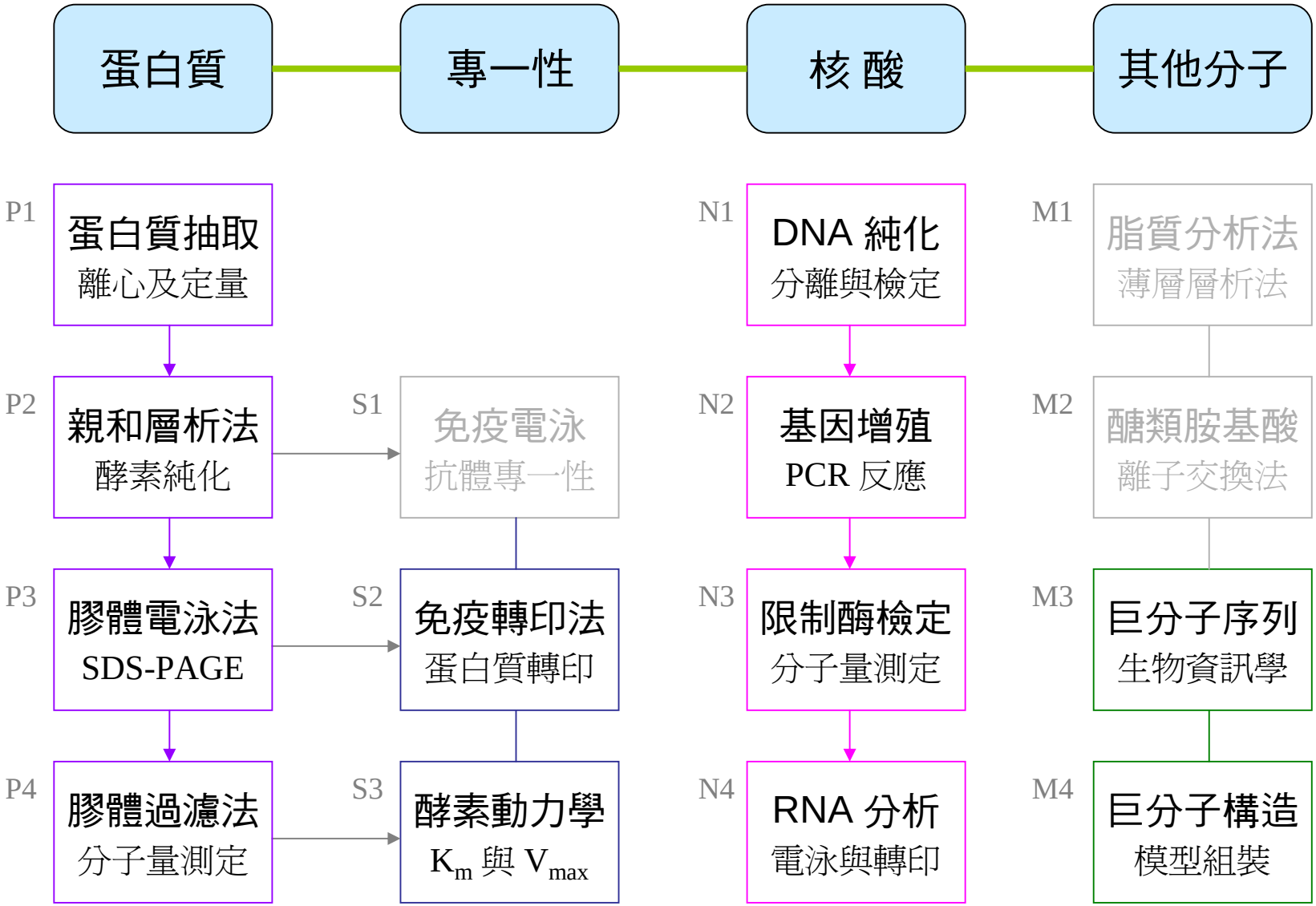
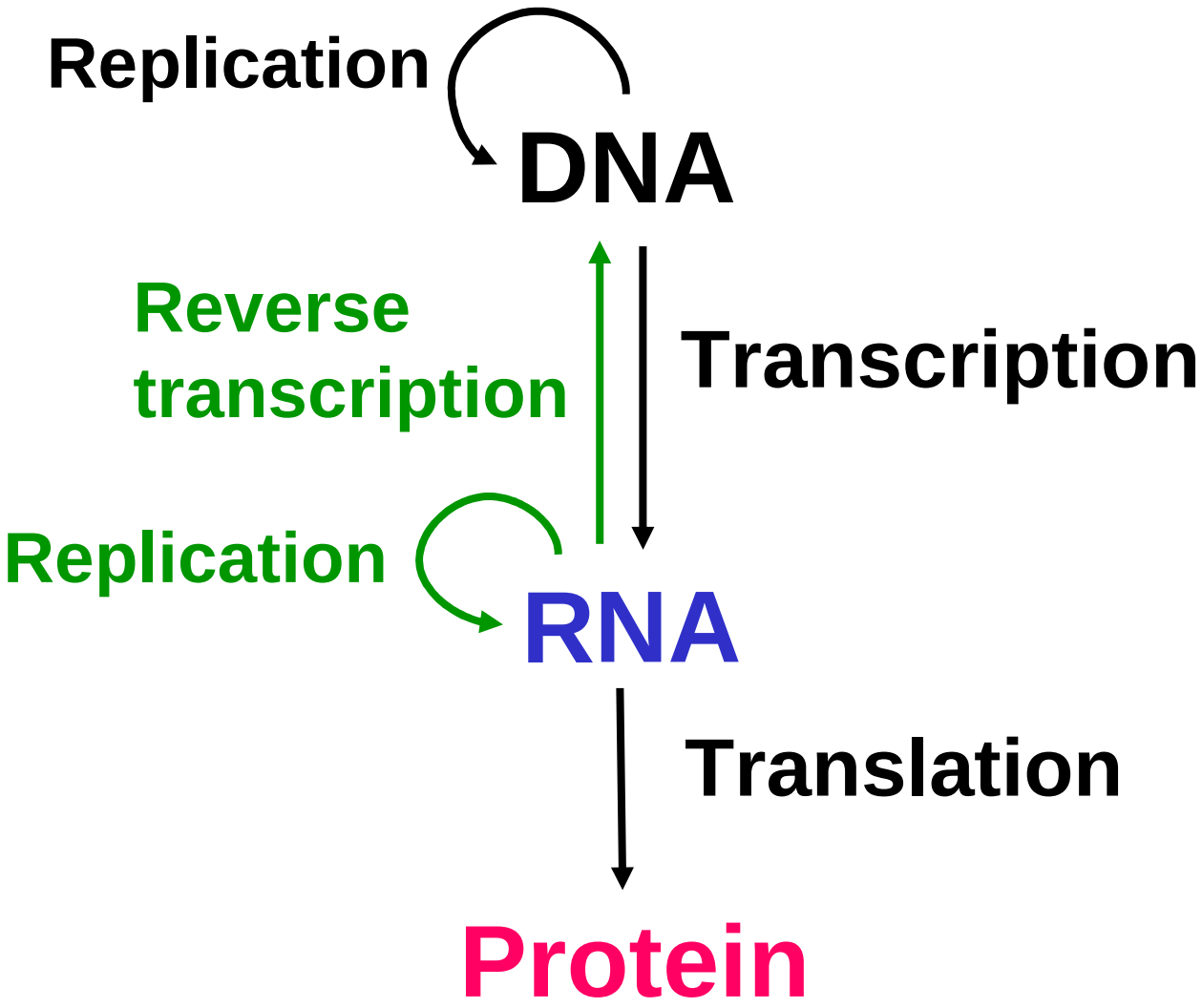
BCX

