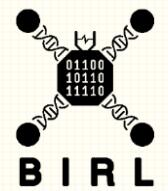




# ATLANTIS

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

## 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 an environment rich 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 a minimum of 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

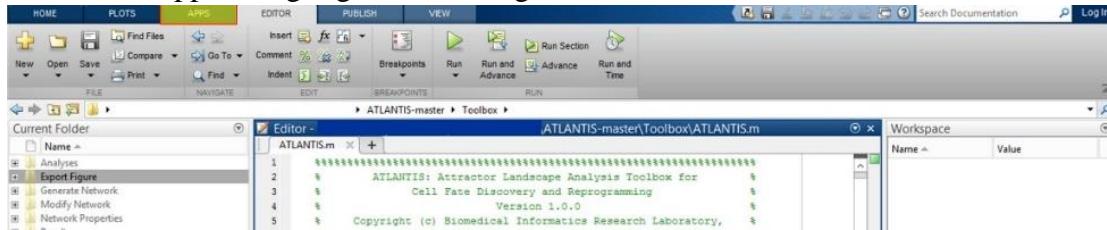
#### 2.1.4. Running ATLANTIS without MATLAB

ATLANTIS has been developed as a MATLAB toolbox for cell fate discovery and reprogramming. However, for running ATLANTIS without MATLAB, we provide support for executing the toolbox using MATLAB-independent executables. We have provided ATLANTIS executable for [Windows 64-bit](#). In case the user has an operating system or version which is not compatible with the precompiled executables provided herewith, we have also included a tutorial on building custom executables targeting other operating systems.

#### Generating Executable file (.exe) for Windows

For generating the executable (.exe) file the following steps can be followed. A [video tutorial](#) is also presented to help the user build and install the executable file.

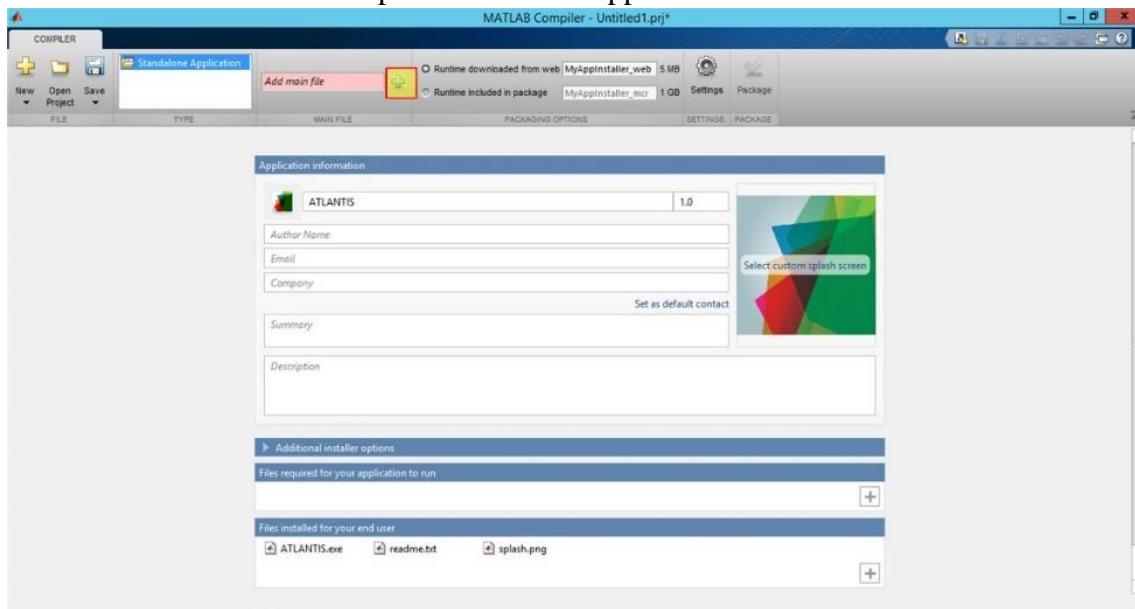
1. Open MATLAB (for compilation use R2017b) and change the current working directory in order to view the ‘ATLANTIS.m’ file.
2. Click on Apps as highlighted in the figure below.



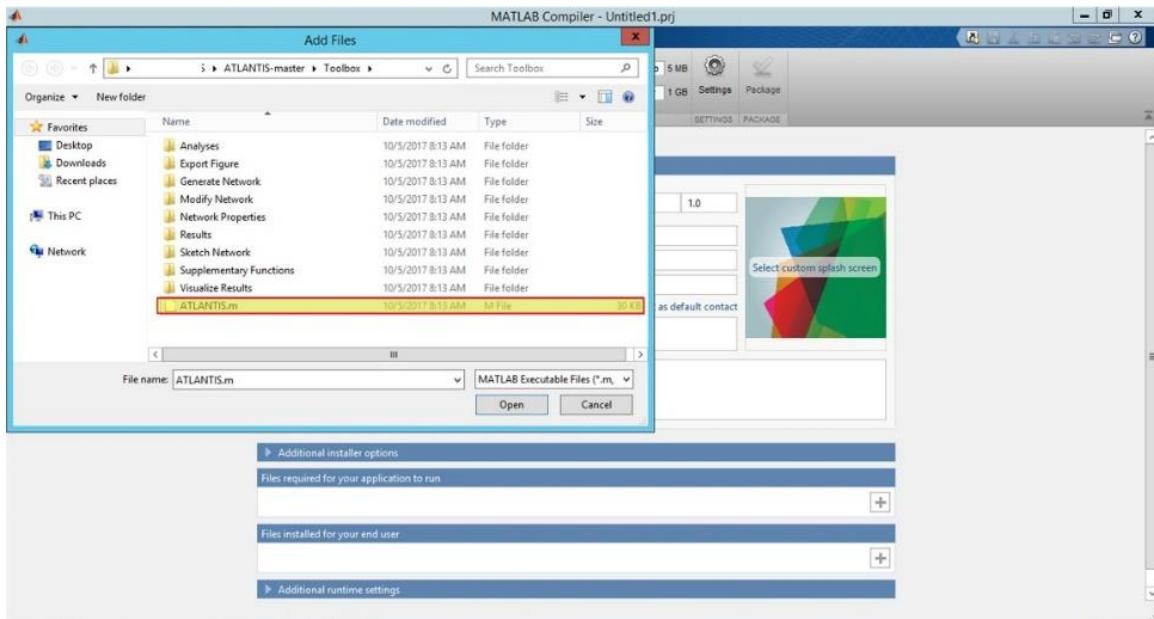
3. Click on ‘Application Compiler’ as highlighted in figure below:



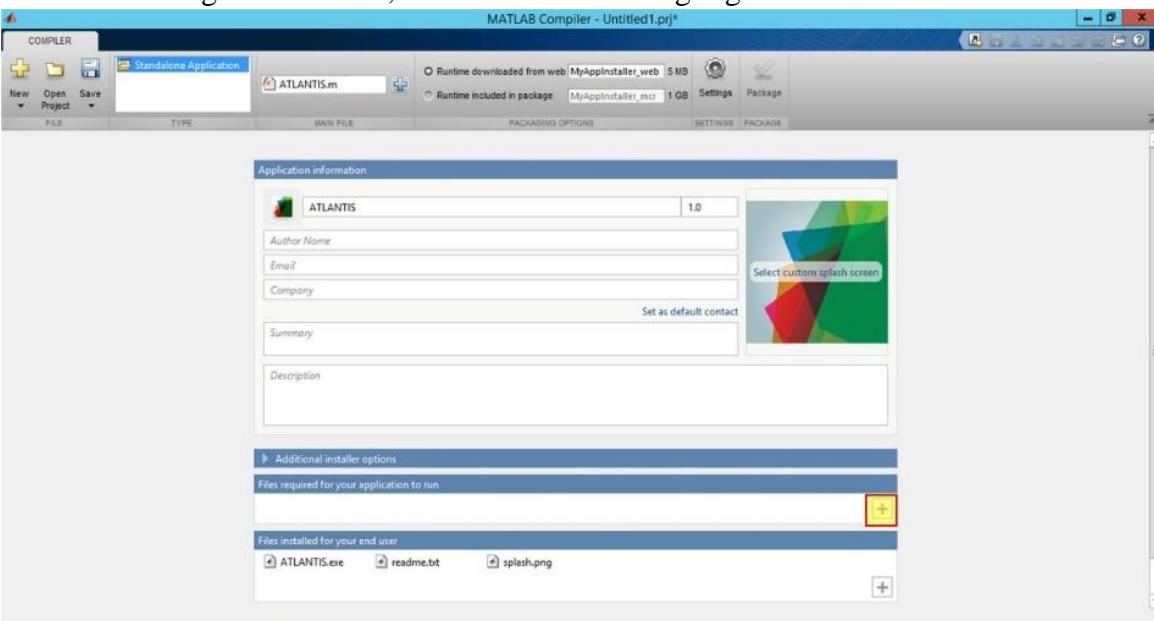
4. Click on the button highlighted in the image below in order to add the main file and write ATLANTIS in the first input field under ‘Application Information’ section.



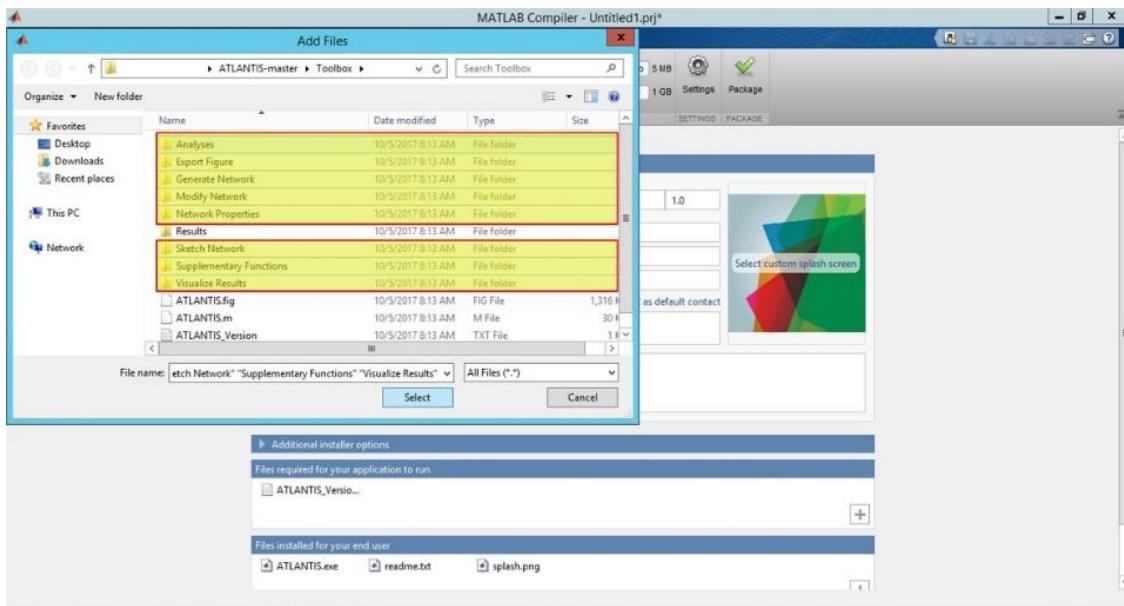
5. Add ‘ATLANTIS.m’ file as shown in the figure below.



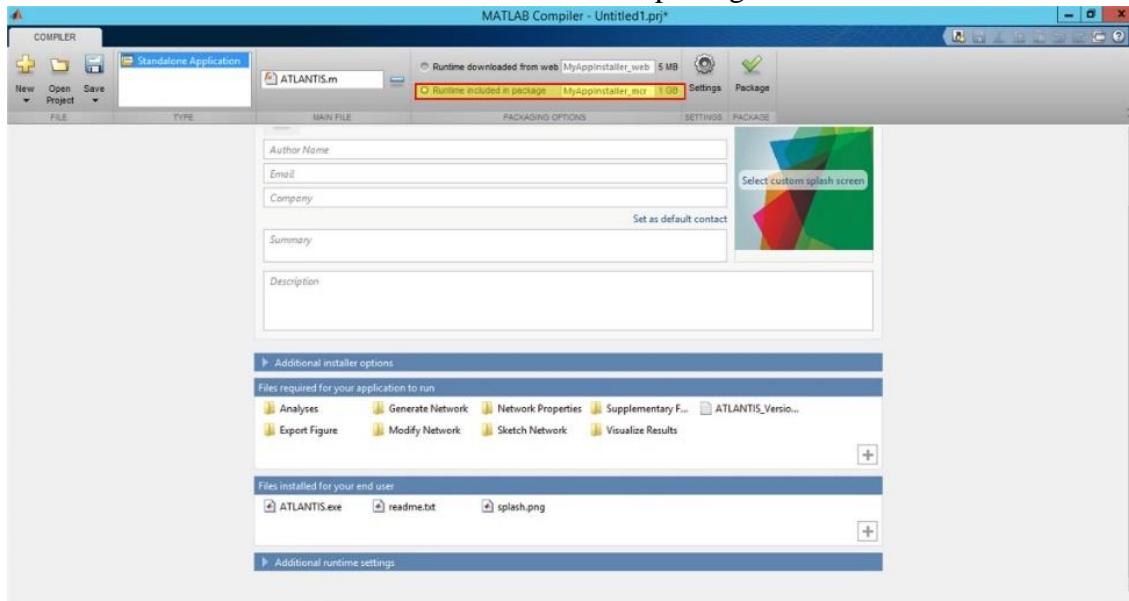
**6.** After adding the main file, click on the button highlighted below.



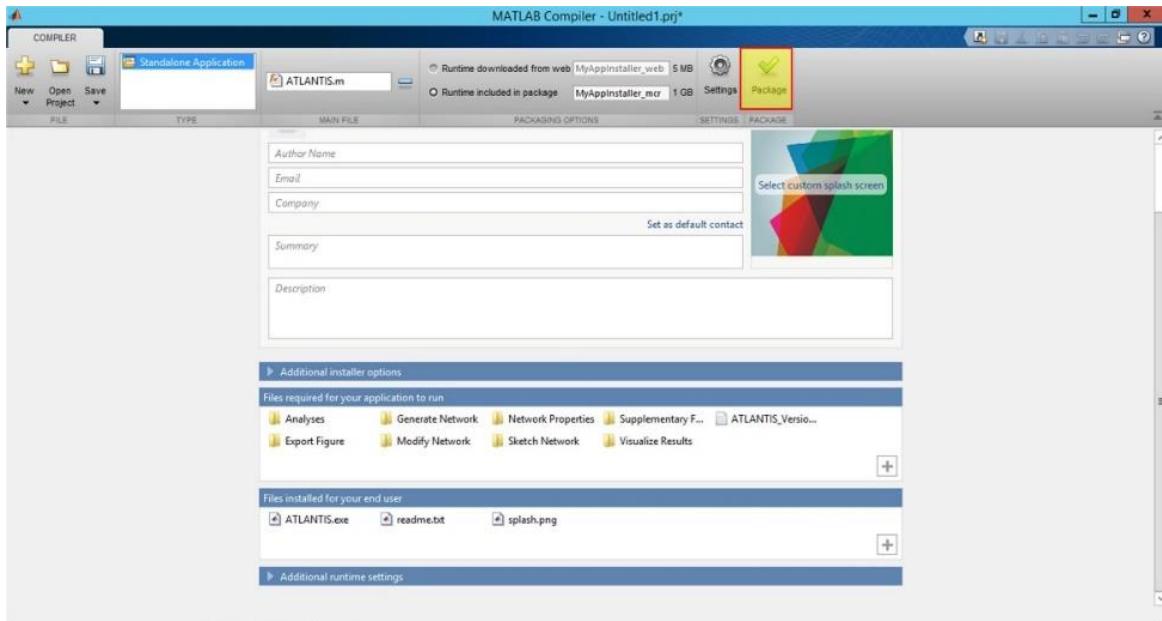
**7.** After clicking, select the files as shown in figure below.



