Viewing exam as superuser/admin

PRACTICAL EXAM 2 MOLECULAR BIOLOGY
實作考試2　分子生物學

GENERAL INFORMATION
一般事項

Max. total points 100
Exam duration 90 minutes
15 questions
總分 100分　考試時間 90分鐘　　15個問題

The exam consists of three parts.
本考試包括3部分

Part 1. Restriction enzyme map of plasmid (48 points)
第一部分 質體限制酶切位圖 (48分)

Part 2. PCR-based genotyping of yeast mutants (37 points)
第二部分 以PCR鑑定酵母菌突變株的基因型(37分)

Part 3. Amino acid auxotrophy of mutant yeast (15 points)
第三部分 酵母菌胺基酸合成缺失突變株(15分)

We suggest you read the entire exam file before you begin the lab work. In order to accomplish the entire exam, you have to run the three parts in parallel (see Figure below).
要完成全部3部分試題，你必須同時進行各部分試題，我們建議你先快速瀏覽全部試題，再開始做實驗，並參考以下的時間安排圖

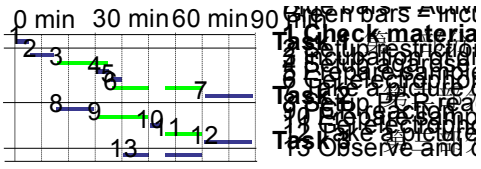


Figure 0.1: Suggested time table for completion of the three parts of the exam.
Figure 0.1 建議時間表以完成所有*3*部分實驗題

1 Check materials and equipment
1. 檢查材料和儀器設備

Task 1
2 Setup restriction enzyme digestion
3 Incubation of digestion reaction
4 Setup agarose gel
5 Prepare samples and load on gel
6 Gel electrophoresis*
7 Take a picture of the gel + Questions
第一部分
2.準備限制酶反應
3.進行限制酶反應
4.準備瓊脂膠
5.準備樣品和注膠
6.電泳
7.膠片照相和回答問題

Task 2
8 Setup PCR reactions
9 PCR reaction (in thermal cycler)
10 Prepare samples and load on gel
11 Gel electrophoresis*
12 Take a picture of the gel + Questions
第二部分
8.準備PCR反應
9.進行PCR反應
10.準備瓊脂膠
11.準備樣品和注膠
12.電泳
13.膠片照相和回答問題

Task 3
13 Observe and deduce + Ouestions
第三部分
13.觀察、推論及回答問題

**Use the same gel, but different wells for the samples*
注意： 第一部分和第二部分使用同一膠片，不同樣品孔和注膠時間

INTRODUCTION
背景介紹

MATERIALS AND EQUIPMENT
材料和儀器設備

In order to do your lab work, you need the materials A-V listed below. Please, ensure that these materials are available to you. **If anything is missing, contact the exam personnel by raising your pink card immediately – and no later than 15 minutes after the beginning of the exam.**
為完成你的實驗，你需要表列中的材料A~V，請確認這些材料都正確，若在考試開始**15**分鐘內發現缺少任何項目，立即舉起粉紅卡，通知試務人員，逾時則不補。

Please notice
1. All liquids are provided in 2X excess amount of what is needed in order to do the analyses. Additional materials (including tubes and agarose gel) cannot be provided in case of spillage or errors during the set-up of experiments.
2. Remember, in order to collect liquids at the bottom of a tube, use the technique that you were shown on Monday.
3. Remember, one of the purposes of the exam is to test your lab work skills.
4. Tubes labeled with a blue line on the label should be **kept on ice at all times**.
5. All solutions are frozen so you will have to **thaw and mix them** before use.
請注意
1. 所有液體會提供使用量**2**倍之量，若有打翻或出錯，都不會再補充**(**包括反應小管和電泳膠**)**。
2. 記住**:** 為吸取管中液體，請先手甩小管，讓液體集中在管底。
3. 記住**:** 本試題目的之一是測驗你的實驗技巧。
4. 標示藍色線的管子要隨時存放於冰上。
5. 所有溶液原本都是冷凍的，確認使用前已完全融化並混合完全。

MATERIAL ANNOTATION 標註	**QUANTITY** 數量	**MATERIAL** 器材	**USED IN PART** 使用的實驗項次
A	1	micropipette 1-10 µL 1-10 µl 微量滴管	1, 2
B	1	micropipette 20-200 µL 20-200 µl 微量滴管	1, 2
C	1	box of pipette tips 1-10 µL 1-10 µl 滴管尖	1, 2

D	1	box of pipette tips 20-200 µL 2-200 µl 滴管尖	1, 2
E	1	rack for PCR tubes PCR管架	1, 2
F	1	rack for 1.5 ml tubes 1.5 ml 管架	1, 2
G	5	1.5 ml tubes (in envelope) 1.5 ml 小管(大信封中)	2
H	2	strips of 0.2 ml PCR tubes (in envelope). Do not break the tubes apart 0.2 ml PCR小管聯條(大信封中)，勿將這些管聯條分開。	1, 2
I	1	White bag with agarose gel with 12 wells. (Do not open the bag until you are ready to load the gel) 白色塑膠袋內有12孔電泳膠(直到你要注膠時，才打開袋子)	1, 2
J	1	OneRun electrophoresis system with running buffer 電泳系統及電泳緩衝液	1, 2
K	1	marker pen 麥克筆	1, 2
L	1	stopwatch/timer 計時器	1, 2
M	1	paper tissue 紙	1, 2
N	1	set of plastic gloves (handed out in the waiting room) 塑膠手套(在等待室發給)	1, 2
O	2	zip-lock bags (in envelope) 夾鏈袋(大信封中)	1
		Booklet with photos of yeast	

