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Supplementary information

Optimization of metabolomic data processing using NOREVA

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Supplementary Information for:

Optimization of Metabolomic Data Processing Using NOREVA

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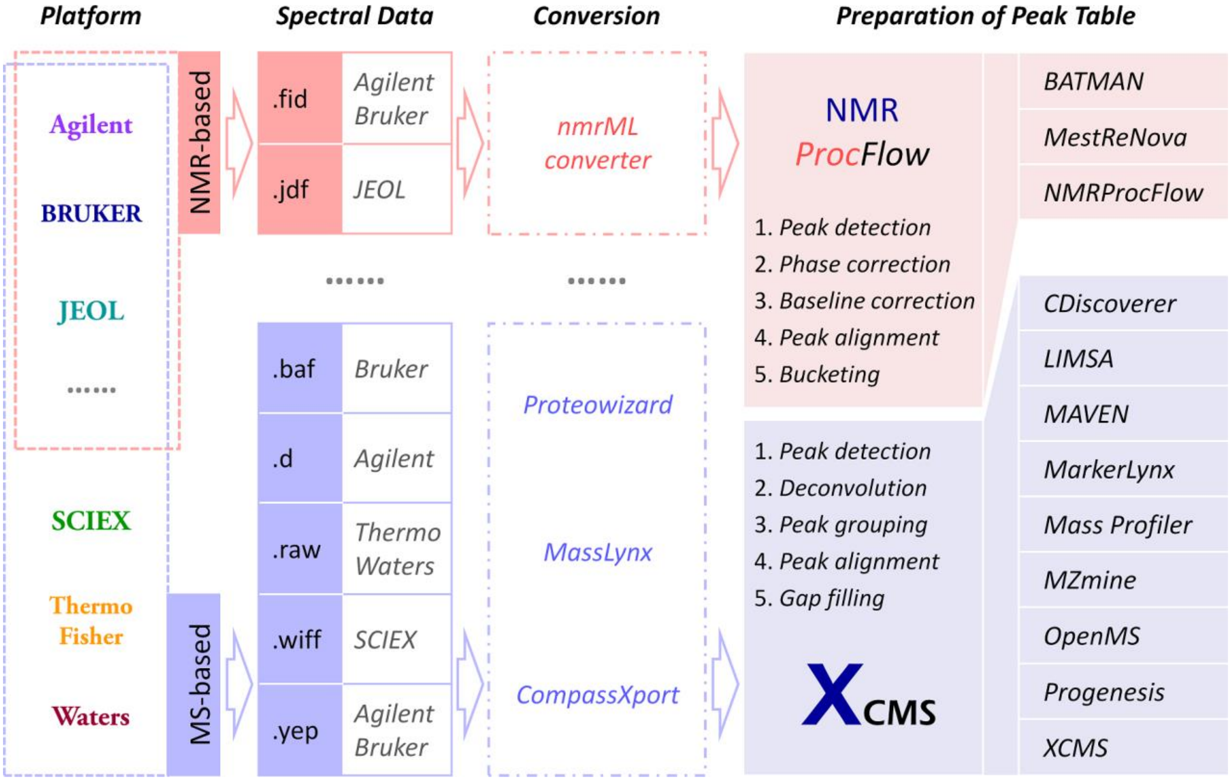


Figure S1. Pre-processing of the spectral data generated based on nuclear magnetic resonance (NMR) or mass spectrometry (MS). Spectral data were first generated using various platforms developed by different vendors, which were then *converted* to the open-source format. Finally, a peak table was *prepared* based on the resulting files of open-source format. The *preparation* process (peak detection, phase correction, baseline correction, peak alignment & bucketing) for NMR-based metabolomics is slightly different from that (peak detection, deconvolution, peak grouping, peak alignment & gap filling) for the MS-based ones. The resulting peak table gives a starting point for the protocol described in this study.

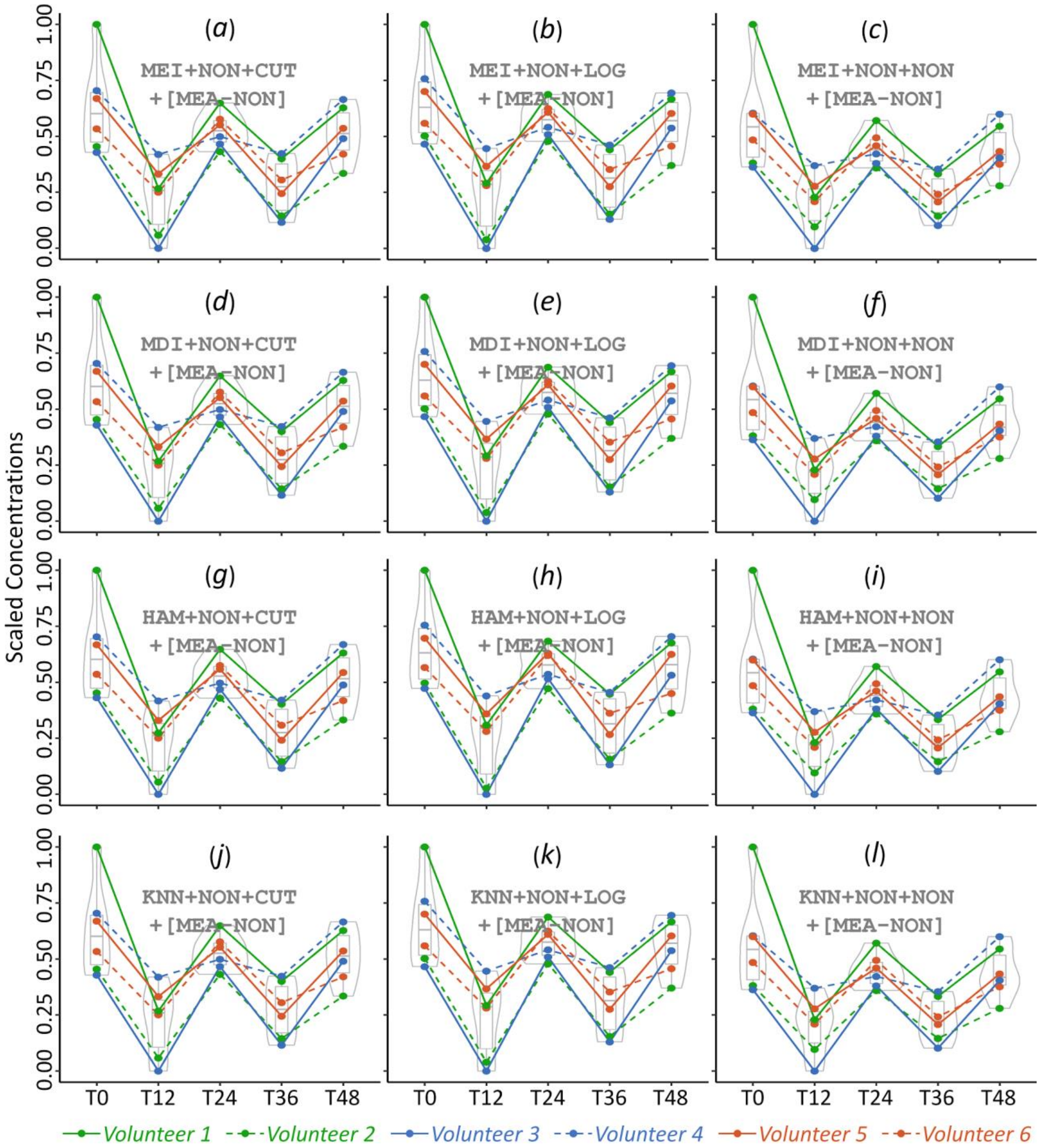


Figure S2. The performances of 12 possible workflows (that were used in *Skarke*'s pioneering study (1) to a time-course dataset PMID29215023) assessed using a well-established metabolic biomarker *Cortisol* (that elevates in the morning and declines in the evening). This dataset is a time-course consecutive sample collection of different time-points (T0: 0 hour in the morning; T12: 12 hours in the evening; T24: 24 hours in the morning; T36: 36 hours in the evening; T48: 48 hours in the morning). The time-dependent fluctuation pattern of metabolic marker *Cortisol* was successfully reproduced by all 12 possible workflows.

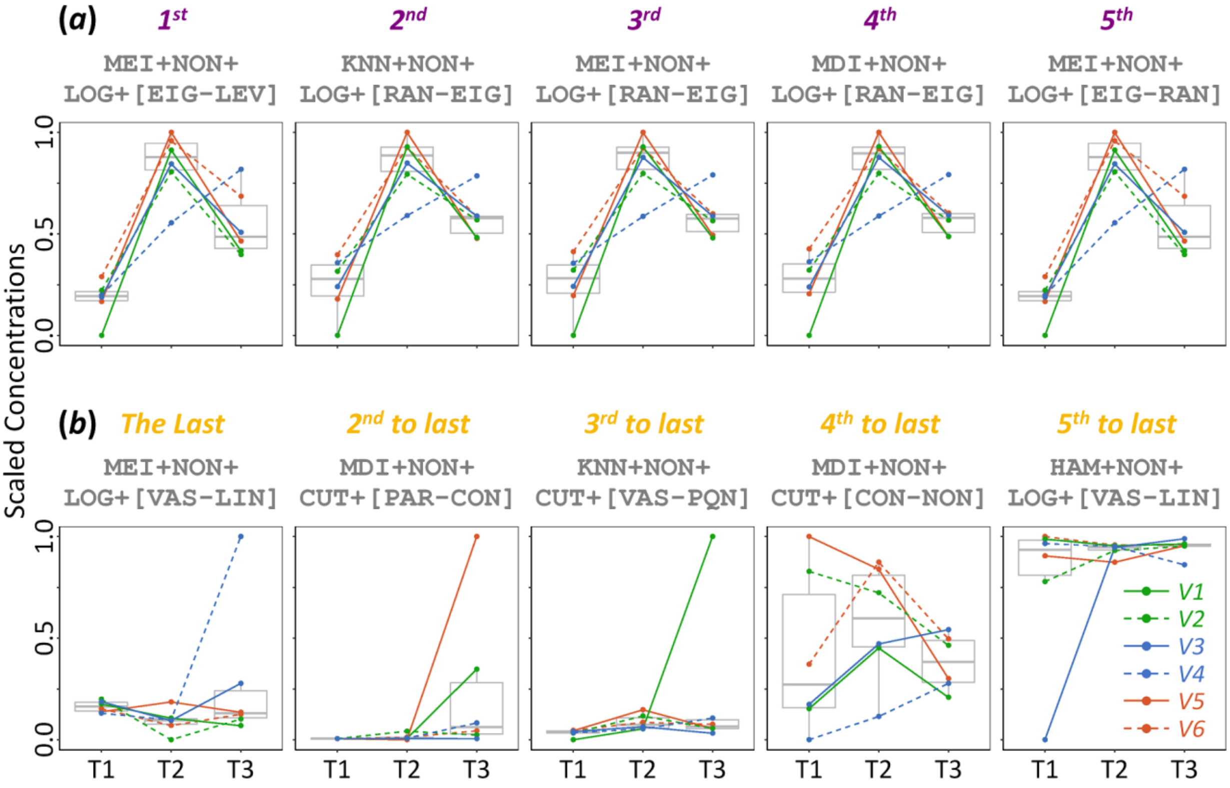


Figure S3. The processing outcomes of (***a***) five top-ranked and (***b***) five last-ranked workflows on the well-established metabolic marker '*kynurenine*' at three different time-points. **T1**: before malaria infection; **T2**: on the day of positive blood smear; **T3**: three weeks after anti-infectious treatment. (***a***) five top-ranked workflows can effectively preserve the 'true' biological variation of '*kynurenine*', which was reported (2,3) to elevate in patient plasma after infection (T1 to T2) and then decline after anti-malaria treatment (T2-T3); (***b***) the last-ranked workflows can hardly reproduce such 'true' biological variation (no statistically significant variation between any two time-points). V1, V2, V3, V4, V5, and V6 referred to six volunteers that were numbered from 1 to 6. Corresponding processing workflow was provided for each plot, and detailed descriptions on the processing methods in these workflows can be found in **Table S4** and **Table S5**.

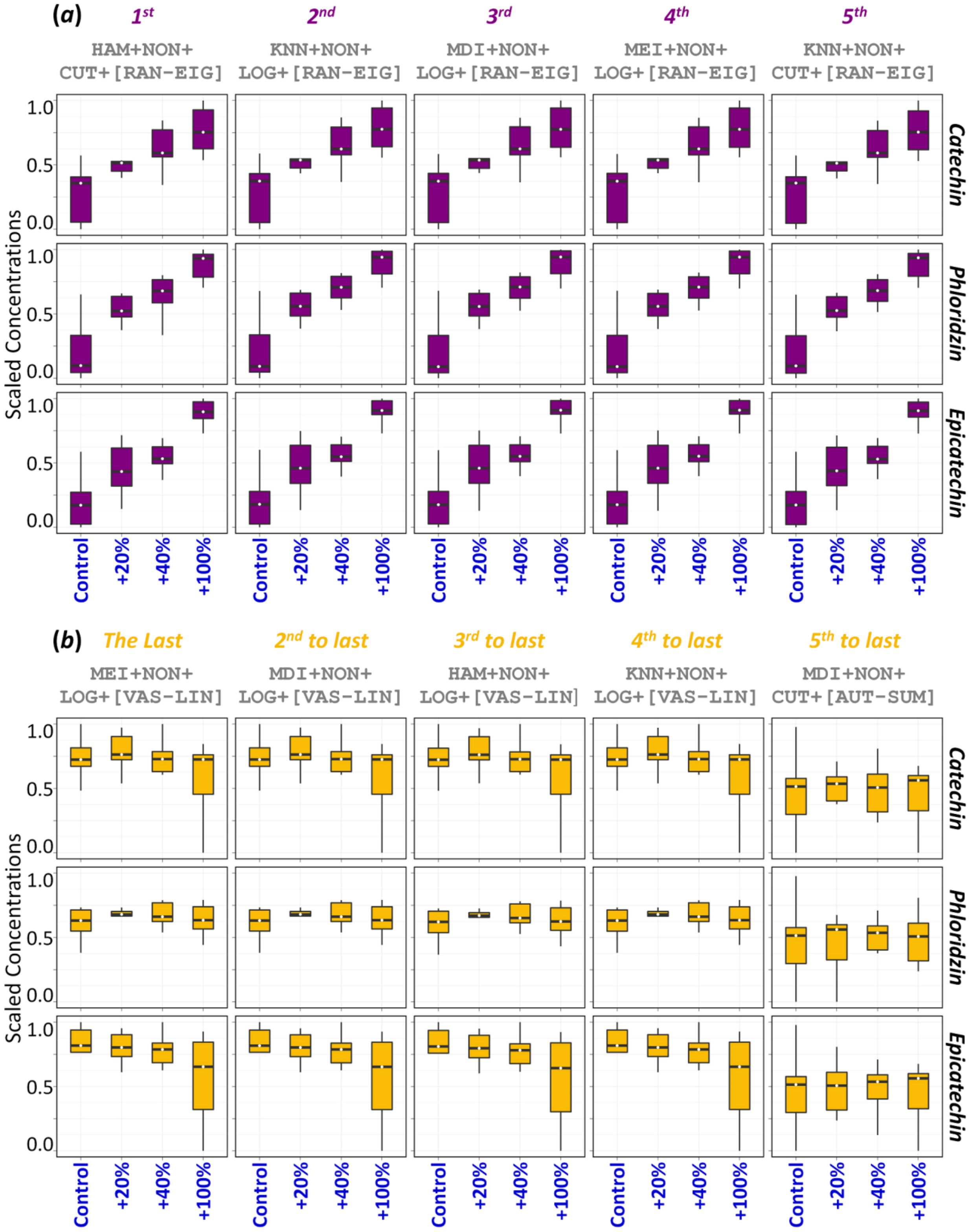


Figure S4. The processing outcomes of (***a***) five top-ranked and (***b***) five last-ranked workflows on three compounds (*catechin*, *phloridzin*, and *epicatechin*) spiked with the gradual increase of concentrations from the control to an increase of 20%, then 40%, and finally 100% (4). (***a***) five top-ranked workflows largely preserved the expected concentration variations of these spike-in compounds (from control to +20%, then to +40%, and finally to +100%); (***b***) five last-ranked workflows could not reproduce the spiked concentration variations for any of the compounds. The corresponding processing workflow was provided for each plot, and detailed descriptions on the processing methods in these workflows can be found in **Table S4** and **Table S5**.

