BIOLOGY 2017

Unit 4
Key Topic Test 4 – DNA manipulation

Recommended writing time*: 45 minutes
Total number of marks available: 45 marks

SOLUTIONS

SECTION A: Multiple-choice questions (1 mark each)

Question 1
Answer: D

Explanation:
A is wrong as PCR is a synthetic process. B is wrong as DNA helicase is not used during PCR. C is wrong as DNA replication does not result in the production of billions of copies of DNA from a single template. D is correct as both processes involve the template strands of DNA being read by DNA polymerase. In PCR, the DNA polymerase is usually taq polymerase.

Question 2
Answer: C

Explanation:
A is wrong as the smallest fragment would be produced by using Hind II only. B is wrong because although 4 fragments would be produced by using PovI only, 4 fragments would also be produced if only Hind III were used. C is correct as 7 fragments would be produced if both enzymes were used. D is wrong as 6 fragments would be produced if both enzymes were used.

Question 3
Answer: A

Explanation:
A vector is used to transfer DNA from one organism to another. The plasmid into which foreign DNA has been placed acts as a vector because when a bacterium takes up the plasmid they will take up the foreign DNA incorporated into the plasmid.

Question 4
Answer: C

Explanation:
It is important to be precise as both DNA ligase and RNA ligase exist and perform different functions. DNA ligase is the enzyme that is used to anneal fragments of DNA.

Question 5
Answer: C

Explanation:
The ends of the DNA would be complementary to each other. If the ends were identical annealing would not be able to occur.

Question 6
Answer: B

Explanation:
DNA ligase is used as a sealing agent, the role of DNA ligase is to anneal strands of DNA.

Question 7
Answer: A

Explanation:
The size of a DNA fragment determines the extent to which it will move through the gel. Smaller fragments move further through the gel, whilst larger fragments remain closer to the originating well.

Question 8
Answer: D

Explanation:
Although bacteria do reproduce very quickly, their value in gene cloning relies on the fact that they can take up, replicate and pass on recombinant plasmids.

Question 9
Answer: B

Explanation:
Plasmids are small, circular pieces of extrachromosomal DNA that are found in bacterial cells.

Question 10
Answer: A

Explanation:
Taq polymerase is the type of polymerase that is used in PCR. It comes from the bacterium *Thermus aquaticus*, which inhabits hot environments. Therefore, it can withstand temperatures over 90 degrees, which are used during PCR, without denaturing.

SECTION B - Short-answer questions

Question 1 (10 marks)

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

1 mark

c. DNA helicase.

1 mark

AND

The role played by DNA helicase is to separate the strands of DNA so that they can be exposed and read. Raising the temperature to about 92°C during the first stage of PCR accomplishes the same purpose, so DNA helicase is not necessary.

1 mark

d. 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

e. 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

Total 10 marks

Question 2 (14 marks)

a. EcoRI is a restriction enzyme (endonuclease)

1 mark

AND

The function of EcoRI is to recognise, bind to and cut specific sequences of DNA, producing fragments with sticky ends.

1 mark

b. EcoRI is shown in conjunction with both the plasmid and the foreign DNA because both pieces of DNA have to be cut with the same restriction enzyme in order for them to have complementary sticky ends which enables the foreign DNA to be inserted into the plasmid.

1 mark

c. The gene for antibiotic resistance is included in the plasmid because it is essential to be able to identify the bacteria that have been transformed. Inserting the resistance gene enables an antibiotic to be used as a screening agent.

1 mark

d. Sticky ends.

1 mark

e. Blunt ends may be made.

1 mark

AND

Sticky ends are the best type of end to produce.

1 mark

AND

If DNA has sticky ends then pieces of DNA may be annealed together. If blunt ends are produced then the pieces of DNA will not anneal together with great efficiency.

1 mark

f. Recombinant.

1 mark

AND

Recombinant DNA contains DNA from two or more different sources. In this case, the plasmid contains the desired gene and the antibiotic resistance gene.

1 mark

g. The bacteria are transformed.

1 mark

AND

As the bacterium now has new genetic material that it can express, the phenotype of the bacterium changes.

1 mark

h. Not all bacteria take up the recombinant plasmid and transform. The two colours represent the transformed bacteria and the non-transformed bacteria.

1 mark

AND

The bacteria that did not transform do not have the gene for antibiotic resistance and will be killed by the antibiotic. Therefore, only the transformed bacteria remain at the end of stage 6.

1 mark

Total 14 marks

Question 3 (11 marks)

a. Starting from the top of the page, the sizes should be 16, 14.5, 13, 11.5, 9, 7.5, 6, 4.5, 3 and 1.5

1 mark

b. The purpose of using a standard solution when performing electrophoresis is to enable the size of unknown fragments to be estimated because DNA fragments of the same size move through the gel to the same extent.

1 mark

c. Standard solutions cannot be used to determine the exact size of a DNA fragment.

1 mark

AND

The strand in lane 2 is somewhere between 7.5 and 9kb in length, but the exact size cannot be stated.

1 mark

d. Lane 2 contains the plasmid that was cut open.

1 mark

AND

The plasmid is 8kb in length. The fragment in lane 2 is the only fragment that is between 7.5 and 9 kb in length.

1 mark

AND

Lane 2 contains the gene.

1 mark

AND

The gene is 2 kb in size. Lane 2 contains the only fragment between 1.5 and 3kb in length.

1 mark

AND

Lane 4 contains the gene annealed to the plasmid.

1 mark

AND

The length of the annealed gene and plasmid is 10kb. The fragment in lane 4 is the only fragment whose length is between 9.5 and 11kb.

1 mark

e. Gel electrophoresis is used to confirm that particular bacteria colonies have taken up the recombinant plasmid. It would be pointless to grow bacteria that do not contain the gene insert.

1 mark

Total 11 marks