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Optimized preparation of Formalin-Fixed Paraffin-Embedded (FFPE) tissue enables flexible and scalable processing for in-depth proteome analysis

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INTRODUCTION

Formalin-fixed, paraffin-embedded (FFPE) tissues represent an invaluable resource for translational and clinical research, enabling retrospective studies across large, well-annotated cohorts. However, chemical crosslinking, limited sample amounts, and heterogeneous sampling formats such as curls and punches complicate robust, scalable proteomic analysis.

The BeatBox–iST FFPE workflow enables high-throughput plate-based processing without separate deparaffinization, supporting retrospective proteomic studies. Still, it was limited to small inputs of up to two FFPE curls.

Here, we present an adapted tube-based FFPE sample preparation workflow that overcomes these limitations. Using heart and liver tissues, we demonstrate flexible, scalable processing of higher input amounts as well as different FFPE formats.

MATERIALS

Tissue:

- FFPE mouse heart and liver.
- **Sectioning:** 10-µm full curls.
- **Punch geometry:** 1x1 mm (dxh).

Workflow scaling:

- **Plate vs. tubes:** 2 curls/well (plate) vs. 2–5 curls/tube (tubes).
- **Curl vs. punch:** 2 curls/tube vs. 1 punch/tube.

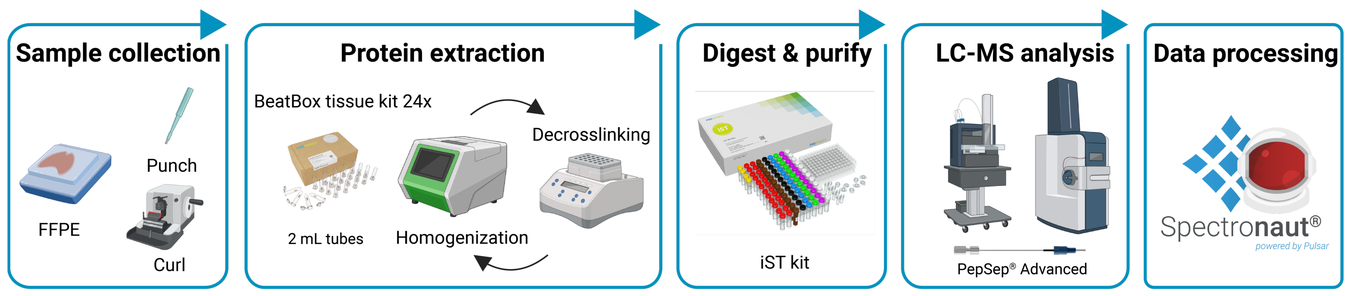
METHODS

Sample preparation:
Quadruplicates of FFPE tissue curls or punches were homogenized using BeatBox® (PreOmics) in the Tissue Kit 96x plate format (curls) or Tissue Kit 24x tube format with iST LYSE buffer, decrosslinked on a heating shaker followed by iST-based digestion and cleanup.

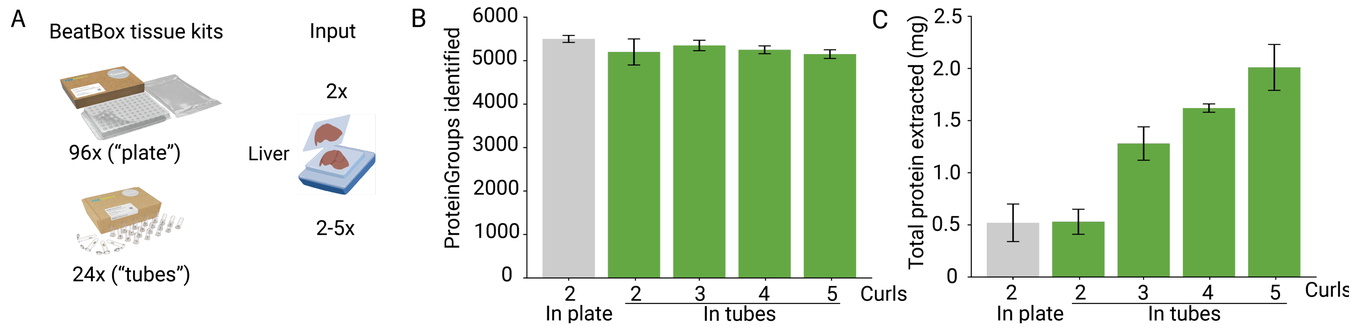
LC-MS analysis:
300 ng of peptides were analyzed using nanoElute® 2 HPLC (Bruker) equipped with either a PepSep® Advanced (Bruker) or an integrated-emitter (Competitor) C18 UHPLC column (25 × 75 for both), operated with a 30-min gradient and coupled to a timsTOF HT mass spectrometer (Bruker) in dia-PASEF® mode.