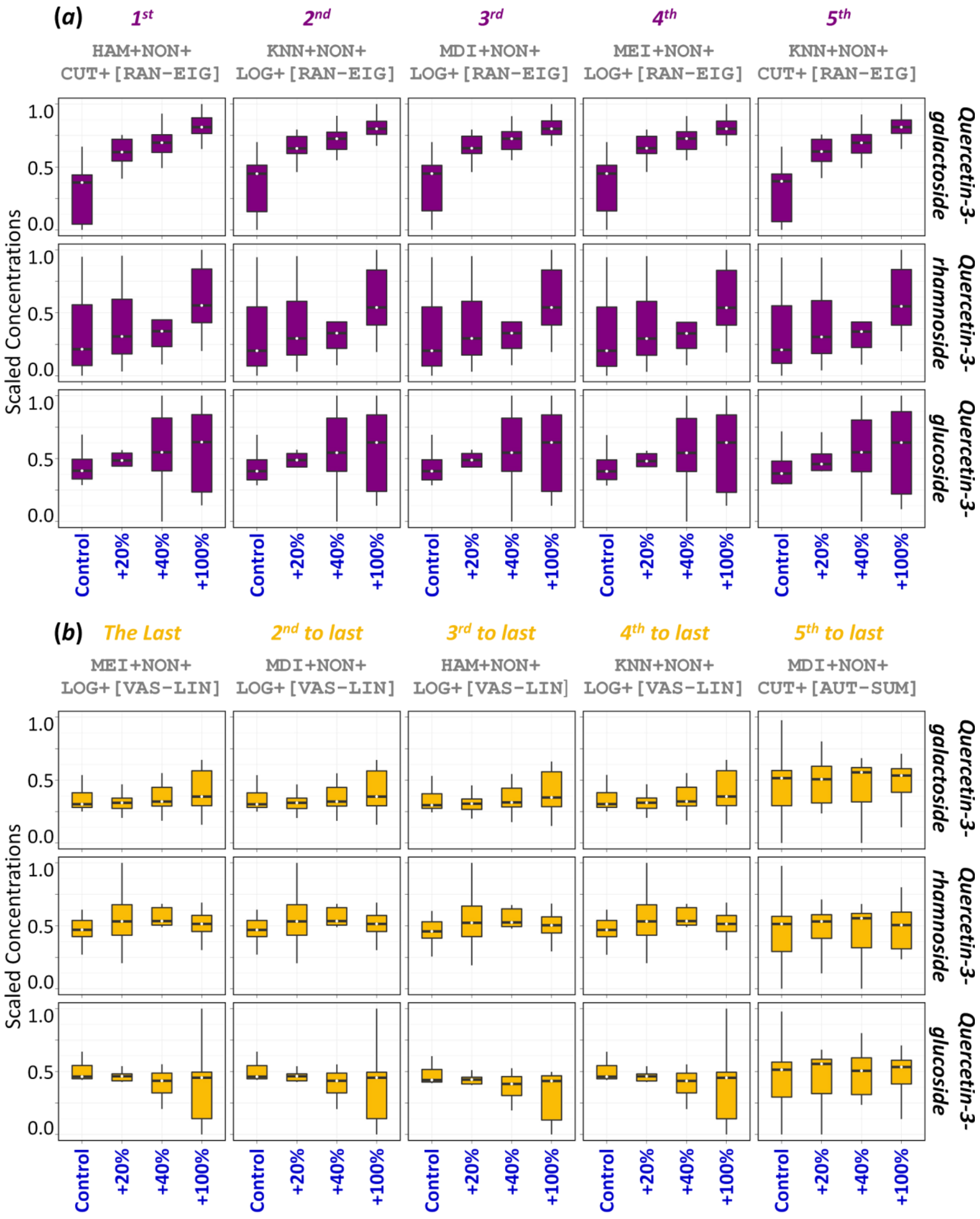


Figure S5. The processing outcomes of (***a***) five top-ranked and (***b***) five last-ranked workflows on three spike-in compounds (*quercetin-3-galactoside*, *quercetin-3-rhamnoside*, and *quercetin-3-glucoside*) spiked with the gradual increase of concentrations from the control to an increase of 20%, then 40%, and finally 100% (4). (***a***) five top-ranked workflows largely preserved those expected concentration variations of these spike-in compounds (from control to +20%, then to

+40%, and finally to +100%); (***b***) five last-ranked workflows could not reproduce those spiked concentration variations for any of those compounds. The corresponding processing workflows applied were indicated for all plots, and the detailed descriptions on the processing methods in these workflows can be found in **Table S4** and **Table S5**.

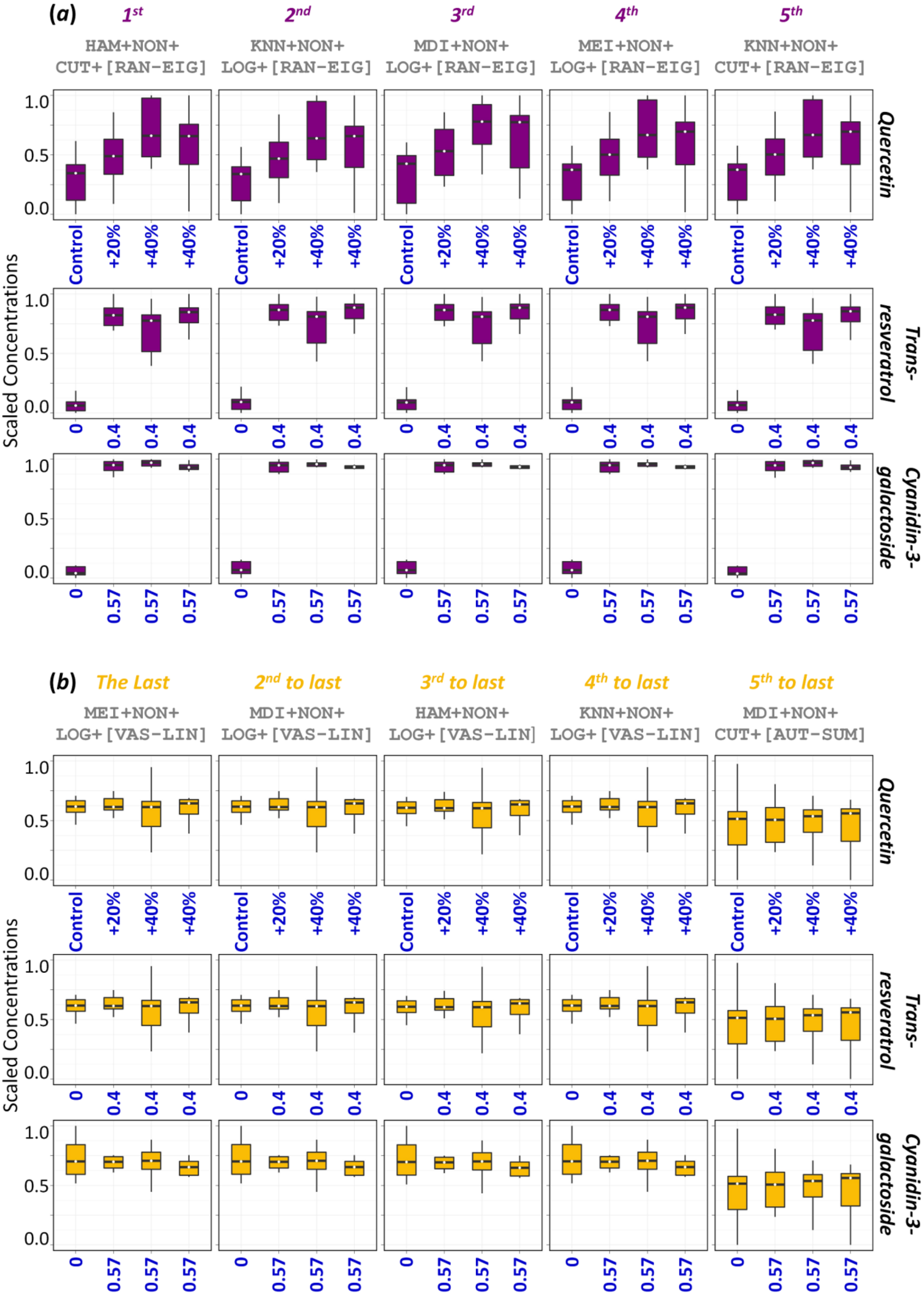


Figure S6. The processing outcomes of (***a***) five top-ranked and (***b***) five last-ranked workflows on three compounds (*quercetin*, *trans-resveratrol*, and *cyanidin-3-galactoside*). *Quercetin* was spiked with a concentration variation from control to an increase of 20%, then 40%, and finally 40% (4); *Trans-resveratrol*, and *Cyanidin-3-galactoside* were naturally unavailable in the first extract type, and spiked via constant concentration (0.4 and 0.57 mg/l, respectively) in another 3 extract types (4). (***a***) five top-ranked workflows largely preserved the expected concentration variations of these spike-in compounds; (***b***) five last-ranked workflows could not reproduce the spiked concentration changes for any of the compounds. Corresponding processing workflows applied were indicated for all plots, and the detailed descriptions on the processing methods in these workflows can be found in **Table S4** and **Table S5**.

Table S1. A variety of typical tools available for the pre-processing of spectral data acquired using various instruments of different vendors. NMR: nuclear magnetic resonance; MS: mass spectrometry. The detailed application of each tool in *spectral data pre-processing* procedure was provided in **Figure S1** (4 and 12 tools were applied to the sections of '*Conversion*' and '*Preparation of Peak Table*', respectively).

Data Pre-processing Tool	**Analytical Platform**	**Compatible Operating System**	**License (*vendor*)**	**Brief Description of Each Data Pre-processing Tool**
BATMAN	NMR	Linux MacOS Windows	General Public License	Preparing metabolomic peak table based on *Bayesian* model, which incorporates information on characteristic peak patterns of metabolites and is able to account for shifts in the position of peaks commonly seen in NMR spectra (5).
CompassXport	MS	Linux MacOS Windows	Apache License	Converting *Bruker* and some *Agilent* raw files to the universal mzXML format. These raw files include the following *Bruker* MS data file formats: analysis.baf, analysis.yep, *etc.*, and the *Agilent* MS data file format: analysis.yep (6)
Compound Discoverer	MS	Windows	Commercial (*Thermo Fisher*)	Preparing peak table for targeted/untargeted metabolomics, which comprises a workflow including peak picking, RT alignment, formula prediction, background annotation, and an automated library scanning for identification purposes (7).
LIMSA	MS	Linux Windows	General Public License	Preparing peak table for quantitative analysis of mass spectrometric metabolomic or lipidomic data, which sequentially carries out peak identification, integration, assignment, isotopic overlap correction, and quantification (8).
MarkerLynx	MS	Windows	Commercial (*Waters*)	Preparing the peak table for mass spectrometry-based metabolomics, which aims at conducting noise filtering, peak detection, raw data deconvolution, removal of isotope masses and alignment of the retention time (9).
MassHunter Profinder	MS	Windows	Commercial (*Agilent*)	Preparing the peak table from profiling and MSD data files, which is optimized to not only extract features from large datasets but also provides with an intuitive user interface to inspect and review each feature across the files (10).
MassLynx	MS	Windows	Apache License	Converting *Waters* and other raw files to mzXML format and controlling *Waters* mass spectrometers using integrated embedded PC technology, which acquires nominal mass, exact mass, MS/MS and exact mass MS/MS data (11).

MAVEN	MS	Linux MacOS Windows	General Public License	Preparing peak table for metabolomic quantitation from high-resolution full-scan mass spectrometry or multiple reaction monitoring datasets, which automatically detects and reports peak intensities for isotope-labeled metabolites (12).
MestreNova	NMR	Linux MacOS Windows	Commercial (*Mestrelab*)	Preparing peak table by aiming primarily at chemists dealing with 1D/2D spectra of small/midsized molecules, which covers all spectra pre-processing steps such as phase correction, baseline correction, peak alignment, and bucketing (13).
MZmine	MS	Linux MacOS Windows	General Public License	Preparing peak table for data processing of mass spectrometric metabolomic and proteomic data, which included noise reduction by filtering in chromatographic direction, cropping raw data range and removing scans by their width (14).
nmrML	NMR	MacOS Windows	MIT License	Converting the exchange syntax from the vendors' raw files into XSD-compliant nmrML by means of mappings from the *Bruker* 'acqus' or *Agilent* 'procpar' raw file to nmrML elements and controlled vocabulary terms (15).
NMRProcFlow	NMR	Linux MacOS Windows	General Public License	Preparing peak table for 1D spectra processing and metabolic fingerprinting of NMR metabolomic data, which covers all spectra pre-processing steps such as phase correction, baseline correction, peak alignment, and bucketing (16).
OpenMS	MS	Linux MacOS Windows	Berkeley Software Distribution	Preparing the peak table for addressing the most common tasks in quantitative metabolomics, which includes isotopic deconvolution, chromatographic peak-picking, RT alignment and feature-linking across multiple runs (17).
Progenesis QI	MS	Windows	Commercial (*Waters*)	Preparing the peak table for targeting the small molecule discovery analysis for metabolomics, which contains a series of pre-processing procedures including baseline correction, smoothing, deconvolution, and peak alignment (18).
Proteowizard	MS	Linux MacOS Windows	Apache License	Converting the obtained original MS data into mzXML format, which supports reading of mzML, mzXML and Thermo RAW files and provides a modular and extensible set of open-source, cross-platform tools and libraries (19).
XCMS	MS	Linux MacOS Windows	General Public License	Preparing peak table for targeted/untargeted LC-MS metabolomics by extracting metabolic features from raw MS data, which comprises chromatographic peak detection, sample alignment and peak correspondence (20).

Table S2. Representative tools available for the *statistical treatment & interpretation* of metabolomic data. ANOVA: analysis of variance; FC: fold change; HCA: hierarchical clustering analysis; *K*-means: *k*-means clustering; OPLS-DA: orthogonal partial least squares-discriminant analysis; PCA: principal component analysis; PLS-DA: partial least squares discriminant analysis; SAM: significance analysis for microarrays; SOM: self-organizing map; SVM-RFE: support vector machine-recursive feature elimination; WRS: wilcox rank sum test with permutation.

