

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

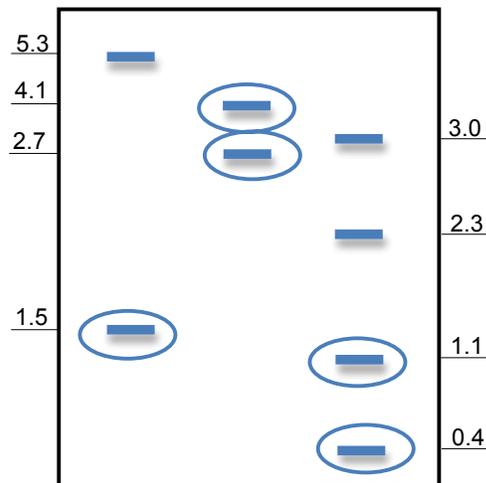
Question 1

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?

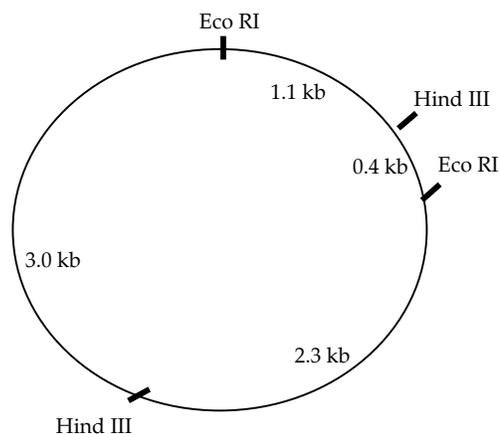
*You would inoculate media with some cells from the colony that you have identified. You would allow many cells to grow, and then isolate the vector from these cells. Once you have the vector DNA, you would digest it with *EcoRI*, and separate the resulting DNA fragments on an agarose gel. If your vector had a cDNA inserted into the *EcoRI* site, you will see at least two bands on your gel.*

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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GGACCGCGGGGCAGGATTGCTCCGGGCTGTTTCATGACTTGTGAGGTGGGATGACTTGGATGGAAAAGTAGAAGGTCATG
1 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CCTGGCGCCCGTCCTAACGAGGCCCGACAAAGTACTGAACAGTCCACCCTACTGAACCTACCTTTTCATCTCCAGTAC

GGGTGGCCAACCTGGGCGAGAAAAGGTATATAAAAGGTCTCTTGCTCCCATCAACTGCCTCAAAAAGTAGGTATTCAGCAG
81 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
CCCACCGGTTGAACCCGCTCTTTCCATATATTTCCAGAGAACGAGGGTAGTTGACGGAGTTTTCATCCATAAGGTCGTC

ATCAGACAACGTCAGGTGGGAGGACTTGGACGAAAAGTAGAAGGTCAAGACCAACCTCTTCCAATCCAACCACAAACAA
161 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TAGTCTGTTGCAGTCCACCCTCCTGAACCTGCCTTTTCATCTTCCAGTTCGGTTGGAGAAGGTTAGGTTGGTGTGTTGTT

AAAATCAGCCAATATGTCCGACTTTCGAGAACAAGAACCCCAACAACGTCCTTGGCGGACACAAGGCCACCCTTACAAC
241 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TTTTAGTCGGTTATACAGGCTGAAGCTCTTGTCTTGGGGTTGTTGCAGGAACCGCCTGTGTTCCGGTGGGAAGTGTGG

CTAGTATGTATCCTCCTCAGAGCCTCCAGCTTCCGTCCTCGTCGACATTTCCCTTTTTTTTCATATTACATCCATCCAAG
321 +-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GATCATACATAGGAGGAGTCTCGGAGGTCGAAGGCAGGGAGCAGCTGTAAAGGAAAAAAAAAGTATAATGTAGGTAGGTT
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Primer 1: 5' G G A C C G C G G G C A G G A 3'

Primer 2: 5' C T T G G A T G G A T G T A A T 3'

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.
The ddATP would be missing the 3' OH that is found on dATP and it would have some marker that will allow visualization.
- Explain why your the Dideoxy Chain Termination method for sequencing DNA would not work if you included too much ddATP, ddCTP, ddGTP, or ddTTP.
If you included too much ddATP, ddCTP, ddGTP, or ddTTP, then each elongation reaction would incorporate a ddNTP very early in the reaction. This would cause all of the products to terminate near their 5' end and you would never generate products that would allow you to determine the sequence of the end of the template.

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