# PREOMICS

# High-throughput and low-input Metabolomics/Lipidomics sample preparation – A new BeatBox® application

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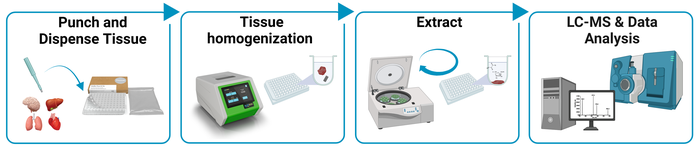
## SPOTLIGHT

- High-throughput approach that enables homogenization and extraction from 1 to 96 samples.
- Fast, easy-to-use, and standardized sample preparation for metabolite and lipid extraction from wide variety of tissue samples.
- Overall, excellent reproducibility determined by CV <20% analytes.
- Facilitates the processing of very small amounts of tissue (wet weight 1-5 mg), ideal for targeted MS approaches.
- Extraction of biologically relevant metabolites and lipids throughout the various tissue types.




## MATERIALS & METHODS

**Tissue and sample preparation:**
Approximately 5 mg wet weight tissue from mouse (cardiac muscle, brain, intestine, liver, lung, muscle, and spleen). Using a 1.0 mm disposable biopsy pen (Integra Lifesciences), a single tissue punch was added to each well of a BeatBox Tissue Kit 96x plate with 100 µl of a cold 80% methanol (MeOH).

The tissue was homogenized for 10-minutes by using the BeatBox at standard power settings.

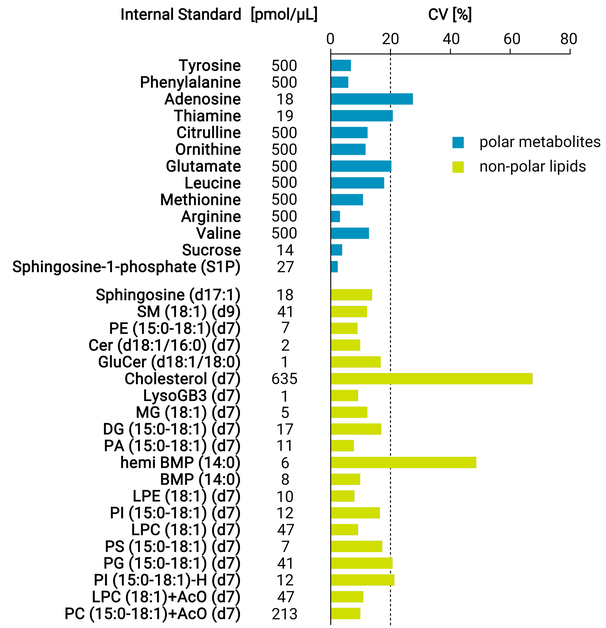
After centrifugation (4,000g for 20-minutes at 4 °C) supernatant was collected and protein precipitate was discarded.

**LC-MS analysis:**
Extracted analytes were separated and analyzed using the Vanquish Duo HPLC (ThermoFisher Scientific) coupled to the Sciex7500 triple quadrupole mass spectrometer (SCIEX).

**Data processing:**
Raw files were processed using Skyline v.23.1. Statistical evaluation of the data was done via MetaboAnalyst v5.0.

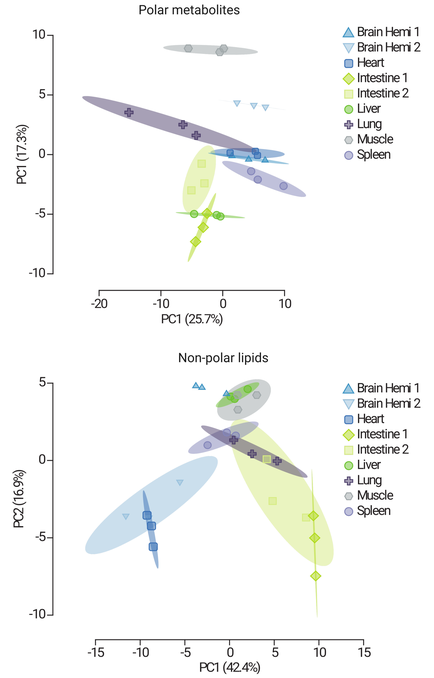
## RESULTS

### List of internal standards, concentrations, and respective CVs



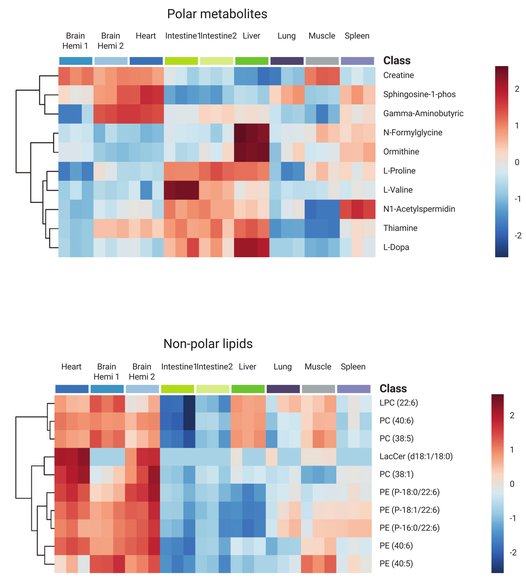

To determine the robustness of metabolite extraction from samples with low-input, a pool (12 polar metabolites, 21 non-polar lipids) of different stable isotope-labeled standards (IS) were spiked to the samples before homogenization. Overall, the extraction efficiency resulted in a CV of the extracted metabolites of below 20%, indicating a robust and reproducible workflow.

### PCA plot of analytes extracted from various tissues




Clustering based on their origin reveals different metabolite and lipid level in different organs indicating information can be conserved by this workflow.

### Heatmap of the 10 most abundant analytes




The top 10 polar metabolites revealed unique regulation levels throughout various tissues. Creatine is upregulated in the brain, muscle, and cardiac muscle tissues, while ornithine is significantly upregulated in the liver and thiamine upregulated in the intestine, liver, and brain. In the lipid fraction, glycerophospholipids, like LPC, PC, and PE are upregulated in cardiac muscle, brain, liver, and muscle tissue.

## KEY TAKEAWAYS

- The BeatBox platform offers a fast, efficient, and space-saving method for homogenizing various tissue and cell types.
- A novel high-throughput BeatBox processing workflow for subsequent LC-MS based metabolomic and lipidomic analysis.
- Highly efficient approach for low-input, high-throughput metabolomics and lipidomics studies.
- Extraction of polar metabolites and non-polar lipids from limited tissue samples offers an attractive solution for the analysis of large clinical cohorts.
- Top 10 selected polar metabolites and non-polar lipids indicated biological trends of the tissue origin.

## More Information & Contact

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Conflict of Interest Disclosure:
K. Limm, S. Wuertenberger, C. Ellis are employed by PreOmics GmbH/PreOmics Inc.
B. Ngo, D. Itzhak, J. Wang are employed by Altos Labs