

# 單株抗體在生物技術上的應用

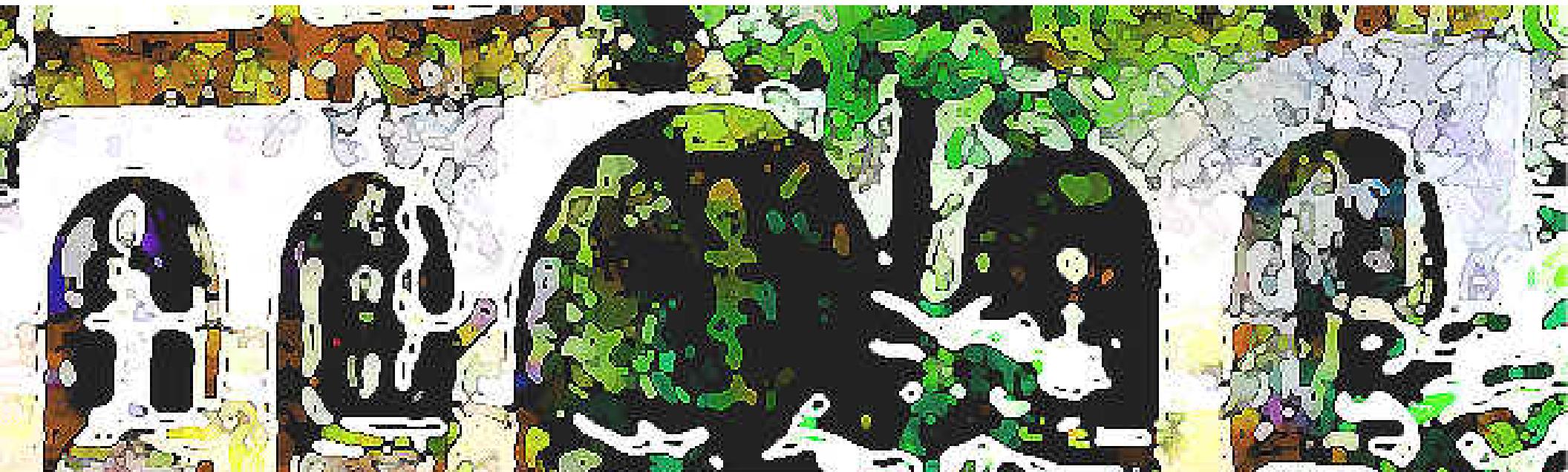
## (1) Monoclonal Antibody 單株抗體

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 - 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



Hybridoma Technique

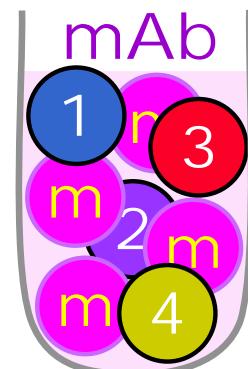
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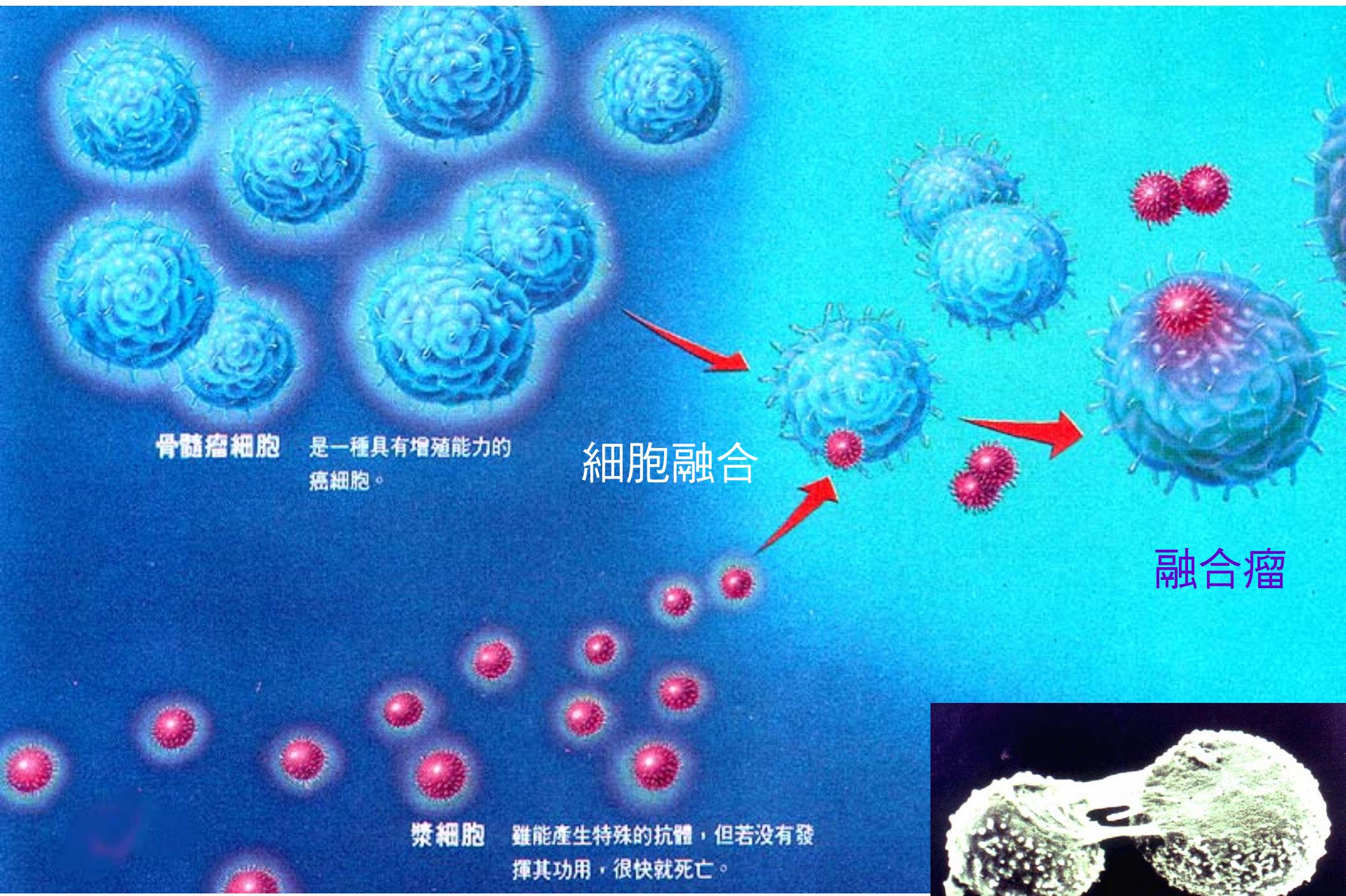
# 單株抗体

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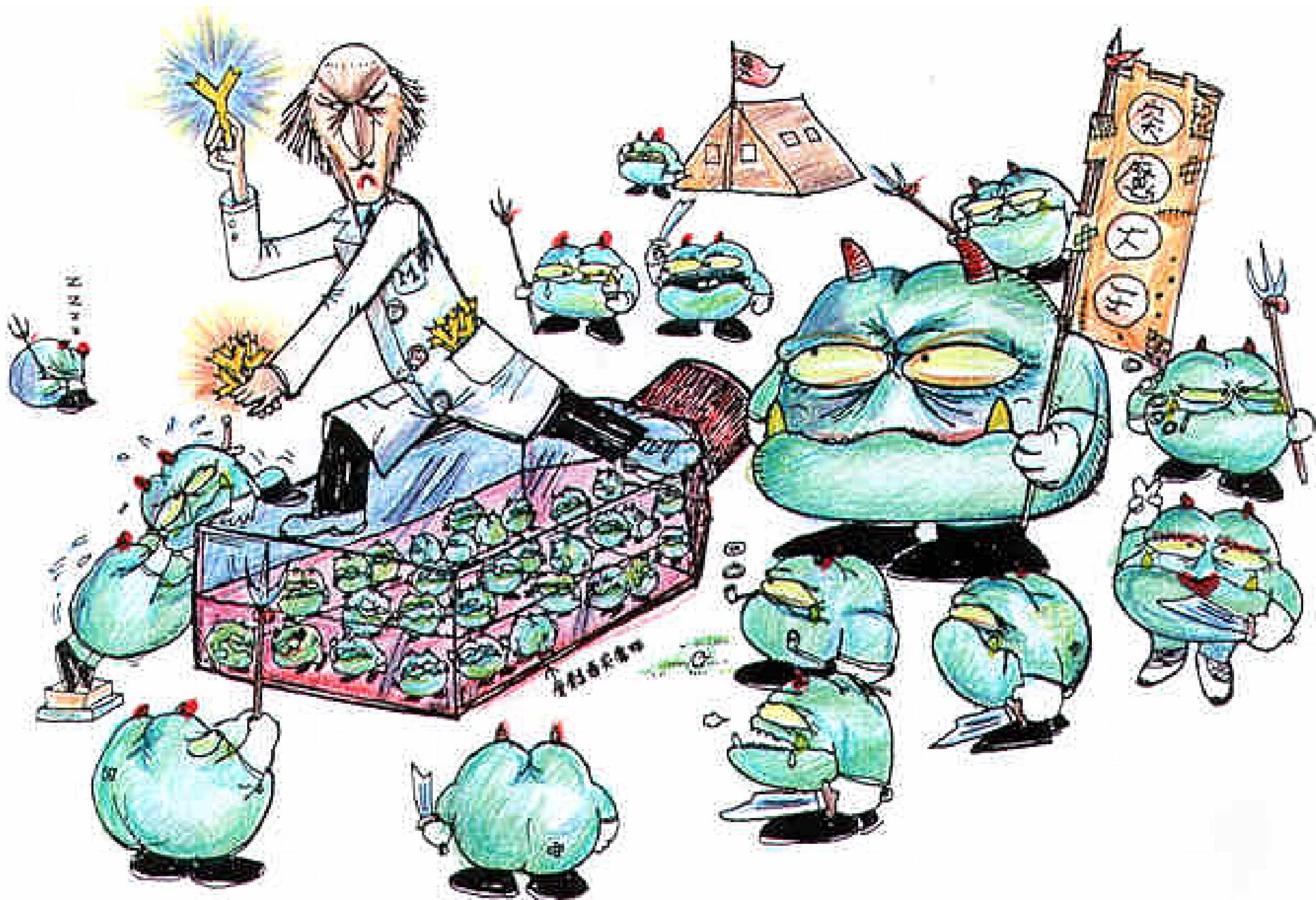
Monoclonal  
Antibody

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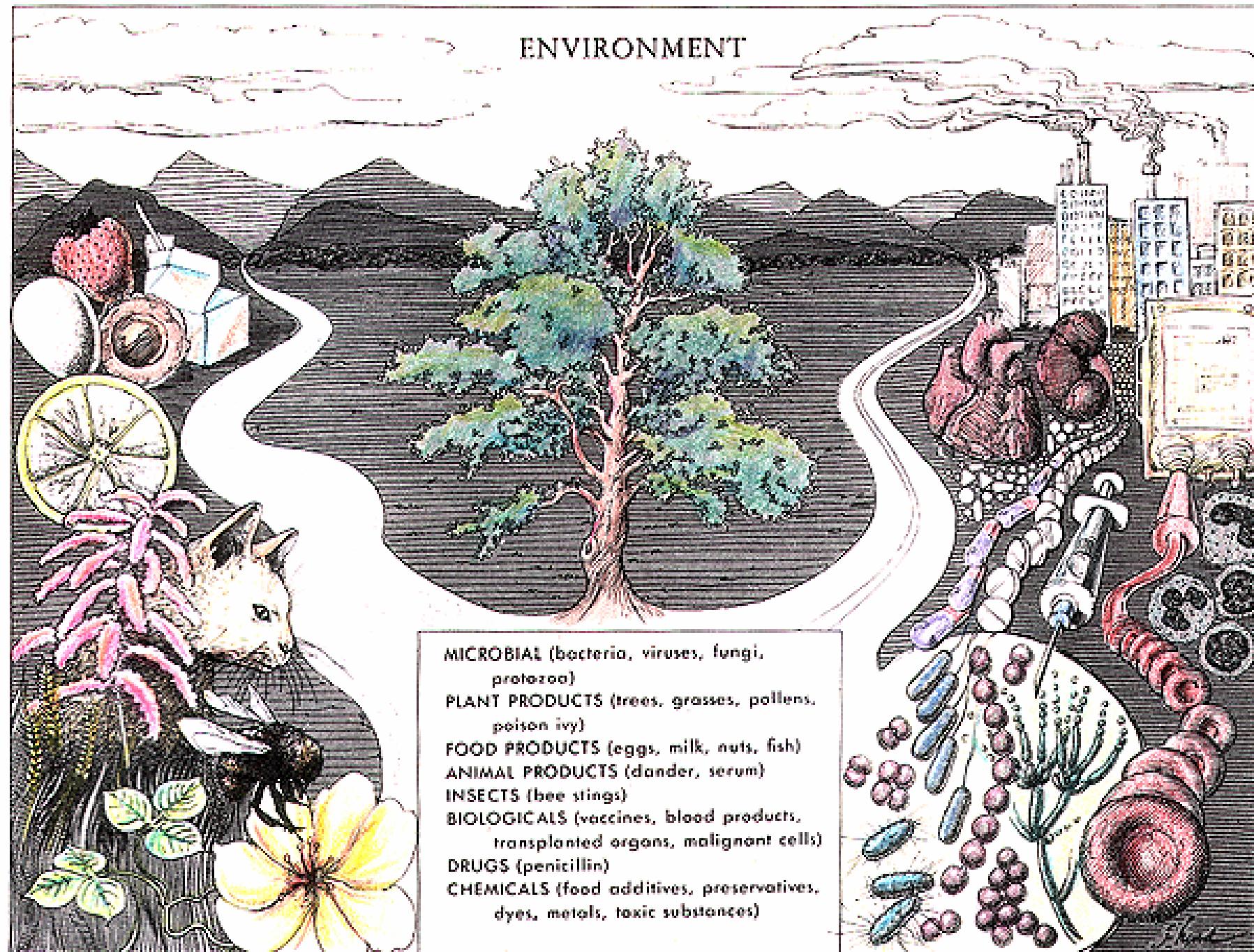




# 迷爾使呆與突魔大王



# 外在環境的許多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

巨噬細胞  
(Macrophage, mφ)  
自然殺手細胞  
(Natural killer cell, NK)

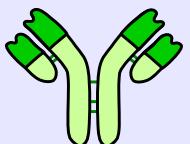
分子

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 →

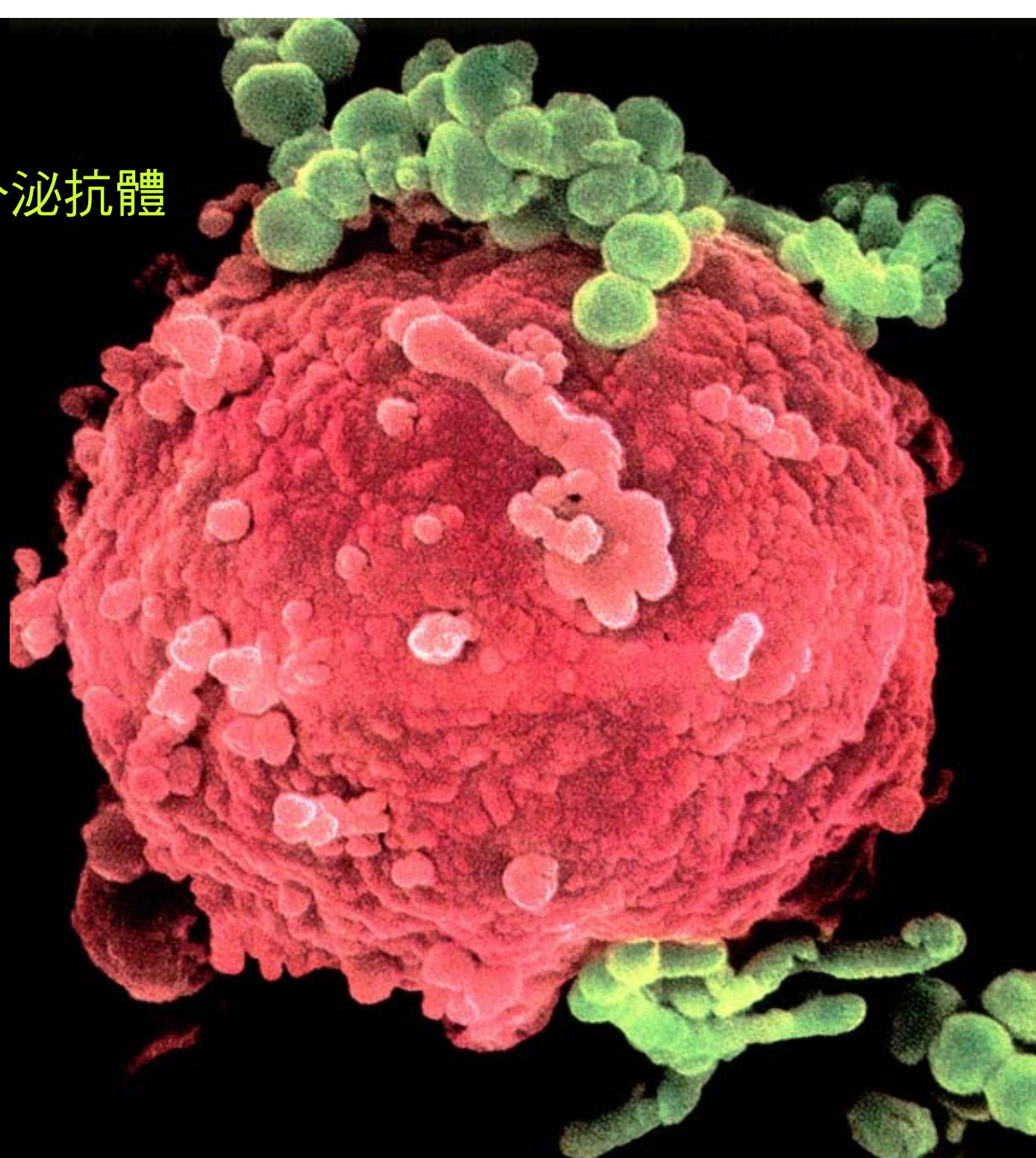
警察系統 (+ 調察局)

軍事系統

B 細胞分泌抗體

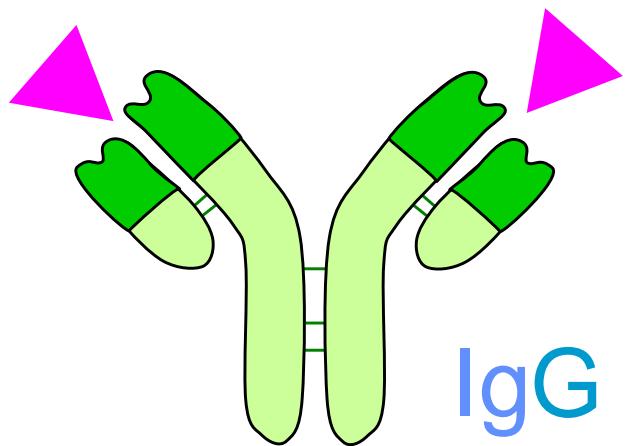
凝集細菌

如何凝集

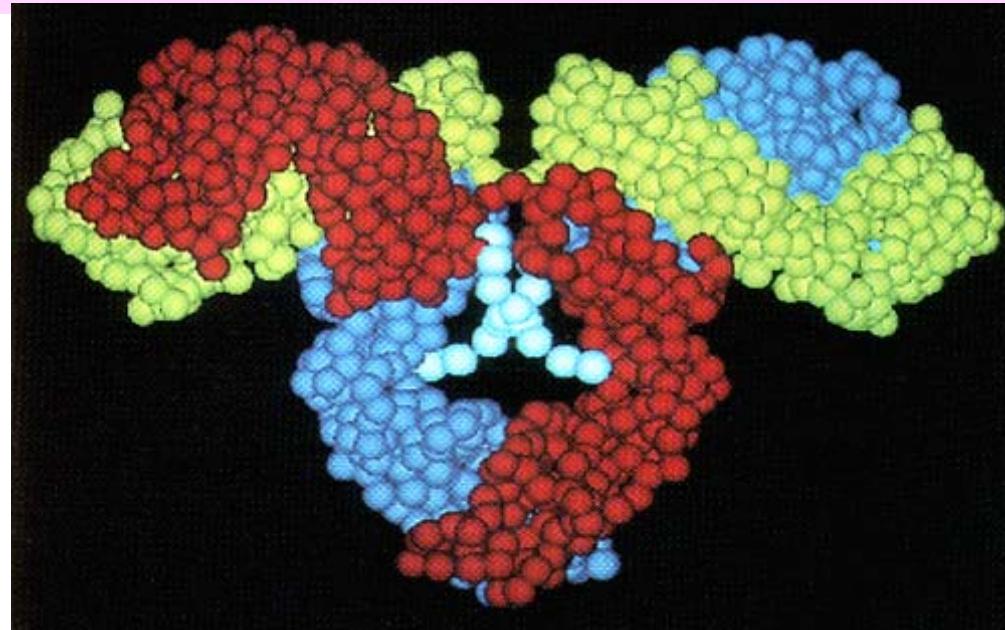


# ■ 抗體 是一種蛋白質

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



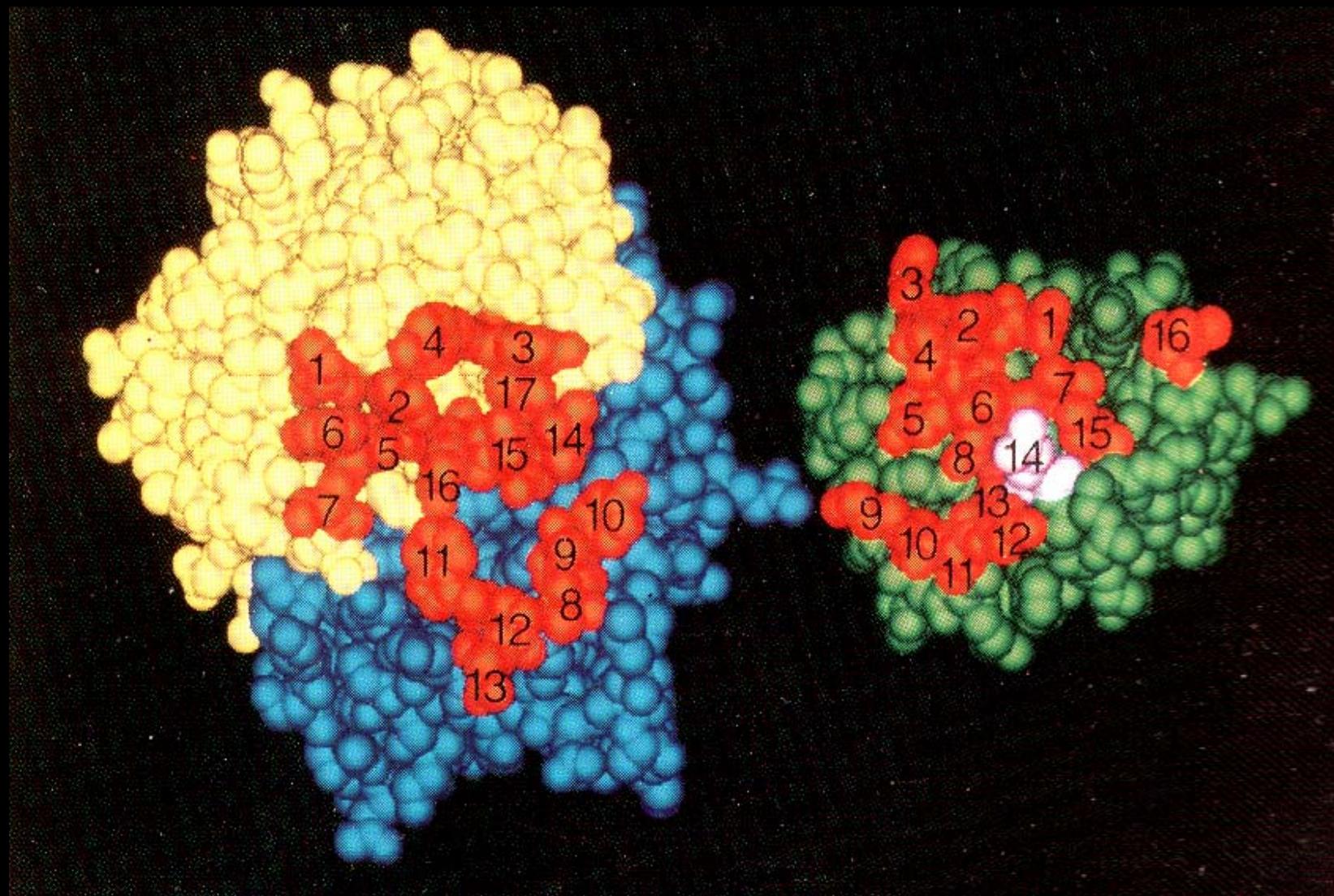
Immunoglobulin



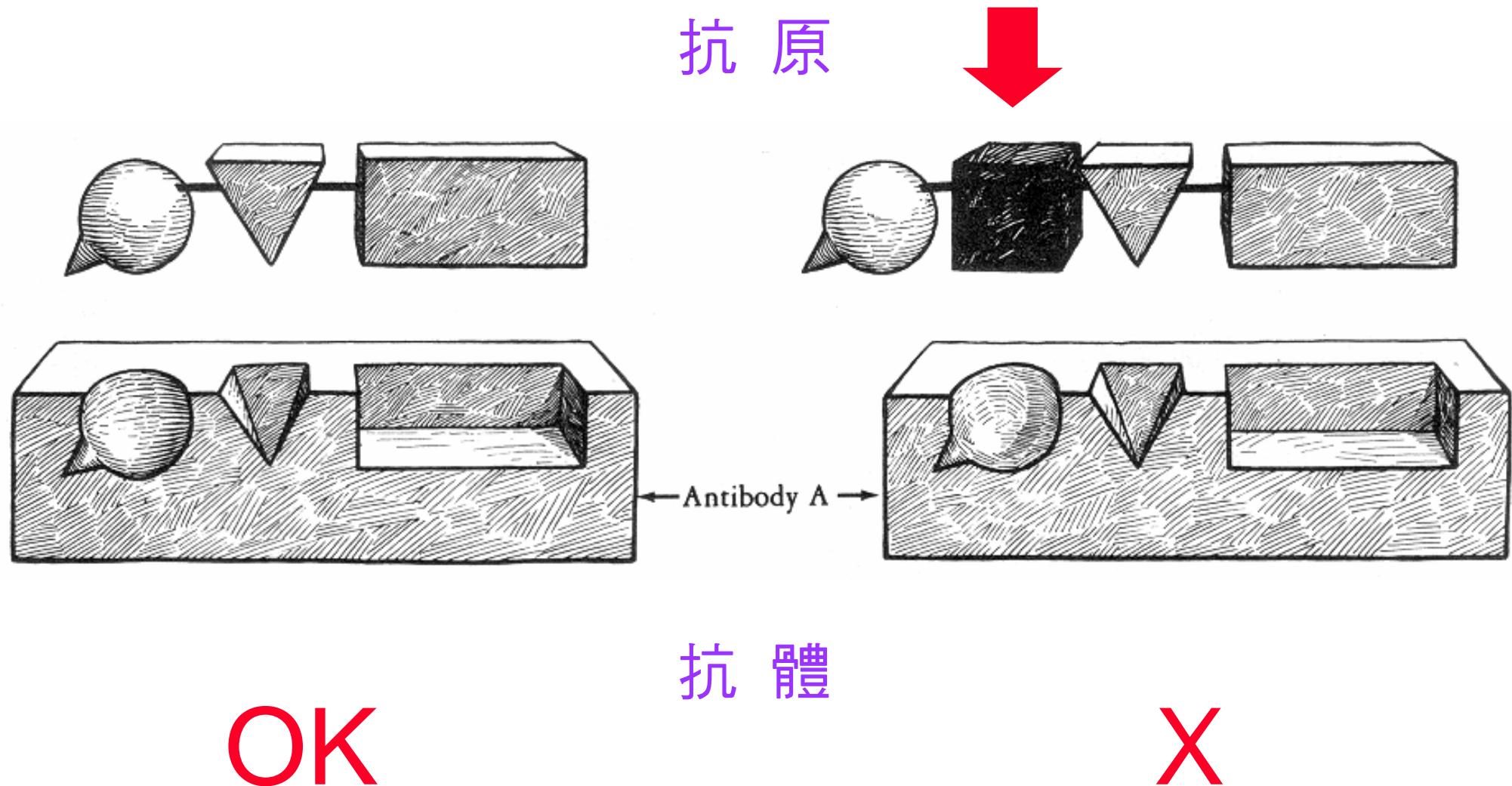
- 抗體由四條 蛋白質 長短鍊所組成 (兩長兩短)
- 抗體分子上有兩個 抗原結合區 (二者相同) ▼
- 抗體與抗原結合是專一性的 (lock & key)

