Metabolomics Pipeline Overview

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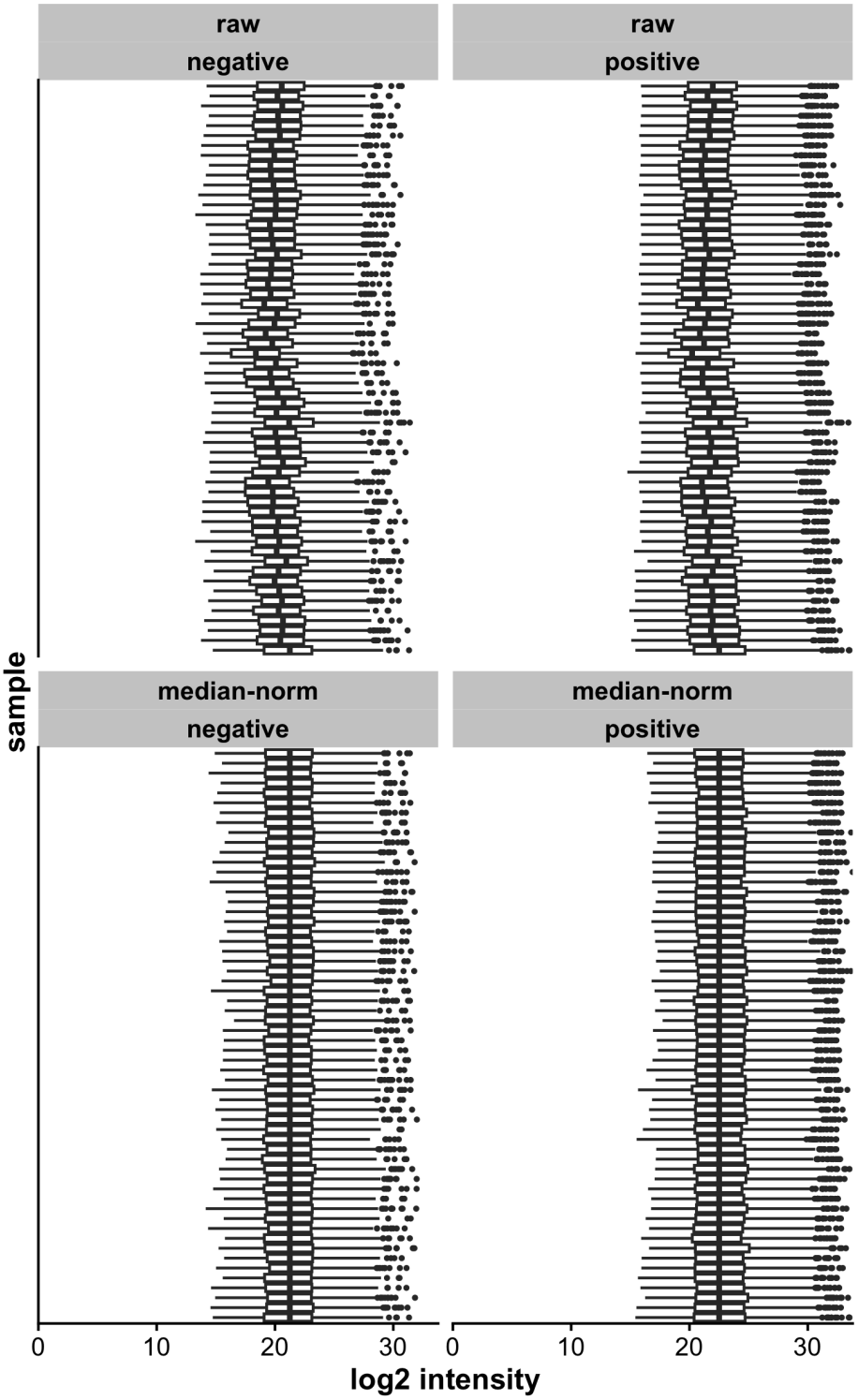
1 Introduction

A basic analysis pipeline in R for untargeted LC-MS metabolomics, including quality control, normalization, PCA overview of sample behavior, and differential feature analysis with linear models. The data are panels of untargeted LC–MS features from positive and negative ionization mode runs, such as metabolites measured from tissue, stool samples, or microbial cultures. In this hypothetical scenario, the data have been structured to resemble samples belonging to control subjects, one of two treatments (tr1 or tr2), or a dual-treatment group (tr1+tr2). The pipeline demonstrates a conventional approach to characterizing metabolomic differences among these groups.

