Theatre for *In Silico* Systems Oncology

Version 1.0

A Next-Generation Multi-Scale Modeling and Simulation Platform for *In Silico* Systems Oncology

Networks Editor User Manual

Version 1.0.20220606

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Contents

Table of Figures

List of Tables

1. Networks Editor

Networks Editor (NE) allows the user to construct biomolecular networks that can be analyzed to predict cell fate outcomes which can then be visualized as three-dimensional landscapes. Several network analysis strategies, such as Deterministic Modeling, Probabilistic Modeling, and Ordinary Differential Equations (ODE) Modeling have been provided via an intuitive and user-friendly Graphical User Interface (GUI). Network analysis results can be viewed as an 'Attractor Landscape', 'Cell Fate Landscape', 'Potential Energy Landscape', 'Probability Landscape', and 'ODE Landscape'. NE's GUI, features list, network analysis, and result visualization have been outlined in the proceeding text.

1.1 Graphical User Interface (GUI)

In **Figure 1.1.1**, various sections and toolbars of Network Editor's intuitive GUI have been labeled, and the functionality of each feature has been explained below.

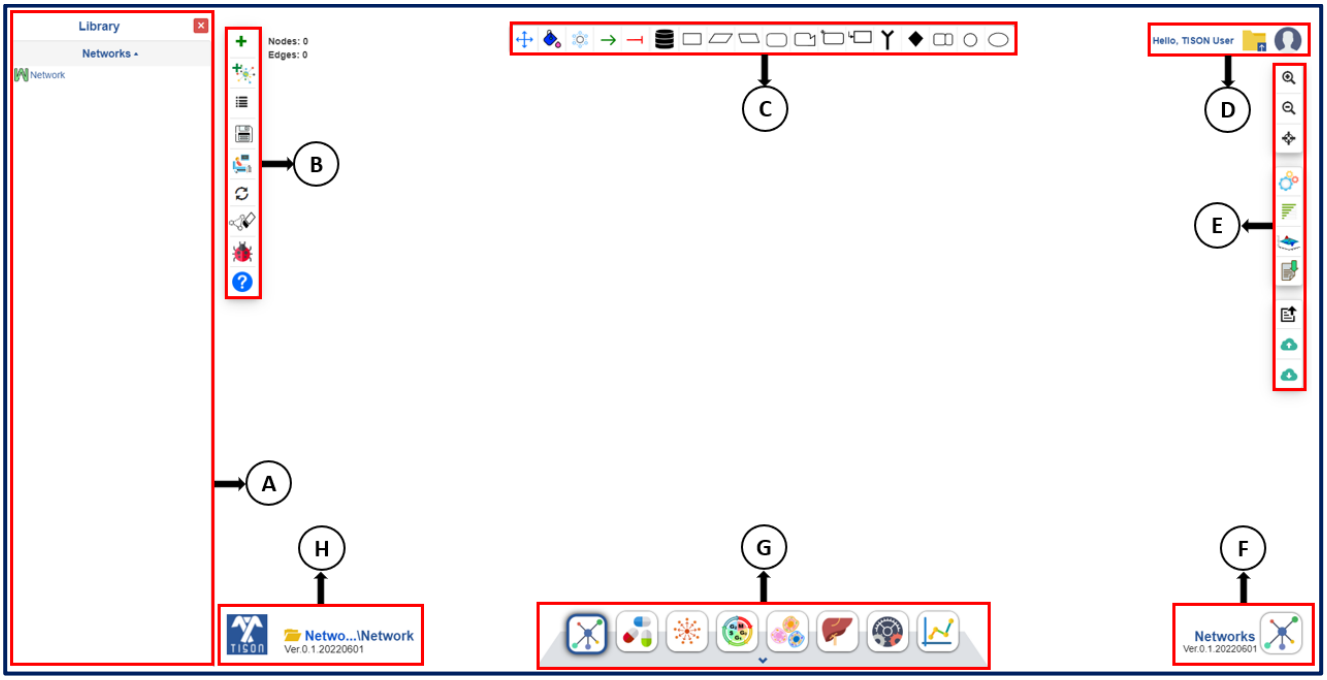


Figure 1.1.1 - The main GUI of TISON's NE.

1.1.1 Library Section

- In **Figure 1.1.1 (A)**, the left 'Networks' panel displays the library of user-constructed and saved networks in the database. A user can open the network by double-clicking the desired network's name or 'Edit Network', 'Edit Properties', 'Duplicate Network', and 'Delete Network' by clicking the icons displayed (**Figure 1.2.3.2**).

1.1.2 Canvas Toolbar (Left)

In **Figure 1.1.1 (B)**,

- 'Create Network' button  in the toolbar creates a new network on the canvas.
- 'Automated Network Construction' button  allows the user to automatically create the network by collecting data from relevant databases and literature.
- 'Show Networks Library' button  displays and hides the library section.
- 'Save Network' button  in the toolbar saves the current network in TISON's database against the user's current project.
- 'Personalize Weight Based Networks' button  allows the user to import biomolecular expression data to calculate basal values for the personalization of networks. Please note that this option is only available for weight-based networks.
- 'Weight-based to Rules-based Conversion' button  allows the user to convert a weight-based network to a rules-based network.
- 'Clear All' button  clears the entire canvas and allows the user to create a new network.
- 'Report a Bug' button  opens http://github.com/BIRL/TISON/issues and allows the user to report any bug/issue in TISON.
- 'Help' button  opens the 'Networks Editor Manual' located on https://tison.lums.edu.pk/Manuals/NetworksManual.pdf.

1.1.3 Canvas Toolbar (Top)

In **Figure 1.1.1 (C)**,

- 'Move Toolbar' button  allows the user to reposition the toolbar.
- 'Change Background Color'  button allows the user to recolor the canvas.
- 'Change Layout' button  allows the user to alter the network layout by opting for either one of the following options: grid, circle, concentric, cose and BFL (Breadthfirst Layout).
- 'Activation Link' button  creates a green activation link between two nodes while drawing a network on the canvas.
- 'Inhibition Link' button  creates a red inhibition link between two nodes while drawing a network on the canvas.
- 'Load Biomolecule from Integrated Databases' button  helps the user find a biomolecule along with its expression from MERAV (Metabolic gEne RApid Visualizer), HPA (Human Protein Atlas), FireBrowse (TCGA), and GTEx (Genotype-Tissue Expression).
- 'Gene' button  draws a gene on the canvas.
- 'RNA' button  draws RNA molecule on the canvas.
- 'Antisense RNA' button  draws an antisense RNA on the canvas.
- 'Protein' button  draws a protein on the canvas.
- 'Truncated protein' button  draws a truncated protein on the canvas.
- 'Cofactor' button  draws a cofactor on the canvas.
- 'Transcription Factor' button  draws a transcription factor on the canvas.
- 'Receptor' button  draws a receptor on the canvas.
- 'Ligand' button  draws a ligand on the canvas.
- 'Ion Channel' button  draws an ion channel on the canvas.
- 'Ion' button  draws an ion on the canvas.
- 'Molecule' button  draws a molecule on the canvas.

1.1.4 Account

In **Figure 1.1.1 (D)**,

- Text at the top right corner of the canvas 

displays the username saved during the registration process.
- 'Return to Project Explorer'  icon in the account settings panel takes the user back to the project explorer and shows a list of all the projects.
- User icon  allows the user to either return to TISON's home page via 'Main' or sign out from TISON via 'Sign Out'.

1.1.5 Canvas Toolbar (Right)

In **Figure 1.1.1 (E)**,

- 'Zoom In' button  zooms in on the canvas and repositions the network to the center of the screen.
- 'Zoom Out' button  zooms out of the canvas and repositions the network to the center of the screen.
- 'Reset to Default' button  resets the zoom level/canvas size to default.
- 'Perform Network Analysis' button  allows the user to perform network analysis using Deterministic Analysis (DA), Probabilistic Analysis (PA), and ODE Analysis.
- 'Analysis Progress and Status'  button allows the user to check the status of analyses running in the network.
- 'View Network and Results' button  allows the user to switch the view between the network and network results (visual plots including 'Attractor Landscape', 'Cell Fate Landscape', 'Potential Energy Landscape', 'Probability Landscape', and 'ODE Landscape').
- 'Download Results' button  allows the user to download network analysis results.
- 'Upload Case Study' button  allows the user to upload template networks from the database.
- 'Import Network' button  allows the user to upload a network file.

- 'Export Network' button  downloads a (.zip) folder containing input files provided by the user and output files produced after analysis. Folder may include network file, parameters file, custom states file, fixed nodes file, cell fate logic file, and the result files.

1.1.6 Editor Logo

- In **Figure 1.1.1 (F)**, the editor logo and text 

at the bottom right corner of the canvas display the editor being used.

1.1.7 Canvas Toolbar (Bottom)

- In **Figure 1.1.1 (G**), the toolbar at the bottom of the canvas allows the user to switch between different editors in TISON.

1. Networks button  navigates to "Networks Editor."
2. Therapeutics button  navigates to "Therapeutics Editor."
3. Environments button  navigates to "Environments Editor."
4. Cell Circuits button  navigates to "Cell Circuits Editor."
5. Cell Lines button  navigates to "Cell Lines Editor."
6. Organoids button  navigates to "Organoids Editor."
7. Simulations button  navigates to "Simulations Editor."
8. Analytics button  navigates to "Analytics Editor."

1.1.8 Project Panel

In **Figure 1.1.1 (H)**,

- 'TISON Home' button  on the bottom left corner of the canvas takes the user back to TISON's home page.
- 'Project Information' button 

displays the project name at the bottom left corner of the canvas and takes the user back to the 'Project Explorer' page.

1.2 Features List

A guide to the functionality of each GUI button and the parameters required from the user to build a basic network is provided in the following sections.

1.2.1 'Create Network' Button

The 'Create Network' button in the left canvas toolbar (highlighted in **Figure 1.2.1.1**) allows the user to create a new weight-based or rules-based network (detailed below).

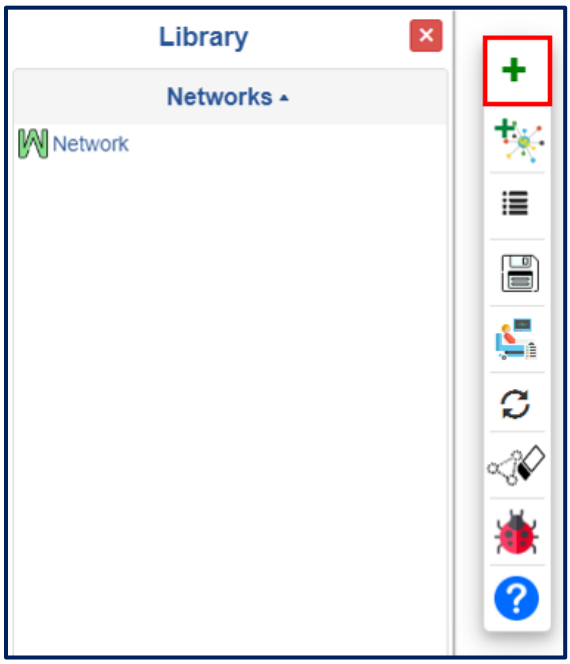


Figure 1.2.1.1 - 'Create Network' button in NE.

Once the user clicks on this button, the following window will appear (**Figure 1.2.1.2**).

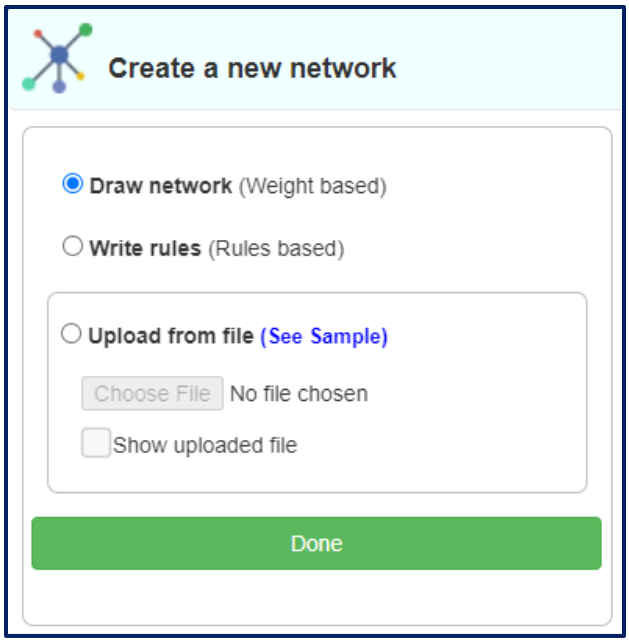


Figure 1.2.1.2 - Window for selecting the type of network.

Networks can either be created manually or uploaded in the form of an excel file (format .csv). Manual creation is possible through the 'Draw network' option for a weight-based network and the 'Write rules' option for a rules-based network. Selecting 'Upload from file' will allow the user to upload a network file from their hard drive (For visual reference, see Section 1.4, Table 1.4.1 – Video 3).

Weight-based Networks:

i. Manual Construction of Network

Selecting the 'Draw network' option will open the main GUI (**Figure 1.2.1.3**); the user must choose their biomolecule of interest, then click on the canvas to begin constructing their weight-based network.

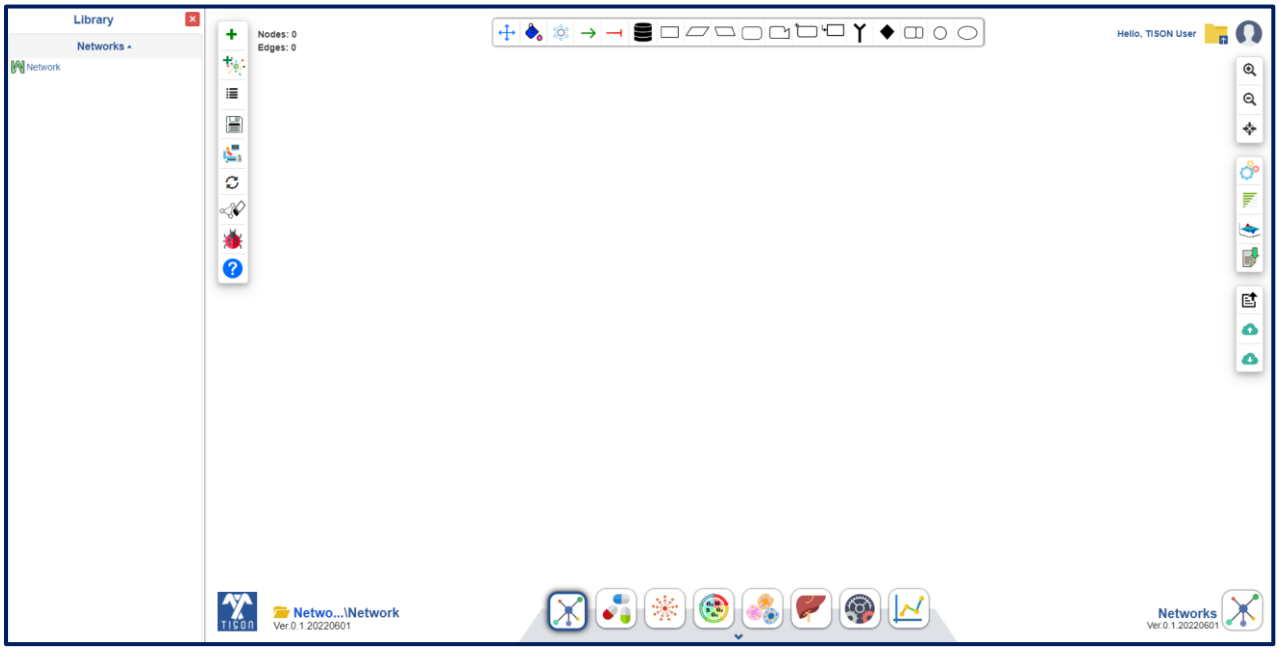
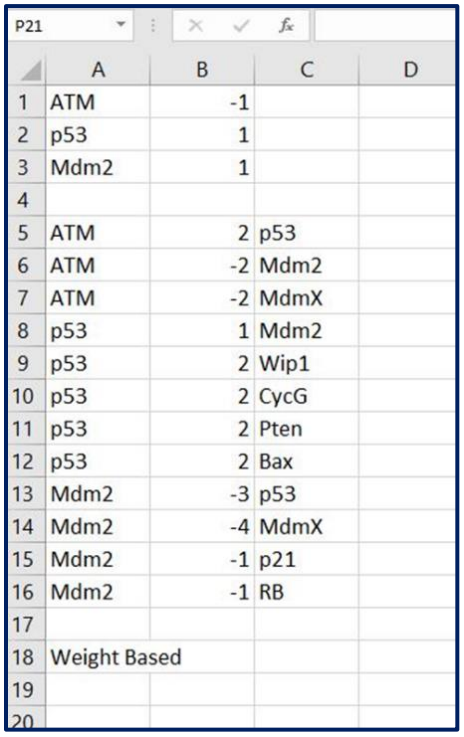


Figure 1.2.1.3 - Weight-based editor window that appears upon selecting the 'Draw Network' option.

ii. Uploading a File

A comma-separated values file (format .csv) must be created; the file should include the node names and their associated basal values (this indicates the minimum expression of each node). Following initialization, a row should be left empty, and then the node interactions should be stated along with their interaction weights. The comma-separated file's first, second, and third columns must contain the source nodes, interaction weights, and target nodes, respectively (**Figure 1.2.1.4**).



	A	B	C	D
1	ATM	-1		
2	p53	1		
3	Mdm2	1		
4				
5	ATM	2	p53	
6	ATM	-2	Mdm2	
7	ATM	-2	MdmX	
8	p53	1	Mdm2	
9	p53	2	Wip1	
10	p53	2	CycG	
11	p53	2	Pten	
12	p53	2	Bax	
13	Mdm2	-3	p53	
14	Mdm2	-4	MdmX	
15	Mdm2	-1	p21	
16	Mdm2	-1	RB	
17				
18	Weight Based			
19				
20				

Figure 1.2.1.4 - Weight-based network file format.

Selecting the 'Show uploaded file' option (**Figure 1.2.1.2**) will display the network file once it gets uploaded (**Figure 1.2.1.5**).

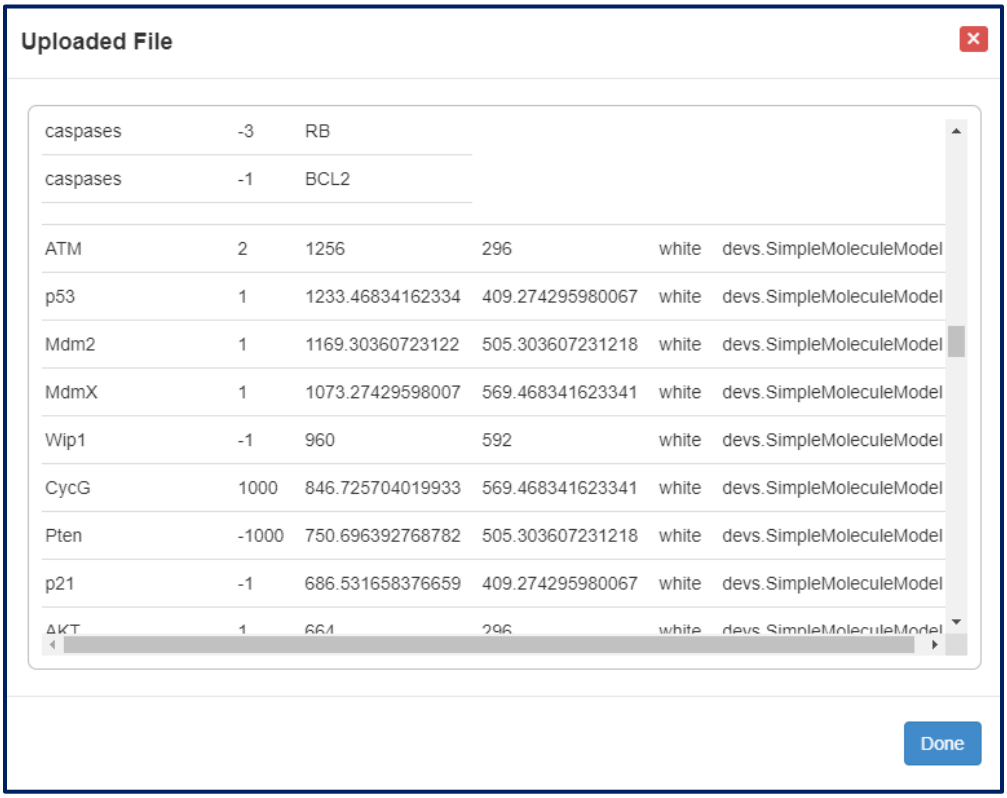


Figure 1.2.1.5 - The window displaying the contents of a weight-based network: *The content uploaded as a (.csv) file appears upon selecting the 'Show uploaded file' option.*

Rules-Based Networks:

i. Manual Construction of Network

The 'Rules Editor' window (**Figure 1.2.1.6**) appears when the user selects the 'Write rules' option highlighted in **Figure 1.2.1.2**; this editor includes a word processor and instructions for writing rules in JavaScript syntax (For visual reference, see Section 1.4 – **Table 1.4.1**)

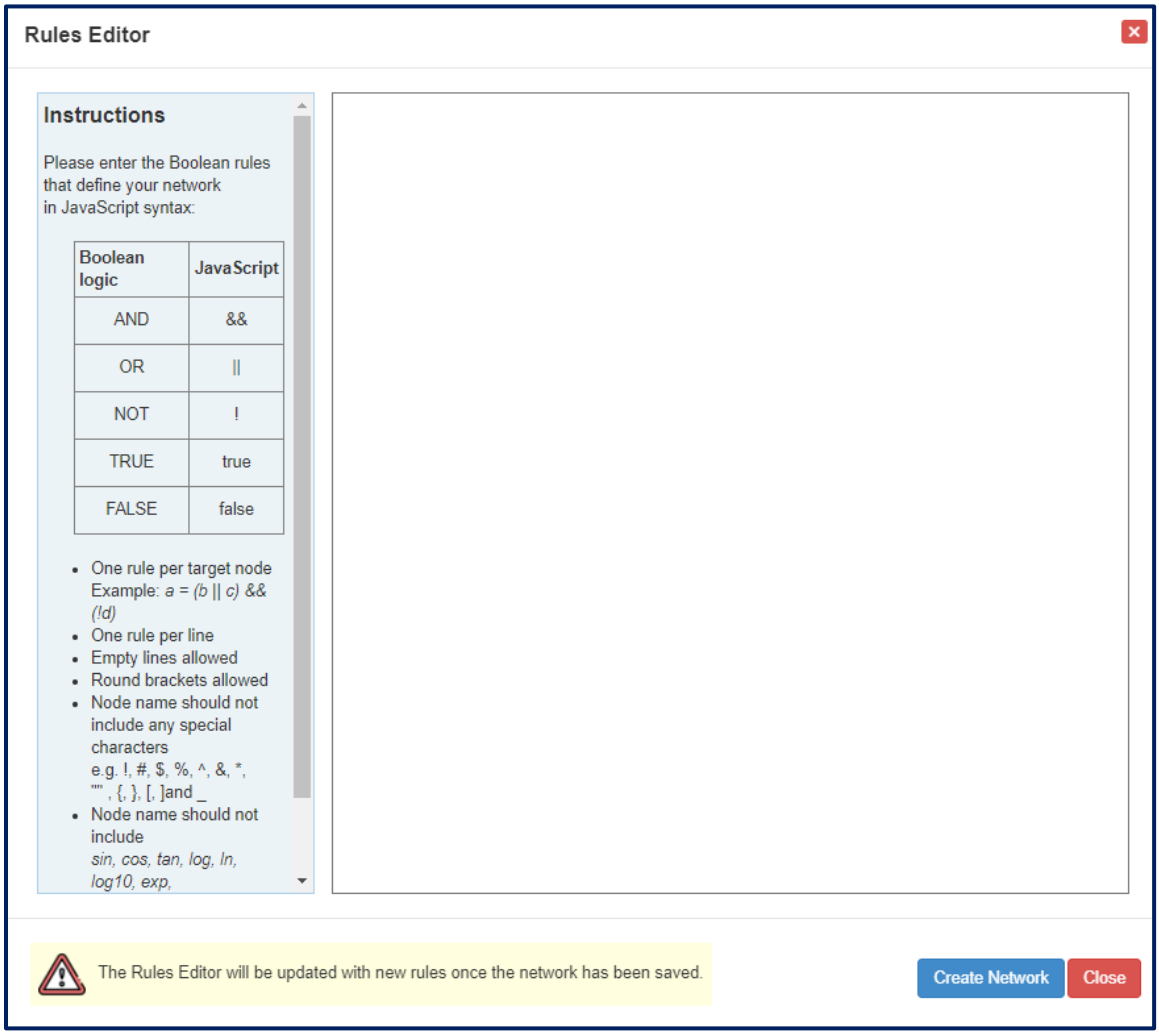


Figure 1.2.1.6 - Rules-based editor window that appears upon selecting the 'Write rules' option.

Each target node can be assigned its interaction logic by a '=' symbol. Moreover, Boolean operators like AND, OR, and NOT represented as '&&', '||' and '!' respectively, can be used to describe the simple dynamics of activation and inhibition between nodes.

If the regulators form a complex and regulate a target node, the interaction should be represented by an 'AND operator', if the regulators independently regulate the target node, the interaction should be represented by an 'OR operator', and if the regulator negatively regulates the target node and inhibits its activity, the interaction should be represented by a 'NOT operator'.

ii. Uploaded from File

A comma-separated file (format .csv) must be created; each row contains a target node followed by its regulators/source nodes and the rules defining the target node's activity (**Figure 1.2.1.7**). The convention to be followed for creating interaction logic is shown in the 'Rules editor' window (**Figure 1.2.1.6**) (For visual reference, see Section 1.4, Table 1.4.1 – Video 1).

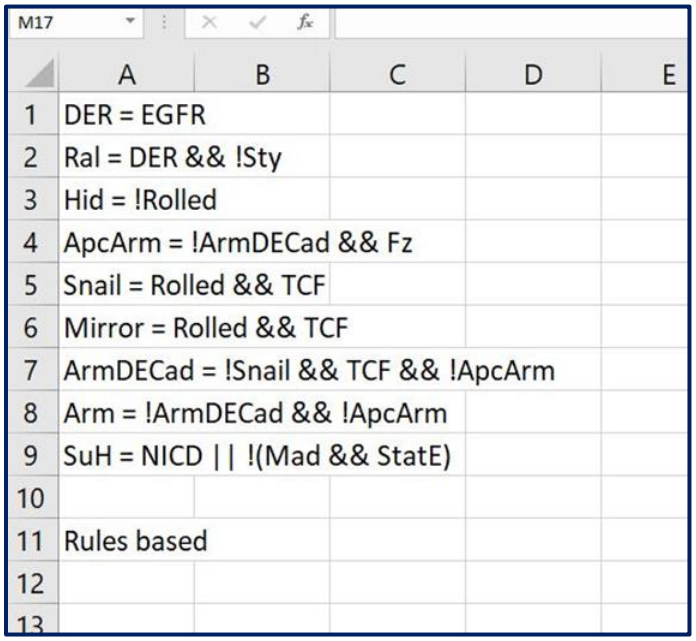


Figure 1.2.1.7 *-* ***Rules-based network file format:*** *Node name followed by '=' and interaction logic.*

Selecting the 'Show uploaded file' option (**Figure 1.2.1.2**) will display the network file once it gets uploaded (**Figure 1.2.1.8**).

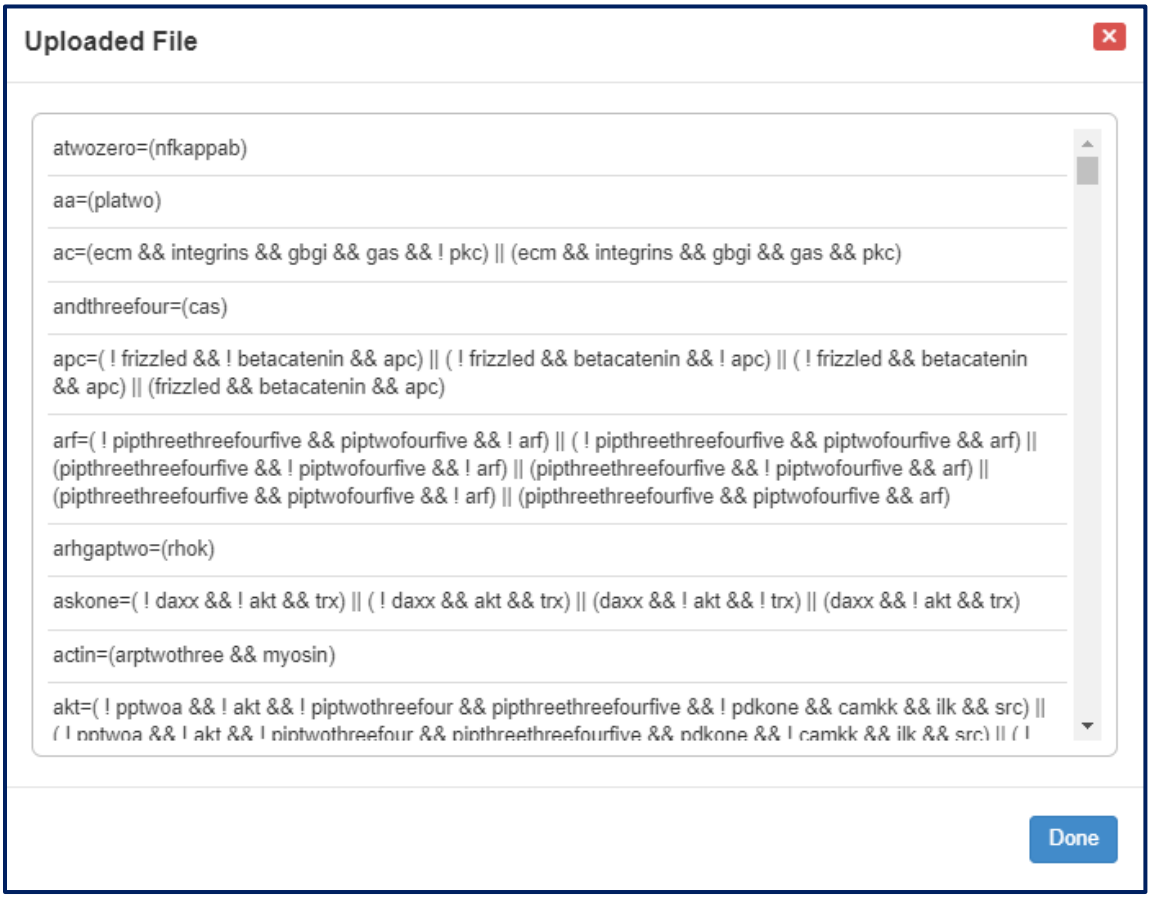


Figure 1.2.1.8 - The window displaying the contents of a rules-based network: *The content uploaded as a (.csv) file appears upon selecting the 'Show uploaded file' option.*

If the user switches to another network or editor while editing an existing network, the following window will appear to ensure that the user saves any unsaved changes (**Figure 1.2.1.9**).

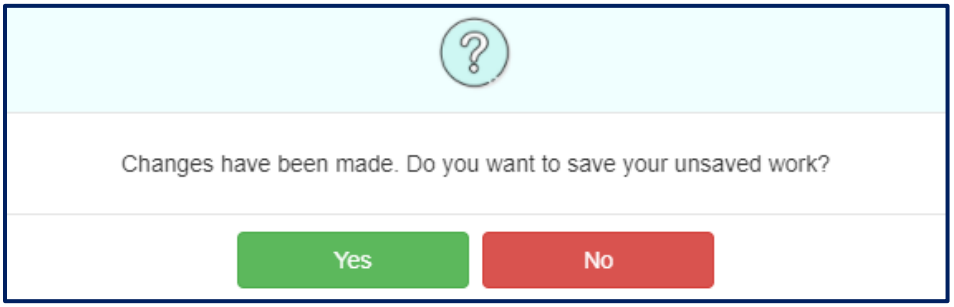


Figure 1.2.1.9 - Prompt for unsaved changes.

If the user does not have anything drawn on the canvas or the network changes are already saved, it will automatically create a new canvas without any prompt.

1.2.2 'Automated Network Construction' Button

The 'Automated Network Construction' button (highlighted in **Figure 1.2.2.1**) allows the users to construct their network automatically.

