Browsing and analysing SalmoNet 2 data in Cytoscape

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***Prerequisites* :**

- An up-to-date version of Cytoscape, available from: https://cytoscape.org/

Downloading data

- Download SalmoNet data from the website: http://salmonet.org/download
- Select the *Strain*, *Layer*, and output *Format*, and click on the *Download* button.

***Importing data* :**

- If you downloaded a *.cys* format network file, open it with **File → Open …**
- If you downloaded a *.csv* or *PSI-MITAB* file, import it with the **File → Import → Network from File** option
  - After selecting the import option, you will be greeted by a window like the following:
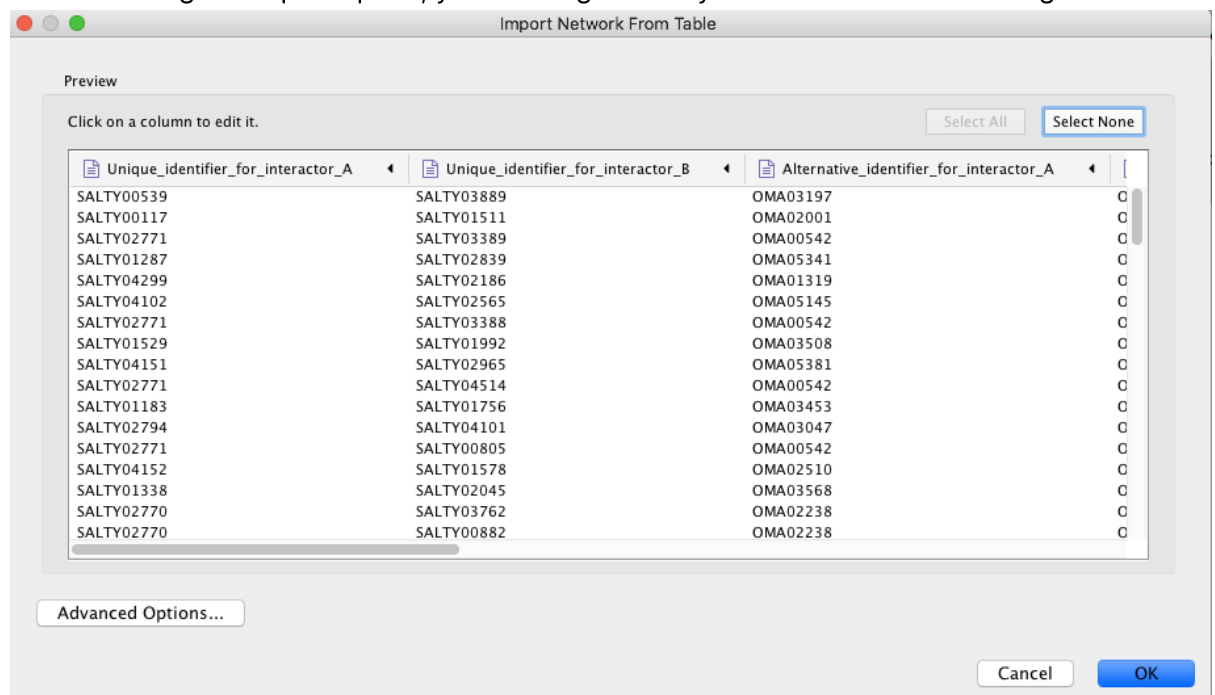
  - The pictograms show the category you would like to place the individual columns into. The green and orange dot are the "main" source and target nodes, while the green and orange file icons will be your node attributes, like alternative IDs.

- Make sure you select the green dot for the source interactor

- And select the orange circles for the target interactor.