● 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



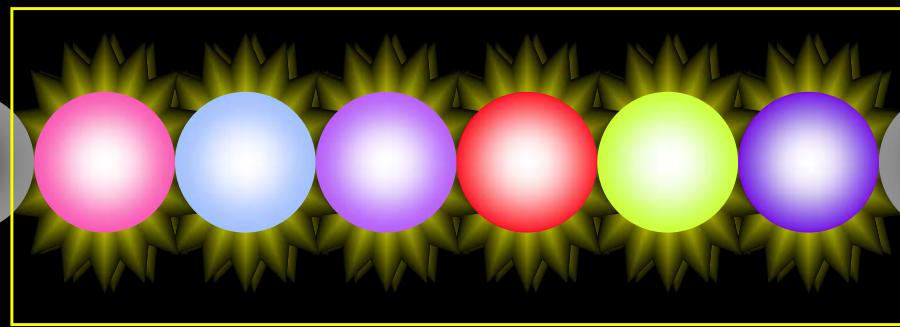
# 抗體與抗原的結合面有如互補的積木



# Epitope, Antigenic determinant

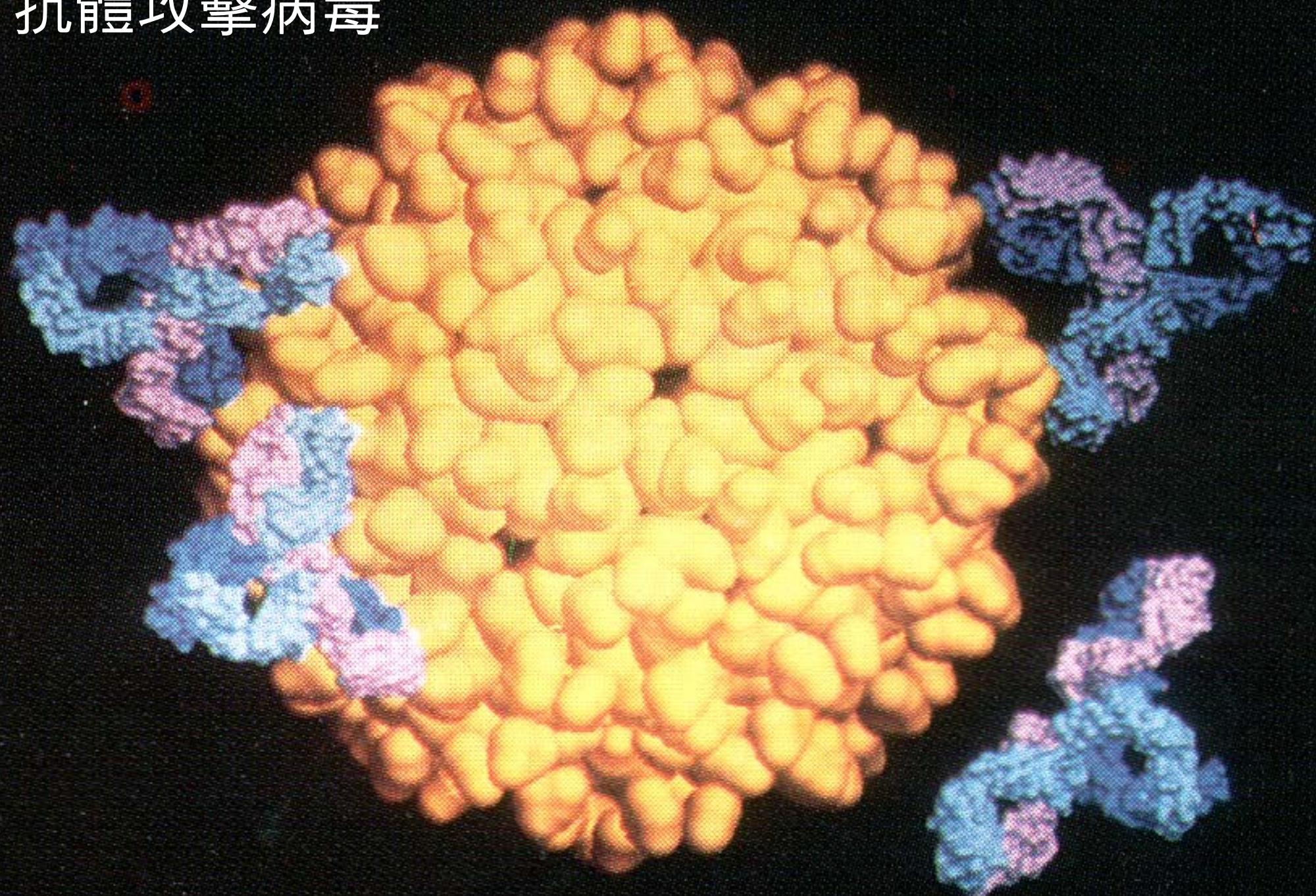
## 抗原決定基

- 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

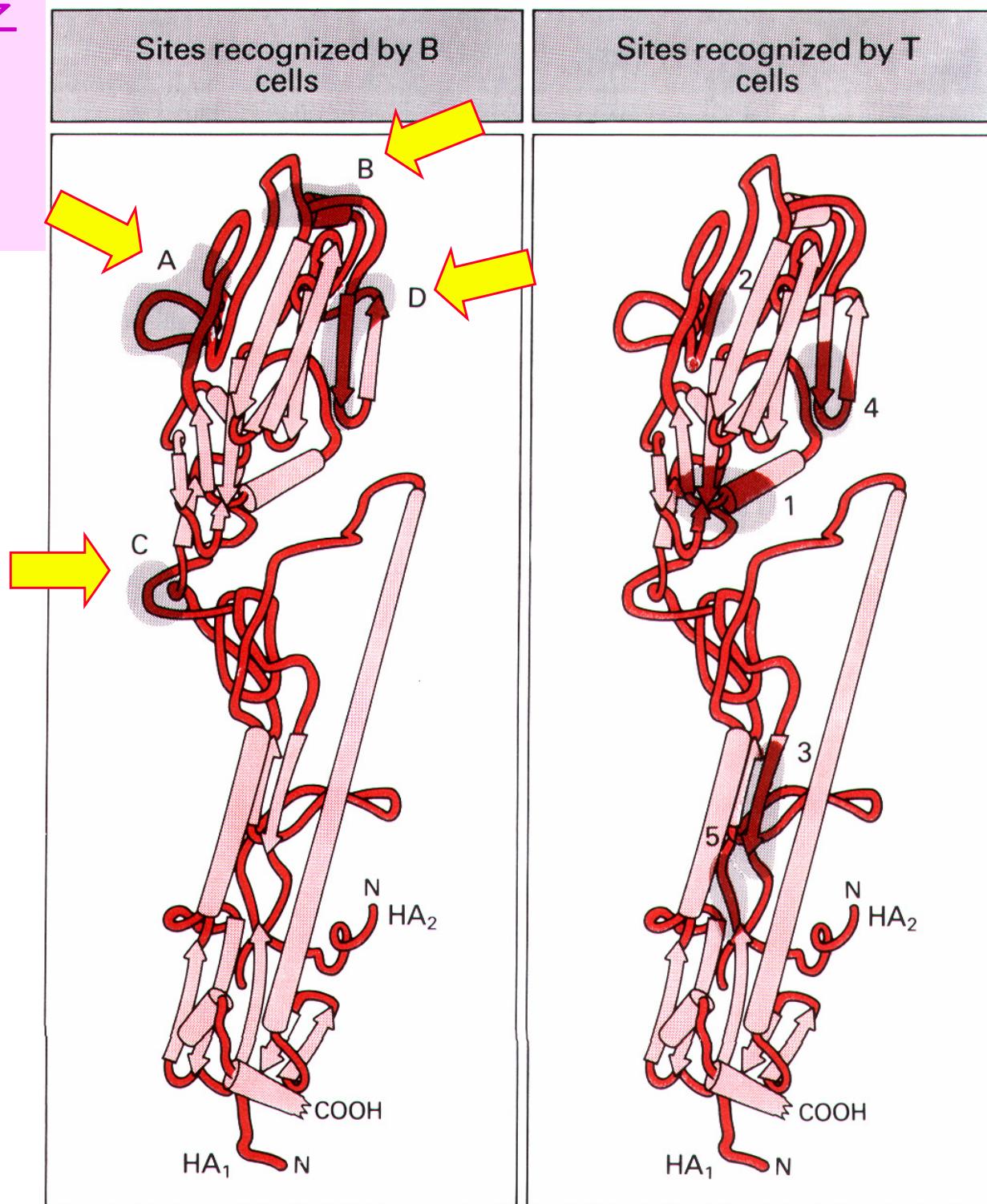


- 蛋白質性 抗原決定基 含有六個以上胺基酸

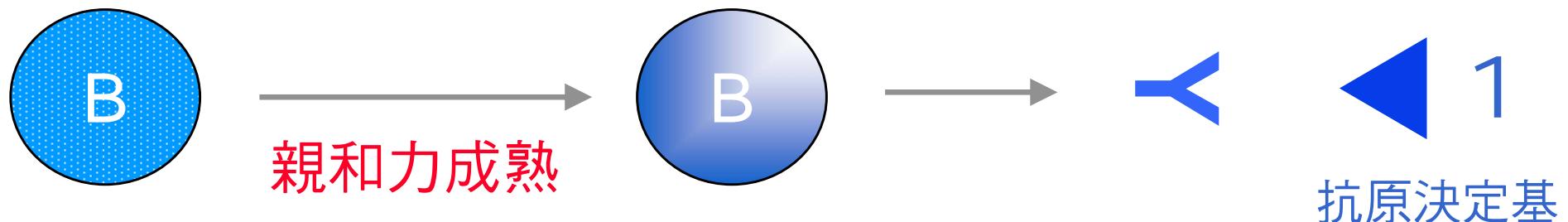
# 抗體攻擊病毒



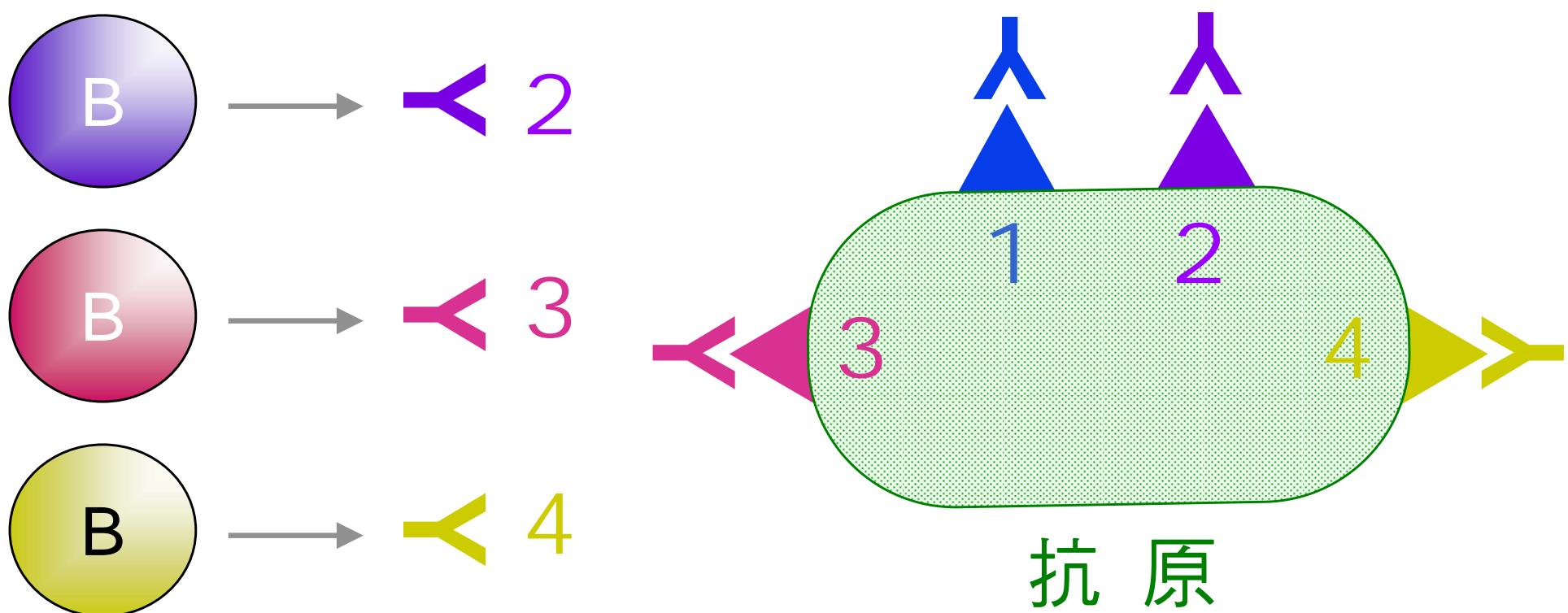
病毒表面分子  
上有許多個  
抗體結合區



- 一個 B 細胞只能生產一種抗体，對付某一抗原決定基。

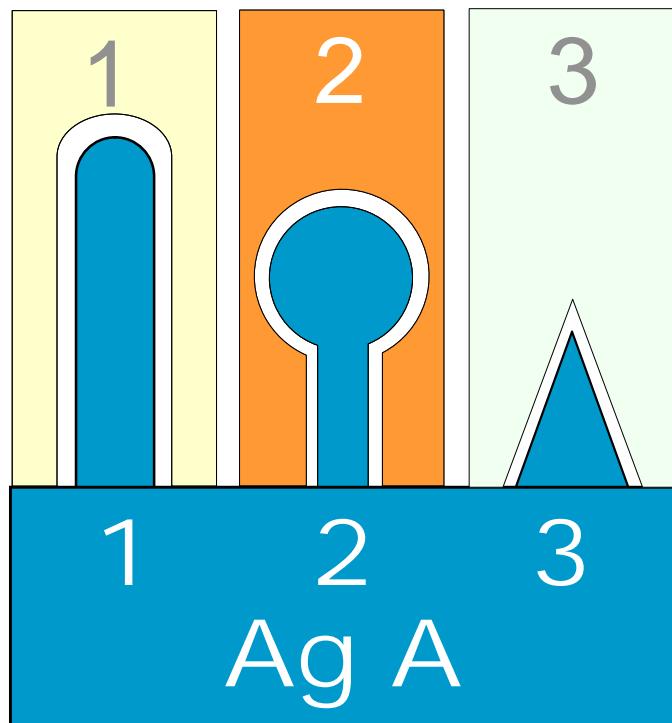


- 若有許多抗原決定基，則需許多株 B 細胞分別生產許多抗体。

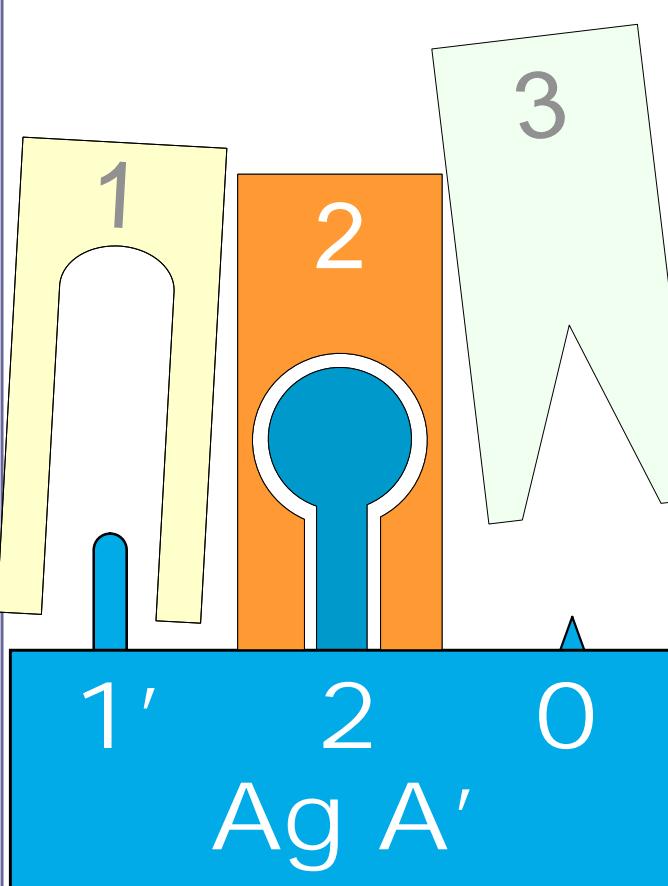


# 傳統抗血清的交叉反應

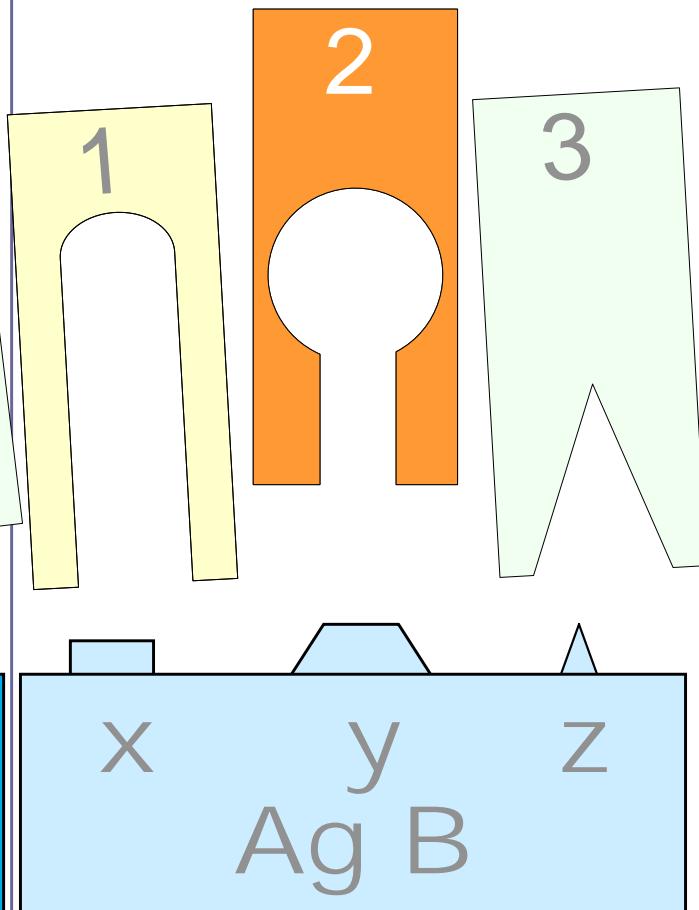
專一性反應



交叉反應



沒有反應

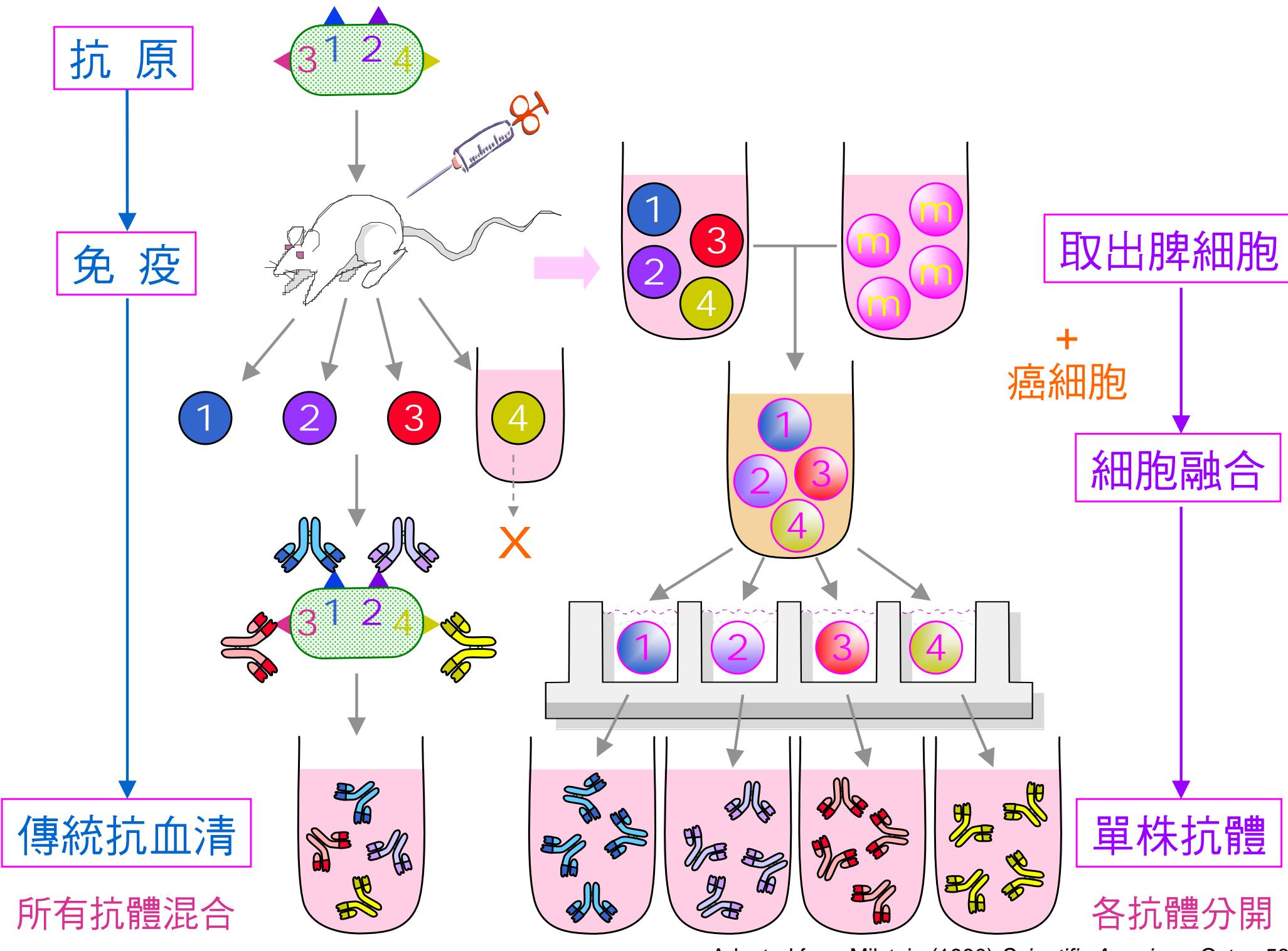


+

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-

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



實驗步驟

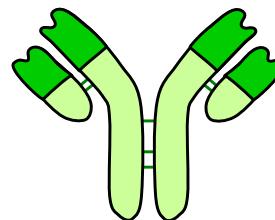
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# 細胞融合法

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## Cell Fusion

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細胞融合 → 初步篩選 → 專一性篩選 → 單株化 → 抗體生產

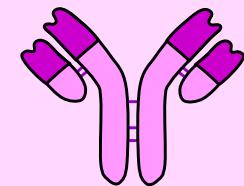
PEG

HAT

ELISA

Limiting  
dilution

Ascites  
fluid

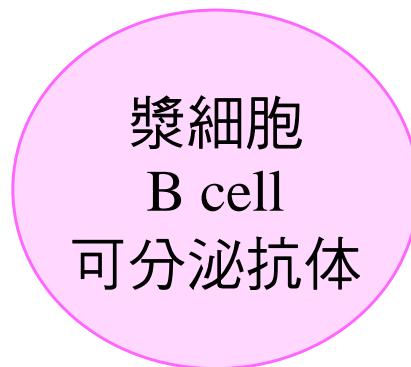


# 單株抗體

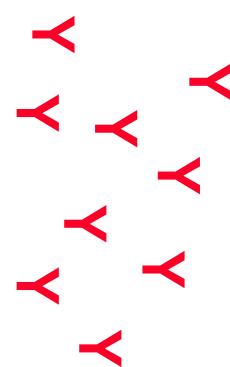
可生產有用抗體的 **淋巴細胞** 若與 **癌細胞** 融合，則形成穩定而可培養的細胞株。



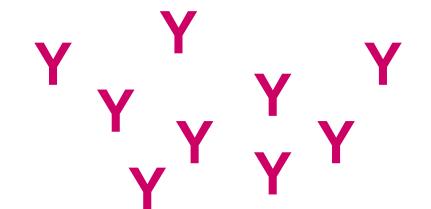
都是白血球



一個 B cell 只  
產生一種抗體

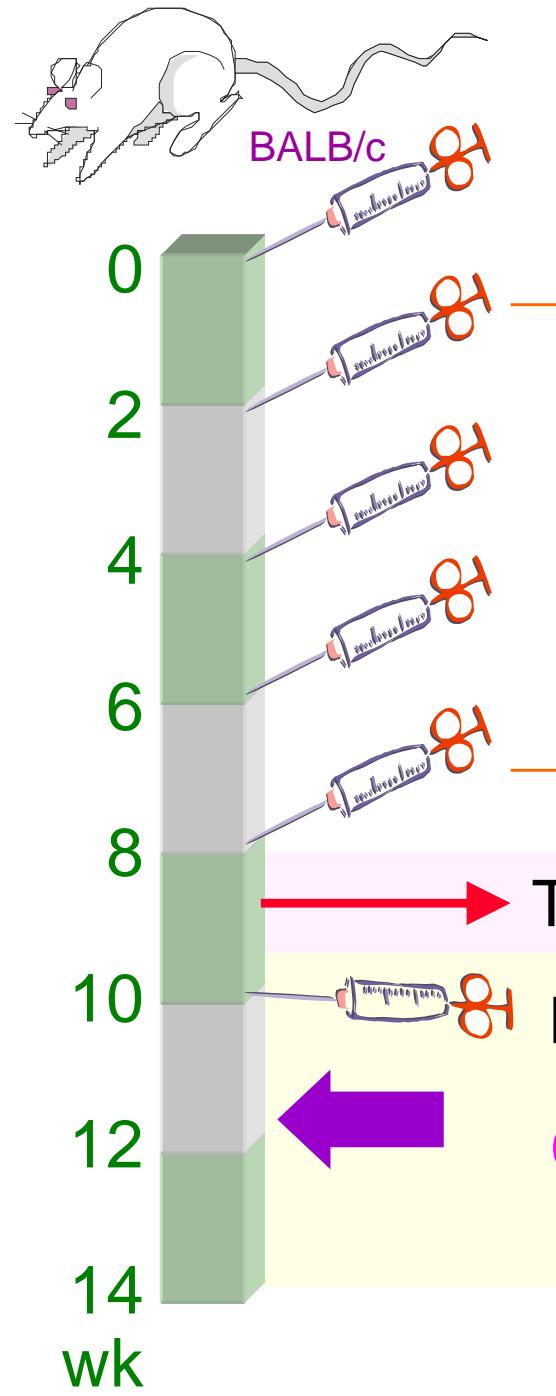


兩組染色體混在一起



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)

