Array Quality Metrics

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Microarray data quality into question

- Microarrays are widely/routinely used
- Technology and protocol improvements → trustworthy
- Variance and noise
  - Technical causes:
    - Platform
    - Lab, experimentalist
    - RNA extraction
    - Amplification, labeling, hybridization, scanning...
  - Biological causes:
    - Tissue itself (cell lines, biopsies, blood...)
    - Tissue contamination
    - Clinical covariates (age, sex, race...)
    - Cell cycle...

At which step of the analysis?

- Importing the data
- Preprocessing: background correction, normalisation, summarisation of probesets
- Differential Expression
- Gene set enrichment analysis

At which step of the analysis?

- Importing the data
- Quality Assessment
- Preprocessing: background correction, normalisation, summarisation of probesets
- Quality Assessment
- Differential Expression
- Gene set enrichment analysis

At which step of the analysis?

- Importing the data
- Quality Assessment
- Preprocessing: background correction, normalisation, summarisation of probesets
- Quality Assessment
- Remove outlier(s)
- Differential Expression
- Gene set enrichment analysis

What aspects to be evaluated? Which quality metrics?

Per Slide

- What are we looking at?
  - Intensity-dependent ratio
  - Detection of spatial effects
- How?
  - MAplots
  - Representation of the chip

Between Slides

- What are we looking at?
  - Homogeneity
  - Outlier samples
  - Biological meaning
- How?
  - Boxplots, density plots
  - Heatmap, PCA

How to easily perform quality assessment?

- **arrayQualityMetrics,** Bioconductor package for:
  - Affymetrix, Agilent, Illumina, homemade arrays etc...
- From an R object $\Rightarrow$ HTML report
- Plots:
  - MA plot and spatial representations
  - Boxplots and density
  - Heatmap and PCA
  - Variance-mean dependency
  - GC content and probe mapping studies
  - Affymetrix only: NUSE, RLE, RNA degradation, QCstats, PM/MM
- Outlier identification

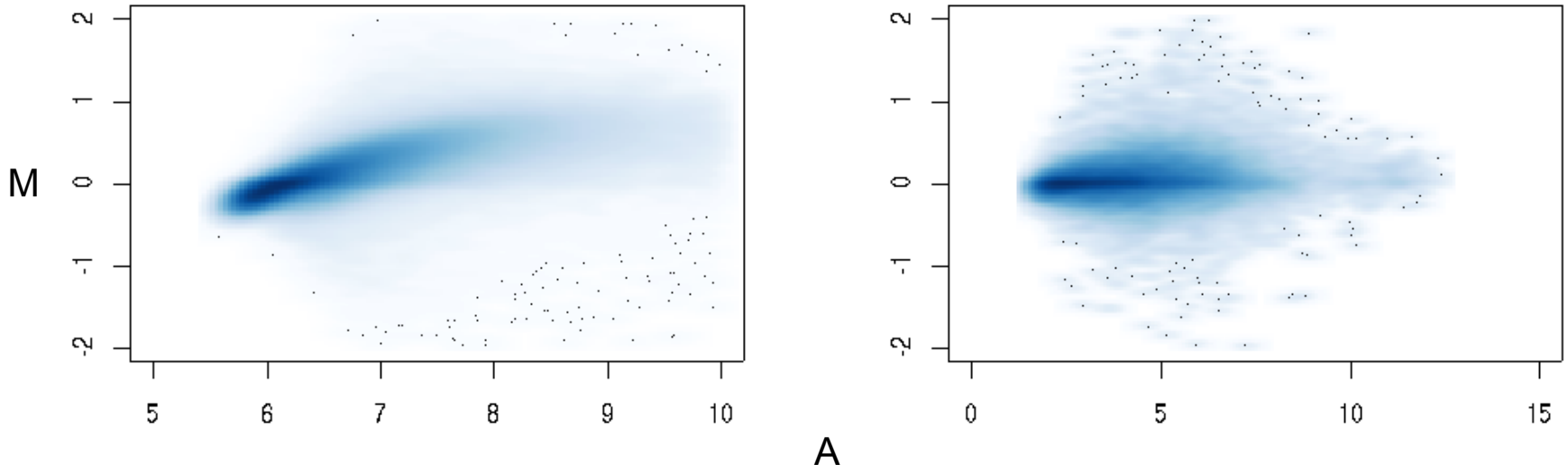
Functions

- **arrayQualityMetrics**(expressionset, outdir, force, do.logtransform, intgroup, grouprep, spatial, sN)
- aQM « modules »:
  - aqm.prepdata()
  - aqm.maplot()
  - aqm.density()
  - …
  - aqm.plot()
  - aqm.writereport()

MA plot

Before normalisation

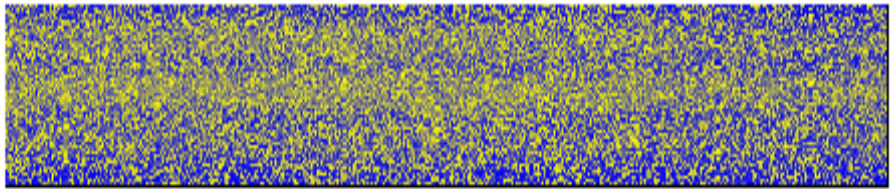
After normalisation

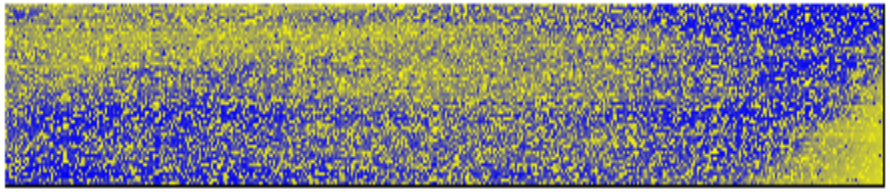


$$M = log_2(I_1) - log_2(I_2)$$

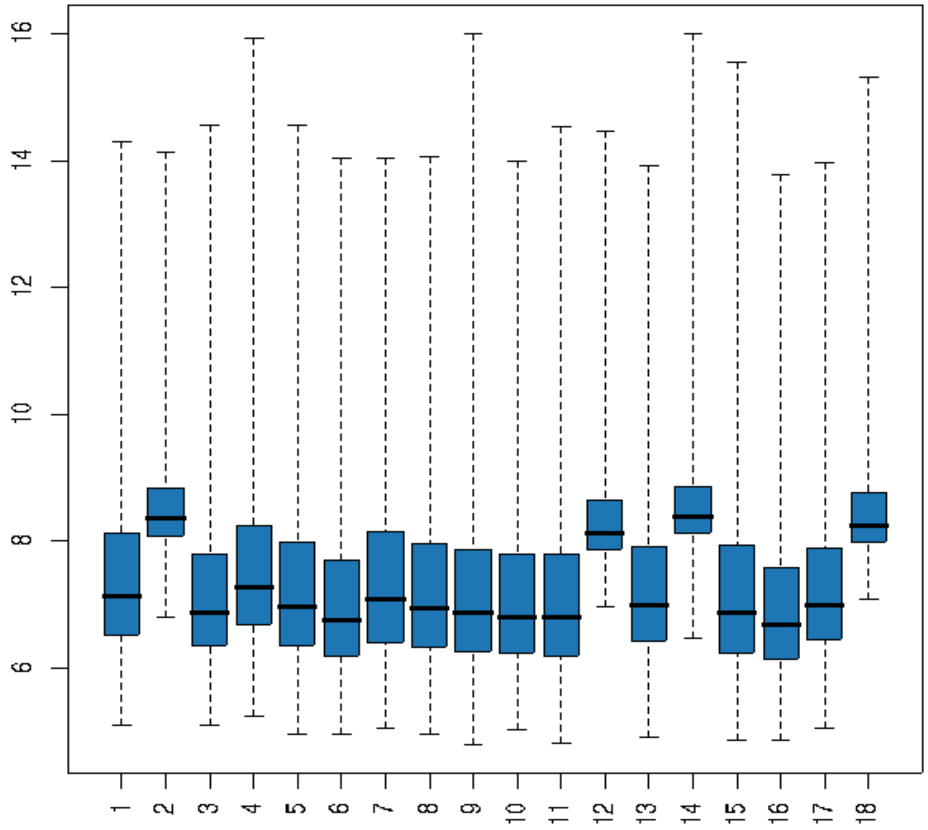
$$A = 1/2\ (log_2(I_1) + log_2(I_2))$$

Spatial representations



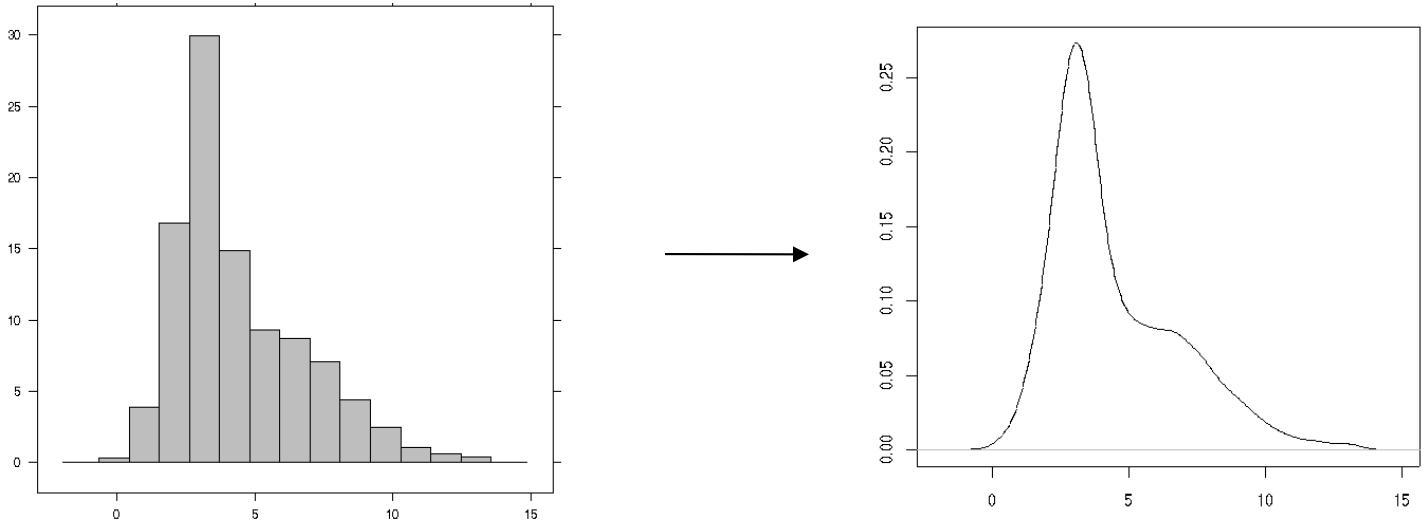


Boxplot



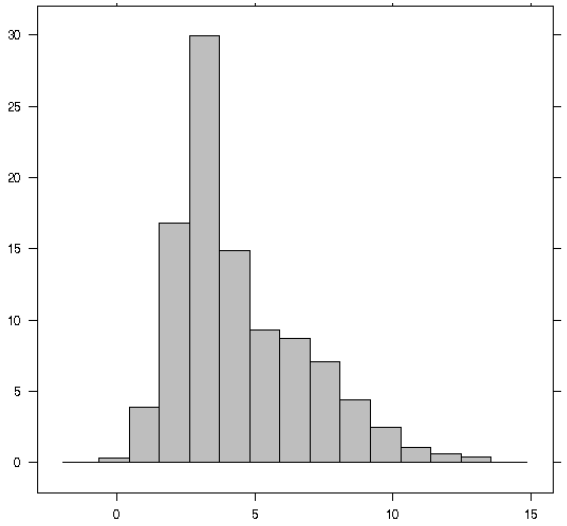
Density plot

- Histograms: graphical representation of frequencies, discrete values
- Density: estimate of the histogram, continuous values