P	1	grown on eight different media 酵母菌在8種培養基的生長照片冊	3
Q	1	Chart of biochemical reactions for formation of various amino acids (on the wall of the workspace) 實驗台牆上的胺基酸生化合成路徑圖	3
R	1	1 Kb Plus DNA ladder (in envelope) 1 kb plus 表尺DNA照片((大信封中)	1, 2
S	1	Pink card for contat with exam personnel 通知試務人員的粉紅卡片	1, 2, 3
T	2	Small ID tags 小ID標籤	1,2
U	1	Big ID tags 大ID標籤	1
V	1	Water 540 µL 540 µl水	1,2

Figure 0.2: *Material H: Tubes and ID label filled out with country code and student number.*
Figure 0.2: 器材*H :* 小管聯條及含國家代碼和學生*ID*的標籤

Ice bucket containing 1.5 ml tubes with a set of liquids.
在冰桶內一組裝有各種溶液的1.5 ml 小管

LIQUID ANNOTATION 溶液	QUANTITY 數量	LABEL 標示	VOLUME (µL) 體積(µL)	CONTENT 內容物
1	1	dNTPs	60	dATP, dGTP, dCTP and dTTP mix
2	1	DNApol buffer	140	DNA polymerase buffer (5X) DNA聚合酶緩衝液(5X)
3	1	Loading buffer	100	Loading buff for agarose g electrophore 電泳注膠緩衝液
4	1	Prime A	30	Primer pair A 引子對 A
5	1	Prime B	30	Primer pair B 引子對 B
6	1	Prime C	30	Primer pair C 引子對 C
7	1	Prime D	30	Primer pair D 引子對 D
8	1	Prime E	30	Primer pair E 引子對 E
9	1	DNApol	14	DNA polymerase DNA聚合酶
10	1	1 Kb ladder	12	DNA ladder f agarose gel electrophore

				電泳尺標DNA
11	1	Buffer 1	8	Buffer 1 (10X 緩衝液 1 (10
12	1	Buffer 2	8	Buffer 2 (10X 緩衝液 2 (10
13	1	Buffer 3	8	Buffer 3 (10X 緩衝液 3 (10
14	1	TemplateWT	6	Template DN wild type 正常基因型 DNA模板
15	1	Template mutant	6	Template DN mutant 突變體DNA模板
16	1	Plasmid tube 1	6	Plasmid tube 質體 1
17	1	Plasmid tube 2	6	Plasmid tube 質體 2
18	1	ApaI	6	ApaI restricti enzyme ApaI限制酶
19	1	EcoRI	6	EcoRI restriction enzyme EcoRI限制酶
20	1	SmaI	6	SmaI restricti enzyme SmaI限制酶

1. RESTRICTION ENZYME MAP OF PLASMID (48 POINTS)

1. 質體限制酶切位圖 (48分)

An experiment was designed to determine the subcellular localization of protein Alb in *Saccharomyces cerevisiae*. The experimental strategy required that the gene alb (999bp) was fused with the sequences encoding two different fluorescent proteins. This was achieved by cloning the gene into two plasmid backbones (pX and pZ) resulting in plasmid pX:alb and pZ:alb.
為偵測酵母菌Alb蛋白在細胞內之位置，欲將表達此蛋白之alb基因 (999bp) 與2種表達不同螢光蛋白之基因結合，需要將alb基因插入含此2螢光蛋白基因之質體(pX和pZ)上，以得到質體pX: alb和pZ: alb。

E. coli was transformed with the two ligation mixes. Plasmids from two resulting E.coli transformants were purified, yielding DNA found in the tubes “Plasmid tube 1” and “Plasmid tube 2”. Your task is now to determine which two of the four possible plasmids (pX, pX:alb, pZ or pZ:alb) are contained in each of the two tubes (one in each tube)
將2種質體建構的黏合反應混合液轉植入E.coli，從2個E.coli轉植株中抽出之質體，分別為”Plasmid tube 1” 和 ” ”Plasmid tube 2”。你要檢定此2管的質體，分別為4種可能質體pX、pX: alb、pZ及pZ: alb中的哪2種?

