Name ____________________

Date ____________________

Restriction Mapping of Plasmid DNA

Background

You are a molecular biologist working in a small university town. Things are normally very quiet around here, and that's how the residents like it.

Last night, however, something unusual happened. While driving home late from your research lab, you saw something in the sky over Hwy. 1175. A glowing red cloud surrounded a small black object in the northern part of the sky. As you watched, the glow became more intense, and the black object hurtled toward the ground. You braked your car and stared intently toward the black object. It passed behind the trees, and you could not see whether or not it struck the ground.

Suddenly, a military truck filled with soldiers carrying firearms zoomed past you, and came to a halt in front of you, forcing you to stop as well. A soldier jumped out of the cab of the truck and told you that they were cordoning off the road and that you would have to find another way home.

The next day, you are working at your lab bench when suddenly a top military officer enters the room. He is carrying a small tube filled with a tiny amount of clear liquid. The officer tells you that the tube contains DNA of uncertain origin and it must be characterized immediately. He posts a guard outside your lab door and leaves you to begin your work.

You know that restriction mapping is the first step in characterizing a novel DNA sequence. A restriction map of a piece of DNA is like a fingerprint of the DNA. You set up different restriction enzyme digests using three restriction enzymes that you already have on hand—*Pst*I, *Hpa*I, and *Ssp*I. Because you want to know not only the number of cut sites present in the DNA sequence for each restriction enzyme, but also the positions of those cut sites relative to one another, you set up a series of four single, double, and triple digests. First, you digest the unknown DNA with *Pst*I alone. Then, you digest the unknown DNA with *Pst*I and either *Hpa*I or *Ssp*I. Finally, you digest the unknown DNA with all three restriction enzymes.

You also set up a restriction enzyme digest of lambda DNA with *Pst*I, to make a size marker to compare to the unknown DNA.

Now you are ready to load the DNA onto the gel and begin gel electrophoresis. After the gel electrophoresis is finished, your real work will begin when you analyze the unknown DNA to determine the number of cut sites for each restriction enzyme and the positions of those cut sites relative to one another.

Procedure

A: Cast Agarose Gel

1. Seal the ends of the gel-casting tray with tape, and insert the well-forming comb. Place the gel-casting tray out of the way on the lab bench, so that the agarose poured in the next step can set without being disturbed.
2. Carefully pour enough agarose solution into the casting tray to fill to a depth of about 5 mm. The gel should cover only about one-third the height of the comb teeth. Use a pipet tip or toothpick to move large bubbles or solid debris to the sides or ends of the tray while the gel is still liquid.
3. The gel will become cloudy as it solidifies (about 10–15 min). Do not move or jar the casting tray while the agarose is solidifying.

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4. When the agarose has set, unseal the ends of the casting tray. Place the tray in the gel box, so that the comb is at negative (black) end.

5. Fill the box with 1× tris-borate-EDTA (TBE) buffer to a level that just covers the entire surface of the gel.

6. Gently remove the comb, taking care not to rip the wells.

7. Make certain that the sample wells left by the comb are completely submerged. If dimples appear around the wells, slowly add buffer until they disappear.

8. The gel is now ready to load with DNA.

Note: If this will be your stopping point for the lab period, cover the electrophoresis chamber to prevent the gel from drying out.

B: Load Gel

Use a needlepoint pipet (or other gel-loading device) to load the contents of each reaction tube into a separate well in the gel. Use a fresh pipet for each reaction tube. Write down the order in which you load the samples. This is very important! If the loading order is lost, you will not be able to analyze your results.

1. Draw the sample into the pipet.

2. Steady the pipet over the well using two hands.

3. Be careful to expel any air in the pipet tip end before loading the sample. (If an air bubble forms a cap over a well, the DNA/loading dye will flow into the buffer around the edges of the well.)

4. Dip the pipet tip through the surface of the buffer, position it over the well, and slowly expel the sample. Sucrose in the loading dye weighs down the sample, causing it to sink to the bottom of the well. Be careful not to punch the pipet tip through the bottom of the well.

C: Electrophorese

1. Close the top of the electrophoresis chamber, and connect the electrical leads to an approved power supply, anode to anode (red-red) and cathode to cathode (black-black). Make sure both the electrodes are connected to the same channel of the power supply.