生化實驗課程單元

工作目標

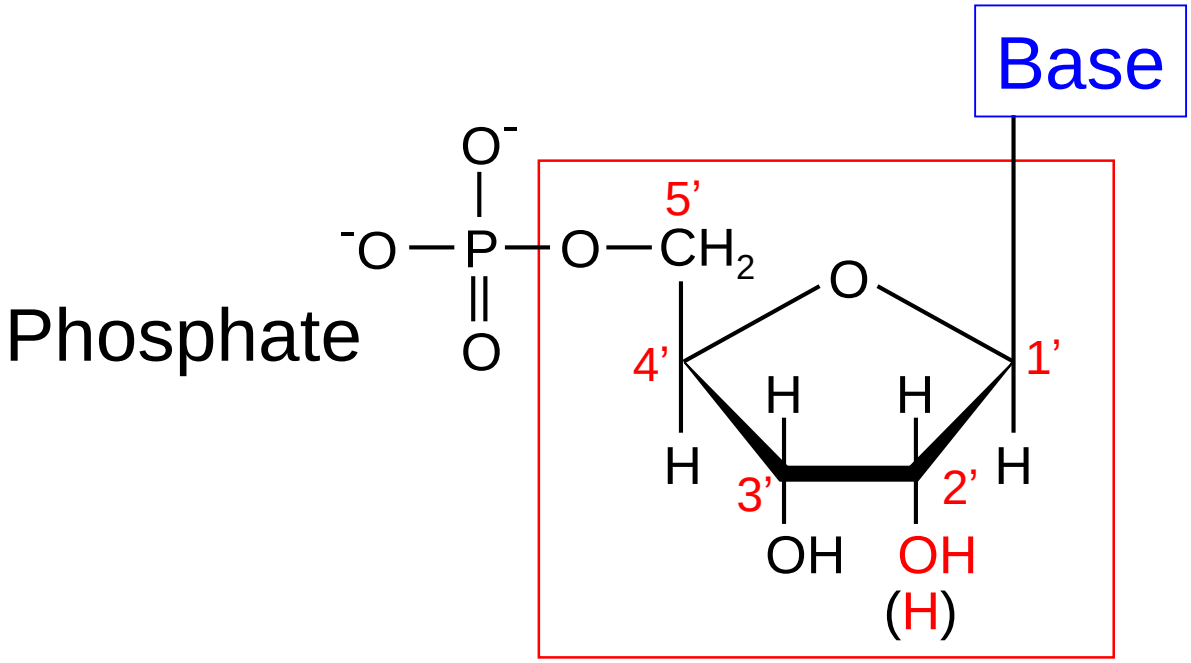
1




Replication
DNA
Reverse
transcription
Transcription
Replication
RNA
Translation
Protein

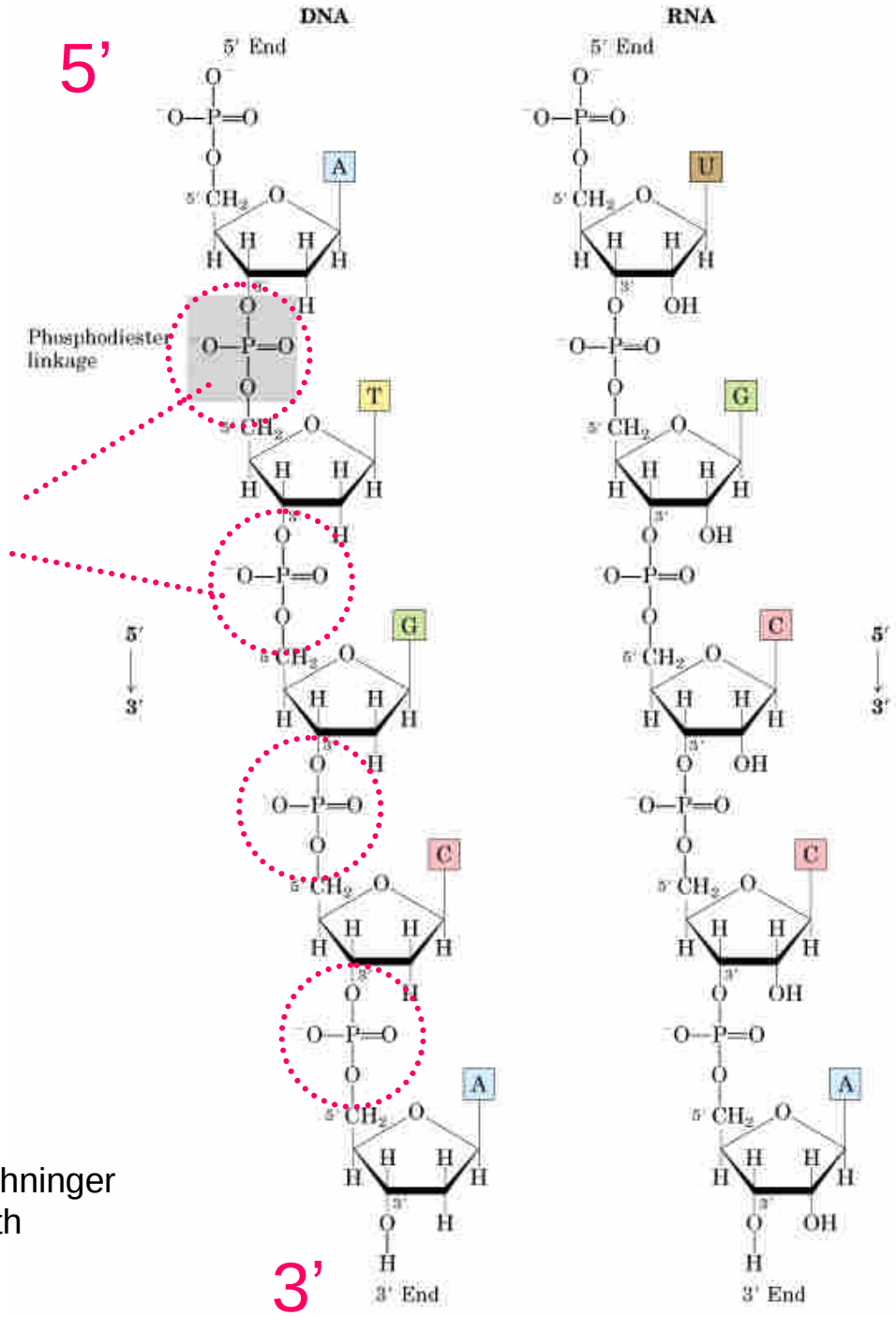
核酸的基本組成：

Nucleotide –



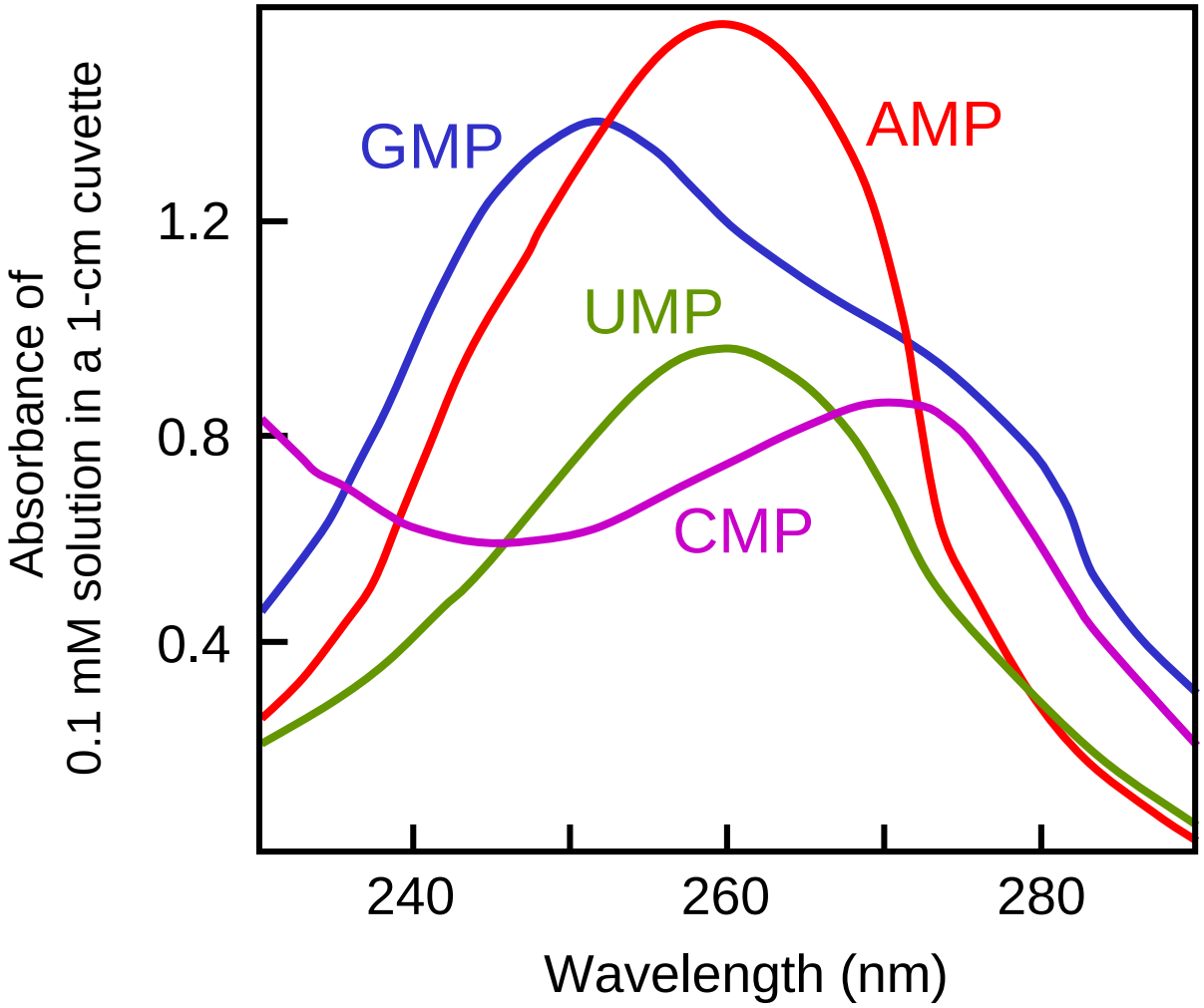
Ribose
(Deoxyribose)

