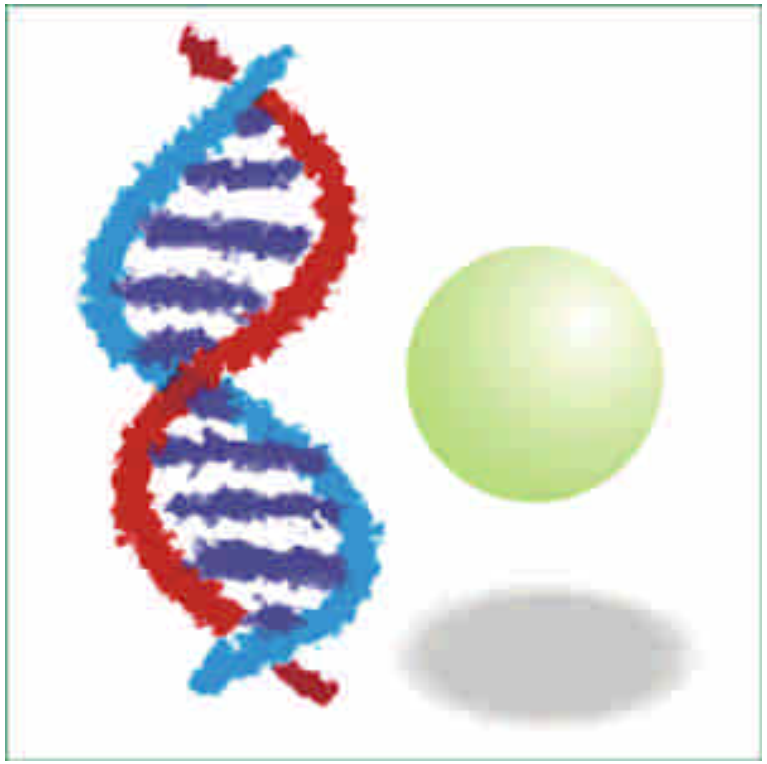
BST

生化科技系

BCX

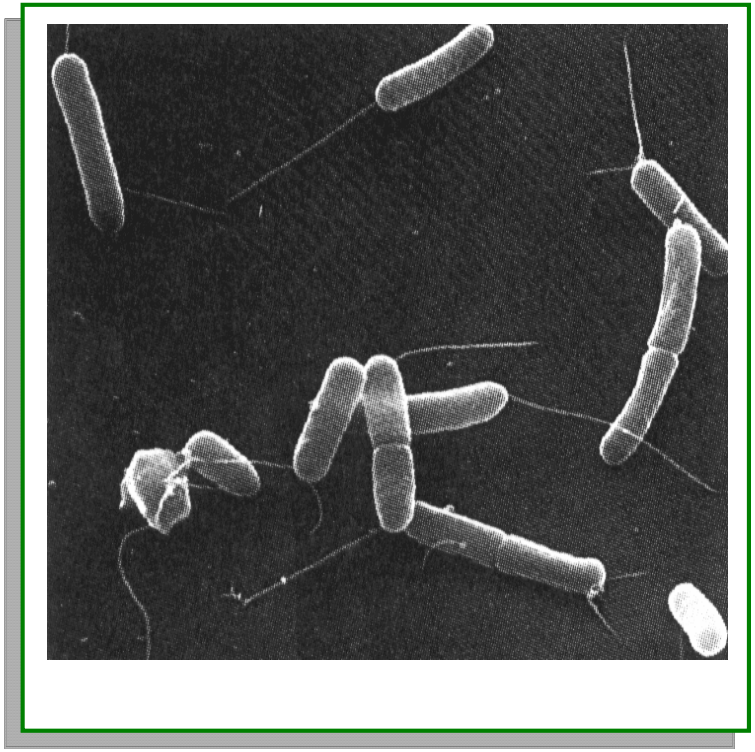


N1

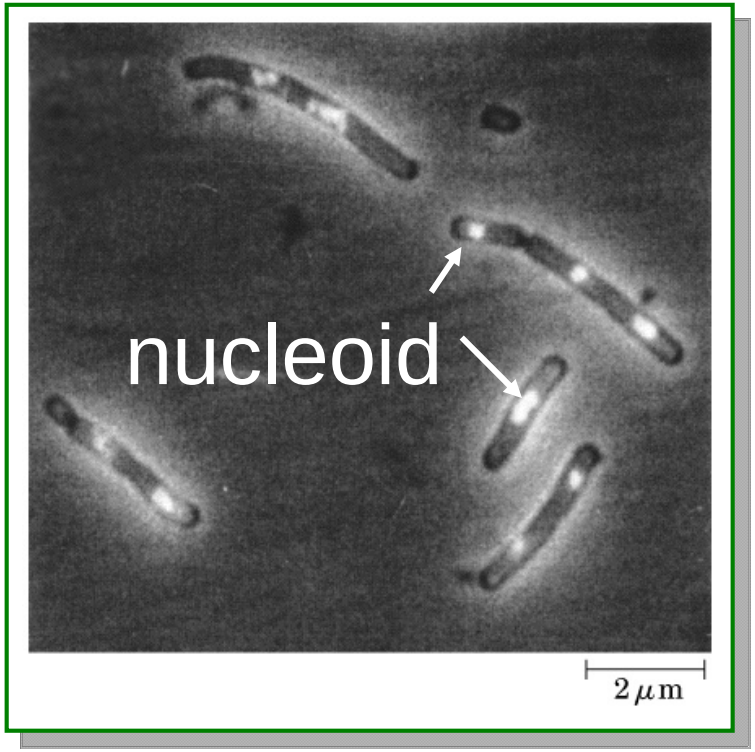
生物化學實驗

染色體 DNA 分離與檢定

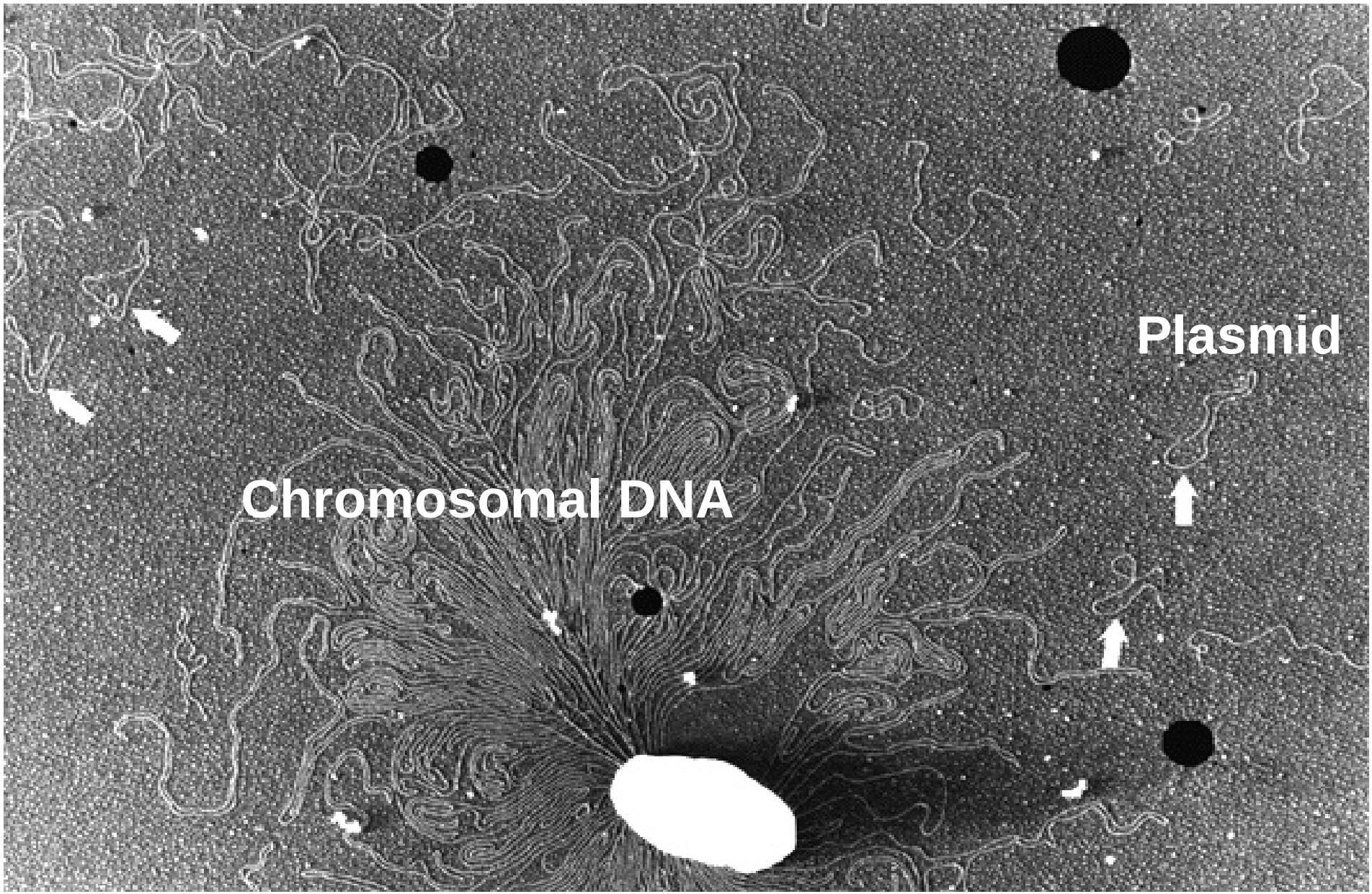
實驗材料：*E. coli*



Alcamo, I. E. (2000) DNA Technology: The Awesome Skill. 2nd Ed. Academic Press. Fig. 5.9

