clusterProfiler: an R package for Statistical Analysis and Visualization of Functional Profiles for Genes and Gene Clusters

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July 20, 2011

1 Introduction

In recently years, high-throughput experimental techniques such as microarray and mass spectrometry can identify many lists of genes and gene products. The most widely used strategy for high-throughput data analysis is to identify different gene clusters based on their expression profiles. Another commonly used approach is to annotate these genes to biological knowledge, such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG), and identify the statistically significantly enriched categories. These two different strategies were implemented in many bioconductor packages, such as *Mfuzz* and *BHC* for clustering analysis and *GOstats* for GO enrichment analysis.

After clustering analysis, researchers not only want to determine whether there is a common theme of a particular gene cluster, but also to compare the biological themes among gene clusters, which have different expression profiles. There is no existing tools to bridge this gap, and we designed *clusterProfiler*, for comparing functional profiles among gene clusters.

This document presents an introduction to the use of *clusterProfiler*, an R package for the analysis of lists of genes and gene clusters based on their GO annotation distribution or enrichment categories of GO and KEGG, and provides methods for visulization.

2 Quick start

The following lines provide a quick and simple example on the use of *clusterProfiler* to explore gene list and compare gene clusters.

The analysis proceeds as follows:

- First a sample dataset is loaded. This dataset contains 5 gene clusters.

```
> require(clusterProfiler)
> data(gcSample)
> gcSample

$C1
 [1] "23753" "57222" "5036"  "5037"  "10111" "10856" "6228"
 [8] "9361"  "1537"  "3376"  "6124"  "4175"  "2539"

$C2
```

```
> print(plot(xx, type = "dot", title = "MF Ontology Distribution Comparison"))
```

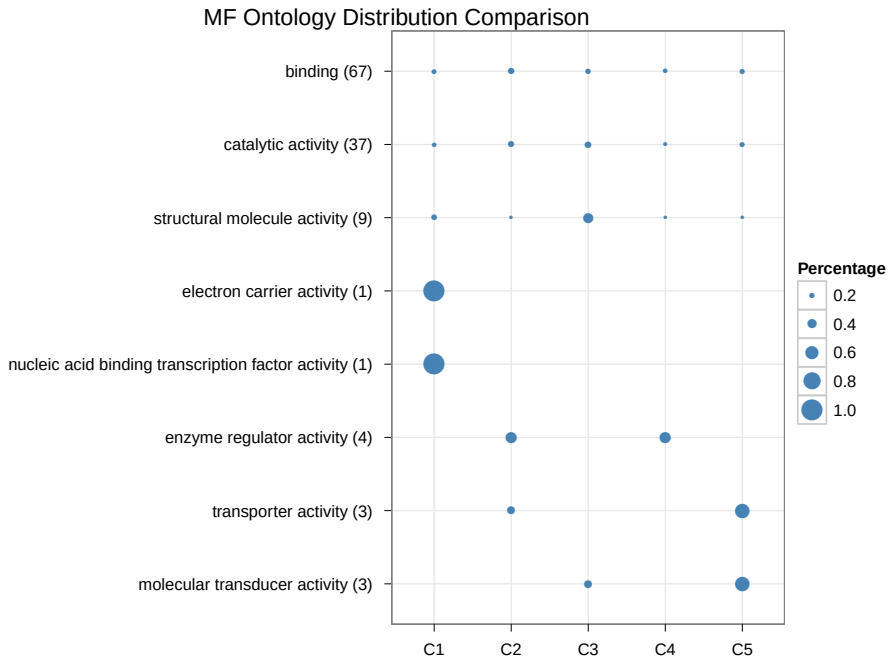

Figure 1: Example of comparing MF ontology distribution using dotplot.

```
 [1] "6629"  "10291" "7094"  "3843"  "6611"  "10399" "10576"
 [8] "4705"  "5216"  "6697"  "5868"  "80777" "1973"  "1938"
[15] "23450" "9343"  "1917"  "9520"

$C3
 [1] "4905"   "10383"  "10953"  "645958" "7280"   "10381"
 [7] "5869"   "5985"   "23197"  "290"    "309"    "10577"
[13] "23071"  "121504" "2495"   "653226" "84617"

$C4
 [1] "51552" "8336"  "302"   "5984"  "50814" "8813"  "871"
 [8] "81"    "23344" "4134"  "10262" "22919" "159"

$C5
 [1] "11171"  "8243"   "112464" "2194"   "9318"   "79026"
 [7] "1654"   "65003"  "6240"   "3476"   "6238"   "3836"
[13] "4176"   "1017"   "249"
```

