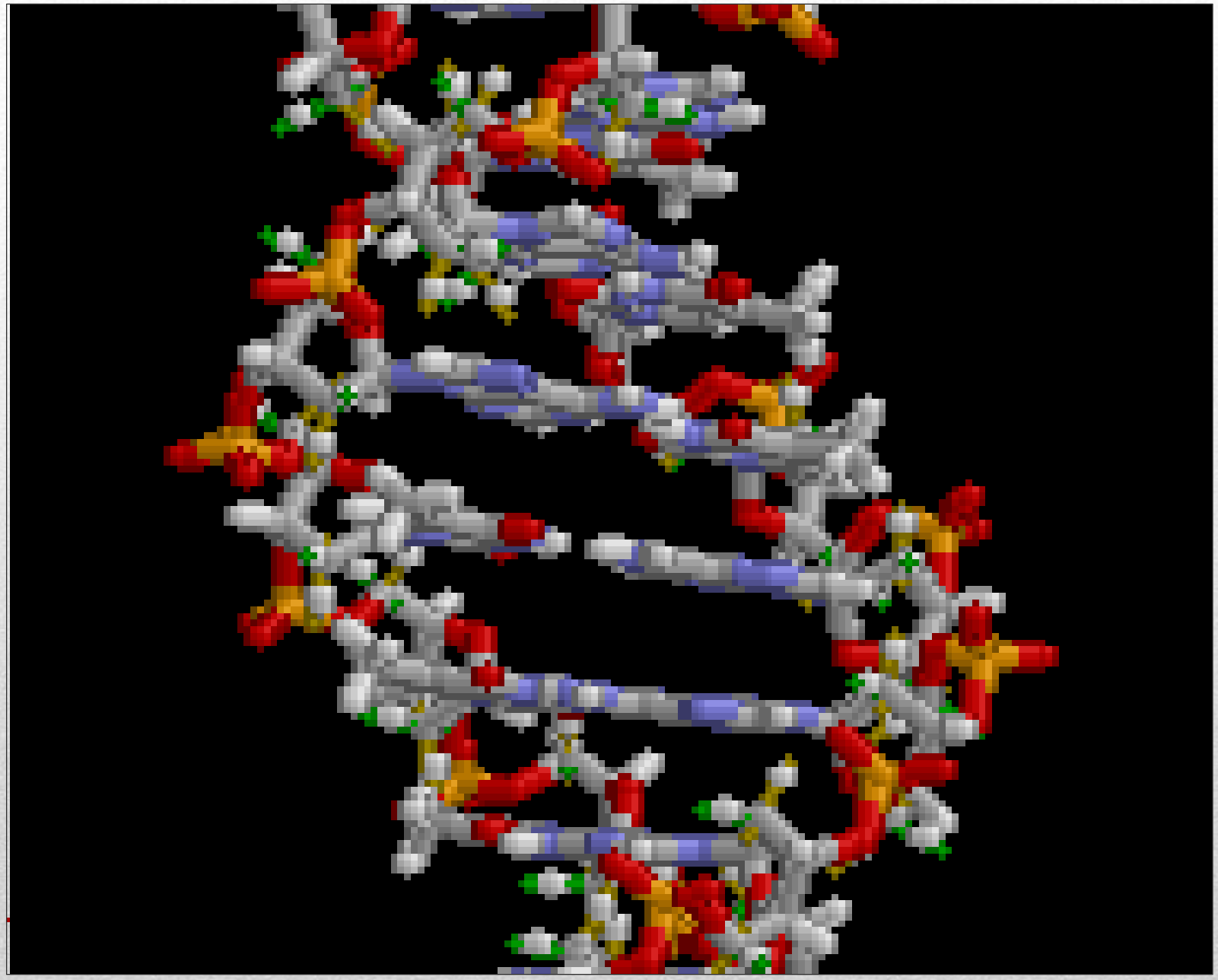
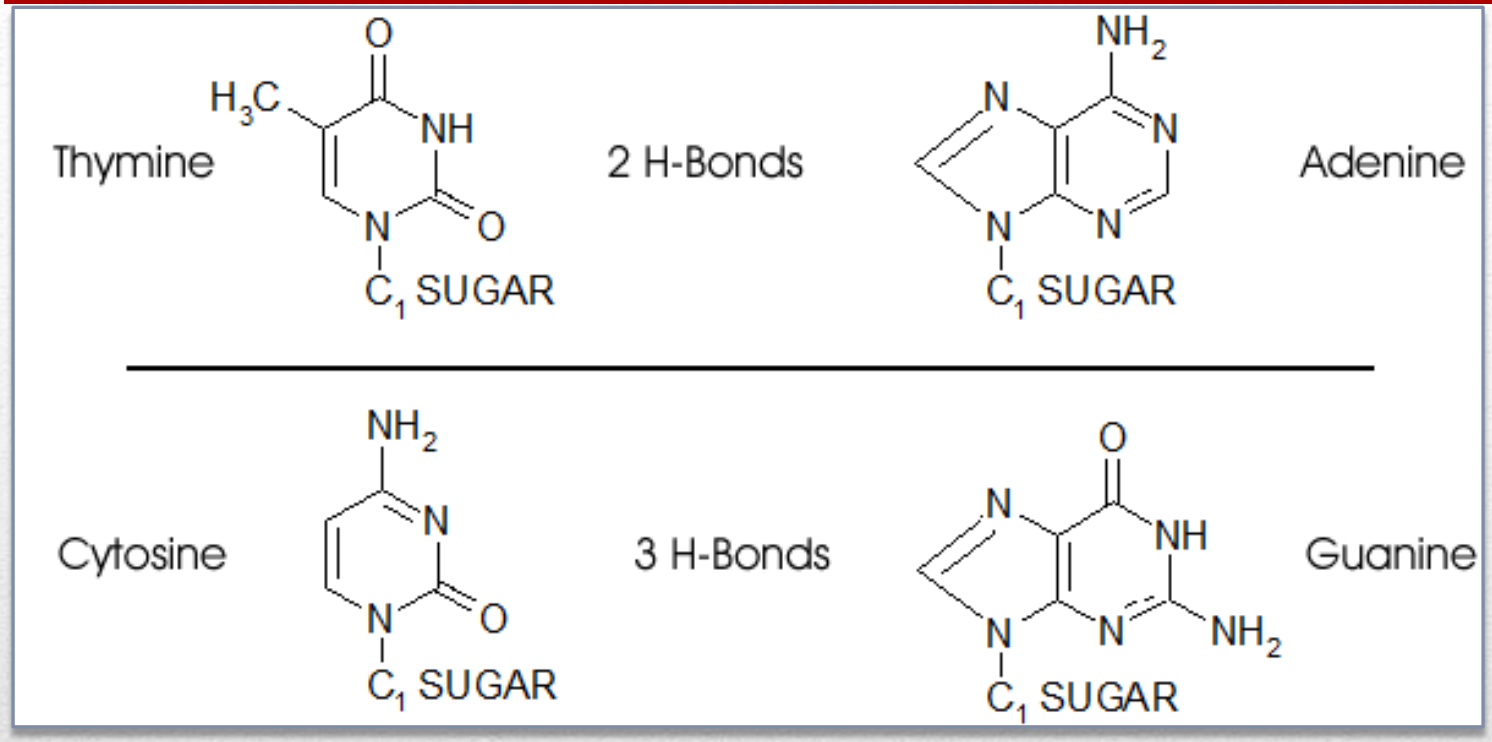
基因定序法

