

# CrosstalkNet Documentation

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## Getting Started

CrosstalkNet is a web-based network visualization tool to retrieve and mine interactions in large scale co-expression networks, which are bipartite in nature. An important application of co-expression networks in biology is to uncover the effects of tumor microenvironment in the context of tumor epithelial-stromal interactions. Below is a tutorial of the web application. The manual is written according to the functionality of each tab in the application. The application

For guest users, the application has a case study from Oh et al. work (Oh et al. 2015). In order to upload customized network files, users have to register and the credentials can be obtained from [bhaibeka@uhnresearch.ca](mailto:bhaibeka@uhnresearch.ca) or [benjamin.haibe.kains@utoronto.ca](mailto:benjamin.haibe.kains@utoronto.ca)

Status: Waiting for user to query...BHK Lab

MAIN GRAPH

Navigation ☐ Graph

DATA

Network Type:  
☐ Normal  
☒ Tumor  
☐ Delta

Select Tumor Matrix File ▾

GET GENES

Optional File Upload

CHOOSE TUMOR FILE

Normal File: No file selected

UPLOAD FILES

## Main Graph

### Data

#### Selecting a Dataset

Before any analysis of the network can be done, a network type and associated file(s) must be chosen from Main Graph → Data. Once a the network type and file(s) are selected, the “Get Genes” button needs to be clicked in order to enable the other tabs and load the list of genes associated with the specified file(s).

#### Upload

Users can upload their own adjacency matrices to be used for visualization. The adjacency matrix should be converted to a sparse matrix using Matrix package in *R*, and the file should be saved as an RData file using saveRDS function in *R* before uploading the files.

Status: Waiting for user to query...
BHK Lab

MAIN GRAPH

Navigation
Graph

DATA

Network Type:

☐ Normal
Select a network type

☒ Tumor

☐ Delta

Select Tumor Matrix File

TNBCGenWideEpiStromt.05.Rdata

Select the file(s) to be used

GET GENES
Click me to get genes for this

Optional File Upload
Upload own files if desired

CHOOSE TUMOR FILE

Normal File: No file selected

UPLOAD FILE'S

## Types of Networks

The user can upload normal and tumor co-expression networks which are sparse. In addition, the sparse delta networks (tumor-normal) can also be uploaded, which are obtained from matched tumor and normal epi-stroma pairs.

## Query

### Selection of genes

From the Query sub-tab, the user can find out information about the first and second neighbours of a group of selected genes. To select a gene, click on the "Choose Genes" box and start typing in the name of a gene. The box will filter down results based on what you've typed so far. You will notice that all gene names have a -E or -S appended to them followed by a number. The -E indicates epi, the -S indicates stroma, and the number indicates the number of first neighbours for that gene.

As an example, let us select CD8A in the stroma, GZMB in the stroma, and CXCL13 expressed in the epithelial. As you begin typing CD8A, you will notice that the drop down list has fewer and fewer results. To choose CD8A-S, simply click on it.

Status: Graph finished

BHK Lab

MAIN GRAPH

INTERACTION EXPLORER

PATH EXISTENCE VIEWER

Navigation

Graph

DATA

QUERY

STYLING

Choose Genes

RESET

Select genes here

Min Degree

0

Selected Genes:

X

CD8A-S

X

GZMB-S

X

CXCL13-E

REMOVE ALL GENES

Genes selected so far appear here

FILTER

GET FIRST NEIGHBOURS

Click to get the first neighbours

First Neighbour Filters

Min Negative Correlation |

Lowest: -1

0

Min Positive Correlation |

Highest: 1

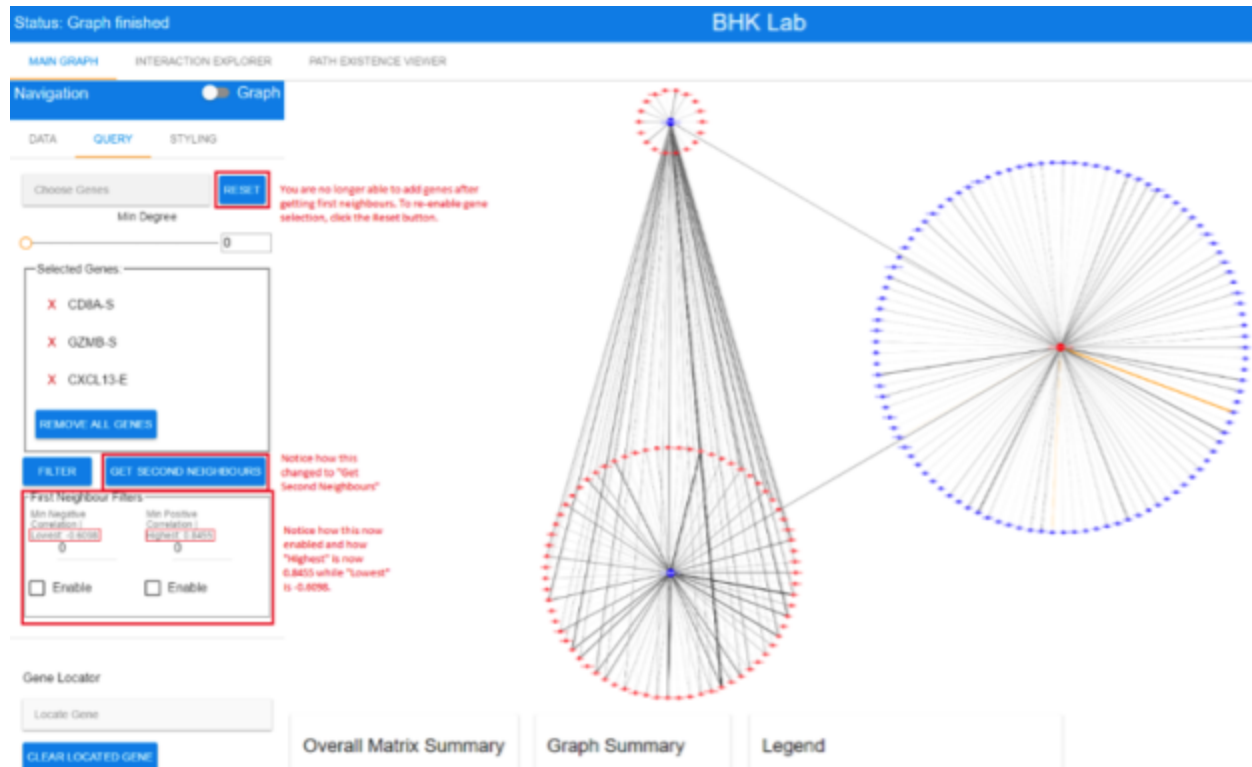
0

Enable

Enable

## First and Second neighbors

Once the user selects the genes of interest and clicks on the “Get First neighbors” button, all the interactions pertained to those selected genes will appear as a graph along with a tabular view of results. Once the “Get First neighbors button” is clicked, the “Get Second neighbors” button will be automatically enabled. When the user clicks on the “Get Second neighbors” button, all the interactions pertained to the selected genes along with their first neighbors will be displayed in a graph.



## Gene locator

The user has the ability to enter a gene of interest to highlight it in the displayed graph. This functionality is provided in the “Main Graph” and “Interaction Explorer” tabs

## Edge inspector

This functionality displays information about the edge weight, which is the strength of interaction between any two nodes in a given network. In the case of delta networks, the edge weight between two nodes provides the difference between the tumor and normal interaction along with the edge weight of normal and tumor networks.

## Filter

After obtaining the first neighbours, they can be filtered so that only interactions of a specified strength are seen. There are 2 input boxes available for filtering first neighbours interactions.

1. The box labeled "Min Negative Correlation | -1" is used to control only the negative interactions. If one types -0.5 in the box, and selects enable, then only negative interactions of  $\leq -0.5$  will be shown. The number after the "|" indicates the lowest interaction in the entire graph. Entering a number lower than this or entering a positive number are both prohibited.

2. The box labeled "Min Positive Correlation | 0.8455" is used to control only the positive interactions. If one types 0.7 in the box, and selects enable, then only positive interactions of  $\geq 0.7$  will be shown. The number after the "|" indicates the highest interaction in the entire graph. Entering a number higher than this or entering a negative number are both prohibited.

If one enables both filters, then all interactions  $\leq -0.5$  and  $\geq 0.7$  will be returned. To get the filtered results, simply click the "Filter" button. If the user doesn't want to filter, then the checkboxes should remain unchecked.

The filter option works for the second neighbors as well.

