

PRO-Simat: Protein network simulation and design tool

USER MANUAL



Introduction

Gene lists are mostly obtained by studies on genomics, transcriptomics, proteomics, or metabolomics. Enrichment analysis helps to correlate these gene lists with underlying molecular pathways and functional categories using databases such as gene ontology (GO) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway.

In this study, we present our new integrative web tool “PRO-Simat” designed by using the R Shiny package. This tool provides GO enrichment and KEGG pathway analyses and network visualization from an integrated database of more than 8 million protein-protein interactions of organisms such as *Arabidopsis thaliana*, *Bacillus subtilis*, *Bacillus anthracis*, *Bos taurus*, *Caenorhabditis elegans*, *Cavia porcellus*, *Danio rerio*, *Drosophila melanogaster*, *Escherichia coli*, *Gallus gallus*, *Homo sapiens*, *Helicobacter pylori*, *Listeria monocytogenes*, *Mus musculus*, *Oryzias latipes*, *Ovis aries*, *Plasmodium falciparum*, *Rattus norvegicus*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* (strain 972 / ATCC 24843), *Staphylococcus aureus*, *Treponema pallidum*, *Vaccinia virus*, *Vaccinia virus Copenhagen*, *Vaccinia virus WR*, *Vaccinia virus L-IPV*, *Vaccinia virus Ankara*, *Vaccinia virus GLV-1h68*, *Xenopus laevis*. In this tool, we also integrated the dynamical network simulation from the Jimena framework, which efficiently and fast simulates Boolean genetic regulatory networks. It enables the user to get simulation outputs with attached explanatory notes directly on the website. This integration also results in the creation of PRO-Simat signaling networks for any process of interest such as design of an oncolytic virus evaluating host cell and virus interactions. The users evaluate the consistency of their network models using the simulation graphs, activity heatmaps, as well as principal component analysis. The PRO-Simat as a graphical web tool will help researchers to establish and analyze dynamical cellular networks from gene clusters.

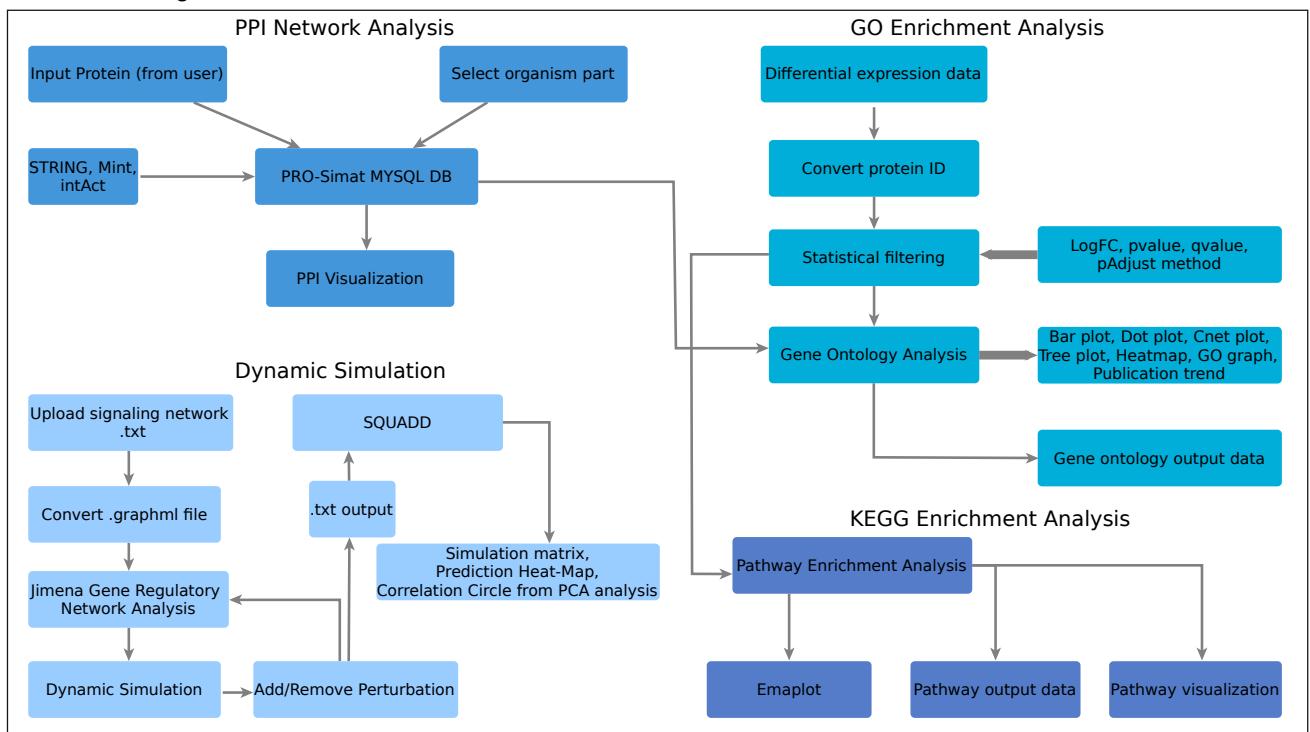


Fig 1. The workflow of PRO-Simat web tool



NETWORK ANALYSIS

Steps for Protein-Protein interaction visualization;

- ① When the users want to refresh the page, they can use the refresh button.
- ② It guides you how to use PRO-Simat.
- ③&④ Select **Organism 1** and **Organism 2** for which the users would like to display protein-protein interactions.
- ⑤ Select the **node shape** where your proteins will be displayed.
- ⑥ Select one of the **layouts** from the select input box to see the network.

③&④	⑤	⑥
<div style="background-color: #2e3436; color: white; padding: 10px;"> ① Refresh ② User Guide Organism 1 Nothing selected Organism 2 Nothing selected Node Shape Circle Layout Nicely Search Protein <input style="width: 150px; border: 1px solid #2e3436; padding: 2px; margin-right: 10px;" type="text"/> Delete Search ⑦ Submit </div>	<div style="border: 1px solid #2e3436; padding: 5px; width: fit-content;"> <i>Arabidopsis thaliana</i> <i>Bacillus anthracis</i> <i>Bacillus subtilis</i> <i>Bos taurus</i> <i>Caenorhabditis elegans</i> <i>Cavia porcellus</i> <i>Danio rerio</i> <i>Drosophila melanogaster</i> <i>Escherichia coli</i> <i>Gallus gallus</i> <i>Homo sapiens</i> <i>Helicobacter pylori</i> <i>Listeria monocytogenes</i> <i>Mus musculus</i> <i>Oryzias latipes</i> <i>Ovis aries</i> <i>Rattus norvegicus</i> <i>Plasmodium falciparum</i> <i>Saccharomyces cerevisiae</i> <i>Schizosaccharomyces pombe</i> <i>Staphylococcus aureus</i> <i>Treponema pallidum</i> <i>Vaccinia virus</i> <i>Vaccinia virus Copenhagen</i> <i>Vaccinia virus WR</i> <i>Vaccinia virus L-IPV</i> <i>Vaccinia virus Ankara</i> <i>Vaccinia virus GLV-1h68</i> <i>Xenopus laevis</i> </div>	<div style="border: 1px solid #2e3436; padding: 5px; width: fit-content;"> Circle Database Diamond Dot Star Ellipse Circle Box Text Triangle Triangle Down Square Hexagon Sugiyama Circle Nicely Grid Sphere Randomly Dh Fr Gem Graphopt KK LGL MDS </div>

- ⑦ The users can search in the database by typing the “**Gene Symbol**” of the protein they want to search in the box and see the related interactions.
- ⑧ The submit button is clicked to use the selected parameters and visualize the protein-protein interaction.

