

High-Throughput Single Cell Proteomics Analysis with Nanodroplet Sample Processing, Multiplex TMT labeling, and Ultra-Sensitive LC-MS

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ABSTRACT

Understanding heterogeneity at single cell level is of great interest for biomedical research. MS-based proteomics is a promising technique for single cell analysis by enabling identification and quantification of thousands of proteins in unbiased manner. However, due to inefficient single cell isolation, large sample losses during sample preparation and low throughput, the extension to single cell studies has been largely ineffective. To address these challenges, we combined nanoPOTS (Nanodroplet Processing in One-pot for Trace Samples) technology with Thermo Scientific™ Tandem Mass Tag™ (TMT™) isobaric labeling to efficiently process and analyze single mammalian cells containing <0.2 ng total proteins on new Thermo Scientific™ Orbitrap Eclipse™ Tribrid™ mass spectrometer with Real-Time Search to improve single cell proteome coverage and enhance quantification accuracy.

MATERIALS AND METHODS

Single cells were isolated from cultured murine cells via fluorescence-activated cell sorting and nanoPOTS platform¹. Single cells and 5 ng boost sample² were digested and labeled on nanoPOTS chip. The Thermo Scientific™ UltiMate™ 3000 RSLCnano system was used with 30 μm i.d., 30 cm, C18, integrated electrospray emitter at 20 nL/min coupled to Orbitrap Eclipse Tribrid mass spectrometer. MS² and SPS MS³ with Real-Time Search³ analysis was done on 5 single cell batches (total 40 cells from three different cell types, epithelial, endothelial and raw immune cells). Thermo Scientific™ Proteome Discoverer™ 2.4 software was used for data analysis. Thermo Scientific™ TMT10plex™ reagent based protein quantifications were evaluated with a focus on protein coverage, reproducibility in quantification and throughput.

Figure 1. Workflow for nanoPOTS-based single cell proteomics sample preparation and nanoLC-MS/MS analysis.

