Computing and plotting agreement among ranked vectors of features with matchBox.

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Contents

1	In	troduction	2
2	Preparing the Data		3
	2.1	Data structure	:
	2.2	Removing redundancy	4
	2.3	Merging the data	6
3	\mathbf{C}	orrespondance at the Top Curves	6
	3.1	Computing CAT curves without a reference ranking	7
	3.2	Computing CAT curves using a reference ranking	7
	3.3	Computing Probability Intervals	8
4	Plotting the results		11
	4.1	CAT curves based on equal ranks using a reference.	11
	4.2	CAT curves based on equal statistics using a reference	12
	4.3	CAT curves based on equal ranks without using a reference	13
	4.4	CAT curves based on equal ranks, unspecified expected proportion of corresponding features	14
5	Sy	stem Information	15

1 Introduction

The matchBox package allows to compare ranked vectors (i.e. by differential expression) of features (i.e. genes, or probe sets), using Correspondence-At-the-Top (CAT) curves. A CAT curve displays the overlapping proportion between two ranked vectors of identifiers against the number of considered features. This techiques was originally envisaged for comparing differential gene expression results obtained from distinct microarray platforms in different laboratories (see Irizarry et al, Nat Methods (2005) [1]), and further used to compare differential gene expression across distinct studies (see Ross et al, Prostate (2011) [2], and Benassi et al., Cancer Discovery (2012) [3]).

The matchBox package contains several utilities enabling the following:

- 1. To filter redundant features in a data.frame (i.e. filtering multiple probe sets pointing to the same gene);
- 2. To merge multiple data sets together based on a common set of identifiers;
- 3. To compute the CAT curves probability intervals;
- 4. To nicely plot the CAT curves.

Briefly, CAT curves enable to compare the correspondence of two vectors ordered by a predefined ranking statistics at their top. This is accomplished through:

- 1. Ordering the two vectors according to a desired statistics (*i.e.* differential gene expression, significance, probability, . . .);
- 2. Computing the proportion of top ranking elements in common between the two vector for a given vector size (*i.e.* top 5 features);
- 3. Reiterating the two steps above increasing the vector size up to all common elements (*i.e.* the top 6 identifiers, then the top $7, 8, 9, \ldots$, all features);
- 4. Plotting the proportion of common elements against the increasing vector size.

Since CAT curves evaluate agreement at the top of ranked vectors they are particularly useful in gene expression analysis, where only a small fraction of genes is expected to be differentially expressed over the large total number of analyzed genes.

2 Preparing the Data

2.1 Data structure

Download and install the package matchBox from Bioconductor.

```
> source("http://bioconductor.org/biocLite.R")
> biocLite("matchBox")
```

Load the library.

> require(matchBox)

Load the example data contained in the matchBox package.

> data(matchBoxExpression)