Tool	**Statistical Treatment**		**Interpretation**	
	No. of Methods for Sample Separation	**No. of Methods for Marker Identification**	**Availability of Pathway Analysis**	**Availability of Functional Enrichment**
KIMBLE (21)	≥2 (HCA, *etc.*)	≥1 (SVM-RFE)	NO	NO
MeltDB (22)	≥2 (PCA, *etc.*)	≥3 (ANOVA, *etc.*)	YES	YES
MetaboAnalyst (23)	≥4 (SOM, *etc.*)	≥11 (SAM, *etc.*)	YES	YES
Metabolomics Workbench (24)	≥2 (HCA, *etc.*)	≥5 (OPLS-DA, *etc.*)	YES	NO
metaP-server (25)	≥1 (PCA)	≥1 (Student's *t*-test)	YES	NO
metaX (26)	≥1 (PCA)	≥6 (PLS-DA, *etc.*)	YES	NO
MetDAT (27)	≥2 (HCA, *etc.*)	≥4 (FC, *etc.*)	YES	NO
MetFlow (28)	≥2 (HCA, *etc.*)	≥6 (WRS, *etc.*)	YES	NO
MMEASE (29)	≥4 (*K*-means, *etc.*)	≥13 (Relief, *etc.*)	YES	NO
muma (30)	≥1 (PCA)	≥4 (OPLS-DA, *etc.*)	NO	NO
W4M (31)	≥2 (HCA, *etc.*)	≥6 (FC, *etc.*)	NO	NO
WebSpecmine (32)	≥3 (*K*-means, *etc.*)	≥5 (SVM-RFE, *etc.*)	YES	NO

Table S3. Twenty representative studies that explicitly described the application of NOREVA in their metabolomic studies. These studies covered a very wide range of research fields, such as: *Microbiology*, *Molecular Biology*, *Pharmaceutical Science*, *Medical Science*, *Food & Environmental Science*, *Analytical Chemistry*, *Chromatography & Spectrometry*, and *Chemometrics* (for each field, 2~3 representative studies were described). CE-MS: capillary electrophoresis-mass spectrometry; GC-MS: gas chromatography-MS; LC-HR-MS/MS: liquid chromatography-high resolution-tandem MS; LC-HRMS: LC-high resolution MS; UPLC-HRMS: ultra-performance LC-high resolution MS; LC-MS/MS: LC-tandem MS.

Representative Publications	**The Application of NOREVA in Current Metabolomics as Described in Each Representative Publication**	**Research Field (*sub-field of research*)**	**Metabolomic Study Type**	**Platform**
(01) *Gut Microbes*. 11: 882, 2020	NOREVA was used to process the multi-class metabolomic data of 9 strains/combinations, which revealed the beneficial effects of microbiome regulation in the amelioration of non-alcoholic fatty liver disease (33).	Microbiology (*microbiome regulation*)	*Multi-class*	GC-MS
(02) *Front Microbiol*. 10: 1996, 2019	NOREVA was applied to process the metabolomic data that were generated based on the knockout of key biosynthetic gene, which revealed the mechanism of metabolite synthesis in the survival of a bacterial pathogen (34).	Microbiology (*metabolite biosynthesis*)	*Binary Classification*	UPLC-HRMS
(03) *Sci Rep*. 10: 17931, 2020	NOREVA was adopted to process time-course metabolomic data across 5 time-points, which helped the characterization of the bacterial and fungal diversity of three phyto-thermal baths performed in different months (35).	Microbiology (*microbiota dynamics*)	*Time-course*	GC-MS
(04) *Nucleic Acids Res*. 48: 385, 2020	NOREVA was employed as a data treatment module in the development of an interactive tool for single cell omics data interpretation, which facilitated the biological interpretation of single-cell multi-omics data by bench scientists (36).	Molecular Biology (*single-cell multi-omics*)	*Multi-class*	LC-MS GC-MS
(05) *Anal Chim Acta*. 1143: 124, 2021	NOREVA was recognized as one of the 'popular tools' for the processing of metabolomic data, which could transform complex raw data to a simplified data matrix, and reduced the effect from extreme outliers (37).	Molecular Biology (*single-cell metabolomics*)	*Multi-class* *Time-course*	LC-MS GC-MS

(06) *Aging Cell*. 19: e13213, 2020	NOREVA was utilized to remove systematic variations among samples in metabolomics study, which helped to discover the functional effect and metabolic profile of a drug combination for the treatment of cardiac aging (38).	Pharmaceutical Science (*drug synergistic efficacy*)	*Multi-class Time-course*	LC-MS/MS
(07) *Front Pharmacol*. 10: 127, 2019	NOREVA was used to process the metabolomic dataset for subsequent binary classification, which helped to assess the effectiveness of direct data merging strategy in long-term & large-scale pharmacometabonomics (39).	Pharmaceutical Science (*pharmaco-metabolomics*)	*Binary Classification*	LC-HRMS
(08) *J Proteome Res*. 19: 1913, 2020	NOREVA was adopted to process the metabolomic data and remove unwanted variations between samples, which assisted in determining whether urinary volatile terpenes levels could monitor breast cancer treatment efficacy (40).	Medical Science (*disease diagnosis*)	*Binary Classification*	GC-MS
(09) *Sci Rep*. 10: 16142, 2020	NOREVA was applied to process the metabolomic dataset for subsequent binary classification, which helped to discover the metabolic markers capable of predicting the development of a typical pregnancy complication (41).	Medical Science (*disease development*)	*Binary Classification*	LC-MS GC-MS
(10) *J Chromatogr B Analyt Technol Biomed Life Sci*. 1114: 119, 2019	NOREVA enabled the process of a lipidomic profile dataset for the subsequent binary classification, which facilitated the successful characterization of several novel lipidomic markers specific to the internet-gaming disorder (42).	Medical Science (*disease marker discovery*)	*Binary Classification*	LC-MS
(11) *Sci Total Environ*. 718: 137267, 2020	NOREVA facilitated the process of the metabolomic data in prior to binary classification, which helped to identify brain region-specific variations in metabolic pathway associated with the exposure to environmental ultrafine particles (43).	Food & Environmental Science (*environmental pollutant*)	*Binary Classification*	LC-MS GC-MS
(12) *LWT-Food Sci Technol*. 129: 109454, 2020	NOREVA realized the processing of a metabolomic dataset consisting of different sesame seeds from 6 countries, which quantified metabolic markers and enabled the evaluation of nutrition composition (44).	Food & Environmental Science (*nutrition composition*)	*Multi-class*	LC-MS GC-MS

(13) *Anal Chem*. 92: 203, 2020	NOREVA was regarded as one of the 'advanced tools' for the processing of metabolomic data, which facilitated the comparative evaluation of the performance of methods in processing the studied data matrix (45).	Analytical Chemistry (*instrument methodology*)	*Multi-class* *Time-course*	GC-MS CE-MS
(14) *Anal Chim Acta*. 1061: 60, 2019	NOREVA was used to check the effectiveness of a novel tool in improving the classification accuracy in metabolic marker discovery, which facilitated the removal of batch effects for large-scale untargeted metabolomics (46).	Analytical Chemistry (*quantitative analysis*)	*Binary Classification*	LC-MS GC-MS
(15) *Metabolomics*. 14: 54, 2018	NOREVA was employed as a data-processing module in the construction of a novel metabolomic tool, which allowed the visualization and comparative evaluation between different normalization algorithms (47).	Analytical Chemistry (*analytical software*)	*Multi-class* *Time-course*	LC-MS GC-MS
(16) *Anal Chem*. 91: 9836, 2019	NOREVA was considered to 'simplify' the investigation and selection of optimum processing workflow, which provided the platforms to perform and evaluate different normalization techniques on metabolomic dataset (48).	Chemometrics (*chemical calibration*)	*Multi-class* *Time-course*	LC-HR-MS/MS
(17) *Bioessays*. 40: e1700210, 2018	NOREVA was 'encouraged' to be applied for assessing the potential performance of different metabolomic processing methods on their empirical profiles, which could reflect the structure of empirical data in question (49).	Chemometrics (*cheminformatic pattern*)	*Multi-class* *Time-course*	GC-MS
(18) *Mass Spectrom Rev*. doi: mas.21672, 2020	NOREVA was considered as a 'user-friendly' metabolomic implementation with graphical interface for assessing the performances of both batch effect removal and biological information retention for mass spectrometry (MS) (50).	Chromatography & Spectrometry (*mass spectrometry*)	*Multi-class* *Time-course*	LC-MS GC-MS
(19) *Mass Spectrom Rev*. 40: 162, 2021	NOREVA was recognized as an MS-based batch processing service, which combined data-driven normalizations with internal standard/quality control-based methods and evaluated the performance for multiple testing (51).	Chromatography & Spectrometry (*mass spectrometry*)	*Multi-class* *Time-course*	LC-MS

(20) *Metabolites*. 9: 292, 2019	NOREVA was adopted to process the metabolomic data for subsequent binary classification, which helped to evaluate the performance of ammonium fluoride as additive salt in the hydrophilic interaction liquid chromatography (52).	Chromatography & Spectrometry (*liquid chromatography*)	*Binary Classification*	LC-HRMS

Table S4. A list of methods for data filtering, data imputation, quality control (QC) sample correction, and data transformation in the metabolomic *peak table processing*. A three-letter abbreviation (Abb.) code was used to represent each method of the different step (**Figure 1**) of metabolomics *peak table processing*. In a particular step of a processing workflow, if none of the above methods was applied, a three-letter code NON was used to indicate the non-application of any method in the corresponding step. Both method's introduction and research application(s) were provided.

Abb.	Method Name	Brief Introduction to Each Method and Its Application(s) in Metabolomic Studies
		Data Filtering
TPM	Tolerable Percent of Missing Values	**Method's Introduction**: This method calculates the percentage of missing values for each metabolite, and discards the one whose percent of missing values among all samples is higher than a threshold (53). **Research Application(s)**: It was used to filter the raw metabolomic data in hippocampal study for revealing neuroinflammatory factors in the transgenic hippocampus tissues of mice with *Alzheimer*'s disease (54).
TRS	Tolerance of Relative Standard Deviation	**Method's Introduction**: This method deletes the metabolite whose relative standard deviation (RSD) across all samples is higher than a threshold, since a lower RSD indicates a better reproducibility (55,56). **Research Application(s)**: It was integrated in a pipeline for the processing of both GC-MS and LC-MS open metabolomic profiling data based on the KNIME analytics platform (53).
		Data Imputation
HAM	Half of the Minimum Imputation	**Method's Introduction**: This method substitutes missing values with the half of the minimum value of non-missing values in the corresponding metabolites to reduce variation among experimental groups (57,58). **Research Application(s)**: It was adopted by the Q-TOF and HPLC-QqQ-MS metabolomics for identifying alterations of the exo-/endo-metabolite profiles in breast cancer cell lines (59).
KNN	*K*-nearest Neighbor Imputation	**Method's Introduction**: This method identifies *K* metabolites that are similar to the metabolite with missing value, and the missing values are imputed with the weighted average values of these neighboring ones (60). **Research Application(s)**: It was adopted in a metabolomic study, and facilitated the early identification of vincristine-induced peripheral neuropathy in pediatric leukemia patients (61).

MDI	Column Median Imputation	**Method's Introduction**: This method uses the median values, which are not easily affected by outliers, of non-missing values to impute those missing values in the corresponding metabolites (62). **Research Application(s)**: It was applied to discover the metabolic markers induced by metformin exposure and response, and to understand the metabolic mechanisms of metformin in ammonia detoxification (63).
MEI	Column Mean Imputation	**Method's Introduction**: This method replaces missing values with a median value of non-missing values in the corresponding metabolite, and tends to increase differences between diverse experimental groups (62). **Research Application(s)**: It was applied to a pharmacometabolomic assessment study for the discovery of novel drug response phenotypes of both atenolol and hydrochlorothiazide (64).
QC Sample Correction		
LLR	Local Linear Regression	**Method's Introduction**: This method corrects signals based on a local linear regression model which looks linear in small regions of input-space if the function has sufficient smoothness (65). **Research Application(s)**: It was utilized to process peak areas for quality control correction, and identify potential metabolic markers from a large-scale metabolomic dataset of hepatocellular carcinoma (66).
LPF	Local Polynomial Fits	**Method's Introduction**: This method is a nonparametric approach integrated in the QC-based LOESS signal correction (QC-RLSC) method for smoothing scatter plots and modeling functions (67). **Research Application(s)**: It was applied for metabolomic batch correction and the subsequent detection of significant metabolic variations in the athletes that were applied with growth hormone (68).
NWE	Nadaraya-Watson Estimator	**Method's Introduction**: This method provides a regression model which estimates the regression function by a weighted average of the raw data where the weights are a decreasing function of distance (69). **Research Application(s)**: It was integrated into some statistical *R* packages, which are the streamlined tools for signal drift correction and interpretations of quantitative MS-based metabolomic data (70).
Data Transformation		
CUT	Cube Root Transformation	**Method's Introduction**: This method increases the weight of metabolites of relatively lower concentrations and compresses the weight of metabolites of higher ones to an approximate normal distribution (71).

		Research Application(s): It was used to reveal metabolomic alterations in invasive ductal carcinoma of breast and help to identify diagnostic markers as well as potential therapeutic targets (72).
LOG	Log Transformation	**Method's Introduction**: This method tends to transform the distribution of metabolite abundance ratio to a more symmetrical (almost normal) distribution by minimizing the metabolites of extreme abundance (73). **Research Application(s)**: This method was utilized to enhance small signals in the metabolomics spectrum and facilitate the identification of metabolic markers for the early-stage diagnosis of oral cancer (74).

Table S5. A list of methods for data normalization in the metabolomic *peak table processing*. A three-letter abbreviation (Abb.) code was adopted to represent each method. If none of the methods was applied, a three-letter code NON was used to indicate the non-application of any method in normalization. For normalization method, there are three types of study assumption: (**SAα**) all metabolites should be equally important, which is the prerequisite for applying scaling methods (75-77); (**SAβ**) the level of metabolite intensity should be constant among all samples, which is the priori hypothesis for some normalizations, such as MED and SUM (78,79); and (**SAγ**) the intensity of the vast majority of the metabolites should be unchanged under the studied condition, which are demanded by some other normalization methods, such as PQN, VSN and QUA (78,80,81). It is essential to emphasize that due to the distinct assumptions, some methods are fundamentally inappropriate for certain dataset and thus cannot be assessed using NOREVA (81,82). Therefore, before any performance assessment, the nature of the studied dataset should be analyzed and whether the study assumption held for these data should be clarified.

Abb.	Method Name	Method's Introduction, Reported Applicable Domain(s), and Research Application(s)
		Sample-based Normalization
CON	Contrast	**Method's Introduction**: as a popular normalization, this method selects a baseline sample, to which other samples are normalized by fitting a nonlinear smooth curve (83,84). **Study Assumption *Gamma* (SAγ)**: the intensities of most metabolites are not changed under the studied conditions in the analyzed data (84). **Metabolomic Application**: it has been applied to improve data quality and remove unwanted variations in 1H NMR metabolite fingerprinting data in the case of unbalanced metabolite regulation (85).
CUB	Cubic Splines	**Method's Introduction**: this method aims to make the distribution of metabolite concentrations (geometric or arithmetic mean) among all samples comparable using the nonlinear baseline (84,86). **Study Assumption *Gamma* (SAγ)**: the intensities of most metabolites are not altered under the studied conditions in the analyzed data (87). **Metabolomic Application**: it has been used to eliminate unwanted biases and experimental variance for correctly classifying samples regardless of the dataset size (88).
EIG	EigenMS	**Method's Introduction**: this method is an adaptation of surrogate variable analysis, which identifies trends attributable to bias by utilizing singular value decomposition on model residuals (89,90).

