

Systematic Comparison of Label-free, Metabolic Labeling, and Chemical Labeling Approaches for Quantitative Proteomics on LTQ-Orbitrap-Velos



Zhou Li^{1,2}, Rachel Adams^{1,2}, Karuna Chourey¹, Robert Hettich¹, Chongle Pan¹

¹Oak Ridge National Laboratory, Oak Ridge, TN, ²University of Tennessee, Knoxville, TN

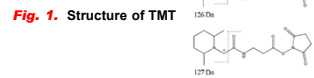


OVERVIEW

- Various quantitative proteomics methods have been developed, and range from label-free to metabolic and chemical labeling.
- LTQ-Orbitrap-Velos represents the latest generation of high performance mass spectrometer with improved ion extraction efficiency and higher-energy collisional dissociation (HCD) capability.
- The three aforementioned quantitative methods were compared in terms of proteome coverage, quantification accuracy, precision, and reproducibility on an LTQ-Orbitrap-Velos.
- The goal was to provide guidance for selecting the appropriate method for a proteomics study where several methods are applicable

INTRODUCTION

- Label-free quantification can be achieved by correlating protein abundance with either mass spectrometric signal intensities of peptides or the number of MS/MS spectra matched to peptides and proteins.
- Metabolic labeling is realized by measuring the abundance ratios of mass-different isotopic variants of peptides using their signal intensities in full scans of LC-MS/MS analysis.
- Chemical labeling via iTRAQTM and TMTTM is accomplished by labeling each sample with an isobaric tag (Fig. 1) and measuring intensities of reporter ion dissociated from the tag in MS/MS scan.



Quantification Mechanism of the Methods

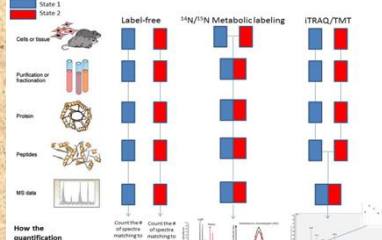


Fig. 2. Sample preparation workflow and quantification scheme of label-free, metabolic labeling and iTRAQ/TMT

EXPERIMENTAL DESIGN

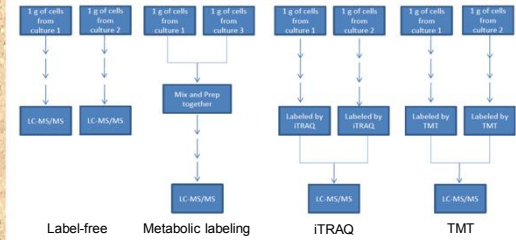


Fig. 3. Experimental setup for the four quantitative methods. Three cultures of *Pseudomonas putida* were grown under the same condition but for culture 3, ¹⁵NH₄Cl, instead of NH₄Cl, was used for introducing ¹⁵N into proteins. For each method, the abundance ratio of proteins between two cultures was expected to be 1:1.

Analytical Instrument Configuration

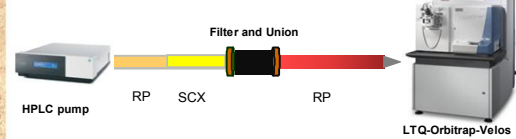
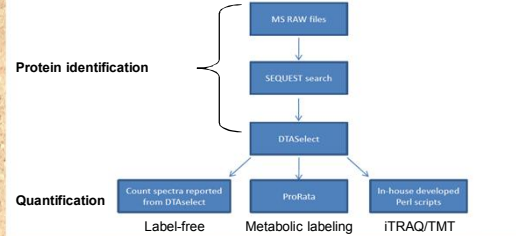


Fig. 4. 2D Nano LC-MS/MS arrangement. The two-dimensional LC separation was performed with eleven consecutive increasing (0-500mM) pulses of ammonium acetate salt. Each salt pulse was followed by a ~2 hrs reverse phase gradient

Bioinformatics Pipeline



PROTEIN IDENTIFICATION

	Label-Free				Metabolic Labeling		iTRAQ		TMT	
	Culture1* Run1**	Culture2* Run1**	Culture1* Run2**	Culture2* Run2**	Run1**	Run2**	Run1**	Run2**	Run1**	Run2**
Spectrum count	58674	61440	43695	49389	52348	64972	29926	29328	35826	32897
Peptide count	12391	12727	11472	11184	9862	9618	7317	8248	6464	6795
Protein count	1687	1607	1598	1516	1447	1394	1202	1353	1239	1233
Average Spectrum count per peptide	4.7	4.8	3.8	4.4	5.3	6.7	4.0	3.6	5.5	4.8
Average peptide count per protein	7.3	7.9	7.2	7.4	6.8	6.9	6.1	6.1	5.2	5.5
Average sequence coverage	24.6%	25.8%	23.6%	24.1%	22.3%	23.2%	19.9%	19.3%	16.5%	17.3%
Genome coverage	32.1%	30.6%	30.4%	28.9%	27.6%	26.6%	22.9%	25.8%	23.6%	23.5%

Table 1. Protein identification from label-free, metabolic labeling, iTRAQ and TMT. *: Culture 1 and Culture 2 were the same in Fig. 3. **: Run 1 and Run 2 were technical replications of each method.

