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Quality Control Report

GLOBAL QC RESULT..... PASSED

Sample Quality Metrics..... PASSED

Hybridization Quality Metrics PASSED

Labeling Quality Metrics PASSED

GenoSplice estimates that your data are good enough to be further analyzed.

Details of the main QC analyses are detailed in the following pages. Please note that we performed complementary QC analyses that are not shown below (*e.g.*, analyses of JPEG from CEL files, RLE Box Plot...).

GenoSplice Quality Control policy

In the case of the "GLOBAL QC" value is "PASSED", GenoSplice estimates that data quality is good enough to be further analyzed.

In the case of the "GLOBAL QC" value is "MEDIUM" or "FAILED", GenoSplice estimates that data quality is not good and that potential analysis results will not be relevant. Therefore, from our experience, we strongly advise you to perform new experiments to provide us new sample(s). If you don't, we decline all responsibilities relating to the analyses results.

Do not hesitate to contact us if you need more information:

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- Fax: +33 (0) 1 57 27 68 31

The GenoSplice team

Sample Quality Metrics

Metric 1: *pos_vs_neg_auc*

What does it measure?

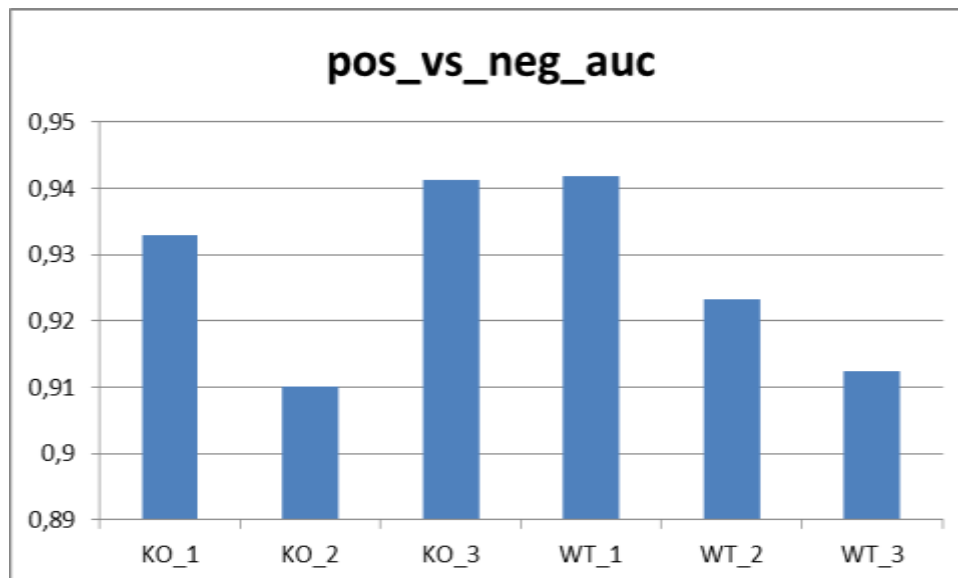
This is the “area under the curve”, or AUC value for a ROC curve which plots the detection of positive controls against the false detection of negative controls. This metric is a robust measurement for overall data quality (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”).

Expected value (EV) > 0.8

Typical values range between 0.8 and 0.9, with a value of 1.0 being perfect and a value of 0.5 illustrating no discernable difference between the positive and negative controls (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”).

Values

| Sample | Value | Result compared to EV |
|--------|----------|-----------------------|
| KO_1 | 0.932941 | passed |
| KO_2 | 0.910074 | passed |
| KO_3 | 0.941230 | passed |
| WT_1 | 0.941861 | passed |
| WT_2 | 0.923199 | passed |
| WT_3 | 0.912336 | passed |



Conclusion: **PASSED**

All sample values are above 0.8.

Metric 2: *all_probeset_rle_mean*

What does it measure?

