Extension Activity 1: Plasmid Mapping

Reading a plasmid map

1. From the map of plasmid S2 list all the restriction enzymes would cut this plasmid.

   ***Pvu*II, *Eco*RI, *Bam*HI, *Pst*I, *Eco*RV, *Hin*dIII, *Sca*I**

2. Which plasmid, S2 or S5, is the biggest and what is its size?

   S5 is the largest (9481 bp)

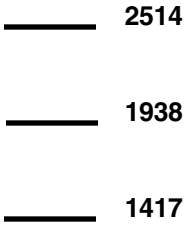
3. Using plasmid S2 as an example, find the restriction sites for the enzyme *Pvu*II. How many sites are there? What is their location? If *Pvu*II was used to cut (digest) this plasmid, how many fragments would it make?

   There are three sites for *Pvu*II on plasmid S2. They are at position 55, 1993 and 3410. Three fragments would be created if plasmid S2 was digested with *Pvu*II.

4. Next determine the size of the fragments created when plasmid S2 is cut by *Pvu*II. Size is calculated by subtracting the site locations from each other. (Note: if a fragment contains the 0 point of the plasmid, it is not just a simple subtraction!). How big are the fragments from plasmid S2 that is cut with *Pvu*II? The fragment sizes should add up to the total for that plasmid (5869 base pairs).

   The fragment sizes are 1417, 1938 and 2514 bp.

5. If the fragments from the plasmid S2 digested with *Pvu*II were run on an agarose gel, what would they look like? Draw the gel and label the fragments and their sizes.

6. Now you can determine the fragment sizes of the plasmids when cut with the two enzymes, *Eco*RI and *Pst*I. Indicate the sizes of the fragments that would be generated if the plasmid were a digest by *Pst*I alone, *Eco*RI alone or by both *Pst*I and *Eco*RI.

Plasmid S2 (5869 bp)			
Enzymes	*Eco*RI	*Pst*I	Both
Fragments	5869	2860	2817
		1700	1700
		1159	1159
		150	150
			43
Total			

7. If plasmid S2 was digested and run on an agarose gel, what would the gel look like? Draw a gel and the fragment sizes if digested by *Eco*RI alone, *Pst*I alone and by *Eco*RI and *Pst*I together.

***Eco*RI**	***Pst*I**	**Both Enzymes**
5809		
	2860	2817
	1700	1700
	1159	1159
	150	150
	43	43

8. How does your diagram in question 7 compare to what was observed in your gel after the experiment? Indicate a reason for why your data in question 7 might be different from the actual experimental data seen from lesson 2.

   They should be similar but the bands at 150 and 43 bp are too small to be observed on the gel.

Mapping the Plasmid Questions

1. How big is plasmid S5? Add the fragments in each column. The total should add up to the size of the plasmid. Why?

   The plasmid is 9481 bp in size. The fragments are cut from the plasmid and all the pieces together should be equal to the size of the original plasmid.

2. Look at the data from the *Eco*RI digest of plasmid S5. How many fragments are there? Did the enzyme cut the plasmid, or did it remain as a circle? How could you tell?

   There should be only one fragment. At first glance, it is not possible to tell if the plasmid was cut although sometimes a circular (and possibly twisted) plasmid does not run through an agarose gel at the same place as a linear piece of DNA of the same size.

3. Compare the data from the *Pst*I digest of plasmid S5 with that of the *Eco*RI digest. How many fragments are there? How many restriction sites are there for *Pst*I?

   There should be 7 fragments and therefore 7 restriction sites.

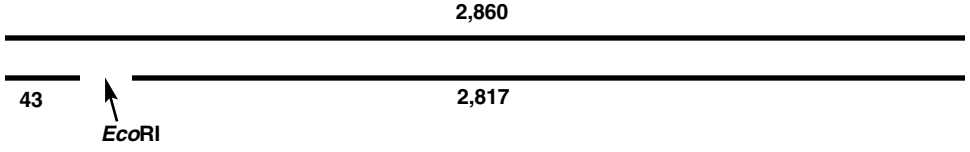
4. How many fragments are there when *Eco*RI and *Pst*I are used to digest plasmid S5? Does that answer the question of whether or not *Eco*RI cut the plasmid? Why?

