Quality assessment, single channel (Affymetrix) arrays

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Probe level QC

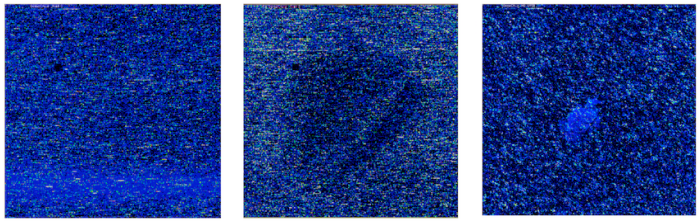
Visual inspection of image file

- Any spot that comes in contact with a streak will be of unusually high intensity, making its value suspect. Flag and exclude such spots.
- Air bubbles and pockets prevent the sample from hybridizing, so that these regions will appear as dark regions on the image plot. Flag and exclude such spots.
- General haze – location based normalization may alleviate this problem.

Visual defects

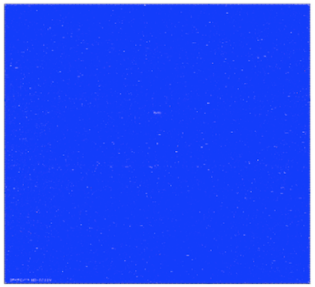
- Dimness/brightness, high background, high/low intensity spots, scratches, high
- Regional abnormalities, overall background, unevenness, spots, haze band, scratches, crop circle, cracks
- As long as these areas do not represent more than 10% of the total probes for the chip, then the area can be masked and the data points thrown out as outliers, or set as NAs.

Haze Band | Crop Circles | Spots, Scratches, etc.

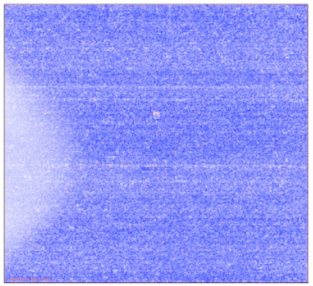


Spatial biases

Images of probe level data

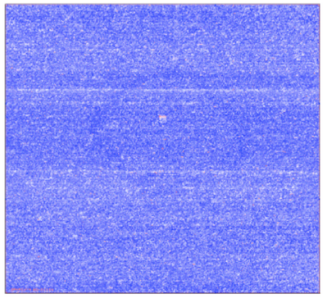


This is the raw data

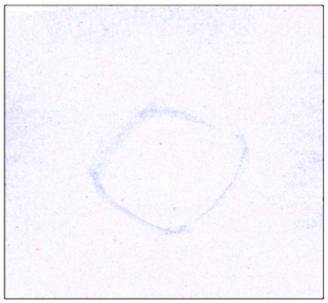


Log scale much more informative

Images of probe level data

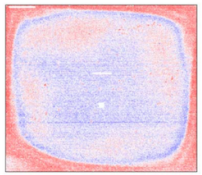


Here is a more subtle artifact. The strong probe effect makes it hard to detect



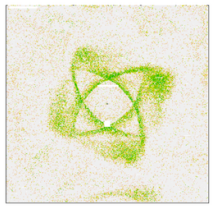
Probe level fit residuals really show it

Images of probe level data

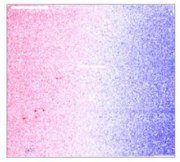


"Ring of Fire"

"Crop Circles"



"Tricolor"



http://plmimagegallery.bmbolstad.com/

Probe quality based on duplicate spots

- For arrays $j = 1, \ldots, J$, suppose there are various spots ($k1$ and $k2$) which interrogate the same gene g.
- Let x_{gjk} represent the log ratio for gene g, spot k, on array j
- The *mean squared difference* between the log ratios is

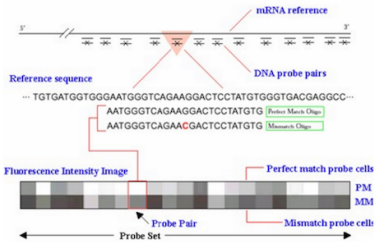
$$\frac{1}{J}\sum_{j=1}^{J}(x_{gjk1} - x_{gjk2})^2$$

- A reasonable threshold would be to say that the multiple probes from the same gene disagree if this mean squared difference is greater than 1 on the log2 scale.

Overall RNA quality control

RNA degradation plot

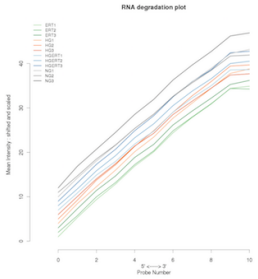
In Affymetrix arrays, a probe-set is dedicated to each target. A probe-set is composed by several probes (classically, 11), all targeting the mRNA target sequence. The RNA degradation plot proposes to plot the average intensity of each probes across all probe-sets, ordered from the 5' to the 3' end.