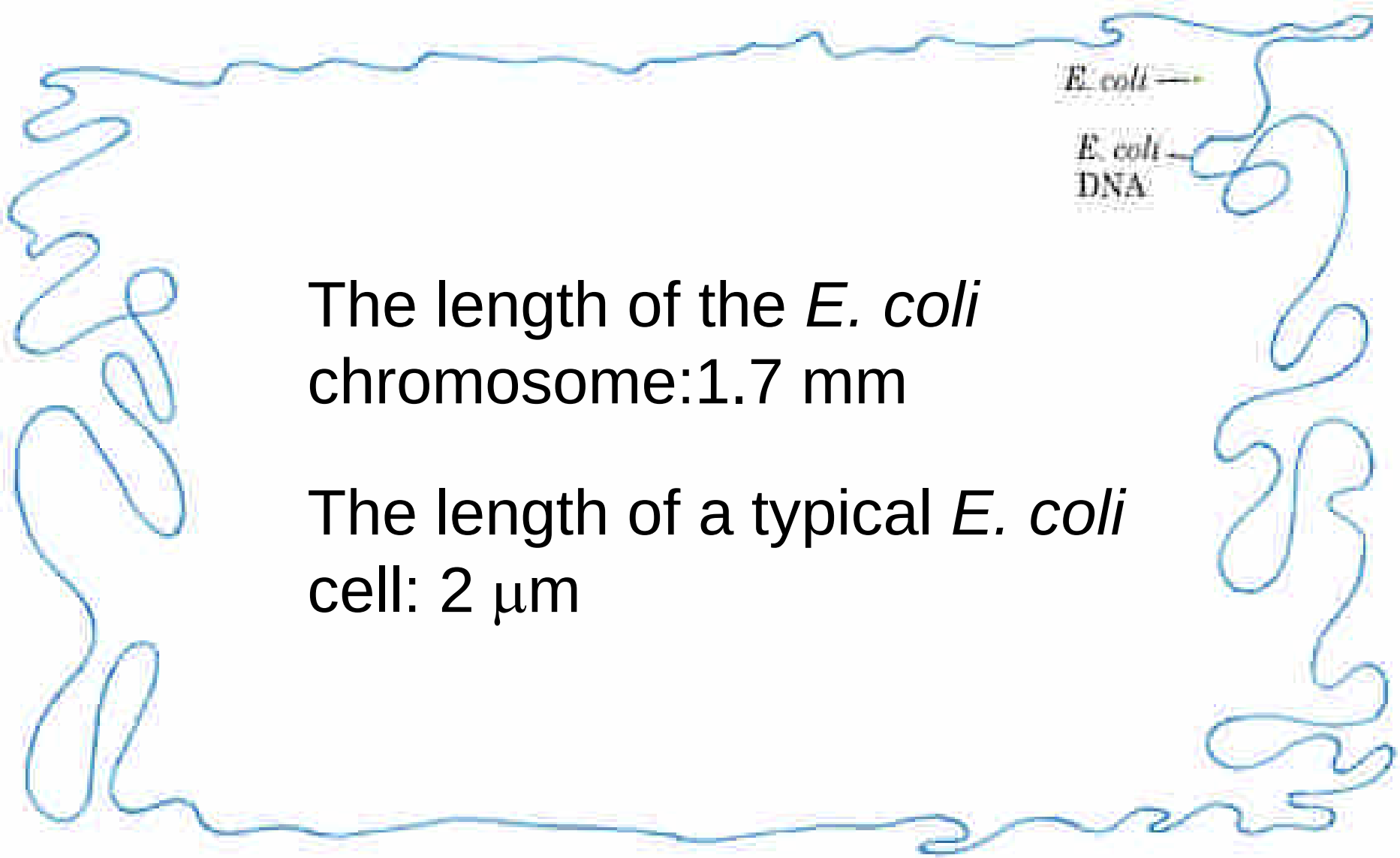


Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 24-31



DNA from a lysed *E. coli* cell

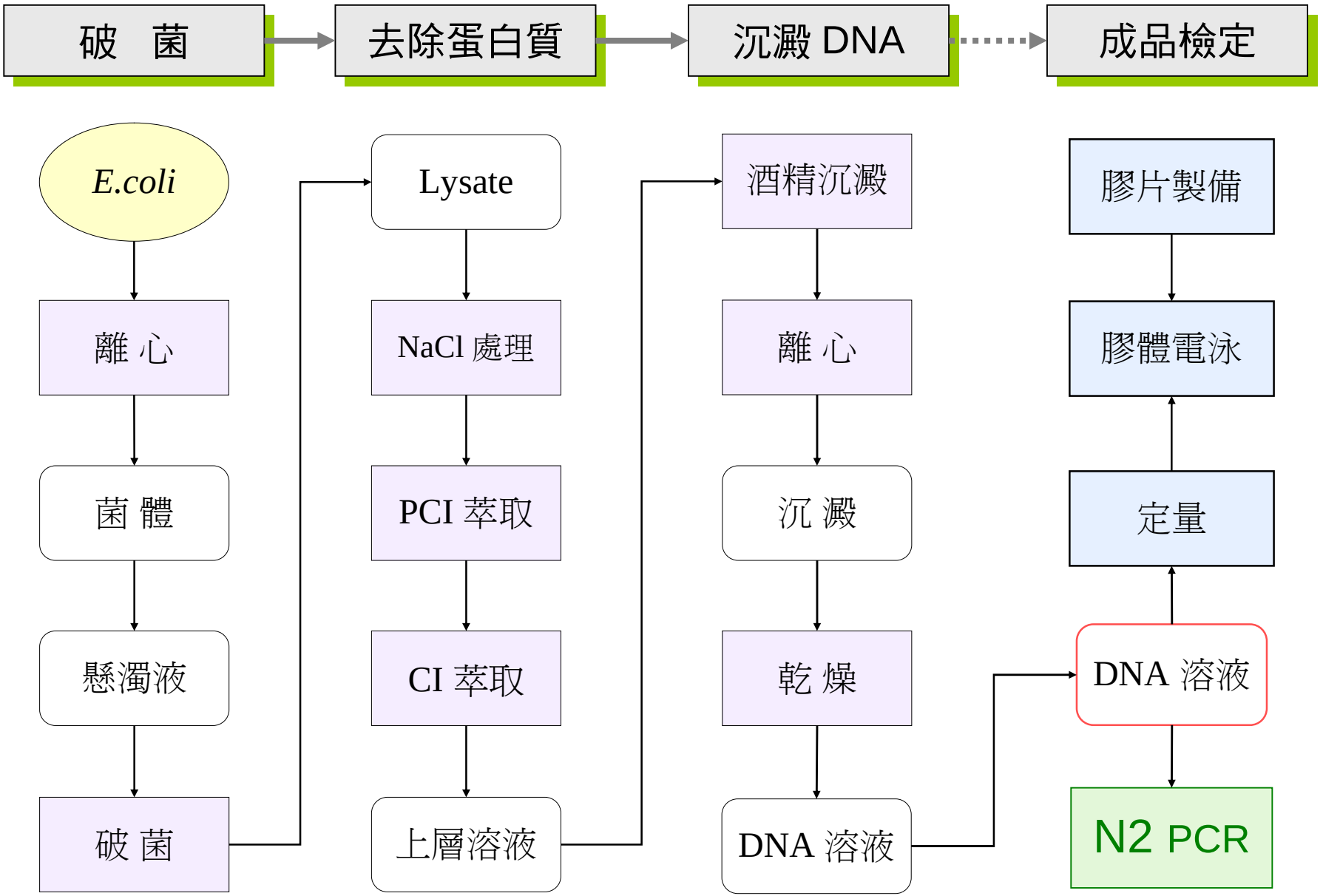
圖取材自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 24-6