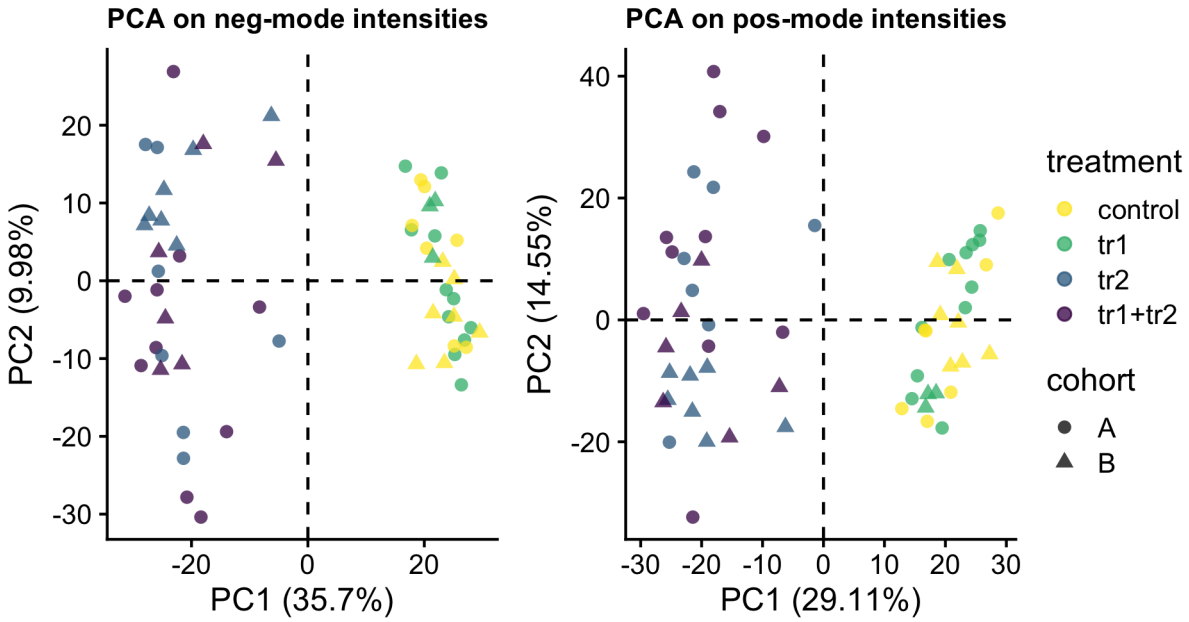
2 Data Distribution

The LC-MS intensities of metabolomics data typically skew to the right. Data normalization is required to improve comparability across samples and help stabilize variance before actual analysis can begin. The figure below demonstrates how median normalization reduces between-sample differences in overall intensity while preserving the underlying variability that's of interest in the data. The data are also transformed into a log2 scale to reduce domination by extreme values and bring the data closer to normality.



