

BeatBox® and iST for streamlined FFPE-tissue processing: A xylene-free, robust, and high-throughput sample preparation for in-depth proteomic analysis

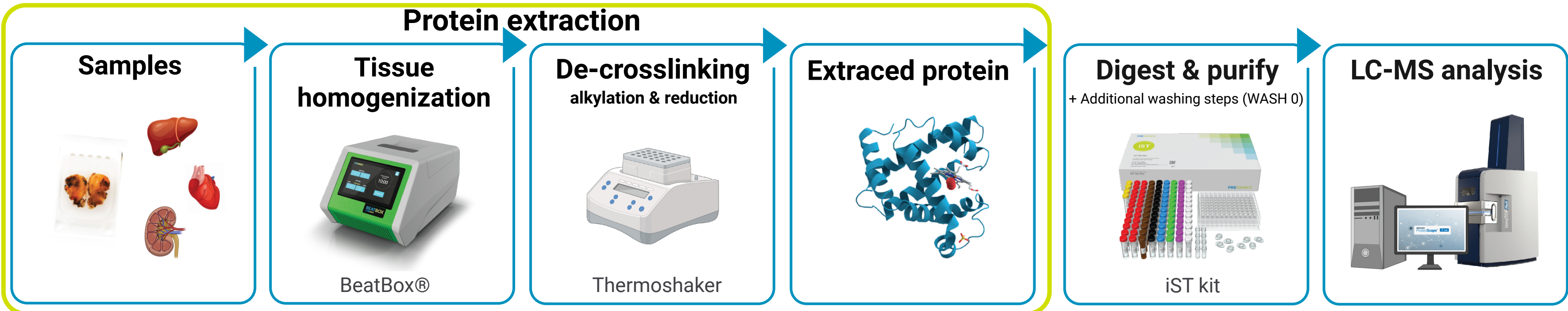
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SPOTLIGHT

LC-MS-based proteomics workflow from sample homogenization to data analysis

- Streamlined FFPE sample preparation workflow combining BeatBox® and iST technology
- High-throughput processing of up to 96 samples/day to clean peptides
- Optimized xylene-free approach without deparaffinization for LC-MS based proteomics
- BeatBox®-iST workflow performance with FFPE samples is equivalent to working with fresh frozen tissue
- Coupled to Bruker ProteoScope™ for improved and accelerated data processing

MATERIALS & METHODS



Input:

- Formalin-fixed, paraffin-embedded (FFPE) mouse tissue (cardiac muscle, kidney and liver; 10 µm curls)
- Fresh frozen mouse tissue (cardiac muscle, kidney and liver; 1-2 mg tissue)

Homogenization:

- BeatBox® + BeatBox Tissue Kit 96x (10 min, standard power settings for fresh frozen tissue as "Control Method" or high power settings for FFPE samples)

De-crosslinking / Denaturation&Alkylation

- FFPE: boiling for 1h at 95 °C
- Fresh frozen: boiling for 10 min at 95 °C

Sample digestion/peptide clean-up:

- iST workflow with optimized washing for FFPE full curls
- iST workflow for xylene-based deparaffinized tissue and fresh frozen tissue

LC-MS analysis:

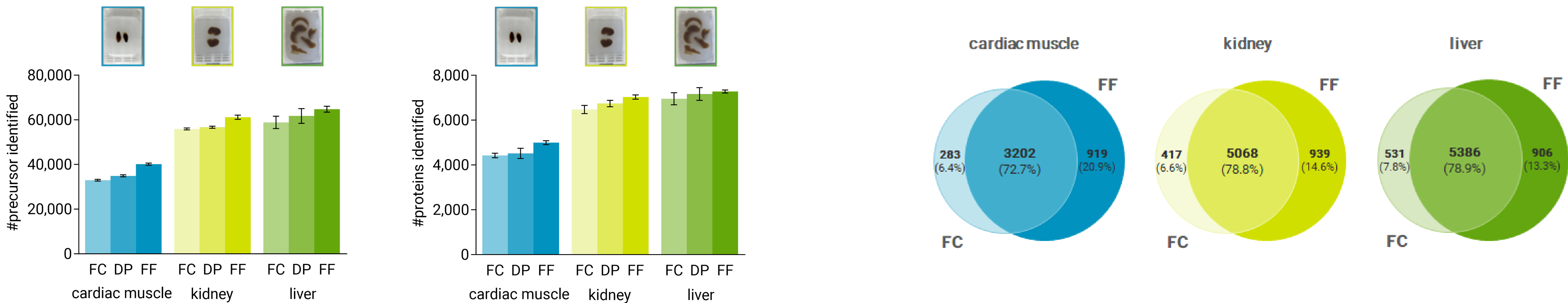
- EASY-nLC™ 1200 - TimsTOF HT
- DIA-PASEF acquisition; 30-min gradient

