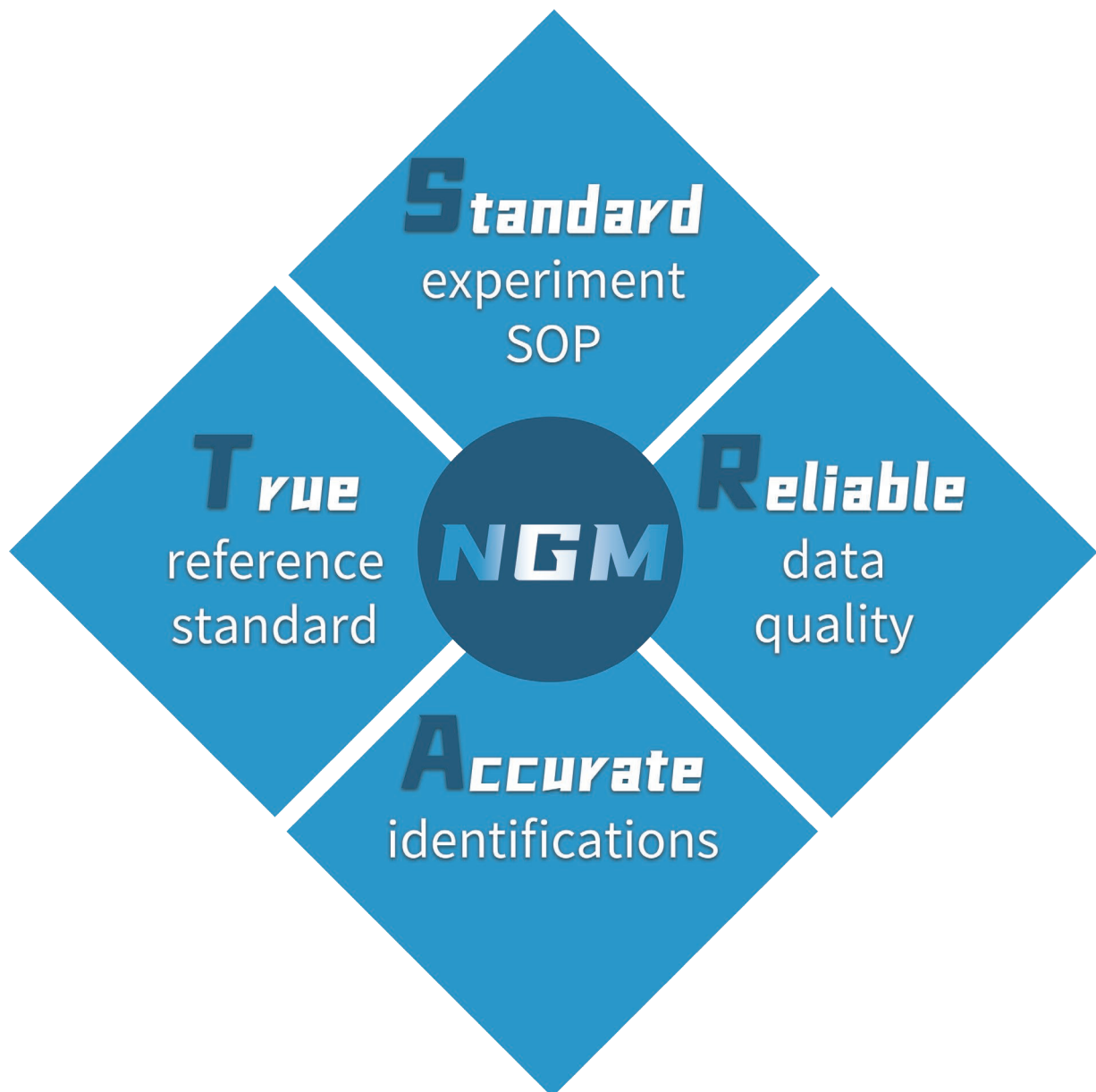


Next Generation Metabolomics

Accurate Identifications

Economize Your Time



The purpose of metabolomics is to study the different types of small molecule substances in various biological samples, such as amino acids, organic acids, hormones, etc. In order to improve the accuracy of metabolite identification, Biotree builds a standards database, referring to the guidelines of the International Metabolomics Standards Initiative (MSI), and has established a new generation of testing technology for metabolomics (Next Generation Metabolomics, NGM).

NGM untargeted metabolomics possesses a database of over 20,000 standards, and is equipped with ultra-high-resolution mass spectrometers. It uses advanced algorithms to achieve the identification of substances in samples, introduces automated preprocessing to reduce errors caused by manual operation, and sets up quality control systems at every stage of sample testing, thereby comprehensively enhancing the accuracy of metabolite annotation results.

