

Gene Set Data for Pathway Analysis in Mouse

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1 Data Introduction

Gene Set Knowledgebase (GSKB) is a comprehensive knowledgebase for pathway analysis in mouse. GSKB is similar to MSigDB (molecular signature database), developed at Broad Institute (<http://www.broadinstitute.org/gsea/msigdb>). GSKB is intended for mouse, while MSigDB is for human. GSKB is created to support pathway analysis using software like Gene Set Enrichment Analysis (GSEA), etc.

The GSKB contains various gene sets, corresponding to pathways and functional categories. There are seven different types of gene sets:

- mm_GO: gene sets from Gene Ontology for mouse (*Mus musculus*)
- mm_location: Gene sets based on chromosomal location
- mm_metabolic: metabolic pathways
- mm_miRNA: Target genes of microRNAs, predicted or experimentally verified
- mm_pathway: Currated pathways
- mm_TF: Transcription factor target genes.
- mm_other

In addition, we also compiled a large collection of gene lists representing differentially expressed genes manually collected from literature. This dataset is too big and is only available on our web site. At the end of this vignette, we show how these gene sets can be used.

2 Data Description

Interpretation of high-throughput genomics data based on biological pathways constitutes a constant challenge, partly because of the lack of supporting pathway database. We created a functional genomics knowledgebase in mouse, which includes 33,261 pathways and gene sets compiled from 40 sources such as Gene Ontology, KEGG, GeneSetDB, PANTHER, microRNA and transcription factor target genes, etc. Detailed information on these 40 sources and the citations is available <http://ge-lab.org/gskb/Table%201-sources.pdf>.

In addition, we also manually collected and curated 8,747 lists of differentially expressed genes from 2,526 published gene expression studies to enable the detection of similarity to previously reported gene expression signatures. These two types of data constitute a comprehensive Gene Set Knowledgebase (GSKB), which can be readily used by various pathway analysis software such as gene set enrichment analysis (GSEA).

More information about this data is available here <http://ge-lab.org/gskb/>. A paper describing these data are currently in revision by Database: The Journal of Biological Databases and Curation.

3 Loading the data

The datasets can be loaded using the `data` function. Here we load the specific types of gene sets, microRNA target genes. We also show a portion of the first gene set. Users can change "mm_miRNA" to any of the 7 categories such as "mm_pathway" to load other types of gene sets.

```
> library(gskb)
> data(mm_miRNA)
> mm_miRNA[[1]][1:10]

[1] "MIRNA_MM_BETEL_MMU-LET-7A"
[2] "BETEL_MMU-LET-7A; Good mirSVR score Conserved; The microRNA.org resource: targets and
[3] "NSUN4"
[4] "DCX"
[5] "KCNK6"
[6] "PBX1"
[7] "PHF8"
[8] "RACGAP1"
[9] "EFHD2"
[10] "DCBLD2"
```

4 Using the Data

The following examples use the knowledge base gene sets to run pathway analysis on a microarray gene expression data obtained from Gene Expression Omnibus(GSE40261). In this experiment, microRNA-29b was blocked by anti-sense oligonucleotides. We will first use the PAGE: Parametric Analysis of Gene Set Enrichment method (<http://www.biomedcentral.com/1471-2105/6/144>), as implemented in the PGSEA bioconductor package. Then we will use GSEA to analyze it.

