CASE STUDY

Automating Complex Manual Sample Preparation for Mass-Spectrometry-Based Proteomics Using the Opentrons OT-2 Platform

Written by
Opentrons, based on data from Dr. Joao Paulo, Harvard University

As quantitative proteomics experiments increase in scale and complexity, greater consistency and precision are required, which is best delivered by automation. Dr. Joao Paulo, PhD, and his laboratory at the Harvard Medical School Department of Cell Biology employ mass spectrometry-based proteomics (MSP) analysis to better understand the effects of molecular mechanisms and cell signaling pathways on the regulation of the proteome of various model systems. Dr. Paulo sought to replace labor-intensive manual sample preparation with automation, and his work serves as a demonstrated case study for successful automation of sample preparation for mass spectrometry-based proteomics using the Opentrons OT-2.

Mass Spectrometry-Based Proteomics

Mass spectrometry (MS) is an analytical chemistry technique that identifies the quantity and type of molecules present within a sample by measuring the mass-to-charge ratio of gas-phase ions.[1] This technology is used in proteomics to profile protein quantity, structure, function, and molecular interactions in biological samples[1] and it is a preferred method for protein detection, identification, and quantitation. Mass spectrometry-based proteomics (MSP) is increasing in use for peptide-spectra matching and protein identification. MSP detects peptides at the attomole (10^{-18}) level and supports multiplex processing of hundreds to thousands of proteins from a single analysis. Multiplexing tagged peptides provides insight into quantitative differences observed among samples of varied conditions processed simultaneously. Isobaric tandem mass tag (TMT) barcoding labels every protein with a sample-specific tag. Samples are mixed, processed, and demultiplexed by reading the intensity of the reporter ions on the peptide. The MS then measures and sorts the various peptides detected. Multiplexing on a single instrument removes run-to-run variability, improves precision of resulting data, and increases throughput.

Sample Preparation for MSP Analysis

MS sample preparation can be complex, tedious, and time-consuming **(Figure 1)**. Cells are lysed, and the proteins are isolated and purified to remove irrelevant cellular components such as membranes and nucleic acids and to prevent protein degradation from proteases. Protein purification can be performed manually using chloroform-methanol precipitation or by paramagnetic bead precipitation. Methanol chloroform precipitation is a common source of variability in sample preparation as it is performed manually using a series of centrifugation and pipette aspiration steps. The methanol-chloroform process is labor-intensive and error prone. An automation friendly method would be preferable to provide repeatability and free up technician time.

