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Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units

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Human BioMolecular Atlas Program (HuBMAP) Method Development Community

PNNL-TTD

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ABSTRACT

The protocol describes how to use laser capture microdissection (LCM) to cut small regions of interest (~200-300 µm) from tissue sections. This is followed by top down proteomics analysis by liquid chromatography - mass spectrometry (LC-MS).

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PROTOCOL CITATION

James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic 2022. Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units. **protocols.io** https://protocols.io/view/top-down-proteomics-data-collection-for-microdisse-b8aarsae

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OWNERSHIP HISTORY

Apr 26, 2022 Kevin J. Zemaitis

Aug 12, 2022  Mowei Zhou Pacific Northwest National Laboratory

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61474

PARENT PROTOCOLS

In steps of

Overall protocol for MicroPOTS LCMS top down proteomics of kidney tissue sections

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

MATERIALS TEXT

- LC solvents

Mobile phase A (MPA): 0.2% formic acid in water (LCMS grade)
Mobile phase B (MPB): 0.2% formic acid in acetonitrile (LCMS grade)

- Instrumentation

NanoAcquity
liquid chromatography
Waters **186016002**
Dual pump configuration with autosampler
186016007

Orbitrap Lumos
Mass spectrometer
Thermo **IQLAAEGAAPFADBMBHQ**

- QC sample

Shewanella oneidensis MR-1 cell culture
Homogenization buffer (HB) : 8M urea solution (480 mg/mL) in 50 mM ammonium bicarbonate with 15 mM TCEP
Wash Buffer (WB): 0.2% formic acid, 5% acetonitrile (LC-MS grade solvents)

Liquid chromatography (LC) method setup

1 Set up reversed-phase LC system with online trapping for desalting.

Dual pump configuration
Mobile phase A (MPA): 0.2% formic acid in water (LCMS grade)
Mobile phase B (MPB): 0.2% formic acid in acetonitrile (LCMS grade)

NanoAcquity
liquid chromatography
Waters **186016002**
Dual pump configuration with autosampler
186016007

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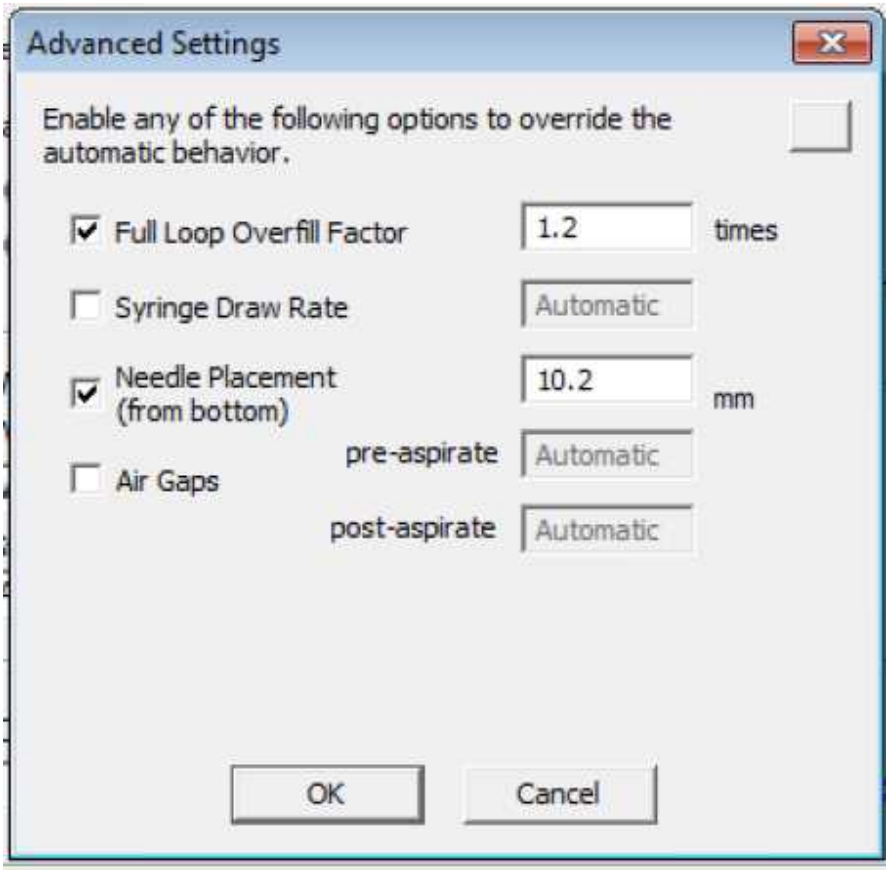
1.1 ⚠

Prepare the method for autosampler for microPOTS samples.

