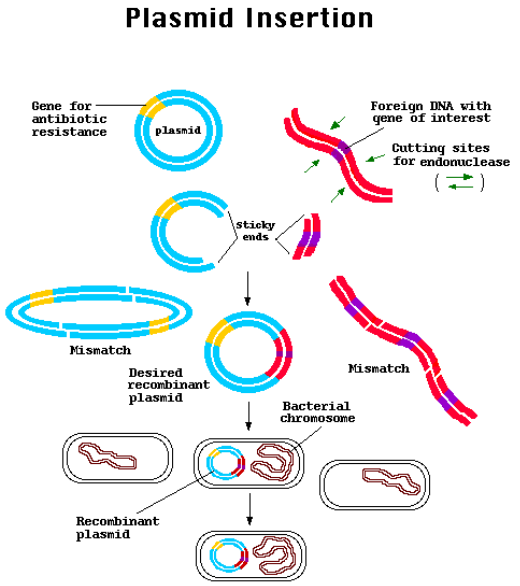
Name…….. Date…… Period…….

Student Laboratory Packet
Molecular Biology
A Laboratory Activity for the Living Environment

Introduction Bacteria is an ideal organism for the molecular geneticist to manipulate and has been used extensively in recombinant DNA research.

Some bacteria may contain small circular DNA molecules (1,000 to 200,000 base pairs) called **plasmids**, which also carry genetic information. Certain plasmids, called R plasmids, carry genes for resistance to antibiotics such as ampicillin, kanamycin, or tetracycline.

Bacterial Transformation using a Restriction Enzyme



Part A: Bacterial Transformation

Background Information

Genes control the traits that living organisms possess. Bacteria have genes on their chromosomes and on a small circular piece of DNA called a plasmid. Genes can be transfered from one bacteria to another on the plasmid by a process known as transformation. In bacterial transformation, a plasmid with a gene (DNA) for resistance to the antibiotic ampicillin (AMP) is used to transfer a resistant gene into a susceptible strain of the bacteria. The same technique is used to transfer genes (DNA) for production of insulin, growth hormones, and other proteins into bacteria. The transformed bacteria are used in fermentation to produce commercial quantities of the protein for treating diabetes, dwarfism, or other uses.

Procedure Part A

Observe photographs of bacterial plates below. The bacterial colonies are represented by the white dots on each plate. Some of the bacteria is resistant to ampicillin, an antibiotic that is lethal to many bacteria.

<u>Count the number of individual colonies.</u>

1

2

3

4

1 _____ 3_____

2 _____ 4_____

2. Compare and contrast the number of colonies on each of the above plates. Which plates contain the ampicillin resistant bacteria?

_____

_____

Part B: Restriction Enzyme Cleavage of DNA and Electrophoresis

Background Information Restriction enzymes or restriction enzymes are essential tools in recombinant DNA technology. Restriction enzymes recognize specific DNA sequences and digest the DNA at these sites. The result is the production of fragments of DNA of various lengths.

Samples of DNA obtained have been incubated with different restriction enzymes. The resulting fragments of DNA were separated by using gel electrophoresis. The DNA samples were loaded into wells of an agarose gel and separated by the process of electrophoresis. After migration of the DNA through an electrical field, the gel was stained with methylene blue, a dye which binds to DNA.

When any molecule enters an electric field, the speed at which it will move is influenced by the charge of the molecule, the strength of the electrical field, the size and shape of the molecule, and the density of the medium (gel) through which it is migrating. When all molecules are positioned at a uniform starting site on a gel and a gel is placed in a solution and electricity is applied, the molecules will migrate or move and appear as bands. Nucleic acids, like DNA and RNA, move because they are negatively charged and will migrate through the gel toward the positive electrode.

In this exercise, we will use a photograph of an **agarose gel**. In agarose, the migration rate of fragments of DNA is proportional to their size; the smaller the DNA molecules, the faster it migrates through the gel.

