Introduction to the Bioconductor marray package: Classes structure component (short)

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Contents

1	Overview	1		
2	Microarray classes	1		
3	Creating and accessing slots of microarray objects			
4	Basic microarray methods			
	4.1 Printing methods for microarray objects	3		
	4.2 Subsetting methods for microarray objects	4		
	4.3 Methods for accessing slots of microarray objects	7		
	4.4 Methods for assigning slots of microarray objects			
	4.5 Methods for coercing microarray objects	9		
	4.6 Functions for computing layout parameters	10		

1 Overview

This document provides a short tutorial on the basic class definitions and associated methods used in the marray package. To load the marray package in your R session, type library(marray).

- > library(marray)
- > data(swirl)

2 Microarray classes

The three main classes for cDNA microarray data are:

marrayLayout: This class is used to keep track of important layout parameters for two-color cDNA microarrays. It contains slots for: the total number of spotted probe sequences on the

array, the dimensions of the spot and grid matrices, the plate origin of the probes, information on spotted control sequences (e.g. probe sequences which should have equal abundance in the two target samples, such as housekeeping genes).

marrayRaw: This class represents pre—normalization intensity data for a batch of cDNA microarrays. A batch of arrays consists of a collection of arrays with the same layout ("marrayLayout"). The class contains slots for the green (Cy3) and red (Cy5) foreground and background intensities, the layout of the arrays, and descriptions of the target samples hybridized to the arrays and probe sequences spotted onto the arrays.

marrayNorm: This class represents post–normalization intensity data for a batch of cDNA microarrays. The class contains slots for the average log–intensities $A = \log_2 \sqrt{RG}$, the normalized log–ratios $M = \log_2 R/G$, the location and scale normalization values, the layout of the arrays, and descriptions of the target samples hybridized to the arrays and probe sequences spotted onto the arrays.

Other classes are marrayInfo which can be used to represents the Target or the Probes information. The function slotNames can be used to get information on the slots of a formally defined class or an instance of the class. For example, to get information of the slots for the marrayLayout class or on the slots for the object swirl use

```
> slotNames("marrayLayout")
[1] "maNgr"
                  "maNgc"
                                "maNsr"
                                              "maNsc"
                                                            "maNspots"
[6] "maSub"
                  "maPlate"
                                "maControls" "maNotes"
> slotNames(swirl)
[1] "maRf"
                 "maGf"
                              "maRb"
                                           "maGb"
                                                        "maW"
                                                                     "maLayout"
[7] "maGnames"
                 "maTargets" "maNotes"
```

3 Creating and accessing slots of microarray objects

[1] "Swirl array 1" "Swirl array 2" "Swirl array 3" "Swirl array 4"

Creating new objects. The function new from the methods package may be used to create new objects from a given class. For example, to create an object of class marrayInfo describing the target samples in the Swirl experiment, one could use the following code

```
> zebra.RG <- as.data.frame(cbind(c("swirl", "WT", "swirl", "WT"),
+ c("WT", "swirl", "WT", "swirl")))
> dimnames(zebra.RG)[[2]] <- c("Cy3", "Cy5")
> zebra.samples <- new("marrayInfo", maLabels = paste("Swirl array ",
+ 1:4, sep = ""), maInfo = zebra.RG, maNotes = "Description of targets for Swirl experiment
> zebra.samples
An object of class "marrayInfo"
@maLabels
```

```
@maInfo
        Cy3     Cy5
1 swirl     WT
2     WT swirl
3 swirl     WT
4     WT swirl
```

@maNotes

[1] "Description of targets for Swirl experiment"

Slots which are not specified in **new** are initialized to the prototype for the corresponding class. These are usually "empty", e.g., matrix(0,0,0). In most cases, microarray objects can be created automatically using the input functions and their corresponding widgets in the marrayInput package. These were used to create the object swirl of class marrayRaw.

Accessing slots. Different components or slots of the microarray objects may be accessed using the operator @, or alternately, the function slot, which evaluates the slot name. For example, to access the maLayout slot in the object swirl and the maNgr slot in the layout object L:

```
> L <- slot(swirl, "maLayout")
> L@maNgr
[1] 4
```

4 Basic microarray methods

The following basic methods were defined to facilitate manipulation of microarray data objects. To see all methods available for a particular class, e.g., marrayLayout, or just the print methods

```
> showMethods(classes = "marrayLayout")
> showMethods("summary", classes = "marrayLayout")
```

4.1 Printing methods for microarray objects

Since there is usually no need to print out fluorescence intensities for thousands of genes, the print method was overloaded for microarray classes by simple report generators. For an overview of the available microarray printing methods, type methods? print, or to see all print methods for the session

```
> showMethods("print")
Function "print":
  <not a generic function>
```

For example, summary statistics for an object of class marrayRaw, such as swirl, can be obtained by summary(swirl)

> summary(swirl)

Pre-normalization intensity data: Object of class marrayRaw.

