

Optimized FFPE tissue processing: Enhancing flexibility and scalability with BeatBox® Tissue Kit 24x for in-depth proteome analysis

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INTRODUCTION

FFPE tissues are invaluable for translational medicine. The BeatBox FFPE workflow for the Tissue Kit 96x provides a high-throughput solution for FFPE tissue processing, eliminating the need for separate de-paraffinization steps. However, it is limited to small sample inputs (up to 2 FFPE curls per reaction), making it unsuitable for workflows requiring higher input (e.g., PTM analysis). The BeatBox FFPE workflow for the Tissue Kit 24x addresses this limitation, enhancing compatibility with various analytical needs.

MATERIALS & METHODS



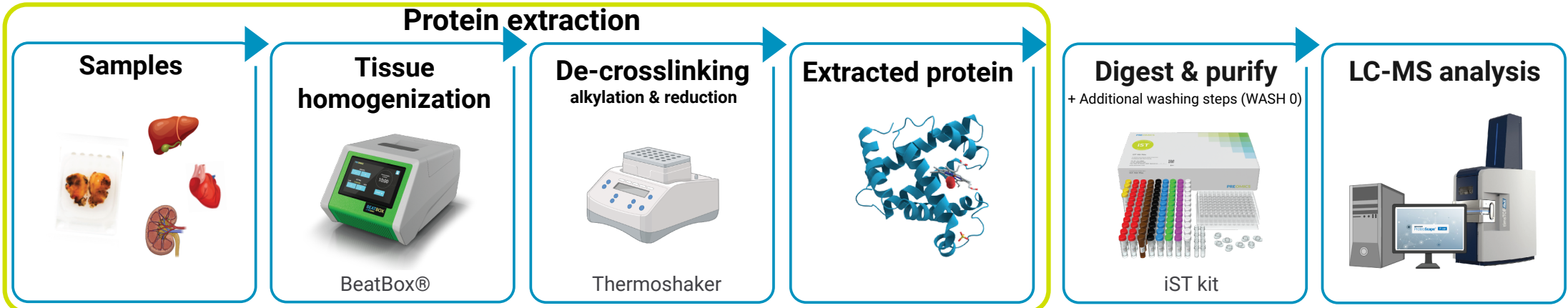
The BeatBox Tissue Kit 24x ("tubes") was tested with full curl ("FC") FFPE samples.



The BeatBox Tissue Kit 96x ("plate") was used as control workflow.



A full (not deparaffinized) FFPE curl in a 2 mL tube.

