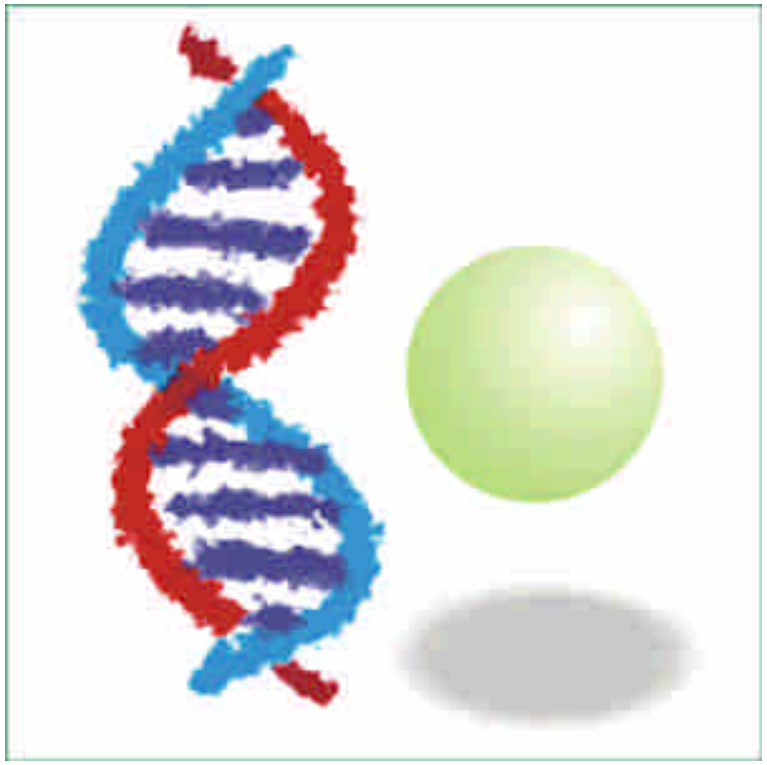
BST

生化科技系



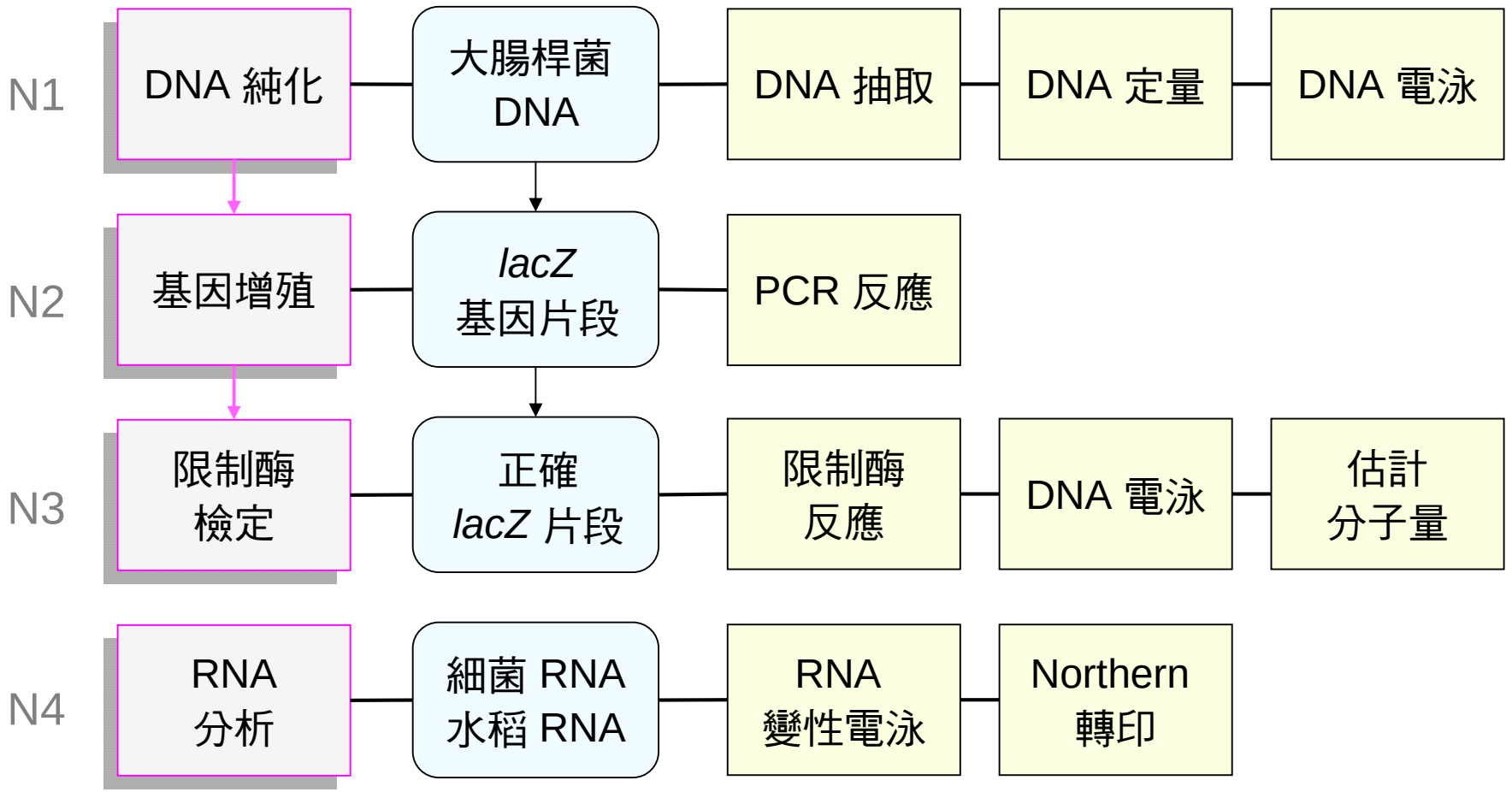
BCX

N3

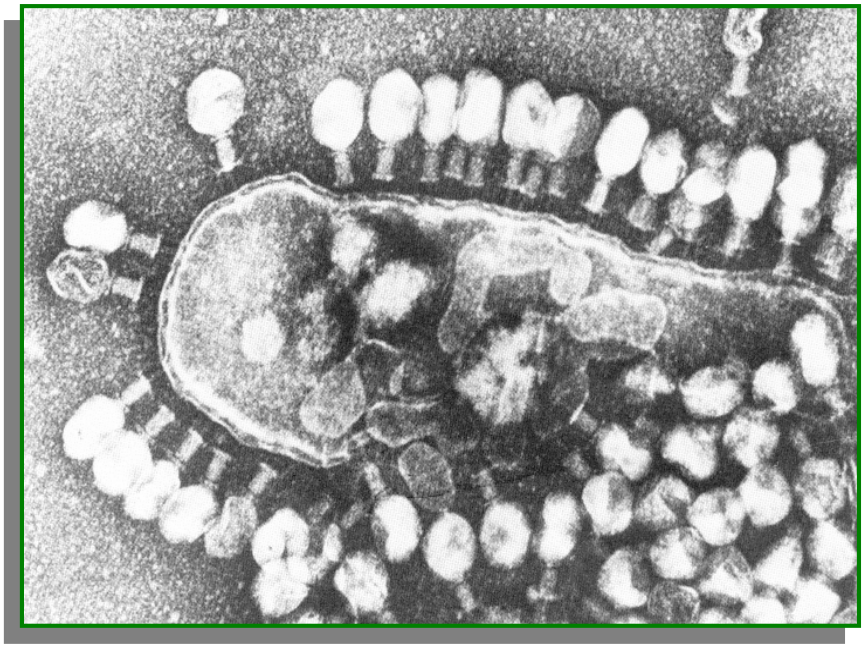
生物化學實驗

Restriction Analysis of DNA

