goCluster - User Manual

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http://www.biozentrum.unibas.ch/personal/primig/gocluster/

1 Introduction

The *goCluster* package provides a modular framework that allows to combine a variety of clustering approaches with different statistical approaches. It also allows to correct for multiple testing problems. *goCluster* uses S4-methods to provide an object oriented structure which can be easily extended by new clustering algorithms, statistical procedures as well as visualization methods.

This vignette provides a short overview on *goCluster* and explains how the package can be used to combine clustering with gene ontology (GO) term analysis.

For a deeper understanding of the statistical aspect of such an analysis the interested reader is referred to the "GOStats" vignette, which explains the concept (as well as the statistical problems) of such a GO term analysis.

2 Getting started

```
> library("goCluster", verbose = FALSE)

Loading required package: Biobase

Welcome to Bioconductor

        Vignettes contain introductory material.
        To view, simply type 'openVignette()' or start with 'help(Biobase)'.
        For details on reading vignettes, see the openVignette help page.

Loading required package: YEAST

> data(benomylsetup)
> benomyldata <- benomylsetup$data$dataset[1:400, ]
```

This first step loads the package together with a prepared analysis of the "benomyl dataset" (`benomylsetup`). This dataset characterizes the stress response of yeast to the micro-tubule destabilizing drug benomyl. The original expression values are included in the configuration object. A reduced dataset (400 genes) is being extracted and stored in the variable `benomyldata`. This dataset is an `exprSet` object as defined in the core *Biobase*-package and it will be the sample dataset to demonstrate the *goCluster*-setup and analysis process. The selection of the first four hundred gene is intended to reduce the execution time for this vignette.

At this point it would be possible to run the complete analysis using the following two statements:

```
> benomyl <- new("goCluster")
> execute(benomyl) <- benomylsetup
```

This is possible because the `benomylsetup` variable is a list that holds all necessary configuration information for the *goCluster*-analysis. But since this vignette is meant to explain the usage of *goCluster* it is not very instructive to use a preconfigured setup. Instead the `config`-method provided by the *goCluster*-package will be demonstrated in the next section.

goCluster provides functions for an interactive configuration. Since interactivity is something the vignette cannot provide the configuration process in the following sections will just contain a static transcript of the steps to take.

If you do not like the commandline configuration procedure you can also test the very simple GUI provided with goCluster. It is not yet complete but you can use the following call in order to perform a complete *goCluster* analysis:

```
> result <- goClusterTk()
```

3 Configuring *goCluster*

The following six sections have to be configured for a *goCluster*-analysis:

1. Data
2. Annotation
3. Clustering
4. Significance analysis
5. Statistical analysis
6. Visualization

All steps will be performed using a single *goCluster*-object that will be created with the following statement:

```
> benomyl <- new("goCluster")
```

Using the `config`-method with this object will start the configuration process. The result of the config call is a complete *goCluster* setup object that is stored in the *benomylConfigured* variable.

```
> benomylConfigured <- config(benomyl)
```

3.1 Data

The following sections provide a transcript of the dialog after calling the `config`-method. It has been splitted to the six sections mentioned above for better readability.

```
Please select a name or title for the dataset.

# Benomyl

Please specify the variable name of the dataset to be analyzed.

# benomyldata
```

```
Please specify the variable name of the unique ID for the rows
of the dataset. Alternatively you can specify "rownames" and
the current rownames of the dataset will be used as unique ID.

# rownames
```

Here you can specify a title for the analysis. The dataset to be analyzed is specified by the name of the variable that holds the expression values. The benomyl data has been stored in the `benomyldata`-variable (see above).

Finally the unique ID has to be specified so that *goCluster* will be able to link each gene to its corresponding annotation data. Often the rows of a dataset will be labeled with the unique ID. In that case you can specify *rownames* here and *goCluster* will retrieve the information from the dataset. But you may also specify the name of a character vector that holds the unique IDs.

In case of the benomyl dataset the rownames have already been replaced with the corresponding yeast symbolic names which provides a convenient link to the *YEAST*-annotation-package available on the bioconductor website.

goCluster will check for the correct class of the dataset variable and it will also verify the length of the unique ID vector by comparing it to the size of the dataset.

