

Using *clusterProfiler* to identify and compare functional profiles of gene lists

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1 Introduction

In recently years, high-throughput experimental techniques such as microarray, RNA-Seq and mass spectrometry can detect cellular moleculars at systems-level. These kinds of analyses generate huge quantities of data, which need to be given a biological interpretation. A commonly used approach is via clustering in the gene dimension for grouping different genes based on their similarities [1].

To search for shared functions among genes, a common way is to incorporate the biological knowledge, such as Gene Ontology (GO) and Kyoto Encyclopedia of genes and Genomes (KEGG), for identifying predominant biological themes of a collection of genes.

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. The manual step to choose interesting clusters followed by enrichment analysis on each selected cluster is slow and tedious. To bridge this gap, we designed *clusterProfiler* [2], for comparing and visualizing functional profiles among gene clusters.

2 Citation

Please cite the following articles when using *clusterProfiler*.

G Yu, LG Wang, Y Han, QY He. *clusterProfiler*: an R package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology*. 2012, 16(5), 284-287.

3 Supported organisms

At present, *clusterProfiler* about 20 species as shown below:

- *Arabidopsis*
- *Anopheles*
- *Bovine*
- *Canine*
- *Chicken*
- *Chimp*
- *E coli strain K12*

- *E coli strain Sakai*
- *Fly*
- *Human*
- *Malaria*
- *Mouse*
- *Pig*
- *Rat*
- *Rhesus*
- *Worm*
- *Xenopus*
- *Yeast*
- *Zebrafish*

These species are all supported by GO and KEGG analyses.
GO analyses also support *Coelicolor* and *Gondii*.

4 Gene Ontology Classification

In *clusterProfiler*, `groupGO` is designed for gene classification based on GO distribution at a specific level.

```
require(DOSE)
data(geneList)
gene <- names(geneList)[abs(geneList) > 2]
head(gene)
```

```
## [1] "4312" "8318" "10874" "55143" "55388" "991"
```

```
ggo <- groupGO(gene = gene, organism = "human", ont = "BP",
  level = 3, readable = TRUE)
head(summary(ggo))
```

##	ID	Description	Count	GeneRatio
##	GO:0019953 GO:0019953	sexual reproduction	10	10/207
##	GO:0019954 GO:0019954	asexual reproduction	0	0/207
##	GO:0032504 GO:0032504	multicellular organism reproduction	11	11/207
##	GO:0032505 GO:0032505	reproduction of a single-celled organism	0	0/207

```
## GO:0051321 GO:0051321 meiotic cell cycle 5 5/207
## GO:0006807 GO:0006807 nitrogen compound metabolic process 76 76/207
##
## GO:0019953
## GO:0019954
## GO:0032504
## GO:0032505
## GO:0051321
## GO:0006807 CDC45/MCM10/S100A9/FOXM1/KIF23/CENPE/MYBL2/S100A8/TOP2A/NCAPH/E2F8/CX
```

5 Enrichment Analysis

5.1 Hypergeometric model

Enrichment analysis [3] is a widely used approach to identify biological themes. Here we implement hypergeometric model to assess whether the number of selected genes associated with disease is larger than expected.

To determine whether any terms annotate a specified list of genes at frequency greater than that would be expected by chance, *clusterProfiler* calculates a p-value using the hypergeometric distribution:

$$p = 1 - \sum_{i=0}^{k-1} \frac{\binom{M}{i} \binom{N-M}{n-i}}{\binom{N}{n}}$$

In this equation, N is the total number of genes in the background distribution, M is the number of genes within that distribution that are annotated (either directly or indirectly) to the node of interest, n is the size of the list of genes of interest and k is the number of genes within that list which are annotated to the node. The background distribution by default is all the genes that have annotation.

P-values were adjusted for multiple comparison, and q-values were also calculated for FDR control.

5.2 Gene set enrichment analysis

A common approach in analyzing gene expression profiles was identifying differential expressed genes that are deemed interesting. The enrichment analysis we demonstrated previous were based on these differential expressed genes. This approach will find genes where the difference is large, but it will not detect a situation where the difference is small, but evidenced in coordinated way in a set of related genes. Gene Set Enrichment Analysis (GSEA) [4] directly addresses this limitation. All genes can be used in GSEA; GSEA aggregates the per gene statistics across genes within a gene set, therefore making it possible to detect situations where all genes in a predefined set change in a small but coordinated

way. Since it is likely that many relevant phenotypic differences are manifested by small but consistent changes in a set of genes.

Genes are ranked based on their phenotypes. Given a priori defined set of genes S (e.g., genes sharing the same *GO* or *KEGG* category), the goal of GSEA is to determine whether the members of S are randomly distributed throughout the ranked gene list (L) or primarily found at the top or bottom.

There are three key elements of the GSEA method:

- **Calculation of an Enrichment Score.**
The enrichment score (ES) represents the degree to which a set S is over-represented at the top or bottom of the ranked list L . The score is calculated by walking down the list L , increasing a running-sum statistic when we encounter a gene in S and decreasing when it is not. The magnitude of the increment depends on the gene statistics (e.g., correlation of the gene with phenotype). The ES is the maximum deviation from zero encountered in the random walk; it corresponds to a weighted Kolmogorov-Smirnov-like statistic [4].
- **Estimation of Significance Level of ES .**
The p -value of the ES is calculated using a permutation test. Specifically, we permute the gene labels of the gene list L and recompute the ES of the gene set for the permuted data, which generates a null distribution for the ES . The p -value of the observed ES is then calculated relative to this null distribution.
- **Adjustment for Multiple Hypothesis Testing.**
When the entire *GO* or *KEGG* gene sets are evaluated, *clusterProfiler* adjusts the estimated significance level to account for multiple hypothesis testing and also q -values were calculated for FDR control.