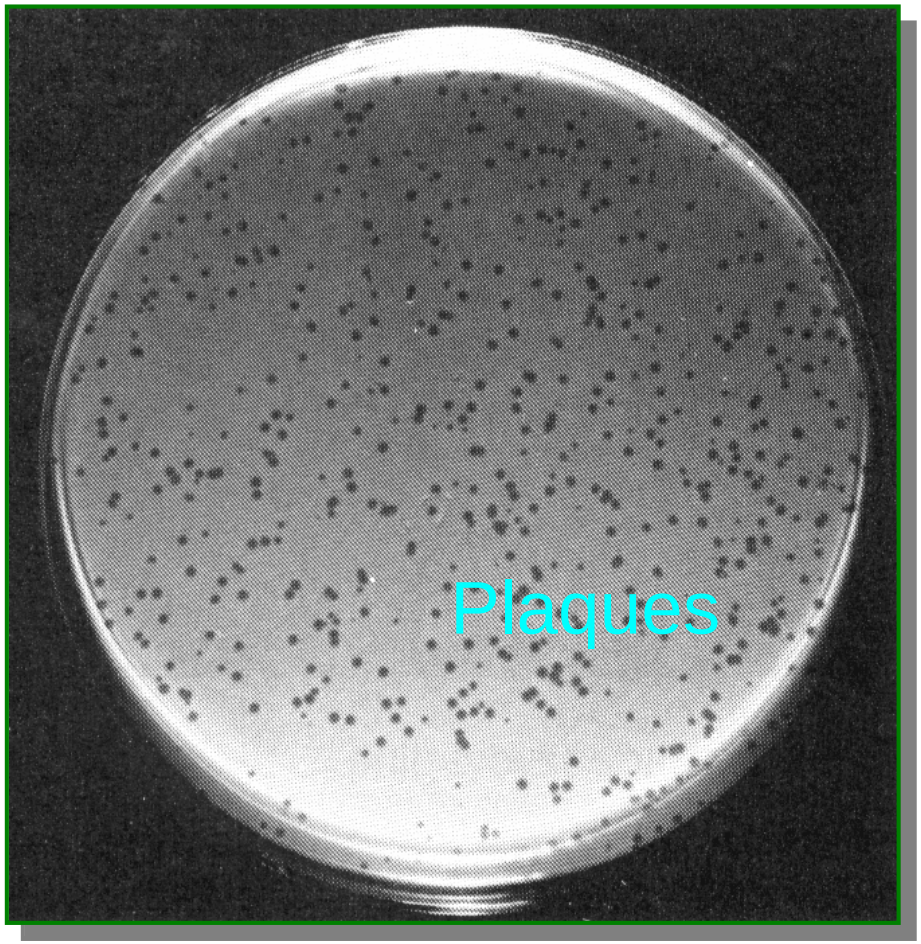
核酸部分的學習目的：


N1
DNA 純化
大腸桿菌 DNA
DNA 抽取
DNA 定量
DNA 電泳
N2
基因增殖
lacZ 基因片段
PCR 反應
N3
限制酶檢定
正確 lacZ 片段
限制酶反應
DNA 電泳
估計分子量
N4
RNA 分析
細菌 RNA 水稻 RNA
RNA 變性電泳
Northern 轉印

