Czech Centre for Phenogenomics

Phenotyping module

MALDI imaging unit

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Impact of instrument, detection method, and statistical methods in mouse plasma metabolite profiling

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Abstract

Changes in the metabolic profile of plasma reflect the metabolic state of an organism and may reveal disease biomarkers or depict the phenotype of an animal model. For a thorough study of the said changes, reproducible and, more so, fast methods for high-throughput non-targeted screening of plasma are essential. The aim of this study was the comparison of mass spectrometric plasma metabolite measurements among a group of wild-type and Klk8 knockout mice across three methods; ultra-high resolution instrument (scimaX MRMS 7T) coupled with a) ESI using flow injection analysis, b) MALDI and c) MALDI-TOF-MS.

Mass spectrometric results were analyzed by Metaboanalyst statistical toolkit (Metaboanalyst 4.0, McGill University, Quebec, Canada). Number of discriminative peaks identified by each instrument and number of suggested enriched pathways were compared.

In negative ion mode, the ultra-high resolution instrument (scimaX MRMS 7T, Bruker Daltonics Inc.) detected the highest number of peaks (5964 by ESI-MRMS and 6728 by MALDI-MRMS) compared to 520 peaks detected with a MALDI-TOF instrument (rapifleX, Bruker Daltonics Inc.). Statistical analysis by t-test revealed highly different number of discriminative peaks between MRMS and TOF machines (fold change > 2 with FDR score < 0.05; 1067 by ESI-MRMS, 930 by MALDI-MRMS compared to only 53 peaks by MALDI-TOF). With every method we were able to putatively annotate hundreds of peaks and thus depict enrichment in particular number of metabolic pathways, namely with data obtained with MRMS instrument (34 pathway hits with MALDI-MRMS and 20 hits by ESI-MRMS). Nevertheless, only the MRMS instrument showed significantly higher number of metabolites to depict changes in the metabolome.

Consequently, ultra-high mass resolution flow injection analysis can be used for fast metabolite profiling of plasma.

Materials and Methods

Plasma withdrawal

Klk8-deficient (n=7) and littermate wildtype (n=6) mice were anesthetized and plasma was withdrawn into heparinized tube by eye puncture. After centrifugation plasma was collected and divided into aliquots and kept at -80°C

FIA

Plasma was diluted 100times with methanol and precipitated at -20°C, supernatant was further diluted 2.5times with methanol and 20 µl introduced into FIA and eluted by methanol. ESI ion source coupled to scimaX MRMS instrument was used to detect metabolites in negative ion mode. Three technical replicates were included in the study for each animal (n=3).

MALDI

Plasma was diluted 100times with methanol and 0.5 µl was spotted onto ground steel target plate, left dried and sprayed with 9-Aminoacridine matrix (7 mg/ml in 70% EtOH; HTX TM-Sprayer M3, HTX Technologies, 75°C, 8 cycles, Flow 0.125 ml/min). MALDI-MRMS and MALDI-TOF-MS instruments were used for metabolite detection in negative ion mode. Six technical replicates were included in the study for each animal (n=6).

Statistics

For t-test followed by fold change calculations (threshold<2, FDR<0.05; in Metaboanalyst 4.0 (*McGill University, Quebec, Canada) Statistical Analysis, MS Peak to Pathway and Pathway Analysis modules were used)*, technical replicates were averaged for each subject. VANTED software (University of Konstanz, Germany) with simplified KEGG pathway template (Kyoto Encyclopedia of Genes and Genomes) was used for pathway mapping.

Rationale for the usage of multiple instruments and detection methods

Our established pipeline with LC-MS instrument coupled to C18 column requires 440 min to finish MTBE extracts and data acquisition. To fulfil the requirements for high-throughput measurement we need to shorten the time of data acquisition and reduce the volume of sample to be used.

FIA-MRMS, MALDI-MRMS and MALDI-TOF methods were tested in order to asses whether with shortened data acquisition time it is still possible to mine sufficient amount of information from the samples.

Results

Processing time per one animal

LC-MS: Sample preparation 180 min, data acquisition 240 min, 20 µl of plasma per sample.

FIA-ESI-MRMS: Sample preparation 20 min, data acquisition 104 min, in total 124 min, 24 µl of plasma per sample.

MALDI-MRMS: Sample preparation 70 min, data acquisition 182 min, in total 252 min, 6 µl of plasma per sample.

MALDI-TOF: Sample preparation 70 min, data acquisition 65 min, in total 135 min , 6 µl of plasma per sample.

