Include this Attachment in all Emails

Include a Copy of this Form Along with your Freezer Box and Samples

Referencing Us: The NIH requires the Core to submit a usage report for scientific equipment purchased though NIH grant mechanisms. Part of this usage report contains publications that reference these grants in the acknowledgment section. These data are part of the review process when applying for additional instrument funding and are critical for funding new instrumentation. **Please cite "Mass Spectrometers were purchased through NIH Office of the Director, grants 1S10OD016232-01, 1S10OD018210-01A1 and 1S10OD021505-01.**

Core Contact Info

URL: http://cihd.cores.utah.edu/metabolomics
Shipping Address: 15 N. Medical Dr. East, Bldg 565 Room A306, Salt Lake City, Ut 84112
Phone: (801) 587-7779
Director: Dr. James Cox

Chartfield or PO

If new to the HSC Cores please fill out a work authorization form on the HSC Cores website http://cores.utah.edu/ under the WORK AUHT FORMS link. We will not process samples until this is completed. All new grants must be added to the system even if previous work has been done with the Core.

Investigator Contact Details

Date Submitted:
Chartfield:
PI:
PI Email:
Researcher:
Researcher Email:
Institution:
Contact Phone #:

Experiment Details: **FILL IN THE TABLE ON THE ACCOMPANYING SPREADSHEET**

General Sample Description:

Goal(s)

General Goal of the Analysis:

Metabolites/Pathways of Primary Interest:

Metabolites/Pathways of Secondary Interest:

Groups:
Total Number of Samples:
Additional Details:

Metabolomics Analysis and Type Desired

GC-MS	Targeted
LC-MS	Non-Targeted
BOTH	BOTH

Pricing Table*

Please fractionate all tissues and other materials into appropriate amounts prior to submission (contact us for details)

Service	CIHD Internal	CIHD External	University of Utah	External Academic	Commercial
GC-MS Metabolomics	$35.00	$53.38	$55.00	$83.88	$110.00
LC-MS Metabolomics	$40.00	$61.00	$60.00	$91.51	$120.00
Lipidomics	$40.00	$61.00	$68.00	$103.70	$136.00
Data Analysis cost/hour	$0.00	$0.00	$100.00	$152.50	$200.00
GC-MS Instrument cost/hour	$40.00	$61.01	$40.00	$61.00	$80.00
LC-MS Instrument cost/hour	$45.00	$68.63	$45.00	$69.00	$90.49

*Additional preparatory services such as weighing, fractionating, etc. will accrue a $100/hr charge.

Cost Estimate (see below for explanation of cost):

Guidelines and Explanations for the Above:

Types of Metabolomics; GC/MS, LC/MS or Both?: For a detailed explanation of our services, please see our webpage. http://cihd.cores.utah.edu/metabolomics/#1465330998552-1e79904c-ec8a. The Metabolomics Services tab has a more complete explanation of services. In brief, GC/MS is optimal for the analysis of central carbon metabolism (TCA cycle and parts of the glycolytic pathway), organic acid, amino acids, free fatty acids, carbohydrates, and purine and pyrimidine bases. LC/MS is optimal for redox metabolites, coenzymes, nucleotides, and a number of other metabolites.

Targeted, Non-Targeted or Both: The Core uses Quadrupole Time-of-Flight mass spectrometers for most metabolomics analysis. Data is recorded for 1000's of molecular features, many are known metabolites but unknown metabolites are detected at a high rate as well. Targeted data analysis uses quantification software that records the area under the curve for known metabolites. These metabolites have been validated using pure purchased standards. This type of analysis is optimal for determining alterations in known pathways. Non-targeted analysis only looks for altered metabolites between the submitted samples and disregards unaltered metabolites. This approach is useful for biomarker discovery. A combination of the two produces a powerful data set but is very labor intensive and thus costly to generate.

Experimental Goals: Briefly describe your experimental goals in terms of biological type, treatment, GC/MS, LC/MS or both, non-targeted, targeted or both analysis. An example would be "We desire to profile the metabolome of a MEL cell culture line treated by siRNA by GC/MS to determine if TCA cycle intermediates are altered."

Sample Type: Examples are cell culture, fruit fly, mouse liver, zebrafish brain etc.

Groups: The number and name of experimental groups i.e. control, treated etc.

Sample Total: The total number of submitted samples.