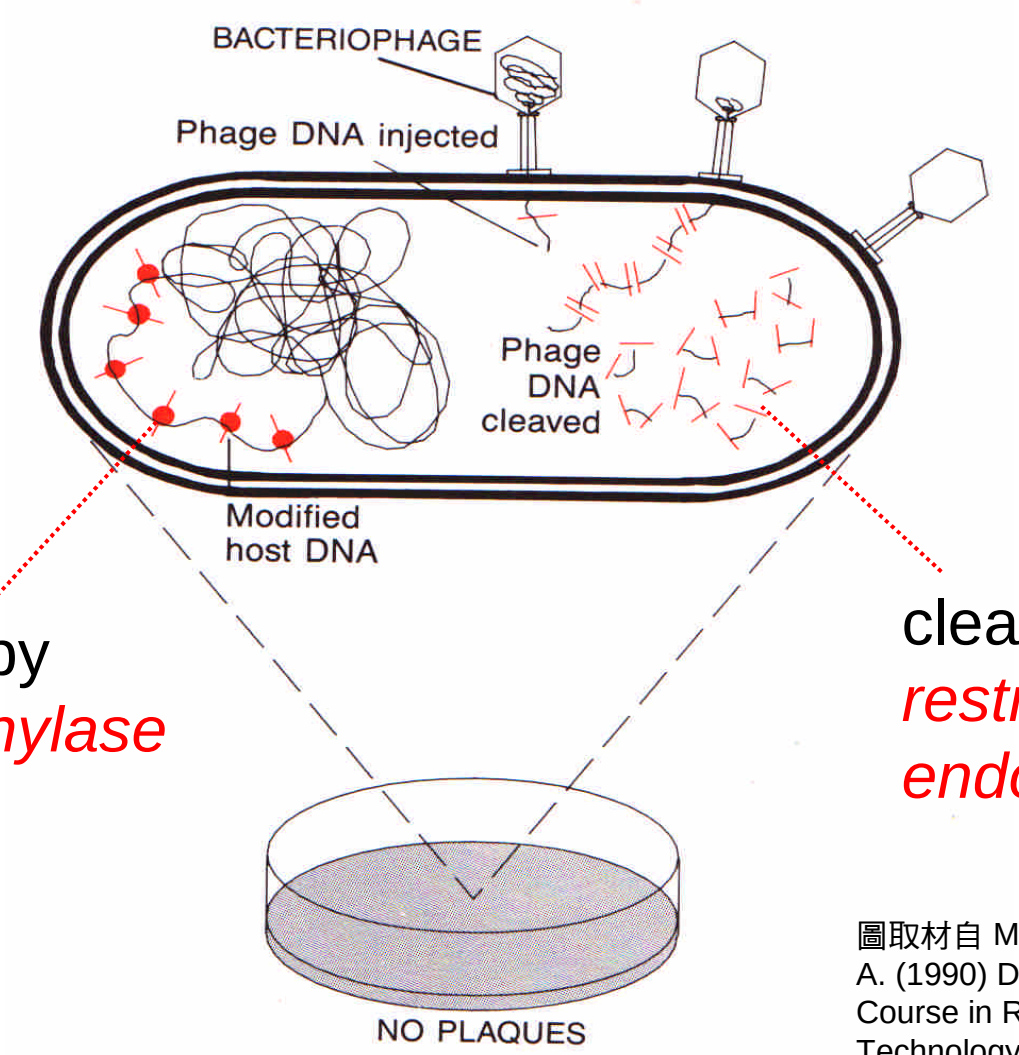
E. coli 受到噬菌體感染



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



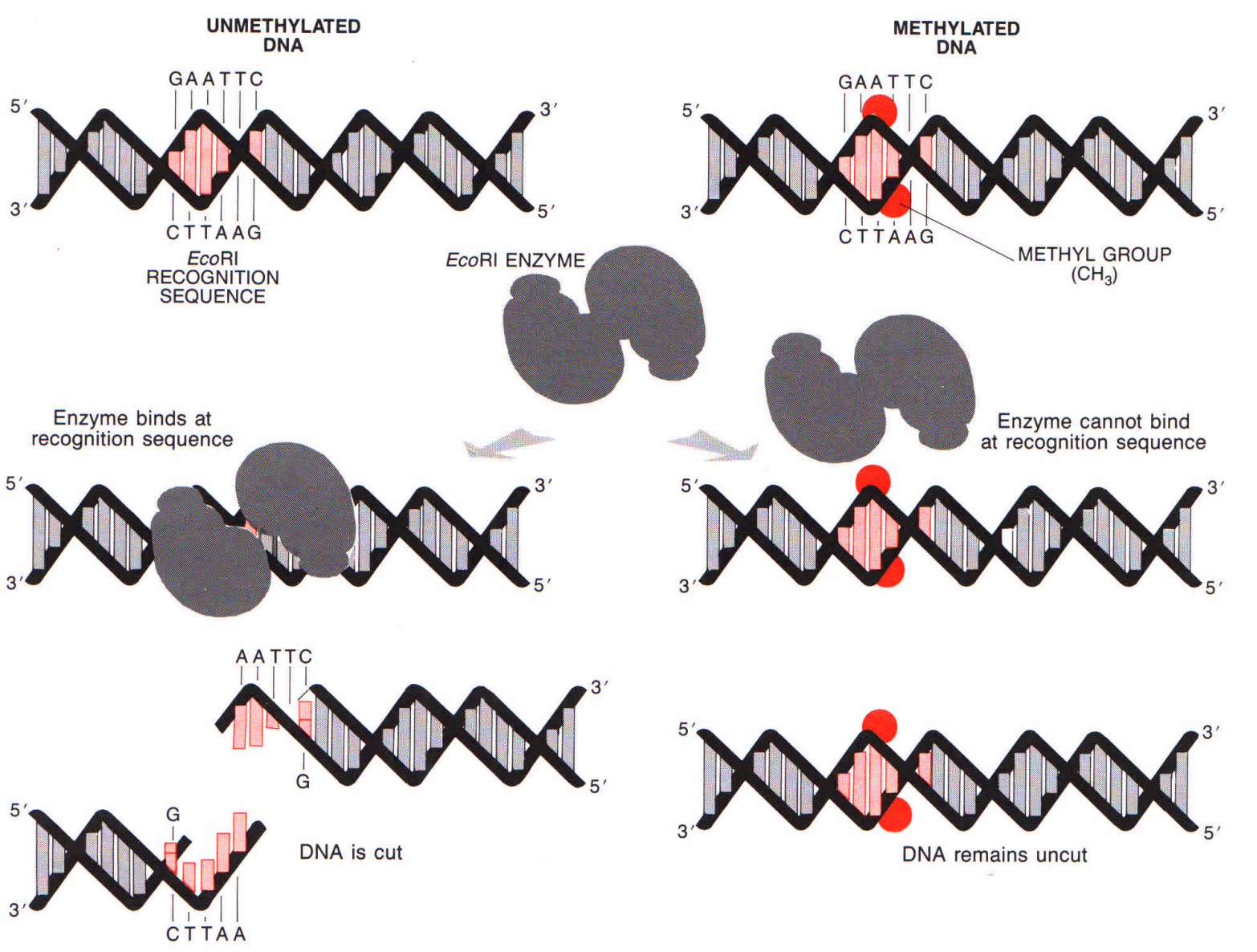
Restriction-modification 系統可防禦噬菌體感染：



modified by
DNA methylase

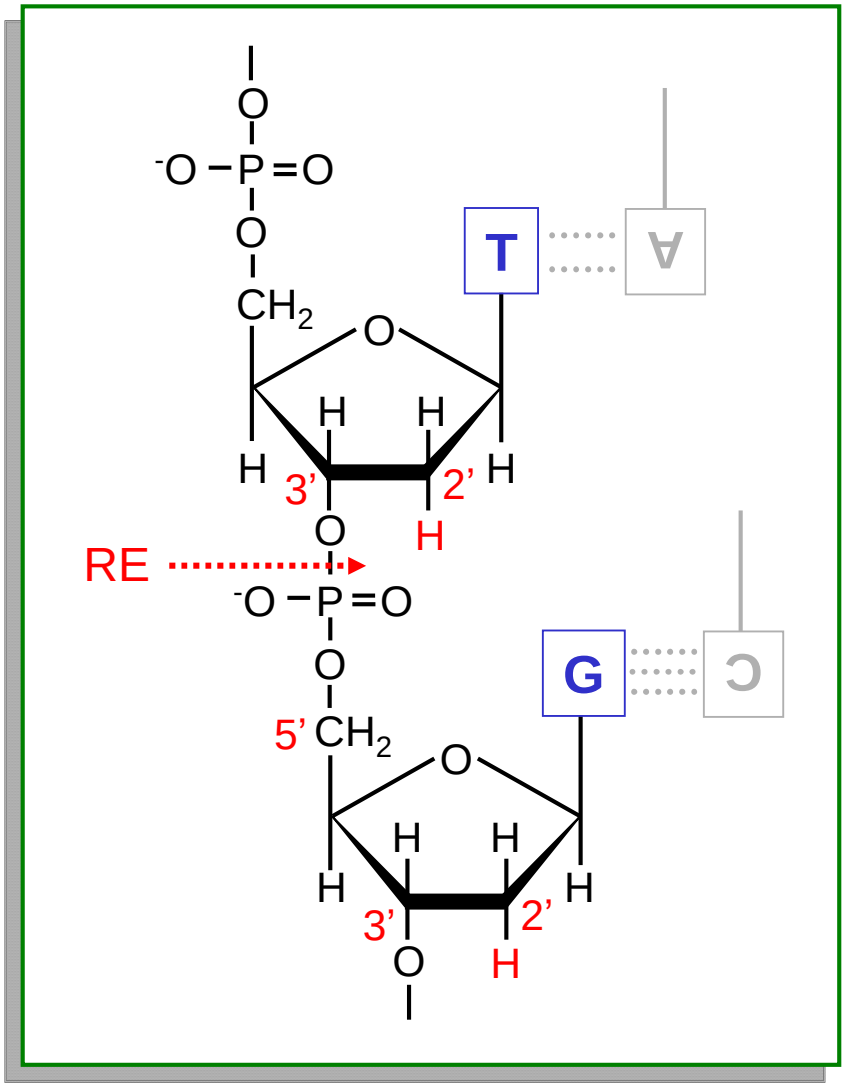
