Browsing and analysing SalmoNet 2 data in Cytoscape

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***Prerequisites* :**

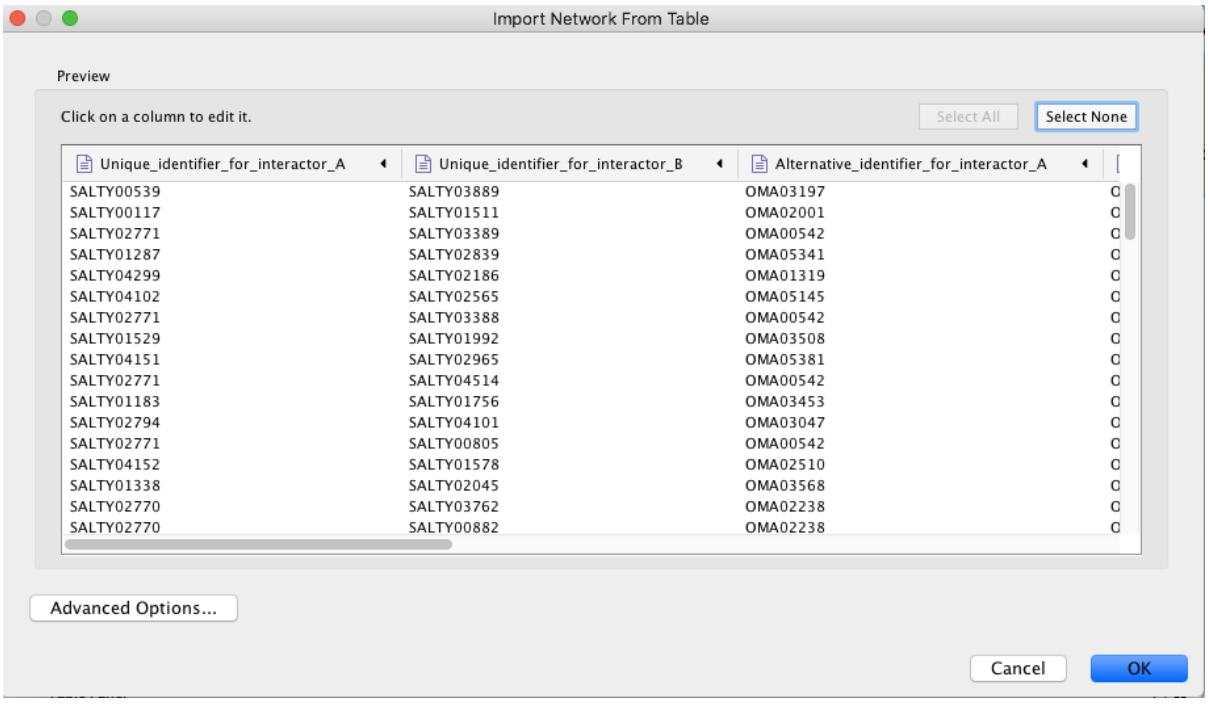
- An up-to-date version of Cytoscape, available from: https://cytoscape.org/

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.

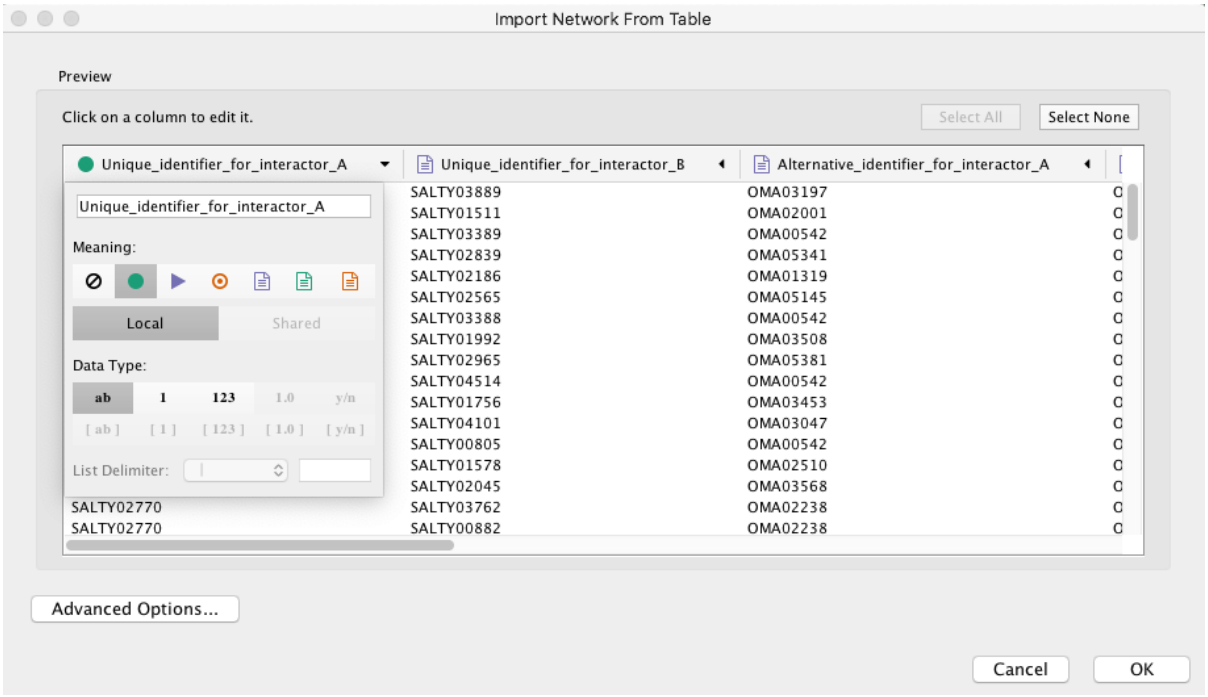
Importing data :