FIGURE 1

MSP Sample Preparation Workflow

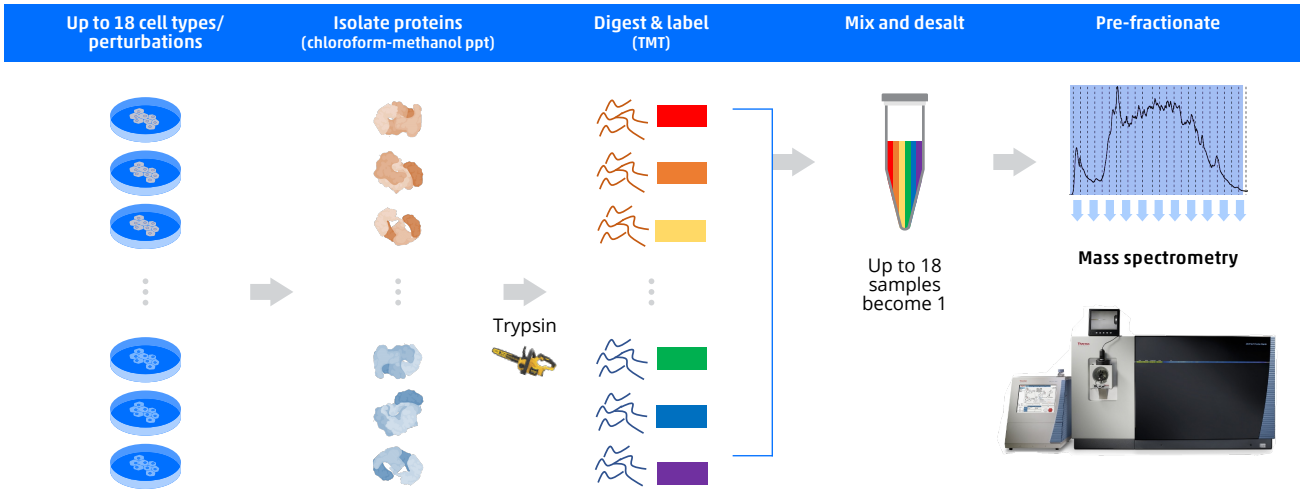


Figure 1: MSP sample preparation workflow. The standard MSP sample preparation workflow includes protein isolation (usually by methanol-chloroform precipitation), digestion and sample-specific TMT labeling, pooling, desalting, and pre-fractionating.

FIGURE 2

SP3 Bead-Based Sample Preparation Workflow

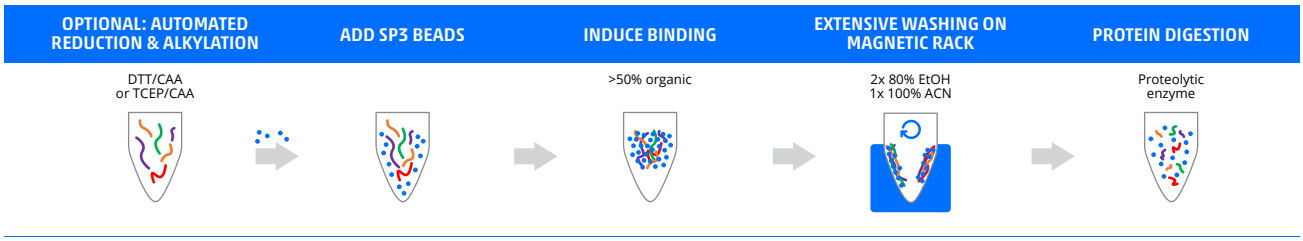


Figure 2: Example of the SP3 bead-based sample preparation workflow.

Bead purification is an alternative method to chemical precipitation that is automatable and can facilitate a range of sample throughput requirements. Single-pot solid phase-enhanced sample preparation (SP3) beads are paramagnetic microspheres that permit on-bead protein binding, precipitation, and clean-up using magnet-assisted aspiration **(Figure 2)**.

Following precipitation of proteins, steps to digest and label resulting peptides are conducted. These methods can be performed manually or by automated methods. SP3 beads streamline the process further by providing on-bead digestion and labeling with peptide elution into an aqueous buffer that is ready to be further fractionated, labeled, or placed on the MS instrument. Alternatively, peptides can be processed using a liquid chromatography (LC) instrument for peptide separation based on hydrophobicity.

Once samples are prepared, electrospray ionization (ESI) is used to ionize peptides and move them from a liquid phase to a gas phase. Mass spectrometry profiles the peptides based on concentration and size, and these peptides can be fragmented into smaller ions and further analyzed.

Processing samples individually using this complex workflow introduces opportunities for error and inconsistency. Automation was desired to enable processing of dozens of samples simultaneously with improved repeatability. Investigation of available automation options by Dr. Paulo's laboratory led to the discovery of the Opentrons OT-2 system.[1]

Automation of Sample Preparation with Opentrons OT-2

The Opentrons OT-2 system is a simple, affordable, open-source liquid handling platform with the capability to streamline complex processing workflows. Dr. Paulo's laboratory used the OT-2 system for automation of MSP sample preparation using SP3 paramagnetic beads **(Figure 3)**.

The OT-2 system is customizable with various modules, including multichannel pipettors (P20 and P200), a magnetic module, and a temperature-controlled module.