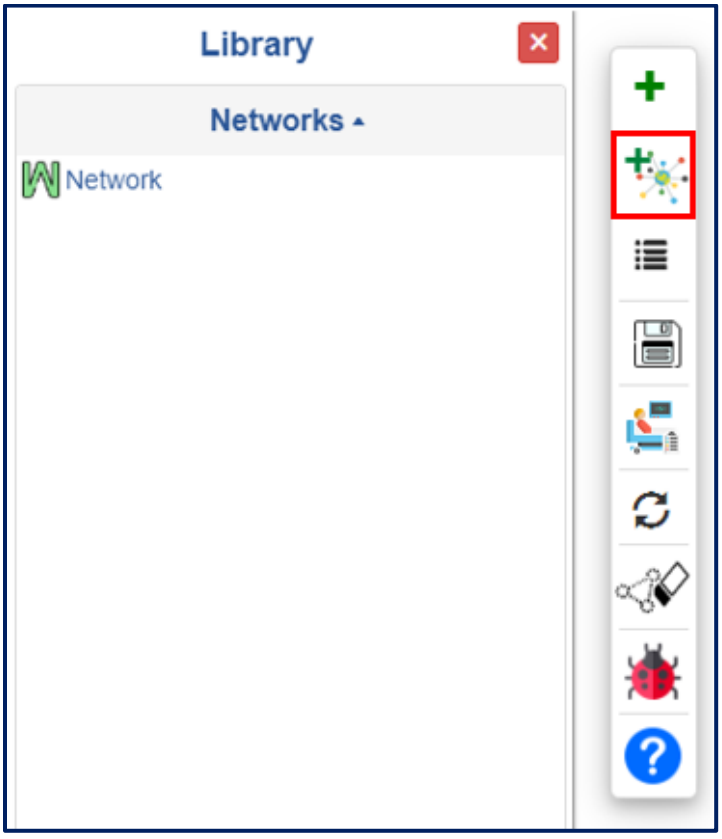


Figure 1.2.2.1 - 'Automated Network Construction' button in NE.

Once the user selects the option shown above, a window for 'Automated Network Construction' settings appears (**Figure 1.2.2.2**).

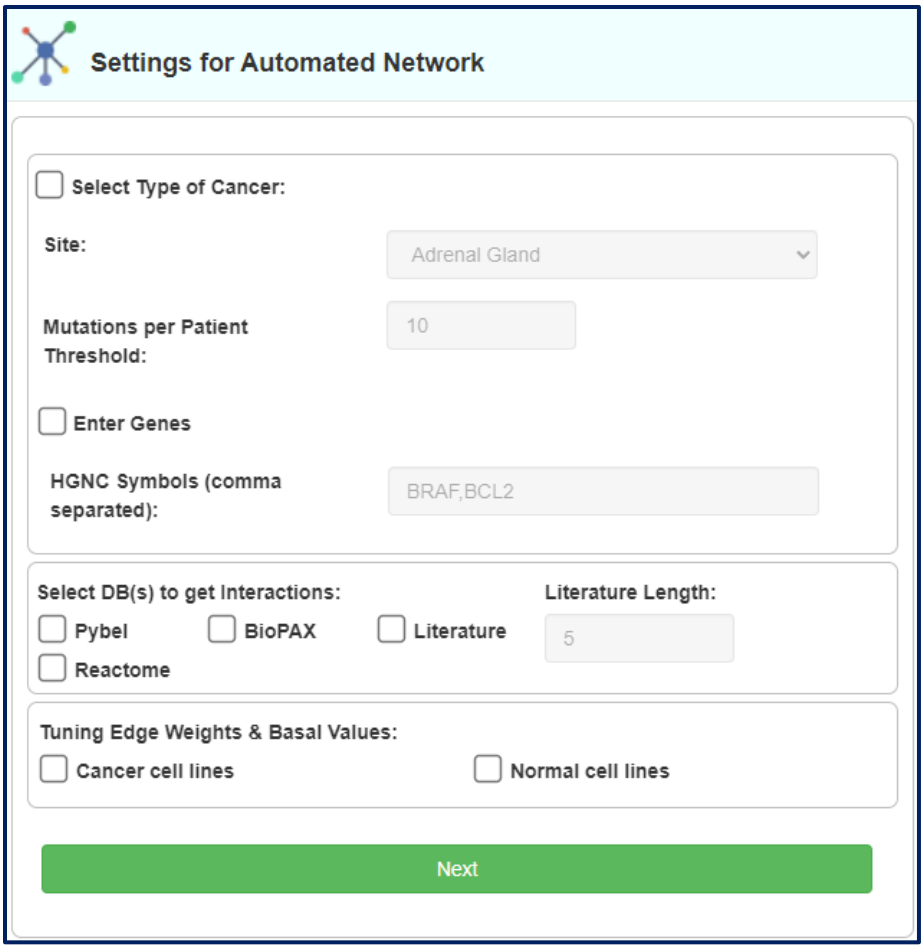


Figure 1.2.2.2 - Settings for 'Automated Network Construction'.

In the first panel, the user may check either one or both boxes labeled 'Select Type of Cancer' and 'Enter Genes', respectively. A drop-down list of various sites of cancers will open up on choosing 'Select Type of Cancer'. The user must select the site of cancer and specify an appropriate threshold value for 'Mutations per Patient Threshold' (default value of 10). Moreover, if the 'Enter Genes' option is selected, the names of the relevant genes must be entered by the user in HGNC format (**Figure 1.2.2.3**).

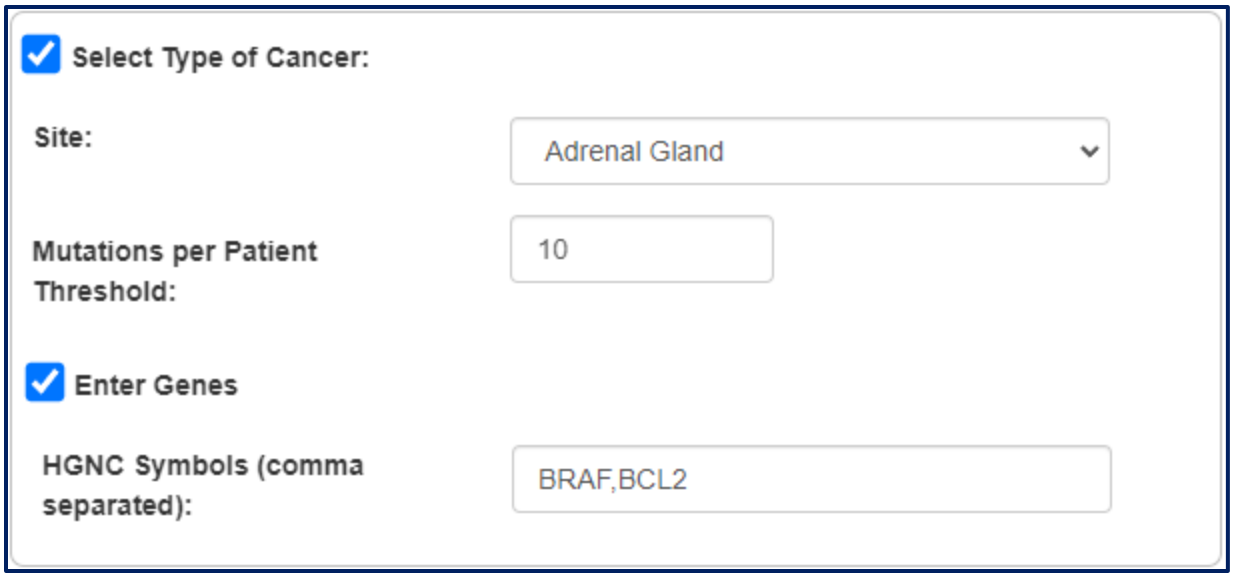


Figure 1.2.2.3 - First panel of 'Automated Network Construction'.

In the second panel, the user may select one or many databases, for example: Pybel, Reactome, and BioPAX to obtain their network interactions. The user may also select literature and specify the literature length, which refers to the number of literature studies that will be referred to in order to obtain the interactions (**Figure 1.2.2.4**).

Figure 1.2.2.4 - Second panel of 'Automated Network Construction'.

In the third panel, the user can choose whether they want to tune the edge weights and obtain the basal values using cancer cell lines, normal cell lines, or both (**Figure 1.2.2.5**).

Figure 1.2.2.5 - Third panel of 'Automated Network Construction'.

1.2.3 'Show Networks Library' Button

The 'Show Networks Library' button (highlighted in **Figure 1.2.3.1**) has a toggling action that provides the option to display or hide the library panel (i.e., if the library is open, it will close it and vice versa).

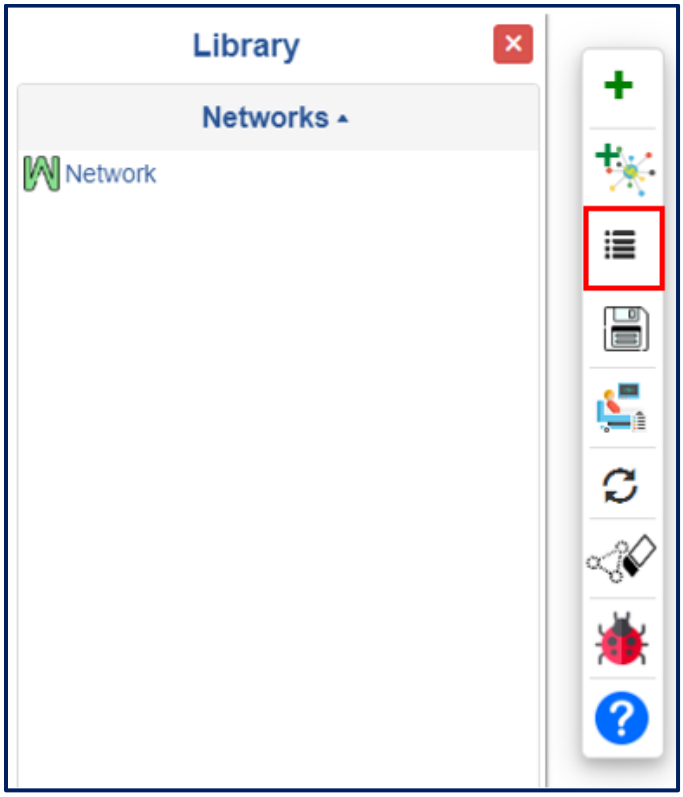


Figure 1.2.3.1 - 'Show Networks Library' button in NE.

The library panel allows users to view a previously saved network from the database. Any of the user's pre-constructed and saved networks can be loaded from the library section by double-clicking on their respective names. The selected network gets highlighted in grey to help the user locate the network in use. Moreover, the options to 'edit network', 'edit properties', 'duplicate network' and 'delete network' are displayed for *weight-based networks*, whereas, an additional option to 'edit network' is available for *rules-based networks* (**Figure 1.2.3.3**). Note that, when the user clicks on the 'edit network' option they are directed to the 'Rules Editor' window (**Figure 1.2.7.2**) where they can alter the rules manually.

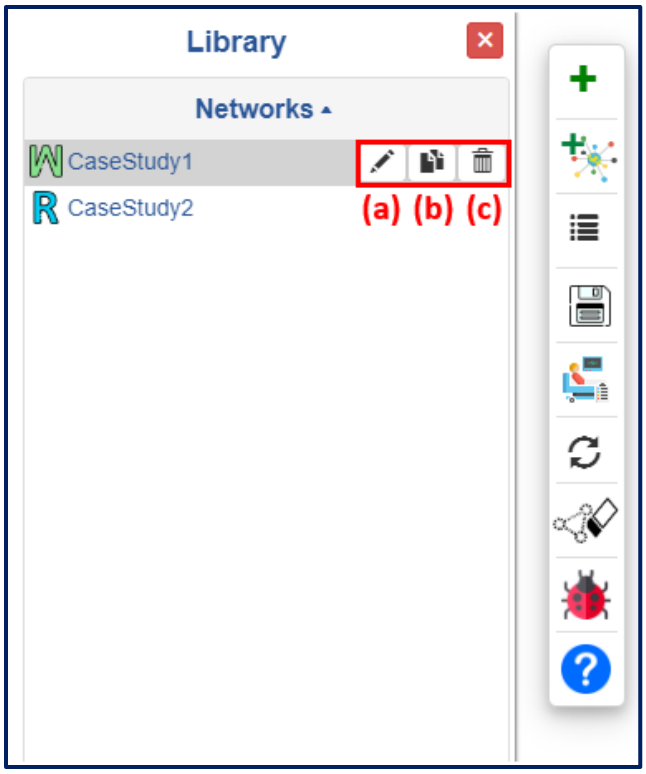


Figure 1.2.3.2 - Options to modify a weight-based network: (a) 'Edit Properties', (b) 'Duplicate Network', and (c) 'Delete Network'.

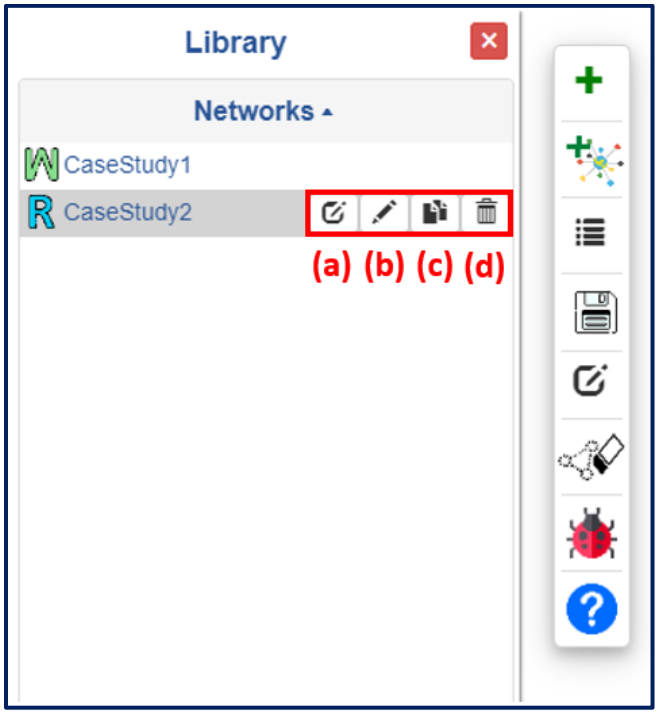


Figure 1.2.3.3 - Options to modify a rules-based network. (a) 'Edit Network', (b) 'Edit Properties', (c) 'Duplicate Network', and (d) 'Delete Network'.

1.2.4 'Save Network' Button

After drawing or uploading a network, the user may save the network in TISON's database through the 'Save Network' button highlighted in **Figure 1.2.4.1.**

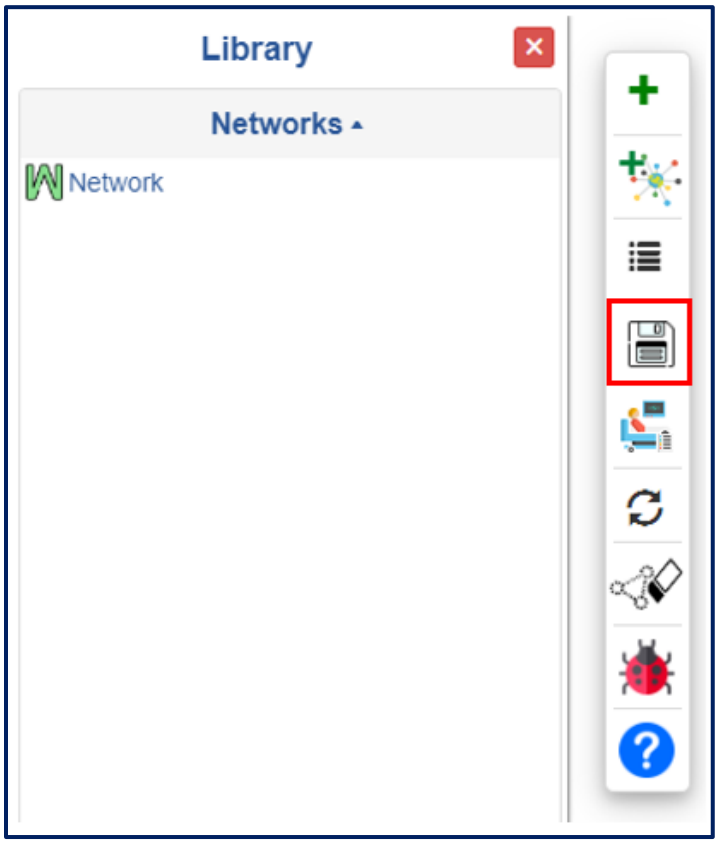


Figure 1.2.4.1 - 'Save Network' button in NE.

If the network in use is not saved in the database, the user will be asked to enter the name and description of the network (**Figure 1.2.4.2**). The network will only be saved if its name is not greater than 50 characters and has not been used for a previously saved network; moreover, the description of the network cannot exceed 250 characters.

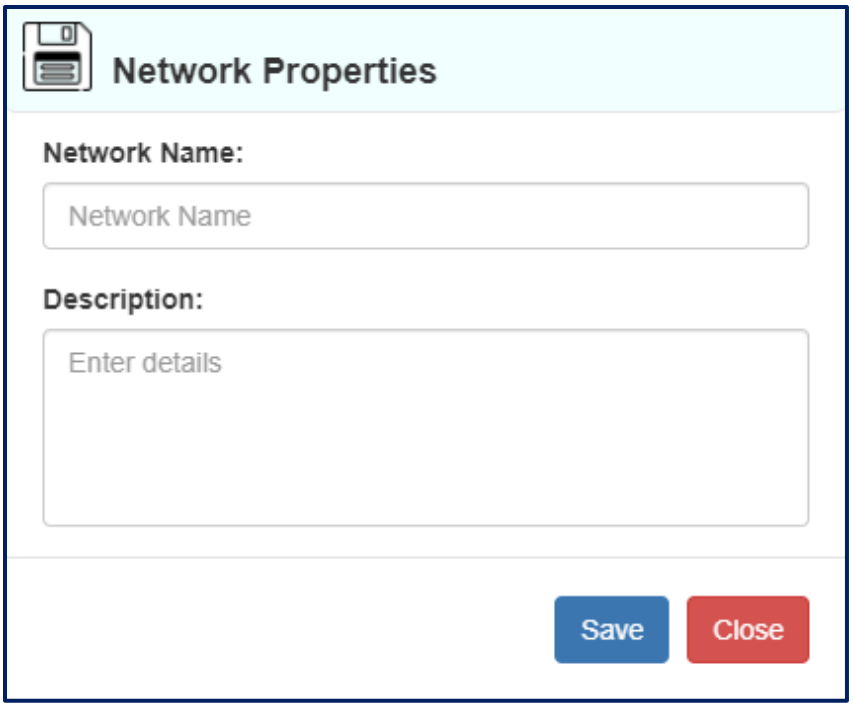


Figure 1.2.4.2 - Save network window for a newly created network.

If the user tries to switch to another network or editor, the prompt shown in **Figure 1.2.4.3** will appear to ensure that any unsaved changes are saved in the database.

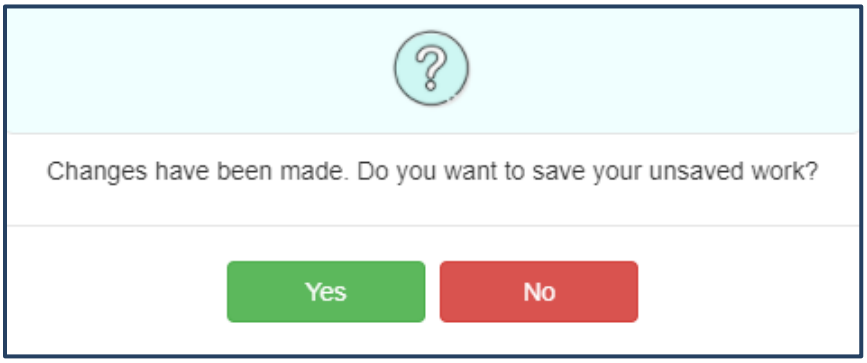


Figure 1.2.4.3 - Save network prompt.

1.2.5 'Personalize Weight Based Networks' Button

A *weight-based network* can also be personalized using the 'Personalize Weight Based Networks' button in NE (**Figure 1.2.5.1**).

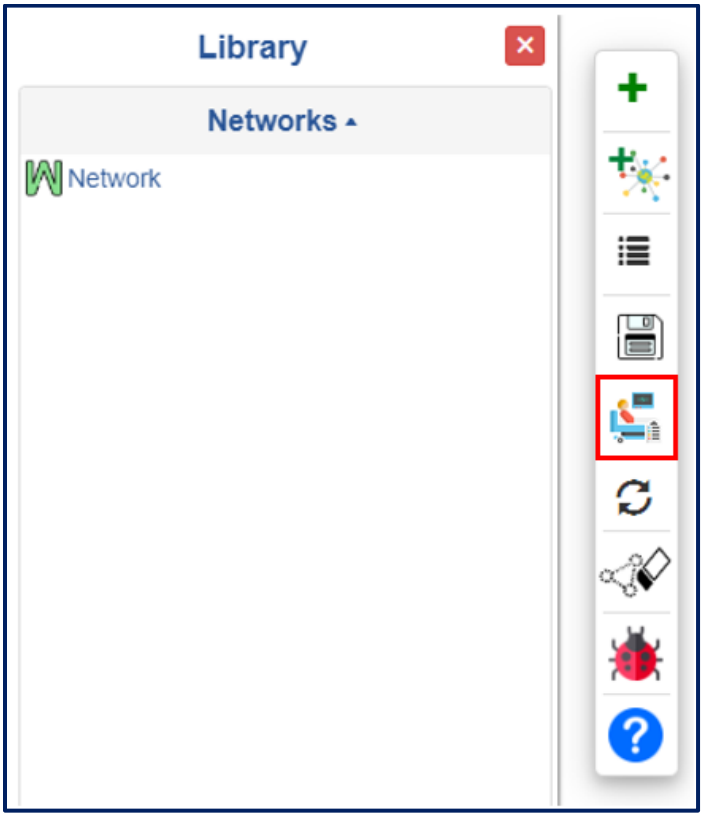
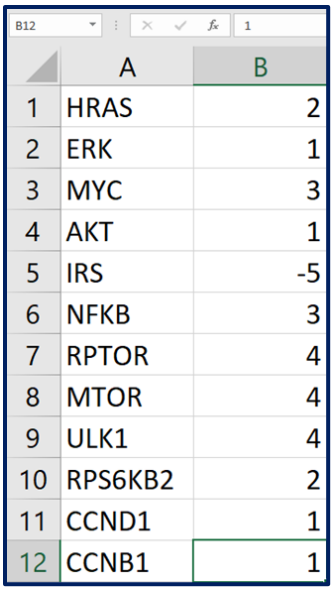


Figure 1.2.5.1 - 'Personalize Weight Based Networks' button.

This option allows the user to customize the network by employing patient-specific gene expression data such as RNA-seq or microarray expression data for calculating and updating network basal values. The user must upload an expression file (format .csv) that contains raw or normalized RNA-seq or raw or normalized microarray expression values extracted from a database such as The Cancer Genome Atlas (TCGA) or Gene Expression Omnibus (GEO) (**Figure 1.2.5.2**). The gene expression file can be uploaded by clicking the 'Choose File' option displayed in **Figure 1.2.5.3**.



	A	B
1	HRAS	2
2	ERK	1
3	MYC	3
4	AKT	1
5	IRS	-5
6	NFKB	3
7	RPTOR	4
8	MTOR	4
9	ULK1	4
10	RPS6KB2	2
11	CCND1	1
12	CCNB1	1

Figure 1.2.5.2 - Example expression file for basal value calculation.

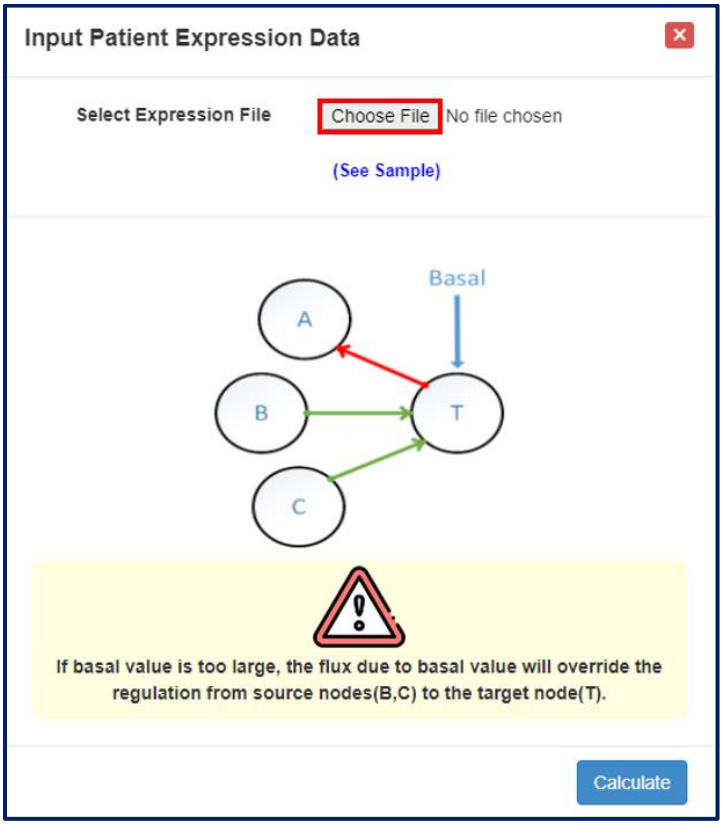


Figure 1.2.5.3 - Network personalization window for basal value calculation.

The details of the backend computations required for basal value calculation are given below.

i. Basal Value Calculation: The b_i is the basal value of the node, x_j is the experimental value, and w_{ij} represents the network's adjacency matrix.

$$x_i(t+1) = \begin{cases} 1 & if \sum_j \left(w_{ij}x_j(t)\right) + b_i > 0 \\ 0 & else\ if \sum_j \left(w_{ij}x_j(t)\right) + b_i < 0 \\ x_i & else\ if \sum_j \left(w_{ij}x_j(t)\right) + b_i = 0 \end{cases}$$

$$b_i = x_i(t+1) - \sum_j \left(w_{ij}x_j(t)\right)$$

This feature allows the users to employ expression data to calculate the basal expression of each node, thus, personalizing the resultant network with real patient data; this is exemplified in **Figure 1.2.5.4**.

$$b_i = 3.65 - \left((2.22 \times 0.65) + (0.56 \times 0.91) + (7.43 \times -0.81) + (4.61 \times 4.61)\right) = -13.5364$$

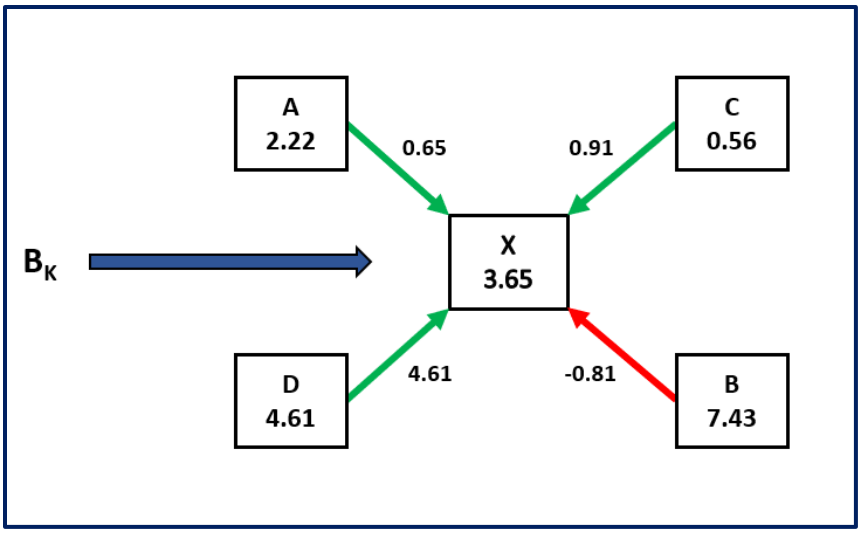


Figure 1.2.5.4 - Example of basal value estimation.

The output basal value results, computed after calculations, can be viewed in **Figure 1.2.5.5**. On selecting 'Update Network' button, the basal values of the network will be updated on the canvas as well.

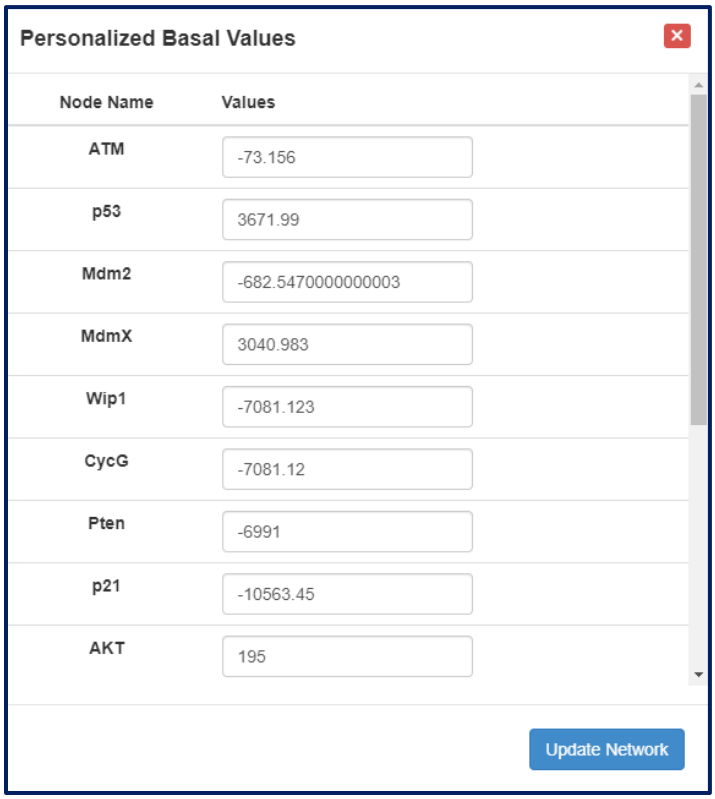


Figure 1.2.5.5 - Example of TISON's basal value estimation results window

ii. Expression Value Normalization (*recommended*): Please note that in the case of personalizing a network with expression data, it is recommended that users should not use the raw expression data. Instead, users must normalize the expression values for all genes between 0 and 1, e.g., for a particular gene, divide the expression values across all patients by the maximum expression value for that gene. Next, the basal values will be computed with respect to normalized gene expressions**.** The raw RNA-seq gene expression data and its normalized values are displayed in **Figure 1.2.5.6** and **Figure 1.2.5.7** respectively.

Network_Genes	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Max
ATM	1.510623	2.518765	0.908262	2.058787	1.316602	1.522188	1.622792	1.86019032	2.518765
Tp53	5.022743	7.702509	42.84711	32.09936	18.91071	59.49285	34.63199	7.22958871	59.49285
MDM2	4.552624	5.701428	5.859361	8.787163	4.90684	5.118764	3.646182	4.24858655	8.787163
CDKN1A	29.53484	60.16466	47.50169	60.80388	89.00106	44.90698	116.884	28.0266711	116.884

Figure 1.2.5.6 - Raw expression file.

Network_Genes	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
ATM	0.599747	1	0.360598	0.817379	0.522717	0.604339	0.644281	0.73853272
Tp53	0.084426	0.129469	0.720206	0.53955	0.317865	1	0.58212	0.12152029
MDM2	0.518099	0.648836	0.666809	1	0.55841	0.582528	0.414944	0.48349926
CDKN1A	0.252685	0.514738	0.4064	0.520207	0.761448	0.384201	1	0.23978203

Figure 1.2.5.7 - Normalized expression file.

1.2.6 'Weight-based to Rules-based Conversion' Button

To change the network from *weight-based to rules-based*, the user should select the button highlighted in **Figure 1.2.6.1**.

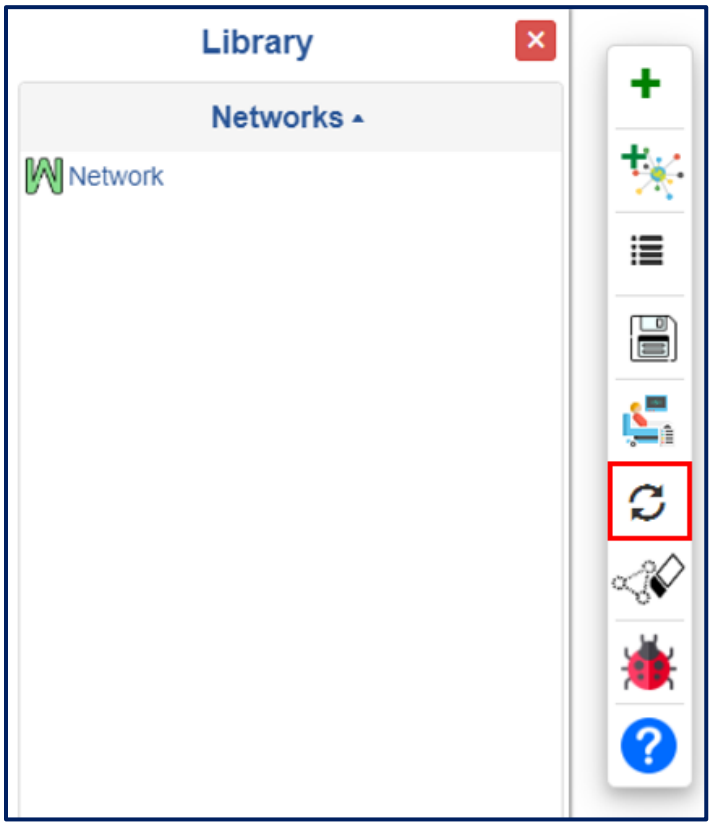


Figure 1.2.6.1 - 'Weight-based to Rules-based Conversion' button in NE.

1.2.7 'Edit Network' Button

For a *rules-based networks* only, the user can select the 'Edit Network' option highlighted in **Figure 1.2.7.1**; this opens the 'Rules Editor' modal that allows the user to edit the network rules directly (**Figure 1.2.7.2**). 'Edit Network' button is also available for *rules-based networks* in networks library section (**Figure 1.2.3.3**).

