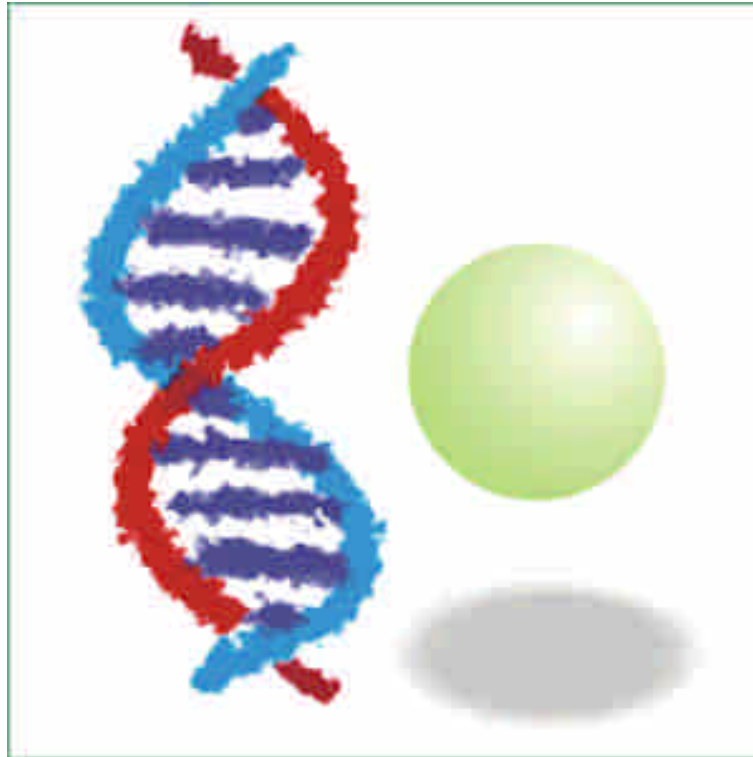


BST  
生化科技系

BCX

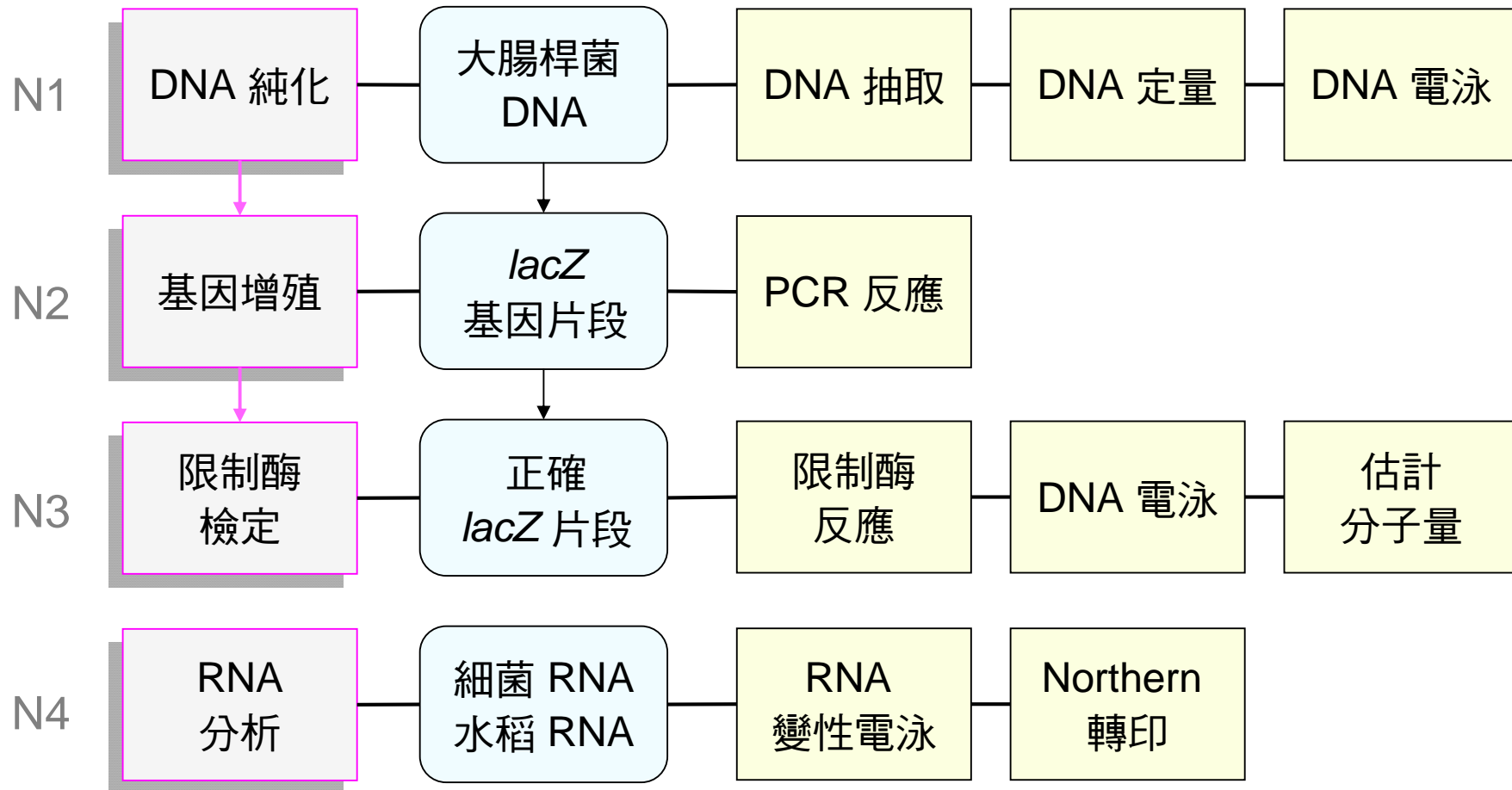


N3

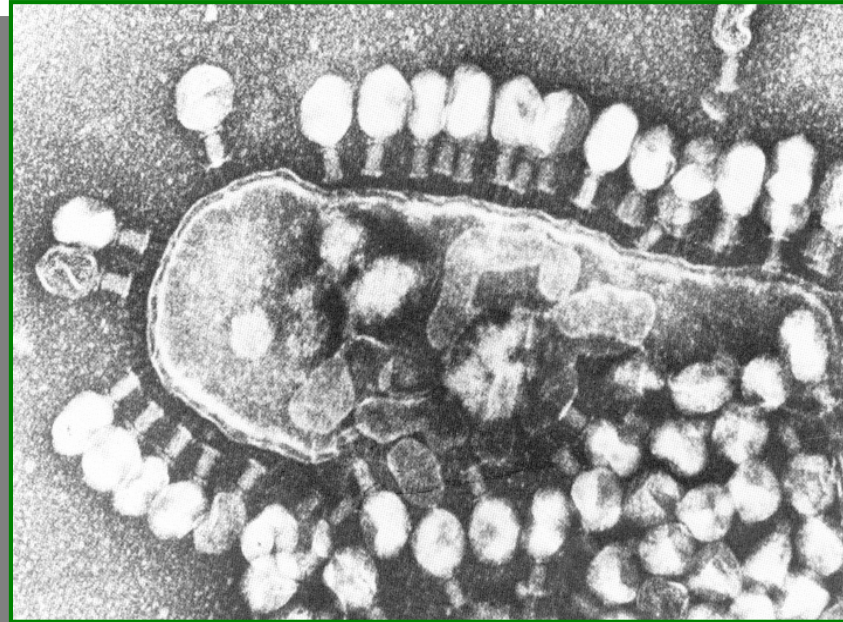
