Codelink

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January 29, 2007

1 Introduction

Codelink^{TM} is a platform for the analysis of gene expression on biological samples using short (30 base long) oligonucleotide probes. There is a proprietary software for reading scanned images, spot intensity quantization and some diagnostics. Quality flags (Table 1) are assigned to the spot based on signal to noise ratio (SNR) computation (Eq: 1) and other morphological characteristics as irregular shape of the spots, saturation of the signal or manufacturer spots removed. The results can be exported in many formats as XML, Excel, plain text, etc. This library allows to read Codelink plain text exported data into R [3] for analysis of gene expression with any of the available tools in R+Bioconductor[1]. A new class is defined for convenient storing Codelink data as `exprSet` class is not convenient for this purpose.

Flag	*Meaning*
G	Good signal (SNR >= 1)
L	Limit signal (SNR < 1)
I	Irregular shape
S	Saturated signal
M	MSR spot
C	Background contaminated
X	User excluded spots

Table 1: Quality Flag description. SNR: Signal to Noise Ratio.

$$SNR = \frac{Smean}{(Bmedian + 1.5 * Bstdev)} \tag{1}$$

2 Reading data

Currently only data exported as plain text from Codelink software can be used. The Codelink text format can have arbitrary columns and header fields so depending of what you have exported you can read it or not. The suggestion

Probe type	*Meaning*
DISCOVERY	Gene expression testing probes
POSITIVE	Positive control probes
NEGATIVE	Negative control probes
FIDUCIAL	Grid alignment probes
OTHER	Other controls and housekeeping gene probes

Table 2: Probe types for Codelink arrays.

is that you put on the files Spot_mean and Bkgd_median values so you can do background correction and normalization in R. If you put Raw_intensity or Normalized_intensity columns then you can also read it directly and avoid background correction and/or normalization but this is not recommended. To read some Codelink files you do:

```
> library(codelink)
> data <- readCodelink()
```

This suppose that your files have the extension “TXT” (uppercase) and they are in your working directory. If this is not the case you can specify files to be read with the ’file’ argument. The function `readCodelink` returns and object of `Codelink`:

```
> library(codelink)
> data(codelink.example)
> codelink.example

An object of class "Codelink"
$product
[1] "UniSet Human 20K I"

$sample
[1] "Sample 1" "Sample 2"

$file
[1] "T001-2006-12-25_Sample 1.TXT" "T002-2006-12-25_Sample 2.TXT"

$name
[1] "NM_012429.1_PROBE1" "NM_003980.2_PROBE1" "AY044449_PROBE1"
[4] "NM_005015.1_PROBE1" "AB037823_PROBE1"
20464 more elements ...

$type
```

```
[1] "DISCOVERY" "DISCOVERY" "DISCOVERY" "DISCOVERY" "DISCOVERY"
20464 more elements ...

$flag
  1   2
1 "L" "L"
2 "L" "L"
3 "G" "L"
4 "G" "M"
5 "G" "G"
20464 more rows ...

$method
$background
[1] "NONE"

$normalization
[1] "NONE"

$merge
[1] "NONE"

$log
[1] FALSE


$snr
          1         2
1 0.7920656 0.7856675
2 0.8160216 0.7862189
3 1.0744066 0.9331916
4 3.8865153        NA
5 4.6647164 1.7993536
20464 more rows ...

$logical
  row col
1   1   9
2   1  10
3   1  11
4   1  12
5   1  13
20464 more rows ...

$Smean
         1        2
```

```
1  49.0588  46.7304
2  48.6395  45.8333
3  65.0781  52.4917
4 243.3116       NA
5 267.6458 102.0803
20464 more rows ...

$Bmedian
   1  2
1 43 42
2 42 42
3 42 40
4 44 NA
5 42 42
20464 more rows ...
```

Slot	*Description*
product	Chip name description
sample	Sample names vector
file	File names vector
name	Probe names vector
type	Probe types vector
method	Methods applied to data
method$background	Background correction method used
method$normalization	Normalization method used
method$merge	Merge method used
method$log	Logical: If data is in log scale
flag	Quality flag matrix
Smean	Mean signal intensity matrix
Bmedian	Median background intensity matrix
Ri	Raw intensity matrix
Ni	Normalized intensity matrix
snr	Signal to Noise Ratio matrix
cv	Coefficient of Variation matrix

Table 3: Description of Codelink object slots.

