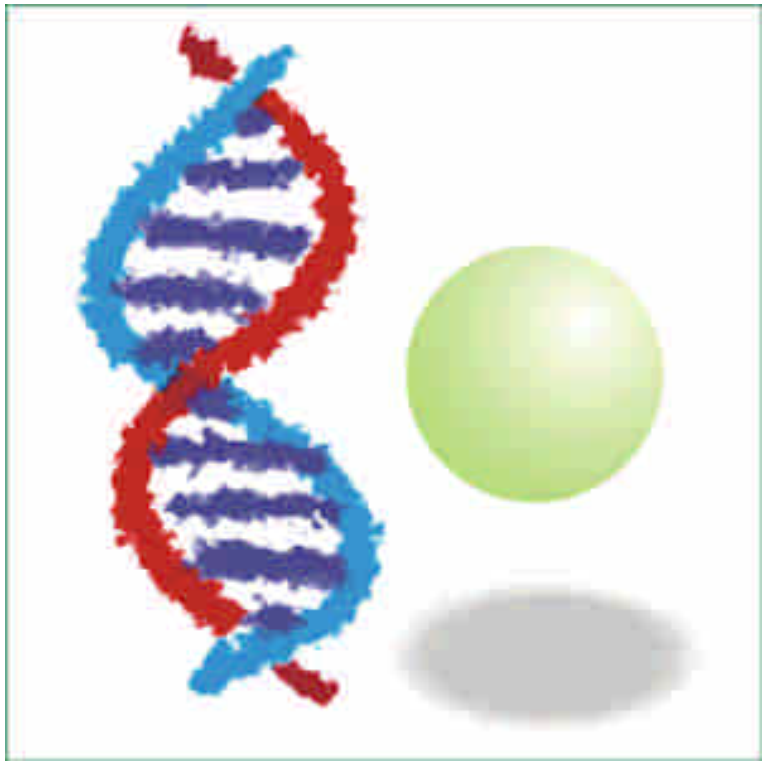
BST

生化科技系

BCX

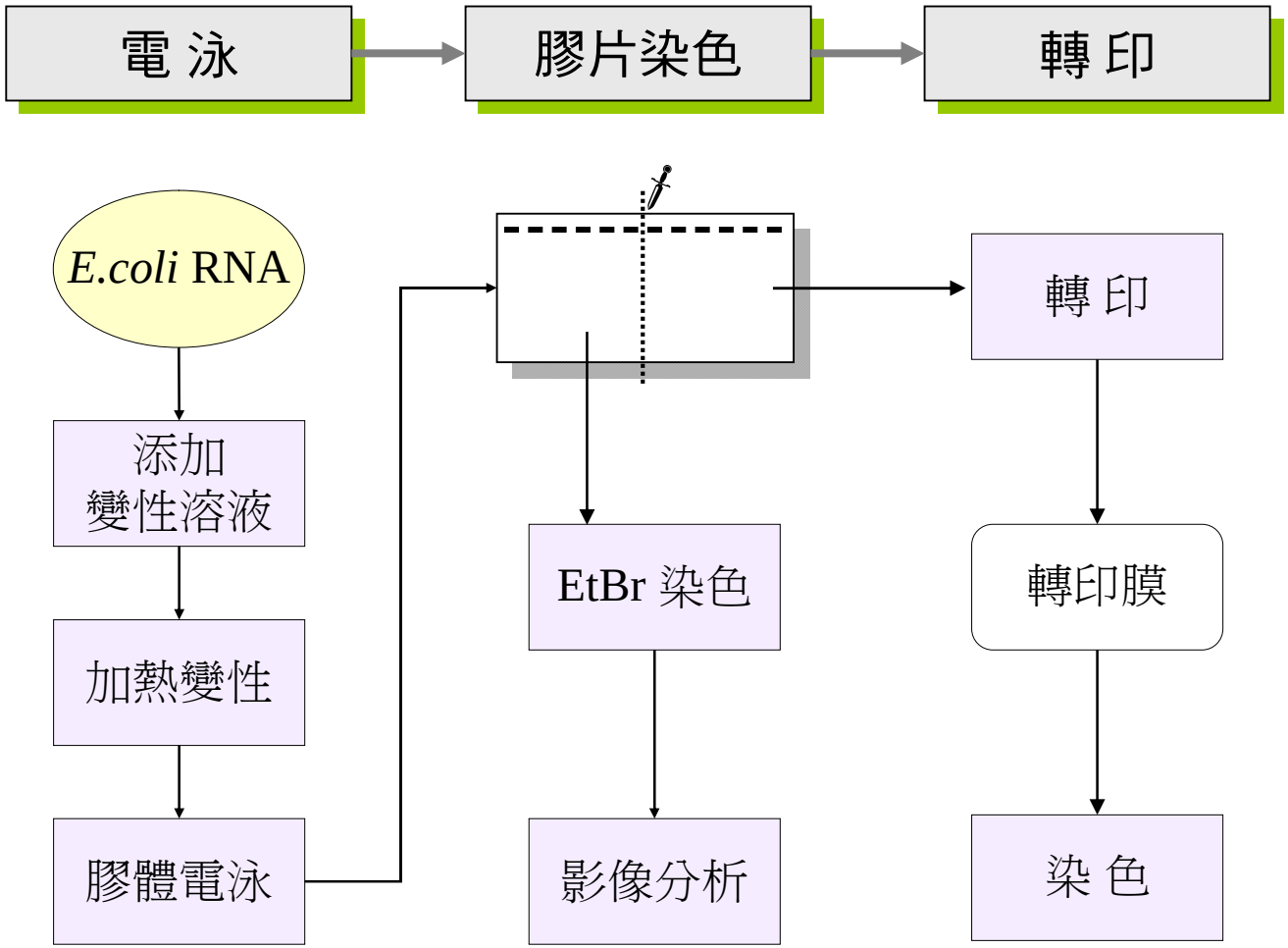


N4

生物化學實驗

RNA 電泳與 Northern 轉印

N4 RNA 電泳與 Northern 轉印



先加入 RNA 變性溶液後再上課：

4.2.3 操作步驟：

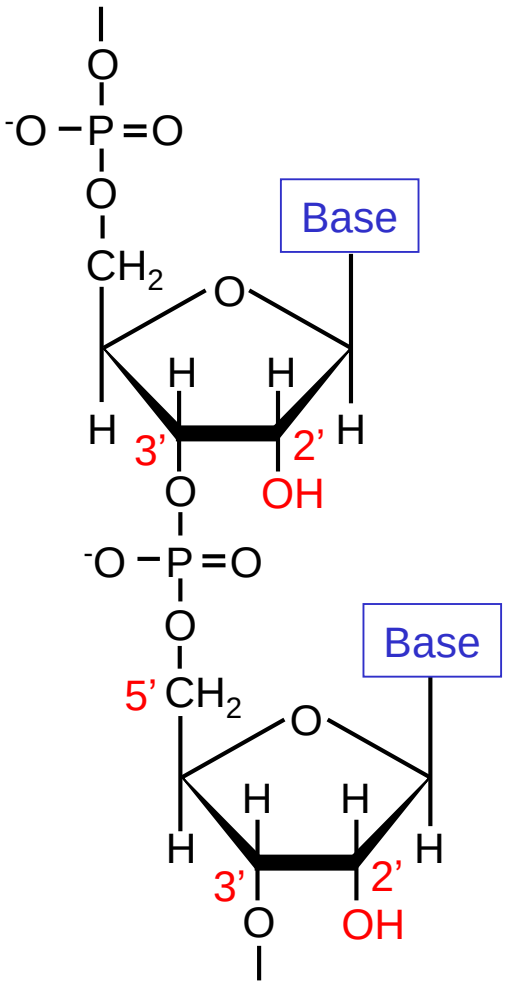
準備RNA 樣品：

5) 每一組有兩管 RNA 樣品，分別標示為 *E. coli* RNA 與 Rice RNA，其內各含 4 μL RNA (RNA 含量為 30 μg)。

6) 每管加入 20 μL RNA 變性溶液，以 tip 吸放溶液使混合均勻。

