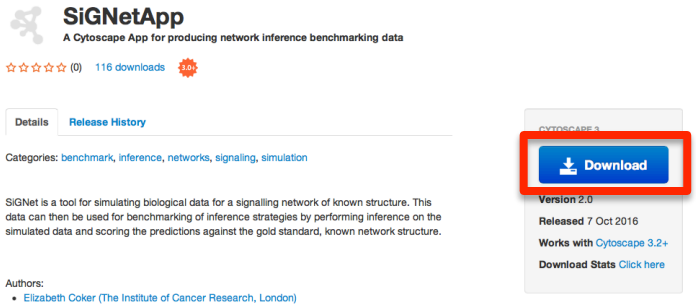
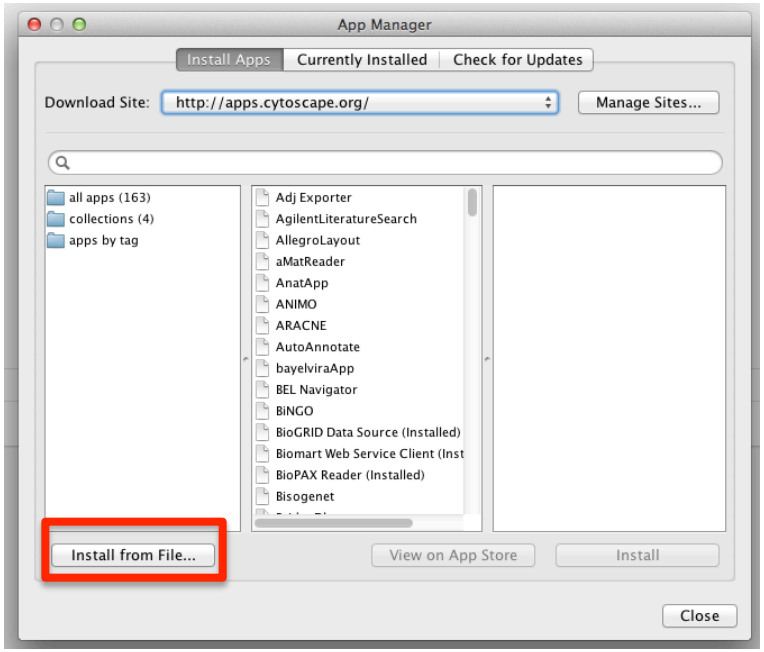
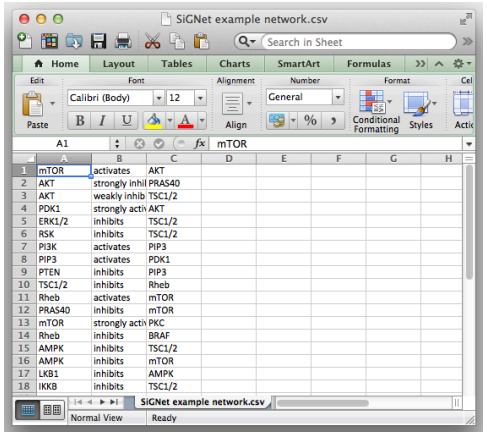
SiGNet Benchmarking Example

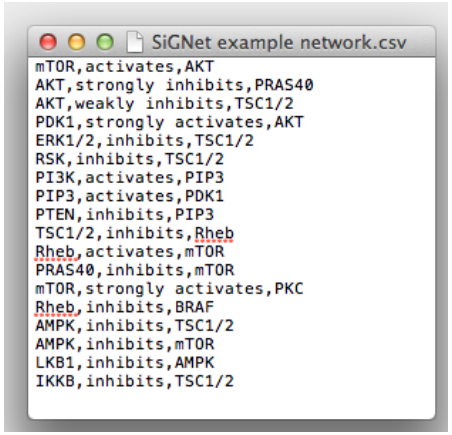
SiGNet is a tool for simulating biological data for a signalling network of known structure. This data can then be used for benchmarking of inference strategies by performing inference on the simulated data and scoring the predictions against the gold standard, known network structure. Here we illustrate how this can be achieved.