- 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:

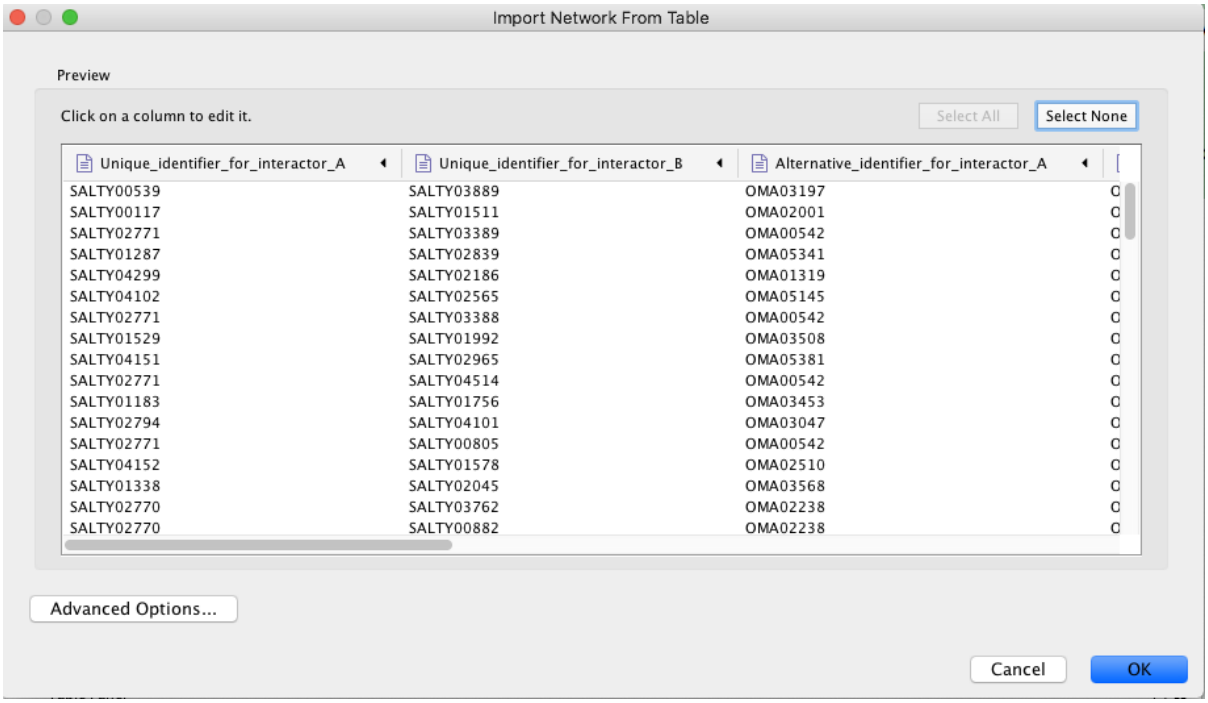


    - The pictograms show the category you would like to place the individual columns into. The green and orange dot are the "main" source and target nodes, while the green and orange file icons will be your node attributes, like alternative IDs.

