Olink®

Olink® Normalization and Bridging Service

EXAMPLE

PROJECT NAME	Example Report - 91062
CUSTOMER	Customer Organization customer email address
BUSINESS DEVELOPMENT MANAGER	BDM Name BDM/S email address
DATA SCIENCE SERVICE ITEM	Olink® Normalization and Bridging Service 91062 biostat@olink.com
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Contents

EXAMPLE

Product Overview

Olink Normalization and Bridging Service is used to normalize together two NPX^{TM} datasets using bridging samples, also known as reference samples, which have been run on both projects. This process allows for subsequent statistical analysis to be performed on both datasets together without the need for batch to be used as a covariate or the need for a meta-analysis.

The inputs needed for this product include:

- An NPX file per project as exported from an Olink NPX Software with overlapping samples across each product. Note that the overlapping samples should have the same SampleID. The NPX files should be from the same product and panel.
  - If the SampleIDs are not consistent for the overlapping samples, an additional file is required to map the sample IDs across projects.

The deliverables for this product include:

- A report summarizing the analysis and results
- An NPX file of both adjusted NPX values of the normalized data and un-adjusted NPX values of the reference project
- Separate PDFs for the figures within the report

EXAMPLE

Project Overview

The goal of the analysis is to use the bridging samples common between the reference dataset and the new dataset to normalize overall assay medians in the new project Q-N to the overall assay medians in the project Q-R (as the reference). Panels and number of samples run for new project (Q-N) and reference project (Q-R) can be seen in Tables 1 and 2, respectively.

Table 1: Panels and number of samples ran in project Q-R.

Panel	No. Samples
Explore_HT	156

Table 2: Panels and number of samples ran in project Q-N.

Panel	No. Samples
Explore_HT	172

EXAMPLE

QC status for bridging samples

Table 3 below shows the list of bridging samples together with their SampleQC status. A sample gets a PASS status if it passes all Panels, otherwise the sample gets a WARN status. The percentage in the parenthesis represent the percentage of passing Panels.

Table 3: List of bridge samples with their SampleQC status (Percentage indicates percent passing).

SampleID	Q-R	Q-N
A13	WARNING (63.37%)	WARNING (63.37%)
A29	WARNING (63.37%)	WARNING (63.37%)
A30	WARNING (63.37%)	WARNING (63.37%)
A36	WARNING (63.37%)	WARNING (63.37%)
A45	WARNING (63.37%)	WARNING (63.37%)
A46	WARNING (63.37%)	WARNING (63.37%)
A52	WARNING (63.37%)	WARNING (63.37%)
A63	WARNING (63.37%)	WARNING (63.37%)
A71	WARNING (63.37%)	WARNING (63.37%)
A73	WARNING (63.37%)	WARNING (63.37%)
B3	WARNING (63.37%)	WARNING (63.37%)
B37	WARNING (63.37%)	WARNING (63.37%)
B4	WARNING (63.37%)	WARNING (63.37%)
B45	WARNING (63.37%)	WARNING (63.37%)
B63	WARNING (63.37%)	WARNING (63.37%)
B75	WARNING (63.37%)	WARNING (63.37%)

EXAMPLE

Preliminary Analysis

Interquartile Range (IQR) vs. Sample Median plot and Principal Component Analysis (PCA) plot along the first two principal components were generated per project to determine if any bridging samples appeared to be outlier in either of the projects (Figures 1 and 2).

