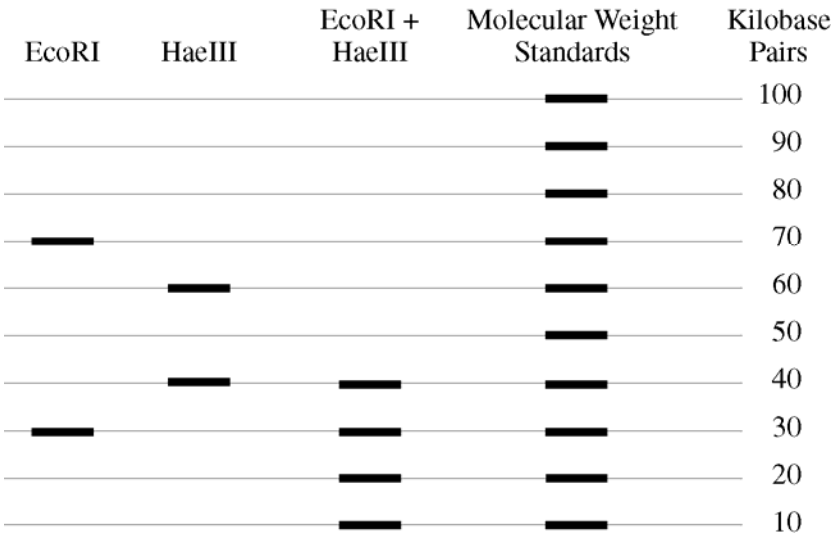
AP® BIOLOGY
2007 SCORING GUIDELINES

Question 4

A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double digest). The fragments were then separated with electrophoresis, as shown.

RESULTS OF GEL ELECTROPHORESIS



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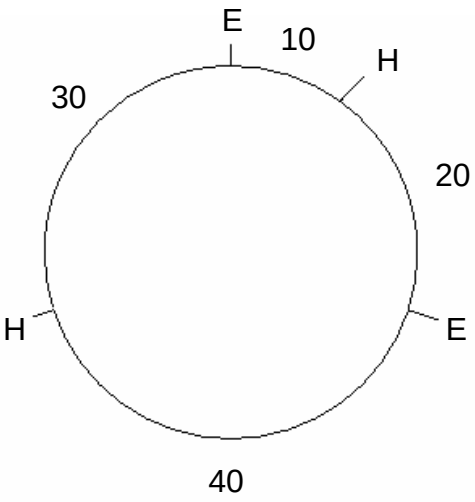
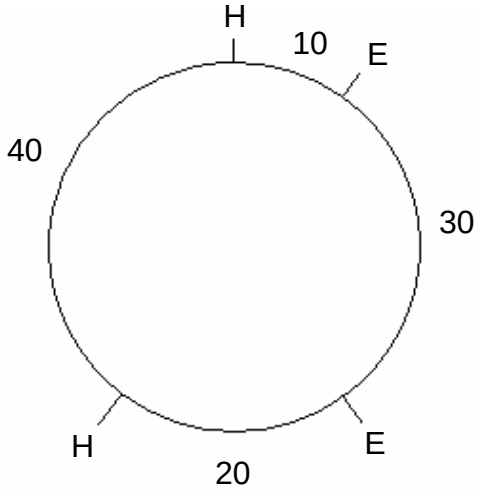
Question 4 (continued)

(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

Construct a labeled map and **explain (3 points maximum)**





E = EcoRI Restriction Point
H = HaeIII Restriction Point

- Restriction sites correctly placed and kilobase sizes shown **(2 points)**

- Explanation **(1 point)**
  (NO POINTS for explanation with incorrect or missing map OR for interpreting gel only)
  - trial and error discussion
  - restriction site within larger fragment

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Question 4 (continued)

(b) **Describe** how:

- Recombinant DNA technology could be used to insert a gene of interest into a bacterium
- Recombinant bacteria could be identified
- Expression of the gene of interest could be ensured

Describe how to: (6 points maximum)

(1) Insert gene of interest (4 points maximum)

- Cut gene of interest from source and/or cut plasmid with restriction enzyme
- Use SAME restriction enzyme on both
- Anneal/ligate/mix/combine gene of interest with vector (plasmid/virus/phage)
- "Sticky ends"/bp matches/complementarity
- Treatment for competent cells ($CaCl_2$/heat shock); incubate together
- Chemical modification can prevent restriction enzyme activity (e.g., methylation)
- Gene = cDNA (without introns) to fit into plasmid