cleaved by
restriction endonucleases

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)



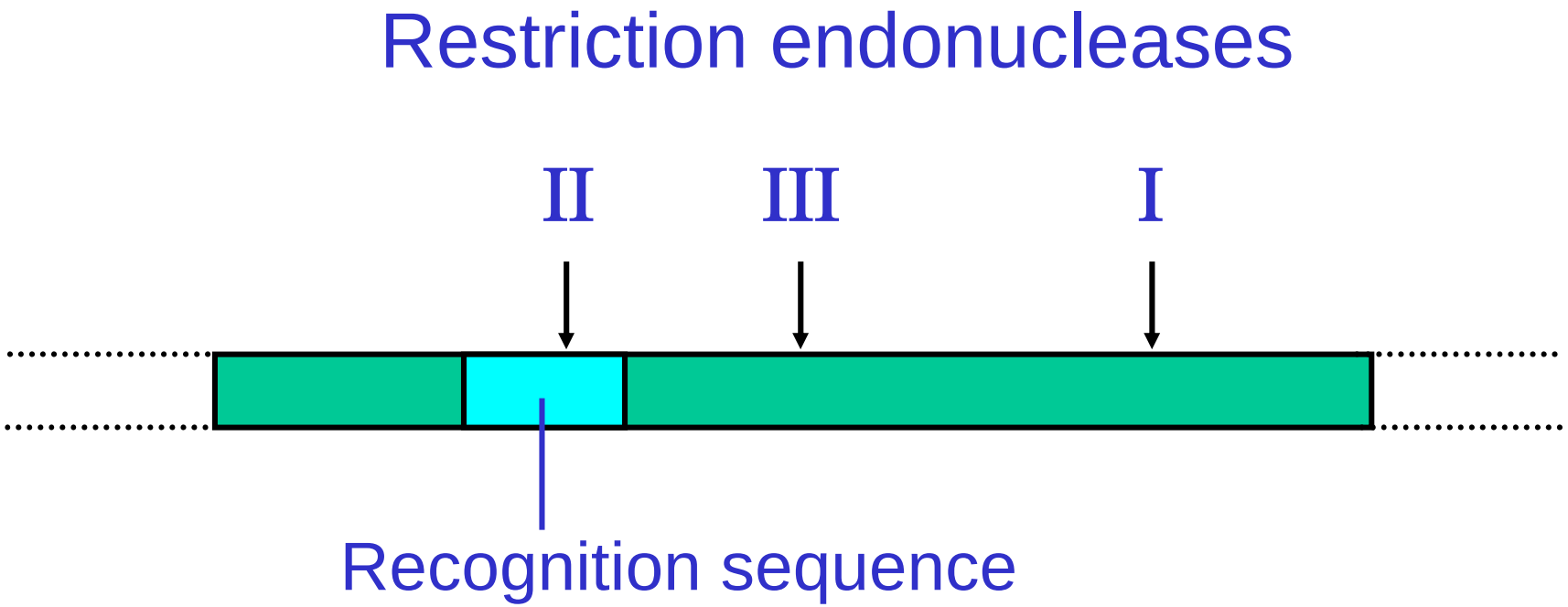
Micklos, D. A. and Freyer, G. A. (1990) DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory Press. (p.46)