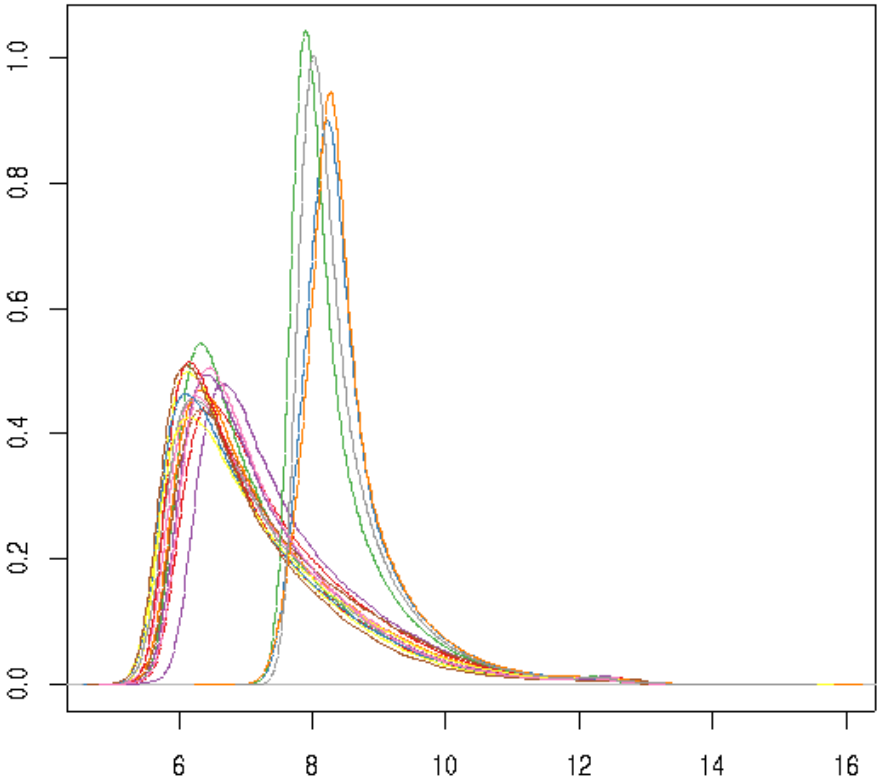


Density plot

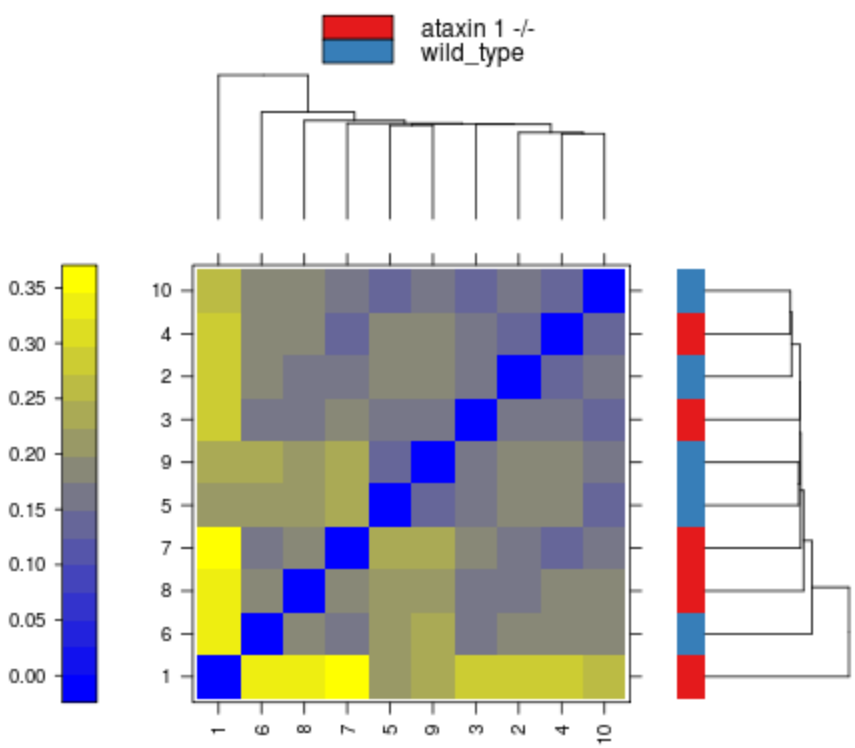
- Histograms: graphical representation of frequencies, discrete values

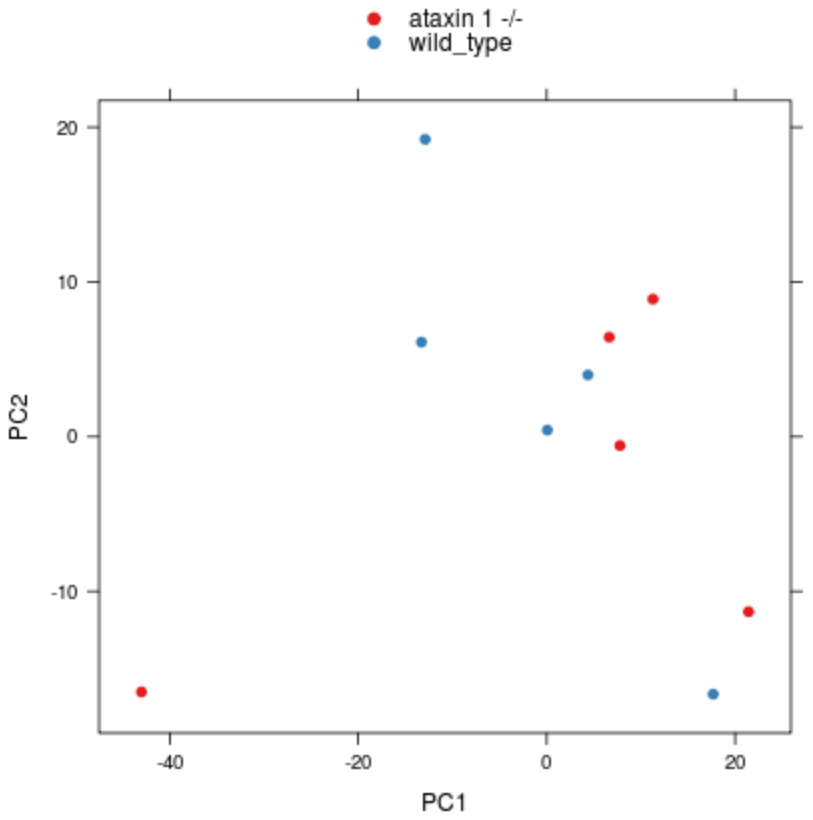


- Density: estimate of the histogram, continuous values

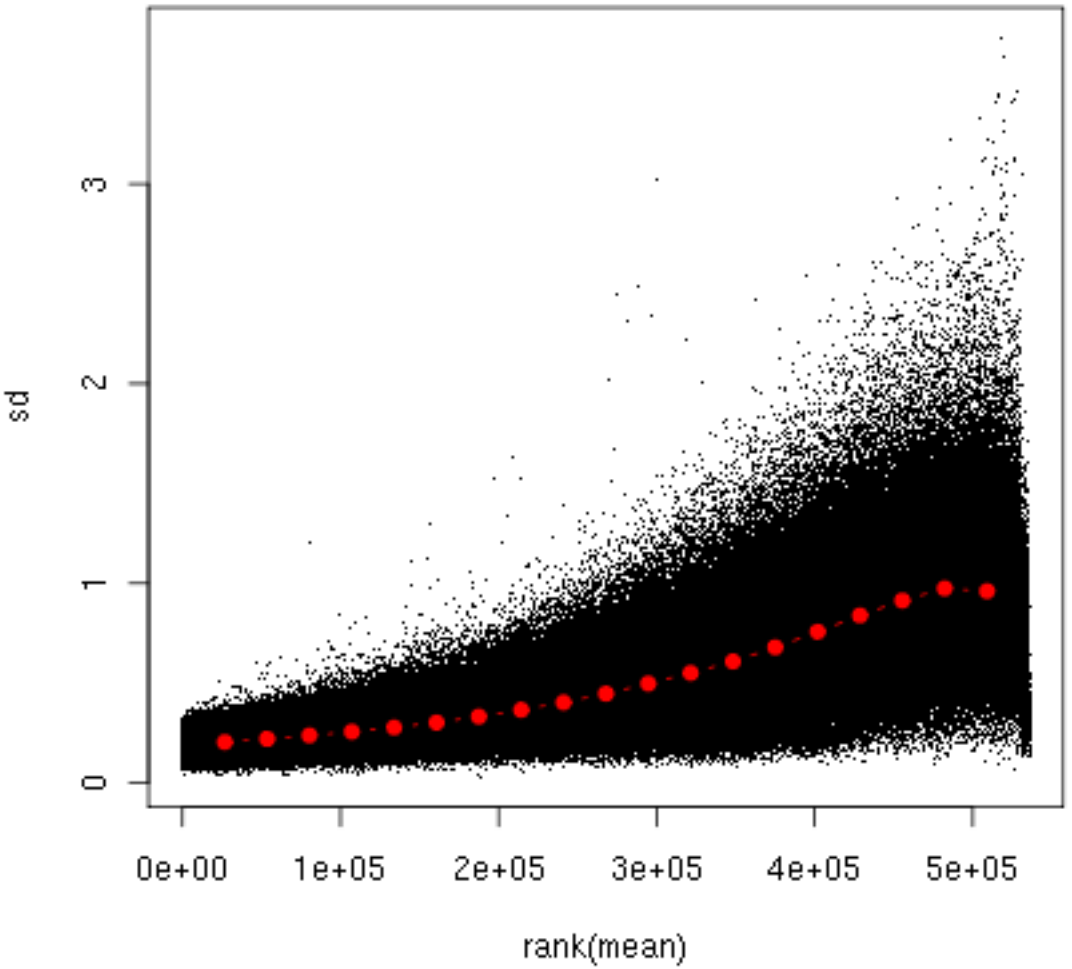


Heatmap and PCA





Variance Mean Dependency



arrayQualityMetrics report - *Outlier detection*

QMreport.html - Mozilla Firefox

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file:///home/audrey/tmp/testaqm/EMEXP886raw/QMreport.html

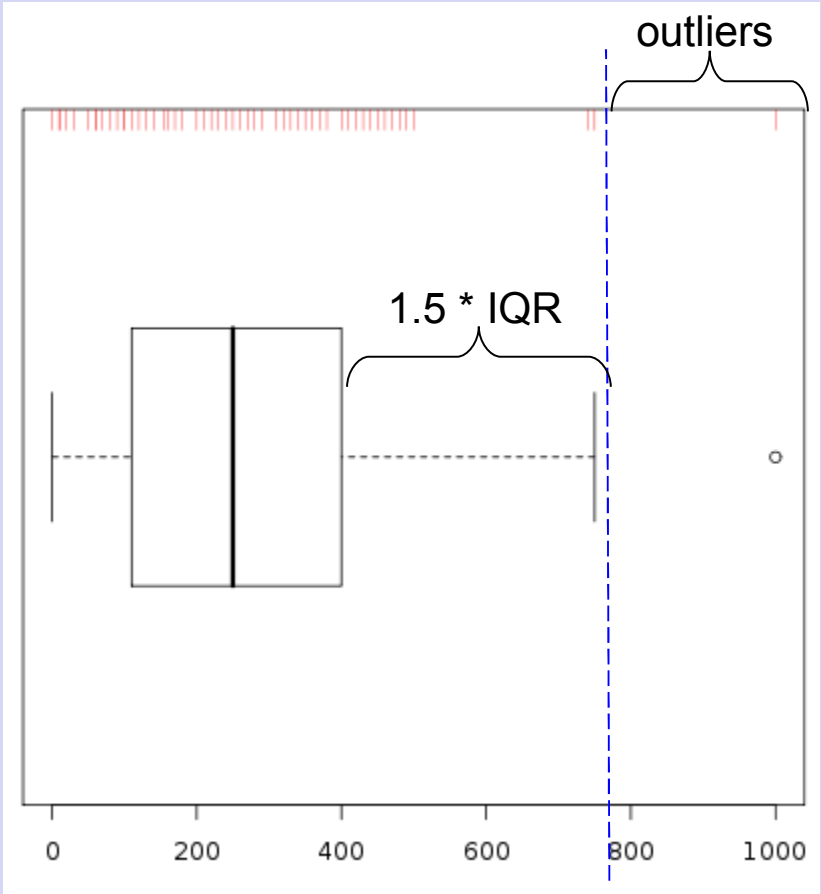
EMEXP886 quality metrics report

Summary

Array #	Array Name	MA plots	Spatial distribution	Boxplots	Heatmap	RLE	NUSE
1	E-MEXP-886-raw-cel-1227302827.cel						*
2	E-MEXP-886-raw-cel-1227302800.cel						
3	E-MEXP-886-raw-cel-1227302845.cel		*				
4	E-MEXP-886-raw-cel-1227302836.cel						
5	E-MEXP-886-raw-cel-1227302791.cel						
6	E-MEXP-886-raw-cel-1227302809.cel	*		*	*		
7	E-MEXP-886-raw-cel-1227302867.cel						
8	E-MEXP-886-raw-cel-1227302889.cel	*		*			
9	E-MEXP-886-raw-cel-1227302818.cel		*				
10	E-MEXP-886-raw-cel-1227302782.cel						