The chip type (product slot) is read from the PRODUCT field in the header of Codelink files. If it is not found then a warning message is shown and product slot is set to ”Unknown”. If one product type disagree with the others an error message is shown and reading of files is terminated.

By default, all spots flagged with M, I, and S flags are set to NA. This can be changed with the flag argument in `readCodelink`. The flag argument is a list that can contain a valid flag identifier and a value for that flag. For example, if you want to set all M flagged spots to 0.01 and let other spot untouched you do:

```
> data <- readCodelink(flag=list(M=0.01) )
```

It is possible to find probes wit more that one flag assigned, i.e. CL for a probe labeled as C and L, CLI for a probe labeled as C, L and I, and so on. As a regular expression is used to find flag types it is possible to manage all this situations. When two user modified flags fall in the same probe the smallest (or NA if applicable) is assigned.

3 Background correction

Smean intensity values can be processed into Ri through background correction:

```
> data <- bkgdCorrect(data, method="half")
```

The default method used is based on the *half* method from *limma* [4] package. Median background intensity (Bmedian) is subtracted from mean spot intensity (Smean) and if the result is less than 0.5 then it is set to 0.5 to ensure no negative numbers are obtained.

4 Normalization

Normalization of Ri values are done with the wrapper function normalize(). The default method is *quantile* normalization that in fact call `normalizeQuantiles()` from *limma* package (that allows for NAs). There is also the possibility to use a modified version of cyclic loess from *affy* [2] package that also allows NA values.

```
> data <- normalize(data, method="quantile")
```

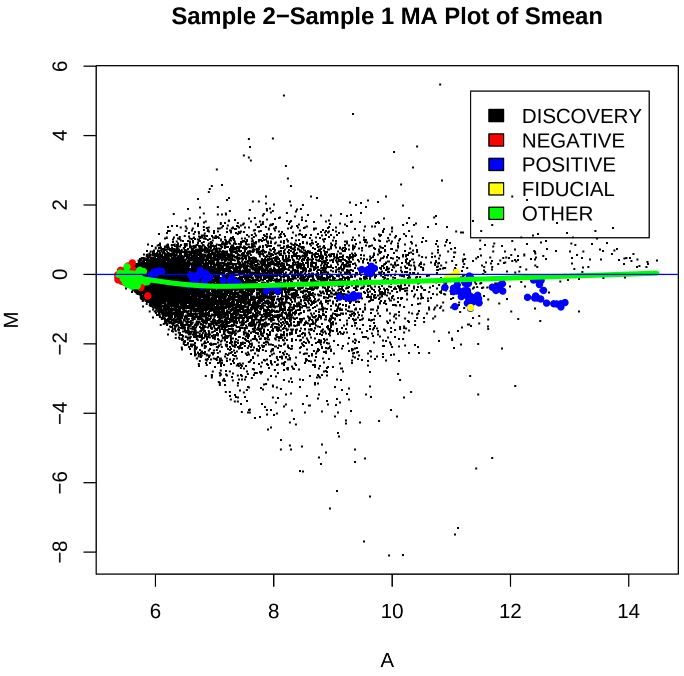
By default, `normalize` return log2 intensity values. This could be changed setting the parameter log.it to FALSE.

5 Plotting

There are some plotting functions available that can use directly the Codelink objects. These are functions for producing MA plots (`plotMA`), scatterplots (`plotCorrelation`) and density plots (`plotDensities`). All functions use the available intensity value (i.e. Smean, Ri or Ni) to make the plot.