Cost estimate:
The pricing for metabolomics is based upon two parameters; a) the cost for extraction and instrument analysis, and b) the cost for data analysis and report generation. The per sample cost is fixed and based upon the actual cost to extract, prepare, and run each sample on the instrument. The cost of data analysis is highly variable depending on the number of comparisons to be made and the type of data analysis (targeted, non-targeted or both). Labor cost ranges from $50/hour to $100/hour depending on the analyst and the desired product. A simple example is two sample groups consisting of six biological replicates each. The desired instrument is GC/MS with targeted data analysis. The per sample rate is $45/each, it would take approximately 4 hours to generate a report by a junior analyst for a total of $740. If non-targeted analysis were desired for this sample set the number of hours needed would be approximately eight by a senior analyst at $100/hour for a total of $1340. If both targeted and non-targeted analysis desired the cost would be approximately $1540.
We will provide a cost estimate prior to sample submission.

How to Submit: Fill out the Excel spread sheet "Investigator Submission Form Sample Details" fully with the name of each sample that matches that of the sample submission tube (this much be legible!) along with other needed descriptor such as sample type, cell number, weight, etc. Important: if submitting tissue all samples must be weighed prior to submission, we will not weigh them. Bring samples to the core frozen on dry ice or ship on dry ice to address at top of submission form.

Turnaround Time: This is highly variable and is dependent on the number of samples submitted, the number of groups to be compared, the type of data analysis, the current backlog and acts of God. We will provide a best estimate of project completion barring instrument failure at time of submission.

What type of data to expect
For targeted analysis we will provide an excel file and report describing all the steps taken to prepare the report. The actual values in the excel file will be normalized Area Under the Curve for each metabolite. The final sheet contains data that can be further analyzed by MetaboAnalyst.ca. We will perform partial or full MetaboAnalyst report generation depending on the investigators desired outcome. A sample report is from /MS is below.
For untargeted analysis only altered chemical entities are reported. These can be either know or unknown depending if the retention time and MS/MS pattern matches with a database. Many times this is not the case and the findings are reported as a chemical entity described by retention time and characteristic mass/charge ratio. We make every attempt to identify altered metabolites using MS/MS fragmentation pattern and purchased standards but not all altered molecular features are identifiable.

Sample Metabolomics Report

GC-MS Metabolomics Analysis of mouse heart tissue for Johnny Johnson from the Tim Thomson lab.

I. Experimental Goal

The goal of this work was to analyze 2 groups of mouse heart tissue, mutant and wild type, for metabolic changes using gas chromatography-mass spectrometry (GC-MS).

II. Introduction to Metabolomics

Metabolomics is the unbiased survey of metabolites found within a tissue, biological fluid, organism, culture or other biological source. Metabolomics is a comparative science; we analyze the differences found between biological samples that have been subjected to a treatment. This can be due to a genetic mutation, drug treatment, etc. Because this is a relative analysis, we can only make judgments on individual metabolites, such as comparing the amounts of succinate found in a mutant and a wild type. For example, we cannot compare the levels of succinate and fumarate within the same group or between different groups.

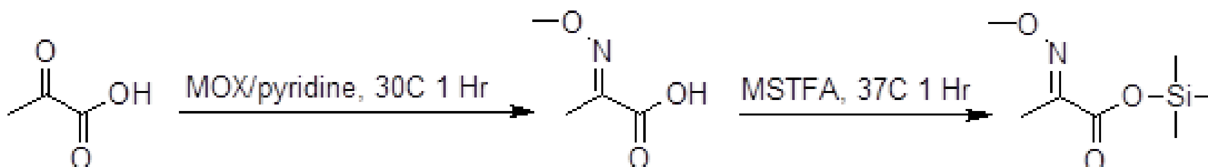
III. Experimental

Metabolite Extraction

For the heart extraction, each sample was weighed and broken into roughly equal, manageable pieces and their masses were recorded. Each tissue sample was then transferred to 2.0ml bead mill tubes (bio Express). To each sample was added cold 90% methanol (MeOH) solution containing the internal standard d4-succinic acid (Sigma 293075) to give a final concentration of 80% MeOH to each tissue sample. The samples were then homogenized in an OMNI Bead Ruptor 24. Homogenized samples were then incubated at -20 ˚C for 1 hr. After incubation the samples were centrifuged at 20,000 x g for 10 minutes at 4˚C. 450ul of supernatant was transferred from each bead mill tube into a labeled, fresh micro centrifuge tubes. Another internal standard, d27-myristic acid, was then added to each sample. Pooled quality control samples were made by removing a fraction of collected supernatant from each sample. Process blanks were made using only extraction solvent and went through the same process steps as each sample. The samples were then dried en vacuo.