   There should be 8 fragments when the plasmid is cut by both enzymes. The additional fragment shows that *Eco*RI also cut the plasmid one time.

5. Which fragment of *Pst*I digested plasmid S5 was shortened by an *Eco*RI cut?

   The 2860 bp fragment was cut into two fragments (2817 bp and 43 bp)

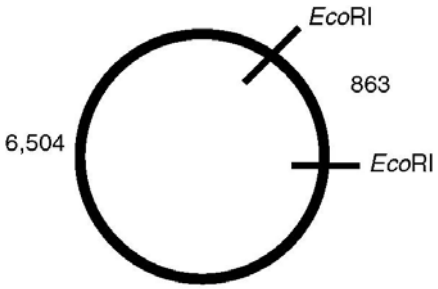
6. Draw the *Pst*I fragment that is cut with *Eco*RI in plasmid S5 to demonstrate how the fragment was cut with *Eco*RI.



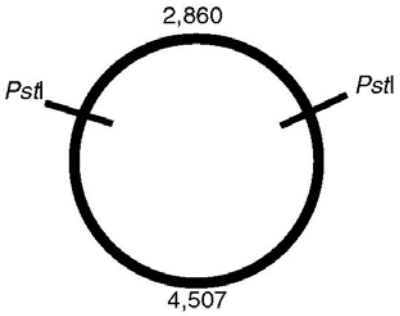
7. Restriction mapping is an exercise in critical thinking and logic. Plasmid S5 is difficult to completely map because of the numerous *Pst*I restriction sites. With the data, it would be very difficult to place all the restriction sites in order. It is easier to map plasmid S3. How many times did *Eco*RI cut plasmid S3? What are the fragment sizes?

   ***Eco*R1 cut plasmid S3 twice. The fragments are 863 and 6505 bp in size.**

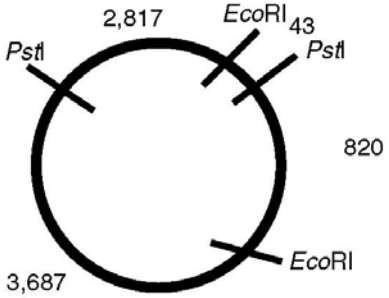
8. The data from the *Eco*RI digest of plasmid S3 indicate that the fragments are not equal. Draw a possible map and label the *Eco*RI sites and the sizes of the fragments.



9. Now draw an approximate map of the *Pst*I sites on plasmid S3 and label the *Pst*I sites and the sizes of the fragments.

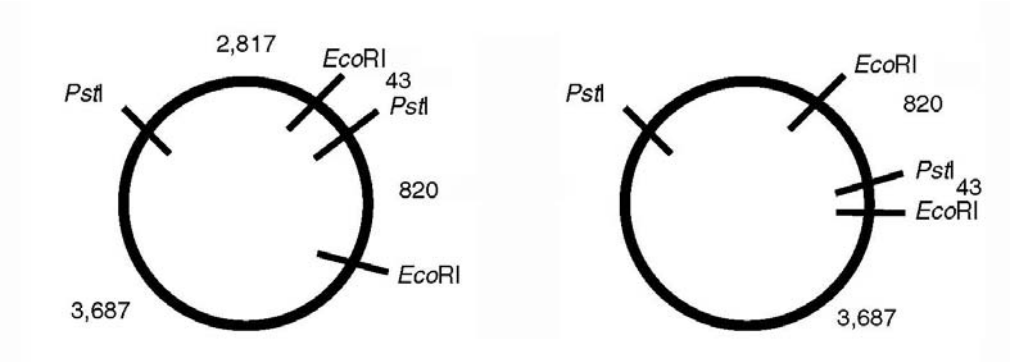


10. Draw a circular map of plasmid S3 digested with both *Pst*I and *Eco*RI? Mark sizes of each fragment and name the restriction sites on your figure.



11. Is there another possible order of restriction sites on plasmid S3 digested with both *Pst*I and *Eco*RI? How might you resolve these possibilities?

    Using the data from the double digest there are two possibilities (see diagram). However the single digest with *Pst*I would have fragments of 3687 and 3637 bp (not 4507 and 2860 bp as was seen).



