1 Introduction

Codelink is a platform for the analysis of gene expression on biological samples property of Applied Microarrays, Inc. (previously was GE Healthcare and Amersham). The hybridization reagents are still supplied by GE Healthcare.

The system uses 30 base long oligonucleotide probes for expression testing. There is a proprietary software for reading scanned images, doing spot intensity quantization and some diagnostics. The software assigns quality flags (see Table 1) to each spot on the basis of a 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. By default, the software performs background correction (subtract) followed by median normalization. The results can be exported in several formats as XML, Excel, plain text, etc. This library allows to read Codelink plain text exported data into R [3] for the analysis of gene expression with any of the available tools in R+Bioconductor[1].

For storing the available data, a new class Codelink was designed. The now defunct exprsSet in the Biobase package was not a convenient store class for Codelink data, because it didn’t allow probes with duplicated names, as those names are stored as rownames in the expression matrix.

Currently there is experimental support for a ExpressionSet derived class that can accomodate duplicated probe names in an AnnotatedDataFrame whereas the feature ids would be used for row names when available.

<table>
<thead>
<tr>
<th>Flag</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>Good signal (SNR &gt;= 1)</td>
</tr>
<tr>
<td>L</td>
<td>Limit signal (SNR &lt; 1)</td>
</tr>
<tr>
<td>I</td>
<td>Irregular shape</td>
</tr>
<tr>
<td>S</td>
<td>Saturated signal</td>
</tr>
<tr>
<td>M</td>
<td>MSR spot</td>
</tr>
<tr>
<td>C</td>
<td>Background contaminated</td>
</tr>
<tr>
<td>X</td>
<td>User excluded spots</td>
</tr>
</tbody>
</table>

Table 1: Quality Flag description. SNR: Signal to Noise Ratio.
\begin{align*}
SNR &= \frac{S_{mean}}{(B_{median} + 1.5 \times B_{stdev})} 
\end{align*}

<table>
<thead>
<tr>
<th>Probe type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>DISCOVERY</td>
<td>Gene expression testing probes</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>Positive control probes</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>Negative control probes</td>
</tr>
<tr>
<td>FIDUCIAL</td>
<td>Grid alignment probes</td>
</tr>
<tr>
<td>OTHER</td>
<td>Other controls and housekeeping gene probes</td>
</tr>
</tbody>
</table>

Table 2: Probe types for Codelink arrays.

2 Reading data

Currently only data exported as plain text from Codelink software is supported. Unfortunately the Codelink exported text format can have arbitrary columns and header fields so depending of what you have exported you can read it or not. The suggestion is that you put on the files everything you can, including Spot\_mean and Bkgd\_median values so you can do background correction and normalization in R. In addition, Bkgd\_stdev is needed to compute the SNR. 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:

\begin{verbatim}
# NOT RUN #

> library(codelink)
> foo <- readCodelink()
> summaryFlag(foo) # will show a summary of flag values.

# NOT RUN #
\end{verbatim}

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

\begin{verbatim}
> library(codelink)
> data(codelink.example)
> codelink.example
\end{verbatim}
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
The `Codelink-class` is basically a list that stores information in several slots (see Table 3). The chip type (product slot) is read from the PRODUCT field if found 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 the product is not the same in all the files the reading is canceled with an error message.

By default, all spots flagged with M, I, and S flags are set to NA. This can be controlled with the flag argument of `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:

```r
# NOT RUN #

> foo <- readCodelink(flag = list(M = 0.01) )
```
It is possible to find probes with more than 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. A regular expression is used to find flag types in an attempt to manage all the possible situations. When two user modified flags fall in the same probe the more restricting (NA being the most) is assigned.

### 3 Background correction

If you have Spot mean values Bkgd median values you can apply one of the several background correction methods interfaced. This is done by the function `bkgdCorrect`. To see the different options look at `?bkgdCorrect`. For instance, if you want to apply half method you do:

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

The default method used is half and is based in the same method applied in the `limma` package to two channel microarrays. In this method, the median background intensity (Bmedian) is subtracted from mean spot intensity (Smean) and any value smaller than 0.5 is shifted to 0.5 to ensure no negative numbers are
obtained that would prevent to transform the data into log scale. Other available methods are none that let the spot intensities untouched, subtract that is analog to the default method used in the Codelink software and normexp and interface to the method available in the limma package.