GC-MS analysis

All GC-MS analysis was performed with an Agilent 7200 GC-MS QTOF and an Agilent 7693A automatic liquid sampler. Dried samples were suspended in 40 µL of a 40 mg/mL O-methoxylamine hydrochloride (MOX) (MP Bio #155405) in dry pyridine (EMD Millipore #PX2012-7) and incubated for one hour at 37 °C in a sand bath. 13 µL of this solution was added to auto sampler vials. 60 µL of N-methyl-N-trimethylsilyltrifluoracetamide (MSTFA with 1%TMCS, Thermo #TS48913) was added automatically via the auto sampler and incubated for 30 minutes at 37 °C. After incubation, samples were vortexed and 1 µL of the prepared sample was injected into the gas chromatograph inlet in the split mode with the inlet temperature held at 250°C. A 50:1 split ratio was used for analysis. For highly abundant metabolites that saturated at the 50:1 split, a split ratio of 100:1 was used for analysis. The gas chromatograph had an initial temperature of 60°C for one minute followed by a 10°C/min ramp to 325°C and a hold time of 2 minutes. A 30-meter Agilent Zorbax DB-5MS with 10 m Duraguard capillary column was employed for chromatographic separation. Helium was used as the carrier gas at a rate of 1 mL/min. Below is a description of the two step derivatization process used to convert non-volatile metabolites to a volatile form amenable to GC-MS. Pyruvic acid is used here as an example.



Analysis of GC-MS data

Data was collected using MassHunter software (Agilent). Metabolites were identified and their peak area was recorded using MassHunter Quant. This data was transferred to an Excel spread sheet (Microsoft, Redmond WA). Metabolite identity was established using a combination of an in-house metabolite library developed using pure purchased standards, the NIST library and the Fiehn library. There are a few reasons a specific metabolite may not be observable through GC-MS. The metabolite may not be amenable to GC-MS due to its size, or a quaternary amine such as carnitine, or simply because it does not ionize well. Metabolites that do not ionize well include oxaloacetate, histidine and arginine. Cysteine can be observed depending on cellular conditions. It often forms disulfide bonds with proteins and is generally at a low concentration. Metabolites may not be quantifiable if they are only present in very low concentrations.

Data Analysis

Data Pretreatment

Data was exported to an Excel file and the following steps taken to prepare a data set. A number of steps were performed. I included all the data for transparency purposes but the only tabs of interest are the Finalized Data and MA tabs. The steps below describe the data pretreatment.

1. Raw Data: No treatment of any kind was performed in this tab. Any sample removed from analysis was marked in red.
2. Normalized Data to d4: The data was then corrected by mean centering to the internal standard d4-succinate. Any blanks where MassHunter Quant failed to find a peak were filled with a value of 0 for statistical ease-of-use.
3. Normalized to weight: This is done if supplied with tissue.
4. Data QC: The fourth tab tests for individual metabolite reliability. Each metabolite was tested by determining the %CV of the QC samples. Values less than 30% were considered passing. Important metabolites that failed, but were near the threshold were reviewed and kept or removed based on peak quality and consistency. We next tested contribution of background and contaminate peaks by testing the average of the QC samples divided by the average of the process blank (PB) samples. Values greater than 1.5 were considered passing.
5. Parsed: The fourth tab has data that did not pass the QC tests mentioned above removed. Most metabolites with %CV of QC greater than 30% were removed. Also the identity of each peak was ensured by visualizing each peak in Mass Hunter Qual. False positives were removed.
6. MetaboAnalyst: This tab is a combined data set that is ready for analysis by Metaboanlyst (see below) after making a new .csv file and removing false positives. Metadata describing the grouping is added.

Statistical analysis

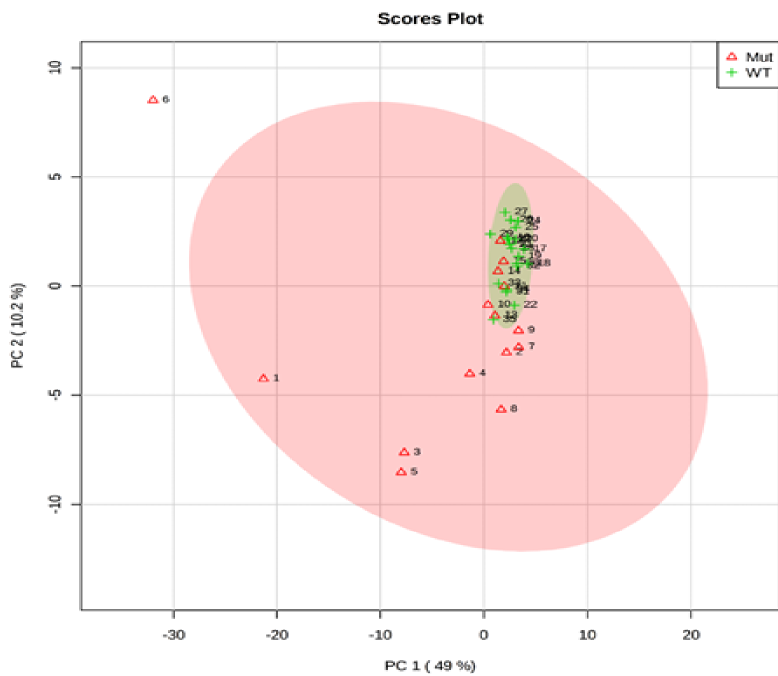
Further analysis was performed using Metaboanalsyt 3.0 (http://www.metaboanalyst.ca/). This is a freely available web-based program used for significance testing and pathway mapping. The attached .csv file is in the appropriate format and is a copy of the data from the original Excel file. To further analyze the data, I performed the following steps.

