

# NetworkAnalyst: Tutorial 1 Overview

-- Gene expression, meta-analysis, functional profiling and network visualization, leading to system-level insights

# Intro to NetworkAnalyst

- Web application that enables complex meta-analysis and visualization
- Designed to be accessible to biologists rather than specialized bioinformaticians
- Integrates <u>advanced statistical methods</u> and <u>innovative data visualization</u> to support:
  - Efficient data comparisons
  - Biological interpretation
  - Hypothesis generation







NetworkAnalyst -- a web-based platform for gene expression profiling & biological network analysis

# Overview

- Goal: To provide an integrative web-based framework for data processing, functional analysis and visualization of various forms of gene expression data.
  - Gene list(s)
  - Gene expression data (raw and processed)
  - Multiple gene expression data sets
  - Network files
- Overall approaches:
  - <u>Advanced statistical</u> methods coupled with <u>interactive data visualization</u> system.
- 50MB limit for data upload
  - ~300 samples for gene expression data with 20 000 genes

#### Single gene expression analysis



#### Gene expression meta-analysis



# New features

- Process <u>raw RNA-seq</u> data through our Galaxy server
- Enable users to save work state
- Additional functional profiling methods (GSEA)
- Enrichment networks for visualization of functional profiling results
- Larger collection of interaction networks (beyond PPI)
- Support for <u>3D visualization and VR</u>

# **Functional profiling**

- Overrepresentation analysis (ORA)
- Cut-off free functional enrichment analysis methods
  - Gene Set Enrichment Analysis (GSEA)
- Interactive <u>volcano</u> plot
- Advanced <u>diagnostic plots</u> for quality check purposes
- Dimension reduction: <u>3D PCA, t-sne</u>

#### Interactive volcano plot



## **Diagnostic plots**

0.5 -

0.0-

5.0

7.5

10.0

mean

count

15.0

12.5



PCA plot



### **Dimension reduction: PCA**



Synchronized view of score and loading plot

#### **GSEA Heatmap Clustering**



# **Network Analysis**

- Knowledgebase expansion including additional types of interaction:
  - Tissue specific PPI
  - Tissue and cell specific co-expression
  - TF and miRNA regulation
  - Gene-chemical, gene-drug, gene-disease networks
- <u>Prize Collecting Steiner Forest</u> (PCSF) algorithm to identify high confidence subnetwork relevant to the context.
- Additional <u>customization</u> features for 2D network visualization
- Added support for  $\underline{3D}$  and  $\underline{VR}$

#### Interaction databases



### 2D vs 3D



#### Implementation of 3D and VR



# Galaxy server for processing raw RNA-seq data

- Galaxy is a web-based platform for integrating various computational tools and resources into a cohesive workspace for comparative genomics.
- NetworkAnalyst Galaxy server
  - You can easily upload your own data
  - 34 workflows (i.e. customized pipelines for RNA-seq raw data processing) are provided for 17 species and 2 different RNA-seq alignment programs
  - Results and step-by-step analysis can be recorded (Data Libraries and Histories)

## Galaxy.networkanalyst.ca main page

#### Galaxy / NetworkAnalyst

#### Welcome to NetworkAnalyst Galaxy Server

This NetworkAnalyst Galaxy server is designed for mapping and quantification of raw RNA-seq data and it is integrated with NetworkAnalyst for statistical analysis and visualization.

#### How does it work?

If this is your first time to visit a Galaxy server and would like to gain more familiarity, please visit Galaxy 101 for an introduction of several fundamental concepts.

1) Account registration: you need to first register in order to upload files to our server.

2) Data upload: to upload your RNA-seq fastq.gz files using FTP (details).

3) Building collection: this is a very practical step when you have tens or more of RNA-seq samples to process (details).

4) Import workflow: you need to first specify alignment program (details), sequencing type and organism. Click "View workflow". Then, just click on 🛟 icon at the top right corner to import the workflow.

| Alignment program: Kallisto (fa                                                                                                | ter) 📀 Sequencing type: Paired-end 📀 | Organism: H. sapiens (hur         |                          |
|--------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|-----------------------------------|--------------------------|
| <ul><li>5) Run workflow: after importin</li><li>From the drop down menu attac</li><li>6) Download your gene count ta</li></ul> | Choose alignment                     | ou can visit the list of all impo | Click on "View workflow" |
|                                                                                                                                |                                      |                                   |                          |

### New user registration system



- Allows recurrent users to register and save their work state.
  - The projects are saved for one year upon creation
- Total number of 10 projects are allowed.



For more information, visit the **FAQs**, **Tutorials**, **Resources**, and **Contact** pages on <u>www.networkanalyst.ca</u>