AP® BIOLOGY
2009 SCORING GUIDELINES (Form B)

Question 1

Describe how a plasmid can be genetically modified to include a piece of foreign DNA that alters the phenotype of bacterial cells transformed with the modified plasmid. **Describe** a procedure to determine which bacterial cells have been successfully transformed.

Describe plasmid modification (8 points maximum):

Topic	Description (1 point each)
Plasmid vector	Describes plasmid as small circular DNA
Cut (cleave) DNAs	Use of restriction endonucleases (RE) Plasmid and inserted DNA must have same RE cut ends or be cut by same RE
Sticky ends	Ends of DNA should be sticky, wanting to bond with matching ends Generate ends for attachment using endonucleases
Ligase	For joining of sticky ends
Orientation	Correct orientation of insertion to ensure expression
Gene of interest	DNA cut should be a complete sequence of gene Attach piece with a promoter or insert next to promoter
Reporter gene	Gene used to identify insertion of desired DNA Insert DNA with a gene that produces a new phenotype
Selective marker	Inserted to help identify the DNA insertion (e.g., antibiotic resistance)
AUG in place	Ensure proper start codon
Uptake of plasmid	Calcium chloride and heat shock, electroporation to make competent
Alternative procedures	Blunt cuts; T4 ligase; add terminal transferase to add poly (A) to 3' end

Describe plasmid uptake and how transformation is **determined (6 points maximum)**:

Topic	Description (1 point each)
Transformation	Defined process of transformation of a plasmid
Isolation	Isolate plasmids/agar plate that grows only colonies of resistance gene
Antibiotic	Use of antibiotic resistance/sensitivity genes Detailed description of antibiotic resistance lab procedure
Gel electrophoresis	Isolate plasmid using electrophoresis Detailed description of gel electrophoresis for isolation
Retrieval	Retrieve altered plasmid
Protein	Identification of new protein, possible glowing marker protein Detailed description of retrieval or protein method
Tag	Fluorescent marker, etc. Detailed description of alternate method

BIOLOGY
SECTION II
Time—1 hour and 30 minutes

Directions: Answer all questions.

Answers must be in essay form. Outline form is not acceptable. Labeled diagrams may be used to supplement discussion, but in no case will a diagram alone suffice. It is important that you read each question completely before you begin to write. Write all your answers on the pages following the questions in this booklet.

1. **Describe** how a plasmid can be genetically modified to include a piece of foreign DNA that alters the phenotype of bacterial cells transformed with the modified plasmid. **Describe** a procedure to determine which bacterial cells have been successfully transformed.

Plasmids are circular DNA fragments of bacteria which replicate independently from the organism. Plasmids can be used in order to modify organisms with inserting a gene. In order to do so, both the plasmid and gene of interest, the gene that will be inserted into the plasmid to modify the bacterium, should be cut with a restriction enzyme. A restriction enzyme is a special enzyme that cuts the DNA ~~in~~ on a specific sequence forming sticky ends. Sticky ends are complementary to each other; therefore they stick to each other easily. When both the plasmid and the gene of interest are cut, they should be brought together and sticked to each other with the enzyme ligase. The gene of interest should be cut ~~that~~ at the places (sequences of nucleotides): one before the gene of interest and one after. Ligase forms covalent bonds between the sticky ends which have already formed hydrogen bonds between the complementary sequences of nucleic acids. (such as A with T and G with C). Then the vector, a special plasmid that has been inserted with a gene of interest, should be inserted into the bacterium. This

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can happen by transformation (the uptake of foreign DNA by the bacterium itself.) Whether the plasmid has been successfuly taken in or not can be determined in two ways. First one is through gene expression. If the bacteria has taken in the plasmid, it contains the gene of interest. Therefore, if the favorable conditions are met, such as the PH level, temperature and food supply, and the cell is supplied with ribosomes, the gene of interest will be expressed, if it is inserted in an operon., which is a subunit of DNA that is involved in gene expression in prokaryotes. It has a operator and the gene of interest, in this case the gene that was inserted in the plasmid. Thus, if the protein product of this gene is produced (this can be resistance to a antibiotic eg: ampicilin), the gene has been successfully taken in and the bacterium has been transformed. However this method takes a lot of time. The second and easier method is to use radioactive probes, which are complementary DNA sequences (cDNA) that are radioactive. Probes glow in darkness. Therefore, special probes for the gene of interest can be used to identify the inserted gene. Probes should be put into the same environment with the bacterium (they can be transformed into it or transduction can take place which is a virus inserting its DNA into a bacterium.) Then the probes will fit into the gene of interest because they're complementary. When all the [redacted] probes that do not complement a gene or nucleotide sequence is rinsed away, the glowing part is (washed)

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the gene of interest. Thus, if the bacteria grows, it is because it contains the gene of interest. And therefore, it has been successfully transformed.