Number of acquired peaks

FIA-ESI-MRMS: 5964 peaks; MALDI-MRMS: 6728 peaks; MALDI-TOF: 520 peaks.

Number of significant peaks

FIA-ESI-MRMS: 1067 peaks; MALDI-MRMS: 930 peaks; MALDI-TOF: 53 peaks.

Number of potentially enriched pathways in Metaboanalyst web-tool:

FIA-ESI-MRMS: 20 pathways; MALDI-MRMS: 34 pathways; MALDI-TOF: 7 pathways.

Number of hits in KEGG Pyrimidine Metabolism Pathway :

FIA-ESI-MRMS: 6 hits; MALDI-MRMS: 5 hits; MALDI-TOF: 1 hit.

Conclusion

Flow injection analysis with electrospray ionization (FIA-ESI) and matrix-assisted laser desorption-ionization (MALDI) magnetic resonance mass spectrometry (MRMS) outperformed MALDI time-of-flight (TOF) both in acquired peaks as well as number of potential hits in pathway enrichment.

At the same time these methods significantly shorten the time for analysis.

Acknowledgment

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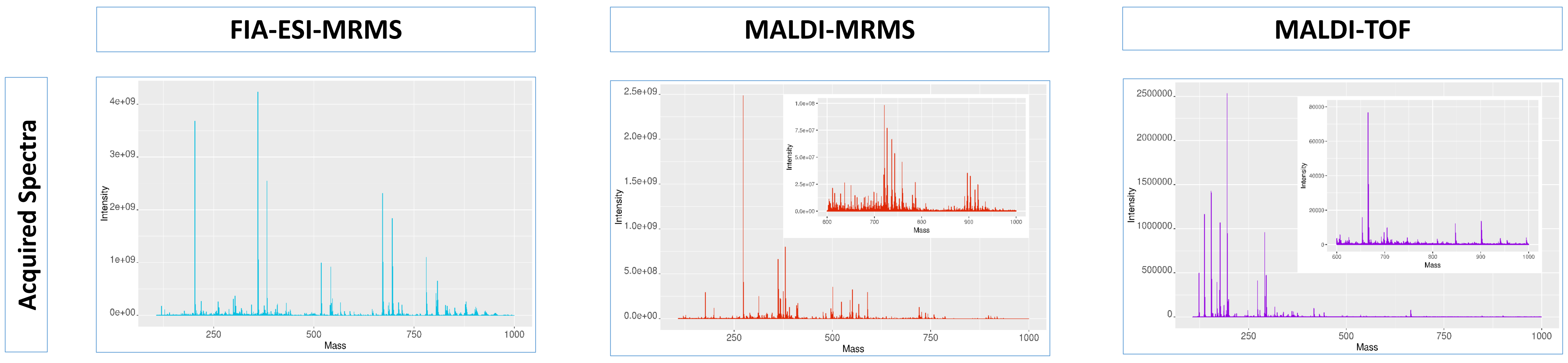
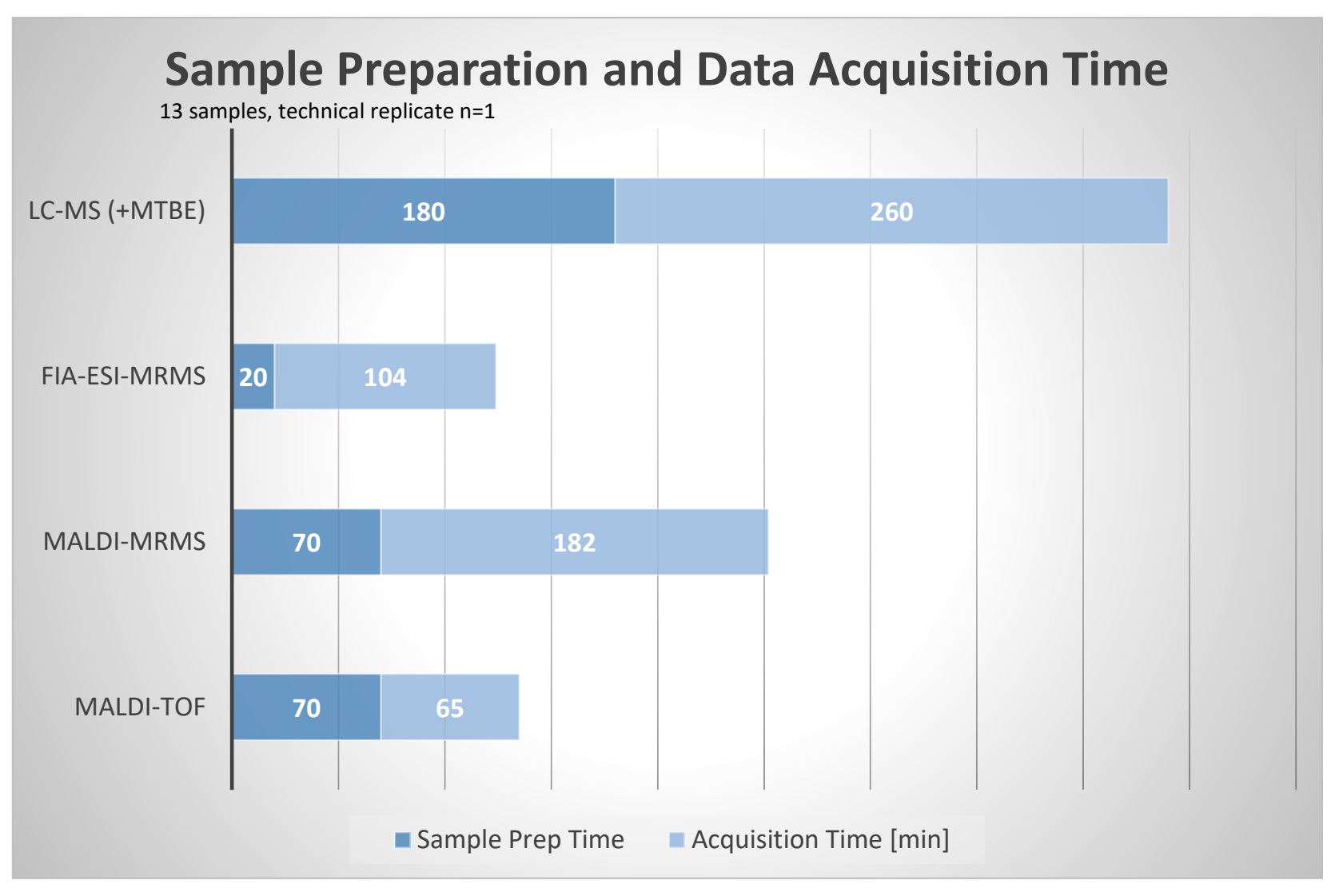
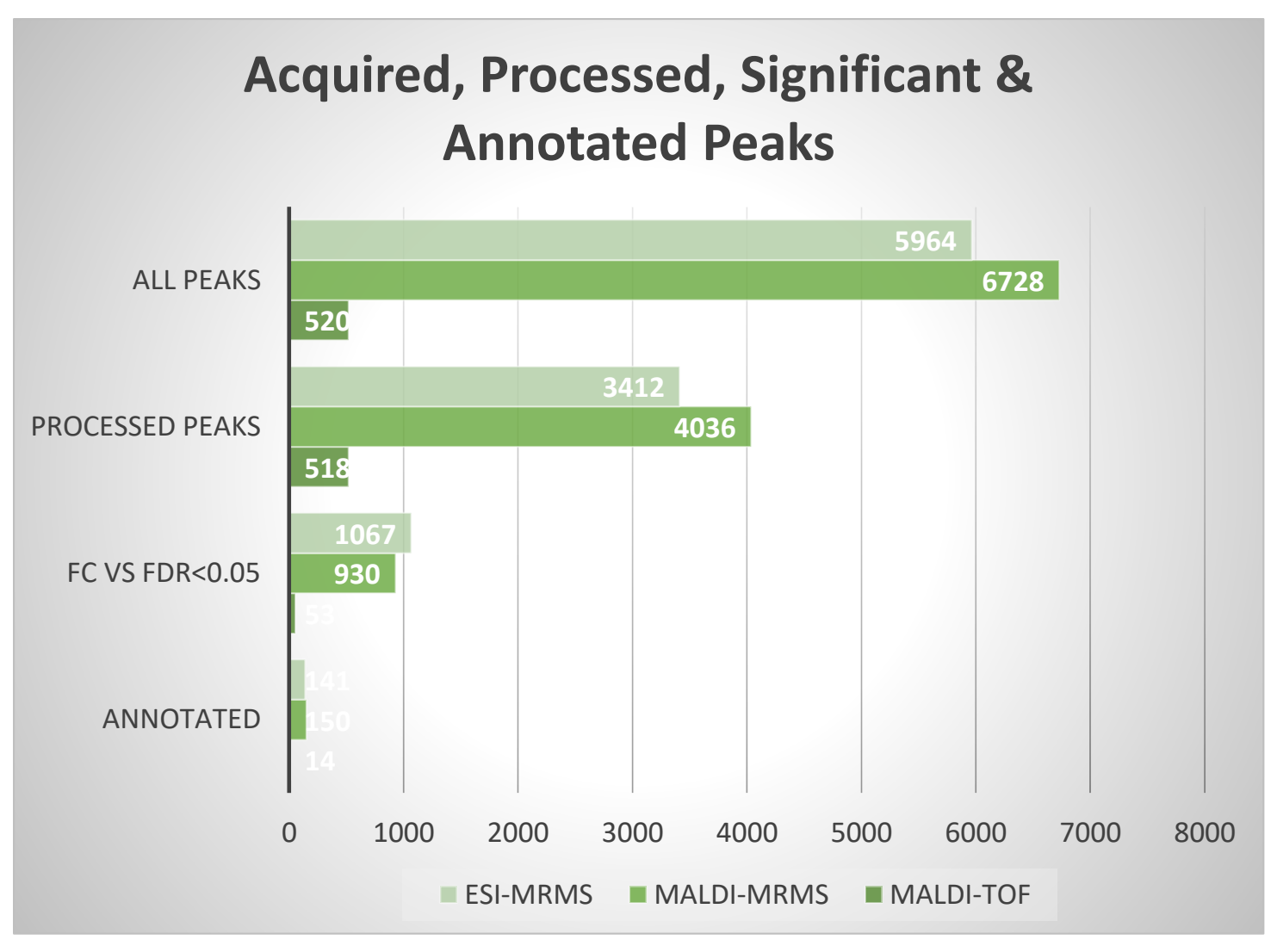
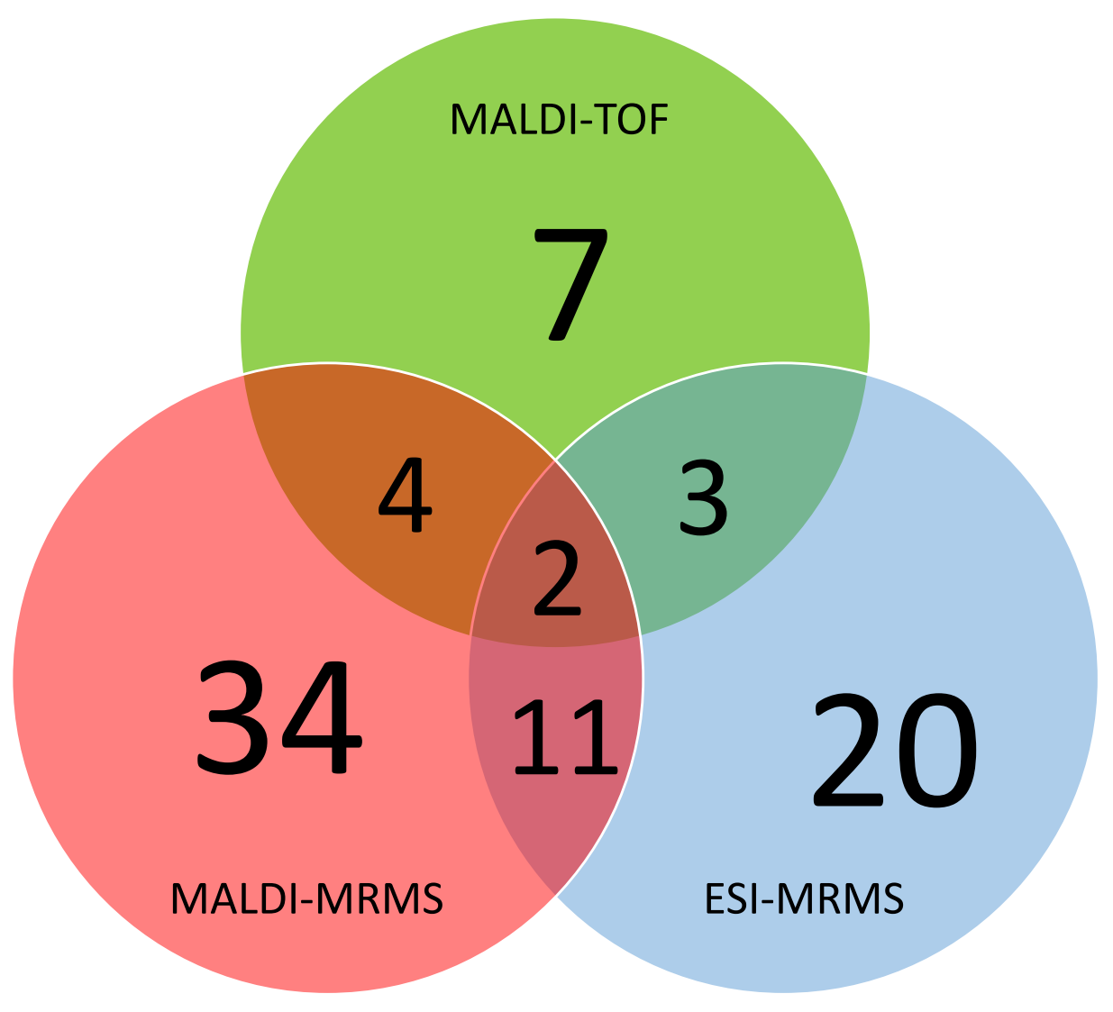
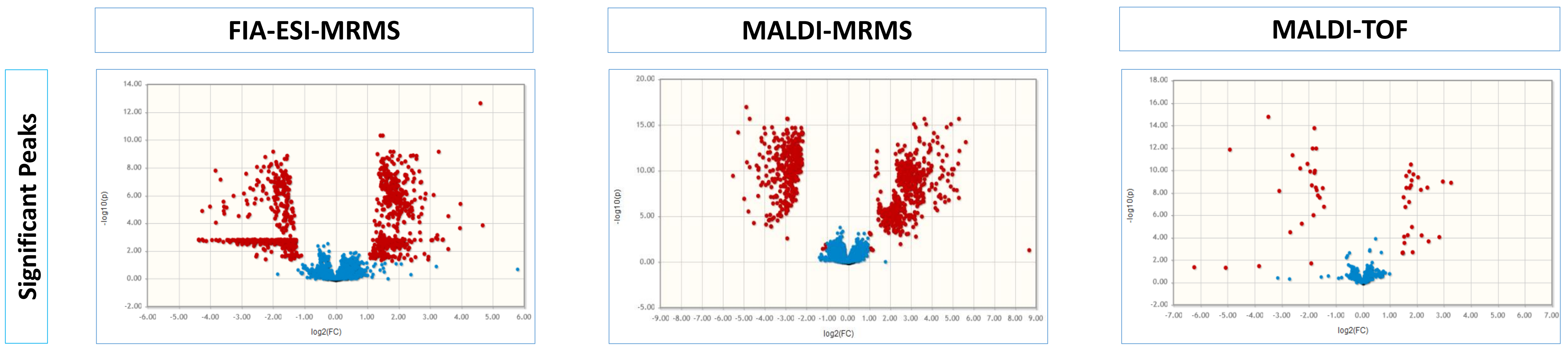


