*Knowledge that will change your world*

# Searching for metabolites and the Metabolomics Workbench

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http://www.metabolomicsworkbench.org/

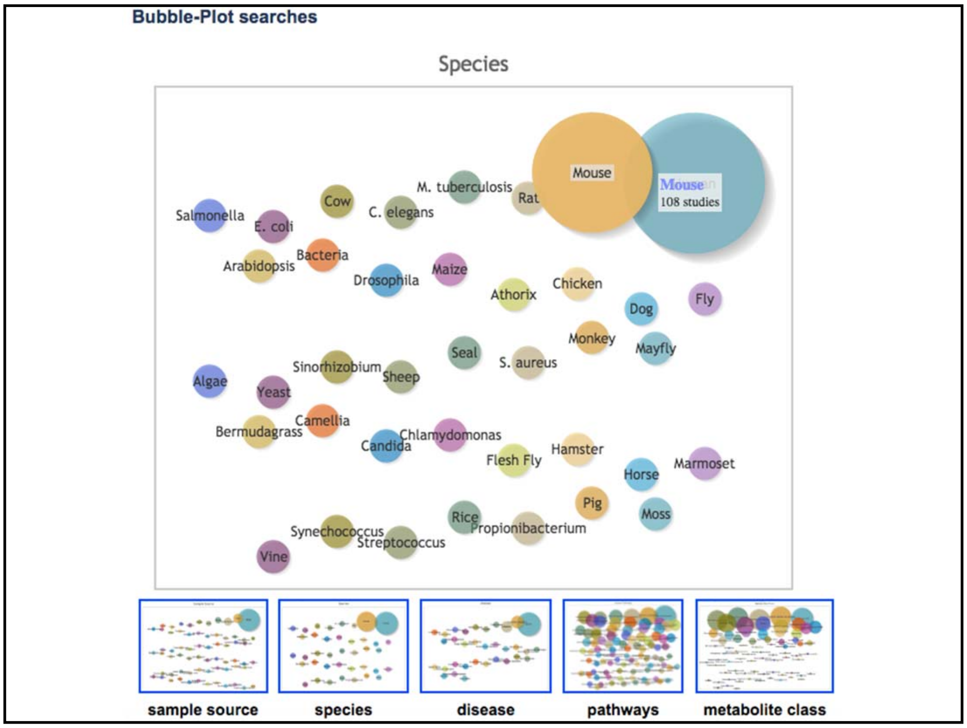
*Welcome to the UCSD Metabolomics Workbench, a resource sponsored by the Common Fund of the National Institutes of Health.*

05-04-2016 Free Agilent and West Coast Metabolomics Center workshop; please register at http://www.agilent.com/chem/ucdavis .

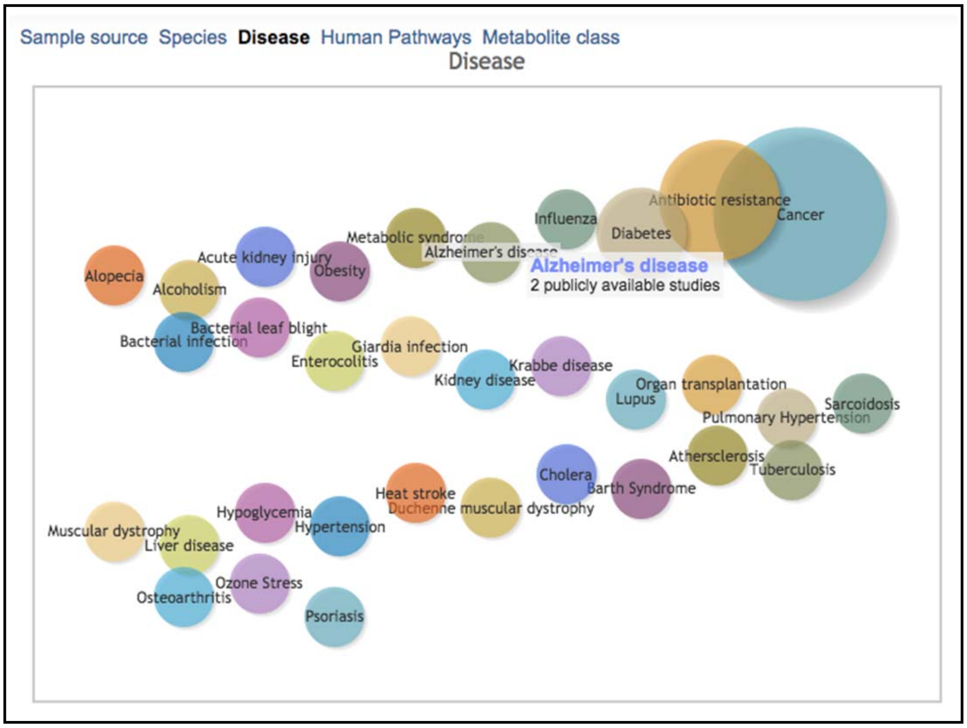
02-25-2016 UAB 4th Annual Workshop on Metabolomics July 17 - 21, 2016, Birmingham, AL, USA
This course is jointly sponsored by the National Institute of General Medical Sciences (NIGMS) as part of the NIH Common Fund Metabolomics Initiative, and the Departments of Chemistry and Pharmacology and Toxicology at UAB. Topics covered range from design of a metabolomics experiment to advanced elective sessions (imaging mass spectrometry, isotope ratio analysis, ion mobility, command line and R programs)

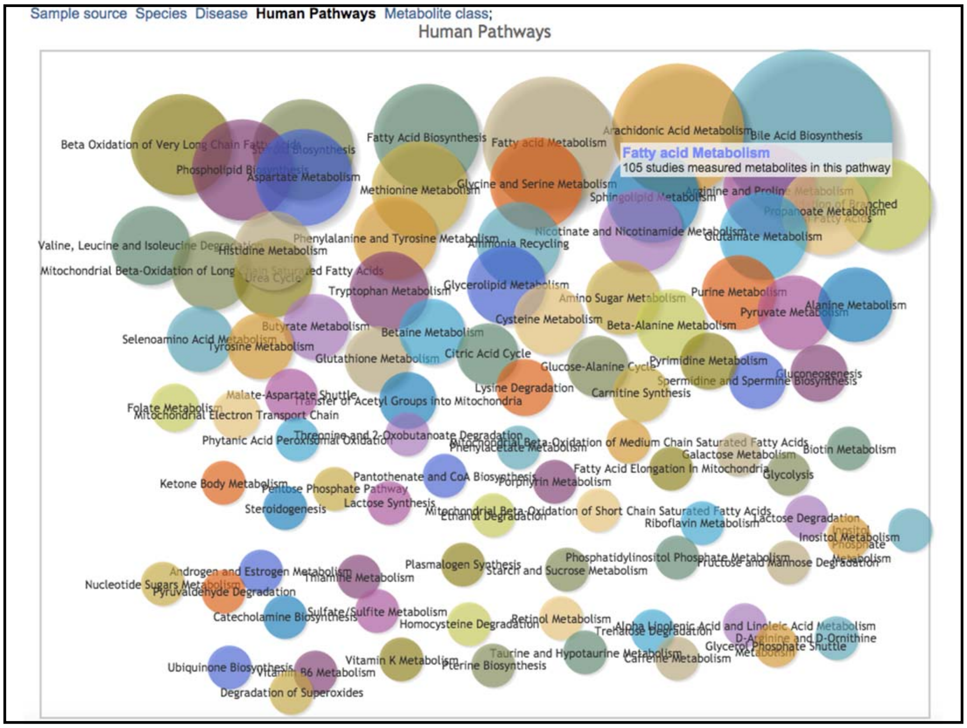
YOUR FEEDBACK- Please tell us about your experiences with the **Metabolomics Workbench**. We also welcome your comments regarding our **Ring Trial.**

UAB 4th Annual Workshop on Metabolomics
July 17-21, 2016
Birmingham, AL, USA



**Bubble-Plot searches**

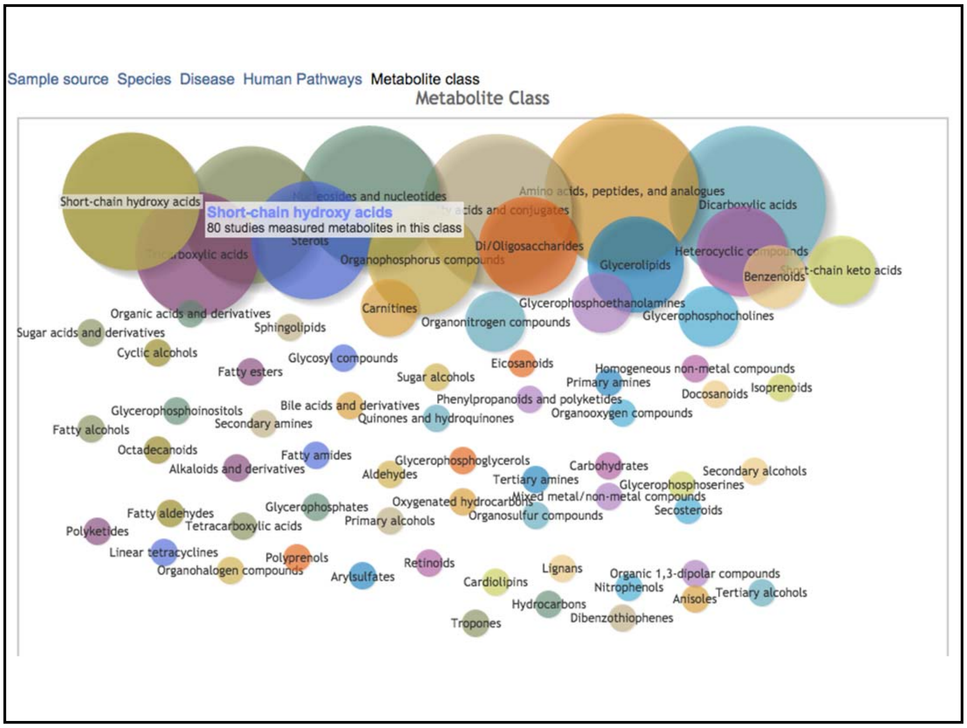




Sample source Species Disease **Human Pathways** Metabolite class;

Human Pathways

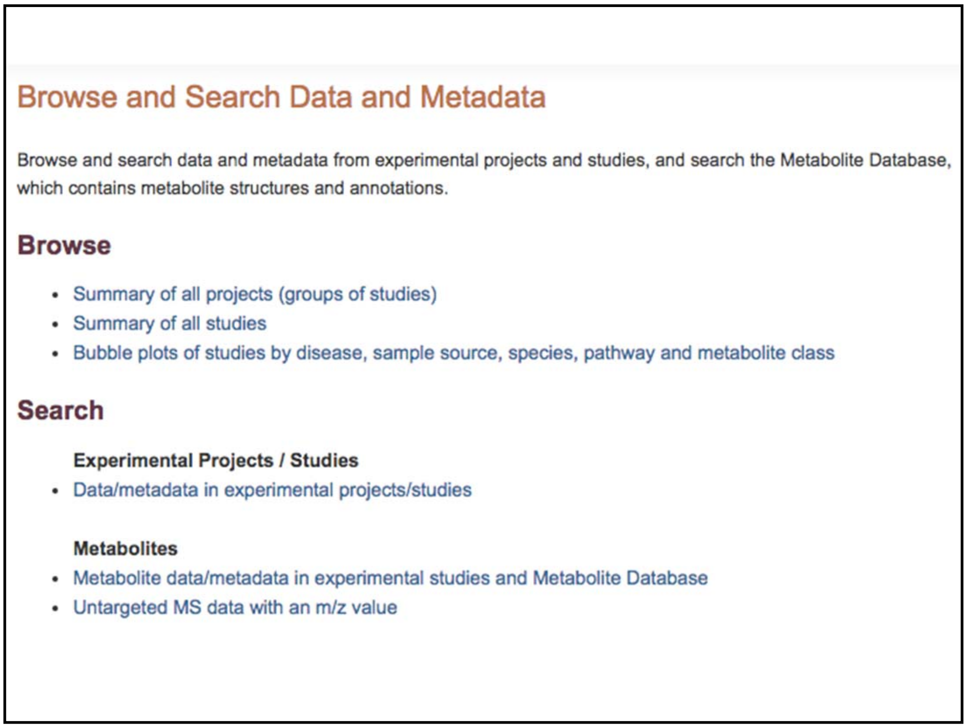
**Fatty acid Metabolism**
105 studies measured metabolites in this pathway



Sample source Species Disease Human Pathways **Metabolite class**

Metabolite Class

**Short-chain hydroxy acids**
80 studies measured metabolites in this class



## Browse and Search Data and Metadata

Browse and search data and metadata from experimental projects and studies, and search the Metabolite Database, which contains metabolite structures and annotations.

### Browse

- Summary of all projects (groups of studies)
- Summary of all studies
- Bubble plots of studies by disease, sample source, species, pathway and metabolite class

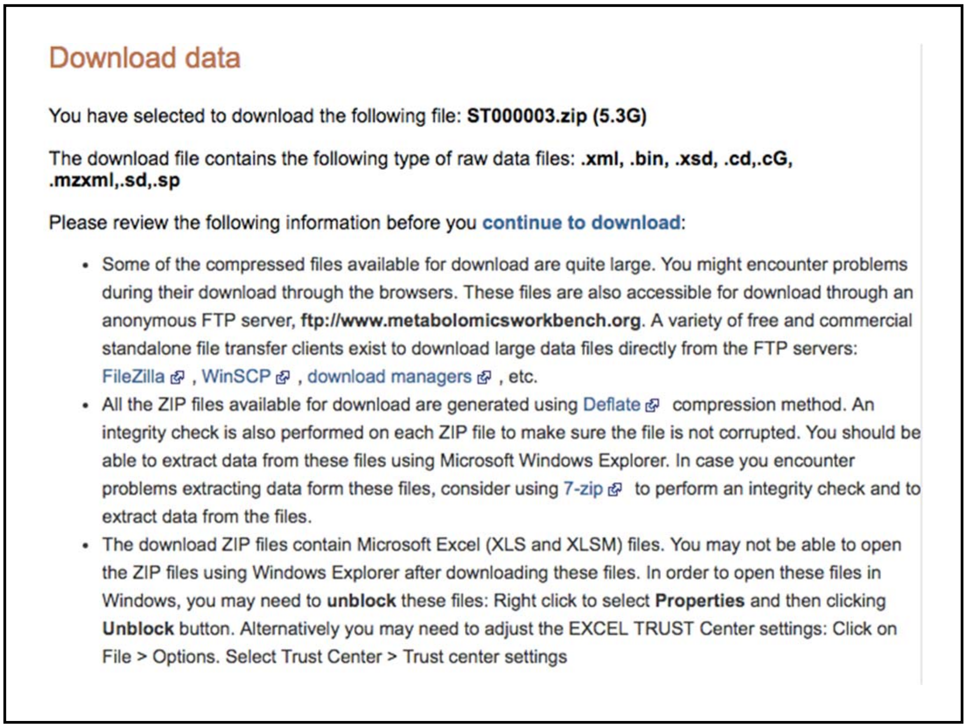
### Search

**Experimental Projects / Studies**

- Data/metadata in experimental projects/studies

**Metabolites**

- Metabolite data/metadata in experimental studies and Metabolite Database
- Untargeted MS data with an m/z value



## Download data

You have selected to download the following file: **ST000003.zip (5.3G)**

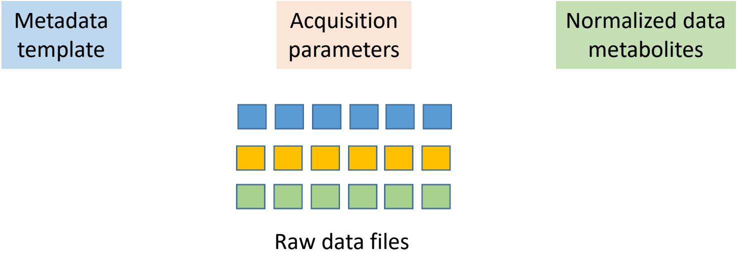
The download file contains the following type of raw data files: **.xml, .bin, .xsd, .cd,.cG, .mzxml,.sd,.sp**