- If everything goes well after a bried loadtime you should see a network like this:
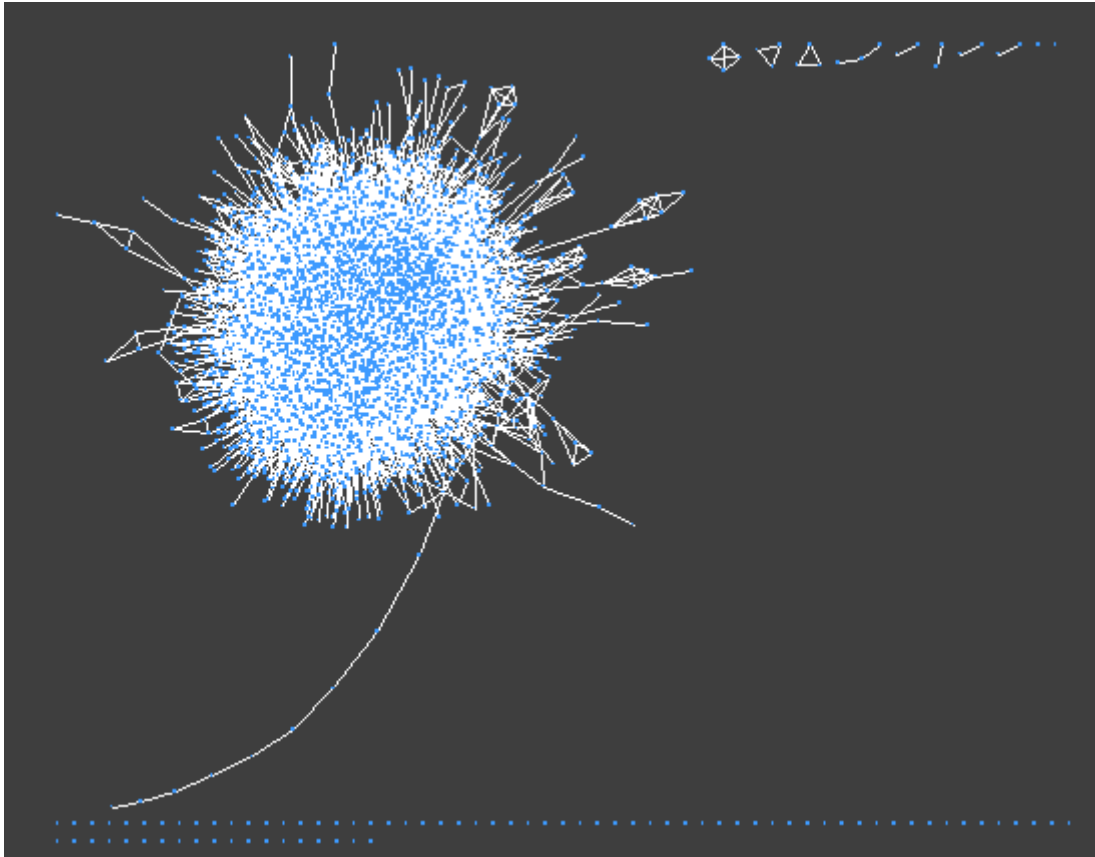
The Cytoscape GUI

The user interface has 3 main elements:

- 1: The panel on the left lets you select the visual mapping options (Node, Edge, and Network tabs on the bottom)