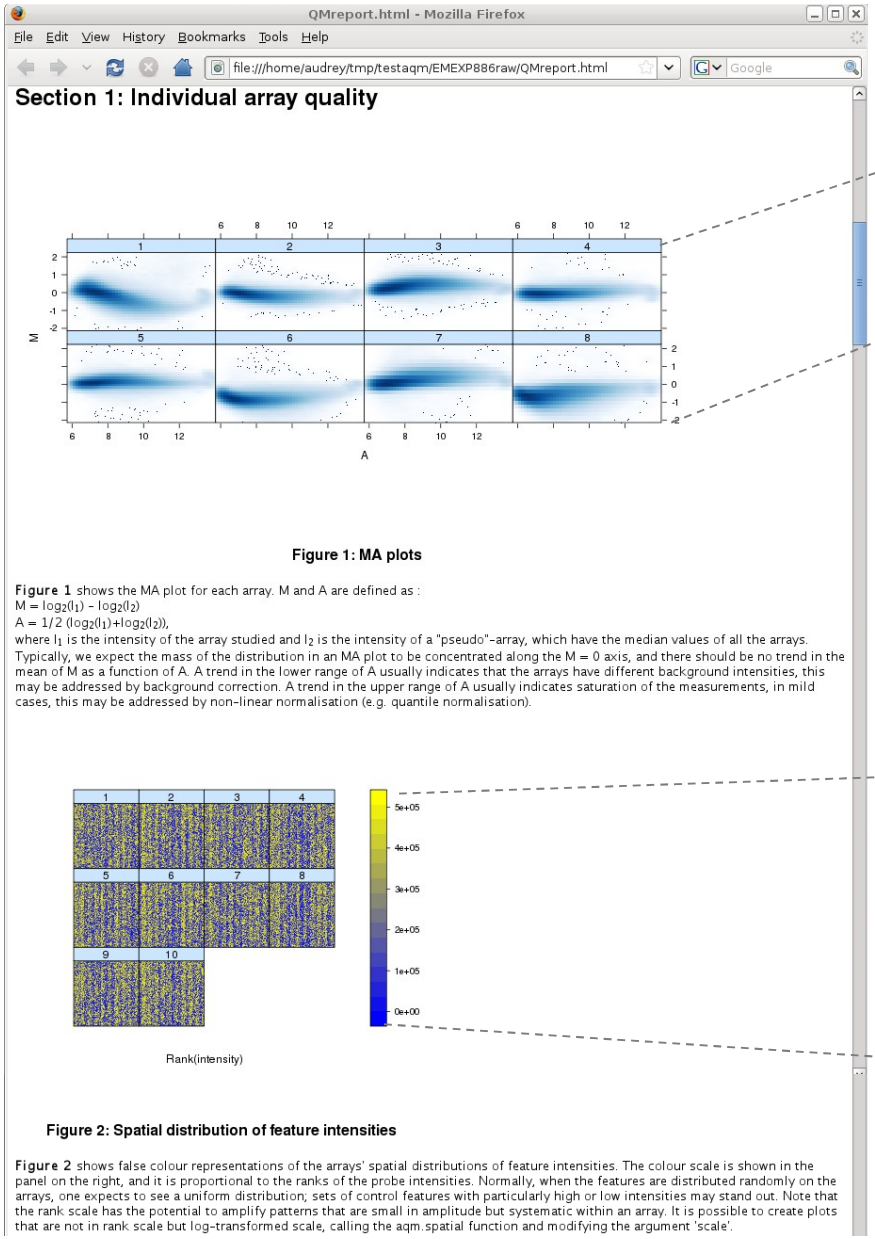
*outlier array

Index



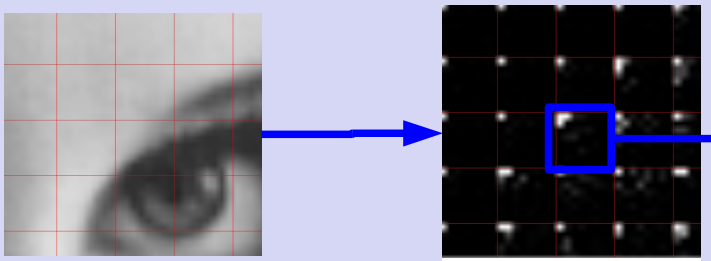
Boxplot of the scores S

arrayQualityMetrics report - *Per array*



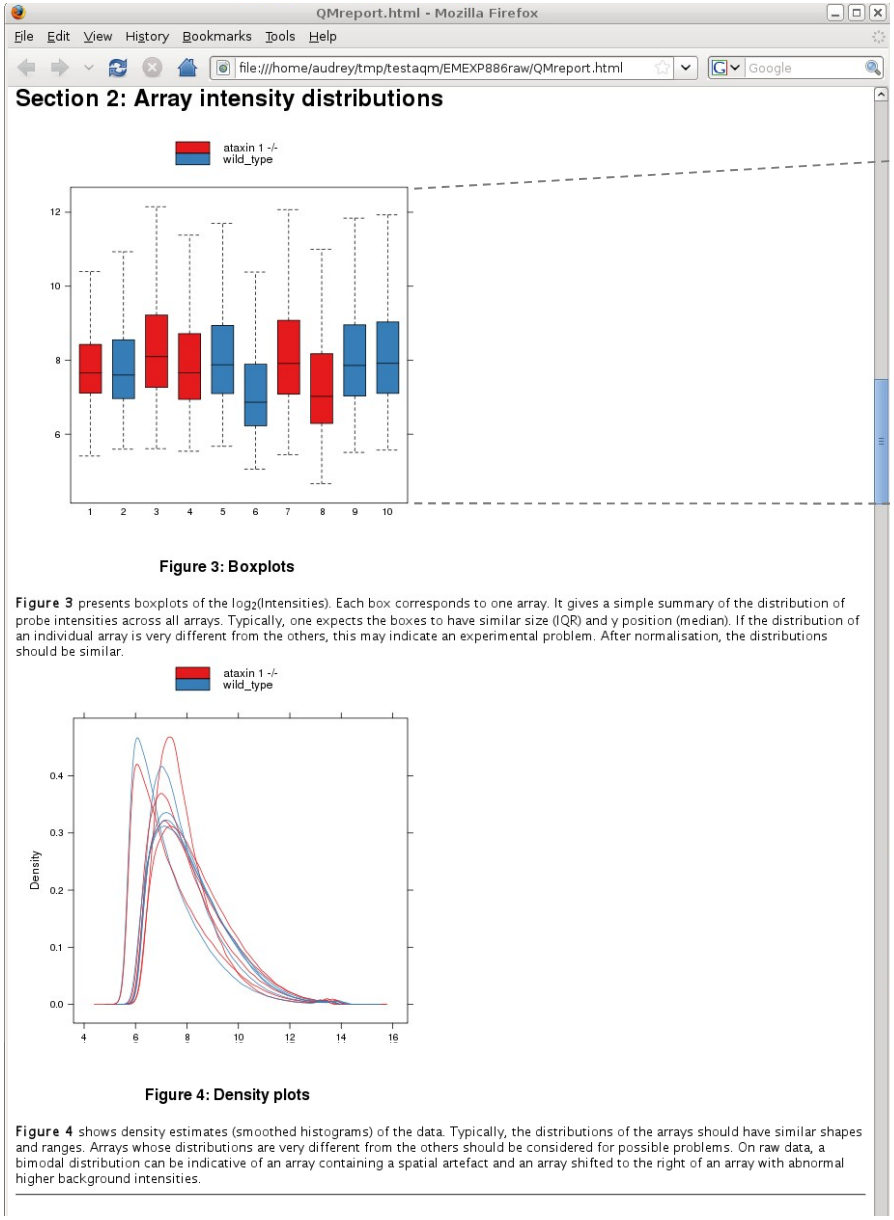
$$S_i = \sum |M_{ik}|$$

Fourier Transform



$$S_i = \frac{\sum lowFreq_{ik}}{\sum highFreq_{ik}}$$

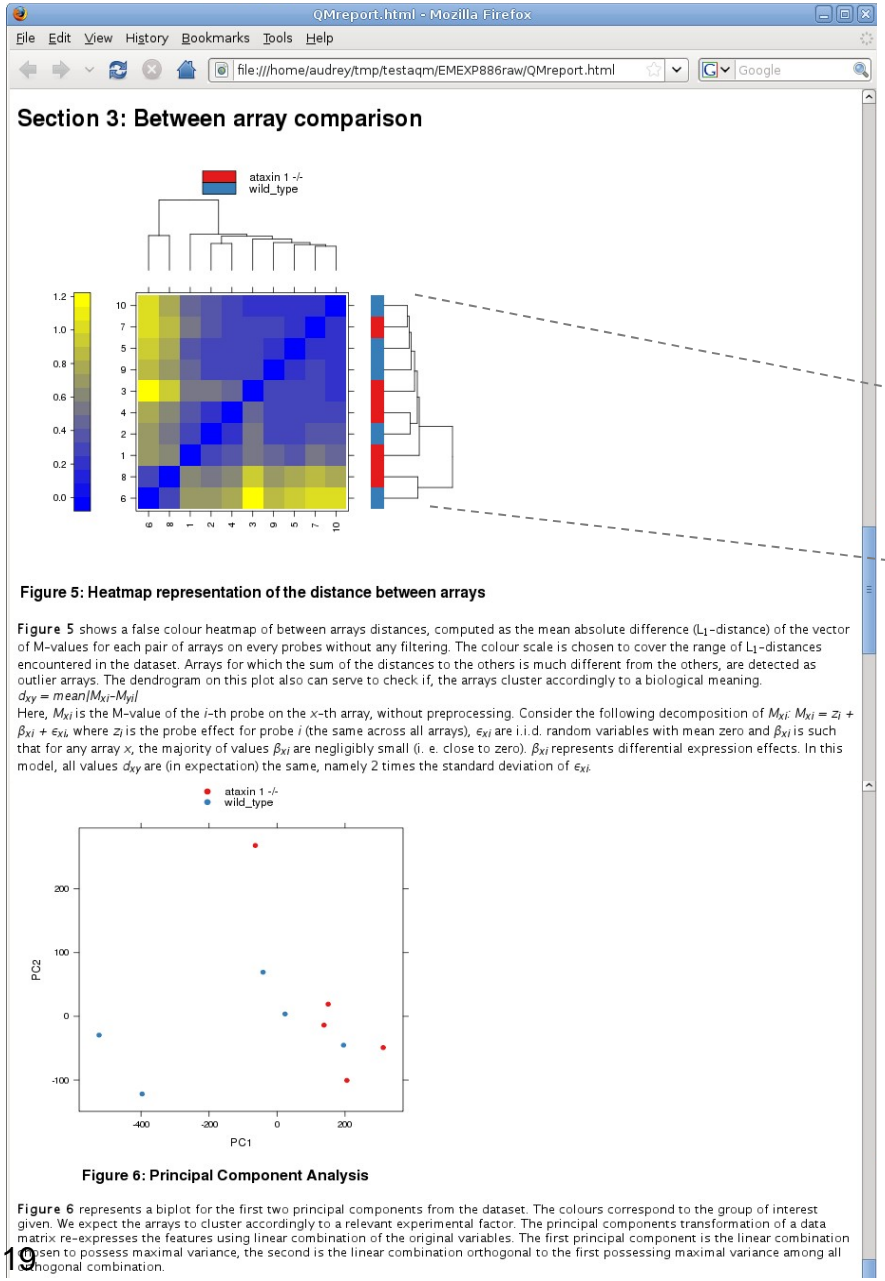
arrayQualityMetrics report - *Intensity distributions*



$$S_i = median(I_{ik})$$

$$S_i = IQR(I_{ik})$$

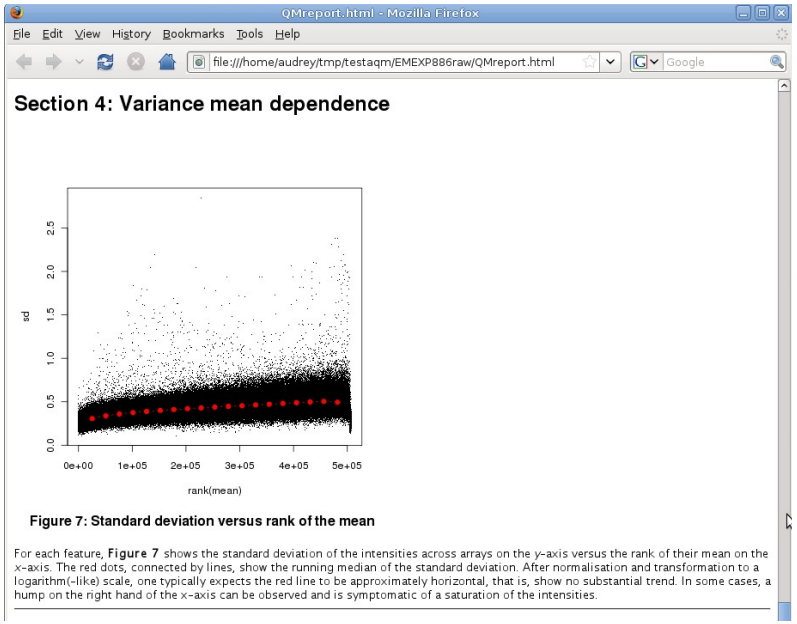
arrayQualityMetrics report - *Between arrays*



For each couple of arrays i and j, k is a probe and the distance between the arrays is:

$$d_{ij} = mean_k\left(\left| I_{ik} - I_{jk} \right|\right)$$

$$S_i = \sum d_{ij}$$



arrayQualityMetrics report - *Affymetrix plots*

QMreport.html - Mozilla Firefox

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Section 5: Affymetrix specific plots

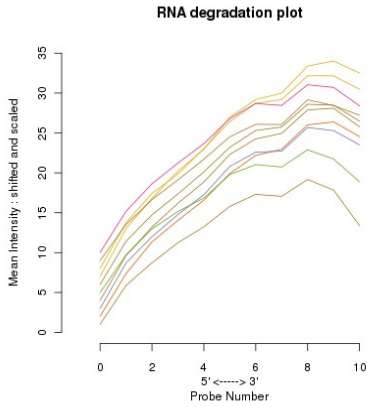


Figure 8: RNA degradation plot

In **Figure 8** , a RNA digestion plot is computed on normalised data (so that standard deviation is equal to 1). In this plot each array is represented by a single line. It is important to identify any array(s) that has a slope which is very different from the others. The indication is that the RNA used for that array has potentially been handled quite differently from the other arrays. This diagnostic plot is based on tools provided in the **affy** package.