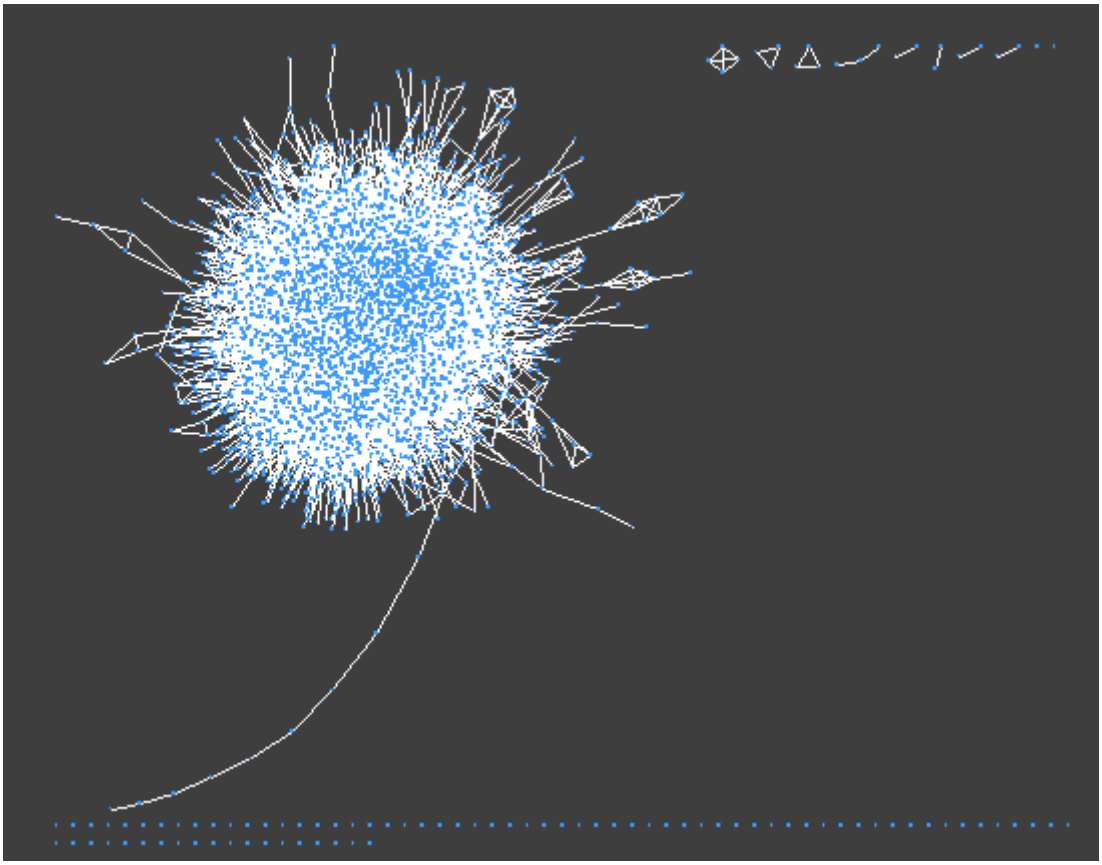
- Make sure you select the green dot for the source interactor



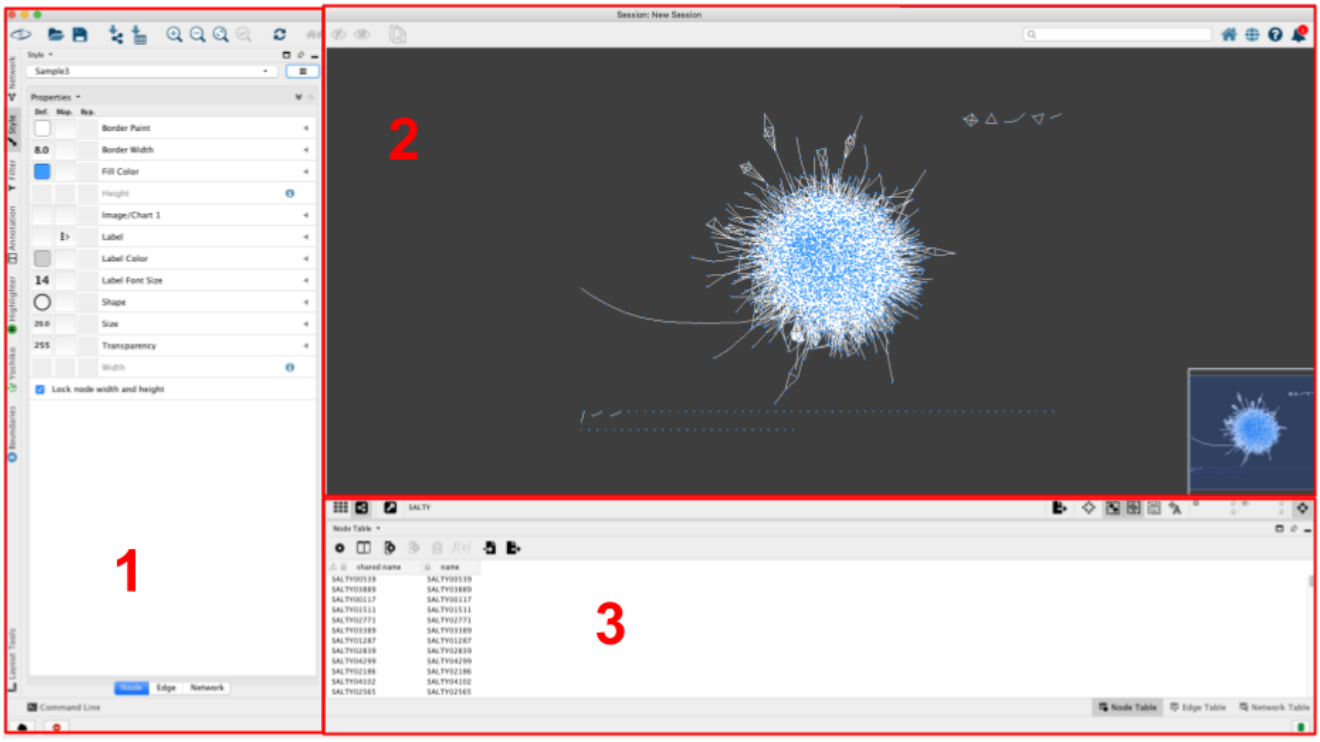
- And select the orange circles for the target interactor.



- 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.