An example *Homo sapiens*- *Vaccinia virus GLV-1h68* protein interaction

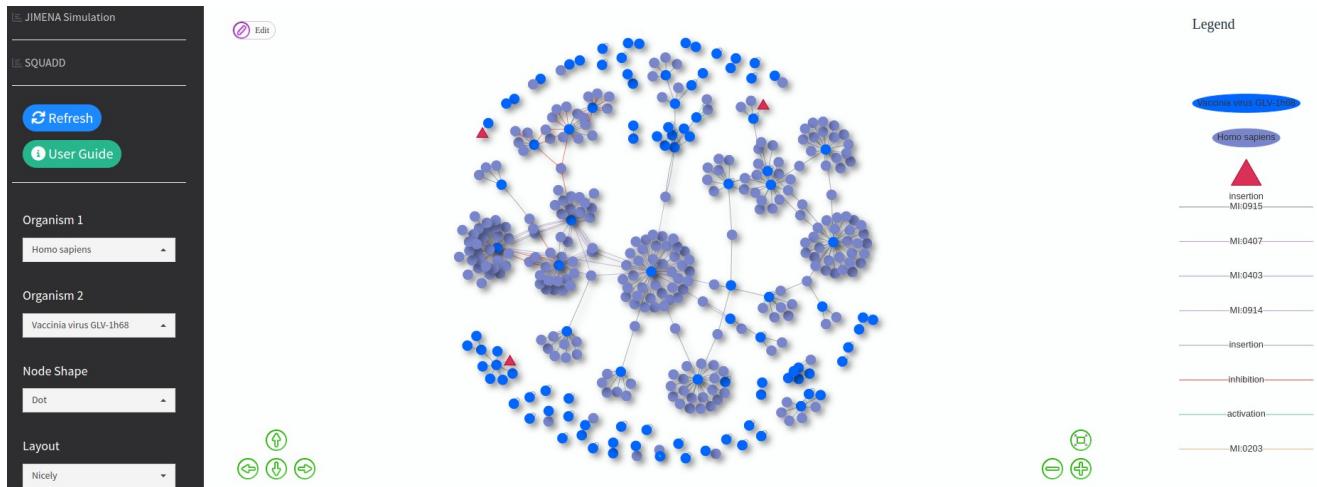


Fig 2. PPI network between *Homo sapiens*- *Vaccinia virus GLV-1h68* modified version. Insertion refers to the parts of the genes that have been changed.

It can be done on PRO-Simat when it is desired to add or remove a new node/edge on the network. When the **edit** button in Network is clicked, add node and add edge options are offered (Fig 2). You can name the added node/edge. (Node/edge added or deleted by the user does not change the PRO-Simat database. Only each users can make changes on their own network).

If you hover over any protein, users can see information about the protein such as Gene, UniprotKB, Species, Taxonomy (Fig 3). Users can either select the protein they would like to search in the network or search by typing owing to the auto-complete feature.

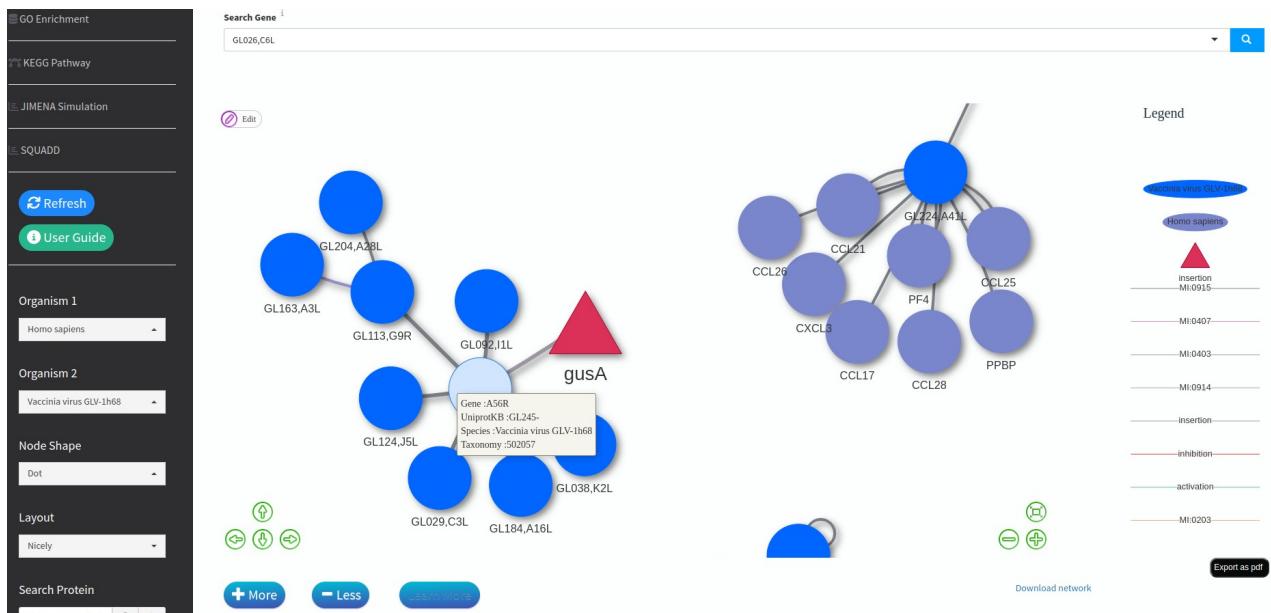


Fig 3. A zoomed-in image of the PPI network between *Homo sapiens*- *Vaccinia virus GLV-1h68*. Protein information is presented as a pop-up.

The interaction information of the protein-protein interaction created (Fig 2) in the PRO-Simat network analysis is presented in a table. The table is in searchable form and it is also allowed to download the table (Fig 4). Thus, the file downloaded in csv format can be used in software programs such as Cytoscape for visualizing complex networks.

	From_A	To_B	Interaction_Type	Gene_A	Gene_B	Taxonomy_A	Taxonomy_B	Organism_A	Organism_B	Description_A	Search:
1	P17362	ATMCY6	Mi:0915	GL026,C6L	TBKBP1	502057	9606	Vaccinia virus GLV-1h68	Homo sapiens	Protein C6	
Description_A TANK-binding kinase 1-binding protein 1											
2	B9U153	B9U153	Mi:0915	GL001,GL290,C23L	GL001,GL290,C23L	502057	502057	Vaccinia virus GLV-1h68	Vaccinia virus GLV-1h68	Chemokine-binding protein	
3	OT5152	B9U153	Mi:0915	ZC3H11A	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Zinc finger CCCH domain-containing protein 11A	
4	POCG48	B9U153	Mi:0915	UBC	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Polyubiquitin-C	
5	P19013	B9U153	Mi:0915	KRT4	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Keratin, type II cytoskeletal 4	
6	P20042	B9U153	Mi:0915	EIF2S2	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Eukaryotic translation initiation factor 2 subunit 2	
7	P46778	B9U153	Mi:0915	RPL21	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	60S ribosomal protein L21	
8	Q13427	B9U153	Mi:0915	PPIG	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Peptidyl-prolyl cis-trans isomerase G	
9	Q8WV24	B9U153	Mi:0915	PHLDA1	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Pleckstrin homology-like domain family A member 1	
10	Q99729	B9U153	Mi:0915	HNRNPAR	GL001,GL290,C23L	9606	502057	Homo sapiens	Vaccinia virus GLV-1h68	Heterogeneous nuclear ribonucleoprotein A/R	
Showing 1 to 10 of 512 entries											

Fig 4. PPI network table between *Homo sapiens*- *Vaccinia virus GLV-1h68*

When users click on any protein, the gene ontology information of the protein is shown in a tabular form (Fig 5). It is allowed to search for the desired term in the table using the Search Box.