第六組 邱奕誠 陳冠璋 林佳勳





- 兩股**DNA**長鏈上的鹼基以氫鍵相互吸引，使雙螺旋形態得以維持。
- 這些鹼基可分為嘌呤和嘧啶。分別是腺嘌呤（**A**）、胞嘧啶（**C**）、鳥嘌呤（**G**）與胸腺嘧啶（**T**）。

- 基因是指一段含有遺傳訊息，且可影響生物體表現型的**DNA**序列。

- 人類有23 對染色體，約 30 億個鹼基對
- 人類和黑猩猩**DNA**之間的差異只有1.24%
- 人類和其他靈長類動物基因之間的差異源自基因突變。基因突變是基因複製過程中產生的錯誤

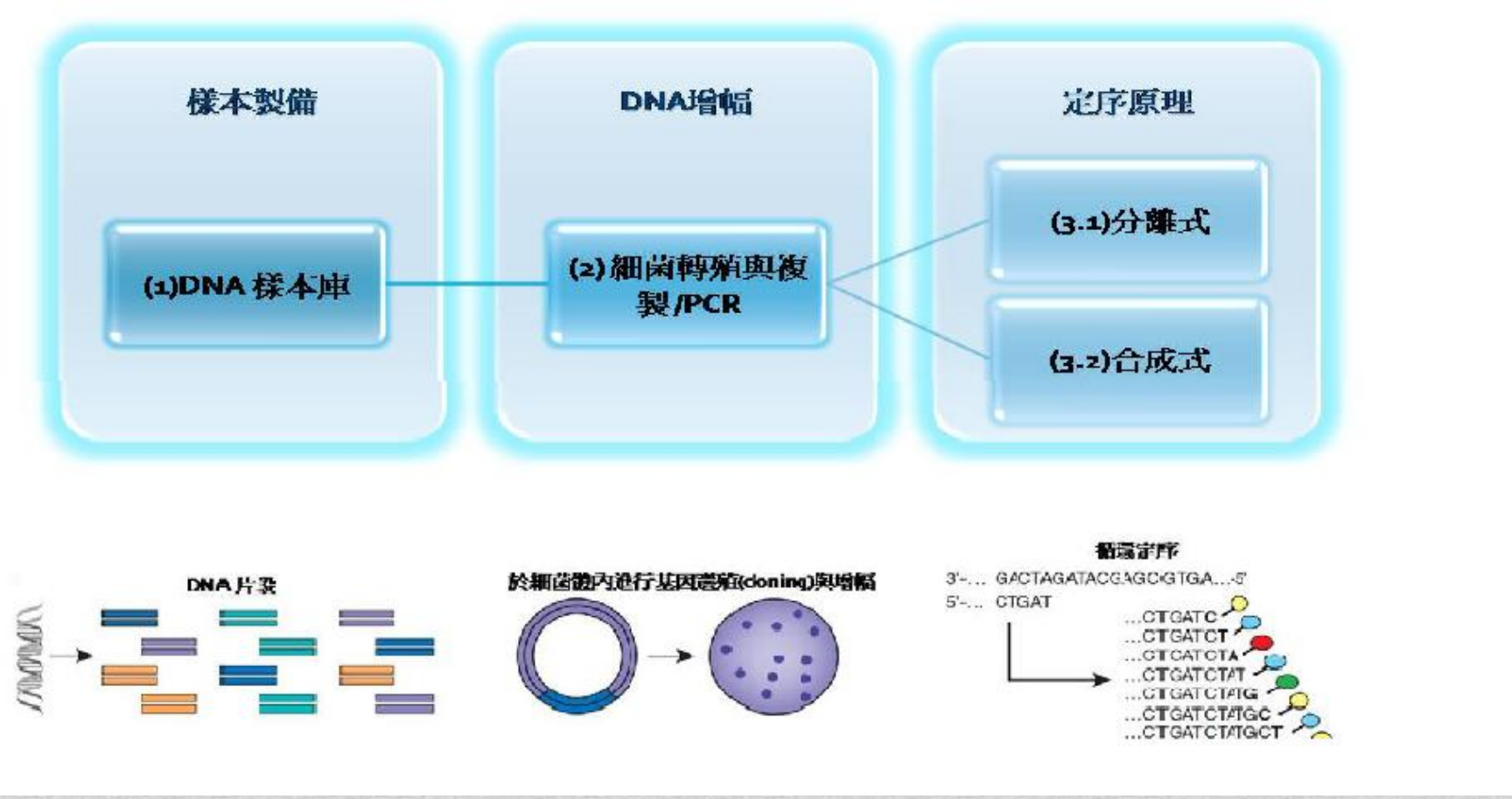
為什麼需要知道基因序列

- 醫療上根據病人遺傳背景量身訂做治療藥物
  - 提高治療效率，並可大大減 少副作用
- 取代目前粗略的疾病分類
  - 例如同為乳癌，會因為病患基因背景不同而有不同的疾病分類及處方。
- 對基因工程的掌握：現代鍊金學
  - 扮演上帝的角色