Data analysis:
Raw files were searched in Spectronaut® 20 (Biognosys) using directDIA+™. GO enrichment was performed using significantly regulated genes, with all quantified genes serving as the background set in STRING-DB.

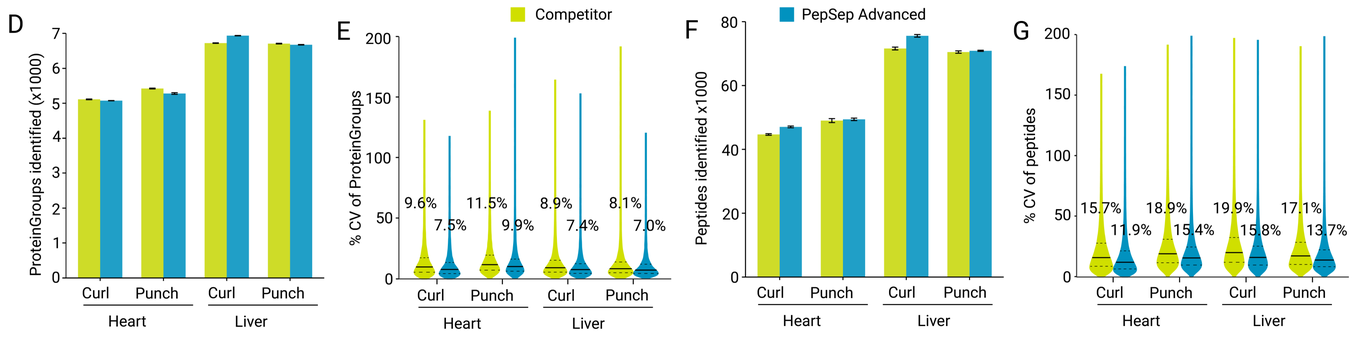
RESULTS



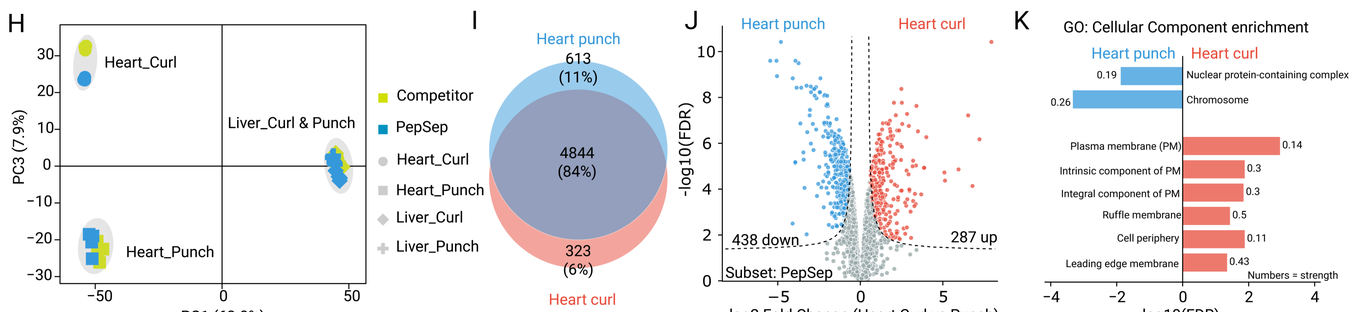
End-to-end FFPE proteomics workflow for curls and punches, combining the BeatBox and iST sample preparation with PepSep Advanced–coupled LC-MS analysis and Spectronaut data processing.