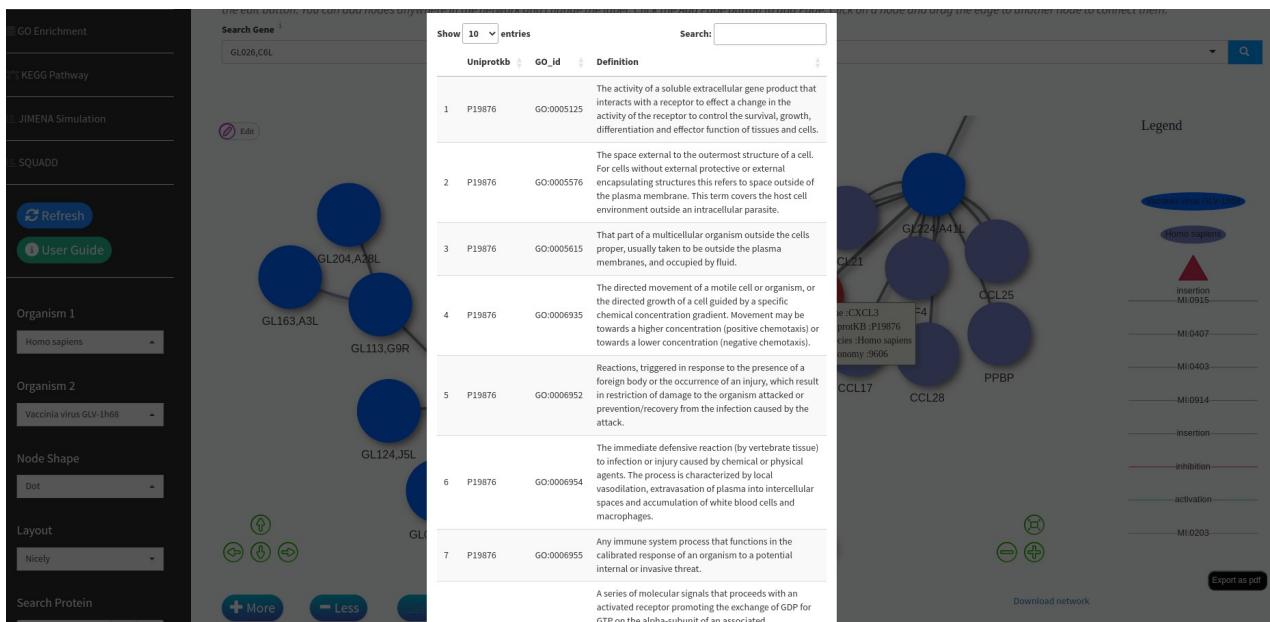


Fig 5. The created PPI network between *Homo sapiens*- *Vaccinia virus GLV-1h68* is clicked on the protein to see the gene ontology information of each protein.

GO ENRICHMENT ANALYSIS



Before starting the GO Enrichment and KEGG pathway analysis, users should decide whether they would like to use their own differential expression genes (DEGs) data or continue with the protein list that they created in the PRO-Simat Network Analysis tab.

The screenshot shows the 'GO Enrichment Analysis' interface. On the left is a sidebar with links: Home, Protein Network, GO Enrichment (selected), KEGG Pathway, JIMENA Simulation, SQUADD, Refresh, and User Guide. Below these are sections for 'Choose:' (Continue with PPI data, Use example data, Upload your data, all with radio buttons; 'Upload your data' is selected), 'Choose CSV File' (Browse... button, No file selected, Header checked, Delete csv file link), and a 'Run Analysis' button.

1a and **1b** are circled in black at the top left of the sidebar.

2 is circled in black at the top right of the 'Select Data Columns' section.

3 is circled in black at the top right of the 'Parameters for GO and KEGG analysis' section.

4 is circled in black at the bottom right of the 'Run Analysis' button.

Fig 6. Box with parameters required for GO and KEGG analyzes

1

- a) If users want to continue the analysis using the protein list in the Network Analysis tab, they should select the "continue with PPI data" option from the "choose" section. In line with this selection, the second part (number 2) is skipped, and the third part (number 3) where can specify the analysis parameters.
- b) If users would like to continue with their DEGs data, they should upload the data in a ".csv" format.

2

Please select the columns with Gene, Log2FC and pAdjust values in the data that you have loaded. Pre-processing is applied to the uploaded data. A Volcano plot will be created together with the thresholds of the parameters users will be able to specify here. In addition, GO and KEGG analysis are continued using only filtered genes.

③

Pvalue, qvalue, keytype, GO/KEGG species (which organism's genes are used) and which padjust method the analysis is desired should be selected.

④

Click the **Run Analysis** button and start the analysis.

Detailed instructions for each tab can be obtained from the “**User Guide**” button, where users will be prompted to make the analysis easily (Fig 7).

The screenshot shows the JIMENA web interface. On the left, there is a sidebar with various options like KEGG Pathway, JIMENA Simulation, SQUADD, Refresh, and User Guide. The User Guide button is highlighted with a green box. The main panel has two tabs: "Select Data Columns" and "Gene Expression Data". The "Select Data Columns" tab contains fields for "Select Gene Column", "Select log2FC Column", and "Select pAdj Column", along with sliders for "min log2FC" (set to 1) and "cut pAdjust" (set to 0.05). A modal window titled "Skip" is open, asking "Select the column with Gene in the data you have loaded." Below it are "Prev" and "Next" buttons. The "Gene Expression Data" tab displays a table of gene expression data with columns: SYMBOL, GeneID, Median.Tumor., Median.Normal., Log2FC, and adjP. The table lists 14 rows of data. At the bottom of the main panel is a "Run Analysis" button.

Fig 7. “User Guide” button guides the user interactively

ⓘ

When the information icon next to the box headings is clicked, the user is informed about how to use the web page (Fig 8).

The screenshot shows the JIMENA web interface. The sidebar includes Protein Network, GO Enrichment, KEGG Pathway, JIMENA Simulation, SQUADD, Refresh, and User Guide. The User Guide button is highlighted with a green box. The main panel has a "GO Enrichment Analysis" header with a sub-instruction: "Load your differential gene expression data or you can use example data (LUAD) and select the columns containing the Gene names and log2FC values. Then click the Run Analysis button." Below this is a "Select Data Columns" tab with fields for "Gene column" and "log2FC column", both set to "Nothing selected". It also has sliders for "min log2FC" (1) and "cut pAdjust" (0.05). A modal window with an information icon (ⓘ) is open over the "Parameters for GO and KEGG analysis" tab. The modal text reads: "For GO and KEGG analysis, you can upload your own DEGs file in csv format or select sample LUAD DEGs data. Specify the desired columns of the csv file you have uploaded/selected from the select input box. For example, select the column with gen symbols from the 'select gene column' tab. Repeat this procedure for all desired columns. Your genes are filtered according to the Log2FC and padjust threshold you will determine and the filtered genes are used in the analysis." At the bottom of the modal are "OK" and "Cancel" buttons.

Fig 8. Information button guides the user interactively

Users can display the Biological Process (BP), Molecular Function (MF) and Cellular Component (CC) gene ontology categories and associated genes (Fig 9). In addition, PRO-Simat subcategorizes apoptotic process, proliferation, and positive and negative regulation (Fig 9).