Overall RNA quality control

RNA degradation plot

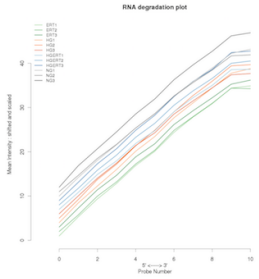
Since RNA degradation starts from the 5` end of the molecule, we would expect probe intensities to be globally lowered at that end of a probe set when compared to the 3’ end. The RNA degradation plot aims at visualizing this trend.



Overall RNA quality control

RNA degradation plot

RNA which is too degraded will have a very high slope from 5' to 3'. The standardized slope of the curves is used as a quantitative indicator of the RNA degradation. An array with unexpected degradation is identified because it has a bigger slope and should stand out.



Overall RNA quality control

RNA degradation plot

- Because β-actin and GAPDH are expressed is most cell types and are relatively long genes, Affymetrix chips use them as controls of the RNA quality.
- Three probe-sets are designed on 3 regions of these genes (5', middle (called M) and 3' extremities).
- Similar intensities for their 3 regions indicate that the transcripts were not truncated and labeled equally along the sequence.

For an array of good quality, Affymetrix recommends that the 3'/5' ratio should not exceed :

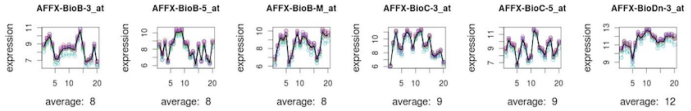
- 3 for beta-actin
- 1.25 for GAPDH

Present/Margin/Absent calls

- **Percent present**: The present calls are defined with significant PM (perfect match) values regarding the MM (mismatch) values. The percentage of present calls should be similar for replicate arrays and within a range of 10% over the arrays

Profiles and boxplots of all controls

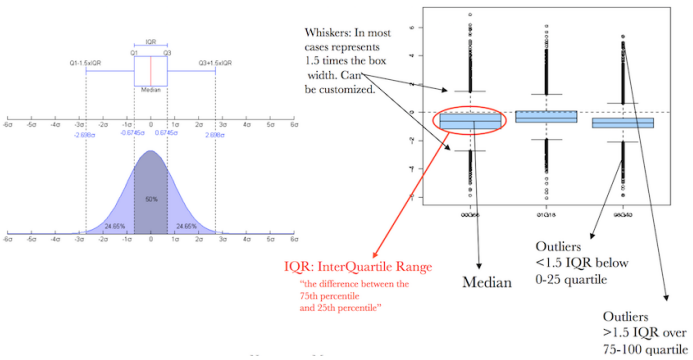
- Affymetrix arrays contain several control probesets, most of them annotated with the "AFFX" prefix. Oulier arrays may have different intensity profiles compared to other arrays.
- The probe calls AFFX-r2-Ec-bioB, bioC, and bioD are *E. coli* genes that are used as internal hybridization controls and must always be present (P)
- The poly-A controls AFFX-r2-Bs-Dap, AFFX-r2-Bs-Thr, AFFXr2-Bs-Phe and AFFX-r2-Bs-Lys are modified *B. subtilis* genes and should be called present at a decreasing intensity



Signal distribution: Scale factor

- A main assumption behind most of the normalization methods for high-throughput expression arrays is that most of the genes are unchanged.
- Affymetrix MAS5 algorithm applies a scale factor to each array in order to equalize their mean intensities.
- A dataset of arrays of good quality should not have very different scale factors.
- Affymetrix recommends that their scale factors should be within 3-fold of one another.

Boxplots

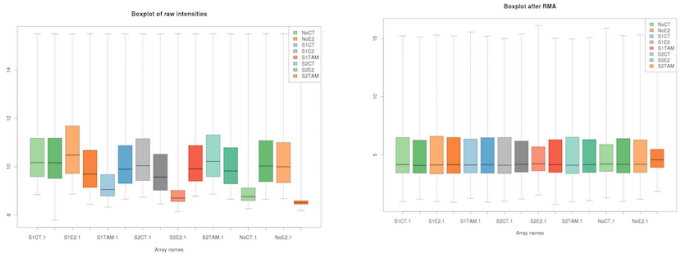


The box plot can answer the following questions:

- Does signal distribution/variation differ between subgroups?
- Are there any outliers?

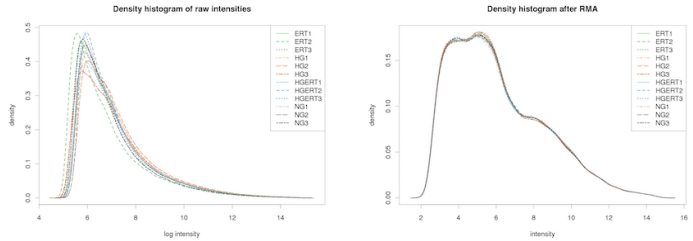
Boxplots of log-intensities

The distributions of raw PM log-intensities are not expected to be identical but still not totally different while the distributions of normalized (and summarized) probe-set log-intensities are expected to be more comparable.



