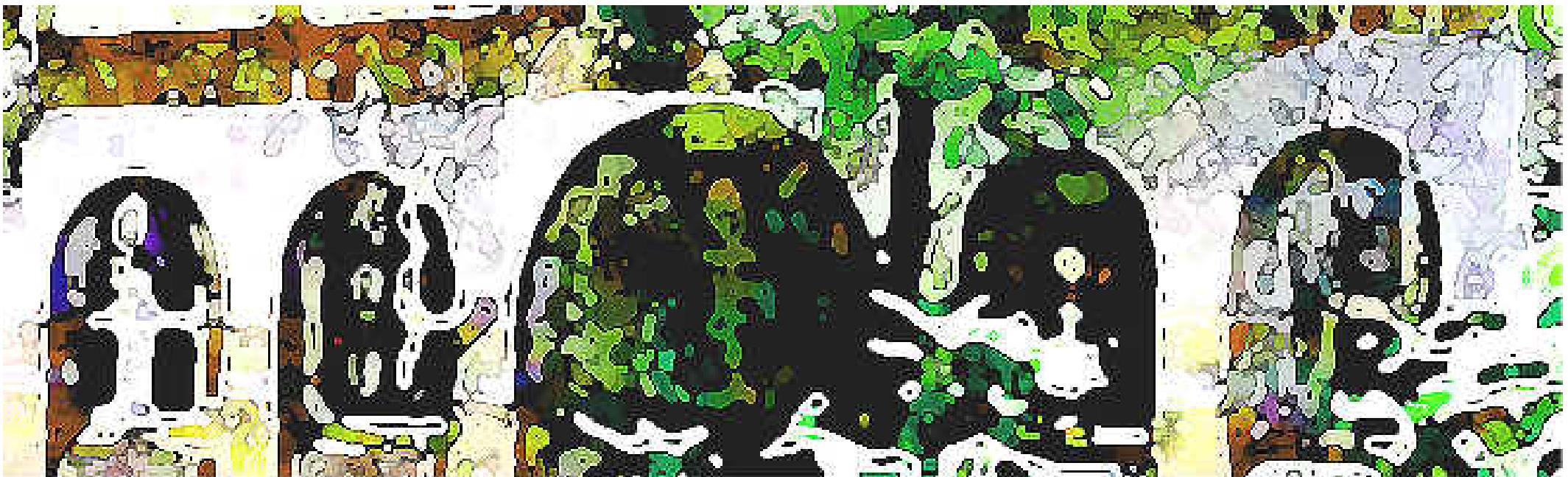


Proteomics and Antibody Bank

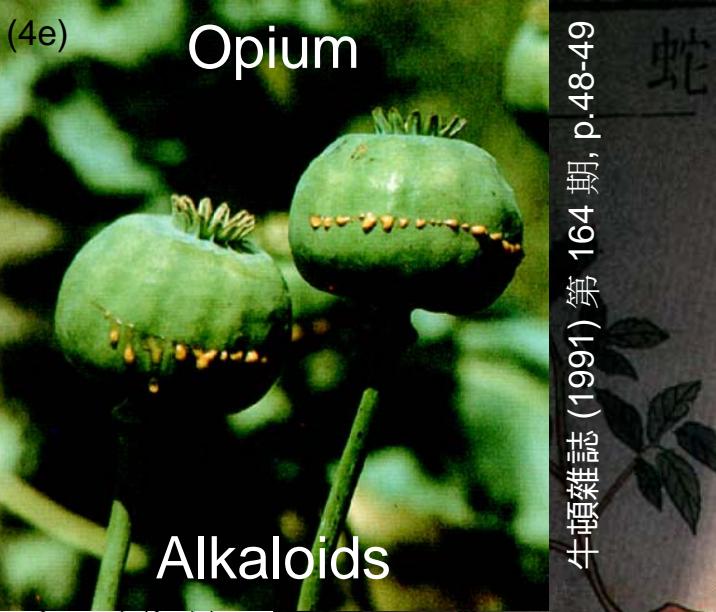
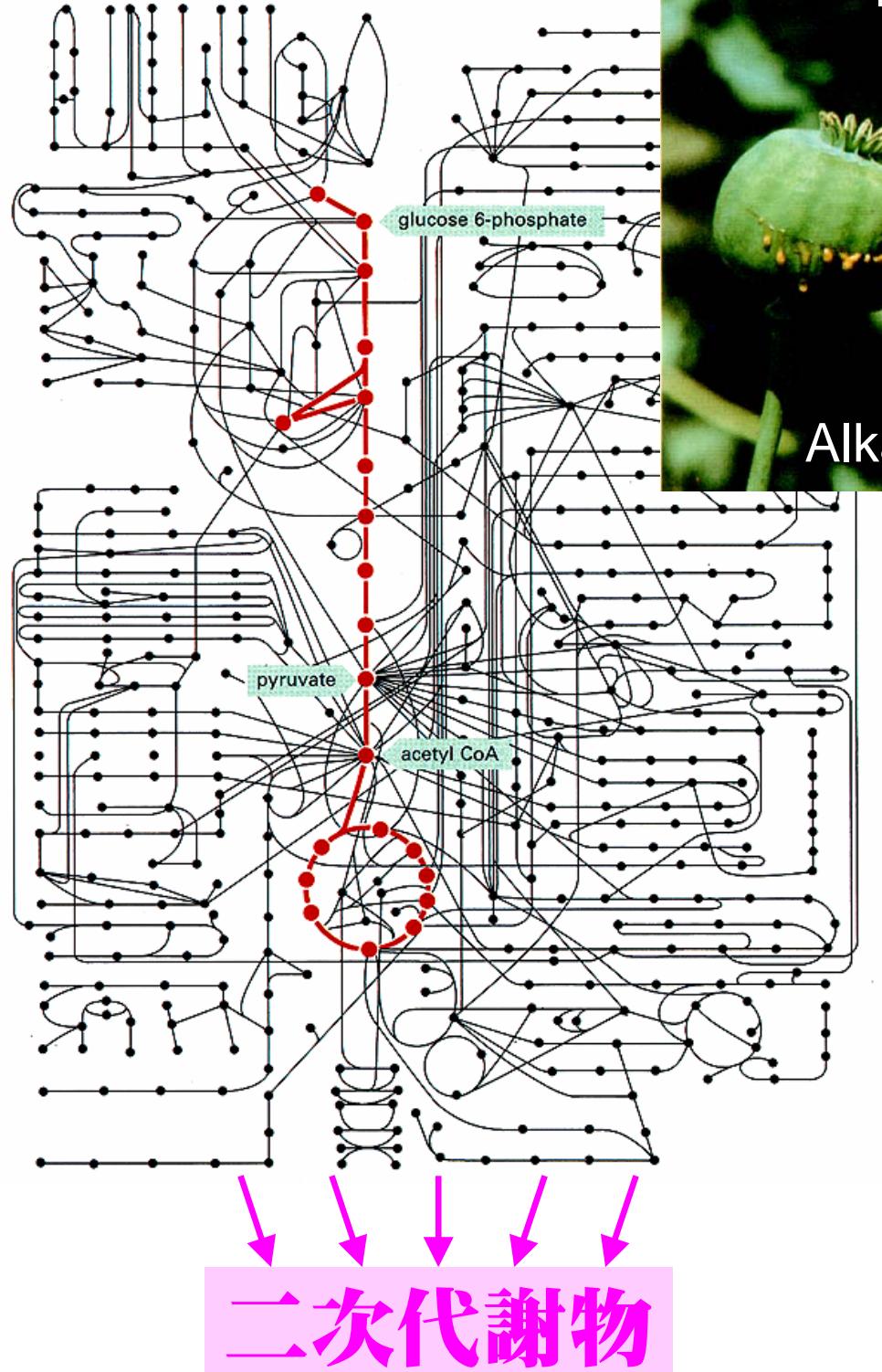
蛋白質體與抗體庫