7) 置 55℃ 反應 1 小時（等待期間，請至 B01 教室上課）

RNA 實驗要點：



(1) 避免被 RNase 作用

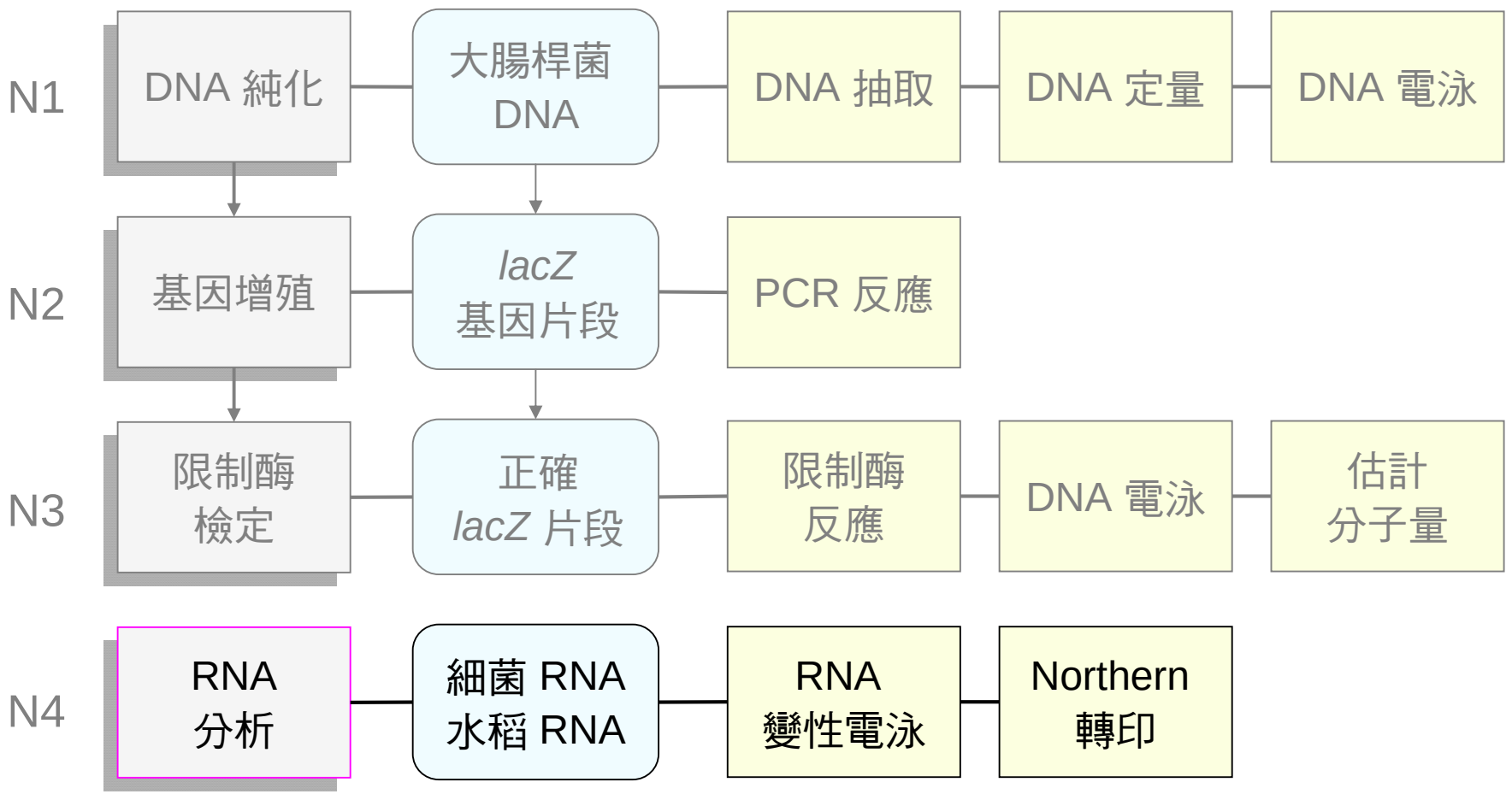
(2) 避免污染

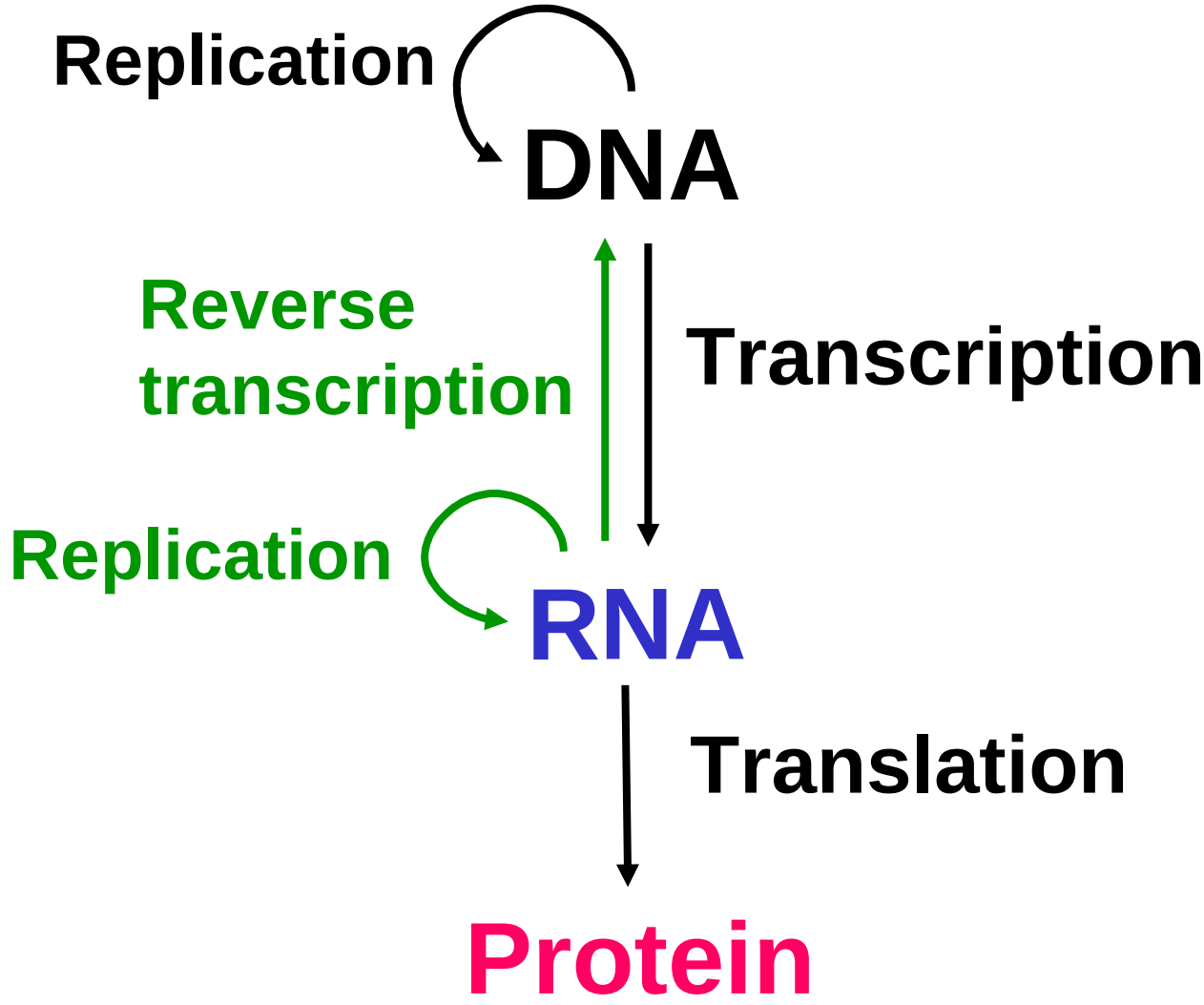
(3) 保持清潔

避免 RNase 作用

- 操作區域保持清潔，維持良好操作習慣。
- 戴手套操作。
- 準備一套 RNA 專用試劑及用具。
- 容器需先經高溫滅菌。
- 水及各種溶液需以 diethylpyrocarbonate (DEPC) 處理後再滅菌。
- 不能用 DEPC 處理的試劑，需以滅過菌的容器及 DEPC 處理過的水配製。