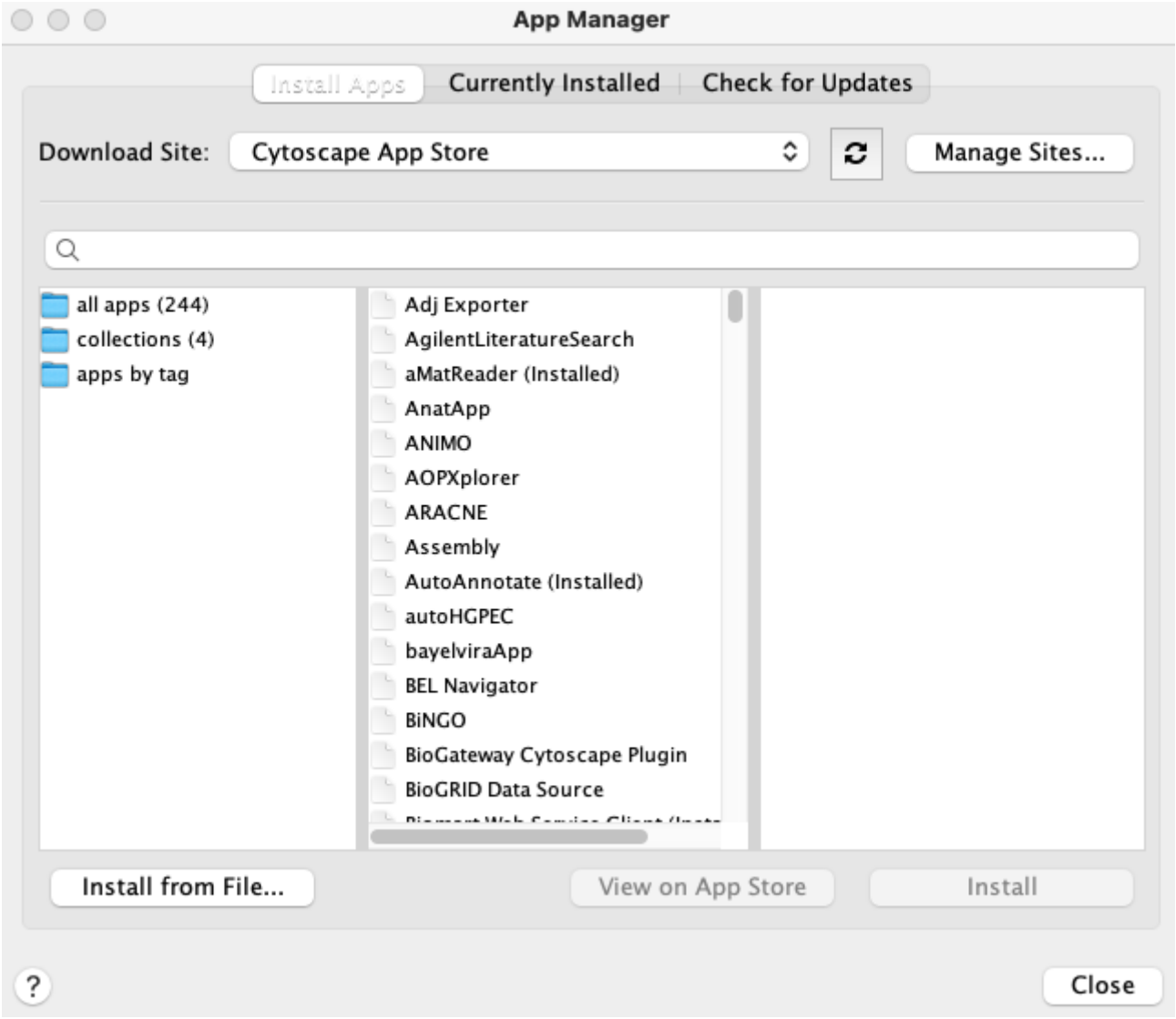
Degree	BetweennessCentrality	ClusteringCoefficient	AverageShortestPathL	ClosenessCentrality
69	0.09798571041737145	0.0315430520034...	3.2276595744680...	0.3098220171390903
68	0.09512288712938682	0.0324846356453...	3.2297872340425...	0.3096179183135705
55	0.16836018172039355	0.0087542087542...	3.5446808510638...	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 I recommend the freely available online version of the Network Science textbook by Albert-Laszlo Barabasi (http://networksciencebook.com/).