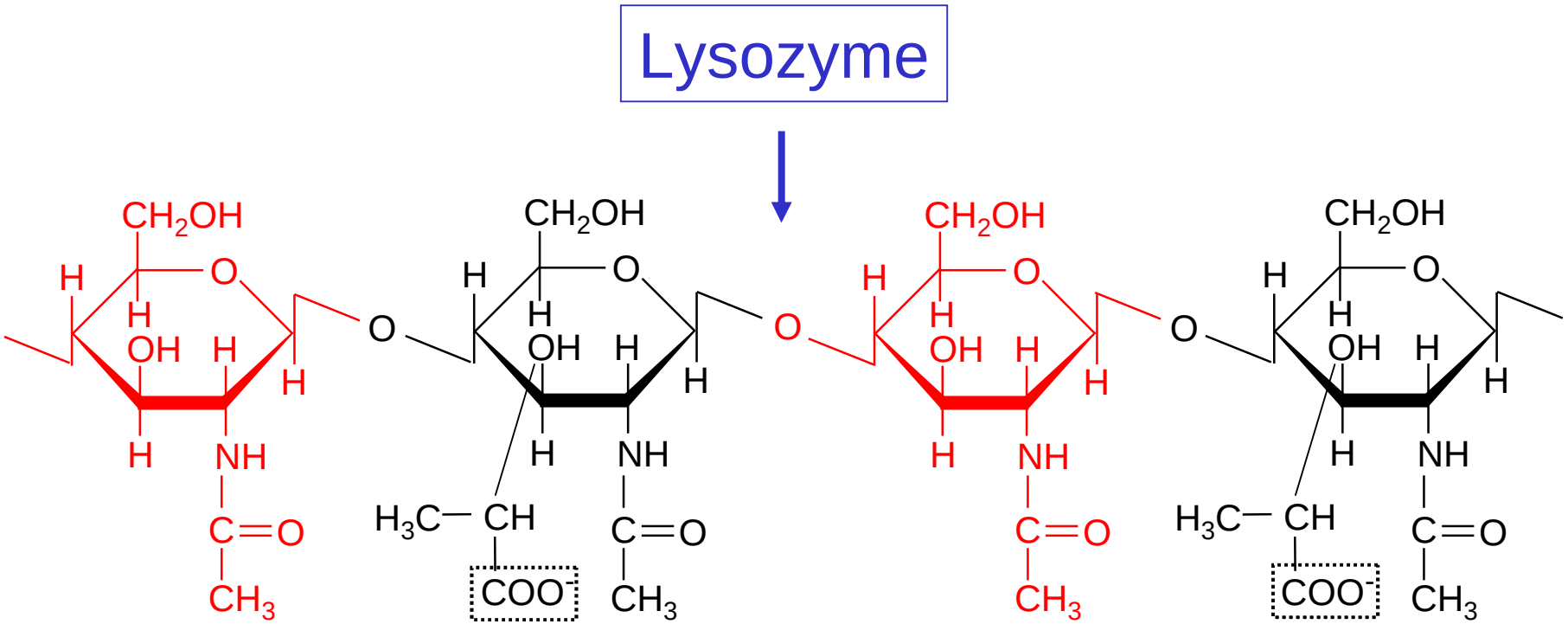
The length of the *E. coli* chromosome:1.7 mm

The length of a typical *E. coli* cell: 2 μm

Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 24-5


破　菌
去除蛋白質
沉澱 DNA
成品檢定
E.coli
離 心
菌 體
懸濁液
破 菌
Lysate
NaCl 處理
PCI 萃取
CI 萃取
上層溶液
酒精沉澱
離 心
沉 澱
乾 燥
DNA 溶液
膠片製備
膠體電泳
定量
DNA 溶液
N2 PCR

Lysozyme：作用於 peptidoglycan 中的醣苷鍵



去除核酸中的蛋白質：

DNA or RNA solution

↓ Extracted with phenol/chloroform/IAA (25:24:1)

↓ cfg

Aqueous phase

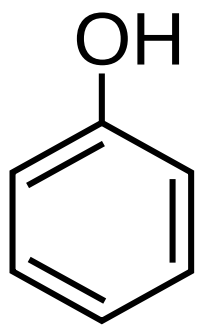
↓ Extracted with chloroform/IAA (24:1)

