

snpGeneSets: an R Package for a Unified Pathway Enrichment Analysis with Integrated Genetic Annotation Database and Fast Genomic Mapping Annotation

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1. Introduction

The *snpGeneSets* package integrates local copies of parsed NCBI dbSNP [1] and Entrez Gene [2] databases based on two recent genome builds of GRCh37/hg19 and GRCh38/hg38 and MSigDB gene sets V4.0 [3], and provides a unified pathway analysis with computationally efficient genomic mapping and annotation for a large number of GWS SNPs and genes and pathways. The *snpGeneSets* supports 4 measures of gene effects from genome-wide study (GWS) of SNP associations, and enables two types of enrichment analyses for genome-wide association study (GWAS) and genome-wide expression study (GWES). The general type */* analysis investigates pathway enrichment for a list of candidate genes, and the specialized type *//* analysis applies the Uniform-Score Gene-Set Analysis (USGSA) method [4] to examine pathway enrichment for GWS significant genes.

2. Installation

Before the installation of new version, an old version of *snpGeneSets* can be removed by system command:

```
R CMD REMOVE snpGeneSets
```

Or by R command

```
>remove.packages("snpGeneSets")
```

The package source file of [snpGeneSets_1.10.tar.gz](http://www.umc.edu/biostats/software/snpGeneSets_1.10.tar.gz) and windows binary file of [snpGeneSets_1.10.zip](http://www.umc.edu/biostats/software/snpGeneSets_1.10.zip) can be downloaded from <https://www.umc.edu/biostats/software/> .

Installation from the source file of *snpGeneSets_1.10.tar.gz* can be completed through the system command:

```
R CMD INSTALL snpGeneSets_1.10.tar.gz
```

Installation from the binary file of *snpGeneSets_1.10.zip* for Windows can be completed through the GUI interface: “Packages”→” Install package(s) from local zip files...” .

Notes: The package is integrated with parsed NCBI dbSNP 138 (GRCh37/hg19) and 142 (GRCh38/hg38) [1], Entrez gene 105 (GRCh37/hg19) and 106 (GRCh38/hg38) [2]. The installation will automatically download and install the integrated databases based on GRCh37 and GRCh38, which requires high-speed internet access. The SNP annotation data based on GRCh37/hg19 includes common variants with unique position from NCBI dbSNP and those low-frequency variants from 1000 Genome project. The SNP annotation data based on GRCh38/hg38 includes common variants with

unique position from NCBI dbSNP, but does not have low-frequency variants from 1000 Genome project.

3. Installation of MSigDB gene sets

Due to the license issue of MSigDB gene sets, the data is not directly provided by the *snpGeneSets* package. Instead, the user needs to visit the MSigDB download web at <http://www.broadinstitute.org/gsea/downloads.jsp> and registers with the email.

To install the MSigDB 4.0, the zipped file of [msigdb_v4.0_files_to_download_locally.zip](#) (“MSigDB version 4.0 - zipped msigdb.xml, gmt and chip files”) has to be downloaded and extracted locally. All required *gmt* files can be founded at the extracted directory of “msigdb_v4.0_GMTs”. The installation can be completed by function *msigdb_build*:

```
> library(snpGeneSets)
> msigdb_build(gmt_dir=~"/tmp/msigdb_v4.0_files_to_download_locally/msigdb_v4.0_GMTs")
```

The argument of *gmt_dir* shows where all the extracted *gmt* files can be found. The function will parse all *gmt* files and build the integrated database.

4. Identification of SNP and gene map positions from an updated reference genome

Many GWAS of SNP associations were based on an old reference genome build, e.g. NCBI36. The *snpGeneSets* can quickly convert old map positions for a large number of GWAS SNPs to updated positions based on a recent genome build, GRCh37 or GRCh38, simultaneously by function of *getSNPMap()*. Map positions of GRCh37 and GRCh38 for genes can be identified by function of *getGeneMap()*.

The *snpGeneSets* comes with two GWS data, T2D-GWAS and T2D-GWES. The T2D-GWAS contains GWAS SNP association for T2D in Finnish population from dbGaP (Analysis ID: pha002839) [5], and T2D-GWES presents differential expression p-values at pancreases of 10 control and 10 T2D human subjects [6], which we obtained by analysis of GEO expression data (GDS3782) using the linear models with empirical Bayes adjusting method [7].

4.1 Example: Identification of T2D-GWAS SNP map positions

```
> library(snpGeneSets)
> data("T2DGWAS")
```

```

> class(T2DGWAS)
[1] "data.frame"

> dim(T2DGWAS)
[1] 306368  2

> head(T2DGWAS)
      snp      p
1  rs4649592 0.95773144
2  rs41332249 0.98747972
3  rs1079109 0.42112743
4  rs3934834 0.38536813
5  rs3737728 0.64311534
6  rs6687776 0.08061468

```

The T2DGWAS results can be loaded into R by the function `data()`. There are total 306,368 SNPs with GWAS association p-values available. Identifiers of these SNPs and their map positions are obtained based on old genome build. Genomic map positions of these SNPs based on a recent map build can be obtained simultaneously by function of `getSNPMap()` and reference genome build can be specified by parameter `GRCh=37` (in default) or `GRCh=38`.

```

> snpMapAnn<- getSNPMap(T2DGWAS$snp)
> snpMapAnn38<- getSNPMap(T2DGWAS$snp, GRCh=38)

```

Depending on the computer performance, the map annotation may take up to 1 minute for completing the process.

```

> names(snpMapAnn)
[1] "rsid_map" "other"

```

The returned result variables of `snpMapAnn` and `snpMapAnn38` are a list and it contains two components, a data frame of `'rsid_map'` and a character vector of `'other'`. The `'rsid_map'` contains all SNP identifiers that can be found for their genomic positions. The `'other'` contains the SNP identifiers that cannot be found for map positions.

```

> class(snpMapAnn$rsid_map)
[1] "data.frame"

> dim(snpMapAnn$rsid_map)
[1] 306252  3

> dim(snpMapAnn38$rsid_map)
[1] 306045  3

> head(snpMapAnn$rsid_map)
  chr      pos      snp
1   4 21618674 rs10000010
2   4  95733906 rs10000023
3   4 103374154 rs10000030
4   2 237752054  rs1000007
5   4  21895517 rs10000092
6   4 157574035 rs10000121

```

```

> head(snpMapAnn38$rsid_map)
  chr    pos      snp
1   4 21617051 rs10000010
2   4  94812755 rs10000023
3   4 102452997 rs10000030
4   2 236843411  rs1000007
5   4  21893894 rs10000092
6   4 156652883 rs10000121

> class(snpMapAnn$other)
[1] "character"

> length(snpMapAnn$other)
[1] 116

> length(snpMapAnn38$other)
[1] 323

> head(snpMapAnn$other)
[1] "rs4649592" "rs41332249" "rs1079109" "rs7549320" "rs7412106"
[6] "rs12619064"

> head(snpMapAnn38$other)
[1] "rs4649592" "rs41332249" "rs1079109" "rs41511844" "rs17559902"
[6] "rs4297265"

```

The mapping annotation based on GRCh37 showed 306,252 SNPs have been identified for genomic map positions and 116 SNPs cannot be identified, which may be due to alteration or obsolete of these rs ids. For reference genome GRCh38, total 306,045 SNPs have been identified, but 323 SNPs are not.

4.2 Example: Identification of gene map positions for T2D-GWES

```

> data("T2DGWES")

> class(T2DEExpression)
[1] "data.frame"

> dim(T2DEExpression)
[1] 20185  3

> head(T2DEExpression)
  symbol gene_id      p
9199  MDFIC   29969 6.399265e-07
3613  PPP2CB   5516 1.209549e-06
3503  FXYD3    5349 1.955109e-06
2292  IGFBP3   3486 2.853953e-06
6984  UNC13B   10497 2.876275e-06
11673 RRAGD   58528 5.047322e-06

