

GeneAnswers, Integrated Interpretation of Genes

Gang Feng^{‡,*}, Pan Du^{†,†}, Warren A. Kibbe^{‡,‡}, Simon Lin^{‡,§}

October 17, 2016

[‡]Northwestern University Biomedical Informatics Center
Northwestern University, Chicago, IL, 60611, USA

1 Overview of GeneAnswers

Microarray techniques have been widely employed in genomic scale studies for more than one decade. The standard analysis of microarray data is to filter out a group of genes from thousands of probes by certain statistical criteria. These genes are usually called significantly differentially expressed genes. Recently, next generation sequencing (NGS) is gradually adopted to explore gene transcription, methylation, etc. Also a gene list can be obtained by NGS preliminary data analysis. However, this type of information is not enough to understand the potential linkage between identified genes and interested functions. The integrated functional and pathway analysis with gene expression data would be very helpful for researchers to interpret the relationship between the identified genes and proposed biological or medical functions and pathways.

The *GeneAnswers* package provides an integrated solution for a group of genes and specified categories (biological or medical functions, such as Gene Ontology, Disease Ontology, KEGG, etc) to reveal the potential relationship between them by means of statistical methods, and make user-friendly network visualization to interpret the results. Besides the package has a function to combine gene expression profile and category analysis together by outputting concept-gene cross tables, keywords query on NCBI Entrez Gene and application of human based Disease ontology analysis of given genes from other species can help people to understand or discover potential connection between genes and functions.

2 Citation

For the people using *GeneAnswers* package, please cite the following papers in your publications.

* For DOLite:

*g-feng (at) northwestern.edu

†dupan (at) northwestern.edu

‡wakibbe (at) northwestern.edu

§s-lin2 (at) northwestern.edu

Du, P., Feng, G., Flatow, J., Song, J., Holko, M., Kibbe, W.A. and Lin, S.M., (2009) 'From disease ontology to disease-ontology lite: statistical methods to adapt a general-purpose ontology for the test of gene-ontology associations', *Bioinformatics* 25(12):i63-8

* For GeneAnswers:

Feng, G., Du, P., Krett, N.L., Tessel, M., Rosen, S., Kibbe, W.A., and Lin, S.M., (submitted) 'Bioconductor Methods to Visualize Gene-list Annotations',
Thanks for your help!

3 Installation of GeneAnswers package

In order to install the *GeneAnswers* package, the user needs to first install R, some related Bioconductor packages. You can easily install them by the following codes.

```
source("http://bioconductor.org/biocLite.R")
biocLite("GeneAnswers")
```

For the users want to install the latest developing version of *GeneAnswers*, which can be downloaded from the developing section of Bioconductor website. Some additional packages might be required to be installed because of the update the Bioconductor. These packages can also be found from the developing section of Bioconductor website. You can also directly install the source packages from the Bioconductor website by specify the developing version number, which can be found at the Bioconductor website. Suppose the developing version is 2.5, to install the latest *GeneAnswers* package in the Bioconductor developing version, you can use the following command:

```
install.packages("GeneAnswers", repos="http://www.bioconductor.org/packages/2.5/bioc", type=
```

4 Object models of major classes

The *GeneAnswers* package has one major class: **GeneAnswers**. It includes the following slots:

1. *geneInput*: a data frame containing gene Entrez IDs with or without any related values. The values could be foldChange, p value, or other values. These data can be used for concept-gene network. Genes with positive values will be represented as red nodes, while negative value genes are green nodes.
2. *testType*: statistical test method. Current version supports hypergeometric test to test relationship between genes and specified categories.
3. *pvalueT*: the cutoff of statistical test. Any categories will not be reported if the p value is more than the cutoff.
4. *genesInCategory*: a list containing genes belonging to categories. The names of the list are categories.
5. *geneExpProfile*: a data frame to store gene expression data. If not available, it could be NULL.
6. *annLib*: annotation database used for statistical test.
7. *categoryType*: functional or medical category used for statistical test.



Figure 1: Flow chart of GeneAnswers

8. *enrichmentInfo*: a data frame containing filtered categories with statistical results by specified pvalueT.

The figure, 'Flow chart of GeneAnswers', shows how *GeneAnswers* package works. A group of genes are essential. We use unique Entrez gene IDs to represent genes. Any relative feature values of these genes can also be optional input information, like fold changes, p values, etc. If the gene expression profile of these genes are available, it can be considered as input, too. Since we want to find the potential connections between genes and categories, category type is also need to be specified. *GeneAnswers* currently supports Gene Ontology (GO), Pathway (KEGG) and developing Disease Ontology (DOLite) in our team. Furthermore, *GeneAnswers* supports Entrez eUtilis so that users can make customized annotation library based on interested keywords. If users have own annotation library, *GeneAnswers* can use it to build relationship between it and given genes.

Besides usual barplot and pie chart of top categories, *GeneAnswers* also provides four types of visualization. One is concepts-genes network, which show the concepts and genes on a network layout. The second one is concepts-genes cross table that integrated gene expression profile and corresponding categories together. The third one is a concepts-network shows connections between categories only. The last one is a table, which contains all of information of categories and genes. Combining all of these presentations can be helpful to find and explain the possible linkages between genes and categories.

5 Data preprocessing

First of all, load the *GeneAnswers* package.

```
> library(GeneAnswers)
```

5.1 Build a GeneAnswers instance

The key point of *GeneAnswers* package is to build a GeneAnswers instance. The essential input for *GeneAnswers* is an Entrez gene IDs vector (a character vector). However, if users have any interested values associated with genes, these values can also be as optional inputs. In this case, the input, *geneInput*, could be a matrix or a dataframe. The first column is always for Entrez gene IDs. Other columns are used to store those interested values. Rownames for the matrix or dataframe are not necessary, but colnames are recommended for further usage. We use two internal datasets, one is from human and another is from mouse, as examples to show how to implement *GeneAnswers* package. The human and mouse datasets coming with the *GeneAnswers* package are from human and mouse Illumina beadarray experiments. Each dataset contains two dataframes. For example, *humanGeneInput* is a dataframe containing Entrez gene IDs with fold changes and p values, while the data frame, *humanExpr*, includes two types, control and treatment, of gene expression profile of the genes in *humanGeneInput*.

```
> data('humanGeneInput')
> data('humanExpr')
> ## build a GeneAnswers instance with statistical test based on biological process of GO
> x <- geneAnswersBuilder(humanGeneInput, 'org.Hs.eg.db', categoryType='GO.BP', testType='h

[1] "geneInput has built in ..."
[1] "annLib and categoryType have built in ..."
[1] "genesInCategory has built in ..."
[1] "Enrichment test is only performed based on annotated genes"
[1] "testType, pvalueT and enrichmentInfo have built in ..."
[1] "geneExpressionProfile has been built in ..."
[1] "GeneAnswers instance has been successfully created!"

> class(x)

[1] "GeneAnswers"
attr("package")
[1] "GeneAnswers"

> ## build a GeneAnswers instance with statistical test based on KEGG and saved example da
> y <- geneAnswersBuilder(humanGeneInput, 'org.Hs.eg.db', categoryType='KEGG', testType='h

[1] "GeneAnswers instance has been successfully created!"

> ## build a GeneAnswers instance with statistical test based on DOLite and saved example
> z <- geneAnswersBuilder(humanGeneInput, 'org.Hs.eg.db', categoryType='DOLITE', testType=

[1] "GeneAnswers instance has been successfully created!"

> w <- geneAnswersBuilder(humanGeneInput, 'org.Hs.eg.db', categoryType='GO.BP', testType='h

[1] "GeneAnswers instance has been successfully created!"
```

