

單株抗體在生物技術的應用

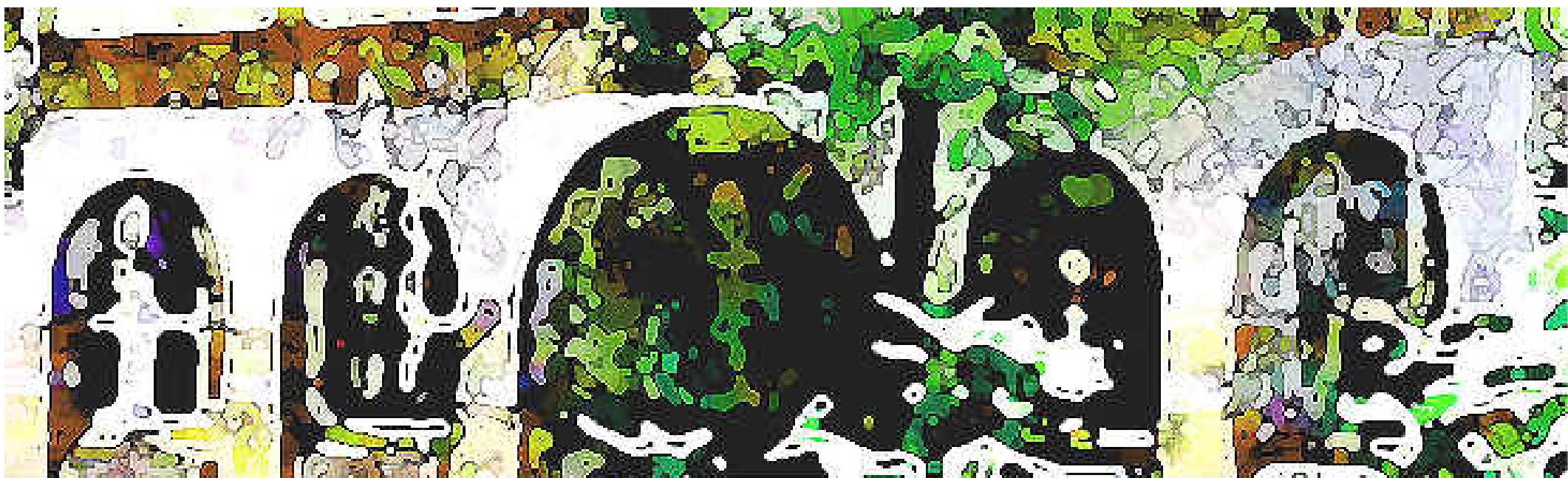
(1) **Monoclonal Antibody** 單株抗體 (奈米探針)

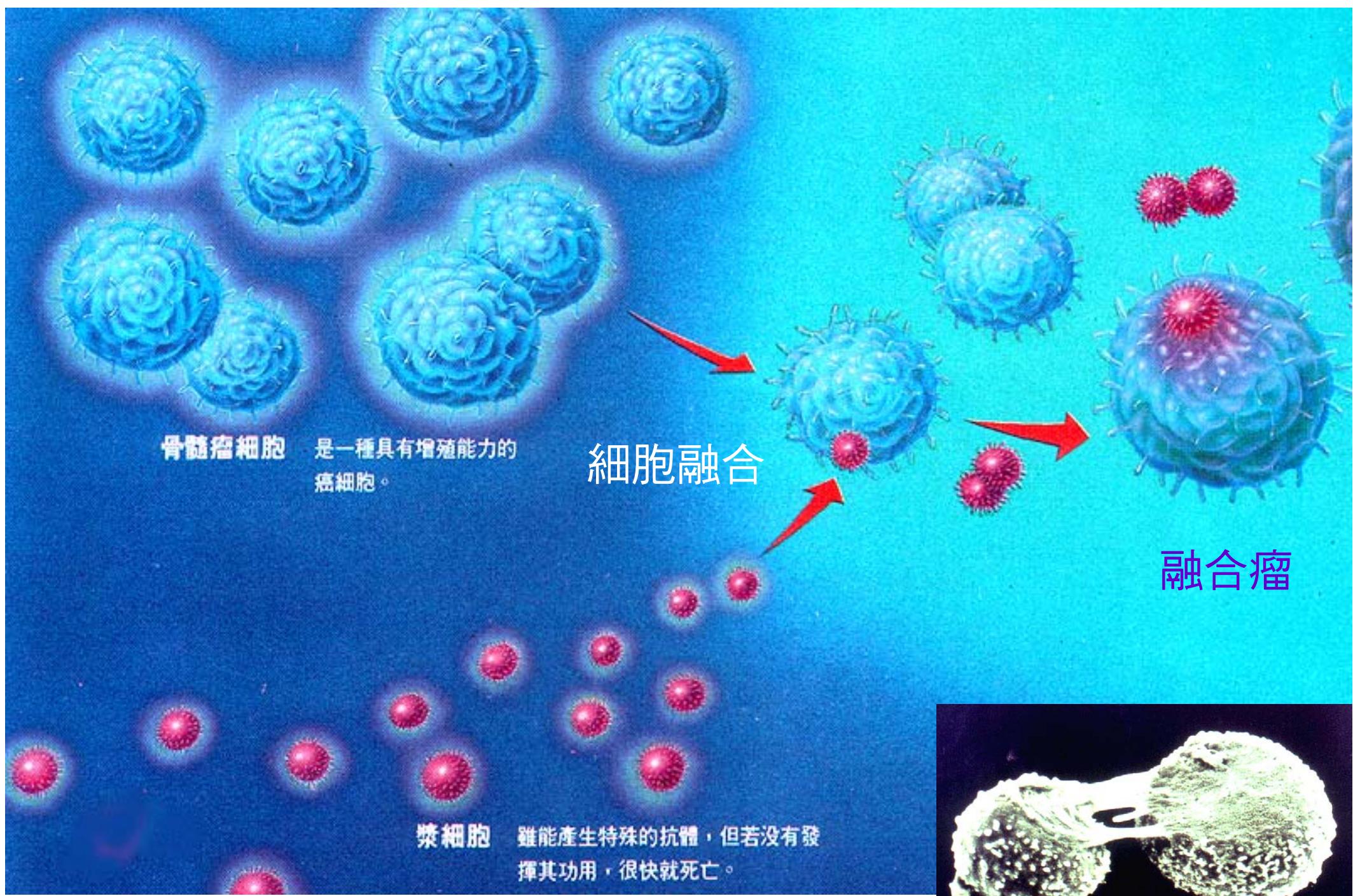
免疫反應 - 抗體的專一性 - 細胞融合法

(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

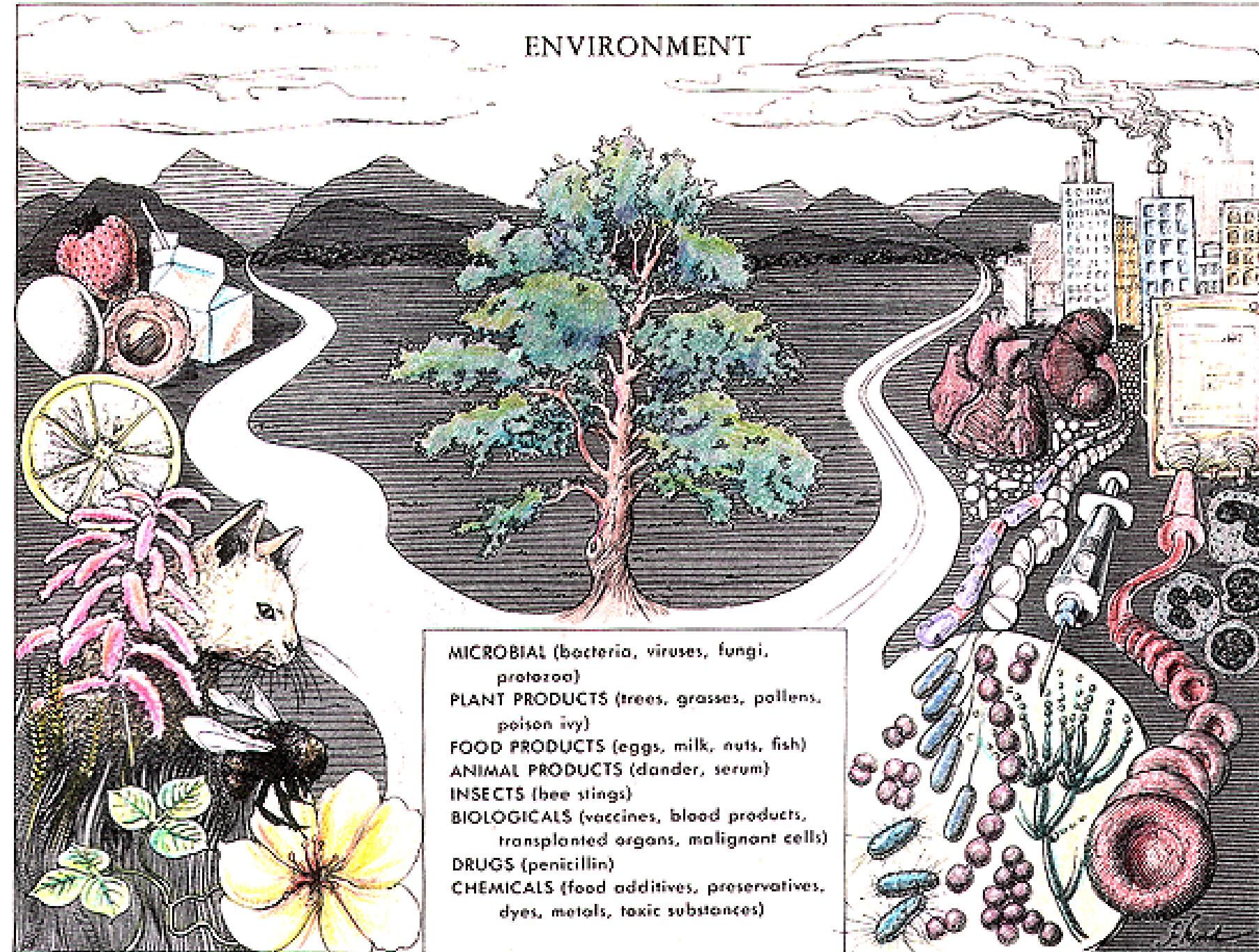
國立台灣大學 生化科技學系 莊榮輝





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)

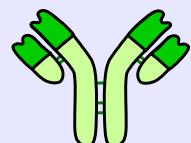
溶菌酶 (Lysozyme)



T 細胞 (T_H , T_S , T_K)

B 細胞

生 產



抗体 (Antibody, Ab)

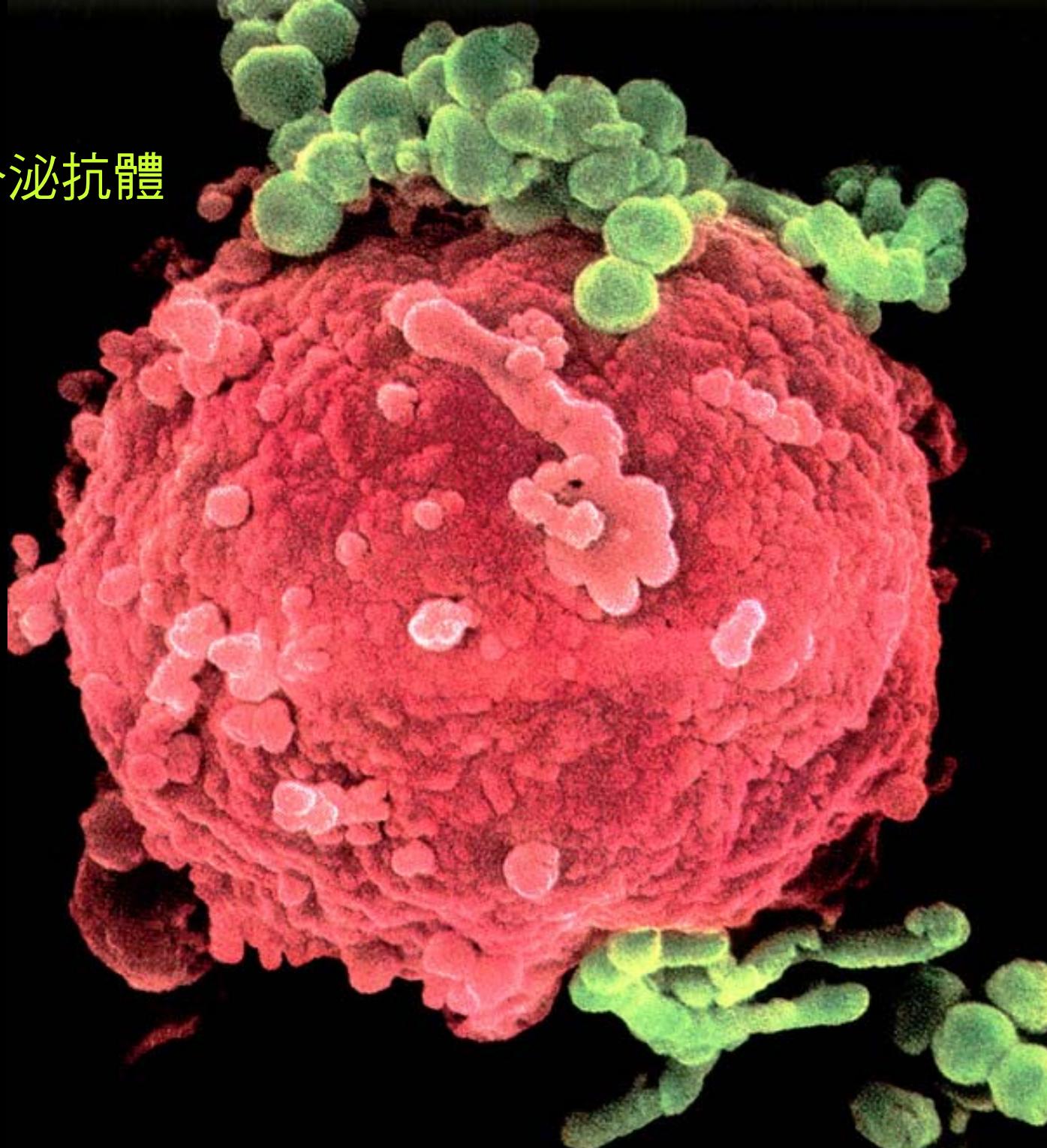
兩大系統 →

警察系統 (+ 調察局)

軍事系統

B 細胞分泌抗體

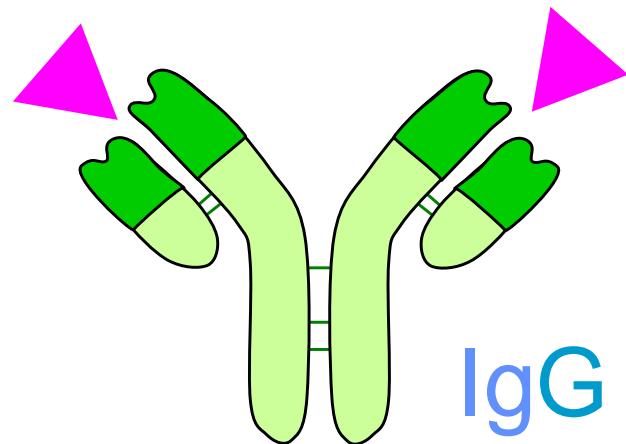
凝集細菌



如何凝集

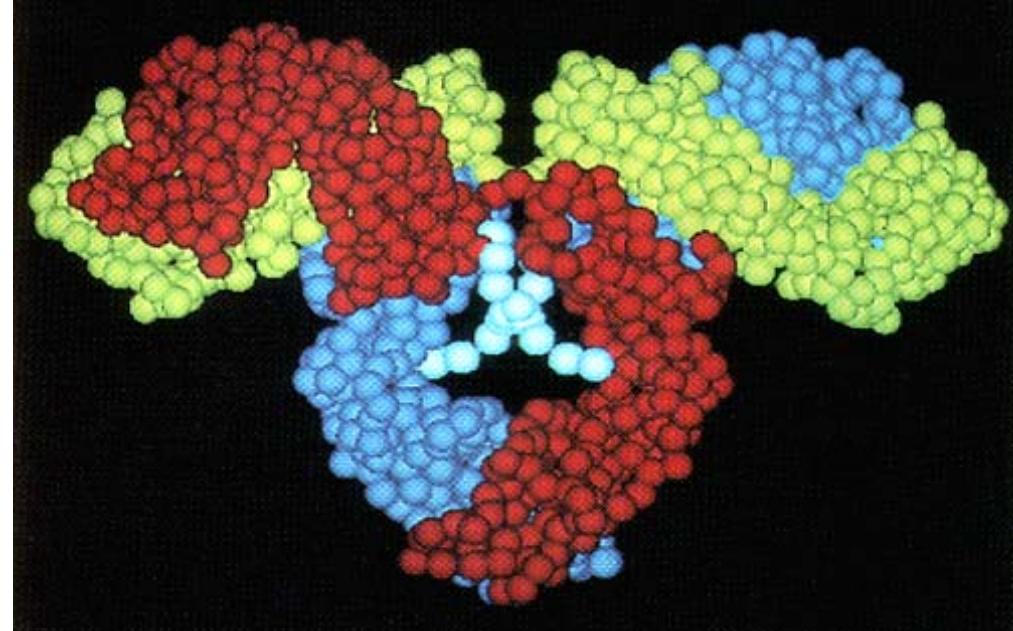
■ 抗體 是一種蛋白質

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)