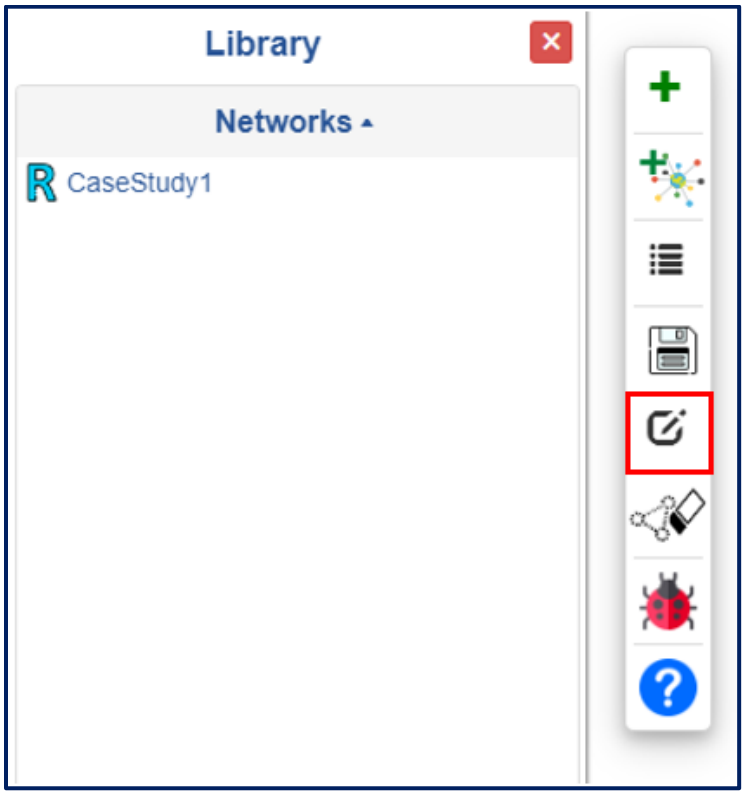


Figure 1.2.7.1 - 'Edit Network' button in NE.

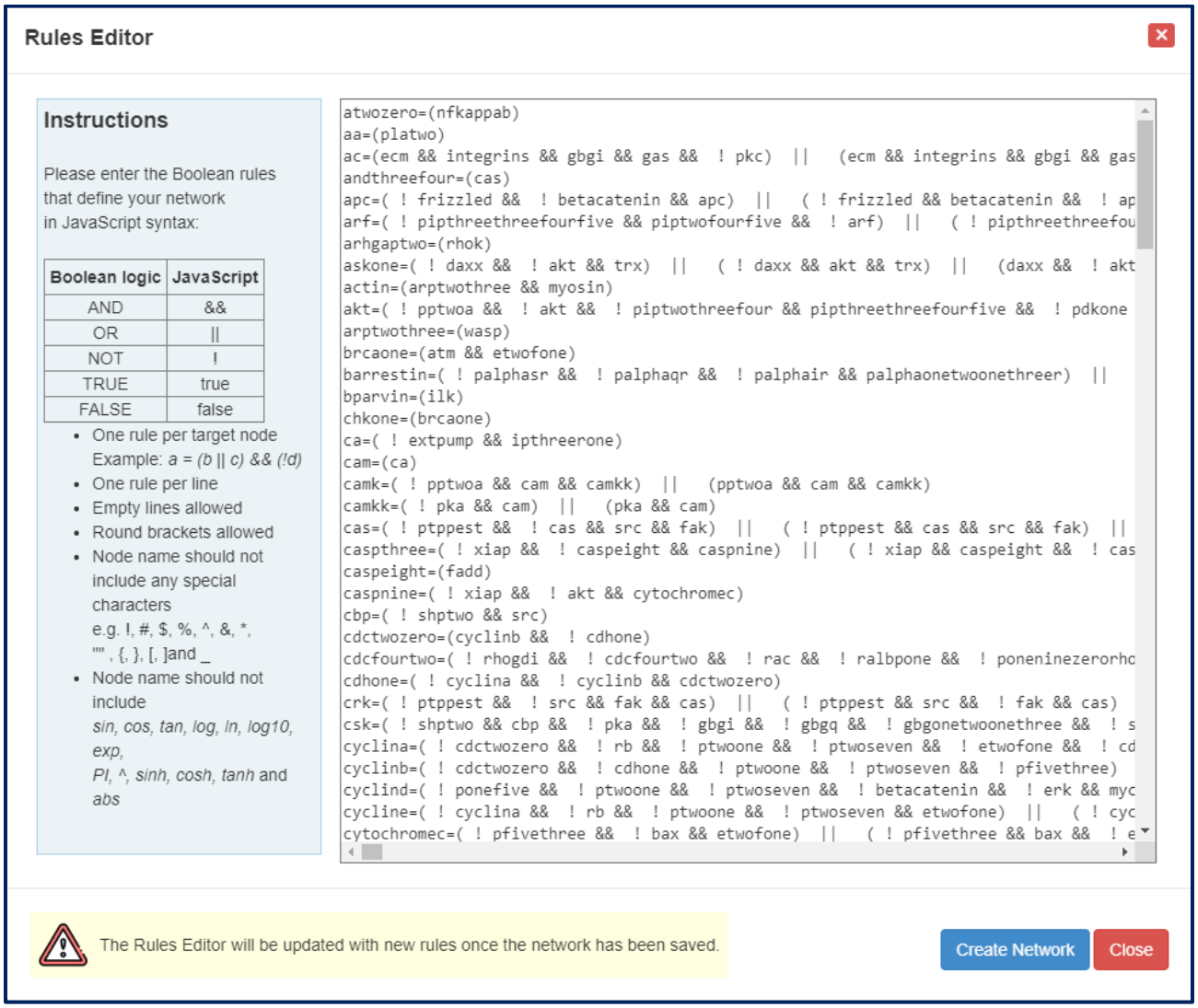


Figure 1.2.7.2 - 'Rules Editor' window.

1.2.8 'Clear All' Button

After creating a network, the user might want to clear the canvas to draw a new network; the 'Clear All' button, highlighted in **Figure 1.2.8.1** allows the user to do this.

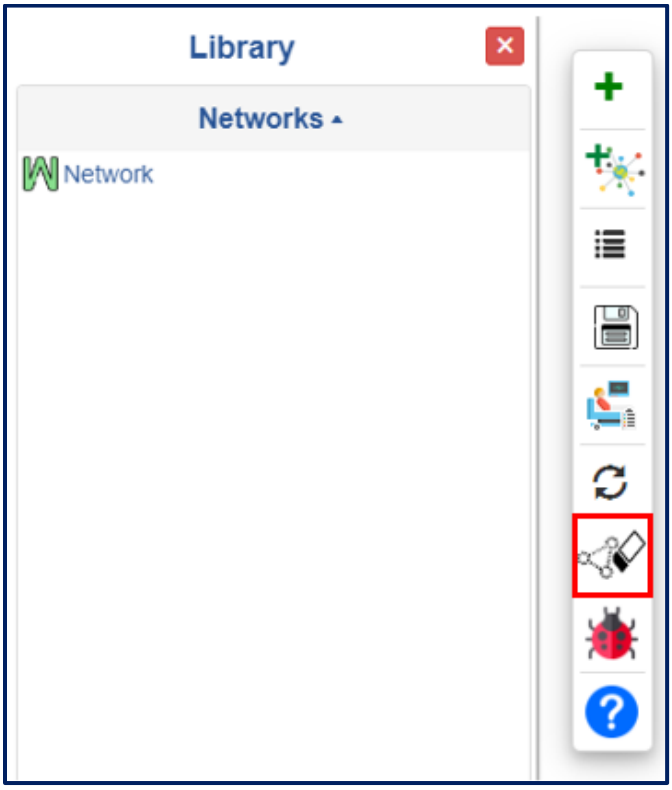


Figure 1.2.8.1 - 'Clear All' button in NE.

The prompt shown in **Figure 1.2.8.2** will appear when the 'Clear All' button is selected to ask the user for confirmation before the canvas is cleared.

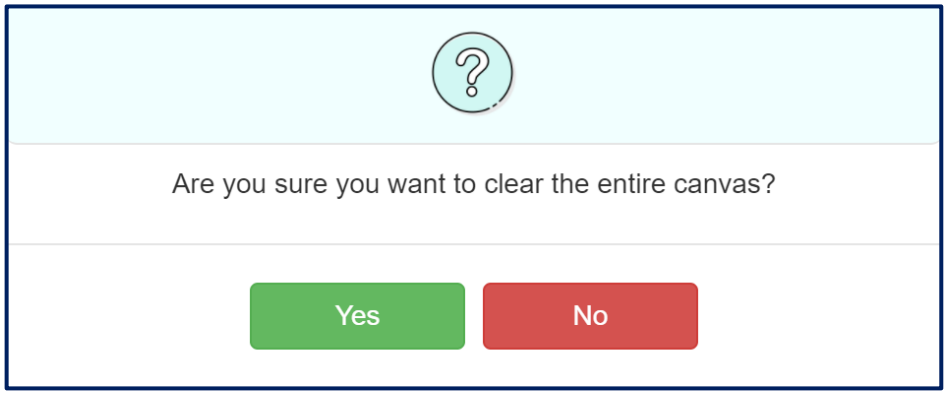


Figure 1.2.8.2 - Warning window that appears upon clicking the 'Clear All' button.

1.2.9 'Report a Bug' Button

Upon encountering any problem in the software, the user can report the bug by clicking on the 'Report a Bug' button highlighted in **Figure 1.2.9.1**.

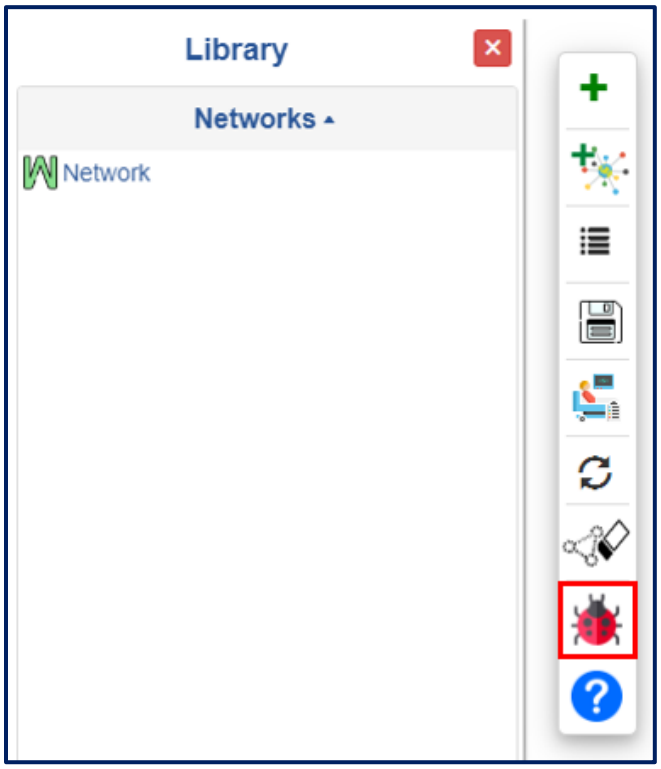


Figure 1.2.9.1 - 'Report a Bug' button in NE.

This button directs the user to a page on GitHub (Issues – BIRL/TISON – GitHub), where the user can report their issue (**Figure 1.2.9.2**).

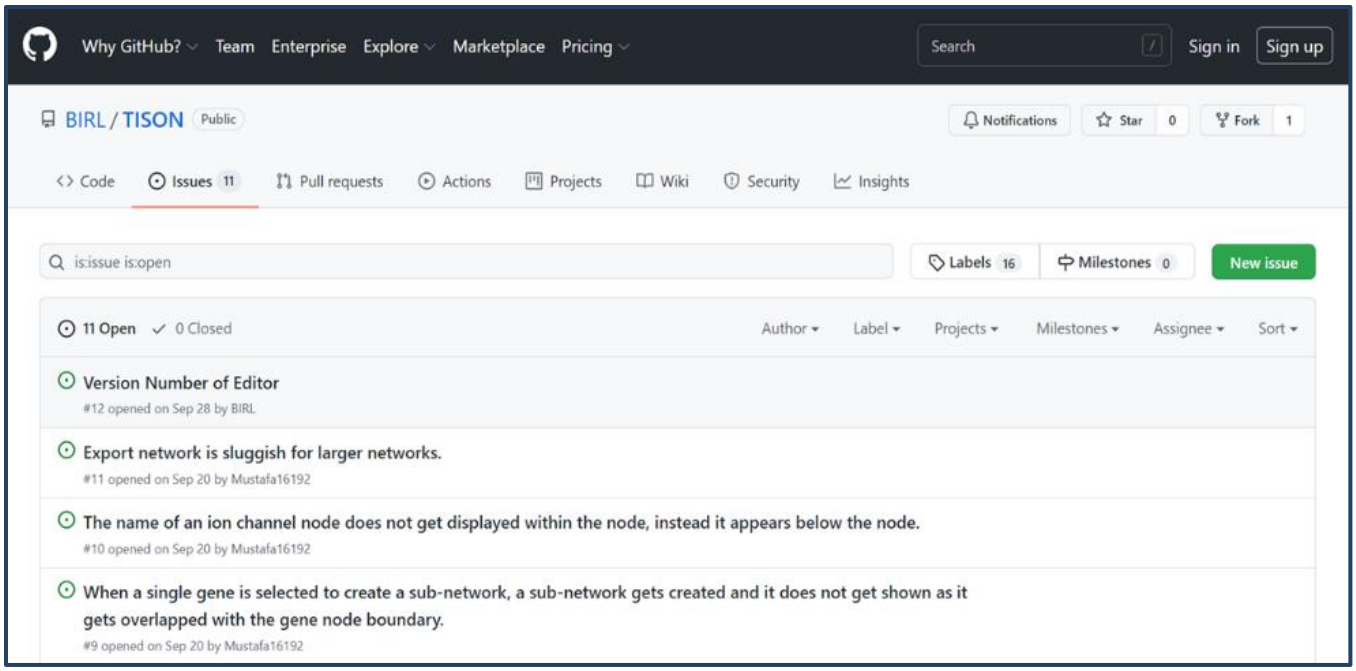


Figure 1.2.9.2 - GitHub page for reporting TISON's bugs.

1.2.10 'Help' Button

Clicking on the 'Help' button highlighted in **Figure 1.2.10.1** opens a user manual of the editor currently in use (https://tison.lums.edu.pk/Manuals/NetworksManual.pdf).

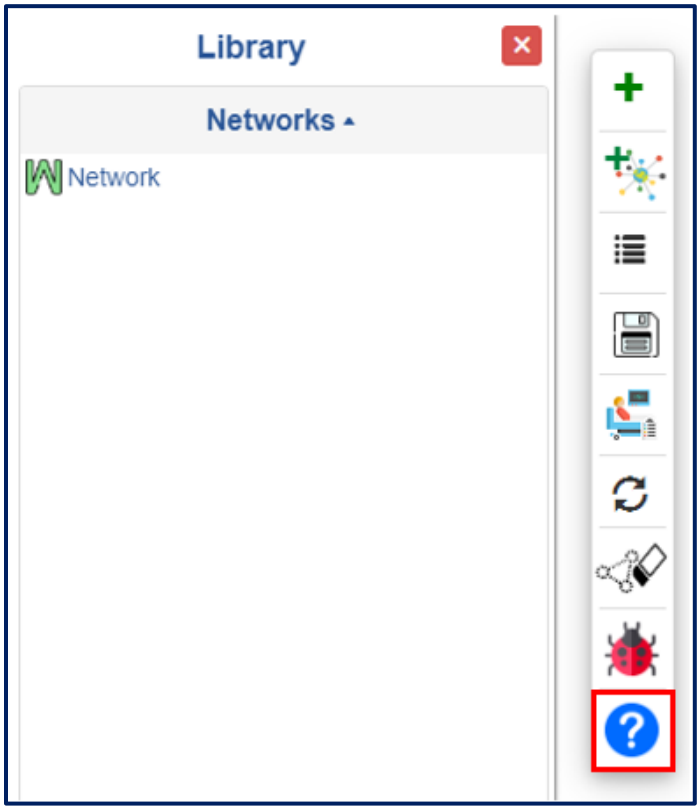


Figure 1.2.10.1 - 'Help' button in NE.

1.2.11 'Move Toolbar' Button

The 'Move Toolbar' button (**Figure 1.2.11.1**) helps to reposition the toolbar displayed at the top of the canvas (**Figure 1.1.1**) to any position on the canvas for the ease of visualizing the created network.

Figure 1.2.11.1 - 'Move Toolbar' button in NE.

1.2.12 'Change Background Color' Button

The canvas color can be changed according to the user's choice by selecting the 'Change Background Color' button shown in **Figure 1.2.12.1**.

Figure 1.2.12.1 - 'Change Background Color' button in NE.

A window will appear next to the button, allowing the user to select a specific color for the background (**Figure 1.2.12.2**).

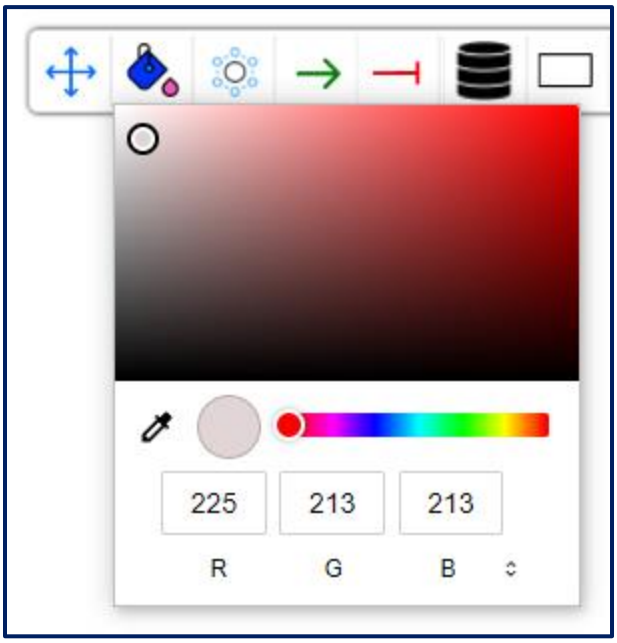


Figure 1.2.12.2 - 'Change Background Color' display.

1.2.13 'Change Layout' Button

The network layout can be changed using the button highlighted in **Figure 1.2.13.1**.

Figure 1.2.13.1 - 'Change Layout' button in NE.

The user can change the conformation of network upon clicking any one of the options provided: 'Grid', 'Circle', 'Concentric', 'Cose', and 'BFL' (Breadthfirst Layout) (**Figure 1.2.13.2**) for facilitating the visualization of network on the canvas.

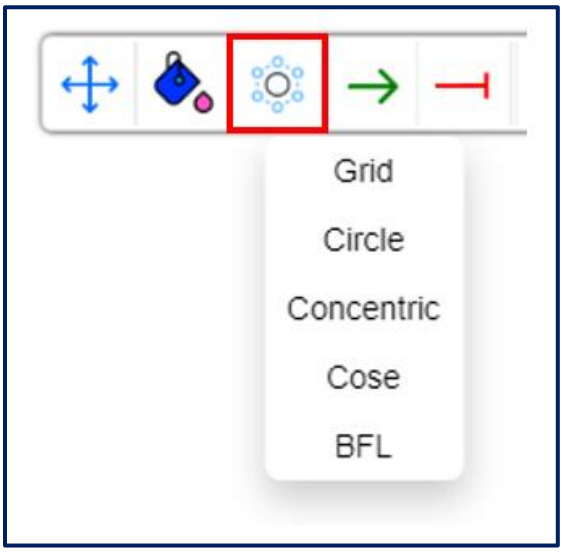


Figure 1.2.13.2 - Various network conformations.

1.2.14 'Link Type Selection' Buttons

A user can draw two types of links while creating a *weight-based network*: 'Activation Link' (**Figure 1.2.14.1**) and 'Inhibition Link' (**Figure 1.2.14.2**).

Figure 1.2.14.1 - 'Activation Link' button in NE's toolbar.

Figure 1.2.14.2 - 'Inhibition Link' button in NE's toolbar.

The links can only be made when at least two nodes of any type (gene, RNA, antisenseRNA, protein, truncated protein, cofactor, transcription factor, receptor, ligand, ion channel, ion, or molecule) have already been drawn on the canvas; the links will not function on an empty canvas.

The activation link implies that the source node is activating the target node; its use has been shown in **Figure 1.2.14.3**.

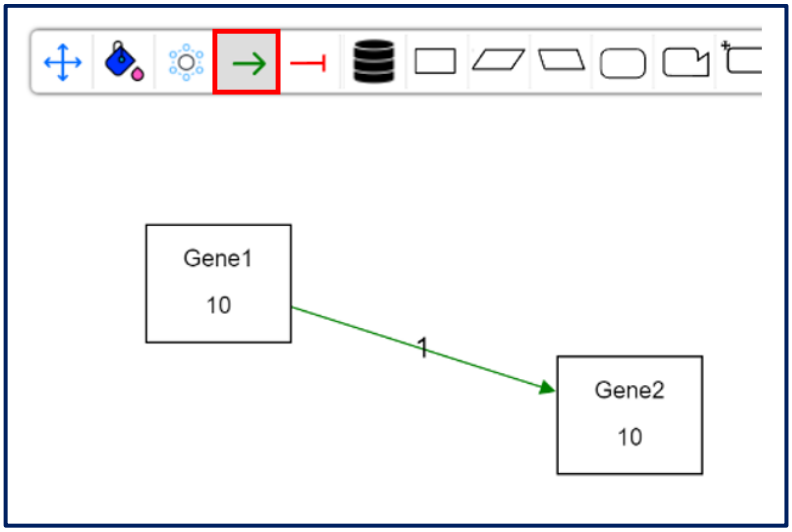


Figure 1.2.14.3 - Activation link in NE.

The inhibition link implies that the source node is inhibiting the target node; its use is shown in **Figure 1.2.14.4**.

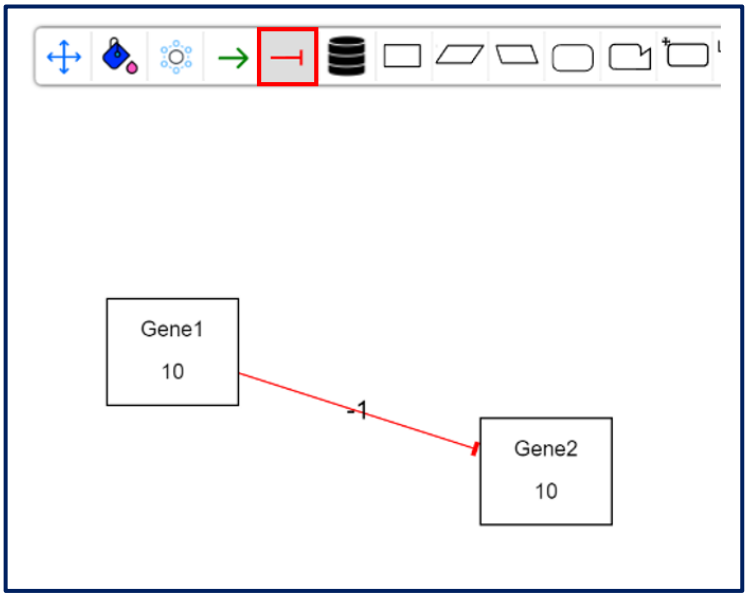


Figure 1.2.14.4 - Inhibition link in NE.

The user can define the interaction strength/weight (**Figure 1.2.14.5**) in a *weight-based network* by double-clicking on the activation/inhibition link. In the case of an inhibition link, the interaction weight should be less than 0, while in the case of an activation link, the interaction weight should be greater than 0.

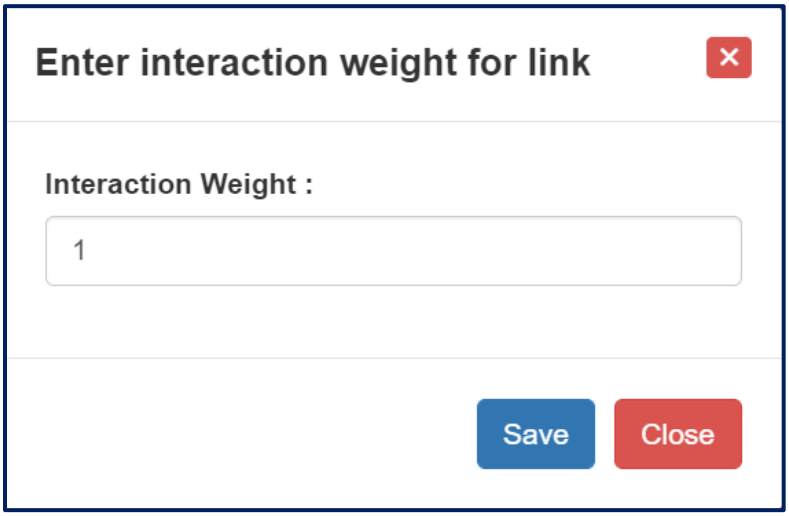


Figure 1.2.14.5 - Defining interaction weight on a link.

1.2.15 Loading Biomolecule from Integrated Databases

In a *weight-based network*, the user can load biomolecules and their expressions directly from cancer databases integrated into TISON by using the button highlighted in **Figure 1.2.15.1** (For visual reference, see Section 1.4, Table 1.4.1 – Video 2).

Figure 1.2.15.1 - 'Load Biomolecule from Integrated Databases' button in NE toolbar.

Upon clicking it, the following window will appear:

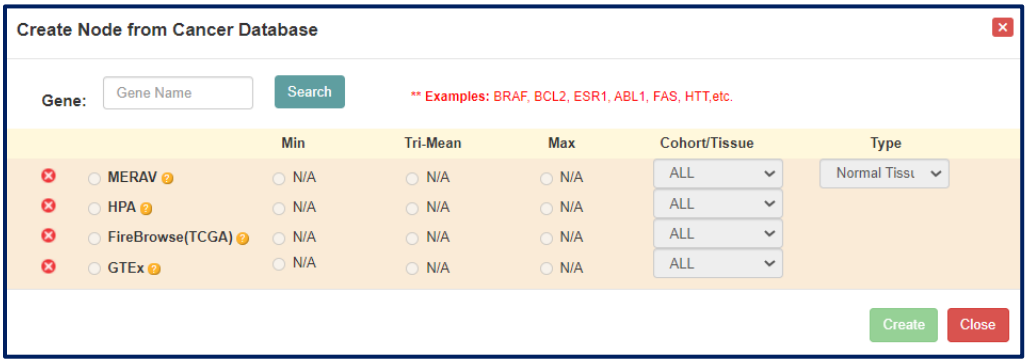


Figure 1.2.15.2 - 'Create Node from Cancer Database' window in NE.

The user must write the name of any gene (e.g. BRAF, BCL2, ESR1, etc.) in the search box given and click 'Search'. Following the search, the user must select from any one of the four integrated and available databases: MERAV (Metabolic gEne Rapid Visualizer), HPA (Human Protein Atlas), FireBrowse (TCGA) and GTEx (Genotype-Tissue Expression). The user must also select the gene's minimum, tri-mean, or maximum expression to create a node on the canvas. Furthermore, they may also choose a cohort/tissue and tissue type. Please note that 'Type' option is available for MERAV database only. After selecting the database type, expression value, and cohort/tissue type, the 'Create' button will work, creating a node on the canvas.

The following window displays the gene expression data found for BRCA1 gene:

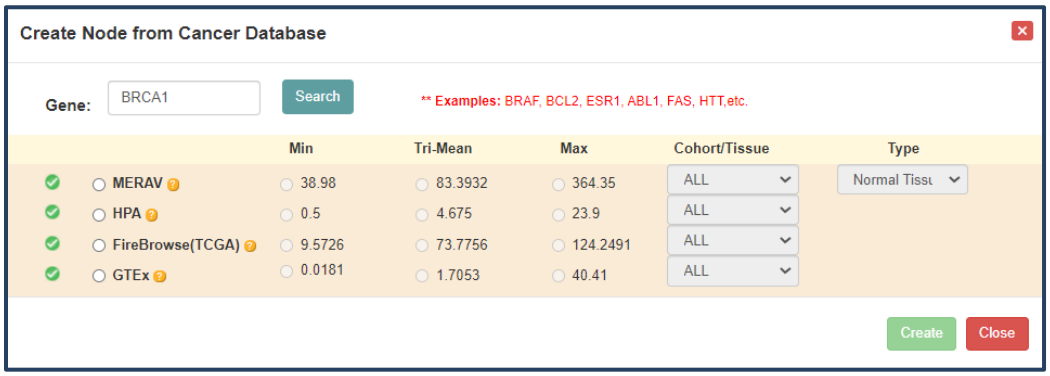


Figure 1.2.15.3 - Searching for a gene and selecting a database, expression, and cohort/tissue.

1.2.16 Drawing Biomolecules

While creating a *weight-based network*, the user can draw twelve different types of biomolecules: gene, RNA, antisense RNA, protein, truncated protein, cofactor, transcription factor, receptor, ligand, ion channel, ion, and molecule, respectively (For visual reference, see Section 1.4, Table 1.4.1 – Video 2).

Figure 1.2.16.1 - Buttons for biomolecules in NE's toolbar.

The user can draw any of the aforementioned biomolecules by selecting the desired biomolecule from the toolbar and then clicking on the canvas; the icons for the biomolecules must be clicked once, followed by a single click on the canvas. Each biomolecule has been labeled in **Figure 1.2.16.2**.

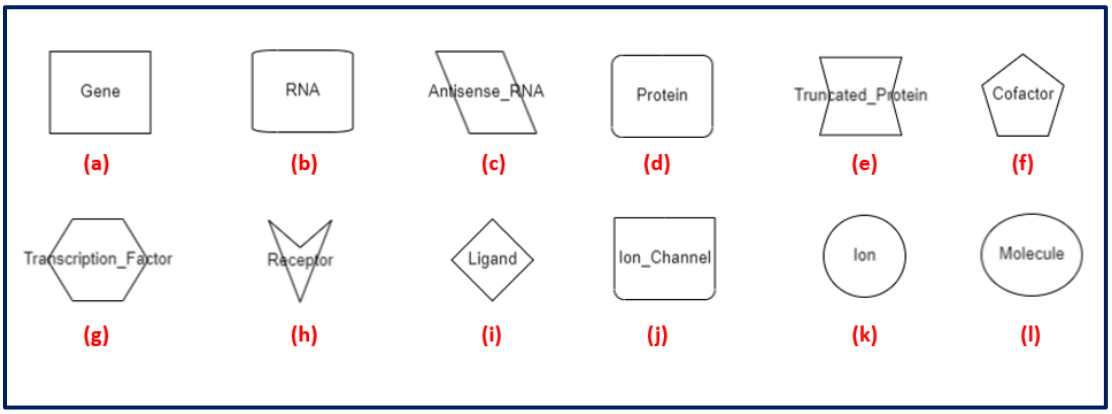


Figure 1.2.16.2 - Nodes drawn on canvas: *(a) 'Gene' (b) 'RNA' (c) 'Antisense RNA' (d) 'Protein' € 'Truncated Protein' (f) 'Cofactor' (g) 'Transcription Factor' (h) 'Receptor' (i) 'Ligand' (j) 'Ion Channel' (k) 'Ion' (l) 'Molecule'.*

Once the user selects the biomolecule and clicks on the canvas, the 'Node Properties' window shown in **Figure 1.2.16.3** appears. The user must enter the node name and its basal value; moreover, the user may alter the color, define the self-activation type, i.e., either activation or inhibition and its associated edge weight, as well as provide a description for the created node.

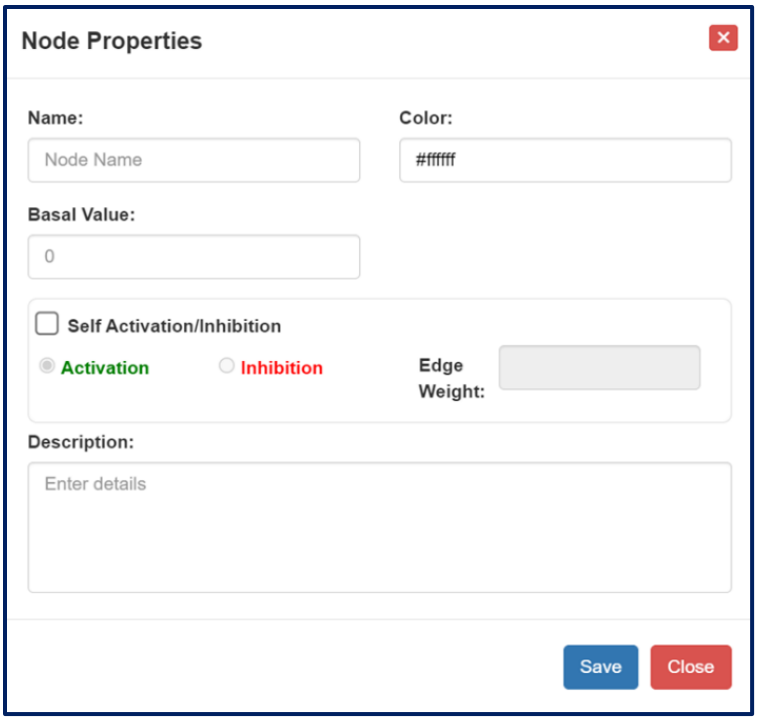


Figure 1.2.16.3 - Node properties for a newly created biomolecule.

