Facilitate Analysis of Proteomic Data from Olink with Olink® Analyze

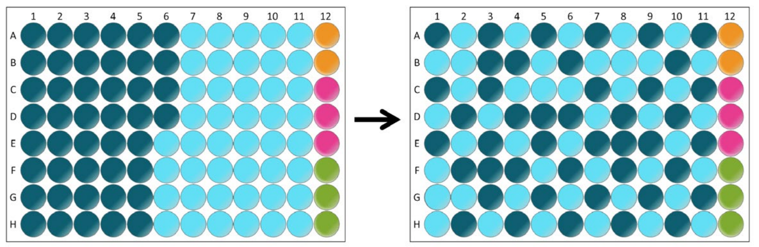
Olink® Analyze R Package Cheatsheet

0

Before generating data

Facilitate plate randomization and bridge sample selection

olink_plate_randomizer(*manifest*) generates a scheme for how to plate samples with an option to keep subjects on the same plate for longitudinal randomization using **SubjectColumn** argument



Visualize plate randomization:

- **olink_displayPlateDistributions(*data, fill.color*)**
- **olink_displayPlateLayout(*data, fill.color*)**

Note about Randomization:

- When a subject has more than one sample, these should be kept together on the same plate
- Samples should still be randomized within a plate
- Groups of interest should be randomized across plates as evenly as possible

olink_bridgeselector*(df, sampleMissingFreq, n)* selects **n** number of bridge samples based on input data. The samples are chosen based on passing QC, covering a large range of the data, and having less than **sampleMissingFreq** data below LOD per sample.

i

Contact us:

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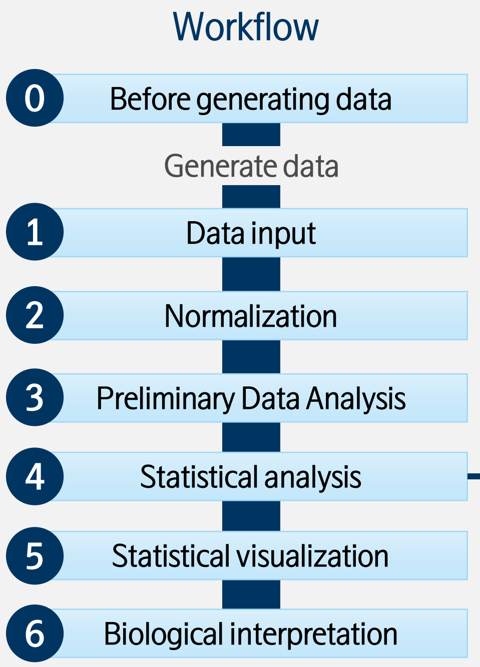
biostattools@olink.com *Olink Analyze and Shiny app support*

support@olink.com *Olink lab product and technical support*

info@olink.com *for more information*

Workflow



0 Before generating data

Generate data

1 Data input

2 Normalization

3 Preliminary Data Analysis

4 Statistical analysis

5 Statistical visualization

6 Biological interpretation

Choosing a Statistical Model

The statistical model will depend on the study design. When choosing an analysis method it is important to answer following questions:

- ? *What is the question I am trying to answer? Ex: What proteins increase with time and with treatment?*
- *What is the sample size and assumed distribution? For sufficient samples sizes, NPX data can be used as is. To use parametric methods with absolute quantification, the data should be log transformed. Non-parametric methods also work for NPX or absolute quantification.*
- *How many variables are there? Ex: Time, Treatment*
- *How many levels are in each group? Ex: 3 timepoints, 2 treatments*
- *Is the data paired? Are their multiple samples for each subject?*
- *Are there any covariates to adjust for? (Other factors that may impact output variable unrelated for variables of interest) Ex: Age, Sex*
- *What comparisons are important? Ex: Time 1 vs Time 2 in Treated patients or Treated vs untreated patients at Time 2*

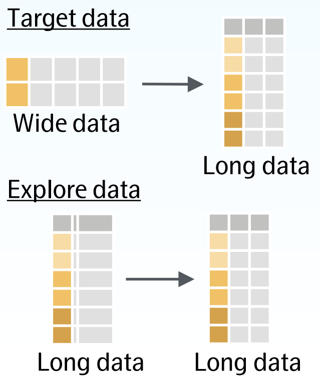
1

Data input

Reading in Olink data

read_NPX(*filename*) imports an NPX^{TM} or Quant file exported from Olink NPX Signature, Olink NPX Explore, or MyData. The file should not be altered prior to import and will be imported in long format

Column name	Description
SampleID	Sample ID name
Index	Unique number for each SampleID
OlinkID	Unique OlinkID for each protein
UniProt	UniProt ID
Assay	Protein symbol
MissingFreq	Proportion of samples below LOD
Panel_Version	Panel version
PlateID	Plate ID
QC_Warning	QC status
LOD	Limit of detection
NPX	Normalized Protein Expression
Normalization	Normalization method used



2

Normalization

Since NPX is a relative measurement, normalization is necessary to combine data across plates or across projects. An adjustment factor is calculated for each assay (protein) which is added to the NPX values from the second plate or project.