## Styling

### Layouts

This option is located in the Mani Graph ---> Styling. There are 3 layouts to display the graph, namely, concentric, random and bipartite. The user can select a layout of choice and the graph gets refreshed automatically. Zoom in and out features are enabled on the graph and the resize button resets the zoom level (in case the user zooms in too much or too far away and has lost the graph).

### Edge Attributes

The color and thickness that an edge has, gives it semantic meaning. Grey to black edges represent positive interactions. Light orange to orange edges represent negative interactions. The darkness and thickness of an edge represents its relative magnitude to other interactions. For example, say that a graph has only 4 edges in it with the following correlations: -0.8, -0.6, 0.5, 0.65. The -0.8 edge will be dark orange and will be thick whereas the -0.6 interaction will be light orange and will be thin. The 0.65 edge will be dark black and will be thick whereas the 0.5 interaction will be light grey and will be thin.

### Network Statistics

There are a total of 4 cards at the bottom of the graph, 3 of which are visible by default and the fourth can be seen when an edge is clicked.

1. **Overall network Summary:** Displays the number of self loops and significant interactions.
2. **Graph Summary:** Shows statistics about the graph that is currently being displayed.
3. **Edge Inspector:** Shows information about a selected edge. Appears only when an edge is clicked.

4. **Legend:** Shows information about the styling in the graph and the meaning of it.

## Views

### Tabular

In addition to the graph, the interactions and self-loops can also be seen in a table-view which is more convenient for doing analysis with. To go to the table view, click on the switch shown in the below example:

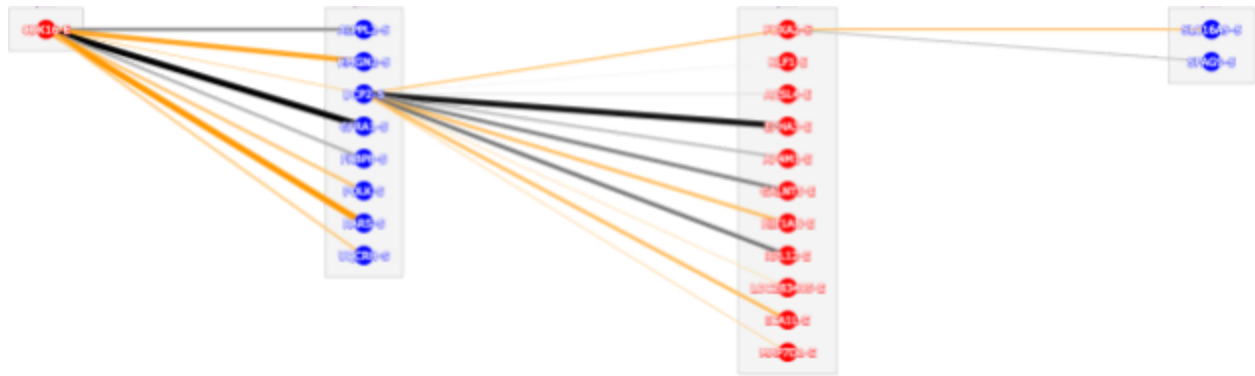
The screenshot shows the BHK Lab Interaction Explorer interface. The 'Tables' view is selected in the navigation bar. The table displays interactions between genes, with columns for gene names and interaction scores. A red box highlights the 'Filter Self Loops' button in the top right corner.