To edit the properties of a particular node, the user can right-click on the node and then point the cursor towards the 'Edit' option (**Figure 1.2.16.4**); this will also open the window shown in **Figure 1.2.16.3**.

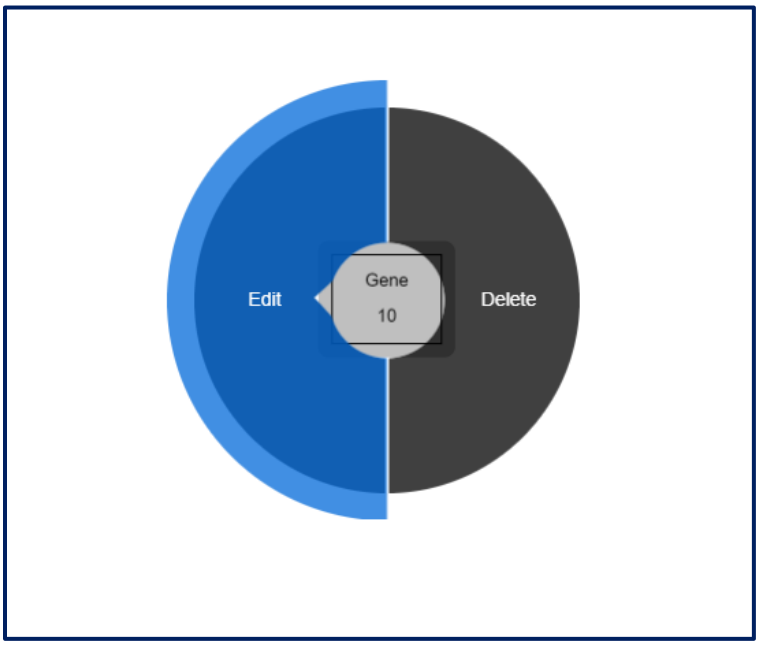


Figure 1.2.16.4 - 'Edit Node Properties' option.

To delete the node, the user can right-click on the node and then point the cursor toward the 'Delete' option, as shown in **Figure 1.2.16.5**.

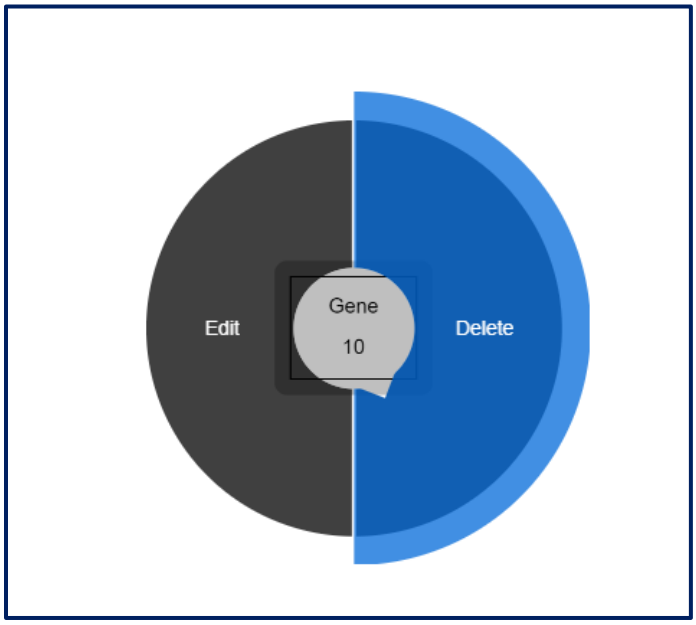


Figure 1.2.16.5 - Deleting a single node.

1.2.17 Username

The username entered by the user during the registration process appears in the highlighted area in **Figure 1.2.17.1**.

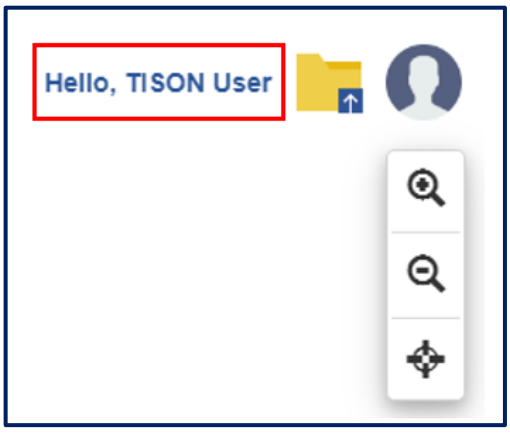


Figure 1.2.17.1 - Username displayed in NE.

1.2.18 Return to Project Explorer

The 'Return to Project Explorer' icon highlighted in **Figure 1.2.18.1** allows the user to return to the project explorer page and view the list of created and saved projects in TISON's database.

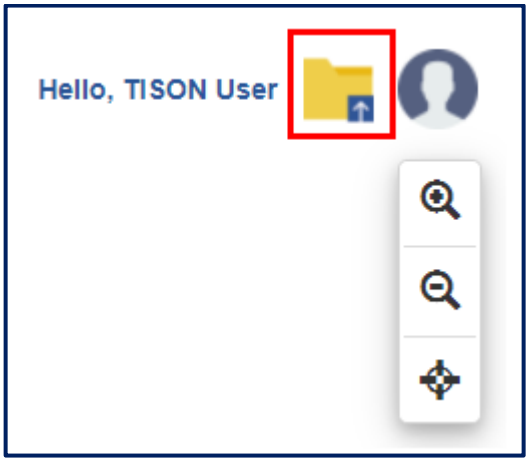


Figure 1.2.18.1 - 'Return to Project Explorer' icon in NE.

1.2.19 Return to TISON Home Page or Sign Out

The highlighted icon in **Figure 1.2.19.1** displays the current TISON user. Upon clicking it, the user is given the option to either return to the TISON home page by selecting 'Main' or sign out from TISON by selecting 'Sign Out'.

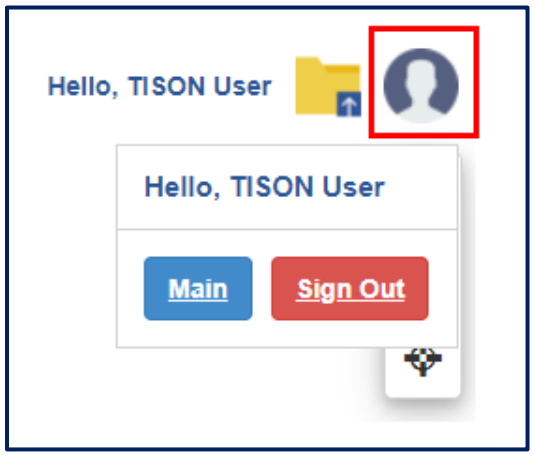


Figure 1.2.19.1 - Return to TISON home page or Sign Out: *'Main' and 'Sign Out' buttons are displayed.*

1.2.20 'Zoom In', 'Zoom Out', and 'Reset to Default' Buttons

The icon bar highlighted in **Figure 1.2.20.1** allows the user to zoom in on the canvas **(a)**, zoom out of the canvas **(b),** and reset the canvas to default size **(c)**. Moreover, the zoom level can also be adjusted with the mouse scroll wheel.

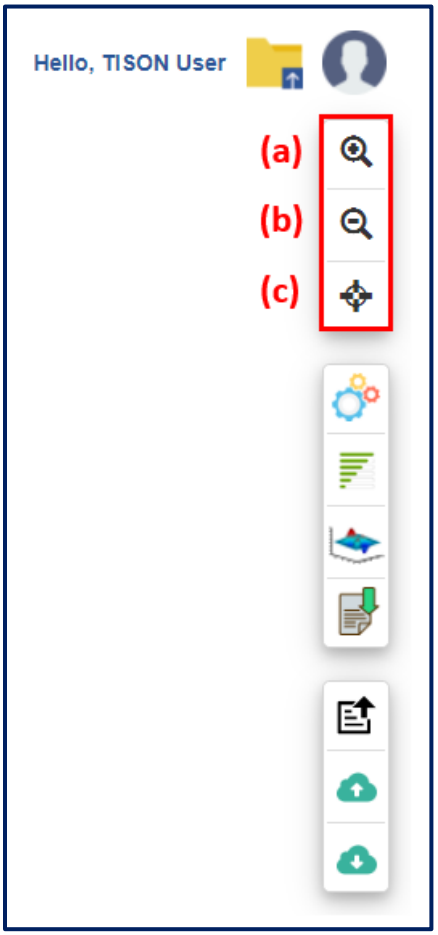


Figure 1.2.20.1 - Zoom control buttons: *(a) 'Zoom In' (b) 'Zoom Out' (c) 'Reset to Default'.*

1.3 Network Analysis

The highlighted toolbar (**Figure 1.3.1**) provides access to the various features of network analysis. The 'Perform Network Analysis' button (**Figure 1.3.1 (d)**) provides options to perform Deterministic Analysis (DA), Probabilistic Analysis (PA), and Ordinary Differential Equations (ODE) Analysis. 'Analysis Progress and Status' button (**Figure 1.3.1 (c)**) shows the status of analyses running on the network. 'View Network and Results' button (**Figure 1.3.1 (f)**) toggles the view between the network and various landscapes. 'Download Results' button (**Figure 1.3.1 (g)**) allows the user to download result files.

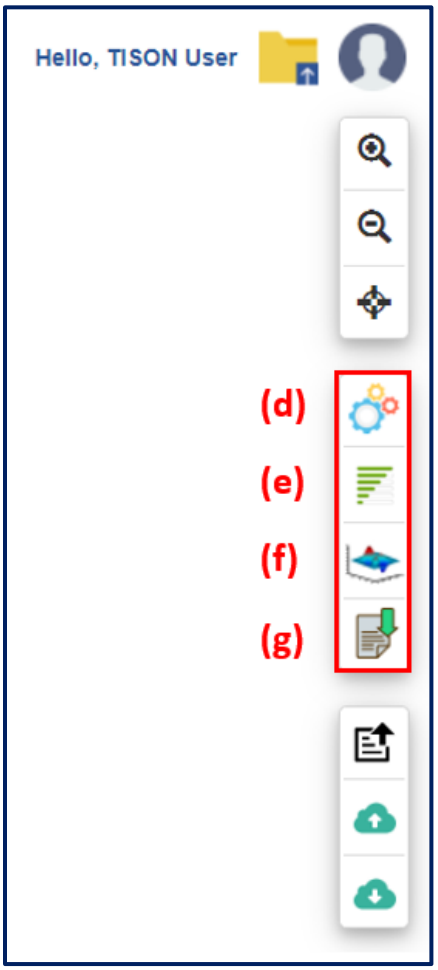


Figure 1.3.1 - Network analysis toolbar in NE.

1.3.1 'Perform Network Analysis' Button

For a *rules-based network* the user can perform DA only, while for a *weight-based network*, DA, PA, and ODE Analysis can be performed by clicking on the button highlighted in **Figure 1.3.1.1**.

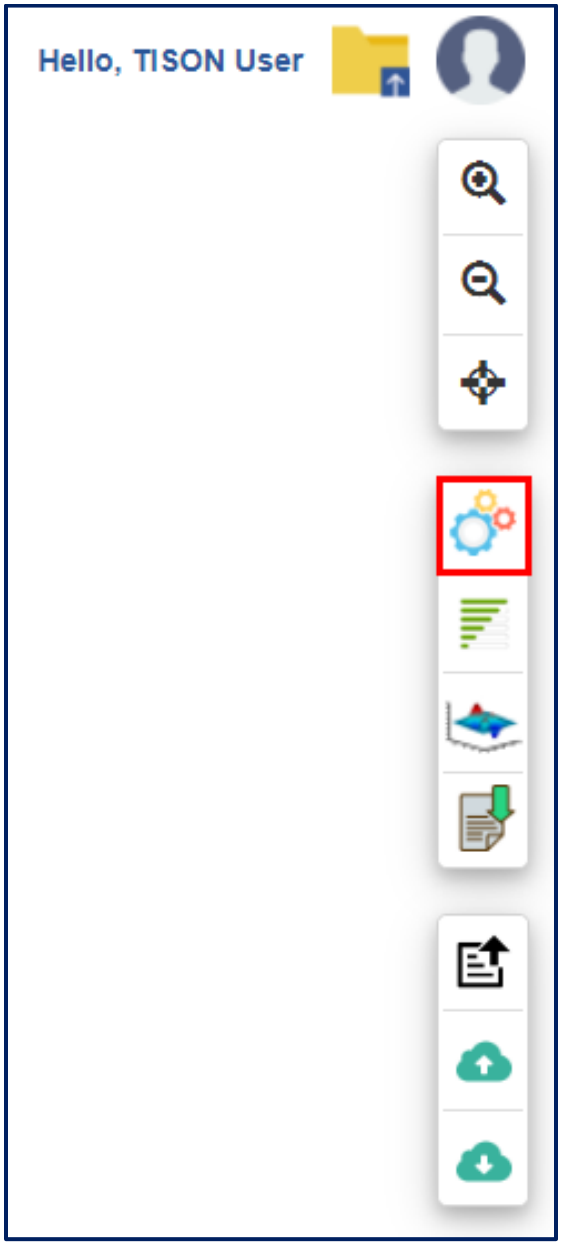


Figure 1.3.1.1 - 'Perform Network Analysis' button.

a) Deterministic Analysis:

In Deterministic Analysis (DA), a biomolecular network is represented as a closed system, although a node's basal value can incorporate external influence. The DA pipeline assumes a lack of external noise or extracellular signaling perturbations and makes use of a basal value vector, $B = [b_1, b_2, \dots, b_n]$, provided by the user or obtained from integrated databases. This vector contains biomolecular expression values that indicate the basal activity of each node, where the basal activity values signify the node expressions without any inputs from the network. The interaction weight matrix, $I_w = [w_{ij}]_{n \times n}$ represents the nature and effective mass of interaction between two participating nodes. The node-interaction model can simulate the node state transition function.

In order to update the node states using DA, a transition function is used; this integrates each model component, including basal values, interaction weights, and node states[2], as given by the piecewise equation below:

$$x_i(t+1) = \begin{cases} 1 & if \sum_j \left(w_{ji} x_j(t)\right) + b_i > 0 \\ 0 & else\ if \sum_j \left(w_{ji} x_j(t)\right) + b_i < 0 \\ x_i & else\ if \sum_j \left(w_{ji} x_j(t)\right) + b_i = 0 \end{cases}$$

The equation above defines the node (x_i) state transition from time step t to $t+1$. The summation, $\sum_j \left(w_{ji} x_j(t)\right) + b_i$, adds weighted interactions and the basal value. The result from the expression is translated using a *sign* (*Signum* function) which is defined as follows[3]:

$$x_i(t+1) = f\left(\sum_j \left(w_{ji} x_j(t)\right) + b_i\right)$$

where f is the *Signum* (or *Sign*) function defined over any input variable, z , as follows[3]:

$$f(z) = \begin{cases} 1 & if\ z > 0 \\ 0 & if\ z = 0 \\ -1 & if\ z < 0 \end{cases}$$

However, in deterministic analysis, an analog of this function is implemented:

$$f(z) = \begin{cases} 1 & if\ z > 0 \\ 0 & if\ z = 0 \\ 0 & if\ z < 0 \end{cases}$$

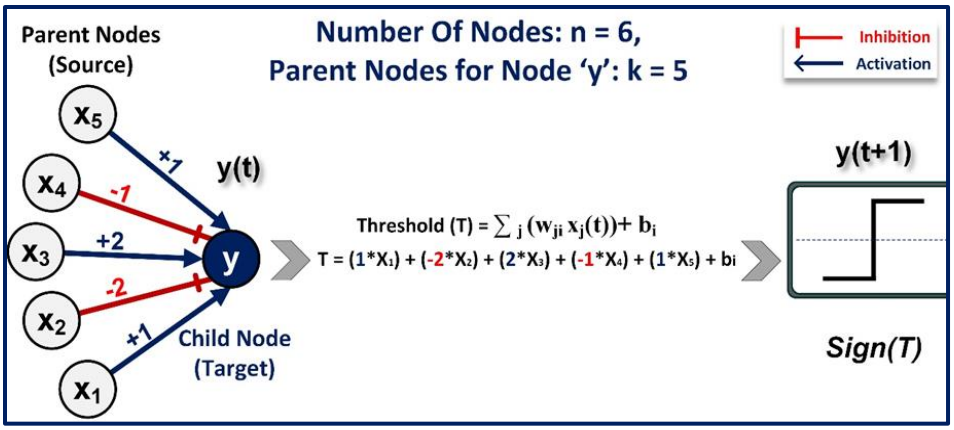


***Figure 1.3.1.2 - Node update for DA in TISON**. The example network has one target node, y, with five parent nodes (k=5) regulating it. Three of these parents activate the node, while the remaining two inhibit its activity.*

Figure 1.3.1.3 outlines the workflow of the DA pipeline implemented in TISON. The DA pipeline starts with a set of initial network states that can be generated by (1) exhaustive sampling (ES) of the state-space, (2) random sampling (RS) of the state-space, or (3) providing a custom state-space (CS) file. ES generates all (2^n) possible states for onward analysis. RS selects a user-specified number of states from the complete state-space, thereby making the analysis of more extensive networks feasible. Using the CS option, TISON's user can selectively incorporate specific network states for further analysis. To achieve a steady-state, network states and transition functions are used. Once the system attains a steady state, the frequently recurring states (or attractors) are identified for attractor landscape plotting.

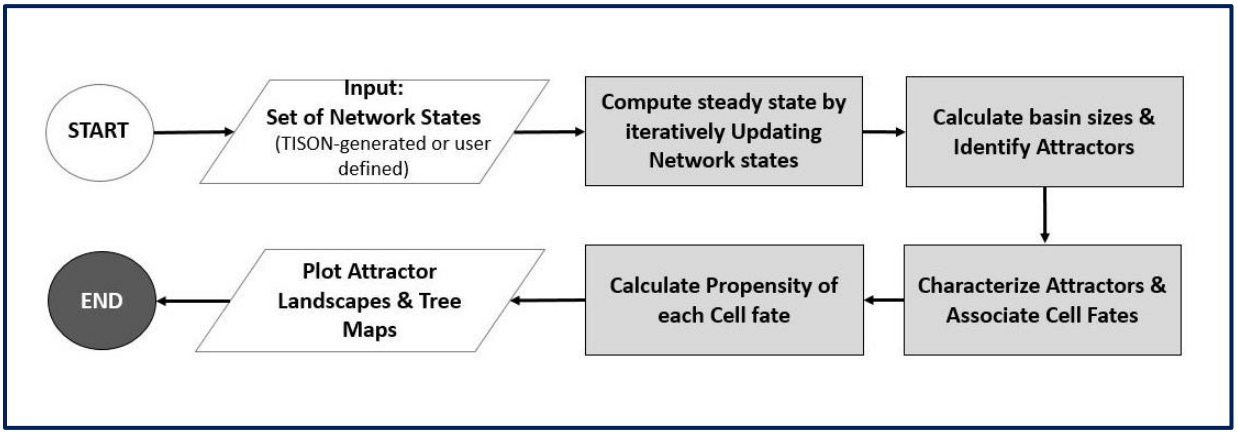


Figure 1.3.1.3 - DA pipeline implemented in TISON.

To assist in the cell fate discovery process, TISON's user can incorporate specific node update rules into the DA pipeline; node update rules are employed during the network update and stored in the form of truth tables. State transition rules are constructed from the exhaustive state space, and only the

biologically plausible node state transitions are employed onwards. Therefore, each node state gets updated from the rules table. An example of rules-based DA is given below in **Figure 1.3.1.1**. The child node 'C' with parent nodes 'A' and 'B' is updated to state '0' by applying the rule '0 1 0' (in the red box).

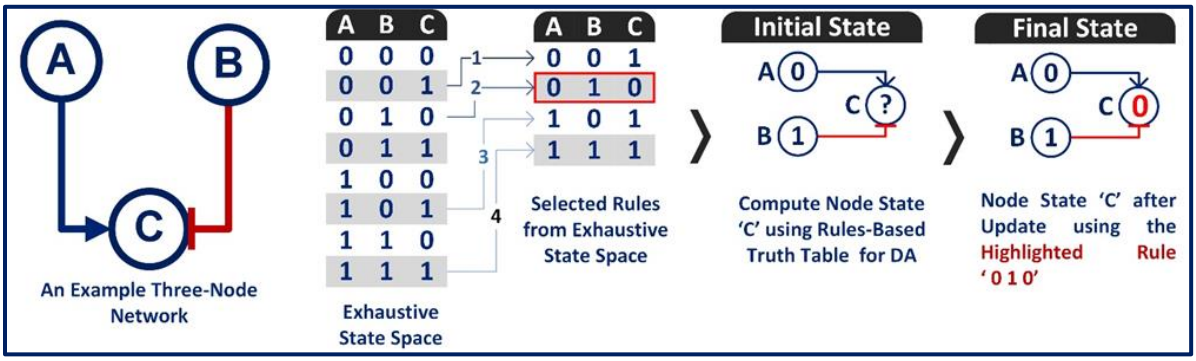


Figure 1.3.1.1 - Rules-based DA methodology: *A three-node network with two-parent nodes, 'A' and 'B,' and one child node, 'C', is considered for rules-based DA. The state of node 'C' ('0') is computed by using rules-based logic defined (red box) from within the exhaustive state space.*

The following window (**Figure 1.3.1.2**) will open when the user selects the 'Perform Network Analysis' button for a *rules-based network*, for which the 'Deterministic Analysis' tab is selected by default (For visual reference, see Section TISON's Networks Editor Video Tutorials 1.4, Table 1.4.1 - Video 1).

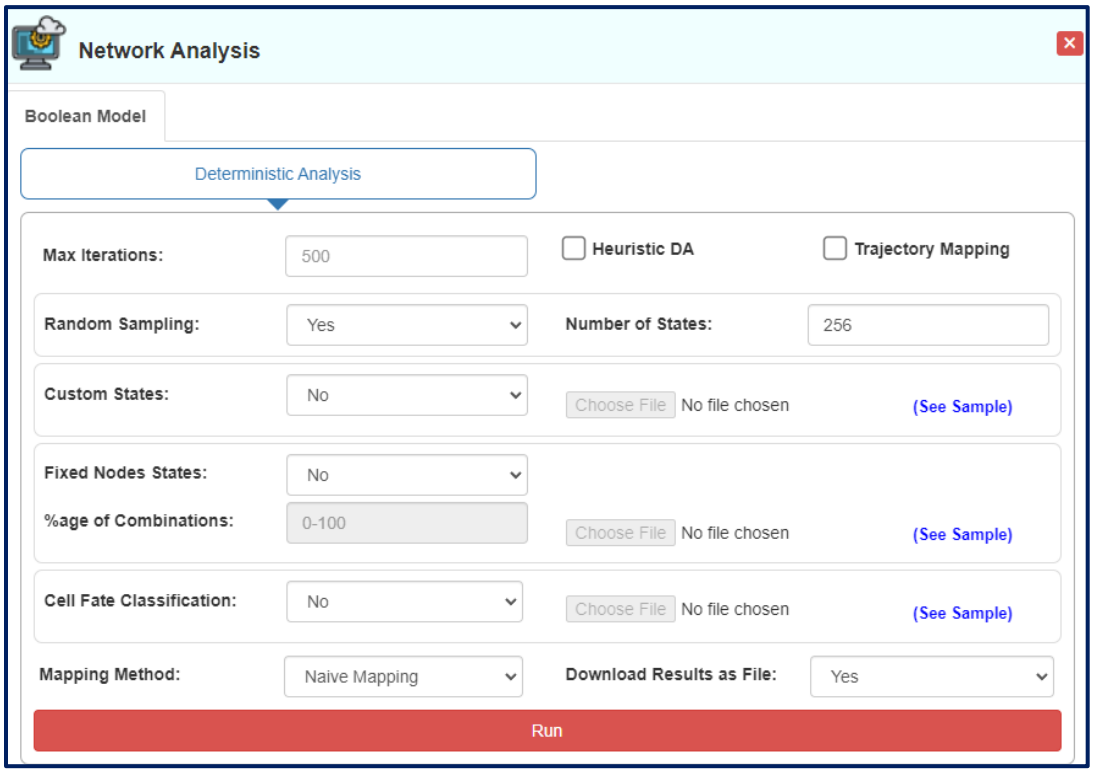


Figure 1.3.1.2 - Network Analysis window.

- **Steps for average node activity/propensity calculation:**

The result file provides the average node activity (see 1.3.2‘Analysis Progress and Status’ Button

In order to keep track of the analyses, Deterministic Analysis (DA), Probabilistic Analysis (PA) and/or Ordinary Differential Equation Analysis (ODE), that are being run on one or multiple networks, the ‘Analysis Progress and Status’ button (highlighted in **Figure 1.3.2.1**) can be used.

Figure 1.3.2.1 - ‘Analysis Progress and Status’ button in NE.

The window shown in **Figure 1.3.2.2** appears upon clicking the 'Analysis Progress and Status' button. This allows the user to view the name of the networks that are being analyzed, the stage that is being processed, the percentage of progress and the type of analysis along with the type of network (weight-based or rules-based). Moreover, the user may also stop a specific analysis of a network by clicking on the 'Stop' button for that network analysis.

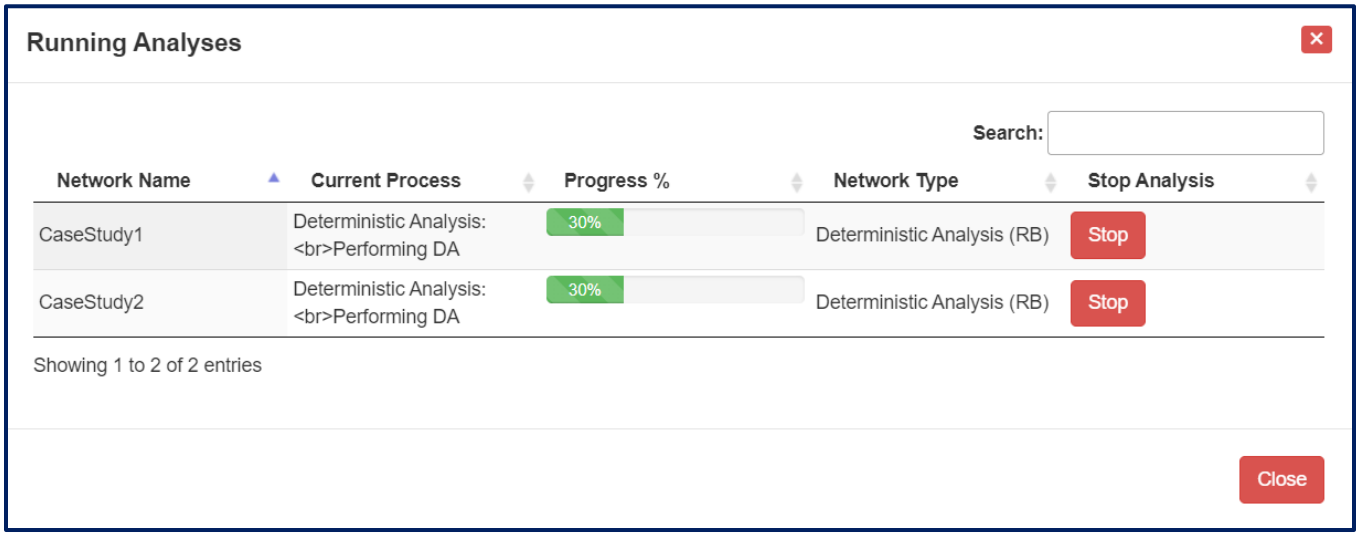


Figure 1.3.2.2 - Window that shows the progress of the analyses that are in process.

1.3.3 'View Network and Results' Button section), which is computed by the following steps.

Step 1: Multiply the basin size ratio with its corresponding node state for all attractors (shown in **Figure 1.3.1.3**).

Step 2: Perform Step 1 for all attractors (shown in **Figure 1.3.1.4**).

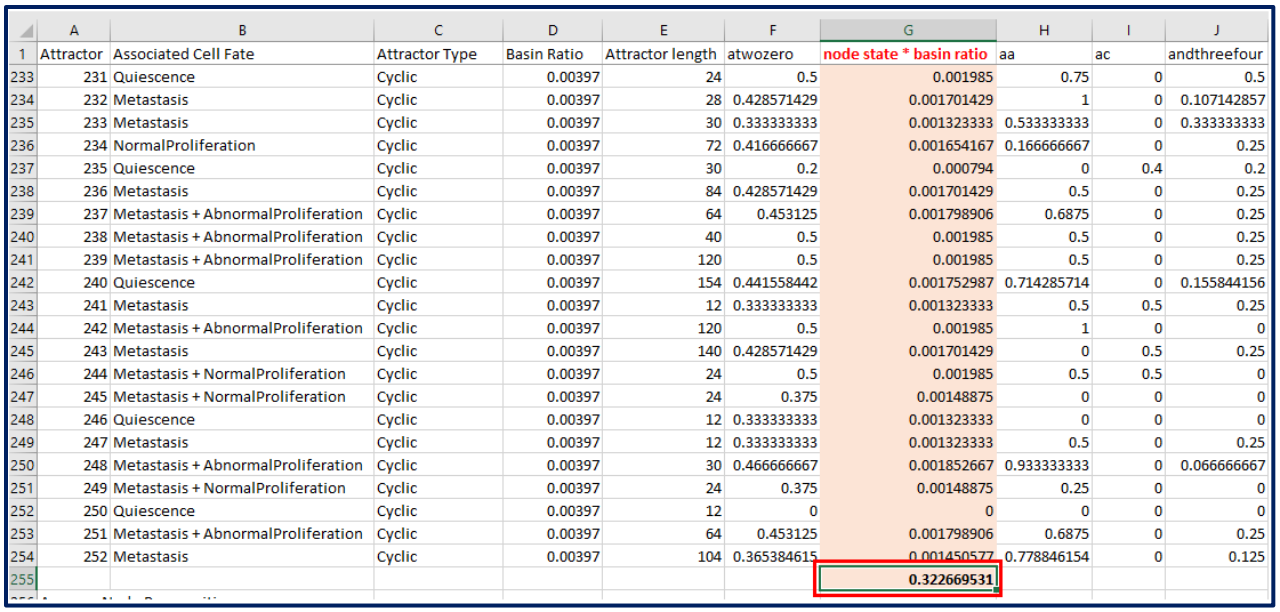
Step 3: Sum the entire column (shown in **Figure 1.3.1.5**).

	A	B	C	D	E	F	G	H	I	J
1	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	atwozero	node state * basin ratio	aa	ac	andthreefour
2										
3	1	Quiescence	Cyclic	0.00397	8	0.375	=F3*D3	0.5	0	0
4	2	Quiescence	Cyclic	0.00397	12	0.333333333		0.5	0	0.25
5	3	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5		0.5	0	0.25
6	4	Metastasis	Cyclic	0.00397	28	0.428571429		1	0	0.25
7	5	Quiescence	Cyclic	0.00397	84	0		0	0	0
8	6	Quiescence	Cyclic	0.00397	12	0		0	0	0
9	7	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5		1	0	0
10	8	Quiescence	Cyclic	0.00397	58	0.137931034		0.413793103	0	0.172413793
11	9	Quiescence	Cyclic	0.00397	24	0.5		0.5	0.5	0
12	10	Metastasis	Cyclic	0.00397	30	0		0	0	0
13	11	Quiescence	Cyclic	0.00397	84	0		0	0	0
14	12	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5		0.5	0	0.25
15	13	Metastasis	Cyclic	0.00397	12	0.333333333		0	0	0
16	14	Quiescence	Cyclic	0.00397	12	0		0	0	0
17	15	Quiescence	Cyclic	0.00397	60	0.1		0	0	0.2
18	16	Quiescence	Cyclic	0.00397	8	0.5		0	0	0
19	17	Metastasis	Cyclic	0.00397	28	0.428571429		1	0	0.107142857
20	18	NormalProliferation	Cyclic	0.00397	40	0.5		0	0	0

Figure 1.3.1.3 - Step 1 for average node activity calculation.

