

cogena, a workflow for co-expressed gene-set enrichment analysis

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2015-10-14

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1 Abstract

Co-expressed gene-set enrichment analysis, *cogena*, is a workflow for gene set enrichment analysis of co-expressed genes. The co-expression analysis is made based on various clustering methods. And The gene set analysis can be pathway analysis, GO analysis, drug repositioning and so on. The type of analysis *cogena* can is dependent on the gene set used in *cogena*. Lots kinds of gene sets are integrated into *cogena* from Msigdb and Connectivity Map. See the workflow of *cogena* in Figure 1.

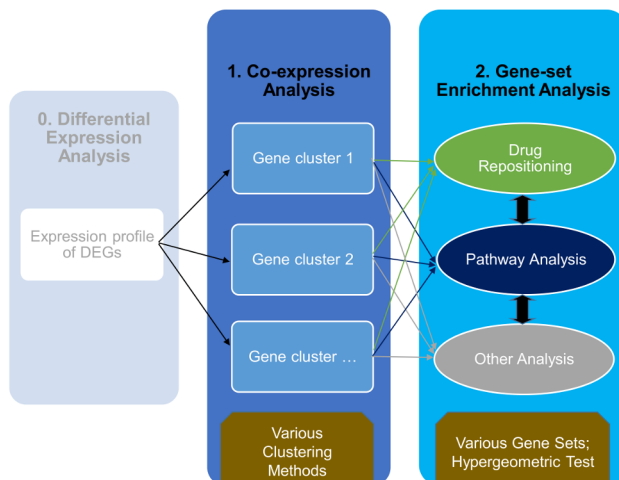


Figure 1: Overview of the *cogena* workflow

2 A quick start

See examples in `?cogena` please.

3 Data Input

Note: all the gene names should be gene SYMBOLs since they are used in the gene set files. Other kind of gene names can be used according to the kind of gene names of user-defined gene-set files.

3.1 Input data required

- the gene expression profiling of differentially expressed genes. It can be matrix with gene in row and sample in column, `data.frame` or `ExpressionSet` object.
- the sample labels indicating the labels, like control and disease, of each sample. A vector with sample names.

3.2 Example dataset

There is an example dataset in `cogena` package. This dataset, from [GSE13355](#), is about psoriasis. There are two objects in the Psoriasis dataset. See `?Psoriasis` for more details.

```
library(cogena)
data(Psoriasis)
# objects in the Psoriasis dataset.
# Note: lable of interest should be behind of control label as this will affect
# the direction of gene regulation. For instance.
# Use factor(c("Normal", "Cancer", "Normal"), levels=c("Normal", "Cancer")),
# instead of factor(c("Normal", "Cancer","Normal")) since "Cancer" is the
# lable of interest.
```

```
## [1] "DEexprs"      "sampleLabel"
```

4 Various Analyses

4.1 What kind of analysis can be done?

The gene set used determines the type of analysis.

There are a variety of gene sets in the `cogena` package, partly collected from [MSigDB](#) and [CMap](#). They are `CmapDn100.gmt.xz`, `CmapUp100.gmt.xz`, `c2.cp.biocarta.v5.0.symbols.gmt.xz`, `c2.cp.kegg.v5.0.symbols.gmt.xz`, `c2.cp.reactome.v5.0.symbols.gmt.xz`, `c2.cp.v5.0.symbols.gmt.xz`, `c5.bp.v5.0.symbols.gmt.xz`, `c5.mf.v5.0.symbols.gmt.xz`. Here the gene-sets can be user-defined gene-sets formatted gmt and compressed by xz, too (such as `c2.cp.kegg.v5.0.symbols.gmt.xz`). Copy it into the `extdata` directory in the installation directory of `cogena`, such as `~/R/x86_64-pc-linux-gnu-library/3.2/cogena/extdata` in Linux), or kindly send to the author of `cogena` to share with others.

4.2 Types of analyses

- Pahtway Analysis
- GO Analysis
- Drug repositioning
- ...