12. When the gels were run for this experiment, there were only three bands for plasmid S3. Which band is missing from your gel? Why?

    The 43 bp band is so small that it does not bind enough Fastblast stain to be visible or it may have run off the gel.

Extension Activity 2: Constructing a Plasmid Questions

1. Where is the *Pst*I site on the pTZ18U plasmid?

   Position 298

2. Look at plasmid S4. What segment of the lambda bacteriophage has been inserted?

   Lambda fragment 5,218–9,617 was added.

3. After looking at the plasmid map and also the lambda phage map, can you determine how many *Pst*I restriction sites were added to the plasmid because of the inserted lambda phage DNA fragment? Note that it is possible for these extra *Pst*I sites to have been added if the original restriction digestion was done for a short time so that not all PstI sites would have been completely cut in every piece of lambda phage DNA.

   Two restriction sites for *Pst*I were added: site 766 and site 3,604.

4. Look at plasmid S1. What segment of lambda was added to that plasmid? Were any *Pst*I restriction sites added to the plasmid with the inserted fragment of lambda DNA?

   Lambda fragment 20,285–22,425 was added to plasmid S1. No additional *Pst*I sites were added.

5. Now let us create a different plasmid from the parent plasmid. You will use *Eco*RI for the construction and need to refer to the lambda bacteriophage genome map that includes the *Eco*RI sites. You must make a plasmid that is at least 5,000 base pairs but not more than 10,000 base pairs in size. Remember that the parent plasmid is 2,860 bp in size. Where is the *Eco*RI site on the parent pTZ18U plasmid?

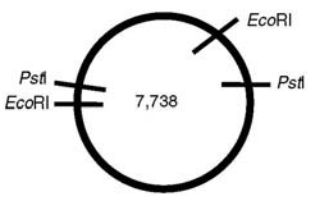
   The *Eco*RI site is at 255 bp.

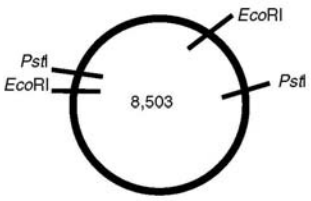
6. Choose a segment of lambda bacteriophage genome that could be cut out by the *Eco*RI enzyme. Which segment will you use?

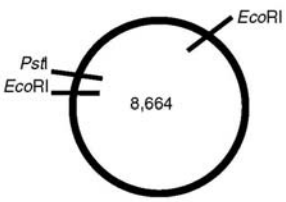
   There are three possibilities: lambda 21,226–26,104; lambda 26,104–31,747; lambda 39,168–44,972. All other options would make the plasmid larger than 10,000 bp.

7. Draw your new plasmid with the insert of your choice. Be sure to include the restriction sites for *Pst*I and *Eco*RI in your drawing. How big is your new plasmid? Give the positions of the restriction sites in your new plasmid a number indicating the location. Remember that the first *Eco*RI site will still be position 255 as it is in the parent pTZ18U plasmid map.

POSSIBILITY 1	**POSSIBILITY 2**	**POSSIBILITY 3**
Lambda 21,226–26,104	**Lambda 26,104–31,747**	**Lambda 39,168–44,972**
4,878 bp insert	**5,643 bp insert**	**5,804 bp insert**
7,738 bp total	**8,503 bp total**	**8664 bp total**
***Eco*EI 255, 5,133**	***Eco*RI 255, 5,898**	***Eco*RI 255, 5,059**
***Pst*I 1,454, 5,176**	***Pst*I 1,083, 5,943**	***Pst*I 6,102**







8. How many restriction sites are there now for *Pst*I in your new plasmid? Predict what fragments you would generate if you were to digest your plasmid with:

i. *Eco*RI alone

Two fragments in each case since there are two EcoRI sites.

POSSIBILITY 1	POSSIBILITY 2	POSSIBILITY 3
Lambda	Lambda	Lambda
21,226-26,104	26,104-31,747	39,168-44,972
4878	5643	5804
2860	2860	2860

ii. *Pst*I alone

Two fragments for possibility 1 & 2 and one fragment with possibility 3.