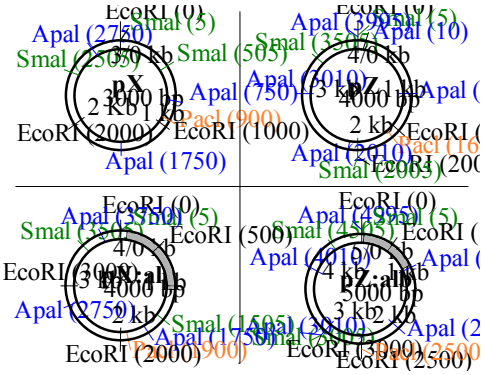
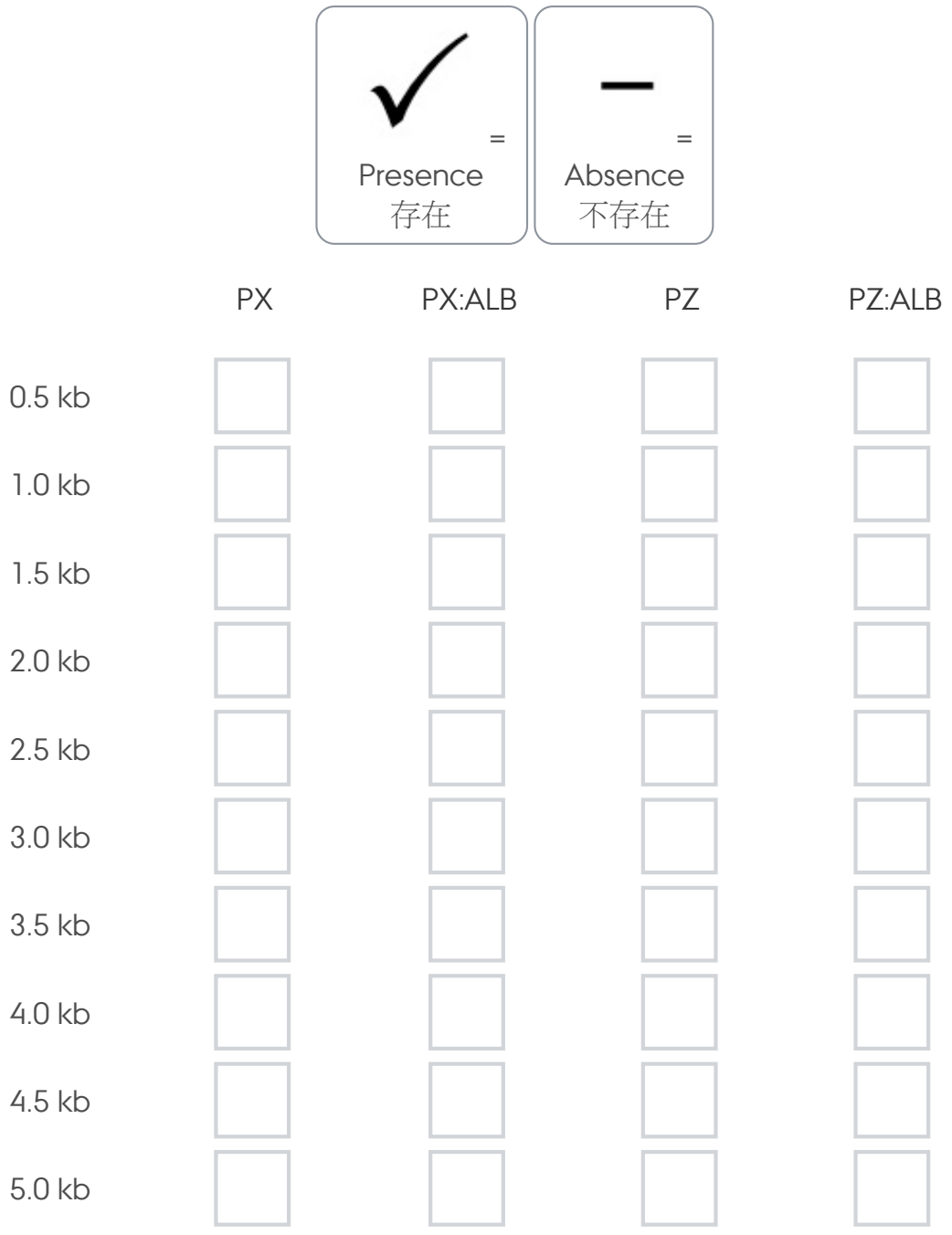


Figure 1.1: *Restriction digestion map for ‘pX’, ‘pZ’, ‘pX:alb’ and ‘pZ:alb’.*
Figure 1.1: 質體pX、pX: alb、pZ及pZ: alb的限制酶切位圖

Q. 1 SIZES OF DNA FRAGMENTS (2 POINTS)
DNA限制酶片段大小 (2分)

Node Id: **b494ba5c17b6eddea97b2778**

Indicate here by a tag, the presence of expected sizes of DNA fragments obtained by a complete PacI digestion of the four plasmids
勾選以**PacI**完全切割後，**4**種質體的預期片段大小。”**V**”表示會出現；”**-**“表示不會出現



Different restriction enzymes require different reaction conditions (Table 1.1)
不同的限制酶需要不同的反應條件 (Table 1.1)

Table 1.1. *Restriction enzymes and their optimal reaction conditions.*
Table 1.1 各種限制酶的最適反應條件

Enzyme 限制酶	% activity in Buffer 1 在buffer 1 中的活性%	% activity in Buffer 2 在buffer 2 中的活性%	% activity in Buffer 3 在buffer 3 中的活性%	Optimal reaction temperature °C 最適作用溫度℃
ApaI	25	50	100	25

EcoRI	10	100	10	37
PacI	100	75	10	37
SmaI	0	0	100	25

RESTRICTION ENZYME DIGESTION OF THE TWO PLASMIDS
2種質體的限制酶切割

Q. 2 CHOICE OF RESTRICTION ENZYME (4 POINTS)
選擇使用何種限制酶 (4分)

Node Id: **2a3e13184a7a07ed1aff6caf**

You have three restriction enzymes available, ApaI, EcoRI, and SmaI. Tap the one of these three enzymes that will allow you to distinguish between the four possible plasmids. Please note, that DNA fragments with a size smaller than 100 bp will result in very faint bands and that their size cannot be determined accurately.
你有**3**種限制酶可以使用：**Apa**、**IEcoRI**、**SmaI**，請選擇使用哪一種限制酶可以讓你區分這**4**種質體。請注意，小於**100bp**的**DNA**片段之訊號會很微弱，且無法準確判定。

1. APAI
2. ECORI
3. SMAI

Q. 3 DIGESTION SOLUTIONS (4 POINTS)
限制酶反應緩衝液 (4分)

Node Id: **6c455f6de8153f592c46f58b**

Restriction enzyme digestion of the two plasmids
限制酶切割**2**個質體

Protocol
實驗步驟

1. Label a strip of 0.2 mL PCR-tubes with S1, S2, S3 and S4 respectively.
1. 分別標示**0.2 ml PCR**小管聯條上的**4**個小管為**S1**、**S2**、**S3**、和**S4**

2. Design your digestion experiment to identify the two plasmids using one of the enzymes for all digestions. Include a control with uncut plasmid DNA. The total volume for each reaction should be 10 µL. A typical restriction enzyme digestion includes the following: 2 µL plasmid DNA, 1 µL of restriction enzyme, 1 µL Buffer and water to a final volume of 10 µL.
2. 只用一種限制酶設計你對**2**個質體的切割實驗：包括各質體的不切割控制組。
反應總體積為 **10 µl**，通常限制酶切割反應包括：**2 µl**質體**DNA**、**1 µl**限制酶、
1 µl反應緩衝液，加水至最後總體積**10 µl**

Indicate with integers, what you add to each of the four tubes (all amounts are in µL). Write '0' if you do not want to add this specific ingredient
決定這**4**個小管中要加的各種溶液及其體積**(**整數量 **µl)**.