5 Pathway Analysis

KEGG Pathway Analysis, as an example, will be done to show the utility of `cogena`.

5.1 Parameter setting

```
# KEGG Pathway gene set
annoGMT <- "c2.cp.kegg.v5.0.symbols.gmt.xz"
# GO biological process gene set
# annoGMT <- "c5.bp.v5.0.symbols.gmt.xz"
```

```

annofile <- system.file("extdata", annoGMT, package="cogena")

# the number of clusters. It can be a vector.
# nClust <- 2:20
nClust <- 10

# the number of cores.
# ncore <- 8
ncore <- 2

# the clustering methods
# clMethods <- c("hierarchical", "kmeans", "diana", "fanny", "som", "model",
# "sota", "pam", "clara", "agnes") # All the methods can be used together.
clMethods <- c("hierarchical", "pam")

# the distance metric
metric <- "correlation"

# the agglomeration method used for hierarchical clustering
# (hierarchical and agnes)
method <- "complete"

```

5.2 Cogena running

```

# Co-expression Analysis
genecl_result <- coExp(DEexprs, nClust=nClust, clMethods=clMethods,
                      metric=metric, method=method, ncore=ncore, verbose=TRUE)

# Enrichment (Pathway) analysis for the co-expressed genes
clen_res <- clEnrich(genecl_result, annofile=annofile, sampleLabel=sampleLabel)

```

5.3 Results of pathway analysis

5.3.1 Summary of cogena result

After completing the cogena analysis, user can use `summary` to see the summary of the result of cogena. And `enrichment` calculate the enrichment score of certain clustering methods and certain number of cluster.

Which clustering method and the number of cluster should be choose? There is no an automatical way. Here we show some principles:

- Different clusters should account for different gene sets.
- A gene set should enriched only in one cluster but not two or more.
- The number of gene in a gene set enriched cluster should be as smaller as it can when the enrichment score is high.

```
summary(clen_res)
```

```
##
## Clustering Methods:
```

```
## hierarchical pam
##
## The Number of Clusters:
## 10
##
## Metric of Distance Matrix:
## correlation
##
## Agglomeration method for hierarchical clustering (hclust and agnes):
## complete
##
## Gene set:
## c2.cp.kegg.v5.0.symbols.gmt.xz
```

```
# Here we consider the "pam" method and 10 clusters.
# Always make the number as character, please!
enrichment.table <- enrichment(clen_res, "pam", "10")
```

5.3.2 Heatmap of expression profiling with clusters

heatmapCluster is developed to show it. Based on Figure 2, it is obvious to know which cluster contains up-regulated or down-regulated genes.

```
# Always make the number as character, please!
heatmapCluster(clen_res, "pam", "10", maintitle="Psoriasis")
```

```
## The number of genes in each cluster:
## upDownGene
## 1 2
## 468 238
## cluster_size
## 1 2 3 4 5 6 7 8 9 10
## 158 65 38 92 50 67 63 94 61 18
```

5.3.3 Enrichment heatmap of co-expressed genes

heatmapPEI can be used to show the enrichment graph. See Figure 3. See ?heatmapPEI for more details.

```
# The enrichment score for 10 clusters, together with Down-regulated,
# Up-regulated and All DE genes. The values shown in Figure 2 is the -log2(FDR).
#
# Always make the number as character, please!
heatmapPEI(clen_res, "pam", "10", printGS=FALSE, maintitle="Pathway analysis for Psoriasis")
```

5.4 Others

5.4.1 Querying genes in a certain cluster

User can obtain the genes in a certain cluster via geneInCluster.

