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Metandem: An Online Software Tool for Mass Spectrometry-based Isobaric Labeling Metabolomics

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Abstract

Mass spectrometry-based stable isotope labeling provides advantages of multiplexing capability and accurate quantification but requires tailored bioinformatics tools for data analysis. Despite the rapid advancements in analytical methodology, it is often challenging to analyze stable isotope labeling-based metabolomics data, particularly for isobaric labeling using MS/MS reporter ions for quantification. We report Metandem, a novel online software tool for isobaric labeling-based metabolomics, freely available at http://metandem.com/web/. Metandem provides a comprehensive data analysis pipeline integrating feature extraction, metabolite quantification, metabolite identification, batch processing of multiple data files, online parameter optimization for custom datasets, data normalization, and statistical analysis. Systematic evaluation of the Metandem tool was demonstrated on UPLC-MS/MS, nanoLC-MS/MS, CE-MS/MS and MALDI-MS platforms, via duplex, 4-plex, 10-plex, and 12-plex isobaric labeling experiments and the application to various biological samples.

Graphical Abstract

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The authors declare no competing financial interests.

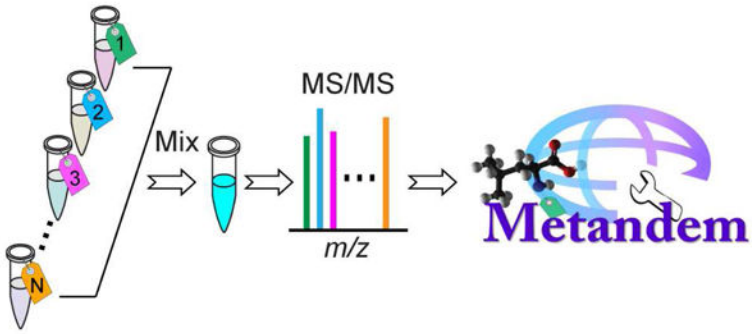
The Supplementary Information is available: Reporter ion information of the custom isobaric DiLeu reagents; Reporter ion information of commercial isobaric tags; Metandem online tool user manual.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of Interest Statement

The authors do not have any conflict of interest to disclose.



Keywords

Metabolomics; Software; Isobaric Labeling; Metandem; DiLeu; Stable Isotope Labeling

1. Introduction

Metabolomics is an essential component of systems biology and has embraced rapid advancements over the past decades. The development of mass spectrometry (MS)-based techniques allows metabolomics to be carried out with sophisticated methodologies, as well as wide applications to biological and clinical studies [1–9]. MS-based metabolomics generates multidimensional datasets where thousands of features can be measured in a single instrument run, pressing significant challenges on data processing and analysis. Particularly for large-scale metabolomics studies, state-of-the-art analytical techniques must be paired up with proper bioinformatics software for automated and efficient data analysis [10–14].

MS-based metabolomic analysis is typically performed by label-free or stable isotope labeling approaches. Stable isotope labeling, in particular isobaric labeling, has gained substantial popularity in proteomic and peptidomic studies and has also been successfully adopted to quantify small molecules in recent years [15–25]. Biomolecules derivatized by multiplexed isobaric labels have nearly identical mass shift of precursor ions, which can be fragmented into a panel of MS/MS reporter ions. Each reporter ion intensity represents the relative abundance of the same molecule from the original sample. Therefore, relative quantification of molecules from different samples can be achieved by calculating the reporter ion ratios in a single LC-MS/MS injection. Isobaric labeling experiments can be conducted using commercial isobaric tags TMT [26, 27] and iTRAQ [28] or custom synthesized reagents like DiLeu [20, 29, 30], DiAla [31], DiVal [31], and DiART [32]. Besides amine-reactive isobaric labels, aminoxyTMT was also developed to target molecules with carbonyl groups such as carbohydrates and steroids.

Stable isotope labeling provides advantages of multiplexing capability and accurate quantification but requires tailored bioinformatics tools for data analysis. Despite the variety of metabolomics software packages, such as XCMS [33–35], MZmine [36], Compound Discoverer (Thermo), Progenesis QI (Waters), and MetaboScape (Bruker), only few software packages can process data with stable isotope labeling, particularly for MS/MS-

based isobaric labeling metabolomics [37–40]. Proteomics software platforms cannot be easily adapted for small molecule analysis because of their distinct isotope distribution and identification algorithms. Scientists have to write their own program or script in order to process MS/MS-based isobaric labeling datasets [20, 27, 32], which are often not freely available to the public, impeding the progress and applicability of isobaric labeling-based metabolomics.

To address this limitation and critical technological gap, we developed Metandem, a novel online software platform for the data analysis of isobaric labeling-based metabolomics. Metandem is freely available at http://metandem.com/web/ and is very easy to use through its graphic interface design. It provides a comprehensive data analysis pipeline that integrates feature extraction, metabolite relative quantification, metabolite identification, batch processing of multiple data files, parameter optimization, data normalization, and statistical analysis (Figure 1). We evaluated the Metandem software tool using duplex, 4-plex, 10-plex, and 12-plex isobaric labeling on different LC-MS and MALDI-MS platforms, and applied the complete isobaric labeling metabolomics workflow to various biological samples.

2. Materials and Methods

2.1. Metabolite Sample Preparation

Metabolite standard mixtures and metabolite fractions from various biological samples were prepared in this study. Detailed sample preparation procedures were described in our previous publications [5, 21, 41, 42]. Briefly, commercially-available pancreatic cancer cells (PANC1) and breast cancer cells (MCF7) were purchased from ATCC and routinely cultured in the lab in DMEM supplement with 10% fetal bovine serum (Gibco). Cells were washed twice with phosphate buffer saline before quenched with methanol, pelleted, and snap-froze in liquid nitrogen. Cellular metabolites were extracted with a methanol/chloroform/water extraction method. Mouse urine samples were collected in metabolic cages and urine samples were centrifuged at 10,000g for 10 min to remove cell debris. Urinary metabolite fractions were obtained using 3 kDa molecular weight cut-off ultracentrifugation filters [42]. Metabolite standard mixtures were prepared by mixing individual stock solutions of 12 representative metabolites including histidine, valine, tyrosine, leucine, lysine, phenylalanine, tryptophan, alanine, serotonin, dopamine, γ-aminobutyric acid, and norepinephrine. Quality control samples were generated by mixing a small aliquot from each sample from the same sample type, which can be used to monitor instrument stability and determine the data analysis parameters via parameter optimization graphs in Metandem. Then these parameters can be used to process the real sample set for the best performance.