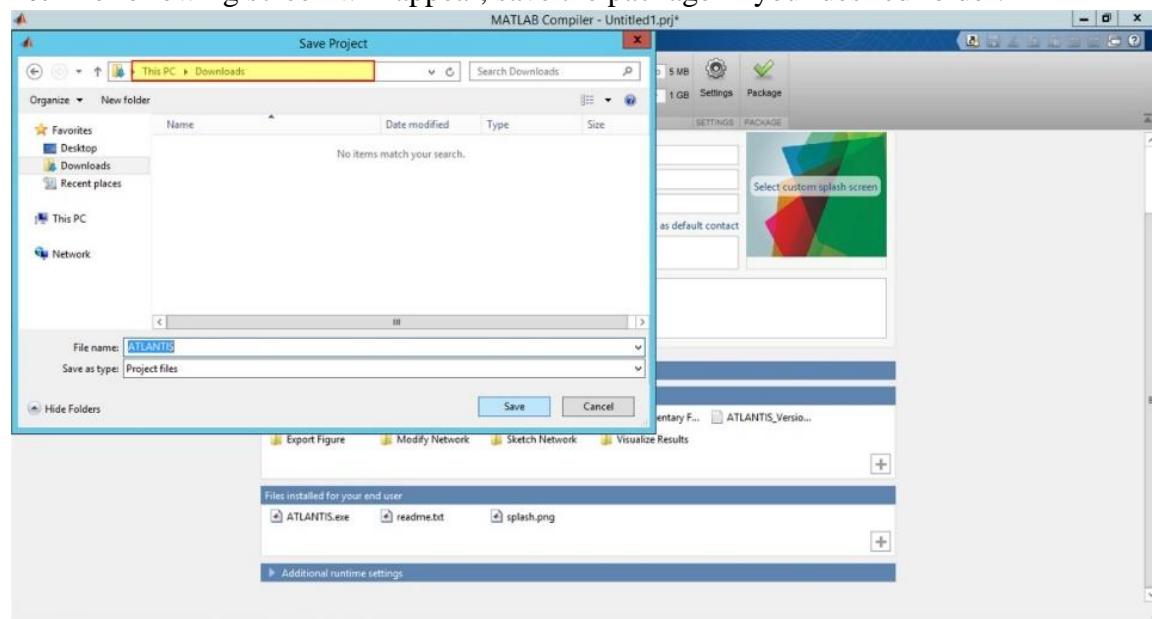
**8. Click on the radio button ‘Runtime included in package’.**



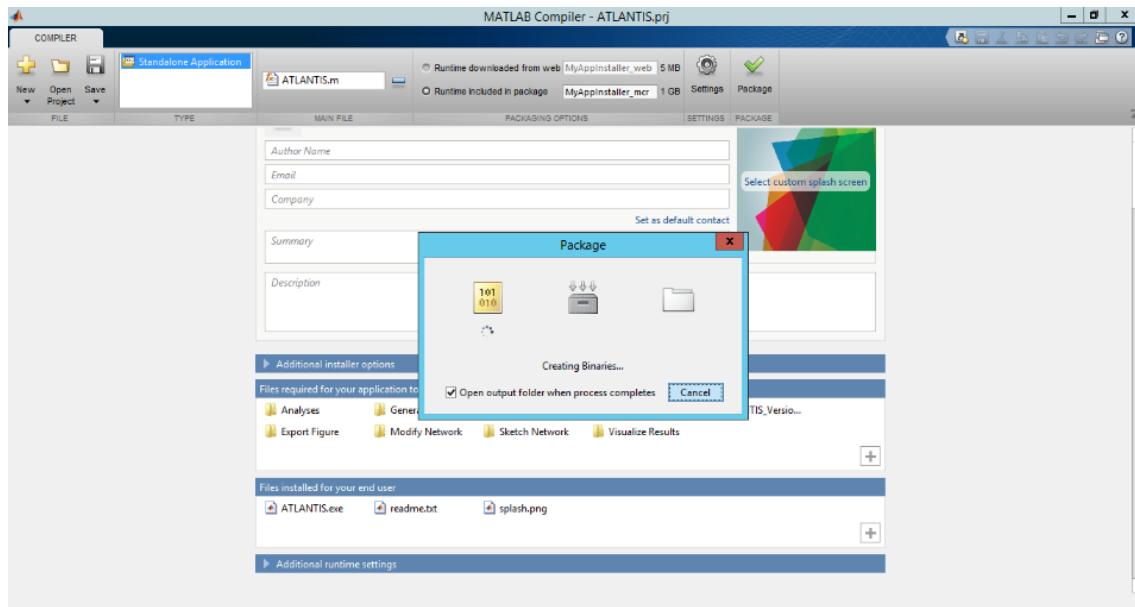
**9. Click on ‘Package’ as shown in the figure below.**



**10.** The following screen will appear, save the package in your desired folder.



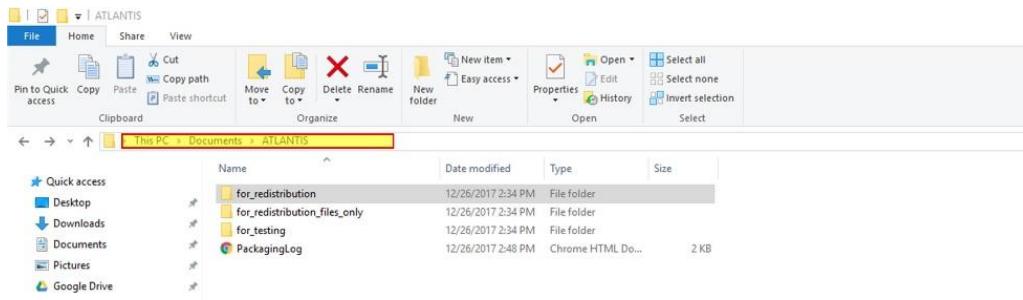
**11.** After the user has selected the desired folder, the following screen will appear. After the process is complete, close the MATLAB window.



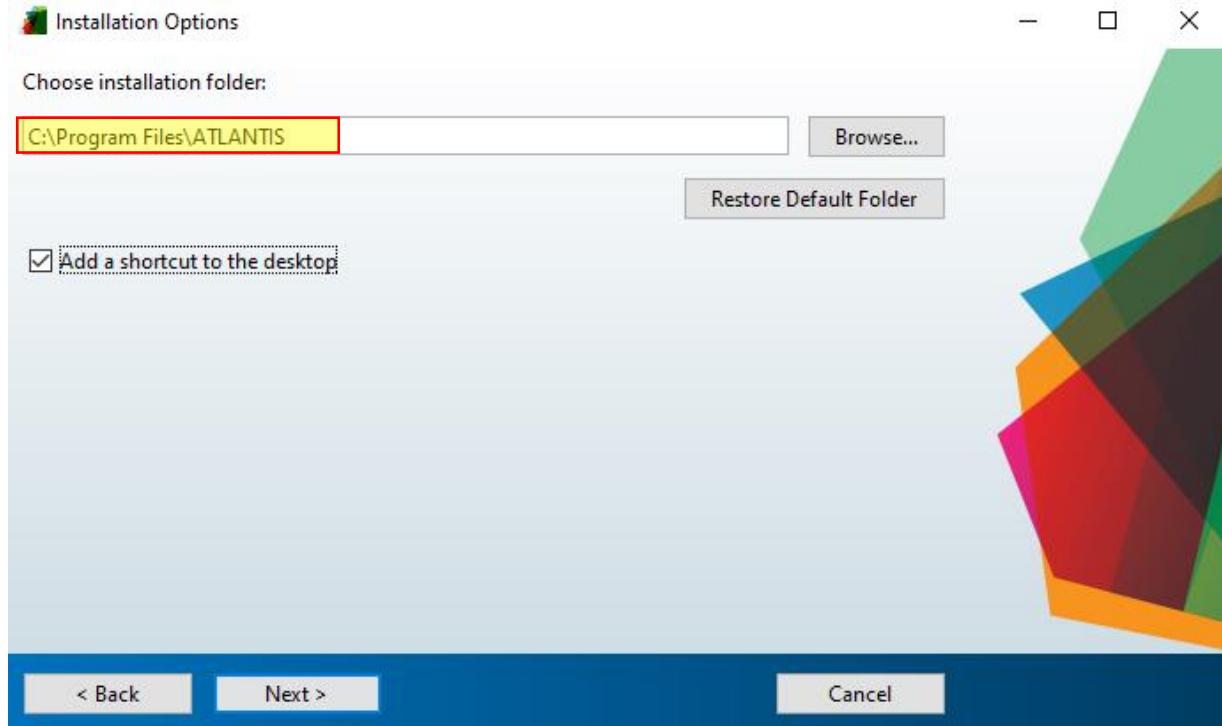
## Installing Executable file (.exe) for Windows (PC with MATLAB Runtime)

For installing the (.exe) file in a computer with MATLAB, the following steps can be followed:

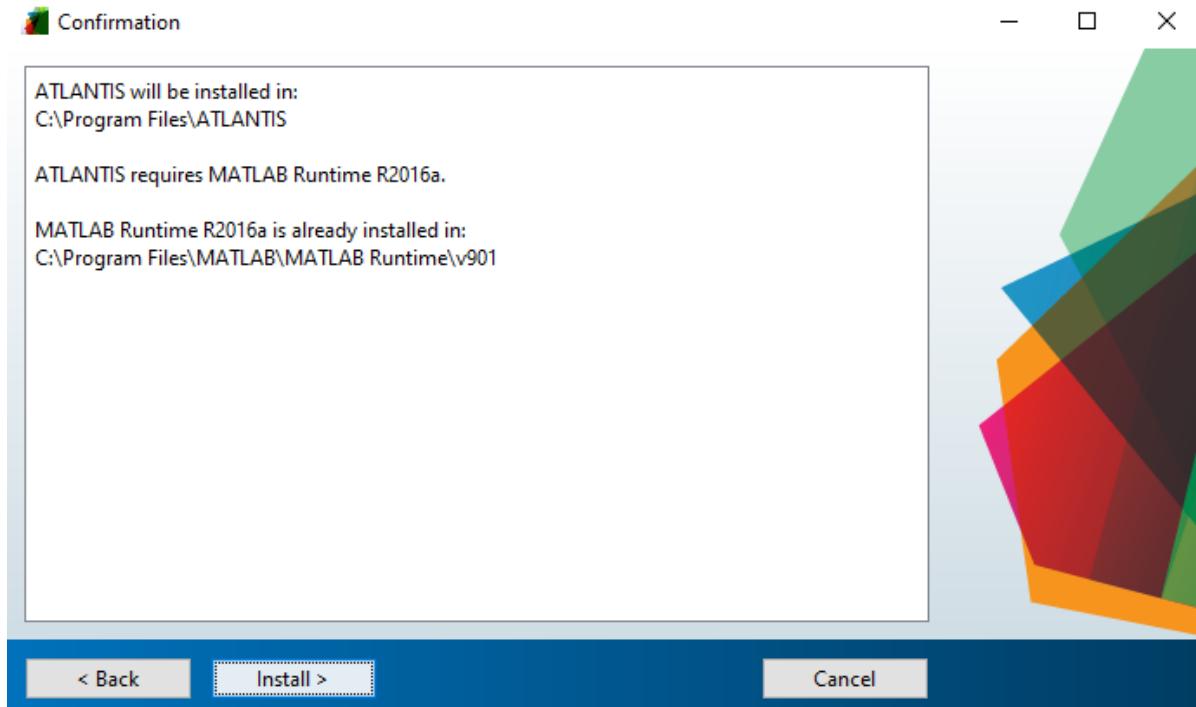
1. Open the folder where ‘.exe’ file is downloaded and go to ‘for redistribution’ folder.



2. Click on ‘MyAppInstaller\_mcr.exe’ and grant access to install the executable file. Keep the installation folder ‘C:\Program Files\Atlantis’ and click on ‘Next’.



3. The following window will appear, click on 'Install'.



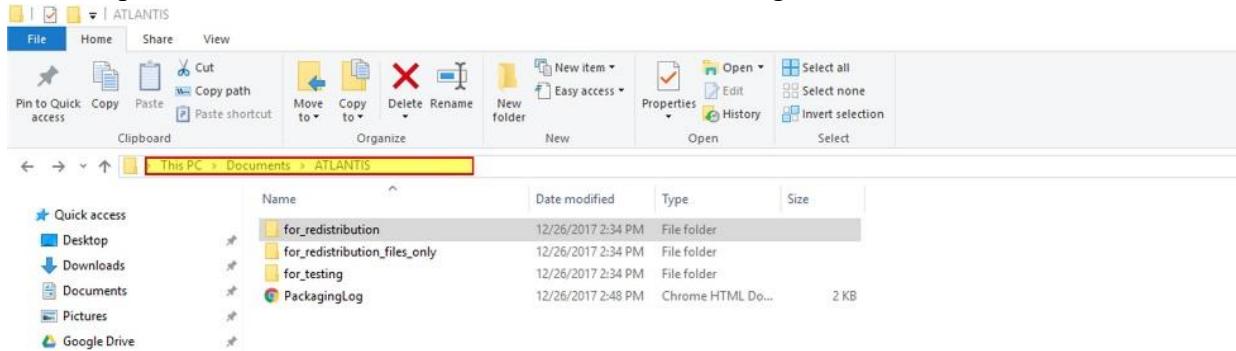
4. After the installation is complete, check desktop for the shortcut generated and click on the shortcut to run ATLANTIS.



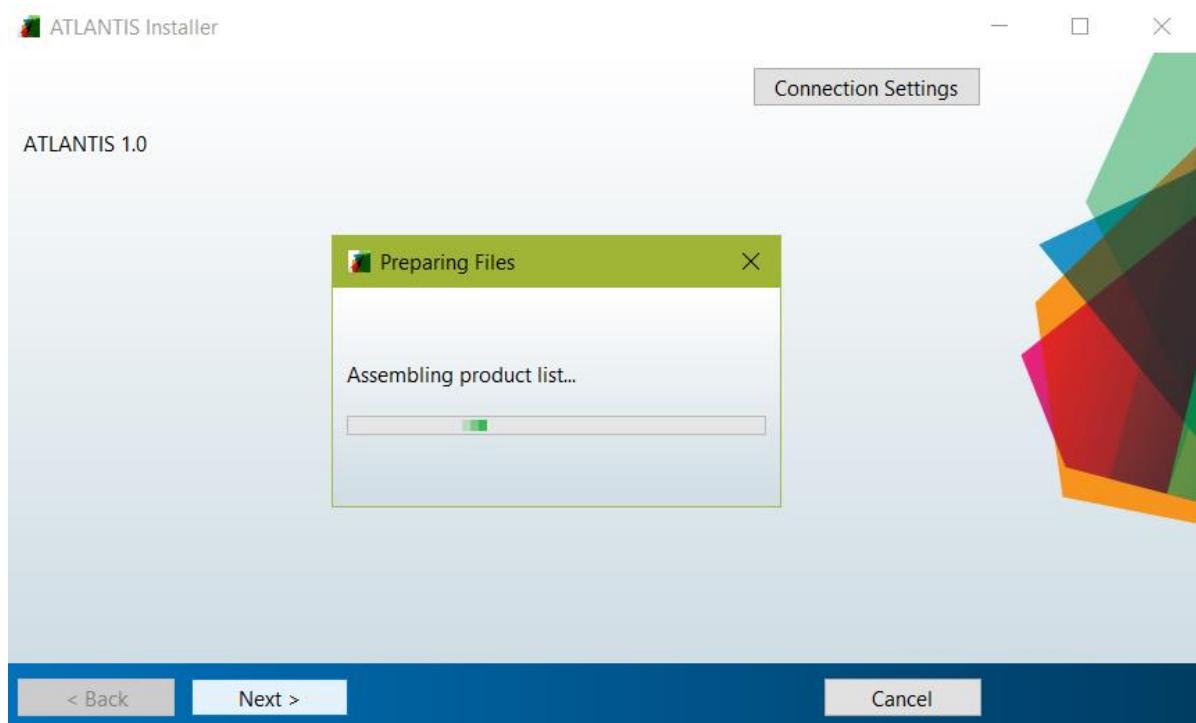
## Installing Executable file (.exe) for Windows (PC without MATLAB Runtime)

For installing the (.exe) file in a computer with MATLAB the following steps can be followed:

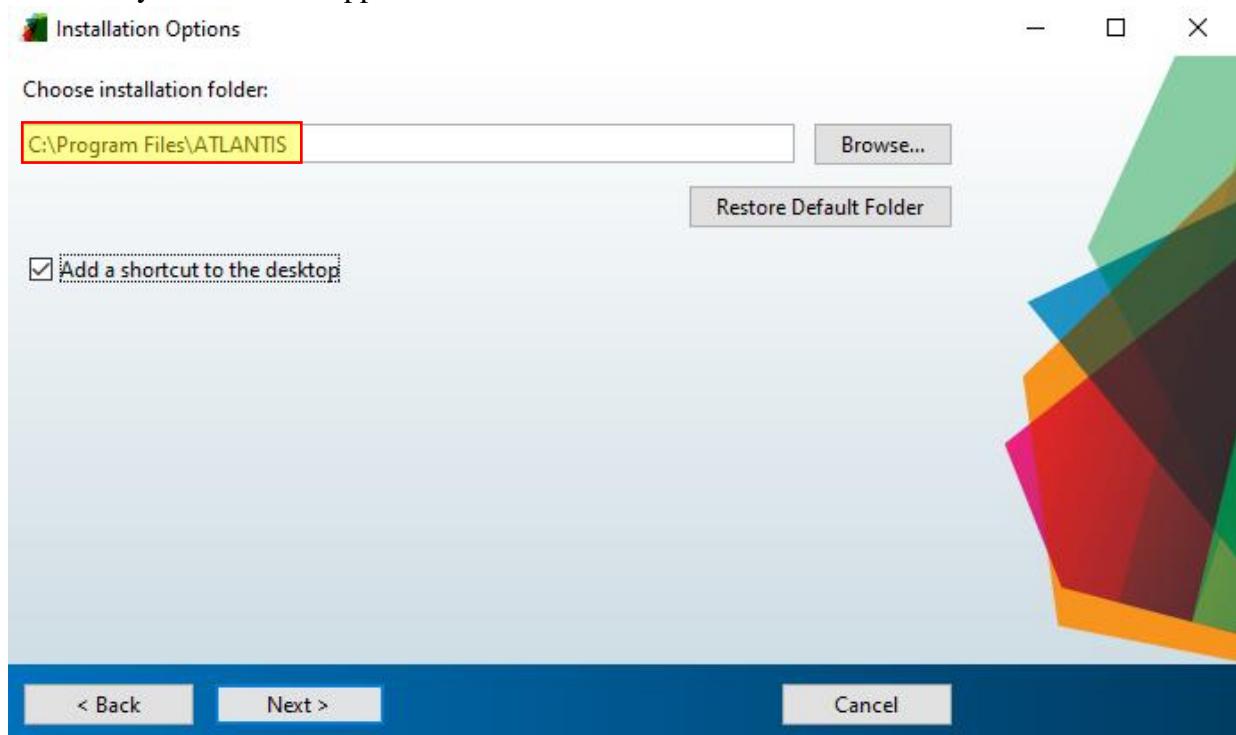
1. Open the folder where ‘.exe’ file is downloaded and go to ‘for redistribution’ folder.