生物化學實驗

Restriction Analysis of DNA

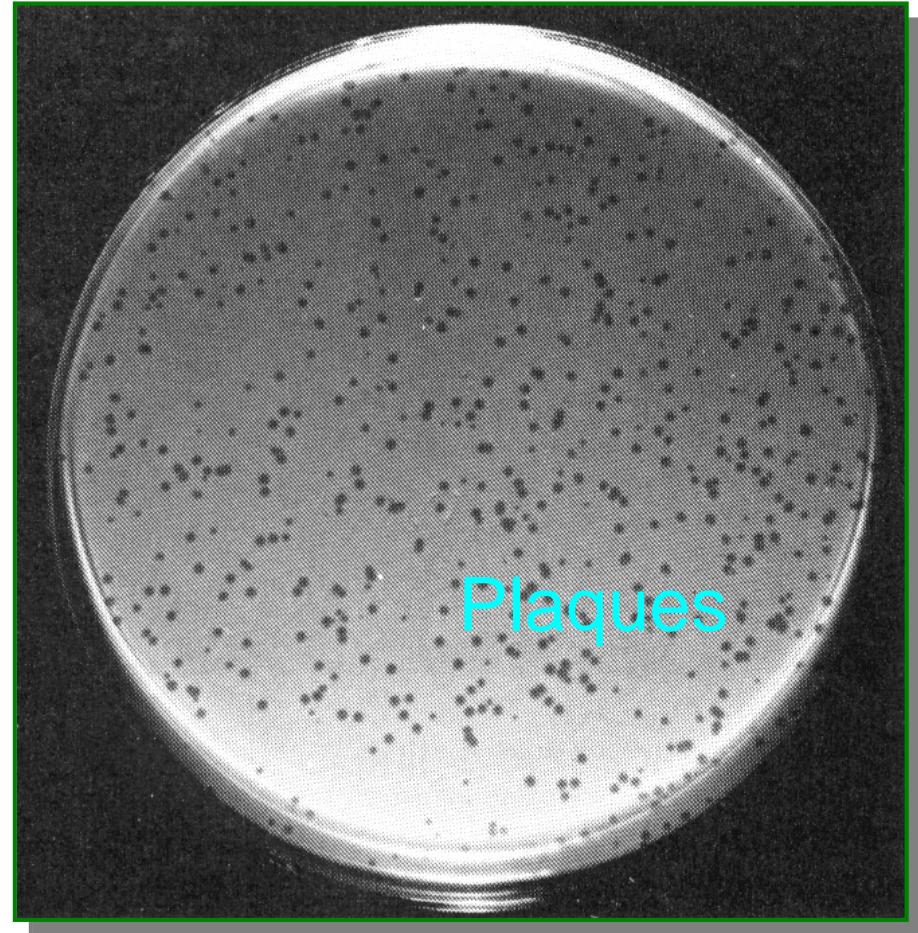
# 核酸部分的學習目的：



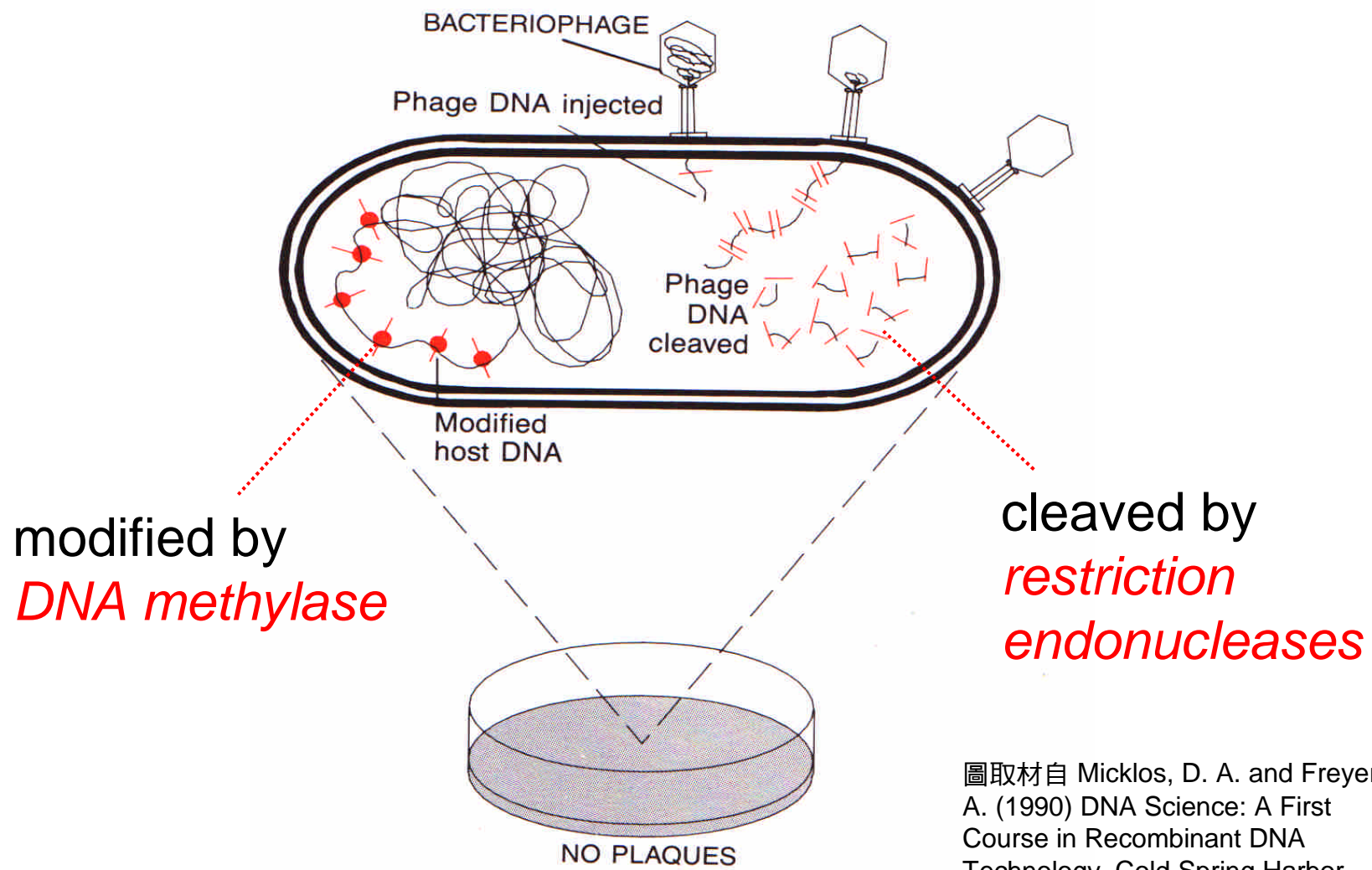
# *E. coli* 受到噬菌體感染



Micklos, D. A. and Freyer, G. A. (1990) DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory Press. (p22)

