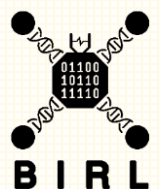




ATLANTIS

Attractor Landscape Analysis Toolbox
for Cell Fate Discovery and Reprogramming
For Use with MATLAB® (R2016a)

User Manual



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1. Introduction to ATLANTIS

1.1. About ATLANTIS

ATLANTIS is a MATLAB toolbox for attractor landscape analysis of biomolecular networks. This interactive toolbox provides a rich environment for creation, modification, visualization and analysis of Boolean regulatory networks. It can be used to analyze user-created networks and to study them under the influence of different mutations and perturbations. This toolbox also provides various analyses options where a user can perform both Deterministic and Probabilistic analysis of a Boolean network. Results are made available in the form of visual plots including potential energy landscapes, attractor landscapes and area-based phenotype classification maps.

1.2. License and Copyright Information

ATLANTIS is an open source, open architecture and freely available MATLAB toolbox. The toolbox is available under the [GNU GENERAL PUBLIC LICENSE](#) (ver. 3.0).

1.3. Features

ATLANTIS includes the following features:

- User defined network import
- Network visualization
- Network modification
- Deterministic analysis of networks
- Biological interpretation of networks
- Probabilistic analysis of networks
- Cell fate prediction using potential energy landscaping
- Phenotype landscaping

2. Getting Started with ATLANTIS

2.1. System Requirements

2.1.1. Hardware

ATLANTIS requires minimum 2 GB of disk space and 4 GB RAM for functioning. Note that MATLAB installation might take an additional amount of space depending on the number of toolboxes selected for installation.

2.1.2. Software

ATLANTIS has been tested to work optimally with “Windows 7, Windows 8, Windows 10 or Windows Server 2012 R2” and “MATLAB R2016a” for functioning.

2.1.3. Compatibility with Windows and MATLAB

All testing is based upon the following Operating System Releases:

- Windows 7, 64 bit
- Windows 10, 64 bit
- Windows Server 2012 R2, 64 bit

All testing is based upon the following MATLAB versions:

- MATLAB R2014a
- MATLAB R2015a
- MATLAB R2016a
- MATLAB R2016b
- MATLAB R2017a

2.2. Installation

2.2.1. Setup ATLANTIS

ATLANTIS can be downloaded from this [link](#) in ‘.zip’ format. After downloading is complete, extract the files in a folder. Note the path to this folder for later use.

2.2.2. Install Graphviz

Download and install the latest release of Graphviz¹ (Graph visualization software) [here](#).

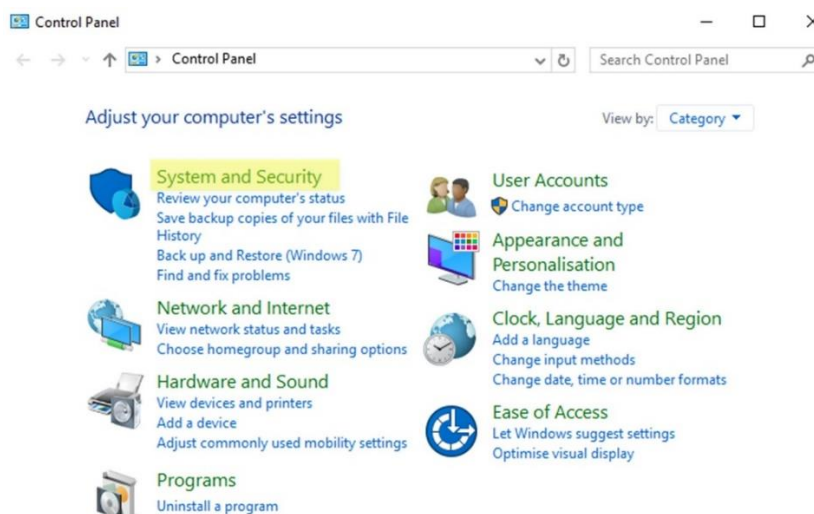
To make Graphviz compatible with MATLAB follow the steps below:

Setting Up Graphviz

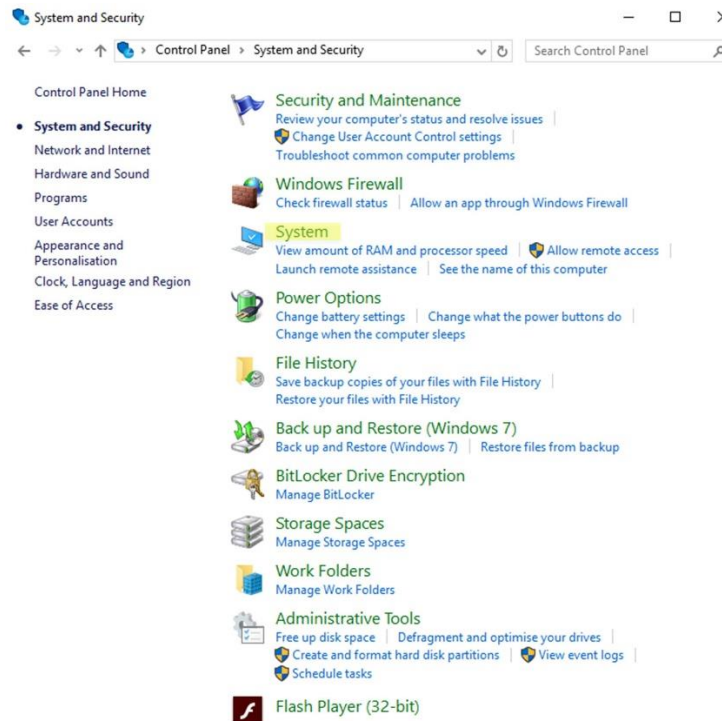
1. Go to the directory where Graphviz is installed and select 'bin' folder. Copy path to this folder for use later. It should resemble "C:\Program Files\Graphviz2.38\bin".



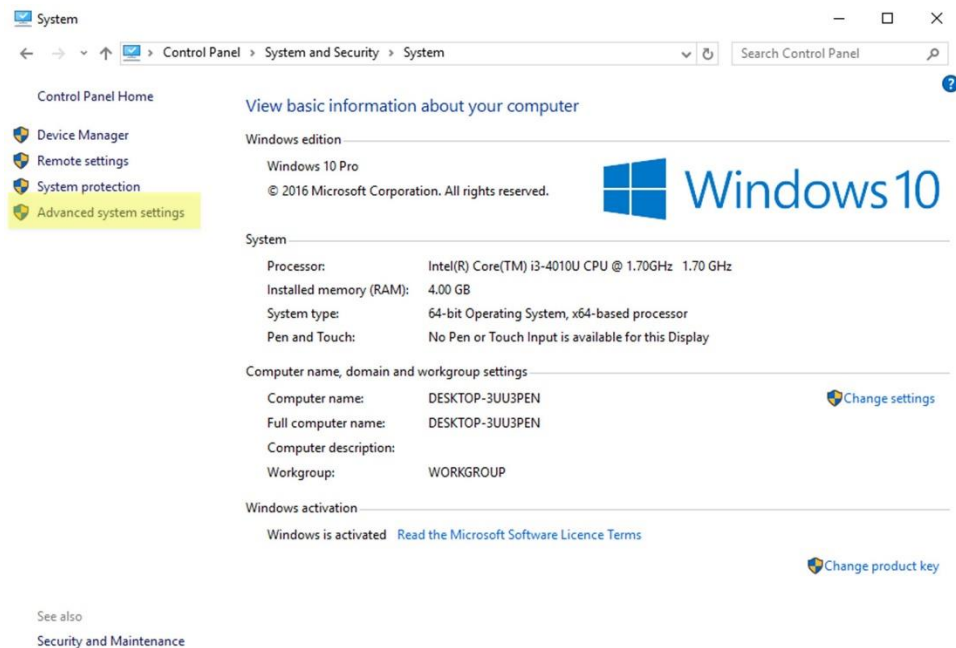
2. Next, go to Start, select Control Panel and then double click 'System and Security'.



