Protein Qualitative Analysis Services

Creative Proteomics

www.creative-proteomics.com

Mass Spectrometry Platform Assists Protein Qualitative Analysis

Protein qualitative analysis based on mass spectrometry is used to explore protein expression within organisms. Mass spectrometry offers highly efficient, robust, and accurate results and is one of the core technologies for proteomic research.

Our Mass Spectrometry Platform

Agilent 6530/6545 Q-TOF 	Bruker AutoFlex MALDI TOF/TOF 	Thermo LTQ Orbitrap Velos 
Thermo Q-Exactive Orbitrap 	Thermo Q-Exactive Plus Orbitrap 	Thermo Orbitrap Fusion 

Protein Gel Band/Mixed Solution Identification

Protein identification is a common topic for biochemistry research, and mass spectrometry is considered one of the most useful techniques that solve this issue. Two major strategies that are widely used for protein identification by mass spectrometry are MALDI-TOF-based protein fingerprinting and LC-MS/MS-based peptide sequencing. Meanwhile, LC-MS/MS reserved higher sensitivity and ability than MALDI-TOF and can accurately identify multiple protein components from a single sample.

Based on the LC-MS/MS technology, Creative Proteomics enables protein identification of various samples, covering gel samples (SDS-PAGE, native PAGE), IP eluates, body fluids, tissues, etc. The basic principle of protein identification by mass spectrometry involves protein digestion with proteases into a peptide mixture and ionizing it by electron bombardment or other means to form charged ions with different mass-to-charge ratios. Then, the mass analyzer is used to separate peptide ions according to their specific mass-to-core ratios. Finally, protein identification is performed by comparing the mass spectrum of digested proteins with the theoretical primary and secondary mass spectrum in a database. This technique is suitable for protein identification based on protein mixtures separated by the SDS-PAGE gel followed by in-gel enzymatic digestion or direct in-solution enzymatic digestion of protein mixtures.

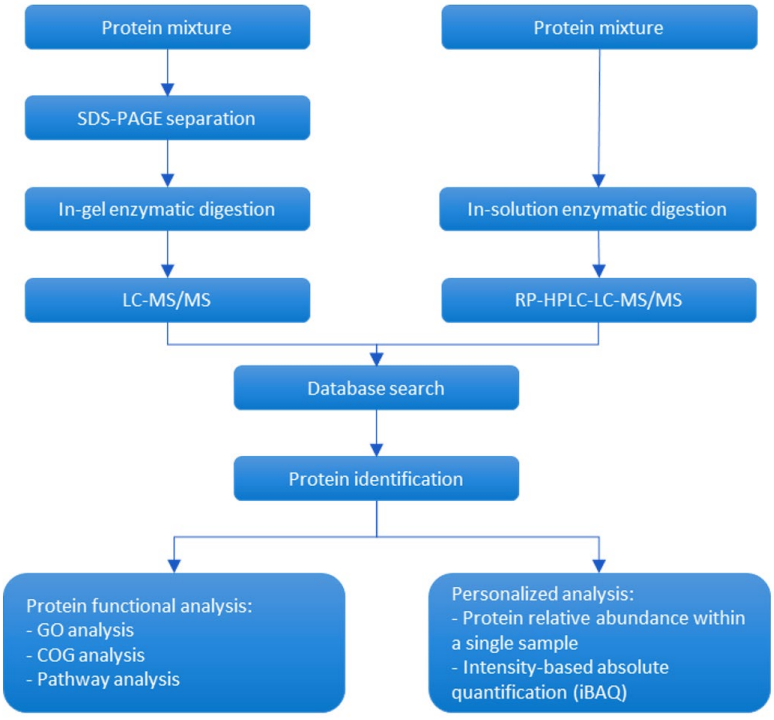


Fig 1. Process of protein gel band/mixed solution identification

Technical Features

- High throughput: Identifying dozens to thousands of proteins within each experiment
- Accurate and reliable: Achieving high mass accuracy (less than 1 ppm) and resolution (over 4×10^4) and providing high-quality data
- Wide range of applications: No species requirements restriction and applicable to all species

Scope of Application: Species with available protein databases or known target protein sequences
For samples that cannot be identified by available database analysis, *de novo* protein sequence analysis can be used.