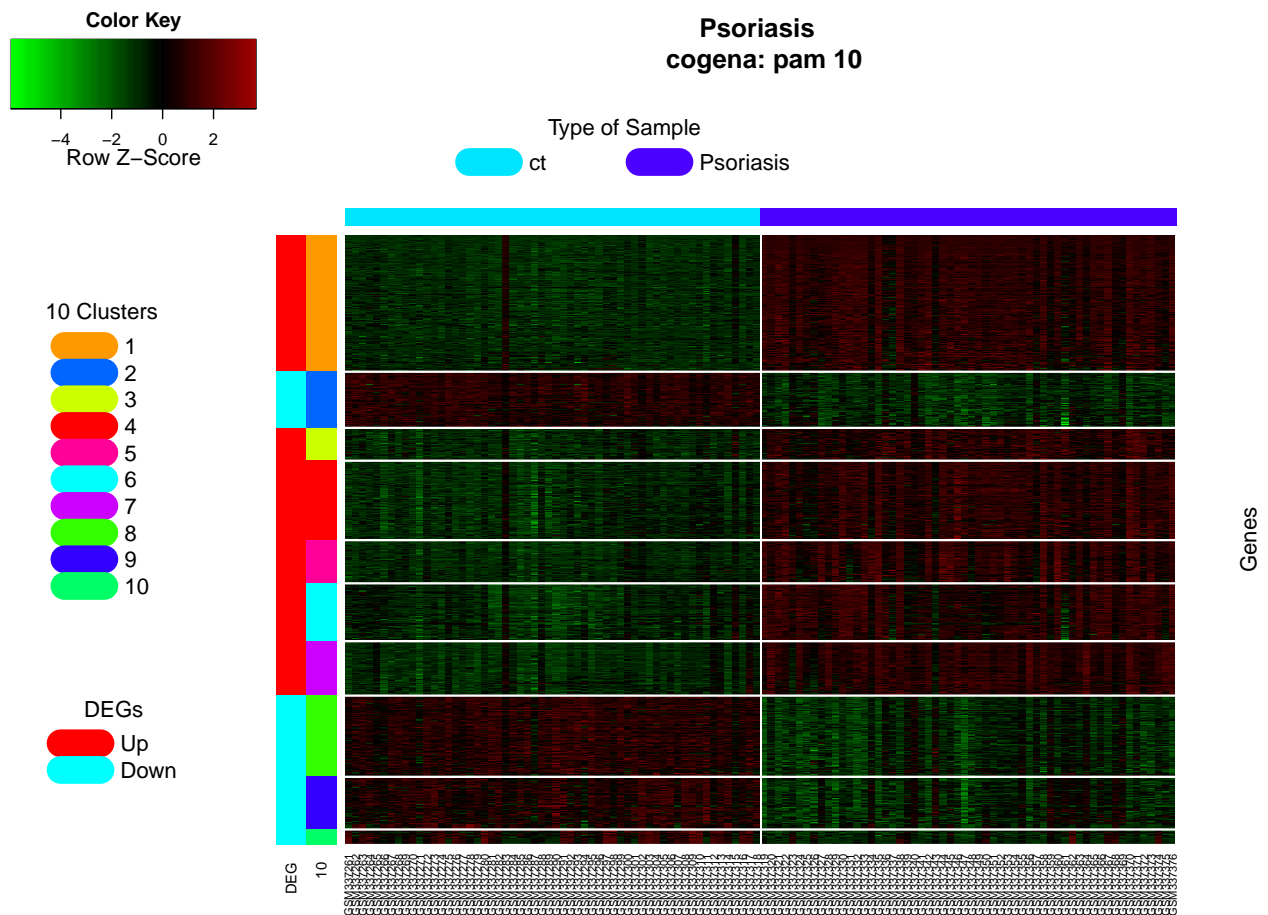


Figure 2: Heatmap of expression profiling with clusters

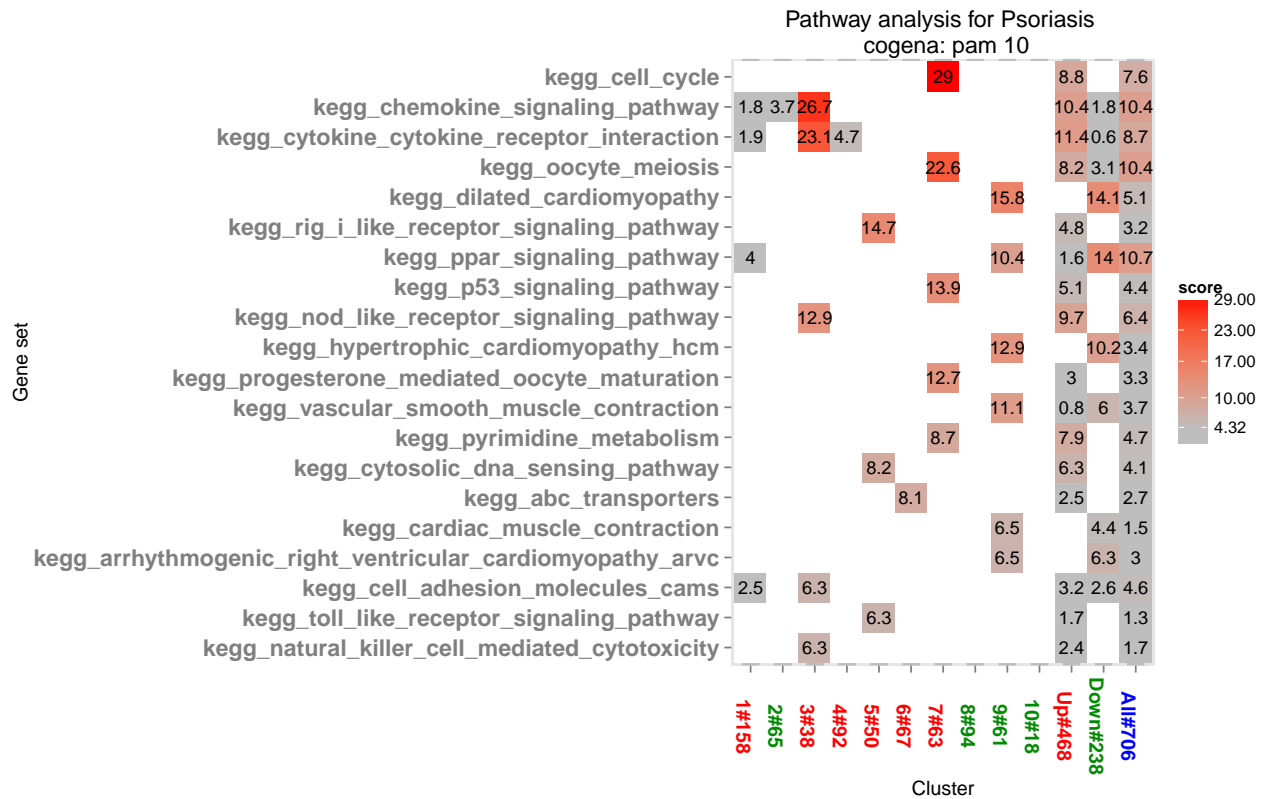


Figure 3: KEGG pathway enrichment

```
# Always make the number as character, please!
geneC <- geneInCluster(clen_res, "pam", "10", "4")
head(geneC)
```

```
## [1] "CD47" "SERPINB13" "PNP" "MPZL2" "KCNJ15" "SOX7"
```

5.4.2 Gene expression profiling with cluster information

It can be obtained by `geneExpInCluster`. There are two items, `clusterGeneExp` and `label`, in the returned object of `geneExpInCluster`. It can be used for other application.

```
# Always make the number as character, please!
gec <- geneExpInCluster(clen_res, "pam", "10")
gec$clusterGeneExp[1:3, 1:4]
```

```
##      cluster_id GSM337261 GSM337262 GSM337263
## PI3          1  6.556556  6.040479  7.033708
## S100A7A       1  4.989918  4.971686  5.677227
## S100A12       1  4.873823  5.168421  5.255036
```

```
gec$label[1:4]
```

```
## GSM337261 GSM337262 GSM337263 GSM337264
##      ct      ct      ct      ct
## Levels: ct Psoriasis
```

5.4.3 The gene correlation in a cluster

The correlation among a cluster can be checked and shown by `corInCluster`. See Figure 4.

```
# Always make the number as character, please!  
corInCluster(clen_res, "pam", "10", "10")
```