(2) Identify recombinant bacteria (1 point)

- Phenotypic selection (antibiotic resistance/blue-white colony selection/"glo" gene, product produced [e.g., insulin])
- Radioactively/fluorescently labeled probe (tag/dye) / mRNA
- Electrophoresis of cut recombinant vs. original (gene/plasmid) **OR** with sequence comparison of recombinant vs. original (gene/plasmid) **(Not bacterial genome)**

(3) Ensure expression of gene of interest (1 point)

- Promoter [for prokaryote]
- cDNA/removal of introns for prokaryotic expression
- Operon (e.g., nutrient/arabinose induced)

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem. **(3 points maximum)**

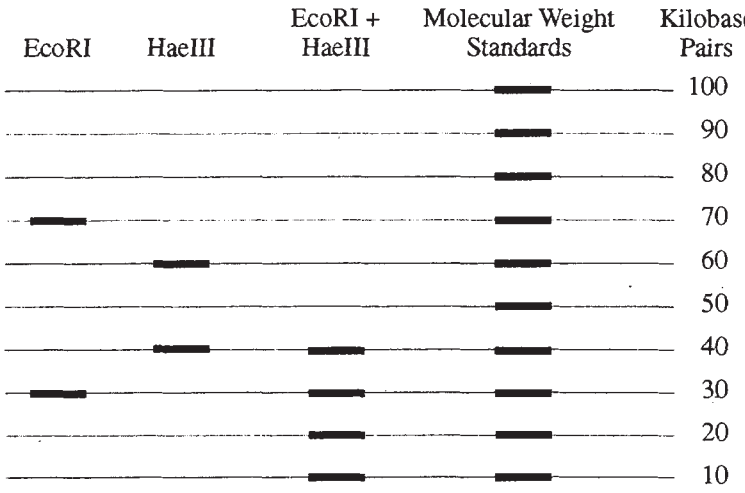
Discuss GM, benefit to humans, and threat to population/ecosystem

- Nonhuman organism with specific, heritable GM trait
- Plausible benefit to humans related to the GM trait
- Plausible or unknown threat to population/ecosystem related to GM trait/modified organism

4A,

4. A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double digest). The fragments were then separated with electrophoresis, as shown.

RESULTS OF GEL ELECTROPHORESIS

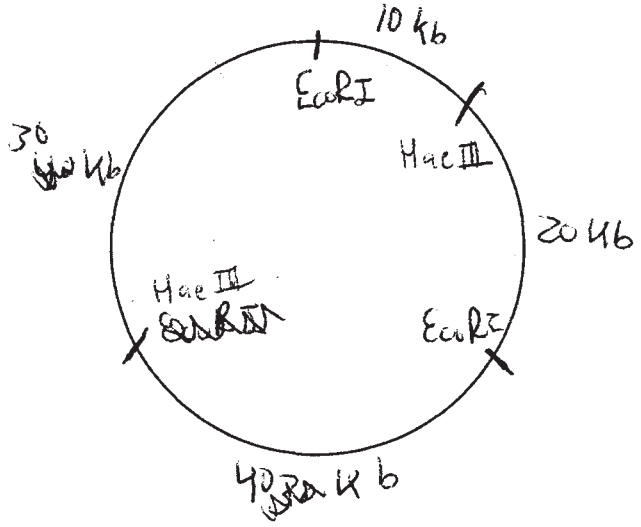


(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

(b) **Describe** how:

- recombinant DNA technology could be used to insert a gene of interest into a bacterium
- recombinant bacteria could be identified
- expression of the gene of interest could be ensured

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem.



1 = place where restriction enzyme cut

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ADDITIONAL PAGE FOR ANSWERING QUESTION 4

B) Recombitant DNA technology could be used to insert a gene of interest into a bacteria. First, restriction enzymes would be use to cut out the gene of interest from another organism's DNA. Then, restriction enzymes would also be used to cut a plasmid, forming "sticky ends." The gene of interest would be spliced into the ~~plg~~ plasmid and the sticky end of the gene would attach to the sticky ended the plasmid. Ligase would then join the pieces together. Next, the scientist would just have to expose the bacterium to an ~~antibiotic~~ solution with the plasmids in it; bacteria often take up plasmids from the environment in transduction. Thus, the gene of interest would be incorporated into the bacteria.