國立台灣大學 生化科技學系

莊榮輝

Opium

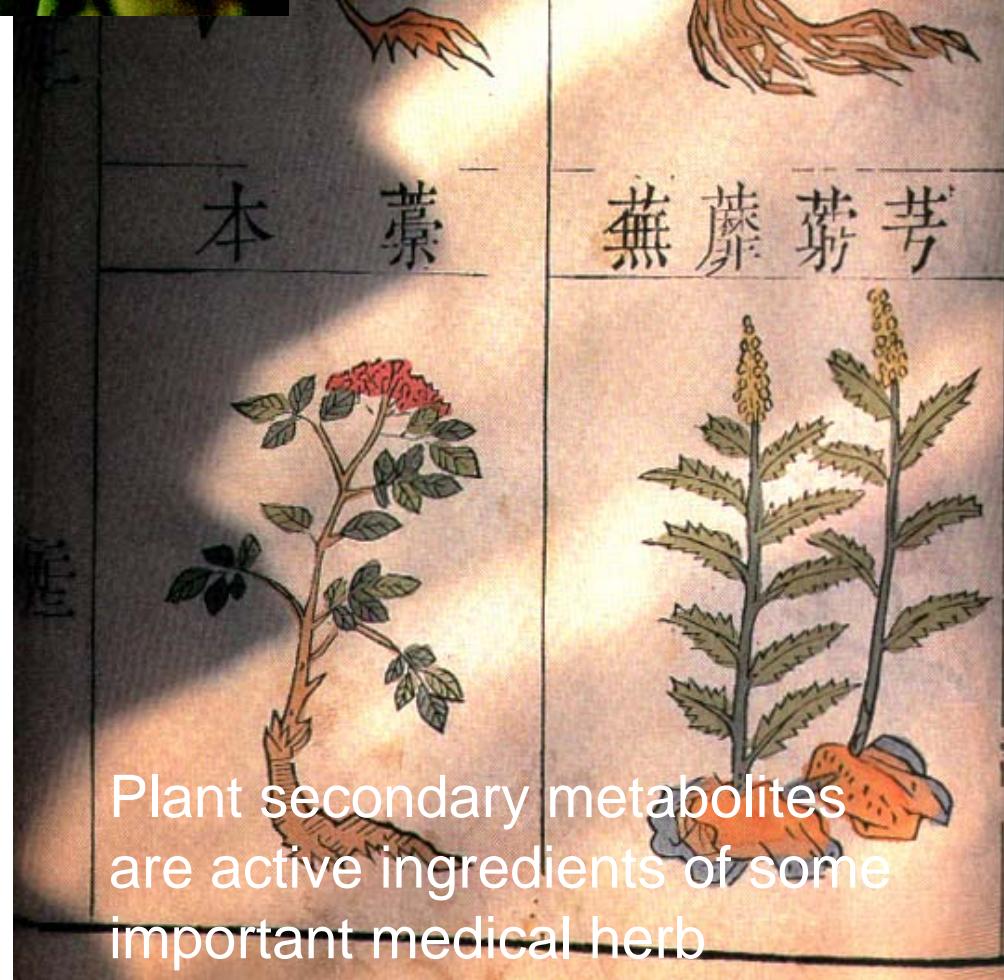


牛頓雜誌 (1991) 第 164 期, p.48-49

蛇

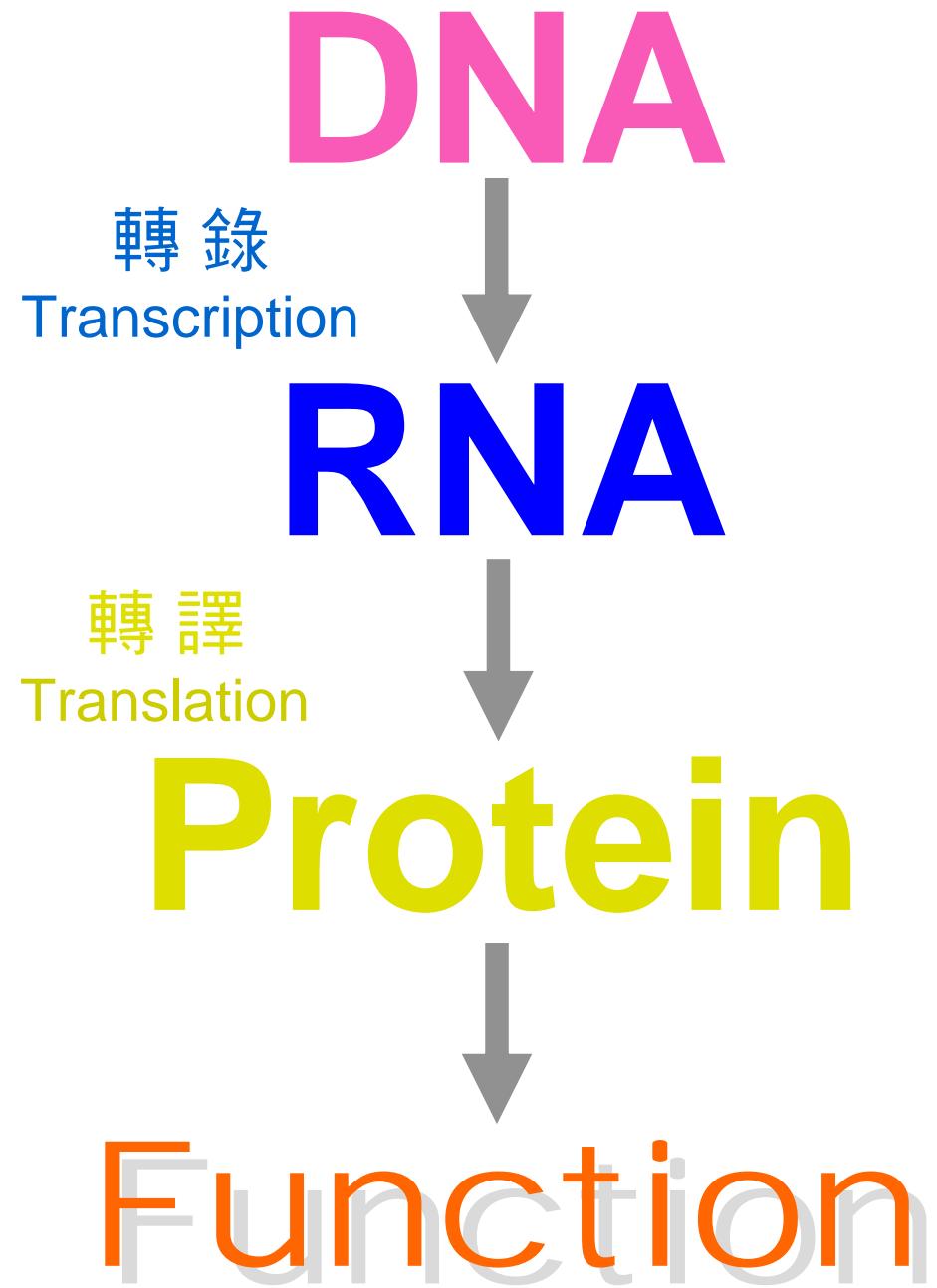
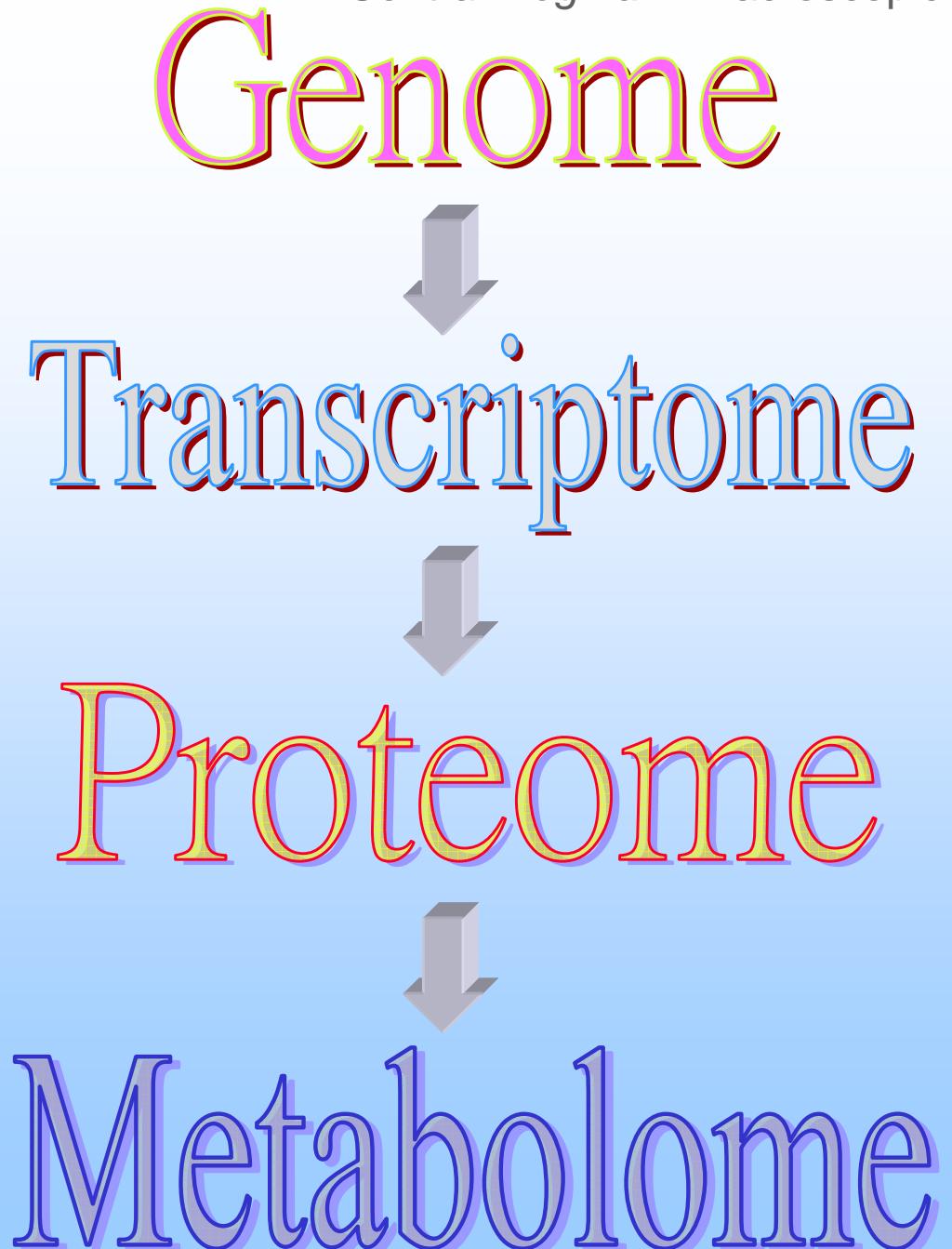


蕪 蕃 芎



Proteomics and the Proteome

Central Dogma in macroscopic



基因表現不一定完全反應在蛋白質圖譜

One Gene, One Protein?

細胞中

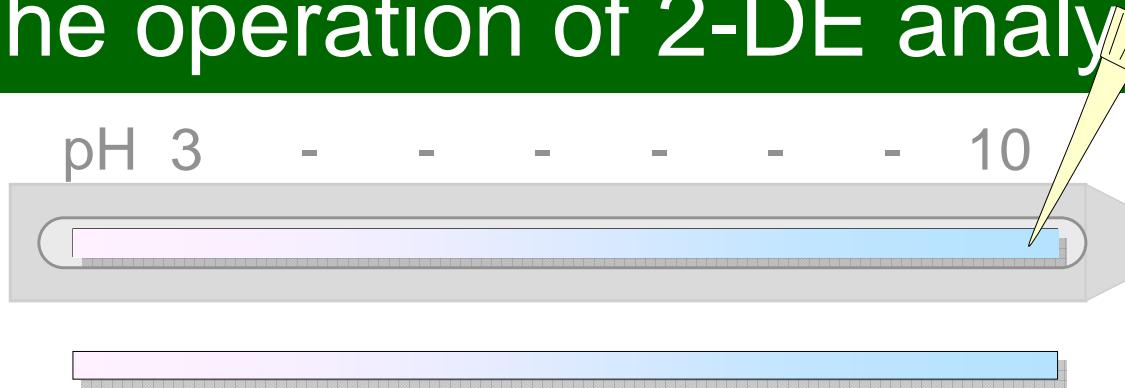
- (1) 細胞內的每個基因不一定都在表現
- (2) 各器官、組織的基因表現都不相同
- (3) 基因表現隨著生長時期而有改變
- (4) 蛋白質表現後有進一步的修飾與調控
- (5) 蛋白質在細胞內的代謝速率不同

試管中

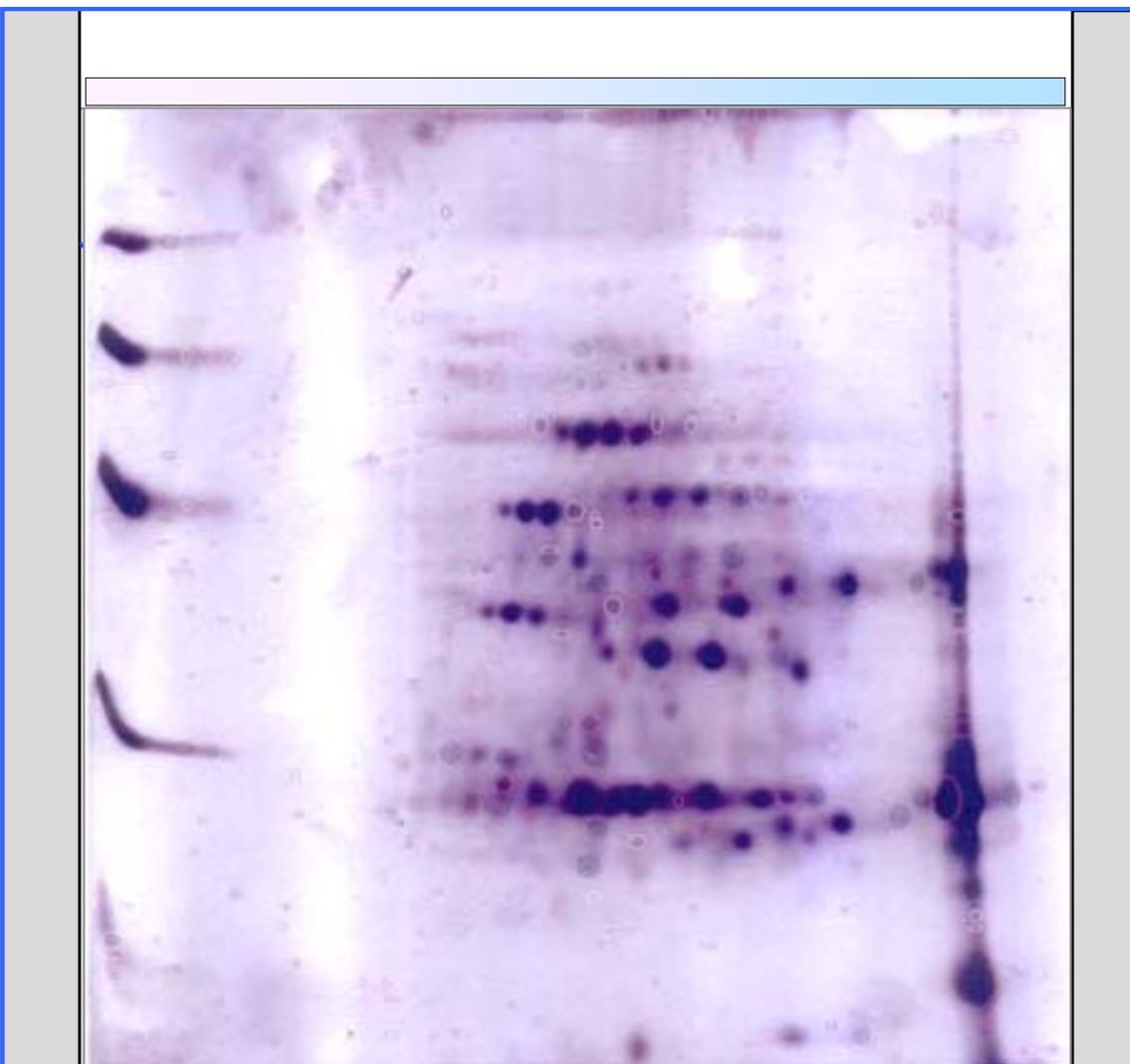
- (1) 蛋白質的水溶性會影響抽取效率
- (2) 蛋白質的含量差異很大
- (3) 蛋白質在抽取後的安定性與半衰期不同

