

Simple Programs for iTRAQ Bioinformatics

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OVERVIEW

Purpose: The purpose of this presentation is to describe our software programs for processing iTRAQ data from QTOF2 LC MS/MS.

Methods: Scripts and macros written in Perl and Microsoft VBA were developed to extract iTRAQ reporter ion intensities and attached the values to the Mascot search. Our scripts are modeled after the strategy used in the GPS Explorer program (Applied Biosystems).

Results: Step-by-step instructions and examples are provided for using our scripts and macros available at <http://www.lerner.ccf.org/eye/crabb/software/>.

INTRODUCTION

Applications of iTRAQ quantitative proteomics technology (Ross et al., 2004) are growing in the literature and this methodology has facilitated our research. However, applying iTRAQ technology with mass spectrometers other than those sold by the iTRAQ reagent vendor can be problematic because of a paucity of appropriate software. To overcome this problem for our research, we have developed a step-by-step workflow of compartmentalized programs in Perl script and in Visual Basic for Applications (VBA) Macros. The purpose of this presentation is to describe our programs and to make them available.

METHODS

On-line LC MS/MS analyses were performed with a QTOF2 mass spectrometer and Cap LC system (Waters). Protein identification utilized MASSLYNX 4.1 software (Waters), the Mascot search engine and the SwissProtein sequence database. Merged database search results were exported in .csv format. Custom procedures written in Perl script and VBA macros extracted peak intensities of the iTRAQ reporter ions from each MSMS spectrum and attached the values to the protein identification based on query number in the Mascot search. Thresholds for quantitation included a minimum of two unique peptides per protein, minimum iTRAQ tag ion intensities of 7-15 and peptide Mascot ion scores ≥ 25 .

Our script for iTRAQ quantitation is modeled after the bioinformatic strategy used in the GPS Explorer program from the iTRAQ reagent vendor (Applied Biosystems). After filtering away peptides that do not meet threshold criteria, a global median peptide iTRAQ ratio was calculated from all the remaining data. Individual peptide iTRAQ ratios were normalized to the global median [normalized iTRAQ ratio = un-normalized iTRAQ ratio/median], then for each protein a mean ratio, standard deviation (STD) and relative standard deviation (RSD = STD/mean x 100) was calculated. A median protein iTRAQ ratio was then calculated and each data set was smoothed (re-normalized) to median = 1.0. Recent application of our iTRAQ software programs are presented on ASMS'07 posters MP530 (Yuan et al., 2007) and MP531 (Gu et al., 2007).

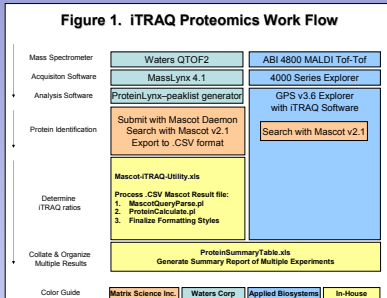


Figure 2. QTOF2 LC MS/MS Peak List

Our peak list generation from QTOF2 LC MS/MS is done with ProteinLynx, a component of MassLynx software (Waters). Each peak identified during a QTOF analysis is appended to a peak list file (example shown). The observed m/z, intensity, and charge state (shown in bold) is followed by multiple lines of MS/MS ions and intensity pairs. Peak lists are the input files searched by the Mascot Protein Identification search engine.

Our software extracts the iTRAQ reporter ion intensities from the Mascot Result file and correlates it with the peptide sequence. The program scans the peak lists submitted for the Mascot search and records the intensity of each observed iTRAQ tag (eg. m/z 114, 115, 116, 117). iTRAQ tags 116 and 117 were used for the data shown. The program allows minimum threshold values to be implemented for iTRAQ reporter ion intensity and Mascot ion score.

473.8440 238.688 2
115.0623 1.9663
116.1220 12.1863
117.0626 76253
117.1149 39.3379
109.0506 1.6689
202.1388 2.2843
194.0328 1.9463
551.3802 21.5964
432.0308 1.9463
542.1712 2.2866
264.7206 2.1126
264.7672 2.1271
473.3896 2.2869
573.5499 668.1897
573.4456 2.0623
574.3393 467.7292
574.4352 1.8462
574.5310 19.4266
574.6267 1.7652
575.5227 1.8462
575.6183 1.1993
473.5895 1.9463
464.7928 20.7591
463.7813 1.2866
731.3802 2.0999
742.6472 1.9463
900.2109 1.9463
109.0506 1.6689
444.3314 472.2928 2
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117.5856 2.2286
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117.7948 2.2866
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