	S1 (µL)	(S2 (µL) (Control) 對照	S3 (µL)	S4 (µL) (control)對照
Plasmid tube 1				
Plasmid tube 2				
ApaI				
EcoRI				
SmaI				
10 x Buffer 1				
10 x Buffer 2				
10 x Buffer 3				
Water				
Total volume 總體積				

Q. 4 REACTION CONDITIONS FOR DIGESTION (2 POINTS)

限制酶反應條件 (2分)

Node Id: **d753f5302f0bf03853b72487**

Protocol continued
繼續實驗步驟

3. Mix the necessary components for the restriction digestion reactions as you specified in Question 2.
3. 依照你在問題**2**中的設計，混合限制酶反應各項成分溶液

4. Set the volume on the pipette to 5 microliter and mix thoroughly by pipetting up and down 5-10 times in each tube. Avoid creating bubbles by keeping the pipette tip below the surface of the liquid at all times.
4. 以微量滴管設定至**5 µl**，吸排**5~10**次混合均勻，保持滴管尖在液面下以避免產生氣泡

5. Place the strip of 0.2 ml tubes in the small Zip lock bag. Close the bag and stick on label with your ID number to the outside of the bag
5. 將此反應小管條放在夾鏈袋中，封好並在袋子外面標示你的**ID**號碼

Indicate here, the reaction conditions you use for the digestion.
回答你要使用的反應溫度

1. 25°C

2. 37°C

Protocol continued
繼續實驗步驟

6. Raise your pink card and an Official will bring your samples to an incubator of your choice.
Complete digestion of the plasmid DNA will require 15 minutes of incubation
6. 舉起你的粉紅卡，試務人員會將你的樣品放入你選擇溫度的恆溫箱，完全切割質體DNA需要15分鐘。

7. After 20 min raise your pink card and ask an Official to return your samples. Your samples will **NOT** be returned unless you ask for it.
7. 20分鐘後，再次舉起你的粉紅卡，請試務人員取回你的樣品，注意，除非你舉卡要求，否則不會取得你的PCR反應產物

8. Check that you have received your own samples.
8. 確認試務人員送回的樣品，確實是你的

9. Add 3 µL of loading buffer to each of the samples

9. 在每一管中加入3μl的電泳注膠緩衝液

10. Mix by pipetting up and down 10 times (avoid making bubbles)
10. 以微量滴管吸排10次混合均勻，避免產生氣泡

Use the Agarose gel electrophoresis' protocol, and analyze the restriction enzyme digestions by gel electrophoresis.
依照瓊脂膠電泳步驟，進行限制酶反應的電泳分析

AGAROSE GEL ELECTROPHORESIS
瓊脂膠電泳步驟

WARNING: ELECTRICAL HAZARD – Do NOT move the RunOne electrophoresis apparatus once it has been started! Do NOT insert objects through the lamella in the lid of the apparatus when it has been started!
警告：電器危險，一旦啟動電泳，不要移動電泳槽系統，不要在上蓋間隙插入異物

WARNING: Potentially HAZARDOUS COMPOUNDS. ALWAYS wear gloves when handling the agarose gel as it is prestained with a DNA binding dye.
警告：危險藥品，因為瓊脂膠已預先以*DNA*染劑處理，操作電泳膠時，一定要

戴手套

Suggested workflow:
建議流程

Your samples from Part 1 and/or 2 should be analyzed by agarose gel electrophoresis.
在本考試第一部分和第二部分的樣品都需要做電泳分析

a. Load the samples from Part 1 and run for 15–20 minutes.
b. Stop the apparatus
c. Load the samples from Part 2
d. Restart the apparatus with all the samples and run for an additional 15–20 minutes.
a. 第一部分樣品先注膠，進行電泳15~20分鐘
b. 暫停電泳
c. 第二部分樣品注膠
d. 重新啟動電泳，所有樣品(第一部分和第二部分)再進行電泳15~20分鐘

Electrophoresis Protocol
電泳步驟

1. Put on rubber gloves.
2. Take the lid off the RunOne electrophoresis chamber by lifting it straight up.
3. Prepare the agarose gel for electrophoresis by unpacking it from the white bag. Remove the transparent lid and carefully lift tray with the gel out of the transparent casing. Handle the gel carefully as it easily breaks. Keep the white bag for later use.
1. 戴上塑膠手套
2. 將電泳槽系統的上蓋取下(垂直向上)
3. 打開裝膠的白色塑膠袋，移開蓋子，小心地將膠片盤移出鑄膠盒，不要弄破膠片，保留白色塑膠袋，等一下有用

4. Examine the gel for visible damage such as cracks, missing wells or bubbles trapped inside the gel. If you identify such problems report it immediately to the Officials by raising your pink card.
5. Place the gel as shown in Fig. 1.2.
4. 檢查膠片是否有損傷(裂開、氣泡)，如果發現有缺損，立刻舉粉紅卡向試務人員報告
5. 如Fig. 1.2. 放置膠片

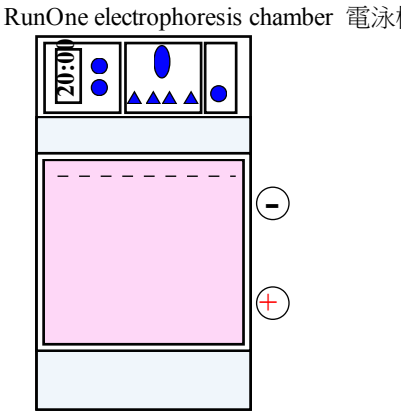


Figure 1.2: *RunOne electrophoresis chamber (left) and posssible orientations of the agarose gel.*
Figure 1.2: 電泳槽系統(左)和電泳膠片放置方向

Electrophoresis protocol continued
繼續電泳步驟

7. The gel should be completely submerged in the running buffer. **If this is NOT the case** please raise your pink card and an Official will help you.
7. 膠片必須完全浸在電泳緩衝液面以下，如果不是，立刻舉粉紅卡，試務人員會協助

