# Hands-Free Sample Preparation for Proteomics Using Universal Chemistry and a Microfluidic Benchtop Instrument

Greg Foster[1], Aaron Robitaille[1], Michael O. Krawitzky[1], Daniel Lopez-Ferrer[1]. [1]Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134

## ABSTRACT

Mass spectrometry has rapidly emerged as a powerful tool for identification and quantification of proteins. Most sample preparation is performed manually using non-standardized homebrew protocols and thus is prone to user error, which makes comparing results among labs impossible. We have engineered a hands-free microfluidic device that performs the entire sample preparation workflow within 1 hour.

## INTRODUCTION

Typically, proteomics samples are processed via a series of steps including reduction of disulfide bonds, alkylation, and proteolytic digestion of proteins into small peptide sequences. While this bottom-up proteomics approach provides comprehensive peptide and protein identification, poor sample preparation technique can significantly decrease protein identification or cause the LC-MS to fail. To address this challenge, we integrated a unique one-pot chemistry into a microfluidic device to perform hands-free proteomic sample preparation.

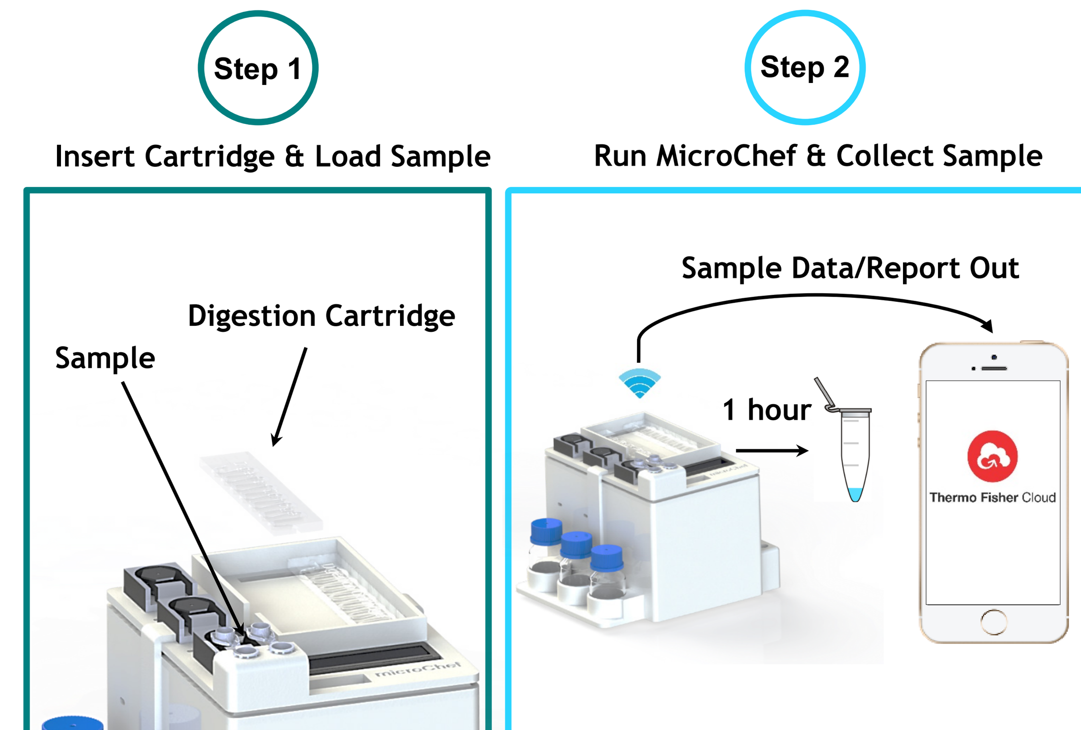
## MATERIALS AND METHODS

The microfluidic device (MicroChef) was assembled using a combination of peristaltic pumps, solenoid valves, microcontrollers, and 3D printed parts. A UV detector and capillary flow cell was placed in the fluid path to quantify peptide concentration. Detergent, reducing agent, alkylating agent, and proteolytic immobilized enzyme were loaded prior to sample injection. HeLa cells were used to evaluate the precision and accuracy of the system in preparing samples for bottom-up analysis with LC-MS. Following processing in the device, samples were briefly dried down in a speed vacuum and reconstituted in 0.1% formic acid. HeLa digest was then injected into a Thermo Scientific™ EASY-nLC™ 1200 system coupled to a Thermo Scientific™ Q Exactive™ HF MS and analyzed using Thermo Scientific™ Proteome Discoverer™ 2.2 software.

