

# 單株抗體在生物技術上的應用

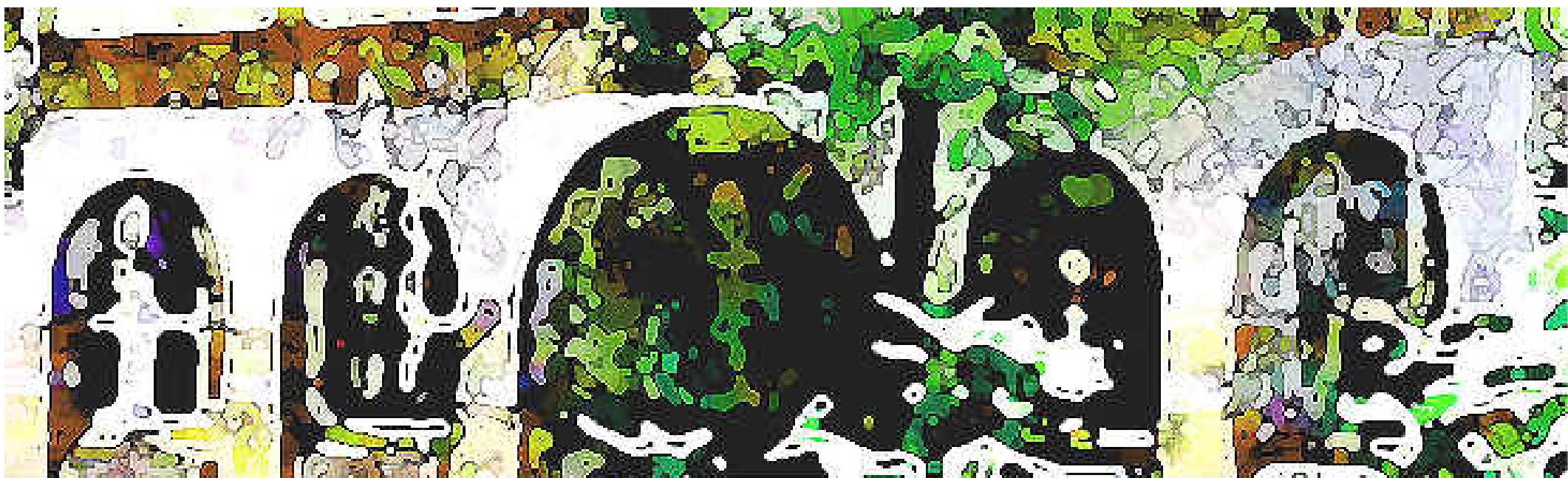
## (1) **Monoclonal Antibody** 單株抗體 (奈米探針)

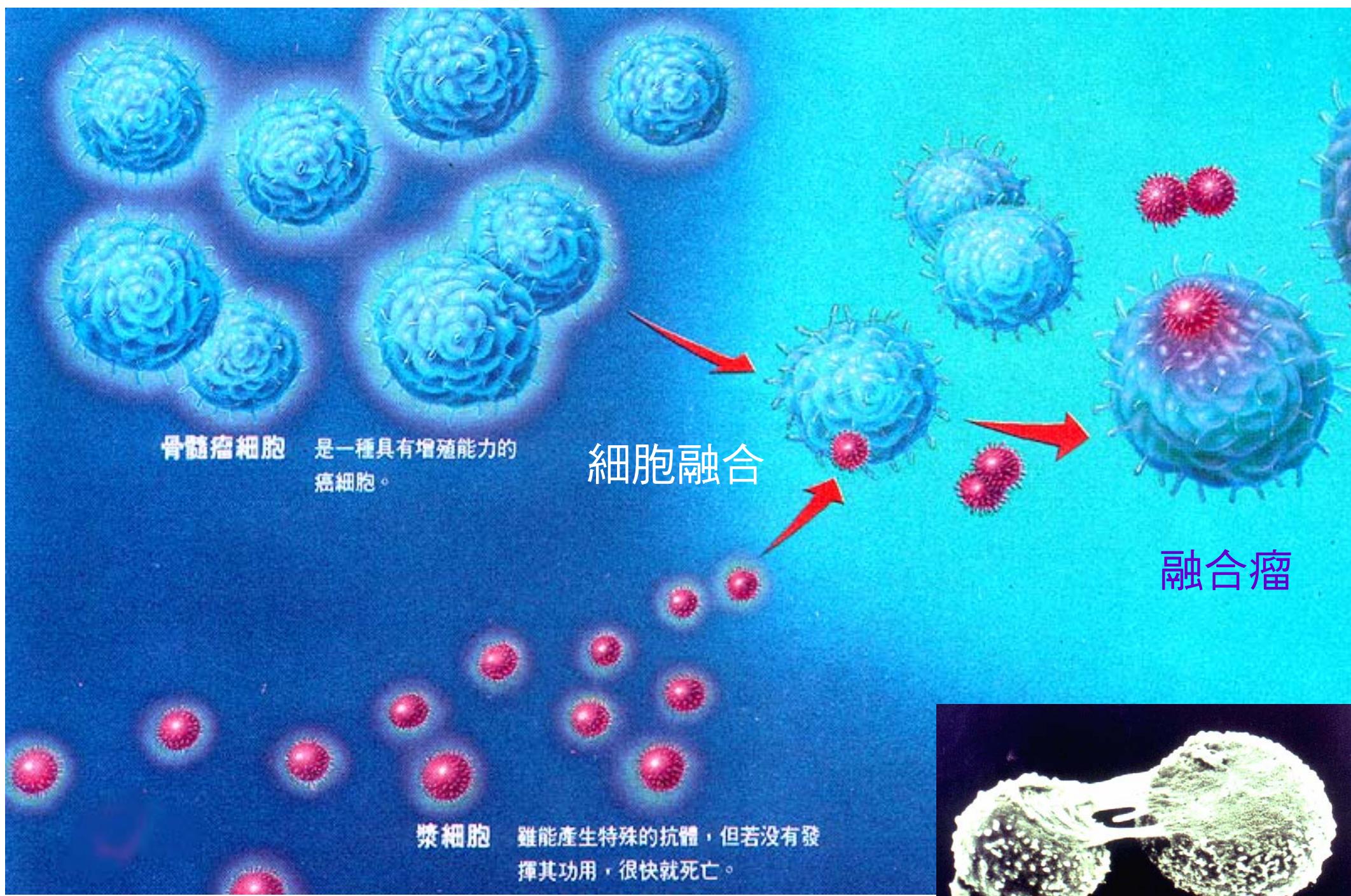
免疫反應 - 抗體的專一性 - 細胞融合法

## (2) **Proteomics and Antibody Bank** 蛋白質體與抗體庫

蛋白質體學 - 建立抗體庫 - 抗體庫的應用

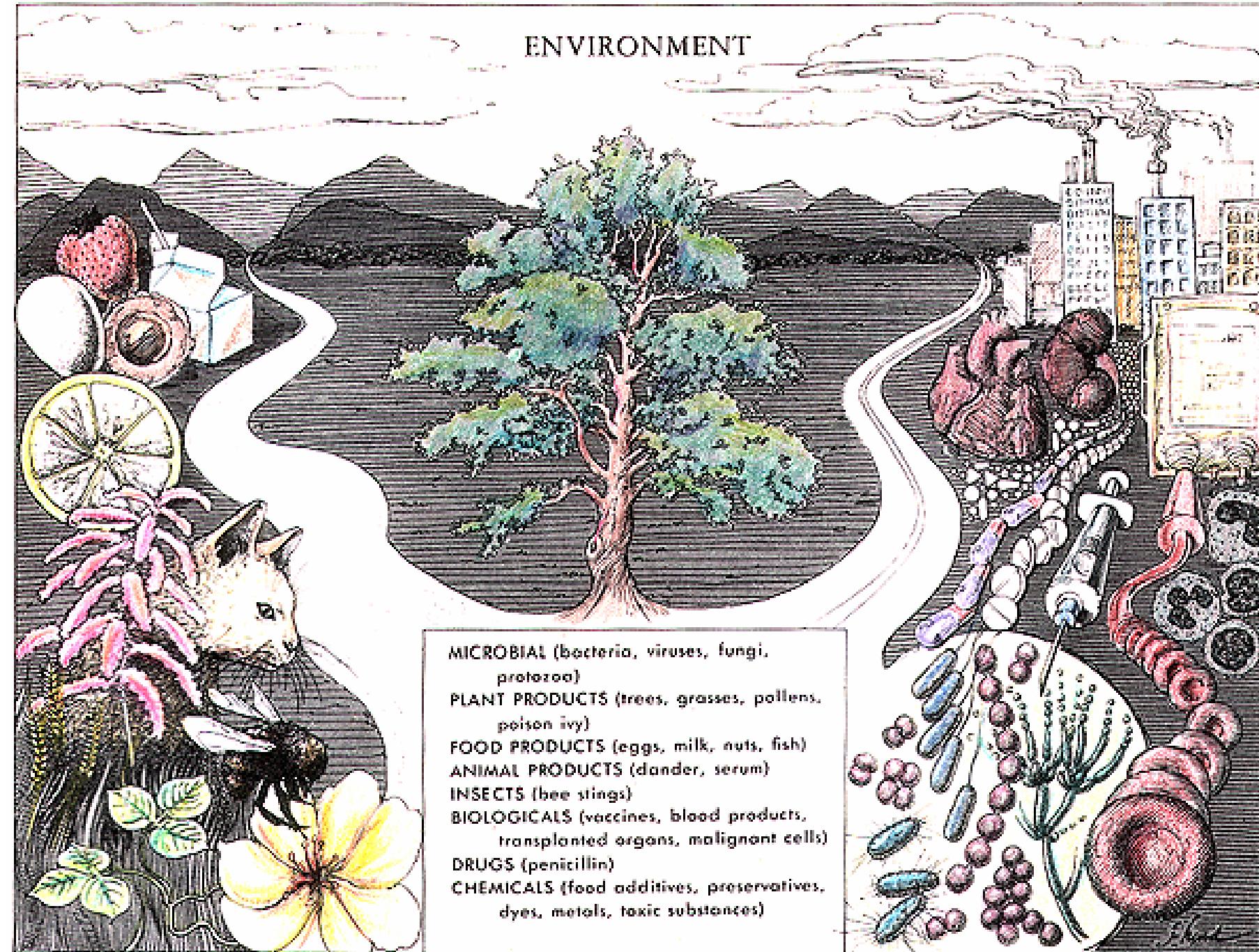
國立台灣大學 生化科技學系 莊榮輝





# 許多外在環境物質可引發免疫反應

過敏  
食物  
動物  
植物  
花粉  
昆蟲



污染  
移植  
藥物  
輸血  
細菌  
病毒

# 免疫系統

脊椎動物體內有摧毀外來入侵物體的免疫系統

兩大系統 →

先天免疫系統

後天免疫系統

兩種方式

細胞

分子

巨噬細胞

(Macrophage, mφ)

自然殺手細胞

(Natural killer cell, NK)

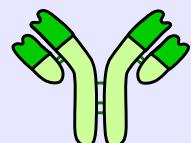
干擾素 (Interferon)

溶菌酶 (Lysozyme)

T 細胞 ( $T_H$ ,  $T_S$ ,  $T_K$ )

B 細胞

生 產



抗体 (Antibody, Ab)

兩大系統 →

警察系統 (+ 調察局)

軍事系統

B 細胞分泌抗體

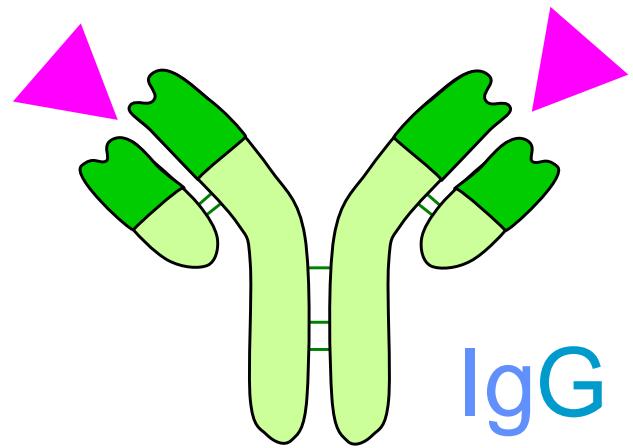
凝集細菌



如何凝集

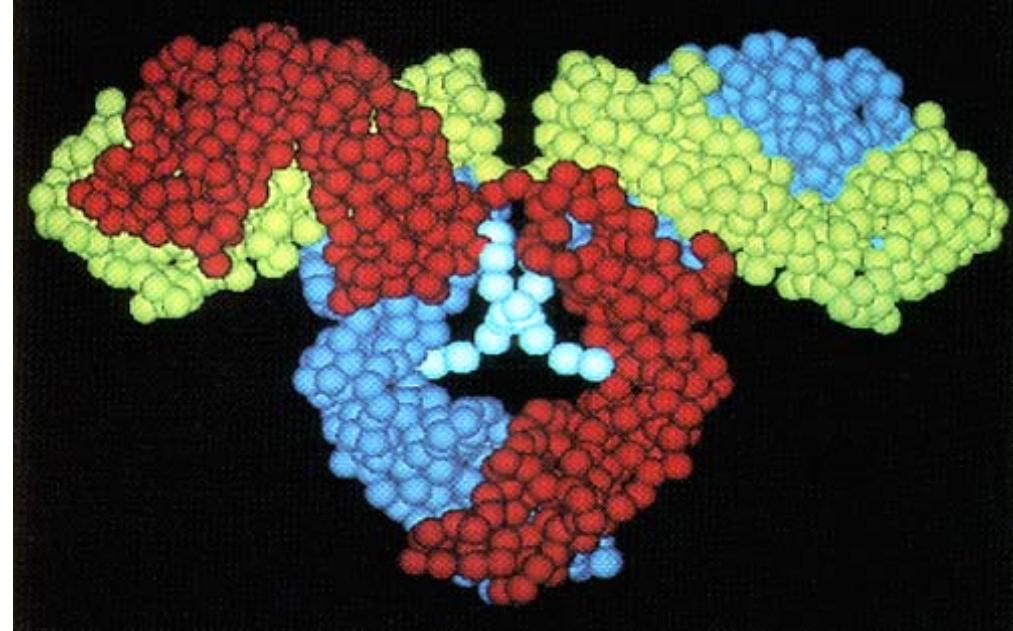
# ■ 抗體 是一種蛋白質

Davies et al (1977) PNAS / Roitt et al (2001) Immunology. p.73



IgG

Immunoglobulin



- 抗體由四條 蛋白質 長短鍊所組成 (兩長兩短)
- 抗體分子上有兩個 抗原結合區 (二者相同) ▼
- 抗體與抗原結合是 專一性的 (lock & key)

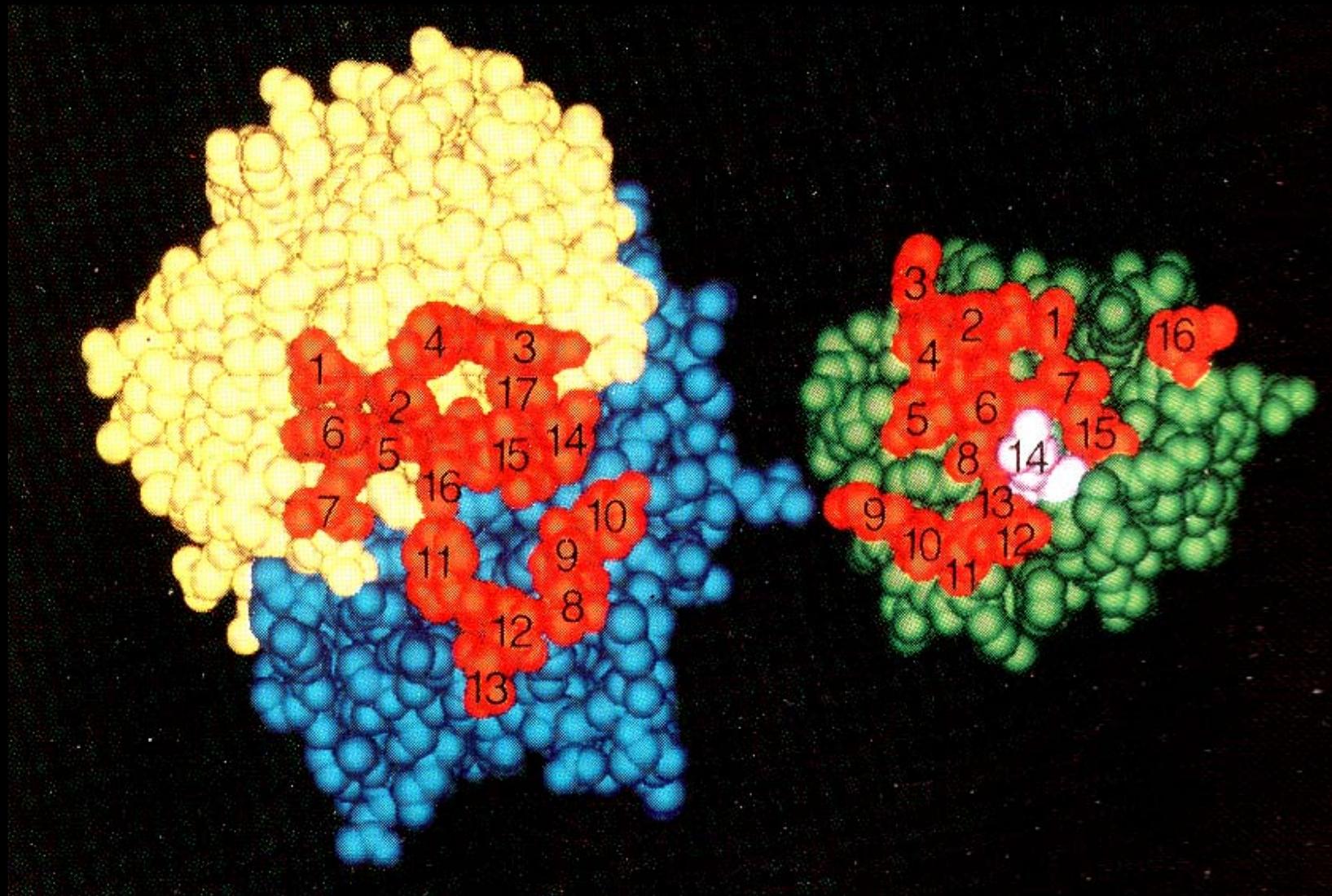
● IgG 是單一個抗體分子，另有 IgM (五元體) 及 IgA (二元體)