5.3 GO enrichment analysis

```
ego <- enrichGO(gene = gene, universe = names(geneList),
  organism = "human", ont = "CC", pvalueCutoff = 0.01,
  readable = TRUE)
head(summary(ego))
```

##	ID	Description	GeneRatio
##	GO:0005819 GO:0005819	spindle	24/196
##	GO:0015630 GO:0015630	microtubule cytoskeleton	37/196
##	GO:0005876 GO:0005876	spindle microtubule	10/196
##	GO:0000793 GO:0000793	condensed chromosome	16/196
##	GO:0000779 GO:0000779	condensed chromosome, centromeric region	12/196
##	GO:0044430 GO:0044430	cytoskeletal part	43/196
##	BgRatio	pvalue p.adjust	qvalue

```
## GO:0005819 211/11884 6.96e-14 6.68e-12 4.39e-12
## GO:0015630 710/11884 3.09e-10 1.49e-08 9.77e-09
## GO:0005876 40/11884 6.53e-10 2.09e-08 1.37e-08
## GO:0000793 141/11884 1.29e-09 3.09e-08 2.03e-08
## GO:0000779 73/11884 2.21e-09 4.24e-08 2.79e-08
## GO:0044430 1005/11884 4.18e-09 6.68e-08 4.40e-08
##
## GO:0005819
## GO:0015630 KIF20A/TACC3/CENPE/CHEK1/KIF18B/
## GO:0005876
## GO:0000793
## GO:0000779
## GO:0044430 KIF20A/TACC3/CENPE/CHEK1/KIF18B/SKA1/ABLIM3/TPX2/PSD3/KIF4A/ASPM/AK5/
## Count
## GO:0005819 24
## GO:0015630 37
## GO:0005876 10
## GO:0000793 16
## GO:0000779 12
## GO:0044430 43
```

```
ego2 <- gseGO(geneList = geneList, organism = "human",
  ont = "CC", nPerm = 100, minGSSize = 120, pvalueCutoff = 0.01,
  verbose = FALSE)
head(summary(ego2))
```

##	ID	Description	setSize	enrichmentScore
## GO:0000228	GO:0000228	nuclear chromosome	291	0.414
## GO:0000775	GO:0000775	chromosome, centromeric region	133	0.636
## GO:0000785	GO:0000785	chromatin	300	0.341
## GO:0000790	GO:0000790	nuclear chromatin	175	0.318
## GO:0000793	GO:0000793	condensed chromosome	141	0.625
## GO:0005575	GO:0005575	cellular_component	11479	0.201
##	pvalue	p.adjust	qvalues	
## GO:0000228	0	0	0	
## GO:0000775	0	0	0	
## GO:0000785	0	0	0	
## GO:0000790	0	0	0	
## GO:0000793	0	0	0	
## GO:0005575	0	0	0	

5.4 KEGG pathway enrichment analysis

```
kk <- enrichKEGG(gene = gene, organism = "human", pvalueCutoff = 0.01,
  readable = TRUE)
head(summary(kk))
```

##	ID	Description	GeneRatio	BgRatio
##	hsa04110 hsa04110	Cell cycle	11/74	128/5894
##	hsa04114 hsa04114	Oocyte meiosis	10/74	114/5894
##	hsa03320 hsa03320	PPAR signaling pathway	7/74	70/5894
##	hsa04914 hsa04914	Progesterone-mediated oocyte maturation	6/74	87/5894
##	hsa04062 hsa04062	Chemokine signaling pathway	8/74	189/5894
##	hsa04060 hsa04060	Cytokine-cytokine receptor interaction	9/74	265/5894
##	pvalue	p.adjust	qvalue	
##	hsa04110	4.31e-07	3.02e-06	4.54e-07
##	hsa04114	1.25e-06	4.38e-06	6.59e-07
##	hsa03320	2.35e-05	5.49e-05	8.25e-06
##	hsa04914	7.21e-04	1.26e-03	1.90e-04
##	hsa04062	2.37e-03	3.32e-03	5.00e-04
##	hsa04060	5.58e-03	6.51e-03	9.79e-04
##		geneID	Count	
##	hsa04110	CDC45/CDC20/CCNB2/CCNA2/CDK1/MAD2L1/TTK/CHEK1/CCNB1/MCM5/PTTG1	11	
##	hsa04114	CDC20/CCNB2/CDK1/MAD2L1/CALML5/AURKA/CCNB1/PTTG1/ITPR1/PGR	10	
##	hsa03320	MMP1/FADS2/ADIPOQ/PCK1/FABP4/HMGCS2/PLIN1	7	
##	hsa04914	CCNB2/CCNA2/CDK1/MAD2L1/CCNB1/PGR	6	
##	hsa04062	CXCL10/CXCL13/CXCL11/CXCL9/CCL18/CCL8/CXCL14/CX3CR1	8	
##	hsa04060	CXCL10/CXCL13/CXCL11/CXCL9/CCL18/IL1R2/CCL8/CXCL14/CX3CR1	9	

```
kk2 <- gseKEGG(geneList = geneList, organism = "human",
  nPerm = 100, minGSSize = 120, pvalueCutoff = 0.01,
  verbose = FALSE)
head(summary(kk2))
```

