Country _____

Competitor# _____

th

16 International Biology Olympiad

Beijing

July 2005

Practical Examination

Part i

Total time available: 90 minutes

The 16th IBO Practical Tests

First name: Last name Country:

Code: Important:

1. Write your name and code on both task paper and answer paper sheets.
2. Make sure that all the results should be written on the answer paper unless otherwise instructed.
3. There are 4 parts in practical test. Each part has 90 min. You should start your **first** test according to last digit of your competitor code. For example, if you have a code of 221, your first practical test will be part I, if you have a code of 223, your first practical test will be part III.
4. Your **second** practical test is as follows: competitors from part I and part II switch labs; competitors from part III and part IV switch labs;
5. You go to your **third** practical test according to the following rules:

If the last digit of your competitor code is 1, you go to practical test part III. If the last digit of your competitor code is 2, you go to practical test part IV. If the last digit of your competitor code is 3, you go to practical test part I. If the last digit of your competitor code is 4, you go to practical test part II. You should follow the instructions from your guides when switching labs.

Practical tests Part I:

Biochemistry and Molecular Biology

Very important notice: you should start task 1 first and finish task 2 while the gel electrophoresis of task 1 is running!

Task l:Separation of plasmid DNA restriction fragments by Agarose Gel Electrophoresis

Instruments: Centrifuge, Agarose gel electrophoresis apparatus and Fluorescence gel imaging systems.

Important:

Raise the blue card on the bench table to ask for help when you want to use the electrophoresis power supplies.

Introduction

Plasmids are circular double-stranded DNA molecules, which can exist and replicate independently in bacteria cells. Restriction enzymes can cut the plasmid DNA into fragments. In the experiment a plasmid and three restriction enzymes *BamW\> Pst* and *Hindlll* are provided. You will use the three restriction enzymes to digest the plasmid DNA and run agarose gel electrophoresis. You need to determine the

restriction enzyme sites and calculate the size of restriction fragments between cutting sites according to migration distance of DNA fragment, which is inversely correlated to the logarithm of the length of fragment.

Reagents

1. 1 xTAE buffer -Tris-acetate-EDTA
2. DNA dye - GeneFinder™ containing anthocyanin and sucrose
3. *BamHl*
4. *Pstl*
5. *Hindltt*
6. PlasmidDNA
7. DNA size standard
8. Distilled water

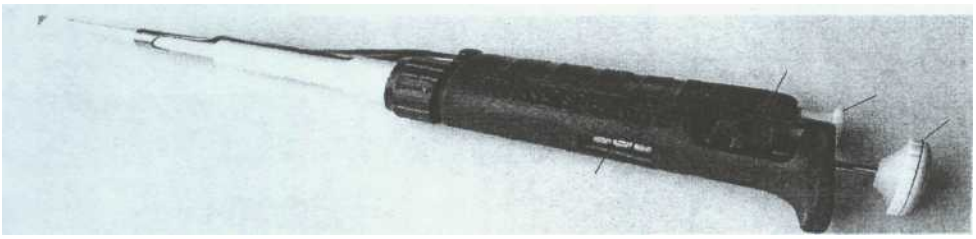
Equipment

1. Lab gloves
2. Marker pen
3. 0.5 ml centrifuge tubes
4. Centrifuge tube holder
5. Pipettes
6. Centrifuge
7. Incubator
8. Agarose gel electrophoresis apparatus

9. Fluorescence gel imaging systems (use it with lab assistants),

Procedure and operation of equipments

1. Pipette:



A 0-lOQjl pipette is provided for the experiment. The volume is adjusted by turning the setting ring. The digits of the volume display should be read from top to bottom. After attaching an appropriate tip, press the control button down to the first stop and insert the tip in liquid. Slowly release the button until it reaches a compete stop to aspirate the sample. Then, insert the tip with the liquid to the target places and press the button down slowly to the second stop until all collected liquid is completely out of the tip. Eject the used tip to the trash by pressing the ejector button.