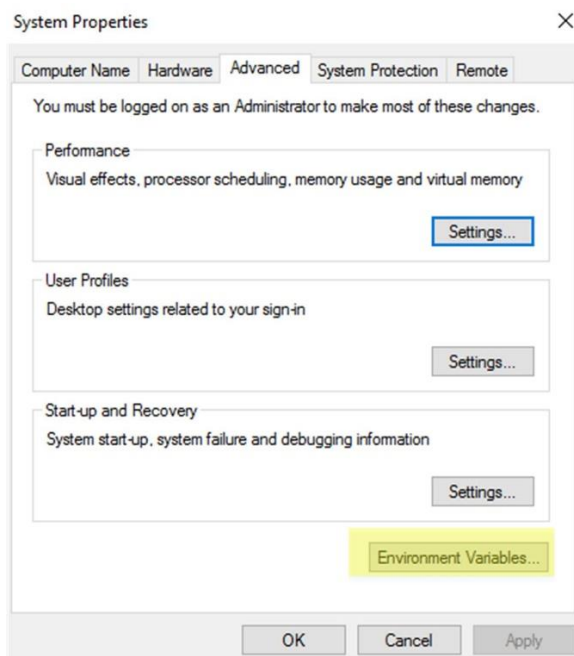
3. Next, click on 'System'.



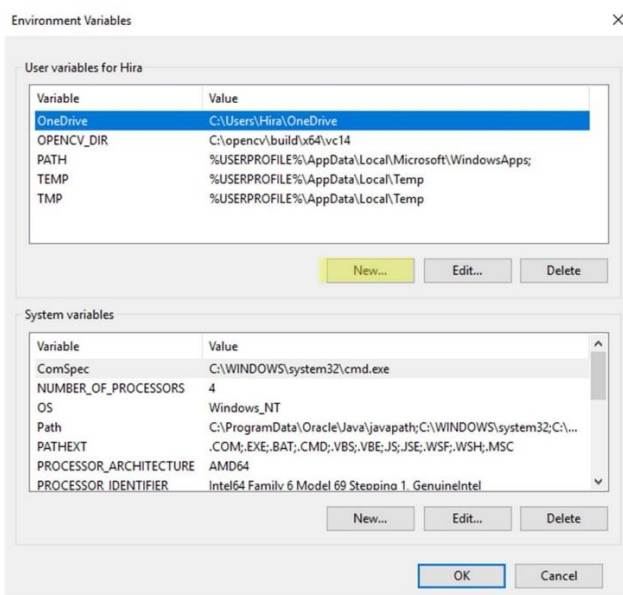
4. Select 'Advanced system settings' on the left side of the page.



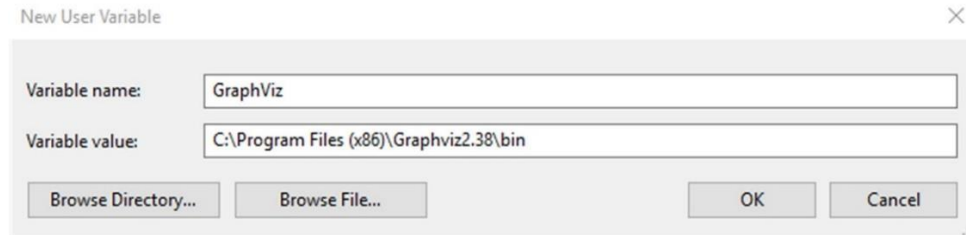
5. A window titled 'System Properties' pops up. Click 'Environment Variables' at the bottom left of the window.



6. A new window titled 'Environment Variables' appears. Click 'New...' to create a new variable for Graphviz in your PC.



7. In the Variable name field type 'Graphviz' and in the Variable value field copy the path to Graphviz/bin saved earlier.

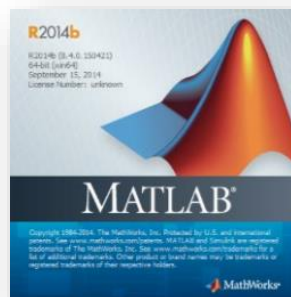


8. Repeat this for 'Systems Variables' Section in the 'Environment Variable' window and click 'OK' to save changes.

3. Using ATLANTIS

3.1. Setting Up MATLAB and Launching the Application

1. Launch MATLAB on your computer.

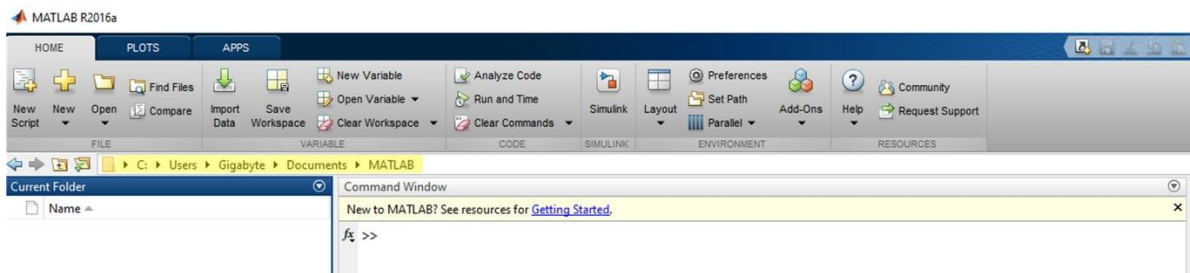


2. Copy full path to the folder that contains ATLANTIS. Assuming that this folder is kept in 'C'

drive, the path will resemble this “C:\Users\User\Desktop\ATLANTIS.”



3. Select the space highlighted in the figure below and paste the path copied in the previous step.



4. Check the “Current Folder” window to ensure that you have changed the current directory successfully.



5. Type ‘ATLANTIS.m’ in MATLAB command line to start the toolbox.

3.2. Data Preparation

ATLANTIS works off a flat text file (‘.txt’) or a comma separated file (‘.csv’) containing description of biomolecular networks. Flat text files (‘.txt’) can be generated using Notepad, Notepad++, Sublime Text or any suitable text editor. Comma separated files (‘.csv’) can be created using Microsoft Excel®, LibreOffice® and Apple Numbers® spreadsheet software. Below, a description of the data preparation process is provided (for both .txt and .csv files) using a simple example.

1. Network description file

Network description file for use with ATLANTIS contains two data elements, (i) node interaction weights matrix and (ii) a vector of node basal values indicating background expression of each node. Figure (a, b) below shows the contents of a sample tab-delimited text and spreadsheet data file for generating network. The file contents include components-interaction weights and basal values. The interaction weights matrix contains the names of nodes and strengths of their interactions (*termed interaction weights*).

a	A	B	C	D	E	F	G	H	I	J	K	L
1		Cln3	MBF	SBF	Cln1-2	Cdh1	Swi5	Cdc20	Clb5-6	Sic1	Clb1-2	Mcm1
2	Cln3		-1	1	1	0	0	0	0	0	0	0
3	MBF			0	0	0	0	0	0	1	0	0
4	SBF				0	1	0	0	0	0	0	0
5	Cln1-2					0	-1	-1	0	0	-1	0
6	Cdh1						0	0	0	0	0	-1
7	Swi5							0	-1	0	1	0
8	Cdc20								1	1	-1	-1
9	Clb5-6									0	-1	1
10	Sic1										0	-1
11	Clb1-2											0
12	Mcm1											
13												
14												
15												
16	basal											
		0	0	0	0	0	0	0	0	0	0	0

b	Cln3	MBF	SBF	Cln1-2	Cdh1	Swi5	Cdc20	Clb5-6	Sic1	Clb1-2	Mcm1
Cln3		-1	1	1	0	0	0	0	0	0	0
MBF			0	0	0	0	0	1	0	0	0
SBF				0	1	0	0	0	0	0	0
Cln1-2					0	-1	-1	0	0	-1	0
Cdh1						0	0	0	0	0	-1
Swi5							0	-1	0	1	0
Cdc20								1	1	-1	-1
Clb5-6									0	-1	1
Sic1										0	-1
Clb1-2											0
Mcm1											
basal											
	0	0	0	0	0	0	0	0	0	0	0