## RESULTS

We first evaluated the total peptide amount recovered from 1 million HeLa cells after processing with our device and following a traditional manual sample preparation. Interestingly, we recovered ~40% more peptides in the microfluidic device, which we hypothesize is due to the low flow rate in the microfluidic device that permits optimal binding of peptides. We further evaluated other quality control metrics and we obtained similar amounts of missed cleavages, alkylated peptides, deamidation, and oxidation with our device compared to manual sample preparation and a HeLa standard. Furthermore, we did not observe differences when comparing the number of peptide and protein identifications. Finally, we observed very high reproducibility in qualitatively metrics such peptide and protein identifications with variation less than 10% among different sample preps, and when we performed label-free quantitation, average CVs for the peptide intensities among five different sample preparation batches were below 10%, thus validating this technology to perform quantitative proteomics experiments.

**Figure 1. Graphical representation of the microfluidic device and workflow for proteomic sample preparation. Samples are loaded into a vessel that then mixes with the one-pot chemistry in a microfluidic cartridge (left). After sample preparation is complete, the user is then sent the peptide concentration via Wi-Fi.**




## Instrument Design

**Figure 2. Schematic describing the components and fluidic path in the microfluidic device. The system operates using a combination of peristaltic pumps and solenoid pinch valves. In total, four buffers are used to perform the sample preparation that includes: one-pot lysis/digestion buffer, two wash buffers, and an elution buffer. Peptide quantitation is determined during the elution step, which triggers the instrument to divert sample from waste to a collection vial.**

