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LCM-NanoPOTS workflow for spatial proteome mapping

Forked from LCM-NanoPOTS workflow for spatial proteome mapping

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We use this protocol and it's working

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Abstract

Spatial proteomics holds great potential to transform our understanding of the role of cell populations and their spatial organizations in human tissues related to diseases. However, in-depth proteome measurement with high spatial resolution has been challenged by the low sample input and inefficient proteomics workflow.
In this protocol, we outlined the detailed procedure for high-resolution analysis of human pancreas tissue sections using a spatial proteomic platform by combining laser capture microdissection (LCM), nanoPOTS (nanodroplet processing in one pot for trace samples), and nanoflow liquid chromatography mass spectrometry (LC-MS). This protocol has been applied to human pancreas specimens for HubMAP Tissue Mapping Centers.

Expected outcome:
The unbiased spatial proteome mapping of >3000 proteins at 50-μm spatial resolution depending on sample types and LC-MS instrumentation.

Materials

1. Reagents

1.1. LCM collection

- Dimethyl sulfoxide (DMSO)

1.2. Proteomic sample processing

- Nanopure water
- n-Dodecy-ß-D-maltoside (DDM) (e.g. Thermo Fisher Scientific Cat# 89902)
- HEPES (pH 8.5)
- TCEP (tris(2-carboxyethyl)phosphine)-HCL (Thermo Fisher scientific, Cat# A35349)
- Chloroacetamide (CAA) (Thermo Fisher Scientific, Cat# A39270)
- Lys-C, (Promega, cat. no. V1671)
- Trypsin, (Promega, cat. no. V5280)
- Formic acid

2. Equipment

- Laser Capture Microdissection System (Zeiss PALM MicroBeam)
- Home-built nanoPOTS sample preparation system
- Home-built nanoPOTS LC autosampler system
- LC-MS/MS system (Orbitrap Eclipse Tribrid MS, Thermo Scientific)

3. Buffer preparation

3.1. Extraction buffer (Desired conc.: 1 mM TCEP, 0.1% DDM, 0.1 M HEPES)

- 10 µL, 1% DDM (Aliquoted 1% DDM is stored in -20 °C)
- 88 µL, 0.1 M HEPES (pH 8.5)
    - Prepare 0.1 M HEPES using premade 0.1 HEPES.
    - Check pH before use 0.1 M HEPES
- 2 µL, 50 mM TCEP
    - No-Weigh, single-use TCEP, 1 mg (stored in 4 °C)
    - Dissolve TCEP with 70 µL of 0.1 M HEPES
    - Vortex several times ~5 min to make sure it is completely dissolved

3.2. Alkylation buffer (Desired conc.: 10 mM CAA in 0.1 M HEPES)

- No-Weigh, single-use CAA, 2 mg (stored in 4 °C)
- Dissolve CAA with 214 µL 0.1 M HEPES to obtain 0.1 M CAA
- Dilute to 10 mM CAA with 0.1 M HEPES

3.3. Digestion buffer (Desired conc.: 0.01 ng/nL Lys-C and 0.04 ng/nL Trypsin)

- 10 µL, 0.1 ng/nL Lys-C (Aliquoted in -80 °C)
- 10 µL, 0.4 ng/nL Trypsin (Aliquoted in -80 °C)
- 80 µL, 0.1 M HEPES

Laser capture microdissection (LCM)

1 Before cut and collection: Load DMSO as capturing media on the nanoPOTS chip

1.1 Set the nanoPOTS robot to be ready (Temperature, humidity).

1.2 Align the nanoPOTS chip.

1.3 Set 100 µL of DMSO (Dimethyl sulfoxide) in the sample plate.

1.4 Dispense 200 nL of DMSO into each well on the nanoPOTS chip

1.5 Cover the chip with the glass slide and cover with aluminum foil.

2 Cut and tissue collection

2.1 Turn on the LCM (PALM MicroBeam).

2.2 Scan the tissue slide.

2.3 Find the target region on scanned image

2.4 Navigate to the region of interest (ROI).

2.5 Draw 4 × 9 grid lines (1 voxel size= 2500 μm^2) on ROI.

2.6 Load the laser setting (Speed 1, Cut energy 40, LPC Energy delta15).

2.7 Load the nanoPOTS chip on the collector (slide collector 48). Align A1 corner of slide collector and A2 well corner of the nanoPOTS chip.