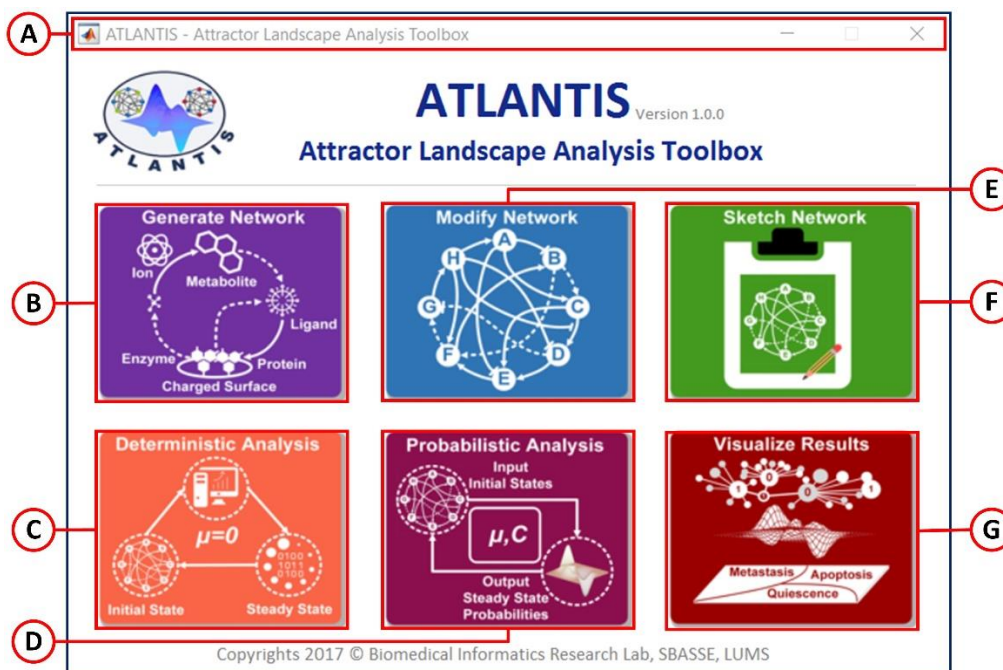
2. Network Modification File for Modifying Node States and Interactions

Networks can be modified by inclusion of 'mutation' information. These mutations can be introduced into the network by modifying it in two different ways. Firstly, the network can be modified by altering node states (either from 0 to 1 or from 1 to 0). Secondly, network modification can also be made by deleting the interaction link between two nodes. To modify a user-defined network in ATLANTIS, a comma separated file (Figure c below) or a simple text file (Figure d below) is required. These files can be used to mutate a node by providing node name and its new (i.e. mutated) state (Figure c, d below) or by removing the interaction between two nodes through providing names of two interacting nodes (e.g. A and B) in the following format 'A>B 2' (Figure c, d below). In case of link deletion, ATLANTIS identifies the type of modification by node names followed by the label '2'.

c		A	B	d	
1	BCL2		0	BCL2	0
2	RAS		1	RAS	1
3	MDM2>P53		2	MDM2>P53	2

3.3. Using ATLANTIS Graphical User Interface

1. ATLANTIS - Main Menu



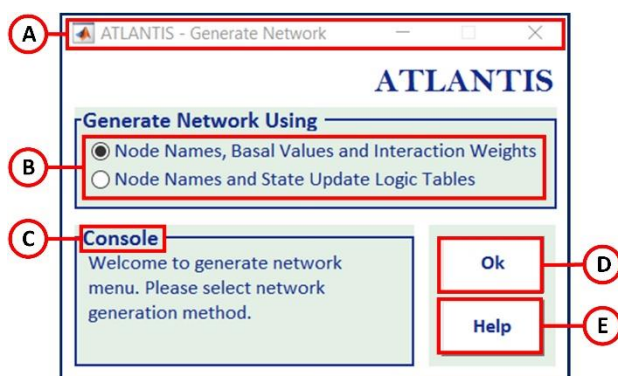
A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.

B. Click on 'Generate Network' to provide user with options to create Boolean models of

biological networks using node name, interaction weights and basal values or node names and node state update logic tables

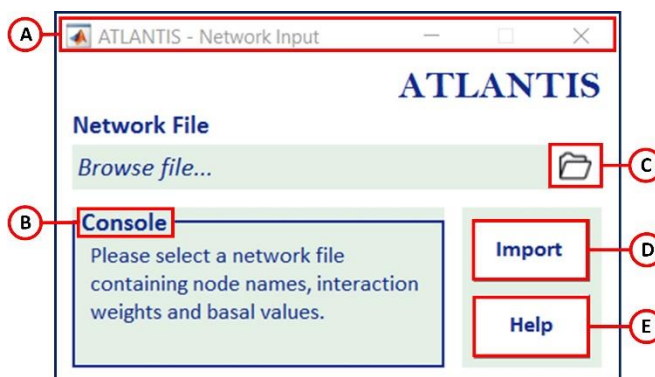
- C. Click on 'Modify Network' to add mutations (knock down and overexpression of nodes and deletion of links) to the network.
- D. Click on 'Sketch Network' to provide the option to display their input network.
- E. Click on 'Deterministic Analysis' to update the network states overtime to find stable state(s) [attractor(s)] and their occurrence frequency (basin size) in absence of noise.
- F. Click on 'Probabilistic Analysis' to associate probabilities with each network state and update these probabilities to find steady state probability of each network state in presence of external noise.
- G. Click on 'Visualize Results' to plot attractor landscape using potential energies or basin ratios. Plot Treemaps to visualize the phenotype landscape.

2. ATLANTIS - Generate Network



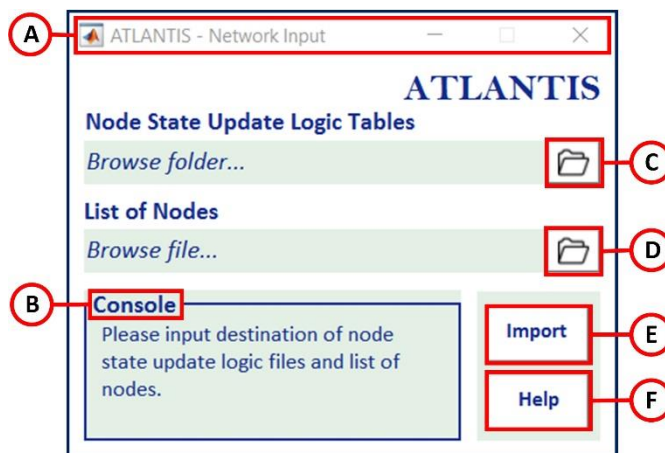
- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Select between generating network using interaction weights and basal values or Node state update logic tables
- C. Console indicates the progress within the GUI.
- D. Click on 'Ok' to proceed to GUI 3 or 4 based on your selected network input type.
- E. Click on 'Help' for a detailed User Manual in pdf format.

3. ATLANTIS – Network Input as node Interaction Weights Matrix



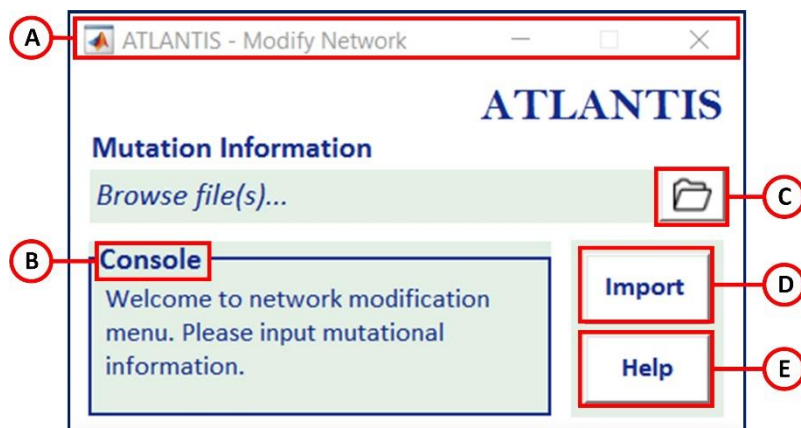
- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Console indicates the progress within the GUI.
- C. Press to browse network file containing node names, interaction weights and basal values.
- D. Press 'Import' to generate network using the input information.
- E. Click on 'Help' for a detailed User Manual in pdf format.

