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Proteome analysis

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Protocol status: Working

We use this protocol and it's working

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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.

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

LINK

- 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 α -synuclein protein sequence.
- 3 Searching parameters included full tryptic or Asp-N restriction, precursor mass tolerance (± 20 ppm), and fragment mass tolerance (± 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>)⁹⁷.



- 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/>).

Citations

Step 1

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

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