Part IV. Object Oriented Programming

`Biobase`, `affy`, and `marrayXXX` packages

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`Biobase` package

- The `Biobase` package provides class definitions and other infrastructure tools that will be used by other packages.
- The two important classes defined in `Biobase` are
  - `phenoData`: sample level covariate data.
  - `exprSet`: the sample level covariate data combined with the expression data and a few other quantities of interest.

`exprSet` class

Slots for the **`exprSet`** class

- **`exprs`**: a matrix of expression measures, genes are rows, samples are columns.
- **`se.exprs`**: standard errors for the expression measures, if available.
- **`phenoData`**: an object of class **`phenoData`** that describes the target samples.
- **`annotation`**: a character vector.
- **`description`**: an object of class `MIAME`
  `notes`: a character vector.

exprSet class

exprs	Matrix of expression measures, genes x samples
se.exprs	Matrix of SEs for expression measures
phenoData	Sample level covariates, instance of class **phenoData**
annotation	Name of annotation data
description	MIAME information
notes	Any notes

`exprSet` class

- One of the most important tasks is to **align** the expression data and the phenotypic data (and to keep that alignment through the analysis).

- To achieve this, the **`exprSet`** class combines these two data sources into one object, and provides subsetting and access methods that make it easy to manipulate the data while ensuring that they are correctly aligned.

`exprSet` class

- A design principle that was adopted for the **`exprSet`** and other classes was that they should be closed under the subset operation.
- So any subsetting, either of rows or columns, will return a valid **`exprSet`** object.
- This makes it easier to use **`exprSet`** in other software packages

exprSet class

Some methods for the **exprSet** class

- **show** controls the printing (you seldom want a few hundred thousand numbers rolling by).
- subset, **[** and **$**, are both designed to keep correct subsets of the **exprs**, **se.exprs**, and **phenoData** objects.
- **split** splits the **exprSet** into two or more parts depending on the vector used for splitting.

`exprSet` class

- **`geneNames`**, retrieves the gene names (row names of **`exprs`**).

- **`phenoData`**, **`pData`**, and **`sampleNames`** provide access to **`phenoData`** slots.

- **`write.exprs`** writes the expression values to a file for processing or storage.

`phenoData` class

Slots for the **`phenoData`** class

- **`pData`**: a dataframe, where the samples are rows and the variables are columns (this is the standard format).

- **`varLabels`**: a vector containing the variable names (as they appear in **`pData`**) and a longer description of the variables.

`phenoData` class

- Methods for the **`phenoData`** class include
  - `[`, the subset operator, this method ensures that when a subset is taken, both the **`pData`** and the **`varLabels`** objects have the appropriate subsets taken.
  - `$`, extracts the appropriate column of the **`pData`** slot (as for a dataframe).
  - **`show`**, a method to control printing, we show only the **`varLabels`** (and the size).

`Biobase` package

- The data package **`golubEsets`** contains instances of the **`exprSet`** class for the ALL AML study of Golub et al. (1999).
- Try

```
library(golubEsets)
data(golubTrain)
show(golubTrain)
golubTrain[1:100,1:4]
pData(golubTrain)
```

Pre-processing cDNA microarray data

- **marrayClasses**:
  - class definitions for cDNA microarray data;
  - basic methods for manipulating microarray objects: printing, plotting, subsetting, class conversions, etc.
- **marrayInput**:
  - reading in intensity data and textual data describing probes and targets;
  - automatic generation of microarray data objects;
  - widgets for point & click interface.
- **marrayPlots**: diagnostic plots.
- **marrayNorm**: robust adaptive location and scale normalization procedures.

`marrayClasses` package

- Based on *Minimum Information About a Microarray Experiment - MIAME -* document.
- Microarray classes should represent
  - – gene expression measures
    - scanned images, i.e., raw data,
    - image quantitation data, i.e., output from image analysis,
    - normalized expression levels, i.e., log-ratios M;
  - – reliability information for these measurements;
  - – information on the probe sequences spotted on the arrays;
  - – information on the target samples hybridized to the arrays.

marrayLayout class

Array layout parameters

maNspots	Total number of spots
maNgr maNgc	Dimensions of grid matrix
maNsr maNsc	Dimensions of spot matrices
maSub	Current subset of spots
maPlate	Plate IDs for each spot
maControls	Control status labels for each spot
maNotes	Any notes

marrayRaw class

Pre-normalization intensity data for a batch of arrays

maRf	maGf	Matrix of red and green foreground intensities
maRb	maGb	Matrix of red and green background intensitie
maW		Matrix of spot quality weights
maLayout		Array layout parameters - **marrayLayout**
maGnames		Description of spotted probe sequences - **marrayInfo**
maTargets		Description of target samples - **marrayInfo**
maNotes		Any notes

marrayNorm class

Post-normalization intensity data for a batch of arrays

maA	Matrix of average log-intensities, A
maM	Matrix of normalized intensity log-ratios, M
maMloc maMscale	Matrix of location and scale normalization values
maW	Matrix of spot quality weights
maLayout	Array layout parameters - **marrayLayout**
maGnames	Description of spotted probe sequences - **marrayInfo**
maTargets	Description of target samples - **marrayInfo**
maNormCall	Function call
maNotes	Any notes

`marrayInput` package

- `marrayInput` provides functions for reading microarray data into R and creating microarray objects of class **`marrayLayout`**, **`marrayInfo`**, and **`marrayRaw`**.
- Input
  - Image quantitation data, i.e., output files from image analysis software.

    E.g. **`.gpr`** for **`GenePix`**, **`.spot`** for **`Spot`**.
  - Textual description of probe sequences and target samples.

    E.g. gal files, god lists.

