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- About SoyNet (2016)

SoyNet is a functional gene network and co-expression network database for a legume crop, **soybean** (*Glycine max*). Soybean is a major sources of oil and protein for human and livestock, and accounts for more than 50% of globally consumed edible oil. To facilitate genetic dissection of economically important traits of soybean via network biology approaches, we developed a network wiring **40,812 soybean genes** (~73% of the coding genome) with **~2 million functional links**, which were inferred by Bayesian statistics framework. Details about network construction methods previously developed are described in *Nature Biotechnology 2010*.

SoyNet web database can generate functional hypotheses for soybean genes using two network algorithms: 'Find new members of a pathway', 'Find context associated genes', and 'Find functional modules'.

Licensing

Contents of this database are **freely available for academia and public entities**. If you are a commercial entity, please send an email for further information about licensing.

- Network-search tools

Network search allows users to identify predict new candidate genes for a phenotype or biological pathways by prioritizing soybean genes. Functionally associated genes are wired to another in the network. Thus, genes that are highly connected to genes of known function (user submitted guide genes: genes that are known to participate in certain biological pathways or that lead to certain phenotypic outcome) are candidate genes of the pathway.

Option 1. Find new members of a pathway



Overview

If you want to identify new candidate genes for a function/pathway/phenotype, you can start search by submitting a set of known genes for the function/pathway/phenotype. These submitted genes are used as 'guide genes' for candidate gene prioritization. This page collects closely connected genes to the guide genes on SoyNet by direct neighborhood and list the prioritized genes by total edge weight score (sum of log likelihood score) to the guide gene set. Highly ranked genes are good candidates for new members of a function/pathway/phenotype of interest.

A. Submit your guide genes



Option II. Find context associated genes

This option allows to use differentially expressed genes (DEGs) as input Click 'Find new members of a pathway' on Network-search page.

Find new members of a pathway



Example gene sets



107 *G. max* Fatty acid biosynthetic process. From GO:0006633 (AgriGO) G.max Fatty acid biosynthetic process V

Available Plant Species

Organism	Common name	Annotation database	Example gene name
Glycine max version 2	Soybean version 2	Glycine max Wm82.a2.v1	Glyma.01G081900
Glycine max version 1.1	Soybean version 1.1	Glycine max genome version 1.1	Glyma01g20720
Arabidopsis thaliana	thale cress	TAIR10	AT1G01010

1. Insert your guide genes in the text box. The gene names must be separated by tab, comma, white space or new line. A maximum of 500 genes are available. User can input not only *Glycine max* genes and *Arabidopsis thaliana* genes for porting well studied pathways to Soybean.

2. Organism selection box. User can select an organism among *Glycine max* version 2 genome, *Glycine max* version 1.1 genome, *Arabidopsis thaliana*, and auto-detection, which option will automatically recognize your species of 'Guide gene set'.

3. Click 'submit' button to start the analysis. This may take about 1~3 minutes.

4. You could select our example guide genes for a test run. In this tutorial, *Glycine max* Fatty acid biosynthetic process genes were selected.

B. Result page - Area Under ROC curve



Area under ROC curve by 107 guide genes

File format: [False_positive_rate] [True_positive_rate] [Area_under_ROC_curve] with tab delimiters

Statistics of query genes. This table shows the number of guide genes input, valid guide genes in *Glycine max*, guide genes in SoyNet, AUC score, and P-value. AUC is 'Area Under ROC Curve' which is shown below and represents closeness of guide genes. Generally, AUC above 0.7 means those genes form closed in SoyNet.

C. Result page - Network visualization of guide genes

Guide genes in SoyNet



1. This plot shows network of guide genes using Cytoscape Web. This requires installation of adobe flash player. Users can click 'Node' or 'Edge' to show additional information of edges or nodes.

2. Additional information of edges or nodes is shown here.

3. Users can view this graph in a new window, or download Cytoscape Network Files to see the network using Cytoscape standard alone program.

D. Result page - Prioritization of guide genes



Guide genes connected to one another in SoyNet (ranked by total connectivity)

This table shows a list of guide genes. Those genes are prioritized using network connectivity scores. Therefore higher rank genes could be considered more important for pathways.

1. Score is calculated by sum of LLS (Log Likelihood Scores) of network links to all other guide genes. Evidence is contribution source of the link.

2. SoyNet also presents various pathway terms of genes to provide comprehensive analysis of pathways. The annotation of each gene are provided using AgiGO, UniprotGOA and Arabidopsis Gene ontology biological process term.