步兵單兵

裝甲兵

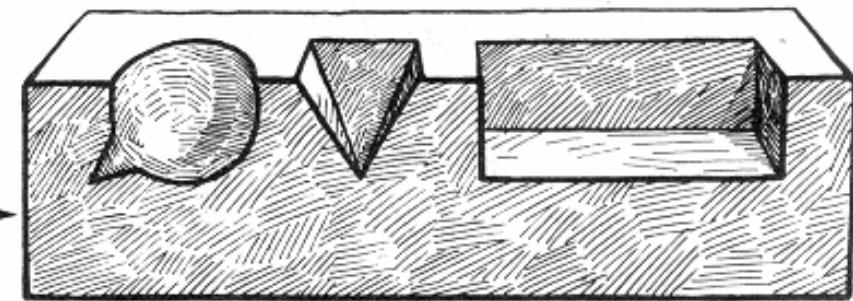
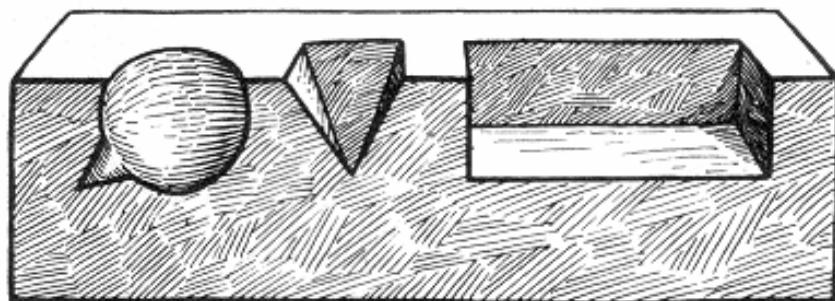
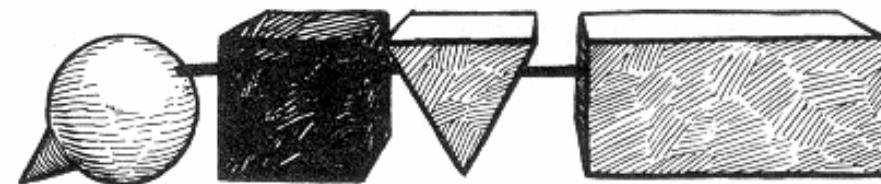
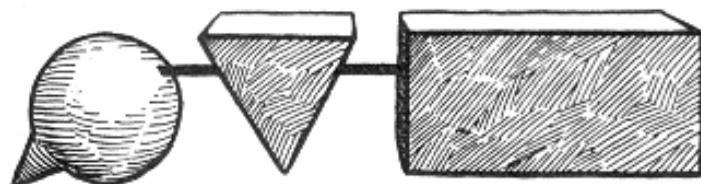
兩棲部隊