Data analysis:

- Bruker ProteoScope™
- timsDIA-NN, predicted Library from FASTA

RESULTS

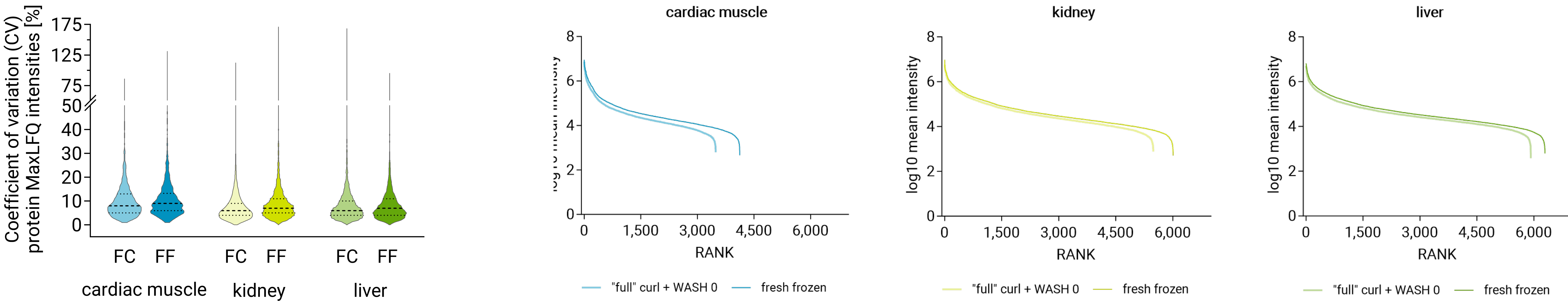
BeatBox®-iST FFPE workflow enables in-depth proteomic analyses without prior xylene-based deparaffinization



The novel BeatBox®-iST workflow for full FFPE curls (FC) was benchmarked against matching deparaffinized FFPE curls (DP) and fresh-frozen tissue, both processed with the BeatBox® homogenizer and iST sample preparation. Bruker ProteoScope™ was used for simplified and accelerated data processing, revealing similar precursor and protein identifications for all three starting conditions.

A high overlap of protein identifications (72-78%) was achieved for 'full' curl FFPE and fresh-frozen samples for all three mouse tissue types.

Comparison of repeatability and dynamic range obtained from FFPE tissue and fresh frozen tissue homogenized on BeatBox®



Compared to fresh frozen tissue, the BeatBox®-iST workflow with FFPE tissue shows similar overall variability. The CVs for FC and FF were below 10%, indicating high repeatability within quadruplicates for all sample types.

Protein quantification demonstrated a comparable dynamic range with approx. 4 orders of magnitude and a similar proteomic depth for 'full' curl FFPE tissue and fresh frozen tissue.

KEY TAKEAWAYS

- Optimized solution combining the BeatBox® and iST technology provides a simple, fast, and robust way to process FFPE tissue for LC-MS based proteomics.
- Preparing FFPE samples with BeatBox®-iST technology saves valuable time by reducing the number of steps and eliminates the need for xylene.
- Innovative FFPE workflow that provides similar overall performance and proteomic depth to fresh-frozen tissue making it ideal for large-scale retrospective studies of FFPE tissue.