- 在反應中加入RNase 抑制劑。

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


Replication
DNA
Reverse
transcription
Transcription
Replication
RNA
Translation
Protein

Northern hybridization 常用於分析基因表現：

General procedures for northern hybridization --

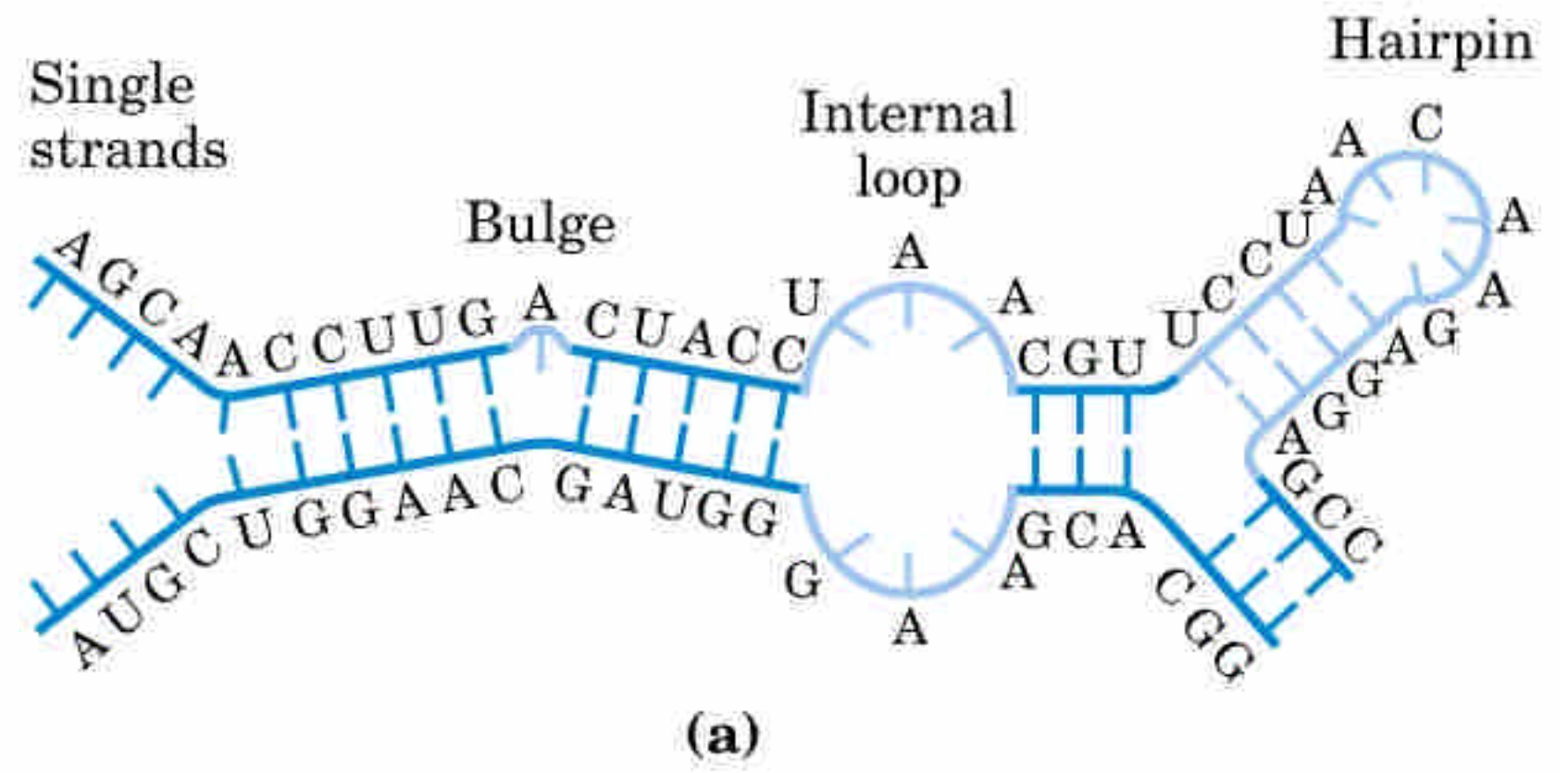
Gel electrophoresis — Separation of RNA according to size