3. Clicking the locus names lead to corresponding annotation databases for detailed descriptions.

E. Result page - Network visualization of new candidates of pathway



New candidate pathway genes associated to 107 valid guide gene(s) in SoyNet

1. Yellow ovals indicate new candidates of submitted pathway and green ovals indicate guide genes.

2. This plot shows network of new candidates of pathway using Cytoscape Web. Users can click 'Node' or 'Edge' to show additional information of edges or nodes. Additional information of edges of nodes is shown below.

4. Users can view this graph in a new window, or download Cytoscape Network Files to see the network using Cytoscape standard alone program.

F. Result page - A list of new candidates of pathway

all predictions report text file
File format: [Rank] [ORF_ID] [Paralogs] [Symbol] [score] [Evidences(with fractions of contribution)] [#_linked_query/#_valid_query] [Linked_query] [GO descriptions] with
tab delimiters
Paralog information is based on gene duplication events in plants Lee et al. Nucleic Acids Research 41(D1):D1152-D1158 (2013)

* Here only top 100 predictions are shown.

(ATTRE	ATTRE1)							
Rank	Glycine max Wm82.a2.v1	Paralogs	Score	Evidences:Contribution	# connected guide gene / # valid guide gene	Connected guide gene		
	·	、	Glycine max AgriGO biological process Arabidopsis GO biological process Uniprot GO					
1	Glyma.11G245600 3. Click!	<u>Glyma.18G011500</u>	65.91	AT-CC:0.69 SC-LC:0.17 OS-CX:0.14	20/107	[+] (Click for connected guide genes)		
		1		dopsis GO: yo development very long-chain fatty acid biosynthetic process				
2	<u>Glyma.18G011600</u> (na)		60.32	AT-CC:0.80 SC-LC:0.20	19/107	[+] (Click for connected guide genes)		
			 AgriGO-BP: na Arabidopsis GO: embryo development very long-chain fatty acid biosynthetic process Uniprot GO: na 					
3	Glyma.05G219800 (ATTRE1)	No Paralog	57.88	SC-CC:1.00	16/107	[+] (Click for connected guide genes)		

This table shows a list of new candidates of input pathway. Those genes are prioritized using network connectivity scores. Therefore higher rank genes could be considered more important for pathways.

1. Score is calculated by sum of LLS (Log Likelihood Scores) of network links to all other guide genes. Evidence is contribution source of the link.

2. We also present various pathway terms of genes to provide comprehensive analysis of pathways. The annotation of each gene are provided using AgiGO, UniprotGOA and Arabidopsis Gene ontology biological process term.

3. Clicking the locus names lead to corresponding annotation databases for detailed descriptions.

Option 2. Find context-associated genes



Overview

This option allows to use differentially expressed genes (DEGs) as input genes for gene prioritization. For example, crop stress response is regulated by many genes. Genome-wide expression profiling upon stress condition may elucidate some stress response regulators (SRR) by DEGs. However, many of DEGs are simply effector genes, which are regulated by actual SRR. In addition, many SRR do not change their expression levels at the stress condition. Therefore, we define subnetworks that are composed of a central hub and their neighbors. If we observe significant overlap between DEGs and neighbor genes of a subnetwork, the central hub of the subnetwork is a hub associated to the stress context (a context-associated hub). The context-associated hubs are often not DEGs.

A. Submit your input differentially expressed genes (DEGs).



Click "Context-associated genes" on Network-search page.

Find context-associated genes



1. Insert your DEGs in the text box. The gene names must be separated by tab, comma, white space or new line. A maximum of 2000 genes are available.

2. Organism selection box. The default setting is auto-detection which option auto-detection will automatically recognize your input gene if the genes are submitted in correct form. Or, User can select between *Glycine max version 2* and *Glycine max version 1*.

3. Click 'submit' button to start the analysis. This may take about 1~2 minutes.

4. You could select our example guide genes for a test run. In this tutorial, *Glycine max* up-regulated genes 8 days after root inoculation of nematode (Race 14, log2 ratio > 2)