# 抗體結合區



# 抗體與抗原的結合面有如互補的積木

抗 原



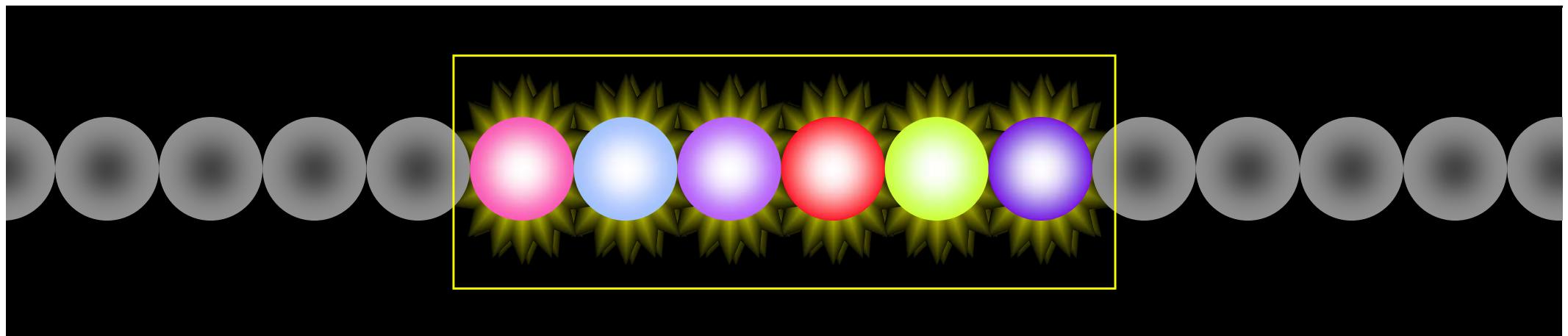
抗 體

OK

X

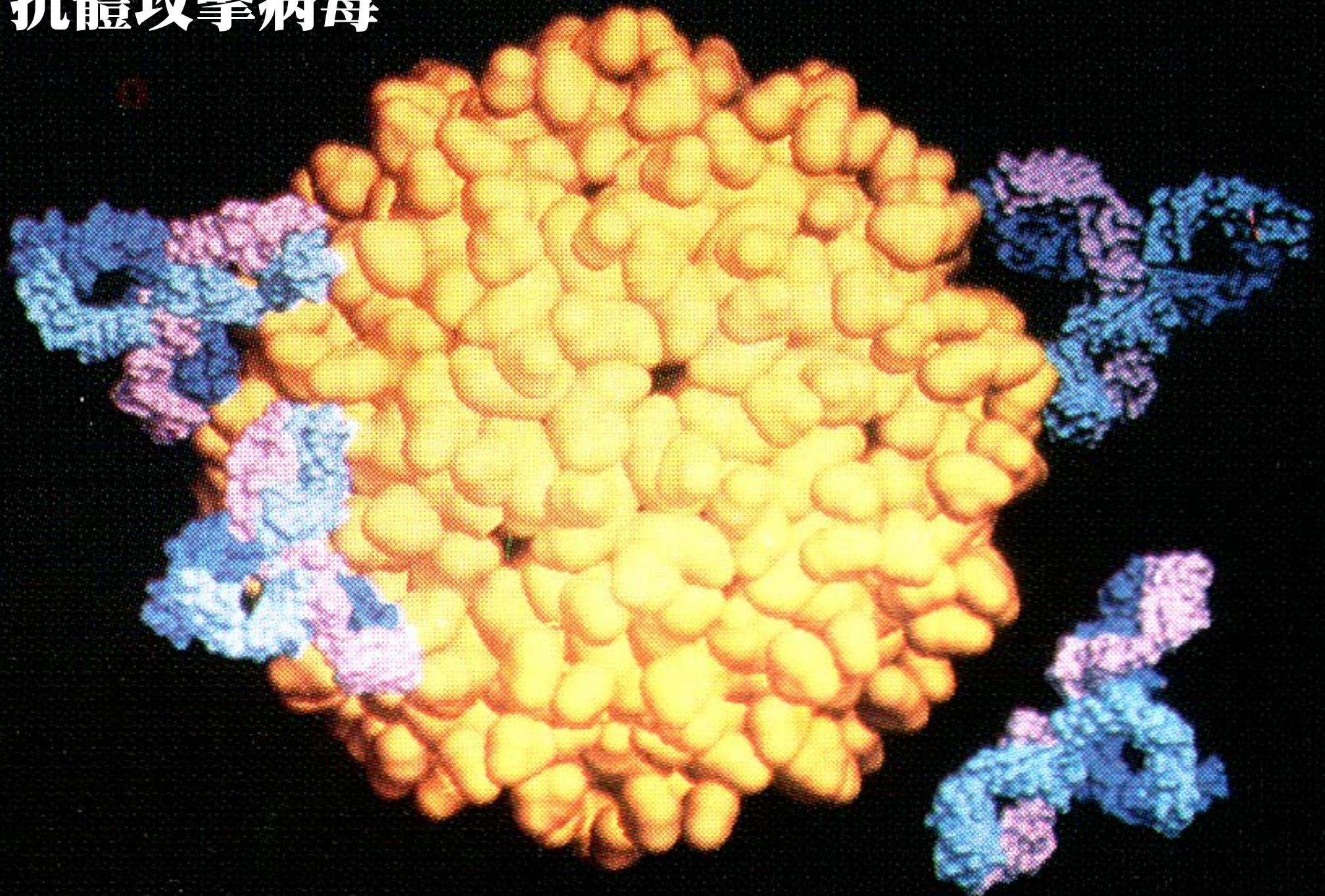
## 抗原決定基

- 一個抗原分子上可能有數個 抗原決定基
- 每個 抗原決定基 至少誘生一種專一性抗体

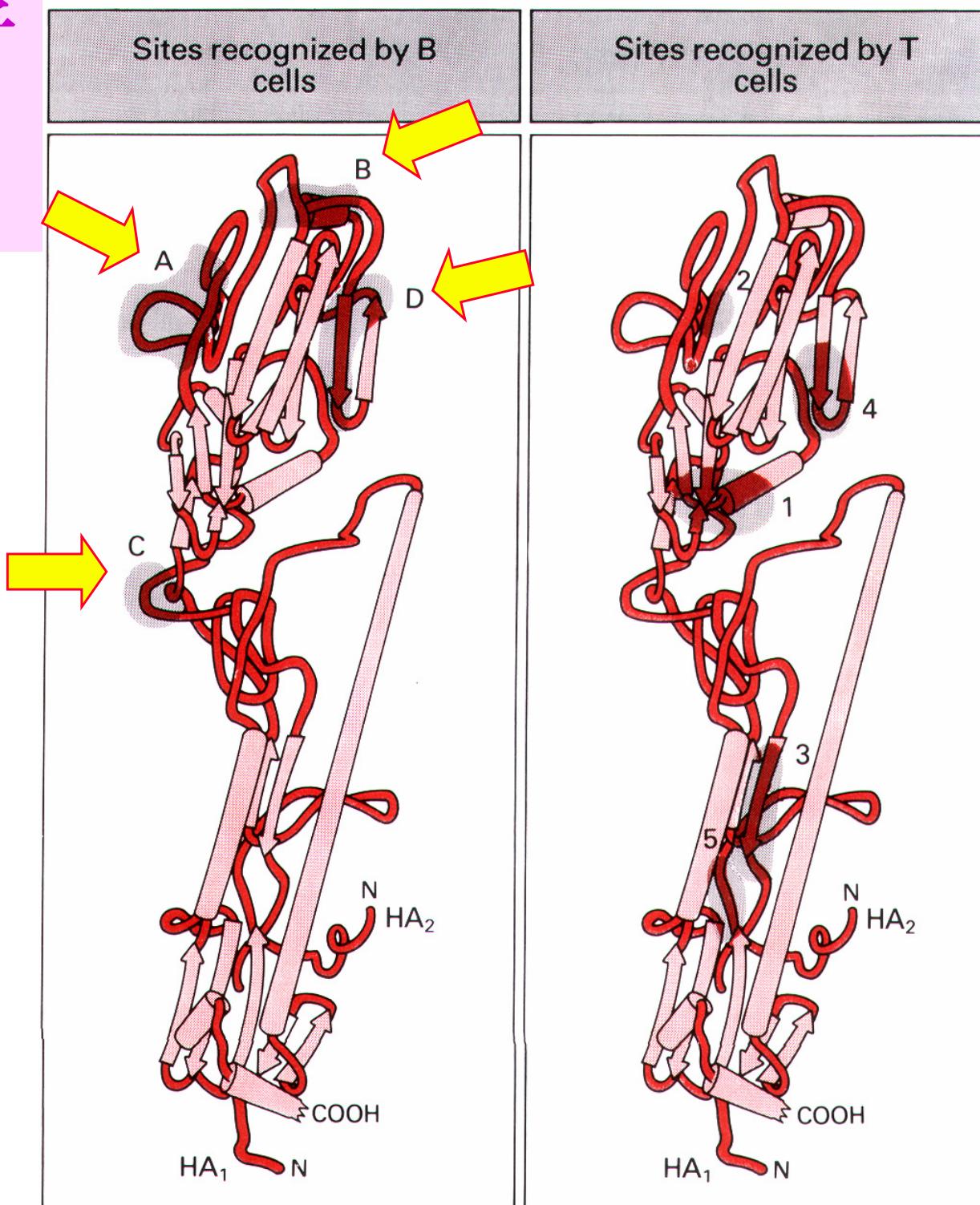


- 蛋白質性 抗原決定基 含有六個以上胺基酸

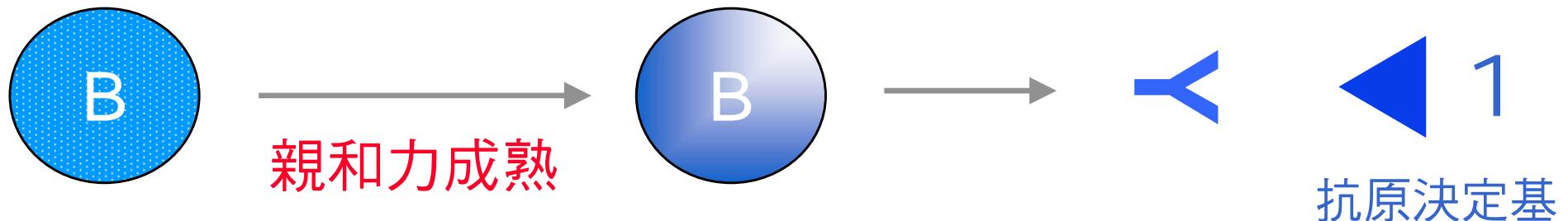
# 抗體攻擊病毒



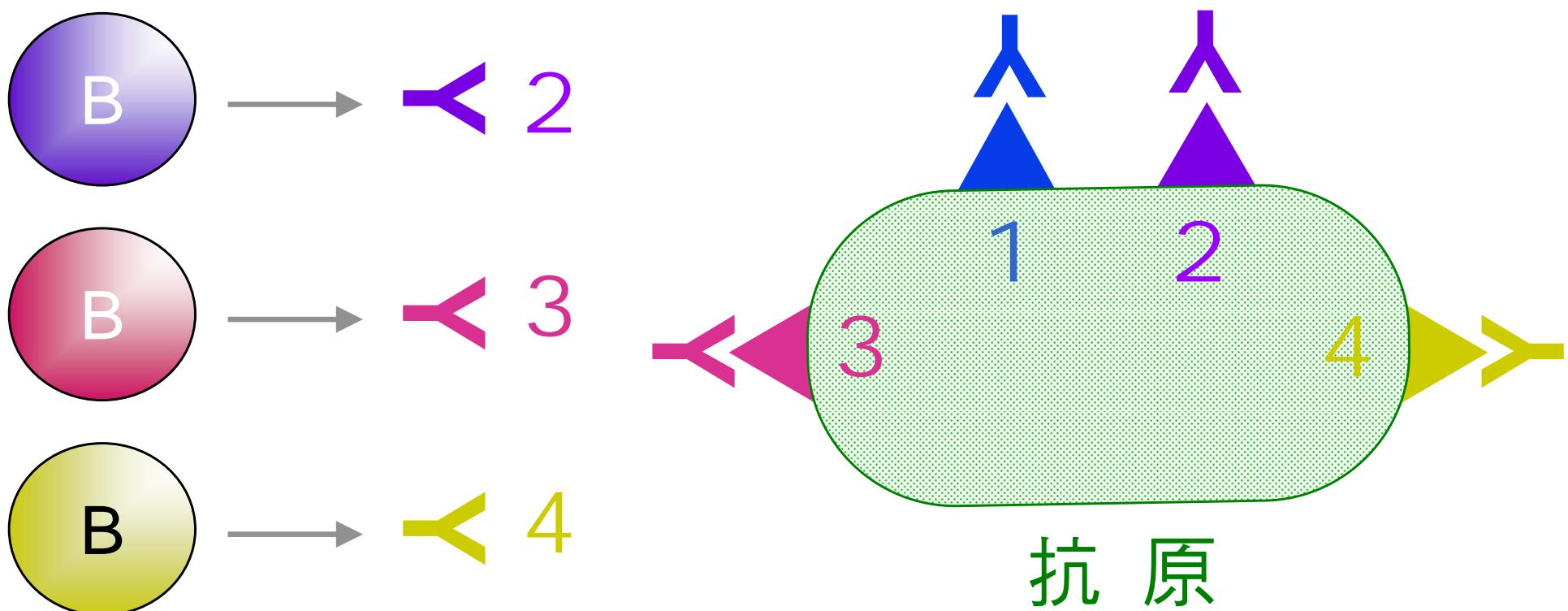
# 病毒表面分子 上有許多個 抗體結合區



- 一個 B 細胞只能生產一種抗体，對付某一抗原決定基。



- 若有許多抗原決定基，則需許多株 B 細胞分別生產許多抗体。

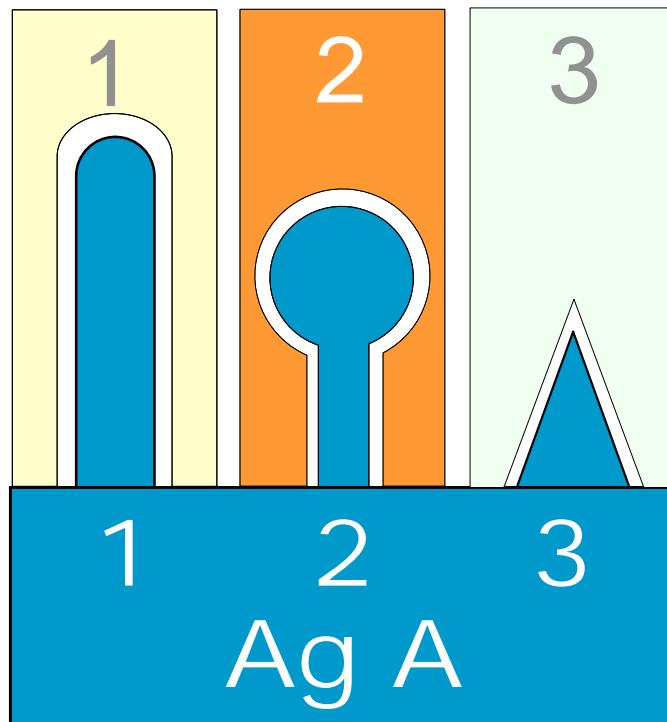


# 傳統抗血清的交叉反應

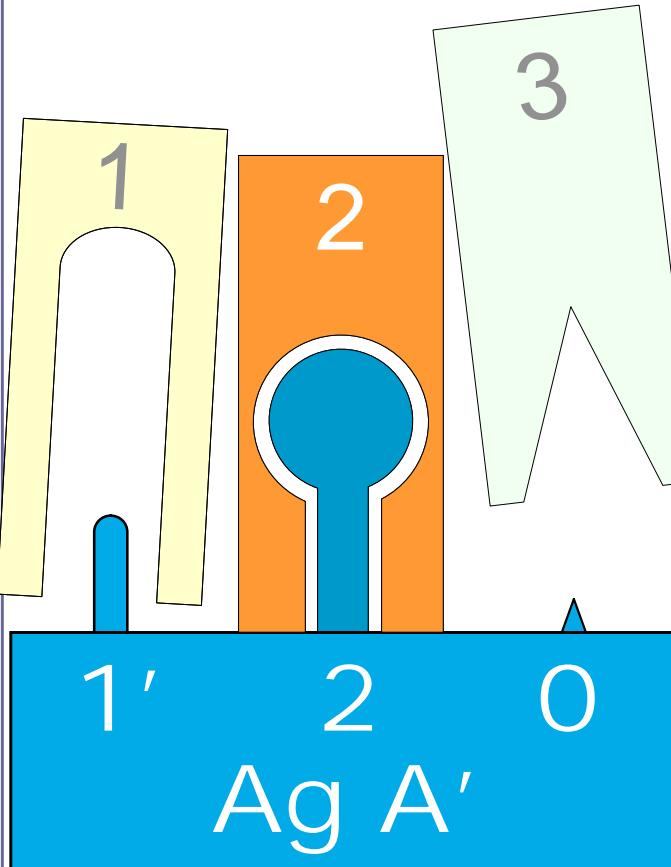
專一性反應

交叉反應

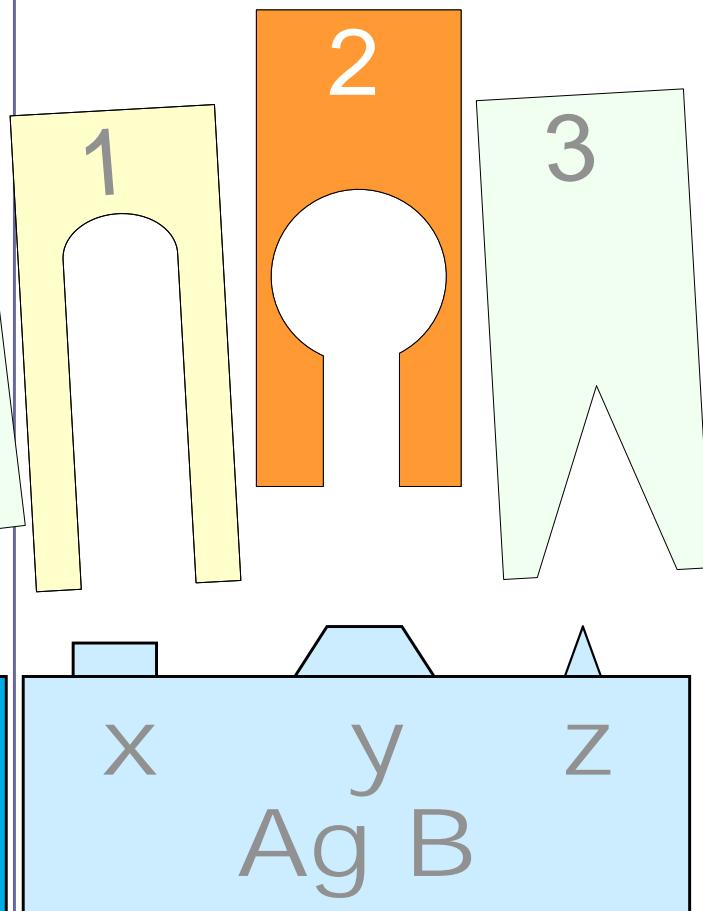
沒有反應



+

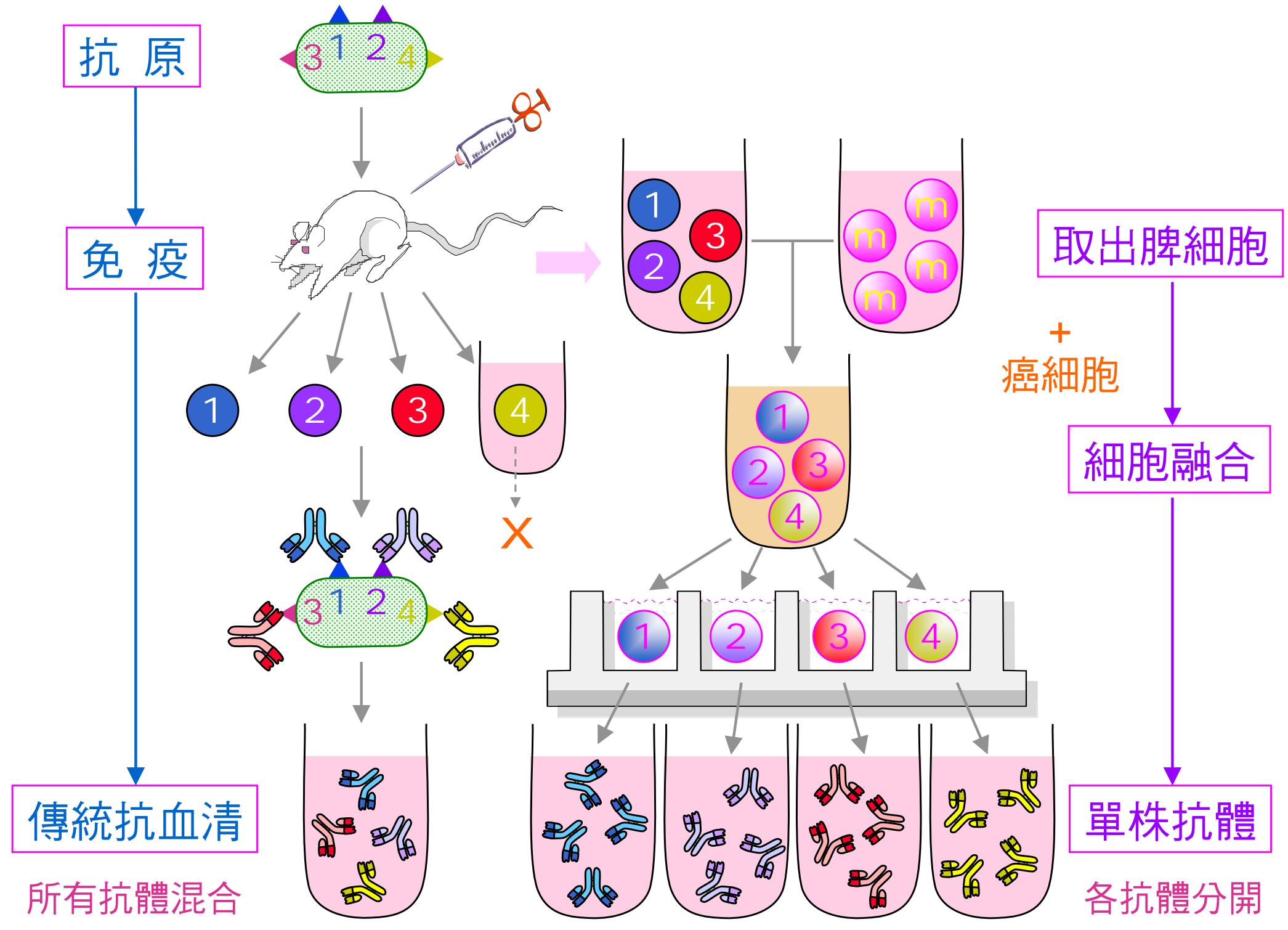


?



-

Adapted from  
Roitt et al (1985) *Immunology*. 6.7

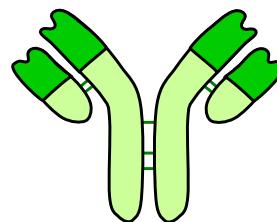


Adapted from Milstein (1980) *Scientific American*, Oct. p.58

實驗步驟

# 細胞融合法

## Cell Fusion



細胞融合 → 初步篩選 → 專一性篩選 → 單株化 → 抗體生產

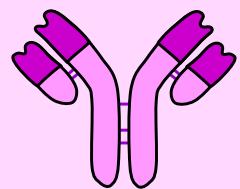
PEG

HAT

ELISA

Limiting  
dilution

Ascites  
fluid

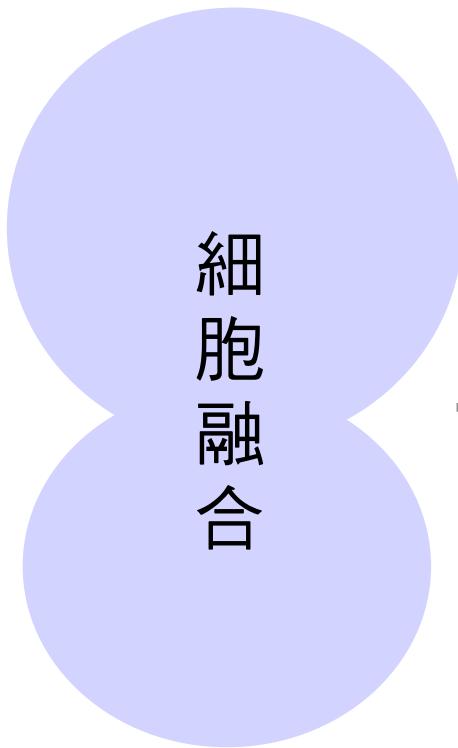
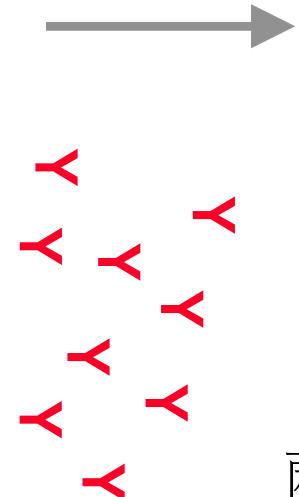


# 單株抗體

可生產有用抗體的 淋巴細胞 若與 癌細胞 融合，則形成穩定而可培養的細胞株。



一個 B cell 只  
產生一種抗體

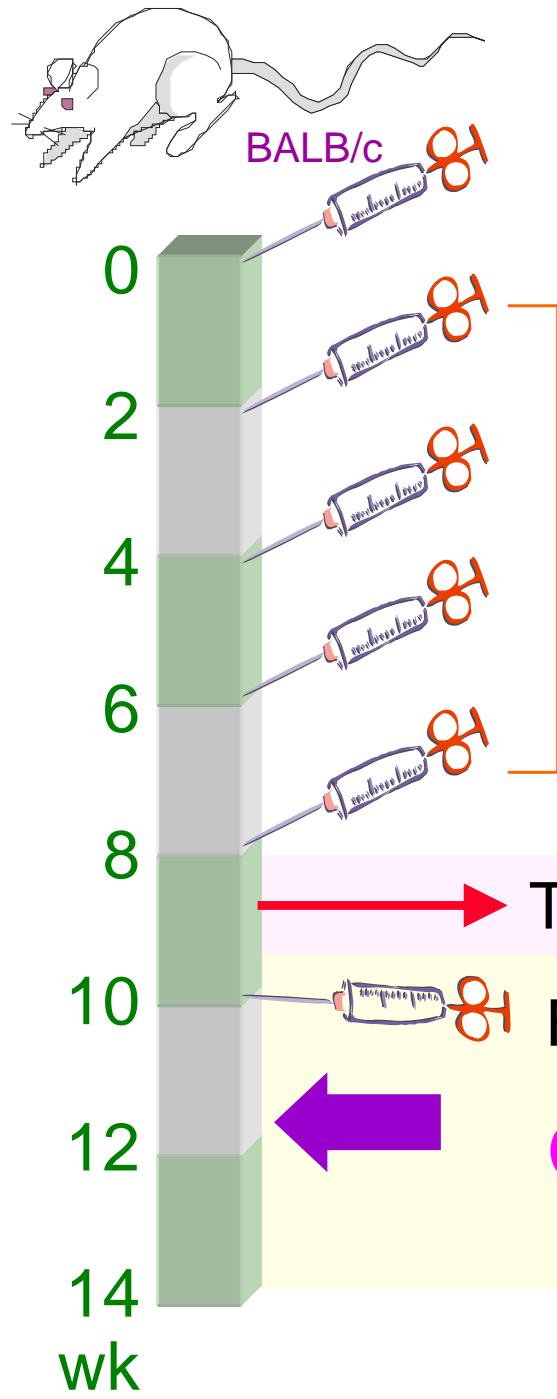


兩組染色體混在一起



也可以培養生長  
產生專一性抗體

# 小白鼠免疫流程



Antigen (50 µg/mouse)

加佐劑製成乳劑

Emulsified in 0.5 mL

Freund's Complete Adjuvant

At least three booster shots,  
same dose in 0.5 mL

Freund's Incomplete Adjuvant

Booster shots might be reduced  
if TiterMax is used as adjuvant

試採血

Trial Bleeding → Titer Determination

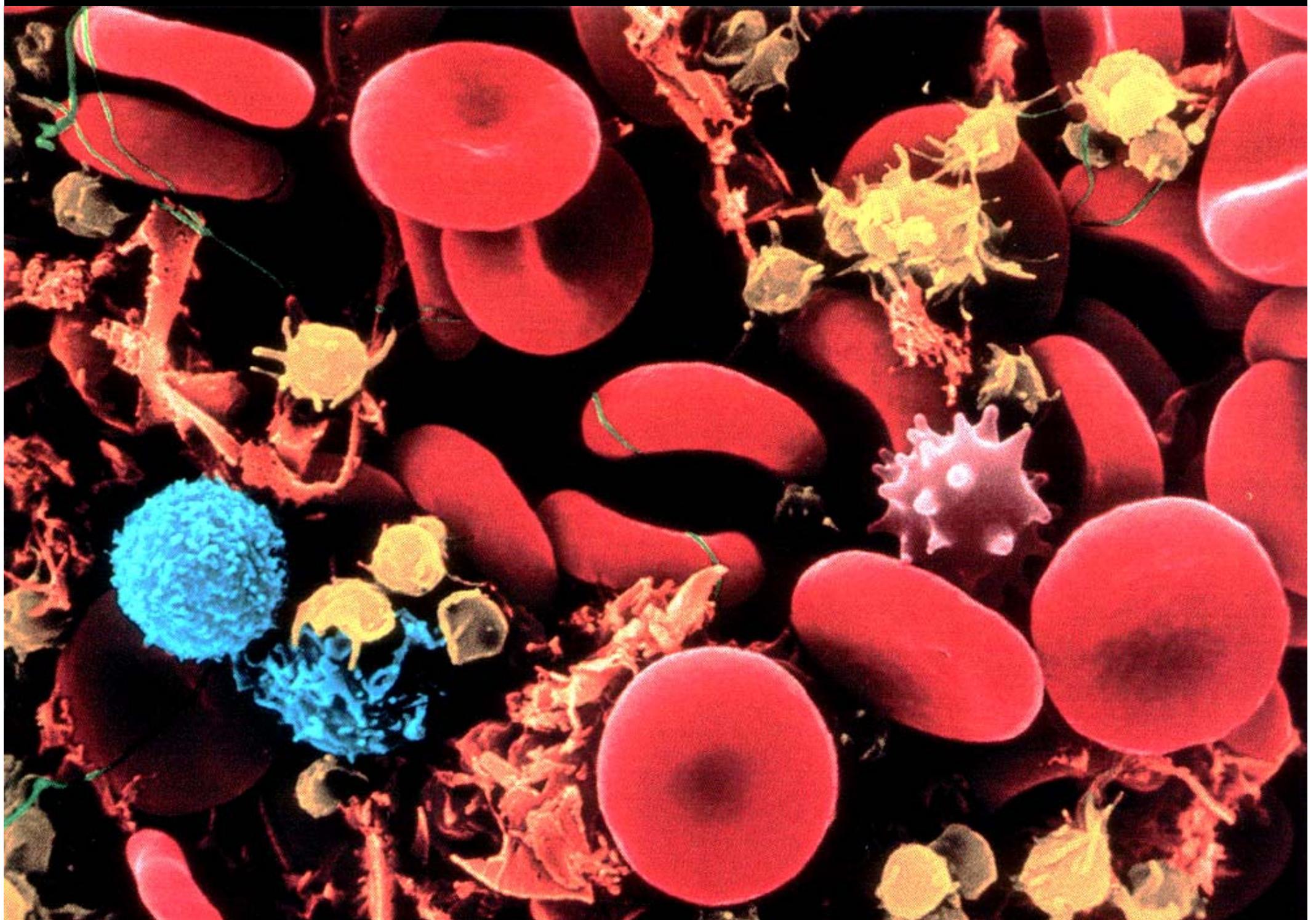
Final booster (soluble antigen)

