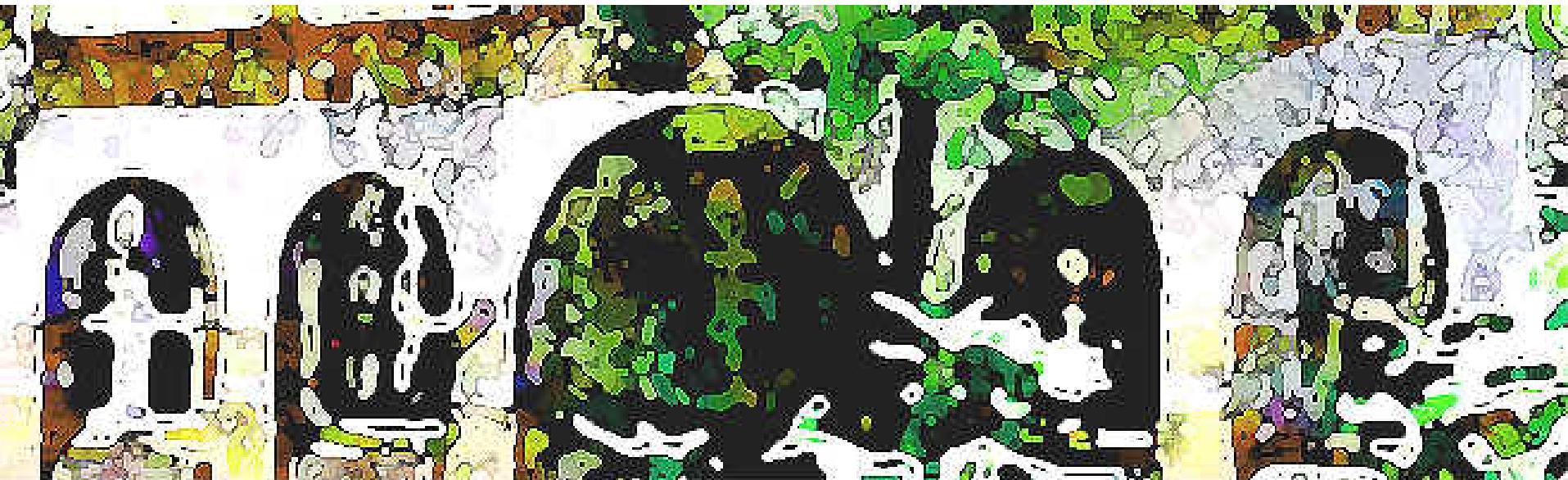


Proteomics and Antibody Bank

蛋白質體與抗體庫

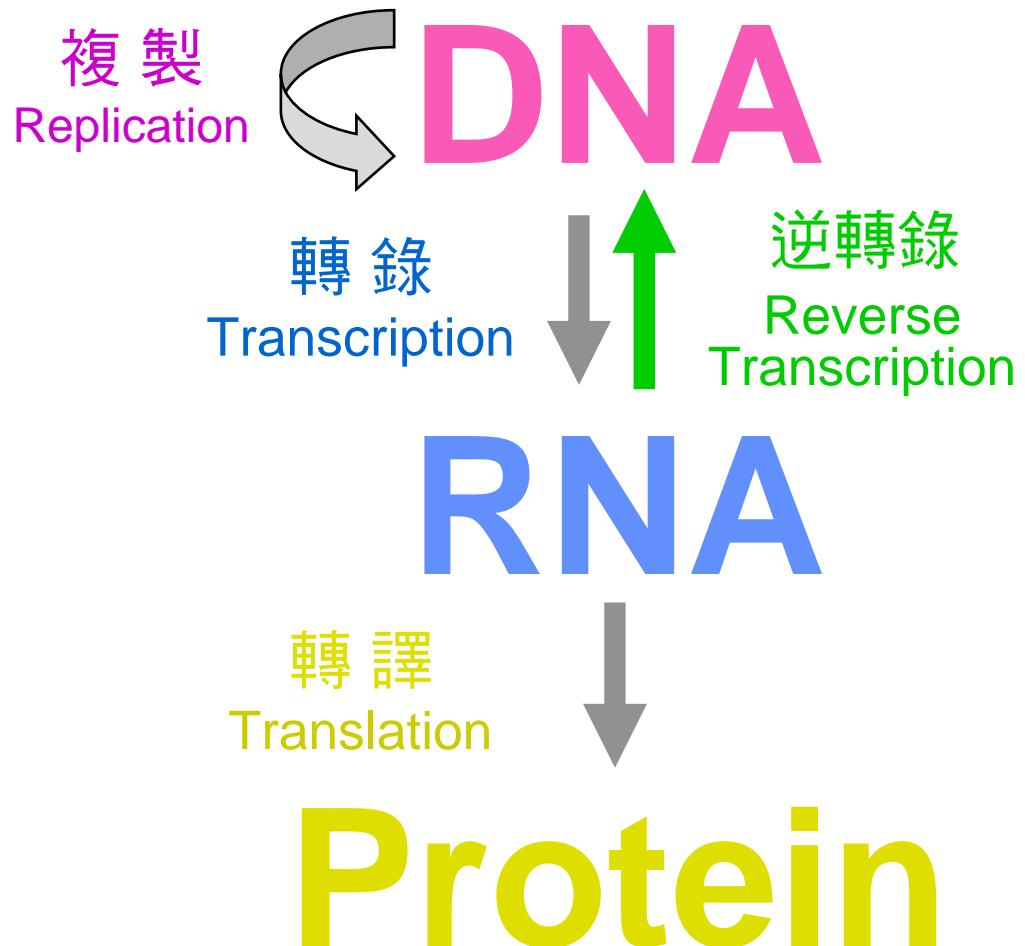
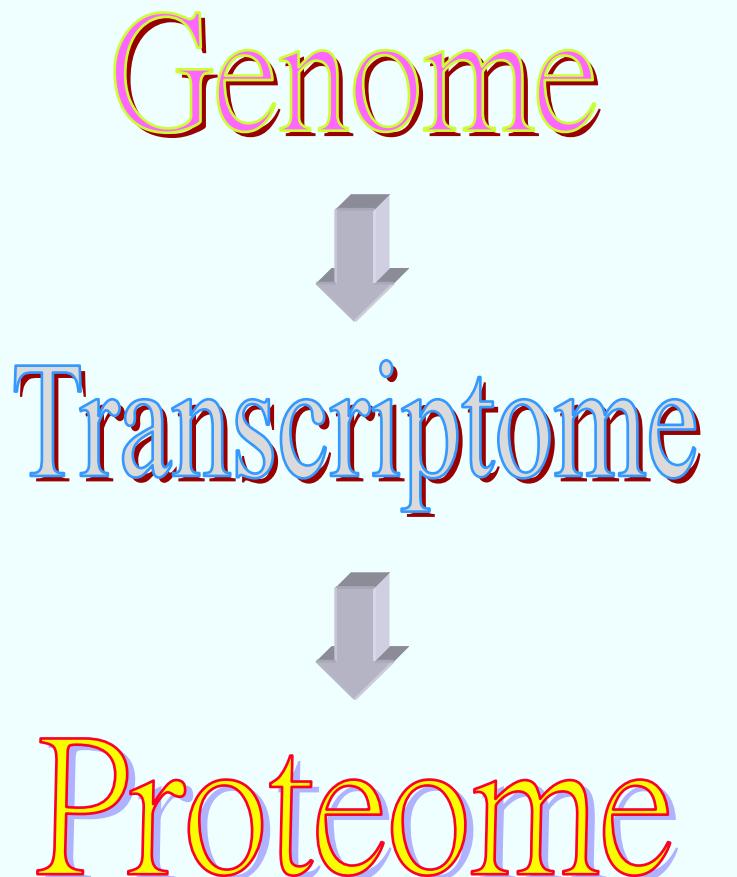


國立台灣大學 生化科技學系

莊榮輝

Central Dogma – 所有生物學的基本教條

蛋白質的胺基酸排列是由其基因的核苷酸序列所決定



Genome

基因表現不一定完全反映在蛋白質

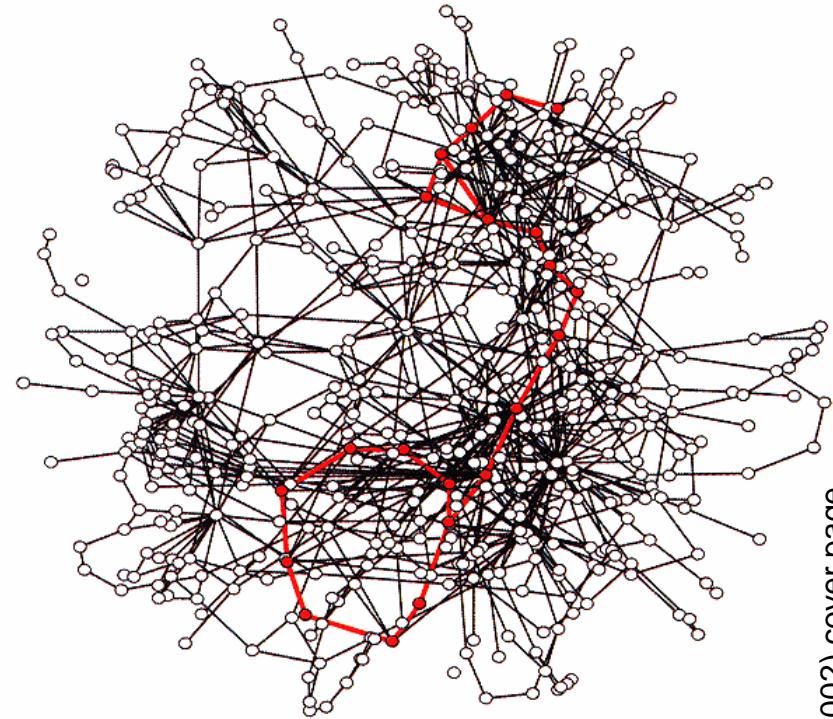
由基因體較難預測蛋白質的修飾及調控

也無法預測蛋白質間的交互作用

Proteome

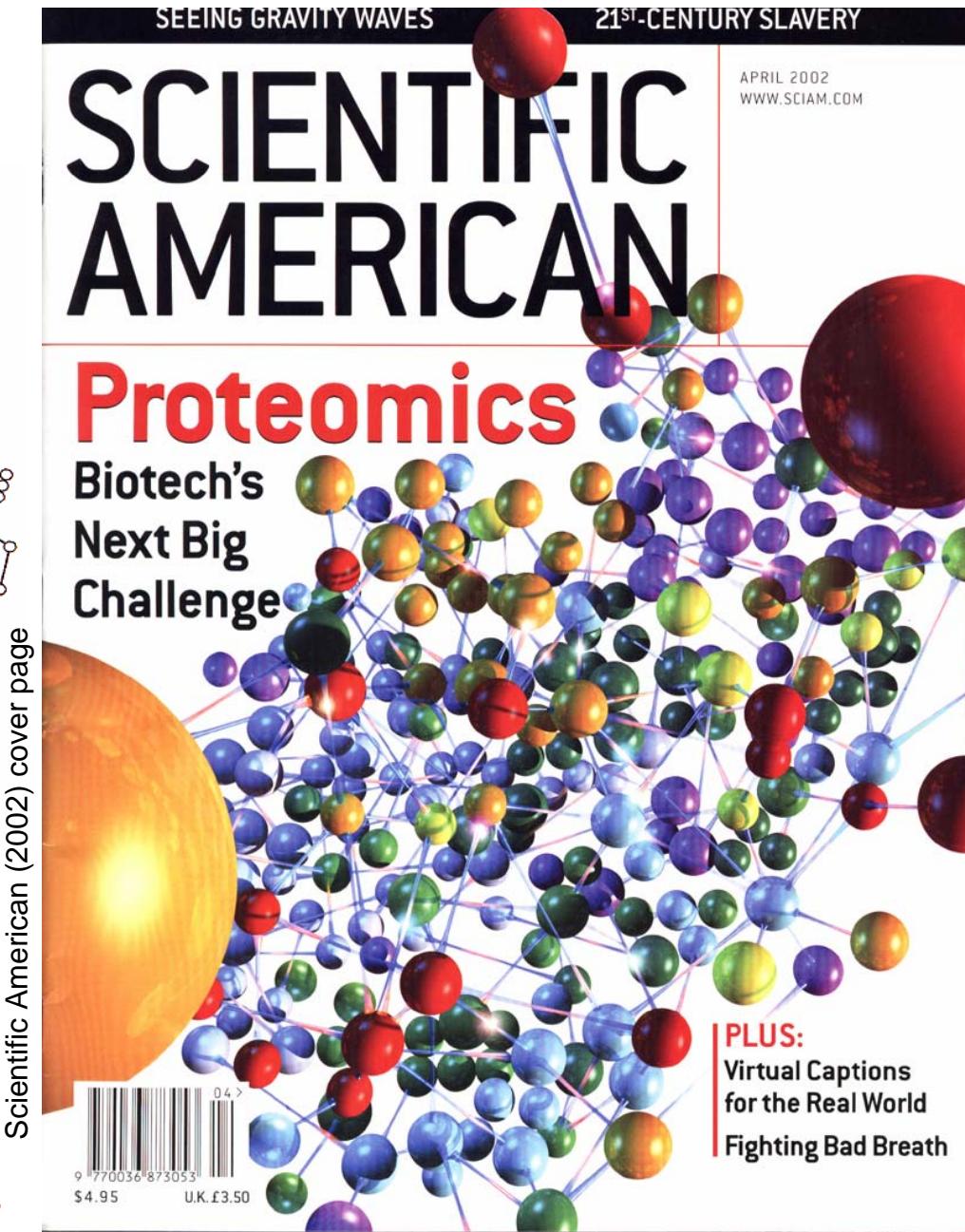
由蛋白質體學延伸到系統生物學

代謝路徑立體圖



Systems Biology

整體性的生物學觀念與工具



基因表現不一定完全反應在蛋白質圖譜

One Gene, One Protein?

