Example of building an epigenetic network in Cytoscape

Cytoscape is a powerful graphics editor and many complex procedures are described in the user manual.

PathEpigen generates 5 files, which can be open by Cytoscape.

epigen.sif – file of correlations: first file read by Cytoscape.
names.na – names of nodes (molecular events, the levels, frequencies & nb. samples)
attributes.mrna – node attributes (frequencies, nb. samples, occurrences)
proba.eda – edge attributes, frequencies.
samples.eda – edge attributes, number of samples
occur.eda – edge attributes, occurrences (number of datasets involved in the edge)

1. Building the network.

- File -> Import Networks (Multiple Files Types).
- Import the network directly from the epigen.sif file, generated by PathEpigen.
- Go to Layout -> Cytoscape Layouts -> Edge-Weighted Spring Embedded.

Your network will appear on the Cytoscape canvas. Use the magnifying glass from the upper horizontal menu of Cytoscape to adjust viewing magnification of the network and the navigation tool from the same menu to move through the network.

At this point, the edges have no directionality.

2. Customising the network.

- Go to VizMapper.
- Find the options icon the the right of Current Visual Stile. Clich on it and choose Copy existing Visual Style. Name this style, for example, Epigenetics.
- Click on Defaults image. A window will pop up.
- Unlock Node Width/Height (left bottom corner of the pop-up window)
- Find the list of visual parameters at the right of the pop-up window. Decrease the size of node font size by clicking NODE_FONT_SIZE.
- Click Edge (right bottom of the pop-up window).
- Activate Edge_TGARROW_SHAPE -> Choose an arrow shape -> Apply.
- The arrows will appear from the source (Event1: the event which happens in all samples) to the target (Event2: the event which happens in a fraction of the samples).

At this point, the network shows too many nodes and links and thus it is difficult to extract any pertinent knowledge from it. Search for hub-nodes on the network. For example, mutation of KRAS in a hub.

- Select nodes that interact directly with this node by going to Select -> Nodes -> First Neighbours of selected nodes. You should see a network with several yellow nodes in the centre.

- Copy the selected nodes and their edges into a separate network by selecting File -> New -> Network -> From selected nodes, all edges.
-

This step can be performed after steps 3-6 are performed.

You can also delete en edge which you do not want to appear on the network by mouse right clicking -> Visual Mapping Bypass -> Set Edge Opacity to 0. The edge will become transparent and disappear.

3. Loading node names

PathEpigen saves the names of the attributres together with main info about the nodes in the file names.**na.**

Official HUGO Symbol
CACNA1G_+Meth_CpGisland_YES = CACNA1G\n+Meth_CpGisland YES\n 0.000 8
CDKN2A:p16_+Meth_CpGisland_YES = CDKN2A:p16\n+Meth_CpGisland YES\n 0.282 394
CRABP1_+Meth_CpGisland_YES = CRABP1\n+Meth_CpGisland YES\n 0.300 110

Line breaks are used through this file if the names are too long.

Now we can modify the visual styles to display the official node names as node labels.

- Go to File -> Import -> Node Attributes.
- Select the file names.na, and click Open.
- A popout window labelled Loading Node Attributes will appear.
- In the Cytoscape desktop, enter in VizMapper. It is located in the control panel to the left. In the Vizual Mapper Browser (down the screen in the VizMapper**)**, click the Node Label left tab.
- To the right from Node Label tab, locate the first pull-down menu, and select Official HUGO Symbol**.**

4. Loading node attributes

To the difference to edge attributes, node attributes can be loaded in the same tame from one single file: attributes.mrna.

- Go to File -> Import -> Attribute/Expression Matrix and import file with node attributes attributes.mrna.
- Go to Node Attribute Browser (bottom of the screen), click the Select Attributes button, and select the attributes of probaexp and samplesexp (Cytoscape attaches suffix *–exp* to the end of the names of the attributes).

5. Colour nodes in gradients according to their properties, using “Visual Styles”

This is an interesting procedure when one is interested in visually representing the frequencies of molecular events (nodes) in the studied phenotype. To do this:

- go to VizMapper, Visual Mapping Browser.

- Select “Node Color” in the left column of the Visual Mapping Browser.
- Double-click on the right column cell corresponding to “Node Color” and select probaexp.
- A section called Mapping will drop down. Set the Mapping type to Continuous. This specifies that each node will be coloured on a colour continuum according to the probability of occurrence.
- By default, you will get black for nodes with proba=0 and white for nodes with proba=1. Invert that, because (i) there are more nodes with smaller probability (ii) because there are nodes for each the probabilities are unknown or can be deduced from the other nodes. Chose a shade of grey for proba=1, so that the nodes labels remain visible.

Other visual mappings can be also done. For example, the nodes can be set higher or narrower in function of *sample* (samplesexp in Cytoscape). This is done with Visual Mapping Browser using Node Size. Or node shape can be mapped in function of the number of datasets used to obtain a node. If the number of datasets is 0, it means that this molecular event appears mentioned only in relation to another event (in table Double relations), hence there is no direct statistical information on the frequency of the event as such in the context of the studied phenotype. In the example, we have represented such nodes as rectangles, whereas the other nodes appear as ovals. This was also done using VizMapper, Visual mapping Browser, and Node Shape has been mapped.

6. Load edge attributes and colour edges in gradients.

Unlike the node attributes, which can all be downloaded in one go, the edge attributes can be loaded only in a one by one fashion. The files containing the edge attributes end in .eda and probably the most interesting edge attribute is edge frequencies in file proba.eda.

- Go to File->Import -> Edge Attributes-> choose the file (proba.eda)
- To map each edge to its attribute, go to Data Panel (bottom part of the screen, chose Edge Attribute Browser and when you click on each edge, you can see it’s attribute.)
- But it is more useful to see the frequencies displayed as edge-labels. Go to Viz-Mapper, Visual Mapping Browser.
- Choose Edge Label tab at the left, choose proba at the right, and set the mapping type to Passthrough.

In the example network, the edges are coloured in gradients according to their frequencies. The darker edges connect highly correlated nodes: displaying molecular events which are likely to occur simultaneously in the context of the given phenotype, while the lighter edges show that correlation between the nodes at their ends has been verified, but found to be weak or non-existent. This means that the given nodes represent different pathways to the same phenotype.

Other edges characteristics can be exploited to make the networks more informative, such as Edge Opacity, Edge Target Arrow Colour, Edge Target Arrow Opacity etc.

7. Exporting Networks.

- Set the desired zoom-out or zoom-in.
- File->Export->Network View as Graphics

The network will be exported as a .pdf.