Overview

1. Introduction
2. Pathway analysis
3. Pathways as tools for network analysis
4. Workshop setup and dataset

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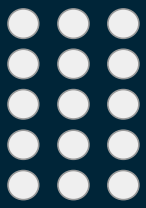
Introduction



Quantitative measurements

Isolated data points

Introduction

Comparative statistics

Isolated lists

Clustering

Isolated groups

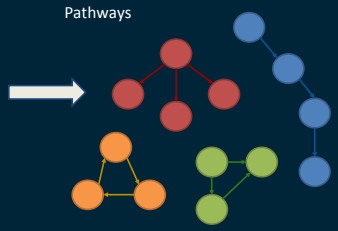
Gene sets

Isolated lists

Introduction

Functional organization

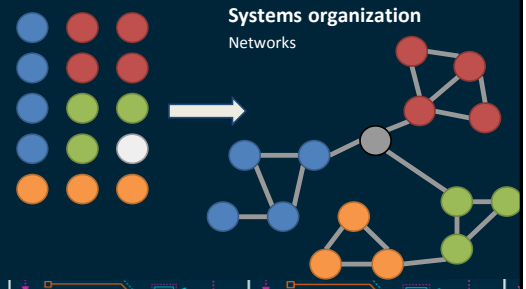
Pathways



Introduction

Systems organization

Networks



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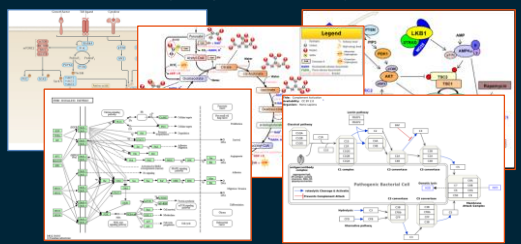
Biological pathways

Pathway diagrams are everywhere



Biological pathways

Pathways are found everywhere

⇩

Utility to biologists as conceptual models is obvious

⇩

If modeled properly - immensely useful for computational analysis and interpretation of large-scale experimental data

Why pathway analysis?

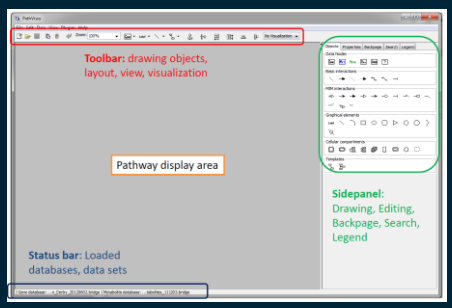
- "A picture is worth a thousand words."
  - Intuitive and simple
  - Puts data into a biological context
  - More efficient than looking up single gene information
  - Reduces complexity → several hundred pathways instead of thousands of genes
  - Higher explanatory power than a simple gene list
  - Visual representation

PathVisio

- Pathway editor, analysis and visualization toolbox
- Java standalone desktop application
- Functionality
  - Design biological pathway models ← Workshop 3
  - Visualize your experimental data
  - Perform pathway statistics

  } Workshop 1

PathVisio



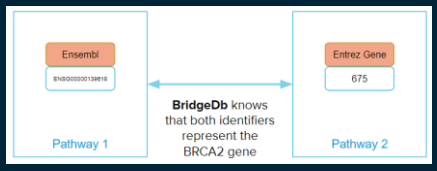
Pathway elements

- Data nodes
  - GeneProducts, Proteins, Metabolites, Pathways, etc.
- Interactions
  - Activation, Inhibition, Binding, Stimulation, etc.
- Literature references
  - For every element
- Annotations
  - External identifiers added for data nodes and interactions

Annotation of biological entities

- Challenge:
  - Many different biological online databases → different identifiers for the same biological element
  - Manual mapping of identifiers is very time-consuming and error-prone
- Solution:
  - BridgeDb framework → identifier mapping databases for gene products, metabolites and interactions

Annotation of biological entities

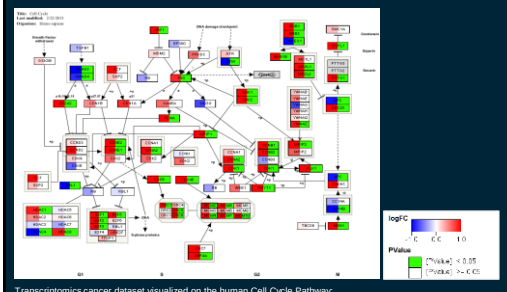


Data visualization

- Data can be visualized on data nodes, interactions
- Color gradient and color-rules