4. ATLANTIS - Network Input as a Vector of node Basal Values



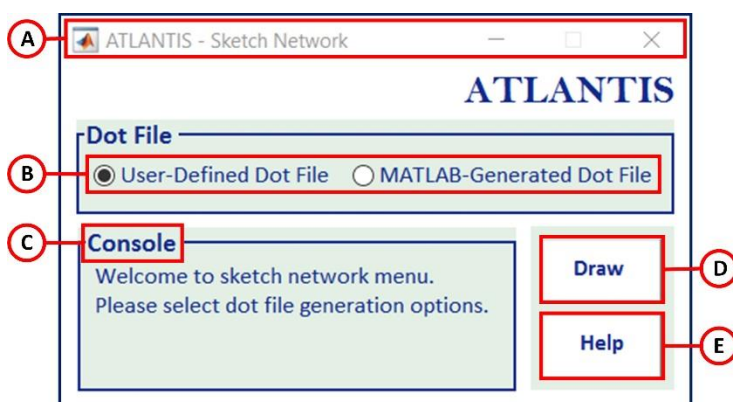
- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Console indicates the progress within the GUI.
- C. Press to browse the folder containing Node update logic table files.
- D. Press to browse Node list file.
- E. Press 'Import' to generate network using the input information.
- F. Click on 'Help' for a detailed User Manual in pdf format.

5. ATLANTIS – Modify Network



- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Console indicates the progress within the GUI.
- C. Press to browse file containing mutational information (Node deletion or Node overexpression or Link deletion information. (Can be left empty to simulate network in absence of mutation when performing batch simulations).
- D. Press 'Import' to incorporate mutations into the network.
- E. Click on 'Help' for a detailed User Manual in pdf format.

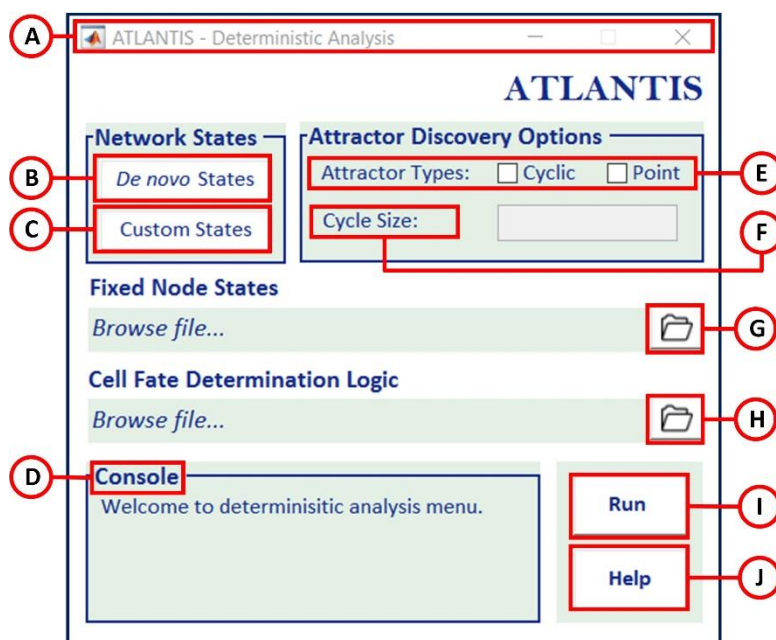
6. ATLANTIS – Sketch Network



- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Select the option to sketch network using user-selected dot file or MATLAB-generated dot file for visualization of the network.

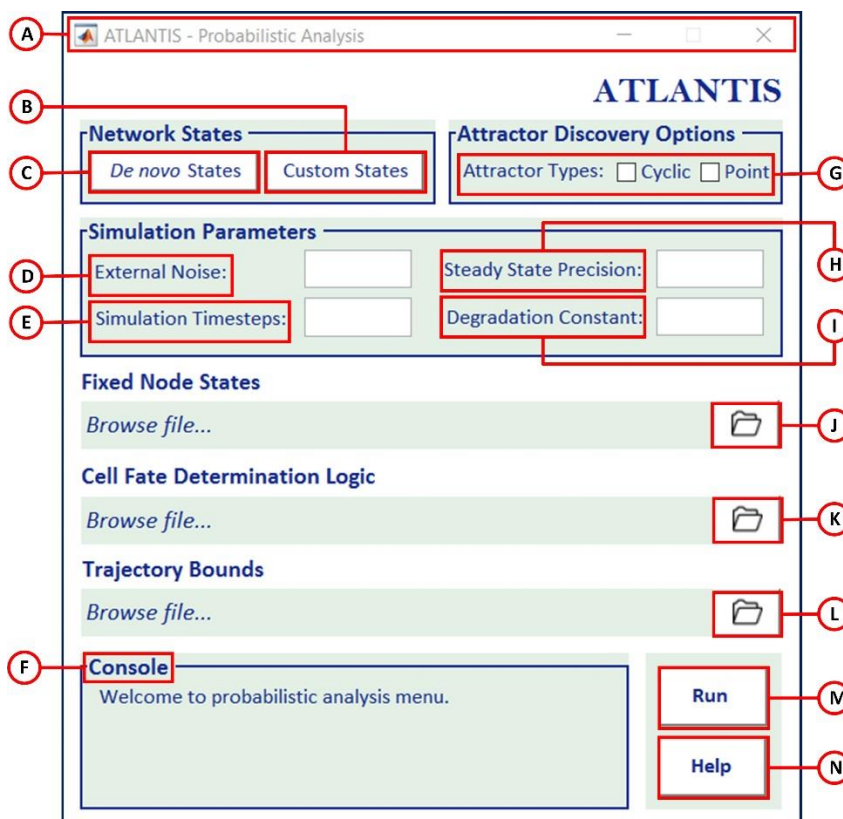
- C. Console indicates the progress within the GUI.
- D. Press draw to display the current network.
- E. Click on 'Help' for a detailed User Manual in pdf format.

7. ATLANTIS – Deterministic Analysis



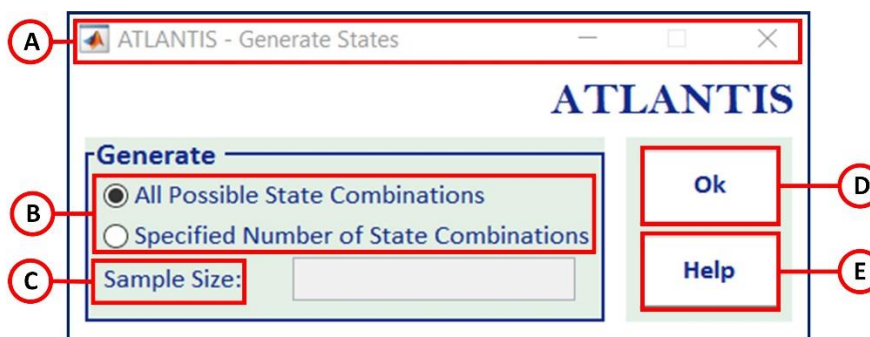
- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Press to generate De novo initial network states list (GUI 9 will pop up for further options).
- C. Press to select .mat file containing custom initial network states.
- D. Console indicates the progress within the GUI.
- E. Check attractor types to be searched for. (Atleast one of the types must be checked).
- F. Defines the maximum number of states present in a cyclic attractor. Cyclic attractors containing more states than cycle size will not be detected. Large cycle sizes will take make deterministic analysis slower but will allow to detect most if not all cyclic attractors.
- G. Browse file containing node names and the state values in which to fix their activity states.
- H. Browse file containing information about the output nodes and their values in each cell fates.
- I. Press 'Run' to start the determinisitc analysis.
- J. Click on 'Help' for a detailed User Manual in pdf format.