台灣的基因解碼

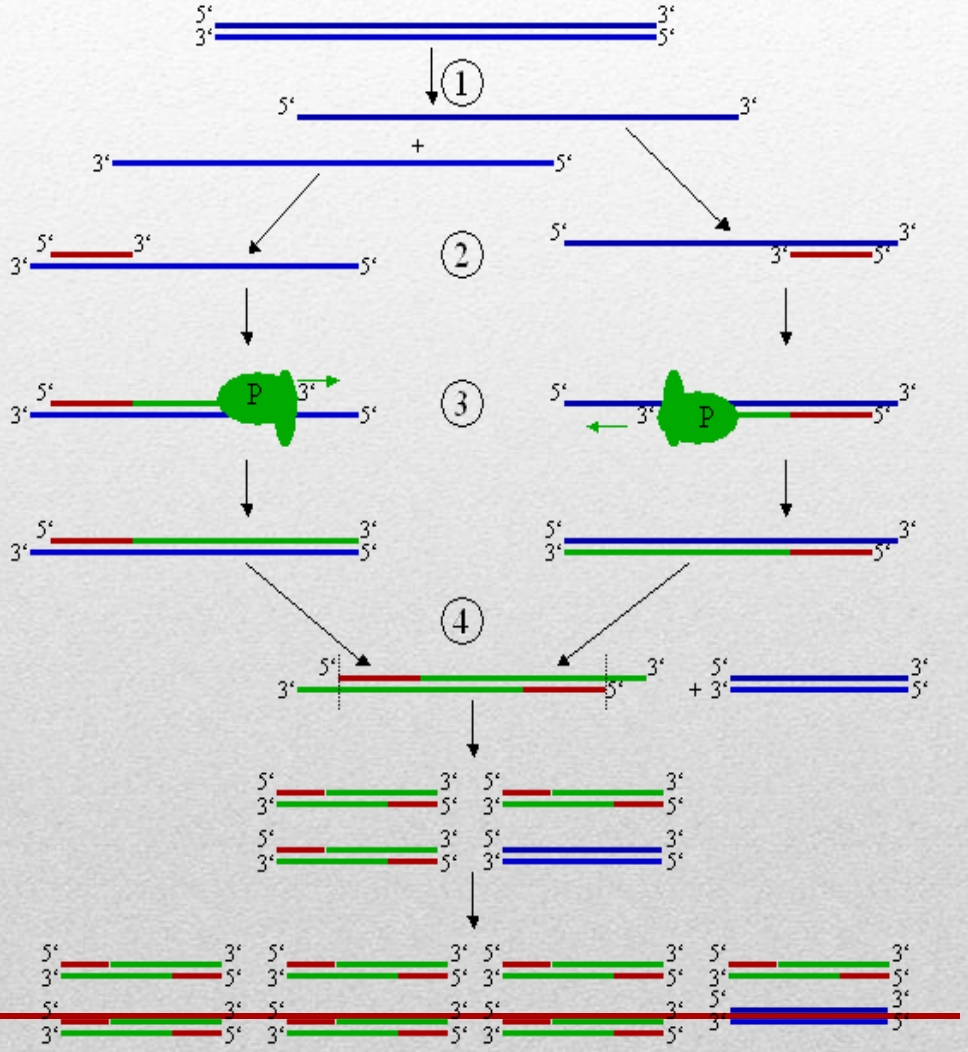
- 人類基因體研究計劃(Human Genome Project，HGP)
  - 1990年開始，為期15年
  - 榮陽團隊
  - 第四號染色體(2000年解碼完畢)
    - 起因：國人常見的肝癌
    - 癌症是細胞基因體發生突變產生的結果
- 黑猩猩、靈芝、水稻的基因解碼

第一代DNA定序 策略


樣本製備
DNA增幅
定序原理
(1)DNA 樣本庫
(2)細菌轉殖與複製/PCR
(3-1)分離式
(3-2)合成式
DNA片段
於細菌體內進行基因選殖(doning)與增幅
GACTAGATACGAGCGTGA...5'
CTGAT
...CTGATC
...CTGATCT
...CTGATCTA
...CTGATCTAT
...CTGATCTATG
...CTGATCTATGC
...CTGATCTATGCT