3 Sample Analysis — PCA

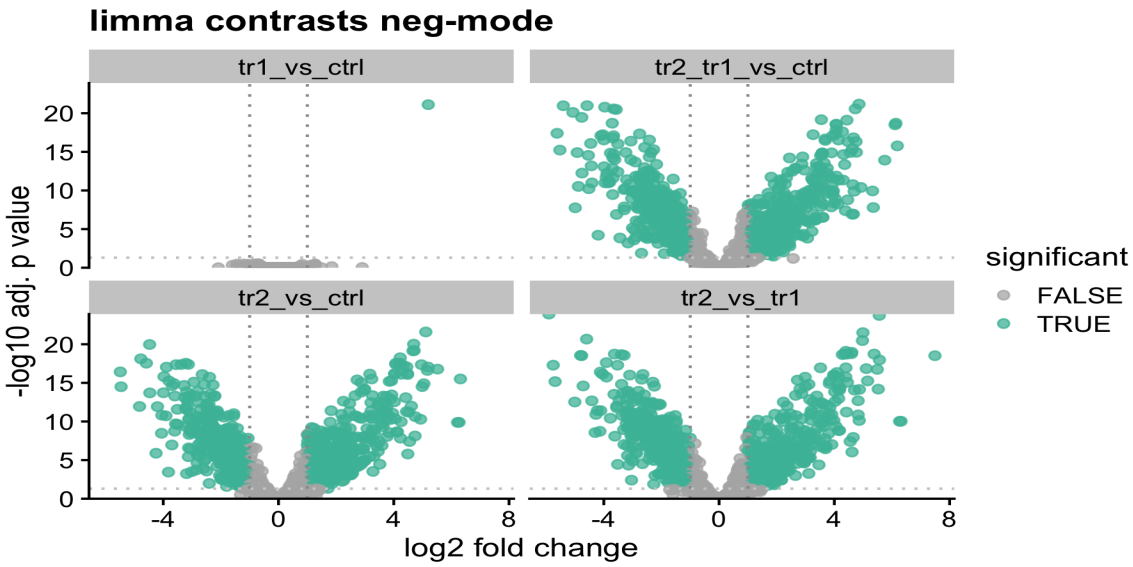
To gauge the overall structure of the metabolomic profiles (samples), a Principal Components Analysis is used. The median-normalized, log2-transformed intensities are next centered and scaled, then decomposed into a set of orthogonal principal components that capture the dominant sources of variance in the dataset.