Cell fusion

# 脾臟為 B 細胞集中地

取出脾臟內細胞

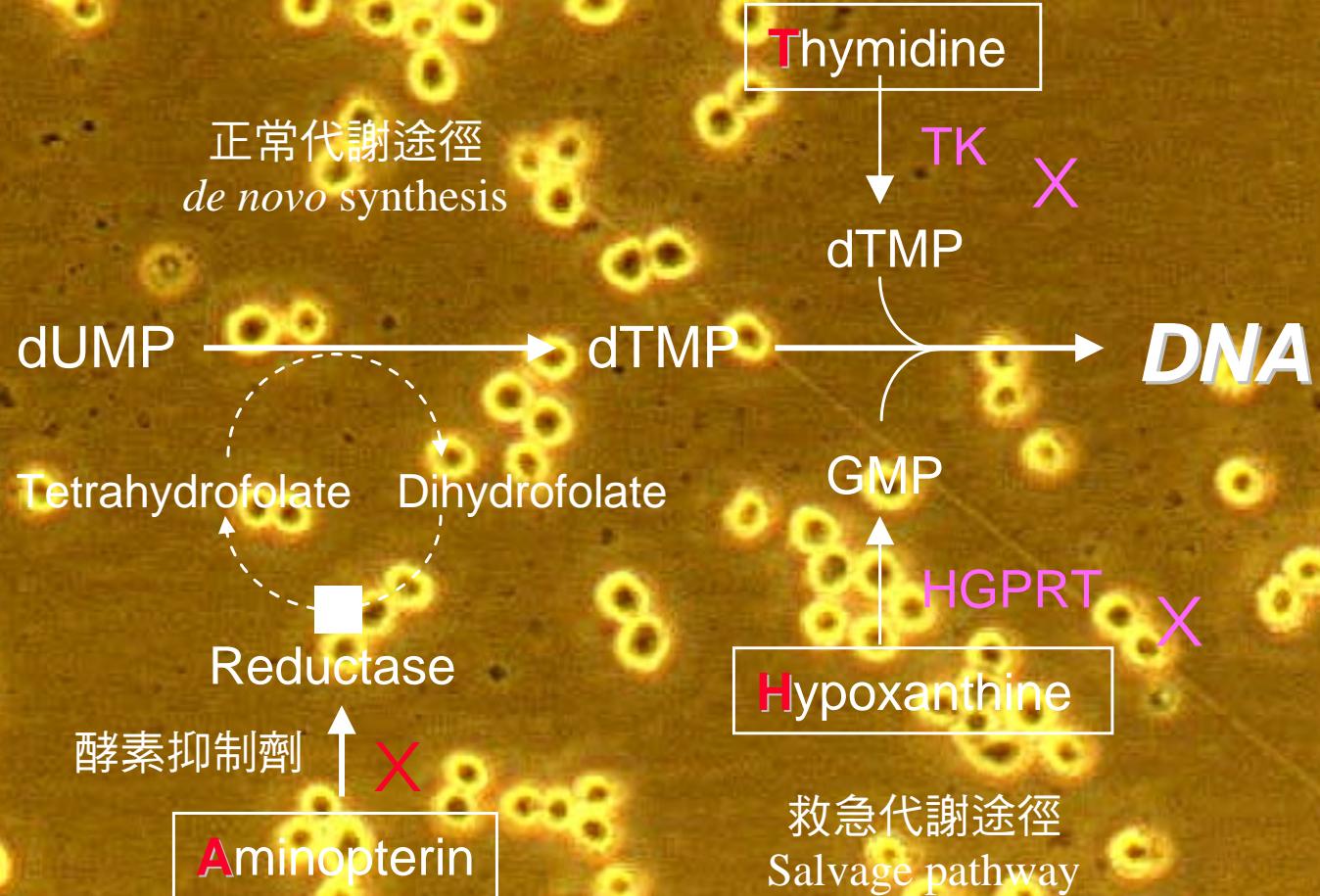




Dennis Kunkel / *Everyday Science Explained*. p.243

# 癌細胞可以在培養基中長久生長

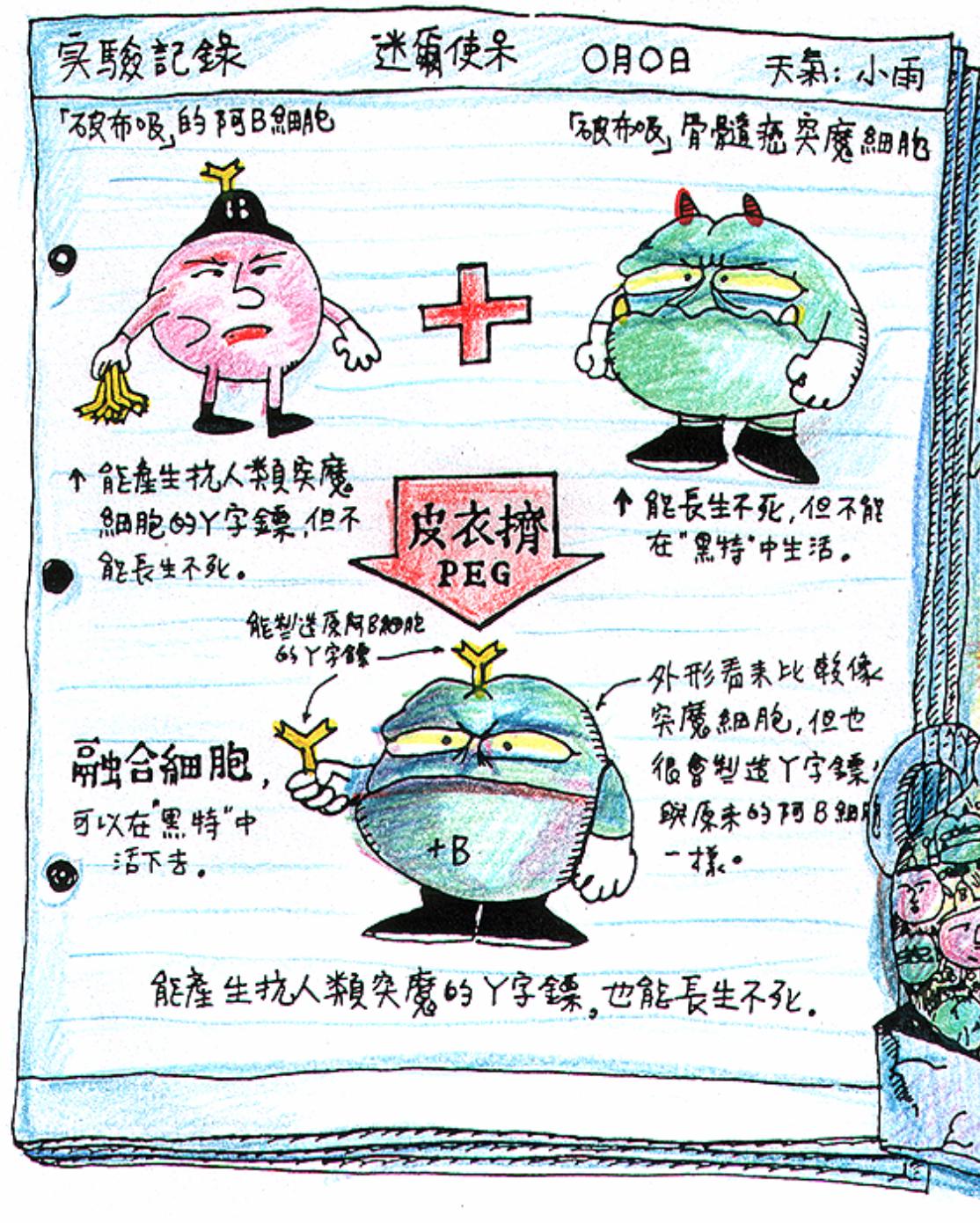
但是骨髓癌細胞 NS-1 無法在 HAT 中生長



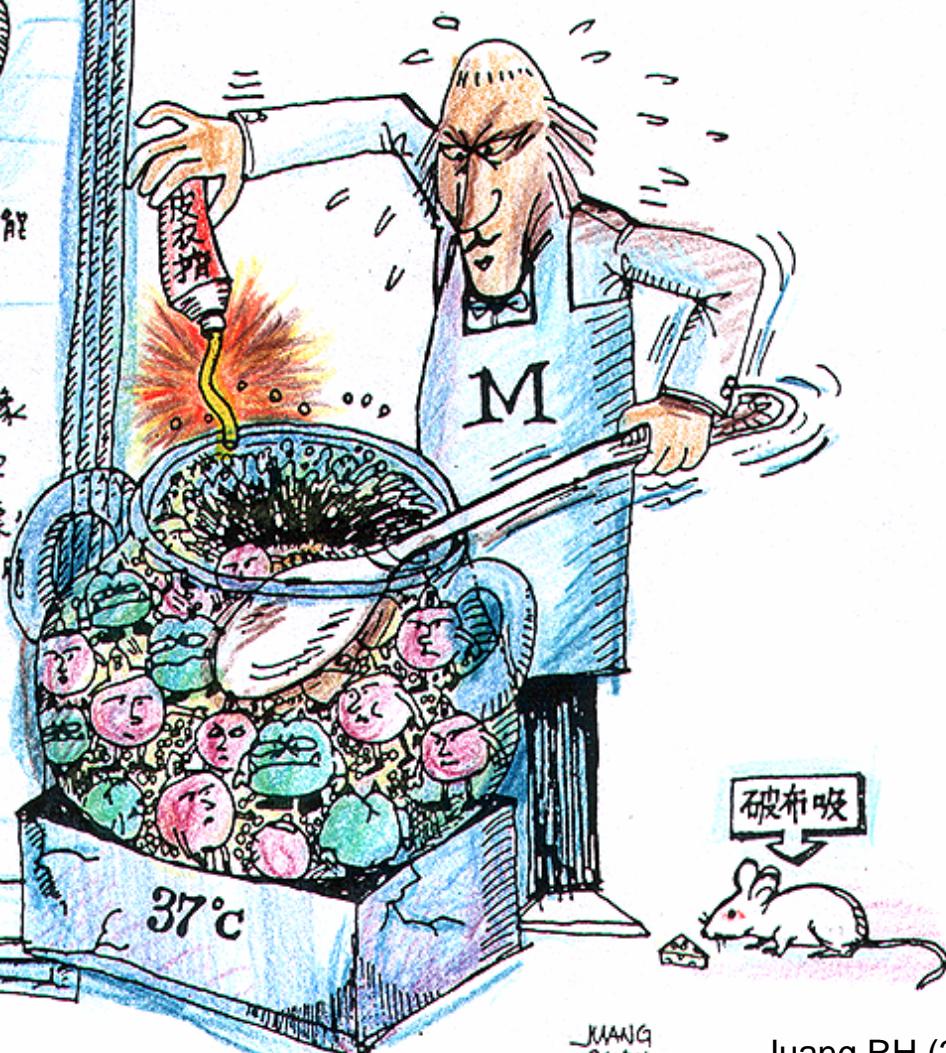
# 以化學試劑將細胞融合



# 迷爾使呆的研究日誌

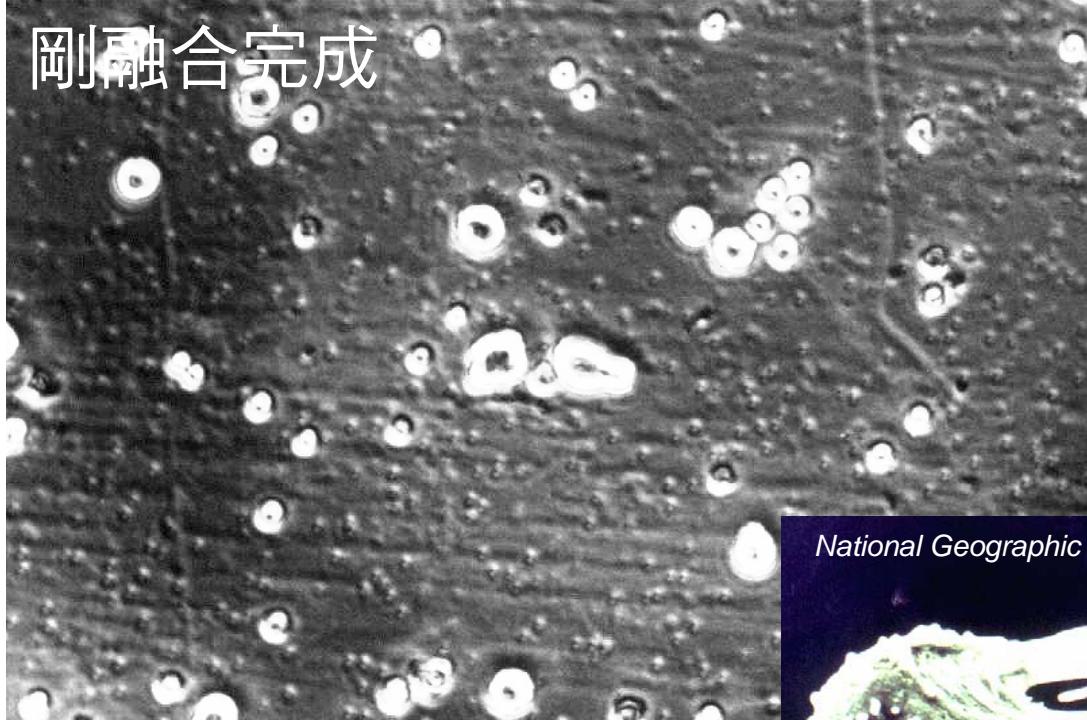


Kohler & Milstein (1984)

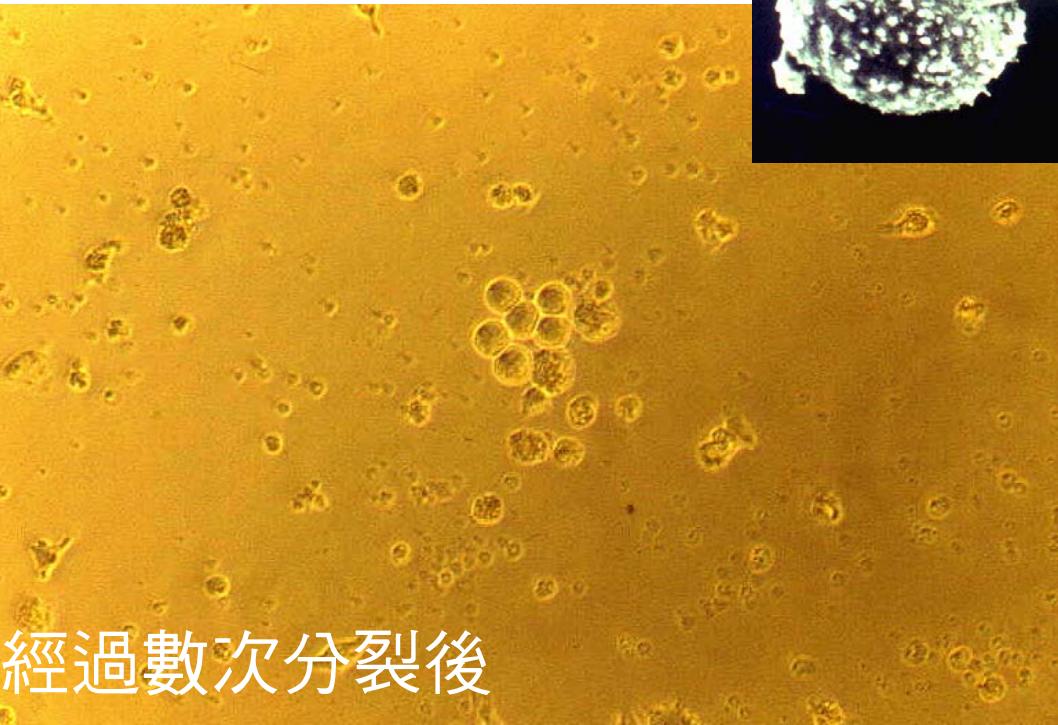
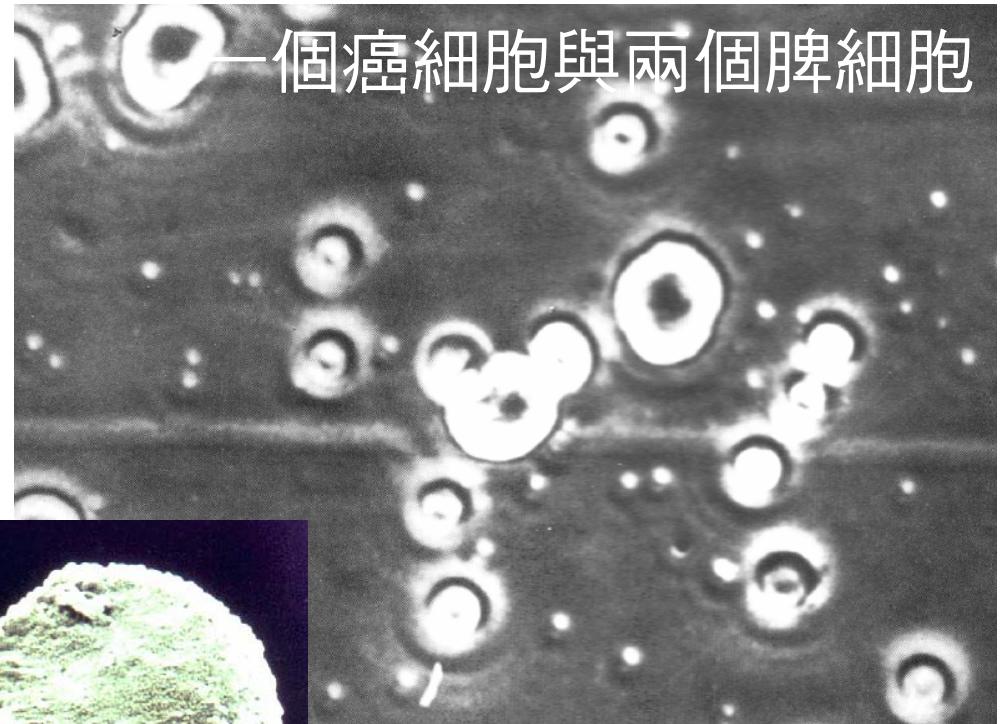


# 融合後的細胞生長情形

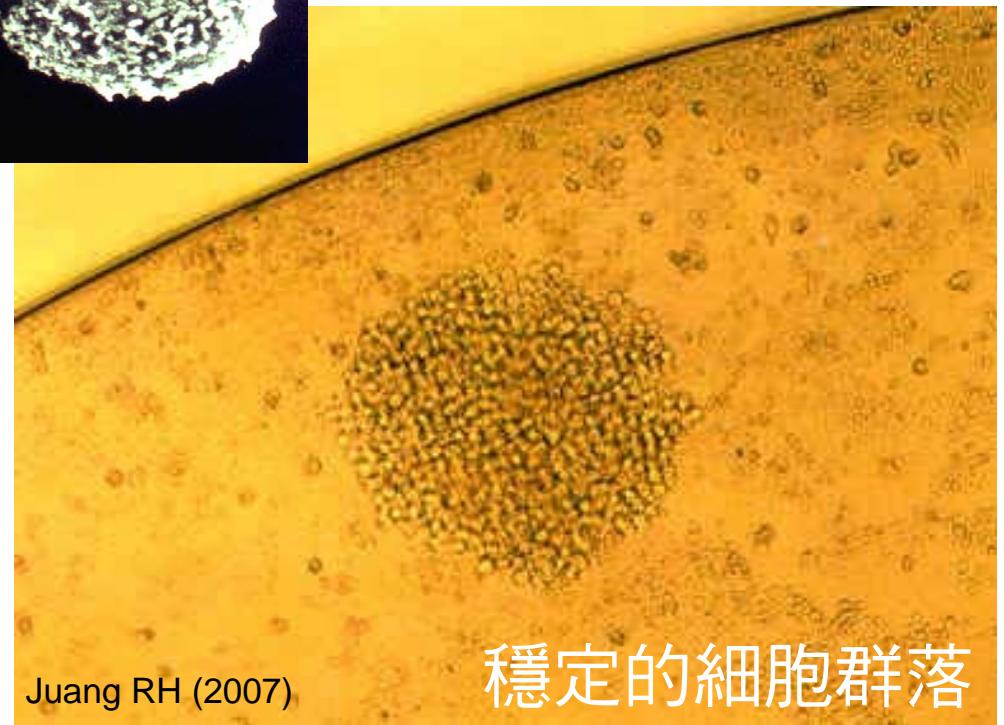
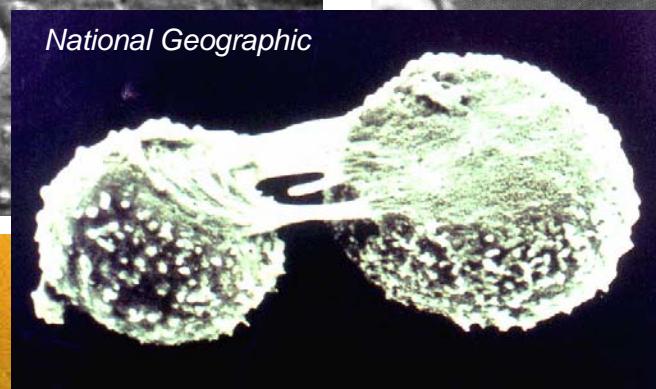
剛融合完成