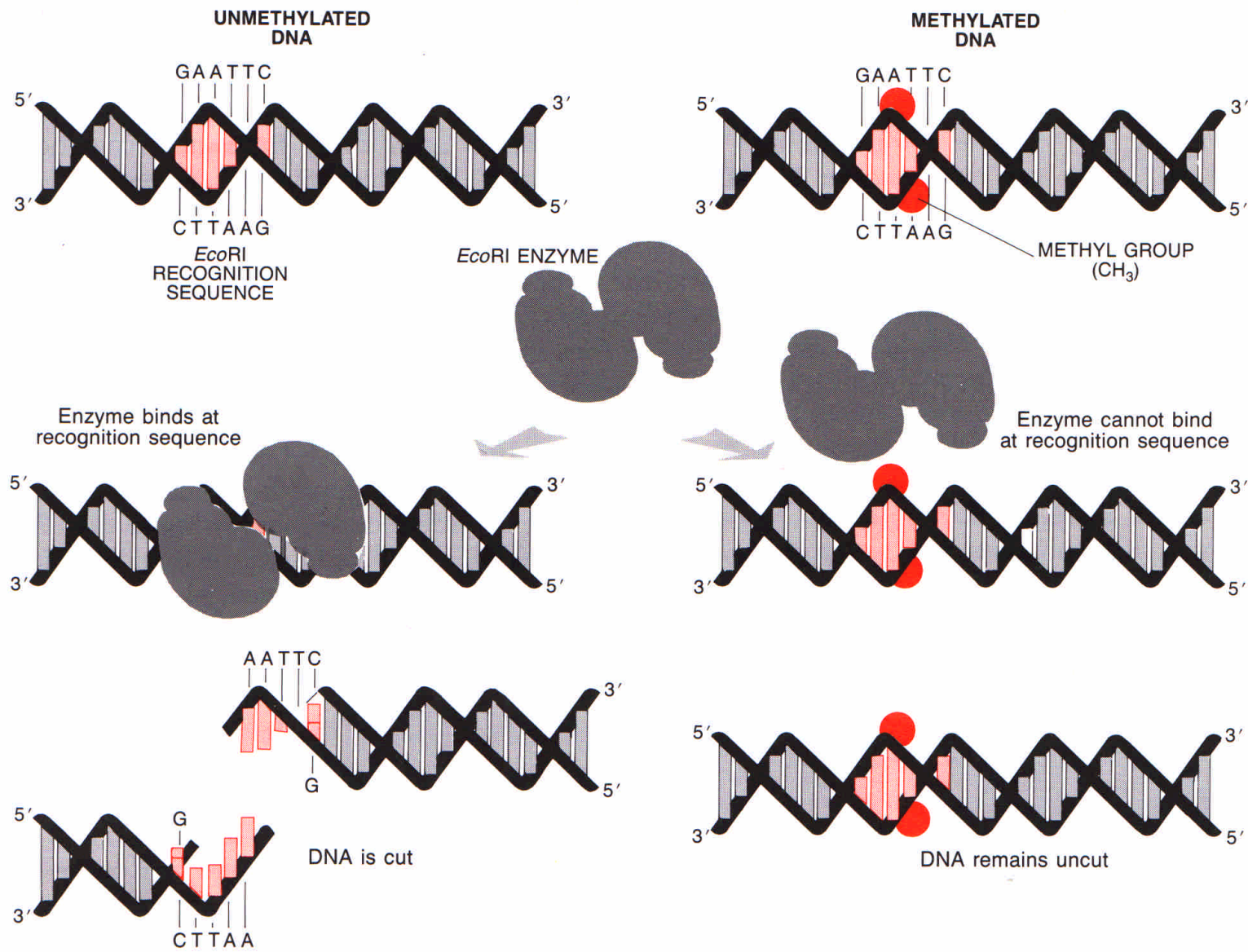


# Restriction-modification 系統可防禦噬菌體感染：



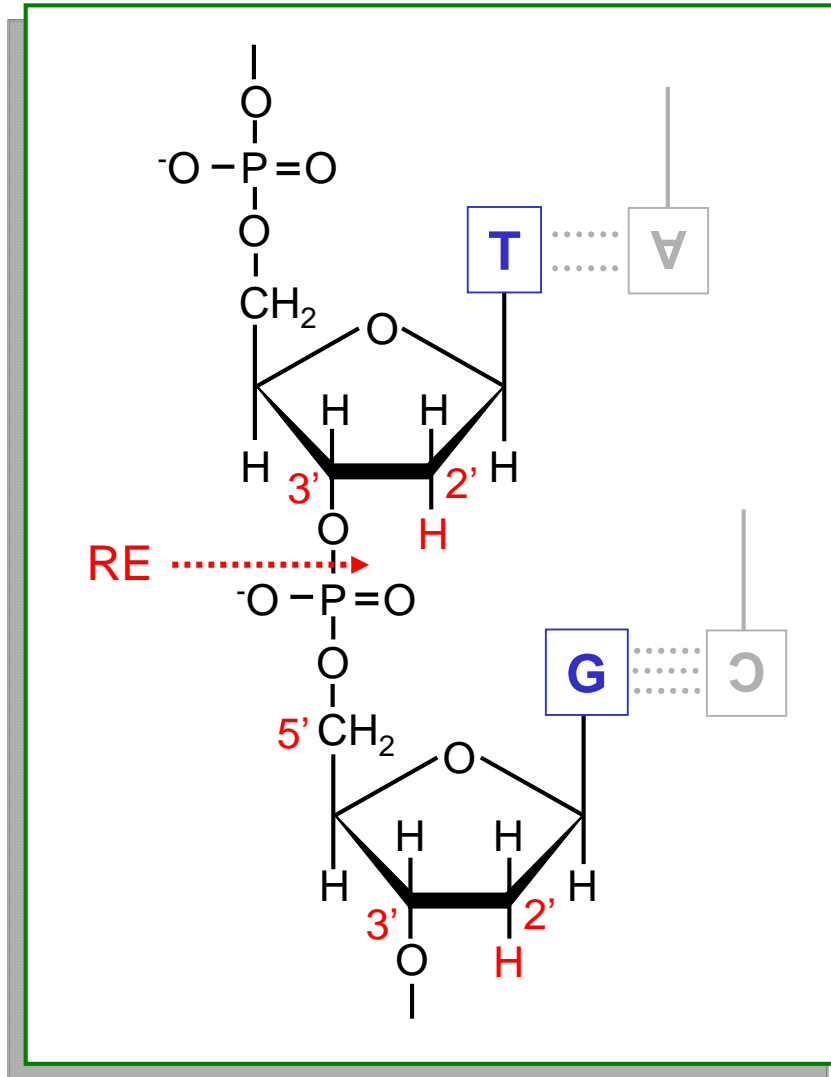
圖取材自 Micklos, D. A. and Freyer, G. A. (1990) DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory Press. (p.40)