		Study Assumption *Gamma* (SAγ): most of the metabolite intensities are not altered among samples (89), and this method reduces the sample-to-sample variations of unknown complexity (91,92). **Metabolomic Application**: it has been used to identify metabolomic biomarkers and dietary factors for characterizing the maternal metabolome with gestational diabetes (93).
LIN	Linear Baseline Scaling	**Method's Introduction**: this method maps linearly from each metabolite spectrum to a baseline through multiplying the metabolite intensities in all spectra using a particular scaling factor (84,94). **Study Assumption *Gamma* (SAγ)**: the intensities of most of the metabolites among samples are unchanged in the studied metabolomic dataset (95,96). **Metabolomic Application**: it facilitates the prediction of capecitabine-induced toxicity in patients with inoperable colorectal cancer based on pharmaco-metabonomic profiling (97).
LIW	Li-Wong	**Method's Introduction**: this method selects a baseline spectrum and normalizes other spectra by fitting a smooth curve on the level of metabolic feature intensities (83). **Study Assumption *Gamma* (SAγ)**: the intensities of the majority of the metabolites among samples are not changed in the studied data (95). **Metabolomic Application**: it has been utilized to eliminate unwanted sample-to-sample bias in 1H NMR metabolite fingerprinting datasets with unbalanced metabolite regulation (85).
LOE	Cyclic Loess	**Method's Introduction**: this method combines MA-plot and *Bland-Altman* plot by assuming the existence of non-linear bias (84), and it estimates a regression surface using multivariate smoothing procedure (98). **Study Assumption *Gamma* (SAγ)**: the majority of the intensities are unchanged in all samples (82,95), and the systematic bias nonlinearly depends on intensities (78). **Metabolomic Application**: it has been used in high throughput metabolomic studies to identify the underlying pathological mechanisms of the APP/PS1 model constructed for *Alzheimer*'s disease (99).
MEA	Mean Normalization	**Method's Introduction**: this method reduces variability among replicates by calculating the intensity of each metabolite in a given sample as the mean of intensities of all variables in samples (100,101). **Study Assumption *Beta* (SAβ)**: the mean level of intensities is consistent among all samples (78), and it ensures the metabolite intensity values in all samples comparable with each ones (102).

		Metabolomic Application: it has been utilized to normalize the pharmaco-metabolomics data and helped to differentiate L-carnitine outcomes in patients treated with septic shock (103).
MED	Median Normalization	**Method's Introduction**: this method removes unwanted variation among samples (101) by calculating the intensity of each metabolite in a given sample as the median of intensities of all variables in samples (78). **Study Assumption *Beta* (SAβ)**: the median level of intensities is consistent among all samples (78,102), and the metabolite intensity of each sample has the same median (78). **Metabolomic Application**: it has been applied to normalize metabolomic data generated by the quadrupole time-of-flight mass spectrometer for facilitating the analysis of human breath (104).
MST	MS Total Useful Signal	**Method's Introduction**: this method divides the intensity of each spectrum by the sum of intensities of all spectra, and makes metabolite intensities among all samples comparable (86,105). **Study Assumption *Beta* (SAβ)**: the level of metabolite intensity is constant among all samples by assuming that there is an equivalence between increased intensities and decreased intensities (86,106). **Metabolomic Application**: it has been used to correct the ionization efficiencies of the detected metabolite peaks and enhance accuracy for untargeted LC-MS based metabolomics data (107).
PQN	Probabilistic Quotient Normalization	**Method's Introduction**: this method integrally normalizes each spectrum and calculates a quotient between test and reference spectra, then all variables of the test spectrum are divided by the median quotient (108). **Study Assumption *Gamma* (SAγ)**: most metabolite intensities are unchanged among all samples (80), and it ensures the metabolite intensity values in all samples comparable with each ones (102). **Metabolomic Application**: it has been utilized in the 1H NMR-based metabolomics and identified as a robust method for complex biological mixtures attributing to various dilution concentration levels (108).
QUA	Quantile Normalization	**Method's Introduction**: this method replaces each point in the samples with the mean of the corresponding quantile and the distribution of the sample is made consistent on the basis of the sample quantile (94). **Study Assumption *Gamma* (SAγ)**: most metabolite intensities is unchanged among all samples (96), and it ensures the metabolite intensities in all samples comparable with each ones (102). **Metabolomic Application**: it was found as a well-performing normalization in processing 1D 1H urinary metabolomic data (109) and assisted the discovery of antihypertensive medication (110).

SUM	Total Sum Normalization	**Method's Introduction**: with the aim of reducing sample-to-sample variations, this method normalizes the metabolite intensities by assigning an appropriate weight to each sample (111). **Study Assumption *Beta* (SAβ)**: the average level of intensities is constant among all samples (112), and it ensures the metabolite intensity value in all samples comparable with each other (102). **Metabolomic Application**: it facilitates the identification of metabolites that associate with the different responses to gemcitabine-carboplatin chemotherapy in patients with metastatic breast cancer (113).
		Metabolite-based Normalization
AUT	Auto Scaling	**Method's Introduction**: this method is one of the simplest methods to adjust the metabolomic variances, which scales metabolite intensities based on the standard deviation of the metabolomic data (84,114). **Study Assumption *Alpha* (SAα)**: all metabolites are equally important (75), and it changes the emphasis from metabolites of high concentrations to those of moderate/small intensities (115,116). **Metabolomic Application**: it has been used in LC/MS-based metabolomics to facilitate the identification of urinary nucleosides as potential urogenital cancer markers (117).
LEV	Level Scaling	**Method's Introduction**: this method transforms the metabolic signal variations to that related to the mean metabolic signal, which are changed to values in percentages relative to the mean concentration (75). **Study Assumption *Alpha* (SAα)**: all metabolites are equally important, that is to say, metabolites with high intensities are not necessarily more important than those with low intensities (75). **Metabolomic Application**: it was applied to remove technical and biological variations in the UPLC-MS based untargeted metabolomic dataset, and to facilitate the investigation of differences in the liver metabolic profiles between distinct animal groups in the toxicology studies (118).
PAR	Pareto Scaling	**Method's Introduction**: this method uses the square root of the standard deviation of the data as the scaling factor, which can reduce the weight of a large fold change in metabolite intensities (84). **Study Assumption *Alpha* (SAα)**: all metabolites are equally important (75), and its disadvantage lines in its high sensitivity to the large fold changes (75). **Metabolomic Application**: it has been used to eliminate the mask effects in metabolomics and assisted the revealing of different responses to *Streptozotocin* and diet intervention in rat models (119).

POW	Power Scaling	**Method's Introduction**: this method aims at correcting the heteroscedasticity and pseudo-scaling through calculating the square root value of the metabolite intensity in different samples (75). **Study Assumption *Alpha* (SAα)**: all metabolites are equally important in the analyzed data, and it converts skewed metabolomics data to symmetric by non-linear transformation (75). **Metabolomic Application**: it has been used to facilitate the identification of serum metabolic changes and the investigation of their associations with colorectal cancers (120).
RAN	Range Scaling	**Method's Introduction**: this method scales the metabolite intensities for a systematic variance according to the intensity range of metabolites of all samples as the scaling factor (121). **Study Assumption *Alpha* (SAα)**: all metabolites are equally important (75), and it is usually applied for transforming the high concentration of metabolites to medium/small intensity (122). **Metabolomic Application**: it has helped to remove instrumental response factors from the metabolomics data and improve value comparability in prior to data fusion (121).
VAS	Vast Scaling	**Method's Introduction**: this method is an extension of auto scaling that focuses on stable variables and uses standard deviation and the so-called coefficient of variation as the scaling factor (75,123). **Study Assumption *Alpha* (SAα)**: all metabolites are equally important (75), and it is suitable for intensities of small fluctuations, but not suited for large variations without group structure (75). **Metabolomic Application**: it has been widely applied to both supervised and unsupervised learning from metabolomic data, and is discovered as a well-performing method for normalizing data and improving the multivariate models for metabolic feature selection and sample classification (114,124).
	Sample & metabolite-based Normalization	
VSN	Variance Stabilization Normalization	**Method's Introduction**: this method approaches the logarithm for large values to remove heteroscedasticity using the inverse hyperbolic sine (84), and keeps the variance constant over the entire data range (125). **Study Assumption *Gamma* (SAγ)**: most metabolites in different samples are not differentially expressed, and it makes the individual observations more directly comparable (126,127). **Metabolomic Application**: it has been adopted to metabolic profiling for processing the urine 1H NMR spectra signals with factors such as diseases, drugs and toxins (128).

Internal Standard-based Normalization		
CCM	Cross-contribution Compensating Multi-ISs Normalization	**Method's Introduction**: this method is capable of monitoring the systematic error and removing unwanted experimental variations based on multiple internal standards (129). **Study Assumption *Gamma* (SAγ)**: the intensities of most metabolites should not change under the studied conditions in the analyzed metabolomic dataset (129). **Metabolomic Application**: it has been applied to the MS-based metabolomics data from randomized and designed experiments that use internal standards to monitor the systematic error (129).
NOM	Normalization using Optimal Selection of Multiple ISs	**Method's Introduction**: this method removes unwanted systematic error via finding optimal normalization factor based on multiple internal standard compounds (130). **Study Assumption *Gamma* (SAγ)**: the intensities of most metabolites do not alter among samples, and it helps to remove unwanted systematic error in the analyzed data (129,131). **Metabolomic Application**: it has been applied to the UPLC/HRMS based mouse liver metabolomics data for removing the effect of systematic error across the full spectrum of metabolite peaks (130).
RUV	Remove Unwanted Variation-Random	**Method's Introduction**: this method utilizes the quality control metabolites (QCMs) that are only associated with unwanted variations to construct a linear mixed effects model for obtaining normalized data (111). **Study Assumption *Gamma* (SAγ)**: the intensities of most metabolites are assumed to be unchanged under the studied conditions in the analyzed metabolomic dataset (129). **Metabolomic Application**: it has facilitated the investigation of associations between metabolite patterns during late childhood and the exposure to maternal gestational diabetes mellitus (132).
SIS	Single Internal Standard	**Method's Introduction**: this method subtracts the log abundance of single internal standard from that of all metabolites in each sample of the analyzed dataset (101,133). **Study Assumption *Gamma* (SAγ)**: the intensities of most metabolites do not alter among samples, and this method is capable of removing unwanted variations in metabolomics data (129). **Metabolomic Application**: it has been integrated into a strategy which is applicable for dealing with large-scale human metabolomics studies, including data processing and validation (134).

Table S6. The time spent on applying NOREVA protocol measured by minute(s). Three metabolomics datasets of different sizes (PMID28528106, PMID21962342, and PMID22647087; as described in **Table 1**) are evaluated using four popular operating systems (CentOS, macOS, Ubuntu, and Windows). Hardware detail for time evaluation is shown under each system. Since the parallel computing together with its corresponding memory management are realized in NOREVA protocol, a comparison on time-cost between the application and non-application of 'parallel computing and memory management' is provided (indicated by 'YES' or 'NO', and measured by minutes).

Tested Datasets	**Application of Parallel Computing and Memory Management**	**CentOS Linux 7**	**macOS High Sierra**	**Ubuntu 20.04 LTS**	**Windows 10**
		2.5 GHz, 16 cores Xeon Platinum 8163	2.2 GHz, 4 cores Intel Core i5-8259U	3.0 GHz, 6 cores Intel Core i5-8500	2.9 GHz, 8 cores Intel Core i7-10700F
		64 GB RAM	8 GB RAM	16 GB RAM	16 GB RAM
PMID28528106 (135)	YES	~200 minutes	~600 minutes	~250 minutes	~200 minutes
	NO	~2,200 minutes	~3,300 minutes	~2,000 minutes	~2,100 minutes
PMID21962342 (4)	YES	~100 minutes	~400 minutes	~150 minutes	~100 minutes
	NO	~1,250 minutes	~2,100 minutes	~1,000 minutes	~1,100 minutes
PMID22647087 (136)	YES	~15 minutes	~60 minutes	~25 minutes	~15 minutes
	NO	~120 minutes	~160 minutes	~60 minutes	~70 minutes

Table S7. A comprehensive list of functions provided in NOREVA together with their descriptions. For each function, its argument names, default values and the allowable argument values are described. In total, 22 different functions are provided in NOREVA and discussed in this table.

(func1). Name of **Function 1**: ***PrepareInuputFiles***()

Description: this function enables the preparation and input of peak table which facilitate the subsequent application of other NOREVA functions. It could process not only a standardized format, but also the customized formats from available tools for peak table preparation (**Figure S1**).

Argument	Value Type	Description of the Argument and the Allowable Argument Values
dataformat	*numeric*	Allows the user to specify the FORMAT of their input peak table (default = *null*) “**1**” denotes a standardized format of peak table accepted by NOREVA “**2**” denotes the customized formats of peak table generated by 12 popular tools (such as XCMS)
rawdata	*character*	Allows the user to indicate the NAME of their input peak table file (default = *null*)
label	*character*	Allows the user to indicate the NAME of their input label file (default = *null*)

(func2). Name of **Function 2**: ***normulticlassqcall***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***with*** quality control sample but ***without*** internal standard) using four criteria, and can scan thousands of processing workflows and rank them based on their performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)

SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func3*). Name of **Function 3**: ***normulticlassnoall***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***without*** quality control sample and ***without*** internal standard) using four independent criteria, and can comprehensively scan thousands of processing workflows and rank all these workflows based on their performances (assessed from four different perspectives).

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)

SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func4*). Name of **Function 4**: ***normulticlassisall***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***with*** internal standards but ***without*** quality control sample) using four criteria, and can scan thousands of processing workflows and rank them based on their performances.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “2,6,8,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+1)*th* columns of your input peak table should be considered to be the IS metabolites.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)

SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func5*). Name of **Function 5**: ***nortimecourseqcall***()

Description: this function enables the performance assessment of metabolomic data processing for the time-course dataset (***with*** quality control sample but ***without*** internal standard) using four independent criteria, and can comprehensively scan thousands of processing workflows and rank all these workflows based on their performances (assessed from four different perspectives).

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func6*). Name of **Function 6**: ***nortimecoursenoall***()

Description: this function enables the performance assessment of metabolomic data processing for time-course dataset (***without*** quality control sample and ***without*** internal standard) using four independent criteria, and can comprehensively scan thousands of processing workflows and rank all these workflows based on their performances (assessed from four different perspectives).

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func7*). Name of **Function 7**: ***nortimecourseisall***()

Description: this function enables the performance assessment of metabolomic data processing for time-course dataset (***with*** internal standards but ***without*** quality control sample) using four independent criteria, and can comprehensively scan thousands of processing workflows and rank all these workflows based on their performances (assessed from four different perspectives).

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “1,5,7,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+2)*th* columns of your input peak table should be considered to be the IS metabolites.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func8*). Name of **Function 8**: ***normulticlassqcallgs***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***with*** quality control sample but ***without*** internal standard) using five independent criteria, and can comprehensively scan thousands of processing workflows and rank all these workflows based on their performances (assessed from five different perspectives).

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
GS	*character*	Allows the user to indicate the name of the file that contains the spike-in compounds (default = *null*) The file should be in a .csv format, which provides the concentrations of spike-in compounds.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Alpha* (SAα) "**N**" denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Beta* (SAβ) "**N**" denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Gamma* (SAγ) "**N**" denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(func9). Name of **Function 9**: ***normulticlassnoallgs***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***without*** quality control sample and ***without*** internal standard) using five criteria, and can scan thousands of workflows and rank them based on their performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)

GS	*character*	Allows the user to indicate the name of the file that contains the spike-in compounds (default = *null*) The file should be in a .csv format, which provides the concentrations of spike-in compounds.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Alpha* (SAα) "**N**" denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Beta* (SAβ) "**N**" denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Gamma* (SAγ) "**N**" denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func10*). Name of **Function 10**: ***normulticlassisallgs***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***with*** internal standards but ***without*** quality control sample) using five criteria, and can scan thousands of processing workflows and rank them based on their performances.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
GS	*character*	Allows the user to indicate the name of the file that contains the spike-in compounds (default = *null*) The file should be in a .csv format, which provides the concentrations of spike-in compounds.

IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to "2,6,8,n" indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+1)*th* columns of your input peak table should be considered to be the IS metabolites.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Alpha* (SAα) "**N**" denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Beta* (SAβ) "**N**" denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Gamma* (SAγ) "**N**" denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(func11). Name of **Function 11**: ***nortimecourseqcallgs***()

Description: this function enables the performance assessment of metabolomic data processing for the time-course dataset (***with*** quality control sample but ***without*** internal standard) using five criteria, and can scan thousands of workflows and rank them based on their performances.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)

GS	*character*	Allows the user to indicate the name of the file that contains the spike-in compounds (default = *null*) The file should be in a .csv format, which provides the concentrations of spike-in compounds.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Alpha* (SAα) "**N**" denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Beta* (SAβ) "**N**" denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Gamma* (SAγ) "**N**" denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func12*). Name of **Function 12**: ***nortimecoursenoallgs***()

Description: this function enables the performance assessment of metabolomic data processing for time-course dataset (***without*** quality control sample and ***without*** internal standard) using five criteria, and can scan thousands of workflows and rank them based on their performances.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
GS	*character*	Allows the user to indicate the name of the file that contains the spike-in compounds (default = *null*) The file should be in a .csv format, which provides the concentrations of spike-in compounds.

SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Alpha* (SAα) “**N**” denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Beta* (SAβ) “**N**” denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = “Y”) “**Y**” denotes that the peak table satisfies the study assumption *Gamma* (SAγ) “**N**” denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func13*). Name of **Function 13**: ***nortimecourseisallgs***()

Description: this function enables the performance assessment of metabolomic data processing for time-course dataset (***with*** internal standards but ***without*** quality control sample) using five criteria, and can scan thousands of processing workflows and rank them based on their performances.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “1,5,7,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+2)*th* columns of your input peak table should be considered to be the IS metabolites.

GS	*character*	Allows the user to indicate the name of the file that contains the spike-in compounds (default = *null*) The file should be in a .csv format, which provides the concentrations of spike-in compounds.
SAalpha	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Alpha* (SAα, all metabolites are assumed to be equally important) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Alpha* (SAα) "**N**" denotes that the peak table does not satisfy the study assumption *Alpha* (SAα)
SAbeta	*character*	Allows the user to specify whether the input peak table satisfies the study assumption *Beta* (SAβ, the level of metabolite abundance is constant among all samples) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Beta* (SAβ) "**N**" denotes that the peak table does not satisfy the study assumption *Beta* (SAβ)
SAgamma	*character*	Allows the user to specify whether the input table satisfies study assumption *Gamma* (SAγ, the intensities of most metabolites are not changed under the studied conditions) (default = "Y") "**Y**" denotes that the peak table satisfies the study assumption *Gamma* (SAγ) "**N**" denotes that the peak table does not satisfy the study assumption *Gamma* (SAγ)

(*func14*). Name of **Function 14**: ***normulticlassqcpart***()

Description: this function enables performance assessment of metabolomic data processing for multi-class dataset (***with*** quality control sample but ***without*** internal standard) using four criteria, and can scan the customized workflows selected by user and rank them based on their performances.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
selectedMethods	*character*	Allows the user to indicate the NAME of the file containing the customized workflows selected by user. The file should be in a .csv format, and the exemplar files are provided in the NOREVA *R* package and available for download at *https://idrblab.org/noreva/NOREVA_exampledata.zip*).

(*func15*). Name of **Function 15**: ***normulticlassnopart***()

Description: this function enables performance assessment of metabolomic data processing for multi-class dataset (***without*** quality control sample and ***without*** internal standard) using four criteria, and can scan the customized workflows selected by user and rank them based on performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
selectedMethods	*character*	Allows the user to indicate the NAME of the file containing the customized workflows selected by user. The file should be in a .csv format, and the exemplar files are provided in the NOREVA *R* package and available for download at *https://idrblab.org/noreva/NOREVA_exampledata.zip*).

(*func16*). Name of **Function 16**: ***normulticlassispart***()

Description: this function enables the performance assessment of metabolomic data processing for multi-class dataset (***with*** internal standards but ***without*** quality control sample) using four criteria, and can scan the customized workflows selected by user and rank them based on performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “2,6,8,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+1)*th* columns of your input peak table should be considered to be the IS metabolites.
selectedMethods	*character*	Allows the user to indicate the NAME of the file containing the customized workflows selected by user. The file should be in a .csv format, and the exemplar files are provided in the NOREVA *R* package and available for download at *https://idrblab.org/noreva/NOREVA_exampledata.zip*).

(*func17*). Name of **Function 17**: ***nortimecourseqcpart***()

Description: this function enables performance assessment of metabolomic data processing for time-course data (***with*** quality control sample but ***without*** internal standard) using four criteria, and can scan the customized workflows selected by user and rank them based on their performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
selectedMethods	*character*	Allows the user to indicate the NAME of the file containing the customized workflows selected by user. The file should be in a .csv format, and the exemplar files are provided in the NOREVA *R* package.

(*func18*). Name of **Function 18**: ***nortimecoursenopart***()

Description: this function enables performance assessment of metabolomic data processing for time-course data (***without*** quality control sample and ***without*** internal standard) using four criteria, and can scan the customized workflows selected by user and rank them based on performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
selectedMethods	*character*	Allows the user to indicate the NAME of the file containing the customized workflows selected by user. The file should be in a .csv format, and the exemplar files are provided in the NOREVA *R* package.

(*func19*). Name of **Function 19**: ***nortimecourseispart***()

Description: this function enables the performance assessment of metabolomic data processing for time-course dataset (***with*** internal standards but ***without*** quality control sample) using four criteria, and can scan the customized workflows selected by user and rank them based on performances.

Argument	Value Type	Description of the Argument and the Allowable Argument Values
fileName	*character*	Allows the user to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)

IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “1,5,7,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+2)*th* columns of your input peak table should be considered to be the IS metabolites.
selectedMethods	*character*	Allows the user to indicate the NAME of the file containing the customized workflows selected by user. The file should be in a .csv format, and the exemplar files are provided in the NOREVA *R* package.

(*func20*). Name of **Function 20**: ***normulticlassmatrix***()

Description: based on a particular processing workflow (especially the one identified as well-performing for the studied metabolomic multi-class dataset), this function outputs the resulting levels of all metabolites among all samples after the data processing based on that workflow. Quality control sample (QCS) and internal standard (IS) are considered in this function.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
datatype	*numeric*	Allows the users to specify the data type of their input peak table (default = *null*) “**1**” denotes the multi-class metabolomic dataset without QCSs and without ISs “**2**” denotes the multi-class metabolomic dataset with QCSs but without ISs “**3**” denotes the multi-class metabolomic dataset with ISs but without QCSs
fileName	*character*	Allows the users to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “2,6,8,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+1)*th* columns of your input peak table should be considered to be the IS metabolites.

impt	*numeric*	Allows the users to specify the NAME of the imputation method (default = 1) “**1**” denotes the method of MEI (*mean imputation*) “**2**” denotes the method of MDI (*median imputation*) “**3**” denotes the method of HAM (*half of the minimum imputation*) “**4**” denotes the method of KNN (*k-nearest neighbor imputation*)
qcsn	*numeric*	Allows the users to specify the NAME of the quality control sample correction method (default = 1) “**1**” denotes the method of NWE (*Nadaraya-Watson estimator*) “**2**” denotes the method of LLR (*local linear regression*) “**3**” denotes the method of LPF (*local polynomial fits*)
trsf	*numeric*	Allows the users to specify the NAME of the transformation method (default = 1) “**1**” denotes the method of CUT (*cube root transformation*) “**2**” denotes the method of LOG (*log transformation*) “**3**” denotes the NON application of any transformation method
nmal	*numeric*	Allows the users to specify the NAME of the normalization method (default = *null*) “**1**” denotes the NON application of any normalization method “**2**” denotes the sample-based method of PQN (*probabilistic quotient normalization*) “**3**” denotes the sample-based method of LOE (*cyclic loess*) “**4**” denotes the sample-based method of CON (*contrast*) “**5**” denotes the sample-based method of QUA (*quantile normalization*) “**6**” denotes the sample-based method of LIN (*linear baseline scaling*) “**7**” denotes the sample-based method of LIW (*Li-Wong*) “**8**” denotes the sample-based method of CUB (*cubic splines*) “**9**” denotes the metabolite-based method of AUT (*auto scaling*) “**10**” denotes the metabolite-based method of RAN (*range scaling*)

		“**11**” denotes the metabolite-based method of PAR (*pareto scaling*) “**12**” denotes the metabolite-based method of VAS (*vast scaling*) “**13**” denotes the metabolite-based method of LEV (*level scaling*) “**14**” denotes the sample & metabolite-based method of VSN (*variance stabilization normalization*) “**15**” denotes the metabolite-based method of POW (*power scaling*) “**16**” denotes the sample-based method of MST (*MS total useful signal*) “**17**” denotes the sample-based method of SUM (*total sum normalization*) “**18**” denotes the sample-based method of MED (*median normalization*) “**19**” denotes the sample-based method of MEA (*mean normalization*) “**20**” denotes the sample-based method of EIG (*EigenMS*)
nmal2	*numeric*	Allows the users to specify the NAME of the normalization method (default = *null*) “**1**” denotes the NON application of any normalization method “**2**” denotes the sample-based method of PQN (*probabilistic quotient normalization*) “**3**” denotes the sample-based method of LOE (*cyclic loess*) “**4**” denotes the sample-based method of CON (*contrast*) “**5**” denotes the sample-based method of QUA (*quantile normalization*) “**6**” denotes the sample-based method of LIN (*linear baseline scaling*) “**7**” denotes the sample-based method of LIW (*Li-Wong*) “**8**” denotes the sample-based method of CUB (*cubic splines*) “**9**” denotes the metabolite-based method of AUT (*auto scaling*) “**10**” denotes the metabolite-based method of RAN (*range scaling*) “**11**” denotes the metabolite-based method of PAR (*pareto scaling*) “**12**” denotes the metabolite-based method of VAS (*vast scaling*) “**13**” denotes the metabolite-based method of LEV (*level scaling*) “**14**” denotes the sample & metabolite-based method of VSN (*variance stabilization normalization*)

		"**15**" denotes the metabolite-based method of POW (*power scaling*) "**16**" denotes the sample-based method of MST (*MS total useful signal*) "**17**" denotes the sample-based method of SUM (*total sum normalization*) "**18**" denotes the sample-based method of MED (*median normalization*) "**19**" denotes the sample-based method of MEA (*mean normalization*) "**20**" denotes the sample-based method of EIG (*EigenMS*) In combination with the argument *nmal* above, this argument (*nmal2*) helps to enable a normalization of combination strategy. Particularly, if a sample-based normalization is selected for the argument *nmal*, a metabolite-based one should be chosen for this argument (*nmal2*). If a metabolite-based normalization is selected for the argument *nmal*, a sample-based one should be chosen for this argument (*nmal2*).
nmals	*numeric*	Allows the users to specify the NAME of the IS-based normalization method (default = *null*) "**1**" denotes the method of SIS (*single internal standard*) "**2**" denotes the method of NOM (*normalization using optimal selection of multiple ISs*) "**3**" denotes the method of CCM (*cross-contribution compensating multi-ISs normalization*) "**4**" denotes the method of RUV (*remove unwanted variation-random*)

(*func21*). Name of **Function 21**: ***nortimecoursematrix***()

Description: based on a particular processing workflow (especially the one identified as well-performing for the studied metabolomic time-course dataset), this function outputs the resulting levels of all metabolites among all samples after the data processing based on that workflow.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**
datatype	*numeric*	Allows the users to specify the data type of their input peak table (default = *null*) "**1**" denotes the multi-class metabolomic dataset without QCSs and without ISs "**2**" denotes the multi-class metabolomic dataset with QCSs but without ISs "**3**" denotes the multi-class metabolomic dataset with ISs but without QCSs

fileName	*character*	Allows the users to indicate the NAME of peak table resulted from *PrepareInuputFiles*() (default = *null*)
IS	*character*	Allows the user to indicate the column number(s) where the internal standard(s) locate (default = *null*) If there is only one internal standard (IS), the column number of this IS should be listed If there are multiple ISs, the column numbers of all ISs should be listed and separated using comma For example, the value of argument IS that is set to “1,5,7,n” indicates that the metabolites in the 3*rd*, 7*th*, 9*th*, and (n+2)*th* columns of your input peak table should be considered to be the IS metabolites.
impt	*numeric*	Allows the users to specify the NAME of the imputation method (default = 1) “**1**” denotes the method of MEI (*mean imputation*) “**2**” denotes the method of MDI (*median imputation*) “**3**” denotes the method of HAM (*half of the minimum imputation*) “**4**” denotes the method of KNN (*k-nearest neighbor imputation*)
qcsn	*numeric*	Allows the users to specify the NAME of the quality control sample correction method (default = 1) “**1**” denotes the method of NWE (*Nadaraya-Watson estimator*) “**2**” denotes the method of LLR (*local linear regression*) “**3**” denotes the method of LPF (*local polynomial fits*)
trsf	*numeric*	Allows the users to specify the NAME of the transformation method (default = 1) “**1**” denotes the method of CUT (*cube root transformation*) “**2**” denotes the method of LOG (*log transformation*) “**3**” denotes the NON application of any transformation method
nmal	*numeric*	Allows the users to specify the NAME of the normalization method (default = *null*) “**1**” denotes the NON application of any normalization method “**2**” denotes the sample-based method of PQN (*probabilistic quotient normalization*) “**3**” denotes the sample-based method of LOE (*cyclic loess*)

		“**4**” denotes the sample-based method of CON (*contrast*) “**5**” denotes the sample-based method of QUA (*quantile normalization*) “**6**” denotes the sample-based method of LIN (*linear baseline scaling*) “**7**” denotes the sample-based method of LIW (*Li-Wong*) “**8**” denotes the sample-based method of CUB (*cubic splines*) “**9**” denotes the metabolite-based method of AUT (*auto scaling*) “**10**” denotes the metabolite-based method of RAN (*range scaling*) “**11**” denotes the metabolite-based method of PAR (*pareto scaling*) “**12**” denotes the metabolite-based method of VAS (*vast scaling*) “**13**” denotes the metabolite-based method of LEV (*level scaling*) “**14**” denotes the sample & metabolite-based method of VSN (*variance stabilization normalization*) “**15**” denotes the metabolite-based method of POW (*power scaling*) “**16**” denotes the sample-based method of MST (*MS total useful signal*) “**17**” denotes the sample-based method of SUM (*total sum normalization*) “**18**” denotes the sample-based method of MED (*median normalization*) “**19**” denotes the sample-based method of MEA (*mean normalization*) “**20**” denotes the sample-based method of EIG (*EigenMS*)
nmal2	*numeric*	Allows the users to specify the NAME of the normalization method (default = *null*) “**1**” denotes the NON application of any normalization method “**2**” denotes the sample-based method of PQN (*probabilistic quotient normalization*) “**3**” denotes the sample-based method of LOE (*cyclic loess*) “**4**” denotes the sample-based method of CON (*contrast*) “**5**” denotes the sample-based method of QUA (*quantile normalization*) “**6**” denotes the sample-based method of LIN (*linear baseline scaling*) “**7**” denotes the sample-based method of LIW (*Li-Wong*)

		“**8**” denotes the sample-based method of CUB (*cubic splines*) “**9**” denotes the metabolite-based method of AUT (*auto scaling*) “**10**” denotes the metabolite-based method of RAN (*range scaling*) “**11**” denotes the metabolite-based method of PAR (*pareto scaling*) “**12**” denotes the metabolite-based method of VAS (*vast scaling*) “**13**” denotes the metabolite-based method of LEV (*level scaling*) “**14**” denotes the sample & metabolite-based method of VSN (*variance stabilization normalization*) “**15**” denotes the metabolite-based method of POW (*power scaling*) “**16**” denotes the sample-based method of MST (*MS total useful signal*) “**17**” denotes the sample-based method of SUM (*total sum normalization*) “**18**” denotes the sample-based method of MED (*median normalization*) “**19**” denotes the sample-based method of MEA (*mean normalization*) “**20**” denotes the sample-based method of EIG (*EigenMS*) In combination with the argument *nmal* above, this argument (*nmal2*) helps to enable a normalization of combination strategy. Particularly, if a sample-based normalization is selected for the argument *nmal*, a metabolite-based one should be chosen for this argument (*nmal2*), and vice versa.
nmals	*numeric*	Allows the users to specify the NAME of the IS-based normalization method (default = *null*) “**1**” denotes the method of SIS (*single internal standard*) “**2**” denotes the method of NOM (*normalization using optimal selection of multiple ISs*) “**3**” denotes the method of CCM (*cross-contribution compensating multi-ISs normalization*) “**4**” denotes the method of RUV (*remove unwanted variation-random*)

(*func22*). Name of **Function 22**: ***norvisualization***()

Description: this function enables the visualization of the overall ranking of all processing workflows using a circular bar plot.