Calculating network rewiring

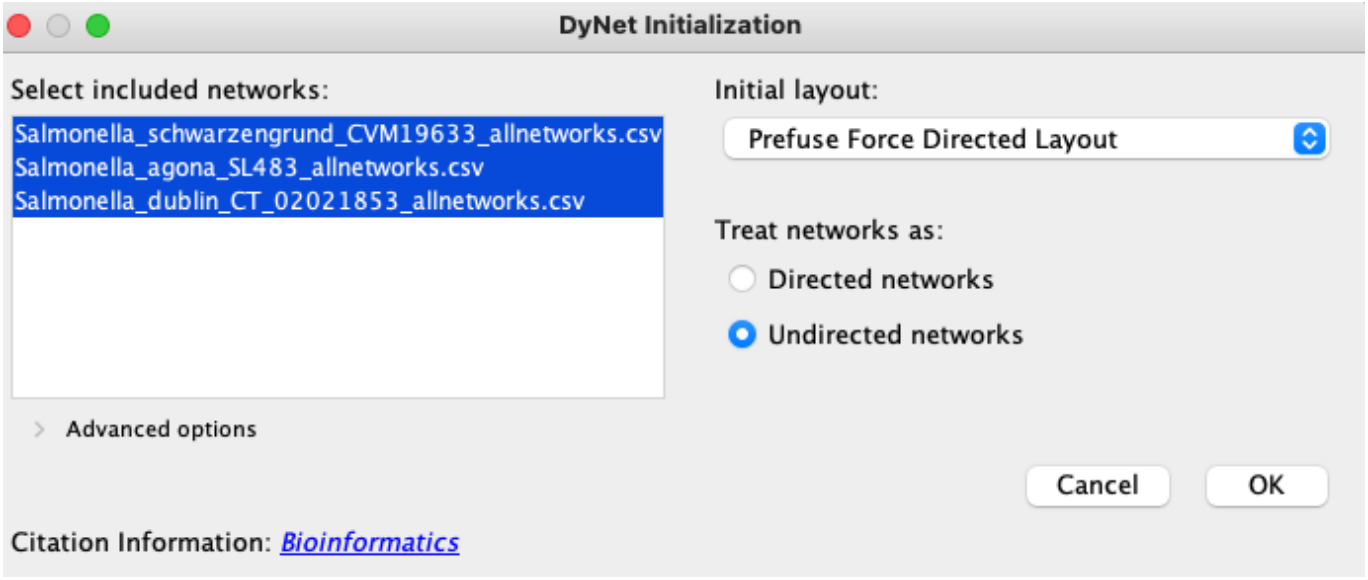
Installing and loading DyNet

Network rewiring entails many approaches aimed at quantifying changes between interaction networks, and has been utilised in the past to highlight the specific differences between similar interaction networks (Mehta et al. 2021; Treveil et al. 2020). In this work, we compared the degree of interaction rewiring between the interactomes of host adapted typhoidal Salmonella strains and gastrointestinal Salmonella strains to explore the utility of a multi-layered network resource such as SalmoNet2. To calculate network rewiring in the same manner as shown in the SalmoNet2 publication, one has to download the DyNet application from the Cytoscape app store first (https://apps.cytoscape.org/apps/dynet). This can be done directly from the webpage (which will show a prompt to open Cytoscape), or from within Cytoscape. In the latter case, select **Apps → App Manager ...**, which will return the following window:

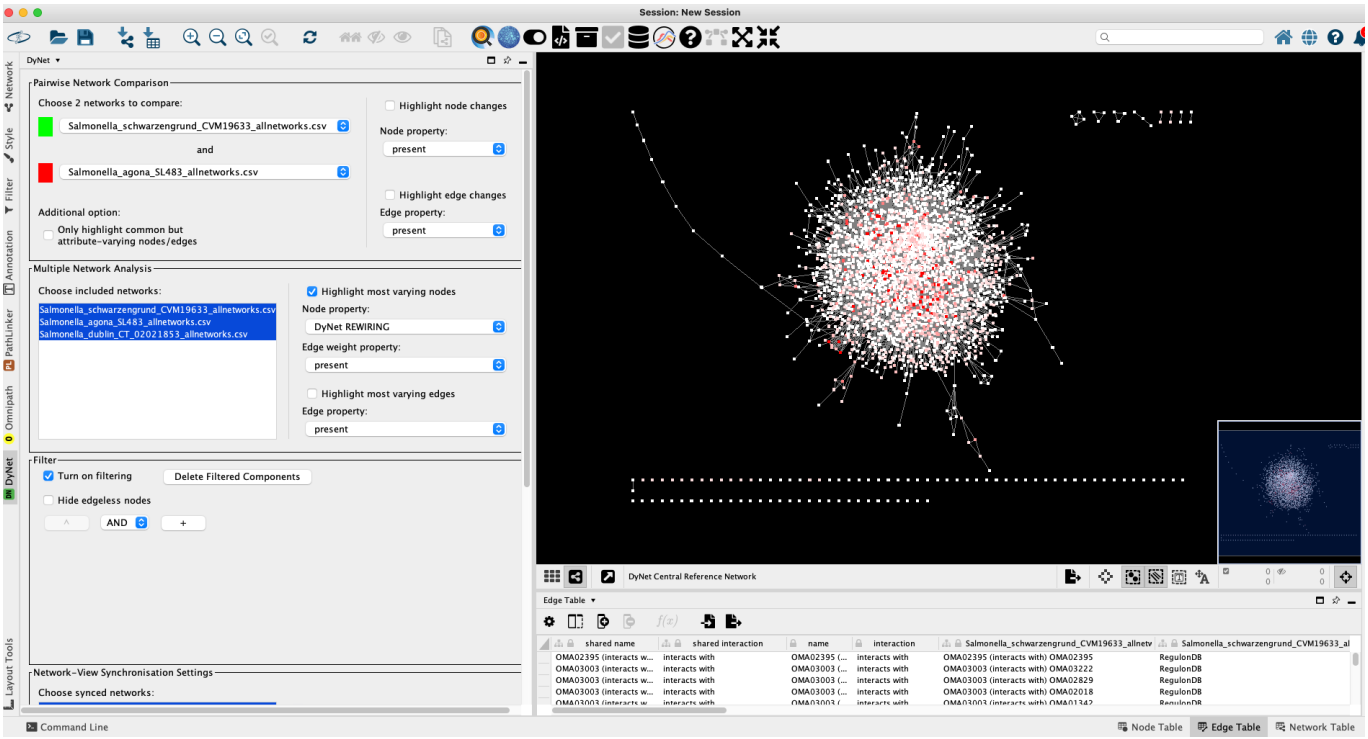


Typing "DyNet" into the search box will list the application, and it can be installed by clicking on the 'Install' button.