IQR vs. Sample Median

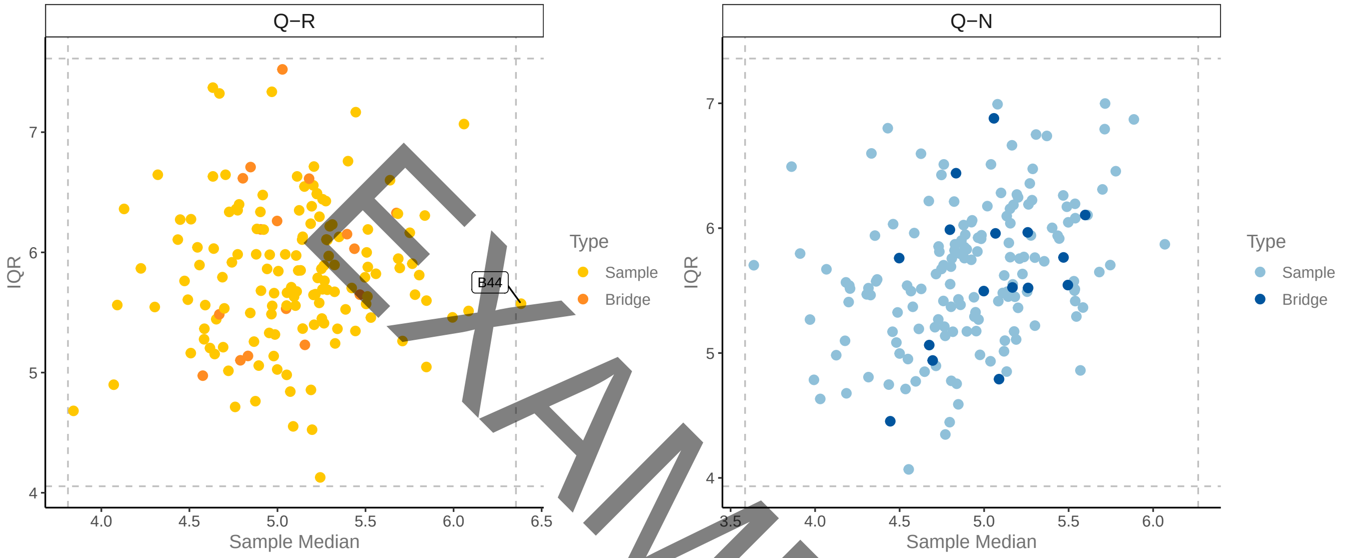


Figure 1: Interquartile range vs. sample median of Q-R and Q-N, per project. Horizontal dashed lines indicate +/-3 standard deviations from the mean IQR. Vertical dashed lines indicate +/-3 standard deviations from the mean sample median.