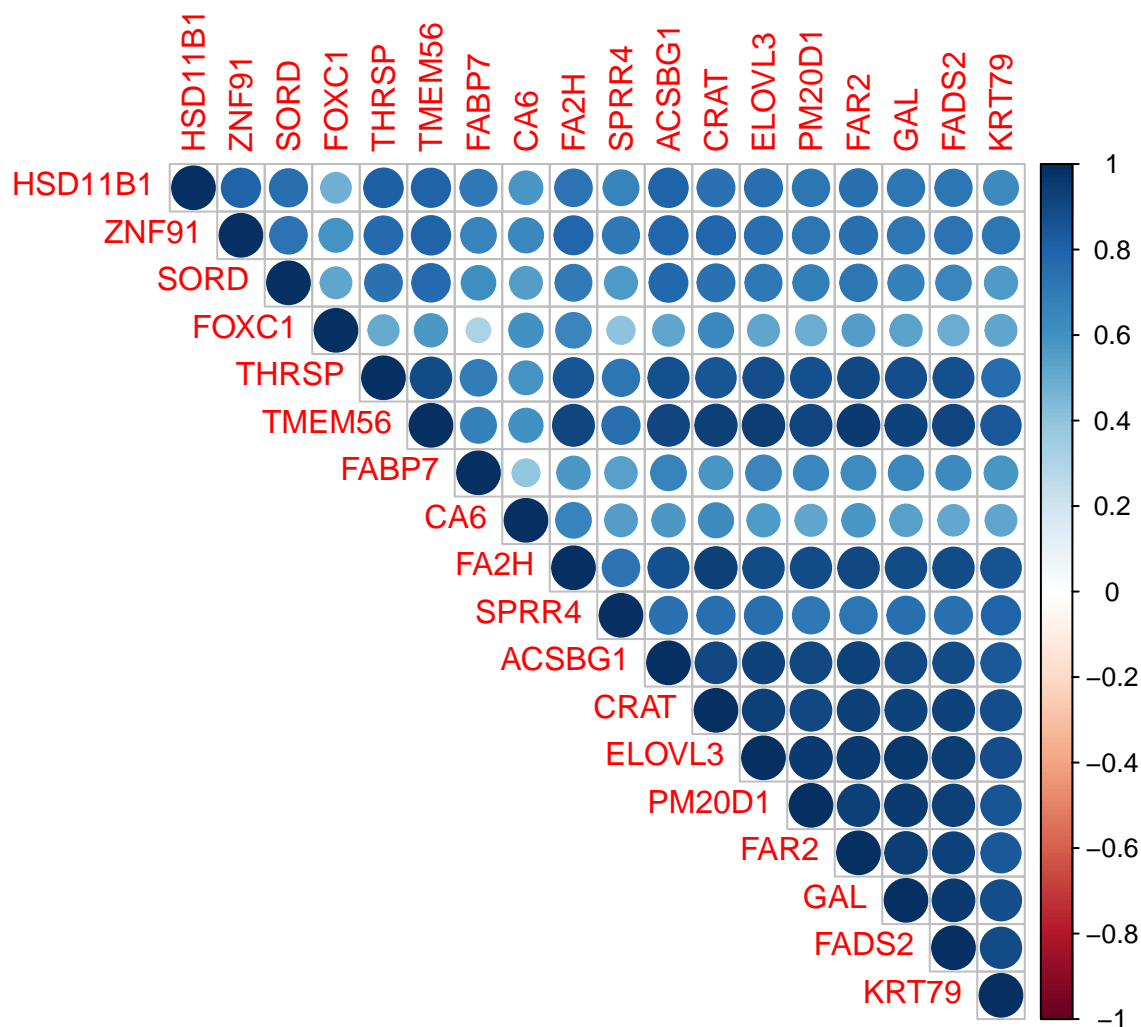


Figure 4: Correlation of genes in a cluster

6 Drug repositioning

Drug repositioning is made based on co-expressed genes instead of all the differentially expressed genes. If the input of cogenia is a disease related data, the drugs enriched should recovery the gene expression changed by the disease, while if the input is drug related, the enriched drugs should show similar gene expression changes caused by the drug studied. Here we show drugs for treating psoriasis, an autoimmune disease.