One step of the manual protocol specified the use of an orbital shaker. To meet this need, a simple program was entered in the OT-2 Protocol Designer software to successfully replace the shaking step with a pipette mixing step. Additional optimizable parameters of the OT-2 include, but are not limited to, pipette aspirate/dispense speeds, addition of an air gap to discourage droplet formation, pipette height and X-Y alignment, incubation times, and placement of labware on the work deck to protect sample integrity.

FIGURE 3

OT-2 Module Arrangement for Sample Processing

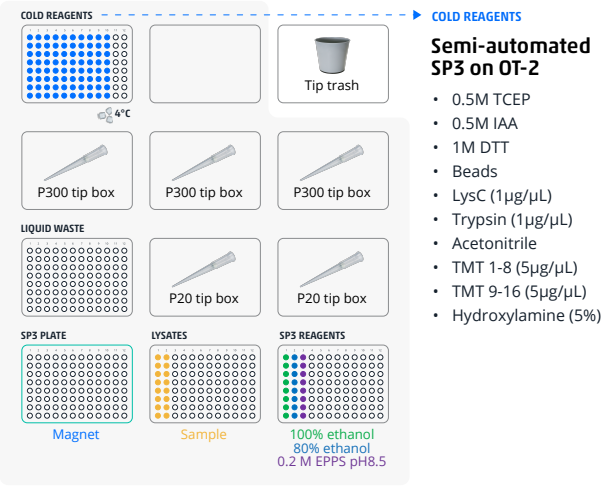


Figure 3: OT-2 module arrangement for sample processing. The OT-2 was set up for the MSP sample preparation workflow to include SP3 bead-based protein separation instead of methanol-chloroform precipitation.

OT-2 Automation VS Manual Sample Processing

The ability of Dr. Paulo's laboratory to perform high-throughput MSP analysis was hampered by manual sample preparation. A side-by-side comparison of manual and OT-2 sample preparation methods was conducted using the proteomes of 6 yeast parental strains prepared in triplicate **(Figure 4)**. Samples were TMT-labeled, multiplexed, and processed by LC-MS.

Comparable Performance Between Automated and Manual Sample Preparation

The number of proteins and peptides detected in samples prepared using the OT-2 were comparable to those detected in samples prepared with manual methods **(Figure 5A)**. Additionally, the data showed a 95.1% protein overlap and a 58.4% peptide overlap, both comparable performance of the OT-2 compared to manual processing **(Figure 5B)**.

The researchers then looked at the number of proteins identified by all replicates in samples prepared by the OT-2 and manual methods. The data in **Figure 6** demonstrates an 84.3% and 85.1% overlap between automated and manual methods, indicating comparable repeatability.

Proteomic profiles obtained from each method were compared. Data clustered by sample type and quantification shows comparable results between the methods **(Figure 7)**.

The data from quantification of control samples **(Figure 8)** demonstrates comparable results between the manual and OT-2 preparation methods.

Automation of sample preparation with the OT-2 offers clear benefits to manual methods for mass spectrometry-based quantitative proteomic analysis. Dr. Paulo's entire sample preparation workflow, from lysate to peptide labeling, was conducted on the OT-2 system using magnetic SP3 beads, which were key to automation. By running methods in a series and replenishing pipette tips during natural pauses in the workflow, 96 samples can be processed simultaneously to increase throughput. Ultimately, results obtained by comparing the proteomes of each sample type processed by both methods gave

FIGURE 4

Experimental Design: OT-2 vs Manual Processing

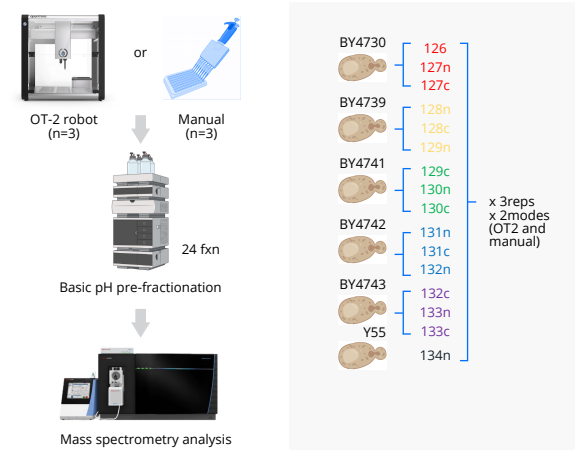


Figure 4: Experimental design: OT-2 vs manual processing. The experiment used TMT-labeled yeast lines that were processed for MS analysis using automated (via OT-2) or manual methods.