```

The T2D-GWES data can be loaded by the `data("T2DGWES")` command, and the results of `T2DEExpression` variable are stored as a data frame that contains differential expression p-values for 20,185 genes. The `T2DEExpression` contains gene symbol ('symbol'), its Entrez gene ID ('gene_id') and

the differential expression p-value ('p'). Map positions of T2D-GWES genes can be identified by `getGeneMap()` function with reference genome specified at parameter of `GRCh` that is 37 in default.

```
> geneMapAnn<-getGeneMap(T2DExpression$gene_id)

> names(geneMapAnn)
[1] "gene_map" "other"

> class(geneMapAnn$gene_map)
[1] "data.frame"

> dim(geneMapAnn$gene_map)
[1] 19299 6

> head(geneMapAnn$gene_map)
  chr   start   end strand gene_name gene_id
1  19 58858172 58864865    -    A1BG      1
2  12 9220304 9268558    -    A2M      2
3   8 18027971 18081198    +   NAT1      9
4   8 18248755 18258723    +   NAT2     10
5  14 95078639 95090395    + SERPINA3    12
6   3 151531769 151546276    +   AADAC     13

> class(geneMapAnn$other)
[1] "numeric"

> length(geneMapAnn$other)
[1] 920

> head(geneMapAnn$other)
[1] 100130051 100129513 5558 727770 100132999 100134017

> geneMapAnn38<-getGeneMap(T2DExpression$gene_id, GRCh=38)

> dim(geneMapAnn38$gene_map)
[1] 19283 6

> head(geneMapAnn38$gene_map)
  chr   start   end strand gene_name gene_id
1  19 58346806 58353499    -    A1BG      1
2  12 9067708 9115962    -    A2M      2
3   8 18170462 18223689    +   NAT1      9
4   8 18391245 18401213    +   NAT2     10
5  14 94612377 94624053    + SERPINA3    12
6   3 151813974 151828488    +   AADAC     13

> length(geneMapAnn38$other)
[1] 927

> head(geneMapAnn38$other)
[1] 100130051 100129513 727770 100132999 100134017 730184
```

The returned annotation variables of `geneMapAnn` and `geneMapAnn38` are a list with two components: "`gene_map`" and "`other`". The "`gene_map`" is a data frame with 19,299 genes from GRCh37 and 19,283 genes from GRCh38, and the map position of a gene is defined by chromosome ('chr'), transcription start position ('start') and transcription termination position ('end'). The "`other`" component is a numeric

vector and it contains 920 Entrez gene IDs for GRCh37 and 927 genes for GRCh38 that are not identified for their map positions.

The `getGeneMap()` function has a second argument of logical variable, `isGeneID`, that determines if the searched genes are character vector of gene symbol or numerical vector of Entrez Gene ID.

5. Two-way mapping between SNP, gene and pathway

5.1 Mapping between SNP and Gene

Fast mapping of GWAS SNPs to genes is important for interpreting and understanding GWAS results. `snp2Gene` identifies genes spanning the target SNPs, based on user-defined gene boundary and SNP positions.

```
> T2DGWAS[T2DGWAS$p==min(T2DGWAS$p),]
      snp      p
70765 rs886374 2.37573e-06
```

The top SNP hit of the T2D-GWAS is the `rs886374` with association p-value of $2.4E-06$. We can apply the `snp2Gene()` function to obtain the genes that cover this SNP based on either GRCh37 (in default) or GRCh38.

```
> rs886374_map<-getSNPMap("rs886374")$rsid_map
> rs886374_map
      chr      pos      snp
1     4     7738369 rs886374
> rs886374_gene<-snp2Gene(rs886374_map)
> rs886374_gene
$map
      snp      gene_id
1     rs886374     57537

$other
character(0)
> getGeneMap(57537)$gene_map
      chr      start      end      strand gene_name  gene_id
1     4     7194374     7744564      +     SORCS2     57537

> rs886374_map38<-getSNPMap("rs886374", GRCh=38)$rsid_map
> rs886374_map38
      chr      pos      snp
1     4     7736642 rs886374
```

```
> rs886374_gene38<-snp2Gene(rs886374_map38, GRCh=38)
```

```
> rs886374_gene38
```

```
$map
```

```
  snp gene_id
```

```
1 rs886374 57537
```

```
$other
```

```
character(0)
```

```
> getGeneMap(57537,GRCh=38)$gene_map
```

	chr	start	end	strand	gene_name	gene_id
1	4	7192647	7742837	+	SORCS2	57537

The *snp2Gene()* function requires a data frame of SNP map including 'chr', 'pos' and 'snp' as the input to perform the SNP-Gene mapping. We first obtained the data frame of *rs886374_map* (GRCh37) and *rs886374_map38* (GRCh38) by *getSNPMap()* function. The *rs886374_gene* and *rs886374_gene38* returned by *snp2Gene()* function showed that SNP *rs886374* mapped to Entrez gene ID *57537*. The *getGeneMap()* function showed that the gene ID of *57537* is *SORCS2*, which is at Chromosome 4 from *7,194,374* to *7,744,564* bp for GRCh37 and from *7,192,647* to *7,742,837* bp for GRCh38.

The *snp2Gene()* function can be applied to map all T2D-GWAS SNPs to genes simultaneously. The mapping may take >1 hour depending on the number of GWAS SNPs. To speed the process, GWAS SNPs can be splitted to map *100,000* SNPs every time.

```
> snpGeneMapAnn<-snp2Gene(snpMapAnn$rsid_map)
```

```
> names(snpGeneMapAnn)
```

```
[1] "map" "other"
```

```
> class(snpGeneMapAnn$map)
```

```
[1] "data.frame"
```

```
> dim(snpGeneMapAnn$map)
```

```
[1] 172041 2
```

```
> head(snpGeneMapAnn$map)
```

	snp	gene_id
1	rs10000010	80333
2	rs10000023	658
5	rs10000092	80333
10	rs10000169	57619
11	rs1000022	171425
14	rs10000300	54502

```
> length(unique((snpGeneMapAnn$map$gene_id)))
```

```
[1] 24339
```

```
> class(snpGeneMapAnn$other)
```



```

[1] "character"
> length(snpGeneMapAnn$other)
[1] 146506
> head(snpGeneMapAnn$other)
[1] "rs10000030" "rs1000007" "rs10000121" "rs1000014" "rs10000141"
[6] "rs1000016"
> snpGeneMapAnn38<-snp2Gene(snpMapAnn$rsid_map, GRCh=38)
> head(snpGeneMapAnn38$map)

```

The *snp2Gene()* function returned the SNP-gene mapping annotation results of *snpGeneMapAnn* (GRCh37) and *snpGeneMapAnn38* (GRCh38) for 306,252 GWAS SNPs. The *snpGeneMapAnn* and *snpGeneMapAnn38* are a list with two components: a data frame of "map" and a character vector of "other". The *snpGeneMapAnn\$map* showed that 172,041 SNPs were successfully mapped to 24,339 genes and *snpGeneMapAnn\$other* indicated that 146,506 SNPs are out of gene boundary. The gene boundary is defined by two arguments, 'up' for the upstream region and 'down' for the downstream region with default value of 2,000 bp for both. Depending on the computer performance, the SNP-gene mapping for all T2D-GWAS SNPs may take up to 30 minutes. The mapping results can be directly found at 'snpGeneMap' variable from "T2DGWAS" data, which is the same as *snpGeneMapAnn\$map*.

In contrast to the *snp2Gene()* function, the *getRegionSNP()* function performs the reverse mapping and it shows annotated common SNPs spanned by the target gene or genomic region. The *getRegionSNP()* function takes a data frame including 'chr', 'start' and 'end' as the input.

```

> chr=c("14","1","18","16","16")
> start=c(78786077, 213910494, 57850422, 53813450, 53820527)
> end=start+1000
> regionDF=data.frame(chr=chr, start=start, end=end, stringsAsFactors=FALSE)
> regionSNPs<-getRegionSNP(regionDF)
> class(regionSNPs)
[1] "data.frame"
> dim(regionSNPs)
[1] 73 3
> head(regionSNPs)

```

```

      chr      pos      snp
1     1 213910494 rs1704198
2     1 213910566 rs10864067
3     1 213910585 rs141152028
4     1 213910675 rs79688837
5     1 213910826 rs182273155
6     1 213910983 rs186584814

>regionSNPs38<-getRegionSNP(regionDF, GRCh=38)

> dim(regionSNPs38)
[1] 24 3

> head(regionSNPs38)
  chr      pos      snp
1   1 213910556 rs75780458
2   1 213910610 rs853744
3   1 213910621 rs59335652
4   1 213910799 rs701894
5   1 213911041 rs12087028
6   1 213911081 rs919894

```

For the example above, the `getRegionSNP()` function returned the results to a data frame variable of `regionSNPs` for mapping annotations of 73 SNPs (GRCh37) and `regionSNPs38` for mapping annotations of 24 SNPs (GRCh38)

5.2 Mapping between Gene and Pathway

For a significant gene from GWAS or GWES, identification of its implicated pathways may shed light on novel gene function for disease genetics, and the mapping of gene to pathway is implemented by the `gene2Set()` function.

```

> T2DExpression[T2DExpression$p==min(T2DExpression$p),]
      symbol      gene_id      p
19199 MDFIC      29969      6.399265e-07

```

The top gene of the T2D-GWES is MDFIC (Entrez gene ID: `gene_id=29969`) with p-value of $6.4E-07$, which acts as a transcriptional activator or repressor. The `gene2Set()` function can be applied to identify the MSigDB gene sets that include the MDFIC gene.

```

> gid29969_C2<-gene2Set(29969, setType=2)
> length(gid29969_C2)
[1] 45

> head(gid29969_C2)
[1] 4074 4926 4928 4973 5029 5074

> gid29969_C5<-gene2Set(29969, setType=14)
> length(gid29969_C5)
[1] 49

> head(gid29969_C5)

```

[1] 239 280 301 305 307 343

Application of *gene2Set()* function shows that *MDFIC* gene is the component gene of 45 MSigDB gene sets at the category of “C2: curated gene sets” and the component gene of 49 MSigDB gene sets at the category of “C5: GO gene sets”. The category of gene sets can be specified by the argument of ‘*setType*’, which takes the value of category ID from 0 to 19. The 20 gene-set categories and their description can be shown by *getSetType()* function. The Table 1 below summarizes all categories and their IDs, and *setType=2* and *setType=14* correspond to category of “C2: curated gene sets” and “C5: GO gene sets” respectively.

