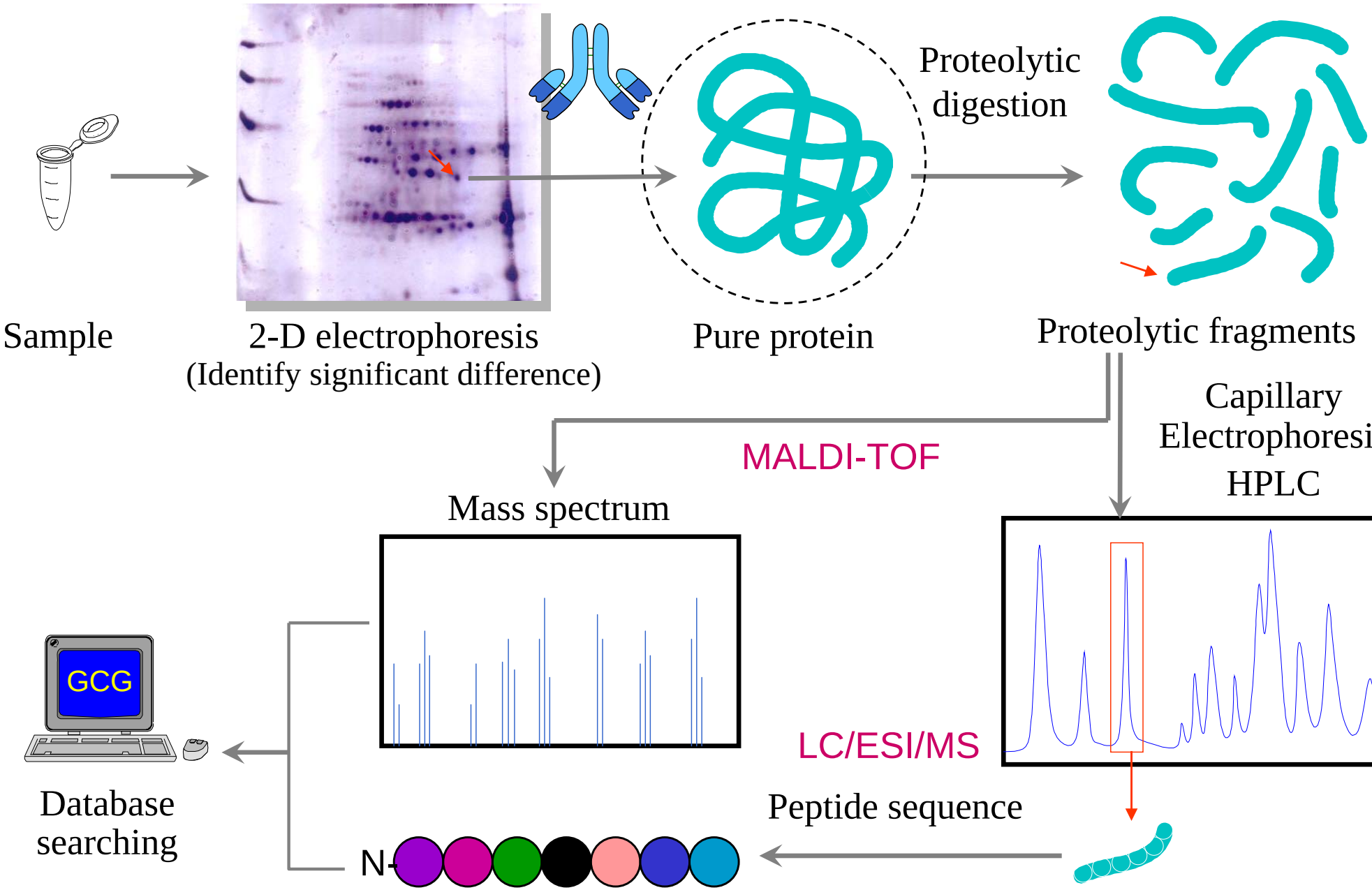
Preparation and Application of Proteomic Antibody Bank against Whole Water-Soluble Proteins from Rapid-Growing Bamboo Shoots

Yu-Jen Wu, Dai-Jer Wu, Jiann-Shing Wu and Rong-Huay Juang*

* *Major affiliation:*
Department of Biochemical Science and Technology (BST),
National Taiwan University, Taipei, Taiwan 106

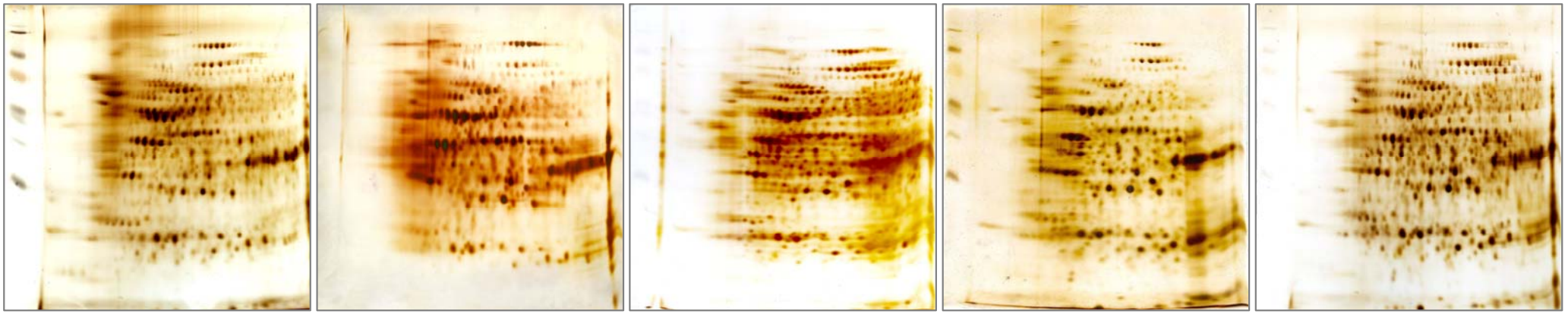
The power of proteomic tools could be enhanced