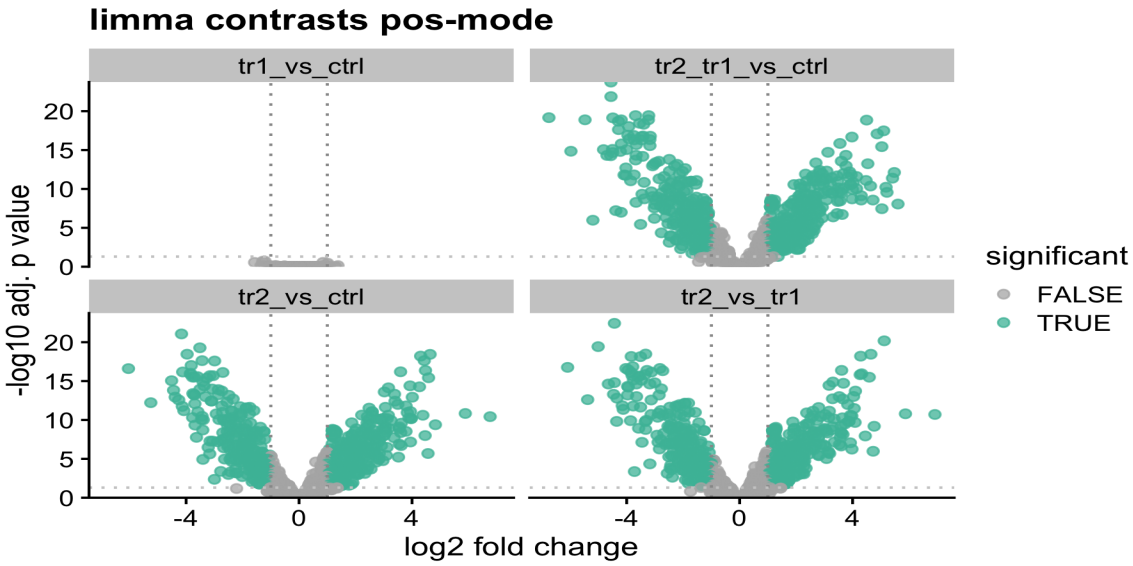


Principal component (PC) 1 illustrates a divide amongst the samples based on the treatment group, with controls and tr1 contrasted from tr2 and the dual-treatment (tr1+tr2). Additionally, PC 1 captures 35.7% of the variance in the data for the negative-mode intensities and 29.11% for the positive-mode intensities, meaning the treatment variable has substantial influence on the overall data structure (as might be expected). PC 2 does not appear to highlight any meaningful secondary structure in the data.

4 Feature Analysis — limma

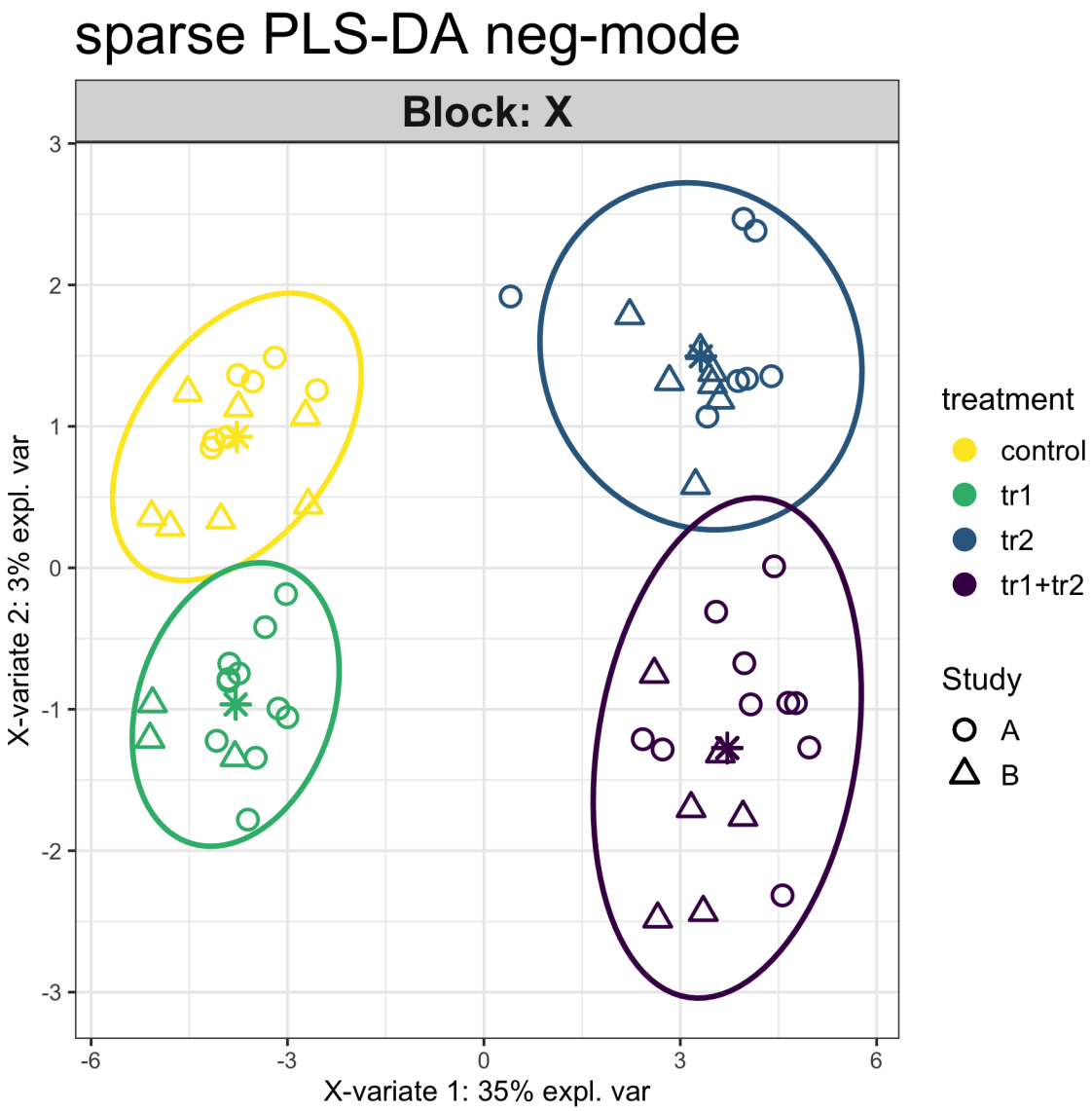
To evaluate the underlying feature fluctuations responsible for the observed sample differences, linear models for microarrays (limma) were applied to the median-normalized, log2-transformed intensities. This approach tests each LC-MS feature for differential abundance across treatment groups while controlling the false discovery rate using multiple-testing correction. The figures below depict the differentially abundant features for each pairwise comparison, highlighting metabolites that most strongly distinguish the control, single-treatment (tr1, tr2), and dual-treatment (tr1+tr2) conditions.

