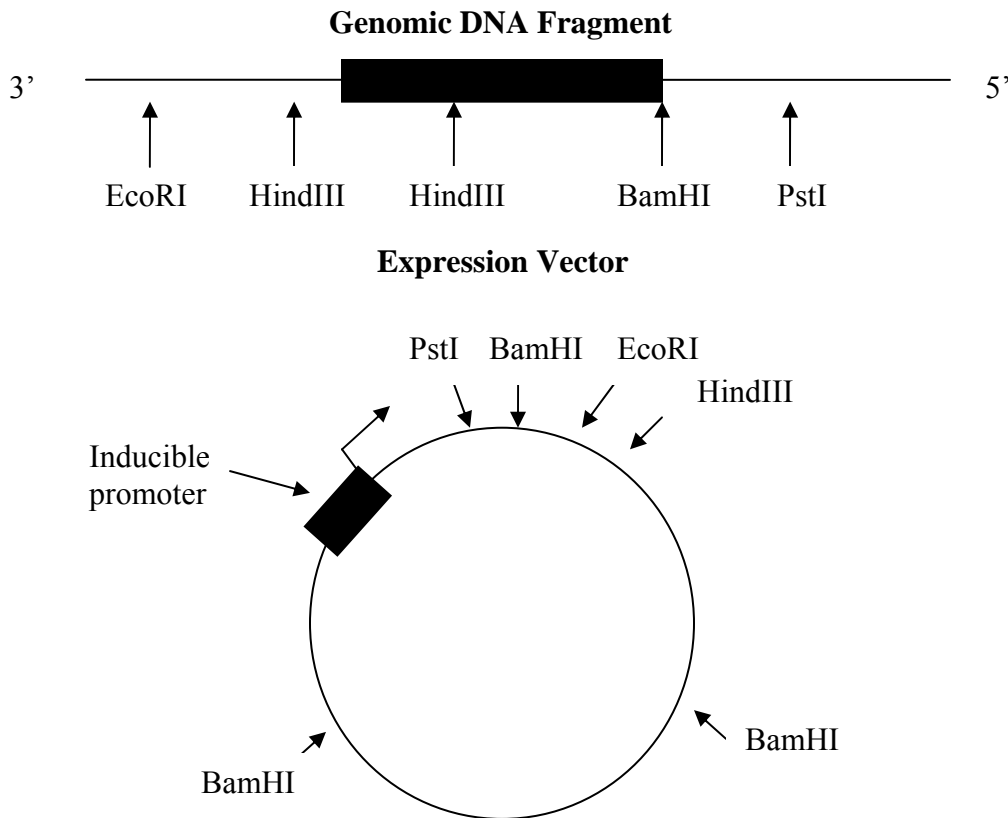


Quiz 6 ANSWERS

NAME: _____

SECTION: _____

1. The illustration below shows a fragment of DNA that has been isolated from a genomic DNA library. The fragment contains a protein coding region (black box) and the sites at which a number of restriction enzymes cut are indicated. Also shown is a plasmid that has been engineered to be used as an expression vector (arrow indicates the direction of translation from the promoter) and its restriction sites are indicated.



- a. If you cut the vector with the restriction enzyme BamHI, how many pieces would you get? 1pt **3**
- b. You wish to insert the coding region of the DNA fragment into the expression vector so that the gene will be expressed (produce the protein it encodes for). What enzyme(s) would you cut the fragment and vector with to achieve this? Indicate where the gene fragment must be inserted into the vector and the orientation it must have. 1pt

The gene fragment must be inserted in the polylinker region, adjacent to and to the right of the promoter. It must be oriented with its 5' end to the left and its 3' end to the right (flipped 180 degrees relative to the illustration above). To achieve this both the plasmid and DNA fragment should be cut with PstI and EcoRI.