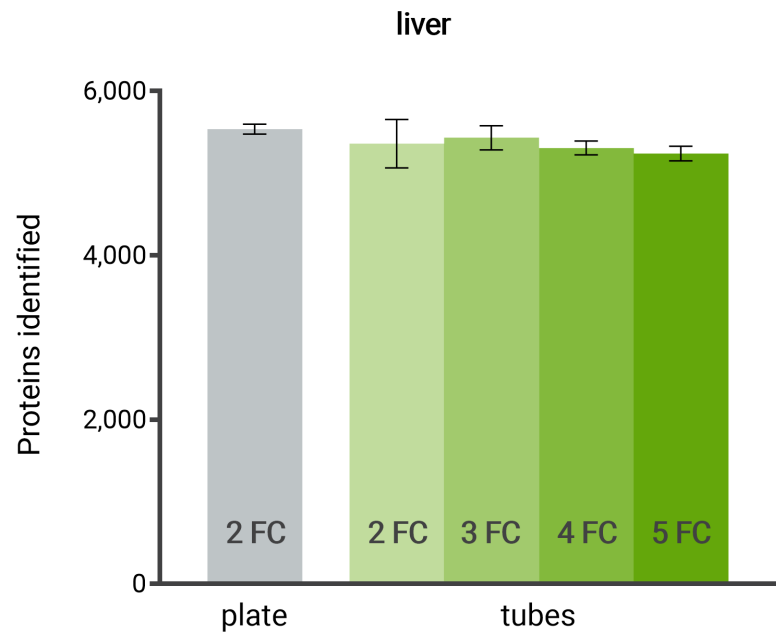
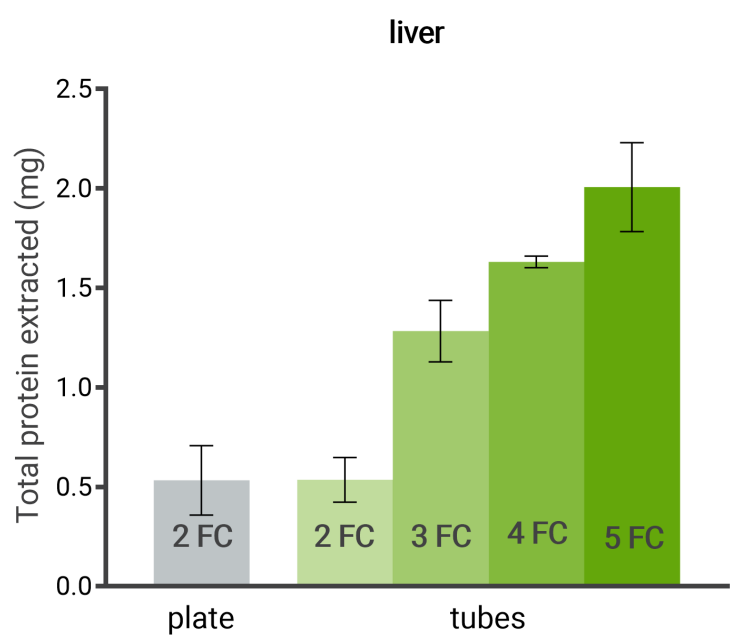
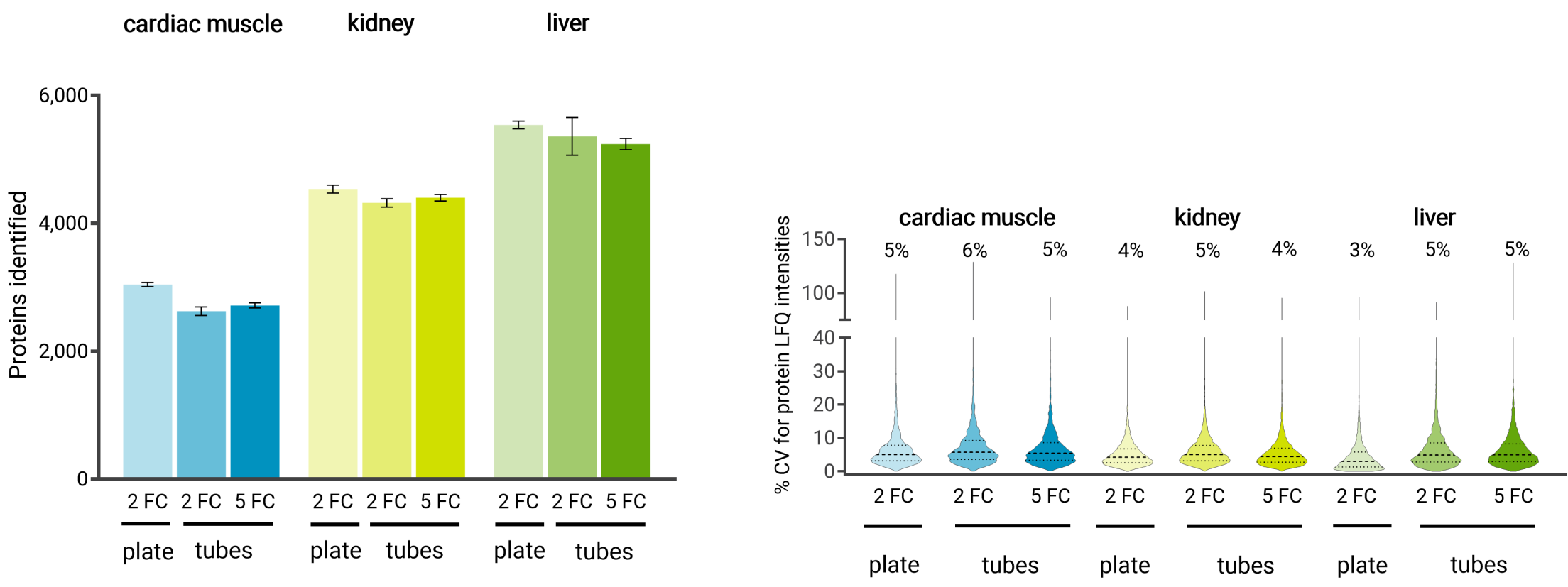


Input: Formalin-fixed, paraffin-embedded (FFPE) mouse tissue: 10-µm sections of full curls and 1-mm diameter punches.

