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Forked from nPOP

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Human Cell Atlas Method Development Community Andrew Leduc

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ABSTRACT

Protocol for preparing single cells for mass-spec analysis by nPOP using the CellenONE system liquid handling and cell sorting system as described by Leduc et al. DOI. This protocol assumes basic level of familiarity and training on the CellenONE. If there are any questions, please email me! leduc.an@northeastern.edu

PROTOCOL CITATION

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Carrier and Reference preperation

1 Prepare cell pellets of all relevant cell types of at least 500,000 cells. Add 100% DMSO to cells to a cellular concentration of 6000 cells/ul. Incubate cells in DMSO for 20 minutes to lyse cells. Add mass spectrometry grade water to bring solution to 2000 cells/ul.

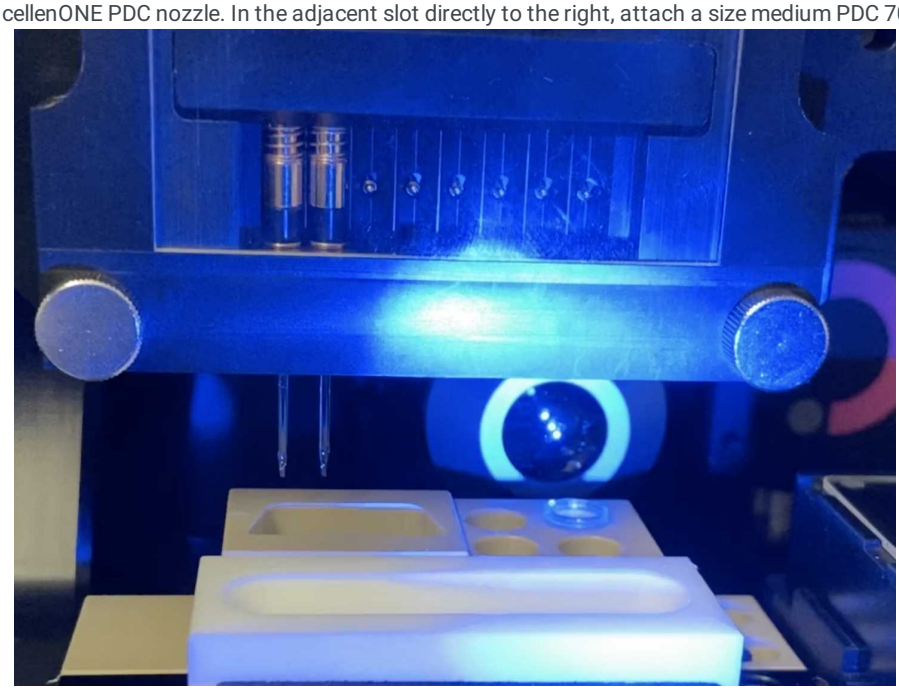
2 Add 1x benzonase, 100mM TEAB and 20 ng/uL of trypsin. Digest sample at 37 degrees C overnight.

3 Label carrier with 126C and reference with 127N. Dilute sample to 200 cells/uL of carrier and 5 cells/uL of reference.

Preparing CellenONE

4 Download the required field files from this link and upload to your CellenONE. The options available are for preparing 572 or 1584 single cells using TMT 16-plex multiplexing reagents. If you are using a different number of multiplexing reagents or performing label free analysis, feel free to use available file as a guide and design own droplet layouts.

5 Follow start up procedure for CellenONE X1 with two nozzles attached. In the far left position, attach a size medium cellenONE PDC nozzle. In the adjacent slot directly to the right, attach a size medium PDC 70 nozzle with type 2 coating.



6 In the target spotting location, place the glass slide holder with four fluorocarbon carbon coated glass slides (CellenONE H1 Slides). In the prob location, place any desired 384 well plate for storing and pickup of cell suspensions and reagents used in sample preparation. On the CellenONE "Main" tab, set the probe location to the relevant 384 well plate and set the target location to dispense on full glass slides.

Preparing cell suspension

7 Suspend cells in 1X PBS at a concentration of 200-300 cells/ul. If cells are prone to clumping together, first filter with a 30 micron filter to ensure fast pace of cell sorting.