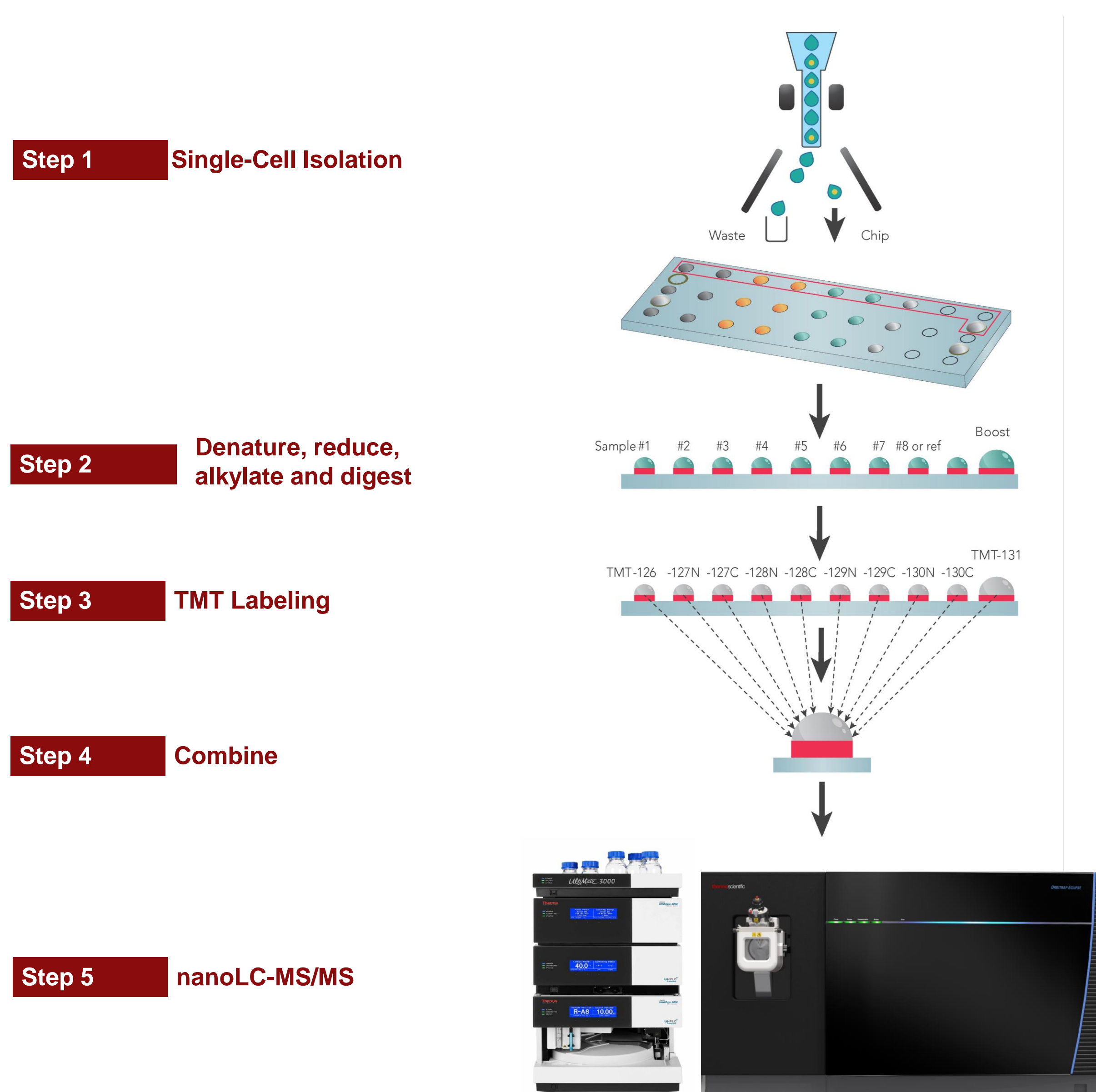
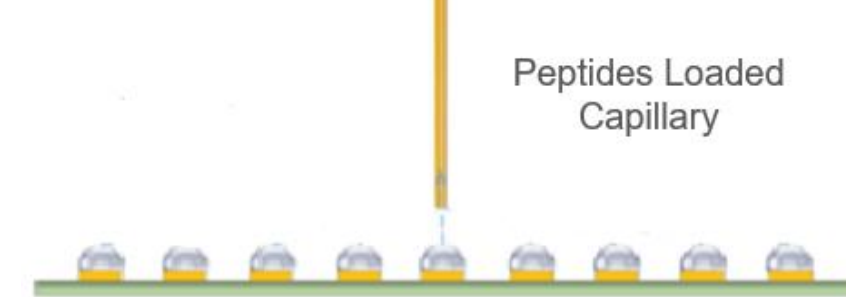
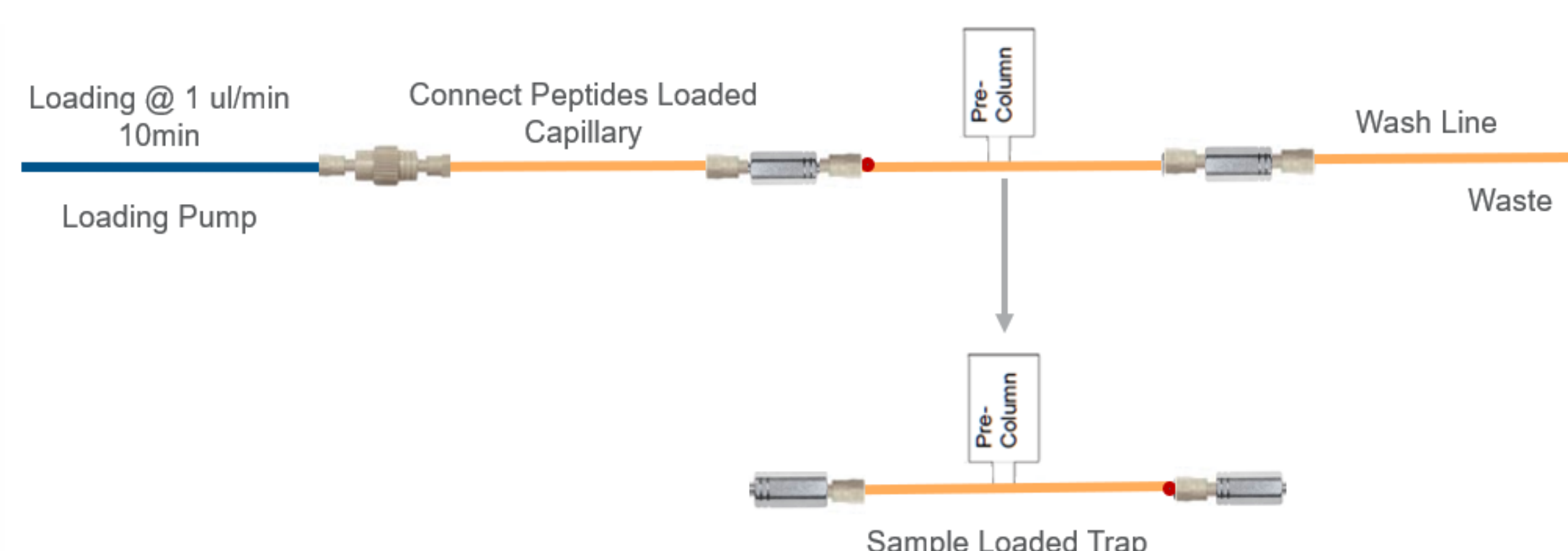