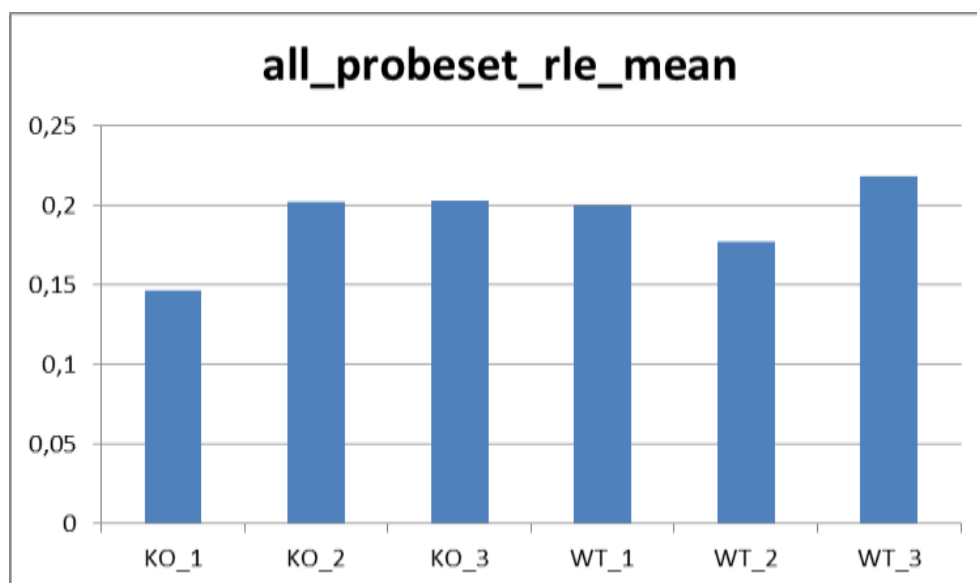
Mean absolute relative log expression (RLE). The signal of each probeset is compared to the median value of this probeset in the study. The metric is the mean of these differences from all the probesets (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”).

Expected value (EV) < 0.6

Unusually high values indicate that the signals on the array are different from others in the study, i.e., big values are bad (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”).

Values

| Sample | Value | Result compared to EV |
|--------|----------|-----------------------|
| KO_1 | 0.146411 | passed |
| KO_2 | 0.201810 | passed |
| KO_3 | 0.202880 | passed |
| WT_1 | 0.199822 | passed |
| WT_2 | 0.177395 | passed |
| WT_3 | 0.218174 | passed |



Conclusion: PASSED

All sample values are below 0.6.

Metric 3: %P

What does it measure?

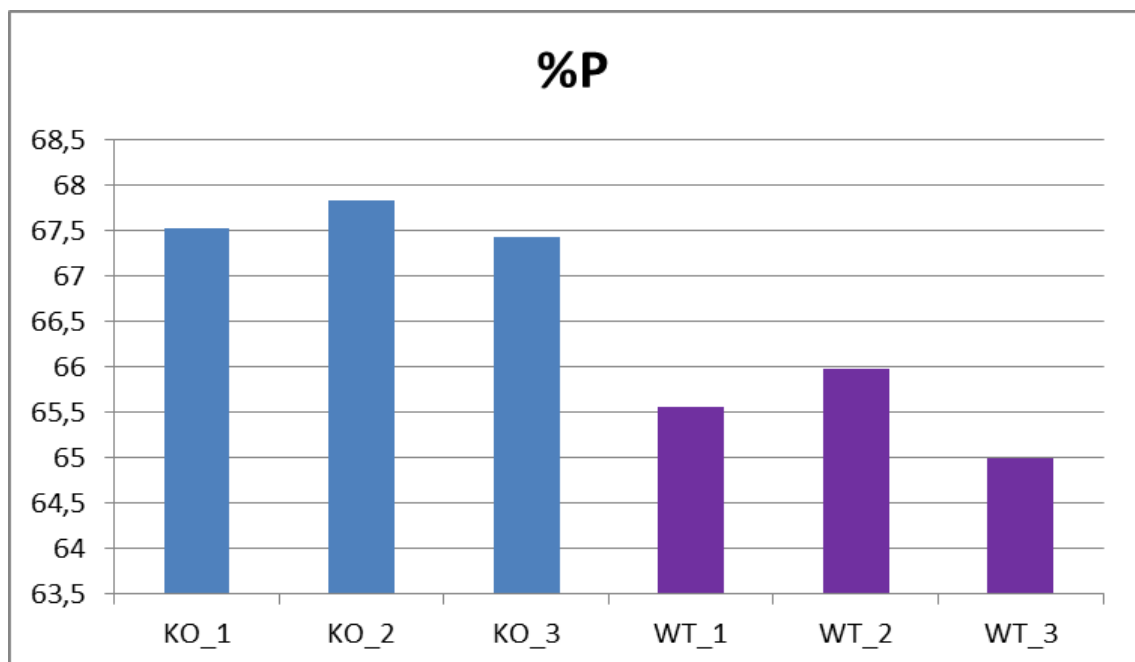
Percent of probesets detected based on the DABG (detection above background) algorithm (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”).

Expected value (EV): $\Delta < 10$ between samples from the same condition

The value should be similar for samples from same condition. Samples are potentially problematic when %P is more than 10 percentage points lower than that of similar sample types. Absolute number varies greatly between different conditions (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”).

