

CfE Higher Biology

Unit 1 DNA and the Genome

Key Area 2 Replication of DNA

- Which of the following are required in a polymerase chain reaction (PCR)?
1.
 - A DNA polymerase, template strand and primers
 - B RNA polymerase, template strand and primers
 - C DNA polymerase, template strand and ligase
 - D RNA polymerase, ligase and primers

 2. Each cycle of a polymerase chain reaction (PCR) takes 5 minutes.
If there are 1000 DNA fragments at the start of the reaction, how long will it take for the number of fragments produced by the reaction to be greater than 1 million?
 - A 15 minutes
 - B 35 minutes
 - C 50 minutes
 - D 55 minutes

 3. Which of the following molecules are required in the replication of the lagging strand of a DNA molecule?
 - A DNA polymerase and ligase only
 - B DNA polymerase and primers only
 - C Ligase and primers only
 - D DNA polymerase, ligase and primers

 4. 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

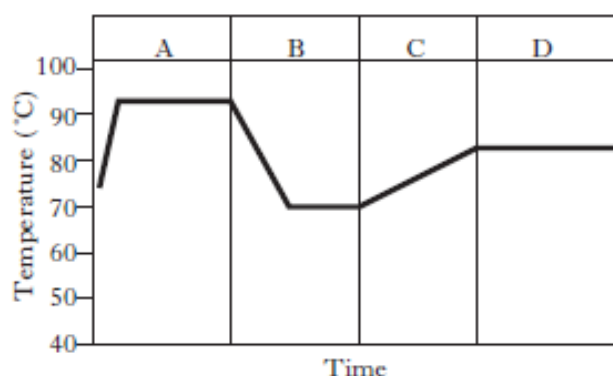
Which of the following is the correct order of the occurrence of these stages in PCR?

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

 5. 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.

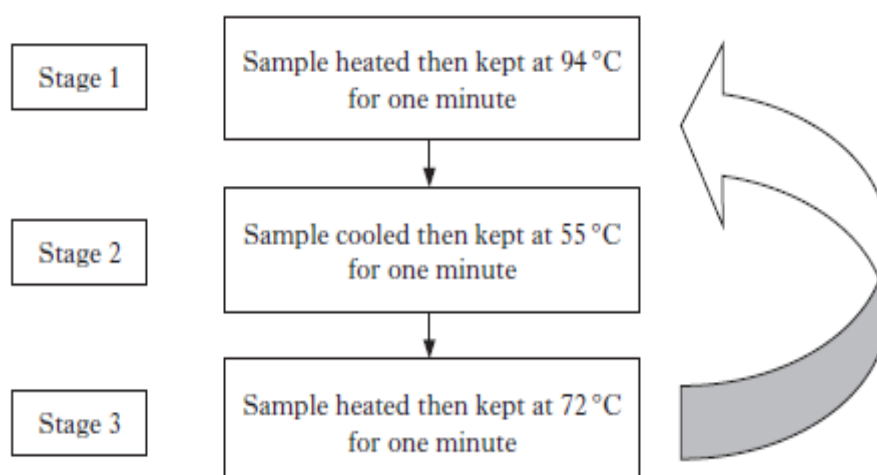
6.

1. 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?

7. The polymerase chain reaction (PCR) amplifies specific sequences of DNA *in vitro*. The flow chart below shows how a sample of DNA was treated during a cycle of the PCR procedure.



- (a) Describe the effect of heating the DNA at Stage 1. (1)
- (b) Give the reason for decreasing the temperature to 55 °C at Stage 2. (1)
- © (i) Name the enzyme used to replicate DNA. (1)
- (ii) State the role of primers in DNA replication during PCR and explain why two different primers are needed.

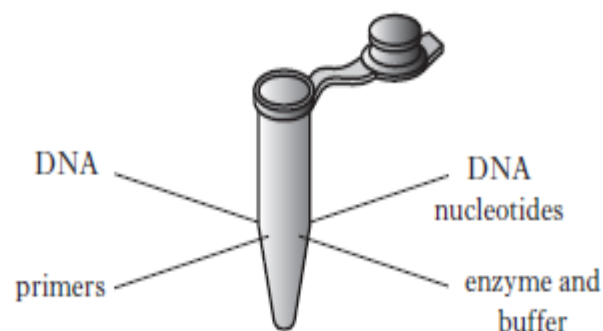
Role _____ (1)

Explanation _____ (1)

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

Calculate the number of cycles needed to produce 128 copies of a single DNA molecule. (1)

