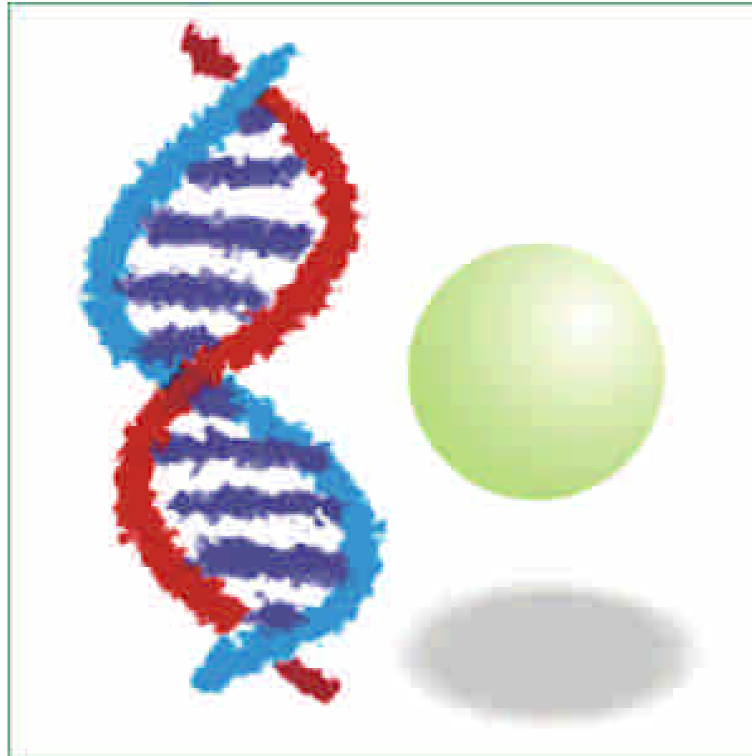
BST

生化科技系

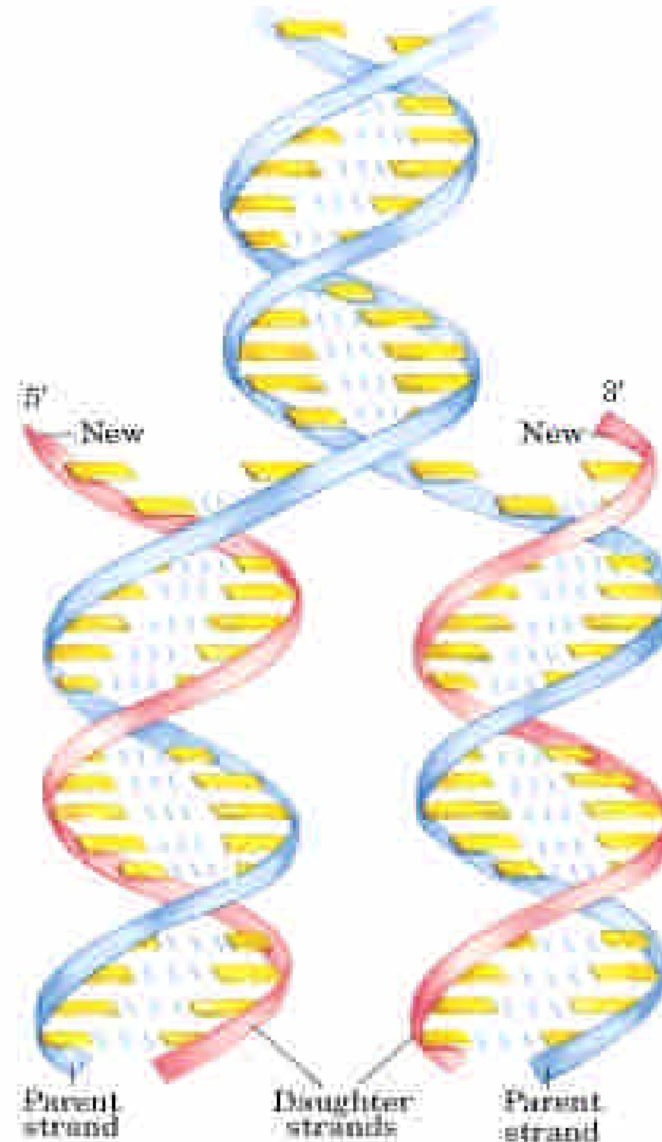
BCX  N2

生物化學實驗

Polymerase Chain Reaction

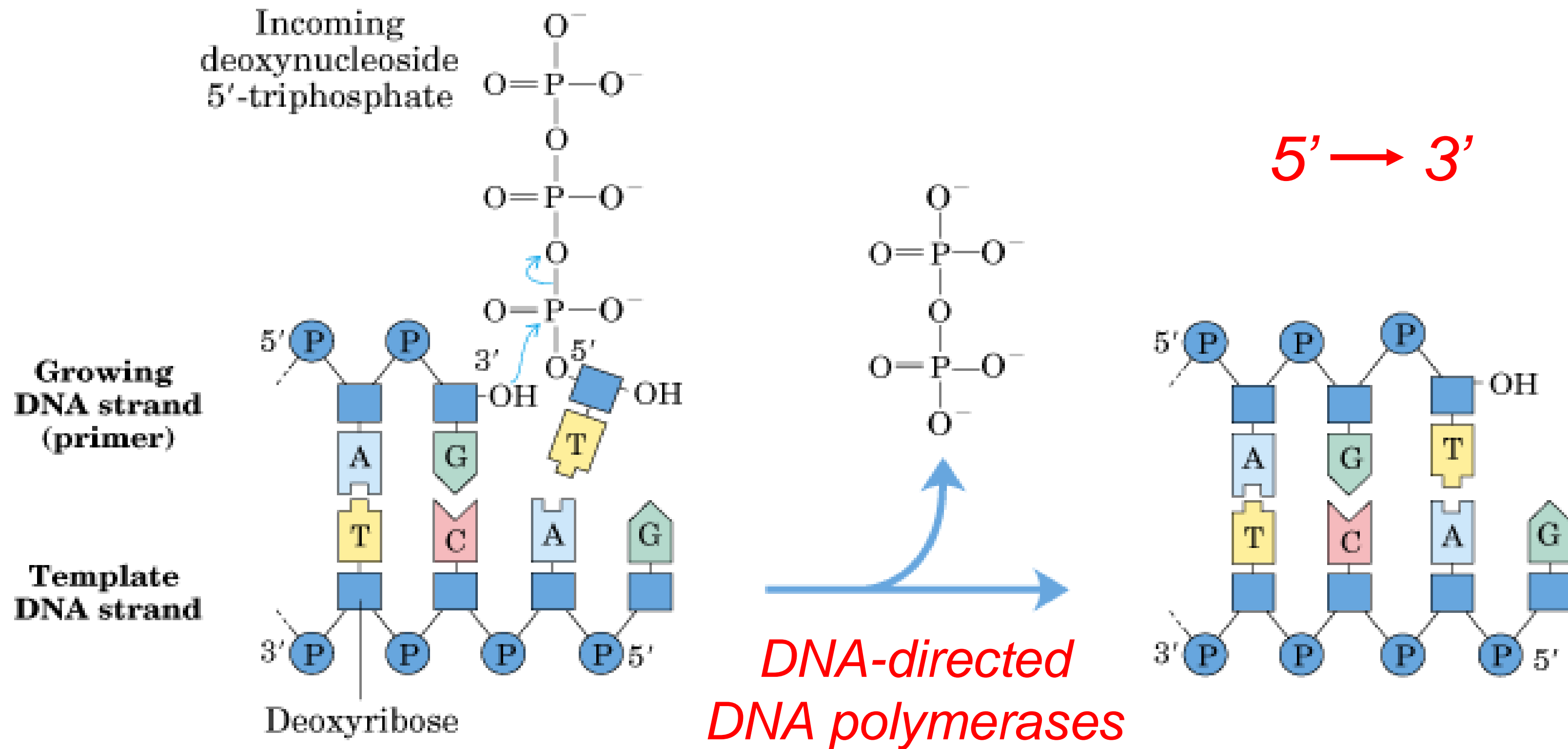
細胞中 DNA 的複製模式：

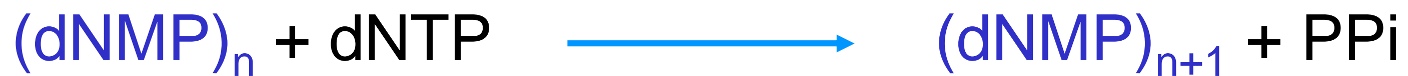
Semiconservative replication



圖引用自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd Ed., Worth Publishers. Fig. 10-17

