Humanizing Phenomics 120
psomagen

Advanced Analysis

Report

2025-01-14

Dr. John Doe

Olink Advanced Analysis

Olink

Project Information

Customer	**Dr. John Doe**
Institute	Example University
Project ID	AN00021767
Olink Panel	Explore HT
Target Proteins	5,416
Number of Samples	172
Number of Plates	2
Normalization Method	Intensity

Table of Contents

1. Data Access and Downloading

1.1 Download Links:

File name	File size	md5sum
AB00000XYZ	xxx	xxxx

Report.zip - This is a zip file of analysis results.

md5sum - In order to verify the integrity of files, md5sum is used. If the values of md5sum are the same, there is no forgery, modification or omission.

Your data will be retained in our server for 3 months. Should you wish to extend the retention period, please contact us.

1.2 Download Instructions:

Data hasbeen transferred via sFTP/Globus/hard drive. Log-in instructions are provided below:

1.3 Folder and Data Structure:

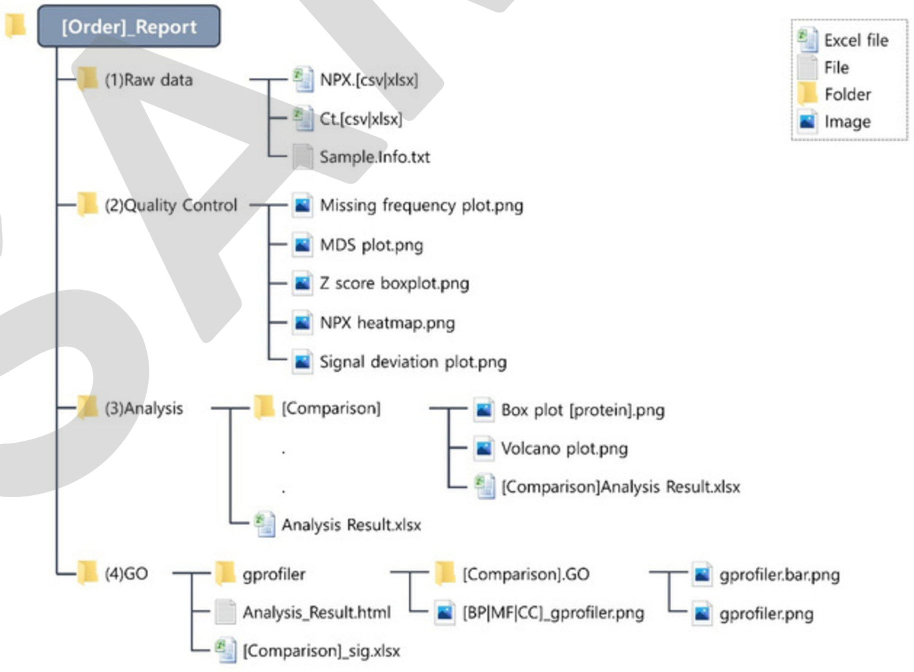

Figure 1: Example data organization.

1.4 Provided Files:

1. Differentially Expressed Protein:
    a. DEP_ttest_results.txt
    b. DEP_volcano.png
    c. DEP_samples_heatmap.png
    d. GSEA_results.txt
    e. GSEA_heatmap.png
    f. Survival_KM_plot.png

2. Sample Results Summary

2.1 Differentially Expressed Proteins (DEP)

Differential protein expression can be conducted on two or more groups. All DEP results will include a table of significant (p<0.05) DEPs with p-value and adjusted p-value. In addition, DEPs will also include a volcano plot and a heatmap of the top 50 DEPs. Note that a single volcano plot is not possible for ≥3 groups (ANOVA), as these plots depict 1 to 1 comparisons. That said, it is possible to provide individual volcano plots for all 2-group subsets (e.g. A vs. B, A vs. C, B vs. C). Upon request, we can provide expression plots of randomly selected proteins or customer-provided proteins of interest as validation of the DEP calculations.