The operation of 2-DE analysis

(1) IEF
等電焦集電泳

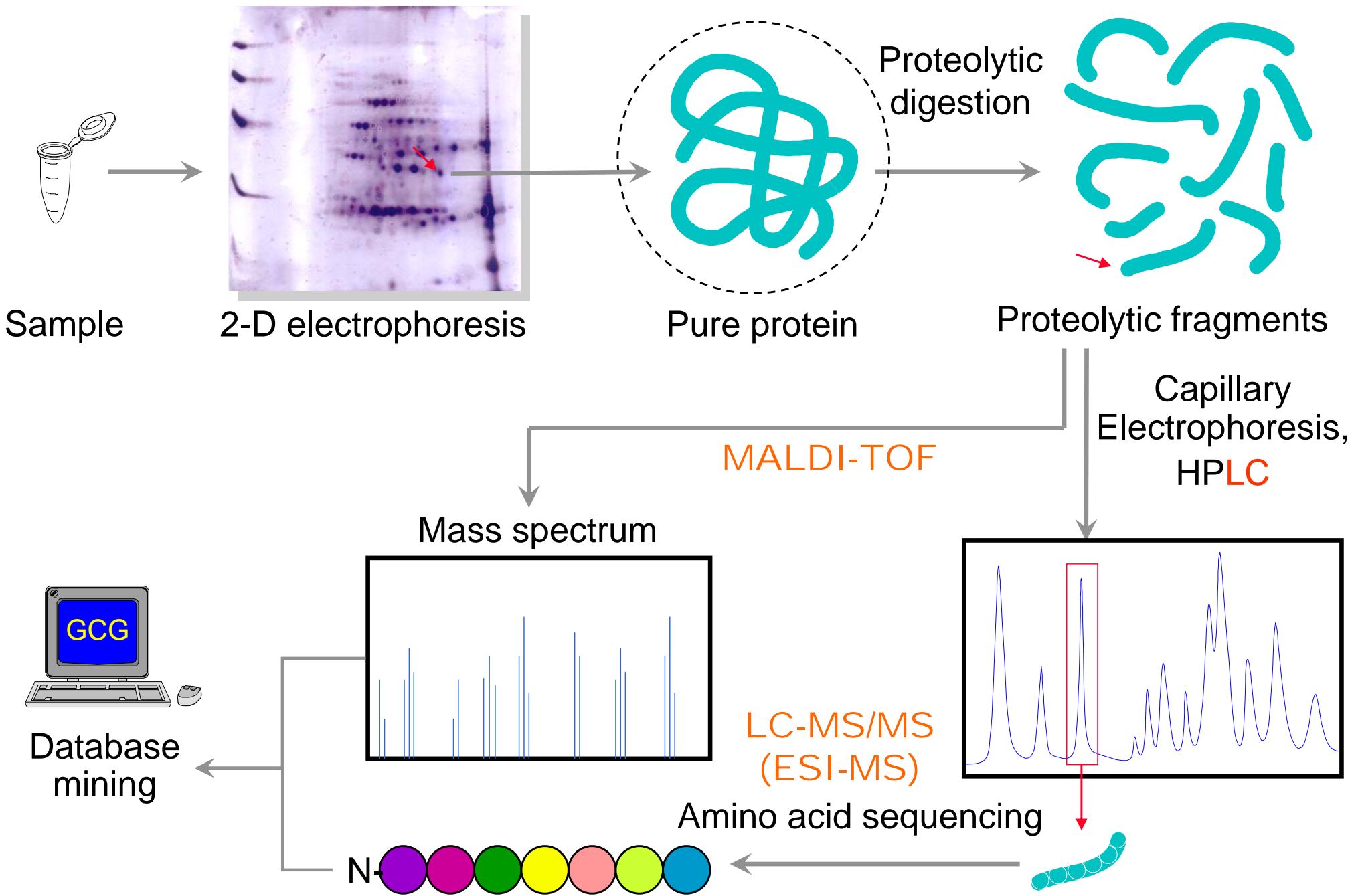


(2)
SDS-PAGE
分離膠體



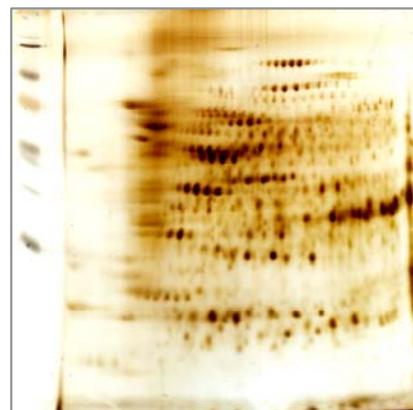
(3) Staining
染色脫色

Quick identification of an unknown protein

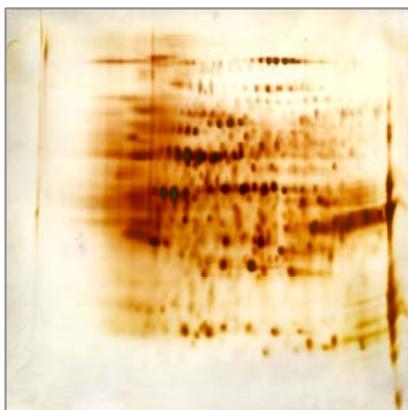


Proteomic pattern changes during growth

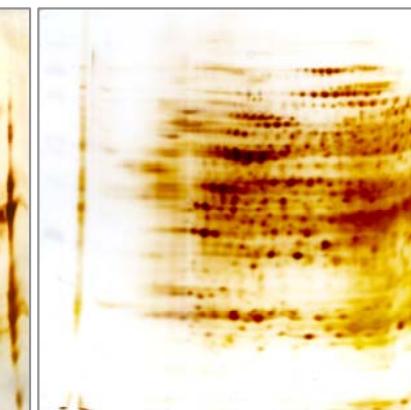
0 cm



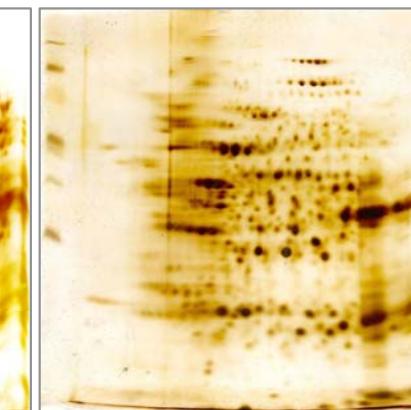
10 cm



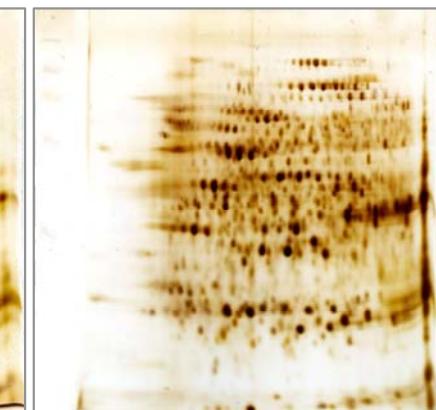
20 cm



40 cm



60 cm



綠竹筍



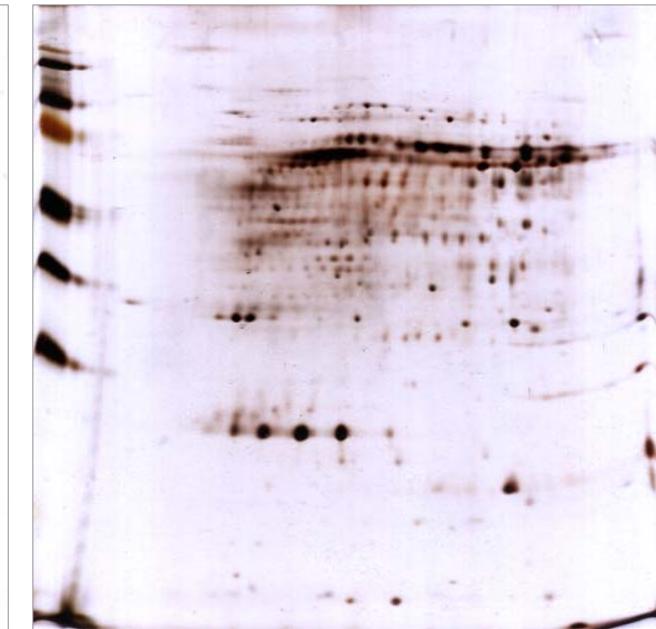
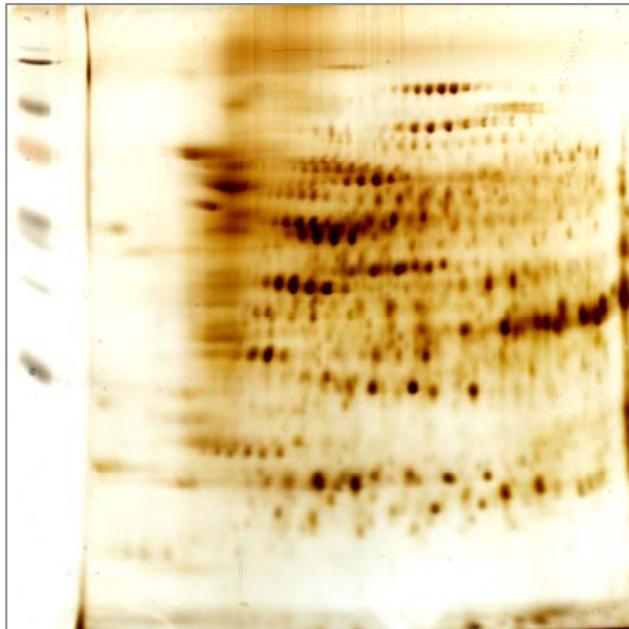
Cellulose synthesis

Juang RH (2006) Proteomics (Wu YJ)

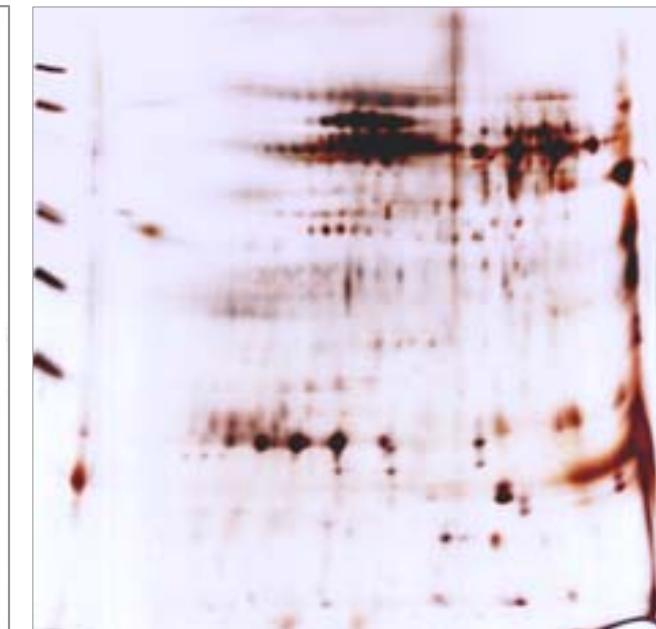
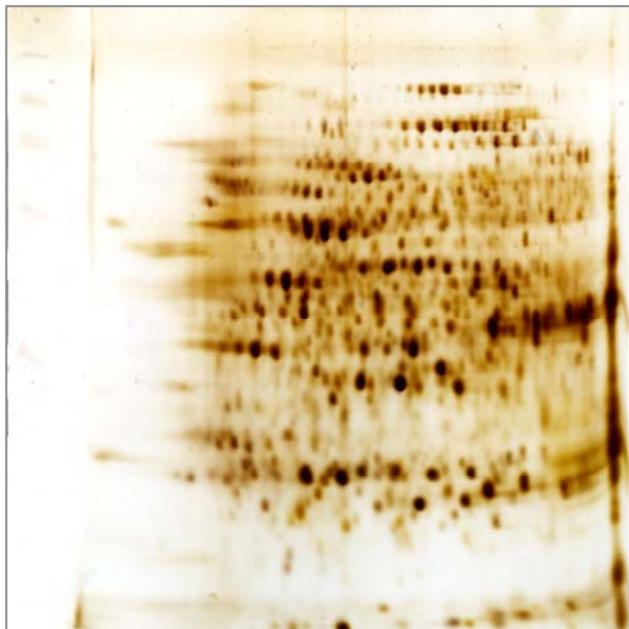
	Protein ID	Accession no.	Calculated Mr (kD) / pI	Sequence coverage (%)	Score (MASCOT)	Match
79	Sucrose synthase	AAV64256 (<i>Bambusa oldhamii</i>)	92.8 / 6.03	35	402	14
80	Sucrose synthase	AAV64256 (<i>Bambusa oldhamii</i>)	92.8 / 6.03	35	245	7
82	Sucrose synthase	AAV64256 (<i>Bambusa oldhamii</i>)	92.8 / 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