	A	B	C	D	E	F	G	H	I	J
1	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	atwozero	node state * basin ratio	aa	ac	andthreefour
233	231	Quiescence	Cyclic	0.00397	24	0.5	0.001985	0.75	0	0.5
234	232	Metastasis	Cyclic	0.00397	28	0.428571429	0.001701429	1	0	0.107142857
235	233	Metastasis	Cyclic	0.00397	30	0.333333333	0.001323333	0.533333333	0	0.333333333
236	234	NormalProliferation	Cyclic	0.00397	72	0.416666667	0.001654167	0.166666667	0	0.25
237	235	Quiescence	Cyclic	0.00397	30	0.2	0.000794	0	0.4	0.2
238	236	Metastasis	Cyclic	0.00397	84	0.428571429	0.001701429	0.5	0	0.25
239	237	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
240	238	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5	0.001985	0.5	0	0.25
241	239	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	0.5	0	0.25
242	240	Quiescence	Cyclic	0.00397	154	0.441558442	0.001752987	0.714285714	0	0.155844156
243	241	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0.5	0.25
244	242	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	1	0	0
245	243	Metastasis	Cyclic	0.00397	140	0.428571429	0.001701429	0	0.5	0.25
246	244	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.5	0.001985	0.5	0.5	0
247	245	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0	0	0
248	246	Quiescence	Cyclic	0.00397	12	0.333333333	0.001323333	0	0	0
249	247	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0	0.25
250	248	Metastasis + AbnormalProliferation	Cyclic	0.00397	30	0.466666667	0.001852667	0.933333333	0	0.066666667
251	249	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0.25	0	0
252	250	Quiescence	Cyclic	0.00397	12	0	0	0	0	0
253	251	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
254	252	Metastasis	Cyclic	0.00397	104	0.365384615	0.001450577	0.778846154	0	0.125
255										

Figure 1.3.1.4 - Step 2 for average node activity calculation.



	A	B	C	D	E	F	G	H	I	J
1	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	atwozero	node state * basin ratio	aa	ac	andthreefour
233	231	Quiescence	Cyclic	0.00397	24	0.5	0.001985	0.75	0	0.5
234	232	Metastasis	Cyclic	0.00397	28	0.428571429	0.001701429	1	0	0.107142857
235	233	Metastasis	Cyclic	0.00397	30	0.333333333	0.001323333	0.533333333	0	0.333333333
236	234	NormalProliferation	Cyclic	0.00397	72	0.416666667	0.001654167	0.166666667	0	0.25
237	235	Quiescence	Cyclic	0.00397	30	0.2	0.000794	0	0.4	0.2
238	236	Metastasis	Cyclic	0.00397	84	0.428571429	0.001701429	0.5	0	0.25
239	237	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
240	238	Metastasis + AbnormalProliferation	Cyclic	0.00397	40	0.5	0.001985	0.5	0	0.25
241	239	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	0.5	0	0.25
242	240	Quiescence	Cyclic	0.00397	154	0.441558442	0.001752987	0.714285714	0	0.155844156
243	241	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0.5	0.25
244	242	Metastasis + AbnormalProliferation	Cyclic	0.00397	120	0.5	0.001985	1	0	0
245	243	Metastasis	Cyclic	0.00397	140	0.428571429	0.001701429	0	0.5	0.25
246	244	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.5	0.001985	0.5	0.5	0
247	245	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0	0	0
248	246	Quiescence	Cyclic	0.00397	12	0.333333333	0.001323333	0	0	0
249	247	Metastasis	Cyclic	0.00397	12	0.333333333	0.001323333	0.5	0	0.25
250	248	Metastasis + AbnormalProliferation	Cyclic	0.00397	30	0.466666667	0.001852667	0.933333333	0	0.066666667
251	249	Metastasis + NormalProliferation	Cyclic	0.00397	24	0.375	0.00148875	0.25	0	0
252	250	Quiescence	Cyclic	0.00397	12	0	0	0	0	0
253	251	Metastasis + AbnormalProliferation	Cyclic	0.00397	64	0.453125	0.001798906	0.6875	0	0.25
254	252	Metastasis	Cyclic	0.00397	104	0.365384615	0.001450577	0.778846154	0	0.125
255							0.322669531			

Figure 1.3.1.5 - Step 3 for average node activity calculation.

To perform DA, the user will have to enter various parameters, each of which is explained below:

- **Max iterations:** Number of iterations for which the algorithm searches the network for an attractor.
- **Heuristic DA:** The network sample can be considerably large for extensive networks and may require a large system memory by TISON. To address this issue, we have implemented a heuristic DA pipeline that works seamlessly on low-memory and large-memory systems. It is implemented by performing a one-step DA and retaining the unique network, after which an n-step DA is performed on these unique states.
- **Trajectory Mapping:** Trajectory mapping traces the route from the initial to the final boundary state, passing through all the intermediate transient states. Users can select 'Trajectory Mapping' from the analysis modal in **Figure 1.3.1.2**, allowing TISON to store and then download the trajectory mapping file. This file shows the complete state transition trajectory from initial network states at iteration 0 to This file shows the complete state transition trajectory from initial network states at iteration 0 to the final iteration number.
- **Random Sampling:** This option caters to insufficient system memory (if the system cannot store 2^n states). Exhaustive sampling, in which 'Random Sampling' is turned *OFF*, generates all (2^n) possible states for onward analysis. However, when 'Random Sampling' is turned *ON*, a user-specified number of states is selected from the complete state-space, thus, making the analysis of more extensive networks feasible. Biological systems tend to be robust, so the final basin size ratio remains accurate within a small error range for a large sampling rate.
- **Number of States:** This parameter is needed when 'Random Sampling' has been selected; it refers to the user-specified number of states from the complete state-space, thus making the analysis of larger networks feasible. The default number of states is 256, while the maximum number of states is 10,000.

If any state, i.e., exhaustive, random, or user-specified custom states, is not converged, then result file will indicate the number of unconverged states and their values in binary form.

- **Custom States:** The Custom States file (format .csv) contains network states defining the states of individual nodes present in the entire network. TISON users can cue the network analyses by utilizing custom states as the initial set of network states thereby arriving at the steady state corresponding to the input custom state. Selecting the 'Heuristic DA' option alongside also allows the user to prune the network state-space using one-step DA, which is especially useful in large networks as it enables traceable computation.

  To exemplify, if the user has a network of ten nodes consisting of input, processing, and output nodes, customized state space for each node must be provided and the values should be in binary (0 or 1). This will allow the analysis of the network towards the generation of steady state outcomes. Assuming that the user has input custom states (**Figure 1.3.1.6**) along with a random sample of size '2', the custom state file of these ten nodes will resemble the sample shown in **Figure 1.3.1.7**.

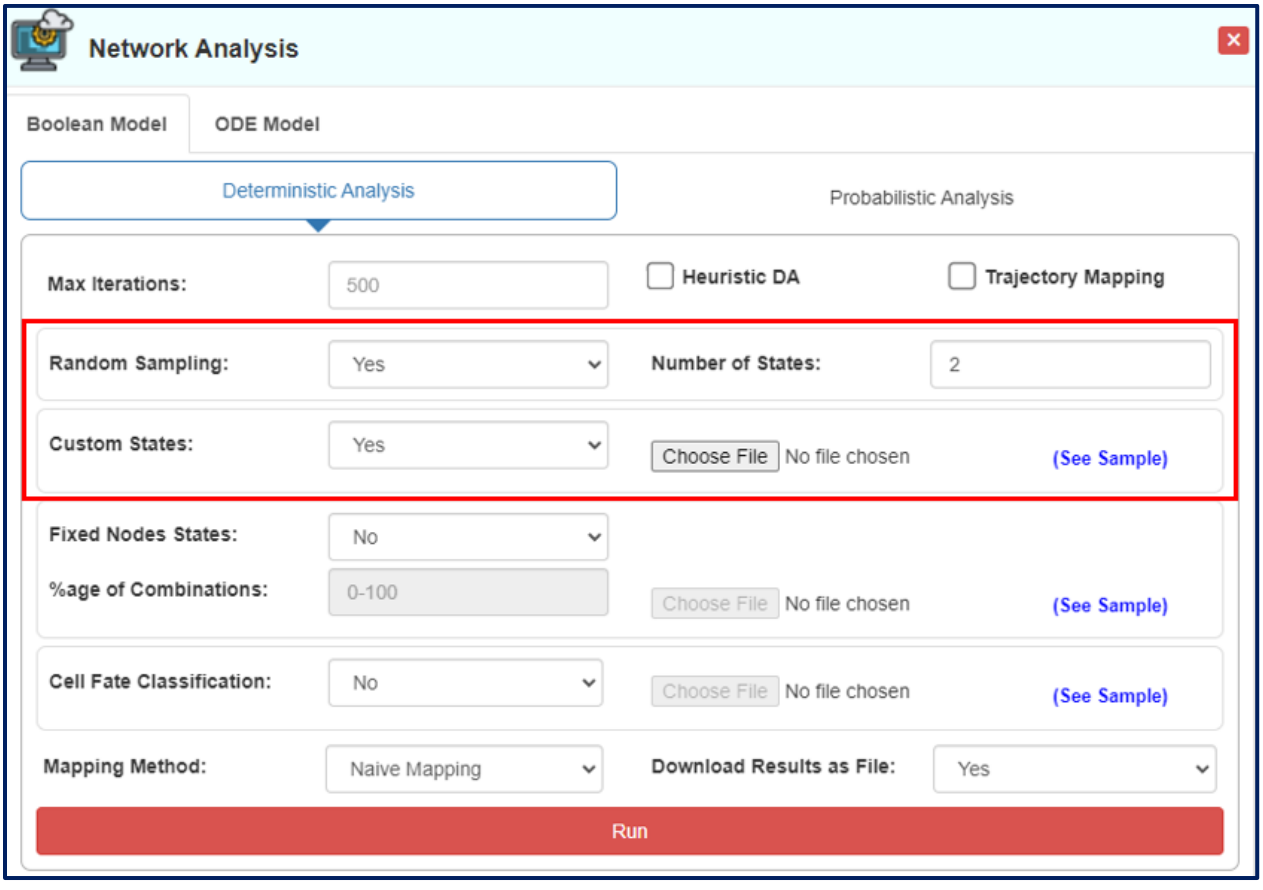


Figure 1.3.1.6 - Setting up the input parameters.

	A	B	C	D	E	F	G	H	I	J	K
1	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	
2	0	0	1	0	0	0	0	0	0	0	
3	1	0	1	0	0	0	0	0	0	1	
4											

Figure 1.3.1.7 - ***Custom states file (.csv):*** *User specifies the names of intermediate nodes in the first row, followed by their custom node values in the second row.*

- **Fixed Nodes States:** Nodes are assigned 'static' fixed values provided by the user after each iteration, i.e., time step, to imitate the effect of a drug, biological mutation, input condition, or other external conditions on a network. Hence, the value of that input node is fixed throughout the analysis. For instance, a value of 0.2 means that, after each iteration, 20% of the fixed nodes will be in the ON state (set to 1), and 80% of the nodes will be in the OFF state (set to 0). The Networks Editor provides the user with two options for 'Fixed Node States' as shown in **Figure 1.3.1.8**. (i) Row-wise (ii) Combinational. In the first option, the user can give single or multiple rows of inputs, and the analysis is run for each of the rows. Whereas in the second option, the user can define a range for inputs, and the analysis is run for each combination.

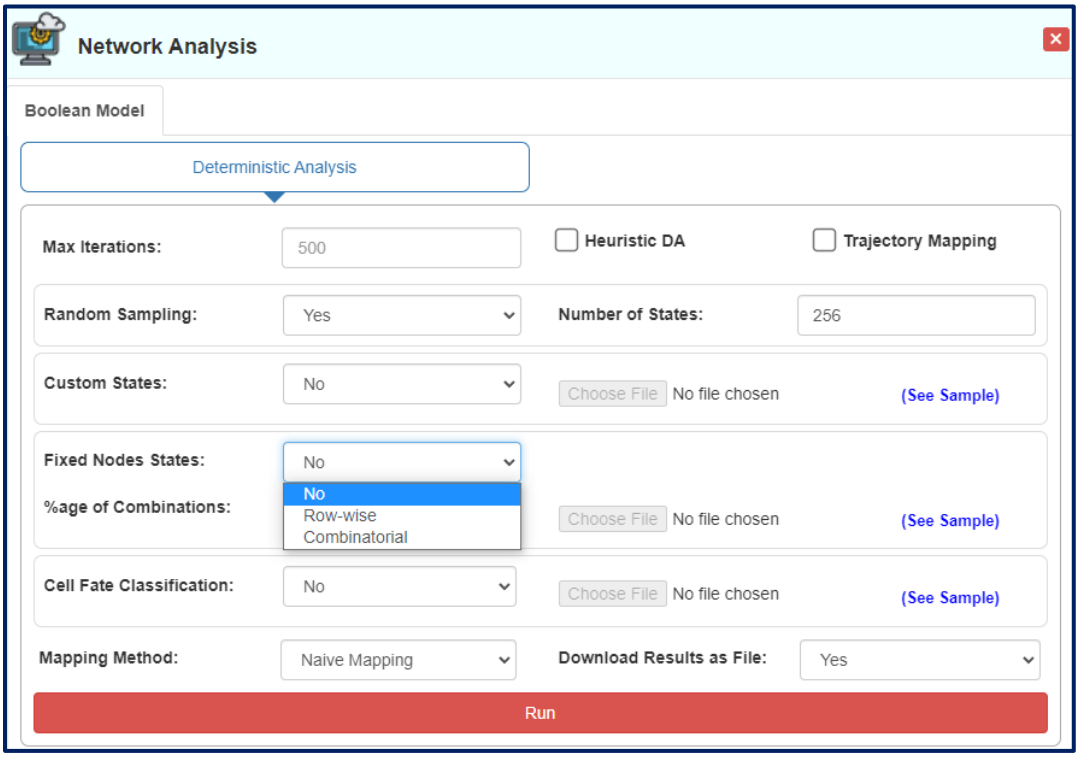


Figure 1.3.1.8 - Fixed nodes state options.

In an analysis that takes in a row-wise formatted fixed nodes state file, the Networks Editor iteratively takes in a single input (a single row) at a time from the input file containing one or multiple inputs (rows) and analyzes the network considering that input. The process is iterated until the network is analyzed under all inputs (rows) individually.

The row-wise fixed nodes states file format is shown in **Figure 1.3.1.9**.

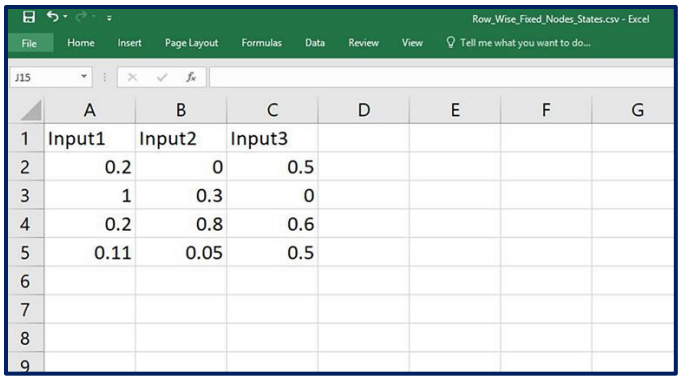


Figure 1.3.1.9 - Row-wise fixed nodes (.csv) file: *The user can give single or multiple rows of inputs, and the analysis is performed for each row.*

In the combinatorial fixed nodes state scenario, the user can provide ranges of input values within a single row of the input file. The format for each input is as follows: the minimum value of the input range, followed by the step size for each iteration, and lastly, the maximum value of the input range, each value separated by a colon. Networks Editor then generates a vector of all possible values for each input, following the user-defined input range and step size specification. Input combinations are generated from these vectors and passed to the network analysis pipeline. The combinations are generated using the following method, where n represents a node and x is the number of inputs for each node:

$$Total\ Input\ Combinations = \prod_{n=1}^{n} x_n$$

However, if the process generates a large number of inputs that are computationally un-scalable, the editor also provides a random sampling feature wherein the user can specify the percentage of combinations to be employed in the analysis. In this case, the total input combinations are first generated using the user-specified range and step size, followed by a random selection based upon the percentage specified. The selected input combinations are then provided to the network analysis pipeline. The precision of the input range for NE is up to two decimal places. The format and contents of a combinational fixed nodes states are shown in the figure below **Figure 1.3.1.10**.

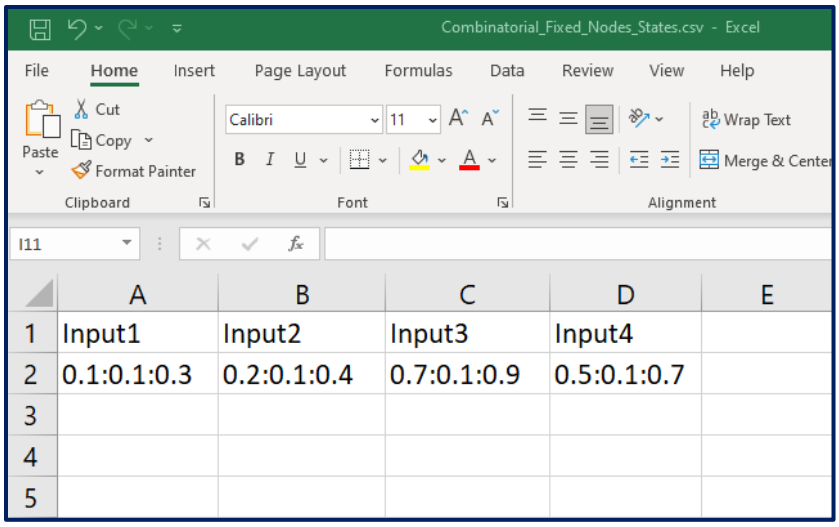


Figure 1.3.1.10 - Combinational fixed nodes (.csv) file: *Input combinations allow for multiple input configurations for fixed nodes values after each iteration. Each input following the format (minimum value: step size: maximum value). In this case, there are n input conditions in each node and a total of N nodes. The total input combinations will, therefore, n^N, i.e., $3^4 = 81$.*

**The precision of the inputs range is up to two decimal places.*

- **Fixed Nodes Combinations:** The user can restrict the number of input node states while using combinatorial input by specifying a percentage of allowable combinations; this value will ensure that only the specified percentage of results for those inputs is generated. This is termed as 'Robustness Analysis'.
- **Cell Fate Classification:** Attractors are mapped to user-defined cell fates such as apoptosis, cell cycle arrest, uncontrolled proliferation, etc. The user must provide a cell fate classification file (format .csv) **Figure 1.3.1.11**, wherein the first column carries cell fate names in each cell (the first cell of the first column is left empty). The node names must be defined in the first row of proceeding columns, followed by the 'node state' in the rows corresponding to each cell fate. The 'node state' in a specific cell fate can be one of the following:

i. '0' includes the node expressions from 0.00 to 0.19. It represents that the node is turned *OFF,* and its biological interpretation can either be an absence of expression or minimal and ineffective expression.
ii. '2' includes the node expressions from 0.20 to 0.80. It represents that the node is in a transient state and its biological interpretation can be a cyclic or fluctuating form of expression.
iii. '1' includes the node expressions from 0.81 to 1.00. It represents that the node is turned O*N* and its biological interpretation can be a high and effective form of expression.

If separated by a semi-colon, multiple node states can be defined in the same cell. The cell fate classification file format is shown in the figure below (**Figure 1.3.1.11**). Please note that no white spaces should be left in the cell fates or node names, i.e., in first column and first row.

	A	B	C	D	E	F	G	H	I
1		Rho	CyclinD	CyclinE	CyclinA	CyclinB	MMP	Ecad	
2	Metastasis	2					1	0	
3	NormalProliferation		2	2	2	2			
4	AbnormalProliferation		1	1;2	1;2	1;2			
5	Quiescence		0					1;2	
6	Quiescence			0				1;2	
7	Quiescence				0			1;2	
8	Quiescence					0		1;2	
9	Quiescence		0				0;2		
10	Quiescence			0			0;2		
11	Quiescence				0		0;2		
12	Quiescence					0	0;2		
13	Quiescence	0;1	0						
14	Quiescence	0;1		0					
15	Quiescence	0;1			0				
16	Quiescence	0;1				0			

Figure 1.3.1.11 - Cell fate classification file (.csv): *Cell fate names are defined in the first column and node names in the first row of proceeding columns. Each cell fate contains specific node states in the rows corresponding to each node, '0', '1', and '2'. If a semi-colon is used to separate each state, multiple node states can be defined in the same cell.*

- **Mapping Method:** Mapping methods employed to map a hyper-dimensional space to a three-dimensional space include 'Naive Bayes mapping' (**Figure 1.3.1.12**) and 'Sammon mapping' (**Figure 1.3.1.13**).'Naive mapping' maps the state-space onto a Cartesian plane without considering the spatial distance between different steady-states. 'Sammon mapping', on the other hand, clusters the related network states before their projection onto a Cartesian plane.

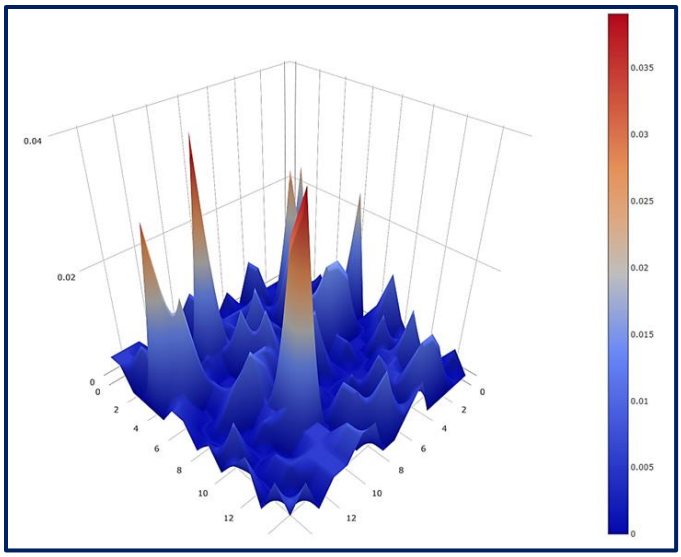


Figure 1.3.1.12 - Naive Bayes mapping basin ratio landscape.

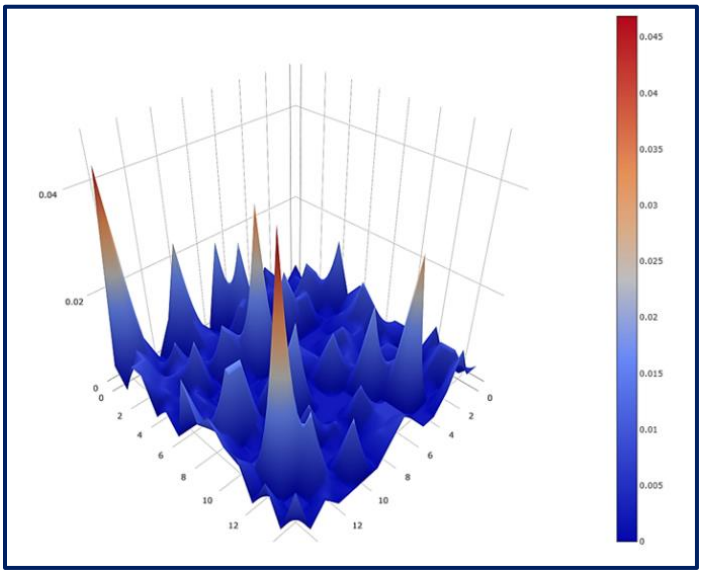


Figure 1.3.1.13 - Sammon mapping basin ratio landscape.

- **Download Results as File:** This allows the user to download the analysis results as a (.zip) file.

b) Probabilistic Analysis:

The probabilistic analysis (PA) pipeline of TISON (**Figure 1.3.1.14**) works off a biomolecular network input with nodes and interaction weights. As in the case of DA, PA can also employ exhaustive, randomly sampled, or user-defined custom state-space for onwards analysis. However, unlike DA[4], the transitions between network states follow a *Markovian* model in PA. Network state at the next time step, $t+1$, is determined only by its state at the current time step, t, with no dependence on earlier time steps. As a result, the state transition probability from t to $t+1$ is a product of individual node transition probabilities from t to $t+1$ [3,4]:

$$\mathrm{T}\{x_i(t+1), x_2(t+1), \dots x_n(t+1) | x_1(t), x_2(t), \dots x_n(t)\} = \prod_{i=1}^{n} T\{x_i(t+1) | x_1(t), x_2(t), \dots x_n(t)\}$$

To integrate the effect of background basal-level activity of nodes, ($B = [b_1, b_2, \dots, b_n]$), in absence of any input, the cumulative inputs to each node are added with its basal expression. Intrinsic and extrinsic noise is incorporated with the use of noise parameter[4], μ. An additional parameter *c* is used to quantify the self-degradation of a node[3]. Given that the nodes lie within a dynamic system, the output from a single node is computed using the net effect of its interaction with the parent nodes, noise, basal values, and self-degradation constant. The input can be either positive, negative, or zero, according to which the node transition probability is updated. The transition matrix *T* for each node[5] is defined as follows:

$$T\{x_i(t+1) = 1 | x_1(t), x_2(t), \dots x_n(t)\} = \frac{1}{2} + \frac{1}{2} \tanh\left[\mu\left(\sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i\right)\right] \quad if \sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i > 0$$

$$T\{x_i(t+1) = 0 | x_1(t), x_2(t), \dots x_n(t)\} = \frac{1}{2} - \frac{1}{2} \tanh\left[\mu\left(\sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i\right)\right] \quad if \sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i < 0$$

$$T\{x_i(t+1) = x_i(t) | x_1(t), x_2(t), \dots x_n(t)\} = 1 - c \qquad if \sum_{j=1}^{n}\left(w_{ji}x_j(t)\right) + b_i = 0$$

If the input $\sum_{j=1}^{n}\left(w_{ji}S_j(t)\right) + b_i$ received by a node *I* is positive (>0), the probability of transitioning to an ON state is high and its probability of transitioning to an OFF state is low. Conversely, if the input $\sum_{j=1}^{n}\left(w_{ji}S_j(t)\right) + b_i$ is negative (<0), the node will transition to an OFF state with a high probability but its transition to an ON state will have a low probability. The last condition defines the probability of a node staying in the same state at the next time step, as there is essentially no change in the input coming into the node. In such a case, the transition probability is dependent on the self-degradation of the node with a value equal to 1 – *c*.

Following the computation of network transition probabilities, it is important to see how the states evolve to reach a steady state, such that there is no further change in their probabilities. This requires the use of kinetic master equations to update state probabilities over time and is given below:

$$\frac{dP_i}{dt} = -\sum_j \mathrm{T}_{ij} P_i + \sum_j \mathrm{T}_{ji} P_j$$

The state transition probabilities change over time due to the effect of neighboring network states according to transitions in both directions from *i* to *j*, and *j* to *i*. T_{ij} is the probability of a network state transitioning from a network state *i* to *j*, and the *vice versa* for T_{ji}. *i* and *j* iterate over all the states (i.e., from 1 to 2^n). The steady-state probabilities are stored in variables, P_i and P_j, wherein all states are initially assigned equal probabilities($P_i = \frac{1}{2^n}$). After that, each P_i is dynamically updated until a steady state, i.e., P_i (*t*+1) equals P_i (*t*) to a good approximation. For tractable computation in large networks, the state-space can also be pruned using one-step DA (termed Heuristic PA). Finally, a potential energy (PE) value is assigned to each state to represent its stability, which is then used to construct PE landscapes for visualizing PA results.

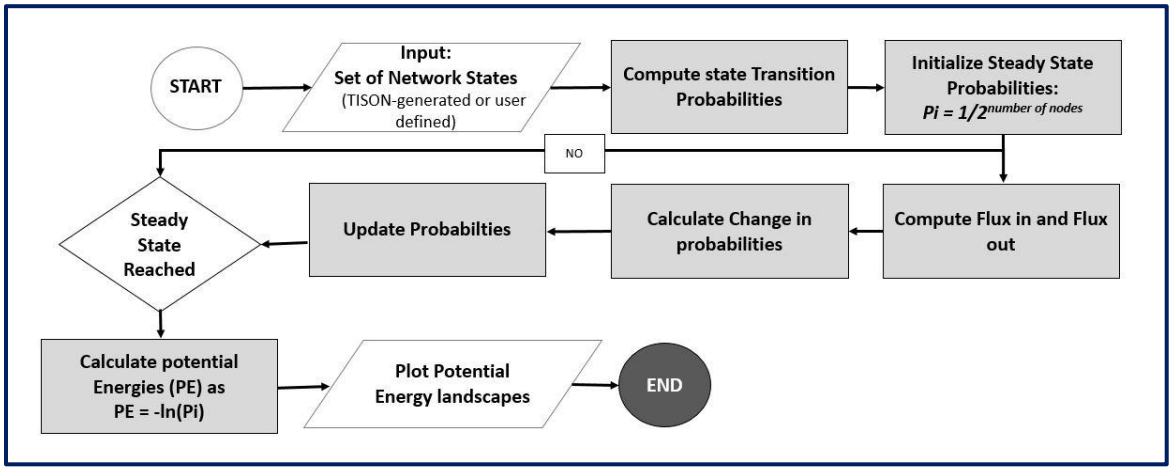


Figure 1.3.1.14 - PA pipeline implemented in TISON.

The window for probabilistic analysis is shown in the figure below (For visual reference, see Section 1.4, Table 1.4.1 - Video 4).

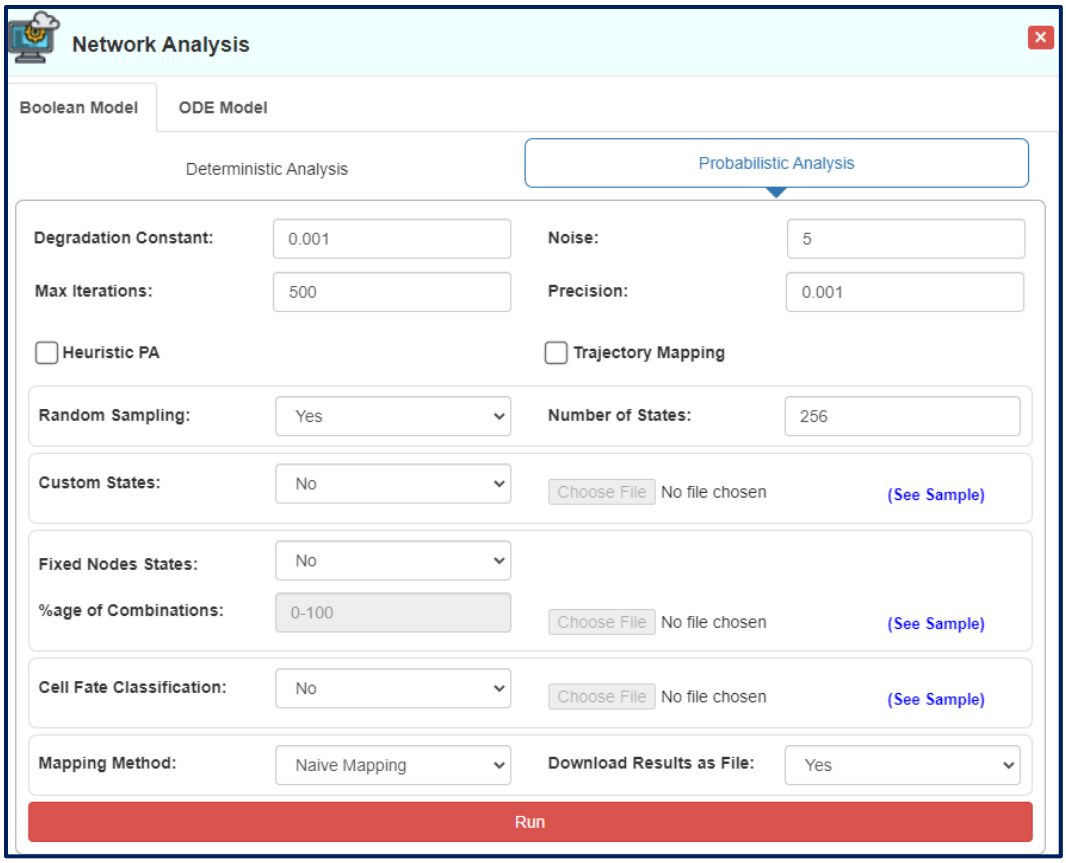


Figure 1.3.1.15 - Network analysis window for PA.

Parameters for Probabilistic Analysis are as follows:

- **Degradation Constant:** Used to quantify the self-degradation of the node.
- **Noise**: Intrinsic and extrinsic noise is incorporated with the use of noise parameter 'Environment Induced Noise' or 'μ'.
- **Max Iterations**: Number of iterations when solving the master equations to obtain steady-state transition probabilities.
- **Precision**: Maximum difference in transition probabilities of nodes between each iteration, for which a steady state is declared.
- **Heuristic PA:** The network sample can be considerably large for extensive networks and may require a large system memory by TISON. To address this issue, we have implemented a heuristic PA pipeline that works seamlessly on low-memory and large-memory systems. It is implemented by performing a one-step DA and retaining the unique network, after which an n-step PA is performed on these unique states.
- **Trajectory Mapping:** Trajectory mapping traces the route from the initial to the final boundary state, passing through all the intermediate transient states. Users can select 'Trajectory Mapping' from the analysis modal in **Figure 1.3.1.2**, allowing TISON to store and then download the trajectory mapping file.

This file shows the complete state transition trajectory from initial network states at iteration 0 to the final iteration number.