Values

| Sample | Value | Δ within condition | Result compared to EV |
|--------|-----------|---------------------------|-----------------------|
| KO_1 | 67.529976 | 0.405517 | passed |
| KO_2 | 67.833572 | | passed |
| KO_3 | 67.428055 | | passed |
| WT_1 | 65.556816 | 0.995293 | passed |
| WT_2 | 65.985863 | | passed |
| WT_3 | 64.990570 | | passed |



Conclusion: PASSED

All sample values from the same condition are comprised within a 10 percent interval. It is interesting to see that the three WT samples have less global intensity than KO samples (about -2%). Many up-regulated genes expected in KO?

Hybridization Quality Metrics

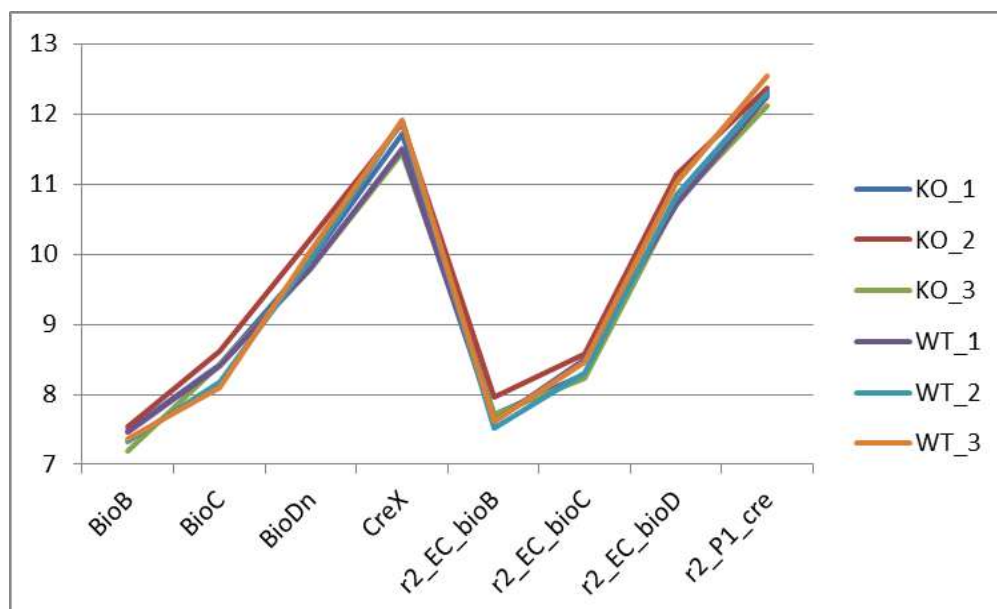
The bacterial spikes (*BioB*, *BioC*, *BioD* and *Cre*) are best for monitoring the quality of the hybridization, similar to 3'-based expression. These spikes are added to the hybridization cocktail but are labeled independently of the rest of the sample. The four spikes are input with increasing concentration (from the Affymetrix Whitepaper "Quality Assessment of Exon and Gene Arrays"). A plot of the signal values associated with these spikes should show a trend where:

BioB* < *BioC* < *BioD* < *Cre

Values

| Sample | BioB | BioC | BioDn | CreX | Order | Result |
|--------|----------|----------|----------|----------|--------------------------|--------|
| KO_1 | 7.471625 | 8.42624 | 9.90456 | 11.70735 | BioB < BioC < BioD < Cre | passed |
| KO_2 | 7.54689 | 8.606755 | 10.217 | 11.87455 | BioB < BioC < BioD < Cre | passed |
| KO_3 | 7.18982 | 8.417845 | 9.793435 | 11.4499 | BioB < BioC < BioD < Cre | passed |
| WT_1 | 7.46761 | 8.39536 | 9.78855 | 11.51 | BioB < BioC < BioD < Cre | passed |
| WT_2 | 7.335385 | 8.177315 | 9.928525 | 11.90165 | BioB < BioC < BioD < Cre | passed |
| WT_3 | 7.361935 | 8.087305 | 10.0451 | 11.8984 | BioB < BioC < BioD < Cre | passed |

| Sample | r2_EC_bioB | r2_EC_bioC | r2_EC_bioD | r2_P1_cre | Order | Result |
|--------|------------|------------|------------|-----------|--------------------------|--------|
| KO_1 | 7.7141 | 8.28415 | 10.83355 | 12.3174 | BioB < BioC < BioD < Cre | passed |
| KO_2 | 7.96866 | 8.58037 | 11.1342 | 12.36725 | BioB < BioC < BioD < Cre | passed |
| KO_3 | 7.707295 | 8.2261 | 10.7426 | 12.12325 | BioB < BioC < BioD < Cre | passed |
| WT_1 | 7.622035 | 8.492905 | 10.71365 | 12.26065 | BioB < BioC < BioD < Cre | passed |
| WT_2 | 7.52252 | 8.300985 | 10.8395 | 12.2902 | BioB < BioC < BioD < Cre | passed |
| WT_3 | 7.61782 | 8.45733 | 11.02495 | 12.54125 | BioB < BioC < BioD < Cre | passed |