3.2 Annotation

```
Please select one of the
following classes for the
annotation data:

1 ) Chromosome-abp
2 ) GO-abp

# 2

Please specify the name of the Bioconductor
meta package that applies to your dataset.
This has to be an AnnBuilder package that
holds all information to your array.
If you are using Affymetrix arrays there is
a high chance you will find a prepackaged
dataset at the Bioconductor website and for
spotted array you will have to create your
own AnnBuilder package.

# YEAST

If the unique id you specified for the
dataset is not the true linker between
genes and annotation, please specify the
linking annotation here. This is the
case for packages like hgu95av2 where the
affymetrix probe ids are the ids that
link all annotations together, but the locus
link ids are the actual ids that have been
```

```
used to establish the package in the first
place. The name you specify here needs to
yield the name of an environment together with
the package you specified the step before.
If you don't specify anything this option is disabled.

#

Please choose the type of ontology to use.
You can use the following abbreviations:
Molecular function (MF),
Biological Process (BP),
Cellular component (CC),
all three (GO).
You can select several ontologies
by separating them with a comma.

# GO

Please choose the type of GO annotations
that you are willing to accept:
IC:  inferred by curator,
IDA: inferred from direct assay,
IEA: inferred from electronic annotation,
IEP: inferred from expression pattern,
IGI: inferred from genetic interaction,
IMP: inferred from mutant phenotype,
IPI: inferred from physical interaction,
ISS: inferred from sequence or structural similarity,
NAS: non-traceable author statement,
ND:  no biological data available,
TAS: traceable author statement,
NR:  not recorded,
ALL: accept all types given above.
You can select several evidence types
by separating them with a comma. If
you need more information on the
different types, please read this page:
http://www.geneontology.org/GO.evidence.html

# ALL
```

The initial class selection lets you specify the type of annotation you want to use for the analysis. The "abp"-suffix signifies that the annotation type is dependant on an *AnnBuilder*-package corresponding to the array type the data was generated with. In the second step you will need to specify the name of this *AnnBuilder*-package.

As mentioned in the previous section the correct package for the benomyl dataset is the *YEAST*-meta-package. The gene ontology has been selected as the annotation type and the corresponding *goCluster*-class allows to exclude certain parts of the gene ontology. Here we chose to include all available information.

goCluster will automatically validate that the meta package you specified provides the necessary environments which hold all requested type of annotation data.

3.3 Clustering

```
Please select one of the
following classes for the
gene selection or clustering algorithm:

1 ) Clara
2 ) Hclust
3 ) Kmeans
4 ) Pam

# 2

Please select the agglomeration method
that should be used. For details see the
documentation to the "hclust" function (?hclust).
1 ) complete
2 ) ward
3 ) single
4 ) average
5 ) mcquitty
6 ) median
7 ) centroid

# 1

Please select the distance measure to be
used. For details see the documentation to
the "hclust" function (?hclust).
1 ) euclidean
2 ) maximum
3 ) manhattan
4 ) canberra
5 ) binary
6 ) minkowski

# 1
```

This section allows to specify the algorithm employed in order to partition the genes into groups. While *goCluster* currently only provides four different clustering algorithms it can easily be extended to utilize any kind of gene selection algorithm that will return one or several gene lists. These can then be analysed within the *goCluster*-framework.

Here we select the hierarchical clustering algorithm. The distance measure is set to euclidian and agglomeration is according to the complete linkage algorithm.

3.4 Significance Analysis

```
Please select one of the
following classes for the
```

```
significance analysis:

1 ) Base
2 ) Bonferroni
3 ) FDR

# 3

Please select how often randomization
should be performed. [0, 10000]

# 4

Please select the false discovery rate
for the selection of GO terms. [0, 1]

# 0.05
```

Here we choose the false discovery rate in order to correct for the massive multiple testing performed when testing the gene ontology terms for each of the clusters identified.

The procedure will replace each of the clusters identified with the same number of randomly selected genes. This will be repeated as often as given in the second parameter. We selected only four repetitions here in order to increase the speed of calculation but in a standard analysis the randomization should be performed around a hundred times.

The significance analysis will use the statistical function configured in the next section in order to establish a histogram over the p-values obtained. This distribution can then be combined with the FDR threshold specified by the user in order to select significant annotation terms.

Please note that this type of statistical approach is not entirely valid for an annotation type like the gene ontology (directed acyclic graph). The results should be considered to be an indication rather than mathematically precise values (the interested reader is referred to a discussion of the problem written by R. Gentleman [`http://bioconductor.org/Docs/Papers/2003/Compendium/GOstats.pdf`]). This problem does not occur in case you use an annotation type that provides terms which are independent of each other.