竹文化 Oriental bamboo culture

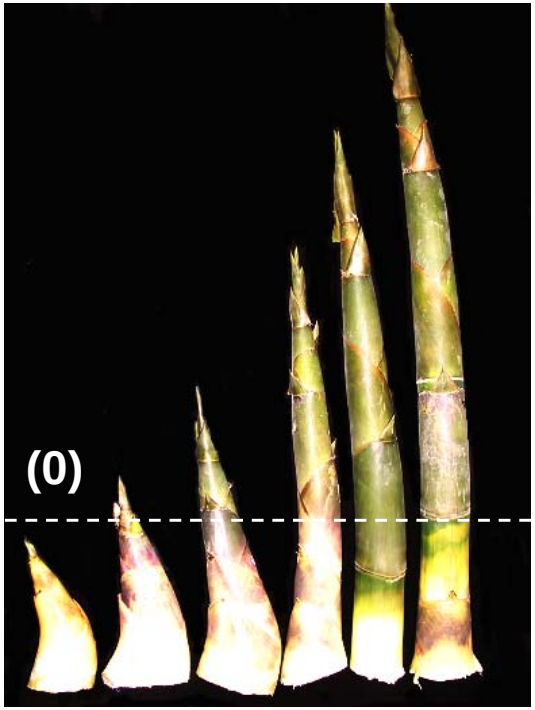
Protein pattern changes during rapid-growth period

Underground (0) | 10 cm | 20 cm | 40 cm | 60 cm



Green bamboo

Bambusa oldhamii



Cellulose biosynthesis

Unpublished (2007)

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

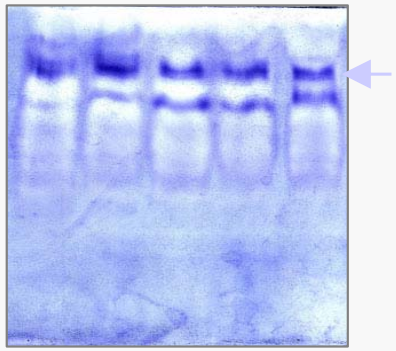
Compile 2-DE results into metabolic pathway flux

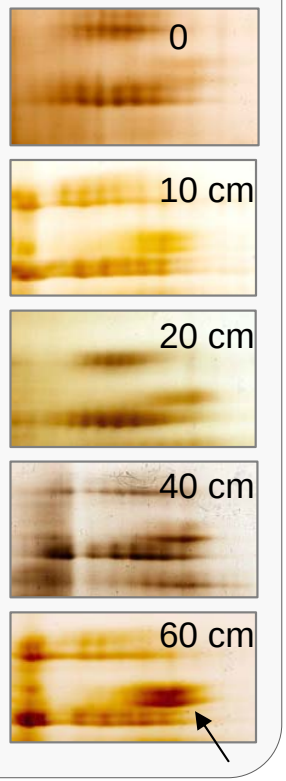
Unpublished (2007)

Sucrose synthase

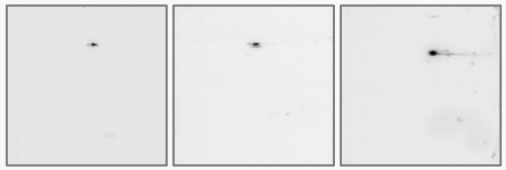
Activity staining

0 10 20 40 60 cm





Immunostaining

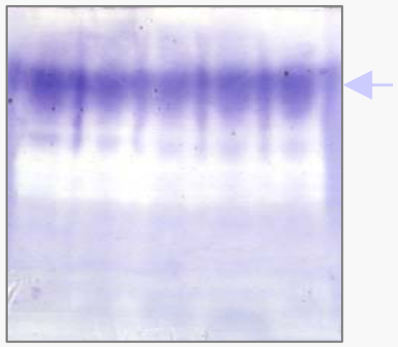


0 20 60 cm

Invertase

Activity staining

0 10 20 40 60 cm



Starch

▲ H-SP

▼ L-SP

Glc-1-P

▲ UDPGase

UDPG

▲ Sucrose synthase (SuSy)

=Invertase

Glucose

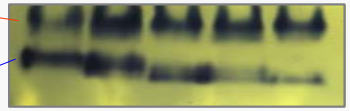
Fructose

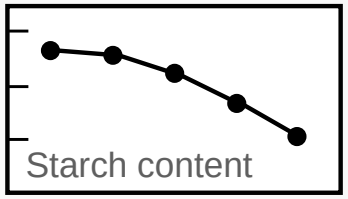
Sucrose

Starch phosphorylases

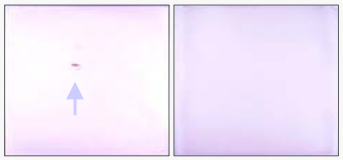
Activity staining

0 10 20 40 60 cm





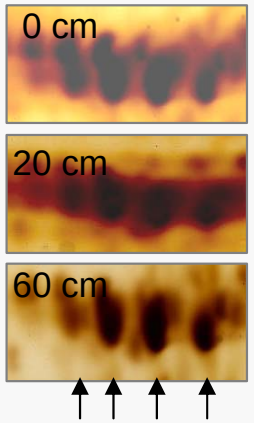
Immunostaining (L-SP)



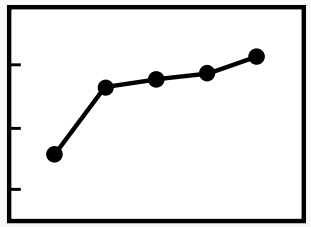
0 60 cm

UDPGase

(UDPG pyrophosphorylase)