Micklos, D. A. and Freyer, G. A. (1990) DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory Press. (p.46)

# 限制酶為 nuclease 之一種：

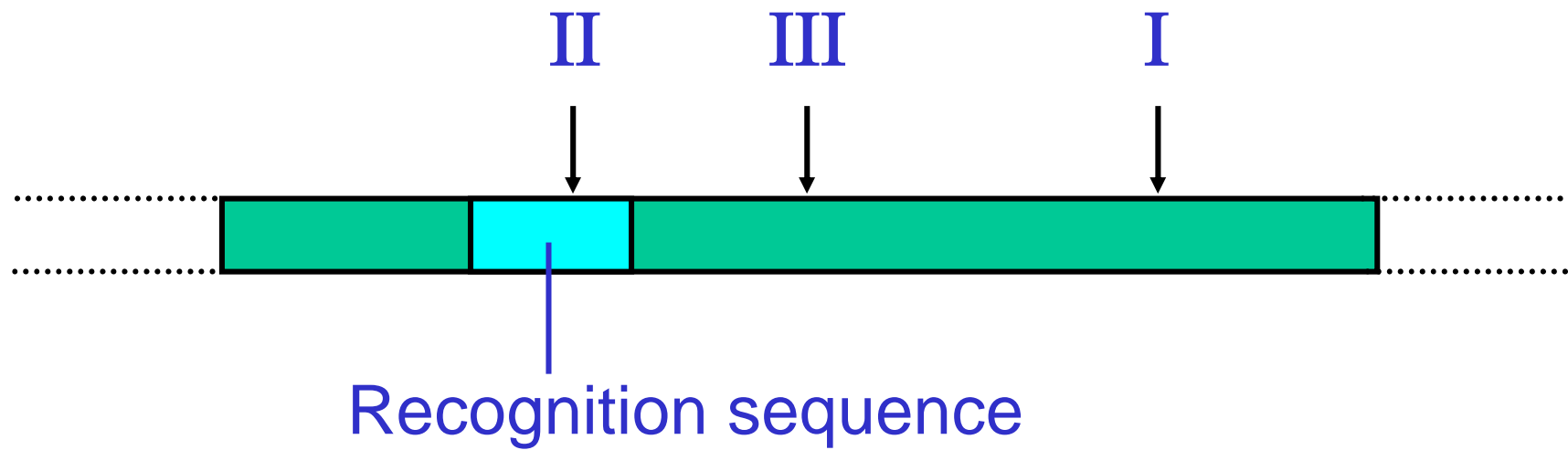


- ▶ 屬於內切酶
- ▶ 作用於雙股 DNA
- ▶ 對序列具專一性

■ 限制酶的種類：



## Restriction endonucleases



## ■ 限制酶的命名：



*EcoRI*     *E* = genus *Escherichia*  
*co* = species *coli*  
R = strain RY13  
I = first endonuclease isolated

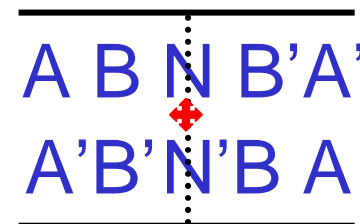
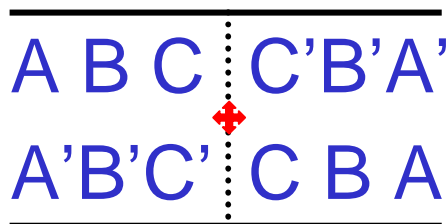
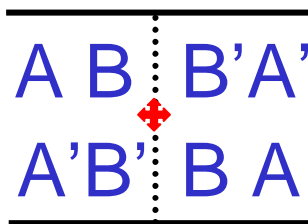
*BamHI*     *B* = genus *Bacillus*  
*am* = species *amyloliquefaciens*  
H = strain H  
I = first endonuclease isolated



## ■ Type II 限制酶的辨認序列：



- ▶ 1. Most type II enzymes recognize symmetric sequences (*Palindromes*).



**Table 4.1** Recognition sequences of some restriction endonucleases

<b>Enzyme</b>	<b>Recognition site</b>	<b>Type of cut end</b>
<i>EcoRI</i>	G ↓ A—A—T—T—C C—T—T—A—A ↑ G	5'-phosphate extension
<i>BamHI</i>	G ↓ G—A—T—C—C C—C—T—A—G ↑ G	5'-phosphate extension
<i>PstI</i>	C—T—G—C—A ↓ G G ↑ A—C—G—T—C	3'-hydroxyl extension
<i>Sau3AI</i>	↓ G—A—T—C C—T—A—G ↑	5'-phosphate extension
<i>PvuII</i>	C—A—G ↓ C—T—G G—T—C ↑ G—A—C	Blunt end
<i>HpaI</i>	G—T—T ↓ A—A—C C—A—A ↑ T—T—G	Blunt end
<i>HaeIII</i>	G—G ↓ C—C C—C ↑ G—G	Blunt end
<i>NotI</i>	G ↓ C—G—G—C—C—G—C C—G—C—C—G—G—C ↑ G	5'-phosphate extension

Glick, B. R. and Pasternak, J. J. (1998) *Molecular Biotechnology: Principles and Applications of Recombinant DNA*. 2<sup>nd</sup> ed., ASM Press. Table 4.1

## ■ Type II 限制酶的辨認序列：



- ▶ 2. Some type II enzymes recognize asymmetric sequences.