EXAMPLE

PCA

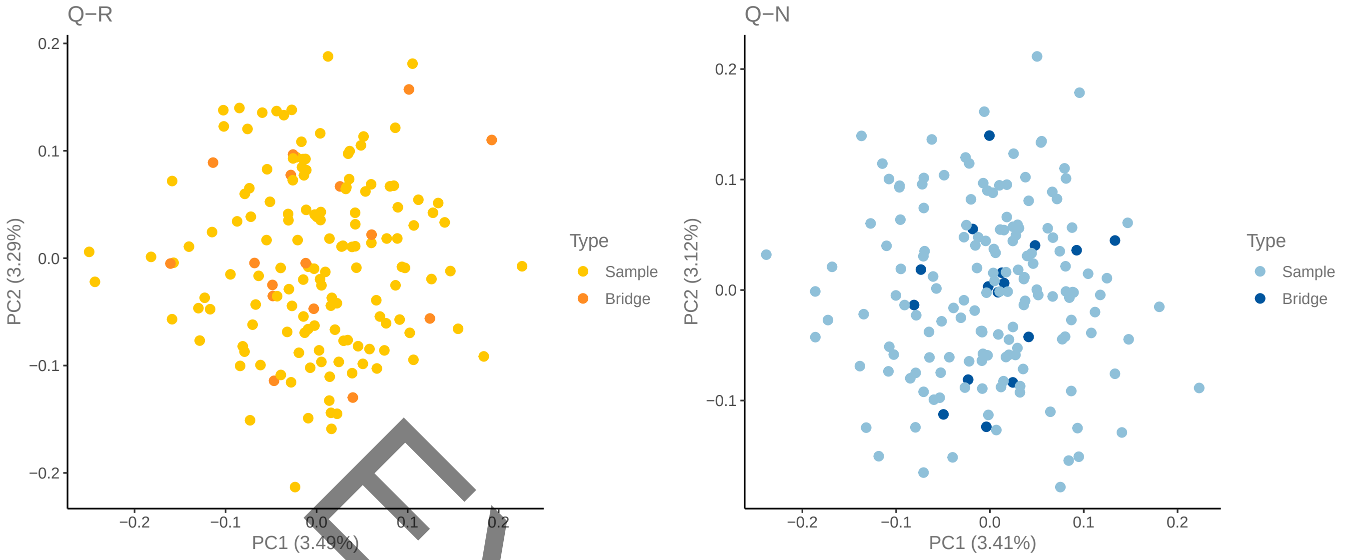


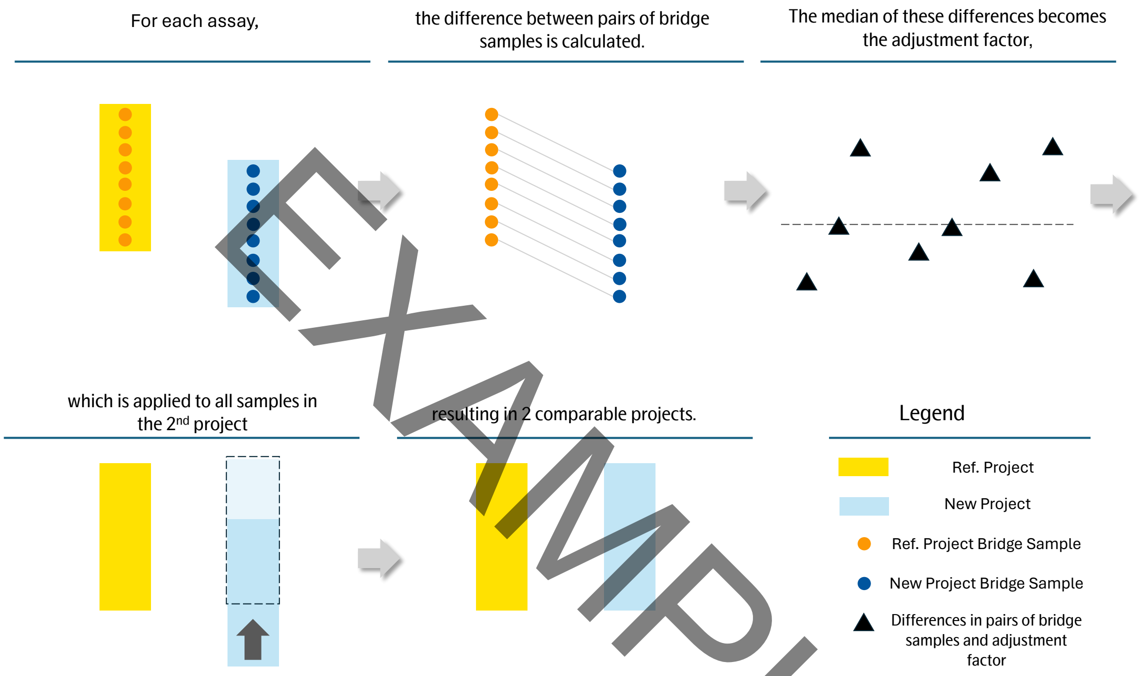
Figure 2: PCA plot along the first two principal components from Q-R and Q-N, in their respective spaces. Bridging sample are highlighted by a different color.

Per this project, the bridging samples appear to be representative of the samples in their respective projects and no bridging samples appear to be outliers.

EXAMPLE

Normalization

The purpose of bridging is to normalize two projects together. To do so, the NPX values of all assays of project Q-N were adjusted to project Q-R using bridging samples. The pair-wise differences were calculated per assay from the 16 bridging samples run in both studies. The median of these differences was used as an adjustment factor *between* the projects and added to each assay of project Q-N. After the normalization, the global NPX distributions in the two projects are comparable.



EXAMPLE

Figure 3: For each assay, there may be a shift in NPX values between studies. Bridging samples can be used as reference points to adjust the new project to the reference values. The pairwise differences between studies is calculated for each reference sample and the median is applied as an adjustment factor to the new study. After bridging the samples are shifted to be closer to the reference sample values. The NPX values of the reference study are not adjusted, an adjustment factor for each assay is applied to the samples from the second study

Post-Normalization Evaluation

The normalization was evaluated by PCA plot, NPX density plot, Coefficient of Variation density plot.

PCA

The PCA plots of the data pre- and post-normalization can be seen in Figure 4. The quality of bridging can be assessed by comparing the results in Figure 4 to two examples of unsuccessful bridgings shown in Figure 5. In Figure 5 the batch effect is not ameliorated and the bridge samples do not appear closer together. Figure 6 further highlights the differences between bridge samples pre and post normalization in these example projects.

