Semi-automated, high-throughput homogenization technique for in-depth analysis of tissue proteome

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Tissue homogenization by BeatBox

- **Semi-automated, high-throughput workflow:**
  Parallel homogenization of 96 samples in 10 minutes
- **For a wide range of tissue types, optimized for low input samples,** such as needle biopsies
- **Works seamlessly in combination with LC-MS sample preparation:**
  ~4 hrs from intact sample to finished data acquisition
- **Easily implemented into automation platforms**

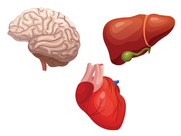
Key points

- **BeatBox vs. traditional tissue homogenization technique:**
  - Tissue lysis shortened to 10 mins
  - Excellent proteomic coverage with a short LC-MS run
  - Improved protein identification
  - Excellent technical variability across different tissue types
- **Fractionation incorporated with BeatBox iST workflow:**
  Increased protein identifications by, on average, 40% compared to unfractionated samples

Workflow

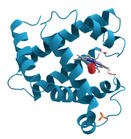
BeatBox tissue homogenization

Intact tissue



Tissue homogenization

Extracted proteins



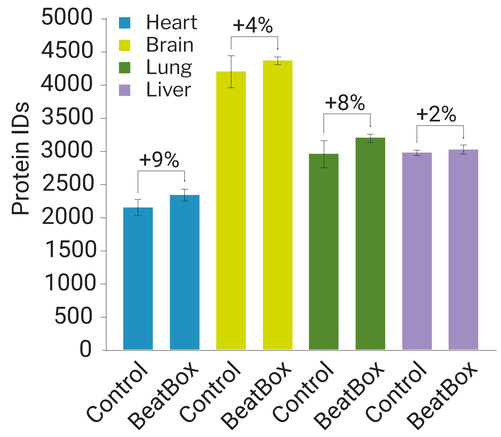
iST digestion & clean-up

LC-MS analysis

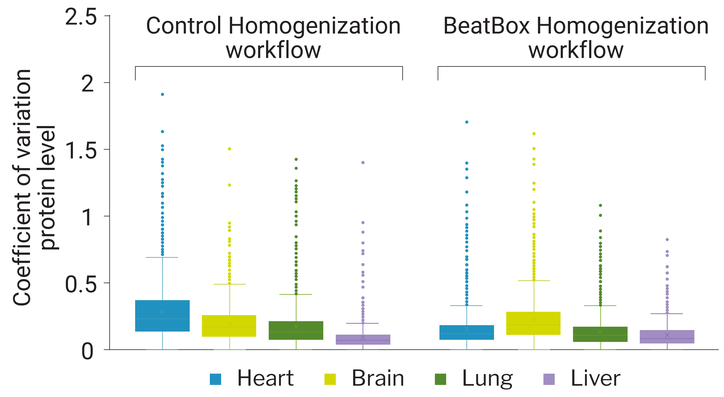
From homogenization to in-depth protein identification using a novel BeatBox instrument and established iST fractionation technology.

Comparison of BeatBox and traditional tissue homogenization for iST protein sample preparation

Gain in protein IDs

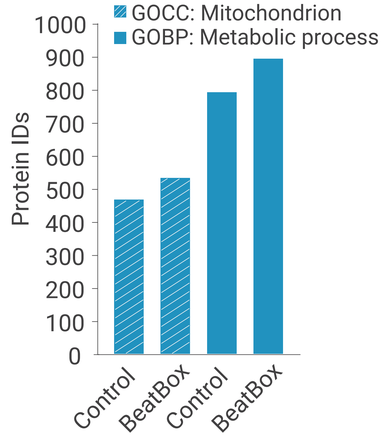


Improved technical variability

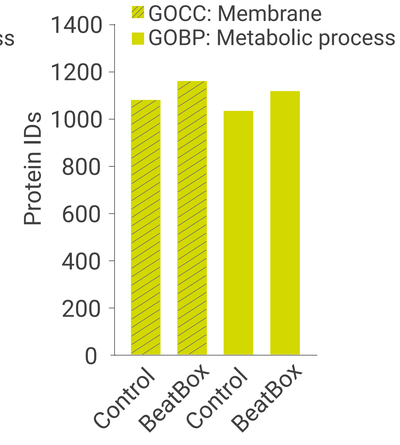


Deeper look into the protein biological relevance

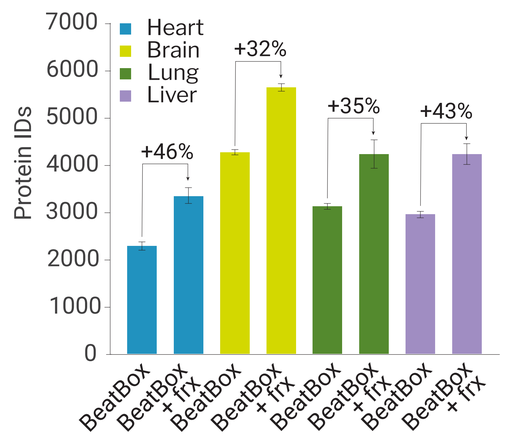
Cardiac mouse muscle



Mouse brain



Substantial expand of various proteomes by fractionation step



Material and Methods

- **Input:** 1-2 mg wet-weight tissue of mouse lung, brain, liver and cardiac muscle (3 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 one hour and purified applying the iST workflow. If specified, peptides were eluted in three fractions applying the three-step 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 v. 2.0.1.0