Protein Identification Using the NanoMate™ 100 System Following 2-D Gel Separation

Automated Nanoelectrospray for Proteomics

Advion BioSciences, Inc. (Ithaca, NY) has demonstrated use of the NanoMate™ 100 system for protein identification from excised two-dimensional gel spots. The NanoMate 100 is an automated sample-handling system coupled with the ESI Chip™ (a 10 x 10 array of nano-electrospray nozzles etched in silicon).

2-D gel electrophoresis is a common means of resolving complex protein mixtures. Nanoelectrospray mass spectrometry is ideal for analyzing 2-D gel spots due to its low sample consumption and increased sensitivity through enhanced ionization efficiency. The manual nature of conventional nanospray, however, limits practical application of this technique for high throughput protein identification. The NanoMate 100 system automates nanoelectrospray to increase sample throughput and eliminate carryover between samples for this application.

Identification of Yeast Proteins

Yeast crude cell extract was prepared and run by 2-D gel separation. Excised spots were in-gel digested, extracted, and analyzed by automated nanoelectrospray MS/MS.

2-D Gel Separation

- 30 µg yeast crude cell extract
- 2-D gel (13 cm, pH 4-7 IPG strip followed by 10% SDS-PAGE gel)
- Silver staining

2-D Gel Spot Preparation for Nanoelectrospray

- Excise spots and wash
- In-gel tryptic digestion
- Peptide extraction
- ZipTip™ C₁₈ sample desalting and cleaning
- Elute in 4 µL 75:25 methanol:water with 0.1% formic acid for infusion analysis

MS Analytical Conditions

- Analyte: Tryptic digests from 2-D gel separated crude extract spots
- Solvent: 75:25 methanol:water with 0.1% formic acid
- Flow Rate: 100 nL/minute
- Spray Voltage: 1.6 kV
- Pressure: 0.2 psi
- Acquisition Time: 5 minutes
- Instruments: NanoMate 100 with ESI Chip, ThermoFinnigan LCQ™ Deca
- Scan Modes: MS survey scan and data-dependent MS/MS scan
- Searches: SEQUEST/yeast.fasta database
Results
The MS/MS results for the 2-D gel yeast crude cell extract spots were used to search the yeast.fasta database using the SEQUEST algorithm (see table below). A representative spot was identified as fructose-bisphosphate aldolase 1 with a score of 471.6. The mass spectrum used to identify the protein is shown below. The analysis time was 8.8 hours for 96 samples with 5 minutes acquisition for each sample.

Representative Results for Yeast Protein Spots

<table>
<thead>
<tr>
<th>Spot No.</th>
<th>Protein Accession</th>
<th>Protein Name</th>
<th>MW(Kd)</th>
<th>pI</th>
<th>Protein Coverage (%)</th>
<th>Number of Peptides Detected</th>
<th>Scores (SEQUEST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NP_013145</td>
<td>Pyruvate decarboxylase</td>
<td>61.50</td>
<td>5.8</td>
<td>5.2</td>
<td>3</td>
<td>85.2</td>
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<tr>
<td>2</td>
<td>P00925</td>
<td>Enolase 2</td>
<td>46.78</td>
<td>5.67</td>
<td>9.8</td>
<td>5</td>
<td>450.5</td>
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<tr>
<td>3</td>
<td>NP_012863</td>
<td>Fructose-bisphosphate aldolase 1</td>
<td>39.58</td>
<td>5.51</td>
<td>23.1</td>
<td>6</td>
<td>471.6</td>
</tr>
<tr>
<td>4</td>
<td>P00924</td>
<td>Enolase 1 (2-phosphoglycerate dehydratase)</td>
<td>46.78</td>
<td>6.17</td>
<td>13.3</td>
<td>8</td>
<td>275.7</td>
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<tr>
<td>5</td>
<td>P00330</td>
<td>Alcohol dehydrogenase I</td>
<td>36.69</td>
<td>6.26</td>
<td>10.9</td>
<td>5</td>
<td>89.0</td>
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<tr>
<td>6</td>
<td>P17819</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
<td>35.32</td>
<td>6.40</td>
<td>9.4</td>
<td>3</td>
<td>219.5</td>
</tr>
</tbody>
</table>

MS/MS Results for Gel Spot 3
Identified Peptide: SPIILQTSNGGAAYFAGK

Acknowledgments
We thank ThermoFinnigan for the generous loan of the LCQ Deca.

Summary
The NanoMate 100 system has demonstrated unambiguous identification of proteins from prepared, 2-D gel spots with increased sensitivity and throughput over current methods. Automated sample handling followed by automated nanoelectrospray analysis produces results rapidly with minimal effort.

Advantages
- Rapid results
- Low sample volume consumption
- No carryover
- Increased sensitivity through ionization efficiency