Extension Activity 2: Constructing a Plasmid

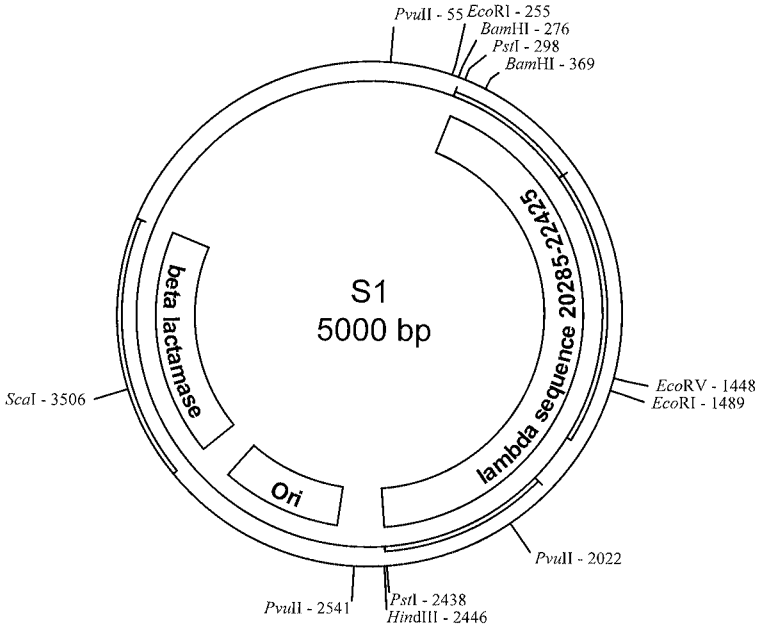
Some plasmids replicate when the bacterial genomic DNA replicates, but others replicate independently producing hundreds of copies of the plasmid within one cell. A self-replicating plasmid contains its own origin of replication (Ori). Many plasmids also contain genes that give antibiotic resistance to bacteria.

Plasmids are made of DNA just like the bacterial genome. Since DNA is universal in all living things, plasmids with inserted foreign DNA may be put into a bacteria (transforming them), causing the bacteria to transcribe and translate that message into a protein product. The biotech industry is in part based on this principle. Bacteria can be given human genes via a plasmid, and once transformed can produce a human protein product.

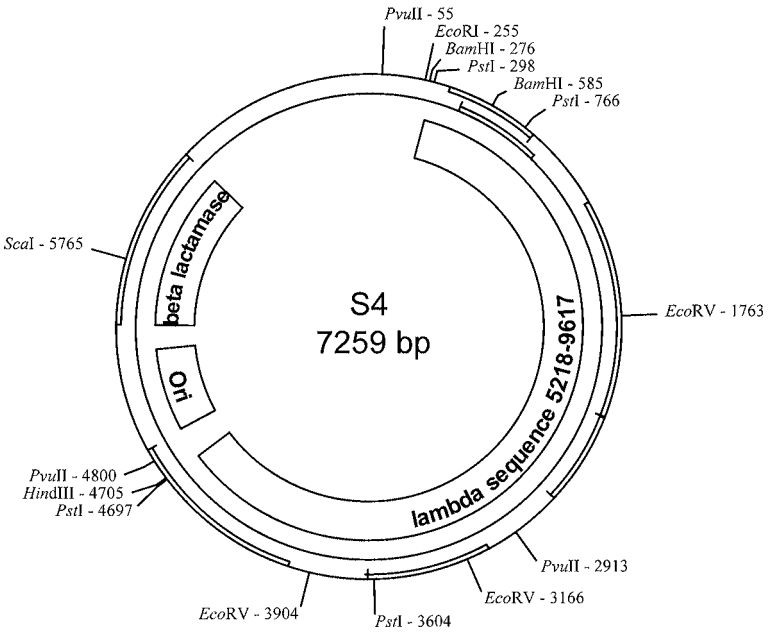
This exercise is based on plasmids used in the forensic DNA fingerprinting lab. Five plasmids were constructed from a parent plasmid, and then were cut (digested) to make fragments of different sizes for a gel analysis. How were the plasmids constructed? Could one have made the plasmids differently? Can one predict fragment sizes using plasmid maps constructed from the lambda bacteriophage genome? In this activity you will design a new plasmid.

The parent plasmid pTZ18U used for plasmid construction is 2860 bp in size. It has numerous restriction sites (see diagram on pages 56–57). The five plasmids constructed for the DNA fingerprinting lab were all based on this plasmid.

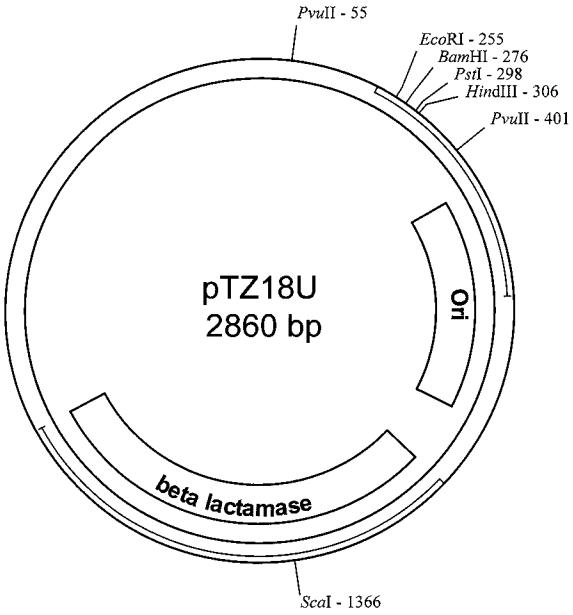
The pTZ18U plasmid was digested with the *Pst*I restriction enzyme. Lambda phage DNA was also digested with the *Pst*I restriction enzyme and the resulting fragments were then inserted into the *Pst*I – digested pTZ18U plasmid. As you look at the different plasmids, notice that each plasmid contains different fragments of lambda phage DNA. For example, plasmid S1 contains lambda sequence 20285–22425.



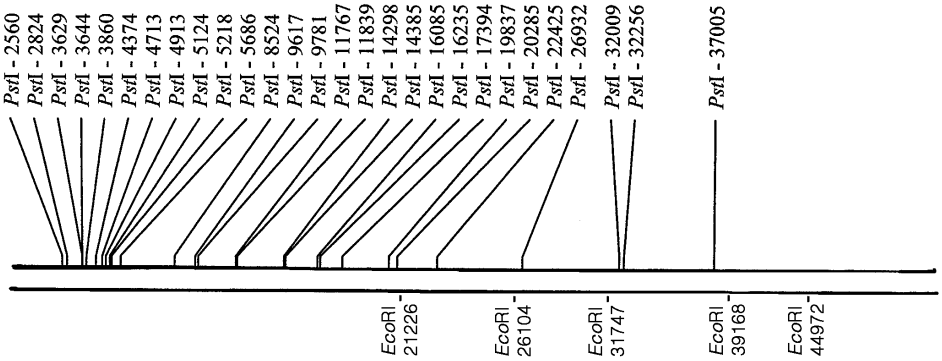
Plasmid S1



Plasmid S4



Parent Plasmid pTZ18U



lambda bacteriophage genome
48502 bp

Constructing a Plasmid Questions

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

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

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 *Pst*I sites would have been completely cut in every piece of lambda phage DNA.

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?

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 2860 bp in size. Where is the *Eco*RI site on the parent pTZ18U plasmid?

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

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.

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

   ii. *Pst*I alone

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

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

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.