↓

Blotting — Transfer and fixation of denatured RNA to membranes

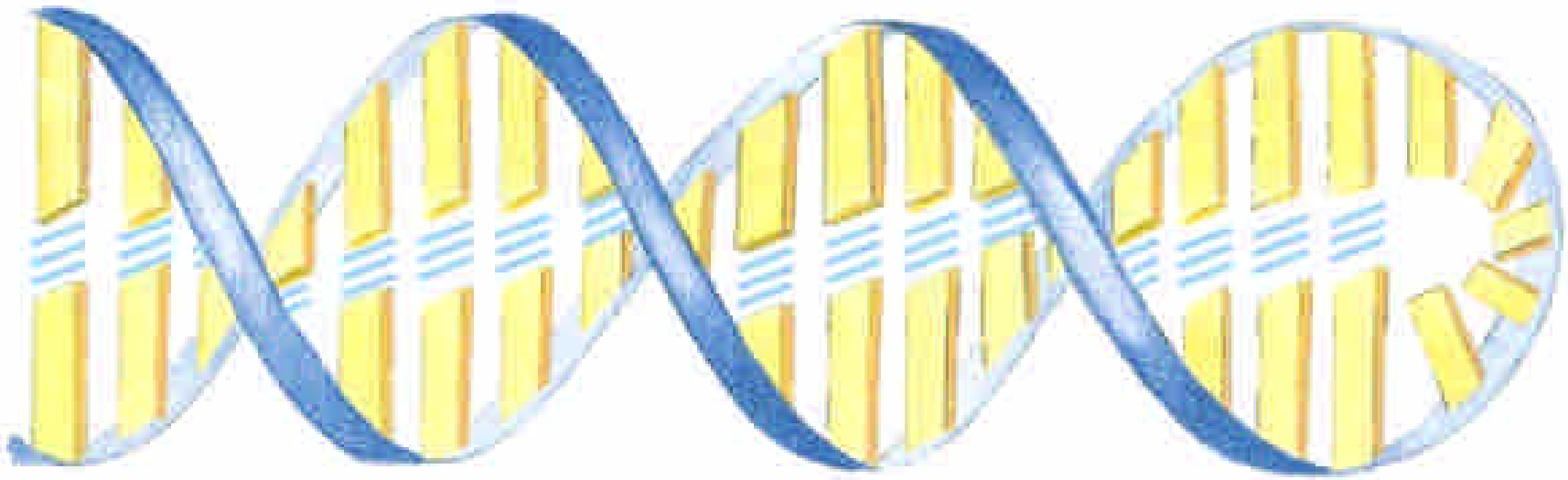
↓

Hybridization — Detection of target RNA by DNA or RNA probes



Secondary structure of RNA

圖取材自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 10-26 (a)

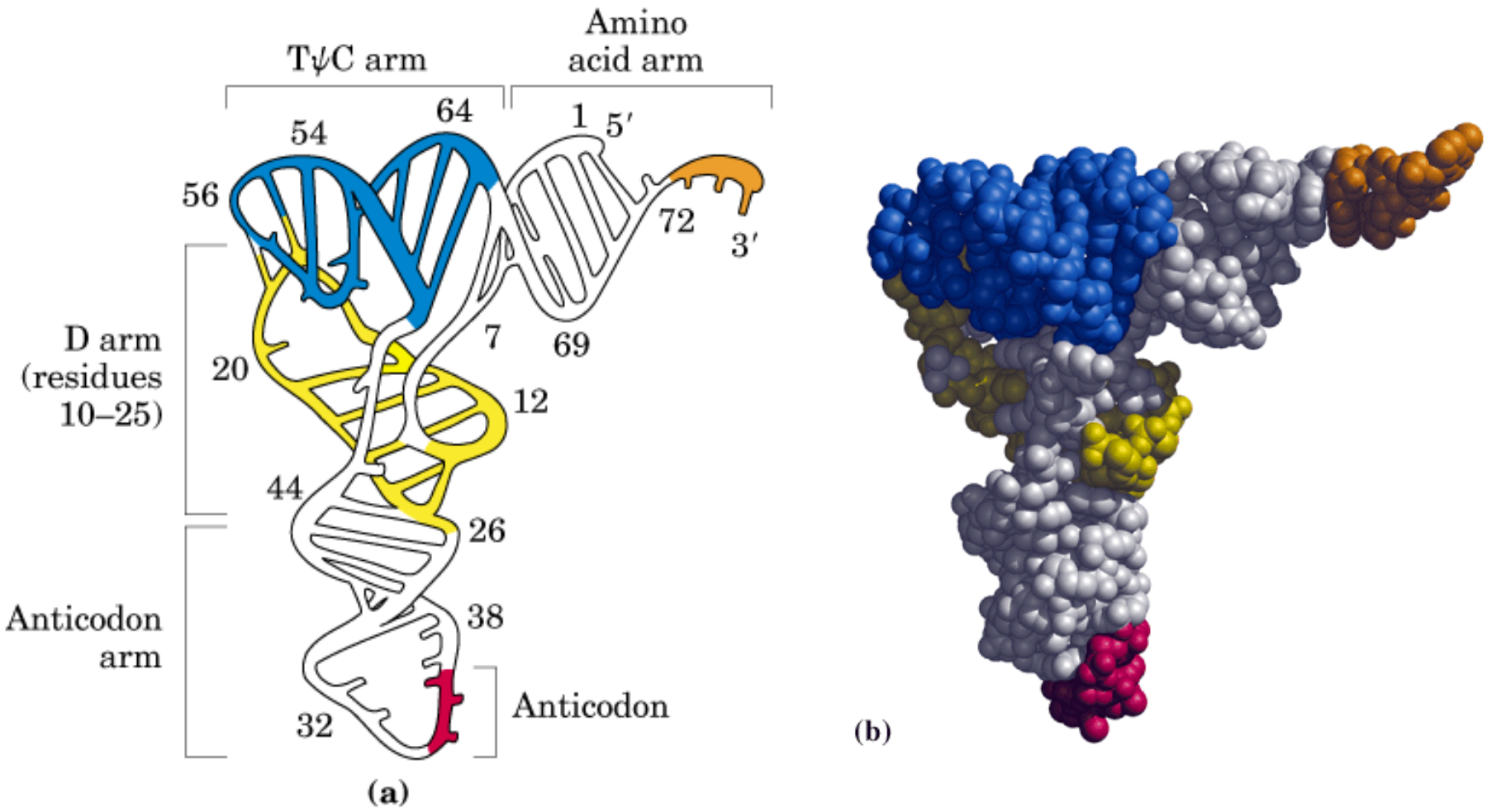


Hairpin double helix
(b)

Secondary structure of RNA

圖取材自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 10-26 (b)

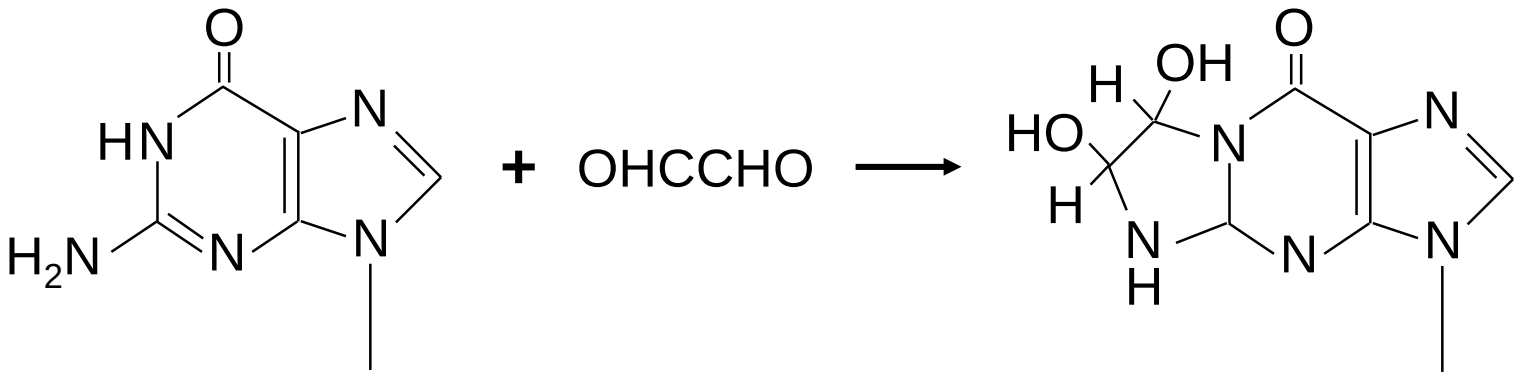
L-shape tertiary structure of yeast $tRNA^{Phe}$:



圖引用自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd Ed., Worth Publishers. Fig. 27-15

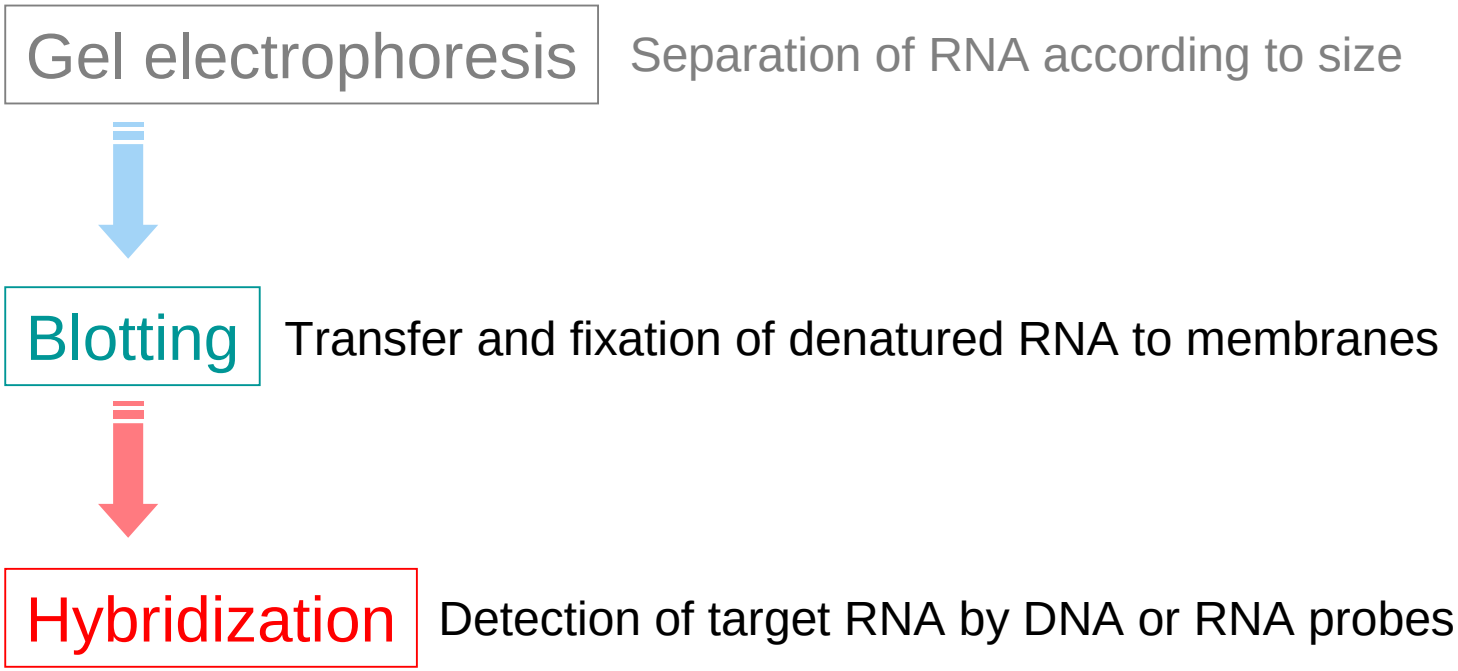
RNA 電泳常用的變性劑：

- Formaldehyde: HCHO
- Glyoxal (diformyl): OHCCHO

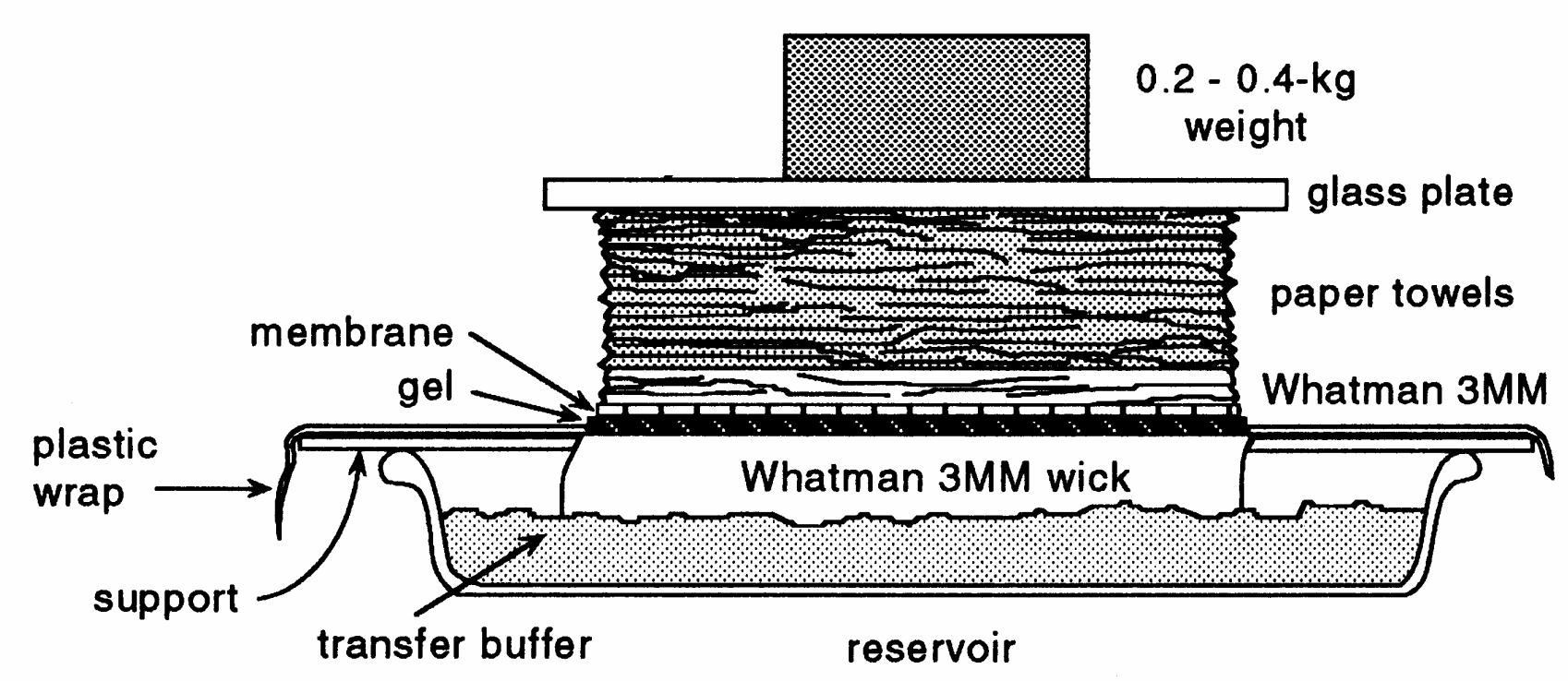


Northern hybridization 常用於分析基因表現：

General procedures for northern hybridization --



轉移膠體上的核酸至轉印膜上：



圖引用自：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. 2.9.1.

