

Biology 1113
Lab 6: Restriction mapping of plasmid DNA

Objectives

1. Learn about the structure of plasmid DNA
2. Explore the action and use of restriction endonucleases.
3. Learn to separate DNA fragments using gel electrophoresis
4. Become familiar with basic molecular biology lab equipment (micro pipettes, gel rigs, electrophoresis equipment)
5. Learn to interpret electrophoretic gels to determine the size of fragments
6. Learn to map relative positions of restriction sites to a plasmid.

Reading

Read section 20.1 in your etext.

Background

Plasmids are naturally-occurring circular DNA molecules found in some bacterial cells. Plasmids can carry genes whose products give bacteria special functions, such as antibiotic resistance. They can be replicated within the bacterial cell and transferred between cells. Plasmids are used as a means for transferring genes between cells in the field of genetic engineering.

Restriction endonucleases are enzymes (also called “restriction enzymes”) that cut DNA in specific locations. Each restriction endonuclease recognizes, binds and cuts a specific sequence of DNA. Restriction endonucleases recognize a different base sequence and cut DNA between specific bases. As such, they serve as part of the bacterial immune system, protecting bacterial cells from foreign DNA, such as viruses.

These enzymes are an important tool in genetic engineering when scientists want to insert a gene of interest into a plasmid. Because each plasmid has a different DNA sequence, the presence and location of specific restriction sites differs between plasmids. The locations of recognition sites can be mapped onto a plasmid using plasmid mapping techniques that you will learn in this laboratory exercise. Mapping is an essential step for a plasmid to be used in genetic engineering.

When plasmid DNA is mixed with restriction endonucleases, each endonuclease recognizes its specific sequence of DNA and cuts **both DNA strands** at that location, creating DNA pieces, or fragments. Scientists refer to this process as “digesting” or “restricting” the DNA. A particular plasmid may have one or more recognition sites for a given endonuclease. Because plasmids are circular, the number of recognition sites and the number of cuts will equal the number of fragments generated. *Convince yourself of this fact by drawing a circle to represent a plasmid then “cutting” it in several places and counting up the number of pieces of DNA that result.*

To visualize the fragments generated by a digest and to estimate fragment size, we use gel electrophoresis. DNA samples that have been cut are loaded onto one end of a gel made of agarose, a polysaccharide. An electrical current is applied to the gel. The negatively charged DNA fragments are pulled toward the positive end. Larger fragments of DNA move through the gel more slowly than smaller fragments, thus DNA migrates distances through the gel based on size. A stain that adheres to DNA is applied to the gel to visualize the positions of the fragments. Comparing the distance traveled to the distance traveled by known standards will help estimate size of the unknown fragments.

Synopsis of laboratory exercise

In this lab you will map recognition sites of a bacterial plasmid by digesting the plasmid with different combinations of three restriction endonucleases and separating the resulting DNA fragments with gel electrophoresis. You will count the fragments in the single digests to determine the number of recognition sites for each restriction enzyme. Then, you will use the double and triple digests, which use two or three restriction enzymes at a time, to determine the relative positions of the restriction sites. You will also practice interpretation of gels, estimation of DNA fragment sizes, and mapping with a series of practice problems.

The plasmid that that you will work with is called pMAP. The plasmid samples have already been exposed to different combinations of three restriction endonucleases to produce DNA fragments. Each sample of plasmid exposed to one, two, or three restriction endonucleases is called a digest. The number and sizes of the fragments remaining in the sample after the digest will depend on which restriction endonucleases were used. Estimates of the sizes of the fragments of the plasmid will help you to map the restriction sites of the different restriction enzymes onto the plasmid.

You will complete the hands on steps while in lab, but because the gels need to de-stain over night, you will not obtain full results immediately. You will analyze the results of your gel at a later time.

Resources

Please watch the following videos in order (on Carmen) before coming to lab.

1. Restriction endonucleases <http://youtu.be/pDHCHa1C85Y>

2. Gel electrophoresis <http://youtu.be/vq759wKCCUQ>

3. Plasmid mapping <http://youtu.be/v2T8Y3-8674> and <https://youtu.be/kRUBRrd6Lyc>

Procedure

The pMAP plasmid is 5615 bp long and has been cut with the restriction endonucleases: *Pst*I, *Hpa*I, and *Ssp*I, each of which has a different recognition sequences, so will cut the plasmid in different places.

Materials

Each team will have:

1 vial Lambda plasmid DNA/ <i>Pst</i> I size markers	Gel in an electrophoresis box
1 vial pMAP/ <i>Pst</i> I	Pipette (10-100 μ l)
1 vial pMAP/ <i>Pst</i> I/ <i>Hpa</i> I	Pipette tips
1 vial pMAP/ <i>Pst</i> I/ <i>Ssp</i> I	Practice gel
1 vial pMAP/ <i>Pst</i> I/ <i>Hpa</i> I/ <i>Ssp</i> I	Practice "DNA" in dye.

Shared resources: Power supplies on each lab bench.

Procedure:

1. Practice loading gels with your pipette using the practice gel and practice "DNA".
2. Spin down your DNA samples in the bench-top centrifuge for ~30 s to concentrate the DNA sample at the base of the tube. Make sure to balance the centrifuge, which can be done by having a symmetrical arrangement of tubes in the rack.
3. If your gel has not been aligned so that the wells are at the negative terminal, do so now. Lift the tray holding the gel and turn it around so that the wells on the gel are at the black end (negative end) of the electrophoresis box. Fill box and cover entire gel and with TBE buffer if that has not been done for you.
4. Situate your gel box near a power supply **before loading your DNA**. Then do not move it.
5. Load samples into the gel using your micro pipette in the order they are listed in the materials list. Use a clean tip for each sample. Discard used tips in the trash.
6. Make a note or sketch of your gel so you can remember which well contains which sample.
7. Place the lid on the electrophoresis box and hook up electrical connections. Set the power supply to run at **75 v for 1 h 30 min**. Please wait until all groups have finished hooking up their gels before turning on. Have your instructor check the settings.
8. When the gels are finished, the power supply shuts off. Carefully remove gel from box and place on the UV light box to visualize the stained bands of DNA in each well.

9. Photograph or sketch a diagram of your gel.

10. Clean your area, put equipment on cart.

While you wait:

While gel is running you should complete the practice problems on a separate handout.

Upload the completed handout to Carmen along with this handout.

Results and Analysis:

1. Photograph your gel and insert the photo below. Provide a scientific figure number and caption for it.
2. Assign fragment lengths to the size standards using these numbers (units are base pairs, bp) 514, 805, 1093, 1159, 1700, 1986, 2140, 2450, 2838, 4700, 11,497. If your gel is not very clear, you can use the unknown fragments as a guide. The smallest band in the first lane should fall between 805 and 1093.
3. Estimate the size of these “unknown” restriction fragments. Label the fragments in your photo with the fragment lengths.
4. Approximate the size of the pMAP fragment by adding up the sizes of the individual fragments in each lane and averaging them across lanes by filling out the table below.

Fragment lengths	PST	PST/HPA	PST/SSP	PST/HPA/SSP
1.				
2.				
3.				
4.				
TOTALS				
AVERAGE				

5. How many restriction sites does each enzyme have?

Restriction Endonuclease	Number of sites	Fragments generated by single digest
PstI		
HpaI		
SspI		

6. Sketch the approximate locations of the restriction sites for each digest and label the restriction sites with the appropriate enzyme. The map of the triple digest represents a composite map of the plasmid restriction sites for all three endonucleases.