2. Centrifuge

Press the stop lever down to open the lid. Load tubes on the rotor. Be sure to balance the load properly. Close and firmly press down the lid until the lid locks into its position. The rotor will begin spinning when the lid is completely closed. Let the

centrifuge last 20 seconds. Push the stop lever, open the lid and remove the tubes after the rotor has stopped spinning.

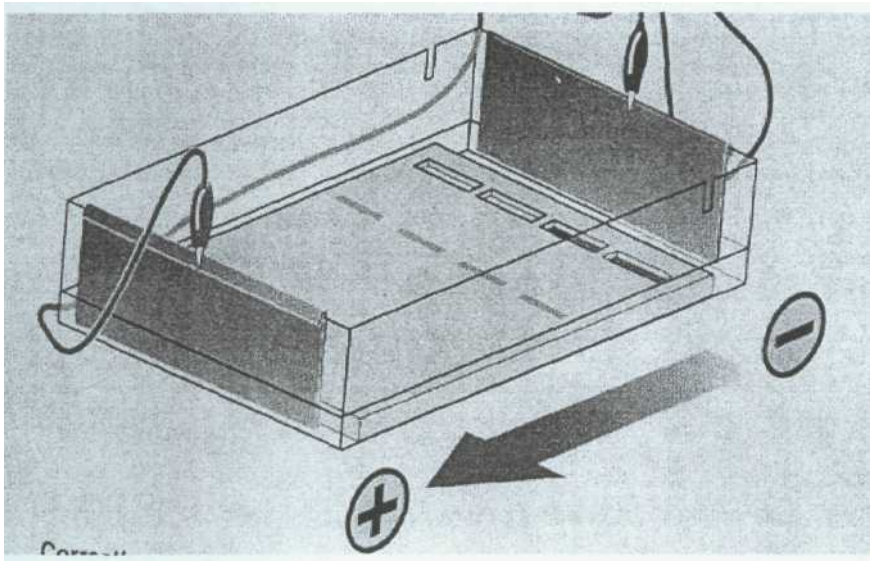
3. Restriction enzyme digestions

Type II restriction enzymes recognize certain DNA sequences and digest DNA at the recognition sites. The plasmid DNA provided to you should be digested by three different enzymes: *BamW\, Pst* and *Hindlll.* Add Inappropriate amount of enzyme(s) to the plasmid DNA in centrifuge tube and close the lid. Mix it well by gently tapping the bottom of the centrifuge tube. Incubate the centrifuge tubes at 37°C for 15 min.

4. Agarose gel electrophoresis apparatus

The 0.8% Agarose gel with wells is ready for use. Filling the electrophoresis tank with 1 xTAE buffer and let the buffer cover the gel. The buffer surface should be about 3-4mm above the agarose gel surface. Load 10 µl of the sample, which contain (1) plasmid DNA cleaved with restriction enzymes and (2) DNA dye, into the wells of the gel. **Please note that** the tip should be 1-2 mm above the bottom of the well so that you could load all sample into the wells without puncturing the bottom of the wells. After loading samples, close the cover of the electrophoresis tank. Note that Red wire connects anode and Black wire connects cathode. Call the laboratory assistant to turn on the power supply by raising the blue card. Run the samples at 100 volt for 40 min. After that call the assistant to turn off the power supply by raising the blue card. Every competitor will use one electrophoresis tank, while every 2

competitors share one power supply.



5. Gel imaging system

This system is operated by lab assistants. Your samples contain a non-toxic dye that binds DNA fragments for visualization.