8. ATLANTIS – Probabilistic Analysis



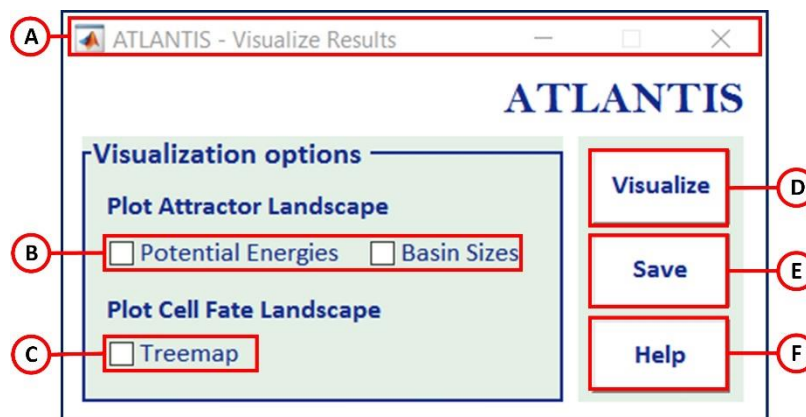
- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Press to select .mat file containing custom initial network states.
- C. Press to generate De novo initial network states list (GUI 9 will pop up for further options).
- D. Enter a value of external noise between (between 0-5, default: 5).
- E. Enter a value to indicate that how long the simulation will run (between 1 and 10000, default: 1000).
- F. Console indicates the progress within the GUI.
- G. Check attractor types to be searched for. (Atleast one of the types must be checked).
- H. Enter a value to define how precise the steady state probability will be. (Between 1-10, default: 1). Higher the precision longer the time taken to complete probabilistic analysis.
- I. Enter a value for degradation constant (Between 0-1, default: 0.001).
- J. Browse file containing node names and the state values in which to fix their activity states.
- K. Browse file containing information about the output nodes and their values in each cell fates.
- L. Browse file containing the start state and end state of the trajectory to be mapped.
- M. Press 'Run' to start the probabilistic analysis.
- N. Click on 'Help' for a detailed User Manual in pdf format.

9. ATLANTIS – Generate States



- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Select the de novo state generation method. Generate all possible combinations of network states or generate a specified number of network state combinations.
- C. Specify the number of states to generate in the Sample size edit box.
- D. Click on 'Ok' to proceed.
- E. Click on 'Help' for a detailed User Manual in pdf format.

10. ATLANTIS – Visualize Results



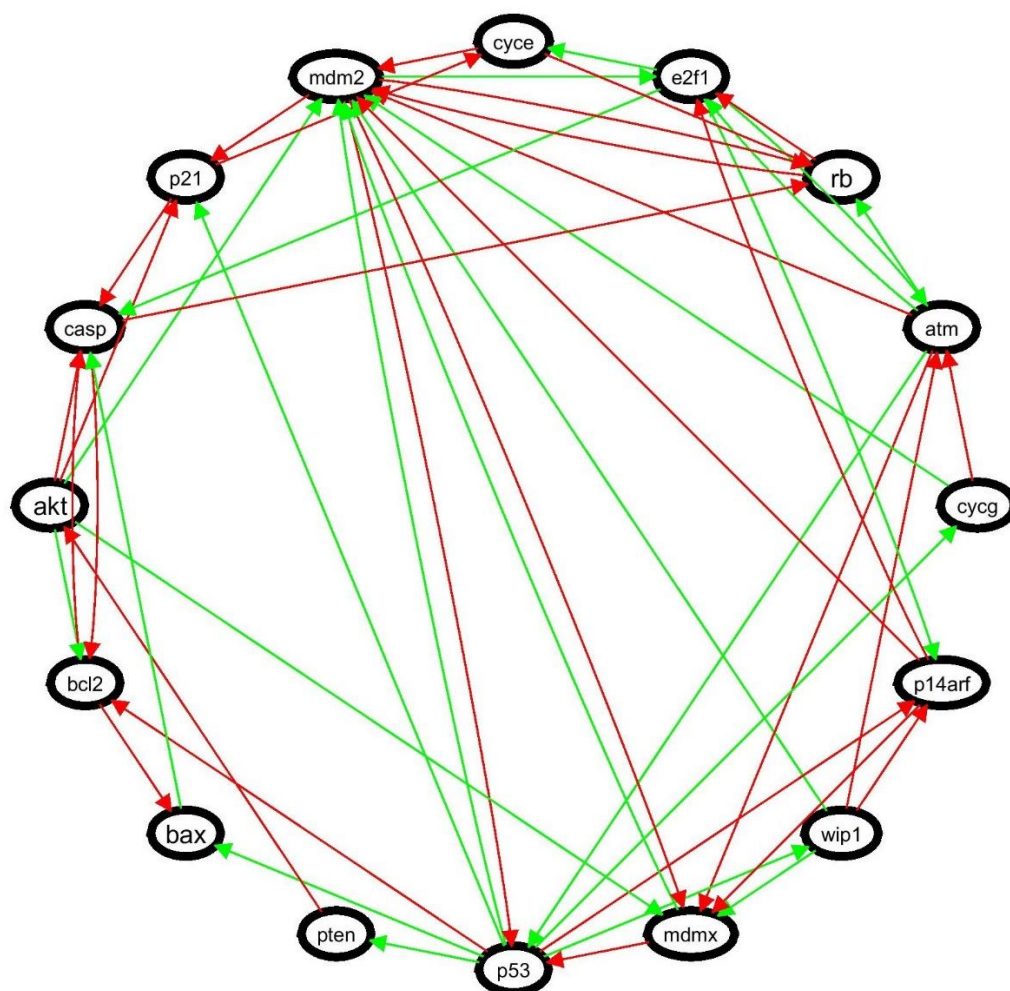
- A. At the top of the ATLANTIS interface is the 'Title bar', which displays the name of the program and the title of the current document.
- B. Select the check boxes to plot attractor landscape using only basin sizes or potential energies or both.
- C. Plot phenotype landscape using treemaps.
- D. Press 'Visualize' to plot the selected plots.
- E. Save the data to an 'Excel file'.
- F. Click on 'Help' for a detailed User Manual in pdf format.

4. Interpreting Results

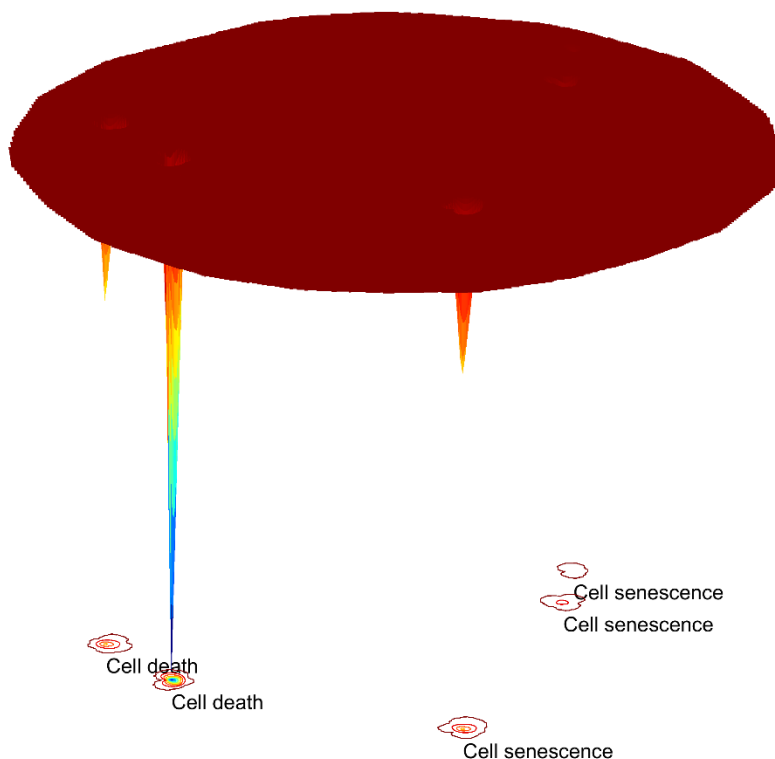
The results shown below involve the Boolean model of p53 regulatory network, as studied by Minsoo Choi et al. in their work on “Attractor landscape analysis reveals feedback loops in the p53 network that control the cellular response to DNA damage”. ATLANTIS was used to construct its gene regulatory network, potential energy landscape and phenotype classification.