(GO and KEGG analyzes were performed using proteins belonging to *Homo sapiens-Vaccinia virus GLV-1h68* organisms obtained in ‘Network Analysis’ tab.)

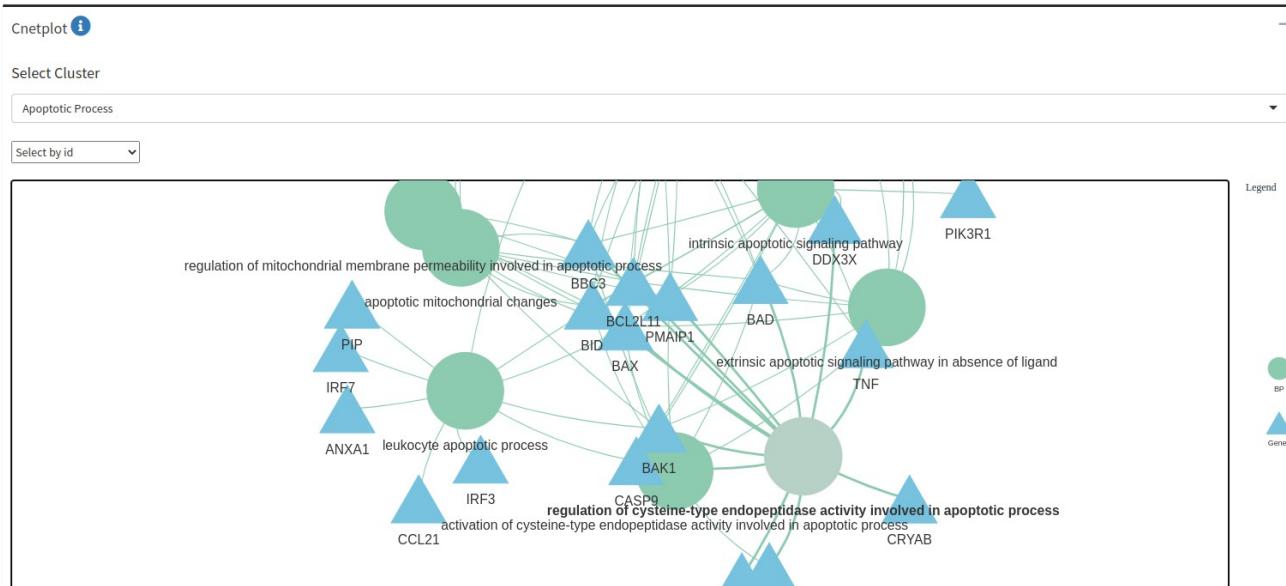


Fig 9. Cnetplot visualization of GO enrichment analysis result obtained in “Network Analysis” tab using proteins belonging to *Homo sapiens- Vaccinia virus GLV-1h68* organisms

Gene ontology has 17 different zoomable graphics options that can be downloaded as “.svg” format using the related button. Thanks to the numeric input of **Show Category** options, users can specify the desired number of categories.

Gene ontology terms are displayed in treeplot according to the number of categories selected by the user (Fig 10).

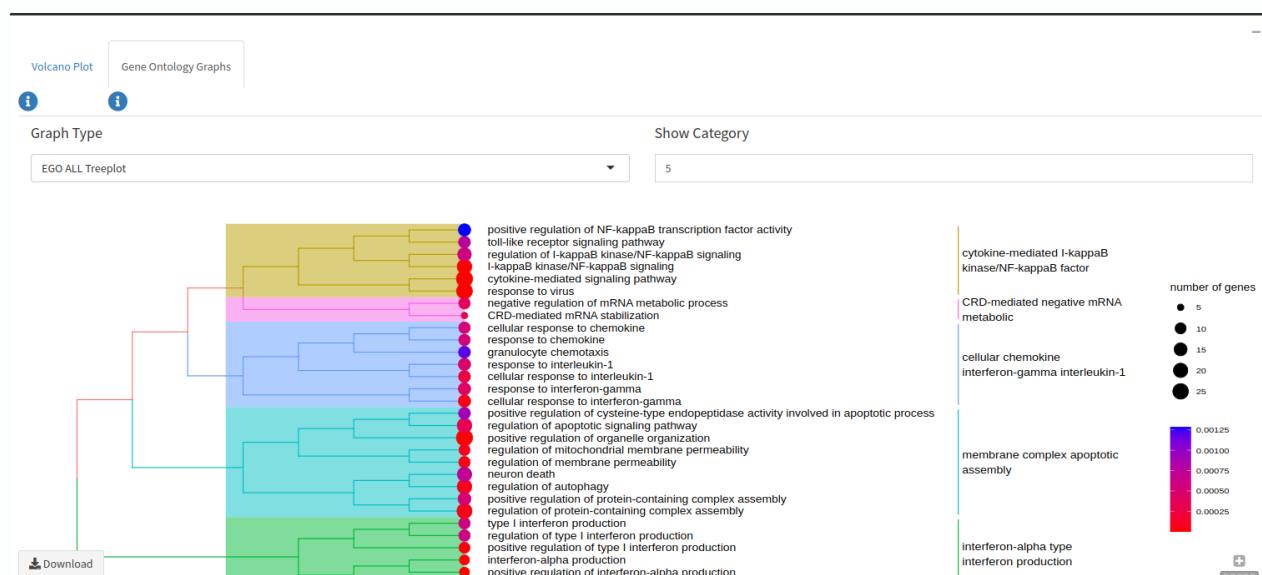


Fig 10. Hierarchical clustering of enriched terms of biological process, cellular component and molecular function

By displaying the result of gene ontology enrichment analysis as a heat map, it provides the user to understand the expressions more easily. As an example, significant 5 categories were selected (Fig 11). User can increase/decrease the number of categories. Increasing the number of categories too much can cause clutter on the heatmap network.

