Country _____	Competitor# __ __ __

16th International Biology Olympiad

Beijing

July 2005

Practical Examination

Part I

Total time available: 90 minutes

The 16th IBO Practical Tests (實作題)

First name (名):

Last name (姓):

Country (國):

Code (考生編號):

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.

重要指示：

1. 在試卷及答案卷上都必須要寫姓名及考生編號。

2. 除非另有指示，所有答案必須要寫到答案紙上。

3. 實作題分為四個部分，每部分 **90** 分鐘。考生編號的最後一位數字，就是你應該開始的第一個實作部分。例如編號 **221** 的考生，第一個實作題是第一部分，編號 **223** 的考生，第一個實作題是第三部分。

4. 有關你第二個實作題的指示如下：第一部分與第二部分的考生交換實驗室；第三部分與第四部分的考生交換實驗室。

5. 有關你第三個實作題，必須遵守的指示如下：

如果考生編號的最後一位數字是 **1**，你應該做第三部分。

如果考生編號的最後一位數字是 **2**，你應該做第四部分。

如果考生編號的最後一位數字是 **3**，你應該做第一部分。

如果考生編號的最後一位數字是 **4**，你應該做第二部分。

在轉換實驗室時，必須遵守助教的指示。

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!

非常重要：你必須從 作業 **1** 開始，當你在跑電泳時，完成 作業 **2** ！

Task 1: Separation of plasmid DNA restriction fragments by Agarose Gel Electrophoresis

作業 1：利用洋菜膠電泳法分離質體 **DNA** 的限制片段

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

儀器：離心機、洋菜膠電泳裝置、螢光膠體顯像系統

注意 (Important)：

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

當你需要使用電泳槽電源供應器時，請舉起桌上的藍牌。

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 *Bam*HI、*Pst*I and *Hind*III 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.

說明

質體是環狀雙股的 DNA 分子，它能存在於細菌細胞中，並獨立複製；限制酶可以將質體 DNA 切割成片段 。本實驗提供一個質體和三種限制酶（*Bam*HI、*Pst*I 和 *Hind*III），你將用這三種限制酶去切割質體 DNA 並進行洋菜膠電泳。你須要決定限制酶的切割點；因為 DNA 片段長度的對數和此片段在膠體中移動距離成反比，所以根據 DNA 片段移動距離去計算位於切割點之間的限制片段的大小。

試劑（Reagents）

1. 1×TAE 緩衝液

   1×TAE buffer – Tris-acetate-EDTA

2. 5×DNA 染劑 （內含 anthocyanin 和蔗糖）

   5×DNA dye - GeneFinder™ containing anthocyanin and sucrose

3. 限制酶 *Bam*HI
4. 限制酶 *Pst*I
5. 限制酶 *Hind*III
6. 質體 DNA (Plasmid DNA)
7. DNA 大小標準樣本 (DNA size standard)
8. 蒸餾水 (Distilled water)

器材 (Equipment)

1. 實驗用手套 Lab gloves
2. 標記用的筆 Marker pen
3. 0.5 ml 離心管 0.5 ml centrifuge tubes
4. 離心管架 Centrifuge tube holder
5. 微量滴管 Pipettes

6. 離心機　　Centrifuge
7. 培養箱　　Incubator
8. 洋菜膠電泳裝置　　Agarose gel electrophoresis apparatus
9. 螢光膠體顯像系統(使用時由助教協助)
   Fluorescence gel imaging systems (use it with lab assistants),

器材的操作步驟 (Procedure and operation of equipments)

1. 微量滴管 (Pipette)

A 0-10 μl 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.
實驗中提供一支 0-10 μl 的微量滴管，藉由轉動設定環可以調節吸取的容量。

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 to the trash by pressing the ejector button.
在插上適當的 tip 後，大拇指壓下控制鈕至第一段，並將 tip 伸進液體中，慢慢放開控制鈕，直到樣本液體完全吸入。接著，將含有液體的 tip 插入標的容器中，大拇指再次緩慢地壓下控制鈕至第二段，直到液體完全由 tip 排出。壓下推出器按鈕，推出並丟棄使用過的 tip。

2. 離心 (Centrifuge)

Press the stop lever down to open the lid. Load tubes on the. 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.