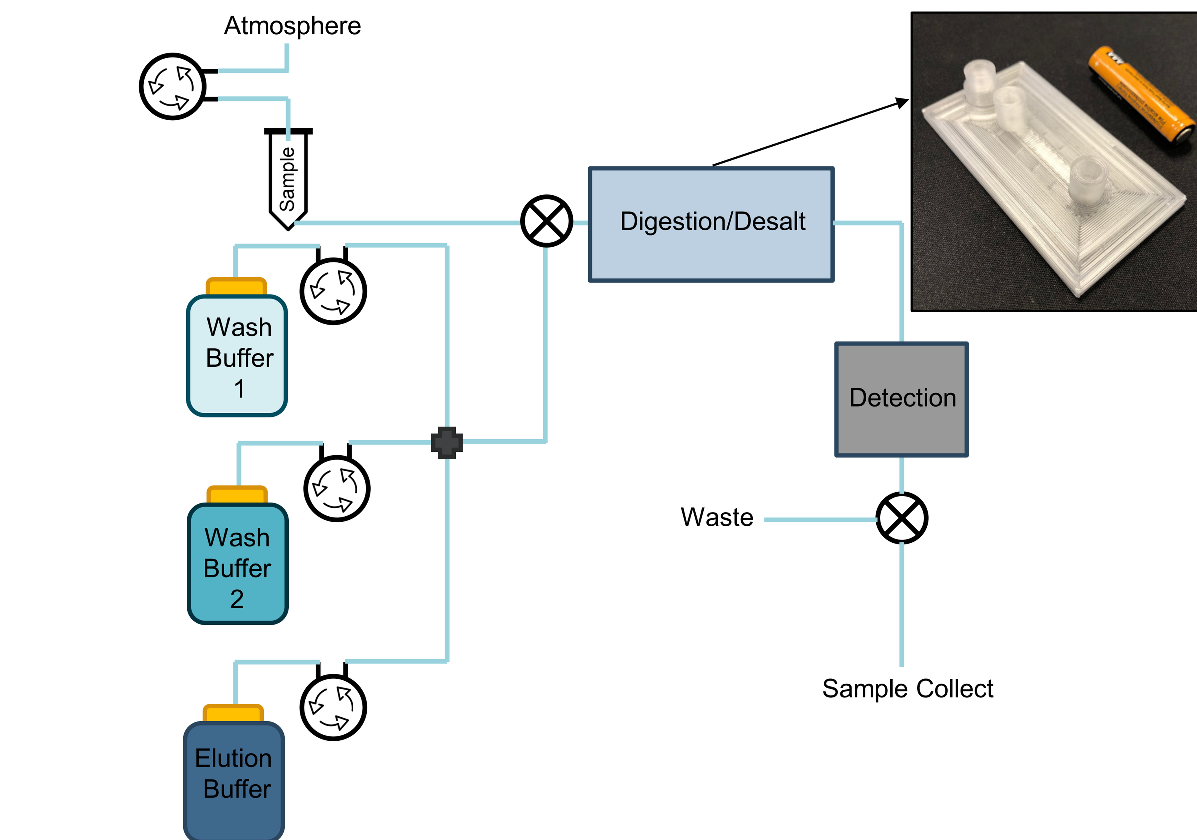
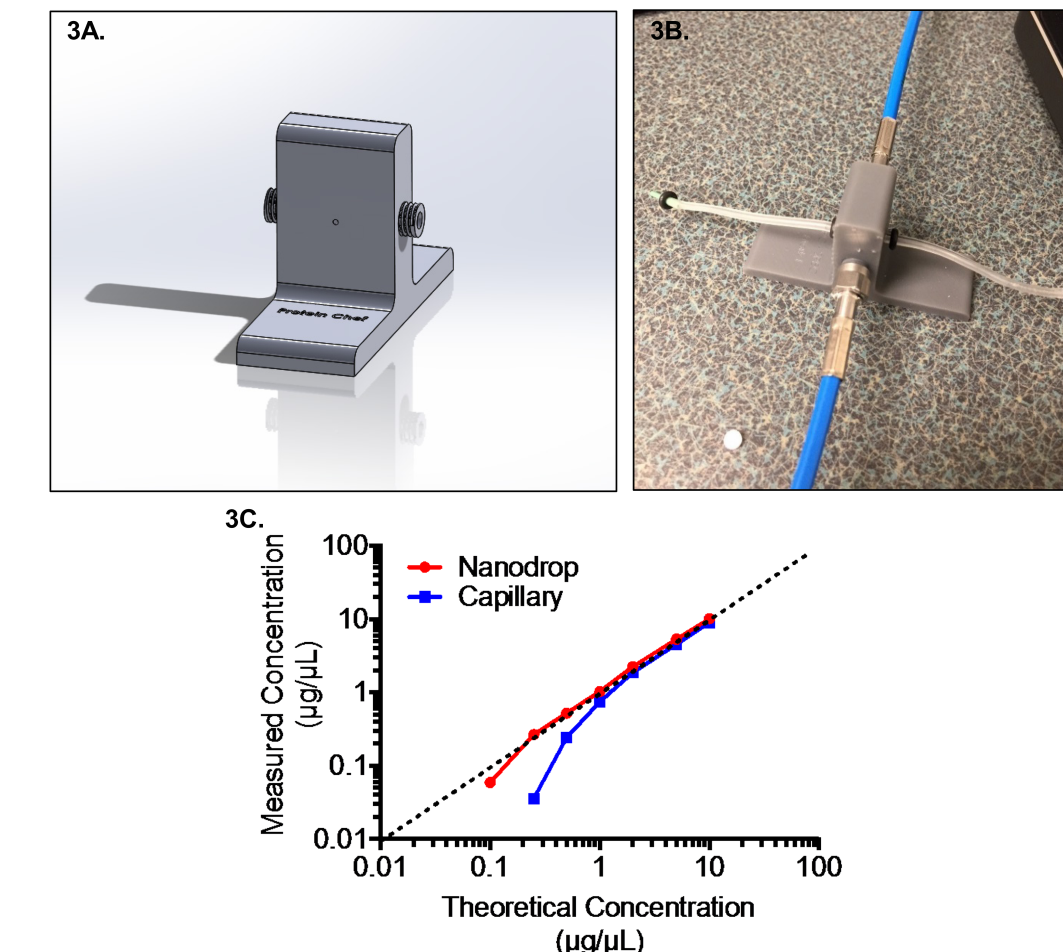