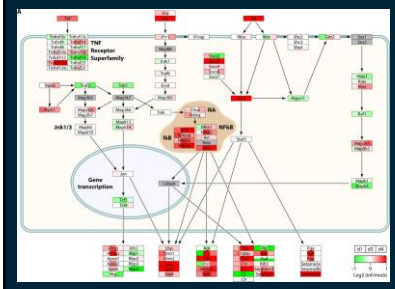
- Basic and advanced options
  - Multi-omics data visualization
  - Time-series data visualization
  - …

Data visualization



Transcriptomics cancer dataset visualized on the human Cell Cycle Pathway:
https://www.wikipathways.org/instance/WP179

Data visualization



Time-series data visualization (virus infection over multiple days)
Into the eye of the cytokine storm. https://doi.org/10.1128/MMBR.05015-11

Pathway statistics

Gene expression

Pathway database

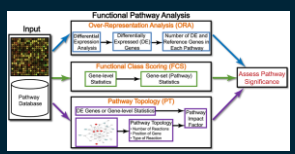
Differentially expressed genes
⇩
Z-score per pathway

PathVisio

Ranking of pathways based on Z-score

Pathway statistics

- Overrepresentation analysis (ORA)
- Functional Class Scoring (FCS)
- Pathway Topology Based (PT)



Khatri, P., Sirota, M., & Butte, A. J. (2012). Ten years of pathway analysis: current approaches and outstanding challenges. PLoS Computational Biology, 8(2), e1002375.

Pathway statistics

- **Overrepresentation analysis:**
  - Input list → e.g. significantly changed genes
  - Background list → e.g. all measured genes
  - Statistical test → e.g. Fisher's exact test (hypergeometric test)

$$\text{Z-score} = \frac{(r - n\frac{R}{N})}{\sqrt{n\frac{R}{N}(1-\frac{R}{N})(1-\frac{n-1}{N-1})}}$$

- Z-Score is calculated for each pathway
  - Results in ranked list of pathways
- Four variables in the formula: N, R, n, r

Z-Score ORA

$$\text{Z-score} = \frac{(r - n\frac{R}{N})}{\sqrt{n\frac{R}{N}(1-\frac{R}{N})(1-\frac{n-1}{N-1})}}$$

N = 25

background list (total number of measured genes in experiment)

Z-Score ORA

$$\text{Z-score} = \frac{(r - n\frac{R}{N})}{\sqrt{n\frac{R}{N}(1-\frac{R}{N})(1-\frac{n-1}{N-1})}}$$

N = 25

background list (total number of measured genes in experiment)

R = 9

input list (number of changed genes in experiment)

Z-Score ORA

$$\text{Z-score} = \frac{(r - n\frac{R}{N})}{\sqrt{n\frac{R}{N}(1-\frac{R}{N})(1-\frac{n-1}{N-1})}}$$

N = 25
background list (total number of measured genes in experiment)

R = 9
input list (number of changed genes in experiment)

n = 9
total number of genes in pathway

Z-Score ORA

$$\text{Z-score} = \frac{(r - n\frac{R}{N})}{\sqrt{n\frac{R}{N}(1-\frac{R}{N})(1-\frac{n-1}{N-1})}}$$

N = 25
background list (total number of measured genes in experiment)

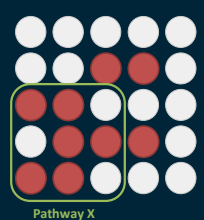
R = 9
input list (number of changed genes in experiment)

n = 9
total number of genes in pathway

r = 6
number of changed genes in pathway

Z-Score ORA

$$\text{Z-score} = \frac{(r - n\frac{R}{N})}{\sqrt{n\frac{R}{N}(1-\frac{R}{N})(1-\frac{n-1}{N-1})}}$$



N = 25
background list (total number of measured genes in experiment)

R = 9
input list (number of changed genes in experiment)

n = 9
total number of genes in pathway

r = 6
number of changed genes in pathway

Z-Score for pathway X = 2.347

Z-Score ORA

Be aware!!

