

Gene set and data preparation

Weijun Luo (luo_weijun AT yahoo.com)

October 30, 2025

1 Introduction

In this short tutorial, we cover a few practical issues we frequently come cross in GAGE (Luo et al., 2009) and other types of gene set analysis. These issues include: gene set and expression data input, probe set ID, transcript and gene ID conversion. This tutorial is written for those who are less familiar with R/Bioconductor basics. If you still have difficulties and you don't need to work on pathways or gene sets other than KEGG, you may use the Pathview Web server: pathview.uncc.edu. The server implemented GAGE based pathway analysis and Pathview based data visualization in a comprehensive pathway analysis workflow. Please check the overview page and the NAR web server paper (Luo et al., 2017) for details.

First we get started as deccribed in the main vignette. Under R, first we load the *gage* package:

```
> library(gage)
```

2 Expression data input

The gage package provides dedicated functions for data input, partically reading in the experimental or gene expression data and gene set data. First, let's look at how to read in a gene expression dataset in tab-delimited text format. You may open it in Excel for a better view. Now that genes are rows and samples are columns.

```
> filename=system.file("extdata/gse16873.demo", package = "gage")
> demo.data=readExpData(filename, row.names=1)
> #check the data
> head(demo.data)
```

	HN_1	DCIS_1	HN_2	DCIS_2	HN_3	DCIS_3	HN_4
10000	6.765984	6.458339	6.921720	6.774493	7.010564	6.986779	6.958761
10001	6.339474	6.755342	7.177369	6.842597	7.392611	6.879474	6.296571
10002	6.591755	6.790304	6.735359	6.773255	6.700016	7.041881	6.586285
10003	6.822092	6.590539	6.508452	6.411859	6.575640	6.470913	6.896886

```

100048912 7.356051 7.311144 7.385513 7.333481 7.392233 7.428623 7.314579
10004      6.941935 6.854373 6.883973 6.833695 6.855043 6.856864 7.021072
          DCIS_4      HN_5      DCIS_5      HN_6      DCIS_6
10000      6.888199 6.949912 6.948589 7.220427 7.070159
10001      6.130034 7.831222 7.942344 7.665248 7.799255
10002      6.501011 6.884931 7.652490 7.342505 7.500791
10003      6.848872 6.615143 6.407087 6.618174 6.651619
100048912 7.362658 7.370044 7.397249 7.383196 7.437643
10004      7.051310 6.767242 6.775276 6.873855 6.805247

```

```
> str(demo.data)
```

```

'data.frame':      100 obs. of  12 variables:
 $ HN_1   : num  6.77 6.34 6.59 6.82 7.36 ...
 $ DCIS_1 : num  6.46 6.76 6.79 6.59 7.31 ...
 $ HN_2   : num  6.92 7.18 6.74 6.51 7.39 ...
 $ DCIS_2 : num  6.77 6.84 6.77 6.41 7.33 ...
 $ HN_3   : num  7.01 7.39 6.7 6.58 7.39 ...
 $ DCIS_3 : num  6.99 6.88 7.04 6.47 7.43 ...
 $ HN_4   : num  6.96 6.3 6.59 6.9 7.31 ...
 $ DCIS_4 : num  6.89 6.13 6.5 6.85 7.36 ...
 $ HN_5   : num  6.95 7.83 6.88 6.62 7.37 ...
 $ DCIS_5 : num  6.95 7.94 7.65 6.41 7.4 ...
 $ HN_6   : num  7.22 7.67 7.34 6.62 7.38 ...
 $ DCIS_6 : num  7.07 7.8 7.5 6.65 7.44 ...

```

```

> #convert the data.frame into a matrix as to speed up the computing
> demo.data=as.matrix(demo.data)
> str(demo.data)

```

```

num [1:100, 1:12] 6.77 6.34 6.59 6.82 7.36 ...
- attr(*, "dimnames")=List of 2
 ..$ : chr [1:100] "10000" "10001" "10002" "10003" ...
 ..$ : chr [1:12] "HN_1" "DCIS_1" "HN_2" "DCIS_2" ...

```

Then it is ready for running GAGE analysis on **demo.data**. Please check the main vignette: Generally Applicable Gene-set/Pathway Analysis, for details. Please do not actually run GAGE analysis on **demo.data** as it only has 100 genes, too few for any meaningful analysis.