細胞中

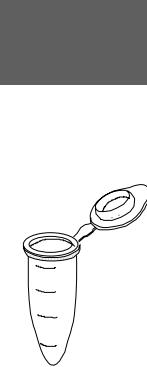
- (1) 細胞內的每個基因不一定都在表現
- (2) 各器官、組織的基因表現都不相同
- (3) 基因表現隨著生長時期而有改變
- (4) 蛋白質表現後有進一步的修飾與調控
- (5) 蛋白質在細胞內的代謝速率不同

試管中

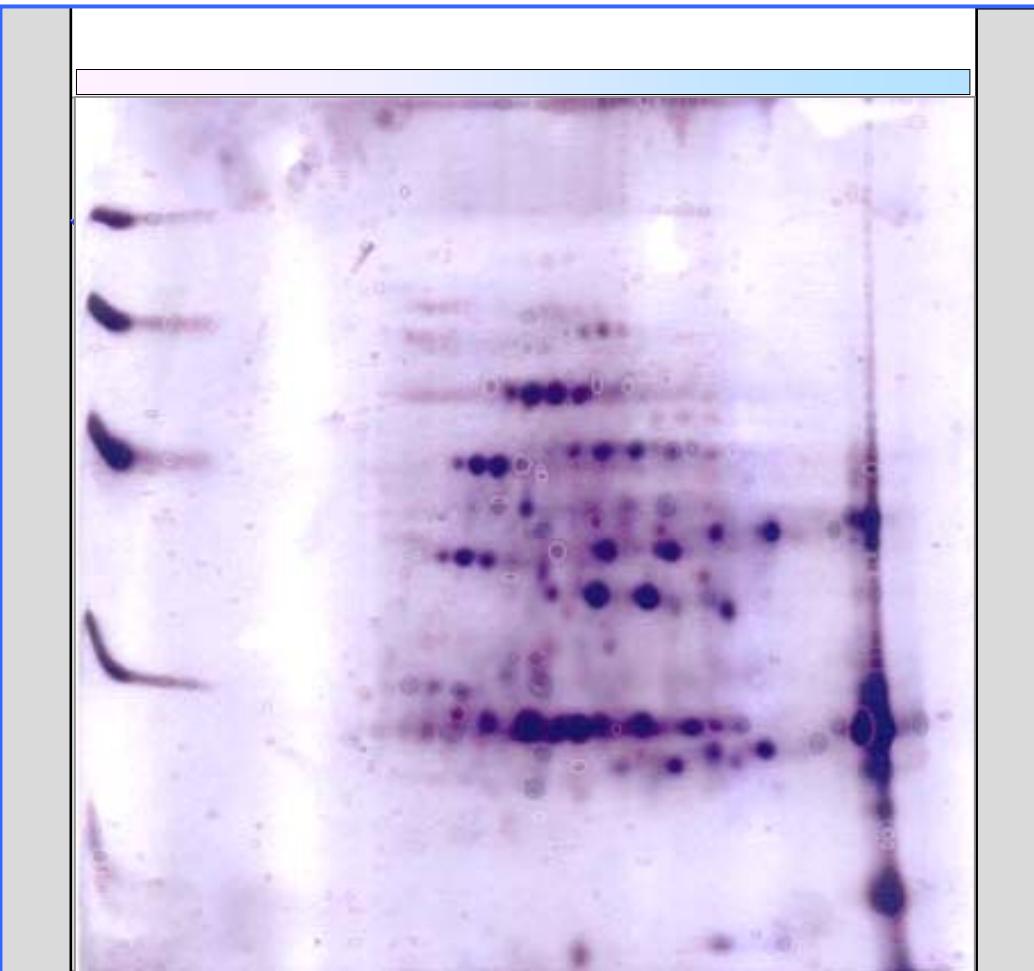
- (1) 蛋白質的水溶性會影響抽取效率
- (2) 蛋白質的含量差異很大
- (3) 蛋白質在抽取後的安定性與半衰期不同

二次元電泳的操作過程

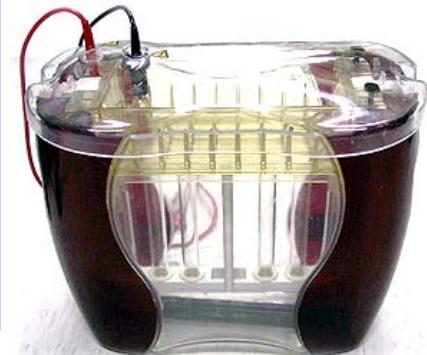
(1) IEF
等電焦集電泳



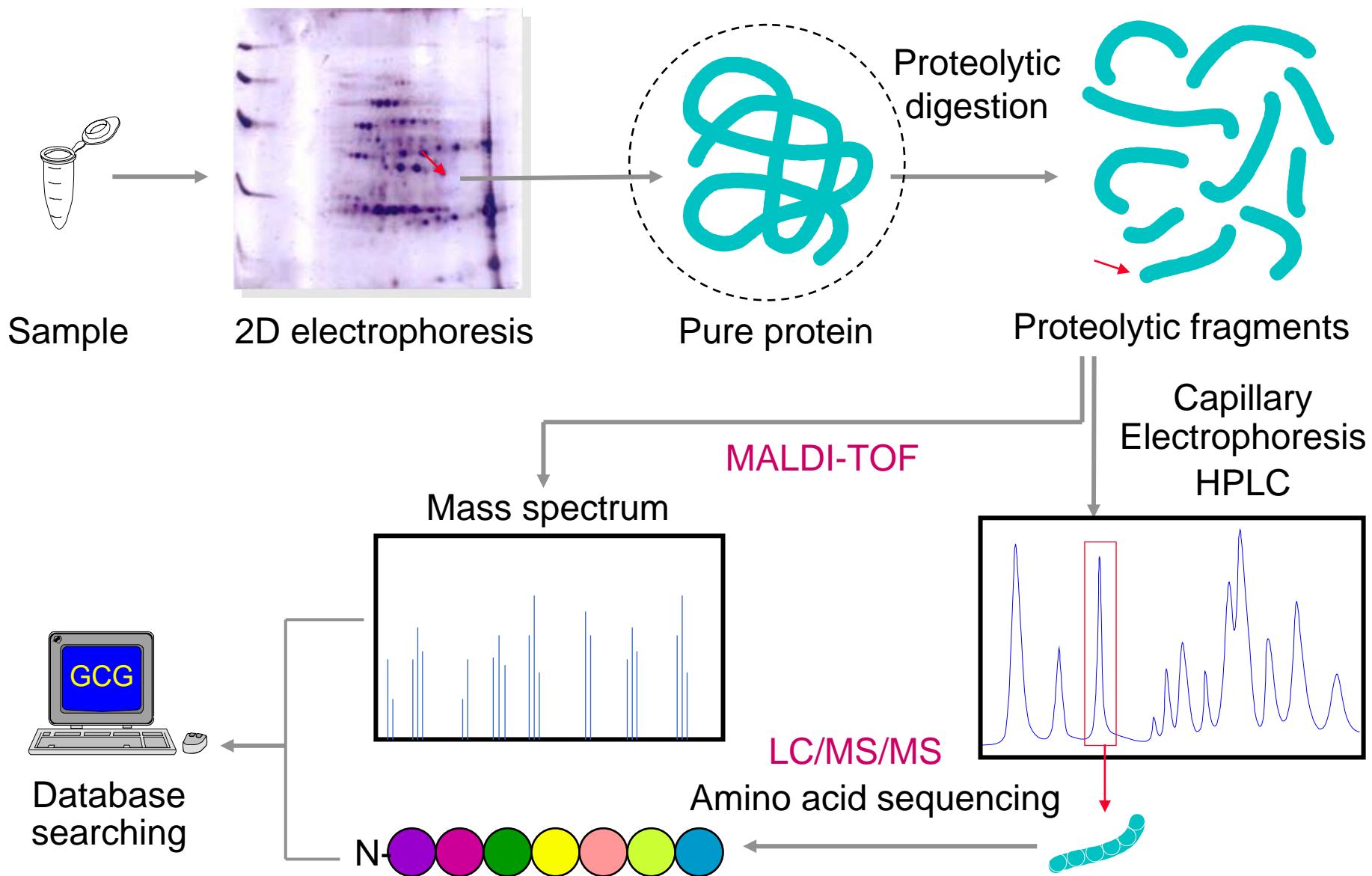
(3)
染色脫色



(2)
SDS-PAGE
分離膠體



■ 蛋白質體可綜觀蛋白質的消長與身分：



蛋白質構造與組成分析

.....

- N-端或 C-端胺基酸決定：

通常都直接定序，C-端較為困難

- 胺基酸組成分析

- 胺基酸定序方法：

cDNA 間推法

Edman 直接定序法

質譜儀定序

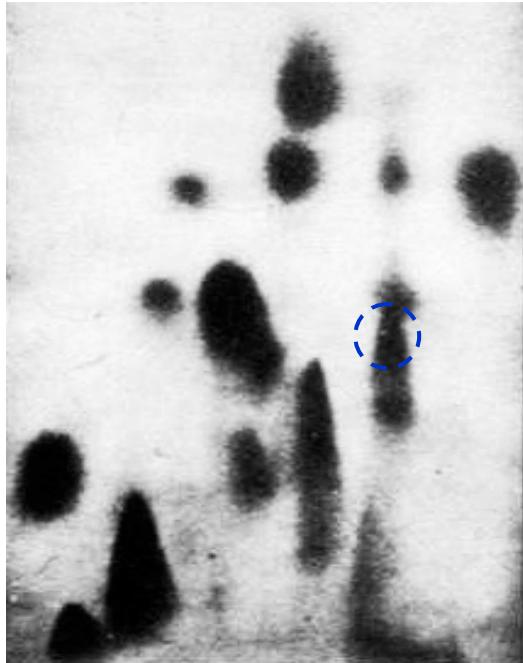
- 胜肽圖譜

以雙向層析電泳鑑定勝肽：



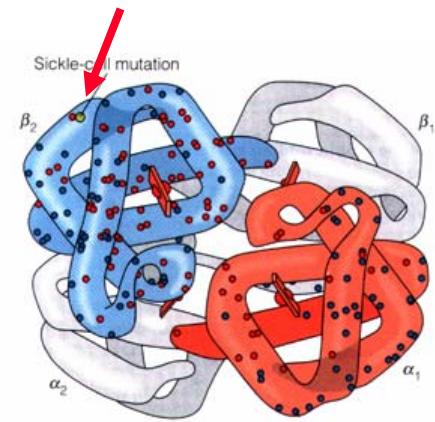
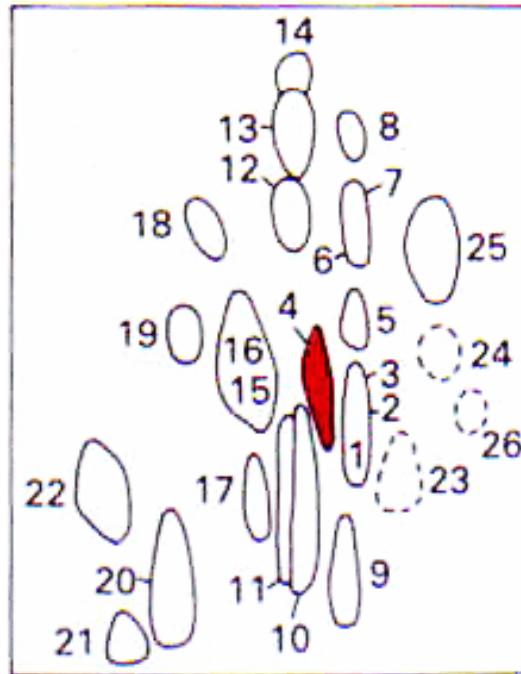
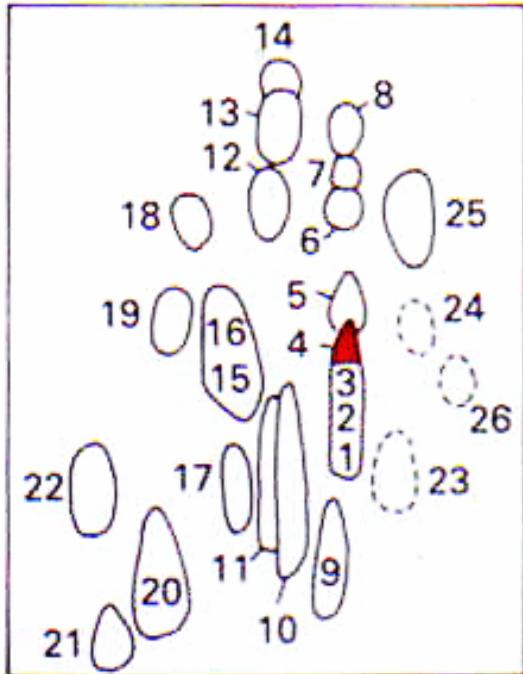
血紅蛋白四號片段