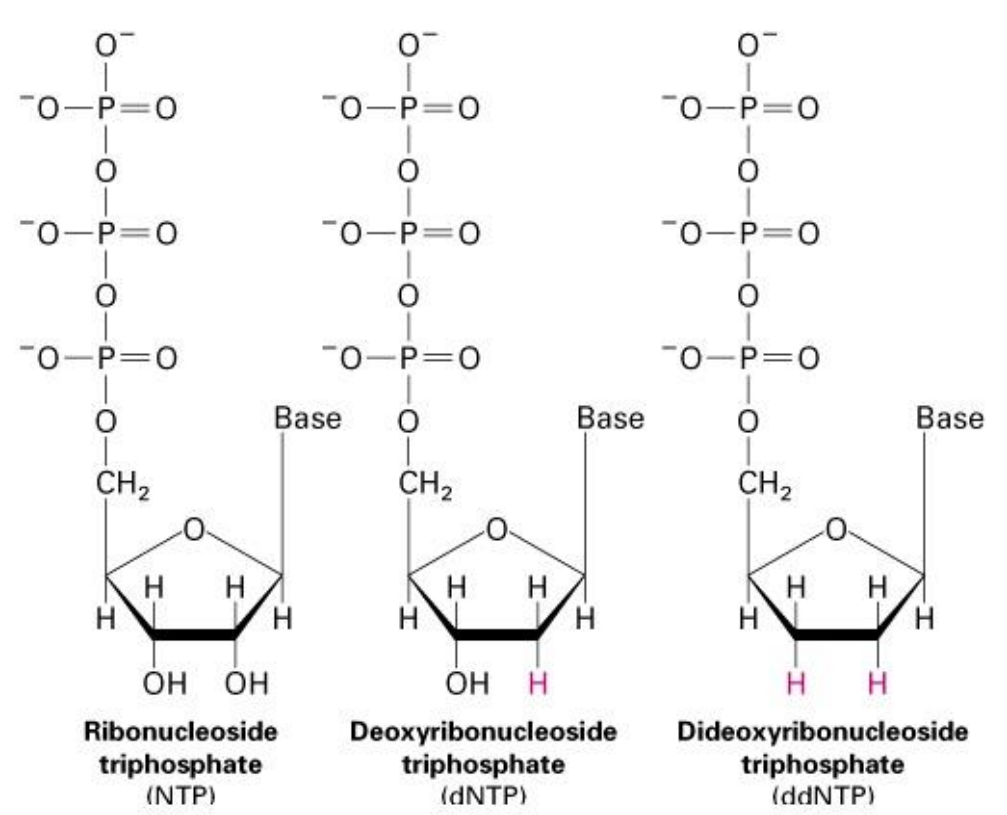
PCR

- Polymerase Chain Reaction

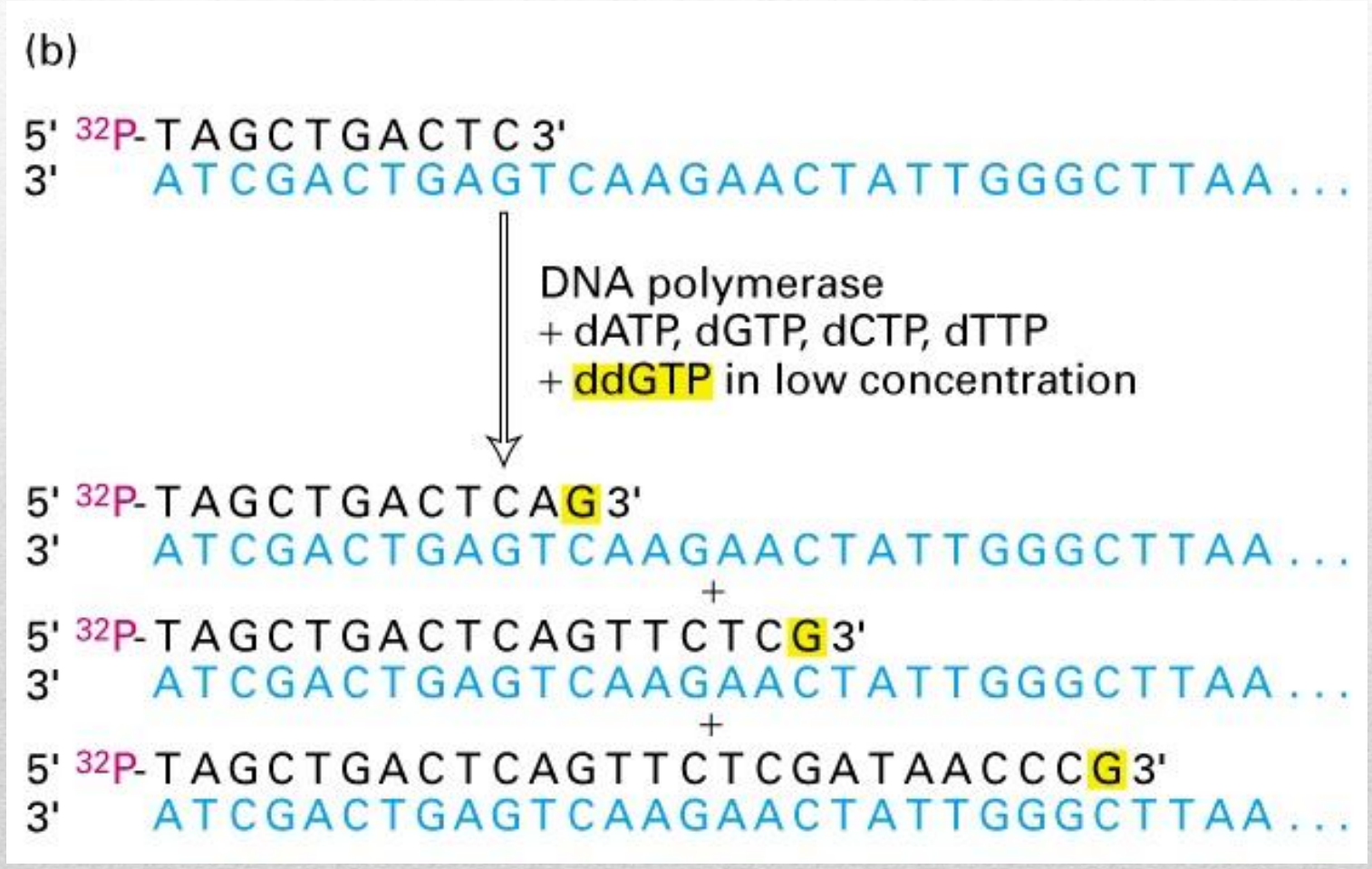


Sanger sequencing

- 分離式定序法
- 放射標定ddNTPs
- 電泳



Sanger sequencing



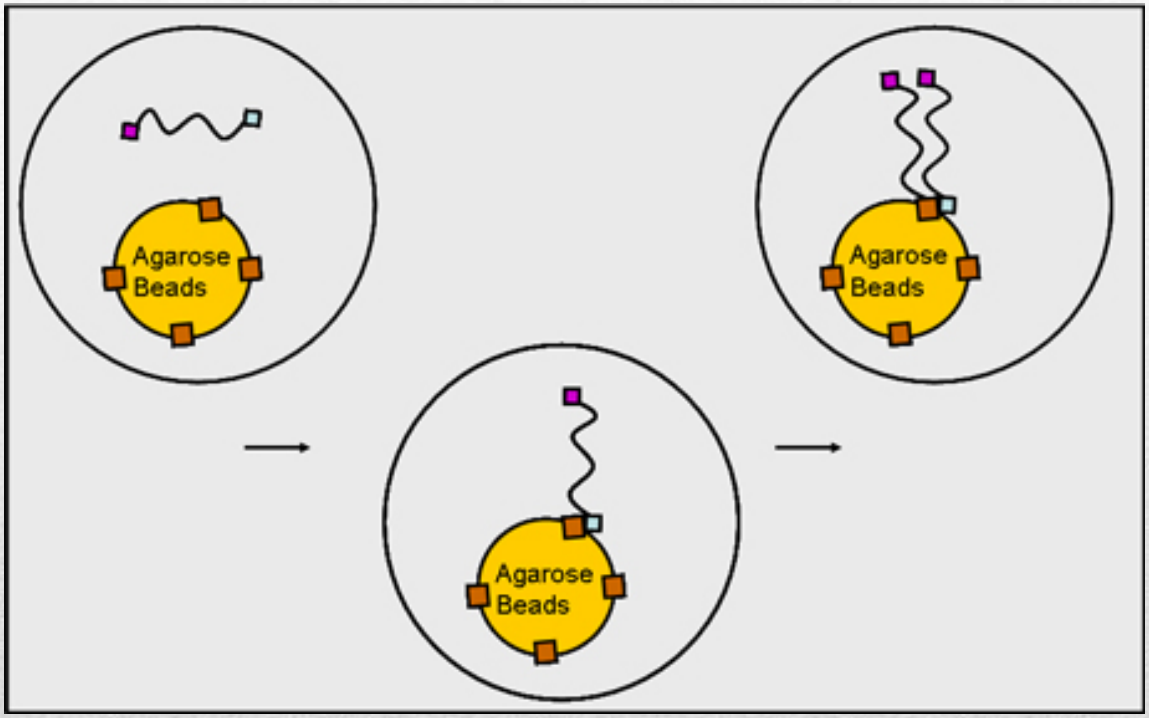
Maxam-Gilbert sequencing

- Concept is mostly the same as that of Sanger sequencing…
- But through a chemical way!