Specific activity



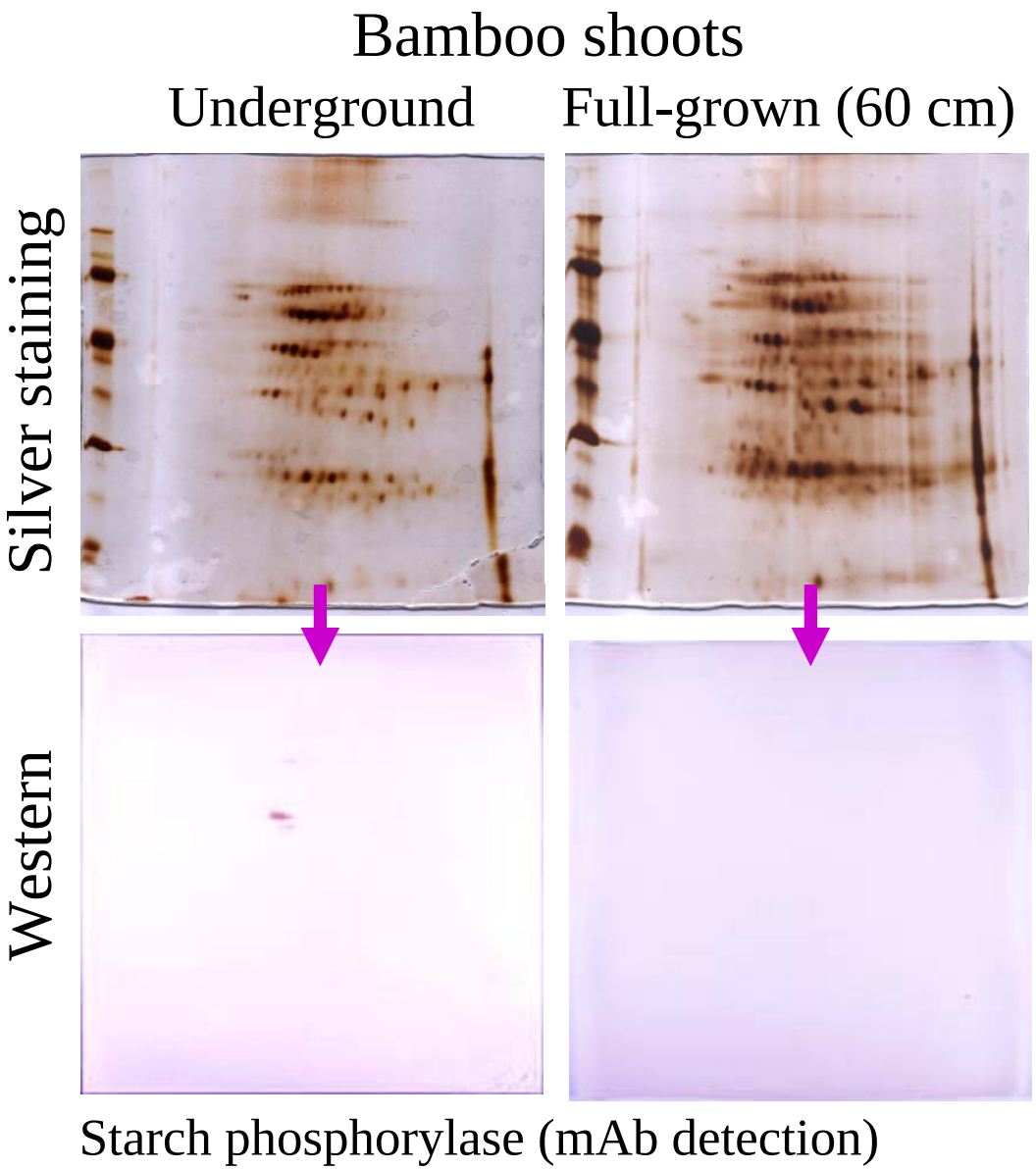
0 60 cm

UDP-glucuronate → UDP-xylose

UDP-xylose → Xyloglucan

Cell wall biosynthesis

Specific probe for every single protein?



Three possible approaches:

(1) Monoclonal antibody

(2) Phage display (phage antibody)

(3) DNA or RNA aptamer

Challenges for hybridoma technique:

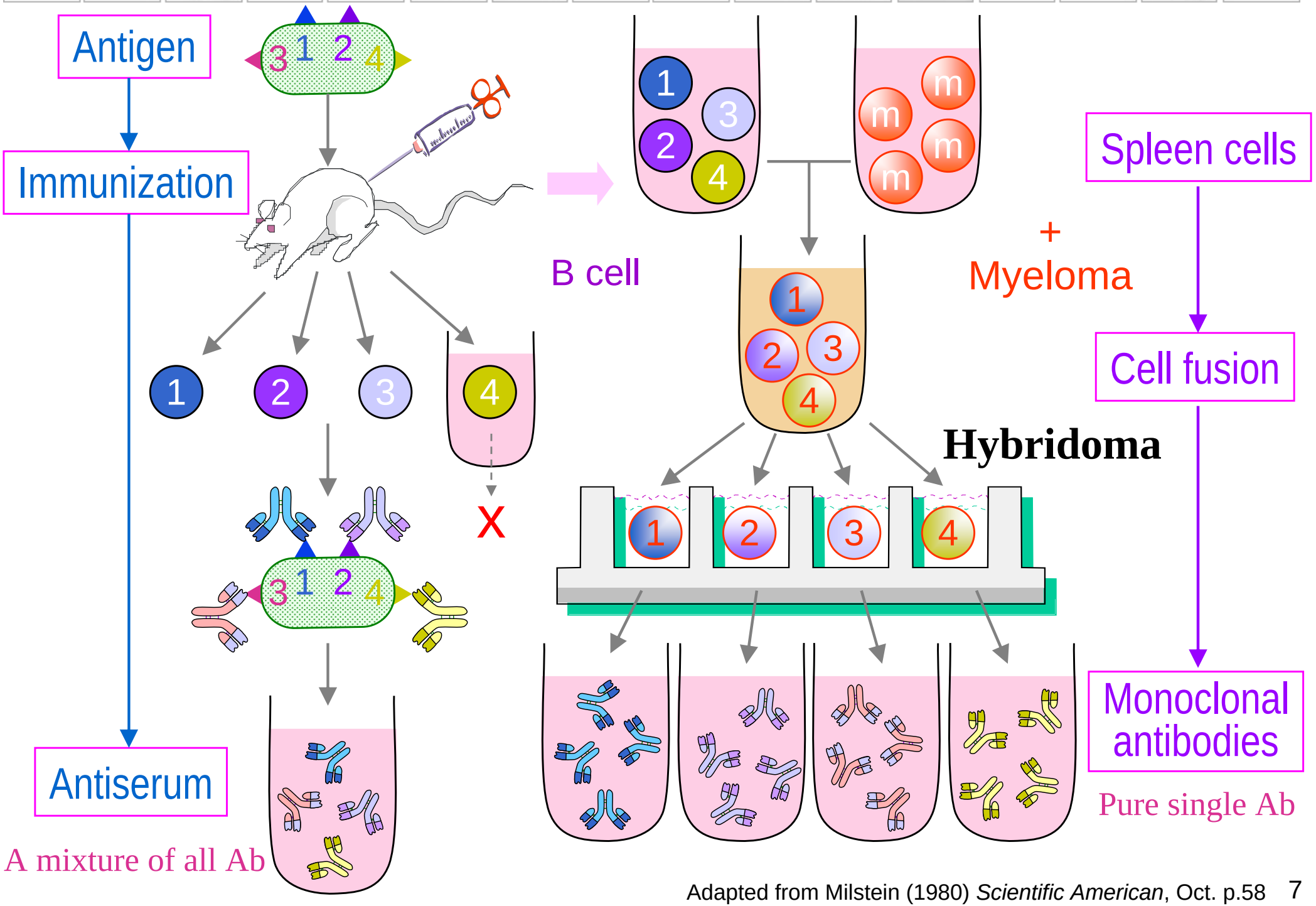
(1) Redundant and time-consuming

(2) Poor immunogenic proteins

(3) Some proteins are less abundant

Pure Ag → Single mAb → **Proteome → Ab bank**

Production of hybridoma by cell fusion



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