Hemoglobin A



Hemoglobin S

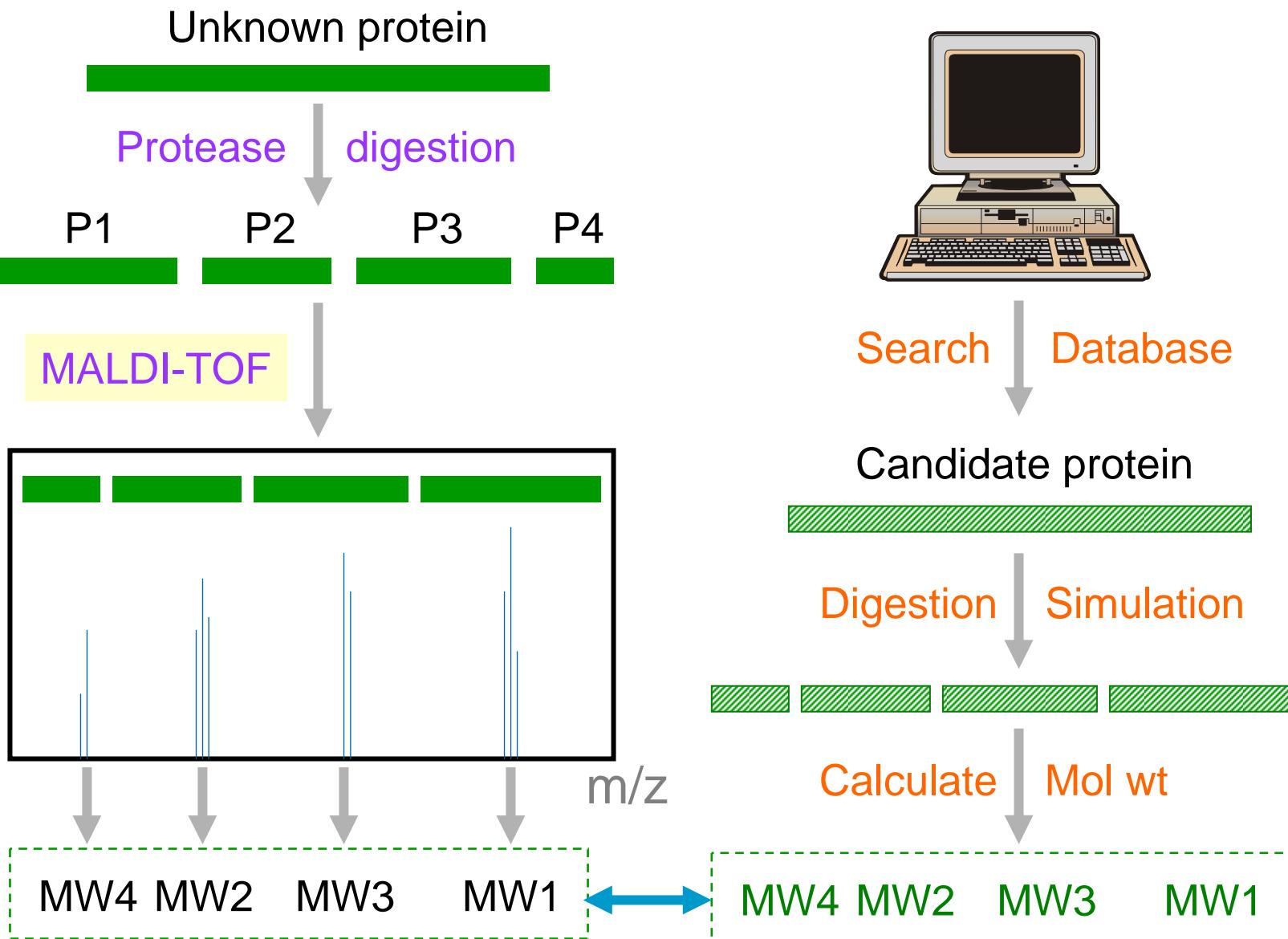
镰型血球



色析

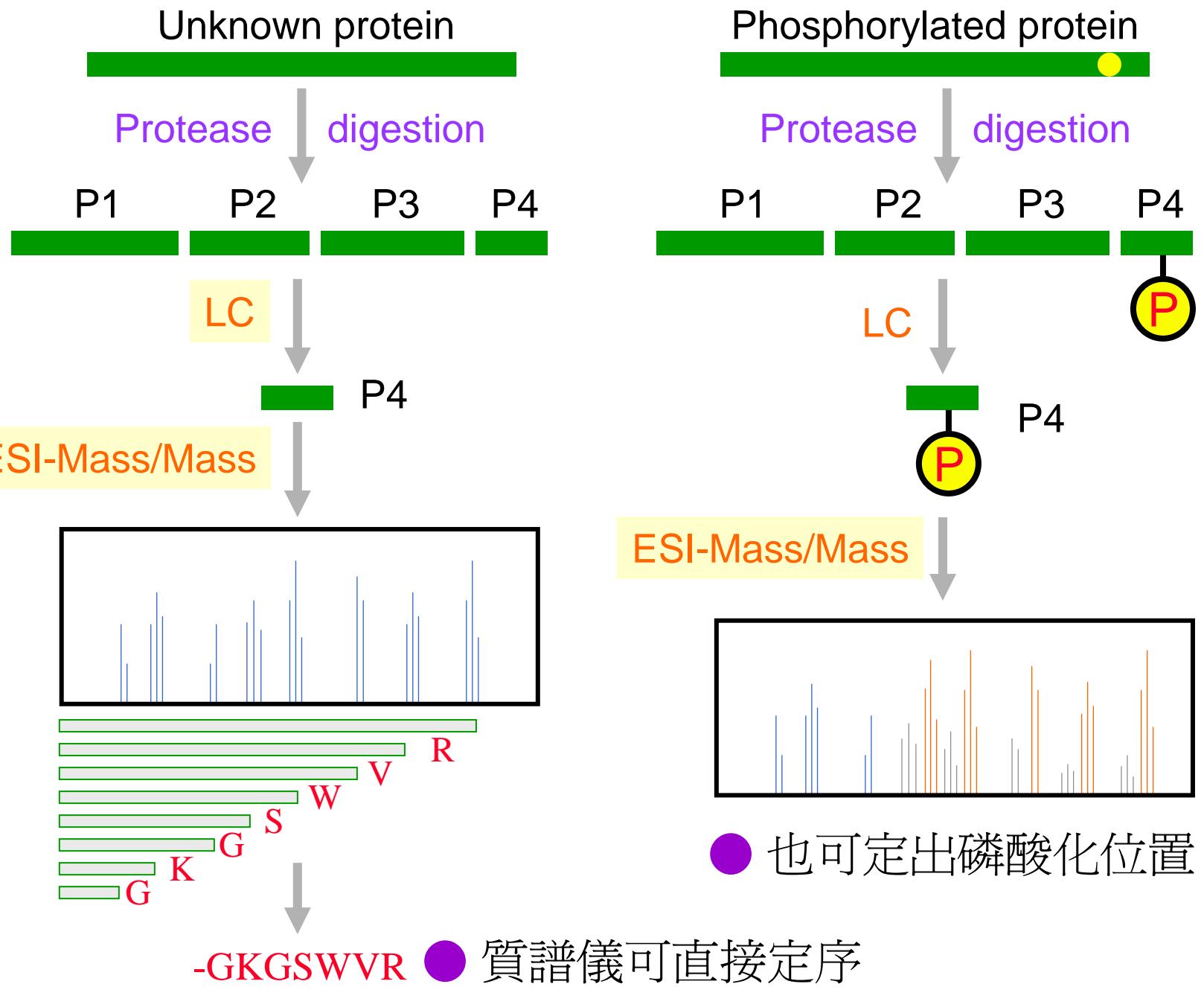
電泳

質譜儀可檢定蛋白質身分：



● 比對各片段分子量可確定該蛋白質身分

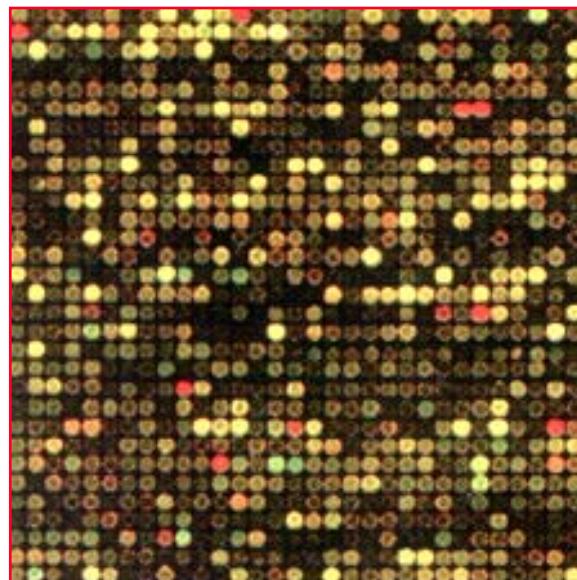
質譜儀可進行蛋白質序列分析：



■ 現代蛋白質科技的特點：

.....

- 高產能 High-through put
- 快速 High-speed
- 微量 Micro-scaled



綠竹筍生長與老化機制之探討及相關功能基因之利用

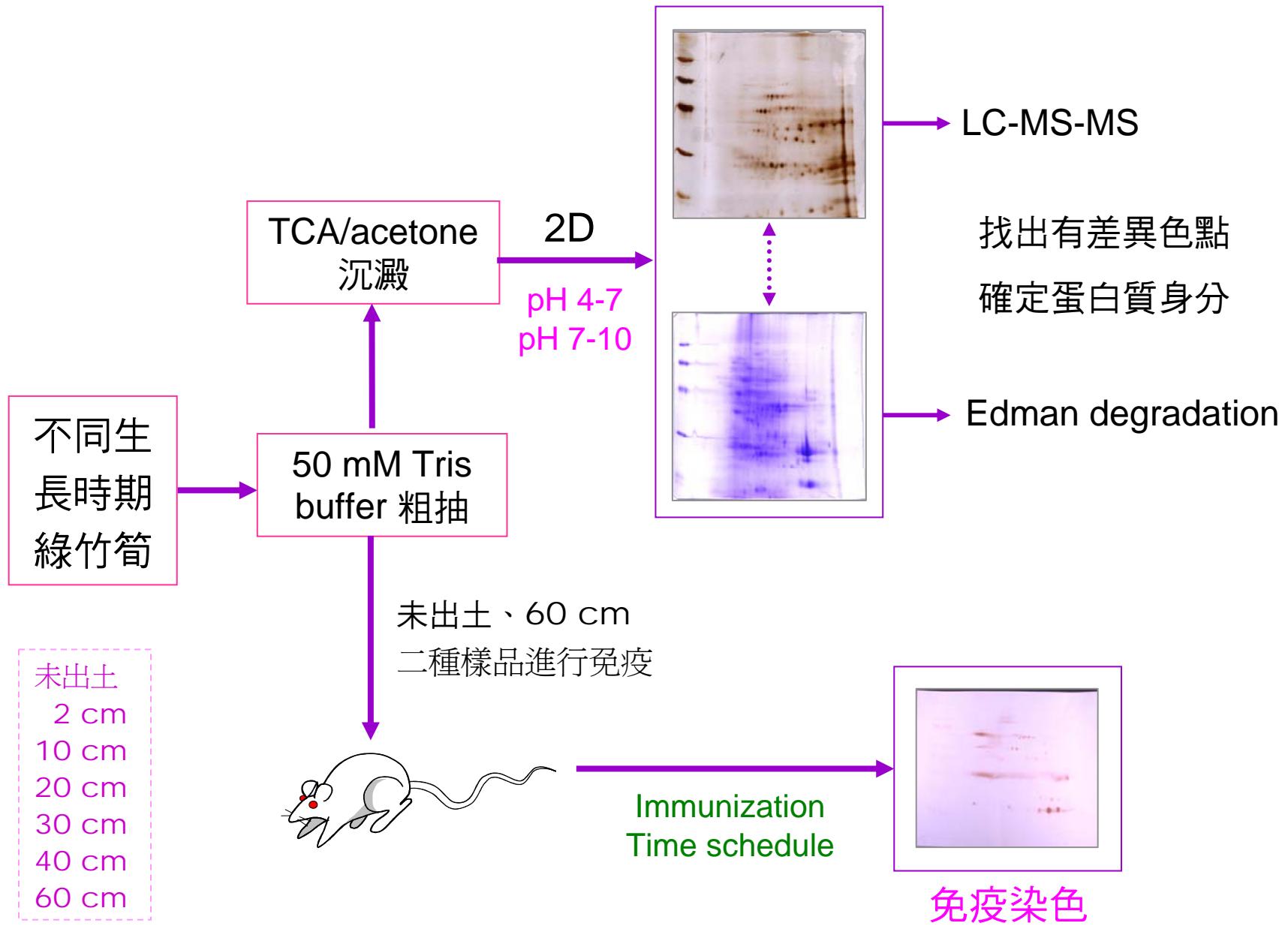
2004/6/1

主持人：莊榮輝
博士班：吳裕仁
及竹筍小組

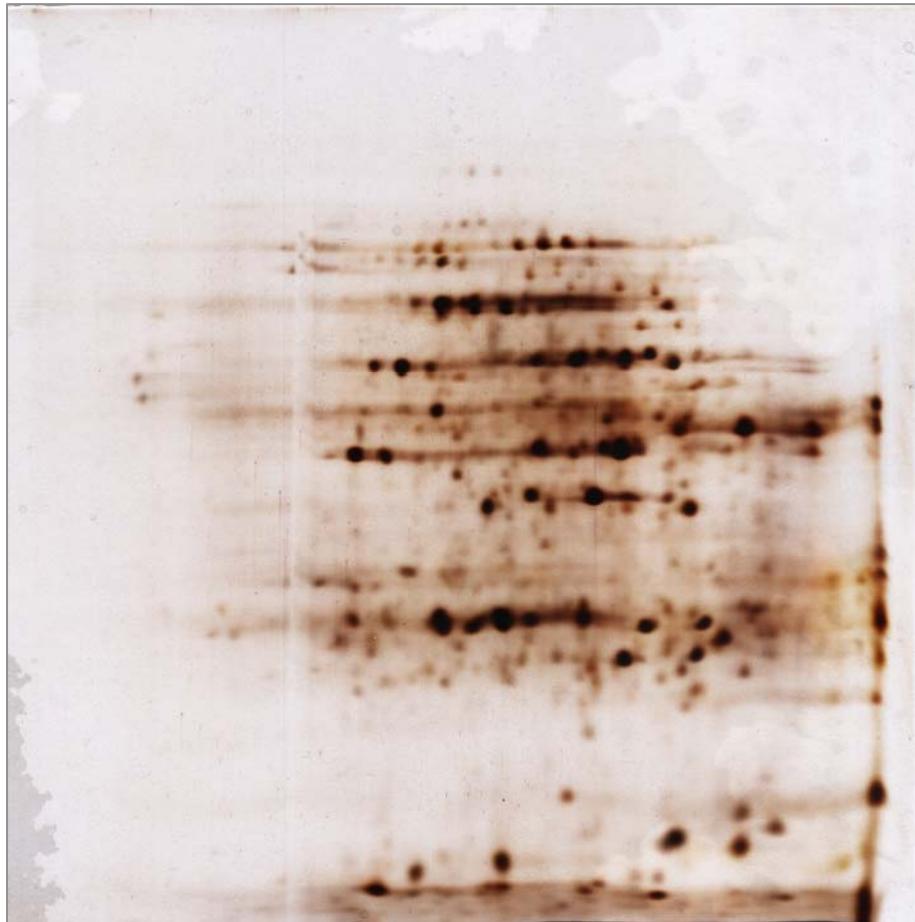


綠竹筍
(*Bambusa oldhami*)

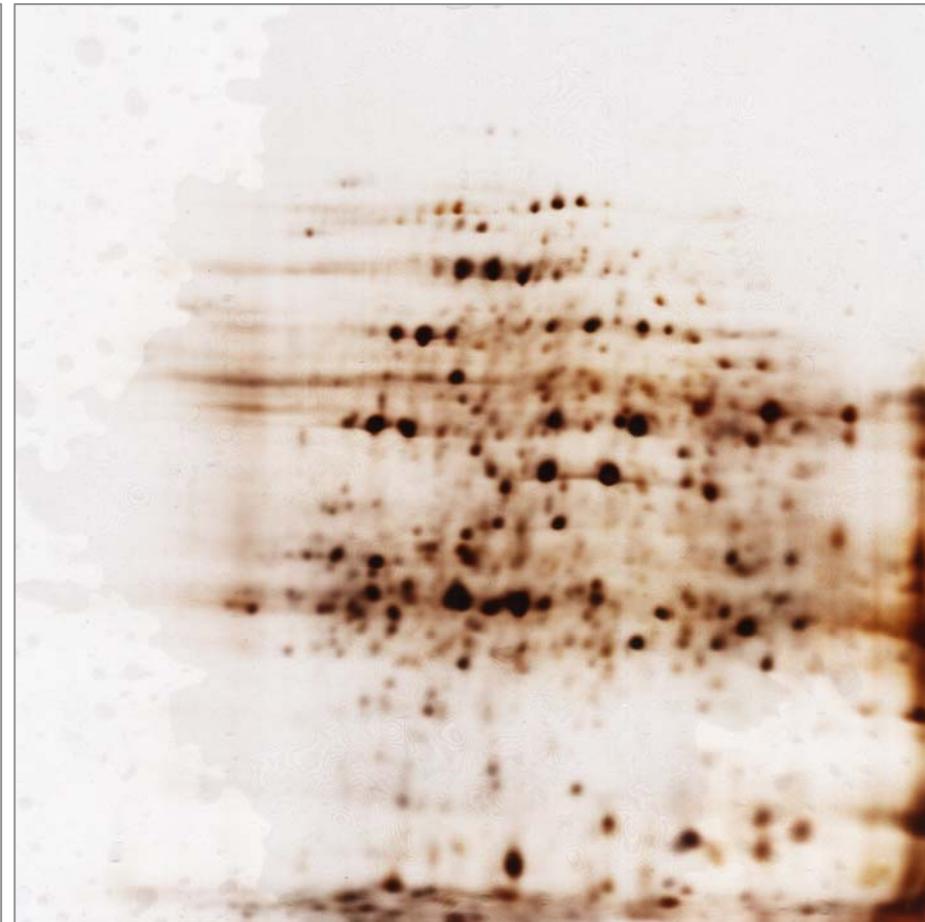
整體研究流程大綱



竹筍快速生長前後的蛋白質表現確有差異



未出土



出土 60 cm

漸增蛋白質色點的身分

編號	Protein ID	分析方法	代謝途徑
B16	Heat-shock protein 70 Heat-shock protein 82	LC-MS/MS	Chaperone response
R26	Heat-shock protein 70	LC-MS/MS	Chaperone response
B17	20S Proteasome alpha subunit F	LC-MS/MS	Proteasome response
R25	20S Proteasome alpha subunit E	LC-MS/MS	Proteasome response
B21	Glutathione-S-transferase	N-terminal	Stress induced protein
R27	Glutathione-S-transferase	LC-MS/MS	Stress induced protein