Experimental procedure

1. Label eight 0.5-ml centrifuge tubes 1 through 8 with a marker pen, Add solutions to each tube the mixture as follows:

Table 1. Digestion of plasmid DNA with restriction enzymes

No.	Plasmid DNA *fa\)*	*BamHl fal)*	*Pstlfal)*	*Hindlllfal)*	ddH_2O *(\il)*
1	5			1 9	
2	5			1 9	
3	5		1	9	
4	5		1	1 8	
5	5			1 1 8	
6 O^	**5**			**1 1**	**1**
7	**/?"**			**10**	

2. Mix well and incubate 1-6 tubes at 37°C for 15 minutes. Leave tube 7 in the tube holder. If you found droplets of the solution on the inside tube wall, you may used the centrifuge to spin them to the bottom of the tube. The centrifuges are provided on your table.
3. Put the agarose gel (previously prepared for you) into the electrophoresis tank, pour 1 xTAE buffer into the tank and let the buffer cover the gel about 3-4mm. The gel has 10 wells for sample loading.
4. Add 6 µl DNA size standards into the No.8 centrifuge tube.
5. Add 3 µl of 5X dye to each tube, mix them well.
6. Load 5 µl of DNA size standards (tube No. 8) into the **First** well of the gel. Load all of your plasmid samples from the second well through the eighth well in the order of Table lUse a clean tip for each load. Close the cover of the electrophoresis tank. Call the assistant by raising the blue card to turn on the

power supply. Run the samples at 100 volt for 40 min.

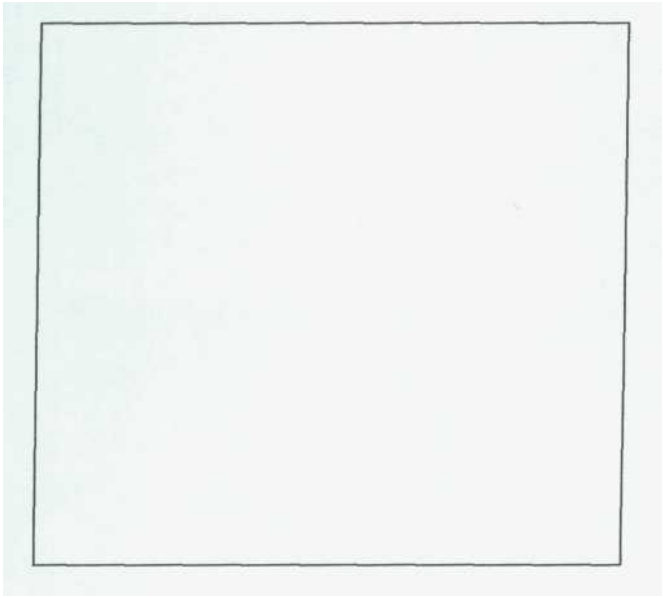
7. After the electrophoresis, call the assistant to turnoff the power supply by raising the blue card. Ware gloves and take out the gel holder. (Note: **during your waiting time for electrophoresis, please go to task 2 and** finish **it.)**

8. Put your gel into the box with your competitor's number. Close the lid and leave the box on the table. A lab assistant will take the gel image and print a copy for you.

Separation of plasmid DNA restriction fragments with agarose gel electrophoresis (24 points: 3 points for each lane). The score of this task will be given by a professor in charge of this test.