壓下停止桿，打開離心機蓋子；將離心管放入轉盤中，並確認放下時保持平衡。穩固的蓋下蓋子，直到蓋子鎖上。當蓋子正確的蓋好後，轉盤將開始旋轉。持續離心 20 秒。壓下停止桿，等轉盤停止後，打開蓋子並取出離心管。

3. 限制酶的切割 (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: *Bam*HI, *Pst*I and *Hind*III. Add one appropriate amount of enzyme(s) to the plasmid DNA in centrifuge tube and close the lid.

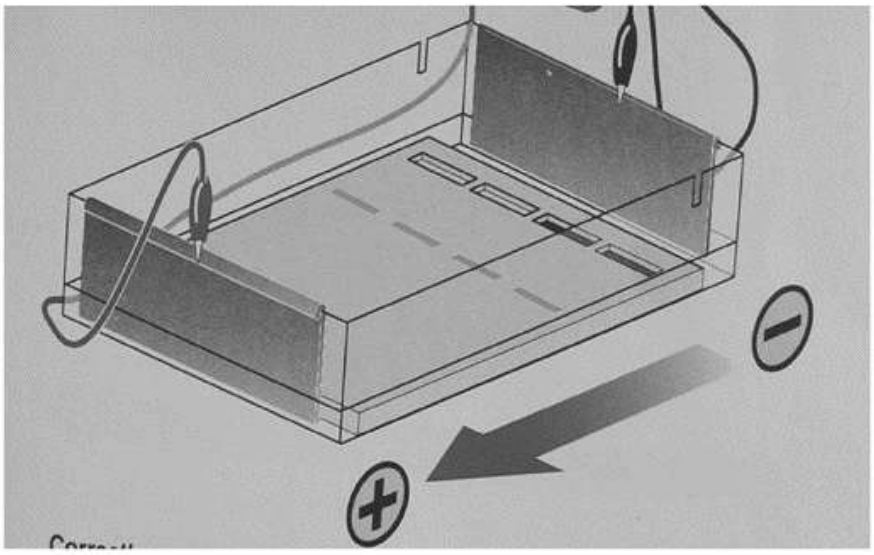
Mix it well by gently tapping the bottom of the centrifuge tube. Incubate the centrifuge tubes at 37℃ for 15 min.

Type II 限制酶可辨識特定的 DNA 序列，並在它的辨識位切割 DNA。提供給你的質體 DNA 將被三種不同的限制酶（*Bam*HI, *Pst*I 和 *Hind*III）切割。加入適量的酵素到含有質體 DNA 的離心管中，並蓋好蓋子。以輕敲離心管底部的方式，將其混合均勻後，離心管於 37℃ 反應 15 分鐘。

4. 洋菜膠電泳裝置 Agarose gel electrophoresis apparatus

The 0.8% Agarose gel with wells is ready for use. Filling the electrophoresis tank with 1×TAE buffer and let the buffer cover the gel. The buffer surface should be about 3-4 mm above the agarose gel surface. Load 10 μl of the sample, which contain (1)

plasmid DNA cleaved with restriction enzymes and (2) 5×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.



（備註：每名選手將使用一個電泳槽，而每 2 名選手共用一部電源供應器。）

實驗中，在樣本槽中已提供一個製備好的 0.8% 洋菜電泳膠。將 1×TAE緩衝液注滿電泳槽，並蓋過膠體和超過膠體表面 3-4 mm。 取 10 µl 樣本注入膠體的樣本槽中，此樣本包含 (1) 限制酶切割後的質體DNA和 (2) DNA染劑。 請注意，注入樣本時，tip頂端應該放在離樣本槽底部之上 1-2 mm，使你能把全部樣本注入樣本槽中，而不致於刺穿樣本槽的底部，破壞膠體。 注入樣本後，蓋上電泳槽的蓋子。請注意，紅色電線連結陽極，黑色電線則連結至陰極。舉起藍牌，請助教打開電源。以100伏特進行電泳40分鐘。電泳結束後，舉起藍牌，請助教關上電源。