Protein N/C-Termini Identification

Protein N/C-Termini Identification by LC-MS/MS

Almost all protein synthesis begins from the N-terminus. Thus, the sequential composition of the N-terminus has a great influence on the overall biological function of the protein. N-terminal sequencing analysis can help analyze the complex protein structure and reveal biological functions.

The C-terminus is an important structural and functional site of the protein. Specific post-translational modifications, i.e., C-terminal cleavage, can affect the organization of the protein structure and, thus, affect their biological functions. Therefore, elucidating the amino acid compositions and sequences of the C-terminus can help reveal the structure and function of the protein.

Creative Proteomic utilized advanced LC-MS/MS technology combined with labeling methods to identify protein N/C-termini with high confidence.

Fig 2. Process of protein N/C-termini identification based on LC/MS-MS

Protein N/C-Termini Identificationby MALDI-ISD

MALDI-ISD is a top-down proteomics approach that breaks inter-residue bonds of the target protein, which mainly cleaves the N-Cα bond of the main chain, and obtaining molecular and fragment ion mass data by ionizing and dissociating a protein in the mass spectrometer. The mass difference between the generated series of consecutive fragment ions is used to identify the N/C-terminal sequence of the target protein.

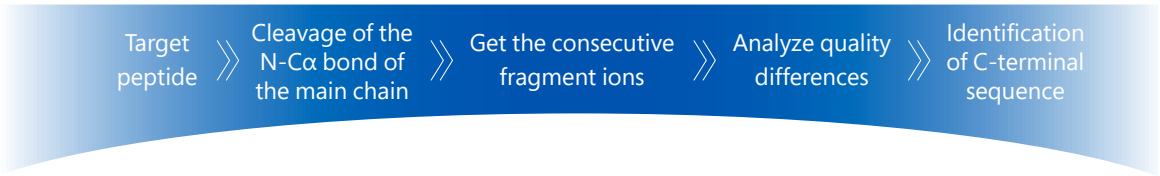


Fig 3. Process of protein N/C-termini identification based on MALDI-ISD

Technical Features

- Enables the sequencing of protein with blocked N-terminus
- Low sample volume
- High precision and accurate results