2. Click on ‘MyAppInstaller\_mcr.exe’ and grant access to install the executable file. The following window will appear.



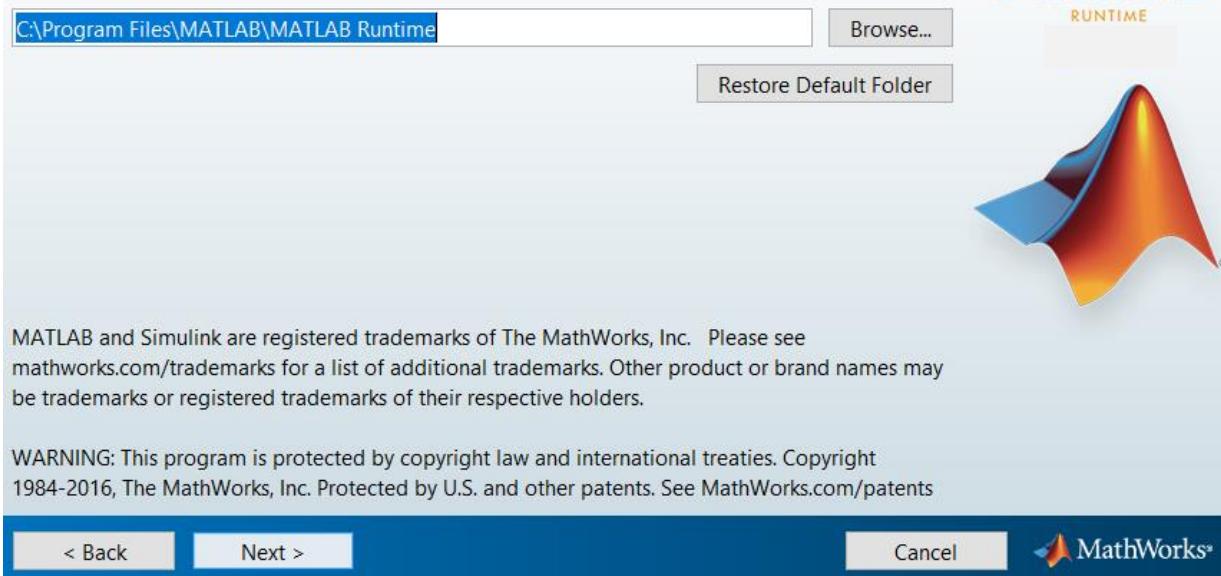
3. Click on 'Next' button and proceed with the installation path given as 'C:\Program Files\ATLANTIS'. Make sure to check the 'Add a shortcut to the desktop' checkbox for easy access to the application.



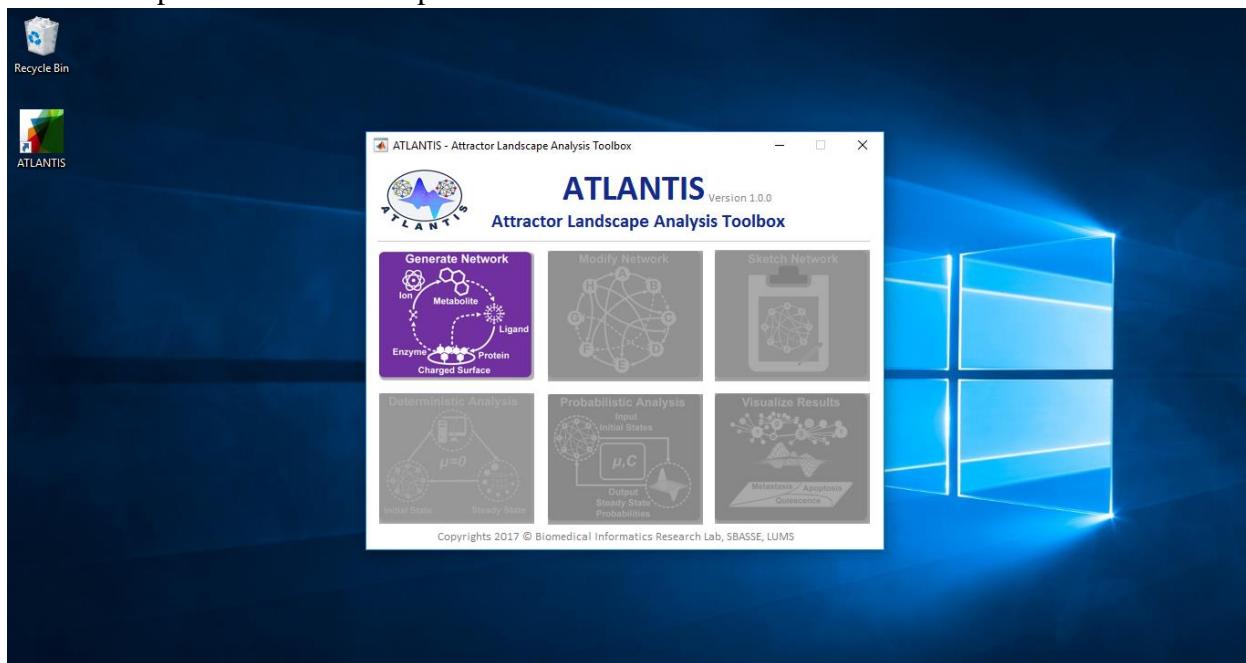
4. Since your PC does not have MATLAB installed, the following window will appear. The path for installation should be ‘C:\Program Files\MATLAB\MATLAB Runtime’. Click on ‘Next’ to proceed.

**MATLAB Runtime is required.**

Choose installation folder:



5. Accept the License Agreement and proceed with installation. Once the installation is complete find the desktop shortcut and click on it.



## 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 for Windows

Download and install the latest release of Graphviz<sup>1</sup> (Graph visualization software) [here](#).

To make Graphviz compatible with MATLAB, follow the steps provided in the next subsection below.

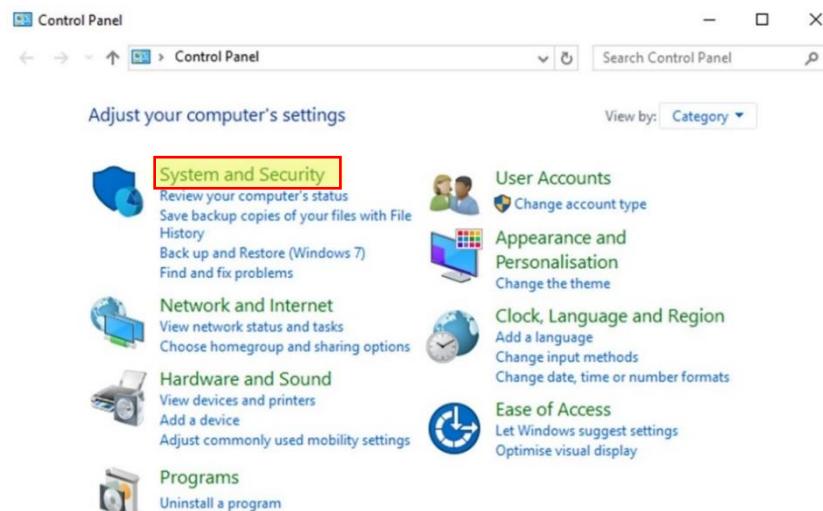
### 2.2.3. Setting Up Graphviz for Windows

The following steps should be followed in order to set up Graphviz. A detailed [video tutorial](#) is also presented to set up Graphviz.

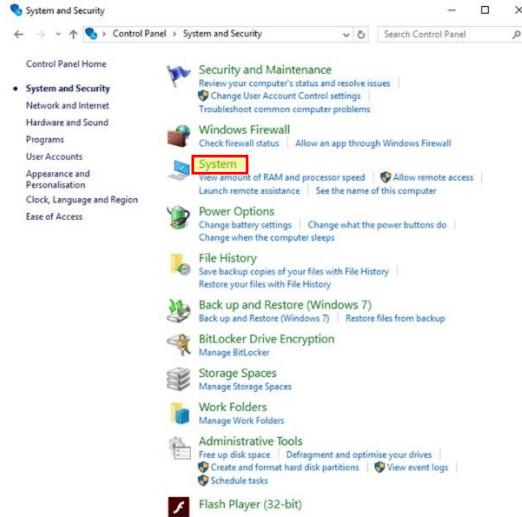
1. Go to the directory where Graphviz is installed and select ‘bin’ folder. Copy path to this folder for use later. It should resemble ‘C:\Program Files\Graphviz2.38\bin’.



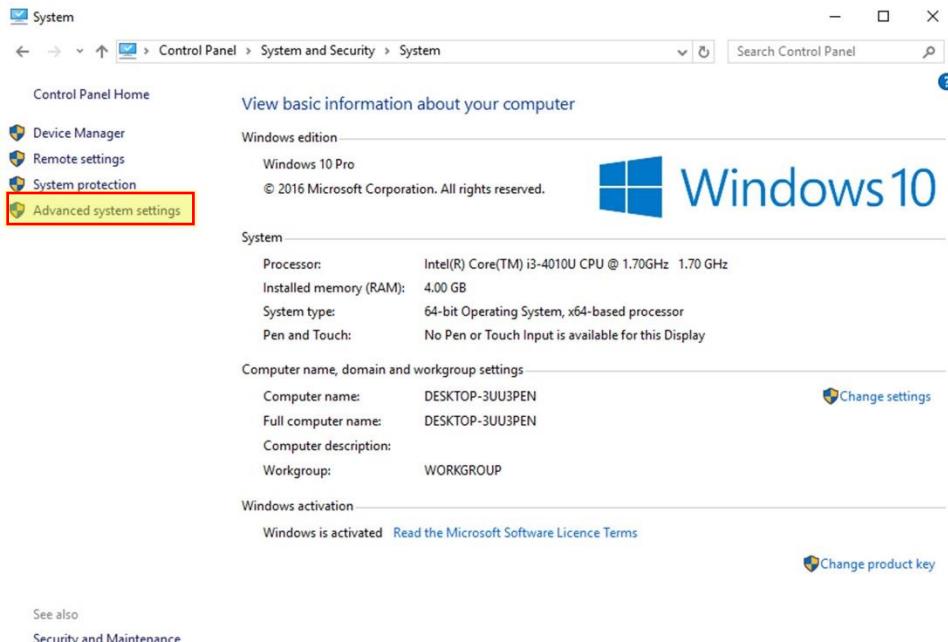
2. Next, go to Start, select Control Panel and then double click ‘System and Security’.



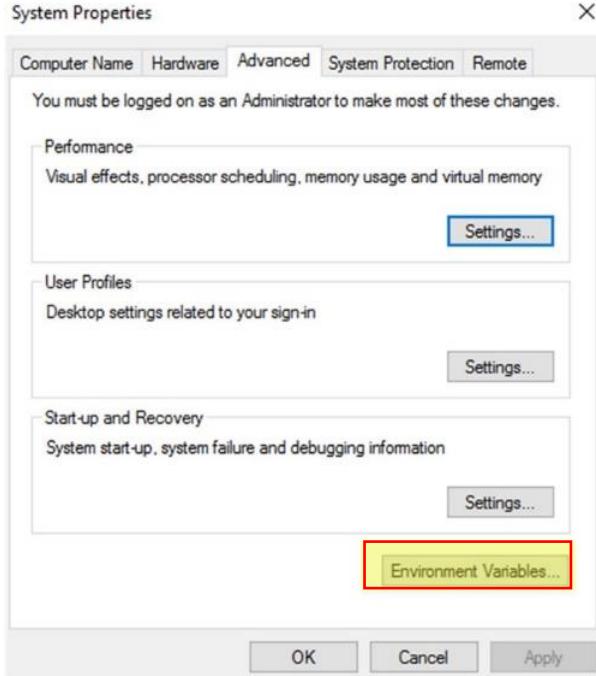
3. Next, click on ‘System’.



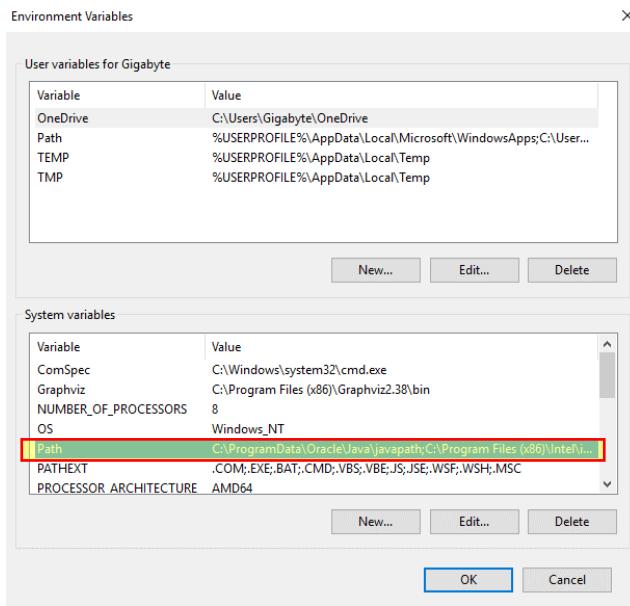
#### 4. Select ‘Advanced system settings’ on the left side of the page.



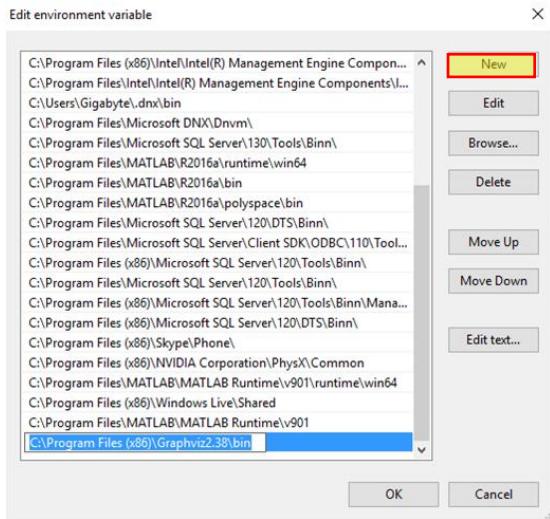
#### 5. A window titled ‘System Properties’ will pop up. Click ‘Environment Variables’ at the bottom left of the window.



6. A new window titled ‘Environment Variables’ appears. Double click on the specified path.



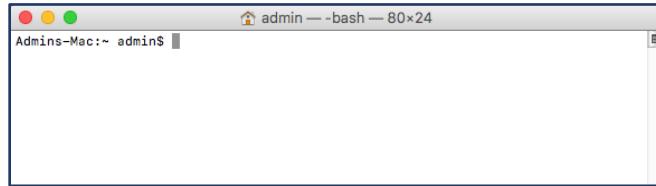
7. Click on ‘New’ to add the path copied earlier ‘C:\Program Files\Graphviz2.38\bin’ and then press ‘OK’.



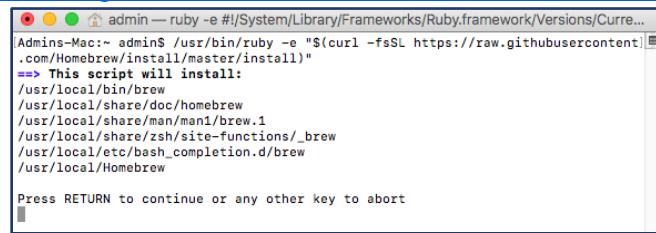
8. For further assistance, you can view the YouTube [tutorial](#).

#### 2.2.4. Setting Up Graphviz for macOS

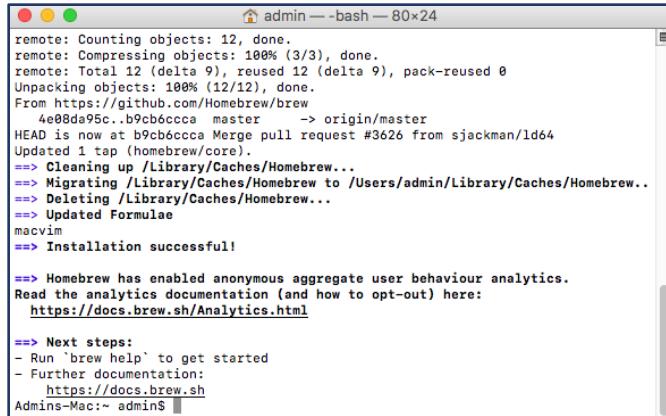
1. Open ‘Terminal’ on your computer.



