Rethink tissue lysis: High-throughput tissue lysis workflow using the BeatBox platform for in-depth proteomic coverage

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Novel tissue homogenization workflow on the BeatBox platform

- High-throughput workflow: Parallel homogenization of 96 samples in less than 10 min
- For a wide range of tissue types: From soft brain to rigid heart muscle
- Optimized for low input samples: 50 - 500 µg protein input
- Seamlessly combinable with LC-MS sample preparation: 4 h from intact sample to finished data acquistion

Workflow

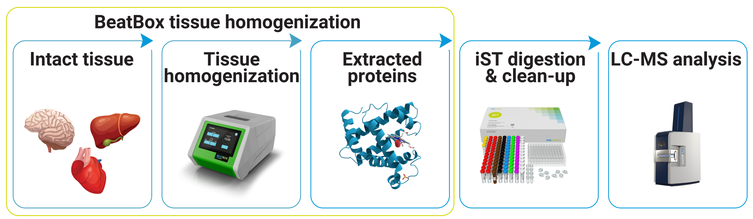

Fig. 1 | BeatBox-based tissue sample preparation.

Discussion

- Excellent proteomic coverage:
  timsTOF Pro; 45 min total acquistion time, DDA
  - > 4000 protein IDs for mouse lung
  - > 3700 protein IDS for mouse brain
  - ~ 3000 protein IDs for mouse liver
  - ~ 2300 protein IDs for mouse heart muscle
- Low technical variability
- > 40% increase of proteomic depth with three-step fractionation approach

Results

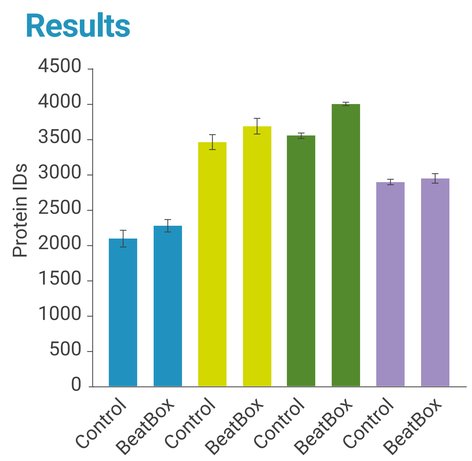

Fig. 2 | Comparison of protein IDs of the BeatBox tissue homogenization workflow coupled to iST sample preparation vs. the control workflow.

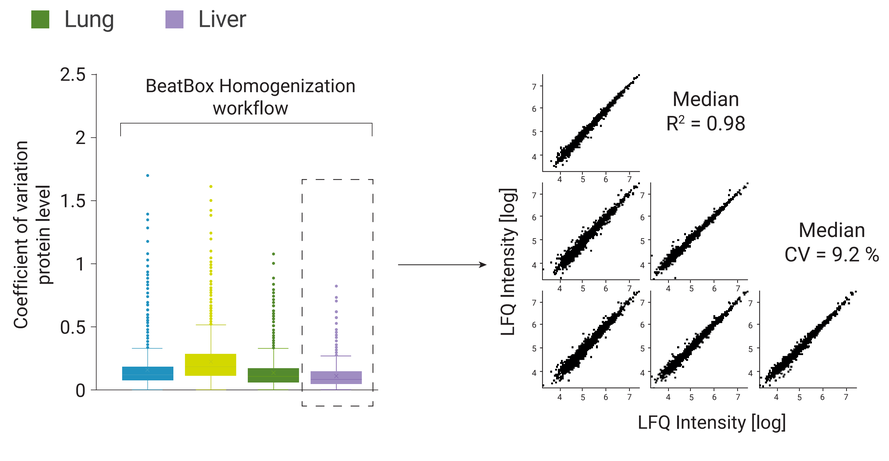

Fig. 3 | Technical variability for the BeatBox tissue homogenization workflow coupled to iST sample preparation. Left: CV. Right: Quantitative reproducibility for mouse liver.

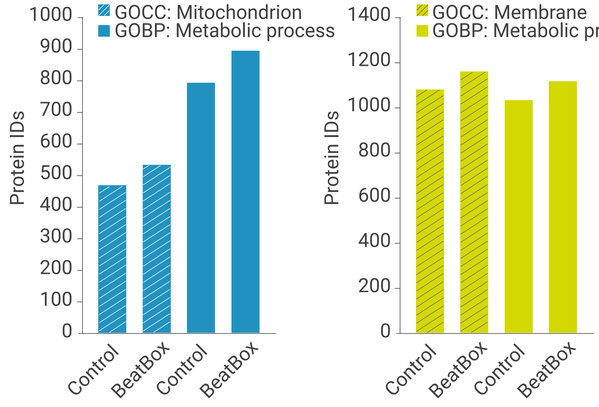

Fig. 4 | Protein IDs of examplary selection of biological relevant protein subsets.
Left: Mouse heart muscle.
Right: Mouse brain.

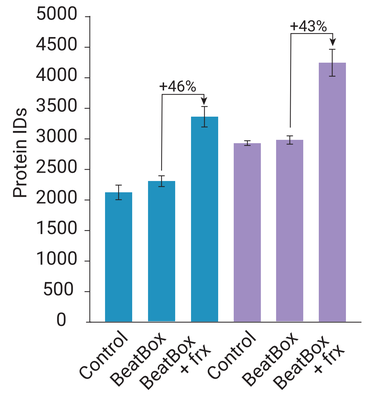

Fig. 5 | Comparison of protein IDs of the BeatBox tissue homogenization workflow coupled to iST sample preparation with vs. w/o fractionation vs. the control workflow.

Material and Methods

- **Input:** 1-2 mg wet-weight tissue of mouse lung, mouse brain (3 replicates each), mouse liver or mouse heart muscle (4 replicates each)
- **Workflow:** Tissue samples were homogenized in a 96-well plate on the BeatBox for 10 min. As control for the homogenization step, optimized bead-based sonication (10 cycles, 30 sec on/off) was performed followed by a boiling step (95°C, 10 min). Next, extracted proteins were digested for 1 h and purified applying the iST workflow. If specified, peptides were eluted in three fractions applying the iST-Fractionation workflow
- **LC-MS analysis:** Easy nLC 1200 coupled to timsTOF Pro; DDA mode; 45 min total acquisition time; data analyzed by MaxQuant software