The object matchBoxExpression is a list of data frames containing differential gene expression analysis results from three distinct experiments. These data frames will be used to retrieve the identifiers and the ranking statistics for computing overlapping proportions displayed by the CAT curves. Below is shown the structure of matchBoxExpression:

```
> sapply(matchBoxExpression, class)
    dataSetA
                dataSetB
                              dataSetC
"data.frame" "data.frame" "data.frame"
> sapply(matchBoxExpression, dim)
     dataSetA dataSetB dataSetC
        1375
                  2358
                          1800
[1,]
[2,]
> str(matchBoxExpression)
List of 3
$ dataSetA: 'data.frame':
                                 1375 obs. of 9 variables:
  ..$ SYMBOL : chr [1:1375] "KLK11" "CDKN1B" "LPAR1" "JAKMIP2" ...
  ..$ GENENAME : chr [1:1375] "kallikrein-related peptidase 11" "cyclin-dependent kinase inhibitor 1B
  ..$ ENTREZID : chr [1:1375] "11012" "1027" "1902" "9832" ...
              : num [1:1375] -1.99 0.974 -1.027 1.376 1.376 ...
  ..$ AveExpr : num [1:1375] 10.1 10.6 10.7 11.4 11.4 ...
              : num [1:1375] -8.13 6.96 -6.73 6.42 6.42 ...
  ..$ P.Value : num [1:1375] 8.44e-11 6.02e-09 1.37e-08 4.32e-08 4.32e-08 ...
  ..$ adj.P.Val: num [1:1375] 1.36e-07 4.45e-06 7.02e-06 1.67e-05 1.67e-05 ...
              : num [1:1375] 15.53 11.71 10.96 9.92 9.92 ...
 $ dataSetB:'data.frame':
                                 2358 obs. of 9 variables:
  ..$ SYMBOL : chr [1:2358] "KLK11" "CDKN1B" "LPAR1" "JAKMIP2" ...
  ..$ GENENAME : chr [1:2358] "kallikrein-related peptidase 11" "cyclin-dependent kinase inhibitor 1B
  ..$ ENTREZID : chr [1:2358] "11012" "1027" "1902" "9832" ...
  ..$ logFC : num [1:2358] 0.885 1.532 0.599 0.714 0.408 ...
```

```
..$ AveExpr : num [1:2358] 8.72 8.49 12.29 7.37 10.79 ...
..$ t : num [1:2358] 13.5 12.08 12.07 9.76 9.36 ...
..$ P.Value : num [1:2358] 1.88e-19 2.18e-17 2.23e-17 8.62e-14 3.81e-13 ...
..$ adj.P.Val: num [1:2358] 6.96e-16 4.06e-14 4.06e-14 6.12e-11 2.31e-10 ...
        : num [1:2358] 33.7 29.5 28.6 22.2 20.8 ...
$ dataSetC: 'data.frame':
                              1800 obs. of 9 variables:
 ..$ SYMBOL : chr [1:1800] "CD38" "C15orf61" "PECI" "PSMC2" ...
..$ GENENAME : chr [1:1800] "CD38 molecule" "chromosome 15 open reading frame 61" "peroxisomal D3,D
..$ ENTREZID : chr [1:1800] "952" "145853" "10455" "5701" ...
..$ logFC : num [1:1800] -0.699 0.24 0.366 0.405 0.442 ...
..$ AveExpr : num [1:1800] 12.2 10.8 11.5 12.1 12.6 ...
         : num [1:1800] -10.77 10.11 9.13 9.09 8.82 ...
..$ P.Value : num [1:1800] 1.14e-13 7.96e-13 1.59e-11 1.79e-11 4.11e-11 ...
..$ adj.P.Val: num [1:1800] 2.29e-10 1.05e-09 1.22e-08 1.33e-08 2.28e-08 ...
       : num [1:1800] 22.1 20.4 17.6 17.5 16.7 ...
```

2.2 Removing redundancy

In order to compute the CAT curves, and then compare the results obtained from the three experiments, it is necessary to identify the set of **non-redundant** features in common among the three data sets. The **filterRedundant** function enables to remove the redundant features within each **data.frame** prior computing the subset of common identifiers across the three data sets. The argument **idCol** specifies the index or the name of the column containing redundant identifiers, while the other arguments enable to control the method used to remove the redundancy, as explained in the help for this function.

By default the filterRedundant function will keep the feature with the largest absolute ranking statistics, as defined by the byCol argument. The method argument enables to control which feature to keep and which to discard. Currently available methods are: maxORmin, geoMean, random, mean, and median (see help for filterRedundant).

In the example below we are using an lapply call to remove the redundant features from all the three data frames at once. To this end we will use gene symbols to identify the redundant features and the t-statistics to select which feature to keep.

Each data frame now contains only unique features.

```
> sapply(allDataBySymbolAndT, dim)
```

```
dataSetA dataSetB dataSetC
[1,] 1075 2058 1500
[2,] 9 9 9
> sapply(allDataBySymbolAndT, function(x) any(duplicated(x[, 1])))
dataSetA dataSetB dataSetC
   FALSE   FALSE   FALSE
```

In the example below we are removing the redundant features using Enrez Gene identifiers to select the redundant features and the **signed** log2 fold-change to select which feature to keep.

Each of the data.frame now contains only unique features.

In the example below we are removing the redundant features using Enrez Gene identifiers to select the redundant features, and the median adjusted P-value will be used to summarize the redundant features.

Each of the data.frame now contains only unique features.