Total proteins extracted by different methods

Underground shoot



60-cm shoot

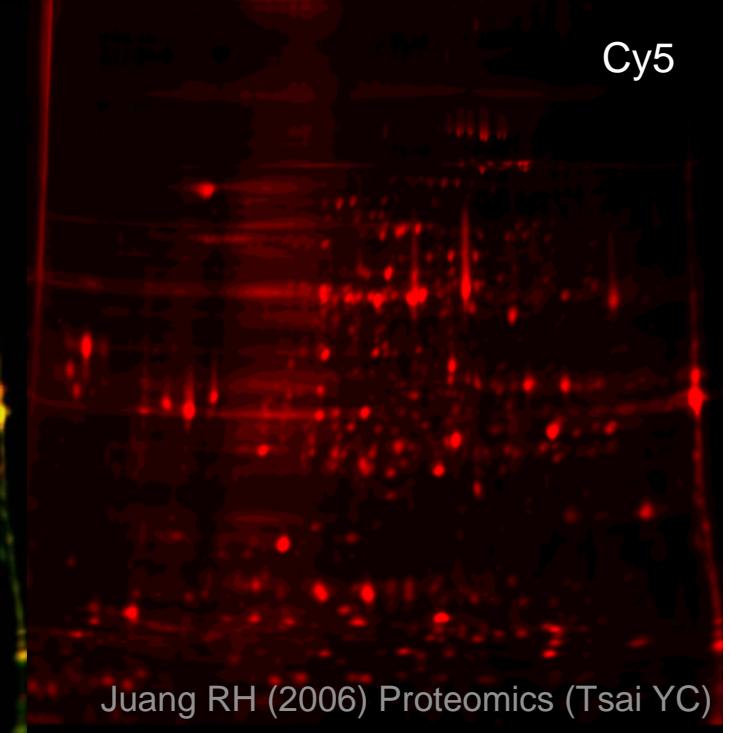
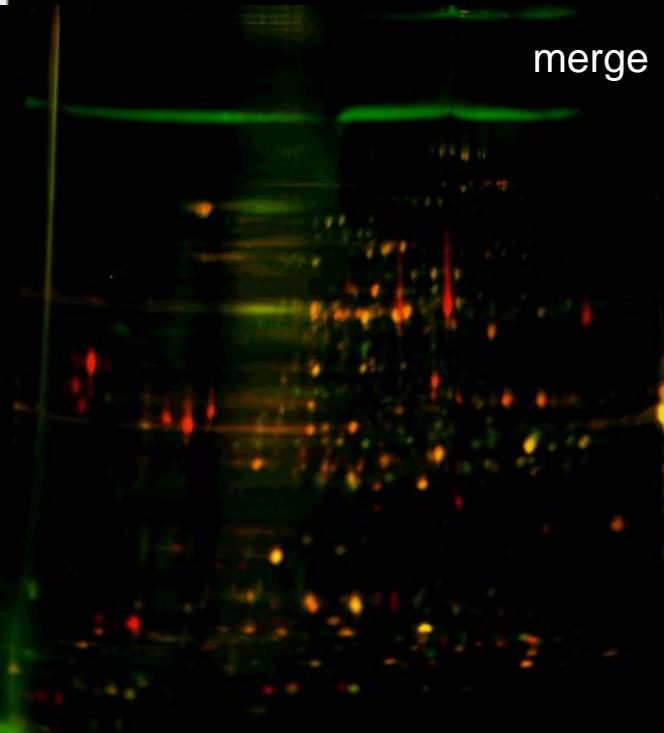
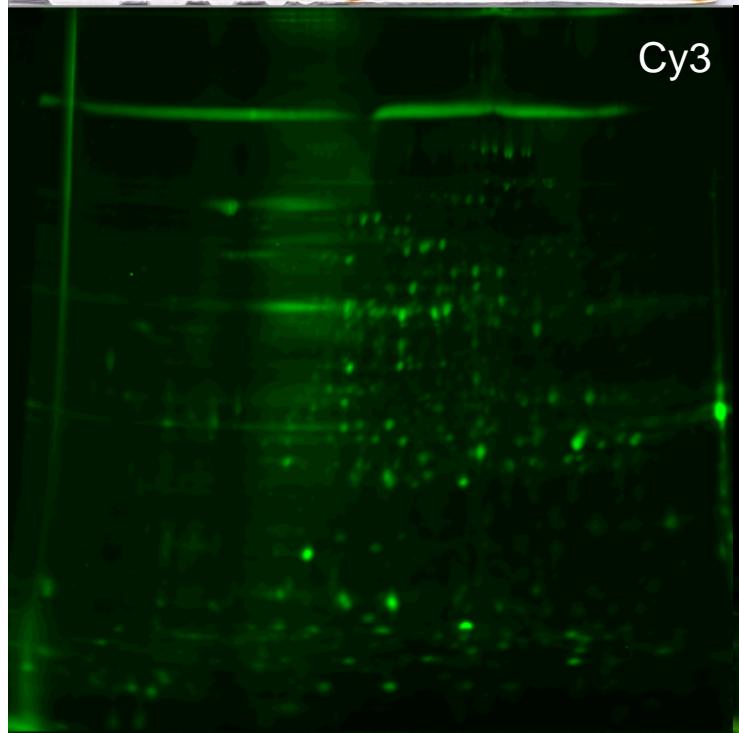
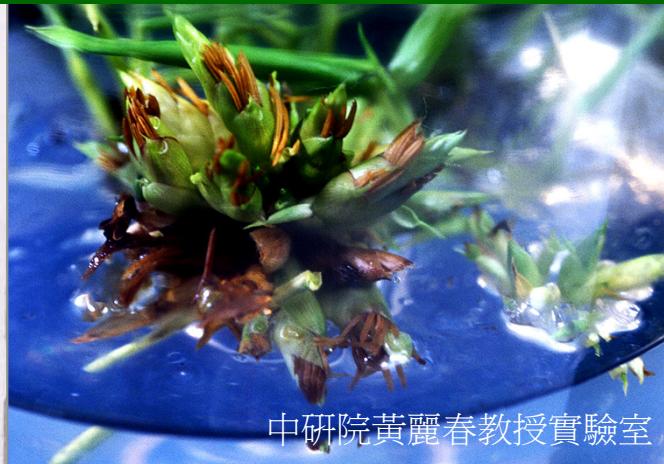
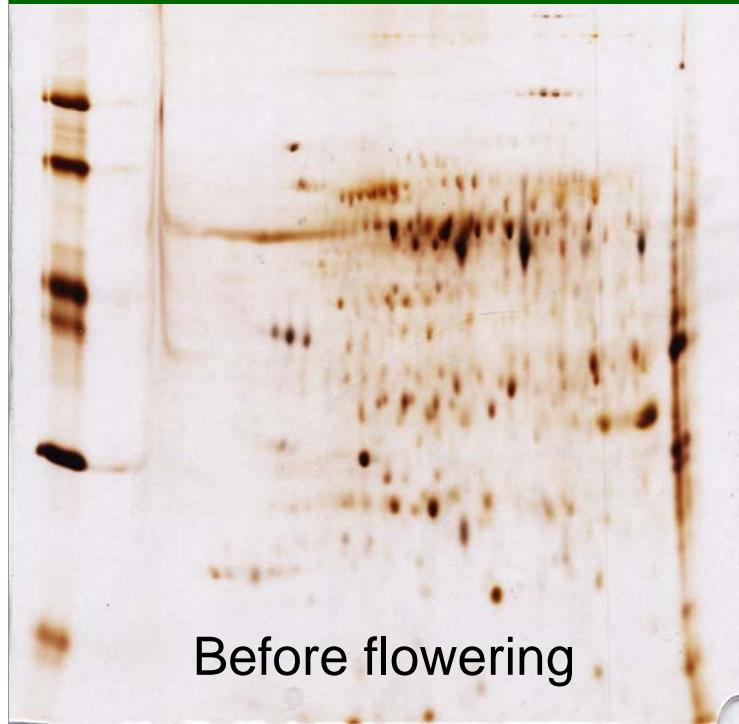