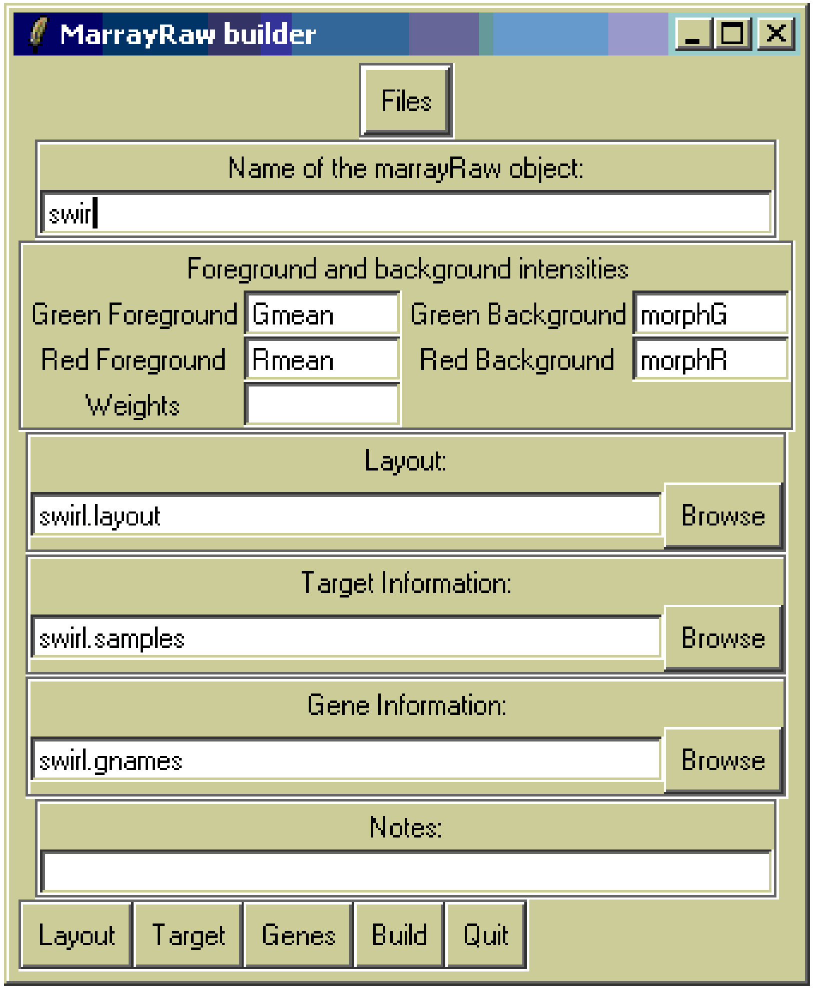
marrayInput package

- Widgets for graphical user interface

  widget.marrayLayout,

  widget.marrayInfo,

  widget.marrayRaw.



Name of the marrayRaw object: swirl

Green Foreground: Gmean

Green Background: morphG

Red Foreground: Rmean

Red Background: morphR

Weights:

Layout: swirl.layout

Target Information: swirl.samples

Gene Information: swirl.gnames

Notes:

marrayPlots package

- Diagnostic plots of spot statistics.

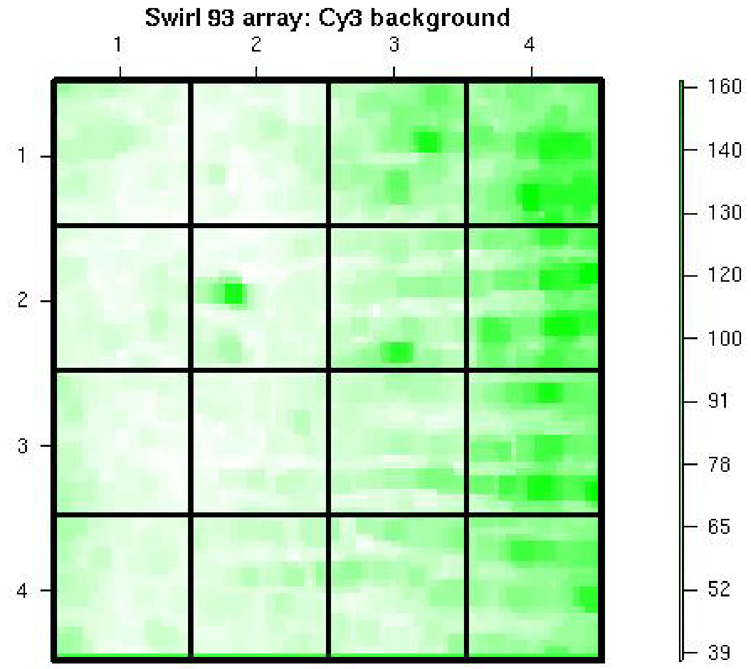
  E.g. red and green log-intensities, intensity log-ratios M, average log-intensities A, spot area.

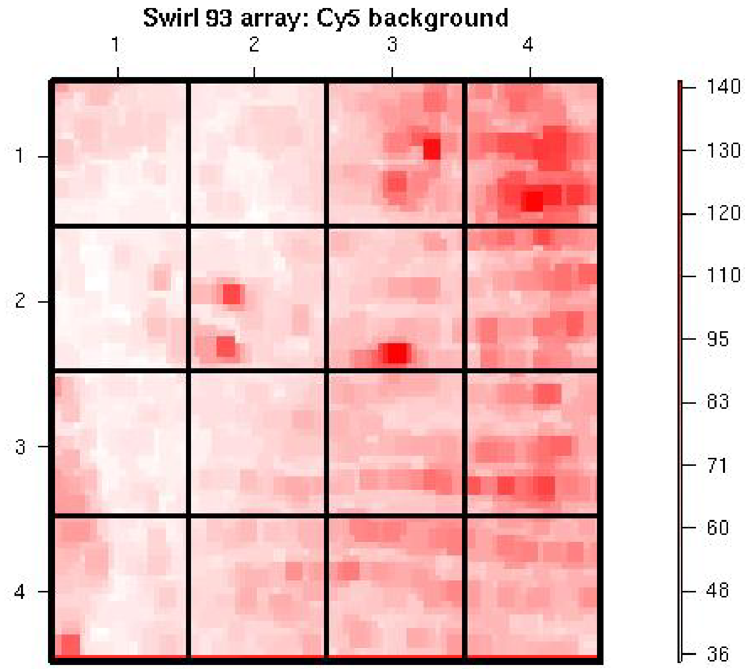
  - **maImage**: 2D spatial images.
  - **maBoxplot**: boxplots.
  - **maPlot**: scatter-plots with fitted curves and text highlighted.
- Stratify plots according to layout parameters such as print-tip-group, plate.

  E.g. MA-plots with loess fits by print-tip-group.
- See **demo(marrayPlots)**.