5. 螢光膠體顯像系統 (Gel imaging system)

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

此系統由助教操作。你的樣本中含有無毒性的染劑，此染劑可結合DNA片段便於觀查。

實驗步驟 (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:
使用標記筆標記 8 支 0.5 ml 的離心管1 至8 號。每一管中加入以下的物質，如表一所示： (表一中未列出編號8離心管的資料，詳見步驟 4)

Table 1. Digestion of plasmid DNA with restriction enzymes
表一、以限制酶切割質體 DNA

No.	Plasmid DNA (µl)	*Bam*HI (µl)	*Pst*I (µl)	*Hind*III (µl)	ddH_2O (µl)
1	5	1			9
2	5		1		9
3	5			1	9
4	5	1	1		8
5	5	1		1	8
6	5	1	1	1	7
7	5				10

1. Mix well and incubate 1-6 tubes at 37℃ 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.
均勻混合後，編號第1-6管置於37℃反應15分鐘，但將第7管置於試管架。如果發現在管壁內側有小水滴殘留，你可以用離心機將其離心約20秒，離至試

管底部；離心機已放置於你的桌面以供使用。

2. Put the agarose gel (previously prepared for you) into the electrophoresis tank, pour 1×TAE buffer into the tank and let the buffer cover the gel about 3-4mm. The gel has 10 wells for sample loading.
將你之前準備好的洋菜膠體放入電泳槽中，將1×TAE緩衝液倒入電泳槽內，並超過膠體表面 3-4 mm。此膠體具有10個樣本槽，供注入樣本之用。

4. Add 6 μl DNA size standards into the No.8 centrifuge tube.
取 6 μl DNA 大小的標準樣本加至編號第 8 的離心管。

5. Add 3 μl of 5×DNA dye to each tube, mix them well.
每一管中加入 3 μl 的 5×DNA 染劑，均勻混合。

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 1. Please note that tube numbers differ from lane numbers in which they are loaded. Use 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.（note：during your waiting time for electrophoresis, please go to task 2 and finish it.）
將 DNA 大小標準樣本（編號第 8 的離心管） 5 μl 注入膠體的**第一個**樣本槽中。根據表一，依序將你所有的質體樣本注入到第 2 至第 8 的樣本槽中。**請特別注意**：原先離心管的編號與膠體上樣本槽的編號不同。
蓋上電泳槽的蓋子後，舉起藍牌，請助教打開電源。以 100 伏特進行電泳 40 分鐘。（備註：等待電泳當中，請進行並完成**作業二**）

7. After the electrophoresis, call the assistant to turn off the power supply by raising the blue card. Ware gloves and take out the gel holder.
電泳完成後，舉起藍牌，請助教關閉電源。戴上手套，取出膠體。

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.

將膠體放置在有你的編號的盒子中，關上盒蓋，將盒子放置於桌面。助教將會在取得膠體影像後，影印出此影像給你。

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.

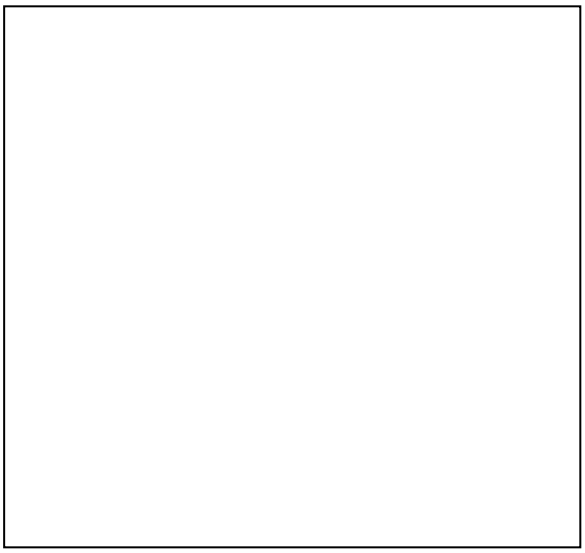
使用洋菜膠電泳分離質體 DNA 的限制片段（24 分：每一條的結果 3 分）。作業一的分數將由監試的教授評分。

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.