		Market level untargeted metabolomics	NGM
Performance	Annotation level	Level 1 to 4	Level 1 to 4
	Algorithm	Single algorithm	Multiple algorithm, including MetDNA 3
	Instrument	Triple TOF 6600, Sciex/Q Exactive HFX, Thermo	Orbitrap Exploris 120, Thermo
	Standard library	2000+	20,000+
	Level 1 results (serum)	200+	1,200+
	Total results (serum)	1500+	5,000+
	Total features (serum)	12000+	80,000+
	Stable detection rate in multicenter study	30%	60%
Quality Control	QC samples	Gather in 2-dimensional PCA	Gather in 2-dimensional PCA ≤2std in 1-dimensional PCA Correlation of QC samples >0.90
	Internal standard (I/S)	1 isotope internal standard Small difference between I/S in QC samples	6 isotope internal standard I/S peak height difference <30% in QC samples I/S RSD<10% in QC samples
	Blank samples	×	No I/S detection in blank samples
Data Analysis	Standard analysis	14	20
	Cohort sample correction	×	✓
Pricing		Low	High
Turn around time		From 1 to 3 months	≤1.5 month
After-sale service		≤6 months	Until publication

*Performance data from human serum samples

ACCURATE

The Metabolomics Standards Initiative (MSI) was jointly established by Imperial College London in the UK and the National Institutes of Health (NIH) in the United States in 2005. Over the past decade, it has sequentially proposed consensus statements on various aspects of metabolomics, including the identification of metabolites, data analysis, and sample processing. Regarding the identification of metabolites, it emphasized that the identification of metabolites must be very rigorous to facilitate following validation experiment.

According to the consensus of the MSI (Metabolomics, 2007), Biotree has fully aligned with international metabolomics standards and annotated 4 levels of metabolite identification:

Level 1: RT, MS1 and MS2 all match with reference standards

Level 2: MS1 and MS2 match with public library

Level 3: MS1, MS2 and forecasted RT match with theoretical database

Level 4: Unknown

Tips:

RT: Retention Time, refers to the time for a substance to pass through chromatographic separation and be detected by mass spectrometry. The smaller the deviation of RT, the more accurate the identification of the substance.

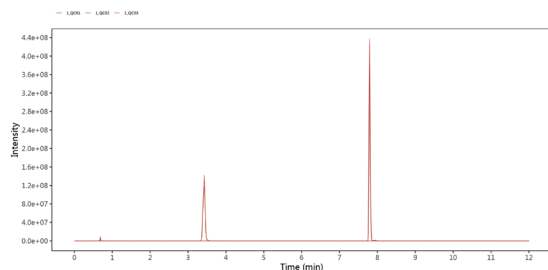
MS1: Primary mass spectrometry, refers to the mass-to-charge ratio (M/Z) of a substance after it has been charged. The smaller the mass deviation, the more accurate the molecular formula information obtained.

MS2: Secondary mass spectrometry, refers to the fragmented ions of a substance after it enters the mass spectrometer and is broken apart. Typically, a substance will have multiple fragment ions. The more fragments matched, the more accurate the substance information obtained. The accuracy of matching only one fragment ion is not sufficient. From the above standards, we can see that level 1 identification results are the most accurate.

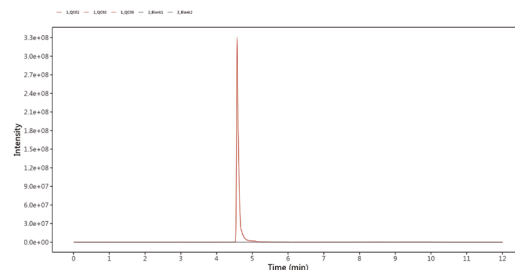
NGM boasts an in-house library of over 20,000+ standard metabolites, strict material identification algorithm, and comprehensive quality control system. It ensures data quality from the source, process, and result stages.