Table 1. Summary of 20 MSigDB gene-set categories

ID	symbol	name
0	c0	C0: all gene sets
1	c1	C1: positional gene sets
2	c2	C2: curated gene sets
3	c2_cgp	C2_CGP: chemical and genetic perturbations
4	c2_cp	C2_CP: Canonical pathways
5	c2_biocarta	C2_CP:BIOCARTA: BioCarta gene sets
6	c2_kegg	C2_CP:KEGG: KEGG gene sets
7	c2_reactome	C2_CP:REACTOME: Reactome gene sets
8	c3	C3: motif gene sets
9	c3_mir	C3_MIR: microRNA targets
10	c3_tft	C3_TFT: transcription factor targets
11	c4	C4: computational gene sets
12	c4_cgn	C4_CGN: cancer gene neighborhoods
13	c4_cm	C4_CM: cancer modules
14	c5	C5: GO gene sets
15	c5_bp	C5_BP: GO biological process
16	c5_cc	C5_CC: GO cellular component
17	c5_mf	C5_MF: GO molecular function
18	c6	C6: oncogenic signatures
19	c7	C7: immunologic signatures

In contrast to *gene2Set()* function, the *getGeneSetInfo()* function identifies all member genes of a pathway and provides the mapping of pathway to genes. The *gid29969_C2* showed that the *MDFIC* gene is a member gene of gene-set *ID=5029*. Description of the pathway can be shown by the *getGeneSetInfo()*.

The *getGeneSetInfo()* function below returns the results to *pid5029Ann* which contains 5 components: the ‘*setID*’ of gene set identifier, the ‘*set_name*’ of the gene set name, the ‘*set_link*’ of the MSigDB web link

describing the gene set, the 'set_type' of the gene-set category including the gene set and the 'set_geneid' of Entrez gene IDs belonging to the gene set.

```
> pid5029Ann<-getGeneSetInfo(5029)
> names(pid5029Ann)
[1] "setID" "set_name" "set_link" "set_type" "set_geneid"
> pid5029Ann

$setID
[1] 5029

$set_name
[1] "AKL_HTLV1_INFECTIION_DN"

$set_link
[1] "http://www.broadinstitute.org/gsea/msigdb/cards/AKL_HTLV1_INFECTIION_DN"

$set_type
      c1          c2      c2_cgp      c2_cp c2_biocarta      c2_kegg
      FALSE      TRUE      TRUE      FALSE      FALSE      FALSE
c2_reactome      c3      c3_mir      c3_tft      c4          c4_cgn
      FALSE      FALSE      FALSE      FALSE      FALSE      FALSE
c4_cm          c5      c5_bp      c5_cc      c5_mf          c6
      FALSE      FALSE      FALSE      FALSE      FALSE      FALSE
      c7
      FALSE

$set_geneid
 [1] 22934 84525 5774 1848 7273 54504 25957 10632 5367 917
[11] 5771 163486 9218 892 55076 57515 51646 158471 10656 3001
[21] 5175 1846 5743 9659 2015 10129 942 3002 23097 3725
[31] 2534 10578 29103 4212 27230 29909 10314 51761 51465 301
[41] 23376 55125 84864 4128 55540 10049 51389 999 23266 3688
[51] 1288 3490 10142 5168 8477 4208 10447 5476 3359 26123
[61] 7259 5569 29969 51150 28683 10225 26228 5980
```

6. Gene measures by SNP associations and U-score calculation for gene effects

A gene typically contains associations of multiple SNPs from a GWAS, and the *getGeneMeasure()* function provides four measures (*minP*, *2ndP*, *simP* and *fishP*) of the gene effect by summarizing SNP association *p*-values. *U*-score of a gene measure represents percentage of genome-wide genes with effects stronger than the given gene and it can be calculated by *uscore()* function.

For *K* SNPs mapped to a gene with GWAS *p*-values (p_1, p_2, \dots, p_K), the ordered *p*-value is defined as $p_{(1)} \leq p_{(2)} \leq \dots \leq p_{(K)}$, where $p_{(1)} = \min\{p_1, p_2, \dots, p_K\}$ and $p_{(K)} = \max\{p_1, p_2, \dots, p_K\}$. Four gene measures are calculated respectively as $\text{minP} = p_{(1)}$, $\text{2ndP} = p_{(2)}$, $\text{simP} = \min_i\{Kp_{(i)}/i\}$ and $\text{fishP} = \Pr(X \geq x = -2 \sum_{i=1}^K \log(p_i)) = \Psi(x)$, where Ψ is the chi-square distribution function with $df=2K$. Uniform score (*U*-score) is calculated as $U_i = (\sum_j I(M_j < M_i) + 0.5 \cdot \sum_j I(M_j = M_i))/L$, where M_i is gene measure of the *i*-th gene and *L* is the total number of genes.

The `getGeneMeasure()` takes an arguments of `'snpGeneP'`. The `'snpGeneP'` is a data frame containing column of `'snp'` for rs id, column of `'gene_id'` for Entrez gene IDs spanning the `'snp'`, and column of `'p'` for SNP association p-value.

```
> snpGeneMap <- snpGeneMapAnn$map #snpGeneMap can be found from data T2DGWAS
> snpGeneP<-merge(snpGeneMap, T2DGWAS, all=FALSE)
> head(snpGeneP)
      snp gene_id      p
1 rs10000010  80333 0.2489708
2 rs10000023   658 0.2059405
3 rs10000092  80333 0.7070708
4 rs10000169  57619 0.5055075
5 rs1000022  171425 0.8532224
6 rs10000300  54502 0.5191723

> T2DGWASGene0<-getGeneMeasure(snpGeneP)
> head(T2DGWASGene0)
  gene_id      minp      sndp      simp      fishp
1      1 0.14992377 0.61819639 0.29984753 0.092682331
2      2 0.63210108 0.65196227 0.79051801 0.585242462
3      3 0.33866379 0.33866379 0.33866379 0.141139178
4      9 0.28229107 0.43147721 0.80126634 0.265557328
5     10 0.04538995 0.05860277 0.08790415 0.003165650
6     12 0.10190141 0.13136668 0.19705002 0.008034187

> minp_uscore<-uscore(T2DGWASGene0$minp)
> head(minp_uscore)
[1] 0.3713382 0.8397428 0.6154937 0.5545215 0.1592300 0.2841735

> T2DGWASGene <- T2DGWASGene0
> for (ms in c("minp", "sndp", "simp", "fishp")) T2DGWASGene[[ms]]<-uscore(T2DGWASGene[[ms]])
> head(T2DGWASGene)
  gene_id      minp      sndp      simp      fishp
1      1 0.3713382 0.7145733 0.28760426 0.4086035
2      2 0.8397428 0.7440528 0.74729857 0.8902790
3      3 0.6154937 0.4576400 0.32357533 0.4921936
4      9 0.5545215 0.5503307 0.75900818 0.6498418
5     10 0.1592300 0.1039279 0.08642508 0.1212252
6     12 0.2841735 0.2105469 0.19181150 0.1590246
```

The `'snpGeneMap'`, `'snpGeneP'` and `'T2DGWASGene'` can be manually created as above. These variables are also pre-generated and automatically loaded with `'T2DGWAS'` data. The `T2DGWASGene0` contains measures of `minP`, `2ndP`, `simP` and `fishP` for every T2DGWAS gene. The `minp_uscore` is the uniform score for `minp` measure and U-score can also be similarly generated for other three measures. The `T2DGWASGene` contains U-scores for every gene measure.

We examined 9 genes that were previously reported to have associations with T2D, and their measures (*gmeasure*) and U-scores (*gscore*) were shown in the Figure 1.

