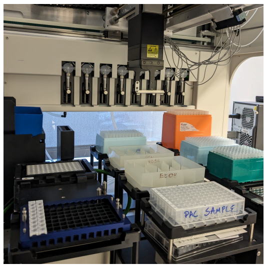
Dec 20, 2023 Version 1

Modular automated sample processing of biological samples using ultrasonication and a workstation for high-throughput proteomics V.1



DOI
dx.doi.org/10.17504/protocols.io.j8nlkoy56v5r/v1

ronan o'cualain[1]

[1]University of Manchester

protocols.io Ambassadors BioMS CRF, UoM

ronan o'cualain
Inoviv

DOI: https://dx.doi.org/10.17504/protocols.io.j8nlkoy56v5r/v1

Collection Citation: ronan o'cualain 2023. Modular automated sample processing of biological samples using ultrasonication and a workstation for high-throughput proteomics. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkoy56v5r/v1

Protocol status: Working
We use this collection and it's working

Created: December 12, 2023

Last Modified: April 10, 2024

Collection Integer ID: 92197

Keywords: throughput proteomics sample preparation for mass spectrometry analysis, throughput proteomics sample preparation, proteomic, qi for proteomic, ultrasonication of biological sample, sample preparation for mass spectrometry analysis, processing of biological sample, protein extraction, sample processing of biological sample, mass spectrometry analysis, progenesis qi for proteomic, biological sample, sample preparation, using ultrasonication, sample lysi, automated sample processing, sample processing, ultrasonication, biomek nxp workstation, using hela extract, protein, hela extract at the entry stage, extraction, peptide, enzymatic digestion, using progenesis qi, using progenesi, data processing stage

Abstract

Sample preparation for mass spectrometry analysis involves **numerous** liquid transfer steps.

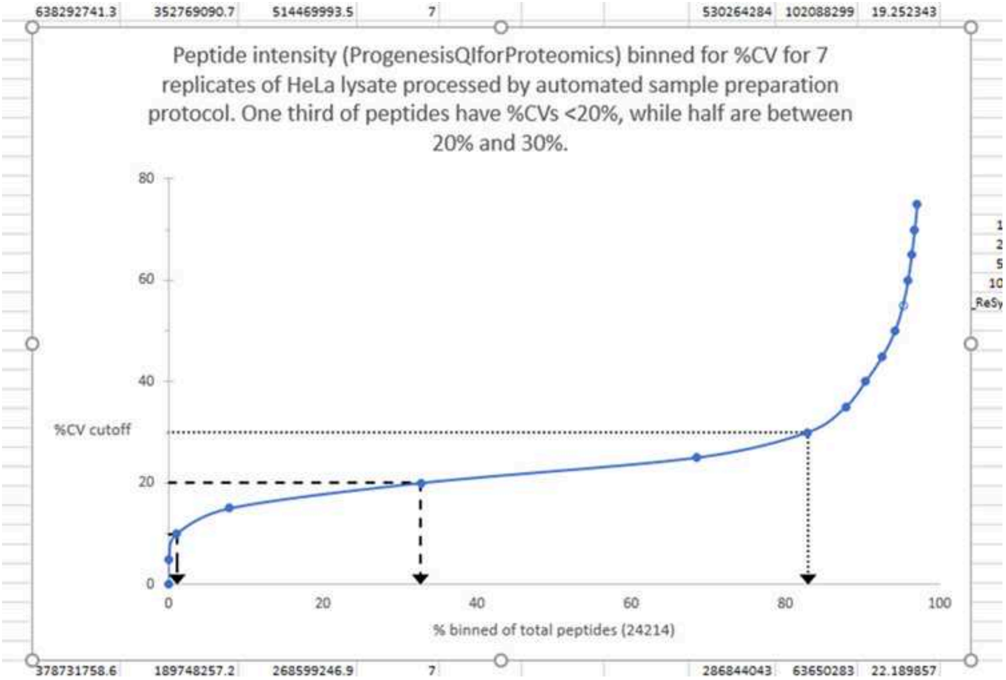
These include
- **sample lysis,**
- **protein extraction,**
- **solubilisation,**
- **estimation,**
- **reduction and alkylation,**
- **normalisation,**
- **clean-up,**
- **enzymatic digestion,**
- **and desalting.**

Adapting these steps onto an automated workstation can increase efficiency, throughput, and reduce coefficients of variance (**%CV**) thereby providing reliable reproducible data for statistical comparisons.

This protocol is part of a modular collection for the processing of biological samples for proteomics.

The entry point is ultrasonication of biological samples (cells, tissues, laser captured FFPE sections) using a plate based LE220+ system from Covaris, followed by sample processing with a Biomek NxP workstation.

Technical measurement of workflow percentage coefficients of variation (%CVs) using HeLa extract at the entry stage, to measurement of the ion intensities at the data processing stage with data collected by DDA using a QE-HF and processed using Progenesis-QI for proteomics has shown that one third of peptides have **%CVs** below **20%**, and with 80% of peptides having %CVs below **30%**. Using Progenesis QI for proteomics indicates that 98% of peptides are found in all 7 replicates processed.



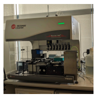
In addition, the whole procedure (80 samples) may be completed in a day, if shorter digestion times are utilised.

Troubleshooting

Files

SEARCH

Protocol



NAME

Automated 96 well plate based protein reduction and alkylation using a Beckman Biomek™ NxP workstation

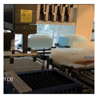
VERSION 1

CREATED BY

ronan o'cualain
Inoviv

OPEN →

Protocol



NAME

Automated 96 well plate based protein quantitation using a Beckman Biomek™ NxP workstation and a Pierce™ 660nm Protein Assay Kit

VERSION 1

CREATED BY

ronan o'cualain
Inoviv

OPEN →

Protocol

NAME

Protein aggregation capture (PAC) and minimal automated processing for proteomics using magnetic beads and a Beckman Biomek™ NxP workstation for 96 well plates.

VERSION 1

CREATED BY

ronan o'cualain
Inoviv

OPEN →