

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

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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* (Falcon et al., 2007) 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. To bridge this gap, 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 visualization.

2 Quick start

The following lines provide a quick and simple example on the use of *clusterProfiler* to explore a set of genes 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
```

```
[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"
```

- Use `groupGO` for genes classification based on GO distribution at a specific level.

```
> x <- groupGO(gene=gcSample[[1]], organism="human", ont="CC", level=2, readable=T)
> summary(x)
```

	GOID	Description	Count
GO:0005576	GO:0005576	extracellular region	1
GO:0005623	GO:0005623	cell	13
GO:0019012	GO:0019012	virion	0
GO:0030054	GO:0030054	cell junction	1
GO:0031974	GO:0031974	membrane-enclosed lumen	7
GO:0032991	GO:0032991	macromolecular complex	6
GO:0043226	GO:0043226	organelle	13
GO:0044421	GO:0044421	extracellular region part	1
GO:0044422	GO:0044422	organelle part	12
GO:0044423	GO:0044423	virion part	0
GO:0044456	GO:0044456	synapse part	1
GO:0044464	GO:0044464	cell part	13
GO:0045202	GO:0045202	synapse	1
GO:0055044	GO:0055044	symplast	0

```
GO:0005576
GO:0005623 SDF2L1/ERGIC1/PA2G4/PEBP1/RAD50/RUVBL2/RPS23/LONP1/CYC1/IARS/RPL4/MCM6/
GO:0019012
GO:0030054
GO:0031974 SDF2L1/PA2G4/RAD50/RUVBL2/LONP1/RPL4/
GO:0032991 PA2G4/RAD50/RUVBL2/RPS23/RPL4/
GO:0043226 SDF2L1/ERGIC1/PA2G4/PEBP1/RAD50/RUVBL2/RPS23/LONP1/CYC1/IARS/RPL4/MCM6/
GO:0044421 P
GO:0044422 SDF2L1/ERGIC1/PA2G4/PEBP1/RAD50/RUVBL2/RPS23/LONP1/CYC1/RPL4/MCM6/
GO:0044423
GO:0044456 P
GO:0044464 SDF2L1/ERGIC1/PA2G4/PEBP1/RAD50/RUVBL2/RPS23/LONP1/CYC1/IARS/RPL4/MCM6/
```

GO:0045202
GO:0055044

P

- Use `enrichGO` for GO enrichment analysis.

```
> y <- enrichGO(gene=gcSample[[2]], organism="human", ont="MF", pvalueCutoff=0.01,
```

- Use `enrichKEGG` for KEGG pathway enrichment analysis.

```
> z <- enrichKEGG(gene=gcSample[[3]], organism="human", pvalueCutoff=0.05, readable=TRUE)  
> summary(z)
```

	pathwayID		Description	
05130	hsa05130	Pathogenic	Escherichia coli infection	
04145	hsa04145		Phagosome	
04540	hsa04540		Gap junction	
04962	hsa04962	Vasopressin-regulated	water reabsorption	
04614	hsa04614		Renin-angiotensin system	
	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
04614	1/17	17/5894	4.798133e-02	0.1111146614
	geneID	Count		
05130	TUBB2C/TUBB2A/TUBB3/TUBB6	4		
04145	TUBB2C/TUBB2A/TUBB3/RAB5B/TUBB6	5		
04540	TUBB2C/TUBB2A/TUBB3/TUBB6	4		
04962	NSF/RAB5B	2		
04614	ANPEP	1		

The input parameters of `gene` is a vector of entrez genes or ORF IDs (for yeast), and `organism` must be one of "human", "mouse", and "yeast", according to the gene IDs. For GO analysis, `ont` must be assigned to one of "BP", "MF", and "CC" for biological process, molecular function and cellular component, respectively. In `groupGO`, the `level` specify the GO level for gene projection. In enrichment analysis, the `pvalueCutoff` is to restrict the result based on their pvalues. Consider multiple testing, qvalues are also provided, for estimating FDR. The `readable` is a logical parameter, if TRUE, the gene IDs will map to gene symbols.

In addition, these results can be visualized by our `plot` function. For example:

```
> plot(x, title="CC Ontology Classification, level 2", font.size=12)
```

```
> plot(z, title="KEGG Enrichment")
```

- 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)  
> plot(xx, title="MF Ontology Distribution Comparison")
```

```
> yy <- compareCluster(gcSample, fun=enrichGO, organism="human", ont="CC", pvalueCutoff=0.05, readable=TRUE)  
> plot(yy, title="CC Ontology Enrichment Comparison")
```

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

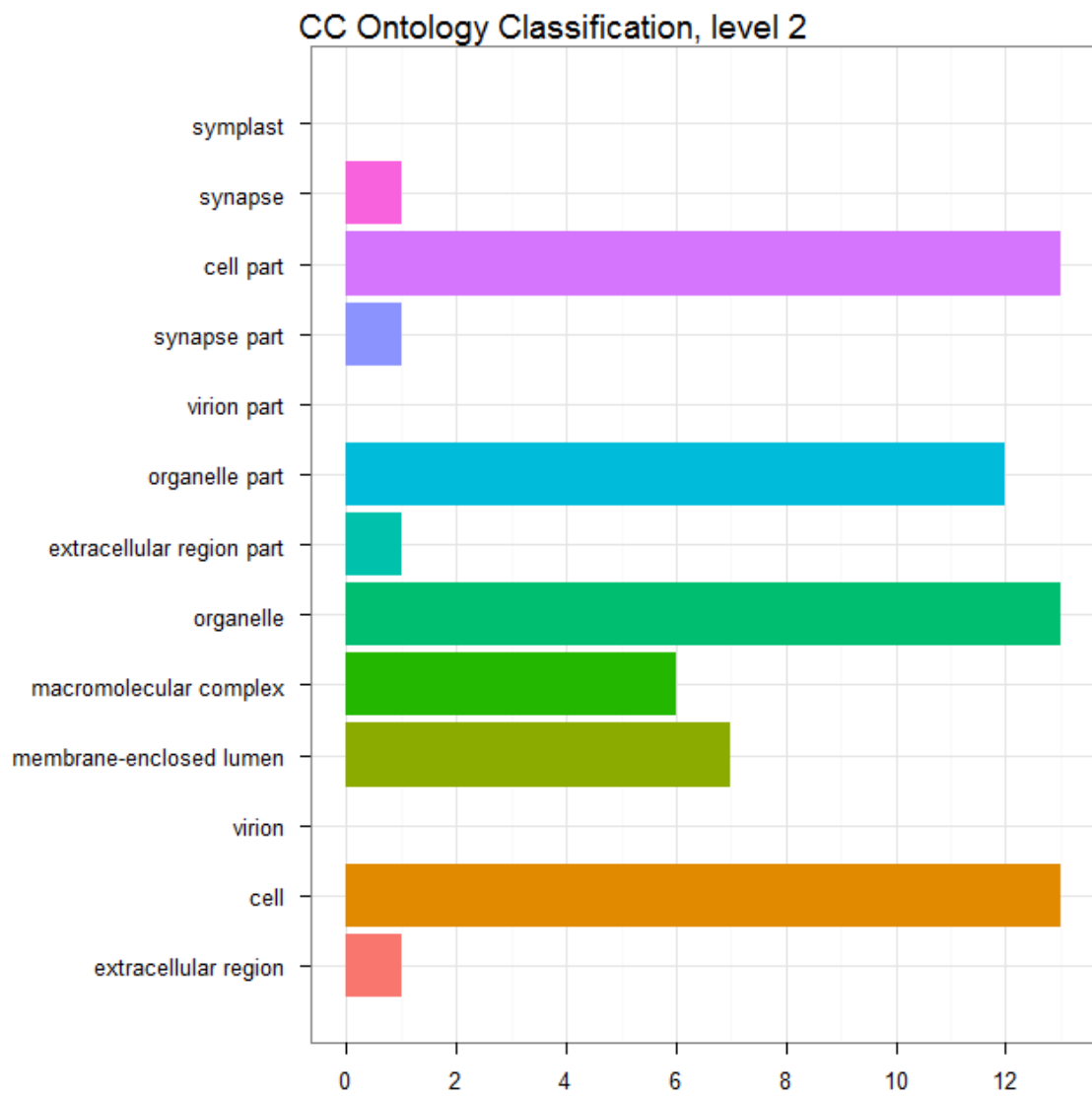


Figure 1: Example of gene classification

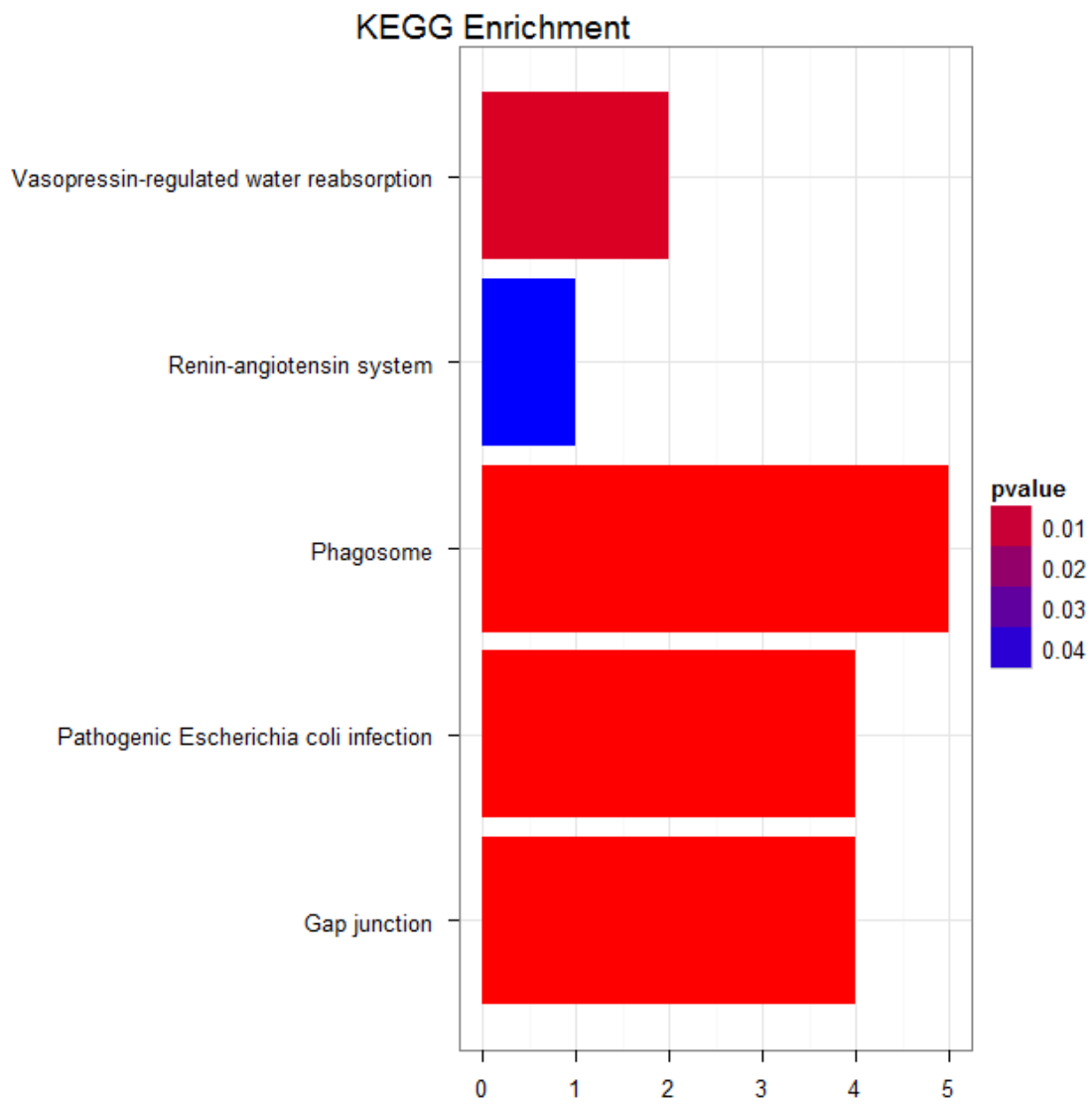


Figure 2: Example of KEGG enrichment analysis

MF Ontology Distribution Comparison

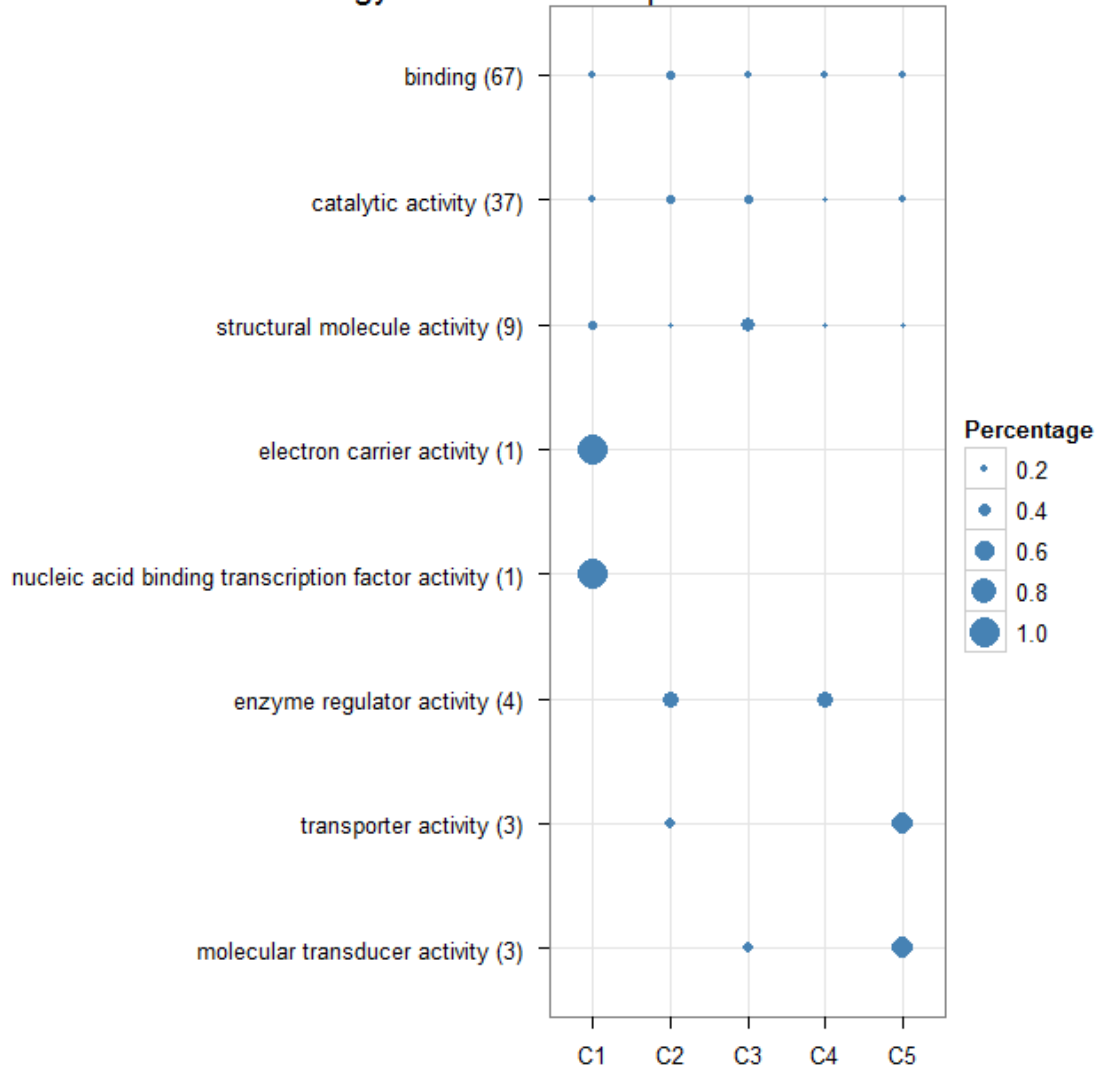


Figure 3: Example of comparing MF ontology distribution using dotplot

