PCR was first used to help solve a crime in 1986.

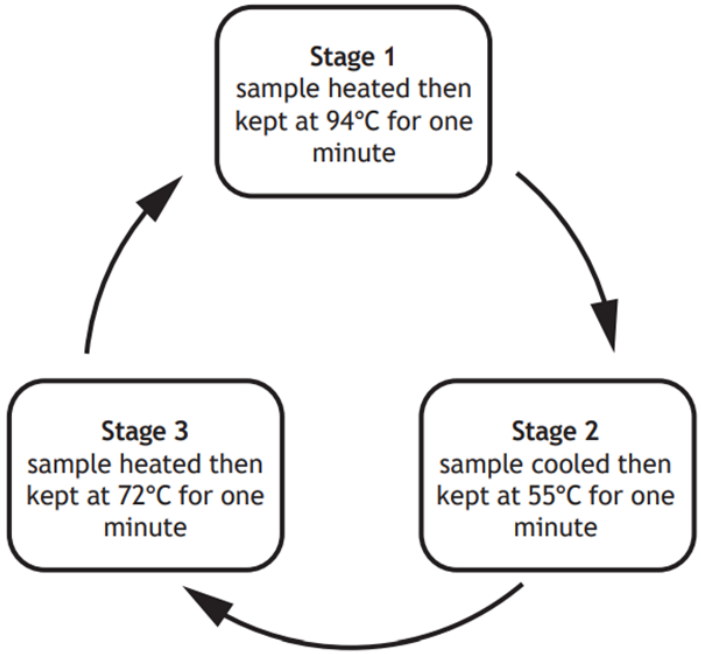
Suggest why PCR can now be used to help solve a crime committed in 1980, where only a small blood spot was found as evidence. **1**

_____

_____

PCR

7.



(a) Explain the purpose of the different heat treatments in Stage 1 and Stage 2. **2**

Stage 1 _____

_____

Stage 2 _____

_____

(b) The number of DNA molecules doubles during each cycle of the PCR procedure.

Caculate the number of cycles needed to produce 128 copies of a single DNA molecule. **1**

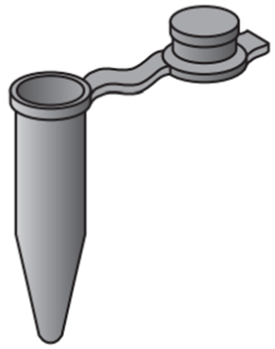
Space for calculation

_____ cycles

(c) The diagram shows the contents of a tube used in PCR.

Contents of tube

— DNA
— DNA nucleotides
— primers
— enzyme and buffer



Describe the contents of a suitable control tube designed to show that primers are needed in the reaction. **1**

_____

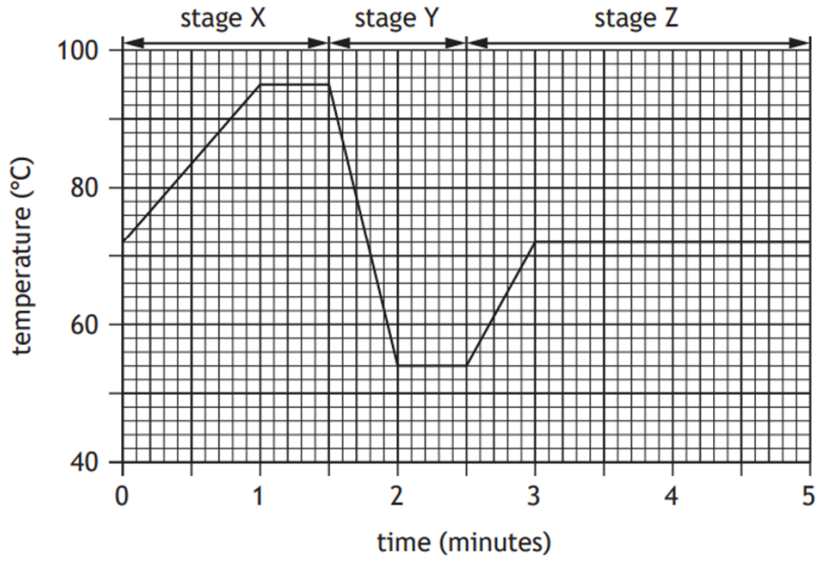
_____

(d) State **one** practical application of PCR. **1**

_____

PCR

8.



a). State the function of PCR.