竹筍快速生長時可能啟動抗逆境機制。

漸減蛋白質色點的身分

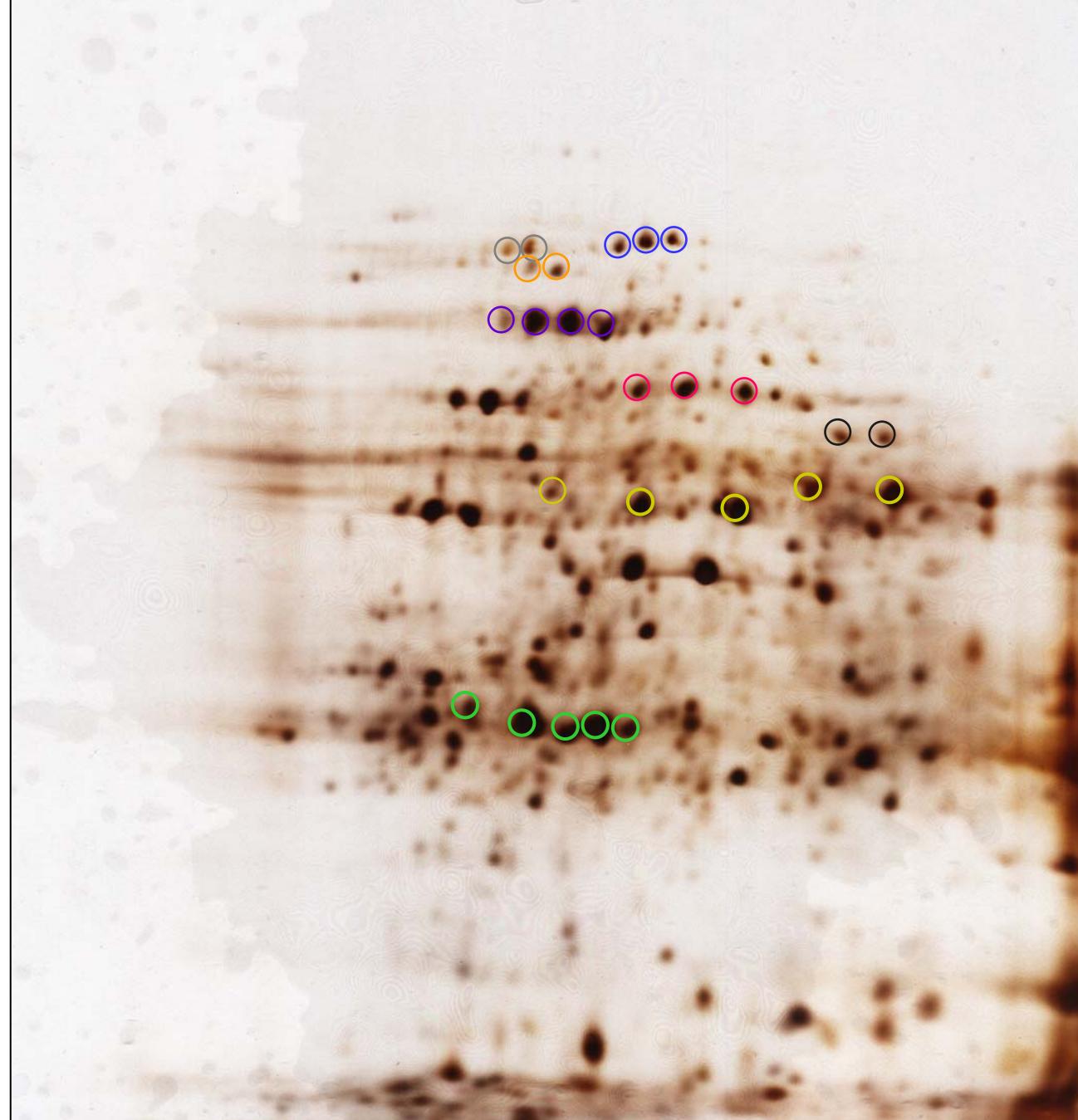
編號	Protein ID	分析方法	代謝途徑
B2	Enolase	LC-MS/MS	Glycolysis
B3	Enolase	LC-MS/MS	Glycolysis
B4	Enolase	LC-MS/MS N-terminal	Glycolysis
R4	Phosphoglycerate mutase	LC-MS/MS	Glycolysis
R5	Phosphoglycerate mutase	LC-MS/MS	Glycolysis

竹筍快速生長時醣解作用可能降低。

恆定蛋白質色點的身分

編號	Protein ID		代謝途徑
B19	Glyceraldehyde-3-phosphate dehydrogenase	LC-MS/MS N-terminal	Calvin cycle Glycolysis
B20	β 1,3-Glucanase	N-terminal	Defense protein
BI	Triosephosphate isomerase	LC-MS/MS N-terminal	Calvin cycle Glycolysis
BH	Triosephosphate isomerase	LC-MS/MS N-terminal	Calvin cycle Glycolysis

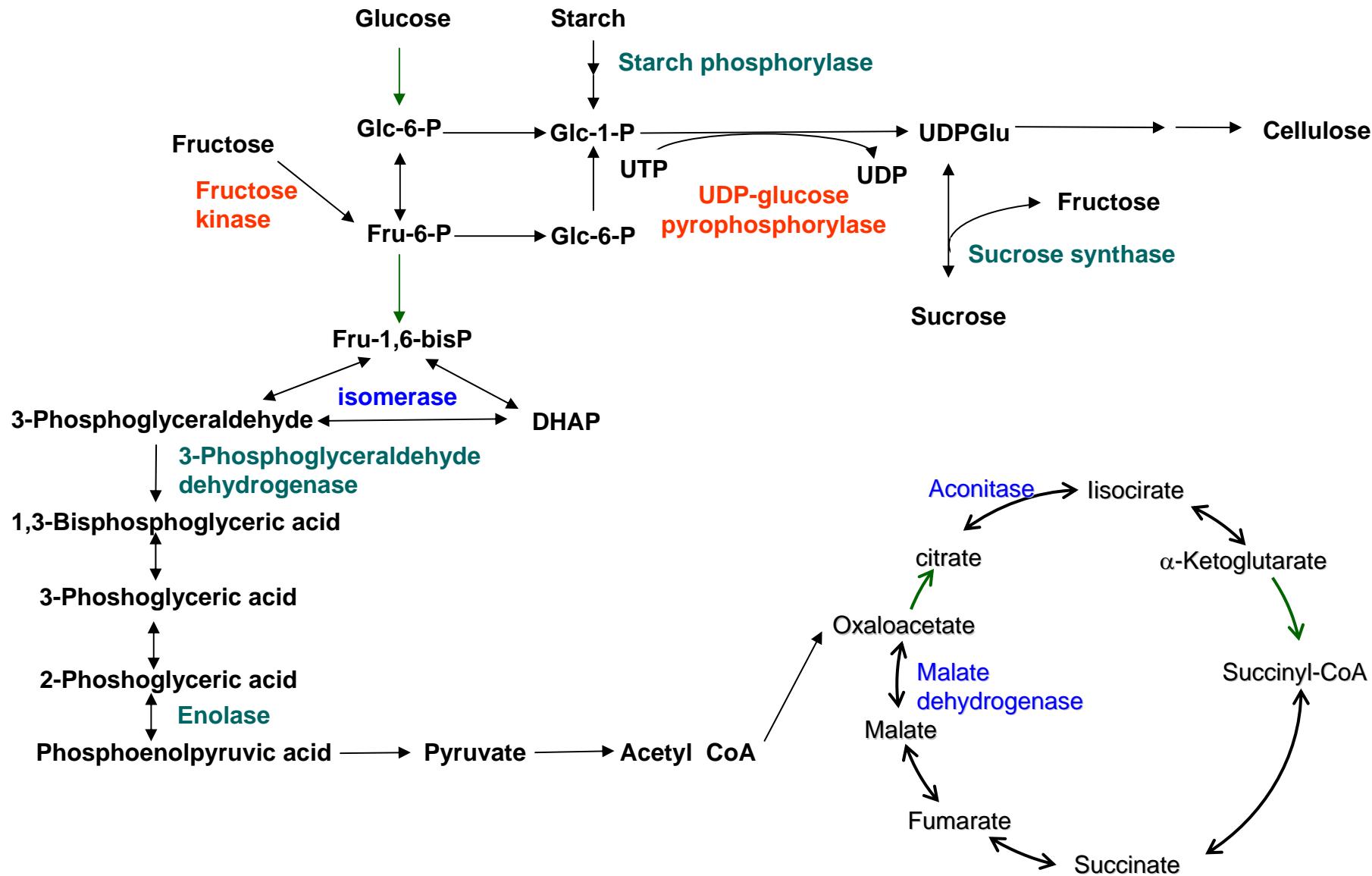
綠竹與水稻基因有相當高的同質性，因此可以用水稻的基因體進行比對。



- Phosphoglycerate mutase
- Malate dehydrogenase
- Triosephosphate isomerase
- UTP-glucose-pyrophosphorylase
- Enolase
- Alcohol dehydrogenase
- Vacuolar ATP synthase catalytic subunit A
- Chaperonin (HSP60-1, mitochondrial)