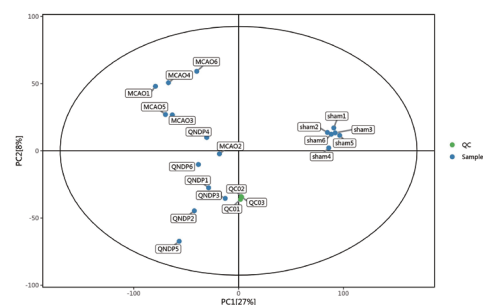
Quality Control System



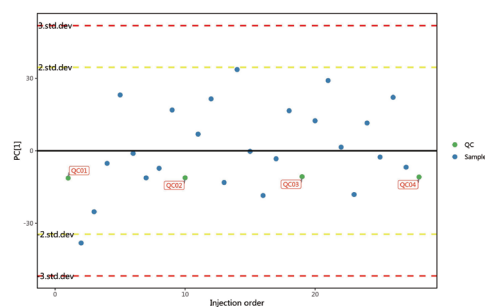
1. The peak height difference of internal standards in QC samples is within 30%



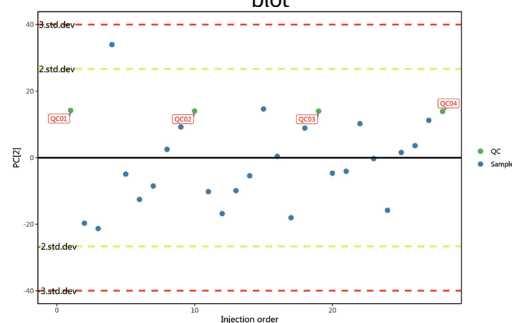
2. No significant detection of internal standards in blank samples



3. QC samples are clustered in the two-dimensional PCA plot



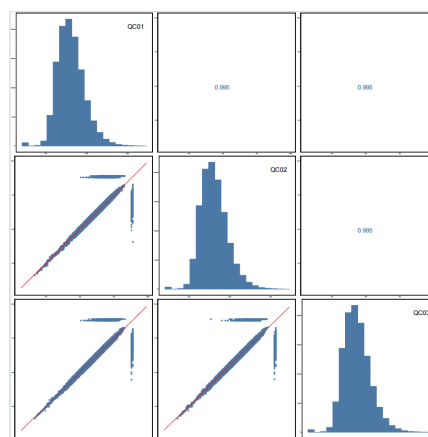
4. The distribution of QC samples along PCA1 is within 2 times the standard deviation(STD)



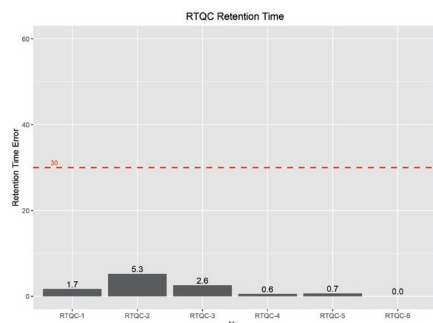
5. The distribution of QC samples along PCA2 is within 2 times the standard deviation(STD)

	rt	mz	rsd
IS1	198.2	183.0819	0.0045
IS2	275.3	133.1058	0.0055
IS3	468	190.2577	0.0013
IS4	275.2	135.1204	0.0048
IS5	205.2	200.047	0.004
IS6	467.8	283.1835	0.0085

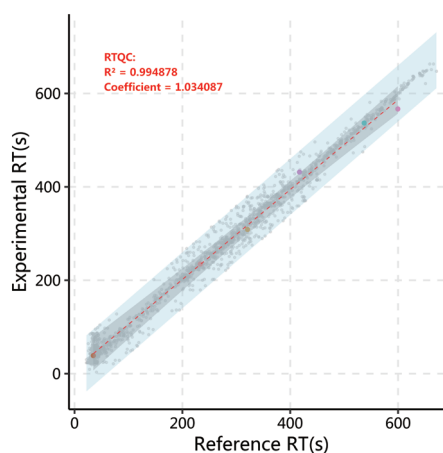
7. The RSD of internal standard responses in QC samples is less than 10%



6. The correlation coefficient of QC samples within the batch is greater than 0.90



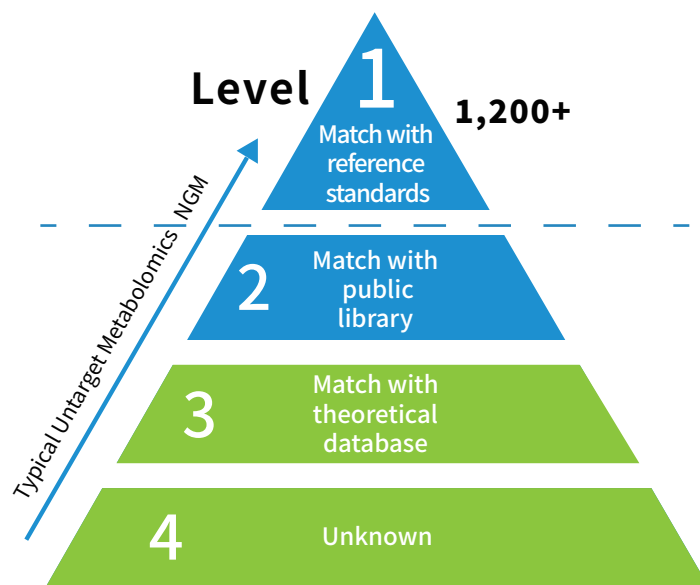
8. The retention time error of RTQC mixed standards is within 30