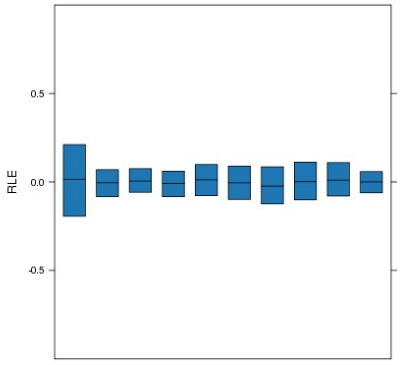


Figure 9: Relative Log Expression plot

Figure 9 is a Relative Log Expression (RLE) plot. RLE are performed on preprocessed data (background correction and quantile normalisation). An array that has problems will either have larger spread, or will not be centred at M = 0, or both. This diagnostic plot is based on tools provided in the **affyPLM** package.

QMreport.html - Mozilla Firefox

File Edit View History Bookmarks Tools Help

file:///home/audrey/tmp/testaqm/EMEXP886raw/QMreport.html

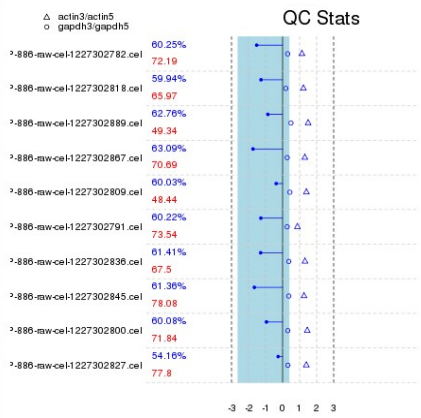


Figure 11: Diagnostic plot recommended by Affymetrix

Figure 11 represents the diagnostic plot recommended by Affymetrix. It is fully described in the package **simpleaffy**'s vignette. Any metrics that is shown in red is out of the manufacturer's specific boundaries and suggests a potential problem, any metrics shown in blue is fine.

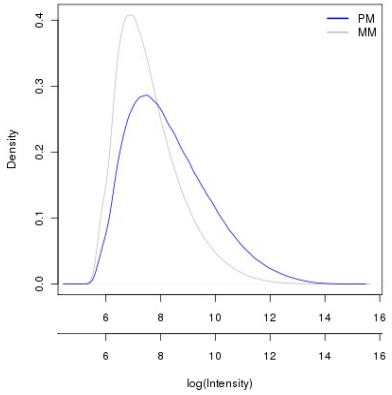
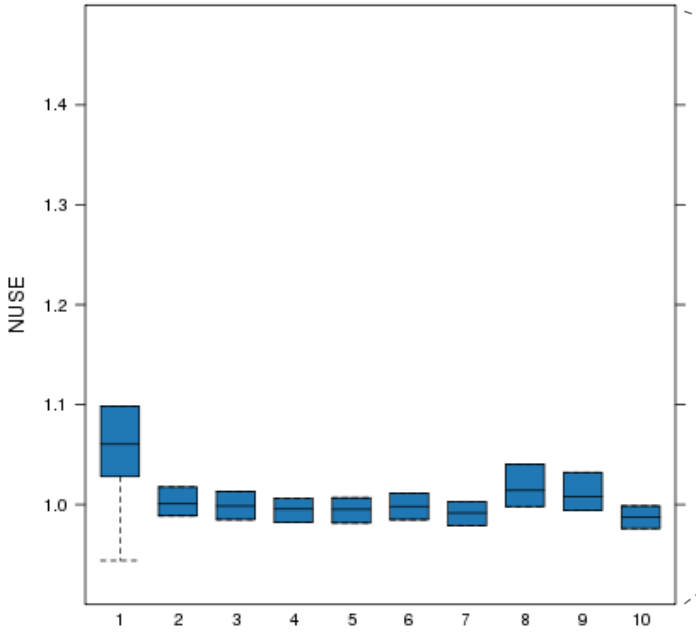


Figure 12: Perfect matches and mismatches

Figure 12 shows the density distributions of the $\log_2$ intensities grouped by the matching of the probes. Blue, density estimate of intensities of perfect match probes (PM) and grey the mismatch probes (MM). We expect that, MM probes having poorer hybridization than PM probes, the PM curve should be shifted to the right of the MM curve.

This report has been created with arrayQualityMetrics 2.0.23 under R version 2.10.0 Under development (unstable) (2009-03-31 r48256)

arrayQualityMetrics report - *Affymetrix NUSE: Normalised Unscaled Standard Error*



$$S_i = median(I_{ik})$$
$$S_i = IQR(I_{ik})$$

$$NUSE(\beta_{ik}) = \frac{SE(\beta_{ik})}{med_i(SE(\beta_{ik}))}$$

- Fitting a probe level model (gene *k*, array *i*)
- Differences in variability between genes. An array with elevated SE (standard error) relative to the other arrays is of lower quality

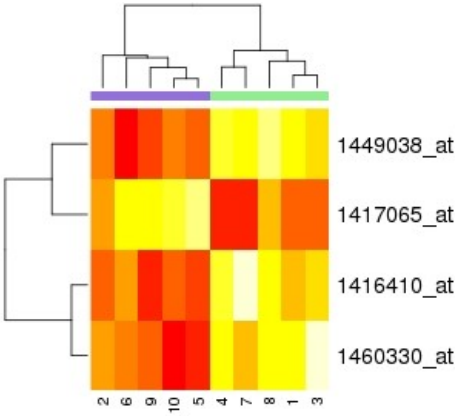
Why is outlier detection important? Example

- ArrayExpress experiment E-MEXP-886, cerebellar gene expression:
  - 5 WT mice (15 weeks of age)
  - 5 Atnx1 KO mice (15 weeks of age)
- Affymetrix MOE-430A (mouse) Genechip
- Ataxin 1 (Atxn1): protein of unknown function associated with cerebellar neurodegeneration in SpinoCerebellar Ataxia type 1 (SCA1), which impairs the of eye movement

E-MEXP-886 analysis

- Moderated t-test

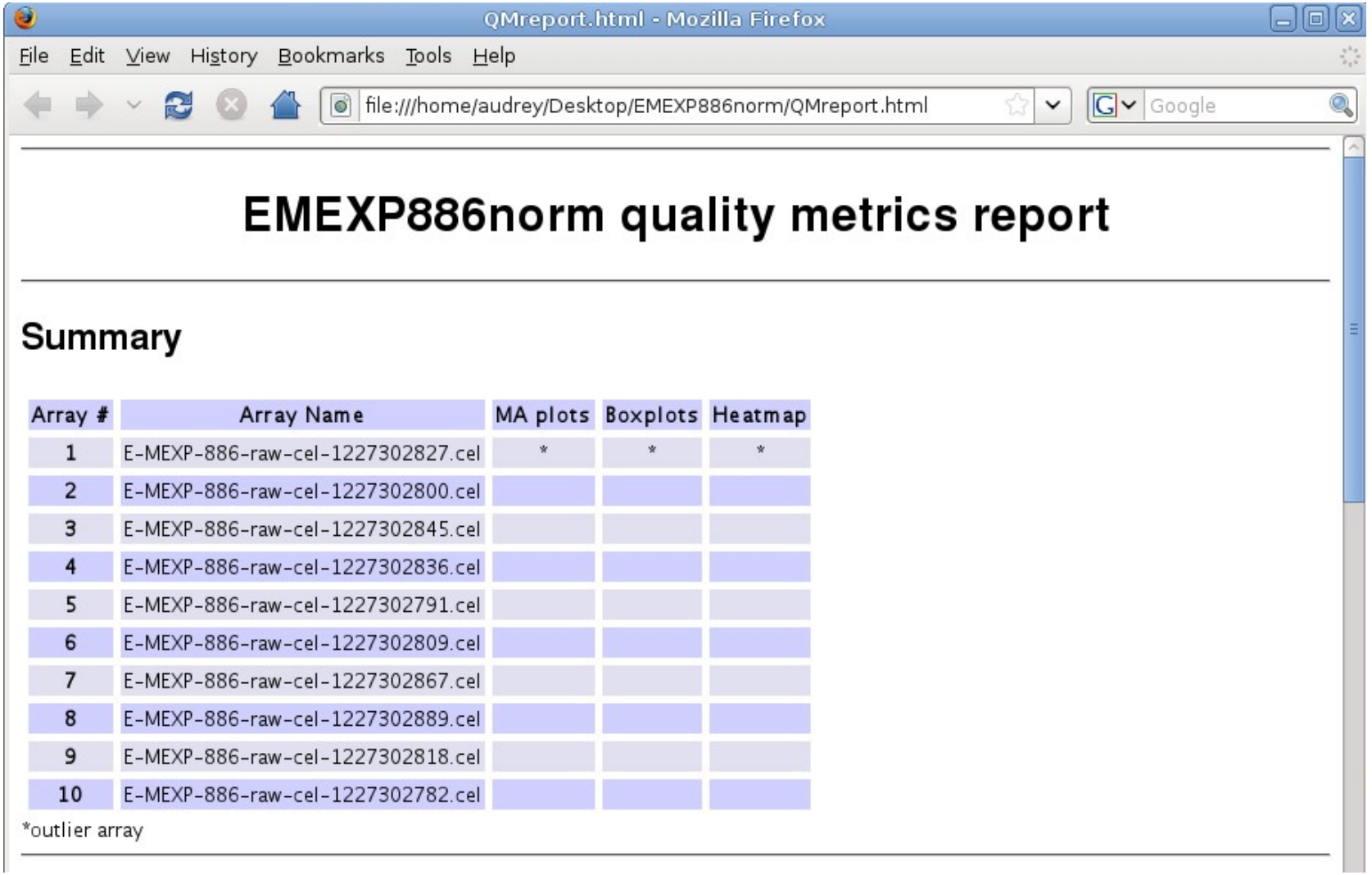
	# Genes	
	P < 0.01	P < 0.001
10 samples	34	4



- Most enriched KEGG Pathway

	Neuroactive ligand-receptor interaction	
	# Significant genes	Corrected t-value
10 samples	4	-5.65

Quality report after normalisation - *outlier detection*



EMEXP886norm quality metrics report

Summary

Array #	Array Name	MA plots	Boxplots	Heatmap
1	E-MEXP-886-raw-cel-1227302827.cel	*	*	*
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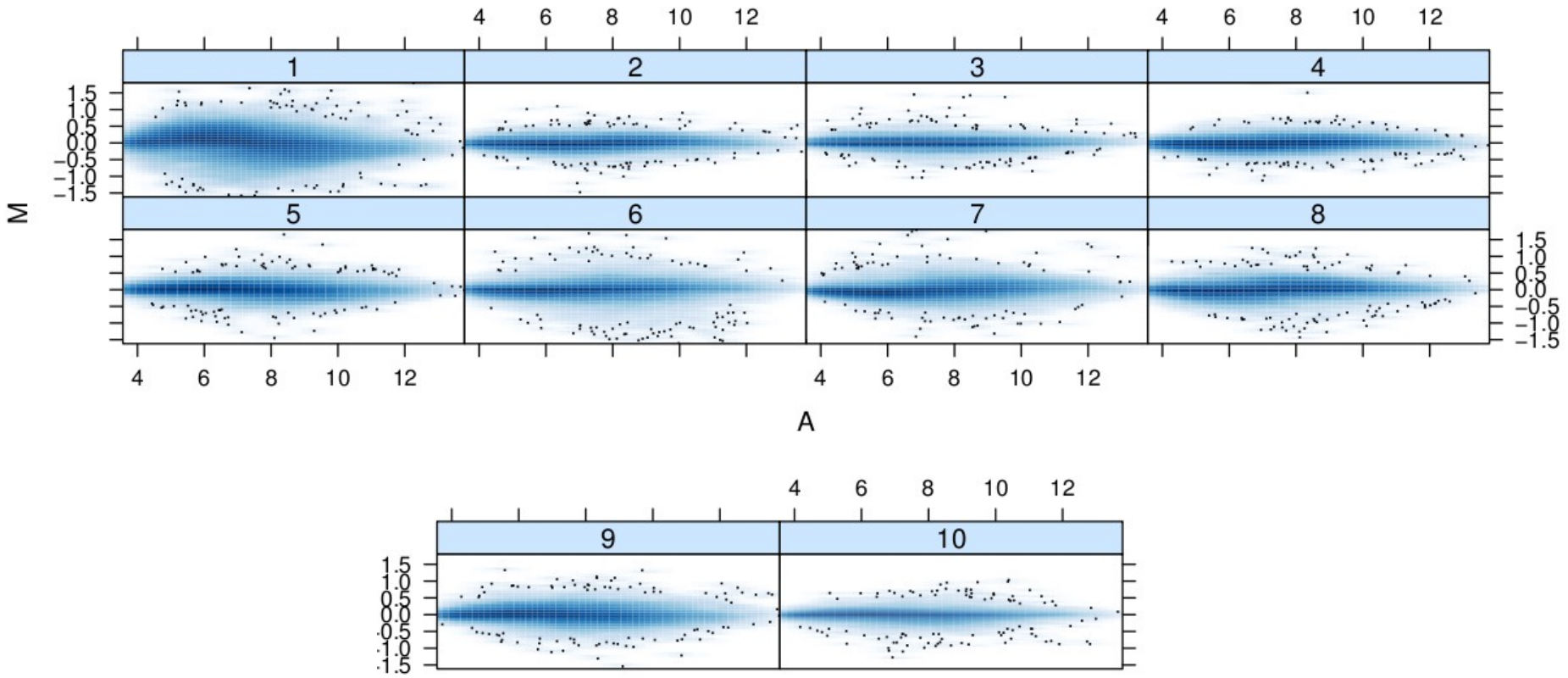
3	E-MEXP-886-raw-cel-1227302845.cel			
4	E-MEXP-886-raw-cel-1227302836.cel			
5	E-MEXP-886-raw-cel-1227302791.cel			
6	E-MEXP-886-raw-cel-1227302809.cel			
7	E-MEXP-886-raw-cel-1227302867.cel			
8	E-MEXP-886-raw-cel-1227302889.cel			
9	E-MEXP-886-raw-cel-1227302818.cel			
10	E-MEXP-886-raw-cel-1227302782.cel			