We have four *GeneAnswers* objects, *x*, *y*, *z* and *w*, containing the statistical test of biological process of GO, KEGG, DOLite and GO (The first two level nodes are removed), respectively. For Gene Ontology, sometimes, users think some nodes are too general and not very relative to their interests. So we provide parameter *level* to determine how many top levels of GO nodes are removed. The instances have included the relationship between given genes and specified categories.

GeneAnswers package also provides a function *searchEntrez* to retrieve Entrez genes for given keywords by Entrez XML query. National Center for Biotechnology Information (NCBI) provides many powerful online query systems. One of them is Entrez Programming Utilities (eUtils). Users can query NCBI databases by simple keywords logical operations based on XML protocol. This is very helpful to find potential or interested biological functions or pathways. Hence, the retrieved information can be considered as a customized annotation library to test whether the given genes are relative to interested keywords. Here is a case to build a customized *GeneAnswers* instance.

```
> ## before running the following codes, make sure that you can connect the internet.
> ##keywordsList <- list(Apoptosis=c('apoptosis'), CellAdhesion=c('cell adhesion'))
> ##entrezIDList <- searchEntrez(keywordsList)
> ##q <- geneAnswersBuilder(humanGeneInput, entrezIDList, testType='hyperG', totalGeneNumb
> ##class(q)
> ##getAnnLib(q)
> ##getCategoryType(q)
```

Customized *GeneAnswers* instances have NULL at *annLib* slot and "User defend" in *categoryType* slot.

5.2 Visulization

Besides barplot and pie chart, *GeneAnswers* package can generate a network (concept-gene network) show how genes are connected to specified categories as well as general barplot and piechart. Function *GeneAnswersConceptNet* can generate a common R canvas or tcl/tk interactive canvas to draw the network by calling *igraph*. Genes are presented as red nodes, if specified values are positive, and the gene nodes are green with negative values. The category nodes are yellow nodes, the sizes are relative to user-specified values. Currently, if function *GeneAnswersBuilder* successfully returns a *GeneAnswers* instance, the genes are represented as entrez IDs and categories are also category IDs. User can map them to gene symbols and categories terms by function *GeneAnswersReadable*. Function *GeneAnswersReadable* reads slot *annLib* to map Entrez IDs to gene symbols, so make sure slot *annLib* is correct before mapping.

```
> ## mapping gene IDs and category IDs to gene symbols and category terms
> xx <- geneAnswersReadable(x)

[1] "Mapping geneInput ..."
[1] "Mapping genesInCategory ..."
[1] "Mapping enrichmentInfo rownames ..."
[1] "Mapping geneExprProfile rownames ..."
```



Figure 2: Screen shot of pie chart of top categories

```
> yy <- geneAnswersReadable(y, verbose=FALSE)
> zz <- geneAnswersReadable(z, verbose=FALSE)
> ww <- geneAnswersReadable(w, verbose=FALSE)
> ## before running the following codes, make sure that you can connect the internet.
> ##q <- setAnnLib(q, 'org.Hs.eg.db')
> ##qq <- geneAnswersReadable(q, catTerm=FALSE)
```

Since function *geneAnswersReadable* implements mapping based on annotation database in slot *annLib*, we assign 'org.Hs.eg.db' to customized *GeneAnswers* instance *annLib* slot at first for make it readable.

```
> ## plot barplot and / or piechart
> geneAnswersChartPlots(xx, chartType='all')
```

The nodes could be represented by different colors for different specified values, like fold change. In this case, overexpressed genes are represented as red nodes while green dots stand for underexpressed genes. If the node is more red, which means its fold change is larger. It's same for green nodes, but different direction.

```
> ## plot interactive concept-gene network
> geneAnswersConceptNet(xx, colorValueColumn='foldChange', centroidSize='pvalue', output=''
```



Figure 3: Screen shot of barplot of top categories

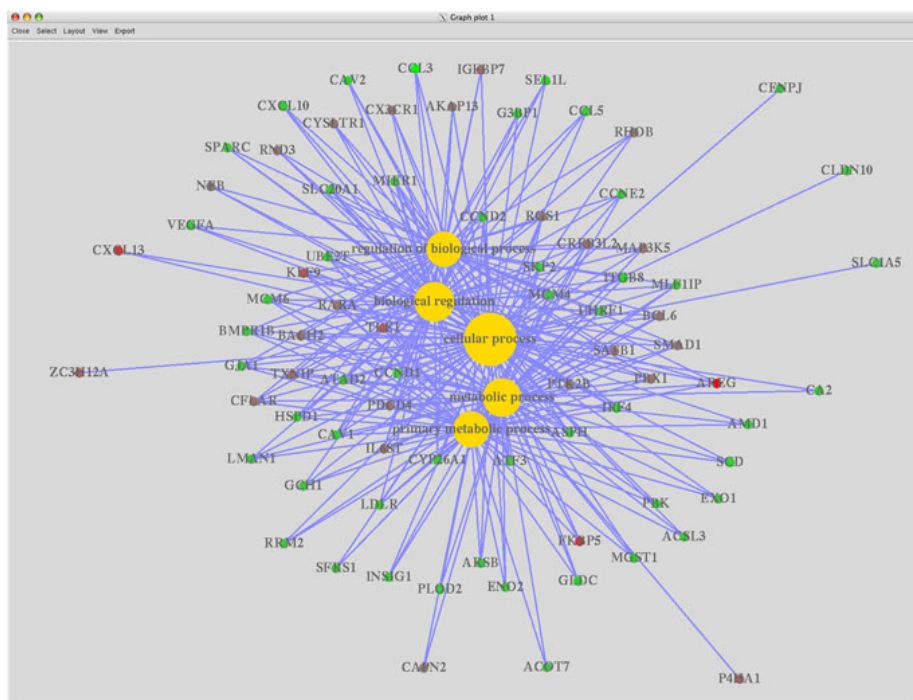


Figure 4: Screen shot of concept-gene network

The top 5 categories might not be very specific. Users might get a tree view to see relative category terms by calling function `geneAnswersConceptRelationIf` if the category has an ontology structure. The size of nodes is proportional to number of genes in these GO categories. The color of nodes stand for how relative the given genes are to the GO categories. More red, more relative. The given GO categories are yellow framed dots with dark purple edges connetions.

```
> ## plot interactive go structure network
> geneAnswersConceptRelation(x, direction='both', netMode='connection', catTerm=TRUE, catI
```