2D spatial images

`maImage`

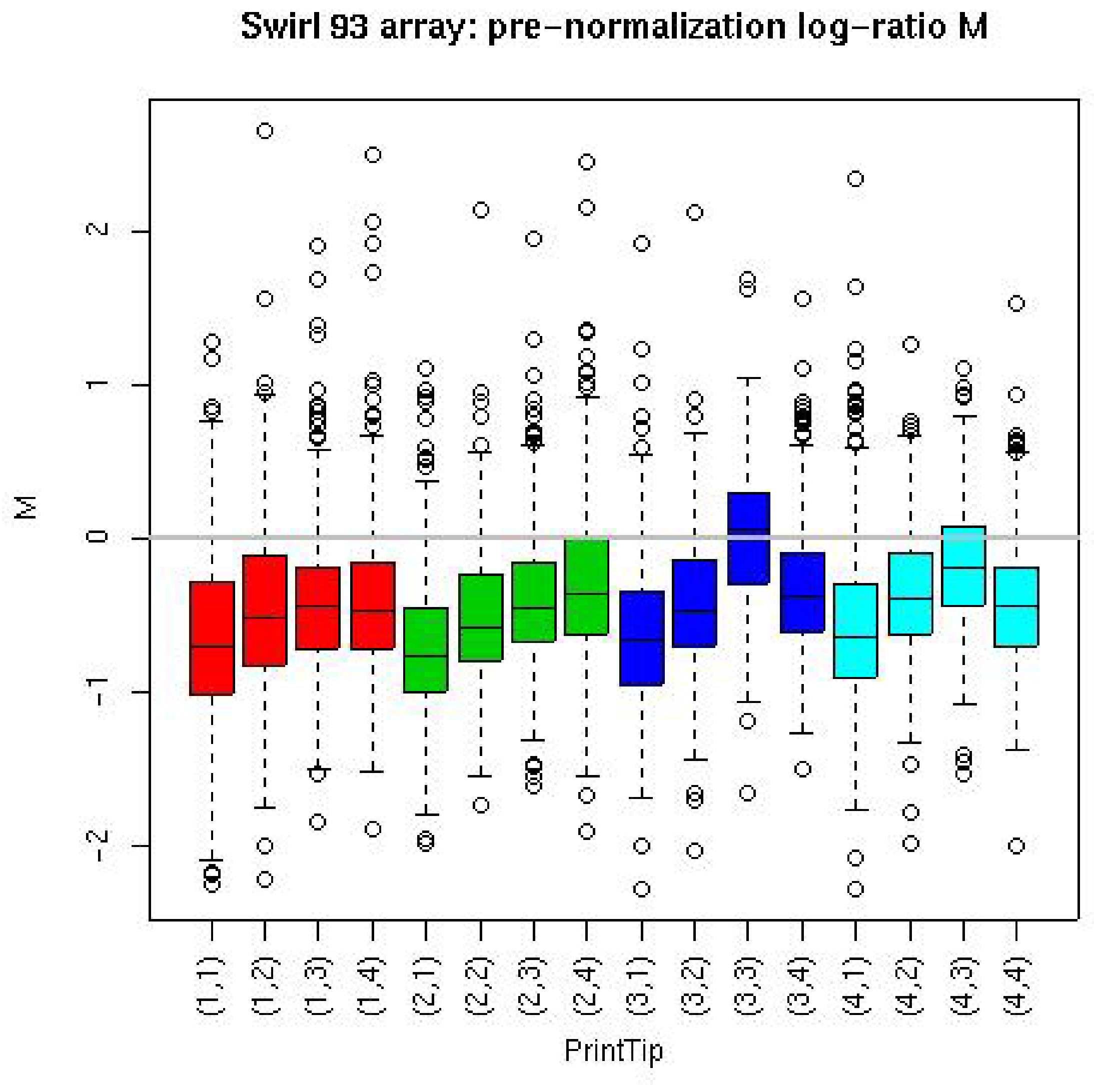




Cy3 background intensity

Cy5 background intensity

Boxplots by print-tip-group
`maBoxplot`

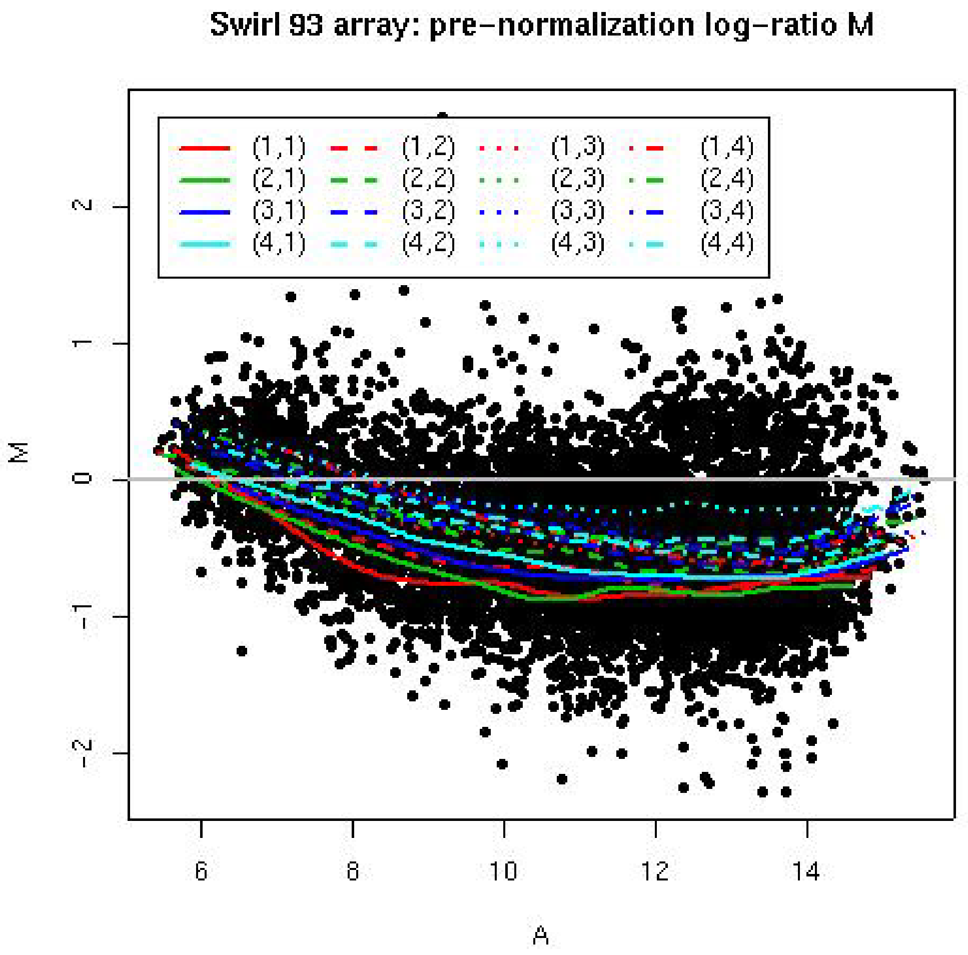


Intensity log-ratio, M

MA-plot by print-tip-group

`maPlot`

$M = \log_2 R - \log_2 G$, $A = (\log_2 R + \log_2 G)/2$



Intensity log-ratio, M

Average log-intensity, A

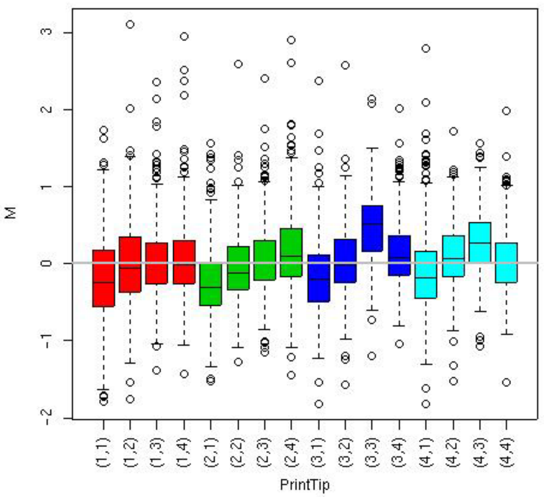
marrayNorm package

- **maNormMain**: main normalization function, allows robust adaptive location and scale normalization for a batch of arrays
  - intensity or A-dependent location normalization (**maNormLoess**);
  - 2D spatial location normalization (**maNorm2D**);
  - median location normalization (**maNormMed**);
  - scale normalization using MAD (**maNormMAD**);
  - composite normalization;
  - your own normalization function.
- **maNorm**: simple wrapper function.
  maNormScale: simple wrapper function for scale normalization.