↓ cfg

Aqueous phase

↓ Precipitated by ethanol

Deproteinated DNA or RNA



Phenol

酒精沈澱：濃縮 DNA 的方法

DNA 溶液

↓ 調整鹽類濃度 ▸

↓ 加入 2 倍體積酒精

↓ 靜置至少 30 分鐘

↓ 離心

DNA 沈澱

↓ 以 70% 酒精清洗

↓ 乾燥

↓ 溶解於少量適當溶液

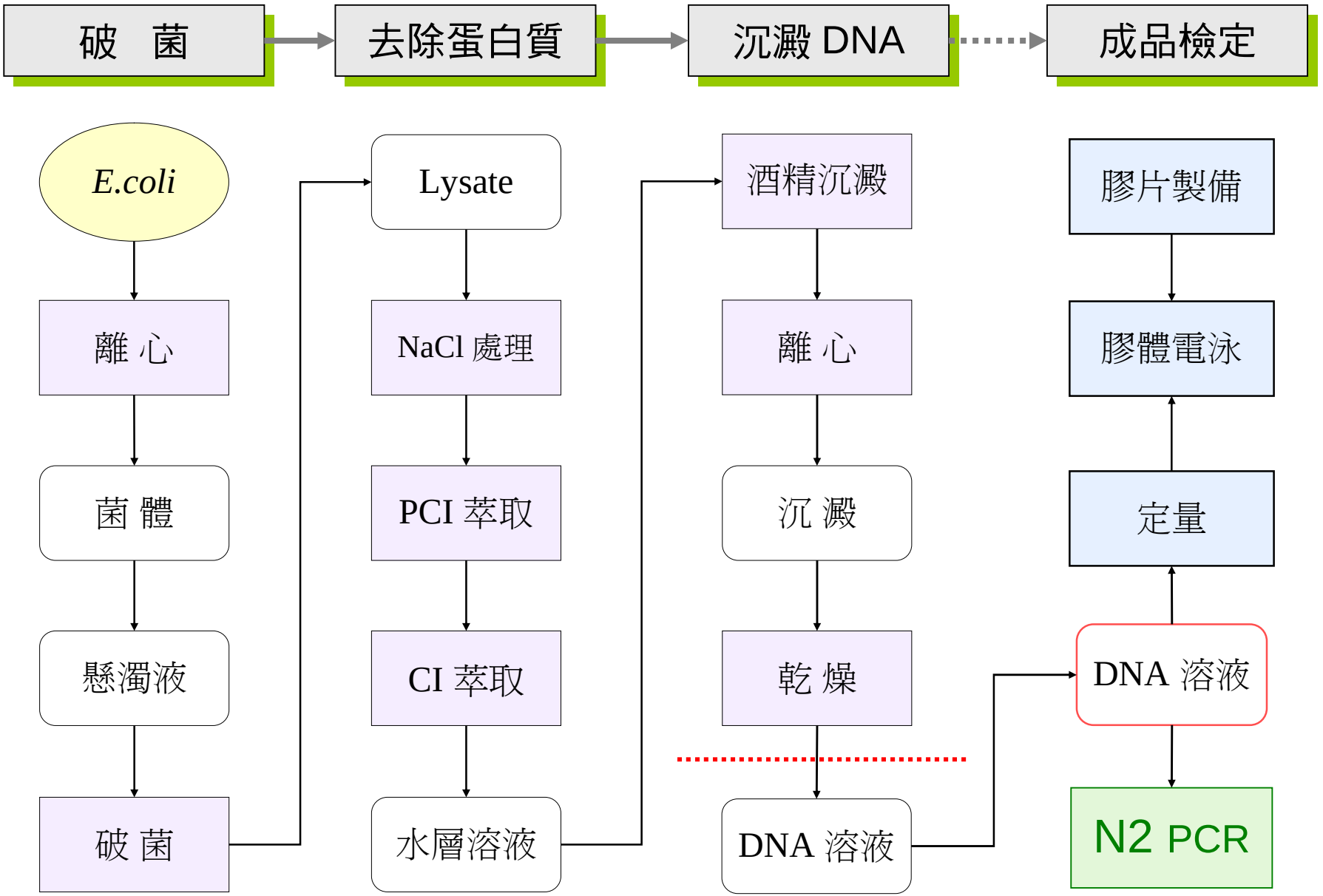
濃縮 DNA 溶液

▸ 鹽類及最終濃度：

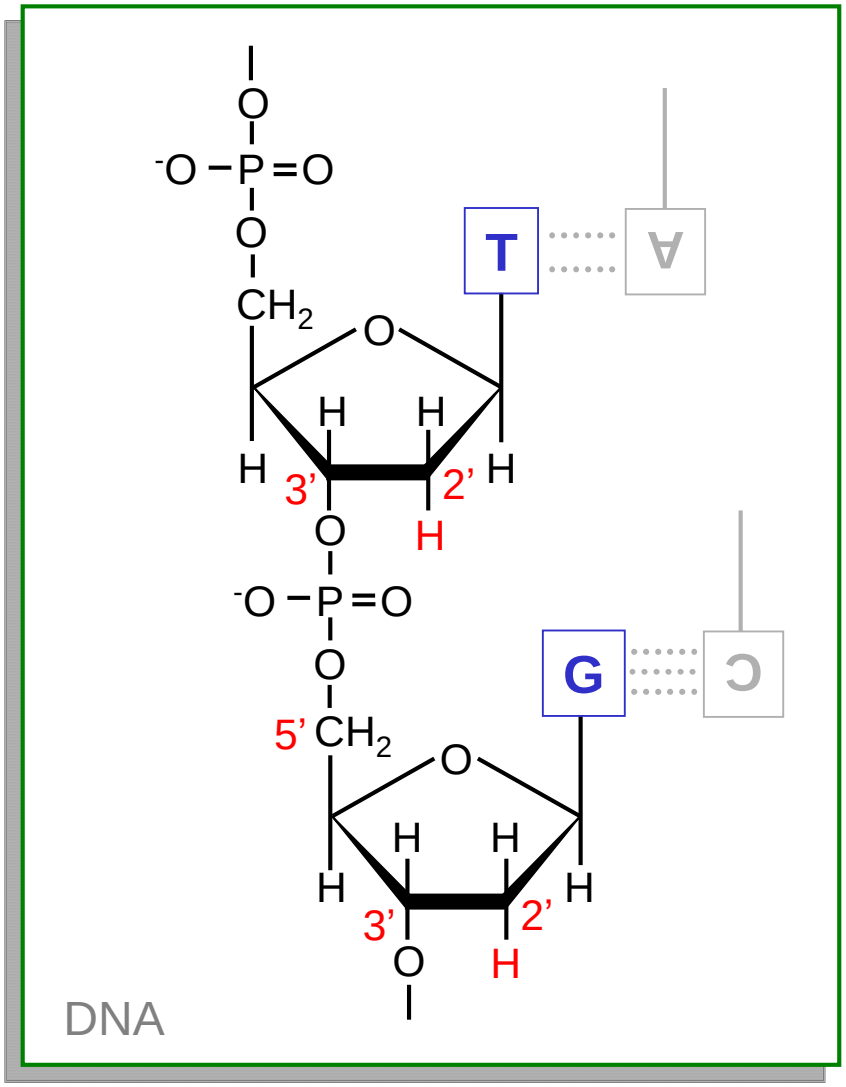
NaOAc (pH 5.2), 0.3 M

NH_4OAc, 2.0-2.5 M

NaCl, 0.2 M


破　菌
去除蛋白質
沉澱 DNA
成品檢定
E.coli
離 心
菌 體
懸濁液
破 菌
Lysate
NaCl 處理
PCI 萃取
CI 萃取
水層溶液
酒精沉澱
離 心
沉 澱
乾 燥
DNA 溶液
膠片製備
膠體電泳
定量
DNA 溶液
N2 PCR