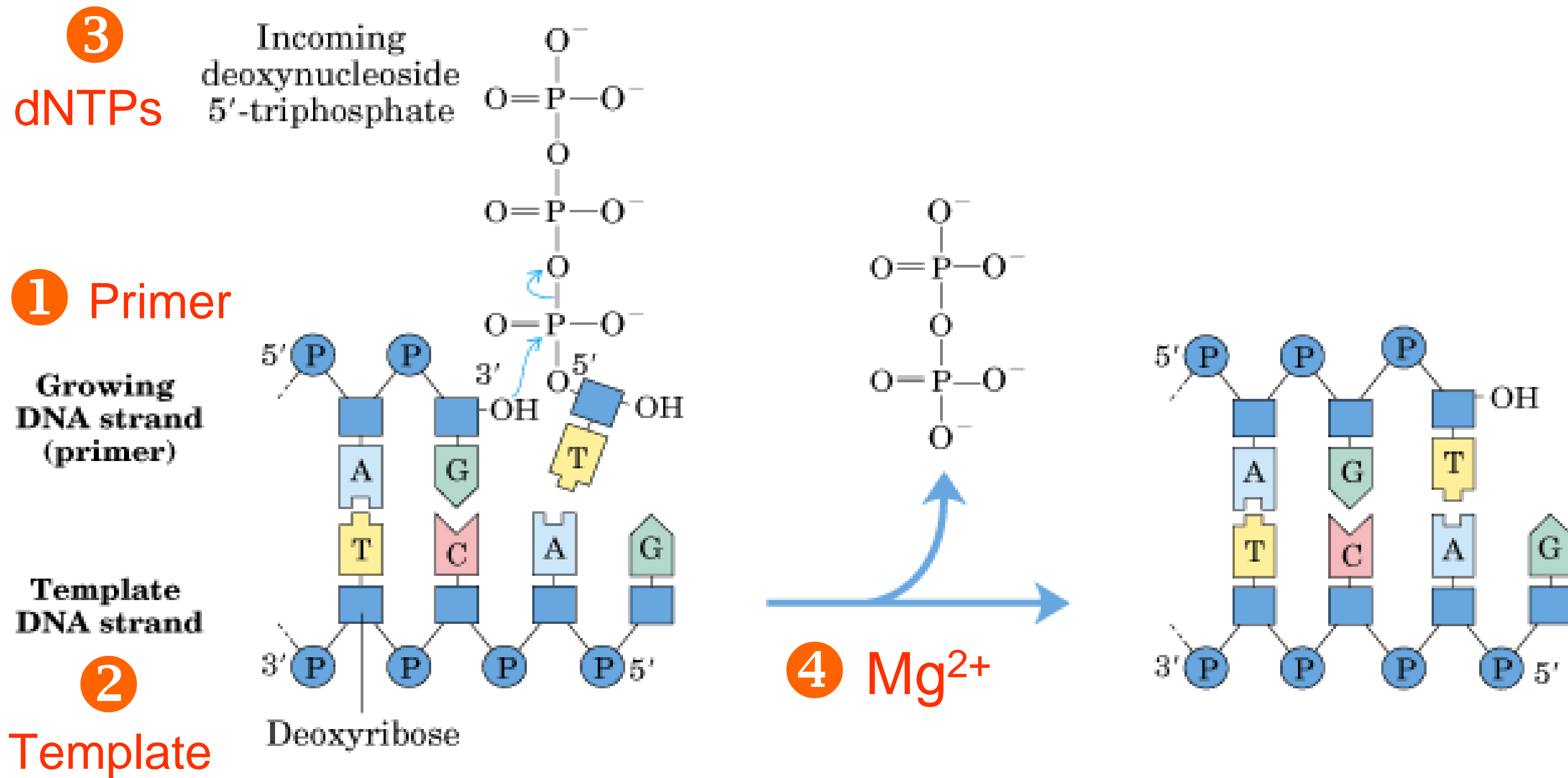
DNA polymerase 催化細胞中 DNA 的複製：





圖引用自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd Ed., Worth Publishers. Fig. 25-5