Multiplexed isobaric DiLeu reagents were custom synthesized following the procedure described previously with steps of reductive dimethylation of leucine and ^{18}O exchange [30]. Dried DiLeu reagents were stored in a desiccator at 4 °C and activated to the triazine ester form right before the labeling reaction with amine groups. A 20-fold molar excess of activated DiLeu was reacted with metabolite samples at a 70% of organic: aqueous ratio. Each plex of labeled sample was combined, dried, and purified by SCX Ziptip as described previously [20].

2.2. Mass spectrometry analysis

Multiple MS instrument platforms were used in this study to demonstrate the applicability of the Metandem tool, including a Dionex UltiMate 3000 nanoLC system coupled with a Fusion™ Lumos™ Orbitrap MS, a Dionex nanoLC coupled with a Q Exactive™ HF Orbitrap MS, a Dionex UHPLC coupled with a Q Exactive™ Orbitrap MS, and a MALDI LTQ Orbitrap MS. For nanoLC-MS analysis, a C18 column was fabricated in-house with an integrated emitter (75.1 μm × 150 mm, 1.7 μm, 100 Å) under a 40 min LC gradient separation and a flow rate of 0.3 μL/min as described previously [21]. For standard flow LC-MS, labeled metabolites were separated with a 20 min gradient on a Phenomenex biphenyl column (2.1 × 100 mm, 2.6 μ m, 100 Å) at a flow rate of 0.3 ml/min as described previously [5]. Mobile phase A was 0.1% formic acid in water, and mobile phase B was 0.1% formic acid in acetonitrile. LC separation was coupled with a top 20 data-dependent acquisition on a mass spectrometer. Full MS scans were acquired from *m/z* 100 to 1000 at a resolution of 60 K, automatic gain control at 5×10^5, and maximum injection time of 100 ms. MS/MS scans were acquired at a resolution of 60 K, an isolation window of 1 m/z, and a lower mass limit of 110 *m/z*. Normalized collision energy for MS/MS fragmentation was 30% with higher-energy collisional dissociation (HCD). CE-MS/MS analysis was conducted on an HP Agilent G1600AX 3D CE system coupled with a Synapt-G2 mass spectrometer as described previously [20]. Samples were dissolved in the background electrolyte solution (0.2% FA in 50% MeOH). Sample injection was achieved by applying 50 mbar for 20 s at the inlet end of a capillary column, which was followed by applying 30 kV at the inlet for sample separation. MALDI MS/MS analysis was carried out with a top 5 data-dependent acquisition and 30% HCD collision energy.

2.3. Data analysis using Metandem

Data analysis was achieved using the Metandem software tool, which is freely available at http://metandem.com/web/. Detailed step-by-step user manual is provided on the website and also in Supplementary information. The web interface allows users to select the number of reporter ions, input accurate mass and purity of each reporter ion, and upload data sets. For Thermo .raw data file, we recommend using COMPASS [43] to convert into .txt format. For other vendor data files, ProteoWizard [44] can be used to convert into .mgf format. After uploading the data files, reporter ion information need to be defined based on the isobaric labeling experiment. Reporter ion information of isobaric tags is summarized in Supplementary Table S1 (DiLeu) and Table S2 (TMT, aminoxyTMT, and iTRAQ). Data analysis parameters for the demonstration data set were optimized using the parameter optimization graphs (POGs) and optimal settings were set as the following: reporter ion mass tolerance of 0.4 mDa, batch processing mass tolerance of 6 ppm, and batch processing retention tolerance of 0.5 min. Reporter ion intensities were extracted from the dataset for relative quantification and statistical analysis. Molecular weight of the detected compound can be calculated based on the charge and mass shift caused by labeling, which was then searched against Human Metabolome Database [45] for putative metabolite identification. Metabolite identification can be further confirmed by backbone fragmentations in MS/MS spectra.

3. Results and Discussion

3.1. Metandem functionality and web interface

Metandem tool integrates feature extraction, relative quantification, metabolite identification, batch processing of multiple data files, parameter optimization, median normalization, and statistical analysis (Figure 1). Relative quantification is achieved at the MS/MS level by extracting reporter ion intensities from the isobaric labeling experiment. The data analysis pipeline of Metandem for isobaric labeling-based metabolomics can be summarized into five steps: 1) upload data; 2) define reporter ion information; 3) optimize parameters (optional); 4) submit job; and 5) generate output and interpret results. Metandem can process both individual data file and multiple data files as batch processing. The graphic interface of Metandem is shown in Figure 2. Metandem also provides the function of isotopic purity correction to account for the isotopic interference and impurities from synthetic isotope reagents [20, 46, 47]. In most cases, the option of “only output data containing all reporter ions” is selected to ensure the best data quality (Figure 2). However, if some target molecules are known to be not present in particular samples or with an extremely high fold change compared to other samples, this option can be unselected.

Customization of parameters for data analysis software is crucial to ensure the best performance, but it is often difficult and time-consuming. Metandem provides straightforward online parameter optimization function for custom datasets. By simply checking the box of Output Parameter Optimization Graphs (POGs) and submitting the job, Metandem runs with a range of parameter values to generate POGs and allows users to select the best parameters for their specific custom datasets. Three POGs are illustrated using the demonstration dataset for the optimization of reporter ion mass tolerance, batch processing mass tolerance, and batch processing retention time tolerance (Figure 3). The total number of features containing reporter ions increases with a reporter ion mass tolerance-dependent manner and then reaches a plateau, indicating an optimal reporter ion mass tolerance of 0.4 mDa for the demo dataset (Figure 3A). With the increase of batch processing mass tolerance (from 0.1 to 20 ppm), the total number of features dropped as more features can be merged together. The shared features among multiple input files rises and then reaches a plateau at a mass tolerance of 6 ppm (Figure 3B). For batch processing retention time tolerance (0.1–2 min), if the tolerance is too high, distinctive features are incorrectly merged together, causing a decreased in the number of both total features and shared features. The optimal retention time tolerance is 0.5 min for the demo dataset (Figure 3C). Depending on the size of the dataset, the parameter optimization step can take from several seconds up to 10 min to complete. Test run function is also available to provide a quick evaluation of a subset of data.