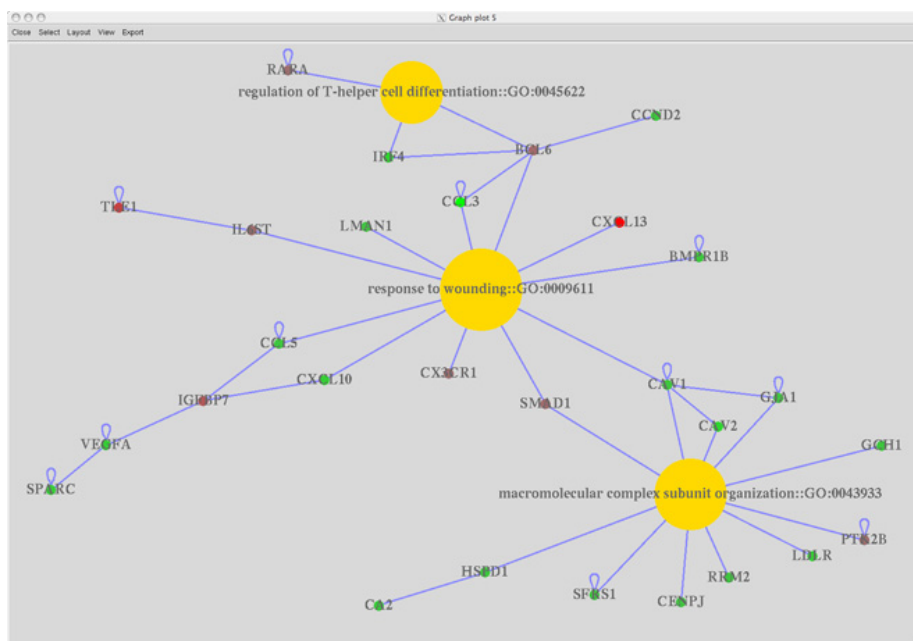
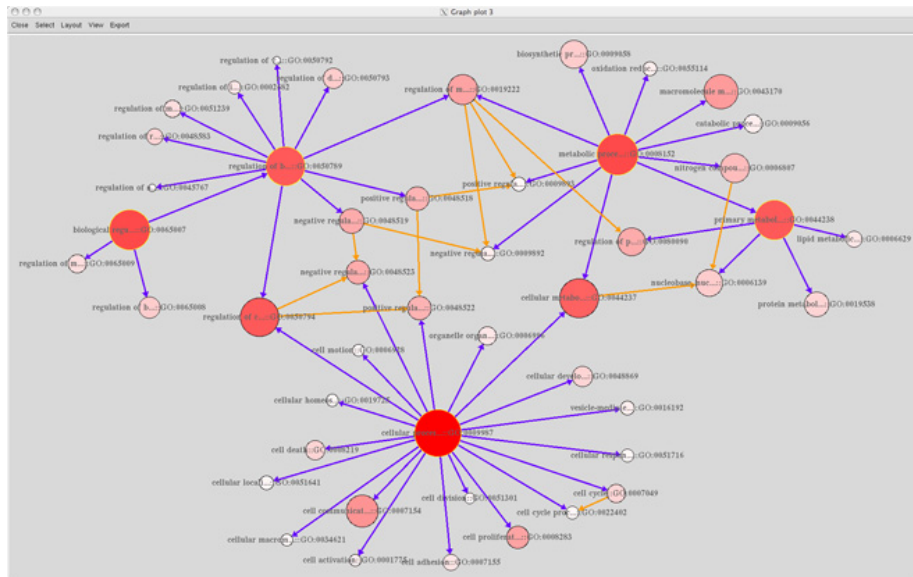





Figure 7: Screen shot of the given gene interaction network

represent the other genes from `getGeneInput(x)`. Small white dots are genes that are not in the genes from `getGeneInput(x)`, but interact with these genes.

If there are some certain values associate with genes in `geneInput` of `x`, like the example data, you can represent any one column by colors. For example, if users want to show how genes interaction with gene expression information, the following command can show this:

```
> ## plot the given gene interaction
> buildNet(c('444', '3638', '5087', '55835'), idType='GeneInteraction', layers=2, filterGrap
```

The following one is based on expression p-values, we often use $-\log_2$ to transform p-values.

```
> ## plot the given gene interaction
> buildNet(c('444', '3638', '5087', '55835'), idType='GeneInteraction', layers=2, filterGrap
```

Users can also define customized color scheme and transfer it into 'buildNet' function by parameter 'colorMap'. Details are available in 'buildNet' manual page. If users want to have a overview for the interaction of all of the given genes from `getGeneInput(x)`, the following command could be used:

```
> ## plot the given gene interaction
> buildNet(getGeneInput(x)[,1], idType='GeneInteraction', layers=2, filterGraphIDs=getGene
```

If there are a lot of genes, the network could be very complicated. We strongly recommend to use 'interactive' mode to show the network since it's easy to manually change layout. The default setting is 'interactive', but users can change it to 'fixed' for a small or simple network.

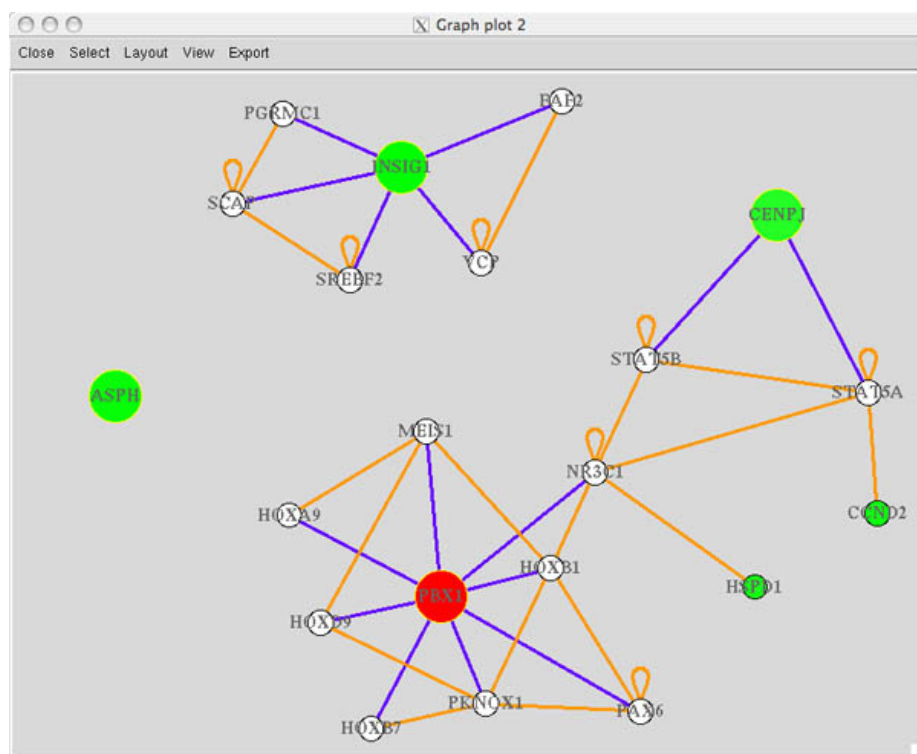


Figure 8: Screen shot of the given gene interaction network with expression information