3.5 Significance Analysis

```
Please select one of the
following classes for the
statistical analysis algorithm:

1 ) Hyper

# 1
```

Currently you can only choose the hyper geometric distribution in order to identify the enrichment of functionally related genes within the clusters.

3.6 Visualization

```
Please select one of the
following classes for the
visualization:
```

```
1 ) Hclust
2 ) Heatmap
3 ) None

# 1
```

Since hierarchical clustering has been chosen as clustering method the corresponding visualization method is being selected.

3.7 Retrieving the configuration

The `setup`-method allows you to retrieve the configuration stored in a *goCluster*-object. The value returned will be a list that contains all relevant parameters. The following command demonstrates how you can retrieve the options set for the clustering algorithm:

```
> setup(benomylConfigured)[["algo"]]

$method
[1] "complete"

$distance
[1] "euclidean"
```

To get an overview of a *goCluster* object you can simply print the variable.

```
> print(benomylConfigured)

goCluster-Object:
-----------------

  Dataset:
  --------

    Type: Bioconductor ExprSet object

    Details:

      -> Expression Set (exprSet) with
      ->         400 genes
      ->         16 samples
      ->                  phenoData object with 0 variables and 0 cases
      ->          varLabels

  Annotation:
  -----------

    Type: Gene Ontology (GO)

    Details:

      -> GO information retrieved from meta package YEAST
      -> GO ontologies selected: MF, BP, CC
```

```
    -> Accepted type of evidence: IC, IDA, IEA, IEP, IGI, IMP, IPI, ISS, NAS, ND, TAS, NR


  Algorithm:
  ----------

    Type: Hclust

    Details:

      -> Distance measure: euclidean
      -> Clustering method: complete
      -> The object holds no result yet.


  Significance:
  -------------

    Type: False Discovery Rate

    Details:

      -> The object holds no result yet.

  Statistic:
  ----------

    Type: Hypergeometric distribution

    Details:

      -> The object holds no result yet.


  Visualization:
  --------------

    Type: Hclust

    Details:

      -> The object holds no result yet.
```

4 Executing *goCluster*

The configured object can be analysed by calling its `execute`-method.

```
> benomylExecuted <- execute(benomylConfigured)

Loading required package: GO
Building GO annotation...
```

```
Done!
Clustering the dataset.
Randomizing groups.
25 %..50 %..75 %..100 %..
Analyzing random data.

Going to analyze 1596  groups.
This might take a while...
33 %..67 %..100 %..
Analyzing original data.

Going to analyze 399  groups.
This might take a while...
Selecting significant annotations.
```

The selected (significant) gene ontology terms can be found in the `selection`-slot of the significance analysis object. This object is stored in the `sign`-slot of the main `goCluster`-object.

The object can be printed again in order to get an overview of the result.