The Next-Generation Sequencing

454-Sequencing

Procedure 1 _ Emulsion PCR



fragmented

↓

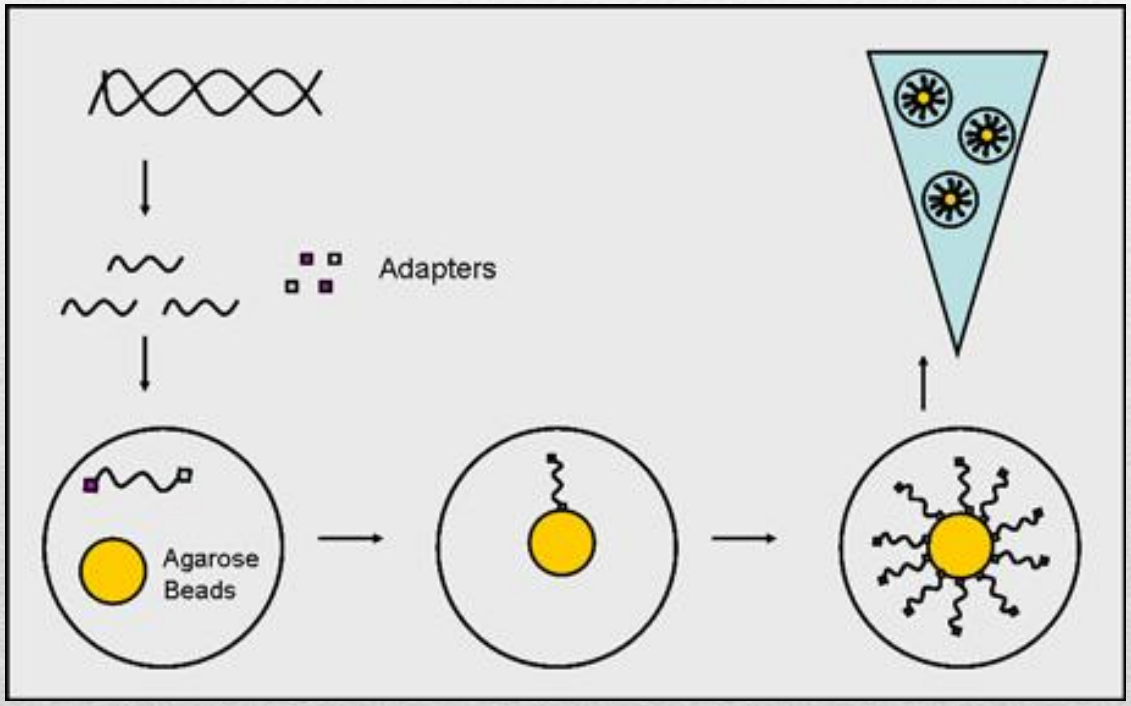
joined to adaptors

↓

include beads

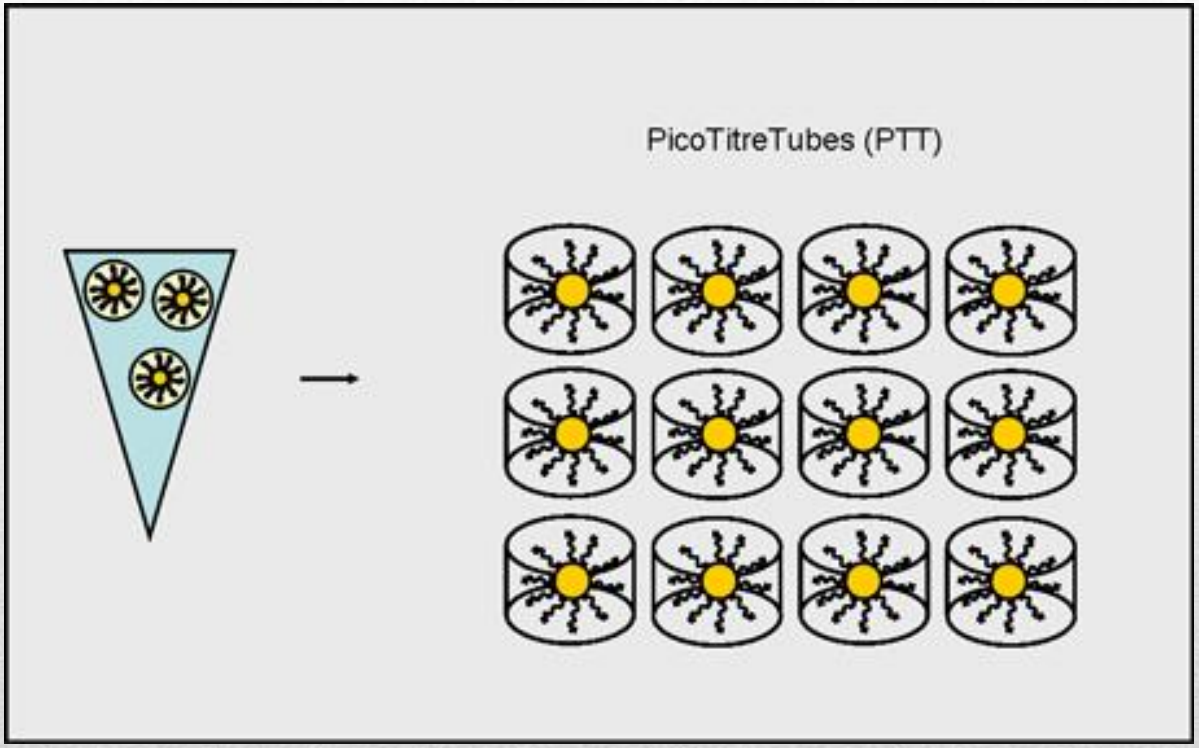
↓

Procedure 2 _ PCR Amplification



amplified
(28 um bead contributes to 1 million identical fragments)