- GO distribution among a set of gene clusters can be compared by *compareCluster*, and plotted by bar chart or dot chart.

```
> xx <- compareCluster(gcSample, fun = groupGO,
+     organism = "human", ont = "MF", level = 2)
```

By default, only top 5 categories of each cluster was plotted. User can change the parameter *limit* to specify how many categories of each cluster to be plotted, and if *limit* set to NULL, the whole result will be plotted. By default, the dot sizes were based on their corresponding row percentage, and user can set the parameter *by* to "count" to make the comparison based on gene counts.

We chose "percentage" as default parameter to represent the sizes of dots, since some categories may contain a large number of genes, and make the dot sizes of those small categories too small to compare. To provide the full information, we also provide number of identified genes in each category (numbers in parentheses), as shown in Figure 1. If the dot sizes were based on "count", the parentheses will not shown.

- GO or KEGG enrichment analysis among a set of gene clusters can also be compared by *compareCluster* as shown in the following examples.

```
> xx <- compareCluster(gcSample, fun = enrichGO,
+     organism = "human", ont = "CC", pvalueCutoff = 0.01)
> head(summary(xx))
```

```
  Cluster       GOID                   Description
1      C1 GO:0044446  intracellular organelle part
2      C1 GO:0044422               organelle part
3      C1 GO:0030529     ribonucleoprotein complex
4      C1 GO:0070013 intracellular organelle lumen
5      C1 GO:0043233              organelle lumen
6      C1 GO:0031974       membrane-enclosed lumen
  GeneRatio    BgRatio       pvalue       qvalue
1     12/13 5351/16589 1.149368e-05 0.0005024969
2     12/13 5424/16589 1.344710e-05 0.0005024969
3      4/13  517/16589 5.327956e-04 0.0079149313
4      7/13 2172/16589 5.441960e-04 0.0079149313
5      7/13 2214/16589 6.132116e-04 0.0079149313
6      7/13 2252/16589 6.816604e-04 0.0079149313
                                                        geneID
1 23753/57222/5036/5037/10111/10856/6228/9361/1537/6124/4175/2539
2 23753/57222/5036/5037/10111/10856/6228/9361/1537/6124/4175/2539
3                                          5036/10856/6228/6124
4                          23753/5036/10111/10856/9361/6124/4175
5                          23753/5036/10111/10856/9361/6124/4175
6                          23753/5036/10111/10856/9361/6124/4175
  Count
1    12
2    12
3     4
4     7
5     7
6     7
```

The p-values indicate that which categories are more likely to have biological meanings. The dots in the image are color-encoded based on their corresponding p-values. Color gradient ranging from blue to red correspond to in order of increasing p-values. Blue indicate lower p-values, and red indicate higher p-values. P-values were filtered out by the threshold giving by parameter *pvalueCutoff*.

We also provide q-values, which were calculated by *qvalue*, for user to control false discovery rate. FDR control is necessary since enrichment analysis carrying out hundreds, if not thousands, of tests.

```
> print(plot(xx, title = "CC Ontology Enrichment Comparison"))
```