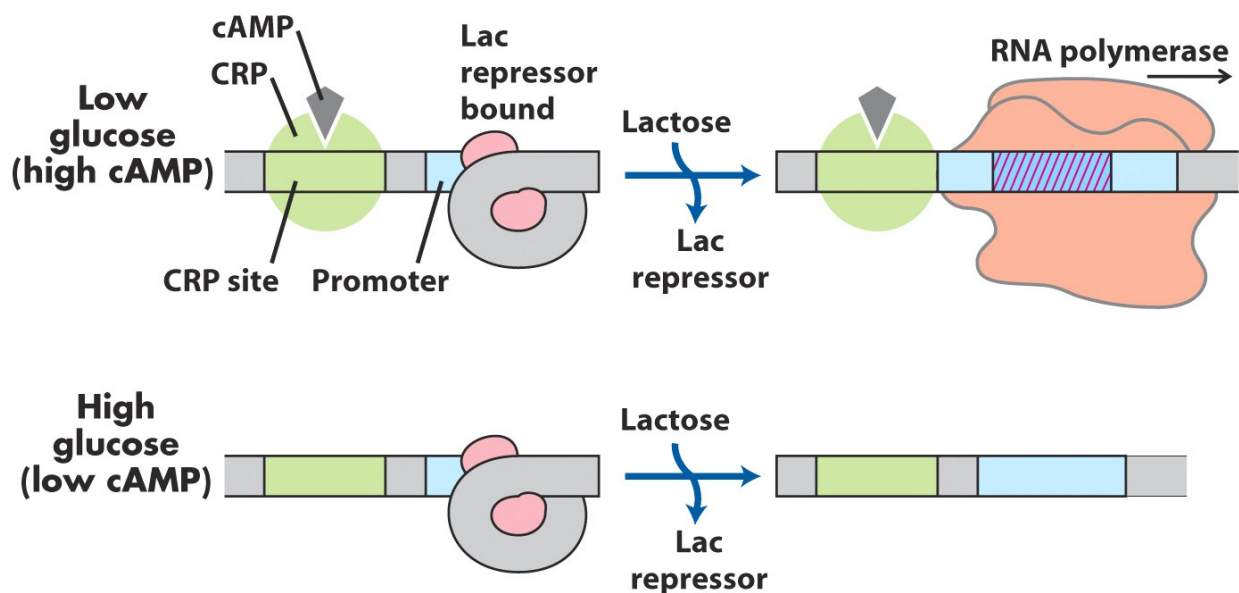
2. What happens to cAMP/CRP when:

a. High glucose is present: 1pt

ATP cannot be turned into cAMP, cAMP levels are low. CRP is unable to bind cAMP and thus unable to bind DNA to turn on lac genes.

b. Low glucose is present: 1pt

ATP is turned into cAMP. cAMP and CRP form a complex and bind DNA to turn on lac genes.



3. You want to identify the promoter for your favorite gene. What is the name of the technique you would use to determine the sequence of the promoter? Briefly describe how the technique works. 1 pts

DNA footprinting can be used to determine the region bound by RNA Polymerase.

- 1. Label DNA with radioactivity at one end of strand**
- 2. Separate into two tubes, one with DNA + RNA Pol, one with DNA alone**
- 3. Treat with DNase**
- 4. Isolate labeled DNA and denature**
- 5. Separate fragments by PAGE, compare two samples**
- 6. Missing bands indicate where RNA Pol binds**

4. DNA is extracted from blood cells of two individuals (1 and 2). In separate experiments, the DNA from each individual is cleaved by restriction endonucleases A, B and C, and the fragments are separated by gel electrophoresis. A hypothetical map of a 10,000 bp segment of a human chromosome is shown (1kbp = 1,000bp). Individual 2 has point mutants that eliminate restriction sites A* and C*. You probe the gel with a radioactive oligonucleotide complementary to the indicated sequence and expose a piece of xray film to the gel. Indicate where you would expect to see bands on the film. 1pt