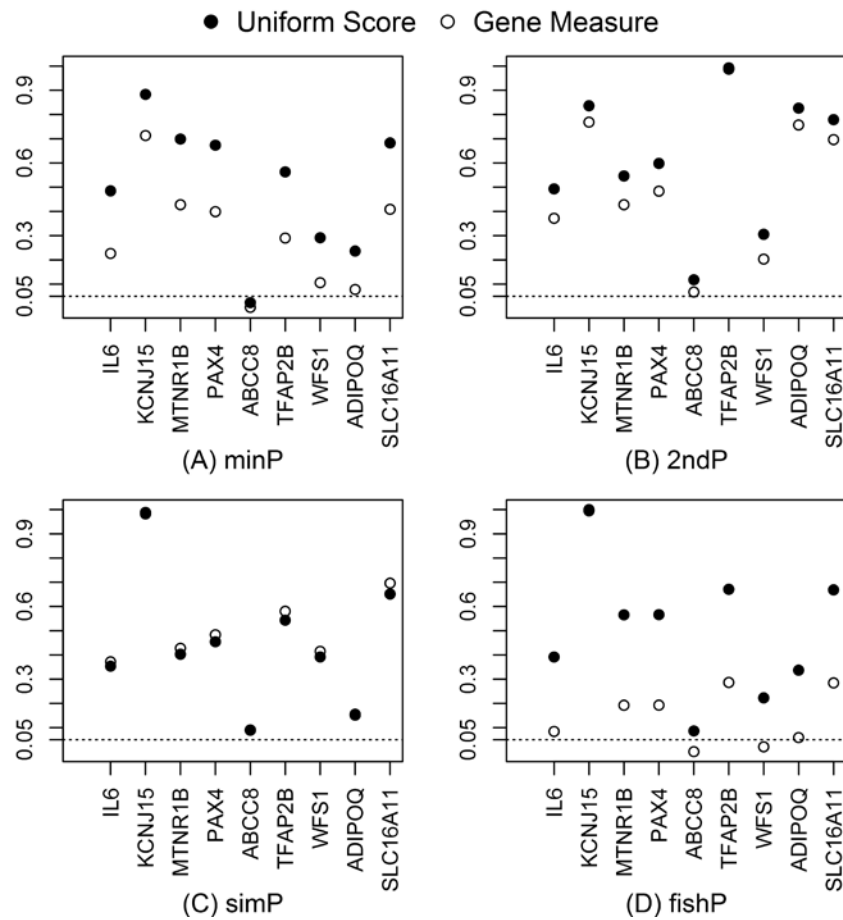


Figure 1. Gene measures and uniform scores of 9 T2D-GWAS genes

```
> genes<-c("IL6", "KCNJ15", "MTNR1B", "PAX4", "ABCC8", "TFAP2B", "WFS1", "ADIPOQ", "SLC16A11")
> genes<-getGeneMap(genes, FALSE)$gene_map[,c("gene_name", "gene_id")]
> gmeasure<-merge(genes, T2DGWASGene0, all=FALSE)

> gmeasure
  gene_id gene_name      minp      sndp      simp      fishp
1    3569      IL6  0.226553618 0.37160095 0.37160095 0.0841875404
2    3772    KCNJ15  0.713388908 0.76853023 0.98839315 0.9947997402
3    4544    MTNR1B  0.427681378 0.42768138 0.42768138 0.1924510584
4    5078      PAX4  0.398935200 0.48357556 0.48357556 0.1929153130
5    6833    ABCC8  0.004314517 0.06752818 0.09060486 0.0008465779
6    7021    TFAP2B  0.290199840 0.98718080 0.58039968 0.2864797108
7    7466      WFS1  0.105901871 0.20330600 0.41484572 0.0209583156
8    9370    ADIPOQ  0.077799163 0.75677862 0.15559833 0.0588767430
9   162515 SLC16A11  0.408816921 0.69633535 0.69633535 0.2846736745

> gscore<-merge(genes, T2DGWASGene, all=FALSE)
> gscore
```

	gene_id	gene_name	minp	sndp	simp	fishp
1	3569	IL6	0.48488023	0.4930564	0.35365052	0.39165537
2	3772	KCNJ15	0.88304778	0.8361272	0.98206582	0.99969185
3	4544	MTNR1B	0.69865237	0.5464275	0.40295411	0.56549160
4	5078	PAX4	0.67338428	0.5981141	0.45464070	0.56635441
5	6833	ABCC8	0.02368626	0.1180205	0.08909569	0.08630182
6	7021	TFAP2B	0.56331402	0.9924607	0.54346933	0.67104236
7	7466	WFS1	0.29148691	0.3056617	0.39186080	0.22287276
8	9370	ADIPOQ	0.23651341	0.8262459	0.15142364	0.33705165
9	162515	SLC16A11	0.68275196	0.7788734	0.65144418	0.66870044

The calculation showed that only ABCC8 has all 4 gene measures and U-scores around or smaller than 0.05. The results presented that a stronger gene measure (i.e. smaller p-values) tends to have a smaller *U*-score. However, different gene measures for the same gene have varied *U*-scores, showing inconsistent measures of gene effects over genome. The calculation of *U*-score will unify these gene measures for comparability with the same interpretability. For example, the *minP*, *2ndP*, *simp* and *fishP* presented summary SNP association p-values of 0.004, 0.068, 0.091 and 0.0008 for ABCC8 gene respectively, and the corresponding *U*-scores indicated that 2.4%, 11.8%, 8.9% and 8.6% GWAS genes have stronger gene effects than ABCC8.

For T2D-GWES, differential expression p-value is used to directly measure gene effect and calculate *U*-scores of the selected 9 genes. The p-value of ABCC8 is $3.4E-04$, showing only 0.4% of genes over genome with stronger measured effect than the ABCC8.

```
>data(T2DGWES)
> escore<-uscore(T2DExpression$p)
> T2DExpression$us<-escore
> T2DExpression[T2DExpression$symbol %in% genes$gene_name,]
      symbol gene_id      p      us
4560   ABCC8    6833 0.0003363277 0.004235819
2490   KCNJ15    3772 0.0268452946 0.091825613
3293    PAX4    5078 0.1437856302 0.270027248
16017 SLC16A11 162515 0.1471156303 0.273792420
2934   MTNR1B    4544 0.1978929899 0.331904880
4994    WFS1    7466 0.2134392425 0.346569235
2330    IL6    3569 0.3036799699 0.440351746
4685   TFAP2B    7021 0.4280112100 0.552068368
6062   ADIPOQ    9370 0.8169270613 0.865320783
```

7. Pathway Enrichment Analysis I of candidate genes

The type I analysis is a generalized pathway enrichment analysis that aims to identify gene sets enriched for a candidate list of genes. The list can be previously identified susceptibility genes or top genes from a GWAS or GWES. The analysis can be performed by function *enrichTest1()*.

7.1 Example: Enrichment analysis I of T2D-GWAS

For *T2DGWAS* data, the top 5% genes were selected as candidate genes by measures of *minP*, *2ndP*, *simP* and *fishP* respectively, and they were tested for pathway enrichment at 186 KEGG gene sets.

```
> topMinpGenes<-
T2DGWASGene[order(T2DGWASGene$minp),][1:trunc(nrow(T2DGWASGene)*0.05),"gene_id"]
> topsndpGenes<-
T2DGWASGene[order(T2DGWASGene$sndp),][1:trunc(nrow(T2DGWASGene)*0.05),"gene_id"]
> topsimpGenes<-
T2DGWASGene[order(T2DGWASGene$simp),][1:trunc(nrow(T2DGWASGene)*0.05),"gene_id"]
> topfishpGenes<-
T2DGWASGene[order(T2DGWASGene$fishp),][1:trunc(nrow(T2DGWASGene)*0.05),"gene_id"]
```

The *enrichTest1()* function takes an argument of 'genes' for the candidate genes tested for pathway enrichment, and the argument 'setType' takes the category ID that defines which category of gene sets will be tested for enrichment. For example, *setType=6* defines the KEGG gene-sets for enrichment test. Description of the category ID can be found at Table 1.

```
> minpGeneSets_KEGG<-enrichTest1(topMinpGenes,setType=6)
> names(minpGeneSets_KEGG)
[1] "enrich_test" "useGenes" "nGenes" "nTopGenes" "setTypeInfo"
> head(minpGeneSets_KEGG$enrich_test)
  pid size genesSize      effect      sd      pval
1 2718  62          4  0.009646184 0.02892126 0.252203325
2 2719  32          0 -0.054869945 0.04025669 0.836564565
3 2720  27          2  0.019204129 0.04382593 0.182359889
4 2721  28          0 -0.054869945 0.04303621 0.794908305
5 2722  34          3  0.033365349 0.03905473 0.113359667
6 2723  26          5  0.137437747 0.04466079 0.002345904
> length(minpGeneSets_KEGG$useGenes)
[1] 289
> minpGeneSets_KEGG$nGenes
[1] 5267
> minpGeneSets_KEGG$nTopGenes
[1] 289
> minpGeneSets_KEGG$setTypeInfo
 $id
[1] 6
 $symbol
[1] "c2_kegg"
 $name
[1] "C2_CP:KEGG: KEGG gene sets"
 $description
[1] "Gene sets derived from the KEGG pathway database, http://www.genome.jp/kegg/pathway.html"
```