Figure 2. Ultrasensitive low nanoflow LC-MS workflow for single cell analysis. The three steps loading, and LC setup shown below; provides direct introduction of tryptic digested single cell proteins to analytical column for peptide separation and followed by MS analysis.

Step 1 Loading Peptides Into Short Capillary Tube



Step 2 Loading Peptides Into the SPE Column



Step 3 Connect the SPE online and start 160 min LC-MS Analysis

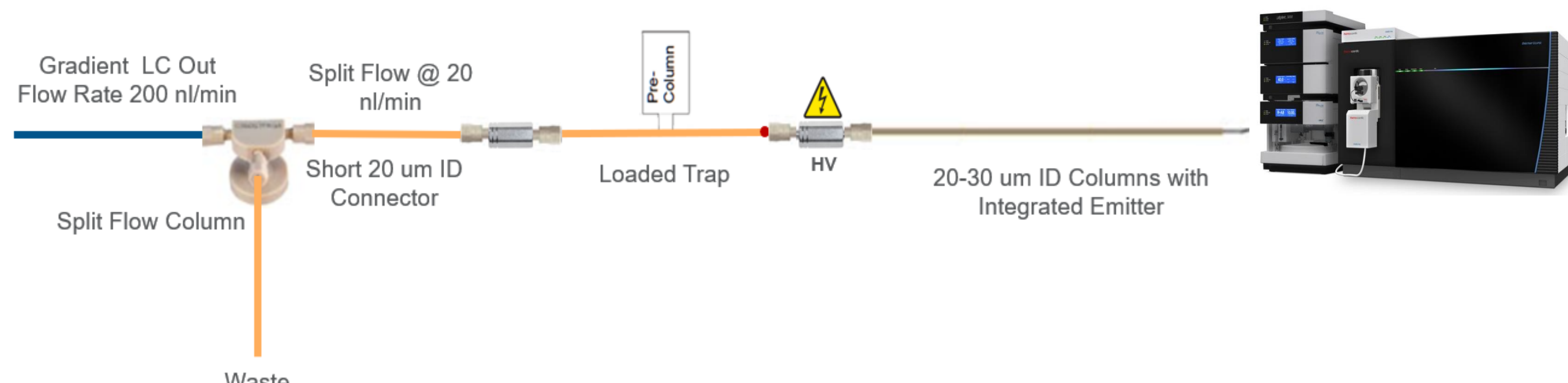


Figure 3. High-throughput murine cell classification with TMT isobaric labeling at MS² level. Total 24 single cells processed on three nanoPOTS chips were analyzed in three LC-MS analysis. The heat map (left) and PCA analysis (middle) of TMT10plex data shows clear differentiation between the three different cell types (raw immune cells, epithelial and Endothelial cells). Total 1676 protein and 8234 peptide groups were identified with TMT isobaric labeling at MS² level (right).

