

BeatBox and iST for standardized FFPE tissue processing: A robust, high-throughput, xylene-free sample preparation for proteomic analysis.

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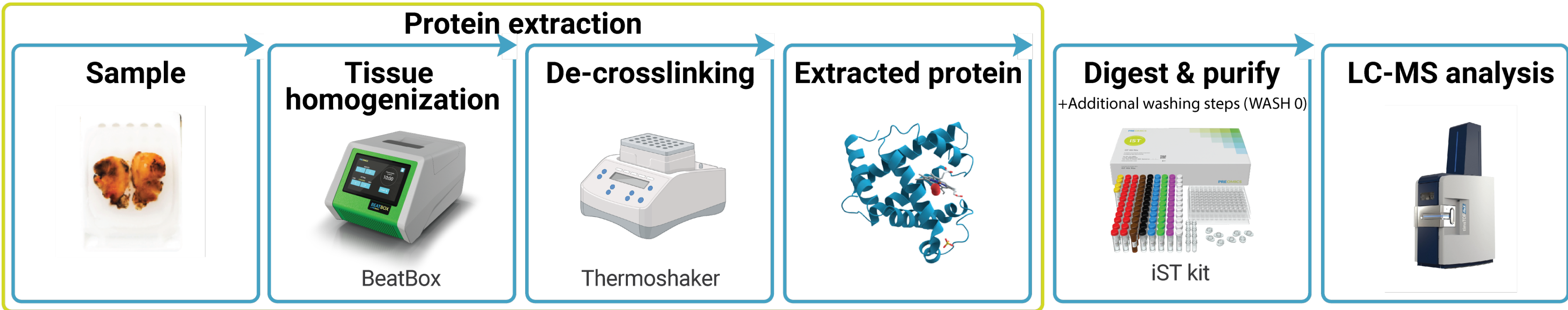
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SPOTLIGHT

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 ready-to-measure 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

