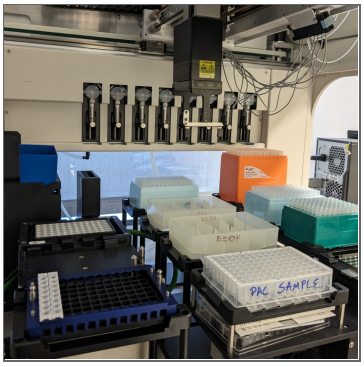

Modular automated sample processing of biological samples using ultrasonication and a workstation for high-throughput proteomics V.1

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VERSION 1

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

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**

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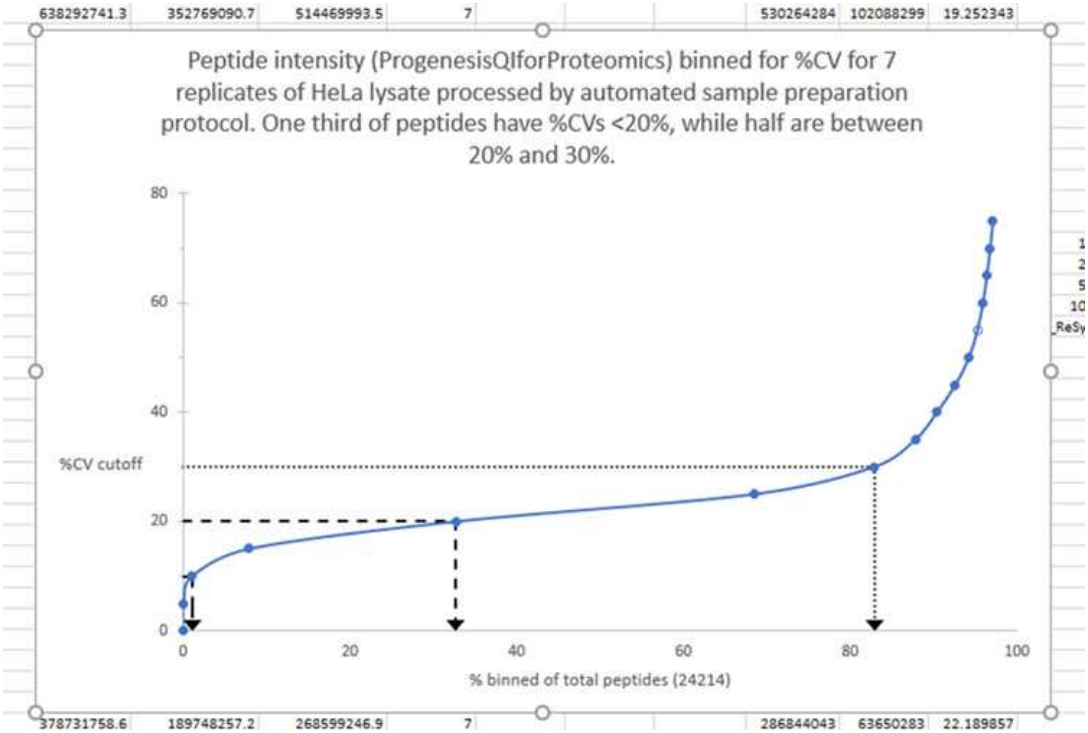
below **20%**, and with 80% of peptides having %CVs below **30%**. Using Progenesis QI for proteomics indicates that 98% of peptides are found in all 7 replicates processed.

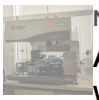


In addition, the whole procedure (80 samples) may be completed in a day, if shorter digestion times are utilised.

FILES

SEARCH

Protocol



NAME

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

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NAME

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

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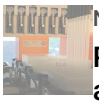

NAME

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

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