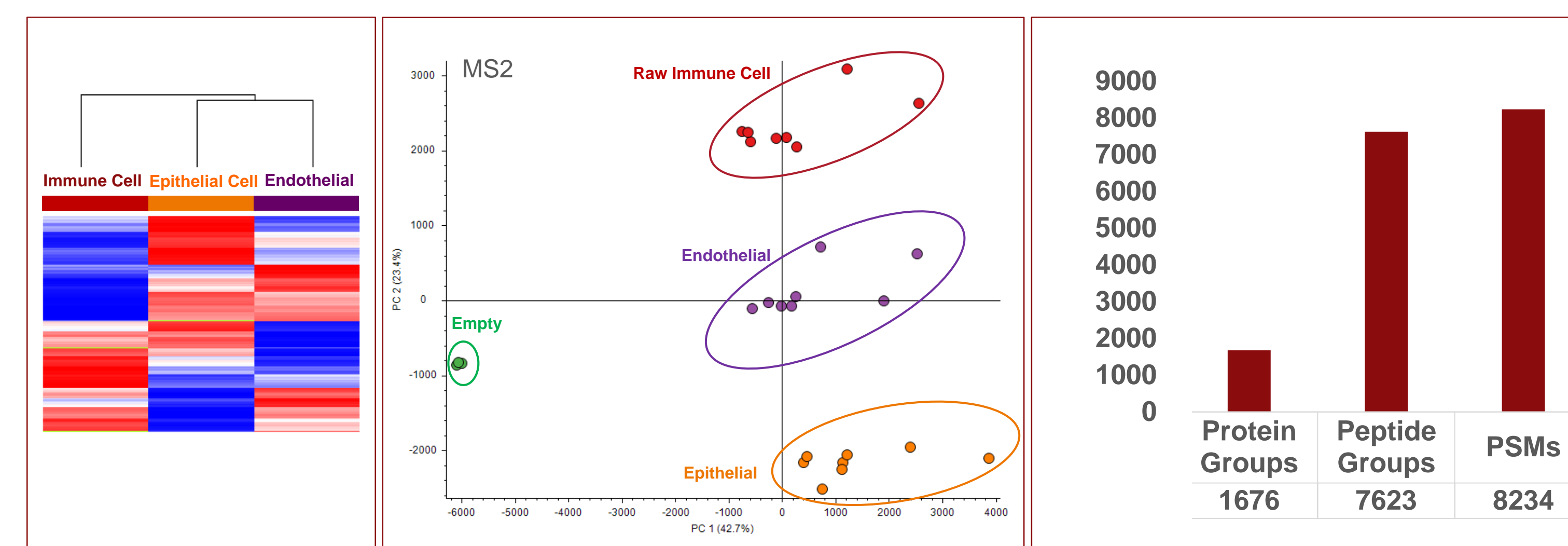


Figure 4. Overview of SPS MS³ Method with Real-Time Search on Orbitrap Eclipse Tribrid MS.

