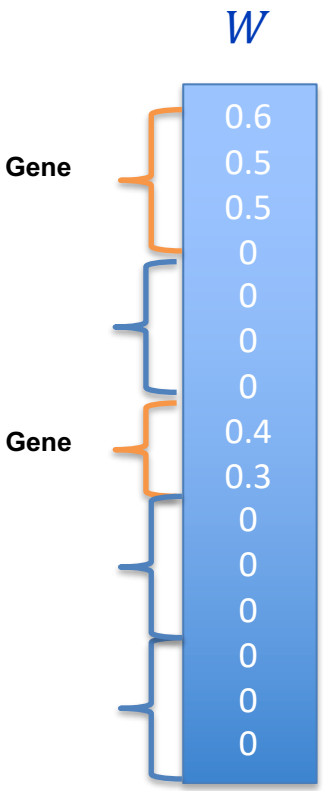
Part 1: overview

- Principal Component Analysis (PCA)
- Partial Least Squares (PLS)
- Two-way Orthogonal PLS (O2PLS)
- Post-hoc analyses using external databases
  - String-DB and gene enrichment

Background

- Data integration with OmicsPLS
- Using the methylation and glycomics datasets, relevant genes estimates
- These genes are important to consider



- **What do they tell us?**

Genes and their meaning

- Vast amount of information available on genes, DNA mutations, proteins, other biomolecules
  - Functionality, location, gene sets, interactions, etc
- Many bioinformatics databases organize this information
- Use these databases to understand significance of the selected genes