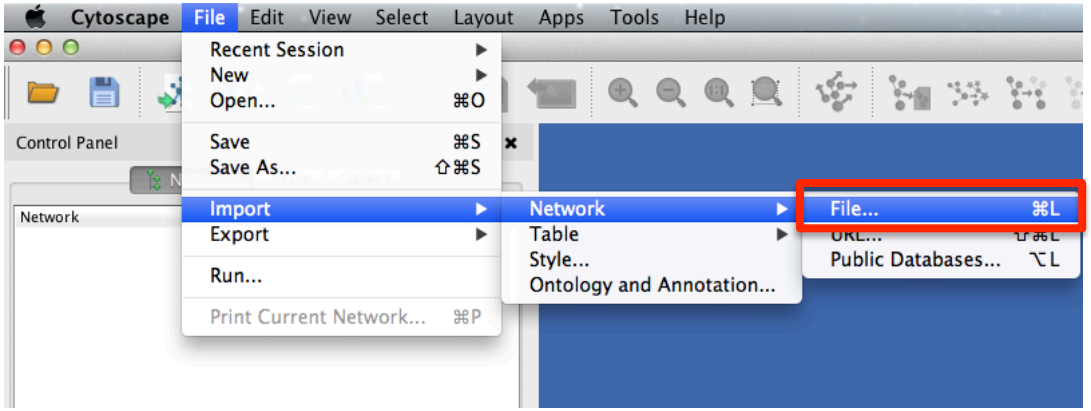


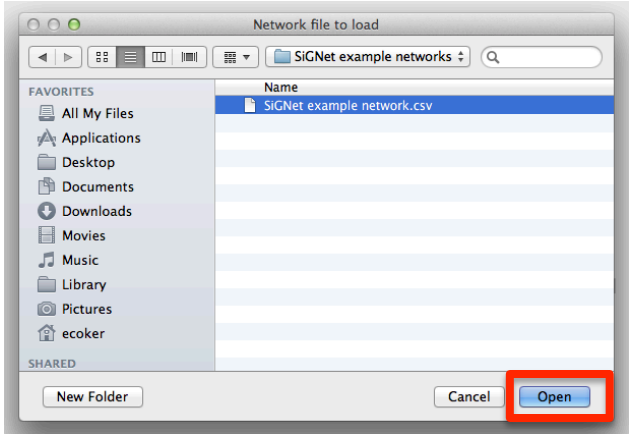


1. Download the SiGNet plugin from either the Cytoscape App store or from http://signet.icr.ac.uk. Open Cytoscape version 3.2+ and select ‘Apps’ then ‘App Manager’ from the Cytoscape toolbar. In the App Manager click ‘Install from File’ and select the file you have just downloaded.