a. Differentially Expressed Proteins (DEPs)

Samples are tested for DEPs based on experimental design [treatment versus control]. The full expression table is provided in /Results/DEP_[ttest]_results.txt/

Protein	UniProt.ID	Treated	Untreated	p.value	adj.p.value	method Welch Two	alternative
TRAIL	P50591	7.4	10	1.85E-06	0.000171	Sample t-test	two.sided
SERPINA7	P05543	9.06	12.3	1.86E-06	0.000171	Welch Two Sample t-test	two.sided
CXCL11	O14625	5.62	3.9	2.96E-05	0.00181	Welch Two Sample t-test	two.sided
MMP-10	P09238	9.2	11.3	8.49E-05	0.0039	Welch Two Sample t-test	two.sided
CD6	Q8WWJ7	2.73	1.84	1.06E-04	0.0039	Welch Two Sample t-test	two.sided
Flt3L	P49771	6.13	4.17	1.57E-04	0.00481	Welch Two Sample t-test	two.sided
DPP4	P27487	4.23	6.22	2.37E-04	0.00622	Welch Two Sample t-test	two.sided
TWEAK	O43508	8.13	10.1	4.36E-04	0.01	Welch Two Sample t-test	two.sided
EFEMP1	Q12805	2.09	2.78	1.66E-03	0.0329	Welch Two Sample t-test	two.sided
REG3A	Q06141	7.23	9.49	2.00E-03	0.0329	Welch Two Sample t-test	two.sided
DEFA1	P59665	4.31	3.47	2.01E-03	0.0329	Welch Two Sample t-test	two.sided
ICAM1	P05362	2.5	3.09	2.15E-03	0.0329	Welch Two Sample t-test	two.sided
IL-22 RA1	Q8N6P7	8.99	11.5	2.72E-03	0.0385	Welch Two Sample t-test	two.sided
TCN2	P20062	5.54	8.46	3.49E-03	0.0459	Welch Two Sample t-test	two.sided

DEPs were visualized by volcano plot with significant results are labeled in red and by heatmap (NPX values).

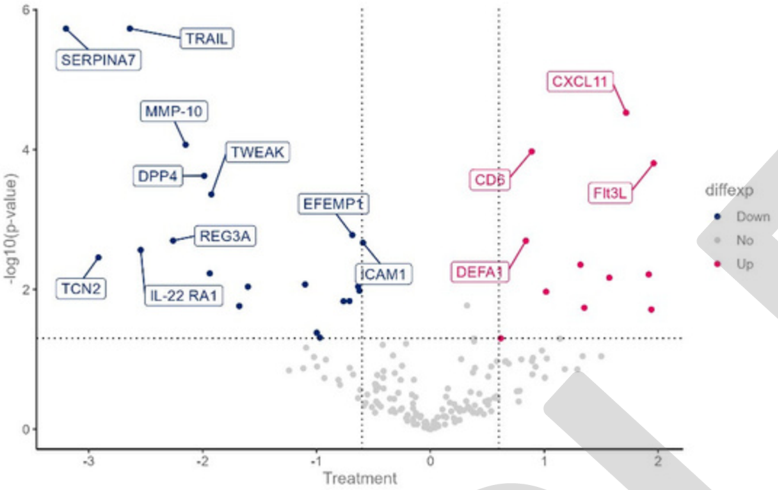

Figure 2: Volcano plot of DEPs

DEPs were visualized by heatmap and classified by treatment group.