2. Enter the following command on your terminal: ‘/usr/bin/ruby -e "\$(curl -fsSL



3. Enter the password to install Homebrew.



```

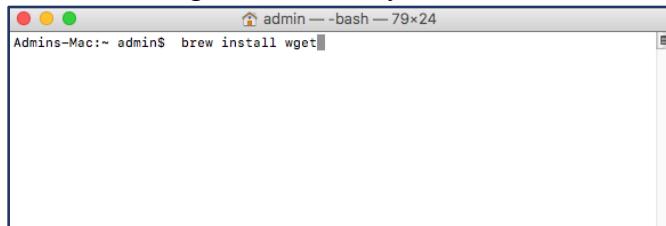
remote: Counting objects: 12, done.
remote: Compressing objects: 100% (3/3), done.
remote: Total 12 (delta 9), reused 12 (delta 9), pack-reused 0
Unpacking objects: 100% (12/12), done.
From https://github.com/Homebrew/brew
  4e08da95c..b9cb6ccca master -> origin/master
HEAD is now at b9cb6ccca Merge pull request #3626 from sjackman/ld64
Updated 1 tap (homebrew/core).
==> Cleaning up /Library/Caches/Homebrew...
==> Migrating /Library/Caches/Homebrew to /Users/admin/Library/Caches/Homebrew...
==> Deleting /Library/Caches/Homebrew...
==> Updated Formulae
macvim
==> Installation successful!

==> Homebrew has enabled anonymous aggregate user behaviour analytics.
Read the analytics documentation (and how to opt-out) here:
  https://docs.brew.sh/Analytics.html

==> Next steps:
- Run `brew help` to get started
- Further documentation:
  https://docs.brew.sh
Admins-Mac:~ admin$ 

```

4. Enter the following command on your terminal: ‘`brew install wget`’



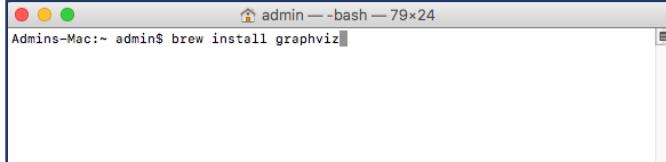
```

admin — bash — 79x24
Admins-Mac:~ admin$ brew install wget

```

### 2.2.5. Install Graphviz for macOS

1. Enter the following command on your terminal: ‘`brew install graphviz`’

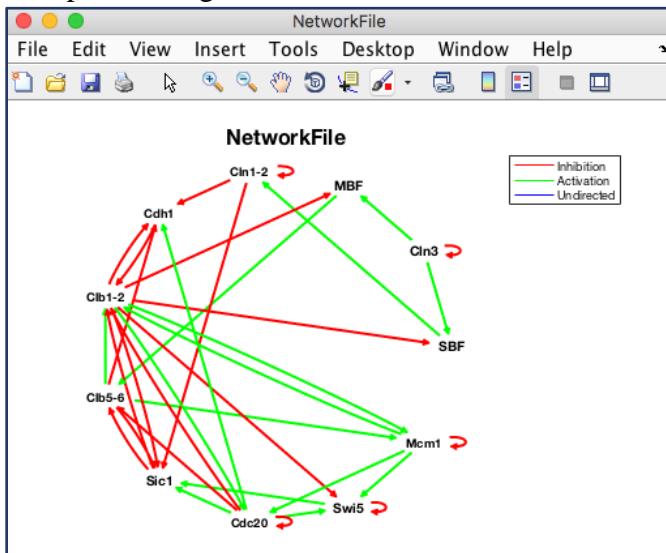


```

admin — bash — 79x24
Admins-Mac:~ admin$ brew install graphviz

```

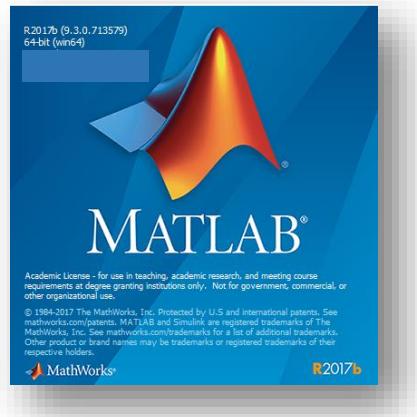
2. Test Graphviz using ATLANTIS.



## 3. Using ATLANTIS

### 3.1. Setting Up MATLAB and Launching the Application

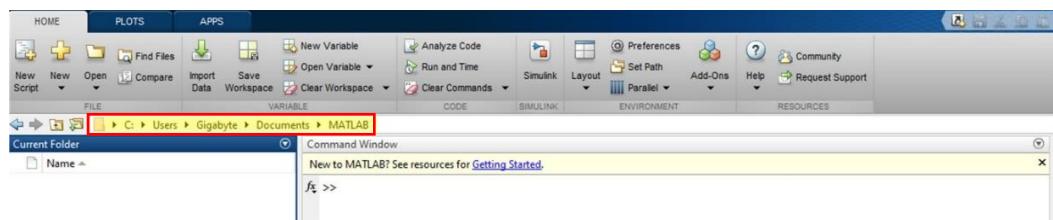
1. Launch MATLAB on your computer.



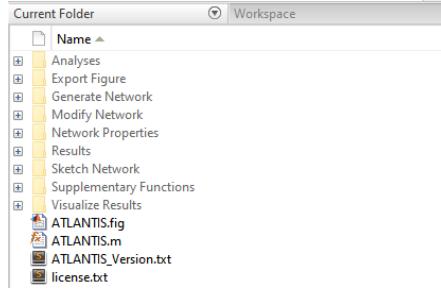
2. Copy full path to the folder that contains ATLANTIS. Assuming that this folder is kept in 'C' drive, the path will resemble this 'C:\Users\User\Desktop\ATLANTIS'



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 or simply run the ATLANTIS.m file.
6. A detailed [video tutorial](#) is also presented to help users launch ATLANTIS.

## 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	0
3	MBF	0	0	0	0	0	0	0	1	0	0	0
4	SBF	0	0	0	1	0	0	0	0	0	0	0
5	Cln1-2	0	0	0	-1	-1	0	0	0	-1	0	0
6	Cdh1	0	0	0	0	0	0	0	0	0	-1	0
7	Swi5	0	0	0	0	0	-1	0	0	1	0	0
8	Cdc20	0	0	0	0	1	1	-1	-1	1	-1	0
9	Clb5-6	0	0	0	0	-1	0	0	0	-1	1	1
10	Sic1	0	0	0	0	0	0	0	-1	0	-1	0
11	Clb1-2	0	-1	-1	0	-1	-1	1	0	-1	0	1
12	Mcm1	0	0	0	0	0	1	1	0	0	1	-1
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	0	0
MBF	0	0	0	0	0	0	0	1	0	0	0	0
SBF	0	0	0	1	0	0	0	0	0	0	0	0
Cln1-2	0	0	0	-1	-1	0	0	0	-1	0	0	0
Cdh1	0	0	0	0	0	0	0	0	0	-1	0	0
Swi5	0	0	0	0	0	-1	0	0	1	0	0	0
Cdc20	0	0	0	1	1	-1	-1	1	-1	0	0	0
Clb5-6	0	0	0	0	-1	0	0	0	-1	1	1	1
Sic1	0	0	0	0	0	0	0	0	-1	0	-1	0
Clb1-2	0	-1	-1	0	-1	-1	1	0	-1	0	1	1
Mcm1	0	0	0	0	0	1	1	0	0	1	-1	0
basal	0	0	0	0	0	0	0	0	0	0	0	0

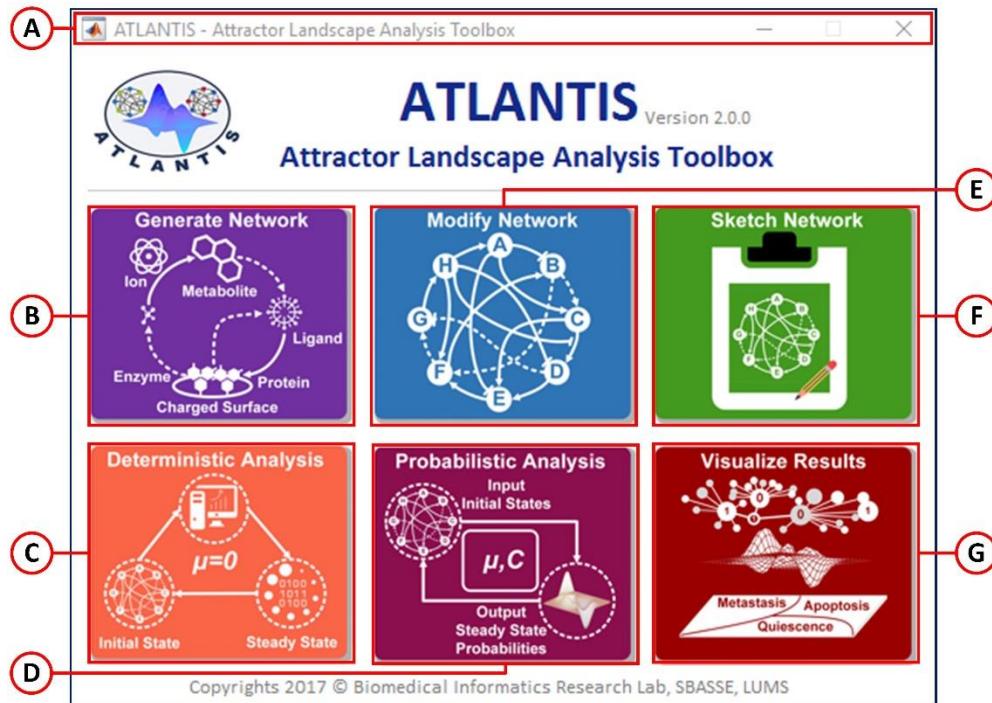
## 2. Network Modification File for Modifying Node States and Interactions

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

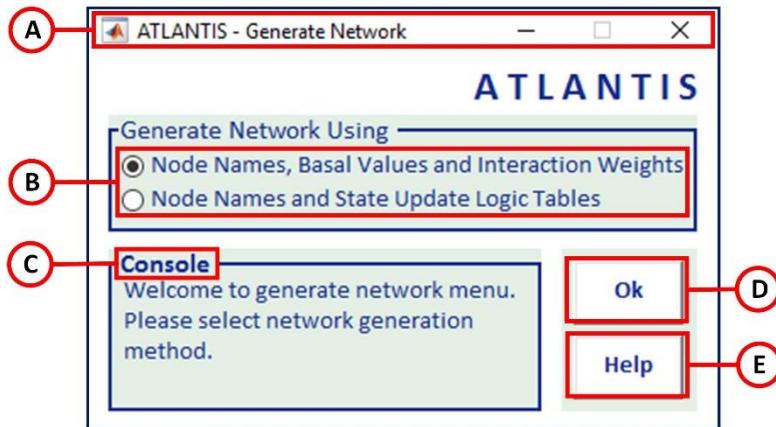
### 3.3. Using ATLANTIS Graphical User Interface

#### 1. ATLANTIS - Main Menu



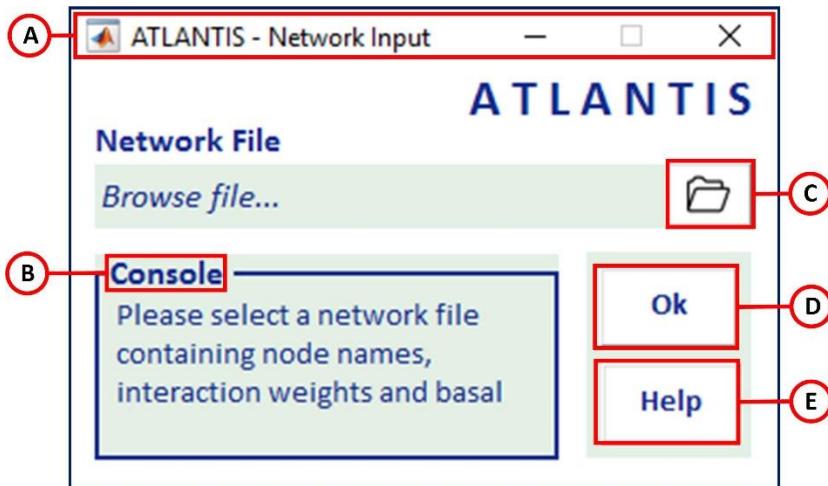
- At the top of the ATLANTIS interface is the ‘Title bar’, which displays the name of the program and the title of the current document.
- Click on ‘Generate Network’ to provide user with options to create Boolean models of biological networks using node name, interaction weights and basal values or node names and node state update logic tables
- 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.
- 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.
- Click on ‘Modify Network’ to add mutations (knock down and overexpression of nodes and deletion of links) to the network.
- Click on ‘Sketch Network’ to provide the option to display their input network.
- 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.

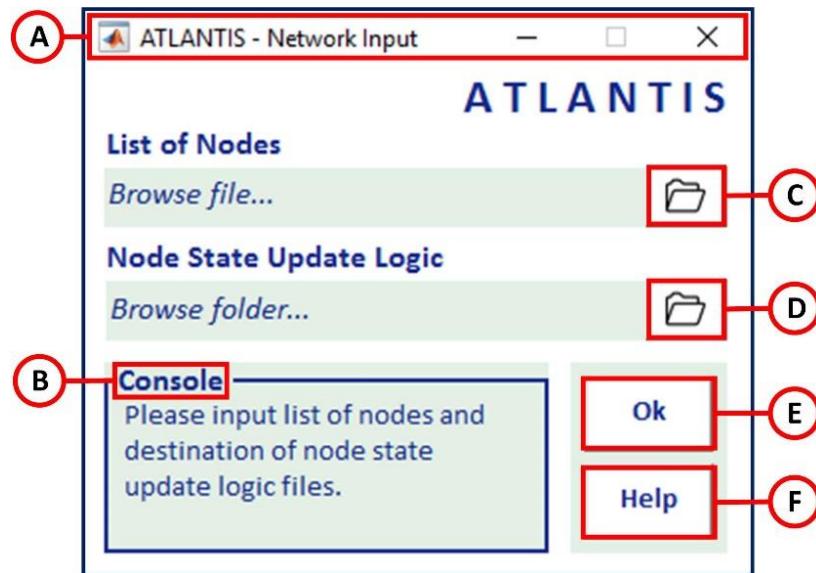
### 3. ATLANTIS – Network Input as node Interaction Weights Matrix



- 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.

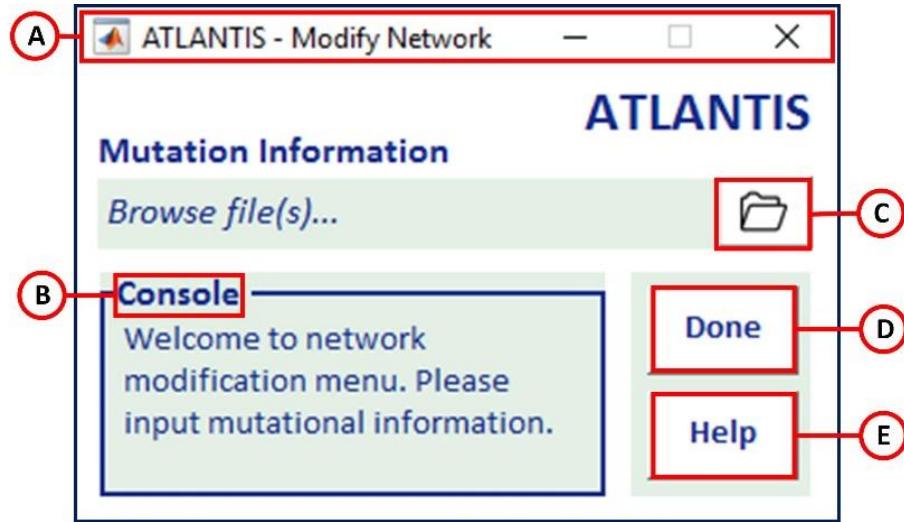
- D. Press ‘OK’ to generate network using the input information.
- E. Click on ‘Help’ for a detailed User Manual in PDF format.