4.1. Network visualization using Graphviz

Biomolecular Network with 16 nodes and 50 links



4.2. Cell fate prediction using potential energy landscape



4.3. Classifying phenotypes

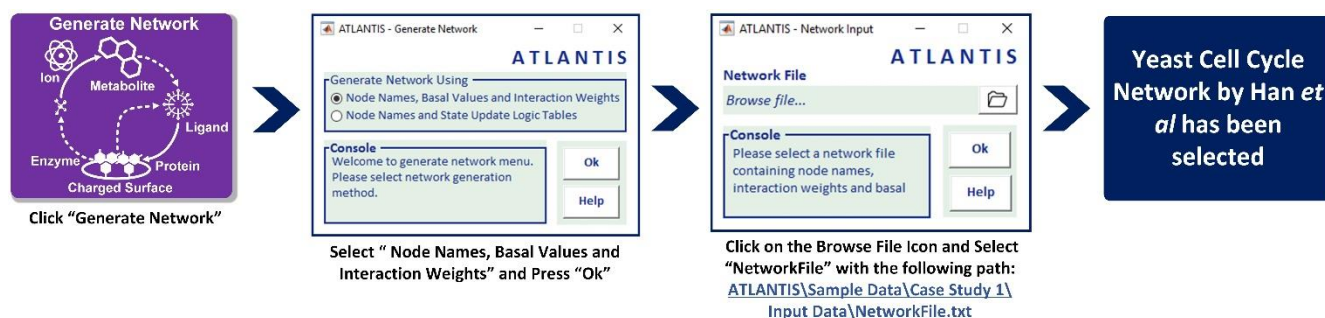


5. Worked Examples

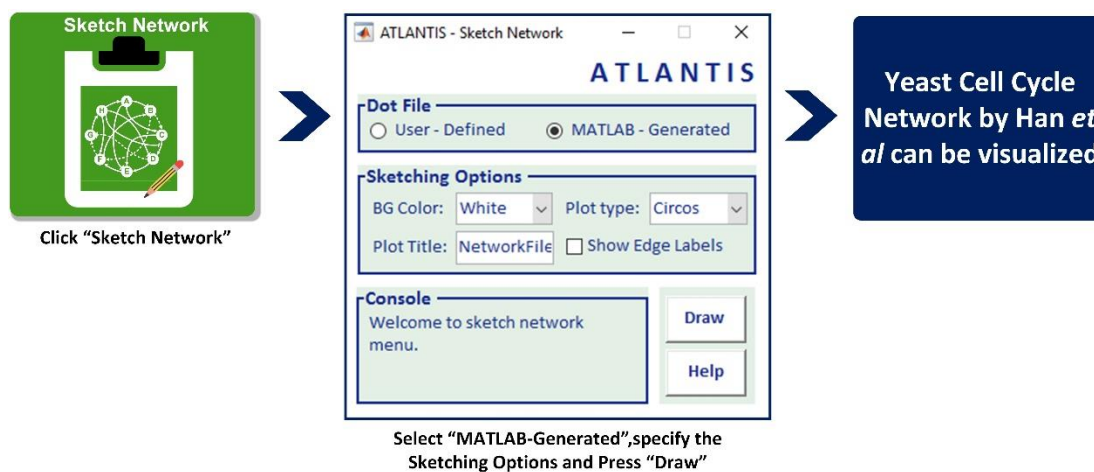
To facilitate the ease of use, we present two elaborate worked out Attractor Landscape Analysis (Case Study-1 and Case Study-3) examples using ATLANTIS. The first worked out example details the procedure for performing the DA and PA of yeast cell cycle network presented by Han et al². The second example illustrates the rules-based deterministic analysis of the 201-node network presented by Cho et al³.

1. Decoding Yeast Cell Cycle Progression using Attractor Landscape Analysis

Step-1. Select Network Information file for generating the network. Click the ‘Generate Network’ button, select the option with ‘Node Names, Basal Values and Interaction Weights’. Next, browse the file at given link for further analysis. The network file for Yeast Cell Cycle Network can be found at: [ATLANTIS\Sample Data\Case Study 1\Input Data\NetworkFile.txt](#).

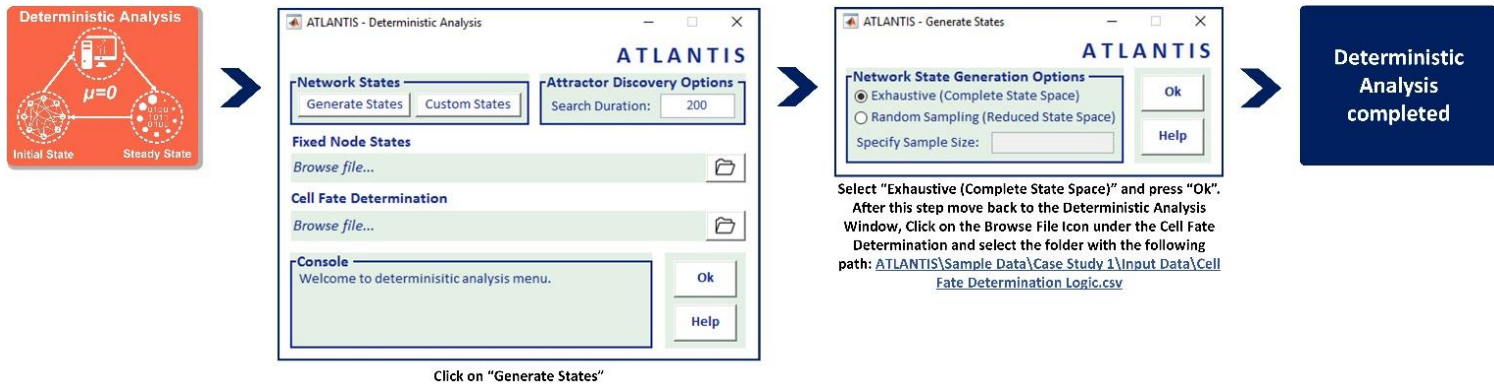


Step-2. After selecting the Network Information file, click on ‘Sketch Network’ Button. Select ‘MATLAB-Generated’, specify the Sketching Options and Press “Draw” to visualize the specified Network. You can export the generated figure by right clicking on the Window.