Recombitant bacteria could be identified by ~~putting~~ testing for the phenotype you hoped to get. For example, if a gene for Ampicillin resistance was put into the bacteria, you would test to see which bacteria picked up the plasmid by putting all the bacteria onto a plate with ampicillin. The bacteria that live and reproduce, forming colonies are the ones that picked up the gene.

Expression of the gene of interest could be ensured if the scientist turned off the ~~intact~~ genes of the bacteria that are interfering with the expression of the gene of interest. Or, scientists could ensure the expression of the gene by ~~cutting~~ first identifying the gene of the bacteria that might interfere with the

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ADDITIONAL PAGE FOR ANSWERING QUESTION 4

expression of the gene of interest and the splice the gene of interest into the cell. The scientist would also want to turn on the gene by giving the bacteria the transcription factor needed to activate that gene.

C) Genetically modified organisms can provide benefits for humans and at the same time pose a threat to a population or ecosystem. For example, scientists might genetically alter bacteria to secrete insulin. This is beneficial for humans with diabetes because they will be able to take supplements of the bacteria produced insulin and thus can regulate their blood sugar level. However, this can also be ~~toxic~~ harmful to a population or an ecosystem. If, for example, some of the genetically altered bacteria get ~~out~~ out of the lab, they may be ingested ~~by~~ or somehow infect another animal. If ~~a~~ a bacteria such as E. Coli was modified then the bacteria will have an affinity ~~for~~ for infecting mammals and will be able to survive in the mammals. However, because these bacteria are still secreting insulin, the mammal will have elevated insulin levels. This will cause low blood sugar levels, and could harm or even kill the infected mammals. ~~There~~ Thus, a population or ecosystem could be severely affected by genetically modified organisms as a domino effect occurs when one type of mammal dies, causing their predators to die, and

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On.

ADDITIONAL PAGE FOR ANSWERING QUESTION 4

A) I developed my map by first looking at how many base pairs the 2 fragments after treatment with EcoR I had. I then put these on my map. Then, I observed how many fragments there were after a double digest with EcoRI and HaeIII. As I already had mapped out how many fragments with how many base pairs were made with EcoRI, I knew that there must be a sequence that HaeIII cut 40 base pairs into the larger fragment cut by EcoRI since 40 base pairs was larger than the other fragment (30 kb). Then, when I looked at the fragments caused by HaeIII, I knew there had to be another sequence that HaeIII would cut to form a 60 kb and 40kb fragment. So, I realized that there must be a sequence that HaeIII cuts 10 kb into the smaller fragment caused by EcoRI treatment. This then satisfied the number of fragments and base pair lengths generated from each of the 3 treatments.

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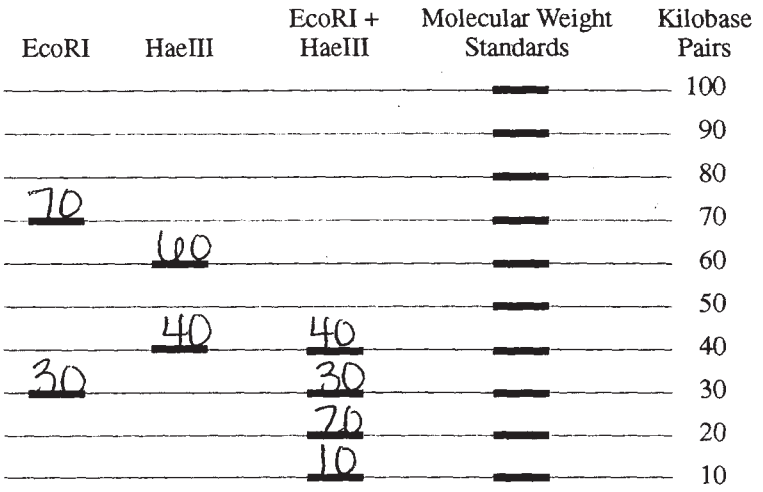
4B

4. A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double digest). The fragments were then separated with electrophoresis, as shown.