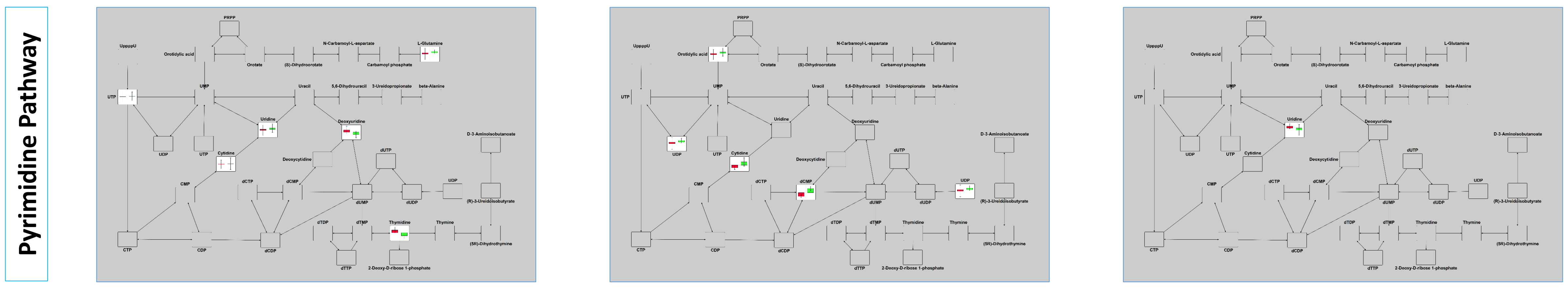






Metaboanalyst Peak to Pathway Tool
Number of Hits





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