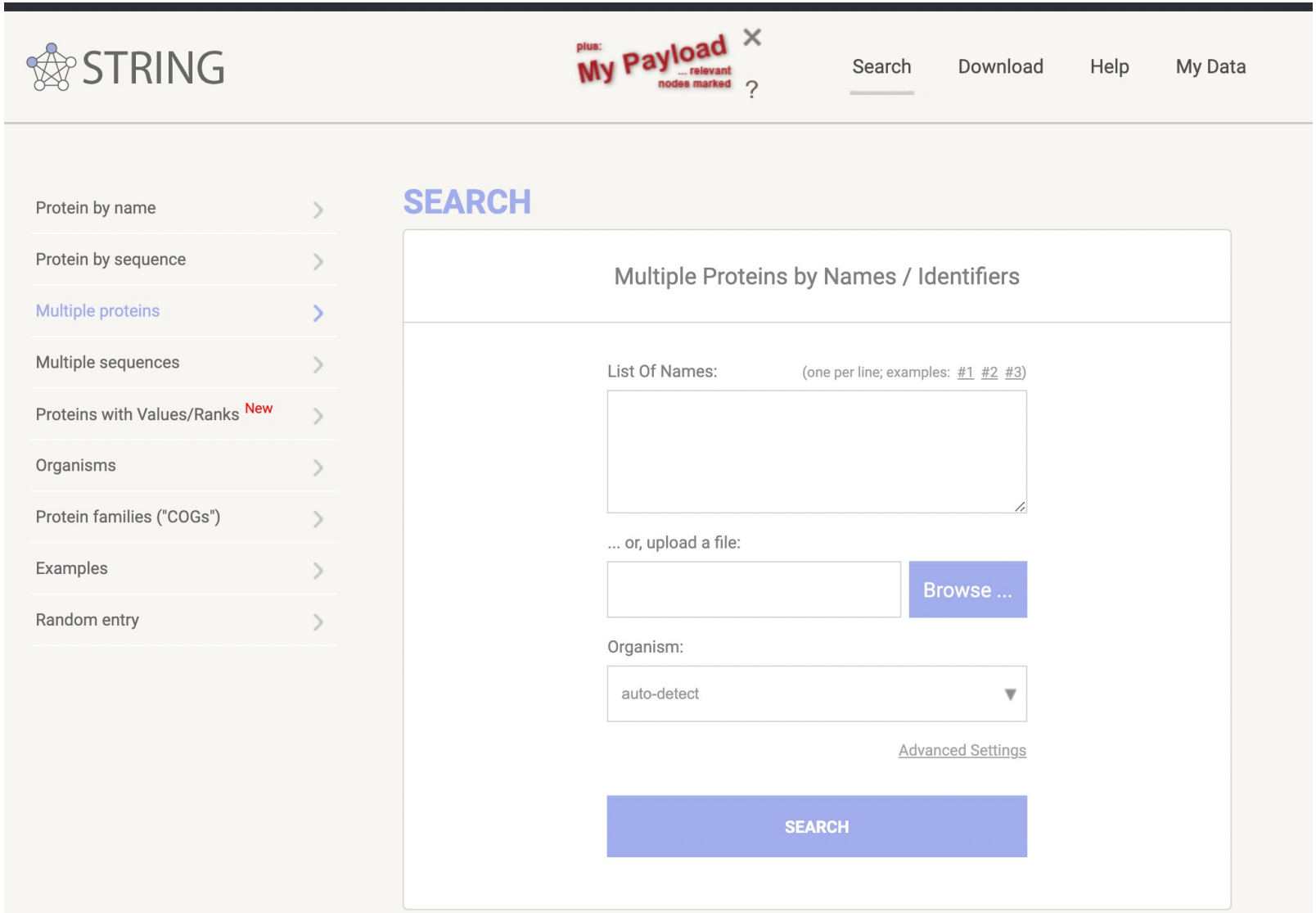
Bioinformatics database: String-DB

- We will focus on String-DB
  - A collection of gene-gene interactions based on several sources of evidence
  - https://string-db.org/
  - Also available as an R package
    - Connects to an online server

String-DB: entering input genes

1. Open website, go to “multiple proteins”
   - Try an example list, click on #1
   - Or: Input your list of names, and organism
2. Review the gene ID mapping and continue
3. An interaction network appears
   - Interactive, so almost everything is clickable
4. Review basic settings to customize the network
   - Most important: Interaction source and confidence
   - Typically: exclude text-mining; set confidence = 700

String-DB: input and output



String-DB: input and output

Version: 11.0 LOGIN | REGISTER

STRING

plus: My Payload ... relevant nodes marked ?

Search Download Help My Data

The following proteins in *Escherichia coli K12 MG1655* appear to match your input.
Please review the list, then click 'Continue' to proceed.

<- BACK | MAPPING | CONTINUE ->

1) 'trpA':

☑ trpA - Tryptophan synthase alpha chain; The alpha subunit is responsible for the aldol cleavage of indoleglycerol phosphate to indole and glyceraldehyde 3-phosphate; Belongs to the TrpA family

2) 'trpB':

☑ trpB - Tryptophan synthase beta chain; The beta subunit is responsible for the synthesis of L- tryptophan from indole and L-serine; Belongs to the TrpB family