2.3 Merging the data

After removing the redundant features it is now possible to obtain the common unique features across all the data sets using the function mergeData. This function finds the intersection across all the data frames, and extract the desired ranking statistics from each one.

```
> data <- mergeData(allDataBySymbolAndT, idCol="SYMBOL", byCol="t")</pre>
```

The object data is a data.frame containing only the common set of features across all three data.frames and the ranking statistics values of choice collected from each data.frame.

```
> sapply(allDataBySymbolAndT, dim)
    dataSetA dataSetB dataSetC
[1,]
        1075
              2058
                       1500
[2,]
           9
                    9
> dim(data)
Γ17 506
> str(data)
'data.frame':
                    506 obs. of 4 variables:
$ commonID : chr "A1CF" "AARS2" "ABCB1" "ABCG8" ...
 $ dataSetA.t: num 2.82 1.91 -2.27 2.26 -1.93 ...
$ dataSetB.t: num -3.61 -2.8 -3.07 3.07 -2.81 ...
$ dataSetC.t: num -3.454 -0.319 1.324 -1.797 -4.777 ...
```

3 Correspondence at the Top Curves

The computeCat R function enables to compute the overlapping proportions of features among ranked vectors of identifiers. Such proportions will be subsequently used to produce the CAT curves. When computing CAT curves a number of parameters must be specificied to control the behaviour of the computeCat function. In particular the following information will determine how the vectors of will be ordered, and which vectors will be compaired, and therefore must be carefully considered:

- Whether or not the ranking statistics used to order the features is signed (*i.e.* t-statistics or F-statistics like);
- Whether the ranking statistics should be used to order the features by decreasing or increasing order. For instance in the case of differential gene expression between group A and B:

- Ordering by **decreasing signed** t-statistics will compute the CAT curve for the genes up-regulated in group A compared to group B;
- Ordering by **increasing signed** t-statistics will compute the CAT curve for the genes down-regulated in group A compared to group B;
- Ordering by **decreasing absolute** t-statistics will compute the CAT curve for the differentially expressed genes between the two groups;
- Ordering by **increasing absolute** t-statistics will compute the CAT curve for the genes that **are not differentially expressed** between the two groups;
- Whether to compare all possible vector combinations, or to select one of the vectors as the reference;
- Whether the overlapping proportion should be computed using equal ranks or equal statistics;

3.1 Computing CAT curves without a reference ranking

The example below shows how to compute CAT curves **without** selecting one of the vectors as the reference, using **decreasing** ranks (*i.e.* up-regulated genes are at the top of the list):

```
> catHigh2LowNoRefByEqualRanks <- computeCat(data = data, idCol = 1, method="equalRank", decreasing=TRUE)
```

The example below shows to compute CAT curves **without** selecting a vector as the reference, using **increasing** ranks (*i.e.* down-regulated genes are at the top of the list):

3.2 Computing CAT curves using a reference ranking

The example below shows how to compute CAT curves selecting one of the vectors as the reference, using decreasing ranks:

The catHigh2LowWithRefByEqualRanks contains the computed overlaps, for decreasing ranks (i.e. up-regulated genes are at the top of the list):

```
> str(catHigh2LowWithRefByEqualRanks)
```

```
List of 2
$ dataSetA.t.vs.dataSetB.t:List of 1
...$ cat: num [1:506] 0 0.5 0.333 0.5 0.6 ...
$ dataSetA.t.vs.dataSetC.t:List of 1
...$ cat: num [1:506] 0 0 0 0 0 0 0 0 0 ...
```

The example below shows to compute CAT curves selecting one of the data set as reference, using decreasing t-statistics:

```
> catHigh2LowWithRefByEqualStats <- computeCat(data = data, idCol = 1, ref="dataSetA.t", method="equalStat", decreasing=TRUE)
```

The catHigh2LowWithRefByEqualStats contains the computed overlaps: for decreasing t-statistics (i.e. down-regulated genes):

> str(catHigh2LowWithRefByEqualStats)

```
List of 2
$ dataSetA.t.vs.dataSetB.t:List of 2
...$ cat: num [1:506] 0 0 0 0 0 0 0 0 0 0 ...
..$ deg: num [1:506] 2 2 2 2 2 2 2 2 2 2 ...
$ dataSetA.t.vs.dataSetC.t:List of 2
...$ cat: num [1:506] 0 0 0 0 0 0 0 0 0 0 ...
..$ deg: num [1:506] 2 2 2 2 2 2 2 2 2 2 ...
```