Screening and identification procedures

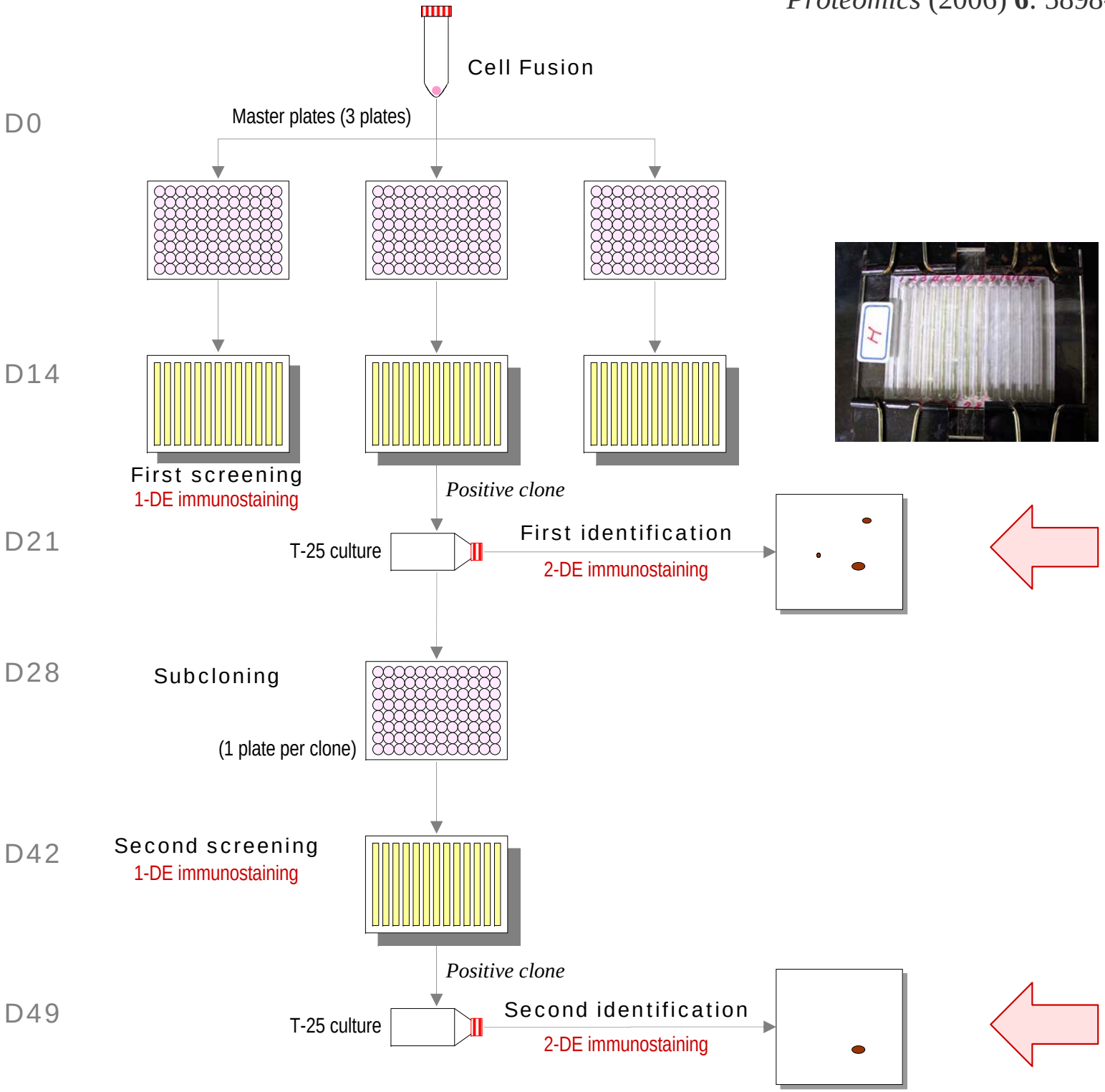


Figure 1 b

Stage-wise immunization and cell fusion protocol

Proteomics (2006) **6**: 5898-5902

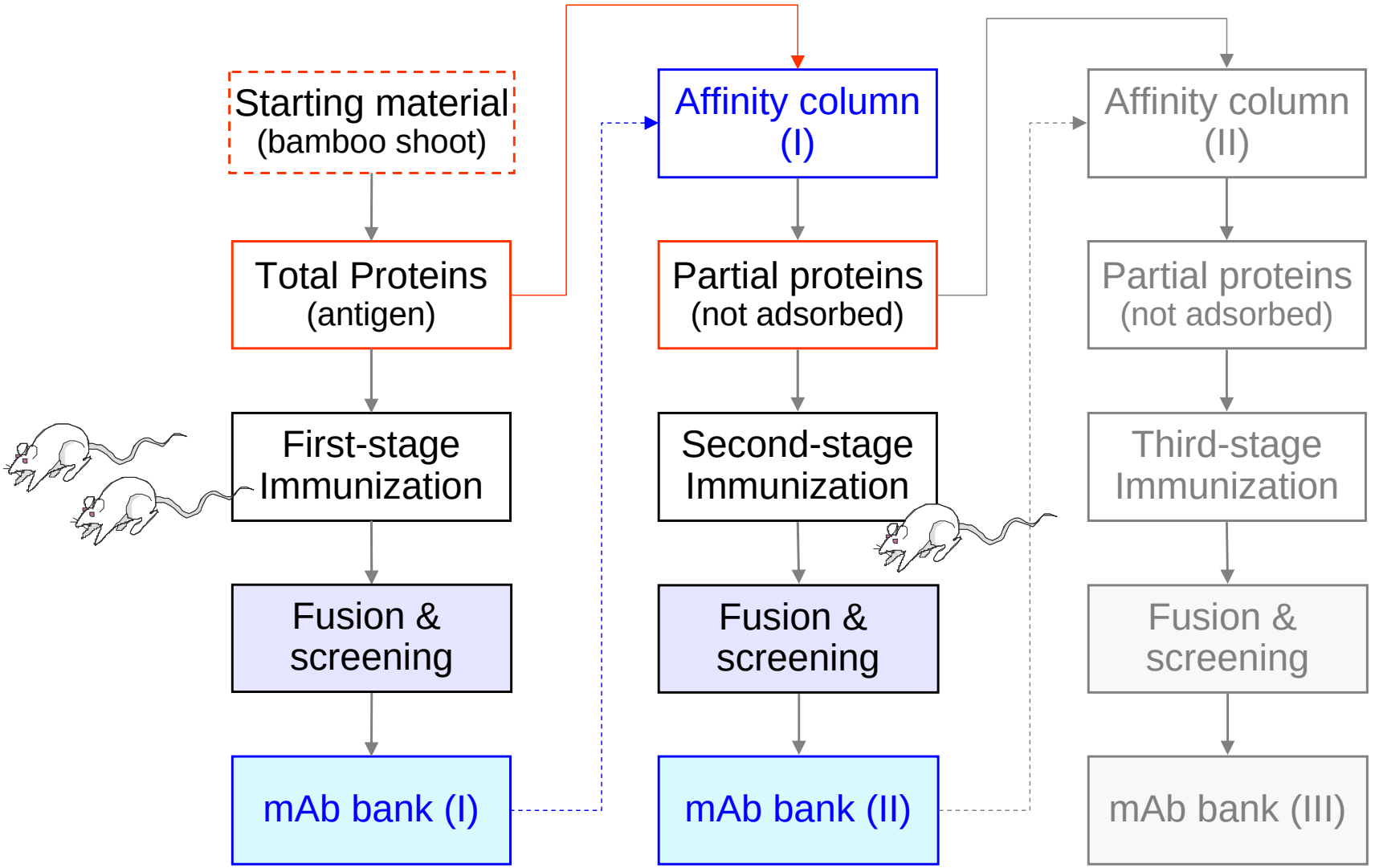
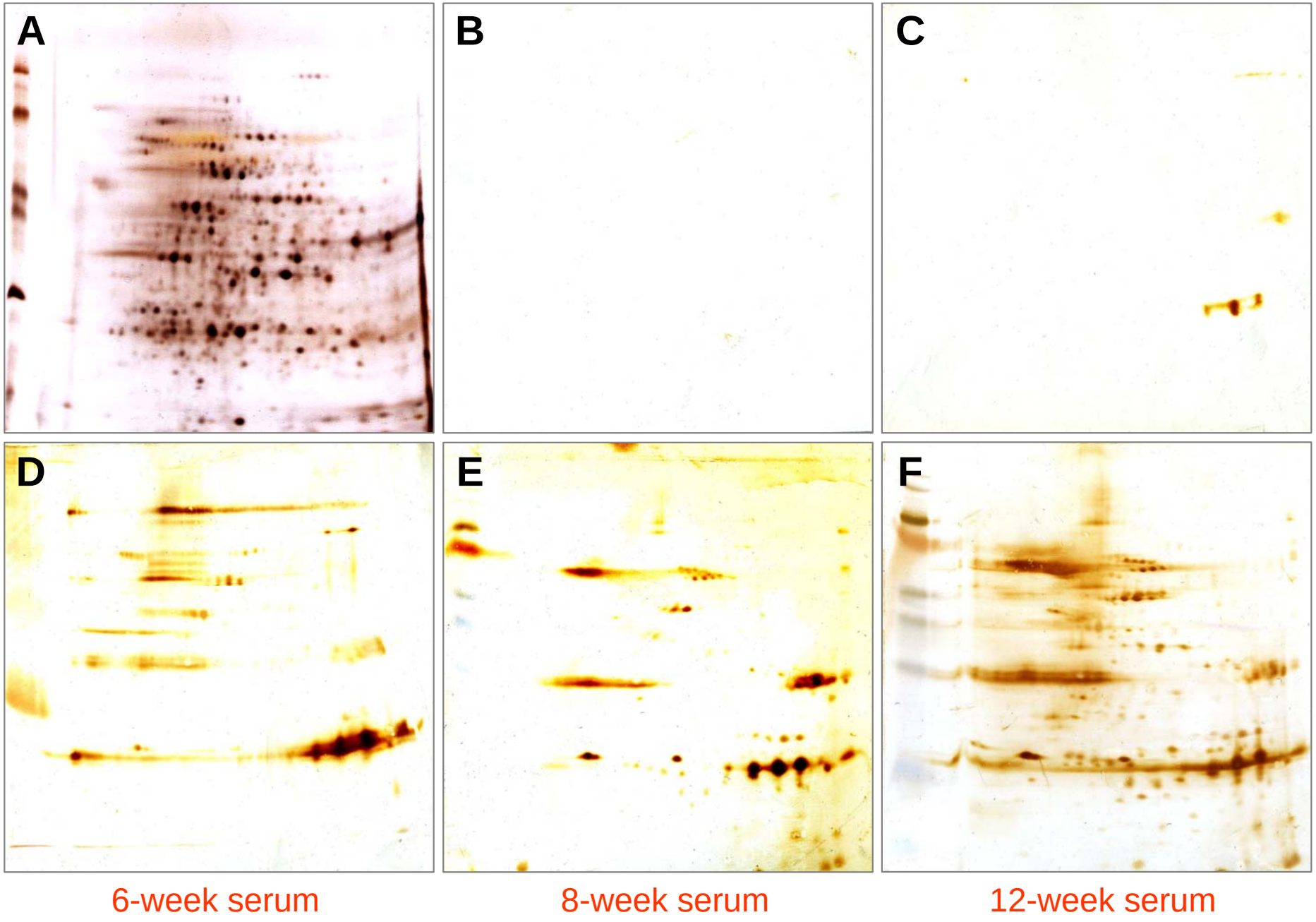


