I am pleased to let you know that version 2 of your project PJW001, Proteomic analysis of primary cells from Down’s syndrome patients, is now completed and can be viewed from our secure online website at the following address, using the following credentials.

<https://fios1.fiosgenomics.com/reports/PJW001>

Username: PJW001

Password: depth\_Valley

Version 2 of this report includes the new set of contrasts using the following linear model that was specified:

“~0 + Group + Age + Gender”

where Group represents sample treatment group, Age was converted to a numeric in years and Gender was a categorial.

A total of 6 extra contrasts were generated using this model:

1 - Trisomy-21 Control vs. Unaffected Control

2 - Trisomy-21 AOAA vs. Unaffected AOAA

3 - Unaffected AOAA vs. Unaffected Control

4 - Trisomy-21 AOAA vs. Trisomy-21 AOAA

5 - (Trisomy-21 AOAA – Trisomy-21 Control) vs. (Unaffected AOAA – Unaffected Control)

6 - All trisomy-21 individuals vs. All unaffected individuals

In brief, using this model returned a limited number of differentially abundant proteins in contrasts 3, 4 and 5 whereas contrasts 1, 2 and 6 returned 53, 128 and 456 significantly differentially abundant proteins at a significance cut-off of adjusted P < 0.05 respectively. There appeared to be some degree in overlap in significant features between these three contrasts, which likely reflects the differences in proteome between individuals with and without Trisomy-21.

We hope that you find these updates useful and we would be happy answer any questions you have via conference call or email.

Kind regards,

Jack

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Hi Lazslo and Csaba

I am glad you have found the result to be useful and as expected. A table of the batch-corrected normalised dataset can be downloaded from the Downloads section of the report (<https://fios1.fiosgenomics.com/reports/PJW001/supplementary_downloads.html>) alongside tables for the raw intensities and imputed raw intensities data. The exact data processing steps taken on the raw intensities data were as follows:

1 – Initial pre-processing where 1,947 proteins that were missing in 2 out of 8 replicates were removed leaving 5,538 proteins remaining for further analysis

2 – Imputation was performed on these 5,538 proteins used the LOD2 approach where for each protein a single unique value equal to half the lowest intensity detected across all samples for that protein was used for imputation

3 – The dataset was then log2 transformed and batch corrected using Combat correcting for “TMT”

Csaba with regards to your questions, I could not find CSB (P35520) in the original raw intensities file so this was not filtered out during my initial pre-processing of the original data. It is therefore possible that this particular protein was not detected during the data generation stages. With relation to your other question, currently the feature overlap between contrasts section can give you some sense of the overlapping proteins that are significantly up or down regulated across different comparisons. However, unfortunately your specific question about proteins that are up regulated in all DS samples vs. control but down-regulate in DS AOAA vs. DS Control is not directly answered within the current report. The interactive charts in the delta-delta contrast (<https://fios1.fiosgenomics.com/reports/PJW001/association_tests_adjusting_for_age_and_gender_5_trisomy_21_individuals_aoaa_control_vs_unffected_individuals_aoaa_control_.html>) association table can give you some idea of the expression across treatment groups. In addition, all of the data including FC and pvalues for each contrast are downloadable from the report, which may be useful for you to answer this question.

Thanks again

Jack

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