Emulsified in 0.5 mL

Freund's Complete Adjuvant

加佐劑製成乳劑

At least three booster shots,  
same dose in 0.5 mL

Freund's Incomplete Adjuvant

Booster shots might be reduced  
if TiterMax is use as adjuvant

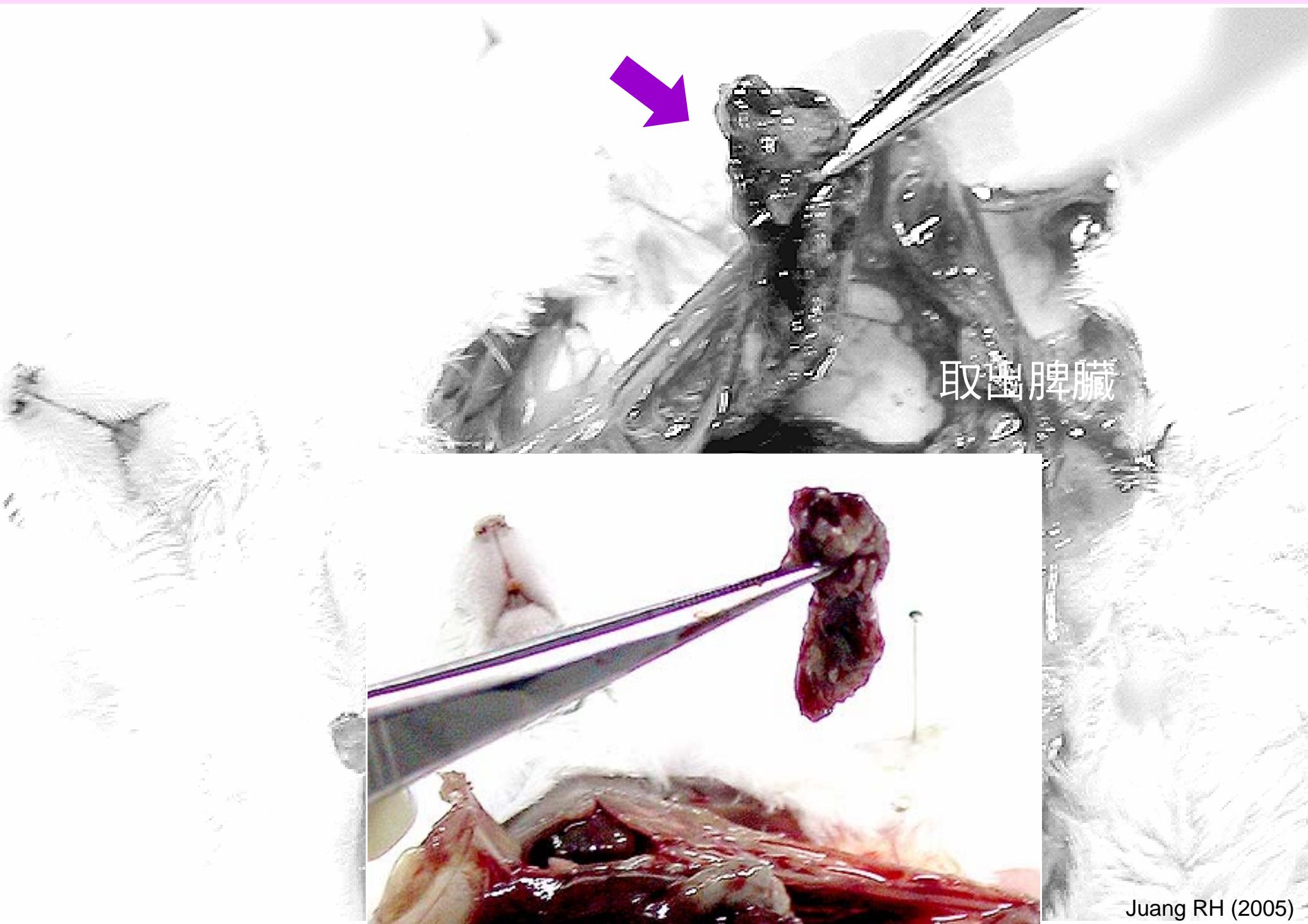
試採血

Trial Bleeding → Titer Determination

Final booster (soluble antigen)

Cell fusion

# 免疫後取出脾臟

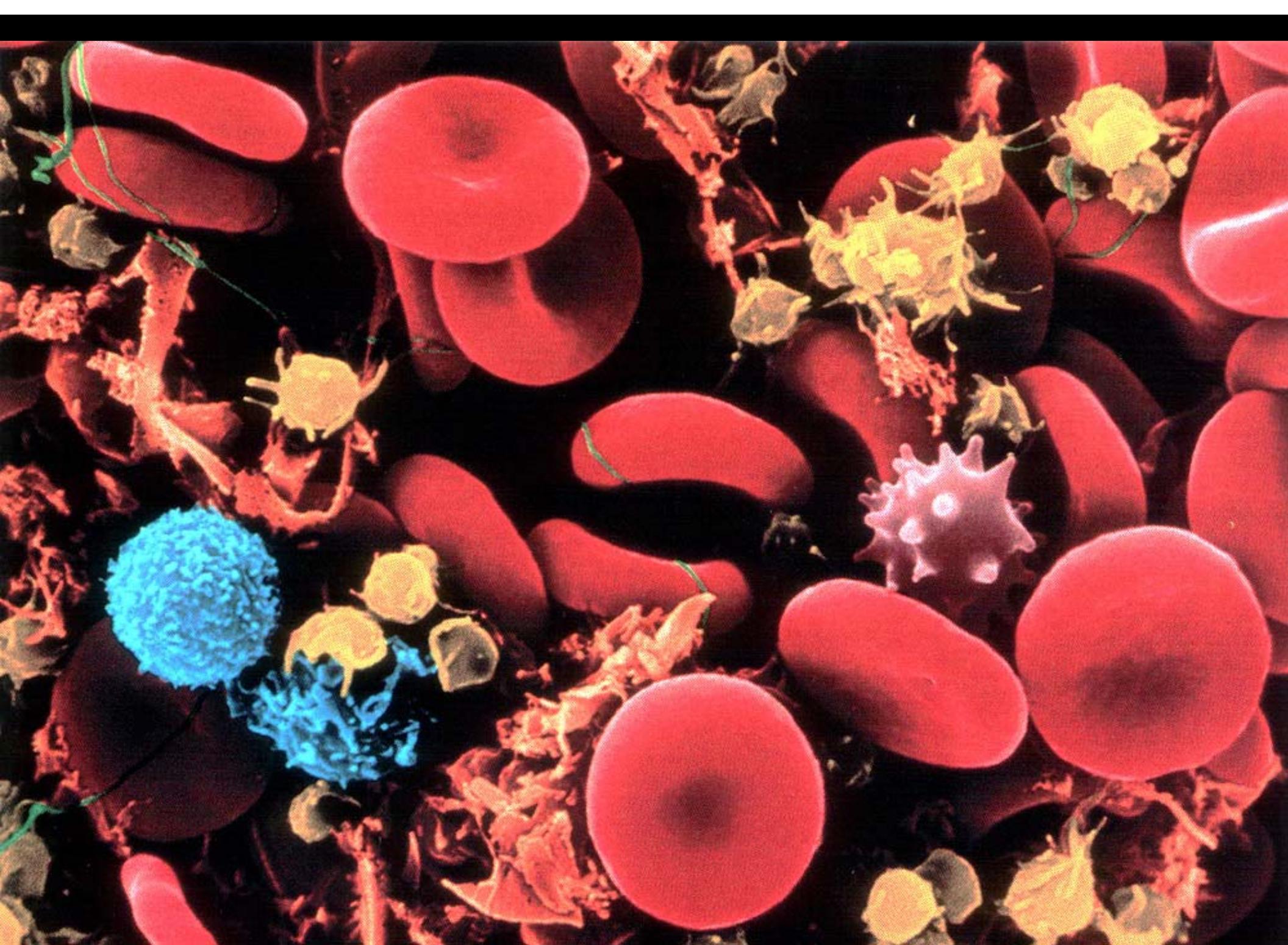


Juang RH (2005)

# 脾臟為 B 細胞集中地

取出脾臟內細胞

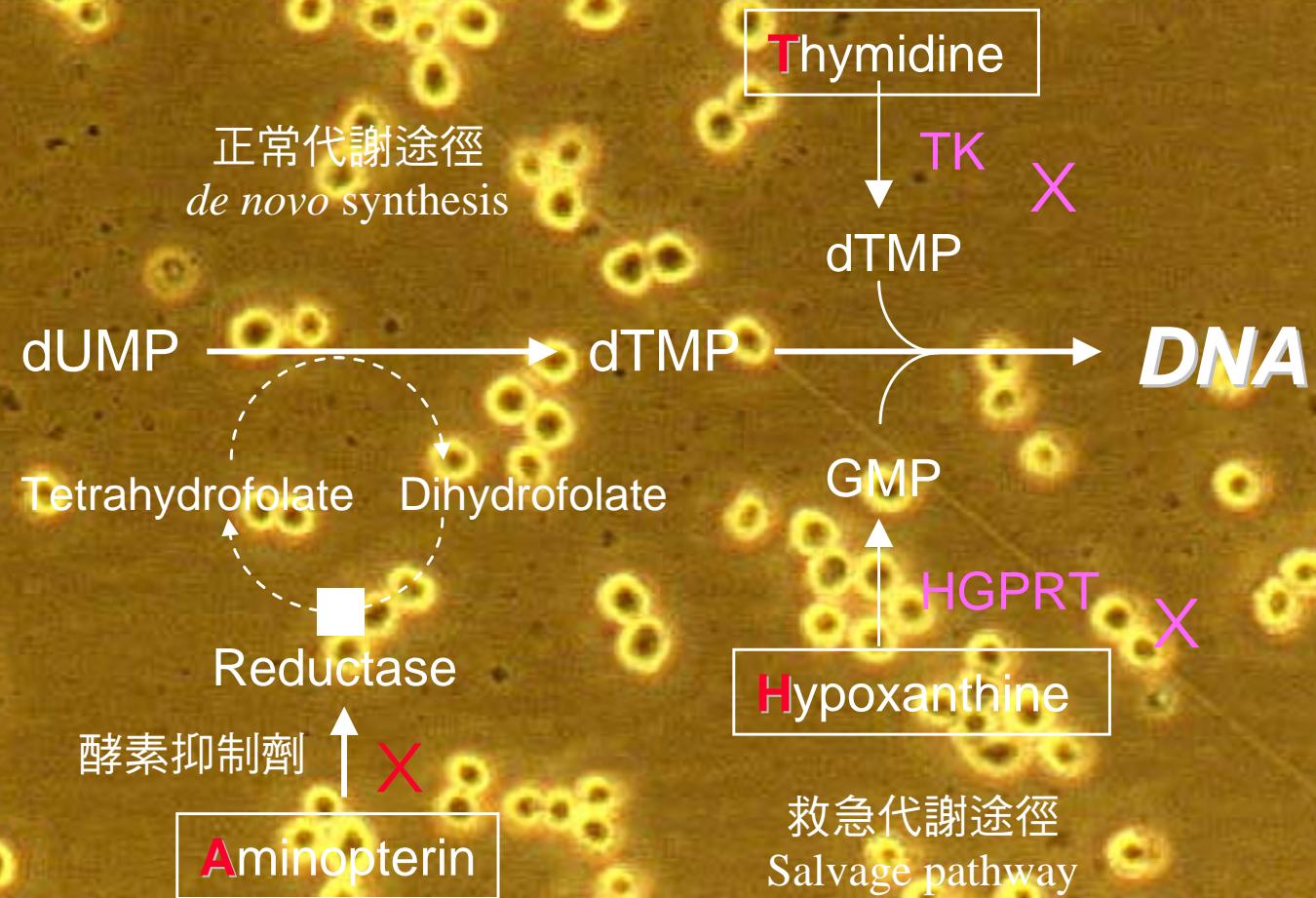




Dennis Kunkel / *Everyday Science Explained*. p.243

# 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

加入 PEG

細胞融合  
2 min

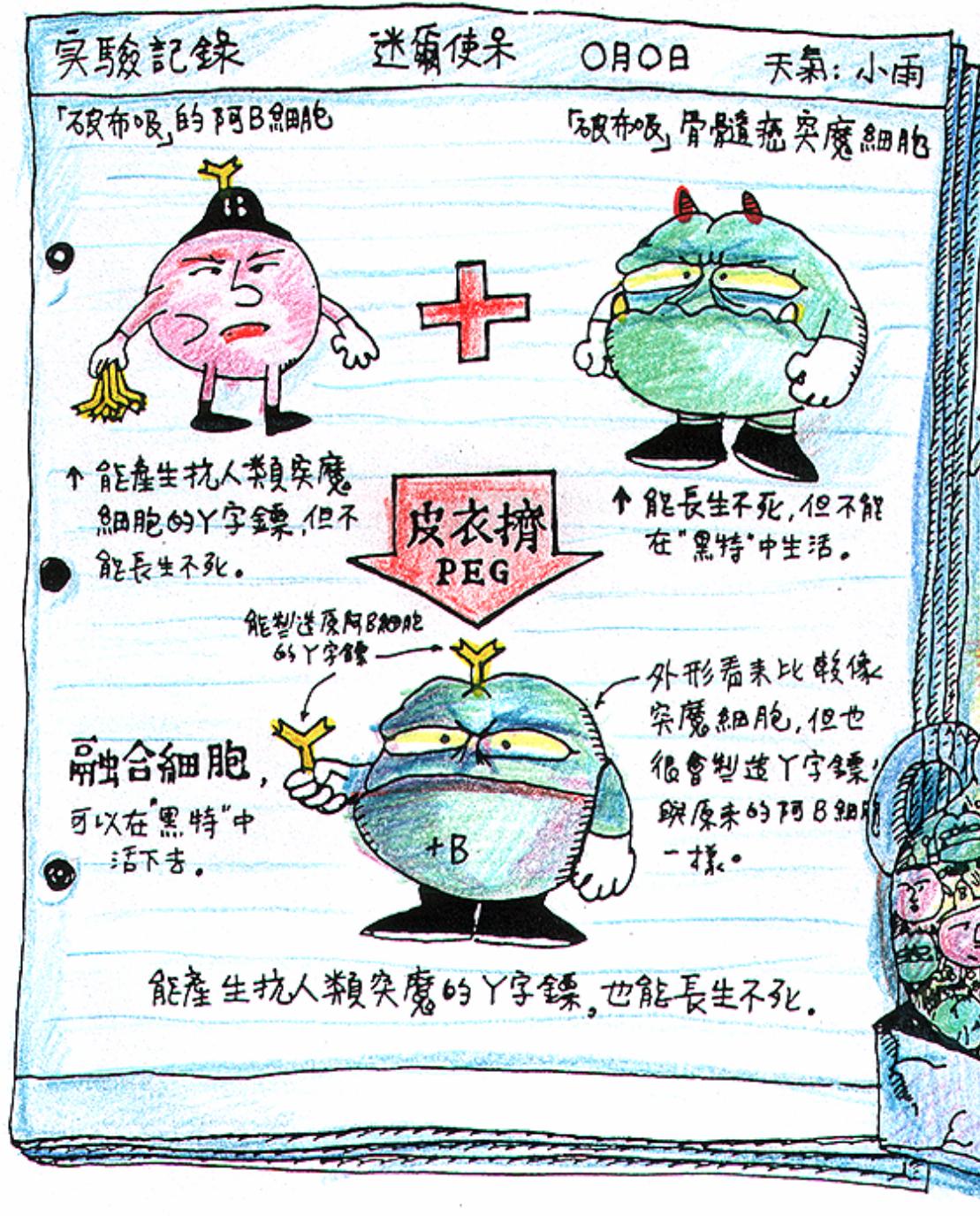


# 96 槽細胞培養盤

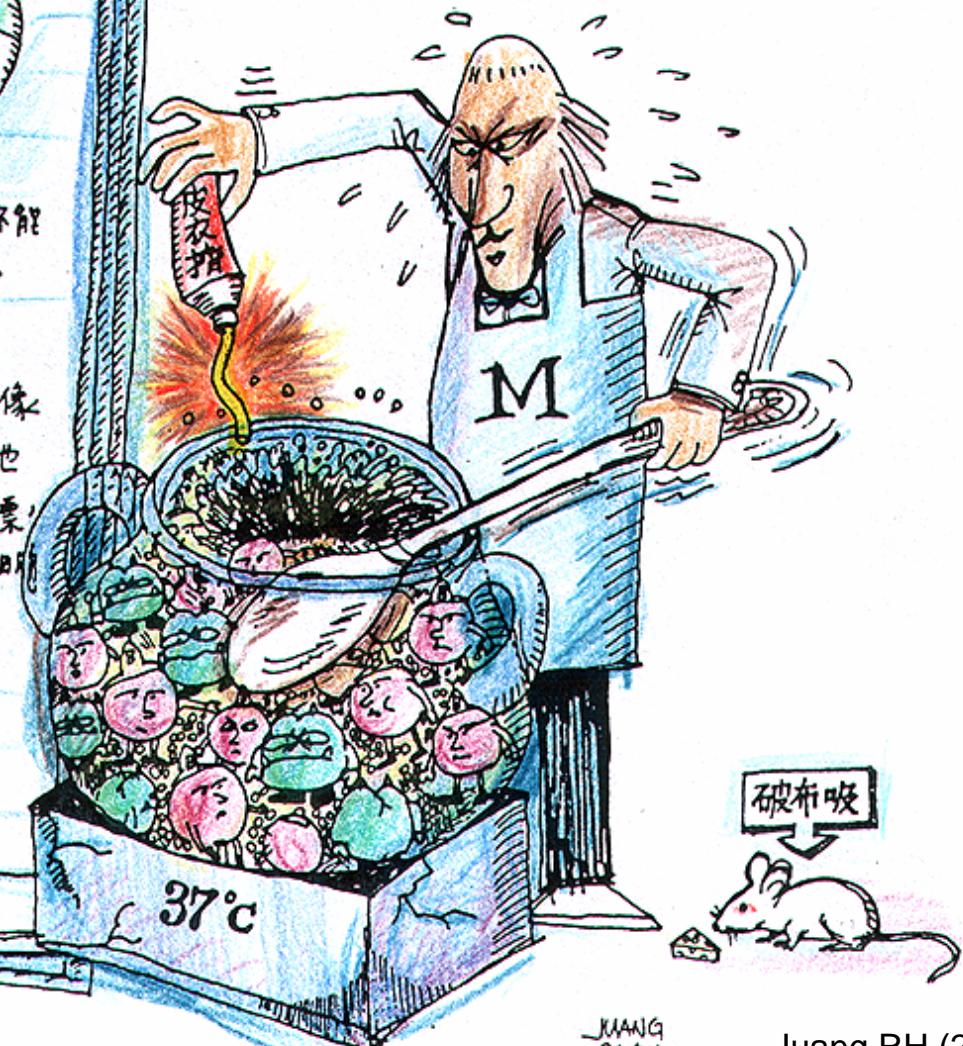


把融合後的細胞均分在培養盤的小槽中

# 迷爾使呆的研究日誌



Kohler & Milstein (1984)

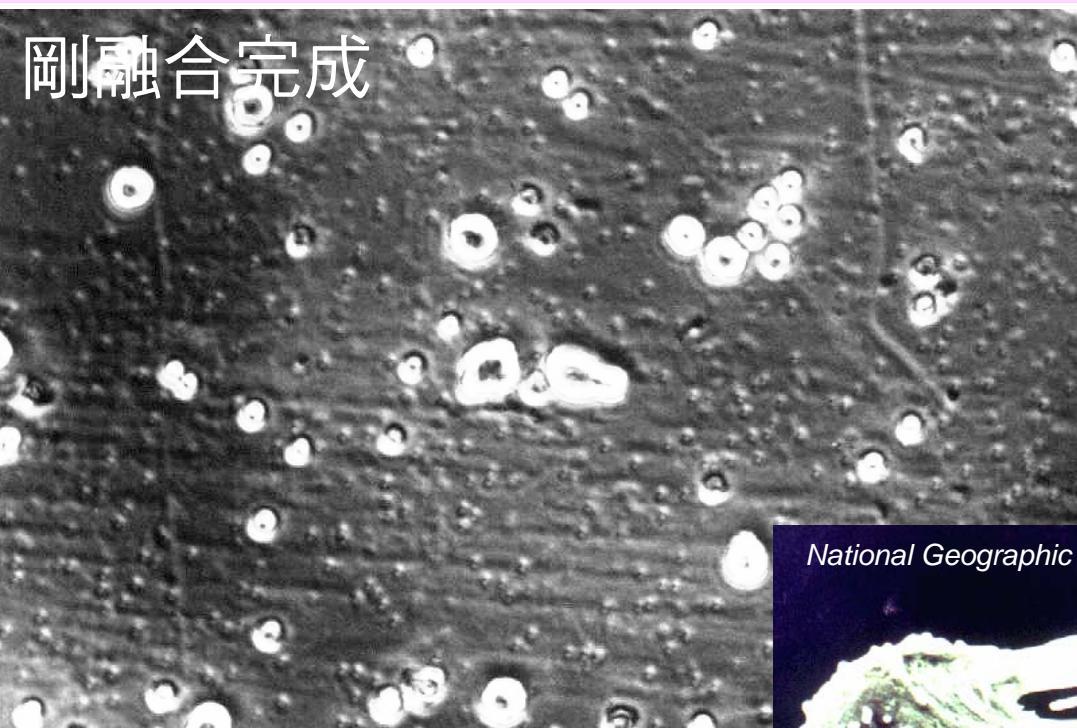


JUANG  
840311

Juang RH (2005)

# 融合後的細胞生長情形

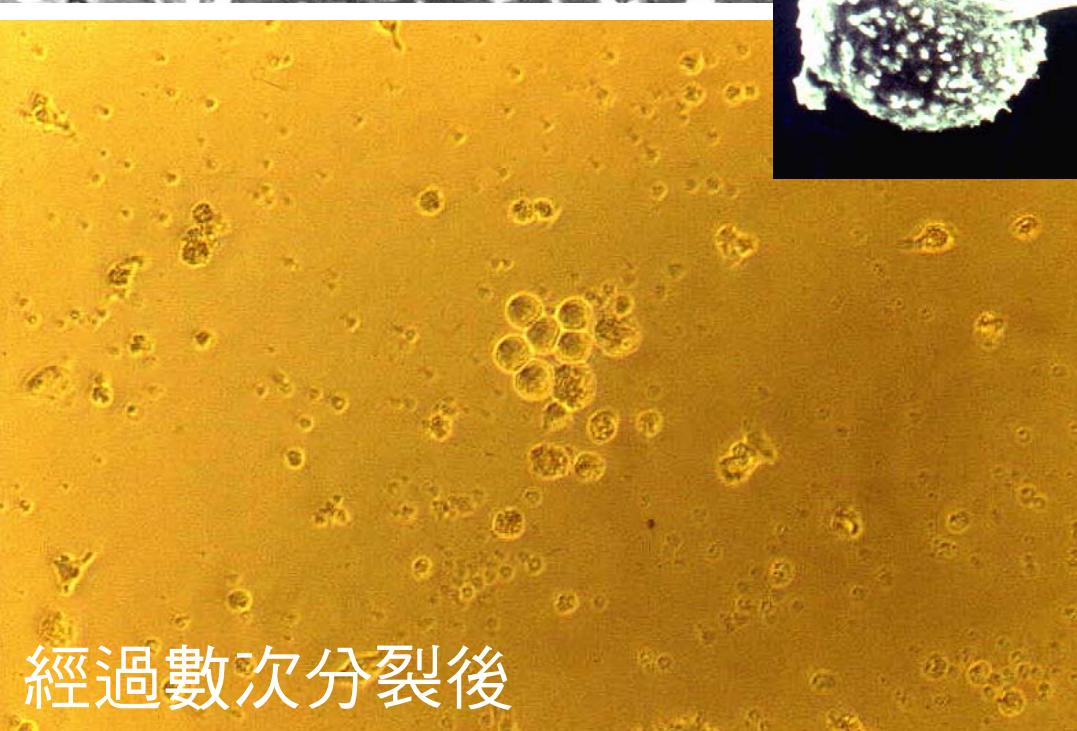
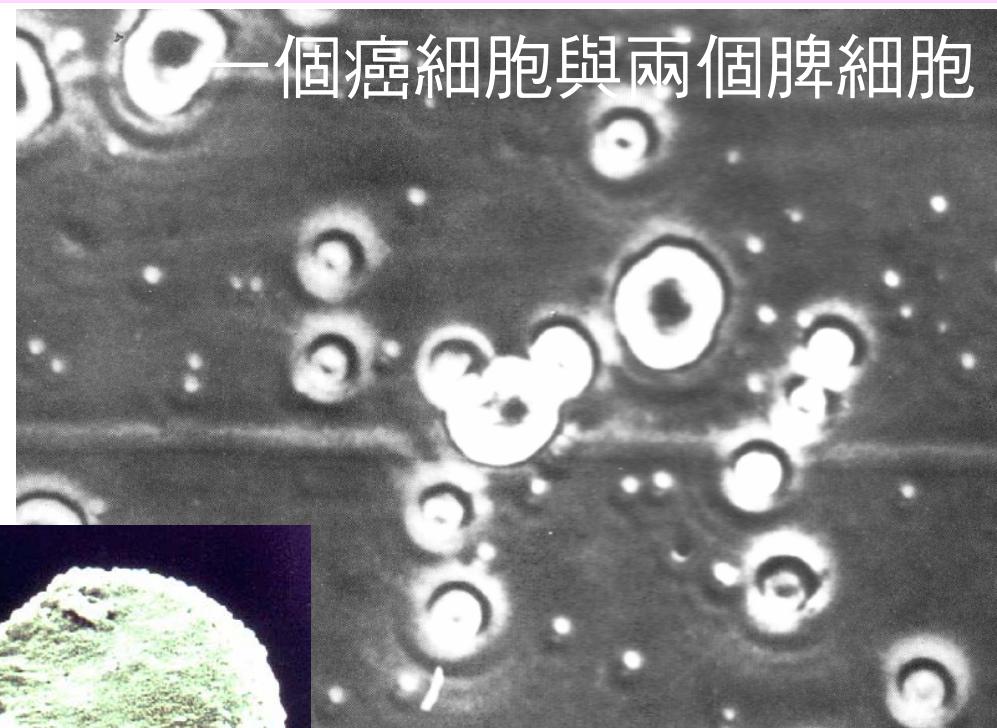
剛融合完成