一個癌細胞與兩個脾細胞



經過數次分裂後



Juang RH (2007)

穩定的細胞群落

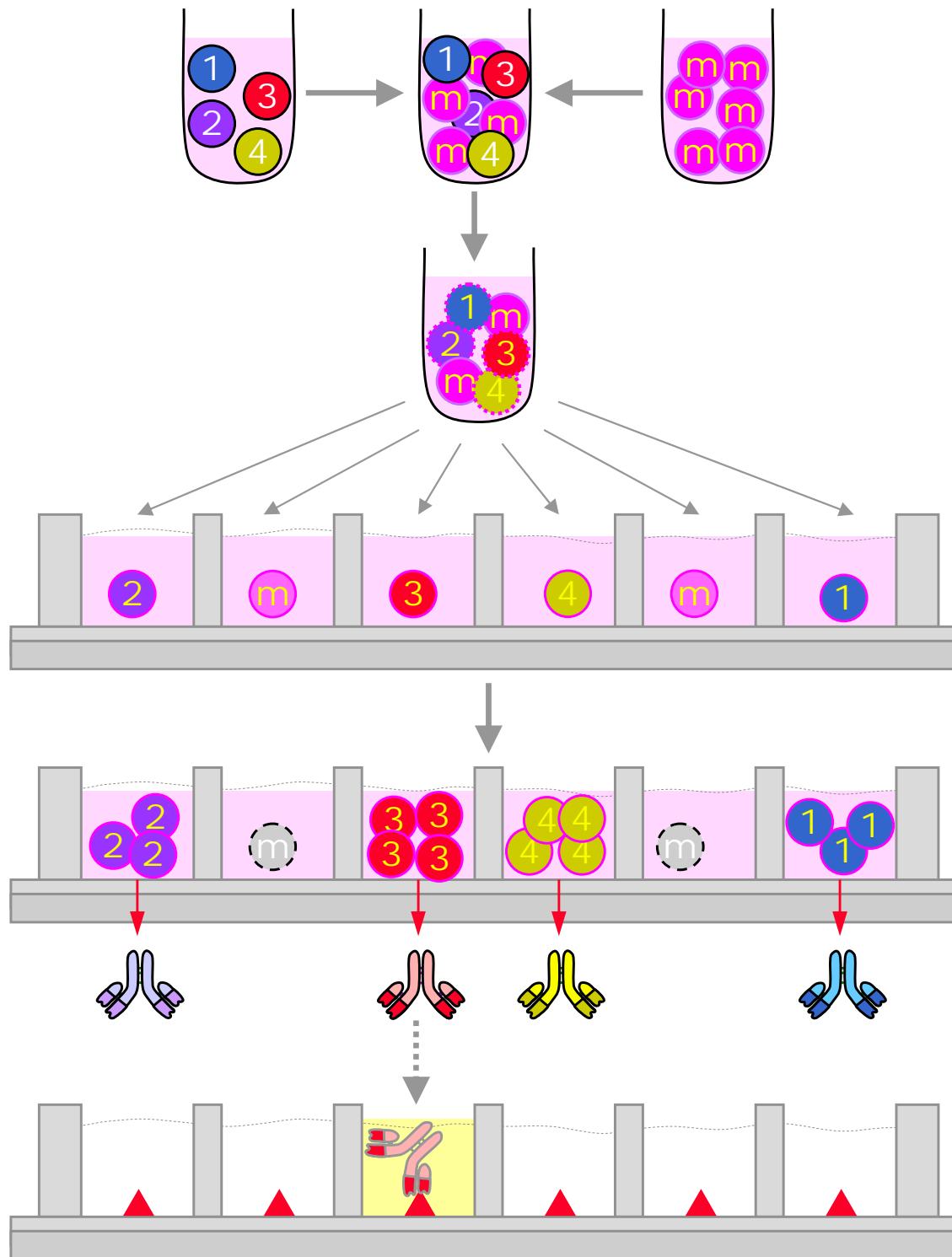
脾細胞

癌細胞

細胞融合

HAT  
初步篩選

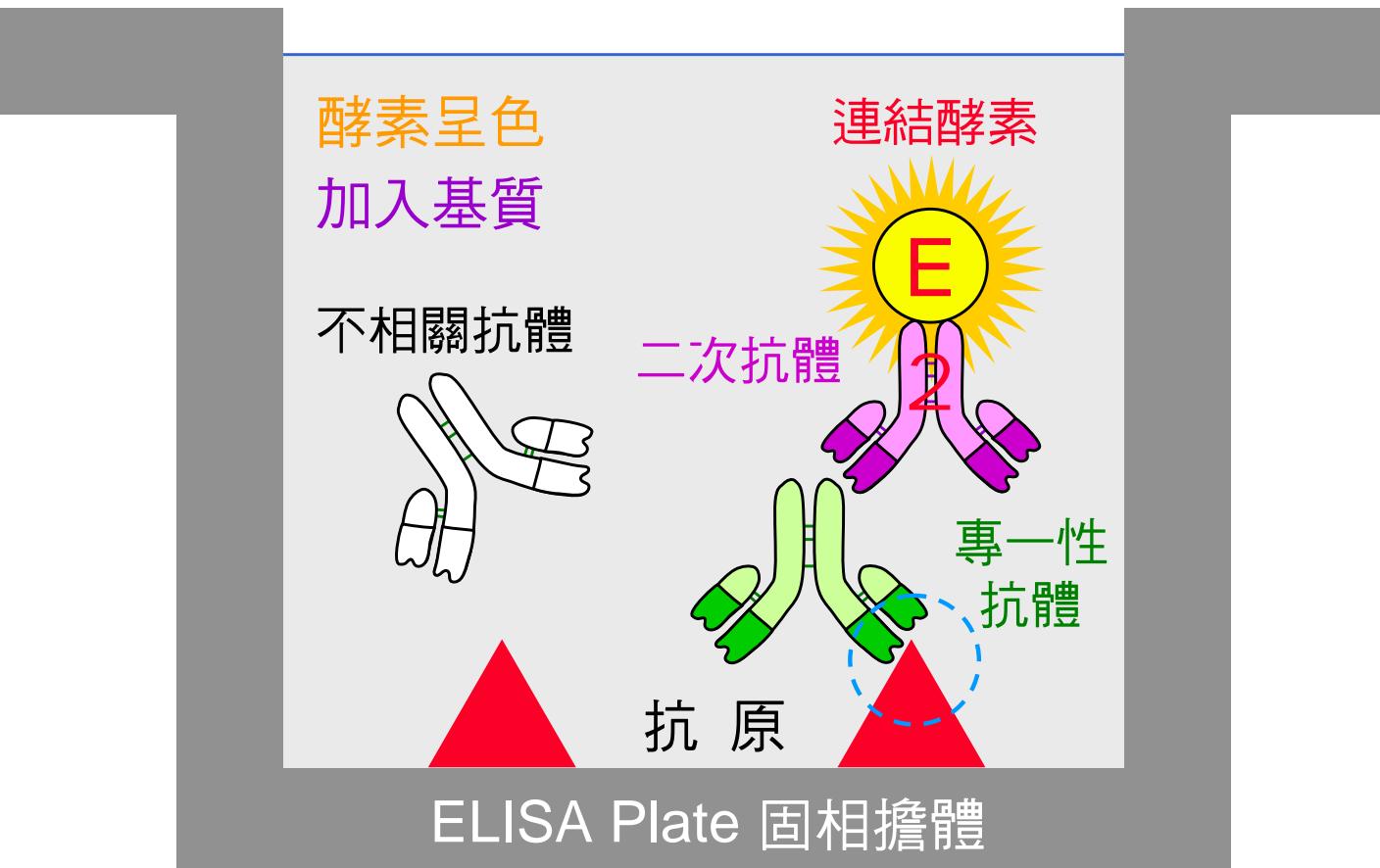
ELISA  
抗體篩選



# 以酵素免疫分析法檢測樣本中的專一性抗體

## ELISA

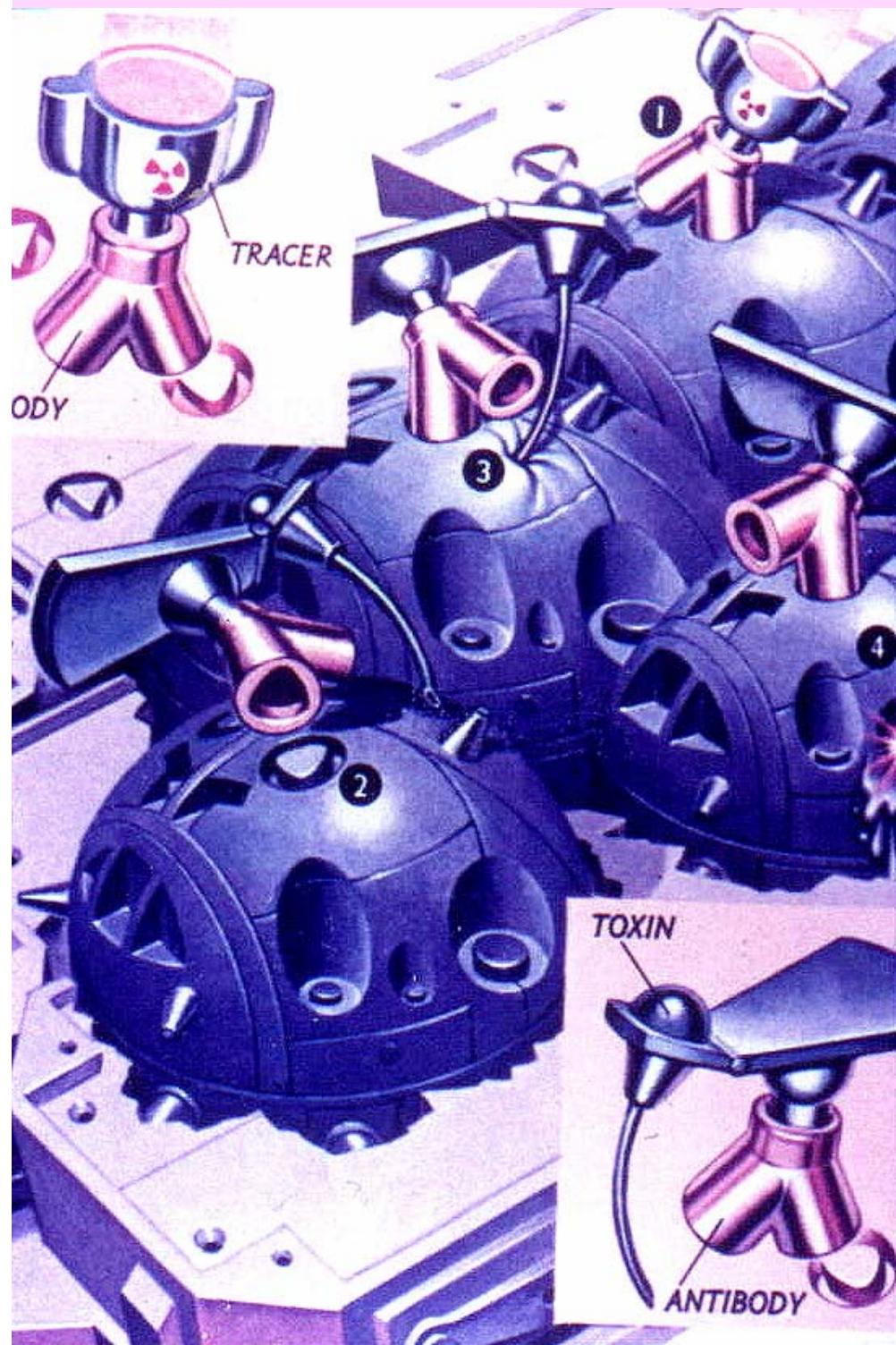
Enzyme-Linked Immunosorbent Assay



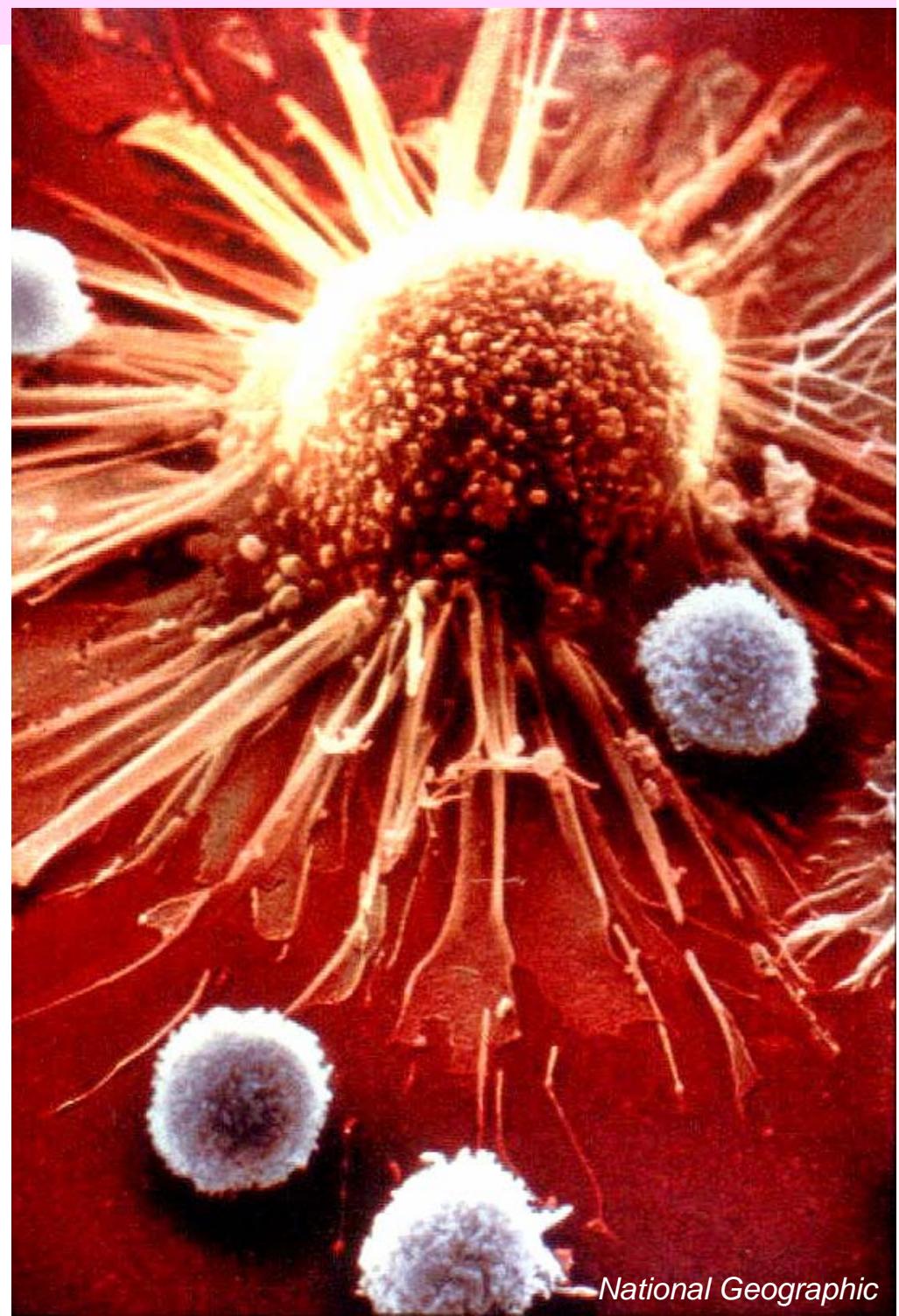
# 細胞穩定後擴大至 T-25 培養瓶



在 T-75 培養小瓶中擴大細胞數目

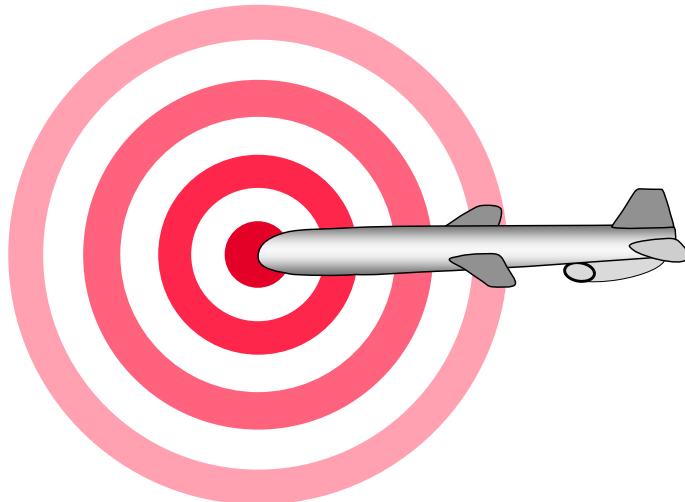


以單株抗體來清除體內癌細胞



# 單株抗體的高度專一性有很多用途

單株抗体是對其抗原有極強 **專一性** 的  
**魔彈**(Magic bullet)或**巡弋飛彈**



- |    |                         |
|----|-------------------------|
| 研究 | 以免疫轉印法偵測 <b>特定抗原</b>    |
| 醫療 | 以毒素連結抗体攻擊 <b>病變細胞</b>   |
| 檢驗 | 以 ELISA 偵測 <b>特定病原體</b> |