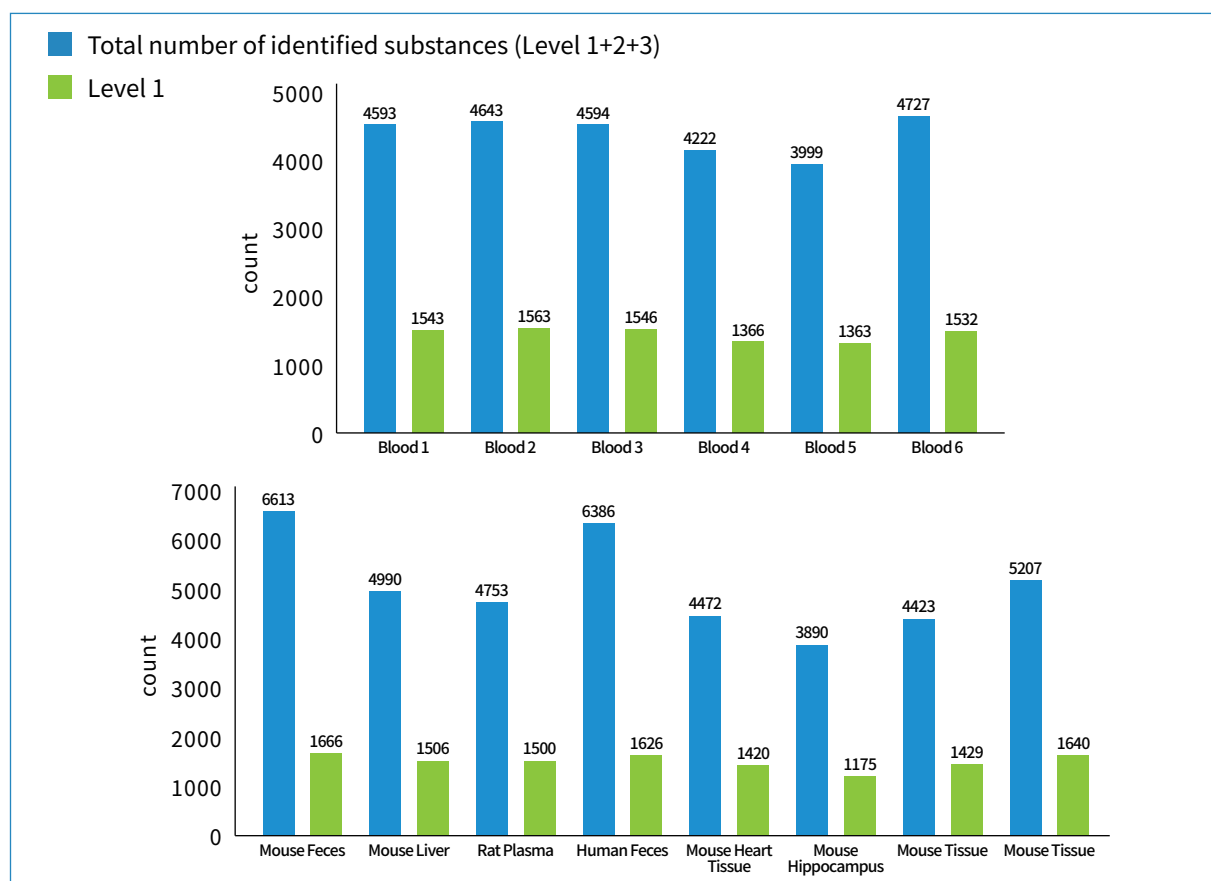
9. The R^2 value of the retention time calibration curve for RTQC mixed standards is greater than 0.9

ABUNDANT

According to the guidelines set by the Metabolomics Standards Initiative (MSI), Biotree creates an in-house standard library including over 20,000+ standard metabolites. Equipped with ultra-high-resolution mass spectrometers, and an innovative quadruple-core matching algorithm, Biotree has comprehensively improved the accuracy of metabolomics testing results, providing 1,200+ level 1 metabolite results in one experiment.