Please review the following information before you **continue to download**:

- Some of the compressed files available for download are quite large. You might encounter problems during their download through the browsers. These files are also accessible for download through an anonymous FTP server, **ftp://www.metabolomicsworkbench.org**. A variety of free and commercial standalone file transfer clients exist to download large data files directly from the FTP servers: FileZilla , WinSCP , download managers , etc.
- All the ZIP files available for download are generated using Deflate compression method. An integrity check is also performed on each ZIP file to make sure the file is not corrupted. You should be able to extract data from these files using Microsoft Windows Explorer. In case you encounter problems extracting data form these files, consider using 7-zip to perform an integrity check and to extract data from the files.
- The download ZIP files contain Microsoft Excel (XLS and XLSM) files. You may not be able to open the ZIP files using Windows Explorer after downloading these files. In order to open these files in Windows, you may need to **unblock** these files: Right click to select **Properties** and then clicking **Unblock** button. Alternatively you may need to adjust the EXCEL TRUST Center settings: Click on File > Options. Select Trust Center > Trust center settings

## Downloading the ST00003 file

- 5.3 GB
- Took about 6 hours to download
- When unzipped, it contained three Excel files and the raw data files



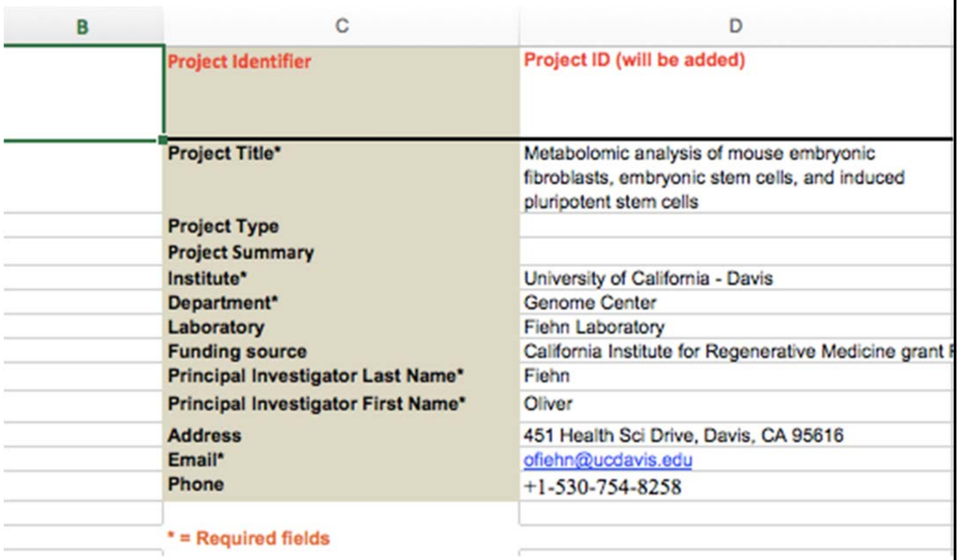
Metadata template

Acquisition parameters

Normalized data metabolites

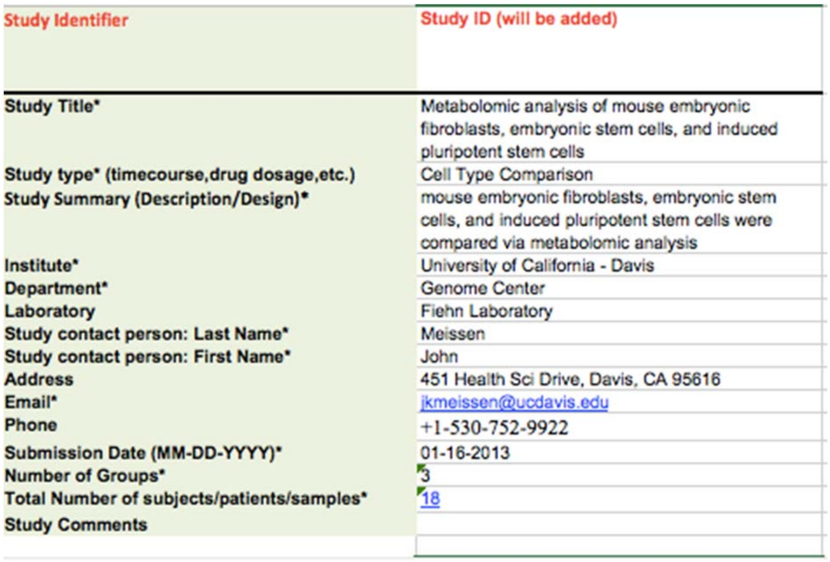
Raw data files

## Metadata 1



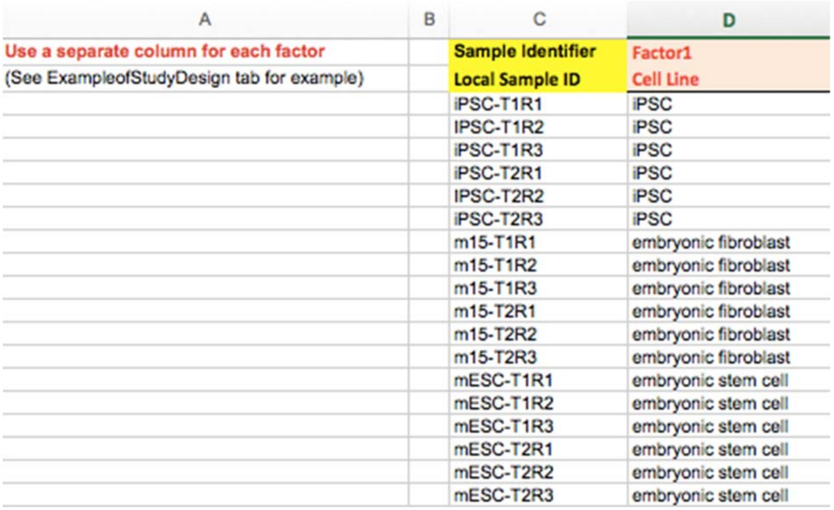
| B | C | D |
|---|---|---|
| | Project Identifier | Project ID (will be added) |
| | Project Title* | Metabolomic analysis of mouse embryonic fibroblasts, embryonic stem cells, and induced pluripotent stem cells |
| | Project Type | |
| | Project Summary | |
| | Institute* | University of California - Davis |
| | Department* | Genome Center |
| | Laboratory | Fiehn Laboratory |
| | Funding source | California Institute for Regenerative Medicine grant |
| | Principal Investigator Last Name* | Fiehn |
| | Principal Investigator First Name* | Oliver |
| | Address | 451 Health Sci Drive, Davis, CA 95616 |
| | Email* | ofiehn@ucdavis.edu |
| | Phone | +1-530-754-8258 |
| | | |
| | * = Required fields | |

# Metadata 2



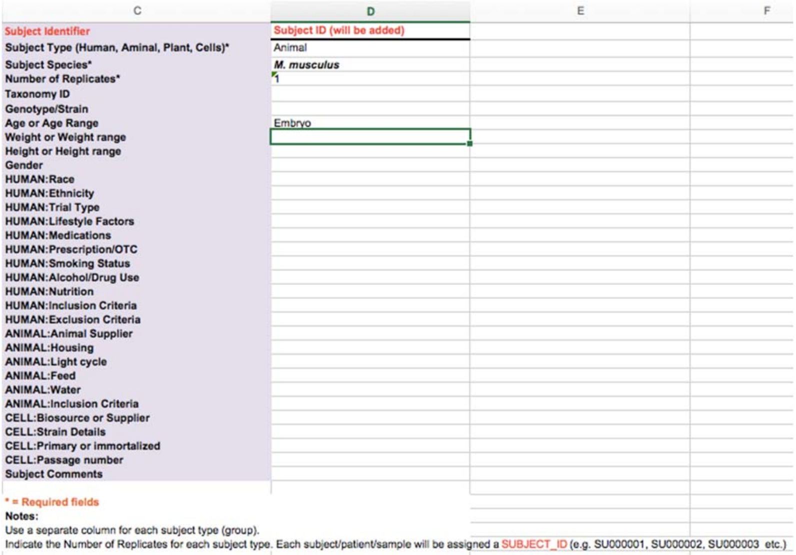
| | |
|---|---|
| Study Identifier | Study ID (will be added) |
| Study Title* | Metabolomic analysis of mouse embryonic fibroblasts, embryonic stem cells, and induced pluripotent stem cells |
| Study type* (timecourse,drug dosage,etc.) | Cell Type Comparison |
| Study Summary (Description/Design)* | mouse embryonic fibroblasts, embryonic stem cells, and induced pluripotent stem cells were compared via metabolomic analysis |
| Institute* | University of California - Davis |
| Department* | Genome Center |
| Laboratory | Fiehn Laboratory |
| Study contact person: Last Name* | Meissen |
| Study contact person: First Name* | John |
| Address | 451 Health Sci Drive, Davis, CA 95616 |
| Email* | jkmeissen@ucdavis.edu |
| Phone | +1-530-752-9922 |
| Submission Date (MM-DD-YYYY)* | 01-16-2013 |
| Number of Groups* | 3 |
| Total Number of subjects/patients/samples* | 18 |
| Study Comments | |

# Metadata 3



| A | B | C | D |
|---|---|---|---|
| Use a separate column for each factor | | Sample Identifier | Factor1 |
| (See ExampleofStudyDesign tab for example) | | Local Sample ID | Cell Line |
| | | iPSC-T1R1 | iPSC |
| | | IPSC-T1R2 | iPSC |
| | | iPSC-T1R3 | iPSC |
| | | iPSC-T2R1 | iPSC |
| | | IPSC-T2R2 | iPSC |
| | | iPSC-T2R3 | iPSC |
| | | m15-T1R1 | embryonic fibroblast |
| | | m15-T1R2 | embryonic fibroblast |
| | | m15-T1R3 | embryonic fibroblast |
| | | m15-T2R1 | embryonic fibroblast |
| | | m15-T2R2 | embryonic fibroblast |
| | | m15-T2R3 | embryonic fibroblast |
| | | mESC-T1R1 | embryonic stem cell |
| | | mESC-T1R2 | embryonic stem cell |
| | | mESC-T1R3 | embryonic stem cell |
| | | mESC-T2R1 | embryonic stem cell |
| | | mESC-T2R2 | embryonic stem cell |
| | | mESC-T2R3 | embryonic stem cell |

# Metadata 4



| C | D | E | F |
|---|---|---|---|
| Subject Identifier | Subject ID (will be added) | | |
| Subject Type (Human, Aminal, Plant, Cells)* | Animal | | |
| Subject Species* | *M. musculus* | | |
| Number of Replicates* | 1 | | |
| Taxonomy ID | | | |
| Genotype/Strain | | | |
| Age or Age Range | Embryo | | |
| Weight or Weight range | | | |
| Height or Height range | | | |
| Gender | | | |
| HUMAN:Race | | | |
| HUMAN:Ethnicity | | | |
| HUMAN:Trial Type | | | |
| HUMAN:Lifestyle Factors | | | |
| HUMAN:Medications | | | |
| HUMAN:Prescription/OTC | | | |
| HUMAN:Smoking Status | | | |
| HUMAN:Alcohol/Drug Use | | | |
| HUMAN:Nutrition | | | |
| HUMAN:Inclusion Criteria | | | |
| HUMAN:Exclusion Criteria | | | |
| ANIMAL:Animal Supplier | | | |
| ANIMAL:Housing | | | |
| ANIMAL:Light cycle | | | |
| ANIMAL:Feed | | | |
| ANIMAL:Water | | | |
| ANIMAL:Inclusion Criteria | | | |
| CELL:Biosource or Supplier | | | |
| CELL:Strain Details | | | |
| CELL:Primary or immortalized | | | |
| CELL:Passage number | | | |
| Subject Comments | | | |

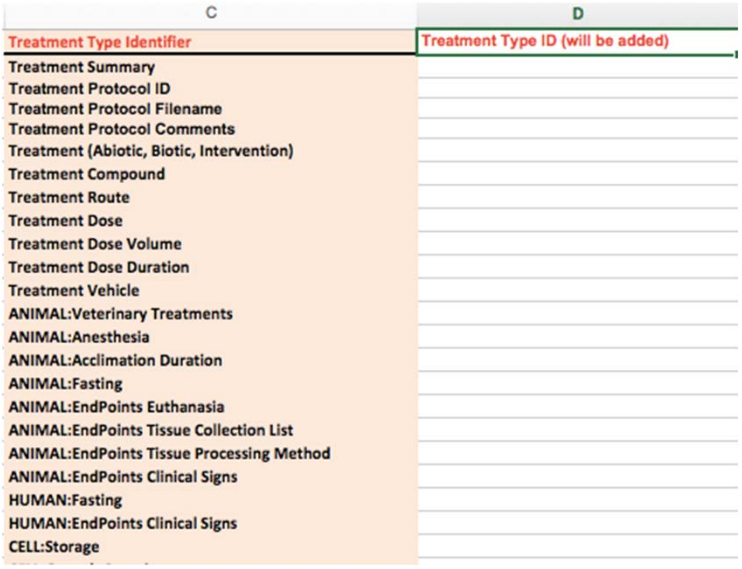
* = Required fields

**Notes:**

Use a separate column for each subject type (group).

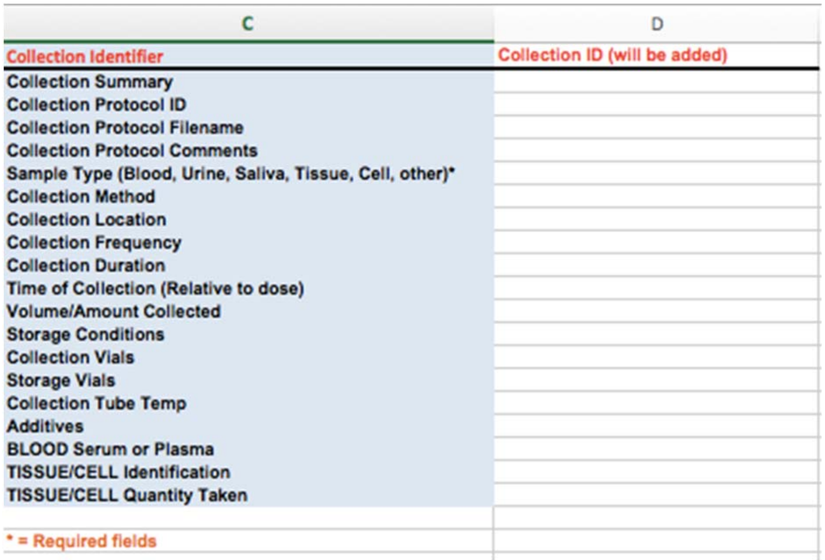
Indicate the Number of Replicates for each subject type. Each subject/patient/sample will be assigned a SUBJECT_ID (e.g. SU000001, SU000002, SU000003 etc.)

