How Useful Is the Product Ion Mass Accuracy for Peptide Identification?

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Overview

Purpose: To evaluate high-resolution mass spectrometry data for peptide identification.
Methods: Database searching and de novo sequencing are investigated with Orbitrap and Linear Trap MS/MS data.
Results: High product ion accuracy is very useful for de novo sequencing.

Introduction

The LTQ-Orbitrap (Thermo Scientific, San Jose, CA) instrument provides excellent precursor ion accuracy by using the Orbitrap analyzer, allowing users to choose between high and low product ion accuracies by using the Orbitrap and Linear Trap analyzers, respectively. It has been widely reported that high precursor ion accuracy greatly improves the peptide identification. [1] It is unclear whether the high product ion accuracy is worthy, because the Orbitrap analyzer is slower than Linear Trap for producing MS/MS scans.

The combined effect of high product ion accuracy and slower scan speed on peptide identification is studied. Both database search and de novo sequencing are utilized.

Methods

The same sample was analyzed on an LTQ-Orbitrap instrument twice, once by using Orbitrap to measure the MS/MS scans, and the other with Linear Trap. Each of the two datasets was analyzed with PEAKS software via both database search and de novo sequencing. The database search performance is evaluated by the number of peptide-spectrum matches (PSM) at 0.5% false discovery rate (FDR). To evaluate the de novo sequencing performance, the top de novo sequence of each spectrum is compared with the highly-confident database search PSM. An amino acid is considered correct if it matches the database search result. The matches between K and Q, L and I, and GG and N, are also considered correct.

Results

Each of the two datasets was analyzed by database search and de novo sequencing with PEAKS 6, respectively. The Uniprot-Swissprot database was used for database searching. For both database searching and de novo sequencing, the mass error tolerances used are: 20 ppm for precursor ions, 0.05 Da for the product ions of Orbi-Orbi dataset and 0.8 Da for the product ions of Orbi-Trap dataset.

The Orbi-Orbi dataset contains 900 MS/MS spectra. 302 PSMs were identified by PEAKS database search with 0.5% FDR. The Orbi-Trap dataset contains 1723 MS/MS spectra (91% more than Orbi-Orbi). 405 PSMs were identified by PEAKS database search with 0.5% FDR (34% more than Orbi-Orbi). The comparison of peptide identification by database search at 0.5% of FDR is shown in Figure 1. Less peptides were reported with high resolution data due to slower scan speed.

Among these 302 PSMs from Orbi-Orbi dataset, de novo sequencing was able to correctly compute 90% of the amino acids. Moreover, the de novo sequencing peptides completely match the database search peptides for 102 of the 302 spectra (shown in Figure 2 & 3).

Among these 405 PSMs from Orbi-Trap, de novo sequencing was able to correctly compute 74% of the amino acids (lower than Orbi-Orbi). Moreover, the de novo sequencing peptides completely matched the database search peptides for 51 of the 405 PSMs (50% lower than Orbi-Orbi) as shown in Figure 2 & 3.

Correspondingly, Orbi-Trap dataset has higher coverage of protein sequence by MS/MS spectra than Orbi-Orbi dataset. The comparison of the coverage of ALBU_Human 78% vs 75% is shown in Figure 4.

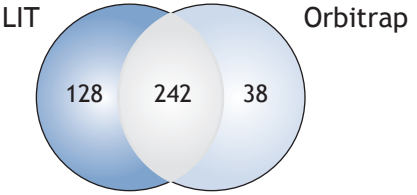

Figure 1. Peptide Identification by Database Search with LIT vs Orbitrap

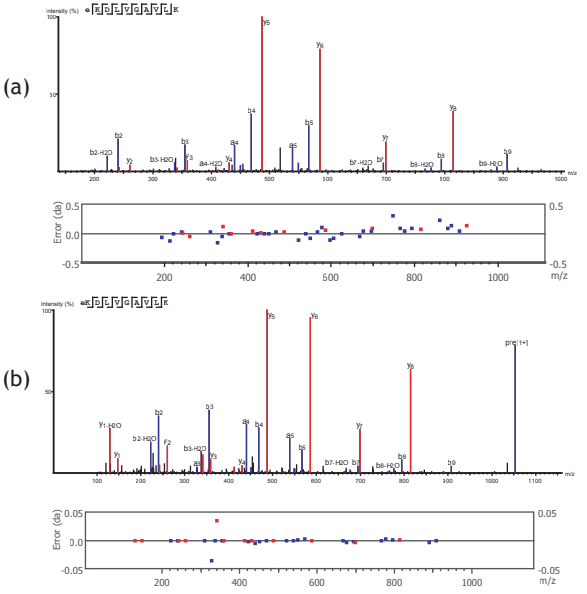

Figure 2. Same Identification by Database Search and de novo with LIT vs Orbitrap

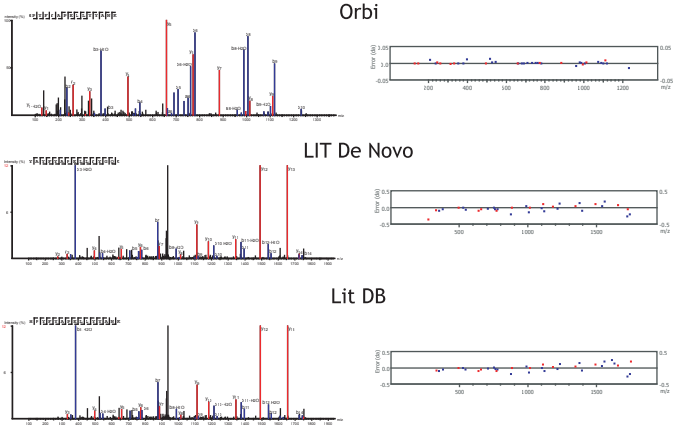

Figure 3. Improvement of de novo sequencing performance by high resolution MS

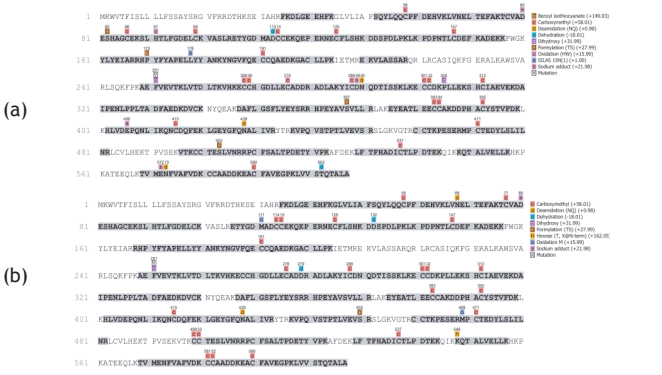

Figure 4. Characterization of amino acids of the sequence of ALBU_BOVIN with (a) Orbi vs (b) LIT

Conclusions

The high product ion accuracy in the Orbi-Orbi data did not improve the database search due to the lower number of MS/MS; but significantly improved the de novo sequencing performance despite the lower number of MS/MS.

Reference

Z. W. Chen, K. Fuchs, W. Sieghart, R. R. Townsend, A. S. Evers (2012) Deep Amino Acid Sequencing of Native Brain GABAA Receptors using High-Resolution Mass Spectrometry. Mol. Cell. Proteomics. 11(1):M111.011445.