1 B1

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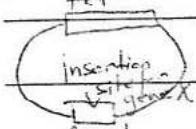
1. **Describe** how a plasmid can be genetically modified to include a piece of foreign DNA that alters the phenotype of bacterial cells transformed with the modified plasmid. **Describe** a procedure to determine which bacterial cells have been successfully transformed.

To genetically modify a plasmid to include a piece of foreign DNA. We first have to select (or use) a specific kind of plasmid. A plasmid with tetracycline resistance gene (or any other antibacterial resistance gene) and along with β-gal gene.



Then we select a restriction enzyme that can cut open a site in the β-gal gene. We use the same restriction enzyme to cut the foreign DNA (gene X). Next step is to put the open plasmid and the foreign DNA in one place and add a special enzyme called ligase, which helps the bonding of phosphate di ester bonding. The next step is to put the plasmid from the step before and the bacterial cell in one place. Give a slight heat shock or electric shock to help get the plasmid in to the bacterial cell. (This stage can be avoided if the foreign DNA was inserted directly in to the bacterial cell, then bonded in to the plasmid while still in side the bacterial cell.) When the all of the steps are done you will be left with bacterial cells containing genetically remodified plasmids.

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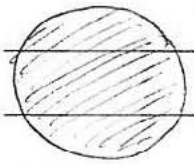
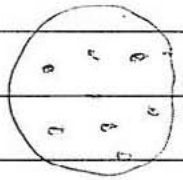
There are three steps required in determining which bacterial cell has the modified plasmid.

First, Use non-genetically modified bacterial cells and culture them in agar gel. The bacteria will probably cover the entire surface.

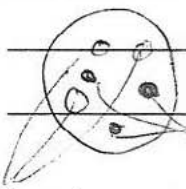


This will be used as reference.

Second, add tetracycline in to the agar gel and culture the genetically modified bacterial cells. In contrast to the experiment above only a few colony will appear. These colonies means the bacterial cells that have a tetracycline resistant plasmid inside of them. However we cannot be sure that all of these bacteria cell colonies have the foreign DNA in the plasmid. This can be determinded in the third step.



Third, add X-gal to the colonies in the step two.
The plasmid with the foreign DNA in the β-gal gene will not be able to transcript the β-gal gene. Thus will not be able to use X-gal. However plasmid with out the foreign DNA will be able to use the X-gal and light up blue. So selecting the bacterial cell colonies that don't light up blue will be same as selecting the bacterias that has ff foreign DNA.



Bacterial cell without foreign DNA.

Bacterial cell with foreign DNA ⟹ Successfully transformed.

1B3

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The phenotype of the bacterial cells are altered when foreign DNA is inserted because bacterial cells with foreign DNA in them can not use X-gal.

1C,

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1. **Describe** how a plasmid can be genetically modified to include a piece of foreign DNA that alters the phenotype of bacterial cells transformed with the modified plasmid. **Describe** a procedure to determine which bacterial cells have been successfully transformed.

A plasmid is a circular DNA segment ring that often exists outside of a living cell and alters a living cell when inserted. It is often used by modern scientists as a tool for genetic engineering. To modify a piece of plasmid, it is first placed within a "vector" bacterium that was made "competent," or able to uptake DNA, through processes such as heat-shock, where the bacterium is placed in an environment of alternating coldness and hotness. ~~This vector bacterium is then~~ The desired DNA strand is ~~[illegible]~~ inserted into this competent bacterium, ~~[illegible]~~ and as a result the bacterium "transformed." This bacterium is then allowed to ~~[illegible]~~reproduce, and the daughter bacteria that resulted would have copies of the plasmid with the addition of the inserted DNA on it.

