NOV 19, 2022

WORKS FOR ME 1

Untargeted metabolomics analysis for Golgi immunopurification (Golgi-IP)

COMMENTS 0

DOI
dx.doi.org/10.17504/protocols.io.n2bvj83exgk5/v1

Wentao Dong[1], Eshaan S Rawat[1], Monther Abu-Remaileh[1]

[1]Department of Chemical Engineering, Department of Genetics, The Institute for Chemistry, Engineering & Medicine for Human Health (ChEM-H), Stanford University, Stanford, CA 94305, USA.

Monther Abu-Remaileh: monther@stanford.edu

M Monther Abu-Remaileh

ABSTRACT

The Golgi apparatus functions as a central hub in the cell that processes, packages, and distributes proteins. Despite its critical cellular function, there has been challenges to quantitatively assess Golgi metabolite profiles. To overcome this hurdle, we developed a rapid harvesting and purification method using immunoprecipitation (GolgiIP). This protocol provides details for analyzing GolgiIP metabolomics samples using liquid chromatography mass spectrometry (LC-MS) for polar metabolite profiling.

DOI

dx.doi.org/10.17504/protocols.io.n2bvj83exgk5/v1

PROTOCOL CITATION

Wentao Dong, Eshaan S Rawat, Monther Abu-Remaileh 2022. Untargeted metabolomics analysis for Golgi immunopurification (Golgi-IP). **protocols.io** https://dx.doi.org/10.17504/protocols.io.n2bvj83exgk5/v1

FUNDERS ACKNOWLEDGEMENT

Aligning Science Across Parkinson's
Grant ID: ASAP-000463

NIH
Grant ID: DP2-CA271386

KEYWORDS

Golgi, immunoprecipitation, metabolomics, lipidomics

CREATED

Nov 18, 2022

LAST MODIFIED

Nov 19, 2022

PROTOCOL INTEGER ID

72963

MATERIALS TEXT

Reagents

- Optima LC/MS water (Fisher, cat. no. W6-4)
- Optima LC/MS acetonitrile (Fisher, cat. no. A955-4)
- Ammonium carbonate
- Ammonium hydroxide
- EASYIC^{TM}

Equipment

- ID-X Orbitrap Tribrid Mass Spectrometer (Thermo Fisher Scientific) with an electrospray ionization (ESI) probe
- SeQuant® ZIC®-pHILIC 150 x 2.1 mm column (Millipore Sigma 1504600001)
- 20 x 2.1 mm guard (Millipore Sigma 1504380001)

SAFETY WARNINGS

Please refer to Safety Data Sheets (SDS) for health and environmental hazards.

LC/MS metabolomics settings

1 Set an ID-X tribrid mass spectrometer (Thermo Fisher Scientific) with an electrospray ionization (ESI) probe, for initial polar metabolite profiling.

Prepare a SeQuant® ZIC®-pHILIC 150 x 2.1 mm column (Millipore Sigma 1504600001) coupled with a 20 x 2.1 mm (Millipore Sigma 1504380001) guard, to carry out hydrophilic interaction chromatography (HILIC) for metabolite separation prior to mass spectrometry. Use EASYIC^{TM} for internal calibration.

2 For HILIC metabolite separation, use [M] 20 millimolar (mM) ammonium carbonate and [M] 0.1 % (v/v) ammonium hydroxide dissolved in [M] 100 % (v/v) LC/MS grade water for Buffer A, and [M] 100 % (v/v) LC/MS grade acetonitrile for Buffer B.

3 Run the chromatographic gradient at a flow rate of 0.150 mL/min. Operate the mass spectrometer in full-scan, polarity-switching mode at *m/z* 70-1000, with Orbitrap resolution set at 120,000, RF lens at 40%, AGC target at 1x10^6, and maximum injection time at 80 ms. Set positive ion voltage to 3000 V, negative ion voltage to 2500 V, ion transfer tube temperature to 275 °C, and vaporizer temperature to 350 °C. Set sheath gas flow to 40 units, auxiliary gas flow to 15 units, and sweep gas flow to 1 unit.

Citation: Wentao Dong, Eshaan S Rawat, Monther Abu-Remaileh Untargeted metabolomics analysis forÃÂ Golgi immunopurification (Golgi-IP) https://dx.doi.org/10.17504/protocols.io.n2bvj83exgk5/v1

Untargeted metabolomics workflow

4 For unbiased differential analysis, extract ion chromatograms using Compound Discoverer (Thermo Fisher Scientific) with a mass tolerance of 5 ppm.

Rigorously quantify metabolite abundance using TraceFinder (Thermo Fisher Scientific) in conjunction with an in-house library of known metabolite standards (MSMLS, Sigma-Aldrich).

5 Compound Discoverer (Thermo Fisher Scientific) was used for initial unbiased differential analysis. In addition to online databases,we also included a local library with both masslist and mzVault spectral archives. Mass tolerance for untargeted discovery, 10 ppm; minimum and maximum precursor mass, 0-5,000 Da; retention time limit, 0-20 min; Peak filter signal to noise ratio, 1.5; retention time alignment maximum shift, 0.5 min; minimum peak intensity, 10,000; compound detection signal to noise ratio, 3. Isotope and adduct settings were kept at default values. Gap filling and background filtering were performed by default settings. Area normalization was performed by constant median after blank exclusion. Compound annotation priority: #1, MassList Search; #2, mzVault Search; #3, mzCloud Search; #4, Predicted Compositions; #5, Chemspider Search; #6, Metabolika Search.

6 The MassList Search was customized with 5 ppm mass tolerance and 1 minute retention time tolerance. The mzVault Search was customized with 10 ppm precursor and fragment mass tolerance and 1 minute retention time tolerance. The mzCloud Search was customized with 10 ppm precursor and fragment mass tolerance. The other searches were performed with default parameters specified in the default workflow “Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic” provided by Compound Discoverer.

7 Raw features were further filtered by the following algorithm: 1) MS2 fragmentation spectra were obtained, and 2) at least 1 annotation match in the mzVault, mzCloud or Chemspider Search. To further improve the rigor of our discovery workflow, we performed additional manual filtering based on the following criteria: 1), features with retention time earlier than 3 minutes on this HILIC column, which are nonpolar and should be quantified by a C18 column, were removed, 2) features with predicted compositions containing chemical elements rarely found in human metabolome (e.g. certain halogens) were removed, and 3) features enriched in the Golgi from only one independent experiment were removed.

8 Rigorous quantification of metabolite abundance was performed by TraceFinder (Thermo Fisher Scientific) in conjunction with an in-house library of known metabolite standards (MSMLS, Sigma-Aldrich). Isotopically labelled amino acids were used as internal standards. Mass tolerance for extracting ion chromatograms, 5 ppm.

Citation: Wentao Dong, Eshaan S Rawat, Monther Abu-Remaileh Untargeted metabolomics analysis forÃÂ Golgi immunopurification (Golgi-IP) https://dx.doi.org/10.17504/protocols.io.n2bvj83exgk5/v1