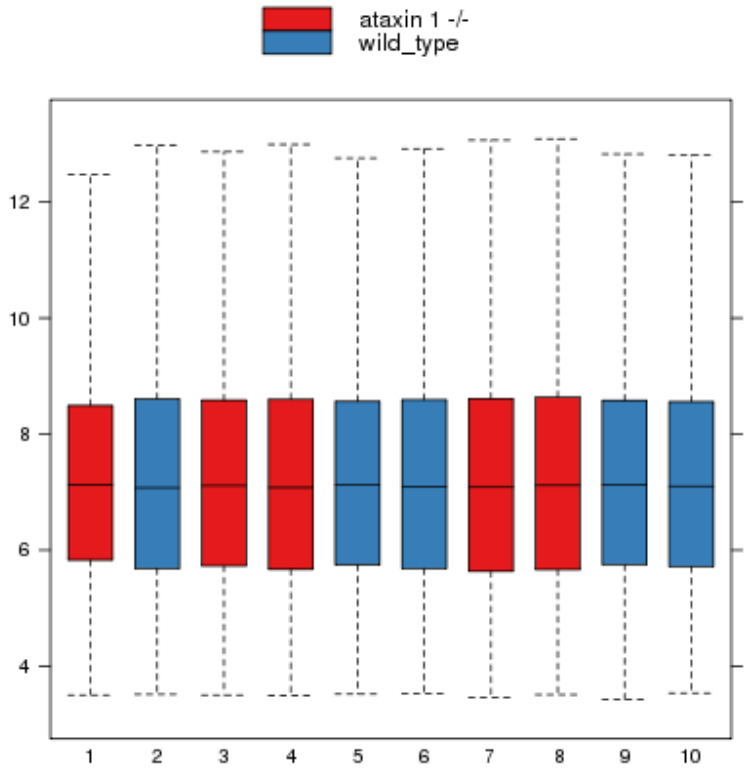
*outlier array

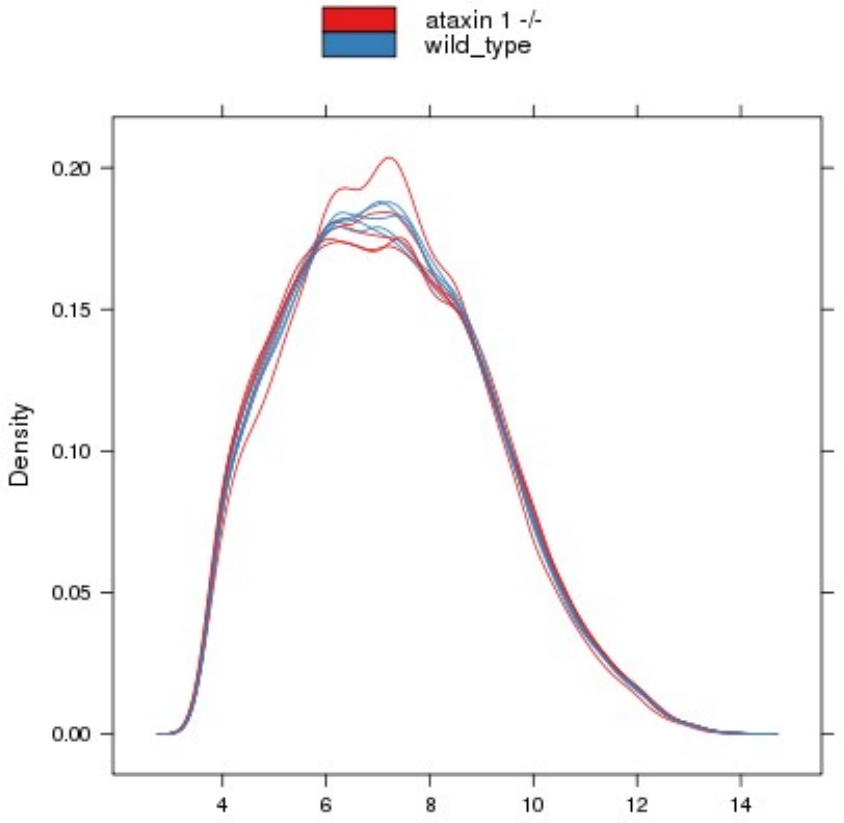
Quality report after normalisation - *per array*



- Excellent M vs A plots except for array #1

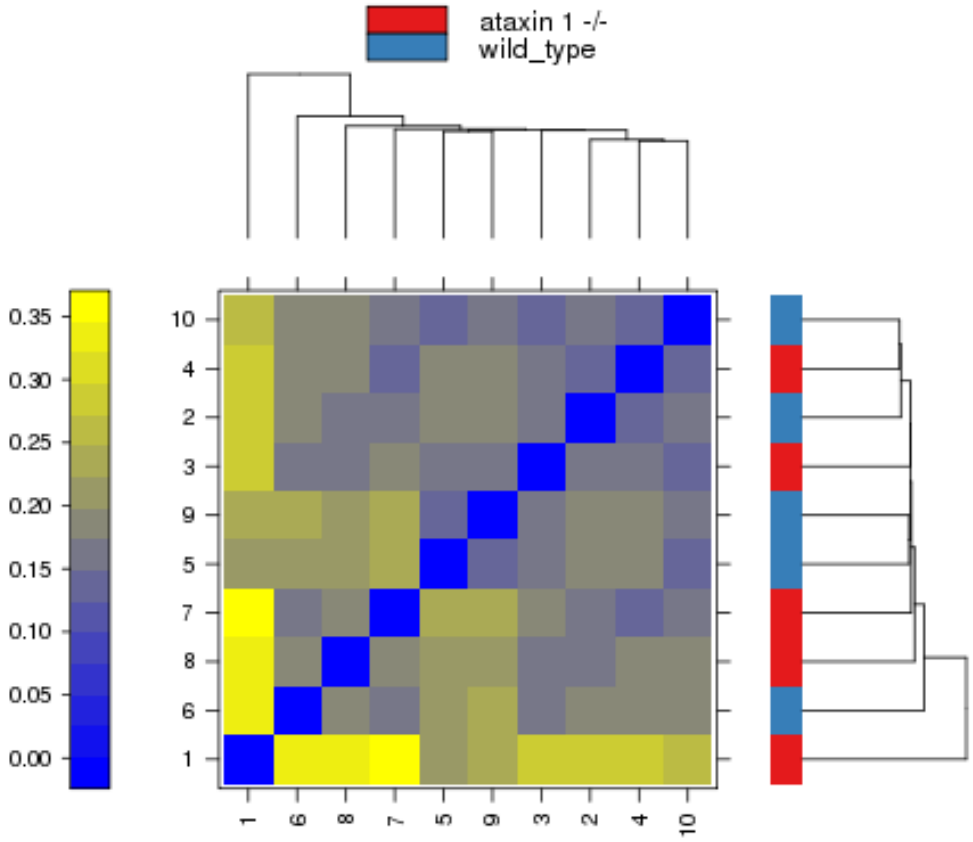
Quality report after normalisation - *intensity distributions*

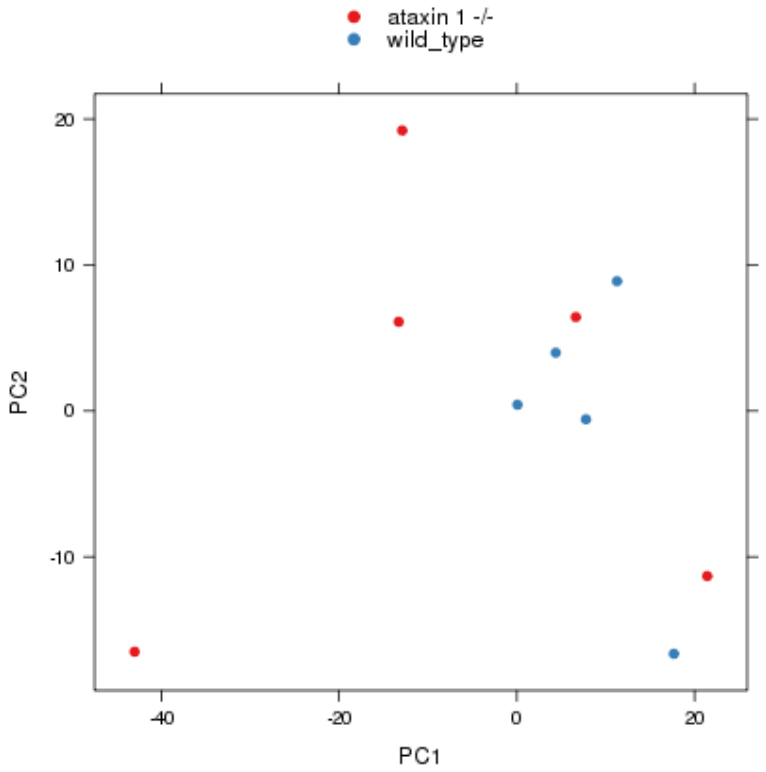




- Very homogeneous intensity distribution
- Smaller wide of the box for array #1

Quality report after normalisation - *array comparison*



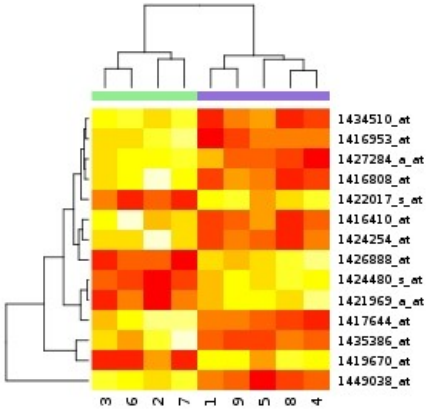


- No clustering of the samples according to biological meaning
- Array #1 further distance from all the other arrays

Outlier array's impact on results

- Moderated t-test

	# Genes	
	P < 0.01	P < 0.001
10 samples	34	4
Without array 1	190	14



- Most enriched KEGG Pathway

	Neuroactive ligand-receptor interaction	
	# Significant genes	Corrected t-value
10 samples	4	-5.65
Without array 1	23	-11.53

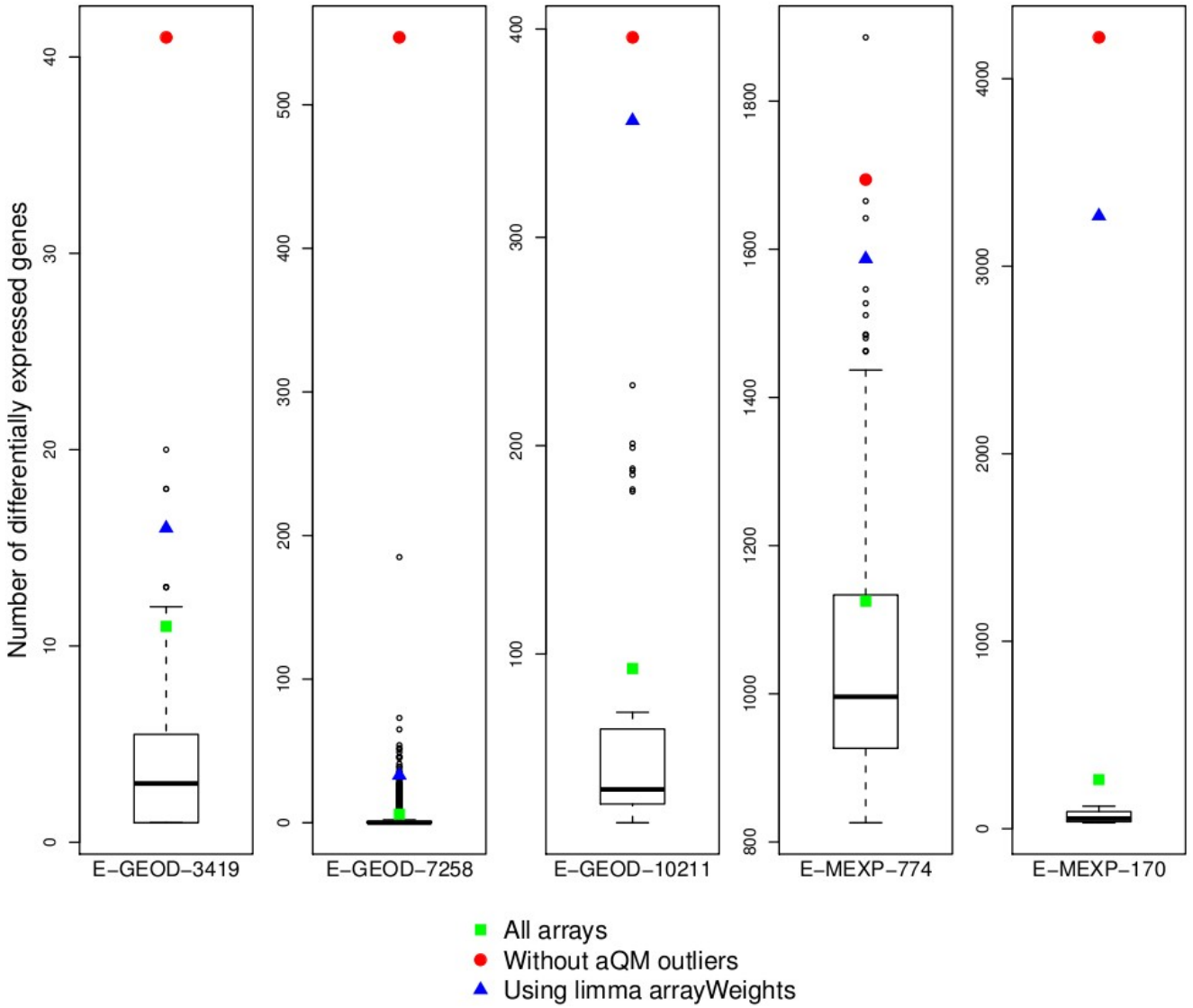
Validation

- Specific effect of array 1?