- **What does the Z-Score tell you?**
  - Z-Score > 1.96
    - Significantly more genes than expected are changed in the pathway → altered pathways in the experiment (different between the groups)
  - **Z-Score = 0**
    - Distribution of changed genes in the pathway is the same as in the complete dataset
  - **Z-Score = < -1.96**
    - Significantly less genes than expected are changed in the pathway → very stable pathway (not affected in experiment)

Z-Score ORA

- **Be aware!**
  - ORA and FCS **do not** take pathway topology into account!
  - You don't know yet where the changes occur in the pathway.
  - Always look at the pathway diagrams and study the changes to make the right conclusions!

Pathway analysis methods

- **Overrepresentation analysis:**
  - Ranked list of pathways

Up-regulated pathways (log2FC > 2, p-value < 0.05)	Z-score	Perm. p-value
Cell Cycle	6.12	0.001
G1 to S cell cycle control	4.26	0.002
Synaptic Vesicle Pathway	3.89	0.001
DNA Damage Response	3.88	0.002
ATM Signaling Pathway	3.80	0.001

Down-regulated pathways (log2FC < -2, p-value < 0.05)	Z-score	Perm. p-value
Complement and Coagulation Cascades	5.87	0.001
Complement Activation	5.84	0.001
Adipogenesis	5.49	0.001
Differentiation of white and brown adipocyte	5.44	0.001
Triacylglyceride Synthesis	4.53	0.001

Same method is also used for Gene Ontology analysis

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WikiPathways app

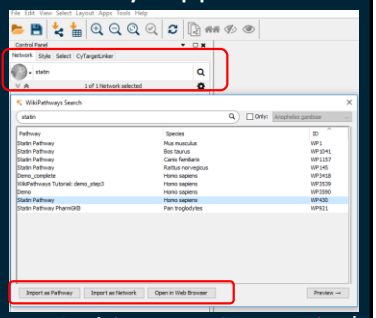
- WikiPathways app
  - Load pathways as networks in Cytoscape
  - Use online database query in Cytoscape or open local GPML file
  - apps.cytoscape.org/apps/wikipathways

WikiPathways app

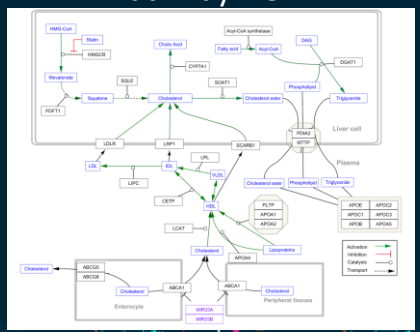
- App supports two views:
  - Pathway view
    - complete visual appearance
    - ideal for data visualization
  - Network view
    - simplified networks without any of the graphical elements of the original pathway diagram
    - ideal for topological analyses, network merging and automatic layout

WikiPathways app

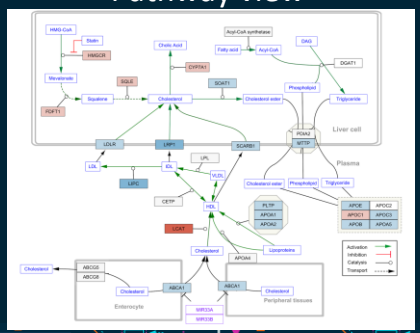
Cytoscape 3.6.0+
WikiPathways app 3.3.0+