Gene	CD8A	GZMB	PDCD1	CRCL13	SAMD3	SELL	CD46LG	SCML4
CD8A-E	0.8154	0.791	0.6566	0.6034	0.8851	0.6885	0.6743	0.5635
GZMA-E	0.7553	0.768	0.7429	0.5892	0.8589	0.6158	0.6162	0
CD8-E	0.7728	0.7179	0.6954	0.6017	0.8398	0.6775	0.6722	0
CD52-E	0.7437	0	0.6271	0	0.8523	0.6022	0.5888	0
GZMB-E	0.7482	0.8455	0.7262	0.6212	0.8627	0.6531	0.6288	0
GZMH-E	0.7995	0	0.6948	0.6117	0.8389	0.6512	0.5905	0.5968
KC9-E	0.7282	0	0.6593	0.5878	0.583	0.6748	0.7113	0
CRCL9-E	0.7328	0.7068	0.7211	0.706	0.726	0.7028	0.6913	0.577
CCR9-E	0.7418	0	0.6324	0	0.8143	0.677	0.6602	0
GBP5-E	0.7455	0.7502	0.6874	0.6562	0.5956	0.6831	0.6986	0
TBC1D18C-E	0.7182	0	0.6005	0	0.808	0.6205	0.6097	0
HC11-E	0.7224	0.7371	0.659	0	0.5899	0.573	0.6061	0
RASAL3-E	0.729	0	0.6601	0	0.5938	0.6145	0.5632	0
SLRG-E	0.7585	0	0.7143	0	0.647	0.7188	0.7075	0
CD8A-E	0.82	0.7797	0.7033	0.678	0.8877	0.6876	0.6453	0.6271
NKG7-E	0.7255	0.7485	0.6974	0	0.5911	0.6071	0.623	0
ARHGAP9-E	0.7235	0	0.5896	0	0.5958	0.5892	0.5629	0
ZNF433-E	0	0	0.7213	0	0.6969	0	0.6313	0

The user can download the table of interactions in a CSV file, using the download button located at the bottom of the page.

## Interaction Explorer

This tab is used for a more targeted approach to explore the network. First, a user must choose a single gene of interest and then click the "Get Data" button. Once the graph is loaded, the genes available for selection are simply the neighbours of the selected gene. This process can be repeated over and over again to go beyond the 2nd neighbour level available in the main graph. An example is presented below, in which the interactions are displayed for a depth of 3 levels.



## Path Existence Explorer

This feature displays all the existing interactions between any two selected genes in the network. The results are presented in a tabular view along with the strength of interactions.

Status: All paths have been obtained BHK Lab

MAIN GRAPH INTERACTION EXPLORER **PATH EXISTENCE VIEWER**

Navigation

QUERY

Path Existence Checker

CD8A-E 53 ×

STAT1-E 27 ×

**GET ALL PATHS**

**All Paths Between CD8A-E and STAT1-E**

Total: 12

Source	Correlation	Intermediate Gene	Correlation	Destination
CD8A-E	0.6248	PDCD1-E	0.6059	STAT1-E
CD8A-E	0.6057	GZMB-E	0.6056	STAT1-E
CD8A-E	0.6895	GZMB-E	0.6045	STAT1-E
CD8A-E	0.6375	IL12RB2-E	0.6022	STAT1-E
CD8A-E	0.6196	GZMB-E	0.6046	STAT1-E
CD8A-E	0.6289	TRAF3-E	0.606	STAT1-E
CD8A-E	-0.6037	WDR5-E	-0.6376	STAT1-E
CD8A-E	0.7089	WDR5-E	0.606	STAT1-E
CD8A-E	0.7156	WDR5-E	0.6733	STAT1-E
CD8A-E	0.6846	WDR5-E	0.6196	STAT1-E
CD8A-E	0.683	TAP1-E	0.7942	STAT1-E
CD8A-E	0.705	LAG3-E	0.6889	STAT1-E

**DOWNLOAD INTERACTIONS**

## Degree Explorer

This functionality allows the user to identify genes expressed in epithelium and stroma according to the degree. The lists of genes can be downloaded in a simple csv format.

## Community Explorer

This functionality allows the user to display all the communities in the network. The interactions in each community can be downloaded in a simple csv format. The users can upload their own data frames of communities to be used for visualization. The file must contain a single data



frame, it must be saved using the `saveRDS()` function, and it needs to have a `.RData` extension. Here is the expected schema of a user-uploaded file:

epi	stroma	interaction	epiCommunity	stromaCommunity
S100A7-E	S100A7-S	0.06765134	13	13
FAM5C-E	FAM5C-S	0.07024922	57	57
BEX1-E	BEX1-S	0.07874761	5	5
IFIH1-E	IFIH1-S	0.09094819	3	3
AGT-E	AGT-S	0.09310902	47	47

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## Export

The graphs can be exported in a PNG format, and the results displayed in the tabular view can be exported as a CSV file

## References

Oh, Eun-Yeong, Stephen M. Christensen, Sindhu Ghanta, Jong Cheol Jeong, Octavian Bucur, Benjamin Glass, Laleh Montaser-Kouhsari, et al. 2015. "Extensive Rewiring of Epithelial-Stromal Co-Expression Networks in Breast Cancer." *Genome Biology* 16 (June): 128.