# Metadata 5



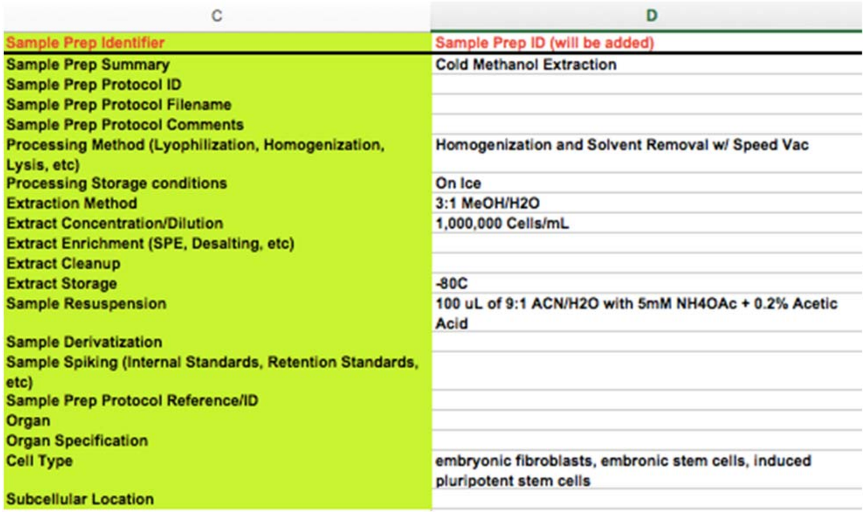
| C | D |
|---|---|
| Treatment Type Identifier | Treatment Type ID (will be added) |
| Treatment Summary | |
| Treatment Protocol ID | |
| Treatment Protocol Filename | |
| Treatment Protocol Comments | |
| Treatment (Abiotic, Biotic, Intervention) | |
| Treatment Compound | |
| Treatment Route | |
| Treatment Dose | |
| Treatment Dose Volume | |
| Treatment Dose Duration | |
| Treatment Vehicle | |
| ANIMAL:Veterinary Treatments | |
| ANIMAL:Anesthesia | |
| ANIMAL:Acclimation Duration | |
| ANIMAL:Fasting | |
| ANIMAL:EndPoints Euthanasia | |
| ANIMAL:EndPoints Tissue Collection List | |
| ANIMAL:EndPoints Tissue Processing Method | |
| ANIMAL:EndPoints Clinical Signs | |
| HUMAN:Fasting | |
| HUMAN:EndPoints Clinical Signs | |
| CELL:Storage | |

# Metadata 6



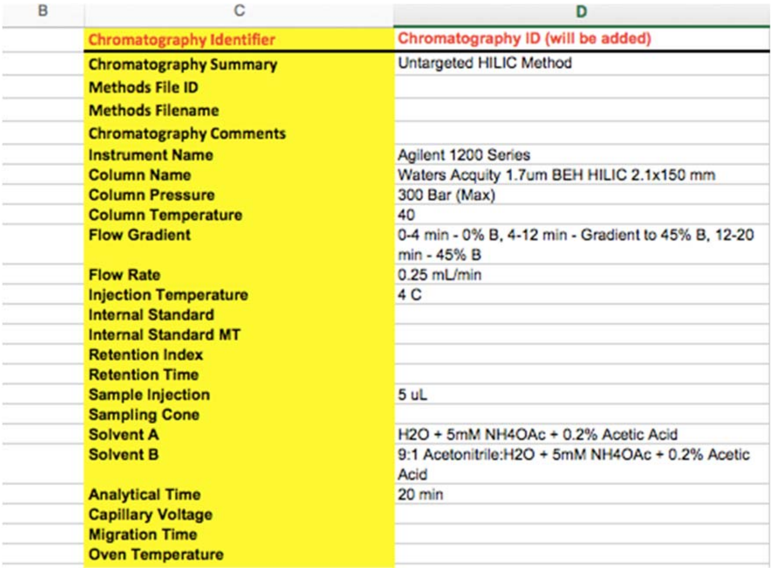
| C | D |
|---|---|
| Collection Identifier | Collection ID (will be added) |
| Collection Summary | |
| Collection Protocol ID | |
| Collection Protocol Filename | |
| Collection Protocol Comments | |
| Sample Type (Blood, Urine, Saliva, Tissue, Cell, other)* | |
| Collection Method | |
| Collection Location | |
| Collection Frequency | |
| Collection Duration | |
| Time of Collection (Relative to dose) | |
| Volume/Amount Collected | |
| Storage Conditions | |
| Collection Vials | |
| Storage Vials | |
| Collection Tube Temp | |
| Additives | |
| BLOOD Serum or Plasma | |
| TISSUE/CELL Identification | |
| TISSUE/CELL Quantity Taken | |
| | |
| * = Required fields | |

# Metadata 7



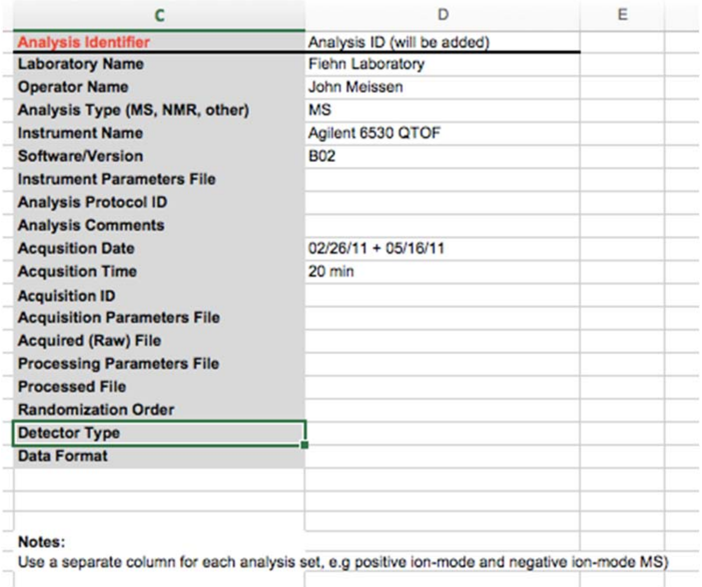
| C | D |
|---|---|
| Sample Prep Identifier | Sample Prep ID (will be added) |
| Sample Prep Summary | Cold Methanol Extraction |
| Sample Prep Protocol ID | |
| Sample Prep Protocol Filename | |
| Sample Prep Protocol Comments | |
| Processing Method (Lyophilization, Homogenization, Lysis, etc) | Homogenization and Solvent Removal w/ Speed Vac |
| Processing Storage conditions | On Ice |
| Extraction Method | 3:1 MeOH/H2O |
| Extract Concentration/Dilution | 1,000,000 Cells/mL |
| Extract Enrichment (SPE, Desalting, etc) | |
| Extract Cleanup | |
| Extract Storage | -80C |
| Sample Resuspension | 100 uL of 9:1 ACN/H2O with 5mM NH4OAc + 0.2% Acetic Acid |
| Sample Derivatization | |
| Sample Spiking (Internal Standards, Retention Standards, etc) | |
| Sample Prep Protocol Reference/ID | |
| Organ | |
| Organ Specification | |
| Cell Type | embryonic fibroblasts, embronic stem cells, induced pluripotent stem cells |
| Subcellular Location | |

# Metadata 8



| B | C | D |
|---|---|---|
| | Chromatography Identifier | Chromatography ID (will be added) |
| | Chromatography Summary | Untargeted HILIC Method |
| | Methods File ID | |
| | Methods Filename | |
| | Chromatography Comments | |
| | Instrument Name | Agilent 1200 Series |
| | Column Name | Waters Acquity 1.7um BEH HILIC 2.1x150 mm |
| | Column Pressure | 300 Bar (Max) |
| | Column Temperature | 40 |
| | Flow Gradient | 0-4 min - 0% B, 4-12 min - Gradient to 45% B, 12-20 min - 45% B |
| | Flow Rate | 0.25 mL/min |
| | Injection Temperature | 4 C |
| | Internal Standard | |
| | Internal Standard MT | |
| | Retention Index | |
| | Retention Time | |
| | Sample Injection | 5 uL |
| | Sampling Cone | |
| | Solvent A | H2O + 5mM NH4OAc + 0.2% Acetic Acid |
| | Solvent B | 9:1 Acetonitrile:H2O + 5mM NH4OAc + 0.2% Acetic Acid |
| | Analytical Time | 20 min |
| | Capillary Voltage | |
| | Migration Time | |
| | Oven Temperature | |

# Metadata 9

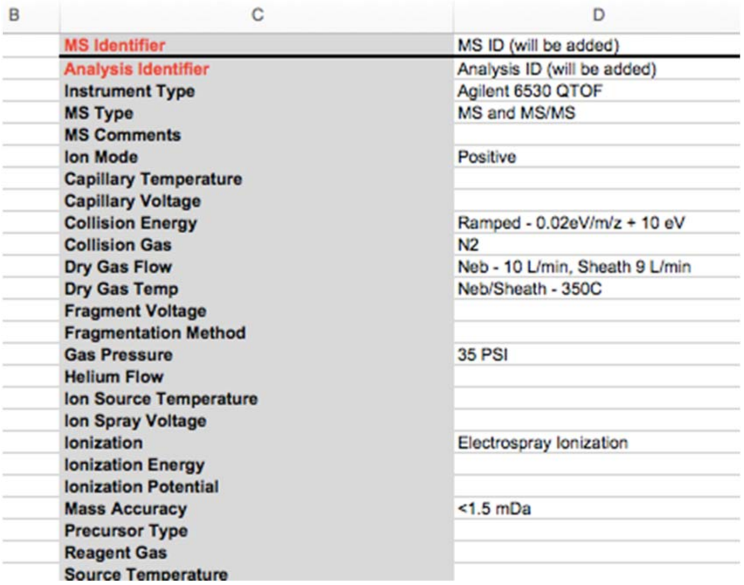


| C | D | E |
|---|---|---|
| Analysis Identifier | Analysis ID (will be added) | |
| Laboratory Name | Fiehn Laboratory | |
| Operator Name | John Meissen | |
| Analysis Type (MS, NMR, other) | MS | |
| Instrument Name | Agilent 6530 QTOF | |
| Software/Version | B02 | |
| Instrument Parameters File | | |
| Analysis Protocol ID | | |
| Analysis Comments | | |
| Acqusition Date | 02/26/11 + 05/16/11 | |
| Acqusition Time | 20 min | |
| Acquisition ID | | |
| Acquisition Parameters File | | |
| Acquired (Raw) File | | |
| Processing Parameters File | | |
| Processed File | | |
| Randomization Order | | |
| Detector Type | | |
| Data Format | | |

**Notes:**

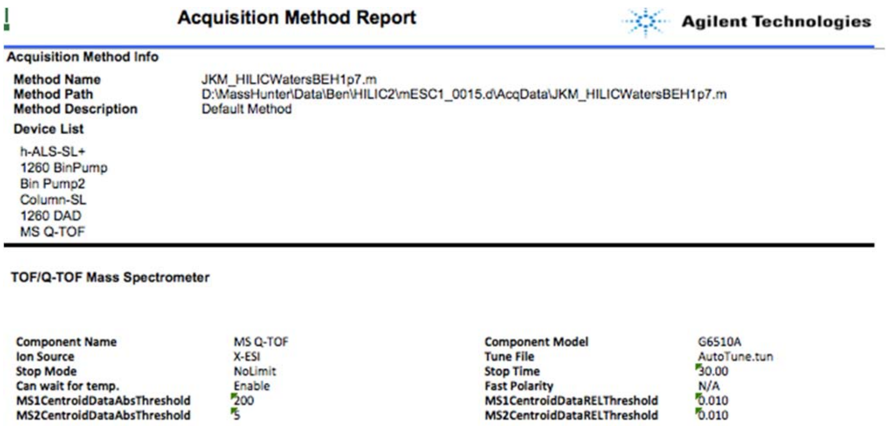
Use a separate column for each analysis set, e.g positive ion-mode and negative ion-mode MS)

# Metadata 10



| B | C | D |
|---|---|---|
| | MS Identifier | MS ID (will be added) |
| | Analysis Identifier | Analysis ID (will be added) |
| | Instrument Type | Agilent 6530 QTOF |
| | MS Type | MS and MS/MS |
| | MS Comments | |
| | Ion Mode | Positive |
| | Capillary Temperature | |
| | Capillary Voltage | |
| | Collision Energy | Ramped - 0.02eV/m/z + 10 eV |
| | Collision Gas | N2 |
| | Dry Gas Flow | Neb - 10 L/min, Sheath 9 L/min |
| | Dry Gas Temp | Neb/Sheath - 350C |
| | Fragment Voltage | |
| | Fragmentation Method | |
| | Gas Pressure | 35 PSI |
| | Helium Flow | |
| | Ion Source Temperature | |
| | Ion Spray Voltage | |
| | Ionization | Electrospray Ionization |
| | Ionization Energy | |
| | Ionization Potential | |
| | Mass Accuracy | <1.5 mDa |
| | Precursor Type | |
| | Reagent Gas | |
| | Source Temperature | |

# LC-MS parameters - overall



**Acquisition Method Report**

Agilent Technologies

**Acquisition Method Info**

| | |
|---|---|
| **Method Name** | JKM_HILICWatersBEH1p7.m |
| **Method Path** | D:\MassHunter\Data\Ben\HILIC2\mESC1_0015.d\AcqData\JKM_HILICWatersBEH1p7.m |
| **Method Description** | Default Method |

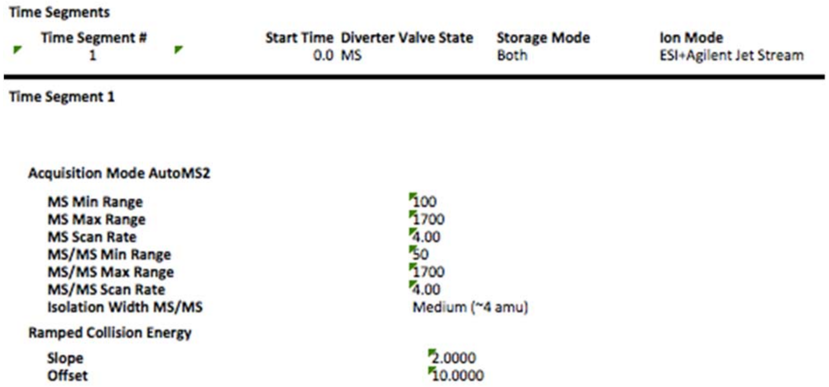
**Device List**

h-ALS-SL+
1260 BinPump
Bin Pump2
Column-SL
1260 DAD
MS Q-TOF

**TOF/Q-TOF Mass Spectrometer**

| | | | |
|---|---|---|---|
| **Component Name** | MS Q-TOF | **Component Model** | G6510A |
| **Ion Source** | X-ESI | **Tune File** | AutoTune.tun |
| **Stop Mode** | NoLimit | **Stop Time** | 30.00 |
| **Can wait for temp.** | Enable | **Fast Polarity** | N/A |
| **MS1CentroidDataAbsThreshold** | 200 | **MS1CentroidDataRELThreshold** | 0.010 |
| **MS2CentroidDataAbsThreshold** | 5 | **MS2CentroidDataRELThreshold** | 0.010 |

# LC-MS parameters – MS conditions



Time Segments

| Time Segment # | Start Time | Diverter Valve State | Storage Mode | Ion Mode |
|---|---|---|---|---|
| 1 | 0.0 | MS | Both | ESI+Agilent Jet Stream |

Time Segment 1

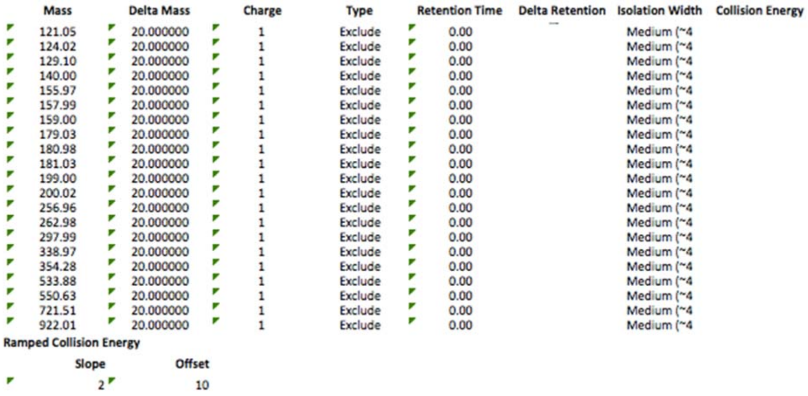
Acquisition Mode AutoMS2

| | |
|---|---|
| MS Min Range | 100 |
| MS Max Range | 1700 |
| MS Scan Rate | 4.00 |
| MS/MS Min Range | 50 |
| MS/MS Max Range | 1700 |
| MS/MS Scan Rate | 4.00 |
| Isolation Width MS/MS | Medium (~4 amu) |