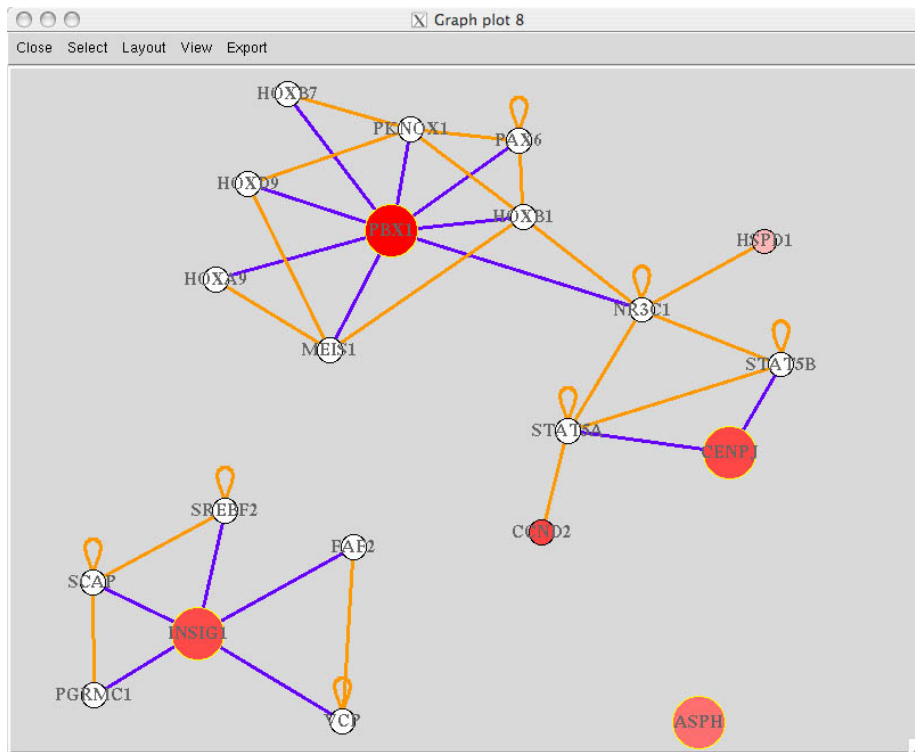


Figure 9: Screen shot of the given gene interaction network with p-value information

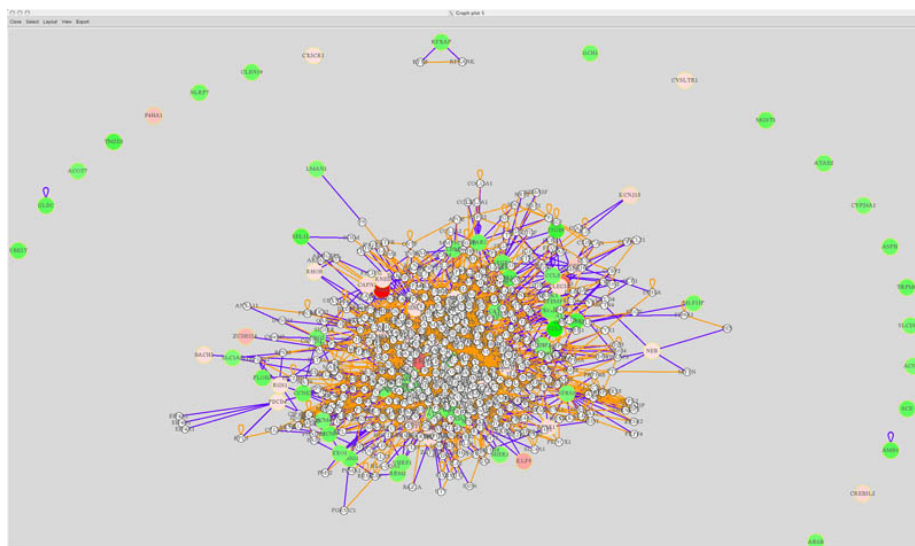


Figure 10: Screen shot of all of the given gene interaction network with expression information



Figure 11: Screen shot of concept-gene network for top 2 GO level nodes removal

The following example is to show top 5 GO-gene network for the first 2 level GO nodes removal.

```
> ## plot Go-concept network for 2 level nodes removal
> geneAnswersConceptNet(wv, colorValueColumn='foldChange', centroidSize='pvalue', output='')
```

Also, users can sort enrichment test information and plot it.

```
> ## sort enrichmentInfo dataframe by fdr adjusted p value
> xxx <- geneAnswersSort(xx, sortBy='correctedPvalue')
> yyy <- geneAnswersSort(yy, sortBy='pvalue')
> zzz <- geneAnswersSort(zz, sortBy='geneNum')

> geneAnswersConceptNet(yyy, colorValueColumn='foldChange', centroidSize='geneNum', output='')
> geneAnswersConceptNet(zzz, colorValueColumn='foldChange', centroidSize='pvalue', output='')
```

If users provide a gene expression profile, *GeneAnswers* package can generate a table or heatmap labeling relationship between genes and categories with a heatmap of these genes expression. We call this type of representation as concept-gene cross tabulation.

```
> ## generate GO-gene cross tabulation
> geneAnswersHeatmap(x, catTerm=TRUE, geneSymbol=TRUE)

> geneAnswersHeatmap(yyy)
```

