Massively parallel sample preparation for multiplexed single-cell proteomics using nPOP

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Abstract

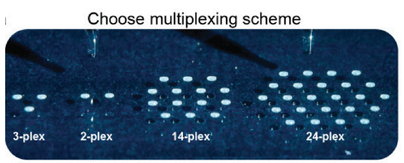
Single-cell proteomics (scP) by mass spectrometry (MS) allows quantifying proteins with high specificity and sensitivity. To increase its throughput, we developed nPOP, a method for parallel preparation of thousands of single cells in nanoliter volume droplets deposited on glass slides. An implementation with plexDIA allowing >1500 cells to be prepared in each experiment demonstrates accurate quantification of about 3,000 - 3,700 proteins per human cell at a rate of over 110 single cells per day. A separate implementation with isobaric mass tags and prioritized data acquisition enabling over 3500 cells to be prepared in each experiment demonstrates analysis of 1,827 single cells at a rate of over 1,000 single cells per day at a depth of 800-1,200 proteins per human cell. The protocol is implemented on the CellenONE instrument and the nPOP Partnership Program is available to new and current cellenONE customers to aid in quick implementation of this powerful method.

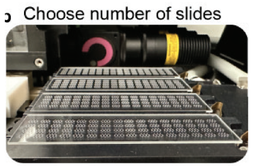
Introduction

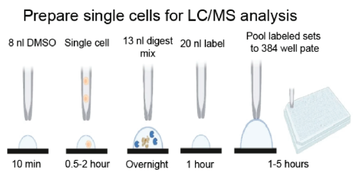
Single cell proteomics by LC/MS is an evolving discipline where new Mass spectrometers and LC tools are emerging that allow more peptides to be detected and more proteins to be identified. One limitation to this technique is the sample cycle time. Most recent papers detailing high sensitivity label free data acquisition have a throughput between 40-80 samples/day (spd) with a large drop (>50%) in Protein ID for 80 spd operation. This means that to process 100s-1000s of single cells, often necessitated by complex biologic inquiries, requires either dedicated LC/MS or 10s of thousands of dollars of core facility costs just to acquire data for each experiment.
To increase scP throughput, we developed nPOP, a method for parallel preparation of thousands of single cells in nanoliter volume droplets on glass slides using the cellenONE single cell and reagent dispenser. The use of an un-patterned surface allows full flexibility of droplets locations. This enables the prep to scale with the changing landscape of MS multiplexing reagents and allows for support of multiple complementary workflows without the need for specialized fabricated microchips. First introduced in Leduc et al. 2022, we have since significantly improved upon the automation and reproducibility of the protocol.

Methods

Because the nPOP method can be performed with various labeling strategies without the exchange of any consumables, the first step is to select the desired multiplexing method for the experiment. These decisions can be made based on available MS, required throughput, depth of coverage desired, cell size, or other factors. Next, select the number of slides to prep which will dictate the total number of cells in the experiment. Follow the onscreen prompts to perform the various process steps.







Improved automation and built in quality controls

nPOP Partnership Program featrures:

Users of nPOP are encouraged to sign up for the nPOP and will receive...

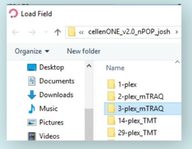
- Access to all the latest software protocols
- End to end protocols from cell suspension generation to basic LC/MS data analysis
- Custom hardware for high throughput robust operation
- Prioritized remote support in event of any issues
- Collaboration for generating custom workflows
- Support on latest workflow from Slavov Lab

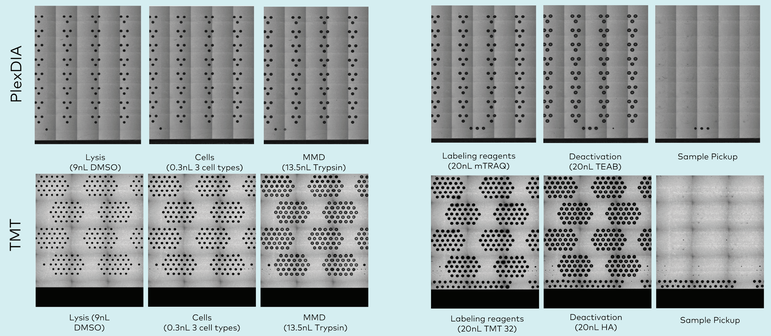
Choose multiplexing scheme:

Considerations (discussed more in Leduc et al. 2024)

- Size of cells
- Heterogeneity of sample
- Intended analysis

Select droplet layout





Day 1: (2-5 hours hands on time)
- Select and clean PDCs and prime the cellenONE (20-30 min)
- DMSO dispensing for cell lysis (10-20 minutes)
- Dispensing cells into DMSO droplets(50 min per thousand cells)
- Prepare and dispense digest mix for overnight incubation (20-40 minutes)
- Overnight incubation

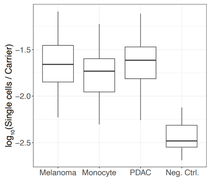
Day 2: (3 hours hands on time)
- Prepare and dispense multiplexing labels (1.5-2 hours)
- Walk away incubation for TMT (1hr)
- Dispense TEAB for mTRAQ labels or HA for TMT labels (30 minutes)
- Walk away incubation for mTRAQ (1hr)
- Sample pickup and transfer to 384 well plate for LC/MS analysis (30 minutes hands on, 1-3 hours wait time on labeling choice)

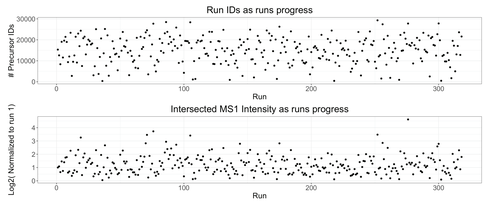
Results

QuantQC computational pipline for interpreting LC/MS results

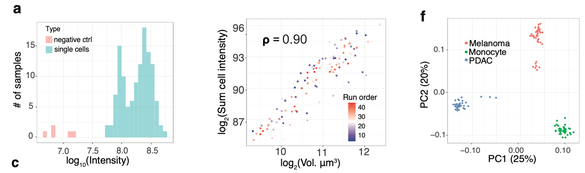
QuantQC is an R package that analyzes searched LC/MS data from cell maps metadata from nPOP experiments

- Provides valuable QC metrics to evaluate prep success
- Correlates single cell image data to MS data
- Generates quick reports in HTML format that allow you to check results and easily share with collaborators.

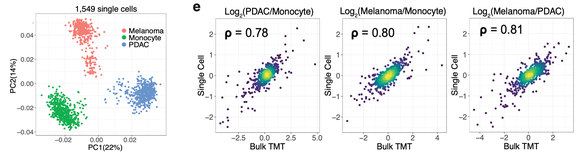




plexDIA – 3,200-3,700 proteins per single cell, > 100 cells per day on TimsTOF Ultra



pSCoPE – 1,000 proteins per single cell, > 1,000 cells per day on Exploris 480



Conclusions

- nPOP has been shown to automate sample preparation for multiplexed single cell proteomics with both isobaric and non-isobaric labels ranging from 3-29 single cells in each LCSM run.
- Cellenion's nPOP Partnership Program offers access to extensive documentation and easy to use cellenONE protocols, process specific hardware and labware, and expert training and remote support to ensure quick tech transfer and high quality results.
- To learn more, email **j.cantlon@scienion.com**

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