

Pathway Analysis

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Call libraries

```
library(clusterProfiler)
```

```
## Warning: replacing previous import 'utils::findMatches' by  
## 'S4Vectors::findMatches' when loading 'AnnotationDbi'
```

```
##
```

```
## Registered S3 method overwritten by 'ggtree':  
##   method      from  
##   identify.gg ggfun
```

```
## clusterProfiler v4.8.1 For help: https://yulab-smu.top/biomedical-knowledge-mining-book/  
##  
## If you use clusterProfiler in published research, please cite:  
## T Wu, E Hu, S Xu, M Chen, P Guo, Z Dai, T Feng, L Zhou, W Tang, L Zhan, X Fu, S Liu, X Bo, and G Yu. clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. The Innovation. 2021, 2(3):100141
```

```
##  
## Attaching package: 'clusterProfiler'
```

```
## The following object is masked from 'package:stats':  
##  
##   filter
```

```
library(DOSE)
```

```
## DOSE v3.26.1 For help: https://yulab-smu.top/biomedical-knowledge-mining-book/  
##  
## If you use DOSE in published research, please cite:  
## Guangchuang Yu, Li-Gen Wang, Guang-Rong Yan, Qing-Yu He. DOSE: an R/Bioconductor package for Disease Ontology Semantic and Enrichment analysis. Bioinformatics 2015, 31(4):608-609
```

```
library(R.utils)
```

```
## Loading required package: R.oo
```

```
## Loading required package: R.methodsS3
```

```
## R.methodsS3 v1.8.2 (2022-06-13 22:00:14 UTC) successfully loaded. See ?R.methodsS3 for help.
```

```
## R.oo v1.25.0 (2022-06-12 02:20:02 UTC) successfully loaded. See ?R.oo for help.
```

```
##  
## Attaching package: 'R.oo'
```

```
## The following object is masked from 'package:R.methodsS3':  
##  
##      throw
```

```
## The following objects are masked from 'package:methods':  
##  
##      getClasses, getMethods
```

```
## The following objects are masked from 'package:base':  
##  
##      attach, detach, load, save
```

```
## R.utils v2.12.2 (2022-11-11 22:00:03 UTC) successfully loaded. See ?R.utils for help.
```

```
##  
## Attaching package: 'R.utils'
```

```
## The following object is masked from 'package:utils':  
##  
##      timestamp
```

```
## The following objects are masked from 'package:base':  
##  
##      cat, commandArgs, getOption, isOpen, nullfile, parse, warnings
```

```
library(gtools)
```

```
##  
## Attaching package: 'gtools'
```

```
## The following object is masked from 'package:R.utils':  
##  
##   capture
```

```
library(org.Hs.eg.db)
```

```
## Loading required package: AnnotationDbi
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
##  
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:stats':  
##  
##   IQR, mad, sd, var, xtabs
```

```
## The following objects are masked from 'package:base':  
##  
##   anyDuplicated, append, as.data.frame, basename, cbind, colnames,  
##   dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,  
##   grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,  
##   order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,  
##   rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,  
##   union, unique, unsplit, which.max, which.min
```

```
## Loading required package: Biobase
```

```
## Welcome to Bioconductor  
##  
##   Vignettes contain introductory material; view with  
##   'browseVignettes()'. To cite Bioconductor, see  
##   'citation("Biobase")', and for packages 'citation("pkgname")'.
```

```
## Loading required package: IRanges
```

```
## Loading required package: S4Vectors
```

```
##  
## Attaching package: 'S4Vectors'
```

```
## The following object is masked from 'package:clusterProfiler':  
##  
##   rename
```

```
## The following object is masked from 'package:utils':  
##  
##   findMatches
```

```
## The following objects are masked from 'package:base':  
##  
##   expand.grid, I, unname
```

```
##  
## Attaching package: 'IRanges'
```

```
## The following object is masked from 'package:R.oo':  
##  
##   trim
```

```
## The following object is masked from 'package:clusterProfiler':  
##  
##   slice
```

```
## The following object is masked from 'package:grDevices':  
##  
##   windows
```

```
##  
## Attaching package: 'AnnotationDbi'
```

```
## The following object is masked from 'package:clusterProfiler':  
##  
##   select
```

```
##
```

```
library(enrichplot)  
library(ggplot2)  
library(stringr)  
library(tibble)  
library(ggupset)  
library(DOSE)  
library(AnnotationDbi)  
library(pathview)
```

```
## #####
## Pathview is an open source software package distributed under GNU General
## Public License version 3 (GPLv3). Details of GPLv3 is available at
## http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
## formally cite the original Pathview paper (not just mention it) in publications
## or products. For details, do citation("pathview") within R.
##
## The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG
## license agreement (details at http://www.kegg.jp/kegg/legal.html).
## #####
```