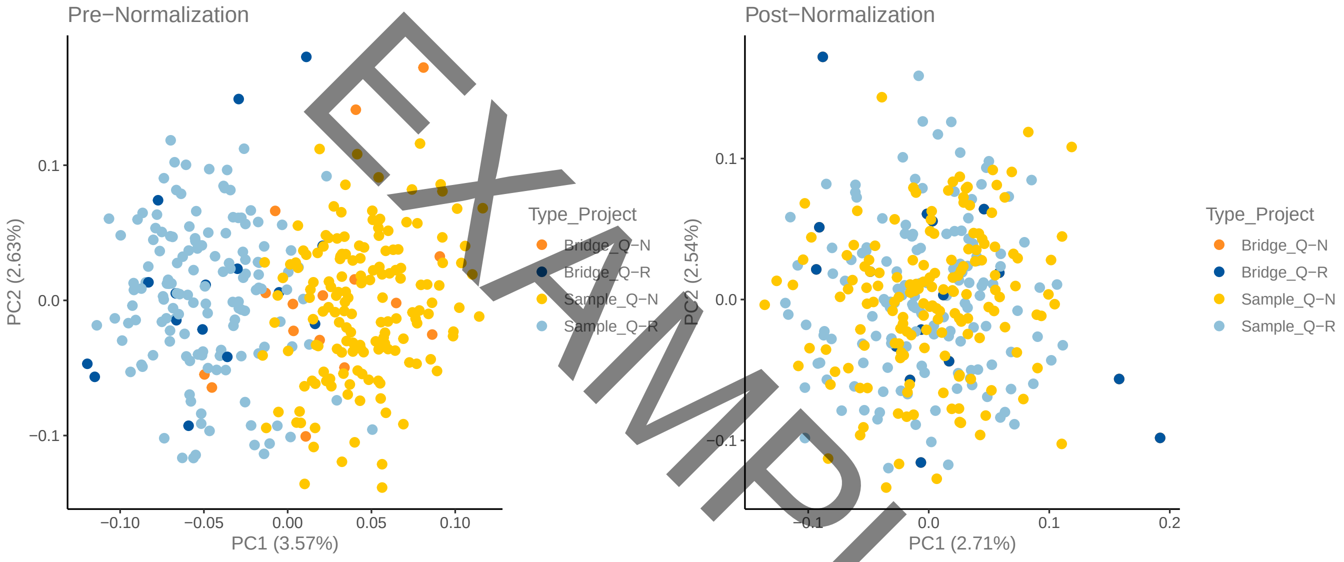


Figure 4: PCA plot along the first two principal components of all samples from Q-R and Q-N, pre- and post-normalization. Bridging sample are highlighted by a different color.