# 魔彈 再起



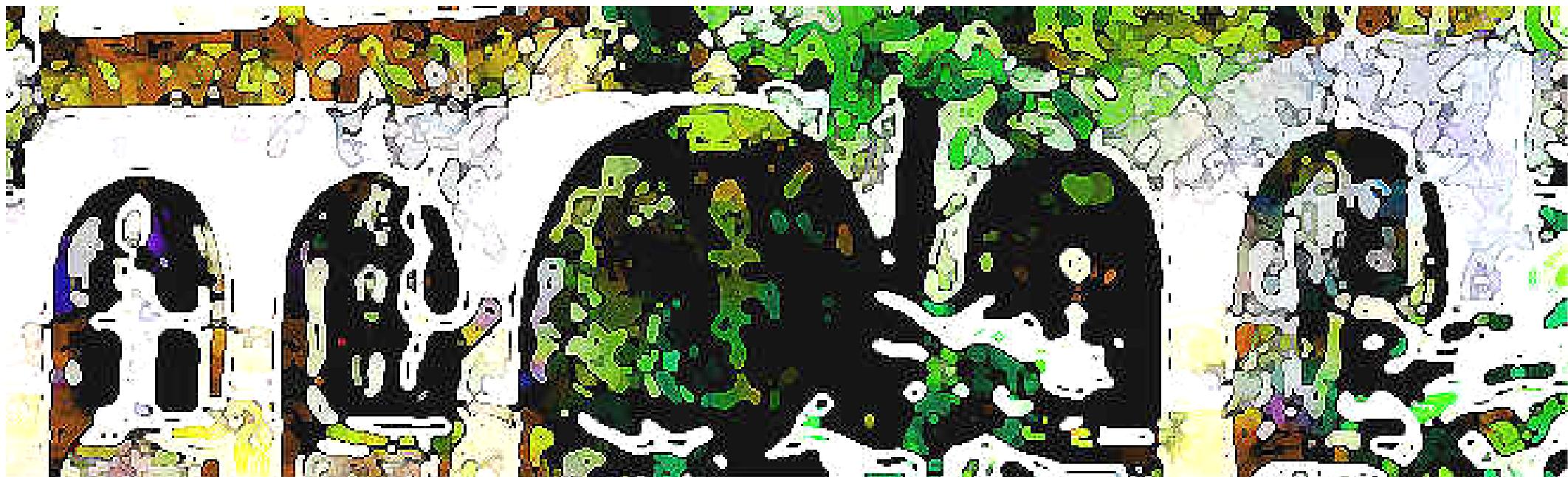
Molecular guided missiles called monoclonal antibodies were poised to shoot down cancer and a host of other diseases—until they crashed and burned. Now a new generation is soaring to market

By Carol Ezzell

# Proteomics and Antibody Bank

## 蛋白質體與抗體庫

蛋白質體學 - 綠竹筍抗體庫 - 抗體庫的應用



國立台灣大學 生化科技學系  
莊榮輝

# Genome

基因表現不一定完全反映在蛋白質

由基因體較難預測蛋白質的修飾及調控

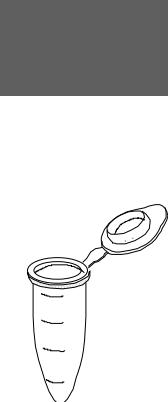
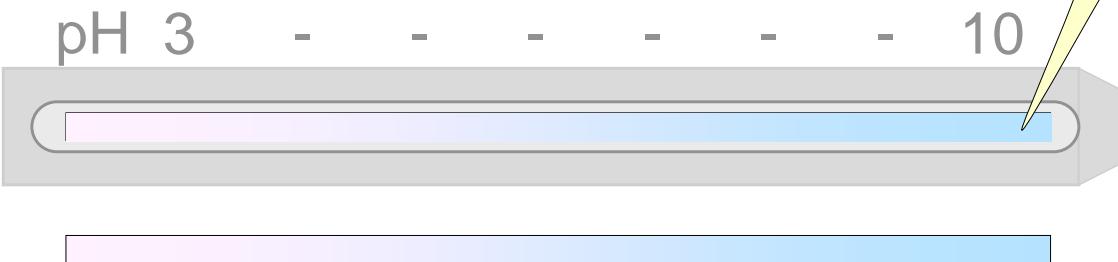
也無法預測蛋白質間的交互作用