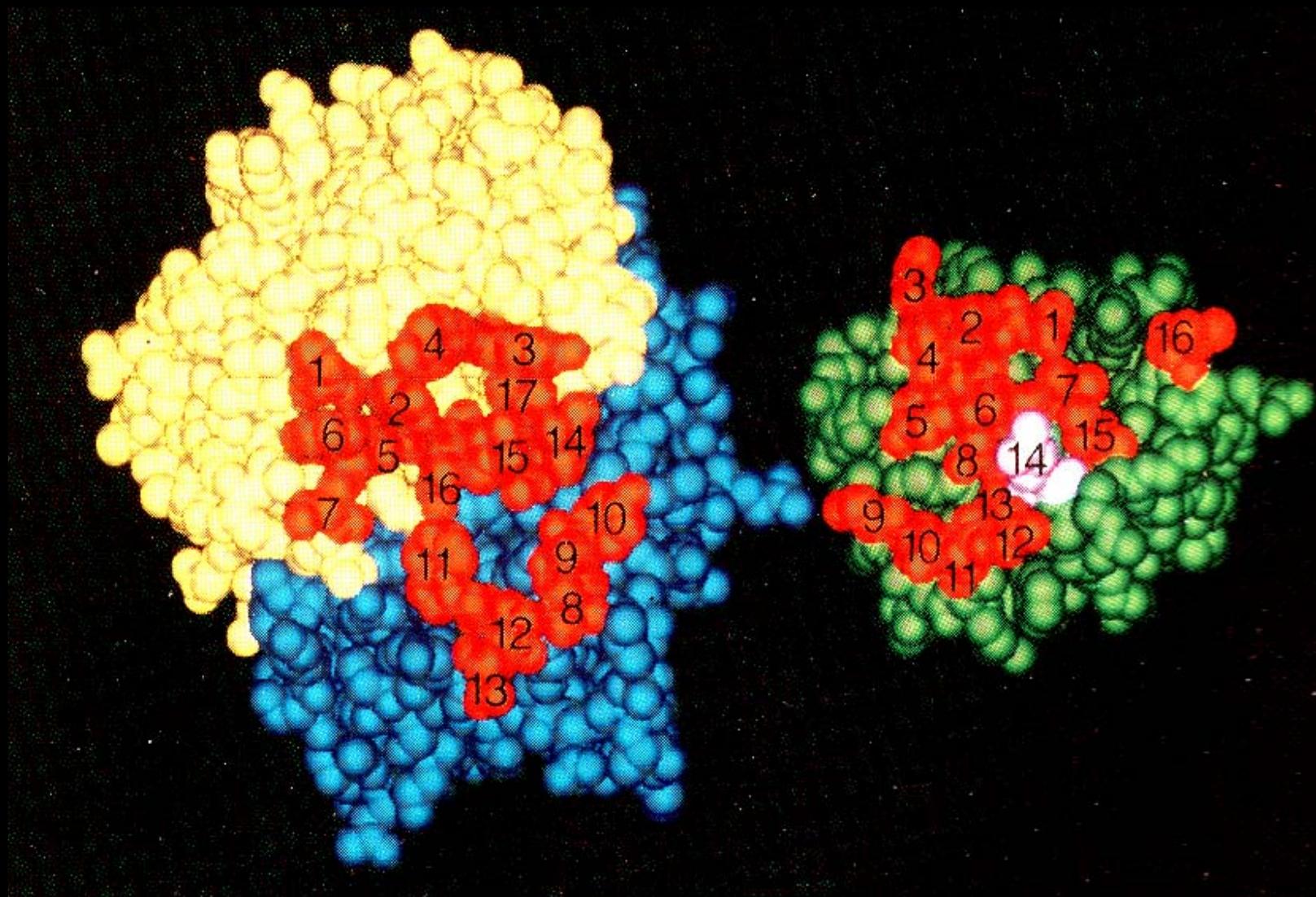
- IgG 是單一個抗體分子，另有 IgM (五元體) 及 IgA (二元體)

步兵單兵

裝甲兵

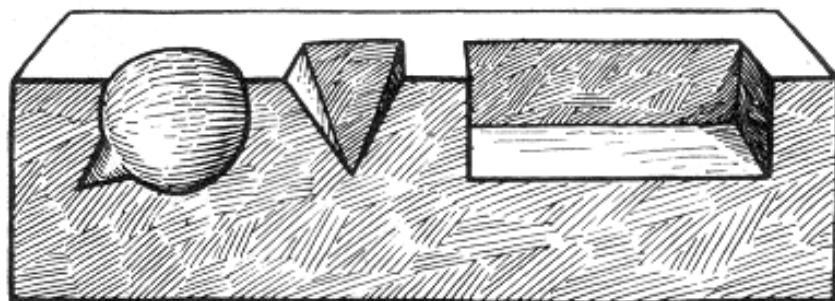
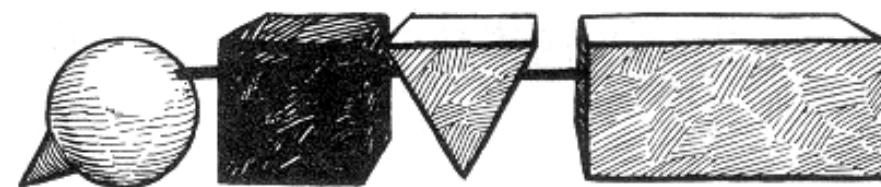
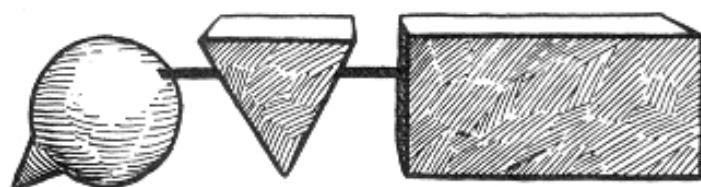
兩棲部隊