Ramped Collision Energy

| | |
|---|---|
| Slope | 2.0000 |
| Offset | 10.0000 |

# LC-MS parameters – excluded ions

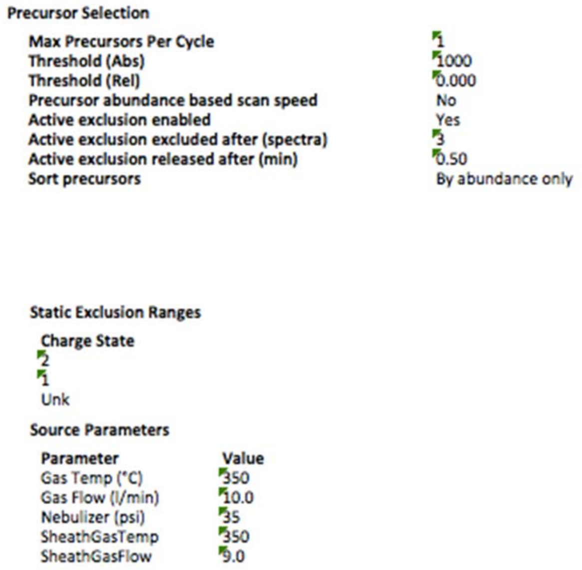


| Mass | Delta Mass | Charge | Type | Retention Time | Delta Retention | Isolation Width | Collision Energy |
|---|---|---|---|---|---|---|---|
| 121.05 | 20.000000 | 1 | Exclude | 0.00 | — | Medium (~4 | |
| 124.02 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 129.10 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 140.00 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 155.97 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 157.99 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 159.00 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 179.03 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 180.98 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 181.03 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 199.00 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 200.02 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 256.96 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 262.98 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 297.99 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 338.97 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 354.28 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 533.88 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 550.63 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 721.51 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |
| 922.01 | 20.000000 | 1 | Exclude | 0.00 | | Medium (~4 | |

Ramped Collision Energy

| Slope | Offset |
|---|---|
| 2 | 10 |

# LC-MS parameters 4



**Precursor Selection**

| | |
|---|---|
| Max Precursors Per Cycle | 1 |
| Threshold (Abs) | 1000 |
| Threshold (Rel) | 0.000 |
| Precursor abundance based scan speed | No |
| Active exclusion enabled | Yes |
| Active exclusion excluded after (spectra) | 3 |
| Active exclusion released after (min) | 0.50 |
| Sort precursors | By abundance only |

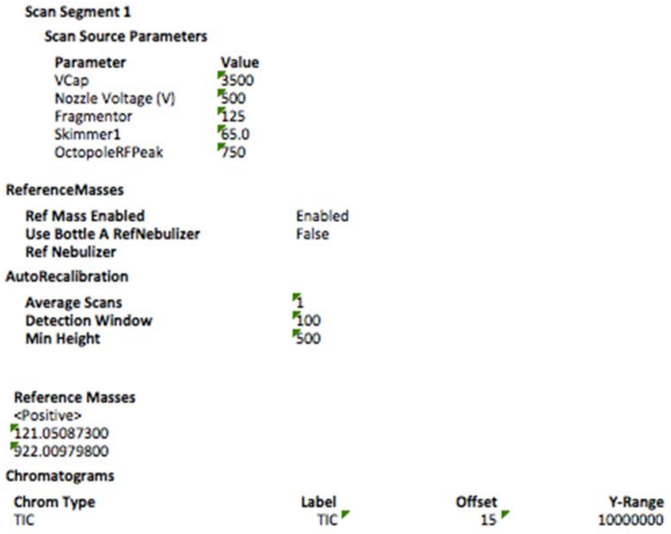
**Static Exclusion Ranges**

| Charge State |
|---|
| 2 |
| 1 |
| Unk |

**Source Parameters**

| Parameter | Value |
|---|---|
| Gas Temp (°C) | 350 |
| Gas Flow (l/min) | 10.0 |
| Nebulizer (psi) | 35 |
| SheathGasTemp | 350 |
| SheathGasFlow | 9.0 |

# LC-MS parameters 5



**Scan Segment 1**

**Scan Source Parameters**

| Parameter | Value |
|---|---|
| VCap | 3500 |
| Nozzle Voltage (V) | 500 |
| Fragmentor | 125 |
| Skimmer1 | 65.0 |
| OctopoleRFPeak | 750 |

**ReferenceMasses**

| | |
|---|---|
| Ref Mass Enabled | Enabled |
| Use Bottle A RefNebulizer | False |
| Ref Nebulizer | |

**AutoRecalibration**

| | |
|---|---|
| Average Scans | 1 |
| Detection Window | 100 |
| Min Height | 500 |

**Reference Masses**

<Positive>

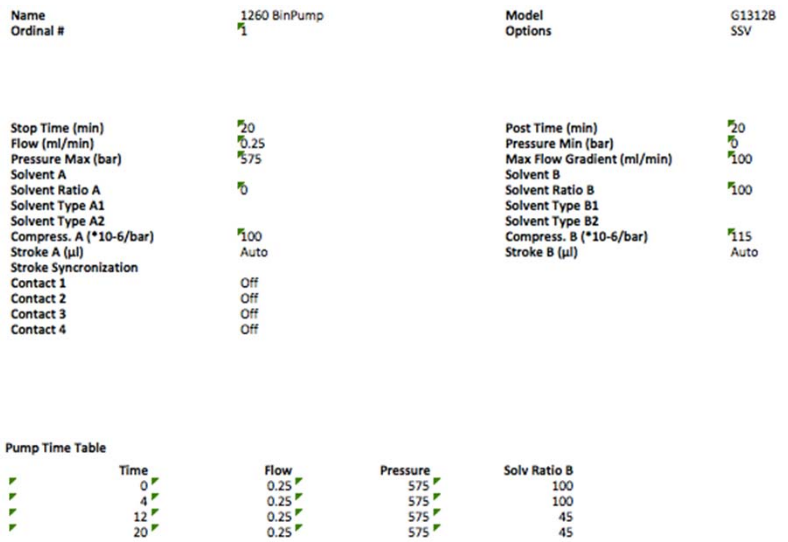
121.05087300

922.00979800

**Chromatograms**

| Chrom Type | Label | Offset | Y-Range |
|---|---|---|---|
| TIC | TIC | 15 | 10000000 |

# LC-MS parameters – pump 1



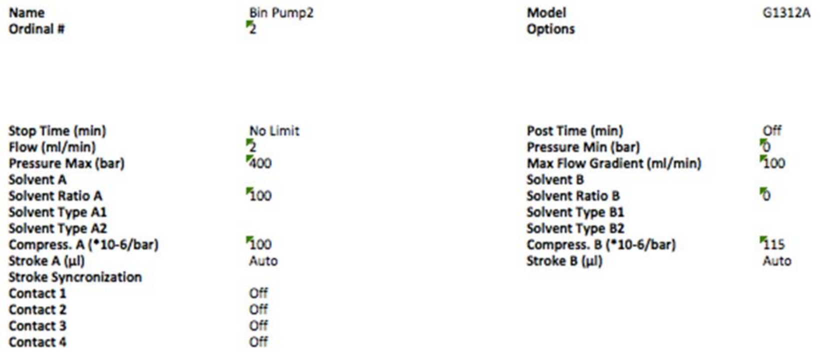
| | | | |
|---|---|---|---|
| Name | 1260 BinPump | Model | G1312B |
| Ordinal # | 1 | Options | SSV |

| | | | |
|---|---|---|---|
| Stop Time (min) | 20 | Post Time (min) | 20 |
| Flow (ml/min) | 0.25 | Pressure Min (bar) | 0 |
| Pressure Max (bar) | 575 | Max Flow Gradient (ml/min) | 100 |
| Solvent A | | Solvent B | |
| Solvent Ratio A | 0 | Solvent Ratio B | 100 |
| Solvent Type A1 | | Solvent Type B1 | |
| Solvent Type A2 | | Solvent Type B2 | |
| Compress. A (*10-6/bar) | 100 | Compress. B (*10-6/bar) | 115 |
| Stroke A (µl) | Auto | Stroke B (µl) | Auto |
| Stroke Syncronization | | | |
| Contact 1 | Off | | |
| Contact 2 | Off | | |
| Contact 3 | Off | | |
| Contact 4 | Off | | |

Pump Time Table

| Time | Flow | Pressure | Solv Ratio B |
|---|---|---|---|
| 0 | 0.25 | 575 | 100 |
| 4 | 0.25 | 575 | 100 |
| 12 | 0.25 | 575 | 45 |
| 20 | 0.25 | 575 | 45 |

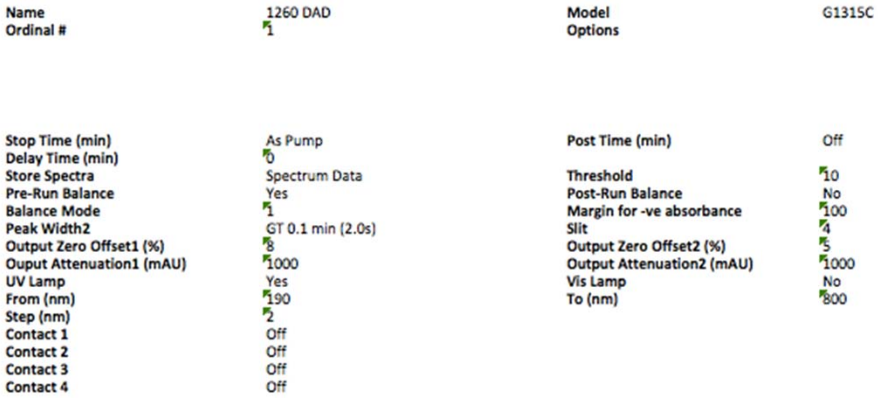
# LC-MS parameters – pump 2



| | | | |
|---|---|---|---|
| Name | Bin Pump2 | Model | G1312A |
| Ordinal # | 2 | Options | |

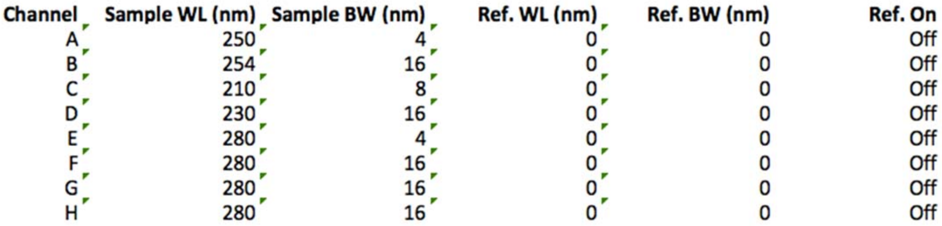
| | | | |
|---|---|---|---|
| Stop Time (min) | No Limit | Post Time (min) | Off |
| Flow (ml/min) | 2 | Pressure Min (bar) | 0 |
| Pressure Max (bar) | 400 | Max Flow Gradient (ml/min) | 100 |
| Solvent A | | Solvent B | |
| Solvent Ratio A | 100 | Solvent Ratio B | 0 |
| Solvent Type A1 | | Solvent Type B1 | |
| Solvent Type A2 | | Solvent Type B2 | |
| Compress. A (*10-6/bar) | 100 | Compress. B (*10-6/bar) | 115 |
| Stroke A (µl) | Auto | Stroke B (µl) | Auto |
| Stroke Syncronization | | | |
| Contact 1 | Off | | |
| Contact 2 | Off | | |
| Contact 3 | Off | | |
| Contact 4 | Off | | |

# LC-MS parameters – diode array

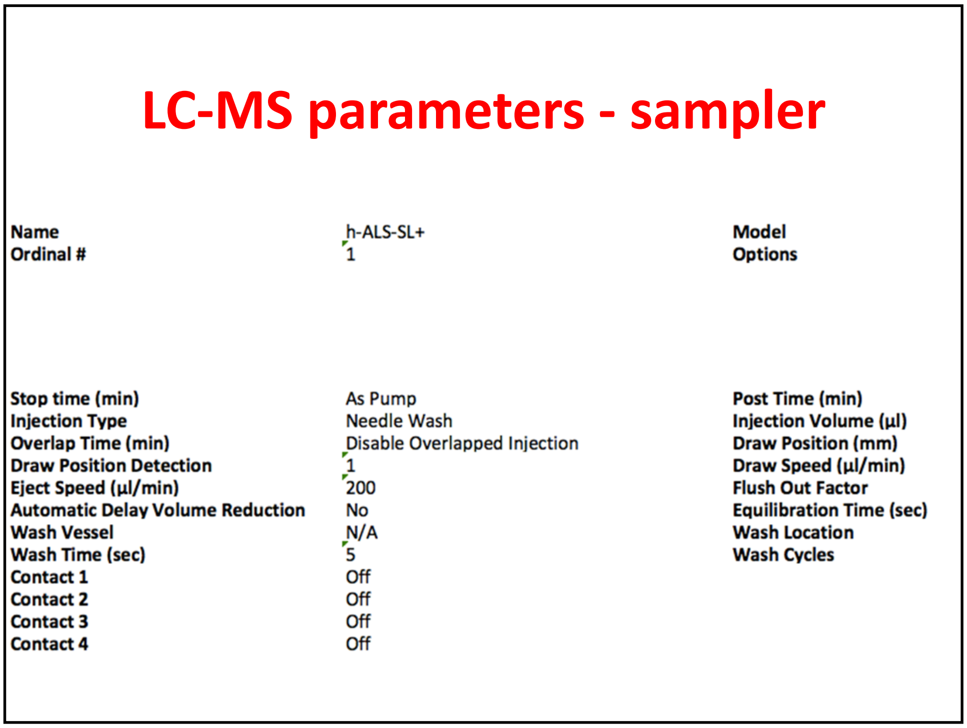


| | | | |
|---|---|---|---|
| **Name** | 1260 DAD | **Model** | G1315C |
| **Ordinal #** | 1 | **Options** | |
| **Stop Time (min)** | As Pump | **Post Time (min)** | Off |
| **Delay Time (min)** | 0 | | |
| **Store Spectra** | Spectrum Data | **Threshold** | 10 |
| **Pre-Run Balance** | Yes | **Post-Run Balance** | No |
| **Balance Mode** | 1 | **Margin for -ve absorbance** | 100 |
| **Peak Width2** | GT 0.1 min (2.0s) | **Slit** | 4 |
| **Output Zero Offset1 (%)** | 8 | **Output Zero Offset2 (%)** | 5 |
| **Ouput Attenuation1 (mAU)** | 1000 | **Output Attenuation2 (mAU)** | 1000 |
| **UV Lamp** | Yes | **Vis Lamp** | No |
| **From (nm)** | 190 | **To (nm)** | 800 |
| **Step (nm)** | 2 | | |
| **Contact 1** | Off | | |
| **Contact 2** | Off | | |
| **Contact 3** | Off | | |
| **Contact 4** | Off | | |

