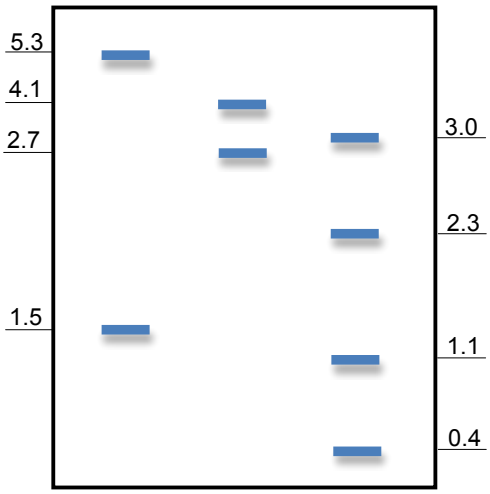
Practice Problems for Recombinant DNA, Session 5: Agarose Gel Electrophoresis, DNA Sequencing, and PCR

Question 1

You make a cDNA library by cloning the cDNA fragments into a unique EcoRI restriction site in the vector. You identify a recombinant vector that you believe has the gene of interest.

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

Suppose you find that the gene of interest is in the vector, but now you want a restriction map of the recombinant plasmid. You take three individual samples of the plasmid and digest one sample with *EcoRI,* the second sample with *HindIII,* and the third sample with both *EcoRI* and *HindIII.* Then you run the digested DNA on an agarose gel to see the fragments.



b) Considering that the cDNA fragment is smaller than the vector…

- Circle the fragments on the gel that contain all or part of the cDNA.
- Draw the restriction map of this recombinant plasmid.

Question 2

a) Given the sequence below, design primers, each 16 nucleotides long, which would allow you to generate many copies of a PCR product that was 400 base pairs long

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    GGACCGCGGGGCAGGATTGCTCCGGGCTGTTTCATGACTTGTCAGGTGGGATGACTTGGATGGAAAAGTAGAAGGTCATG
  1 +---------+---------+---------+---------+---------+---------+---------+---------
    CCTGGCGCCCCGTCCTAACGAGGCCCGACAAAGTACTGAACAGTCCACCCTACTGAACCTACCTTTTCATCTTCCAGTAC

    GGGTGGCCAACTTGGGCGAGAAAAGGTATATAAAGGTCTCTTGCTCCCATCAACTGCCTCAAAAGTAGGTATTCCAGCAG
 81 +---------+---------+---------+---------+---------+---------+---------+---------
    CCCACCGGTTGAACCCGCTCTTTTCCATATATTTCCAGAGAACGAGGGTAGTTGACGGAGTTTTCATCCATAAGGTCGTC

    ATCAGACAACGTCAGGTGGGAGGACTTGGACGGAAAAGTAGAAGGTCAAGACCAACCTCTTCCAATCCAACCACAAACAA
161 +---------+---------+---------+---------+---------+---------+---------+---------
    TAGTCTGTTGCAGTCCACCCTCCTGAACCTGCCTTTTCATCTTCCAGTTCTGGTTGGAGAAGGTTAGGTTGGTGTTTGTT

    AAAATCAGCCAATATGTCCGACTTCGAGAACAAGAACCCCAACAACGTCCTTGGCGGACACAAGGCCACCCTTCACAACC
241 +---------+---------+---------+---------+---------+---------+---------+---------
    TTTTAGTCGGTTATACAGGCTGAAGCTCTTGTTCTTGGGGTTGTTGCAGGAACCGCCTGTGTTCCGGTGGGAAGTGTTGG

    CTAGTATGTATCCTCCTCAGAGCCTCCAGCTTCCGTCCCTCGTCGACATTTCCTTTTTTTTCATATTACATCCATCCAAG
321 +---------+---------+---------+---------+---------+---------+---------+---------
    GATCATACATAGGAGGAGTCTCGGAGGTCGAAGGCAGGGAGCAGCTGTAAAGGAAAAAAAAGTATAATGTAGGTAGGTTC
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Primer 1*:*

Primer 2:

b) When using the Dideoxy Chain Termination method for sequencing DNA you include only a small amount of ddATP, ddCTP, ddGTP, and ddTTP.

- Give two differences between the ddATP used in the sequencing reaction and the regular dATP.

- Explain why your the Dideoxy Chain Termination method for sequencing DNA would not work if you included too much ddATP, ddCTP, ddGTP, or ddTTP.

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