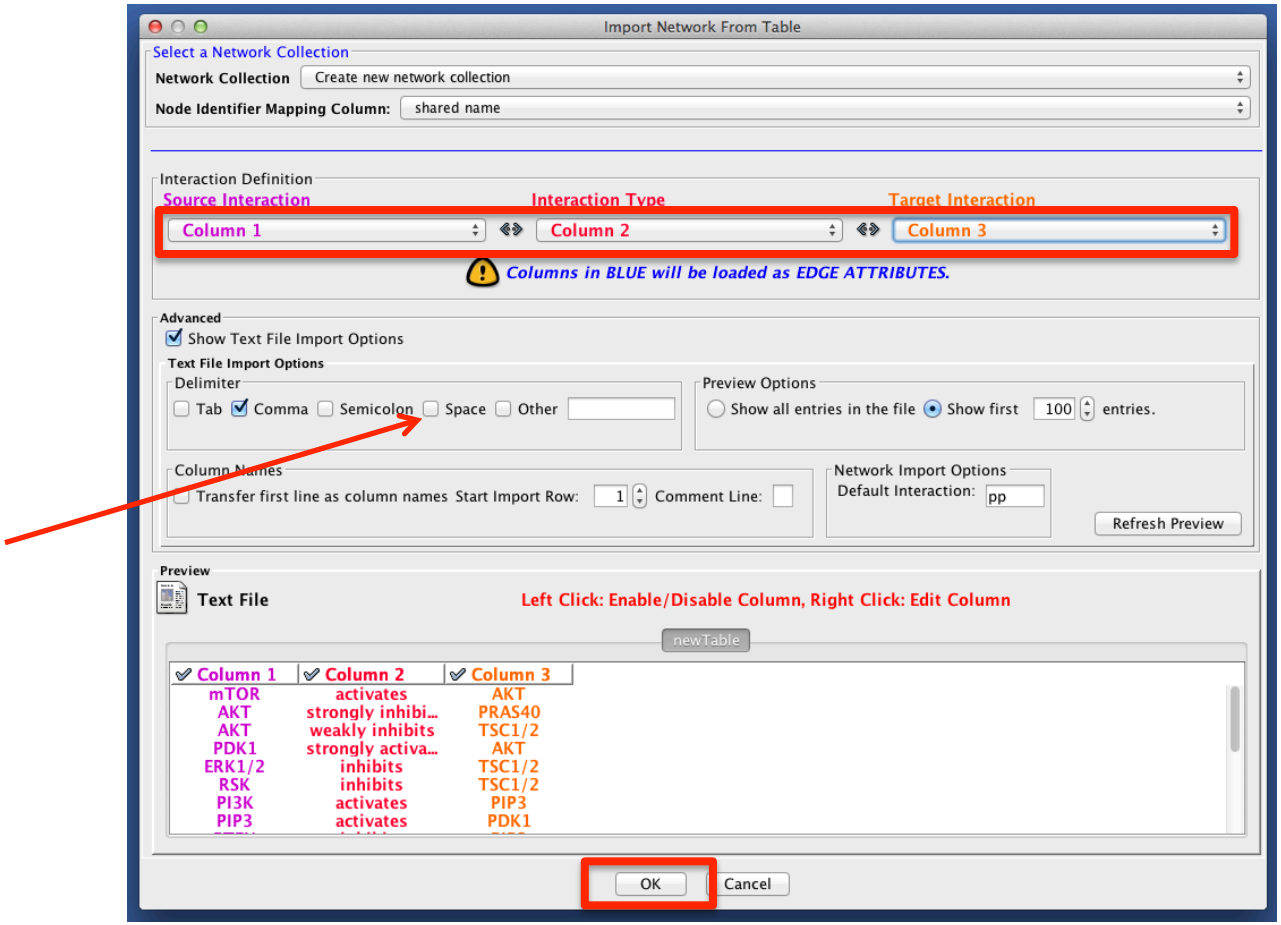








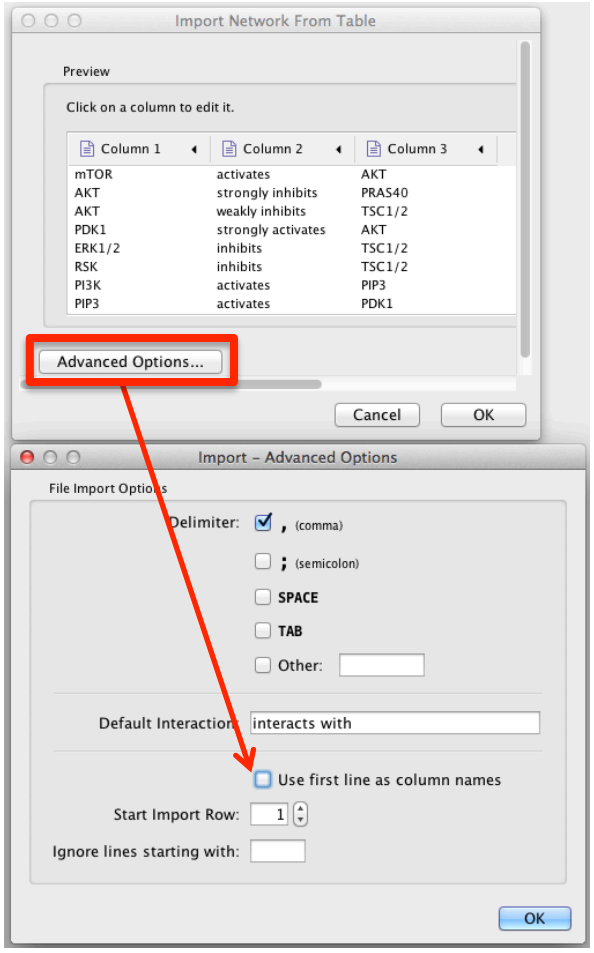
2. Create a file of interactions to be present in the network SiGNet will use for simulation. Edges should be described using one of the following terms: ‘activates’, ‘strongly activates’, ‘weakly activates’, ‘inhibits’, ‘strongly inhibits’, ‘weakly inhibits’ or ‘binds’ (for interactions that do not affect the activity of the target node). If the interaction is left blank or does not match this controlled vocabulary, SiGNet will later replace the interaction with ‘activates’ and warn the user that this has occurred. In Cytoscape, select ‘File’, ‘Import’, ‘Network’ and then ‘File’, and select the file you have just created.