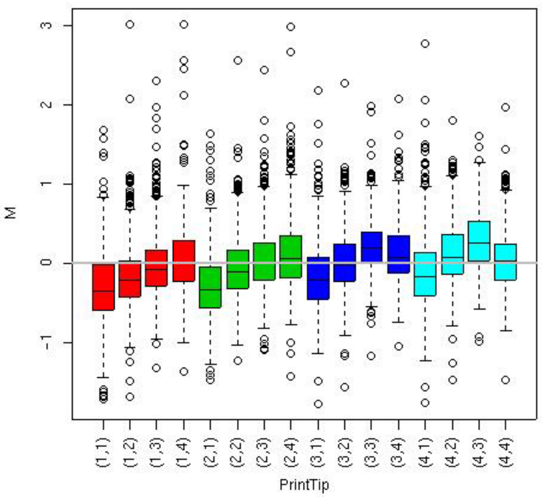
Boxplots of normalized M

Global median normalization

Swirl 93 array: global median normalization log−ratio M



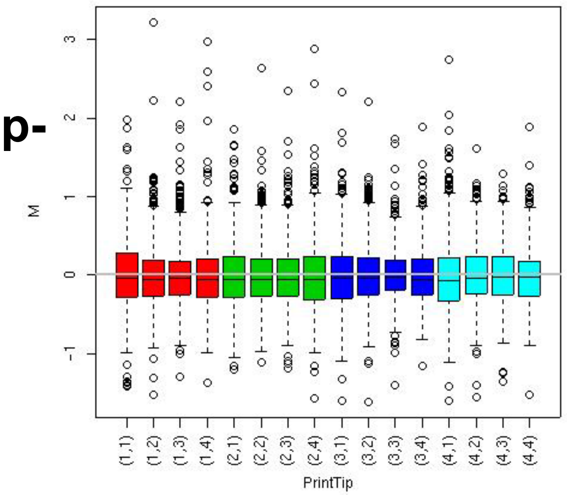
Swirl 93 array: global loess normalization log−ratio M



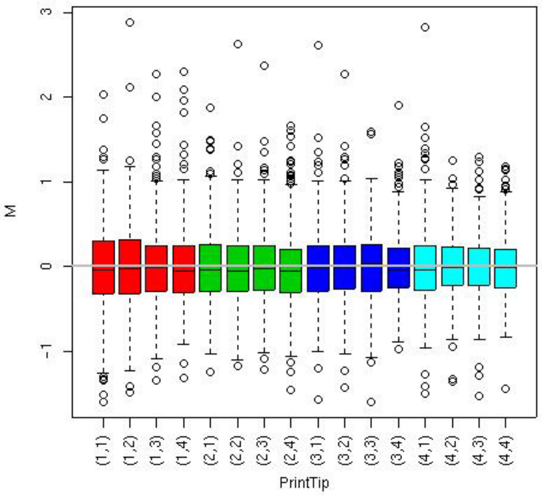
Global loess normalization

Within-print-tip-group loess normalization

Swirl 93 array: within−print−tip−group loess normalization log−ratio



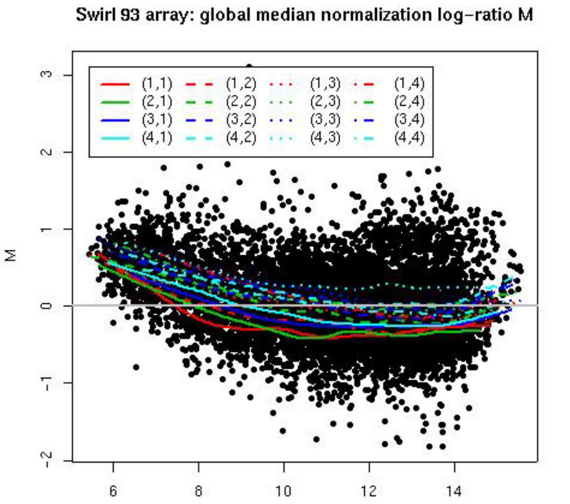
Swirl 93 array: 2D spatial loess normalization log−ratio M

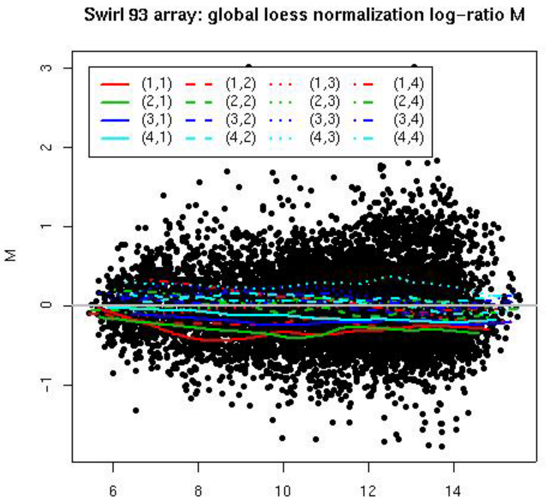


2D spatial normalization

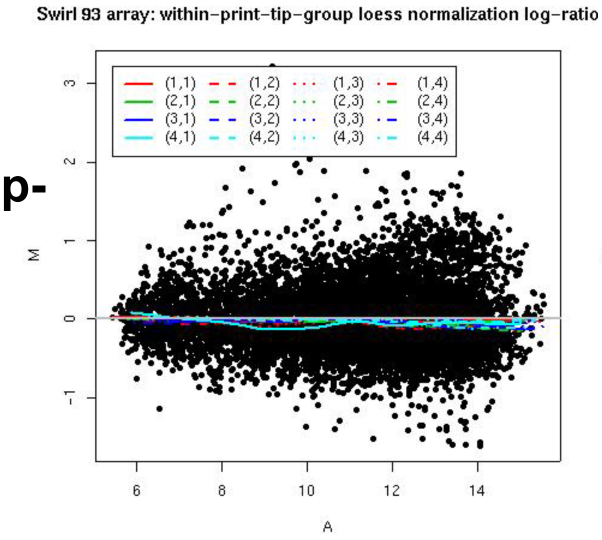
MA-plots of normalized M

Global median normalization

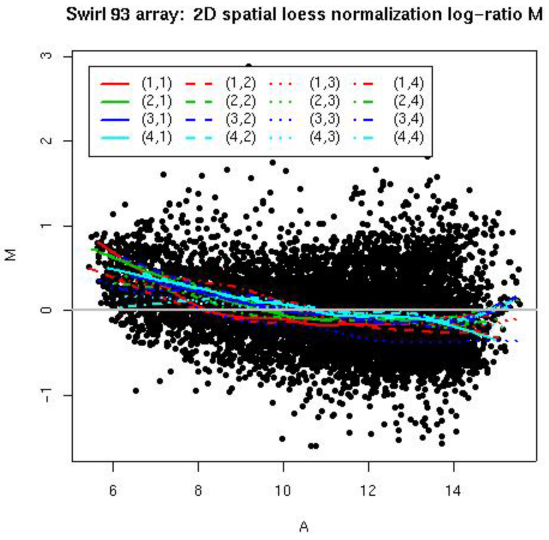

Swirl 93 array: global median normalization log−ratio M


Swirl 93 array: global loess normalization log−ratio M

Global loess normalization


Swirl 93 array: within−print−tip−group loess normalization log−ratio

Within-print-tip-group loess normalization


Swirl 93 array: 2D spatial loess normalization log−ratio M

2D spatial normalization

Pre-processing Affymetrix data

- Bioconductor R package `affy`.
- Background estimation.
- Probe-level normalization: quantile, curve-fitting.
- Expression measures: AvDiff, Signal, Li & Wong (2001), RMA.
- Two main functions: `ReadAffy`, `express`