Number of arrays: 4 arrays.

- A) Layout of spots on the array:
- B) Samples hybridized to the array:
- C) Summary statistics for log-ratio distribution:

```
Min. 1st Qu. Median
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.1.spot -2.73
                                                               -0.79 -0.58
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.2.spot -2.72
                                                               -0.15
                                                                       0.03
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.3.spot -2.29
                                                               -0.75 -0.46
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.4.spot -3.21
                                                               -0.46 -0.26
                                                        Mean 3rd Qu. Max.
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.1.spot -0.48
                                                               -0.29 4.42
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.2.spot 0.03
                                                                0.21 2.35
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.3.spot -0.42
                                                               -0.12 2.65
C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.4.spot -0.27
                                                               -0.062.90
```

D) Notes on intensity data: Spot Data

4.2 Subsetting methods for microarray objects

In many instances, one is interested in accessing only a subset of arrays in a batch and/or spots in an array. Subsetting methods "[" were defined for this purpose. For an overview of the available microarray subsetting methods, type methods? "[" or to see all subsetting methods for the session showMethods("[")). When using the "[" operator, the first index refers to spots and the second to arrays in a batch. Thus, to access the first 100 probe sequences in the second and third arrays in the batch swirl use

```
> swirl[1:100, 2:3]
```

```
An object of class "marrayRaw"
@maRf
     C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.2.spot
                                                    16138.720
[1,]
[2,]
                                                     17247.670
[3,]
                                                     17317.150
[4,]
                                                     6794.381
[5,]
                                                     6043.542
     C:/GNU/R/R-2.4.1/library/marray/swirldata/swirl.3.spot
[1,]
                                                    2895.1600
```

[2,]	2976.6230
[3,]	2735.6190
[4,]	318.9524
[5,]	780.6667
95 more rows	700.0007
95 more rows	
@maGf	
C:/GNU/R/R-2.4.1/library/ma	
[1,]	19278.770
[2,]	21438.960
[3,]	20386.470
[4,]	6677.619
[5,]	6576.292
C:/GNU/R/R-2.4.1/library/mag	rray/swirldata/swirl.3.spot
[1,]	2727.5600
[2,]	2787.0330
[3,]	2419.8810
[4,]	383.2381
[5,]	901.0000
95 more rows	
@maRb	
C:/GNU/R/R-2.4.1/library/mag	_
[1,]	136
[2,]	133
[3,]	133
[4,]	105
[5,]	105
C:/GNU/R/R-2.4.1/library/mag	rrav/swirldata/swirl.3.spot
[1,]	82
[2,]	82
[3,]	76
[4,]	61
[5,]	61
95 more rows	
@maGb	
C:/GNU/R/R-2.4.1/library/mag	rray/swirldata/swirl.2.spot
[1,]	175
[2,]	183
[3,]	183
[4,]	142
[5,]	142
C:/GNU/R/R-2.4.1/library/max	
[1,]	86
L-,J	00

```
[2,]
                                                           86
[3,]
                                                           86
[4,]
                                                           71
[5,]
                                                           71
95 more rows ...
@maW
<0 x 0 matrix>
@maLayout
An object of class "marrayLayout"
@maNgr
[1] 4
@maNgc
[1] 4
@maNsr
[1] 22
@maNsc
[1] 24
@maNspots
[1] 8448
@maSub
[1] TRUE TRUE TRUE TRUE TRUE
8443 more elements ...
@maPlate
[1] 1 1 1 1 1
Levels: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22
95 more elements ...
@maControls
[1] 1 1 1 1 1
Levels: 0 1
95 more elements ...
@maNotes
[1] "No Input File"
```