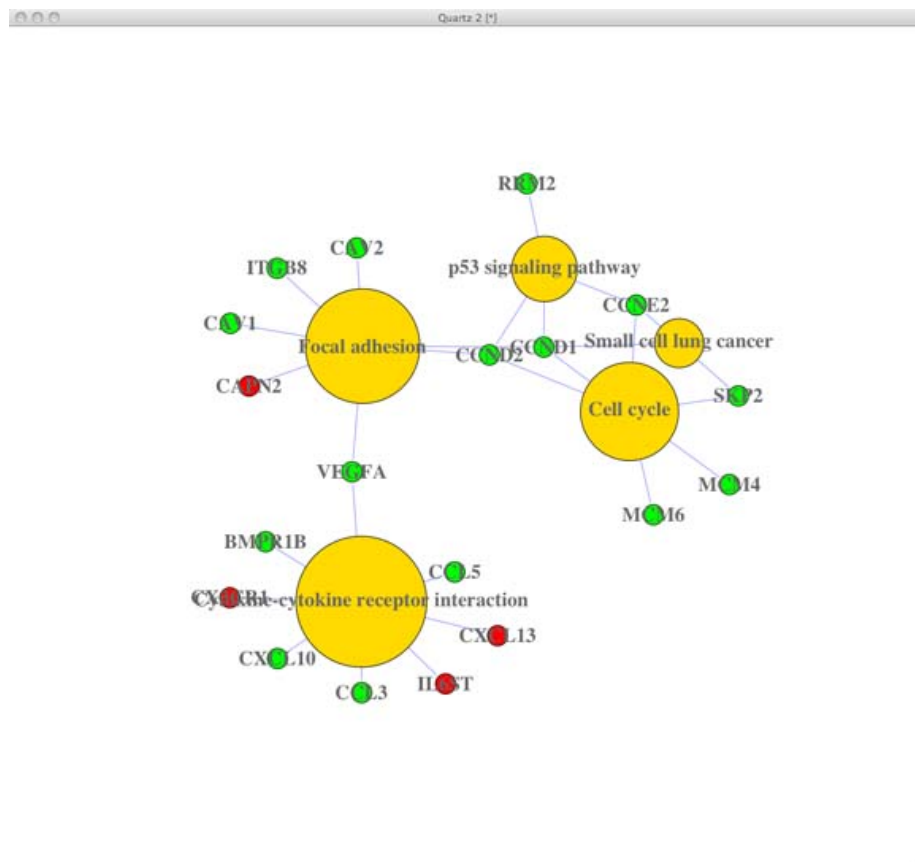


Figure 12: Screen shot of KEGG-gene network

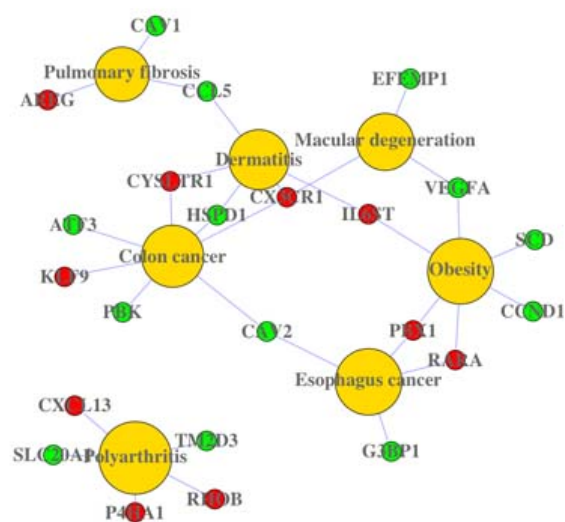
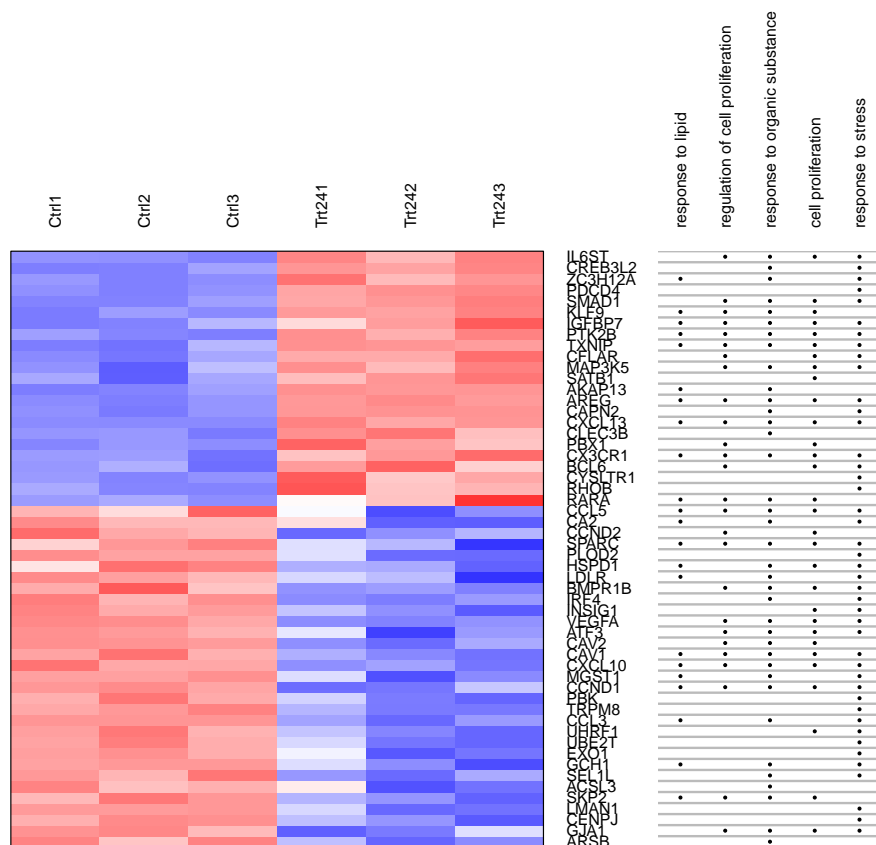


Figure 13: Screen shot of DOLite-gene network

```
initial value 2.140431
iter 5 value 0.734694
iter 10 value 0.336663
iter 15 value 0.196071
iter 20 value 0.104715
iter 25 value 0.024182
iter 30 value 0.017921
final value 0.009853
converged
initial value 19.281277
iter 5 value 0.588247
iter 10 value 0.076872
final value 0.006537
converged
```




```
> geneAnswersHeatmap(yyy)
```

```
initial value 1.716305
iter 5 value 0.407656
iter 10 value 0.112360
iter 15 value 0.072109
iter 20 value 0.052017
iter 25 value 0.029419
final value 0.006019
converged
initial value 4.466884
iter 5 value 1.114859
iter 10 value 0.043826
final value 0.005340
converged
```



Figure 15: KEGG-gene cross tabulation

For cross table, there are two types of representations. One is a table, which is better for few genes, and another one is a two-color heatmap that is adopted for a lot of genes. In the latter, the default setting is that white bar stands for that a gene in that category.

Besides top categories, users can also show interested categories.

```
> GOBPIDs <- c("GO:0043627", "GO:0042493", "GO:0006259", "GO:0007243")
> GOBPTerms <- c("response to estrogen stimulus", "response to drug", "protein kinase casc
> ## generate concept-gene cross tabulation
> geneAnswersConceptNet(x, colorValueColumn='foldChange', centroidSize='pvalue', output='f
> geneAnswersHeatmap(x, showCats=GOBPIDs, catTerm=TRUE, geneSymbol=TRUE)
```

Function *geneAnswersConcepts* shows the linkages of specified categories. The width of edge stands for how overlapping between two categories.

```
> ## generate concept-gene cross tabulation
> geneAnswersConcepts(xxx, centroidSize='geneNum', output='fixed', showCats=GOBPTerms)
```