POSSIBILITY 1	POSSIBILITY 2	POSSIBILITY 3
Lambda	Lambda	Lambda
21,226-26,104	26,104-31,747	39,168-44,972
4016	4858	8664
3722	3645	

iii. *Eco*RI and *Pst*I together (a double digest)

Four fragments for possibility 1 and 2 and three fragments with possibility 3.

POSSIBILITY 1	POSSIBILITY 2	POSSIBILITY 3
Lambda	Lambda	Lambda
21,226–26,104	26,104–31,747	39,168–44,972
3679	4815	5804
2817	2817	2817
1199	828	43
43	43	

9. Draw an agarose gel for each of these digests and label the fragment sizes.

POSSIBILITY 1

*Eco*RI	*Pst*I	Both
4878		
	3722	3679
2860		2817
		1199

POSSIBILITY 2

*Eco*RI	*Pst*I	Both
5643		
	4858	4815
2860		2817
		828

POSSIBILITY 3

*Eco*RI	*Pst*I	Both
	8664	
5804		5804
	2860	2817

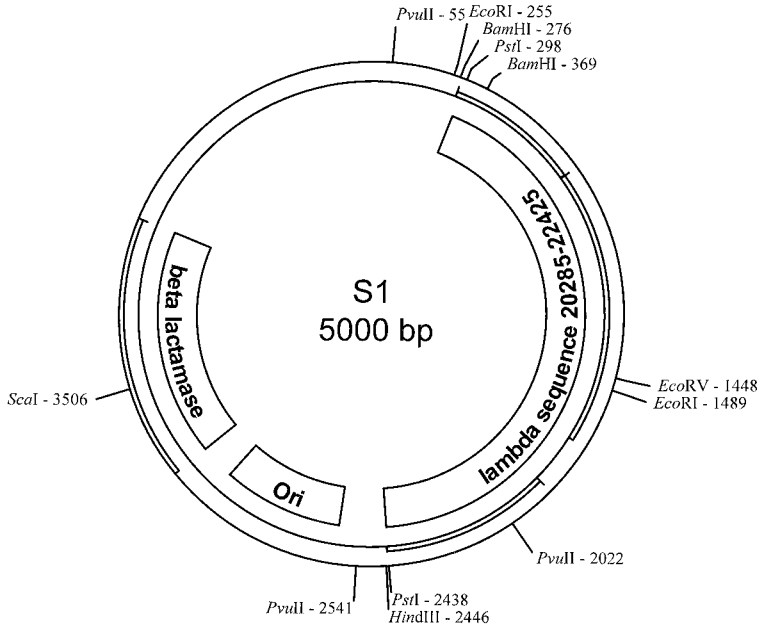
The band at 43 bp is too small to be seen.

10. The lambda phage fragment can be inserted into the host plasmid in either orientation – forwards or backwards. How could you use plasmid mapping to determine in which orientation your fragment was inserted? Use a diagram in your explanation.

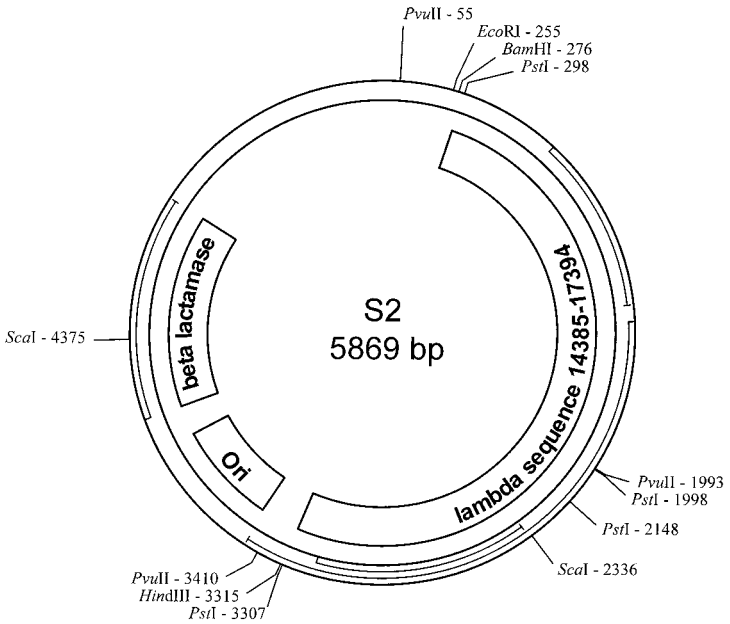
Using possibility 1 from questions 8 and 9 above. The double digests will show the same bands with either orientation but the *Pst*I single digest will be different. In the first orientation it will give two fragments of 4016 bp and 3722 bp. In the second orientation it will give two bands at 6492 bp and 1242 bp.