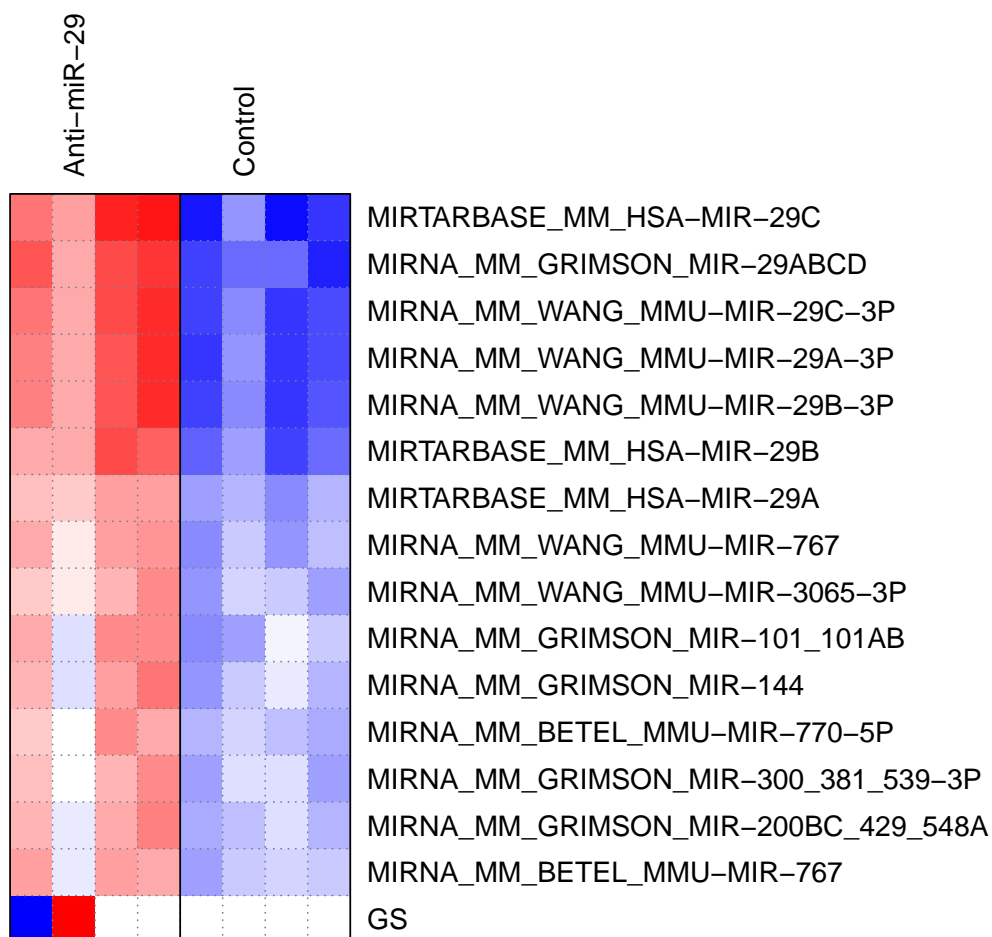
4.1 Pathway analysis using PGSEA

This example is using PGSEA with the microRNA gene sets.

```
> if (!requireNamespace("BiocManager", quietly=TRUE))
+   install.packages("BiocManager")
> BiocManager::install("PGSEA")
```

```
> library(PGSEA)
> library(gskb)
> data(mm_miRNA)
> gse<-read.csv("http://ge-lab.org/gskb/GSE40261.csv",header=TRUE, row.name=1)
> # Gene are centered by mean expression
> gse <- gse - apply(gse,1,mean)
> pg <- PGSEA(gse, cl=mm_miRNA, range=c(15,2000), p.value=NA)
> # Remove pathways that has all NAs. This could be due to that pathway has
> # too few matching genes.
> pg2 <- pg[rowSums(is.na(pg))!= dim(gse)[2], ]
> # Difference in Average Z score in two groups of samples is calculated and
> # the pathways are ranked by absolute value.
> diff <- abs( apply(pg2[,1:4],1,mean) - apply(pg2[,5:8], 1, mean) )
> pg2 <- pg2[order(-diff), ]
> sub <- factor( c( rep("Control",4),rep("Anti-miR-29",4) ) )
> smcPlot(pg2[1:15,],sub,scale=c(-12,12),show.grid=TRUE,margins=c(1,1,7,19),col=.rwb)
```

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This figure shows the top 15 pathways. As expected, miRNA-29 related gene sets are identified as differentially regulated pathway.