##	ID	Description	setSize	
##	hsa04062 hsa04062	Chemokine signaling pathway	166	
##	hsa04510 hsa04510	Focal adhesion	193	
##	hsa03013 hsa03013	RNA transport	124	
##	hsa04060 hsa04060	Cytokine-cytokine receptor interaction	233	
##	enrichmentScore	pvalue	p.adjust	qvalues
##	hsa04062	0.383	0	0
##	hsa04510	-0.446	0	0
##	hsa03013	0.427	0	0
##	hsa04060	0.339	0	0

5.5 DO enrichment analysis

Disease Ontology (DO) enrichment analysis is implemented in *DOSE*, please refer to the package vignettes. The `enrichDO` function is very useful for identifying disease association of interesting genes, and function `gseAnalyzer` function is designed for gene set enrichment analysis of *DO*.

5.6 Reactome pathway enrichment analysis

With the demise of KEGG (at least without subscription), the KEGG pathway data in Bioconductor will not update and we encourage user to analyze pathway using *ReactomePA* which use Reactome as a source of pathway data. The function call of `enrichPathway` and `gsePathway` in *ReactomePA* is consistent with `enrichKEGG` and `gseKEGG`.

5.7 Function call

The function calls of `groupGO`, `enrichGO`, `enrichKEGG`, `enrichDO` and `enrichPathway` are consistent. The input parameters of *gene* is a vector of entrezgene (for human and mouse) or ORF (for yeast) IDs, and *organism* should be supported species (please refer to the manual of the specific function).

For gene set enrichment analysis, the function of `gseGO`, `gseKEGG`, `gseAnalyzer` and `gsePathway` need extra parameter *nPerm* to specify the permutation number.

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 p-values and the adjusted p values. *Q-values* were also calculated for controlling false discovery rate (FDR).

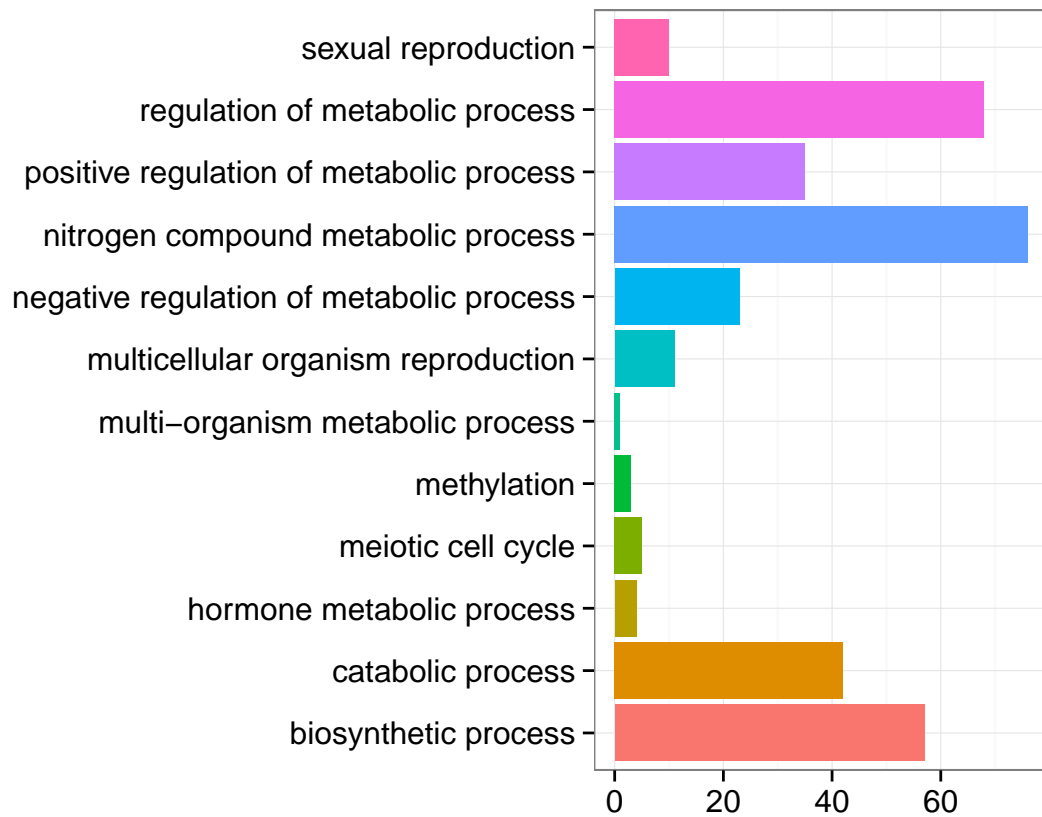
The *readable* is a logical parameter to indicate the input gene IDs will map to gene symbols or not.