*Hgal*      -GACGCNNNNN ↓  
              -CTGCGNNNNNNNNNN ↑

*Bsal*        -GGTCTCN ↓  
              -CCAGAGNNNNN ↑

## ■ Type II 限制酶的辨認序列：

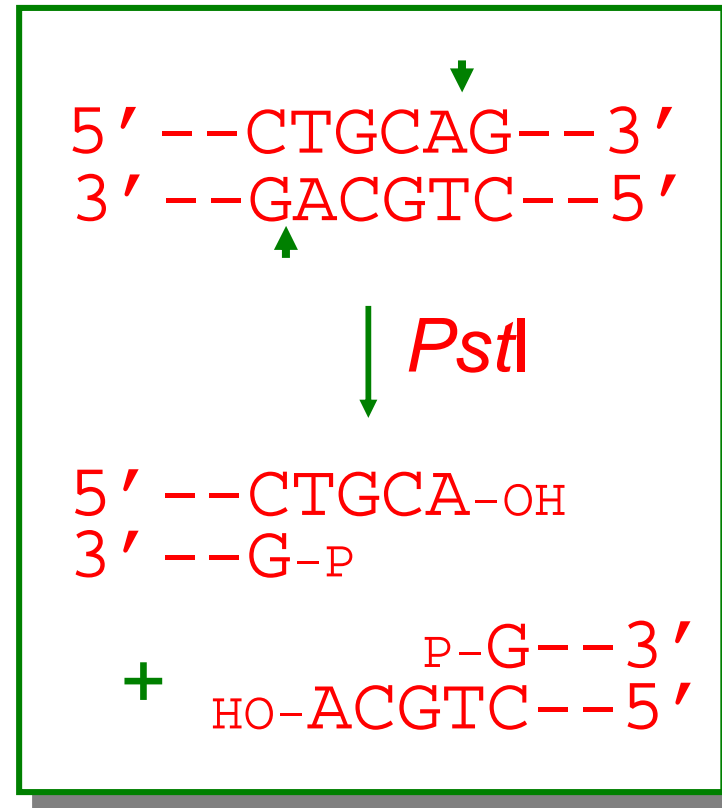
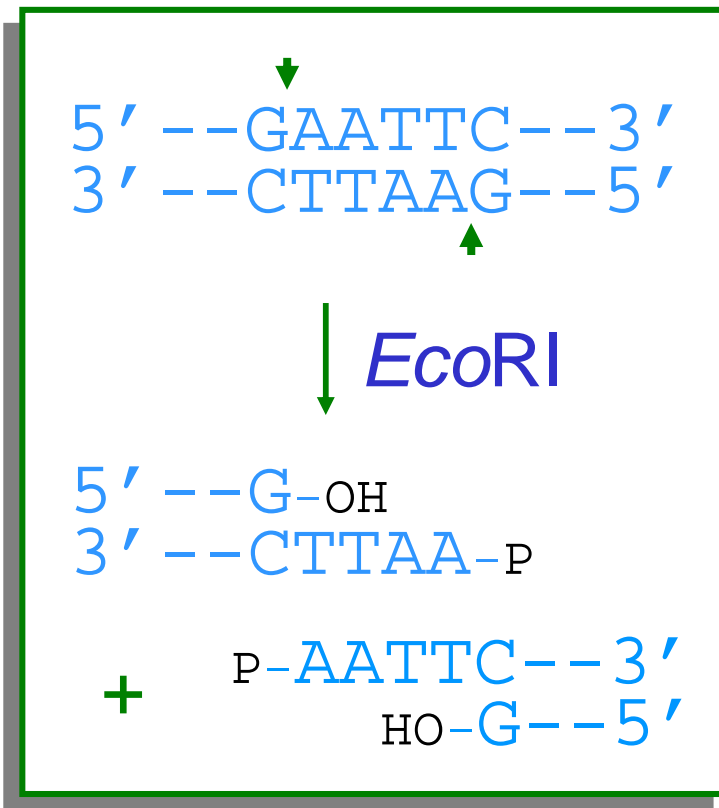
- ▶ 3. Some type II enzymes recognize multiple sequences.