4 Normalization

Normalization of the background corrected intensities is done by the wrapper function normalize. The default method is quantile normalization that in fact call normalizeQuantiles() from limma package (allowing for NAs). There is also the possibility to use a modified version of CyclicLoess from affy [2] package that allow using weights and missing values. Finally, the median normalization allows to normalize using a method analog to the default method in the Codelink software. To normalize you usually do:

```r
> foo <- normalize(foo, method = "quantiles")
```

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

5 Plotting

There are some diagnostic plots available for the Codelink object. 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 functions plotMA and plotCorrelation can highlight points based on the Spot Type, which is the default behavior or using the SNR values. The mode is controlled with argument label. plotCorrelation requires arguments array1 and array2 to be set in order to select which arrays are going to be plotted. For plotMA if only array1 is specified, the values are plotted agains a pseudoarray constructed with the mean of the probe intensities along all available arrays. M and A values are computed following equations 2 and 3.

```r
> plotMA(codelink.example)
```
\[ M = \text{Array2} - \text{Array1} \]  \hspace{1cm} (2)

\[ A = \frac{\text{Array2} + \text{Array1}}{2} \]  \hspace{1cm} (3)

The function \texttt{plotDensities} plot the density of intensity values of all arrays. It can plot only a subset of arrays if the \texttt{subset} argument is supplied.

\texttt{> plotDensities(codelink.example)}
When Logical_row and Logical_col columns are exported into they are stored into the logical slot. This information stores the physical location of each probe in the array, and can be used to plot pseudo images of the array intensities. To plot a pseudo image you should use:

```r
> imageCodelink(foo)
> imageCodelink(foo, what = "snr")
```

It is possible to plot the background intensities (default), the spot mean, raw and normalized intensities and the SNR values. This images are useful to identify spatial artifact that may be affecting the analysis.

## 6 Miscellaneous

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

### 6.1 Export to file

The function `writeCodelink` exports a `Codelink` object to a file. The file will contain probe intensities and, if specified `flag = TRUE`, probe quality flags.
6.2 Using weights

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

\[
\begin{align*}
\text{> w <- createWeights(codelink.example, type = list(FIDUCIAL = 0.01, NEGATIVE = 0.1))} \\
\text{> w[1:10, ]}
\end{align*}
\]

\[
\begin{array}{ccc}
[1,] & 1 & 1 \\
[2,] & 1 & 1 \\
[3,] & 1 & 1 \\
[4,] & 1 & 1 \\
[5,] & 1 & 1 \\
[6,] & 1 & 1 \\
[7,] & 1 & 1 \\
[8,] & 1 & 1 \\
[9,] & 1 & 1 \\
[10,] & 1 & 1
\end{array}
\]

6.3 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 the sample slot using 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.

\[
\begin{align*}
\text{> foo <- mergeArray(foo, class = c(1, 1, 2, 2), names = c("A", "B"))} \\
\text{> plotCV(foo)}
\end{align*}
\]

7 Problems reading data

Some updated version of the Codelink software changed the order in which probes are printed in the exported text files. That makes files from these different software version impossible to be analyzed together. The function readCodelink have a new argument check TRUE by default, that check the Probe_name columns to see if they have the same order in all the arrays at the cost is a little extra loading time. This behaviour can be turn off by setting
check to FALSE. If an ordering problem is found, a warning message is print but the reading of data is not stopped, allowing the visual examination of the data. In this case, the best option is to export the old files again using the updated version of the software. If this is impossible for whatever reason, the codelink package can try to fix the order of the files.

To the aim, a new argument fix = TRUE can be passed to readCodelink. The method will try to order the probes using the Feature_id column, which is a combination of Logical_row and Logical_col and for this, a unique identifier of each probe. This is the optimal fix, and the new data should be perfectly ordered. If that column is missing, the it will try to order the data using the Probe_name column. This is a sub-optimal solution because as the fiducial, control and some discovery probes have duplicated Probe_name, they may be end messed up. In this case, the best solution again, is to try to export again the data with the updated version of the software.

> foo <- readCodelink(fix = TRUE)

8 Future improvements

As the new classes in the Biobase package (eSet and ExpressionSet) allow more flexible data structures, a reimplementation of the Codelink-class based on ExpressionSet-class will be the next major feature added to this package. This will allow a better integration with other tools available through the Bioconductor project.

Right now, there is some experimental implementation of the new codebase. You need to use the wrapper function readCodelink2.

> foo <- readCodelink2()

References


[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,
