A single-sample workflow for joint metabolomic and proteomic analysis of clinical specimens

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Overview

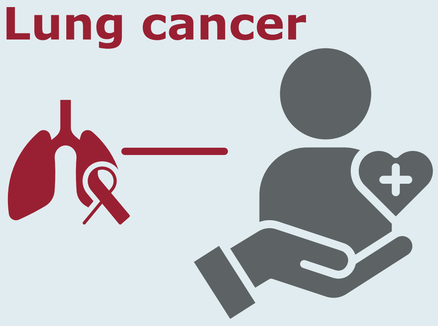
Integrating proteomic and metabolomic data enhances our understanding of biological processes and disease mechanisms. Traditional multi-omics workflows often require separate sample processing, leading to increased variability. To address this, we developed MTBE-SP3, an optimized single-sample workflow that combines a 75% EtOH/MTBE metabolite extraction with automated single-pot solid-phase-enhanced sample preparation for proteins. **This approach reduces variability and improves robustness in multi-omics analyses**, allowing for deeper insights into cellular regulation and disease pathways. Here, we present the MTBE-SP3 workflow and its effectiveness using lung adenocarcinoma patient samples, along with the potential for further automation to boost throughput and standardization in multi-omics research.

Key benefits of MTBE-SP3

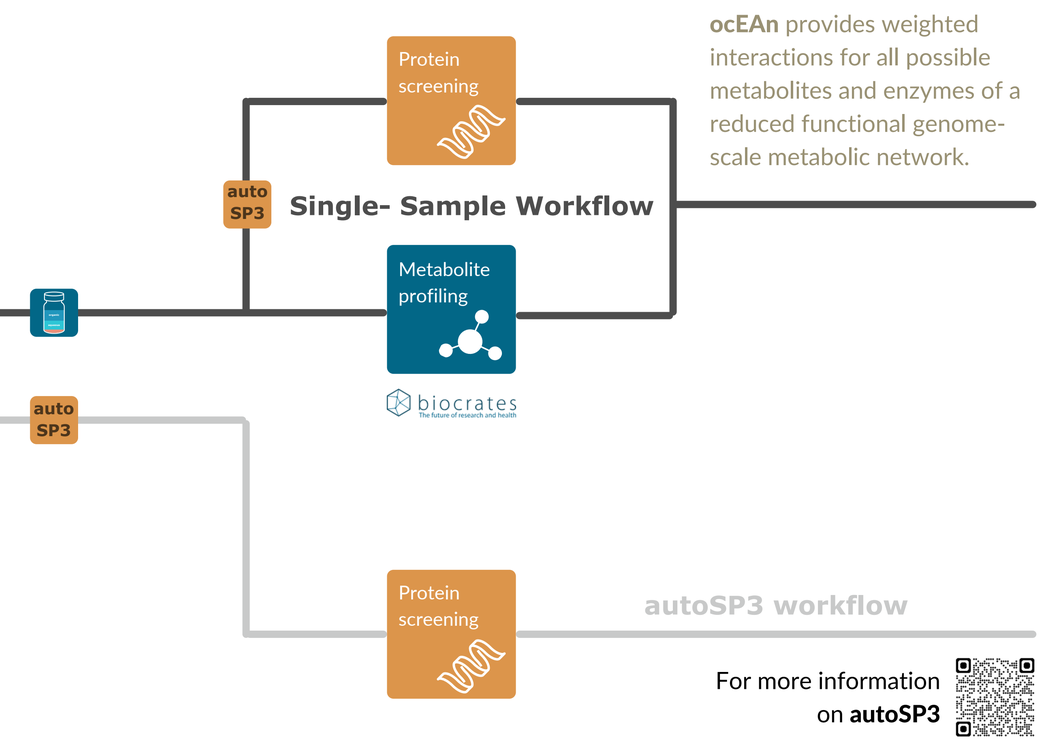
- Single-sample processing for **reduced variability** and **more coherent** multi-omics analysis.
- Broadly tested applicability to various biological matrices like FFPE, fresh-frozen tissues, plasma, serum and cells as well as a pilot study.
- Potential for full automation that minimizes technical variability for clinical workflows.

Pilot Study

Lung cancer



Applied to lung adenocarcinoma patients (10) we identified distinct protein and metabolite changes between tumor (TT) and normal tissues (NT). **Integrating these molecular layers revealed additional dependencies** and emphasized mitochondrial dysfunction in tumor development, demonstrating the value of multi-omics analyses. Finally, we showed that single sample integration results in **better correlation** in the ocEAn analysis.



ocEAn provides weighted interactions for all possible metabolites and enzymes of a reduced functional genome-scale metabolic network.

Single Sample Network Analysis

- Integration revealed **additional pathways** that are affected in Tumor Tissue vs Normal Tissue
- Network Analysis shows better correlation for single sample data

For more information on **autoSP3**

autoSP3
Protein data showed clear differences between Tumor Tissue vs Normal Tissue

Why EtOH/MTBE?

Lower variance than Bligh & Dyer

Broad metabolite **coverage**

Protein pellet at the bottom for **automation**

What is the overlap in percentage?

AutoSP3 vs MTBE-SP3

85%	**FFPE**
89%	**Fresh-frozen**
91%	**Plasma**
98%	**Cells**

See the extraction comparison online.

Next Step: More Automation

Automated adjusted **Matyash extraction (MTBE) with 75% EtOH**

PAL SYSTEM

Explore more automation options

See the open access publication

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