Results of data analysis are summarized into tables (.csv) and statistical graphs. The output result tables include all individual file tables, a merged table, and a metabolite identification table. If median normalization is selected, another merged table after ratio normalization will be generated. Molecular weight of the detected compound can be calculated based on the charge and mass shift caused by labeling, which was then searched against Human Metabolome Database (HMDB) [45] for putative metabolite identification. A major

challenge for isobaric labeling-based metabolites is that the resulting MS/MS fragments cannot be easily matched to the available online database for metabolite identification. To further confirm identification, MS/MS matching needs to be performed by labeling standard compounds since chemical derivatization may alter the fragmentation pattern of target molecules. For the present test dataset, we confirmed the identities of some DiLeu-labeled metabolites by matching to our in-house database containing the MS1, MS/MS, and retention times of amino acids and other common amine-containing metabolite standards. For relative quantification and statistical analysis, reporter ion intensities are extracted from the dataset and reporter ion ratios are calculated from intensities with tag #1 as the denominator. The screenshots of these results tables are provided in Figure 4. Each individual result table consists of precursor ion information, reporter ion intensities, and ratios of all quantified features. When multiple data files are uploaded for analysis, quantitative information is generated for each file and merged among multiple files on the feature level. The average reporter ion ratios and relative standard deviations (RSD) of ratios in the merged file are calculated across all input data files. All graphs are interactive, which can be visualized/edited online or downloaded for offline analysis. Output statistical graphs include histogram distribution of precursor mass (Figure 3D), histogram distribution of retention time (Figure 3E), and box plots of reporter ion ratios before and after median normalization. These graphs are generated with only the shared features among all merged files to ensure the best data quality.

3.2. Evaluation and Applicability

We performed systematic evaluation of Metandem tool using isobaric labeling experiments on UPLC-MS/MS, nanoLC-MS/MS, CE-MS/MS and MALDI-MS platforms. Duplex, 4-plex, 10-plex, and 12-plex isobaric DiLeu labeling was applied to a standards mixture, biofluids, and cancer cell samples for metabolite relative quantification and identification [5, 20, 30, 41, 48].

The accuracy and dynamic range of relative quantification are evaluated by labeling the same sample with multiplexed reagents which were then mixed at known ratios and analyzed by nanoLC-MS/MS. Breast cancer cellular metabolites were labeled with 12-plex isobaric DiLeu, generating a total of 3510 merged features (Figure 5A). Pancreatic cancer cellular metabolites were labeled with duplex isobaric DiLeu and generated a total of 5456 merged features (Figure 5B). As shown in Figure 5C, experimental average ratios can be plotted against theoretical ratios, where slope =1 represents perfect accuracy and R^2 =1 represents perfect consistency and precision. Slopes for 12-plex and duplex labeling are 0.9691 and 1.122, respectively; R^2 for 12-plex and duplex labeling are 0.999 and 0.998, respectively, indicating excellent relative quantification accuracy and precision of the results generated by Metandem. In the theoretical 1, 2, 5, 10, and 20 folds of mixed ratios for DiLeu labeling, the median experimental ratios are 0.98, 2.12, 5.42, 9.69, and 19.59, respectively. At least 20 folds of dynamic range can be accurately quantified by DiLeu labeling. We found that isobaric labeling-metabolomics dataset is less prone to ratio suppression caused by precursor coisolation and cofragmentation, which is a major challenge of isobaric labeling-based bottom-up proteomics. It is probably because labeled metabolites have small

molecular weights and less charges compared to labeled peptide ions, and the injected metabolite samples have less complex chromatography than bottom-up proteomics.

In order to validate feature extraction and metabolite identification functions of Metandem, data files from our previous study using 4-plex DiLeu on CE-MS/MS and nanoLC-MS/MS platforms were re-processed by Metandem [20]. Four-plex DiLeu labeled twelve metabolite standards mixture was also analyzed on a UPLC-MS/MS platform. All 12 metabolite targets were successfully identified and quantified using Metandem. For complex samples like mouse urine, MCF7 breast cancer cells, and PANC1 pancreatic cancer cells, over 2000 features were quantified, and over 500 features can be identified as metabolites (Table 1). After putative identification by Metandem, it is necessary to carefully examine these IDs and further confirm their IDs by labeling metabolite standard compounds. For instance, in PANC1 cells, we confirmed the identities of 153 metabolites either through labeling standard compounds or matching with our in-house DiLeu-labeled metabolite database. The complete list of 153 metabolites can be found in our previous publication [21].

Since stable isotope labels can be used to derivatize both peptides and metabolites, paralleled proteomics and metabolomics can be achieved from the same set of biological samples on the same instrumental platform [21]. Isobaric tags label the N-terminus and lysine side chain of all peptides in bottom-up proteomics experiments. However, due to the heterogeneity of physical and chemical properties of metabolites, a major pitfall of isobaric labeling-based metabolomics is that only subsets of the metabolome can be derivatized depending on the structure of the chemical tags. For instance, TMT, iTRAQ and DiLeu target amine-containing small molecules, and aminoxyTMT targets carbonyl-containing molecules such as carbohydrates and steroids. Therefore, the results of metabolite identification need to be examined to exclude unreasonable matches from the database. On the other hand, these additional functional group requirements also enhance the confidence of metabolite identification.

4. Conclusions

MS-based quantitative analysis has fostered the development of state-of-the-art methodologies in both proteomics and metabolomics studies. There is a pressing need for associated software and computational tools to process complex data automatically and efficiently. Metandem fills a critical gap in the metabolomics field, enabling MS/MS-based isobaric labeling dataset to be processed with simple graphic interface design. Besides basic functions of relative quantification and identification, Metandem also provides unique feature of online parameter optimization to ensure the best performance of such large-scale metabolomic experiments. It is fast, accurate, easy to use, and freely available at http://metandem.com/web/.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Nicholson JK, Lindon JC, Systems biology - Metabonomics, Nature, 455 (2008) 1054–1056. [PubMed: 18948945]

[2]. Patti GJ, Yanes O, Siuzdak G, Metabolomics: the apogee of the omics trilogy, Nature Reviews Molecular Cell Biology, 13 (2012) 263–269. [PubMed: 22436749]

[3]. Theodoridis G, Gika HG, Wilson ID, LC-MS-based methodology for global metabolite profiling in metabonomics/metabolomics, Trac-Trends In Analytical Chemistry, 27 (2008) 251–260.

[4]. Jones CM, Monge ME, Kim J, Matzuk MM, Fernandez FM, Metabolomic serum profiling detects early-stage high-grade serous ovarian cancer in a mouse model, J Proteome Res, 14 (2015) 917–927. [PubMed: 25567202]

[5]. Hao L, Greer T, Page D, Shi YT, Vezina CM, Macoska JA, Marker PC, Bjorling DE, Bushman W, Ricke WA, Li LJ, In-Depth Characterization and Validation of Human Urine Metabolomes Reveal Novel Metabolic Signatures of Lower Urinary Tract Symptoms, Sci Rep-Uk, 6 (2016).