Users can also print top categories and genes on screen and save them in files by specification as well as these two types of visualization. The default file names are "topCategory.txt" and "topCategoryGenes.txt" for top categories with or without corresponding genes, respectively.

```
> ## print top GO categories sorted by hypergeometric test p value
> topGOGenes(x, orderby='pvalue')

[1] "***** response to lipid::GO:0033993    p value : 8.198e-11 *****"
      GeneID foldChange      pValue
11214 AKAP13    2.062521 2.373847e-08
374    AREG    17.553825 1.241073e-11
760    CA2     -2.256972 8.992257e-04
857    CAV1     -2.122246 3.842276e-06
6348   CCL3     -4.031781 1.147014e-07
[1] "***** response to stress::GO:0006950    p value : 1.476e-09 *****"
      GeneID foldChange      pValue
374    AREG    17.553825 1.241073e-11
467    ATF3     -2.014800 5.116121e-05
604    BCL6     2.380046 2.436277e-04
658   BMPR1B    -2.161504 1.308048e-04
760    CA2     -2.256972 8.992257e-04
[1] "***** response to organic substance::GO:0010033    p value : 2.295e-09 *****"
      GeneID foldChange      pValue
2181   ACSL3    -2.049592 5.282896e-04
11214 AKAP13    2.062521 2.373847e-08
374    AREG    17.553825 1.241073e-11
411    ARSB     -2.122207 3.199329e-06
467    ATF3     -2.014800 5.116121e-05
[1] "***** cell proliferation::GO:0008283    p value : 4.459e-09 *****"
      GeneID foldChange      pValue
```

```

initial value 1.851350
iter 5 value 0.390287
iter 10 value 0.146730
iter 15 value 0.120260
iter 20 value 0.092560
iter 25 value 0.046348
iter 30 value 0.035369
iter 35 value 0.028131
iter 40 value 0.021161
iter 45 value 0.011081
iter 45 value 0.008695
iter 45 value 0.005071
final value 0.005071
converged
initial value 19.020185
iter 5 value 2.491750
iter 10 value 0.333367
iter 15 value 0.027846
final value 0.002865
converged

```



Figure 16: DOLite-gene cross tabulation

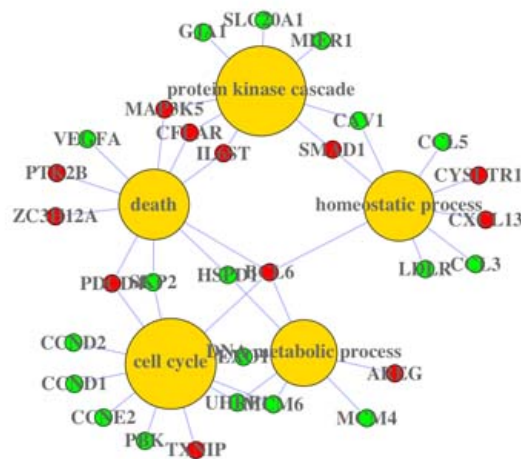


Figure 17: Screen shot of customized GO-gene network

```

374  AREG  17.553825  1.241073e-11
467  ATF3  -2.014800  5.116121e-05
604  BCL6  2.380046  2.436277e-04
658  BMPR1B -2.161504  1.308048e-04
857  CAV1  -2.122246  3.842276e-06
[1] "***** regulation of cell proliferation::G0:0042127    p value : 8.24e-09 *****"
      GeneID foldChange      pValue
374  AREG  17.553825  1.241073e-11
467  ATF3  -2.014800  5.116121e-05
604  BCL6  2.380046  2.436277e-04
658  BMPR1B -2.161504  1.308048e-04
857  CAV1  -2.122246  3.842276e-06

> ## print top KEGG categories sorted by gene numbers and sort genes by fold changes
> topPATHGenes(y, orderby='geneNum', top=4, topGenes=8, genesOrderBy='foldChange')

[1] "***** Cytokine-cytokine receptor interaction::04060    genes in Category : 8 *****"
      GeneID foldChange      pValue
6348  CCL3  -4.031781  1.147014e-07
7422  VEGFA  -2.391115  7.147830e-09
3627  CXCL10 -2.376811  1.315856e-07
658  BMPR1B -2.161504  1.308048e-04
6352  CCL5  -2.154827  1.092361e-03
1524  CX3CR1  2.127494  4.412558e-06
  
```

```

initial value 2.527333
iter 5 value 0.883837
iter 10 value 0.240990
iter 15 value 0.160675
iter 20 value 0.087816
iter 25 value 0.020261
final value 0.003867
converged
initial value 0.000000
final value 0.000000
converged