Phosphodiester linkage

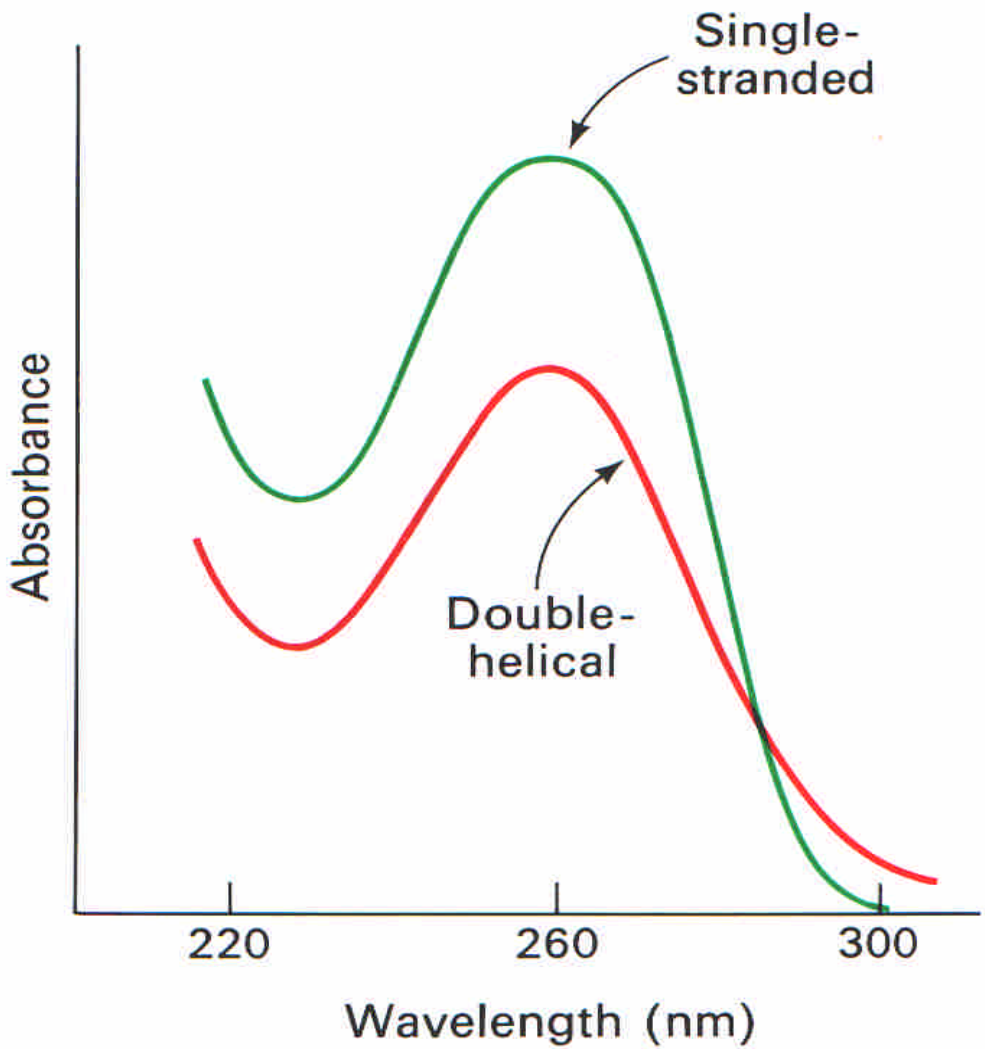


Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd ed., Worth Publishers. Fig. 10-7

