

Title: Lab 6—Molecular Genetics

Purpose:

- In the first part of the lab, you will use antibiotic-resistance plasmids to transform *Escherichia coli*. By introducing the gene for resistance to the antibiotic ampicillin from the plasmids into *E. coli* (*E. coli* is killed by ampicillin). If the *E. coli* incorporate the foreign DNA, they will become ampicillin resistant.
- In the second part, you will use gel electrophoresis to separate fragments of DNA for further analysis. Then you will compare fragments of unknown size to fragments of a known size to calculate the unknown fragment sizes.

Background:

- **Bacterial Transformation**
Genetic transformation occurs when a host organism takes in foreign DNA and expresses the foreign gene.
- **Electrophoresis**
Bacteria have enzymes that cut, or *digest*, the DNA of foreign organisms and thereby protect the cells from invaders such as viruses. These enzymes are known as restriction enzymes. Each is able to recognize and cut at a specific DNA sequence, known as a recognition sequence. The discovery of restriction enzymes made genetic engineering possible because researchers could use them to cut DNA into fragments that could be analyzed and used in a variety of procedures.
- **Restriction Enzymes**
Like all enzymes, restriction enzymes are highly specific. They cut DNA only within very precise recognition sequences.
- **Gel Electrophoresis**
Gel electrophoresis is a procedure that separates molecules on the basis of their rate of movement through a gel under the influence of an electrical field. The direction of movement is affected by the charge of the molecules, and the rate of movement is affected by their size and shape, the density of the gel, and the strength of the electrical field. DNA is a negatively charged molecule, so it will move toward the positive pole of the gel when a current is applied. When DNA has been cut by restriction enzymes, the different-sized fragments will migrate at different rates. Because the smallest fragments move the most quickly, they migrate the farthest during the time the current is on. Keep in mind that the length of each fragment is measured in number of DNA base pairs.

Procedure

Part I: Transformation

In this lab, you use plasmids that carry the amp^{R} gene to transform *E. coli* cells that lack this gene. Also prepare a second group of *E. coli* cells as a control to verify that *E. coli* will not grow on agar with ampicillin unless it is transformed.

1. Transfer ampicillin sensitive *E. coli* cells in their log phase of growth to cold CaCl_2 solution.
2. Add amp^{R} plasmids to the experimental cells only (not to control).
3. Heat-shock cells at 42 degrees C. (Some of the competent cells will take up the amp^{R} plasmid and will be transformed).
4. Spread the treated cells on an agar plate containing ampicillin.
5. Incubate cells for 24 hours.
6. Experiment is finished. You should see that only colonies of *E. coli* that have been transformed by the amp^{R} gene grows.
7. The procedure is the same for the control group except in step 2, where you add amp^{R} plasmids to the experimental cells but not to the control cells.

Part II: DNA Electrophoresis

Three samples of DNA from a virus, the bacteriophage lambda.

One sample will be uncut DNA

One will be incubated with the restriction enzyme HindIII

One will be incubated with EcoRI.

By separating the fragments of DNA by electrophoresis and staining the DNA for visualization you can determine the fragment sizes formed in the EcoRI digest.

modified from <http://www.ekcsk12.org/science/aplabreview/lab06.htm>

Molecular Biology A: Bacterial Transformation—Ampicillin Resistance		
Plate	# Colonies	The total number or antibiotic resistant bacterial colonies on each plate can be used to calculate the transformation efficiency, or the number of antibiotic resistant colonies/ug pAMP. Use your data for the following calculations:
LB+	lawn	
LB-	lawn	
LB/Amp+	18	
LB/Amp-	0	
In this procedure, you used a 0.005 ug/uL concentration. You used a total volume of 10 uL pAMP.		
Total mass of pAMP used:	Mass = volume x concentration = _____	
Total volume of cell suspension:	Total reaction volume from steps 2, 6, 11 = _____	
Fraction spread on plate:	uL spread/total volume = _____	
Mass of pAMP in cell suspension:	Total mass x fraction spread on plate = _____	
Transformation efficiency:	# colonies observed/mass of pAMP in suspension = 1.83×10^3	

Molecular Biology B: Restriction Enzyme Cleavage of DNA and Electrophoresis		
Molecular Weight Standard Lambda DNA Hind III		
DNA Fragment #	Migration Distance (mm)	DNA Fragment Size (bp)
1		23,130
2		9,416
3		6,557
4		4,361
5		2,322
6		2,027
7		564
8		125

Molecular Biology C: Restriction Enzyme Cleavage of DNA and Electrophoresis					
Molecular weights of Unknown DNA Fragments					
Lambda DNA, uncut			Lambda DNA, cut with Eco RI		
DNA Fragment #	Migration Distance (mm)	DNA Fragment Size (bp)	DNA Fragment #	Migration Distance (mm)	DNA Fragment Size (bp)
1			1		
2			2		
3			3		
4			4		
5			5		
6			6		

Discussion, Conclusion, Reflection: See Lab Grading Guidelines
<http://www.jdenuno.com/PDFfiles/LabGuide1.pdf>