2.8 Start the collection with CenterRoboLPC function.

2.9 After collecting all voxels, check on the microscope if every DMSO droplet contains the tissue voxel.

2.10 Remove the nanoPOTS chip carefully from thecollector and cover it with the glass slide.

3 After collection: Dry and scan

3.1 Set incubator temperature to 70 °C.

3.2 Place chips upside down (still wrapped) into incubator for 15 min.

3.3 After 15 min, unwrap the chip and place uncovered in incubator for 5 min intervals until DMSO has evaporate (~19 min).

3.4 After DMSO has evaporated, scan the nanoPOTS chip and check if there is any missing well.

3.5 Wrap with foil and store it in -20 °C until analysis.

NanoPOTS Proteomic Sample Processing for LCM tissue

2d

4 Extraction

4.1 Set the nanoPOTS robot to be ready (Temperature, humidity).

4.2 Align the nanoPOTS chip.

4.3 Place 100 µL of extraction buffer (1 mM TCEP (tris(2-carboxyethyl)phosphine), 0.1% DDM (n-Dodecy-ß-D-maltoside), 0.1 M HEPES (pH 8.5)) in the sample plate.

4.4 Dispense 200 nL of the extraction buffer into each well on the nanoPOTS chip.

4.5 Cover chip with the glass slide and cover with aluminum foil.

4.6 Place the chip upside down in the humidity box in a zipper bag at 70 °C for 60 min.

5 Alkylation

5.1 Set chip at RT and put it into nanoPOTS robot.

5.2 Place 100 µL of alkylation buffer in the sample plate.

5.3 Dispense 50 nL of alkylation buffer (10 mM CAA (2-chloroacetamide) in 0.1 M HEPES) into each well.

5.4 Cover chip with glass slide and wrap with foil.

5.5 Place the chip upside down in the dark for 30 min at RT.

6 Digestion

6.1 Set chip into nanoPOTS robot.

6.2 Place 100 µL of digestion buffer in the sample plate.

6.3 Dispense 50 nL of digestion buffer (0.01 ng/nL LysC, 0.04 ng/nL trypsin in 0.1 M HEPES) into each well.

6.4 Cover chip with a glass slide and cover with foil.

6.5 Place the chip upside down in the humidity box for 10 h at 37 °C.

7 Acid Quenching

7.1 Remove chips from incubator and place into nanoPOTS robot.

7.2 Dispense 50 nL of 5% formic acid in water into each well.

7.3 Remove the chip from the nanoPOTS robot and place it into the desiccator until the droplets have evaporated.

7.4 Cover with a sterile glass slide, wrap with foil and store at -20 °C until analysis.

NanoPOTS-LC-MS/MS for spatial proteome mapping

8 In-house assembled nanoPOTS autosampler with an in-house packed SPE column (100 µm i.d., 4 cm, 5 µm, 300 Å C18 material, Phenomenex) and an LC column (50 µm i.d., 25 cm, 1.7 µm, 190 Å C18 material, Waters) heated to 50 using AgileSleeve column heater (Analytical Sales and services, Inc., Flanders, NJ) was used for sample analysis.

9 Samples were dissolved with Buffer A (0.1% formic acid in water) on the chip, then trapped on the SPE column for 5 min. After washing the peptides, samples were eluted at 100 nL/min and separated using a 60-min gradient from 8% to 35% Buffer B (0.1% formic acid in acetonitrile).

10 An Orbitrap Eclipse Tribrid mass spectrometer (Thermo scientific) with FAIMS-pro interface operated in data-dependent acquisition mode was used for all analyses. Peptides were ionized by applying a voltage of 2,400 V.

11 The ionized peptides were fractionated by the FAIMSpro interface using a 3-CV (-45, -60, -75 V) method. Fractionated ions with a mass range 350-1600 m/z were scanned at 120,000 resolution with an IT of 118 ms and an AGC target of 1E6.

12 For the pooled tissue samples for generating a spectral library, a single CV was used for each LC-MS run. Precursor ions with intensities > 1E4 were selected for fragmentation by 30% HCD and scanned in an Ion trap with an AGC of 2E5 and an IT of 86 ms.

Data analysis

13 Use FragPipe (v 17.1, MSFragger v3.4, Philosopher v 4.1.0) for processing (feature detection, database searching and protein/peptide quantification) of all raw files.

14 For database search, use the latest Uniprot human database.

15 Fixed modification: carbamidomethylation of cysteine / Variable modification: Protein N-terminal acetylation, oxidation of methionine.

16 Cleavage enzyme: strict trypsin, peptide length: 7-50, max missed cleavage: 2, FDR 0.01

17 For quantifications, use match between runs (MBR) and MaxLFQ embedded in the FragPipe (minimum ions:1; minimum scans: 3; m/z tolerance 10 ppm; RT tolerance 0.4 min; MBR FDR at ion level 0.05).