

A streamlined and automated proteomics sample preparation workflow for targeted protein degrader studies and more

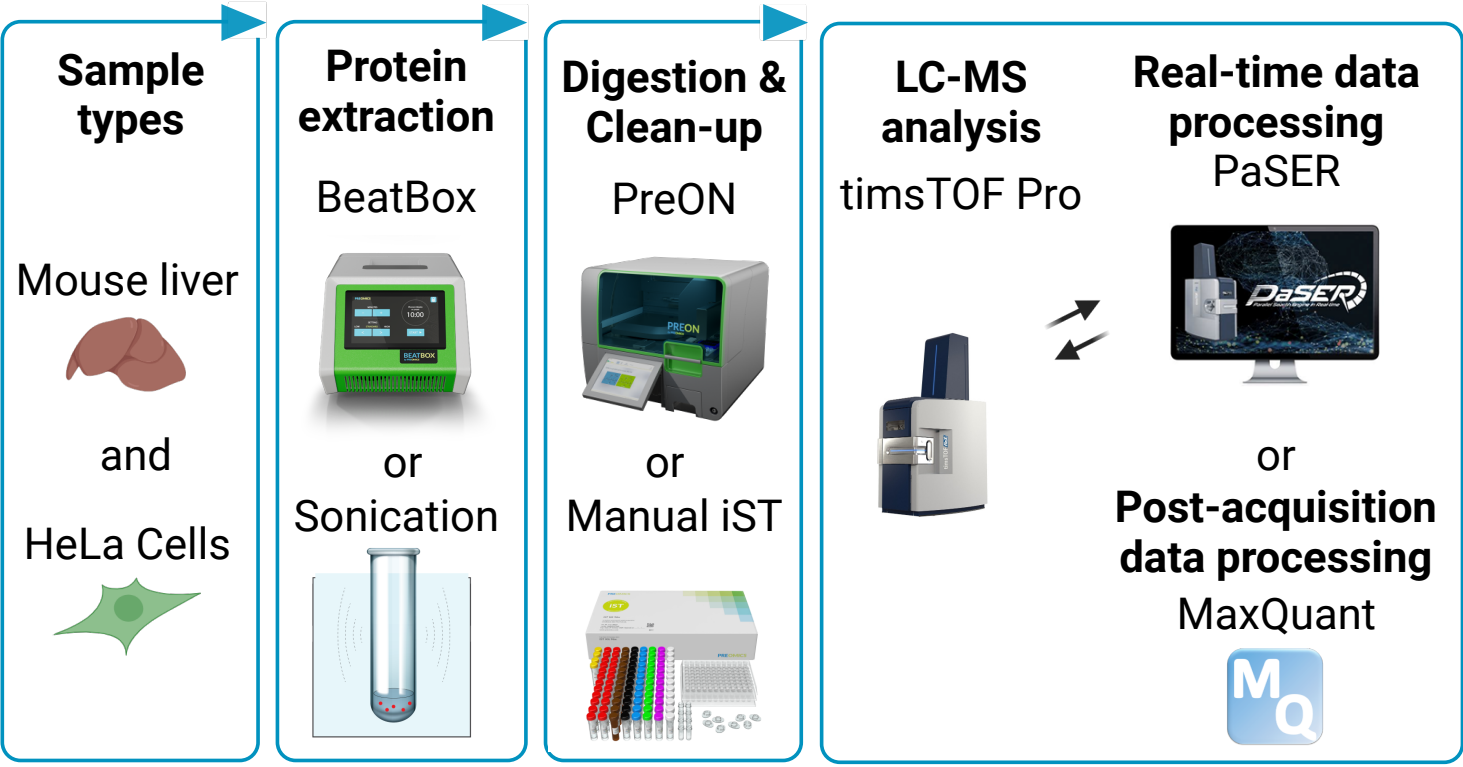
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SPOTLIGHT

Streamlined LC-MS-based proteomics workflow from sample homogenization to data analysis for targeted protein degrader studies and more:

- Efficient protein extraction on the BeatBox platform combined with robust iST sample preparation automated on the PreON platform (PreOmics)
- Measurement on the timsTOF Pro mass spectrometer with real-time analysis using the PaSER™ technology for improved and accelerated data processing (Bruker)