Real testing results from clients' projects:



STABLE

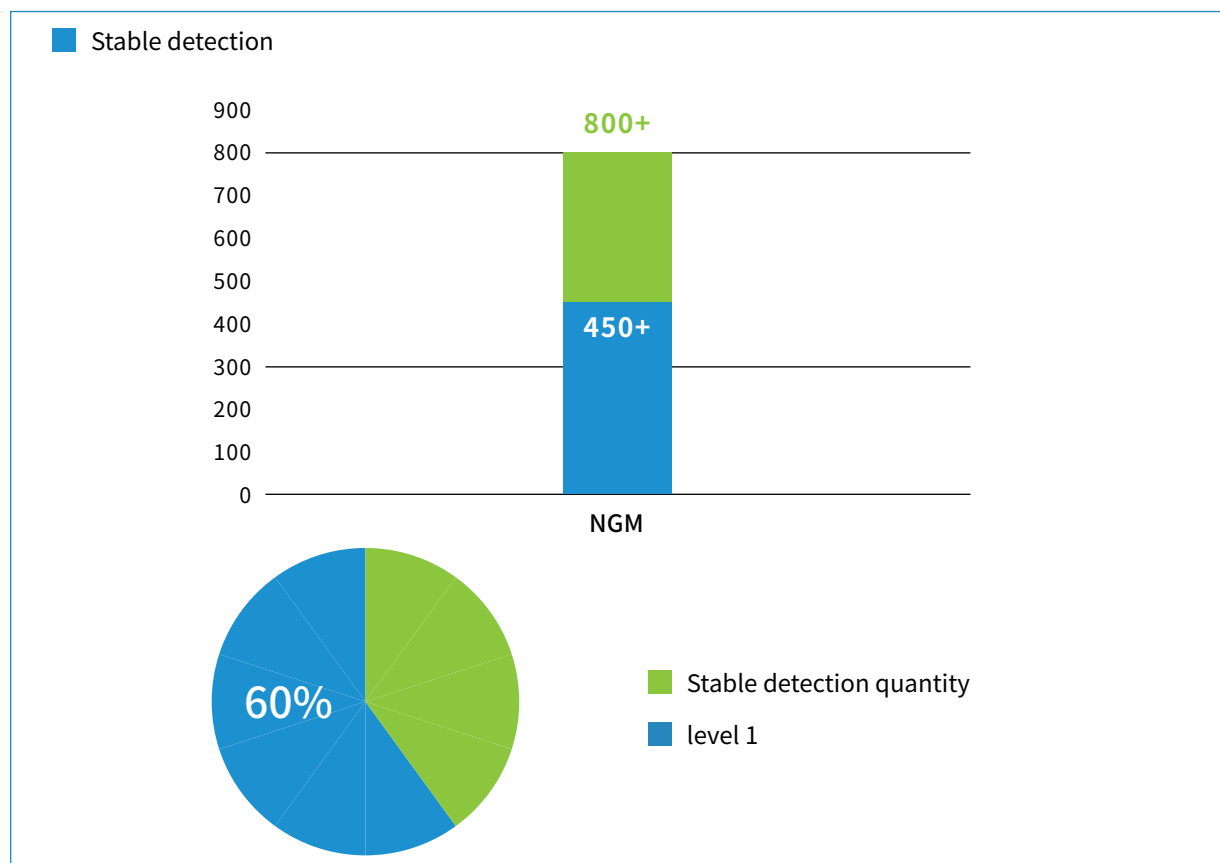
Biomarkers face a long and arduous journey from the initial candidate discovery to clinical application. During this process, we encounter many challenges:

The validation of blood-based candidate biomarkers often requires a significant investment of time and resources. If the candidate biomarkers originate from false positives in the raw data, then much of the subsequent validation work may be rendered and worthless.

Multiple independent studies are necessary to validate the reproducibility of candidate biomarkers. However, due to the differences in sample collection, instrument status, and other factors, it is extremely difficult to ensure the reproducibility of data between different studies.

Biotree NGM technique offers a new approach for the discovery and validation of biomarkers by significantly improving the stability of multi-batch data. Data generated from NGM metabolic testing can be combined with other existing biomarker screening technologies such as proteomics, transcriptomics, and single-cell omics to enhance the accuracy and reliability of biomarkers.