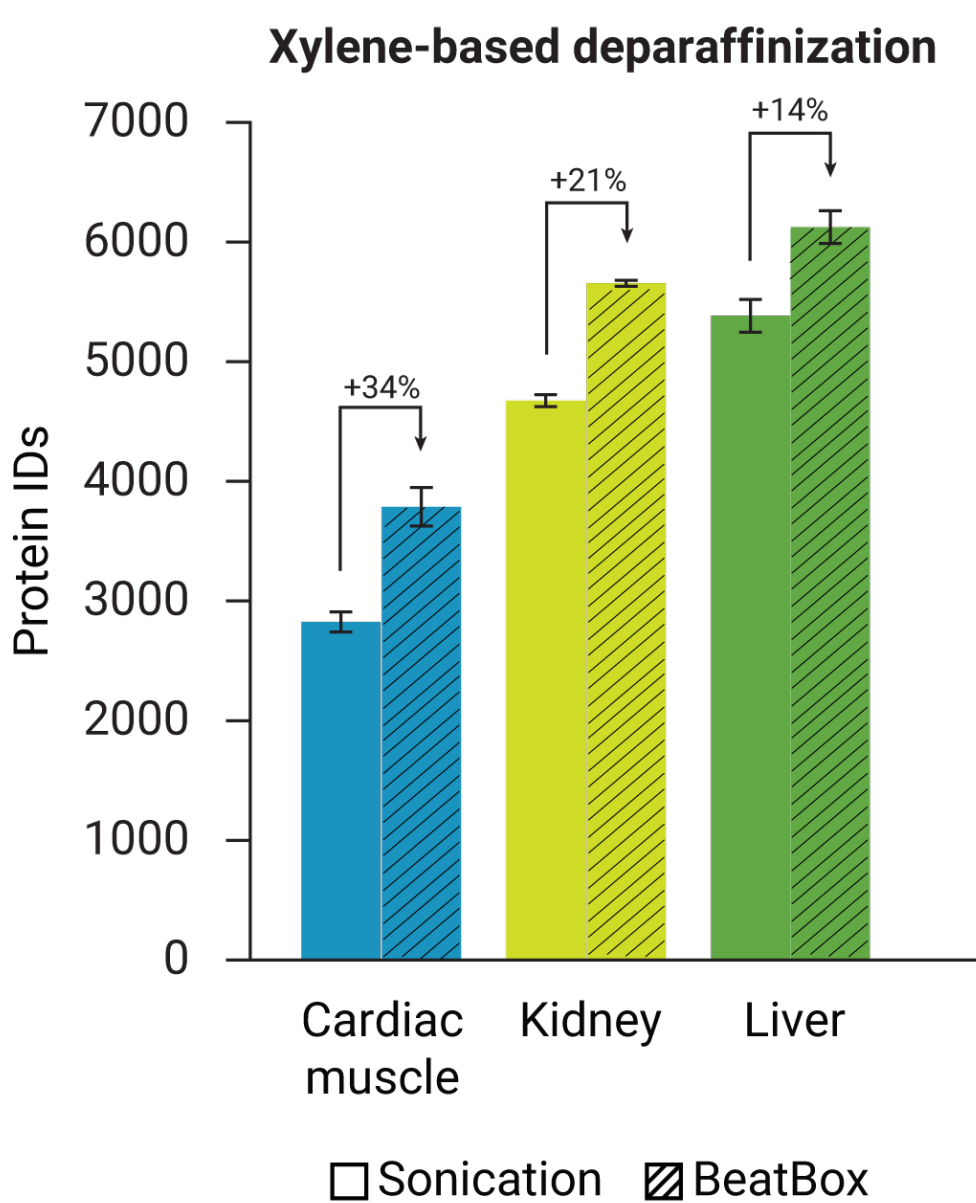
MATERIALS & METHODS



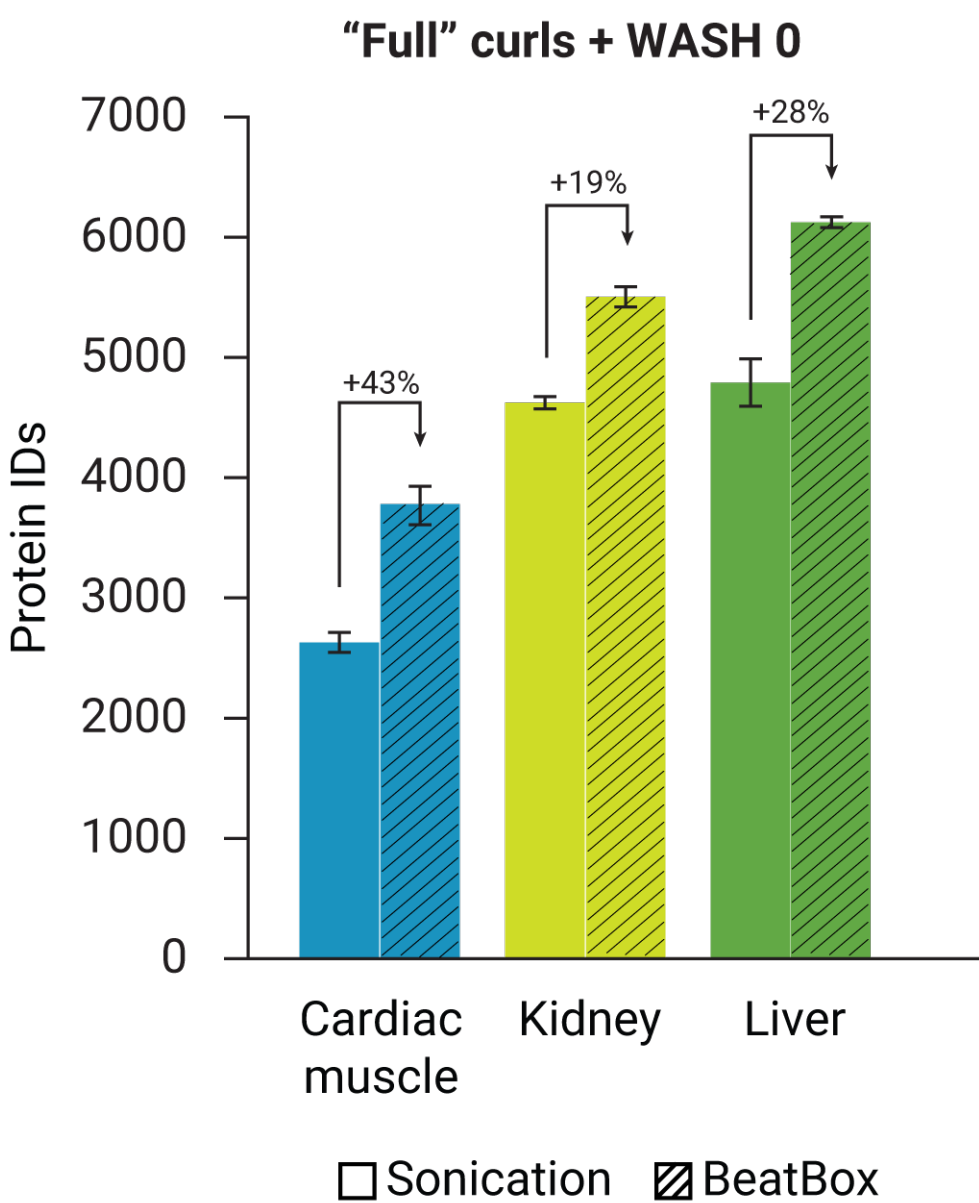
- Input:**
 - Formalin-fixed, paraffin-embedded (FFPE) mouse tissue (cardiac muscle, kidney and liver; 10 µm curls)
 - Fresh frozen mouse tissue (cardiac muscle, kidney and liver; 1-2 mg tissue)
- Homogenization:**
 - BeatBox + BeatBox tissue kit 96x (10 min, standard power settings for fresh frozen tissue as "Control Method" or high power settings for FFPE samples)
 - Bead-based sonication as "Control Workflow" (10 cycles; 30 sec ON/OFF)
- 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:** DIA-NN V1.8 library free

