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

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The power of proteomic tools could be enhanced.

Sample → 2-D electrophoresis (Identify significant difference) → Pure protein → Proteolytic digestion → Proteolytic fragments → Capillary Electrophoresis HPLC

MALDI-TOF → Mass spectrum → LC/ESI/MS → Peptide sequence → Database searching

Proteolytic digestion → GCG
竹文化 Oriental bamboo culture
## Protein pattern changes during rapid-growth period

<table>
<thead>
<tr>
<th>Underground (0)</th>
<th>10 cm</th>
<th>20 cm</th>
<th>40 cm</th>
<th>60 cm</th>
</tr>
</thead>
</table>

### Green bamboo

**Bambusa oldhamii**

Unpublished (2007)

#### Cellulose biosynthesis

<table>
<thead>
<tr>
<th>Protein ID</th>
<th>Accession no.</th>
<th>Calculated Mr (kD) / pI</th>
<th>Sequence coverage (%)</th>
<th>Score (MASCOT)</th>
<th>Match</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>Sucrose synthase</td>
<td>AAV64256 <em>(Bambusa oldhamii)</em></td>
<td>92.8 / 6.03</td>
<td>35</td>
<td>402</td>
</tr>
<tr>
<td>80</td>
<td>Sucrose synthase</td>
<td>AAV64256 <em>(Bambusa oldhamii)</em></td>
<td>92.8 / 6.03</td>
<td>35</td>
<td>245</td>
</tr>
<tr>
<td>82</td>
<td>Sucrose synthase</td>
<td>AAV64256 <em>(Bambusa oldhamii)</em></td>
<td>92.8 / 6.03</td>
<td>35</td>
<td>1112</td>
</tr>
<tr>
<td>8</td>
<td>UDP-glucose-pyrophosphorylase</td>
<td>BAB69069 <em>(Oryza sativa)</em></td>
<td>51.6 / 5.4</td>
<td>18</td>
<td>302</td>
</tr>
<tr>
<td>9</td>
<td>UDP-glucose-pyrophosphorylase</td>
<td>BAB69069 <em>(Oryza sativa)</em></td>
<td>51.6 / 5.4</td>
<td>17</td>
<td>359</td>
</tr>
<tr>
<td>10</td>
<td>UDP-glucose-pyrophosphorylase</td>
<td>BAB69069 <em>(Oryza sativa)</em></td>
<td>51.6 / 5.4</td>
<td>21</td>
<td>408</td>
</tr>
<tr>
<td>11</td>
<td>UDP-glucose-pyrophosphorylase</td>
<td>BAB69069 <em>(Oryza sativa)</em></td>
<td>51.6 / 5.4</td>
<td>20</td>
<td>377</td>
</tr>
</tbody>
</table>
Compile 2-DE results into metabolic pathway flux

**Sucrose synthase**

Activity staining

0 10 20 40 60 cm

Immunostaining

0 20 60 cm

**Starch**

Activity staining

0 10 20 40 60 cm

**Starch phosphorylases**

Immunostaining (L-SP)

**Sucrose synthase**

(SuSy)

**H-SP**

**L-SP**

**UDPGase**

(UDPG pyrophosphorylase)

Specific activity

0 60 cm

**Cell wall biosynthesis**

UDP-glucuronate

UDP-xylose

Xyloglucan

**Invertase**

Activity staining

0 10 20 40 60 cm

**Starch phosphorylases**

**Starch content**

0 60 cm

**UDPG**

**UDP-glucuronate**

**UDP-xylose**

**Xyloglucan**

**Glc-1-P**

**UDPGase**

**Glucose**

**Fructose**

**Invertase**

**Activity staining**

0 10 20 40 60 cm

**Immunostaining**

0 60 cm

**Unpublished (2007)**
Specific probe for every single protein?

Bamboo shoots
Underground      Full-grown (60 cm)

Silver staining

Western

Starch phosphorylase (mAb detection)

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


**Immunization**

Antigen

Antigen is injected into a mouse, resulting in the production of B cells that produce antibodies against the antigen.

**Cell fusion**

Spleen cells from the immunized mouse are mixed with myeloma cells. This mixture is then subjected to cell fusion, leading to the formation of hybridoma cells.

**Hybridoma**

The hybridoma cells are clones of the B cell and myeloma cells, each producing monoclonal antibodies against the antigen.

**Monoclonal antibodies**

The hybridoma cells are cloned to produce a pure single antibody.

**Antiserum**

A mixture of all antibodies is produced from the spleen cells.