Normalizing 2 projects

olink_normalization_bridge() - bridges 2 projects using overlapping samples using the **median of paired differences**

olink_normalization_subset() – normalizes 2 projects using the **difference of group medians.** The group can be a subset or all samples. No overlapping samples required.

olink_normalization() – can be used for bridging, subset, or reference median normalization.

Normalizing more than 2 projects

olink_normalization_n() – normalize multiple projects based on provided normalization schema

For more on bridging and normalization, check out our white paper and vignette.

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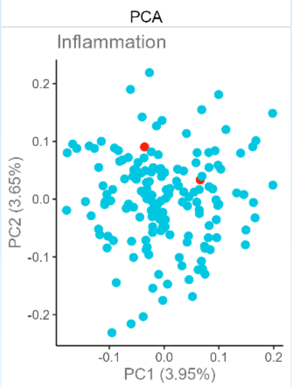
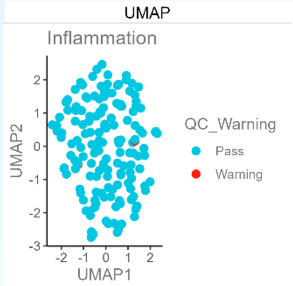
Preliminary Data Analysis and Quality Control

Data overview, outlier detection, and identifying global trends

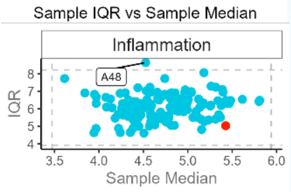
olink_pca_plot(*df*) generates a principal component analysis (PCA) projection of all samples from NPX data along two principal components. Color can be set by **color_g** argument.



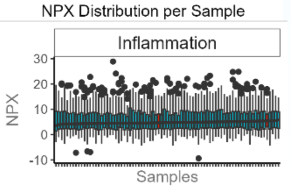
olink_umap_plot(df) generates a uniform manifold approximation and projection (UMAP) for dimension reduction of all samples from NPX data along 2 specified components. Color can be set by **color_g** argument.



olink_qc_plot*(df)* generates a plot of interquartile range (IQR) vs median for all samples for each panel. Outliers can be tagged using **IQR_outlierDef** and **median_outlierDef.** Color can be set using **color_g** argument.



olink_dist_plot(*df*) generates boxplots of NPX vs SampleID. Color can be set by **color_g** argument.



4

Statistical analysis

Number of variables	Number of levels per variable	Support for paired data?	Support for covariates?	**Parametric Statistics** *Statistical analysis of normally distributed data*	**Non-parametric Statistics** *Statistical analysis of non-normally distributed data*
1	2	☑	☒	**olink_ttest(** ***df, variable, pair_id*** **)** performs a t-test for a given grouping variable as defined by **variable** argument. Paired t-test can be performed by defining **pair_id** argument. Variable must have 2 groups with more than 2 samples per group.	**olink_wilcox(** ***df, variable, pair_id*** **)** performs Mann-Whitney U Test per protein for a given grouping variable as defined by **variable** argument. Wilcoxon signed-rank test can be performed by defining **pair_id** argument. Variable must have 2 groups with more than 2 samples per group.
1	2+	☒	☒		**olink_one_non_parametric(** ***df, variable, dependence*** **)** performs a one-way Kruskal-Wallis Test for each protein. When **dependence** = TRUE, a one-way Friedman Test will be performed for each protein. Model is defined by **variable** argument. **olink_one_non_parametric_posthoc(** ***df, olinkid_list, variable*** **)** performs a Wilcox posthoc test per protein listed in **olinkid_list** based on the variable defined by the **variable** argument.
1+	2+	☒	☑	**olink_anova(** ***df, variable*** **)** performs an ANOVA F-test for each protein based on variables and covariates defined by **variable** and **covariate** arguments or by formula defined by **model_formula** argument. **olink_anova_posthoc(** ***df, variable*****,** ***olinkid_list*****,** ***effect*** **)** performs an ANOVA posthoc test per protein listed in **olinkid_list** based on variables and covariates defined by **variable** and **covariate** arguments or by formula defined by **model_formula** argument.	**olink_ordinalRegression(** ***df, variable, covariates*** **)** performs a 2-way ordinal ANOVA for each protein based on variables and covariates defined by **variable** and **covariate** arguments. **olink_ordinalRegression_posthoc(** ***df, olinkid_list, variable, covariates, effect*** **)** performs a 2-way ordinal ANOVA posthoc test per protein listed in **olinkid_list** based on variables and covariates defined by **variable** and **covariate** arguments.
1+	2+	☑	☑	**olink_lmer(** ***df, variable*** **)** performs a linear mixed model per protein based on variables and covariates defined by **variable** and **covariate** arguments or by formula defined by **model_formula** argument **olink_lmer_posthoc(** ***df, variable, olinkid_list*****,** ***effect, random*** **)** performs a linear mixed model posthoc per protein listed in **olinkid_list** based on variables and covariates defined by **variable** and **covariate** arguments or by formula defined by **model_formula** argument.	