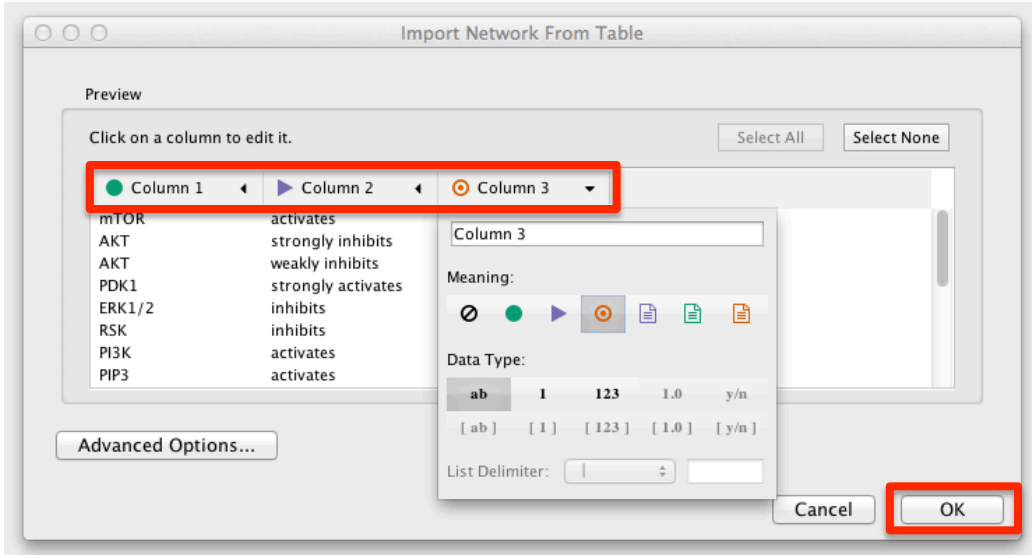


For Cytoscape 3.2.x and 3.3.x:

Click ‘Show Text File Import Options’ and ensure ‘Space’ is **NOT** selected.

For ‘Source Interaction’, select ‘Column 1’. For ‘Interaction Type’, select ‘Column 2’. For ‘Target Interaction’, select ‘Column 3’. Click ‘OK’.