Argument	**Value Type**	**Description of the Argument and the Allowable Argument Values**

data	*character*	Allows the users to specify the NAME of the resulting ranking file that is generated based on NOREVA functions. These functions include *normulticlassnoall*(), *normulticlassqcall*(), *nortimecourseqcall*(), *nortimecoursenoall*(), *et al* (default = *null*).
cutoff	*numeric*	Allows the users to specify the number of the top-ranked processing workflows that are selected by users to be displayed in the circular bar plot (default = 100).
outputtype	*character*	Allows the users to specify the type of output file, and a string indicating the file types of .pdf, .png, .jpg, and .eps are supported in NOREVA (default = "pdf").
outputfile	*character*	Allows the user to specify the NAME of output file (default = *NOREVA-Ranking-Top.%d.workflows.%s*). "%d" is the value of the argument *cutoff* above, and "%s" is the value of the argument *outputtype* above.
maxValue	*numeric*	Allows the users to specify the maximum length of multiple bars, which indicates the performances of the processing workflow under multiple criteria, in the inner layers of the bar plot (default = 40).
colorSet	*character*	Allows the users to specify the colors of the multiple bars that indicate the performances of the workflow under multiple criteria (default = "#FFE699", "#D3E8C7", "#B2B2FF", "#FFCACA").
totalAngle	*numeric*	Allows the users to specify the degree of total angle of rotation of the bar plot (default = 340).
bgColor	*character*	Allows the users to specify the background color of the bar plot (default = "#FFFFFF").
fontColor	*character*	Allows the users to specify the front color of the bar plot (default = "#000000").

Table S8. A comprehensive description on the output files of the software package developed in this study. In total, 13 outputs are generated after the running of NOREVA, which are all provided and described below.

File Name	File Type	Description of the Corresponding Output File
OUTPUT-NOREVA-Overall.Ranking.Data.csv	CSV File	A CSV file providing all results discovered by NOREVA which includes the details of the assessing results, selected criteria. overall ranking, and ranking under each criterion.
NOREVA-Ranking-Top.XXX.workflows.pdf	PDF/EPS/PNG/JPG File	A circular bar plot illustrating the performance and the overall ranking of all processing workflows based on the multiple criteria or a single criterion that are selected by user.
OUTPUT-NOREVA-All.Normalized.Data.Rdata	RDATA File	A RDATA file that provides the resulting outcomes of all processing workflows.
OUTPUT-NOREVA-Criteria.Ca	Folder	A folder of various PDF files that illustrate the performance (using PMAD plot showing intensities among replicates) of each processing workflow assessed by Criterion *Ca.*
OUTPUT-NOREVA-Criteria.Ca.Rdata	RDATA File	A RDATA file that provides the performance (illustrated by intensities among replicates of PMAD plot) of each processing workflow assessed by Criterion *Ca.*
OUTPUT-NOREVA-Criteria.Cb	Folder	A folder of various PDF files that illustrate the performance (using *k*-means clustering between distinct groups) of each processing workflow assessed by Criterion *Cb.*
OUTPUT-NOREVA-Criteria.Cb.Rdata	RDATA File	A RDATA file that provides the performance (shown by the purity of *k*-means clustering ability between distinct groups) of each processing workflow assessed by Criterion *Cb.*
OUTPUT-NOREVA-Criteria.Cc	Folder	A folder of various PDF files that illustrate the performance (using Venn diagram for the marker overlap) of each processing workflow assessed by Criterion *Cc.*
OUTPUT-NOREVA-Criteria.Cc.Rdata	RDATA File	A RDATA file that provides the performance (illustrated by the CWrel value of marker overlap) of each processing workflow assessed by Criterion *Cc.*
OUTPUT-NOREVA-Criteria.Cd	Folder	A folder of various PDF files that illustrate the performance (using the marker classification) of each processing workflow assessed by Criterion *Cd.*

OUTPUT-NOREVA-Criteria.Cd.Rdata	RDATA File	A RDATA file that provides the performance (illustrated by the AUC value for marker classification) of each processing workflow assessed by Criterion *Cd.*
OUTPUT-NOREVA-Criteria.Ce	Folder	A folder of various PDF files that illustrate the performance (using the concentrations of known spike-in compounds) of each processing workflow assessed by Criterion *Ce.*
OUTPUT-NOREVA-Criteria.Ce.Rdata	RDATA File	A RDATA file that provides the performance (illustrated by difference between data and expected logFCs) of each processing workflow assessed by Criterion *Ce*.

Method S1. Processing workflows generated by method combination.

1. ***For the Metabolomic Data with Quality Control (QC) Samples***

Each processing workflow is composed of four sequential steps (S1-S4, as shown in **Figure 1**). For metabolomic data with QC samples, a random, comprehensive, and sequential integration among 4 imputation, 3 QC sample correction, 3 transformation (taking non-transformation into consideration), and 164 normalization (144 combined normalization between sample-based and metabolite-based ones, 19 sample/metabolite/sample & metabolite-based normalization shown in **Table S5**, and 1 non-normalization), can result in a total of **5,904** processing workflows.

2. ***For the Metabolomic Data with Internal Standards (ISs)***

Each processing workflow is composed of four sequential steps (S1-S4, as shown in **Figure 1**). For metabolomic data with ISs, a random, comprehensive, and sequential integration among 4 imputation, non-QC sample correction, 3 transformation (including non-transformation), and 4 IS-based normalization (**Table S5**), can result in a total of **48** processing workflows.

3. ***For the Metabolomic Data without QC Samples and ISs***

Each processing workflow is composed of four sequential steps (S1-S4, as shown in **Figure 1**). For metabolomic data without QC samples and ISs, a random, comprehensive, and sequential integration among 4 imputation, non-QC sample correction, 3 transformation (including non-transformation), and 164 normalization (144 combined normalization between sample-based and metabolite-based ones, 19 sample/metabolite/sample & metabolite-based normalization shown in **Table S5**, and 1 non-normalization), can result in a total of **1,968** workflows.

Method S2. Key steps in metabolomic data processing.

1. ***Data Filtering***

Data filtering aims to remove technical/mathematical uninformative features from the datasets of metabolomics (137). It is realized by setting a pre-specified cutoff based on the intrinsic properties of the metabolomic data, such as the ratio of missing values within the analyzed data (138) and the feature variability among all samples (139). In this protocol, 2 representative data filtering methods are provided, including the TPMV (tolerable percent of missing values) and TRSD (tolerance of relative standard deviation). The former filters metabolites when the ratio of missing values of that metabolite is larger than pre-specified cutoff (138). The latter exerts its effects when the relative standard deviation (the absolute measurement of batch-to-batch variation) of the metabolite among all samples is larger than pre-specified cutoff (139).

2. ***Data Imputation***

Data imputation tends to replace the missing values arising from biological or technical reasons with specific values based on the existing information (e.g., baseline signals or zero values). It accounts for the missingness and reduces the bias while keeping the data structure unchanged (140). In this protocol, 4 representative data imputation methods are provided, including the HAM (half of the minimum imputation), KNN (k-nearest neighbor imputation), MDI (column median imputation) and MEI (column mean imputation). HAM replaces the missing values with the half of the minimum positive values (58). KNN performs imputation by determining k metabolites of interest that are similar to the metabolites containing missing values (141). MDI and MEI replace the missing values with the median and mean value of non-missing values of the corresponding metabolite, respectively (142).

3. ***QC Sample Correction***

QC sample correction targets to correct for signal intensity variations, quality accuracy drifts, and intra- and inter-batch variability based on QC samples (pooled sample mixture by mixing small and equal aliquots from the real samples of interest and are dispersed evenly across the multiple batches to ensure the data quality) (143). It reduces the interference of harmful and uncontrollable signals within metabolomic data and ensures data consistency (144). In this protocol, a representative QC correction strategy named ‘QC-RLSC’ is provided, which is powerful in evaluating signal drifts and other systematic noise using mathematical algorithms (70). Particularly, 3 distinct regression models of the QC-RLSC algorithm including Nadaraya-Watson estimator, local linear regression and local polynomial fits are available here.

4. *Data Transformation*

Data transformation performs the nonlinear conversions of the metabolomic data for correcting heteroscedasticity, for converting multiplicative relations to additive relations, and for making skewed distributions more symmetric (75). It reduces the influence of disturbing factors such as measurement noise by converting the data into different scales (75). In this protocol, 2 representative transformation methods are provided, including CUT (cube root transformation) and LOG (log transformation). The former can improve normality distribution of simple count data by increasing the weight of metabolites of relatively lower concentration and compressing the weight of metabolites of higher ones (145). The latter performs nonlinear data conversions to decrease heteroscedasticity and get symmetric distribution prior to statistical analysis (146).

5. *Data Normalization*

Data normalization adjusts values or statistical distributions of the metabolomic data to remove unwanted variations while preserving biological variability (130). In this protocol, 4 categories of data normalization methods are provided, including sample-based, metabolite-based, sample & metabolite-based and IS-based normalization. Sample-based normalization tends to reduce systematic biases among samples and to make the data from all samples directly comparable to each other (147), while metabolite-based method aims to eliminate the effect of very large metabolite intensities and to make metabolites more comparable or normally distributed (109). Particularly, VSN (variance stabilization normalization), which is a sample & metabolite-based normalization, combines variance stabilization and between-sample normalization by transforming the data through a nonlinear approach to keep the variance of the dataset constant (84). Finally, IS-based normalization removes undesired data fluctuations by using IS(s), which is ideally stable isotopically labelled compounds introduced while sample processing and can be easily distinguished from endogenous metabolites (148). All in all, 12 sample-based, 6 metabolite-based, 1 sample & metabolite-based and 4 IS-based methods are provided here.

Method S3. Performance assessment from multiple perspectives.

As the processing performance of different processing workflows varies considerably and the fact that single criterion is not feasible and sufficient to ensure performance assessment from various perspectives (102), performance assessment conducts quantitative/qualitative evaluation of workflow using multiple criteria (131). In this protocol, five well-established criteria (131) with independent underlying theories are employed. Under each criterion, one specific measure is selected as representative, and a variety of well-defined cutoffs of this measure are used to categorize the processing performance into *Good*, *Fair* and *Poor*.

Criterion C*a*: workflow's ability to reduce intragroup variation among samples.

The PMAD (pooled median absolute deviation) is a frequently used metric under this criterion, which is integrated in several popular pipelines such as NormalyzerDE (149). The lower value of PMAD, the larger biological/experimental induced intragroup variation among samples that is removed by the workflow (150). As reported, PMAD values that fall in the range of ≤0.3, ≤0.7 & >0.3 and >0.7 denote *Good, Fair and Poor* performance, respectively (78,151,152).

Criterion C*b*: workflow's effect on differential metabolic analysis.

To meet the needs of time-course/multi-class metabolomics, the multivariate empirical Bayes statistics and OPLS-DA (orthogonal partial least squares-discriminant analysis) are employed. Specifically, the multivariate empirical Bayes statistics (153) selects time-course metabolic markers using HotellingT2 statistics (154). For multi-class metabolomics, OPLS-DA calculates the number of orthogonal components via cross-validation (155). The parameter of 'crossvalI', 'orthoI' and 'predI' in opls function are set to 'two', 'NA' and 'one', respectively. That is to say, opls function automatically computes the number of orthogonal components and optimizes it based on two-fold cross-validation and one predictive component (155). As a result, differential metabolic markers are selected by setting the VIP (variable influence on projection) value >1 (156) and considered as markers among classes in *K*-means clustering to indicate differential classes among samples (157). Finally, the well-defined metric (*purity*) is measured based on the differential metabolic markers and is chosen as the representative metric for this criterion. *Purity* is a direct metric for assessing clustering performance whose value falls in the range of 0 to 1. The closer the values of *purity* to 0, the poorer the clustering outcome. The closer the value to 1, the better the clustering performance provided by the studied workflow (158,159). *Purity* value of a specific processing workflow within the range of >0.8, ≤0.8 & >0.5 and ≤0.5 are generally accepted as *Good, Fair and Poor* performances, respectively (159).

Criterion C*c*: workflow's consistency in markers discovered from different datasets.

In consideration of the low reproducibility among different sets of markers selected from the same metabolomic dataset by different workflows (160,161), the evaluation of the consistency among workflows is thus adopted in NOREVA and considered as essential assessing criterion (131). First, time-course/multi-class metabolomic data are evenly divided into three subsets using stratified random selection (162,163) and then the selection of differential metabolic markers provided in Criterion C*b* is implemented to each sub-dataset. As expected, three sets of markers are selected from the corresponding sub-datasets and are always somewhat at variance. In this case, CW*rel*, a well-established metric which was discovered powerful in evaluating the consistency among subset-size-biased subsets (164), is now integrated in this criterion. To be more specific, CW*rel* calculates the number of times each feature appears in each single set of markers, which denotes the robustness and reproducibility among selected markers from an overall perspective (164). The value of CW*rel* fluctuates between 0 and 1 in which a larger value refers higher robustness and reproducibility of the different sets of the selected markers (164). Particularly, the value of CW*rel* within the range of >0.3, ≤0.3 & >0.15 and ≤0.15 represent *Good, Fair and Poor* performances, respectively (164).

Criterion C*d*: workflow's influence on classification accuracy.

The general goal of analyzing time-course/multi-class metabolomic data is to identify a variety of markers that can be validated as accurate for revealing biological dynamics or differentiating diverse classes (165,166). In this case, the workflow's influence on classification accuracy is thus evaluated using area under the curve (AUC) and receiver operating characteristic (ROC) analysis (167). This evaluation metric under Criterion C*d* is implemented by three steps, which involves: (1) the identification of the differential metabolic markers as described in Criterion C*b*; (2) the construction of a multiple classification model which is based on SVM (support vector machine) in e1071 *R* package; (3) the calculation of AUC value by running the multi roc function employed in multiROC *R* package. The parameters of 'type', 'kernel' and 'cross' are set as 'classification', 'radial basis' and '5', respectively. In this situation, an RBF-kernel and five-fold cross-validation are implemented to avoid overfitting (168). The 'cost' and 'gamma' parameters are optimized using tune in e1071 R package (169). The output of ROC curve is a graph where x-axis and y-axis represent the 'specificity' and '1-sensitivity', respectively. The higher these two values, the larger the value of AUC. The value of AUC closer to 1 denotes higher classification performance of the classifier, and the AUC within the range of >0.9, ≤0.9 & >0.7 and ≤0.7 refers to *Good, Fair and Poor* performances, respectively (170,171).

Criterion C*e*: level of correspondence between processed and reference data.