1. Start at http://www.metaboanalyst.ca/. Several key papers are listed on the home page; these explain how to use this program.
2. “Click here to start”
3. Click on “Statistical Analysis”
4. In 1) Upload your data, click radio button “Peak intensity table”
5. Choose your file and click “Submit”
6. Click through next screen if no errors
7. Data Integrity Check. Skip, no missing values.
8. In Data Filtering change from Interquantile range to none, click “Submit”
9. In Data Normalization page I selected Norm to SUM in order to reduce variability in data caused by possible heart fibrosis.
10. I clicked on Log Transformation.
11. Under Data scaling, I chose Pareto, then click “Normalize.” Click “View Result” and verify data centering.
12. If Normalization looks centered (see publications listed on Metaboanalyst home page) then click “Proceed.”
13. Next, select an analysis path to explore. For multiple sample types use PCA to determine if there is group separation and ANOVA for significance testing. If two sample types to compare, for example KO vs WT, use PCA and a volcano plot.

Heart Analysis

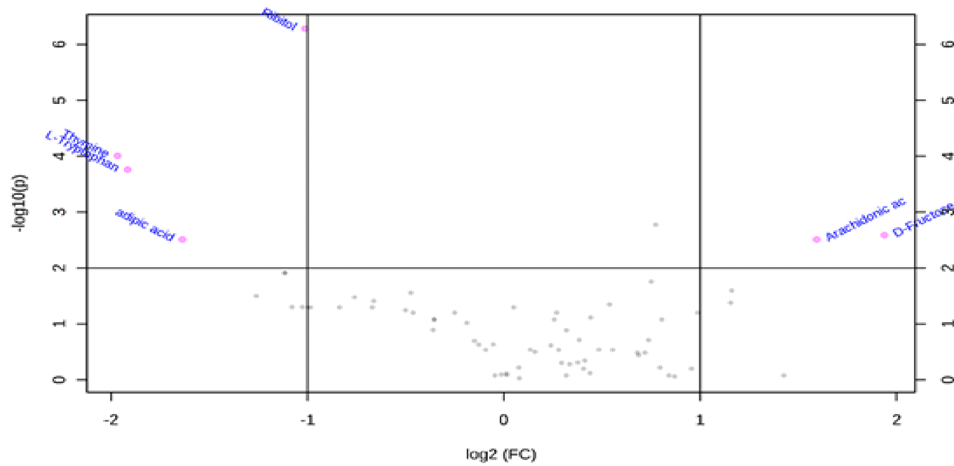
1. PCA of all samples

A Principle Component Analysis (PCA) plot was generated to give a high level over view of the data and to determine if strong outliers were present. This is a non-targeted statistical method in which the algorithm is naïve as to which samples belong to each sample group. Sample 6 is out of the 95% confidence region below.



2. Volcano Plot of Mutant vs WT

Shown below is a volcano plot used find altered metabolites with both a strong p-value and fold change.
Fold change threshold is 2; P-value threshold is 0.01 with FDR



Metabolite	Fold Change Mut/WT	log2(FC)	p.adjusted	-log10(p)
D-Fructose	3.834	1.9389	0.0025632	2.5912
Arachidonic acid	3.0174	1.5933	0.00306	2.5143
Ribitol	0.49562	-1.0127	5.14E-07	6.289
Adipic acid	0.32136	-1.6378	0.00306	2.5143
L-Tryptophan	0.2649	-1.9165	0.00017298	3.762
Thymine	0.25575	-1.9672	9.84E-05	4.0071

1. Pathway analysis-with enough altered metabolites this can be performed in MetaboAnalyst under Pathway Analysis using the same criteria as used in the statistical analysis.