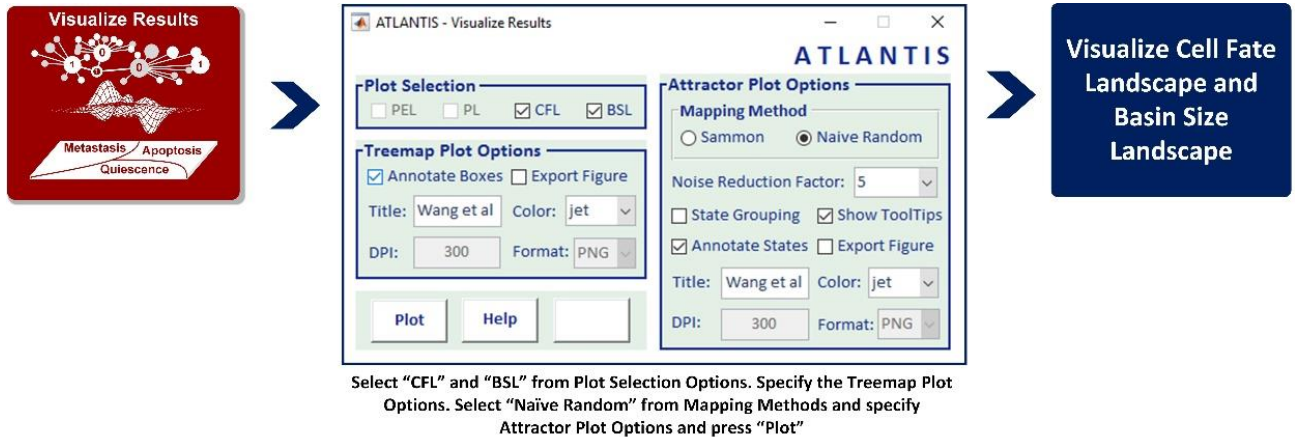


Step-3. After visualizing the Network Information file, click on ‘Deterministic Analysis’ Button. This would open the ‘Deterministic Analysis’ menu. The first step, now, would be selecting

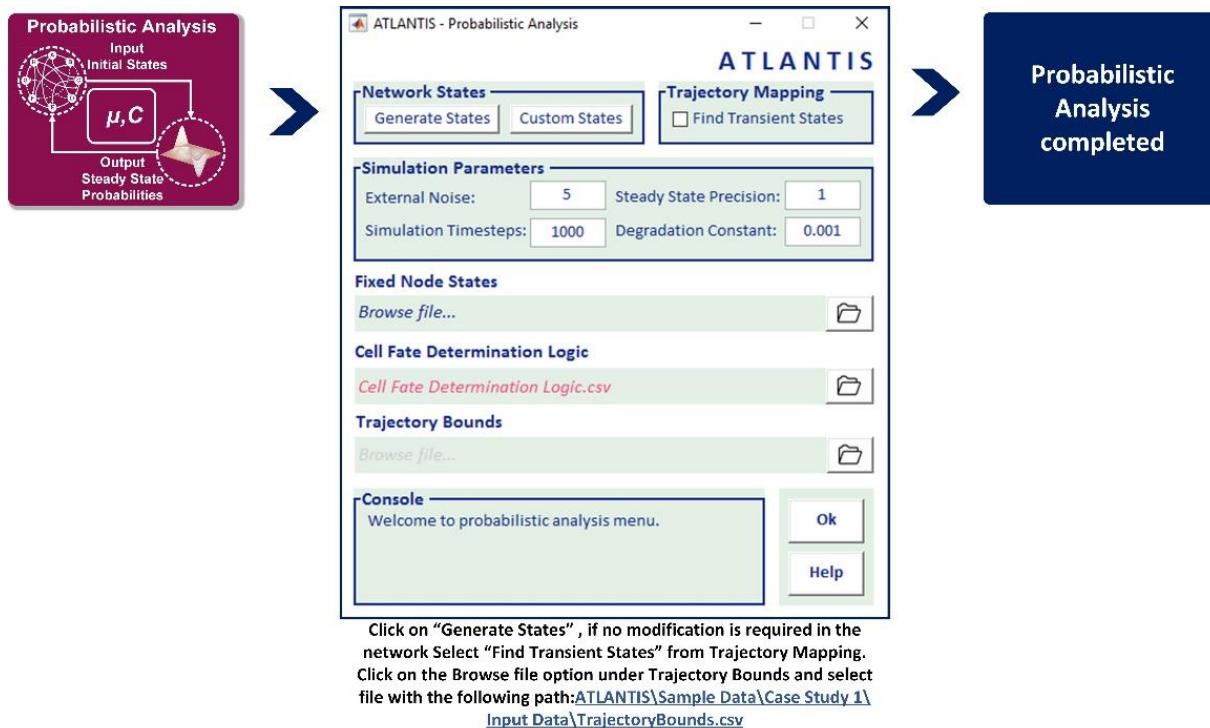
the Network States. Click on 'Generate States' and Select 'Exhaustive (Complete State Space)' and press 'Ok'. After this step move back to the Deterministic Analysis Window, Click on the Browse File Icon under the Cell Fate Determination and select the folder with the following path: [ATLANTIS\Sample Data\Case Study 1\Input Data\Cell Fate Determination Logic .csv](#).



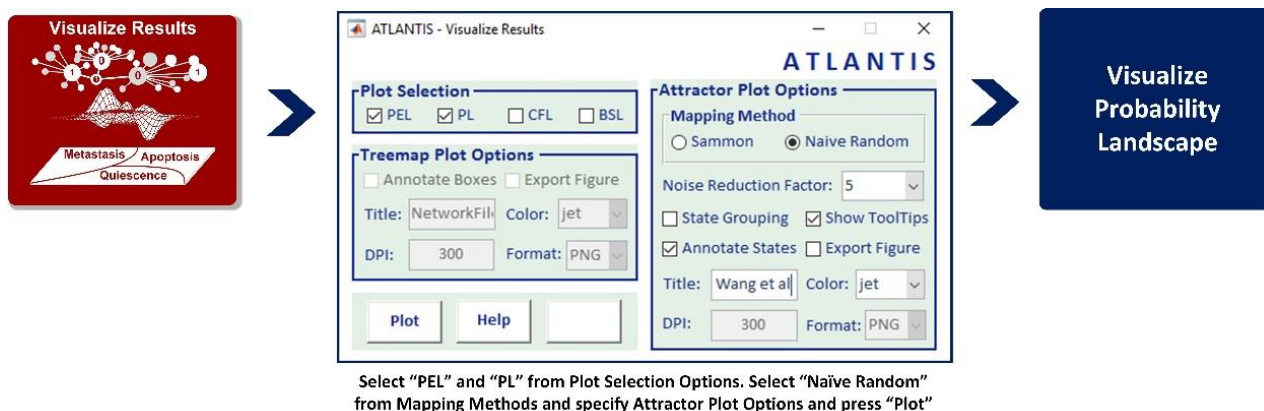
Step-4. After performing the Deterministic Analysis, the next step is to visualize the results. Click on the 'Visualize Result' Button. Select 'CFL' and 'BSL' from Plot Selection Options. Specify the Treemap Plot Options as shown in figure. Select "Naïve Random" from Mapping Methods and specify Attractor Plot Options and press 'Plot'. You can export the generated results as well.



Step-5. In order to perform Probabilistic Analysis, click on 'Probabilistic Analysis' Button. Click on 'Generate States', if no modification is required in the network Select 'Find Transient States' from Trajectory Mapping. Click on the Browse file option under Trajectory Bounds and select file with the following path: [path: ATLANTIS\Sample Data\Case Study 1\Input Data\TrajectoryBounds.csv](#).



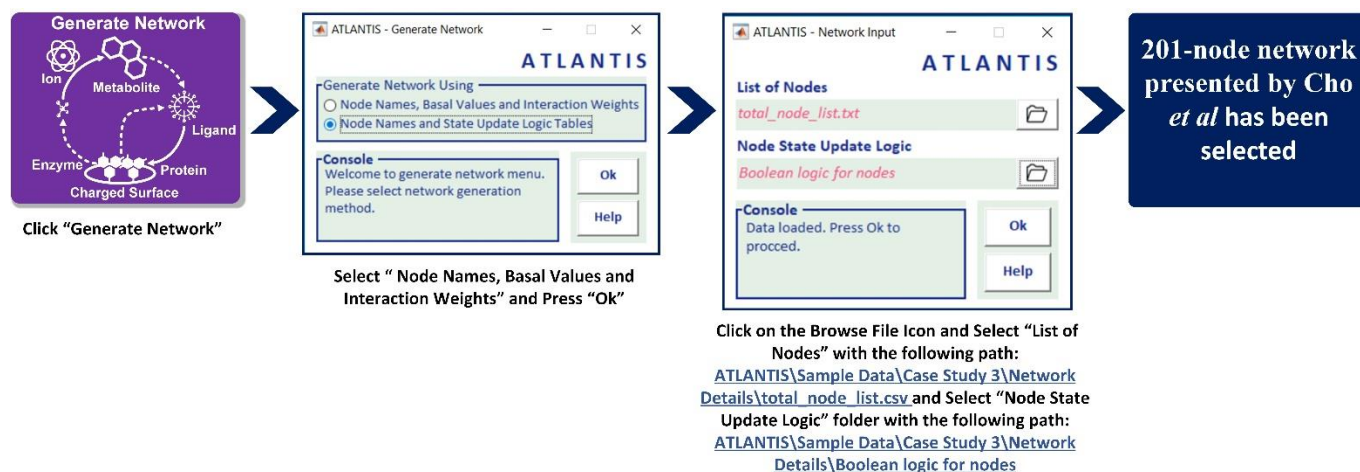
Step-6. After performing the Probabilistic Analysis, the next step is to visualize the results. Click on the 'Visualize Result' Button. Select 'PEL' and 'PL' from Plot Selection Options. Select "Naïve Random" from Mapping Methods and specify Attractor Plot Options and press 'Plot'. You can export the generated results as well.



