High-throughput homogenization technique for deeper analysis of multiple mouse tissue proteomes

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Introduction

Tissue homogenization is still one of the most crucial and time-consuming steps for sample preparation within a proteomics workflow. To release tissue-specific proteins, tissue is homogenized to disrupt the outer cell membrane, intracellular membranes, and the surrounding extracellular structures. The released tissue proteome provides the tissue phenotype, which is particularly important when screening for disease-associated proteins, which can be used as diagnostic markers ("biomarkers") for biopsy analysis and disease diagnosis.

Currently, there are two traditional technologies capable of processing multiple samples simultaneously: bead beating and sonication, but both have limitations.

Bead beating needs careful handling of the beads, is time-consuming, and is not suitable for low-input samples. Sonication can be used for low-input samples, but with it, homogenization isn't consistent between samples.[1] Therefore, neither of these techniques is easily incorporated into high-throughput assays.

Here, we present a novel tissue lysis workflow on the semi-automated 'BeatBox' platform. It enables efficient protein extraction for 96 samples in parallel, in as little as 10 minutes. When BeatBox homogenization is coupled with robust PreOmics iST and fractionation technology, in-depth sample preparation for large-scale applications can be achieved with minimal hands-on time.

Keywords

Proteomics; sample preparation; mass spectrometry; mouse tissue; BeatBox, PreOmics iST, homogenization, fractionation

Key takeaways

Introducing a fast and straightforward workflow suitable for high-throughput homogenization of challenging tissues for deeper proteomic analyses.

Comparison of the novel BeatBox tissue homogenization workflow with the traditional PreOmics iST procedure for low-input samples

iST-Fractionation as a unique tool for biomarker discovery or analyzing lower abundant proteins that could be easily missed in highly complex samples.

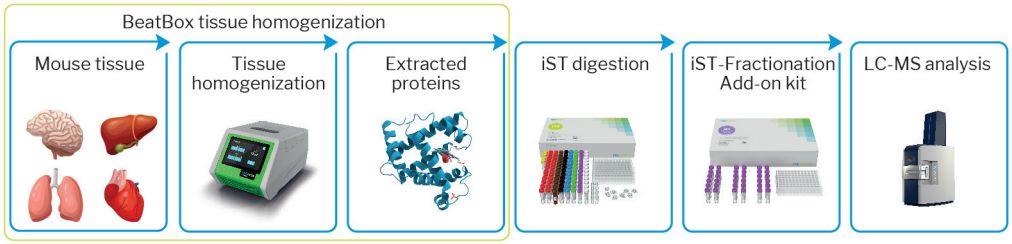

Figure 1 | From mouse tissue homogenization to in-depth protein identification using a novel BeatBox instrument and established iST-Fractionation technology.

Materials and methods

Mouse tissue samples

Studies were performed using 1-2 mg wet-weight of cardiac muscle, brain, liver and lung mouse tissues, collected in triplicates per tissue type.

Tissue homogenization and sample preparation

The samples were prepared using the BeatBox Tissue Kit 96x (PreOmics, P.O.00121), the iST 96x kit (PreOmics P.O.00027) and 96 x 3 reactions iST-Fractionation add-on (PreOmics, P.O.000102).

The iST-Fractionation Add-on kit must be used with iST, iST-NHS, or iST-BCT as it does not contain the cartridge. BeatBox Tissue Kit is compatible with LYSE buffer from iST, iST-NHS, iST-BCT, and iST-SP3.

Control samples were homogenized by optimized bead-based sonication (10 cycles, 30 sec on, 30 sec off) followed by a boiling (95 °C, 10 min) and cool-down step. BeatBox samples were homogenized in a 96-well plate for 10 minutes applying sample-specific BeatBox settings (listed in Table 1).