Sample preparation: 2–5 curls per reaction were placed in a 24x Tissue Kit tube or 2 curls in a well of the 96x Tissue Kit plate. 100–500 µL LYSE was added, followed by heating on a thermoshaker at 95°C for 10 min to ensure complete wetting of samples in tubes. Homogenization was performed using BeatBox (PreOmics) for 10 min at HIGH power, then de-crosslinking for 1h at 95°C. Proteins were processed with the iST 96x kit (PreOmics). FFPE punches were processed similarly using the 24x tube kit with an additional beating and heating step.

LC-MS analysis & data processing: 300 ng peptides were analyzed on nanoElute – timsTOF HT (Bruker Daltonics) in DIA-PASEF® mode with 30-min gradient. Data analysis was performed with Bruker ProteoScape™ (tims DIA-NN). Outlook section: 300 ng peptides were analyzed on Easy-nLC – timsTOF Pro in DIA-PASEF mode with 30-min gradient. Data analysis was performed with Spectronaut™.

RESULTS

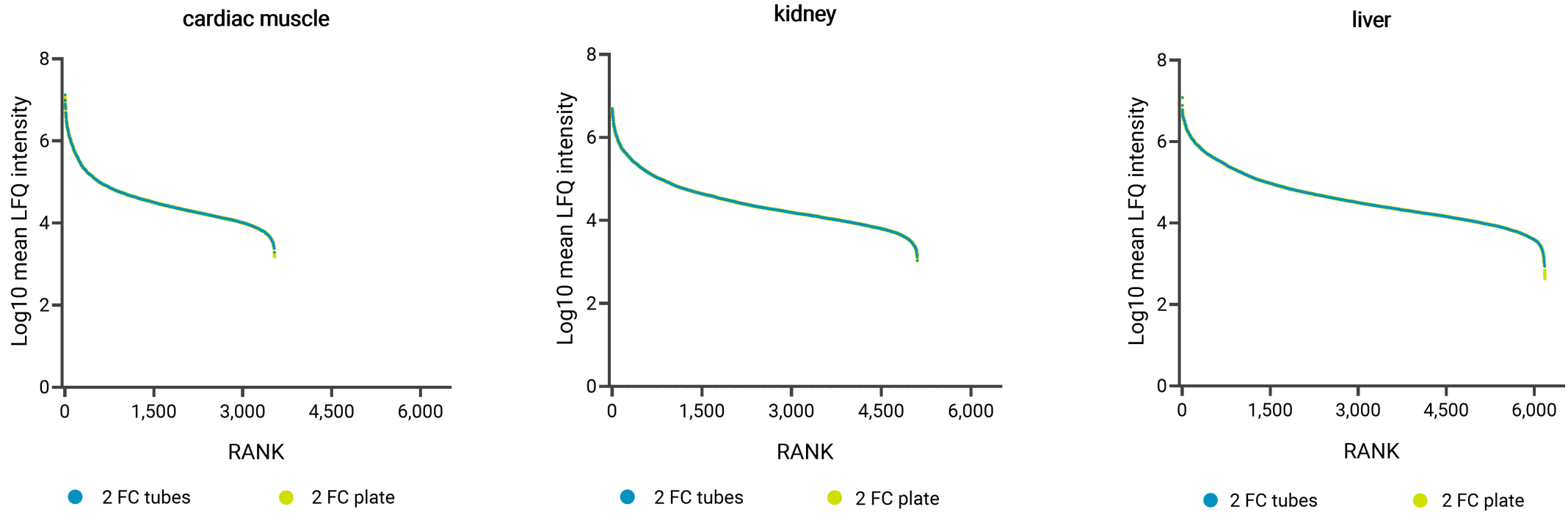
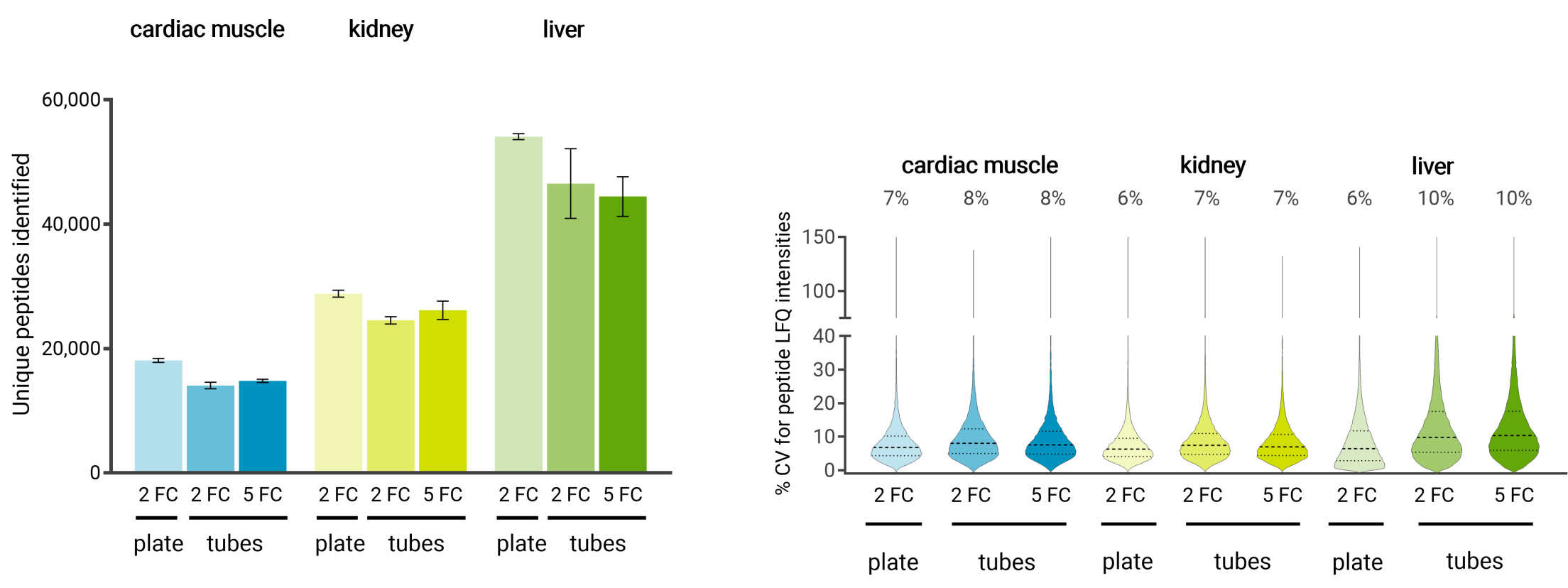


