OPEN ACCESS Freely available online

Journal of Proteomics and Bioinformatics

Perspective

Shotgun Proteomics: A Laboratory Method for Determining the Identities of Proteins in a Mixture

Waleed Abdou*

Department of Cardiology, Menoufia University, Menofia Governorate, Egypt

DESCRIPTION

Shotgun proteomics is a technique that uses a combination of high-performance liquid chromatography and mass spectrometry to detect proteins in complicated mixtures utilising bottom-up proteomics approaches. The name comes from shotgun DNA sequencing, which is named after a shotgun's rapidly expanding, quasi-random discharge pattern. The most typical shotgun proteomics method begins with the digestion of the proteins in the mixture, followed by the separation of the peptides using liquid chromatography. The peptides are then identified using tandem mass spectrometry. In the field of bottom-up proteomics, targeted proteomics using SRM and data-independent collection methods is frequently regarded an alternative to shotgun proteomics. The aforementioned approaches use a deterministic method for acquiring fragment ion scans, whereas shotgun proteomics uses data-dependent selection of precursor ions to generate fragment ion scans.

Shotgun proteomics arose from the difficulty of separating complex combinations using previous approaches. O'Farrell and Klose described two-dimensional polyacrylamide gel electrophoresis claiming that technique could resolve complicated protein combinations. The area of proteomics continued to expand with the introduction of matrix-assisted laser desorption ionisation (MALDI), electrospray ionisation (ESI), and database searching. Low-abundance proteins, abnormal proteins, and membrane proteins were all difficult to identify and separate using these approaches. Shotgun proteomics was discovered for resolving even these proteins. Shotgun proteomics enables for global protein identification as well as the profiling of dynamic proteomes in a systematic manner. It also avoids the poor mass spectral sensitivity and low separation efficiency associated with intact protein analysis. Shotgun proteomics commonly uses dynamic exclusion filtering to increase the number of discovered proteins at the expense of random sampling. The problem may be compounded by shotgun proteomics' intrinsic under sampling.

Cells with the desired protein complement are produced. The mixture's proteins are then isolated and processed with a protease to form a peptide mixture. The peptide mixture is then placed directly onto a micro capillary column, where hydrophobicity and charge are used to separate the peptides. In the first stage of tandem mass spectrometry, the peptides are ionised and separated by m/z as they elute from the column. Collision-induced dissociation or another action causes fragmentation of the targeted ions. In the second stage of tandem mass spectrometry, the charged fragments are separated. By searching against a sequence database with commercially accessible software like SEQUEST or MASCOT, the "fingerprint" of each peptide's fragmentation mass spectrum is utilised to identify the protein from which it derives. The Genpept database and the PIR database are two examples of sequencing databases. Each peptide-spectrum match must be assessed for validity after the database search. Researchers can use this approach to profile a variety of biological systems.

Degenerate peptides make it difficult to determine which protein they belong to without a doubt. Furthermore, certain vertebrate proteome samples have a considerable number of paralogs, and alternative splicing in higher eukaryotes can result in a huge number of identical protein subsequences. Furthermore, many proteins are altered either spontaneously (post-translational modifications) or intentionally (sample preparation artefacts). This complicates the identification of the peptide sequence using traditional database matching methods. This, combined with peptide fragmentation spectra of poor quality or high complexity, leaves many sequencing spectra unidentifiable in a traditional shotgun proteomics experiment.

CONCLUSION

After the human genome has been sequenced, all predicted genes and their protein products must be verified and functionally annotated. Shotgun proteomics can be used to classify and compare these protein products based on their functions. It can be utilised in a variety of applications, from large-scale whole-proteome studies to studies focused on a single protein family. It might be carried out in research labs or in a commercial setting.

Correspondence to : Waleed Abdou, Department of Cardiology, Menoufia University, Menofia Governorate, Egypt, E-mail: waleedabdou@12yahoo.com

Received: 01- Feb-2022, Manuscript No. JBP-22-16034; **Editor assigned**: 03-Feb-2022, PreQC No. JBP-22-16034 (PQ); **Reviewed:** 17-Feb-2022, QC No. JBP-22-16034; **Revised:** 23-Feb-2022, Manuscript No. JBP-22-16034 (R); **Published:** 03-Mar-2022, DOI: 10.35248/ 0974-276X.22.15.570.

Citation: Abdou W (2022) Shotgun Proteomics: A Laboratory Method for Determining the Identities of Proteins in a Mixture. J Proteomics Bioinform.15:570.

Shotgun proteomics was to link protease inhibition and complement activation to high-density lipoprotein's anti-inflammatory effects. Lee et al. found that human hepatoma HepG2 cells expressed higher levels of hnRNP A2/B1 and Hsp90 than wild type cells in a study. This prompted a search for previously described functional activities mediated by both of these multifunctional cellular chaperones.