- **Random Sampling**: This option caters to insufficient system memory (if the system cannot store 2^n states). Exhaustive sampling, in which 'Random Sampling' is turned *OFF*, generates all (2^n) possible states for onward analysis. However, when 'Random Sampling' is turned *ON*, a user-specified number of states is selected from the complete state-space, thus, making the analysis of more extensive networks feasible.
- **Number of States**: User-specified number of states from the complete state-space; this makes the analysis of more extensive networks feasible. The default number of states is 256 whereas the maximum number allowed is 10,000. If any state, i.e., exhaustive, random, or user-specified custom states, is not converged, then result file will indicate the number of unconverged states and their values in binary form.
- **Custom States**: The Custom States file (format .csv) contains the network nodes which have been logically defined in the network file (intermediate nodes). TISON's user can selectively incorporate specific network states of certain intermediate nodes for network analysis through the Custom State option. Selecting the 'Heuristic DA' option along with this allows the user to prune the network state-space using one-step DA, which is especially useful in large networks as it enables traceable computation. (See Section on **Deterministic Analysis:**).
- **Fixed Nodes States**: States in the transition matrix are selected based on the fixed nodes; this is to imitate the effect of a drug, biological mutation, input condition, or other external conditions on a network. A user can choose 'No' to inactivate fixed nodes states. The Networks Editor provides the user with two options for 'Fixed Node States' as shown in **Figure 1.3.1.8**. : (i) Row-wise (ii) Combinational. In the first option, the user can give single or multiple rows of inputs, and the analysis is run for each of the rows. Whereas in the second option, the user can define a range for inputs, and the analysis is run for each combination.

- **Fixed Nodes Combinations**: The user can restrict the number of input node states while using combinational input by specifying a percentage of allowable combinations; this value will ensure that only the specified percentage of results for those inputs is generated. This is termed as 'Robustness Analysis'.
- **Cell Fate Classification**: Attractors are mapped to user-defined cell states such as apoptosis, cell cycle arrest, uncontrolled proliferation, etc.
- **Mapping Method:** Mapping methods employed to map a hyper-dimensional space to a three-dimensional space include 'Naive Bayes mapping' (**Figure 1.3.1.12**) and 'Sammon mapping' (**Figure 1.3.1.13**).'Naive mapping' maps the state-space onto a Cartesian plane without considering the spatial distance between different steady-states. 'Sammon mapping', on the other hand, clusters the related network states before their projection onto a Cartesian plane.
- **Download Results as file:** This allows the user to download the analysis results as a (.zip) file.

c) Ordinary Differential Equation (ODE) Analysis:

The system dynamics of transcriptional regulation driving cellular phenotypic changes can be easily analyzed and predicted with quantitative modeling of probabilistic landscapes using ODE analysis[5]. ODE analysis automates the network modeling and probability landscape visualization pipeline to facilitate cell fate prediction studies.

ODE analysis converts a weight-based network into an ODE model by generating a set of differential equations based on the interaction weights matrix. These ODEs are used to iterate through a set of random initial conditions, to produce time-series data. The data obtained is used to create a set of unique attractors (cell fates), which can be visualized as an attractor landscape.

For performing an ODE analysis, the user selects 'Perform Network Analysis' icon followed by the "ODE Model" tab. The window below will open (For visual reference, see Section 1.4, Table 1.4.1 - Video 6).

$$\frac{d[Gene_N]}{dx} = \sum_{i=1}^{ma} a_i * \frac{Gene_i^{n_a}}{Gene_i^{a} + k_a^{n_a}} + \sum_{i=1}^{mb} b_i * \frac{k_i^{n_i}}{Gene_i^{n_i} + k_i^{n_i}}$$

Where N = number of the gene, and [GeneN] represents concentration of the products of gene 'N'. In the above equation, m_a and m_b denote activators and inhibitors, respectively. Two coefficients, a_i and b_i represent the strength of activators and inhibitors to their target; k_a and k_i are activation constant and inhibition constant; n_a and n_i and are Hill coefficients. However, there are some restrictions while using the above formula: it is not applicable to different types of biochemical reactions such as metabolic signaling networks, it is only employed in gene regulatory networks.

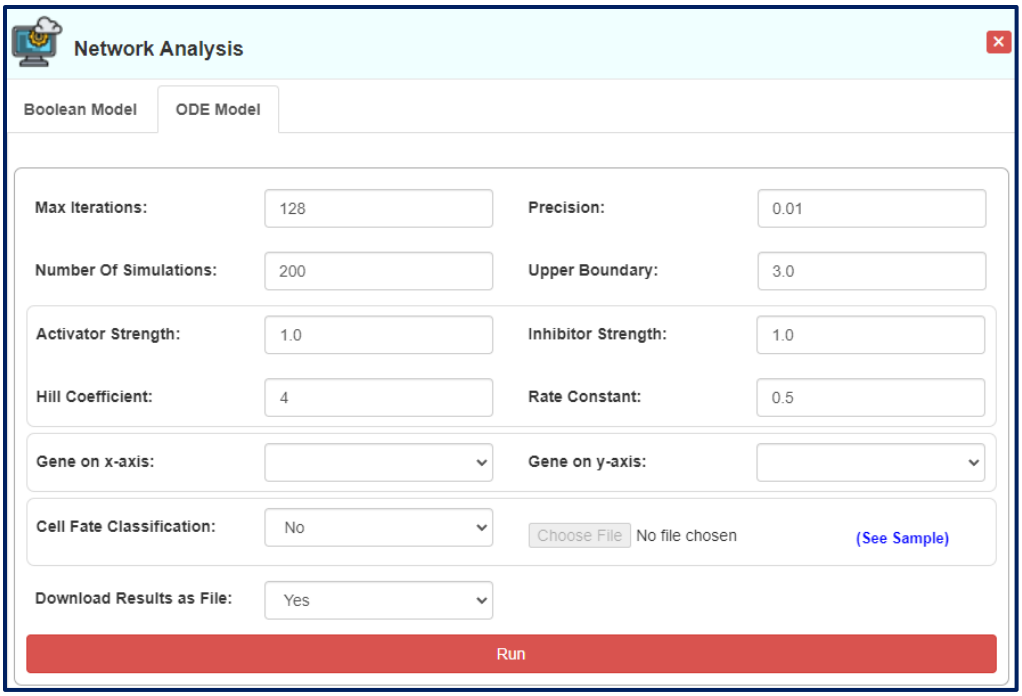


Figure 1.3.1.16 - Network analysis window for ODE analysis.

Parameters for Ordinary Differential Equation (ODE) analysis are as follows:

- **Max Iterations**: Number of iterations when solving the master equations to obtain probability distribution.
- **Precision:** Minimum Euclidean distance between two successive iterations of a simulation for which the simulation will keep running (the solver will calculate successive iterations of each simulation and stop only if a time limit is reached or distance between two successive iterations is less than this value).
- **Number of Simulations:** Number of initial points generated for which the trajectory to an attractor is calculated.
- **Upper Boundary:** The maximum allowed value for each gene in initial points.
- **Activator Strength:** The strength of any positive interaction in the network.
- **Inhibitor Strength:** The strength of any negative interaction in the network.
- **Hill Coefficient:** A measure of cooperative interaction among biomolecules.
- **Rate Constant:** An activation or inhibition constant.
- **Gene on x-axis:** Gene on the x-axis of the ODE landscape.
- **Gene on y-axis:** Gene on the y-axis of the ODE landscape.
- **Cell Fate Classification:** Attractors are mapped to user-defined cell states such as apoptosis, cell cycle arrest, uncontrolled proliferation, etc.
- **Download Result as File:** This allows the user to download the analysis results as a (.zip) file.

A summary of the analyses associated with each network type is provided in **Table 1.3.1**. Moreover, an in-detail workflow for rules and weight-based networks is provided in **Figure 1.3.1.17** and **Figure 1.3.1.18.**

Table 1.3.1 - Network types with their associated analyses

Network Type	Analysis Type	Applicable	Associated Results	Output Result File	Components of Output Result File
Rules-based Network	Deterministic Analysis	✓	Attractor Landscape	Summary and RA Files	1) One output result file with all cell fates, their basin size ratios and each network node's steady state value, etc. Along with average node propensities, individual cell fates and total converged and unconverged states. 2) A robustness analysis (RA) results file with separated cell fates and mean basin size ratios along with their SD and SEM.
		✓	Cell Fate Landscape		
Weight-based Network	Deterministic Analysis	✓	Attractor Landscape	Detail, Summary and RA File	1) If user provides cell fates, they will receive a detailed file with each fate's individual network node propensities in 0s and 1s. 2) Summary file with mean of each network node plotted with the cell fate for cyclic attractors. Along with average node propensities, individual cell fates and total converged and unconverged states. 3) Robustness Analysis (RA) results file with separated cell fates and mean basin size ratios along with their SD and SEM.
		✓	Cell Fate Landscape		
	Probabilistic Analysis	✓	Potential Energy Landscape	Detail and RA Files	1) File containing cell fate and probability along with network node value. 2) A robustness analysis (RA) results file with separated cell fates and mean basin size ratios along with their SD and SEM.
		✓	Probability Landscape		
		✓	Cell Fate Landscape		
	ODE Analysis	✓	ODE Potential Energy Landscape	Summary and Matrix Files	1) ODE result file with node value, name, percentage of attractor contribution. 2) Detailed 2-D matrix file

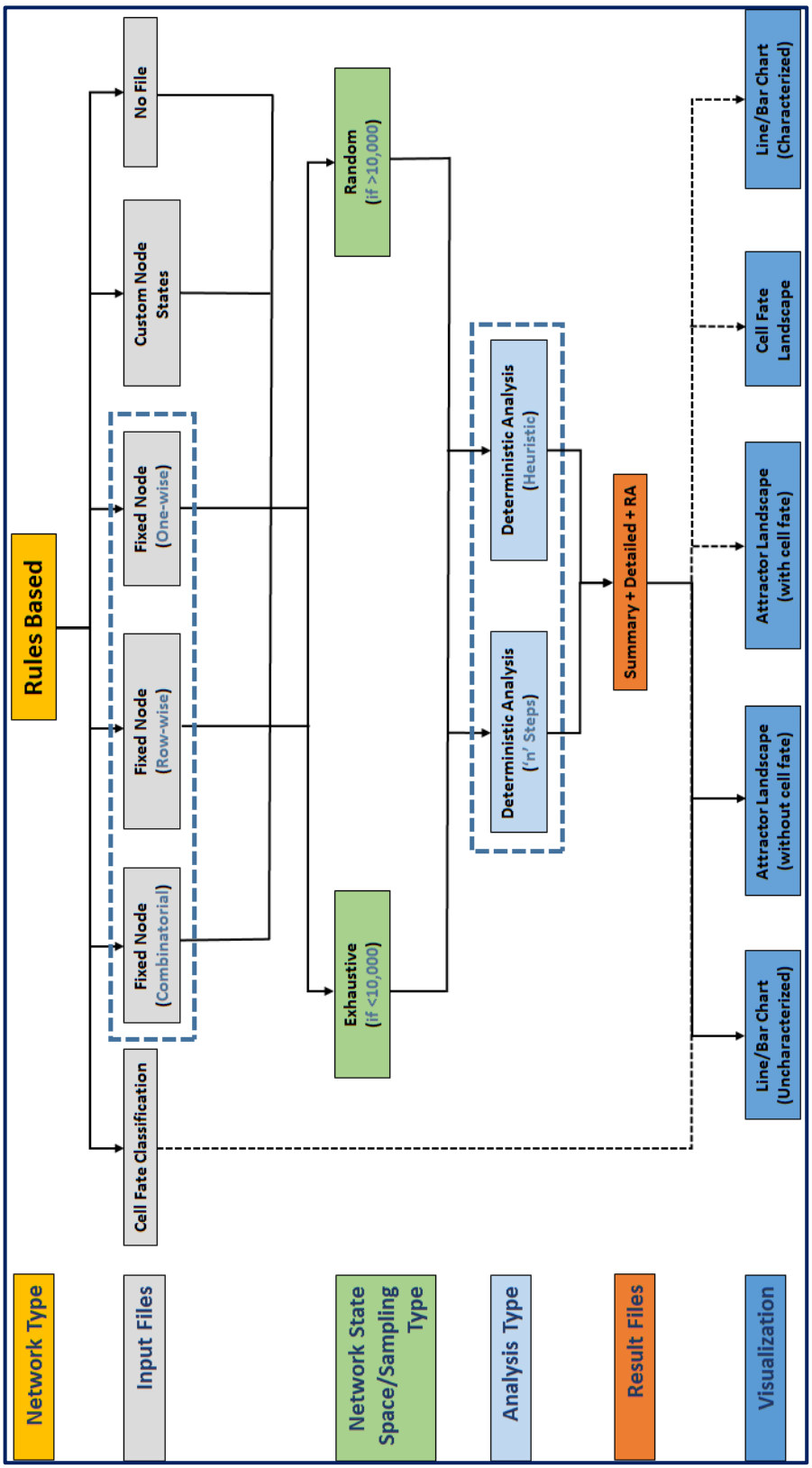


Figure 1.3.1.17 - TISON's rules-based networks pipeline.

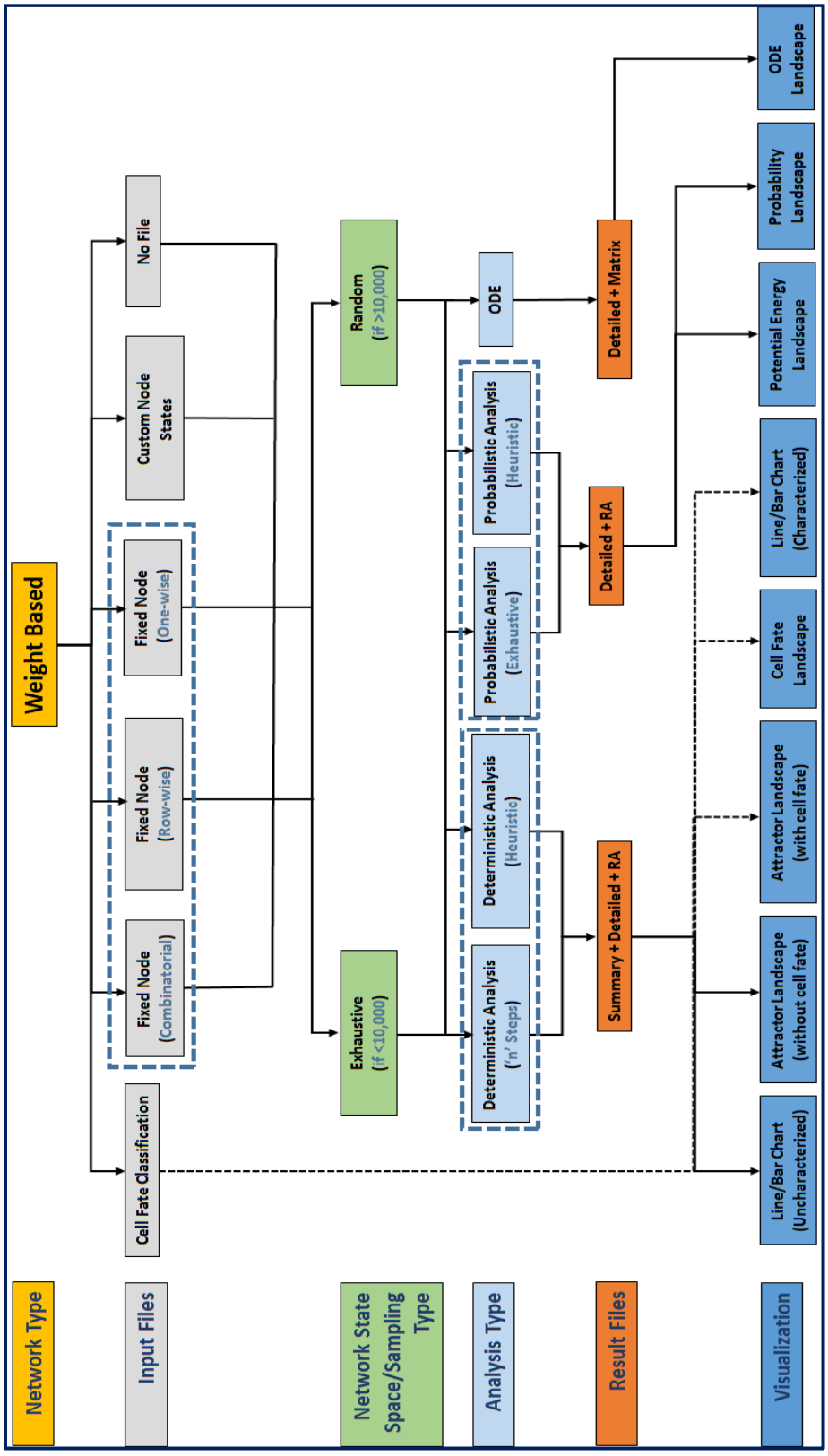


Figure 1.3.1.18 - TISON's weight-based networks pipeline.

1.3.2 'Analysis Progress and Status' Button

In order to keep track of the analyses, Deterministic Analysis (DA), Probabilistic Analysis (PA) and/or Ordinary Differential Equation Analysis (ODE), that are being run on one or multiple networks, the 'Analysis Progress and Status' button (highlighted in **Figure 1.3.2.1**) can be used.

Figure 1.3.2.1 - 'Analysis Progress and Status' button in NE.

The window shown in **Figure 1.3.2.2** appears upon clicking the 'Analysis Progress and Status' button. This allows the user to view the name of the networks that are being analyzed, the stage that is being processed, the percentage of progress and the type of analysis along with the type of network (weight-based or rules-based). Moreover, the user may also stop a specific analysis of a network by clicking on the 'Stop' button for that network analysis.

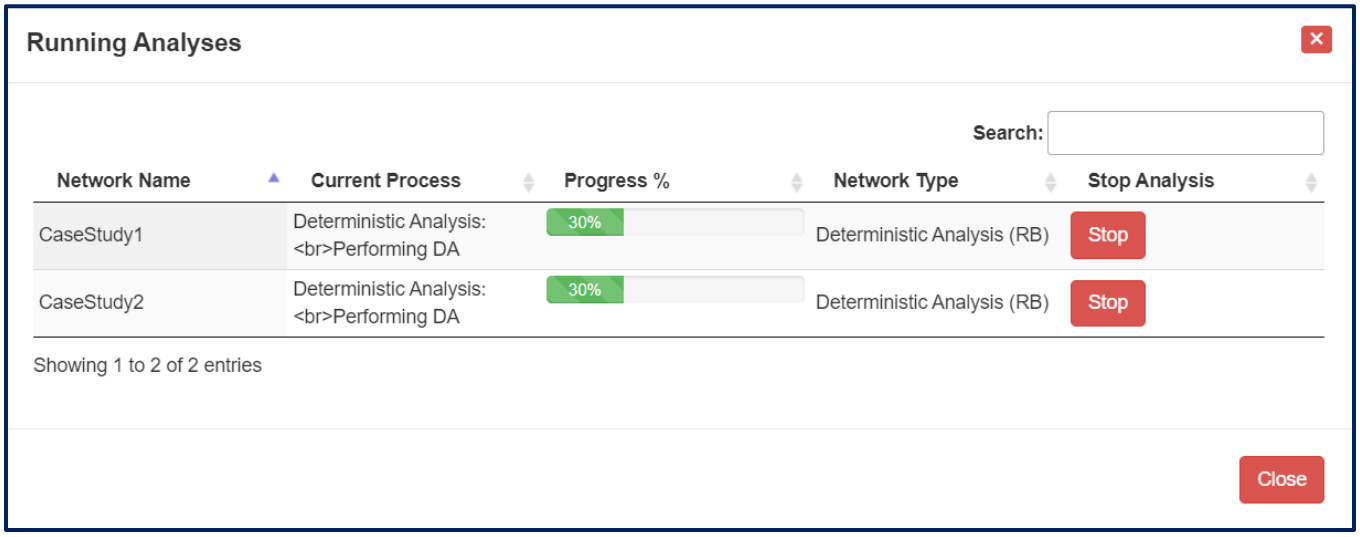


Figure 1.3.2.2 - Window that shows the progress of the analyses that are in process.

1.3.3 'View Network and Results' Button

After performing an analysis, the result can be viewed as an 'Attractor Landscape', 'Cell Fate Landscape', 'Potential Energy Landscape', 'Probability Landscape', and 'ODE Landscape'. Moreover, the results of robustness analysis can also be viewed using 'View Results of Robustness Analysis' and 'View Line Graph of Robustness Analysis' option. The 'View Network and Results' button highlighted in **Figure 1.3.3.1** will help the user to view the network and switch between its various result types.

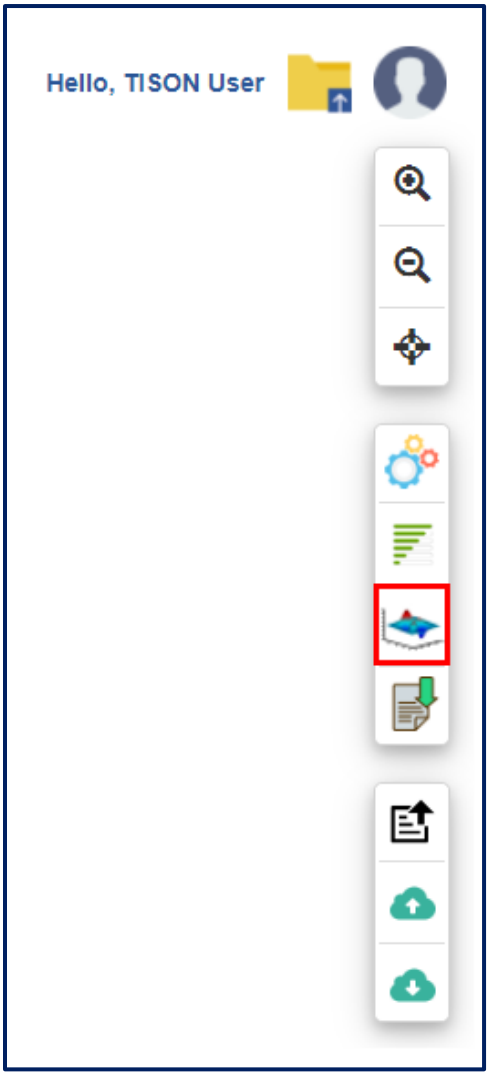


Figure 1.3.3.1 - 'View Network and Results' button.

The context list shown in **Figure 1.3.3.2** will appear upon clicking the 'View Network and Results' button – the user can proceed with any of the mentioned landscapes.

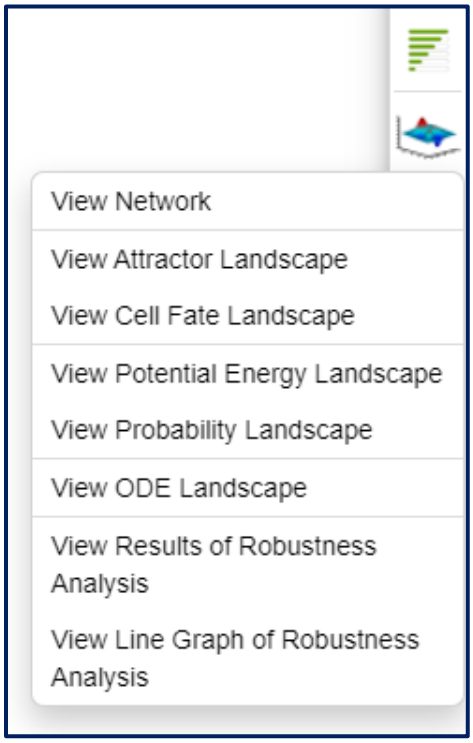


Figure 1.3.3.2 - 'View Network and Results' button with its window.

i. Attractor Landscape

An 'Attractor Landscape' can be viewed in **Figure 1.3.3.3**. The attractor height indicates the propensity of that particular network state.

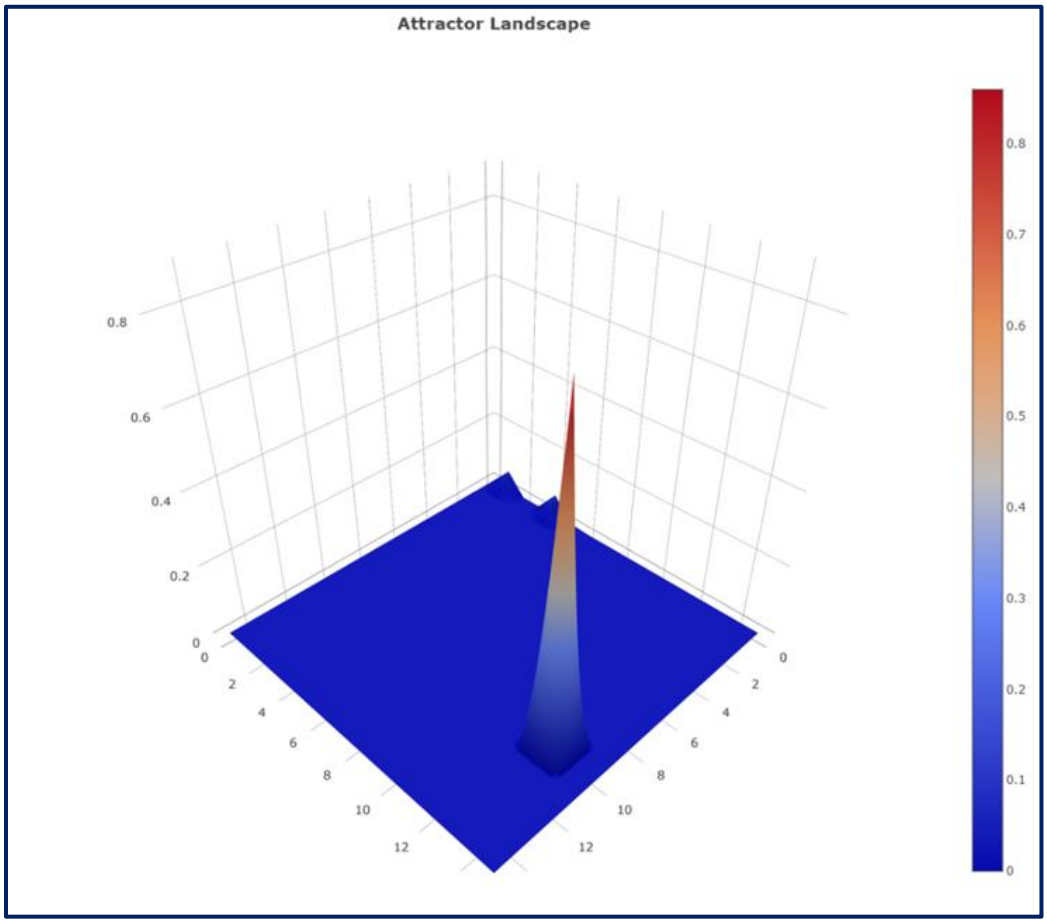


Figure 1.3.3.3 - Attractor landscape.

ii. Cell Fate Landscape

If cell fate classification has been performed by uploading the relevant file, the user can also view their results in the form of a 'Cell Fate Landscape' as shown in **Figure 1.3.3.4**.

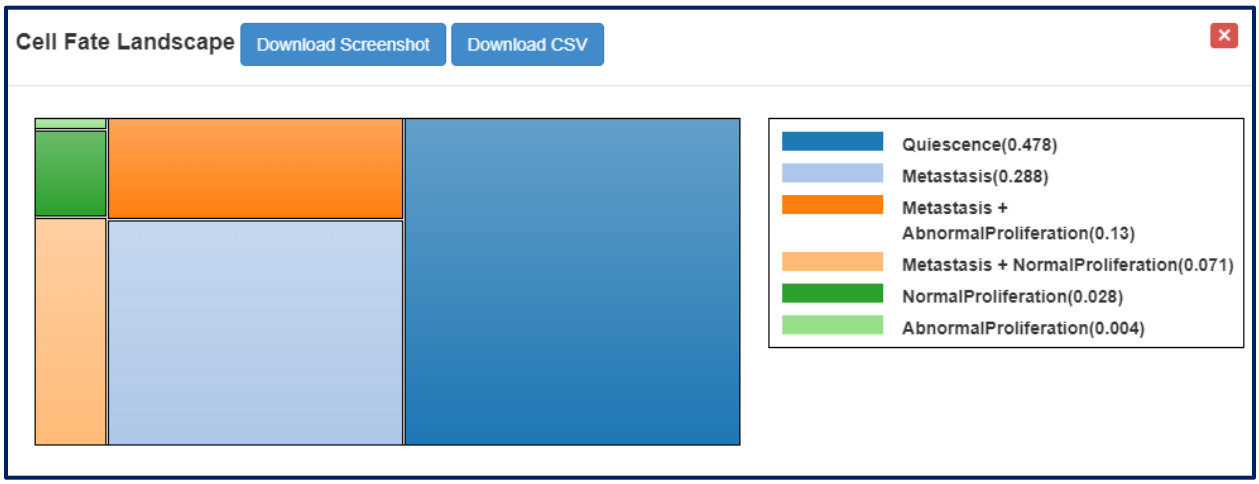


Figure 1.3.3.4 - Cell fate classification landscape.

iii. Potential Energy Landscape

After probabilistic analysis has been performed, the user can view their results in the form of a 'Potential Energy Landscape' where the peaks represent low energy/stable states. A potential energy landscape is shown in **Figure 1.3.3.5**.

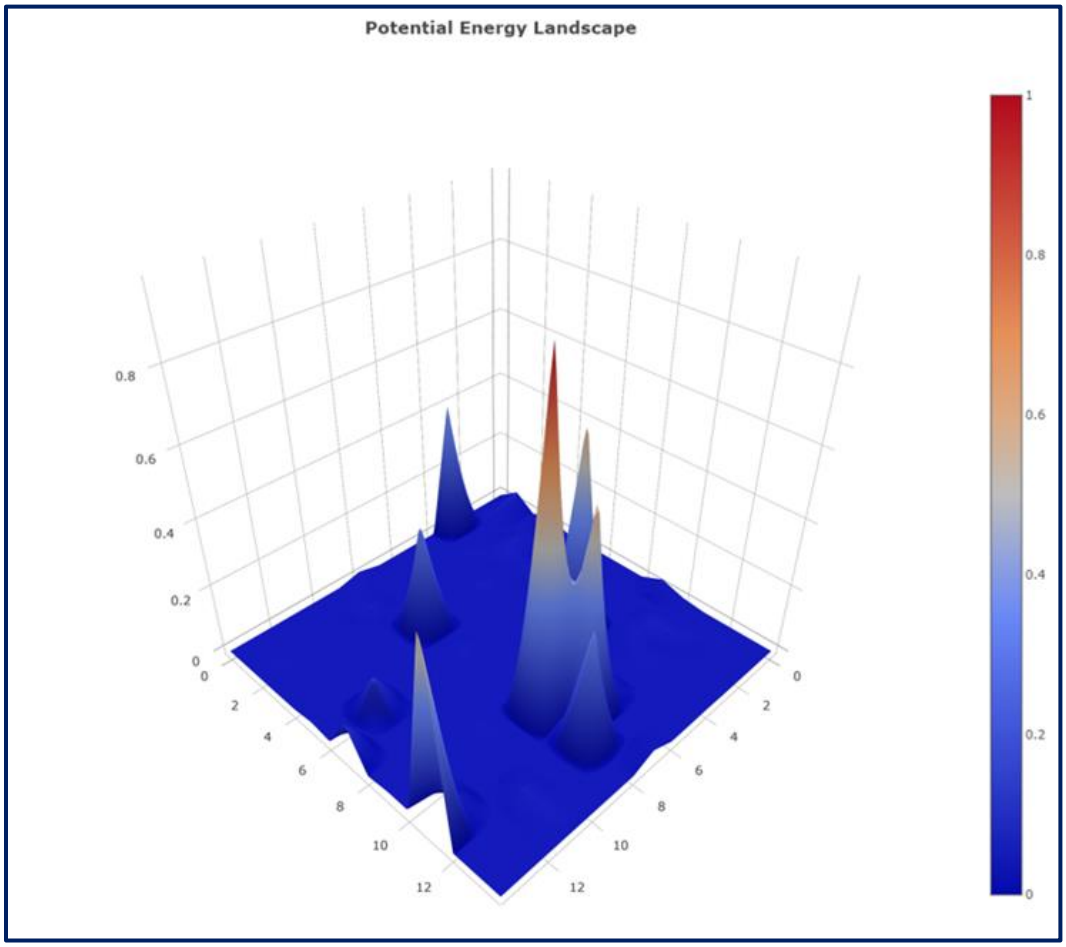


Figure 1.3.3.5 - Potential energy landscape.

iv. Probability Landscape

After probabilistic analysis has been performed, the user can view their results in the form of a 'Probability Landscape' where the peaks represent high probability states. A 'Probability Landscape' is shown in **Figure 1.3.3.6**.