3.3 Computing Probability Intervals

The calcHypPI R function enables to compute the probability intervals for the CAT curves obtained using equal ranks. Such intervals are based on the hypergeometric distribution (see Irizarry et al [1] and Ross et al. [2]). Briefly, the calcHypPI uses qhyper quantile function to compute the expected proportions of common features between two ordered vectors of identifiers for a set of specified quantiles of the hypergeometric distribution.

To understand the way such proportions are obtained we can use the analogy of drawing a certain number of balls from an urn containing black and white balls, where black represents a failure (the vectors are in different order, and therefore the features do not overlap), and white represent a success (the vectors are in the same order, and hence the features overlap). According to this analogy the calcHypPI function uses the total number of features in common between the vectors as the total number of balls in the urn, and the size of the vector being compared as the number of balls drawn from the urn. Thus increasing vectors sizes (1, 2, 3, and so on until all features are used) correspond to an increasing number of balls drawn from the urn at each attempt.

By default the calcHypPI function assumes that the top 10% of the features of the two vectors are similarly ordered. In our analogy, therefore, the number of white balls in the urn

corresponds to 10% of the total common features between the vectors. This expectation can be modified by the user with the expectedProp argument. When expectedProp is set equal to NULL the number of white balls in the urn (i.e. the top ranking features in the correct order) corresponds to the number of balls that are drawn at each attempt (i.e. the increasing size of top features from each vector that are being compared).

The example below shows how to compute probability intervals for CAT curves, using the default 0.1 expected proportion of top ranked features:

```
> PIbyRefEqualRanks <- calcHypPI(data=data)</pre>
```

The PIbyRefEqualRanks contains the computed probability intervals for the CAT curves:

> head(PIbyRefEqualRanks)

```
0.999999 0.000001
                                                                                           0.999000 0.001000 0.990000 0.010000 0.950000 0.050000

      [1,]
      0.01960784
      0
      0.01960784
      0
      0.01960784

      [2,]
      0.03921569
      0
      0.01960784
      0
      0.01960784

      [3,]
      0.05882353
      0
      0.03921569
      0
      0.03921569
      0
      0.01960784

      [4,]
      0.07843137
      0
      0.05882353
      0
      0.03921569
      0
      0.03921569

      [5,]
      0.09803922
      0
      0.07843137
      0
      0.05882353
      0
      0.03921569

      [6,]
      0.09803922
      0
      0.07843137
      0
      0.05882353
      0
      0.03921569

                                                                                                                                                                                                     0 0.01960784
0 0.01960784
                                                                                                                                                                                                                                                                                        0
                                                                                                                                                                                                                                                                                        0
                                                                                                                                                                                                               0 0.03921569
                                                                                                                                                                                                                                                                                        0
                                                                                                                                                                                                                                                                                        0
                                                                                                                                                                                                                                                                                        0
                0.500000
[1,]
[2,]
[3,]
                                        0
\lceil 4.7 \rceil
[5,]
                                       0
[6,]
```

The example below shows how to compute probability intervals for CAT curves, setting the expected proportion of top ranked features to 0.3:

```
> PIbyRefEqualRanks03 <- calcHypPI(data=data, expectedProp=0.3)
```

The PIbyRefEqualRanks03 contains the computed probability intervals for the CAT curves:

> head(PIbyRefEqualRanks03)

```
[2,] 0 0.006578947

[3,] 0 0.006578947

[4,] 0 0.006578947

[5,] 0 0.006578947

[6,] 0 0.013157895
```

The example below shows how to compute probability intervals for CAT curves, using the default 0.1 expected proportion of top ranked features, for a set of specific hypergeometric distribution quantiles:

```
> PIbyRefEqualRanksQuant <- calcHypPI(data=data, prob=c(0.75, 0.9, 0.95, 0.99))
```

The PIbyRefEqualRanksQuant contains the computed probability intervals for the CAT curves:

> head(PIbyRefEqualRanksQuant)

The example below shows how to compute probability intervals for CAT curves, without specifying a proportion of expected top ranked features:

```
> PIbyRefEqualRanksNoExpectedProp <- calcHypPI(data=data, expectedProp=NULL)
```

The PIbyRefEqualRanksNoExpectedProp contains the computed probability intervals for the CAT curves:

> head(PIbyRefEqualRanksNoExpectedProp)

```
0.999999 0.000001 0.999000 0.001000 0.990000 0.010000 0.950000 0.050000

      [1,]
      1.0000000
      0 1.0000000
      0 0.0000000
      0 0.0000000

      [2,]
      1.0000000
      0 0.5000000
      0 0.0000000
      0 0.0000000

      [3,]
      0.6666667
      0 0.33333333
      0 0.33333333
      0 0.0000000

      [4,]
      0.5000000
      0 0.2500000
      0 0.2500000
      0 0.0000000

                                                                                                                                                                       0
                                                                                                                                                                       0
[5,] 0.6000000 0 0.2000000 0 0.2000000 0 0.0000000
[6,] 0.5000000 0 0.3333333 0 0.1666667 0 0.1666667
                                                                                                                                                                         0
          0.500000
[1,]
[2,]
                         0
[3,]
                         0
[4,]
                       0
[5,]
                       0
[6,]
```

4 Plotting the results

The plotCat function can be used to plot the CAT curves along with probability intervals, as derived using the calcHypPI function (see Figures 1 and 2, for CAT curves computed using a reference ranking, Figure 3 for examples in which the reference was not used, and Figure 4 for CAT curves for which the proportion of expected top ranked features was not specified).

4.1 CAT curves based on equal ranks using a reference.

The example below shows how to plot CAT curves based on equal ranks, along with probability intervals based on a fixed expected proportion of similarly ranked features of 30%, using the data set A as the reference.

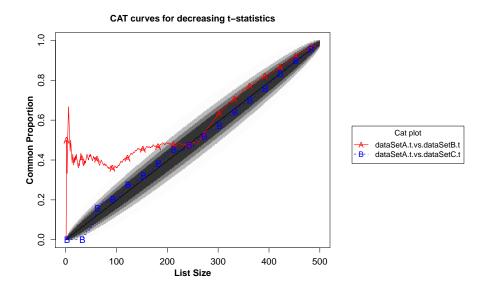


Figure 1: CAT-plot for curves based on equal ranks, using data set A as the reference. Lighter to darker grey shades represent probability intervals for distinct quantiles of the hypergeometric distribution (0.999999, 0.999, 0.99, 0.95), assuming 30% of the features to be similarly ranked.

4.2 CAT curves based on equal statistics using a reference.

The example below shows how to plot CAT curves based on equal statistics, using the data set A as the reference. When equal ranks are used each CAT curve has its own probability intervals, which therefore cannot be shown on the plot.

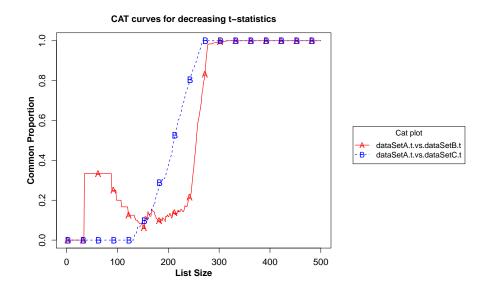


Figure 2: CAT-plot for curves based on equal t-statistics, using data set A as the reference.

4.3 CAT curves based on equal ranks without using a reference.

The example below shows how to plot CAT curves based on equal ranks, along with probability intervals based on a fixed expected proportion of similarly ranked features of 10%, without using any data set as the reference (all pair combinations are shown).

```
> plotCat(catData = catHigh2LowNoRefByEqualRanks,
           preComputedPI=PIbyRefEqualRanks,
            cex=1.2, lwd=1.2, cexPts=1.2, spacePts=30,
            main="CAT curves for decreasing t-statistics",
            where="center", legend=TRUE, legCex=1, ncol=1,
           plotLayout = layout(matrix(1:2, ncol = 2), widths = c(2,1)))
                             CAT curves for decreasing t-statistics
               1.0
               0.8
             Common Proportion
               9.0
                                                                                   Cat plot
                                                                                dataSetA.t.vs.dataSetB.t
                                                                               dataSetA.t.vs.dataSetC.tdataSetB.t.vs.dataSetC.t
               0.4
               0.2
               0.0
                             100
                                      200
                                                300
                                                          400
                                                                   500
                                         List Size
```