抗體結合區

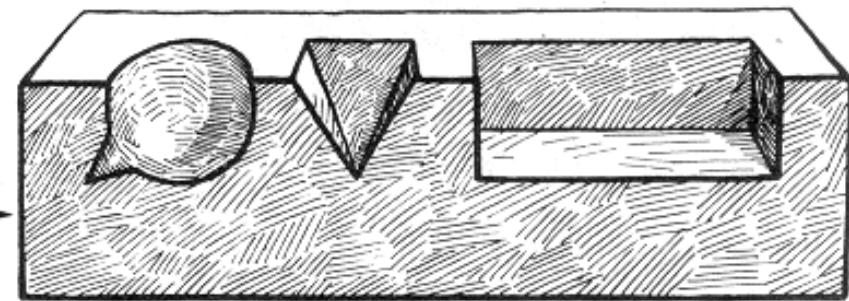


抗體與抗原的結合面有如互補的積木

抗 原



← Antibody A →



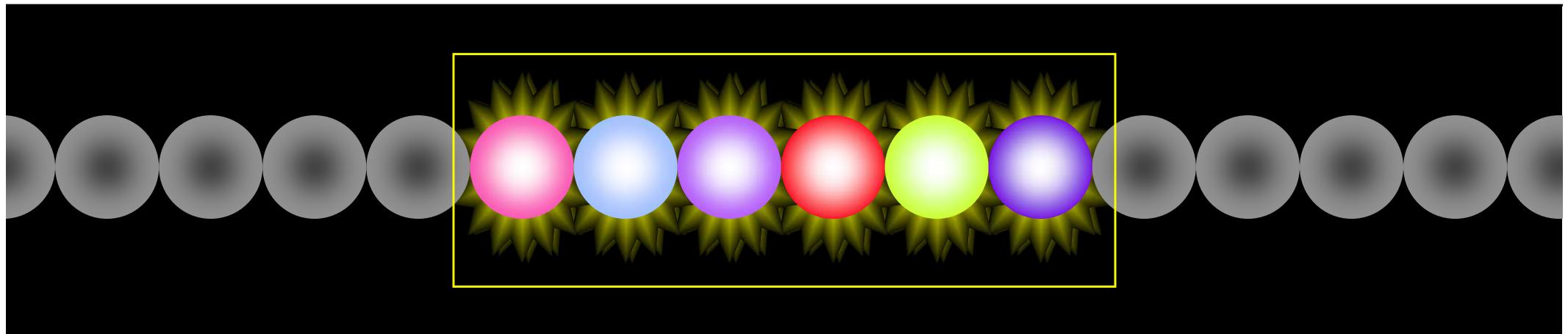
抗 體

OK

X

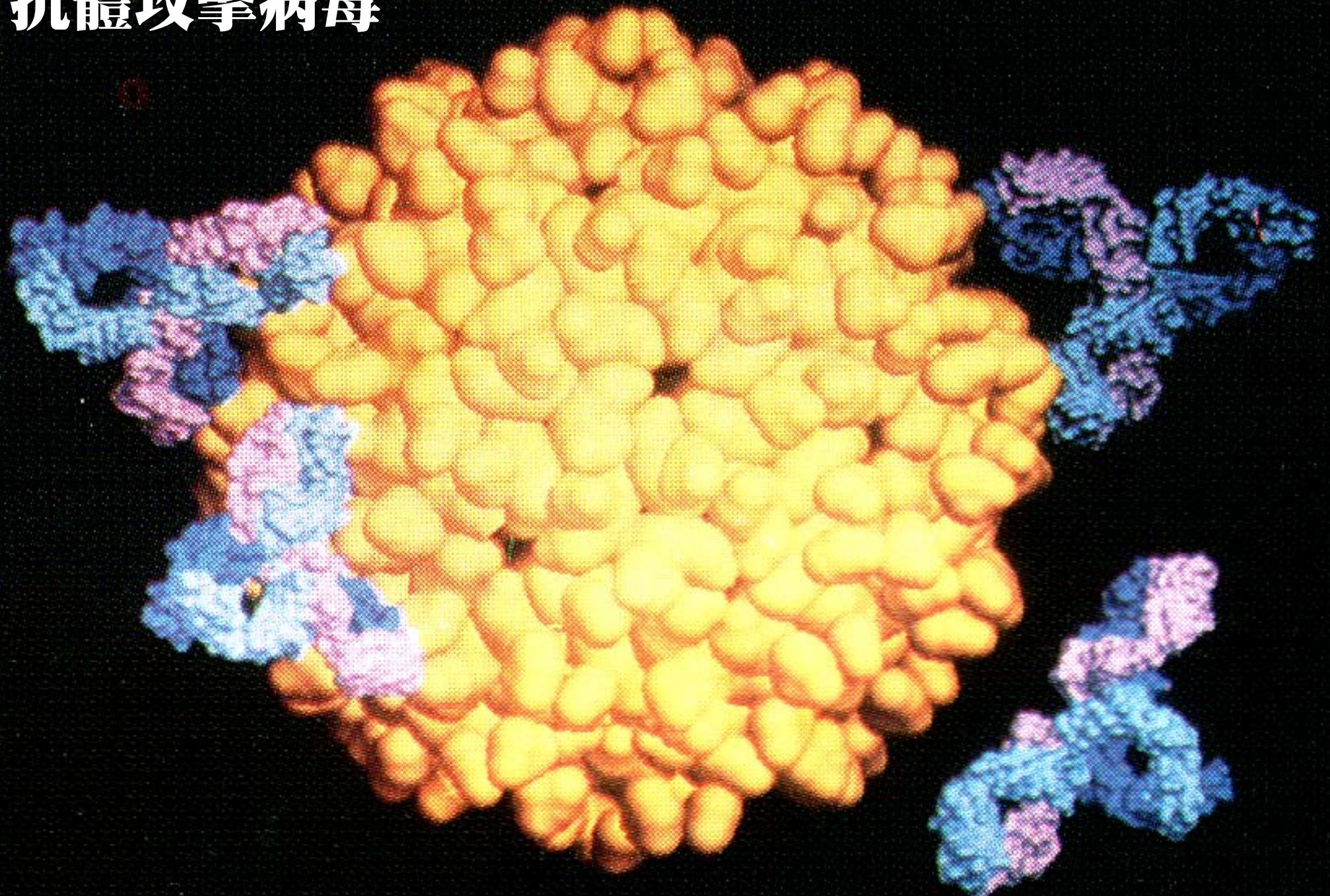
抗原決定基

- 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

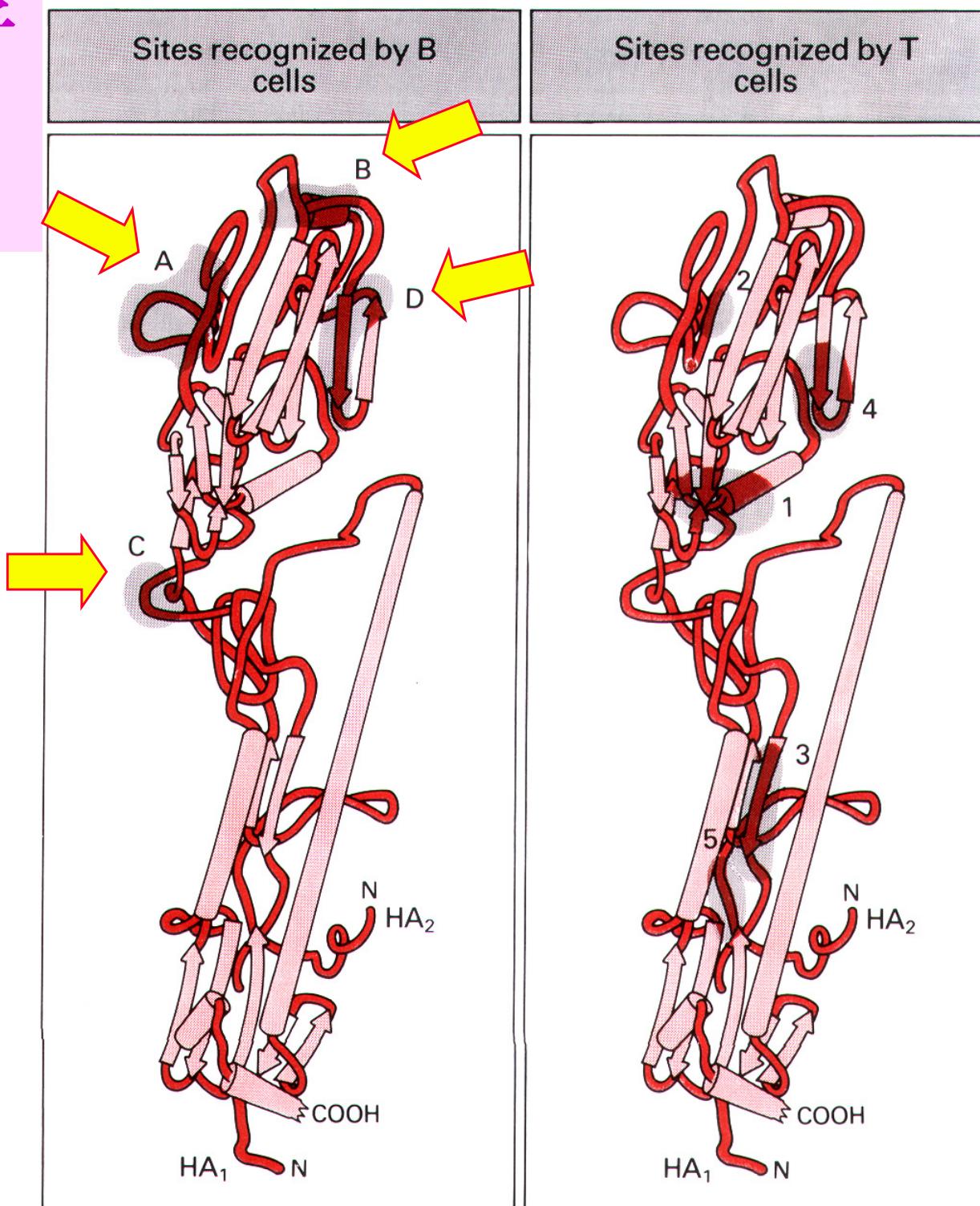


- 蛋白質性 抗原決定基 含有六個以上胺基酸

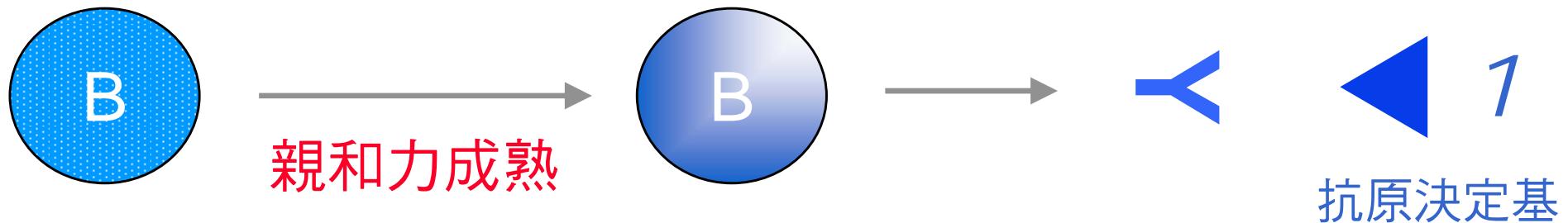
抗體攻擊病毒



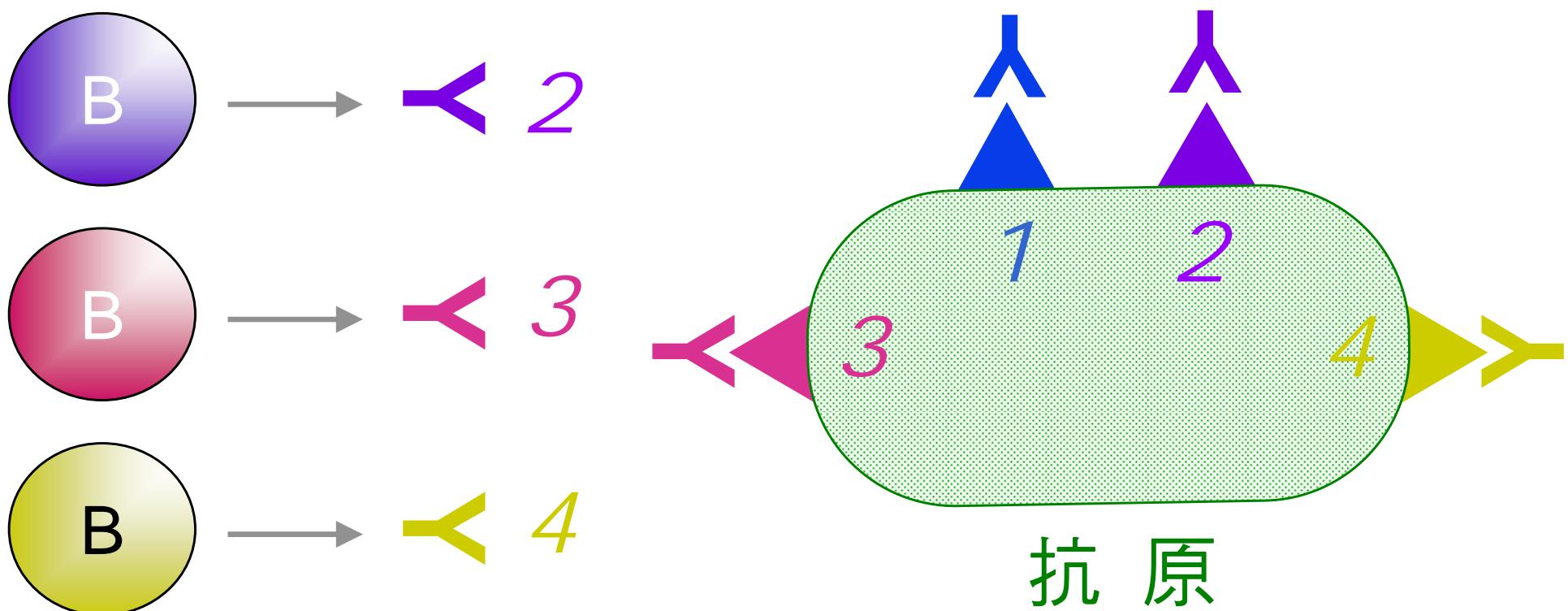
病毒表面分子 上有許多個 抗體結合區



- 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。

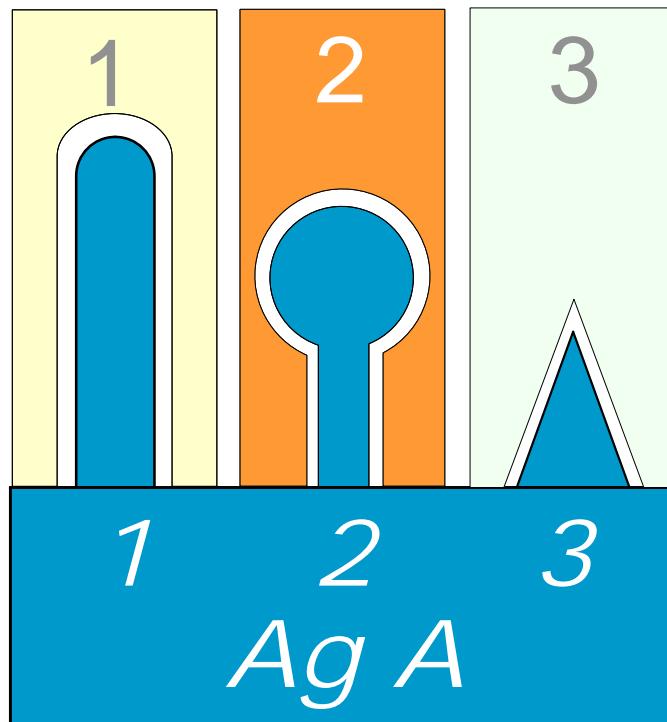


傳統抗血清的交叉反應

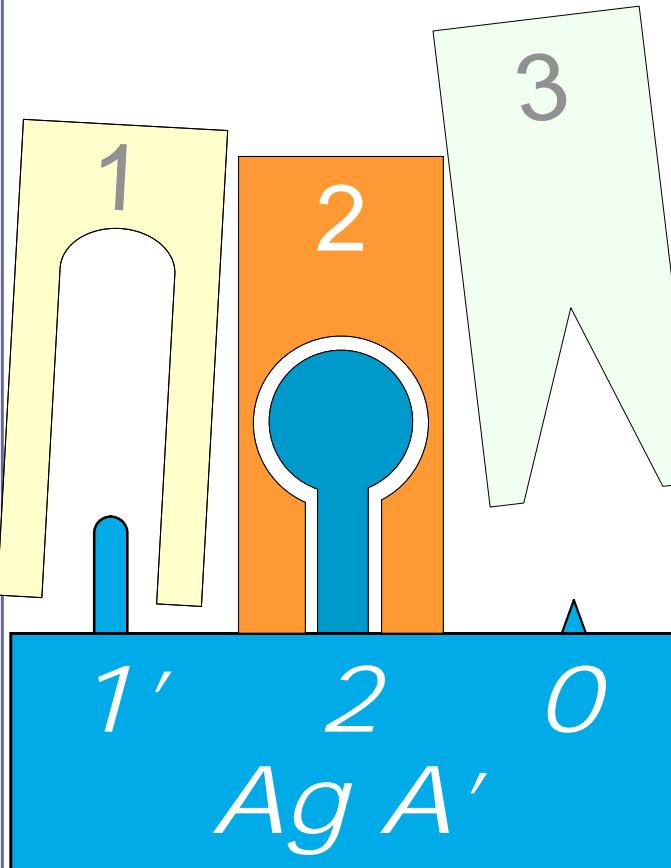
專一性反應

交叉反應

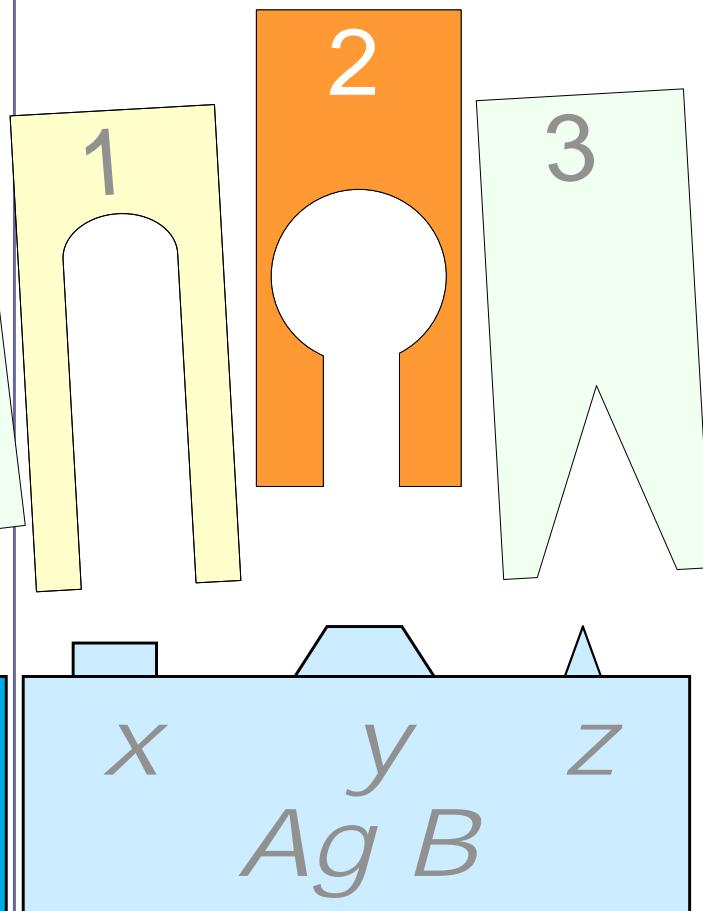
沒有反應



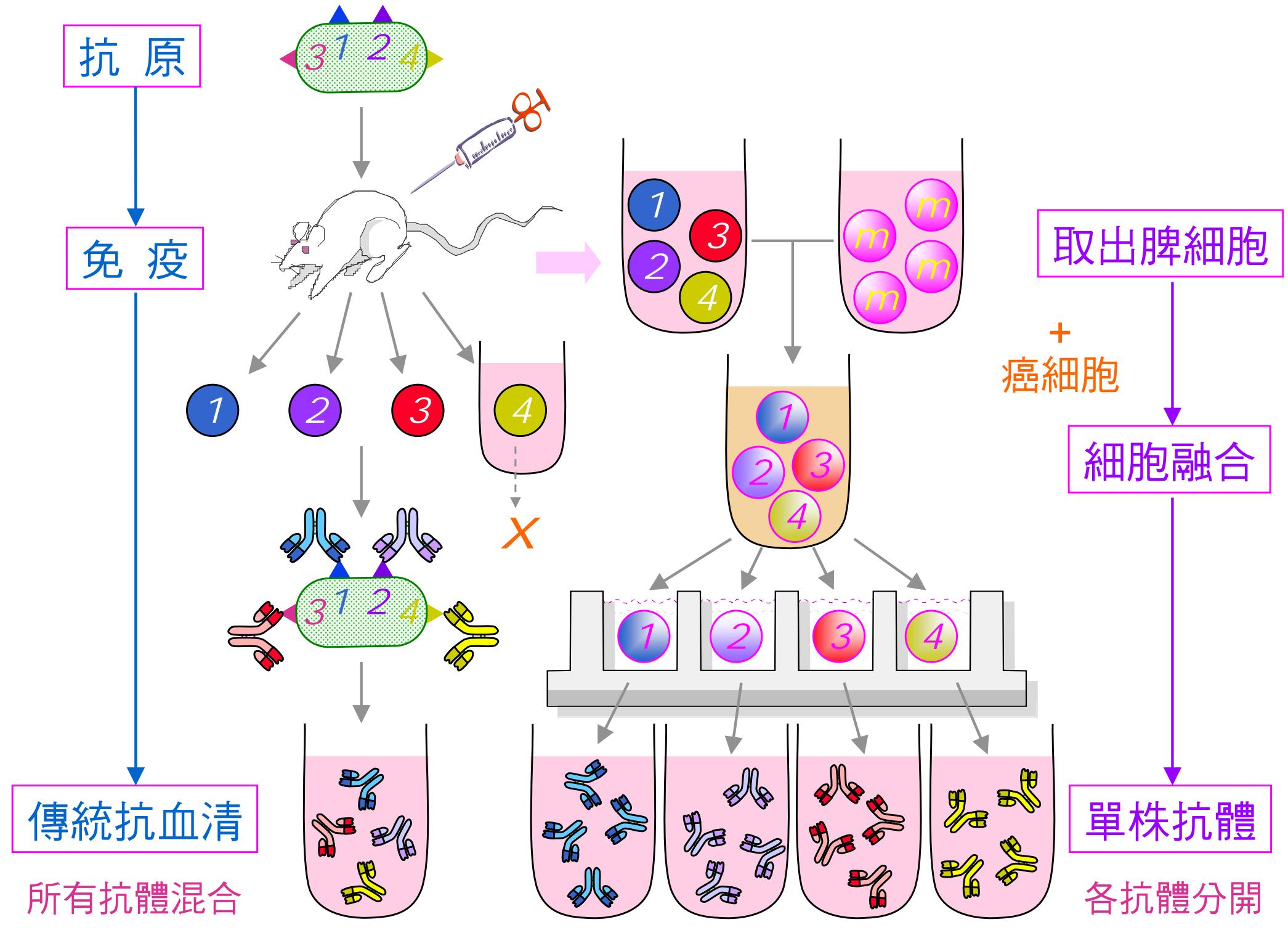
+



?



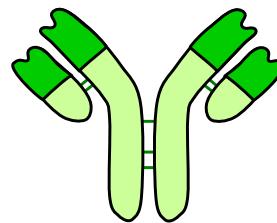
-



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

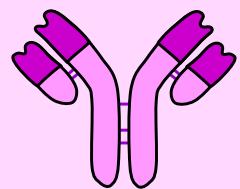
PEG

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。



都是白血球



一個 B cell 只
產生一種抗體

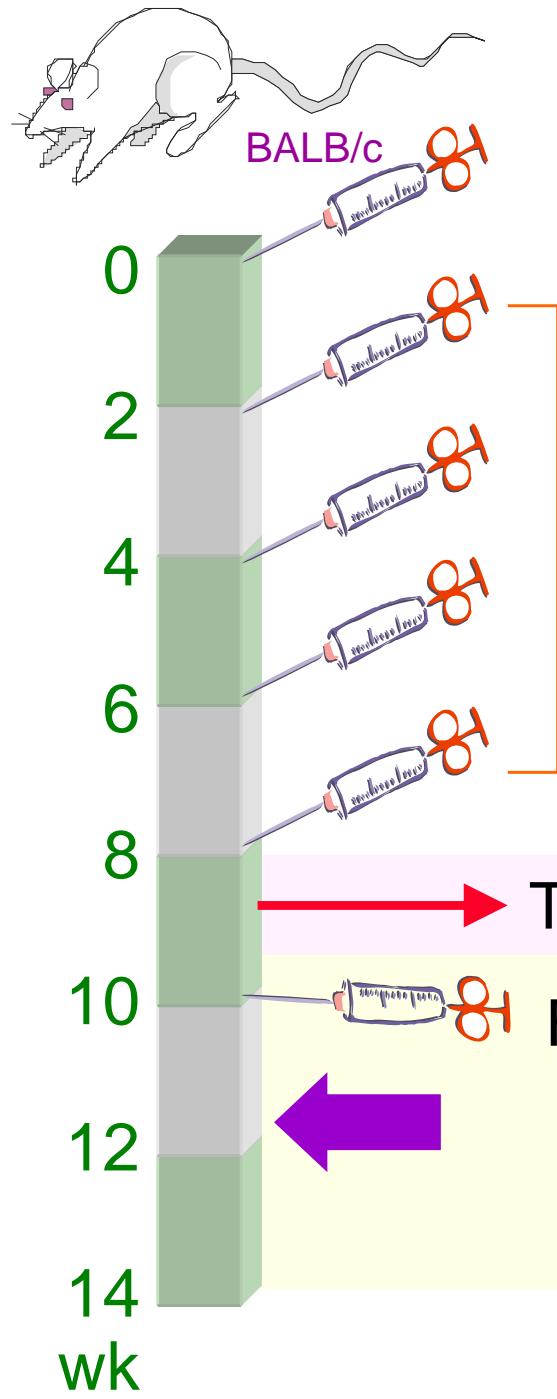


兩組染色體混在一起



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)

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 use as adjuvant

試採血

→

Trial Bleeding

→

Titer Determination

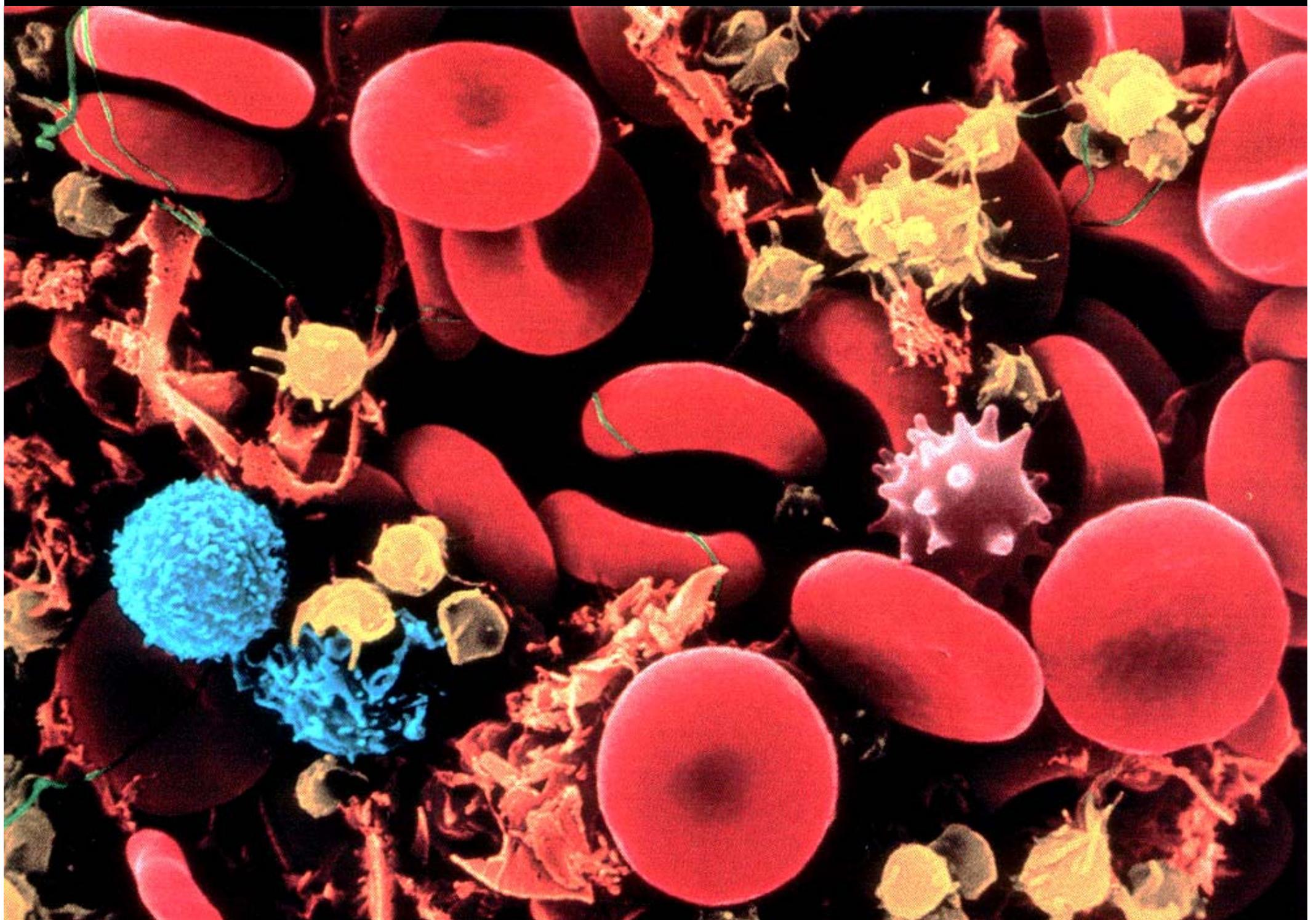
←

Final booster (soluble antigen)