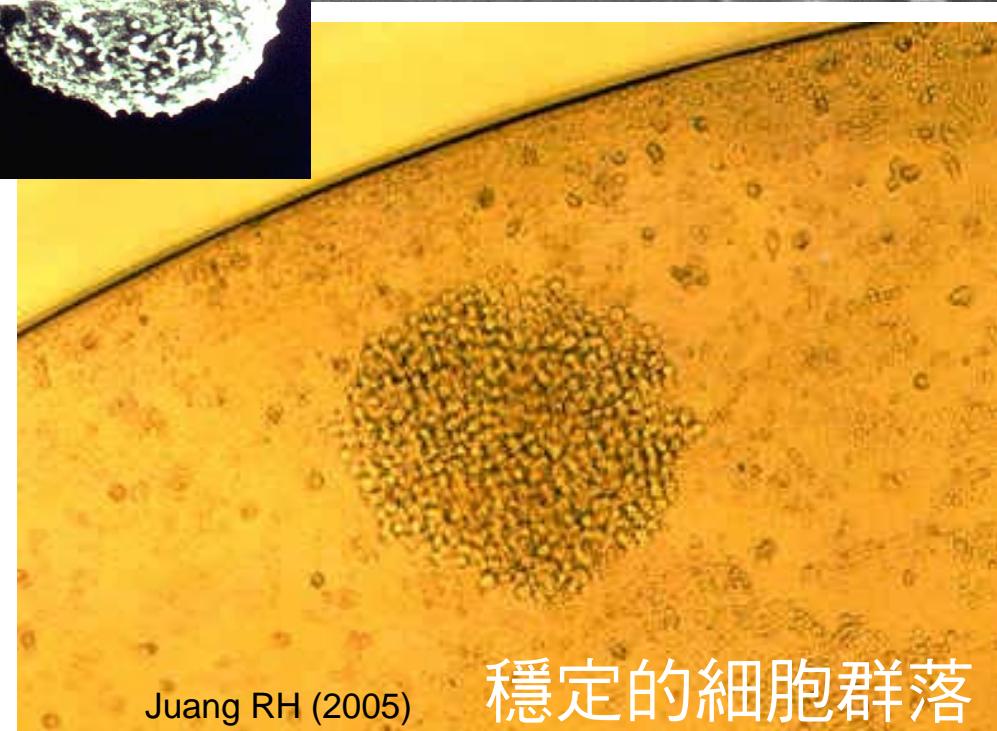
*National Geographic*



一個癌細胞與兩個脾細胞



經過數次分裂後



Juang RH (2005)

穩定的細胞群落

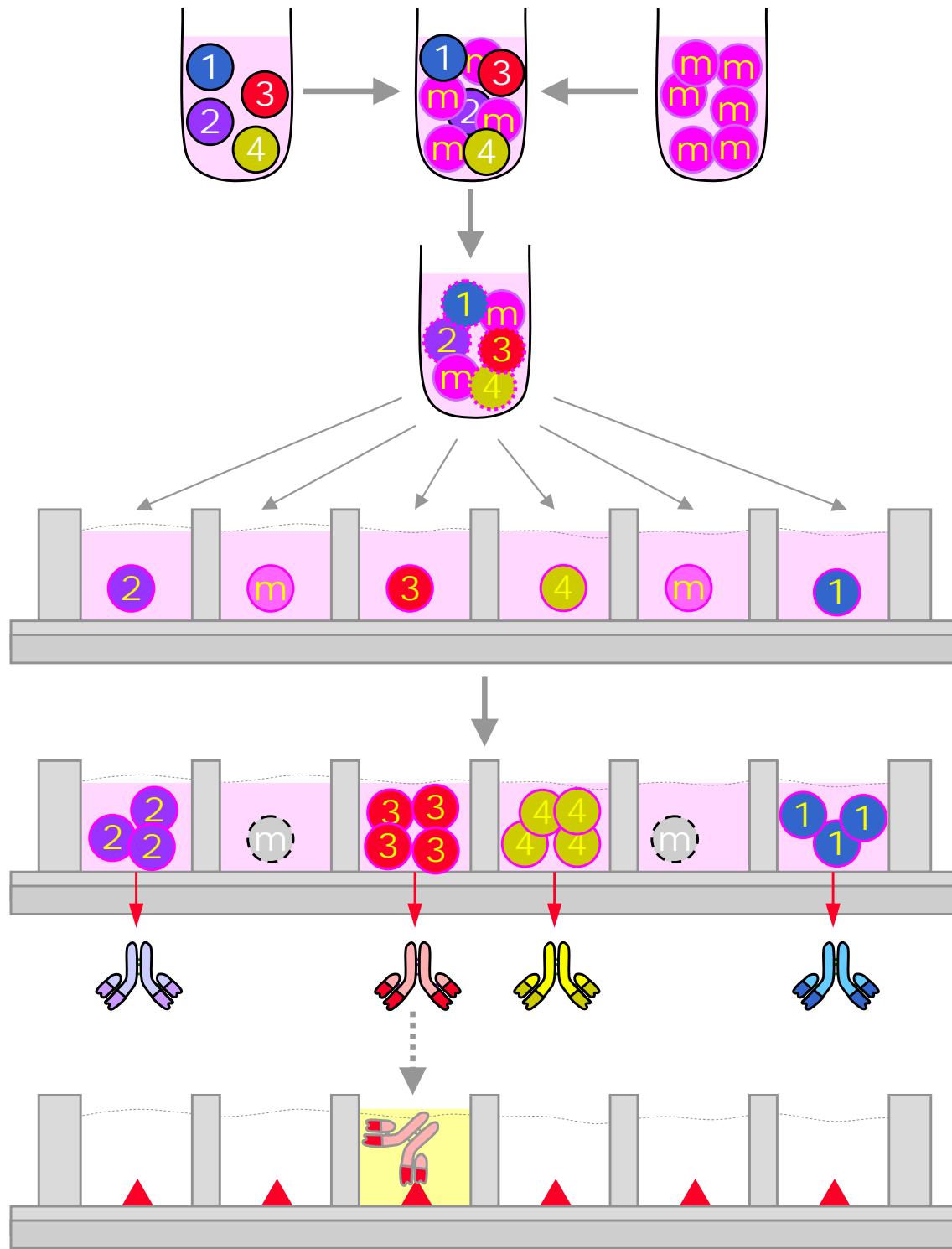
脾細胞

癌細胞

細胞融合

HAT  
初步篩選

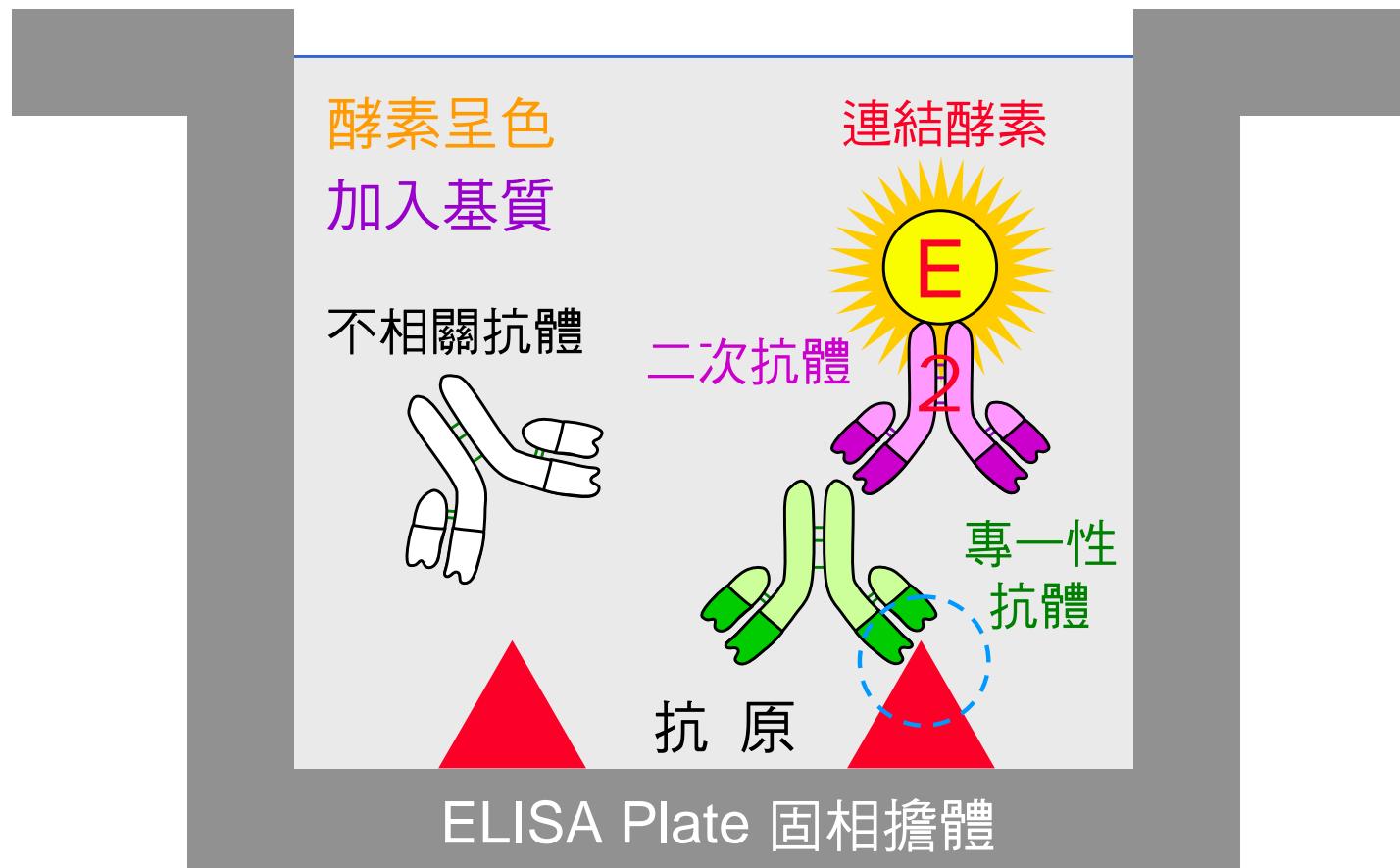
ELISA  
抗體篩選



# 以酵素免疫分析法檢測樣本中的專一性抗體

## ELISA

Enzyme-Linked Immunosorbent Assay

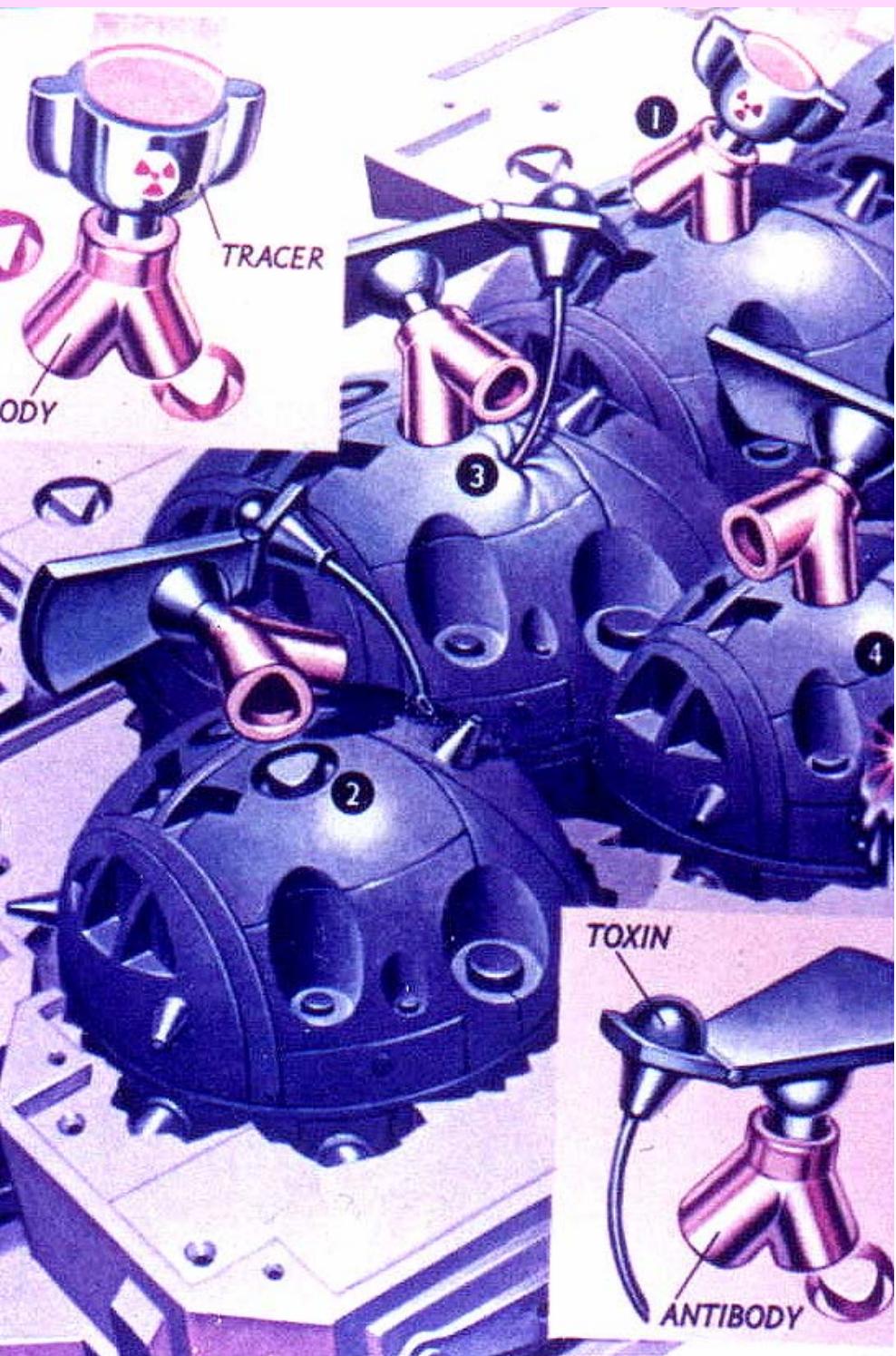


# 細胞穩定後擴大至 T-25 細胞瓶

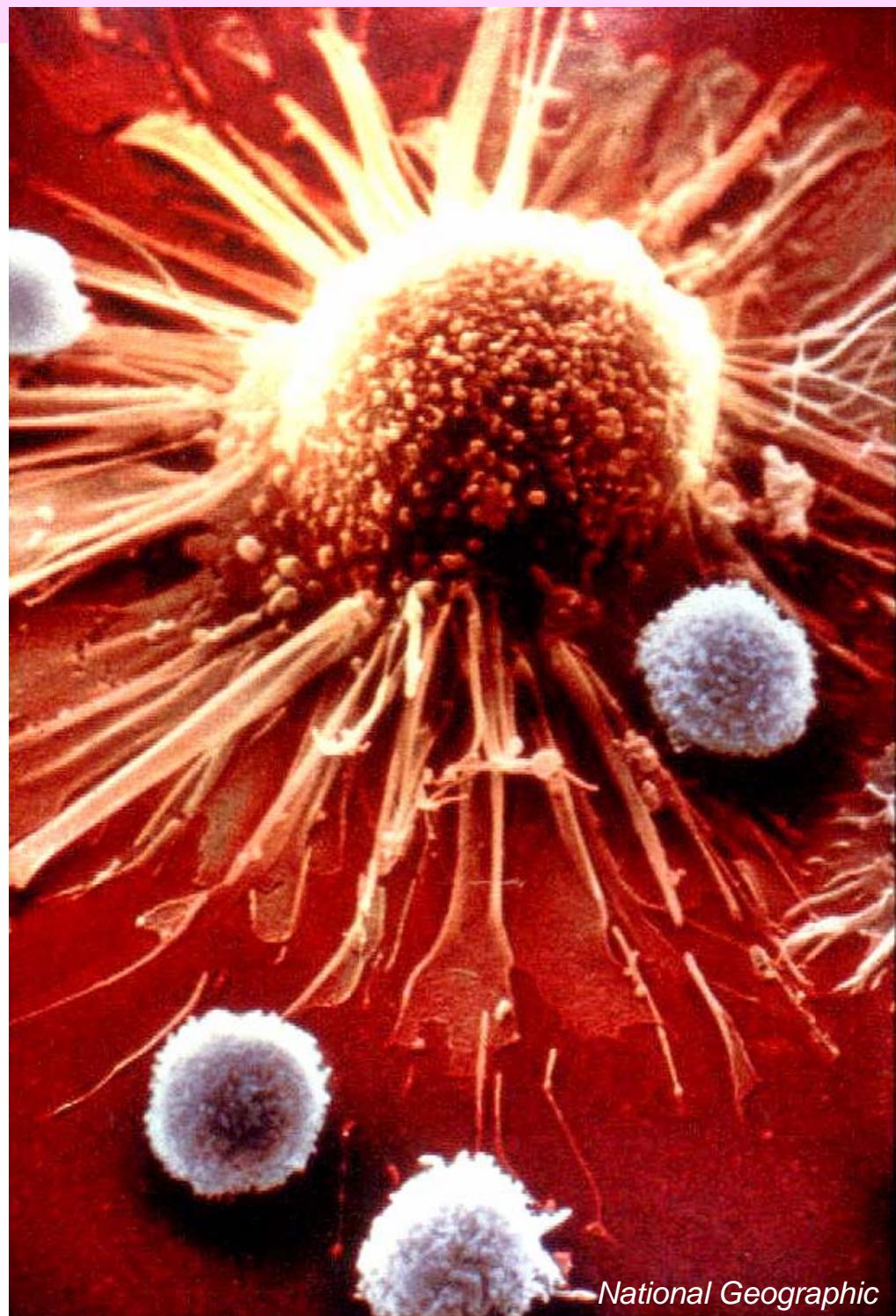


在 T-75 培養小瓶中擴大細胞數目

Juang RH (2005)

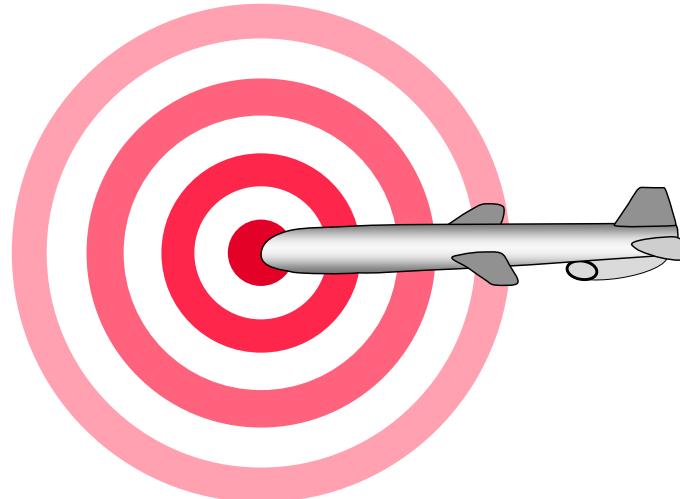


以單株抗體來清除體內癌細胞



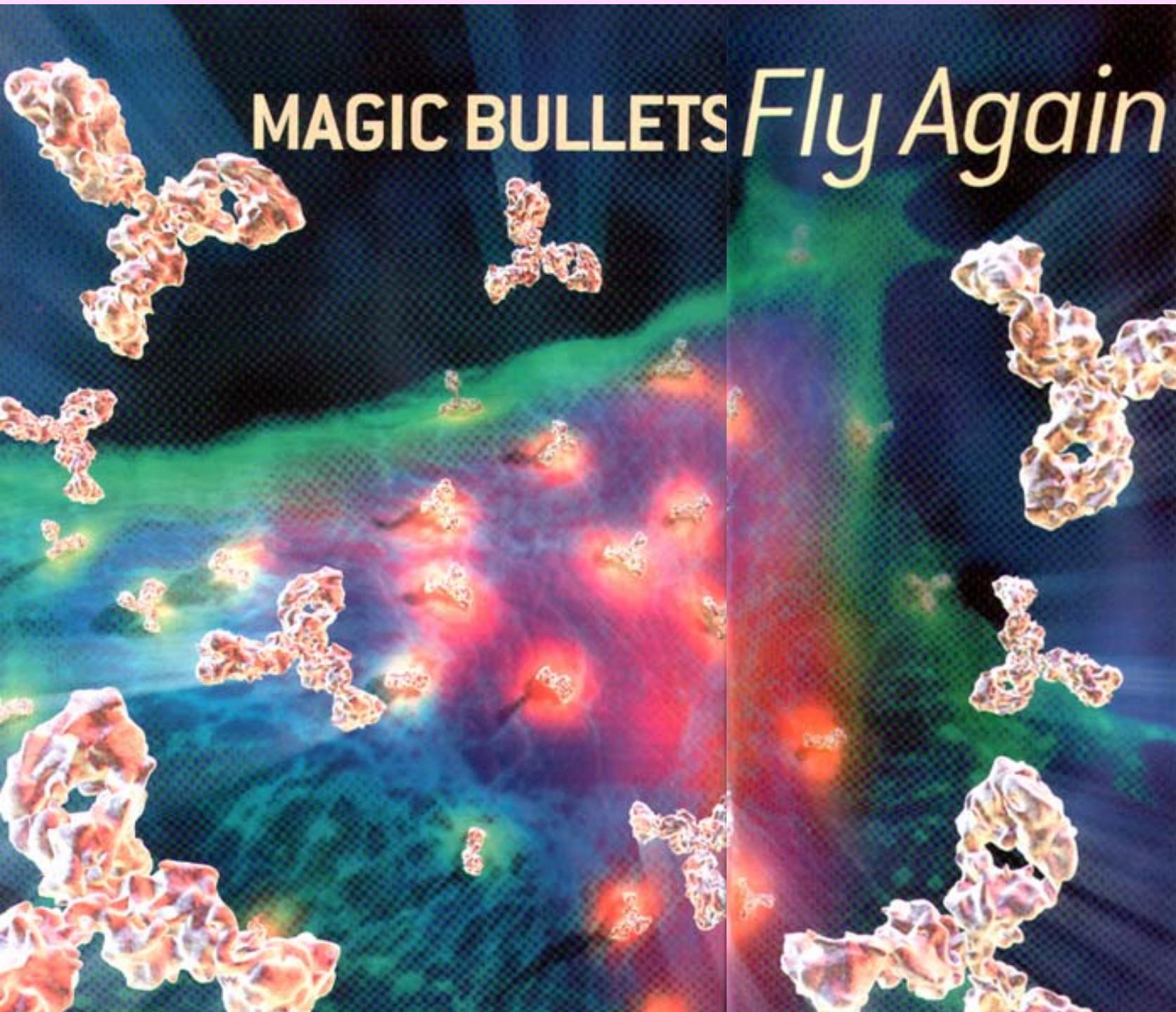
# 單株抗體的高度專一性有很多用途

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 特定抗原    |
| 醫 療 | 以毒素連結抗体攻擊 病變細胞   |
| 檢 驗 | 以 ELISA 偵測特定 病原体 |

魔彈  
再起

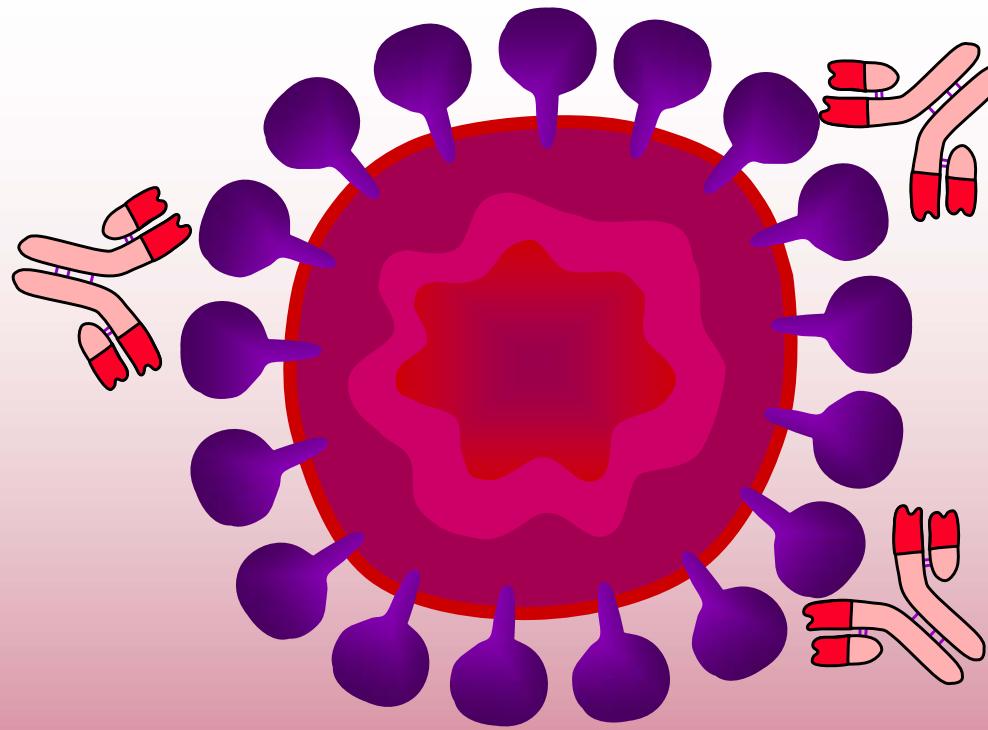


Molecular guided missiles called monoclonal antibodies were poised to shoot down cancer and a host of other diseases—until they crashed and burned. Now a new generation is soaring to market