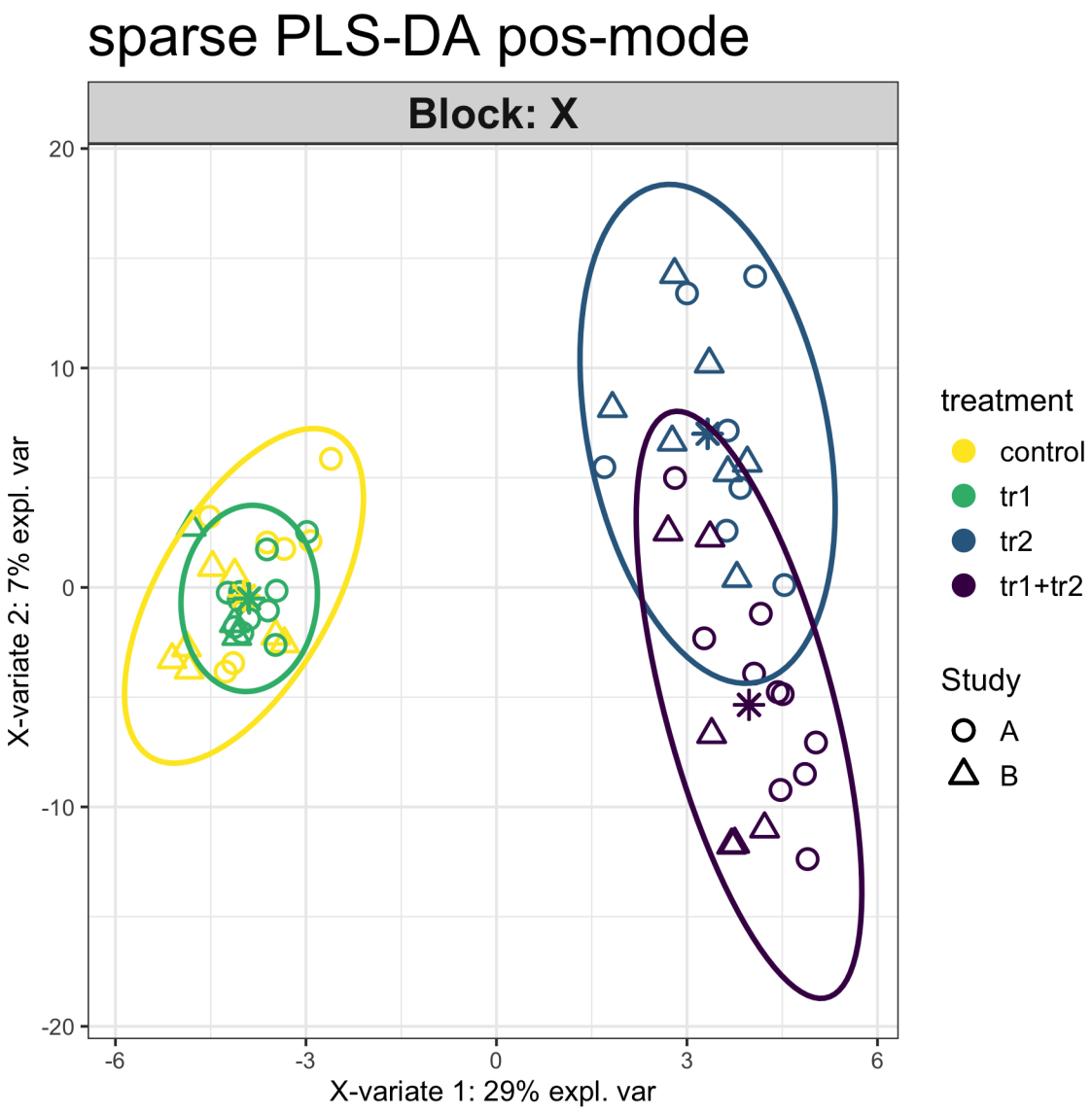




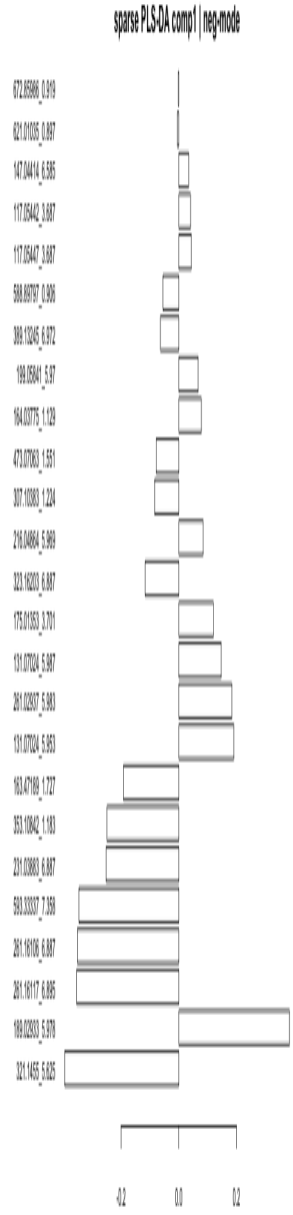
5 Supervised Analysis — sparse PLS-DA

(sparse Partial Least Squares Discriminant Analysis)





(description of sample plots) (introduce the means of exploring LC-MS peak behaviors through plotVar and plotLoadings outputs)