- 2: The main window visualizes the network and its layout, and lets you manipulate nodes/edges directly
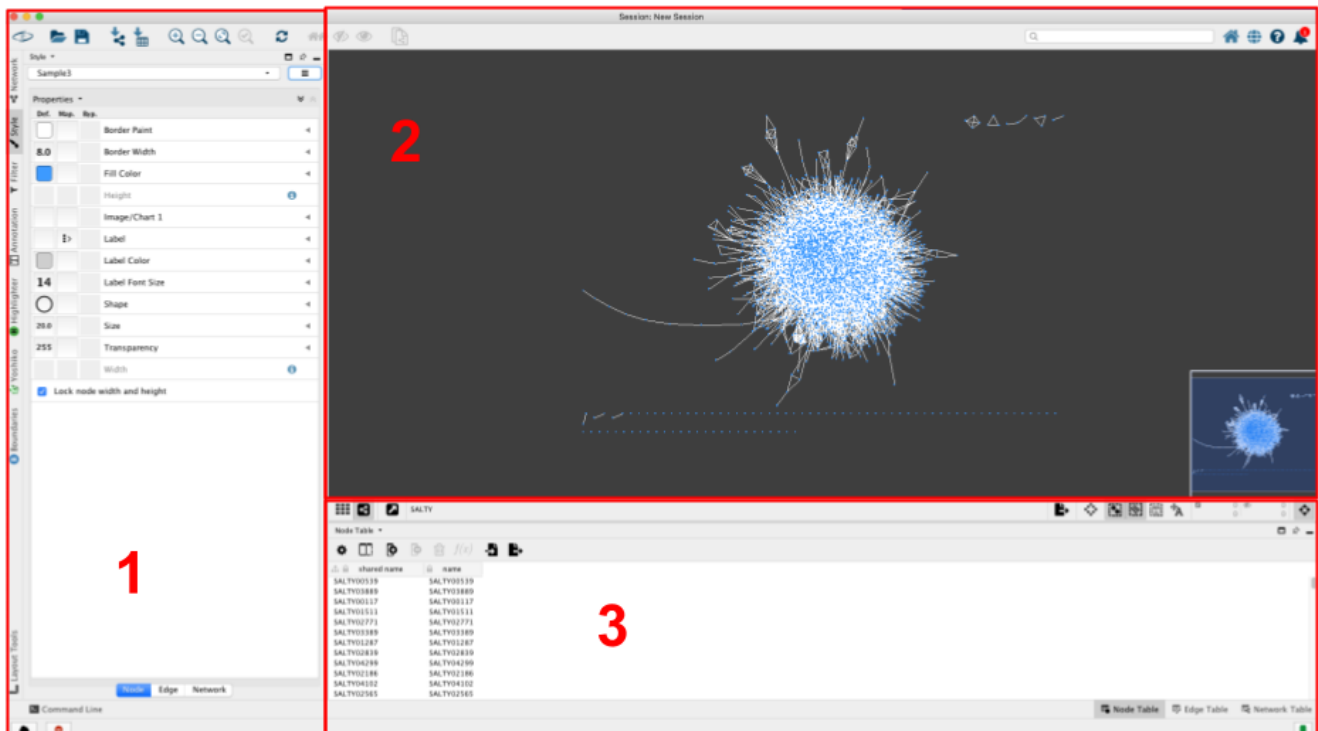- 3: The table pane on the bottom lets you look at the network data directly. Node table contains information pertaining to nodes, the Edge table to edges, and the Network table to the networks.

Network analysis

Our networks only contain interaction and potential annotation data by default. To identify and visualize the inherent characteristics of our network, we will run a group of network analysis methods on them. Thankfully Cytoscape can calculate the most important metrics by default, and there are more methods available through the Cytoscape app store.

To run the network analysis, select the Tools menu point from the toolbar, and click on *Network Analysis*. After a few seconds a number of new columns should show up in the Node Table of our network.

Degree ∨	BetweennessCentrality	ClusteringCoefficient	AverageShortestPathL	ClosenessCentrality
69	0.09798571041737145	0.0315430520034...	3.2276595744680...	0.3098220171390903
68	0.09512288712938682	0.0324846356453...	3.2297872340425...	0.3096179183135705
55	0.16836018172039355	0.0087542087542...	3.5446808510638...	0.28211284513805523
54	0.14389742874030143	0.0146750524109...	3.4255319148936...	0.2919254658385093
54	0.1317381529128289	0.0020964360587...	3.2234042553191...	0.3102310231023102
51	0.11938802798128534	0.0015686274509...	3.3510638297872...	0.2984126984126984
48	0.11527173942301006	0.0345744680851...	3.2638297872340...	0.30638852672750977
41	0.09990411780033899	0.0329268292682...	3.2617021276595...	0.3065883887801696
32	0.1574009224645527	0.0221774193548...	2.8085106382978...	0.3560606060606061
26	0.0573459254088987...	0.0215384615384...	3.6212765957446...	0.27614571092831963
24	0.05625691754417889	0.0434782608695...	3.5851063829787...	0.27893175074183973
24	0.0770571076093298	0.0434782608695...	3.8978723404255...	0.25655021834061137
20	0.04494501316073019	0.1578947368421...	3.0702127659574...	0.3257103257103257

These statistics can tell us a lot about the networks and nodes we are analysing. For example, the degree of a node notes the amount of neighbours it has in the network. These high degree nodes, also known as hubs, are often important biologically, taking part in many processes. To read more about the rest of the metrics we recommend the freely available online version of the Network Science textbook by Albert-Laszlo Barabasi (http://networksciencebook.com/).