相關蛋白質顯現出
水平的色點群落。

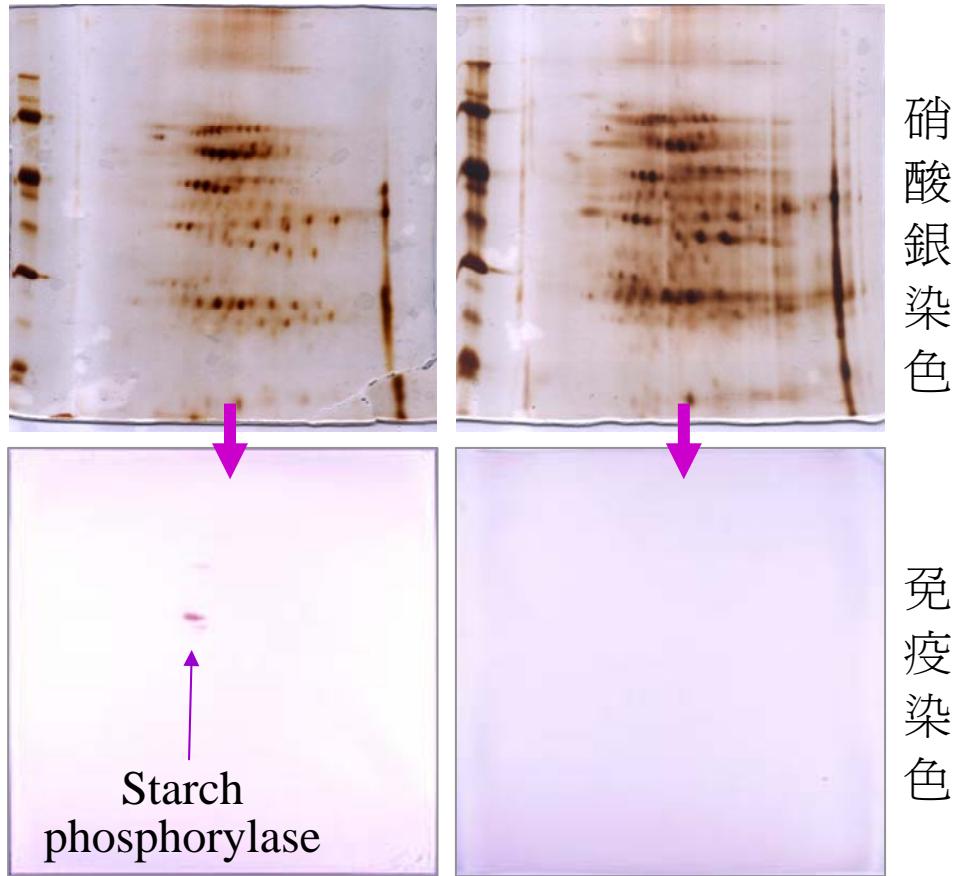
由蛋白質消長推測綠竹快速生長相關代謝



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

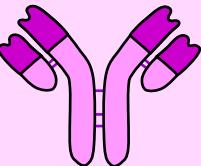


首次提出
高產能抗體製備計畫構想

- (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

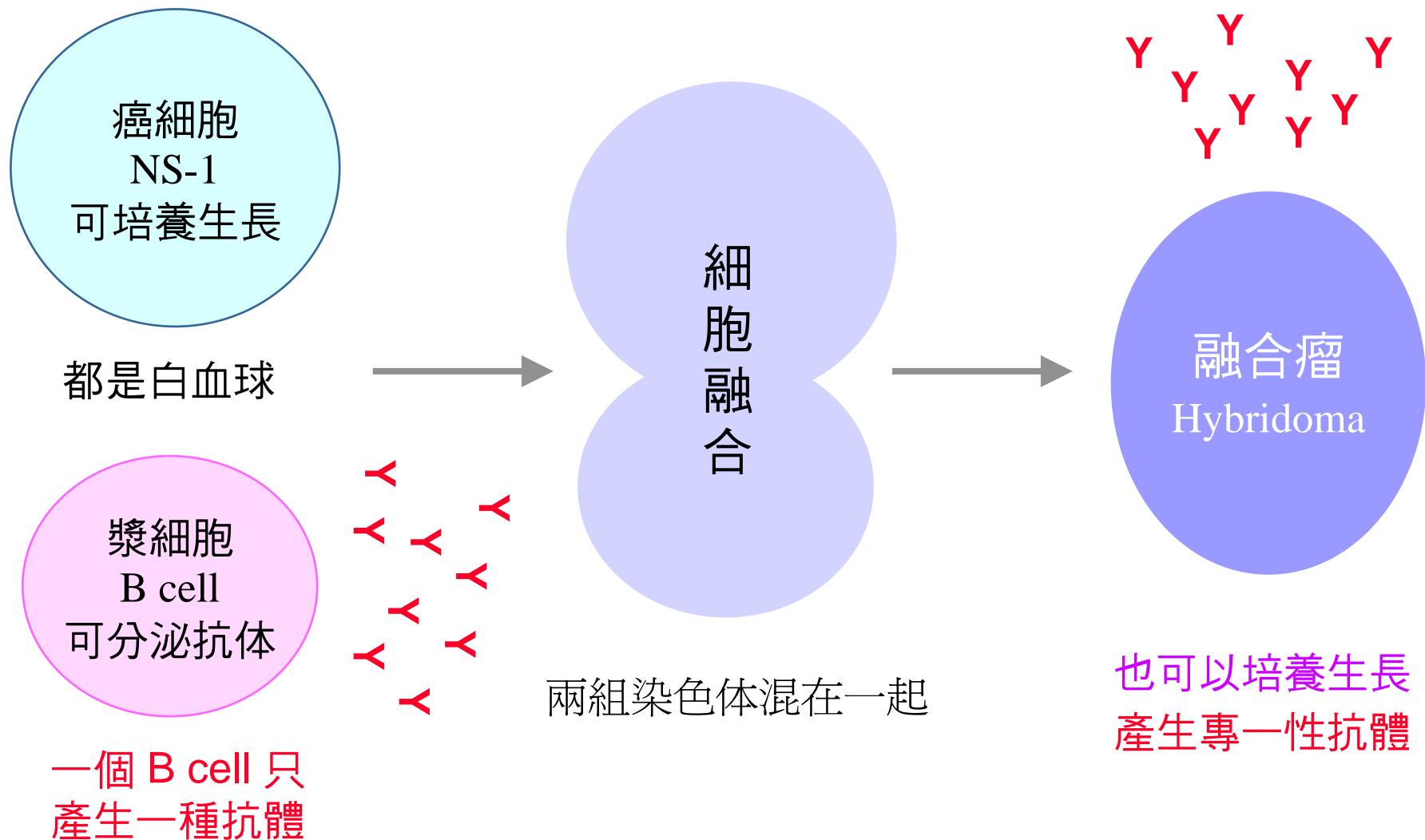
均質抗原 → 單一抗體

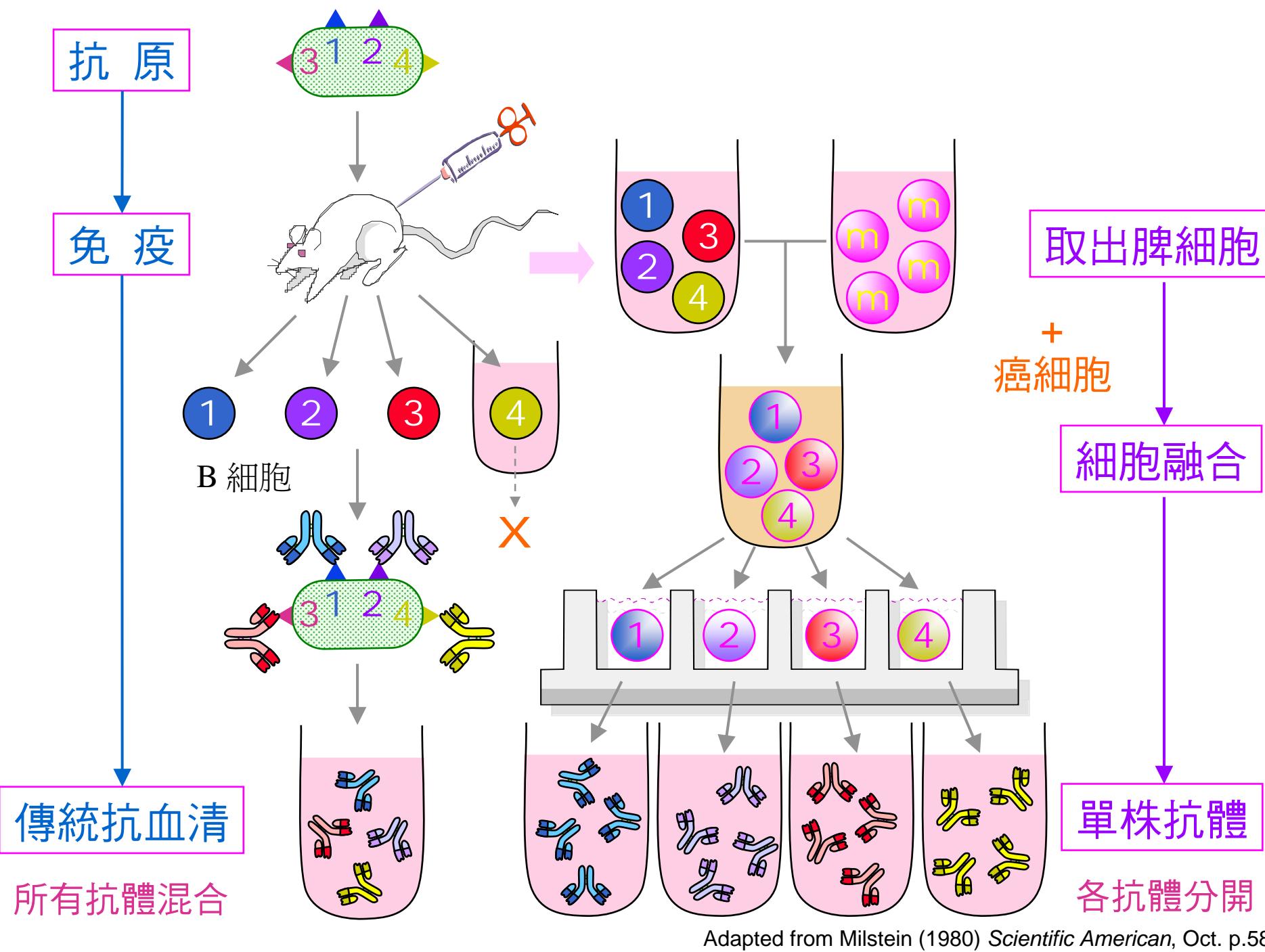
整體抗原 → 全部抗體



單株抗體

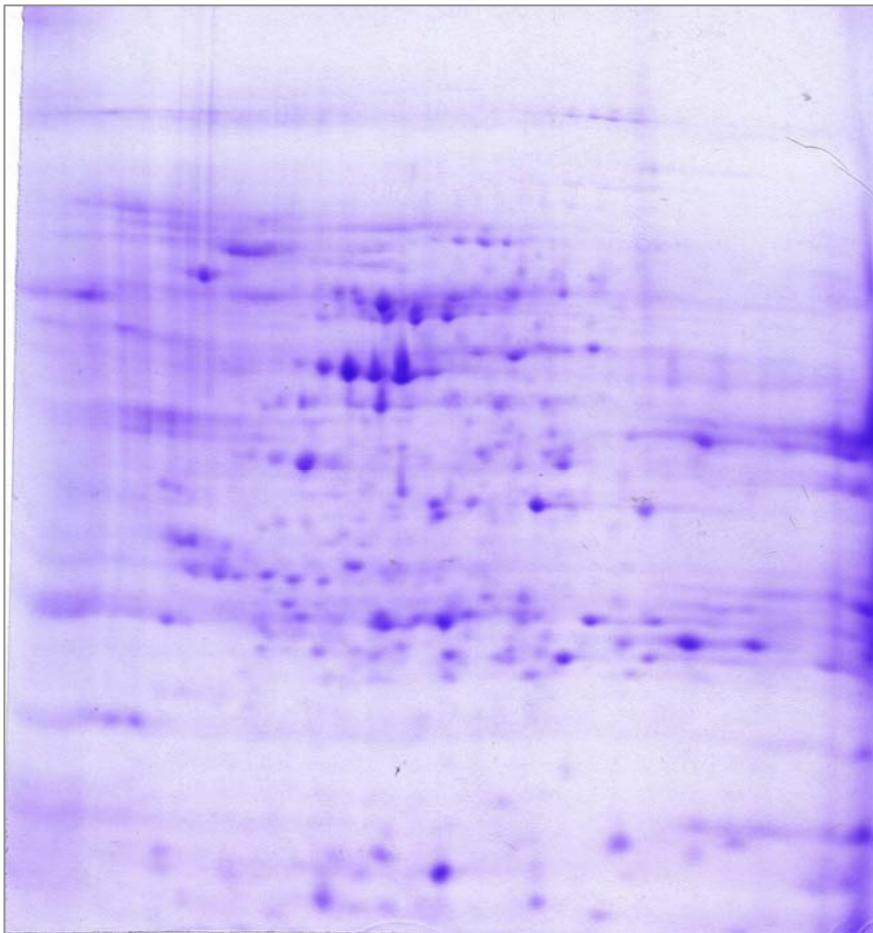
可生產有用抗體的 **淋巴細胞** 若與 **癌細胞** 融合，則形成穩定而可培養的細胞株。





竹筍蛋白質體的免疫反應

未出土竹筍樣本 CBR 染色



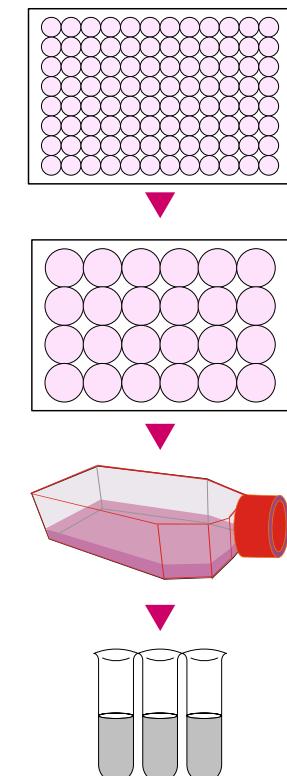
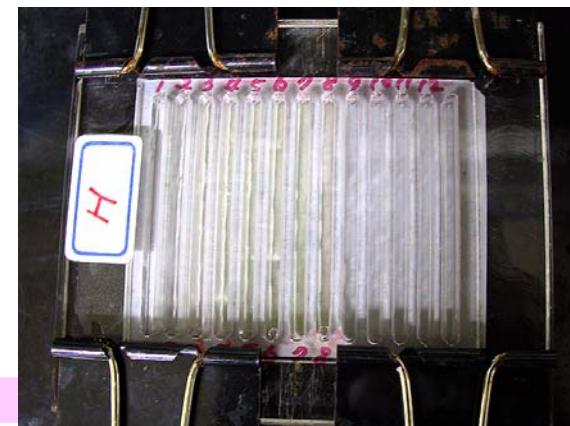
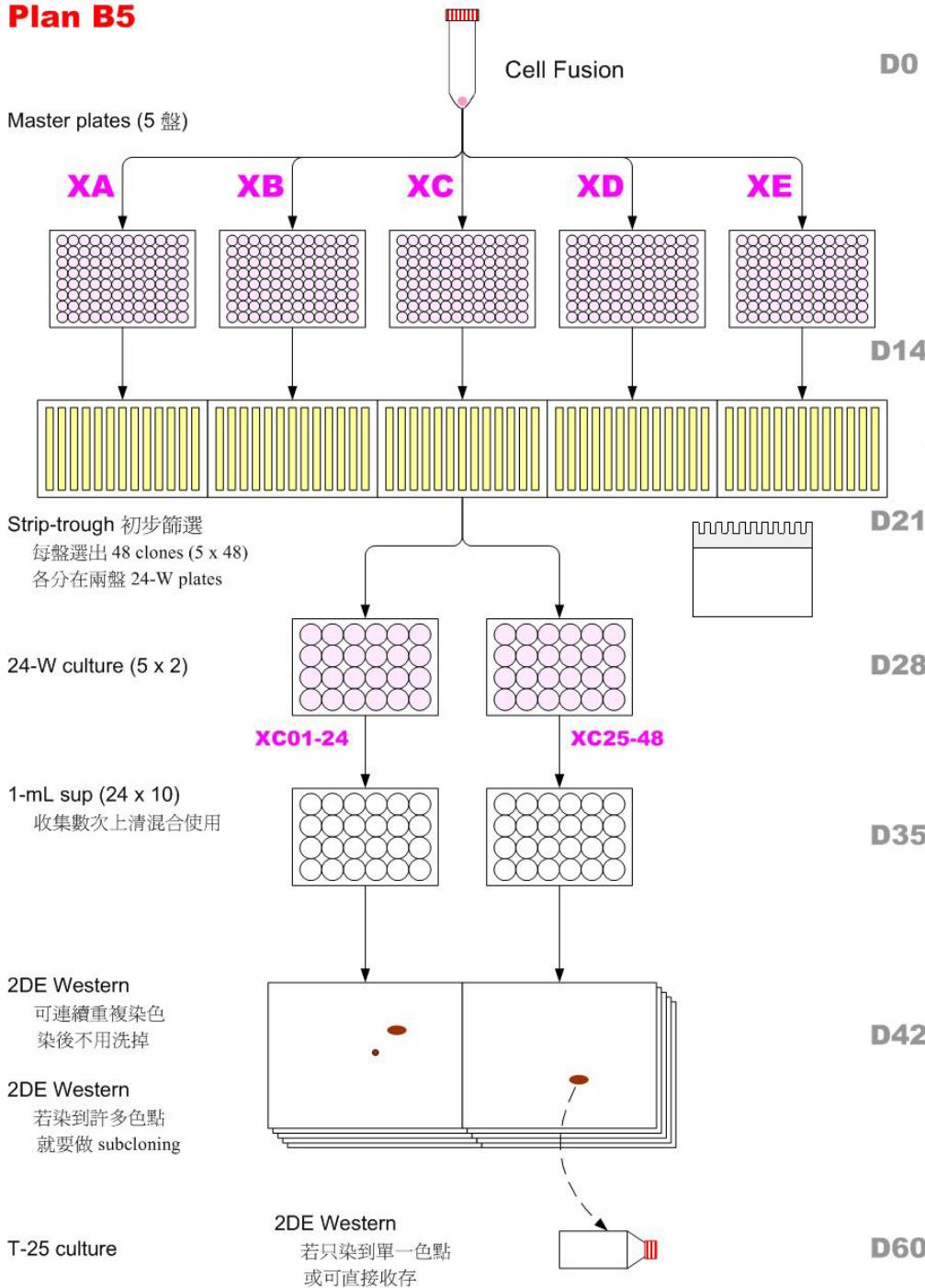
小鼠抗血清免疫染色



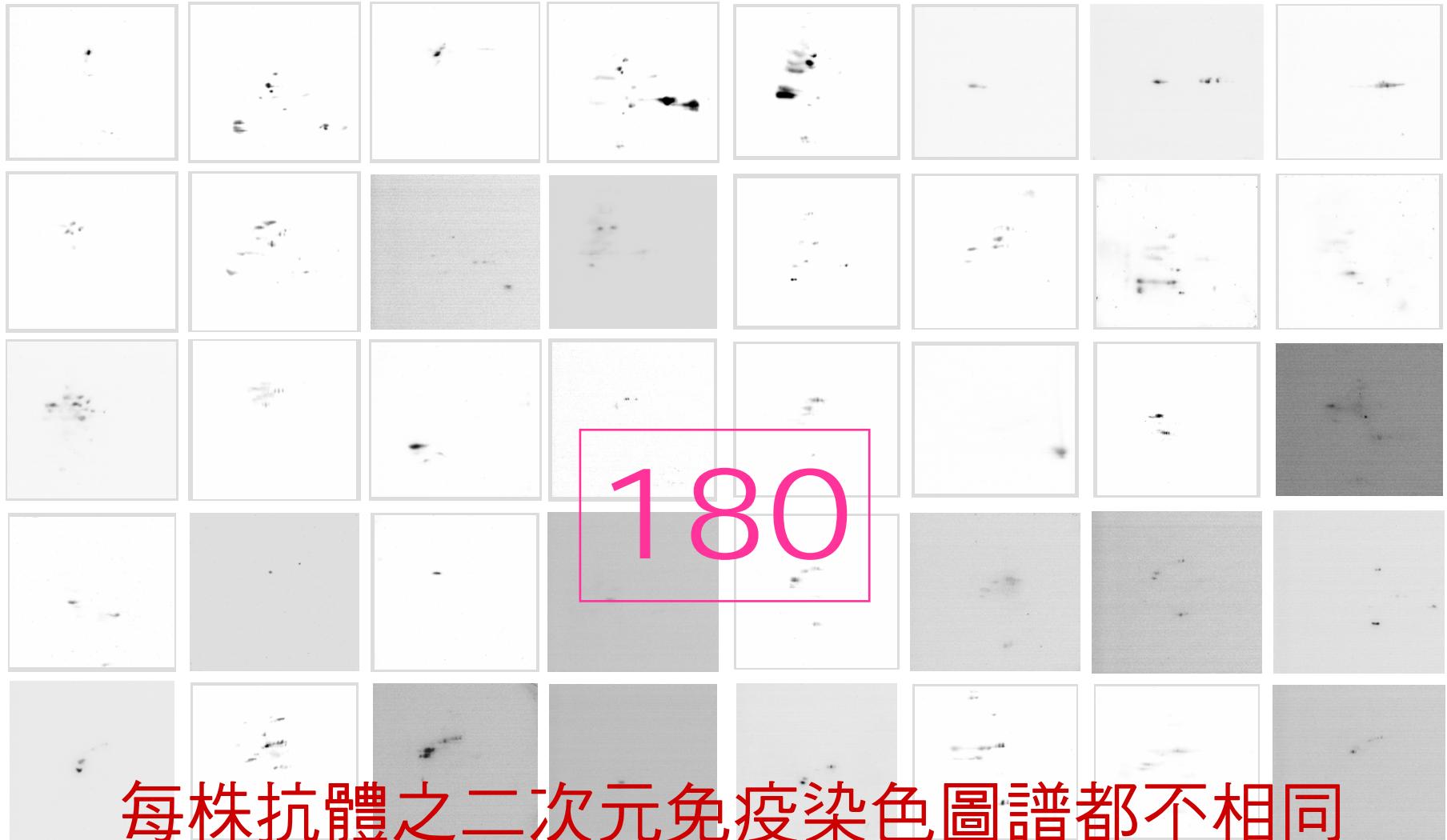
抗原性強弱不同、蛋白質含量多寡不同。

單株抗體高產能製備流程

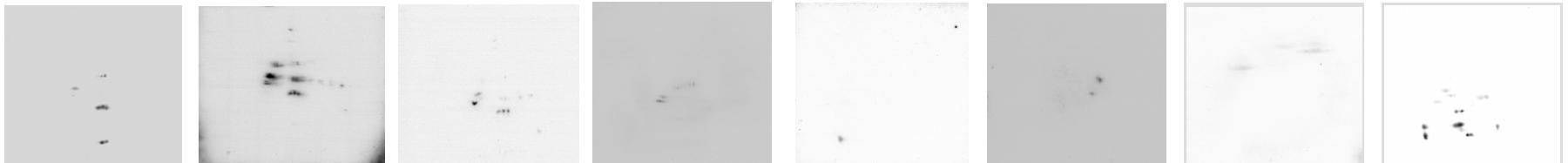
Plan B5



綠竹筍蛋白質體之單株抗體庫 (pH 3-10)