For samples processed by the microPOTS protocol cited below, the LC vials will hold PCR tubes inside. The height of the syringe in the autosampler must be adjusted to avoid damage to the needle. This can be accessed within the nanoACQUITY Sample Manager Software. Select the autosampler and the "advanced" options. Under this tab, check the "Needle Placement (from bottom)" box. Adjust the needle placement to 10.2 mm from the bottom of the LC vial.



Laser Capture Microdissection of Tissue Functional Units for microPOTS Top-Down Proteomics
by james.fulcher

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

1.2 Set up gradient method for samples. 10m

Wash pump: 5 µL/min, 95% MPA, 5% MPB. Loading time 10 min.

Gradient pump: 0.3 µL/min
0 min: 95% MPA, 5% MPB
1 min: 90% MPA, 10% MPB
90 min: 40% MPA, 60% MPB
100 min: 95% MPA, 5% MPB

Mass spectrometer (MS) method setup

2 Calibrate and set up the mass spectrometer method for sample runs.

2.1 Perform both mass and system calibration following instrument vendor's recommendation. 30m

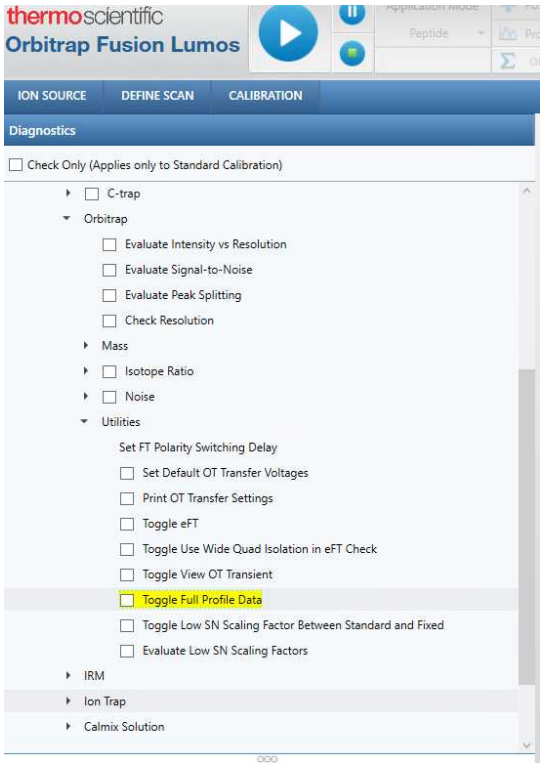
At minimum, "positive polarity" and "Orbitrap mass" calibrations need to be completed. System calibration is strongly recommended to ensure good performance for "Intact protein mode" and ETD.

2.2 ⊛ 1m

(Optional) Turn on full profile mode.

Under "Diagnosis" - "System" - "Orbitrap" - "Utilities", check "Toggle Full Profile Data" before starting the queue.

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1



Full profile mode will generate ~10 GB files per 100 min run. The raw data will save all the baseline signal (including noise), which may increase the likelihood of capturing low abundance species. Remember to toggle off the full profile mode after the queue to reset the instrument for regular experiments.

2.3 Set up the data dependent acquisition method with the following parameters.[5m] Please note that the library methods had 7x more material than the samples for generating high quality MS2 data as "library" for match-between-runs and improve coverage for sample runs. The MS setting was set to have higher numbers of MS2 in the library runs, and lower numbers of MS2 for sample runs.

Kidney sample method

Orbitrap Fusion Lumos Method Summary

Global Settings
Use Static Source Gasses
Use Ion Source Settings from Tune = Checked
Method Duration (min)= 100
Spray Voltage = Static

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

Gas Mode = Static
Infusion Mode (LC)= False
FAIMS Mode = Not Installed
Application Mode = Intact Protein
Pressure Mode = Low Pressure
Default Charge State = 6
Advanced Peak Determination = True
Experiment 1
Experiment Name = MS
Start Time (min) = 0
End Time (min) = 100

Scan MasterScan
Desired minimum points across the peak = 6
MSn Level = 1
Use Wide Quad Isolation = True
Detector Type = Orbitrap
Orbitrap Resolution = 120K
Mass Range = Normal
Scan Range (m/z) = 500-2000
Maximum Injection Time (ms) = 500
AGC Target = 1000000
Normalized AGC Target = 250%
Microscans = 4
Maximum Injection Time Type = Custom
RF Lens (%) = 30
Use ETD Internal Calibration = False
DataType = Profile
Polarity = Positive
Source Fragmentation = True
Energy (V) = 15
Scan Description =
Enhanced Resolution Mode = Off