By Carol Ezzell

# 製備抗 SARS 的單株抗體及應用

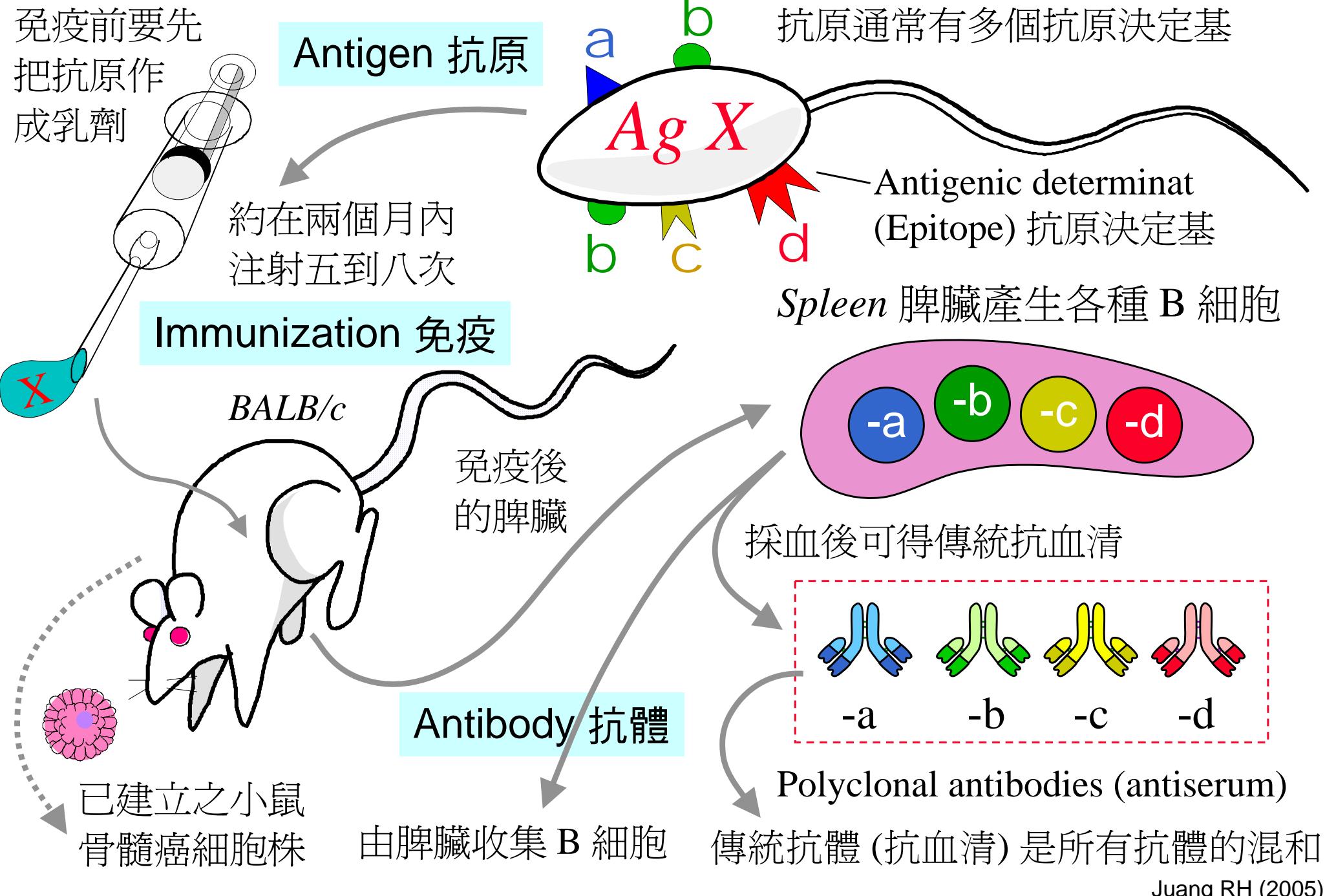
## SARS-CoV



(+) RNA, 29,727 nt, 11 ORF

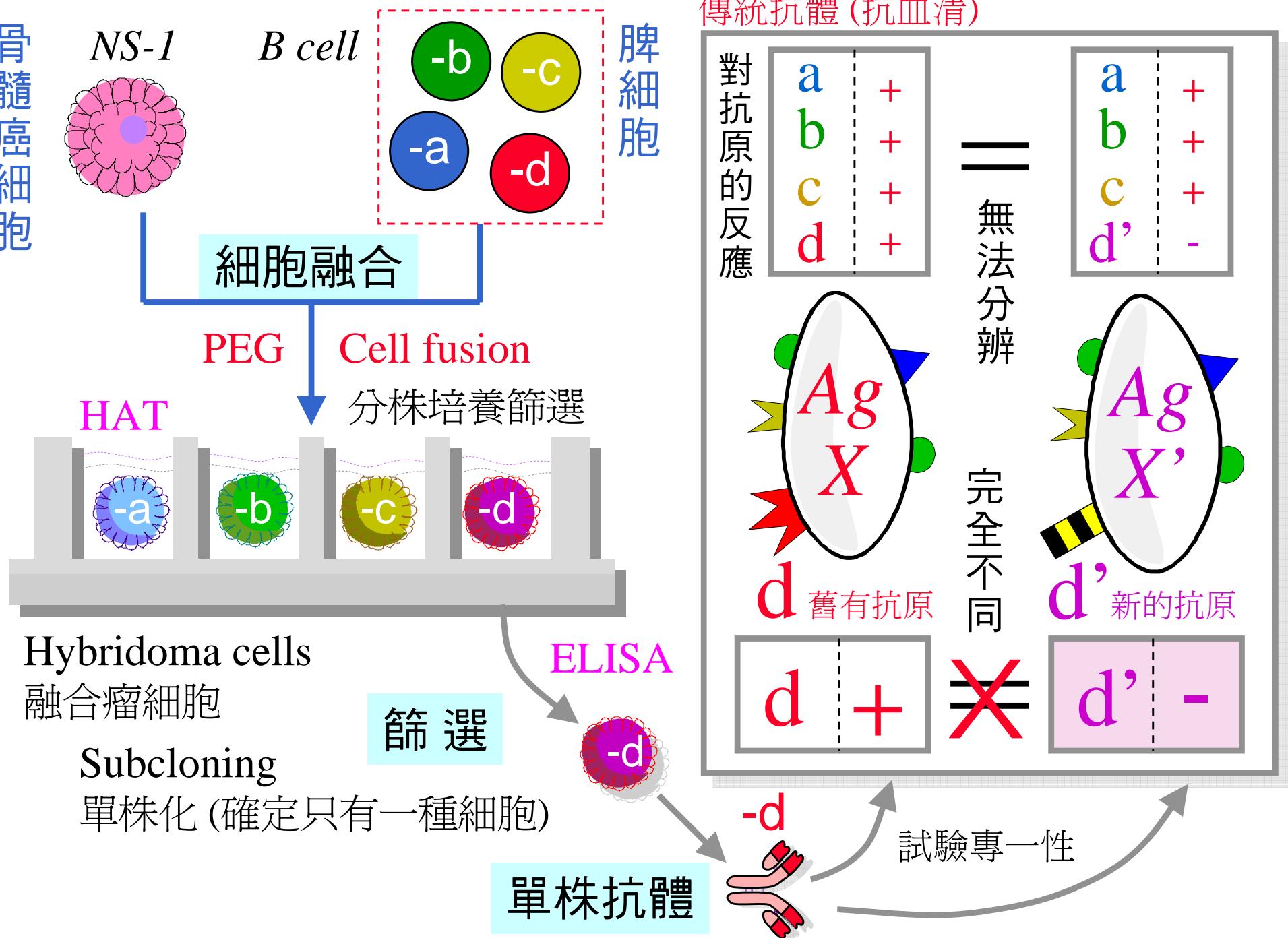
Science (2003) May

# 如何製備單株抗體 – 免疫產生專一性 B 細胞



# 如何製備單株抗體 – 以細胞融合產生融合瘤

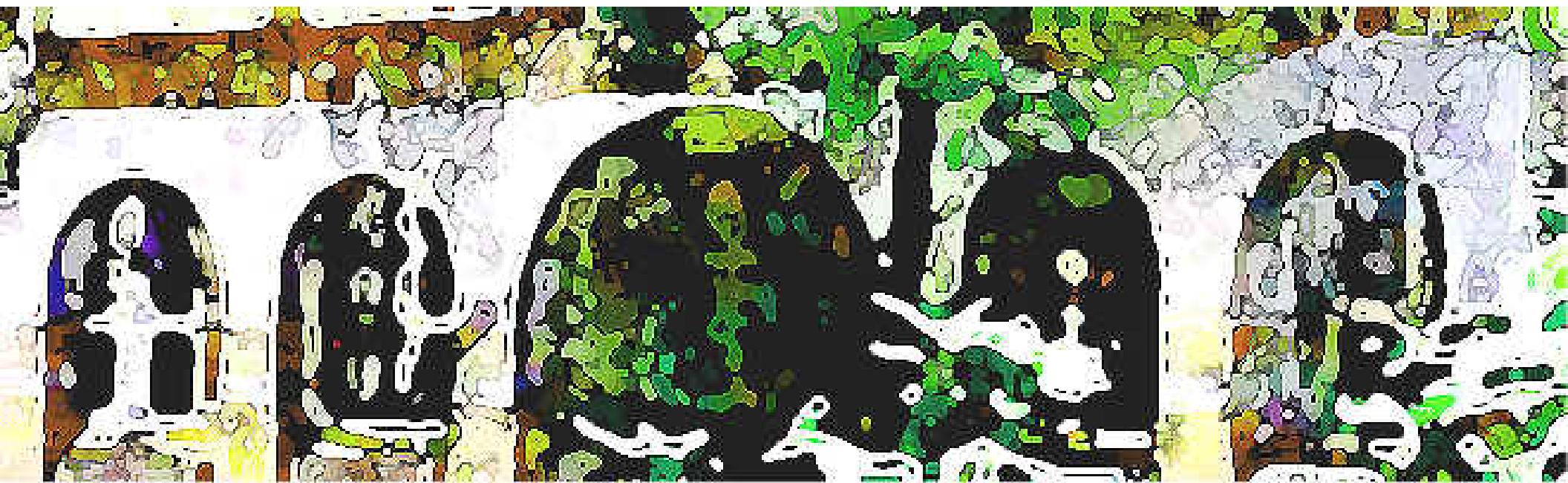
骨髓癌細胞



# 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

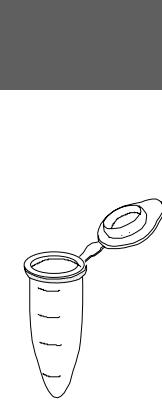
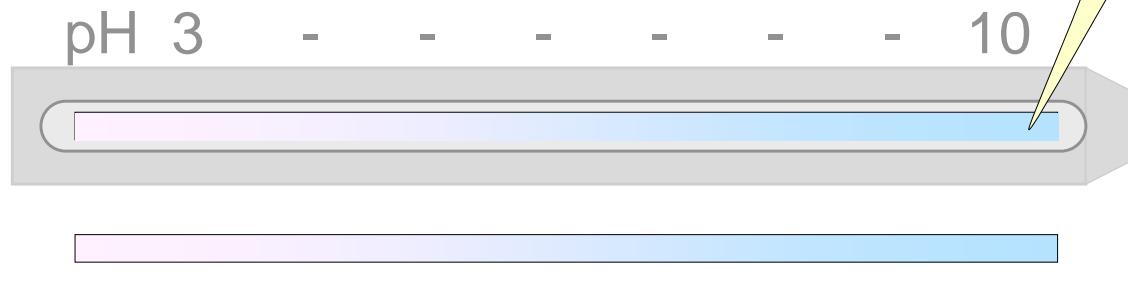
由基因體較難預測蛋白質的修飾及調控

也無法預測蛋白質間的交互作用

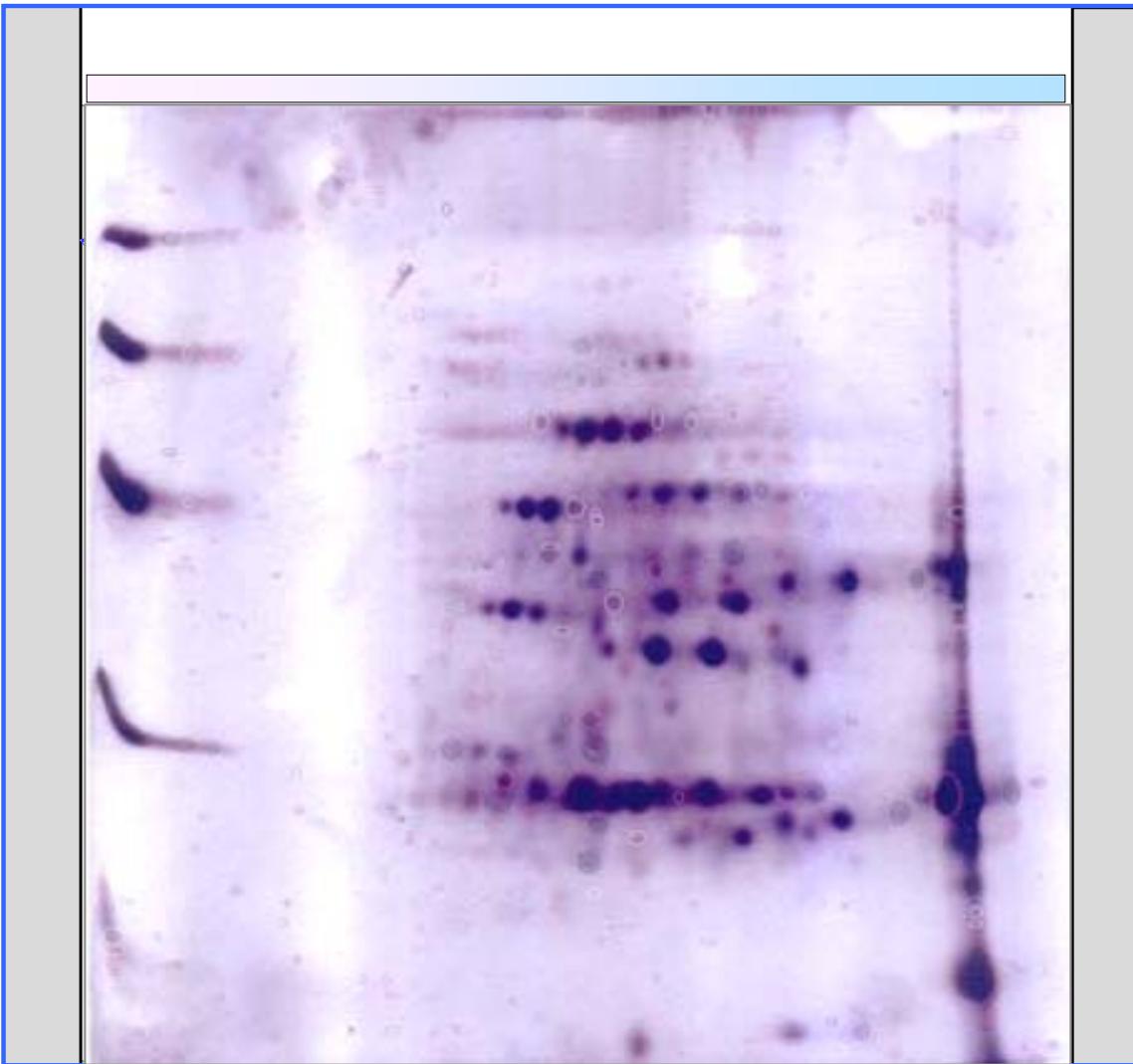
# Proteome

# 二次元電泳的操作過程

(1) IEF  
等電焦集電泳



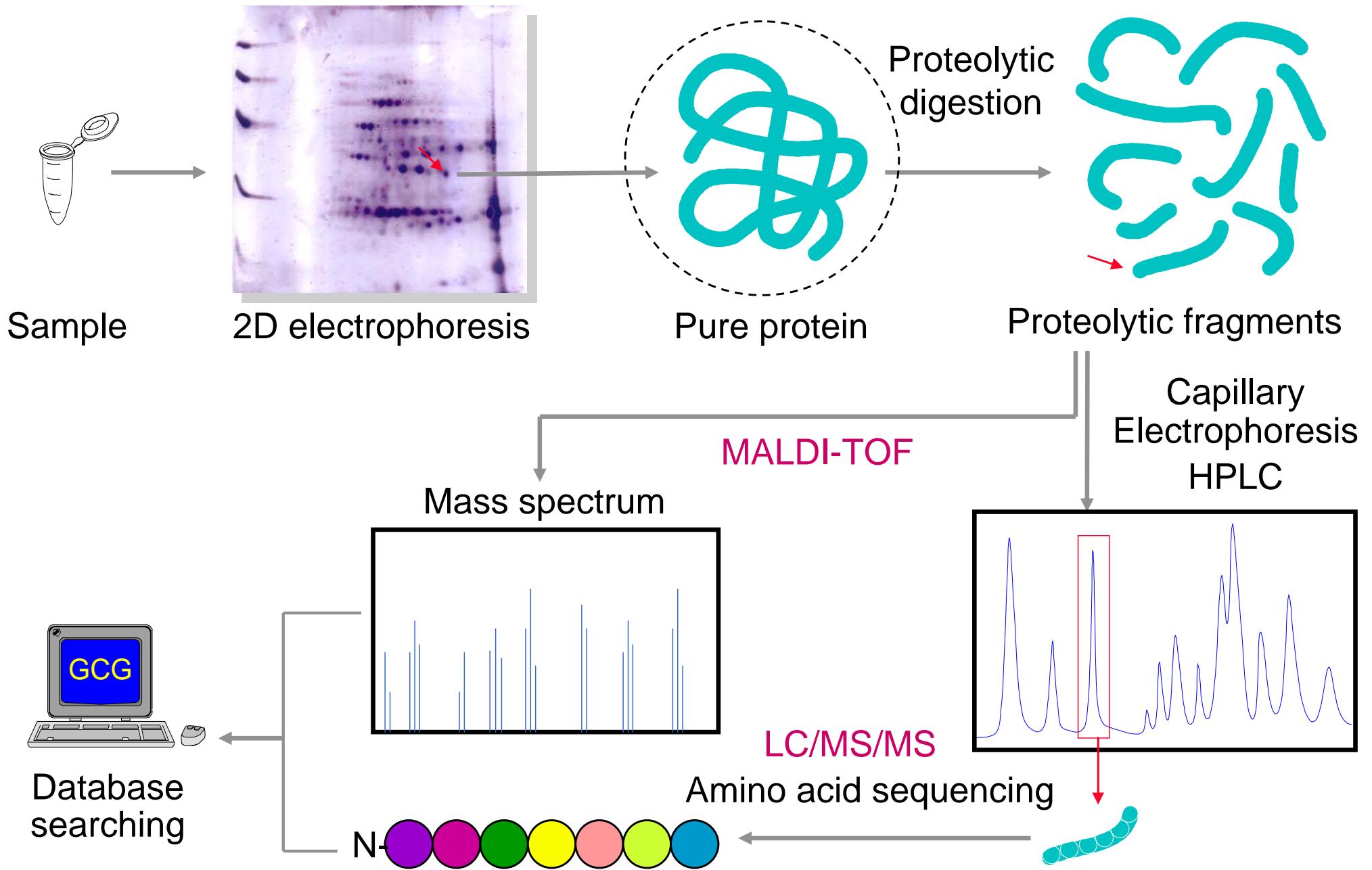
(3)  
染色脫色



(2)  
SDS-PAGE  
分離膠體



## ■ 蛋白質體可綜觀蛋白質的消長與身分：



# 整體研究流程大綱



綠竹筍  
(*Bambusa oldhami*)

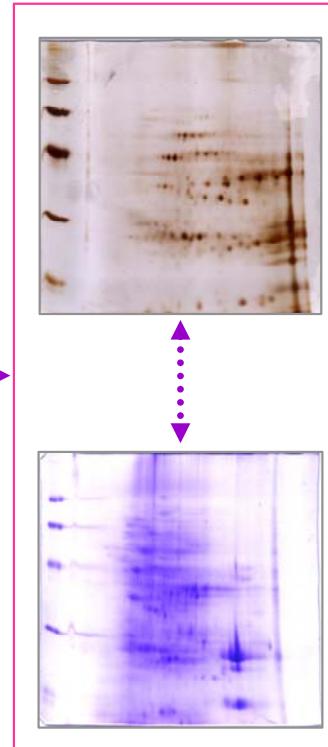
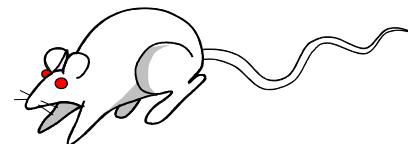
不同生長時期綠竹筍