*AccI*



# ■ Type II 限制酶作用後的末端型式：

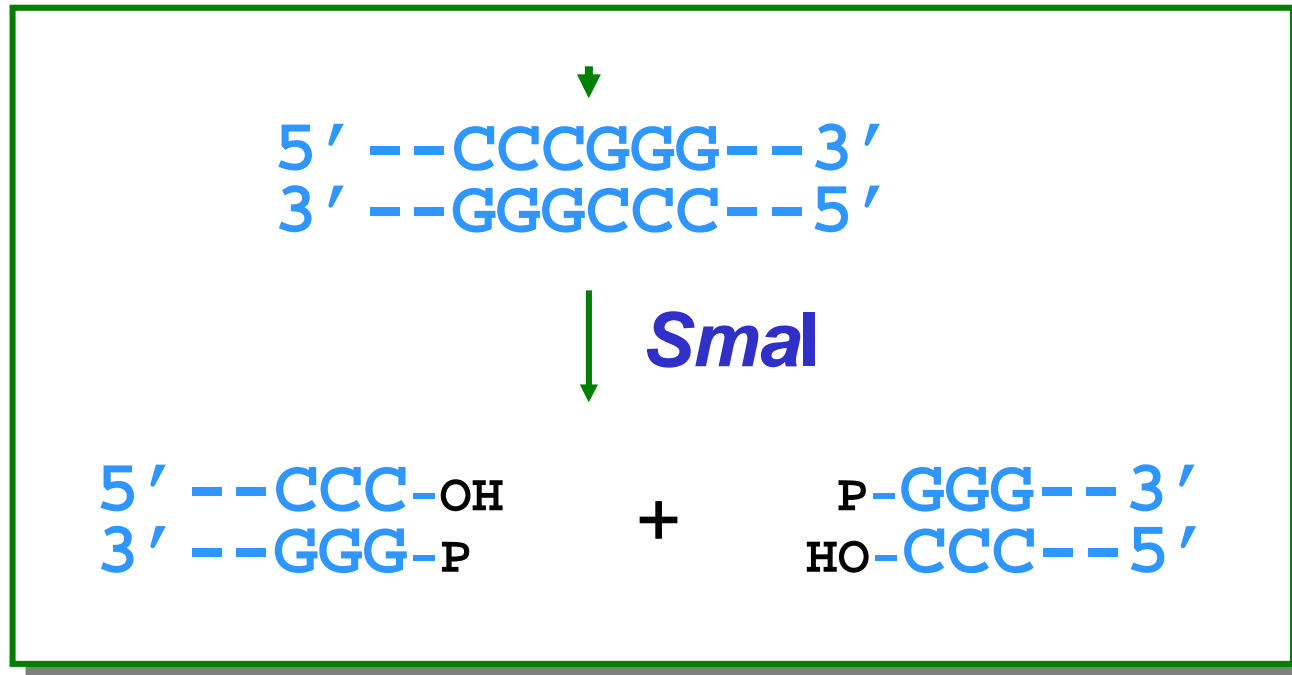
## ▶ 1 Cohesive ends (Staggered ends)：

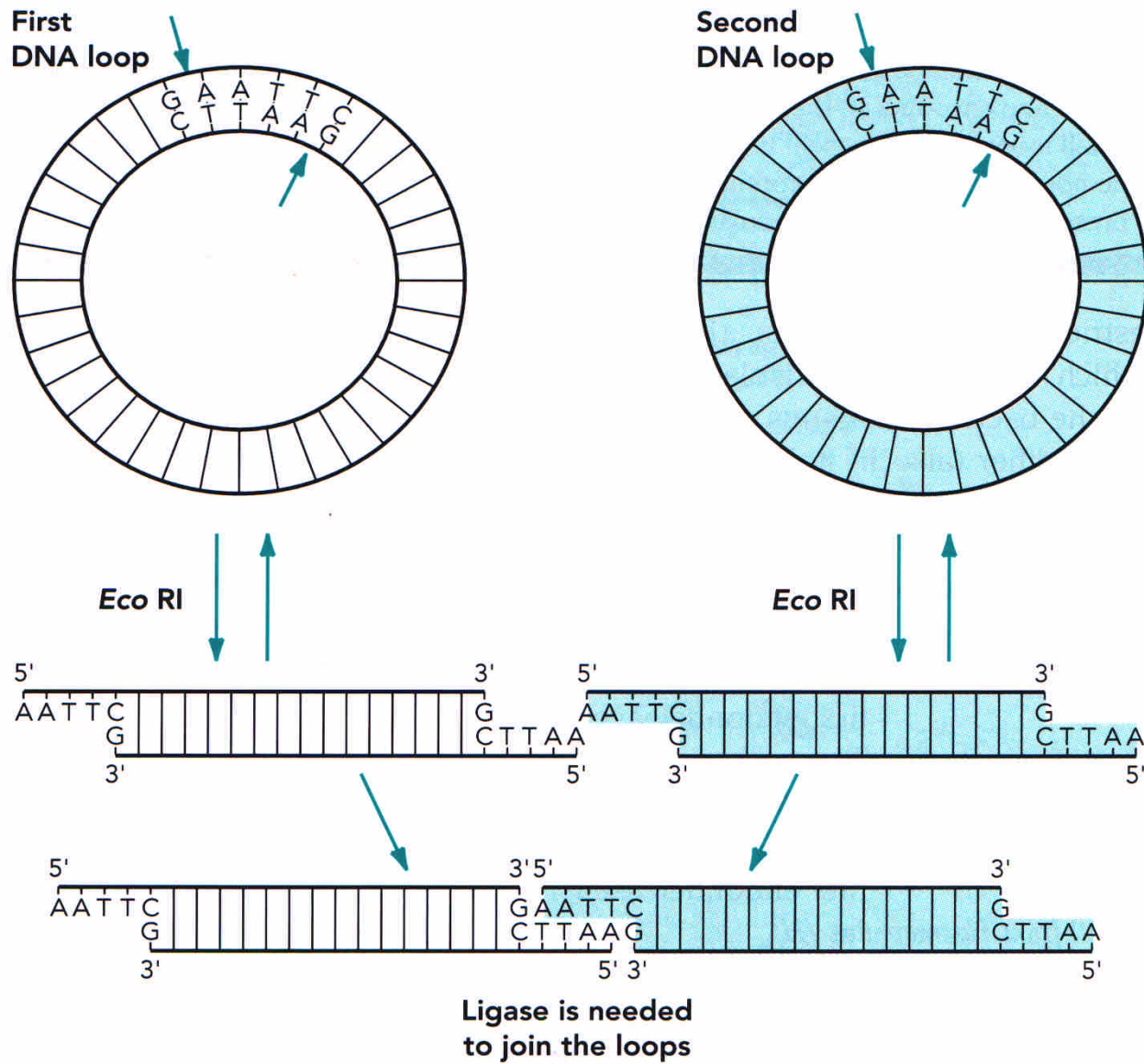




## ■ Type II 限制酶作用後的末端型式：

### ▶ 2 Blunt ends (Flush ends) :





Accamo, I.E. (2000) DNA Technology: The Awesome Skill, 2<sup>nd</sup> ed., Academic Press, Fig. 4.9.

