Quality assessment, spotted (two-channel) arrays

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http://www.ams.org/notices/200202/fea-tukey.pdf

Importance of visualization

“The greatest value of a picture is when it forces us to notice what we never expected to see.”

– John Tukey

RNA sample quality

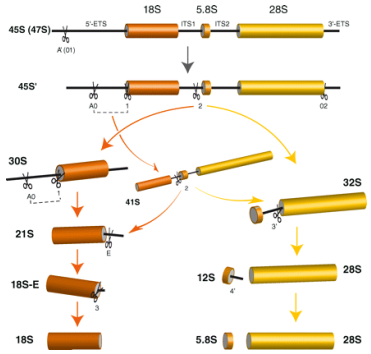
Should be done before any microarray/sequencing experiment

RNA Purity

- Absorbance, optical detection: 260:280 ratios and 260:270 ratios
- Pure RNA has 260:280 ratio is ~2.0
- Contaminations (protein, DNA, phenol) will affect absorbance ratios

RNA sample quality

- Capillary gel electrophoresis: 28S:18S ribosomal RNA ratio
- Pure RNA has 28S:18S ratio >2



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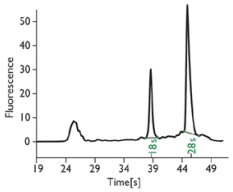


Levels of Quality Control

- **Spot/Probe level**: poor quality of gene expression measurement on one particular array;
- **Gene level**: poor quality of the expression measurement for a single gene across all arrays;
- **Array level**: poor quality of all spots on a particular glass slide or GeneChip.

Overview of quality measures

- Number of spots excluded due to quality problems
- Number of saturated pixels
- Amount of normalization required
- Present/Absent calls for ribosomal RNAs
- 3′:5′ Ratios
- 2D Spatial images
- Boxplots
- MA Plots
- Linearity of signal intensities

Spot Quality Measures

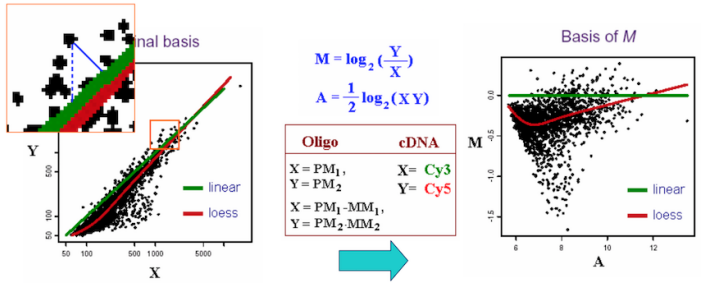
Problematic spots should be flagged and omitted from subsequent analyses

- Spots with too many saturated pixels
- No hybridization
- Negative signal after background adjustment

Intensity-dependent biases

Scatterplots and MA plots

- Compare correlation between the expression values in the two conditions
- Identify genes that are differentially regulated between two conditions
- Drawbacks - Low-intensity values dominate



Intensity-dependent biases

Scatterplots and MA plots

- MA plots used to compare two microarrays (two vectors of intensities)
- MA plots show the dependence between average log2 intensity (x-axis) and ratio of the two intensities (y-axis)
- Better use of the plot's real estate - outliers in logarithm scale spreads the data from the lower left corner to a more centered distribution in which the prosperities of the data are easy to analyze.
- Easier to describe the fold regulation of genes using a log scale. In log2 space, the data points are symmetric about 0.