Density histogram of log-intensities

Density plots of log-intensity distribution of each array are superposed on a single graph for a better comparison between arrays and for an identification of arrays with weird distribution. The density distributions of raw PM log-intensities are not expected to be identical but still not totally different.



QC from probe level model

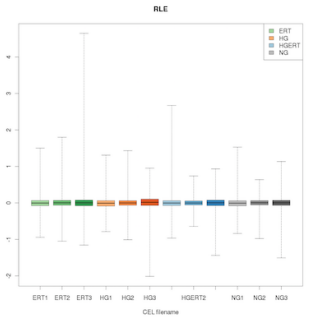
- RMA fits a probe level model
- Additive model, probe effect, array effect, error

$$log_2 y_{ij} = \mu_i + \alpha_j + \epsilon_{ij}$$

- Estimate μ gives RMA
- Use M-estimators
- To avoid showing the variability introduced by expression and probe effect we plot the residuals
- We can also plot the weights used by the regression
- Software available: `affyPLM` Bioconductor package (Ben Bolstad)

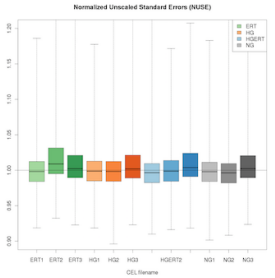
RLE

- The Relative Log Expression (RLE) values are computed by calculating for each probe-set the ratio between the expression of a probe-set and the median expression of this probe-set across all arrays of the experiment.
- It is assumed that most probe-sets are not changed across the arrays, so it is expected that these ratios are around 0 on a log scale.

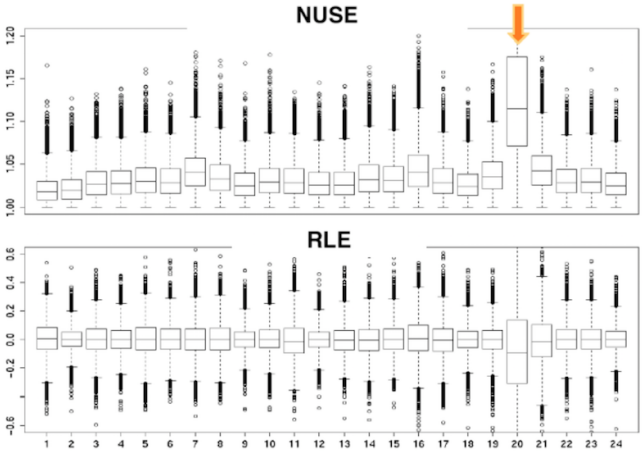


NUSE

- The Normalized Unscaled Standard Error (NUSE) is the individual probe error fitting the Probe-Level Model (the PLM models expression measures using a M-estimator robust regression).
- The NUSE values are standardized at the probe-set level across the arrays: median values for each probe-set are set to 1.

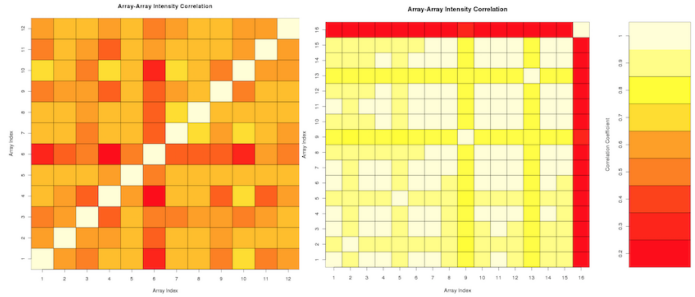


RLE vs. NUSE



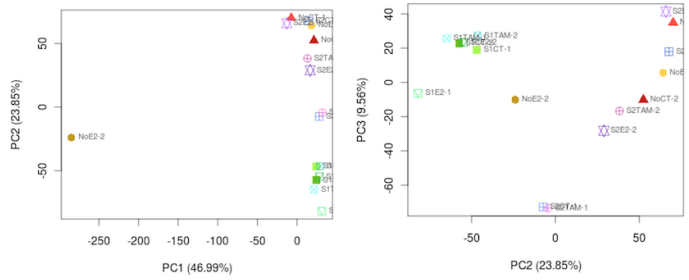
Correlation between arrays

- A correlation coefficient is computed for each pair of arrays in the dataset and is visualized as a heatmap.
- Best to do at each pre-processing step, e.g., before/after normalization



Principal Components Analysis

- Projects arrays onto a coordinate system that emphasizes variability among data



Hierarchical clustering

The Hierarchical Clustering plot is computed in two steps: first it computes an expression measure distance between all pairs of arrays and then it creates the tree from these distances.