脾臟為 B 細胞集中地

取出脾臟內細胞

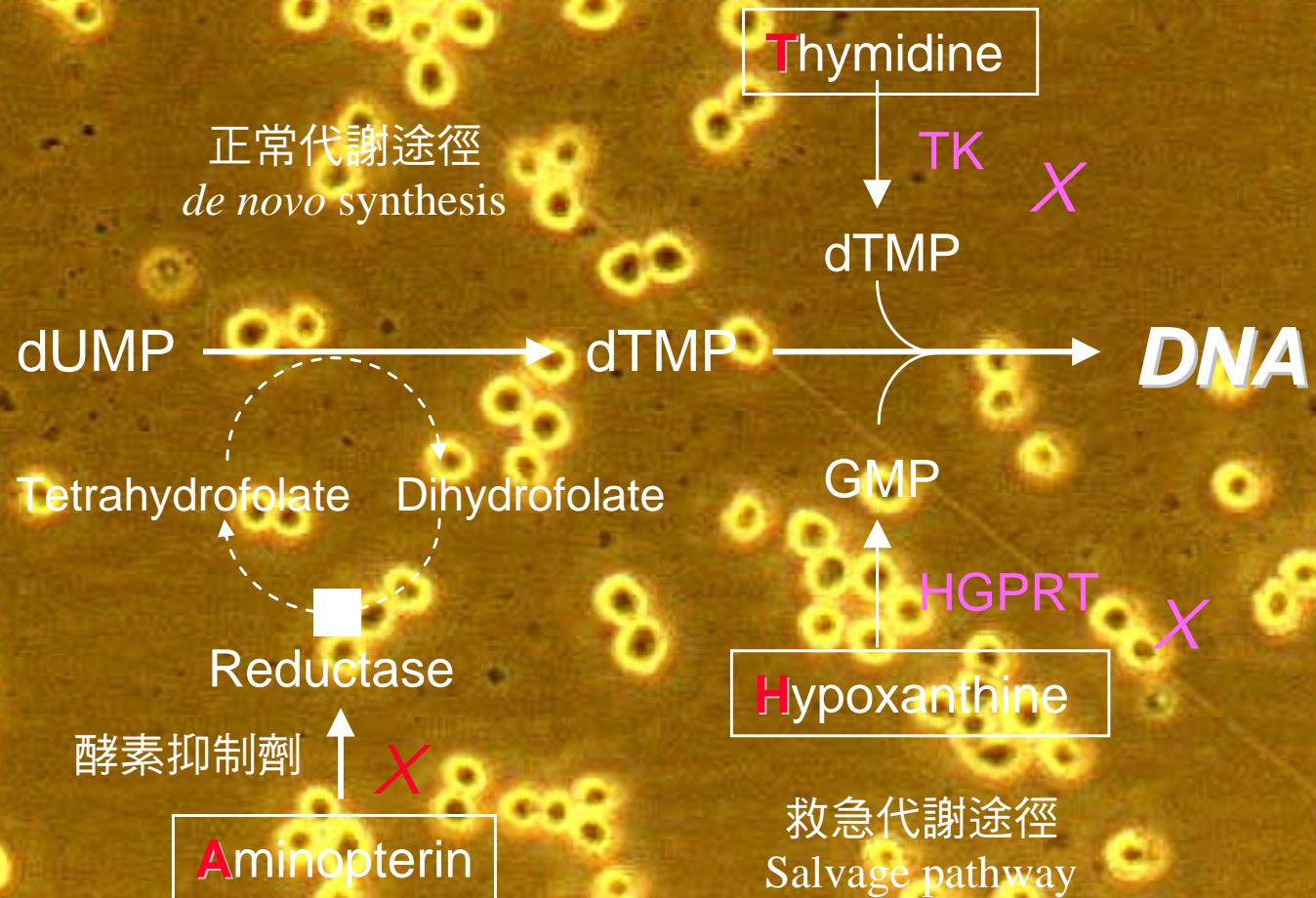




Dennis Kunkel / *Everyday Science Explained*. p.243

癌細胞可以在培養基中長久生長

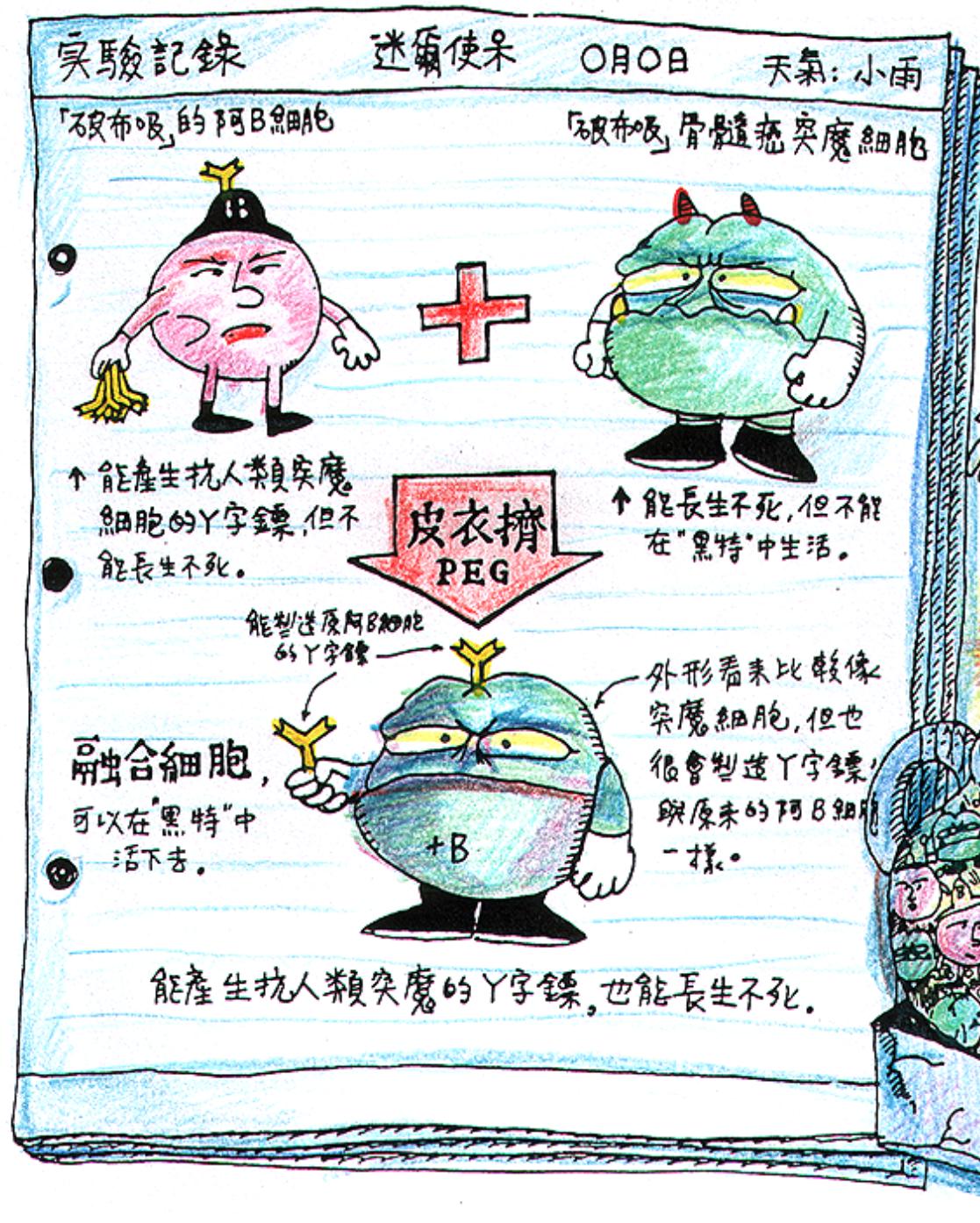
但是骨髓癌細胞 NS-1 無法在 *HAT* 中生長



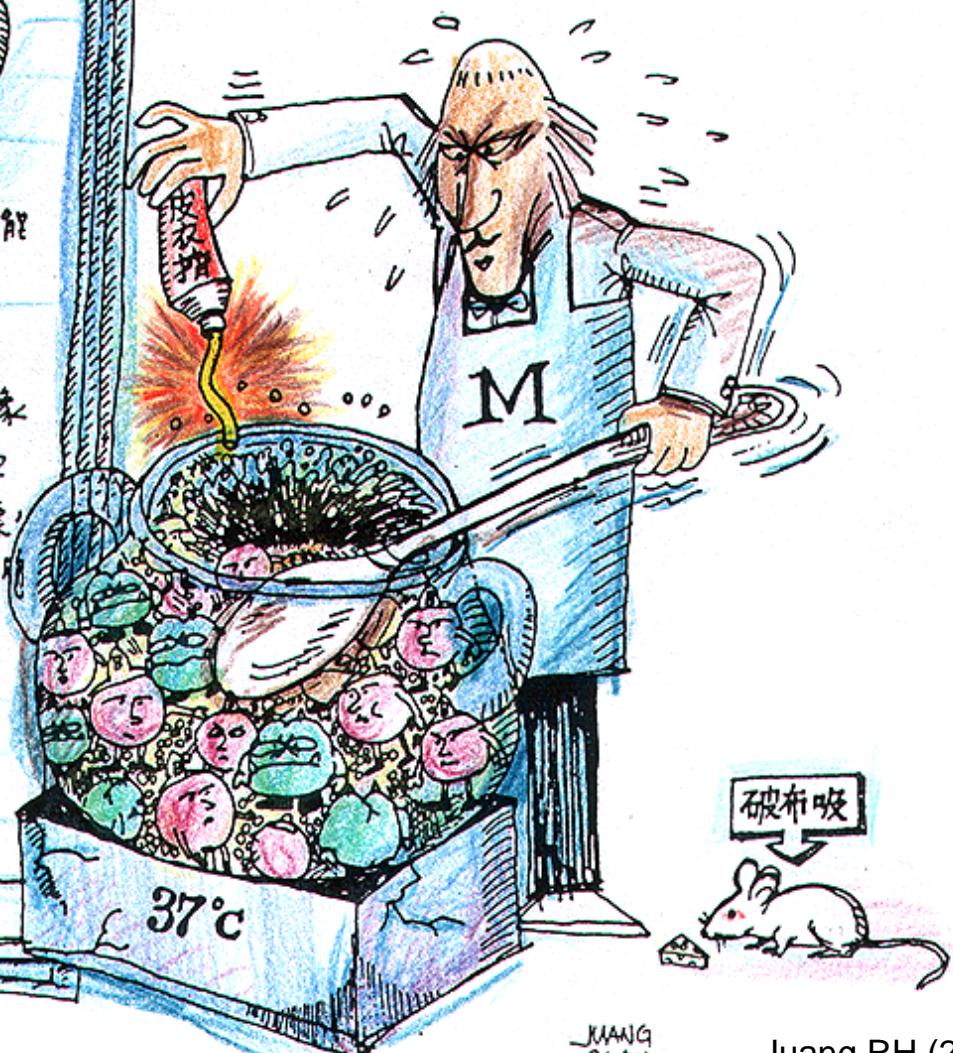
以化學試劑將細胞融合



迷爾使呆的研究日誌

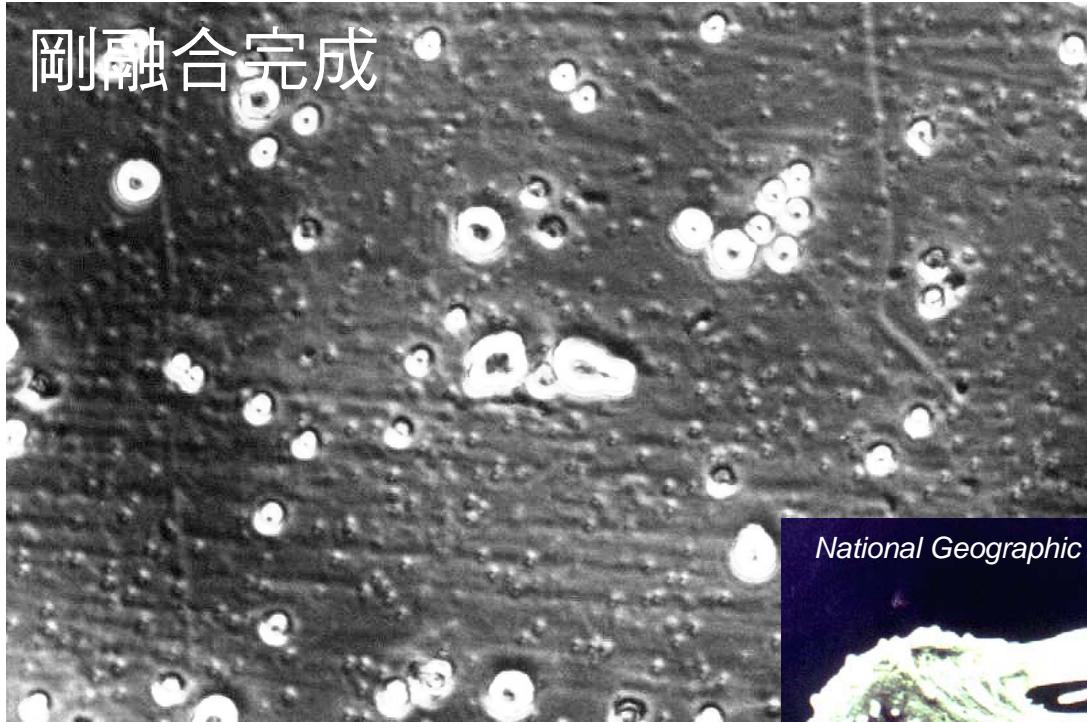


Kohler & Milstein (1984)

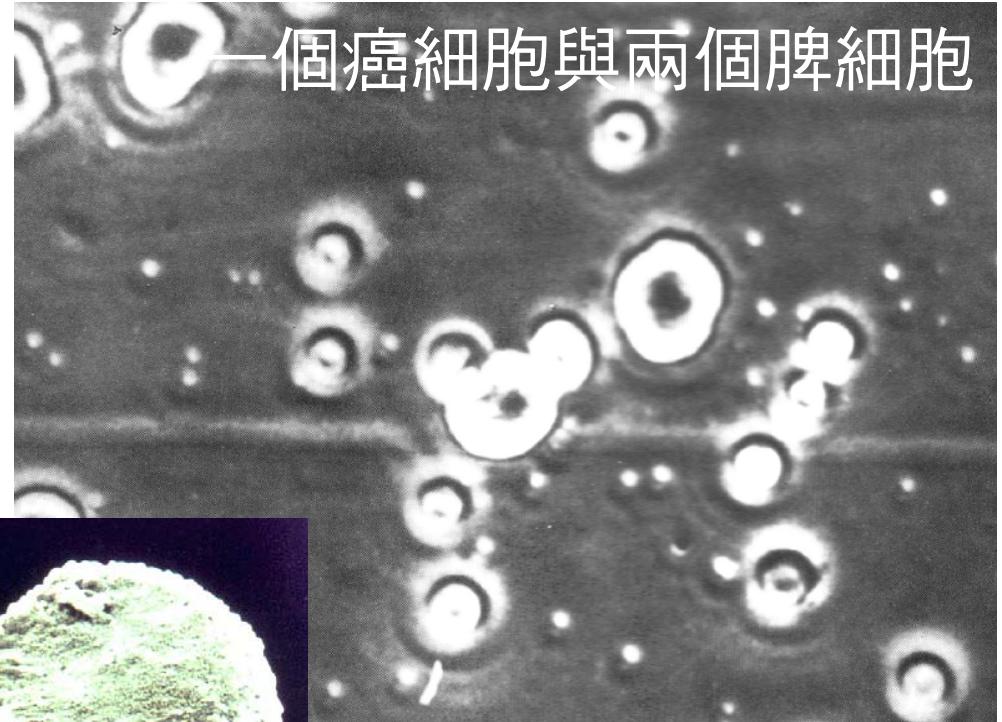


融合後的細胞生長情形

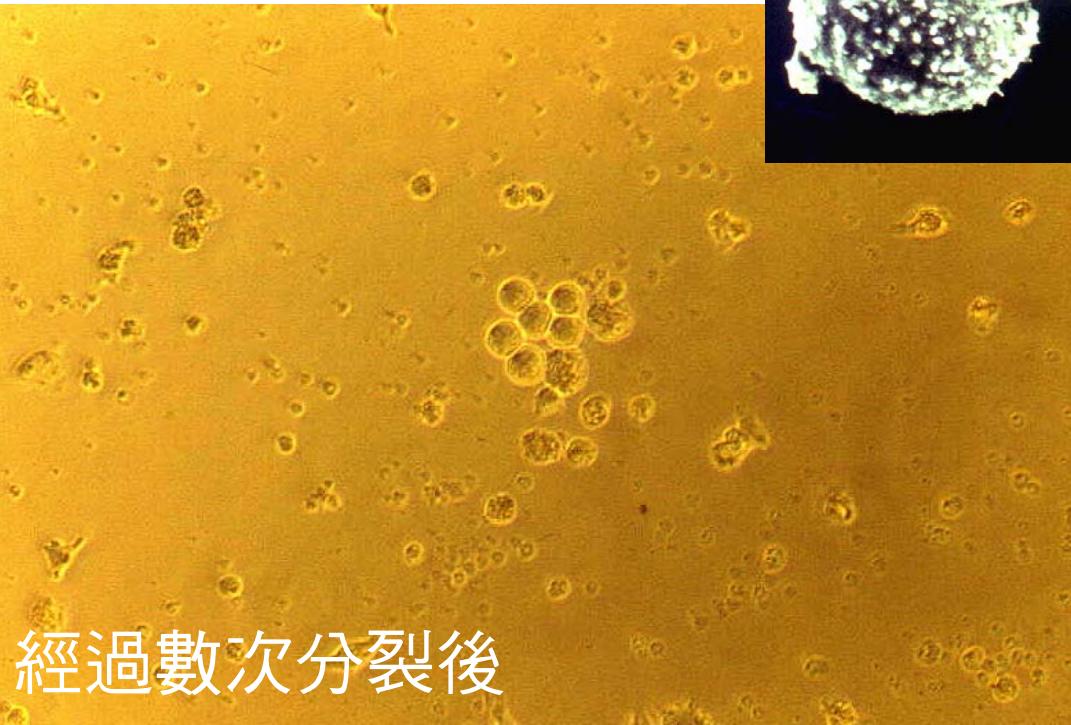
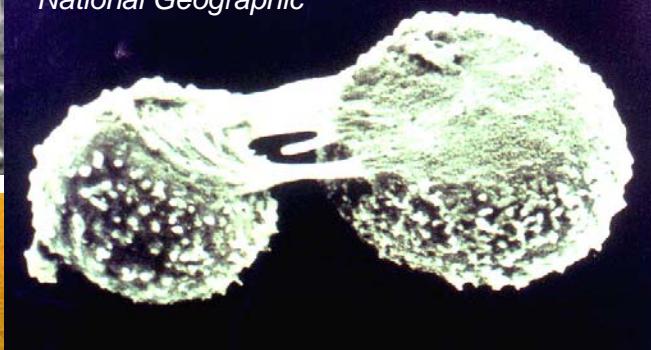
剛融合完成



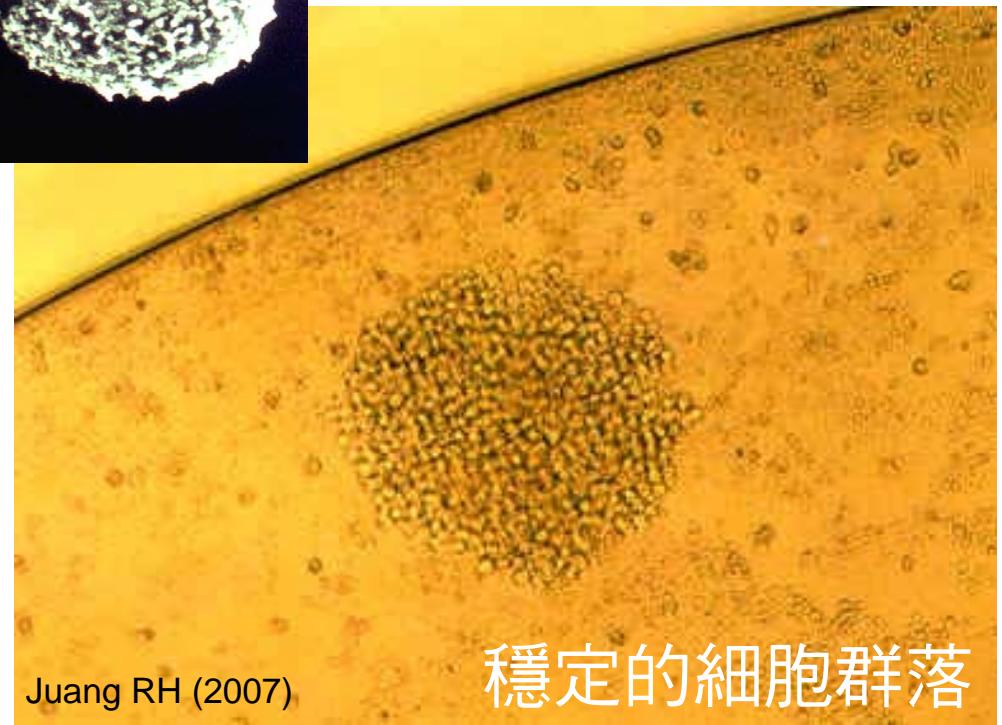
一個癌細胞與兩個脾細胞



National Geographic



經過數次分裂後



Juang RH (2007)

