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Nanostring data- QC explanation and comments

Nanostring data

Congratulations on your new Nanostring data delivered by BioXpedia. Along with the normalized Nanostring data, you will find an "Experiment Properties" report that presents the details of the quality control of the Nanostring data.

It is recommended to use log2 transformed Nanostring data for further statistical analyses. Note that the delivered data is not log2 transformed.

Explanation for the "Experiment Properties" report.

The QC parameters in the table called **QC Tests Performed** are explained below:

Imaging QC. This metric reports the percentage of fields of view (FOVs) the Digital Analyzer or Sprint was able to capture. At least 75% of FOVs should be successfully counted to obtain robust data.

Binding Density QC. This metric is a measurement (in spots per square micron) of the concentration of barcodes seen by the instrument. The Digital Analyzer may not be able to distinguish each probe from the others if too many are present.

Positive Control QC. This metric performs a correlation analysis in log2 space between the known concentrations of positive control target molecules added by Nanostring and the resulting counts. Correlation values lower than 0.95 may indicate an issue with the hybridization reaction and/or assay performance.

Limit of detection QC. This measures the limit of detection of the assay by comparing the results from the positive control probes and those from the negative control probes. Specifically, it is expected that the 0.5 fM positive control probe (Pos_E) will produce raw counts at least two standard deviations higher than the mean of the negative control probes. If not, this means that the limit of detection for the given assay is higher than expected.

The QC parameters in the table **Normalized Data** are explained below:

mRNA Positive Normalization Flag. The data is normalized for technical variability, by scaling the sample values to the geometric mean of the positive controls. This is done for all samples. nSolver raises a warning flag when the geometric mean of positive controls for a sample is more than three-fold different from the geometric mean of the positive control for all samples.

mRNA Content Normalization Flag. The data is normalized for input variability by scaling the sample values to the geometric mean of the following selected reference genes:

NRDE2-mRNA

TBP-mRNA
HPRT1-mRNA
ABCF1-mRNA
PPIA-mRNA
NMT1-mRNA
TUBB-mRNA
CYC1-mRNA
GUSB-mRNA
SDHA-mRNA
POLR2A-mRNA
STK11IP-mRNA
TBC1D10B-mRNA
G6PD-mRNA
BABAM1-mRNA
MRPS7-mRNA

These reference genes were selected using the geNorm algorithm (Vandesompele, 2002).

If CodeSet Content Normalization Factors are outside of the set range (0.1-10), then assay input variability is too large to be corrected for using this normalization approach.

Comments for the QC properties of the Nanostring data

The samples BX0616_014, BX0616_103, and BX0616_110 have an mRNA Content Normalization flag, indicating that the mRNA content in these samples was too low for the normalization method to function as intended. This means that the sample should be used with caution in statistical analysis, if not removed.

No other QC flags were detected for the data. This means that the quality control and normalization of the samples are considered satisfying and within the range of the expected.

Please contact us if further questions arise at stine.rye@bioxpedia.com or maja@bioxpedia.com