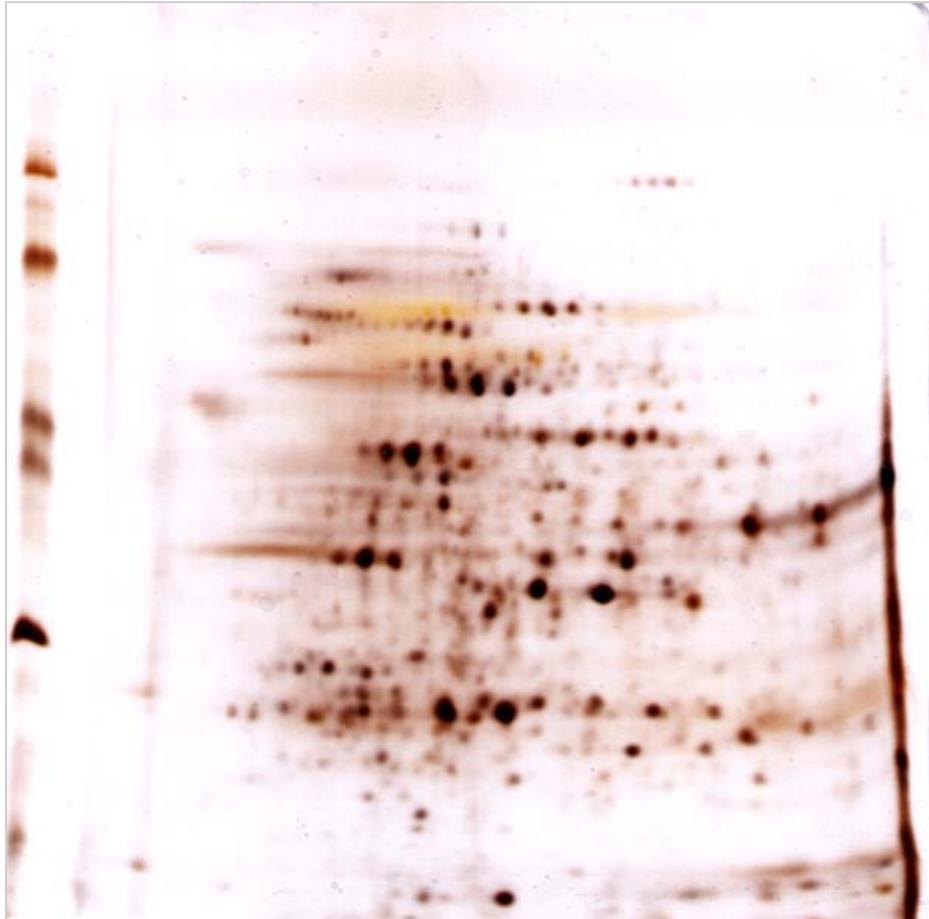


每株抗體之二次元免疫染色圖譜都不相同

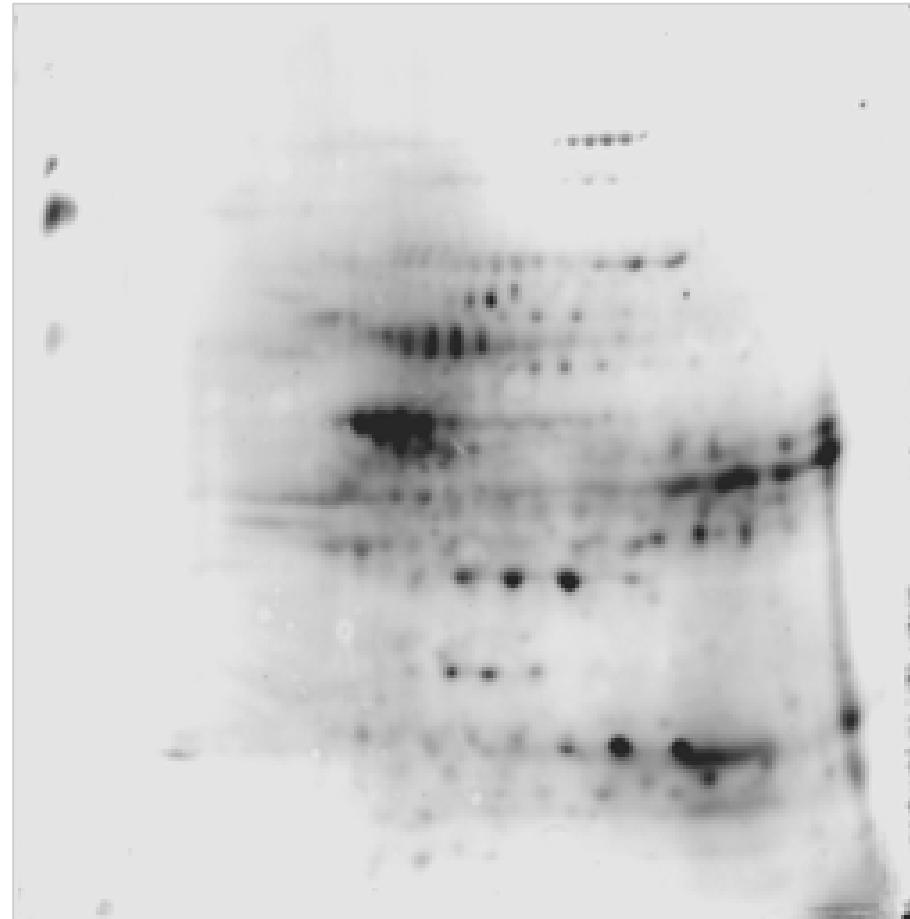


混合八十種單株抗體共同免疫染色

Silver staining



Immunostaining



最終目標：蛋白質染色圖譜 = 免疫染色圖譜

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



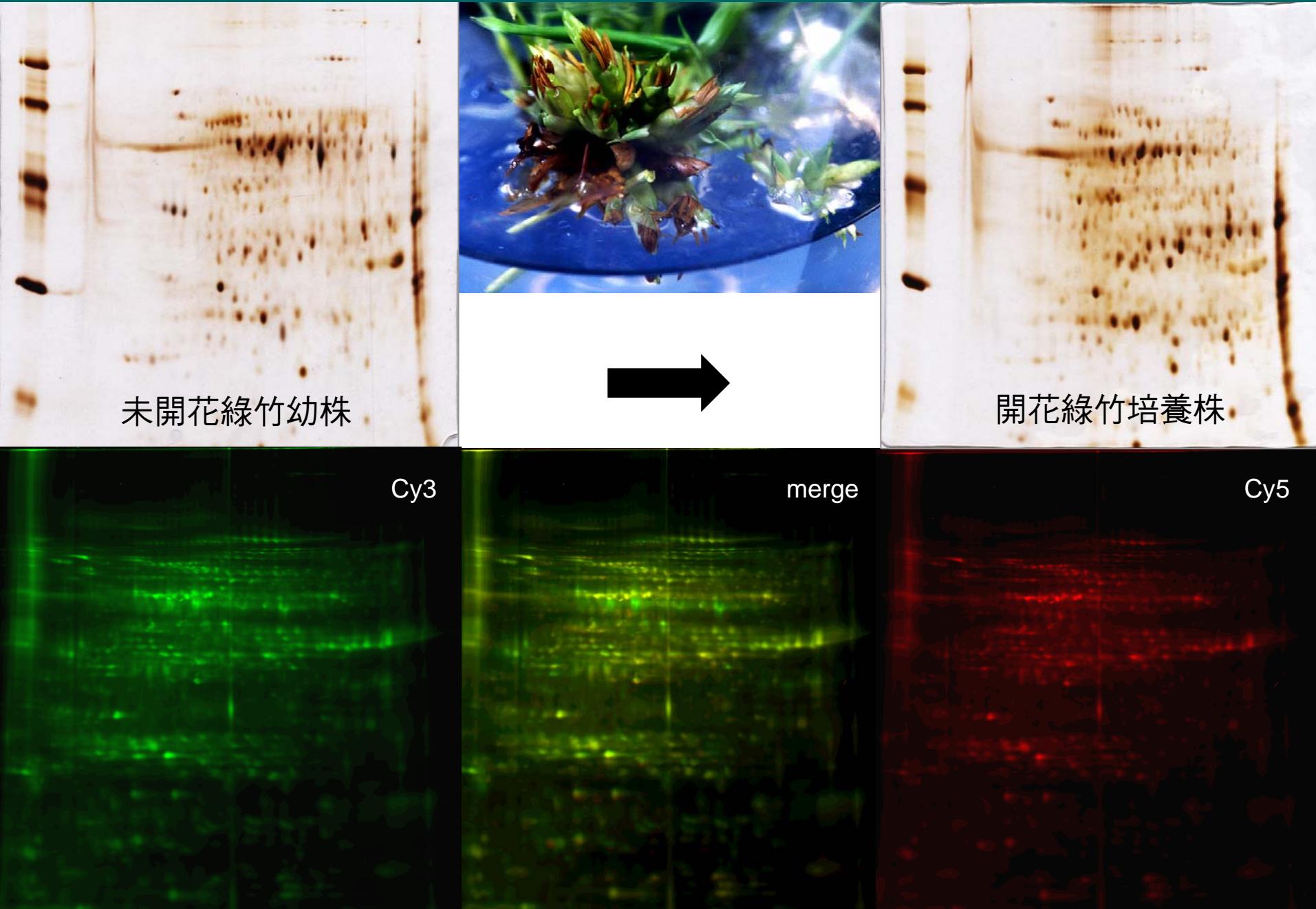
Fuchu He (left) has the task of coordinating China's project to identify liver proteins.



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 individ-

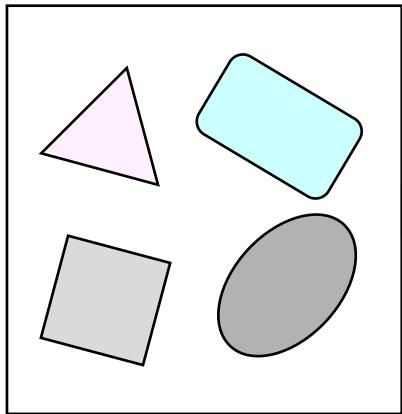
Helmut Meyer of the University of Bochum, Germany, who heads HUPO's brain-proteome project, thinks that the Chinese researchers may be biting off more than they can chew. "Sometimes it's nearly impossible to identify the protein to which an antibody binds," he says. His brain-proteome project plans to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