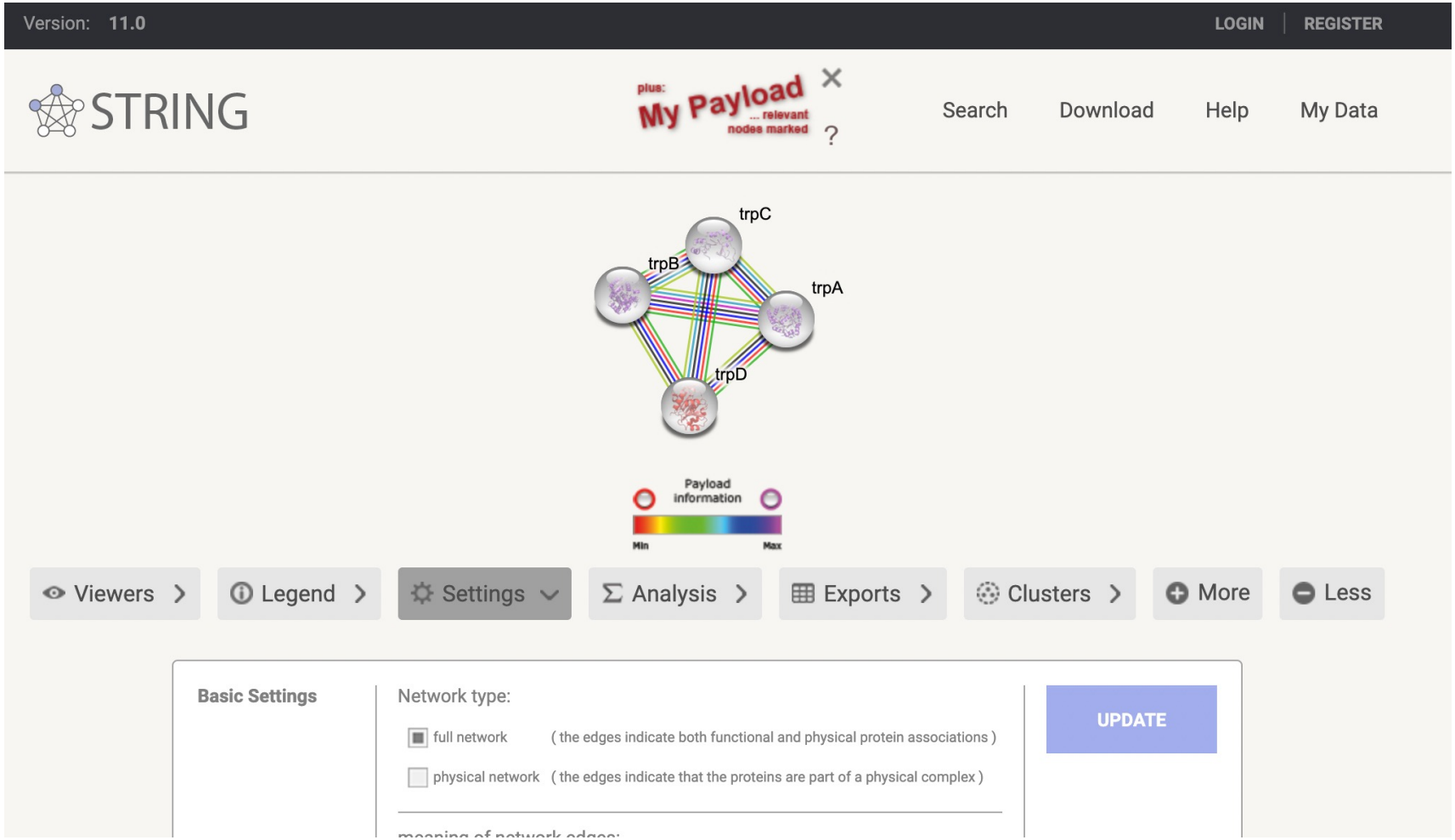
3) 'TRPC_ECOLI':

☑ trpC - Tryptophan biosynthesis protein TrpCF; Bifunctional enzyme that catalyzes two sequential steps of tryptophan biosynthetic pathway. The first reaction is catalyzed by the isomerase, coded by the TrpF domain; the second reaction is catalyzed by the synthase, coded by the TrpC domain *[a.k.a. trpF, b1262, JW1254, TRPC_ECOLI]*

