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Protocol status: Working
We use this protocol and it's working

Quantitative targeted metabolomics for ASO mouse model using Biocrates Q500 Platform

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ABSTRACT

Using Biocrates MxP® -Q500 kit (BIOCRATES Life Science AG, Innsbruck, Austria) which is a commercially available targeted metabolomics platforms, we measured 634 metabolites across 26 biochemical classes from different tissues of ASO and wild type mouse.

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Sample Preparation

1 Plasma samples were centrifuged, and the supernatant was used for analysis.

2 Brain, colon, and duodenum tissue samples were first suspended in 3 µL ethanol/phosphate buffer per mg tissue wet weight.

3 The samples were then sonicated, vortexed and homogenized using a Precellys-24 instrument (Bertin Technologies, Montigny le Bretonneux, France), and the supernatant was used for analysis.

4 For measurement of some metabolites, it was necessary to dilute the duodenum tissue samples 1:5 in buffer before the samples were centrifuged and the supernatant used for analysis.

5 To extract metabolites from duodenal, cecal and colonic contents, samples were resuspended in extraction buffer (85% ethanol in phosphate buffer) and vortexed thoroughly until dissolved.

6 After homogenization, the samples were ultrasonicated in a chilled bath for 5 min.

7 Samples were then centrifuged and the supernatant was used for analysis.

8 An additional 1:1,000 dilution was prepared for the analysis of highly concentrated bile acids.

Quantification of metabolite concentrations

9 A mass spectrometry (MS)-based targeted metabolomics approach was used to determine the concentration of endogenous metabolites in a total of 226 samples: 23 plasma samples, 69 gut content samples (23 cecal contents, 23 colon contents, and 23 duodenum contents), and 134 tissue samples (88 brain, 23 colon, and 23 duodenum)

10 Metabolites were quantified using the commercially available Biocrates MxP® -Q500 kit (BIOCRATES Life Science AG, Innsbruck, Austria). The kit provides measurements of up to 634 metabolites across 26 biochemical classes. Lipids (e.g, acylcarnitines, glycerophospholipids, sphingolipids, triglycerides) and hexoses were measured by flow injection analysis-tandem MS (FIA-MS/MS) using a 5500 QTRAP® instrument (AB Sciex, Darmstadt, Germany) with an electrospray ionization (ESI) source for the plasma and tissue samples, and a Xevo TQ-S (Waters, Vienna, Austria) instrument with an ESI source for the gut content samples. Small molecules were measured by liquid chromatography-tandem MS (LC-MS/MS), also using a 5500 QTRAP® instrument for all samples. Gut tissue and content samples were also measured by LC-MS/MS on a Xevo TQ-S instrument.

11 To quantitatively analyze metabolite profiles in the samples, a 96-well-based sample preparation device was used which consists of inserts that have been impregnated with internal standards. A predefined sample amount was added to the inserts.

12 Next, a phenyl isothiocyanate (PITC) solution was added to derivatize some of the analytes (e.g., amino acids), and after the derivatization was completed, the target analytes were extracted with an organic solvent, followed by a dilution step.

13 The obtained extracts were then analyzed by FIA-MS/MS and LC-MS/MS methods using multiple reaction monitoring (MRM) to detect the analytes.

14 Data were quantified using appropriate mass spectrometry software (Sciex Analyst® and Waters MassLynx™) and imported into Biocrates MetIDQ™ software for further analysis.

Quality Control of Profiles

15 The raw Q500 metabolomic profiles included measurements of 634 metabolites in 226 samples.

16 Separately for each material type, metabolites with >30% of measurements above the lower limit of detection (LOD) in SPF animals were included (n= 539, 459, 503, and 297 remaining metabolites in plasma, GI tissue, gut content, and brain tissue, respectively).

17 Imputation of <LOD values was performed using each metabolite's LOD/2 value to increase statistical power.

18 Since all samples for each material type were measured on one plate, no batch effect removal procedure was conducted. Metabolite concentrations were $\log_2$ transformed to achieve normal distribution.

Statistical analysis

19 All statistical analyses were conducted in R 4.2.2.

20 t-distributed stochastic neighbor embedding (t-SNE) dimensionality reduction scatterplots were generated of $\log_2$-transformed metabolite concentrations using the package Rtsne (v0.1-3.1) with default parameters.

21 To evaluate the overall effect of genotype, microbiome and their interaction on metabolome in each sample type, Permutational Multivariate Analysis of Variance (PERMANOVA) was conducted using the “adonis2” function in the vegan package with 10,000 permutations and Euclidean distance.

22 To investigate the effects of genotype and microbiome status on the metabolome, separate linear regression models were constructed for each sample. These models defined $\log_2$ metabolite concentration as the dependent variable, and body weight, microbiome, genotype and their interaction as independent variables. This allowed us to estimate the effects of genotype and microbiome status while controlling for variability in body weight. The linear model was specified as: ($\log_2$(metabolite [uM]) ~ Genotype + Microbiome + Genotype*Microbiome + Body-weight)