6.1 Drug repositioning analysis running

The drug repositioning gene set choice of *CmapDn100.gmt.xz* or *CmapUp100.gmt.xz* should be made based on the regulation direction of clusters. For example, as the 7th cluster contains up-regulated genes for psoriasis, the *CmapDn100.gmt.xz* is chosen for drug repositioning of psoriasis to recovery the gene expression changes caused by the disease.

```
# A comprehensive way
# cmapDn100_cogena_result <- clEnrich(genecl_result,
# annofile=system.file("extdata", "CmapDn100.gmt.xz", package="cogena"),
# sampleLabel=sampleLabel)

# A quick way
# Based on the pathway analysis results
cmapDn100_cogena_result <- clEnrich_one(genecl_result, "pam", "10",
  annofile=system.file("extdata", "CmapDn100.gmt.xz", package="cogena"),
  sampleLabel=sampleLabel)
```

6.2 Original result of drug repositioning

Showing the results ordered by the 7th cluster in Figure 5.

```
heatmapPEI(cmapDn100_cogena_result, "pam", "10", printGS=FALSE,
  orderMethod = "7", maintitle="Drug repositioning for Psoriasis")
```

```
# Results based on cluster 5.
# heatmapPEI(cmapDn100_cogena_result, "pam", "10", printGS=FALSE,
#           orderMethod = "5", maintitle="Drug repositioning for Psoriasis")

# Results based on cluster 9, containing down-regulated genes.
# heatmapPEI(cmapUp100_cogena_result, "pam", "10", printGS=FALSE,
#           orderMethod = "9", maintitle="Drug repositioning for Psoriasis")
```

6.3 Multi-instance merged result of drug repositioning

Usually there are more than 1 instances for a drug with different doses or time-points in the Cmap gene set. `heatmapCmap` can merge the multi-instance results based on parameter *mergeMethod* (“mean” or “max”). Figure 6 shows the multi-instance merged results ordered by the 7th cluster.

```
heatmapCmap(cmapDn100_cogena_result, "pam", "10", printGS=FALSE,
  orderMethod = "7", maintitle="Drug repositioning for Psoriasis")
```