#### 4. ATLANTIS - Network Input as a set of Update Logic Tables



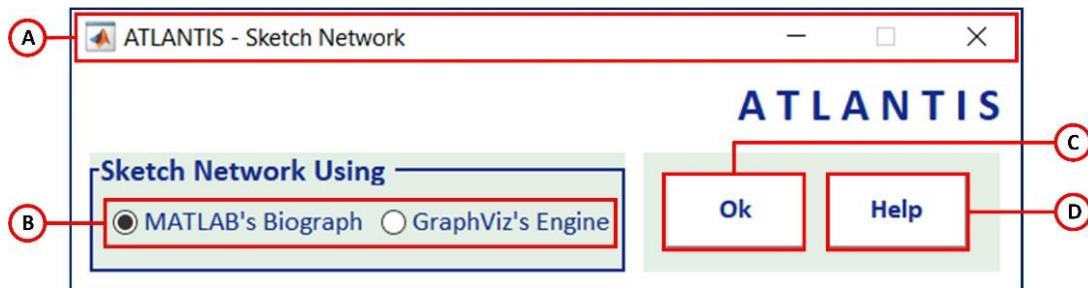
- A. At the top of the ATLANTIS interface is the ‘Title bar’, which displays the name of the program and the title of the current document.
- B. Console indicates the progress within the GUI.
- C. Press to browse Node list file.
- D. Press to browse the folder containing Node update logic table files.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

#### 5. ATLANTIS – Modify Network



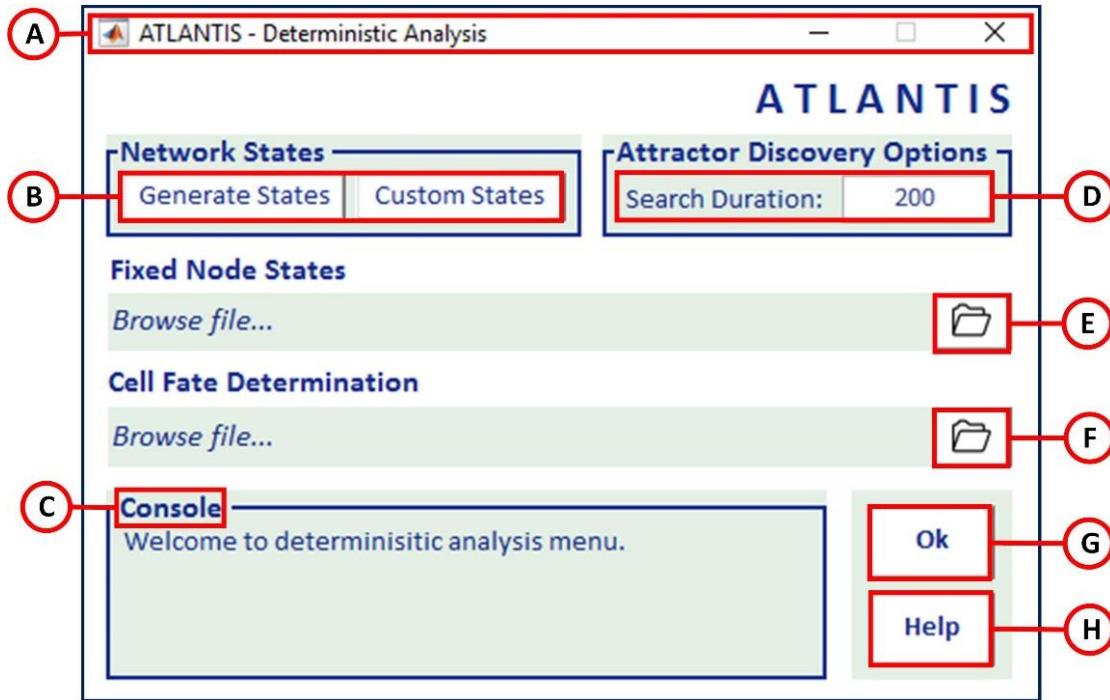
- A. At the top of the ATLANTIS interface is the ‘Title bar’, which displays the name of the program and the title of the current document.
- B. Console indicates the progress within the GUI.
- C. Press to browse file containing information related to mutations (Node deletion or Node overexpression or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

## 6. ATLANTIS – Sketch Network



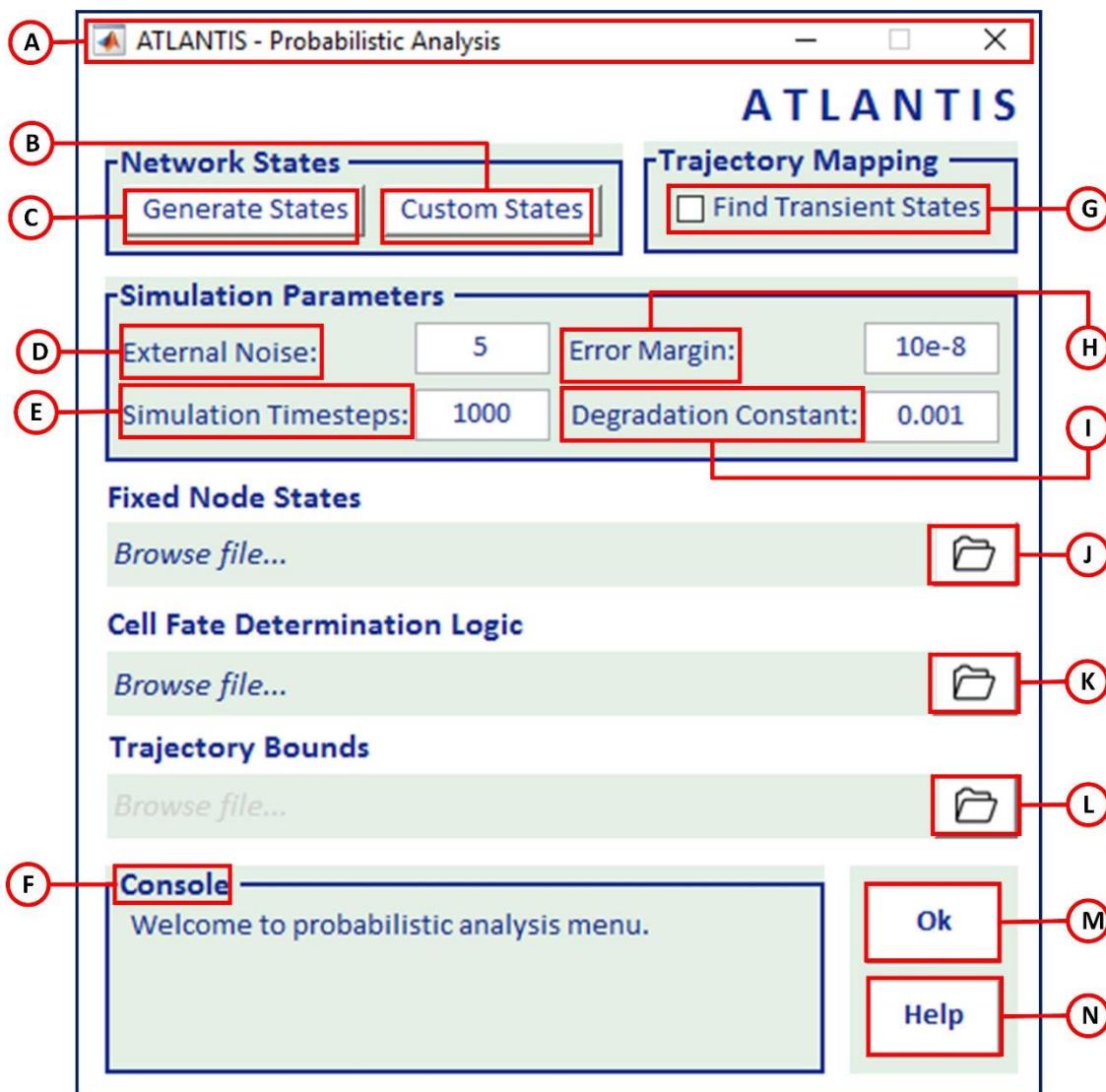
- A. At the top of the ATLANTIS interface is the ‘Title bar’, which displays the name of the program and the title of the current document.
- B. Select the option to sketch network using MATLAB’s Biograph or Graphviz’s Engine for visualization of the network
- C. Press ‘OK’ to display the current network.
- D. 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 initial network states list, whereby the user has two different options, (i) state-space generated by ATLANTIS and (ii) user-specified subset of the complete state-space.
- C. Console indicates the progress within the GUI.
- D. Option to specify time steps utilized towards discovering attractors of various cell fates.
- E. Browse and input the file containing names and values of nodes whose state values need to be fixed for complete simulation.
- F. Browse and Input the Cell Fate Determination Logic Information File.
- G. Press ‘OK’ to start the determinisitic analysis.
- H. Click on ‘Help’ for a detailed User Manual in PDF format.

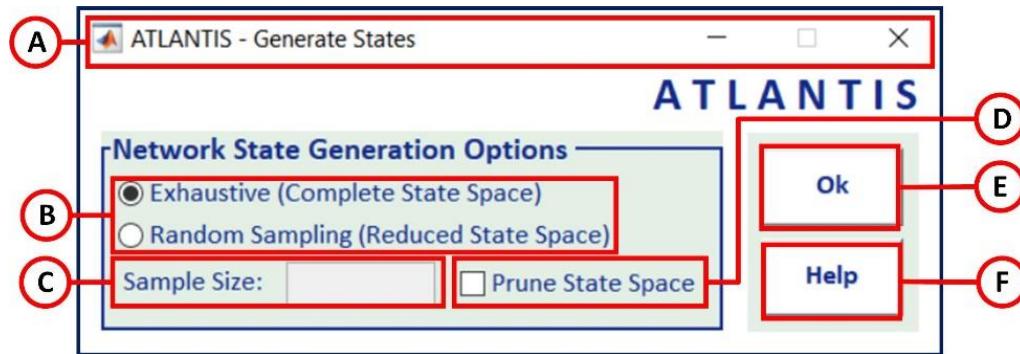
## 8. ATLANTIS – Probabilistic Analysis



- 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.
- Press to generate states space of the network (Exhaustive or Random).
- Press to generate custom states, as a sample from the complete state space specified by user.
- Enter a value of external noise between (between 0-5, default: 5).
- Enter a value to indicate that how long the simulation will run (between 1 and 10000, default: 1000).
- Console indicates the progress within the GUI.
- Check to find the transient states that a network assumes in going from the start boundary state to the final boundary state. These boundary states are specified in the file uploaded under ‘Trajectory Bounds’ (L).
- Enter a value to define what is the error margin. ( $0 - 10^{-4}$ , default:  $10^{-8}$ ). Lower the error margin longer the time taken to complete probabilisitic analysis (PA).

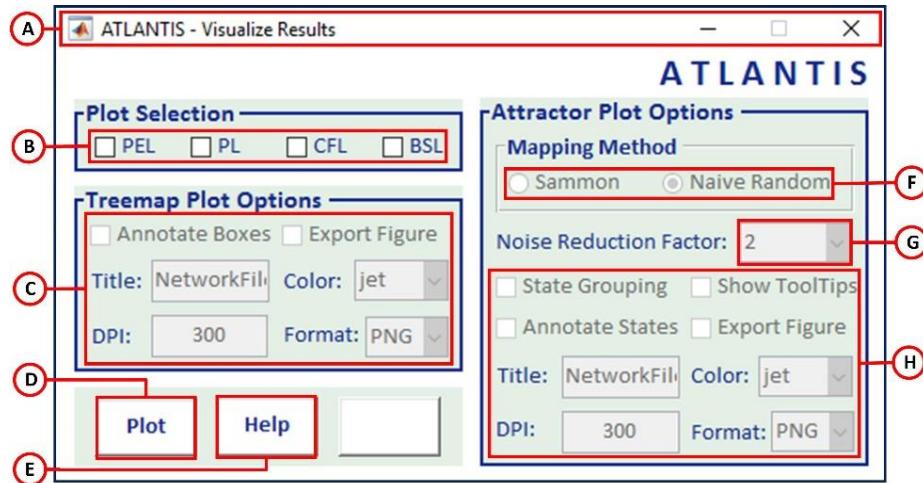
- I. Enter a value for degradation constant (Between 0-1, default: 0.001).
- J. Browse and input the file containing names and values of nodes whose state values need to be fixed for complete
- K. Browse and Input Cell Fate Determination Logic Information File.
- L. Browse file mentioning the initial and final states of the trajectory under consideration.
- M. Press ‘OK’ to start the PA.
- 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. Generate all possible state combinations of network states ( $2^n$  where n is the total number of nodes) or generate a specified number of network state combinations (for Probabilistic Analysis).
- C. Specify the number of states to generate in the Sample size (in case of Random Sampling) edit box.
- D. Prune State Space works for PA only (for Exhaustive state space generation it is optional and for Random Sampling it is always on).
- E. Click on ‘OK’ to proceed.
- F. Click on ‘Help’ for a detailed User Manual in PDF format.

## 10. ATLANTIS – Visualize Results

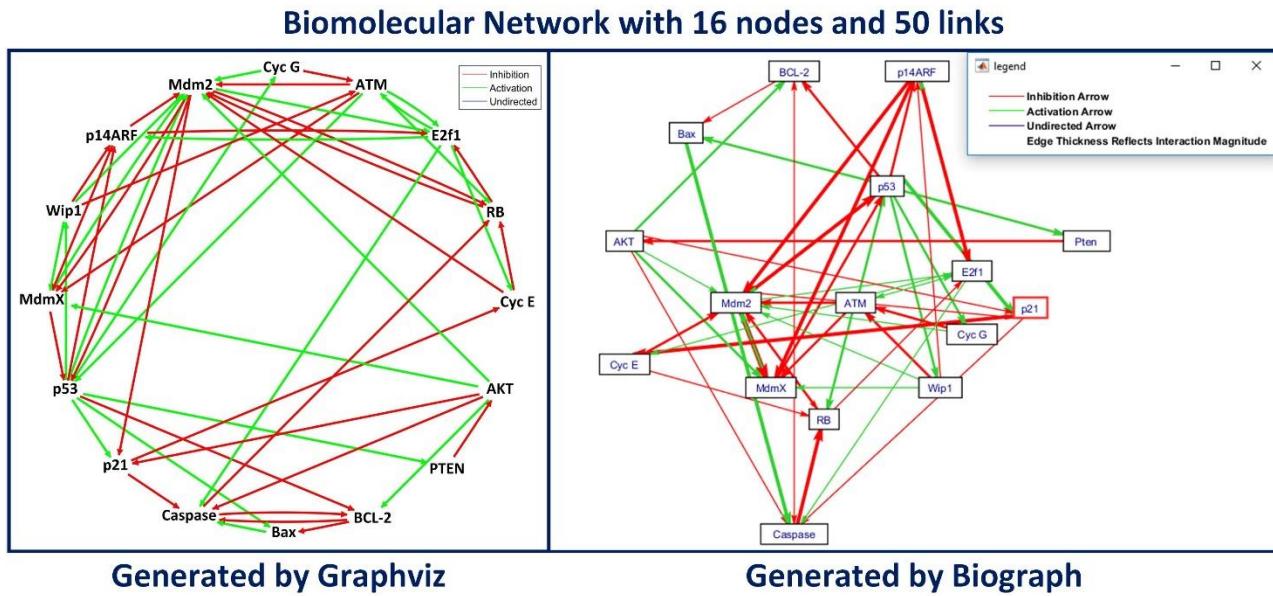


- A. At the top of the ATLANTIS interface is the ‘Title bar’, which displays the name of the program and the title of the current document.
- B. Check boxes to plot different landscapes including Potential Energy Landscape (PEL), Probability Landscape (PL), Cell Fate Landscape (CFL) and Basin Size Landscape (BSL).
- C. Treemap plotting options.
- D. Press ‘Plot’ to plot the selected plots.
- E. Click on ‘Help’ for a detailed User Manual in pdf format.
- F. Choose mapping scheme for Generating Landscapes (Attractor and Potential Energy).
- G. Noise reduction factor for landscape generation.
- H. Landscape format options.

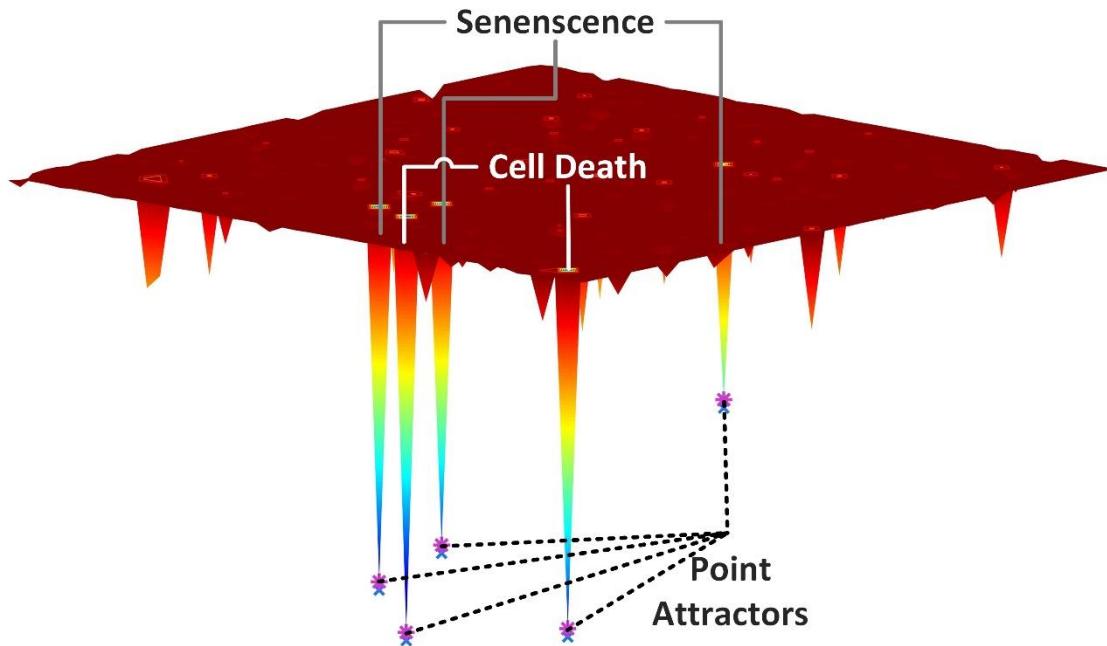
## 4. Interpreting Results

The results shown below involve the Boolean model of P53 regulatory network, as studied by 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.