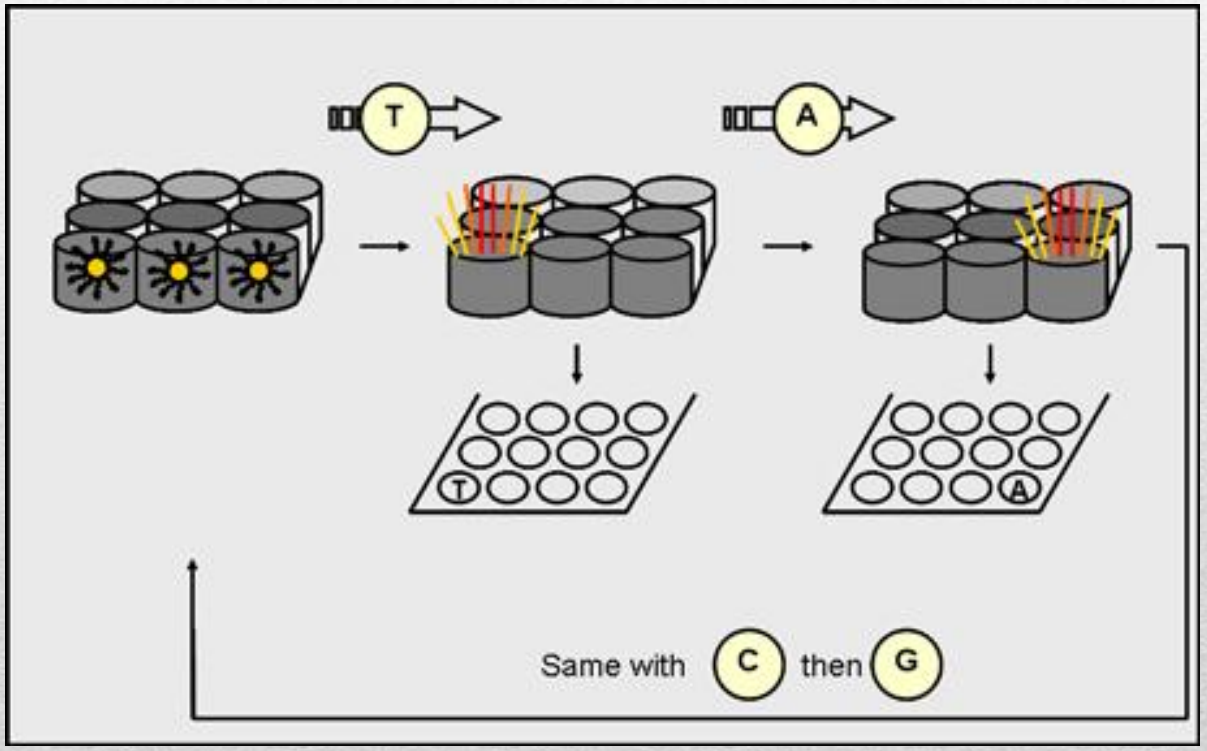
Procedure 3 _ PTT



dropped in to PTT

(with dimensions such that only one bead will fit per well .)