核酸的物理性質：UV absorption of nucleotides

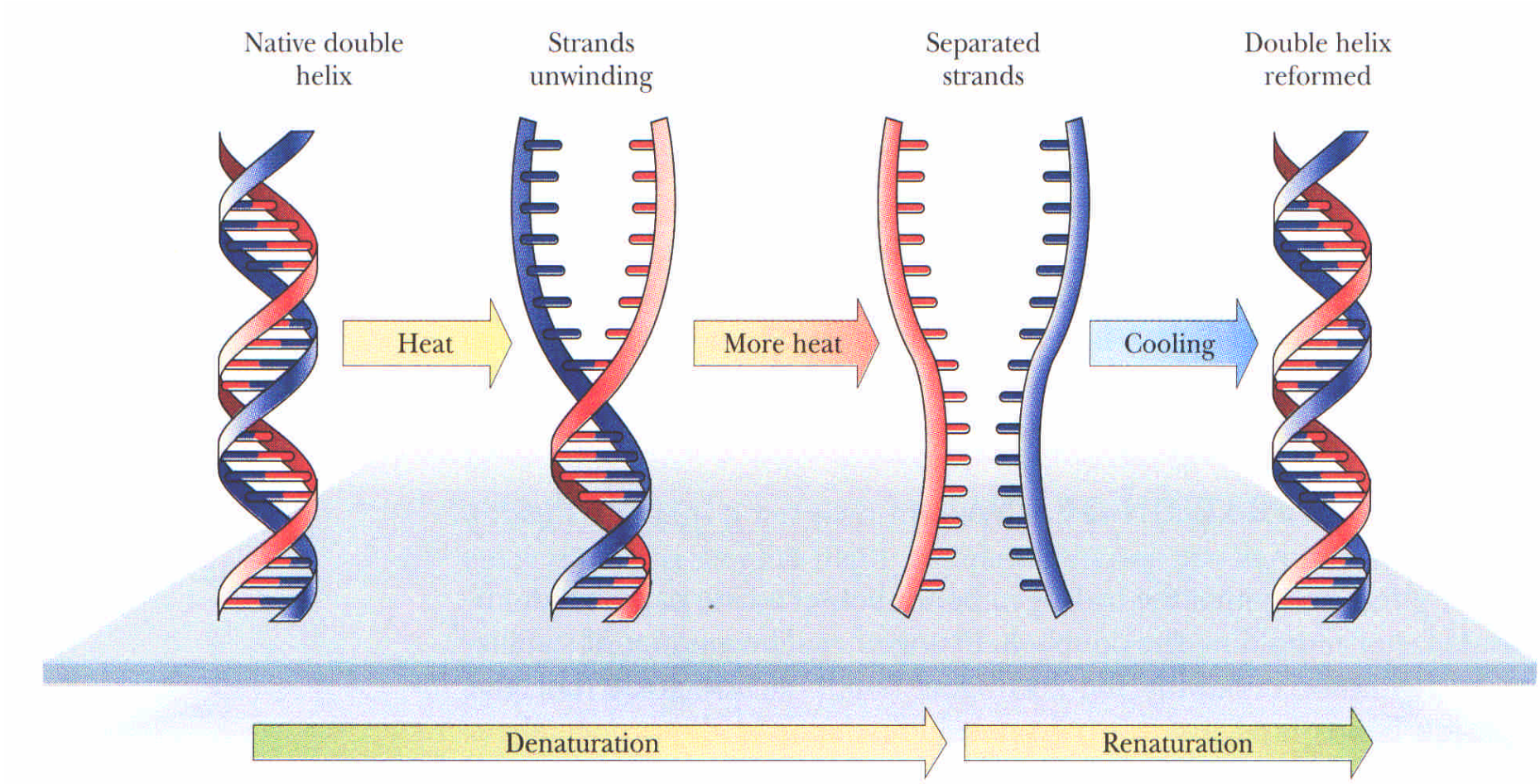


核酸的物理性質：UV absorption of nucleic acids

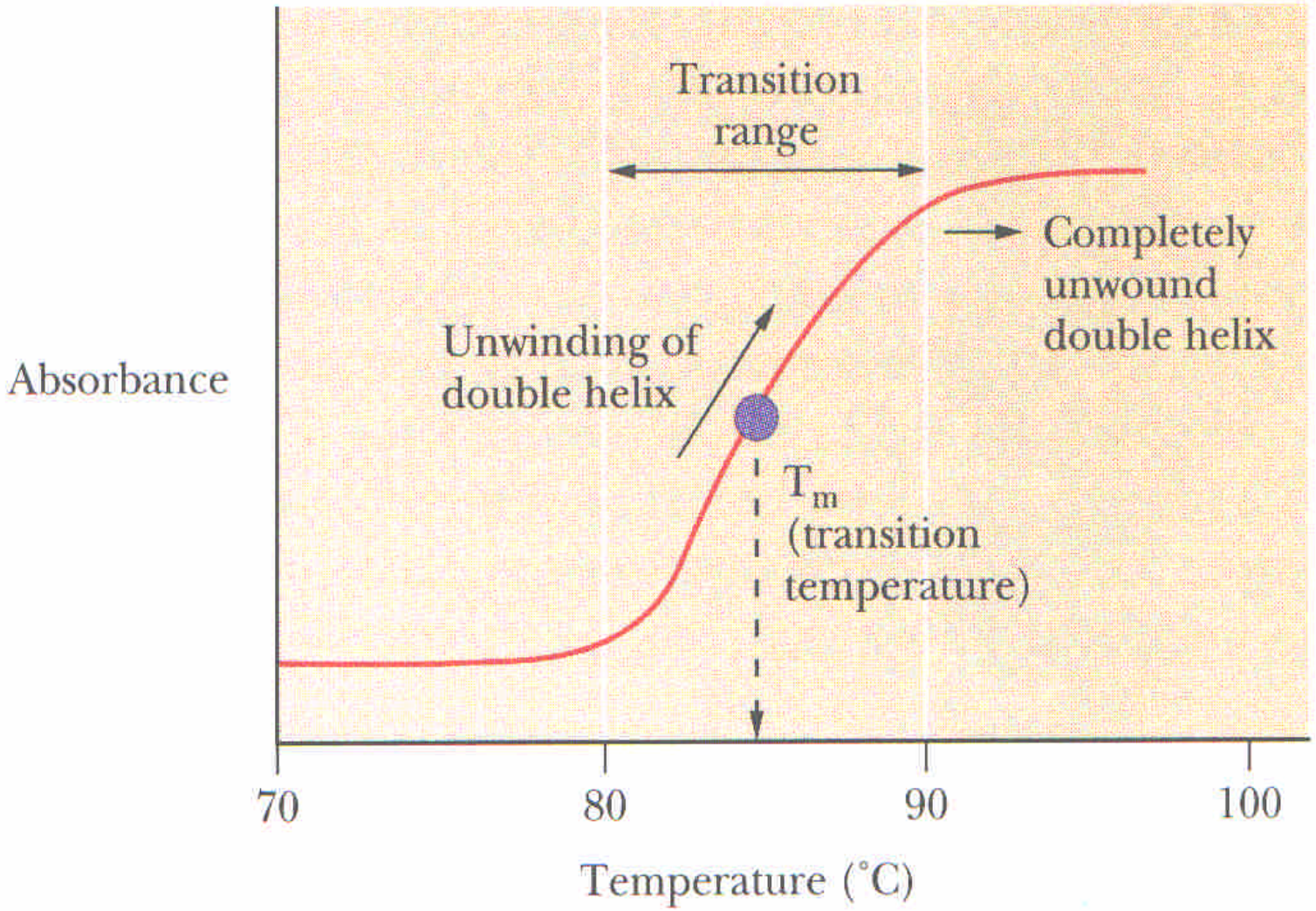


Stryer, L. (1995) Biochemistry. 4th Ed.W. H. Freeman and Company. Fig. 4-16

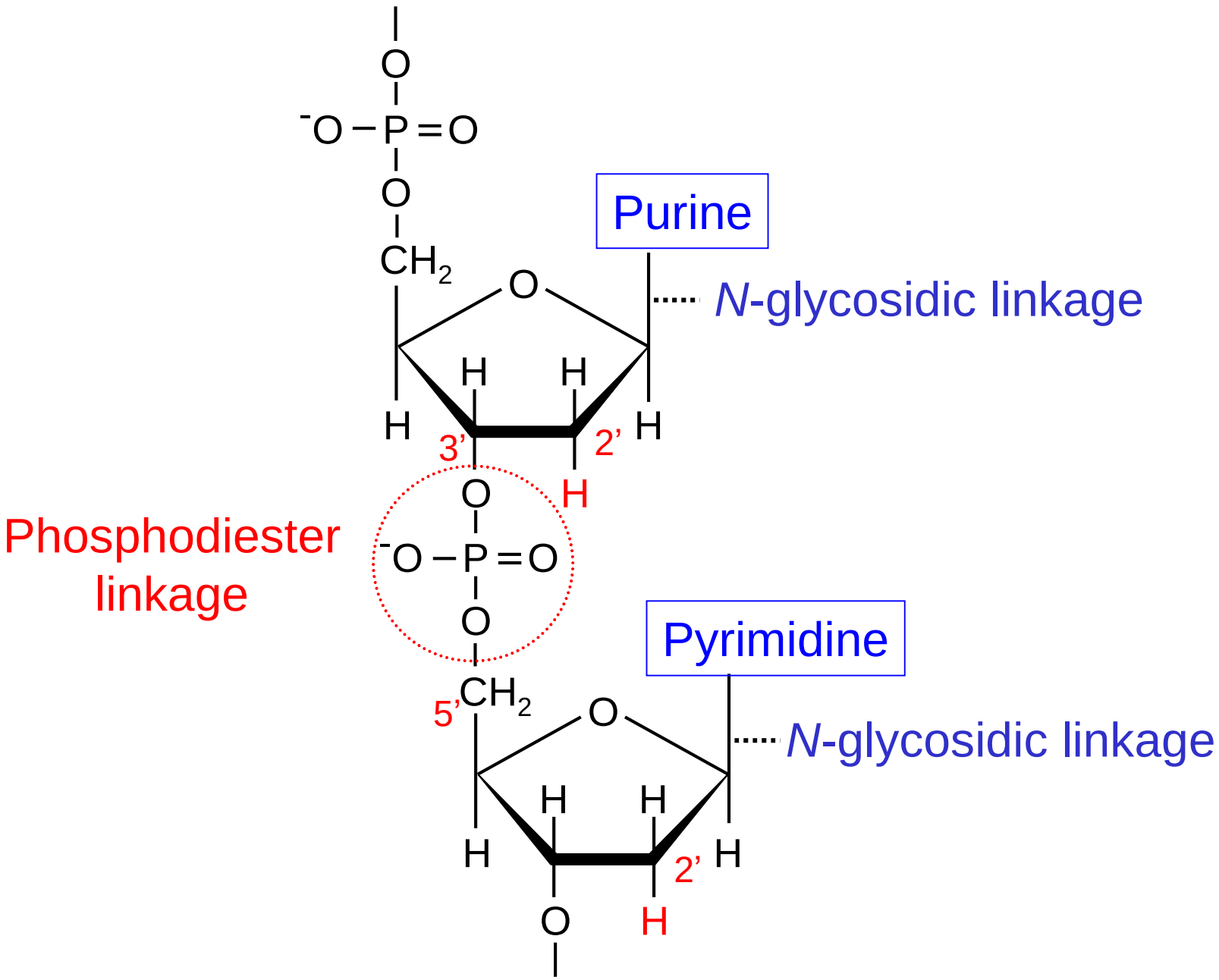
核酸的物理性質：Denaturation & Renaturation



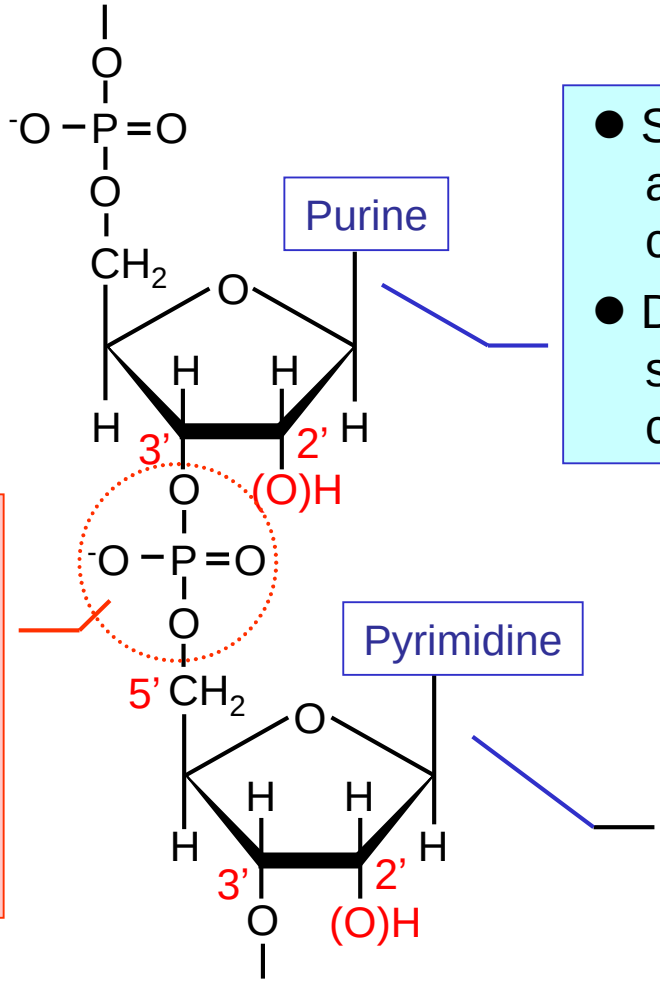
Campbell, M. K. (1999) Biochemistry. 3rd. Harcourt Brace College Publishers. Fig. 7.14.



Campbell, M. K. (1999) Biochemistry. 3rd. Harcourt Brace College Publishers. Fig. 7.13.