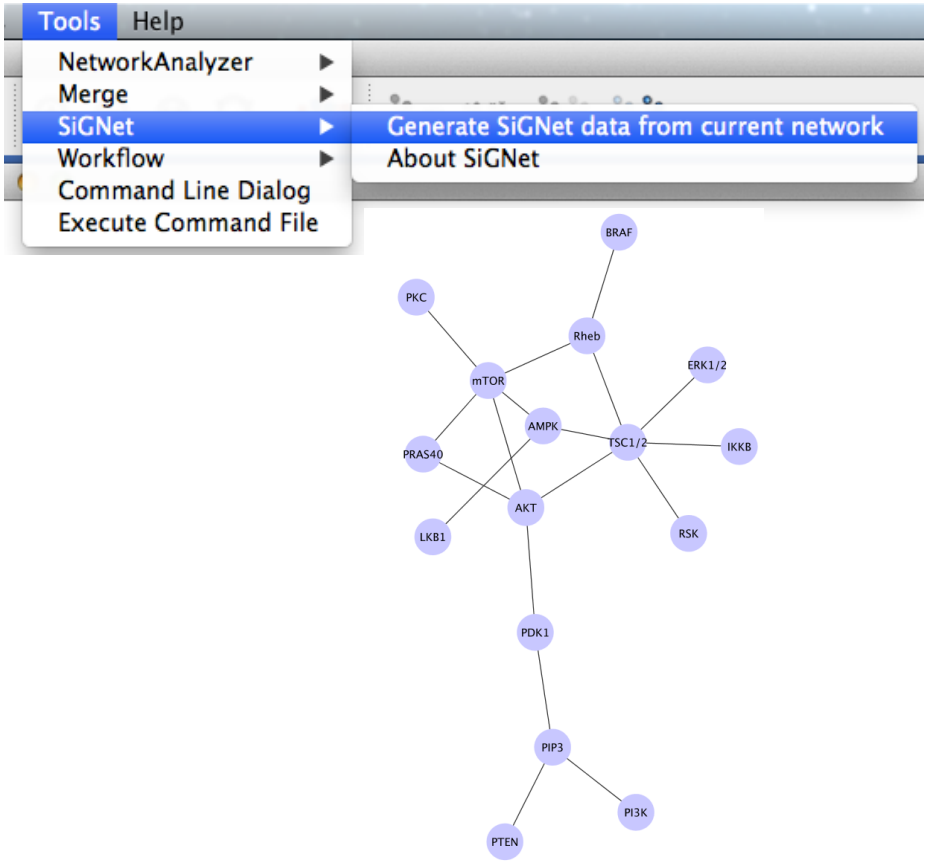


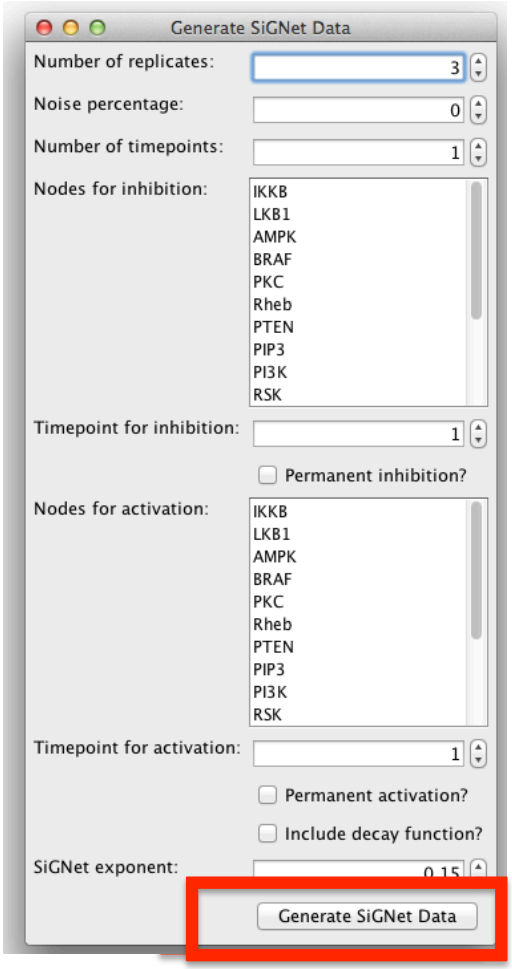


For Cytoscape 3.4.0+:

Select ‘Advanced Options’ and ensure ‘Use first line as column names’ is **NOT** selected.

Click on ‘Column 1’ and select ‘Source node’ as its meaning. Click on ‘Column 2’ and select ‘Interaction type’ as its meaning. Click on ‘Column 3’ and select ‘Target node’ as its meaning. Click ‘OK’.