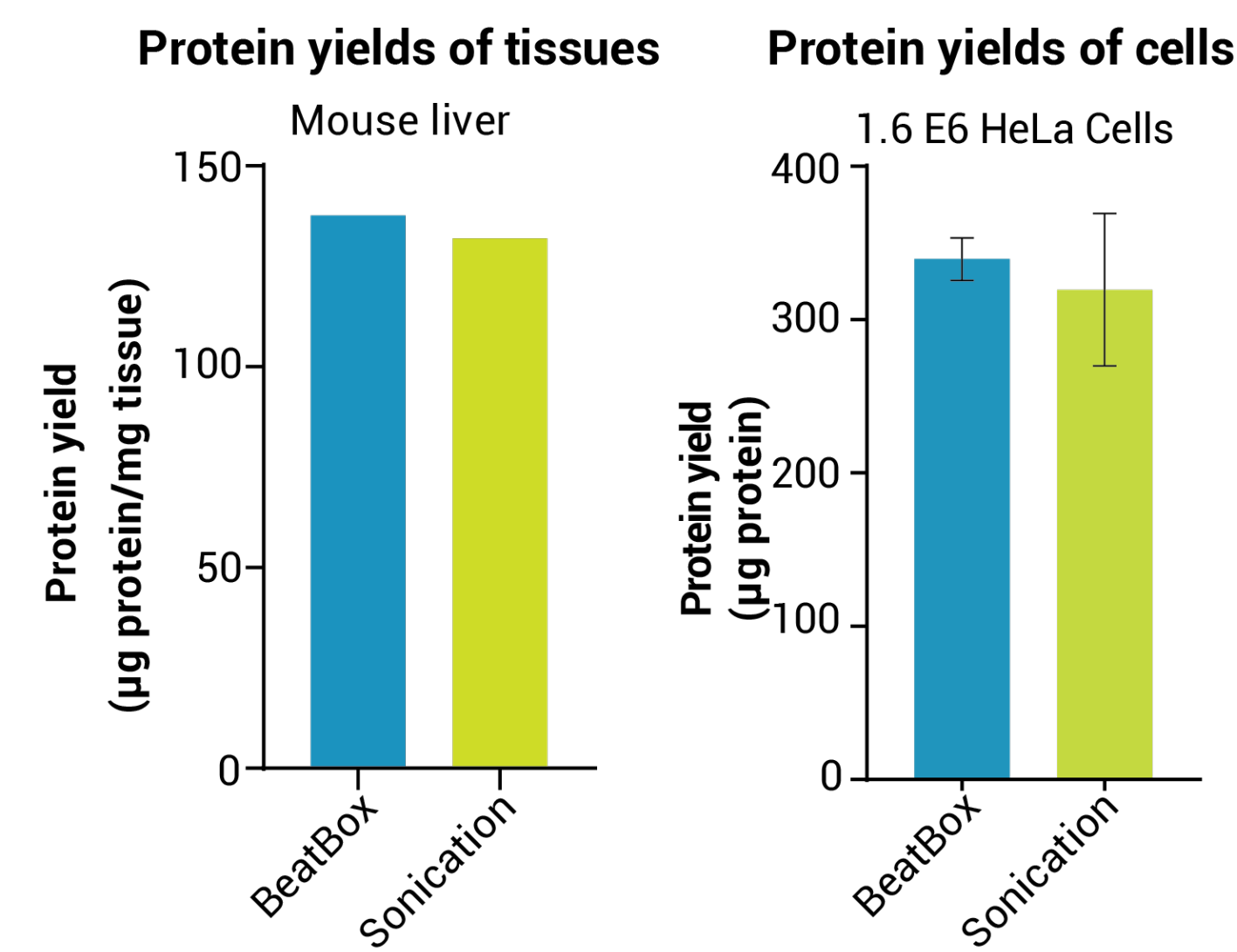
MATERIALS & METHODS



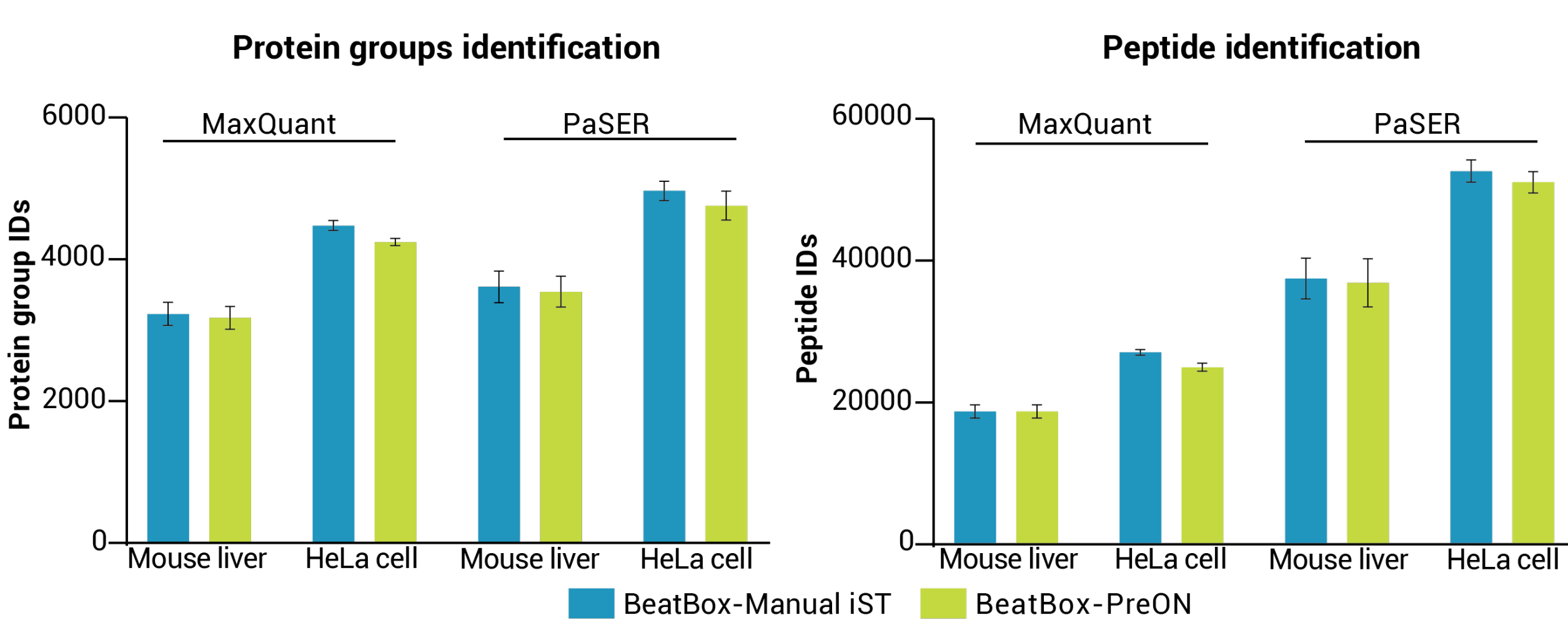
- **Input:** Mouse liver tissue (5 mg for sonication; 50 mg for BeatBox) and HeLa cells (1.6E6 cells); n=3
- **Homogenization:** BeatBox (10 min, standard power settings) with BeatBox Tissue 24x kit or boiling (10 min, 95 °C) plus sonication (10 cycles; 30 sec on / off)
- **Protein assay:** Micro BCA™ Protein Assay (Thermo)
- **Sample digestion/peptide clean-up:** iST workflow processed manually or on PreON
- **LC-MS analysis:** EASY-nLC™ 1200 - TimsTOF Pro; DDA-PASEF acquisition; 30-min gradient
- **Data analysis:** MaxQuant (v 2.0.1.0) or PaSER (v.1.0)