核酸抽取要點：



(1) 避免分解

(2) 避免斷裂

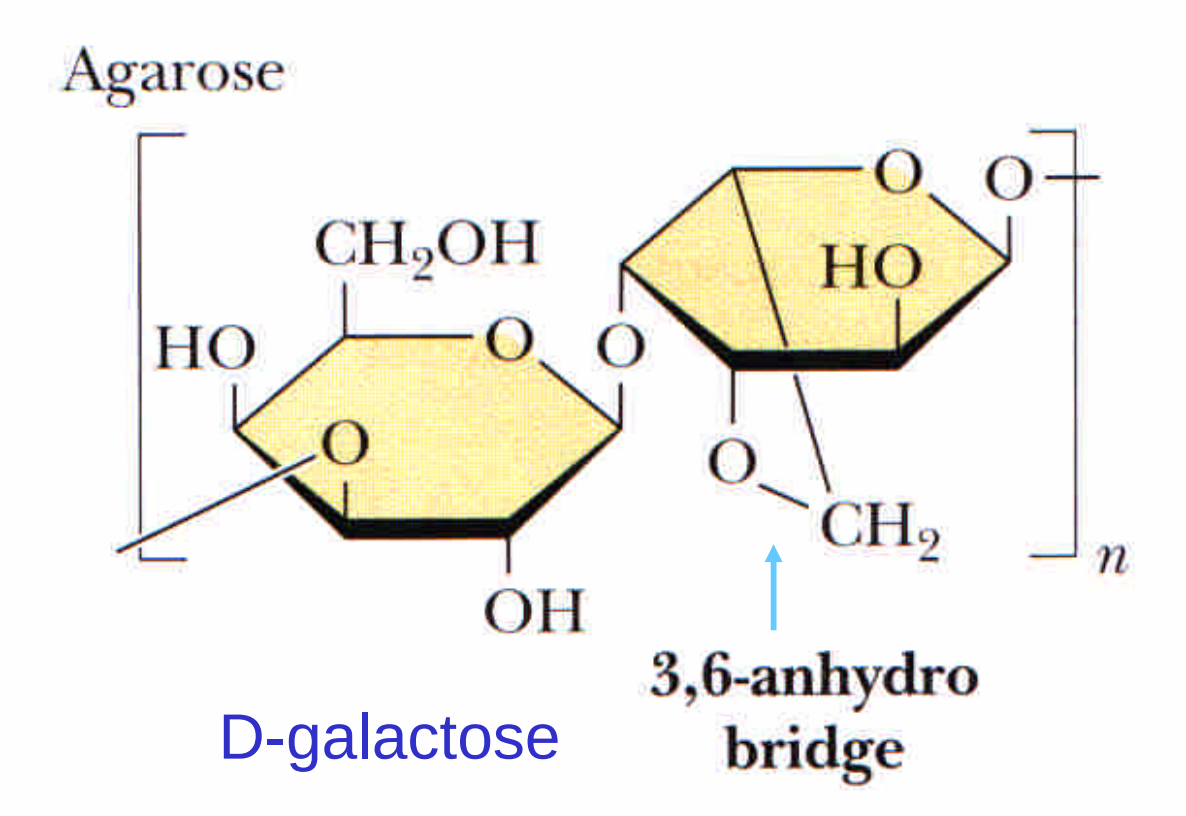
(3) 避免污染

(4) 保持清潔

常用核酸電泳系統：

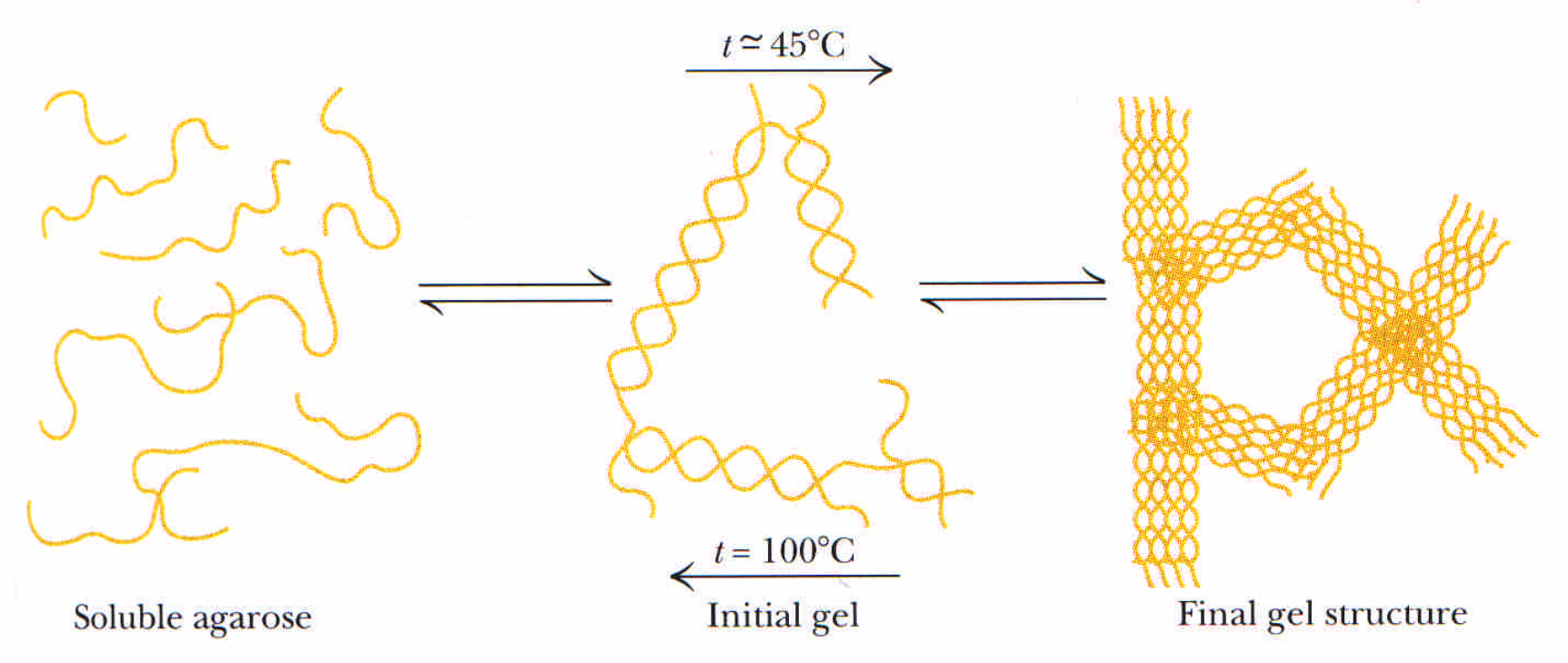
- Agarose gel electroproresis
  - Non-denaturing gel
  - Denaturing gel
- Polyacrylamide gel electrophoresis
  - Non-denaturing gel
  - Denaturing gel

Agarose 基本組成：



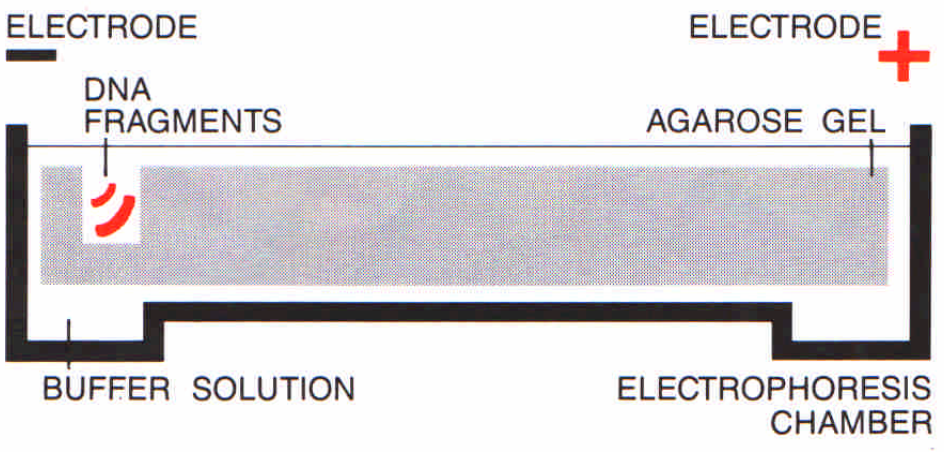
Garrett, R. H. and Grisham, C. M. (1999) Biochemistry. 2nd Ed. Saunders College Publishing. Fig. 7.31

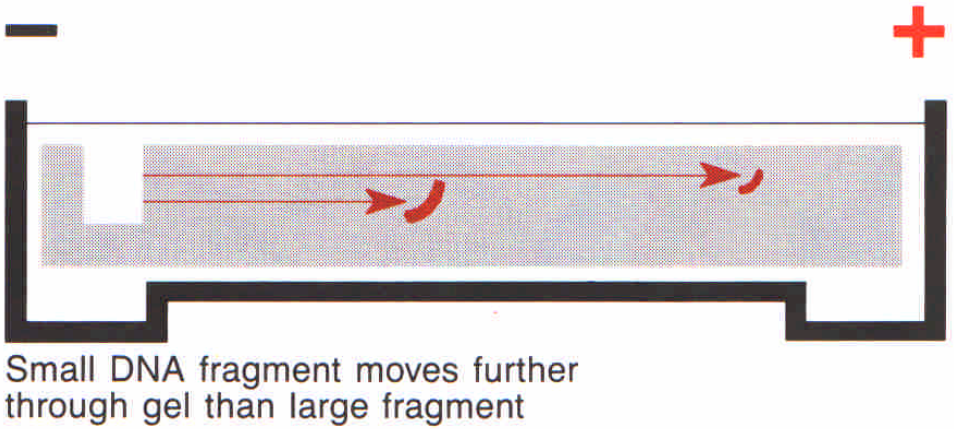
Agarose 溶解與凝膠的結構：



Garrett, R. H. and Grisham, C. M. (1999) Biochemistry. 2nd Ed. Saunders College Publishing. Fig. 7.32