_____

_____

b). Suggest why the temperature is increased during stage Z.

_____

_____

c). Describe what happens to the DNA during stage X. **1**

_____

_____

d). Short sections of DNA called primers are involved in stage Y.

Describe what happens to these primers during stage Y. **1**

_____

_____

e). A forensic scientist discovered a tiny spot of blood at a crime scene.

A sample taken from this spot contained 10 molecules of DNA.

The sample underwent PCR cycles for 30 minutes.

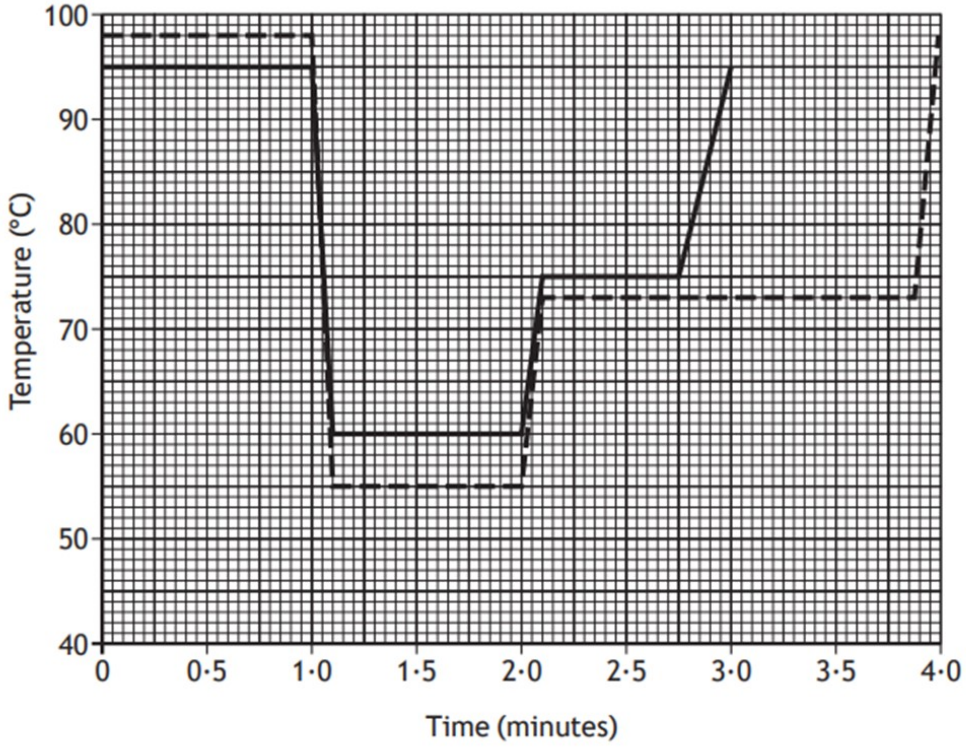
Use data from the graph to calculate how many molecules of DNA would be present after this time. **1**

1 *pace for calculation*

_____ molecules

PCR

9.



(a) (i) Calculate the time taken for 16 copies of the target sequence to be made from one DNA fragment using *Taq* polymerase. **1**

Space for calculation

(ii) Identify the time period during which primers bind to the original DNA fragment. **1**

from _____ to _____ minutes.

(b) A scientist was planning to amplify DNA using PCR.

State which DNA polymerase should be used and describe the advantage of using this polymerase. **1**

DNA polymerase _____

Advantage _____

_____

PCR Answers

1. B
2. A
3. C
4. B
5. A
6. C
7. C
8. D
9.
10.

1a (i) 1 primer for each DNA strand.