# LC-MS parameters – diode array



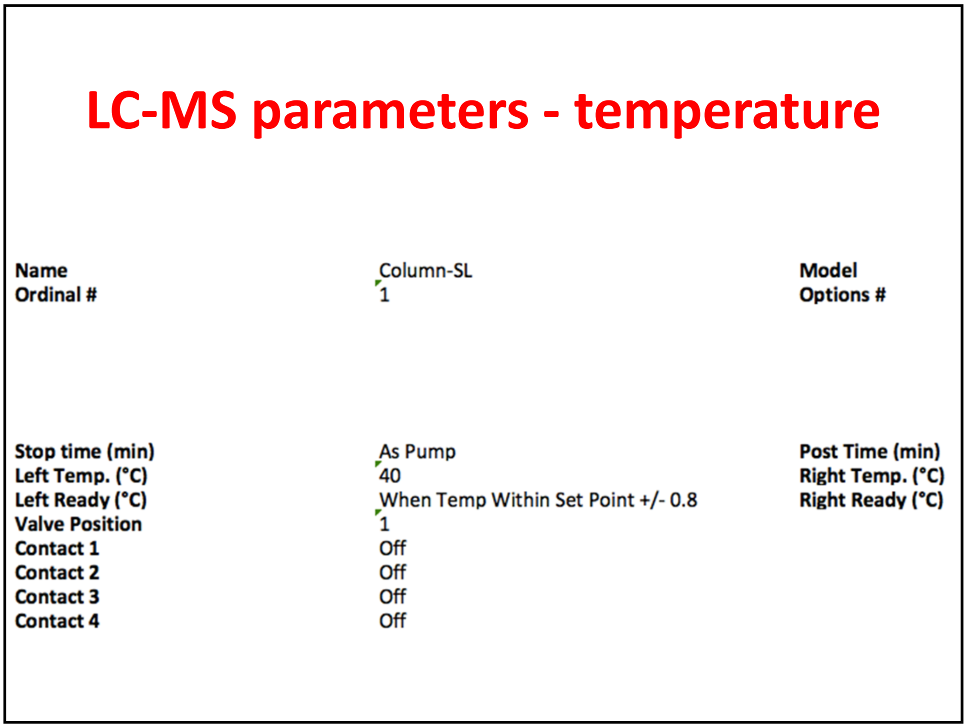
| Channel | Sample WL (nm) | Sample BW (nm) | Ref. WL (nm) | Ref. BW (nm) | Ref. On |
|---|---|---|---|---|---|
| A | 250 | 4 | 0 | 0 | Off |
| B | 254 | 16 | 0 | 0 | Off |
| C | 210 | 8 | 0 | 0 | Off |
| D | 230 | 16 | 0 | 0 | Off |
| E | 280 | 4 | 0 | 0 | Off |
| F | 280 | 16 | 0 | 0 | Off |
| G | 280 | 16 | 0 | 0 | Off |
| H | 280 | 16 | 0 | 0 | Off |



## LC-MS parameters - sampler

| | | |
|---|---|---|
| **Name** | h-ALS-SL+ | **Model** |
| **Ordinal #** | 1 | **Options** |

| | | |
|---|---|---|
| **Stop time (min)** | As Pump | **Post Time (min)** |
| **Injection Type** | Needle Wash | **Injection Volume (µl)** |
| **Overlap Time (min)** | Disable Overlapped Injection | **Draw Position (mm)** |
| **Draw Position Detection** | 1 | **Draw Speed (µl/min)** |
| **Eject Speed (µl/min)** | 200 | **Flush Out Factor** |
| **Automatic Delay Volume Reduction** | No | **Equilibration Time (sec)** |
| **Wash Vessel** | N/A | **Wash Location** |
| **Wash Time (sec)** | 5 | **Wash Cycles** |
| **Contact 1** | Off | |
| **Contact 2** | Off | |
| **Contact 3** | Off | |
| **Contact 4** | Off | |

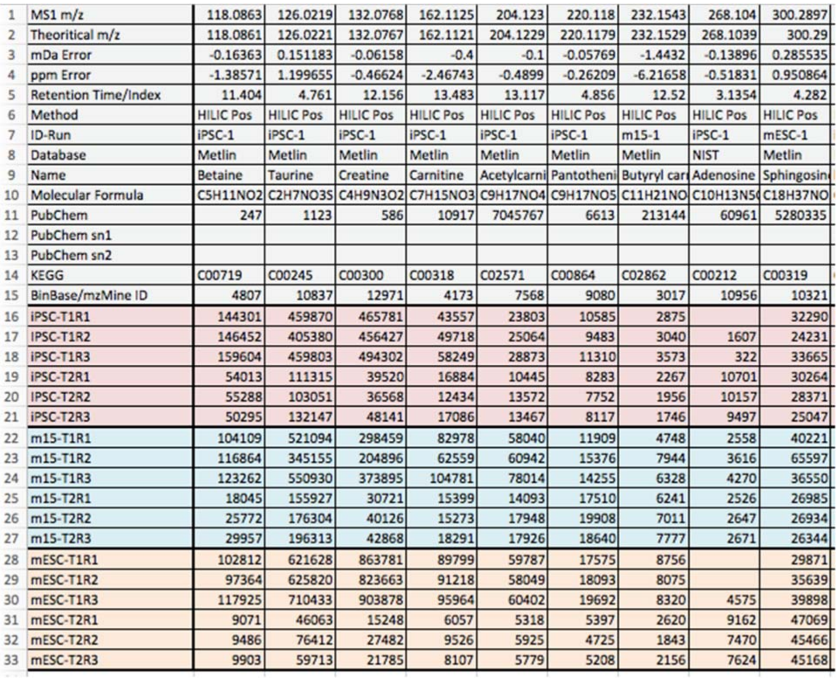


## LC-MS parameters - temperature

| | | |
|---|---|---|
| **Name** | Column-SL | **Model** |
| **Ordinal #** | 1 | **Options #** |

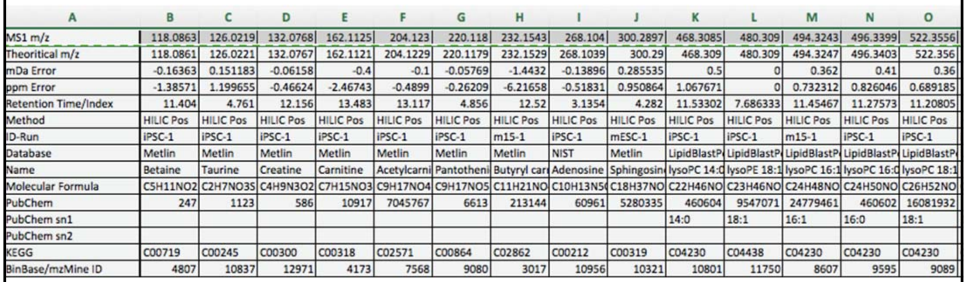
| | | |
|---|---|---|
| **Stop time (min)** | As Pump | **Post Time (min)** |
| **Left Temp. (°C)** | 40 | **Right Temp. (°C)** |
| **Left Ready (°C)** | When Temp Within Set Point +/- 0.8 | **Right Ready (°C)** |
| **Valve Position** | 1 | |
| **Contact 1** | Off | |
| **Contact 2** | Off | |
| **Contact 3** | Off | |
| **Contact 4** | Off | |

# Normalized data set



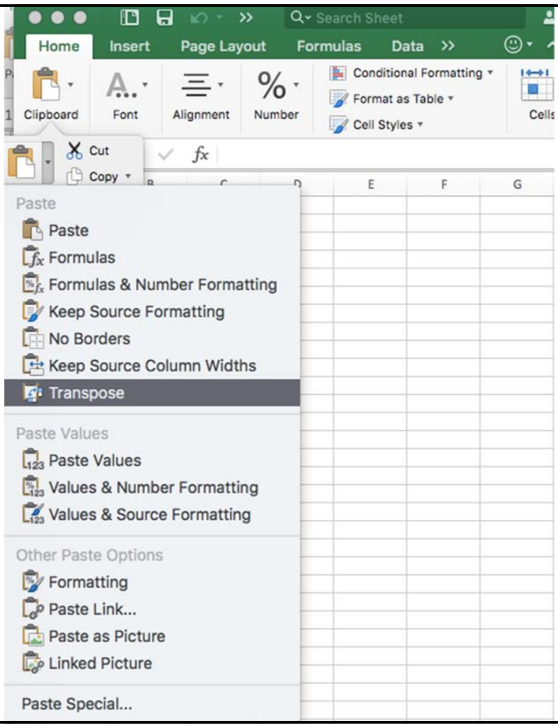
| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MS1 m/z | 118.0863 | 126.0219 | 132.0768 | 162.1125 | 204.123 | 220.118 | 232.1543 | 268.104 | 300.2897 |
| 2 | Theoritical m/z | 118.0861 | 126.0221 | 132.0767 | 162.1121 | 204.1229 | 220.1179 | 232.1529 | 268.1039 | 300.29 |
| 3 | mDa Error | -0.16363 | 0.151183 | -0.06158 | -0.4 | -0.1 | -0.05769 | -1.4432 | -0.13896 | 0.285535 |
| 4 | ppm Error | -1.38571 | 1.199655 | -0.46624 | -2.46743 | -0.4899 | -0.26209 | -6.21658 | -0.51831 | 0.950864 |
| 5 | Retention Time/Index | 11.404 | 4.761 | 12.156 | 13.483 | 13.117 | 4.856 | 12.52 | 3.1354 | 4.282 |
| 6 | Method | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos |
| 7 | ID-Run | iPSC-1 | iPSC-1 | iPSC-1 | iPSC-1 | iPSC-1 | iPSC-1 | m15-1 | iPSC-1 | mESC-1 |
| 8 | Database | Metlin | Metlin | Metlin | Metlin | Metlin | Metlin | Metlin | NIST | Metlin |
| 9 | Name | Betaine | Taurine | Creatine | Carnitine | Acetylcarni | Pantotheni | Butyryl car | Adenosine | Sphingosin |
| 10 | Molecular Formula | C5H11NO2 | C2H7NO3S | C4H9N3O2 | C7H15NO3 | C9H17NO4 | C9H17NO5 | C11H21NO | C10H13N5 | C18H37NO |
| 11 | PubChem | 247 | 1123 | 586 | 10917 | 7045767 | 6613 | 213144 | 60961 | 5280335 |
| 12 | PubChem sn1 | | | | | | | | | |
| 13 | PubChem sn2 | | | | | | | | | |
| 14 | KEGG | C00719 | C00245 | C00300 | C00318 | C02571 | C00864 | C02862 | C00212 | C00319 |
| 15 | BinBase/mzMine ID | 4807 | 10837 | 12971 | 4173 | 7568 | 9080 | 3017 | 10956 | 10321 |
| 16 | iPSC-T1R1 | 144301 | 459870 | 465781 | 43557 | 23803 | 10585 | 2875 | | 32290 |
| 17 | IPSC-T1R2 | 146452 | 405380 | 456427 | 49718 | 25064 | 9483 | 3040 | 1607 | 24231 |
| 18 | iPSC-T1R3 | 159604 | 459803 | 494302 | 58249 | 28873 | 11310 | 3573 | 322 | 33665 |
| 19 | iPSC-T2R1 | 54013 | 111315 | 39520 | 16884 | 10445 | 8283 | 2267 | 10701 | 30264 |
| 20 | IPSC-T2R2 | 55288 | 103051 | 36568 | 12434 | 13572 | 7752 | 1956 | 10157 | 28371 |
| 21 | iPSC-T2R3 | 50295 | 132147 | 48141 | 17086 | 13467 | 8117 | 1746 | 9497 | 25047 |
| 22 | m15-T1R1 | 104109 | 521094 | 298459 | 82978 | 58040 | 11909 | 4748 | 2558 | 40221 |
| 23 | m15-T1R2 | 116864 | 345155 | 204896 | 62559 | 60942 | 15376 | 7944 | 3616 | 65597 |
| 24 | m15-T1R3 | 123262 | 550930 | 373895 | 104781 | 78014 | 14255 | 6328 | 4270 | 36550 |
| 25 | m15-T2R1 | 18045 | 155927 | 30721 | 15399 | 14093 | 17510 | 6241 | 2526 | 26985 |
| 26 | m15-T2R2 | 25772 | 176304 | 40126 | 15273 | 17948 | 19908 | 7011 | 2647 | 26934 |
| 27 | m15-T2R3 | 29957 | 196313 | 42868 | 18291 | 17926 | 18640 | 7777 | 2671 | 26344 |
| 28 | mESC-T1R1 | 102812 | 621628 | 863781 | 89799 | 59787 | 17575 | 8756 | | 29871 |
| 29 | mESC-T1R2 | 97364 | 625820 | 823663 | 91218 | 58049 | 18093 | 8075 | | 35639 |
| 30 | mESC-T1R3 | 117925 | 710433 | 903878 | 95964 | 60402 | 19692 | 8320 | 4575 | 39898 |
| 31 | mESC-T2R1 | 9071 | 46063 | 15248 | 6057 | 5318 | 5397 | 2620 | 9162 | 47069 |
| 32 | mESC-T2R2 | 9486 | 76412 | 27482 | 9526 | 5925 | 4725 | 1843 | 7470 | 45466 |
| 33 | mESC-T2R3 | 9903 | 59713 | 21785 | 8107 | 5779 | 5208 | 2156 | 7624 | 45168 |

# Transposing data in Excel

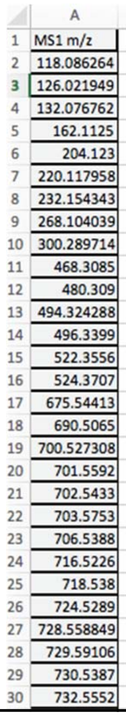


| A | B | C | D | E | F | G | H | I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MS1 m/z | 118.0863 | 126.0219 | 132.0768 | 162.1125 | 204.123 | 220.118 | 232.1543 | 268.104 | 300.2897 | 468.3085 | 480.309 | 494.3243 | 496.3399 | 522.3556 |
| Theoritical m/z | 118.0861 | 126.0221 | 132.0767 | 162.1121 | 204.1229 | 220.1179 | 232.1529 | 268.1039 | 300.29 | 468.309 | 480.309 | 494.3247 | 496.3403 | 522.356 |
| mDa Error | -0.16363 | 0.151183 | -0.06158 | -0.4 | -0.1 | -0.05769 | -1.4432 | -0.13896 | 0.285535 | 0.5 | 0 | 0.362 | 0.41 | 0.36 |
| ppm Error | -1.38571 | 1.199655 | -0.46624 | -2.46743 | -0.4899 | -0.26209 | -6.21658 | -0.51831 | 0.950864 | 1.067671 | 0 | 0.732312 | 0.826046 | 0.689185 |
| Retention Time/Index | 11.404 | 4.761 | 12.156 | 13.483 | 13.117 | 4.856 | 12.52 | 3.1354 | 4.282 | 11.53302 | 7.686333 | 11.45467 | 11.27573 | 11.20805 |
| Method | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos | HILIC Pos |
| ID-Run | iPSC-1 | iPSC-1 | iPSC-1 | iPSC-1 | iPSC-1 | iPSC-1 | m15-1 | iPSC-1 | mESC-1 | iPSC-1 | iPSC-1 | m15-1 | iPSC-1 | iPSC-1 |
| Database | Metlin | Metlin | Metlin | Metlin | Metlin | Metlin | Metlin | NIST | Metlin | LipidBlastP | LipidBlastP | LipidBlastP | LipidBlastP | LipidBlastP |
| Name | Betaine | Taurine | Creatine | Carnitine | Acetylcarni | Pantotheni | Butyryl car | Adenosine | Sphingosin | lysoPC 14:0 | lysoPE 18:1 | lysoPC 16:1 | lysoPC 16:0 | lysoPC 18:1 |
| Molecular Formula | C5H11NO2 | C2H7NO3S | C4H9N3O2 | C7H15NO3 | C9H17NO4 | C9H17NO5 | C11H21NO | C10H13N5 | C18H37NO | C22H46NO | C23H46NO | C24H48NO | C24H50NO | C26H52NO |
| PubChem | 247 | 1123 | 586 | 10917 | 7045767 | 6613 | 213144 | 60961 | 5280335 | 460604 | 9547071 | 24779461 | 460602 | 16081932 |
| PubChem sn1 | | | | | | | | | | 14:0 | 18:1 | 16:1 | 16:0 | 18:1 |
| PubChem sn2 | | | | | | | | | | | | | | |
| KEGG | C00719 | C00245 | C00300 | C00318 | C02571 | C00864 | C02862 | C00212 | C00319 | C04230 | C04438 | C04230 | C04230 | C04230 |
| BinBase/mzMine ID | 4807 | 10837 | 12971 | 4173 | 7568 | 9080 | 3017 | 10956 | 10321 | 10801 | 11750 | 8607 | 9595 | 9089 |

- Highlight all the data on the MS1 m/z values row
- Open a new Excel file



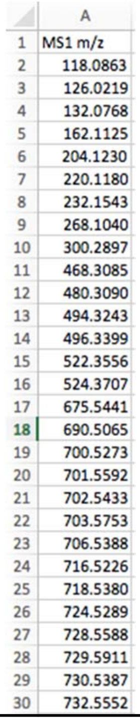
In the new Excel file, click on A column, cell 1 and select "Transpose"