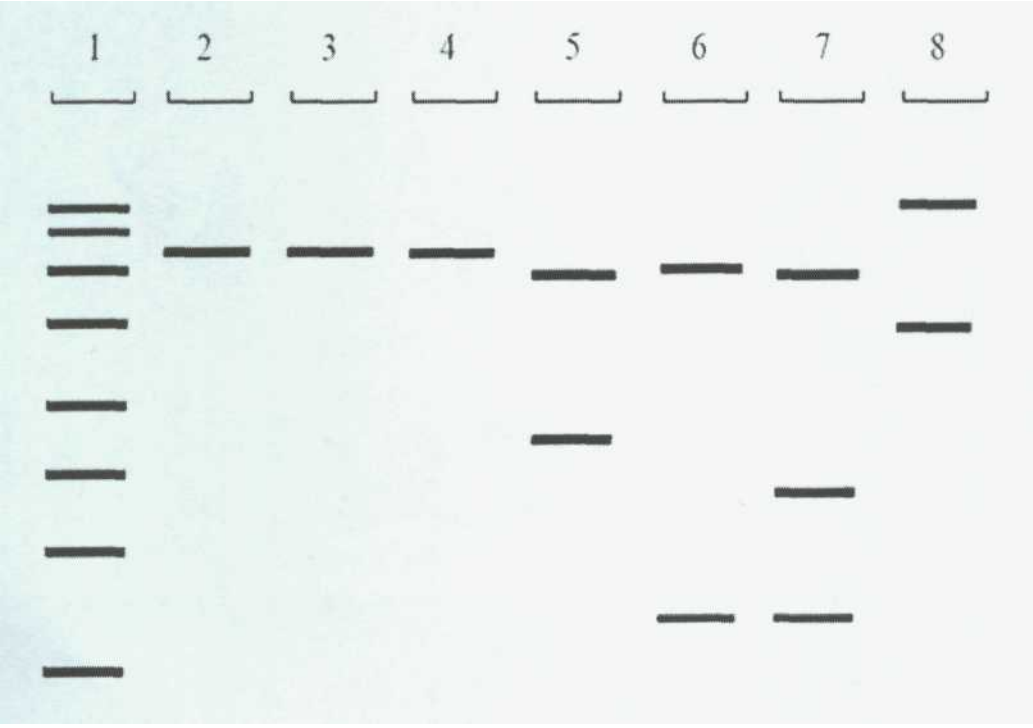
Three points for each lane: No DNA, no point; smearing lane with clear bands, minus 1 point; incomplete digestion, minus 1 point; faint bands, minus 1 point.

Your gel image will be posted below once it is printed by the lab assistant.



Task 2: Determination of restriction enzyme sites and DNA fragment size of restriction fragments. (16 points)

Due to time limitation, you will not be able to your won gel for size analysis. However, the figure below is an agarose gel profile of DNA fragments, in which an identical plasmid was digested with the same three DNA restriction enzymes. The procedure for digestion and loading positions of each digestion in the gel are identical to the instruction in task 1. Please answer the following questions according to the profile.



Question 1. How many sites does this plasmid have for *Pstl, BamHl* and *Hindlll,* respectively? (3 point)

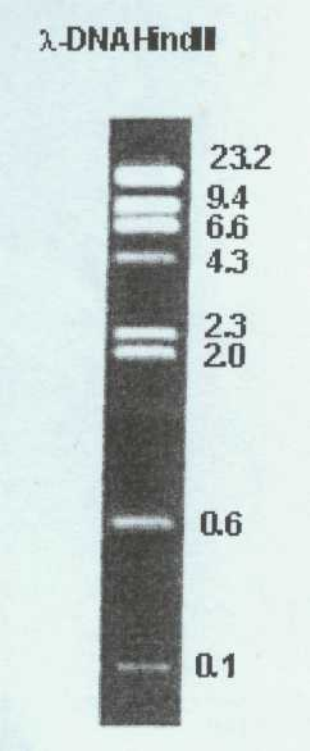
A. ***Pst*I:l,** *BamHl:* 0, *Hindlll*: 2.

B. ***Pstl:2,*** *BamHl*: 0, *Hindlll*: 2.

C. ***Pstl:2,*** *BamHl* : 1, *Hindlll* : 0.

D. Ps**tl:l,** *BamHl* : 1, ///Will: 1.

Question 2. Lambda DNA is often digested with restriction enzymes and used as molecular standard in running agarose gels. The figure below is a profile of lambda viral DNA fragments obtained with *Hindlll* digestion. The numbers on the right side of the gel are fragment sizes in kb.



Which of the following is/are true? (3 points)

(1) There are 8 sites for *Hindlll* on lambda DNA.

(2) Since lambda DNA can be digested by *Hindlll,* the entire molecule of lambda DNA must be double stranded.

