netGO User's Manual

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2019-08-26

1. Introduction

netGO is an R-Shiny package for network-integrated pathway enrichment analysis. It also provides the conventional Fisher's exact test. Specifically, it provides user-interactive visualization of enrichment analysis results and related networks. The netGO package is available at Github (**https://github.com/unistbig/netGO**). Currently, netGO provides network and annotation gene-set data for four species including human, mouse, yeast, and Arabidopsis thaliana. These data are all available from another repository (**https://github.com/unistbig/netGO-Data/**)

2. Installation and Example codes

1) Prerequisite R packages can be installed by simply executing the following R codes:

```
install.packages(c('devtools', 'Rcpp', 'shinyjs', 'DT','doParallel', 'foreach', 'parallel', 'htmlwidgets', 'googleVis', 'V8'))
library(devtools)
install_github('unistbig/shinyCyJS')
```

2) netGO can be installed and executed as follows:

```
library(devtools) # load devtools to use 'install_github' function
install_github('unistbig/netGO') # install netGO
library(netGO) # load netGO
DownloadExampleData() # Download and load example datasets
obj = netGO(genes = brca[1:20], genesets = genesets, network = network, genesetV = genesetV) # Executing netGO
netGOVis(obj = obj, genes = brca[1:20], genesets = genesets, R = 50, network = network) # Visualization of the result
```

This example run takes around 10 min on Desktop, and 15 - 25 min on Laptop (not including downloading data)

3. Data

Human, mouse, yeast and Arabidopsis data are available at https://github.com/unistbig/netGO-Data.

* Tip: Human STRING and MSigDB C2 data are directly loaded using 'DownloadExampleData()' function.

Species	Data Type	File name	Object name
Human	Network	1) Human/networkString.RData 2) Human/networkHumannet.RData	network
	Gene-set (mSigDB C2)	Human/c2gs.RData	genesets
	Pre-calculated interaction data NOTE: After downloading *1.RData and *2.RData, genesetV1 and genesetV2 must be joined by a row! genesetV=rbind(genesetV1,genesetV2)	1) For *STRING*, Human/genesetVString1.RData Human/genesetVString2.RData	genesetV1 genesetV2
		2) For *HumanNet*, Human/genesetVHumannet1.RData Human/genesetVHumannet2.RData	genesetV1 genesetV2
Mouse	Network	networkMousenet.RData	network
	Gene-set (KEGG)	KEGGmouse.RData	genesets
	Pre-calculated interaction data	genesetVMousenet.RData	genesetV
Yeast	Network	networkYeastnet.RData	network
	Gene-set (KEGG)	KEGGyeast.RData	genesets

Arabidopsis	Network	networkAranet.RData	network
	Gene-set (KEGG)	KEGGara.RData	genesets

Table M1. Network and gene-set data provided in netGO

4. Functions

The two main functions of netGO package are ‘**netGO**’ (for enrichment test) and ‘**netGOVis**’ (for visualization of the test results).

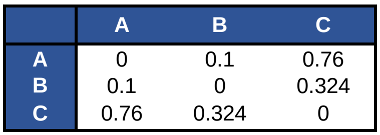
1) netGO

netGO function takes seven arguments: **1) genes, 2) genesets, 3) network, 4) genesetV**, **5) alpha (optional) and 6) beta(optional) 7) nperm (optional)**. It returns the *p*-values and FDR of gene-sets (data.frame) derived from netGO and Fisher’s exact test. Note that the members in **genes** (denoted by A, B, C here) should be given in gene symbols when using the default STRING and MSigDB data. Other types of gene names are also acceptable if the corresponding customized data (network and gene-set data) are uploaded. Descriptions of each argument are as follows:

① **genes**: *A character vector* of input genes (e.g., differentially expressed genes).

② **genesets**: *A list* of gene-sets consisting of groups of genes (e.g., C2 category of MSigDB).