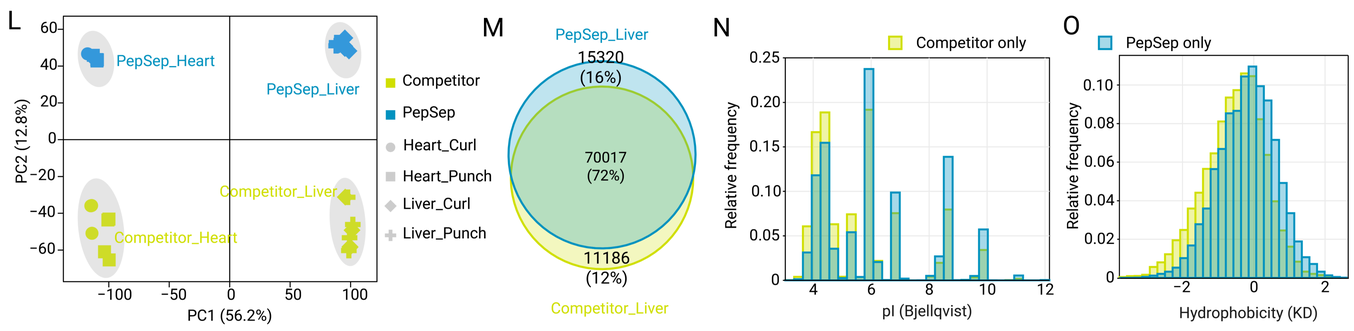
Benchmarking the scalable BeatBox Tissue Kit 24× against the 96× format as the control. Liver curls were processed using both formats (A). The 24× tube format matched the performance of the 96× plate format (B) while enabling higher input scalability (C), supporting flexible implementation without loss of proteome depth.



Comparable depth and improved reproducibility across FFPE sampling modes using the 24× workflow. Across heart and liver FFPE samples, curls and punches delivered similar protein- group (D) and peptide (F) identifications on both columns, achieving deep proteome coverage. PepSep consistently improved quantitative reproducibility, showing lower median CVs at both the protein (E) and peptide (G) levels across all conditions, highlighting its robust performance across FFPE sampling modes. Following benchmarking against the 96× control, the 24× tube format (two curls or one punch per sample) was selected and used for this and all subsequent experiments.



Tissue-dependent subcellular protein enrichment driven by sampling mode. Protein-level PCA is dominated by tissue type (PC1, 63%), with additional contributions from column type (PC2, 10%; discussed below at the peptide-level) and sampling mode (PC3, 8%), with sampling-mode-dependent separation observed in heart but not liver samples (H). In heart, FFPE punches and curls shared 84% of detected proteins (I). Integration of exclusively detected and differentially abundant proteins (PepSep subset; J) revealed distinct subcellular enrichment, with punch-associated proteins enriched for nuclear and chromosome-containing complexes, whereas curl-associated proteins are linked to plasma membrane, cell periphery, and leading-edge compartments (K).



Complementary peptide selectivity between columns. Column-dependent separation was analyzed at the peptide level, with PCA showing clear column-driven separation within each tissue (L). In liver, the majority of peptides are shared between columns (72%), with both columns contributing column-unique peptide fractions, including 16% for PepSep and 12% for the competitor (M). Physicochemical profiling of column-exclusive peptides showed that PepSep-only peptides extend toward higher pI values (more basic peptides; N) and display a more centralized hydrophobicity distribution (O), indicating peptide-level selectivity.

KEY TAKEAWAYS

Streamlined sample prep: The optimized solution combining BeatBox and iST technology enables simple, fast, and robust processing of FFPE tissue for LC-MS-based proteomics.

Flexibility and scalability: The new tube workflow increases flexibility by enabling efficient processing of up to five FFPE curls as well as single FFPE punches.

Robust FFPE proteomics across FFPE formats: Different FFPE tissues show similar protein and peptide depth for curls and punches regardless of the column used, with improved quantitative reproducibility on PepSep.

Tissue-specific biological differences driven by sampling mode: At the protein level, sampling-mode effects are pronounced in heart, with punches preferentially enriched nuclear and chromosome-associated proteins and curls favoring membrane-associated compartments.

Complementary peptide selectivity between columns: Peptide-level profiles show largely overlapping identifications with modest column-dependent selectivity, reflecting complementary physicochemical peptide subsets.

CONTACT & MORE

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Conflict of Interest Disclosure
Abreha, M. is employed by PreOmics Inc.
Hu, Z., Yeoh, K., Wuertenberger, S., Hartinger, K., and Kulak, N.A. are employed by PreOmics GmbH.