3 Gene set data input

We may download gene set definition from third-party databases like MSigDB in "GMT" format, or we may prepare our own gene set data in that format. Please check file "c2.demo.gmt" in the "extdata" folder for details. We may read in such gene set data using `readList` function. You may also modify the function to accomodate your own gene set data format.

```
> #an example GMT gene set data derived from MSigDB data
> filename=system.file("extdata/c2.demo.gmt", package = "gage")
> demo.gs=readList(filename)
> demo.gs[1:3]

$`3AB_GAMMA_DN`
[1] "ANXA3"      "BNIP2"      "COLQ"       "CSAD"       "CSNK1A1"    "DDX3Y"
[7] "DPAGT1"     "FEN1"       "LOC653689" "MED31"      "NAT12"      "PTK9L"
[13] "THOC4"

$`4NQO_ESR_OLD_UNREG`
[1] "EIF3S6IP" "FLOT1"      "GNPNAT1"    "HGF"        "LCK"        "NDRG1"
[7] "SLC18A2"  "SOX9"       "SYK"        "TAF1C"      "TP53I11"

$`4NQO_ESR_WS_UNREG`
[1] "ARPC1B"    "ATP5I"      "CEPT1"      "CNKSR1"     "COL17A1"    "CTCF"
[7] "CTGF"      "FLOT1"      "FUT4"       "GABBR1"     "GALNT2"     "GNPNAT1"
[13] "HADH2"     "HGF"        "HLA-DQA1"   "HNRPC"      "ITGB8"      "KIAA0146"
[19] "LCK"       "LRRFIP2"    "NDRG1"      "PDCD5"      "PRDX4"      "RHOB"
[25] "SGCB"      "SGPL1"      "SOX7"       "SOX9"       "SYK"        "TAF1C"
[31] "TP53I11"   "TTC19"      "TXNRD2"     "UGCG"       "USP1"       "ZNF507"

> #to use these gene sets with gsel6873, need to convert the gene symbols
> #to Entrez IDs first
> data(egSymb)
> demo.gs.sym<-lapply(demo.gs, sym2eg)
> demo.gs.sym[1:3]

$`3AB_GAMMA_DN`
[1] "306"      "663"      "8292"     "51380"     "1452"      "8653"      "1798"      "2237"
[9] NA         "51003"    "122830"   NA          "10189"

$`4NQO_ESR_OLD_UNREG`
[1] NA         "10211"    "64841"    "3082"      "3932"      "10397"     "6571"      "6662"      "6850"
[10] "9013"     "9537"
```

```
$`4NQO_ESR_WS_UNREG`
[1] "10095" "521" "10390" "10256" "1308" "10664" "1490" "10211" "2526"
[10] "2550" "2590" "64841" NA "3082" "3117" NA "3696" "23514"
[19] "3932" "9209" "10397" "9141" "10549" "388" "6443" "8879" "83595"
[28] "6662" "6850" "9013" "9537" "54902" "10587" "7357" "7398" "22847"
```

4 Probe set ID conversion

Gene set or pathway analysis requires that gene sets and expression data use the same type of gene ID (Entrez Gene ID, Gene symbol or probe set ID etc). However, this is frequently not true for the data we have. For example, our gene sets mostly use Entrez Gene ID, but microarray datasets are frequently labeled by probe set ID (or RefSeq transcript ID etc). Therefore, we need to convert or map the probe set IDs to Entrez gene ID. The support data package *gageData* (the latest version: $\geq 2.0.0$) provides a example microarray dataset with Affymetrix probe set IDs (labels with *_at* suffix), which are seen in most Affymetrix microarray datasets. From GEO GSE16873 record page or the CEL data file, we know that it uses Affymetrix Human Genome U133A Array. We need to use the corresponding Bioconductor annotation package, *hgu133a.db*. Of course, you need to have that installed first.

```
> library(gageData)
> data(gse16873.affyid)
> affyid=rownames(gse16873.affyid)
> library(hgu133a.db)
> egids2=hgu133aENTREZID[affyid]
> annots=toTable(egids2)
> str(annots)

'data.frame':      19610 obs. of  2 variables:
 $ probe_id: chr  "1053_at" "117_at" "121_at" "1255_g_at" ...
 $ gene_id : chr  "5982" "3310" "7849" "2978" ...

> gse16873.affyid=gse16873.affyid[annots$probe_id,]
> #if multiple probe sets map to a gene, select the one with maximal IQR
> iqrs=apply(gse16873.affyid, 1, IQR)
> sel.rn=tapply(1:nrow(annots), annots$gene_id, function(x){
+ x[which.max(iqrs[x])])
+ })
> gse16873.egid=gse16873.affyid[sel.rn,]
> rownames(gse16873.egid)=names(sel.rn)
> cn=colnames(gse16873.egid)
```

```

> hn=grep('HN',cn, ignore.case =T)
> dcis=grep('DCIS',cn, ignore.case =T)
> data(kegg.gs)
> gsel16873.kegg.p.affy <- gage(gsel16873.egid, gsets = kegg.gs,
+   ref = hn, samp = dcis)
> #result should be similar to that of using gsel16873