(ii) separates strands

any value 50—65

B) 240

C) So enzyme doesn't denature at high temperatures of PCR

D) TAC TGT TTA GC

(ii) separate strands

2 a)(i) 92-98 (ii) binds to target DNA sequence OR starts DNA replication by DNA polymerase

B (i) ligase no lag stand in PCR with fragments to join

(ii) would denature at high temperatures of PCR

(iii) solve crimes or paternity tests or diagnose **genetic** disease

3a 92-98 degrees cooled down to 50-65 degrees

B (i) DNA polymerase

B (ii) enzyme doesn't denature at high temperatures of PCR

4a (i) separates DNA strands

binds to target DNA sequence OR starts DNA replication by DNA polymerase

(ii) 36 minutes

b) solve crimes or paternity tests or diagnose **genetic** disease

5 (i) 25 minutes

(ii) to separate strands

(iii) to allow primers to bind to target DNA OR to start DNA replication by DNA polymerase

6a) to separate strands

70-80

B) 33 seconds

C) binds to target sequence of DNA

Starts DNA replication by DNA polymerase

D) PCR amplifies DNA

7a to separate strands

For primer to bind to target DNA sequence/to start DNA replication by DNA polymerase

B) 7 cycles

C) exact same setup but no primers & replace with same volume of water

D) solve crimes, paternity tests, diagnose genetic disease.

8.a) amplify DNA

b) to allow DNA replication by DNA polymerase

c) separate strands of DNA

d) to allow primers to bind to target DNA to start DNA replication.

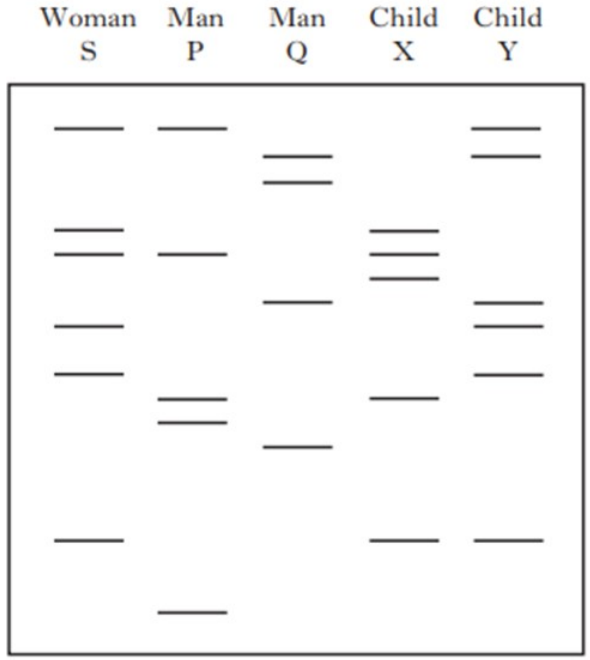
e) 640

9a (i) 12 minutes (ii) 1 minute to 2 minutes

Gel Electrophoresis

1. The diagram below shows the results of a paternity test. It compares DNA samples from five individuals.

Woman S is the mother of child X and child Y. Men P and Q are possible fathers of these children.



Which of the following conclusions can be drawn from these results?

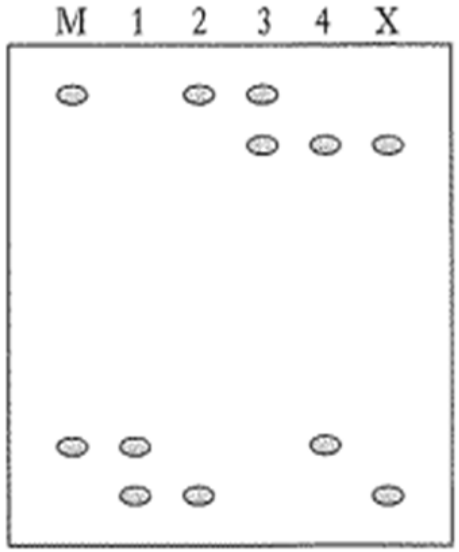
A Man P could be the father of child X

B Man P could be the father of child Y

C Man Q could be the father of child X

D Man Q could be the father of child Y

2. The DNA profile shown below was prepare using a single locus probe to determine if ma X was the father of all four children. Th samples shown are for the mother (M), fou children (1–4) and man (X).



Which of the children have a different father?