③ **network**: *A numeric matrix* of network data. The range of network score is [0,1]**.**

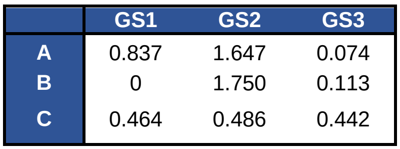


	A	B	C
A	0	0.1	0.76
B	0.1	0	0.324
C	0.76	0.324	0

Figure M1. Example for network data.

④ **genesetV**: *A numeric matrix* of pre-calculated interaction data between genes and gene-sets. The matrix dimension becomes [{# of genes} X {# of gene-sets}]. This matrix can be obtained using **BuildGenesetV** function with the network and gene-set objects as input arguments. Calculating this matrix corresponds to the preprocessing step of netGO.

```
genesetV = BuildGenesetV(network, genesets)
```



	GS1	GS2	GS3
A	0.837	1.647	0.074
B	0	1.750	0.113
C	0.464	0.486	0.442

Figure M2. Example for genesetV.

⑤ **alpha** (optional): *A numeric* parameter weights how much network score will be effected (See (1) in the main text). The value is positive numeric value with > 1 and the default is 20.

⑥ **beta** (optional): A numeric parameter balancing the weights between the relative and absolute network scores (See (1) in the main text). The value is between 0 and 1 and the default is 0.5.

⑦ **nperm** (optional): *The number* of resampling. Default is 10,000.

2) netGOVis

netGOVis also takes six input arguments: **1) obj, 2) genes, 3) genesets, 4) network, 5) R (optional), 6) Q (optional).** This function visualizes the test results on web browser (google chrome is recommended). The resulting network graphs and table are downloadable.

① obj: data frame of the test results obtained from ‘netGO’ function. It consists of five columns including 1) gene-set name and p-values and FDR calculated from 2) netGO (netGOP, netGOFDR) and 3) Fisher’s exact test (FisherP, FisherFDR) and 4) each gene-set’s overlap score (overlap_score) and 5) network score (network_score).

② R (optional): Gene-set rank threshold, default is 50 (Top 50 gene-sets in either method will be displayed). This parameter has higher priority than Q parameter.

③ Q (optional): Gene-set Q-value threshold, default is 1. (Gene-sets with Q-values below this threshold will be displayed)

④ genes, genesets, network: the same arguments in the ‘netGO’ function.

5. Network visualization

netGO visualization page consists of three parts such as **Network, Table,** and **Bubble.**

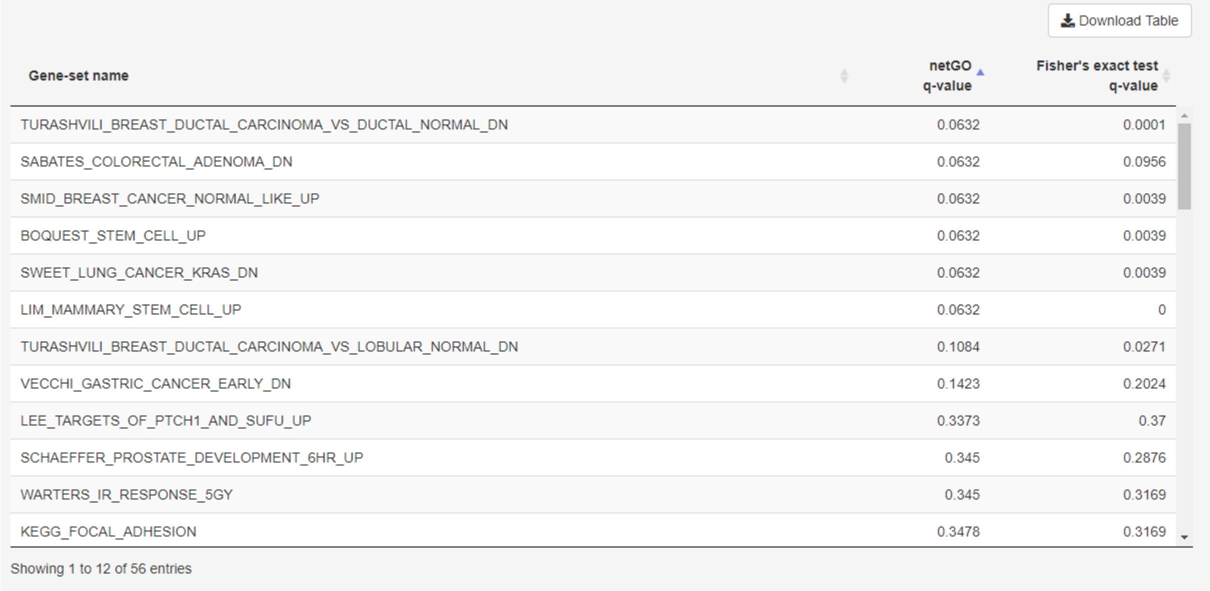
1) **Network** panel displays the input genes, selected gene-set, and the network connections between the two.
   - Sky blue nodes represent input genes (e.g., DE genes)
   - Yellow nodes represent genes in the selected gene-set
   - Green nodes represent the intersection of input genes and the gene-set.
   - The edge width represents the strength of interaction between two nodes.