[6]. Chen S, Kong H, Lu X, Li Y, Yin P, Zeng Z, Xu G, Pseudotargeted metabolomics method and its application in serum biomarker discovery for hepatocellular carcinoma based on ultra high-performance liquid chromatography/triple quadrupole mass spectrometry, Anal Chem, 85 (2013) 8326–8333. [PubMed: 23889541]

[7]. Smith RD, Mass Spectrometry in Biomarker Applications: From Untargeted Discovery to Targeted Verification, and Implications for Platform Convergence and Clinical Application, Clinical Chemistry, 58 (2012) 528–530. [PubMed: 22194635]

[8]. Luan H, Wang X, Cai Z, Mass spectrometry-based metabolomics: Targeting the crosstalk between gut microbiota and brain in neurodegenerative disorders, Mass Spectrom Rev, 38 (2019) 22–33. [PubMed: 29130504]

[9]. Hao L, Wang J, Page D, Asthana S, Zetterberg H, Carlsson C, Okonkwo OC, Li L, Comparative Evaluation of MS-based Metabolomics Software and Its Application to Preclinical Alzheimer's Disease, Sci Rep, 8 (2018) 9291. [PubMed: 29915347]

[10]. Sarpe V, Schriemer DC, Supporting metabolomics with adaptable software: design architectures for the end-user, Curr Opin Biotech, 43 (2017) 110–117. [PubMed: 27870998]

[11]. Kind T, Liu KH, Lee DY, DeFelice B, Meissen JK, Fiehn O, LipidBlast in silico tandem mass spectrometry database for lipid identification, Nat Methods, 10 (2013) 755–758. [PubMed: 23817071]

[12]. Montenegro-Burke JR, Aisporna AE, Benton HP, Rinehart D, Fang M, Huan T, Warth B, Forsberg E, Abe BT, Ivanisevic J, Wolan DW, Teyton L, Lairson L, Siuzdak G, Data Streaming for Metabolomics: Accelerating Data Processing and Analysis from Days to Minutes, Anal Chem, 89 (2017) 1254–1259. [PubMed: 27983788]

[13]. Melamud E, Vastag L, Rabinowitz JD, Metabolomic Analysis and Visualization Engine for LC-MS Data, Anal Chem, 82 (2010) 9818–9826. [PubMed: 21049934]

[14]. Liu Z, Portero EP, Jian Y, Zhao Y, Onjiko RM, Zeng C, Nemes P, Trace, Machine Learning of Signal Images for Trace-Sensitive Mass Spectrometry: A Case Study from Single-Cell Metabolomics, Anal Chem, 91 (2019) 5768–5776. [PubMed: 30929422]

[15]. Griffin TJ, Goodlett DR, Aebersold R, Advances in proteome analysis by mass spectrometry, Curr Opin Biotechnol, 12 (2001) 607–612. [PubMed: 11849943]

[16]. Rauniyar N, Yates JR 3rd, Isobaric labeling-based relative quantification in shotgun proteomics, J Proteome Res, 13 (2014) 5293–5309. [PubMed: 25337643]

[17]. Guo K, Li L, Differential C-12/C-13-Isotope Dansylation Labeling and Fast Liquid Chromatography/Mass Spectrometry for Absolute and Relative Quantification of the Metabolome, Anal Chem, 81 (2009) 3919–3932. [PubMed: 19309105]

[18]. Zhou R, Tseng CL, Huan T, Li L, IsoMS: automated processing of LC-MS data generated by a chemical isotope labeling metabolomics platform, Anal Chem, 86 (2014) 4675–4679. [PubMed: 24766305]

[19]. Li J, Hoene M, Zhao X, Chen S, Wei H, Haring HU, Lin X, Zeng Z, Weigert C, Lehmann R, Xu G, Stable isotope-assisted lipidomics combined with nontargeted isotopomer filtering, a tool to unravel the complex dynamics of lipid metabolism, Anal Chem, 85 (2013) 4651–4657. [PubMed: 23537127]

[20]. Hao L, Zhong XF, Greer T, Ye H, Li LJ, Relative quantification of amine-containing metabolites using isobaric N,N-dimethyl leucine (DiLeu) reagents via LC-ESI-MS/MS and CE-ESI-MS/MS, Analyst, 140 (2015) 467–475. [PubMed: 25429371]

[21]. Hao L, Johnson J, Lietz CB, Buchberger A, Frost D, Kao WJ, Li LJ, Mass Defect-Based N, N-Dimethyl Leucine Labels for Quantitative Proteomics and Amine Metabolomics of Pancreatic Cancer Cells, Anal Chem, 89 (2017) 1138–1146. [PubMed: 28194987]

[22]. Thomas S, Hao L, Ricke WA, Li LJ, Biomarker discovery in mass spectrometry-based urinary proteomics, Proteom Clin Appl, 10 (2016) 358–370.

[23]. Hawkridge AM, Muddiman DC, Mass Spectrometry-Based Biomarker Discovery: Toward a Global Proteome Index of Individuality, Annual Review Of Analytical Chemistry, 2 (2009) 265–277.

[24]. Zhong XF, Hao L, Lu JF, Ye H, Zhang SC, Li LJ, Quantitative analysis of serotonin secreted by human embryonic stem cells-derived serotonergic neurons via pH-mediated online stacking-CE-ESI-MRM, Electrophoresis, 37 (2016) 1027–1030. [PubMed: 26791443]

[25]. Chen X, Sun L, Yu Y, Xue Y, Yang P, Amino acid-coded tagging approaches in quantitative proteomics, Expert Rev Proteomics, 4 (2007) 25–37. [PubMed: 17288513]

[26]. Thompson A, Schafer J, Kuhn K, Kienle S, Schwarz J, Schmidt G, Neumann T, Hamon C, Tandem mass tags: A novel quantification strategy for comparative analysis of complex protein mixtures by MS/MS (vol 15, pg 1895, 2003), Anal Chem, 75 (2003) 4942–4942.

[27]. Murphy JP, Everley RA, Coloff JL, Gygi SP, Combining Amine Metabolomics and Quantitative Proteomics of Cancer Cells Using Derivatization with Isobaric Tags, Anal Chem, (2014).