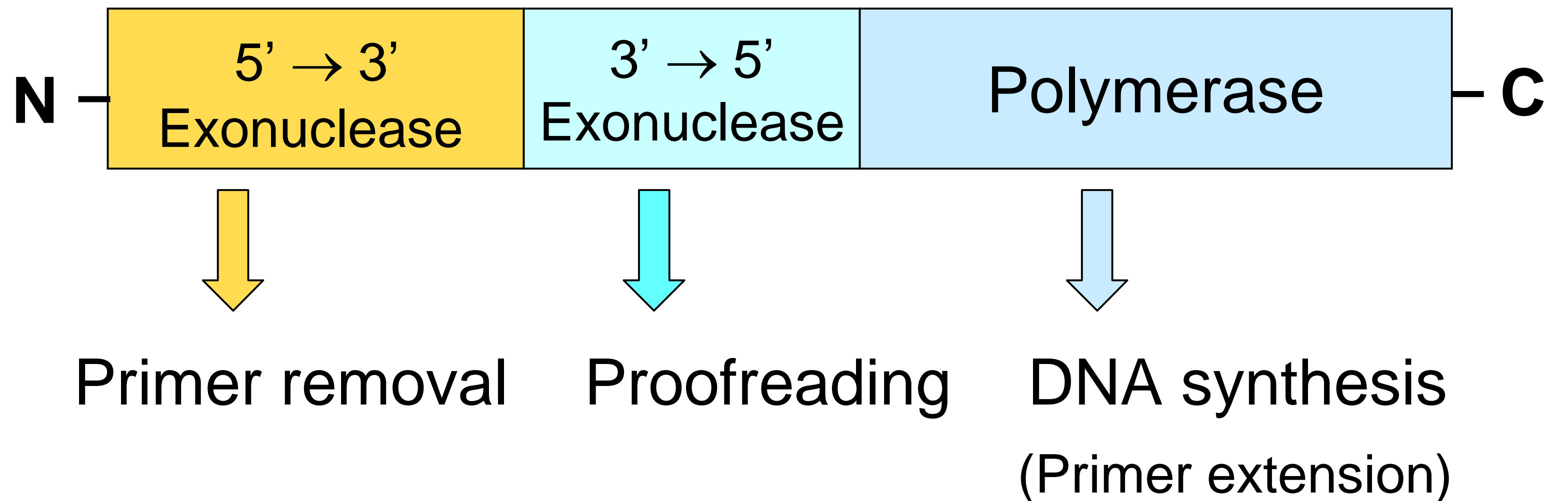
DNA-directed DNA polymerase 反應需求：



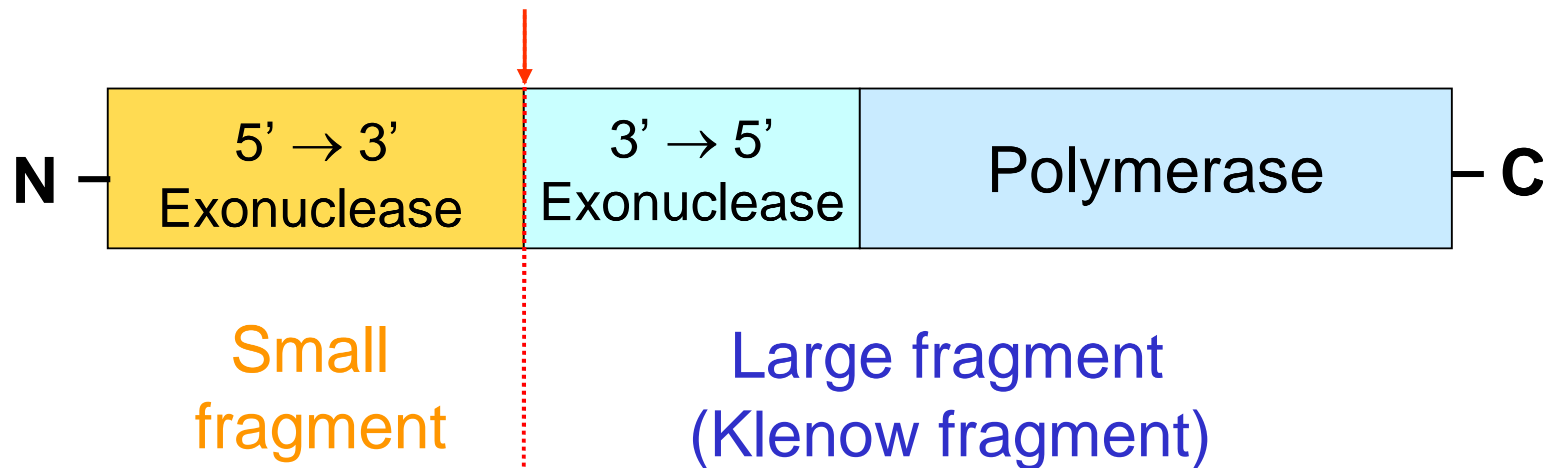
圖引用自：Nelson, D. L. and Cox, M. M. (2000) Lehninger Principles of Biochemistry. 3rd Ed., Worth Publishers. Fig. 25-5

DNA polymerase I of *E. coli* :