EXAMPLE

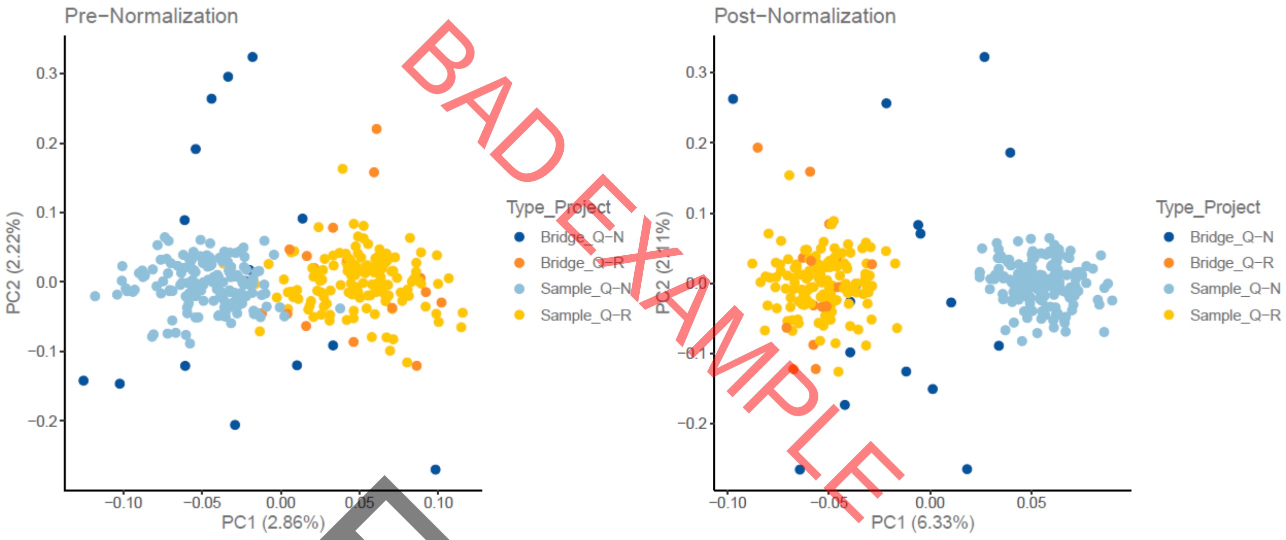


Figure 5: PCA plots along the first two principal components of all samples pre- and post-normalization from example projects to illustrate unsuccessful bridging. Bridging sample are highlighted by a different color.

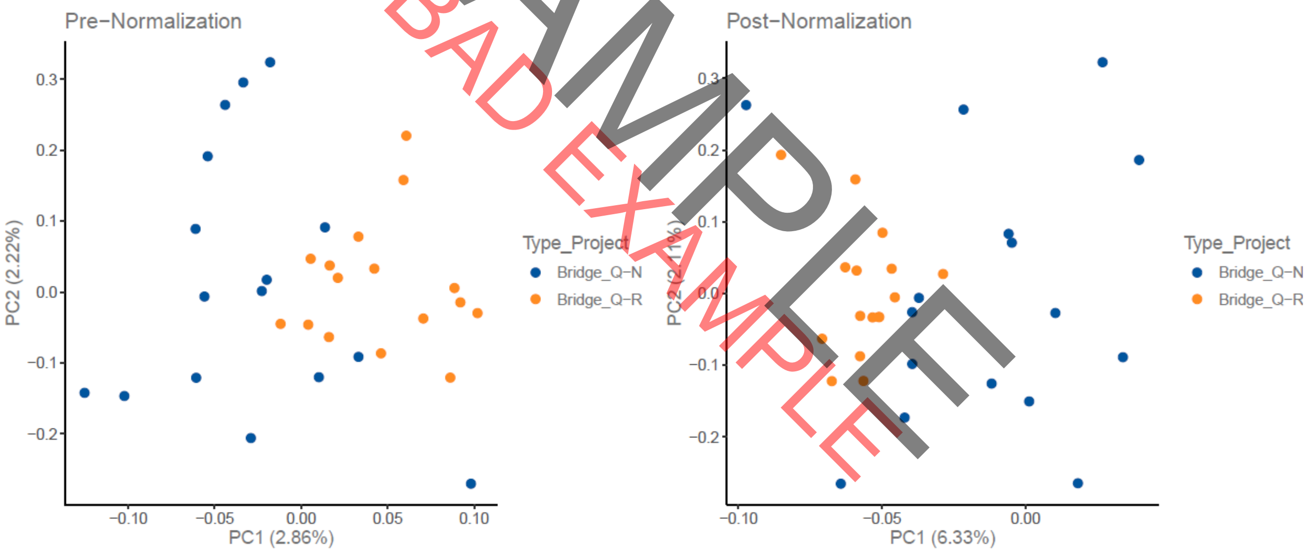


Figure 6: PCA plots along the first two principal components of bridge samples pre- and post-normalization from example projects to illustrate unsuccessful bridging.

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NPX density plot

The normalization was further evaluated by density plots of the data pre- and post-normalization (Figure 7).