For the candidate genes selected by *minP* measure, the *enrichTest1()* function returned the results to the *minpGeneSets_KEGG* variable, which consists of a data frame of "enrich_test", an integer vector of "useGenes", a number of "nGenes", a number of "nTopGenes" and a list of "setTypeInfo". The "enrich_test" shows the enrichment test results for every gene set in the specified category defined by *setType*. The "useGenes" lists the effective candidate genes used for enrichment test. The "nGenes" is the total number of genes in the specified category and the "nTopGenes" is the number of effective candidate genes for enrichment test. The analysis above indicated that the KEGG category contains 5,267 genes, of which 289 genes are candidates, and the test aims to identify which gene set in the KEGG category is significantly enriched for the 289 candidate genes. The "setTypeInfo" presents description of the specified category, *KEGG*.

```
> minpGeneSets_KEGG$enrich_test[order(minpGeneSets_KEGG$enrich_test$pval),][1:10,]
```

	pid	size	genesSize	effect	sd	pval
184	2901	76	17	0.16881427	0.02612199	8.314768e-08
183	2900	85	14	0.10983594	0.02470038	4.694155e-05
185	2902	92	14	0.09730397	0.02374210	1.206243e-04
116	2833	75	11	0.09179672	0.02629556	6.869875e-04
117	2834	134	16	0.06453304	0.01967255	9.446004e-04
138	2855	70	10	0.08798720	0.02721849	1.331522e-03
86	2803	267	26	0.04250833	0.01393662	1.335274e-03
6	2723	26	5	0.13743775	0.04466079	2.345904e-03
147	2864	47	7	0.09406623	0.03321728	3.600516e-03
136	2853	70	9	0.07370148	0.02721849	4.478571e-03

```
> sndpGeneSets_KEGG<-enrichTest1(topsndpGenes,setType=6)
```

```
> sndpGeneSets_KEGG$enrich_test[order(sndpGeneSets_KEGG$enrich_test$pval),][1:10,]
```

	pid	size	genesSize	effect	sd	pval
184	2901	76	19	0.19797798	0.02547334	8.286438e-10
86	2803	267	33	0.07157348	0.01359055	7.224809e-07
185	2902	92	17	0.13276058	0.02315255	8.001061e-07
183	2900	85	15	0.12444856	0.02408703	5.658256e-06
113	2830	201	23	0.06240584	0.01566371	9.876542e-05
117	2834	134	17	0.07484365	0.01918405	1.711858e-04
166	2883	52	9	0.12105490	0.03079577	2.786636e-04
176	2893	54	9	0.11464464	0.03022010	3.843800e-04
167	2884	65	10	0.10182413	0.02754457	4.479005e-04
35	2752	21	5	0.18607321	0.04845997	5.245832e-04

```
> simpGeneSets_KEGG<-enrichTest1(topsimpGenes,setType=6)
```

```
> simpGeneSets_KEGG$enrich_test[order(simpGeneSets_KEGG$enrich_test$pval),][1:10,]
```

	pid	size	genesSize	effect	sd	pval
82	2799	44	6	0.09573330	0.02976404	0.001760317
124	2841	71	8	0.07204572	0.02343090	0.002118911
41	2758	25	4	0.11936966	0.03948646	0.002884391
149	2866	25	4	0.11936966	0.03948646	0.002884391
180	2897	38	5	0.09094861	0.03202775	0.003892430
22	2739	29	4	0.09730069	0.03666226	0.005649881
169	2886	29	4	0.09730069	0.03666226	0.005649881
16	2733	31	4	0.08840192	0.03545989	0.007568922
148	2865	44	5	0.07300602	0.02976404	0.008132063
134	2851	48	5	0.06353633	0.02849690	0.012362053

```
> fishpGeneSets_KEGG<-enrichTest1(topfishpGenes,setType=6)
```

```
> fishpGeneSets_KEGG$enrich_test[order(fishpGeneSets_KEGG$enrich_test$pval),][1:10,]
```

	pid	size	genesSize	effect	sd	pval
	184	2901	76	0.21764862	0.02695643	1.547159e-10
	183	2900	85	0.16486224	0.02548941	5.432837e-08
	185	2902	92	0.12611544	0.02450052	4.564353e-06
	136	2853	70	0.14133283	0.02808796	9.070141e-06
	86	2803	267	0.06492833	0.01438181	1.115548e-05
	113	2830	201	0.07566119	0.01657567	1.306251e-05
	114	2831	84	0.11990426	0.02564068	2.224456e-05
	176	2893	54	0.14503653	0.03197955	4.944214e-05
	167	2884	65	0.12594821	0.02914825	7.779820e-05
	117	2834	134	0.08312387	0.02030097	8.851877e-05

> getGeneSetInfo(2901)

> getGeneSetInfo(2799)

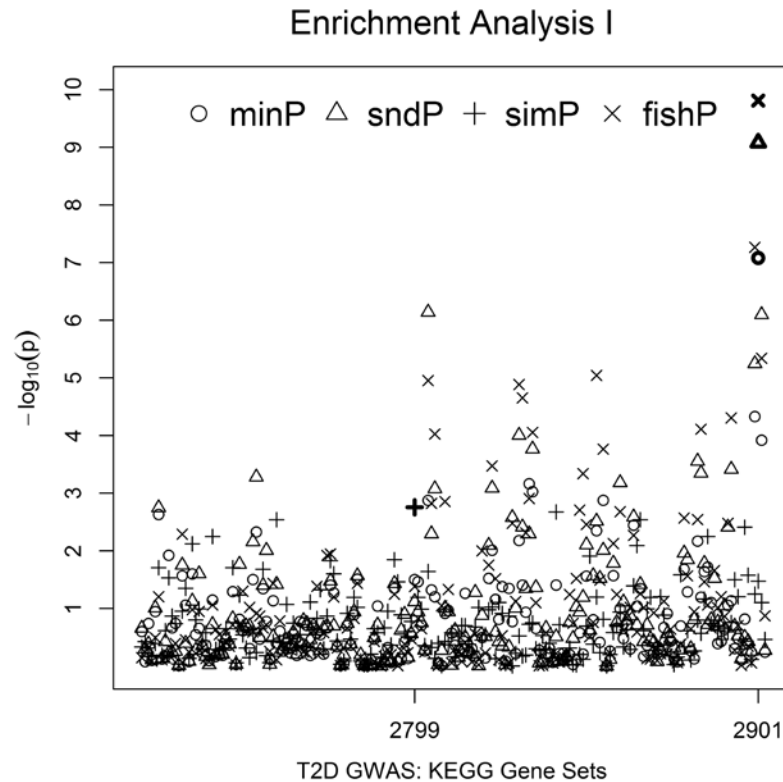


Figure 2. Empirical p-values of KEGG gene sets by enrichment analysis I

The top 10 gene sets for each gene measure were shown above. The $-\log_{10}(\text{empirical } p\text{-values})$ for every gene set was plotted at Figure 2. The four gene measures selected different candidate genes for enrichment test, which caused the pathway test results varied over the measures. The most enriched gene set was pathway of ‘arrhythmogenic right ventricular cardiomyopathy’ (PID=2901) for *minP*, *sndP* and *fishP*, and it was pathway of ‘nucleotide excision repair’ (PID=2799) for *simP*. The pathway of ‘2901’, containing 76 genes, involves 17 candidate genes from *minP* (effect=16.9%, $p_e=8.31E-08$), 19 candidate genes from *2ndP* (effect=19.8%, $p_e=8.29E-10$) and 21 candidate genes from *fishP* (effect=21.8%, $p_e=1.55e-10$); and the pathway of ‘2799’, containing 44 genes, involves 6 candidate genes from *simP* (effect=9.6%, $p_e=1.76E-03$). All component genes for a particular gene set can be

identified by the function `getGeneSetInfo()` function, e.g. `getGeneSetInfo(2901)` where 2901 is the pathway ID.

Different pathways may share common genes and these pathways will be dependent, potentially leading to an inflated type I error. To adjust for this issue and multiple testing, the `enrichTest1_Perm()` function applies a permutation-based test to obtain the adjusted p-value (p_{perm}) for pathway enrichment.