- 第一個被發現的 DNA polymerase
- 103 kD, 928 amino acid residues



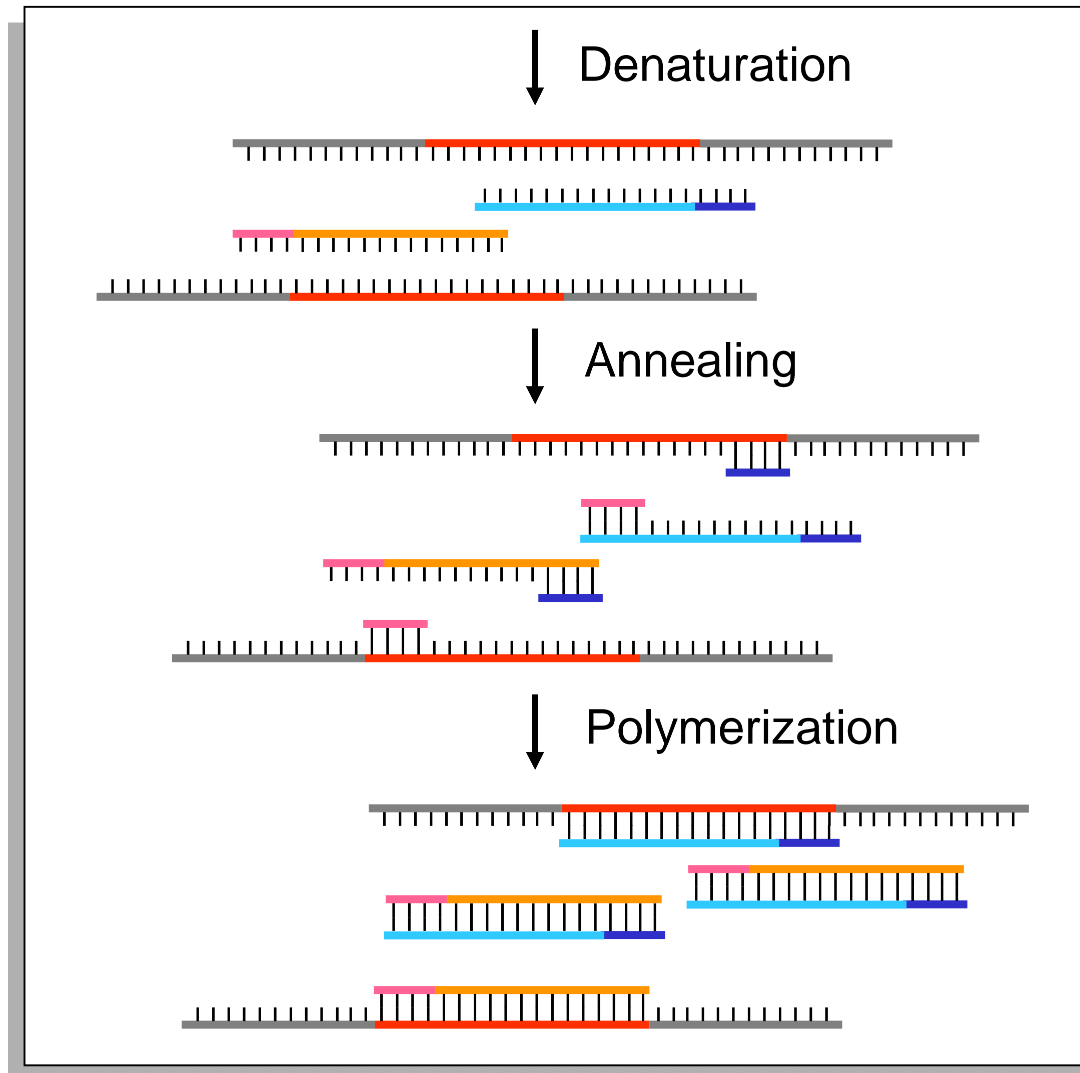
Large fragment of DNA polymerase I:



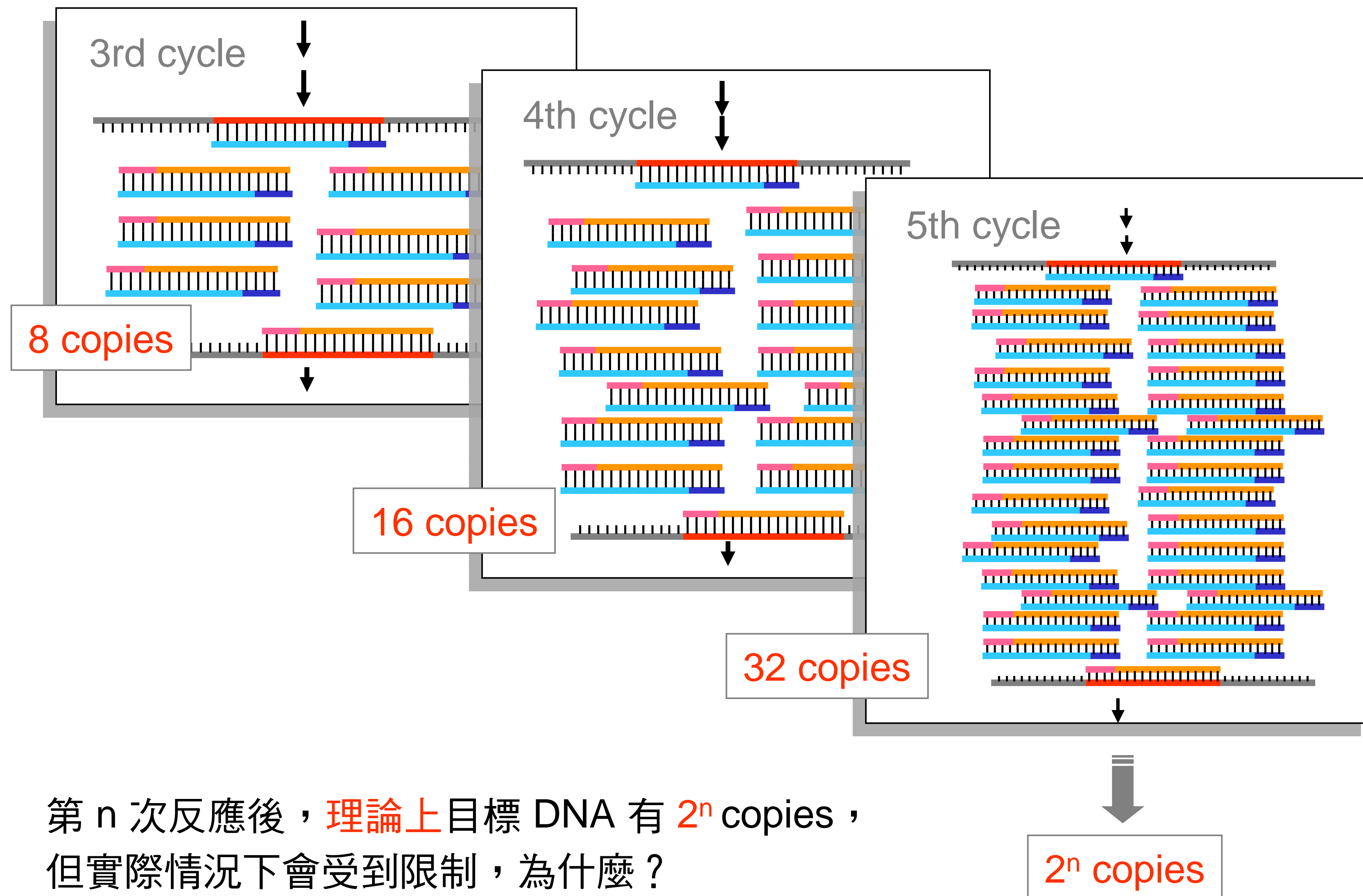
- Klenow fragment 在分子生物實驗中的應用極多，例如，修補 DNA 末端、放射線核酸探針標幟…等等。
- 最初的 polymerase chain reaction 反應即是利用 Klenow enzyme 進行。

Polymerase chain reaction:

Polymerase chain reaction:



Polymerase chain reaction:



第 n 次反應後，理論上目標 DNA 有 2^n copies，
但實際情況下會受到限制，為什麼？

Experimental approach: Standard PCR

Primer design, Preparation of template DNA

Determination of experimental parameters

PCR

Analysis of products

Purification of products

Subcloning

Primer design:

- Primer length: 20-30 nt
- Melting temperature (T_m)：兩引子之差異小於 5°C
- G/C content: ~ 40%-60%
- PolyN stretches
- Complementary sequences
- Specificity
- 3'-end sequence

$$T_m = \Delta H\ [\Delta S + R\ \ln(c/4) - 273.15\ ^\circ C + 16.6\ \log_{10}[K^+]$$

H: the enthalpy for helix formation

S: the entropy for helix formation

R: the molar gas constant

c: the concentration of primer

$$T_m = 2(A+T) + 4(G+C)\ ^\circ C$$

Annealing tempreature = (T_m - 5)°C

Determination of experimental parameters:

- Reaction buffer
- Enzyme
- Denaturation temperature and time
- Annealing temperature and time
- Elongation temperature and time
- Cycle number

Reaction buffer

- Tris-Cl, 10 – 50 mM, pH 8.3 ~8.8
- KCl, < 50 mM
- $MgCl_2$, 0.5 – 2.5 mM over total [dNTP]
- dNTPs, pH 7.0, 20 – 250 μM each
- Primers, 0.2 – 1 μM each
- Additives, e.g., BSA, gelatin, NP-40…etc.

Enzyme

Taq polymerase (from *Thermus aquaticus*)

T. Thermophilus DNA polymerase

Vent polymerase (from *Thermococcus litoralis*)

…………………

是否具 proofreading 功能？

目標基因的長度？

是否在產物末段多加上一個A？

For *Taq* polymerase:

- Concentration: 0.5 – 5 units/100 μl
- Denaturation: 94 – 95 °C, 30 sec - 1 min
- Elongation: 70 – 72 °C, 0.5 – 3 min
  (2 – 4 kb/min)
- Cycle number: 25 - 35