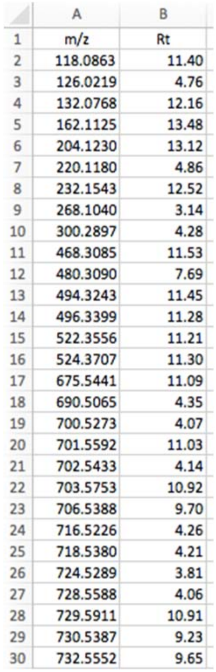
| | A |
|---|---|
| 1 | MS1 m/z |
| 2 | 118.086264 |
| 3 | 126.021949 |
| 4 | 132.076762 |
| 5 | 162.1125 |
| 6 | 204.123 |
| 7 | 220.117958 |
| 8 | 232.154343 |
| 9 | 268.104039 |
| 10 | 300.289714 |
| 11 | 468.3085 |
| 12 | 480.309 |
| 13 | 494.324288 |
| 14 | 496.3399 |
| 15 | 522.3556 |
| 16 | 524.3707 |
| 17 | 675.54413 |
| 18 | 690.5065 |
| 19 | 700.527308 |
| 20 | 701.5592 |
| 21 | 702.5433 |
| 22 | 703.5753 |
| 23 | 706.5388 |
| 24 | 716.5226 |
| 25 | 718.538 |
| 26 | 724.5289 |
| 27 | 728.558849 |
| 28 | 729.59106 |
| 29 | 730.5387 |
| 30 | 732.5552 |

Excel file cleaned up to remove color and lines and to give each *m/z* value four decimal places

Now do the same for the retention time



| | A |
|---|---|
| 1 | MS1 m/z |
| 2 | 118.0863 |
| 3 | 126.0219 |
| 4 | 132.0768 |
| 5 | 162.1125 |
| 6 | 204.1230 |
| 7 | 220.1180 |
| 8 | 232.1543 |
| 9 | 268.1040 |
| 10 | 300.2897 |
| 11 | 468.3085 |
| 12 | 480.3090 |
| 13 | 494.3243 |
| 14 | 496.3399 |
| 15 | 522.3556 |
| 16 | 524.3707 |
| 17 | 675.5441 |
| 18 | 690.5065 |
| 19 | 700.5273 |
| 20 | 701.5592 |
| 21 | 702.5433 |
| 22 | 703.5753 |
| 23 | 706.5388 |
| 24 | 716.5226 |
| 25 | 718.5380 |
| 26 | 724.5289 |
| 27 | 728.5588 |
| 28 | 729.5911 |
| 29 | 730.5387 |
| 30 | 732.5552 |



| | A | B |
|---|---|---|
| 1 | m/z | Rt |
| 2 | 118.0863 | 11.40 |
| 3 | 126.0219 | 4.76 |
| 4 | 132.0768 | 12.16 |
| 5 | 162.1125 | 13.48 |
| 6 | 204.1230 | 13.12 |
| 7 | 220.1180 | 4.86 |
| 8 | 232.1543 | 12.52 |
| 9 | 268.1040 | 3.14 |
| 10 | 300.2897 | 4.28 |
| 11 | 468.3085 | 11.53 |
| 12 | 480.3090 | 7.69 |
| 13 | 494.3243 | 11.45 |
| 14 | 496.3399 | 11.28 |
| 15 | 522.3556 | 11.21 |
| 16 | 524.3707 | 11.30 |
| 17 | 675.5441 | 11.09 |
| 18 | 690.5065 | 4.35 |
| 19 | 700.5273 | 4.07 |
| 20 | 701.5592 | 11.03 |
| 21 | 702.5433 | 4.14 |
| 22 | 703.5753 | 10.92 |
| 23 | 706.5388 | 9.70 |
| 24 | 716.5226 | 4.26 |
| 25 | 718.5380 | 4.21 |
| 26 | 724.5289 | 3.81 |
| 27 | 728.5588 | 4.06 |
| 28 | 729.5911 | 10.91 |
| 29 | 730.5387 | 9.23 |
| 30 | 732.5552 | 9.65 |

- Added the retention time

Now we deal with the areas of each metabolite

Make another new Excel file

# Highlight and copy the metabolite peak areas

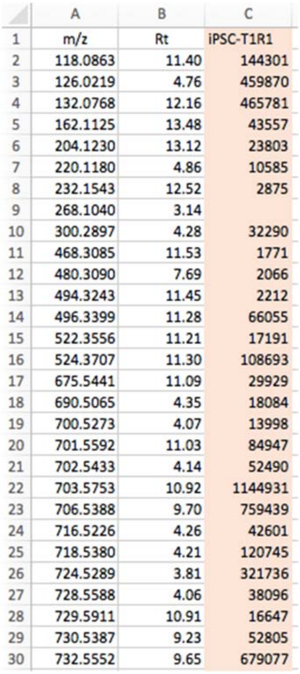
| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| iPSC-T1R1 | 144301 | 459870 | 465781 | 43557 | 23803 | 10585 | 2875 | | 32290 | 1771 | 2066 | 2212 |
| IPSC-T1R2 | 146452 | 405380 | 456427 | 49718 | 25064 | 9483 | 3040 | 1607 | 24231 | 1438 | 1706 | 2663 |
| iPSC-T1R3 | 159604 | 459803 | 494302 | 58249 | 28873 | 11310 | 3573 | 322 | 33665 | 1827 | 2133 | 3297 |
| iPSC-T2R1 | 54013 | 111315 | 39520 | 16884 | 10445 | 8283 | 2267 | 10701 | 30264 | 4500 | 5902 | 14685 |
| IPSC-T2R2 | 55288 | 103051 | 36568 | 12434 | 13572 | 7752 | 1956 | 10157 | 28371 | 2501 | 5823 | 8383 |
| iPSC-T2R3 | 50295 | 132147 | 48141 | 17086 | 13467 | 8117 | 1746 | 9497 | 25047 | 3434 | 6020 | 11877 |
| m15-T1R1 | 104109 | 521094 | 298459 | 82978 | 58040 | 11909 | 4748 | 2558 | 40221 | 3261 | 18040 | 12788 |
| m15-T1R2 | 116864 | 345155 | 204896 | 62559 | 60942 | 15376 | 7944 | 3616 | 65597 | 6972 | 25374 | 17977 |
| m15-T1R3 | 123262 | 550930 | 373895 | 104781 | 78014 | 14255 | 6328 | 4270 | 36550 | 5103 | 22821 | 13343 |
| m15-T2R1 | 18045 | 155927 | 30721 | 15399 | 14093 | 17510 | 6241 | 2526 | 26985 | 5683 | 8912 | 13782 |
| m15-T2R2 | 25772 | 176304 | 40126 | 15273 | 17948 | 19908 | 7011 | 2647 | 26934 | 5496 | 16891 | 14454 |
| m15-T2R3 | 29957 | 196313 | 42868 | 18291 | 17926 | 18640 | 7777 | 2671 | 26344 | 5604 | 8557 | 15070 |
| mESC-T1R1 | 102812 | 621628 | 863781 | 89799 | 59787 | 17575 | 8756 | | 29871 | 3304 | 2420 | 4214 |
| mESC-T1R2 | 97364 | 625820 | 823663 | 91218 | 58049 | 18093 | 8075 | | 35639 | 2519 | 2808 | 4256 |
| mESC-T1R3 | 117925 | 710433 | 903878 | 95964 | 60402 | 19692 | 8320 | 4575 | 39898 | 3752 | 2950 | 5938 |
| mESC-T2R1 | 9071 | 46063 | 15248 | 6057 | 5318 | 5397 | 2620 | 9162 | 47069 | 6486 | 4769 | 19408 |
| mESC-T2R2 | 9486 | 76412 | 27482 | 9526 | 5925 | 4725 | 1843 | 7470 | 45466 | 6668 | 3810 | 15451 |
| mESC-T2R3 | 9903 | 59713 | 21785 | 8107 | 5779 | 5208 | 2156 | 7624 | 45168 | 6925 | 4485 | 18290 |

**Go to the new file**

# Transposed peak areas

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O | P | Q | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | iPSC-T1R1 | IPSC-T1R2 | iPSC-T1R3 | iPSC-T2R1 | iPSC-T2R2 | iPSC-T2R3 | m15-T1R1 | m15-T1R2 | m15-T1R3 | m15-T2R1 | m15-T2R2 | m15-T2R3 | mESC-T1R1 | mESC-T1R2 | mESC-T1R3 | mESC-T2R1 | mESC-T2R2 | mESC-T2R3 |
| 2 | 144301 | 146452 | 159604 | 54013 | 55288 | 50295 | 104109 | 116864 | 123262 | 18045 | 25772 | 29957 | 102812 | 97364 | 117925 | 9071 | 9486 | 9903 |
| 3 | 459870 | 405380 | 459803 | 111315 | 103051 | 132147 | 521094 | 345155 | 550930 | 155927 | 176304 | 196313 | 621628 | 625820 | 710433 | 46063 | 76412 | 59713 |
| 4 | 465781 | 456427 | 494302 | 39520 | 36568 | 48141 | 298459 | 204896 | 373895 | 30721 | 40126 | 42868 | 863781 | 823663 | 903878 | 15248 | 27482 | 21785 |
| 5 | 43557 | 49718 | 58249 | 16884 | 12434 | 17086 | 82978 | 62559 | 104781 | 15399 | 15273 | 18291 | 89799 | 91218 | 95964 | 6057 | 9526 | 8107 |
| 6 | 23803 | 25064 | 28873 | 10445 | 13572 | 13467 | 58040 | 60942 | 78014 | 14093 | 17948 | 17926 | 59787 | 58049 | 60402 | 5318 | 5925 | 5779 |
| 7 | 10585 | 9483 | 11310 | 8283 | 7752 | 8117 | 11909 | 15376 | 14255 | 17510 | 19908 | 18640 | 17575 | 18093 | 19692 | 5397 | 4725 | 5208 |
| 8 | 2875 | 3040 | 3573 | 2267 | 1956 | 1746 | 4748 | 7944 | 6328 | 6241 | 7011 | 7777 | 8756 | 8075 | 8320 | 2620 | 1843 | 2156 |
| 9 | | 1607 | 322 | 10701 | 10157 | 9497 | 2558 | 3616 | 4270 | 2526 | 2647 | 2671 | | | 4575 | 9162 | 7470 | 7624 |
| 10 | 32290 | 24231 | 33665 | 30264 | 28371 | 25047 | 40221 | 65597 | 36550 | 26985 | 26934 | 26344 | 29871 | 35639 | 39898 | 47069 | 45466 | 45168 |
| 11 | 1771 | 1438 | 1827 | 4500 | 2501 | 3434 | 3261 | 6972 | 5103 | 5683 | 5496 | 5604 | 3304 | 2519 | 3752 | 6486 | 6668 | 6925 |
| 12 | 2066 | 1706 | 2133 | 5902 | 5823 | 6020 | 18040 | 25374 | 22821 | 8912 | 16891 | 8557 | 2420 | 2808 | 2950 | 4769 | 3810 | 4485 |
| 13 | 2212 | 2663 | 3297 | 14685 | 8383 | 11877 | 12788 | 17977 | 13343 | 13782 | 14454 | 15070 | 4214 | 4256 | 5938 | 19408 | 15451 | 18290 |
| 14 | 66055 | 62401 | 64752 | 106424 | 79105 | 89241 | 134478 | 225115 | 174603 | 152838 | 172888 | 154558 | 141401 | 142072 | 141988 | 164749 | 161136 | 162089 |
| 15 | 17191 | 17167 | 17290 | 73596 | 42590 | 60631 | 118673 | 145841 | 114514 | 76747 | 81848 | 82498 | 32849 | 30477 | 36011 | 90416 | 84608 | 88758 |
| 16 | 108693 | 103626 | 113683 | 108808 | 93930 | 93300 | 145222 | 204910 | 168732 | 127989 | 146755 | 129175 | 236806 | 223285 | 221103 | 172712 | 162789 | 159881 |
| 17 | 29929 | 26473 | 29008 | 23607 | 20798 | 21648 | 36131 | 28548 | 37382 | 28841 | 23577 | 28006 | 42783 | 42586 | 42049 | 29250 | 33489 | 31004 |
| 18 | 18084 | 16551 | 17398 | 29767 | 30644 | 30625 | 30566 | 18441 | 26919 | 42641 | 40923 | 41504 | 13586 | 15850 | 15377 | 24357 | 23999 | 22333 |
| 19 | 13998 | 15807 | 15077 | 25040 | 25195 | 26792 | 62225 | 37571 | 61289 | 52257 | 48671 | 51941 | 14737 | 15905 | 17202 | 26367 | 25717 | 22935 |
| 20 | 84947 | 79009 | 86398 | 71358 | 62355 | 62814 | 80863 | 75156 | 94941 | 106423 | 108826 | 104474 | 139881 | 142519 | 132411 | 98125 | 96742 | 91781 |
| 21 | 52490 | 59667 | 55648 | 71131 | 75987 | 79332 | 192965 | 81285 | 178041 | 110242 | 93827 | 114506 | 43789 | 46463 | 43294 | 58192 | 69277 | 54190 |
| 22 | 1144931 | 1094742 | 1193675 | 477444 | 460519 | 472838 | 1443859 | 849975 | 1367595 | 360919 | 256003 | 323144 | 1306052 | 1339526 | 1334168 | 436096 | 733519 | 640063 |
| 23 | 759439 | 718293 | 301996 | 568769 | 592863 | 573382 | 839920 | 421555 | 894680 | 978676 | 928944 | 945656 | 757892 | 1205728 | 1054442 | 834070 | 781688 | 797497 |
| 24 | 42601 | 36654 | 41327 | 81300 | 82061 | 81425 | 97897 | 66697 | 90292 | 105311 | 111872 | 106257 | 29825 | 33665 | 30506 | 58031 | 50796 | 49593 |
| 25 | 120745 | 120621 | 125354 | 166662 | 174231 | 179203 | 192310 | 89865 | 177485 | 161255 | 143471 | 164831 | 72027 | 76156 | 73158 | 112985 | 121816 | 104639 |
| 26 | 321736 | 340582 | 322255 | 355237 | 400188 | 383111 | 170502 | 122885 | 161795 | 278712 | 284182 | 280979 | 357660 | 382831 | 385383 | 488145 | 429478 | 405398 |
| 27 | 38096 | 43811 | 40994 | 59224 | 64305 | 65478 | 184483 | 85926 | 183727 | 88323 | 86692 | 98501 | 33222 | 33332 | 33042 | 45930 | 49476 | 40032 |
| 28 | 16647 | 15772 | 15824 | 14530 | 13107 | 13800 | 22316 | 14807 | 23600 | 15367 | 13721 | 15054 | 20411 | 19992 | 17685 | 13698 | 15953 | 13081 |
| 29 | 52805 | 73243 | 58201 | 89078 | 101109 | 101431 | 64893 | 86538 | 41694 | 142324 | 153366 | 137326 | 144965 | 53367 | 55667 | 85413 | 95926 | 101333 |
| 30 | 679077 | 1025469 | 803417 | 902646 | 1071880 | 1068033 | 801055 | 907726 | 528219 | 1451514 | 1484609 | 1413636 | 1521451 | 570753 | 734753 | 1017944 | 1131446 | 1173389 |

Now we can select (one column at a time) the peak area data and add it to the *m/z* and Rt data