⇒ Remove one by one each of the arrays then apply a moderated t-test on the remaining samples

	# Genes	
	P < 0.01	P < 0.001
10 samples	34	4
Without sample 1	**190**	**14**
Without sample 2	39	3
Without sample 3	29	2
Without sample 4	21	1
Without sample 5	12	1
Without sample 6	87	5
Without sample 7	23	4
Without sample 8	34	4
Without sample 9	17	2
Without sample 10	23	2

Importance of outlier detection



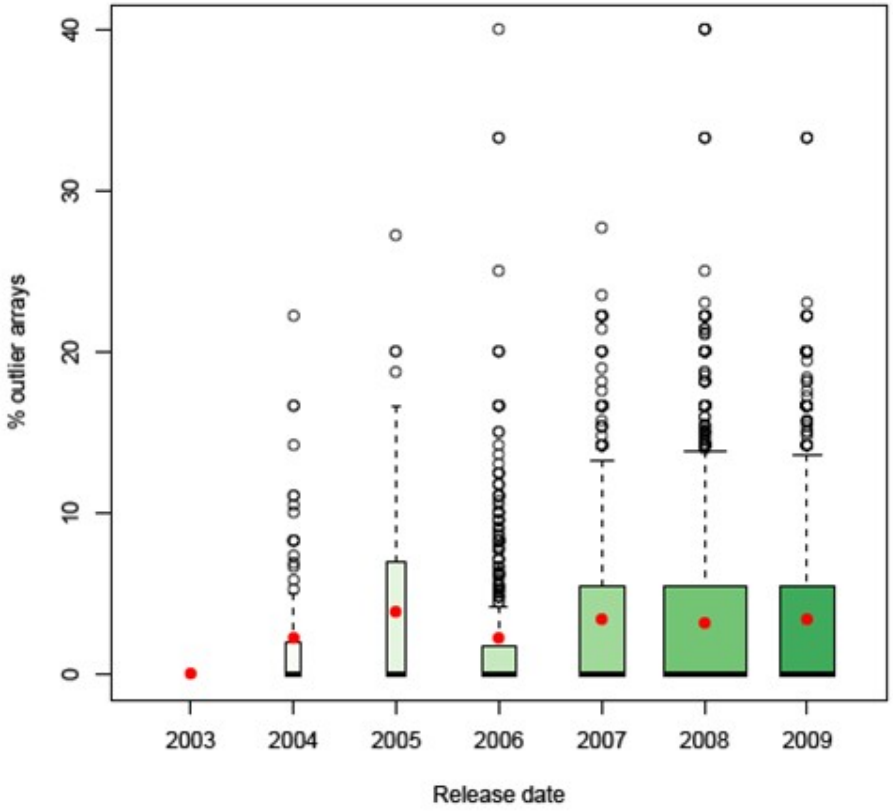
Importance of outlier detection

Pathway name	Genes	p-value when removing outliers	p-value when all arrays
E-GEOD-3419			
Pyrimidine metabolism	37	$<10^{-3}$	0.701
Base excision repair	17	0.001	0.542
DNA replication	19	0.003	0.451
Cell cycle	69	0.009	0.387
TGF-beta signaling pathway	48	0.009	0.558
E-GEOD-7258			
Pentose phosphate pathway	13	0.003	0.588
Fructose and mannose metabolism	28	0.003	0.326
Biosynthesis of steroids	20	0.003	0.012
Oxidative phosphorylation	44	0.003	0.299
Starch and sucrose metabolism	16	0.003	0.317

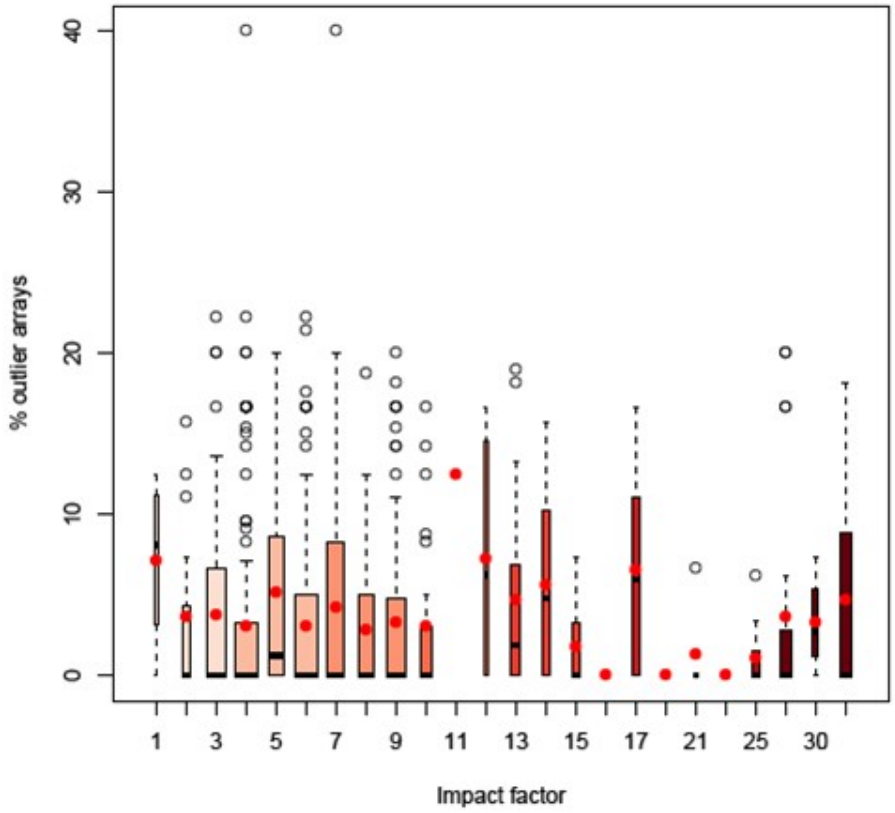
Application to the ArrayExpress database

- ArrayExpress: public repository for microarray data
- Store MIAME-compliant data in accordance with MGED recommendations: MAGE-TAB format
- http://www.ebi.ac.uk/microarray-as/aer
- Build R objects from 7000 datasets using ArrayExpress package
- Run arrayQualityMetrics on these datasets
- Study Array-Distance (heatmap) and NUSE outliers

Outliers per year



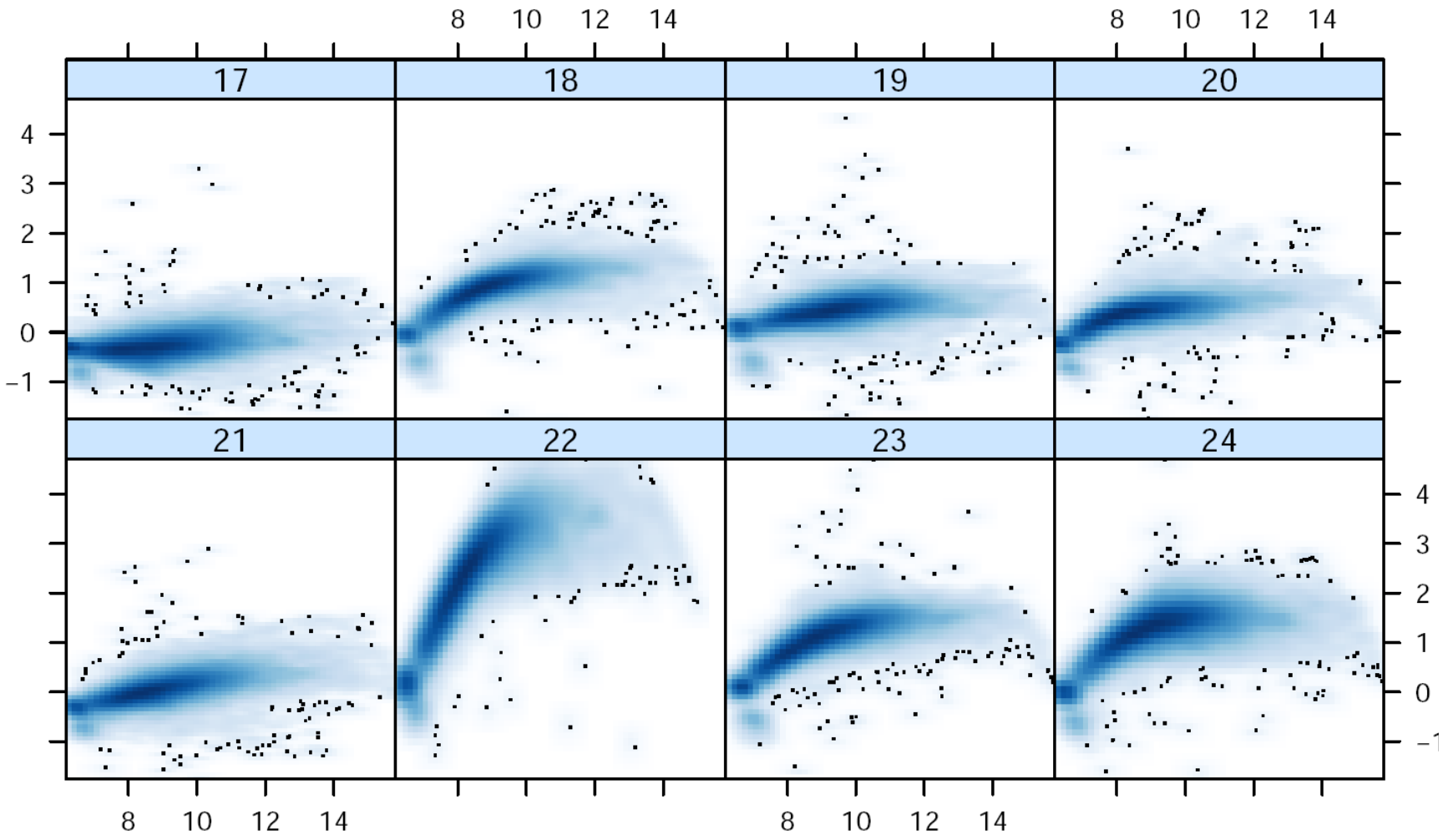
Outliers per impact factor

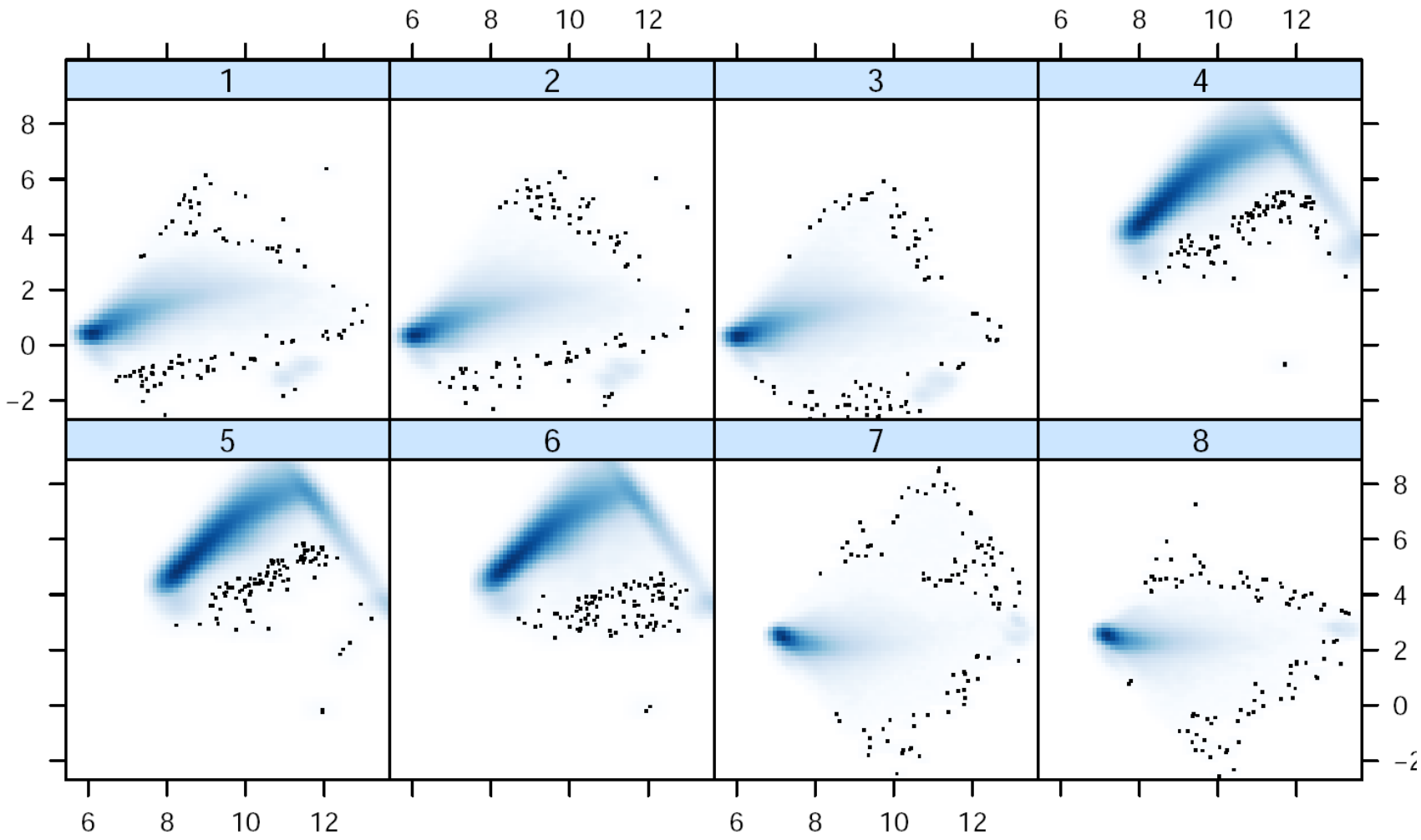


Conclusions

- Quality assessment is important
  - Still needed
  - First “taste” of the data
  - Removing outliers increases statistical power and biological significance
- arrayQualityMetrics
  - Before preprocessing: to choose preprocessing methods
  - After normalisation: to check preprocessing efficiency
  - Comprehensive report
  - Outlier detection
  - Used on any kind of expression array