8. Load the agarose gel as described in Table 1.2 (samples from Part 1) and Table 1.3 (samples from Part 2). Load 3 µL of 1 Kb ladder, 13 µL sample from Part 1 and 15 µL from Part 2.
8. 依照Table 1. 2 (第一部分樣品)和Table 1. 3的描述，注入3µl的1Kb尺標DNA，13µl的樣品 (第一部分樣品)，15µl的樣品 (第二部分樣品)

Table 1.2. *Well 1-5 for Part 1*
Table 1.2. 第一部分樣品使用膠孔*1~5*

Well 樣品膠孔	**1**	**2**	**3**	**4**	**5**
Sample 樣品	1 Kb ladder 1Kb尺標 DNA	S1	S2 (control) 不切割控制組	S3	S4 (control) 不切割控制組
Volume (µL) 體積	3	13	13	13	13

Table 1.3. Well 6–12 for Part 2.
Table 1.3. 第二部分樣品使用膠孔*6~12*

Well 樣品膠孔	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Sample 樣品	1Kb ladder 1Kb尺標 DNA	PCR-1	PCR-2	PCR-3	PCR-4	PCR-5	PCR-6
Volume(μL) 體積	3	15	15	15	15	15	15

Electrophoresis protocol continued
繼續電泳步驟

9. Place the lid on the RunOne apparatus (the tab slide down into the power supply box).
9. 蓋上電泳槽系統上蓋 (卡入電源供應器中)

10. Check that the voltage is set to 100 V otherwise use to "Voltage select" button.
10. 確定電壓是 100 V,，否則使用"Voltage select"調整

11. Switch on the power by pushing the "Run/Stop" button and run the gel for 15 to 20 minutes.
11. 按下"Run/Stop"按鈕以打開電源，電泳15到20分鐘

Note that you will not be able to see the DNA bands migrating through the gel in real time, but that the blue indicator dye in the loading buffer migrates at the same speed as a 300 bp DNA fragment.
注意：電泳進行時，你不會看到DNA條帶，只會看到藍色的指示劑 (約等同300bp DNA片段)

12. After 20 minutes switch off the power by pushing the "Run/Stop" button
12. 15到20分鐘後，按下"Run/Stop"按鈕以關閉電源

.

13. Hold up your pink card and an Official will bring you the equipment required for viewing the DNA in the agarose gel and for documenting this.
13. 立刻舉粉紅卡，試務人員會把DNA檢視設備帶來讓你看到電泳結果

Document the DNA band pattern in the gel by taking a photo of it using your tablet and the equipment the IBO Official brings you. The Official will

assist you setup and photoraph the gel.
用你的平板電腦拍照記錄，試務人員會協助你操作

14. Put on a pair of gloves.
14. 戴上手套

15. Transfer the agarose gel in its tray onto the light-table.
15. 從膠片盤上取出膠片，放在燈箱上

16. Place the black photo hood on top of the light-table.
16. 將拍照黑箱罩放在放在燈箱上

17. Place the tablet on top of the photo chamber in a way that the lens is pointing down into the chamber.
17. 將平板電腦放在黑箱罩上，平板電腦鏡頭朝向平板電腦

18. Capture a picture of your gel.
18. 將膠片拍照

Q. 5 PHOTOGRAPH YOUR DNA BAND PATTERN
將膠片上的DNA條帶拍照

Take three favorable pictures of your DNA gel. These images will be used to judge the success of your PCR and restriction enzyme digestion reactions, loading of a ladder and the validity of the analysis.
拍3張最佳的DNA膠片，照片會作為你的PCR、限制酶反應、注膠及可用性評分的依據

Node Id: **97456e13a3f7a0b2b66a4c29**
Upload the first image of your gel
上傳你拍的第一張照片

Node Id: **2e88ffb3af4dd5b441daefd5**
Upload the second image of your gel
上傳你拍的第二張照片

Node Id: **6398e6f6cb1240fcd6f8afa0**

Upload the third image of your gel
上傳你拍的第三張照片

Once you have documented your gel, please transfer it to the white bag, in which it was delivered
拍照記錄完畢後，將膠片放回原來的白色塑膠袋

Q. 6 DIGESTION OF PLASMID DNA BY RESTRICTION ENZYME
質體DNA的限制酶切割

Node Id: **631ab61f0da876d8b070db01**

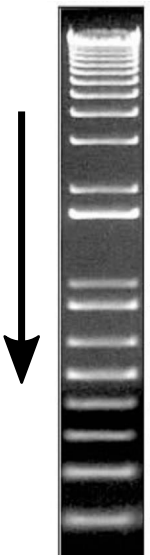
Indicate here for each sample, if the plasmid DNA has been digested by the added restriction enzyme, or not.
勾選每一個樣品是否被限制酶切割

	DIGESTED 切割	**NOT** DIGESTED 無切割
S1	○	○
S2	○	○
S3	○	○
S4	○	○

You will now be provided with a standardized gel picture showing results obtained for the restriction enzyme you chose (Question 3). Use this picture to answer Questions 7 and 8. **NOTE YOU WILL ONLY HAVE ACCESS TO THIS PICTURE ONCE YOU HAVE DOCUMENTED YOUR GEL.**
此時會提供你一張依照你選擇的限制酶反應 (問題3) 應該得到的標準電泳膠照片 ，用這張照片回答問題7和8
注意； 只有完成拍照上傳後，才可看到標準結果照片

Use the provided reference for the DNA ladder as a help.
使用大信封中提供的1 kb plus 尺表DNA照片幫助判讀

1 kb plus 尺標DNA



Contains: 包含

100 bp, 200 bp,
300bp, 400 bp,
500 bp, 650 bp,
850 bp, 1000 bp,
1650 bp, 2 kbp,
3 kbp, 4 kbp, 5 kbp,
6 kbp, 7 kbp, 8 kbp,
9 kbp, 10 kbp,
11 kbp, 12 kbp

Q. 7 DNA FRAGMENT SIZE(S) OF YOUR RESTRICTION ENZYME DIGESTION
你選擇的限制酶反應所得的DNA片段大小

Node Id: **723065e993cf22c5ad91aad4**

Indicate here, with a tag if a DNA fragment of a given size was present in your restriction enzyme digestion
勾選是否出現特定大小的**DNA**片段.