Comparison of protein yields. Increasing the starting amount from two to five curls resulted in a consistent increase in protein yield.

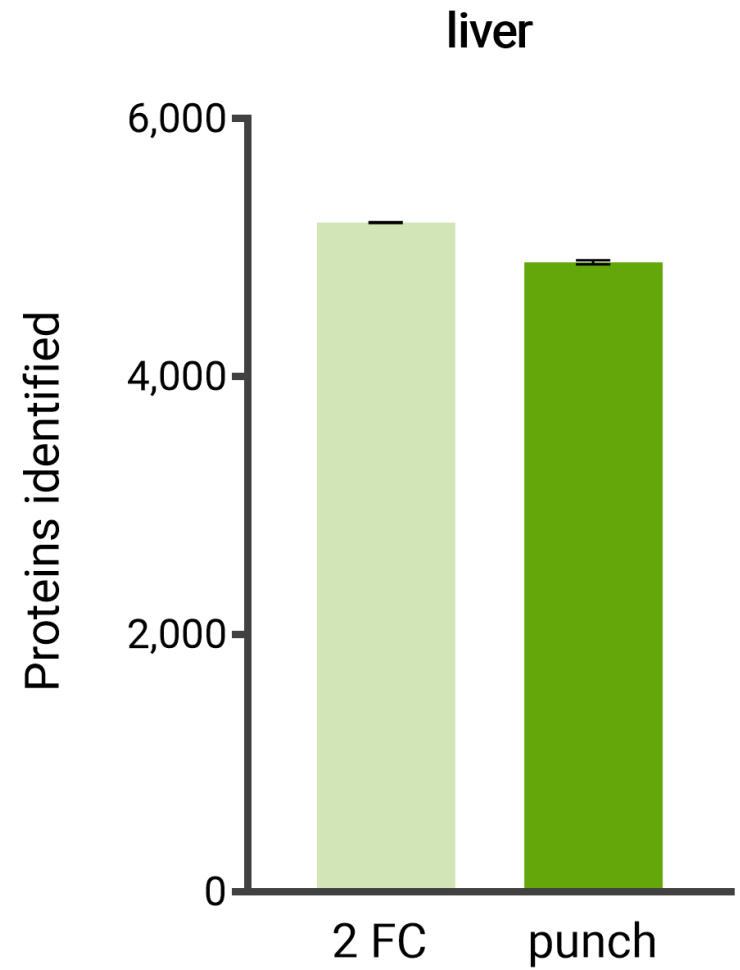
Consistent LC-MS results. The tube workflow provided reliable protein identifications comparable to the plate protocol across all tested input amounts.