5.8 Visualization

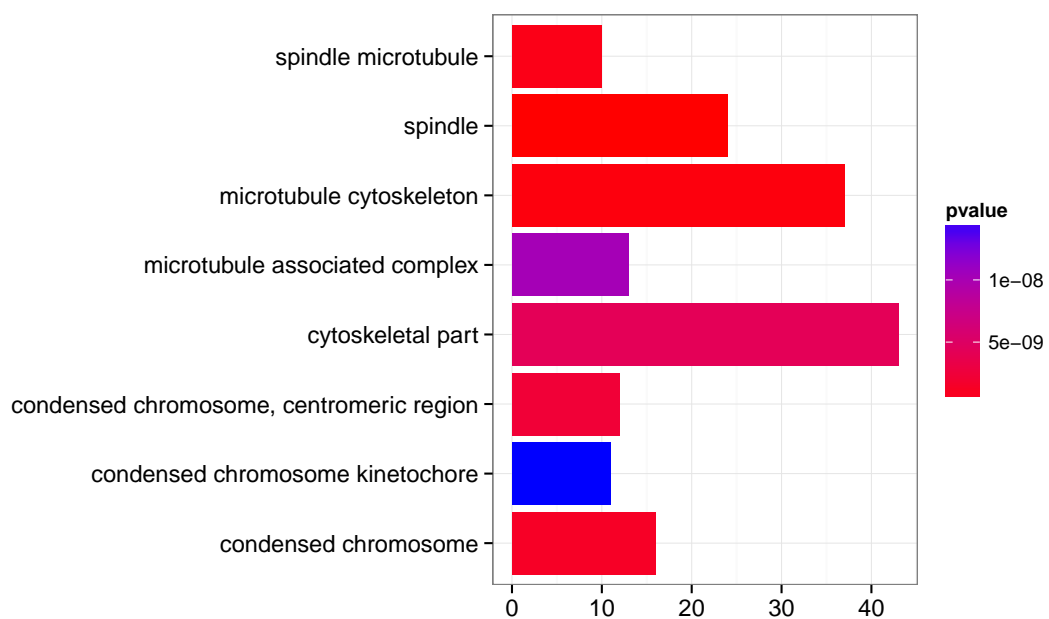
The output of `groupGO`, `enrichGO` and `enrichKEGG` can be visualized by bar plot, enrichment map and category-gene-network plot. It is very common to visualize the enrichment result in bar or pie chart. We believe the pie chart is misleading and only provide bar chart.

5.8.1 barplot


```
barplot(ggo, drop = TRUE, showCategory = 12)
```



```
barplot(ego, showCategory = 8)
```



5.8.2 enrichMap

Enrichment map can be visualized by `enrichMap`, which support results obtained from hypergeometric test and gene set enrichment analysis.

```
enrichMap(ego)
```

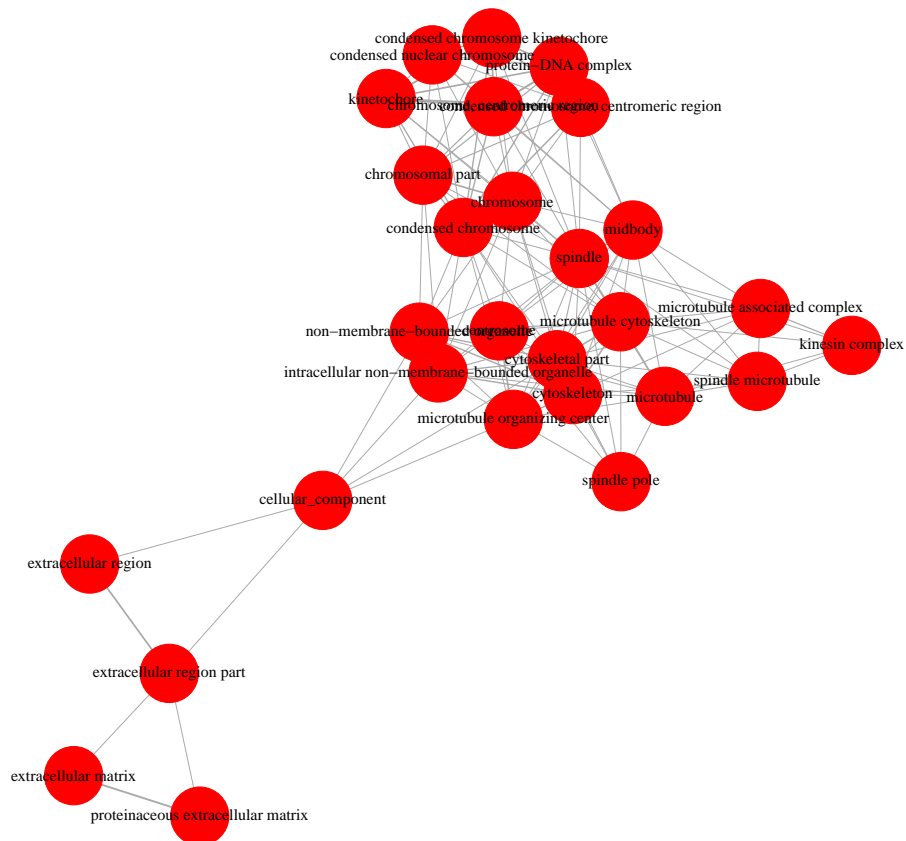


Figure 1: enrichment map of enrichment result

```
enrichMap(ego2)
```

5.8.3 cnetplot

In order to consider the potentially biological complexities in which a gene may belong to multiple annotation categories and provide information of numeric changes if available, we developed `cnetplot` function to extract the complex association.