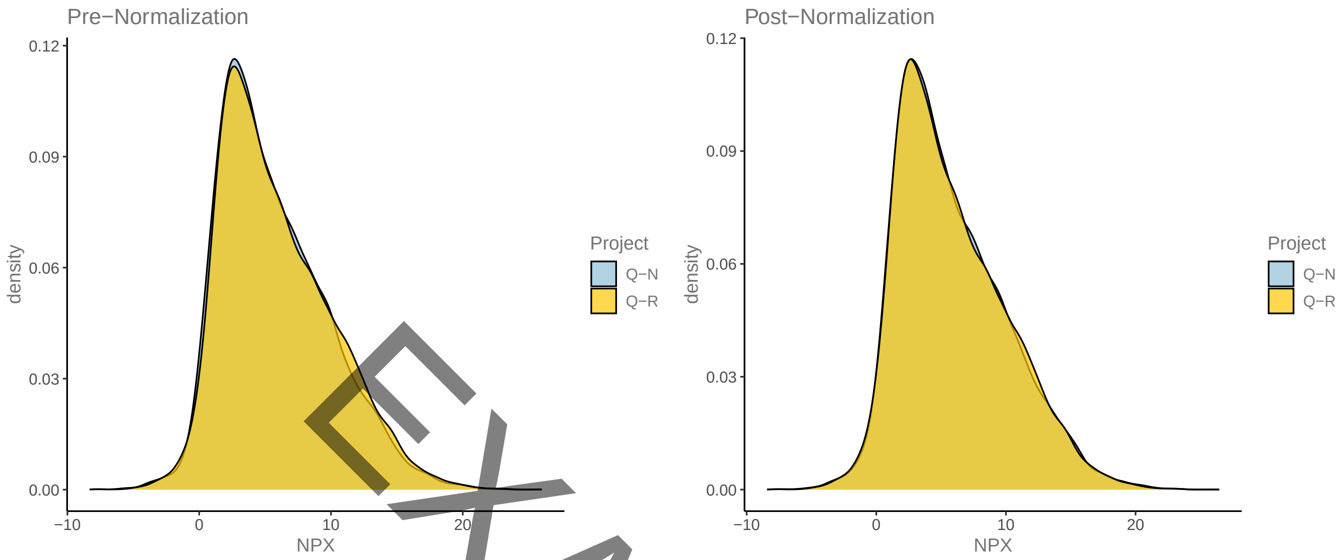

Figure 7: Density plot showing the NPX distribution of the two datasets pre- and post-normalization, colored by project.

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Coefficient of Variation density plot

Inter-project Coefficient of Variation (CV) is calculated pre- and post-normalization using Sample Controls (Figure 8).

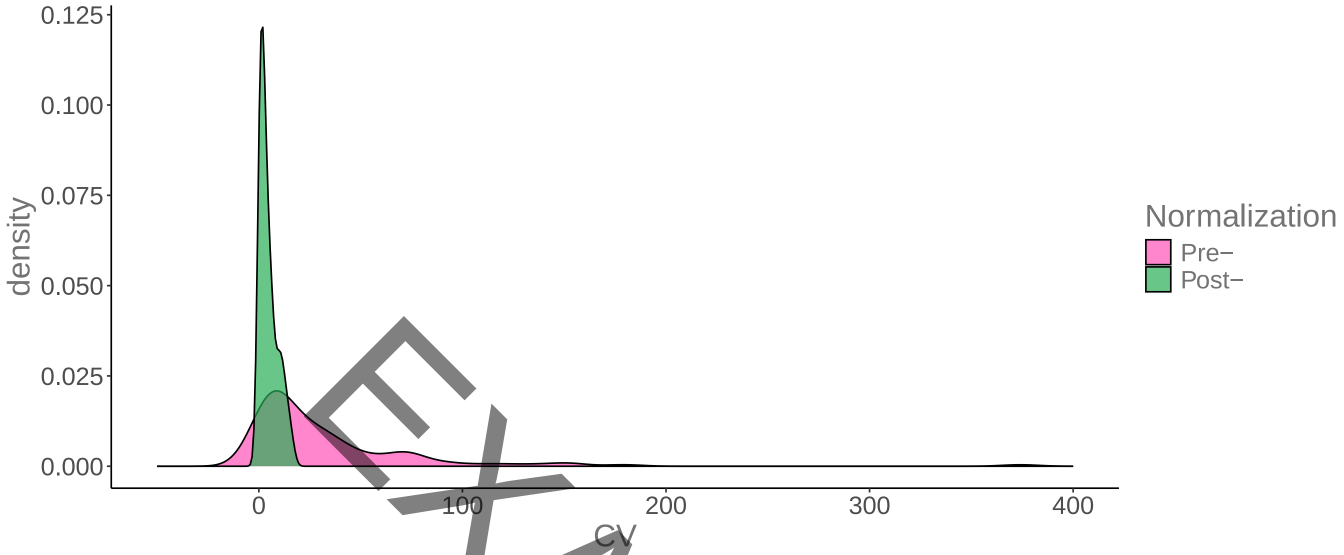

Figure 8: Density plot of inter CV values pre- and post-normalization.