Purine
N-glycosidic linkage
Phosphodiester linkage
Pyrimidine
N-glycosidic linkage
3'
2'
5'
2'

核酸的化學性質：



Purine

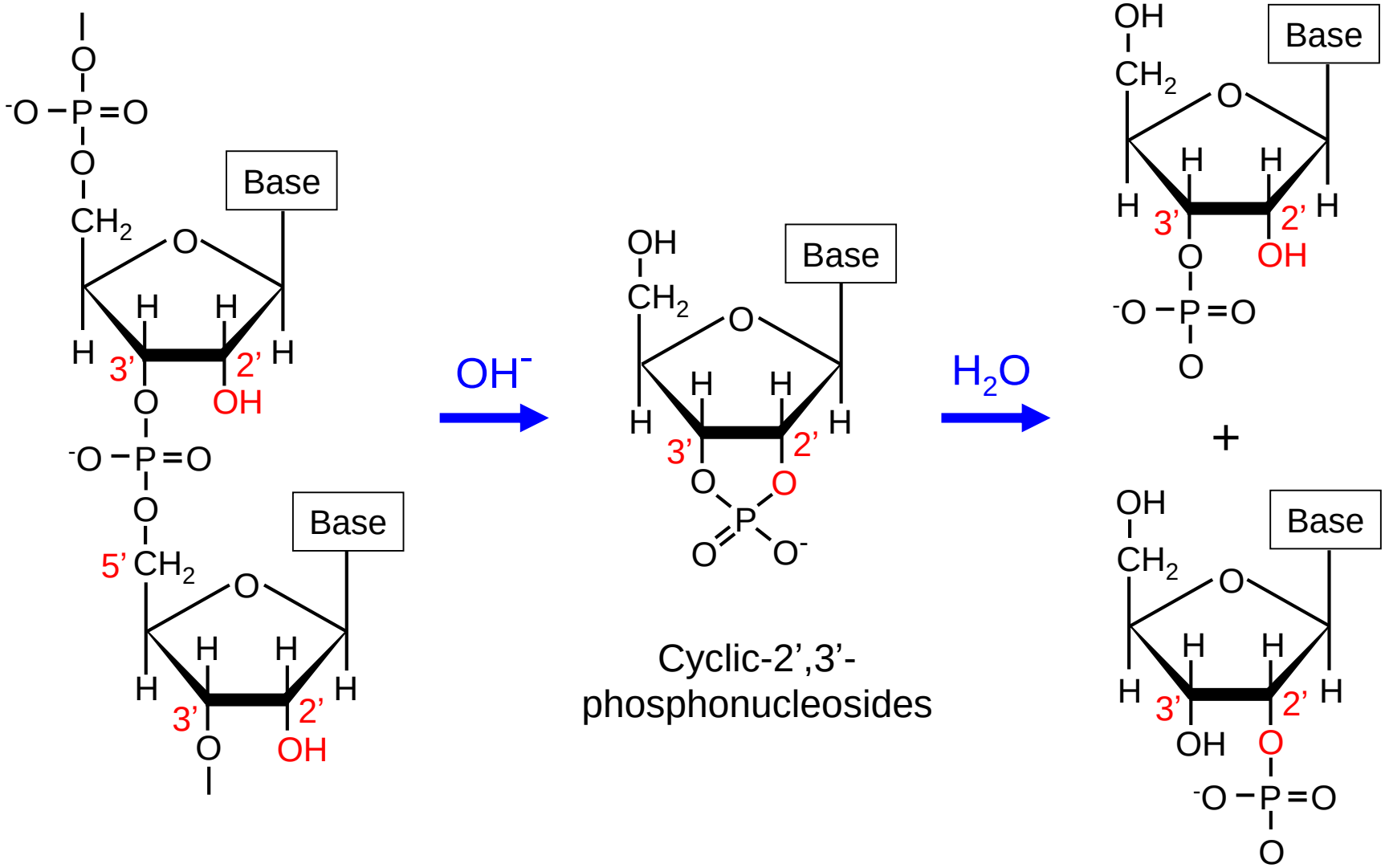
- Stable in mildly alkaline or acidic condition
- Depurinated at strong acidic condition

Pyrimidine

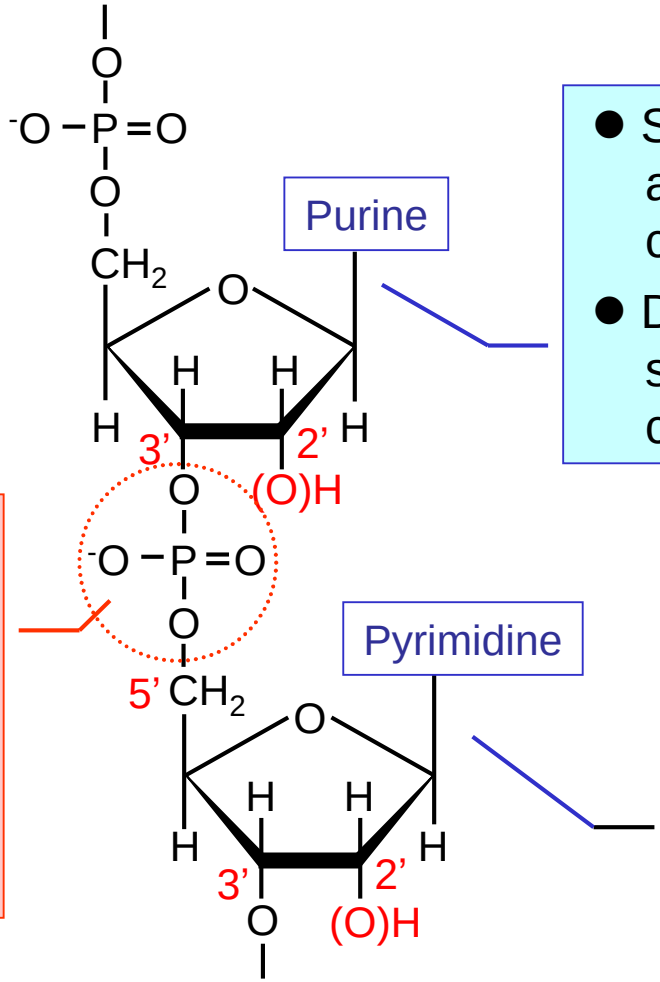
- Stable in mildly alkaline or acidic condition
- Depyrimidinated at harsh acidic condition

- In alkaline condition:
  DNA – stable
  RNA – hydrolyzed
- Unstable during depuration or depyrimidination

Alkaline hydrolysis of RNA



核酸的化學性質：



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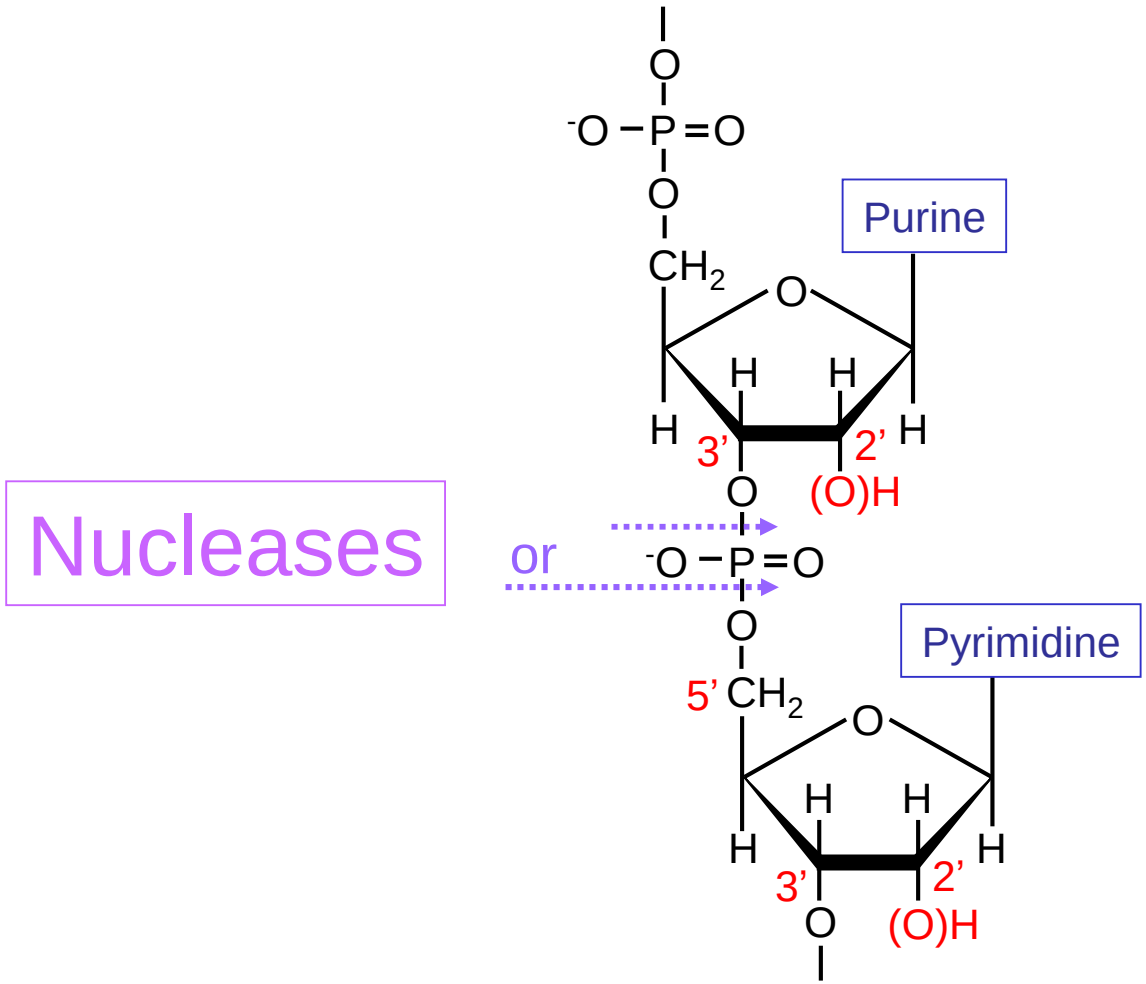