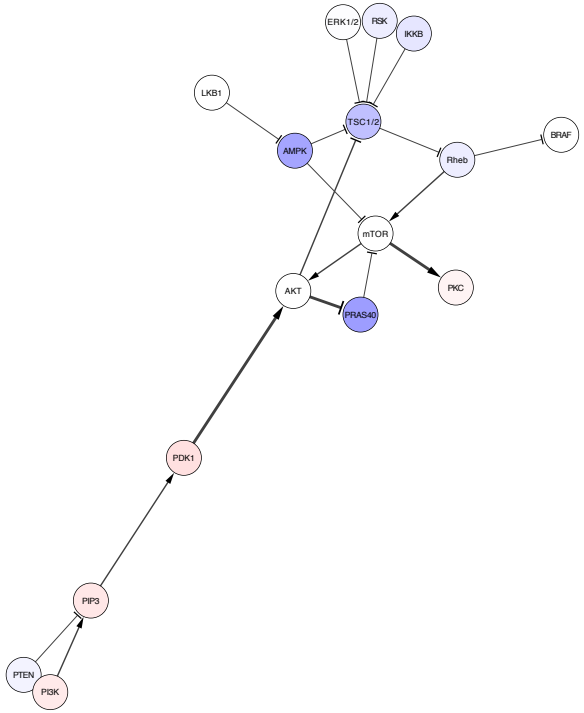


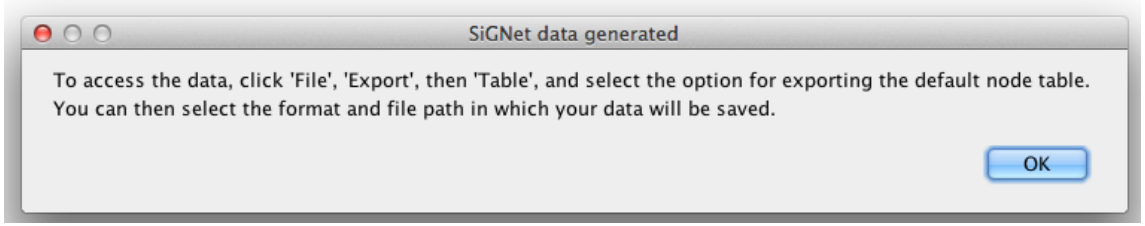


3. Click 'Tools' in the Cytoscape toolbar, then 'SiGNet 'and 'Generate SiGNet data from current network'. In the popup menu, select the number of 'experimental replicates' required, the level of noise in the system (if required), the number of timepoints and whether any nodes should be inhibited or activated, and when. When you are happy with the parameters displayed, click 'Generate SiGNet Data'.

shared...	name	△ SiGNet value average timepoint 1	SiGNet value repeat 1 timepoint 1	SiGNet value repeat 2 timepoint 1	SiGNet value repeat 3 timepoint 1	SiGNet value repeat 4 timepoint 1
mTOR	mTOR	75.8557	77.1068	75.6155	77.5359	76.2225
PKC	PKC	68.1572	77.384	75.1258	75.7901	76.0154
PDK1	PDK1	66.6257	85.5357	45.7638	82.4639	83.114
PIP3	PIP3	57.6546	48.9556	63.2422	49.8048	47.1651
AKT	AKT	56.5174	64.4207	49.1723	62.6577	64.2085
PI3K	PI3K	53.7134	64.144	47.5145	64.4513	45.1646
PTEN	PTEN	45.0414	39.3388	49.6009	40.2854	46.8341
BRAF	BRAF	44.9246	47.6155	42.7305	41.8907	42.0304