The most enriched gene set by every gene measure is prepared for permutation test and the R codes are as below:

```
> KEGG_rst<-
rbind(minpGeneSets_KEGG$enrich_test[minpGeneSets_KEGG$enrich_test$pval==min(minpGeneSets_KEGG$enrich_test$pval)],),
sndpGeneSets_KEGG$enrich_test[sndpGeneSets_KEGG$enrich_test$pval==min(sndpGeneSets_KEGG$enrich_test$pval)],),
simpGeneSets_KEGG$enrich_test[simpGeneSets_KEGG$enrich_test$pval==min(simpGeneSets_KEGG$enrich_test$pval)],),
fishpGeneSets_KEGG$enrich_test[fishpGeneSets_KEGG$enrich_test$pval==min(fishpGeneSets_KEGG$enrich_test$pval)],)
simpGeneSets_KEGG$enrich_test[simpGeneSets_KEGG$enrich_test$pval==min(simpGeneSets_KEGG$enrich_test$pval)],),
fishpGeneSets_KEGG$enrich_test[fishpGeneSets_KEGG$enrich_test$pval==min(fishpGeneSets_KEGG$enrich_test$pval)],)
> KEGG_rst<-cbind(measure=c("minp","2ndp","simp","fishp"),
                 topGenes=c(minpGeneSets_KEGG$nTopGenes,sndpGeneSets_KEGG$nTopGenes,
                             simpGeneSets_KEGG$nTopGenes,fishpGeneSets_KEGG$nTopGenes), KEGG_rst)
> colnames(KEGG_rst)<-c("measure", "topGenes", "pid", "size", "setTopGenes", "effect", "sd", "p")
```

Results of the most enriched pathway for every gene measure were saved to `KEGG_rst` variable and the results were shown below:

```
> KEGG_rst
  measure topGenes pid size setTopGenes effect sd p
184 minp      289 2901  76          17 0.1688143 0.02612199 8.314768e-08
1841 2ndp      274 2901  76          19 0.1979780 0.02547334 8.286438e-10
82  simp      214 2799  44           6 0.0957333 0.02976404 1.760317e-03
1842 fishp     309 2901  76          21 0.2176486 0.02695643 1.547159e-10
```

The `enrichTest1_Perm()` function was applied to get permutation distribution table for calculating permutation p-value of the most enriched pathway. The argument of `geneSize` defines the number of effective candidate genes for enrichment test *I* of the target gene set. The argument of `setType` defines the category of gene sets for permutation adjusting. The argument of `times` specifies the number of permutations for generating distribution table and the argument of `seed` assigns a random seed for permutation.

```
> minp_dist=enrichTest1_Perm(geneSize=KEGG_rst[1,"topGenes"], setType=6,times=1000, seed=1)
> sndp_dist=enrichTest1_Perm(geneSize=KEGG_rst[2,"topGenes"], setType=6,times=1000, seed=1)
> simp_dist=enrichTest1_Perm(geneSize=KEGG_rst[3,"topGenes"], setType=6,times=1000, seed=1)
> fishp_dist=enrichTest1_Perm(geneSize=KEGG_rst[4,"topGenes"], setType=6,times=1000, seed=1)
```

The minimum p-value of the KEGG category (`setType=6`) was extract to construct the distribution table and calculate permutation p-value (`p_perm`)

```
> minp_min=apply(minp_dist,2,min)
> sndp_min=apply(sndp_dist,2,min)
> simp_min=apply(simp_dist,2,min)
> fishp_min=apply(fishp_dist,2,min)
> KEGG_rst$p_perm<-c(sum(minp_min<=KEGG_rst[1,"p"]),sum(sndp_min<=KEGG_rst[2,"p"]),
sum(simp_min<=KEGG_rst[3,"p"]),sum(fishp_min<=KEGG_rst[4,"p"]))/1000
> KEGG_rst
```

The results were summarized at Table 2. The gene set of ‘2901’ has $p_perm < 1e-03$ for enrichment of candidate genes from *minP*, *2ndP* and *fishP*, and the gene set of ‘2799’ has $p_perm = 0.463$ for enrichment of candidate genes from *simP*

Table 2. The most enriched KEGG pathway of T2D-GWAS by enrichment analysis I

Measure	Genes	PID	size	setGenes	effect(%)	sd(%)	p_e	p_perm
minP	289	2901	76	17	16.9	2.6	8.31E-08	<1e-03
2ndP	274	2901	76	19	19.8	2.5	8.29E-10	<1e-03
simP	214	2799	44	6	9.6	3.0	1.76E-03	0.463
fishP	309	2901	76	21	21.8	2.7	1.55E-10	<1e-03

‘Genes’: the number of candidate that is taken for enrichment analysis; ‘PID’: the pathway ID used by `snpGeneSets`. ‘size’: the number of member genes of a pathway; ‘setGenes’: the number of candidate genes contained by the pathway.

7.2 Example: Enrichment analysis I of T2D-GWES

For T2D-GWES, the top 5% genes with the smallest p-values of differential expression were selected as candidate genes and the pathway enrichment test were performed for KEGG gene sets by `enrichTest1()` function.

```
> topExpGenes<-
T2DExpression[order(T2DExpression$p),][1:trunc(nrow(T2DExpression)*0.05),"gene_id"]

> length(topExpGenes)
[1] 1009
```

There are 1,009 candidate genes selected for the enrichment test / of KEGG gene sets. However, only 262 genes belongs to the KEGG gene sets and are effectively used for pathway analysis. The 10 most enriched gene sets were saved to `exp_rst` variable.

```
> expGeneSets_KEGG<-enrichTest1(topExpGenes,setType=6)
> expGeneSets_KEGG$nTopGenes
[1] 262
> exp_rst<-expGeneSets_KEGG$enrich_test[order(expGeneSets_KEGG$enrich_test$pval),][1:10,]
> exp_rst
```

Table 3. Ten most enriched KEGG pathways of T2D-GWES by enrichment analysis I

PID	size	setGenes	effect(%)	sd(%)	p_e	p_{perm}
2872	53	7	8.2	3.0	4.25E-03	0.764
2869	23	4	12.4	4.5	4.71E-03	0.788
2803	267	22	3.3	1.3	6.54E-03	0.874
2866	25	4	11.0	4.3	6.86E-03	0.906
2825	47	6	7.8	3.2	7.92E-03	0.932
2719	32	4	7.5	3.8	1.96E-02	0.995
2751	44	5	6.4	3.3	2.06E-02	0.997
2787	22	3	8.7	4.6	2.15E-02	0.997
2864	47	5	5.7	3.2	2.77E-02	0.999
2874	35	4	6.5	3.7	2.80E-02	1

'PID': the pathway ID used by `snpGeneSets`. 'size': the number of member genes of a pathway; 'setGenes': the number of candidate genes contained by the pathway.

The permutation test was applied to obtain permutation adjusted p-value (p_{perm}) by `enrichTest1_Perm()` function.

```
> exp_dist<-enrichTest1_Perm(geneSize =expGeneSets_KEGG$nTopGenes, setType=6,times=1000,
seed=1)

> exp_min=apply(exp_dist,2,min)

> exp_rst$p_perm<-unlist(lapply(exp_rst$pval, function(x) sum(exp_min<=x)/1000))
```

```

> colnames(exp_rst)=c("pid","size","setTopGenes","effect","sd","p","p_perm")
> exp_rst
> getGeneSetInfo(2872)

```

The enrichment test *I* and its permutation adjustment were summarized at Table 3. The most enriched gene set is the pathway of ‘Amyotrophic lateral sclerosis’ (*PID=2872*) that contains 53 member genes. The pathway presented enrichment effect of 8.2% with empirical $p_e=4.25E-03$, but the test based on 1,000 permutations showed that the adjusted p-value was 0.764.

8. Pathway Enrichment Analysis II of GWS genes

The type II analysis is a specialized pathway enrichment analysis that aims to identify enriched gene sets based on genome-wide association and expression study results. The analysis can be performed by *enrichTest2()* function, which test for pathway enrichment by the *USGSA* method. The test depends the threshold of *U*-score that defines genome-wide significant genes. The default value of threshold is 0.05 for *enrichTest2()*, which assumes that 5% of genome-wide genes are involved in pathway of studied phenotype.

