

INVESTIGATION OF THE PROTEOME OF *E. COLI* WITH AUTOMATED TECHNOLOGIES

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The investigation of a proteome suggests the identification of all the proteins expressed by a genome. This is an enormous task, but it has become significantly easier and faster through the development of new technologies that have enabled automation of sample processing and analysis. We have begun the investigation of the proteome of *E. coli* using 2-D electrophoresis to visualize the expressed proteins and automated high throughput technologies to perform the subsequent sample processing, analysis and identification of the protein spots. In three months 324 protein spots have been identified on a protein map of *E. coli*.

The proteins from an *E. coli* cell lysate were separated with 2-D electrophoresis. Three different *E. coli* loads from 1 mg to 6 mg were loaded onto a 4–7 pH gradient polyacrylamide gel. In collaboration with Advanced Rapid Robotic Manufacturing (Adelaide, SA) an automated protein spot excision robot has been designed and manufactured.¹ This robot was used to cut protein spots from the gels and the gel pieces were transferred to a microtitre plate for further batch processing. Advantages of this robot are it eliminates mistakes, reduces contamination and keeps an electronic record of the protein spots.

The protein spots underwent an in-gel tryptic digest before they were analysed with matrix assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry. A Canberra Packard Multiprobe 104 robot was used for all the liquid handling steps of the tryptic digestion. This robot could also spot the digested protein samples and matrix onto flat MALDI sample plates in preparation for mass spectrometric analysis. A Micromass ToFSpec 2E mass spectrometer was used for the analysis of the digested samples. The instrument was operated in an automated mode, with batches of up to 96 samples analysed at a time. Processing and calibration of the mass spectra was performed automatically with a beta version of PeptideAuto (Micromass). This also generated monoisotopic peak lists that were utilised in the identification of the digested proteins.

A custom program, rapid automated interrogation of databases (RAID), was created to automate a number of data analysis operations that were previously performed manually. RAID submits peaklist files to MS-Fit (<http://prospector.ucsf.edu>), a peptide mass fingerprinting tool, with user defined parameters, and receives the results which are appended to a report.

Over 1000 protein spots have been cut out from 5 gels, digested, analysed and submitted for peptide mass fingerprinting using these automated routines. This has resulted in the identification of 324 protein spots on a 2-D electrophoresis protein map of *E. coli*.

1. M. Traini, A.A. Gooley, K. Ou, M.R. Wilkins, L. Tonella, J.-C. Sanchez, D.F. Hochstrasser and K.L. Williams, *Electrophoresis*, 1998, 19, 1941.