✓ = Present 出現	— = Absent 未出現

	DNA FRAGMENTS FROM DIGESTION OF DNA IN 'PLASMID TUBE 1 PLASMID TUBE 1 的DNA 片段	DNA FRAGMENTS FROM DIGESTION OF DNA IN 'PLASMID TUBE 2 PLASMID TUBE 2 的DNA 片段
0.5 kb	☐	☐
1.0	☐	☐

kb		
1.5 kb		
2.0 kb		
2.5 kb		
3.0 kb		
3.5 kb		
4.0 kb		
4.5 kb		
5.0 kb		
5.5 kb		
6.0 kb		

Q. 8 IDENTIFICATION OF PLASMIDS IN TUBES
判定管中質體DNA為何?

Node Id: **f182a828e66bfcb5be092fde**

Indicate here for each tube which plasmids are present, and which plasmids are absent. Indicate with a '+' in the last column if it was impossible to determine the presence of plasmids for a tube.
指出各管中何種**DNA**存在，何種不存在。若無法判定，則在最後一欄中填入"**+**"

= Plasmid present 質體存在	= Plasmid absent 質體不存在

PLASMID PX	PLASMID PX:ALB	PLASMID PZ	PLASMID PZ:ALB	IMPOSSIBLE TO DETERMINE BASED ON THE CHOSEN ENZYME 依選擇的酵

素，無法判定

Plasmid tube 1	☐	☐	☐	☐	☐
Plasmid tube 2	☐	☐	☐	☐	☐

2. PCR BASED GENOTYPING OF YEAST MUTANTS (37 POINTS)
2. 以PCR鑑定酵母菌突變株的基因型

A mutant yeast strain was isolated. This yeast strain, requires tyrosine and phenylalanine in the growth medium to be able to propagate. The ability of the mutant to grow on a medium without tryptophan has not been tested. A back cross of the mutant strain has shown that only a single gene is affected.
某一個酵母菌突變株的純化過程，需要在培養基中加入酪胺酸(tyrosine)和苯胺酸(phenylalanine)才能生長，此突變株是否需要色胺酸(tryptopan)才能生長則是不清楚。利用回交分析，已得知此突變株中只有單一個基因發生突變。

Your task is now to determine the genetic basis for the observed auxotrophy. You have five primer pairs available (Table 2.1), each capable of detecting dysfunctional (mutant) alleles of the genes encoding five key enzymes in the biosynthetic pathway for formation of aromatic amino acids (Fig. 2.1).
你的任務就是找出此營養突變株的哪一個基因發生突變，有5對引子可以供你使用(Table 2.1)，這5對引子可以分別鑑定5個合成芳香族胺基酸(aromatic amino acids)的關鍵酵素(Fig.2.1)，其相對應基因是正常等位基因或是突變等位基因。

Table 2.1. *The available primer pairs, the genes they amplify and the expected DNA amplicon in the wild type and mutant alleles.*
Table 2.1. 使用這*5*對引子分別進行*PCR*反應時，相對應基因是正常等位基因或是突變等位基因的*PCR*產物大小。

Primer pair 引子對	Gene 基因	Size of PCR product (bp) – wild type 正常型PCR產物大小	Size of PCR product (bp) – mutant 突變體PCR產物大小
A-forward +			

A-reverse	*TYR1*	500	400
B-forward + B-reverse	*PHA2*	500	250
C-forward + C-reverse	*ARO7*	500	300
D-forward + D-reverse	*TRP5*	500	350
E-forward + E-reverse	*ARO2*	500	300

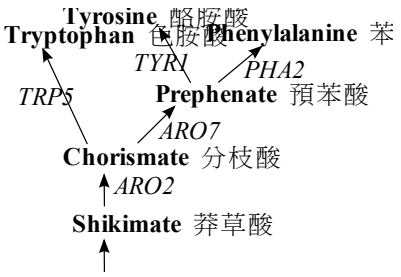


Figure 2.1: *Biosynthetic pathway for the formation of aromatic amino acids (from upper left: Tryptophan, Tyrosine, Phenylalanine, Prephenate, Chorismate and Shikimate) and genes encoding the individual enzymes in the pathways.*
Fig.2.1: 芳香族胺基酸的合成途徑以及各步驟合成酵素的對應基因

Q. 9 CHOICE OF PRIMER PAIRS (5 POINTS)
引子對選擇 (5分)

By combining the results from two of the five available primer pairs (Table 2.1) you will be able to determine the genetic basis for the nutritional requirements observed in the mutant yeast strain.
在這5對引子(Table 2.1)的PCR反應中，其中有2對引子的PCR實驗綜合結果可以讓你判定此突變株的哪1個基因發生突變。

Node Id: **d72a621f704984960a188a6c**

Indicate the combination of two primer pairs (each either A, B, C, D or E from Table 2.1) to be used.
指出可以使用哪**2**種引子對 (從**Table 2.1**中選擇**A**、**B**、**C**、**D**或**E**)

	A	B	C	D	E
First Primer Pair 第一個引子對	○	○	○	○	○

Second Primer Pair
第二個引子對

Protocol
實驗步驟

1. Make a Master-mix for the PCR reactions in a 1.5 ml tube by mixing: 196 µl Water + 70 µl DNA polymerase buffer (x5) + 35 µl dNTP's + 7 µl DNA polymerase.
1. 配製PCR反應混合液，取1.5ml小管，依序加入
196 µl水、
70 µl DNA 聚合酶緩衝液(x5)、
35 µl dNTPs、
7 µl DNA 聚合酶