Table 2.9.1 Properties of Materials used for Immobilization of Nucleic Acids[a]

	Nitrocellulose	Supported nitrocellulose	Uncharged nylon	Positively charged nylon	Activated papers
Application	ssDNA, RNA, protein	ssDNA, RNA, protein	ssDNA, dsDNA, DNA, protein	ssDNA, dsDNA, RNA, protein	ssDNA, RNA
Binding capacity (μg nucleic acid/cm^2)	80-100	80-100	400-600	400-600	2-40
Tensile strength	Poor	Good	Good	Good	Good
Mode of nucleic acid attachment[b]	Noncovalent	Noncovalent	Covalent	Covalent	Covalent
Lower size limit for efficient nucleic acid retention	500 nt	500 nt	50 nt or bp	50 nt or bp	5 nt
Suitability for reprobing	Poor (fragile)	Poor (loss of signal)	Good	Good	Good
Commercial examples	Schleicher & Schuell BA83, BA85; Amersham Hybond-C; PALL Biodyne A	Schleicher & Schuell BA-S; Amersham Hybond-C extra	Amersham Hybond-N; Stratagene Duralon-UV; Du Pont NEN GeneScreen	Schleicher & Schuell Nytran; Amersham Hybond-N^+; Bio-Rad ZetaProbe; PALL Biodyne B; Du Pont NEN GeneScreen Plus	Schleicher & Schuell APT papers

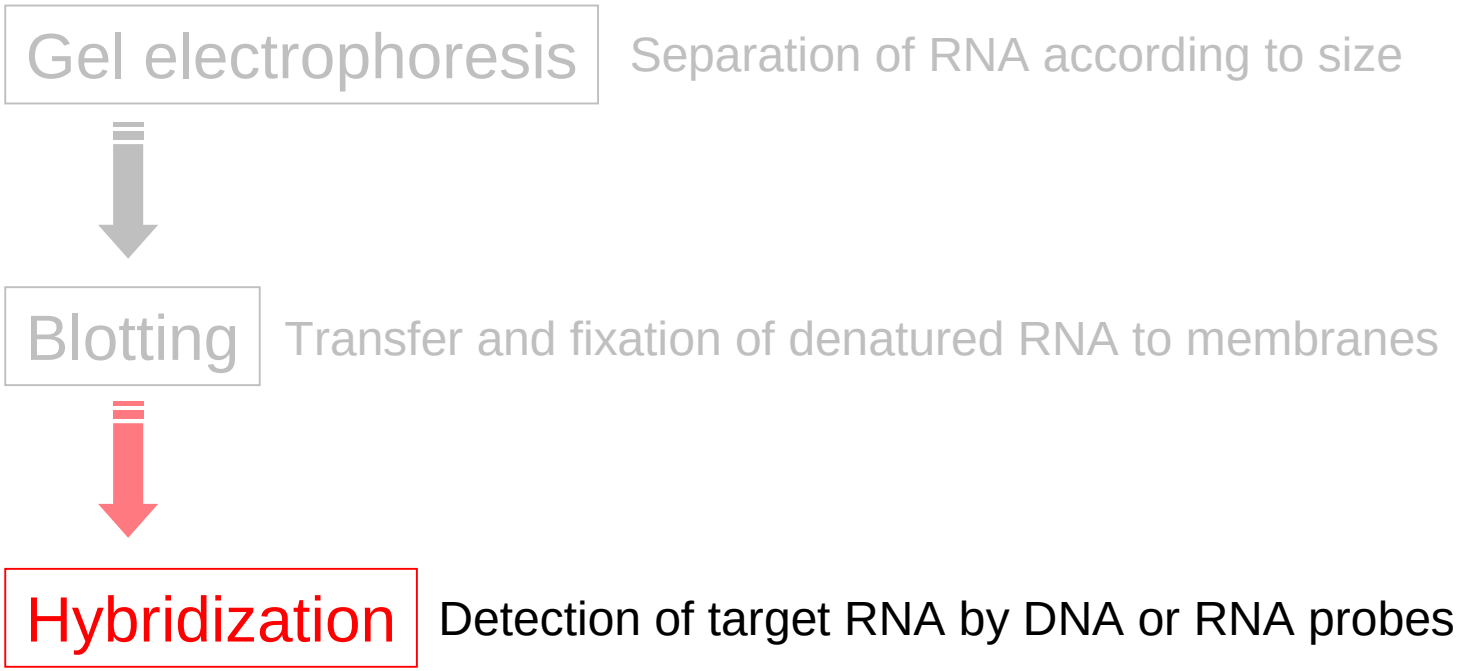
[a]This table is based on Brown (1991), with permission from BIOS Scientific Publishers Ltd.

[b]After suitable immobilization procedure (see text).

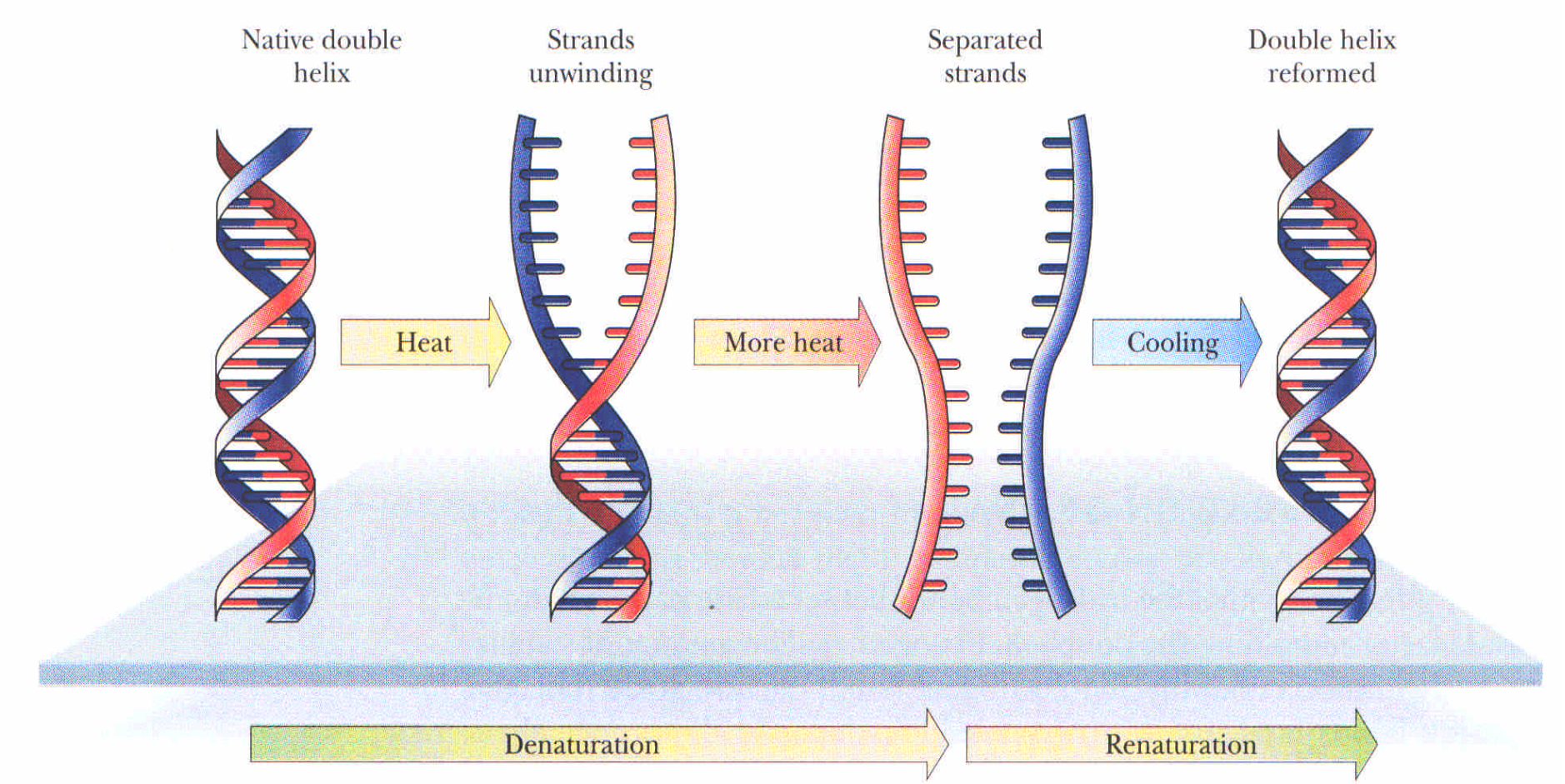
Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. and Struhl, K. (1987) Current protocols in molecular Biology. Table 2.9.1