Horror Picture Show

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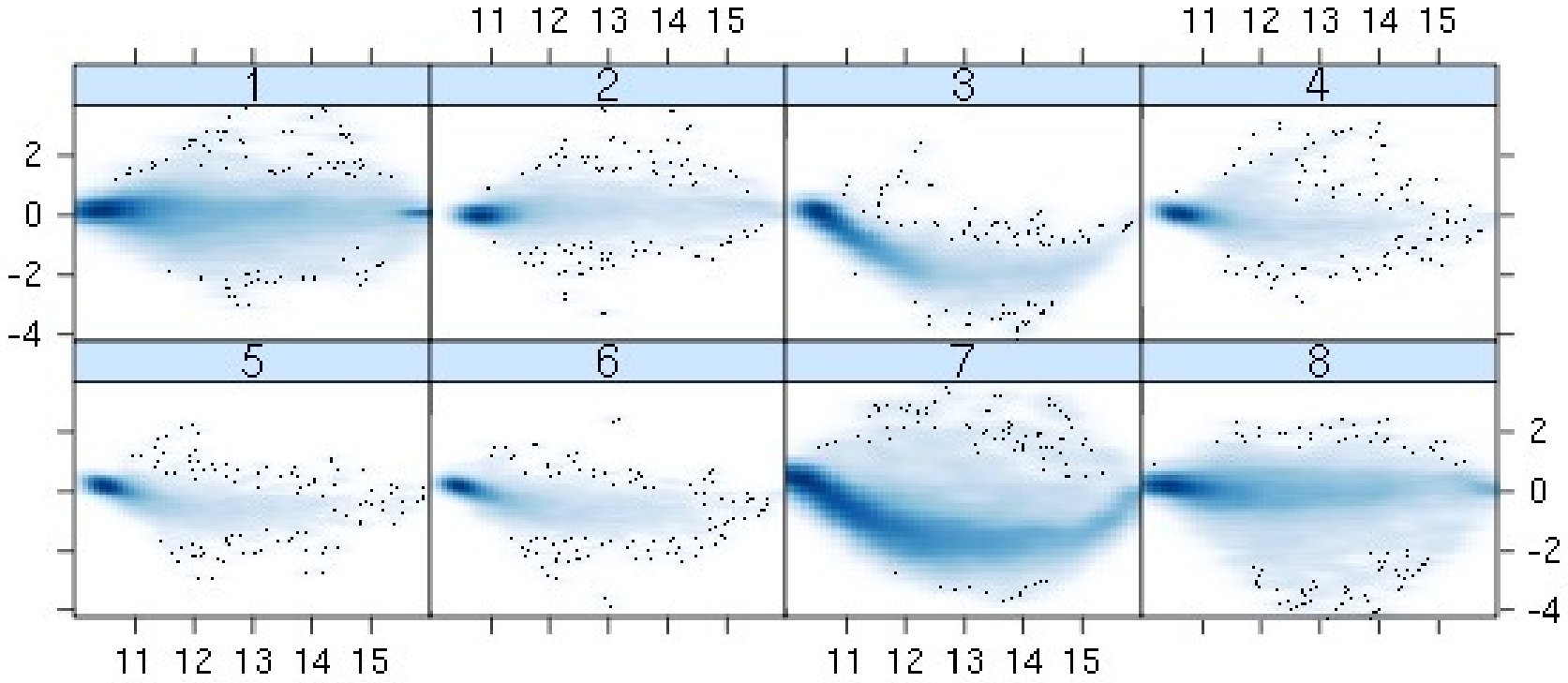
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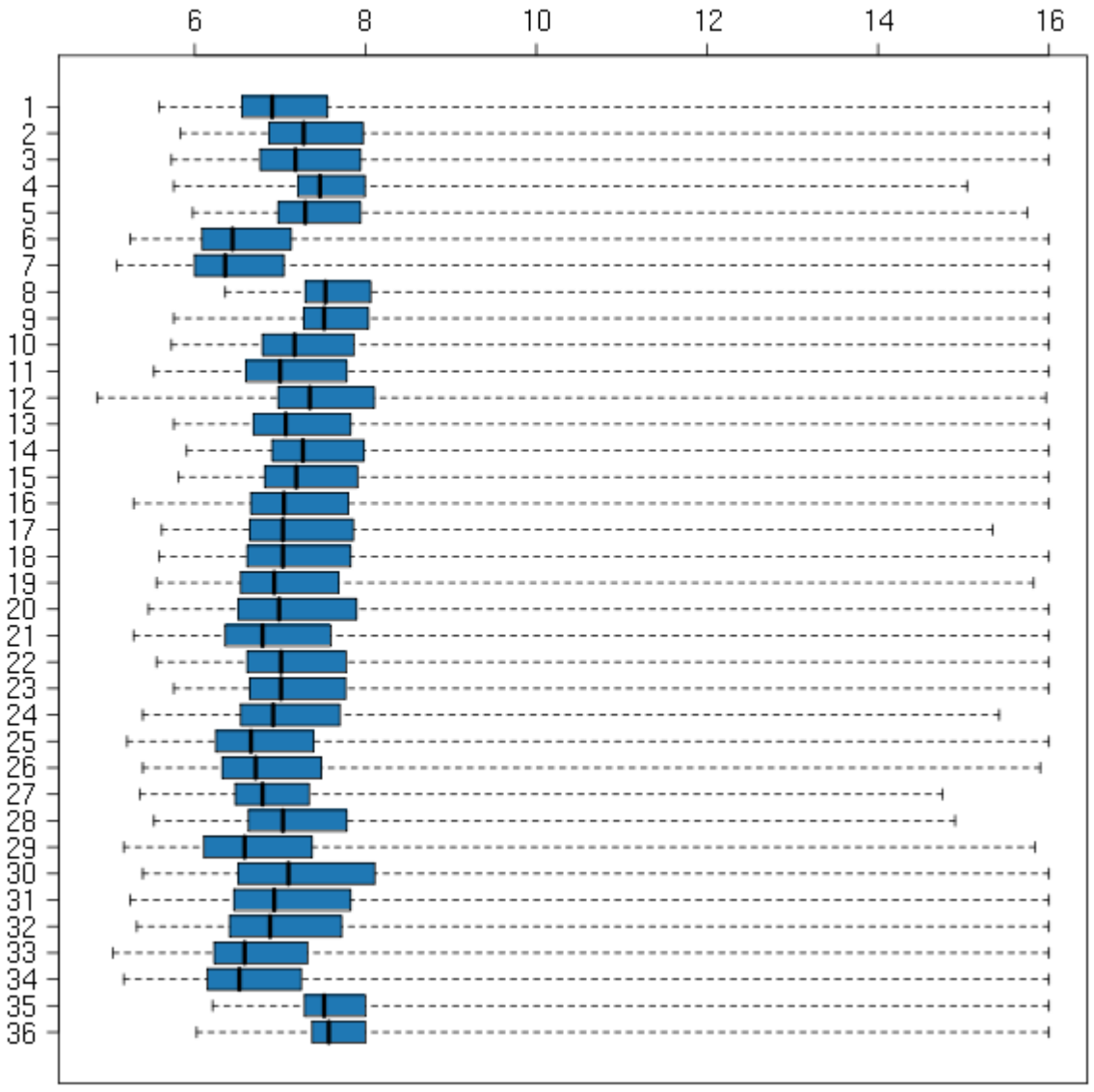
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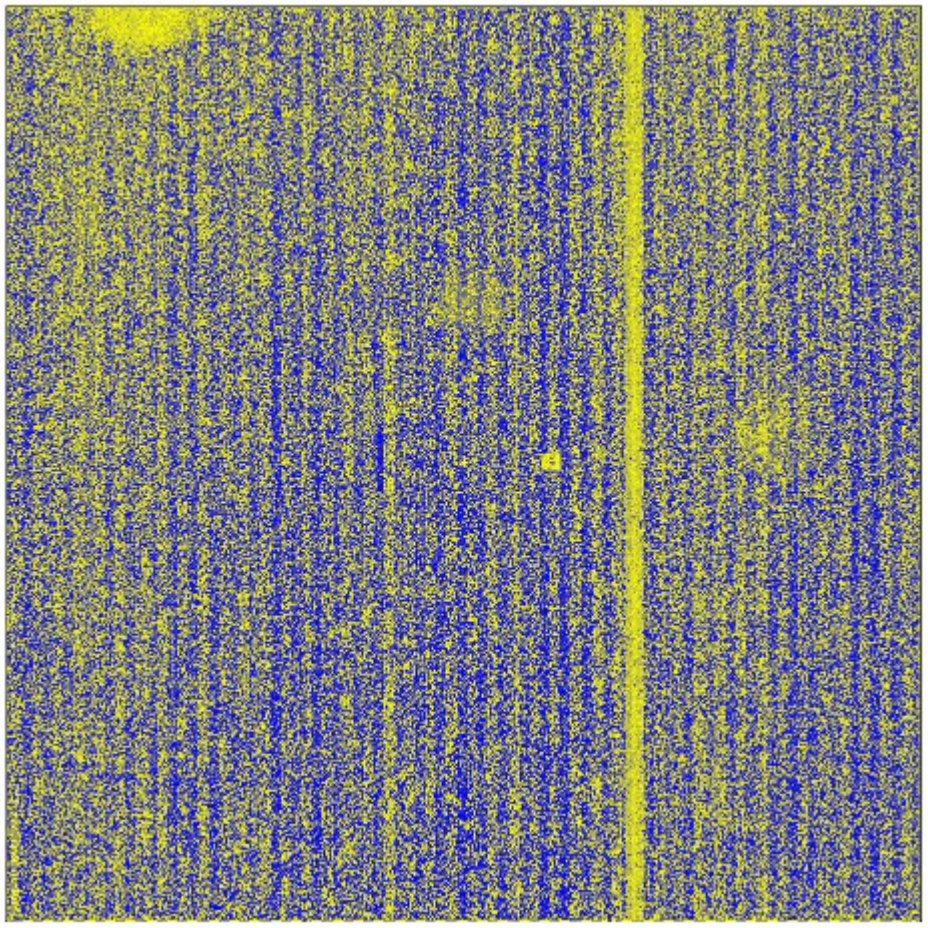