```

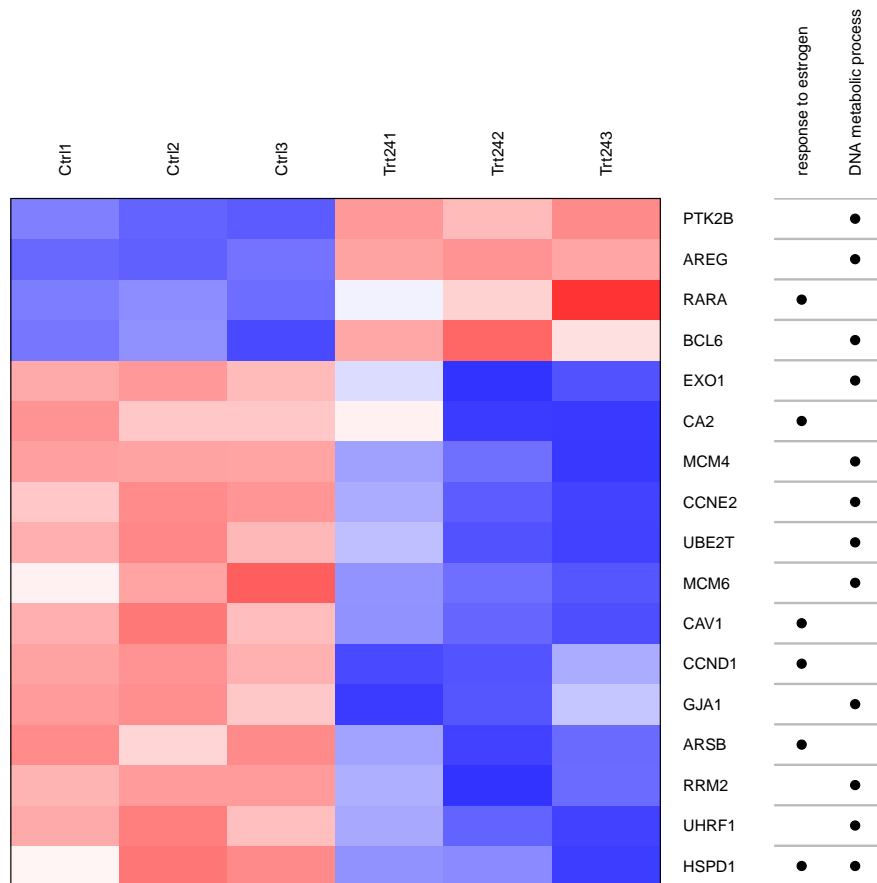


Figure 18: Customized concept-gene cross tabulation



Figure 19: Screen shot of customized GO category linkage

```

3572 IL6ST 2.213384 8.018338e-08
10563 CXCL13 10.688601 1.073456e-10
[1] "***** Focal adhesion::04510 genes in Category : 7 *****"
      GeneID foldChange      pValue
3696 ITGB8 -3.097913 1.178489e-06
858 CAV2 -2.643498 2.625770e-08
7422 VEGFA -2.391115 7.147830e-09
595 CCND1 -2.257123 5.932920e-07
894 CCND2 -2.239806 1.208734e-06
857 CAV1 -2.122246 3.842276e-06
824 CAPN2 2.568772 1.890638e-08
[1] "***** Cell cycle::04110 genes in Category : 6 *****"
      GeneID foldChange      pValue
9134 CCNE2 -2.379943 3.326011e-06
4175 MCM6 -2.356668 2.080808e-04
6502 SKP2 -2.276824 1.013445e-05
595 CCND1 -2.257123 5.932920e-07
894 CCND2 -2.239806 1.208734e-06
4173 MCM4 -2.082429 8.413734e-06
[1] "***** Chemokine signaling pathway::04062 genes in Category : 6 *****"
      GeneID foldChange      pValue
6348 CCL3 -4.031781 1.147014e-07
3627 CXCL10 -2.376811 1.315856e-07
6352 CCL5 -2.154827 1.092361e-03
2185 PTK2B 2.036789 1.970066e-07
1524 CX3CR1 2.127494 4.412558e-06
10563 CXCL13 10.688601 1.073456e-10

> ## print and save top 10 DOLites information
> topDOLITEGenes(z, orderby='pvalue', top=5, topGenes='ALL', genesOrderBy='pValue', file=T

[1] "***** Hyperlipidemia::DOLite:261 p value : 0.001982 *****"
      GeneID foldChange      pValue
10628 TXNIP 3.899457 4.296784e-09
6319 SCD -2.480571 6.976419e-07
2181 ACSL3 -2.049592 5.282896e-04
6352 CCL5 -2.154827 1.092361e-03
[1] "***** Prostate cancer::DOLite:447 p value : 0.003963 *****"
      GeneID foldChange      pValue
374 AREG 17.553825 1.241073e-11
262 AMD1 -2.538954 1.216657e-09
3490 IGFBP7 3.787547 2.688423e-08
5087 PBX1 2.552036 1.120693e-07
3627 CXCL10 -2.376811 1.315856e-07
[1] "***** Alveolar bone loss::DOLite:43 p value : 0.008729 *****"
      GeneID foldChange      pValue
6348 CCL3 -4.031781 1.147014e-07
6678 SPARC -2.289622 7.751533e-04
[1] "***** Leukodystrophy NOS::DOLite:307 p value : 0.01148 *****"
      GeneID foldChange      pValue

```

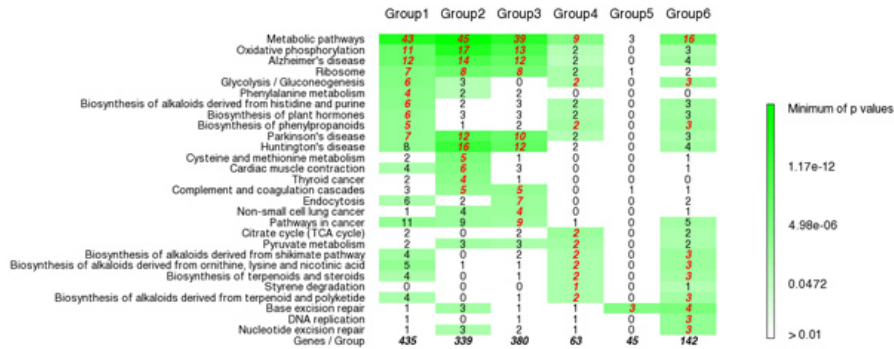


Figure 20: Screen shot of multigroup genes KEGG analysis

```
411 ARSB -2.122207 3.199329e-06
3329 HSPD1 -2.144728 7.145728e-05
[1] "***** Bronchiolitis::DOLite:93 p value : 0.01148 *****"
GeneID foldChange pValue
6348 CCL3 -4.031781 1.147014e-07
6352 CCL5 -2.154827 1.092361e-03
[1] "File topCategoryGenes.txt is successfully generated!"
```

5.3 Multigroup Genes Analysis

Several groups of genes are used to dynamically study biological processes by different treatment or time points. In this case, *GeneAnswers* provide a solution to integrate enrichment test information of different groups of genes.

```
> ##load multigroup genes sample data
> data(sampleGroupsData)
> ##Build a GeneAnswers List
> gAKEGGL <- lapply(sampleGroupsData, geneAnswersBuilder, 'org.Hs.eg.db', categoryType='KEGG')

[1] "GeneAnswers instance has been successfully created!"
[1] "GeneAnswers instance has been successfully created!"
[1] "GeneAnswers instance has been successfully created!"
[1] "GeneAnswers instance has been successfully created!"
[1] "GeneAnswers instance has been successfully created!"
[1] "GeneAnswers instance has been successfully created!"

> ##Output integrated text table
> output<- getConceptTable(gAKEGGL, items='geneNum')
```

Function groupReport can generate a html file including all information.

5.4 Homologous Gene Mapping

Since DOLite is developed for human, any gene from other species can not take advantage of this novel annotation database. Therefore, *GeneAnswers* package provides two functions for this type of data interpretation. *getHomoGeneIDs*