Northern hybridization 常用於分析基因表現：

General procedures for Northern hybridization --

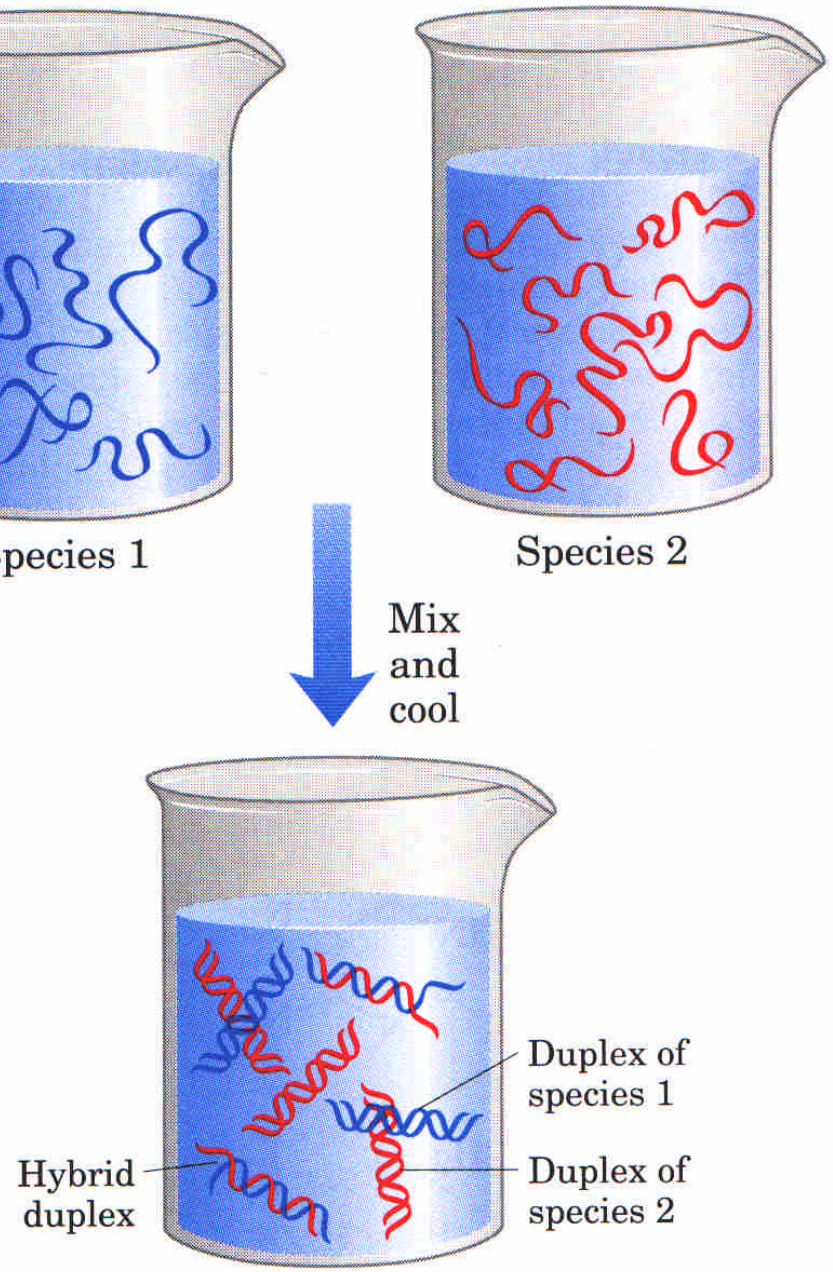


核酸的物理性質：Denaturation & Renaturation



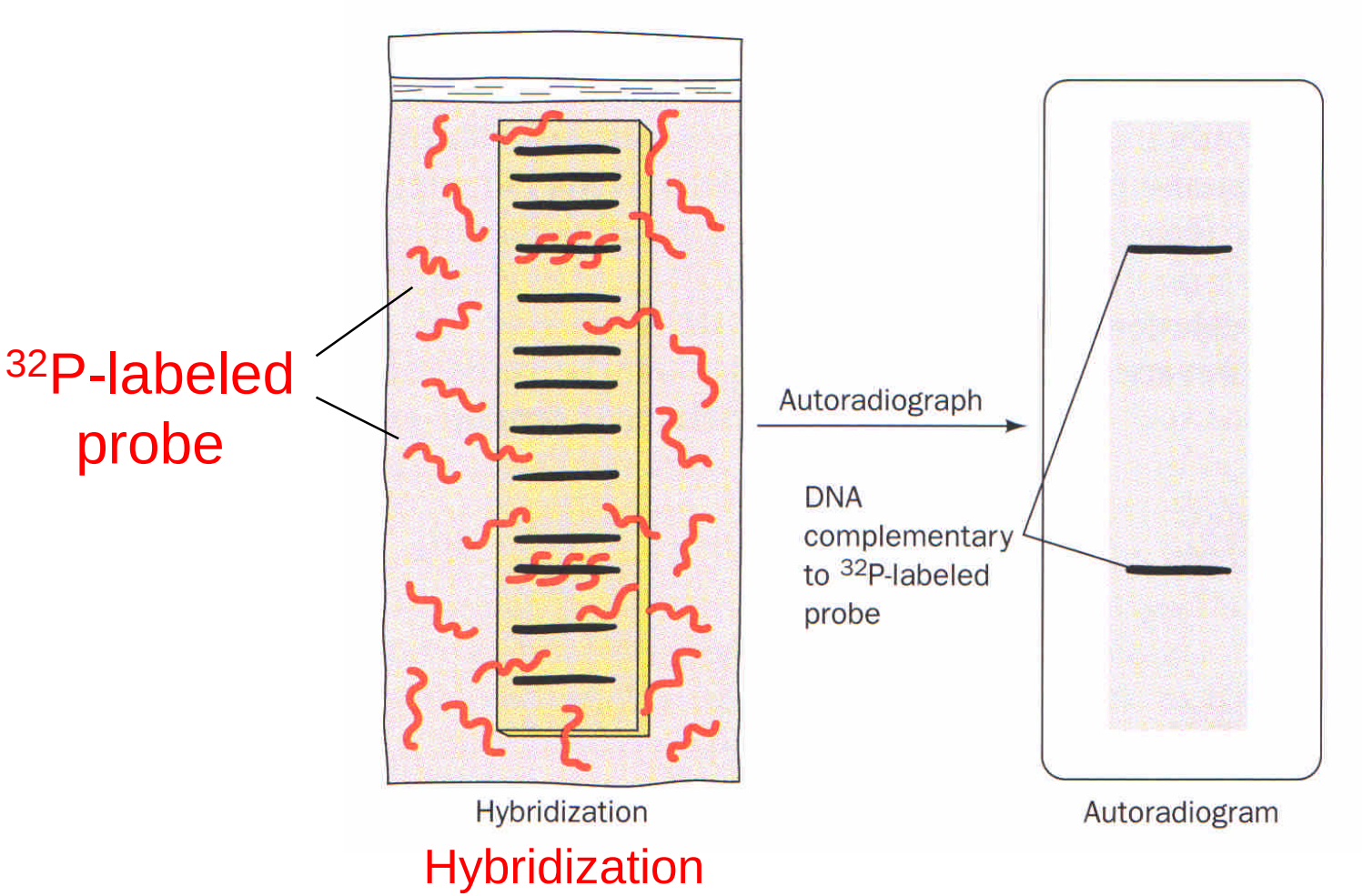
Campbell, M. K. (1999) Biochemistry. 3rd. Harcourt Brace College Publishers. Fig. 7.14.

序列互補的核酸可互相雜合：



圖引用自：Lehninger, A. L., Nelson, D. L. and Cox, M. M. (1993) Biochemistry. 2nd Ed. Worth Publishers, Inc. Fig.12-31

以探針進行雜合反應可偵測到轉印膜上的目標基因：