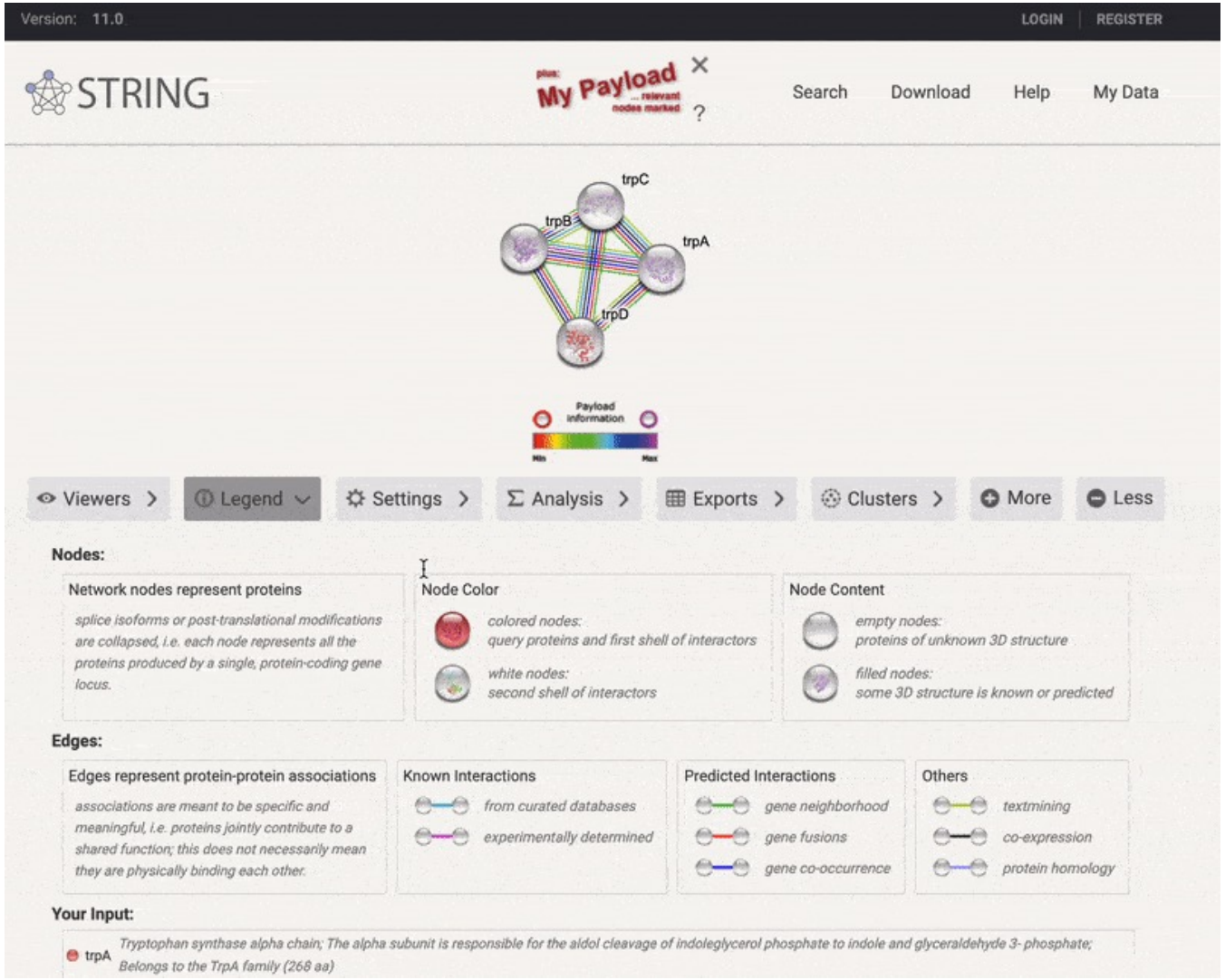
4) 'b1263':

☑ trpD - Bifunctional protein TrpGD; Part of a heterotetrameric complex that catalyzes the two-step biosynthesis of anthranilate, an intermediate in the biosynthesis of L-tryptophan. In the first step, the glutamine- binding beta subunit (TrpG) of anthranilate synthase (AS) provides the glutamine amidotransferase activity which generates ammonia as a substrate that, along with chorismate, is used in the second step, catalyzed by the large alpha subunit of AS (TrpE) to produce anthranilate. In the absence of TrpG, TrpE can synthesize anthranilate directly from chorismate and high concentrations [...] *[a.k.a. trpGD, b1263, JW1255]*