RESULTS OF GEL ELECTROPHORESIS

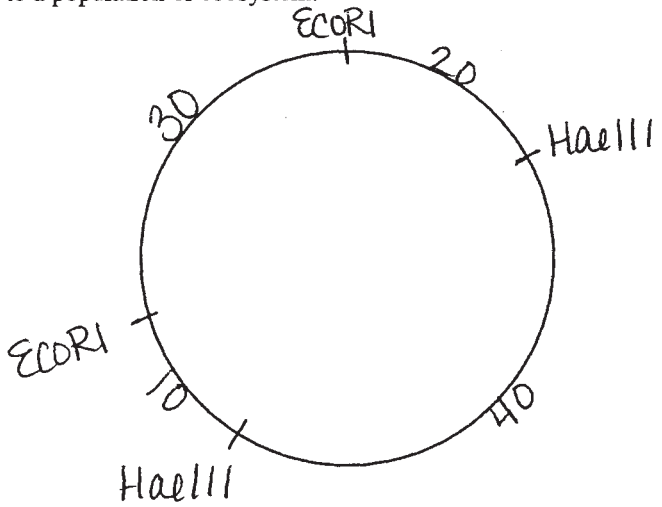


(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

(b) **Describe** how:

- recombinant DNA technology could be used to insert a gene of interest into a bacterium
- recombinant bacteria could be identified
- expression of the gene of interest could be ensured

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem.



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ADDITIONAL PAGE FOR ANSWERING QUESTION 4

In the map above, I developed the cuts by using trial and error. Since the first cut was made with ECORI, I added that to the map first. I then added cuts with HaeIII in order that the rest of the criteria was satisfied.

The process in the above experiment could be used to insert a gene of interest into a bacterium by using the same restriction enzyme to cut both the gene out and the bacterium. The specific gene can then be simply introduced into the bacterium.

The recombinant DNA created can then be identified by using the same restriction enzyme to cut the bacterium, and separating the pieces using gel electrophoresis.

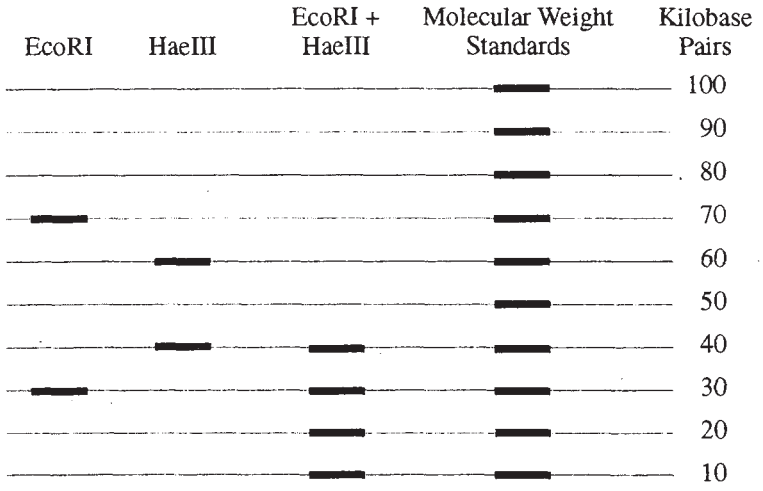
The expression of the gene of interest can be assured by digesting with restriction enzymes all other unwanted kilobase pairs. to assure optimum potential.

While recombinant DNA technology used to genetically modify organisms can be an amazing advance, it could also pose a threat. For example, sheep modified to produce thicker wool to benefit the economy could also prove harmful to the environment. These sheep could now live longer with less susceptibility to cold, and overgraze otherwise fertile land. Before humans genetically modify organisms for themselves, repercussions must be analyzed.

4C,

4. A bacterial plasmid is 100 kb in length. The plasmid DNA was digested to completion with two restriction enzymes in three separate treatments: EcoRI, HaeIII, and EcoRI + HaeIII (double digest). The fragments were then separated with electrophoresis, as shown.

RESULTS OF GEL ELECTROPHORESIS

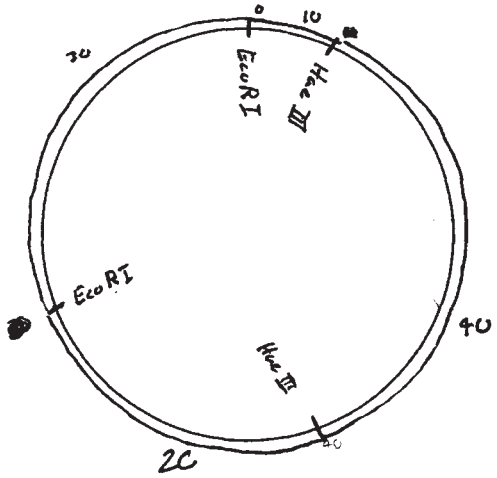


(a) Using the circle provided, **construct** a labeled diagram of the restriction map of the plasmid. **Explain** how you developed your map.