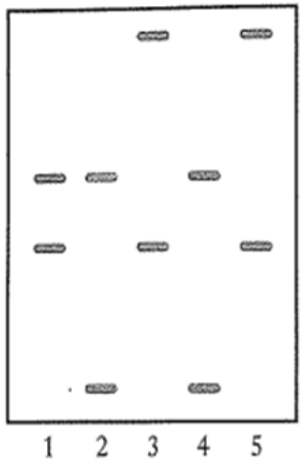
A 1 and 2

B 2 and 3

C 3 and 4

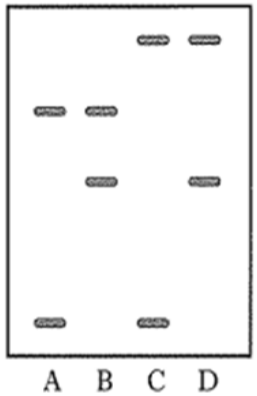
D None of them

3. A forensic scientist is reconstructing the DNA profile of a missing person from analysis of DNA profiles of close relatives. In this case a father of four children is missing. All the children have the same biological mother and father. Results from a single locus probe DNA profile analysis for the four children and their mother are shown below.



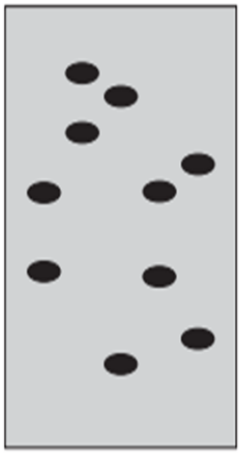
Key	
1	mother
2	child P
3	child Q
4	child R
5	child S

Which of the following is likely to be the DNA profile of the missing father?



Gel Electrophoresis

4. The result of profiling various DNA samples in a criminal investigation is shown below.



1 2 3 4 5

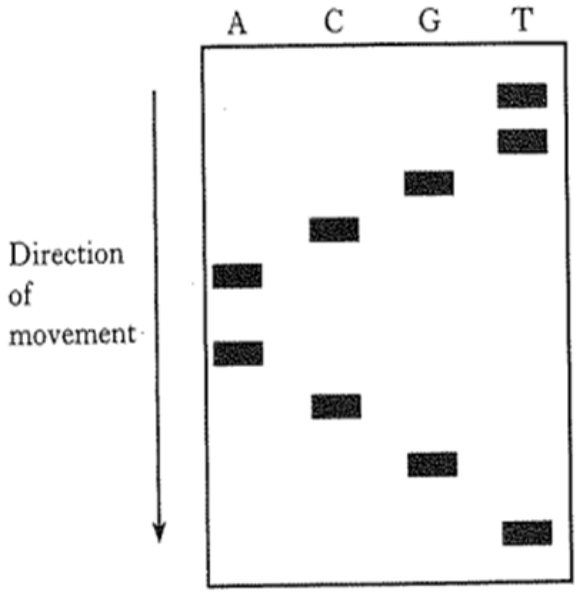
Key:

1 blood sample of victim
2 blood sample of suspect X
3 blood sample of suspect Y
4 first sample from forensic evidence
5 second sample from forensic evidence

Which of the following could the DNA analyst conclude about the crime?

A Only suspect X was involved
B Only suspect Y was involved
C Suspects X and Y were both involved
D Neither suspect X nor Y was involved

5. When sequencing a strand of DNA, DNA fragments are separated by electrophoresis. Larger fragments move more slowly through the gel than the smaller fragments and the gel is normally "read" starting with the smallest fragment. A diagram of the resulting gel is shown below.



What is the base sequence of the DNA strand?