穩定的細胞群落

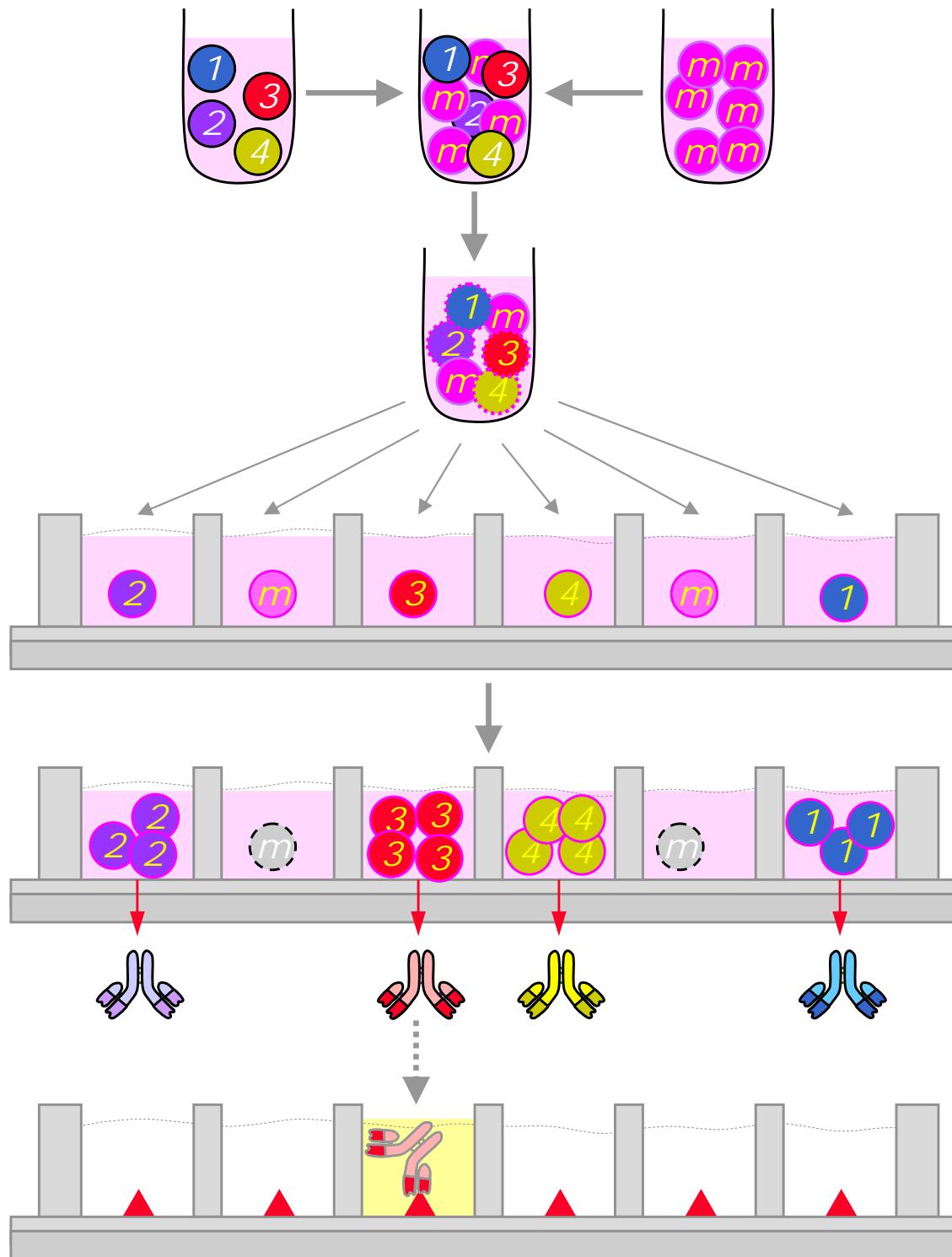
脾細胞

癌細胞

細胞融合

HAT
初步篩選

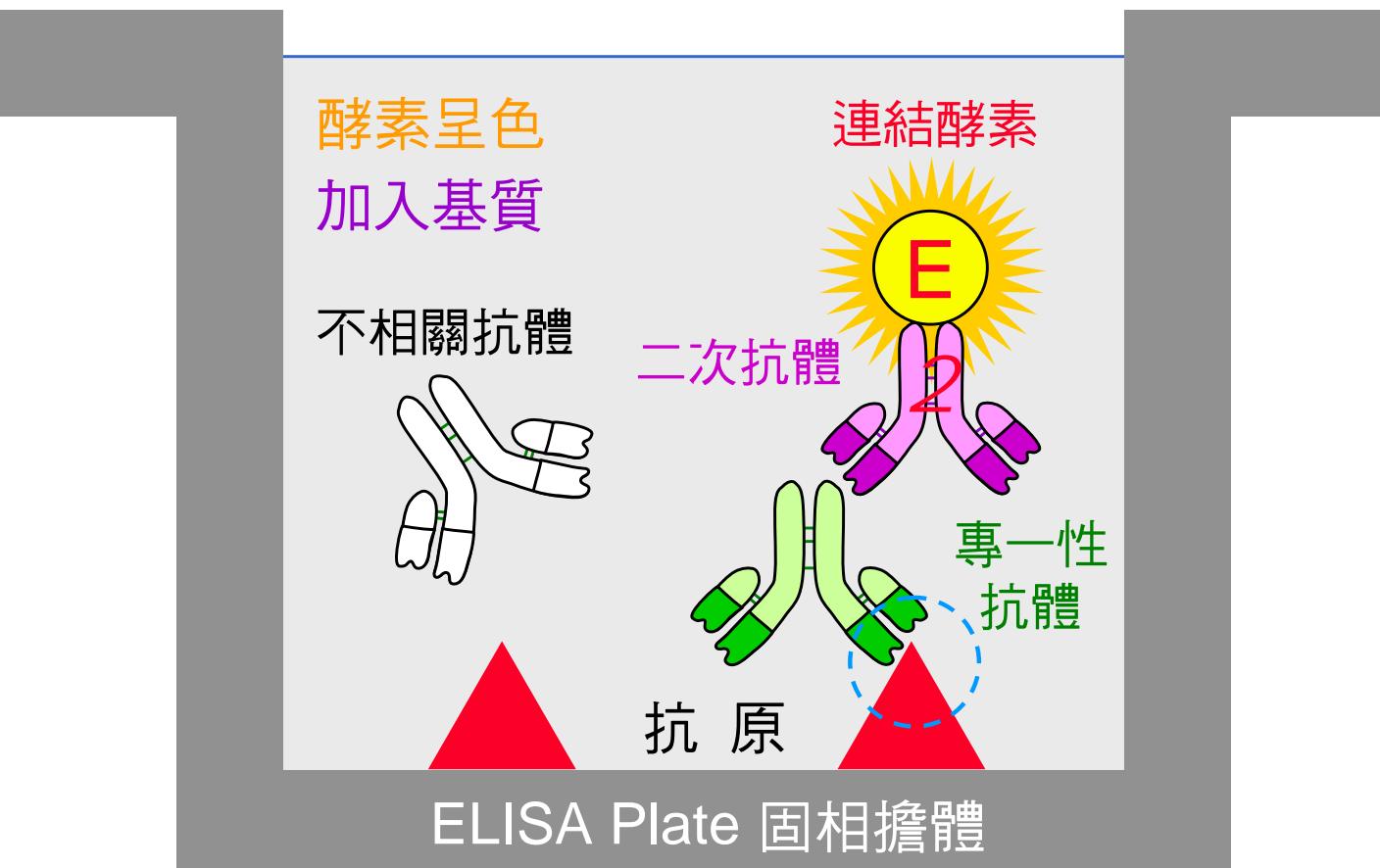
ELISA
抗體篩選



以酵素免疫分析法檢測樣本中的專一性抗體

ELISA

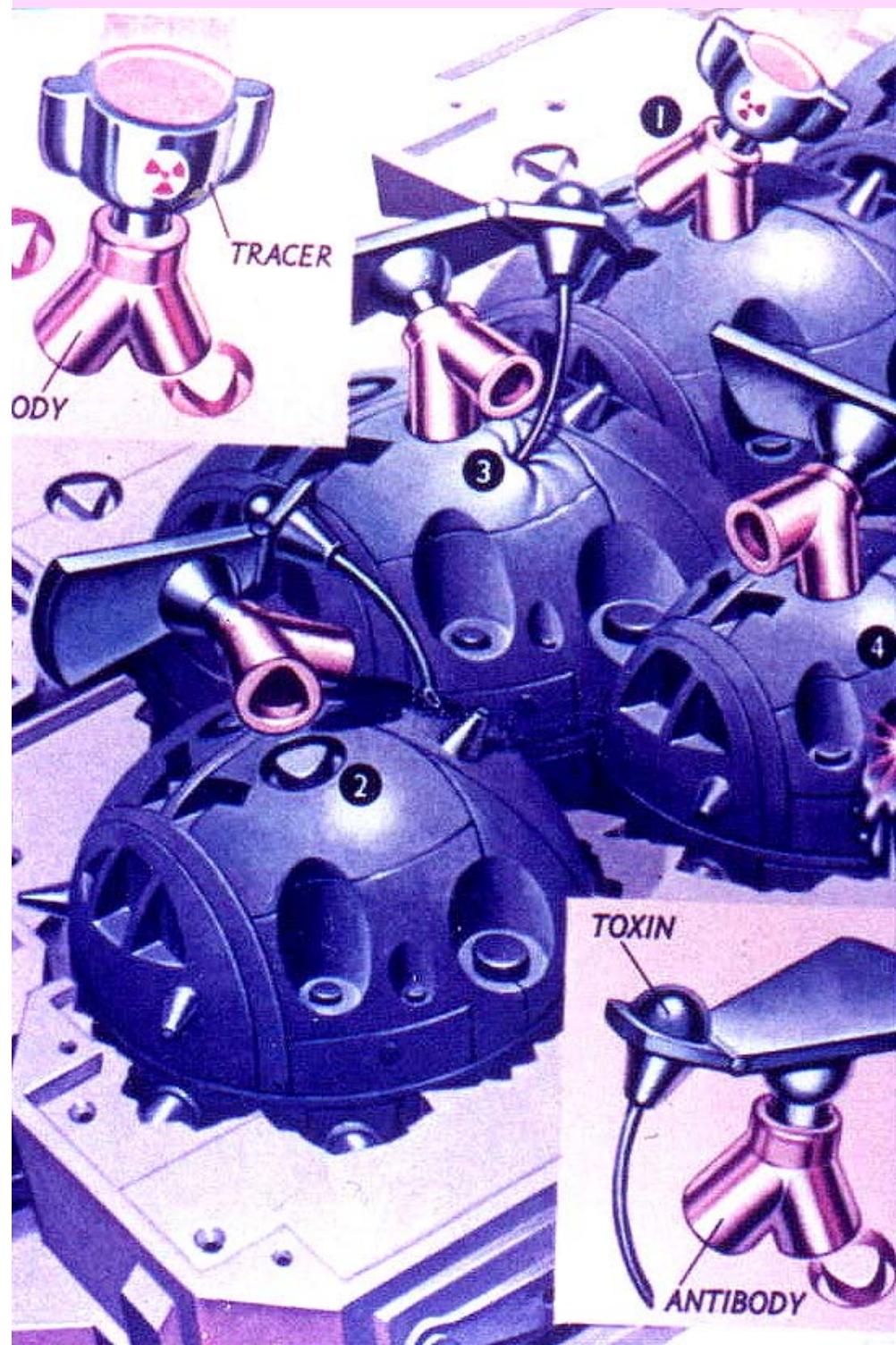
Enzyme-Linked Immunosorbent Assay



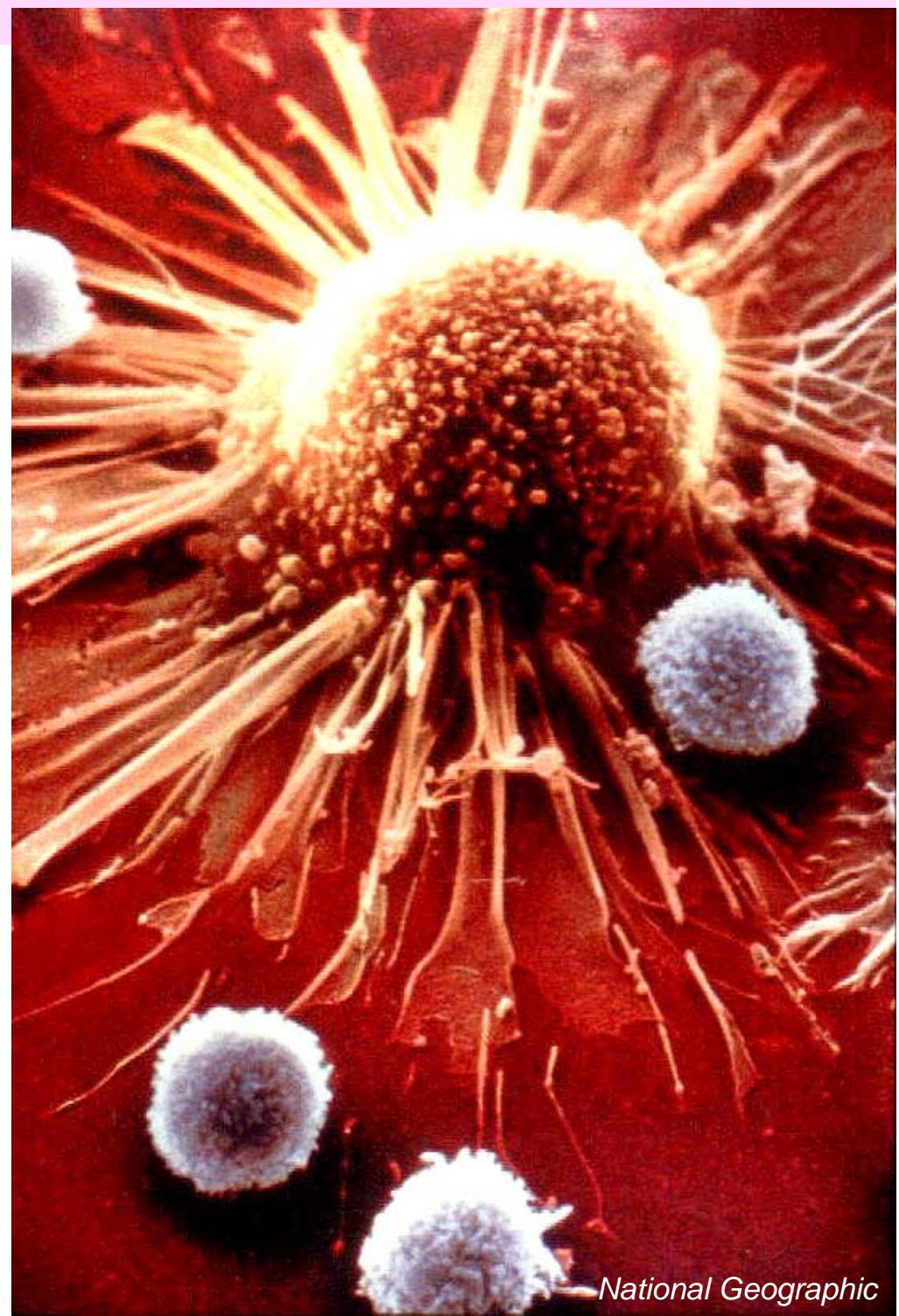
細胞穩定後擴大至 T-25 培養瓶



在 T-75 培養小瓶中擴大細胞數目

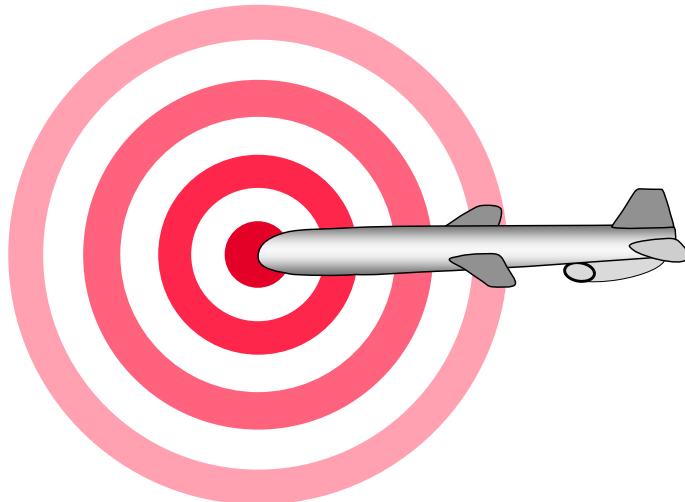


以單株抗體來清除體內癌細胞



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 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 特定病原體 |

魔彈 再起



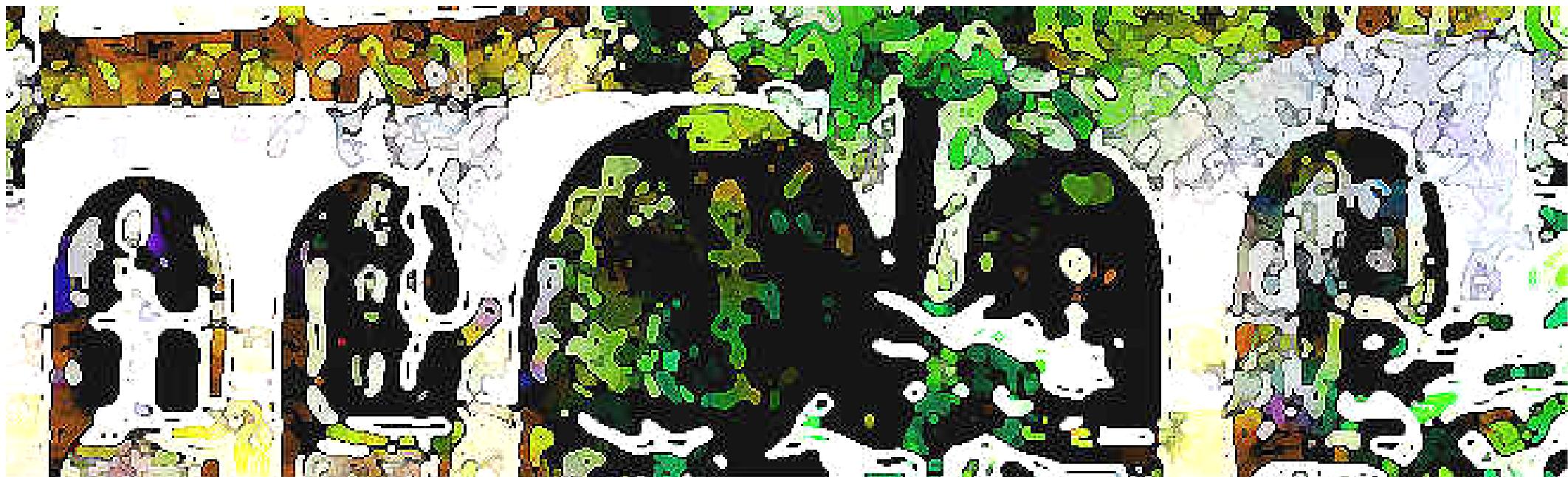
Molecular guided missiles called monoclonal antibodies were poised to shoot down cancer and a host of other diseases—until they crashed and burned. Now a new generation is soaring to market