7 Bug Report

<https://github.com/zhilongjia/cogena/issues>

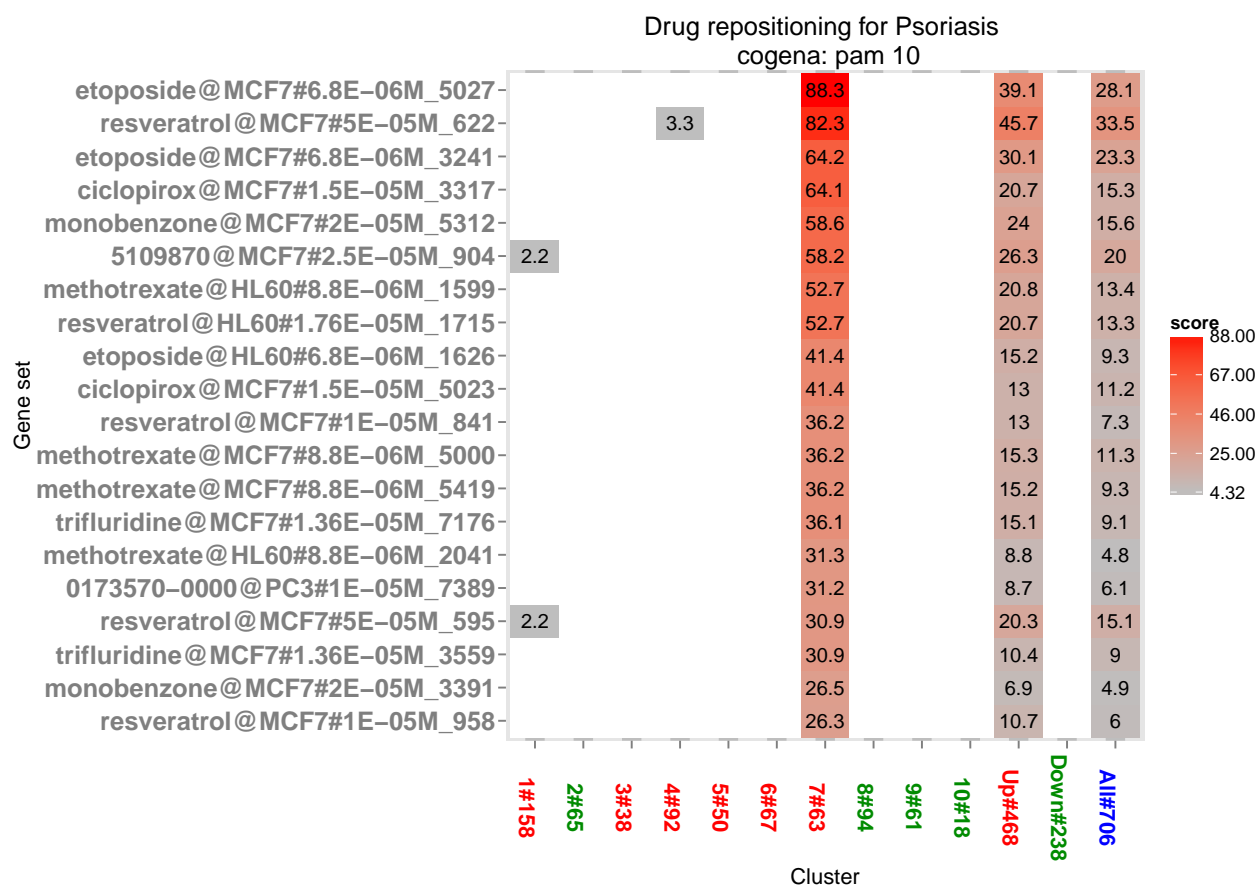


Figure 5: Drug repositioning

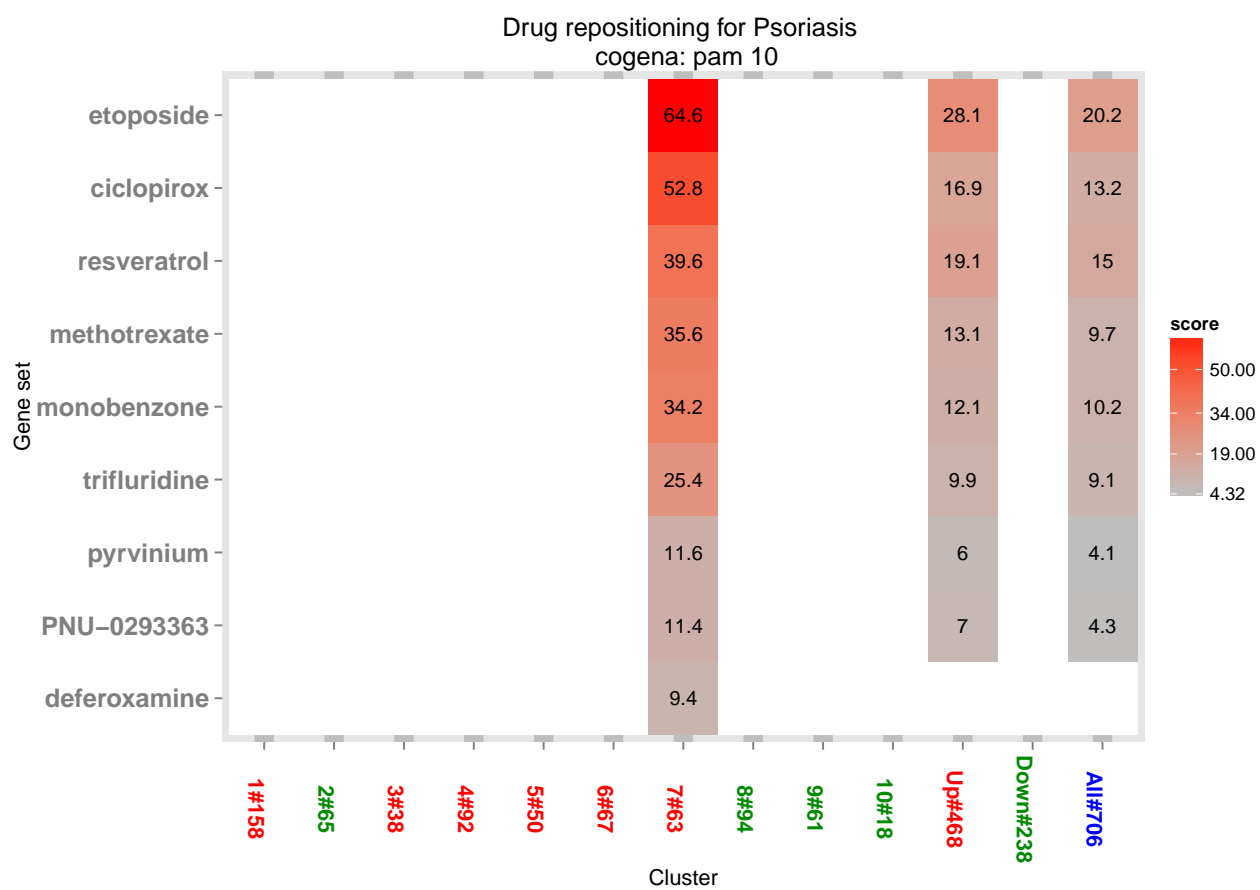


Figure 6: Drug repositioning (multi-instance merged)

8 Citation

Jia Z. et al. *Cogena, a tool for co-expressed gene-set enrichment analysis and visualization.*

9 Other Information

System info

```
## R version 3.2.2 (2015-08-14)
## Platform: x86_64-apple-darwin13.4.0 (64-bit)
## Running under: OS X 10.9.5 (Mavericks)
##
## locale:
## [1] C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats      graphics  grDevices  utils      datasets  methods    base
##
## other attached packages:
## [1] cogena_1.4.0   kohonen_2.0.19 MASS_7.3-44   class_7.3-14
## [5] ggplot2_1.0.1 cluster_2.0.3
##
## loaded via a namespace (and not attached):
## [1] fastcluster_1.1.16  gtools_3.5.0      reshape2_1.4.1
## [4] corrplot_0.73       lattice_0.20-33    pcaPP_1.9-60
## [7] colorspace_1.2-6    