FIGURE 5

Protein and Peptide Identification

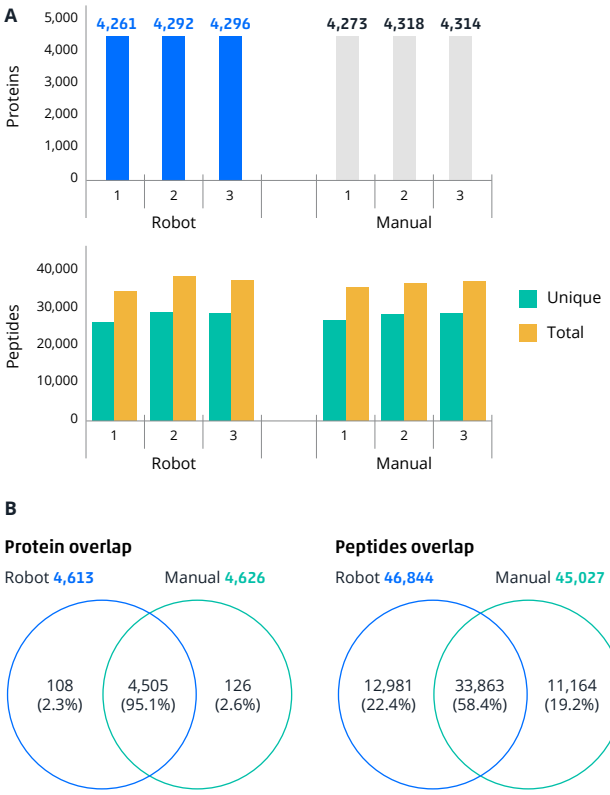


Figure 5: Protein and peptide identification: OT-2 vs. manual processing. Protein and peptide identification was performed using samples prepared with the OT-2 vs. the manual method, and the number of proteins and unique and total peptides in each sample quantitated by MS (left panel). The numbers of proteins and peptides identified by each method were also compared (right panel).

strong correlation in depth plus quantitative accuracy and precision. Automation of tedious, labor-intensive processes promotes laboratory efficiency and returns the focus from sample preparation to result analysis.

FIGURE 6

Repeatability Analysis

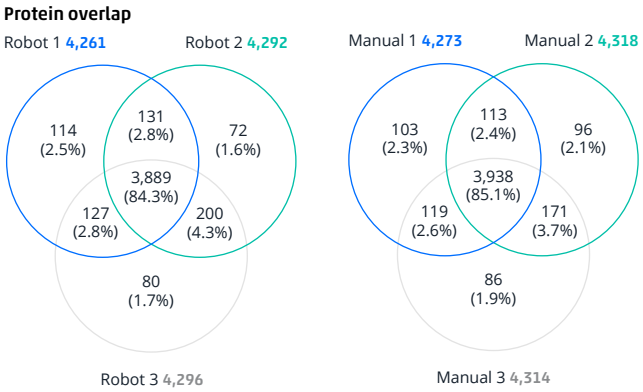


Figure 6: Repeatability analysis: OT-2 vs. manual processing. The number of proteins identified in samples prepared by the OT-2 and manual methods were compared by each replicate.