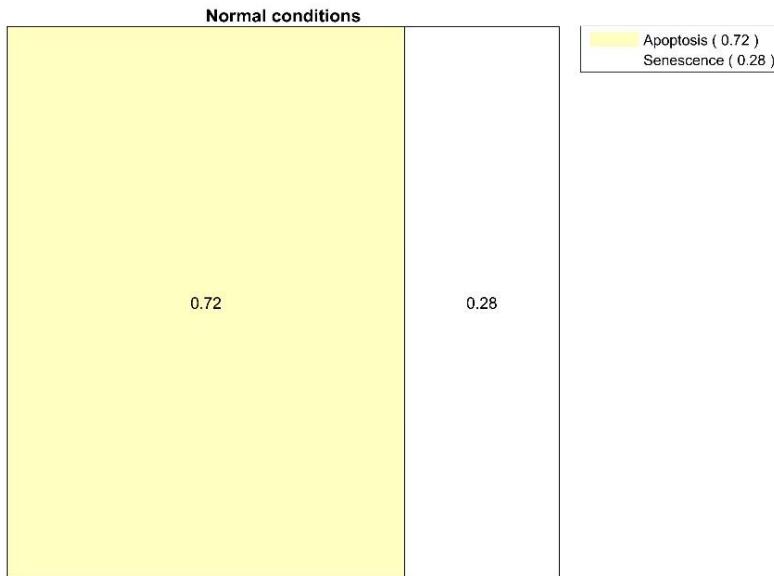
### 4.1. Network Visualization using Graphviz and Biograph



## 4.2. Cell fate prediction using potential energy landscape



### 4.3. Classifying phenotypes

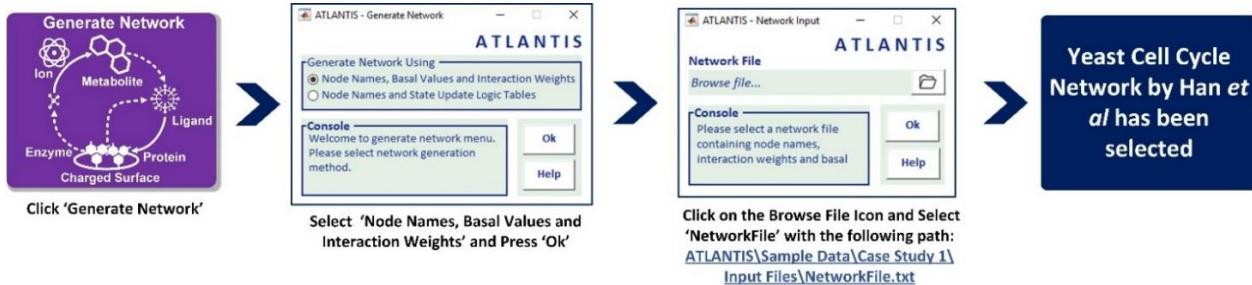


## 5. Worked Examples

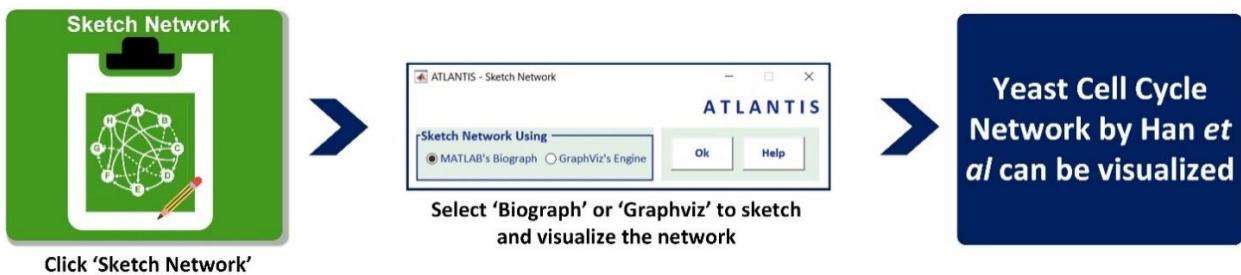
To facilitate the ease of use, we present two elaborate worked out attractor landscape analysis (Case Study-1 and Case Study-3) examples using ATLANTIS. The first worked out example details the procedure for performing the deterministic analysis (DA) and probabilistic analysis (PA) of yeast cell cycle network presented by Han *et al*<sup>2</sup>. The second example illustrates the rules-based DA of the 201-node network presented by Cho *et al*<sup>3</sup>. In addition, a few worked examples have been provided to highlight some specific features presented by ATLANTIS. Detailed tutorials of case studies and novel features of ATLANTIS are available as a YouTube [playlist](#).

### 1. Decoding Yeast Cell Cycle Progression using Attractor Landscape Analysis

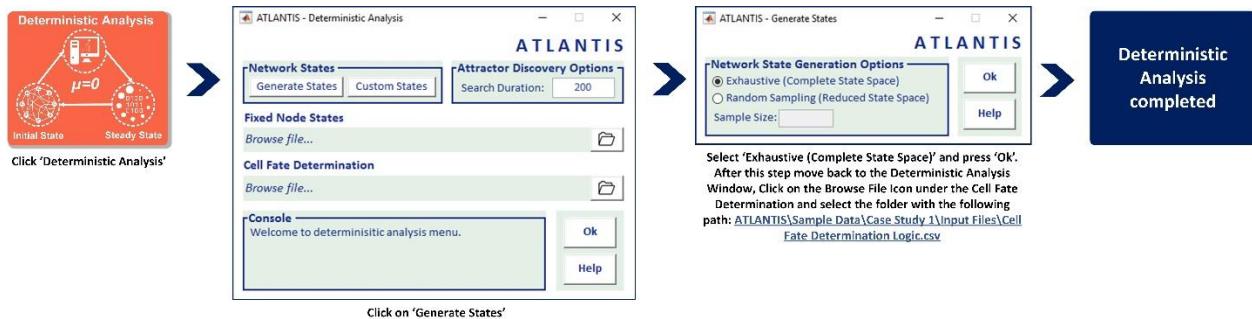
**Step-1.** Click the ‘Generate Network’ button, select the option with ‘Node Names, Basal Values and Interaction Weights’. Next, click Browse file to select the network. The network file for yeast cell cycle network can be found at: [ATLANTIS\Sample Data\Case Study 1\Input Files\NetworkFile.txt](#)



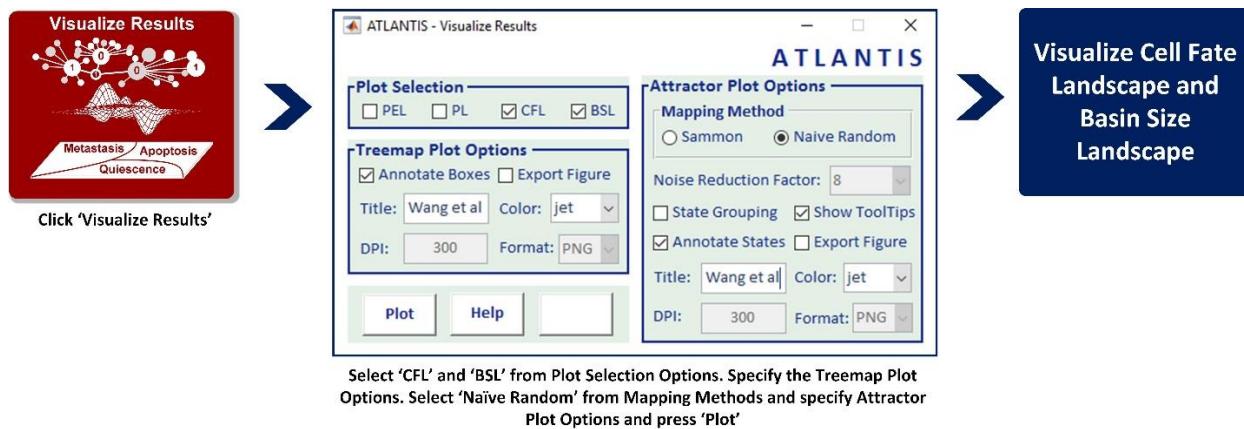
**Step-2.** After loading the network information file, click on ‘Sketch Network’ button. Select Graphviz or Biograph to visualize the specified network. You can export the generated figure by right clicking on the figure.



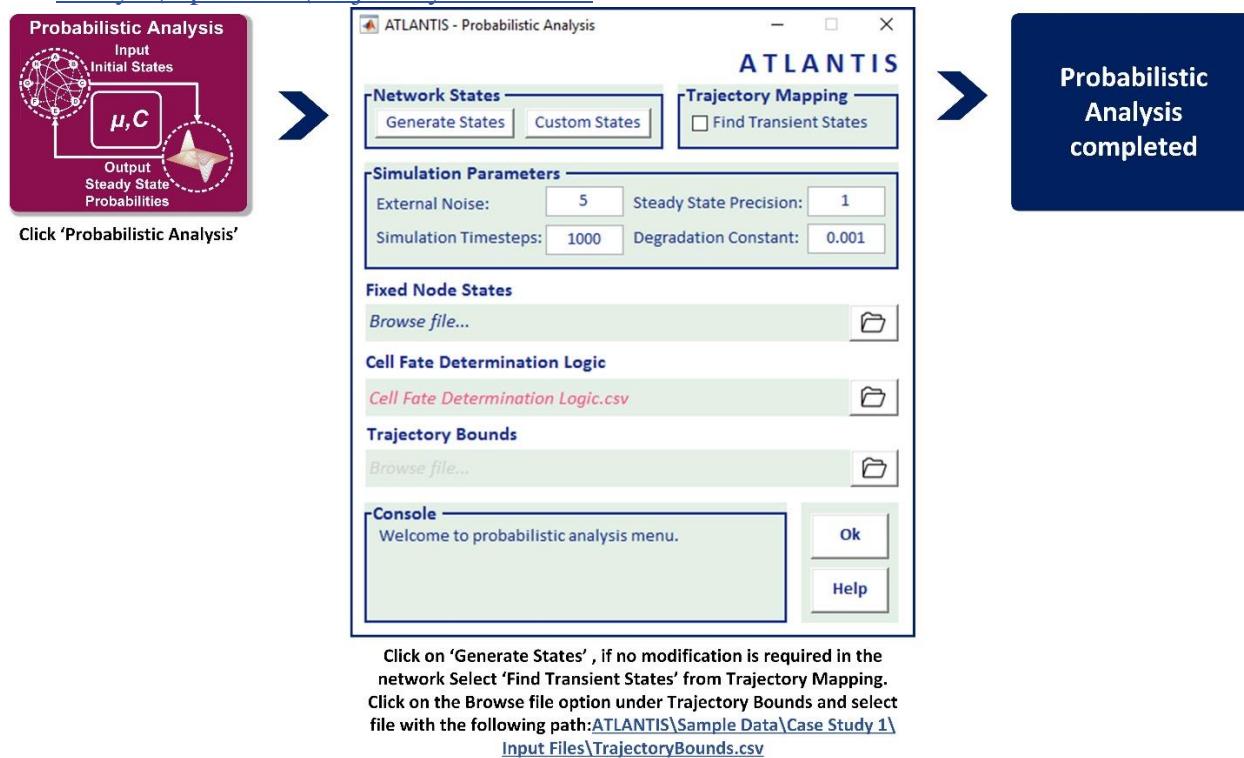
**Step-3.** After visualizing the network, click on ‘Deterministic Analysis’ button. This would open the DA menu. The first step, now, would be generating the network states. Click on ‘Generate States’, select ‘Exhaustive (Complete State Space)’ option and press ‘Ok’. After this step move back to the DA window, click on the Browse file icon under ‘Cell Fate Determination’ and select the folder with the following path: [ATLANTIS\Sample Data\Case Study 1\Input Files\Cell Fate Determination Logic.csv](#)



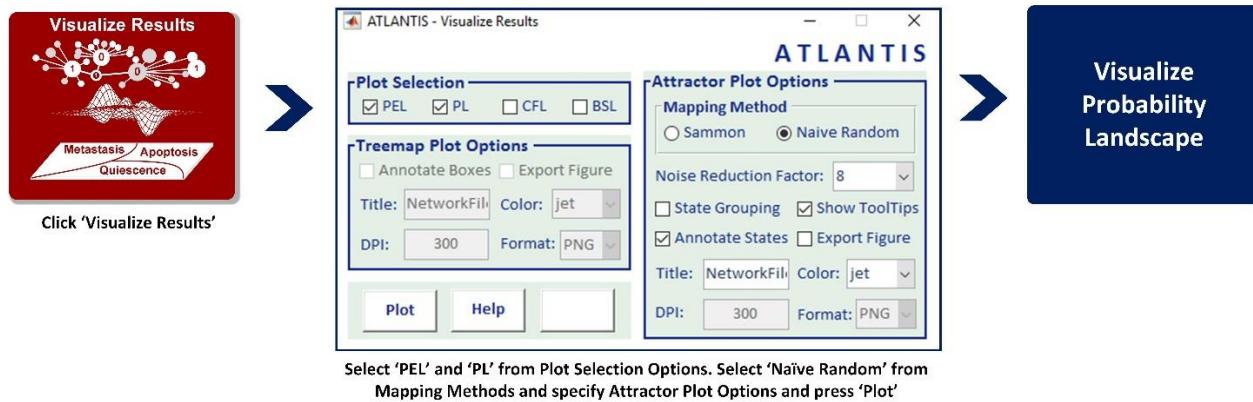
**Step-4.** After performing DA, the next step is to visualize the results. Click on the ‘Visualize Results’ button. Select ‘CFL’ and ‘BSL’ from the ‘Plot Selection’ pane. Specify the ‘Treemap Plot Options’ as shown in figure. Select ‘Naïve Random’ from the ‘Mapping Method’ pane, specify ‘Attractor Plot Options’ and click ‘Plot’. You can export the generated figures as well.



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

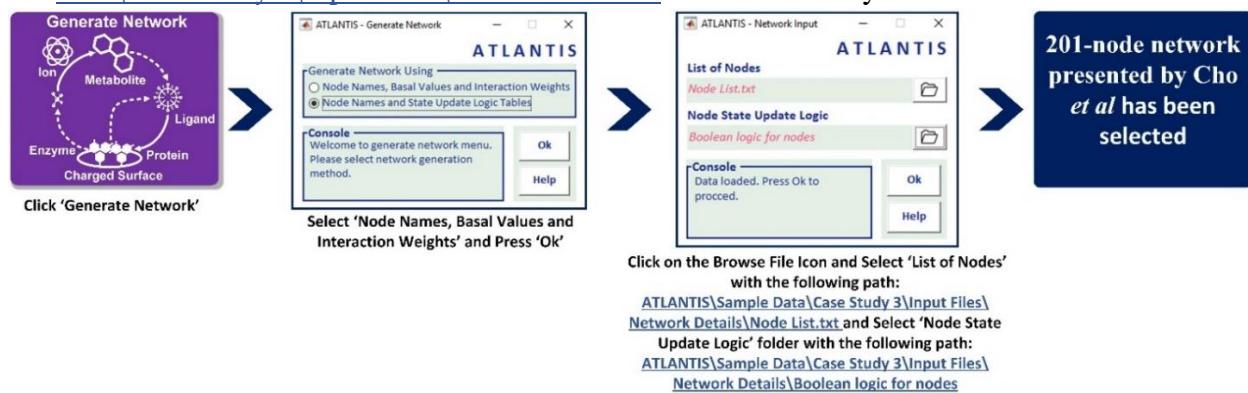


**Step-6.** After performing the PA, the next step is to visualize the results. Click on the ‘Visualize Results’ button. Select ‘PEL’ and ‘PL’ from ‘Plot Selection Options’. Select ‘Naïve Random’ from Mapping Method and specify ‘Attractor Plot Options’ and press ‘Plot’. You can export the generated figures as well.



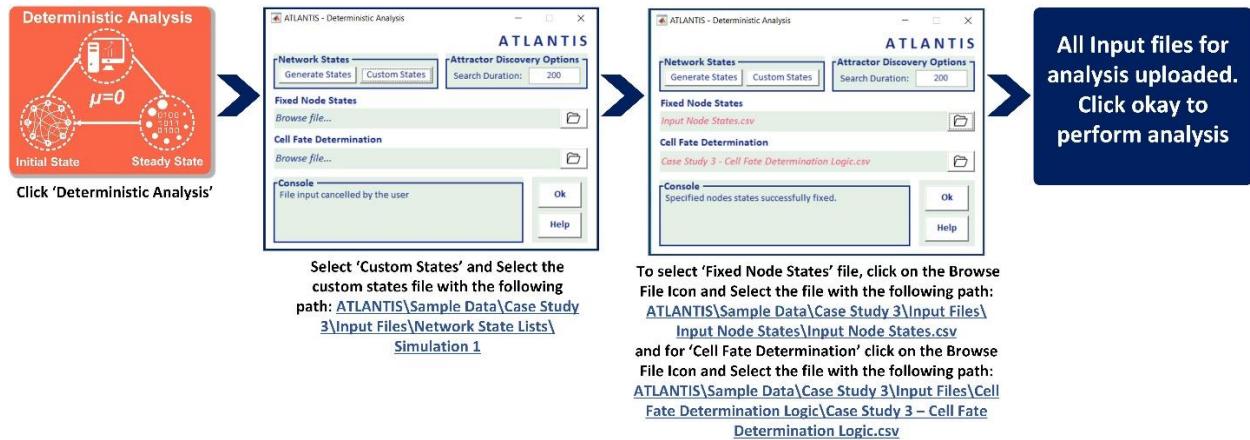
## 2. Investigating the Evolution of Cell Fate Landscape during Colorectal Tumorigenesis

**Step-1.** 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: [ATLANTIS\Sample Data\Case Study 3\Input Files\Network Details](#) for further analysis.

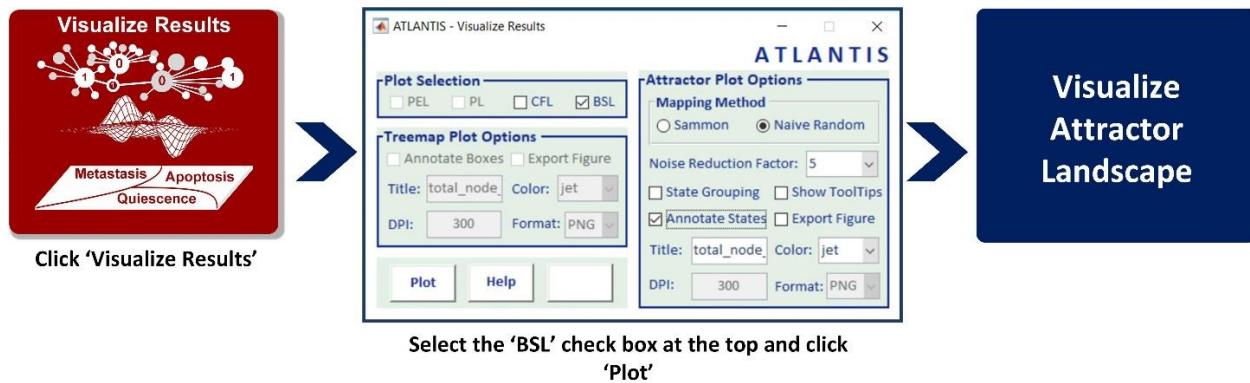


**Step-2.** Click on ‘Deterministic Analysis’ button. This would open the DA menu. The first step, now, would be generating the network state-space. Click on ‘Custom States’ and upload the file containing custom network states. The path of the file is given as: [ATLANTIS\Sample Data\Case Study 3\Input Files\Network State Lists\Simulation 1](#).