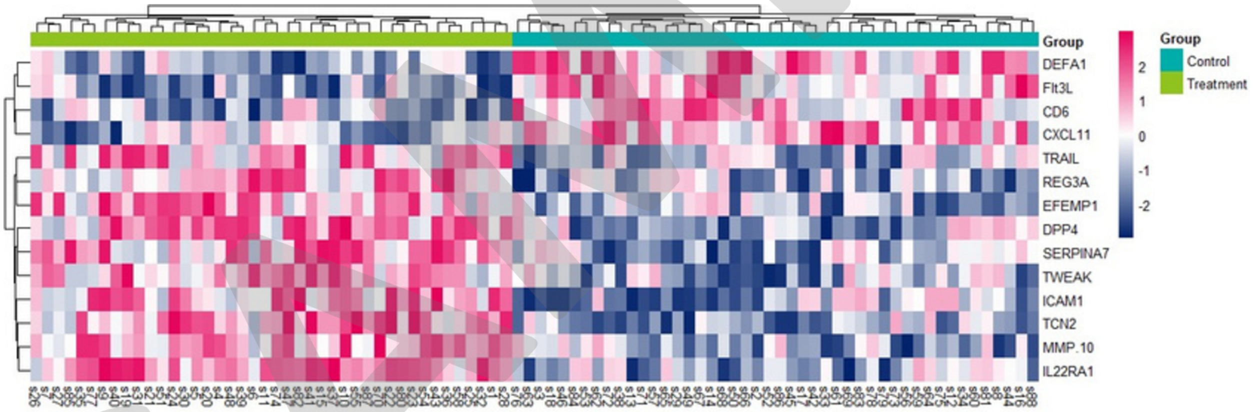

Figure 3: Significant differentially expressed proteins across samples.

2.2 Gene Set Enrichment Analysis (GSEA) or Over-Representation Analysis (ORA)

GSEA will include a table of differentially expressed pathways with p-value and adjusted p-value. A bar plot of the GSEA results is also included. With the appropriate metadata, Kaplan-Meier Survival analysis with Cox multivariate regression can also be delivered.

b. GSEA Pathway Enrichment

DEPs were passed through GSEA for pathway enrichment scores. The top 10 differentially enriched pathways are provided below in table and heatmap format. The full table is provided in /Results/gsea_results.txt/

ID	Set Size	Enrichment Score	NES	P value	P adjust	Q value	rank
LEE_BMP2_TARGETS_UP	18	-0.73251	-1.93225	0.000401	0.224638	0.216615	28
WP_ALLOGRAFT_REJECTION	14	0.689654	1.914638	0.002998	0.29257	0.282121	8
GOBP_RESPONSE_TO_LIPID	26	0.592278	1.891845	0.002019	0.29257	0.282121	40
GOBP_RESPONSE_TO_MOLECULE_OF_BACTERIAL_ORIGIN	22	0.610721	1.87099	0.003738	0.29257	0.282121	30
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	14	0.668808	1.856767	0.005498	0.29257	0.282121	40
GOBP_NEGATIVE_REGULATION_OF_NUCLEOBASE_CONTAINING_COMPOUND_METABOLIC_PROCESS	10	0.721473	1.79663	0.00733	0.29257	0.282121	13
GOMF_G_PROTEIN_COUPLED_RECEPTOR_BINDING	19	0.602718	1.795635	0.006249	0.29257	0.282121	58
GOMF_CHEMOKINE_ACTIVITY	18	0.608482	1.773397	0.008276	0.29257	0.282121	58
GOMF_CHEMOKINE_RECEPTOR_BINDING	18	0.608482	1.773397	0.008276	0.29257	0.282121	58
KEGG_CHEMOKINE_SIGNALING_PATHWAY	18	0.608482	1.773397	0.008276	0.29257	0.282121	58