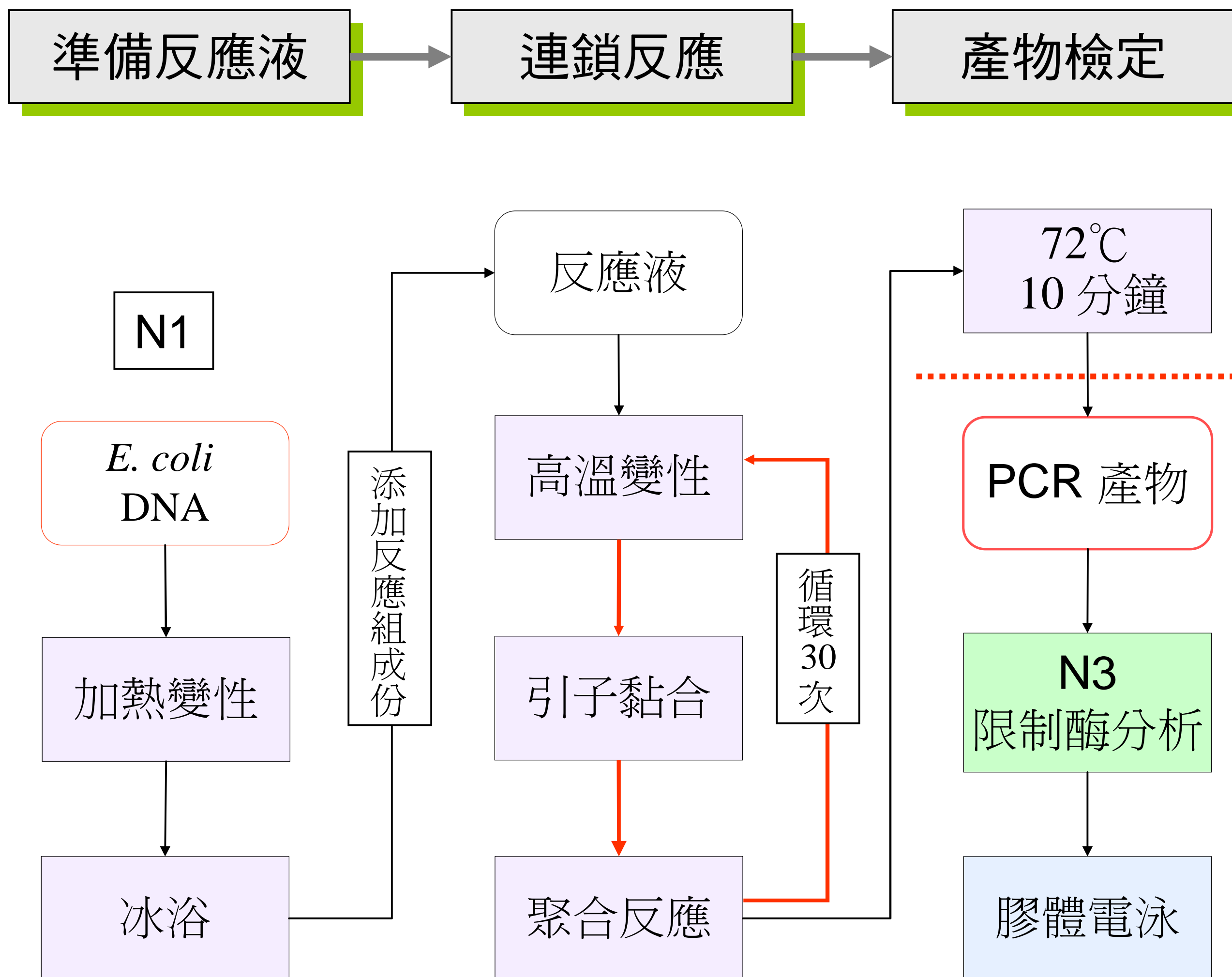
為什麼你的 PCR 失敗？

- $MgCl_2$ 濃度不對：沒有試最適濃度？使用前沒有混合均勻？
- dNTP 濃度太高？已經降解了？
- 反應液中存在 inhibitors？
  例如：EDTA, chloroform, phenol, ethanol, SDS, sarkosyl,
  tracking dyes,….？
- Enzyme 太多？
- Template 過多或過少？
- Primers 濃度不對？ Primers 設計不良？
- Annealing temperature 不對？
- Reagents 有雜質？

