PREOMICS

Unlocking the proteomic potential of FFPE tissues with BeatBox® and iST: A xylene-free, high-throughput workflow for in-depth proteome 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
- Equivalent workflow performance for FFPE samples compared to fresh frozen tissue
- Coupled to Bruker ProteoScape™ for improved and accelerated data processing

MATERIALS & METHODS

Input:

- Formalin-fixed, paraffin-embedded (FFPE) mouse tissue: 10-µm sections prepared with either xylene-based deparaffinized tissue or full curls
- Fresh frozen mouse tissue (cardiac muscle, kidney and liver; 1-2 mg tissue)

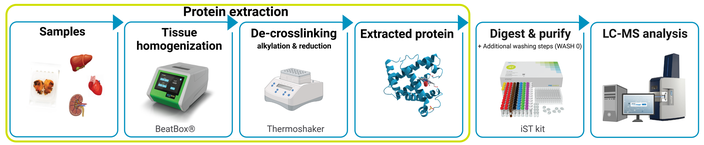
Sample Preparation:

- BeatBox homogenization: BeatBox Tissue Kit 96; 10 min, standard power settings for fresh frozen tissue as "control workflow" or high power settings for FFPE samples)
- Boiling: 1h at 95 °C for FFPE; 10 min at 95 °C for fresh frozen tissue
- iST workflow with optimized washing for FFPE full curls
- iST workflow: Standard workflow for deparaffinized and fresh frozen tissue; with optimized washing for FFPE full curls

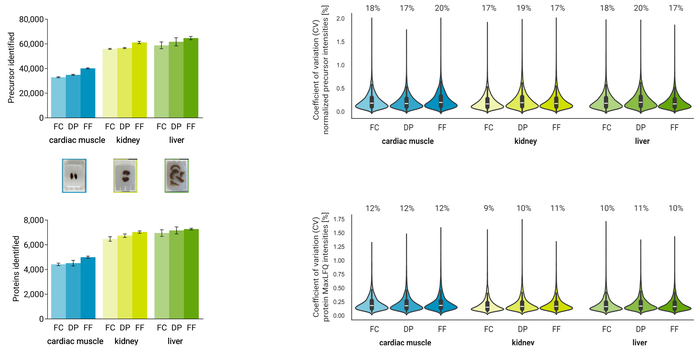
LC-MS analysis & Data processing:

- EASY-nLC™ 1200 - TimsTOF HT
- DIA-PASEF acquisition; 30-min gradient
- Bruker ProteoScape™ (timsDIA-NN, predicted Library from FASTA)

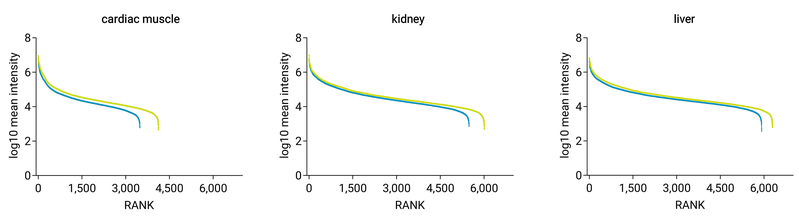
RESULTS



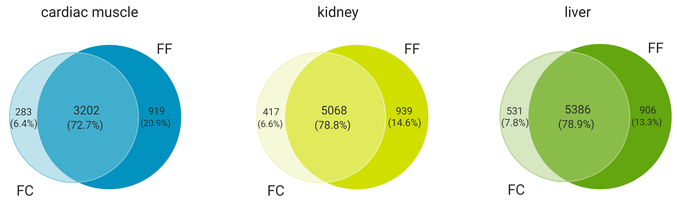
Overview of BeatBox workflow from tissue to ready-to-use peptides for LC-MS measurement.



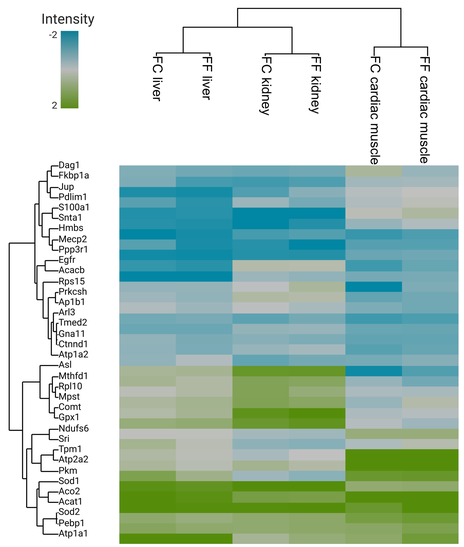
Comparing identified precursor and protein IDs. By using the BeatBox workflow for FFPE tissue sections (10 µm) and fresh frozen tissue (1-2 mg), it could be clearly shown that only minor differences can be observed. Starting from full curl (FC), the identified precursor and protein IDs are almost the same compared to the gold standard - xylene-deparaffinized FFPE tissue (DP). In direct comparison to fresh-frozen (FF) tissue, similar IDs could be determined. When evaluating the respective CVs at precursor and protein level, a similar performance and precision was observed, which was in the range of 17-20% for precursors and 9-12 % for the respective proteins.



Deep proteome coverage. 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 (three valid values in four replicates).



Shared proteins. A high overlap of protein identifications (72-79%) was achieved for full curl FFPE and fresh-frozen samples for all three mouse tissue types (three valid values in four replicates).



Preserved information. An evaluation of the biological information conserved between fresh-frozen and FFPE tissue was performed by applying statistical analysis and fitering for proteins significantly regulated for each tissue type. This resulted in 1259 proteins in FF and 1140 proteins in FC, showing a differentially expression between tissue types, but similar protein abundance level within a tissue specimen. A selection of proteins described for the development or regulation (based on GO-terms) of heart, kidney or liver are shown in the heat map, demonstrating that biological information is preserved between FFPE 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 processing steps and eliminating the need for deparaffinization with xylene.
- An innovative FFPE workflow that offers similar overall performance and proteomic depth to fresh frozen tissue is perfectly suited for large-scale retrospective studies with FFPE tissue.
- A more detailed analysis revealed a conserved biological information on protein level for selected proteins within a tissue type.

This BeatBox-iST workflow simplifies large-scale retrospective proteomic studies by providing a xylene-free, robust and high-throughput solution for FFPE tissue samples.

More Information & Contact

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Conflict of Interest Disclosure
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