

CyNET UI

<https://epicimmuneatlas.org>

The screenshot shows the CyNET UI interface. On the left, a sidebar has 'Analysis' and 'Plots' selected. The main area has two 'Upload' sections: 'Upload freq files' and 'Upload node info files', both with 'No file selected' buttons. Below these are two buttons: 'Upload cell subset frequency file' and 'Upload cell subset Info file'. The 'cal net stats' section contains fields for 'Choose no of samples' (10), 'input cor cutoff' (0.6), and 'Choose color plt' (Blues). A color scale from 0 to 1 is shown below. A callout for 'Choose no of samples' says: 'Change Number of samples for the group to include for network analyses: If n > samples available in frequency file it will show error'. A callout for 'Choose color plt' says: 'Use this if you choose to overlay node intensity (marker expression) (continuous value) on the network'. A 'plot network' button is at the bottom.

The screenshot shows the CyNET UI interface with specific configurations highlighted. The 'cal net stats' section has 'Choose no of samples' set to 10 and 'input cor cutoff' set to 0.6. The 'Choose group for network stats' dropdown is set to 'A_CBNB'. The 'Choose node attribute to color node' dropdown is set to 'Node'. The 'Choose color plt' dropdown is set to 'Blues'. The 'plot network' button is at the bottom. A red arrow points from the text 'Cal net stats: will calculate the network properties and will be shown as table.' to the 'cal net stats' button. Another red arrow points from the text 'Plot network: will plot the network. It can be downloaded as html file where node position can be adjusted for declustering some node' to the 'plot network' button.

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CyNET: requires two file for the network analysis of the cytometry data

1: Node frequency file

2: Node information file

Node frequency file:

Node frequency file can be generated from flow-cytometry as well as mass cytometry data.

Usually mass cytometry panels are more than 35 marker that include most lineage markers.

However if lab just have regular flow-cytometer with 12 color, they can stain using multiple panels each panel focussed on a single major lineage and then merge the cell frequency in one file from each lineage to create network using CyNET. Usually when using high dimensional mass/flow cytometry data we use clustering algorithms to group the cells in an unbiased manner to allow previously unknown cell subsets. Node frequency file must be formated as specified on page 3 of this manual and should be in csv file format.

Node Information file:

Node information file allows overlaying information of nodes (cell subset) on the network diagram.

Any additional information can be visualised on network. Node and celltype information is required columns. Node frequency file must be formated as specified on page 3 of this manual. Node information file must be formated as specified on page 3 of this manual and should be in csv file format.

Correlation cutoff value and number of samples in each group.

Correlation (R) value cutoff: it defaults to 0.6 to include only strongly correlated nodes. However different values can be tested. Suggest to keep it between 0.5-0.8. For number of sample in each group n<7 may not be very robust for correlation values. Statistically significant correlations are used only and the p-value is set to 0.05.

Positively correlated edges in the network are shown in red color and negatively correlated edges are shown in green color

Outputs:

1: Network property table can be copied and saved.

2: Network diagram overlayed with node information can be saved and downloaded as html file.

3: Node centrality bar graph and csv file can be downloaded

4: Node pageRank centrality figure and csv file can be downloaded

Data format : for CyNET App

Cell subset Frequency file

| group | sampleName | Node_1 | Node_2 | Node_3 | Node_4 | Node_5 |
|------------|------------|--------|--------|--------|--------|--------|
| A_CBNB | EPICC011 | 0.0064 | 0.008 | 0.0318 | 0.0559 | 0.0559 |
| A_CBNB | EPICP010 | 0.0175 | 0.0047 | 0.0273 | 0.0787 | 0.0787 |
| F_20yTo55y | EPICP005 | 0.0029 | 0.0104 | 0.01 | 0.0372 | 0.0372 |
| F_20yTo55y | EPICP002 | 0.0044 | 0.0063 | 0.018 | 0.0528 | 0.0528 |
| H_71yToA | EPICC004 | 0.0136 | 0.0119 | 0.0171 | 0.0449 | 0.0449 |
| H_71yToA | EPICC005 | 0.0136 | 0.0119 | 0.0171 | 0.0449 | 0.0449 |

Format for frequency file:

The file must have group and sampleName columns. Names of subsets (nodes) must follow the convention Node_{node_id}. Once the file is uploaded group dropdown (Fig 2.) will appear on the app. Choose the group for network creation and analysis. Input the number of samples that you want to include. If sample number exceed the number of sample present in the uploaded file it will show error. If you are analysing multiple group it's better to keep number of samples balance in all groups. For eg. If you have three group with n number of sample, Healthy Control(n=20), Disease 1(n=17), Disease 2(n=15). We suggest to use n =15 to have equal number of samples in each group.

Node (cell subset) Information file

| Node | celltype |
|--------|----------|
| Node_1 | CD4p |
| Node_2 | CD4p |
| Node_3 | CD8p |
| Node_4 | CD8p |
| Node_5 | NK |
| Node_6 | NK |
| Node_7 | Bell |

Node information file must have Node and celltype columns. Node columns should have all the nodes that are in Node frequency file. Celltype column should have major cell lineage for Nodes in Nodes columns Additional information can also be included in this file to overlay the node information onto the network. Marker expression values can also be plotted on network. All the columns in this

file will have those columns in dropdown menu to choose. If the column values are continuous values then continuous color scale will be applied to overlay node information. However if the column values are categorical then discrete color for each category will be overlayed.