Filter ChargeState
Include charge state(s) = 3-35
Include undetermined charge states = False

Filter DynamicExclusion
Exclude after n times = 1
Exclusion duration (s) = 30
Mass Tolerance = mz
Mass tolerance low = 1
Mass tolerance high = 1
Use Common Settings = False
Exclude isotopes = True

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

Perform dependent scan on single charge state per precursor only = True

Data Dependent Properties
Data Dependent Mode= Number of Scans
Number of Dependent Scans= **1 (for sample run) or 8 (for library run)**
Scan Event 1

Scan ddMSnScan
Desired minimum points across the peak = 6
MSn Level = 2
Isolation Mode = Quadrupole
Enable Intelligent Product Acquisition for MS2 Isolation = False
Isolation Window = 2
Isolation Offset = Off
Reported Mass = Original Mass
Multi-notch Isolation = False
Scan Range Mode = Auto
Scan Priority= 1
Collision Energy Mode = Fixed
ActivationType = CID
Collision Energy (%) = 35
Activation Time (ms) = 10
Activation Q = 0.25
Multistage Activation = False
Detector Type = Orbitrap
Orbitrap Resolution = 60K
Maximum Injection Time (ms) = 200
AGC Target = 500000
Inject ions for all available parallelizable time = False
Normalized AGC Target = 1000%
Microscans = 1
Maximum Injection Time Type = Custom
Use ETD Internal Calibration = False
DataType = Profile
Polarity = Positive
Source Fragmentation = False
Scan Description =
Time Mode = Unscheduled
Enhanced Resolution Mode = Off

Instrument Quality Control (QC) and method setup

3 A QC standard is used to evaluate instrument performance before starting samples. Herein we use a bacterial lysate established in our lab (see reference below for more information), other samples can be used as QC as well.

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

Shen Y, Tolić N, Piehowski PD, Shukla AK, Kim S, Zhao R, Qu Y, Robinson E, Smith RD, Paša-Tolić L (2017). High-resolution ultrahigh-pressure long column reversed-phase liquid chromatography for top-down proteomics.. Journal of chromatography. A. https://doi.org/10.1016/j.chroma.2017.01.008

QC Sample information

Intact protein lysate from cultured Shewanella oneidensis MR-1 cells

Buffer preparation:
Homogenization buffer (**HB**) : 8M urea solution (480 mg/mL) in 50 mM ABC with 15 mM TCEP
Note: Use BondBreaker 0.5 M TCEP stock solution

Wash Buffer (**WB**): 0.2% formic acid, 5% acetonitrile
Note: Use LC-MS grade water

NOTE: Adjust centrifugal filtration speeds and times as appropriate for your sample type and filter size. It is recommended to do all spin steps at **10 °C** (8 M urea will freeze at **4 °C**).

1. Lyse cells or homogenize tissue in homogenization buffer (HB).
2. Incubate sample at room temperature for 30 min to extract and denature proteins
3. Centrifuge lysate at 14,000 x G, 10C for 10 minutes to pellet cell debris
4. Transfer supernatant to 100K MWCO filter and centrifuge at 14,000 x G until minimum volume is reached.
5. Wash 100K spin filter with 1X max volume of HB, spin at 14,000 x G until minimum volume is reached.
6. Transfer filtrate from 100K filter to a fresh 10K filter and centrifuge at 14,000 x G for time needed to get to minimum volume.
   a. If needed, add multiple aliquots of filtrate from 100K filter to the same 10K filter
7. Wash 10K filter three times with wash buffer (WB) and spin to minimum volume each wash.
8. Perform Coomassie or BCA protein assay.
9. Dilute sample to 0.01 ug/uL in WB and aliquot 100 uL into separate 0.6 mL Eppendorf tubes with labels.

