ASSIGNMENT 1

Restriction Enzymes:

I. Restriction enzymes are proteins that recognize specific sequences at which they cut the DNA. Two commonly-used restriction enzymes are HindIII and EcoRI.

    A. **Look up the recognition sequences that these enzymes cut and make a sketch of the pattern of cutting they carry out.**

        To answer this question go to the Enzyme Finder in the New England Biolabs (NEB) website:

        (http://www.neb.com/nebecomm/EnzymeFinderSearchByName.asp)

        Enter the name of the restriction enzyme you are interested in (make sure you search by "name"). Find the double-stranded (two rows) sequence; the forward slashes indicate where the backbone of the DNA is cleaved.

    B. **Consider the roughly 48,000 bp genome of λ (lambda) phage (a bacterial virus) and make an estimate, based on simple probability arguments, of the lengths of the fragments that you would get if the DNA is cut by either HindIII or EcoRI (remember that lambda DNA is linear). There is a precise mathematical way to do this that you should derive, and it depends upon the length of the recognition sequence. Based on your derivation, explain why a 5 cutter (i.e. a restriction enzyme that recognizes a 5 base pair sequence) will have shorter fragments than an 8 cutter.**

II. **We did this digestion experiment with lambda DNA in class. Find the actual fragment lengths obtained after the lambda genome is treated ("digested") with these restriction enzymes by going to the New England Biolabs website:**

    http://www.neb.com/nebecomm/tech_reference/restriction_enzymes/sites/Lambda_sites.pdf

    Look up the tables that they have for identifying the sites on the lambda genome that are cut by these different enzymes. Calculate the fragment sizes for:

        a) a single digestion with HindIII
        b) a single digestion with EcoRI
        c) a double digestion with HindIII and EcoRI simultaneously

III. **Based on the fragment sizes you found in part II (a. and b.) is the prediction that you made in part I, B. a good one?**

IV. **Below is an image of a gel electrophoresis sample showing the following (from the left):**

Lane 1: a double digestion of λ DNA with HindIII and EcoRI simultaneously.
Lane 2: a single digestion of λ DNA with HindIII
Lane 3: 1kb NEB ladder.

Each horizontal band in the gel corresponds to a fragment of a different size. You can estimate the size of the fragments by comparing them to the fragments in the right most lane (lane 3), which is a sequence of fragments of known size, called a ladder. To obtain the fragment sizes for this ladder (called a 1kb NEB) look up the following NEB site:

http://www.neb.com/nebecomm/products/productN3232.asp

A. **Match the fragment lengths for the ladder based on the website above with the actual pattern of fragments in the right most lane**.

B. **Now use the ladder as a reference and try to match the fragment lengths you calculated in part B with the actual bands that appear in the remaining two lanes. Note that for experimental reasons, some of the bands may be too faint to make out. These have been boxed in red on the diagram below. When referring to the gel, make sure you explicitly identify the fragments that you think correspond to your expected results.**

Matlab from Week 1

I. Graticule: For all three magnifications of the graticule that you took images of turn in the image of the graticule, the plot of the pixel intensities along the line you used to calibrate the micron/pixel ratio (you should all have different plots!) and give the value of that ratio.

II. Take your images of prestained fluorescent slides and make an RGB photo out of them. Make sure you have added a scale bar using the ratio determined from the graticule at that magnification.

LANE 1
λ DNA
+HindIII
+EcoRI

LANE 2
λ DNA
+HindIII

LANE 3
NEB 1 kb
ladder