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

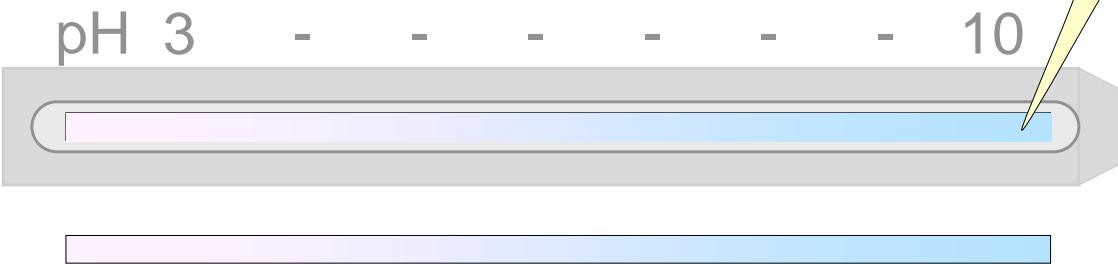
也無法預測蛋白質間的交互作用

Proteome

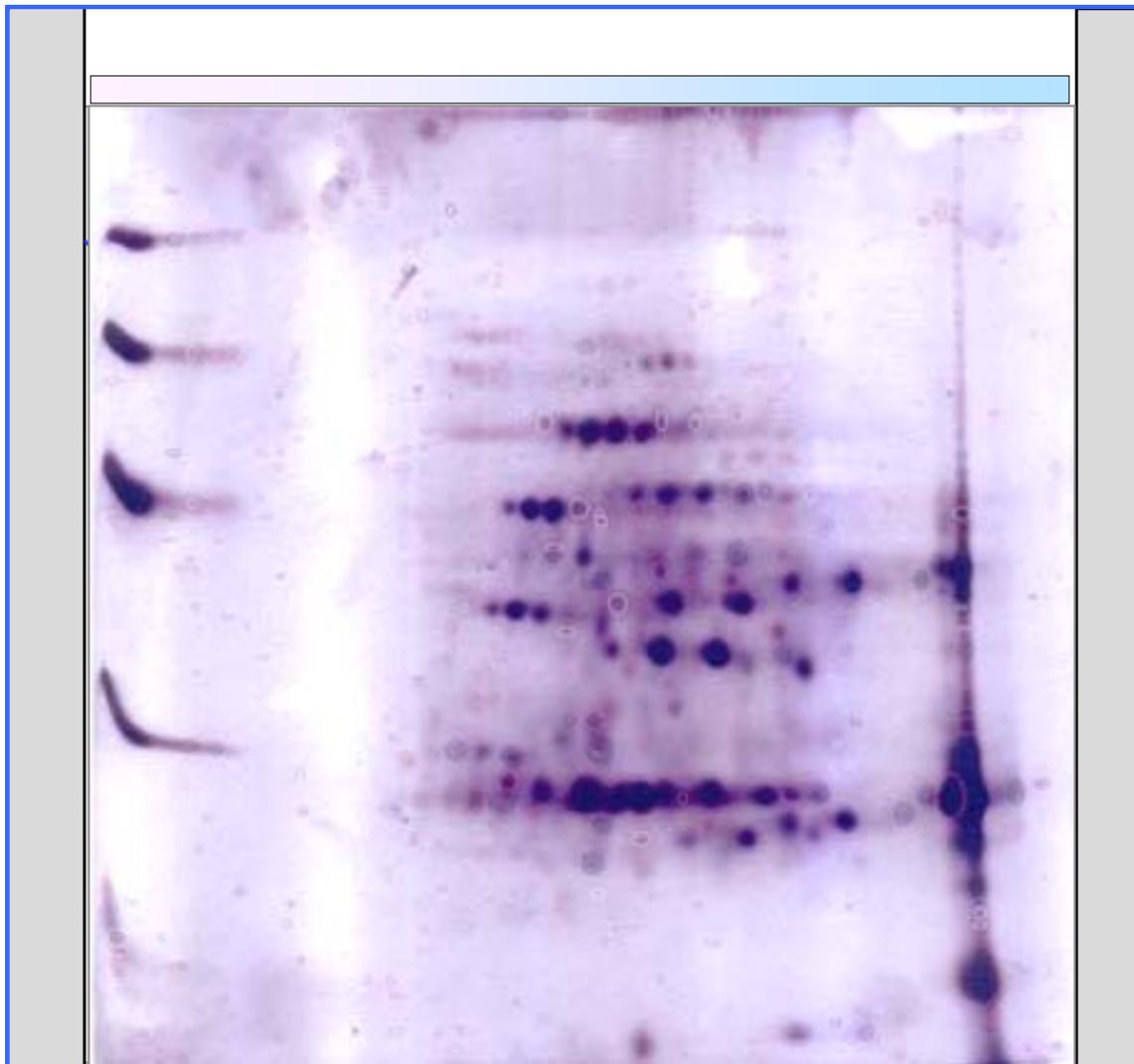
二次元電泳的操作過程

(1) IEF

等電焦集電泳



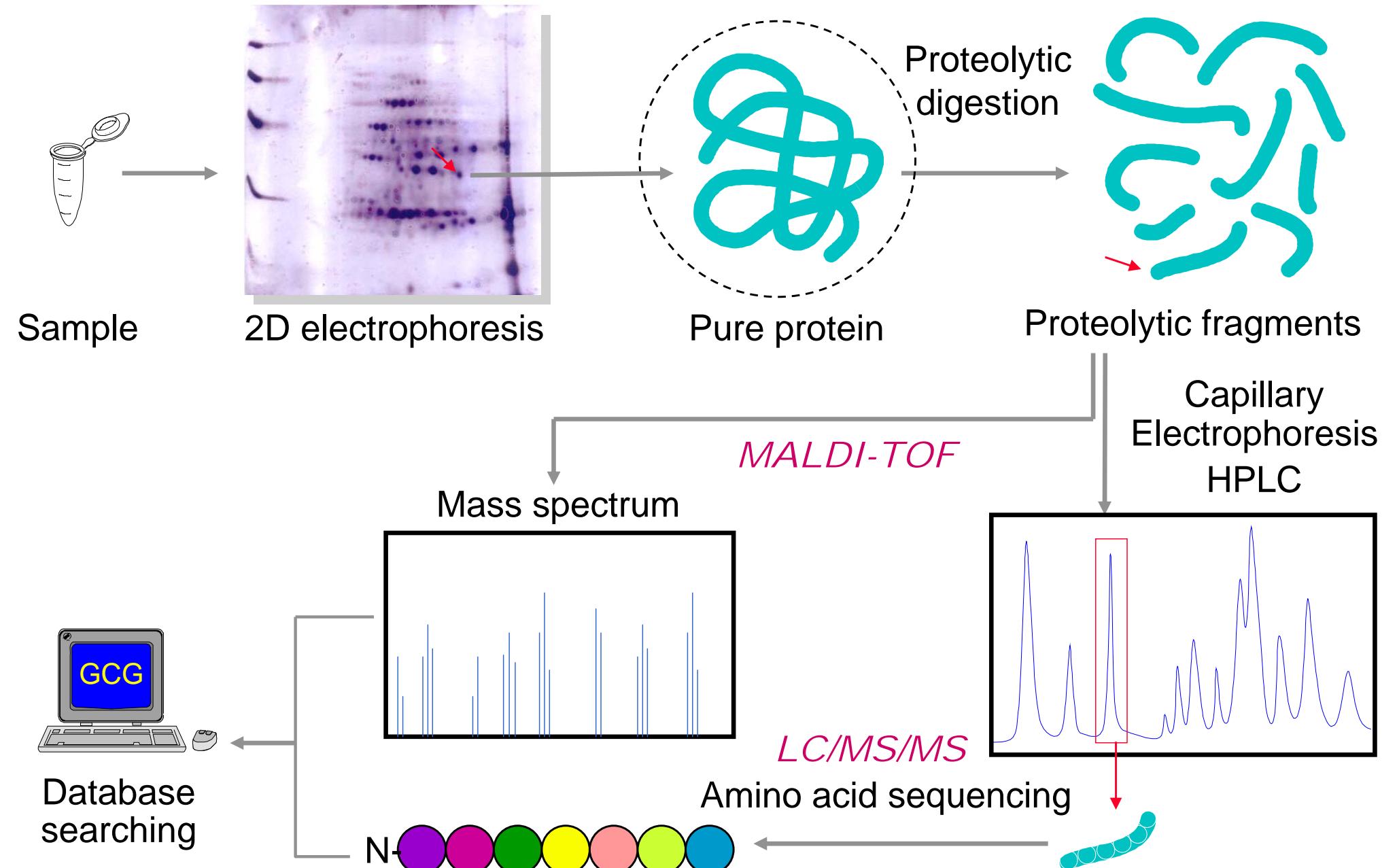
(3)
染色脫色



(2)
SDS-PAGE
分離膠體



■ 蛋白質體可綜觀蛋白質的消長與身分





WIKIPEDIA



竹文化

Oriental bamboo culture

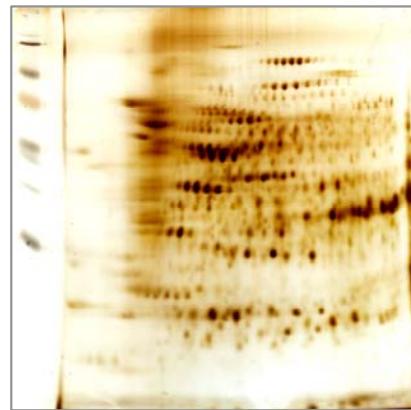


WIKIPEDIA

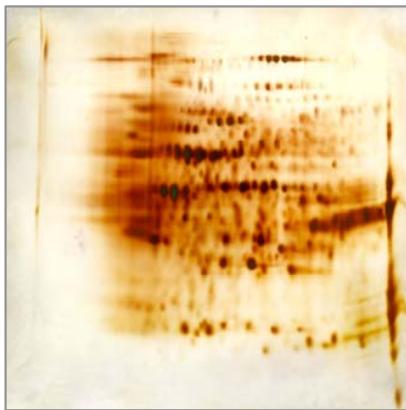


Proteomic pattern changes during growth

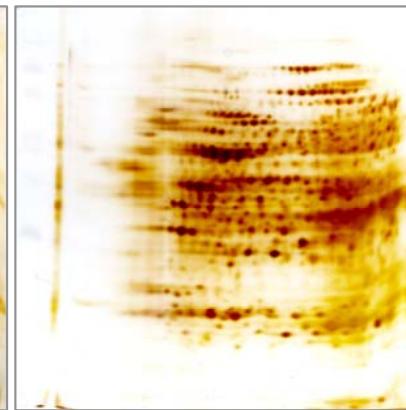
0 cm



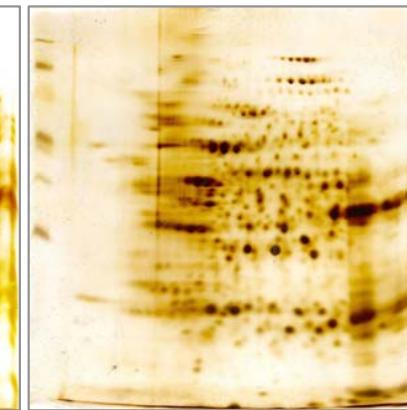
10 cm



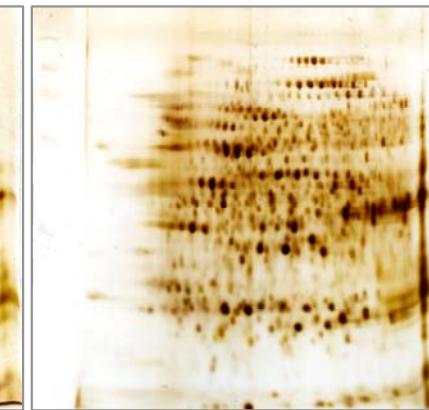
20 cm



40 cm



60 cm



綠竹筍

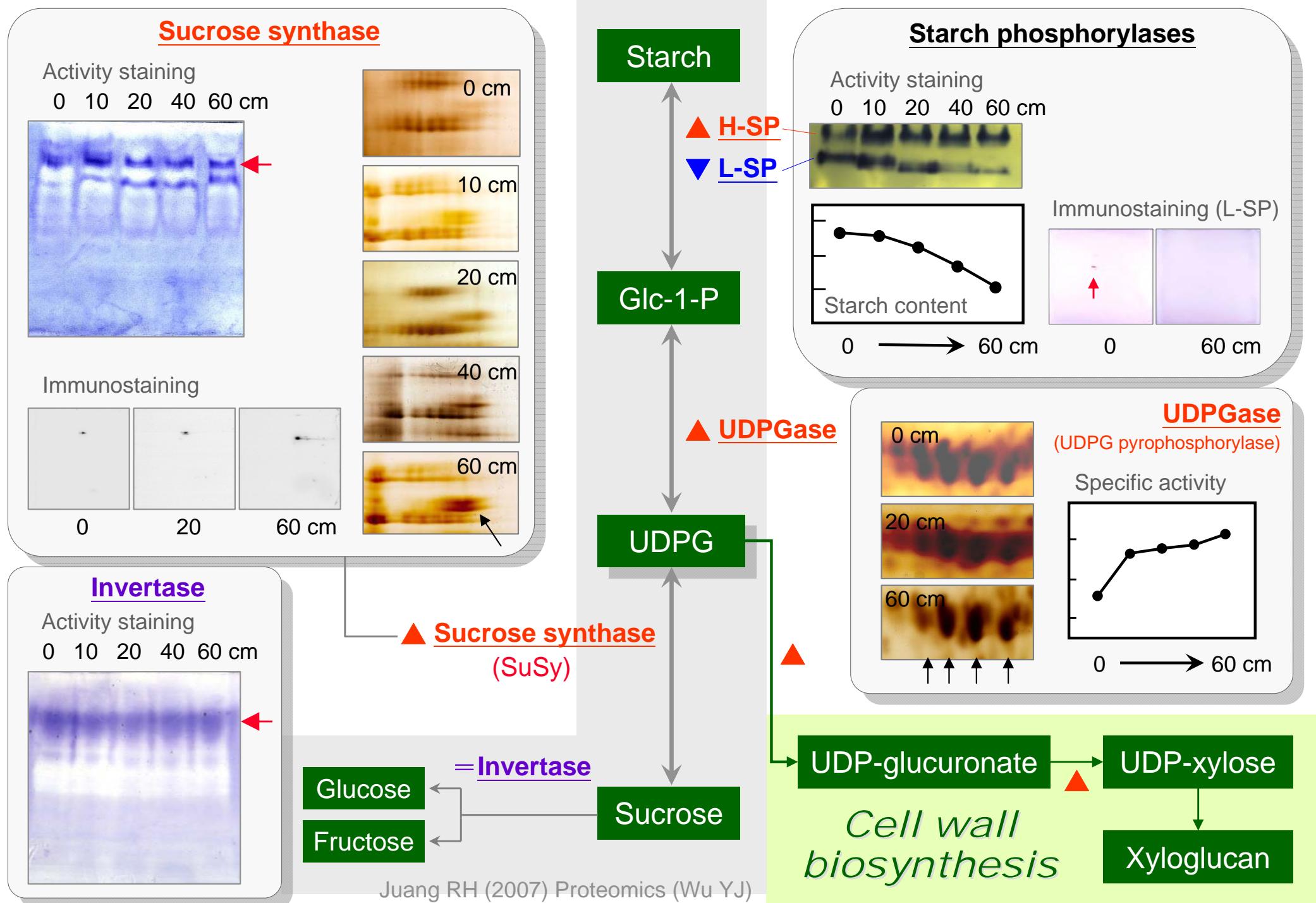
Cellulose synthesis

Juang RH (2007) Proteomics (with Wu YJ)