Procedure 4 _ Pyrosequencing



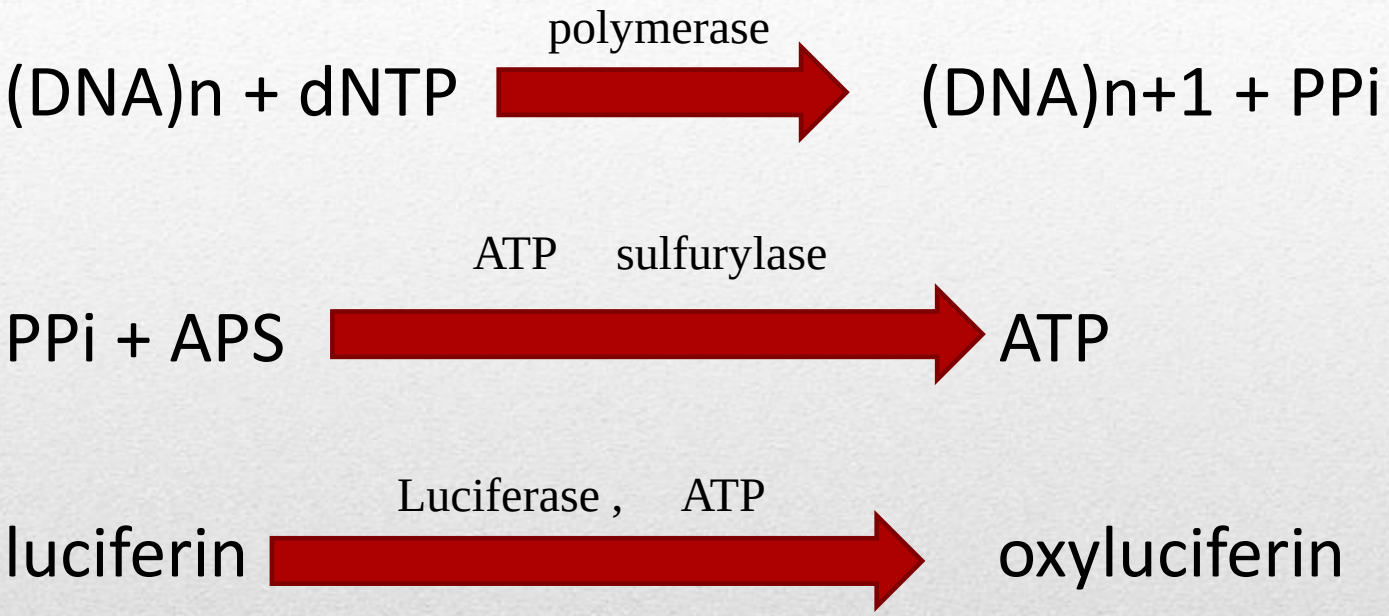
add nucleotide

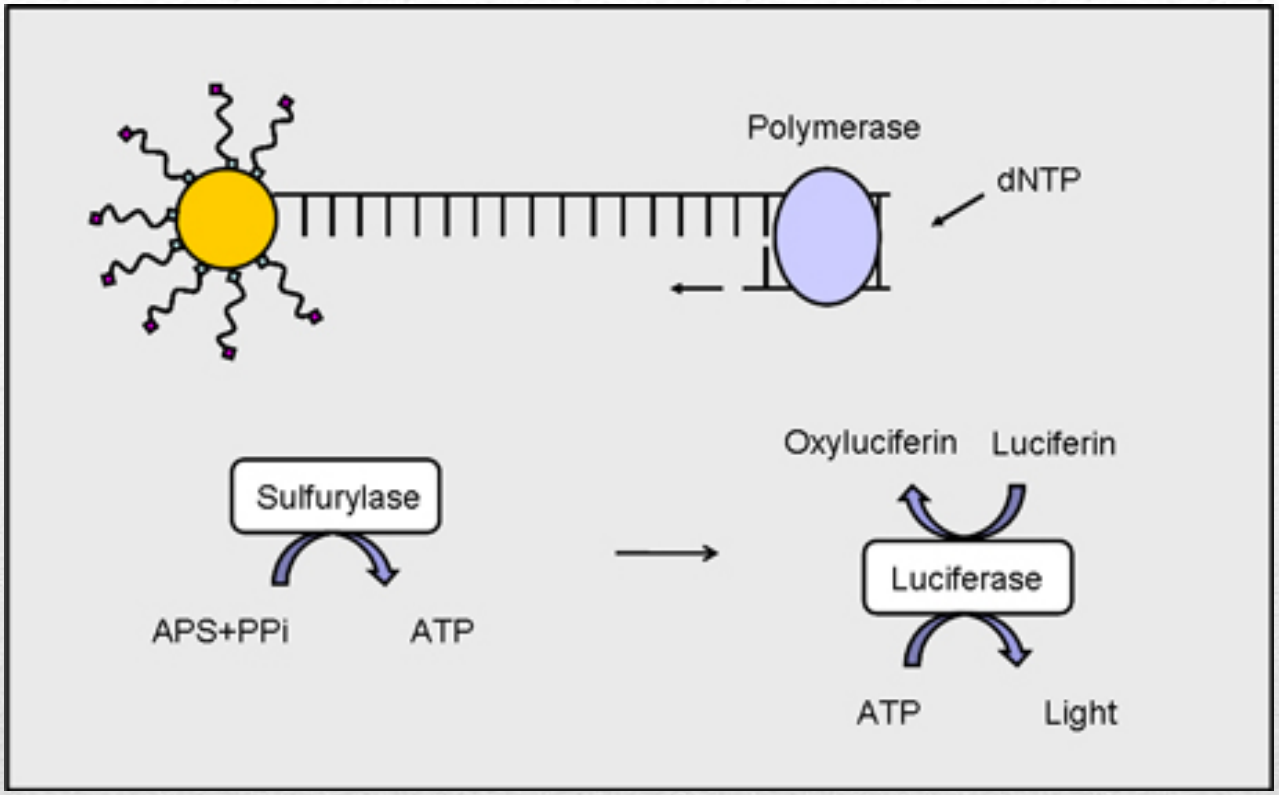
(one of 4 kinds of dNTPs is added per cycle.)

Introduction of Pyrosequensing

- DNA聚合酶（DNA polymerase）
- ATP硫酸化酶（ATP sulfurylase）
- 萤光素酶（luciferase）
- 三磷酸腺苷双磷酸酶（apyrase）
- adenosine 5´ phosphosulfate (APS)
- 萤光素（luciferin）

- 4 kinds of dNTP (dATPS, dTTP, dCTP, dGTP)
- primers.