Figure 1 a

The immune response for complex antigens



Proteomics (2006) **6**: 5898-5902 (supplement)

Supplementary Figure 1

Summary for the bank production

Proteomics (2006) **6**: 5898-5902

(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

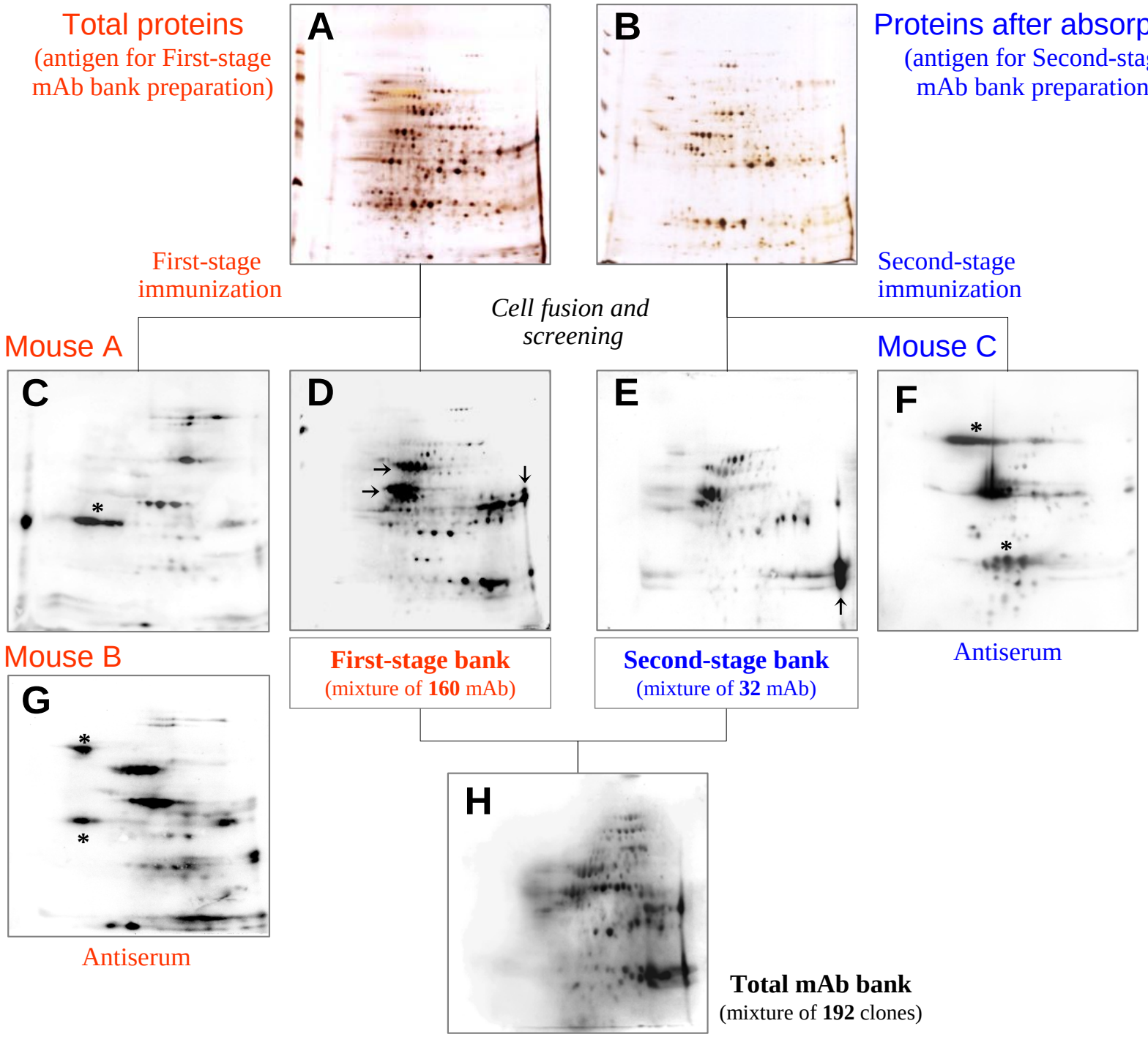
Spleen cell fused with	Sp2/0 (Mouse C)	
Number of clones	screened	positive
First screening	100	40
Second screening (after subcloning)	400	120
Final monoclonal		**32**