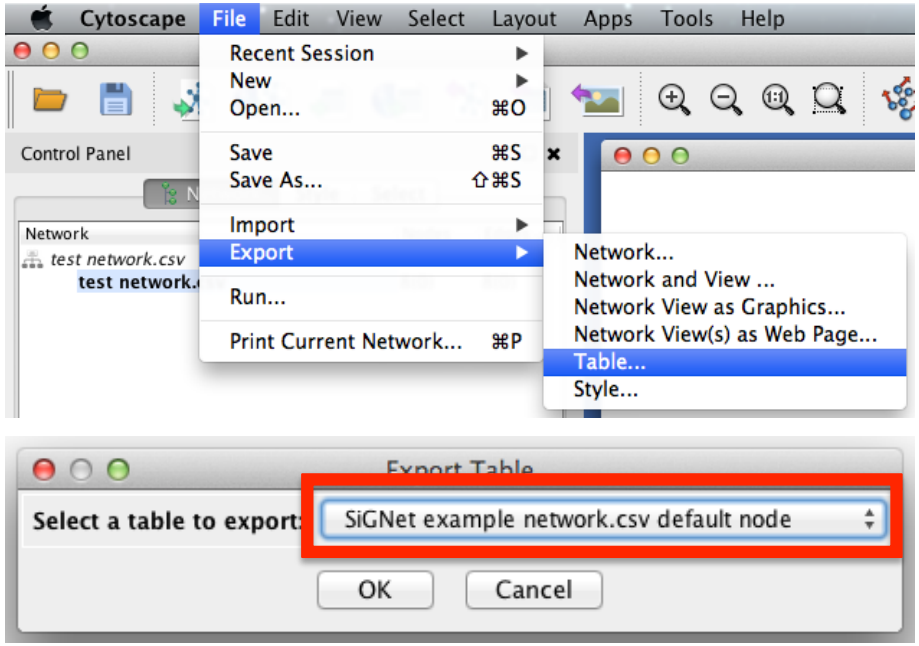
Node Table

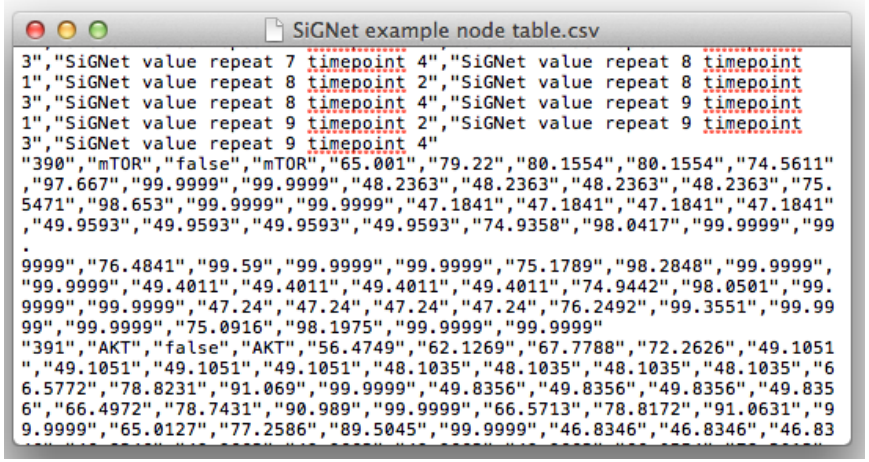




4. The SiGNet algorithm now runs and populates the node table with data for each node at each replicate and at each time point, along with node averages for each time point. A pop up message will appear explaining how to export the data.

In the network shown here, nodes have been coloured according to their SiGNet average value for timepoint 1 – this can be achieved using the 'Style – Node' tab under the Cytoscape Control Panel.





5. By clicking ‘File’, ‘Export’ , ‘Table’ and then selecting the default node table to export, the SiGNet data you have generated will be exported to the location you specify.