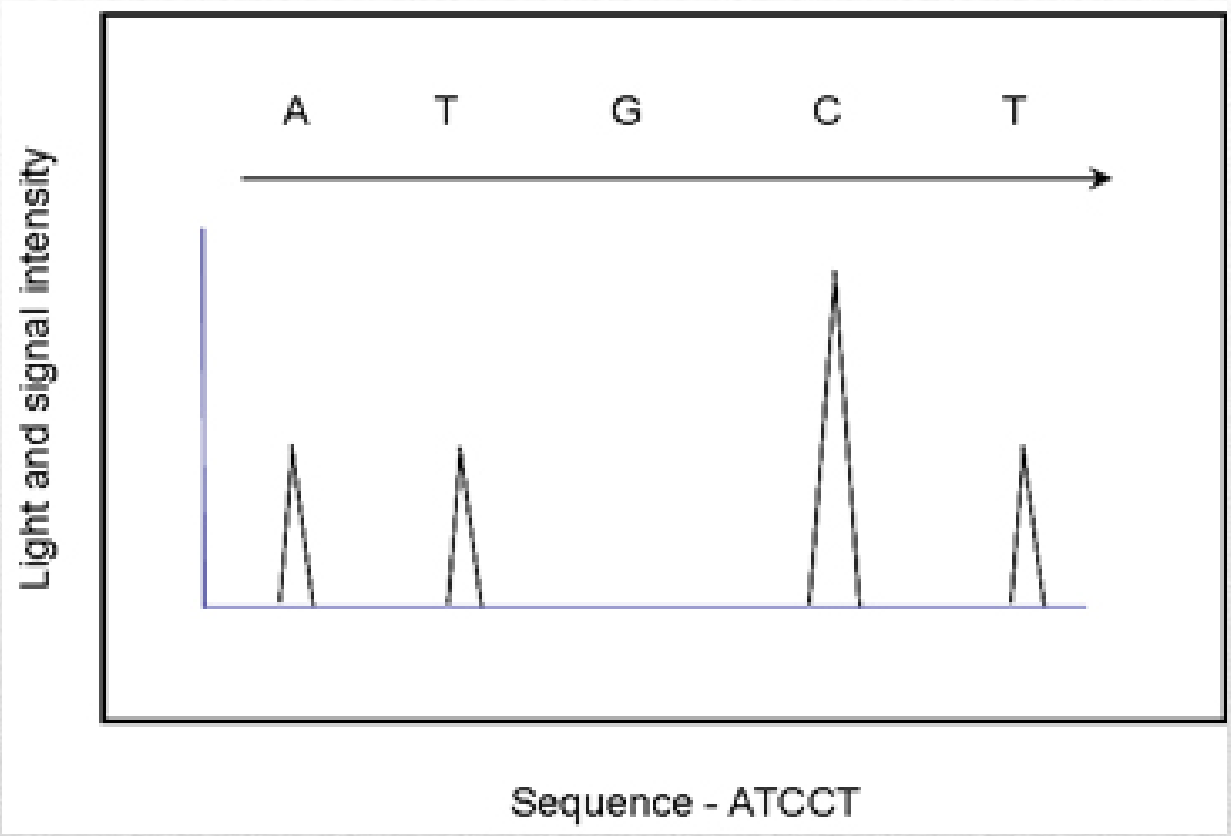

polymerase
(DNA)n + dNTP
(DNA)n+1 + PPi
ATP sulfurylase
PPi + APS
ATP
Luciferase , ATP
luciferin
oxyluciferin



sequentially add dNTPs
(the unbounded dNTP and the redundant ATP are removed by Apyrase.)

dNTP $\xrightarrow{\text{Apyrase}}$ dNDP + dNMP + phosphate

ATP $\xrightarrow{\text{Apyrase}}$ ADP + AMP + phosphate



reading the light signals.

⬇

information processed by software & hardware systems

⬇

sequencing completed !

Compared with 1st generation

	454	Sanger
cost	: ~ $60/megabase	~$500/megabase
speed	: ~1000Mb/day	~0.5Mb/day
accuracy:	99 %	99.999 %
read-length	: ~250bp	~1000 bp

Difficulties 454 faces

- Read length

➡ Higher throughput is an issue !

cannot be "bridged“

hard to “rebuild”

- Nonlinearity

➡ 8A or 7A being read is a problem !

- Not really cheap

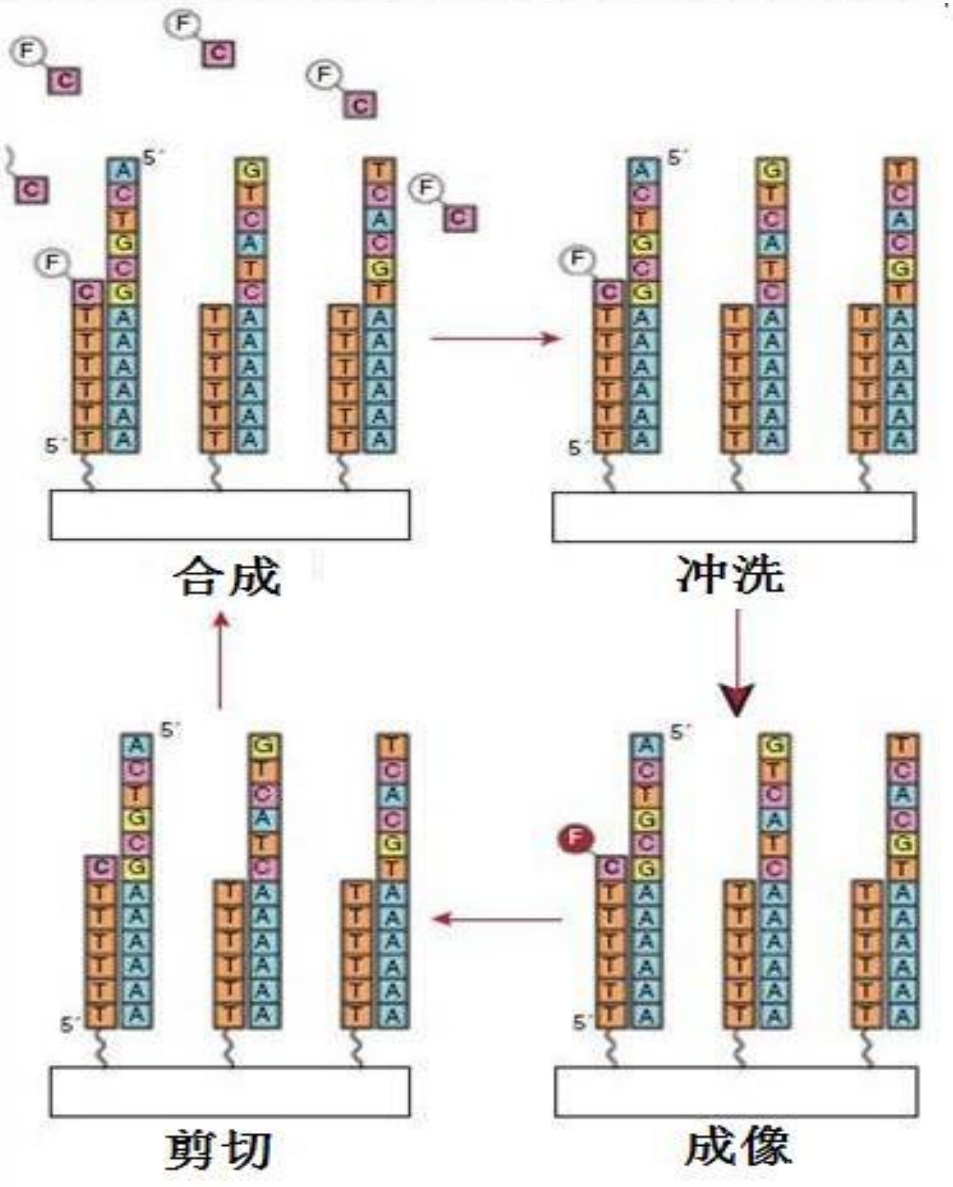
➡ SOLiD or Solexa is an alternative !

The “Next-next generation” sequencing

tSMS : true single molecule synthesis

➡ Cheaper !
Faster !
More accurate !

by accurately reading the 6400 base pair long genome of the M13 virus.


合成
冲洗
剪切
成像

The END

Thank you for your attention.