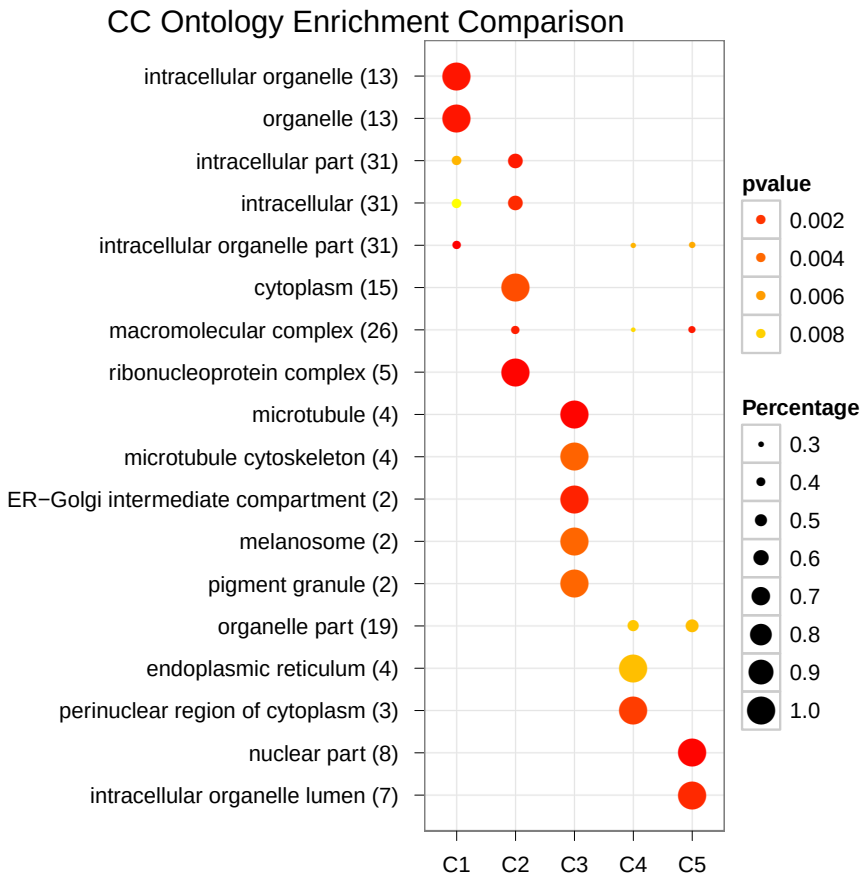

Figure 2: Example of comparing CC ontology enrichment among gene clusters.

```
> print(plot(xx, type = "bar", by = "count", title = "CC Ontology Enrichment Comparison
```