IDENTIFICATION REPRODUCIBILITY

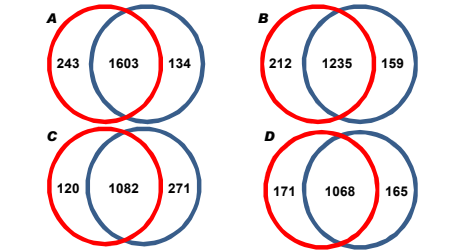


Fig. 5. Venn diagram showing protein identification reproducibility of each method. A: Label-Free; B: Metabolic Labeling; C: iTRAQ; D: TMT; Red: protein uniquely identified from Run 1; Blue: protein uniquely identified from Run 2; Overlapped region: protein shared between duplicate runs.

ARG- VS. LYS-TERMINATED PEPTIDE

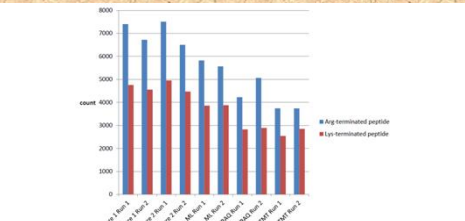


Fig. 6. Frequency of Arg-terminated peptide vs. Lys-terminated peptide identified. Culture 1 and Culture 2 were from label-free. Run 1 and Run 2 were technical replication. ML: Metabolic Labeling. The ratios of Lys-terminated peptides to Arg-terminated peptides were similar among different methods and ranged from 0.57 to 0.76.

PROTEIN QUANTIFICATION

	Label Free		Metabolic Labeling		iTRAQ		TMT	
	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2	Run 1	Run 2
Medium	0.07*	0.10*	0*	0*	-0.02*	0.00*	0.00*	0.00*
Median absolute deviation	0.40*	0.43*	0.3*	0.3*	0.17*	0.17*	0.17*	0.05*
Percentage of protein with log ₂ ratio within [-1,1]	87%	84%	94%	93%	99%	98%	99%	100%
# of quantified protein	1174	1116	1327	1300	1185	1338	1231	1215

Table 2. Protein quantification from label-free, metabolic labeling, iTRAQ and TMT. Median and median absolute deviation were used to evaluate quantification accuracy and precision of each quantitative proteomics method, respectively. Relative abundance ratio of each protein, measured by spectra count, extracted ion chromatogram and reporter ion intensity for label-free, metabolic labeling, and iTRAQ and TMT, were log₂ transformed before the calculation. For label-free, at least 4 spectral count was required for a protein to be accurately quantified. *: the value was calculated at protein level; **: the value was calculated at peptide level

QUANTIFICATION ACCURACY AND PRECISION

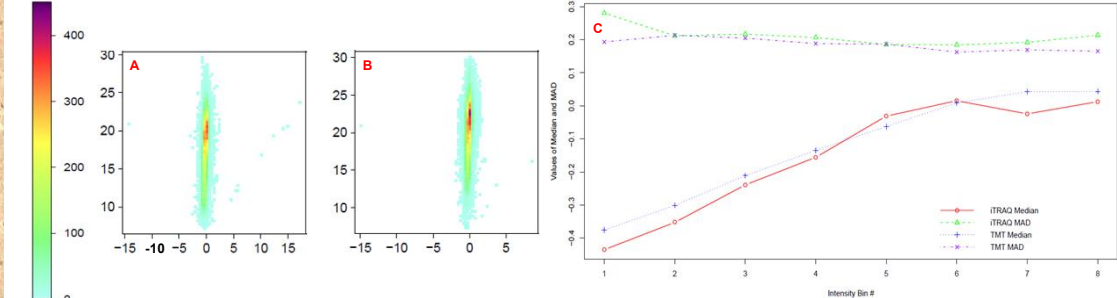


Fig. 7. Two dimensional scatter plot showing log₂ ratio of each peptide against the total reporter ion intensity used to calculate the ratio from iTRAQ (A) and TMT (B). In A and B, horizontal axis is log₂ ratios; vertical axis is log₂-transformed reporter ion intensities. The colors encode the density of the dots on the scatter plots. Then, the entire intensity range was split into eight bins. Median and Median Absolute Deviation (MAD) of each binned intensity range were plotted (C). Intensity range for each bin: Bin 1: 7-9; Bin 2: 10-12; Bin 3: 13-15; Bin 4: 16-18; Bin 5: 19-21; Bin 6: 22-24; Bin 7: 25-27; Bin 8: 28-29

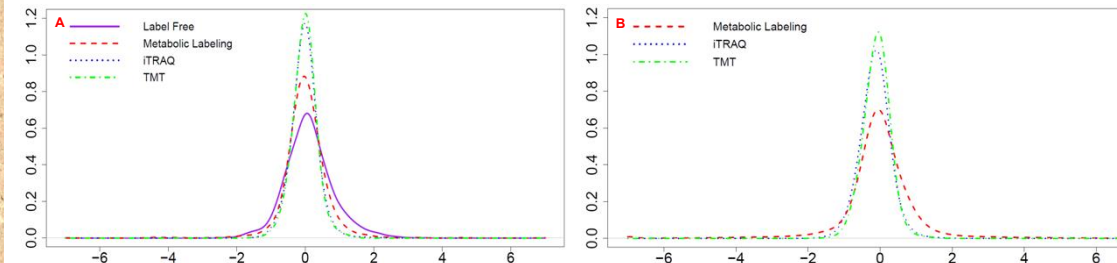


Fig. 8. Density plot illustrating distribution of log₂ ratios from each quantitative proteomics method at protein level (A) and peptide level (B).