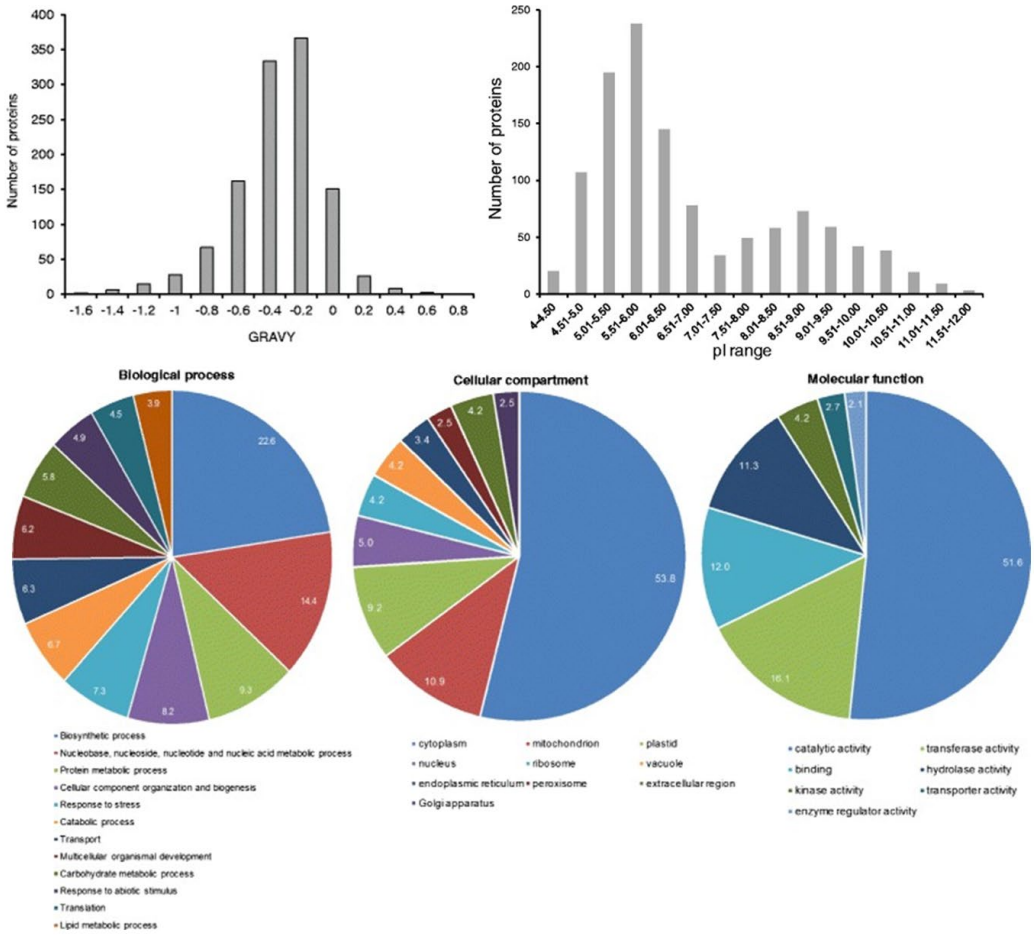
Scope of Application: Suitable for all types of N/C-terminal sequence detection

Application Cases

Case 1. Analysis of protein profiles for different barley seeds

Barley seed proteins are important for brewing, human and animal nutrition, and plant breeding variety identification. In order to obtain comprehensive proteomic data from seeds, total proteins of two-rowed (Conrad) and six-rowed (Lacey) were precipitated in acetone, digested in solution, and resulting peptides were analyzed utilizing the nano-liquid chromatography-tandem mass spectrometry coupling method.

A total of 1168 proteins were identified in the two-rowed and six-rowed seeds using a bottom-up, gel-free proteomics strategy. GO analysis indicated that most barley seed proteins had catalytic activity and were associated with carbohydrate metabolism. Protein profiling revealed 20 differential proteins between the two-rowed and six-rowed barley seeds.



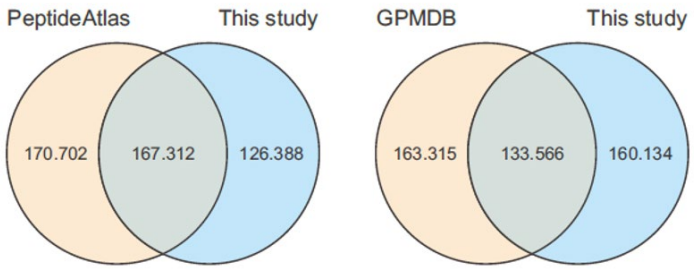
Barley seed shotgun proteomics

Reference: Mahalingam, R. (2017). Shotgun proteomics of the barley seed proteome. BMC genomics, 18(1), 1-11.

Application Cases

Case 2 Drafting the human proteome

In this study, 30 samples of different tissues and organs (17 adult tissues, 7 fetal tissues, 6 primary purified hematopoietic cells) from normal humans were subjected to deep proteomic resolution using the LC-MS/MS technique. As a result, a total of 17,294 proteins were identified, representing 84% of all human genes with annotated coding proteins. This study used both proteomics and genomics in the identification of new coding regions (previously considered non-coding regions). Meanwhile, this vast amount of proteomic data will complement the existing genomic and transcriptomic data to accelerate biomedical research in health and disease.



Comparison of identification results with the PeptideAtlas and GPMDB databases

Reference: Kim, M. S., Pinto, S. M., et al. (2014). A draft map of the human proteome. Nature, 509(7502), 575-581.

Stay in Contact

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