Importing expression data

Download a subset of data from an applicable source. In this example we are using inter-strain relative expression levels from the SalComD23580 database. Select a group of genes, and download the resulting table. (http://bioinf.gen.tcd.ie/cgi-bin/salcom_v2.pl?_HL)

Download and load the *S.* Typhimurium 4/74 network from the SalmoNet website, and load it into Cytoscape using the steps outlined above.

To overlay the expression data open the downloaded expression table file:

- **File → Import → Table from File …**
- Select the expression file from the file system

- Clicking on import will open a window like this:

To match the network information with the expression information we need to find the matching identifiers to combine the data on. The *Key Column for Network* toggle lets you select the column to match on from the network's side, and clicking on the columns from the table below, and selecting the **key** icon lets you indicate the column to match on from the table's side.

After a brief load time the new columns will appear in the node table.
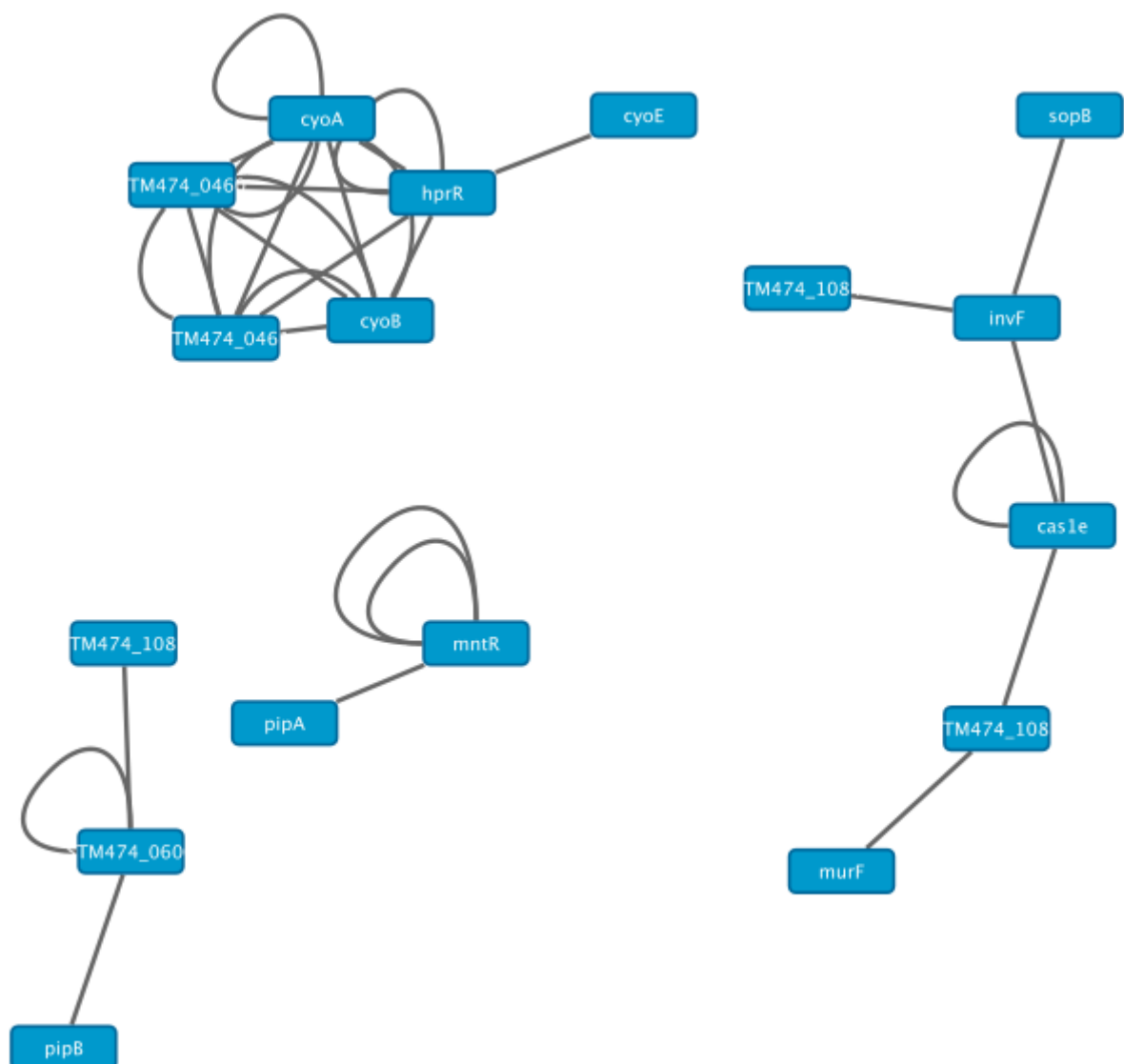
Visualizing expression data