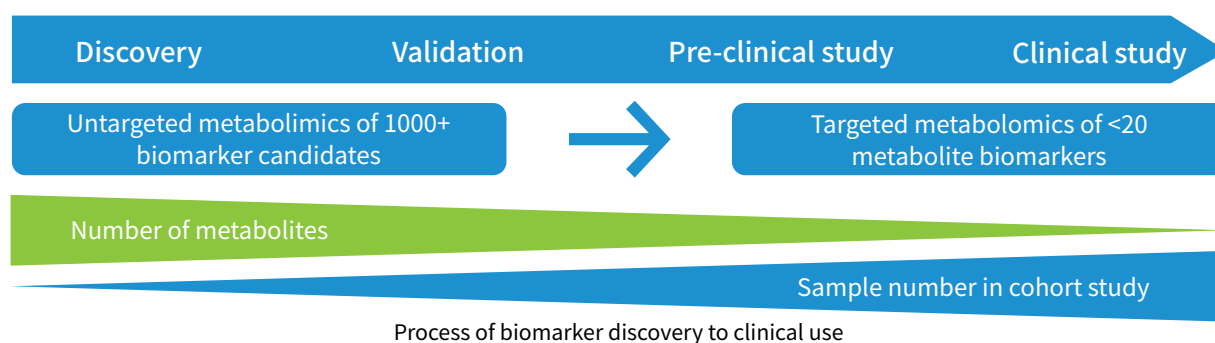
Consistently and stably detection of hundreds of metabolites is one of the most important advantages of Biotree NGM testing. An analysis of blood samples from multiple population cohorts revealed stable detection over 450 Level 1 metabolites (meaning these metabolites are detected in every population cohort), which represents an average stable detection rate of 60% to support following biomarker validation study.



CUSTOMIZABLE

To further increase the detection rate of interested metabolites, Biotree NGM offers personalized solutions tailored to specific research topics.

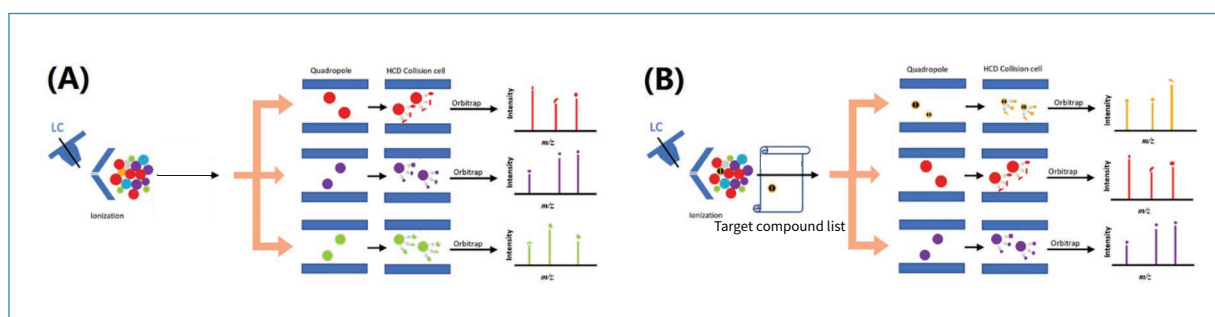
In clinical practice, the validation of biomarkers typically involves Triple Quadrupole Mass Spectrometry for multiple reaction monitoring (MRM) testing. The validation process entails quantitative detection of biomarker candidates in large sample amount to determine which candidates (individually or in combination) can best distinguish the presence of a disease. The development of MRM assays for each substance requires a lot of time and resources. If the candidate biomarkers are false positives in the original discovery cohort, it'll result in a substantial waste of time and resource. Clearly, there is a need for an intermediate step between discovery and validation. Before investing the significant resources required for quantitative assay development, there must be a robust method to prioritize the list of biomarker candidates.



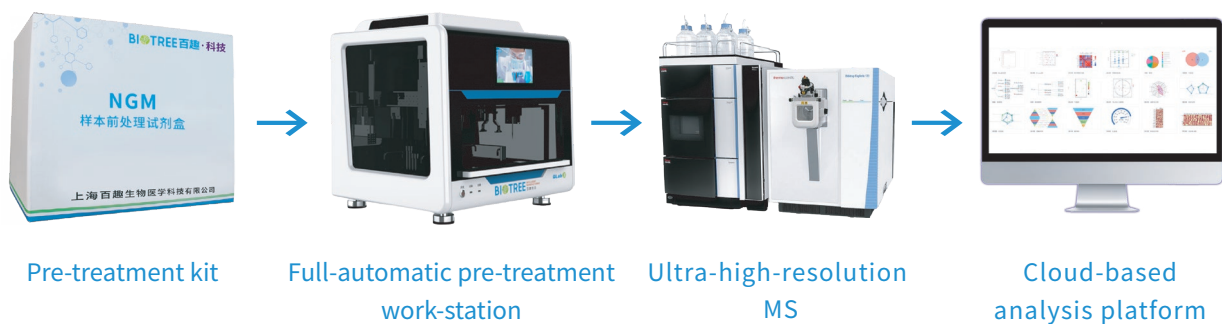
If you are concerning about:

Concentration of interested metabolites is low in samples and may not be detected; Existing publications show some metabolites are related to your study but these metabolites may not be detected in your samples; Each batch in a cohort study requires long time to run on MS, and the detection results may differ in every batch of samples; Biotree NGM offers personalized solution to solve your concern. By providing a list of interested metabolites, Biotree NGM can significantly increase the MS/MS coverage, which in turn enhances the detection of interested metabolites in your samples. Our stable detection rate of interested metabolites can be as high as 60%. Additionally, the MS/MS data obtained from Biotree NGM can be directly utilized for the following development of MRM target methods.