The function `plotMA` can highlight spots based on type values (by default) or SNR values setting the argument label. It requires array1 and array2 arguments and compute M and A values based on equations 2 and 3.

```
> plotMA(codelink.example, legend.x = "topright")
```

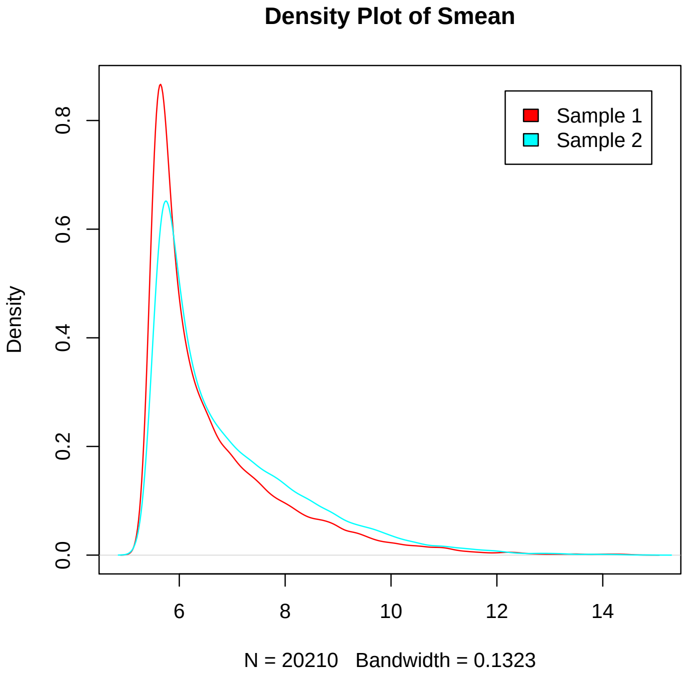


$$M = Array2 - Array1 \tag{2}$$

$$A = \frac{Array2 + Array1}{2} \tag{3}$$

The function `plotDensities` plot the density of intensity values of all arrays. If the subset argument is supplied it can use only a subset of the arrays in the `Codelink` object.

```
> plotDensities(codelink.example)
```



6 Miscellaneus

There are also some miscellaneous functions used in some analysis that could be useful for someone.

6.1 Using weights

The `createWeights` function creates a matrix of weights based on probe type labels to be used, for example, in fitting a linear model with *limma* [4].

```
> w <- createWeights(codelink.example, type = list(FIDUCIAL = 0.01,
+     NEGATIVE = 0.1))
> w[1:10, ]
```

```
      [,1] [,2]
 [1,]    1    1
 [2,]    1    1
 [3,]    1    1
 [4,]    1    1
 [5,]    1    1
 [6,]    1    1
 [7,]    1    1
 [8,]    1    1
 [9,]    1    1
[10,]    1    1
```

6.2 Merging arrays

In case you want to merge array intensities the `mergeArray` function help on this task. It computes the mean of Ni values on arrays of the same class. The grouping is done by means of the 'class' argument (numerical vector of classes). New sample names should be assigned to sample slot with the 'names' argument. The function also returns the coefficient of variation in the 'cv' slot. The distribution of coefficients of variations can be checked with the function `plotCV`.

```
> data <- mergeArray(data, group=c(1,1,2,2),
+ names=c("A","B"))

> plotCV(data)
```

References

[1] Robert C Gentleman, Vincent J. Carey, Douglas M. Bates, Ben Bolstad, Marcel Dettling, Sandrine Dudoit, Byron Ellis, Laurent Gautier, Yongchao Ge, Jeff Gentry, Kurt Hornik, Torsten Hothorn, Wolfgang Huber, Stefano Iacus, Rafael Irizarry, Friedrich Leisch Cheng Li, Martin Maechler, Anthony J. Rossini, Gunther Sawitzki, Colin Smith, Gordon Smyth, Luke Tierney, Jean Y. H. Yang, and Jianhua Zhang. Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biology*, 5:R80, 2004.

[2] Rafael A. Irizarry, Laurent Gautier, Benjamin Milo Bolstad, , Crispin Miller with contributions from Magnus Astrand <Magnus.Astrand@astrazeneca.com>, Leslie M. Cope, Robert Gentleman, Jeff Gentry, Conrad Halling, Wolfgang Huber, James MacDonald, Benjamin I. P. Rubinstein, Christopher Workman, and John Zhang. *affy: Methods for Affymetrix Oligonucleotide Arrays*, 2005. R package version 1.8.1.

[3] R Development Core Team. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria, 2005. ISBN 3-900051-07-0.

[4] Gordon K Smyth. *Limma: linear models for microarray data*, pages 397–420. Springer, New York, 2005.