(b) **Describe** how:

- recombinant DNA technology could be used to insert a gene of interest into a bacterium
- recombinant bacteria could be identified
- expression of the gene of interest could be ensured

(c) **Discuss** how a specific genetically modified organism might provide a benefit for humans and at the same time pose a threat to a population or ecosystem.



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4C2

ADDITIONAL PAGE FOR ANSWERING QUESTION 4

I created my restriction map by comparing the plasmid length of EcoRI (30 and 70) and Hae III (60 and 40) as well as the lengths of the remaining plasmids after a double digestion (40, 30, 20, and 10). I then deduced how the plasmid must have been cut

A circular plasmid, when treated correctly, may result in the gene being integrated into a bacteriums DNA. Adding to the plasmid something such as a gene that produces color or glowing under a black light allows one to see that the gene was successfully incorperated. By exposing the bacteria to ~~the~~ a substance that the added DNA gives them a resistance to allows only the modified ones survive.

Genetically modified organisms may be able to provide us with substances that could be used in medicine. However at the same time if the organism is released it may upset an ecosystem since it could have an advantage over a natural organism, in the same way introduced species do.

AP® BIOLOGY
2007 SCORING COMMENTARY

Question 4

Overview

The intent of this question was to test students' ability to describe biotechnology techniques and interpret the data obtained using these techniques. Students needed a working understanding of Lab 6 recommended in the Course Description (bacterial transformation and gel electrophoresis analysis) to adequately answer the question. In addition, they had to apply critical thinking skills to the task of using the gel electrophoresis data to construct and explain their restriction map in part (a). Part (b) of the question required students to explain the essential steps used to insert a gene of interest into a bacterium. In addition, they were asked to describe how these recombinant bacteria could be identified and how the expression of the gene could be ensured. Part (c) addressed the application of biotechnology to genetically modified organisms (GMOs). Students were expected to name a specific GMO with an identified modified trait and discuss how it would be both beneficial to humans and a potential threat to a population or ecosystem.

Sample: 4A
Score: 10

In part (a) 2 points were earned for correctly constructing the restriction map, showing the restriction sites, and correctly labeling the length of the fragments in kilobases. One point for explaining the method of constructing the map would have been earned on page 4 of the response, but the maximum number of points had already been earned.

In part (b) the student received 1 point for using restriction enzymes to cut the gene of interest from the DNA; 1 point for the formation of sticky ends; 1 point for the use of ligase to join the pieces together; and 1 point for mixing the plasmid with the bacteria for plasmid uptake. One more point was awarded in the identification section for ampicillin resistance in the recombinant bacteria. No point was earned in the gene expression section because transcription factors regulate transcription in eukaryotes, not prokaryotes.

In part (c) 1 point was earned for identifying the genetically modified organism (bacteria) with the trait (insulin production); 1 point for the benefit to humans of helping to regulate blood sugar levels; and 1 point for the potential threat to other mammals of increased insulin levels.

Sample: 4B
Score: 7

In part (a) 2 points were earned for correctly constructing the restriction map, showing the restriction sites, and correctly labeling the length of the fragments. One point was earned for explaining the method of constructing the map (trial and error).

In part (b) 1 point was earned for using restriction enzymes to cut the DNA. No point was granted for incorrectly cutting "the bacterium" with the same enzyme. No point was given for the identification of recombinants by gel electrophoresis, because there is no indication of a comparison of the original plasmid and the recombinant plasmid. No credit was awarded for the gene expression section.

In part (c) the student was given 1 point for correctly identifying a genetically modified organism (sheep) with the trait for "thicker wool"; 1 point for the economic benefit to humans; and 1 point for the potential threat to the ecosystem of overgrazing.

Question 4 (continued)

Sample: 4C
Score: 4

In part (a) 2 points were earned for correctly constructing the restriction map, showing the restriction sites, and correctly labeling the length of the fragments. One point was earned for explaining the method of constructing the map (trial and error).

In part (b) no points were earned for insertion, as the explanation does not discuss how the gene would be integrated into the bacteria. One point was awarded for identifying the recombinant bacteria via a *glo* gene. The student does not attempt the gene expression section.

No points were earned in part (c) because the student does not name a specific genetically modified organism.