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Statistical visualization

A

olink_volcano_plot(***p.val_tbl*** **)** generates a volcano plot using the results from *olink_ttest()*

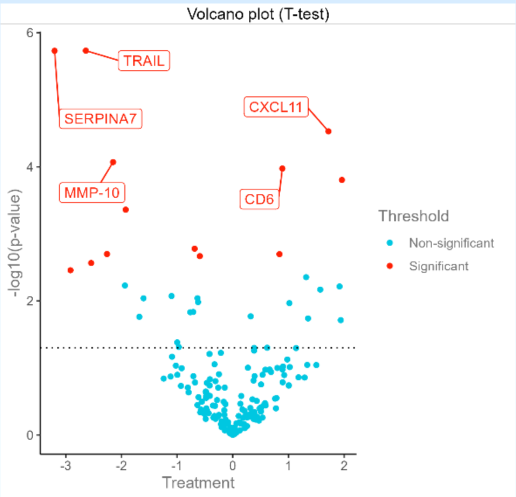
B

olink_boxplot(***df, variable, olinkid_list, posthoc_results, ttest_results*** **)** generates boxplots of **variable** for each protein in **olinkid_list** for **posthoc_results** or **ttest_results**

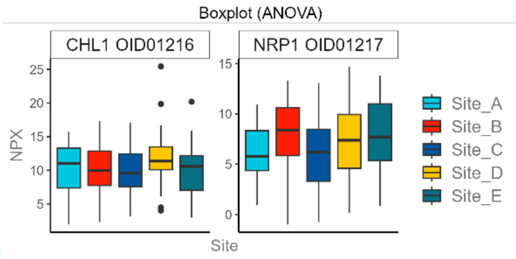
C

olink_lmer_plot ***(df, variable, random, olinkid_list, covariates, x_axis_variable, col_variable)*** generates point-range plots of using **x_axis_variables** (x-axis) and **col_variable** (color) for each protein in **olinkid_list.**

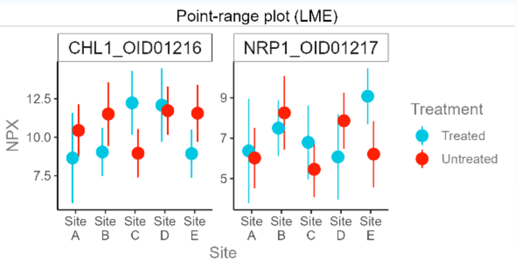
A



B



C



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Biological interpretation

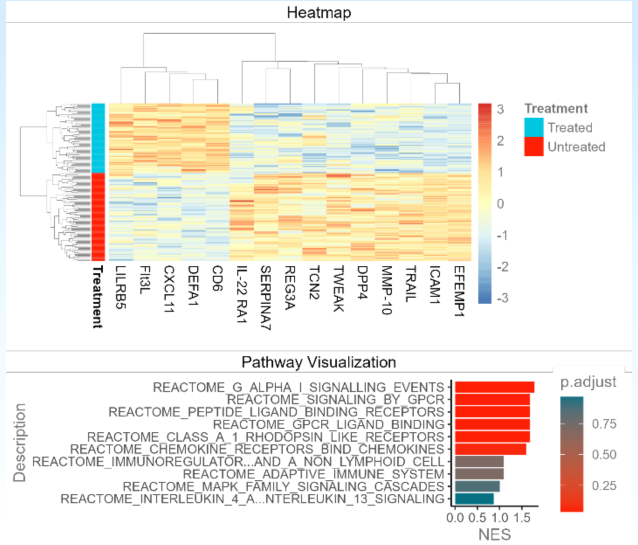
Pathway Enrichment and Heatmap

olink_pathway_enrichment(***data, test_results*** **)** performs pathway enrichment using over-representation analysis or gene-set enrichment analysis (based on **method** argument)

olink_pathway_heatmap(***enrich_results, test_results*** **)** generates a heatmap of proteins related to pathways using enrichment results for **olink_pathway_enrichment()**

olink_pathway_visualization(***enrich_results*** **)** generates bargraph of top/selected enrichment terms based on enrichment results from **olink_pathway_enrichment()**

olink_heatmap_plot ***(df)*** generates a heatmap of all samples from NPX data


Heatmap
Treatment
LILRB5
Flt3L
CXCL11
DEFA1
CD6
IL-22 RA1
SERPINA7
REG3A
TCN2
TWEAK
DPP4
MMP-10
TRAIL
ICAM1
EFEMP1
Treatment
Treated
Untreated
Pathway Visualization
Description
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS
REACTOME_SIGNALING_BY_GPCR
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS
REACTOME_GPCR_LIGAND_BINDING
REACTOME_CLASS_A_1_RHODOPSIN_LIKE_RECEPTORS
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES
REACTOME_IMMUNOREGULATOR...AND_A_NON_LYMPHOID_CELL
REACTOME_ADAPTIVE_IMMUNE_SYSTEM
REACTOME_MAPK_FAMILY_SIGNALING_CASCADES
REACTOME_INTERLEUKIN_4_A...NTERLEUKIN_13_SIGNALING
NES
p.adjust