Bio 181 Homework #1 Questions due ~~Sept. 17th~~ λ

Read Molecular Biology chapter 4.1 (handed out)

You used the following restriction enzymes to digest pBR322. Their recognition sites are shown. Arrows indicate actual cut sites (places where the phosphodiester bond in the DNA backbone is broken).

Bgl II
5’ …AGATCT… 3’
3’ …TCTAGA… 5’

DraI
5’ …TTTAAA… 3’
3’ …AAATTT… 5’

EcoRI
5’ …GAATTC… 3’
3’ …CTTAAG… 5’

HaeIII
5’ …GGCC.. 3’
3’ …CCGG.. 5’

HindIII
5’ …AAGCTT… 3’
3’ …TTCGAA… 5’

Questions.

1. Which of the above enzymes generates
   - sticky ends?
   - blunt ends?

2. Since there are four nucleotide bases, a given restriction site (recognition sequence) should occur once every 4^n base pairs, where n = # base pairs in the restriction site. Calculate the frequency at which you would expect to randomly find a given enzyme’s recognition sequence if it is
   - 4 bases long
   - 6 bases long

3. You have a plasmid (of unknown sequence) that is 4.5 kb in size.

- Statistically, how many EcoRI cut sites do you expect to find? (whole number)

- If you digest this plasmid with EcoRI, how many bands do you expect to see on an agarose gel?

4A. Is this DNA sequence a palindrome? **TCTAGA**

4B. Is this DNA sequence a palindrome? **GGTATC**

Visit & view these web pages:
http://www.promega.com/guides/re_guide/chapone/1_3.htm
http://www.dnalc.org/ddnalc/resources/restriction.html

5. Why do you think most restriction enzymes recognize palindromic sequences? HINT: most restriction enzymes act as homodimers (complexes of two identical enzyme subunits)

6. Following cleavage of the phosphodiester bond in the DNA backbone by a restriction enzyme, where is the phosphate group left: at the **5' end** or **3' end**?

7. Sticky ends can hydrogen bond together, but the DNA fragments joined in this way will fall apart easily as hydrogen bonds are weak. The "nicks" in the backbones must be sealed (i.e., new phosphodiester bonds must be formed). What kind of enzyme can do this?