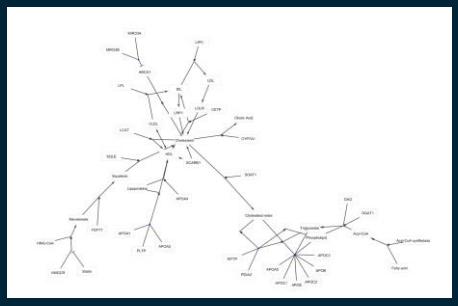
Pathway view



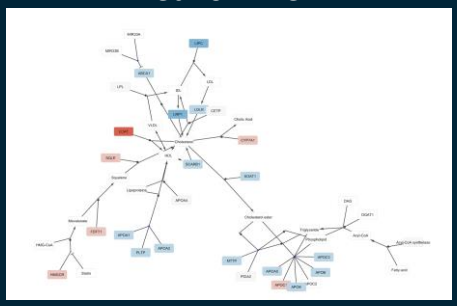
Pathway view

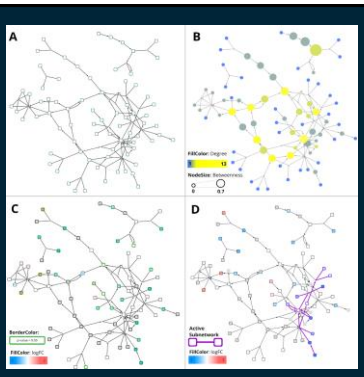


Network view

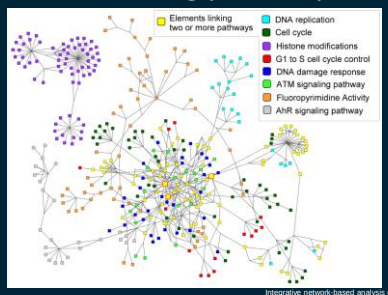


Network view





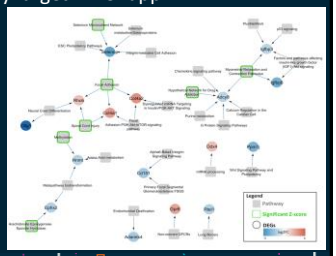
Connecting pathways



Kutmon, M. et al.
Integrative network-based analysis of mRNA and microRNA expression in 1,25-dihydroxyvitamin D3-treated cancer cells
https://doi.org/10.1007/s12263-015-0484-0

Pathway associations

- Extend molecular networks with pathway associations using CyTargetLinker app



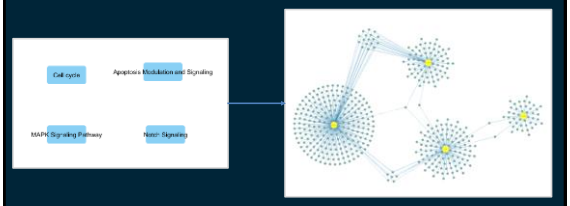
CyTargetLinker: a flexible solution for network extension
https://doi.org/10.12688/f1000research.14613.1

Pathway associations

- Visualize gene overlap between significant pathways with CyTargetLinker

Pathway associations

- Visualize gene overlap between significant pathways with CyTargetLinker



Cytoscape automation

- Available since the last major release (3.6.0+)
- Execute commands from within R or Python
- WikiPathways and CyTargetLinker app both have automation interface

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Workshop setup

- Step-by-step instructions
- Required data provided in a zip file
- https://github.com/PathVisio/tutorials → 2018-WP-Summit

- Feel free to ask questions at any point
- Three instructors will help you out


Tina


Kristina


Denise

Workshop setup

- Data visualization on pathways
- Identifying affected pathways
- Using pathways as networks
- Extension of pathways with regulatory information
- Pathway-gene associations

Dataset


Data retrieved from lung cancer subjects in TCGA

Primary tumor tissue sample

Solid tissue normal sample

RNA-sequencing

RNA-sequencing

Gene expression Raw counts

Gene expression Raw counts

Data pre-processing and statistical analysis with edgeR in R

Differential gene expression Comparison between groups

Dataset

GeneID	GeneName	log2FC	P.Value	adj.P.Value
ENSG00000230657	PRB4	13.27397592	0.003920605	0.097861917

- **GeneID** → identifier in online database
- **GeneName** → official gene symbol
- **log2FC** → log2 of fold change (ratio of the differences between cancer and healthy samples)
- **P.Value** → significance level of comparison
- **adj.P.Value** → corrected P.Value for multiple testing

Dataset

- log2FC
  - Is the gene more or less expressed in the cancer tissue sample compared to the normal tissue sample?

Negative log2FC
downregulation in cancer sample

Positive log2FC
upregulation in cancer sample

Example
log2FC = 1 → fold change = 2^1 = 2
gene is twice as expression cancer compared to healthy tissue sample

Why do we use the log2?
easier for interpretation – 0 = no chance, 1 is upregulation, -1 is downregulation

Questions

https://github.com/PathVisio/tutorials → 2018-WP-Summit

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