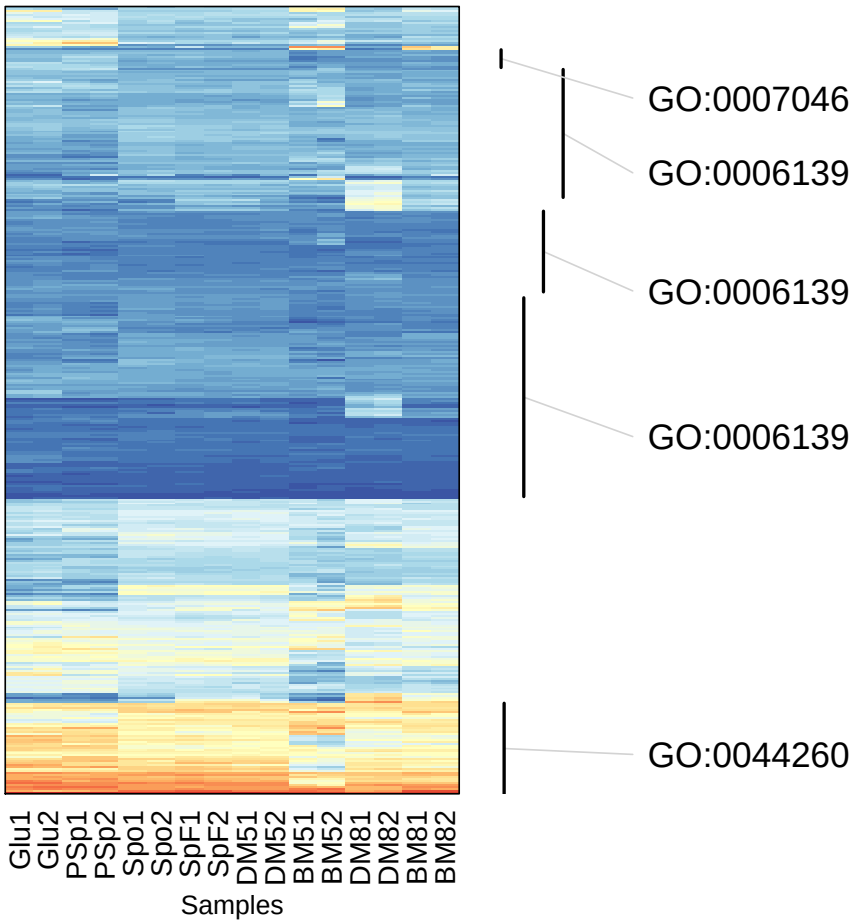
```
> print(benomylExecuted)

goCluster-Object:
-----------------

  Dataset:
  --------

    Type: Bioconductor ExprSet object

    Details:

      -> Expression Set (exprSet) with
      ->         400 genes
      ->         16 samples
      ->                 phenoData object with 0 variables and 0 cases
      ->          varLabels

  Annotation:
  -----------

    Type: Gene Ontology (GO)

    Details:

      -> GO information retrieved from meta package YEAST
      -> GO ontologies selected: MF, BP, CC
      -> Accepted type of evidence: IC, IDA, IEA, IEP, IGI, IMP, IPI, ISS, NAS, ND, TAS, NR


  Algorithm:
  ----------
```

```
  Type: Hclust

  Details:

    -> Distance measure: euclidean
    -> Clustering method: complete
    -> The dataset has been clustered.


Significance:
-------------

  Type: False Discovery Rate

  Details:

    -> The clustered data has been analyzed.
    -> A total of 1288 annotation terms have been selected

Statistic:
----------

  Type: Hypergeometric distribution

  Details:

    -> A total of 1197 clusters (this might include randomized datasets) have been analyzed.


Visualization:
--------------

  Type: Hclust

  Details:

    -> The clustered data has been prepared for visualization.
    -> A total of 3 annotation types can be visualized.
```

5 Visualizing the result

The result can be visualized using the *display* function. The resulting plot depends on the visualization function chosen earlier and will be a hierarchical clustering in this case. The display function can take different arguments depending on the type of function selected. In case of the hierarchical clustering display it is necessary to choose the type of GO annotation that should be displayed alongside the plot.

```
> try(display(benomylExecuted, selection = 2))
```



In this type of plot *goCluster* highlights regions in which an annotation term was found to be significantly overrepresented. In case such regions overlap, only the term with the lowest p-value will be displayed.

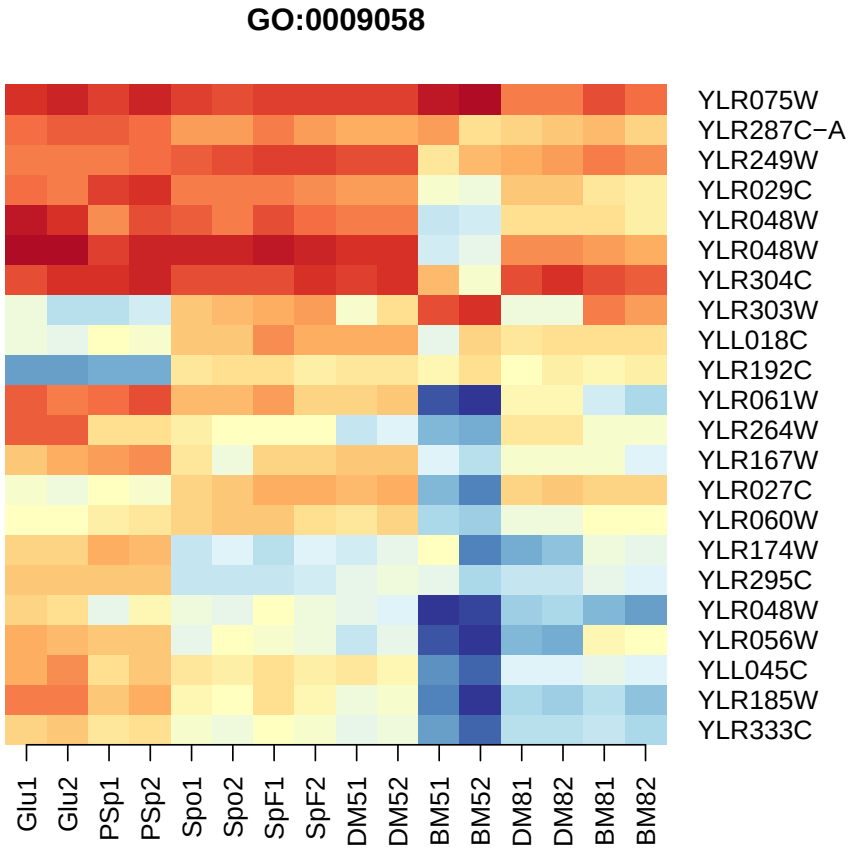
In this case *goCluster* only identifies a small amount of terms since we initially reduced the dataset to only 400 genes.