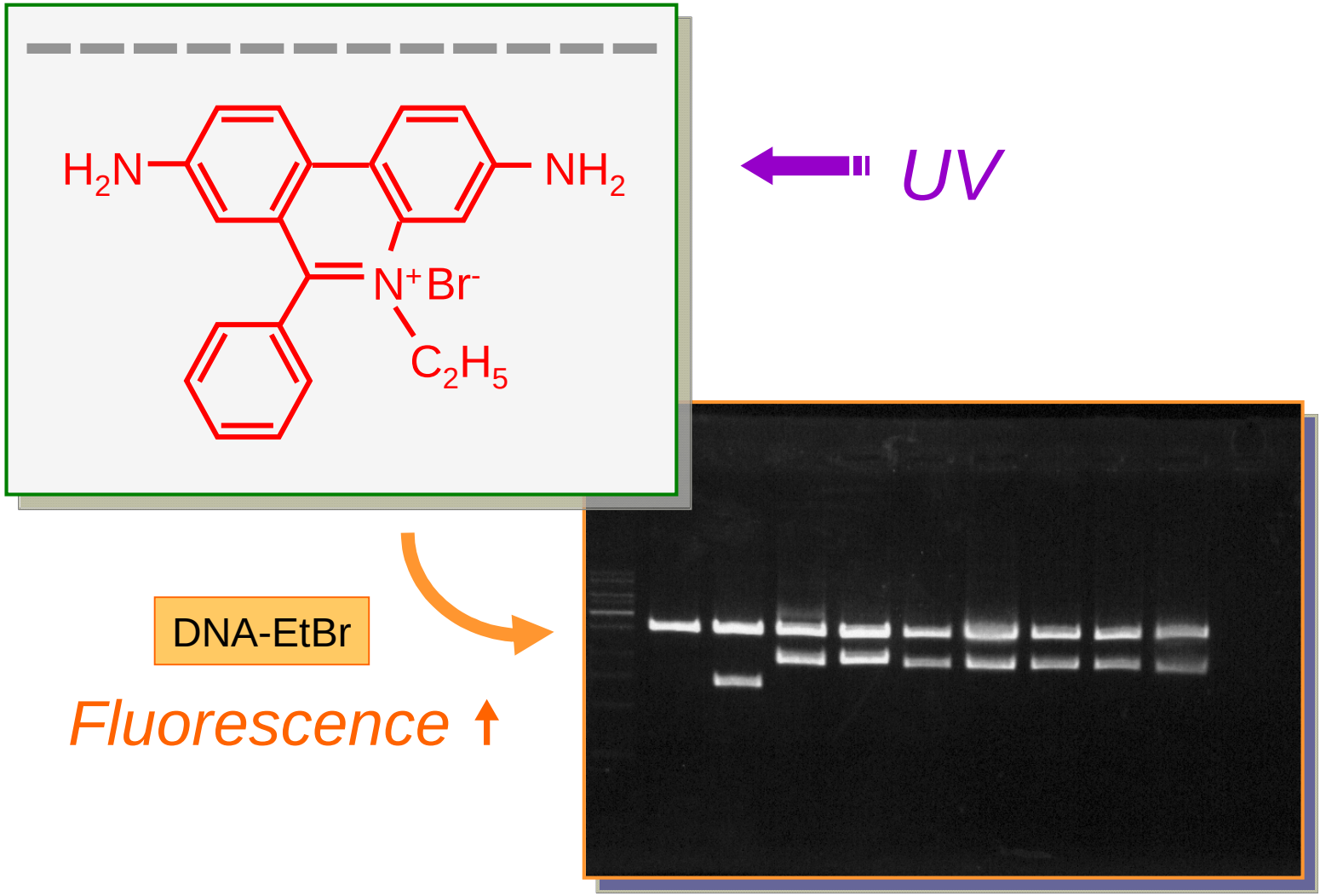
以水平電泳槽進行 agarose 膠體電泳：



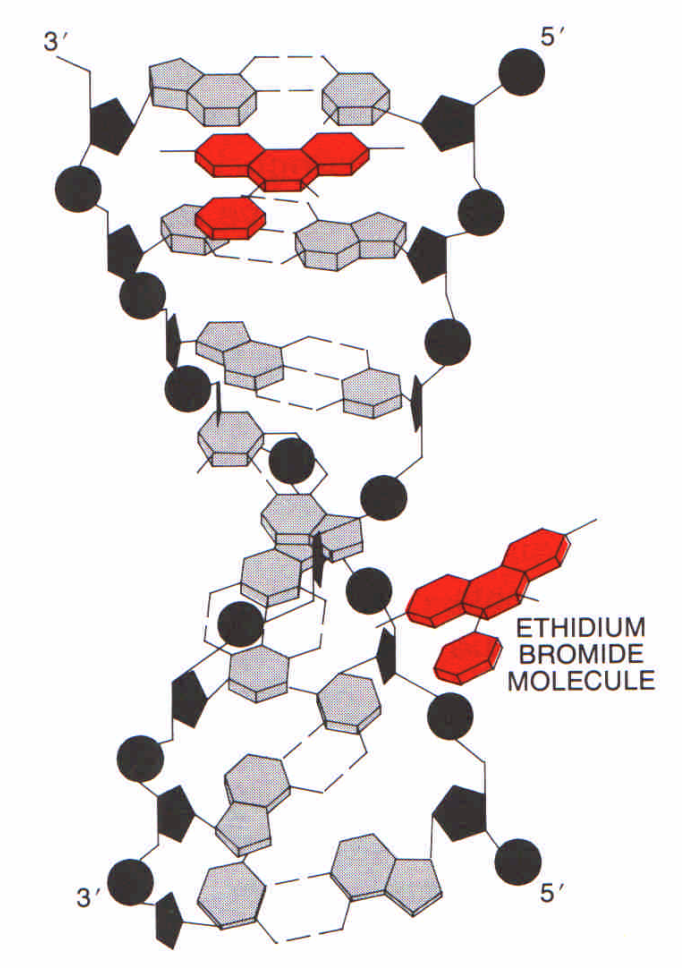


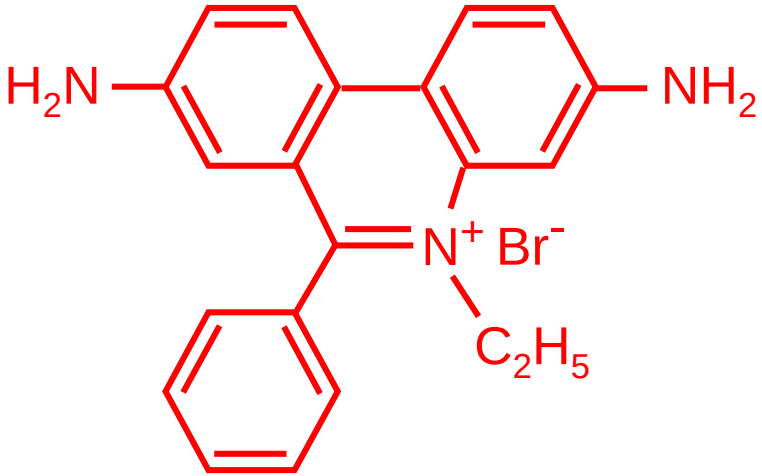
取材自 Micklos, D. A. and Freyer, G. A. (1990) DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory Press. (p.48)

Ethidium bromide 可染出 DNA 色帶：



Ethidium bromide 可嵌入雙股 DNA





Ethidium bromide

突變劑！請小心使用！

左圖取材自 Micklos, D. A. and Freyer, G. A. (1990) DNA Science: A First Course in Recombinant DNA Technology. Cold Spring Harbor Laboratory Press. (p.49)