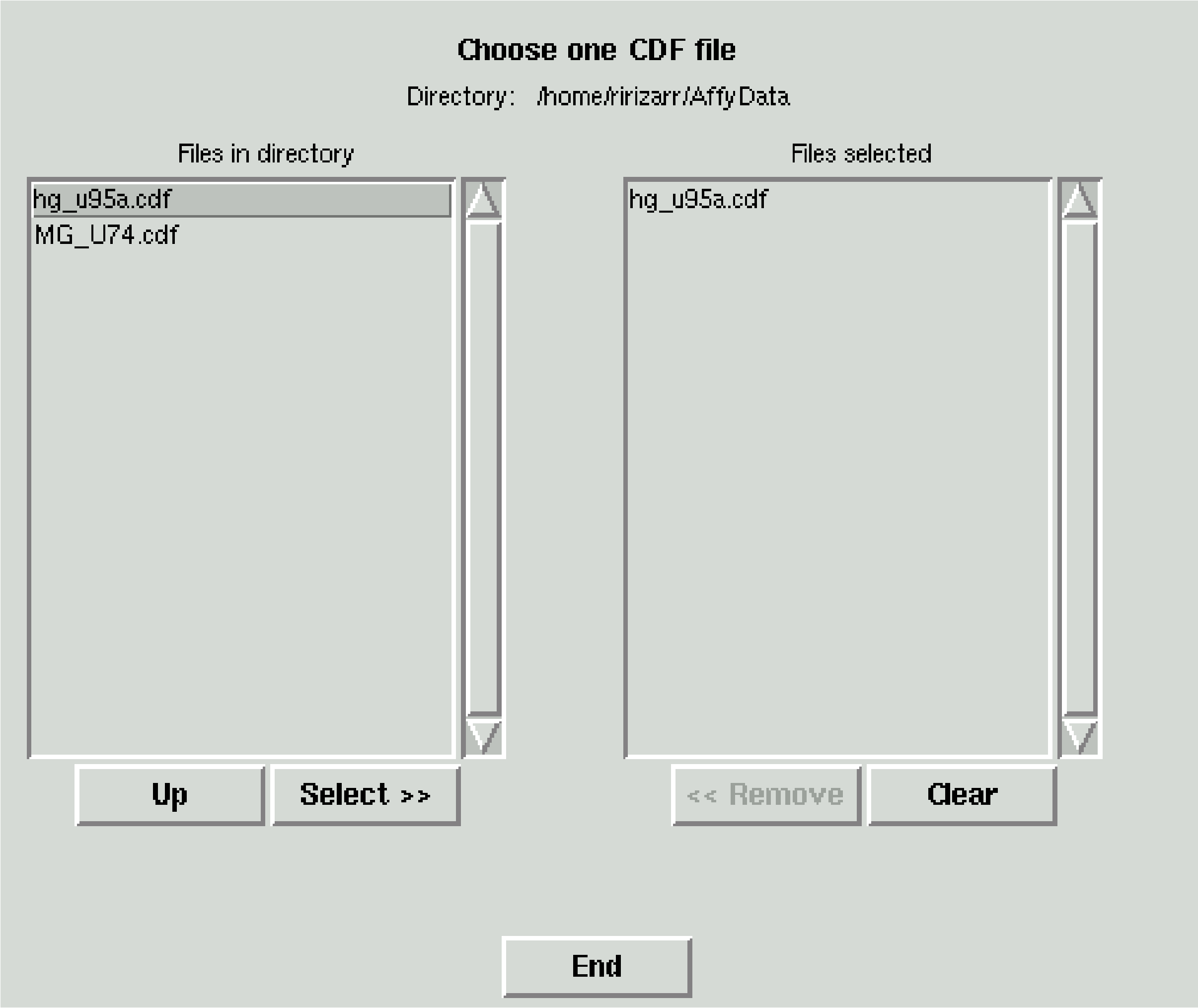
`affy` package

- Pre-processing for Affymetrix chip data.
- Class definitions for probe-level data: **`Cdf`**, **`Cel`**, **`PPSet`**, **`Plob`**.
- Basic methods for manipulating microarray objects: printing, plotting, subsetting.
- Functions and widgets for data input from CDF and CEL files, and automatic generation of microarray data objects.

`affy` package

- Diagnostic plots: 2D spatial images, boxplots, MA-plots, etc.
- Background estimation.
- Probe-level normalization: quantile and curve-fitting normalization.
- Expression measures: MAS 4.0 AvDiff, MAS 5.0 Signal, MBEI (Li & Wong, 2001), RMA (Irizarry et al., 2002).
- Two main functions: **`ReadAffy`**, **`express`**.