RESULTS

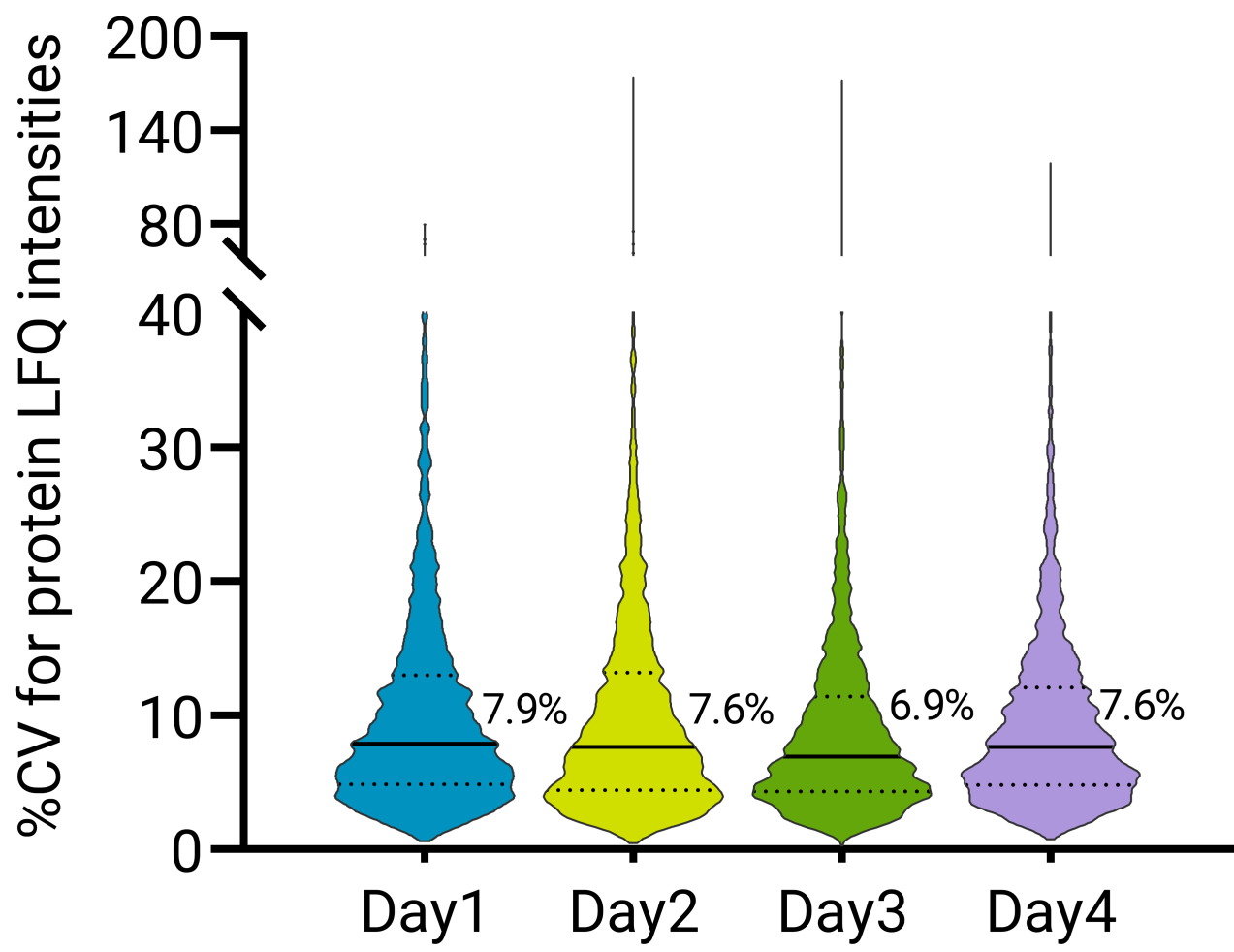
BeatBox-iST FFPE workflow allows for in-depth proteomic analyses without prior xylene-based deparaffinization