4.2 GSEA

This example is using GSEA with the miRNA gene set. Gene expression data is reformatted to a GCT format. And also a phenotype vector file is created in the CLS format. See GSEA help file for more information on these files. <http://www.broadinstitute.org/gsea/>. These files are read from our web site. R program for GSEA is also downloaded and saved on our web site.

```
> library(gskb)
> data(mm_miRNA)
> ## GSEA 1.0 -- Gene Set Enrichment Analysis / Broad Institute
```

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```
>
> GSEA.prog.loc<- "http://ge-lab.org/gskb/GSEA.1.0.R"
> source(GSEA.prog.loc, max.deparse.length=9999)
> GSEA(
+   # Input/Output Files :-----
+
+   # Input gene expression Affy dataset file in RES or GCT format
+   input.ds = "http://ge-lab.org/gskb/mouse_data.gct",
+
+   # Input class vector (phenotype) file in CLS format
+   input.cls = "http://ge-lab.org/gskb/mouse.cls",
+
+   # Gene set database in GMT format
+   gs.db = mm_miRNA,
+
+   # Directory where to store output and results (default: "")
+   output.directory = getwd(),
+
+   # Program parameters :-----
+   doc.string = "mouse",
+   non.interactive.run = T,
+   reshuffling.type = "sample.labels",
+   nperm = 1000,
+   weighted.score.type = 1,
+   nom.p.val.threshold = -1,
+   fwer.p.val.threshold = -1,
+   fdr.q.val.threshold = 0.25,
+   topgs = 10,
+   adjust.FDR.q.val = F,
+   gs.size.threshold.min = 15,
+   gs.size.threshold.max = 500,
+   reverse.sign = F,
+   preproc.type = 0,
+   random.seed = 3338,
+   perm.type = 0,
+   fraction = 1.0,
+   replace = F,
+   save.intermediate.results = F,
+   OLD.GSEA = F,
+   use.fast.enrichment.routine = T
+ )
```

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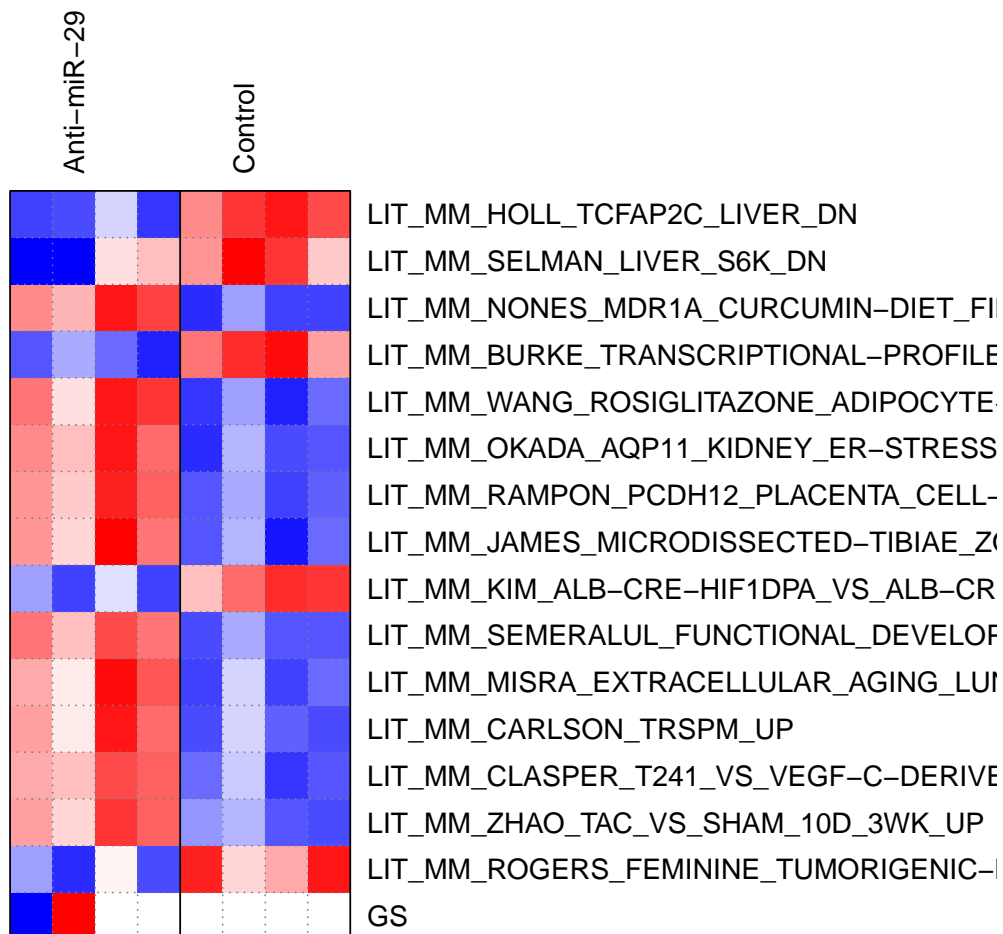
This will produce many output files in the current folder and the user can examine these figures and tables.

4.3 Additional Data

In addition to the 7 types of gene sets listed above, we also compiled a large collection of gene lists representing differentially expressed genes manually collected from literature. This dataset is too big and is only available on our web site. http://ge-lab.org/gskb/2-MousePath/MousePath_Co-expression_gmt.gmt Users can read these files directly from our website and use it in pathway analysis.