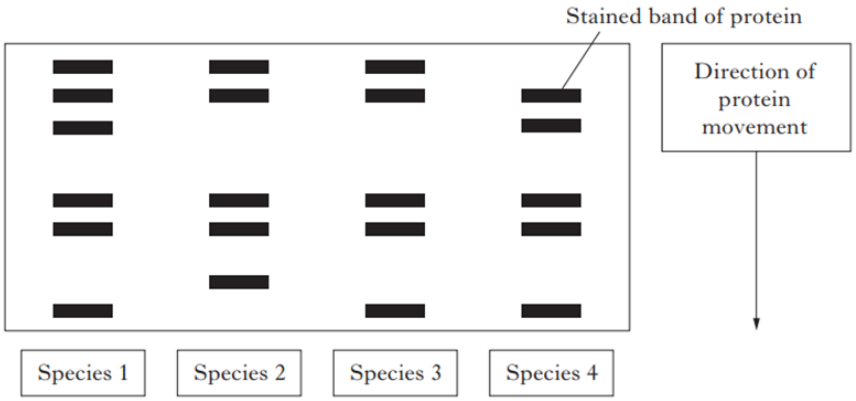
A TGCAACGTT
B TTGCAACGT
C ACGTTGCAA
D AACGTTGCA

Gel Electrophoresis

Protein fingerprinting is a technique used to compare proteins from different species. Species which are closely related have similar protein fingerprints.

Samples of protein are extracted from cells, separated by gel electrophoresis, then stained to make each type of protein show up as a band. The smaller the protein molecules the further they travel through the gel.

The diagram below shows protein fingerprints for four different species.



6. Which species contain protein with the smallest molecules?

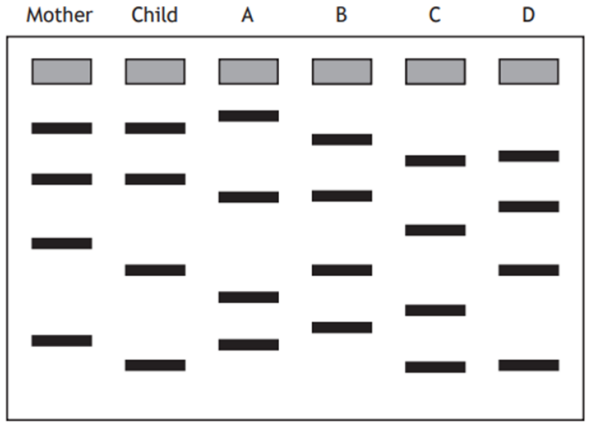
A 1, 3 and 4 only
B 2 only
C 4 only
D 1, 2 and 3 only

7. Which two species show the **greatest** difference in their protein fingerprints?

A Species 1 and 4
B Species 1 and 2
C Species 1 and 3
D Species 3 and 4

8. DNA from a mother, child and four men (A, B, C and D) in a paternity suit was analysed. The DNA was amplified using PCR and separated by gel electrophoresis.

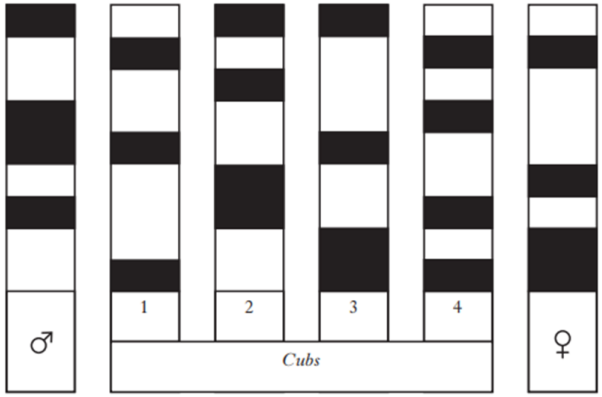
From the results shown in the diagram, identify the likely father of the child.



9. A study was carried out on a lion (♂), a lioness (♀) and a group of cubs.

Samples of DNA were extracted from the animals and analysed using gel electrophoresis.

The results are shown in the diagram below, in which the dark bands represent fragments of DNA of a specific length.



Which of the cubs could be the offspring of the lion and lioness studied?