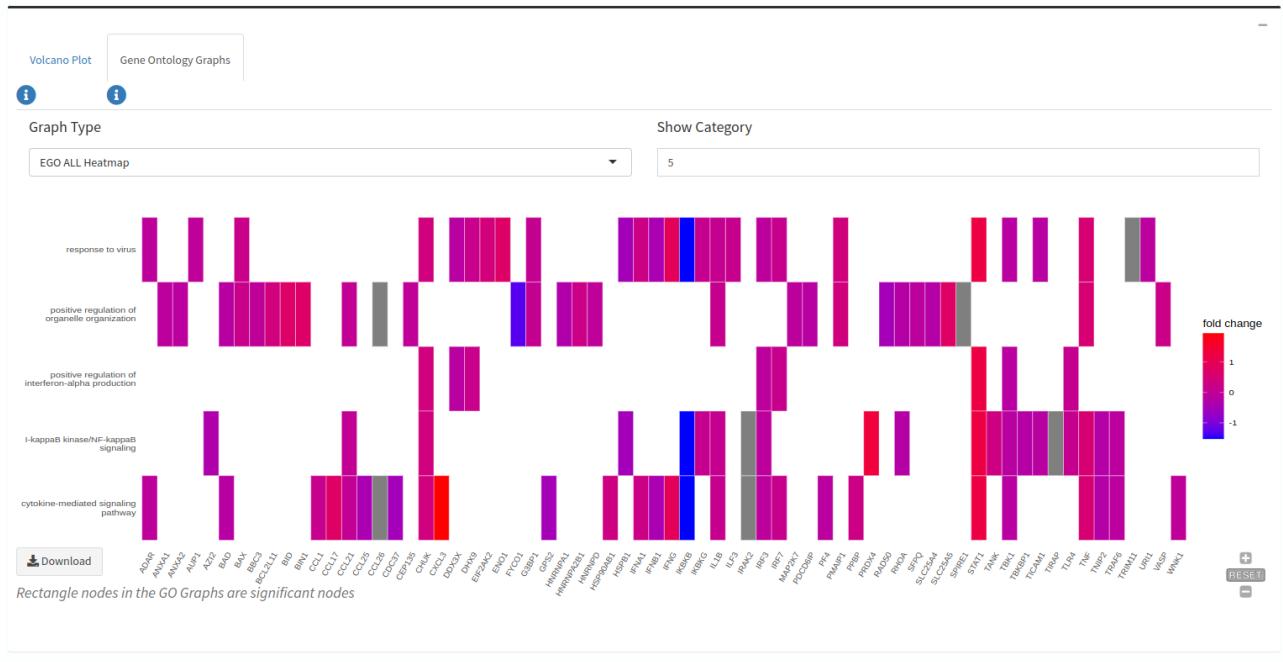


Fig 11. Display of gene and significant terms as heat plots

Gene ontology enrichment results are presented to the user in 4 different tables as BP, CC, MF and all category (Fig 12). Users can download the outputs as PDF, or save them as in a ".csv/excel" format.

Table						
Select Table						
Biological Process						
ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
1	GO:0009615 response to virus	26/267	392/18800	7.67859533131867e-11	3.13056331657862e-7	2.26154839337154e-7
2	GO:0032727 positive regulation of interferon-alpha production	8/267	21/18800	2.58518545257262e-10	0.00000105398010901386	2.76900246892329e-7
3	GO:0019221 cytokine-mediated signaling pathway	28/267	486/18800	3.58919771739539e-10	0.000014633159093821	2.76900246892329e-7
4	GO:0010638 positive regulation of organelle organization	28/267	487/18800	3.76061807788009e-10	0.00000153320399035171	2.76900246892329e-7
5	GO:0007249 I-kappaB kinase/NF-kappaB signaling	21/267	288/18800	1.01082977838937e-9	0.00000412115300649346	5.95431941038623e-7
6	GO:0032607 interferon-alpha production	8/267	27/18800	2.61969450795318e-9	0.0000106804945089251	0.0000011022413884591
7	GO:0032647 regulation of interferon-alpha production	8/267	27/18800	2.61969450795318e-9	0.0000106804945089251	0.0000011022413884591
8	GO:0032481 positive regulation of type I interferon production	10/267	58/18800	8.08226775603301e-9	0.0000329514056413466	0.00000297555068176057
9	GO:0090559 regulation of membrane permeability	11/267	77/18800	1.12627623743875e-8	0.0000459182822003778	0.00000358946741779362
10	GO:0071346 cellular response to interferon-gamma	13/267	118/18800	1.31939526331408e-8	0.0000537917448853151	0.00000358946741779362
11	GO:0010506 regulation of autophagy	21/267	336/18800	1.56343810602082e-8	0.0000637413715824688	0.00000358946741779362

Showing 1 to 11 of 106 entries

Fig 12. Biological process result of GO Enrichment Analysis

If users selected the GO and KEGG analysis using their own DEGs data, the volcano plot is created based on the Log2FC and pAdjust threshold values (Fig 14). Fig 13 displays an example of this step, where the Pancreatic cancer DEGs data from the GEPI database was used.

(Figure 6 shows how the GO and KEGG analysis was performed using user data.)

SYMBOL	GenelD	Median.Tumor.	Median.Normal.	Log2FC	adjp
A2M	ENSG00000175899	183.787	28.3	2.657	1.29e-49
A4GALT	ENSG00000126274	17.14	2.2	2.503	2.69e-48
AACS	ENSG00000081760	15.64	6.74	1.104	5.73e-40
AADAC	ENSG00000114771	4.32	10.68	-1.135	4.15e-8
AAE1	ENSG00000151822	7.5	2.08	1.465	6.02e-57
AAGAB	ENSG00000103591	24.92	7.61	1.59	1.64e-66
AAADC	ENSG00000087884	42.739	12.78	1.666	3.29e-55
AAMP	ENSG00000127837	105.917	34.951	1.572	8.52e-50
AAR2	ENSG00000131043	23.61	6.82	1.654	2.6e-67
AASS	ENSG00000088311	2.85	9.27	-1.415	2.35e-27
AATF	ENSG000000275700	38.239	14.08	1.38	1.24e-42
ABAT	ENSG00000183044	6.34	16.71	-1.271	8.49e-11
ABC7-42404400C24.1	ENSG00000277758	0.17	1.49	-1.09	2.06e-37
ABCA1	ENSG00000165029	4.22	1.43	1.103	2.87e-27

Fig 13. Uploading DEGs data of pancreatic cancer

An interactive volcano plot is created based on the Log2FC and pAdjust values that users set.

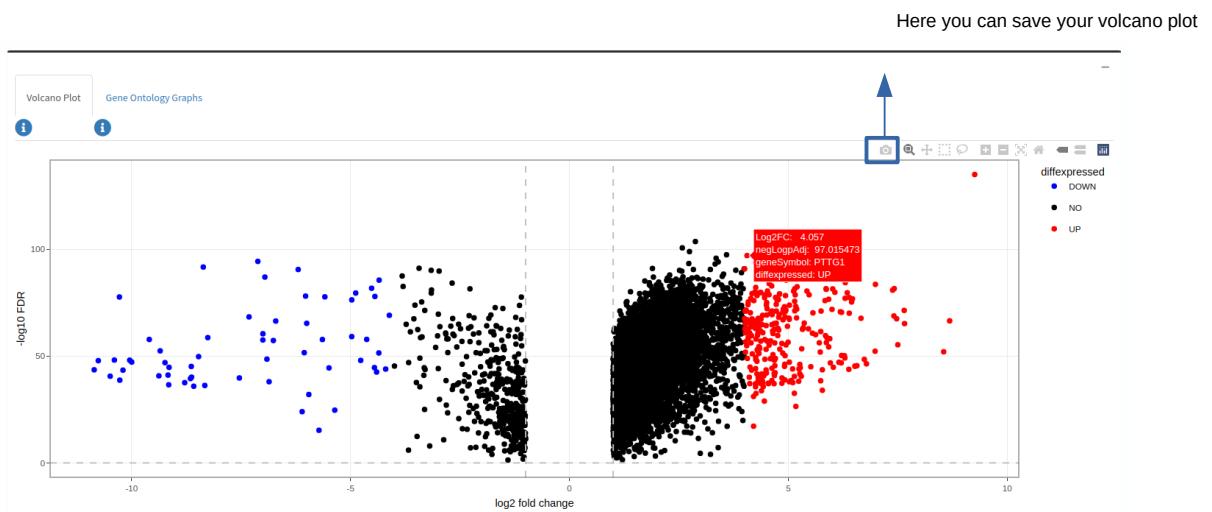


Fig 14. Volcano plot of pancreatic cancer DEGs data



According to the filtering and parameter selections found in the GO enrichment UI tab, the results of the KEGG enrichment analysis are displayed in the KEGG Enrichment Analysis tab. Analysis result is presented in tabular form and can be saved in pdf, csv, excel format (Fig 15 and Fig 16).