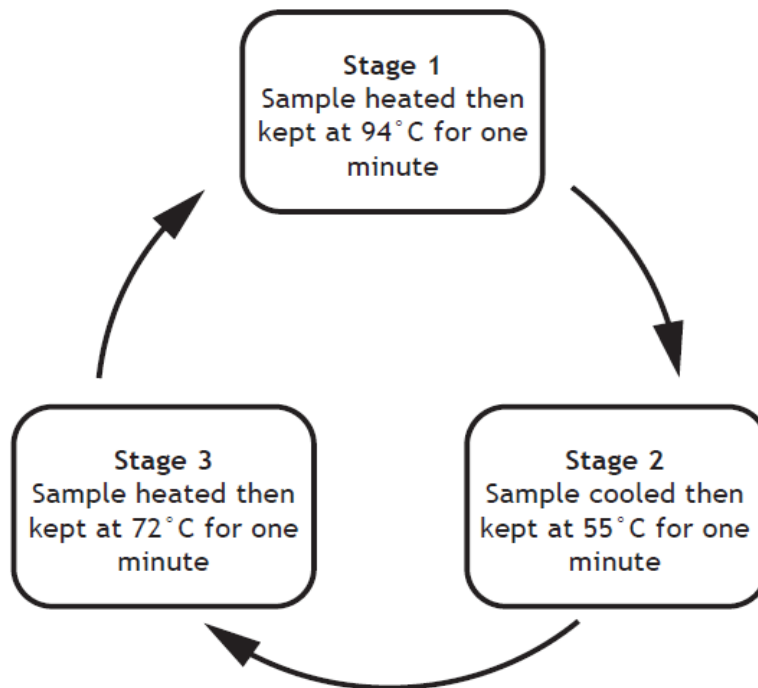
(e) The diagram below shows the contents of a tube used in a PCR procedure.



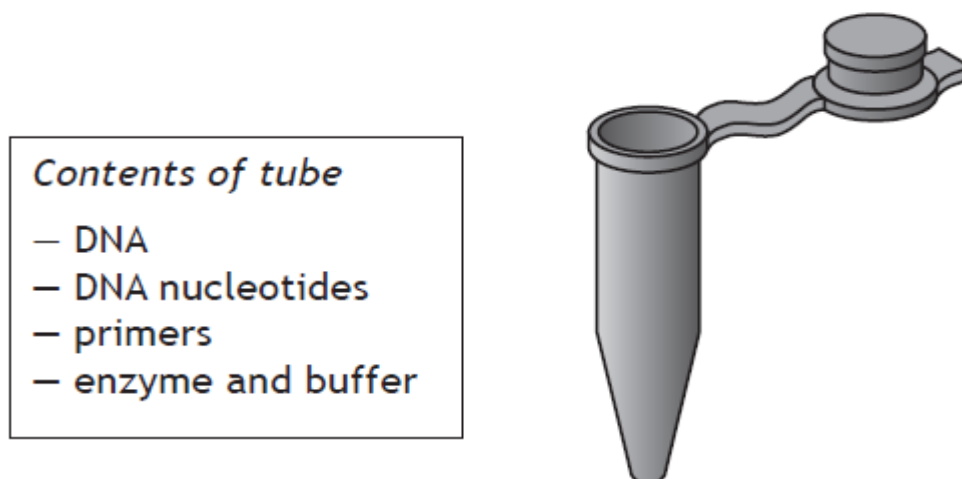
Describe the contents of a suitable control tube designed to show that DNA is needed in a PCR reaction. (1)

8. The polymerase chain reaction (PCR) amplifies specific sequences of DNA.

The flow chart below shows how a sample of DNA was treated during a cycle of the PCR procedure.



- (a) Explain the purpose of the different heat treatments in Stage 1 and Stage 2. (1)
- (b) The number of DNA molecules doubles during each cycle of the PCR procedure. Calculate the number of cycles needed to produce 128 copies of a single DNA molecule. (1)
- (c) The diagram below shows the contents of a tube used in PCR.