Water soluble proteins

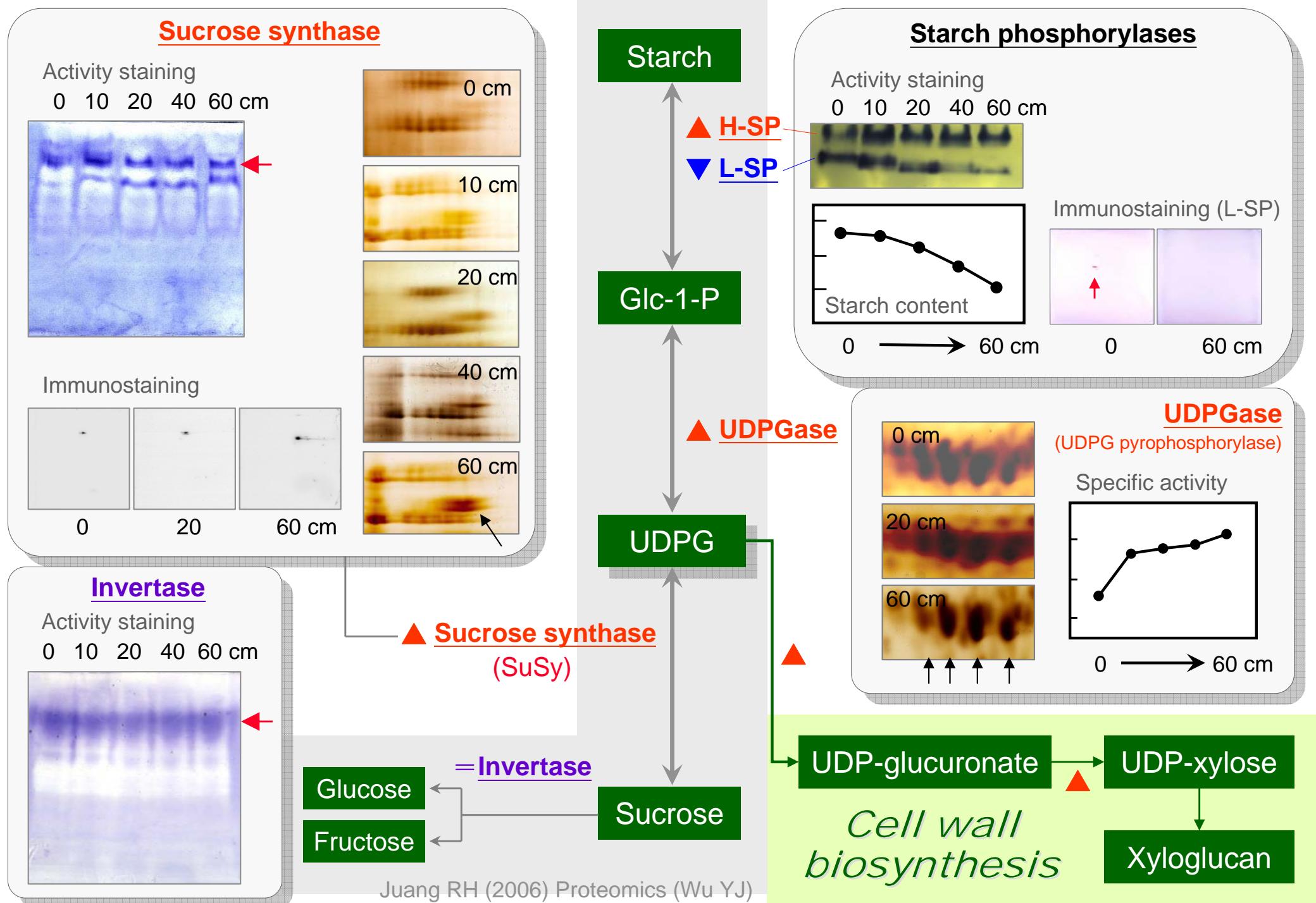
Non-polar proteins

Glycoproteins

Proteomic change during bamboo flowering

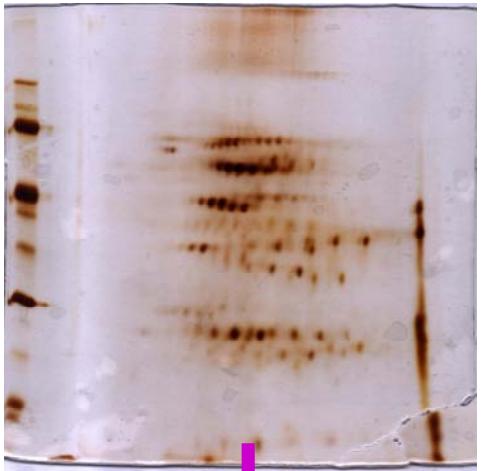


Connect 2-DE results to metabolic pathway

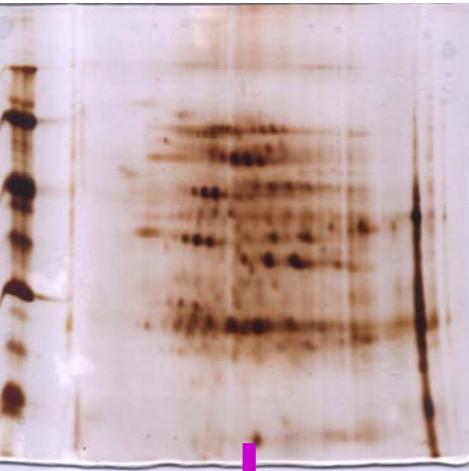


抗體應用在蛋白質體檢定的強大效果

未出土竹筍

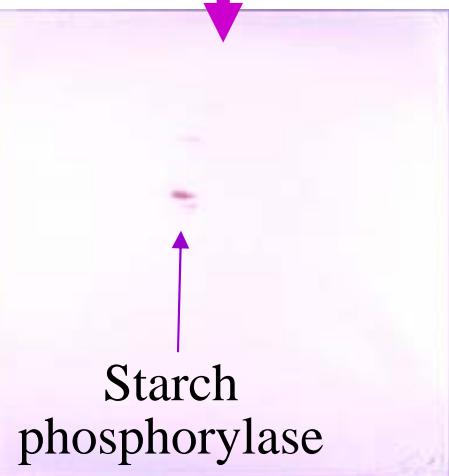


出土 60 cm 竹筍



硝酸銀染色

免疫染色



首次提出
高產能抗體製備計畫構想

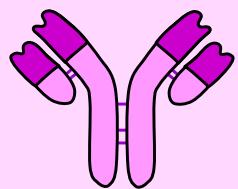
(1) 建立高產能抗體製備
之 標準流程