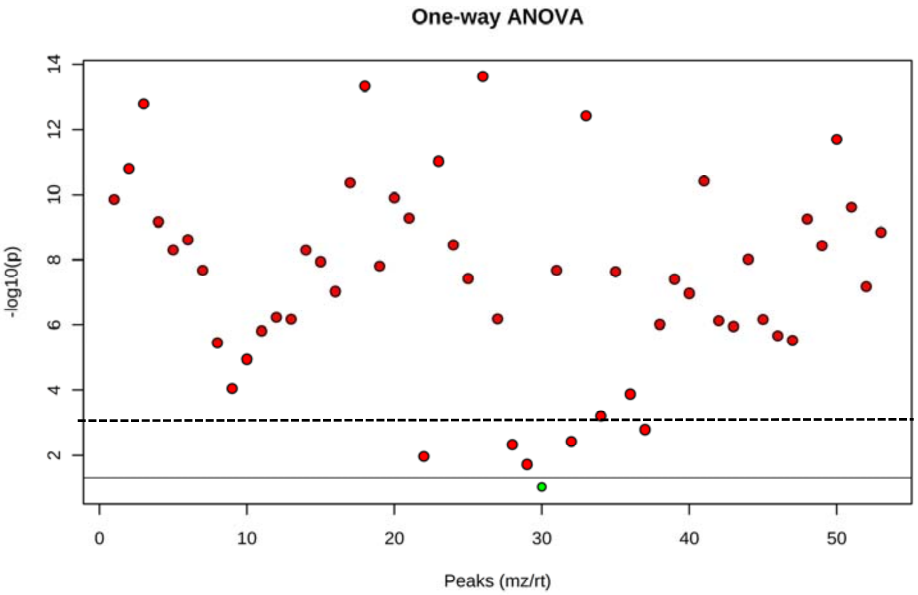
|  | A | B | C |
|---|---|---|---|
| 1 | m/z | Rt | iPSC-T1R1 |
| 2 | 118.0863 | 11.40 | 144301 |
| 3 | 126.0219 | 4.76 | 459870 |
| 4 | 132.0768 | 12.16 | 465781 |
| 5 | 162.1125 | 13.48 | 43557 |
| 6 | 204.1230 | 13.12 | 23803 |
| 7 | 220.1180 | 4.86 | 10585 |
| 8 | 232.1543 | 12.52 | 2875 |
| 9 | 268.1040 | 3.14 |  |
| 10 | 300.2897 | 4.28 | 32290 |
| 11 | 468.3085 | 11.53 | 1771 |
| 12 | 480.3090 | 7.69 | 2066 |
| 13 | 494.3243 | 11.45 | 2212 |
| 14 | 496.3399 | 11.28 | 66055 |
| 15 | 522.3556 | 11.21 | 17191 |
| 16 | 524.3707 | 11.30 | 108693 |
| 17 | 675.5441 | 11.09 | 29929 |
| 18 | 690.5065 | 4.35 | 18084 |
| 19 | 700.5273 | 4.07 | 13998 |
| 20 | 701.5592 | 11.03 | 84947 |
| 21 | 702.5433 | 4.14 | 52490 |
| 22 | 703.5753 | 10.92 | 1144931 |
| 23 | 706.5388 | 9.70 | 759439 |
| 24 | 716.5226 | 4.26 | 42601 |
| 25 | 718.5380 | 4.21 | 120745 |
| 26 | 724.5289 | 3.81 | 321736 |
| 27 | 728.5588 | 4.06 | 38096 |
| 28 | 729.5911 | 10.91 | 16647 |
| 29 | 730.5387 | 9.23 | 52805 |
| 30 | 732.5552 | 9.65 | 679077 |

# Save as a .csv file

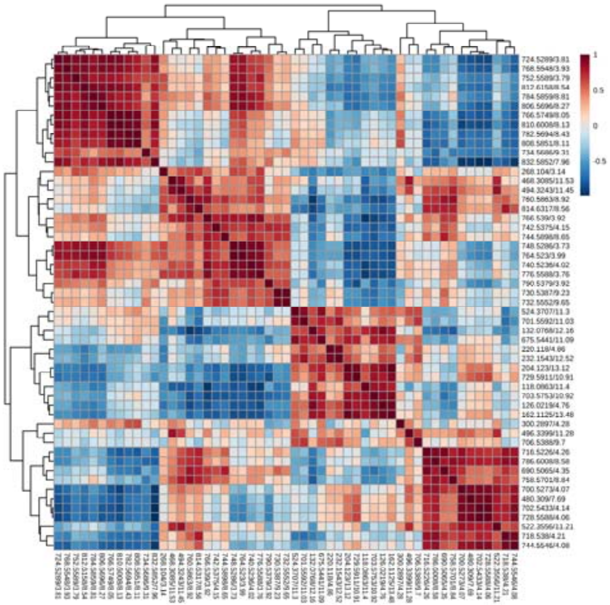
**Repeat this for each biological sample**

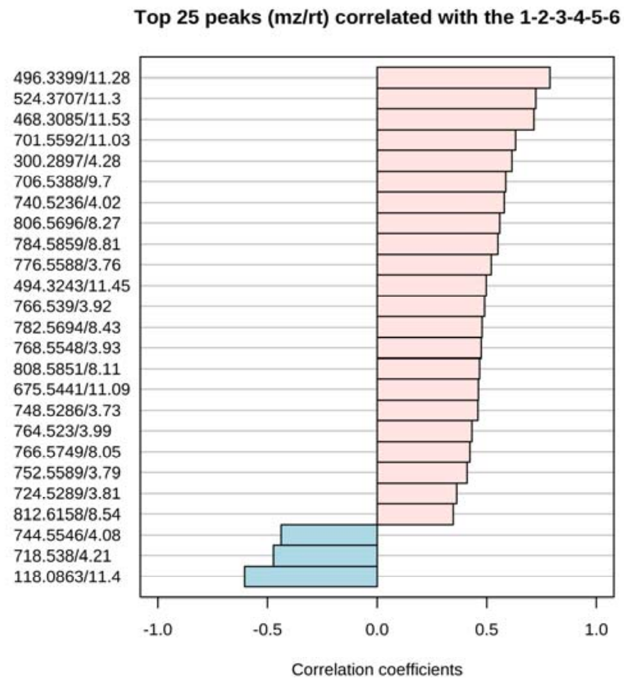
**Combine files for each sample type into a folder**

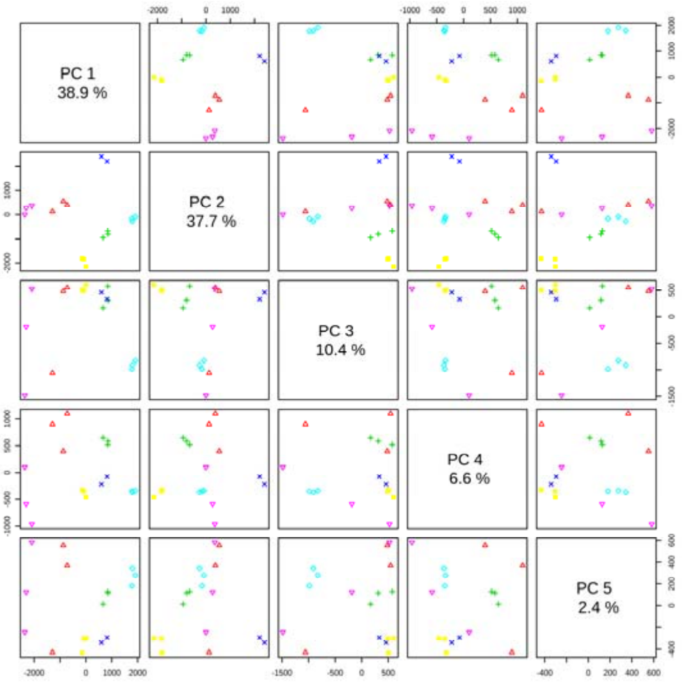
**Combine folders into a .zip file and submit to MetaboAnalyst**



# Correlation analysis






PC 1
38.9 %
PC 2
37.7 %
PC 3
10.4 %
PC 4
6.6 %
PC 5
2.4 %
-2000
0
1000
-1000
-500
0
500
1000
2000
1000
0
-2000
1000
0
-2000
500
0
-500
-1500
1000
500
0
-500
-1000
600
400
200
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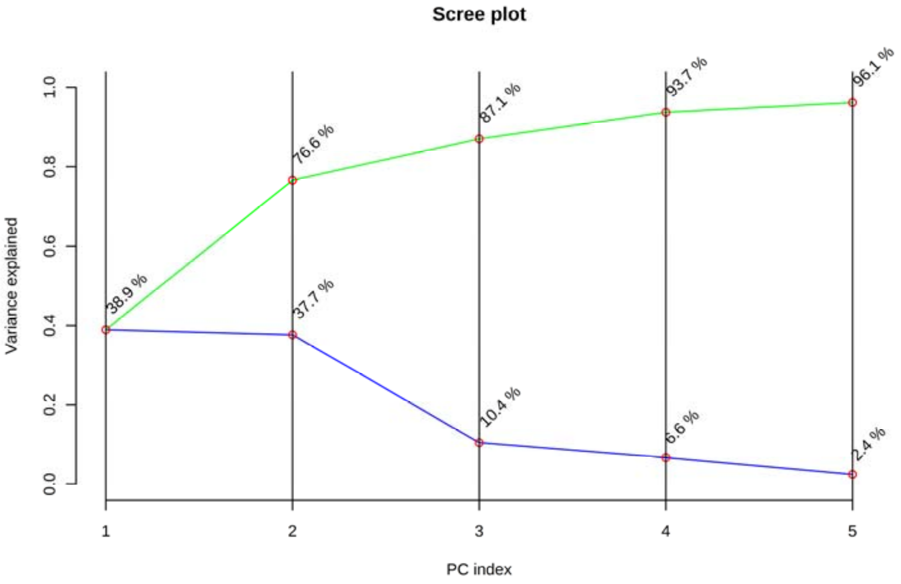
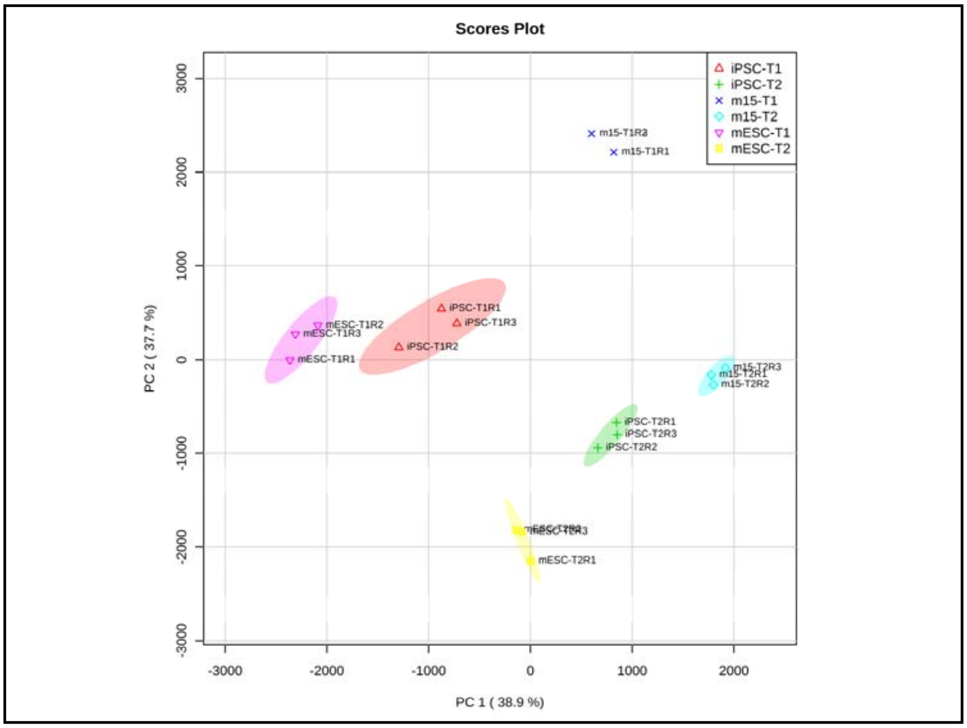
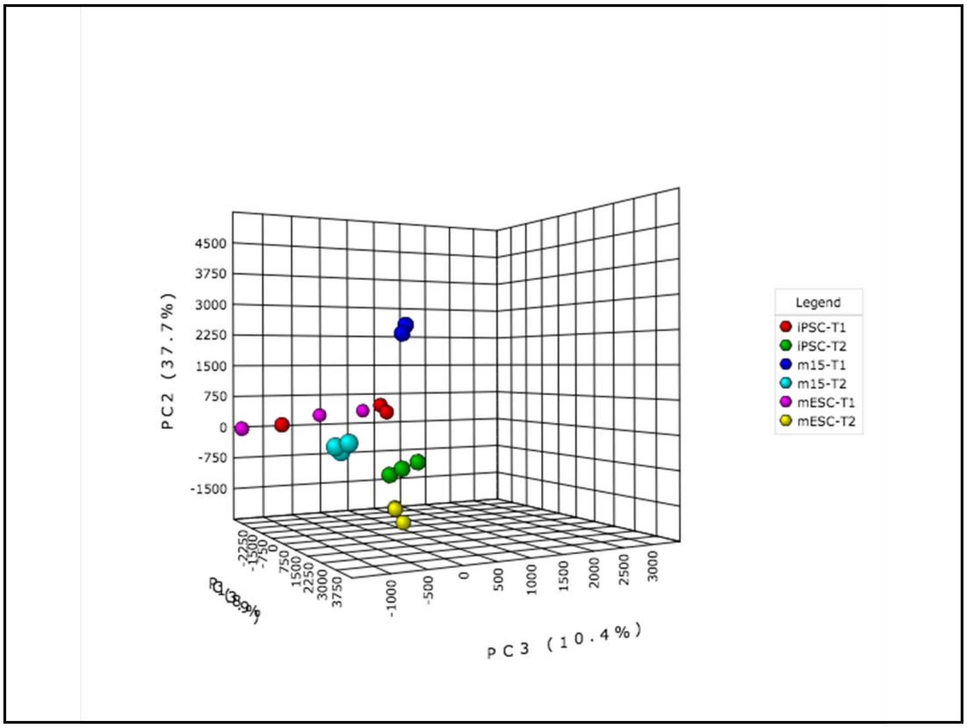
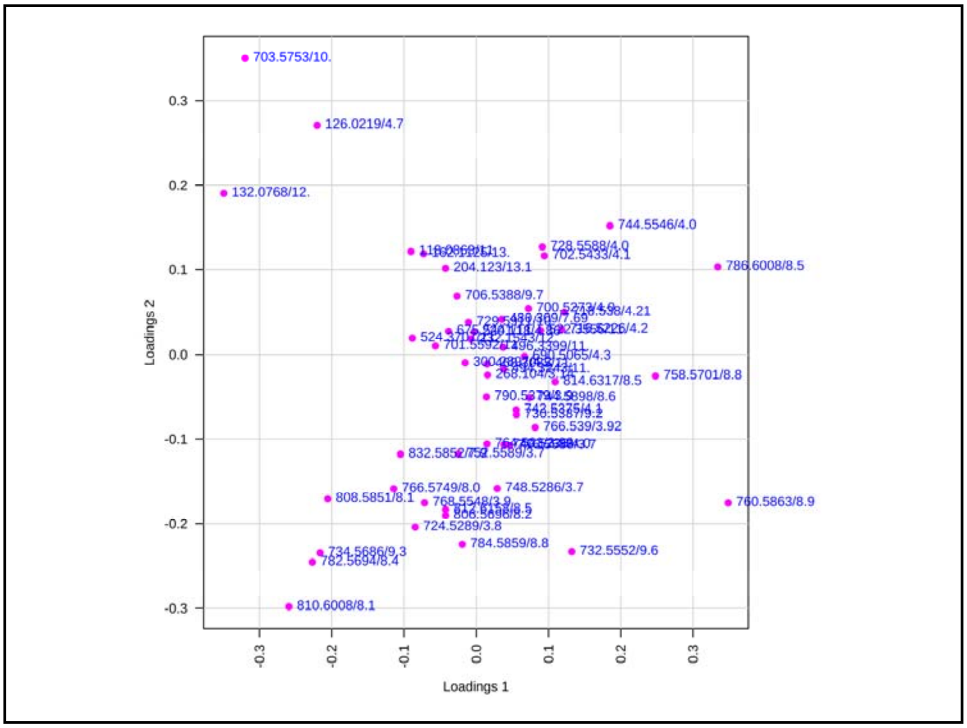
-400
-2000
0
1000
2000
-1500
-500
0
500
-400
0
200
400
600