   * Green Nodes without edges are discarded.

   * The gene-set can be selected by clicking on the gene-set name in the **Table** on the right side.

   * The users can download the graph image as SVG format.

2) **Table** contains the names of gene-sets and their p-values evaluated from netGO and Fisher’s exact test, respectively. It is downloadable by clicking the ‘Download Table’ button in the upper right of the table (Figure M3).



Gene-set name	netGO q-value	Fisher's exact test q-value
TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_DUCTAL_NORMAL_DN	0.0632	0.0001
SABATES_COLORECTAL_ADENOMA_DN	0.0632	0.0956
SMID_BREAST_CANCER_NORMAL_LIKE_UP	0.0632	0.0039
BOQUEST_STEM_CELL_UP	0.0632	0.0039
SWEET_LUNG_CANCER_KRAS_DN	0.0632	0.0039
LIM_MAMMARY_STEM_CELL_UP	0.0632	0
TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_LOBULAR_NORMAL_DN	0.1084	0.0271
VECCHI_GASTRIC_CANCER_EARLY_DN	0.1423	0.2024
LEE_TARGETS_OF_PTCH1_AND_SUFU_UP	0.3373	0.37
SCHAEFFER_PROSTATE_DEVELOPMENT_6HR_UP	0.345	0.2876
WARTERS_IR_RESPONSE_5GY	0.345	0.3169
KEGG_FOCAL_ADHESION	0.3478	0.3169

Showing 1 to 12 of 56 entries

Figure M3. Table panel that shows the list of significant gene-sets

3) **Bubble** module plots the bubble chart of significant gene-sets. Bubble module plots the bubble chart of significant gene-sets. The overlap (x-axis) and network scores (y-axis) of each significant gene-sets are represented. The size of bubbles represents the significance level of each gene-set. The sum of the two

scores is the integrated score $P(T \rightarrow A)$ as follows:

$$P(T \rightarrow A) = \underbrace{\frac{|T \cap A|}{|T|}}_{\text{Overlap score}} + \underbrace{\frac{\alpha}{|T|} \sum_{x \in T-A} AI(x,A)^{\beta} \cdot RI(x,A)^{1-\beta}}_{\text{Network score}}$$