The metric calculated under this criterion is the log value of fold changes (logFC) between the concentrations of any two groups in the analyzed dataset. The level of correspondence between the processed and reference data is calculated. Specifically, the level of correspondence can be evaluated by calculating the logFC between processed data and references (relative intensities of various spike-in metabolites) for performance assessment (78,167). The closer the logFC of the means of normalized data corresponds to that of reference data, the better the performance is. Moreover, Criterion C*e* utilizes boxplots for demonstrating the variations between any two groups, and it is desirable that the medians in boxplot would equal to zero with the narrowed variations (131). The logFC of the standard deviations can be calculated as a supplement.

Method S4. The user manual of NOREVA protocol.

Introduction

The NOREVA package not only enables the pre-processing and assessment of multi-class/time-series metabolomic data but also realize a high-throughput discovery of the well-performing pre-processing workflows. Particularly, five well-established criteria, each with a distinct underlying theory, are integrated to ensure a much more comprehensive evaluation than any single criterion. This study provides guidelines for researchers who will engage in biomarker discovery or other differential profiling “omics” studies with respect selecting the most appropriate preprocessing method for a given dataset. For function descriptions and analysis of sample datasets you can also use “??NOREVA” command in *R*.

Installation

```
# download the source package of NOREVA_0.1.0.tar.gz and install it.

install.packages ("NOREVA_0.1.0.tar.gz", repos = NULL, type = "source",
INSTALL_opts = "--no-multiarch")

# Or the development version from GitHub:

install.packages("devtools")

devtools::install_github("idrblab/NOREVA")

# NOREVA package depends on several packages, which can be installed using the
below commands:

if (!requireNamespace("BiocManager", quietly = TRUE))

install.packages("BiocManager")
BiocManager::install("Biobase")
BiocManager::install("pcaMethods")
BiocManager::install("multtest")
BiocManager::install("limma")
BiocManager::install("impute")
BiocManager::install("statTarget")
BiocManager::install("ProteoMM")
BiocManager::install("timecourse")
BiocManager::install("ropls")
BiocManager::install("vsn")
BiocManager::install("affy")
devtools::install_github("metabolomicstats/NormalizeMets")
devtools::install_github("fawda123/ggord")
install.packages(c('rJava', 'DiffCorr', 'MetNorm', 'ggsci', 'multiROC', 'dummies',
'ggfortify', 'ggpubr', 'sampling', 'VennDiagram', 'RcmdrMisc', 'reshape2',
'futile.logger', 'foreach', 'data.table', 'parallel', 'doSNOW', 'tidyverse',
'iterators'))
```

Usage

```
library(NOREVA)
```

1. This function enables the preparation and input of peak table which facilitate the subsequent application of other NOREVA functions.

```
PrepareInuputFiles(dataformat, rawdata, label)
```

`dataformat` This variable allows the user to specify the FORMAT of their input peak table.

“1” denotes the standardized format of peak table accepted by NOREVA;

“2” denotes the customized format of peak table generated by 12 available software tools.

`rawdata` This variable allows the user to indicate the NAME of their input peak table file.

`label` This variable allows the user to indicate the NAME of their input label file for time-course/multi-class.

2. This function enables the performance assessment of time-course metabolomic study with dataset with QCSs based on 4 distinct criteria. It could automatically assess the performance of all processing workflows from different criteria.

```
nortimecourseqcall(fileName, SAalpha="Y", SAbeta="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`SAalpha` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

3. This function enables the performance assessment of time-course metabolomic study with dataset with ISs based on 4 distinct criteria. It could automatically assess the performance of all processing workflows from different criteria.

```
nortimecourseisall(fileName, IS)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs should be listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

4. This function enables the performance assessment of time-course metabolomic study with dataset without QCSs and ISs based on 4 distinct criteria. It could automatically assess the performance of all processing workflows from different criteria.

```
nortimecoursenoall(fileName, SAalpha="Y", SAbeta ="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function. Sample data of this data type can be downloaded as the following section “Welcome to Download the Sample Data for Testing and for File Format Correcting”.

`SAalpha` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

5. This function enables the performance assessment of multi-class (N>1) metabolomic study with dataset with QCSs based on 4 distinct criteria. It could automatically assess the performance of all processing workflows from different criteria.

```
normulticlassqcall(fileName, SAalpha="Y", SAbeta ="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`SAalpha` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

6. This function enables the performance assessment of multi-class (N>1) metabolomic study with dataset with ISs based on 4 distinct criteria. It could automatically assess the performance of all processing workflows from different criteria.

```
normulticlassisall(fileName, IS)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs should be listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

7. This function enables the performance assessment of multi-class (N>1) metabolomic study with dataset without QCSs and ISs based on 4 distinct criteria. It could automatically assess the performance of all processing workflows from different criteria.

```
normulticlassnoall(fileName, SAalpha="Y", SAbeta="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`SAalpha` This variable allows the user to specify the study assumption of their peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

8. This function enables the performance assessment of time-course metabolomic study with dataset with QCSs based on 5 distinct criteria including the performance of processing workflows could be assessed using criteria (e).

```
nortimecourseqcallgs(fileName, GS, SAalpha="Y", SAbeta="Y", SAgamma="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`GS` The corresponding data of golden standards for performance evaluation using Criterion e. For the detailed information of the correct file format, please use “??NOREVA” and download sample data in the corresponding section “Welcome to Download the Sample Data for Testing and for File Format Correcting”.

`SAalpha` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

9. This function enables the performance assessment of time-course metabolomic study with dataset with ISs based on 5 distinct criteria including the performance of processing workflows could be assessed using criteria (e).

```
nortimecourseisallgs(fileName, IS, GS)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs should be listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

`GS` The corresponding data of golden standards for performance evaluation using Criterion e. For the detailed information of the correct file format, please use “??NOREVA” and download sample data in the corresponding section “Welcome to Download the Sample Data for Testing and for File Format Correcting”

10. This function enables the performance assessment of time-course metabolomic study with dataset without QCSs and ISs based on 5 distinct criteria including the performance of processing workflows could be assessed using criteria (e).

```
nortimecoursenoallgs(fileName, GS, SAalpha="Y", SAbeta ="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`GS` The corresponding data of golden standards for performance evaluation using Criterion e. For the detailed information of the correct file format, please use “??NOREVA” and download sample data in the corresponding section “Welcome to Download the Sample Data for Testing and for File Format Correcting”.

`SAalpha` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

“Y” denotes the peak table satisfies the study assumption.

“N” denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

11. This function enables the performance assessment of multi-class (N>1) metabolomic study with dataset with QCSs based on 5 distinct criteria including the performance of processing workflows could be assessed using criteria (e).

```
normulticlassqcallgs(fileName, GS, SAalpha="Y", SAbeta ="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`GS` The corresponding data of golden standards for performance evaluation using Criterion e. For the detailed information of the correct file format, please use "??NOREVA" and download sample data in the corresponding section "Welcome to Download the Sample Data for Testing and for File Format Correcting".

`SAalpha` This variable allows the user to specify the study assumption of their input peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

12. This function enables the performance assessment of multi-class (N>1) metabolomic study with dataset with ISs based on 5 distinct criteria including the performance of processing workflows could be assessed using criteria (e).

```
normulticlassisallgs(fileName, IS, GS)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`GS` The corresponding data of golden standards for performance evaluation using Criterion e. For the correct file format, please use "??NOREVA" and download sample data from the section "Welcome to Download the Sample Data for Testing and for File Format Correcting".

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs should be listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

13. This function enables the performance assessment of multi-class (N>1) metabolomic study with dataset without QCSs and ISs based on 5 distinct criteria including the performance of processing workflows could be assessed using criteria (e).

```
normulticlassnoallgs(fileName, GS, SAalpha="Y", SAbeta="Y", SAgamma ="Y")
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`GS` The corresponding data of golden standards for performance evaluation using Criterion e. For the detailed information of the correct file format, please use "??NOREVA" and download sample data in the corresponding section "Welcome to Download the Sample Data for Testing and for File Format Correcting".

`SAalpha` This variable allows the user to specify the study assumption of their peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption alpha represents that all metabolites are assumed to be equally important.

`SAbeta` This variable allows the user to specify the study assumption of their input peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption beta represents that the level of metabolite abundance is constant among all samples.

`SAgamma` This variable allows the user to specify the study assumption of their input peak table.

"Y" denotes the peak table satisfies the study assumption.

"N" denotes the peak table satisfies the study assumption.

Study assumption gamma represents that the intensities of the majority of the metabolites are not changed under the studied conditions.

14. This function enables the performance assessment of the processing workflows defined by the preference of NOREVA users based on time-course metabolomic study with dataset with QCSs.

```
nortimecourseqcpart(fileName, selectedMethods)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`selectedMethods` This variable allows the user to indicate the NAME of the file containing processing workflows defined by the preference of NOREVA users. The file should be in the format of Comma-Separated Values (CSV). Exemplar files are provided by NOREVA and available for download at https://idrblab.org/noreva/NOREVA_exampledata.zip).

15. This function enables the performance assessment of processing workflows defined by the preference of NOREVA users based on time-course metabolomics with ISs.

```
nortimecourseispart(fileName, IS, selectedMethods)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs are listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

`selectedMethods` This variable allows the user to indicate the NAME of the file containing processing workflows defined by the preference of NOREVA users. The file should be in the format of Comma-Separated Values (CSV). Exemplar files are provided by NOREVA and available for download at https://idrblab.org/noreva/NOREVA_exampledata.zip).

16. This function enables the performance assessment of processing workflows defined by users' preference based on time-course metabolomics without QCSs and ISs.

```
nortimecoursenopart(fileName, selectedMethods)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`selectedMethods` This variable allows the user to indicate the NAME of the file containing processing workflows defined by the preference of NOREVA users. The file should be in the format of Comma-Separated Values (CSV). Exemplar files are provided by NOREVA and available for download at https://idrblab.org/noreva/NOREVA_exampledata.zip).

17. This function enables the performance assessment of processing workflows defined by users' preference based on multi-class (N>1) metabolomic study with dataset with QCSs.

```
normulticlassqcpart(fileName, selectedMethods)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`selectedMethods` This variable allows the user to indicate the NAME of the file containing processing workflows defined by the preference of NOREVA users. The file should be in the format of Comma-Separated Values (CSV). Exemplar files are provided by NOREVA and available for download at https://idrblab.org/noreva/NOREVA_exampledata.zip).

18. This function enables the performance assessment of processing workflows defined by users' preference based on multi-class (N>1) metabolomic study with dataset with ISs.

```
normulticlassispart(fileName, IS, selectedMethods)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs should be listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

`selectedMethods` This variable allows the user to indicate the NAME of the file containing processing workflows defined by the preference of NOREVA users. The file should be in the format of Comma-Separated Values (CSV). Exemplar files are provided by NOREVA and available for download at https://idrblab.org/noreva/NOREVA_exampledata.zip).

19. This function enables the performance assessment of the processing workflows defined by the preference of NOREVA users based on multi-class (N>1) metabolomic study with dataset without QCSs and ISs.

```
seleranks_non <- normulticlassnopart(fileName, selectedMethods)
```

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`selectedMethods` This variable allows the user to indicate the NAME of the file containing processing workflows defined by the preference of NOREVA users. The file should be in the format of Comma-Separated Values (CSV). Exemplar files are provided by NOREVA and available for download at https://idrblab.org/noreva/NOREVA_exampledata.zip).

20. This function will output a processed peak table of time-course metabolomic study according to the choice of users must provide the processing workflow.

```
nortimecoursematrix(datatype, fileName, IS, impt=NULL, trsf=NULL, nmal=NULL,
nmal2=NULL, nmals=NULL)
```

`datatype` This variable allows the user to specify the Type of their input peak table.

“1” denotes time-course metabolomic study without QCSs and ISs.

“2” denotes time-course metabolomic study with QCSs.

“3” denotes time-course metabolomic study with ISs.

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs are listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

`impt` This variable allows the user to specify the Type of imputation method.

“1” denotes method of column mean imputation.

“2” denotes method of column median imputation.

“3” denotes method of half of the minimum positive value.

“4” denote method of K-nearest neighbor imputation.

`trsf` This variable allows the user to specify the Type of transformation method.

“1” denotes method of cube root transformation.

“2” denotes method of log transformation.

“3” denotes none transformation method.

`nmal` This variable allows the user to specify the Type of normalization method.

“1” denotes none normalization method.

Metabolite-based Normalization:

"2" denotes method of probabilistic quotient normalization.

"3" denotes method of cyclic loess.

"4" denotes method of contrast.

"5" denotes method of quantile.

"6" denotes method of linear baseline.

"7" denotes method of Li-Wong.

"8" denotes method of cubic splines.

"16" denotes method of MS total useful signal.

"17" denotes method of total sum normalization.

"18" denotes method of median normalization.

"19" denotes method of mean normalization.

"20" denotes method of EigenMS.

Sample-based Normalization:

"9" denotes method of auto scaling.

"10" denotes method of range scaling.

"11" denotes method of pareto scaling

"12" denotes method of vast scaling

"13" denotes method of level scaling

"15" denotes method of power scaling

Sample & Metabolite-based Normalization:

"14" denotes method of variance stabilization normalization.

`nmal2` This variable allows the user to specify the Type of normalization method. According to the normalization methods of combination strategy, if you choose sample-based normalization methods for argument “nmal”, “nmal2” should select metabolite-based normalization methods. Similarly, if you choose metabolite-based normalization methods for argument “nmal”, “nmal2” should select sample-based normalization methods. The VSN method you selected is a sample & metabolite-based normalization, which should be applied alone to remove the unwanted signal variations.

`nmals` This variable allows the user to specify the Type of IS-based normalization method.

“1” denotes method of Single Internal Standard.

“2” denotes method of Normalization using Optimal Selection of Multiple ISs

“3” denotes method of Cross-contribution Compensating Multi-ISs Normalization

“4” denotes method of Remove Unwanted Variation-Random.

21. This function will output a processed peak table of multi-class metabolomic study according to the choice of users must provide the processing workflow.

```
normulticlassmatrix(datatype, fileName, IS, impt=NULL, trsf=NULL, nmal=NULL,
nmal2=NULL, nmals=NULL)
```

`datatype` Input the number of data type.

If set 1, the dataset of multi-class (N>1) metabolomic study without QCSs and ISs.

If set 2, the dataset of multi-class (N>1) metabolomic study with QC samples (QCSs).

If set 3, the dataset of multi-class (N>1) metabolomic study with dataset with (ISs).

`fileName` This variable allows the user to indicate the NAME of result obtained from PrepareInuputFiles function.

`IS` This variable allows the user to indicate the column of IS.

If there is only one IS, the column number of this IS should be listed.

If there are multiple ISs, the column number of all ISs are listed and separated by comma (,).

For example, the replacement of IS to 2,6,9,n indicates that the metabolites in the 2st, 6th, 9th, and nth columns of in your peak table should be considered as the ISs metabolites.

`impt` This variable allows the user to specify the Type of imputation method.

“1” denotes method of column mean imputation.

“2” denotes method of column median imputation.

“3” denotes method of half of the minimum positive value.

“4” denote method of K-nearest neighbor imputation.

`trsf` This variable allows the user to specify the Type of transformation method.

“1” denotes method of cube root transformation.

“2” denotes method of log transformation.

“3” denotes none transformation method.

`nmal` This variable allows the user to specify the Type of normalization method.

“1” denotes none normalization method.

Metabolite-based Normalization:

"2" denotes method of probabilistic quotient normalization.

"3" denotes method of cyclic loess.

"4" denotes method of contrast.

"5" denotes method of quantile.

"6" denotes method of linear baseline.

"7" denotes method of Li-Wong.

"8" denotes method of cubic splines.

"16" denotes method of MS total useful signal.

"17" denotes method of total sum normalization.

"18" denotes method of median normalization.

"19" denotes method of mean normalization.

"20" denotes method of EigenMS.

Sample-based Normalization:

"9" denotes method of auto scaling.

"10" denotes method of range scaling.

"11" denotes method of pareto scaling

"12" denotes method of vast scaling

"13" denotes method of level scaling

"15" denotes method of power scaling

Sample & Metabolite-based Normalization:

"14" denotes method of variance stabilization normalization.

`nmal2` This variable allows the user to specify the Type of normalization method. According to the normalization methods of combination strategy, if you choose sample-based normalization methods for argument “nmal”, “nmal2” should select metabolite-based normalization methods. Similarly, if you choose metabolite-based normalization methods for argument “nmal”, “nmal2” should select sample-based normalization methods. The VSN method you selected is a sample & metabolite-based normalization, which should be applied alone to remove the unwanted signal variations.

`nmals` This variable allows the user to specify the Type of IS-based normalization method.

“1” denotes method of Single Internal Standard.

“2” denotes method of Normalization using Optimal Selection of Multiple ISs

“3” denotes method of Cross-contribution Compensating Multi-ISs Normalization

“4” denotes method of Remove Unwanted Variation-Random.

22. Plot circular barplot of overall ranking results. A circular barplot illustrating the performance level and the overall ranking of all calculatable processing workflows based on the multiple criteria or a single criterion that are selected by user..