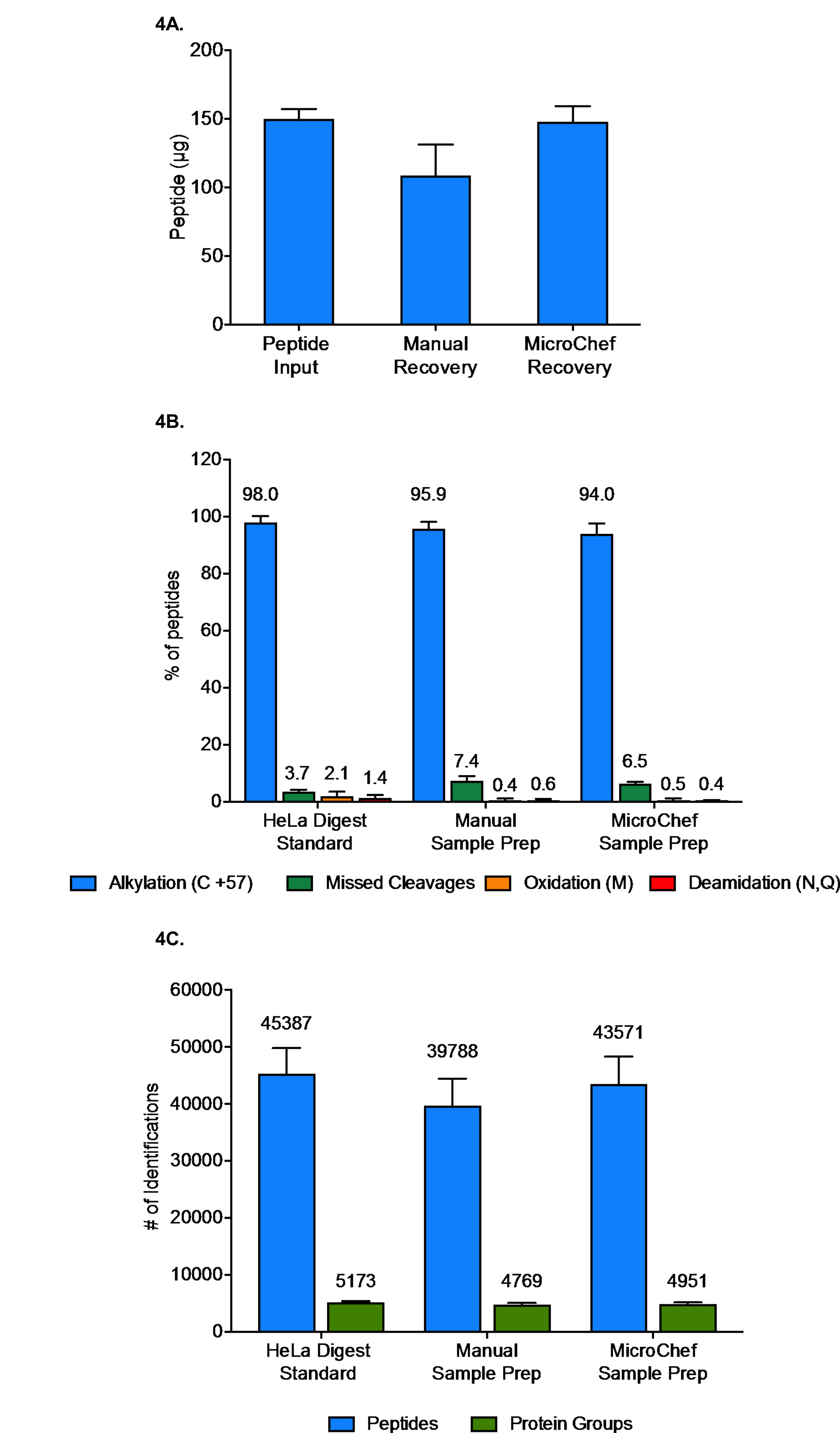