Where

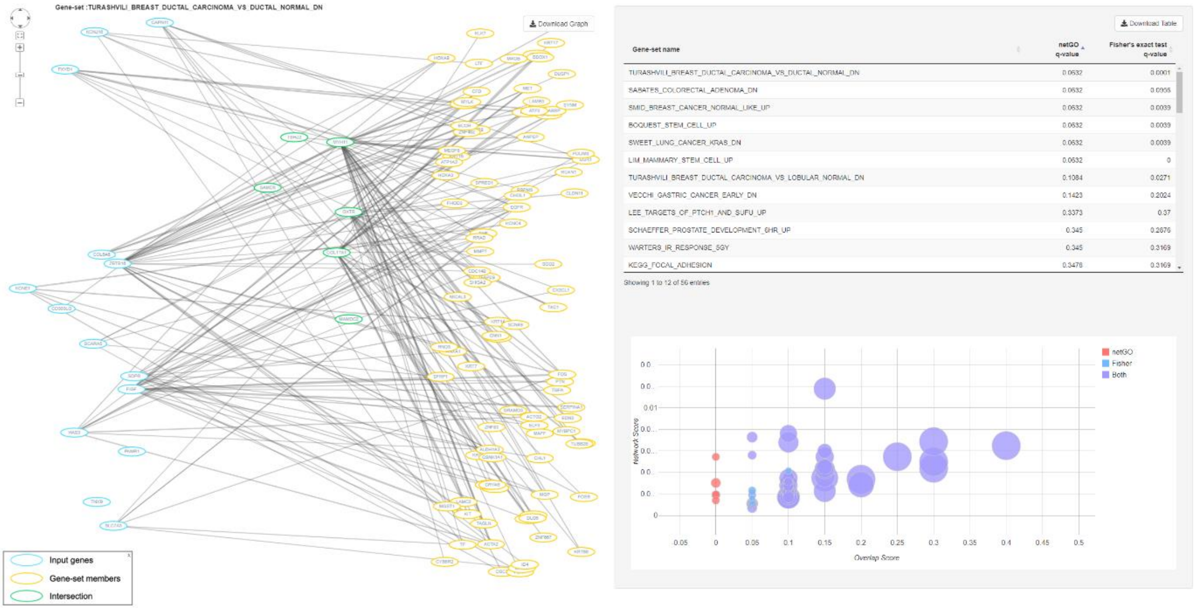
$$AI(x,A) = \frac{1}{|A|} \sum_{y \in A} I(x,y)$$

$$RI(x,A) = \frac{1}{|N(x)|} \sum_{y \in A} I(x,y)$$

T and A are target and annotation gene-sets, respectively; $|\cdot|$ denotes the size of a set; $I(x,y)$ represents the interaction score between x and y genes normalized to unit interval [0,1]; $N(x)$ is the set of all scores of interactions to x and $|N(x)|$ is their summation.

Note that high network score means a dense interaction between input genes and gene-set.

- The size of bubble represents the significance of each gene-set ($-\log_{10} p - value$).
- Colors of bubble indicate the method that detected the gene-set as significant.



Gene-set name	netGO q-value	Fisher's exact test q-value
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SABATES_COLORECTAL_ADENOMA_DN	0.0632	0.0956
SMID_BREAST_CANCER_NORMAL_LIKE_UP	0.0632	0.0039
BOQUEST_STEM_CELL_UP	0.0632	0.0039
SWEET_LUNG_CANCER_KRAS_DN	0.0632	0.0039
LIM_MAMMARY_STEM_CELL_UP	0.0632	0
TURASHVILI_BREAST_DUCTAL_CARCINOMA_VS_LOBULAR_NORMAL_DN	0.1084	0.0271
VECCHI_GASTRIC_CANCER_EARLY_DN	0.1423	0.2024
LEE_TARGETS_OF_PTCH1_AND_SUFU_UP	0.3373	0.37
SCHAEFFER_PROSTATE_DEVELOPMENT_6HR_UP	0.345	0.2676
WARTERS_IR_RESPONSE_5GY	0.345	0.3169
KEGG_FOCAL_ADHESION	0.3478	0.3169

Showing 1 to 12 of 56 entries

Figure M4. netGO interface