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**Protocol status:** Working  
We use this protocol and it's working

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## Proteome analysis

Leonardo A Parra-Rivas<sup>1</sup>

<sup>1</sup>University of California, San Diego

Subhojit Roy Lab



Leonardo A Parra-Rivas  
University of California, San Diego

### ABSTRACT

Proteome analysis

- 1 Mass spectrometry data was analyzed according to a published protocol

#### CITATION

Wingo TS, Duong DM, Zhou M, Dammer EB, Wu H, Cutler DJ, Lah JJ, Levey AI, Seyfried NT (2017). Integrating Next-Generation Genomic Sequencing and Mass Spectrometry To Estimate Allele-Specific Protein Abundance in Human Brain..

LINK

<https://doi.org/10.1021/acs.jproteome.7b00324>

- 2 Spectra were searched using Proteome Discoverer (RRID:SCR\_014477) (<https://www.thermofisher.com/order/catalog/product/IQLAEGABSAKJMAUH>) software version 2.1 against 2020 mouse UniProtKB/Swiss-Prot (RRID:SCR\_021164) (<https://www.expasy.org/resources/uniprotkb-swiss-prot>) (17,042 target sequences) along with the human  $\alpha$ -synuclein protein sequence.
- 3 Searching parameters included full tryptic or Asp-N restriction, precursor mass tolerance ( $\pm 20$  ppm), and fragment mass tolerance ( $\pm 0.05$  Da). Serine, threonine, and tyrosine phosphorylation (+79.9663 Da), methionine oxidation (+15.99492 Da), asparagine and glutamine deamidation (+0.98402 Da), and protein N-terminal acetylation (+42.03670 Da) were variable modifications (up to 3 allowed per peptide); cysteine was assigned a fixed carbamidomethyl modification (+57.021465 Da).
- 4 Percolator was used to filter the peptide spectrum matches to a false discovery rate of 1%.
- 5 Gene ontology was analyzed using The Database for Annotation, Visualization and Integrated Discovery DAVID (RRID:SCR\_001881) (<https://david.ncifcrf.gov/tools.jsp-DAVID>)<sup>97</sup>.

- 6** Biological processes involving synaptic function were selected for grouping analysis. Functional grouping was based on Fisher's exact test ( $p < 0.05$ ). Protein-protein interaction networks were then identified using the STRING (RRID:SCR\_005223) (<https://string-db.org/>) version 11.5, and the protein class was determined using PANTHER (RRID:SCR\_004869) (<http://www.pantherdb.org/>).