Figure 3: CAT-plot for curves based on equal ranks for all possible combinations of vectors. Lighter to darker grey shades represent probability intervals for the distinct quantiles of the hypergeometric distribution (0.999999, 0.999, 0.99, 0.99), assuming 10% of the features to be similarly ranked.

4.4 CAT curves based on equal ranks, unspecified expected proportion of corresponding features.

The example below shows how to plot CAT curves based on equal ranks, along with probability intervals based on an unspecified expected proportion of similarly ranked features, without using any data set as the reference (all pair combinations are shown).

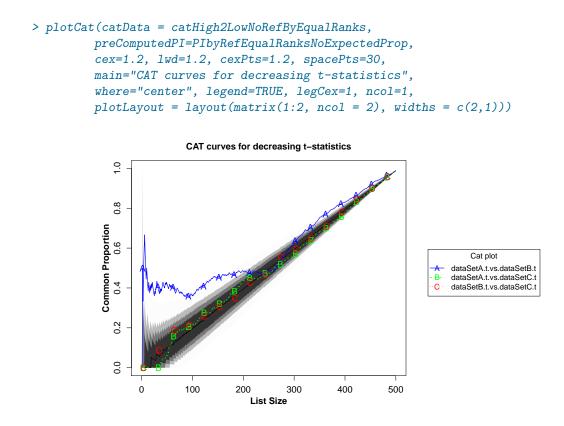


Figure 4: CAT-plot for curves based on equal ranks for all possible combinations of vectors. Lighter to darker grey shades represent probability intervals for distinct quantiles of the hypergeometric distribution (0.999999, 0.999, 0.99, 0.95). No expected proportion of similarly ranked features is specified.

5 System Information

Session information:

- > toLatex(sessionInfo())
 - R version 3.2.2 (2015-08-14), x86_64-pc-linux-gnu
- Locale: LC_CTYPE=en_US.UTF-8, LC_NUMERIC=C, LC_TIME=en_US.UTF-8, LC_COLLATE=C, LC_MONETARY=en_US.UTF-8, LC_MESSAGES=en_US.UTF-8, LC_PAPER=en_US.UTF-8, LC_NAME=C, LC_ADDRESS=C, LC_TELEPHONE=C, LC_MEASUREMENT=en_US.UTF-8, LC_IDENTIFICATION=C
- Base packages: base, datasets, grDevices, graphics, methods, stats, utils
- Other packages: matchBox 1.12.0
- Loaded via a namespace (and not attached): tools 3.2.2

6 Literature Cited

References

- [1] Rafael A Irizarry, Daniel Warren, Forrest Spencer, Irene F Kim, Shyam Biswal, Bryan C Frank, Edward Gabrielson, Joe G N Garcia, Joel Geoghegan, Gregory Germino, Constance Griffin, Sara C Hilmer, Eric Hoffman, Anne E Jedlicka, Ernest Kawasaki, Francisco Martínez-Murillo, Laura Morsberger, Hannah Lee, David Petersen, John Quackenbush, Alan Scott, Michael Wilson, Yanqin Yang, Shui Qing Ye, and Wayne Yu. Multiple-laboratory comparison of microarray platforms. *Nat Methods*, 2(5):345–350, May 2005.
- [2] Ashley E Ross, Luigi Marchionni, Milena Vuica-Ross, Chris Cheadle, Jinshui Fan, David M Berman, and Edward M Schaeffer. Gene expression pathways of high grade localized prostate cancer. *The Prostate*, February 2011.
- [3] B Benassi, R Flavin, L Marchionni, S Zanata, Y Pan, D Chowdhury, M Marani, S Strano, P Muti, and G Blandino. c-Myc is activated via USP2a-mediated modulation of microR-NAs in prostate cancer. *Cancer Discovery*, 2012.