```
> library(PGSEA)
> library(gskb)
> d1 <- scan("http://ge-lab.org/gskb/2-MousePath/MousePath_Co-expression_gmt.gmt", what="")
> mm_Co_expression <- strsplit(d1, "\t")
> names(mm_Co_expression) <- sapply(mm_Co_expression, '[', 1)
> pg <- PGSEA(gse, cl=mm_Co_expression, range=c(15,2000), p.value=NA)
> # Remove pathways that has all NAs. This could be due to that pathway has
> # too few matching genes.
> pg2 <- pg[rowSums(is.na(pg))!= dim(gse)[2], ]
> # Difference in Average Z score in two groups of samples is calculated and
> # the pathways are ranked by absolute value.
> diff <- abs( apply(pg2[,1:4],1,mean) - apply(pg2[,5:8], 1, mean) )
> pg2 <- pg2[order(-diff), ]
> sub <- factor( c( rep("Control",4),rep("Anti-miR-29",4) ) )
> smcPlot(pg2[1:15,],sub,scale=c(-12,12),show.grid=TRUE,margins=c(1,1,7,19),col=.rwb)
```

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5 Session Info

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

```
> sessionInfo()

R version 3.6.0 (2019-04-26)
Platform: x86_64-pc-linux-gnu (64-bit)
Running under: Ubuntu 18.04.2 LTS

Matrix products: default
BLAS: /home/biocbuild/bbs-3.9-bioc/R/lib/libRblas.so
LAPACK: /home/biocbuild/bbs-3.9-bioc/R/lib/libRlapack.so

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=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] parallel stats4 stats graphics grDevices utils datasets
[8] methods base

other attached packages:
 [1] PGSEA_1.58.0      annaffy_1.56.0      KEGG.db_3.2.3
 [4] GO.db_3.8.2      AnnotationDbi_1.46.0 IRanges_2.18.0
 [7] S4Vectors_0.22.0 Biobase_2.44.0      BiocGenerics_0.30.0
[10] gskb_1.16.0

loaded via a namespace (and not attached):
 [1] Rcpp_1.0.1      knitr_1.22        bit_1.1-14        blob_1.1.1
 [5] tools_3.6.0    xfun_0.6          DBI_1.0.0         htmltools_0.3.6
 [9] yaml_2.2.0     bit64_0.9-7      digest_0.6.18     BiocManager_1.30.4
[13] memoise_1.1.0  evaluate_0.13     RSQLite_2.1.1     rmarkdown_1.12
[17] compiler_3.6.0 BiocStyle_2.12.0  pkgconfig_2.0.2
```