DNA Restriction and Gel Electrophoresis

This week, we will learn how restriction enzymes can be used to evaluate genomic and plasmid DNA. We will use the genomic DNA and the plasmid DNA that you isolated last week.

What is a restriction enzyme?

A restriction enzyme is an endonuclease which recognizes a specific, short oligonucleotide (a short piece of DNA, with a specific pattern) between four and 8 base pairs long and cleaves DNA at this site. For example, in the sequence:

5' ...GAATTC...3'
3' ...CTTAAG...5'

the commonly used restriction enzyme **EcoRI** (*Escherichia coli* strain RY13 endonuclease I) will cut

5' ...G'AAATTC...3'
3' ...CTTAA'G...5'

yielding:

5' ...G↓AATTC...3'
3' ...CTTAA↑G...5'

A restriction enzyme will cut a piece of DNA every time it ‘finds’ the sequence where it cuts.

For example, **EcoRI** will cut DNA at every

5' ...GAATTC...3'
3' ...CTTAAG...5'

sequence that is present in that DNA. So, the result may be two pieces of DNA or it may be many more than two pieces of DNA. There are hundreds of restriction enzymes. Restriction enzymes that cut a four-base pair long sequence will usually result in more fragments of DNA than a restriction enzyme that cuts at a specific eight basepair sequence. This is because any specific four base pair combination is more probable than a specific eight base pair combination.

Notice that after the DNA has been cut, a 3'...TTAA 5' is left hanging single stranded on the negative strand and 5'AAATTC... 3' is left hanging single stranded on the positive strand. We call these sticky-ends. It is also possible to cleave DNA in a ‘blunt-ended’ fashion, where there are no single-stranded overhangs. In the examples below, the enzyme **SmaI** would yield blunt-ended products.

<table>
<thead>
<tr>
<th>EcoRI</th>
<th>5'...G'AAATTC...3'</th>
<th>SmaI</th>
<th>5'...CCC'GGG...3'</th>
<th>PstI</th>
<th>5'...CTGCA'G...3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'...CCTAAG'G...5'</td>
<td>3'...GGG'COC...5'</td>
<td>3'...G'ACGTC...5'</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Restriction enzymes usually cannot cut DNA that is methylated (but there are also restriction enzymes that can cut methylated DNA, e.g. **MspI**, see below).
Gel Electrophoresis

Molecules can be separated according to their size and charge. In an electric field, where one side of the field has a positive charge and the other side has a negative charge, molecules migrate towards the charge that is opposite of the molecule. For example, DNA is negatively charged, and will therefore move towards the positive charge. The speed of migration is determined by the size of the DNA and by the base pair composition of that piece of DNA.

However, for DNA, the charge-mass ratio is almost always the same, so that migration speed is mainly determined by DNA length. We suspend the DNA (or protein or RNA) in a gel, and then expose the gel to an electric field. The molecule will migrate through the gel when the electric field is applied. We can use different types of gels that have different pore sizes. In this way, we can choose a gel that will separate DNA (or RNA or protein) fragments of a particular size best. The two types of gels most commonly used are called agarose and acrylamide.

How these techniques are often used:

1. DNA Fingerprinting. People have used restriction enzymes to estimate variation across entire genomes. Because any four to eight basepair sequence is likely to occur multiple times across millions of basepairs, when an entire genome is digested by a restriction enzyme, multiple fragments are usually generated. These fragments will be of various lengths. These pieces of DNA in a gel are usually called ‘bands’. We can compare the whole genomes of two or more individuals by digesting them with restriction enzymes and comparing the patterns of bands that we see. This technique has been used many times for studies of genetic variation in populations as well as for determining paternity and in forensics. Note that the nature of genetic variation is not clear using this method, but some measure of the amount of variation between two or more individuals can be estimated.

2. Mapping. By using different restriction enzymes and combinations of different restriction enzymes to cut the same DNA, a ‘restriction map’ can be generated. This map can help people to identify where restriction sites occur across a piece of DNA, so that some interesting aspect, such as a band that is variable between two individuals, can be mapped to its location in the DNA.