每一條樣本的結果 3 分：沒有 DNA，零分；背景模糊但仍可辨識出清楚的條紋，扣一分；切割不完全，扣一分；微弱的條紋，扣一分。

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

一旦助教將你的膠體影像列印出，將它貼至下列的表格中。

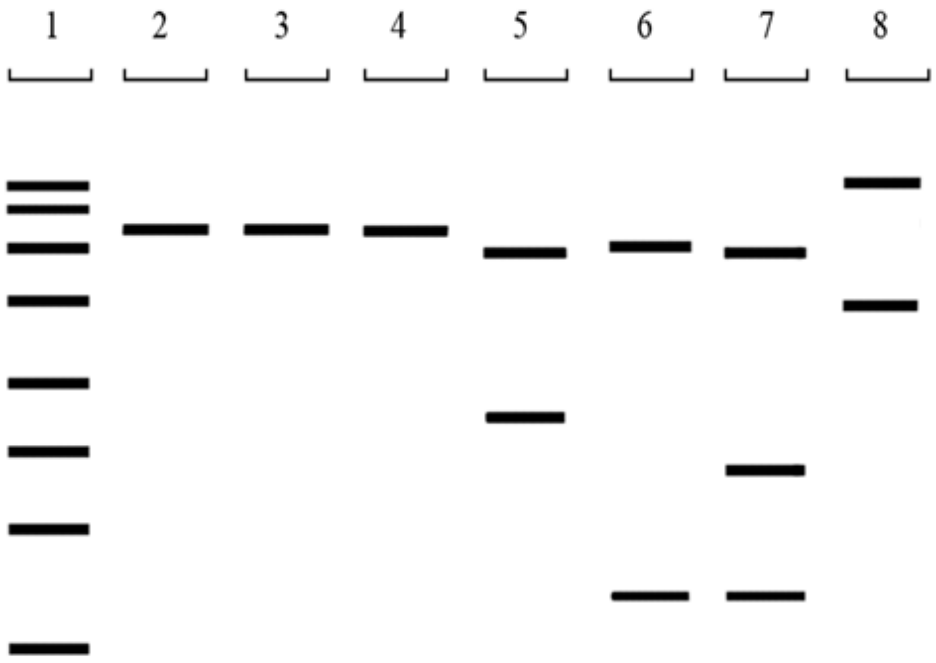


Task 2: Determination of restriction enzyme sites and DNA fragment size of restriction fragments. (16 points)

作業 2：決定限制酶的切點和限制片段的 DNA 片段大小（16 分）。

Due to time limitation, you will not be able to use your own 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.

由於時間有限，你將無法使用自己的膠體進行大小的分析。下列 DNA 片段的膠體圖譜與**作業 1 的實驗中**所有的實驗質體及酵素種類、步驟與結果完全相同。根據此圖譜回答下列 1 至 5 題：



Question 1. How many sites does this plasmid have for *Pst*I, *Bam*HI and *Hind*III, respectively? (3 point)

*Pst*I、 *Bam*HI 和 *Hind*III 對質體分別具有幾個切割點？（3 分）

A. *Pst*I:1, *Bam*HI: 0, *Hind*III : 2.

B. *Pst*I:2, *Bam*HI : 0, *Hind*III : 2.

C. *Pst*I:2, *Bam*HI : 1, *Hind*III : 0.

D. *Pst*I:1, *Bam*HI : 1, *Hind*III : 1.

Question 2. Linear 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 *Hind*III digestion. The numbers on the right side of the gel are fragment sizes in kb.

在洋菜膠電泳中，以限制酶切割的線狀 lambda DNA 經常作為分子標準樣本。下圖是經由 *Hind*III 切割後的 lambda DNA片段圖譜，右側是膠體中的DNA片段大小（kb）。

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

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

(2) Since lambda DNA can be digested by *Hind*III, 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.