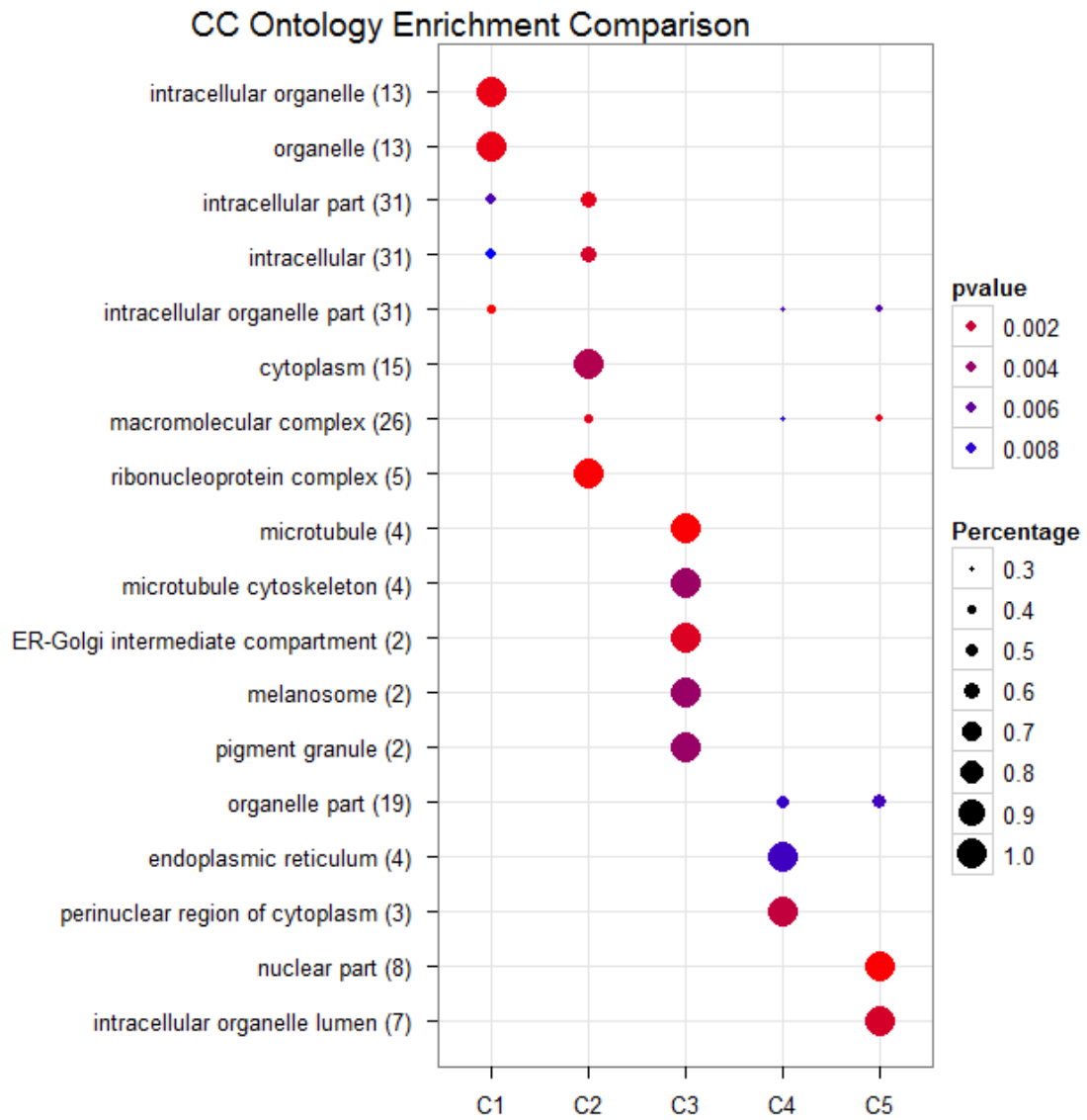


Figure 4: Example of comparing CC ontology enrichment using dot chart

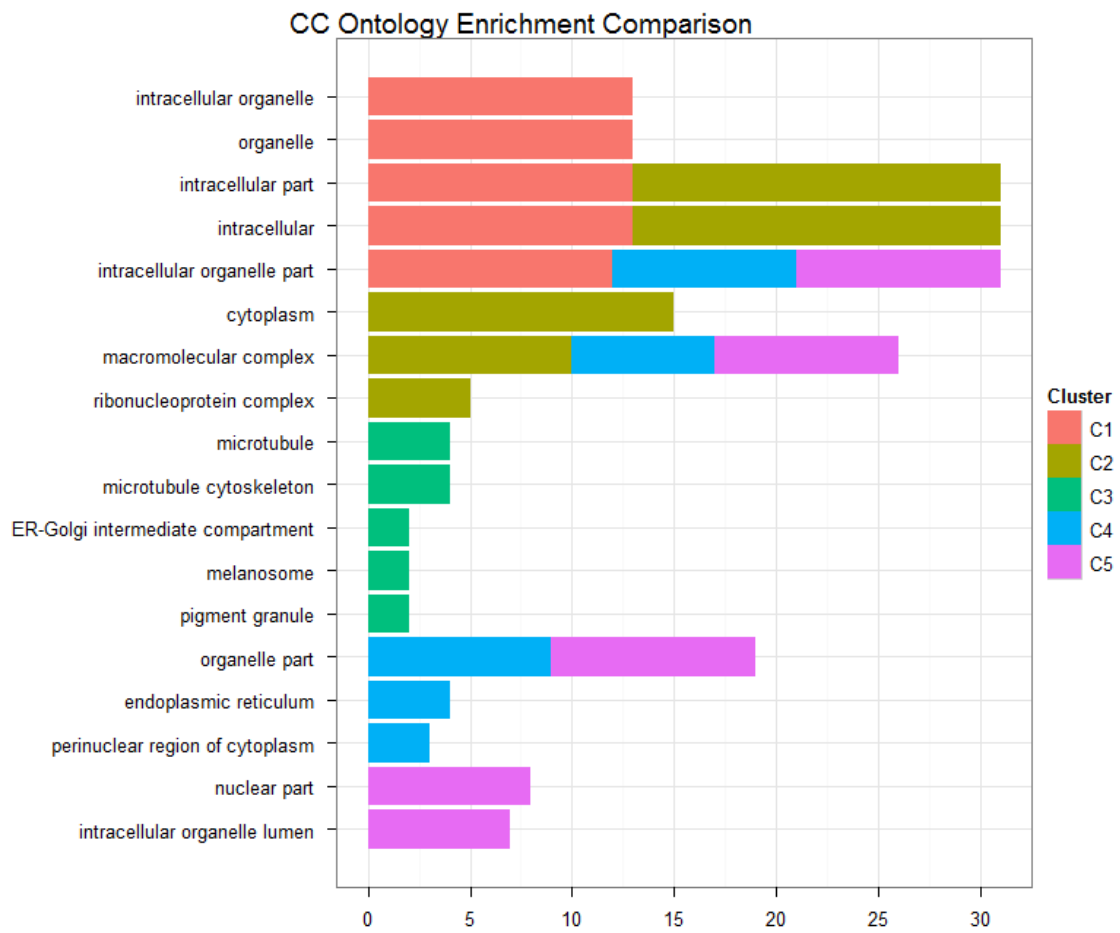


Figure 5: Example of comparing CC ontology enrichment using bar chart

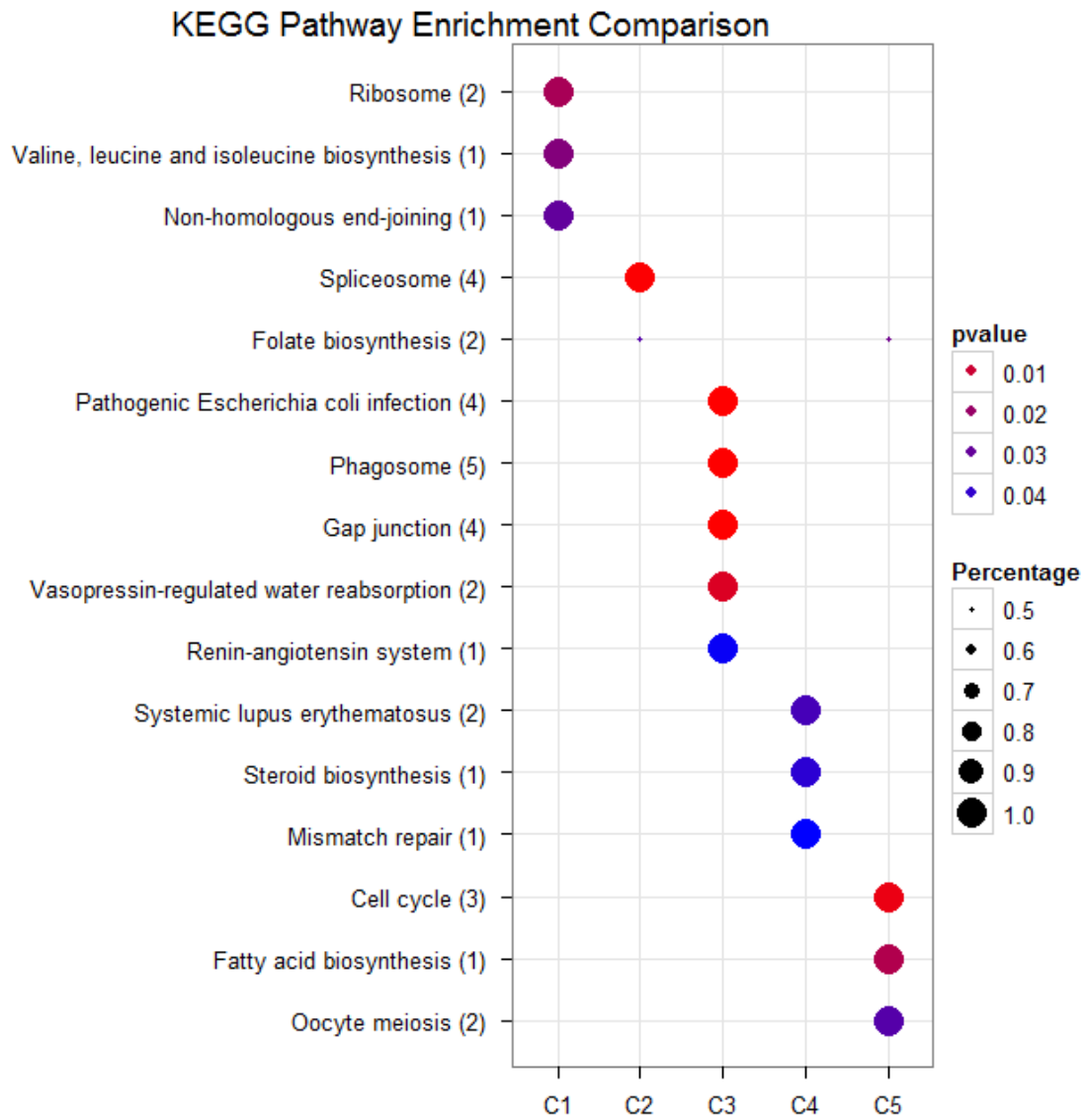


Figure 6: Example of comparing KEGG enrichment among gene clusters

```
> zz <- compareCluster(gcSample, fun=enrichKEGG, organism="human", pvalueCutoff=0.
> plot(zz, title="KEGG Pathway Enrichment Comparison")
```

By default, only top 5 categories of each cluster was plotted. User can changes 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.

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 red to blue correspond to in order of increasing p-values. red indicate low p-values (high enrichment), and blue indicate high p-values (low enrichment). 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.

- Use `compareCluster` as a general function for comparing gene clusters of other kind of gene-ontology associations.

clusterProfiler can cooperates with other R package for comparing other ontology analyzed results.

For example, `compareCluster` can call function provided by *DOSE* to compare gene-disease associations among gene clusters. Comparing gene clusters in the context of disease is a critical step in translating molecular findings from microarray, proteomics and other high-throughput methods into clinical relevance.

```
> require(DOSE)
> do <- compareCluster(gcSample, fun=enrichDO, organism="human", pvalueCutoff=0.05
> plot(do, title="Disease Ontology Enrichment Comparison")
```