A 1, 2 and 3
B 1, 2 and 4
C 2, 3 and 4
D 1, 3 and 4

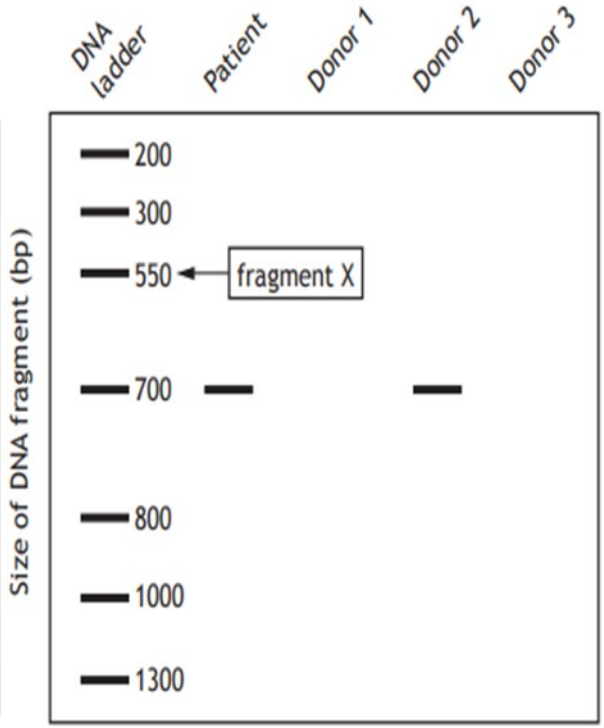
Gel Electrophoresis

1. Patients requiring an organ transplant are tissue typed to match with potential donors. Polymerase chain reaction (PCR) and gel electrophoresis are used to compare DNA sequences of the patient with those of donors. Gel electrophoresis separates mixtures of DNA fragments according to size. The presence of a specific DNA band indicates that a donor is a suitable match.

   Patient and potential donor samples were compared with a DNA ladder.

   The DNA ladder contains fragments of DNA, separated by gel electrophoresis, which are of a known size and measured in base pairs (bp). The distances the DNA fragments travelled were measured and are shown in the table below. The diagram below shows the result of the gel electrophoresis.

Size of DNA fragment (bp)	*Distance travelled* (mm)
200	72
300	58
550	32
700	18
800	12
1000	10
1300	8



a) Using information in the **table** and the **diagram** give the distance travelled by fragment X in the DNA ladder. 1

_____ mm

(d) Give a conclusion about the suitability of the donors. 1

_____

Gel Electrophoresis

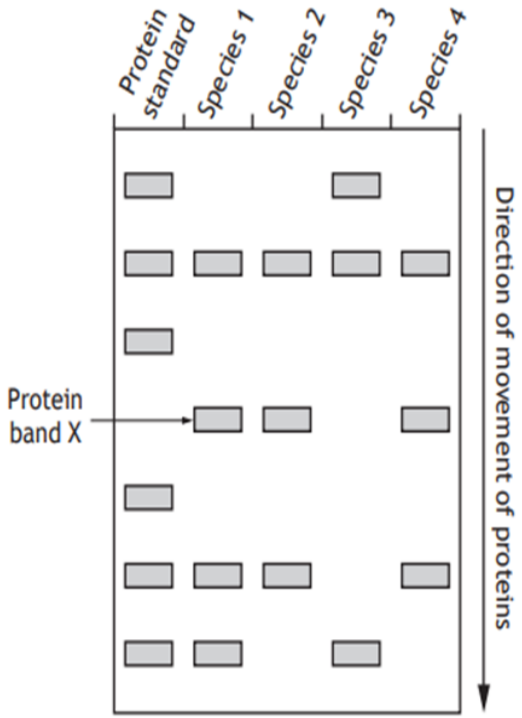
1. An experiment was carried out to investigate the evolutionary relatedness (2.
four species of fish by comparing proteins extracted from the fish. The mo
closely related species are, the more proteins they have in common.

A sample of muscle tissue from each species of fish was heated in a solution to extract proteins.

The protein extracts were analysed by gel electrophoresis which separates proteins according to their mass. A protein standard containing proteins of known masses was also analysed.

The results of the gel electrophoresis are shown in the diagram. Each band represents a protein.

Mass of protein in protein standard (kDa)	*Distance travelled* (mm)
16	50
20	38
26	30
44	24
66	15
108	10



(ii) Band X travelled 28 mm. Use the graph to identify the mass of the protein in band X. 1

_____ kDa

Gel Electrophoresis Answers

1 D
2 D
3 C
4 D
5 A
6 D
7 B
8 D
9 D

1a 32 b) Only donor 2 was suitable OR Donor 1 & 3 were not suitable

2. Any value above 24 and below 30.