**B cell**

The B cells are the antibody-producing cells.

**Myeloma**

The myeloma cells provide an immortal cell line for the hybridoma cells.

**Antigen**

The antigen is the substance that induces the immune response.

**Spleen cells**

These are the cells from the spleen that are used in the cell fusion process.
Screening and identification procedures

**Figure 1 b**

Cell Fusion

**D0**
- Master plates (3 plates)

**D14**
- First screening
  - 1-DE immunostaining

**D21**
- T-25 culture
- First identification
  - 2-DE immunostaining
- Positive clone

**D28**
- Subcloning
  - (1 plate per clone)

**D42**
- Second screening
  - 1-DE immunostaining

**D49**
- T-25 culture
- Second identification
  - 2-DE immunostaining
- Positive clone

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

Note: The diagram illustrates the process of cell fusion, screening, identification, and subcloning over a period from D0 to D49, with specific steps involving 1-DE and 2-DE immunostaining.
Stage-wise immunization and cell fusion protocol

**Starting material (bamboo shoot)**

- **Total Proteins (antigen)**
  - **First-stage Immunization**
    - **Fusion & screening**
      - **mAb bank (I)**
  - **Affinity column (I)**
    - **Partial proteins (not adsorbed)**
      - **Second-stage Immunization**
        - **Fusion & screening**
          - **mAb bank (II)**
  - **Affinity column (II)**
    - **Partial proteins (not adsorbed)**
      - **Third-stage Immunization**
        - **Fusion & screening**
          - **mAb bank (III)**

**First stage**

**Second stage**

(Third stage)
The immune response for complex antigens

Supplementary Figure 1

Proteomics (2006) 6: 5898-5902 (supplement)
# Summary for the bank production

## (A) First-stage

<table>
<thead>
<tr>
<th>Spleen cell fused with</th>
<th>NS0/1 (Mouse A)</th>
<th>Sp2/0 (Mouse B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of clones</td>
<td>screened</td>
<td>positive</td>
</tr>
<tr>
<td>First screening</td>
<td>150</td>
<td>100</td>
</tr>
<tr>
<td>Second screening</td>
<td>320</td>
<td>150</td>
</tr>
<tr>
<td>(after subcloning)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final monoclonal</td>
<td></td>
<td><strong>78</strong></td>
</tr>
</tbody>
</table>

## (B) Second-stage

<table>
<thead>
<tr>
<th>Spleen cell fused with</th>
<th>Sp2/0 (Mouse C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of clones</td>
<td>screened</td>
</tr>
<tr>
<td>First screening</td>
<td>100</td>
</tr>
<tr>
<td>Second screening</td>
<td>400</td>
</tr>
<tr>
<td>(after subcloning)</td>
<td></td>
</tr>
<tr>
<td>Final monoclonal</td>
<td></td>
</tr>
</tbody>
</table>
The library of total 192 blots in the mAb bank

In total 1,360 spots
The immune response & the mAb production

Mouse A

First-stage immunization

Total proteins (antigen for First-stage mAb bank preparation)

A

Cell fusion and screening

B

Proteins after absorption (antigen for Second-stage mAb bank preparation)

Mouse C

Second-stage immunization

C

First-stage bank (mixture of 160 mAb)

D

Antiserum

Mouse B

First-stage immunization

E

Second-stage bank (mixture of 32 mAb)

F

Antiserum

G

Antiserum

H

Total mAb bank (mixture of 192 clones)

Proteomics (2006) 6: 5898-5902
mAb H7-E3

~ GAPN

An example for the application

Fructose-6-phosphate

\[ \text{Glyceraldehyde-3-phosphate} \]

\[ \text{Pyruvate} \]

\[ \text{NADPH} \leftarrow \text{NADP}^+ \]

\[ \text{ADP} \rightarrow \text{ATP} \]

\[ \text{Pi} \rightarrow \text{NAD}^+ \]

\[ \text{mAb H7-E3} \]

\[ \sim \text{GAPN} \]

\[ \text{Phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPDH)} \]

\[ \text{3-Phosphoglycerate kinase} \]

\[ \text{3-Phosphoglycerate} \]

\[ \text{Nonphosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPD)} \]

\[ \text{NADPH} \]

\[ \text{GAPN:} \]

Nonphosphorylating glyceraldehyde-3-phosphate dehydrogenase
Glyceraldehyde-3-P dehydrogenase (GAPN)

mAb H7-E3

ECL staining

DAB staining

Unpublished (2007)
Purified GAPN-Hi was transform into GAPN-Lo

Unpublished (2007)
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)