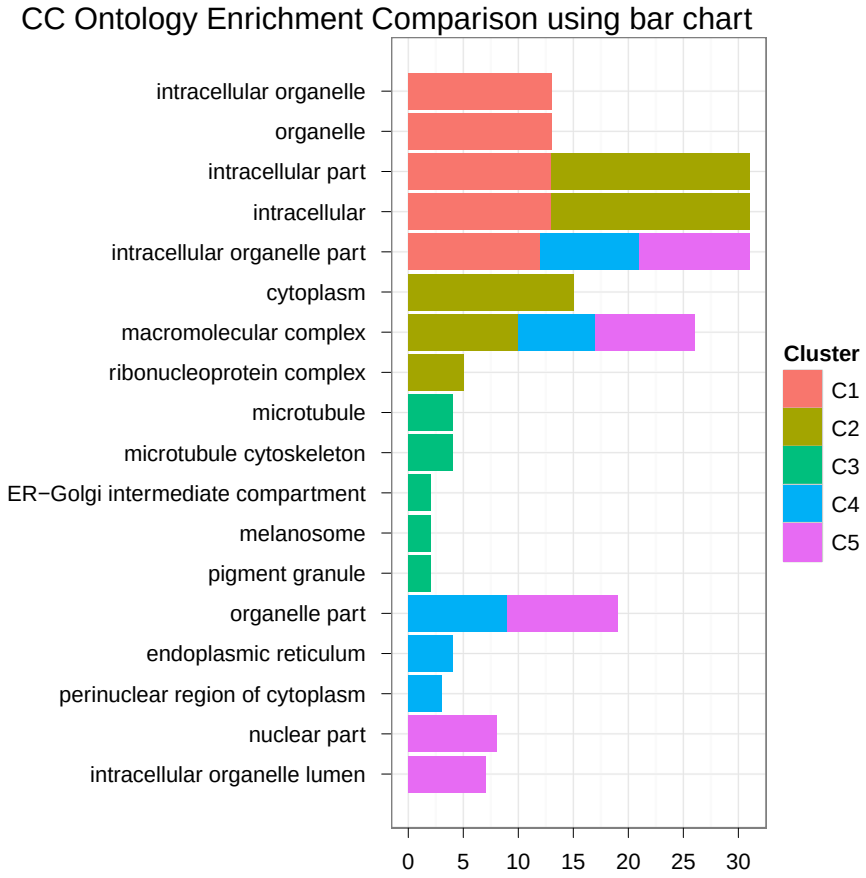

Figure 3: Example of comparing CC ontology enrichment among gene clusters.

```
> xx <- compareCluster(gcSample, fun = enrichKEGG,
+     organism = "human", pvalueCutoff = 0.05)
> head(summary(xx))

  Cluster pathwayID
1      C1  hsa03010
2      C1  hsa00290
3      C1  hsa03450
4      C2  hsa03040
5      C2  hsa00790
6      C3  hsa05130
                                  Description GeneRatio
1                                   Ribosome      2/13
2 Valine, leucine and isoleucine biosynthesis      1/13
3                 Non-homologous end-joining      1/13
4                                 Spliceosome      4/18
5                          Folate biosynthesis      1/18
6       Pathogenic Escherichia coli infection      4/17
   BgRatio       pvalue       qvalue                 geneID
1  92/5894 1.680998e-02 0.1283081428              6228/6124
2  11/5894 2.401632e-02 0.1283081428                   3376
3  14/5894 3.047318e-02 0.1283081428                  10111
4 128/5894 5.127898e-04 0.0097160175  6629/10291/23450/9343
5  11/5894 3.311286e-02 0.3137008120                   6697
6  58/5894 1.826892e-05 0.0002115348 10383/7280/10381/84617
  Count
1     2
2     1
3     1
4     4
5     1
6     4
```

- The internal functions for annotating gene and enrichment analysis was `groupGO`, `enrichGO` and `enrichKEGG`, which was designed to analyze one particular gene list. Gene list can be projected to GO at a given level by `groupGO`. GO enrichment analysis were also provided by `enrichGO` for exploring biological themes of a given gene list. The internal algorithm in `enrichGO` was `hyperGTest` provided by *Category*. `enrichGO` extend *GOstats*(Falcon et al., 2007) by providing corresponding enrichment gene list and the q-values. KEGG enrichment analysis were also supported by `enrichKEGG`.

```
> yy <- groupGO(gcSample[[1]], organism = "human",
+     ont = "BP", level = 2)
> yy <- enrichGO(gcSample[[1]], organism = "human",
+     ont = "BP", pvalueCutoff = 0.01)

> yy <- enrichKEGG(gcSample[[3]], organism = "human",
+     pvalueCutoff = 0.01)
> head(summary(yy))

      pathwayID                          Description
05130  hsa05130    Pathogenic Escherichia coli infection
04145  hsa04145                            Phagosome
```

```
> print(plot(xx, type = "dot", title = "KEGG Enrichment Comparison"))
```

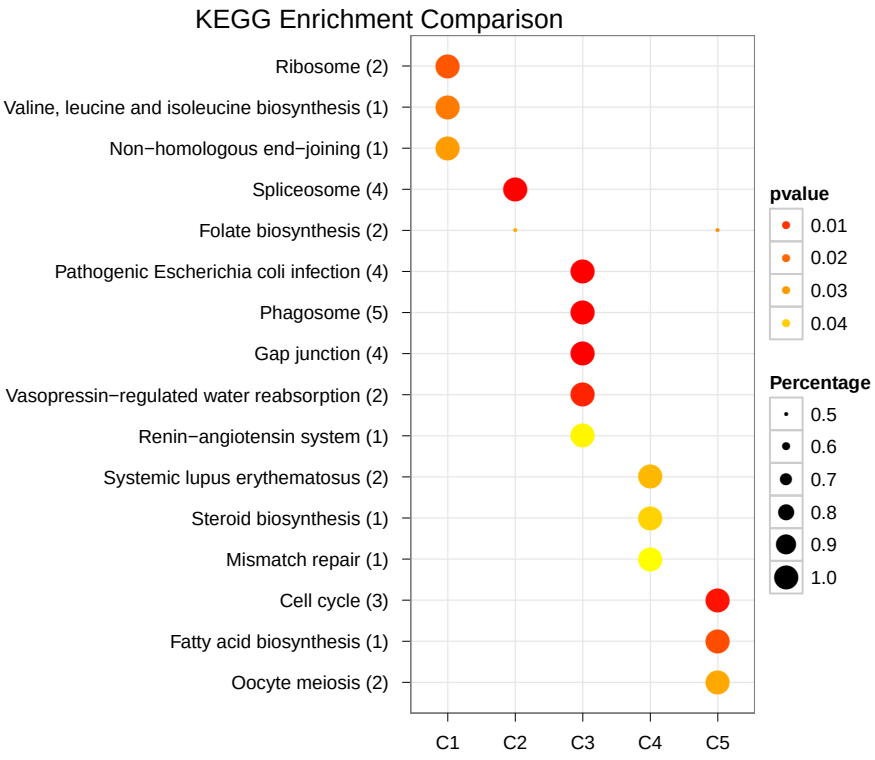

Figure 4: Example of comparing KEGG enrichment among gene clusters.

```
> print(plot(yy, title = "KEGG Enrichment Analysis"))
```