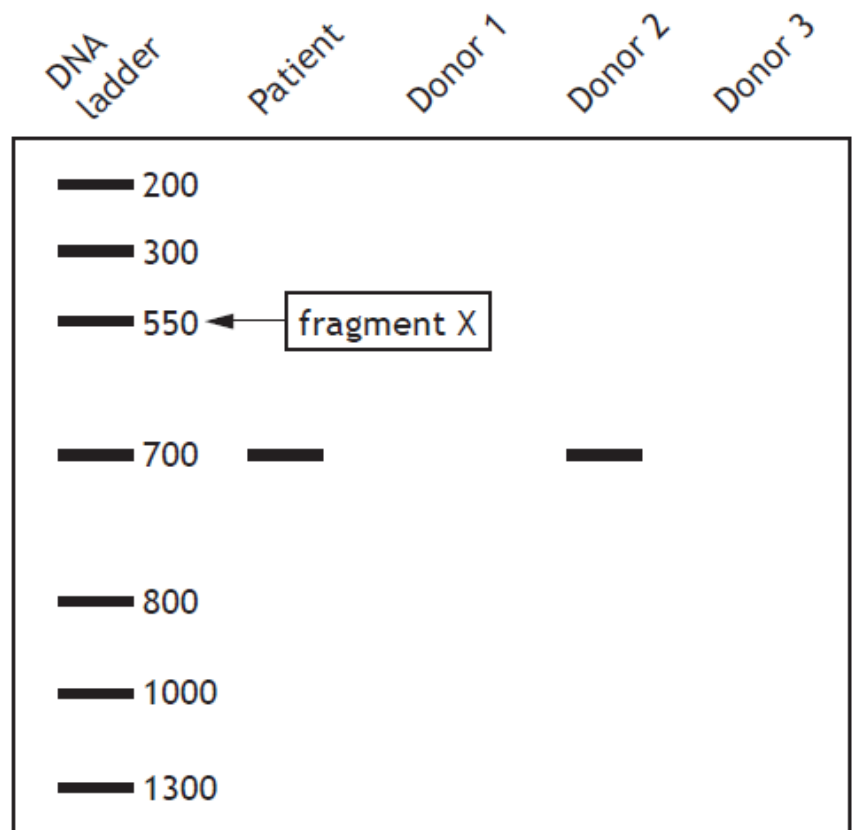
Describe the contents of a suitable control tube designed to show that primers are needed in the reaction. (1)

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

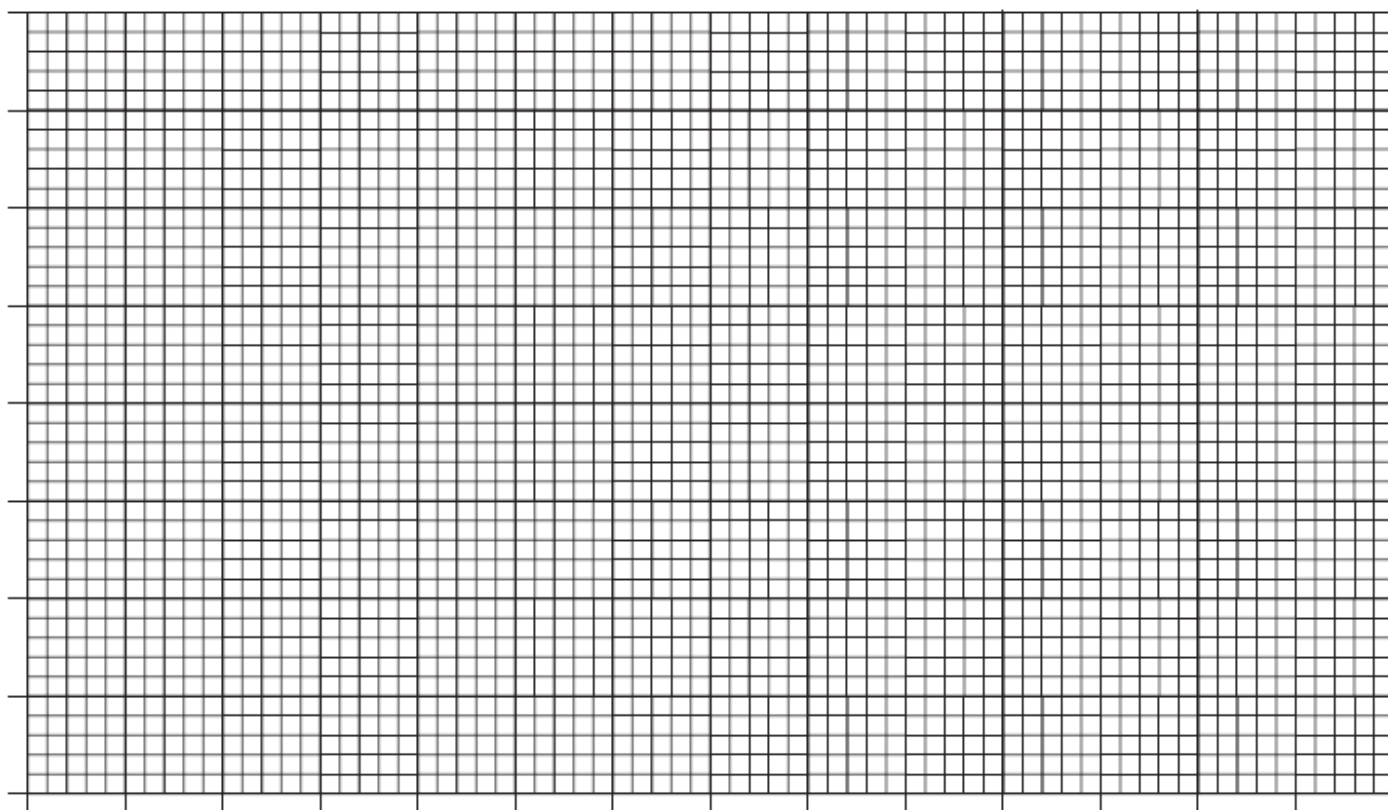
9. Patients requiring an organ transplant are tissue typed to match with potential donors. Polymerase chain reaction (PCR) and gel electrophoresis are used to compare DNA sequences of the patient with those of donors. Gel electrophoresis separates mixtures of DNA fragments according to size. The presence 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.