In order to display one of the identified GO terms the visualization method can be switched to the heatmap. *benomylExecuted* is copied to *benomylExecuted2* and the visualization method is replaced in this new object by swapping the *visu* slot to a new object of the type *clusterVisualHeatmap*.

```
> benomylExecuted2 <- benomylExecuted
> benomylExecuted2@visu <- new("clusterVisualHeatmap")
> benomylExecuted2@visu <- execute(benomylExecuted2@visu, benomylExecuted2)
```

After executing just this subobject again, the same dataset can be visualized in a different manner. This time the selection variable isolates a specific GO term. The genes which were responsible for the identification of the GO term as being overrepresented in one of the clusters will be displayed in a heatmap.

```
> try(display(benomylExecuted2, selection = "GO:0009058"))
```



6 Appendix: Verifying the *goCluster* results against *GOstats*

The central function used in goCluster (`clusterStatisticHyper`) has been derived from the GOHyperG function provided by the `GOstats` package. But the function in `goCluster` had to be modified to work with any type of annotation. The following is a small test script that verifies that the modifications did not impair the result of the calculations and that both functions result in the same values if applied to the same problem.

All necessary libraries are being loaded:

```
> require(GOstats)
> require(goCluster)
> require(hgu95av2)
> require(GO)
```

The example from the `GOHyperG` function is being executed now. This example assumes a that we used a "hgu95av2" chip from Affymetrix and randomly selects 100 genes from the array. The `GOHyperG` function will subsequently be used to determine if any gene ontology terms occur more frequent among the annotation to these 100 genes than expected by chance alone. There should be no such annotations since the genes were randomly selected but this is not important for the comparison since we will directly compare the calculated p-values.

```
> w1 <- as.list(hgu95av2LOCUSID)
> w2 <- unique(unlist(w1))
> set.seed(123)
> myLL <- sample(w2, 100)
```

The list of genes is stored in the myLL variable.

There are only very specific parts of the goCluster framework necessary for the comparison. Since no actual dataset has been defined the necessary parts will be generated seperately so that it is possible to omit specifying any real data for goCluster.

So these will be the parts for the mock goCluster object:

```
> gC <- new("goCluster")
> data <- new("clusterData")
> anno <- new("clusterAnnotationGO-abp")
> cluster <- new("clusterAlgorithm")
> stats <- new("clusterStatisticHyper")
```

It is sufficient to specify the unique ids of the affymetrix array in the data object:

```
> data@uniqueid <- names(w1)
```

The annotation object needs the complete configuration. It is set to the "hgu95av2" array and will only selected entries from the "molecular function" ontology of GO. The object needs to be executed in order to actually prepare the annotation data for analysis.

```
> setup(anno) <- list(meta = "hgu95av2", ontologies = "MF", evidence = EVIDENCECODES,
+     trueid = "LOCUSID")
> gC@data <- data
> anno2 <- execute(anno, gC)
```

The selected list of 100 genes is stored in the clusterAlgorithm object and does contain the indexes of the genes that corresponds to the list of unique ids stored in the data object.

```
> cluster@clusterset <- list(match(myLL, unlist(w1)))
```

The three objects that have been created can now be assembled to a complete goCluster object:

```
> gC@data <- data
> gC@data@anno <- anno2
> gC@algo <- cluster
```

And finally we execute the GOHyperG and the clusterStatisticHyper functions. The results are stored in the res1 and the res2 variable respectively.

```
> xx <- GOHyperG(myLL)
> compare <- execute(stats, gC)
> res1 <- xx$pvalues
> res2 <- compare@statset[[1]][[1]]
```

The final comparison should result in a TRUE statement, indicating that all p-values generated by the two functions match:

```
> all(res2[match(names(res1), names(res2))] == res1)
```

```
[1] NA
```