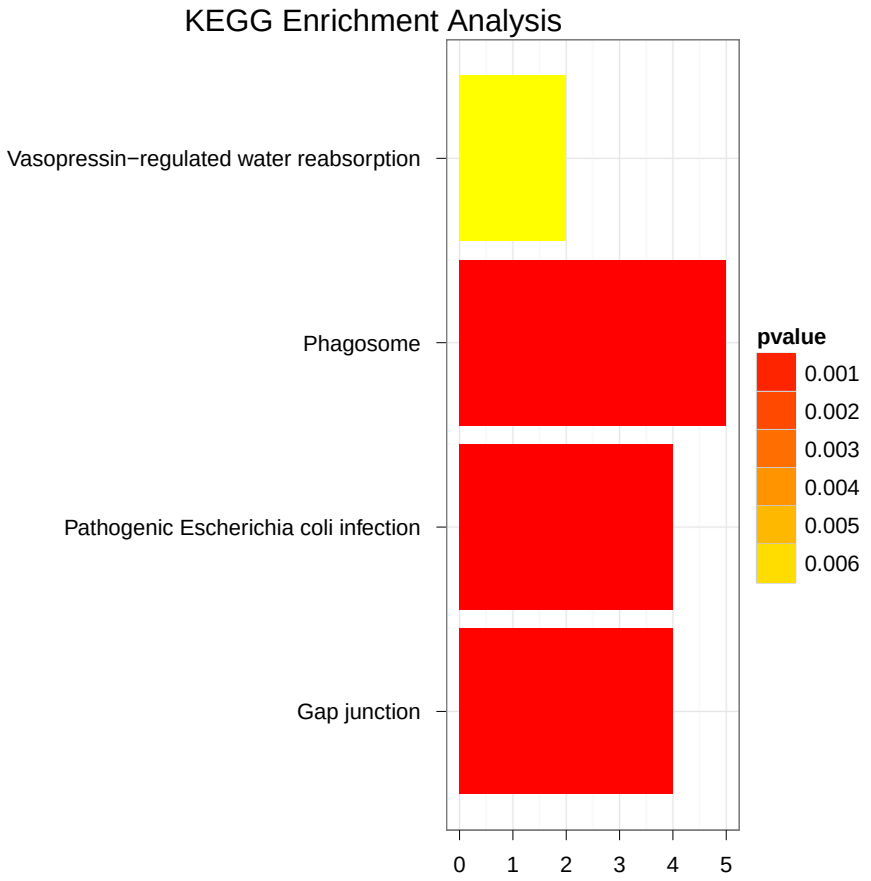

Figure 5: Example of KEGG Enrichment Analysis.

```
04540  hsa04540                                Gap junction
04962  hsa04962 Vasopressin-regulated water reabsorption
      GeneRatio  BgRatio       pvalue       qvalue
05130      4/17  58/5894 1.826892e-05 0.0002115348
04145      5/17 156/5894 5.827611e-05 0.0003373880
04540      4/17  90/5894 1.039489e-04 0.0004012064
04962      2/17  44/5894 6.898981e-03 0.0199707355
                          geneID Count
05130      10383/7280/10381/84617     4
04145 10383/7280/10381/5869/84617     5
04540      10383/7280/10381/84617     4
04962                  4905/5869     2
```

The outputs of `groupGO`, `enrichGO` and `enrichKEGG` can also be visualized by `plot`.

3 Session Information

The version number of R and packages loaded for generating the vignette were:

```
R version 2.13.0 (2011-04-13)
Platform: i686-pc-linux-gnu (32-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
 [5] LC_MONETARY=C              LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] grid      stats     graphics  grDevices utils
[6] datasets  methods   base

other attached packages:
 [1] GO.db_2.5.0            AnnotationDbi_1.14.0
 [3] Biobase_2.12.0         clusterProfiler_1.1.20
 [5] RSQLite_0.9-4          DBI_0.2-5
 [7] ggplot2_0.8.9          proto_0.3-9.1
 [9] reshape_0.8.4          plyr_1.5.1

loaded via a namespace (and not attached):
[1] KEGG.db_2.5.0       digest_0.4.2
[3] org.Hs.eg.db_2.5.0  org.Mm.eg.db_2.5.0
[5] org.Sc.sgd.db_2.5.0 qvalue_1.26.0
[7] tcltk_2.13.0        tools_2.13.0
```

References

S. Falcon, , and R. Gentleman. Using gostats to test gene lists for go term association. *Bioinformatics*, 23: 257–258, 2007.