FIGURE 7

Proteomic Data Compared from Samples Prepared by OT-2 and Manual Methods

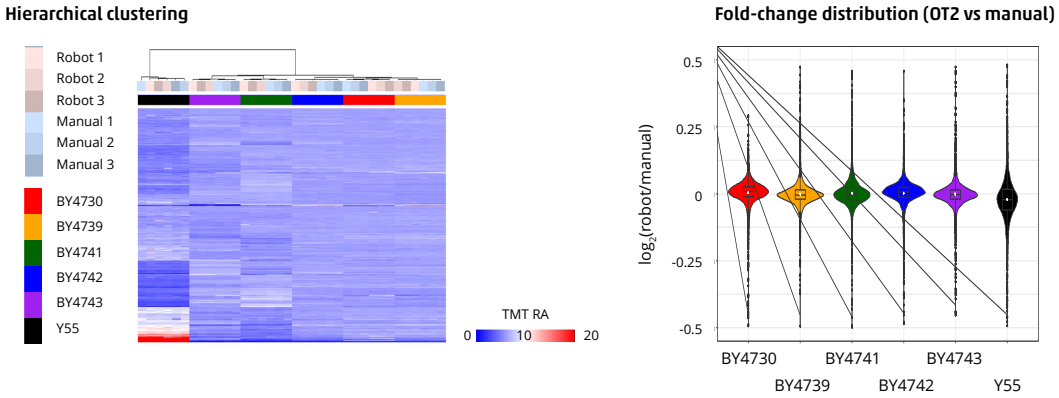
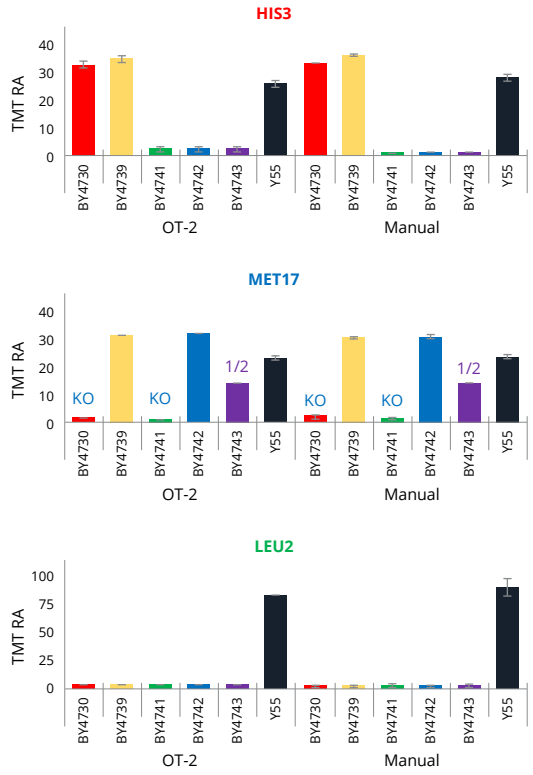


Figure 7: Proteomic data compared from samples prepared by OT-2 and manual methods. Hierarchical clustering was performed, with the data from samples prepared using the OT-2 and manual methods placed side-by-side to identify any differences (left panel). Fold change distribution was also performed to identify any differences in proteomic fold changes between samples prepared using the OT-2 and manual methods.

FIGURE 8

Proteomic Analysis of Control Samples



CODE	STRAIN	BACKGROUND	PARTIAL GENOTYPE
30	BY4730	MATa	leu2Δ0 met17Δ0
39	BY4739	MATalpha	leu2Δ0
41	BY4741	MATa	his3Δ1 leu2Δ0 met17Δ0
42	BY4742	MATalpha	his3Δ1 leu2Δ0
43	BY4743	MATa/MATalpha	4741/4742
55	Y55	MATa/MATalpha	HO/HO

Figure 8: Proteomic analysis of control samples processed using both manual and OT-2 automated methods shows comparable quantitative results.

REFERENCES

1. Liu X, Gygi SP, **Paulo JA**. A Semiautomated Paramagnetic Bead-Based Platform for Isobaric Tag Sample Preparation. J Am Soc Mass Spectrom. 2021 Jun 02; **32(6)**:1519-1529.
2. Müller, Torsten, *et al*. Molecular Systems Biology. **16**: e9111 (2020).