(3) The profile shown in the figure above is likely a fluorescent image of a dye binding to DNA fragments.

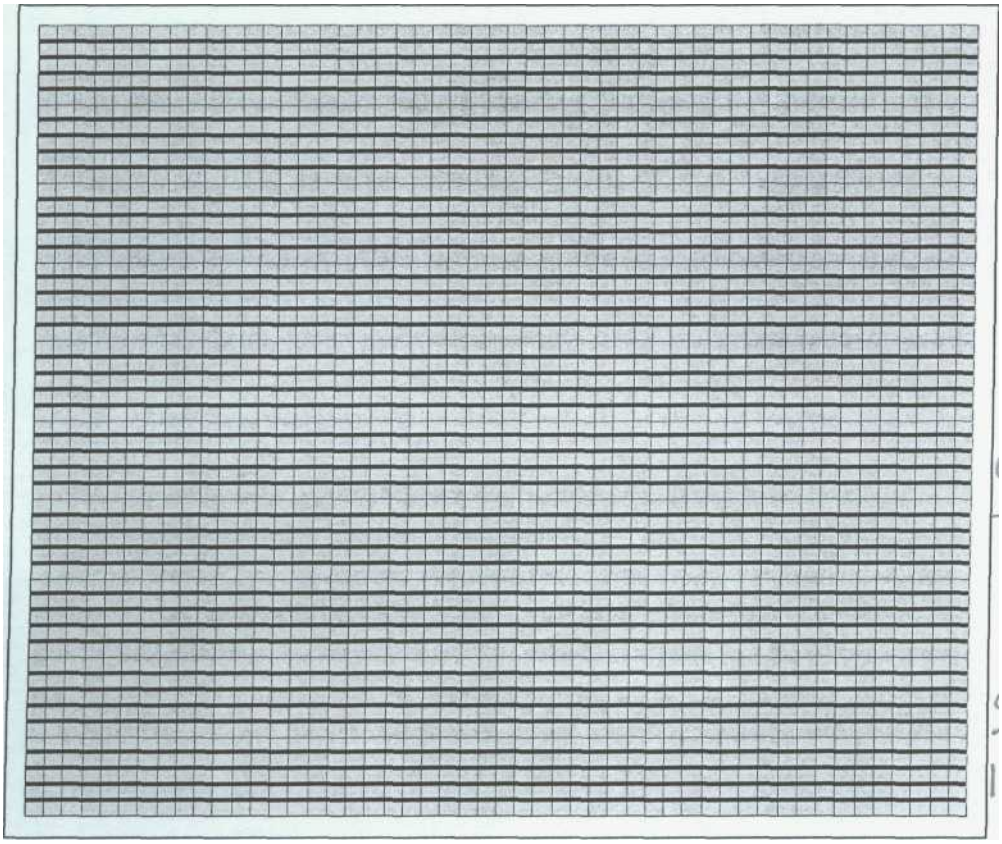
A. 1

B. 1,2,3

C. 2,3

D. 3

Questions 3-5. The gel profile contains six bands of DNA size standards in lane 8 and the sizes of the DNA fragments in lane 8 are labeleb. It is known that migration distance of a DNA fragment is inversely correlated to the logarithm of the fragment length. Please plotting the logarithm of the DNA fragment sizes (kb) versus the migration distances (cm) on the plotting paper below, and calculate the sizes (kb) of the DNA fragments.



Question 3. The size (kb) of the smaller restriction fragment between *Pstl* & *Hindlll* is: (3 points)

A. 2.5

B. 0.8

C. 1.1

D. 0.6

Question 4. The size (kb) of the smaller restriction fragment between *Hind*III & *Bam*HI is: (3 points)

A. 0.8

B. 0.4

C. 0.5

D. 0.6

Question 5. The plasmid length (kb) is: (4 points)

A. 5.2

B. 6.9

C. 4.8

D. 4.3