Evaluating the Performance of an Automated, Inexpensive Hood Robot using an Open-Source, Python-Scripted In-Gel Digestion Workflow for Bottom-Up Proteomics

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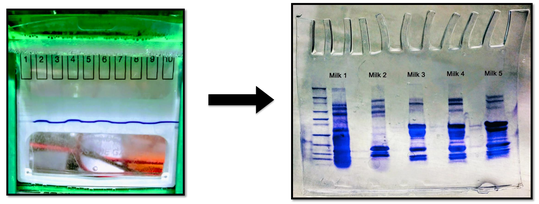
Overview

Opentrons' OT-One Pipetting Hood Robot affordably allows researchers to automate labor-intensive workflows. We present the first application of the OT-One Robot to proteomics, specifically an in-gel digestion workflow. The OT App gives users control of the robot to calibrate pipette positions relative to labware on deck and upload open-source, python-scripted protocols. The open-source format allows easy transfer of protocols to other researchers around the globe for use in replicating experiments with high fidelity. With time and workplace ergonomics in mind, the use of the robot reduces the physical constraints and alleviates researcher time. To test the effectiveness of the robot, a comparative study of sample preparation was performed in parallel by hand and Robot on "sister samples" (N=5).

Method



Five different milk samples were run on SDS-PAGE for 1.5 hours at 100V. A single band from each sample was cut in half and prepped manually or by Robot. Milk was the analyte of choice because of its well characterized difficulty in digestion processes. Samples were reduced with DTT and alkylated with acrylamide. All samples were washed twice to remove additional stain. The Robot used 50 µL (125ng) of a pre-mixed solution of trypsin/lys-C, ProteaseMax and AmBic. For samples prepared by hand, 10 µL (125ng) of Trypsin/Lys-C mixed with ProteaseMax was added first and covered with AmBic ten minutes later. Digestion proceeded overnight. The mass spectrometry analysis was done on an Orbitrap Fusion with a Waters M-Class LC; CID was the fragmentation method. Data was analyzed by both Preview and Byonic (Protein Metrics) using 10.0 ppm precursor and 0.4 Da tolerances.

Results

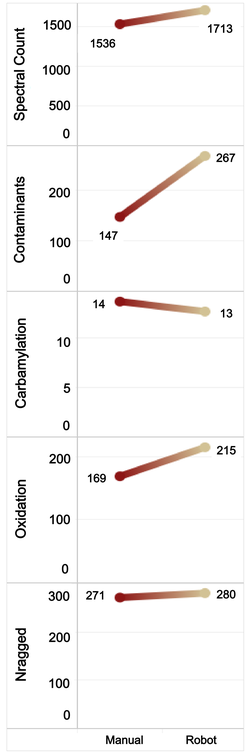


Figure 1. The average values for Manual or Robot prepared samples were calculated for spectral count, exogenous contamination, Carbamylation, oxidation and N-ragged cleavages. On average, the Robot results showed higher oxidation and exogenous contamination: 10% vs. 16% of spectral counts, respectively. Carbamylation and N-ragged differences are negligible.

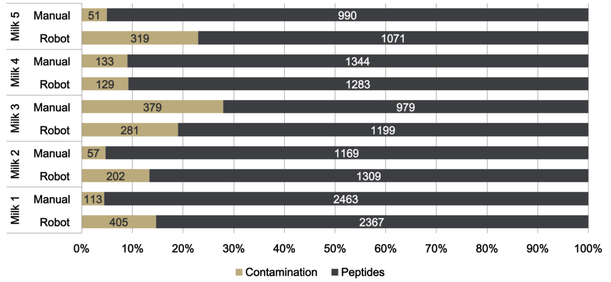
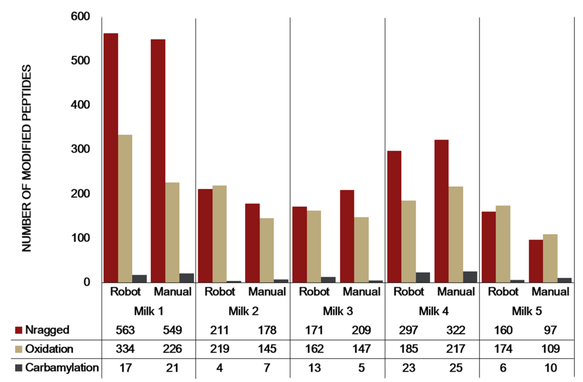


Figure 2. The number of identified peptides and contaminants for each sister sample are compared. Overall, the number of spectral counts between samples was the same (within 15%). Contamination was higher in 3 of 5 samples prepared by Robot and on average 17% higher overall.

Figure 3. Sample to sample characterization of N-ragged, oxidation and carbamylation. The baseline endogenous level of oxidation independent of preparation was high (likely a result of the analyte); even so, our data reflect increased levels in Robot prepared samples. High amount of N-ragged may be due to instability of milk proteins, decreasing the efficiency of Trypsin/Lys-C digestion, a well established caveat for milk samples. Carbamylation in all samples is minor and negligible.



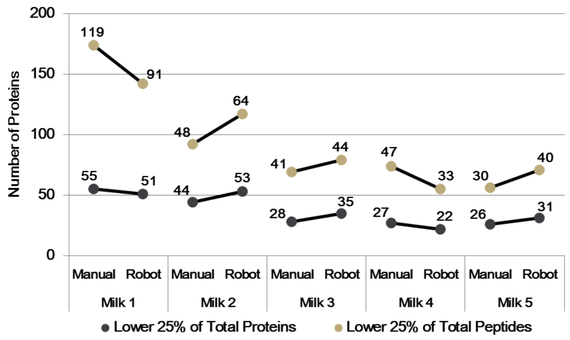


Figure 4. After elimination of all contaminants, isoforms and reverse proteins, the lower 25% of total protein and peptides were calculated. The total protein count between each sister sample is nearly the same. Total peptide count show marked trends between milk samples. These data support the conclusion that there is no loss in proteomic depth as a function of preparation type.

Discussion

The goal of this proteomic study was to assess whether gel samples prepared by an automated robot gave results as robust as manually prepared samples. Comparing the spectral count, contamination level, and modifications between sister samples, our results demonstrate that overall the Robot is as effective at preparing in-gel digest samples as a manual operator with the advantages that it is easy to program and control, enables consistency and reproducibility of any experiment using the open-source protocol, and is ergonomically friendly. Results showed that the top protein hit between each sister sample was always the same and the spectral counts were relatively similar. We did observe a higher degree of exogenous contamination in the Robot prepared samples. We posit this is a result of the eppendorf vial caps being open for the duration of the Robot digestion protocol. The excess exposure to air also explains the higher amount of oxidation in samples prepared by Robot. The alkylation efficiency was the same in all samples with 100% cysteine capping, and difference in carboxylation was minor and negligible. Importantly, proteome depth was not measurably different between preparation type.

Future Considerations

- Use slit-opening, silicone caps for sample tubes being processed by Robot, to allow pipette tip access to the gel while keeping contamination and oxidation levels low.
- Develop robotic digestion protocols for in-solution and magnetic particles.

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This poster may be downloaded from the Stanford University Mass Spectrometry website: https://mass-spec.stanford.edu/publications