DMSO for Cell Lysis

8 Load the DMSO_Lysis field file and ensure that droplet volumes are 5 nl. On the main page, set the task to SpotRun_ImageFields and dispense DMSO. After droplets are imaged and fields are dispensed, check images to ensure DMSO droplets were dispensed consistently for all arrays.

If deviations are noticed, wipe droplets on the relevant slide with a KimWipe and re-dispense for required slide. When removing slide, be careful not to move adjacent slides or this will cause misplacement of cells.

9 Load 20 uL of 100 percent mass spectrometry grade DMSO into any desired well in the 384well plate. Aspirate 10 uL of DMSO with the PDC 70 type 2. Test droplet 3 times to ensure straight and stable stream.

Critical: If you notice residual DMSO on bottom of PDC, this will impact stability of droplet. To resolve this, move PDC to home position and lightly dab bottom of tip with a KimWipe. This will remove DMSO and ensure stream stability.

Dispense droplets for DMSO lysis. If volume of dispensed droplets exceeds 7 uL, dispense half of total spots, flush tip,

10 and repeat step 7.

Cell Dispensing

11 Load the Cells field file and ensure spots correspond to one droplet. If you are dispensing multiple separate cell suspensions, remove a portion of spots from each cluster of 12 to ensure that every set will contain all relevant cell types.

12 On main tab, set run program to CellenONE_Basic. Aspirate 10 ul of your cell suspension from the 384 well plate using the CellenONE PDC.

13 Dispense first cell type.

Repeat steps 11 and 12 for additional cell types

Evaporation Control

14 After cell dispensing is finished, set humidity control to 75 % relative humidity and set cooling control to dew point chase with a deviation of 0 degrees.

15 Load the perimeter field file. Dispense a perimeter of system water to control local evaporation.

Digestion

16 Prepare master mix solution by mixing 20 uL of Promega Trypsin Gold at 200 ng/uL with 1 mM HEPEs buffer at pH 8.5.

17 Aspirate 20 uL of master mix with the PDC type 2 and dispense 10 nL to each spot.

Critical: Make sure to run dip wash immediately after aspirating master mix to ensure stable droplet.

18 Let single cell digestion proceed for 4 hours. Refresh perimeter field every 30-45 minutes throughout digestion to prevent single cell evaporation.

Either PDC should work fine.

19

After digestion turn off humidity and cooling and let single cell peptides dry out on the slide.

This is a pause point. Labeling can proceed the next day as peptide rest dried out on the slide.

Prepare Inserts for Storing Sample

20 Pipette 200 cells worth of combined carrier and reference into the number of mass spectrometry insert vials of planned sets being prepared.

Labeling

21 If multiplexing reagents are stored in Acetonitrile, evaporate off acetonitrile completely in speed vac or lyophilizer. Resuspend labels in 100% DMSO at a concentration of 1/3 maximum strength. For TMT this is a concentration of 29 mM.

Critical: Pipette mix labels vigorously to prevent TMT crystals from forming that can interfere with label dispensing.

22 Load all labels into the 384 well plate and place securely inside CellenONE.

23 Load the field file for the first label. Set the run method to Spot Run. Aspirate 10 ul of the first label and dispense 30 nl for the corresponding spot in each field. Flush remaining label after dispensing. Make sure there is no residual DMSO on the tip of nozzle and brush with Kim wipe to remove any hanging droplets.

Critical: Check droplet stream before and after dispense to ensure the label was accurately dispensed as there will be no imaging validation until end. Imaging each field after each label will significantly increase time for dispensing labels.

24 Repeat step for all subsequent labels and let samples label one hour after last label is dispensed.

Quenching Labeling Reaction

25 Turn on humidity and cooling to previous settings. Step 14.

26 Prepare a solution of 5% hydroxylamine (HA) diluted with mass spectrometry grade water. Add 25 nl of HA to each droplet and incubate for 20 minutes.

27 Repeat step 26.

Sample collection

28 Aspirate 100 ul of Mass spectrometry grade water. Dispense 2 ul into the center of each array.

Critical: Increase frequency parameter for nozzle to 1000 to speed up dispensing.

29 Set a .5 - 10 ul pipette tip to 3 ul and pick up each pooled set one by one dispensing into its own insert vial. Wash each spot with ACN to pick up any residual droplets.

30 Dry down each sample in a speed vacuum for 30 minutes to remove any residual DMSO in the samples and store in -80 until samples are ready to be run on LC/MS.