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：



三類限制酶性質比較

TABLE 25.1 Properties of restriction–modification systems

	Type I	Type II	Type III
Example	*Eco*B	*Eco*RI	*Eco*PI
Recognition site	$TGAN_8TGCT$	GAATTC	AGACC
Cleavage site	Up to 10 kbp away from recognition site	Between G and A (both strands)	24–26 base pairs 3′ to recognition site
Methylation site	$\overset{m}{TGAN_8TGCT}$ $ACTN_8\underset{m}{ACGA}$	$\overset{m}{GAATTC}$ $CTTAA\underset{m}{G}$	$\overset{m}{AGACC}$ (only one strand methylated)
Nuclease and methylase in one enzyme?	Yes	No	Yes
Requirements for cleavage	ATP, Mg^{2+}, AdoMet	Mg^{2+} or Mn^{2+}	Mg^{2+}, AdoMet
Requirements for methylation	ATP, Mg^{2+}, AdoMet	AdoMet	Mg^{2+}, AdoMet

Mathew, C. K., van Holde, K. E. and Ahern, K. G. (2000) Biochemistry. 3rd ed. Addison Weseley Longman, Inc. Table 25.1

限制酶的命名：

*Eco*RI

E = genus *Escherichia*

co = species *coli*

R = strain RY13

I = first endonuclease isolated

*Bam*HI

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*).

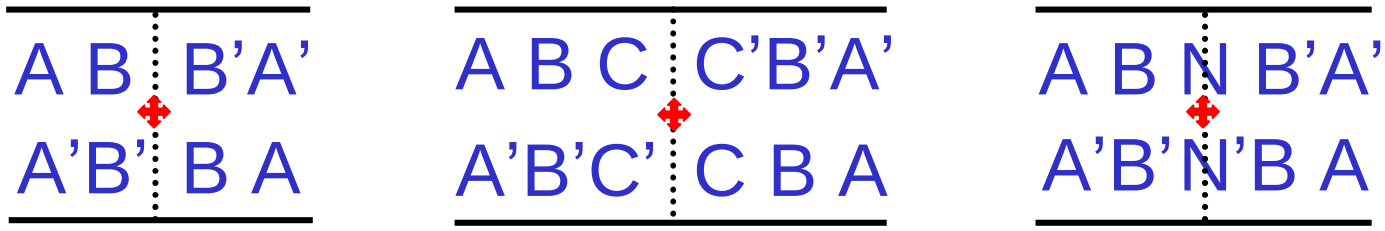


Table 4.1 Recognition sequences of some restriction endonucleases

Enzyme	Recognition site	Type of cut end
*Eco*RI	G↓A—A—T—T—C C—T—T—A—A↑G	5′-phosphate extension
*Bam*HI	G↓G—A—T—C—C C—C—T—A—G↑G	5′-phosphate extension
*Pst*I	C—T—G—C—A↓G G↑A—C—G—T—C	3′-hydroxyl extension
*Sau*3AI	↓G—A—T—C C—T—A—G↑	5′-phosphate extension
*Pvu*II	C—A—G↓C—T—G G—T—C↑G—A—C	Blunt end
*Hpa*I	G—T—T↓A—A—C C—A—A↑T—T—G	Blunt end
*Hae*III	G—G↓C—C C—C↑G—G	Blunt end
*Not*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. 2nd ed., ASM Press. Table 4.1

Type II 限制酶的辨認序列：

- 2. Some type II enzymes recognize asymmetric sequences.