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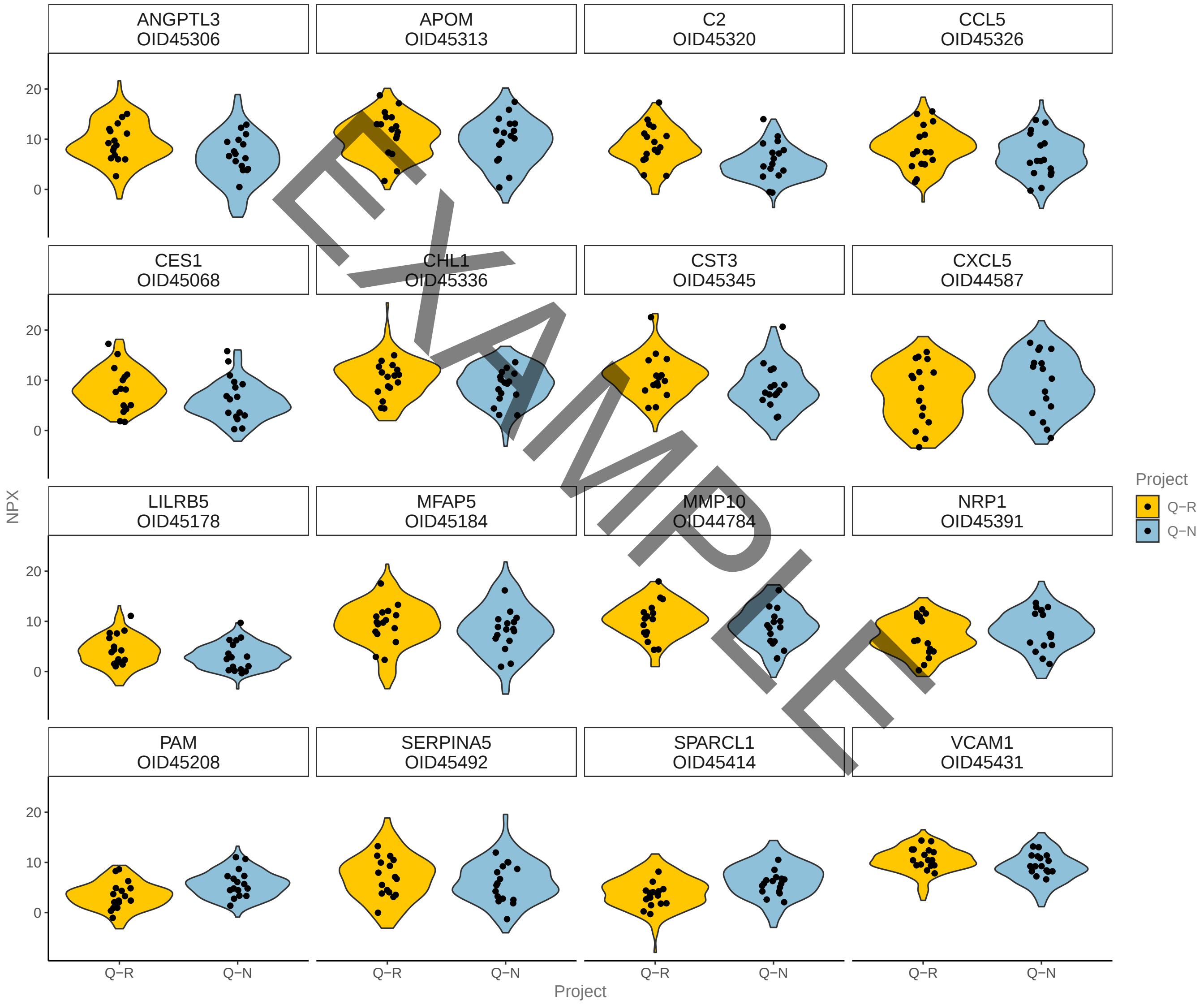
Assay(s) with High Adjustment Factor(s)

Large adjustment factors can indicate differences between panel versions or other technical improvements, as well as any other changes that can occur between the running of two projects. It is useful to verify that assays with high adjustment factors are still comparable between projects. Adjustment factors greater than 1 or less than -1 NPX were further investigated.

Figure 9 displays the violin plots of assay(s) with high adjustment factor(s). In this case, there was(were) 23 assay(s) with high adjustment factor(s).