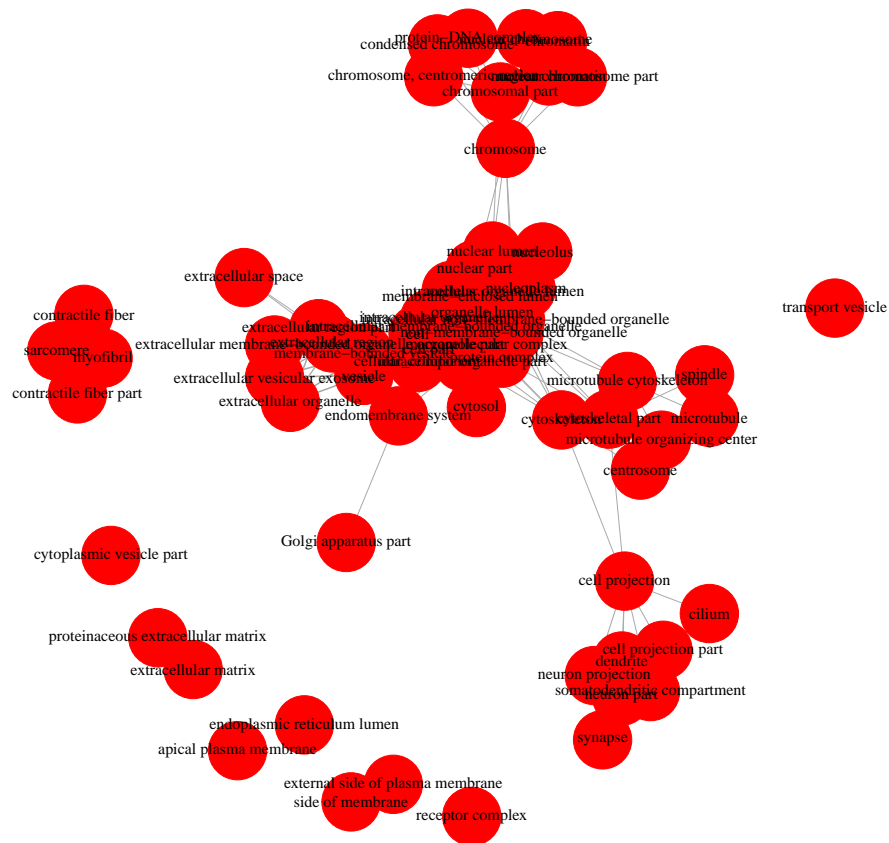
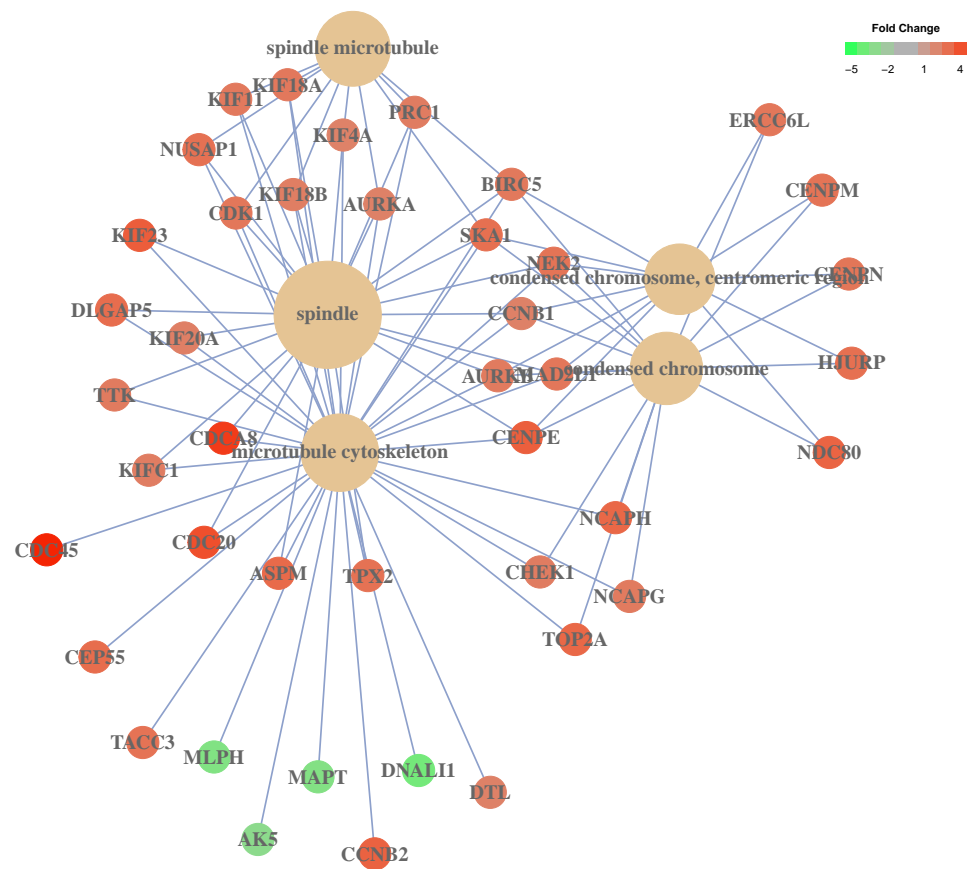
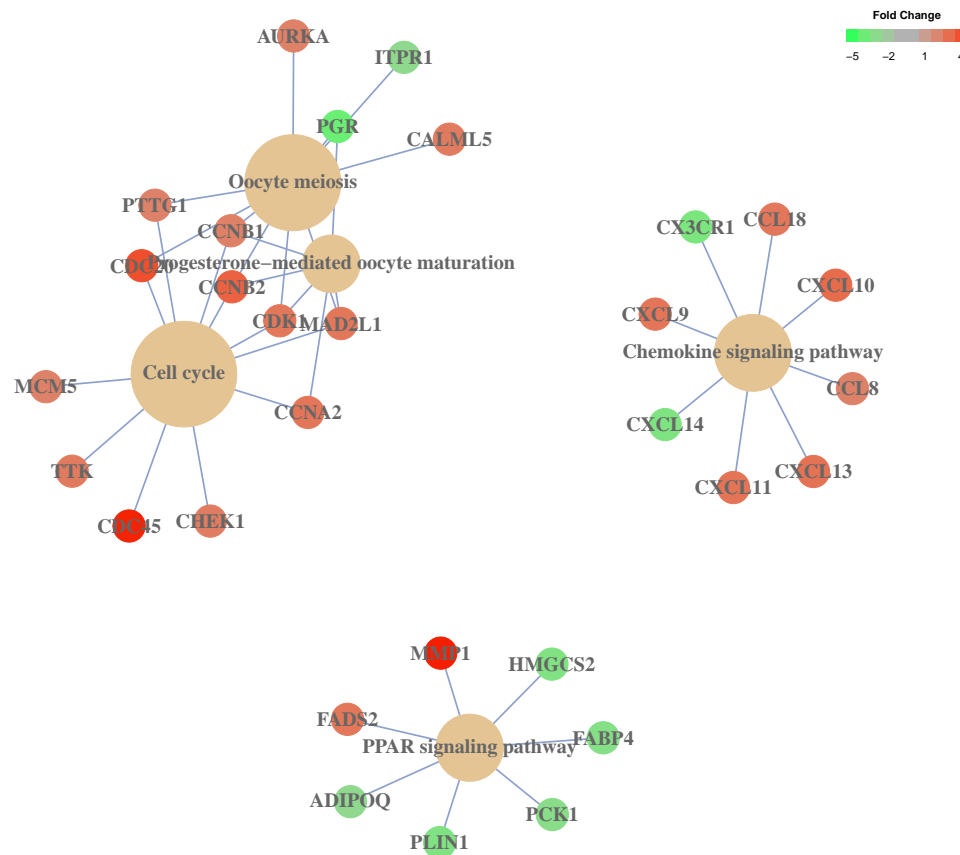