8.1 Example: Enrichment analysis II of T2D-GWAS

Measures of *minP*, *2ndP*, *simP* and *fishP* or their *U*-scores can all be applied for pathway enrichment test. The required parameter of *geneDF* for *enrichTest2()* function is a data frame which contains at least a column of ‘*gene_id*’ for Entrez gene IDs and a column of ‘*score*’ for a gene measure or *U*-score. The argument of ‘*setType*’ defines the pathway category for enrichment test. For the T2D-GWAS, the example below used *U*-score of *minp* measure for the analysis and ‘*setType=6*’ limited enrichment analysis to pathways of the KEGG category.

```

> e2_minp<-enrichTest2(geneDF =
data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$minp), setType=6)
> names(e2_minp)
[1] "enrich_test" "useGenes"  "nGenes"    "nSigGenes"  "setTypeInfo"
> head(e2_minp$enrich_test)
  pid size genes sigGenes      effect      sd      pval
1 2718  62   50         4  0.01146787 0.03573107 0.256079675
2 2719  32   24         0 -0.06853213 0.05157336 0.818895315
3 2720  27   18         2  0.04257898 0.05955179 0.121221901
4 2721  28   22         0 -0.06853213 0.05386662 0.791100789
5 2722  34   24         3  0.05646787 0.05157336 0.077531279
6 2723  26   25         5  0.13146787 0.05053137 0.005729825
> length(e2_minp$useGenes)
[1] 4217

```

```

> e2_minp$nGenes
[1] 4217

> e2_minp$nSigGenes
[1] 289

> e2_minp$setTypeInfo
$tid
[1] 6

$symbol
[1] "c2_kegg"

$name
[1] "C2_CP:KEGG: KEGG gene sets"

$description
[1] "Gene sets derived from the KEGG pathway database, http://www.genome.jp/kegg/pathway.html"

```

```

>e2_minp$enrich_test[order(e2_minp$enrich_test$pval),][1:10,]

```

	pid	size	genes	sigGenes	effect	sd	pval
184	2901	76	69	17	0.17784468	0.03041631	4.626583e-07
183	2900	85	76	14	0.11567839	0.02898173	1.496373e-04
185	2902	92	81	14	0.10430737	0.02807298	3.136454e-04
116	2833	75	70	11	0.08861073	0.03019827	2.482412e-03
117	2834	134	120	16	0.06480120	0.02306431	2.997884e-03
138	2855	70	65	10	0.08531402	0.03133822	4.127198e-03
86	2803	267	237	26	0.04117251	0.01641183	5.435991e-03
6	2723	26	25	5	0.13146787	0.05053137	5.729825e-03
35	2752	21	18	4	0.15369009	0.05955179	5.956215e-03
9	2726	17	12	3	0.18146787	0.07293575	6.886650e-03

For *U*-score of *minP*, the *enrichTest2()* function returned the results to the *e2_minp* variable, which consists of a data frame of "enrich_test", an integer vector of "useGenes", a number of "nGenes", a number of "nSigGenes" and a list of "setTypeInfo". The "enrich_test" shows the enrichment test results for every gene set in the specified category defined by *setType*. The "useGenes" lists GWS genes used for enrichment test. The "nGenes" is the total number of GWS genes in the specified category (i.e. the length of "useGenes") and the "nSigGenes" is the number of GWS significant genes for enrichment test. The "setTypeInfo" presents description of the specified category.

The examples below similarly used *U*-scores of *2ndP*, *simP* and *fishP* for pathway tests.

(Notes: Either a gene measure or its *U*-score can be used for type II pathway test. Since a gene measure will automatically be converted to its *U*-score by *enrichTest2* function, they will present the same results.)

```

> e2_sndp<-enrichTest2(geneDF =
data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$sndp), setType=6)

> e2_sndp$enrich_test[order(e2_sndp$enrich_test$pval),][1:10,]

```

	pid	size	genes	sigGenes	effect	sd	pval
184	2901	76	69	19	0.21038722	0.02967294	5.799842e-09
185	2902	92	81	17	0.14490144	0.02738688	2.677123e-06
86	2803	267	237	33	0.07426541	0.01601072	6.431854e-06
183	2900	85	76	15	0.13239332	0.02827342	2.025952e-05
113	2830	201	180	23	0.06280268	0.01837168	4.965014e-04
117	2834	134	120	17	0.07669157	0.02250062	6.261319e-04
35	2752	21	18	5	0.21280268	0.05809635	6.794323e-04
166	2883	52	47	9	0.12651426	0.03595308	6.859949e-04
176	2893	54	50	9	0.11502490	0.03485781	1.144063e-03
167	2884	65	59	10	0.10451642	0.03208921	1.208255e-03

```
> e2_simp<-enrichTest2(geneDF =
data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$simp), setType=6)
```

```
> e2_simp$enrich_test[order(e2_simp$enrich_test$pval),][1:10,]
```

	pid	size	genes	sigGenes	effect	sd	pval
124	2841	71	50	8	0.10925302	0.03103924	0.000765214
82	2799	44	35	6	0.12068159	0.03709899	0.001563236
149	2866	25	18	4	0.17147525	0.05173207	0.001598354
180	2897	38	28	5	0.12782445	0.04147793	0.002341210
16	2733	31	20	4	0.14925302	0.04907735	0.002661440
41	2758	25	21	4	0.13972921	0.04789459	0.003351116
148	2865	44	35	5	0.09211017	0.03709899	0.007499138
22	2739	29	26	4	0.10309918	0.04304368	0.008809557
134	2851	48	37	5	0.08438816	0.03608238	0.009872187
76	2793	36	27	4	0.09740117	0.04223906	0.010375498

```
> e2_fishp<-enrichTest2(geneDF =
data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$fishp), setType=6)
```

```
> e2_fishp$enrich_test[order(e2_fishp$enrich_test$pval),][1:10,]
```

	pid	size	genes	sigGenes	effect	sd	pval
184	2901	76	69	21	0.23107299	0.03137100	1.276659e-09
183	2900	85	76	19	0.17672516	0.02989139	2.700813e-07
185	2902	92	81	17	0.13660170	0.02895412	1.470682e-05
136	2853	70	61	14	0.15623336	0.03336476	2.143464e-05
114	2831	84	75	15	0.12672516	0.03009001	7.509137e-05
86	2803	267	237	33	0.06596567	0.01692695	8.415314e-05
113	2830	201	180	27	0.07672516	0.01942302	8.888730e-05
88	2805	178	149	23	0.08108758	0.02134813	1.658807e-04
176	2893	54	50	11	0.14672516	0.03685258	1.858624e-04
167	2884	65	59	12	0.13011499	0.03392555	2.533609e-04

The top 10 gene sets for each gene measure were shown above. The $-\log_{10}(\text{empirical } p\text{-values})$ for every gene set was plotted at Figure 3. Consistent with the type *I* analysis, the most enriched gene set is the pathway of *PID=2901* for *minP*, *2ndP* and *fishP* measures (Figure 3). However, the most enriched pathway for *simP* is the ‘RIG-I-like receptor signaling pathway’ (*PID=2841*) in contrast to the pathway of *PID=2799* by enrichment analysis *I*.

```
> KEGG_rst<-rbind(
  e2_minp$enrich_test[e2_minp$enrich_test$pval==min(e2_minp$enrich_test$pval),],
  e2_sndp$enrich_test[e2_sndp$enrich_test$pval==min(e2_sndp$enrich_test$pval),],
  e2_simp$enrich_test[e2_simp$enrich_test$pval==min(e2_simp$enrich_test$pval),],
  e2_fishp$enrich_test[e2_fishp$enrich_test$pval==min(e2_fishp$enrich_test$pval),])
```



```

> KEGG_rst<-cbind(measure=c("minP","2ndP","simp","fishP"),
                  topGenes=c(e2_minP$nSigGenes,e2_sndP$nSigGenes,
                             e2_simp$nSigGenes,e2_fishP$nSigGenes), KEGG_rst)

> colnames(KEGG_rst)<-c("measure",
                        "sigGenes","pid","size","effectGenes","setSigGenes","effect","sd","p")

> KEGG_rst

```

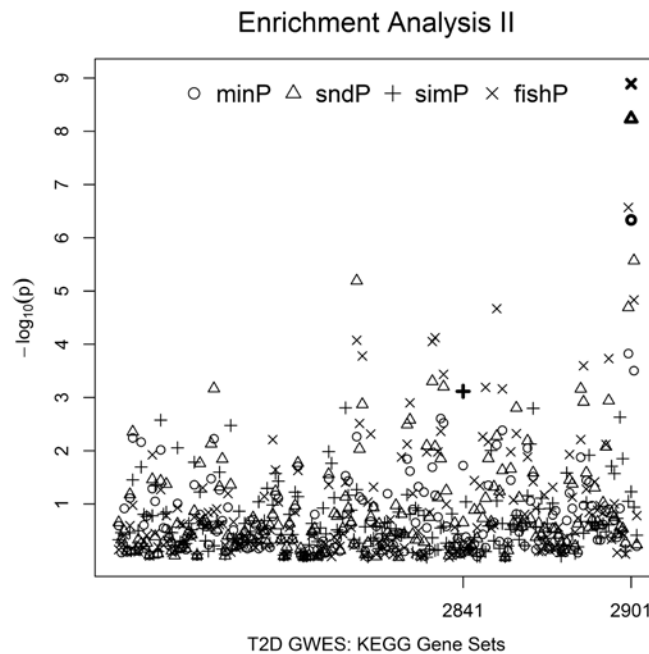


Figure 3. Empirical p-values of KEGG gene sets by enrichment analysis II

For enrichment analysis II, the pathway of '2901', containing 69 GWAS genes, involves 17 significant genes from *minP* (*effect*=17.8%, $p_e=4.63E-07$), 19 significant genes from *2ndP* (*effect*=21.0%, $p_e=5.80E-09$) and 21 significant genes from *fishP* (*effect*=23.1%, $p_e=1.28E-09$); and the pathway of '2841', containing 50 GWAS genes, involves 8 significant genes from *simP* (*effect*=10.9%, $p_e=7.65E-04$) (Table 4).