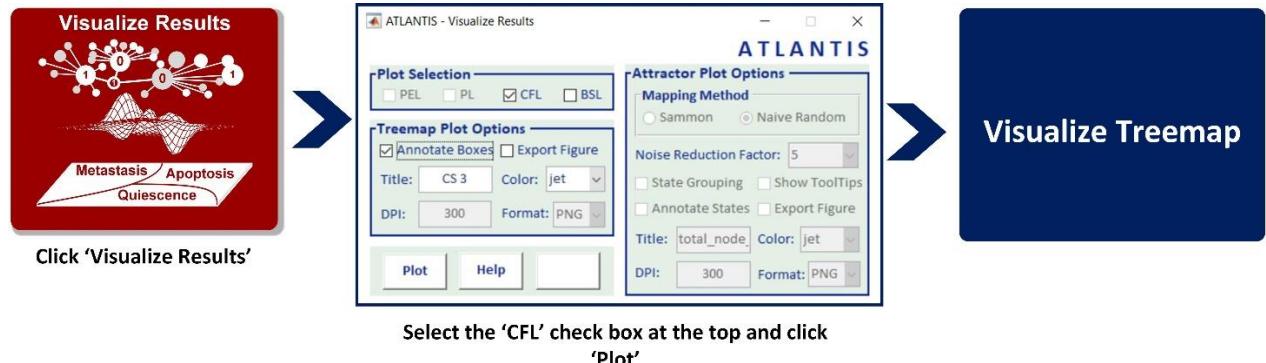
*Important Note: While loading the network, a warning message might pop-up to warn the users about possibly missing or wrongly named logic files nodes present in the node list file. This warning should be ignored if the message mentions names of the nodes which the user wishes not to update during the network analyses. An example of such nodes can be unregulated input nodes (as was the case in case study 3 and 4) which do not require to be updated during simulation.*



**Step-3.** On completion of DA move on to visualizing the results. Here, to visualize the Treemap plot for phenotype classification, check ‘CFL’, enter a title for your plot and then click ‘Plot’.

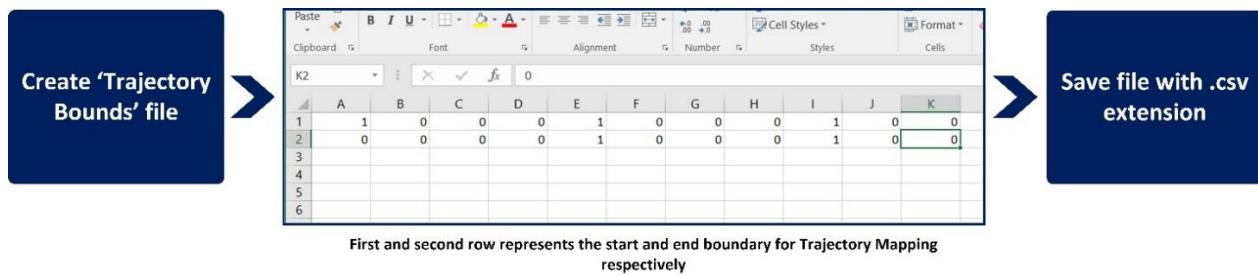


**Step-4.** To visualize the attractor landscape, check ‘BSL’, enter remaining parameters and click ‘Plot’.

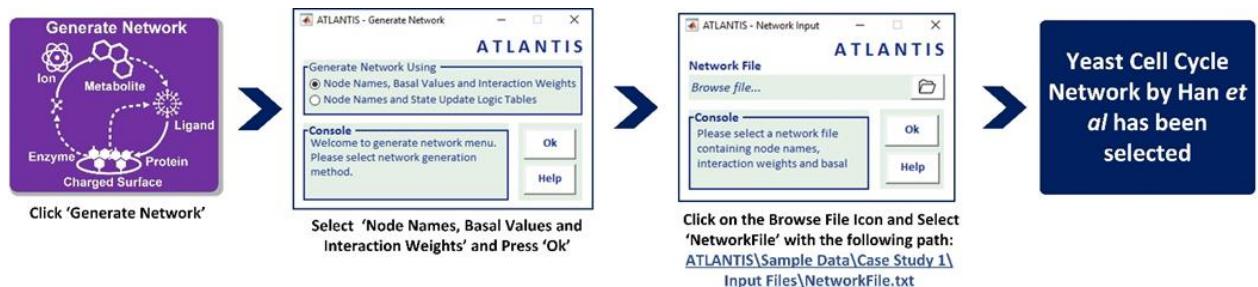


### 3. Demonstrating the use of ‘Trajectory Mapping’ in Yeast Cell Cycle Progression Network

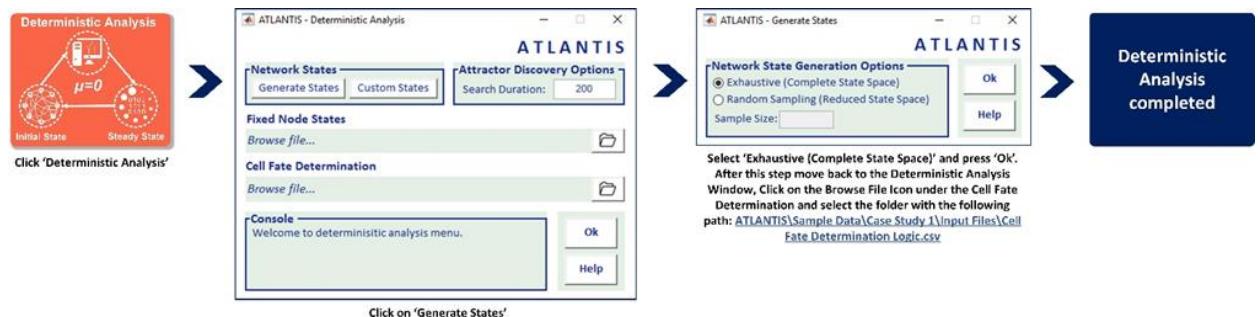
**Step-1.** Create ‘Trajectory Bounds.csv’ file for use in Step-4. The first and second row in the file represents the initial and final boundary state for the complete trajectory respectively.



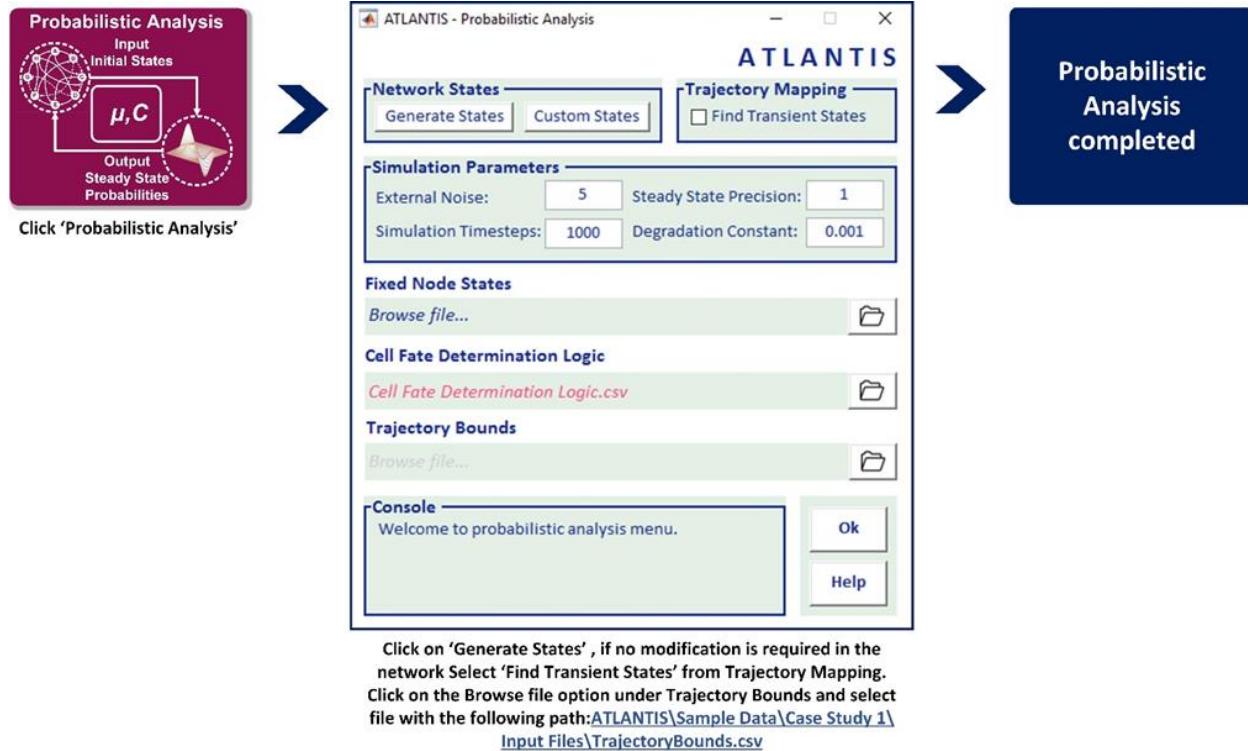
**Step-2.** Click the ‘Generate Network’ button, select the option with ‘Node Names, Basal Values and Interaction Weights’. Next, browse the file at given link for further analysis. The network file for yeast cell cycle can be found at: [ATLANTIS\Sample Data\Case Study 1\Input Files\NetworkFile.txt](#)



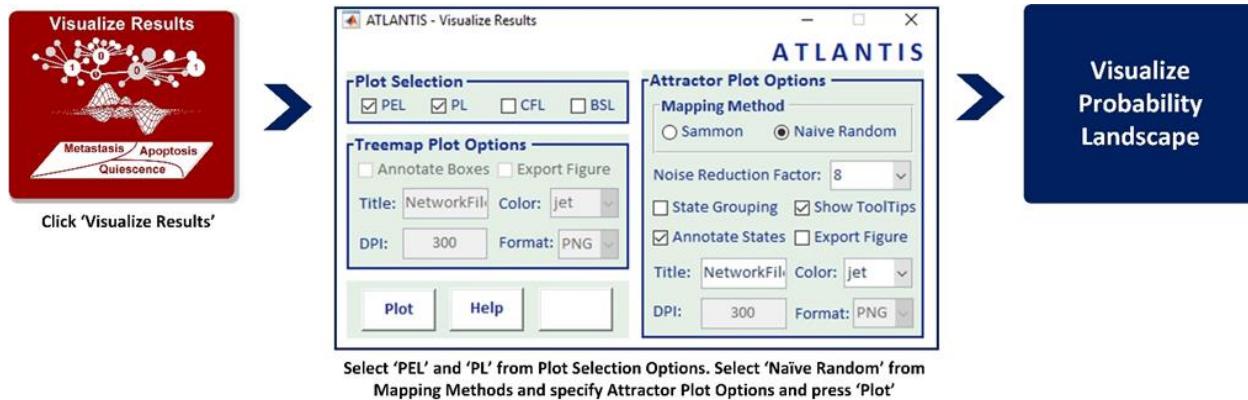
**Step-3.** After generating the network, click on ‘Deterministic Analysis’ button. This would open the DA menu. The first step, now, would generating the network states. Click on ‘Generate States’ and select ‘Exhaustive (Complete State Space)’ and press ‘Ok’. After this step, move back to the DA window, click on the ‘Browse file’ icon under ‘Cell Fate Determination’ and select the folder with the following path: [ATLANTIS\Sample Data\Case Study 1\Input Files\Cell Fate Determination Logic.csv](#)



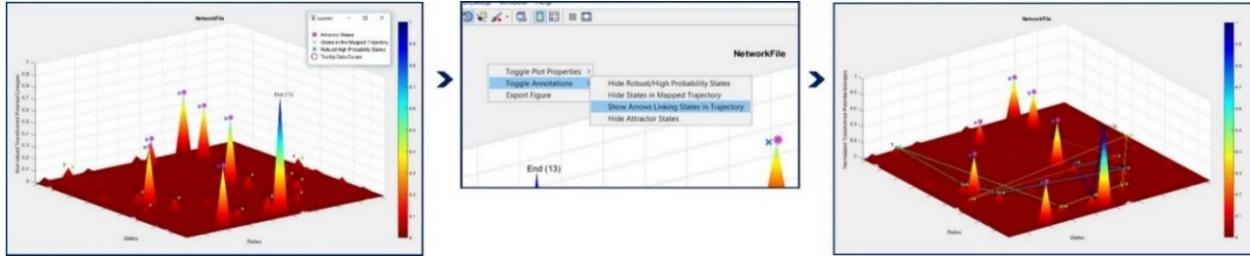
**Step-4.** Next to perform PA, click on ‘Probabilistic Analysis’ button. Click on ‘Generate States’ followed by exhaustive sampling of the initial state-space. Under ‘Trajectory Mapping’, select ‘Find Transient States’ checkbox. Click on the ‘Browse file’ option under ‘Trajectory Bounds’ and select file stored in Step-1.



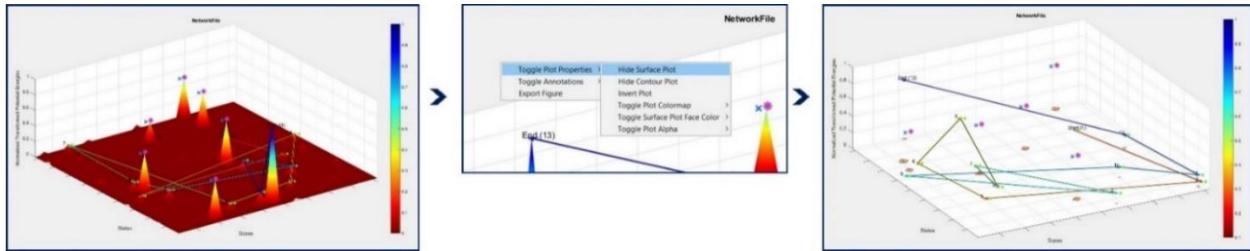
**Step-5.** After performing the analyses, the next step is to visualize the results. Click on the ‘Visualize Results’ button. Select ‘PEL’ and ‘PL’ from ‘Plot Selection’ options. Select ‘Naïve Random’ from ‘Mapping Method’ and check annotation and tooltips options and press ‘Plot’.



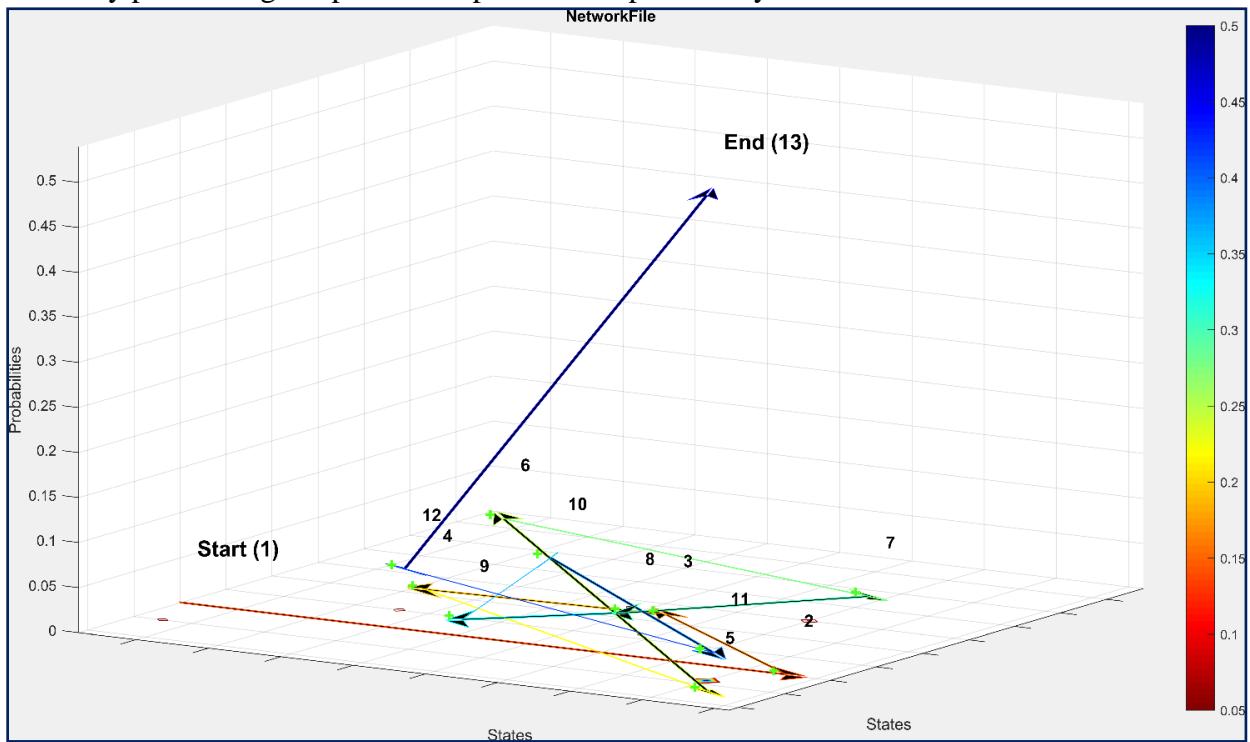
**Step-6.** To display transient states trajectory with in the attractor landscape, right-click on the figure, click ‘Toggle Annotations’ and then click ‘Show Arrows Linking States in Trajectory’.