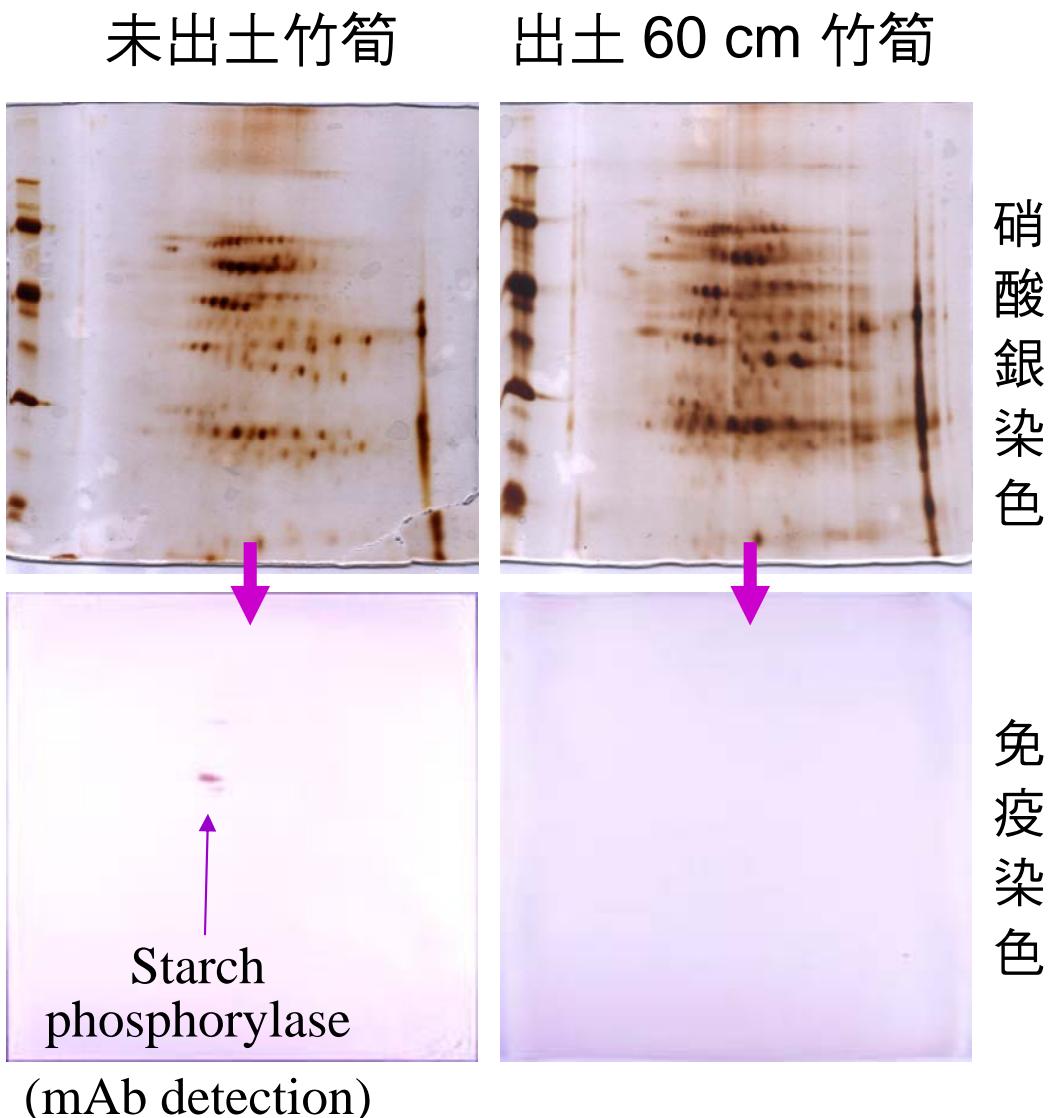


點	Protein ID	Accession no.	Calculated Mr (kD) / pI	Sequence coverage (%)	Score (MASCOT)	Match fragments
79	Sucrose synthase	AAV64256 (<i>Bambusa oldhamii</i>)	92.8 / 6.03	35	402	14
80	Sucrose synthase	AAV64256 (<i>Bambusa oldhamii</i>)	92.8 / 6.03	35	245	7
82	Sucrose synthase	AAV64256 (<i>Bambusa oldhamii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



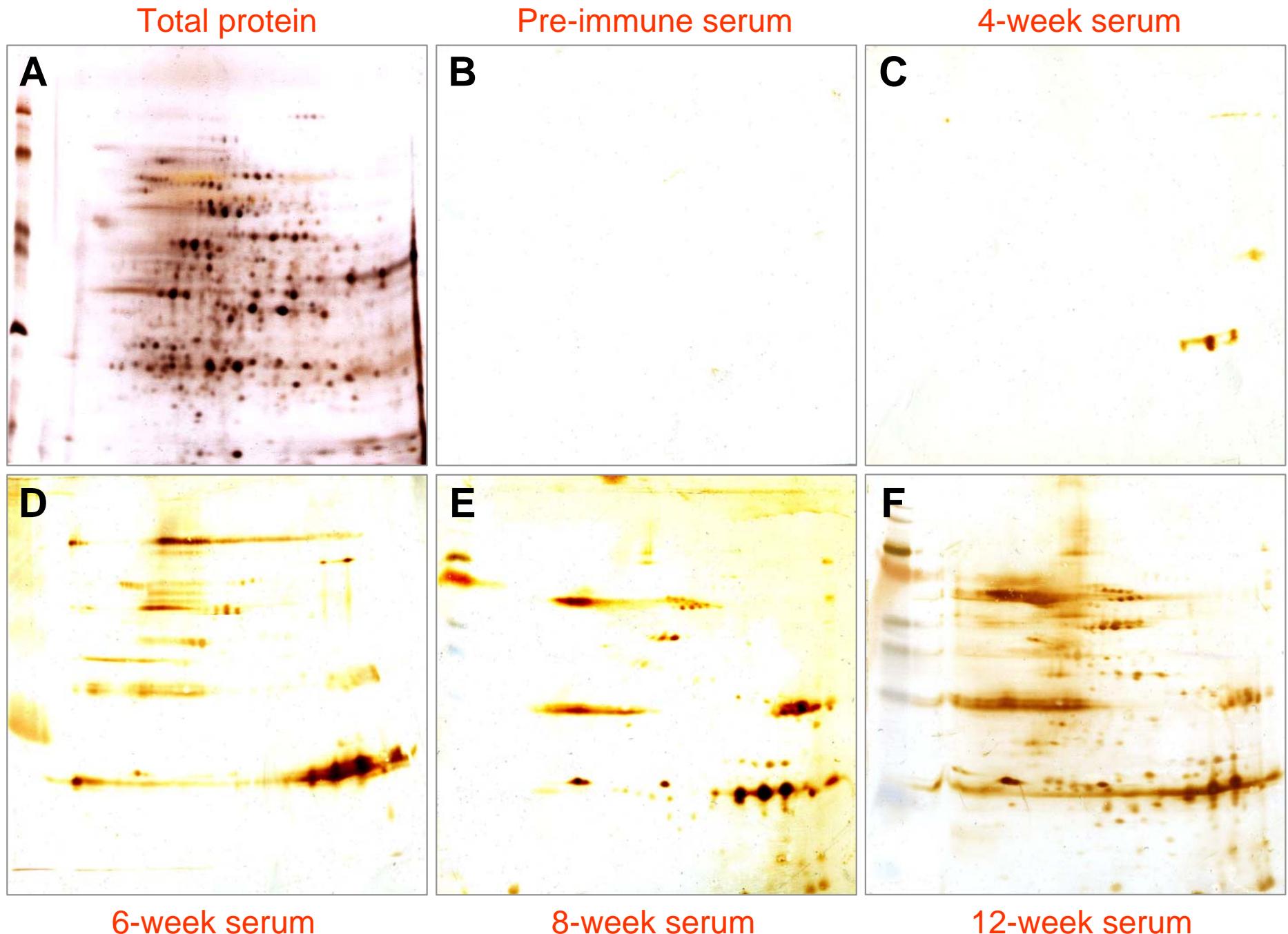
首次提出
高產能抗體製備計畫構想

- (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

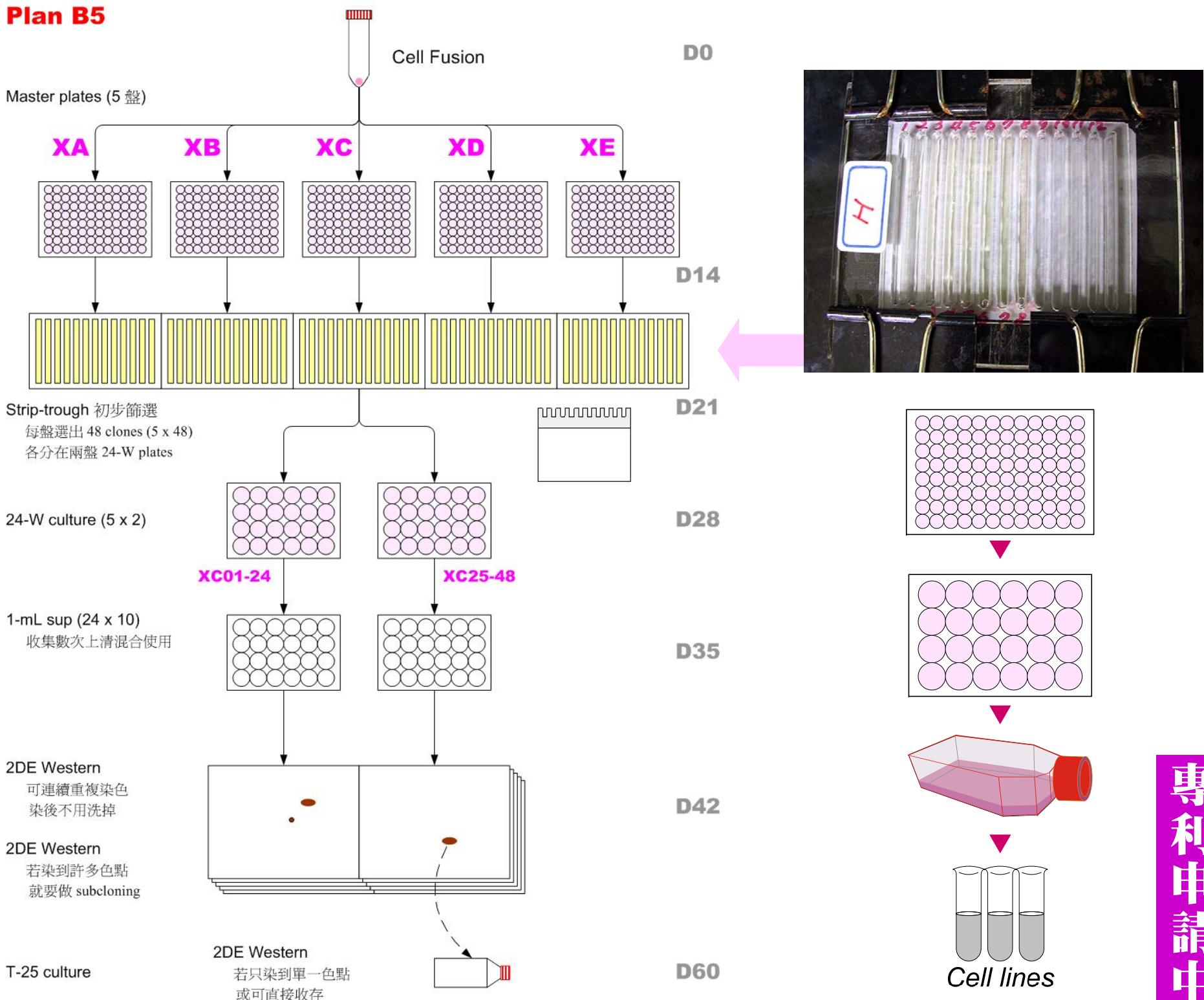
The immune response for complex antigen



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 (after subcloning)	320	150	500	250
Final monoclonal		78		82

(B) Second-stage

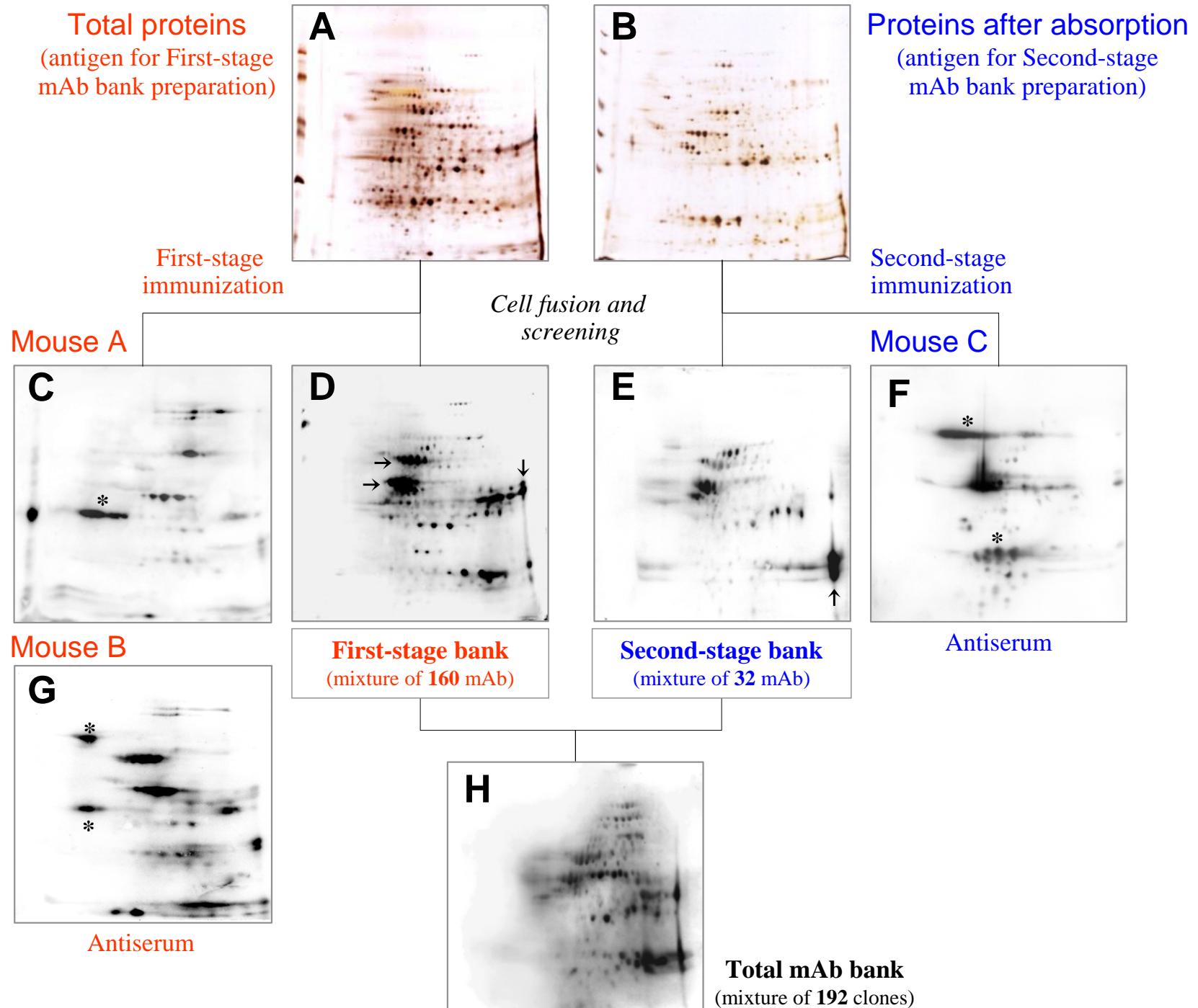
Spleen cell fused with	Sp2/0 (Mouse C)	
Number of clones	screened	positive
First screening	100	40
Second screening (after subcloning)	400	120
Final monoclonal		32

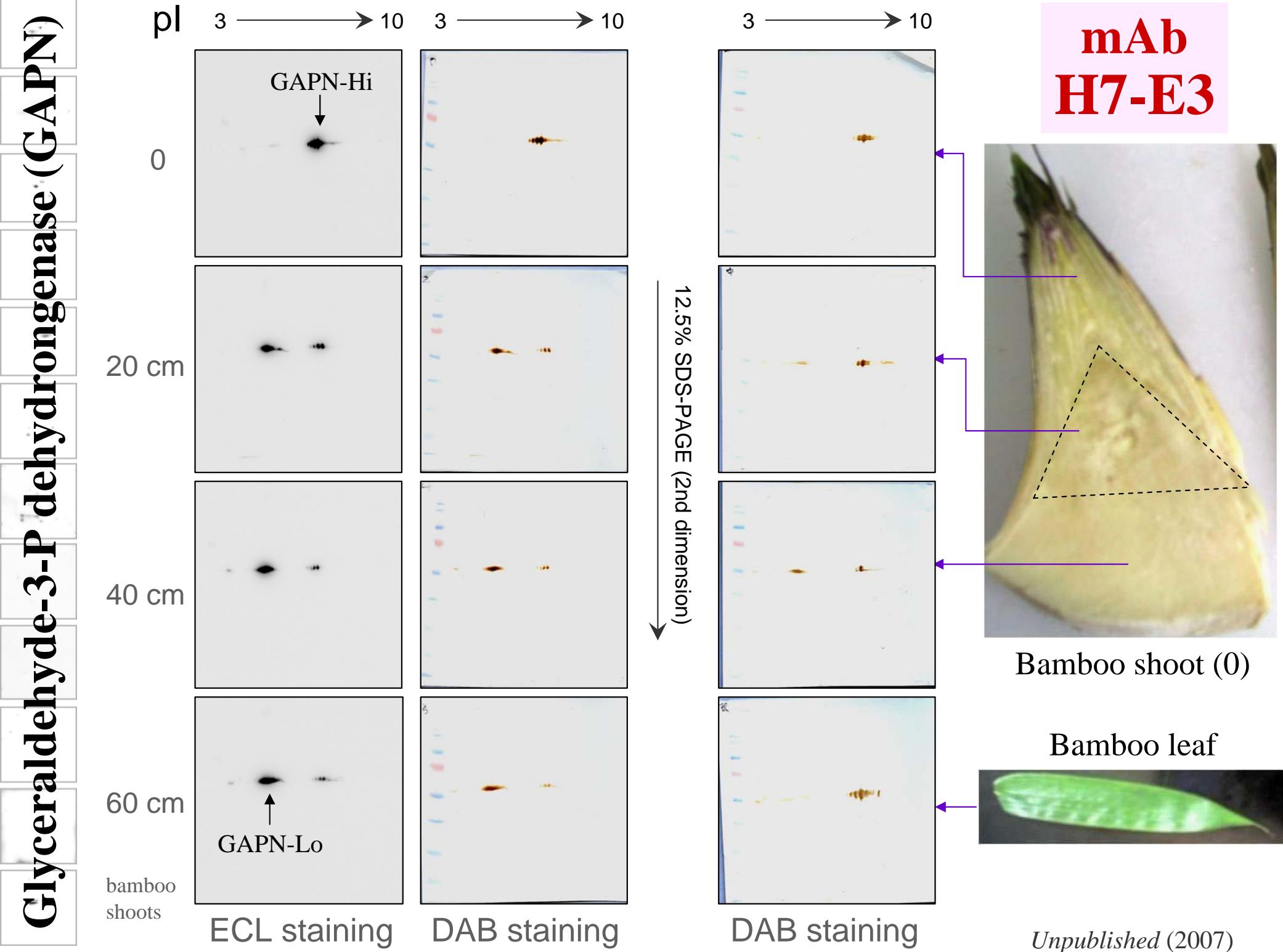
The library of total 192 blots in the mAb bank

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



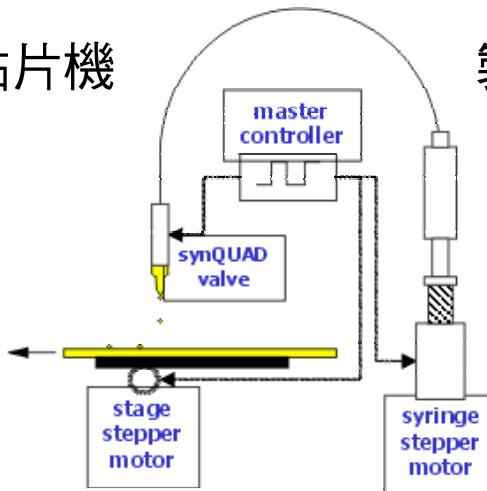


自製抗體晶片 (NC 噴霧塑膠板)