(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



單株抗體

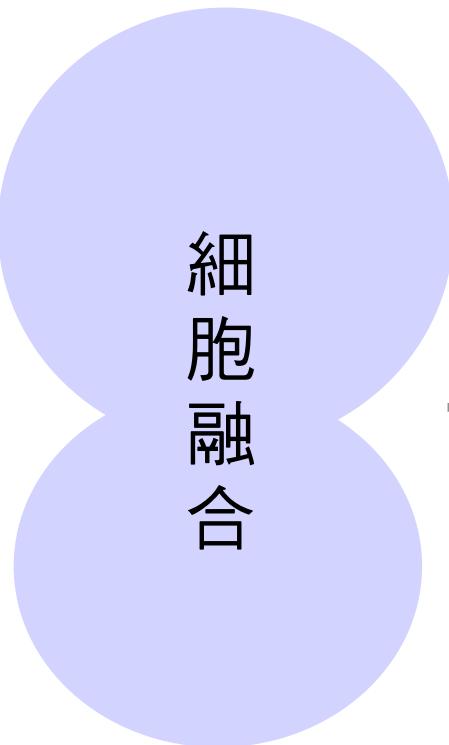
可生產有用抗體的 淋巴細胞 若與 癌細胞 融合，則形成穩定而可培養的細胞株。



都是白血球



一個 B cell 只
產生一種抗體

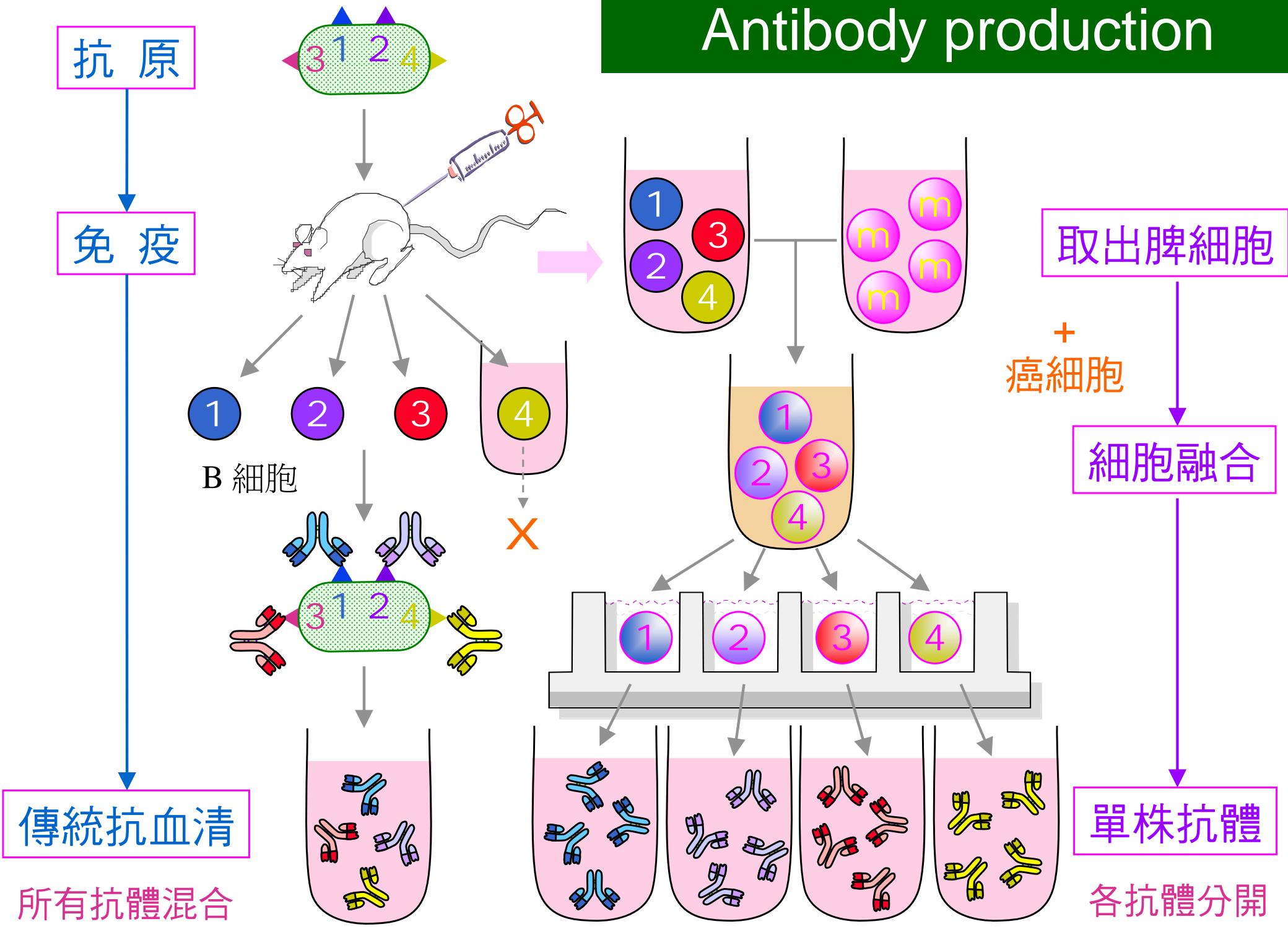


兩組染色体混在一起



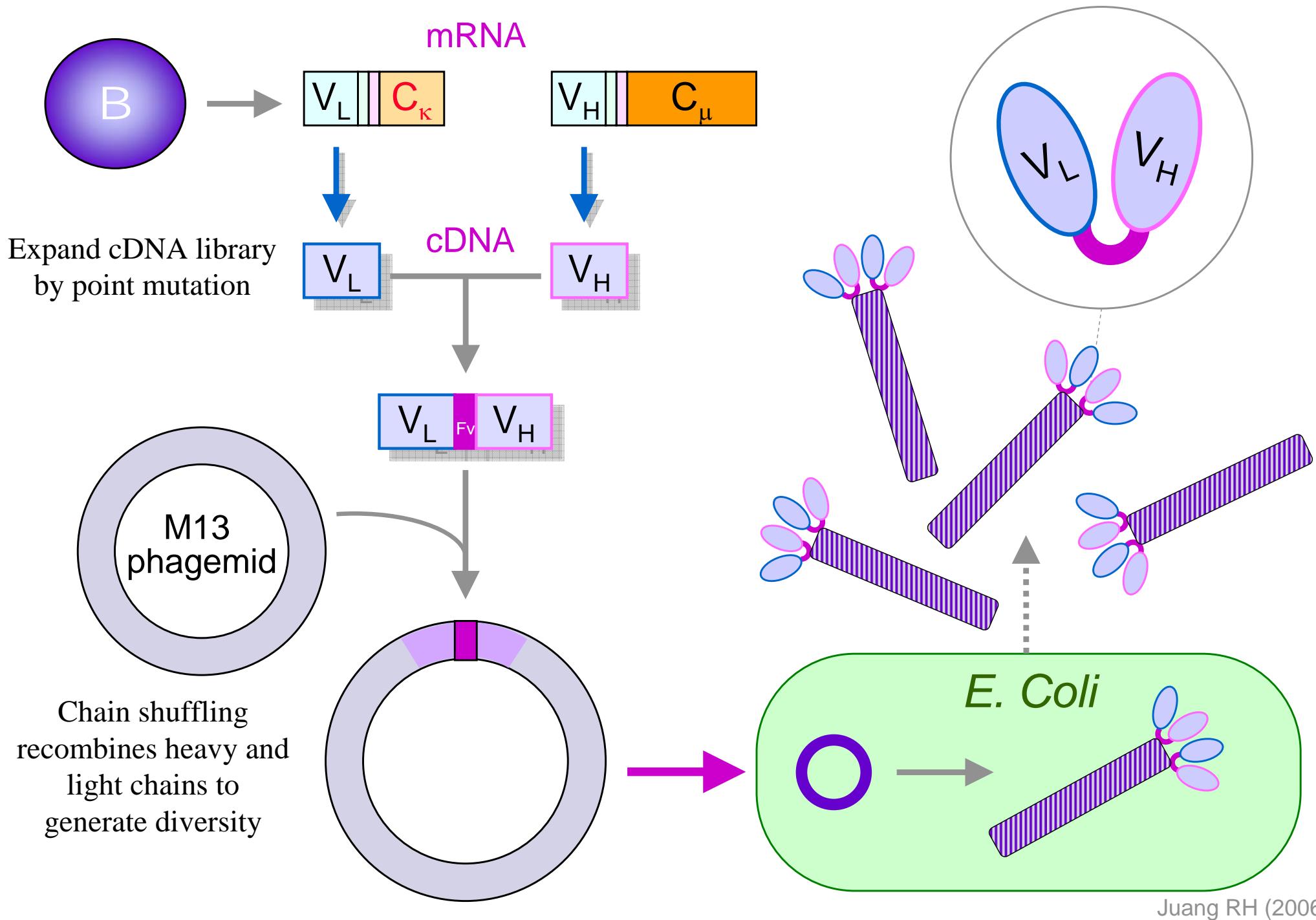
也可以培養生長
產生專一性抗體

Antibody production

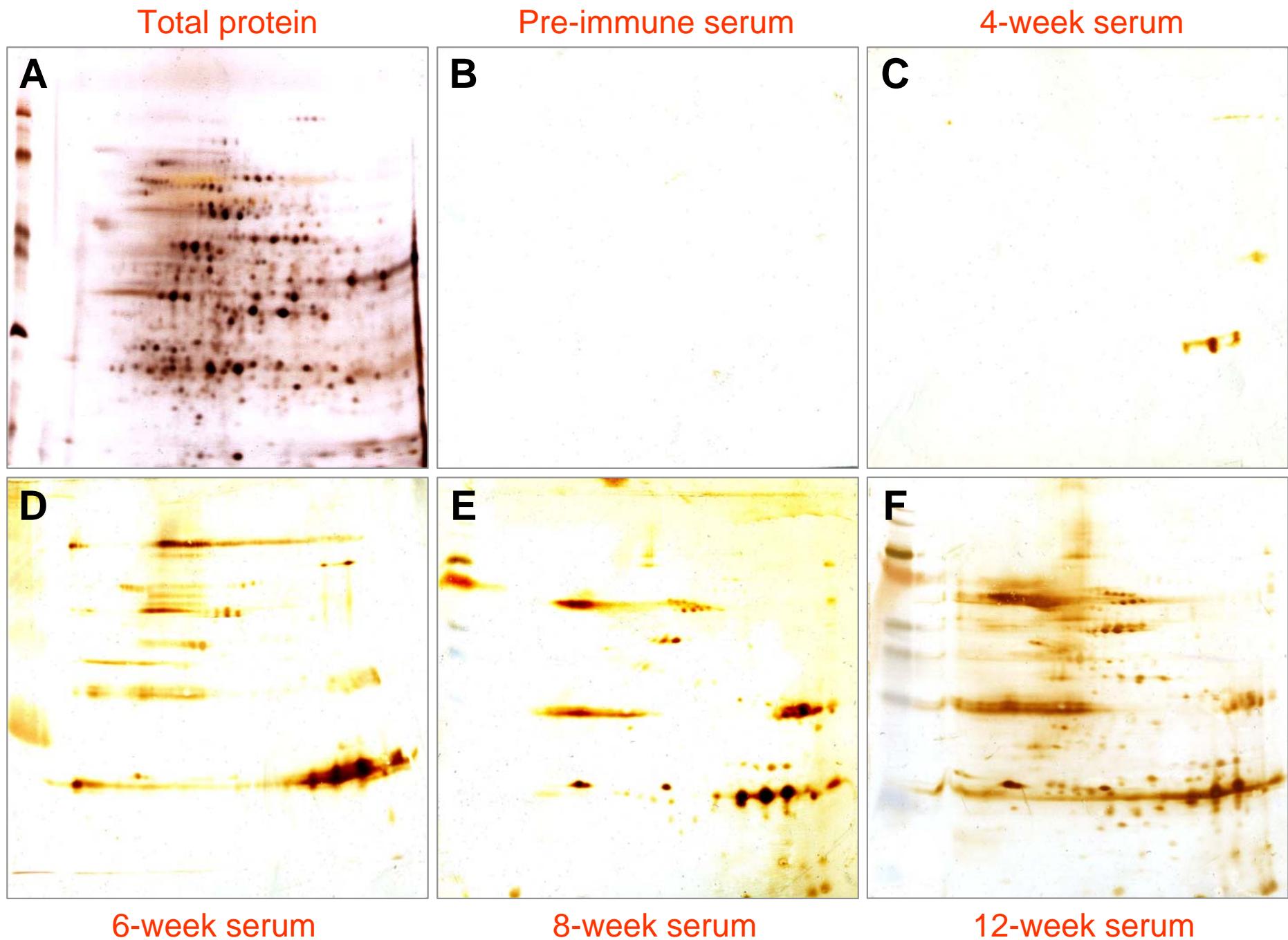


Adapted from Milstein (1980) *Scientific American*, Oct. p.58

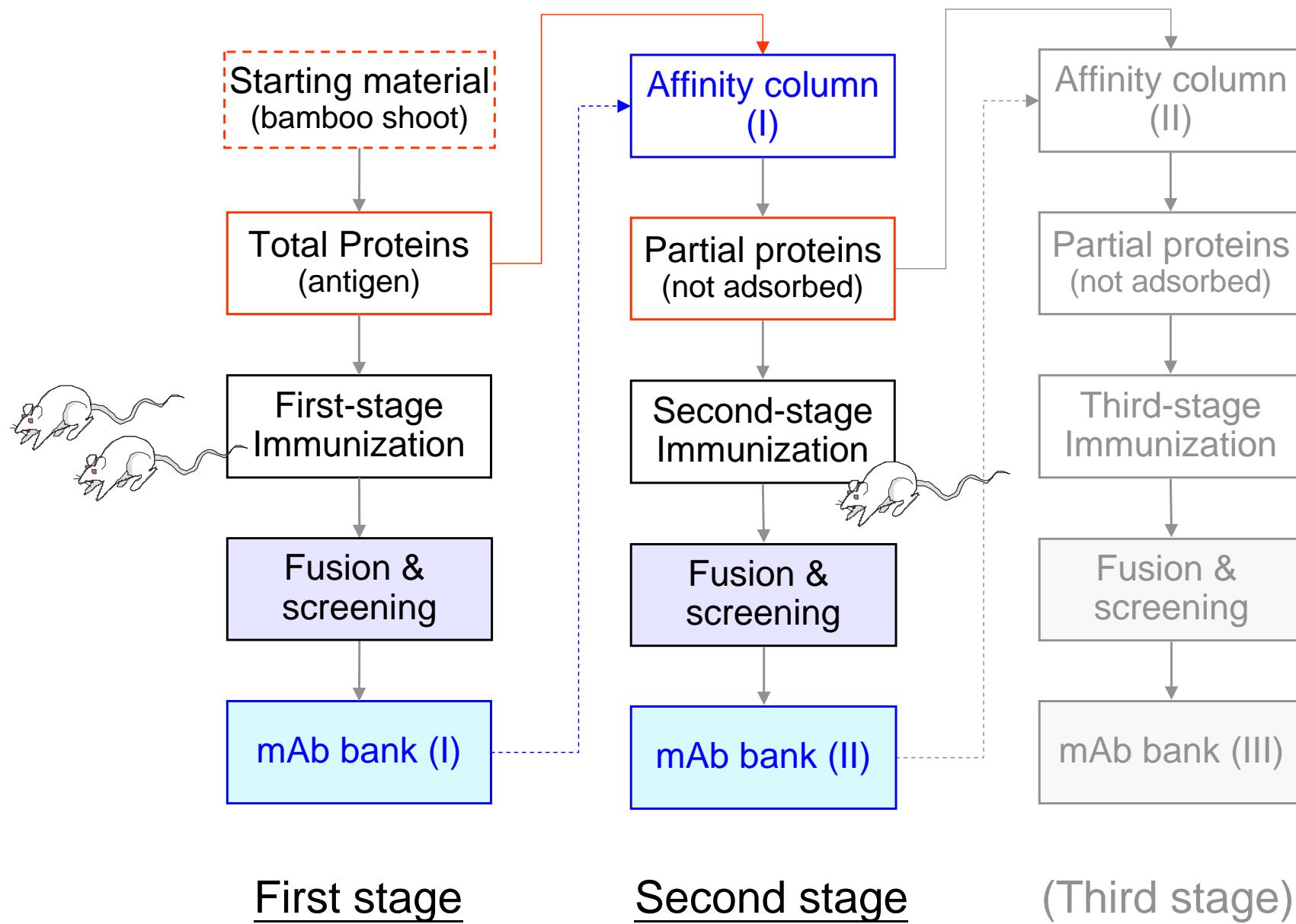
Phage display generates binding diversity



