Differential Expression Analysis using an Unlabelled Approach and a New Software for Relative Quantification of LC-MS Data

John Flensburg[1], Carolina Johansson[2] and Lars Sundström[2]
[1]GE Healthcare, Amersham Biosciences AB, SE-751 84 Uppsala, Sweden
[2]Uppsala University, BMC, IMBIM, Box 582, SE-751 23 Uppsala, Sweden

Introduction

DeCyder™ MS Differential Analysis Software (DeCyder MS) is a new tool for visualization, detection, identification and label-free relative quantification of LC-MS and LC-MS/MS data. To assess its quantification abilities, in a complex background, total soluble trypsin digested proteins extracts from strains of E.coli expressing varying levels of dihydrofolate reducatase (DHFR) were analysed using nanoLC-MS/MS. Full scan MS spectra were matched and quantified using DeCyder MS. A second experiment was performed on a bacterial strain containing an inducible integrase.
A schematic workflow for using DeCyder MS is shown in figure 1.

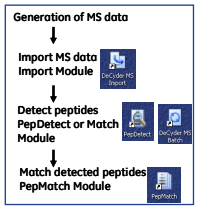

Figure 1. DeCyder MS workflow

Methods

E.coli strains and growth. 1810, HB101(pCV27) and HB101(III:76) expressing varying dihydrofolate reductase levels were grown in Isosensitest medium. BL21(DE3) containing the integrase gene was grown in LB medium at 37 °C to mid exponential phase. The T7-polymerase directed integrase expression was induced by addition of isopropyl-β-D-thiogalalactopyranoside (IPTG) at 1 mM. After induction, retarded growth was continued at 16 °C for four hours. Cells were harvested by centrifugation, washed once with lysis-buffer (20 mM Tris-HCl pH 7.5, 2 mM $MgCl_2$, 1 mM DTT and Protease Inhibitor Mix (Amersham Biosciences AB)). The cells were sonicated and insoluble material separated from soluble proteins by centrifugation at 15000 r.p.m. for 30 min at 4 °C. Protein content was determined using Ettan™ 2-D Quant Kit.
LC-MS/MS analysis. The bacterial extracts were alkylated and trypsin digested and the resulting peptide mixtures were separated either by nanoRPC or by ion exhange chromatography followed by nanoRPC using Ettan MDLC (Amersham Biosciences AB) coupled to a Finnigan LTQ™ Linear Ion Trap (Thermo Electron Corp.) fitted with a nano-spray interface. Full scan mass spectra were collected in profile mode and MS/MS spectra in centroid mode. Detection, profile comparison and quantification was done on the full scan precursor mass spectra in fully automatic mode using DeCyder MS. The peptides were identified using the information in the MS/MS spectra and TurboSEQUEST™. The peptide matches were filtered based on cross-correlation scores (Xcorr) of 1.5, 2, and 2.5 for charge states 1+, 2+, and 3+, respectively.

Results

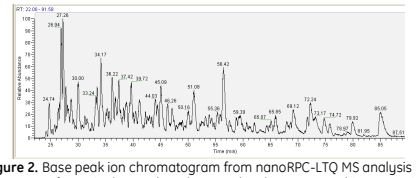

Figure 2. Base peak ion chromatogram from nanoRPC-LTQ MS analysis of 0.25 µg of trypsin digested E.coli 1810. The chromatographic separation was performed on a 0.075 x 150 mm Zorbax C_{18} column (Agilent) at a flow rate of 200 nl/min. Eluent A was 0.1 % formic acid in H_2O and eluent B was 0.1 % formic acid in 84 % acetonitrile. The linear gradient applied was 0-60 % B during 90 min.

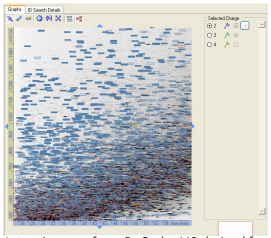

Figure 3. Intensity map from DeCyder MS derived from part of the chromatogram (25-60 min) in fig.2. More than 1200 peptides were automatically detected by the PepDetect module

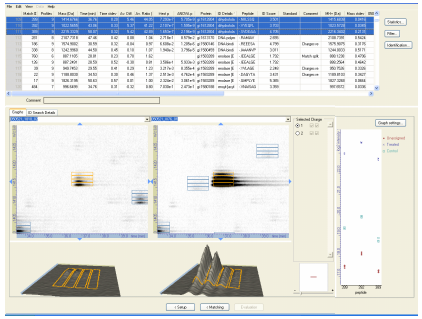

Figure 4. View from the PepMatch module of DeCyder MS after comparing total protein digests from 1810, HB101(pCV27) and HB101(III:76) following LC-MS/MS analysis. The samples were run in triplicates and the tabulated list shows that three different peptides of DHFR were automatically detected and quantified. The 2- and 3D visualizations clearly demonstrate a 12-fold difference in signal intensity for the peptide NIILSSQPGTDDR between the control (1810) and treated sample (HB101(III:76).