RESULTS

Highly efficient protein extraction

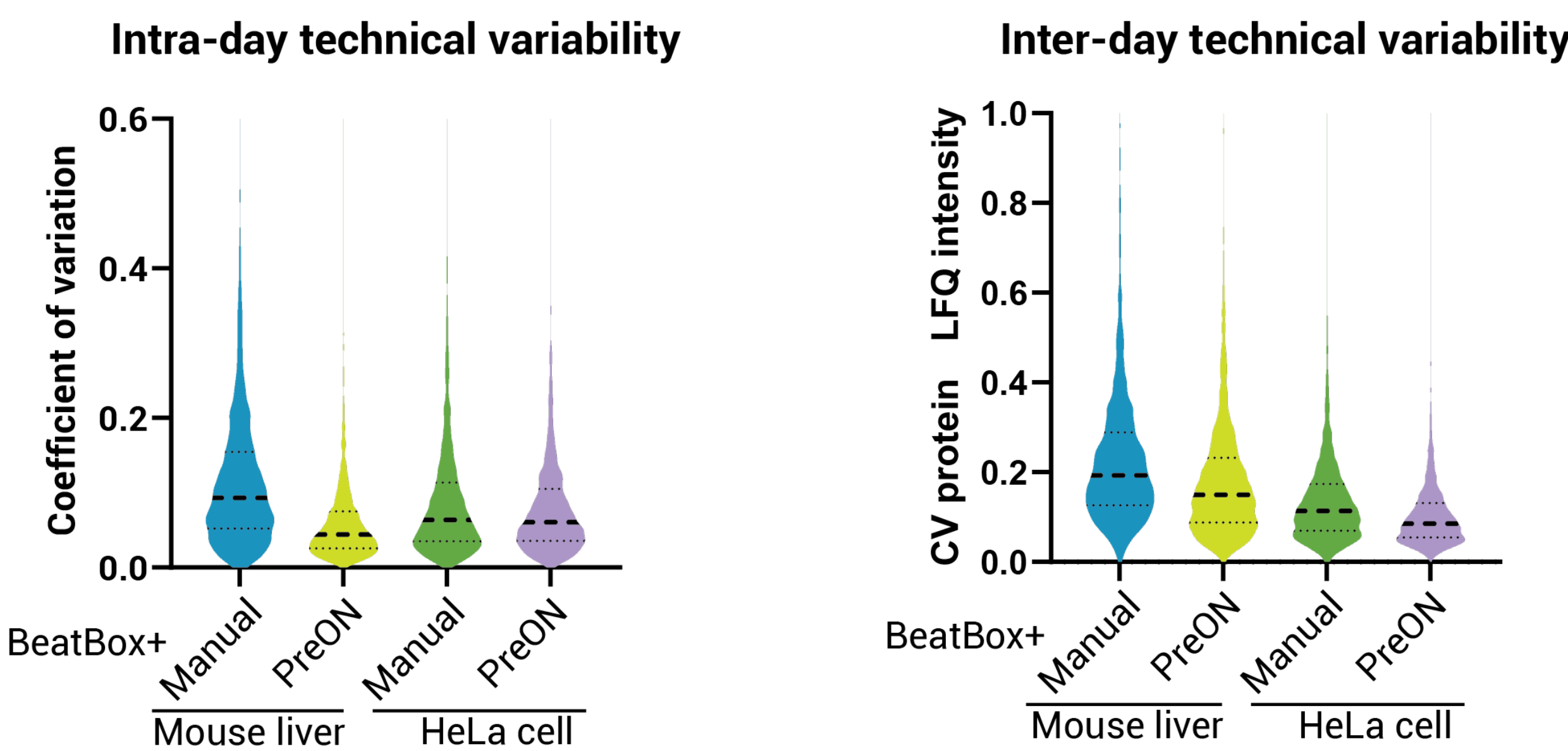


Improved protein and peptide identification



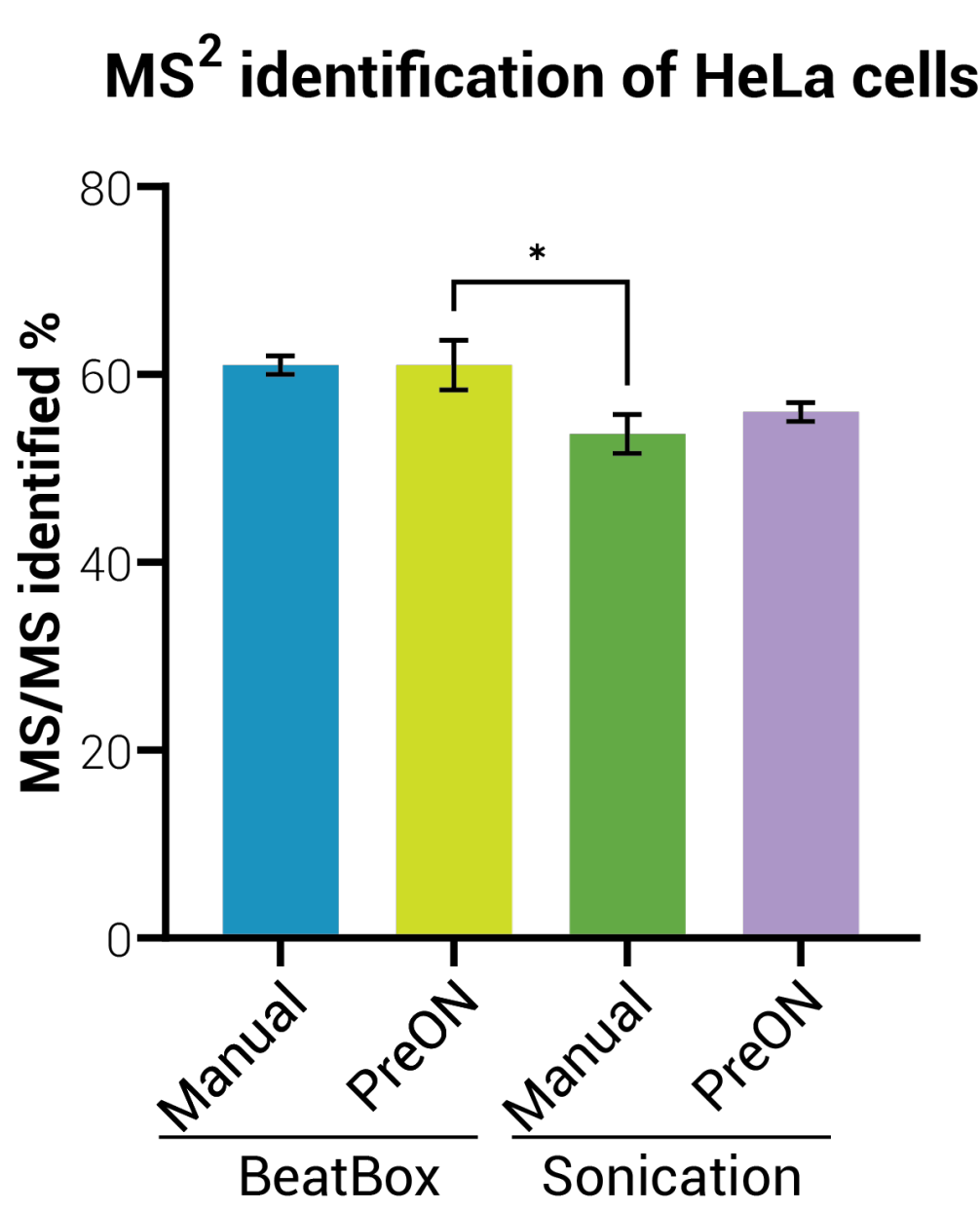
- BeatBox improves protein extraction efficiency compared to sonication-based homogenization.
- Coupling BeatBox homogenization to manual or automated sample processing results in deep proteome coverage with >95 % overlap in proteins for both workflows.
- Increase in peptide identifications applying the optimized PaSER search engine indicating enhanced sequence coverage.

Excellent inter-day and intra-day repeatability for PreON sample processing



- PreON sample processing starting from pooled BeatBox homogenates provides minimal technical variability for both intra-day ($CV_{PreON}:CV_{manual}=7\%:10\%$ for mouse liver and $CV_{PreON}:CV_{manual}=6\%:6\%$ for HeLa) and inter-day ($CV_{PreON}:CV_{manual}=15\%:19\%$ for mouse liver and $CV_{PreON}:CV_{manual}=9\%:11\%$ for HeLa).

Outstanding sample quality



- BeatBox-PreON workflow exceeds 60% in MS/MS identification, which is a significant increase ($p=0.019$) compared to the traditional workflow.

KEY TAKEAWAYS

BeatBox vs. sonication-based homogenization

- BeatBox shortens protein extraction to 10 min with parallel processing of 1 - 96 samples
- BeatBox allows to process large sample quantities
- BeatBox provides excellent protein extraction efficiency

PreON vs. manual iST sample preparation

- Coupling BeatBox to iST sample preparation provides reliable and improved LC-MS proteome analysis
- PreON improves technical variability
- BeatBox-PreON saves valuable time with less hands-on steps

PaSER vs. MaxQuant data processing

- PaSER accelerates data processing by making the results available immediately after the run
- PaSER enhances peptide identifications by an optimized search algorithm