```
norvisualization(data, outputfile="NOREVA-Ranking-
Top.%d.workflows.%s",cutoff="100", outputtype="pdf", maxValue="40", colorSet =
c("#EA4335", "#4285F4", "#FBBC05", "#800080"), totalAngle = "340", bgColor =
"#FFFFFF", fontColor="#000000")
```

`data` This variable allows the user to specify the NAME of the file (.csv) containing the names of processing workflows, their ranking value and representative measurement values under differential criteria, which is obtained from the functions such as the “normulticlassnoall”, “normulticlassqcall”, “nortimecourseqcall”, or “nortimecoursenoall” et al.

`outputfile` This variable allows the user to specify the NAME of the output file. A format string containing the cutoff value and data type to generate formatted file name.

`cutoff` This variable allows the user to specify the cutoff value. Integer for the number of strategies ranking at the top of the list, which is used to filter the results. Integer, which means to filter the results, the default is 100.

`outputtype` String, indicating the output type, support pdf, eps, default is pdf.

`MaxValue` Double-precision floating-point number, representing the characteristic value represented by the maximum length of the rectangle, the default is 40.

`colorSet` Hexadecimal color string group, representing the four-layer color setting of the graphics from the inside to the outside, the default is "#FFE699", "#D3E8C7", "#B2B2FF", "#FFCACA".

`totalAngle` Double-precision floating-point number, representing the total angle of rotation of the drawing, in degrees, the default value is 340.

`bgColor` Hexadecimal color string, representing the background color of the graphic drawing, the default is white (#FFFFFF).

`FontColor` Hexadecimal color string, representing the font color, the default is black (#000000).

Welcome to Download the Sample Data for Testing and for File Format Correcting

```
# Time-course Metabolomic Study
```

Dataset with quality control samples (QCSs) could be downloaded and the corresponding data of golden standards for performance evaluation using Criterion e could be downloaded.

Dataset with internal standards (ISs) could be downloaded and the corresponding data of golden standards for performance evaluation using Criterion e could be downloaded.

Dataset without QCSs and ISs could be downloaded and the corresponding data of golden standards for performance evaluation using Criterion e could be downloaded.

```
# Multi-class Metabolomic Study
```

Dataset with quality control samples (QCSs) could be downloaded and the corresponding data of golden standards for performance evaluation using Criterion e could be downloaded.

Dataset with internal standards (ISs) could be downloaded and the corresponding data of golden standards for performance evaluation using Criterion e could be downloaded.

Dataset without QCSs and ISs could be downloaded and the corresponding data of golden standards for performance evaluation using Criterion e could be downloaded.

Sequential step for the performance assessment of time-course metabolomic study with dataset with QCSs. For other types of study, replace the function related to the types.

```
Step 1: time_qcs_data <- PrepareInuputFiles(dataformat = 1, rawdata =
"Timecourse-QC-XXX.csv")

Step 2: normulticlassqcall(fileName = time_qcs_data, SAalpha = "Y", SAbeta = "Y",
SAgamma = "Y")

Step 3: norvisualization(data = "OUTPUT-NOREVA-Overall.Ranking.Data.csv", cutoff
= "100")
```

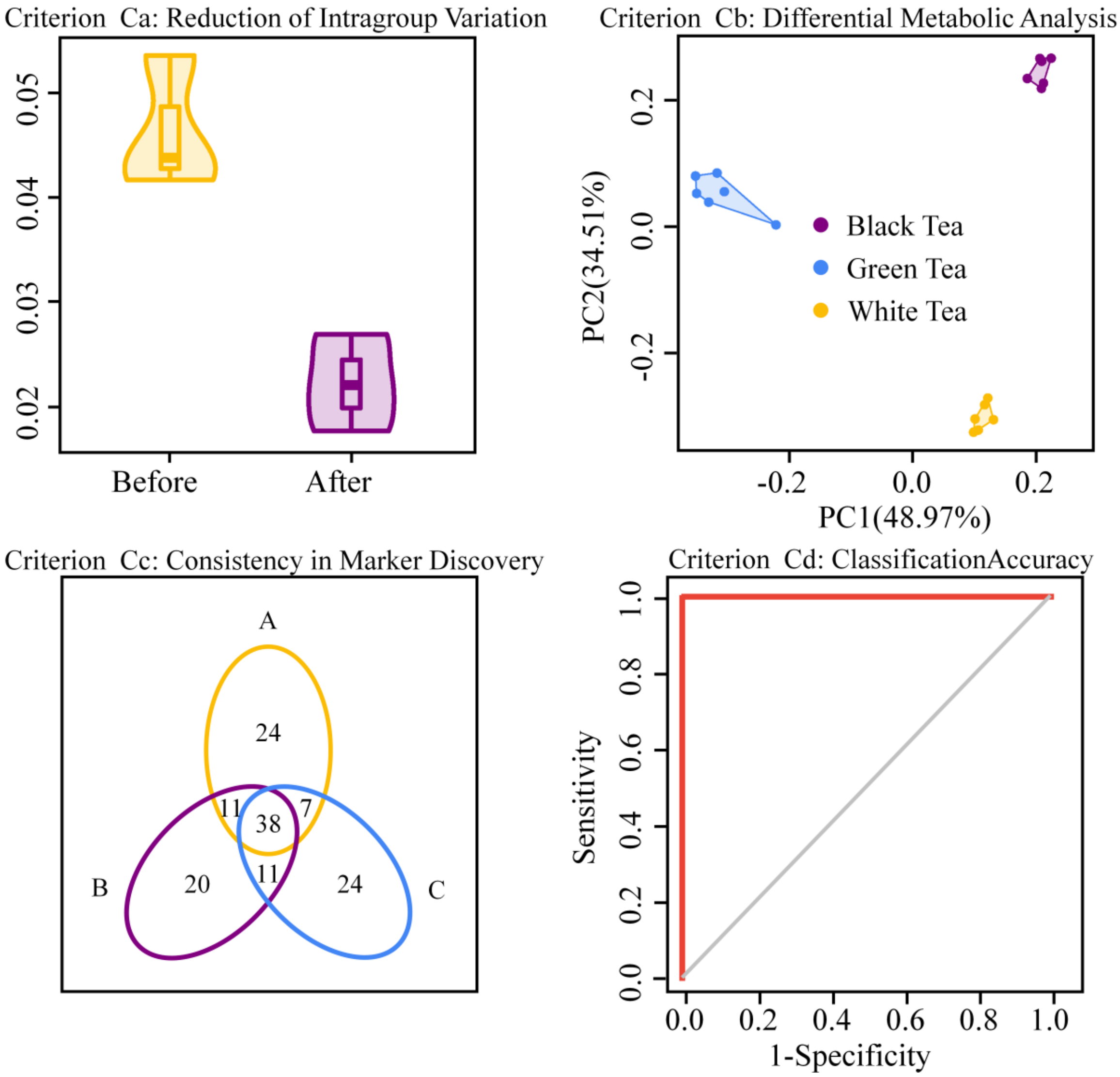
Sequential step for the performance assessment of multi-class metabolomic study with dataset with QCSs. For other types of study, replace the function related to the types.

```
Step 1: multi_qcs_data <- PrepareInuputFiles(dataformat = 1, rawdata =
"Multiclass-QC-XXX.csv")

Step 2: normulticlassqcall(fileName = multi_qcs_data, SAalpha = "Y", SAbeta =
"Y", SAgamma = "Y")

Step 3: norvisualization(data = "OUTPUT-NOREVA-Overall.Ranking.Data.csv", cutoff
= "100")
```

Examples

Step 1: Prepare input of peak table for assessing normalization for metabolomic data

```
multi_qcs_data <- PrepareInuputFiles(dataformat = 1, rawdata = "Multiclass-QC-
MTSL403.csv")
```



NOREVA-All-Criteria-Output-Figures

Note: the file should be in the format of Comma-Separated Values (CSV), which provides the intensity data of metablites. Different functions require different data types. Please refer to the section “Welcome to Download the Sample Data for Testing and for File Format Correcting”.

Sample data MTBLS403: untargeted metabolomic dataset of 3 classes with quality control samples (QCSs), which contained 3805 metabolites from 3 types of tea samples (green tea, black tea and white tea), could be downloaded. *Food Res Int*. 96:40-45,2017.

Step 2: Assessing all processing workflows for multi-class metabolomic data

Multi-class (N>1) Metabolomic Study with dataset with Quality Control Samples (QCSs)

```
normulticlassqcall(fileName=multi_qcs_data,SAalpha="Y",SAbeta="Y",SAgamma="Y")
```

```
allrankings <- read.csv(file = "./sampledata/OUTPUT-NOREVA-
verall.Ranking.Data.csv",header = T)
head(allrankings)
```

```
##                X Overall.Rank Criteria.Ca.Rank Criteria.Cb.Rank
## 1 HAM+CUT+SUM+LEV            1              164                1
## 2 HAM+LOG+MST+NON            2               78                1
## 3 HAM+LOG+MST+PAR            3              166                1
## 4 HAM+LOG+SUM+RAN            4              298                1
## 5 KNN+LOG+MST+NON            5               85                1
## 6 HAM+CUT+SUM+RAN            6              305                1
##   Criteria.Cc.Rank Criteria.Cd.Rank Criteria.Ca.Value Criteria.Cb.Value
## 1               50                1            0.0076                 1
## 2              140                1            0.0003                 1
## 3              139                1            0.0079                 1
## 4               13                1            0.0344                 1
## 5              232                1            0.0012                 1
## 6               51                1            0.0365                 1
##   Criteria.Cc.Value Criteria.Cd.Value
## 1            0.7208                 1
## 2            0.6167                 1
## 3            0.6167                 1
## 4            0.7500                 1
## 5            0.5667                 1
## 6            0.7208                 1
```

Step 3: a circular barplot illustrating the performance ranking of all processing workflows.

```
norvisualization(data = "OUTPUT-NOREVA-Overall.Ranking.Data.csv", cutoff = "100")
```

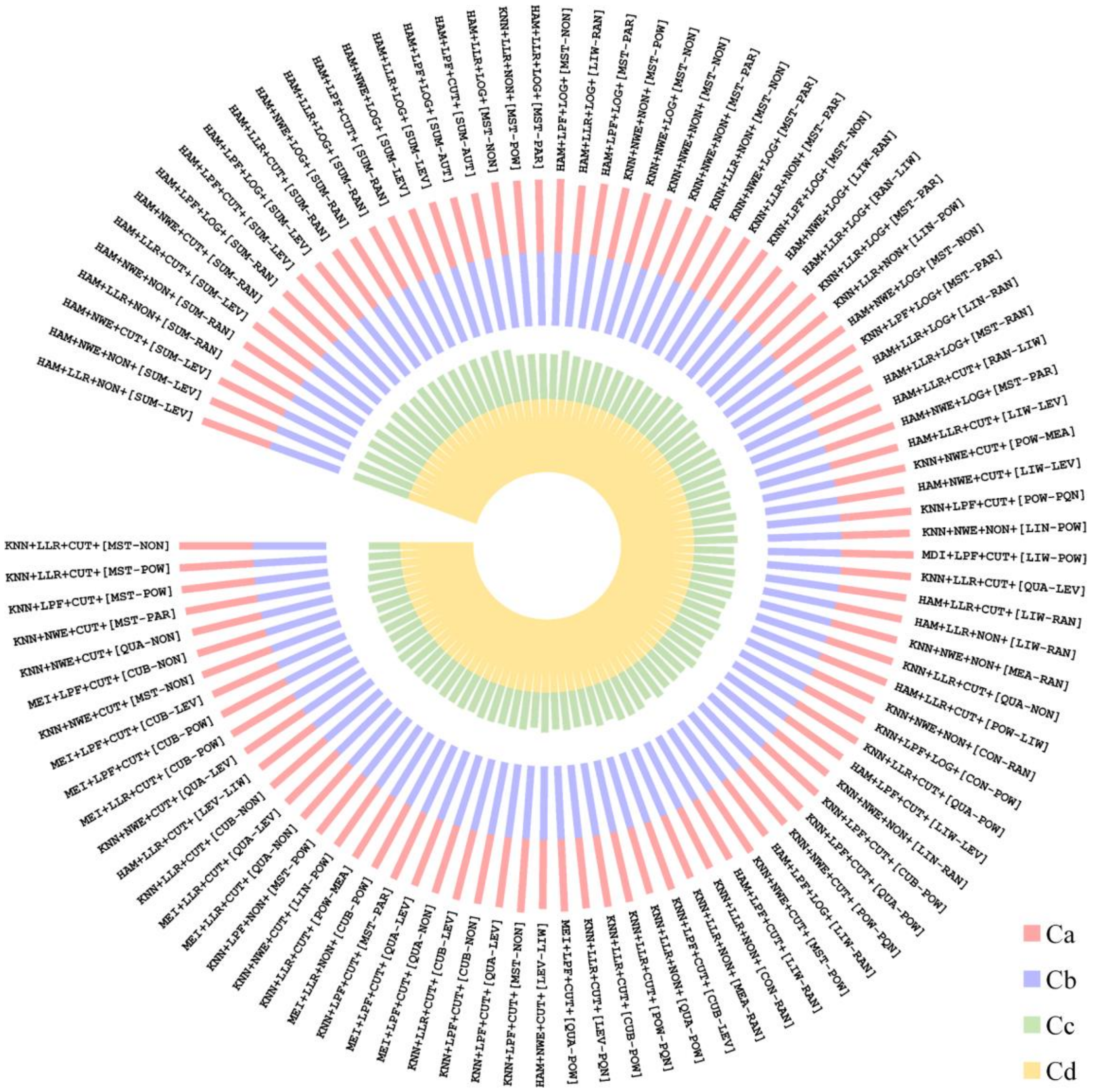
Comprehensive assessment among all processing workflows (the top-100 were shown) based on the collective evaluations using four different criteria.

Step 4: Processing datasets using the processing workflow based on the results of assessment.

Normalization with datasets of multi-class (N>1) metabolomic study

```
nordata <- normulticlassmatrix(datatype = 2, fileName = multi_qcs_data, impt = "3",
trsf = "1", nmal = "17", nmal2 = "13")
```

Note: please select the appropriate number code represents imputation, transformation, normalization methods (See above details).



NOREVA-Ranking-Top.100.workflows

Step 5: Users can also use NOREVA for accessing the part of normalization methods/strategies which you preferred.

Multi-class (N>1) Metabolomic Study with dataset with Quality Control Samples (QCSs)

```
normulticlassqcpart(fileName = multi_qcs_data, selectedMethods =
"selectedMethods.csv")
```

Note: please select the appropriate number code represents imputation, transformation, normalization methods (see above details).

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