3 Session Information

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

```
R version 2.14.1 (2011-12-22)
Platform: x86_64-unknown-linux-gnu (64-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=en_US.UTF-8  LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=C                LC_NAME=C
 [9] LC_ADDRESS=C             LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

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

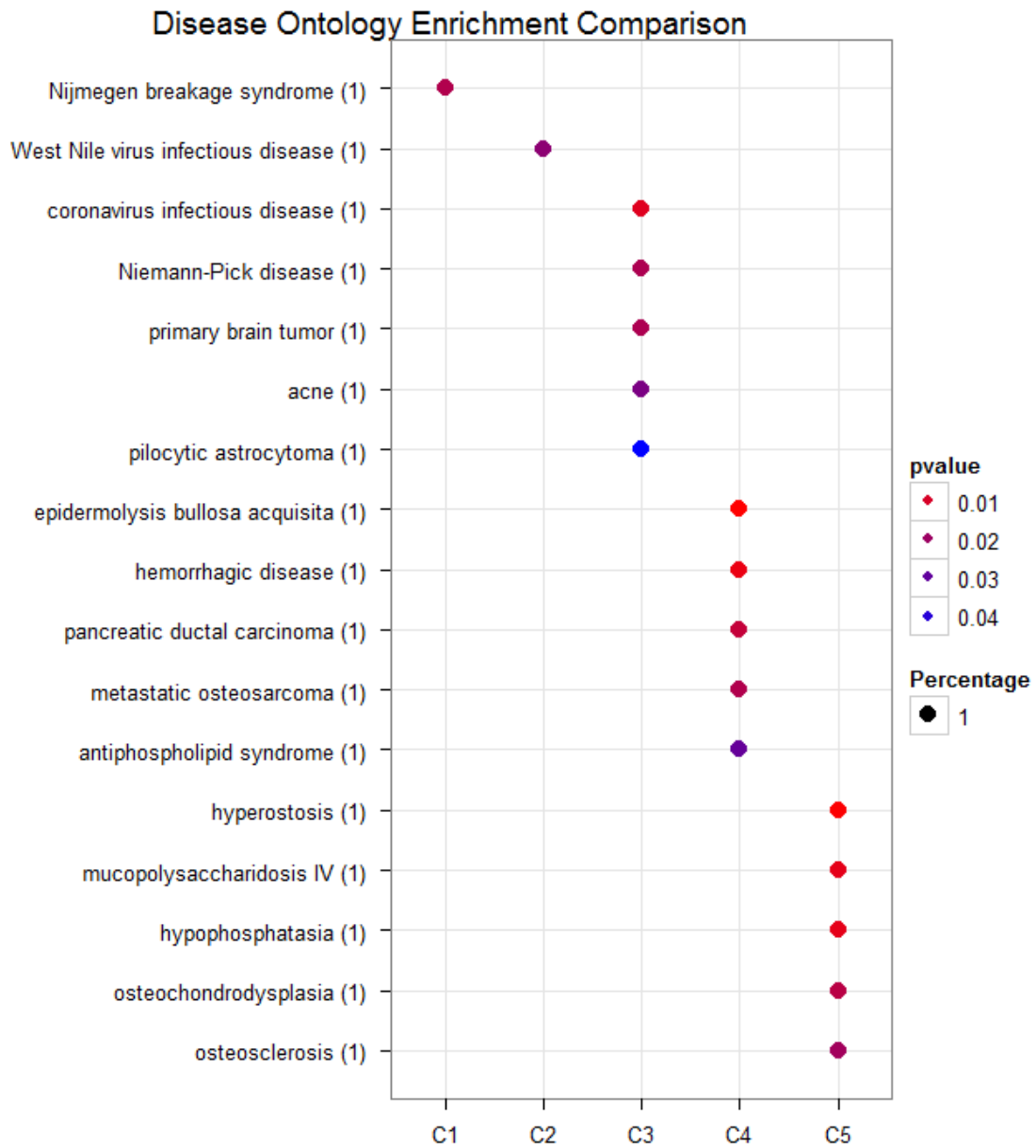


Figure 7: Example of comparing disease ontology enrichment among gene clusters

other attached packages:

```
[1] clusterProfiler_1.2.1 AnnotationDbi_1.16.15
[3] Biobase_2.14.0          RSQLite_0.11.1
[5] DBI_0.2-5               plyr_1.7.1
[7] ggplot2_0.9.0
```

loaded via a namespace (and not attached):

```
[1] GO.db_2.6.1             IRanges_1.12.6
[3] KEGG.db_2.6.1          MASS_7.3-17
[5] RColorBrewer_1.0-5     colorspace_1.1-1
[7] dichromat_1.2-4        digest_0.5.1
[9] grid_2.14.1            memoise_0.1
[11] munsell_0.3            org.Hs.eg.db_2.6.4
[13] org.Mm.eg.db_2.6.4     org.Sc.sgd.db_2.6.4
[15] proto_0.3-9.2          qvalue_1.28.0
[17] reshape2_1.2.1         scales_0.1.0
[19] stringr_0.6            tcltk_2.14.1
[21] tools_2.14.1
```

References

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