2. Turn the power supply on and set the voltage as directed by your instructor. Shortly after the current is applied, the loading dye (bromphenol blue) should move through the gel toward the positive pole of the electrophoresis apparatus.

3. Bromphenol blue migrates through the gel at the same rate as a DNA fragment approximately 300 base pairs long.

4. Allow the DNA to electrophorese until the bromphenol blue band is about 2 cm from the end of the gel. Your instructor may monitor the progress of electrophoresis in your absence; in that case, omit Steps 5 and 6.

5. Turn off the power supply, disconnect the leads from the inputs, and remove the top of the electrophoresis chamber.

6. Carefully remove the casting tray, and slide the gel into the staining tray labeled with your group name. Take your gel to your instructor for staining.

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Data Analysis

1. Examine your stained gel on a light box or overhead projector.
2. Assign sizes to the lambda DNA/*Pst*I size marker bands on your gel. These marker bands are 514, 805, 1093, 1159, 1700, 1986, 2140, 2450, 2838, 4700, and 11,497 bp in size. Remember, small DNA fragments migrate more quickly than large ones.
3. Now, assign approximate sizes to the DNA fragments of unknown size by comparing them to the lambda DNA/*Pst*I size marker. These approximations will not be perfectly accurate. That is all right since exact sizing is NOT required for determination of the number and relative positions of the cut sites of the restriction enzymes.
4. Determine the total size of the digested DNA by adding up the sizes of the fragments from each digest. You should take an average size from the four digests: pMAP/*Pst*I, pMAP/*Pst*I/*Hpa*I, pMAP/*Pst*I/*Ssp*I/, and pMAP/*Pst*I/*Hpa*I/*Ssp*I. Remember, the same DNA was digested in each sample, so the fragment sizes should always add up to the same total.

Now you can begin the real data analysis to determine the number and relative positions of the restriction enzyme cut sites in the DNA of uncertain origin.

Number of *Pst*I sites: Number of *Ssp*I sites: Number of *Hpa*I sites:

Sketch the positions of these sites, relative to one another. Include the approximate distances between the sites in the DNA.

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Student Practice Problems

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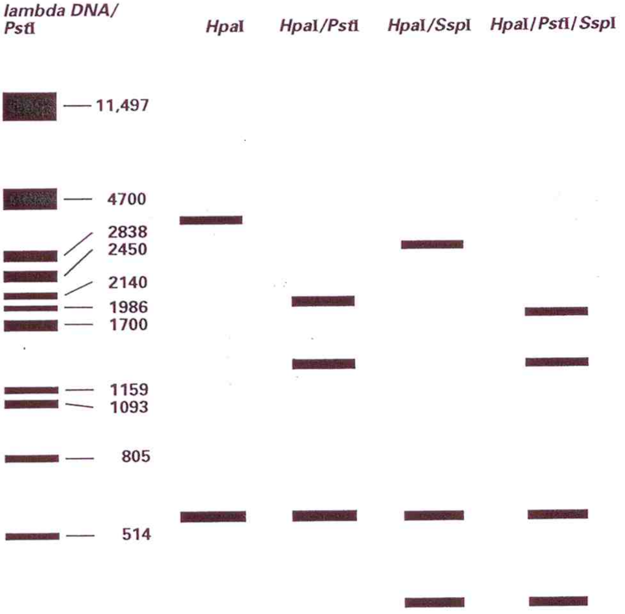
Restriction Mapping of Plasmid DNA

Problem 1: Digested with HpaI, HpaI/PstI, HpaI/SspI, and HpaI/PstI/SspI



1. Estimate the sizes of the DNA fragments (in base pairs) by comparing them with the lambda/*Pst*I size marker. These estimated sizes do not have to be exact. Sizing of the smaller fragments will be more accurate than sizing of the larger fragments.

2. Determine the total size of the digested DNA by adding up the sizes of the fragments from each digest. You may take an average size from the four digests. The same DNA was digested in each sample, so the fragment sizes from the different digests should always add up to the same total.

3. There are two *Hpa*I sites present. Based on the number of fragments obtained from the *Hpa*I digest, is this DNA linear or circular? Draw the DNA with the *Hpa*I sites present.

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4. How many *Pst*I sites are present?