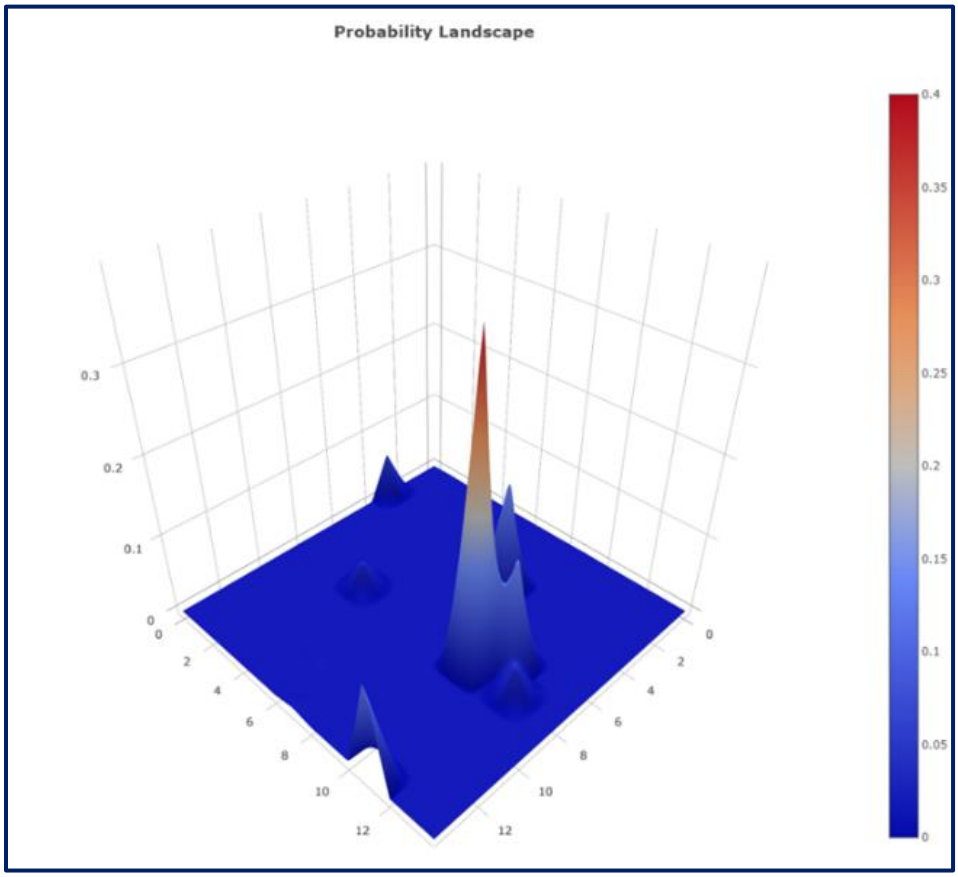


Figure 1.3.3.6 - Probability landscape.

v. ODE Landscape

After performing the ODE analysis, the user can click on 'View ODE Landscape' to view the 'ODE Potential Energy Landscape'; an example is shown in **Figure 1.3.3.7**.

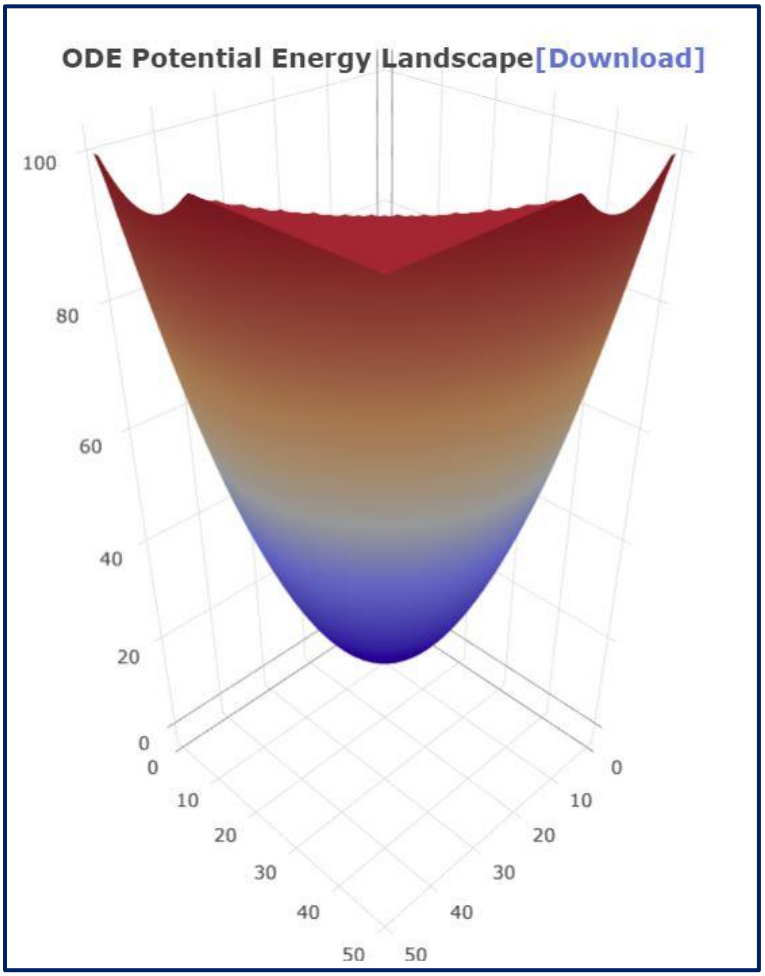


Figure 1.3.3.7 - ODE potential energy landscape.

iv. Robustness Analysis

The user can view two results from robustness analysis (RA) by choosing 'View Results of Robustness Analysis' (**Figure 1.3.3.8**) and 'View Line Graph of Robustness Analysis' (**Figure 1.3.3.9**).The figure below shows the results for 'View Results of Robustness Analysis' (For visual reference, see Section TISON's Networks Editor Video Tutorials, Table 1.4.1 - Video 5).

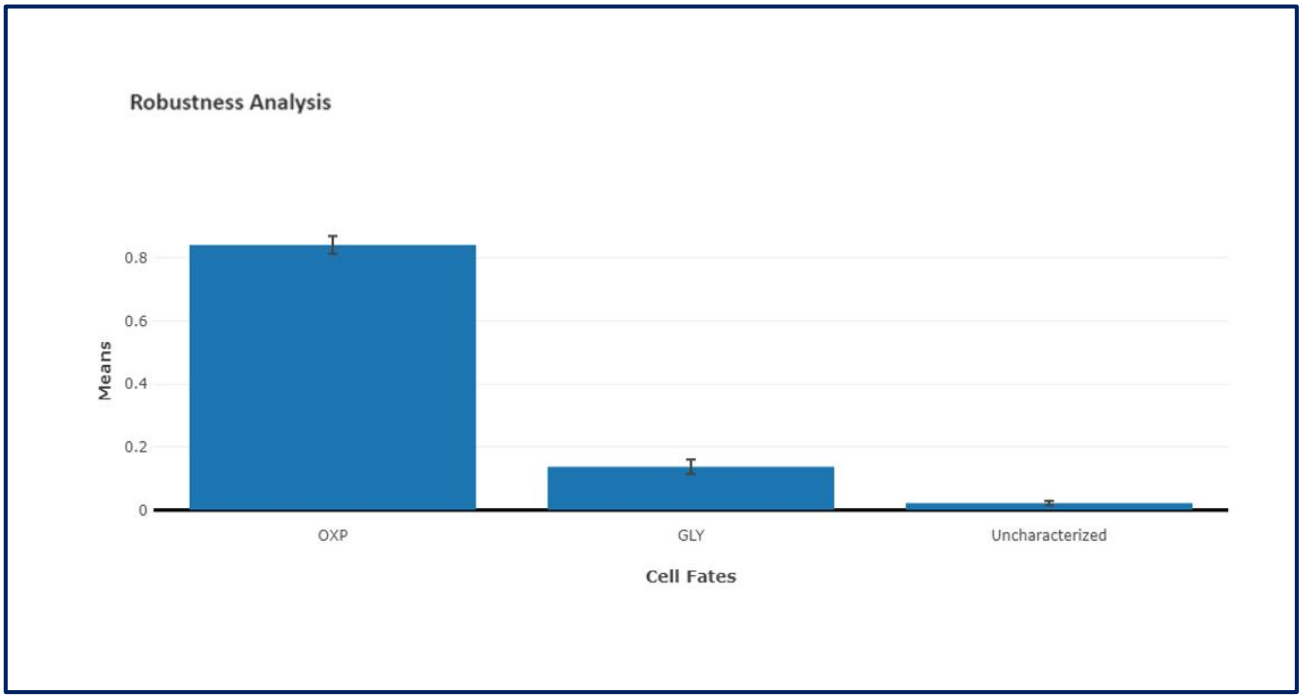


Figure 1.3.3.8 - Robustness analysis showing deviation and sensitivity of cell fates basin size ratios.

The line graph for robustness analysis is illustrated in **Figure 1.3.3.9**.

Figure 1.3.3.9 - Robustness analysis line graph.

1.3.4 'Download Results' Button

After performing network analysis, the user can download the results by clicking on the button highlighted in **Figure 1.3.4.1**.

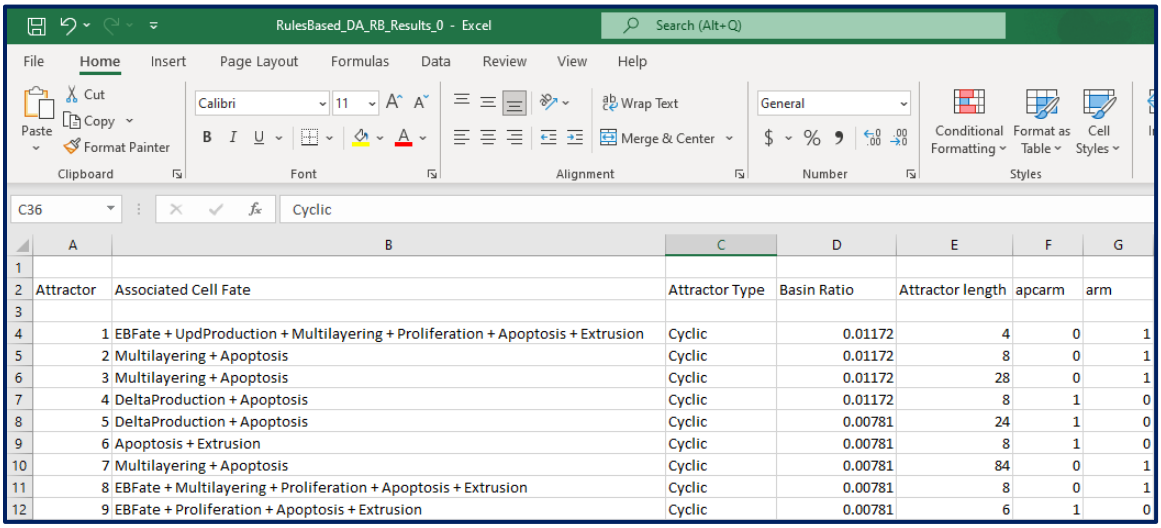
Figure 1.3.4.1 - 'Download Results' button in NE.

Result files for the last analysis performed by the user will be downloaded as comma-separated files (format .csv).

i. Deterministic Analysis (DA)

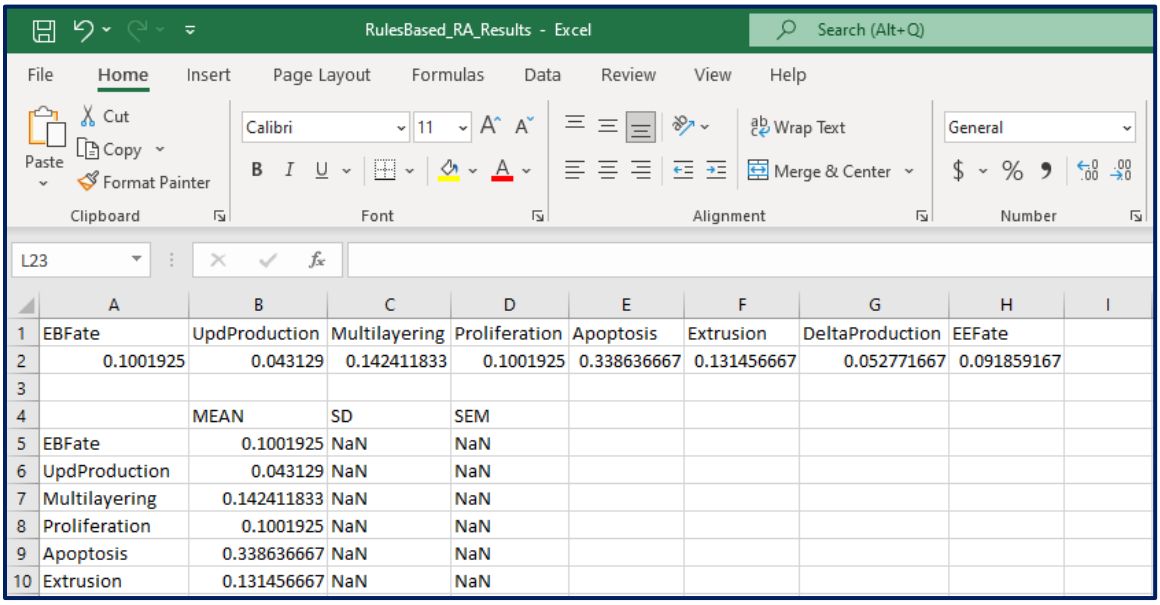
Performing DA will allow the user to download the different files for multi-input analyses.

For a *rules-based network*, a DA file (**Figure 1.3.4.2**) and an RA file (**Figure 1.3.4.3**) are generated.



	A	B	C	D	E	F	G
1							
2	Attractor	Associated Cell Fate	Attractor Type	Basin Ratio	Attractor length	apcarm	arm
3							
4	1	EBFate + UpdProduction + Multilayering + Proliferation + Apoptosis + Extrusion	Cyclic	0.01172	4	0	1
5	2	Multilayering + Apoptosis	Cyclic	0.01172	8	0	1
6	3	Multilayering + Apoptosis	Cyclic	0.01172	28	0	1
7	4	DeltaProduction + Apoptosis	Cyclic	0.01172	8	1	0
8	5	DeltaProduction + Apoptosis	Cyclic	0.00781	24	1	0
9	6	Apoptosis + Extrusion	Cyclic	0.00781	8	1	0
10	7	Multilayering + Apoptosis	Cyclic	0.00781	84	0	1
11	8	EBFate + Multilayering + Proliferation + Apoptosis + Extrusion	Cyclic	0.00781	8	0	1
12	9	EBFate + Proliferation + Apoptosis + Extrusion	Cyclic	0.00781	6	1	0

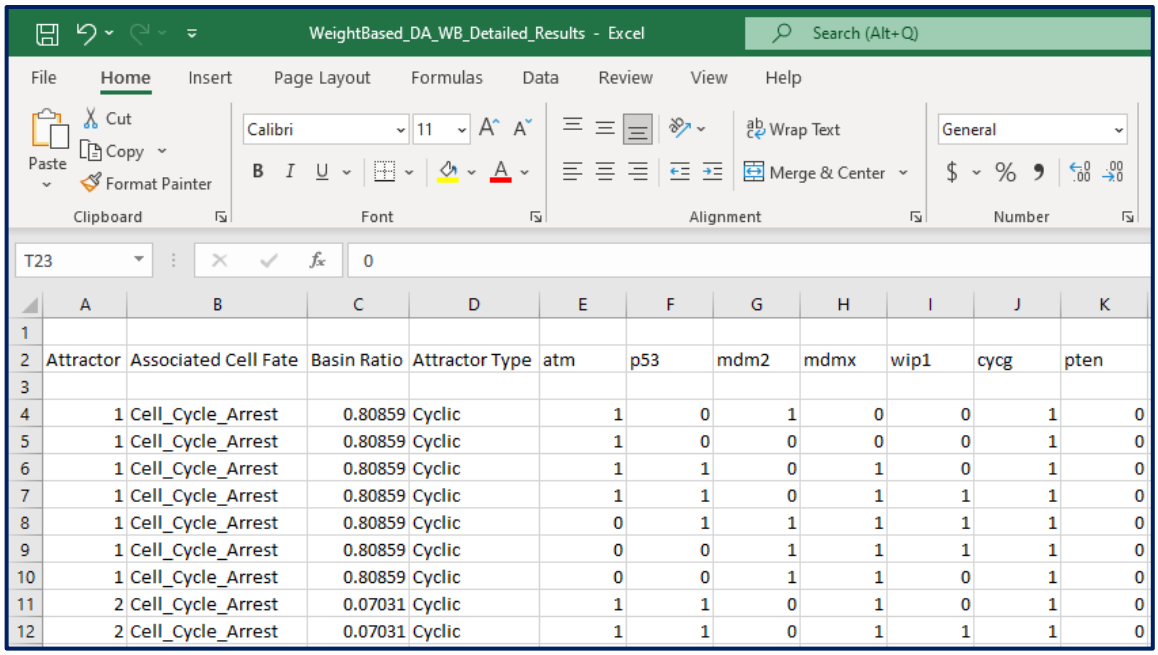
Figure 1.3.4.2 - DA results file for a rules-based DA.



	A	B	C	D	E	F	G	H	I
1	EBFate	UpdProduction	Multilayering	Proliferation	Apoptosis	Extrusion	DeltaProduction	EEFate	
2	0.1001925	0.043129	0.142411833	0.1001925	0.338636667	0.131456667	0.052771667	0.091859167	
3									
4		MEAN	SD	SEM					
5	EBFate	0.1001925	NaN	NaN					
6	UpdProduction	0.043129	NaN	NaN					
7	Multilayering	0.142411833	NaN	NaN					
8	Proliferation	0.1001925	NaN	NaN					
9	Apoptosis	0.338636667	NaN	NaN					
10	Extrusion	0.131456667	NaN	NaN					

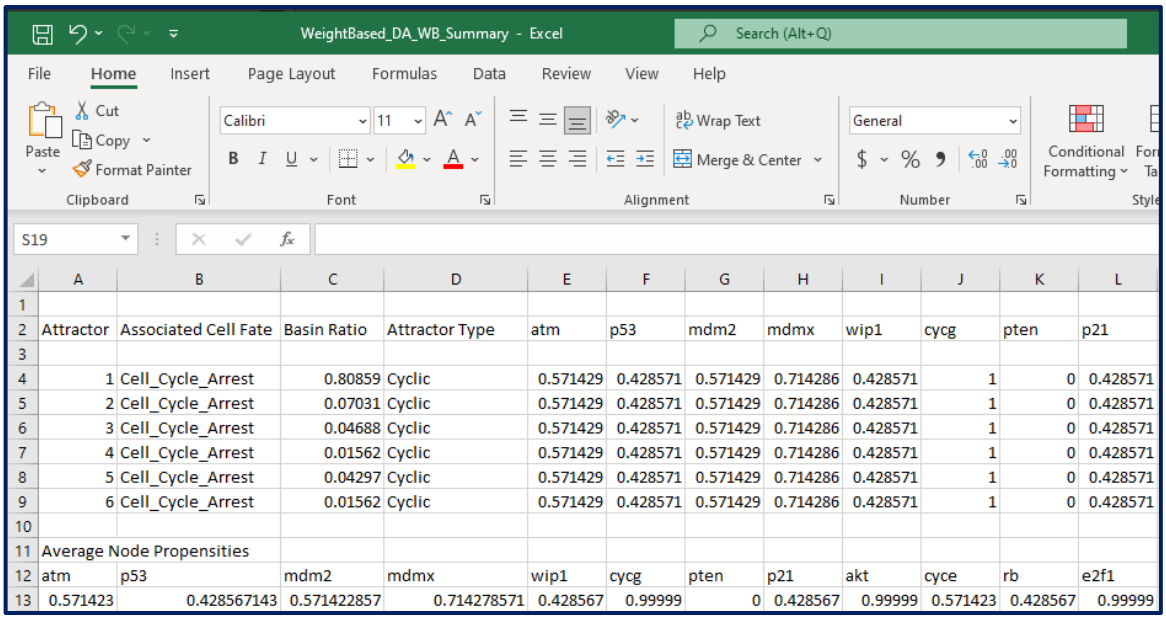
Figure 1.3.4.3 - RA results file for a rules-based DA.

For a *weight-based network*, a detailed result file (**Figure 1.3.4.4**), a summary file (**Figure 1.3.4.5**), and a RA file (**Figure 1.3.4.6**) can be downloaded after performing DA. The detailed file displays every node state in each attractor in terms of 0 or 1, whereas the summary file contains the average node value for each node if the attractor is the same.



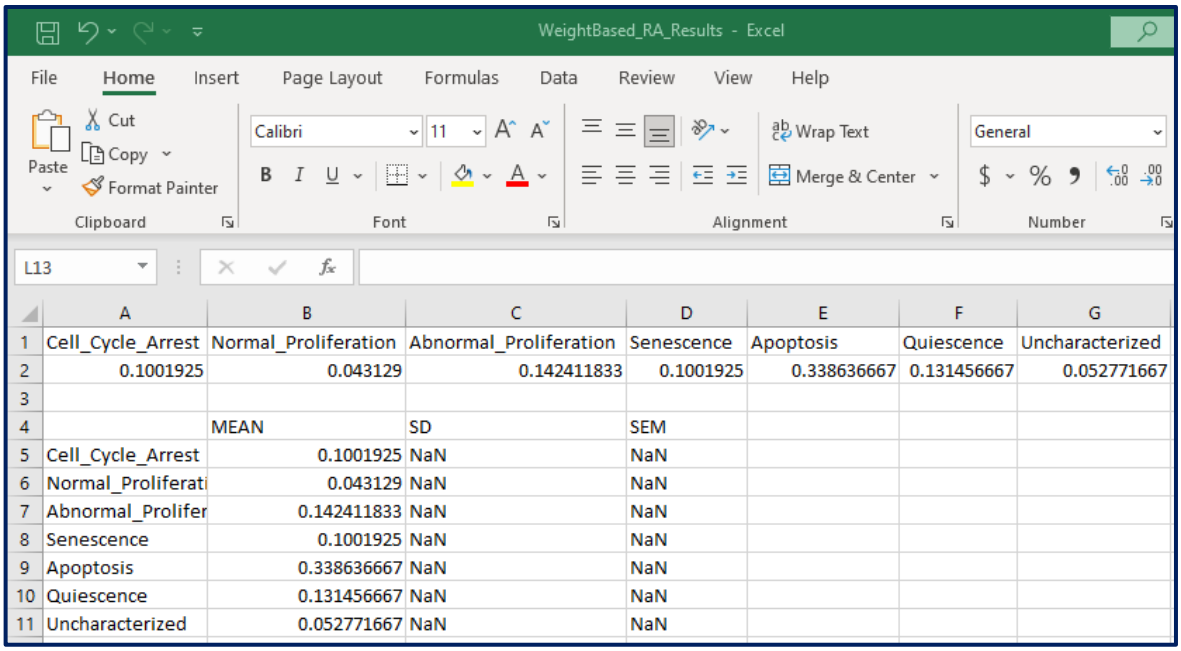
	A	B	C	D	E	F	G	H	I	J	K
1											
2	Attractor	Associated Cell Fate	Basin Ratio	Attractor Type	atm	p53	mdm2	mdmx	wip1	cycg	pten
3											
4	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	0	1	0	0	1	0
5	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	0	0	0	0	1	0
6	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	1	0	1	0	1	0
7	1	Cell_Cycle_Arrest	0.80859	Cyclic	1	1	0	1	1	1	0
8	1	Cell_Cycle_Arrest	0.80859	Cyclic	0	1	1	1	1	1	0
9	1	Cell_Cycle_Arrest	0.80859	Cyclic	0	0	1	1	1	1	0
10	1	Cell_Cycle_Arrest	0.80859	Cyclic	0	0	1	1	0	1	0
11	2	Cell_Cycle_Arrest	0.07031	Cyclic	1	1	0	1	0	1	0
12	2	Cell_Cycle_Arrest	0.07031	Cyclic	1	1	0	1	1	1	0

Figure 1.3.4.4 - Detailed results file for a weight-based DA.



	A	B	C	D	E	F	G	H	I	J	K	L
1												
2	Attractor	Associated Cell Fate	Basin Ratio	Attractor Type	atm	p53	mdm2	mdmx	wip1	cycg	pten	p21
3												
4	1	Cell_Cycle_Arrest	0.80859	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
5	2	Cell_Cycle_Arrest	0.07031	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
6	3	Cell_Cycle_Arrest	0.04688	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
7	4	Cell_Cycle_Arrest	0.01562	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
8	5	Cell_Cycle_Arrest	0.04297	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
9	6	Cell_Cycle_Arrest	0.01562	Cyclic	0.571429	0.428571	0.571429	0.714286	0.428571	1	0	0.428571
10												
11	Average Node Propensities											
12	atm	p53	mdm2	mdmx	wip1	cycg	pten	p21	akt	cyce	rb	e2f1
13	0.571423	0.428567143	0.571422857	0.714278571	0.428567	0.99999	0	0.428567	0.99999	0.571423	0.428567	0.99999

Figure 1.3.4.5 - Summary file for a weight-based DA.



	A	B	C	D	E	F	G
1	Cell_Cycle_Arrest	Normal_Proliferation	Abnormal_Proliferation	Senescence	Apoptosis	Quiescence	Uncharacterized
2	0.1001925	0.043129	0.142411833	0.1001925	0.338636667	0.131456667	0.052771667
3							
4		MEAN	SD	SEM			
5	Cell_Cycle_Arrest	0.1001925	NaN	NaN			
6	Normal_Proliferati	0.043129	NaN	NaN			
7	Abnormal_Prolifer	0.142411833	NaN	NaN			
8	Senescence	0.1001925	NaN	NaN			
9	Apoptosis	0.338636667	NaN	NaN			
10	Quiescence	0.131456667	NaN	NaN			
11	Uncharacterized	0.052771667	NaN	NaN			

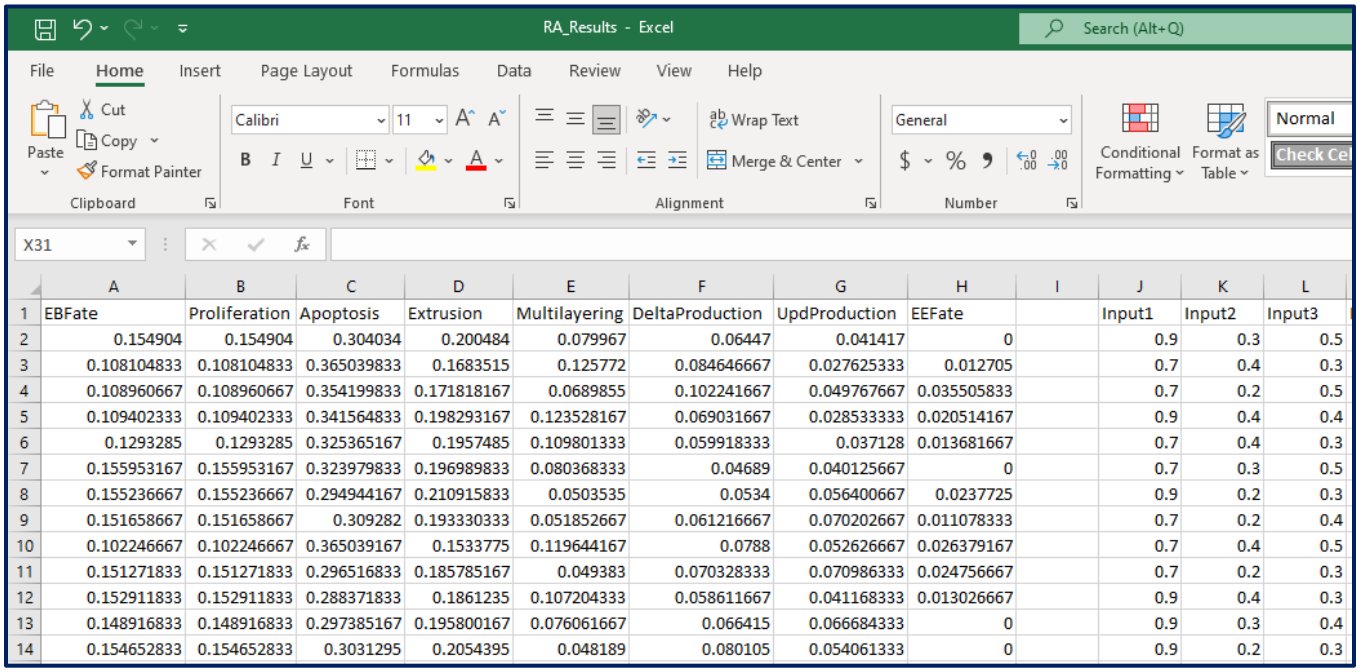
Figure 1.3.4.6 - RA result file for a weight-based DA.

The robustness result file (**Figure 1.3.4.3**) displays the occurrence of each phenotype with respect to the inputs as well as the overall mean, standard deviation (SD), and standard error of means (SEM) of cell fates. The column headers in the files are as explained below:

- **Attractor:** Serial number and rank (in terms of highest basin size ratio) of an attractor.
- **Associated Cell Fate:** The fate assigned to an attractor using its node values and cell fate logic file. If fate is not categorized or if cell fate logic is not assigned, the respective states will be 'Uncharacterized'.
- **Basin Ratio:** The ratio of initial states which converge to an attractor.
- **Attractor Type:** Either cyclic or point. This signifies whether the attractor continuously moves through a finite set of states or remains in a single state.
- **Attractor Length:** The number of states in an attractor. Point attractor has an attractor length of 1, while cyclic attractor toggles between the Boolean states having attractor length > 1 (only for rules-based networks).
- **Average Node Propensities:** The average node activity after analysis is calculated by multiplying node activity for each attractor with its respective basin size ratio and then summed over all attractors (explained in figures).
- **Rest of the columns:** Values of nodes in the respective states.
- **Individual Cell Fates:** The cell fate assigned to an attractor generates individual cell fates which are also summarized at the bottom of file.
- **Total Sample Size:** The user-specified number of states from the total state-space is given along with the number of states converged. If any state is not converged during the analysis, then state is given in

the file.

- **Total States Converged:** The number of state space which converged towards forming attractors.

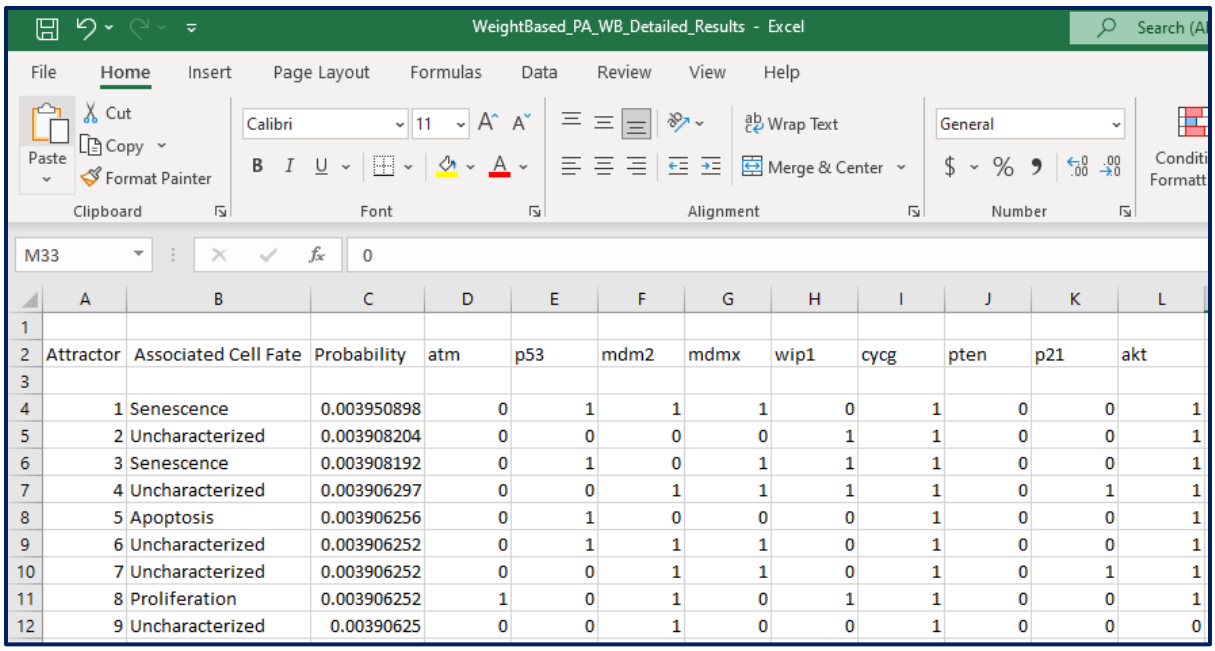


	A	B	C	D	E	F	G	H	I	J	K	L
1	EBFate	Proliferation	Apoptosis	Extrusion	Multilayering	DeltaProduction	UpdProduction	EEFate		Input1	Input2	Input3
2	0.154904	0.154904	0.304034	0.200484	0.079967	0.06447	0.041417	0		0.9	0.3	0.5
3	0.108104833	0.108104833	0.365039833	0.1683515	0.125772	0.084646667	0.027625333	0.012705		0.7	0.4	0.3
4	0.108960667	0.108960667	0.354199833	0.171818167	0.0689855	0.102241667	0.049767667	0.035505833		0.7	0.2	0.5
5	0.109402333	0.109402333	0.341564833	0.198293167	0.123528167	0.069031667	0.028533333	0.020514167		0.9	0.4	0.4
6	0.1293285	0.1293285	0.325365167	0.1957485	0.109801333	0.059918333	0.037128	0.013681667		0.7	0.4	0.3
7	0.155953167	0.155953167	0.323979833	0.196989833	0.080368333	0.04689	0.040125667	0		0.7	0.3	0.5
8	0.155236667	0.155236667	0.294944167	0.210915833	0.0503535	0.0534	0.056400667	0.0237725		0.9	0.2	0.3
9	0.151658667	0.151658667	0.309282	0.193330333	0.051852667	0.061216667	0.070202667	0.011078333		0.7	0.2	0.4
10	0.102246667	0.102246667	0.365039167	0.1533775	0.119644167	0.0788	0.052626667	0.026379167		0.7	0.4	0.5
11	0.151271833	0.151271833	0.296516833	0.185785167	0.049383	0.070328333	0.070986333	0.024756667		0.7	0.2	0.3
12	0.152911833	0.152911833	0.288371833	0.1861235	0.107204333	0.058611667	0.041168333	0.013026667		0.9	0.4	0.3
13	0.148916833	0.148916833	0.297385167	0.195800167	0.076061667	0.066415	0.066684333	0		0.9	0.3	0.4
14	0.154652833	0.154652833	0.3031295	0.2054395	0.048189	0.080105	0.054061333	0		0.9	0.2	0.3

***Figure 1.3.4.7 - Robustness result file for multi-input analysis**: In multiple row-wise and combinatorial fixed nodes states.*

ii. Probabilistic Analysis (PA)

For PA, the following *weight-based* probabilistic result file is produced (**Figure 1.3.4.8**) and an RA file, once the analysis is completed (Error! Reference source not found.).

