**Recombinant DNA and PCR problem set**

**Problems from iGenetics textbook:**

• Ch10 – 3,4,6,13,18,20,26

1. Briefly describe the following methods or tools used in recombinant DNA technology, making sure to use scientific terminology and mention any critically important features:

a) restriction endonuclease-

b) cloning vector

d) expression vector-

e) cDNA library-

f) genomic library-

g) nucleic acid hybridization-

h) Northern blot analysis-

i) polymerase chain reaction (PCR)

2. Discuss the **specificity** and **sensitivity** of the PCR reaction.

3. Describe three techniques in recombinant DNA technology that rely for specificity on **complementary base-pairing**.

4. The partial sequence of one strand of a double-stranded DNA molecule is:

5’------GACGAAGTGCTGCAGAAAGTCCGCGTTATAGGCATGAATTCCTGAGG----3’

The cleavage sites for the restriction enzymes EcoRI and PstI are shown below.

Eco RI 5’-G A A T T C -3’

C T T A A G

Pst I 5’- C T G C A G -3’

G A C G T C

Write the sequence of both strands of the DNA fragment created when this DNA is cleaved with both EcoRI and PstI. The top strand of your duplex DNA fragment should be derived from the strand sequence given above.

5. A bacterial plasmid called pBR322 is cleaved with the restriction endonuclease PstI. An isolated DNA fragment from a eukaryotic genome (also produced by PstI cleavage) is added to the prepared vector and ligated. The mixture of ligated DNAs is then used to transform bacteria, and plasmid-containing bacteria are selected by growth in the presence of an antibiotic to which the plasmid confers resistance due to an encoded resistance gene.

The cloned DNA fragment is 1000 bp long and has an EcoRI site 250 bp from one end. Three different recombinant plasmids are cleaved with EcoRI and analyzed by gel electrophoresis, giving patterns shown below. What does each pattern say about the cloned DNA? Note that in pBR322 the PstI and Eco RI restriction sites are about 750 bp apart. The entire plasmid with no insert has 4361 bp. Size markers in lane 4 have the number of nucleotides noted on the right.

A picture containing graphical user interface

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6. A BAC vector is designed so that large DNA fragments can be inserted into a cleavage site for the enzyme BamHI. To prepare chromosomal DNA from a target organism for cloning into this vector, the target DNA is treated just briefly with BamHI, not long enough to cleave all of the BamHI sties present. Explain why the Bam HI reaction is halted before the chromosomal DNA is completely cleaved.

7. One strand of a chromosomal DNA sequence is shown below. An investigator wants to amplify and isolate a DNA fragment defined by the segment shown in red using PCR.

A) Design two PCR primers, each 20 nucleotides long, that can be used to amplify this DNA segment.

Text, letter

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B) A researcher wants to amplify the same DNA segment, but to make cloning easier she wants to add a short DNA sequence on each end of the amplified segment that includes the restriction site for the enzyme EcoRI. Design the two PCR primers that this researcher needs, incorporating 20 nucleotides complementary to the appropriate target sequences.

8. The restriction enzymes *Xho*I and *Sal*Icut their specific sequences as shown below (cut sites are denoted by∨ and^):

***Xho*I**: 5’-C∨ T C G A G-3’ ***Sal*I**: 5’-G∨ T C G A C-3’

3’-G A G C T^C-5’ 3’ -C A G C T^G-5’

Can the sticky ends created by *Xho*I and *Sal*I sites bet ligated? If yes, can the resulting sequences be cleaved by either *Xho*I or *Sal*I?

9. The bacteriophage φX174 has a single-stranded DNA genome (circular molecule) of 5386 bases. During DNA replication, double stranded forms of the genome are generated. In an effort to create a restriction map of φX174, you digest the genome with several restriction enzymes and obtain the following results. Draw a map of the φX174 genome.

|  |  |
| --- | --- |
| **Enzyme(s) used to digest** | **Size of digestion products (base pairs)** |
| PstI | 5386 |
| PsiI | 5386 |
| DraI | 4307, 1079 |
| PstI + PsiI | 3078, 2308 |
| PstI + DraI | 331, 1079, 3976 |
| PsiI + DraI | 898, 1079, 3409 |

10. You have isolated a genomic clone from the mustard plant *Arabidopsis thaliana* with an EcoRI fragment of 11 kb that encompasses the *CRABS CLAW* gene. You digest the genomic clone with HindIII and note that the 11kb EcoRI fragment is split into three fragments of 9 kb, 1.5 kb, and 0.5 kb.

A) Does this tell you anything about where the CRABS CLAW gene is located within the

11 kb clone?

B) Restriction enzyme sites within a cDNA clone are often also found in the genomic

sequence. Would there ever be a scenario where a restriction site in a cDNA clone would not also be in the genomic sequence? Please explain your answer.

C) Are restriction sites in a genomic clone always in a cDNA clone of a gene? Why or why

not?

11. To further analyze the *CRABS CLAW* gene, you create a map of the genomic clone. The 11 kb EcoRI fragment is ligated into the EcoRI site of the multiple cloning site (MCS) of a vector with the following map:

Chart, bubble chart

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You digest the resulting recombinant plasmid with several restriction enzymes and obtain the following results. Using the available data, draw a map of the plasmid that contains the 11 kb genomic fragment containing *CRABS CLAW*.

\*\*\*HINT NUMBER 1: Remember that the multiple cloning site of a plasmid consists of many consecutive restriction sites for a bunch of different endonucleases, one right after the other. As a result, sometimes cutting with different enzymes can yield some of the same sized fragments because their cut sites in the MCS are very close to each other.

\*\*\*HINT NUMBER 2: When one restriction site is used for cloning (that is, used for inserting a fragment into a plasmid), in the resulting plasmid that contains the insert that site will be duplicated such that the insert is flanked on either end by it.

|  |  |
| --- | --- |
| **Enzyme(s) used to digest** | **Size of digestion products (kilobases)** |
| EcoRI | 11.0. 3.0 |
| EcoRI + XbaI | 4.5, 6.5, 3.0 |
| XbaI | 4.5, 6.5 |
| EcoRI + XhoI | 10.2, 3.0., 0.8 |
| XhoI | 13.2, 0.8 |
| EcoRI + SalI | 6.0, 5.0, 3.0 |
| SalI | 6.0, 8.0 |
| EcoRI + HindIII | 9.0, 3.0, 1.5, 0.5 |
| HindIII | 12.0, 1.5, 0.5 |