Mouse tissue type	BeatBox setting
Cardiac muscle	High
Brain	Standard
Liver	Standard
Lung	Standard

Table 1 | BeatBox sample-specific settings

All BeatBox and control samples were digested for 1 hour according to the iST workflow[2], and iST peptide purification was performed by applying a three-step peptide iST-Fractionation[3] if indicated. The iST and iST-Fractionation workflows are graphically represented in Figure 2.

The concentration of resulting peptides was measured by NanoDro™Lite (Thermo Fisher Scientific).

Before the liquid chromatography-mass spectrometry (LC-MS) analysis, the complex peptide mixture was diluted with LC-LOAD to a concentration of 150 ng/µL.

LC-MS/MS and data analysis

The Easy nLC 1200 system (ThermoFisher Scientific) was coupled to the timsTOF Pro mass spectrometer (Bruker Daltonics). Samples containing 300 ng of peptides were loaded on to in house packed column (length 50 cm, i.d. 75 µm, C18 particle with 1.9 µm diameter). Peptides were sepa rated using a binary gradient at a 450 nL/min constant flow rate. Mobile phase A consisted of 0.1 % formic acid and mobile phase B consisted of 0.1 % formic acid in 80% acetonitrile. The 45 min LC-MS analysis started by ramping the mobile phase B from 5 to 35% in 25 min, then to 60% in 15 min and to 95% in 2.5 min, where it was kept for an additional 2.5 min prior to column equilibration.

Peptides were ionized with the electrospray voltage at 1.5 kV, and the capillary temperature was 180°C. The TimsToF Pro was operated in parallel accumulation—seral fragmentation (PASEF) mode over an MS and MS/MS range of 100 to 1700 m/z using an accumulation and ramp time of 100 ms. Ion mobility range was 0.8 – 1.4 1/k0, and MS/MS spectra were acquired using stepped collision energy. Data were analyzed and statistically evaluated by MaxQuant software, version 2.0.1.0.

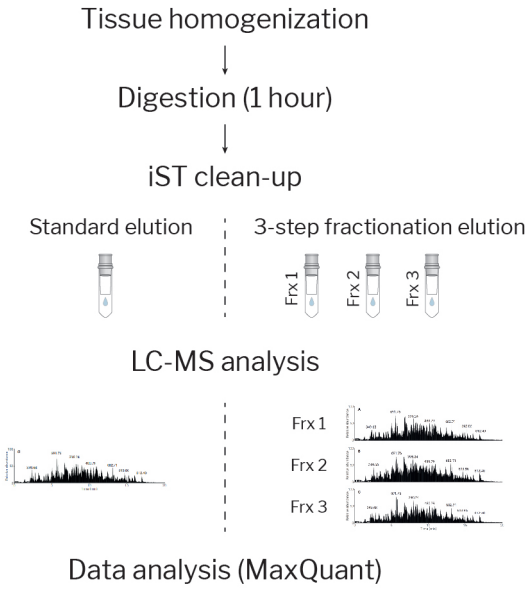

Figure 2 | The iST sample preparation with and without peptide fractionation. A three-step fractionation replaces the standard iST elution step for the cartridge-based peptide fractionation. Individual fractions are measured separately and processed together as a single data file.

Results and discussion

The use of the BeatBox platform speeds up and simplifies tissue preparation for proteomics analysis. The BeatBox platform combined with iST workflow allowed for the identification of ~2,300 proteins in mouse cardiac muscle, ~ 4,300 proteins in mouse brain tissue, ~3,100 proteins in mouse lung tissue, and ~ 3,000 proteins in mouse liver tissue. In comparison to tissue preparation by homogenization and boiling in the traditional iST workflow, this demonstrates an increase of 9% (cardiac muscle), 4% (brain), 8% (lung), and 2% (liver) in protein identifications when using the BeatBox for tissue homogenization. The protein identifications achieved by both tissue homogenization techniques are illustrated in Figure 3. Figure 4 shows improved quantitative reproducibility using BeatBox technology over the traditional tissue lysis workflow.