[28]. Ross PL, Huang YLN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlet-Jones M, He F, Jacobson A, Pappin DJ, Multiplexed protein quantitation in Saccharomyces cerevisiae using amine-reactive isobaric tagging reagents, Molecular & Cellular Proteomics, 3 (2004) 1154–1169. [PubMed: 15385600]

[29]. Xiang F, Ye H, Chen RB, Fu Q, Li LJ, N,N-Dimethyl Leucines as Novel Isobaric Tandem Mass Tags for Quantitative Proteomics and Peptidomics, Anal Chem, 82 (2010) 2817–2825. [PubMed: 20218596]

[30]. Frost DC, Greer T, Li L, High-Resolution Enabled 12-Plex DiLeu Isobaric Tags for Quantitative Proteomics, Anal Chem, 87 (2015) 1646–1654. [PubMed: 25405479]

[31]. Yu Q, Shi XD, Greer T, Lietz CB, Kent KC, Li LJ, Evaluation and Application of Dimethylated Amino Acids as Isobaric Tags for Quantitative Proteomics of the TGF-beta/Smad3 Signaling Pathway, Journal of Proteome Research, 15 (2016) 3420–3431. [PubMed: 27457343]

[32]. Yuan W, Zhang JX, Li SW, Edwards JL, Amine Metabolomics of Hyperglycemic Endothelial Cells using Capillary LC-MS with Isobaric Tagging, Journal Of Proteome Research, 10 (2011) 5242–5250. [PubMed: 21961526]

[33]. Smith CA, Want EJ, O'Maille G, Abagyan R, Siuzdak G, XCMS: Processing mass spectrometry data for metabolite profiling using Nonlinear peak alignment, matching, and identification, Anal Chem, 78 (2006) 779–787. [PubMed: 16448051]

[34]. Mahieu NG, Genenbacher JL, Patti GJ, A roadmap for the XCMS family of software solutions in metabolomics, Curr Opin Chem Biol, 30 (2016) 87–93. [PubMed: 26673825]

[35]. Llufrio EM, Cho K, Patti GJ, Systems-level analysis of isotopic labeling in untargeted metabolomic data by X(13)CMS, Nat Protoc, (2019).

[36]. Pluskal T, Castillo S, Villar-Briones A, Oresic M, MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data, Bmc Bioinformatics, 11 (2010).

[37]. Huang X, Chen YJ, Cho K, Nikolskiy I, Crawford PA, Patti GJ, X13CMS: global tracking of isotopic labels in untargeted metabolomics, Anal Chem, 86 (2014) 1632–1639. [PubMed: 24397582]

[38]. Capellades J, Navarro M, Samino S, Garcia-Ramirez M, Hernandez C, Simo R, Vinaixa M, Yanes O, geoRge: A Computational Tool To Detect the Presence of Stable Isotope Labeling in LC/MS-Based Untargeted Metabolomics, Anal Chem, 88 (2016) 621–628. [PubMed: 26639619]

[39]. Chokkathukalam A, Jankevics A, Creek DJ, Achcar F, Barrett MP, Breitling R, mzMatch-ISO: an R tool for the annotation and relative quantification of isotope-labelled mass spectrometry data, Bioinformatics, 29 (2013) 281–283. [PubMed: 23162054]

[40]. Bueschl C, Kluger B, Neumann NKN, Doppler M, Maschietto V, Thallinger GG, Meng-Reiterer J, Krska R, Schuhmacher R, MetExtract II: A Software Suite for Stable Isotope-Assisted Untargeted Metabolomics, Anal Chem, 89 (2017) 9518–9526. [PubMed: 28787149]

[41]. Liu F, Ma F, Wang Y, Hao L, Zeng H, Jia C, Wang Y, Liu P, Ong IM, Li B, Chen G, Jiang J, Gong S, Li L, Xu W, PKM2 methylation by CARM1 activates aerobic glycolysis to promote tumorigenesis, Nat Cell Biol, 19 (2017) 1358–1370. [PubMed: 29058718]

[42]. Hao L, Shi Y, Thomas S, Vezina CM, Bajpai S, Ashok A, Bieberich CJ, Ricke WA, Li L, Comprehensive urinary metabolomic characterization of a genetically induced mouse model of prostatic inflammation, Int J Mass Spectrom, 434 (2018) 185–192. [PubMed: 30872949]

[43]. Wenger CD, Phanstiel DH, Lee MV, Bailey DJ, Coon JJ, COMPASS: a suite of pre- and post-search proteomics software tools for OMSSA, Proteomics, 11 (2011) 1064–1074. [PubMed: 21298793]

[44]. Holman JD, Tabb DL, Mallick P, Employing ProteoWizard to Convert Raw Mass Spectrometry Data, Curr Protoc Bioinformatics, 46 (2014) 13 24 11–19.

[45]. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu YF, Djoumbou Y, Mandal R, Aziat F, Dong E, Bouatra S, Sinelnikov I, Arndt D, Xia JG, Liu P, Yallou F, Bjorndahl T, Perez-Pineiro R, Eisner R, Allen F, Neveu V, Greiner R, Scalbert A, HMDB 3.0-The Human Metabolome Database in 2013, Nucleic Acids Res, 41 (2013) D801–D807. [PubMed: 23161693]

[46]. Shadforth IP, Dunkley TPJ, Lilley KS, Bessant C, i-Tracker: For quantitative proteomics using iTRAQ (TM), Bmc Genomics, 6 (2005).

[47]. Sturm RM, Lietz CB, Li LJ, Improved isobaric tandem mass tag quantification by ion mobility mass spectrometry, Rapid Commun Mass Sp, 28 (2014) 1051–1060.

[48]. Greer T, Hao L, Nechyporenko A, Lee S, Vezina CM, Ricke WA, Marker PC, Bjorling DE, Bushman W, Li LJ, Custom 4-Plex DiLeu Isobaric Labels Enable Relative Quantification of Urinary Proteins in Men with Lower Urinary Tract Symptoms (LUTS), Plos One, 10 (2015).

Highlights:

- A novel online software tool, Metandem, was developed for isobaric labeling-based metabolomics.
- Metandem provides highly accurate metabolite quantification on the MS/MS level.
- Online parameter optimization, identification, and statistics can be conducted simultaneously.
- Metandem was evaluated by various MS platforms and multiplex isobaric labeling experiments.