Reading in data: `ReadAffy()`



Choose one CDF file

Directory: /home/ririzarr/AffyData

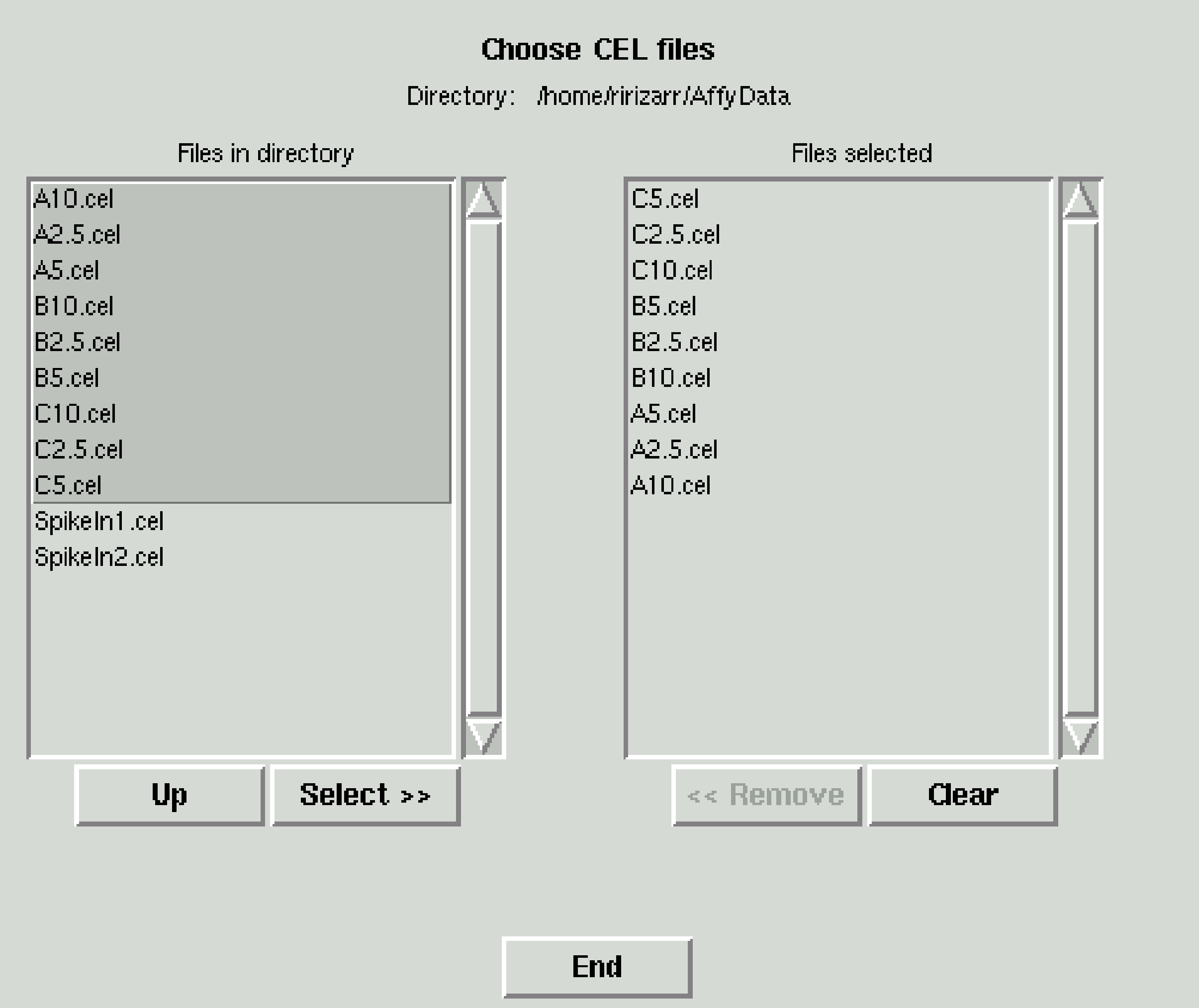
Files in directory

hg_u95a.cdf
MG_U74.cdf

Files selected

hg_u95a.cdf

Reading in data: `ReadAffy()`



Choose CEL files

Directory: /home/ririzarr/AffyData

Files in directory

A10.cel
A2.5.cel
A5.cel
B10.cel
B2.5.cel
B5.cel
C10.cel
C2.5.cel
C5.cel
SpikeIn1.cel
SpikeIn2.cel