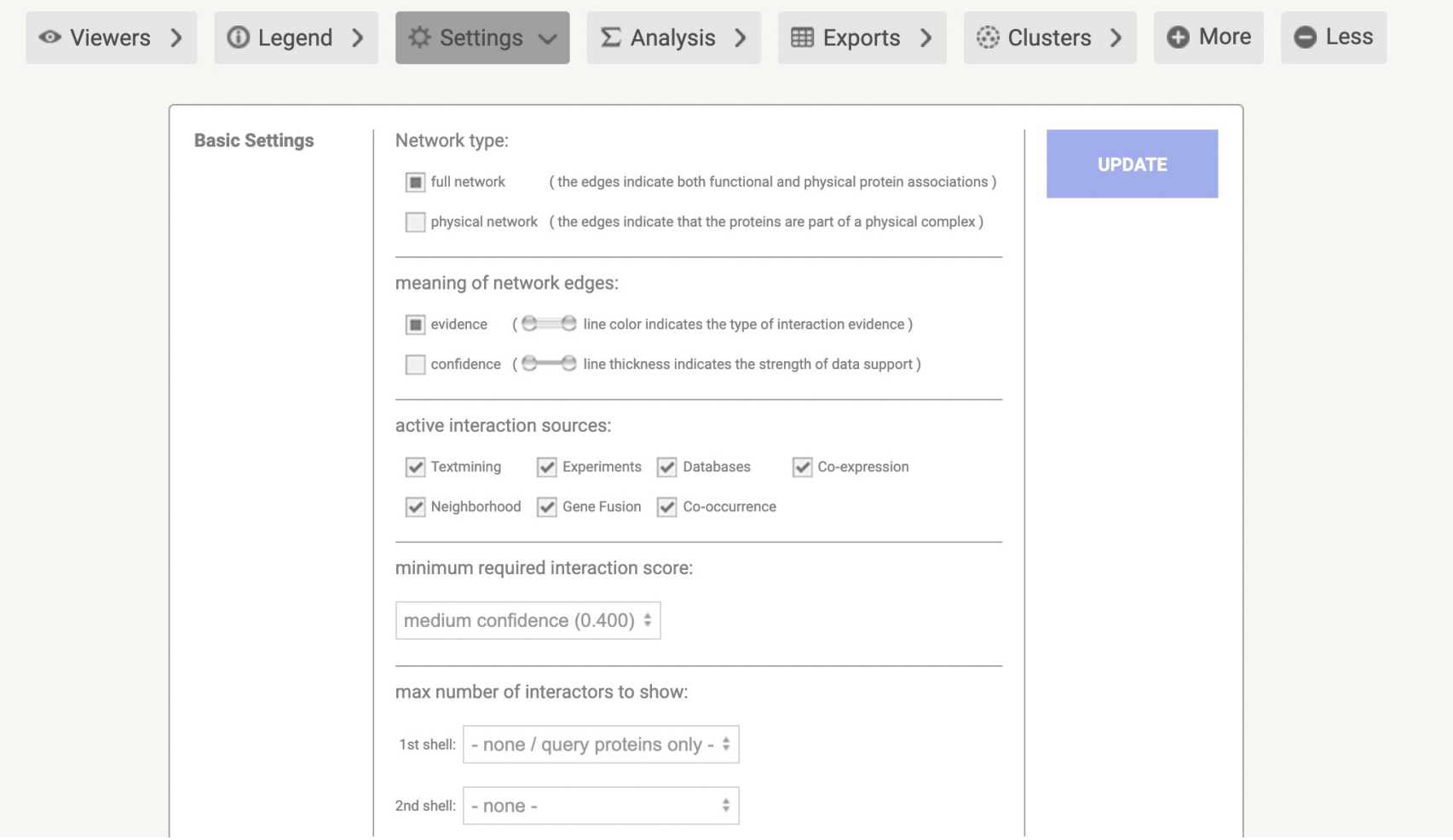
String-DB: input and output



String-DB: output



String-DB: input and output



String-DB: output

- String network
  - Network of interactions between genes
  - 7 metrics that form a combined score
  - How likely is it that this link is biologically relevant?
- Enrichment analysis
  - Are certain pre-defined gene categories overrepresented in our gene list?
  - Many types of categories (biophysical, experimental, text-mining, location)

String-DB: input and output

Viewers | Legend | Settings | Analysis | Exports | Clusters | More | Less

Network Stats

number of nodes:	4	expected number of edges:	1
number of edges:	6	PPI enrichment p-value:	1.75e-05
average node degree:	3	*your network has significantly more interactions than expected (what does that mean?)*	
avg. local clustering coefficient:	1		

Functional enrichments in your network

explain columns

Biological Process (Gene Ontology)

GO-term	*description*	*count in network*	*strength*	*false discovery rate*
GO:0000162	tryptophan biosynthetic process	4 of 9	2.66	2.66e-09

Molecular Function (Gene Ontology)

GO-term	*description*	*count in network*	*strength*	*false discovery rate*
GO:0004834	tryptophan synthase activity	2 of 2	3.01	0.00013
GO:0016830	carbon-carbon lyase activity	2 of 71	1.46	0.0167
GO:0016829	lyase activity	4 of 195	1.33	0.00013

Reference publications (PubMed)

publication	*(year) title*	*count in network*	*strength*	*false discovery rate*
PMID:2838460	(1988) Cosmid cloning of five Zymomonas trp genes by com...	4 of 4	3.01	5.33e-09
PMID:2113923	(1990) Nucleotide sequences and genomic constitution of fiv...	4 of 4	3.01	5.33e-09
PMID:6276387	(1982) Yeast gene TRP5: structure, function, regulation.	3 of 3	3.01	3.14e-07
PMID:403178	(1977) Immunochemical comparison of phosphoribosylanthr...	3 of 3	3.01	3.14e-07
PMID:3146017	(1988) Cloning of the trp genes from the archaebacterium M...	3 of 3	3.01	3.14e-07