Figure 2: enrichment map of gsea result

```
cnetplot(ego, categorySize = "pvalue", foldChange = geneList)
```



```
cnetplot(kk, categorySize = "geneNum", foldChange = geneList)
```



5.8.4 gseaplot

Running score of gene set enrichment analysis and its association of phenotype can be visualized by `gseaplot`.

```
gseaplot(kk2, geneSetID = "hsa04145")
```

5.8.5 pathview from pathview package

`clusterProfiler` users can also use `pathview` from the `pathview` [5] to visualize KEGG pathway.

The following example illustrate how to visualize "hsa04110" pathway, which was enriched in our previous analysis.

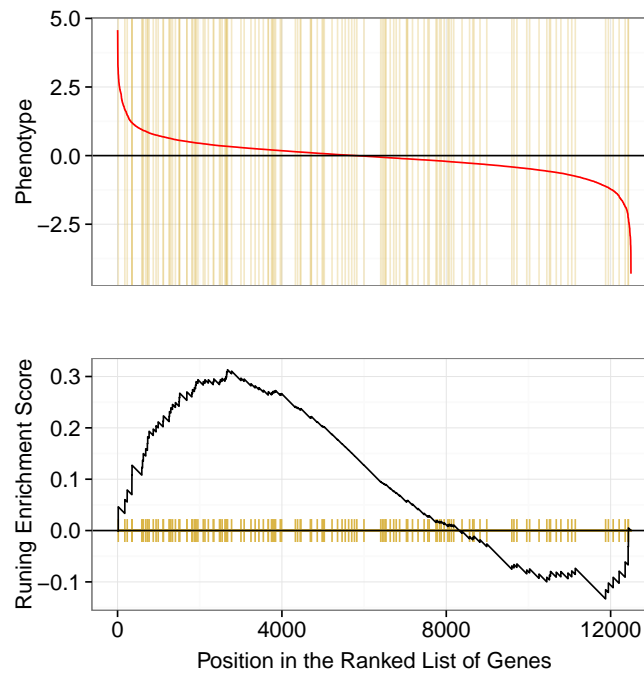


Figure 3: plotting gsea result

```
require(pathview)
hsa04110 <- pathview(gene.data = geneList, pathway.id = "hsa04110",
  species = "hsa", limit = list(gene = max(abs(geneList)),
    cpd = 1))

## Info: Downloading xml files for hsa04110, 1/1 pathways..
## Info: Downloading png files for hsa04110, 1/1 pathways..
## Info: Working in directory /private/tmp/Rtmpkdh5JJ/Rbuild48d7463dc810/cluster
## Info: Writing image file hsa04110.pathview.png
```

For further information, please refer to the vignette of *pathview* [5].