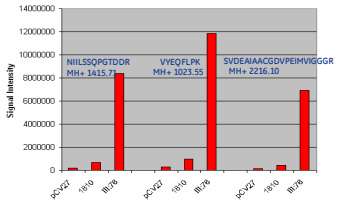

Figure 4. Graph showing the average signal intensities for three different peptides of DHFR extracted from the PepMatch module for the three different strains of E.coli. The average difference in gene expression was 13-fold between HB101(III:76) and 1810 while it was 43-fold between HB101(III:76) and HB101(pCV27).

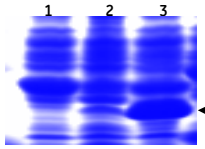

Figure 5. SDS-PAGE of undigested protein extracts stained with Coomassie Brilliant Blue. Lanes: 1) 10 µg HB101(pCV27; 2) 10 µg 1810; 3) 10 µg HB101(III:76).

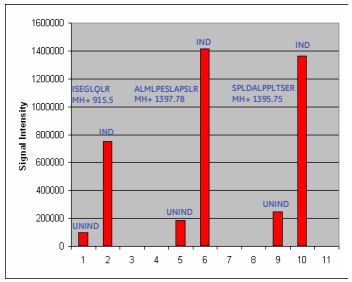

Figure 6. Graphs extracted from DeCyder MS data representing signal intensities for three different peptides from induced and uninduced integrase. The LC-MS conditions were as described in fig. 2. The average difference in peptide content was 7-fold.

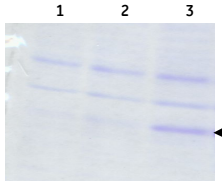

Figure 7. SDS-PAGE of protein extracts from BL21(DE3) containing the integrase. Lanes: 1) uninduced; induced 1.5 hours; 3) induced 4 hours.

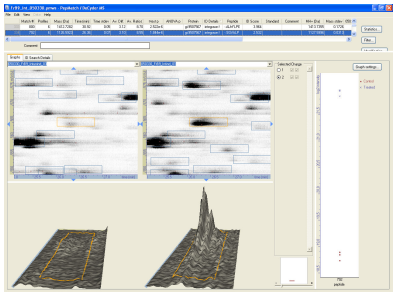

Figure 8. View from the Pep Match module of DeCyder MS. The 2- and 3D graphs represent the peptide SGVALPDALER from integrase. In this case the tryptic peptides were separated by offline fractionation using Ettan MDLC. 40 µg of sample was injected onto a 2.1 x 250 mm SCX column (BioBasic, Thermo Electron) and eluted with a linear salt gradient (A: 20 mM citric acid, 25% acetonitrile, B: A+ 1 M NH4Cl) where fractions were collected and analyzed by RPC. The conditons for RPC were as in fig.2 with the exception that the linear gradient was 50 min.

Peptide	MH+	ID Score	Average Ratio	t test p
SGVALPDALER	1127.60	2.5318	8.55	1.84E-06
ALM*LPESLAPSLR	1413.70	3.9644	8.70	2.52E-06
LPVVLTFPDEVVR	1336.90	3.3572	5.32	2.38E-04
LFAQLLYGTGM*R	1385.73	4.2503	11.89	8.40E-06
ISEGLQLR	915.56	3.0096	15.23	4.42E-06
RLPVVLTPDEVVR	1493.20	4.1727	16.94	7.16E-06
ILGFLEGEHR	1170.73	3.565	11.71	0.0632
PATPHTLR	892.50	2.2646	9.94	5.69E-5

Table 1. List of peptides from Induced and uninduced BL21(DE3) containing the integrase gene that was quantified using DeCyder MS following MDLC purification and MS/M analyses of total trypsin digested protein extracts.

Conclusions

Accurate quantification results are obtained using DeCyder MS even in highly complexed backgrounds.

The quantified peptides in this study reflect a wide dynamic range of protein abundance.

This user-friendly and none-labelled approach to relative quantification of MS-data will have wide implications on the LC-MS based proteomics workflow for differential analysis.

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Correspondence should be addressed to: john.flensburg@ge.com