Xylene-deparaffinized tissue was homogenized via bead-based sonication or BeatBox resulting in an increase of identified proteins with BeatBox

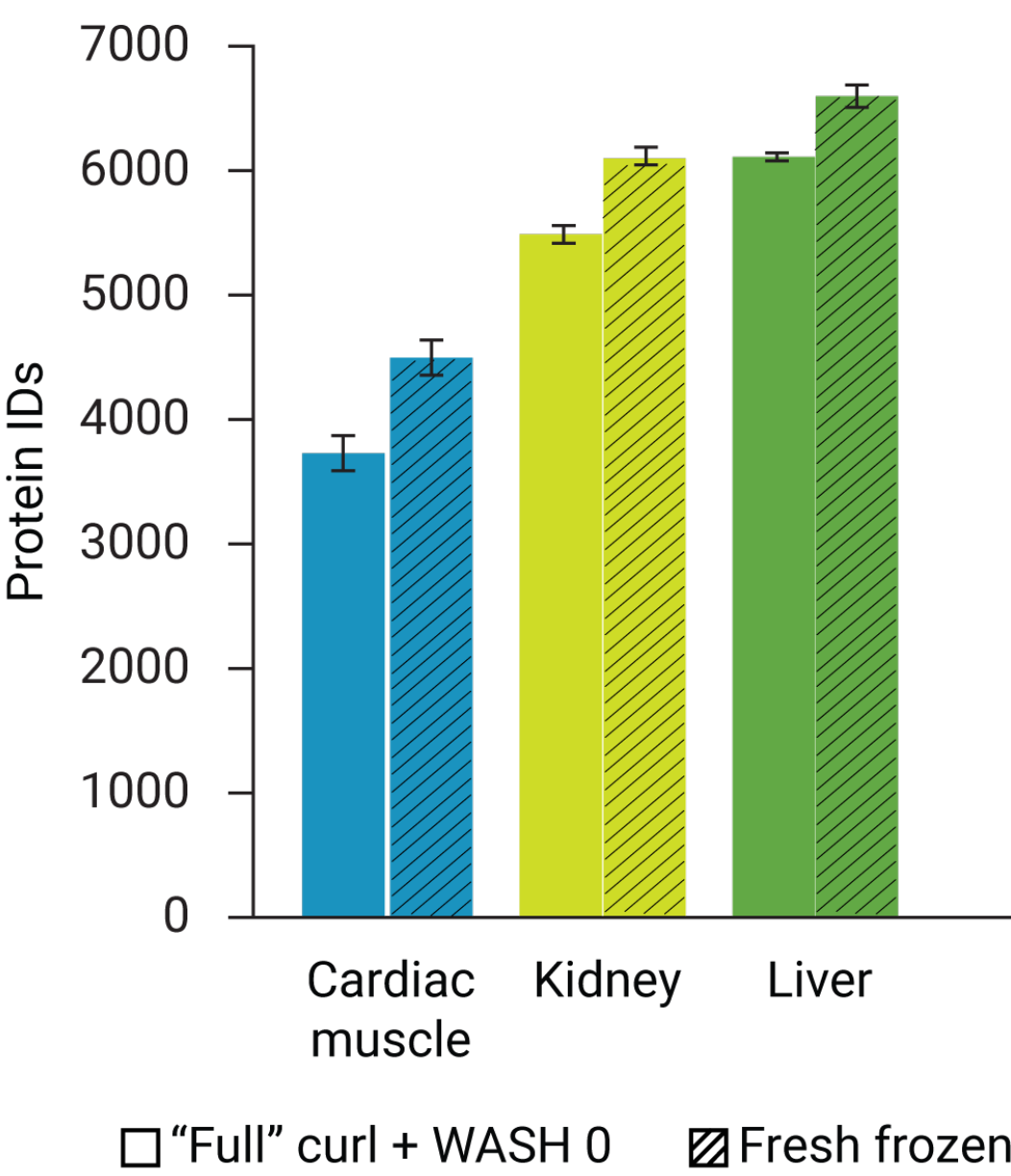


The optimized peptide purification using WASH 0 renders a separate xylene-based deparaffinization step