Conclusion: **PASSED**

Signal values associated with the bacterial spikes show a trend where *BioB* < *BioC* < *BioD* < *Cre* for all samples.

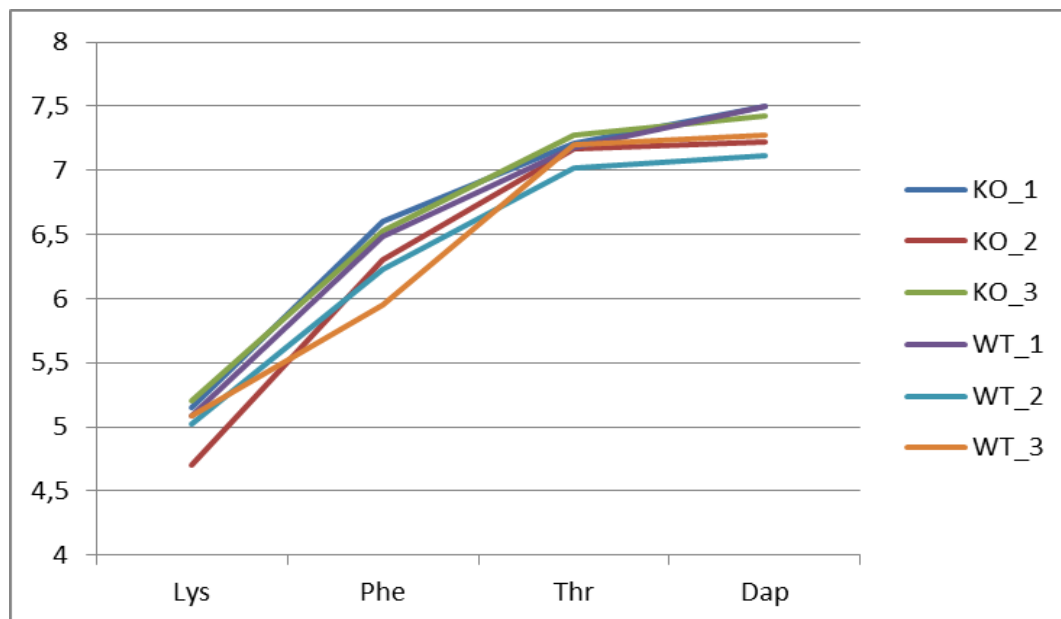
Labeling Quality Metrics

The polyA-control RNAs (*Lys*, *Phe*, *Thr* and *Dap*) are best for monitoring the quality of the labeling reaction. These polyA RNAs are spiked into the sample prior to amplification and labeling. Consequently, these molecules are amplified and labeled with the rest of the biological sample (from the Affymetrix Whitepaper “Quality Assessment of Exon and Gene Arrays”). The rank order of the signal values for these probesets should be:

Lys < Phe < Thr < Dap.

Values

| Sample | Lys | Phe | Thr | Dap | Order | Result |
|--------|----------|----------|----------|----------|---------------------------------------|--------|
| KO_1 | 5.149605 | 6.59961 | 7.21392 | 7.495805 | <i>Lys < Phe < Thr < Dap</i> | passed |
| KO_2 | 4.699375 | 6.303665 | 7.1649 | 7.21917 | <i>Lys < Phe < Thr < Dap</i> | passed |
| KO_3 | 5.20945 | 6.523475 | 7.273115 | 7.42624 | <i>Lys < Phe < Thr < Dap</i> | passed |
| WT_1 | 5.08746 | 6.487475 | 7.174845 | 7.497175 | <i>Lys < Phe < Thr < Dap</i> | passed |
| WT_2 | 5.022195 | 6.22882 | 7.022325 | 7.1189 | <i>Lys < Phe < Thr < Dap</i> | passed |
| WT_3 | 5.086835 | 5.9542 | 7.19967 | 7.280705 | <i>Lys < Phe < Thr < Dap</i> | passed |



Conclusion: PASSED

For all samples, signal values associated with the polyA-control RNAs show a trend where *Lys < Phe < Thr < Dap*.