*Hga*I

-**GACGC**NNNNN↓
-**CTGCG**NNNNNNNNNN↑

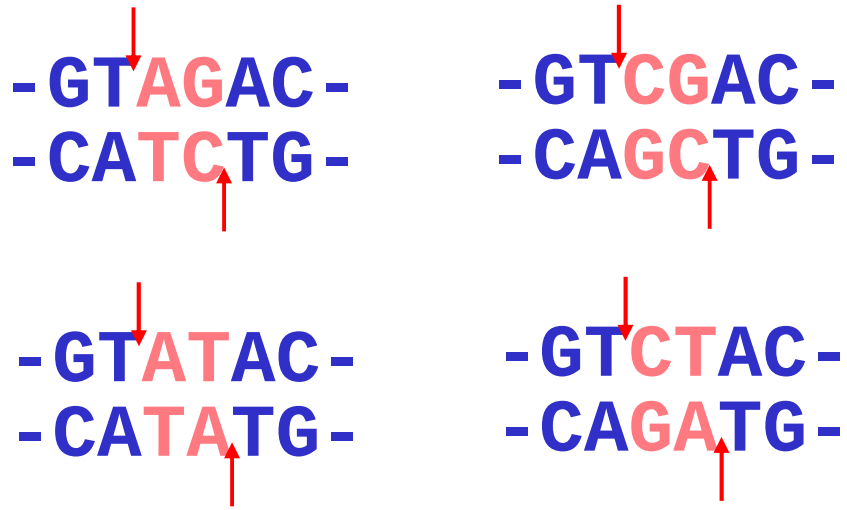
*Bsa*I

-**GGTCTC**N↓
-**CCAGAG**NNNNN↑

Type II 限制酶的辨認序列：

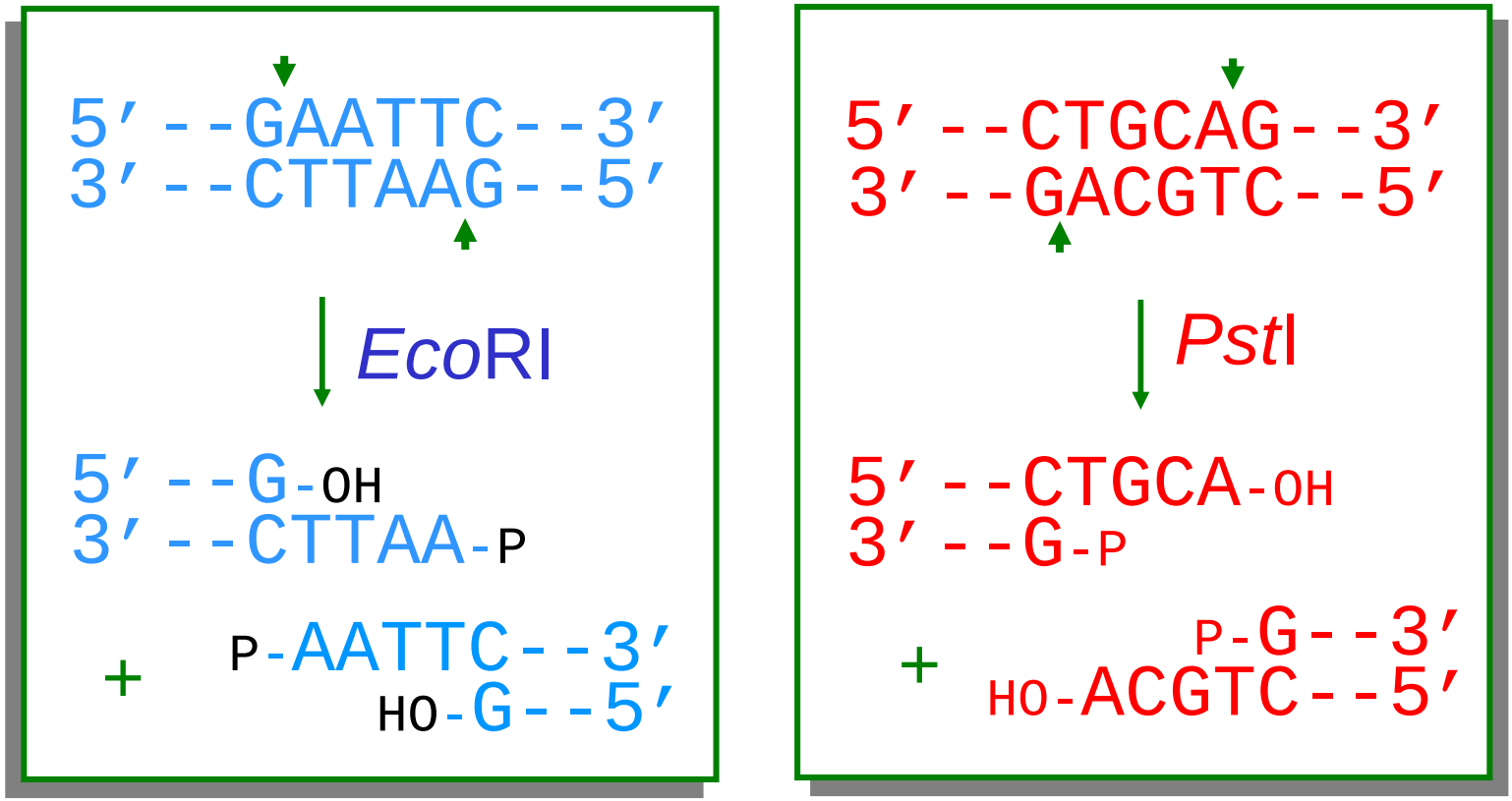
- 3. Some type II enzymes recognize multiple sequences.