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

Size of DNA fragment (bp)



- (a) The gel used for electrophoresis contains agarose. Calculate the mass of agarose required to make 30 cm³ of a 0.8% agarose gel.
- (b) Using information in the **table** and the **diagram** give the distance travelled by fragment X in the DNA ladder.
- (c) On the grid below, draw a line graph to show the distance travelled against the size of DNA fragment (2)



- (e) (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.

<i>Temperature (°C)</i>	<i>Reason</i>
94	
	Allows primer to bind to target sequence

10. DNA holds the genetic information in both prokaryotic and eukaryotic cells.

(a) (i) Describe **one** organisational difference between prokaryotic and eukaryotic chromosomal DNA. (1)

(ii) Name the substance with which DNA is packaged in eukaryotes. (1)

(b) State **one** location, other than the nucleus, where DNA is found in eukaryotic cells. (1)

(c) During DNA replication two new daughter strands are synthesised using the original strands as templates.

(i) State why the antiparallel nature of the DNA molecule results in one of the strands being synthesised in short fragments. (1)

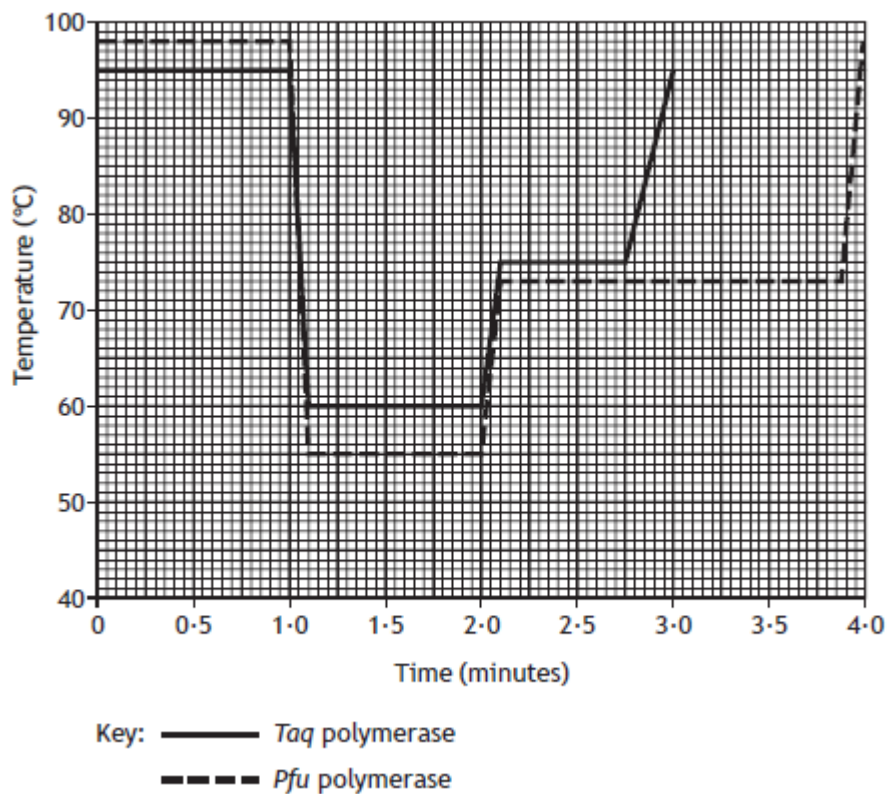
(ii) Template DNA, enzymes and ATP are necessary for DNA replication.

State **one** other substance required. (1)

(d) Explain why cells need to carry out DNA replication. (1)

11 Two heat-tolerant DNA polymerases used in polymerase chain reactions (PCR) are *Taq* and *Pfu*. *Pfu* has “proof reading” activity. It checks that the correct nucleotides are inserted during replication of a target sequence and then corrects any errors.

The graph shows the temperatures during a single PCR cycle required to amplify a target sequence using *Taq* and *Pfu*.



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

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

From _____ to _____ minutes (1)

(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.

DNA polymerase _____

Advantage _____ (1)

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