## ■ 查詢限制酶：



### ▶ 1 廠商目錄：

例如，

Fermentas AB, Life Technologies Inc.,

New England BioLabs, Promega Corporation,

Roche Molecular Biochemicals,

Sigma Chemical Corporation, Stratagene,

Takara Shuzo Co. Ltd., Toyobo Biochemicals, etc.

■ 查詢限制酶：



- ▶ 2 web site: REBASE



<http://rebase.neb.com>

## ■ 限制酶使用要點：

---

- ▶ 1. **貯存**：一般貯存於-20°C，少數酵素須存放於更低溫；  
避免存放於無霜冰箱。
  
- ▶ 2. **取用**：
  - (1) 由冰箱取出後立即置入冰浴中，短暫離心後再取用
  - (2) 添加不同酵素須更換 tips
  - (3) 一次取出需要量，置微量離心管，再加入各反應中
  - (4) 取用後立即置回冰箱



## ■ 限制酶使用要點：

### ▶ 3. 反應條件：

(1) 選擇適當反應液

(2) 注意甘油濃度

(3) 酵素添加適量

(4) 選擇適當反應溫度

(5) 反應適當時間

(6) 注意 Star activity

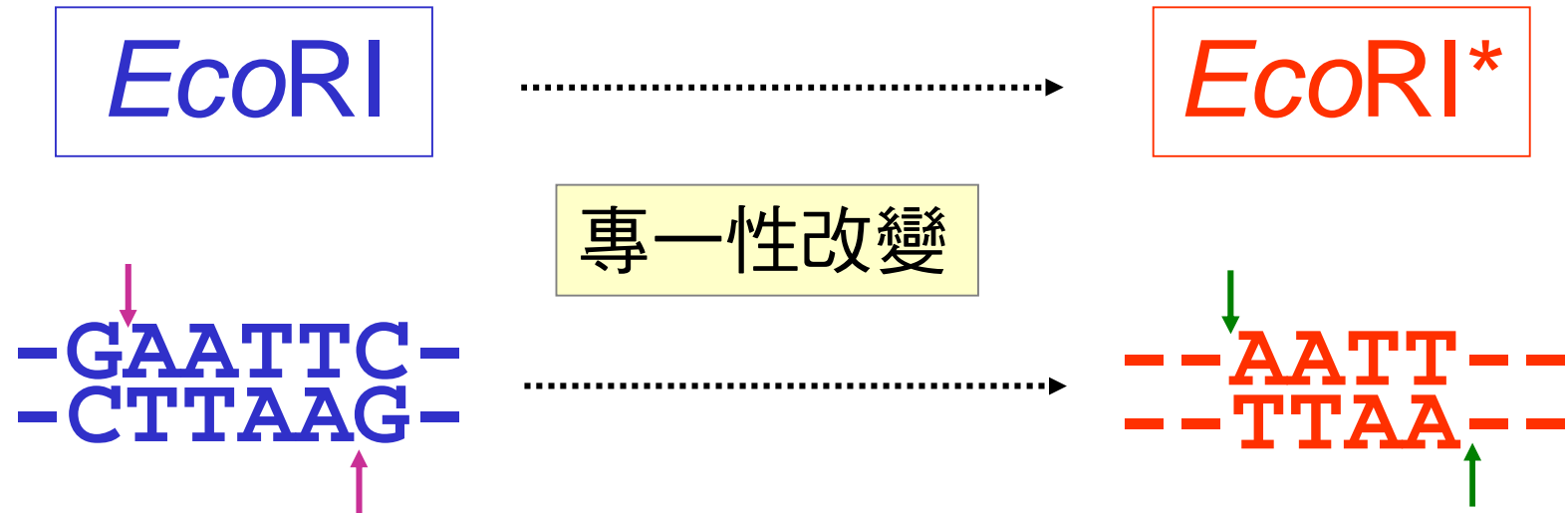
## \* *Star activity*

某些限制酶在非最適反應條件時，  
酵素的專一性發生改變。

?

- 酵素濃度過高
- 甘油濃度過高
- 離子強度過低
- pH 值過高
- 以  $Mn^{2+}$  取代  $Mg^{2+}$
- 有機溶劑存在

**\* Star activity**



## ■ 限制酶使用要點：

▶ 4. 終止反應：

(1) 加熱

(2) 加入 EDTA

(3) 以 phenol/chloroform 萃取

## ■ 限制酶使用要點：

### ▶ 5. 當 DNA 無法被切割或反應不完全時：

- (1) DNA 是否含有雜質、高鹽、酒精、phenol、高濃度 EDTA、大量 RNA ..... ?
- (2) 反應是否不在最適條件？
- (3) 酵素作用處是否距離 DNA 末端太近？
- (4) 兩種酵素的作用序列是否太近？
- (5) 是否 DNA methylation 的問題？