Set wd and read data

```
setwd('E:/R-Programming-Practices/Functional Enrichment/Pathway Analysis')
data= data.frame(read.csv('GBM.csv'))
```

Convert gene symbol to ENTREZID

```
Converted <- bitr(unique(data$Genes), fromType = "SYMBOL",
                  toType = c("ENTREZID"),
                  OrgDb = org.Hs.eg.db)
```

```
## 'select()' returned 1:many mapping between keys and columns
```

```
## Warning in bitr(unique(data$Genes), fromType = "SYMBOL", toType =
## c("ENTREZID"), : 1.62% of input gene IDs are fail to map...
```

```
head(Converted)
```

	SYMBOL <chr>	ENTREZID <chr>
1	YAE1	57002
2	CBX3	11335
3	PSMA2	5683
4	SSBP1	6742
5	BUD31	8896
6	STARD3NL	83930

6 rows

Prepare gene list for KEGG analysis

```
gene_list <- Converted$ENTREZID
gene_list = sort(gene_list, decreasing = TRUE)
head(gene_list)
```

```
## [1] "9997" "9994" "9993" "9992" "9991" "9990"
```

Perform KEGG analysis

```
kegg <- enrichKEGG(gene      = gene_list,
                   organism   = 'hsa',
                   pvalueCutoff = 0.05)
```

```
## Reading KEGG annotation online: "https://rest.kegg.jp/link/hsa/pathway"...
```

```
## Warning in utils::download.file(url, quiet = TRUE, method = method, ...): the
## 'wininet' method is deprecated for http:// and https:// URLs
```

```
## Reading KEGG annotation online: "https://rest.kegg.jp/list/pathway/hsa"...
```

```
## Warning in utils::download.file(url, quiet = TRUE, method = method, ...): the
## 'wininet' method is deprecated for http:// and https:// URLs
```

```
head(kegg)
```

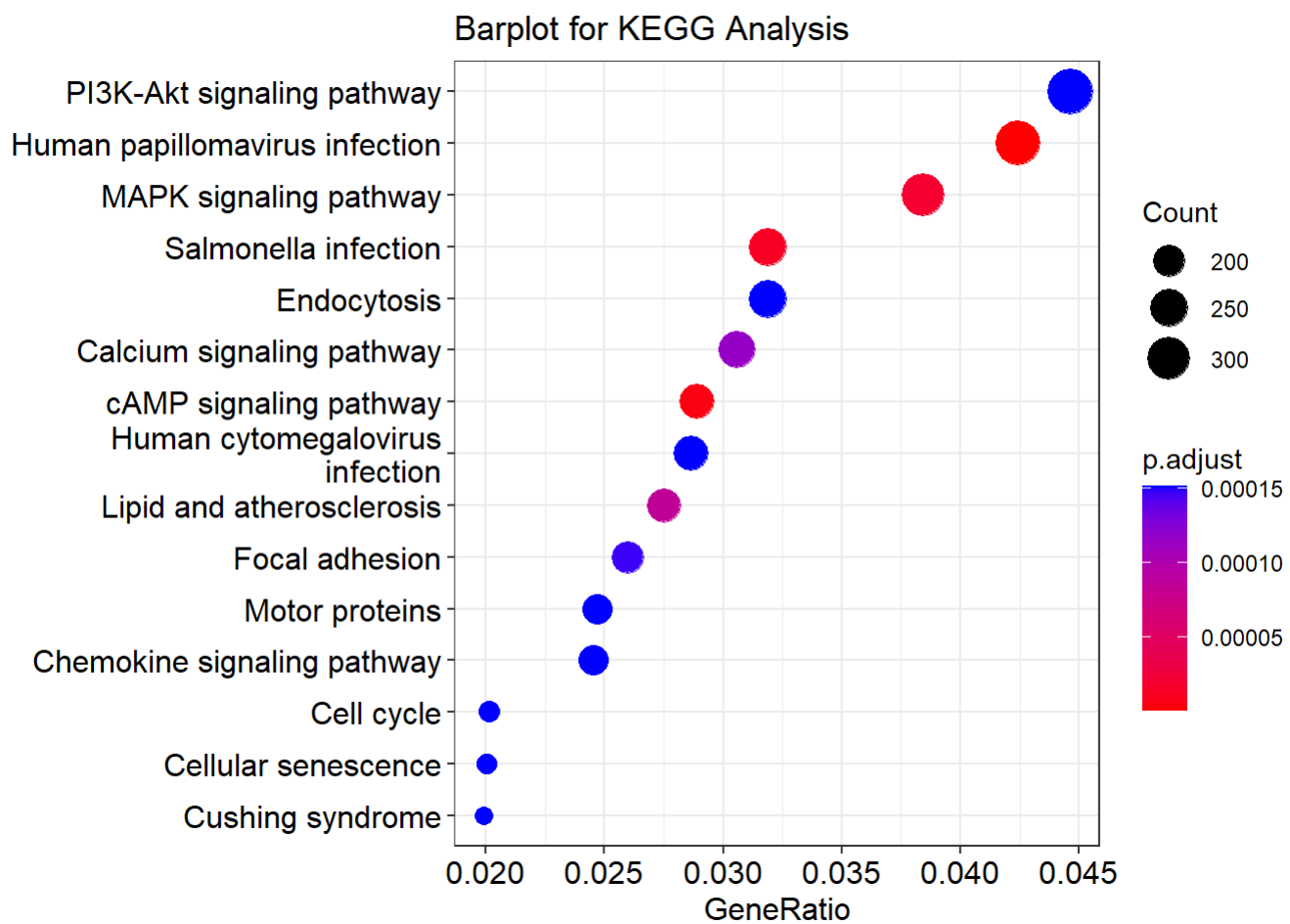
	ID <chr>	Description <chr>	GeneRatio <chr>	BgRatio <chr>	pvalue <dbl>	
	hsa05165	hsa05165 Human papillomavirus infection	330/7779	331/8390	2.247686e-10	7
	hsa04024	hsa04024 cAMP signaling pathway	225/7779	225/8390	3.213740e-08	5
	hsa05132	hsa05132 Salmonella infection	248/7779	249/8390	1.050729e-07	1
	hsa04010	hsa04010 MAPK signaling pathway	299/7779	302/8390	2.186062e-07	1
	hsa05417	hsa05417 Lipid and atherosclerosis	214/7779	215/8390	1.283966e-06	8
	hsa04020	hsa04020 Calcium signaling pathway	238/7779	240/8390	2.083826e-06	1