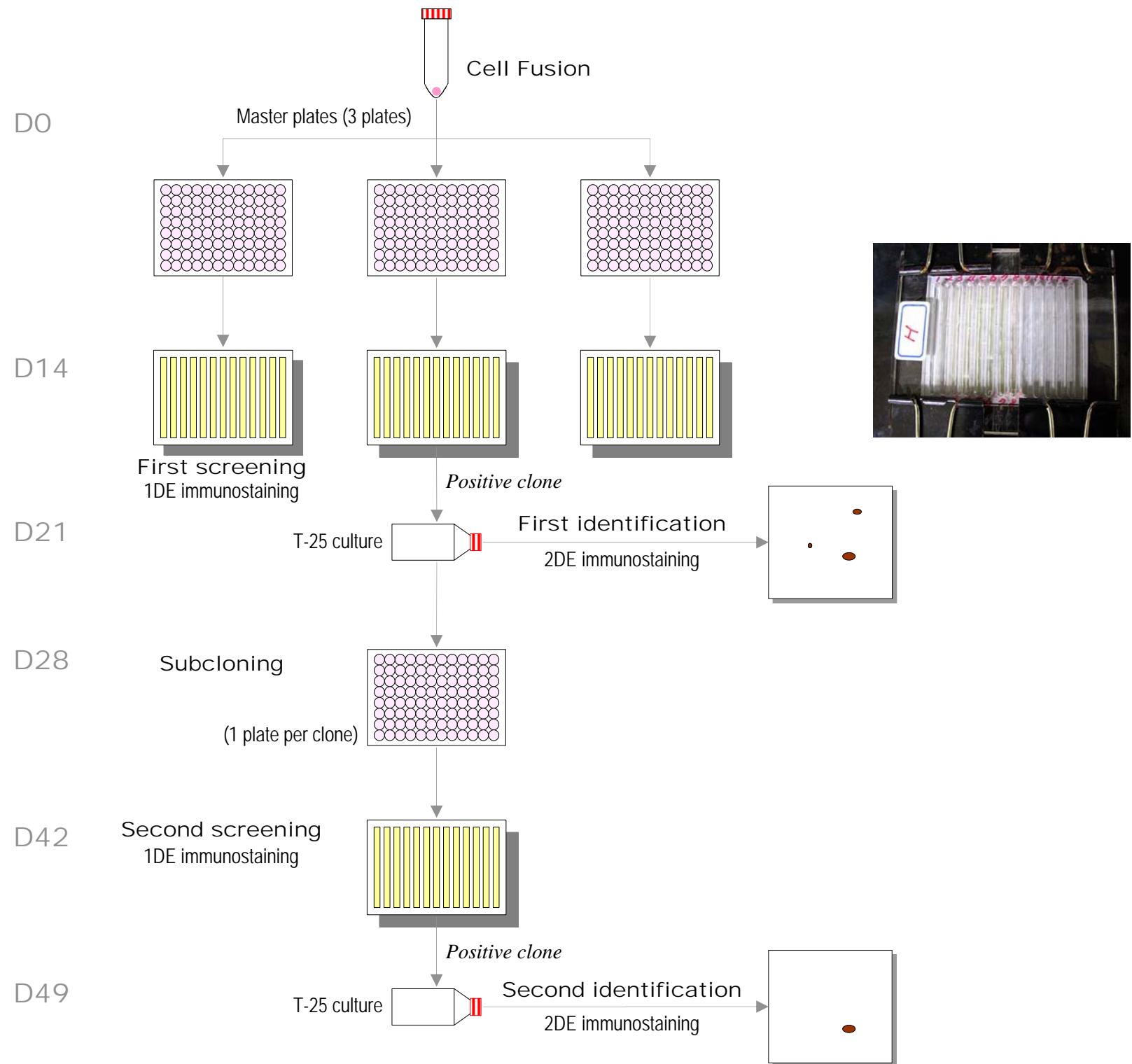
The immune response for complex antigen



Stage-wise immunization and cell fusion protocol



Screening and identification procedures



Summary for the mAb bank production

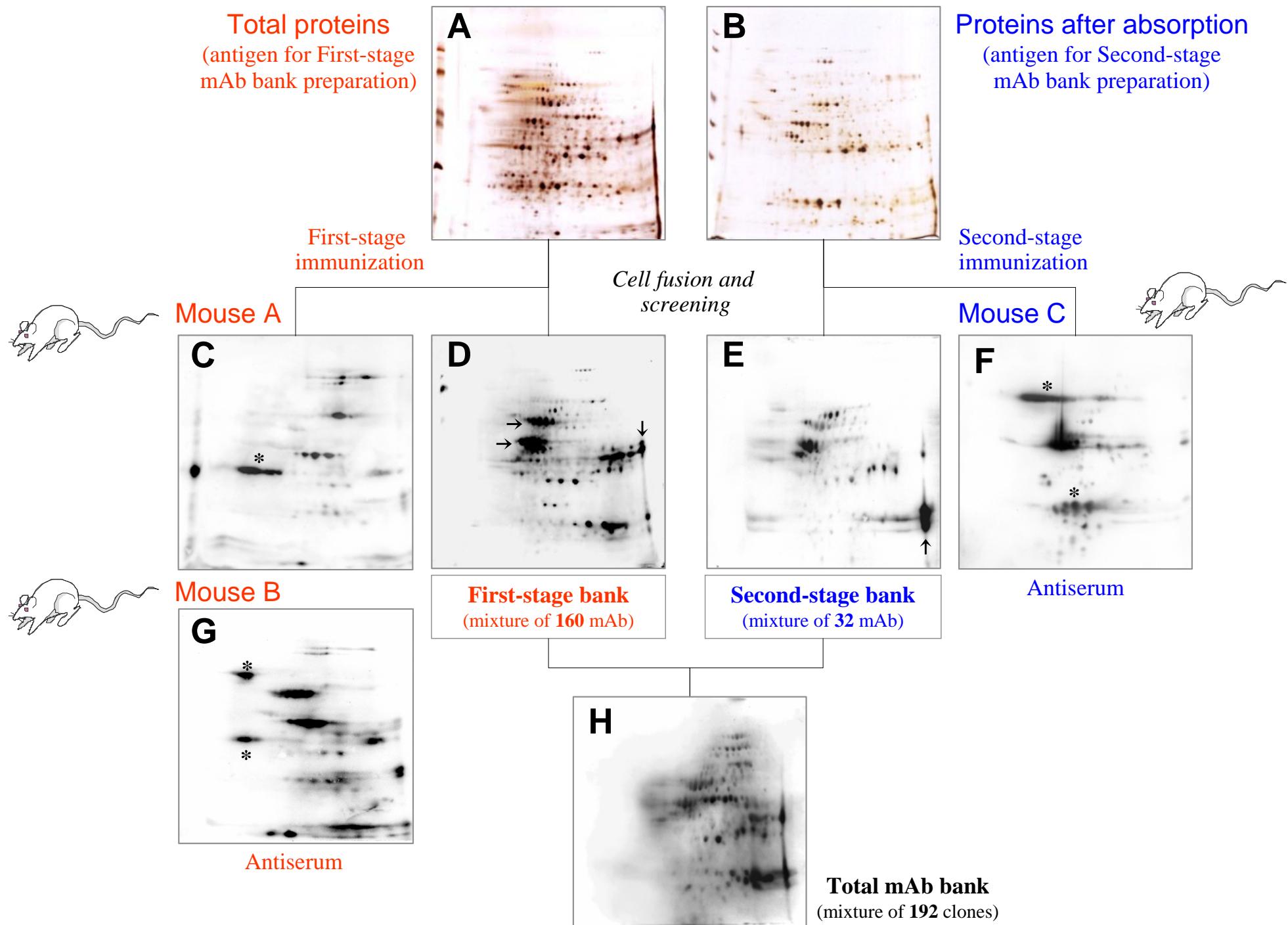
(A) First-stage

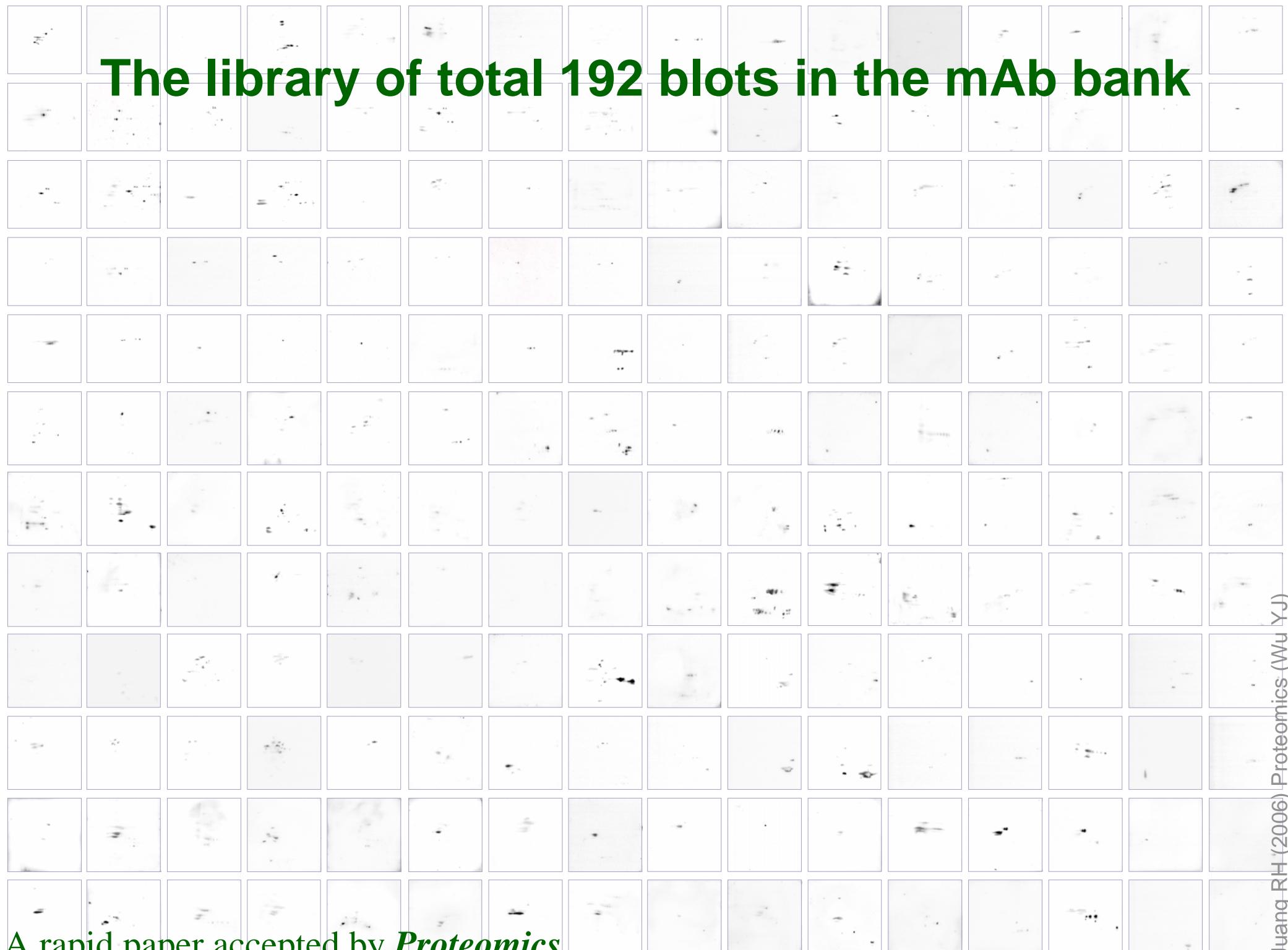
Spleen cell fused with	NS0/1 (Mouse A)		Sp2/0 (Mouse B)	
Number of clones	screened	positive	screened	positive
First screening	150	100	180	120
Second screening (after subcloning)	320	150	500	250
Final monoclonal		78		82

(B) Second-stage

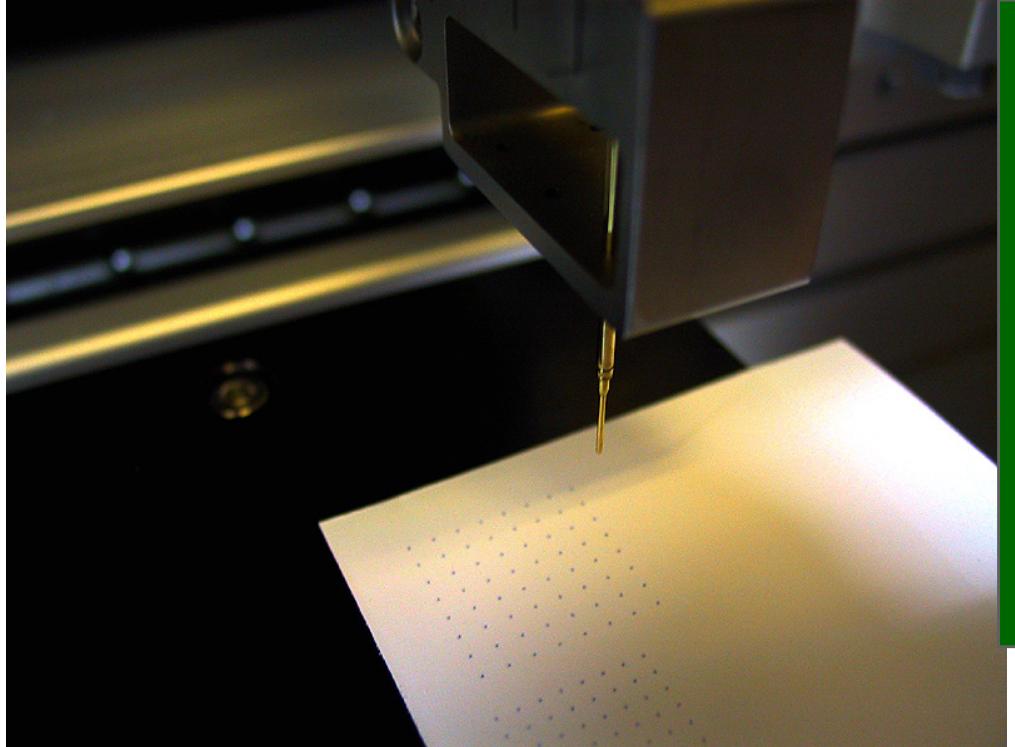
Spleen cell fused with	Sp2/0 (Mouse C)	
Number of clones	screened	positive
First screening	100	40
Second screening (after subcloning)	400	120
Final monoclonal		32