2. Mix by pipetting up and down 5-10 times with the pipette set at 100 µl.
2. 將微量滴管設定至100 µl，吸排5~10次混合均勻

3. Find the strip of 0.2 ml PCR tubes and glue on a small label with your ID number.
3. 取一排0.2 ml的PCR反應小管，貼上你的ID號碼小標籤

4. Label the tubes 1 - 6 (**DO NOT** break the strip of tubes into individual tubes – if you do so, your samples will not be accepted for PCR).
4. 將小管編號1~6，注意不要折斷這排反應小管，如果斷了，將無法進行PCR

5. Pipette 44 µl of the Master-mix to each of the six PCR tubes.
5. 在這6個PCR反應小管中，分別加入44 µl PCR反應混合液

6. To tubes 1 to 3, add 5 µl of the first Primer pair you have chosen.
6. 在編號1~3小管中，分別加入5 µl你選擇的第一個引子對

7. To tubes 4 to 6, add 5 µl of the second Primer pair you have chosen.
7. 在編號4~6小管中，分別加入5 µl你選擇的第二個引子對

8. To Tube 1 and 4, add 1 µl of wild type template DNA
8. 在編號1和4的小管中加入1 µl正常型菌株的DNA，作為反應模板
.
9. To Tube 2 and 5, add 1 µl of mutant template DNA
9. 在編號2和5的小管中加入1 µl 突變菌株的DNA，作為反應模板
.
10. To Tube 3 and 6, add 1 µl of water.
10. 在編號3和6的小管中加1 µl 的水

11. Mix the individual reaction by pipetting up and down 5-10 times with the pipette set at 45 µl.
11. 以微量滴管設定至45 µl，吸排5~10次混合均勻

12. Close the lids.
12. 蓋緊管蓋

Table 2.2. *Three PCR programs (1-3), stored at 10°C.*
Table 2.2. 3種PCR反應程式(1-3), 存於 10°C.下

Program 1 PCR反應程式1	Program 2 PCR反應程式2	Program 3 PCR反應程式3
98°C for 1 min	98°C for 1 min	98°C for 1 min
25 times: 98°C for 15 sec 55°C for 5 sec 72°C for 20 sec	25 times: 72°C for 20 sec 55°C for 5 sec 98°C for 15 sec	25 times: 72°C for 10 sec 98°C for 15 sec 55°C for 5 sec
72°C for 1 min	72°C for 1 min	72°C for 1 min
Store at 10°C	Store at 10°C	Store at 10°C

Q. 10 CHOICE OF PCR PROGRAM (2 POINTS)
選擇PCR反應程式

Node Id: **33418d50512f939d3aeb6813**

Indicate here, which of the three PCR programs (Table 2.2) should be used for successful amplification if the primers have a melting temperature (Tm) of 60 °C and the used DNA polymerase can synthesize DNA at a speed of 25 bp/sec at 72 °C?
如果這些引子的熔點 **(Tm)** 是**60**℃，**DNA**聚合酶可以在**72**℃時以**25 bp/sec** 的速率合成**DNA**。在**Table 2.2**中的**3**種**PCR**反應程式，哪一種應該使用於本實驗？

1. PCR PROGRAM 1
PCR反應程式1

2. PCR PROGRAM 2
PCR反應程式2

3. PCR PROGRAM 3
PCR反應程式3

IMPORTANT
重要事項

1. Assure yourself that Questions 9 and 10 are answered as you intended. Once you are happy with the answers, lock them by clicking "LOCK ANSWERS".
Note: after locking, you will not be able to change your answers anymore!
1. 確定問題9和10已回答：如果你滿意你的答案，點選**"LOCK ANSWER"**。
注意：一但鎖定，你不能再更改。

2. Once your answers are locked, raise your pink card and an official will transfer your samples to the thermal cyclers.The PCR program takes 20 minutes to complete. (In the meantime go to Parts 1 or 3 according to the time table in the introduction).
2. 鎖定答案後，舉起你的粉紅卡，試務人員會將你的樣品送至PCR處進行反應。PCR反應會在20分鐘後完成，此時按照背景介紹的時間表，進行第1和第3部分的實驗

3. After 20 minutes raise your pink card and an official will return your samples. Please note that the sample will NOT be returned unless you ask for it.
3. 20分鐘後，舉起你的粉紅卡，試務人員將會送回你的PCR反應。
注意，除非你舉卡要求，否則不會取得你的**PCR**反應產物

4. Upon receiving your samples please check that they are **your** samples.
4. 請確認試務人員送回的樣品，確實是你的PCR反應

5. Prepare the PCR samples for agarose gel electrophoresis by adding 10 µL of loading buffer to each of the samples and mix by pipetting up and down 5 times (avoid making bubbles).
5. 在每一PCR樣品中加入10 µl注膠緩衝液，以微量滴管吸排5~10次混合，避免氣泡

6. Load your samples as described in the Agarose gel electrophoresis section.
6. 將你的樣品加注到瓊脂電泳膠，如前電泳部分所述

7. Documentate the gel by taking a photo (the photo of this gel will be inspected and a maximum of 22 points will be awarded)
7. 將此電泳膠結果拍照存證，此影像會被檢查評分，最高可得到22分

You will now be provided with a standardized gel picture based on the

primer pairs you previously chose to use for the PCR analysis. use this pciture to answer Questions 11 and 12. **NOTE: YOU WILL GET THIS PICTURE FROM THE ASSISTANT RETURNING YOUR GEL.**
此時試務人員會依照你選擇的引子對，給你一張標準電泳膠圖，你依此回答問題11和問題12

Q. 11 SIZE OF DNA BANDS (4 POINTS)
DNA 條帶(bands)大小

Node Id: **2261c610758917fbc24905ed**

Indicate here if a given size of the obtained DNA (bp) products was present '+'. Report your results column-by-column.
逐項回答是否得到特定大小的**DNA** 條帶，若有，選擇 "**+**"