# Proteome

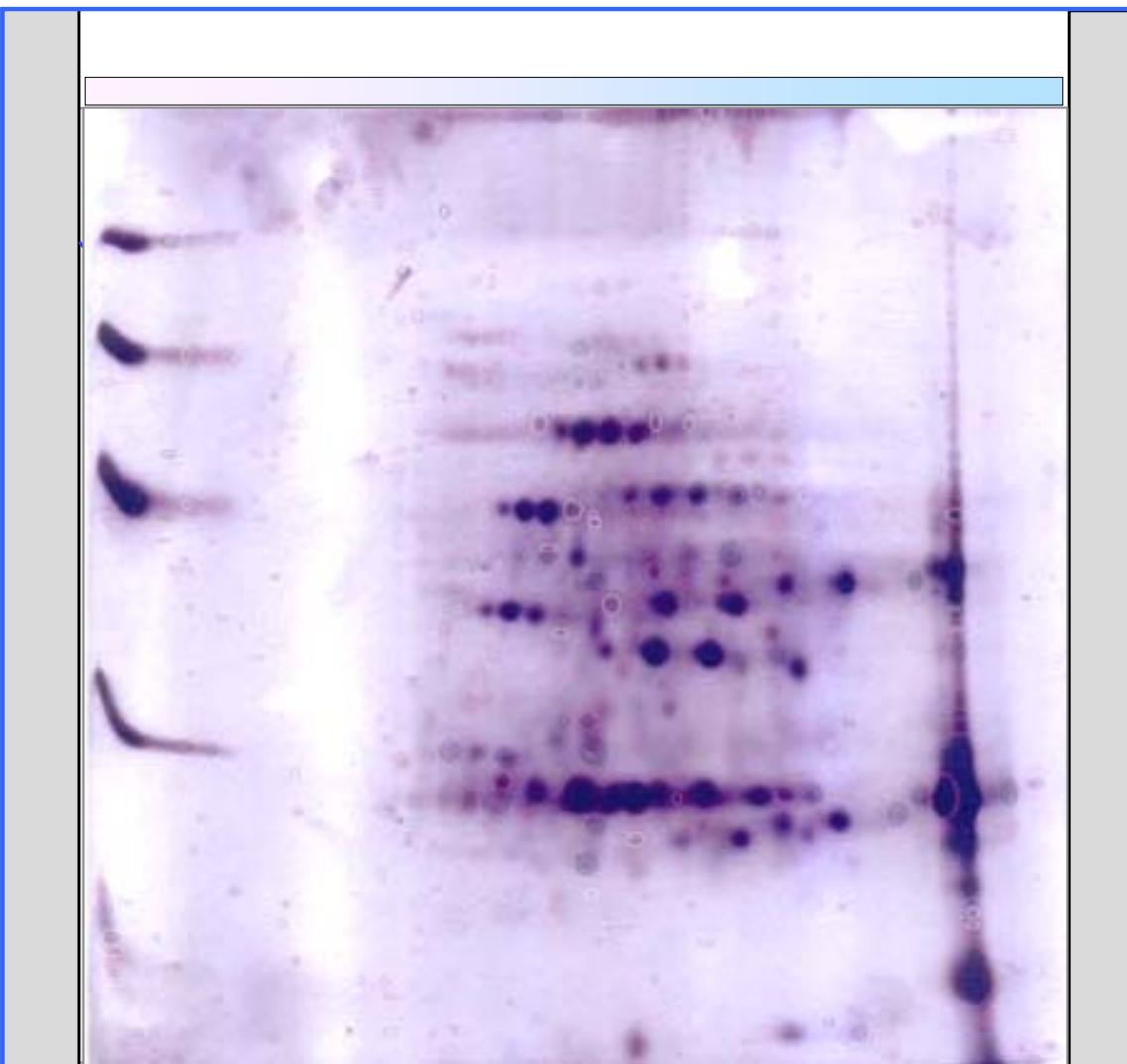
# 二次元電泳的操作過程

(1) IEF

等電焦集電泳

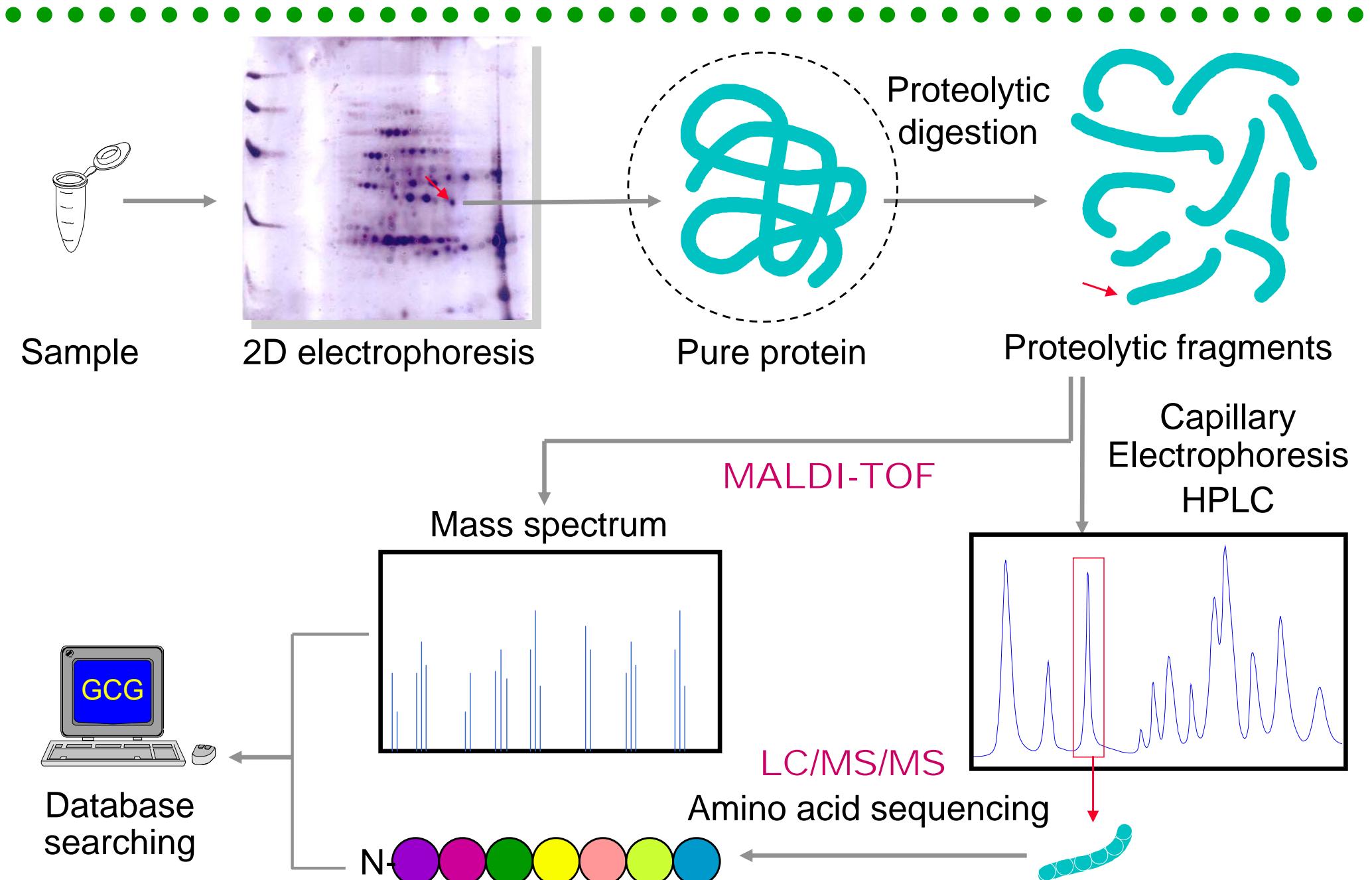


(2)  
SDS-PAGE  
分離膠體



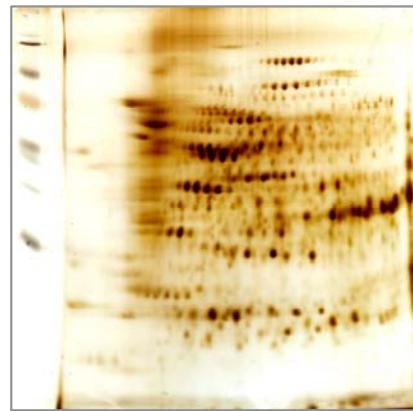
(3)  
染色脫色

## ■ 蛋白質體可綜觀蛋白質的消長與身分

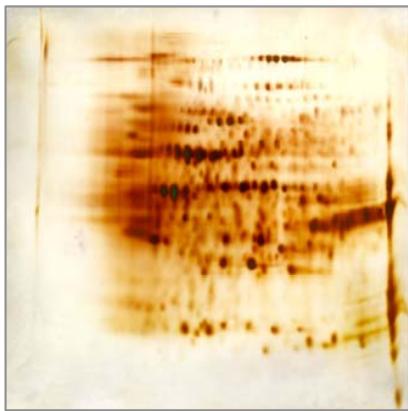


# Proteomic pattern changes during growth

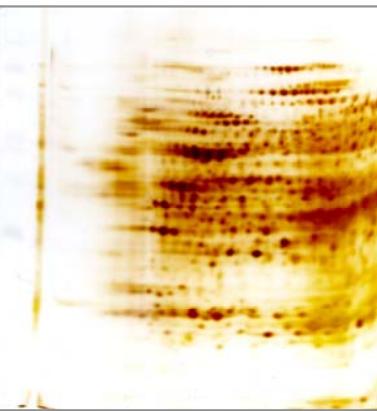
0 cm



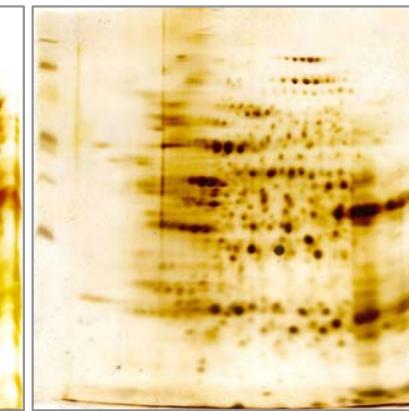
10 cm



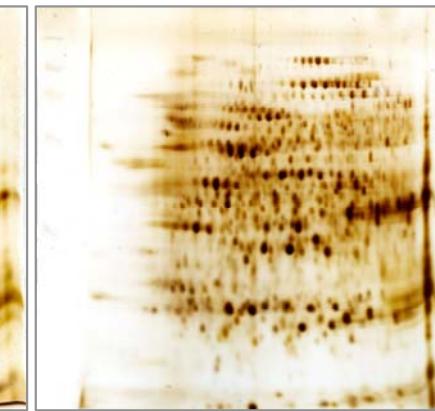
20 cm



40 cm



60 cm



綠竹筍

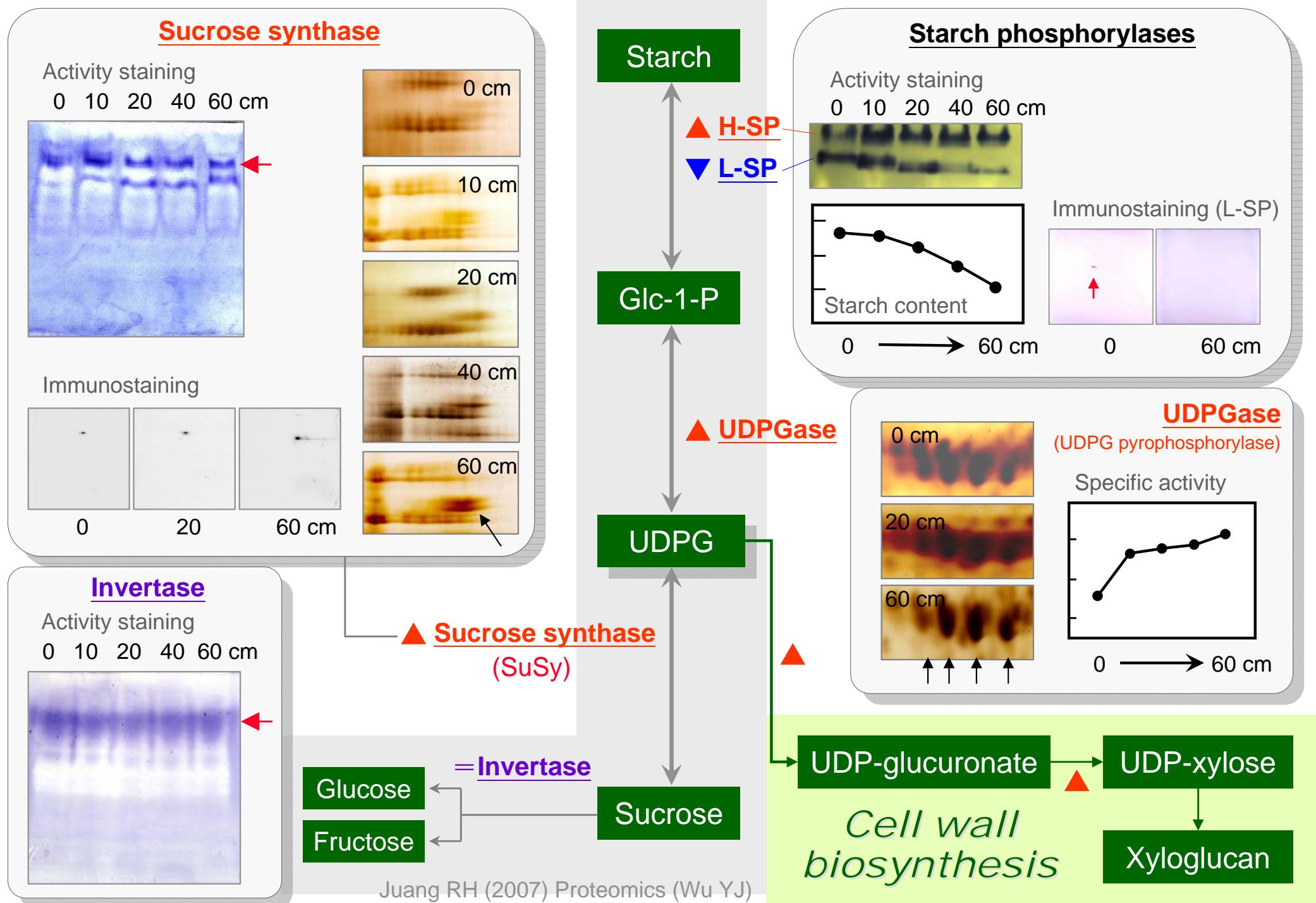


## Cellulose synthesis

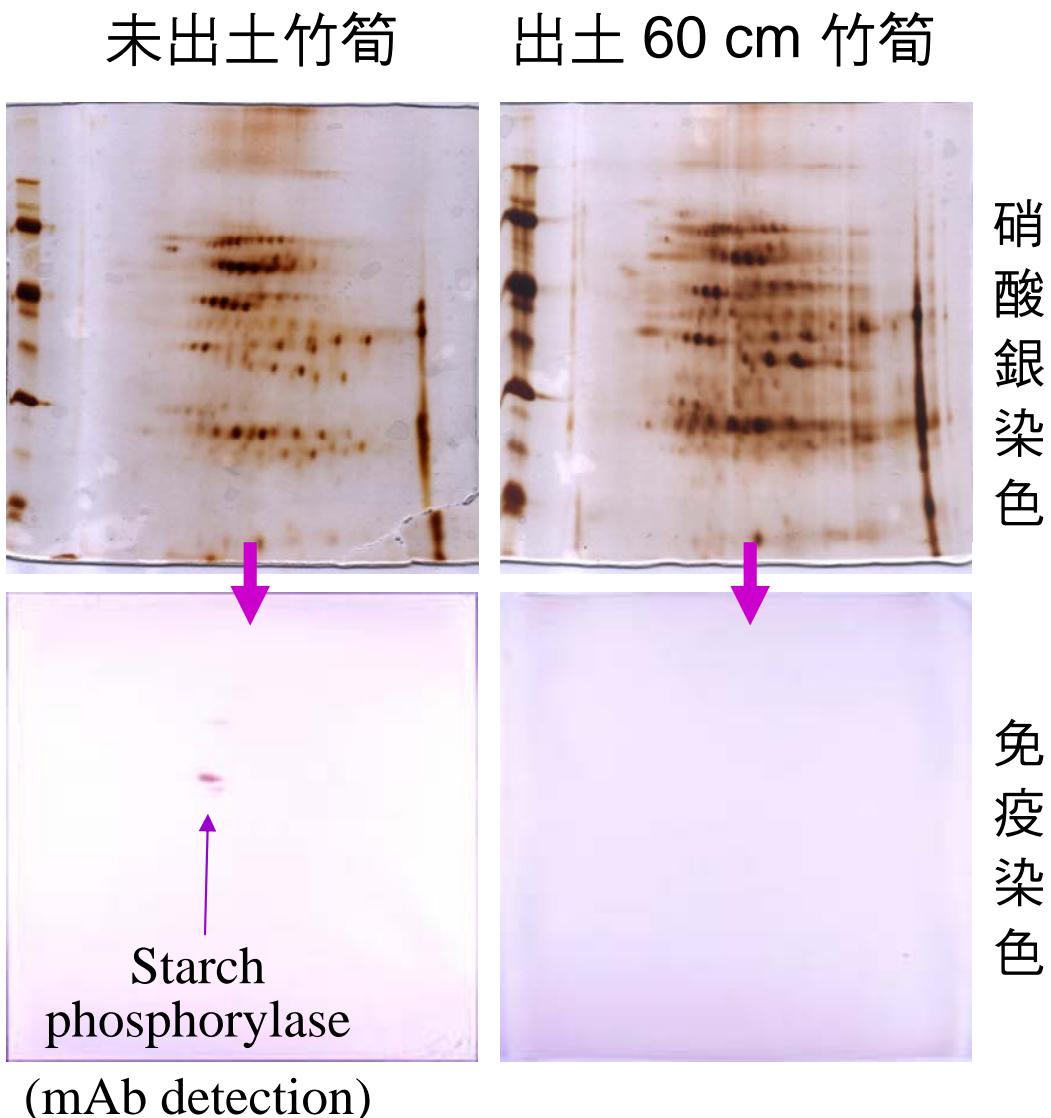
Juang RH (2007) Proteomics (with Wu YJ)

| 點  | Protein ID                    | Accession no.                           | Calculated Mr (kD) / pI | Sequence coverage (%) | Score (MASCOT) | Match fragments |
|----|-------------------------------|---|-------------------------|-----------------------|----------------|-----------------|
| 79 | Sucrose synthase              | AAV64256<br>( <i>Bambusa oldhamii</i> ) | 92.8 / 6.03             | 35                    | 402            | 14              |
| 80 | Sucrose synthase              | AAV64256<br>( <i>Bambusa oldhamii</i> ) | 92.8 / 6.03             | 35                    | 245            | 7               |
| 82 | Sucrose synthase              | AAV64256<br>( <i>Bambusa oldhamii</i> ) | 92.8 / 6.03             | 35                    | 1112           | 45              |
| 8  | UDP-glucose-pyrophosphorylase | BAB69069<br>( <i>Oryza sativa</i> )     | 51.6 / 5.4              | 18                    | 302            | 26              |
| 9  | UDP-glucose-pyrophosphorylase | BAB69069<br>( <i>Oryza sativa</i> )     | 51.6 / 5.4              | 17                    | 359            | 20              |
| 10 | UDP-glucose-pyrophosphorylase | BAB69069<br>( <i>Oryza sativa</i> )     | 51.6 / 5.4              | 21                    | 408            | 38              |
| 11 | UDP-glucose-pyrophosphorylase | BAB69069<br>( <i>Oryza sativa</i> )     | 51.6 / 5.4              | 20                    | 377            | 35              |

# Connect 2-DE results to metabolic pathway



# Antibody is a specific probe against target protein



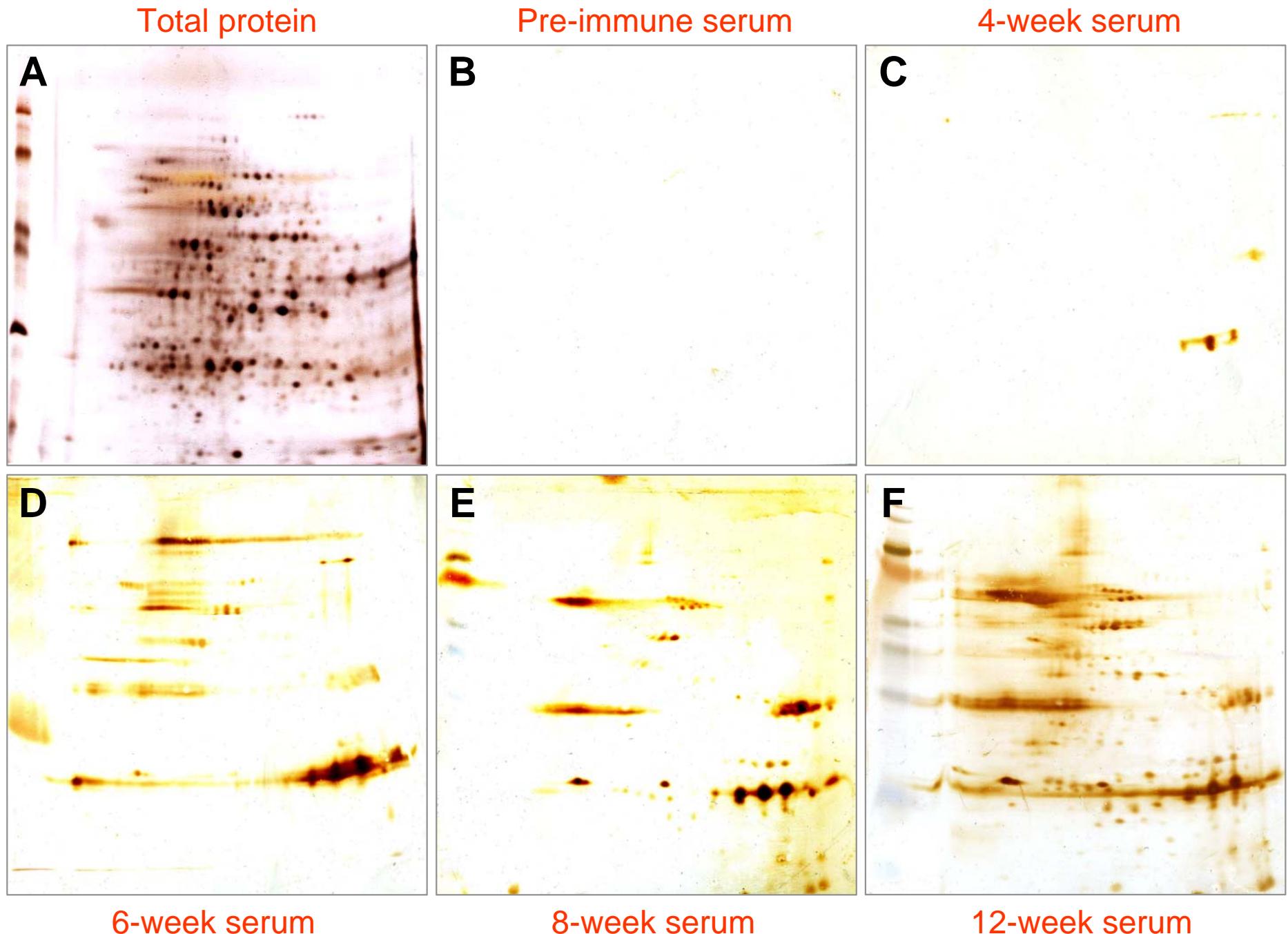
首次提出  
高產能抗體製備計畫構想

- (1) 建立高產能抗體製備之 標準流程
- (2) 大量快速 生產有用的單株抗體庫 mAb bank
- (3) 提供 抗體晶片 所需之抗體庫

均質抗原 → 單一抗體

整體抗原 → 全部抗體

# The immune response for complex antigen



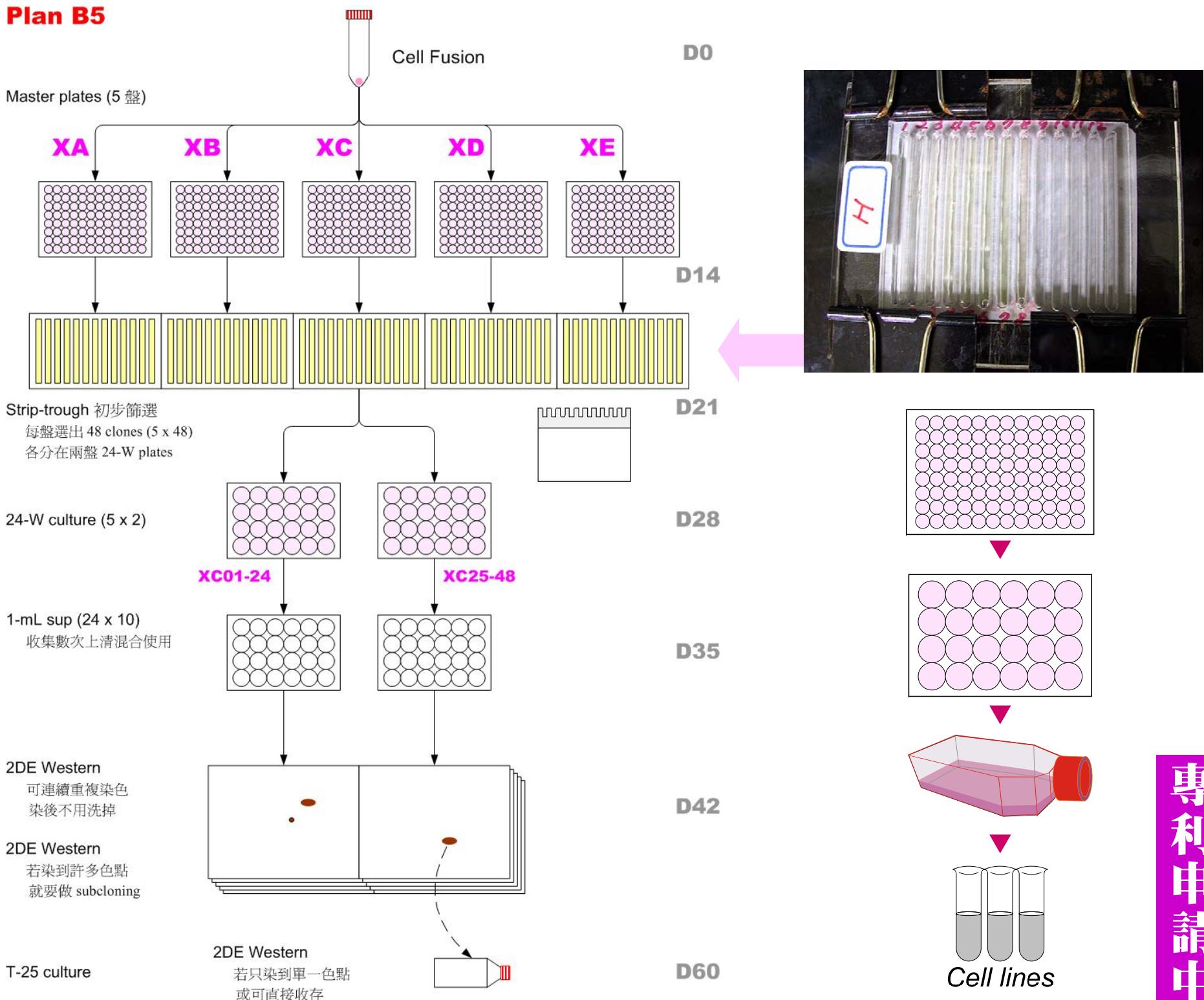
Supplementary Figure 1

Juang RH (2007) Proteomics (with Wu YJ)

# 單株抗體高產能製備流程

Juang RH (2007)

## Plan B5



專利申請中

# Summary for the mAb bank production

## (A) First-stage

| Spleen cell fused with                 | NS0/1 (Mouse A) |           | Sp2/0 (Mouse B) |           |
|--|-----------------|-----------|-----------------|-----------|
| Number of clones                       | screened        | positive  | screened        | positive  |
| First screening                        | 150             | 100       | 180             | 120       |
| Second screening<br>(after subcloning) | 320             | 150       | 500             | 250       |
| Final monoclonal                       |                 | <b>78</b> |                 | <b>82</b> |

## (B) Second-stage

| Spleen cell fused with                 | Sp2/0 (Mouse C) |           |
|--|-----------------|-----------|
| Number of clones                       | screened        | positive  |
| First screening                        | 100             | 40        |
| Second screening<br>(after subcloning) | 400             | 120       |
| Final monoclonal                       |                 | <b>32</b> |