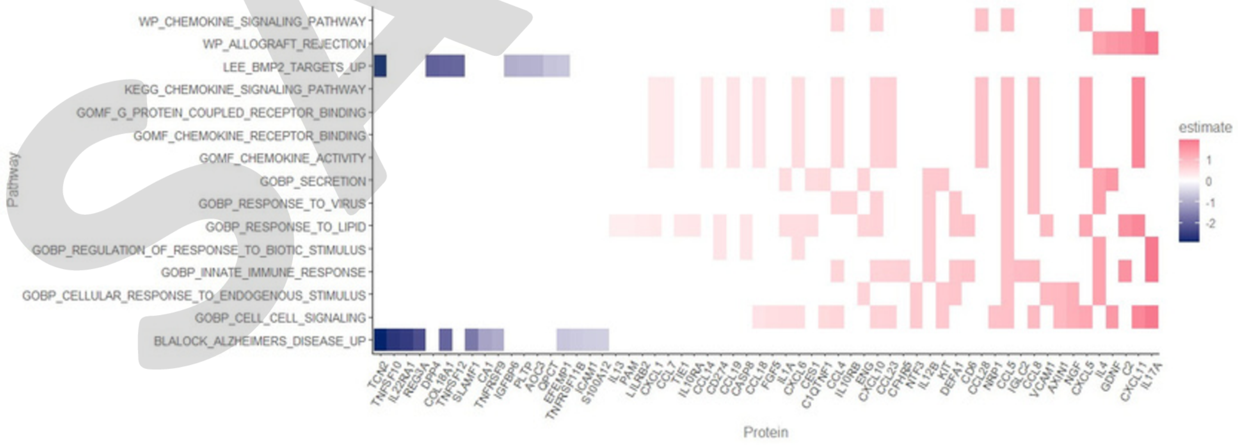

Figure4: Differentially pathways after GSEA by assay.

2.3 Survival Analysis

All differentially expressed proteins were checked for significance in survival status based on median NPX value for each protein. Example plots are provided below.

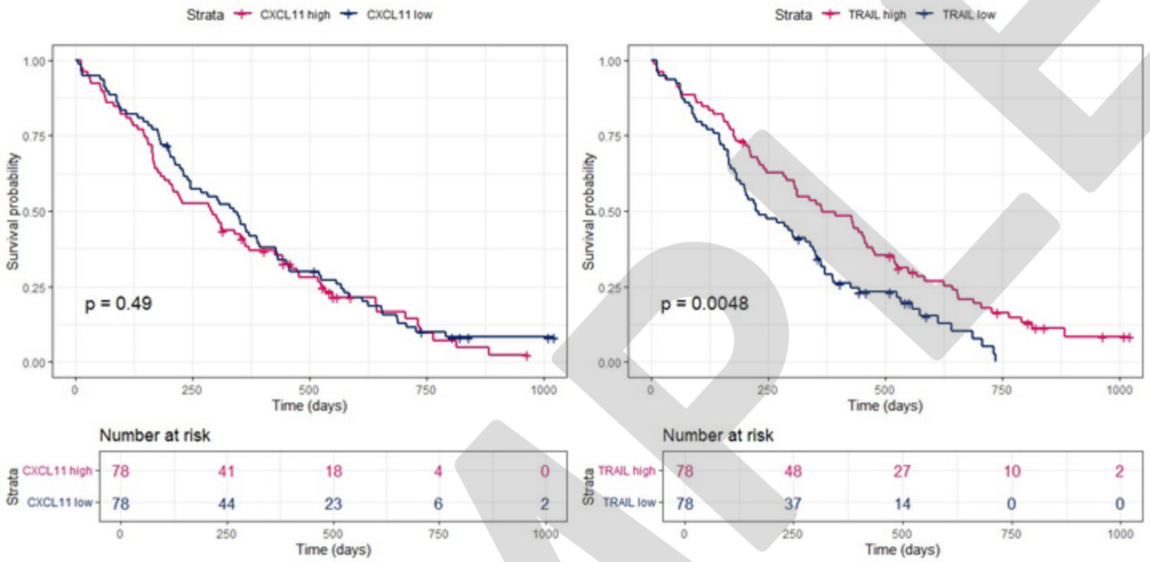

Figure 5: Representative Kaplan-Meier survival plots

c. Cox Regression:

Proteins significantly associated with survival were passed to a Cox multivariate regression model with provided metadata categories.

	estimate	std.error	statistic	p.value
TRAIL	-0.05782	0.025411	-2.27554	0.022874
age	0.017178	0.010452	1.643556	0.100268
sex	-0.53844	0.186449	-2.88787	0.003879
ph.ecog	0.377067	0.122942	3.067035	0.002162

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