*Acc*I



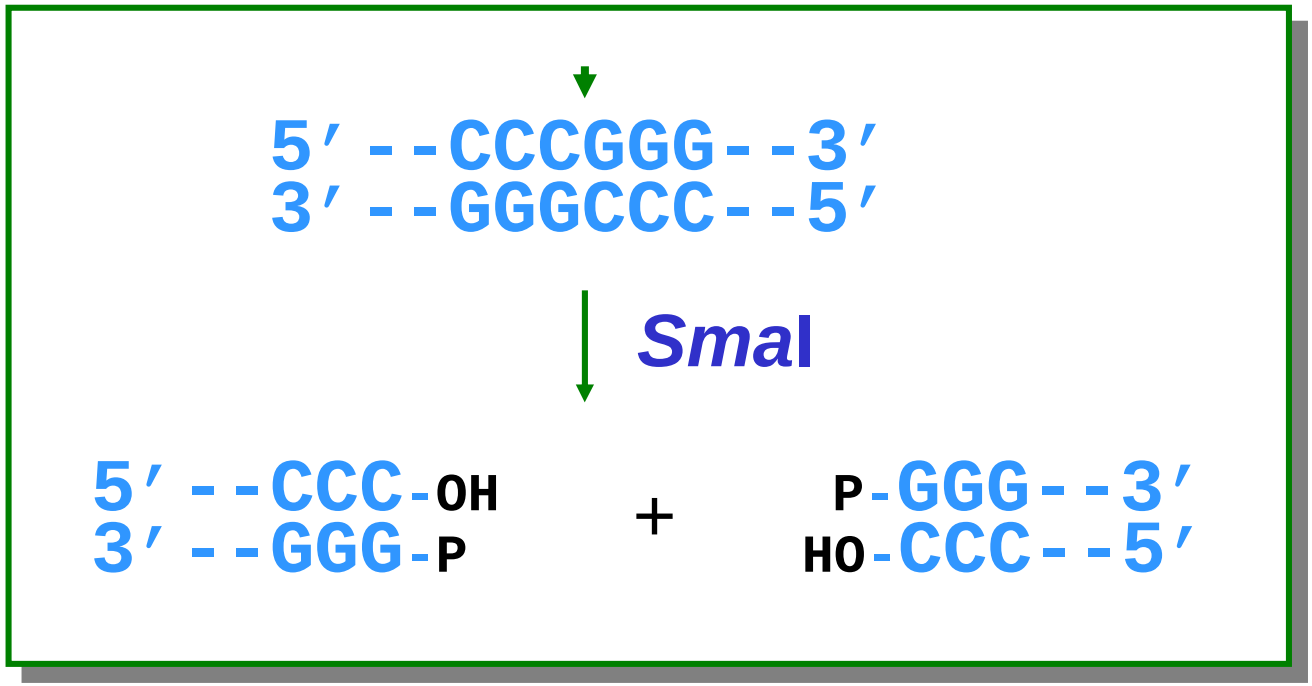
Type II 限制酶作用後的末端型式：

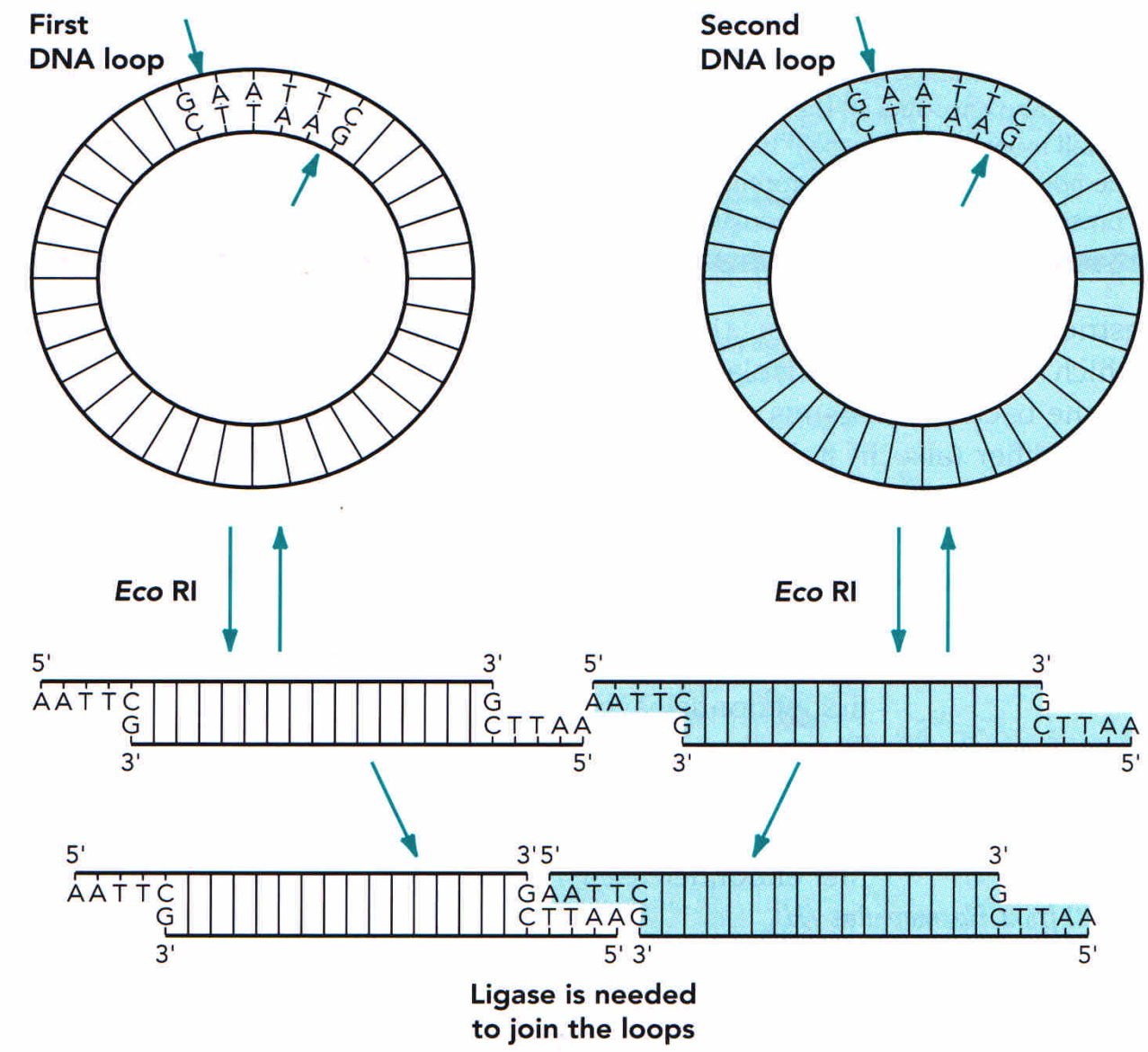
- 1　Cohesive ends (Staggered ends)：



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, 2nd 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℃，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