6 rows | 1-7 of 10 columns

Visualization

Dotplot

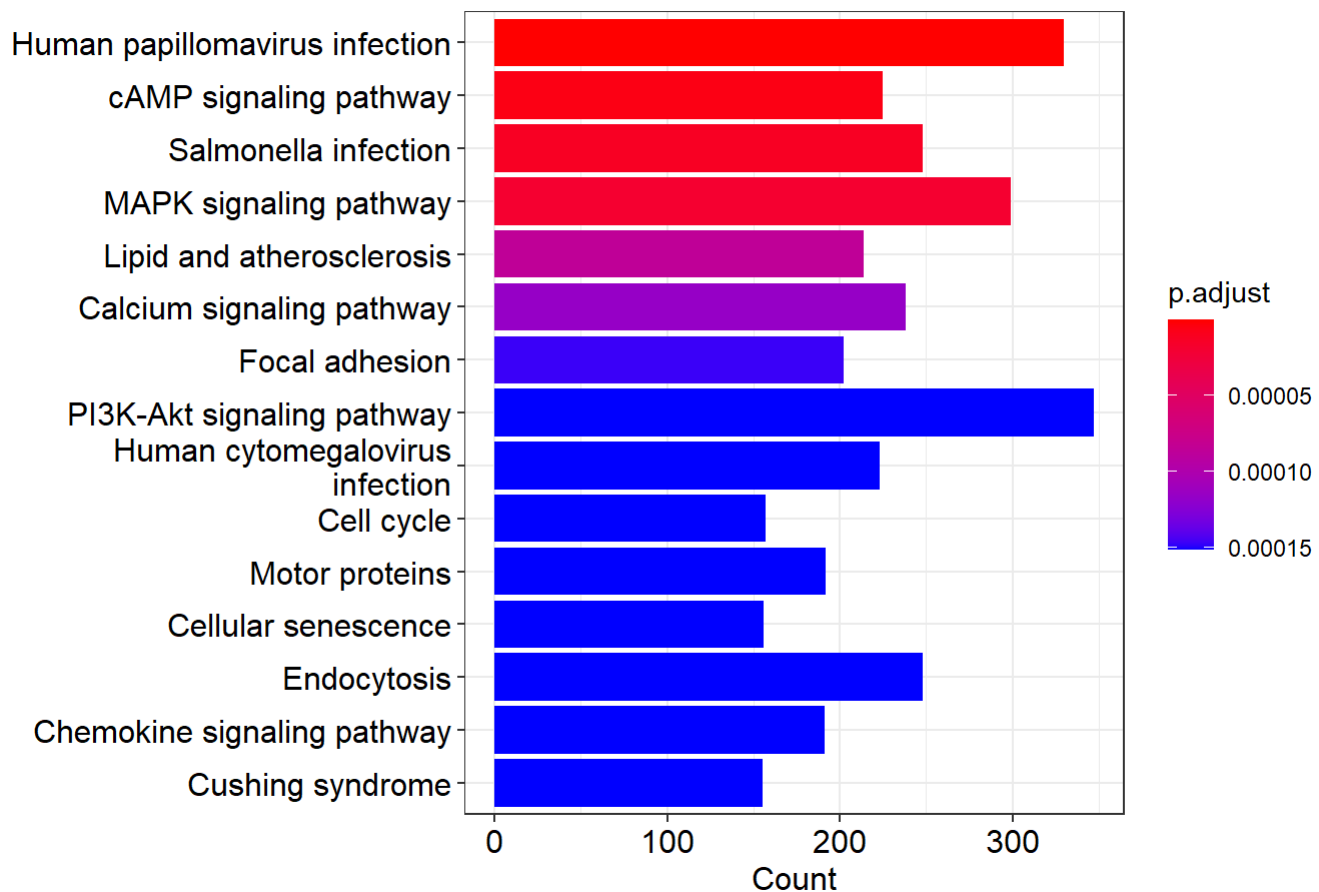
```
dotplot(kegg, showCategory=15) + ggtitle("Barplot for KEGG Analysis")
```