影響 DNA 泳動的主要因素：

- Agarose 濃度
- DNA 構形與大小
- 電壓
- 電泳緩衝液
- Intercalating dye 存在與否

選擇適當膠體濃度

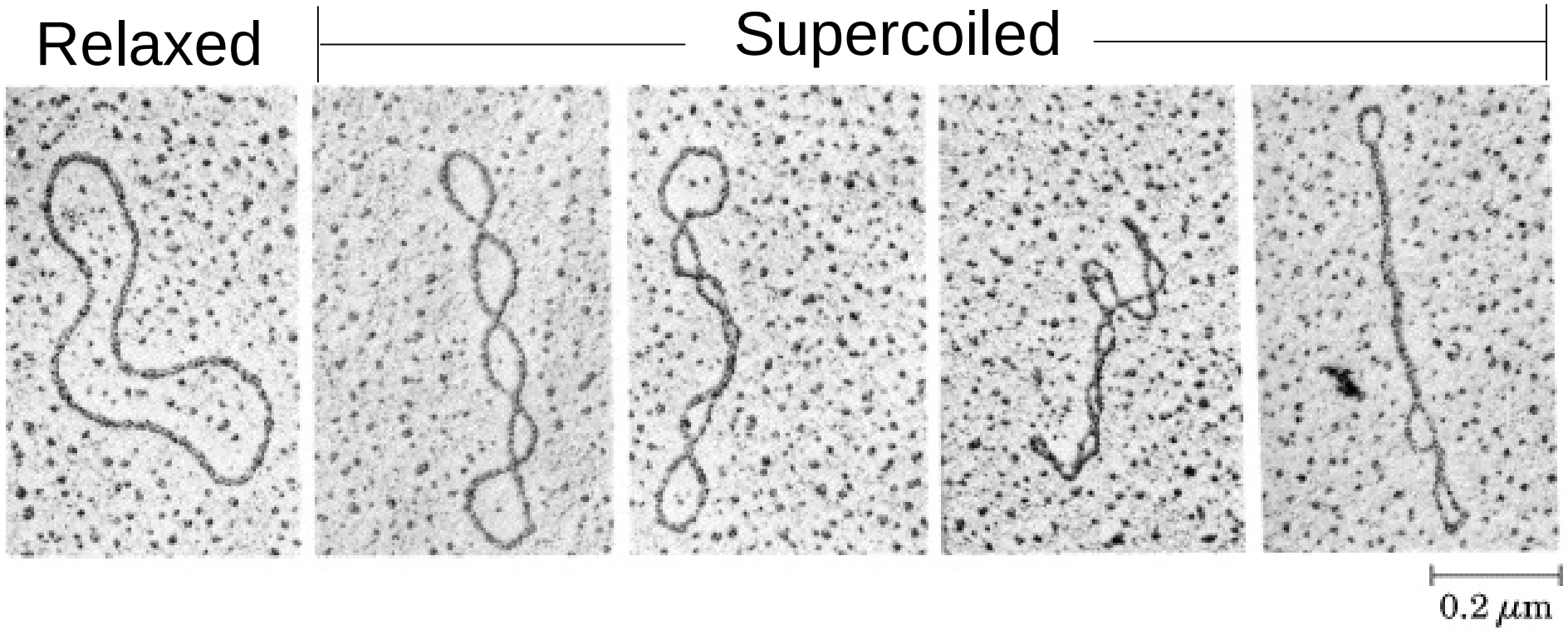
Agarose concentration in gel (% [W/V])	Range of separation of linear DNA molecules (kb)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0.2-3
2.0	0.1-2

資料取材自 Sambrook, J., Russell, D. W. (2001) Molecular Cloning: A Laboratory Manual. 3rd ed., Cold Spring Harbor Laboratory Press. Table 5.5