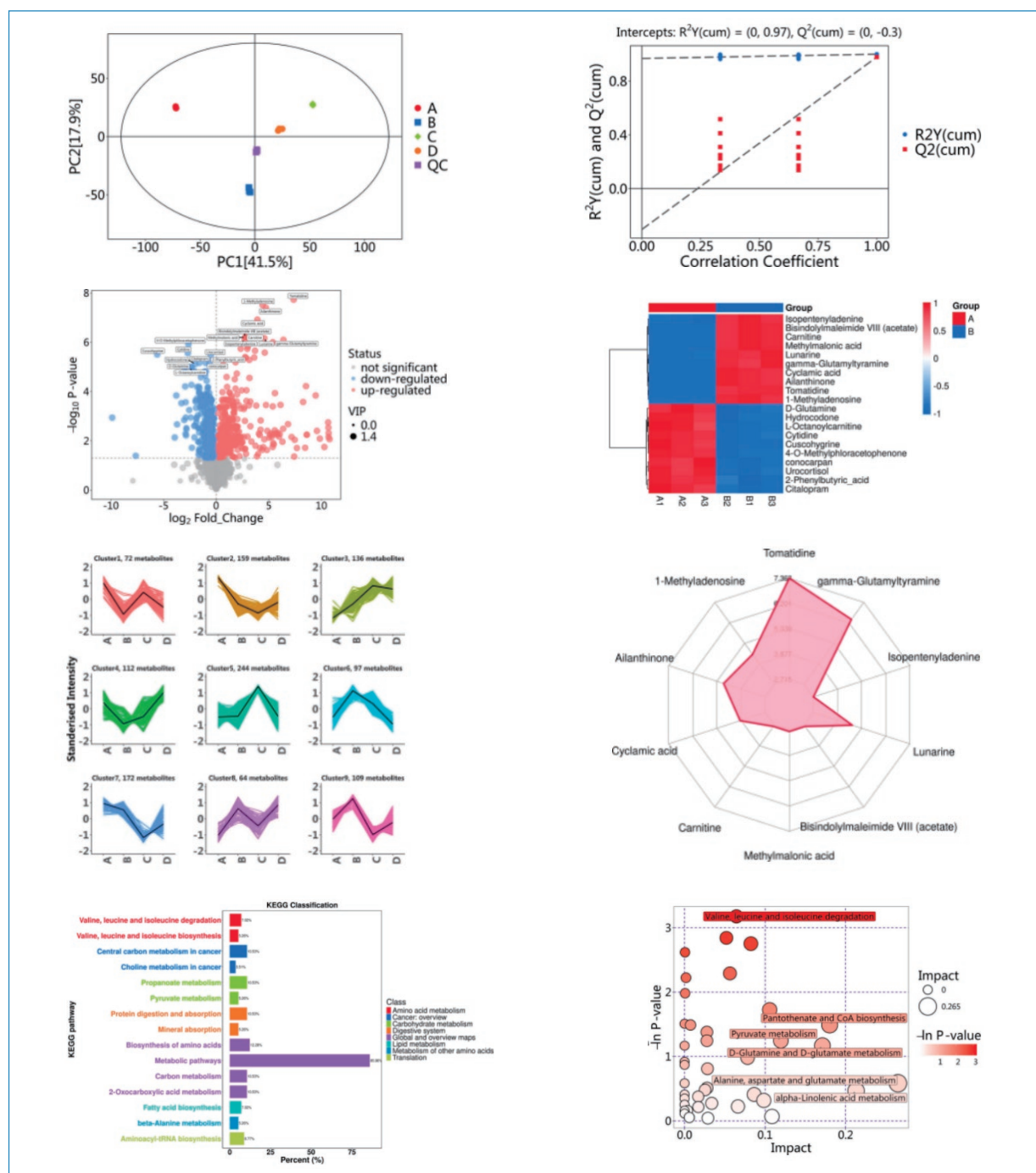
To conclude, Biotree NGM reduces the randomness in MS/MS screening and doubles the stable detection rate (from 30% to 60%) of a certain list of interested metabolites. This technique brings an additional detection of 300-500 metabolites in multicenter validation.



