**Problems in genetic engineering / restriction enzymes**

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**Question 1: Multiple choices:**

**1. Which option BEST describes sticky ends.**

A) Sticky ends are DNA fragments that carry a higher charge than normal after they have been cleaved by restriction enzymes.

B)Sticky ends are DNA fragments cleaved by a restriction enzyme so that one strand is longer than the other.

C)Sticky ends are DNA fragments cleaved by a restriction enzyme so that both strands are the same length.

D) Sticky ends are DNA fragments that attract a carbohydrate molecule to one end after being cleaved by a restriction enzyme.

**2. Restriction enzymes cleave a \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ sequence of bases.**

**3. In nature, the purpose of restriction enzymes is to**

A. Protect the bacterium from virus attack by not allowing the virus to attach to the cell wall.

B. Protect the bacterium from the DNA of other organisms the bacterium infects.

C. Protect the bacterium from replicating its DNA at the wrong time.

D. Protect the bacterium from virus attack by cutting up foreign DNA.

**4. Restriction enzymes**

A. Bind together strands of DNA.

B. Bind RNA fragments together.

C. Cut DNA at specific sites.

D. Stop transcription.

**Question 2**

Restriction enzymes are extensively used in molecular biology. Below are the recognition sites of two of these enzymes, BamHI and BclI.

1. BamHI, cleaves after the first G:

5’ GGATCC 3’

3’ CCTAGG 5’

Does cleavage by BamHI result in a 5’ or 3’ overhang? What is the sequence of this overhang?

1. BclI cleaves after the first T:

5’ TGATCA 3’

3’ ACTAGT 5’

Does cleavage by BclI result in a 5’ or 3’ overhang? What is the sequence of this overhang?

1. Given the DNA shown below …

5’ ATTGAGGATCCGTAATGTGTCCTGATCACGCTCCACG 3’

3’ TAACTCCTAGGCATTACACAGGACTAGTGCGAGGTGC 5’

i) If this DNA was cut with BamHI, how many DNA fragments would you expect?

Write out the sequence of these double-stranded DNA fragments.

ii) If the DNA shown on the previous page in (c) was cut with BclI, how many DNA fragment would you expect? Write out the sequence of these double-stranded DNA fragments.

1. You can ligate a restriction fragment produced in (c, i) to one produced in (c, ii). Write out the sequence of the resulting fragment.
2. Could you cut the fragment from (d) with either BamHI or BclI? Explain.

**Question 3**

You find a plasmid that you think carries the *harE* gene, but you need to confirm that indeed the target gene has been inserted. When you made your library, you cut your genomic DNA with EcoRI and cloned it into a unique EcoRI restriction site in the vector.

How can you use the EcoRI restriction enzyme to tell you if the gene has been inserted?