3.1 QC LCMS method

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

LC method - same as sample

MS method

Global Settings
Use Static Source Gasses
Use Ion Source Settings from Tune = Checked
Method Duration (min)= 90
Spray Voltage = Static
Gas Mode = Static
Infusion Mode (LC)= False
FAIMS Mode = Not Installed
Application Mode = Intact Protein
Pressure Mode = Low Pressure
Default Charge State = 10
Advanced Peak Determination = True
Experiment 1
Experiment Name = MS
Start Time (min) = 0
End Time (min) = 90

Scan MasterScan
Desired minimum points across the peak = 6
MSn Level = 1
Use Wide Quad Isolation = True
Detector Type = Orbitrap
Orbitrap Resolution = 120K
Mass Range = Normal
Scan Range (m/z) = 500-2000
Maximum Injection Time (ms) = 400
AGC Target = 1000000
Normalized AGC Target = 250%
Microscans = 2
Maximum Injection Time Type = Custom
RF Lens (%) = 30
Use ETD Internal Calibration = False
DataType = Profile
Polarity = Positive
Source Fragmentation = True
Energy (V) = 15
Scan Description =
Enhanced Resolution Mode = Off

Filter ChargeState

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

Include charge state(s) = 4-35
Include undetermined charge states = False

Filter DynamicExclusion
Exclude after n times = 1
Exclusion duration (s) = 30
Mass Tolerance = mz
Mass tolerance low = 1
Mass tolerance high = 1
Use Common Settings = False
Exclude isotopes = True
Perform dependent scan on single charge state per precursor only = True

Data Dependent Properties
Data Dependent Mode= Number of Scans
Number of Dependent Scans= 4
Scan Event 1

Scan ddMSnScan
Desired minimum points across the peak = 6
MSn Level = 2
Isolation Mode = Quadrupole
Enable Intelligent Product Acquisition for MS2 Isolation = False
Isolation Window = 2
Isolation Offset = Off
Reported Mass = Original Mass
Multi-notch Isolation = False
Scan Range Mode = Define m/z range
Scan Priority= 1
Collision Energy Mode = Fixed
ActivationType = CID
Collision Energy (%) = 35
Activation Time (ms) = 10
Activation Q = 0.25
Multistage Activation = False
Detector Type = Orbitrap
Orbitrap Resolution = 60K
Scan Range (m/z) = 400-2000
Maximum Injection Time (ms) = 200
AGC Target = 500000
Inject ions for all available parallelizable time = False
Normalized AGC Target = 1000%
Microscans = 2
Maximum Injection Time Type = Custom
Use ETD Internal Calibration = False
DataType = Profile

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Polarity = Positive
Source Fragmentation = False
Scan Description =
Time Mode = Unscheduled
Enhanced Resolution Mode = Off

Data collection and QC Metric

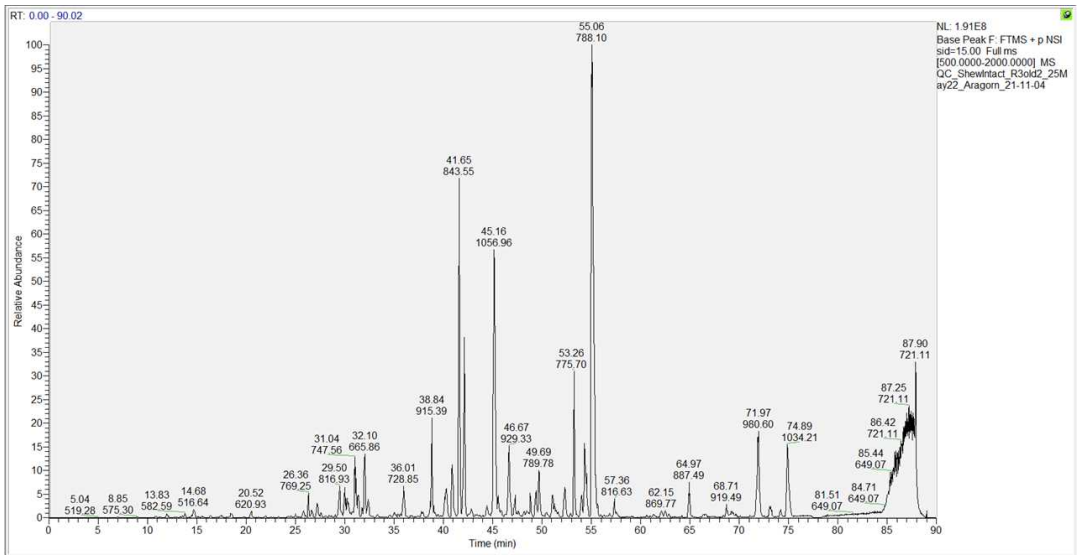
4 Queue the QC before starting the samples using the LCMS method described in section 3. QC runs need to pass the metrics defined below. Once passed, queue the sample runs using the LCMS method described in section 2.

4.1 Evaluation of QC data (*Shewanella* lysate from section 3)

To quickly evaluate the QC data, open them in the Thermo Scientific Freestyle software or Xcalibur QualBrowser. The following metrics are inspected to ensure that the LCMS run meets the expected standard.