Nucleases
or
Purine
Pyrimidine
O
-O−P=O
O
CH2
O
H
H
H
3'
2'
H
O
(O)H
O
-O−P=O
O
5' CH2
O
H
H
H
3'
2'
H
O
(O)H

Nucleases 種類相當多

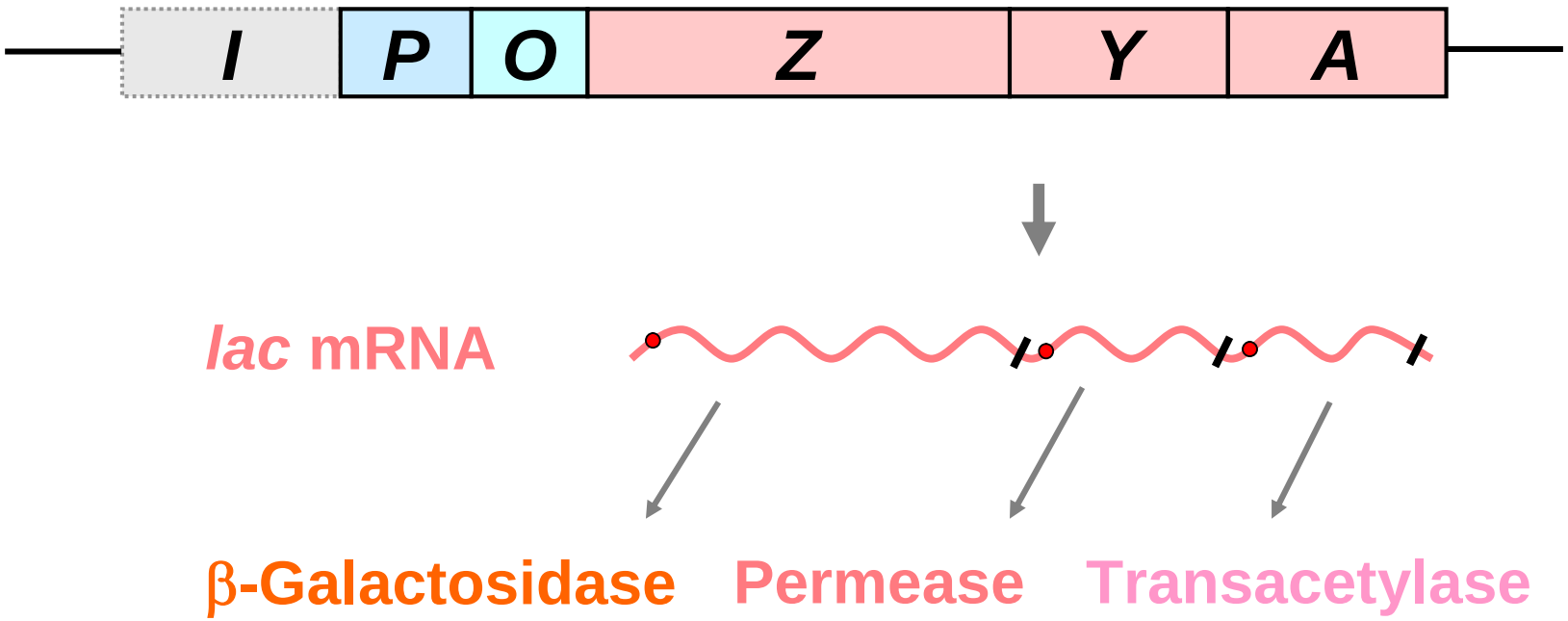
Nucleases –

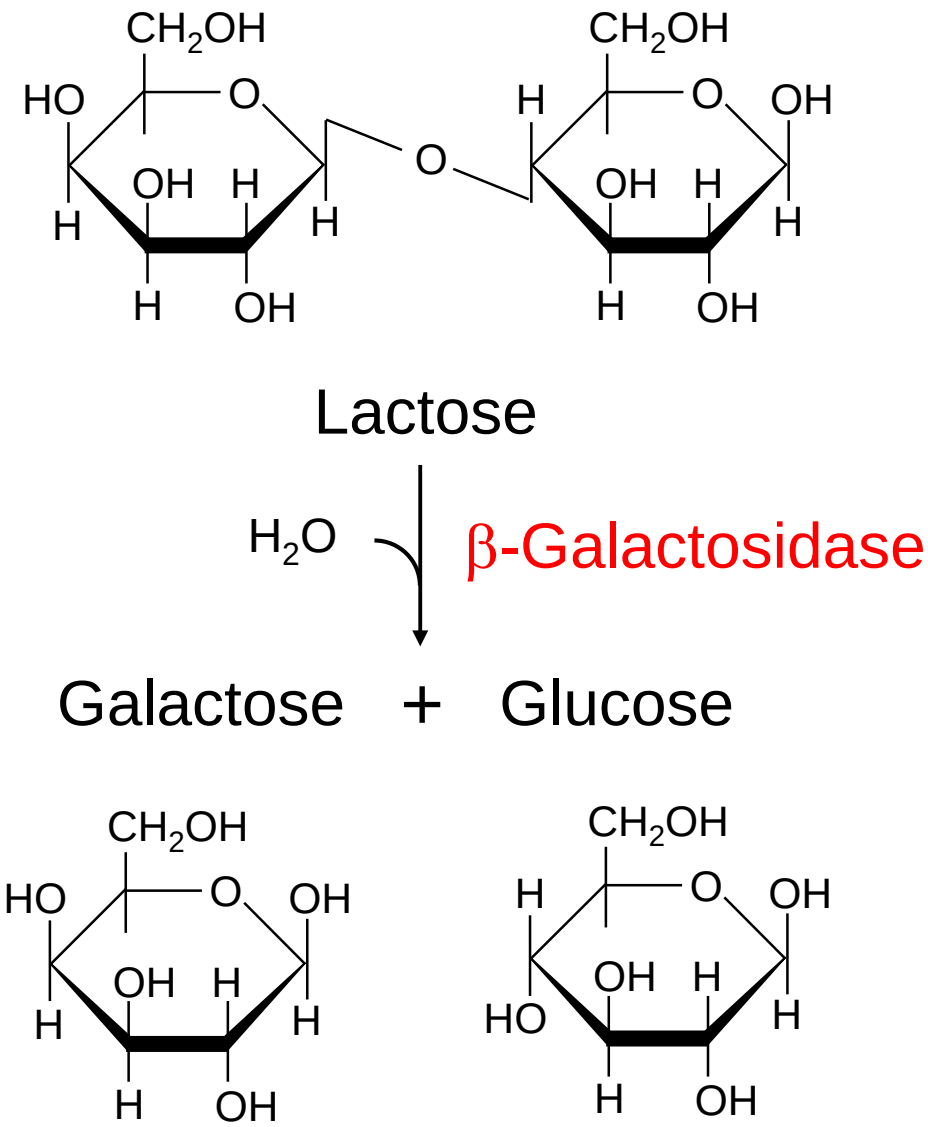
水解 DNA 或 RNA 之 phosphodiester linkages 的酵素

- Non-specific cleavage
- Specific cleavage
  - DNases or RNases
  - Exonucleases or endonucleases
  - Base specificity
  - Sequence specificity