To adjust for pathway dependence and multiple testing, the *enrichTest2_Perm()* function calculates the adjusted p-value (p_{perm}) by 1,000 permutations. The argument of *geneDF* is the data frame for enrichment test II by *enrichTest2()* function. The argument of *setType* defines the category of gene sets for permutation adjusting. The argument of *times* specifies the number of permutations for generating distribution table and the argument of *seed* assigns a random seed for permutation. The permutation

adjusted p-value (p_{perm}) was shown in Table 4. The p_{perm} is $<1E-3$, $<1E-3$, 0.306 and $<1E-3$ for the most enriched pathways based on gene measures of *minP*, *2ndP*, *simpP* and *fishP* respectively.

Table 4. The most enriched KEGG pathway of T2D-GWAS by enrichment analysis II

Measure	Genes	PID	size	setGenes	effect(%)	sd(%)	p_e	p_{perm}	p_{table}
minp	289	2901	69	17	17.8	3.0	4.63E-07	$<1E-3$	0.0003
2ndp	274	2901	69	19	21.0	3.0	5.80E-09	$<1E-3$	$<1E-4$
simp	214	2841	50	8	10.9	3.1	7.65E-04	0.306	0.2617
fishp	309	2901	69	21	23.1	3.1	1.28E-09	$<1E-3$	$<1E-4$

'Genes': the number of GWAS significant genes that is taken for enrichment analysis; 'PID': the pathway ID used by *snpGeneSets*. 'size': the number of GWAS genes of a pathway; 'setGenes': the number of GWAS significant genes contained by the pathway.

```

> minp_dist =
enrichTest2_Perm(data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$minp),
setType=6,times=1000, seed=1)

> sndp_dist =
enrichTest2_Perm(data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$sndp),
setType=6,times=1000, seed=1)

> simp_dist =
enrichTest2_Perm(data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$simp),
setType=6,times=1000, seed=1)

> fishp_dist =
enrichTest2_Perm(data.frame(gene_id=T2DGWASGene$gene_id,score=T2DGWASGene$fishp),
setType=6,times=1000, seed=1)

> minp_min=apply(minp_dist,2,min)
> sndp_min=apply(sndp_dist,2,min)
> simp_min=apply(simp_dist,2,min)
> fishp_min=apply(fishp_dist,2,min)

> KEGG_rst$p_perm<-c(sum(minp_min<=KEGG_rst[1,"p"]),sum(sndp_min<=KEGG_rst[2,"p"]),
sum(simp_min<=KEGG_rst[3,"p"]),sum(fishp_min<=KEGG_rst[4,"p"]))

```

To enable direct calculation of permutation p-value, a pre-generated distribution table based on 10,000 permutations is made [4] and *getEnrich2P()* function is provided to obtain the permutation p-value (p_{table}) directly. The p_{table} is $3.00E-04$, $<1E-4$, 0.2617 and $<1E-4$ for the most enriched pathways based on gene measures of *minP*, *2ndP*, *simpP* and *fishP* respectively (Table 4). The codes are shown below:

```

> KEGG_rst$p_table<-getEnrich2P(setP=KEGG_rst$p, setType=6)$perm$p

```

```
> KEGG_rst
```

8.2 Example: Enrichment analysis II of T2D-GWES

For type II enrichment analysis of GWES, differential expression p-value is typically used as measure of gene effect. As pathway analysis of GWAS, both gene measure and its calculated U -score can be applied to test pathway enrichment by `enrichTest2` function. For the example of T2D-GWES data, the default value of U -score threshold=0.05 were used for enrichment test of KEGG pathways (i.e. `setType=6`).

```
> expGeneSets_KEGG<-  
enrichTest2(data.frame(gene_id=T2DExpression$gene_id,score=uscore(T2DExpression$p)),  
setType=6)
```

The 10 most enriched pathways for significant GWES genes were identified as below and results were saved to `exp_rst` variable.

```
> exp_rst<-expGeneSets_KEGG$enrich_test[order(expGeneSets_KEGG$enrich_test$pval),][1:10,]
```

The permutation test was applied to obtain permutation p-value (p_{perm}) by `enrichTest2_Perm()` function.

```
> exp_dist =  
enrichTest2_Perm(data.frame(gene_id=T2DExpression$gene_id,p=uscore(T2DExpression$p)),  
setType=6,times=1000, seed=1)  
  
> exp_min=apply(exp_dist,2,min)  
  
> exp_rst$p_perm<-unlist(lapply(exp_rst$pval, function(x) sum(exp_min<=x)/1000))
```

To enable direct calculation of permutation p-value, a pre-generated distribution table based on 10,000 permutations [4] is made and `getEnrich2P()` function is provided to obtain the permutation p-value (p_{table}) directly.

```
> exp_rst$p_table<-getEnrich2P(setP=exp_rst$pval, setType=6)$perm$p  
  
> exp_rst
```

The results of enrichment analysis II for T2D-GWES were shown at Table 5, and 9 of them were also shown as the top 10 pathways by enrichment analysis I. The most enriched pathway is the same for both type I and II analysis, which is the pathway of '2872' with effect=7.9% and empirical p-value=7.28E-03. However, the 1,000 permutations got the $p_{perm}=0.889$ and pre-generated distribution table showed the $p_{table}=0.9118$.

Table 5. Ten most enriched KEGG pathways of T2D-GWES by enrichment analysis II

PID	size	setGenes	effect(%)	sd(%)	p_e	p_{perm}	p_{table}
2872	52	7	7.90	3.18	7.28E-03	0.889	0.9118
2866	23	4	11.83	4.78	7.51E-03	0.912	0.9194
2869	23	4	11.83	4.78	7.51E-03	0.912	0.9194
2825	46	6	7.49	3.38	1.25E-02	0.988	0.9808
2803	259	22	2.94	1.42	1.61E-02	0.996	0.9918
2719	29	4	8.24	4.25	2.02E-02	0.998	0.9967
2751	40	5	6.94	3.62	2.17E-02	0.999	0.9977
2787	20	3	9.44	5.12	2.23E-02	0.999	0.998
2746	44	5	5.81	3.45	3.32E-02	1	0.9999
2874	34	4	6.21	3.93	3.78E-02	1	1

PID': the pathway ID used by *snpGeneSets*. 'size': the number of GWAS genes of a pathway; 'setGenes': the number of GWAS significant genes contained by the pathway.

9. Pathway Enrichment Analysis of GWAS by ALIGATOR

The ALIGATOR (Association List Go AnnoTatOR)[8] method is also implemented in the *snpGeneSets* package by the function *alligator()*. The method tests pathway enrichment for GWAS significant gene that is defined through p-value threshold *pcut* of SNP association. The default value of *pcut* is 0.05 for *alligator()*, and any gene with a SNP p-value $< pcut$ is defined as significant. The method applies permutation to obtain empirical unadjusted p-value and the number of permutation is defined through parameter *Nsample* that takes default value of 5000. The adjusted p-value is obtained through bootstrap sampling and the number of bootstrapping is set through parameter *Btimes* that takes default value of 1000.

The example below shows the analysis of pathway enrichment for T2DGWAS by ALIGATOR method. The first parameter *snpGeneP* is a data frame containing at least columns of 'snp' (SNP rsid) , 'gene_id' (Entrez gene ID) and 'p' (SNP association p-value) . The data of *T2DGWAS* comes with the *snpGeneP* data frame and *pcut* of 0.001 is applied to test pathway enrichment.

```
> data(T2DGWAS)
> head(snpGeneP)
> path0=aligator(snpGeneP, pcut=0.001)
> path0[order(path0$p),][1:10,]
```

	pid	p	adj_p
4243	7717	0.0004	0.609
2133	5607	0.0010	0.827
3358	6832	0.0012	0.858
4270	7744	0.0014	0.886
28	2745	0.0022	0.953
4526	8000	0.0030	0.980
4106	7580	0.0032	0.983
4630	8104	0.0044	0.995
4164	7638	0.0046	0.997
16	2733	0.0054	0.999

It was shown that the first pathway with $pid=7717$ has empirical unadjusted p-value of 4E-04, but the permutation adjusted p-value is 0.609.

References:

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