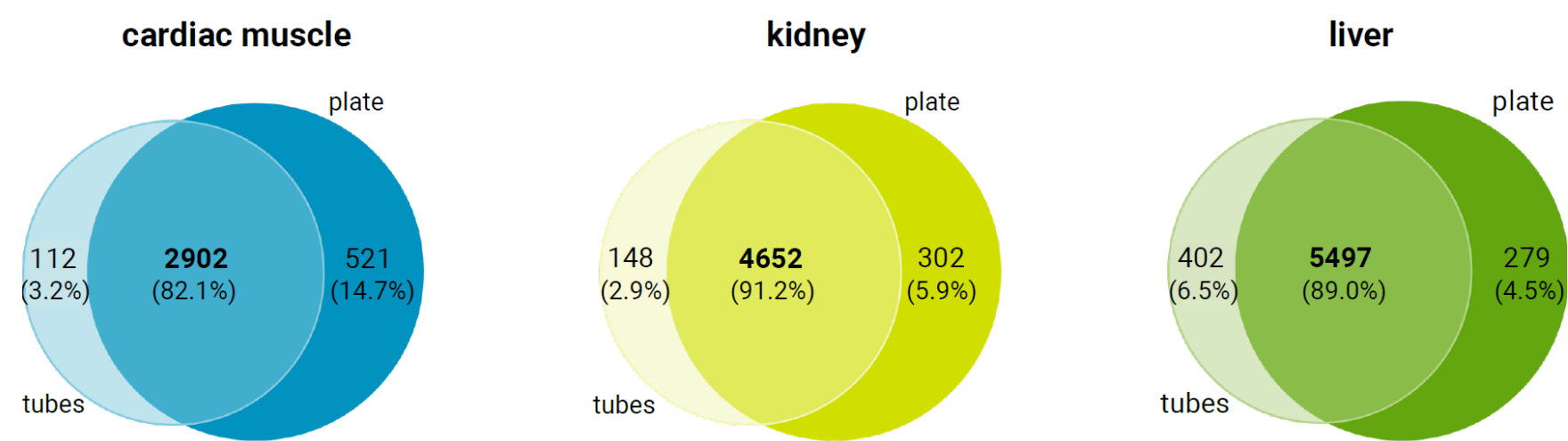
A 1-mm FFPE punch in a 2 mL tube.



Deep proteome coverage. Protein quantification demonstrated an identical dynamic range (~4 orders of magnitude) and similar proteomic depth for full curl FFPE tissue samples processed using either tubes or plate.



Comparison of identified proteins and unique peptides. Using the BeatBox FFPE workflow for 2–5 FFPE tissue sections (10 µm), only minor differences were observed between the plate and tube workflows. When evaluating the respective CVs at the peptide and protein levels, similar performance and precision were observed, with CVs in the range of 7–10% for precursors and 4–6% for proteins.



Shared proteins. A high overlap of protein identifications (82–91%) was achieved for full curl FFPE samples processed with the tube or plate workflow for all three mouse tissue types.

KEY TAKEAWAYS

- The optimized solution combining BeatBox and iST technology enables simple, fast, and robust processing of FFPE tissue for LC-MS-based proteomics.
- The new BeatBox FFPE tube workflow enhances flexibility over the plate-based format by expanding its capacity to efficiently process higher input amounts of FFPE samples.
- With its exceptional repeatability, the BeatBox FFPE workflow for the Tissue Kit 24x ensures efficient homogenization and high sample quality across a range of tissue types, from soft to hard.

With intuitive handling, flexibility regarding sample inputs, and a streamlined, standardized process, this BeatBox FFPE workflow minimizes hands-on time while ensuring consistent, reliable, and reproducible results for both small- and large scale retrospective proteomic analyses.

CONTACT & MORE



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Conflict of Interest Disclosure
Abreha, M, S. Wuertenberger, J. Johansson, K. Hartinger, and N.A. Kulak are employed by PreOmics GmbH.

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