The library of total 192 blots in the mAb bank

Proteomics (2006) **6**: 5898-5902

In total 1,360 spots

The immune response & the mAb production

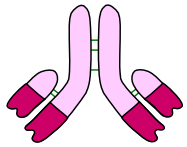


Proteomics (2006) **6**: 5898-5902

Figure 2

An example for the application

mAb H7-E3 ~GAPN



Fructose-6-phosphate

↓

Glyceraldehyde-3-phosphate

Phosphorylating glyceraldehyde-3-phosphate dehydrogenase **(GAPDH)**

1 **NAD⁺ & P_i** → **NADH**

Glyceraldehyde-1,3-bisphosphate

3-Phosphoglycerate kinase

2 **ADP** → **ATP**

3-Phsophoglycerate

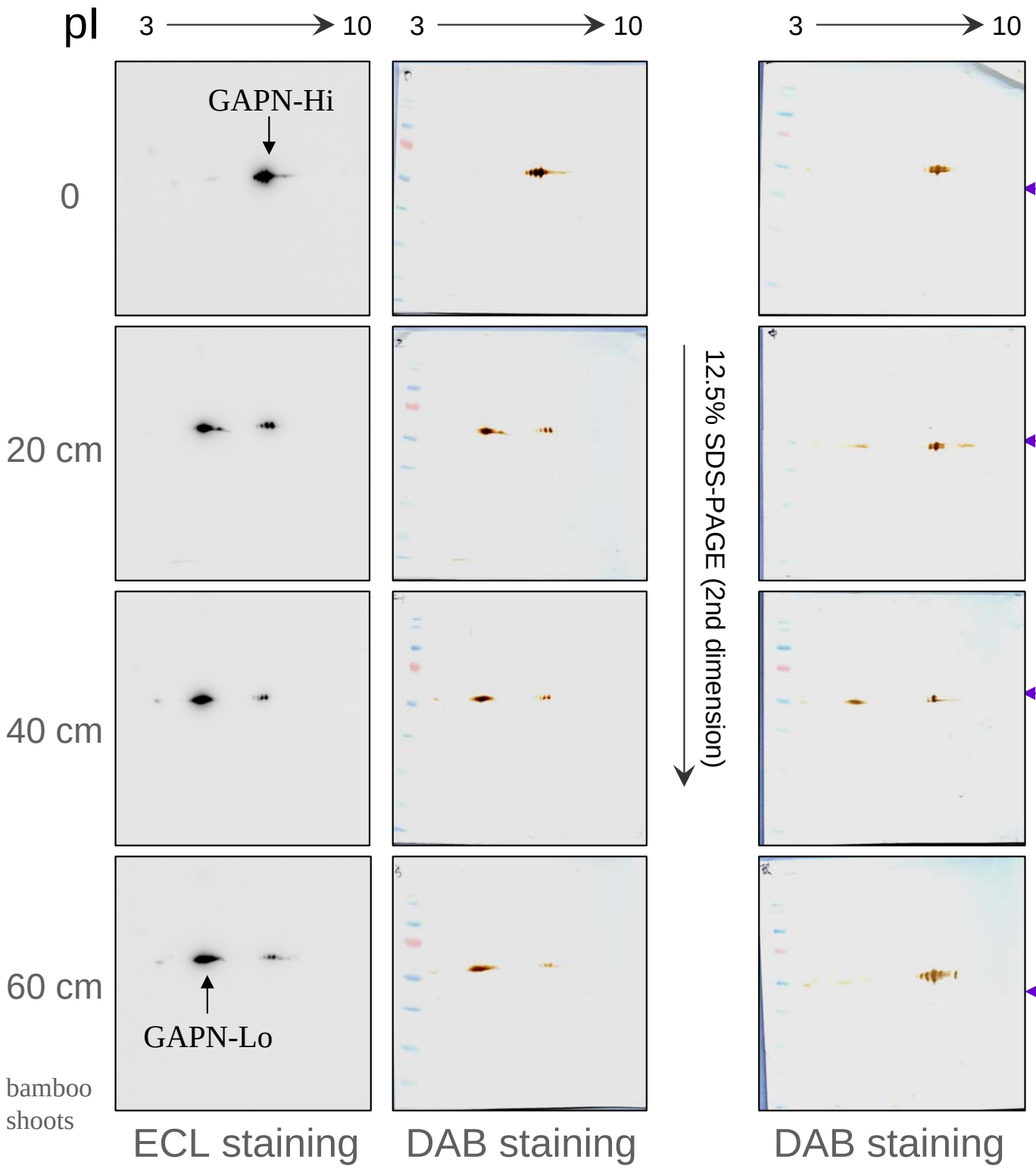
↓

Pyruvate

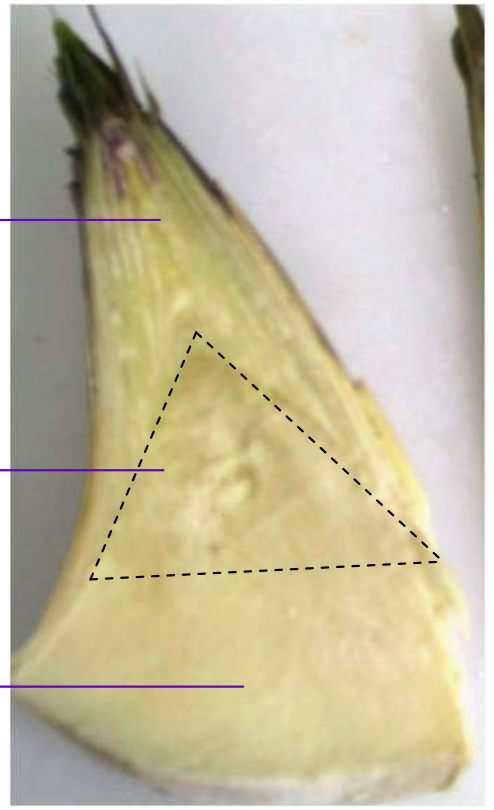
3 **NADP⁺** → **NADPH**

3 GAPN:
Nonphosphorylating Glyceraldehyde-3-phosphate dehydrogenase

Glyceraldehyde-3-P dehydrongenase (GAPN)