影響 DNA 泳動的主要因素：

- Agarose 濃度
- DNA 構形與大小
- 電壓
- 電泳緩衝液
- Intercalating dye 存在與否

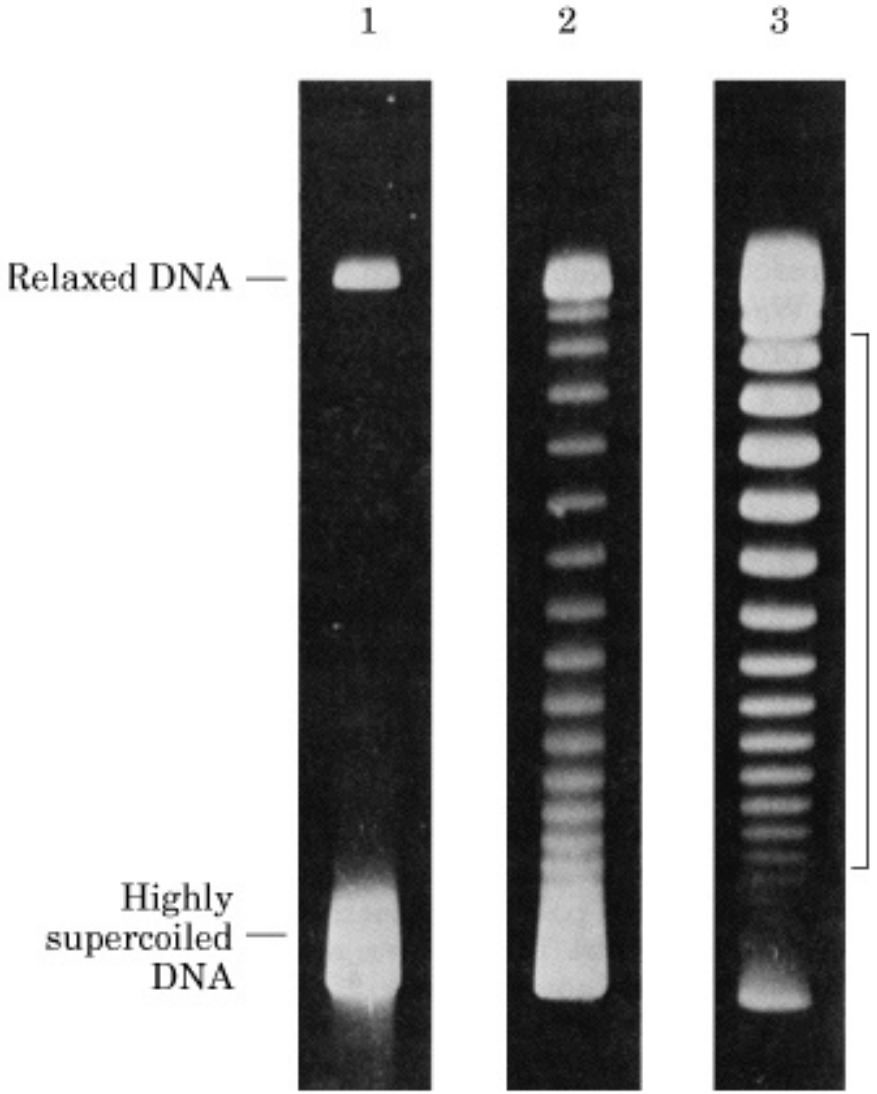
環狀質體的不同構形



Closed circular plasmid DNA

圖取材自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 24-12

不同構形具不同泳動率



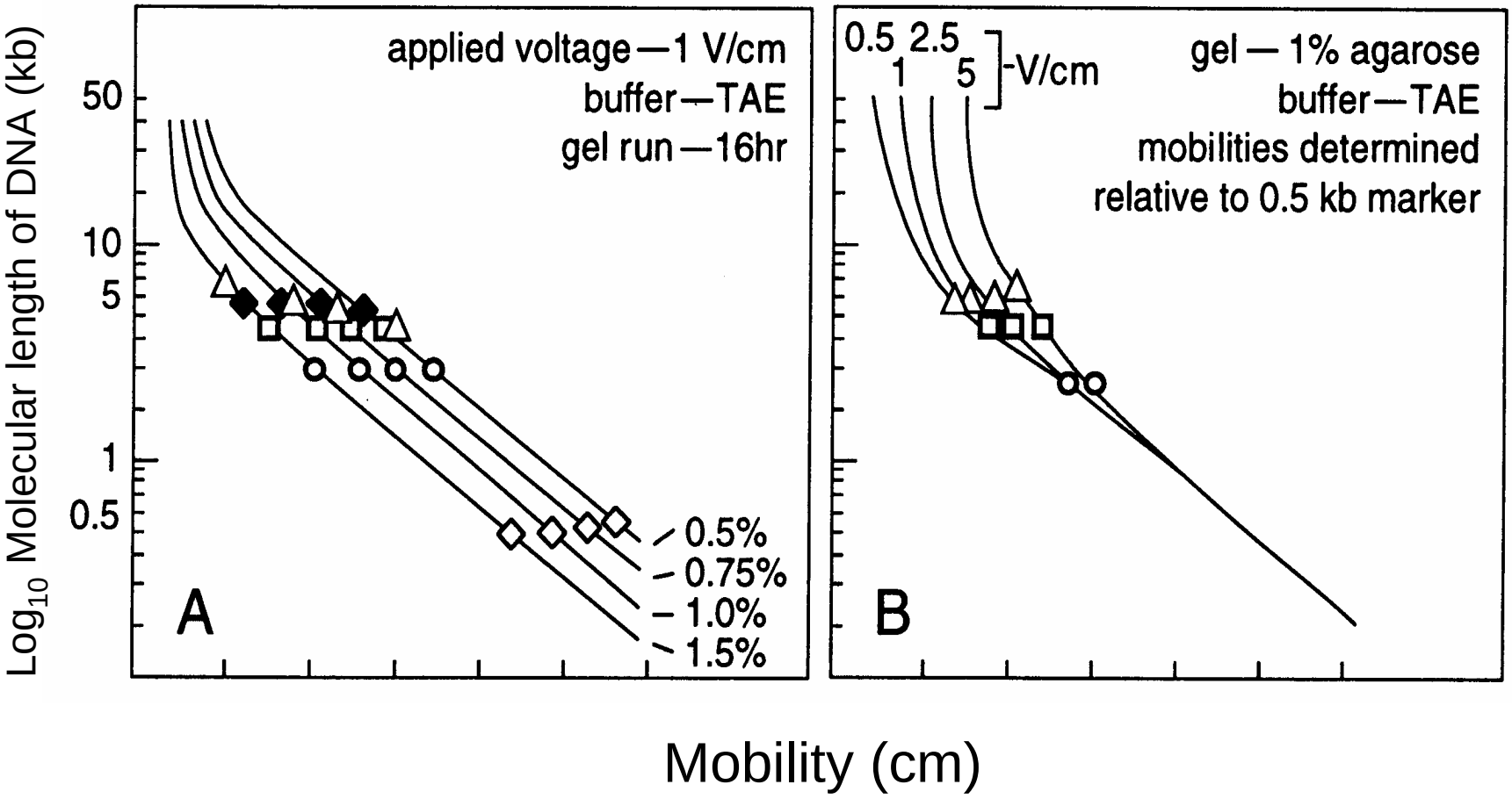
* Lane 2 & 3:
  DNA treated with a type I topoisomerase

圖取材自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 24-19

影響 DNA 泳動的主要因素：

- Agarose 濃度
- DNA 構形與大小
- 電壓
- 電泳緩衝液
- Intercalating dye 存在與否

膠體濃度及電壓的影響



Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) Current protocols in molecular Biology. Fig. 2.5A.2.

常用定量 DNA 的方法：測定 260 nm 吸光值

Beer-Lambert Law: $A = \varepsilon bC$

ε_{260nm} of ss DNA = 0.027 $(\mu g/ml)^{-1}cm^{-1}$

ε_{260nm} of ds DNA = 0.020 $(\mu g/ml)^{-1}cm^{-1}$

ε_{260nm} of ss RNA = 0.025 $(\mu g/ml)^{-1}cm^{-1}$

註：此處之 ε_{260nm} 為平均值，且不適用於 oligonucleotides

常用定量 DNA 的方法： Hoechst 33258 定量法

Hoechst 33258: bis-benzimide

- a non-intercalating dye
- binding to the minor grooves of four consectutive AT bps in DNA

H33258-DNA

excited with 365 nm light

An increase in emission 458 nm

Hoechst 33258 定量法不受 RNA 及蛋白質干擾

Fluorescence of DNA, RNA and protein in the Hoechst 33258 DNA assay:

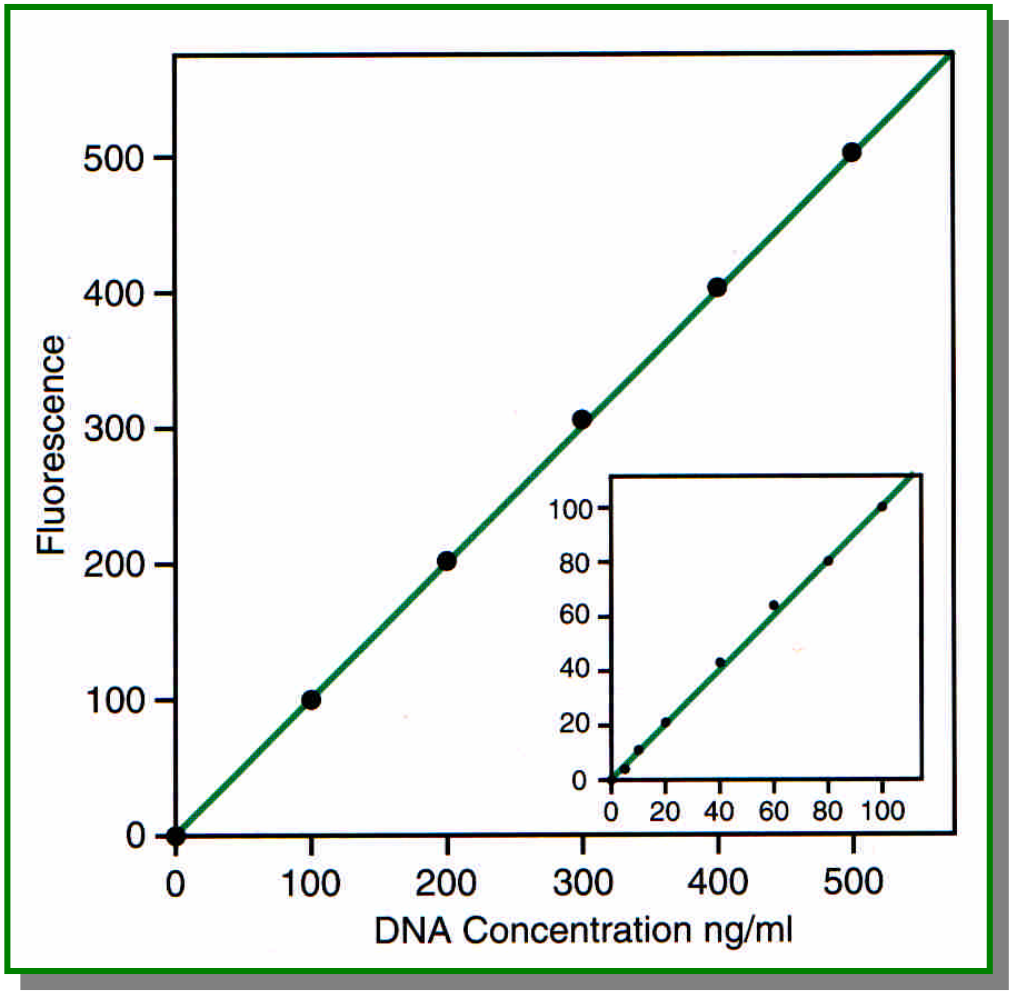
Solution	Actual concentration (μg/mL)	Fluorescence units	Apparent concentration (μg/mL)
DNA (Calf thymus DNA)	1.0	1000	1.000
RNA (tRNA)	1.0	41	0.041
Protein (BSA)	10.0	0	0.000

資料取材自 Nucleic acid purification guide. Pharmacia Biotech.

由標準曲線估計樣品濃度

Standard curve：

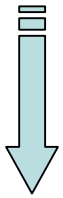
- DNA:
  calf thymus DNA
- Assay buffer:
  0.1 μg/ml H33258 in TNE buffer, pH7.4



Nucleic acid purification guide.
Pharmacia Biotech.

常用定量 DNA 的方法： Ethidium bromide 法

DNA-EtBr



excited with 302 or 546 nm light

An increase in emission at 590 nm

- Measurement of the fluorescene intensity of DNA-EtBr solutions
- Estimation of the fluorescence intensity of EtBr-stained DNA bands or spots

常用定量 DNA 的方法：靈敏度比較

Table A.3D.3 Properties of Absorbance and Fluorescence Spectrophotometric Assays for DNA and RNA

Property	Absorbance (A_{260})	Fluorescence	
		H33258	EtBr
Sensitivity (μg/ml)			
DNA	1-50	0.01-15	0.1-10
RNA	1-40	n.a.	0.2-10
Ratio of signal (DNA/RNA)	0.8	400	2.2

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1994) Current protocols in molecular Biology. John Wiley & Sons, Inc. Table A.3D.3.