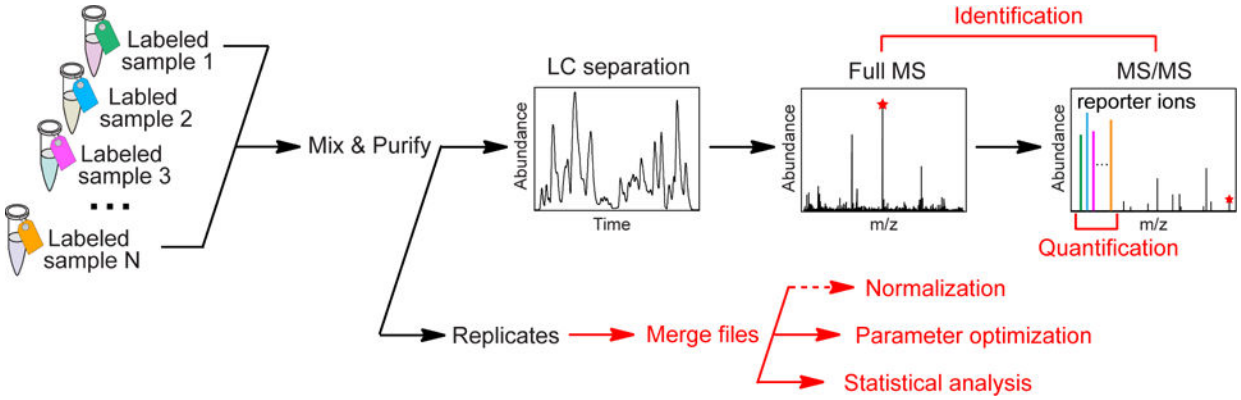

Figure 1.

Pipeline of mass spectrometry-based isobaric labeling metabolomics using Metandem tool. Data analysis capability of Metandem is illustrated and highlighted in red color, including metabolite quantification, identification, parameter optimization, and statistical visualization.

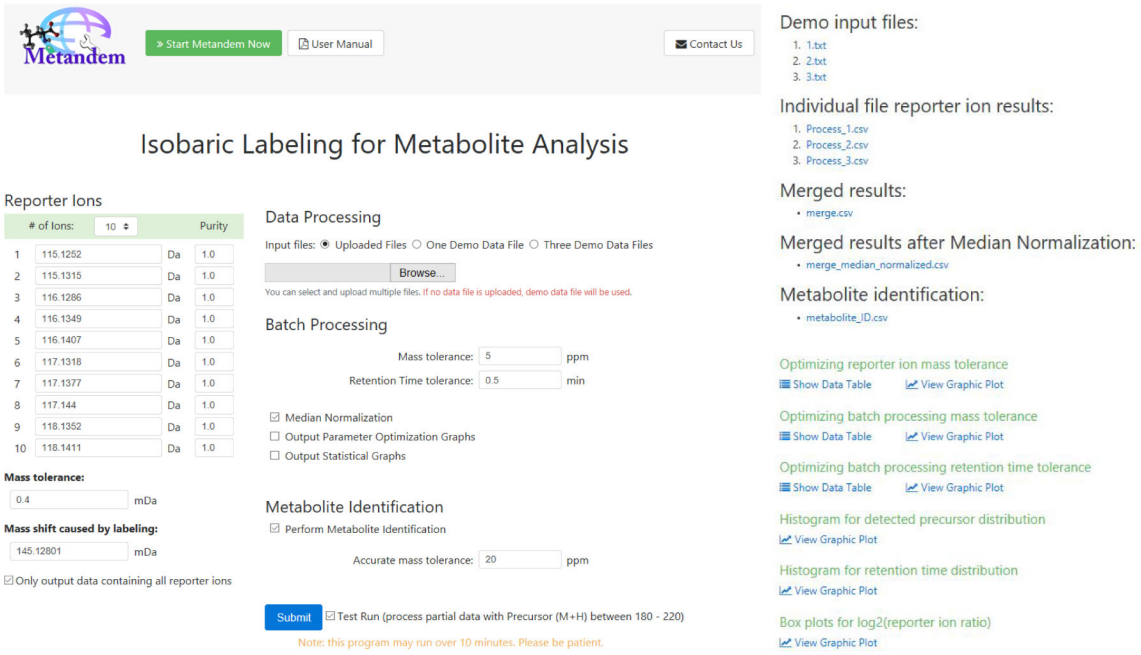

Figure 2.

Screenshot of the Metandem web interface. Output results of three demo data file are illustrated on the right which can be downloaded as .csv files. Parameter optimization and statistical plots can be edited/visualized online. Demo data is available online at http://metandem.com/web/.

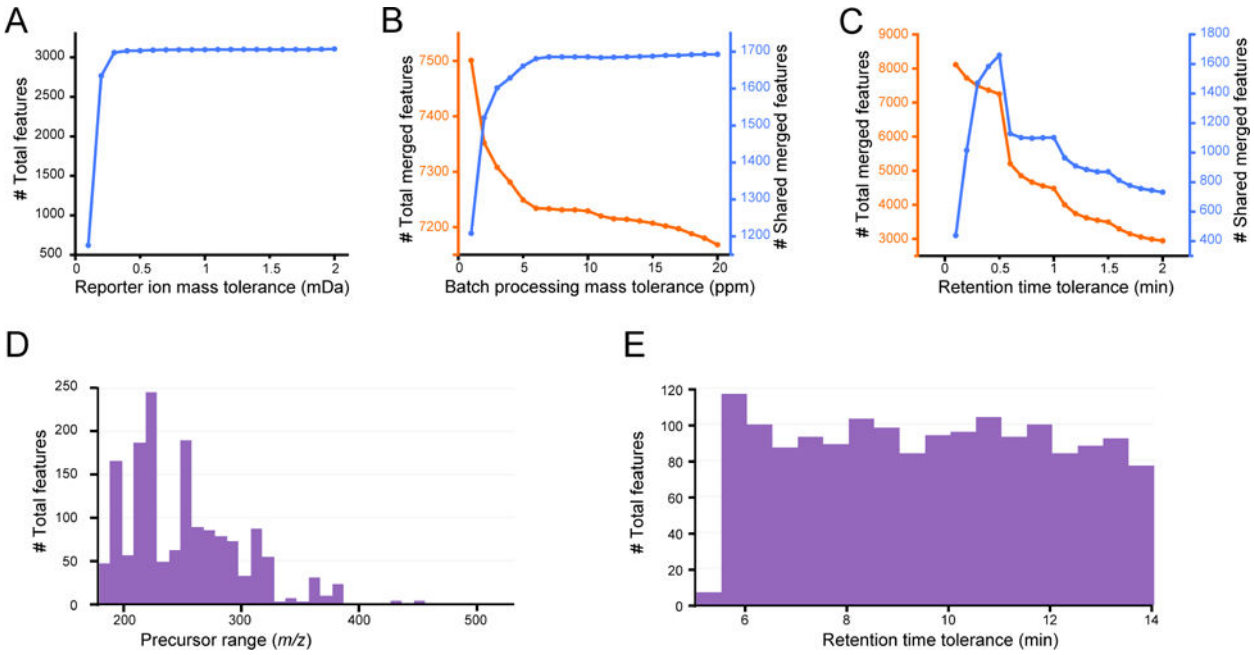

Figure 3.

