ANALYSIS AND DISCUSSION QUESTIONS

Agarose gel electrophoresis combined with ethidium bromide staining allows the rapid analysis of DNA fragments. However, prior to the introduction of this method in 1973, analysis of DNA molecules was a laborious task. The original separation method, involving ultracentifugation of DNA in a sucrose gradient, gave only crude size approximations and took more than 24 hours to complete.

Electrophoresis using a polyacrylamide gel in a glass tube was an improvement, but it could only be used to separate small DNA molecules of up to 2000 bp. Another drawback was that the DNA had to be radioactively labeled prior to electrophoresis. Following electrophoresis, the polyacrylamide gel was cut into thin slices, and the radioactivity in each slice was determined. The amount of radioactivity detected in each slice was plotted versus distance migrated, producing a series of radioactive peaks representing each DNA fragment.

DNA restriction analysis is at the heart of recombinant DNA technology and of the laboratories in this course. The ability to cut DNA predictably and precisely enables DNA molecules to be manipulated and recombined at will. The fact that discrete bands of like-sized DNA fragments are seen in one lane of an agarose gel shows that each of the more than 1 billion l DNA molecules present in each restriction reaction was cut in precisely the same place.

By convention, DNA gels are "read" from left to right, with the sample wells oriented at the top. The area extending from the well down the gel is termed a "lane." Thus, reading down a lane identifies fragments generated by a particular restriction reaction. Scanning across lanes identifies fragments that have comigrated the same distance down the gel and are thus of like size.

1. Why is water added to tube labeled "–" in Part I, Step 7?

2. What is the function of compromise restriction buffer?

3. What are the two functions of loading dye?

4. How does ethidium bromide stain DNA? How does this relate to the need to minimize exposure to humans?

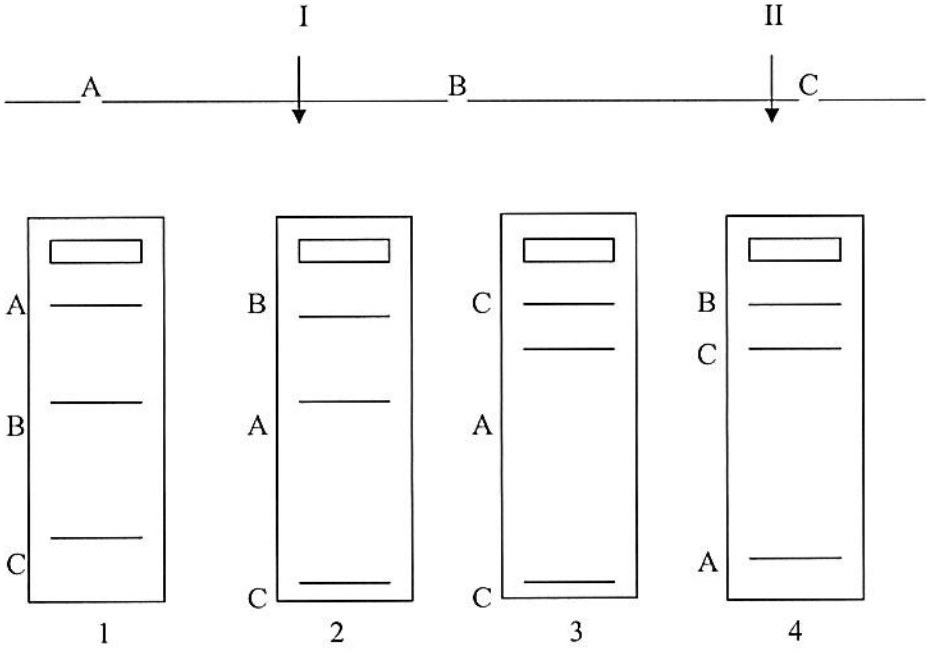
5. Troubleshooting electrophoresis: What would occur if:
   a. the gel box is filled with water instead of TBE buffer?
   b. water is used to prepare the gel instead of TBE buffer?
   c. the electrodes are reversed?

6. Examine the photograph of your stained gel (or view on a light box or overhead projector). Compare your gel with the ideal gel shown below and try to account for the fragments of DNA in each lane. How can you account for differences in separation and band intensity between your gel and the ideal gel?

7. What is the identity of the unknown restriction enzyme? Explain your answer.

8. Troubleshooting gels. What effect will be observed in the stained bands of DNA in an agarose gel:
   a. if the casting tray is moved or jarred while agarose is solidifying in Part II, Step 1?
   b. if the gel is run at very high voltage?
   c. if a large air bubble or clump is allowed to set in agarose?
   d. if too much DNA is loaded in a lane?

9. A segment of DNA has two restriction sites. When digested with restrictions enzymes I and II, three fragments will be formed. (A, B and C). Which of the following gels produced by electrophoresis would represent the separation and identity of these fragments? Select the number of the gel and explain your results.



Dolan DNA Learning Center

Bacterial Transformation

Student Post-Lab Worksheet

Name: _____ **Date:** _____

1. Observe the plates, and record number of colonies on each box in the matrix below. If cell growth is too dense to count individual colonies, record "lawn."

	Transformed cells +plasmid	Nontransformed cells –plasmid
LB/amp		
LB		

Were results as expected? Explain possible reasons for variations from expected results.

2. Compare and contrast the number of colonies on each of the following pairs of plates. What does each pair of results tell you about the experiment?

   a. +LB and –LB

   b. –LB/amp and –LB

   c. +LB/amp and –LB/amp

   d. +LB/amp and +LB

3. Transformation efficiency is expressed as the number of antibiotic resistant colonies per μg of pGFP DNA. The object is to determine the mass of pGFP that was spread on the experimental plate, and was responsible for the transformants observed.

   a. Determine total mass (in μg) of pGFP used in Step 9.
   Concentration x Volume = Mass.

   b. Determine fraction of cell suspension spread onto +LB/amp plate (Step 18).
   Volume Suspension Spread/Total Volume Suspension = Fraction Spread.

c. Determine mass of pGFP in cell suspension spread onto +LB/amp plate.
Total Mass of pGFP (a) x Fraction Spread (b) = Mass of pGFP Spread.

d. Determine number of colonies per μg of pGFP. Express answer in scientific notation.
Colonies Observed / Mass of pGFP Spread (c) = Transformation Efficiency.

4. What factors might influence transformation efficiency?