未出土  
2 cm  
10 cm  
20 cm  
30 cm  
40 cm  
60 cm

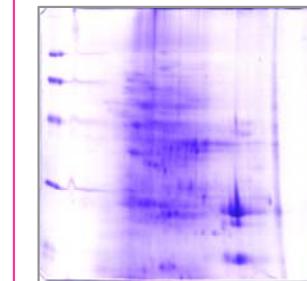
TCA/acetone  
沉澱

50 mM Tris  
buffer 粗抽

未出土、60 cm  
二種樣品進行免疫



2D  
pH 4-7  
pH 7-10



Immunization  
time schedule



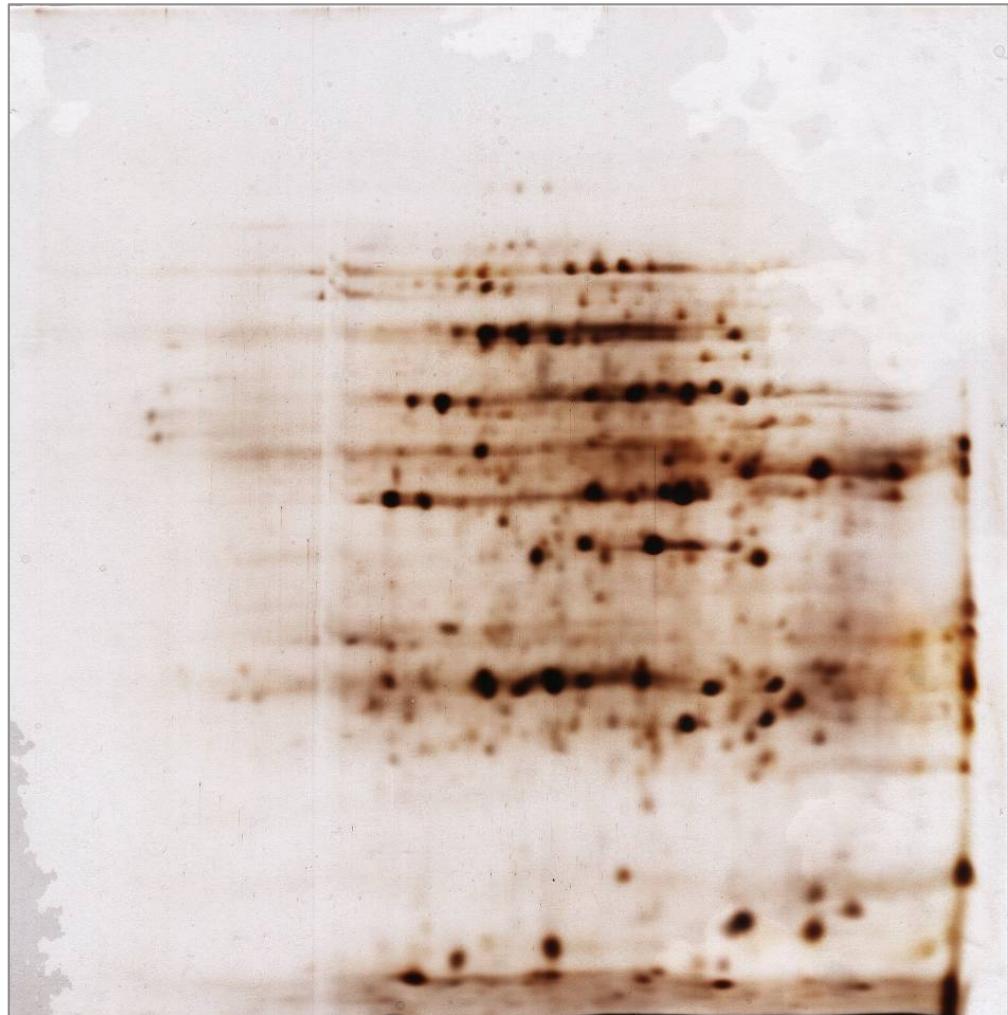
免疫染色

LC-MS-MS

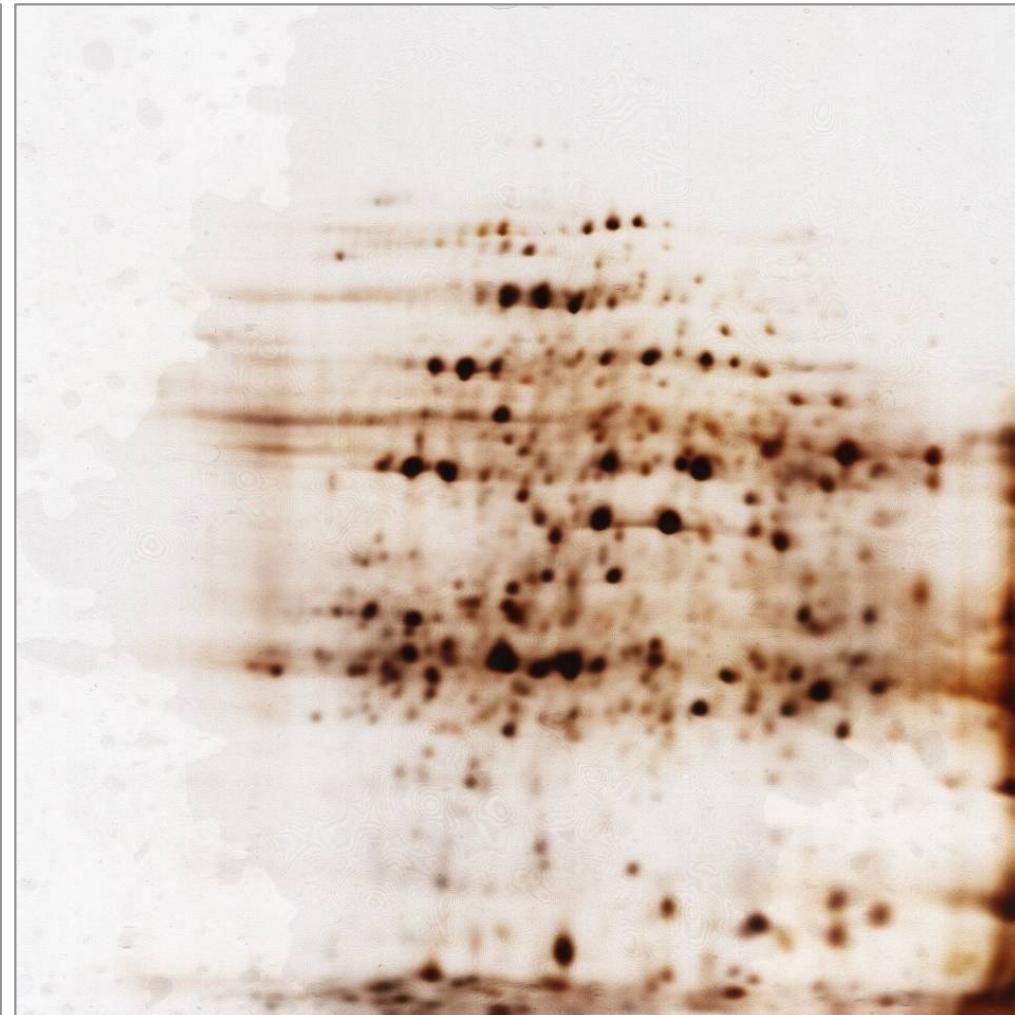
找出有差異色點  
確定蛋白質身分

Edman degradation

# 綠竹筍快速生長前後蛋白質之表現差異

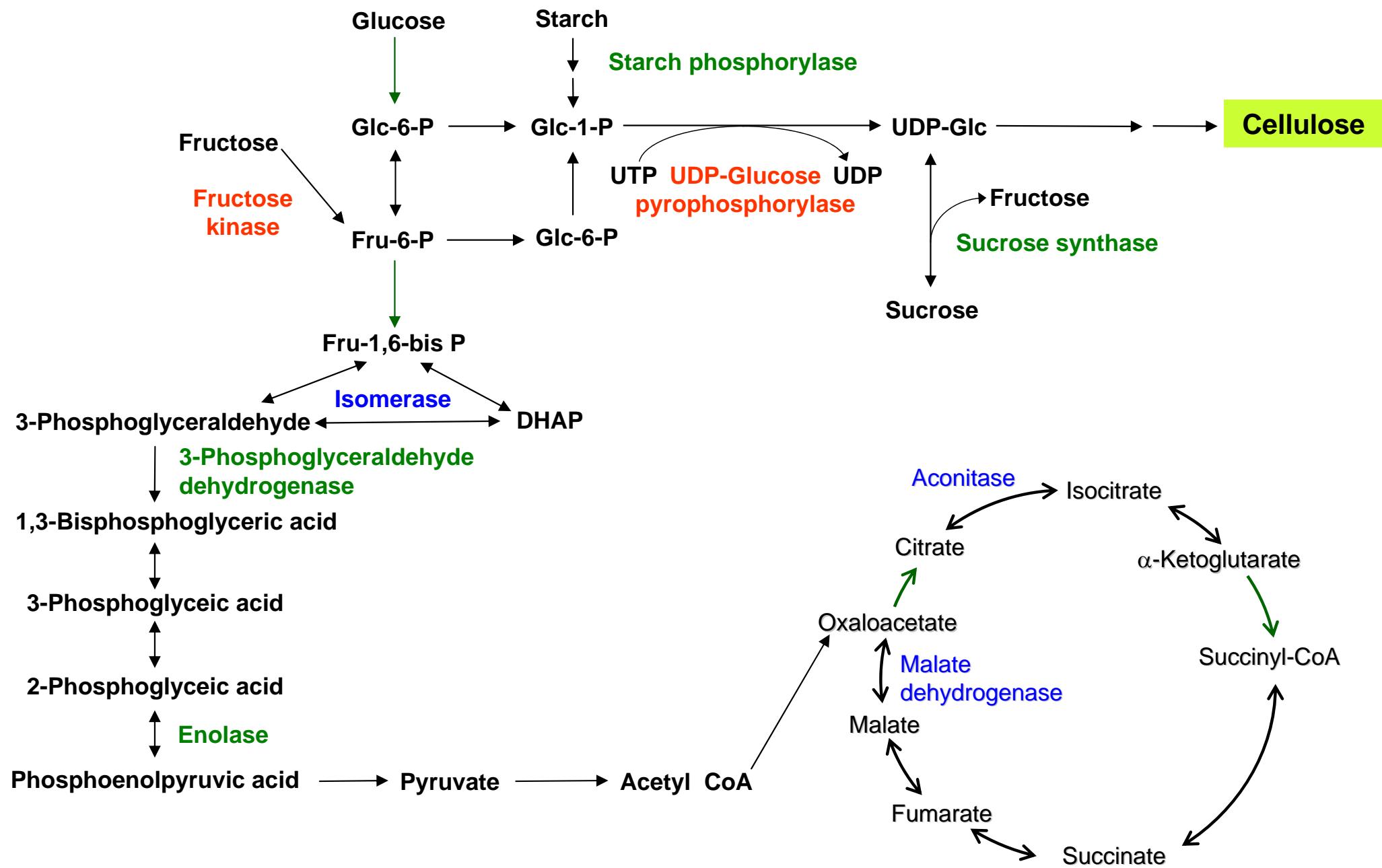


未出土



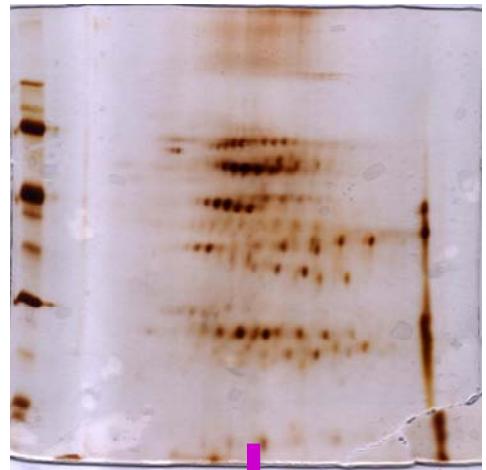
出土 60 cm

# 推測快速生長相關的代謝路徑

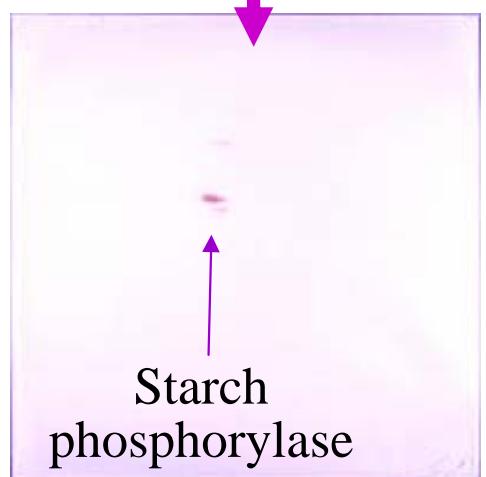


# 抗體應用在蛋白質體檢定的強大效果

未出土竹筍



出土 60 cm 竹筍



首次提出  
高產能抗體製備計畫構想

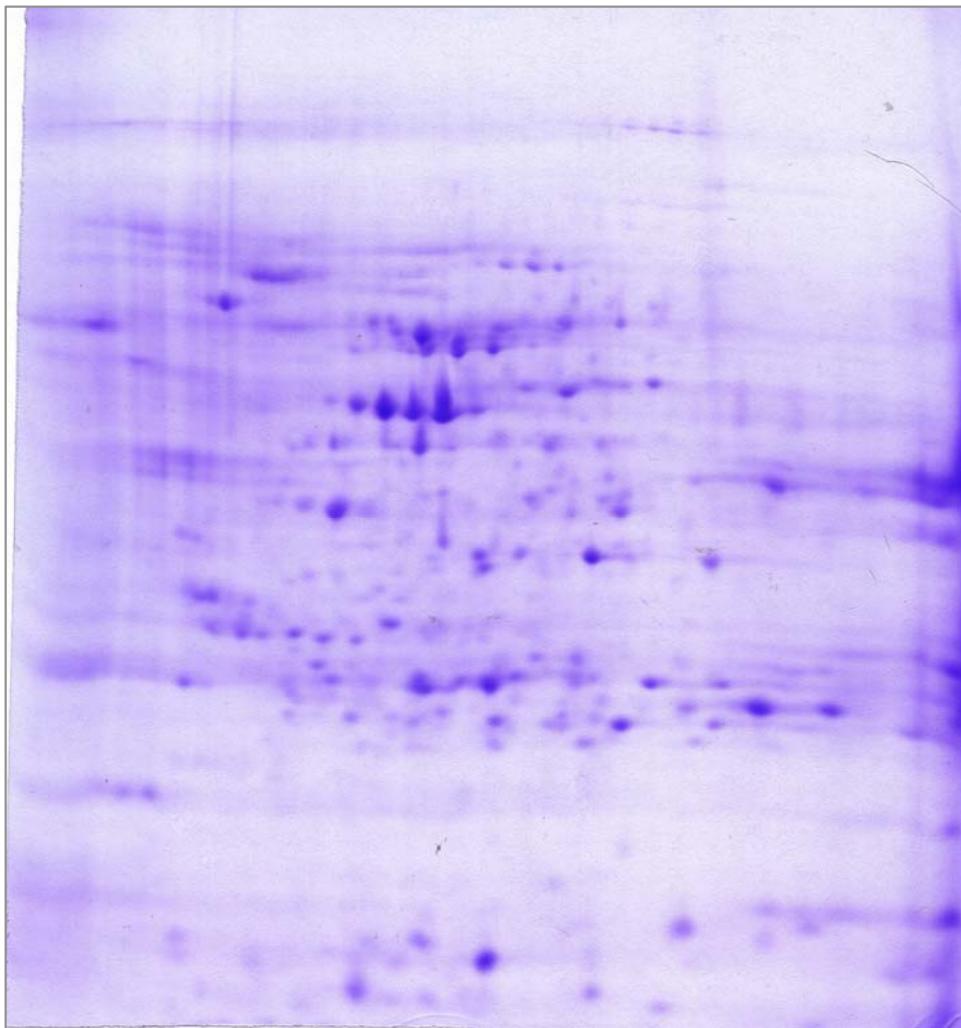
- (1) 建立高產能抗體製備之 標準流程
- (2) 大量快速 生產有用的  
**單株抗體**
- (3) 提供 抗體晶片 所需之  
抗體庫

均質抗原 → 單一抗體

整體抗原 → 全部抗體

# 竹筍蛋白質體的免疫反應

未出土竹筍樣本 CBR 染色



小鼠抗血清免疫染色

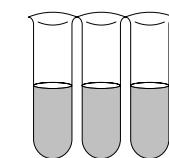
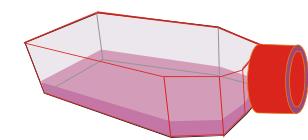
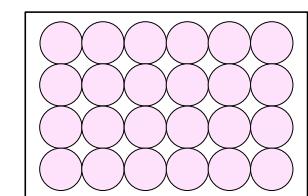
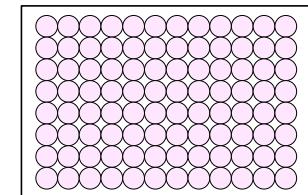
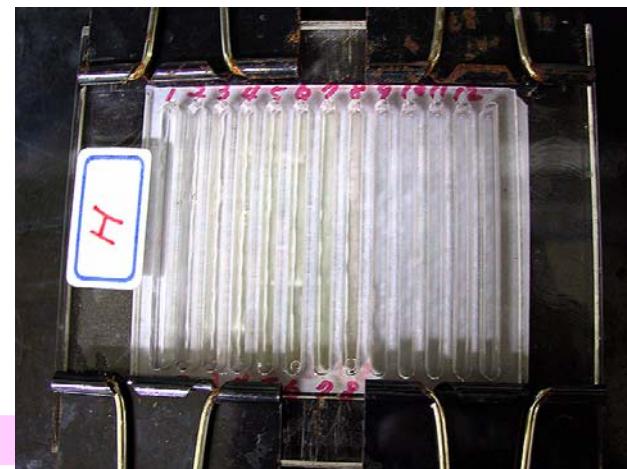
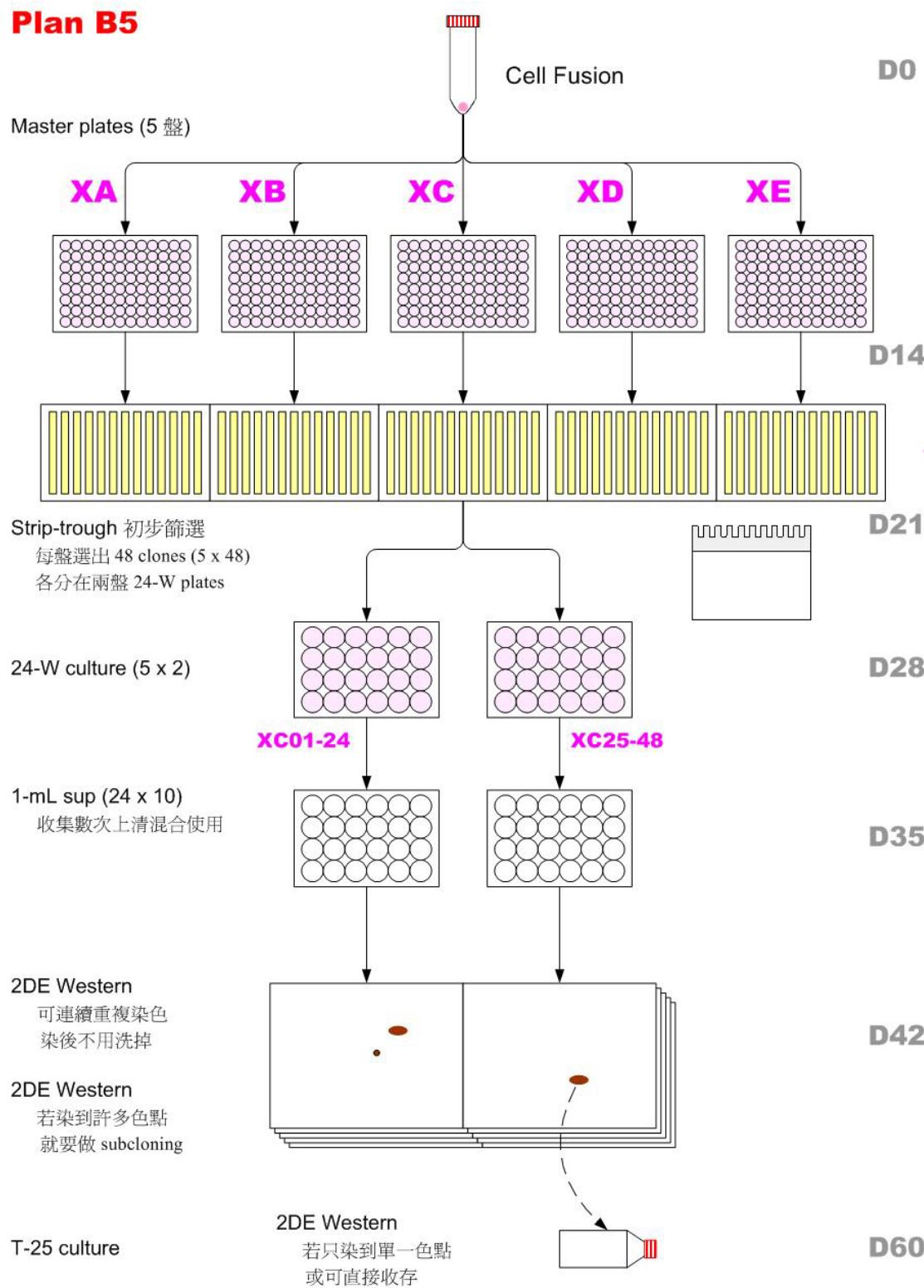


抗原性強弱不同、蛋白質含量多寡不同。

# 單株抗體高產能製備流程

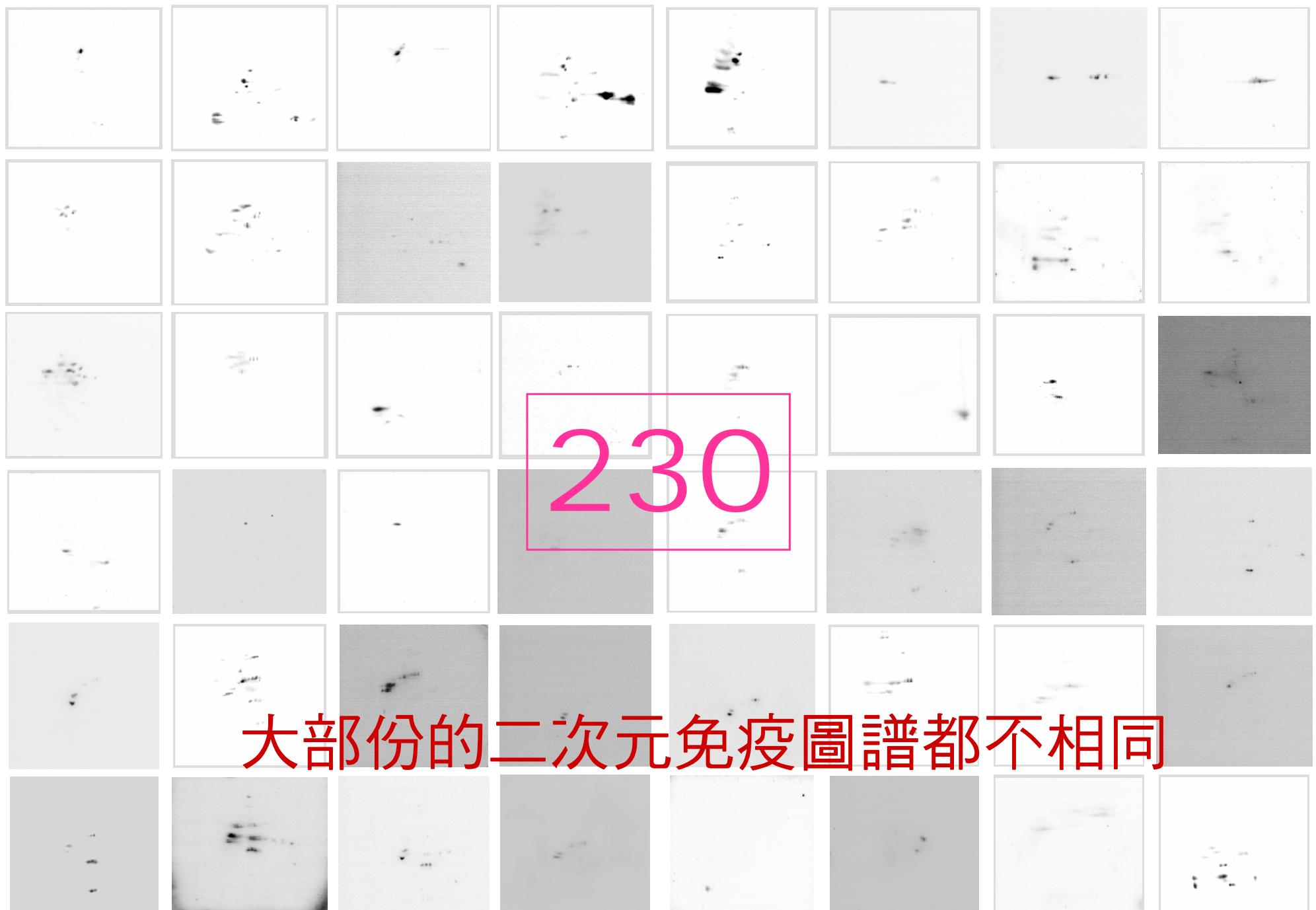
Juang RH (2005)

## Plan B5



專利申請中

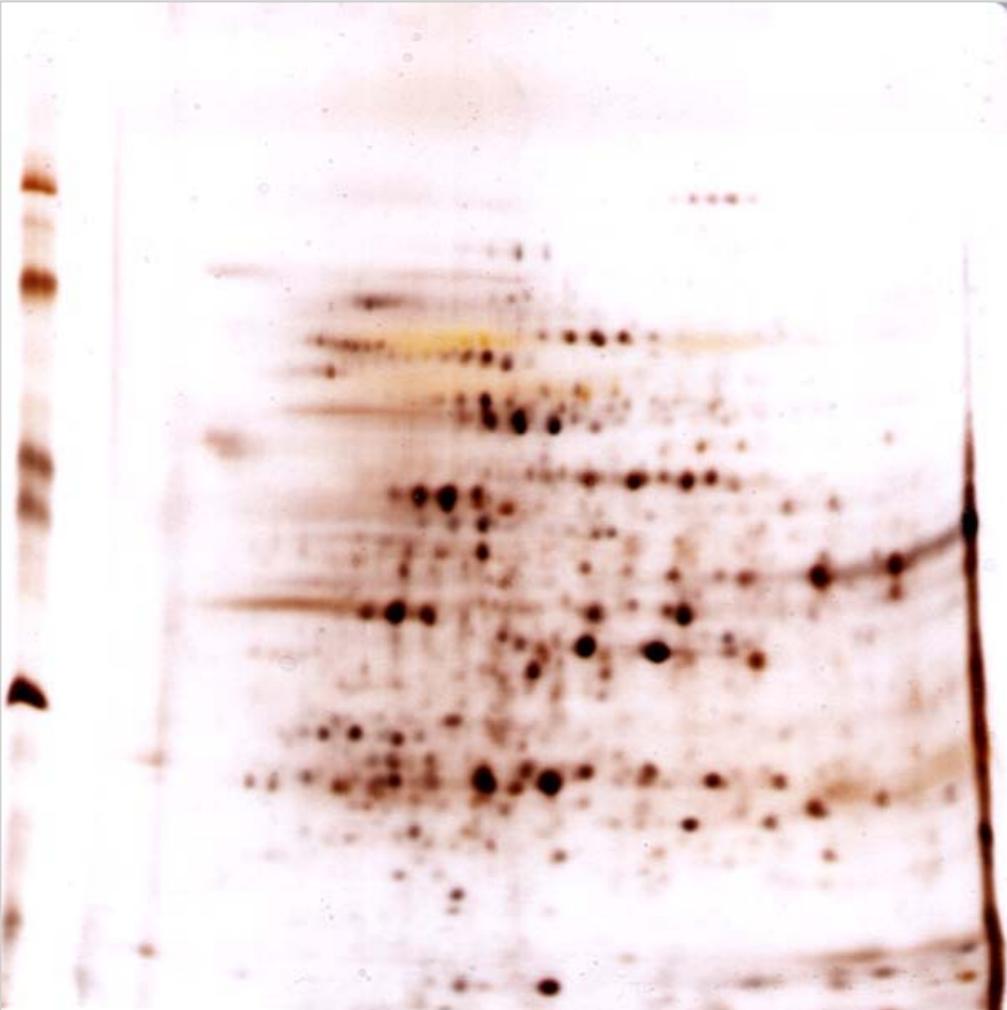
# 2003~2005 建立綠竹筍蛋白質體之單株抗體庫



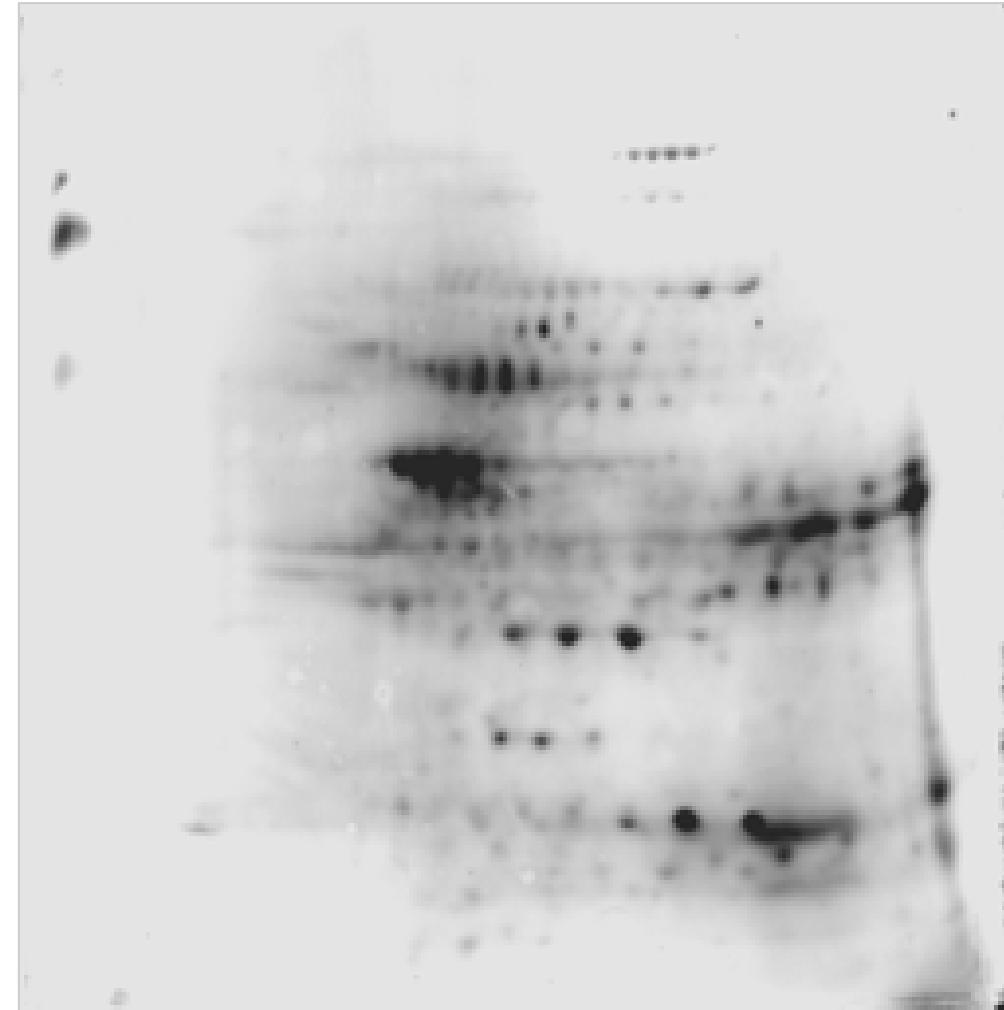
大部份的二次元免疫圖譜都不相同

# 混合八十種單株抗體共同免疫染色

Silver staining

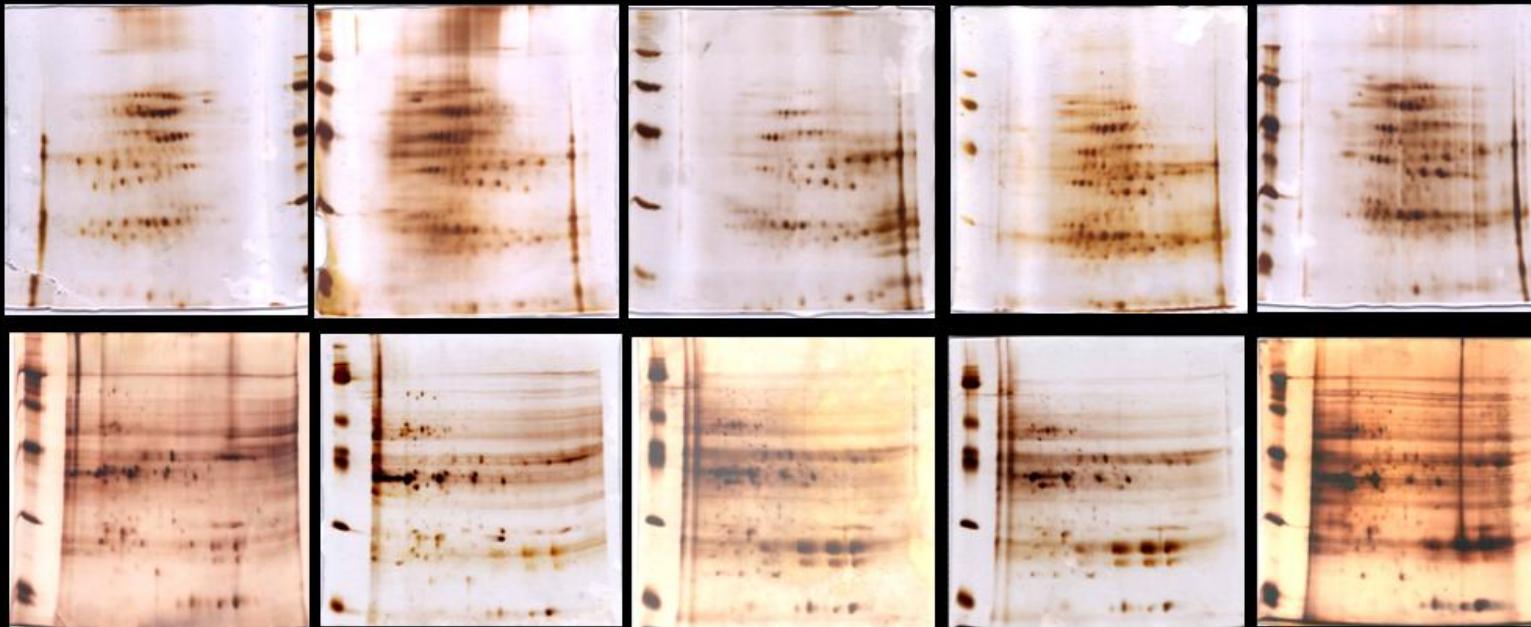


Immunostaining



最終目標：蛋白質染色圖譜 = 免疫染色圖譜

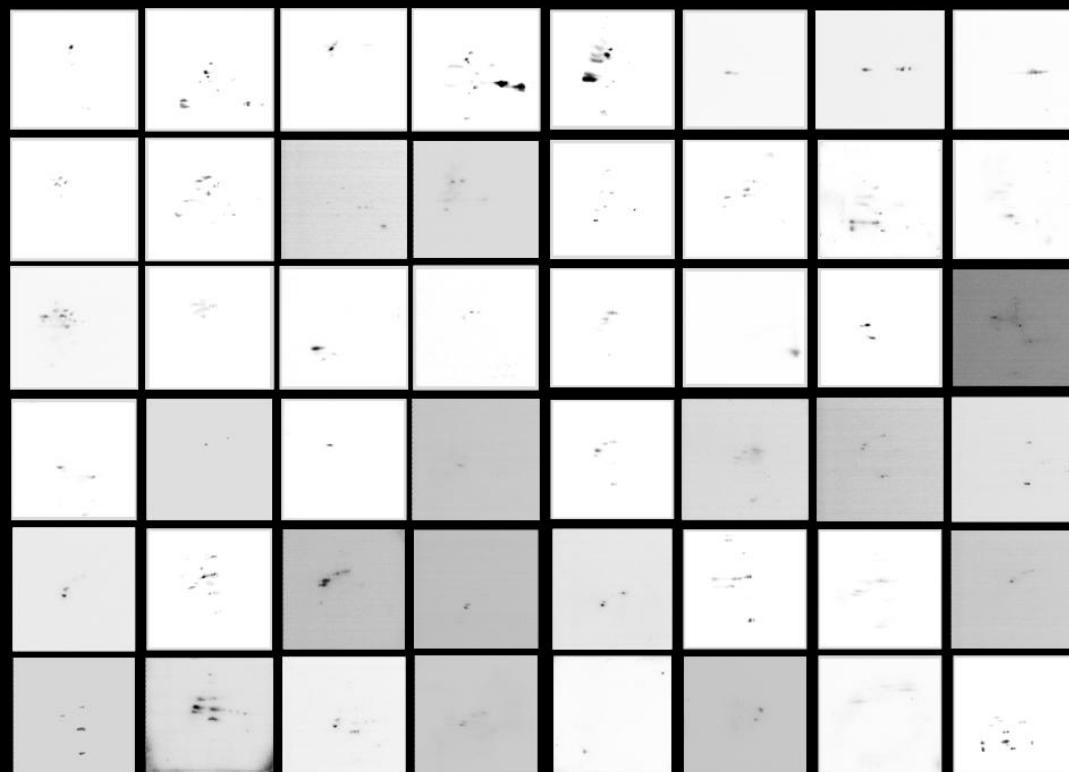
# 綠竹筍快速生長過程蛋白質體有很大的變化 (由左至右)



