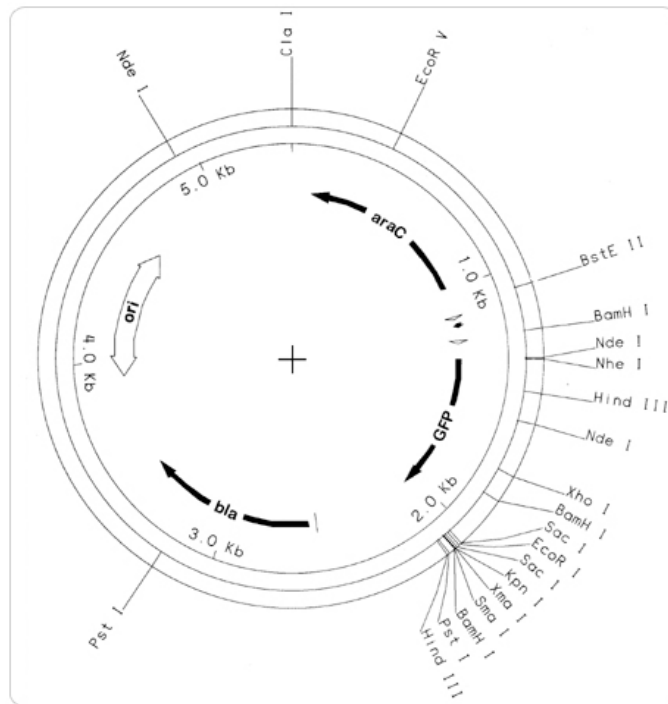


Lab 7 – Restriction Digestion

Background:

Plasmid DNA can come in many forms. When purified using a miniprep kit, plasmid DNA is often supercoiled. This means it looks like a rubber band that is wound really tight. If supercoiled plasmids were run on an agarose gel, they would appear to be much smaller in size than they actually are. To get a true measurement of the size of a purified plasmid, you need to cut the plasmid at least once to unwind the DNA before running the sample on an agarose gel (Brown, 1995).

Read *At the Bench* page 290. Restriction enzymes, or endonucleases, are proteins that cut DNA at specific recognition sequences that are unique for each enzyme. Restriction enzymes are named by the bacteria in which they were first discovered and the order in which they were discovered in that strain of bacteria. For example EcoR1 was the first restriction enzyme found in the bacteria strain *E. coli* RY13. Certain restriction enzymes are more commonly used in the lab than others. The plasmid map of the pGLO plasmid is shown below with indications of where commonly used restriction enzymes cut the plasmid. In today's lab we will be using Pst1 and Kpn1 (New England Biolabs, 2005).



Purpose:

To practice cutting DNA with restriction enzymes and interpreting the results.

Procedure:

1. Obtain and label three eppendorf tubes reaction 1 and reaction 2.
2. Calculate how much of each solution you will need for each reaction using the table below.

Reaction 1

Reagent	Stock Conc.	Final Conc.	μL to add
Buffer	10 X	1 X	
Kpn1	10 X	1 X	
Plasmid DNA			
		Total Volume	10 μL

Reaction 2

Reagent	Stock Conc.	Final Conc.	μL to add
Buffer	10 X	1 X	
Pst1	10 X	1 X	
Plasmid DNA			
		Total Volume	10 μL

- Combine ingredients as calculated for each reaction and incubate at 37°C for 45 minutes.
- While the restriction digestion is incubating, prepare a 50 mL 1% (w/v) agarose gel with TAE buffer.
- Prepare 3 samples to run on an agarose gel using the table below.

Sample 1	Sample 2	Sample 3	Sample 4
10 μL RXN 1	10 μL RXN 2	8 μL plasmid DNA	5 μL DNA ladder
3 μL sample buffer	3 μL sample buffer	2 μL sample buffer	2 μL sample buffer

- Load samples on a 1% agarose and run in TAE buffer at 100 mV until the dye front has moved half the distance of the gel.
- When approved by the instructor, remove the gel and image using the Bio-Doc it imager.
- Tape picture of gel into lab book as data.
- Clean up lab bench and throw away all used samples and tubes.

Data: Label the values of the DNA ladder correctly along the side of the gel picture. Measure and record the size of the DNA bands in reactions 1-3.

Conclusion:

- Looking at the plasmid map of the pGLO plasmid, what size fragments would you expect to see if you digested the plasmid with Kpn1? With Pst1?
- Based on your gel, what is the size of the pGLO plasmid?
- Is your uncut plasmid DNA supercoiled? Why or why not?
- Does your data support your calculations from problem 1 and why?

Works Cited

- Brown, T. A. (1995). *Gene Cloning An Introduction*. London: Chapman & Hall.
- New England Biolabs. (2005). *New England Biolabs 2005-06 Catalog and Technical Reference*. Beverly, MA: New England Biolabs.