To visualise the expression data in the context of the network, we need to select the rows we would like to analyse from the table, and their first neighbours so we add some context to the subset.

- This can be done by holding down the **shift** key and dragging the mouse cursor. Right clicking on the selected rows will bring up a number of options.

- Click on **Select nodes from selected nodes**.

sopB	sopB	SALT401037	STM474_1083	STM474_RS05360	OMA00529	sopB	5.12	1	2.56	1.6
pipB	pipB	SALT401034	STM474_1080	STM474_RS05345	OMA02530	pipB	1	1	1.3	1.23
pipA	pipA	SALT401033	STM474_1079	STM474_RS05340	OMA03373	pipA	0.89	0.83	0.76	1.16
STM474_1084	STM474_10...	SALT401038	STM474_1084	STM474_RS05365	OMA06145	orfX	Apply to entire column			–
STM474_1087	STM474_10...	SALT401041	STM474_1087	STM474_RS05380	OMA01017	copS	Apply to selected nodes			1
hprR	hprR	SALT401042	STM474_1088	STM474_RS05385	OMA03090	copR				1.36
STM474_1081	STM474_10...	SALT401035	STM474_1081	STM474_RS05350	OMA05189	copS	Edit			1
STM474_2632	STM474_26...	SALT402527	STM474_2632	STM474_RS12970	OMA00176		Copy			
STM474_1508	STM474_15...	SALT401445	STM474_1508	STM474_RS07445	OMA00165		Paste			
dmsB	dmsB	SALT402526	STM474_2631	STM474_RS12965	OMA03552					
STM474_1740	STM474_17...	SALT401673	STM474_1740	STM474_RS08610	OMA00669		Select nodes from selected rows			
trpD	trpD	SALT401674	STM474_1741	STM474_RS08615	OMA00636					
wecA	wecA	SALT403937	STM474_4096	STM474_RS20155	OMA01650					

- This will highlight the nodes in your network, and reduce the amount of hits in the table column to only the selected ones.
- To select their first neighbours use **Select → Nodes → First Neighbours of Selected Nodes → Undirected**
- Create a new subnetwork from your selection **File → New Network → From Selected Nodes, All Edges**


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- To visualize the expression differences select the **Style** pane on the side of the window.