One procedure to determine whether the bacterial cells have been successfully transformed would be growing them in labratory dishes that contain different growth factors or chemicals. For example, a certain DNA segment could have been inserted into a bacterium to strengthen its resistance against certain antibiotics. To test whether the transformation was successful, prepare four dishes as following: two without the ~~[illegible]~~ presense of the antibiotic and two with the antibiotic. Make sure the dishes contain enough nutrients to ensure the growth

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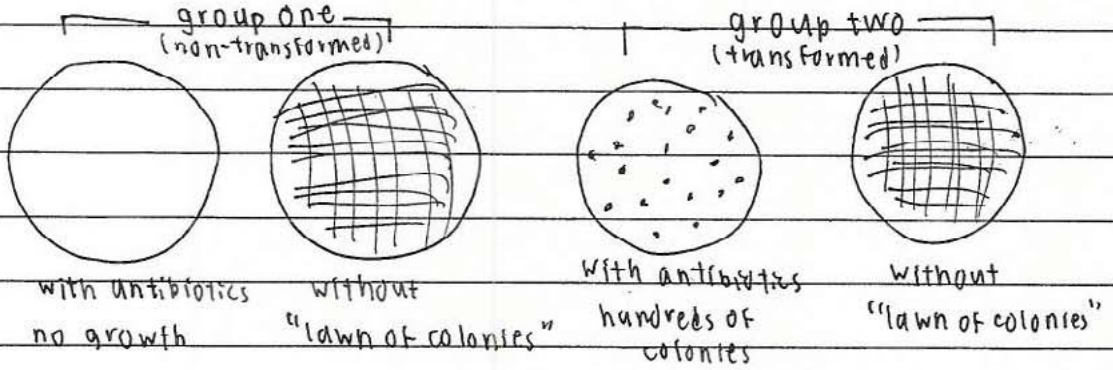
~~type of~~ of the specific bacteria you are transforming. Then, prepare two groups of the same type of bacteria that are both heat-shocked, but one group would be treated with the DNA segment that causes transformation. Place half of the first group (w/o added DNA) in a dish without antibiotics and the other half (~~w/ addd~~ also w/o added DNA) in a dish with the antibiotics. Do the same to the second group, which had been transformed. The resulting bacteria culture should resemble something as follows if transformation was successful:



The transformed bacteria would be able to survive in to presense of the antibiotics due to its strengthening DNA. while the original bacteria that were not transformed would ^added die in the antibiotics.

AP® BIOLOGY
2009 SCORING COMMENTARY (Form B)

Question 1

Sample: 1A
Score: 10

The response is well written and organized according to the question. Key terms are underlined and followed with good descriptions of those terms.

A total of 6 points were earned from the description of how a plasmid can be modified. The first point was earned for providing the definition of the plasmid. The next 3 points were earned for the description of the cutting of the DNA: the plasmid and the gene of interest must be cut with the same (1 point) restriction enzyme (1 point), and these cuts produce sticky ends (1 point) for attachment. The response earned 1 point by providing an appropriate description of insertion of the DNA of interest with the plasmid, indicating that the DNAs are attached to each other using the enzyme ligase. Another point was earned for the statement that the DNA cut should include the whole sequence of the gene, not just a section of DNA.

The response earned 5 points for the description of how the transformation is determined. The response earned the transformation point for providing a good definition of transformation. Three points were earned for providing examples of the modification, with a detailed description of how the new protein will be produced by the insertion and the statement that the procedure can use a possible selective marker of resistance to antibiotics. The response uses the determination method, indicating that a radioactive tag can be used, and thus earned 1 point. The response then provides a good description of how the tag is employed and earned another point. Because a maximum of 10 points could be earned on this question, the last point was not recorded.

Sample: 1B
Score: 8

The response earned a total of 5 points for the description of the plasmid modification. One point was earned by stating that a selective marker needs to be inserted along with the DNA. Three points were earned for the description of how to cut and insert the DNA of interest into the plasmid: 1 point for the indication that a restriction enzyme is used to cut the DNA; another point for the indication that both the plasmid and DNA will be cut by the same restriction enzyme; and the next point for the indication that the insertion of the DNA to the plasmid is sealed using the enzyme ligase. The response earned another point by describing uptake of the plasmid by use of heat shock and/or electric shock. This point was often missed because of inadequate descriptions of how to make the membrane of the bacteria competent to receive the plasmid.

The response earned 3 points for describing how the transformation can be determined to be successful. The first 2 points were earned for identifying the method of antibiotic resistance or a glowing protein. The last point was earned for providing a good description of the determination process.

Sample: 1C
Score: 5

The response is brief but direct, addressing the question in an organized format. It earned 2 points for describing how the plasmid would be modified: 1 point for a proper description of a plasmid and 1 point for a method for uptake of the plasmid (heat shock).

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Question 1 (continued)

The response earned 3 points for a description of the determination of transformation. The first point was earned by providing a proper description of transformation. The next two points were earned for identifying one of the methods used to indicate modification (antibiotic resistance), and for giving a description of the process.