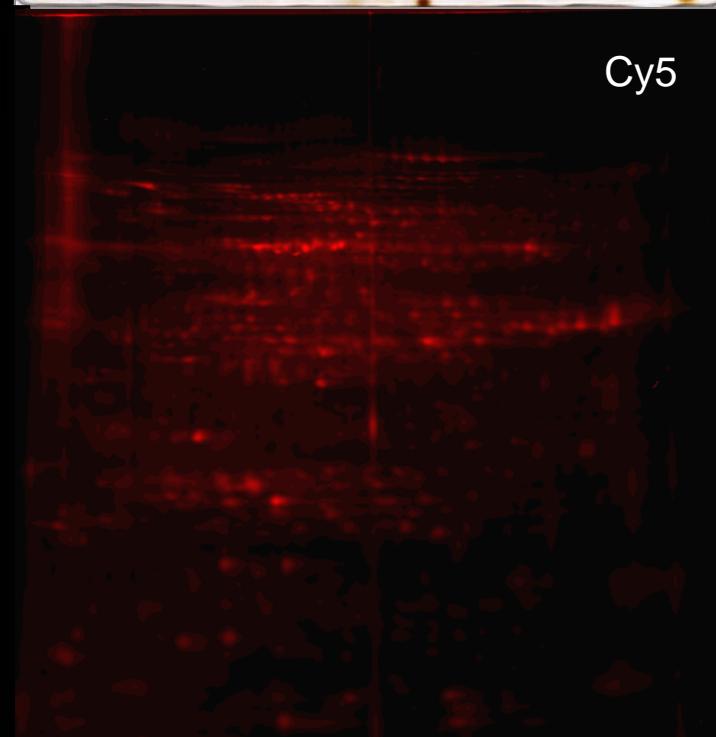
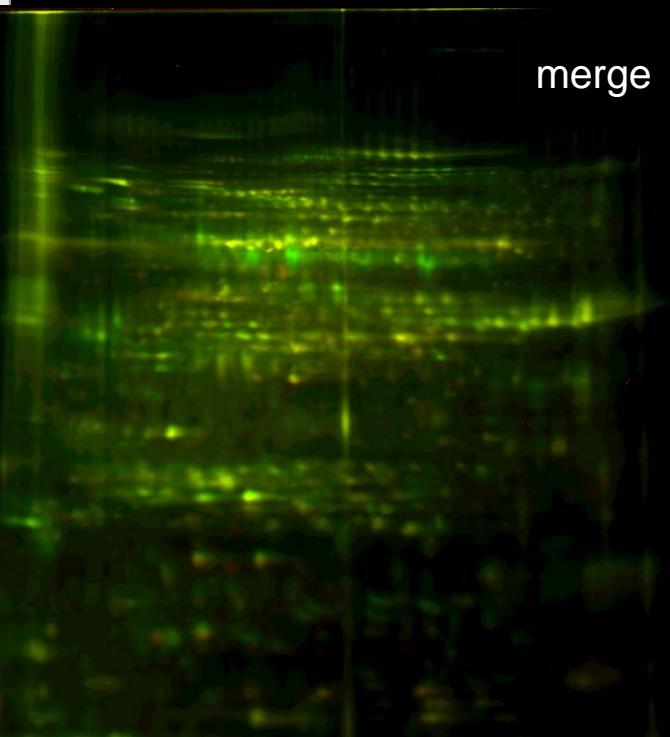
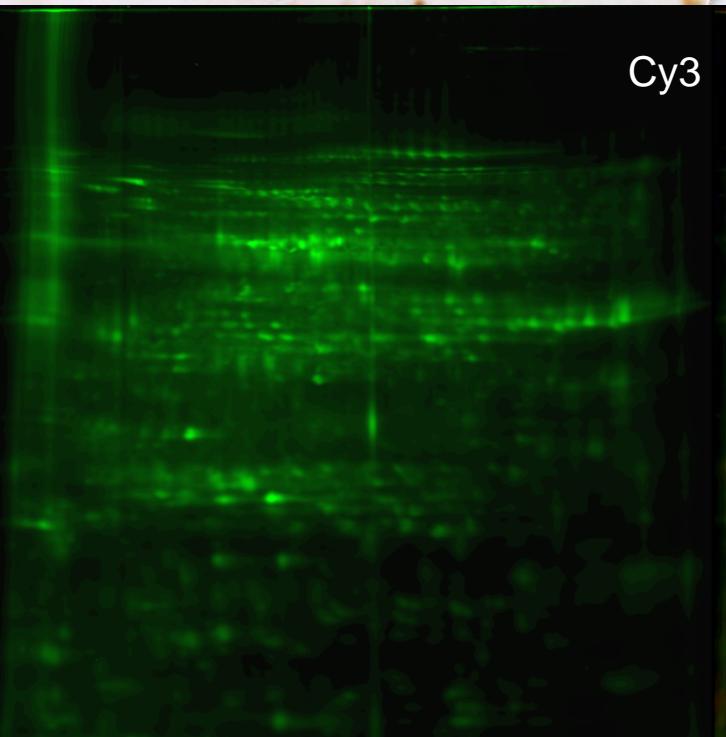
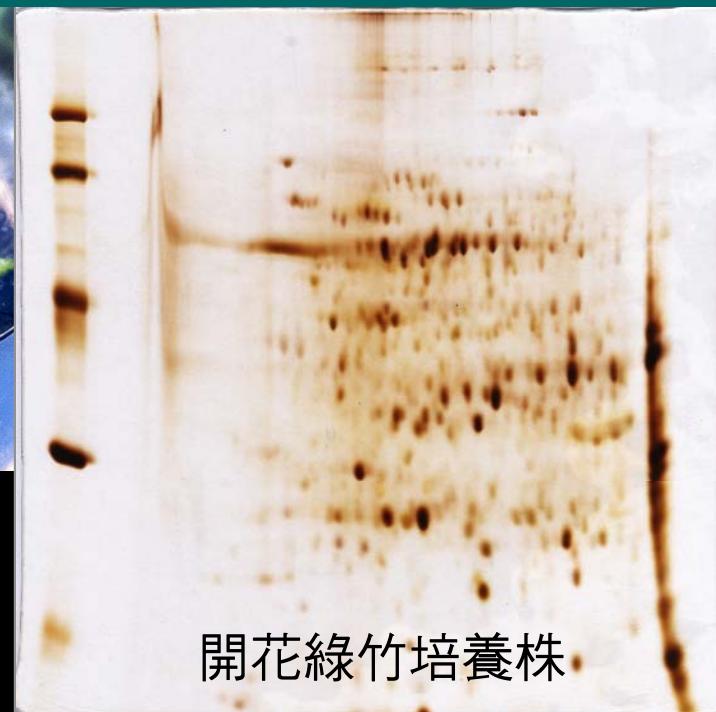
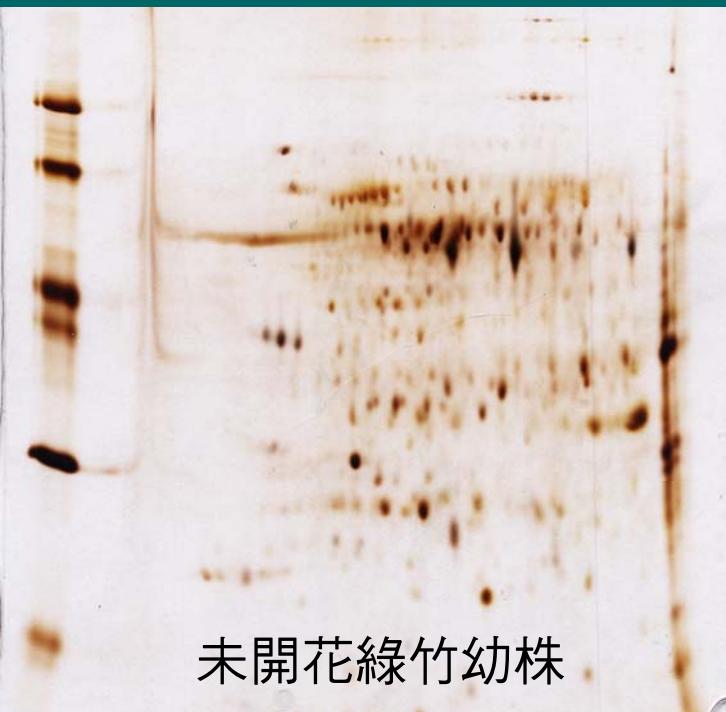
在一週內觀察綠竹快速生長情形，利用『蛋白質體』工具，比對此過程蛋白質總體變化。初步發現糖類代謝轉變，以及抗逆境蛋白質的出現，可能是造成竹筍快速生長的原因。

## 以單株抗體庫概念製備綠竹蛋白質體的抗體庫 (230 株)

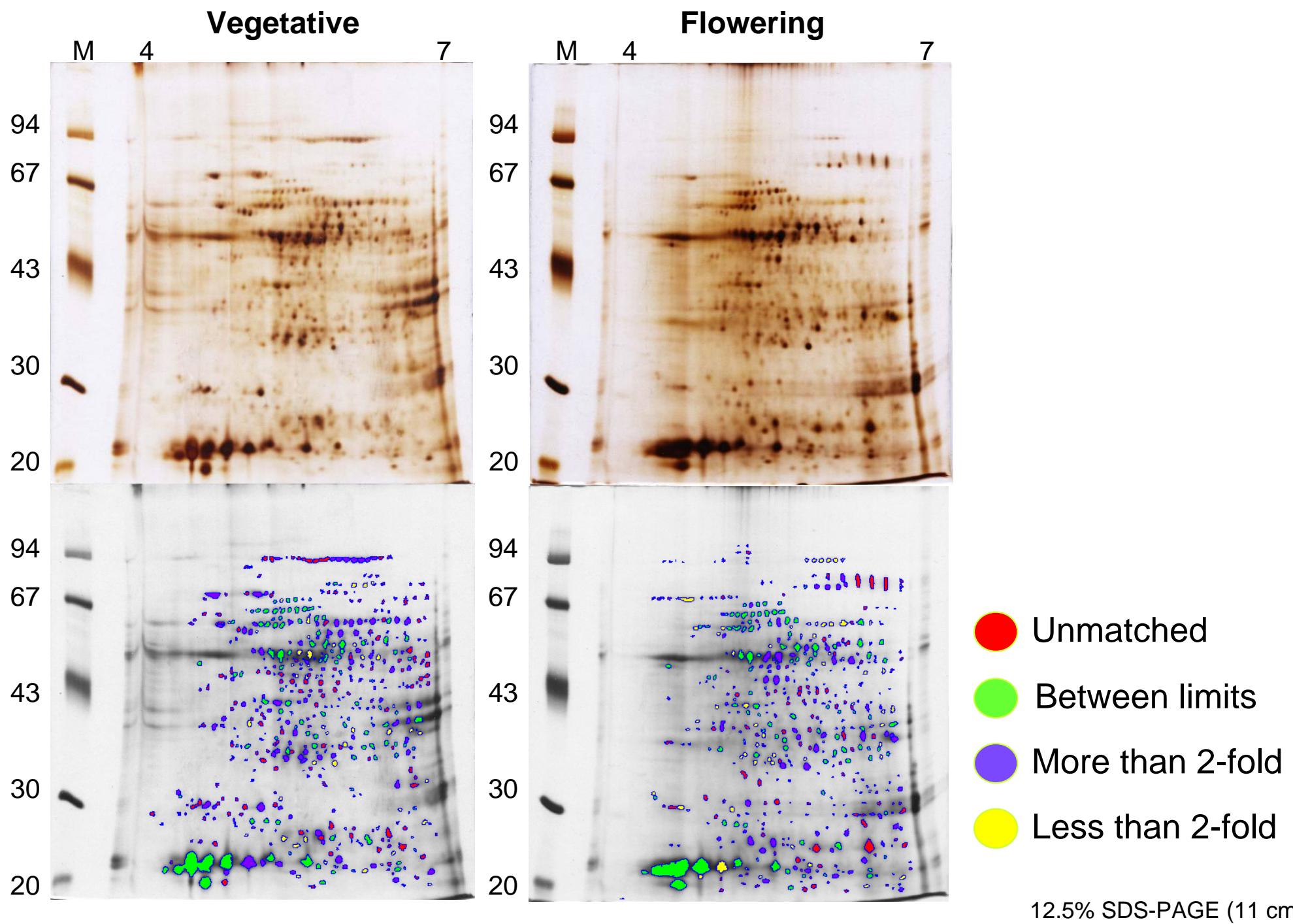
嘗試以綠竹蛋白質體為對象，製備整個筍蛋白質體之抗體庫，目前約累積 230 種不同蛋白質的單株抗體。右圖僅列出抗體庫中的部份圖譜，可發現每一種抗體所能結合的蛋白質，都不相同，未來在蛋白質晶片的製備上，將極有價值。



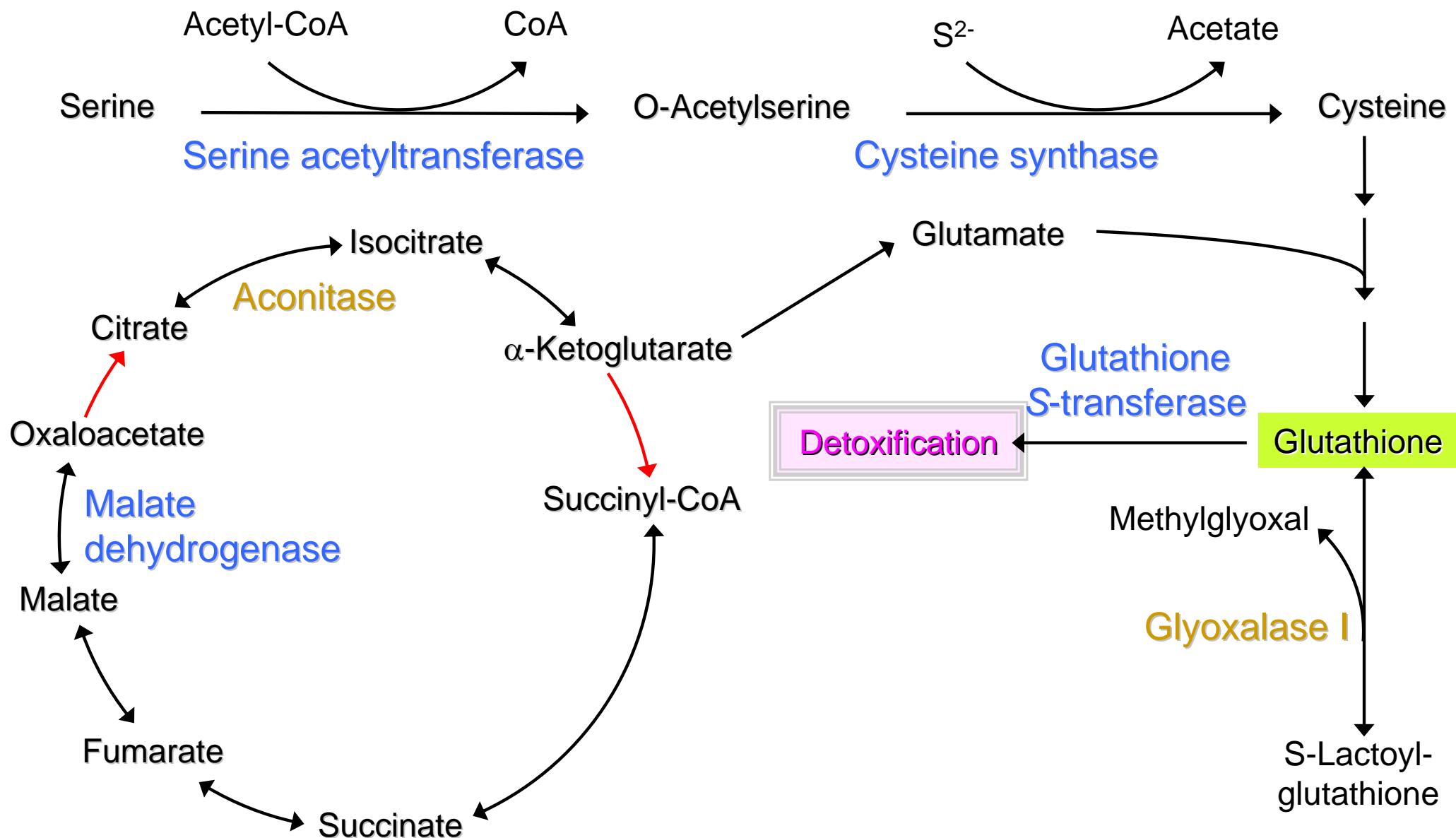
# 綠竹幼株開花前後的蛋白質表現差異



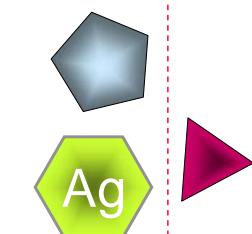
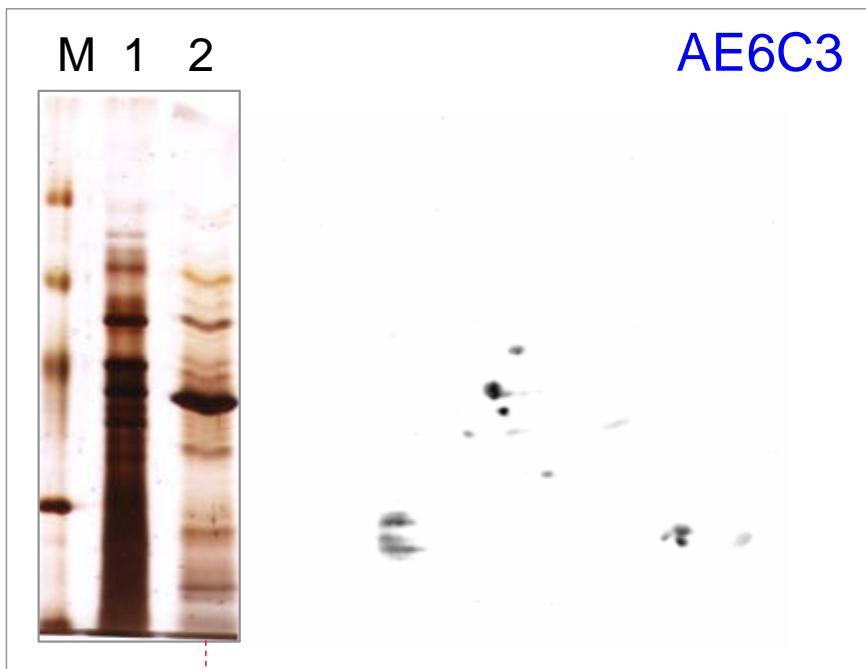
# 綠竹開花非水溶性蛋白質圖譜消長



# 可能影響綠竹開花之代謝途徑

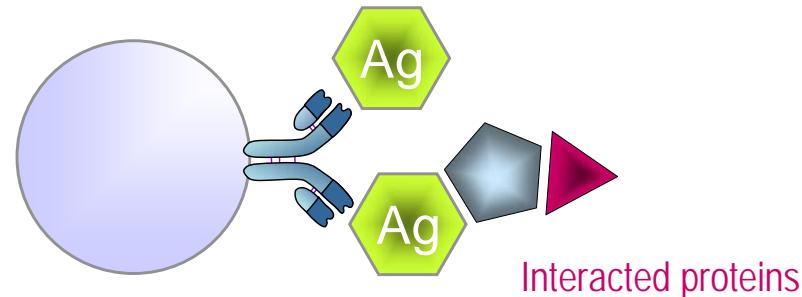


# 抗體免疫沈澱與蛋白質交互作用



Interacted proteins

LC/MS/MS



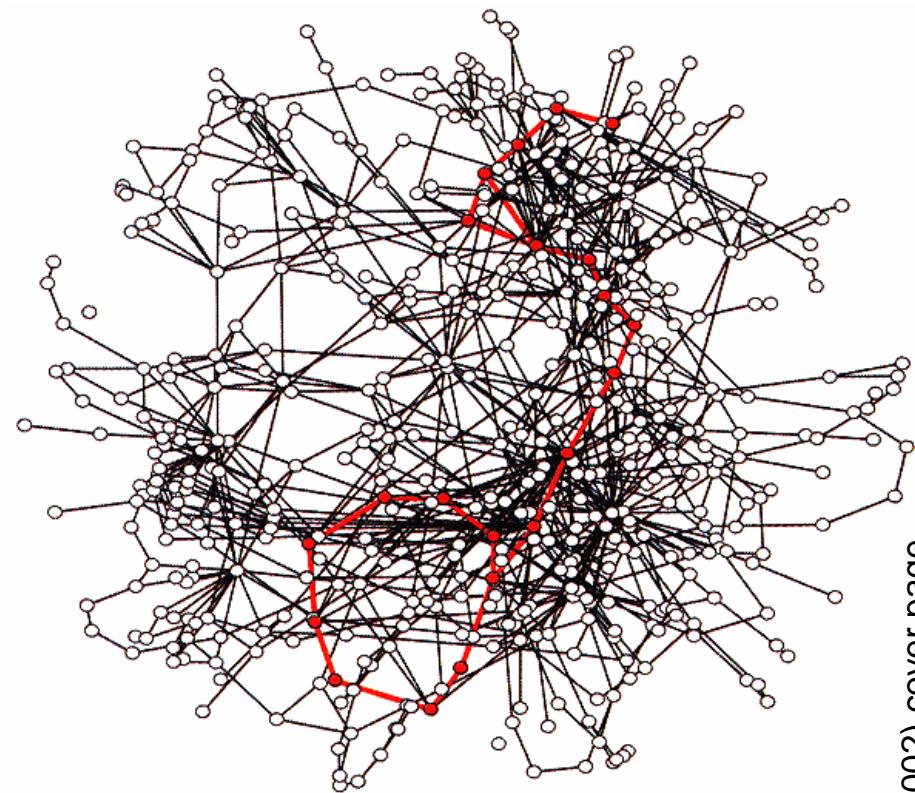
Protein ID	Match peptide
Adenosylhomocysteinase	IVLTIIR DSAAVFAWK HSLPDGLMR LGVSEETTGVK
Histone H4 (wheat)	IFLENVIR IDGLIYEETR TVRAMDVYALKR
Fructose bisphosphate aldolase	VTPEVIAEYTVR IGPNEPSQLAIDLNAQGLAR
Triosephosphate isomerase	TNVSPEVAESTR VIACVGETLEQR
NAD-dependent malate dehydrogenase	DDLFNINAGIVK
Histone H3	ASAPATGGVK
Putative lipase	DQVLEEVRR