(more ...)

local network cluster (STRING)

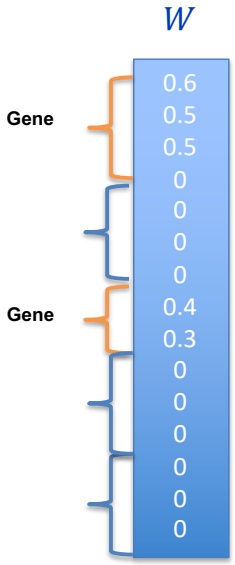
cluster	*description*	*count in network*	*strength*	*false discovery rate*
CL:1345	Tryptophan biosynthesis	4 of 5	2.92	7.28e-11

KEGG Pathways

pathway	*description*	*count in network*	*strength*	*false discovery rate*
eco00400	Phenylalanine, tyrosine and tryptophan biosynthesis	4 of 21	2.29	6.26e-09
eco00260	Glycine, serine and threonine metabolism	2 of 37	1.75	0.00062
eco01230	Biosynthesis of amino acids	4 of 116	1.55	2.03e-06
eco01130	Biosynthesis of antibiotics	4 of 209	1.3	1.38e-05
eco01110	Biosynthesis of secondary metabolites	4 of 301	1.14	4.38e-05

(more ...)

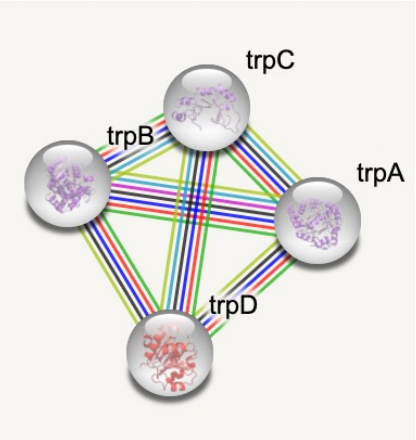
Omics analysis workflow

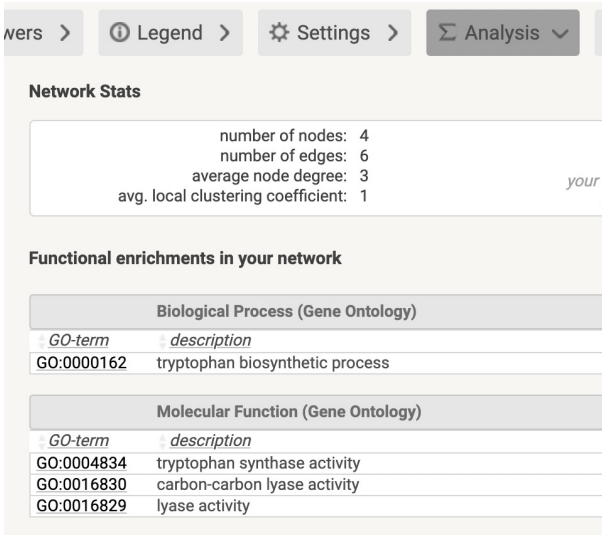


Estimate important genes/proteins

Input in String-DB

Interpret clusters and annotations





wers > Legend > Settings > Σ Analysis

Network Stats

number of nodes: 4
number of edges: 6
average node degree: 3
avg. local clustering coefficient: 1

Functional enrichments in your network

Biological Process (Gene Ontology)	
GO-term	description
GO:0000162	tryptophan biosynthetic process

Molecular Function (Gene Ontology)	
GO-term	description
GO:0004834	tryptophan synthase activity
GO:0016830	carbon-carbon lyase activity
GO:0016829	lyase activity

Other gene enrichment databases available

We will also consider DisGeNet

- A database of gene-disease associations
- Contains information about potential links between gene sets and diseases
- https://www.disgenet.org/
- Also available as an R package
  - Nowadays need to register an account

Exercise: Down syndrome case study

- Apply some of the techniques to investigate the relation between Down syndrome and multi-omics data
- Use statistical measures and bioinformatics databases to interpret the results

Part 1: overview

- Principal Component Analysis (PCA)
- Partial Least Squares (PLS)
- Two-way Orthogonal PLS (O2PLS)
- Post-hoc analyses using external databases
  - String-DB and gene enrichment