Appendix E: Suspect Plasmid Maps

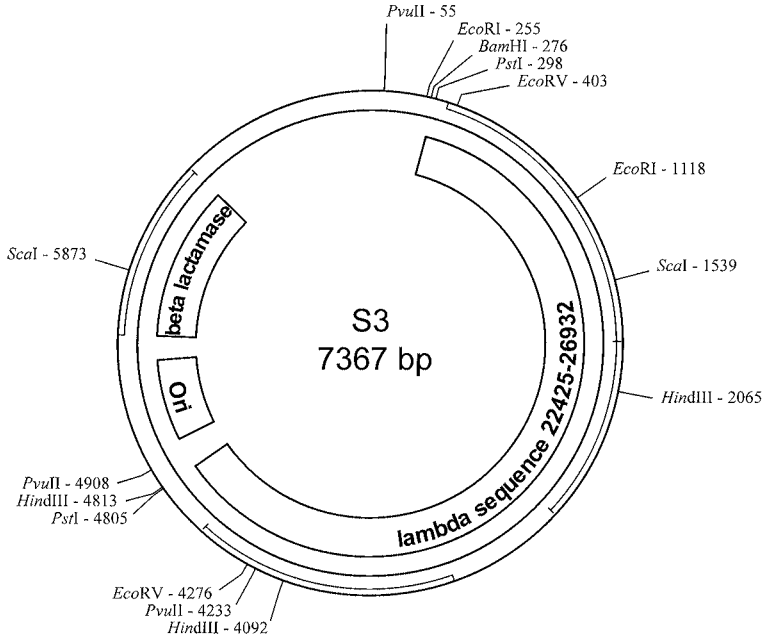
Plasmid Maps



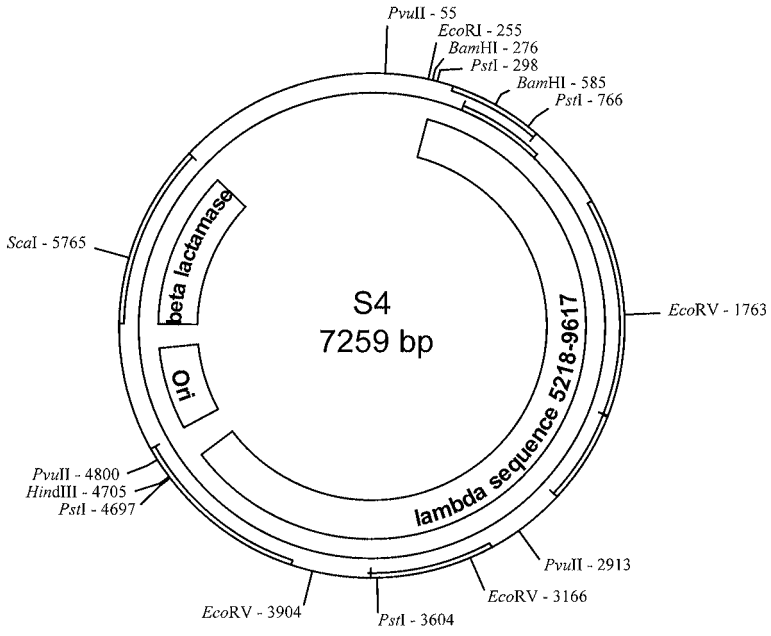
Suspect 1 DNA Sample



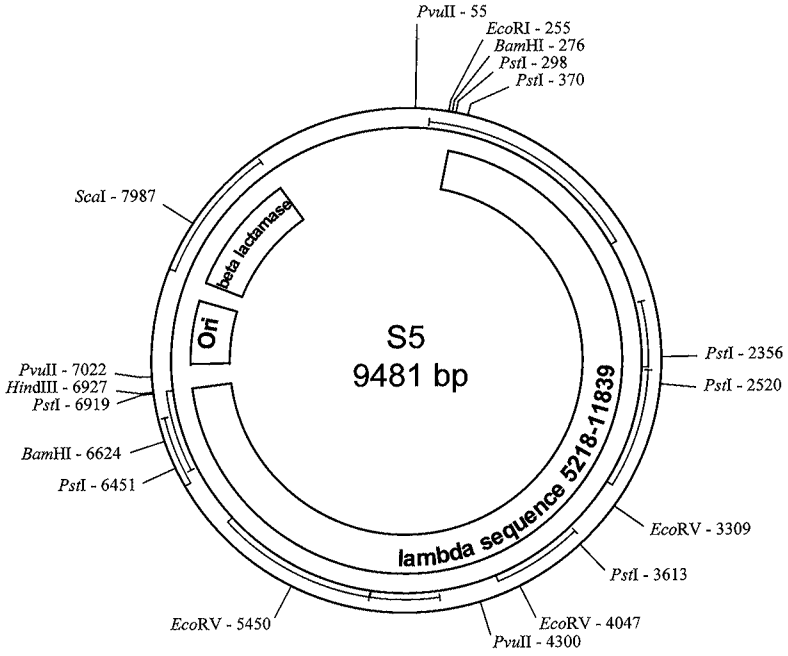
Suspect 2 DNA Sample