Barplot

```
barplot(kegg, showCategory=15) + ggtitle("Barplot for KEGG Analysis")
```

Barplot for KEGG Analysis



Check KEGG enrichment of the input gene list online in one pathway

```
hsa04024<- pathview(gene.data = gene_list,
                    pathway.id = "hsa04024",
                    species    = "hsa")
```

```
## Info: Downloading xml files for hsa04024, 1/1 pathways..
```

```
## Info: Downloading png files for hsa04024, 1/1 pathways..
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## Info: Working in directory E:/R-Programming-Practices/Functional Enrichment/Pathway Analysis
```

```
## Info: Writing image file hsa04024.pathview.png
```

Check KEGG enrichment of the input gene list offline (wd) in one

pathway

```
hsa04110 <- pathview(gene.data = gene_list,
                    pathway.id = "hsa04110",
                    species    = "hsa",
                    )
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## Info: Working in directory E:/R-Programming-Practices/Functional Enrichment/Pathway Analysis
```

```
## Info: Writing image file hsa04110.pathview.png
```

Integrate log2FC in KEGG pathway

Read data

```
DEGs=read.csv('DEGs.csv')
```

```
Converted <- bitr(unique(DEGs$Genes), fromType = "SYMBOL",
                 toType = c("ENTREZID"),
                 OrgDb = org.Hs.eg.db)
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
head(Converted)
```

	SYMBOL <chr>	ENTREZID <chr>
1	CDC25A	993
2	HJURP	55355
3	NCAPG	64151
4	RRM2	6241
5	CCNA2	890
6	BUB1	699
6 rows		

Prepare gene list for KEGG analysis

```
Gene_List <- Converted$ENTREZID
Gene_List = sort(Gene_List, decreasing = TRUE)
head(Gene_List)
```

```
## [1] "993" "991" "983" "9806" "9787" "9540"
```

Create object

```
logFC<- DEGs[,2]  
names(logFC)<-Gene_List  
mypathway<-"hsa04110"
```

View pathway

```
pathview(gene.data=logFC,species="hsa",  
         pathway=mypathway, limit = list(gene=3, cpd=1))
```

```
## 'select()' returned 1:1 mapping between keys and columns
```

```
## Info: Working in directory E:/R-Programming-Practices/Functional Enrichment/Pathway Analysis
```

```
## Info: Writing image file hsa04110.pathview.png
```

Wiki_Pathway Analysis

```
WP=enrichWP(gene_list, organism = 'Homo sapiens')
```

```
## Warning in utils::download.file(url, quiet = TRUE, method = method, ...): the  
## 'wininet' method is deprecated for http:// and https:// URLs
```

```
dotplot(WP, showCategory = 15) + ggtitle('Dotplot for WikiPathway')
```