綠竹幼株開花前後的蛋白質表現差異

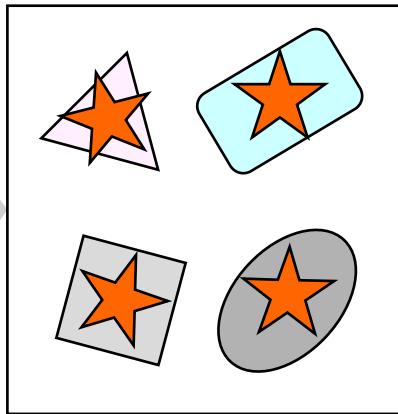


以奈米抗體晶片檢定綠竹開花前後的蛋白質體

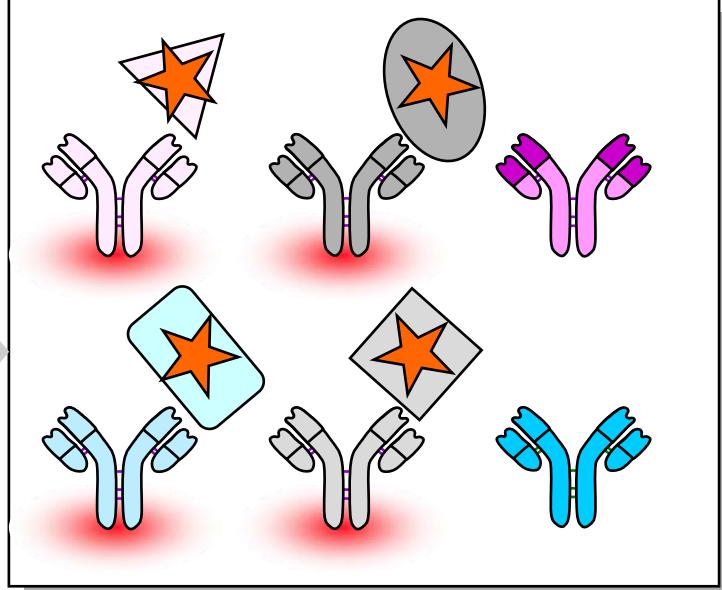
綠竹開花前
的蛋白質體



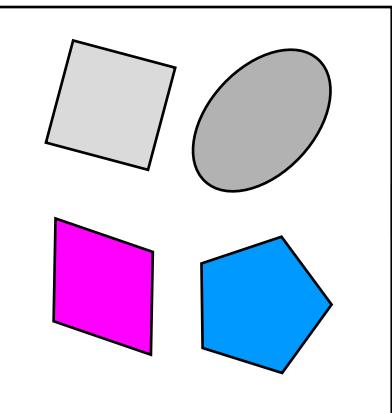
奈米標幟



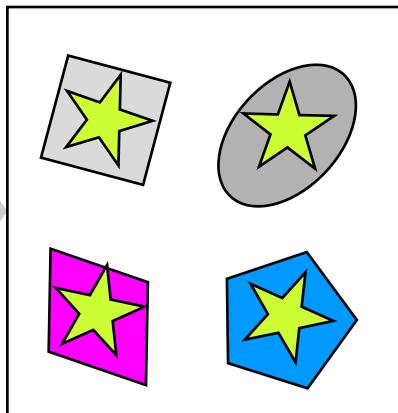
抗體晶片檢測



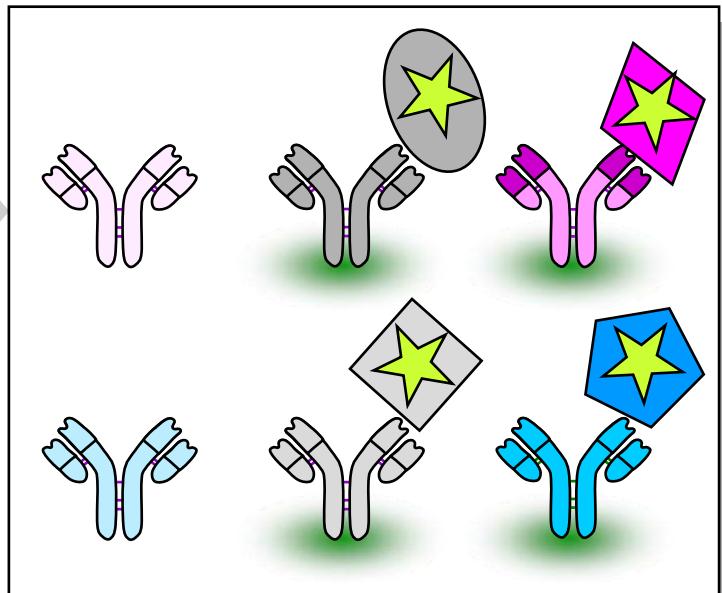
相同晶片有不同的結合圖譜



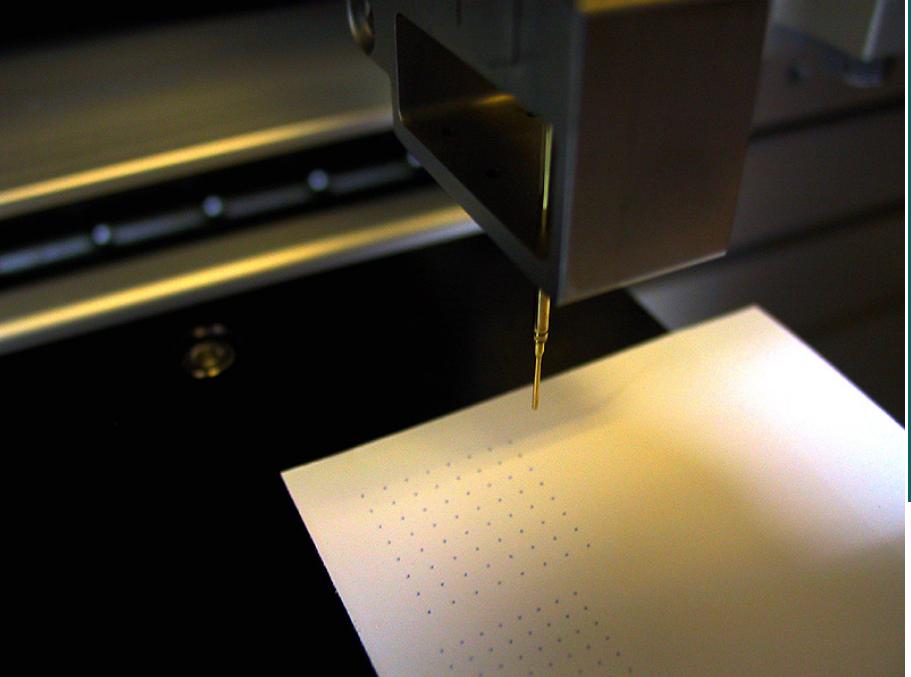
奈米標幟



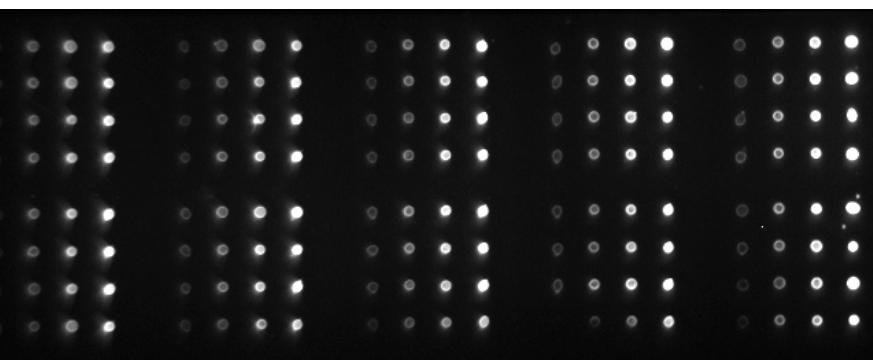
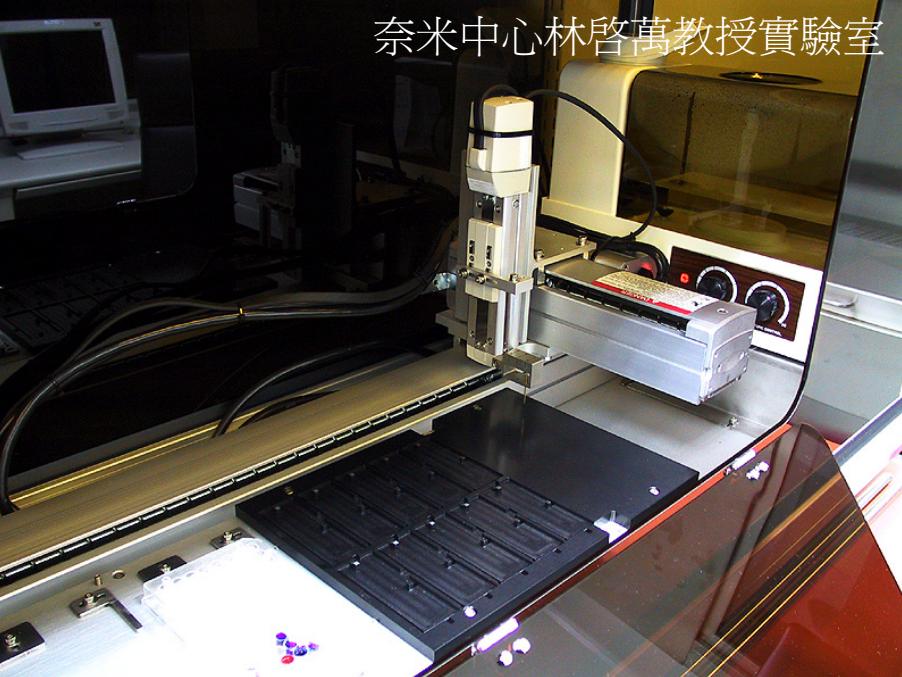
抗體晶片檢測



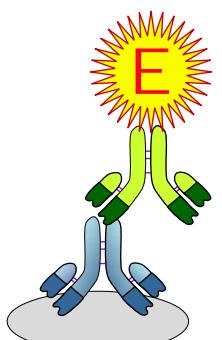
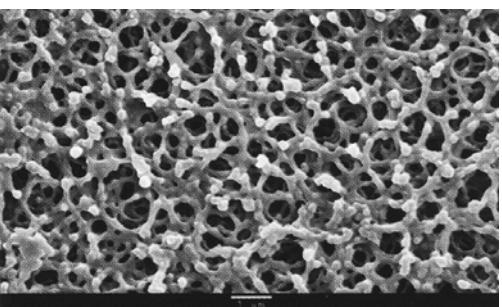
綠竹開花後
的蛋白質體



蛋白質晶片試製

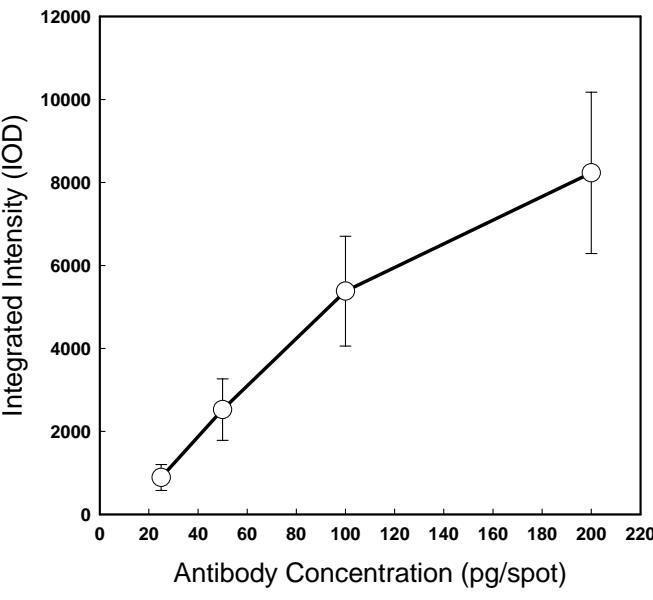


Nitrocellulose

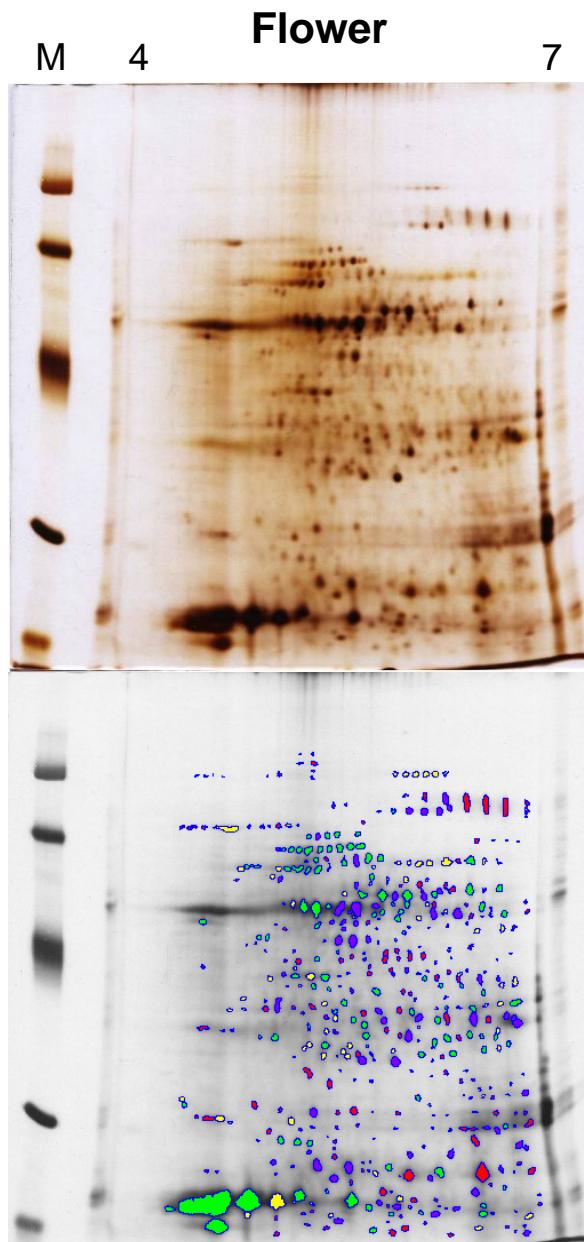
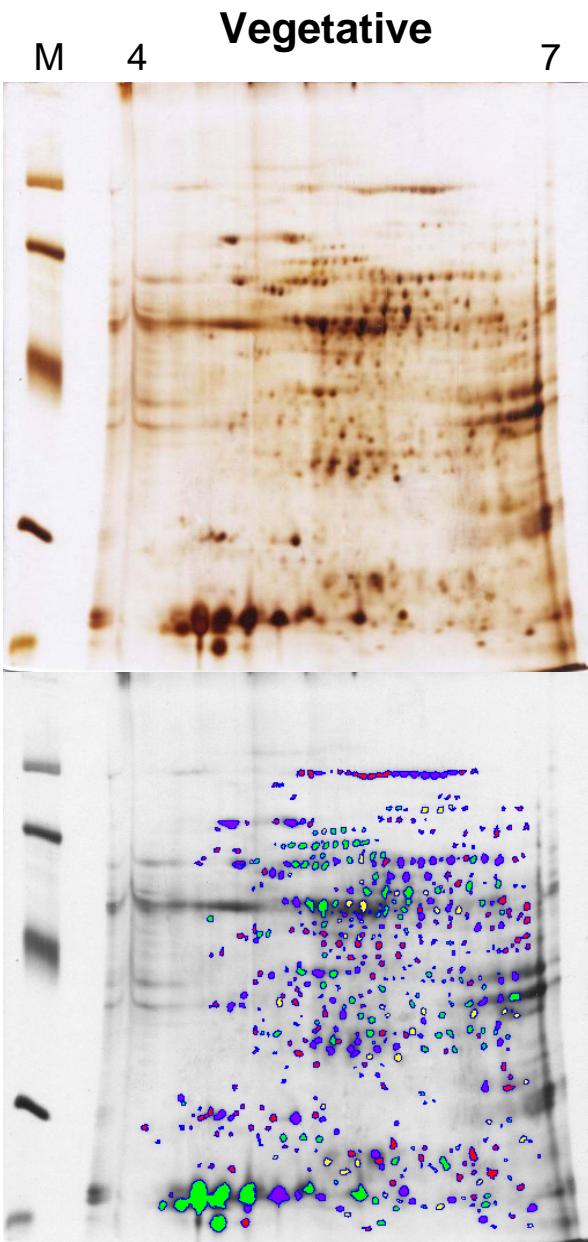


Juang RH (2005)

Schleicher & Schuell



比對綠竹開花非水溶性蛋白質圖譜的消長



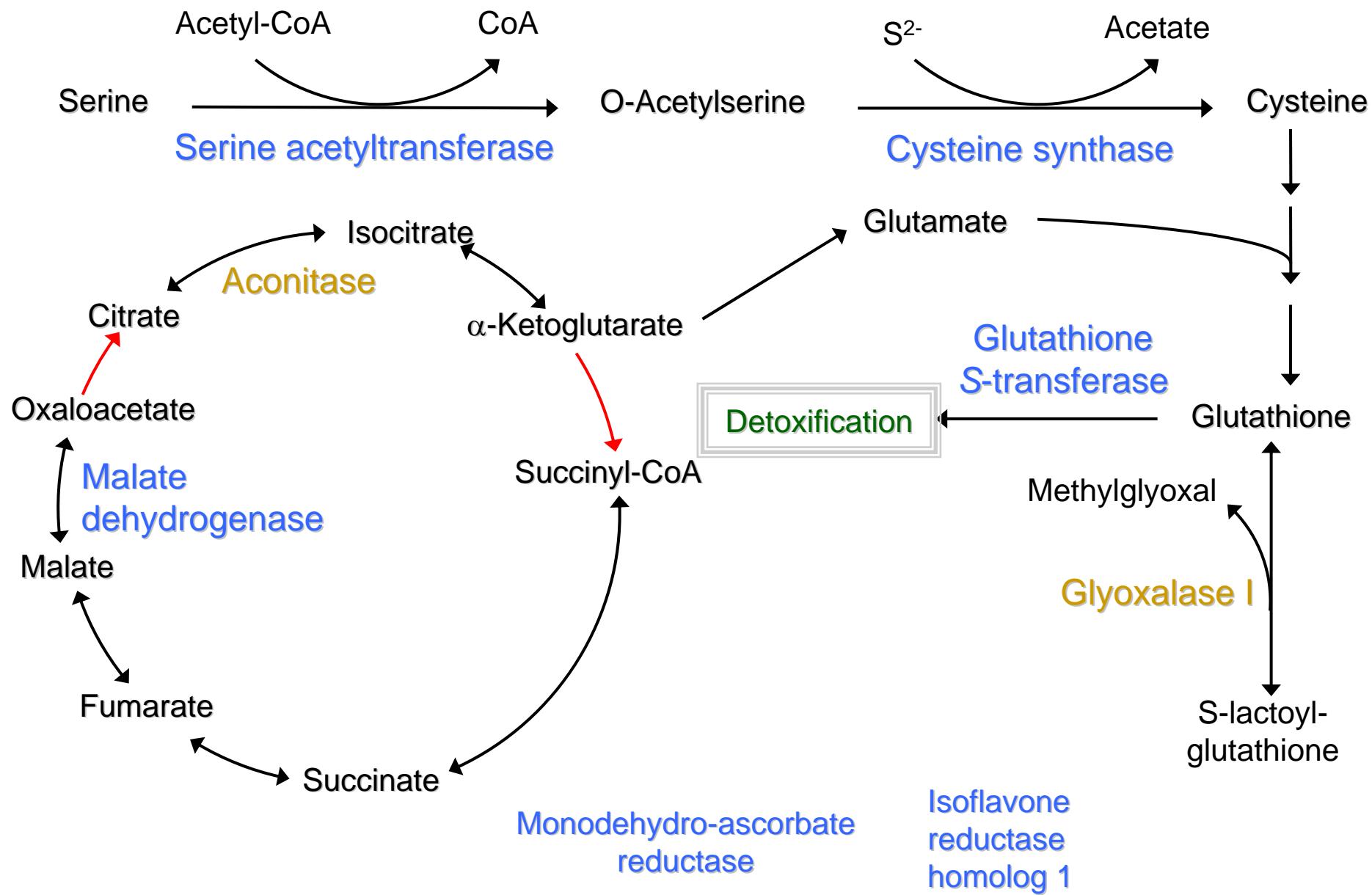
- Unmatched
- Between limits
- More than 2-fold
- Less than 2-fold