下列敘述何者正確？（3 分）

(1) lambda DNA 具有 8 個 *Hind*III 的切割點

(2) 因為 lambda DNA 可以被 *Hind*III 切割，所以整個 lambda DNA 的分子一定是雙股的

(3) 以上顯示的圖譜似乎是由染劑結合到DNA片段的螢光顯像

A. 1

B. 1, 2, 3

C. 2, 3

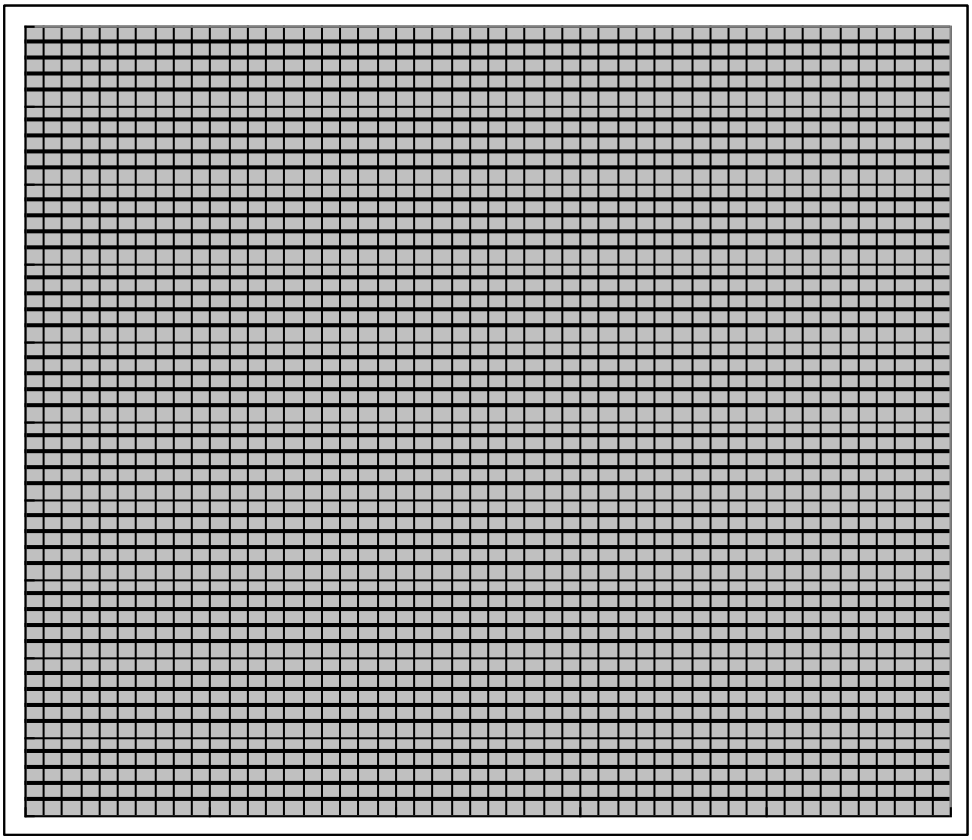
D. 3

Questions 3-5. The gel profile contains eight bands of DNA size standards in lane 1 and the sizes of the DNA fragments in lane 1 are follows（in bp）： 200, 500, 800, 1200, 2000, 3000, 4500, 7000. 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.

問題 3 至 5

在實驗的膠體圖譜中， lane 1 是 DNA 大小的標準樣本，內含 8 條 DNA 片段，它們的大小（bp）分別為 200, 500, 800, 1200, 2000, 3000, 4500, 7000。已知 DNA 片段在膠體中移動的距離與它長度的對數呈反比。

請以 DNA 片段大小 (bp) 的對數對移動距離 (cm) 作圖於所附的作標圖中，並計算 DNA 片段的大小。



Question 3. The size (kb) of the smaller restriction fragment between *Pst*I site & *Hind*III site is: (3 points)

位於 *Pst*I 切割點和 *Hind*III 切割點之間的較小之限制片段的大小為何？

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 site & *Bam*HI site is: (3 points)

位於 *Hind*III 切割點和 *Bam*HI 切割點之間較小的限制酶片段的大小為何？

A. 0.8

B. 0.4

C. 0.5

D. 0.6

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

質體的長度為何？

A. 5.2

B. 6.9

C. 4.8

D. 4.3