Scree plot
Variance explained
0.0
0.2
0.4
0.6
0.8
1.0
PC index
1
2
3
4
5
38.9 %
76.6 %
87.1 %
93.7 %
96.1 %
37.7 %
10.4 %
6.6 %
2.4 %


Scores Plot
iPSC-T1
iPSC-T2
m15-T1
m15-T2
mESC-T1
mESC-T2
m15-T1R3
m15-T1R1
iPSC-T1R1
iPSC-T1R3
iPSC-T1R2
mESC-T1R2
mESC-T1R3
mESC-T1R1
m15-T2R3
m15-T2R1
m15-T2R2
iPSC-T2R1
iPSC-T2R3
iPSC-T2R2
mESC-T2R2
mESC-T2R3
mESC-T2R1
3000
2000
1000
0
-1000
-2000
-3000
PC 2 ( 37.7 %)
-3000
-2000
-1000
0
1000
2000
PC 1 ( 38.9 %)


Legend
iPSC-T1
iPSC-T2
m15-T1
m15-T2
mESC-T1
mESC-T2
4500
3750
3000
2250
1500
750
0
-750
-1500
PC2 (37.7%)
-2250
-1500
-750
0
750
1500
2250
3000
3750
PC1 (38.9%)
-1000
-500
0
500
1000
1500
2000
2500
3000
PC3 (10.4%)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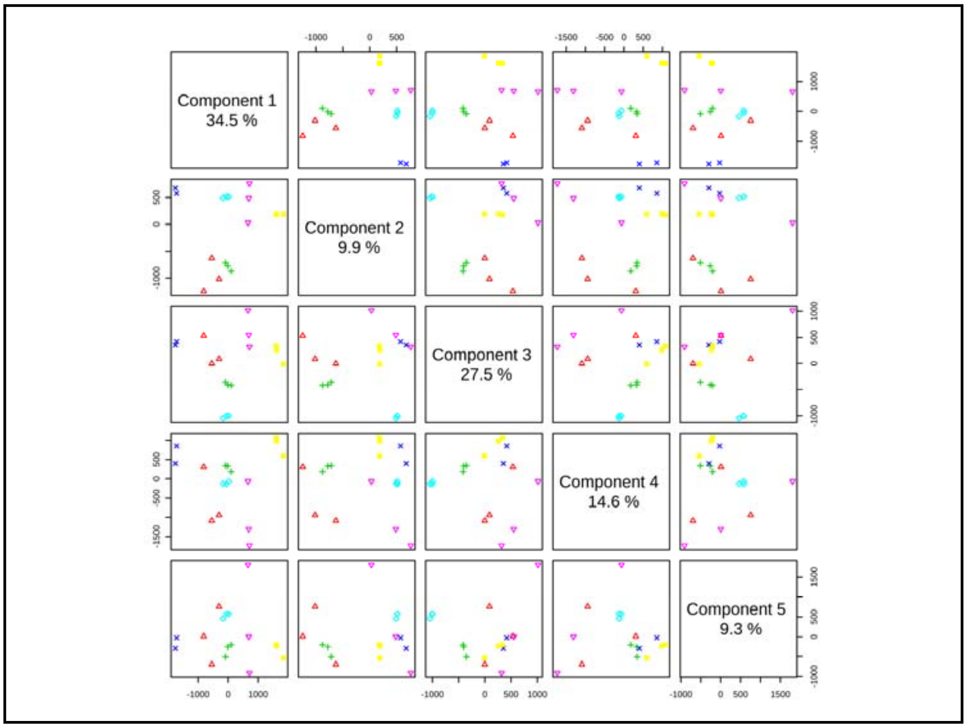
126.0219/4.7
132.0768/12.
744.5546/4.0
728.5588/4.0
702.5433/4.1
204.123/13.1
786.6008/8.5
706.5388/9.7
700.5273/4.0
4.21
488.369/7.69
4.2
524.37
701.5592/14
496.3399/11
690.5065/4.3
268.104/3.14
814.6317/8.5
758.5701/8.8
790.53
898/8.6
742.5375/4.1
736.5387/9.2
766.539/3.92
832.5852/7
2.5589/3.7
766.5749/8.0
748.5286/3.7
808.5851/8.1
768.5548/3.9
806.5696/8.2
760.5863/8.9
724.5289/3.8
784.5859/8.8
732.5552/9.6
734.5686/9.3
782.5694/8.4
810.6008/8.1
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
Loadings 2
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
Loadings 1


Component 1
34.5 %
Component 2
9.9 %
Component 3
27.5 %
Component 4
14.6 %
Component 5
9.3 %
-1000
0
500
-1500
-500
0
500
1000
0
-1000
500
0
-1000
1000
500
0
-1000
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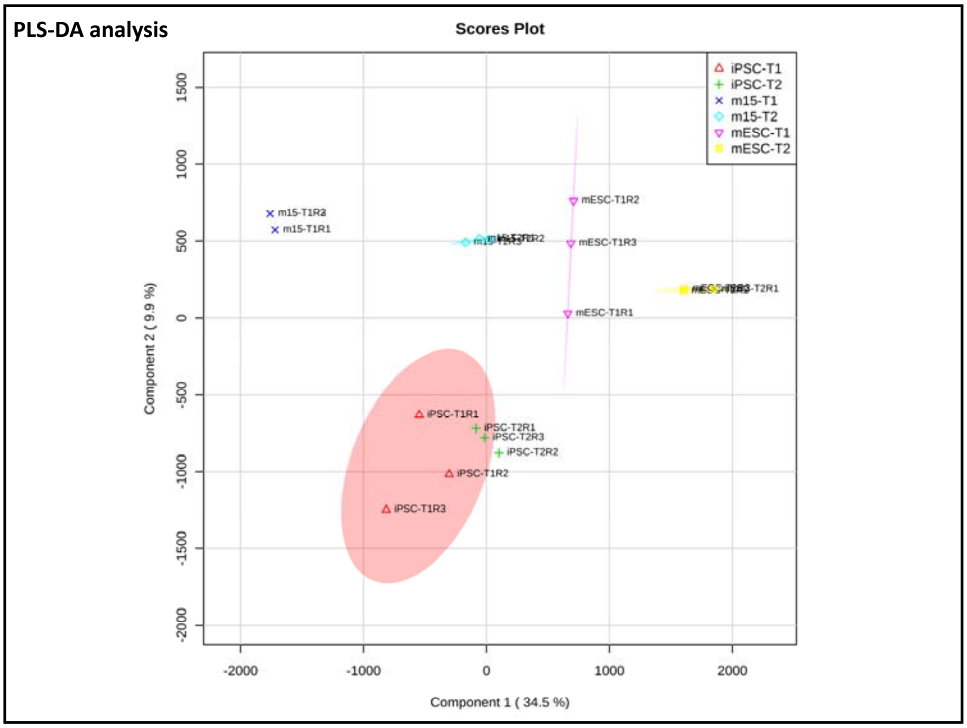
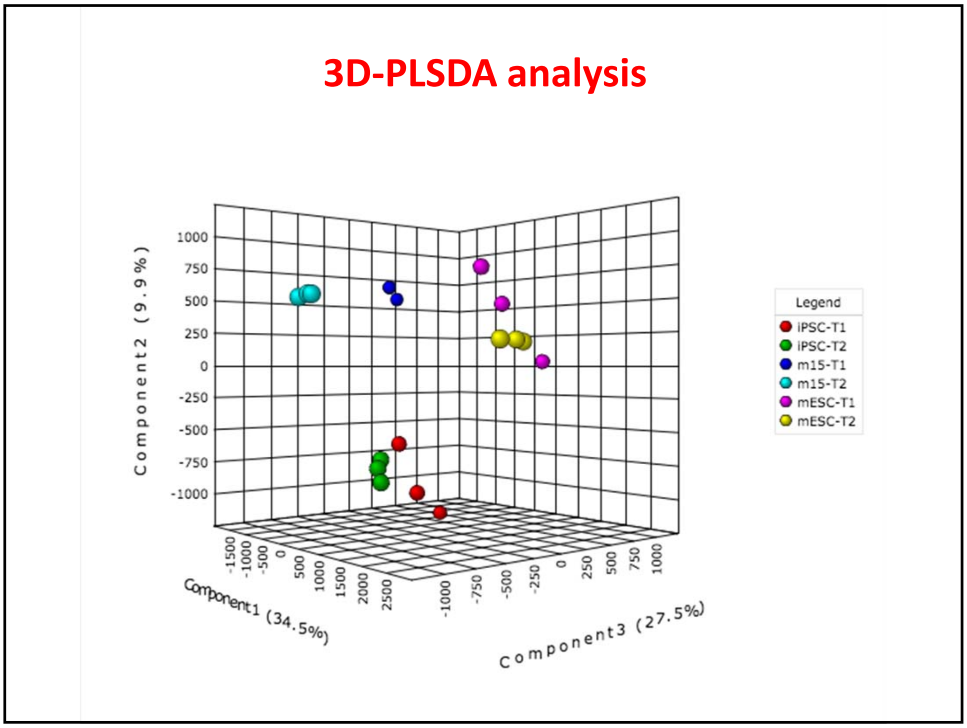
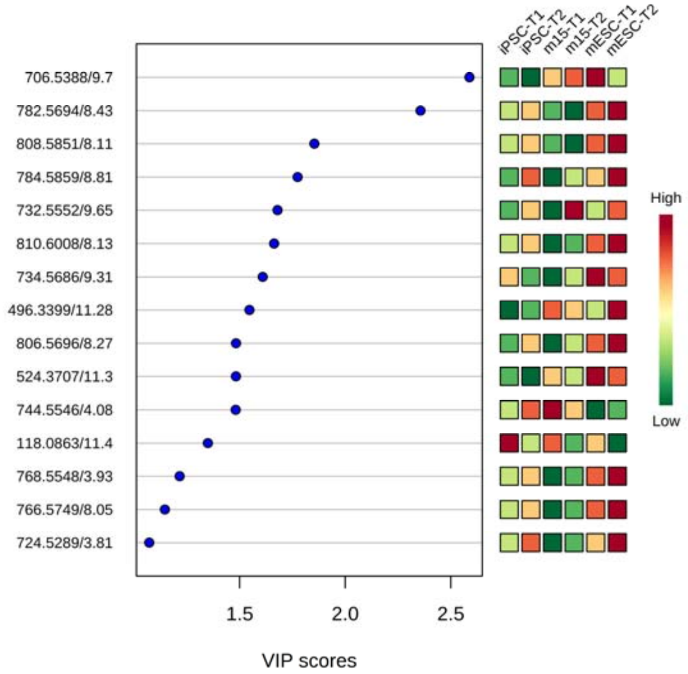
1000
-1000
0
500
1500



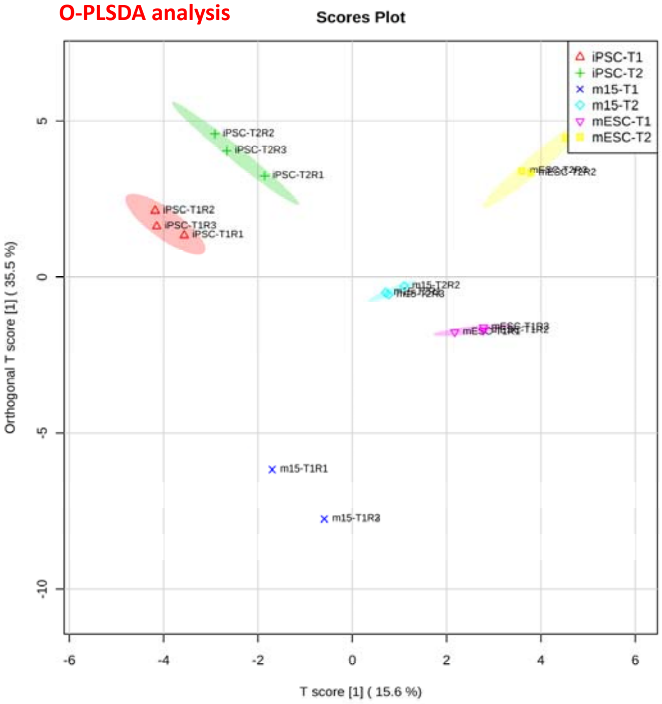
**PLS-DA analysis**

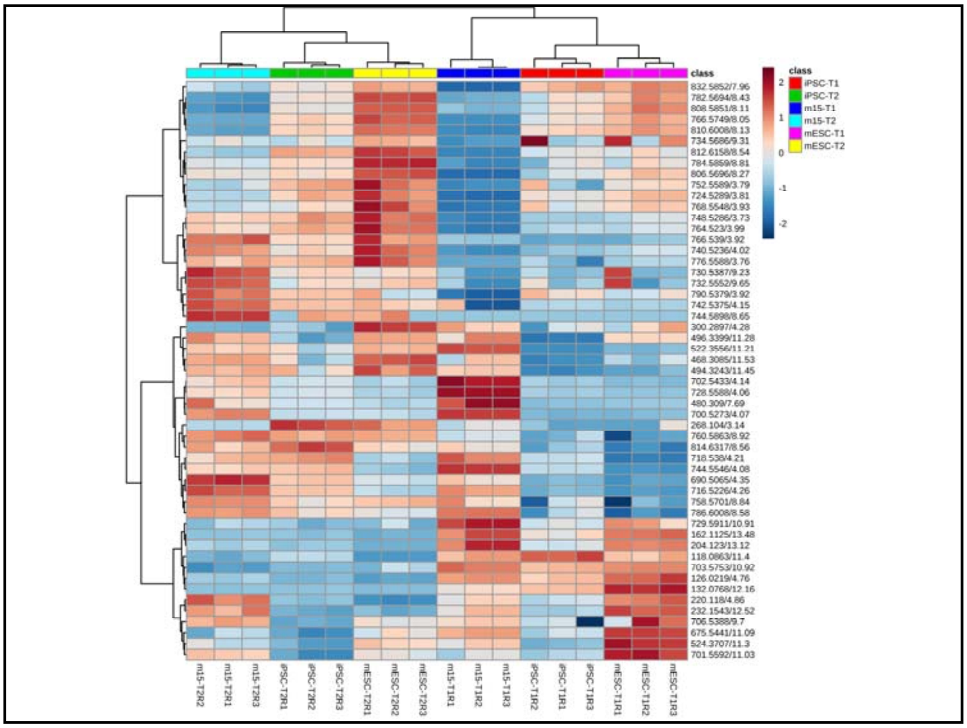


# 3D-PLSDA analysis



**O-PLSDA analysis**

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808.5851/8.11
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734.5686/9.31
812.6158/8.54
784.5859/8.81
806.5696/8.27
752.5589/3.79
724.5289/3.81
768.5548/3.93
748.5286/3.73
764.523/3.99
766.539/3.92
740.5236/4.02
776.5588/3.76
730.5387/9.23
732.5552/9.65
790.5379/3.92
742.5375/4.15
744.5898/8.65
300.2897/4.28
496.3399/11.28
522.3556/11.21
468.3085/11.53
494.3243/11.45
702.5433/4.14
728.5588/4.06
480.309/7.69
700.5273/4.07
268.104/3.14
760.5863/8.92
814.6317/8.56
718.538/4.21
744.5546/4.08
690.5065/4.35
716.5226/4.26
758.5701/8.84
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729.5911/10.91
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703.5753/10.92
126.0219/4.76
132.0768/12.16
220.118/4.86
232.1543/12.52
706.5388/9.7
675.5441/11.09
524.3707/11.3
701.5592/11.03
m15-T2R2
m15-T2R1
m15-T2R3
iPSC-T2R1
iPSC-T2R2
iPSC-T2R3
mESC-T2R1
mESC-T2R2
mESC-T2R3
m15-T1R1
m15-T1R2
m15-T1R3
iPSC-T1R2
iPSC-T1R1
iPSC-T1R3
mESC-T1R1
mESC-T1R2
mESC-T1R3
2
1
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-1
-2
class
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iPSC-T2
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