B. Result page - Candidate context-associated genes

2

	List of candidate context-associated genes.							
D	If you want to see all of the results, see this report file: Report file 1 Description of columns in the report file: [Glycine max Wm82.a2.v1 ID] [Paralog] [Glycine max Wm82.a2.v1 ID] [is TF?] [is submitted vEG?] [p-value (by Hypergeometric test)] [# of to soybean genes] [# of query genes] [# of genes for the predefined gene set] [# of genes for intersection between m and n] [AgriGO biological process term of soybean] * Paralog information is based on gene duplication events in plants Lee et al. Nucleic Acids Research 41(D1):D1152-D1158 (2013) * TFs are transcription factors identified from Zheng Wang et al. BMC Plant Biology 2010, 10:14. * If the predicted gene is the user submitted differentially expressed gene, they are marked as 'DEG' * Clicking the locus ID will visualize the network connections of genes and guery DEGs. This takes a few minutes so please be patient.							
	Rank	Glycine max Wm82.a2.v1 ID	Paralog	is TF?	submitted DEG?	Glycine max AgriGO biological process Arabidopsis GO biological process Uniprot GO	p-value	
	1	Glyma.06G052000 Network view				 AgriGO-BP: metal ion transport Arabidopsis GO: iron ion transport; zinc II ion transport Uniprot GO: metal ion transport; zinc ion transmembrane transport 	1.356e-11	
	2	<u>Glyma.20G022500</u> Network view				 AgriGO-BP: metal ion transport Arabidopsis GO: cadmium ion transport; cellular iron ion homeostasis; iron ion transport; manganese ion transport; metal ion transport; nickel cation transport; zinc II ion transport Uniprot GO: metal ion transport;zinc ion transmembrane transport 	4.008e-11	
	3	Glyma.10G231600 Network view	Glyma.18G235900			 AgriGO-BP: NA Arabidopsis GO: NA Uniprot GO: NA 	1.124e-09	
	4	Glyma.15G036300 Network view				 AgriGO-BP: metal ion transport Arabidopsis GO: zinc II ion transport Uniprot GO: metal ion transport 	3.328e-09	

SoyNet provides list of candidate context associated genes (hubs) that are ranked by p-value significance. The p-value is obtained by calculating hypergeometric p-value. If the network neighbors of a gene is enriched with DEGs, it is likely to show lower p-value providing evidence that the gene is a good context-associated gene. Maximum 100 predictions are shown on the web

1. You can download the full result list by clicking Report file.

2. Hubs are ranked by their significance of p-values. If the gene is a transcription factor, 'is TF?' column is marked as 'TF'. If the gene is one of the DEGs submitted by the user, 'submitted DEG?' is marked as 'DEG'. P-value of each gene is hypergeometric p-value.

C. Result page - Network visualization of a candidate gene (hub)



By clicking the link 'Network view', the web tool provides network visualization of the connections between candidate context-associated genes and their DEG partners as below.

1. Red node: A context-associated gene is denoted in red. The edges to query DEGs are denoted as red lines.

2. Cyan node: Query DEGs are denoted in cyan. Connections of query DEGs are denoted as cyan lines.

3. You can get the information of the nodes or the links by clicking on them.

Option 3. Find functional modules





This option provides functional modules of given functional genes with significant intermediates. If given pathway genes are not connected in SoyNet, it could be connected by intermediates between two pathway genes. Also, these intermediates can complete the module of the given pathway The significance of intermediates is measured by z-score from binomial proportion test described in Berger et al. (BMC Bioninformatics, 2007). When user input pathway genes, 'Find functional modules' tool presents functional modules of pathway genes with significant intermediates. Since a large number of nodes and interactions may generate stop problem due to calculation time, we draw only top 50 intermediates when the number of intermediates is over 50. Users also can control the threshold of zscore to view proper number of intermediates using slide bar on upper panel.



Click!

This option provides functional modules of given functional genes with significant intermediates. If given pathway genes are not connected in SoyNet, it could be connected by intermediates between two pathway genes. Also, these intermediates can complete the module of the given pathway The significance of intermediates is measured by z-score from binomial proportion test described in <u>Berger et al., BMC</u> <u>Bioninformatics, 2007.</u> When user input pathway genes, 'Find functional modules' tool presents functional modules of pathway genes with significant intermediates. Since a large number of nodes and interactions may generate stop problem due to calculation time,

we draw only top 50 intermediates when the number of intermediates is over 50. Users also can control the threshold of z-score to view proper number of intermediates using slide bar on upper panel.

Click "Find functional modules" on Network-search page.

Find functional modules



1. Insert your pathway genes in the text box. The gene names must be separated by tab, comma, white space or new line. A maximum of 200 genes are available.

2. Organism selection box. The default setting is auto-detection which option auto-detection will automatically recognize your input gene if the genes are submitted in correct form. Or, User can select between *Glycine max version 2* and *Glycine max version 1*.

3. Click 'submit' button to start the analysis. This may take about 1~2 minutes.

4. You could select our example guide genes for a test run. In this tutorial, *Glycine max* Phosphorus stress response genes are prepared for the test.



B. Result page - Functional modules of input pathway genes

A network figure of functional modules is shown by cytoscape web (Flash player should be need to view the figure).

1. You can control z-score threshold to adjust the number of intermediates using the slide bar or enter z-score in the text box.

2. You can see all intermediates by clicking 'List of total intermediates'. Also you can download network links as cytoscape sif format.