**Figure 3. Spectrophotometric design and UV detection of peptides. (3A) A capillary housing was designed using 3D modeling software such that fiber optics could be inserted perpendicular to a 1 mm OD x 0.75 mm ID silica flow cell. (3B) The housing was fabricated using a stereolithographic 3D printer and coupled with fiber optics, a spectrometer, and xenon pulse lamp from Ocean Optics. (3C) Peptide concentration was determined using peak absorbance at 280 nm and accuracy was compared with a Thermo Scientific™ NanoDrop™ spectrophotometer.**



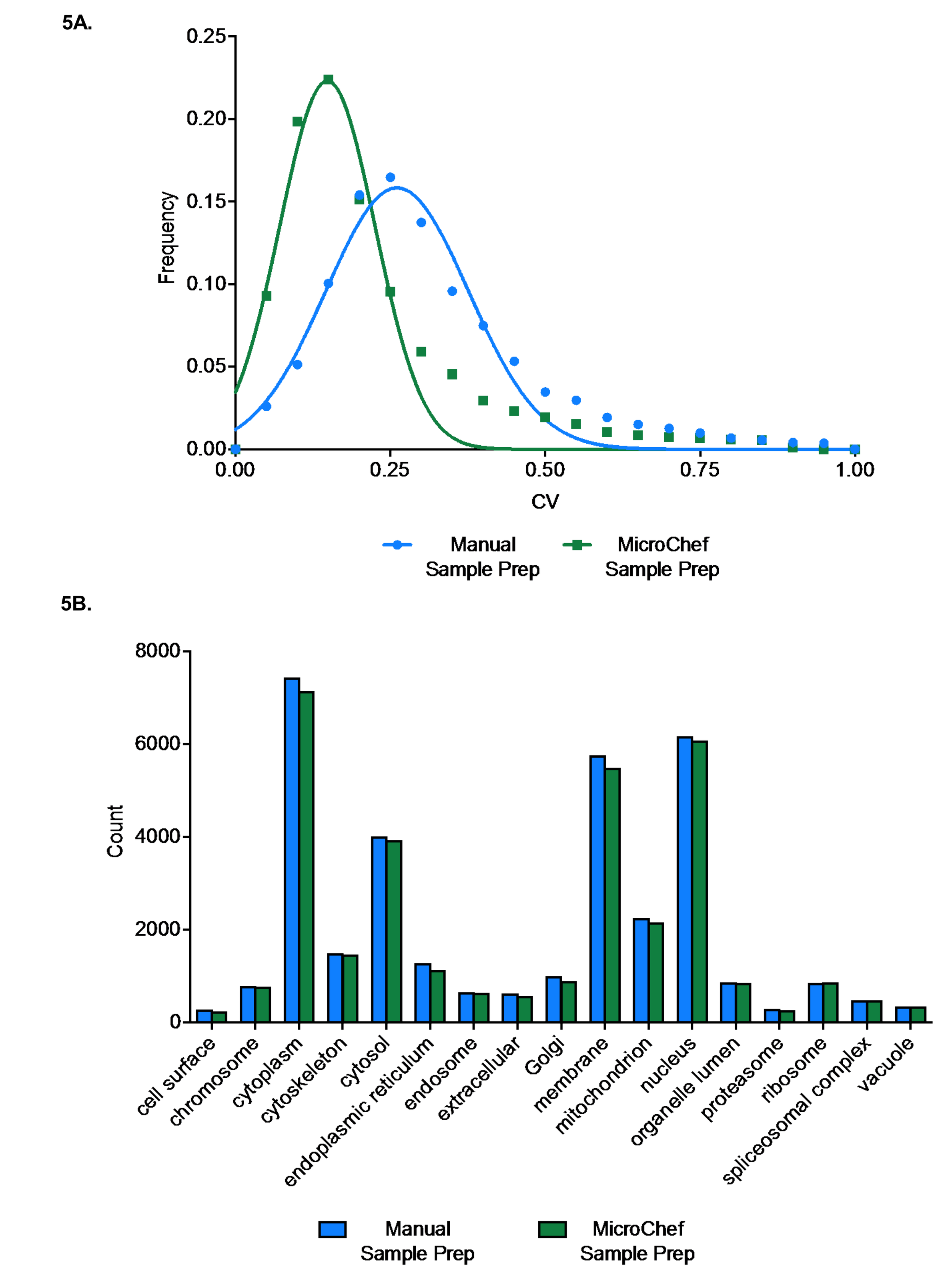

## Results

**Figure 4. HeLa peptide recovery and features using the MicroChef compared to manual sample preparation. (4A) Digested peptides were cleaned up using spin columns and a bench top centrifuge (Manual Recovery) or the MicroChef (n=5). The concentration of peptides was determined using UV absorbance at 280 nm. (4B) Following sample preparation, 1 µg of peptide was analyzed on a 3 hr gradient using an EASY-nLC 1200 system and a Q Exactive HF MS. Searches were performed using PMI-Preview™ or Proteome Discoverer 2.2 software.**