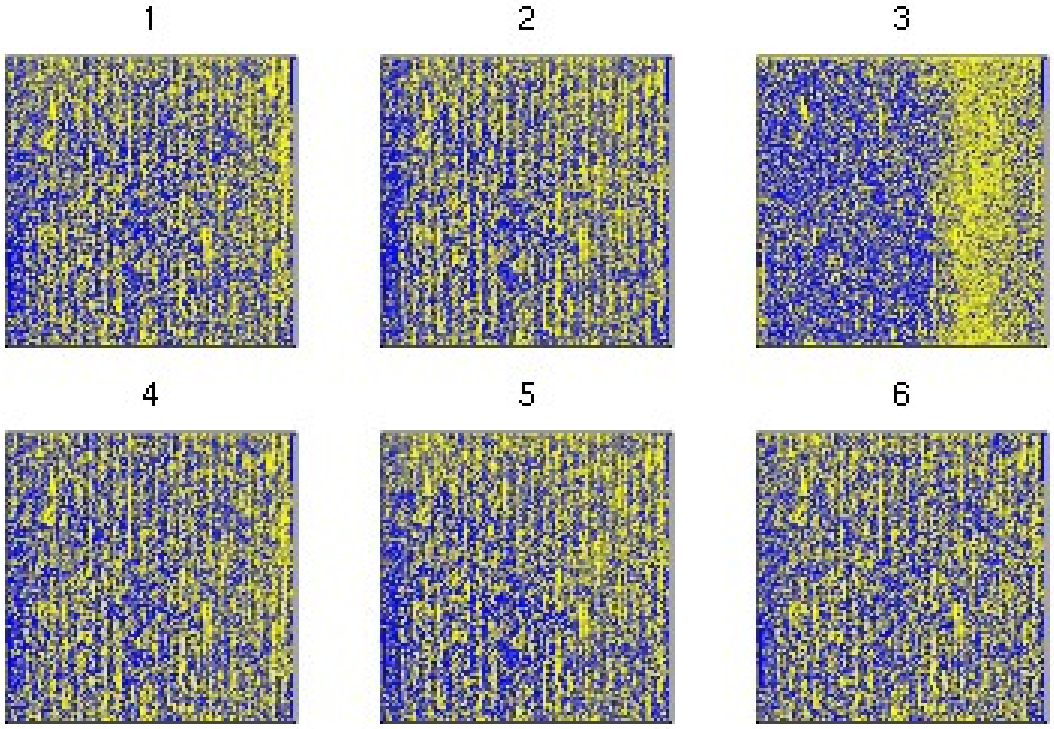
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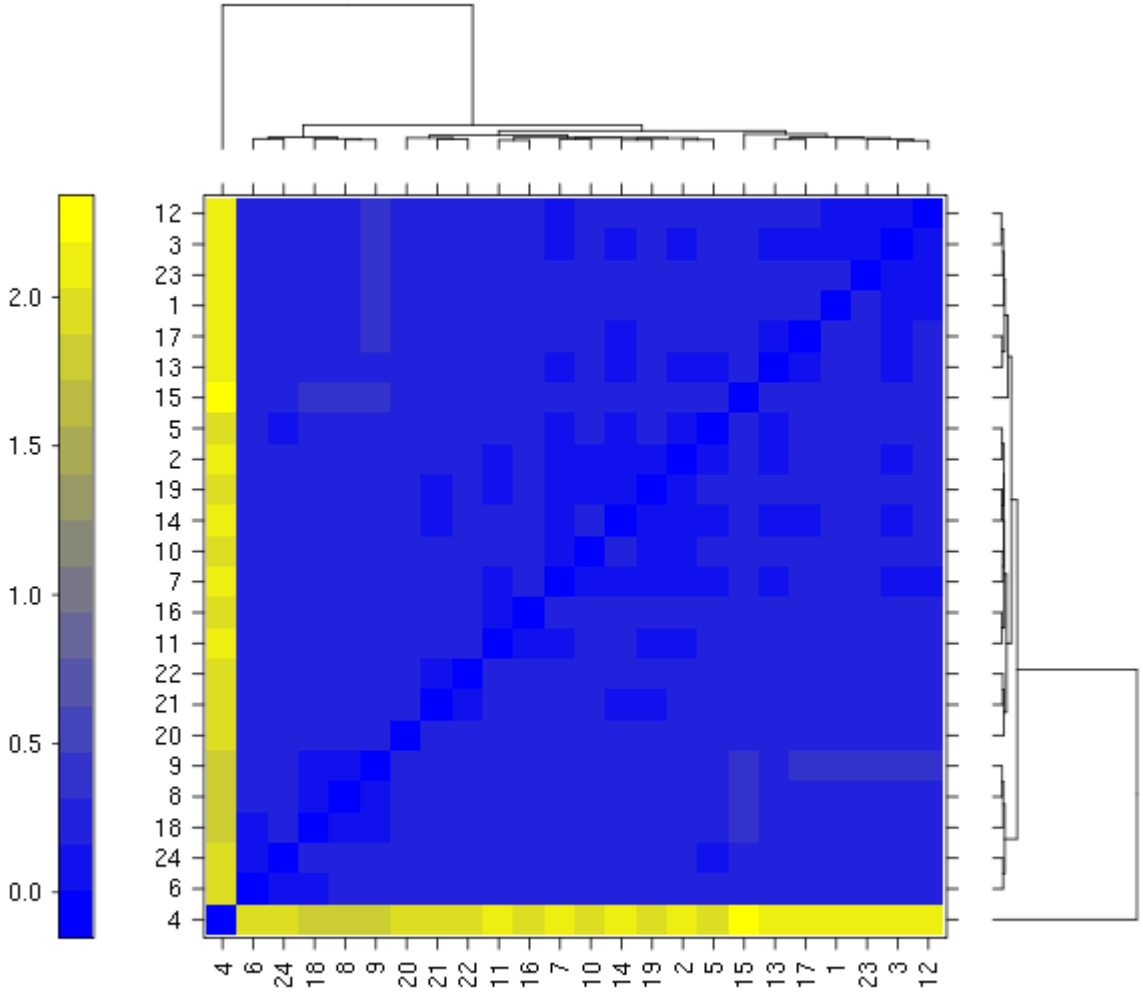
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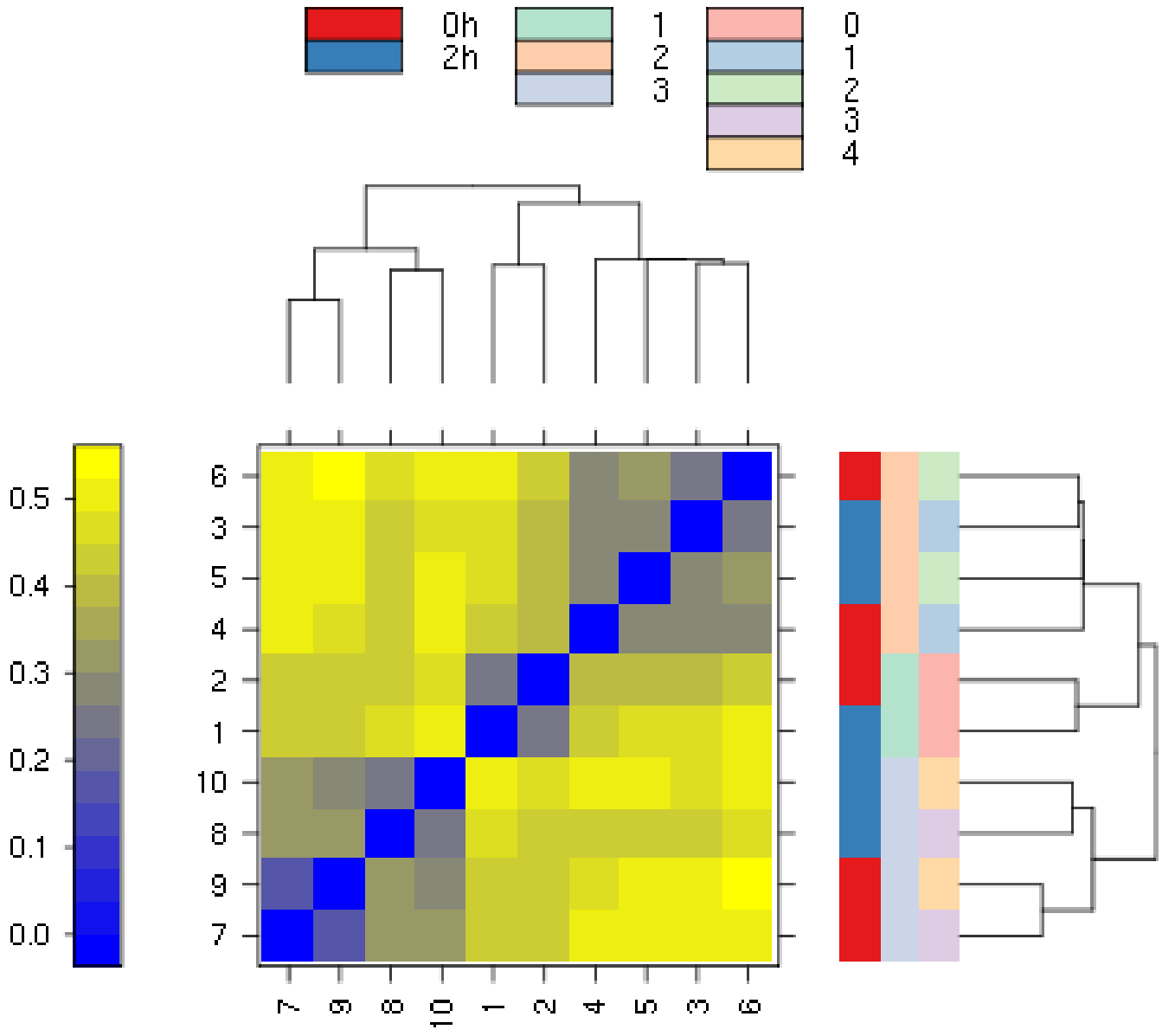
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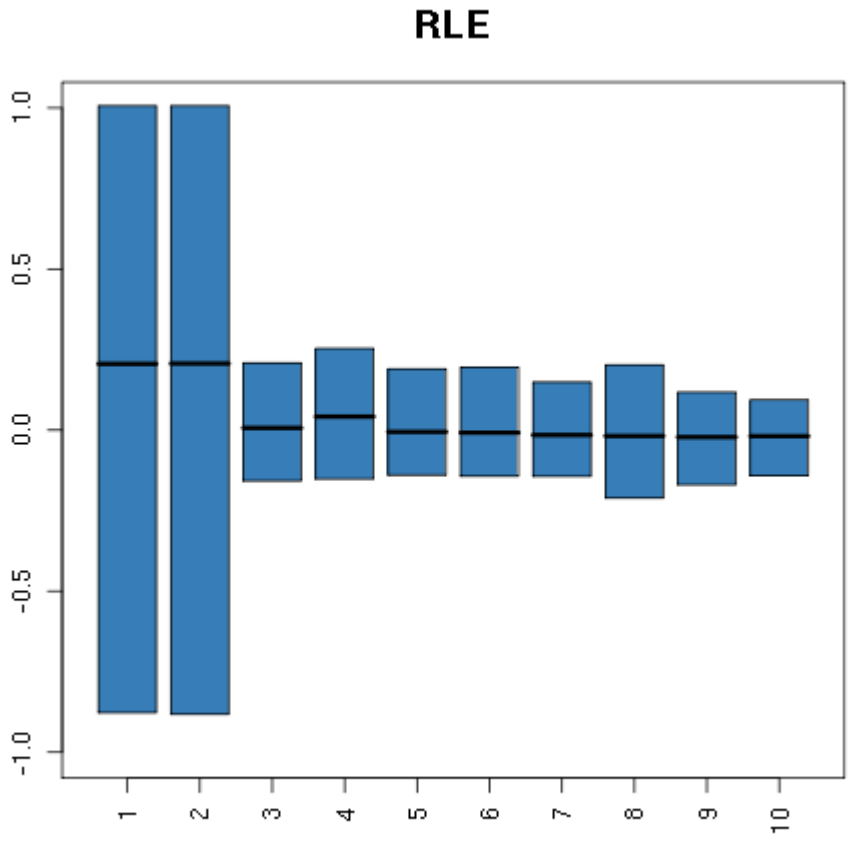
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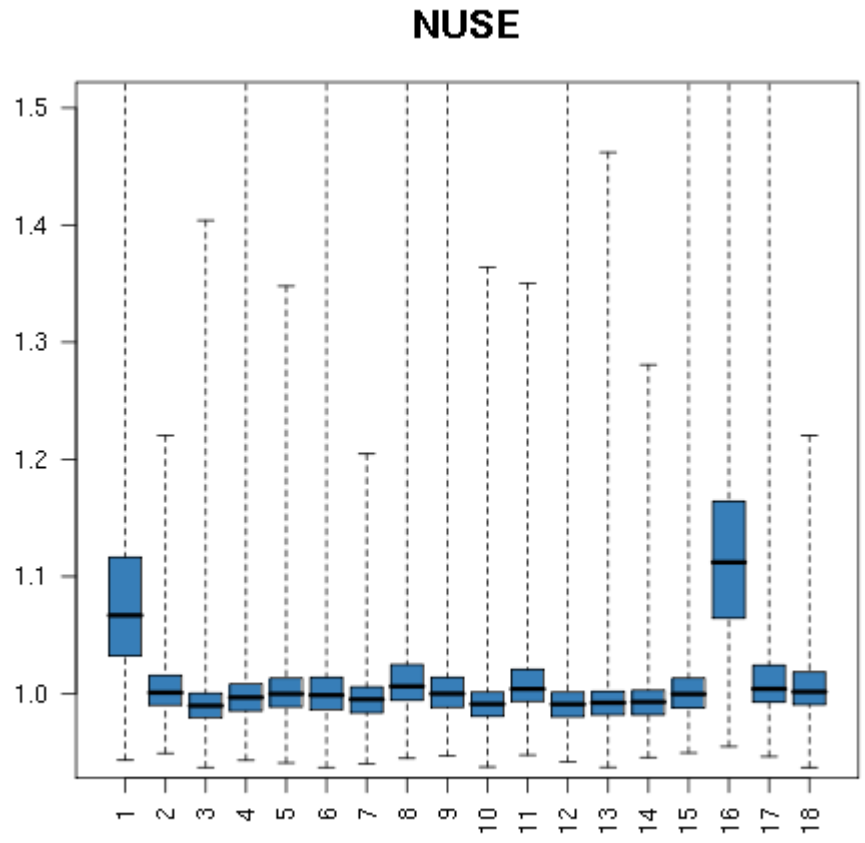
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