Parameter optimization graphs (POGs) and statistical analysis of Metandem. Three demo files provided in Metandem interface were used as input files. Parameter optimization graphs for reporter ion mass tolerance (A), batch processing mass tolerance (B), and batch processing retention time tolerance (C). Statistical graphs were generated using the shared features among all merged input files, including histogram of precursor mass range (D) and histogram of retention time (E).

A

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	Precursor (M+H)	Charge	MW	Time	RP1	RP2	RP3	RP4	RP5	RP6	RP7	RP8	RP9	RP10	Ratio RP1	Ratio RP2	Ratio RP3	Ratio RP4	Ratio RP5	Ratio RP6	Ratio RP7	Ratio RP8	Ratio RP9	Ratio RP10
2	221.1734	1	75.0376	5.40	1.E+07	1.E+07	1.E+07	1.E+07	8.E+06	3.E+07	3.E+07	3.E+07	8.E+07	8.E+07	1	0.93	0.78	0.91	0.54	2.19	1.97	2.12	5.80	5.55
3	251.1782	1	105.0423	5.41	2.E+06	2.E+06	2.E+06	2.E+06	2.E+06	4.E+06	3.E+06	4.E+06	1.E+07	1.E+07	1	0.96	0.84	0.79	0.75	1.80	1.59	1.84	4.65	4.67
4	251.1845	1	105.0487	5.41	2.E+06	2.E+06	2.E+06	2.E+06	2.E+06	4.E+06	3.E+06	4.E+06	1.E+07	1.E+07	1	0.82	0.79	0.70	0.72	1.87	1.56	1.89	4.61	4.76
5	613.1592	2	321.8954	5.50	3.E+04	2.E+04	2.E+04	3.E+04	4.E+04	6.E+04	5.E+04	6.E+04	2.E+05	1.E+05	1	0.73	0.79	0.81	1.15	1.77	1.68	1.76	4.73	3.72
6	219.1555	1	73.0196	5.50	2.E+06	2.E+06	1.E+06	2.E+06	1.E+06	9.E+04	5.E+04	7.E+04	1.E+05	9.E+04	1	0.85	0.71	0.96	0.60	0.05	0.03	0.04	0.08	0.05
7	280.1973	1	134.0614	5.51	1.E+05	1.E+05	1.E+05	1.E+05	1.E+05	3.E+05	2.E+05	3.E+05	7.E+05	6.E+05	1	0.95	1.03	1.04	1.07	2.33	1.96	2.65	6.50	5.66
8	809.2445	2	517.9806	5.51	3.E+04	4.E+04	3.E+04	3.E+04	4.E+04	6.E+04	7.E+04	1.E+05	2.E+05	2.E+05	1	1.19	0.97	1.06	1.36	2.04	2.39	3.27	7.16	7.34

B

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X
1	Number	Average Precursor (M+H)	Charge	Average MW	Average Time	Average _Ratio_ RP1	Average _Ratio_ RP2	Average _Ratio_ RP3	Average _Ratio_ RP4	Average _Ratio_ RP5	Average _Ratio_ RP6	Average _Ratio_ RP7	Average _Ratio_ RP8	Average _Ratio_ RP9	Average _Ratio_ RP10	RSD_ Ratio _RP1	RSD_ Ratio _RP2	RSD_ Ratio _RP3	RSD_ Ratio _RP4	RSD_ Ratio _RP5	RSD_ Ratio _RP6	RSD_ Ratio _RP7	RSD_ Ratio _RP8	RSD_ Ratio _RP9	R R _ 0
2	1	221.1733	1	75.0374	5.48	1	0.95	0.72	0.90	0.52	2.13	1.95	2.06	5.59	5.48	0	0.04	0.09	0.06	0.02	0.02	0.03	0.07	0.03
3	2	251.1781	1	105.0423	5.57	1	0.86	0.79	0.69	0.73	1.68	1.51	1.76	4.26	4.40	0	0.09	0.08	0.11	0.04	0.07	0.07	0.07	0.09
4	3	251.1844	1	105.0486	5.41	1	0.86	0.75	0.69	0.71	1.75	1.56	1.77	4.40	4.60	0	0.03	0.04	0.04	0.02	0.06	0.01	0.05	0.03
5	4	193.1983	1	47.0625	5.47	1	0.14	5.27	43.52	46.35	15.43	13.27	112.67	15.75	23.11	0	0.18	0.05	0.03	0.03	0.05	0.03	0.02	0.07
6	5	231.1706	1	85.0347	5.60	1	1.33	0.81	0.84	1.18	1.47	1.64	1.72	4.70	4.25	0	0.15	0.14	0.08	0.27	0.05	0.13	0.20	0.09
7	6	337.0866	1	190.9508	5.43	1	2.56	2.23	2.67	3.86	5.13	4.29	6.02	10.47	11.75	0	0.77	0.87	0.82	0.91	0.77	0.86	0.81	0.69
8	7	295.1153	1	148.9795	5.63	1	1.09	1.30	1.41	0.79	3.51	3.53	4.00	7.33	10.30	0	0.07	0.07	0.05	0.07	0.01	0.01	0.05	0.04
9	8	190.1782	1	44.0423	5.69	1	0.39	1.26	0.20	0.20	0.24	0.19	0.22	0.32	0.33	0	0.08	0.02	0.01	0.02	0.01	0.05	0.04	0.03
10	9	216.1598	1	70.0239	5.49	1	3.78	2.02	1.79	2.86	2.76	2.49	2.82	6.23	5.62	0	0.36	0.41	0.28	0.32	0.19	0.27	0.22	0.29

C

	A	B	C	D	E	F	G	H	I	J	K	L
1	Input Number	Average Precursor (M+H)	Charge	Average MW	Average Time	dppm	name	chemical_ formula	monisotopic _MW	kegg_id	pubchem_ compound _id	hmdb_id
2	20	301.2075	1	155.0717	0.35	14.03	L-Histidine	C6H9N3O2	155.0695	C00135	6274	HMDB00177
3	20	301.2075	1	155.0717	0.35	11.91	4-Phenylpyridine	C11H9N	155.0735	C11310	13651	HMDB33123
4	32	235.1831	1	89.0473	0.56	4.29	L-Alanine	C3H7NO2	89.0477	C00041	5950	HMDB00161
5	32	235.1831	1	89.0473	0.56	4.29	Sarcosine	C3H7NO2	89.0477	C00213	1088	HMDB00271
6	32	235.1831	1	89.0473	0.56	4.29	Beta-Alanine	C3H7NO2	89.0477	C00099	239	HMDB00056
7	32	235.1831	1	89.0473	0.56	4.29	D-Alanine	C3H7NO2	89.0477	C00133	71080	HMDB01310

Figure 4.