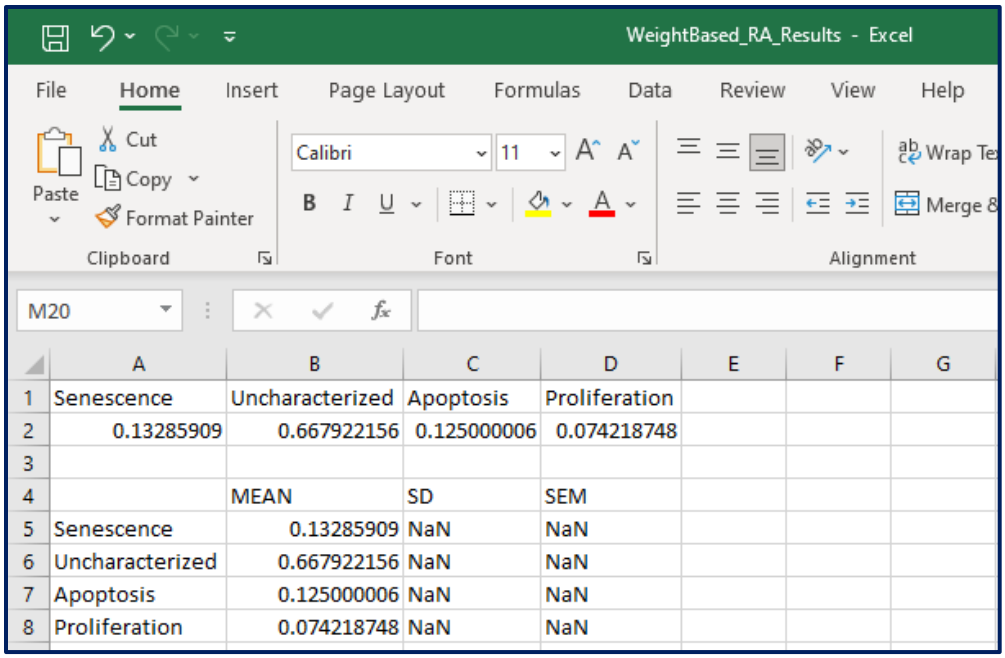


	A	B	C	D	E	F	G	H	I	J	K	L
1												
2	Attractor	Associated Cell Fate	Probability	atm	p53	mdm2	mdmx	wip1	cycg	pten	p21	akt
3												
4	1	Senescence	0.003950898	0	1	1	1	0	1	0	0	1
5	2	Uncharacterized	0.003908204	0	0	0	0	1	1	0	0	1
6	3	Senescence	0.003908192	0	1	0	1	1	1	0	0	1
7	4	Uncharacterized	0.003906297	0	0	1	1	1	1	0	1	1
8	5	Apoptosis	0.003906256	0	1	0	0	0	1	0	0	1
9	6	Uncharacterized	0.003906252	0	1	1	1	0	1	0	0	1
10	7	Uncharacterized	0.003906252	0	0	1	1	0	1	0	1	1
11	8	Proliferation	0.003906252	1	0	1	0	1	1	0	0	1
12	9	Uncharacterized	0.00390625	0	0	1	0	0	1	0	0	0

Figure 1.3.4.8 - Result file for weight-based PA.

In addition to the parameters similar to **i. Deterministic Analysis (DA)** described above, the PA result file has the following:

- **Probability:** Steady-state probabilities of the respective state (probability of finding system in state on random observation).



WeightBased_RA_Results - Excel

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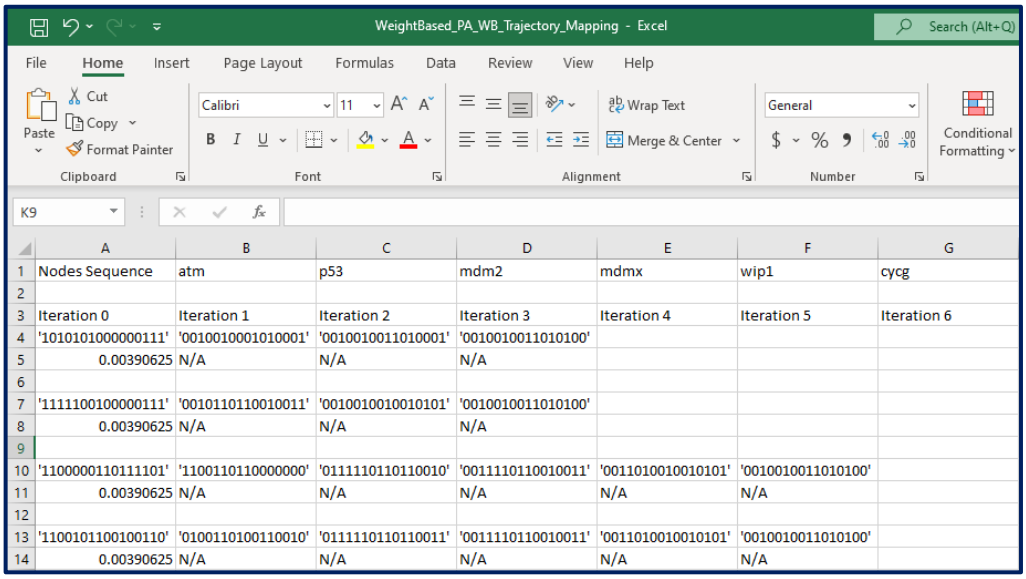
	A	B	C	D	E	F	G
1	Senescence	Uncharacterized	Apoptosis	Proliferation			
2	0.13285909	0.667922156	0.125000006	0.074218748			
3							
4		MEAN	SD	SEM			
5	Senescence	0.13285909	NaN	NaN			
6	Uncharacterized	0.667922156	NaN	NaN			
7	Apoptosis	0.125000006	NaN	NaN			
8	Proliferation	0.074218748	NaN	NaN			

Figure 1.3.4.9 - RA Result file for weight-based PA.

- **Trajectory Mapping**: In case user selects 'Trajectory Mapping' in the analysis modal in **Figure 1.3.1.2**, the following network trajectory file is also downloaded.



WeightBased_PA_WB_Trajectory_Mapping - Excel

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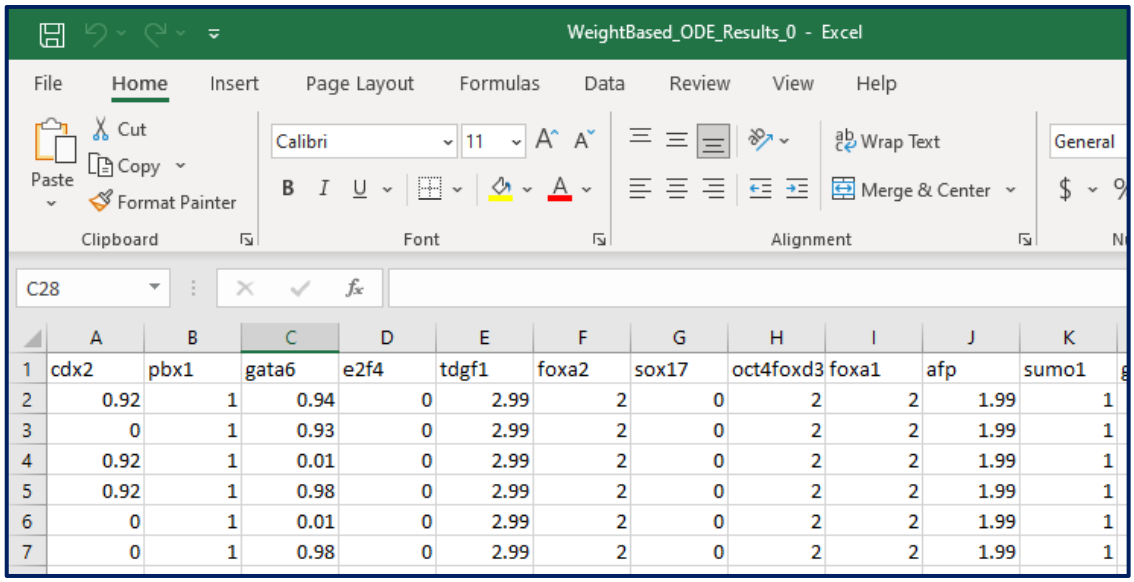
	A	B	C	D	E	F	G
1	Nodes Sequence	atm	p53	mdm2	mdmx	wip1	cycg
2							
3	Iteration 0	Iteration 1	Iteration 2	Iteration 3	Iteration 4	Iteration 5	Iteration 6
4	'1010101000000111'	'0010010001010001'	'0010010011010001'	'0010010011010100'			
5	0.00390625	N/A	N/A	N/A			
6							
7	'1111100100000111'	'0010110110010011'	'0010010010010101'	'0010010011010100'			
8	0.00390625	N/A	N/A	N/A			
9							
10	'1100000110111101'	'1100110110000000'	'0111110110110010'	'0011110110010011'	'0011010010010101'	'0010010011010100'	
11	0.00390625	N/A	N/A	N/A	N/A	N/A	
12							
13	'1100101100100110'	'0100110100110010'	'0111110110110011'	'0011110110010011'	'0011010010010101'	'0010010011010100'	
14	0.00390625	N/A	N/A	N/A	N/A	N/A	

Figure 1.3.4.10 - Output result file for 'Trajectory Mapping'.

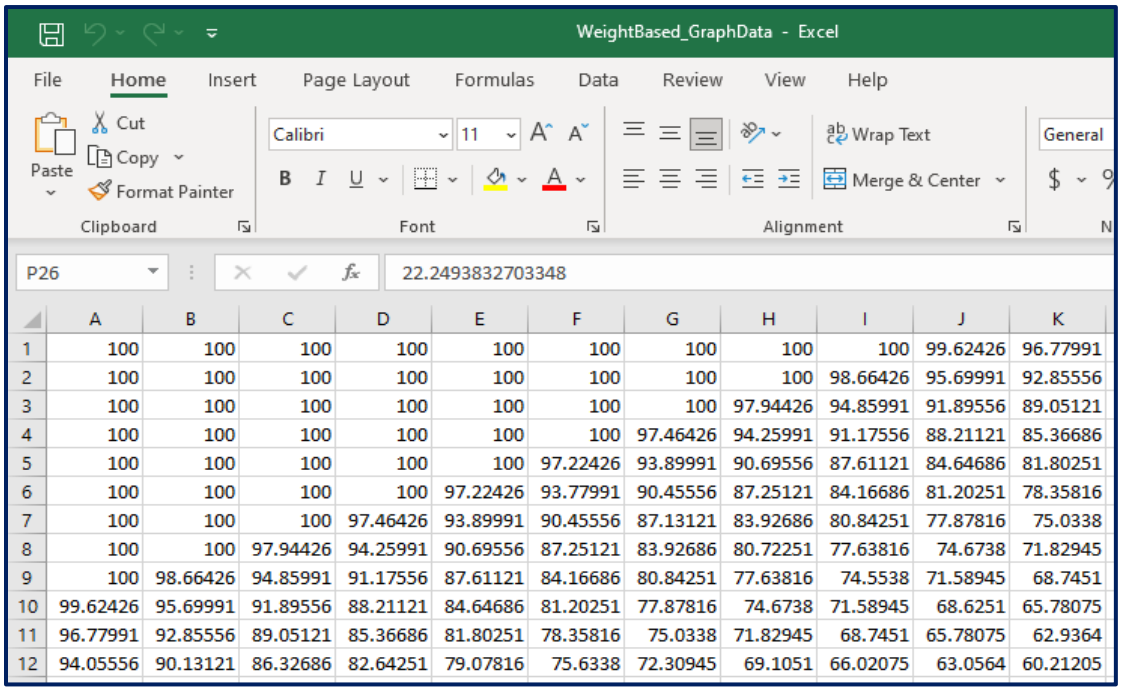
iii. Ordinary Differential Equation (ODE) Analysis

For ODE analysis, the ODE result file (**Figure 1.3.4.11**) and graph data file (**Figure 1.3.4.12**) are downloaded.



	A	B	C	D	E	F	G	H	I	J	K
1	cdx2	pbx1	gata6	e2f4	tdgf1	foxa2	sox17	oct4foxd3	foxa1	afp	sumo1
2	0.92	1	0.94	0	2.99	2	0	2	2	1.99	1
3	0	1	0.93	0	2.99	2	0	2	2	1.99	1
4	0.92	1	0.01	0	2.99	2	0	2	2	1.99	1
5	0.92	1	0.98	0	2.99	2	0	2	2	1.99	1
6	0	1	0.01	0	2.99	2	0	2	2	1.99	1
7	0	1	0.98	0	2.99	2	0	2	2	1.99	1

Figure 1.3.4.11 - Result file for a weight-based ODE analysis.



	A	B	C	D	E	F	G	H	I	J	K
1	100	100	100	100	100	100	100	100	100	99.62426	96.77991
2	100	100	100	100	100	100	100	100	98.66426	95.69991	92.85556
3	100	100	100	100	100	100	100	97.94426	94.85991	91.89556	89.05121
4	100	100	100	100	100	100	97.46426	94.25991	91.17556	88.21121	85.36686
5	100	100	100	100	100	97.22426	93.89991	90.69556	87.61121	84.64686	81.80251
6	100	100	100	100	97.22426	93.77991	90.45556	87.25121	84.16686	81.20251	78.35816
7	100	100	100	97.46426	93.89991	90.45556	87.13121	83.92686	80.84251	77.87816	75.0338
8	100	100	97.94426	94.25991	90.69556	87.25121	83.92686	80.72251	77.63816	74.6738	71.82945
9	100	98.66426	94.85991	91.17556	87.61121	84.16686	80.84251	77.63816	74.5538	71.58945	68.7451
10	99.62426	95.69991	91.89556	88.21121	84.64686	81.20251	77.87816	74.6738	71.58945	68.6251	65.78075
11	96.77991	92.85556	89.05121	85.36686	81.80251	78.35816	75.0338	71.82945	68.7451	65.78075	62.9364
12	94.05556	90.13121	86.32686	82.64251	79.07816	75.6338	72.30945	69.1051	66.02075	63.0564	60.21205

Figure 1.3.4.12 - Graph Data file a weight-based ODE analysis.

The file shown above indicates nodes along with their expression values. These results can also be downloaded by selecting the 'Download Results as File' option in 'Perform Network Analysis' window.

1.3.5 'Upload Case Study', 'Import Network' and 'Export Network' Buttons

The user can upload, import, or export case studies using the buttons shown below:

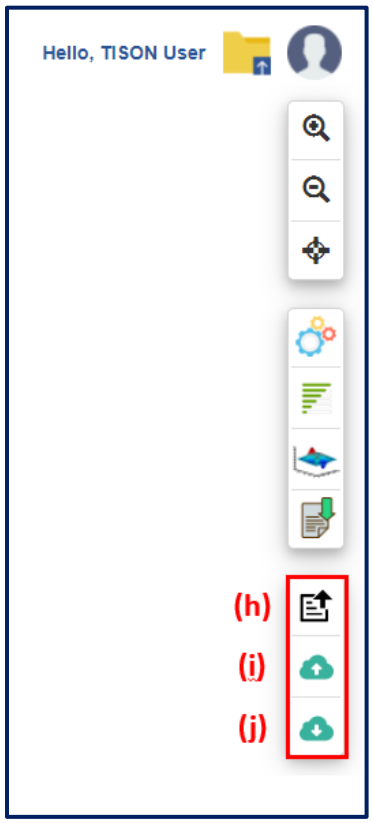


Figure 1.3.5.1 - Case Study, Import and Export buttons: *(h) 'Upload Case Study' button, (i) 'Import Network' button, (j) 'Export Network' button.*

'Upload Case Study' button (**Figure 1.3.5.1** (h)) allows the user to open an editor specific template case study. Predefined case studies have been incorporated into TISON's NE to guide the user towards designing and analyzing the network models. Once the user clicks 'Upload Case Study' button, the following window opens displaying a list of predefined case studies for NE (**Figure 1.3.5.2**). Currently, NE supports four predefined case studies: (i) Case Study 1- Human Signaling Network (rules based), (ii) Case Study 2 - p53 Network with DNA Damage OFF (weight based), (ii) Case Study 2 – p53 Network with DNA Damage ON (weight based), (iii) Case Study 3 – Yeast Cell Cycle (weight based), and (iv) Case Study 4 – Human Stem Cell Network (weight based).

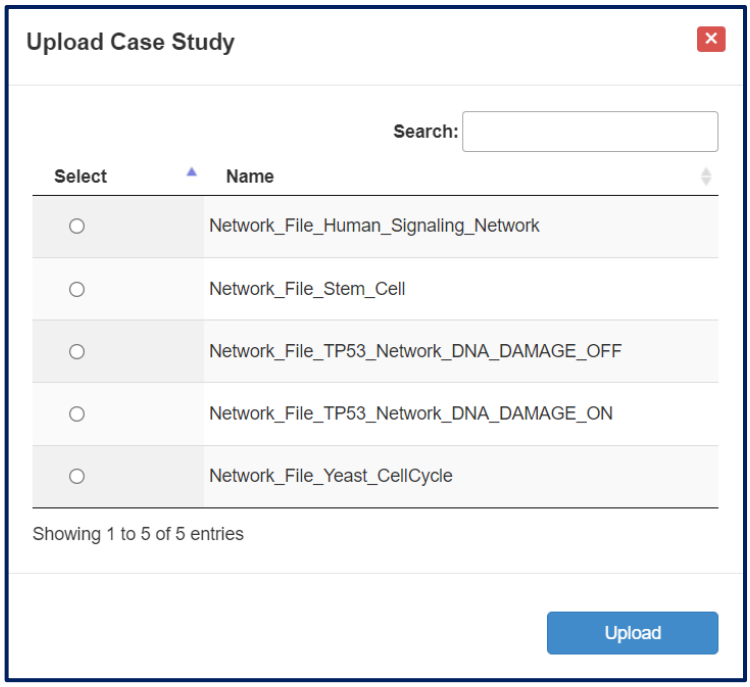


Figure 1.3.5.2 - 'Upload Case Study' window enlisting predefined case studies included in NE.

'Import Network' button (**Figure 1.3.5.1** (i)) allows the user to upload a network file (**Figure 1.3.5.3**).

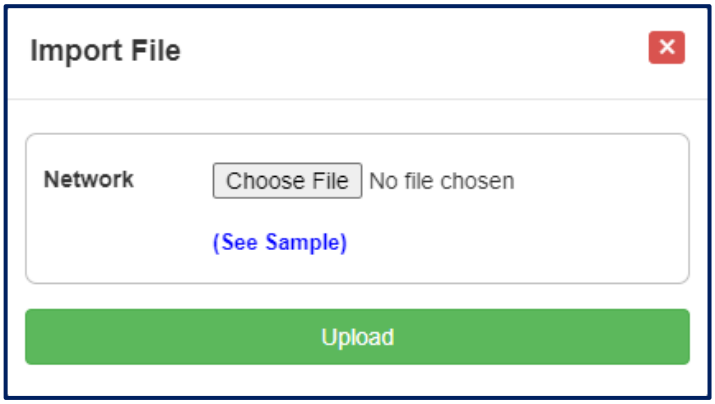
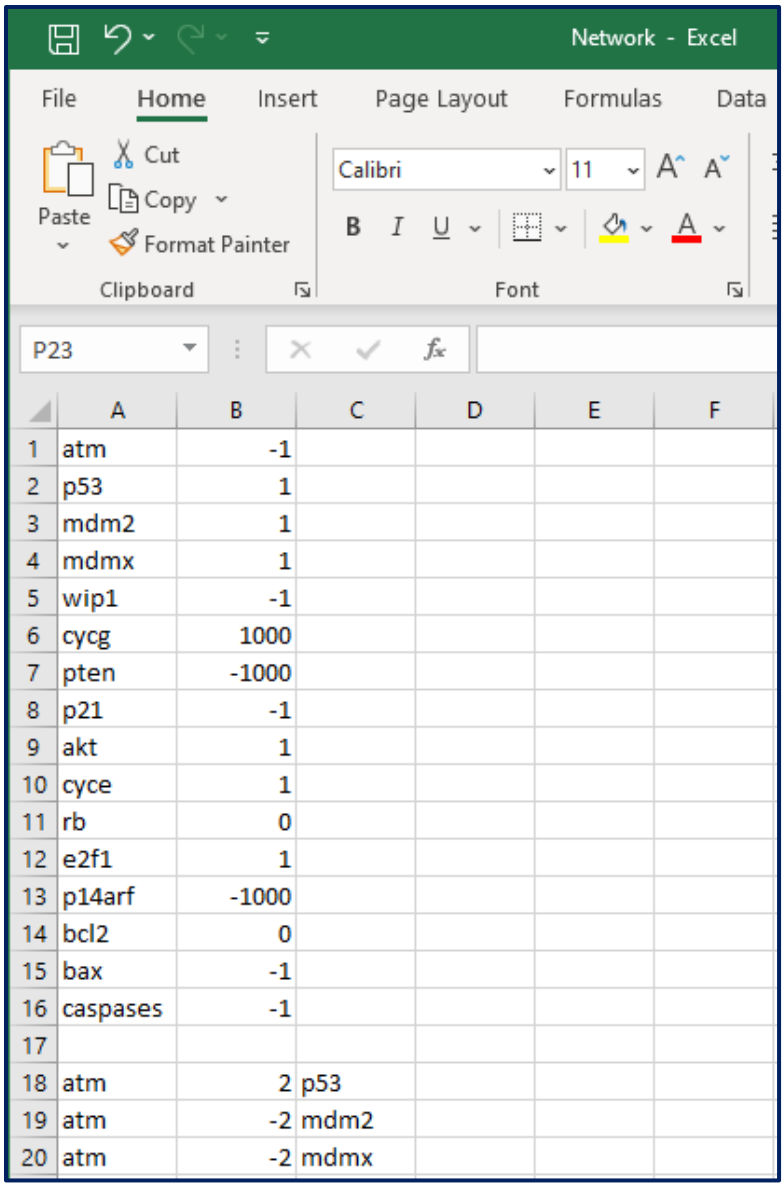


Figure 1.3.5.3 - Upload files prompt for the 'Import Network' button.

'Export Network' button (**Figure 1.3.5.1** (j)) downloads a (.zip) file containing the uploaded network file (**Figure 1.3.5.4**) along with the input parameters file (**Figure 1.3.5.5**), custom states file (**Figure 1.3.5.6**), fixed nodes states file (**Figure 1.3.5.7**), cell fate logic file (**Figure 1.3.5.8**), and result file (**Figure 1.3.4.4**) produced after the network analysis has been performed.



	A	B	C	D	E	F
1	atm	-1				
2	p53	1				
3	mdm2	1				
4	mdmx	1				
5	wip1	-1				
6	cycg	1000				
7	pten	-1000				
8	p21	-1				
9	akt	1				
10	cyce	1				
11	rb	0				
12	e2f1	1				
13	p14arf	-1000				
14	bcl2	0				
15	bax	-1				
16	caspases	-1				
17						
18	atm	2	p53			
19	atm	-2	mdm2			
20	atm	-2	mdmx			

Figure 1.3.5.4 - Export Network: Network file.

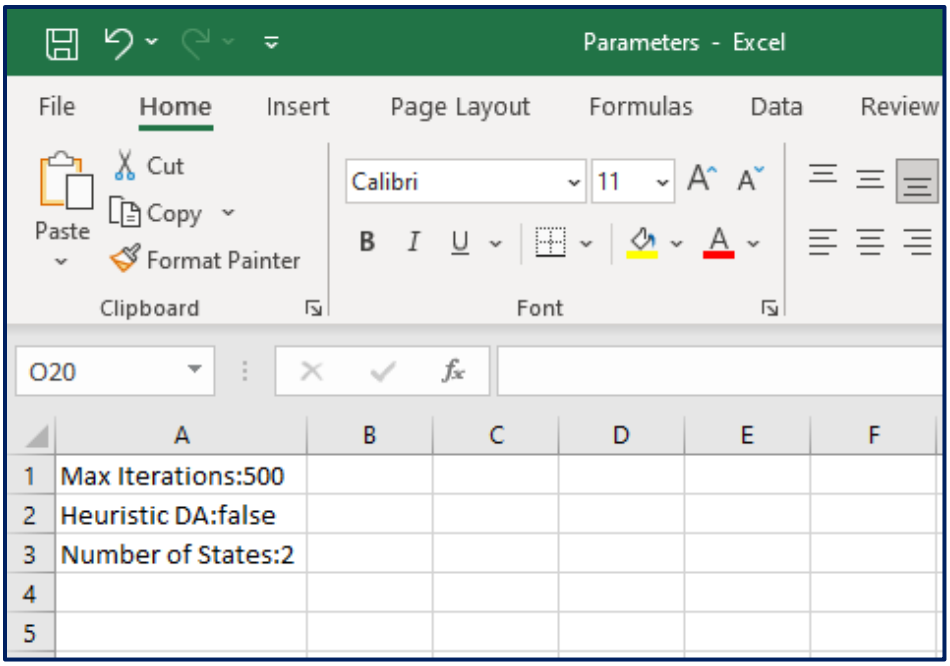


Figure 1.3.5.5 - Export Network: Parameters file.

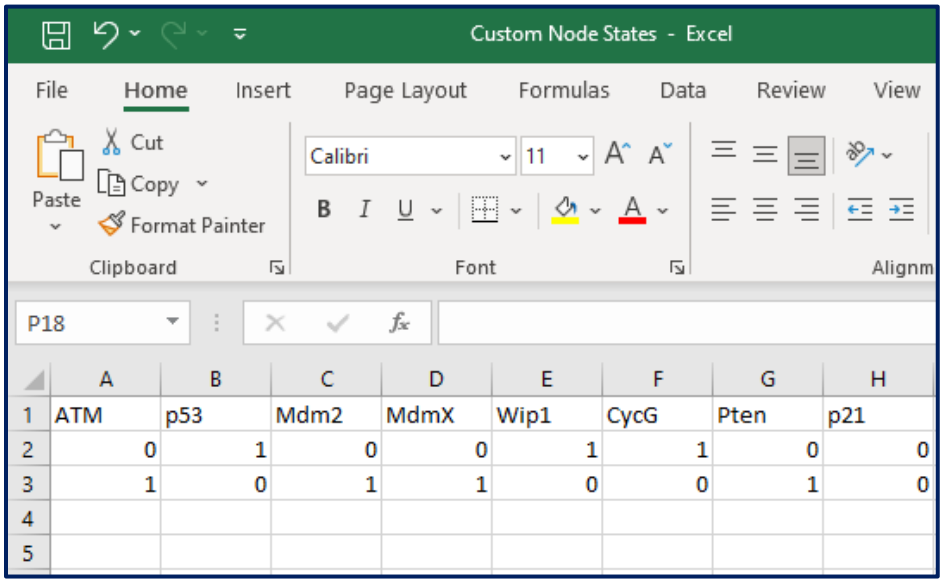
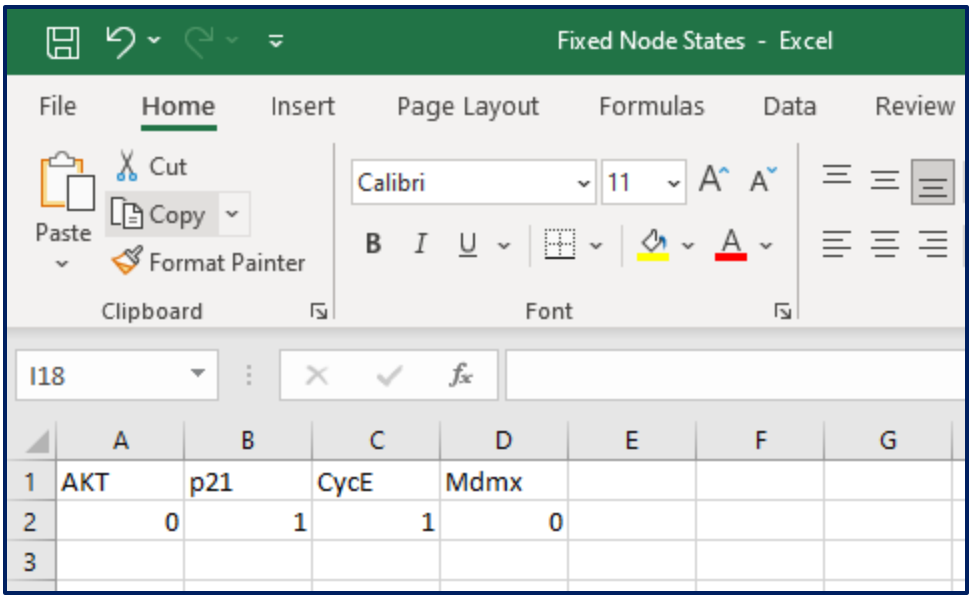
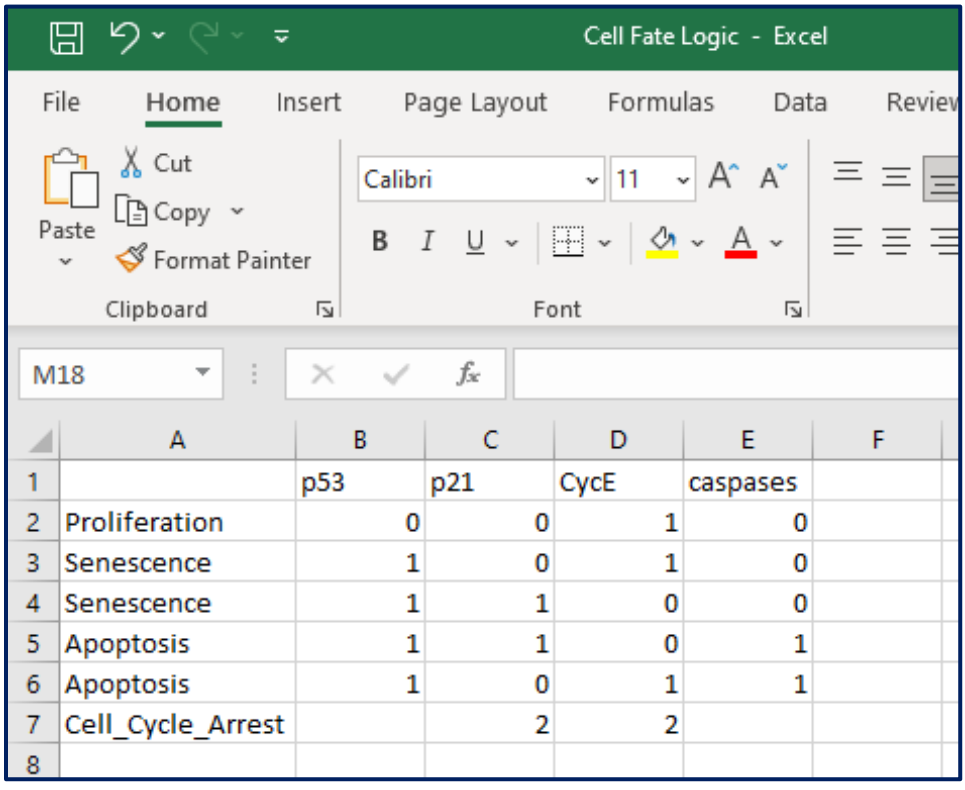


Figure 1.3.5.6 - Export Network: Custom node states file.



	A	B	C	D
1	AKT	p21	CycE	Mdmx
2	0	1	1	0
3				

Figure 1.3.5.7 - Export Network: Fixed node states file.



	A	B	C	D	E
1		p53	p21	CycE	caspases
2	Proliferation	0	0	1	0
3	Senescence	1	0	1	0
4	Senescence	1	1	0	0
5	Apoptosis	1	1	0	1
6	Apoptosis	1	0	1	1
7	Cell_Cycle_Arrest		2	2	
8					

Figure 1.3.5.8 - Export Network: Cell fate logic file.

1.4 TISON's Networks Editor Video Tutorials

Table 1.4.1 - Video tutorials for NE.

Video No.	Video Tutorials
1.	Constructing Rules-based Network and Performing Deterministic Analysis (DA)
2.	Drawing Weight Based Network
3.	Uploading Networks using Template Projects
4.	Performing Weight Based Probabilistic Analysis (PA)
5.	Performing Robustness Analysis on Biomolecular Networks
6.	Performing Ordinary Differential Equation (ODE) Analysis

1.5 Bibliography

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