Data collection in regular untarget testing vs Biotree NGM



Data Analysis



Applications

Clinical

- biomarker screening
- TDM
- disease subtypes



Food & beverage

- clinical nutrition
- food nutrition



Husbandry

- modern feeding
- disease control
- animal growth



Drug

- drug research
- toxic research



Traditional medicine

- mechanization research
- drug target research



Environmentology

- Environmental Exposome



Publication example

◆ Knowledge and data-driven two-layer networking for accurate metabolite annotation in untargeted metabolomics

◆ Publication: *nature communications*

◆ IF: 15.7

◆ Year: 2025

◆ Research Background

Untargeted metabolomics based on liquid chromatography-mass spectrometry (LC-MS) enables the detection of both known and unknown metabolites in samples. However, the annotation of unknown metabolites remains a major challenge in untargeted metabolomics.

◆ Research achievement

In MetDNA3, the authors developed a two-layer interactive network topology to enhance metabolite annotation. Recursive metabolite annotation was achieved through cross-network interaction, which significantly improved the efficiency, coverage and accuracy of metabolite identifica-

tion, providing robust technical support for metabolomics research and related applications in life sciences and medicine.

Application

In tests using metabolomic datasets from diverse biological samples, MetDNA3 successfully annotated more than 1,600 Level 1 metabolites and annotated over 12,000 metabolites via network topology-based strategies. Furthermore, two novel metabolites not included in the Human Metabolome Database were discovered and validated using dual network-driven propagated iterative annotation. The study also demonstrated that highly specific knowledge networks are critical for improving the accuracy and propagation efficiency of network-based annotation.

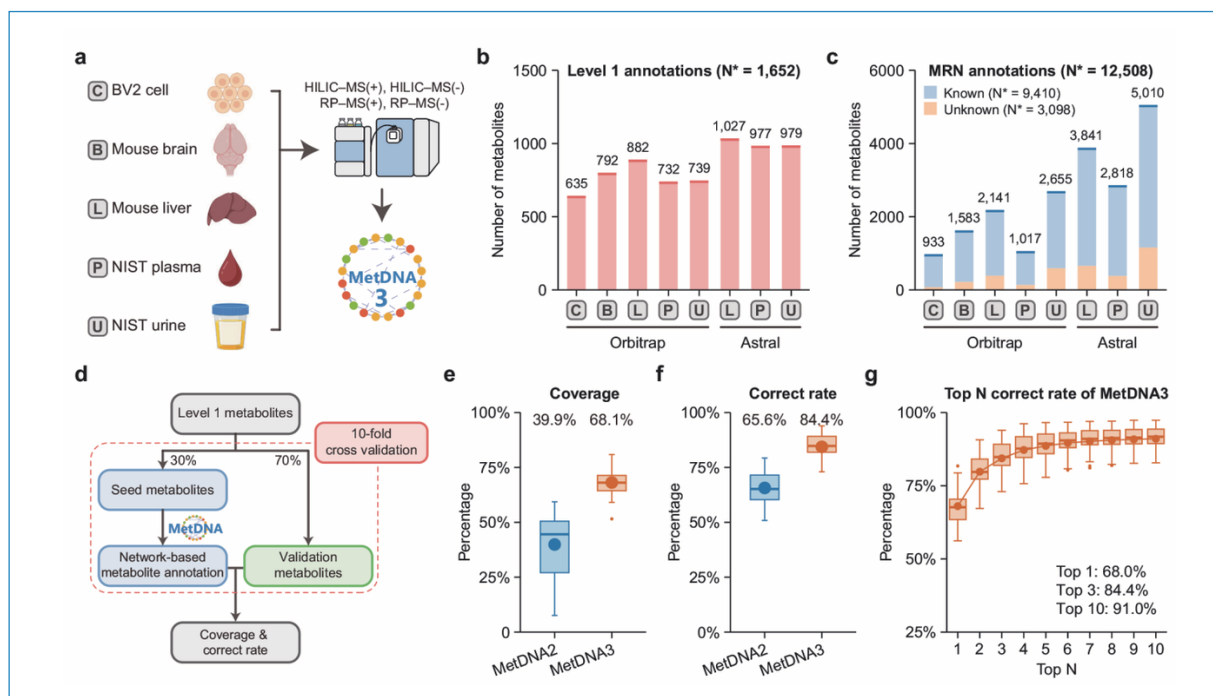


Figure: Improved coverage and accuracy of metabolite annotation

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