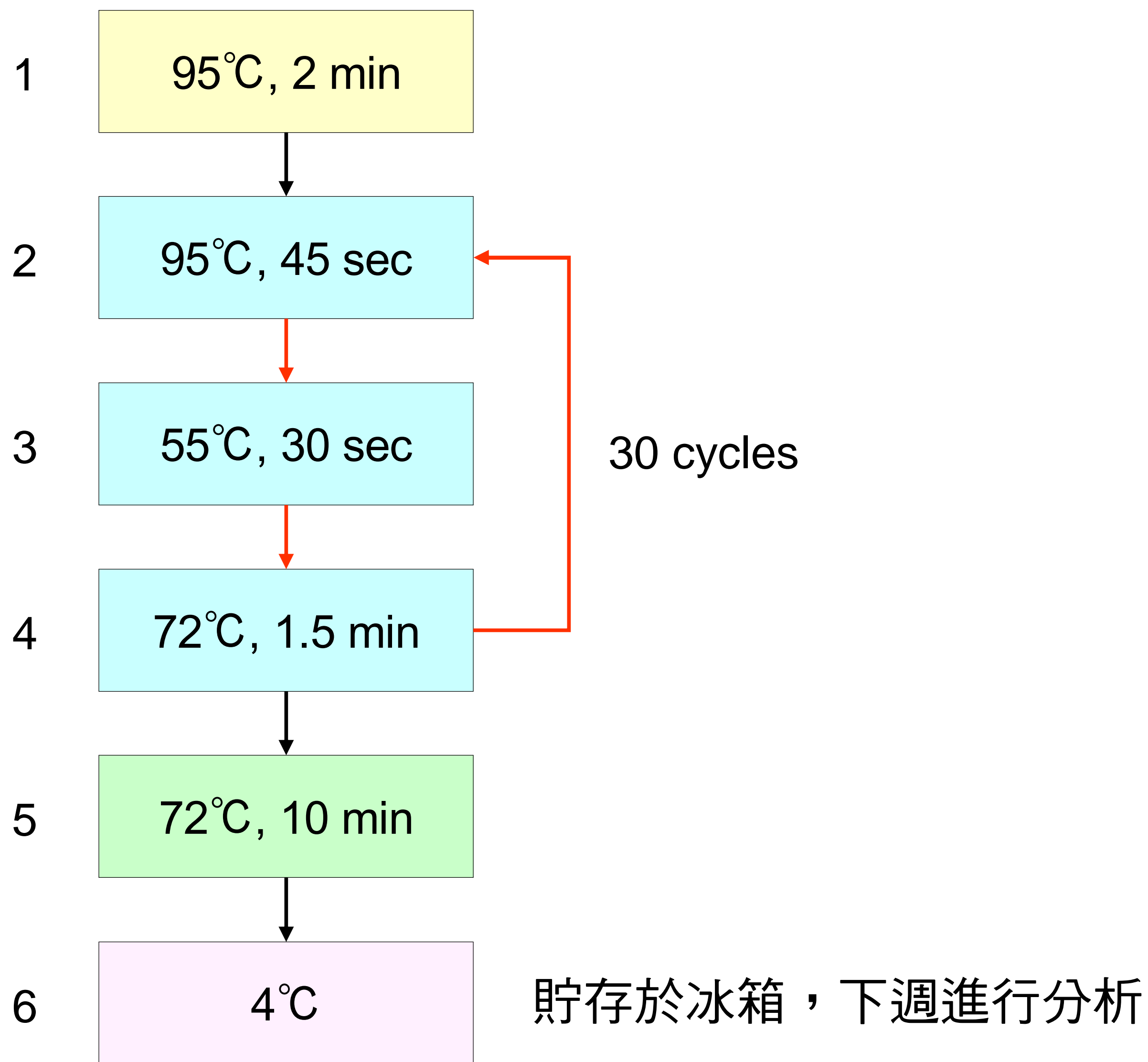
Always remember –

- Work clean.
- Titrate $MgCl_2$.
- Do not use too much template DNA.
- Do not use PCR products in PCR preparation areas.
- Always include negative and positive control in every experiment.
- Wear gloves.

N2 以聚合酶連鎖反應增殖DNA



本次實驗反應程式：



本週實驗進行要點：

樣品準備

- 準備電泳樣品 → 進行加熱 (p.N1-6, 1.4.3)
- TAs：示範鑄膠，說明電泳槽，示範 sample loading

電　泳

- 1, 3 組：進行 DNA 稀釋 [p.N1-5, 1.3.3, step 1]
  → 測定吸光值 [p.N1-5, 1.3.3, steps 2-3]
  → 準備 PCR（DNA 及酵素除外）[p.N2-6, 2.2.3, step 3 及背面]
- 2, 4 組：進行 DNA 稀釋 [p.N1-5, 1.3.3, step 1]
  → 準備 PCR（DNA 及酵素除外）[p.N2-6, 2.2.3, step 3 及背面]
  → 測定吸光值 [p.N1-5, 1.3.3, steps 2-3]

膠片分析

- TAs: 協助判讀結果，決定 DNA 是否可用於 PCR
- TAs：說明 PCR 程式設定

PCR

- 各組添加 PCR 之 DNA 及酵素