@maGnames

```
An object of class "marrayInfo"
@maLabels
[1] "geno1" "geno2" "geno3" "3XSSC" "3XSSC"
95 more elements ...
@maInfo
     "ID" "Name"
1 control geno1
2 control geno2
3 control geno3
4 control 3XSSC
5 control 3XSSC
95 more rows ...
@maNotes
[1] "C:/GNU/R/R-2.4.1/library/marray/swirldata/fish.gal"
@maTargets
An object of class "marrayInfo"
@maLabels
[1] "swirl.2.spot" "swirl.3.spot"
@maInfo
         Names slide number experiment Cy3 experiment Cy5
                                                                date comments
2 swirl.2.spot
                         82
                                 wild type
                                                     swirl 2001/9/20
                                                                            NA
3 swirl.3.spot
                                                 wild type 2001/11/8
                         93
                                      swirl
                                                                            NA
@maNotes
[1] "C:/GNU/R/R-2.4.1/library/marray/swirldata/SwirlSample.txt"
@maNotes
[1] "Spot Data"
```

4.3 Methods for accessing slots of microarray objects

A number of simple methods were defined to access slots of the microarray classes. Using such methods is more general than using the slot function or @ operator. In particular, if the class definitions are changed, any function which uses the @ operator will need to be modified. When using a method to access the data in the slot, only that particular method needs to be modified. Thus, to access the layout information for the array batch swirl one may also use malayout(swirl).

In addition, various methods were defined to compute basic statistics from microarray object slots. For instance, for memory management reasons, objects of class marrayLayout do not store the spot coordinates of each probe. Rather, these can be obtained from the dimensions of the grid and spot

matrices by applying methods: maGridRow, maGridCol, maSpotRow, and maSpotCol to objects of class marrayLayout. Print—tip—group coordinates are given by maPrintTip. Similar methods were also defined to operate directly on objects of class marrayRaw and marrayNorm. The commands below may be used to display the number of spots on the array, the dimensions of the grid matrix, and the print—tip—group coordinates.

```
> swirl.layout <- maLayout(swirl)
> maNspots(swirl)

[1] 8448
> maNspots(swirl.layout)

[1] 8448
> maNgr(swirl)

[1] 4
> maNgc(swirl.layout)

[1] 4
> maPrintTip(swirl[1:10, 3])

[1] 1 1 1 1 1 1 1 1 1
```

4.4 Methods for assigning slots of microarray objects

A number of methods were defined to replace slots of microarray objects, without explicitly using the @ operator or slot function. These make use of the setReplaceMethod function from the R methods package. As with the accessor methods just described, the assignment methods are named after the slots. For example, to replace the maNotes slot of swirl.layout

```
> maNotes(swirl.layout)
[1] "No Input File"
> maNotes(swirl.layout) <- "New value"
> maNotes(swirl.layout)
[1] "New value"
To initialize slots of an empty marrayLayout object
> L <- new("marrayLayout")
> L
```

```
An object of class "marrayLayout"
@maNgr
numeric(0)
@maNgc
numeric(0)
@maNsr
numeric(0)
@maNsc
numeric(0)
@maNspots
numeric(0)
@maSub
[1] TRUE
@maPlate
factor(0)
Levels:
@maControls
factor(0)
Levels:
@maNotes
character(0)
> maNgr(L) <- 4
```

Similar methods were defined to operate on objects of class marrayInfo, marrayRaw and marrayNorm.

4.5 Methods for coercing microarray objects

To facilitate navigation between different classes of microarray objects, we have defined methods for coercing microarray objects from one class into another. A list of such methods can be obtained by methods? coerce. For example, to coerce an object of class marrayRaw into an object of class marrayNorm

```
> swirl.norm <- as(swirl, "marrayNorm")</pre>
```

It is also possible to convert objects of class marrayRaw or marrayNorm into objects of class exprSet (see definition in the Biobase and convert package)

```
> library(convert)
> as(normdata, "exprSet")
```

4.6 Functions for computing layout parameters

In some cases, plate information is not stored in marrayLayout objects when the data are first read into R. We have defined a function maCompPlate which computes plate indices from the dimensions of the grid matrix and number of wells in a plate. For example, the Swirl arrays were printed from 384-well plates, but the plate IDs were not stored in the fish.gal file. To generate plate IDs (arbitrarily labeled by integers starting with 1) and store these in the maPlate slot of the marrayLayout object use

```
> maPlate(swirl) <- maCompPlate(swirl, n = 384)</pre>
```

Similar functions were defined to generate and manipulate spot coordinates: maCompCoord, maCompInd, maCoord2Ind, maInd2Coord. The function maGeneTable produces a table of spot coordinates and gene names for objects of class marrayRaw andmarrayNorm.