11 cm
12.5% SDS-PAGE

開花後非水溶性蛋白質差異點 LC/MS/MS 結果

Spot no.	Protein match to	Organism	Access. No.	Sequence Coverage (%)	Scores	Mw / pl (molecular weight and pl of matched protein)	calculated Mw / pl	Mechanism
F14	NADH dehydrogenase	<i>Plocosperma buxifolium</i>	CAA09900	1%	20	82.4 / 9.14	84.8 / 6.62	Photosynthesis
F15	alpha tubulin	<i>Oryza sativa</i> (japonica cultivar-group)	AAT77077	38%	445	49.8 / 4.88	48.2 / 5.69	Cytoskeleton
F16	ATP synthase CF1 alpha chain	<i>Oryza sativa</i> (japonica cultivar-group)	NP_039380	39%	749	55.6 / 5.95	57.3 / 6.46	Photosynthesis
F17	osr40c1	<i>Oryza sativa</i> (indica cultivar-group)	CAA64683	10%	155	38.8 / 6.3	39.3 / 6.75	Dry stress response
F18	Maf family protein	<i>Arabidopsis thaliana</i> (thale cress)	NP_199091	4%	27	25.8 / 4.81	26.0 / 6.05	Development of nuclear-envelope
F19	putative potassium channel protein	<i>Oryza sativa</i> (japonica cultivar-group)	XP_475262	0%	43	94.7 / 6.64	25.7 / 6.54	Voltage gate
F20	putative malate dehydrogenase	<i>Oryza sativa</i> (japonica cultivar-group)	XP_475913	12%	110	35.4 / 8.22	38.3 / 6.06	TCA cycle
F21	isoflavone reductase homolog 1	<i>Glycine max</i> (soybean)	AAF17577	3%	47	33.9 / 5.75	36.0 / 5.96	Antioxidant
F22	glucanase	<i>Oryza sativa</i>	AAK16694	3	34	34.7 / 5.92	32.1 / 5.71	
F23	actin	<i>Oryza sativa</i> (japonica cultivar-group)	AAO62546	9%	78	41.6 / 5.31	32.6 / 5.04	Cytoskeleton

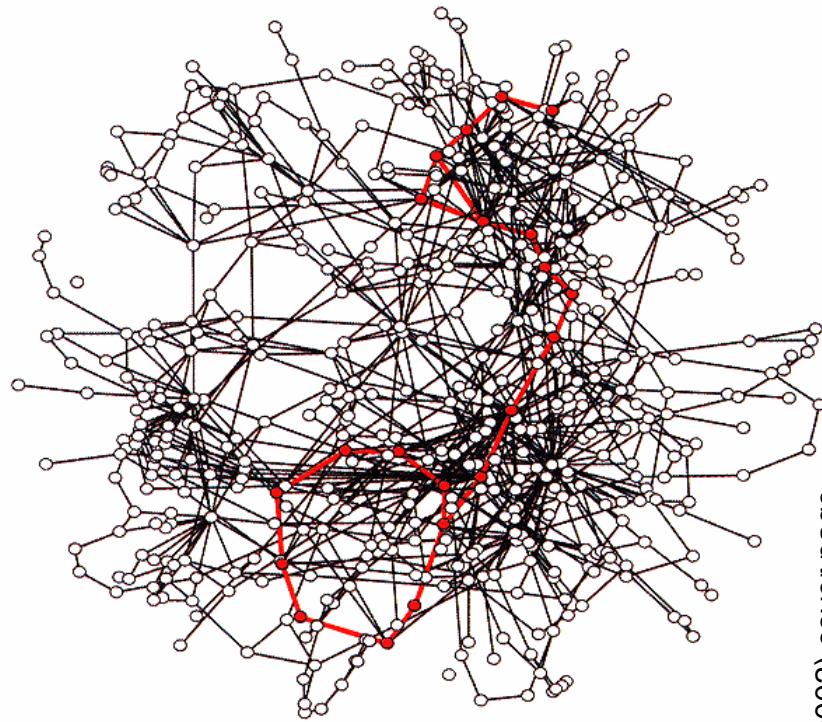
推測與綠竹開花相關之可能代謝途徑



蛋白質分子間的交互作用 Cross-Talk

代謝路徑立體圖

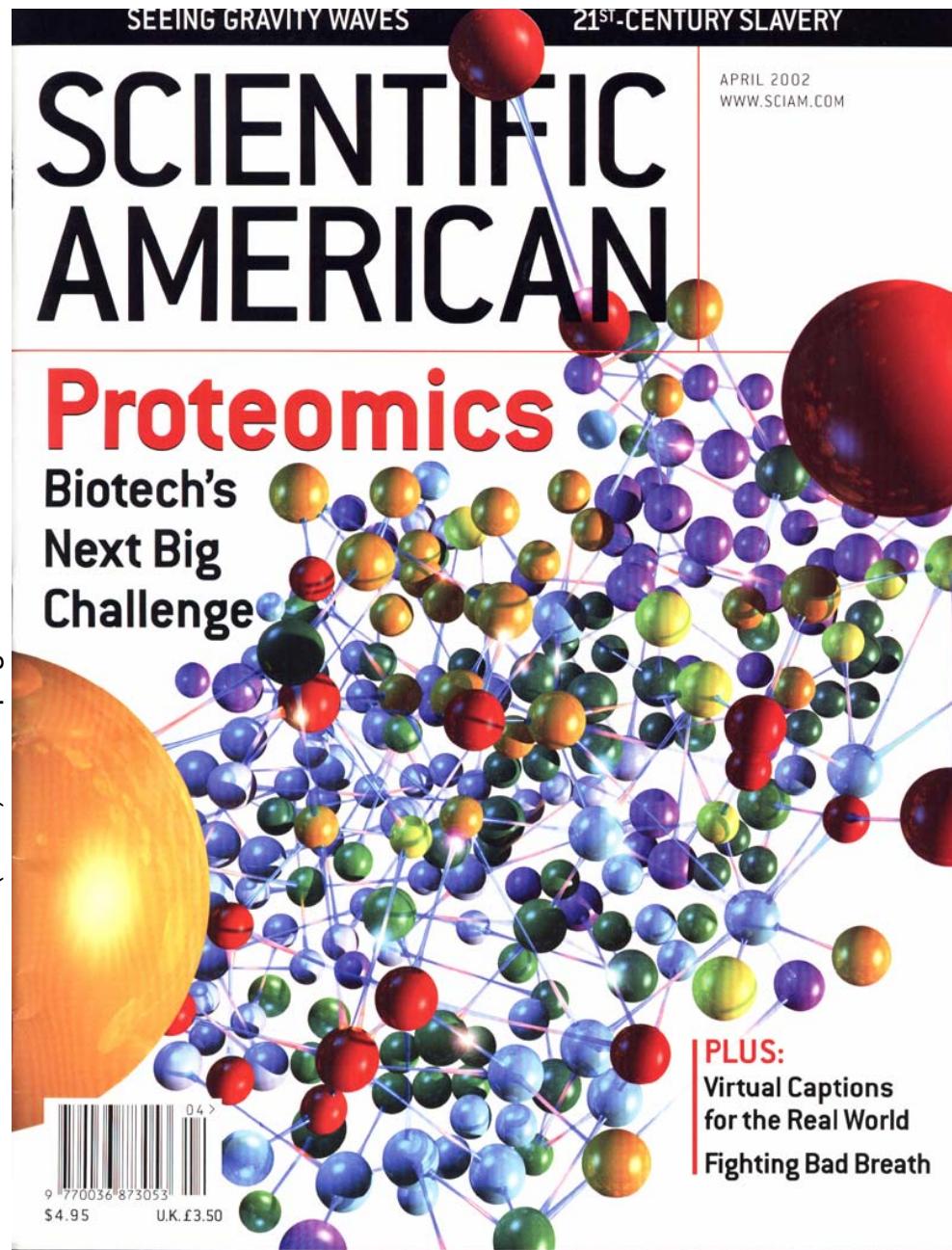
Alberts et al (2002) Molecular Biology of the Cell (4e) p.107



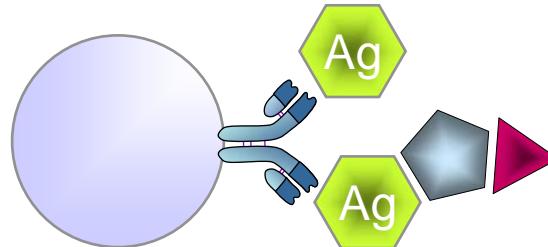
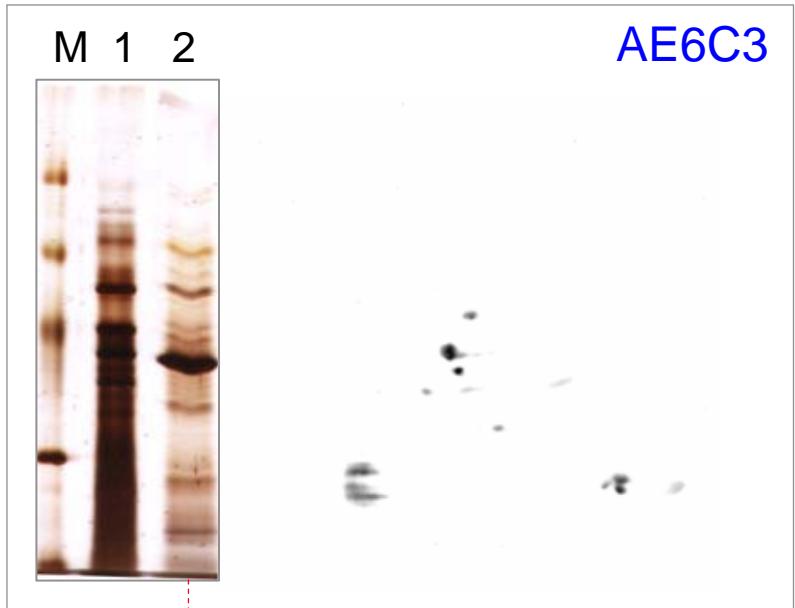
Systems Biology

整體性的生物學觀念與工具

Scientific American (2002) cover page



抗體免疫沈澱與蛋白質交互作用



Interacted proteins

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

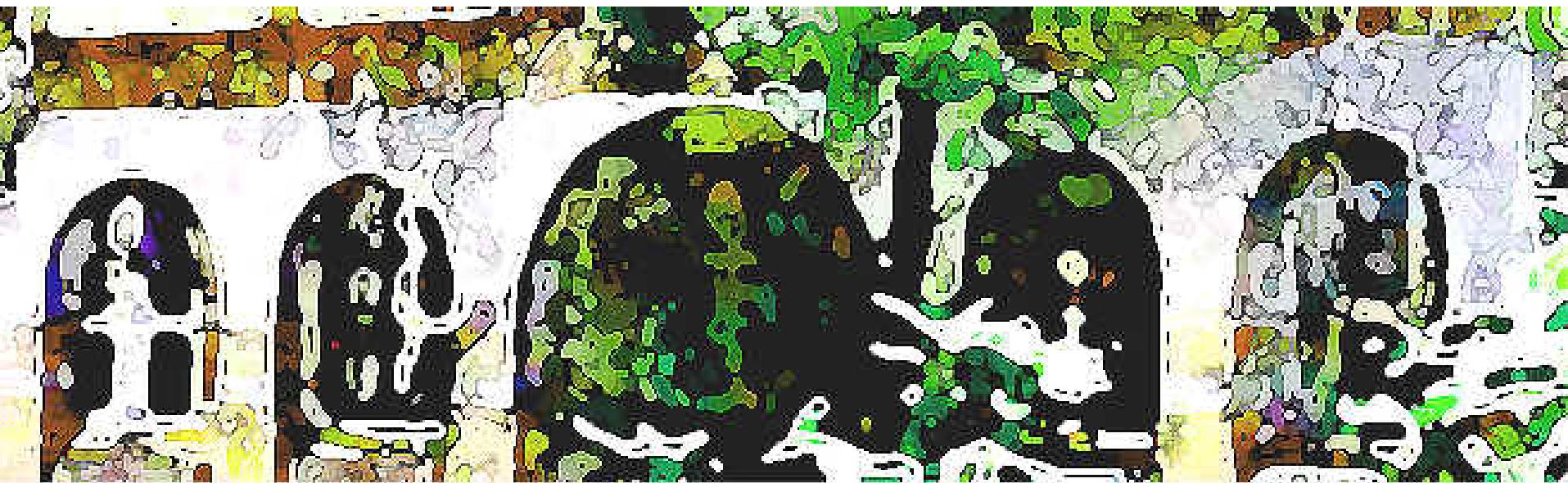
Interacted proteins

LC/MS/MS

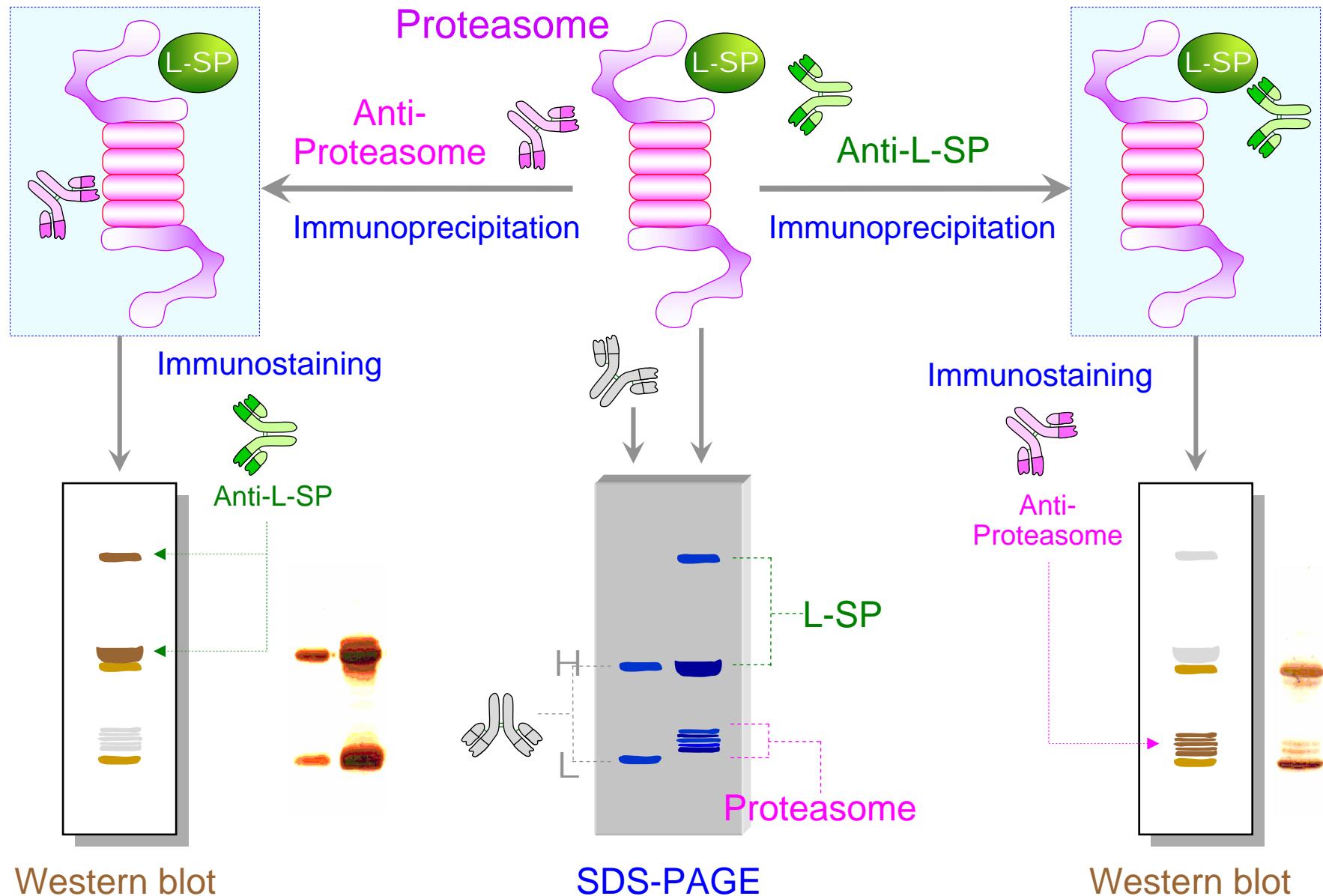
BST

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以免疫沈澱法檢定蛋白質間的交互作用



Western blot

SDS-PAGE

Western blot