6 Biological theme comparison

clusterProfiler was developed for biological theme comparison, and it provides a function, `compareCluster`, to automatically calculate enriched functional categories of each gene clusters.

```
data(gcSample)
ck <- compareCluster(geneCluster = gcSample, fun = "enrichKEGG")
plot(ck)
```

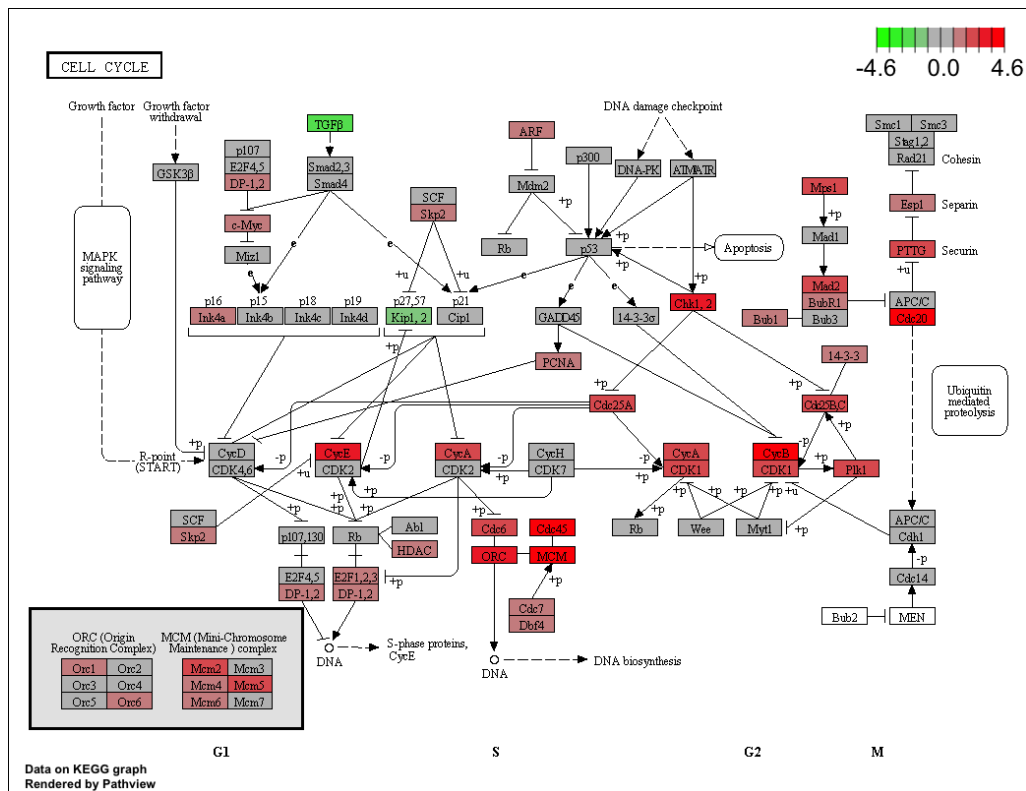
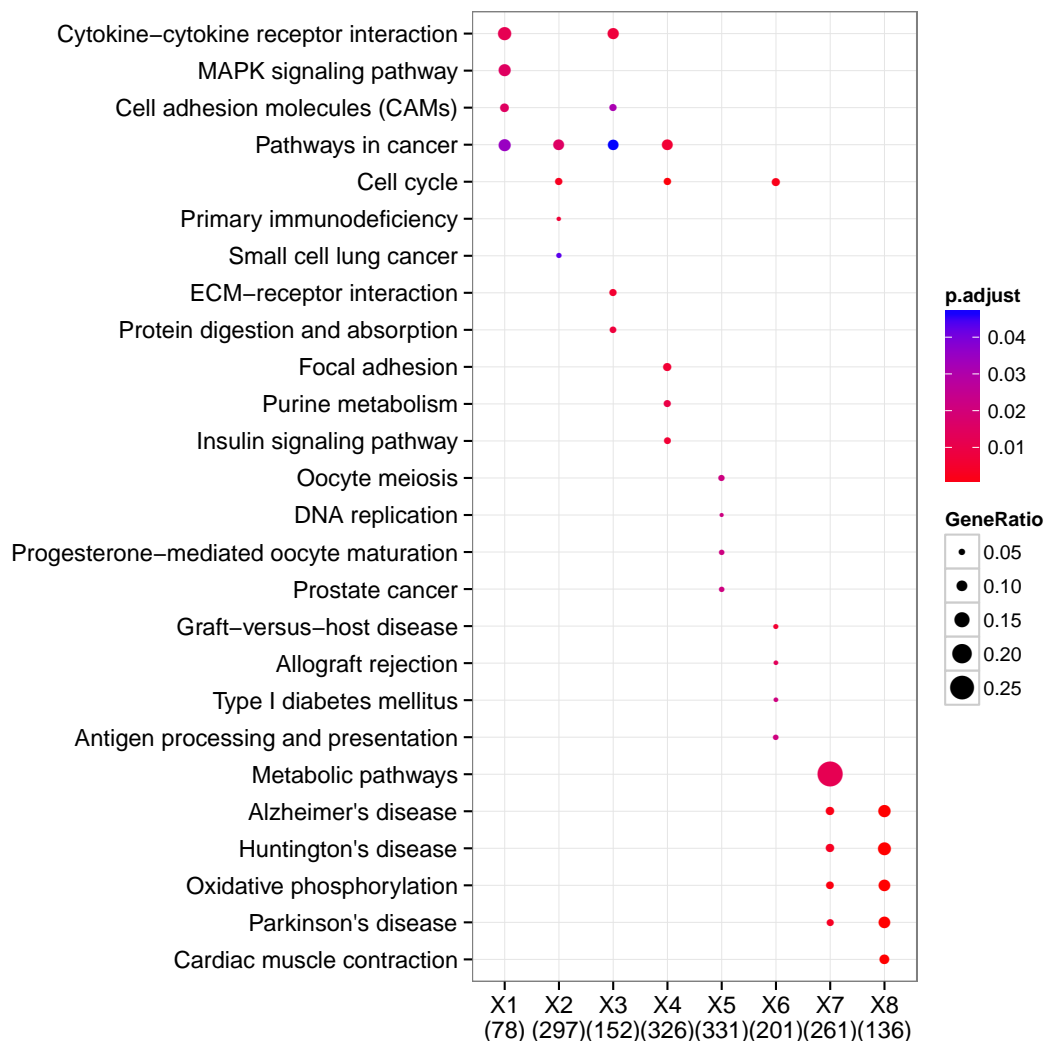