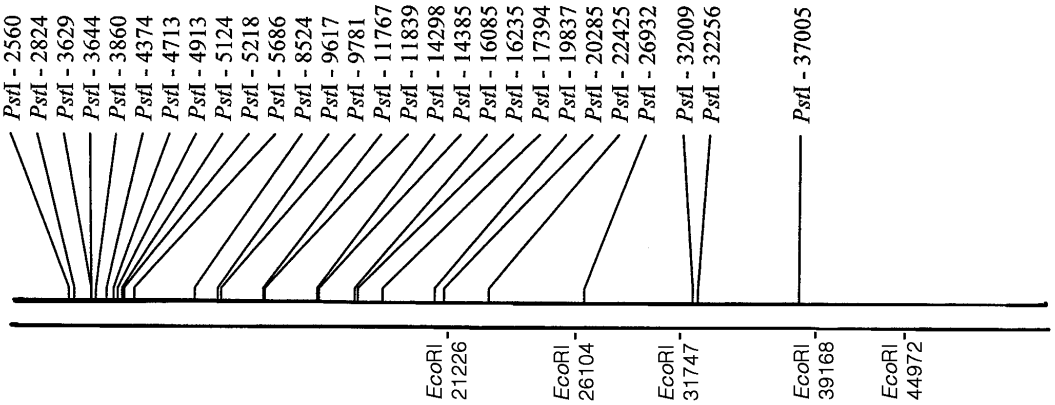
Crime Scene/Suspect 3 DNA Sample



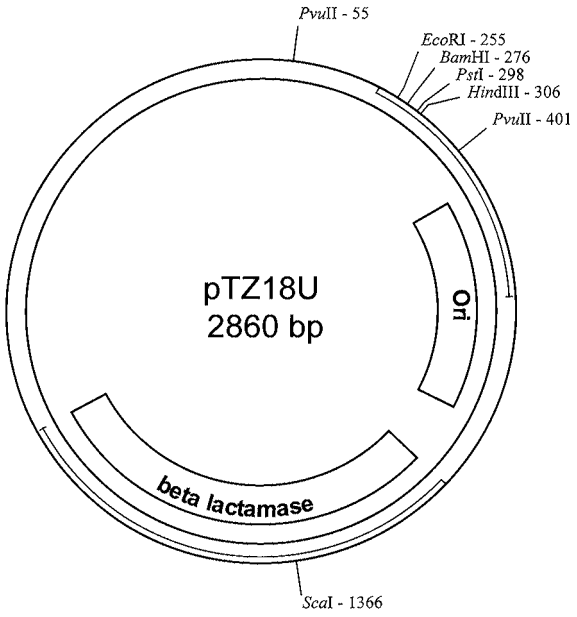
Suspect 4 DNA Sample



Suspect 5 DNA Sample



lambda bacteriophage genome
48502 bp



Plasmid Parent Vector