Output Table Plot Pathway

KEGG Enrich Output Data [i](#)

ID	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
1	Influenza A	31/160	171/8164	6.81965101841415e-22	1.6367162444194e-19	7.03500841899565e-20
2	Measles	27/160	139/8164	5.91474075438099e-20	1.41953778105144e-17	2.50068939847988e-18
3	Lipid and atherosclerosis	32/160	215/8164	7.27241304659966e-20	1.74537913118392e-17	2.50068939847988e-18
4	Toll-like receptor signaling pathway	22/160	104/8164	3.0535821336685e-17	7.32859712080441e-15	7.87502760788193e-16
5	Hepatitis B	26/160	162/8164	4.4918211151148e-17	1.07803706762755e-14	9.26733619539474e-16
6	Necroptosis	25/160	159/8164	3.16031151300602e-16	7.58474763121444e-14	5.43351803990508e-15
7	Epstein-Barr virus infection	27/160	202/8164	1.25351896652088e-15	3.00844551965012e-13	1.84729110855709e-14
8	Hepatitis C	24/160	157/8164	2.53332044518779e-15	6.0799690684507e-13	3.26665004774215e-14
9	Salmonella infection	29/160	249/8164	4.09873005946208e-15	9.83695214270899e-13	4.31795374683448e-14
10	Kaposi sarcoma-associated herpesvirus infection	26/160	194/8164	4.18577148927832e-15	1.0045851574268e-12	4.31795374683448e-14
11	Apoptosis	22/160	136/8164	1.1965257573478e-14	2.87166181763472e-12	1.12210071024004e-13

Showing 1 to 11 of 81 entries

Fig 15. KEGG pathway enrichment analysis result

CSV Description GeneRatio BgRatio pvalue p.adjust qvalue

CSV	Description	GeneRatio	BgRatio	pvalue	p.adjust	qvalue
Excel	Influenza A	31/160	171/8164	6.81965101841415e-22	1.6367162444194e-19	7.03500841899565e-20
PDF	Measles	27/160	139/8164	5.91474075438099e-20	1.41953778105144e-17	2.50068939847988e-18
3	Lipid and atherosclerosis	32/160	215/8164	7.27241304659966e-20	1.74537913118392e-17	2.50068939847988e-18
4	Toll-like receptor signaling pathway	22/160	104/8164	3.0535821336685e-17	7.32859712080441e-15	7.87502760788193e-16
5	Hepatitis B	26/160	162/8164	4.4918211151148e-17	1.07803706762755e-14	9.26733619539474e-16
6	Necroptosis	25/160	159/8164	3.16031151300602e-16	7.58474763121444e-14	5.43351803990508e-15
7	Epstein-Barr virus infection	27/160	202/8164	1.25351896652088e-15	3.00844551965012e-13	1.84729110855709e-14
8	Hepatitis C	24/160	157/8164	2.53332044518779e-15	6.0799690684507e-13	3.26665004774215e-14
9	Salmonella infection	29/160	249/8164	4.09873005946208e-15	9.83695214270899e-13	4.31795374683448e-14
10	Kaposi sarcoma-associated herpesvirus infection	26/160	194/8164	4.18577148927832e-15	1.0045851574268e-12	4.31795374683448e-14
11	Apoptosis	22/160	136/8164	1.1965257573478e-14	2.87166181763472e-12	1.12210071024004e-13

Showing 1 to 11 of 81 entries

Fig 16. KEGG pathway enrichment analysis results table download options

Users can access the Enrichment Map for the result of over-representation analysis (ORA) in the KEGG enrichment emapplot in Plot tab (Fig 17).

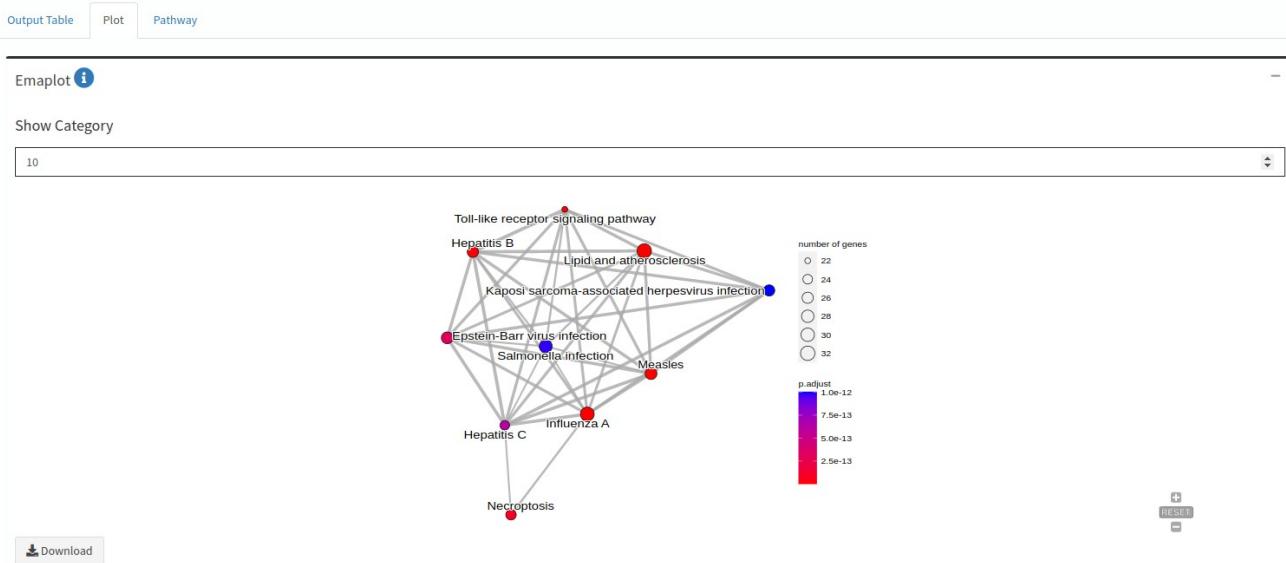


Fig 17. Emap plot with 10 categories

According to the data obtained as a result of ORA, when users write the pathway ID to the text box of input pathway, an enriched KEGG pathway will be created (Fig 18). If users upload their own DEGs data, the coloring will be done using the log2FC scores. If users proceed by using the genes in the PRO-Simat Network Analysis tab, the log2FC values of the DOSE package for *Homo sapiens* will be used.