The coefficient of variance (CV) for different mouse tissue types prepared on the BeatBox was 12% for cardiac muscle, 18% for brain, 11% for lung, and 9% for liver (Figure 4B). This demonstrates that the BeatBox tissue homogenization workflow decreases the sample-to-sample variation compared to the traditional sonication and boiling lysis methods. It was observed with harder tissues, such as heart muscle, that the CV was improved from 24% to 12% using BeatBox for homogenization. This is most likely due to powerful tissue cell disruption using BeatBox technology. Additionally, Beatbox was twice as fast compared to the traditional tissue lysis method compared here.

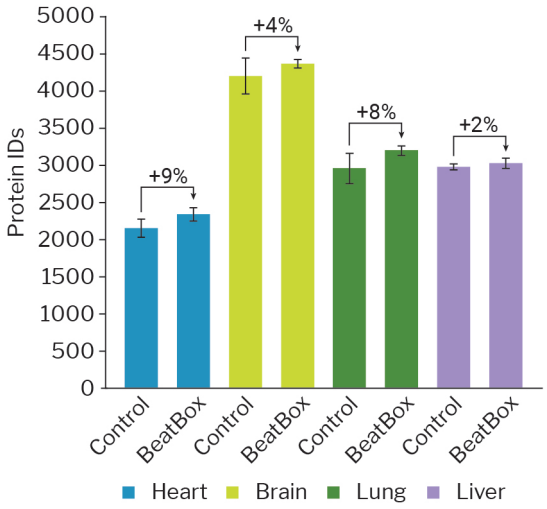

Figure 3 | Comparison of mouse protein identification rate between Control and BeatBox workflows for various mouse tissue homogenates. Control: homogenization by an optimized bead-based sonication and boiling workflow. BeatBox: homogenization by BeatBox platform with sample-specific settings. Mouse tissue types: brain, lung and liver (n=3, BB setting: Standard); cardiac muscle (n=3, setting: High). For LC-MS analysis, homogenates were prepared using the iST workflow.

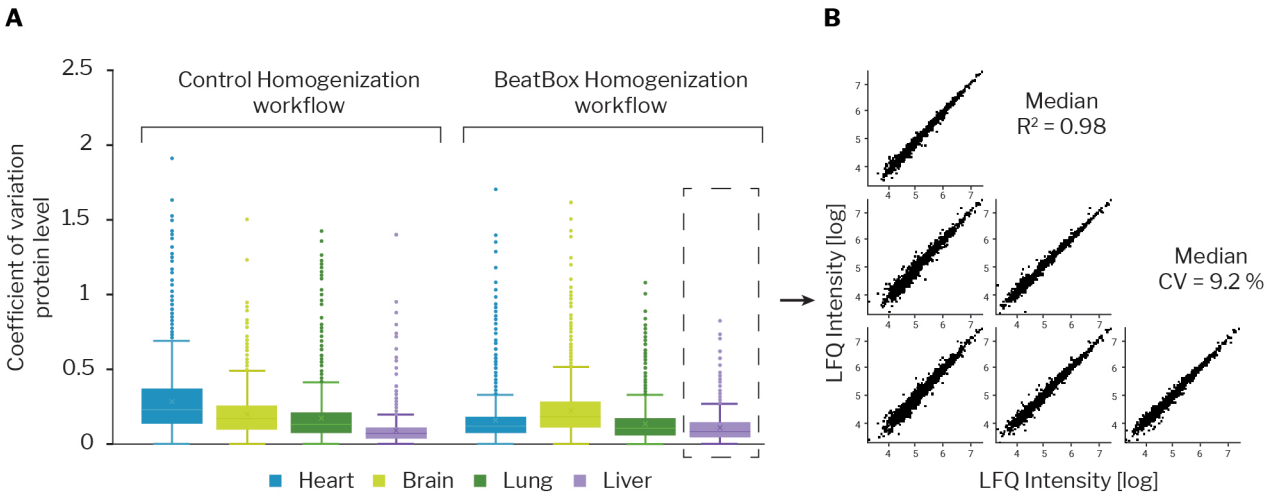

Figure 4 | Comparison of technical variability between Control and BeatBox homogenization workflows.
(A) Coefficient of variation (protein level) of the BeatBox homogenization workflow vs. the Control homogenization workflow. (B) As an example, quantitative reproducibility of mouse liver samples homogenized on the BeatBox platform. Mouse tissue types: brain, lung and liver (n=3, BB setting: Standard); cardiac muscle (n=3, setting: High). For LC-MS analysis, homogenates were prepared using the iST workflow.