BCX 核酸部分的主角：

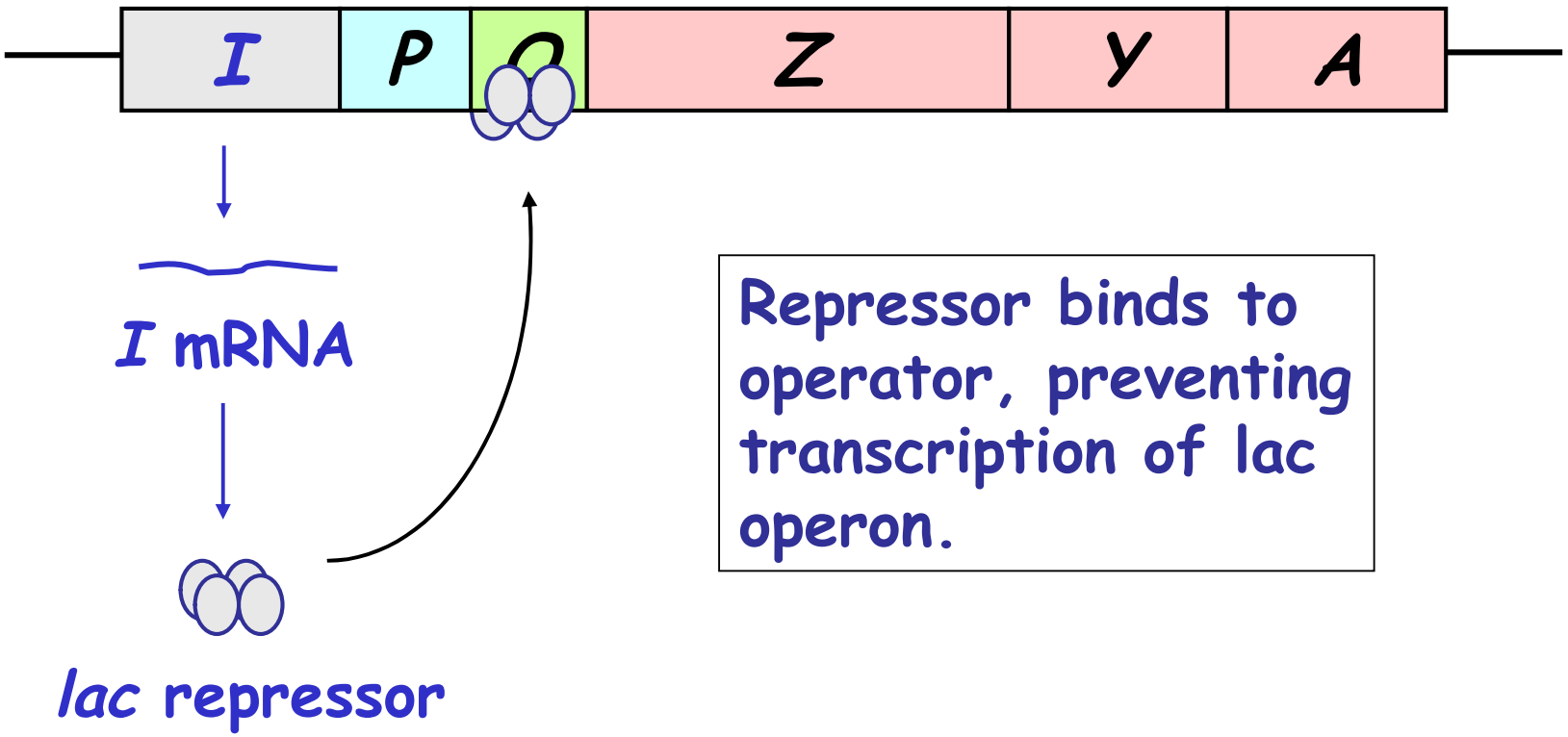
E. coli lac operon




CH2OH
HO
O
OH
H
H
H
OH
O
CH2OH
H
O
OH
OH
H
H
H
OH
Lactose
H2O
β-Galactosidase
Galactose + Glucose
CH2OH
HO
O
OH
OH
H
H
H
H
OH
CH2OH
H
O
OH
OH
H
HO
H
H
OH

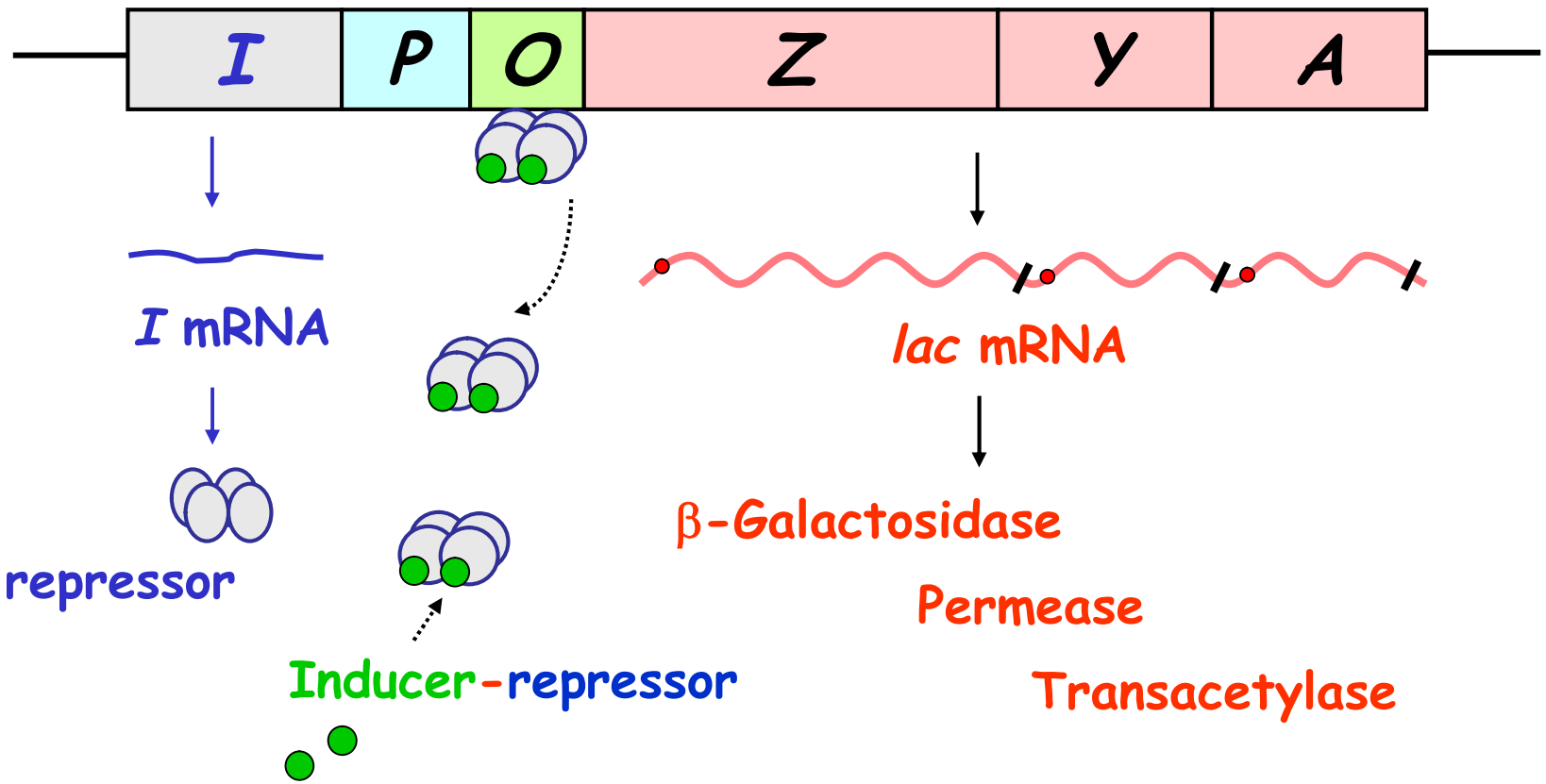
The expression of the lac operon:

(1) Absence of inducer:



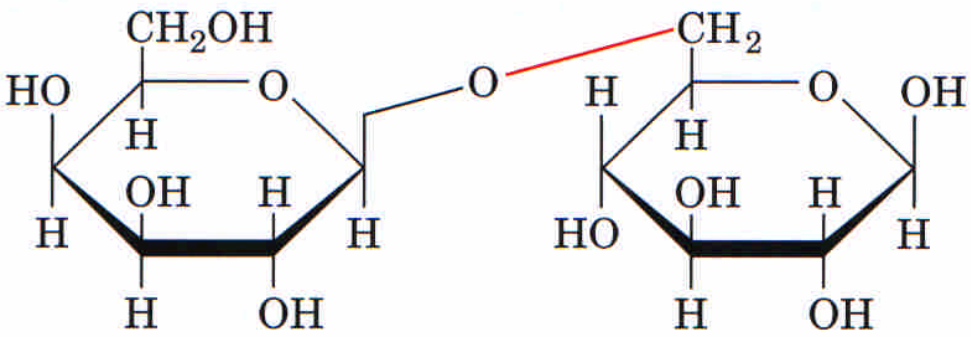
Repressor binds to operator, preventing transcription of lac operon.

(2) Presence of inducer ●



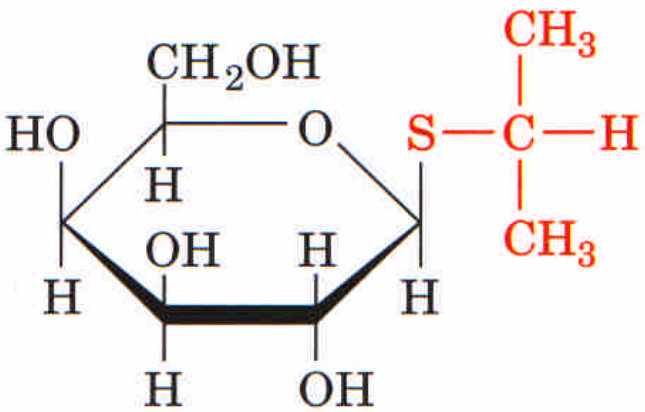
Inducer-repressor complex can not bind to operator. ⇒ Transcription and translation of lac structural genes occur.