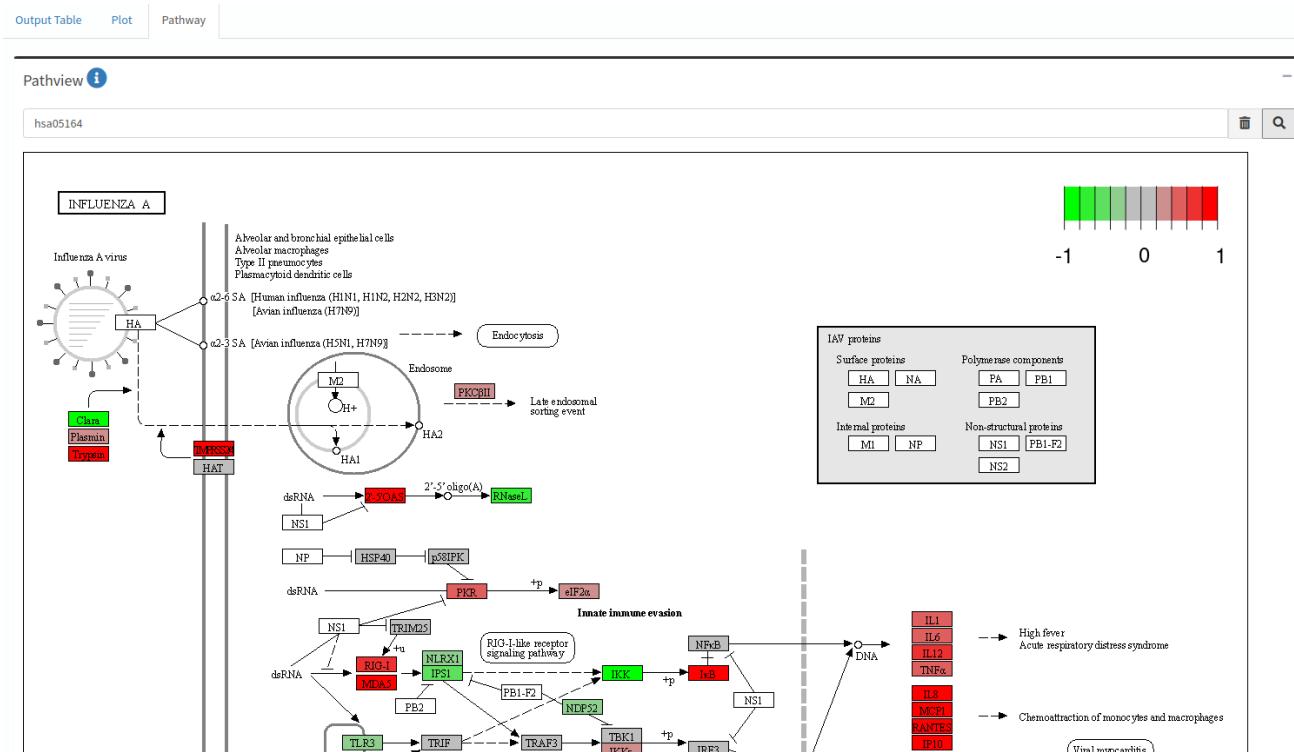


Fig 18. Colored pathway with the help of Pathview package

Users can download the pathway images from the “Download.png” and “Download.pdf” buttons (Fig 19).

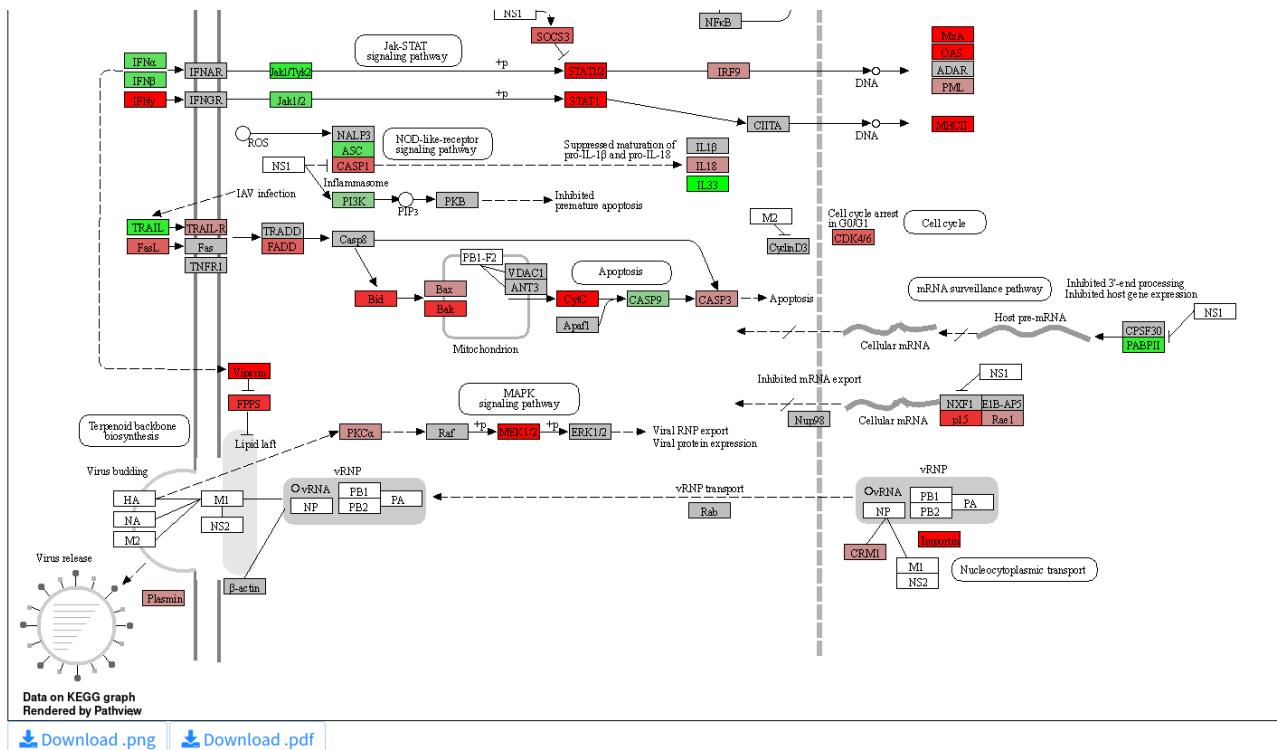
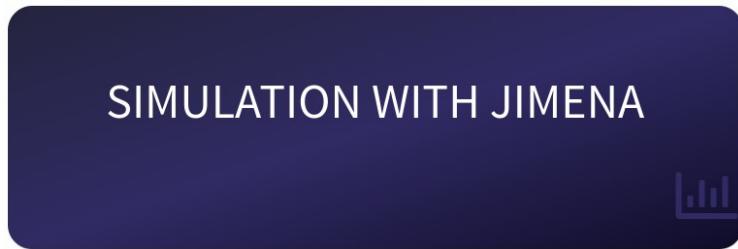


Fig 19. Options to download the colorized path image created with the Pathview package



Users can choose the “Step 1” and “Step 2” information boxes or the **User Guide** button to learn how to use Jimena genetic regulatory simulation with Squad method (Fig 20).

The screenshot shows the Jimena software interface. On the left, a sidebar lists 'Home', 'Protein Network', 'GO Enrichment', 'KEGG Pathway', 'JIMENA Simulation', 'SQUADD', 'Refresh' (blue button), and 'User Guide' (green button). Below these are 'Choose:' options ('Use example data' and 'Upload your data'), 'Choose Txt File' with a 'Browse...' button, and a file named 'test_for_Jimena.txt' with a 'Upload complete' message. The main area is titled 'JIMENA' and contains two tabs: 'Step 1' and 'Perturbation setting Step 2'. The 'Step 1' tab has a table with columns 'node1', 'label', and 'node2', showing 5 rows of data. The 'Perturbation setting' tab shows a table with columns 'Start' (0), 'End' (1000), and 'Value' (1), with buttons for 'Add New Perturbation' and 'Remove Perturbation'. A note at the bottom explains the perturbation parameters. At the bottom of the main area are 'Convert Graphml' and 'Run Jimena' buttons.

Fig 20. Jimena genetic regulatory network simulation tab

- Upload the **.txt file** containing the genes containing Node1, label, Node2 titles and the activation/inhibition information of the signals between them and click the "**Convert Graphml!**" button (Fig 20).
- PRO-Simat will convert the txt file you have uploaded to graphml format and make it suitable for use by the Jimena program (Fig 20).
- You can start the first analysis by clicking the **Run Jimena** button (Fig 20).
- Step 2 includes adding and subtracting Perturbation. From the “Add perturbation” selection input box, select the node you want to change the activation level (overexpression or knockout), the time interval you want this node to change in the simulation, and the activation level (between 0 and 1). You can also give values like 0.5 so that it can be partially active, and click the **Add New Perturbation** button. At the same time, after selecting any node you have added, you can remove the node you have selected by clicking the **Remove Perturbation** button. Thus, you will see the node you want to change and the values added to the table in the analysis (Fig 20).
- By pressing the **Run Jimena** button again, perform the analysis based on the node you have changed. The interactive chart will be updated in a few seconds (Fig 20). Your Jimena dynamic simulation will look like the one below (Fig 21).

