

Worksheet 4

Objective of the Worksheet: In this exercise, you are to retrieve a network from a web service client, use the ClusterONE algorithm to find subnetworks/complexes and do a functional analysis with BinGO and Jepetto plugins.

A) Loading Network

STEP 1: Start Cytoscape, under “File”, select “Import” a “Network”. From “Public Databases”, select “Pathway Commons Web Service Client”.

STEP 2: Enter “NFkB” in the Gene Name/ID box, and select “Human” from the All Organism box. Click the “Search” button.

STEP 3: A new page will show up. In the new page, select “NFkB Complex_Human”. Click “Pathways” button and double click the “TLR4 Cascade” pathway from the reactome database.

After a brief load, a network in the grid structure will show up on the screen.

STEP 4: The default grid structure is ugly and you can change it to a more visible style: From “Layout”, choose “yFiles Layouts” and select “Organic”.

In the control panel, click the “Vizmapper” button. Double clicking the node to edit the network attributes. In the “dependence” option, select “Lock node width and height”.

In the “Node” option, change the node height, node fill color, node border width, *etc.* to your preferred values. Click “Apply” button to refresh the styles.

In the table panel, select the “show selected” and display the selected nodes with the Entrez IDs, which are the IDs employed by NCBI (www.ncbi.nlm.nih.gov), and other relevant information.

B) Find the Subnetworks with ClusterONE Plugin

STEP 5: Follow the procedure of Apps→ClusterONE. In the ClusterONE control page, click the “Start” button. The discovered subnetworks will be displayed in the “Results Panel” on the right hand side of the screen.

STEP 6: The cluster 2 comprises of ATF1, ATF2, pS6K, CREB1, and other MAPK pathway-related genes. Click the Cluster 2 to select it. The selected genes shall be highlighted by yellow in the Cytoscape canvas.

C) Functional Analysis of the Selected Gene Set with BinGO

STEP 7: Follow the procedure of Apps→BinGO.

1) Input “NFkB TLR4 C2” to the Cluster Name box.

- 2) Check the “Paste genes from text” box.
- 3) Go to the Table Panel, display the selected nodes with the Entrez IDs only. Highlight the entries and press ctrl+C to copy the entries. Return to the BinGO’s gene set text box, press ctrl+v to paste the selected Entrez gene IDs into the text box.
- 4) In the Statistical Test, select “Hypergeometric test”.
- 5) In the Multiple Testing Correction, select “Benjamini & Hochberg”.
- 6) Input “0.1” to the Significance Level box.
- 7) Change the Organism to “Homo Sapien”.
- 8) Check the “Checkbox for Saving Files” checking box, and hit “Save BinGO data file in”. Select the directory and hit “Save” button.

STEP 8: Click the “Start BinGO” button. After a few seconds, the results will appear in the new window. Examine these entries, you will find the selected genes to be involved in the GO biological processes of “protein phosphorylation” (75%), signal transduction (87.5%), signal transmission (87.5%), and “signaling pathways” (87%).

D) Analysis of Gene Enrichment in Pathways

Jepetto performs integrated gene set analysis using information from interaction, pathways and processes databases.

STEP 9: Follow the procedure of Apps→Jepetto. Check the “Enrichment” check box. In the Identifier format, select “Entrez Gene”.

STEP 10: Click the Gene/Protein set window, press ctrl+v to paste the selected Entrez gene IDs into the text window. Select the “Kegg” for the annotation database. Click the “Start analysis” button.

STEP 12: After a few seconds, the results will be displayed in the “Jepetto Enrichment Panel” on the right hand side of the screen.

We can discover three pathways that contain more than 10 genes of the selected gene set: “TLR Signaling pathway” (10 genes), “Neurotrophin signaling pathway” (10 genes), and “MAPK signaling pathway” (13 genes).

Q1: Identify the genes and the gene products (proteins) involved in the TLR pathway. Find out the functions of these gene products.

Double click the TLR pathway to bring out a new network, which shows the input genes (grey), pathway genes (green), overlap between input and pathway (blue), pathway expansion (orange). New interactions not present in the input set are highlighted with red color.

Q2: Identify the genes of the blue-color nodes and the relevant gene products (proteins). Find out the functions of these gene products. Discuss the roles of these genes in the TLR pathway.