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.

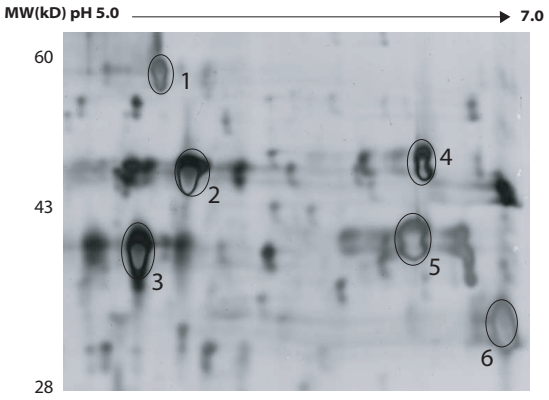
Identification of Yeast Proteins

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

Portion of 2-D Gel for Yeast Crude Cell Extract



2-D Gel Spot Preparation for Nanoelectrospray

- Excise spots and wash
- In-gel tryptic digestion
- Peptide extraction
- ZipTip™ C_{18} 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

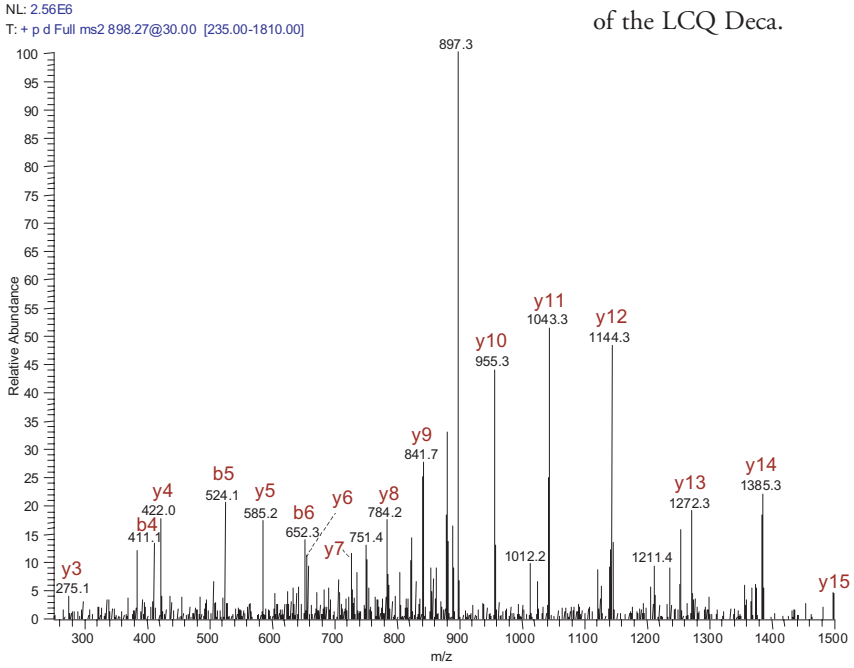
Results

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

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

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



Summary

The NanoMate 100 system has demonstrated unambiguous indentification 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

Acknowledgments

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