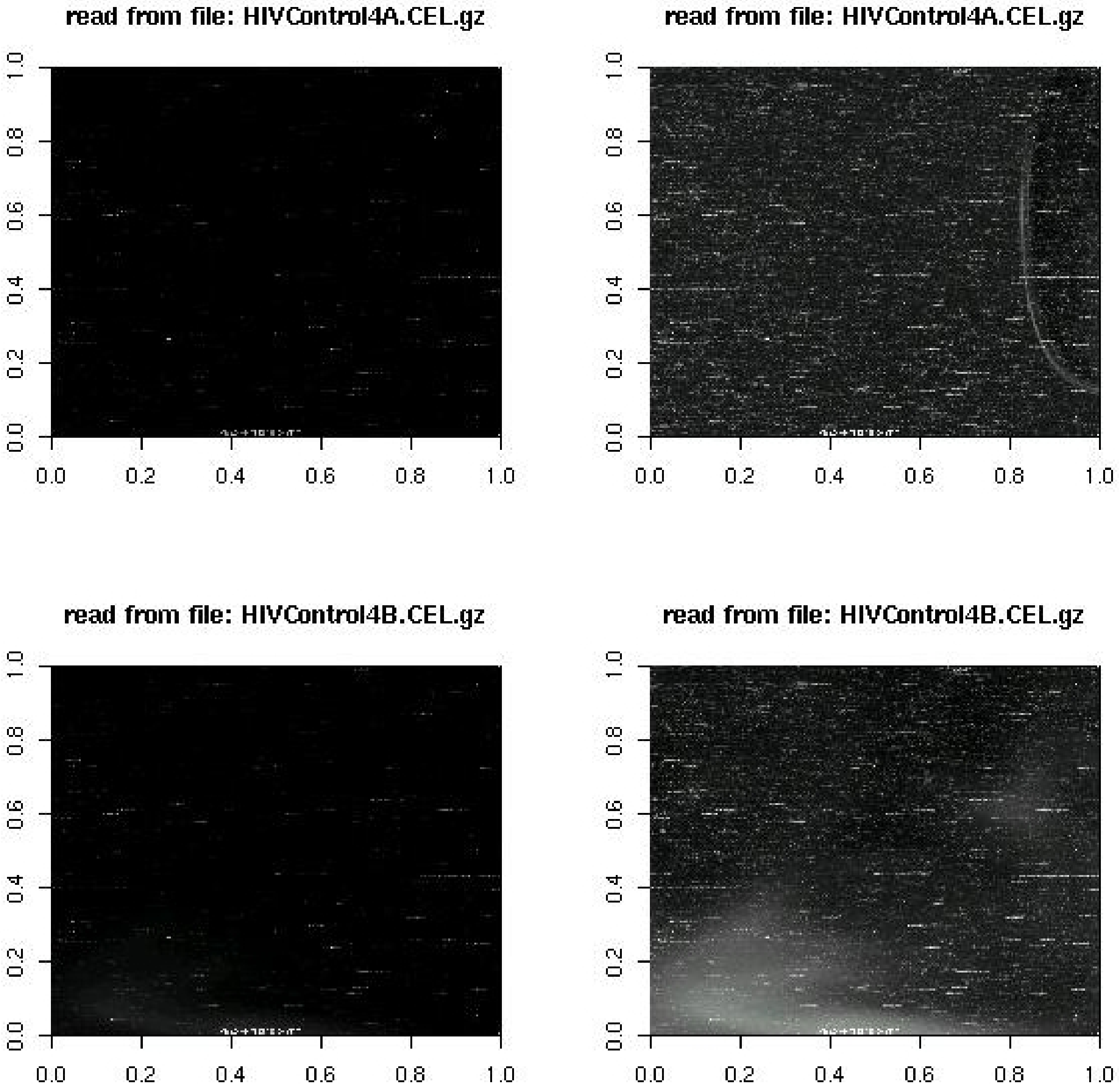
Files selected

C5.cel
C2.5.cel
C10.cel
B5.cel
B2.5.cel
B10.cel
A5.cel
A2.5.cel
A10.cel

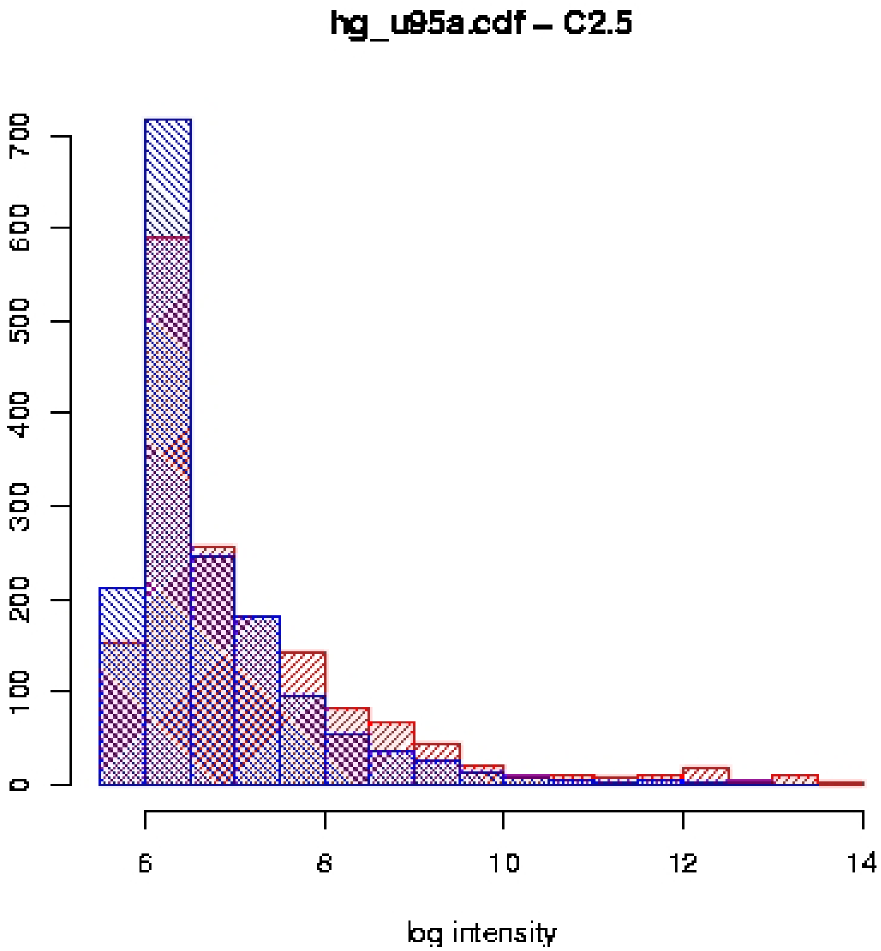
Methods for Quality Control:

`image, hist, boxplot, mva.pairs`

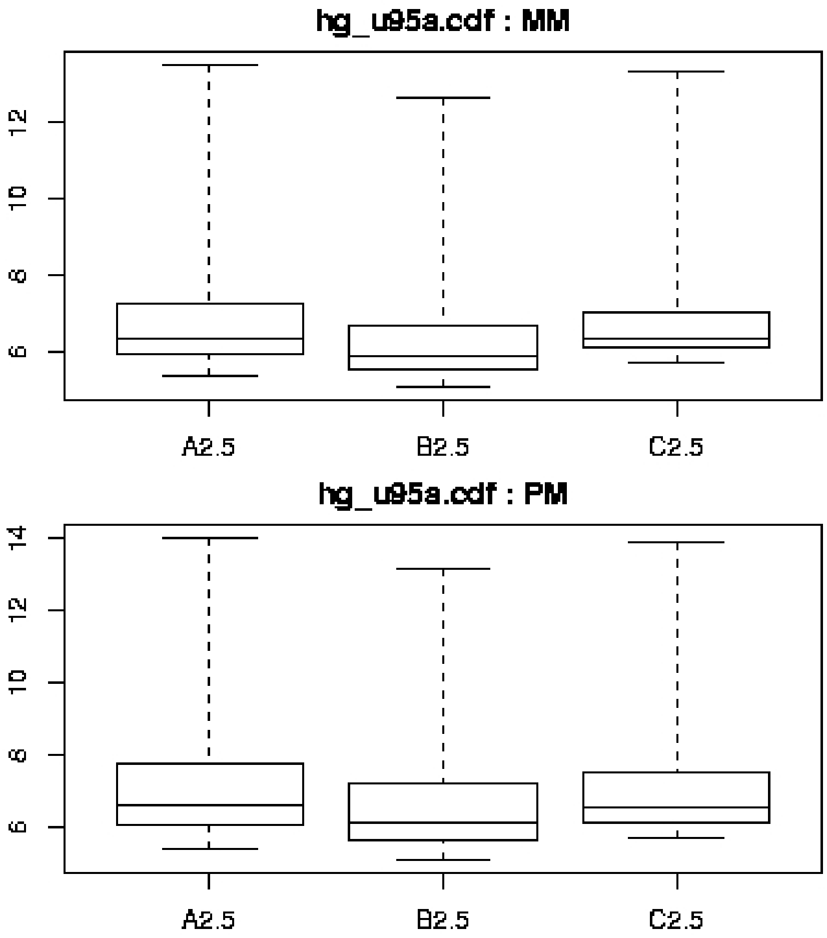
image

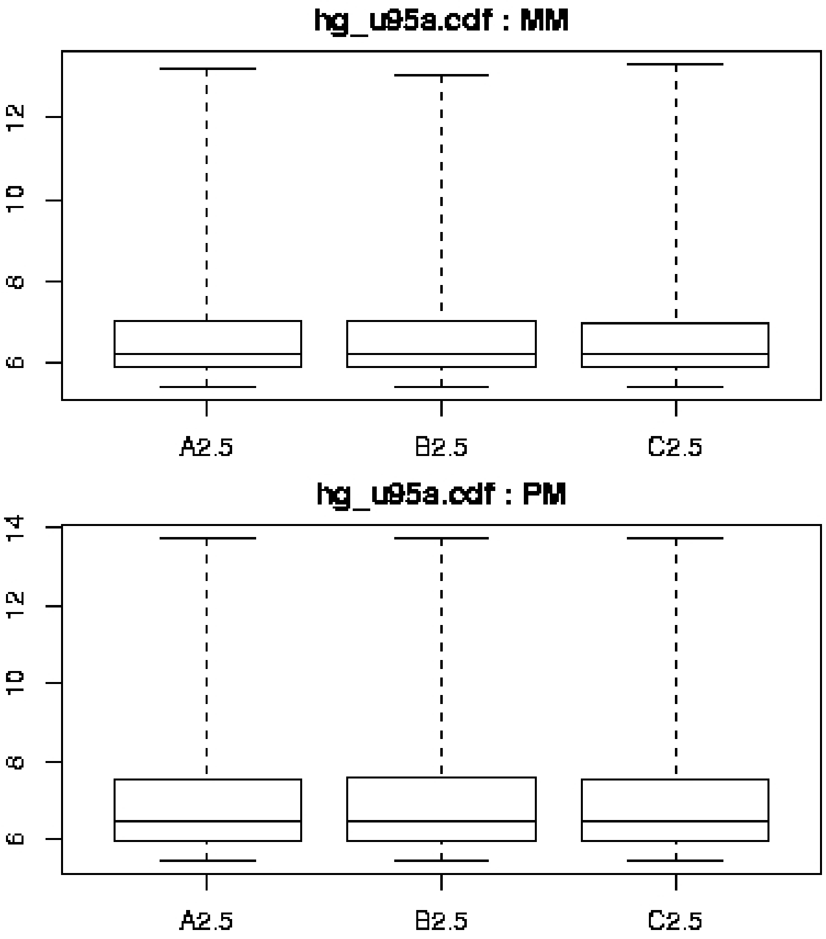

read from file: HIVControl4A.CEL.gz
read from file: HIVControl4A.CEL.gz
read from file: HIVControl4B.CEL.gz
read from file: HIVControl4B.CEL.gz
0.0
0.2
0.4
0.6
0.8
1.0