QUANTIFICATION REPRODUCIBILITY

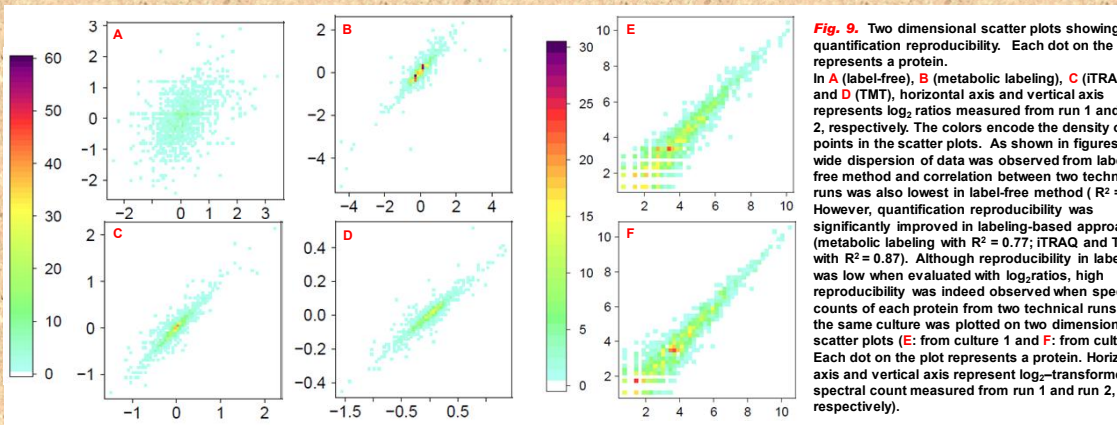


Fig. 9. Two dimensional scatter plots showing quantification reproducibility. Each dot on the plot represents a protein. In A (label-free), B (metabolic labeling), C (iTRAQ), and D (TMT), horizontal axis and vertical axis represents log₂ ratios measured from run 1 and run 2, respectively. The colors encode the density of the points in the scatter plots. As shown in figures, wide dispersion of data was observed from label-free method and correlation between two technical runs was also lowest in label-free method (R² = 0.2). However, quantification reproducibility was significantly improved in labeling-based approaches (metabolic labeling with R² = 0.77; iTRAQ and TMT with R² = 0.87). Although reproducibility in label-free was low when evaluated with log₂ ratios, high reproducibility was indeed observed when spectral counts of each protein from two technical runs of the same culture was plotted on two dimensional scatter plots (E: from culture 1 and F: from culture 2; Each dot on the plot represents a protein. Horizontal axis and vertical axis represent log₂-transformed spectral count measured from run 1 and run 2, respectively).

ABUNDANCE INDICATOR vs. ACCURACY

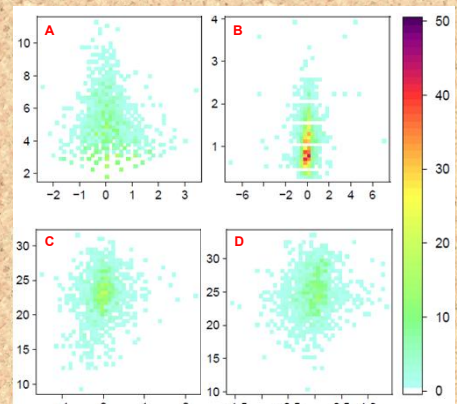


Fig. 6. Two dimensional scatter plot showing log₂ ratio of each protein against associated spectral count (A), width of confidence interval (B), and reporter ion intensities (C, D). The spectrum count and reporter ion intensities were log₂ transformed. The colors encode the density of dots on the scatter plots. A: label-free; B: metabolic labeling; C: iTRAQ; D: TMT.

CONCLUSIONS

- Label-free method provides the deepest proteome coverage. Although quantification is compromised, reasonable quantification performance can be still obtained.
- Metabolic labeling and iTRAQ/TMT are capable of accurate, precise and reproducible quantification, and iTRAQ and TMT surpass metabolic labeling in terms of precision and reproducibility. Although protein identification is compromised, quantitative proteome coverage is higher in the labeling-based approaches.
- iTRAQ and TMT perform very similar in all aspects compared in current study.

ACKNOWLEDGEMENT

- This research was sponsored by the Genomic Science Program, U.S. Department of Energy, Office of Science, Biological and Environmental Research.
- Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.