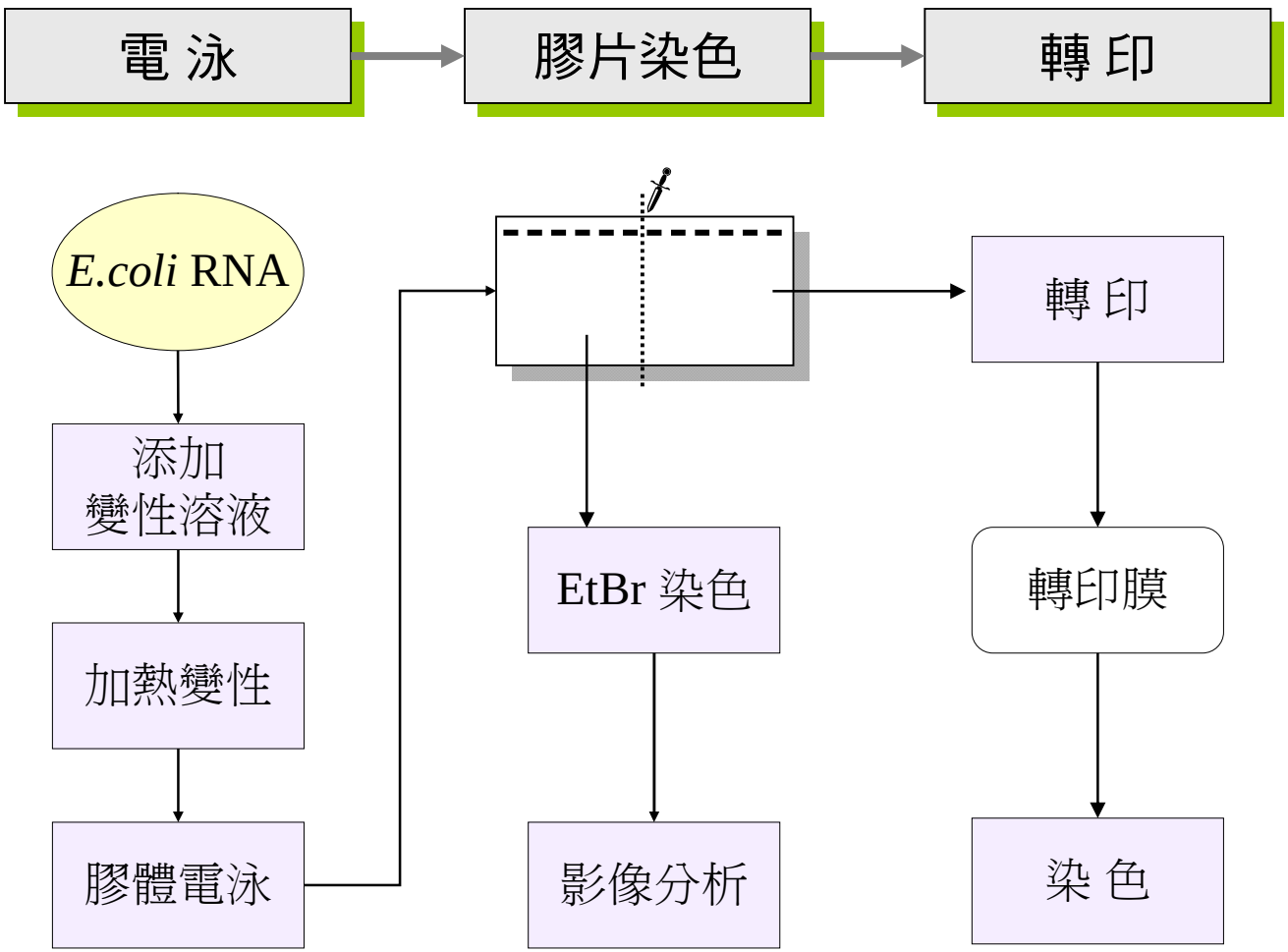


圖引用自：Voet, D., Voet, J. G. and Pratt, C. W. (1999) Fundamentals of Biochemistry. John Wiley & Sons, Inc. Fig. 23-28.

各種轉印分析方法 ：

方法	轉印膜上的分子	偵測工具
Southern	DNA	DNA 或 RNA
northern	RNA	DNA 或 RNA
western	蛋白質	抗體
southwestern	蛋白質	DNA

N4 RNA 電泳與 Northern 轉印



本週實驗進行要點：


RNA 變性
電　泳
轉　印
影像分析
第二天
染　色
• 等待期間請至 B01 上課
• 等待期間請 TAs 示範 membrane 處理及轉印操作
• 各組先處理 membrane
• 雙數組先進行轉印
• 單數組先觀察色帶
• 以 methylene blue 染色
中午 12: 15 到實驗室