Expander v7.1.1– hands-on session:

Launching Expander:

In your Expander directory double click on **Expander.bat**.

- Before starting please create a directory named "results" in the Expander directory.

Human CAL51 cell line analysis:

In this section, we will analyze a dataset published by Rashi-Elkeles, Warnatz and Elkon et al. (Science Signaling 2014) in which HiSEq 200 Illumina RNA-Seq data was used to measure time-series expression profiles from CAL51 cell line under IR-induction for 2 biological replicates with 5 samples each – Control (0h), Control (4h), IR (4h), Control (8h) and IR (8h).

1. Loading the data:

- From the "File" menu, select "New Session" → "Expression Data" → "Tabular Data File".
- In the "Load Study" dialog box, make sure the Organism is set to "**Human**". Use the "Browse" button and search for the file *CAL51_IR_exp.groups.ensid.txt*.
- Add an ID conversion file using "Browse" button and search for the file *ens_ent_map.txt*.
- Make sure that the "Data type" is set to "Relative Intensities".
- Press "OK" in the "Load Study" dialog box.
- If a question pops up asking if you would like to download data for human, press Ok and wait for the download process to end.

2. Preprocessing the data:

- From the "Preprocessing" menu, select "Filter Probes" → "Fold Change". In the dialog box select probes that change in 2 folds in at least 2 conditions. Press "OK". A message will pop-up saying the number of probes will remain. Press "Yes".
- From the "Preprocessing" menu, select "Standardization" → "Mean 0 and Variance 1".

3. Using t-test statistics to detect differential expression:

- From the "Supervised Grouping" menu, select "Differential Expression" → "t-Test". Click "Yes" for performing T-test on standardized values.
  - In the "Requested type of change" combo-box select "Differential".
  - Press the "Select" button for the "Group 1 conditions".
  - In the "Select Conditions" dialog box, check (mark) 'Control' samples (checkbox) press "OK".
  - Press the "Select" button for the "Group 2 conditions".
  - In the "Select Conditions" dialog box, check (mark) 'IR' samples (checkbox) and press "OK".
  - Press "Ok".
- Look at the resulting groups on the left.
- Click on the pattern chart of the up-regulated group. A table will appear on the right pane. It contains the relevant t-test results.
- Click on the p-value column title to sort the probes according to their p-values.
- Click on the t-score column title, results will be sorted according to their t-score.
- Select the "Expression Matrix" tab to view the expression matrix of the up-regulated probes.

- From the “File” menu, select “Export to text”.
- Save the results to “*results/ttest1.1.txt*”
- Open the directory using Windows Explorer and browse through it to see the content of this output file.

4. Performing hierarchical clustering on the data:

- From the “Unsupervised Grouping” menu, select “Hierarchical Clustering”>>”Cluster”.
- In the dialog box, select “Average” as linkage type. Press the “OK” button.
- Look at the resulting display.
- Use the “Zoom in” and "Zoom out" buttons from tool bar to change zoom, and get a closer or more general view of the expression patterns arranged in the hierarchical clustering order.
- From the “File” menu, select “Save As Image”. In the dialog box select only "Dendrogram with matrix" Save the results to folder “*results/Hier1.1*”.
- Open the directory using Windows Explorer and browse through it to see the content of this output file.

5. Operating the Matisse algorithm to detect modules (connected sub-networks whose components exhibit coherent expression profiles):

- From the “Unsupervised Grouping” menu, select “Network” → “Matisse”.
- A window will pop-up asking to merge duplicated probes. Select "Merge Probes by Gene IDs". In the "Confirm operation" window select "Remove previous analysis results" and then "OK". Select "OK" in the "Average Probes" window.
- In the network file path select the file */organisms/human/interactions/Expander.hsa.IntAct.sif*.
- In the Matisse dialog box, press “OK”.
- After running the “Matisse” algorithm, the results will be displayed in a new tab.
- Click on the pattern chart of module #1. The genes assigned to this module will appear in the list on the right pane. Note that the back nodes (i.e. genes that do not appear in the filtered expression data, but are part of the module) are marked in pink.
- Click on the “Interactions” tab on the right to view the module interaction graph. Press on the left tool bar button for automatic layout of the graph. Note that the back nodes (i.e. nodes that correspond to genes that do not appear in the filtered expression data, but are part of the module) are marked in pink.
- From windows start button, search for the "Cytoscape" program and run it. In Expander "Interactions" tab, press on the right Cytoscape tool-button (). Press "OK" in the dialog box. Look inside the Cytoscape program to view the interaction made by Expander.
- Select the “Expression Matrix” tab to view the expression matrix of module #1.
- Select the “Positions” tab to view the chromosomal positions of the genes in the module.
- Optional: Repeat the previous steps for another module of your choice.

6. Identifying enriched miRNA targets within the modules:

- From the “Enrichment Analysis” menu, select “miRNA Analysis” → “FAME”. In the “miRNA Analysis” dialog box select “Matisse 1.1” as the grouping solution on which analysis is to be performed. Set the number of iterations to 100. Select "**Original GE data**" as background genes and press the “OK” button.
- The analysis might take a few minutes.
- Look at the resulting display (diagrams and enrichment table). Which miRNA has been detected as enriched in the modules generated by Matisse?

- Click on one of the columns in the enrichment diagram. The “Enrichment Info” dialog box will appear.
- In the “Enrichment info” dialog box search for the raw p-value.

7. Identifying enriched GO categories within the modules:

- From the “Enrichment Analysis” menu, select “Functional Analysis” → “TANGO”. Make sure that in the “Functional Analysis” dialog box “Matisse 1.1” is selected as the grouping solution on which analysis is to be performed. Press the “OK” button.
- Look at the resulting display (diagrams and enrichment table). Which functional enrichment has been detected?
- Click on one of the columns in the enrichment diagram. The “Enrichment Info” dialog box will appear.
- Click on one of the Gene IDs in the table. An internet browser will open, containing information about the corresponding gene, from the NCBI-Gene site.

8. Identifying enriched KEGG pathways within the modules:

- From the “Enrichment Analysis” menu, select “Pathway Analysis” → “KEGG…”. Make sure that in the “Pathway Analysis” dialog box “Matisse 1.1” is selected as the grouping solution on which analysis is to be performed. Select "**Original GE data**" as background genes. Press the “OK” button.
- Look at the resulting display (diagrams and enrichment table). Which pathway enrichments have been detected? Is "p53 signaling pathway" among the pathways found?
- Click on one of the columns in the enrichment diagram. The “Enrichment Info” dialog box will appear.

9. Browsing results for module 1:

- Go back to “Matisse 1.1” tab.
- Select “Module 1” from the list on the left. The entire set of results produced for module 1 will appear on the right. Browse through the results.
- Open the “Expression Matrix” tab to view the columns added on the left for each enrichment that has been detected.

10. Searching for your VIP gene groups in the results:

- From the “options” menu select Search Gene>>Group.
- In the dialog box, browse for the file *VIPGenesCAL51.txt.*
- Press OK.
- Look at the resulting report.
- Browse through the Expander display tabs and search for the highlighted VIP genes.

References

1) S. Rashi-Elkeles, H.J. Warnatz, R. Elkon, A. Kupershtein, Y. Chobod, A. Paz, V. Amstislavskiy, M. Sultan, H. Safer, W. Nietfeld, H. Lehrach, R. Shamir, M.L. Yaspo and Y. Shiloh. Parallel Profiling of the Transcriptome, Cistrome, and Epigenome in the Cellular Response to Ionizing Radiation. Science Signaling Vol. 7, 325, RS3, 2014.