3. Analysis of DNA methylation. By using different restriction enzymes that can either cut DNA that is methylated or cannot cut DNA that is methylated, DNA methylation can be analyzed. An example: the restriction enzymes HpaII and MspI recognize and cut CCGG sequences. HpaII does not cut when the second cytosin is methylated, MspI cuts at all CCGG positions, independent from the methylation status of the second cytosin. Therefore, DNA methylation can be analyzed by digesting extracted DNA with both HpaII and MspI. A famous method that makes use of these characteristics is reduced representative bisulfite sequencing (RRBS, see for example Meissner et al. 2005)

Overview for today

In today’s lab, you will evaluate the DNA that you isolated last week by digesting it and electrophoresing it through an agarose gel. You will then visualize your results by staining the gel in ethidium bromide and take a picture of it. For the plasmid DNA, you will use different combinations of EcoRI, EcoRV, XhoI and NcoI.

After visualizing the results, you will use the information provided by the different combinations of enzymes to construct a map of the plasmid.

I. Digestion of plasmid DNA extracted from *Escherichia coli*

Use one tube per reaction (Tube 1 = plasmid DNA with 1 enzyme and tube 2 = plasmid DNA with 2 or 3 enzymes). Each person evaluates his/her plasmid DNA. For the double digestion, you will be assigned an enzyme combination. Pipet each reagent into the tube and mix gently.

**Note:**
- The reagents should be added in the given order.
- The enzyme-buffer mixture MUST be kept on ice.
- When pipetting the enzyme-buffer mixture, be very careful.

**Digestion of Plasmid DNA from bacteria:**

<table>
<thead>
<tr>
<th>Sterile water</th>
<th>9μl</th>
</tr>
</thead>
<tbody>
<tr>
<td>template DNA</td>
<td>2μl</td>
</tr>
<tr>
<td>enzyme+10Xbuffer*</td>
<td>4μl</td>
</tr>
</tbody>
</table>

*Note that the buffer you use will depend on the enzyme that you use. Different enzymes work optimally in different salt concentrations, and the buffers are adjusted to optimize the activity of a particular enzyme. When using combinations of enzymes, you must choose the buffer that is optimal for both. We have a chart to help you choose the right buffer for an enzyme or enzyme combination. For this exercise, the enzyme and buffer have already been mixed.*

The samples must then be digested at 37°C for at least one hour!

By assessing the different band lengths produced by different enzyme digest combinations, you will be able to create a restriction map for your plasmid. Details about how this is done will be covered in class.
II. Gel electrophoresis to evaluate your DNA extractions

While the digestions are running, we will check how your DNA extractions worked, by running a small amount of undigested DNA on a gel with Roti®-Safe (see below).

Prepare your samples as follows:

- Label four 1.5 ml tubes with your group number and sample number (i.e. group 5 would label the three tubes 5.1, 5.2, 5.3, and 5.4 corresponding to plasmid extraction 1, plasmid extraction 2, and fly DNA extractions 1 and 2.  
- Be sure to write down in your notes which plasmid corresponds to which partner.)  
- In each tube, put 5μl water and 2μl of the 6X LB. Then add 5μl of the appropriate DNA to these tubes. You will load all 12μl onto the gel.

See diagram on board and instructions in class for exactly which lanes your group will use before you load your gel!

A technician will add the 1000 bp ladder, and we will run each gel for about 45 minutes (until the bottom band of loading dye is approximately 2/3-3/4 across the gel) at a constant voltage of 85V. After this gel is finished, we will visualize it (see step 3 below).

A second gel will be run for the digested plasmid DNA.

Again, we will diagram this on the board before you run the gel.

To each digestion from above, add 2 μl of 6X LB. CAREFULLY load 12μl of each sample with LB onto the appropriate gel.

The technician will add the ladder, and we will run each gel for about 45 minutes (until the bottom band of loading dye is approximately 2/3-3/4 across the gel) at a constant voltage of 85V.

III. Visualization

To visualize the DNA, Roti®-Safe was added to the gel. We will then place the gel on a UV lightbox and take a picture of the bands. Save all good samples of DNA (genomic and plasmid) for use in future labs. They will be kept at -20°C.