Figure 4: visualize KEGG pathway using pathview



By default, only top 5 (most significant) categories of each cluster was plotted. User can change the parameter *showCategory* to specify how many categories of each cluster to be plotted, and if *showCategory* was set to *NULL*, the whole result will be plotted.

The dot sizes were based on their corresponding row percentage by default, and user can set the parameter *by* to "count" to make the comparison based on gene counts. The parameter *by* can also be set to "rowPercentage" to normalize the dot sizes, since some categories may contain a large number of genes, and make the dot sizes of those small categories too small to compare. The default parameter *by* is setting to "geneRatio", which corresponding to the "GeneRatio" column of the output. To provide the full information, we also provide number of identified genes in each category (numbers in parentheses) when *by* is setting to "rowPercentage" and number of gene clusters in each cluster label (numbers in parentheses) when *by* is setting to "geneRatio", as shown in Figure 3. If the dot sizes were based on "count", the row numbers will not be shown.

The p-values indicate that which categories are more likely to have biological meanings. The dots in the plot are color-coded based on their corresponding p-values. Color gradient ranging from red to blue correspond to in order of increasing p-values. That is, red indicate low p-values (high enrichment), and blue indicate high p-values (low enrichment). P-values and adjusted p-values were filtered out by the threshold giving by parameter *pvalueCutoff*, and FDR can be estimated by *qvalue*.

User can refer to the example in [2]; we analyzed the publicly available expression dataset of breast tumour tissues from 200 patients (GSE11121, Gene Expression Omnibus) [6]. We identified 8 gene clusters from differentially expressed genes, and using *compareCluster* to compare these gene clusters by their enriched biological process.

Another example was shown in [7], we calculated functional similarities among viral miRNAs using method described in [8], and compared significant KEGG pathways regulated by different viruses using *compareCluster*.

The comparison function was designed as a general-package for comparing gene clusters of any kind of ontology associations, not only *groupGO*, *enrichGO*, and *enrichKEGG* this package provided, but also other biological and biomedical ontologies, for instance, *enrichDO* from *DOSE* and *enrichPathway* from *ReactomePA* work fine with *compareCluster* for comparing biological themes in disease and reactome pathway perspective. More details can be found in the vignettes of *DOSE* and *ReactomePA*.

7 Session Information

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

- R version 3.1.2 (2014-10-31), x86_64-apple-darwin10.8.0

- Locale:
en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
- Base packages: base, datasets, graphics, grDevices, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.28.1, Biobase 2.26.0, BiocGenerics 0.12.1, clusterProfiler 2.0.1, DBI 0.3.1, DOSE 2.4.0, GenomeInfoDb 1.2.4, GO.db 3.0.0, graph 1.44.1, IRanges 2.0.1, KEGGgraph 1.24.0, knitr 1.9, org.Hs.eg.db 3.0.0, pathview 1.6.0, RSQLite 1.0.0, S4Vectors 0.4.0, XML 3.98-1.1
- Loaded via a namespace (and not attached): Biostrings 2.34.1, codetools 0.2-10, colorspace 1.2-4, digest 0.6.8, DO.db 2.8.0, evaluate 0.5.5, formatR 1.0, ggplot2 1.0.0, GOSemSim 1.24.1, grid 3.1.2, gtable 0.1.2, highr 0.4, httr 0.6.1, igraph 0.7.1, KEGG.db 3.0.0, KEGGREST 1.6.4, labeling 0.3, MASS 7.3-39, munsell 0.4.2, plyr 1.8.1, png 0.1-7, proto 0.3-10, qvalue 1.40.0, Rcpp 0.11.4, reshape2 1.4.1, Rgraphviz 2.10.0, scales 0.2.4, stringr 0.6.2, tools 3.1.2, XVector 0.6.0, zlibbioc 1.12.0

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