MOLECULAR BIOLOGY
PRE-LAB

Name: _____ Date: _____

Total Points: 40 Due Date: _____

1. Define the following terms: (0.5 points per term, total of 4 points)
   a. Plasmid:
   b. R Plasmid:
   c. Transformation:
   d. Competence:
   e. Restriction Endonuclease:
   f. pAMP:
   g. EcoRI:
   h. Electrophoresis:
2. How are competent cells produced? (2 points)
3. What are the steps needed in producing a new plasmid resistant to antibiotics? (2 points)
4. Why do we place the bacteria onto plates treated with ampicillin? (2 points)
5. What are the steps needed in order to clone the DNA? (2 points)
6. Distinguish between 'blunt' and 'sticky' ends. (2 points)
7. Why do we use gels in electrophoresis? (1 point)
8. How does electrophoresis work? (2 points)
9. What determines the distance of the DNA fragments? (2 points)
10. Why is the buffer solution needed? (1 point)

For the following questions and data manipulation, you will use the pictured electrophoresis gel (included).

Information: Linear DNA fragments move at rates inversely proportional to the $\log_{10}$ of their molecular weights. For simplicity, the base—pair length is substituted for molecular weight.

Procedure:

1) For the ideal gel, measure the distance (in mm) of each HindIII fragment that migrated from the well. Measure the front edge of the well to the front edge of each band and record the information in table 1 next to the appropriate band. Remember, the larger the band, the slower the movement in the gel.
2) Set up the semi-log paper with the distance migrated on the X-axis. Plot the distance migrated verses the base-pair length for each HindIII fragment.
3) Connect the points with the best-fit line.
4) Measure the EcoRI fragments in the same manner that you measured the HindIII fragments and record the information in Table 1.

5) Locate on the X-axis the distance migrated for the first EcoRI fragment. Using a ruler, draw a vertical line from this point to the best-fit line. Now extend a horizontal line from this point to the Y-axis. This give the calculated base-pair size of the EcoRI fragment.
6) Record this in Table 2.
7) Repeat steps 5 and 6 for all EcoRI fragments.
8) Compare your data with the real fragment lengths of the EcoRI fragments. (15 points for the table and 5 points for the graph)

TABLE 1: HINDIII DISTANCES AND SIZE

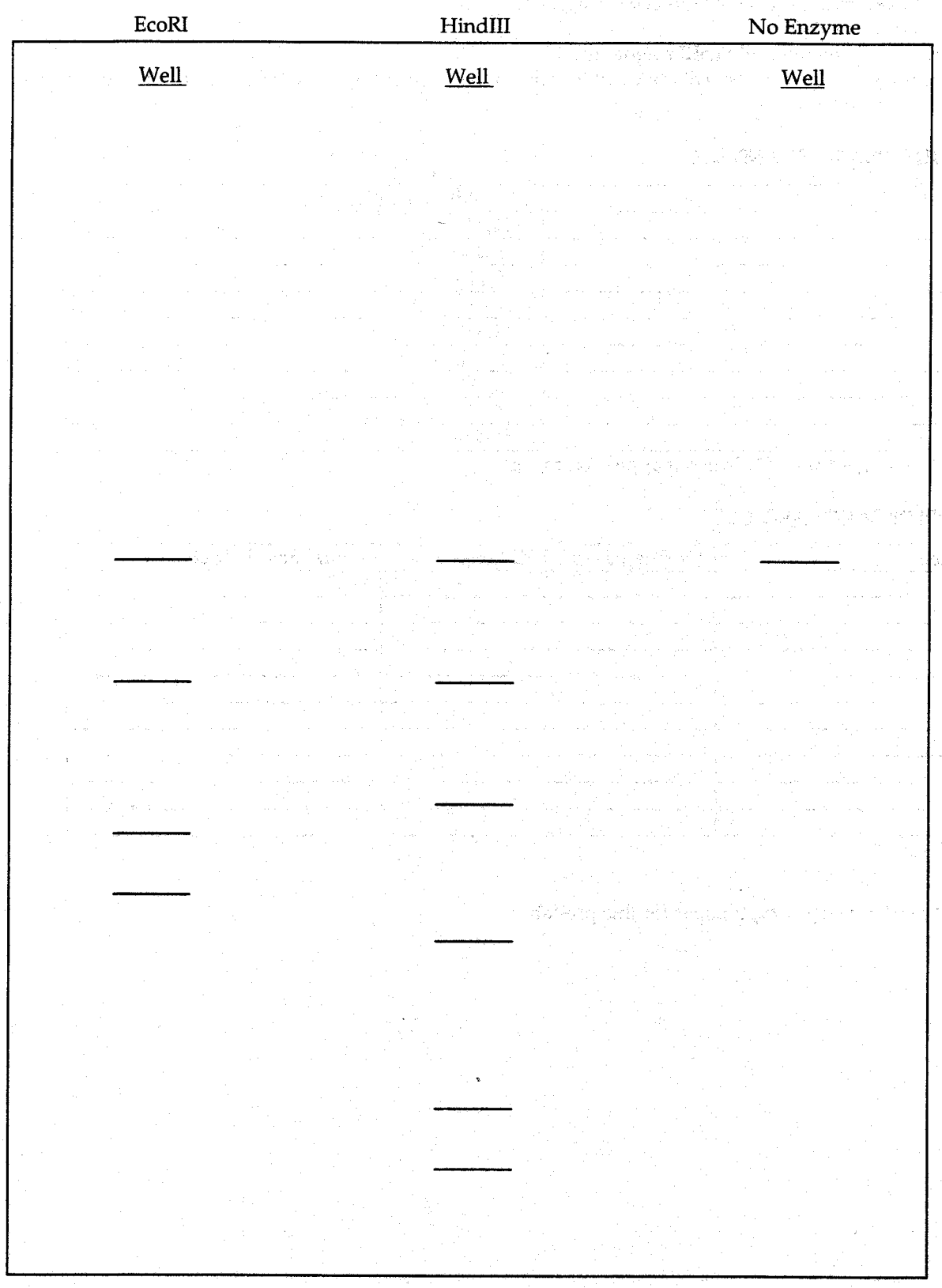
Distance	Actual Base Pair Length
	*27,491
	*23,130
	9,416
	6,682
	4,361
	2,322
	2,027
	**564
	**125

*Pair appears as a single band ** Does not appear on this gel

TABLE 2: ECORI DISTANCE AND SIZE

Distance	Calculated Base Pair Length	Actual Base Pair Length

Note: You will need semi-log graph paper for this pre-lab.


EcoRI
HindIII
No Enzyme
Well
Well
Well