Procedure Part B

1. Measure the migration distance (in cm) for each band on the gel sample below. Measure from the top edge to the bottom of each fragment band (dark rectangle) using the ruler to the right. Record these measurements on **Table 1.1.**
2. Measure the actual base pairs for each band on the gel sample below. Find each fragment band (dark rectangle) and line it up to the Actual base pairs ruler to the right. Record these measurements on **Table 1.1.**

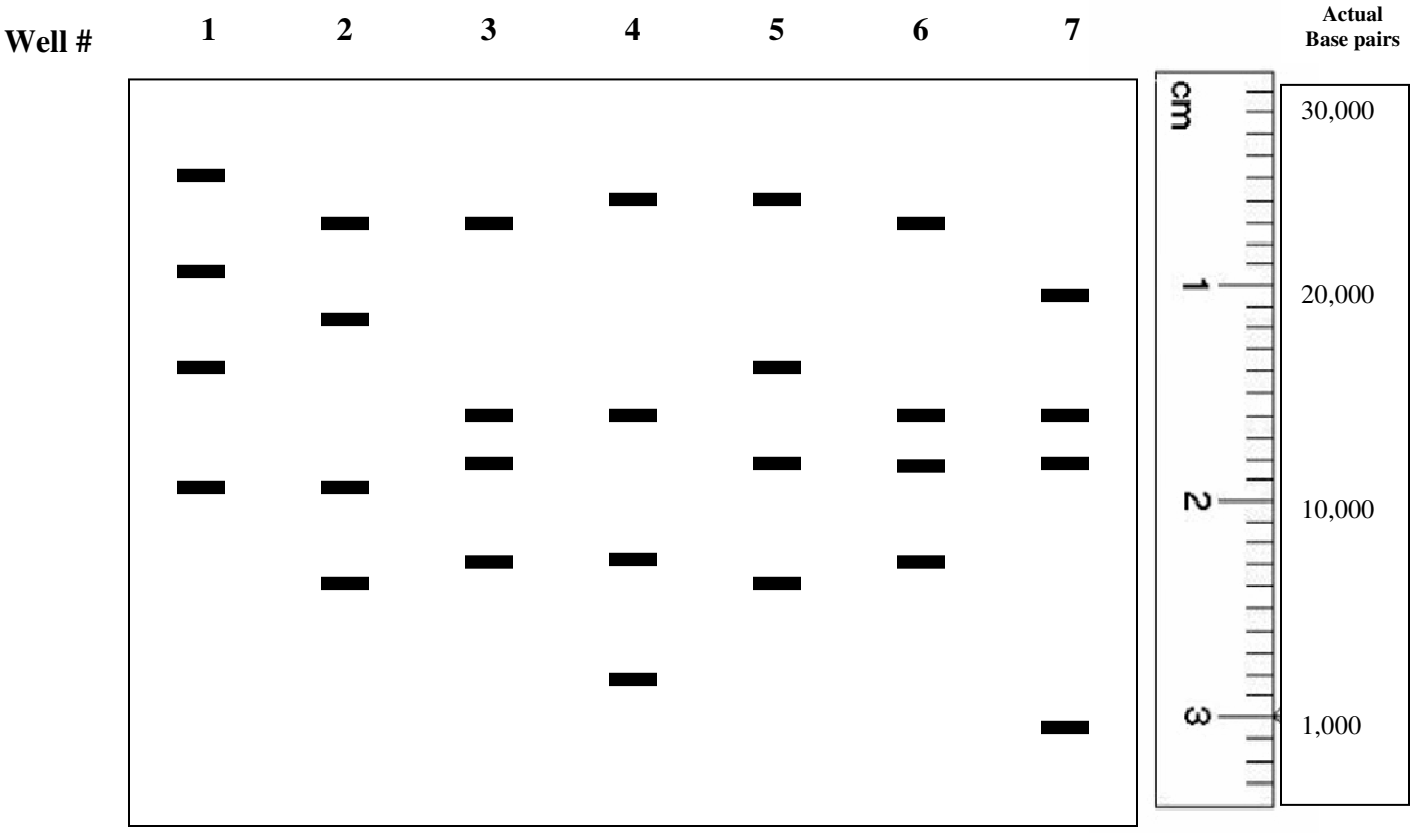
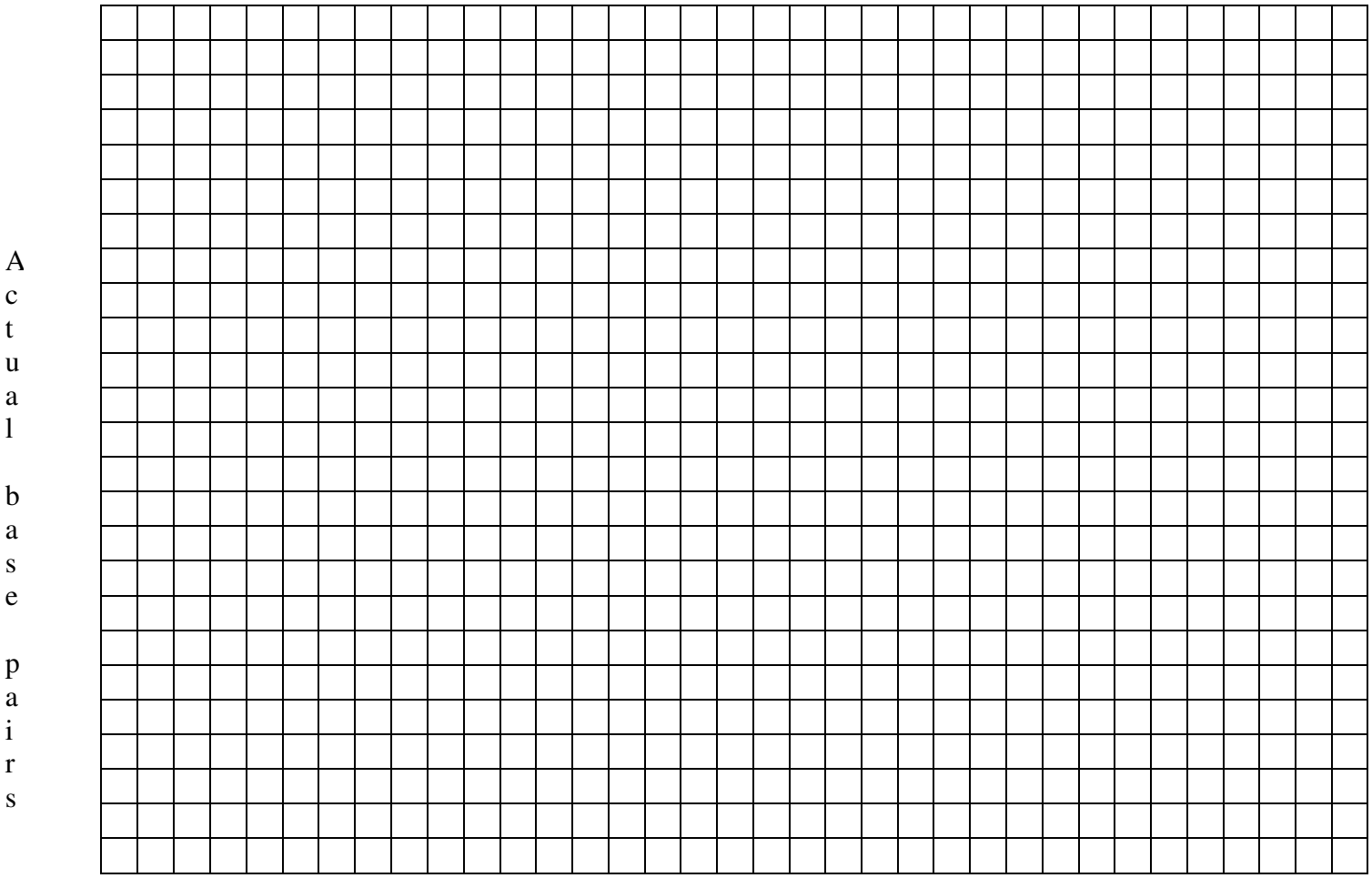


Table 1.1: Distance fragments moved in agarose gel (cm) and fragment size (base pairs in thousands)

Distance fragments moved in (cm)							Actual base pairs (in thousands)						
Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7	Well 1	Well 2	Well 3	Well 4	Well 5	Well 6	Well 7

2. **USING JUST WELLS 3 and 6** Plot on the x axis the distance migrated by each fragment. Plot a point using the y axis and actual base pair size. Circle each point. Connect the points as a line graph.

Graph Title:



Questions

1. What is a plasmid? How are plasmids used in genetic engineering ?

2. What are restriction enzymes? How do they work?

3. Use the graph you will prepare from your lab data to predict how far (in cm) a fragment of 8,000 bp would move.