Pull down proteins interacted with Ag

Juang RH (2005) EPA

# 蛋白質分子間的交互作用 – Cross talk

代謝路徑立體圖

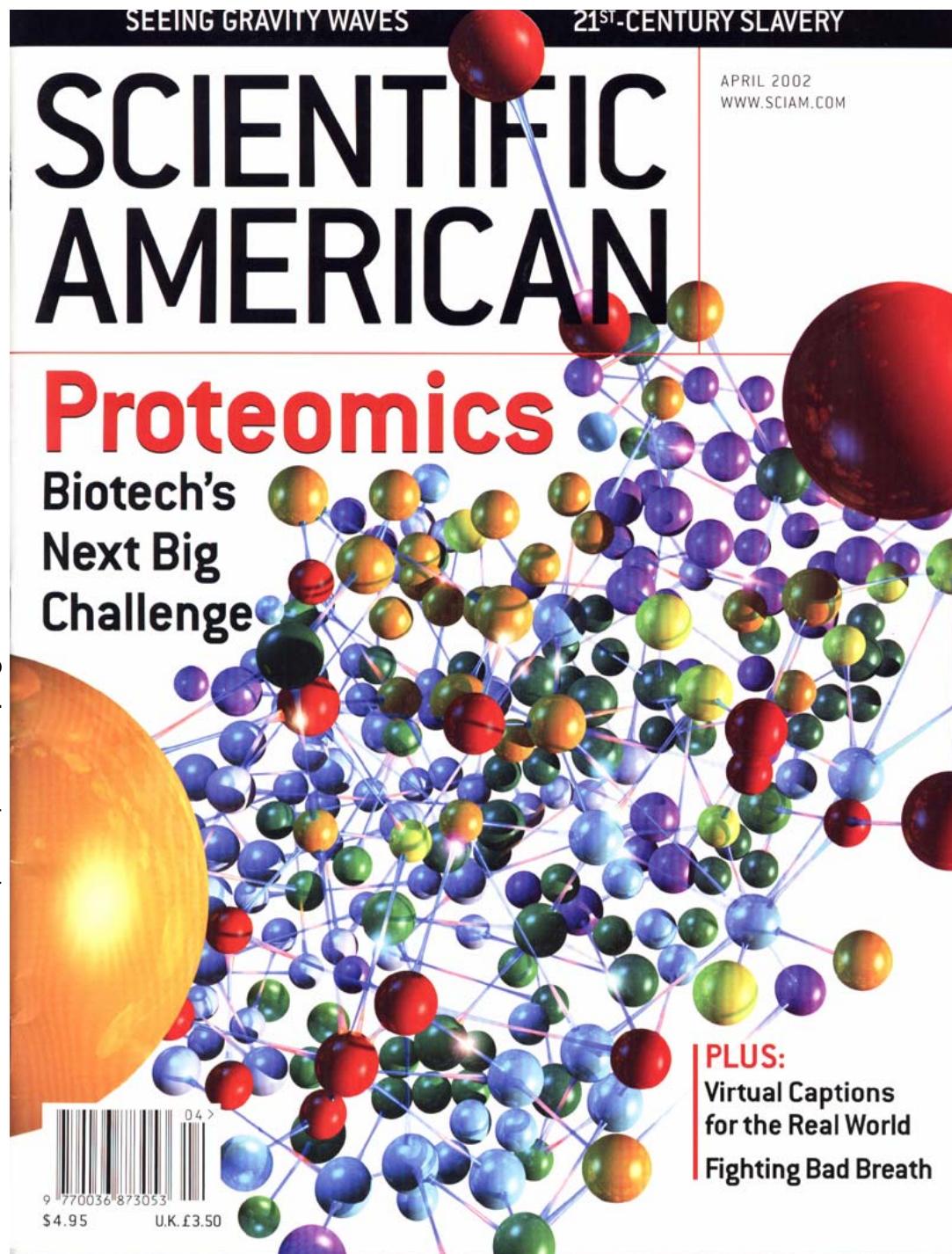


**Systems Biology**

整體性的生物學觀念與工具

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

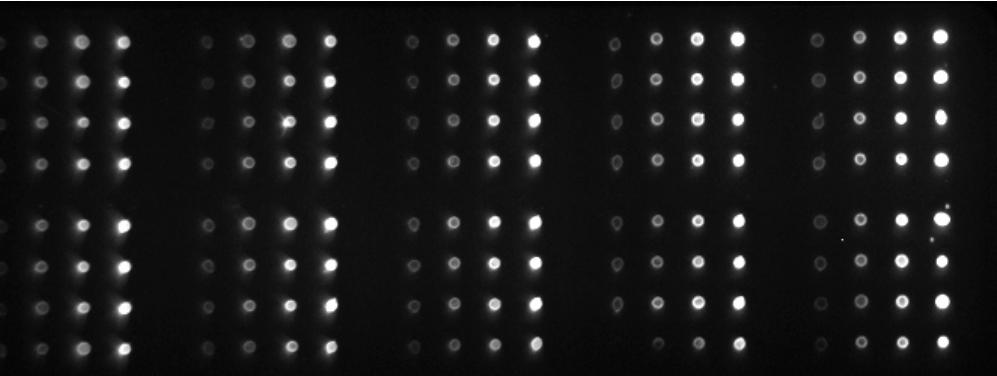
Scientific American (2002) cover page



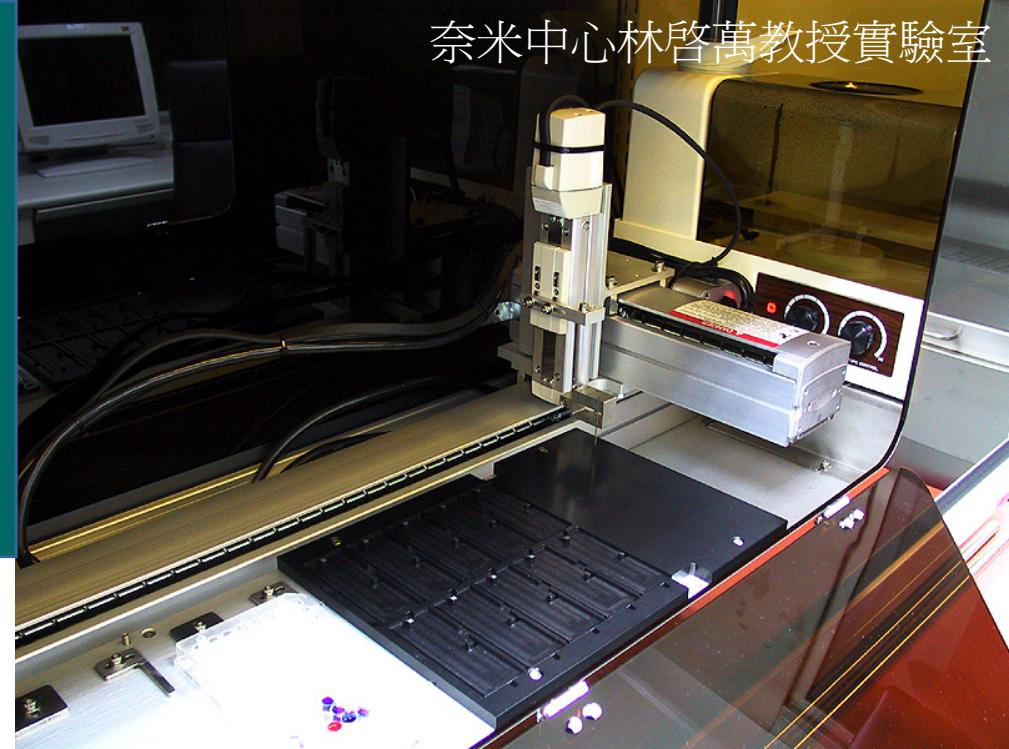
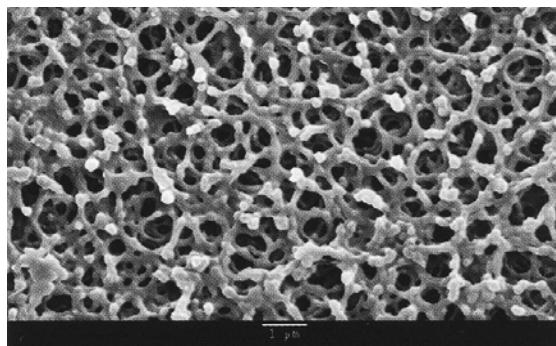
奈米中心林啓萬教授實驗室

## 蛋白質晶片試製

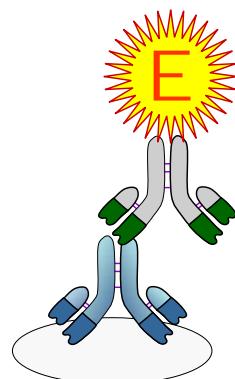
Making  
protein chips



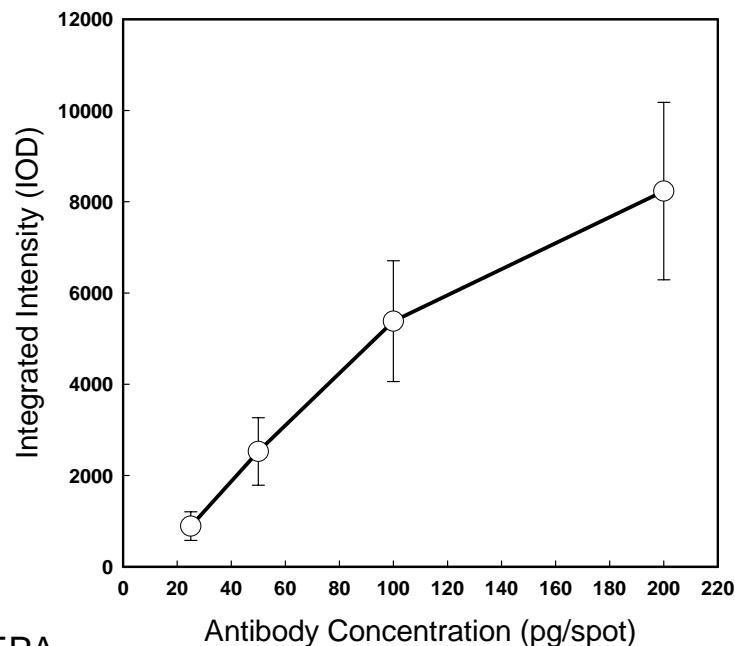
*Nitrocellulose*



Schleicher & Schuell



Juang RH (2005) EPA

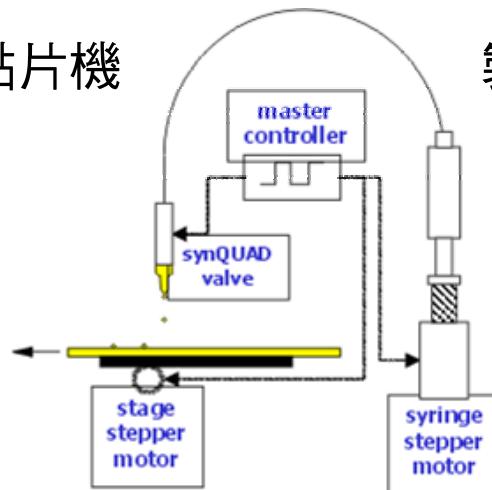


# 自製抗體晶片 (NC 噴霧塑膠板)

硝化纖維塗佈

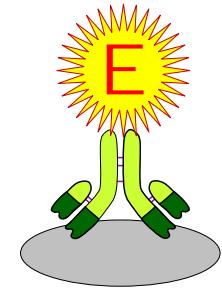


點片機



製作晶片

免疫呈色測試



蛋白質可穩定結合在晶片表面

NET-gelatin  
washing  
0 min

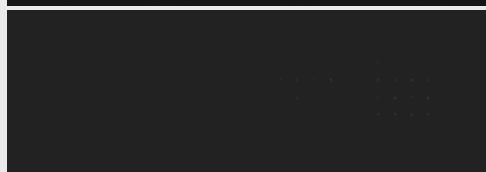
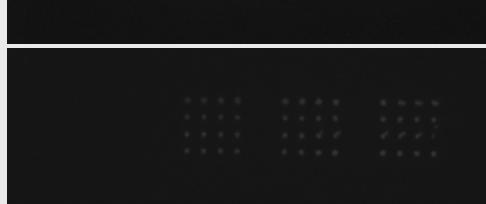
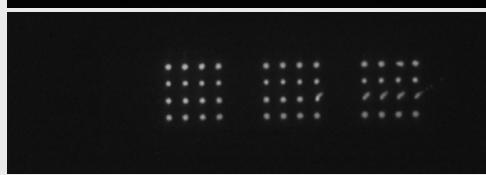
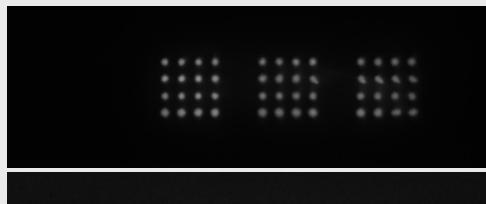
30 min

60 min

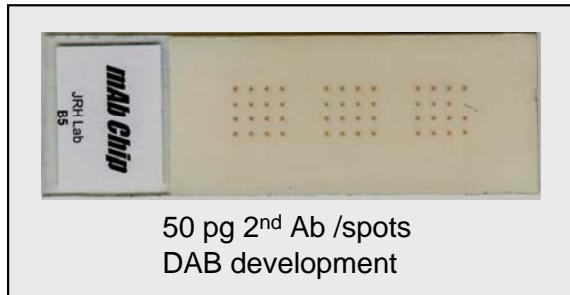
120 min

overnight

PBS  
washing 30 min

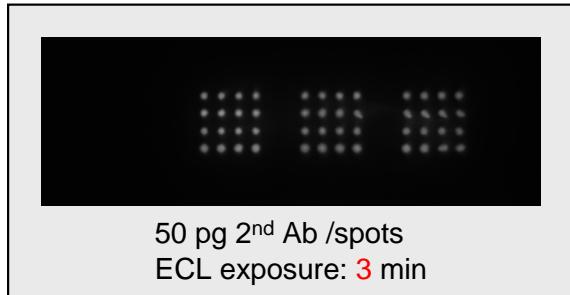


(a) 可用 DAB 均勻呈色



50 pg 2<sup>nd</sup> Ab /spots  
DAB development

(b) 也可用化學螢光偵測

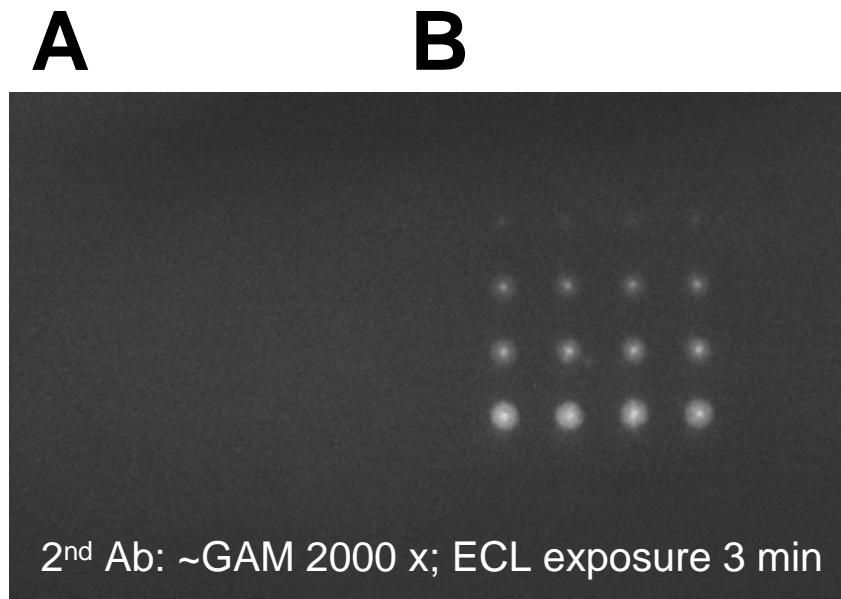
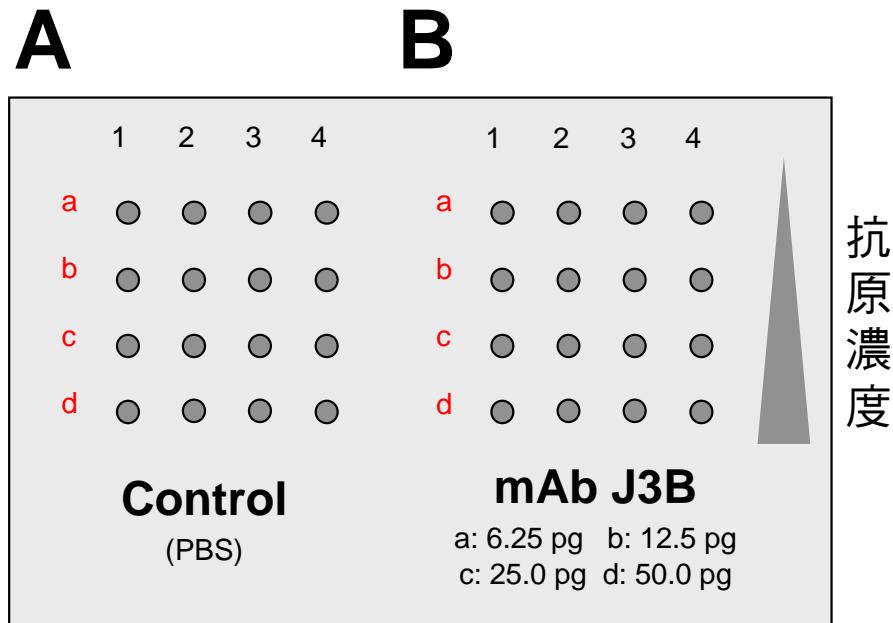


50 pg 2<sup>nd</sup> Ab /spots  
ECL exposure: 3 min

Conclusion:

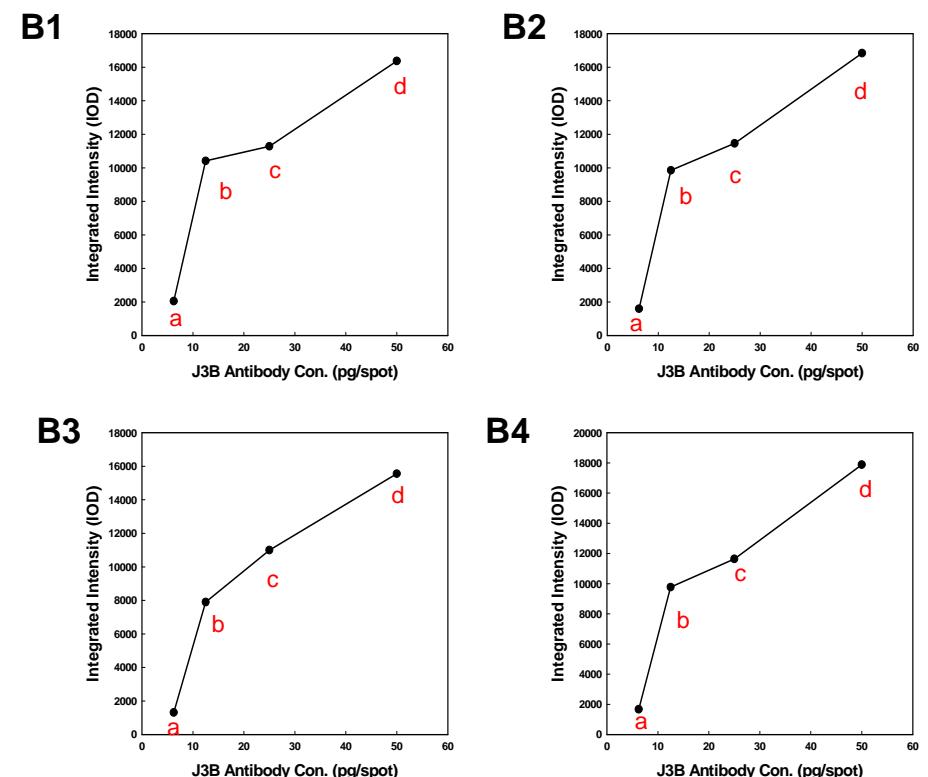
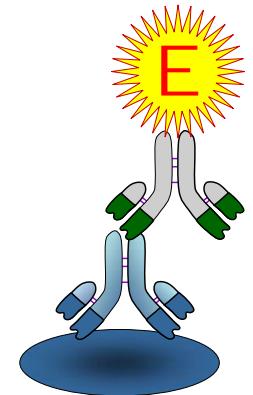
- (1) NC 晶片可自噴霧製作
- (2) NC 可用兩種方式呈色
- (3) 蛋白質與 NC 結合穩定

# 點片抗體濃度有線性關係



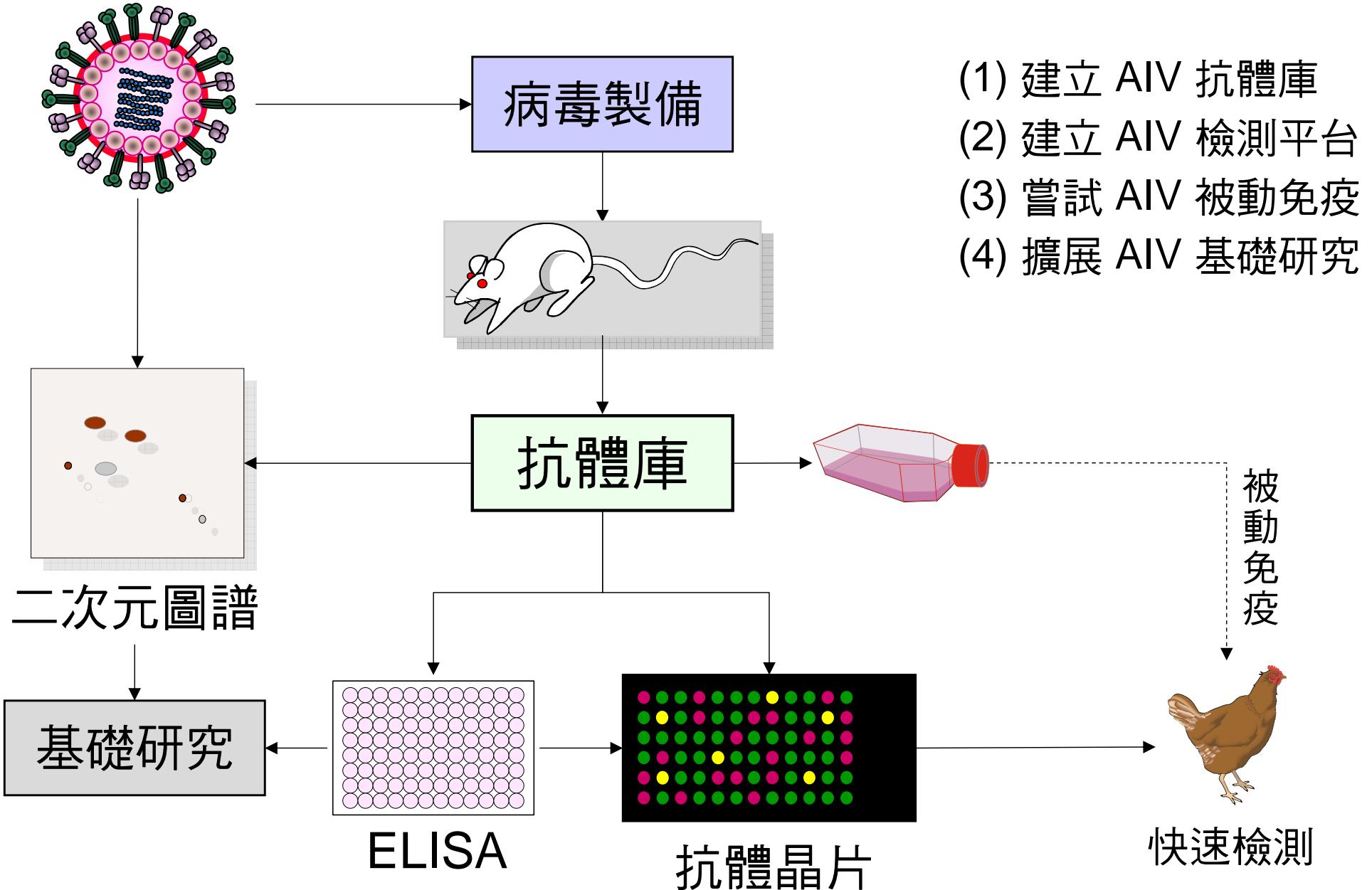
## Conclusion:

- (1) 點片系統再現性不錯
- (2) 點片量有線性關係
- (3) 本晶片系統可行

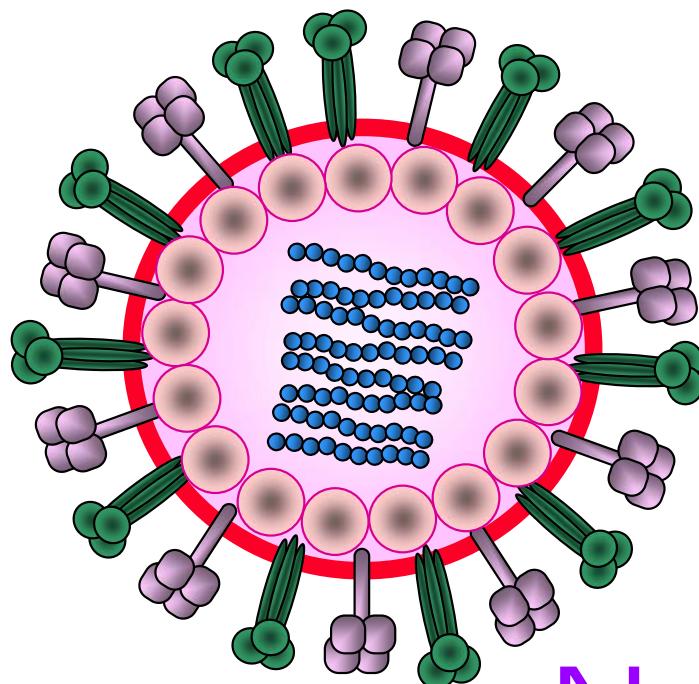


# AIHV 抗體庫及應用

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

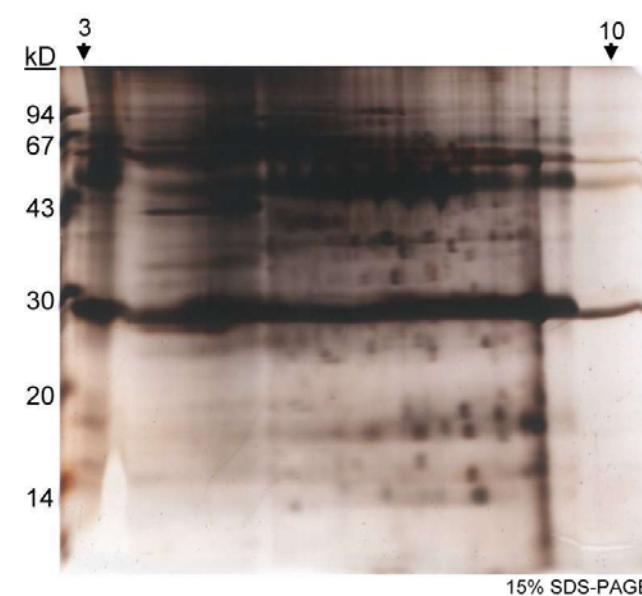


# 製備兩種 AIV 亞型的抗體庫



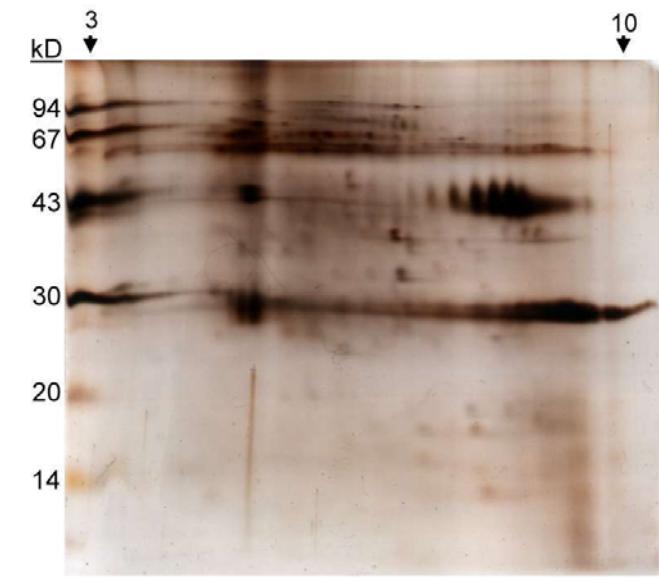
H

N



H6N1

H5N2



Hemagglutinin	HA	61 kD
Neuraminidase	NA	50 kD
Nucleo capsids	NP	56 kD
Matrix proteins	M <sub>1</sub>	27 kD
	M <sub>2</sub>	11 kD
Polymerases	PA	84 kD
	PB <sub>1</sub>	87 kD
	PB <sub>2</sub>	86 kD
Nonstructural proteins	NS <sub>1</sub>	27 kD
	NS <sub>2</sub>	14 kD

Nature (2003 Oct) 425:441

news

## China takes centre stage for liver proteome

David Cyranoski, Changsha, China

China is set to lead a massive research project to describe all of the proteins in the human liver — the liver proteome. The initiative is being coordinated by the Human Proteome Organisation (HUPO), an international group that is also overseeing plasma and brain proteome projects.

But parts of China's plan are controversial — in particular, a project to generate a range of different antibodies in one go.

Last month, China surprised the world of protein chemistry by pledging 200 million yuan (\$24 million) for the three-year pilot phase of the international liver-proteome study. Two weeks later, researchers at China's annual proteomics meeting, on 18–21 September, established a Chinese branch of HUPO and elected as its director the ambitious Fuchu He, a systems-biology researcher at the Beijing Institute of Radiation Medicine.

"This is a golden opportunity for China to lead an international effort," says He, who



Fuchu He (left) has the task of coordinating China's project to identify liver proteins.



Conventionally, antibodies are made one at a time, but Sun's team will inject several different proteins extracted from human livers into mice and then harvest the antibodies made to each of them from the mice's blood. These antibodies will be identified individu-

Helmut Meyer of the University of Bochum, Germany, who heads HUPO's brain-proteome project, thinks that the Chinese researchers may be biting off more than they can chew. "Sometimes it's nearly impossible to identify the protein to which an antibody binds," he says. His brain-proteome project plans to start an antibody project