can map other species gene Entrez IDs to human homologous gene Entrez IDs at first. Then users can perform normal GeneAnswers functions. Finally, function *geneAnswersHomoMapping* maps back to original species gene Entrez IDs. Current version supports two types of homologous gene mapping. One is called "direct", which is simple and only works between mouse and human. Since all of human gene symbols are capitalized, while only first letter of mouse homologous gene symbols is uppercase, this method simply maps homologous genes by capitalized mouse gene symbols. Another method adopts *biomaRt* to do mapping. *biomaRt* contacts its online server to mapping homologous genes. Its database include more accurate information, but it might take longer to do that, while 'direct' method can rapidly do conversation though it is possible to miss some information.

```
> ## load mouse example data
> data('mouseExpr')
> data('mouseGeneInput')
> mouseExpr[1:10,]

      GeneID      S11      S12      S13      S21      S22      S23
1    93695 11.140745 11.555394 11.199022 13.53989 13.684888 13.521658
2    20750 10.378364 10.780340 10.280152 12.51370 12.777766 12.727550
3    16854 10.576541 10.823445 10.539105 12.52568 12.948083 12.752825
4    20210 10.417790 10.503403 10.603501 12.38010 12.643765 12.453697
5    14282  9.392208  9.574147  9.456061 11.47399 11.247493 11.426657
6    17105 10.599174 11.078450 10.565310 12.47790 12.797566 12.508973
7    17110 12.674773 13.153840 12.672851 14.56094 14.891312 14.578346
8    16002 11.766943 12.268368 11.557304 13.42105 13.621636 13.608379
9    21924  8.874513  9.096380  8.860733 10.46360 10.669648 10.626148
10 269994 10.913894 10.330857 10.853911  9.07294  9.076301  9.043659

> mouseGeneInput[1:10,]

      Symbol foldChange      pValue
93695   93695   4.869452 1.864011e-08
20750   20750   4.573777 1.224957e-07
16854   16854   4.274721 6.526113e-08
20210   20210   3.956676 4.098411e-09
14282   14282   3.754383 3.190981e-09
17105   17105   3.597932 1.088294e-06
17110   17110   3.587662 1.035619e-06
16002   16002   3.217968 5.465650e-06
21924   21924   3.122260 2.337725e-08
269994 269994 -3.106423 1.962161e-06

> ## only keep first one for one to more mapping
> pickHomo <- function(element, inputV) {return(names(inputV[inputV == element])[1])}
> ## mapping geneInput to homo entrez IDs.
> homoLL <- getHomoGeneIDs(mouseGeneInput[,1], species='mouse', speciesL='human', mappingM

[1] "Warning: homologues of some input genes can not be found and are removed!!!"

> newGeneInput <- mouseGeneInput[mouseGeneInput[,1] %in% unlist(lapply(unique(homoLL), pick
> dim(mouseGeneInput)
```

```

[1] 71  3

> dim(newGeneInput)

[1] 67  3

> newGeneInput[,1] <- homoLL[newGeneInput[,1]]
> ## mapping geneExpr to homo entrez IDs.
> homoLLExpr <- getHomoGeneIDs(as.character(mouseExpr[,1]), species='mouse', speciesL='hum

[1] "Warning: homogenes of some input genes can not be found and are removed!!!"

> newExpr <- mouseExpr[as.character(mouseExpr[,1]) %in% unlist(lapply(unique(homoLLExpr) ,
> newExpr[,1] <- homoLLExpr[as.character(newExpr[,1])]
> dim(mouseExpr)

[1] 71  7

> dim(newExpr)

[1] 67  7

> ## build a GeneAnswers instance based on mapped data
> v <- geneAnswersBuilder(newGeneInput, 'org.Hs.eg.db', categoryType='DOLITE', testType='h

[1] "geneInput has built in ..."
[1] "annLib and categoryType have built in ..."
[1] "genesInCategory has built in ..."
[1] "Enrichment test is only performed based on annotated genes"
[1] "testType, pvalueT and enrichmentInfo have built in ..."
[1] "geneExpressionProfile has been built in ..."
[1] "GeneAnswers instance has been successfully created!"

> ## make the GeneAnswers instance readable, only map DOLite IDs to terms
> vv <- geneAnswersReadable(v, geneSymbol=F)

[1] "Mapping genesInCategory ..."
[1] "Mapping enrichmentInfo rownames ..."

> getAnnLib(vv)

[1] "org.Hs.eg.db"

> ## mapping back to mouse genes
> uu <- geneAnswersHomoMapping(vv, species='human', speciesL='mouse', mappingMethod='direc

[1] "Change annLib ..."
[1] "Mapping geneInput ..."
[1] "Mapping genesInCategory ..."
[1] "Mapping geneExprProfile ..."

> getAnnLib(uu)

[1] "org.Mm.eg.db"

```



```

initial value 0.561792
iter 5 value 0.185166
iter 10 value 0.055120
iter 15 value 0.051535
iter 20 value 0.035624
final value 0.009132
converged
initial value 16.218142
iter 5 value 9.824398
final value 9.383898
converged

```



Figure 22: homogene DOLite-gene cross tabulation

```

19288 Ptx3 -2.100947 4.364358e-05
[1] "***** Obesity p value : 5.282e-06 *****"
      Symbol foldChange      pValue
11421 Ace 2.897884 4.811431e-10
20525 Slc2a1 -2.590159 6.290580e-09
13614 Edn1 2.504625 1.536084e-08
22339 Vegfa -2.535157 3.651889e-08
17390 Mmp2 2.932919 5.771626e-08
[1] "***** Hypoglycemia p value : 9.692e-06 *****"
      Symbol foldChange      pValue
11421 Ace 2.897884 4.811431e-10
20525 Slc2a1 -2.590159 6.290580e-09
11816 Apoe 2.465045 4.135459e-06
[1] "***** Polyarthrititis p value : 2.853e-05 *****"
      Symbol foldChange      pValue
13614 Edn1 2.504625 1.536084e-08
93695 GpnmB 4.869452 1.864011e-08
18452 P4ha2 -2.584996 3.363470e-07
18792 Plau 2.456354 4.029683e-07
13038 Ctsk 2.220556 1.394015e-06
[1] "***** Macular degeneration p value : 3.459e-05 *****"
      Symbol foldChange      pValue
11421 Ace 2.897884 4.811431e-10
12266 C3 2.580510 1.185513e-09
22339 Vegfa -2.535157 3.651889e-08
11816 Apoe 2.465045 4.135459e-06
[1] "***** Capillaries disease p value : 5.687e-05 *****"
      Symbol foldChange      pValue
13614 Edn1 2.504625 1.536084e-08
22339 Vegfa -2.535157 3.651889e-08
17390 Mmp2 2.932919 5.771626e-08
[1] "File topCategoryGenes.txt is successfully generated!"

```

6 Session Info

```
> toLatex(sessionInfo())
```

- R version 3.3.1 (2016-06-21), x86_64-apple-darwin13.4.0
- Locale: C/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
- Base packages: base, datasets, grDevices, graphics, methods, parallel, stats, stats4, utils
- Other packages: AnnotationDbi 1.36.0, Biobase 2.34.0, BiocGenerics 0.20.0, DBI 0.5-1, GO.db 3.4.0, GeneAnswers 2.16.0, Heatplus 2.20.0, IRanges 2.8.0, KEGG.db 3.2.3, MASS 7.3-45, RColorBrewer 1.1-2, RCurl 1.95-4.8, RSQLite 1.0.0, S4Vectors 0.12.0, XML 3.98-1.4, annotate 1.52.0, bitops 1.0-6, igraph 1.0.1, org.Hs.eg.db 3.4.0, org.Mm.eg.db 3.4.0

- Loaded via a namespace (and not attached): RBGL 1.50.0, digest 0.6.10, downloader 0.4, graph 1.52.0, magrittr 1.5, tools 3.3.1, xtable 1.8-2

7 Acknowledgments

We would like to thank the users and researchers around the world contribute to the *GeneAnswers* package, provide great comments and suggestions and report bugs

8 References

Du, P., Feng, G., Flatow, J., Song, J., Holko, M., Kibbe, W.A. and Lin, S.M., (2009) 'From disease ontology to disease-ontology lite: statistical methods to adapt a general-purpose ontology for the test of gene-ontology associations', *Bioinformatics* 25(12):i63-8

Feng, G., Du, P., Krett, N.L., Tessel, M., Rosen, S., Kibbe, W.A., and Lin, S.M., (submitted) 'Bioconductor Methods to Visualize Gene-list Annotations',