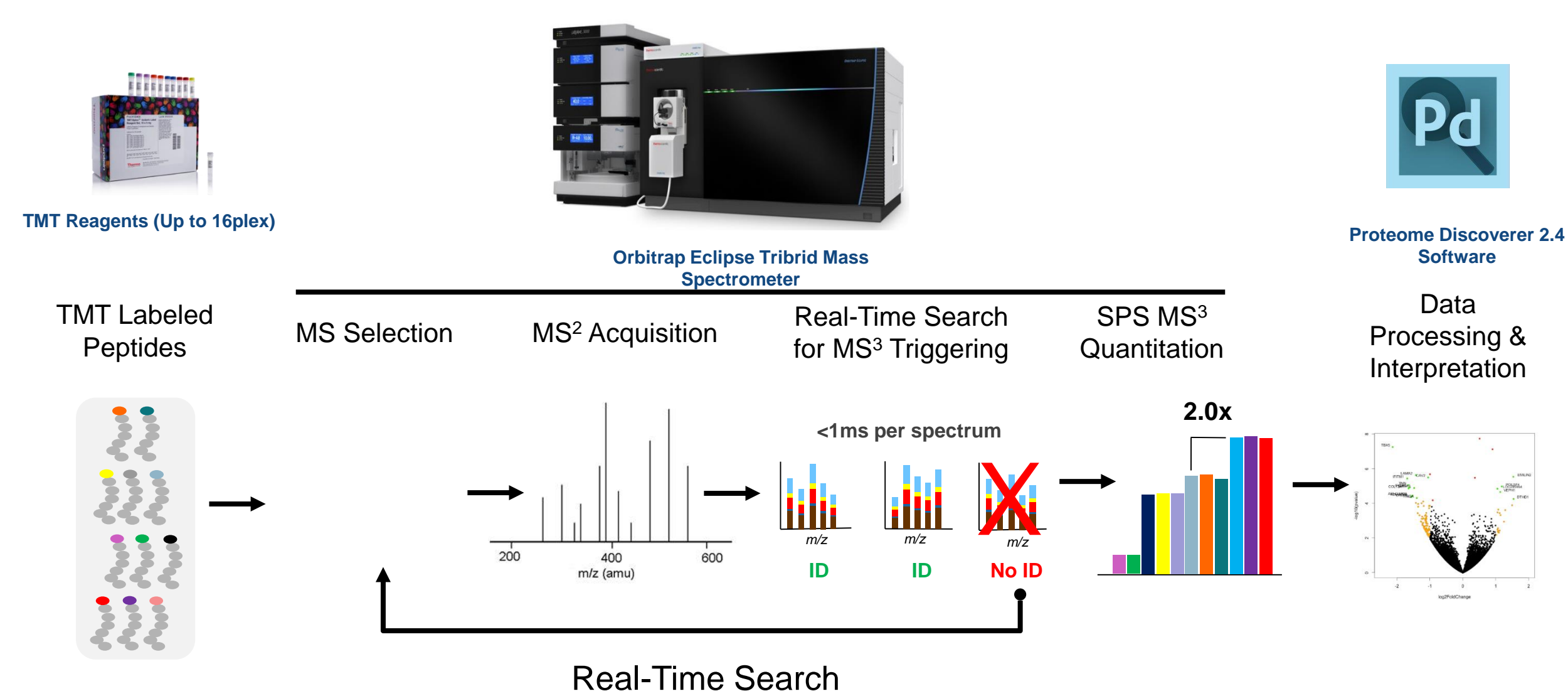
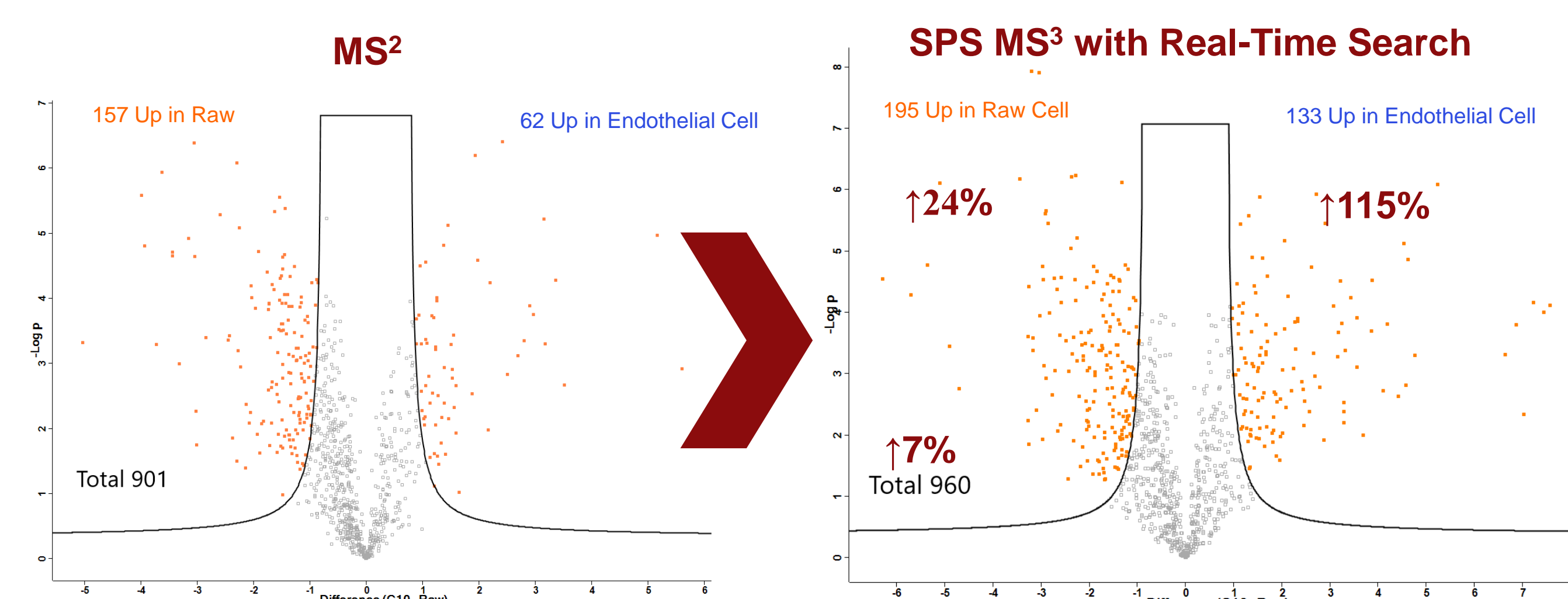