To calculate rewiring we will need to load multiple *.csv* or *PSI-MITAB* networks we would like to compare. When selecting **source** and **target** columns, please select the **orthology group ID** columns, as these are the only identifiers we will be able to match our networks on. Please refer to the **Importing data** section for more details. Once multiple networks have been loaded, activate the DyNet application by clicking on **Apps → DyNet Analyzer** [1]. A window will appear:

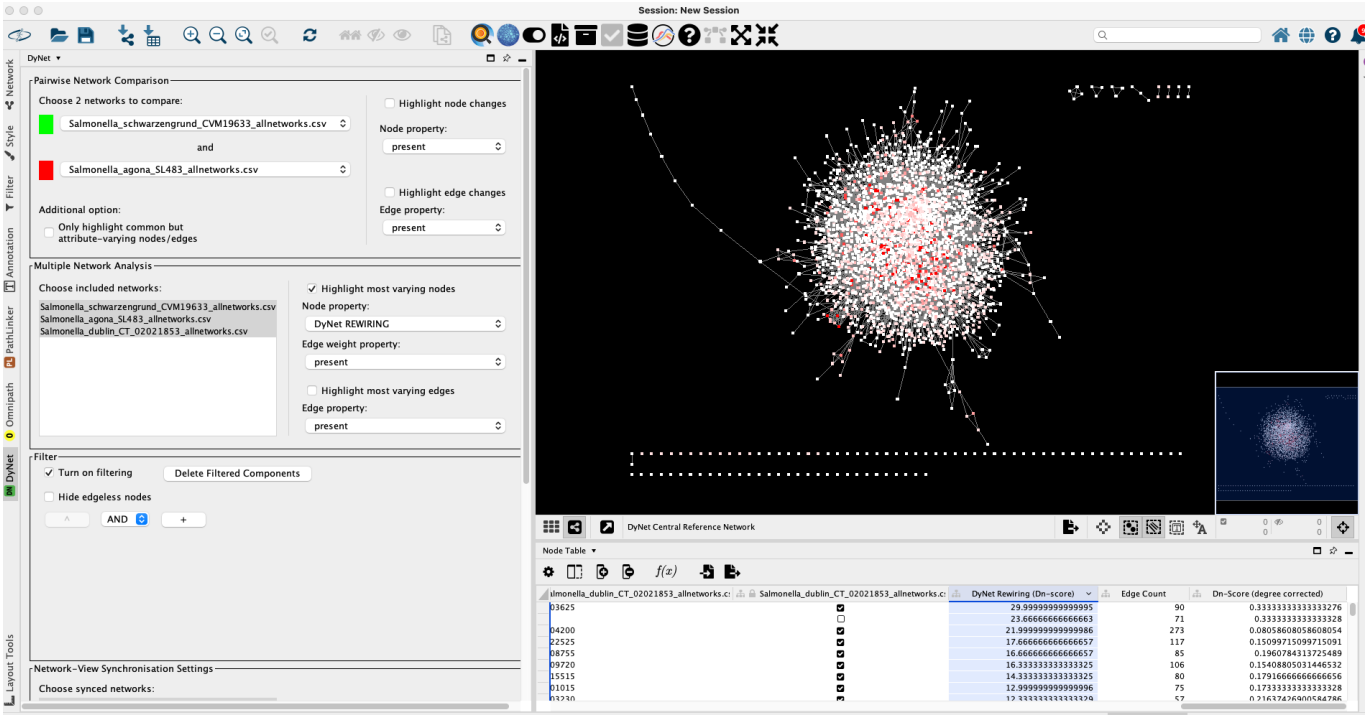


By default all networks will be selected. Networks should be treated as directed or undirected according to the layer type: PPI networks are undirected, regulatory and metabolic layers are directed. Clicking on OK will bring up the main DyNet window:

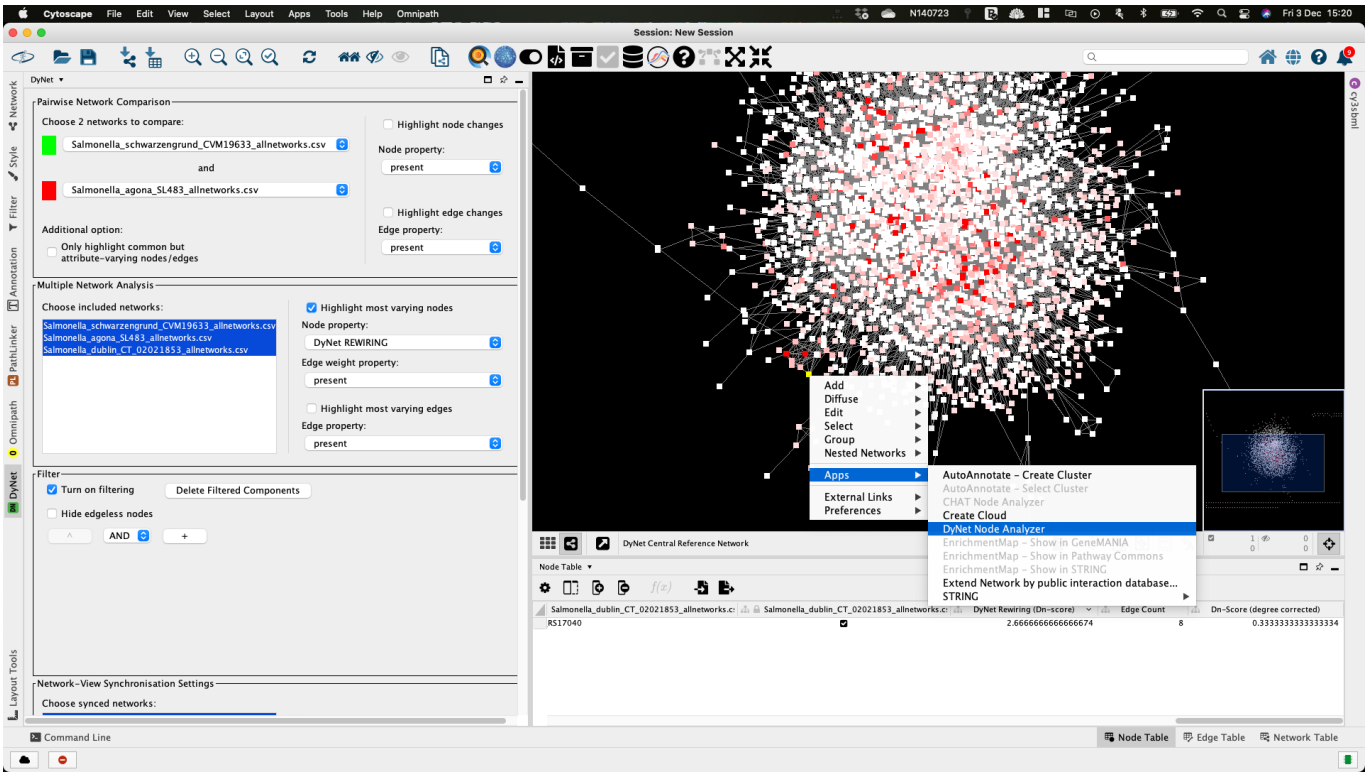


When working with more than 2 networks, by default **Multiple Network Analysis** is selected. However, once loaded, users can select any subset of networks they wish to compare, which updates the rewiring values in real time. By shift-clicking on *S.* Agona and *S.* Schwarzengrund and toggling the **Pairwise Network Comparison** options on the top I can proceed with pairwise network analysis. Here, the colour-coding details the specific binary node and edge differences between the two networks. The Dn rewiring values will be also more discrete: -Inf, 0, and +Inf referring to the absent, matching, and additional interactions when comparing the two networks respectively.

Selecting all three networks again and toggling *Highlight most varying nodes* lets us compare the three *Salmonella* networks simultaneously.