3. Yellow nodes indicate input pathway genes

4. White nodes indicate significant intermediates

5. If you click a node or an edge of interest, detail information will be listed in this panel.

- Network download

Option 1. Download SoyNet



[+] Gold Standard: Positive functional gene associations

[-] Sub-networks: Individual networks derived directly from G. max data

Network	Description	Links
<u>GM-CX</u>	Co-expression patterns of G. max genes	539,521
<u>GM-GN</u>	Gene Neighborhood, Proximity between two Soybean gene orthologs in Prokaryotic genomes	211,084
<u>GM-PG</u>	Phylogenetic profiling, Co-inheritance patterns of Soybean ortholog genes across organisms	30,695

[+] Sub-networks: Individual networks derived from networks of other organisms' (Associalogs)

You can download SoyNet and component networks in the 'Network-download' page.

In section 'Download SoyNet', SoyNet and its component networks are provided.

- **1.** Click an interest network to show details.
- 2. Click the network name to download the network.

Descriptions of Downloadable network files

Network file format

Each downloaded network is a plain text file which contains a set of interactions.

Format : Gene A <tab> Gene B <tab> Log-likelihood score (LLS)

Gene A is connected with Gene B in SoyNet.

We trained co-functional gene pairs using gold-standard data with log-likelihood score (LLS) scheme based on Bayesian statistics framework

Log-likelihood score indicates significance of the link. Links with higher score are more significant than lower score.

- SoyNet: Full integrated network

File Name: SoyNet.txt

Description: SoyNet whole network.

- Gold Standard: Positive functional gene associations

File Name: SoyNet_GS.txt

Description: Gold standard pairs used for training SoyNet. The pairs were consturcted using soybean pathway database, KEGG, Mapman, and SoyCyc.

- Sub-networks: Individual networks derived directly from G.max data

File Name: GM-CX.txt

Description: Co-expression patterns of G. max genes

File Name: GM-GN.txt

Description: Gene Neighborhood, Proximity between two Soybean gene orthologs in Prokaryotic genomes

File Name: GM-PG.txt

Description: Phylogenetic profiling, Co-inheritance patterns of Soybean ortholog genes across organisms

- Sub-networks: Individual networks derived from networks of other organisms' (Associalogs)

File Name: AT-CC.txt

Description: PubMed Central full-text Co-citation links of A. thaliana

File Name: AT-CX.txt Description: Co-expression patterns of A. thaliana genes File Name: AT-HT.txt Description: High-throughput PPI data of A. thaliana File Name: AT-LC.txt Description: Literature curated PPI of A. thaliana File Name: CE-CC.txt Description: Co-citation links in C. elegans network File Name: CE-CX.txt Description: Co-expression links in C. elegans network File Name: DM-CX.txt Description: Co-expression links in D. melanogaster network File Name: DM-HT.txt Description: High-throughput PPI data of D. melanogaster File Name: DM-LC.txt Description: Literature curated PPI of D. melanogaster File Name: DR-CX.txt Description: Co-expression links in D. rerio network File Name: HS-HT.txt Description: High-throughput PPI in H. sapiens network File Name: HS-LC.txt Description: Literature curated PPI in H. sapiens network File Name: SC-CC.txt Description: Co-citation links in S. cerevisiae network File Name: SC-CX.txt Description: Co-expression links in S. cerevisiae network File Name: SC-GT.txt

Description: Genetic interactions in S. cerevisiae network

File Name: SC-HT.txt

Description: High-throughput PPI in S. cerevisiae network

File Name: SC-LC.txt

Description: Literature curated PPI in S. cerevisiae network

File Name: OS-CX.txt

Description: Co-expression links in O. sativa network



Option 2. Download Coexpression Component networks

In the section of 'Download Coexpression Component Networks', you can download each coexpression network used in SoyNet.

1. You can search network using keywords.

2. Gene Expression Omnibus (GEO) accession of the transcriptome data.

http://www.ncbi.nlm.nih.gov/geo/

3. Title of the transcriptome data. Users can click title to show detail description of the study.

4. User can download the network by the link.

5. Detail description of the study

Descriptions of Downloadable network files

Network file format

Each downloaded network is a plain text file which contains a set of interactions.

Format : Gene A <tab> Gene B <tab> Log-likelihood score (LLS)

Gene A is connected with Gene B in SoyNet.

We trained co-functional gene pairs using gold-standard data with log-likelihood score (LLS) scheme based on Bayesian statistics framework

Log-likelihood score indicates significance of the link. Links with higher score are more significant than lower score.

- Component networks for constructing co-expression network

File Name: [Gene Expression Omnibus (GEO) Accession Number].txt

Description: Component co-expression networks