Screenshots of the example result tables. Three demo files provided in Metandem interface were used as input files. A is the quantification result of individual file consisting of precursor ion information and reporter ion intensities and ratios. B is the merge table. For each merged feature, average reporter ion ratios and relative standard deviations (RSD) of ratios are calculated across all input data files, followed by the original reporter ion intensities and ratios from each data file. C is the metabolite identification table with ID numbers from KEGG, PubChem and HMDB databases. Average MW is the average monoisotopic MW across all input files. Note that accurate mass matching can generate multiple IDs from the same mass feature, which requires further examination and confirmation of metabolite identities by labeling metabolite standard compounds.

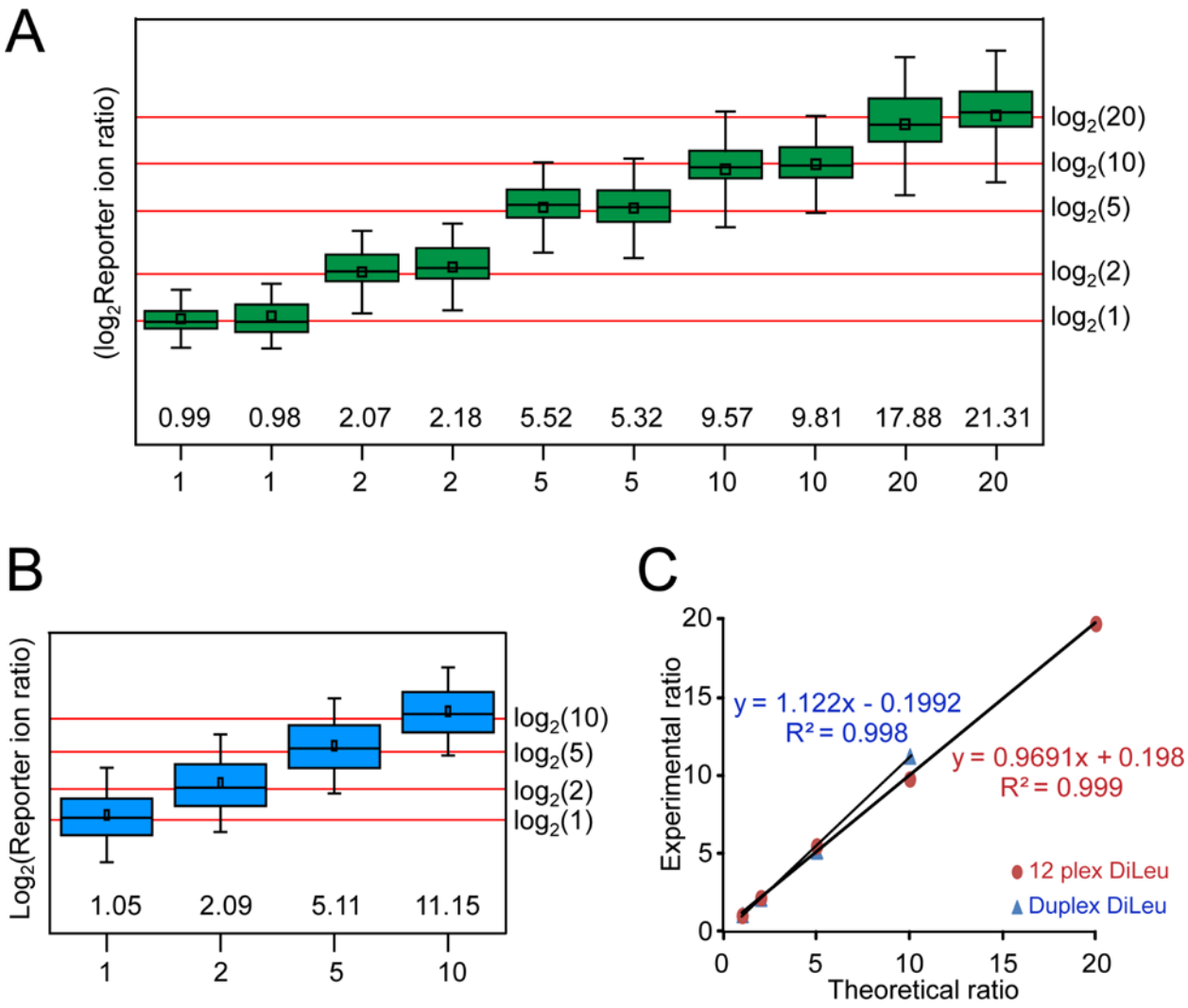

Figure 5.

Quantification accuracy of Metandem tool demonstrated by isobaric DiLeu labeling of cellular metabolites. Boxplots of reporter ion ratios illustrate isobaric 12-plex DiLeu labeling of MCF7 cellular metabolites (A) and isobaric duplex DiLeu labeling of PANC1 cellular metabolites (B). Box denotes 25th and 75th percentiles; line inside the box denotes the median; whisker denotes standard deviation. Excellent linearity and dynamic range of relative quantification was shown in (C) with slope and R^2 close to 1.

Table 1.

Isobaric labeling results of various biological samples analyzed by Metandem.

Biological sample	MS platform	Isobaric labels	Quantified feature[a]	Identified feature
Standards mix	UPLC-MS/MS	4-plex DiLeu	20	12
Standards mix	nanoLC-MS/MS	4-plex DiLeu	14	12
Standards mix	CE-MS/MS	4-plex DiLeu	16	12
Mouse urine	MALDI MS/MS	4-plex DiLeu	55	9
Mouse urine	nanoLC-MS/MS	10-plex DiLeu	2108	510
MCF7 cells	nanoLC-MS/MS	12-plex DiLeu	3510	849
PANC1 cells	nanoLC-MS/MS	Duplex DiLeu	5456	861

[a]Quantified features often contain labeling artifacts that cannot be identified as metabolites.