unnecessary, for both the BeatBox and the sonication-based workflow

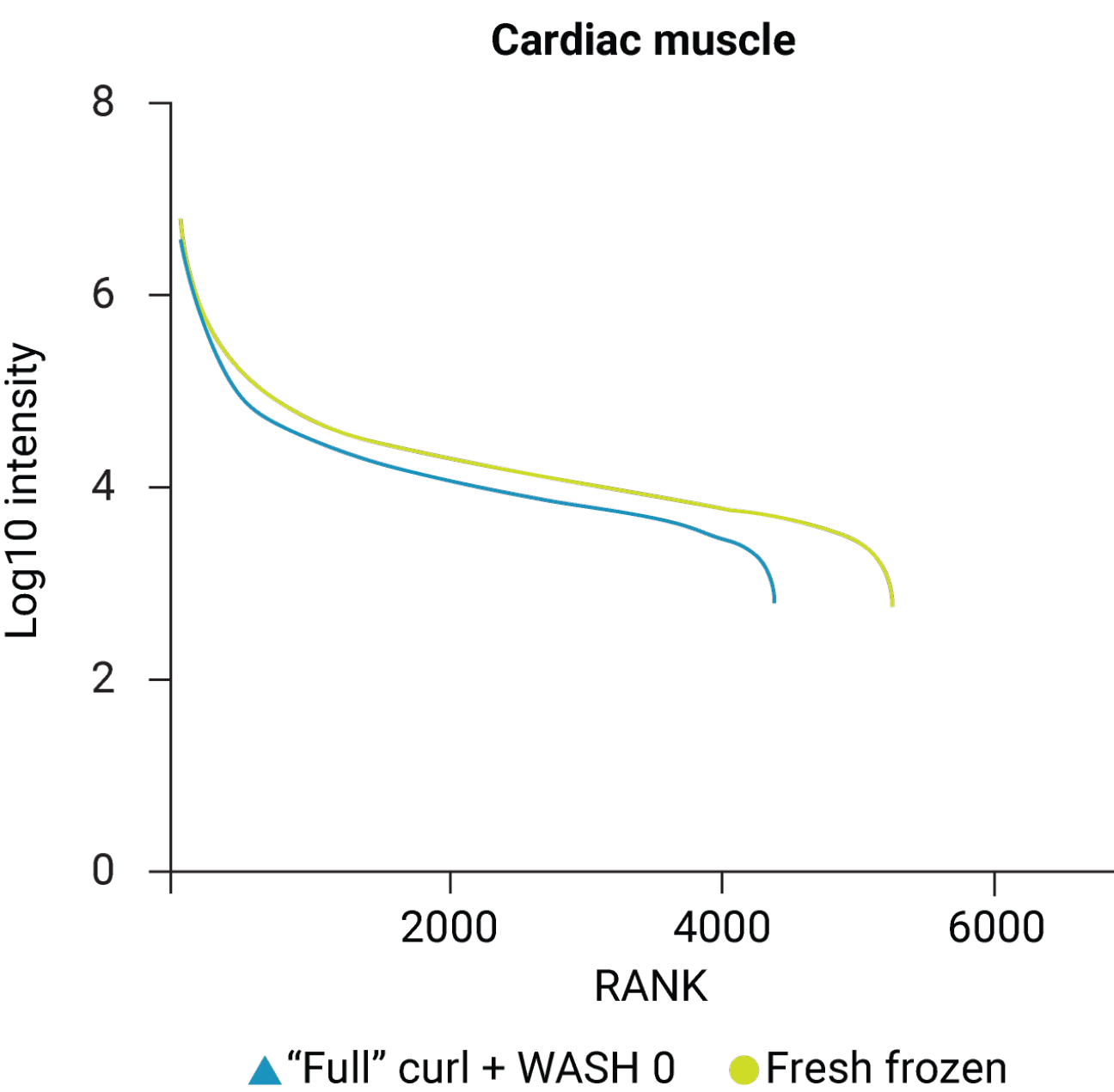


Intra-day repeatability of the BeatBox+iST workflow was assessed using full curl of mouse cardiac muscle which resulted in a median coefficient of variation of less than 10%