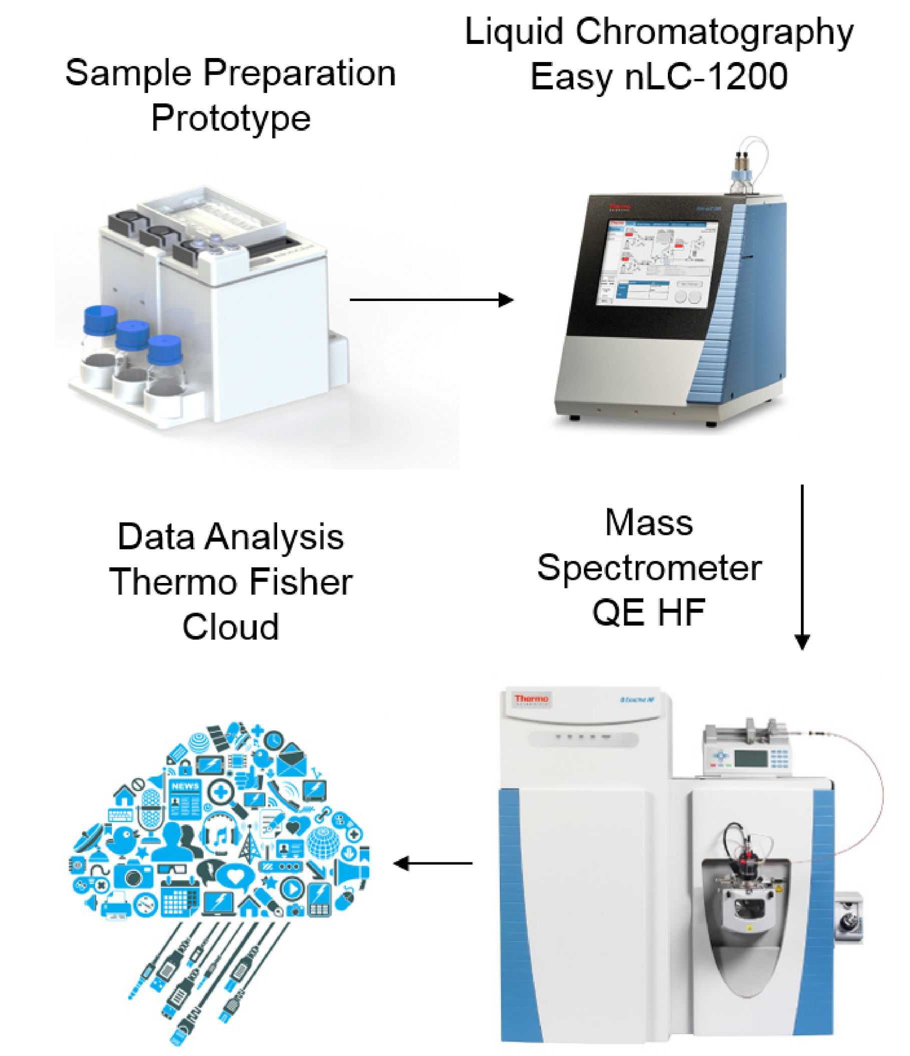

## Reproducibility

**Figure 5. HeLa peptide abundance and distribution in MicroChef compared to manual sample preparation. (5A) Searches were performed using Proteome Discoverer 2.2 software and peptide and protein abundances were calculated using label-free quantitation with the Minora Feature Detector mode. The distribution of CVs for manual sample preparation was plotted versus the CVs for MicroChef and fitted with a normal distribution (n=5). (5B) The distribution of cellular components of proteins were calculated using the Protein Center Annotation node in Proteome Discoverer 2.2 software.**




## Envisioned Workflow

**Figure 6. We envision a universal workflow for proteomics that provides the entire ecosystem to enable all scientists and researchers to perform bottom-up mass spectrometry proteomics. The ecosystem would allow the user to go from sample to knowledge with minimal effort and high confidence.**




## CONCLUSIONS

We demonstrate that our engineered microfluidic prototype exploits the unique properties of our sample preparation formulation to provide optimal peptide and protein quantitation of cells in 1 hour. Furthermore, the hands-free and cloud connectivity feature of the device allows for the potential to integrate the system with downstream LC-MS processing and data analysis.

## TRADEMARKS/LICENSING

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