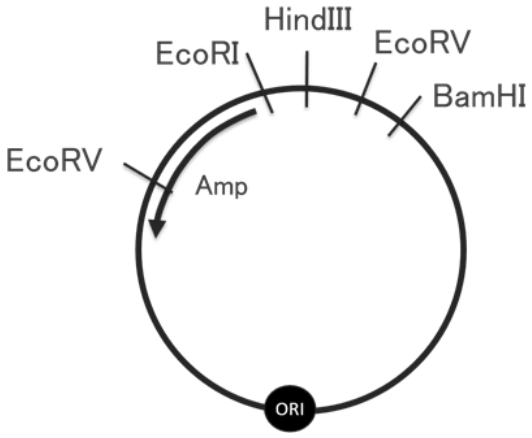
DNA technologies - Gene cloning

SECTION A - Multiple-choice questions

Use the following information to answer questions 1 and 2

The diagram below is a map of a bacterial plasmid showing the origin of replication (ORI) and selected restriction enzyme cutting sites.



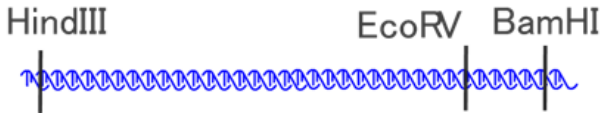
Question 1

The plasmid was mixed with the restriction enzymes EcoRV and HindIII. The number of fragments created by this restriction digest is:

- ☐ A 2
- ☐ B 3
- ☐ C 4
- ☐ D 5

Question 2

A gene was amplified using PCR to prepare it for insertion into the plasmid. The gene has the recognition sites shown in the diagram below.

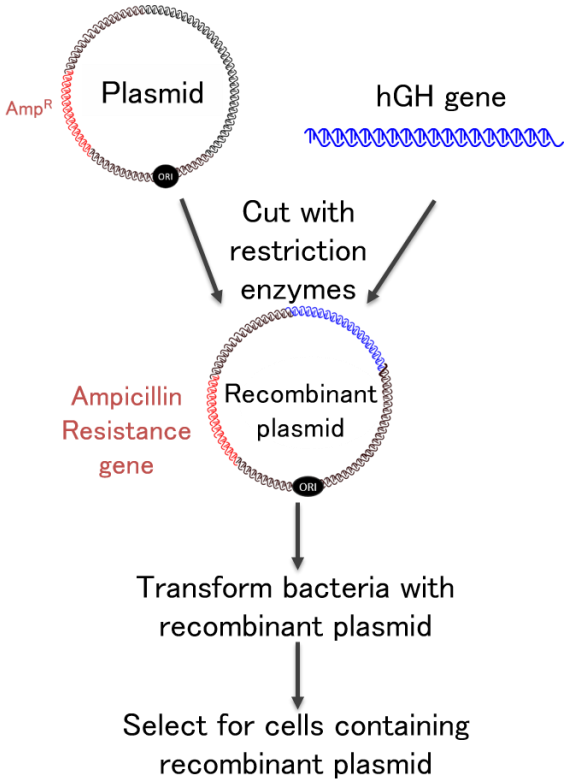


The ideal restriction enzymes to digest the plasmid and the gene insert are :

- ☐ A HindIII, EcoRV and BamHI
- ☐ B HindIII and EcoRV
- ☐ C HindIII and BamHI

Use the following information to answer questions 3 - 5

The diagram below shows the steps involved in gene cloning.



Question 3

The vector used in this gene cloning process is:

- ☐ A The plasmid
- ☐ B The bacteria
- ☐ C The recombinant plasmid
- ☐ D The hGH gene

Question 4

A bacteria cell line is mixed with the recombinant plasmid and a small amount of bacteria are transformed when the recombinant plasmid enters the bacteria. These bacteria can use gene expression to make new proteins. The new proteins they produce are

- ☐ A Plasmid protein and human growth hormone
- ☐ B Ampicillin resistance protein and human growth hormone

Question 5

To select for the bacteria that have been transformed you should:

- ☐ A Add ampicillin so transformed bacteria can survive
- ☐ B Add ampicillin to kill any bacteria that have not been transformed