Combining the BeatBox iST workflow with peptide fractionation allows complex samples, such as tissue proteomes, to be analyzed in greater depth. The iST-Fractionation technology has three fractionation steps with an additional ten minutes of hands-on sample preparation time, delivering deeper proteome coverage with relatively minimal increase in preparation time.

When BeatBox is coupled to iST and fractionation, the single proteomics workflow increases depth by 46% for the mouse cardiac muscle, 32% for mouse brain, 35% for mouse lung, and 43% for mouse liver compared to the unfractionated samples (Figure 5).

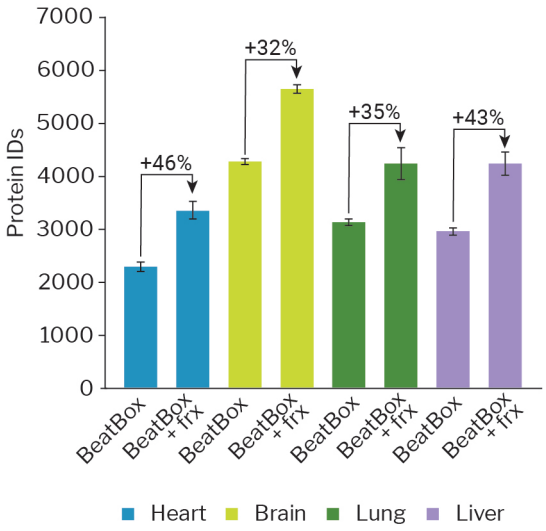

Figure 5 | Improved proteomic depth by the three-step fractionation approach. Comparison of protein IDs of the BeatBox homogenization workflow with and without fractionation. BeatBox: BeatBox lysis followed by iST digestion and clean-up; BeatBox+fractionation: BeatBox lysis followed by iST digestion and clean-up with add-on fractionation kit. Mouse tissue types: brain, lung, and liver (n=3, BB setting: Standard); cardiac muscle (n=3, setting: High).

Conclusions

As demonstrated in these studies, the BeatBox workflow sets a new benchmark for low-input tissue sample preparation. It enables fast and highly efficient protein extraction in a high-throughput manner, which could be easily coupled to common automation platforms for sample handling. It takes less than 4 hours from obtaining the intact tissue sample to data visualization.

This application note compares the novel BeatBox tissue homogenization workflow with the traditional iST procedure for low-input samples, such as tissue needle biopsies. The workflows only differ in the tissue lysis/homogenization step.

The BeatBox technology improved protein identification on average by 6%, with excellent technical variability across different tissue types with an average CV of 12.5%. It also shortens the time for tissue lysis to 10 min. A combination of the BeatBox iST workflow with the iST-Fractionation add-on increased the protein identifications by, on average, 40% compared to unfractionated samples.

Overall, the combination of BeatBox tissue homogenization and protein digestion by iST, followed by fractionation of digested peptides, offers a unique tool for biomarker discovery or analyzing lower abundant proteins that could be easily missed in highly complex samples.

PREOMICS

Products

Product	Manufacturer	Product Code
BeatBox Instrument	PreOmics GmbH	P.0. 00103
BeatBox Tissue kit 96x	PreOmics GmbH	P.0. 00121
iST kit 96x	PreOmics GmbH	P.0. 00027
96x3 iST-Fractionation Add-on kit	PreOmics GmbH	P.0. 00102

Ordering information

http://www.preomics.com/quote

order@preomics.com

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