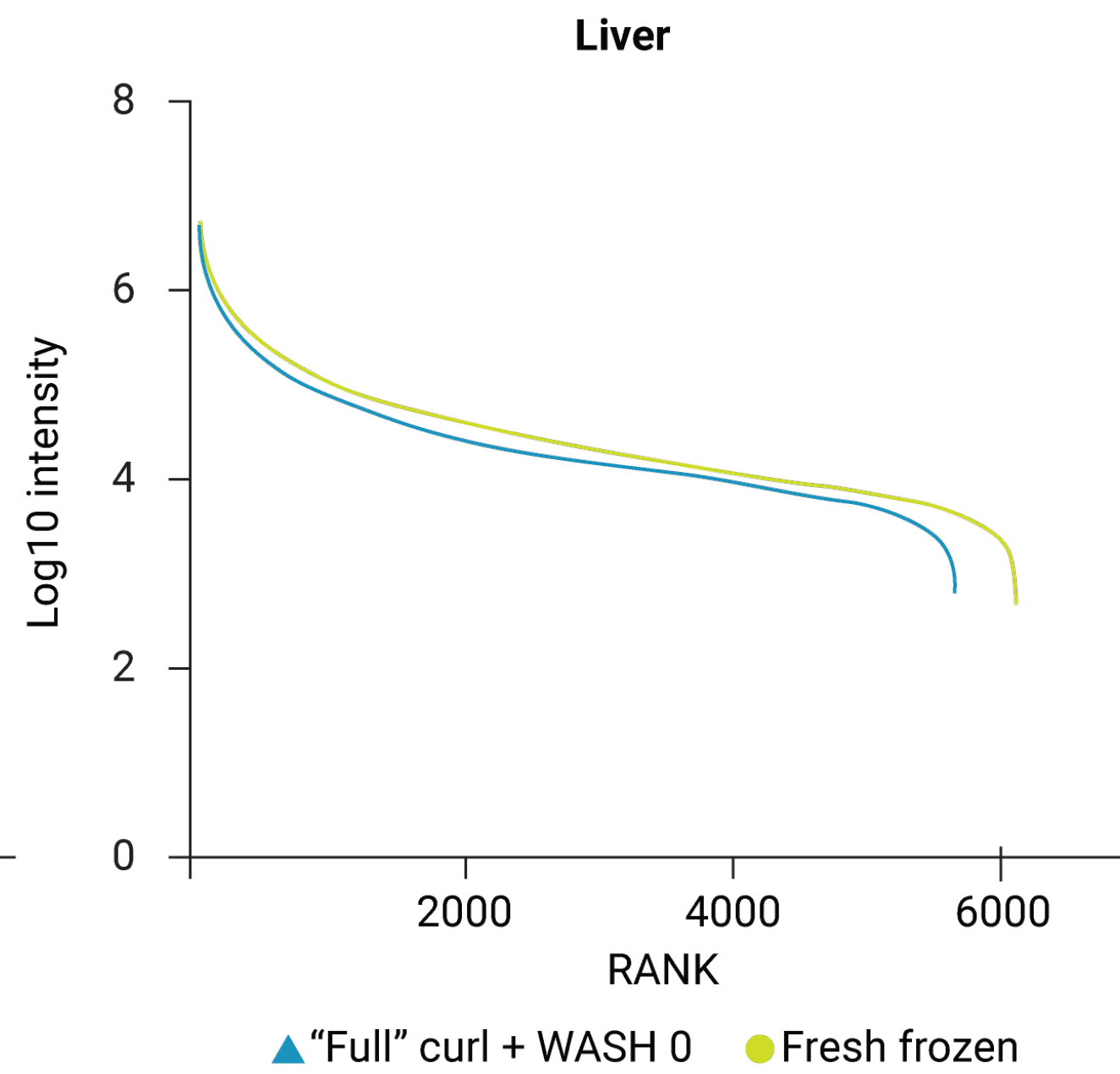
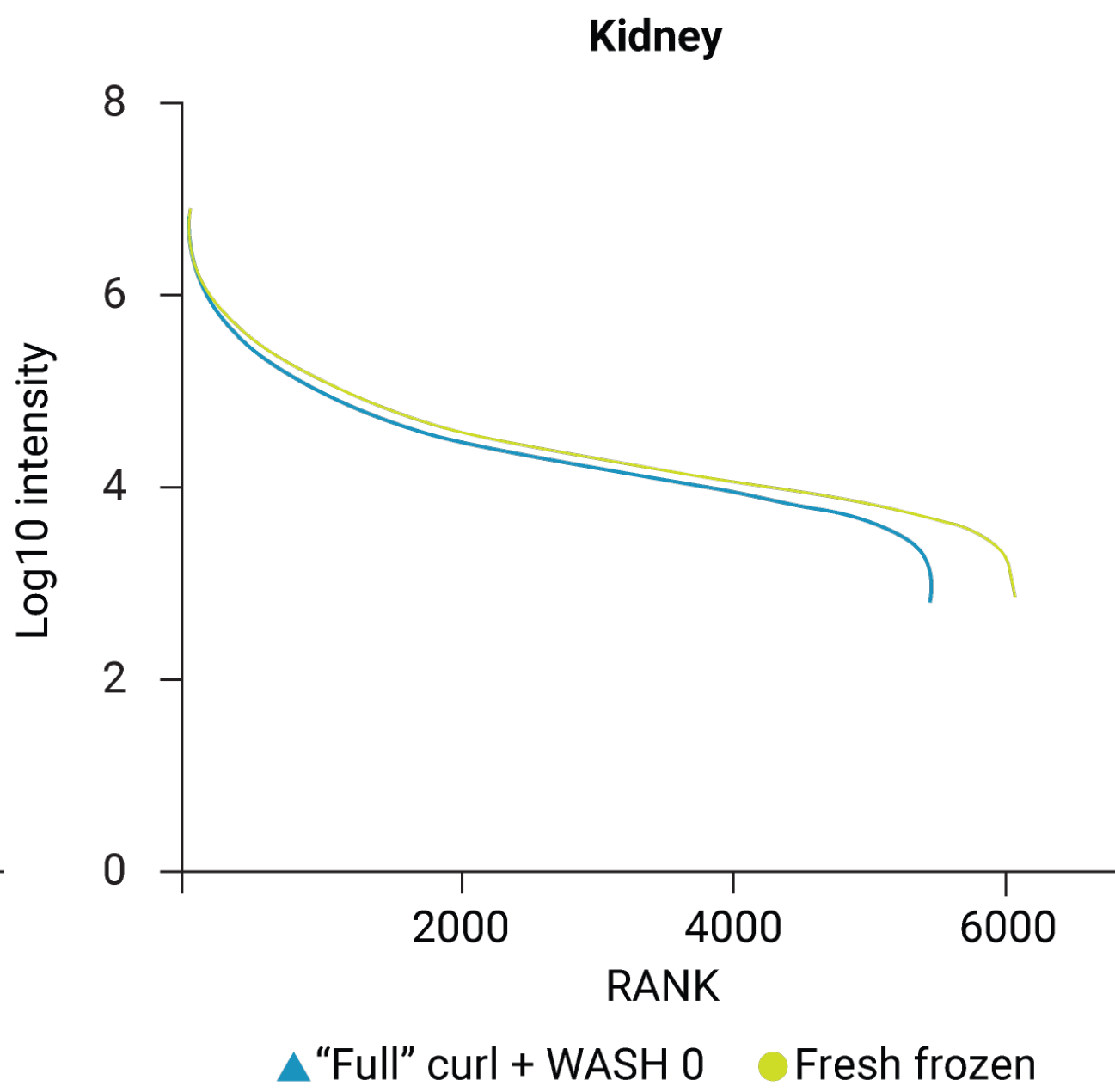
Comparison of identified proteins and dynamic range obtained from FFPE tissue and fresh frozen tissue homogenized on BeatBox



Compared to fresh frozen tissue, the BeatBox-iST workflow with FFPE tissue identifies similarly high numbers of proteins with equivalent variability



Evaluating proteins found in three out of four replicates, protein quantification exhibits similar dynamic ranges with approx. 4 order of magnitudes



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 workflow suitable for large-scale retrospective studies on FFPE tissue