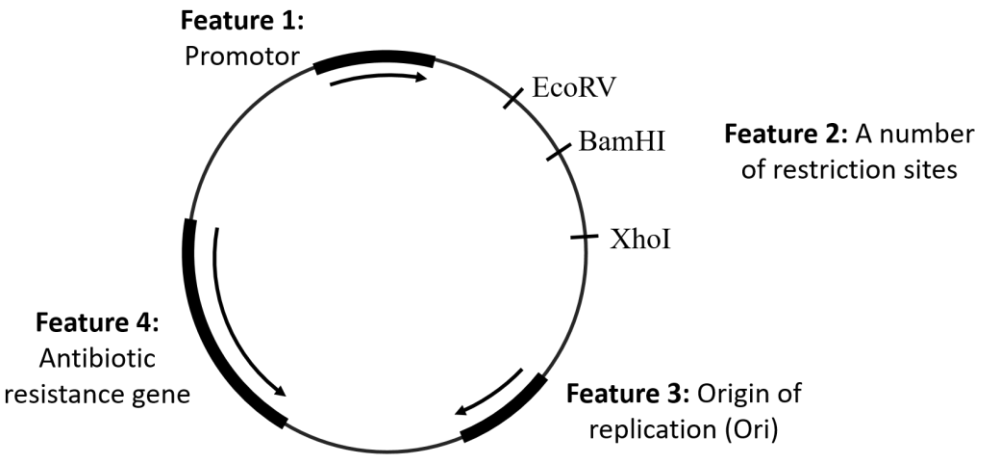
Written responses – Gene Cloning

Question 1 (7 marks)

Type 1 diabetes is a chronic condition in which the pancreas produces little or no insulin, a hormone involved in blood glucose regulation. Since 1922, type 1 diabetes has been treated by injecting insulin into the body. The first marketed insulin treatment was produced by extracting and purifying insulin from the pancreas of pigs and cows. Animal-derived insulin is still available to diabetics, however, the extraction and purification process has improved to reduce health issues that were associated with the early marketed animal-derived insulin treatments.

1a. Suggest a health issue that might be associated with the first animal-derived insulin treatments. (**1 mark**)

In 1982, Humalin became the first therapeutic protein product available that was made using recombinant DNA technology. Diabetics could now be treated with human insulin that was produced on a large scale by bacteria. The diagram on the next page shows the plasmid map for a plasmid vector used in Humalin production.

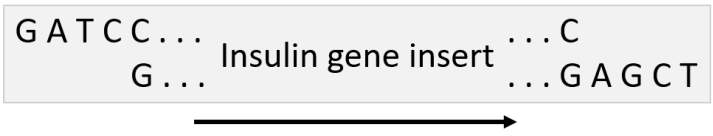


1b. Referring to the diagram, select 2 features of this plasmid vector and describe why each feature is required in gene cloning for insulin production in bacteria. (**2 marks**)

Selection 1:

Selection 2:

DNA containing the human insulin gene was digested with restriction enzymes in preparation for insertion into the plasmid vector. An image of the digested insulin gene insert is shown below.



The recognition sites for the three restriction enzymes shown in the plasmid map are presented in the table below. The results of a DNA digest with that enzyme are also shown.

Restriction enzyme	Recognition site	Digest result
EcoRV	5′... G A T ▼ A T C ...3′ 3′... C T A ▲ T A G ...5′	5′... G A T 3′... C T A A T C ...3′ T A G ...5′
BamHI	5′... G ▼ G A T C C ...3′ 3′... C C T A G ▲ G ...5′	5′... G 3′... C C T A G G A T C C ...3′ G ...5′
XhoI	5′...C ▼ T C G A G ...3′ 3′...G A G C T ▲ C ...5′	5′...C 3′...G A G C T T C G A G ...3′ C ...5′

1c. Identify the restriction enzymes required to digest the plasmid vector and justify your choice. (**2 marks**)

1d. The digested plasmid vectors and the digested insulin gene inserts were combined in a tube. In order to create recombinant plasmids, an enzyme also needs to be added to the tube. Name this enzyme and explain its role in the process. (**2 marks**)

Suggested responses begin on the next page.

DNA technologies – Gene cloning written responses: Suggested responses

In general, note the command terms: suggest, select, describe, identify, justify, name and explain.

1a. **Suggest** a health issue that might be associated with the first animal-derived insulin treatments. (**1 mark**)

Response you wrote:

Suggested response:

Health issues could include, but are not limited to, the following:

- Swelling and pain (inflammation) around the injection site
- Allergic reactions caused by an immune response
- Transmission of diseases
- Side effects leading to other health complications

Tips for answering this question: The command term is "suggest" so the examiner is seeking any plausible health issue that may have been experienced by diabetics injecting the first animal-derived insulin treatments. To receive a mark the student should consider how injecting insulin extracted from the pancreas of cows and pigs may cause health issues. Injecting animal-derived insulin may lead to an adverse immune response causing inflammation and allergic reactions. As stated in the question stem the purification process has improved since early manufacture. Early treatments may have contained impurities including other animal products that could also cause an allergic reaction, or could result in transmission of disease. For example, extractions from cows may transmit the causative agent of Creutzfeldt-Jakob disease.