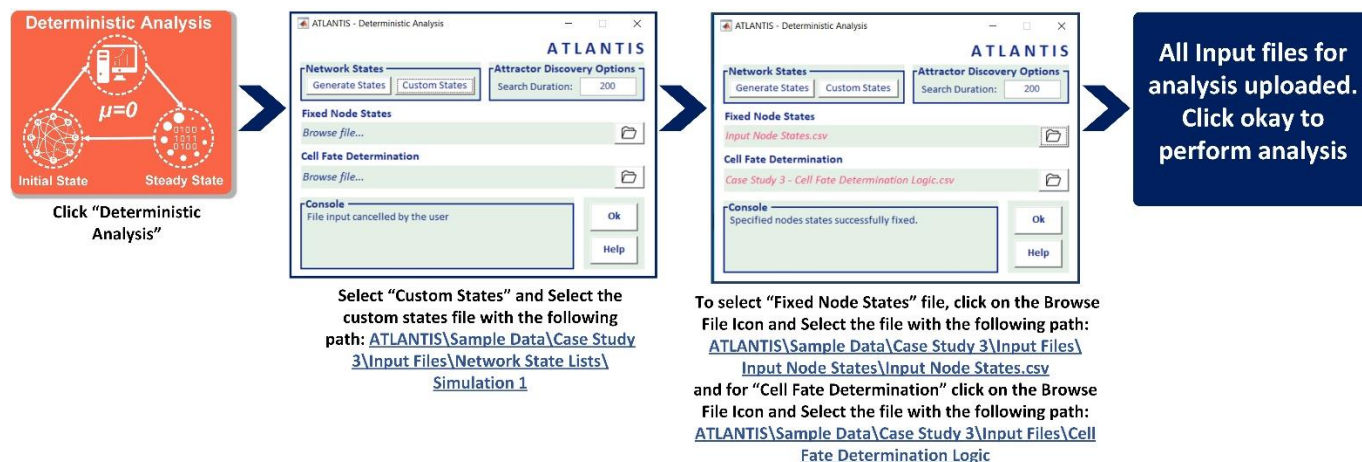
2. Investigating the Evolution of Cell Fate Landscape during Colorectal Tumorigenesis

Step-1. Select Network Information file for generating the network. The network file and update logic folder for Colorectal tumorigenesis network can be found at: ATLANTIS\Sample

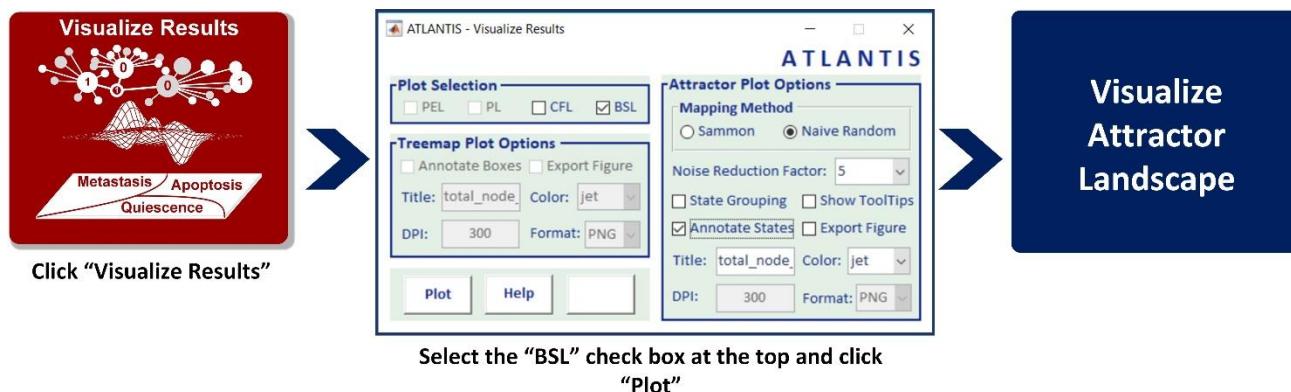
Data\Case Study 1\Input Data\Network Details. After clicking the ‘Generate Network’ button, select the option with ‘Node Names and State Update Logic Tables’. Next, browse the file and folder at given link for further analysis.



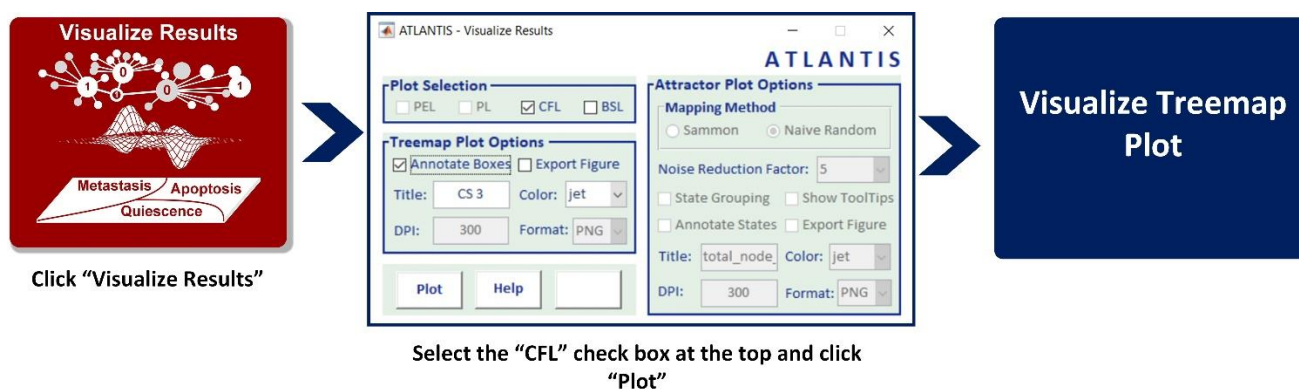
Step-2. After selecting the Network Information file, click on ‘Deterministic Analysis’ Button. This would open the ‘Deterministic Analysis’ menu. The first step, now, would be selecting the Network States. Click on ‘Custom Button’ and upload a file containing custom network states.



Step-3. On completion of deterministic analysis move on to visualizing results. Here, to visualize the Treemap plot for phenotype classification, check “CFL”, enter a title for your plot and then click Plot.



Step-4. To visualize the attractor landscape, check "BSL", enter remaining parameters and click Plot.



6. Bibliography

1. Ellson, J., Gansner, E., Koutsofios, L., North, S. C. & Woodhull, G. *Graphviz—open source graph drawing tools*. *Graph Drawing* (2002). doi:10.1007/3-540-68339-9_34
2. Han, B. & Wang, J. Quantifying robustness and dissipation cost of yeast cell cycle network: the funneled energy landscape perspectives. *Biophys. J.* **92**, 3755–3763 (2007).
3. Cho, S.-H., Park, S.-M., Lee, H.-S., Lee, H.-Y. & Cho, K.-H. Attractor landscape analysis of colorectal tumorigenesis and its reversion. *BMC Syst. Biol.* **10**, 96 (2016).