⊕ = Present 有

	PCR-1	PCR-2	PCR-3	PCR-4	PCR-5	PCR-6
150 bp						
200 bp						
250 bp						
300 bp						
350 bp						
400 bp						
450 bp						
500 bp						
550 bp						

Q. 12 DYSFUNCTIONAL ENZYME STEPS (4 POINTS)
失效酵素作用步驟(4分)

Node Id: **ff6dc825d5bde0184dfec782**

Based on your results, indicate for each of the five possible enzymatic steps shown in Fig. 2.1 if they are functional or dysfunctional, or whether their status can not be determined with the used primers.
根據你的選擇，回答**Fig.2.1**中的**5**個酵素作用步驟是正常、失效、或是你選擇使用的引子無法判定

	FUNCTIONAL 有功能	DYSFUNCTIONAL 無功能	NOT POSSIBLE TO DETERMINE 無法判定
TRP5	○	○	○
TYR1	○	○	○
PHA2	○	○	○
ARO7	○	○	○
ARO2	○	○	○

3. AMINO ACID AUXOTROPHY OF MUTANT YEAST (15 POINTS)
3. 酵母菌胺基酸合成缺失突變株

For many years, the haploid fungus *Saccharomyces cerevisiae* (baker's yeast) has been used as a model organism for elucidating the central metabolism in eukaryotes. Yeast is normally capable of synthesizing all twenty amino acids needed for protein synthesis. Following UV-mutagenesis various amino acid auxotrophic mutants have been isolated.
多年來，單倍體麵包酵母菌被用來做為解答真核生物主要代謝途徑的模式生物。正常酵母菌能自行合成所有20種胺基酸。利用紫外光(UV)突變誘導後，分離出各種不同胺基酸合成缺失突變株。

The booklet with eight photos depicts haploid *S. cerevisiae* strains 1–5 grown on eight different media (A to H). 照片冊中的8張照片呈現了5個單倍體酵母菌株(1~5)在8種培養基(A~H)上的生長情形：

Position 1 = no inoculum, Position 2 = Strain 1; Position 3 = Strain 2; Position 4 = Strain 3; Position 5 = Strain 4; Position 6 = Strain 5. The media composition for plate A to H is given in Table 3.1
第1區沒有培養酵母菌
第2區是1號菌株
第3區是2號菌株
第4區是3號菌株

第5區是4號菌株
第6區是5號菌株
培養基A~H的成分在Table 3.1

DO NOT MAKE ANY NOTES ON THE PHOTOS.
注意：不要在照片上做任何註記

Table 3.1. Growth media

MEDIA 培養基	**COMPOSITION** 成份
A	Rich complex media (yeast extract + peptone + dextrose) 豐富培養基 (酵母菌抽出物 + 蛋白質 + 葡萄糖)
B	Minimal media without any amino acids 最少培養基，不含任何胺基酸
C	Minimal media + homocysteine 最少培養基 + 同半胱胺酸 (homocysteine)
D	Minimal media + isoleucine 最少培養基 + 異白胺酸 (isoleucine)
E	Minimal media + threonine 最少培養基 + 蘇胺酸 (threonine)
F	Minimal media + methionine + threonine 最少培養基 + 甲硫胺酸 (methionine) + 蘇胺酸 (threonine)
G	Minimal media + lysine 最少培養基 + 離胺酸 (lysine)
H	Minimal media + proline 最少培養基 + 脯胺酸 (proline)

Q. 13 GROWTH/LACK OF GROWTH OF YEAST STRAINS (10 POINTS)
酵母菌生長或不生長 (10分)

Node Id: **bbd24126db8bb263c5dc6452**

Record here growth or lack of growth for the various strains, use: "+" for growth and "–" for no growth.

記錄不同菌株的生長或不生長情形：”**+**”代表生長； “**-**“代表不生長

	STRAIN 1 菌株 1	STRAIN 2 菌株 2	STRAIN 3 菌株 3	STRAIN 4 菌株 4	STRAIN 5 菌株 5
MEDIA A 培養基A					
MEDIA B 培養基B					
MEDIA C 培養基C					
MEDIA D 培養基D					
MEDIA E 培養基E					
MEDIA F 培養基F					
MEDIA G 培養基G					
MEDIA H 培養基H					

Q. 14 DYSFUNCTIONAL ENZYME STEP(S) (5 POINTS)
沒有功能的酵素作用步驟(5分)

Node Id: **5d4effda2f48e112ec5b7354**

Based on the recorded growth patterns, deduce which enzymatic step(s) that is/are most likely dysfunctional, if any, in the five strains (1–5). For each mutant write the digit (1–31) for the dysfunctional enzymatic step (see Biochemical chart on the wall of the workspace) or 0 if no step is dysfunctional.

根據記錄之菌株生長情形，推導出在這**5**個菌株中，分別可能是哪一個酵素作用步驟失效。對每一個菌株，以數字 ”**1~31**” 表示失效酵素作用步驟，或 ”**0**” 代表沒有任何酵素作用步驟失效，酵素作用步驟 ”**1~31**” 請看實驗台牆上的生化合成路徑圖

	STRAIN 1 菌株1 STRAIN 1	STRAIN 2 菌株2 STRAIN 2	STRAIN 3 菌株3 STRAIN 3	STRAIN 4 菌株4 STRAIN 4	STRAIN 5 菌株5 STRAIN 5

ENZYME STEP NO. in biochemical chart on the wall 酵素作用步驟					

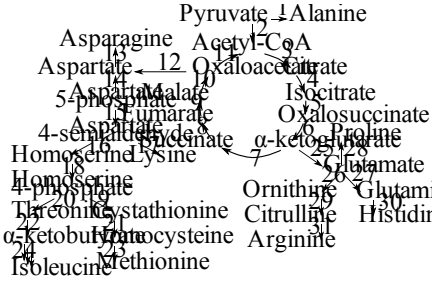


Figure 3.1: Simplified model for amino acid biosynthesis in yeast.

END
結束