MOLEBIO LAB #8: Restriction Enzyme Simulation Using NEB Cutter

IDENTIFICATION OF PHIX174 RF DNA BY RESTRICTION MAPPING

1. Go to http://tools.neb.com/NEBcutter/index.php3.
2. Click on the PhiX174 link listed under "test sequences". (#Viral + phage) Click "Submit".
3. A map of the circular PhiX174 DNA will appear. Several restriction sites are shown; enzyme names are abbreviated in purple. The grey arrows show the location of genes, or **Open Reading Frames** (**ORFs**). The abbreviation "aa" stands for amino acids.
4. Click on "Custom Digest". A complete list of restriction enzymes that can cut PhiX174 DNA at least once appears. For each enzyme, its specificity, or DNA recognition sequence is shown:
    a. Note that the restriction sequence of only one DNA strand is given. That is because we can use base-pairing rules and this sequence to figure out the sequence of the opposite, complementary strand. According to the base-pairing rules, A always base pairs with T, and G always base pairs with C. Note that the two strands are identical when read in opposite directions.
    b. Also note that some enzymes are pickier than others. G, A, T, and C refer to the nucleotide letters in the DNA alphabet. Y stands for **pyrimidine** (either T or C will do), M stands for A or C, K stands for T or G, R stands for **purine** (G or A), and N stands for *any base*.
    c. The list also shows the number of times each enzyme will cut PhiX174 DNA, and the % activity in each NEB buffer.
5. Since we want double digest (cutting with two different enzymes in the same tube), we will want to use enzymes that will perform well together in the same buffer. Click on "Enzymes with compatible buffers" and scroll down to CutSmart Buffer (it should be the first one) for enzymes that cut optimally in the NEB CutSmart Buffer.
6. Select DraI. Click on the digest button at the bottom. The computer will now generate a circular map showing DraI cutting sites.
7. Click on "View gel" to predict what the gel will look like. Note the fragment coordinates (each PhiX174 base is numbered) and predicted fragment lengths on the top right.
8. Choose the Marker "1 kb DNA Ladder" as your known values. This will show how DNA segments of 1000 → 10000 bp will separate on a gel.
9. Go back to the NEB 4 (green) list, and repeat the process for MfeI, then for DraI and MfeI together. Print out the simulated gel with the dual DraI and MfeI digest of PhiX174 with the 1 kb DNA Ladder.
10. Listen for directions on how to graph a standard curve for DNA analysis as well as using the other options when using NEB Cutter. (correlates with question #5)

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1. What is a restriction map, and how does it help identify a particular DNA?

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2. What is a recognition sequence?

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3. Go back to the NEBcutter site and once again custom digest PhiX174 DNA with DraI and MfeI, then view the gel. What happens to your predicted results when the % agarose is increased from 0.7% to 2.0%? Why is it beneficial to use the 0.7% agarose?

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4. Use the NEBcutter program to come up with another set of restriction enzymes that would have also worked well to identify the PhiX174 DNA? What enzymes did you choose? What were your criteria for picking these enzymes?

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5. Use the DNA size markers that run alongside your digest samples to plot the known marker DNA fragment sizes in bases on the y-axis, and band migration of those fragments measured from the well in mm on the x-axis on semi-log graph paper. (Listen for the help I give in class). Use the graph (called a standard *curve*, but should be more or less a **straight** line) to interpolate each PhiX174 DNA fragment size from its corresponding band migration of the double digest. Did all bands in a given lane add up to approximately 5.39 kb? Why or why not?

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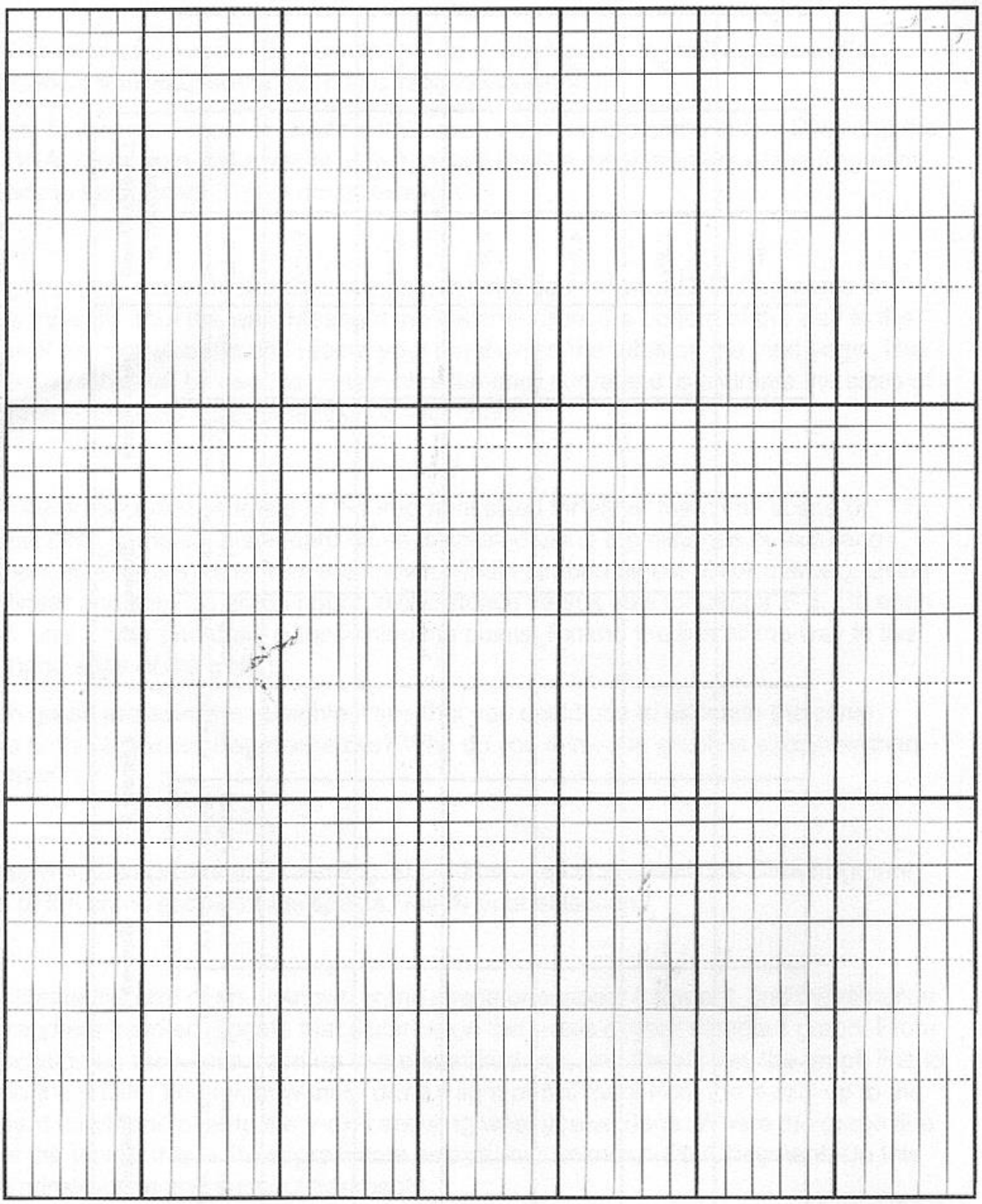

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