EXAMPLE

Figure 9: Violin plots of assay(s) with large adjustment factor(s) greater than 1 or less than -1 NPX. Points on violin plots are values of bridging samples from their respective projects before normalization.

Summary

Bridge normalization was performed on Q-N using 16 bridging samples selected from Q-R to reduce run-to-run variability between the projects. Normalization appears to have performed well have bridge samples that appear the closer after bridging in PCA and there is a similar or larger overlap in the two projects as seen in PCA and NPX distribution plots.

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R Session Information

- R version 4.4.0 (2024-04-24), `x86_64-pc-linux-gnu`
- Locale: `LC_CTYPE=en_US.UTF-8`, `LC_NUMERIC=C`, `LC_TIME=en_US.UTF-8`, `LC_COLLATE=en_US.UTF-8`, `LC_MONETARY=en_US.UTF-8`, `LC_MESSAGES=en_US.UTF-8`, `LC_PAPER=en_US.UTF-8`, `LC_NAME=C`, `LC_ADDRESS=C`, `LC_TELEPHONE=C`, `LC_MEASUREMENT=en_US.UTF-8`, `LC_IDENTIFICATION=C`
- Time zone: `America/New_York`
- TZcode source: `system (glibc)`
- Running under: `Ubuntu 22.04.4 LTS`
- Matrix products: default
- BLAS: `/usr/lib/x86_64-linux-gnu/openblas-pthread/libblas.so.3`
- LAPACK: `/usr/lib/x86_64-linux-gnu/openblas-pthread/libopenblasp-r0.3.20.so`; LAPACK version3.10.0
- Base packages: base, datasets, graphics, grDevices, methods, stats, utils
- Other packages: dplyr 1.1.4, extrafont 0.19, forcats 1.0.0, ggplot2 3.5.1, ggpubr 0.6.0, kableExtra 1.4.0, knitr 1.46, lubridate 1.9.3, OlinkAnalyze 3.7.0, purrr 1.0.2, readr 2.1.5, stringr 1.5.1, tibble 3.2.1, tidyr 1.3.1, tidyverse 2.0.0
- Loaded via a namespace (and not attached): abind 1.4-5, arrow 15.0.1, assertthat 0.2.1, backports 1.4.1, bit 4.0.5, bit64 4.0.5, boot 1.3-30, broom 1.0.5, car 3.1-2, carData 3.0-5, cellranger 1.1.0, cli 3.6.2, codetools 0.2-20, colorspace 2.1-0, compiler 4.4.0, cowplot 1.1.3, digest 0.6.35, emmeans 1.10.1, estimability 1.5, evaluate 0.23, extrafontdb 1.0, fansi 1.0.6, farver 2.1.1, fastmap 1.1.1, generics 0.1.3, ggrepel 0.9.5, ggsignif 0.6.4, glue 1.7.0, grid 4.4.0, gtable 0.3.5, hms 1.1.3, htmltools 0.5.8.1, labeling 0.4.3, lattice 0.22-6, lifecycle 1.0.4, lme4 1.1-35.3, lmerTest 3.1-3, magrittr 2.0.3, MASS 7.3-60.2, Matrix 1.7-0, minqa 1.2.6, multcomp 1.4-26, munsell 0.5.1, mvtnorm 1.2-4, nlme 3.1-164, nloptr 2.0.3, numDeriv 2016.8-1.1, pillar 1.9.0, pkgconfig 2.0.3, R6 2.5.1, Rcpp 1.0.12, readxl 1.4.3, rlang 1.1.3, rmarkdown 2.27, rstatix 0.7.2, rstudioapi 0.16.0, Rttf2pt1 1.3.12, sandwich 3.1-0, scales 1.3.0, splines 4.4.0, stringi 1.8.3, survival 3.5-8, svglite 2.1.3, systemfonts 1.0.6, TH.data 1.1-2, tidyselect 1.2.1, timechange 0.3.0, tinytex 0.50, tools 4.4.0, tzdb 0.4.0, utf8 1.2.4, vctrs 0.6.5, viridisLite 0.4.2, withr 3.0.0, xfun 0.43, xml2 1.3.6, xtable 1.8-4, yaml 2.3.8, zip 2.3.1, zoo 1.8-12

EXAMPLE