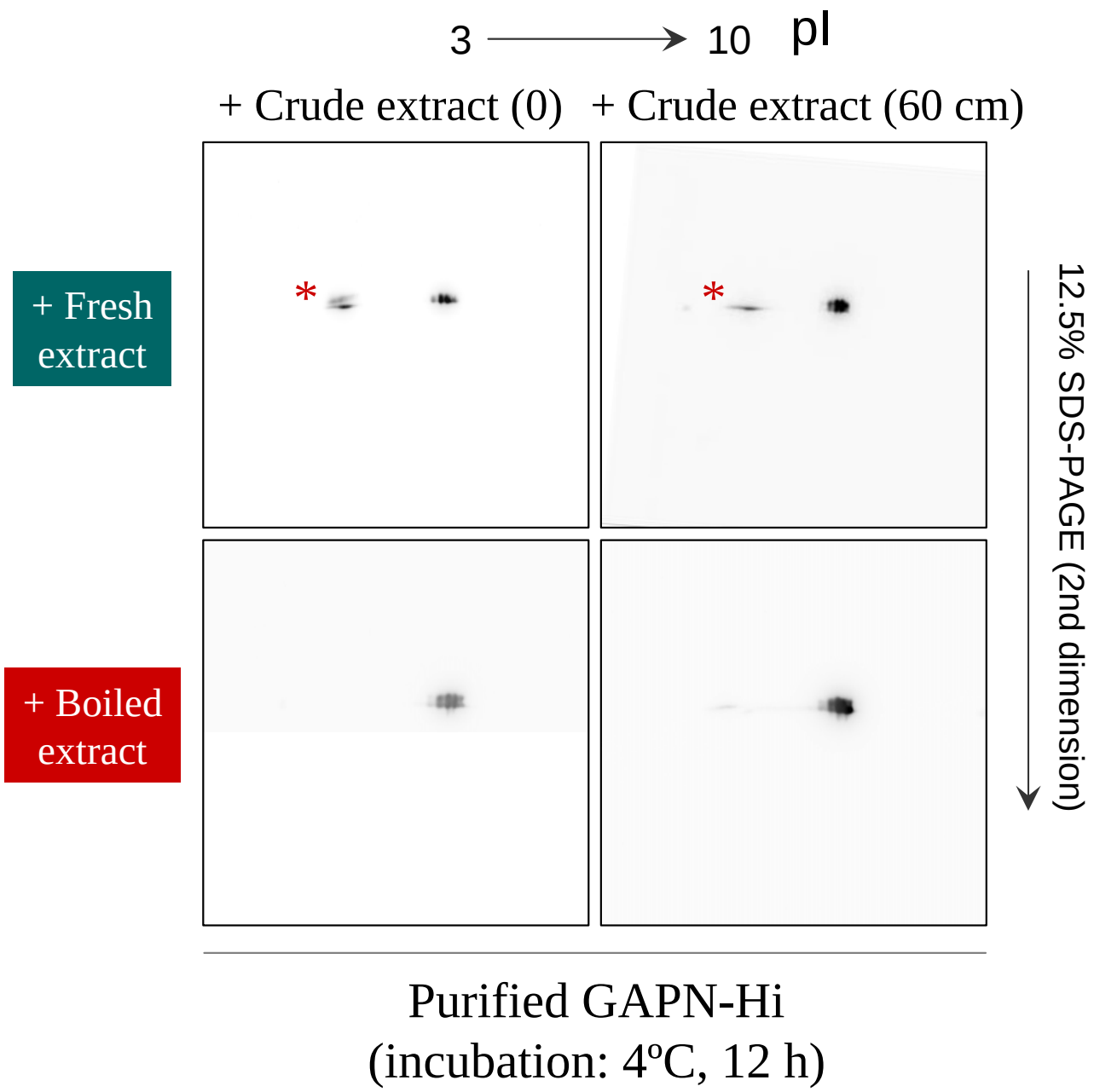
mAb H7-E3



Bamboo shoot (0)

Bamboo leaf

Purified GAPN-Hi was transform into GAPN-Lo



* GAPN-Lo

Proof of the concept "The proteomic mAb bank"

Conclusions:

(1) We have successfully prepared 192 hybridoma cell lines which produce specific mAb against complex proteins in a proteome

(2) The screening and identification procedures were critical in selecting useful mAb lines

(3) Stage-wise immunization and cell fusion worked successfully for the poor antigens

(4) This work was accomplished by two full-time research staffs in 18 months

(5) The number of antibodies in the final bank could be increased if more resource is invested and automation is implemented

Possible limitations

(1) The reliability of the technique depends on how the total protein are prepared, since proteins are heterogeneous in properties

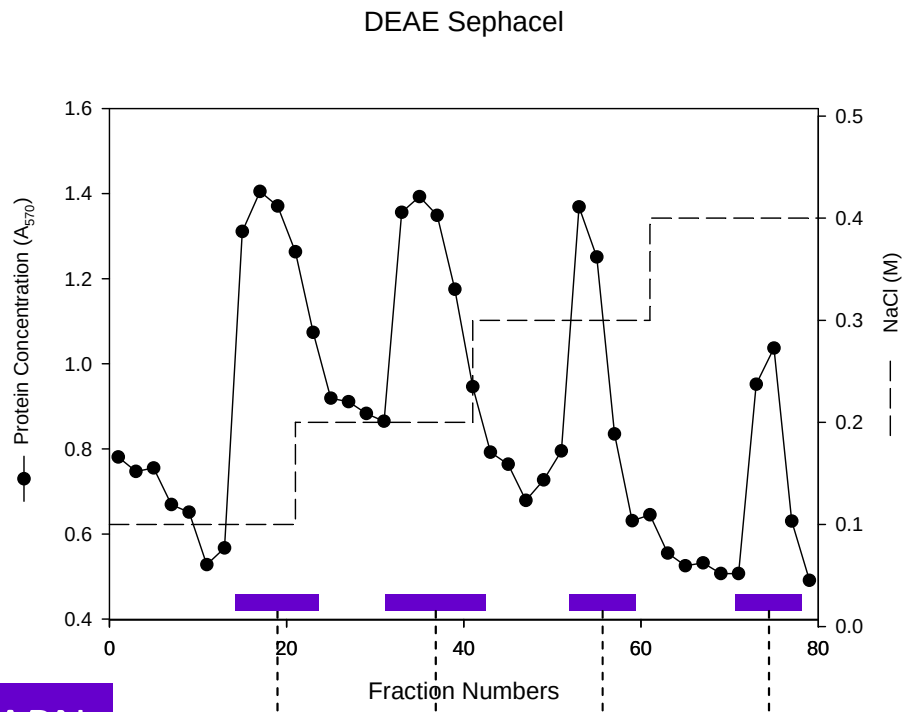
(2) 2-DE is not perfect which could not faithfully display all proteins in a gel at the same time

(3) Immune system can not distinguish between proteins with high homogeneity in their sequences

(4) Some antibodies might not recognize the native conformation of their antigen

(5) Automation for the 2-DE and Western detection is urgently needed, however, the current progress is very limiting

Purification of enzyme X by DEAE Sephacel



13 cm strip, pH 3-10
12.5% SDS-PAGE
First Ab 1:2,000 (H7-E3)
Second Ab 1:5,000
(Goat anti-mouse IgG)

Fractions added to GAPN