Figure 5. High Throughput murine cell classification with TMT isobaric labeling with Real-Time Search for SPS MS³. Total 16 single cell processed on two nanoPOTS chips were analyzed in two LC-MS analysis. The heat map (left) and PCA analysis (middle) of TMT10plex analysis shows clear differentiation between the three different cell types (Raw Immune cells, epithelial and Endothelial cells). Total 2346 protein and 4781 peptide groups were identified by TMT isobaric labeling (right) with improved quantitative accuracy and differential protein coverage with Real-Time Search for SPS MS³.



Figure 6. Improved coverage and accuracy of single cell proteomics analysis using SPS MS³ with Real-Time Search on Orbitrap Eclipse Tribrid MS. SPS MS³ with Real-Time Search method provided improved coverage of differentially expressed protein between the three cell types and improved quantitative accuracy without compromising in total number of proteins identified.



RESULTS

The TMT10plex analysis of the three cultured murine cell populations (C10, SVEC and Raw cells) enabled identification of 2346 proteins and 1300 quantifiable among 40 single cells. We have demonstrated that single cell proteome can be quantified with TMT workflows by combining nanoPOTS with Orbitrap Eclipse Tribrid mass spectrometer, enabling researchers to investigate cell heterogeneity as well as rare cells.

CONCLUSIONS

- The nanoPOTS platform combined with TMT multiplexed isobaric labeling provides a robust, high-throughput proteomic preparation method for handling extremely small biological samples like single cells.
- Reproducible quantitative proteome measurement with coverage of 2000 protein groups was achieved among a total of 40 single cells obtained from cultured murine cell populations.
- NanoPOTS integrated with multiplexed isobaric labeling represents a highly promising platform towards; single cell typing, understanding of stem cell development, proteomic studies of isolated clinical specimens (circulating tumor cells) and proteome imaging of tissue heterogeneity.

REFERENCES

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TRADEMARKS/LICENSING

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