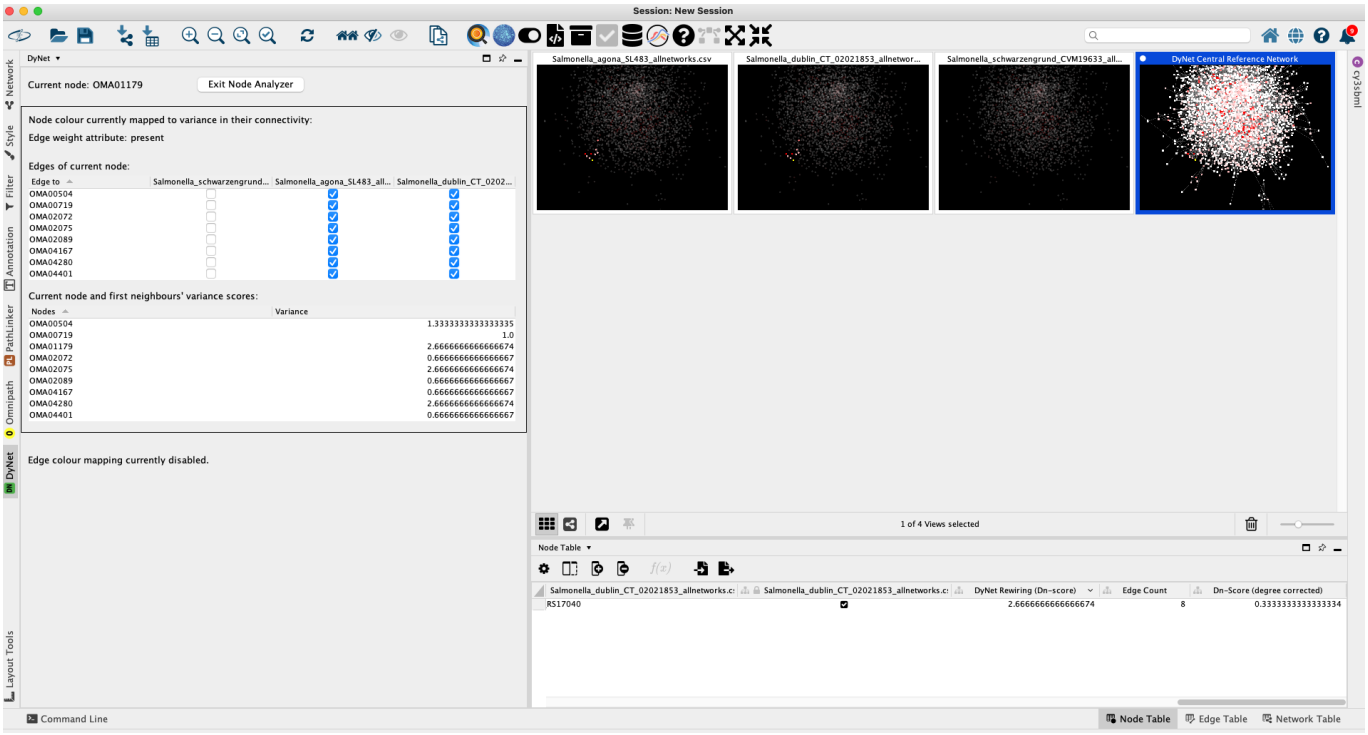


The resulting rewiring values can be sorted in the **Node table**, and the top *n* hits can be further analysed in detail. Selecting a node of interest, and right clicking on it brings up a context menu, where after selecting **Apps → DyNet Node Analyzer** the immediate interactome of the selected node can be analysed in detail.

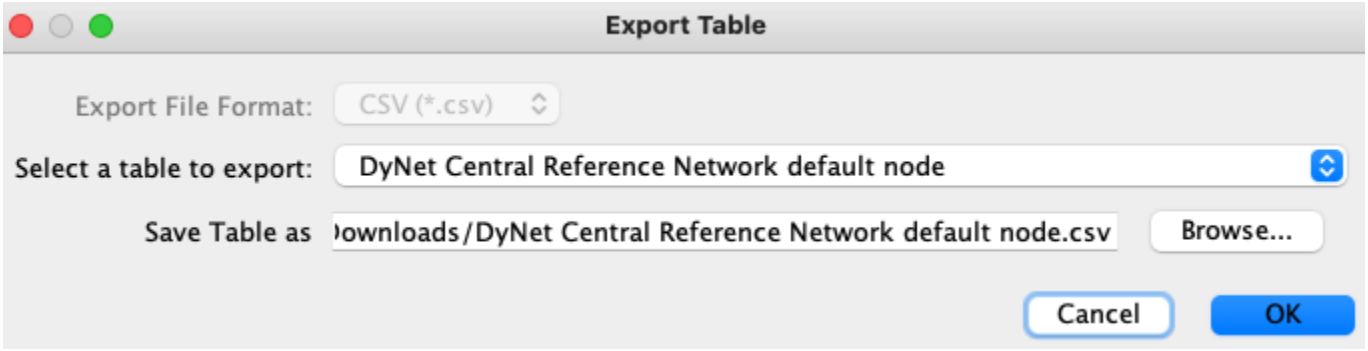


The **Node analyzer** pane lists the interactions present between the selected node and its first neighbours, and the status of these interactions in the compared networks. In this specific example **OMA01179** is missing from *S.* Schwarzengrund altogether, and as such all further interactions are absent. DyNet synchronises the positions of the nodes and edges across all networks, making visual comparisons easier, as seen on the right of the figure. This multi-network pane can be toggled by clicking on the **Grid** icon on

the bottom of the main window.



The results of the rewiring analysis can be exported from Cytoscape by clicking on **File → Export → Table to File ...**. Make sure to select the **DyNet Central Reference Network default node** table to import, as this table holds the node rewiring data. The results will be saved in a convenient *.csv* format readable by Microsoft Excel and other similar software.



[1]: There is an option titled **DyNet Network Importer** among the addons, allowing the user to import multiple networks at the same time. However, this requires a special file format that SalmoNet2 does not supply. For more details please refer to the DyNet documentation at https://apps.cytoscape.org/apps/dynet

Mehta TK, Koch C, Nash W, Knaack SA, Sudhakar P, Olbei M, Bastkowski S, Penso-Dolfin L, Korcsmaros T, Haerty W, Roy S, Di-Palma F. **Evolution of regulatory networks associated with traits under selection in cichlids.** *Genome Biol*. 2021 Jan 8;22(1):25. doi: 10.1186/s13059-020-02208-8. PMID: 33419455; PMCID: PMC7791837.

Treveil A, Sudhakar P, Matthews ZJ, Wrzesiński T, Jones EJ, Brooks J, Ölbei M, Hautefort I, Hall LJ, Carding SR, Mayer U, Powell PP, Wileman T, Di Palma F, Haerty W, Korcsmáros T. **Regulatory network analysis of Paneth cell and goblet cell enriched gut organoids using transcriptomics approaches.** *Mol Omics*. 2020 Feb 17;16(1):39-58. doi: 10.1039/c9mo00130a. PMID: 31819932.