硝化纖維塗佈

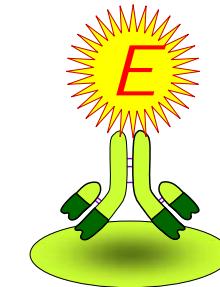


點片機

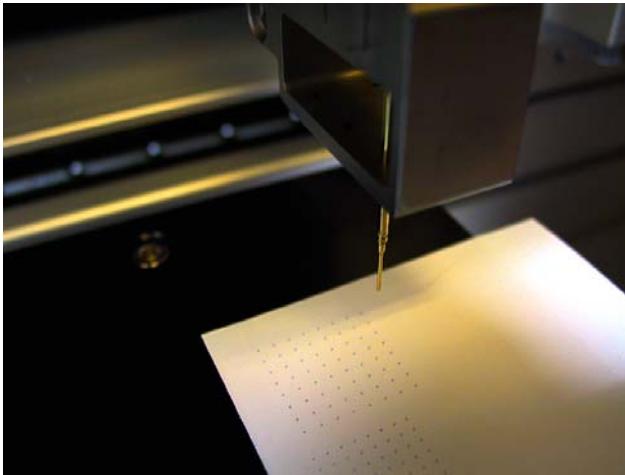
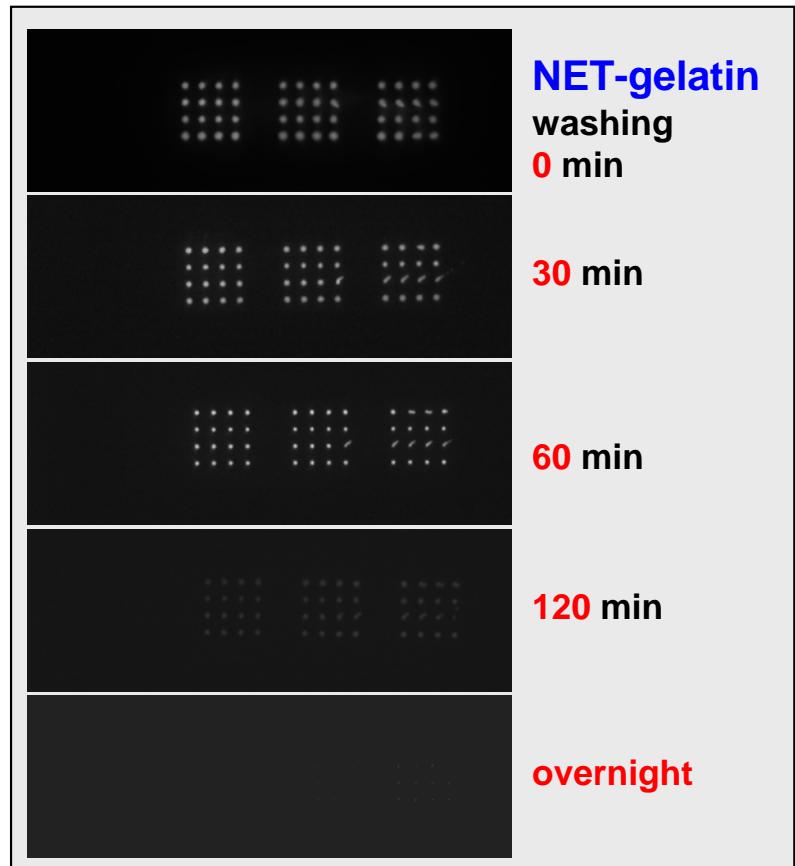


製作晶片

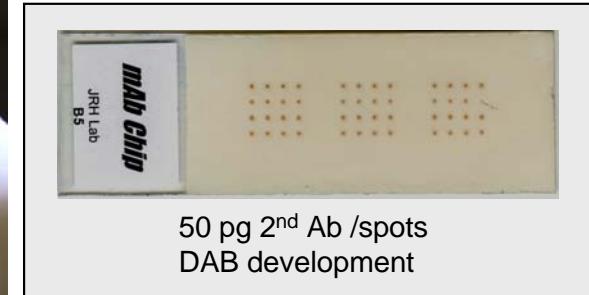
免疫呈色測試



蛋白質可穩定結合在晶片表面



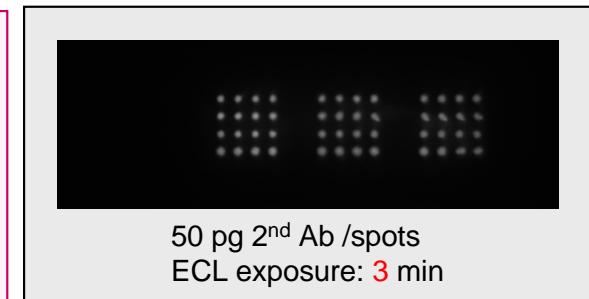
(a) 可用 DAB 均匀呈色



(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



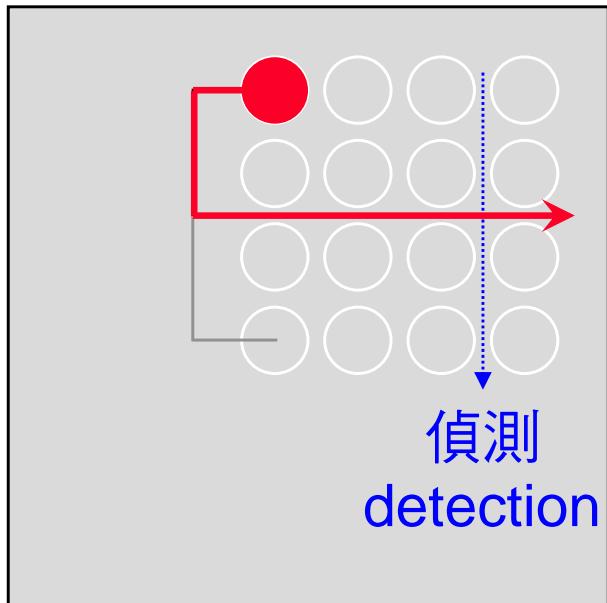
Microfluidics and Lab-on-a-chip applications

Agilent 所有蛋白質純化與活性分析均予微小化

Agilent HPLC-Chip/MS

Agilent 2100 bioanalyzer

樣本 sample well



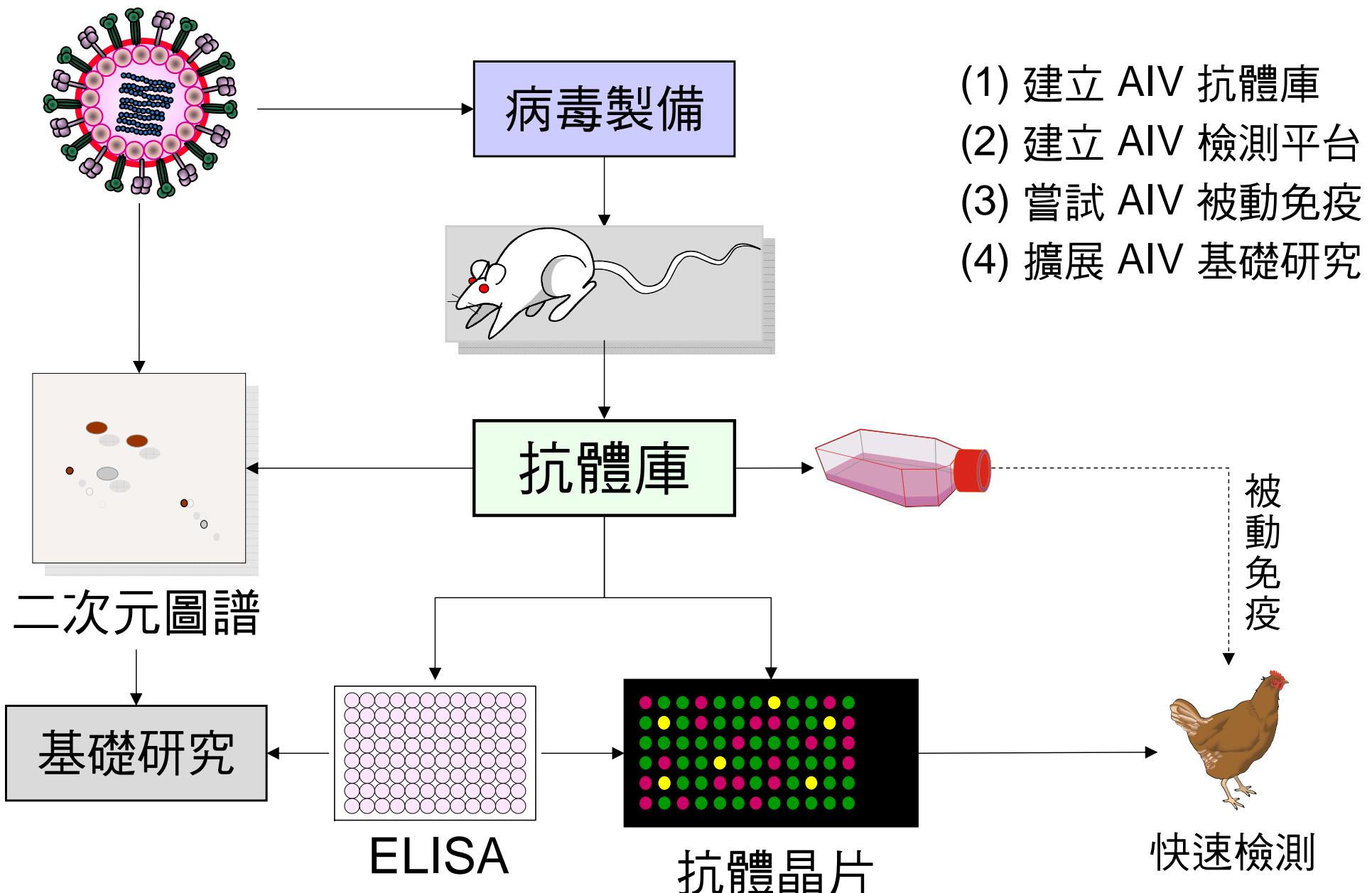
<http://www.chem.agilent.com/Scripts/Phome.asp>

Minimize protein purification and analysis in one chip

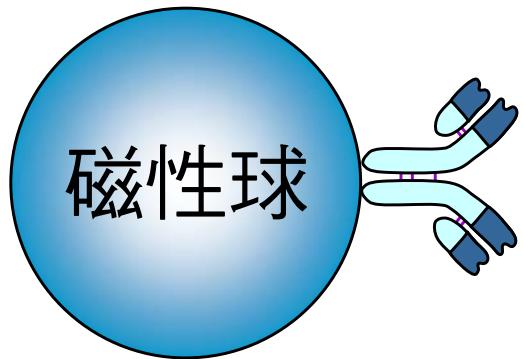


AIIV 抗體庫及應用

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



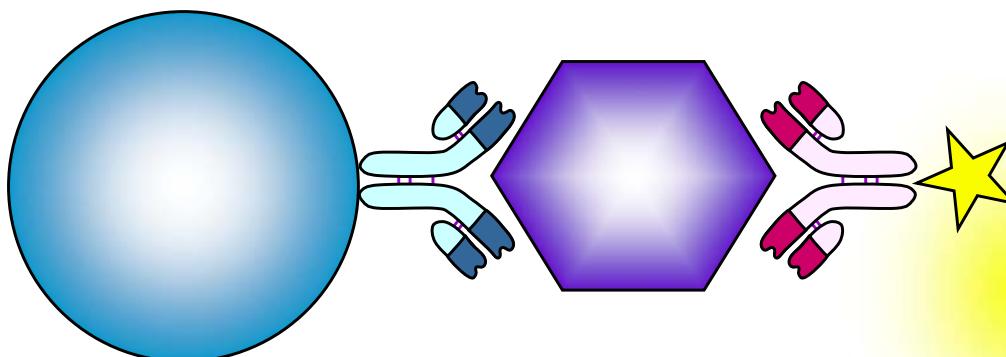
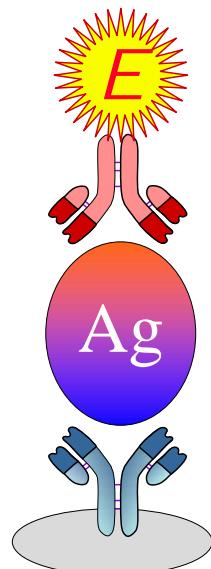
奈米磁性球檢測平台 (三明治法)



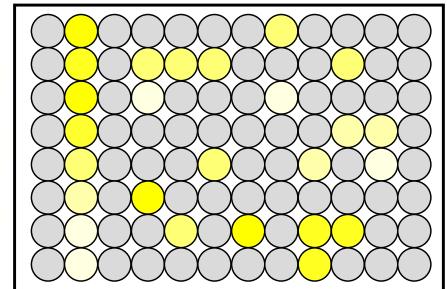
也可直接測量磁性球
與病毒結合後的改變

Incubation

抗體晶片

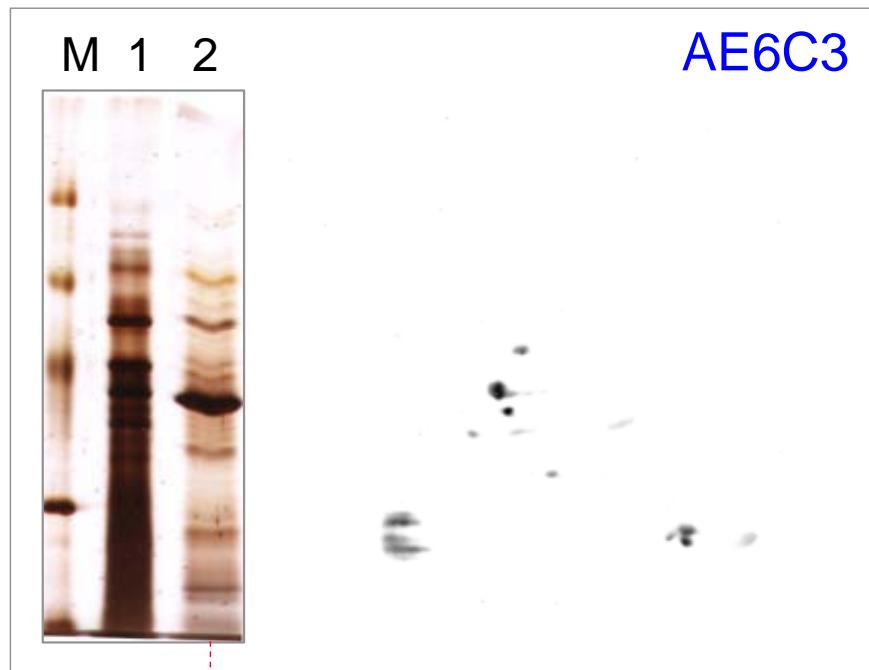


ELISA 方式

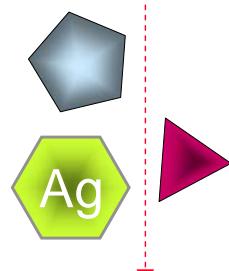
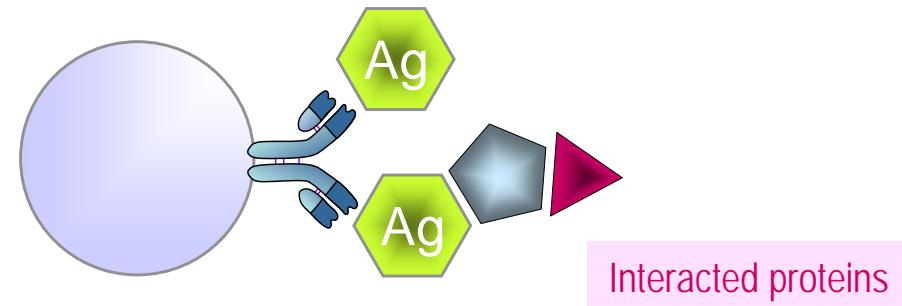


Magnetic Plate

Immunoprecipitation of interacted proteins



AE6C3



LC-MS/MS

Interacted proteins

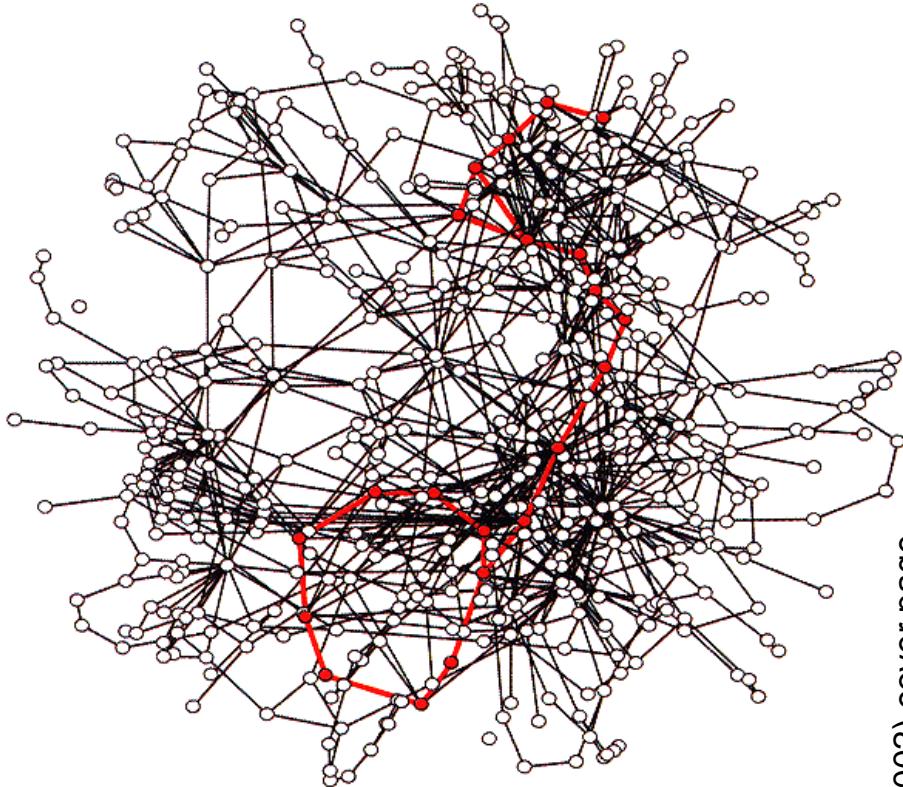
Validation!

Protein ID	Match peptide
Adenosylhomocysteinase	IVLTIIR DSAAVFAWK HSLPDGLMR LVGVSEETTGVK
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

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