- Click on **Fill color**, select an environment (e.g. EEP), and select **Continuous mapping** from the
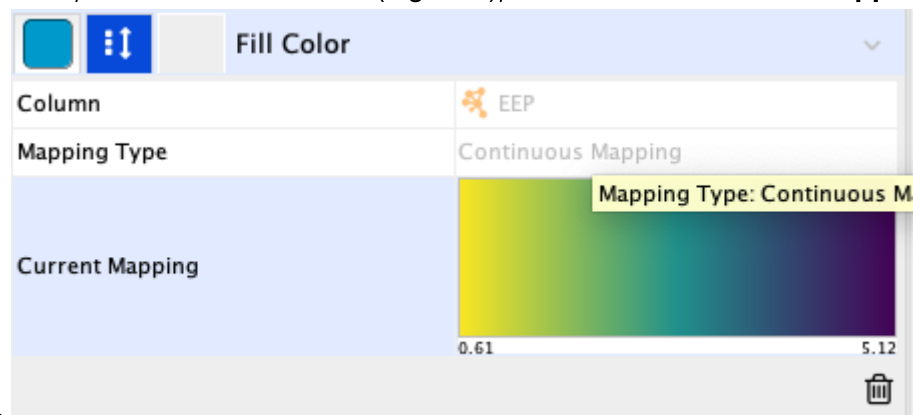
  second row.
- This mapping function takes the values out of our data (i.e. the EEP column), and maps them to a visual value, in this case the node fill colour, visualizing the expression values of the impacted nodes.
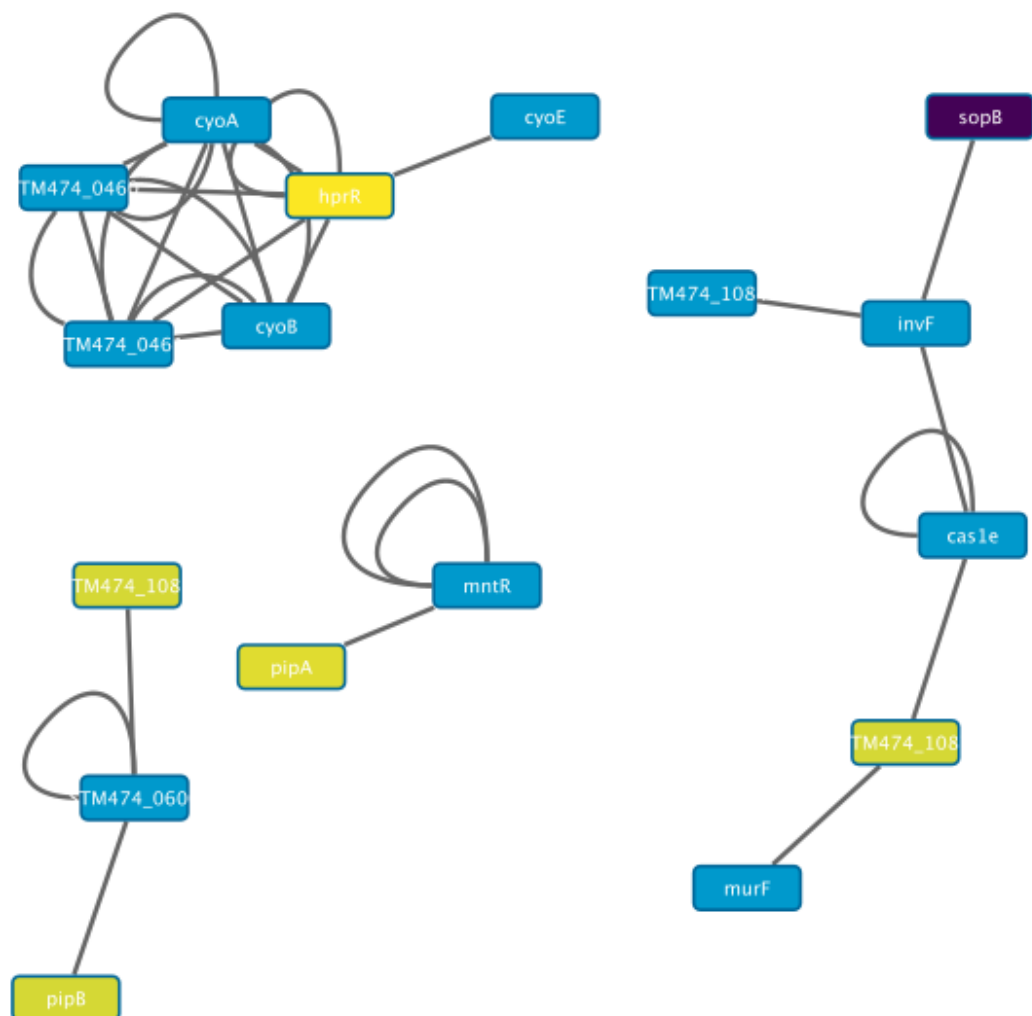
- For nodes where no such data was imported, the default color remains, blue in this case.