The immune response vs the mAb production

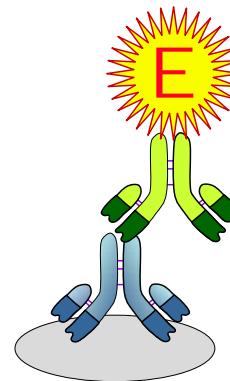
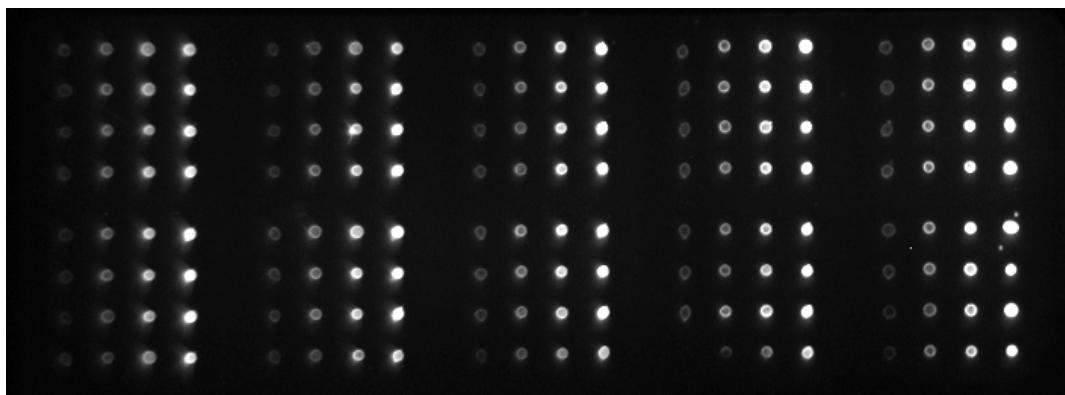
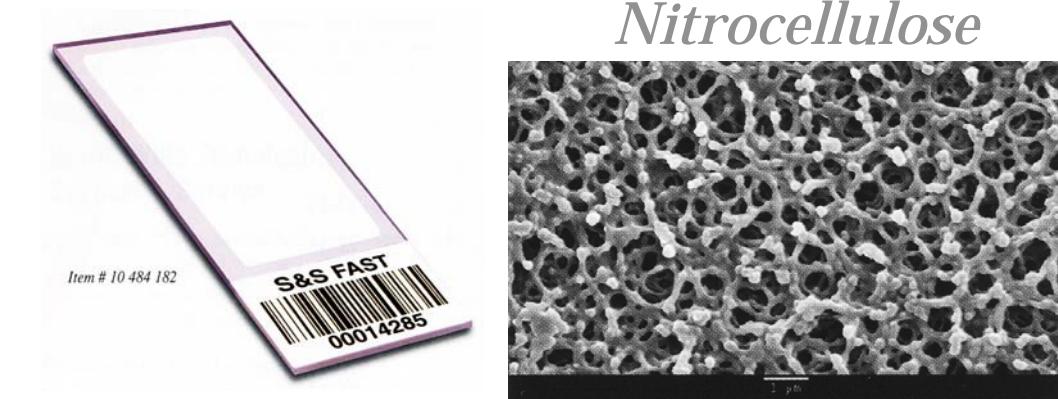
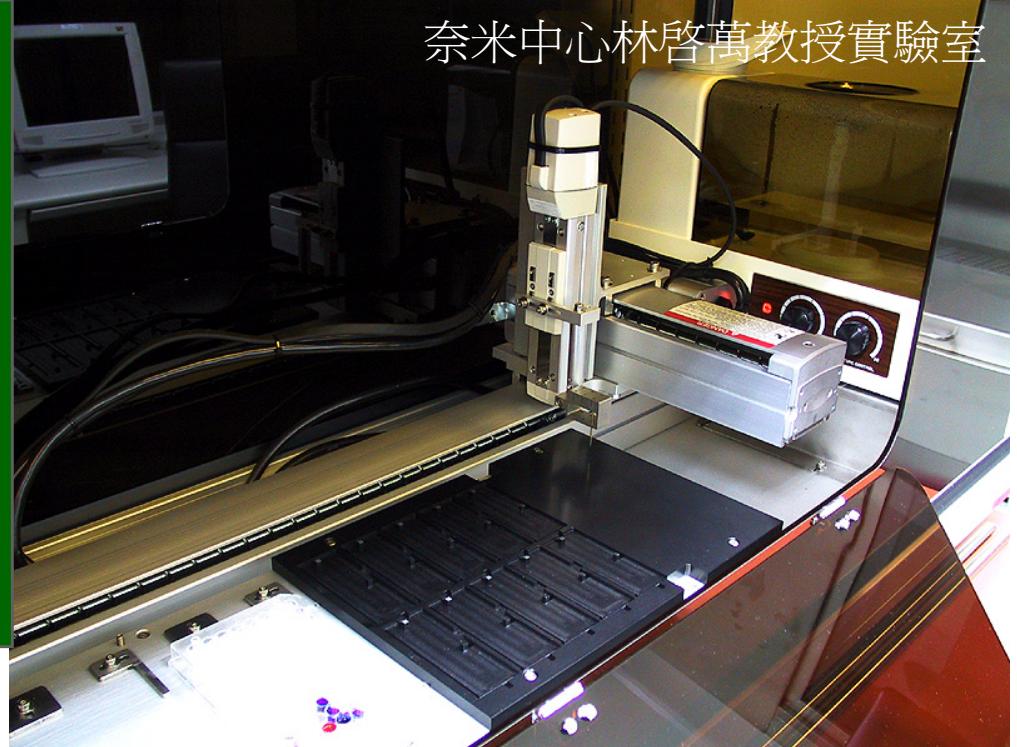




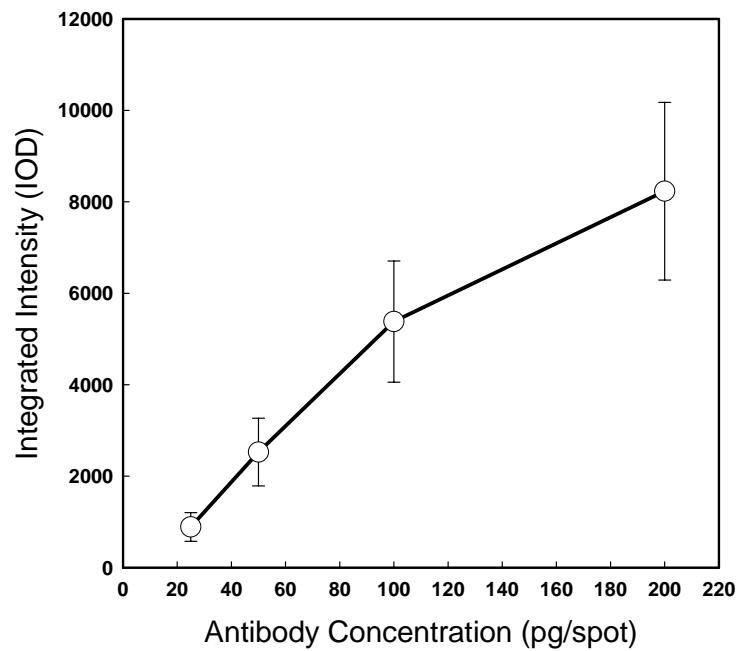
The library of total 192 blots in the mAb bank



蛋白質晶片試製



Schleicher & Schuell

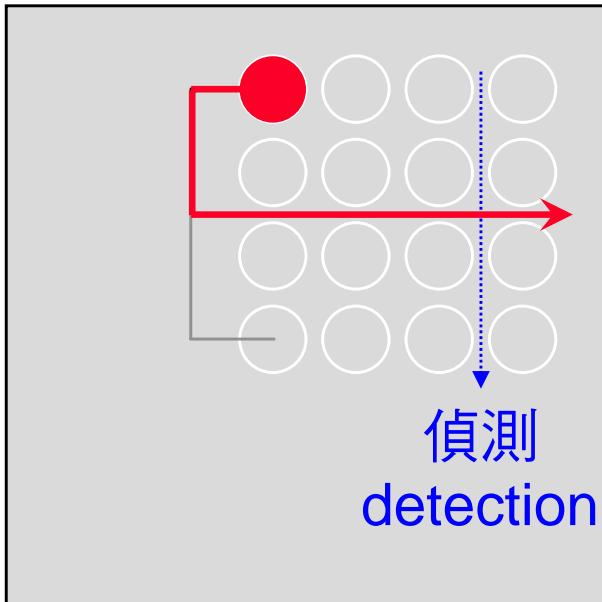


Microfluidics and Lab-on-a-chip applications

Agilent 所有蛋白質純化與活性分析均予微小化

Agilent 2100 bioanalyzer

樣本 sample well



毛細管電泳
Capillary electrophoresis



Agilent HPLC-Chip/MS

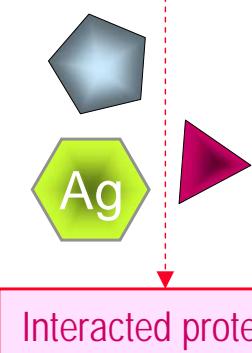
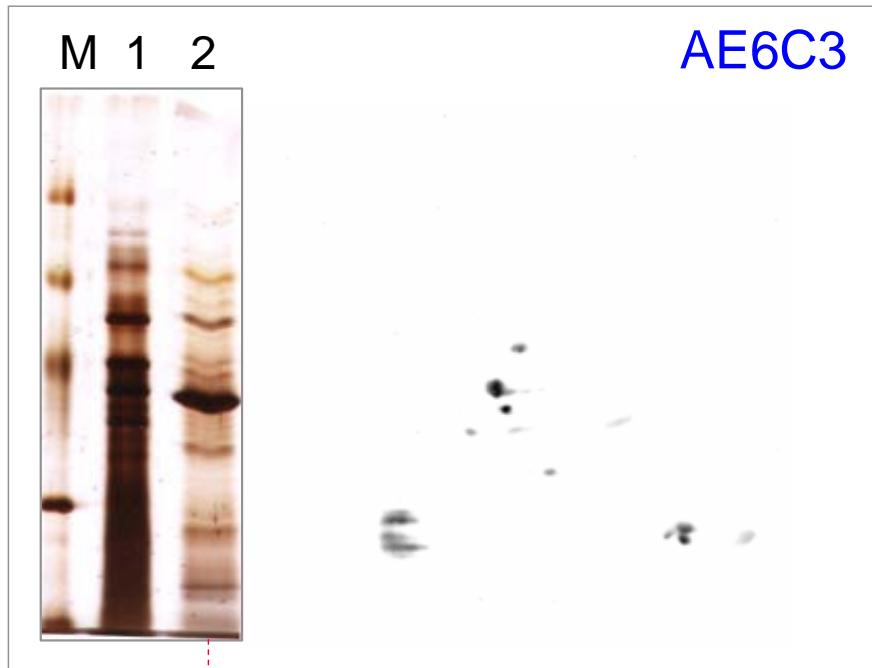
前處理
pretreatment

分離管柱

質譜儀分析
Mass analysis

Minimize protein purification and analysis in one chip

Immunoprecipitation of interacted proteins



LC-MS/MS

Interacted proteins

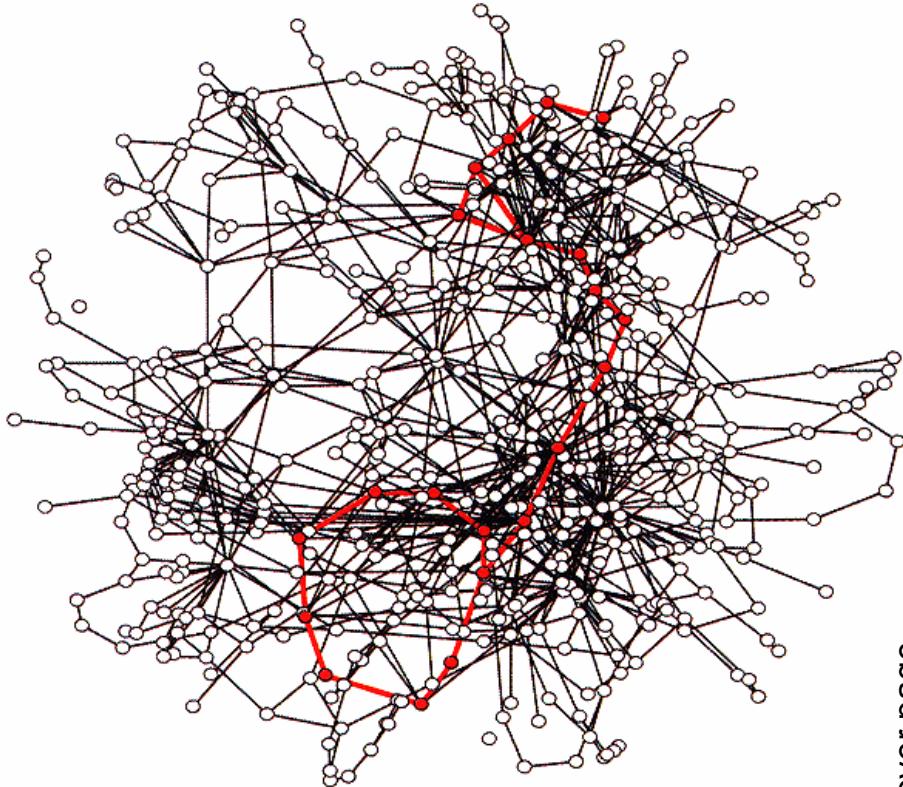
Validation!

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	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