1. Signal levels
The intensity levels at the total ion current (TIC), base peak intensity (BPI), and the MS2 spectra are inspected to ensure that they meet the expected intensity levels. The TIC intensity levels are typically expected to be above the 1e10 level, BPI at or above 1e8 level, and the MS2 spectra ion current from 1e5 to 1e7 levels.
2. Chromatography
2a.
We look at the chromatography distribution using the base peak FTMS scan as filter to ensure that the eluted peaks are well distributed through the LC run. The chromatogram is filtered to show the BPI peaks and inspected to ensure no peak broadening (early or late) is observed.

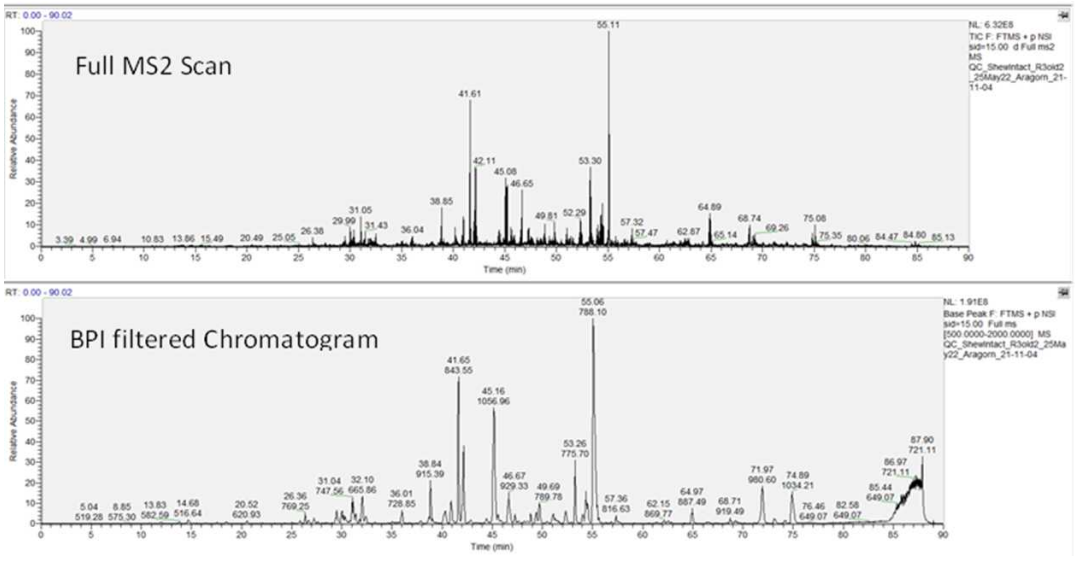
Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1



Chromatogram filtered using the BPI to show the peak distribution.

2b.

The distribution of the MS2 peaks (fragmentation of peaks selected at the MS1 level) is inspected to evaluate how similar the distribution is to that of the MS1 level. The MS2 distribution is expected to emulate what is observed for the peak distribution of the MS1 when the BPI filter is applied, indicating that peak selection for fragmentation was performed at an appreciable level. To assess the MS2 fragmentation signal, the “full MS2” is applied as the filter to show the MS2 distribution through the whole experiment.



2c.

Finally, QC samples are analyzed with TopPIC to ensure appropriate number of proteoforms and proteoform spectrum matches (PrSMs) are being identified.

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1

TopPIC Suite 1.4.13.1

source by Xiaowen Liu

Proteoforms are counted by opening the exported "..._proteoforms.tsv" file and PrSMs through the "..._PrSMs.tsv" file. The QC passing threshold for proteoforms is 1,000 and 2,000 for PrSMs.

Quality Assurance (QA) of HubMAP Samples

5 Perform proteoform identification using the "TopPIC processing" section in the following protocol.

James M Fulcher, Yen-Chen Liao, Mowei Zhou, Ljiljana.PasaTolic. Proteoform Identification and Quantitation with TopPIC and TDPortal for Human Tissues.
http://dx.doi.org/10.17504/protocols.io.3byl4bpj2vo5/v1

5.1 Using the TopPIC PrSM results (after TopPICR post-processing), kidney samples are filtered based on the total number of PrSMs. A cutoff of 100 PrSMs was used to remove samples that were of lower quality.

Citation: James M Fulcher, Isaac Kwame Attah, Mowei Zhou, Ljiljana.PasaTolic Top Down Proteomics Data Collection for Microdissected Kidney Tissue Functional Units https://dx.doi.org/10.17504/protocols.io.rm7vzy5e5lx1/v1