1b. Referring to the diagram, **select** 2 features of this plasmid vector and **describe** why each feature is required in gene cloning for insulin production in bacteria. (**2 marks**)

Response you wrote:

Suggested response:

Select and describe any two of the following features:

- *Feature 1: The promotor region* – this is required so that the inserted insulin gene is transcribed once the plasmid is inside the host bacteria.
- *Feature 2: A number of restriction sites* – this is required so restriction enzymes can be used to cut open the plasmid allowing for the insulin gene to be inserted into the plasmid.
- *Feature 3: The origin of replication* – this is required so plasmid DNA replication will occur when host bacteria cells divide producing new bacteria that contain the plasmid.
- *Feature 4: Antibiotic resistance gene* – this is required as a selective agent as host bacteria transformed with the plasmid will survive in the presence of that antibiotic while all other bacteria die.

Tips for answering this question: The command terms are "select" and "describe". Students should refer to the diagram of a plasmid map and select any two of four possible features shown. Students are awarded marks for their descriptions. When describing, students must provide a detailed account of how the selected feature functions in gene cloning and insulin production. Students should draw on knowledge of gene cloning. The aim of gene cloning, in this example, is to get bacteria to use the human insulin gene insert to produce insulin. For gene cloning to be successful the human insulin gene must be inserted into the plasmid vector. This recombinant plasmid must then enter host bacteria. Host bacteria must copy the plasmid and pass it on when they divide. Any host bacteria that don't contain the plasmid are removed by growing bacteria in the presence of the specific antibiotic that transformed cells are no longer sensitive to. To receive 2 marks select two features and describe the purpose of each.

1c. Identify the restriction enzymes required to digest the plasmid vector and **justify** your choice. (**2 marks**)

Response you wrote:

Suggested response:

- BamHI and XhoI
- The sticky ends of the gene insert show that it was digested with BamHI and XhoI and restriction sites for these enzymes are present on the plasmid map. Digesting the plasmid with the same restriction enzymes will result in compatible sticky ends to insert the gene.

Tips for answering this question: The command term here is “identify” and “justify”. When identifying, the student must single out the correct answer from the list of possible restriction enzymes shown on the plasmid map. To choose the correct restriction enzymes, students must draw on knowledge of recombinant DNA technology to understand that the ends of the insulin gene insert and the plasmid DNA will need to be complementary, so they need to be digested by the same enzymes. Looking at the recognition sites in the restriction enzyme table, and at the sticky ends produced by these enzymes and by the digest of the insulin gene insert, students can determine it was digested with BamHI and XhoI. The plasmid map shows the restriction sites for these enzymes. When justifying, students must give a valid reason or evidence to support their answer. They should refer to the enzymes that created the sticky ends of the insulin gene insert and the presence of restriction sites for these enzymes on the plasmid map. Digesting the plasmid with the same restriction enzymes will result in complementary sticky ends so the gene insert will bind to the sticky ends of the plasmid. Students receive 1 mark for identifying the correct restriction enzymes and 1 mark for providing a justification related to complementary base pairing between ends of plasmid DNA and the insulin gene insert.

1d. The digested plasmid vectors and the digested insulin gene inserts were combined in a tube. In order to create recombinant plasmids, an enzyme also needs to be added to the tube. **Name** this enzyme and **explain** its role in the process. (**2 marks**)

Response you wrote:

Suggested response:

- DNA ligase
- The role of DNA ligase is to attach the insulin gene DNA to the plasmid DNA by restoring bonds in the DNA backbone.

Tips for answering this question: The command terms are “name” and “explain” so the student must draw on knowledge of recombinant DNA technology to identify that DNA ligase is required to join pieces of DNA to construct a recombinant plasmid, and then explain the role of the enzyme. When explaining, the student is required to provide the reason(s) or the cause(s) for an observation or an event. In this case the student should communicate the need for DNA ligase to join the breaks in the DNA backbone between the gene insert and the digested ends of the plasmid vector. Students receive one mark for naming the enzyme and one mark for explaining the role of the enzyme.