The library of total 192 blots in the mAb bank

192

最後所挑出來的二次元免疫圖譜都不相同

# The immune response vs. the mAb production

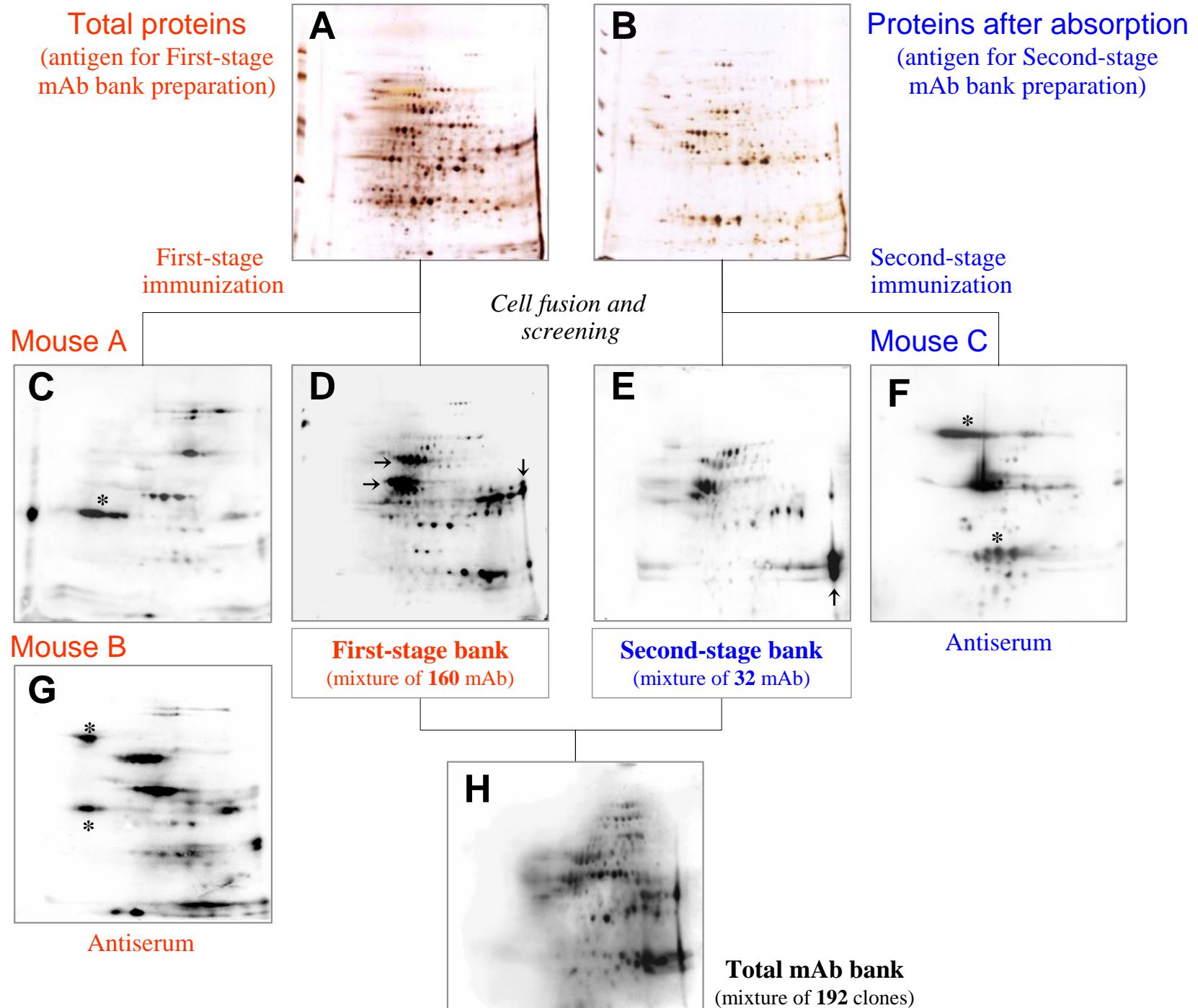


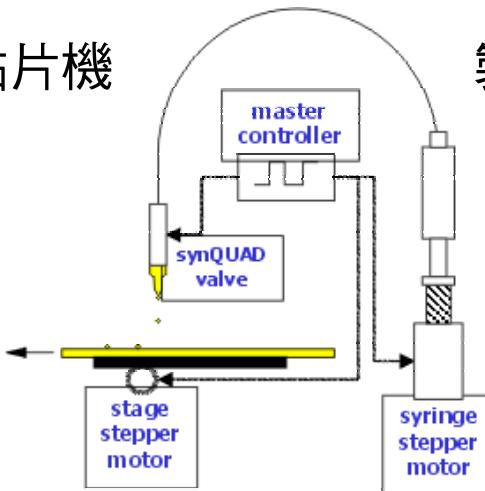
Figure 2

# 自製抗體晶片 (NC 噴霧塑膠板)

硝化纖維塗佈

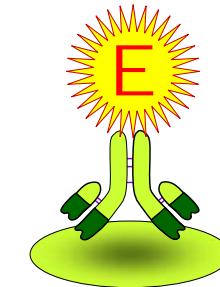


點片機

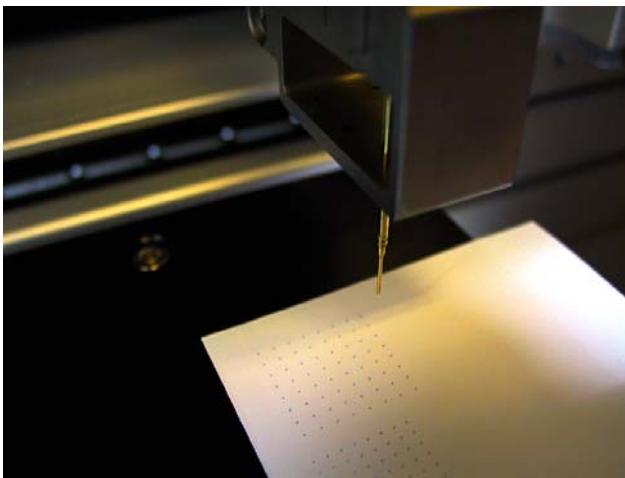
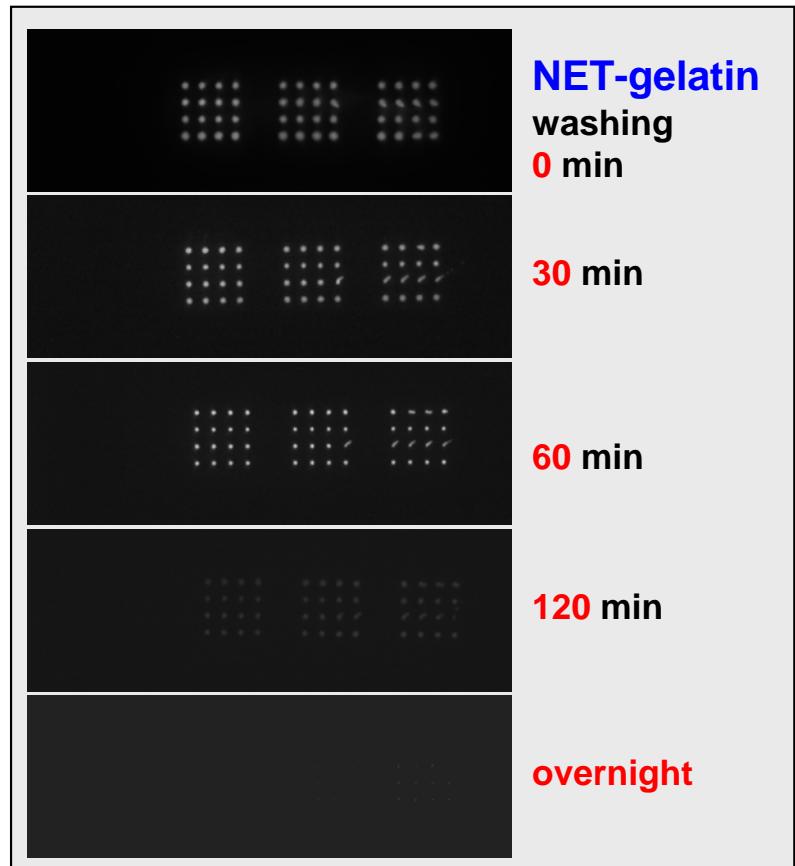


製作晶片

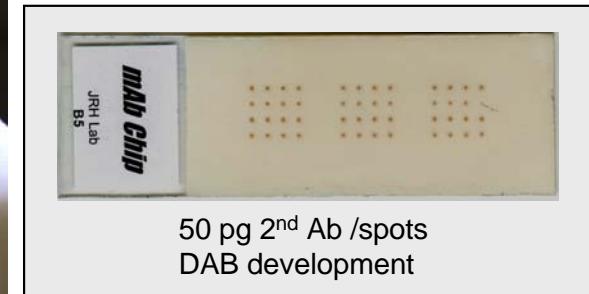
免疫呈色測試



蛋白質可穩定結合在晶片表面



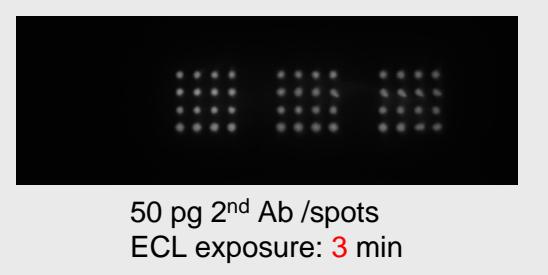
(a) 可用 DAB 均匀呈色



(b) 也可用化學螢光偵測

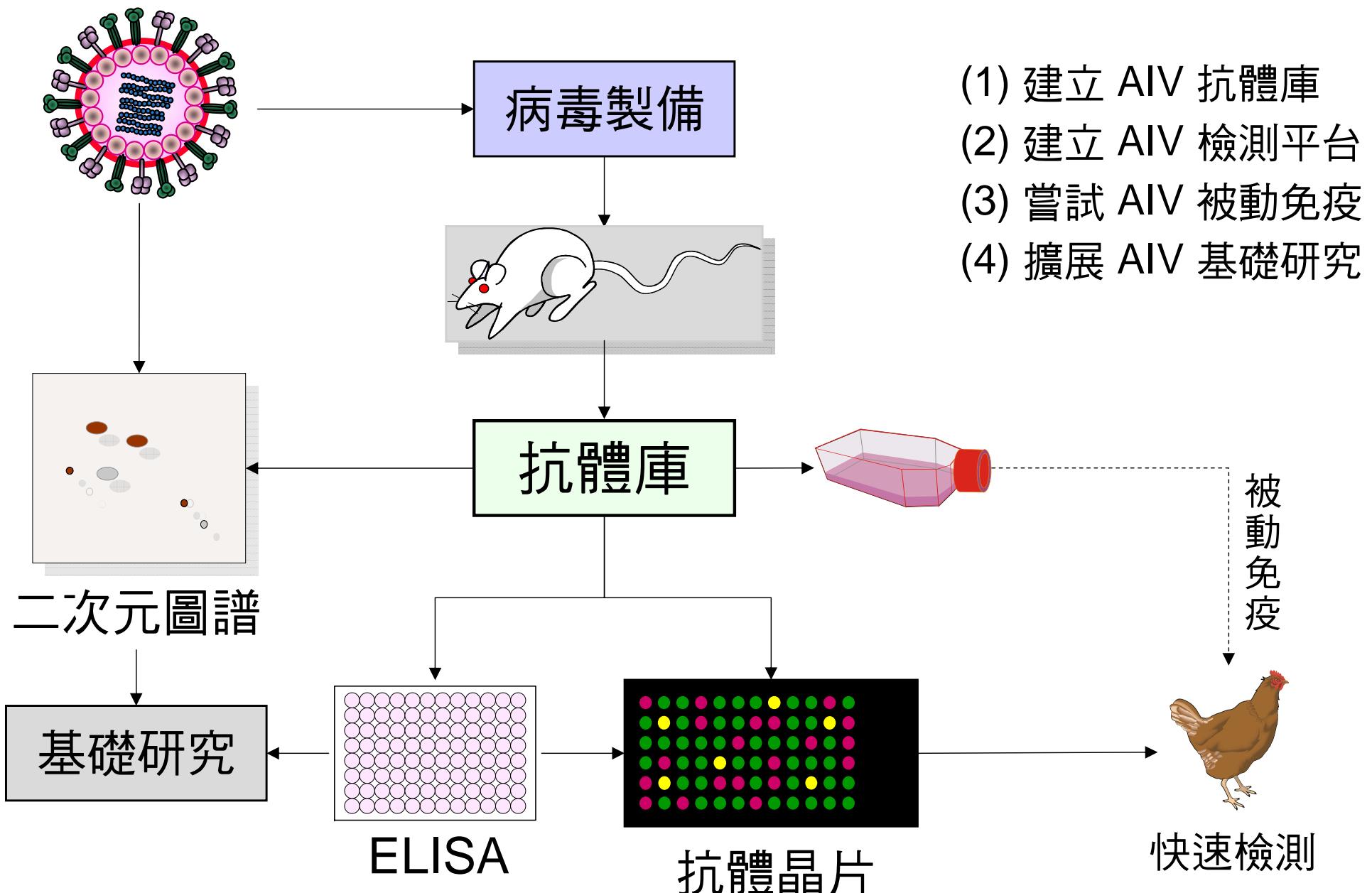
## Conclusion

- (1) NC 晶片可自噴霧製作
- (2) NC 可用兩種方式呈色
- (3) 蛋白質與 NC 結合穩定

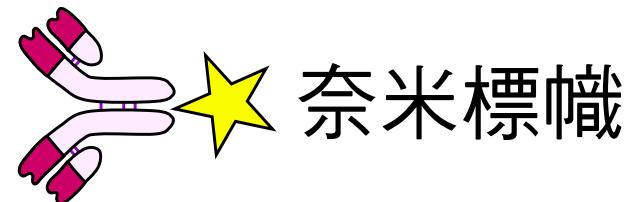
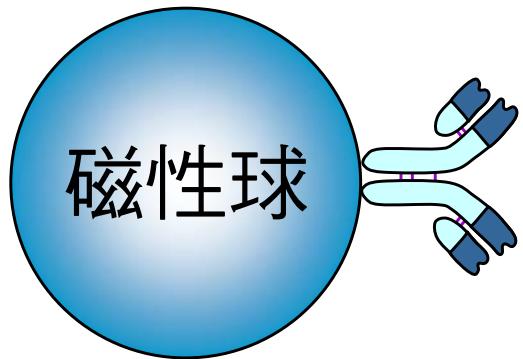


# AIIV 抗體庫及應用

建立蛋白質體抗體庫及晶片平台以應用於新近惡性傳染病之快速診斷



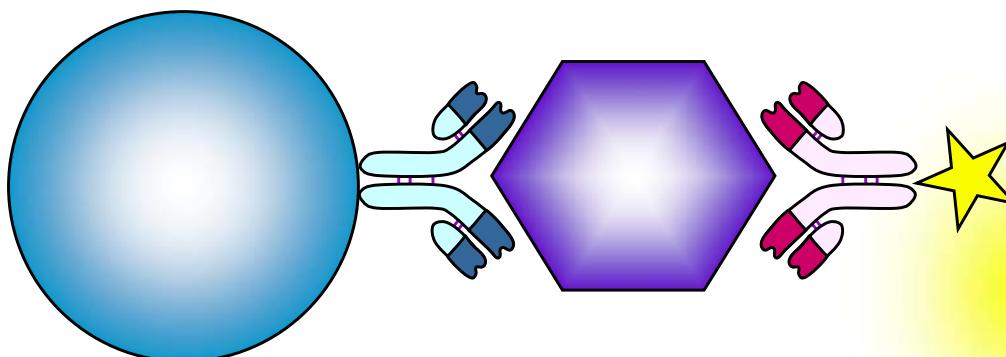
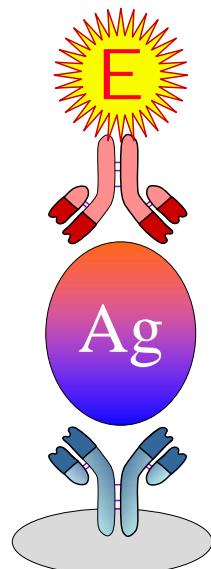
# 奈米磁性球檢測平台 (三明治法)



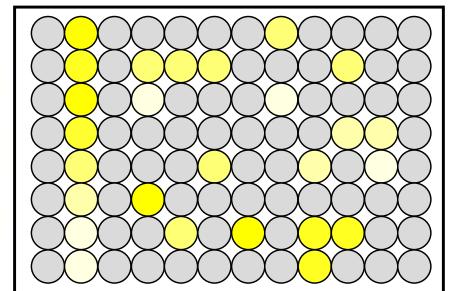
也可直接測量磁性球  
與病毒結合後的改變

↓  
Incubation

抗體晶片

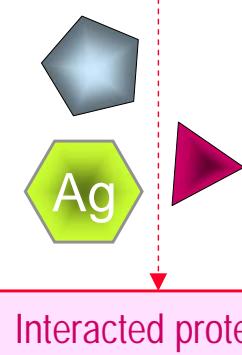
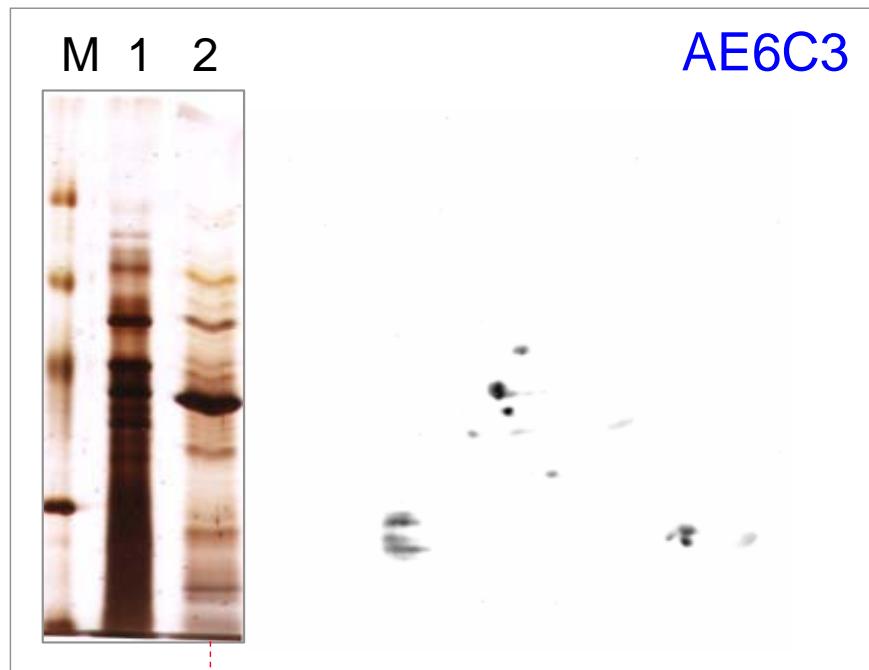


ELISA 方式



Magnetic Plate

# Immunoprecipitation of interacted proteins



LC-MS/MS

Interacted proteins

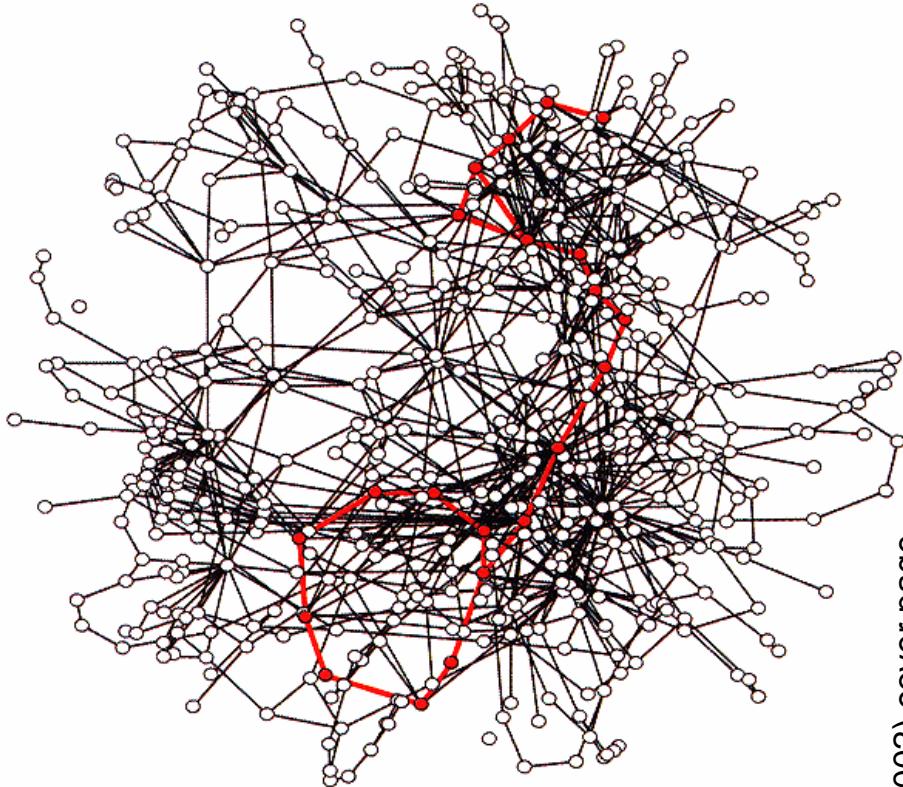
Validation!

| Protein ID                         | Match peptide                                     |
|------------------------------------|---|
| Adenosylhomocysteinase             | IVLTIIR<br>DSAAVFAWK<br>HSLPDGLMR<br>LVGVSEETTGVK |
| Histone H4 (wheat)                 | IFLENVIR<br>IDGLIYEETR<br>TVRAMDVYALKR            |
| Fructose bisphosphate aldolase     | VTPEVIAEYTVR<br>IGPNEPSQLAIDLNAQGLAR              |
| Triosephosphate isomerase          | TNVSPEVAESTR<br>VIACVGETLEQR                      |
| NAD-dependent malate dehydrogenase | DDL FNINAGIVK                                     |
| Histone H3                         | ASAPATGGVK  |
| Putative lipase                    | DQVLEEVRR   |

# From proteomics to systems biology

代謝路徑立體圖

Alberts et al (2002) Molecular Biology of the Cell (4e) p.107



Systems Biology

整體性的生物學觀念與工具