5. Where is the *Pst*I site? Draw the position of the *Pst*I site on the plasmid, relative to the *Hpa*I sites.

6. How many *Ssp*I sites are present?

7. Where is the *Ssp*I site? Draw the position of the *Ssp*I site on the plasmid, relative to the *Hpa*I sites. It might be best if this is done in a separate sketch from the *Pst*I site sketch since we have not yet determined where the *Ssp*I and *Pst*I sites are relative to one another.

8. Will the 600-bp *Hpa*I fragment remain unchanged after digestion with either*Pst*I or *Ssp*I? (Check the gel.)

9. Which fragments are unchanged from the *Hpa*I/*Pst*I digest to the *Hpa*I/*Pst*I/*Ssp*I digest? Which fragments disappeared? Why did those fragments disappear?

10. Which fragments are unchanged from the *Hpa*I/*Ssp*I digest to the *Hpa*I/*Pst*I/*Ssp*I digest? Which fragments disappeared? Why did those fragments disappear?

11. Is there a fragment that appears only in the *Hpa*I/*Pst*I/*Ssp*I digest? What does this mean?

12. Draw the full plasmid map, with all restriction enzyme recognition sites present in their relative locations.

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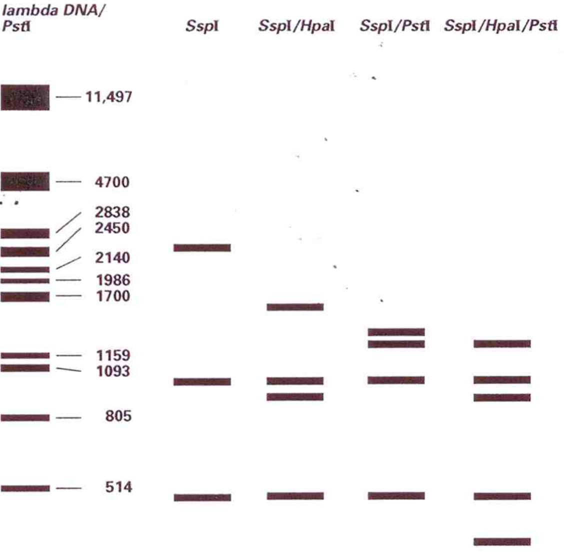
Restriction Mapping of Plasmid DNA

Problem 2: Digested with SspI, SspI/HpaI, SspI/PstI, and SspI/HpaI/PstI



1. Estimate the sizes of the DNA fragments (in base pairs) by comparing them to the lambda/*Pst*I size marker. These estimated sizes do not have to be exact. Sizing of the smaller fragments will be more accurate than sizing of the larger fragments.

2. Determine the total size of the digested DNA by adding up the sizes of the fragments from each digest. You may take an average size from the four digests. The same DNA was digested in each sample so the fragment sizes from the different digests should always add up to the same total.

3. This is plasmid DNA, which is circular. How many *Ssp*I sites are present? Draw the relative positions of the *Ssp*I restriction sites on the plasmid.

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4. How many *Hpa*I sites are present?

5. Where is the *Hpa*I site? Draw the position of the *Hpa*I sites on the plasmid, relative to the *Ssp*I sites.

6. How many *Pst*I sites are present?

7. Where is the *Pst*I site? Draw the position of the *Pst*I site on the plasmid, relative to the *Ssp*I sites. It might be best if this is done in a separate sketch from the *Hpa*I site sketch, since we have not yet determined where the *Hpa*I and *Pst*I sites are relative to one another.

8. Will the 500- and 1000-bp *Ssp*I fragments remain unchanged after digestion with either *Pst*I or *Hpa*I? (Check the gel.)

9. Which fragments are unchanged from the *Ssp*I/*Hpa*I digest to the *Ssp*I/*Pst*I/*Hpa*I digest? Which fragment disappeared? Why did that fragment disappear?

10. Which fragments are unchanged from the *Ssp*I/*Pst*I digest to the *Ssp*I/*Hpa*I/*Pst*I digest? Which fragment disappeared? Why did that fragment disappear?

11. Which fragment appears only in the *Ssp*I/*Hpa*I/*Pst*I digest? Why is it present only in this digest?

12. Draw the full plasmid map with all restriction enzyme recognition sites present in their relative locations.

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