amap_0.8-14        htmltools_0.2.6
## [10] stats4_3.2.2        yaml_2.1.13        DBI_0.3.1
## [13] BiocGenerics_0.16.0 biwt_1.0           foreach_1.4.3
## [16] plyr_1.8.3          robustbase_0.92-5  stringr_1.0.0
## [19] munsell_0.4.2       gtable_0.1.2       devtools_1.9.1
## [22] caTools_1.17.1      mvtnorm_1.0-3      codetools_0.2-14
## [25] memoise_0.2.1       evaluate_0.8        Biobase_2.30.0
## [28] knitr_1.11          doParallel_1.0.8   parallel_3.2.2
## [31] DEoptimR_1.0-3      proto_0.3-10       Rcpp_0.12.1
## [34] KernSmooth_2.23-15  scales_0.3.0       formatR_1.2.1
## [37] gdata_2.17.0        apcluster_1.4.1    gplots_2.17.0
## [40] digest_0.6.8        stringi_0.5-5      dplyr_0.4.3
## [43] grid_3.2.2          tools_3.2.2        bitops_1.0-6
## [46] magrittr_1.5         lazyeval_0.1.10    rrcov_1.3-8
## [49] Matrix_1.2-2        assertthat_0.1     rmarkdown_0.8.1
## [52] iterators_1.0.8     R6_2.1.1           mclust_5.0.2
## [55] compiler_3.2.2
```