Bio 230 General Genetics Lab Exercise 5

Restriction digests of phage lambda (λ)

Background
The bacteriophage (phage) virus lambda (λ) was discovered by Esther Lederberg in 1950, and has been a favorite tool of geneticists ever since. It infects the bacterium *E. coli* by attaching to the coat of the bacterium and injecting its DNA into the host. Once inside the viral DNA either integrates into the bacterium’s genome, or it takes over the cell and makes it synthesize up to a hundred new viruses. Once done, the host cell ruptures and releases the new viruses, all ready to infect new cells. Read pp 773-781 of your text for general information on how viruses work.

The lambda genome
Lambda contains a single DNA molecule that is 48502 base pairs long. While it is inside the phage head it is linear, but it becomes circular once it enters the host cell. The entire sequence of bases is known, and the location of every known restriction recognition site has been mapped. See [http://www.fermentas.com/techinfo/nucleicacids/siteslambda.htm](http://www.fermentas.com/techinfo/nucleicacids/siteslambda.htm) for a list of cut sites in lambda for almost 100 different restriction enzymes.

Restriction enzymes
Restriction endonucleases are a series of enzymes that cut both strands of double stranded DNA at specific recognition sites. The recognition sites vary from 4 to about 8 base pairs in length for different restriction endonucleases (RE), and the cuts are made at specific sites within the recognition sequence. These characteristics of specific sequence recognition and specific cleavage products have made REs useful for a variety of experimental techniques used to characterize genes and genomes. A given piece of DNA, when treated with a specific restriction enzyme, will always be cut into the same number of unique pieces (restriction digest fragments) and each piece will have a specific number of bases.
Agarose gels
In order to separate the fragments, the restriction digest is run on an agarose gel. Agarose is a polysaccharide derived from seaweed that will dissolve in liquid when boiled and then solidify to form a gel when it cools. DNA fragments will move through such a gel when exposed to an electric current, and the rate at which they move depends on their size: the larger the fragment, the slower it moves. For a given digest, all the fragments of the same size will move at the same rate, and form a band. Each digest will produce several bands (depending on how many cut sites there are for the enzyme) and each will move a different distance, forming a characteristic pattern for that digest. Most agarose gels use a buffer called TAE (Tris/acetate/EDTA). Tris is a commonly used buffering agent since it is biologically inert. In this case it is buffered with acetic acid, though other acids would also work. EDTA is a compound that binds divalent metals, such as Mg++. Since most nucleases capable of attacking DNA need Mg++, this effectively inactivates them.

Seeing the bands
DNA is not visible to the human eye, so we need some way of identifying where the bands are. Typically this is done using a dye that binds to DNA. One common dye is methylene blue but it has two drawbacks: it is not very sensitive and getting rid of the background staining takes a long time. You will be using ethidium bromide, a sensitive dye that fluoresces orange when it binds to DNA and is exposed to ultraviolet light.

Determining fragment size
Although it is true that the bigger the fragment, the slower it moves, you need some way of knowing just ‘how big’ a fragment is. To do this you run a sample containing a series of fragments of known size. By measuring how far each of the bands moves and plotting that against its know size, you can create a standard curve that will allow you to estimate the size of your fragments.

In today’s lab you will cut lambda DNA with a variety of restriction enzymes and then separate the fragments on an agarose gel. By comparing the distance moved by each of the fragments, you should be able to determine how big each of them is and compare the results from cutting with different enzymes.
**Procedure**

1) Start the restriction digests. Each group will work with a different combination of enzymes.

<table>
<thead>
<tr>
<th>Group</th>
<th>Enzyme 1</th>
<th>Enzyme 2</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EcoR1</td>
<td>PvuI</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>EcoR1</td>
<td>Mlu1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>HindIII</td>
<td>PvuI</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>HindIII</td>
<td>Mlu1</td>
<td>2</td>
</tr>
</tbody>
</table>

Add the following ingredients to each tube. Be very careful when measuring and use a fresh tip every time. Keep the restriction enzymes on ice at all times. All volumes are in microliters.

<table>
<thead>
<tr>
<th></th>
<th>Lambda</th>
<th>Enzyme 1</th>
<th>Enzyme 2</th>
<th>Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lambda DNA</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme 1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Enzyme 2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Buffer</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Water</td>
<td>16</td>
<td>13</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Standard</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>30</td>
</tr>
</tbody>
</table>

Place the tubes with restriction enzymes in a 37°C water bath for one hour. When finished, add 5 microliters of loading buffer to the tube containing uncut lambda and the restriction digests. Place all four tubes in a 65°C water bath for five minutes and place on ice for two minutes. Load the entire contents of each tube into the gel.

**Making agarose gels.**

Each gel needs 70 mls of TAE buffer and 0.56 g of agarose. This will make a 0.8% gel. The higher the agarose concentration, the stronger the gel, but the slower the DNA will move. Measure out both into a 250 Ehrlenmeyer flask and microwave on full power for 30 seconds. Swirl the flask and look to see if all of the agarose is fully dissolved. This will take several rounds:
the solution will need to boil strongly for some time. If you can still see small flecks in the liquid, then it is not ready. 

Cool down the liquid until you can just hold the base of the flask in the palm of your hand. Assemble the gel holder as shown. Be sure you are using the 6 place comb and that you have the thick side down.

Pour all of the gel into the apparatus and leave to cool. The gel will become milky and opaque. Once it has solidified, wiggle the comb a few times and lift straight up. Lift the gel holder out of the container, turn it 90° and place back on the shelf with the wells closer to the black (-ve) electrode. CAUTION: the gel is often slippery and will slide off the holder. Keep your fingers over the ends of the holder to prevent this.

Pour enough gel running buffer to completely cover the gel by a couple of millimeters. Because of this, these are often referred to a submarine gels.

**Loading and running the gel**

Load the samples into the wells in the following order: Blank, Lamba, Enzyme 1, Standard, Enzyme 2, Blank. When loading, place the tip of the micropipet just above the well and slowly depress the plunger all the way. Do not release the plunger until the tip is back out of the buffer. Place the lid on the apparatus and connect the leads to the power supply.
Press the K button until Amps is lit and then use the arrows to adjust to 100 mAmps. Then set Voltage to 200. Press the Run button. The display will cycle through Voltage, Amperage and Time of run. Voltage should always be 200, the others will vary. You should see bubbles coming off the electrodes in the apparatus.

Once the run is complete, turn off the power supply, disconnect the lead and remove the top of the gel apparatus. Carefully (Slippery!) remove the gel holder and place on a paper towel. Cover the gel with an ethidium bromide staining strip and gently press, to ensure good contact. After 2 minutes, remove the strip and place it in the disposal bag. Bring the holder and gel to the GelDoc camera system to get a picture of your gel. Once done, the gel can go in the disposal bag. Plot the distance moved against size for each of the bands in the standard sample. Use the graph to estimate the sizes of the bands in your two enzyme treated samples.
Questions
For each digest, how many bands did you see? What size are they? What does the total length add up to? How does this compare with the original length of lambda DNA?

How would the results change if the enzyme did not have time to cut all of the sites?

Why is it safer for the virus to have its DNA become circular when it enters the host, rather than stay linear?