sparse PLS-DA comp1 | neg-mode
672.85986 0.919
621.01035 0.897
147.04414 6.595
117.05442 3.687
117.05447 3.687
568.89797 0.936
369.13245 6.972
188.05841 5.97
164.03775 1.129
473.07063 1.551
107.10083 1.224
216.04864 5.999
323.19203 6.887
175.01553 3.701
131.07024 5.987
261.03637 5.983
131.07024 5.953
163.47188 1.727
353.10842 1.103
231.03803 6.887
593.33337 7.359
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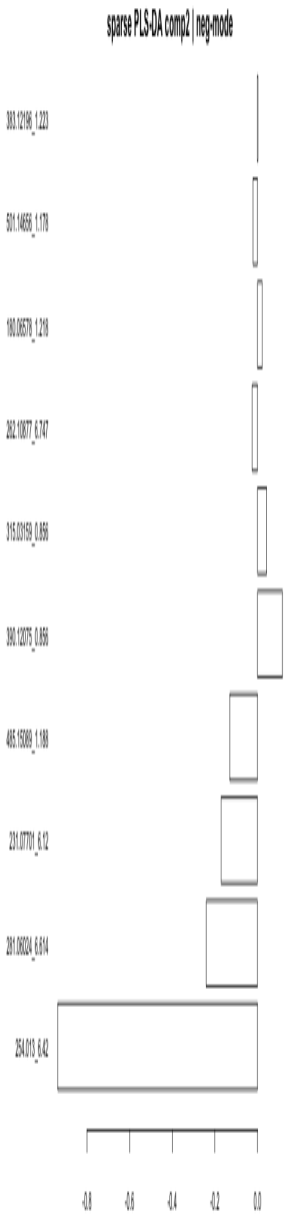
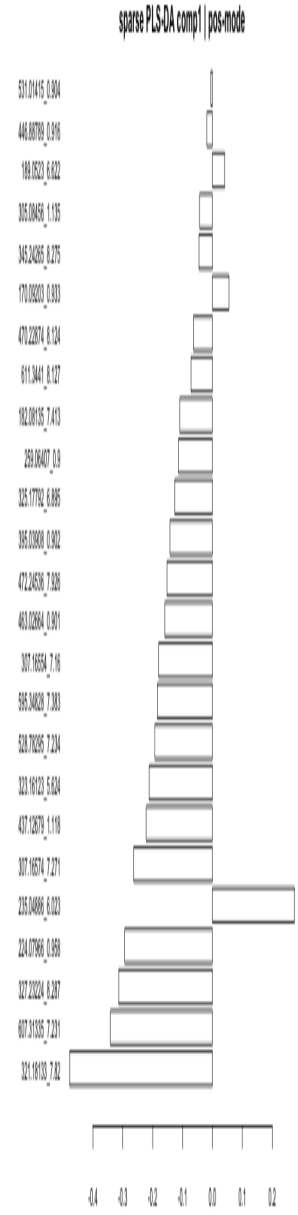
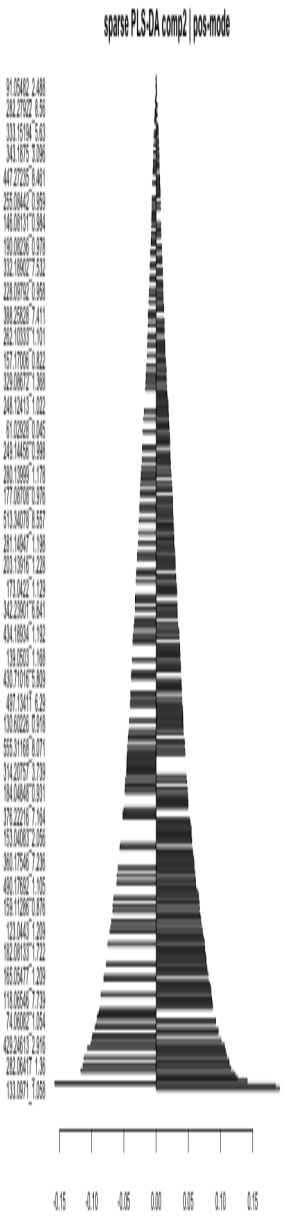
261.16117 6.895
189.02833 5.979
321.1455 5.835
-0.2
0.0
0.2



(explanatory text for neg-mode sPLSDA)


sparse PLS-DA comp1 | pos-mode
531.01415_0.804
446.88786_0.916
188.0523_6.822
305.08456_1.135
345.24265_8.275
170.09203_0.933
470.23074_8.124
611.3441_8.127
182.09116_7.413
259.06407_0.9
325.17792_6.895
305.03806_0.932
472.24536_7.926
463.03664_0.951
307.16554_7.16
595.34828_7.383
526.79285_7.234
323.19123_5.624
437.12679_1.118
307.16574_7.271
235.04886_6.023
224.07966_0.958
327.23224_6.887
607.31359_7.231
321.19193_7.82
-0.4
-0.3
-0.2
-0.1
0.0
0.1
0.2



(explanatory text for pos-mode sPLSDA)

6 Upcoming

- Add table containing differential feature counts per contrast and percent-of-features exhibiting significant differences.
- Incorporate more methodology discussion
- Putative feature annotation using KEGG (example workflow).