Super-Omics: Liquid-Liquid Extraction for Proteomics, Lipidomics and Metabolomics from a Single Sample, Towards a Method for Biopsies

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**Abstract**

Traditionally, omics technologies have been performed by starting with separate biological material for the sole purpose of extracting or enriching for a specific molecule class while typically discarding the remaining sample. However, common liquid-liquid extraction methods used for metabolomics and lipidomics can be used to phase separate polar molecules, non-polar molecules and proteins/DNA. We can take advantage of this by utilizing each phase for separate omics experiments. We will show how we can take several tumor samples from breast and lung cancers as well as a single plate of cells to perform “omics” (lipidomics, metabolomics and phosphoproteomics). This process will especially useful for medical needle biopsies since little material is collected for pathological evaluation.

**Cancer tissue samples were extracted using methanol tert-butyl ether (MTBE). 80% methanol or dichloromethane. Using MTBE phase separation, the upper phase was used for lipidomics after specific evaporation, middle phase for polar metabolomics and the lower phase for proteomics. Lipids were profiled by reversed-phase LC-MS/MS on a Q Exactive Plus Orbitrap using HCD with positive/negative ion switching with Data Dependent Acquisition (DDA) on the middle layer of the Q Exactive extracting showing polarity of the polypeptide pathways.**

**Methods**

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**Targeted Metabolomics and Untargeted Metabolomics**

**Targeted Metabolomics from middle phase (≈ 300 polar molecules)**

**Untargeted Lipidomics**

**Conclusions**

- We used tissue from a mouse breast tumor compared to normal mammary gland tissue.
- We then performed a MTBE extraction and utilized all three phases of the extraction for performing a 3-omics (SuperOmic) analysis.
- We could also gain biological insight into mechanisms that drive these tumors.
- The above-mentioned studies demonstrate that no sample was wasted from previous tumors and in one liquid-liquid extraction step, we can prepare samples for metabolomics, lipidomics and proteomics.