hist

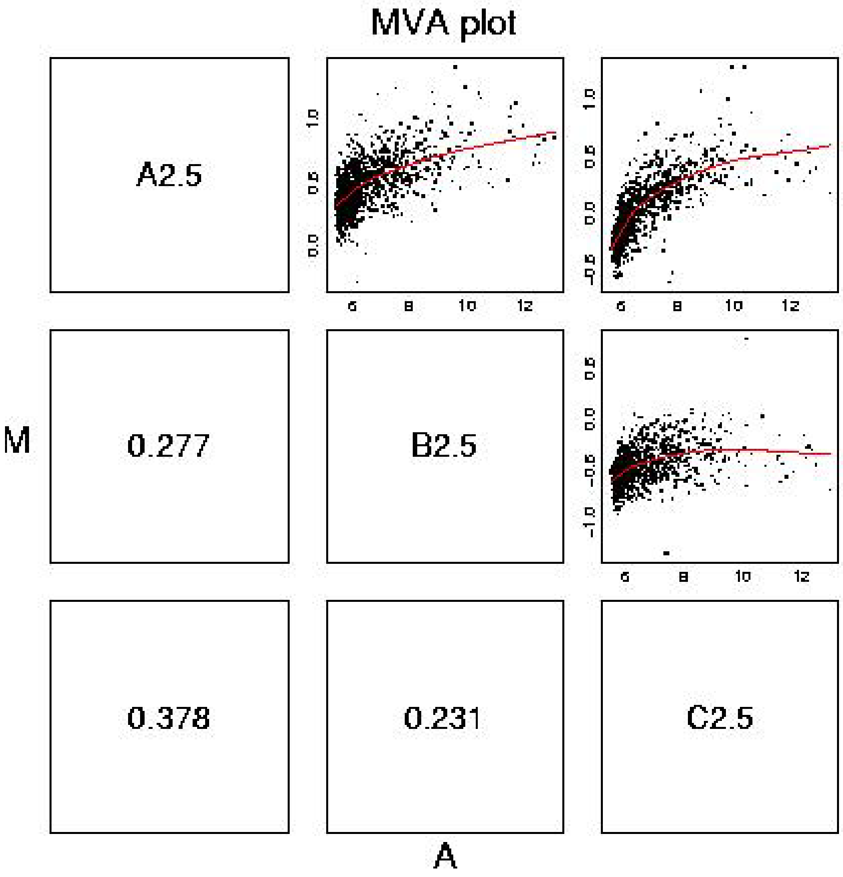

hg_u95a.cdf – C2.5
0
100
200
300
400
500
600
700
6
8
10
12
14
log intensity

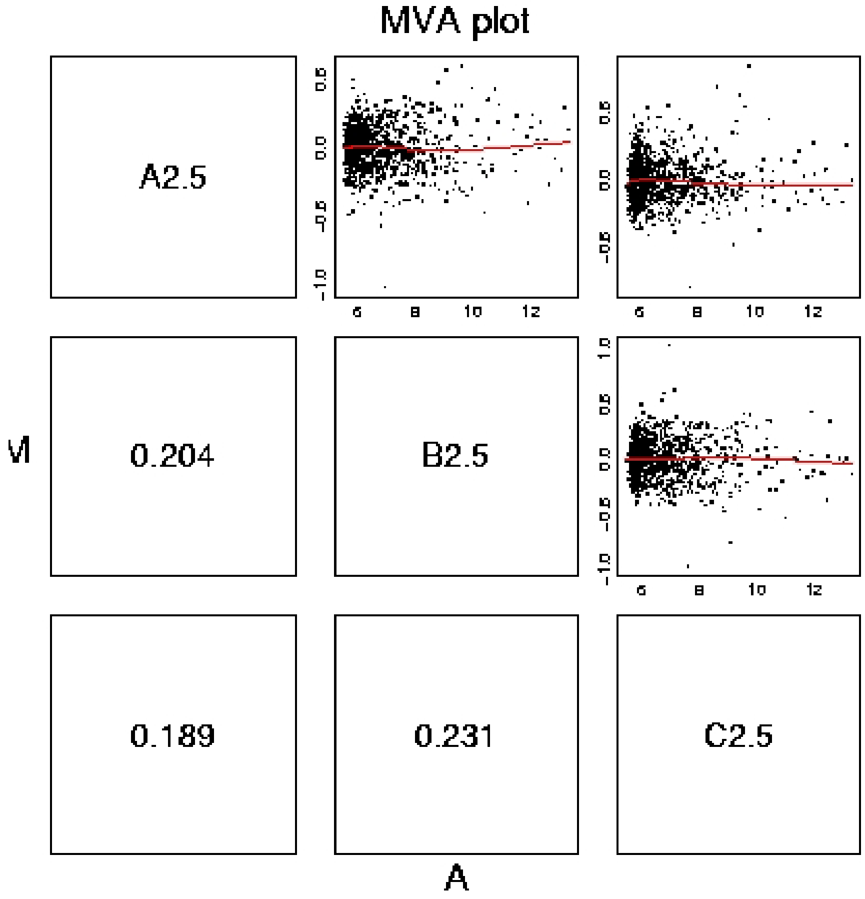
boxplot


hg_u95a.cdf : MM
6
8
10
12
A2.5
B2.5
C2.5
hg_u95a.cdf : PM
6
8
10
12
14
A2.5
B2.5
C2.5


hg_u95a.cdf : MM
6
8
10
12
A2.5
B2.5
C2.5
hg_u95a.cdf : PM
6
8
10
12
14
A2.5
B2.5
C2.5

mva.pairs


MVA plot
A2.5
1.0
0.5
0.0
6
8
10
12
1.0
0.5
0.0
-0.5
6
8
10
12
M
0.277
B2.5
0.5
0.0
-0.5
-1.0
6
8
10
12
0.378
0.231
C2.5
A


MVA plot
A2.5
0.5
0.0
-0.5
-1.0
6
8
10
12
0.5
0.0
-0.5
6
8
10
12
M
0.204
B2.5
1.0
0.5
0.0
-0.5
-1.0
6
8
10
12
0.189
0.231
C2.5
A

Computing Expression `express`

- Works on probe level objects
- Takes as arguments
  - `Normalize (TRUE or FALSE)`
  - `Method` (method of normalization)
  - `bg` (arbitrary function that perfoms an action on pm, mm matrix)
  - `Summary` (arbitrary function to summarize columns, e.g. medpolish)