Inducers of the lac operon:



1,6-Allolactose

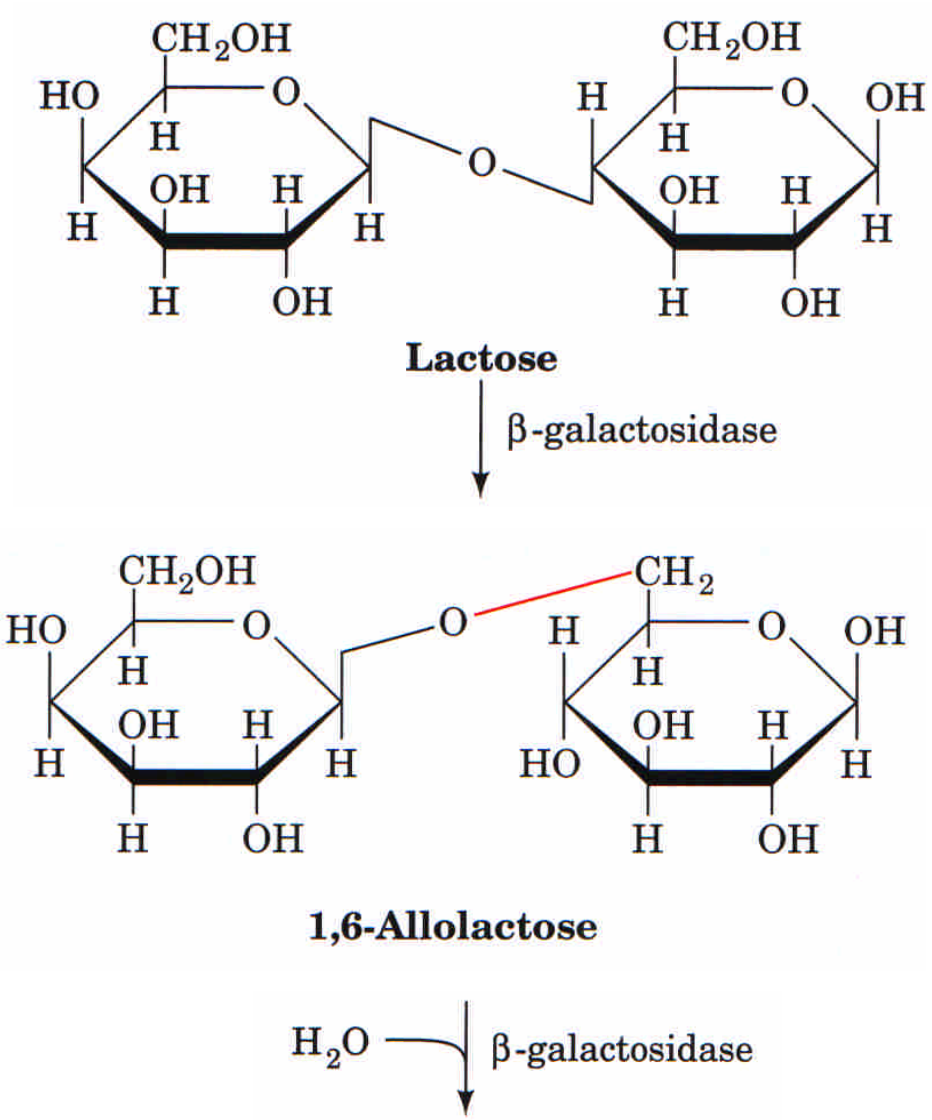
Physiological inducer



Isopropylthiogalactoside (IPTG)

Synthetic inducer

圖引用自：Voet, D., Voet, J. G. and Pratt, C.W. (1999) Fundamentals of Biochemistry. John Wiley & Sons, Inc. p.895



transglycosylation

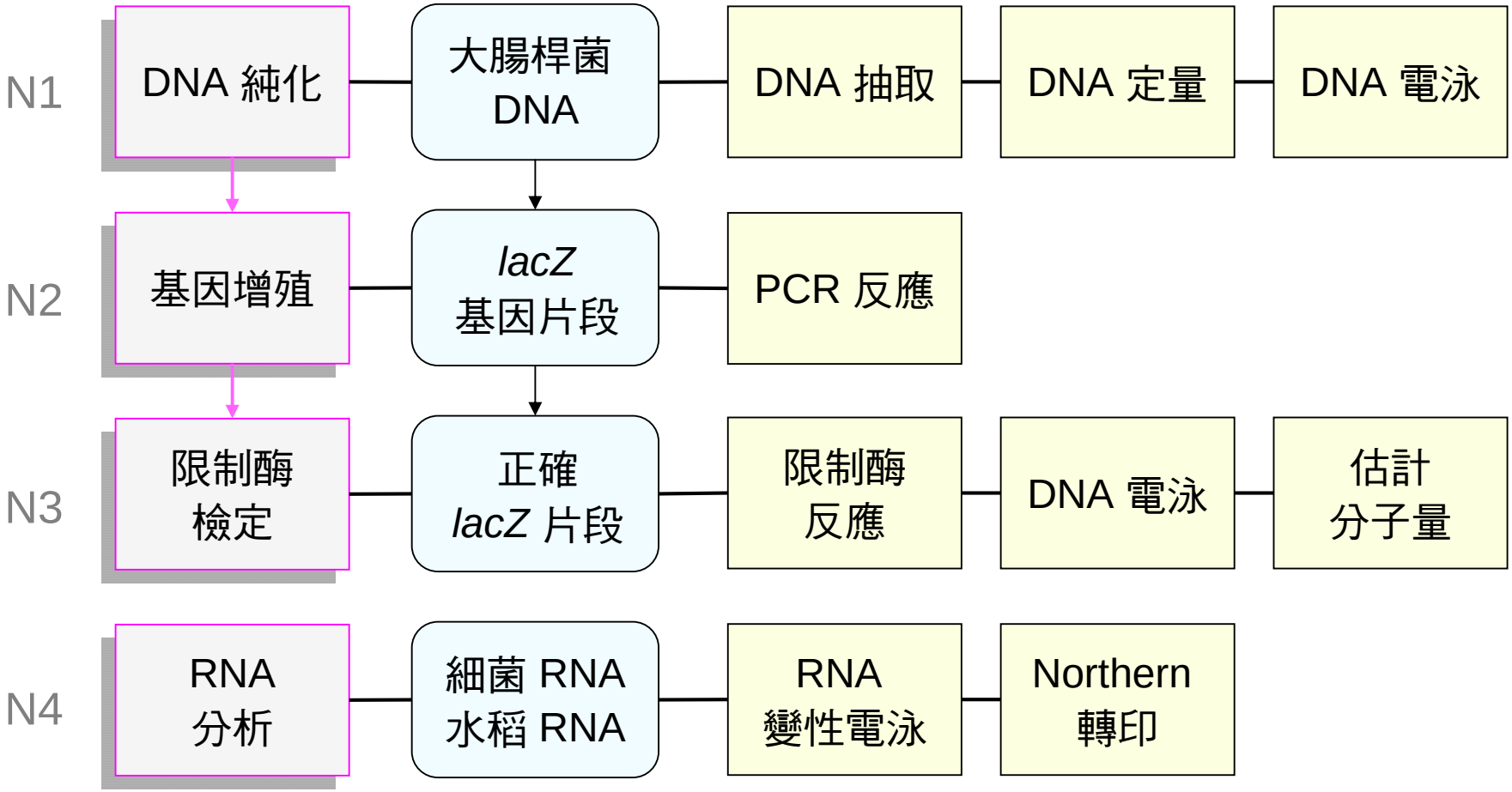
Galactose + Glucose

What happens to the expression of the lac operon when both glucose and lactose are present?

Catabolite repression

Glucose	Lactose	Transcription
+	+	off
+	-	off
-	+	on
-	-	off

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

核酸部分成績評量：

- 平常上課表現　30%
- 實驗記錄本　25%
- 實驗報告　45%

報告繳交期限

報告	週一班	週三班
N1	12/27	12/29
N2/N3	1/3	1/5
N4	1/10	1/12

你準備好了嗎？

- ☐ 我熟知實驗室安全守則。
- ☐ 我還記得生化在講些什麼。
- ☐ 我還記得核酸的結構與物理、化學性質。
- ☐ 老師會隨時提出問題或請同學上台授課。
- ☐ 不能缺席。遲到要請客、罰勞役或回答問題。
- ☐ 核酸實驗很繁瑣並會用到突變劑，要全神貫注。
- ☐ 實驗失敗不能氣餒，一定要找出原因，累積經驗。