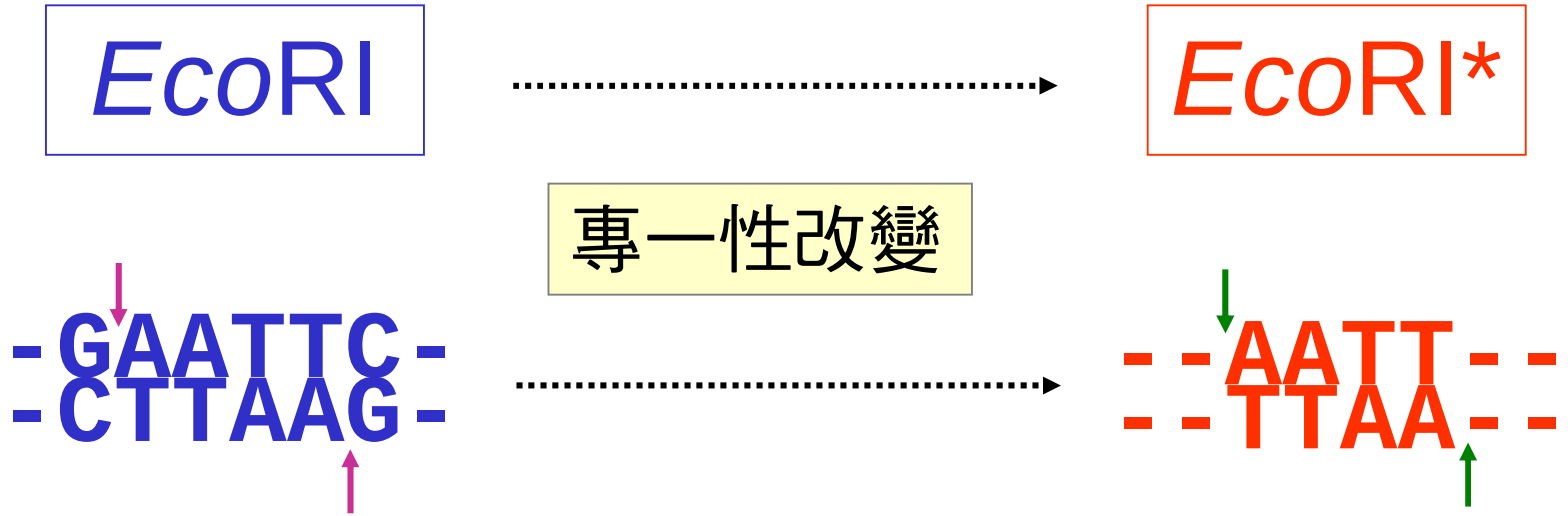
某些限制酶在非最適反應條件時，

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：

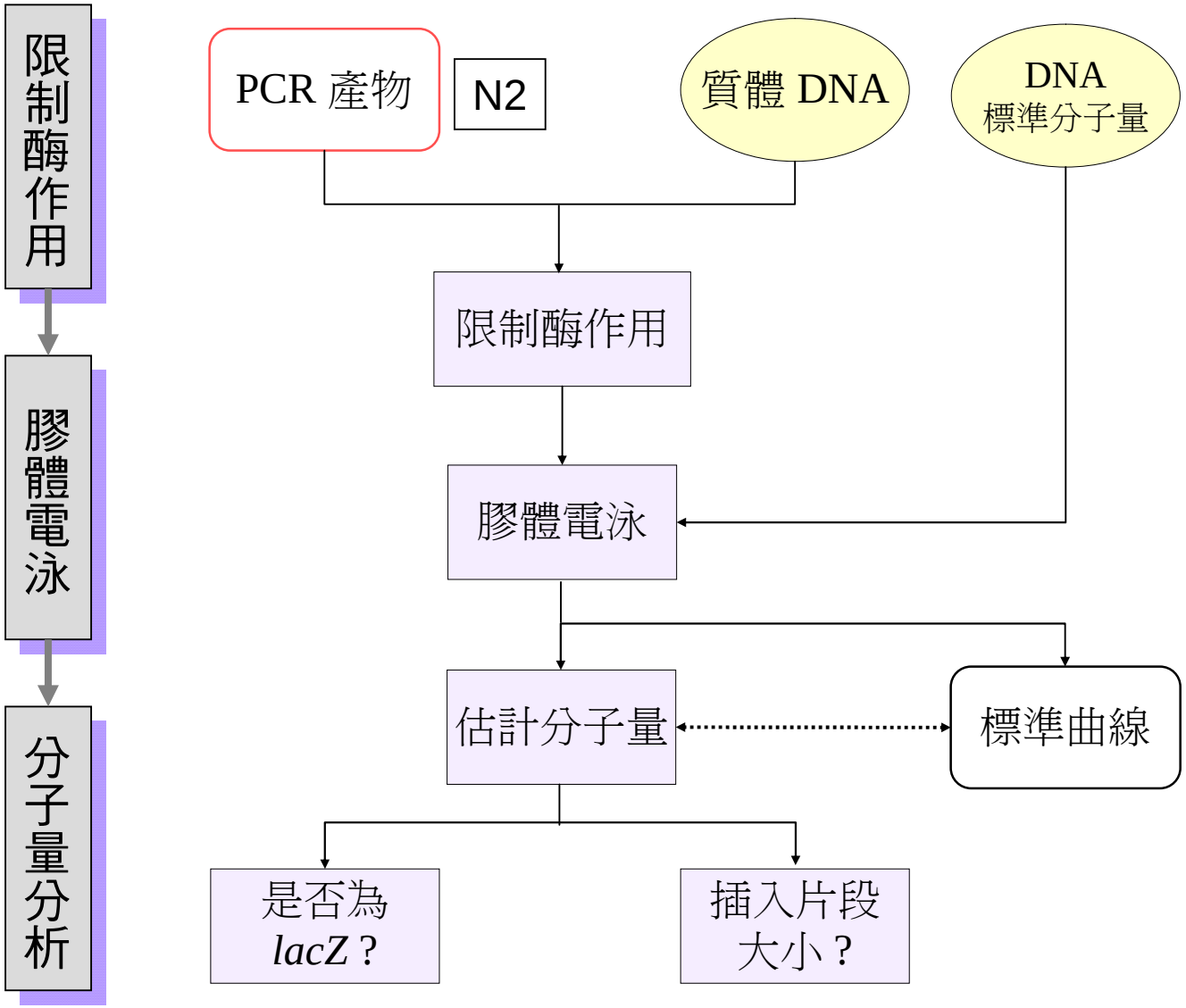
  (1) DNA 是否含有雜質、高鹽、酒精、
  phenol、高濃度 EDTA、大量 RNA ……？

  (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？


限制酶作用
膠體電泳
分子量分析
PCR 產物
N2
質體 DNA
DNA
標準分子量
限制酶作用
膠體電泳
估計分子量
標準曲線
是否為
lacZ ?
插入片段
大小 ?