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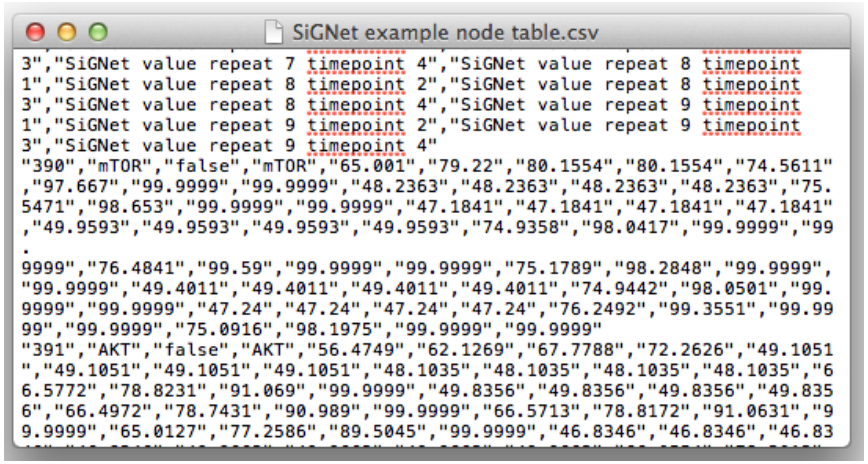

Input file into inference algorithm
e.g. Parmigene CLR algorithm, Parmigene MRNET algorithm

6. The files of simulated data generated by SiGNet can now be used for inference of network structure. Many inference techniques are available, including those based on correlations, Bayesian inference, mutual information and hybrid approaches. Here we are using two algorithms from the Parmigene R Package[1] to generate inferred interactions between nodes for timepoint 3.

1. Sales G and Romualdi C. *parmigene*—a parallel R package for mutual information estimation and gene network reconstruction.Bioinformatics (2011) 27 (13): 1876-1877 doi:10.1093/bioinformatics/btr274

Gold_standard

mTOR	AKT
AKT	PRAS40
AKT	TSC1/2
PDK1	AKT
ERK1/2	TSC1/2
RSK	TSC1/2
PI3K	PIP3
PIP3	PDK1
PTEN	PIP3
TSC1/2	Rheb
Rheb	mTOR
PRAS40	mTOR
mTOR	PKC
Rheb	BRAF
AMPK	TSC1/2
AMPK	mTOR
LKB1	AMPK
IKKB	TSC1/2

Gold standard interactions

Compare and score

$$\text{Sensitivity} = \frac{\text{true positive}}{\text{true positive} + \text{false negative}}$$

$$\text{Precision} = \frac{\text{true positive}}{\text{true positive} + \text{false positive}}$$

Timepoint_3_Parmigene_MRNET_hits.txt

AKT	PRAS40	
AKT	TSC1/2	
AMPK	mTOR	
ERK1/2	TSC1/2	
IKKB	TSC1/2	
LKB1	AMPK	
mTOR	AKT	
mTOR	PKC	✓
PIP3	PDK1	✓
PRAS40	mTOR	✓
PTEN	PIP3	✗
Rheb	mTOR	✗
RSK	TSC1/2	✗
TSC1/2	Rheb	
AKT	AMPK	
AKT	ERK1/2	
AKT	mTOR	
AKT	PI3K	
AKT	PIP3	
AKT	PKC	
AKT	PTEN	
AMPK	AKT	
AMPK	ERK1/2	
AMPK	IKKB	
AMPK	LKB1	
AMPK	PI3K	
AMPK	RSK	
BRAF	ERK1/2	
BRAF	IKKB	
BRAF	mTOR	

Edges inferred by Parmigene MRNET algorithm

Technique	Number in SiGNet Gold Standard	Number in inferred file	True positives	False positives	False negatives	Sensitivity	Precision	F1
Parmigene MRNET	18	170	14	156	4	0.778	0.082	0.149
Parmigene ARACNE A	18	130	10	120	8	0.556	0.077	0.135
Parmigene CLR	18	158	11	147	7	0.611	0.070	0.125

7. Inferred edges can now be checked against the gold standard, “correct” network structure we used in Step 1. For example, the user can score the results of inference in terms of true positives (i.e. interactions correctly identified by the inference approach), true negatives, false positives and false negatives. These scores can then be used to calculate the accuracy, precision, sensitivity, false discovery rate and so on for the inference techniques used. In this example we can see that the Parmigene MRNET algorithm has a higher sensitivity and precision than other Parmigene algorithms for the network we simulated in SiGNet.