```

5 gene or transcript ID conversion

Frequently, we need to convert other types of gene/transcript IDs to Entrez Gene ID or the reverse. The *gage* package provides functions `eg2sym` and `sym2eg` for such ID conversions on human genes, which uses a integrated ID mapping matrix, to access it do: `data(egSymb); head(egSymb)`. The *pathview* package (Luo and Brouwer, 2013) provides two more comprehensive functions: `eg2id` and `id2eg`, which make use of the Bioconductor gene annotation packages or similar custom annotation packages. These functions not only cover the latest gene annotations but also convert gene IDs for many common model organisms, for a list of model organisms and corresponding Bioconductor gene annotation packages, check `data(bods)` as below. These functions derive a ID mapping matrix, which then can be used to map the data in a seperate step because multiple transcript ID may map to the same Entrez Gene ID.

```

> library(pathview)
> data(bods)
> print(bods)

```

	package	species	kegg code	id.type
[1,]	"org.Ag.eg.db"	"Anopheles"	"aga"	"eg"
[2,]	"org.At.tair.db"	"Arabidopsis"	"ath"	"tair"
[3,]	"org.Bt.eg.db"	"Bovine"	"bta"	"eg"
[4,]	"org.Ce.eg.db"	"Worm"	"cel"	"eg"
[5,]	"org.Cf.eg.db"	"Canine"	"cfa"	"eg"
[6,]	"org.Dm.eg.db"	"Fly"	"dme"	"eg"
[7,]	"org.Dr.eg.db"	"Zebrafish"	"dre"	"eg"
[8,]	"org.EcK12.eg.db"	"E coli strain K12"	"eco"	"eg"
[9,]	"org.EcSakai.eg.db"	"E coli strain Sakai"	"ecs"	"eg"
[10,]	"org.Gg.eg.db"	"Chicken"	"gga"	"eg"
[11,]	"org.Hs.eg.db"	"Human"	"hsa"	"eg"
[12,]	"org.Mm.eg.db"	"Mouse"	"mmu"	"eg"
[13,]	"org.Mmu.eg.db"	"Rhesus"	"mcc"	"eg"
[14,]	"org.Pf.plasmo.db"	"Malaria"	"pfa"	"orf"
[15,]	"org.Pt.eg.db"	"Chimp"	"ptr"	"eg"
[16,]	"org.Rn.eg.db"	"Rat"	"rno"	"eg"

```

[17,] "org.Sc.sgd.db"      "Yeast"      "sce"      "orf"
[18,] "org.Ss.eg.db"      "Pig"        "ssc"      "eg"
[19,] "org.Xl.eg.db"      "Xenopus"    "xla"      "eg"

> #simulated human expression data with RefSeq ID
> refseq.data <- sim.mol.data(mol.type = "gene", id.type = "REFSEQ",
+                             nexp = 2, nmol = 1000)
> #construct map between non-Entrez ID and Entrez Gene ID
> id.map.refseq <- id2eg(ids = rownames(refseq.data), category =
+                         "REFSEQ", org = "Hs")
> #Map data onto Entrez Gene IDs, note different sum.method can be used
> entrez.data <- mol.sum(mol.data = refseq.data, id.map = id.map.refseq,
+                         sum.method = "mean")

```

References

- Weijun Luo and Cory Brouwer. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. *Bioinformatics*, 29(14):1830–1831, 2013. doi: 10.1093/bioinformatics/btt285. URL <http://bioinformatics.oxfordjournals.org/content/29/14/1830.full>.
- Weijun Luo, Michael Friedman, Kerby Shedden, Kurt Hankenson, and Peter Woolf. GAGE: generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics*, 10(1):161, 2009. URL <http://www.biomedcentral.com/1471-2105/10/161>.
- Weijun Luo, Gaurav Pant, Yeshvant K Bhavnasi, Steven G Blanchard, and Cory Brouwer. Pathview Web: user friendly pathway visualization and data integration. *Nucleic Acids Research*, Web server issue:gkx372, 2017. doi: 10.1093/nar/gkx372. URL <https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/gkx372>.