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Modular automated bottom-up proteomic sample preparation for high-throughput applications V.1

Yan Chen¹, Nurgul Kaplan Lease¹, Jennifer Gin¹, Tad Ogorzalek¹, Paul D. Adams¹, Nathan Hillson¹, Christopher J Petzold¹

¹Lawrence Berkeley National Laboratory

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Christopher Petzold

Lawrence Berkeley National Laboratory

These protocols are for research purposes only.

Manual proteomic sample preparation methods limit sample throughput and often lead to poor data quality when thousands of samples must be analyzed. Automated workflows are increasingly used to overcome these issues for some (or even all) of the sample preparation steps. Here, we detail three optimised step-by-step protocols to: (A) lyse Gram-negative bacteria and fungal cells; (B) quantify the amount of protein extracted; and (C) normalize the amount of protein and set up tryptic digestion. These protocols have been developed to facilitate rapid, low variance sample preparation of hundreds of samples, be easily implemented on widely-available Beckman-Coulter Biomek automated liquid handlers, and allow flexibility for future protocol development. By using this workflow 50 micrograms of peptides for 96 samples can be prepared for tryptic digestion in under an hour. We validate these protocols by analyzing 47 *E. coli* and *R. toruloides* samples and show that this modular workflow provides robust, reproducible proteomic samples for high-throughput applications. The expected results from these protocols are 94 peptide samples from Gram-negative bacterial and fungal cells prepared for bottom-up quantitative proteomic analysis without the need for desalting column cleanup and with peptide variance (CVs) below 15%.

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Automation, Sample preparation, Proteomics, Protein quantification, Biomek, Lowry assay, Cell lysis, Normalization

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- A Beckman-Coulter Biomek FX liquid handler system with a 96-pod head is used for the protein extraction and quantification protocols. Alternative liquid handlers can be used with appropriate method development.

- A Beckman-Coulter Biomek NX-S8 or NXP liquid handler system with an 8-pod head is used for the normalization protocol. Alternative liquid handlers can be used with appropriate method development.

- A Molecular Devices Spectramax 250 microplate reader is used for the protein quantification assay measurement.

Notes:

- This protocol is set up to measure the amount of protein in duplicate.

For this protocol you will need:

- a Beckman-Coulter Biomek FX liquid handler system with a 96-pod head or similar liquid handler system
- a Beckman-Coulter Biomek NX-S8 or NXP liquid handler system with an 8-pod head or similar liquid handler system
- an Eppendorf 5810R centrifuge with S-4-104 rotor or similar centrifuge

Wear proper PPE (gloves, safety goggles, and lab coat), and prepare solvents in a

chemical fume hood.

Store organic solvents in a flammable storage cabinet when not in use.

Discard used solvents and buffers in appropriate waste containers.

DISCLAIMER:

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Manual proteomic sample preparation methods limit sample throughput and often lead to poor data quality when thousands of samples must be analyzed. Automated workflows are increasingly used to overcome these issues for some (or even all) of the sample preparation steps. Here, we detail three optimised step-by-step protocols to: (A) lyse Gram-negative bacteria and fungal cells; (B) quantify the amount of protein extracted; and (C) normalize the amount of protein and set up tryptic digestion. These protocols have been developed to facilitate rapid, low variance sample preparation of hundreds of samples, be easily implemented on widely-available Beckman-Coulter Biomek automated liquid handlers, and allow flexibility for future protocol development. By using this workflow 50 micrograms of peptides for 96 samples can be prepared for tryptic digestion in under an hour. We validate these protocols by analyzing 47 *E. coli* and *R. toruloides* samples and show that this modular workflow provides robust, reproducible proteomic samples for high-throughput applications. The expected results from these protocols are 94 peptide samples from Gram-negative bacterial and fungal cells prepared for bottom-up quantitative proteomic analysis without the need for desalting column cleanup and with peptide variance (CVs) below 15%.

FILES

- **Automated Chloroform-Methanol Protein Extraction on the Biomek-FX Liquid Handler System**
Version 1
by Jennifer Gin, Lawrence Berkeley National Laboratory
- **Automated Protein Quantification with the Biomek-FX Liquid Handler System**
Version 1
by Jennifer Gin, Lawrence Berkeley National Laboratory
- **Automated Protein Normalization and Tryptic Digestion on a Biomek-NX Liquid Handler System**
Version 1
by Christopher Petzold, Lawrence Berkeley National Laboratory