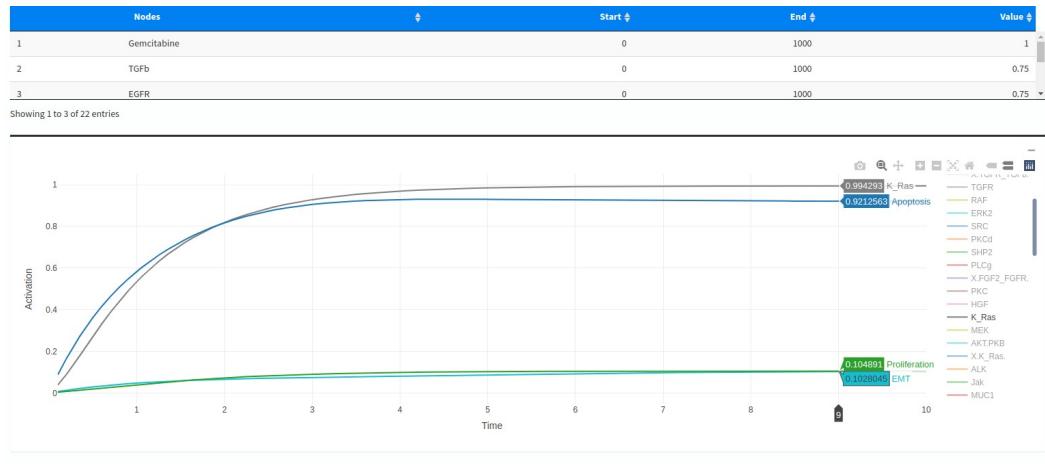


Fig 21. Jimena dynamic simulation output with added perturbations

When you double-click on the node you see on the right and want to select, only the trend of that node will be displayed. Alternatively, after the first double click, you can single click the node you want to compare (Fig 21). In addition, the output obtained as a result of the Jimena simulation is presented in the form of a downloadable table in pdf, csv, and excel formats (Fig 22).

	time	EGFR	CD44	EGF	X.EGFR_EGF.	X.HGFR_HGF.	HGFR	X.TGFR_TGFb.	TGFR	RAF	ERK2	SRC
1	0.1	0.75	0.7	0	0.0931708958197312	0.2	0.0708864721209359	0.034943187053786	0.0936349584418187	0.3	0.15	0.0624126522878274
2	0.2	0.75	0.7	0	0.177475411956323	0.2	0.135027889357698	0.0781834260329228	0.178359411519173	0.3	0.15	0.126491422252987
3	0.35	0.75	0.7	0	0.289131252589446	0.2	0.219980243009601	0.152577885539675	0.290571495349954	0.3	0.15	0.220805205720076
4	0.5	0.75	0.7	0	0.385234332287224	0.2	0.293100920762297	0.231100265578036	0.38715341678805	0.3	0.15	0.309276154170432
5	0.6	0.75	0.7	0	0.441745350864224	0.2	0.336098659444583	0.282914590767679	0.443946055976244	0.3	0.15	0.364077554841279
6	0.7	0.75	0.7	0	0.492878638983402	0.2	0.37500552177079	0.333104162254315	0.495334211952823	0.3	0.15	0.415350483238192
7	0.8	0.75	0.7	0	0.539145956022229	0.2	0.410210975289961	0.381072369725754	0.541832198806452	0.3	0.15	0.463142589385727
8	0.9	0.75	0.7	0	0.581010361256329	0.2	0.442067482731419	0.426476733508196	0.583905390436611	0.3	0.15	0.507595966816856
9	1.05	0.75	0.7	0	0.636457049181418	0.2	0.484262392975485	0.489420321234483	0.639628761378888	0.3	0.15	0.568393997554093
10	1.15	0.75	0.7	0	0.66906109921742	0.2	0.509076654841548	0.527869980012899	0.672395643799792	0.3	0.15	0.605238188452637
11	1.25	0.75	0.7	0	0.69856247467818	0.2	0.531532167233486	0.563547292701024	0.702044494048511	0.3	0.15	0.639295727539628

Fig 22. Jimena dynamic simulation output as a table

VISUAL WITH SQUADD



Users have to **Run Jimena** before using the **SQUADD** visualization package. The reason is when you **Run Jimena** for each node users added by changing the activation level, the results are indexed and kept in PRO-Simat until users close the web application. Different simulation results are re-visualized for the genes users choose with the help of the SQUADD R package. Thus, matrix graphs are created for each gene users have selected for each condition they change.

Please select the nodes from the **Select nodes** box and click the **Run** button.



Fig 23. Index_, result of the first Jimena run simulation without applying add/remove perturbation.
Index_CD44, second simulation result run by changing activation level. In this example, it was tried by blocking CD44. In the **Index_HSF1** sample, active HSF1 was blocked.

The correlation circle was used to assess the consistency of a model. If the angle between each vector is greater than 90 degrees, we can say that there is no correlation (Fig 24). The darker the color, the higher the increase of the node activation in prediction heatmap (Fig 24).

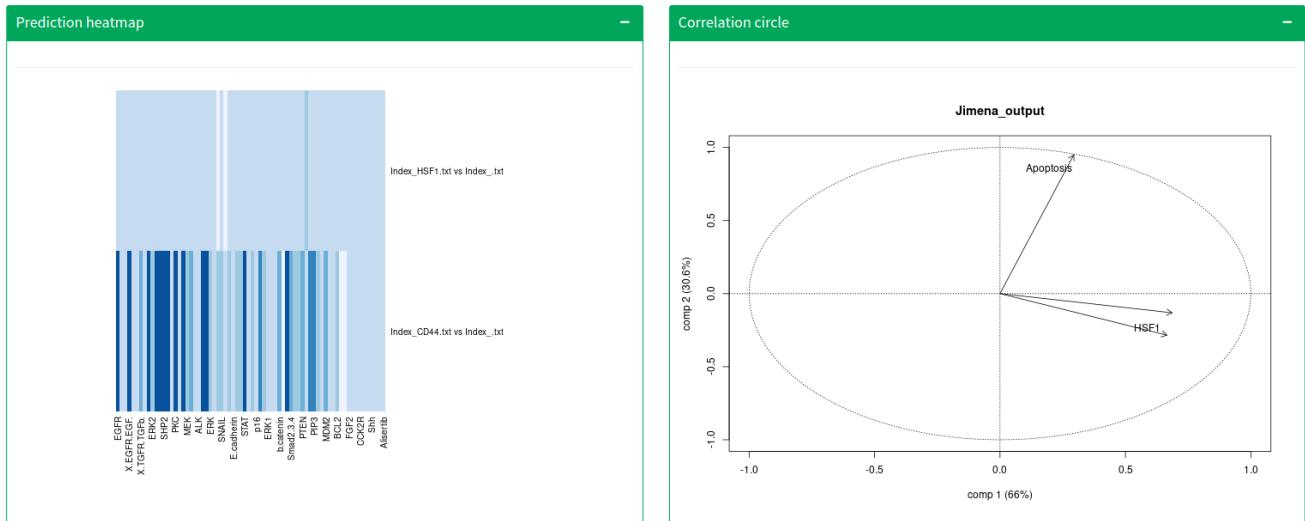


Fig 24. Prediction heatmap and Correlation circle with selected nodes and applied different activation level of genes in Jimena