**Step-7.** To view the trajectory path alone, right-click on the screen, click ‘Toggle Plot Properties’ and then click ‘Hide Surface Plot’.

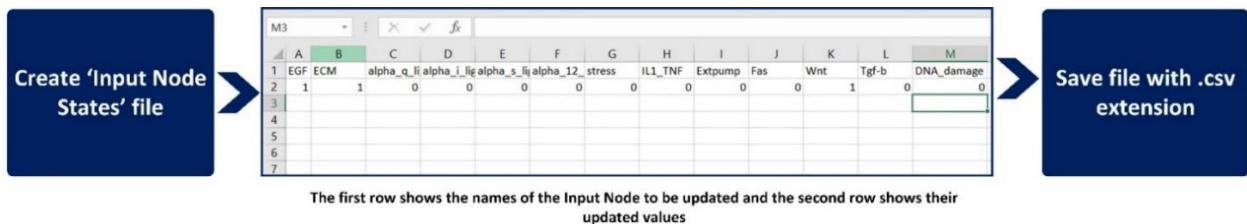


**Step-8.** A variety of visualization options can be used to clearly view trajectory path and transient states. The trajectory path traces the route from the initial boundary state to the final boundary state, passing through all the intermediate transient states. The color intensity at every point along the path corresponds to its probability.



#### 4. Demonstrating the use of ‘Fixed Nodes States’ feature in 201-node network for Colorectal Tumorigenesis

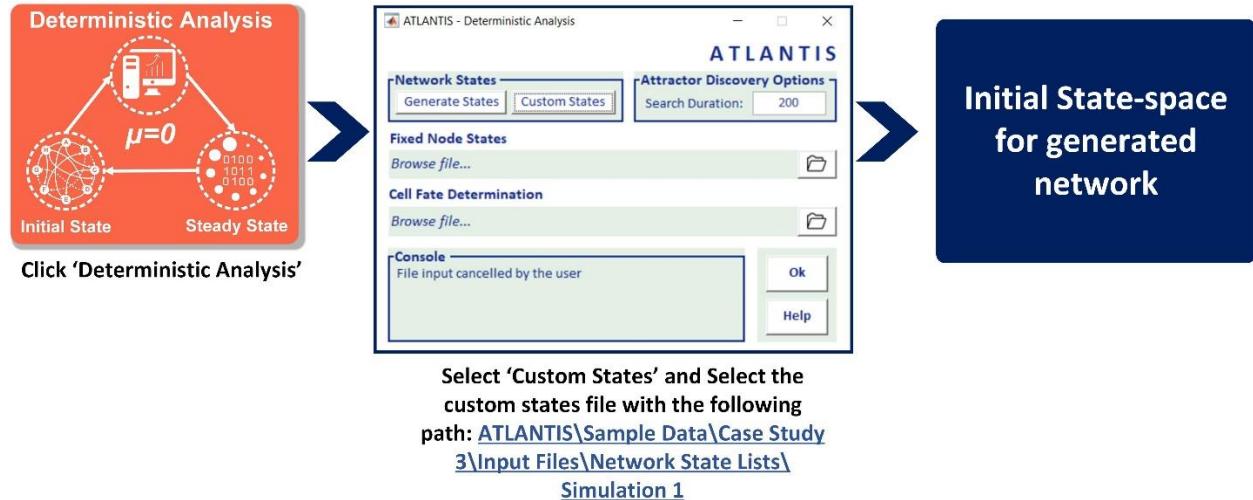
**Step-1.** Create ‘Input Node States.csv’ file for use in Step-4. The first row shows the names of the input nodes to be updated and the second row shows their fixed values. Once fixed, the values for these input nodes will remain constant throughout the simulation.



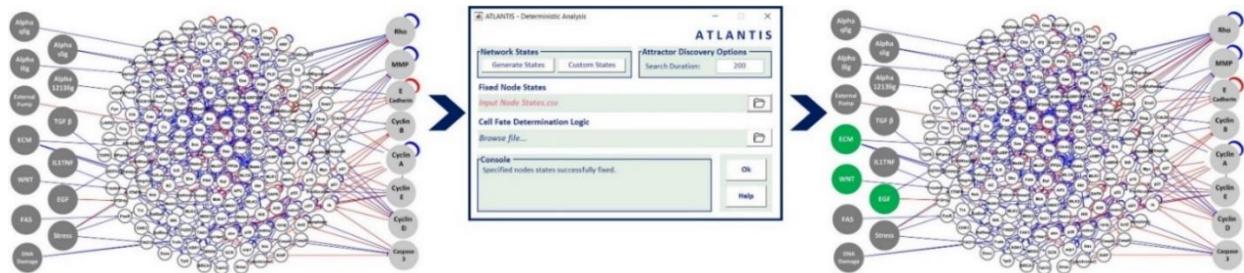
**Step-2.** The network file and update logic folder for colorectal tumorigenesis network can be found at: [ATLANTIS\Sample Data\Case Study 3\Input Files\Network Details](#). After clicking the ‘Generate Network’ button, select the option with ‘Node Names and State Update Logic Tables’. Next, browse the file and folder at given link for further analysis.



**Step-3.** Click on ‘Deterministic Analysis’ button. This would open the DA menu. The first step, now, would be generating the network state-space. Click on ‘Custom States’ and upload the file containing custom network states. The path of the file is given as: [ATLANTIS\Sample Data\Case Study 3\Input Files\Network State Lists\Simulation 1](#)



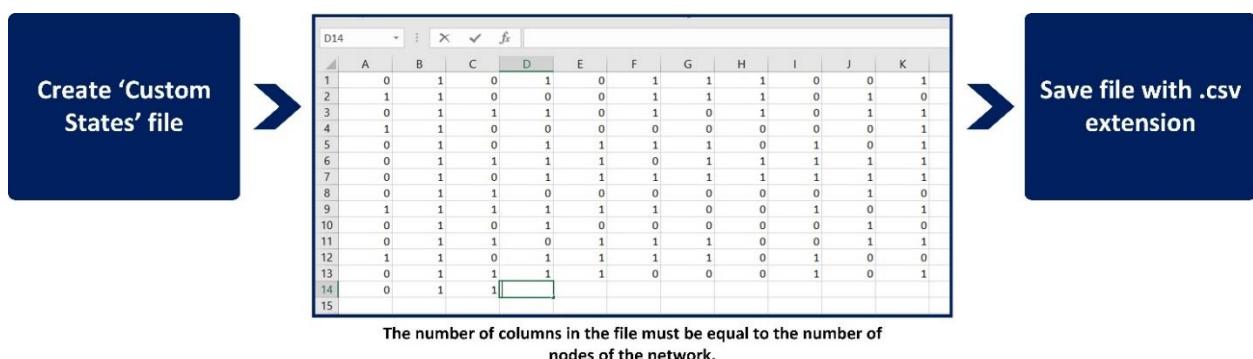
**Step-4.** To set the values of input nodes in a modular network, select the ‘Fixed Node States’ file. Click on the ‘Browse file’ option under ‘Fixed Node States’ and select file stored in Step-1.



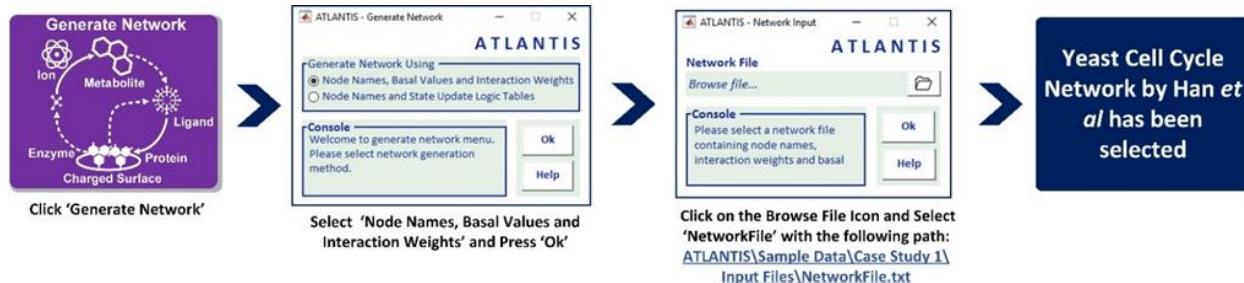
**Step-5.** Click ‘Ok’ on the DA menu to perform onward analysis.

## 5. Demonstrating the use of ‘Custom States’ feature in PA of Yeast Cell Cycle Progression Network

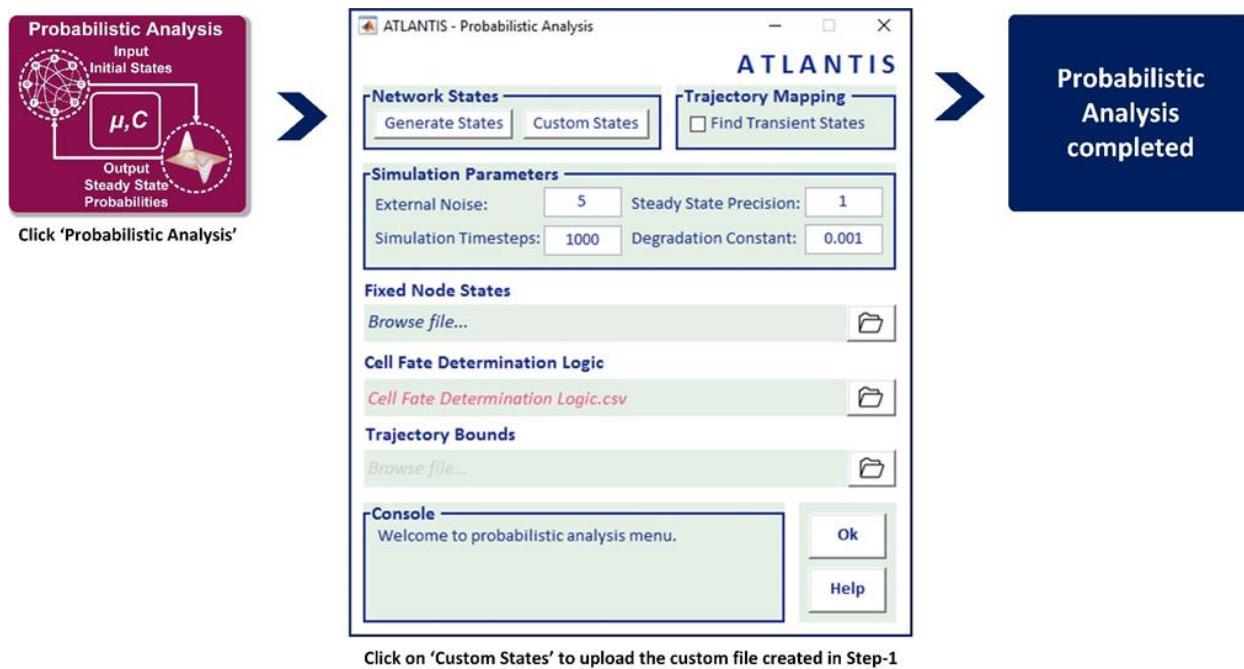
**Step-1.** Create ‘Custom States file.csv’ file for use in Step-3. Each row in the file represents a state and each column represents a node’s value in each state.



**Step-2.** 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 Files\NetworkFile.txt](#)



**Step-3.** Next to perform PA, click on ‘Probabilistic Analysis’ button. Click on ‘Custom States’ to upload the custom states file created in Step-1. Then click ‘Ok’ to perform analysis.

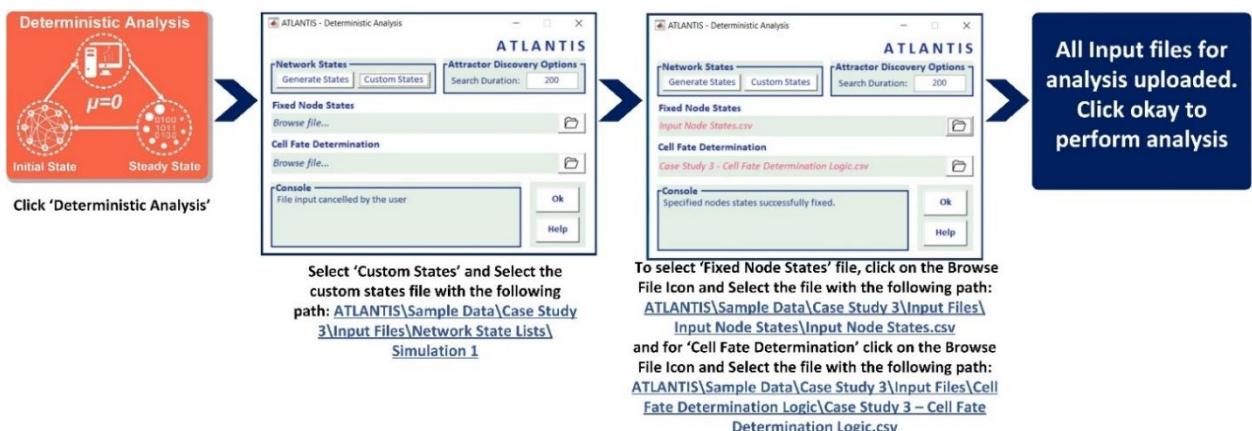


## 6. Cancel Simulation in ATLANTIS

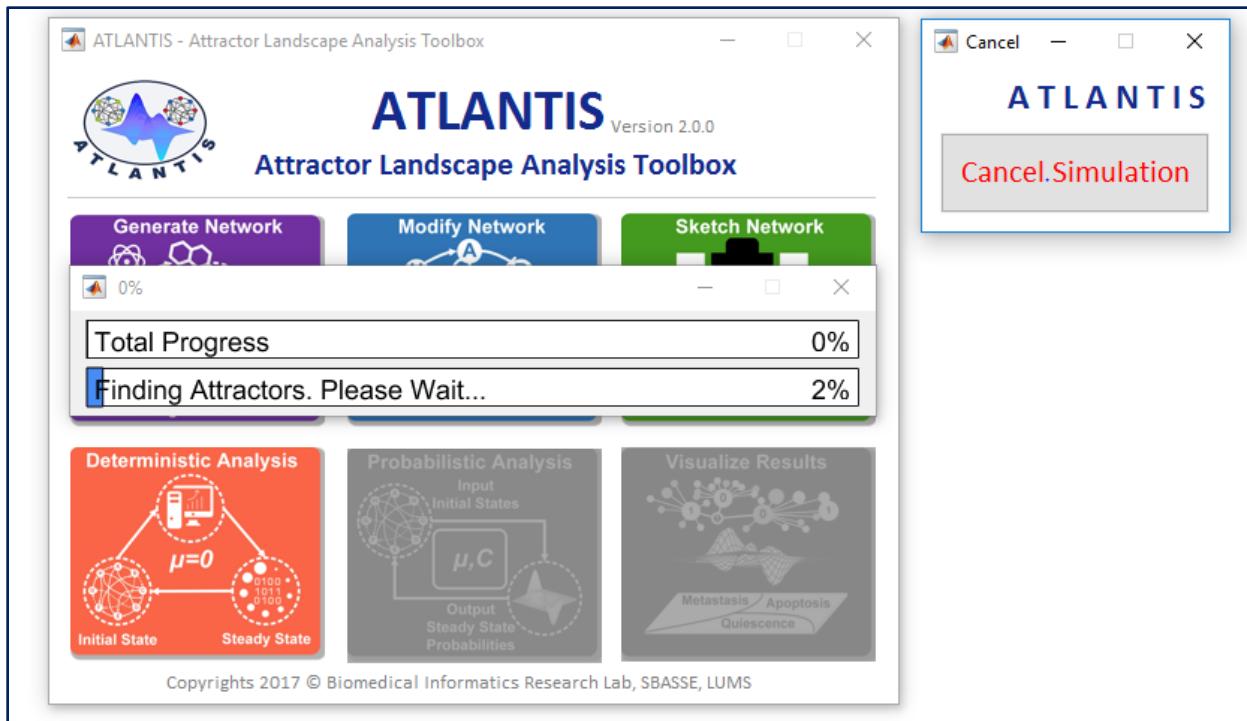
**Step-1.** The network file and update logic folder for colorectal tumorigenesis network can be found at: [ATLANTIS\Sample Data\Case Study 3\Input Files\Network Details](#). After clicking the ‘Generate Network’ button, select the option with ‘Node Names and State Update Logic Tables’. Next, browse the file and folder at given link for further analysis.



**Step-2.** Click on ‘Deterministic Analysis’ button. This would open the DA menu. The first step, now, would be selecting the Network States. Click on ‘Custom States’ button and upload the file containing custom network states. The path of the file is given: [ATLANTIS\Sample Data\Case Study 3\Input Files\Network State Lists\Simulation 1](#)



**Step-3.** Once the analysis starts the following pop up also appears on the screen. In order to stop the simulation, click on ‘Cancel Simulation’. A [video tutorial](#) is also available for this functionality.



## 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).