EXpander

EXPression ANalyzer and DisplayER

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Ron Shamir's Computational Genomics Group

Rani Elkon's Group

Schedule

- Data, preprocessing, grouping (10:15-10:45)
- Hands-on part I (10:45-11:00)
- Coffee Break (11:00 – 11:15)
- Grouping analysis (11:15-11:40)
- Hands-on part II (11:40-13:00)
- Coffee Break (13:00 – 13:15)
- Enrichment analysis (13:15-13:40)
- Hands-on part III (11:40-13:00)
- Coffee Break (13:00 – 13:10)
- Expander new features – GSEA/ChIP-Seq/RNA-Seq (13:10-13:30)
- Hands-on part IV (13:30-14:00)

- **EXPANDER – an integrative package for analysis of gene expression and NGS data**

- **Built-in support for 18 organisms:**

  human, mouse, rat, chicken, fly, zebrafish, C.elegans, yeast (s. cereviciae and s. pombe), arabidopsis, tomato, listeria, leishmania, E. coli (two strains), aspargillus, rice. And v.vinifera (grape)

- Demonstration on human CAL51 cell line experiments*:
  - RNA-Seq data, which contains expression profiles measured in several time points after IR-induction.
  - P53 ChIP-Seq data after 2 hours of IR-induction.

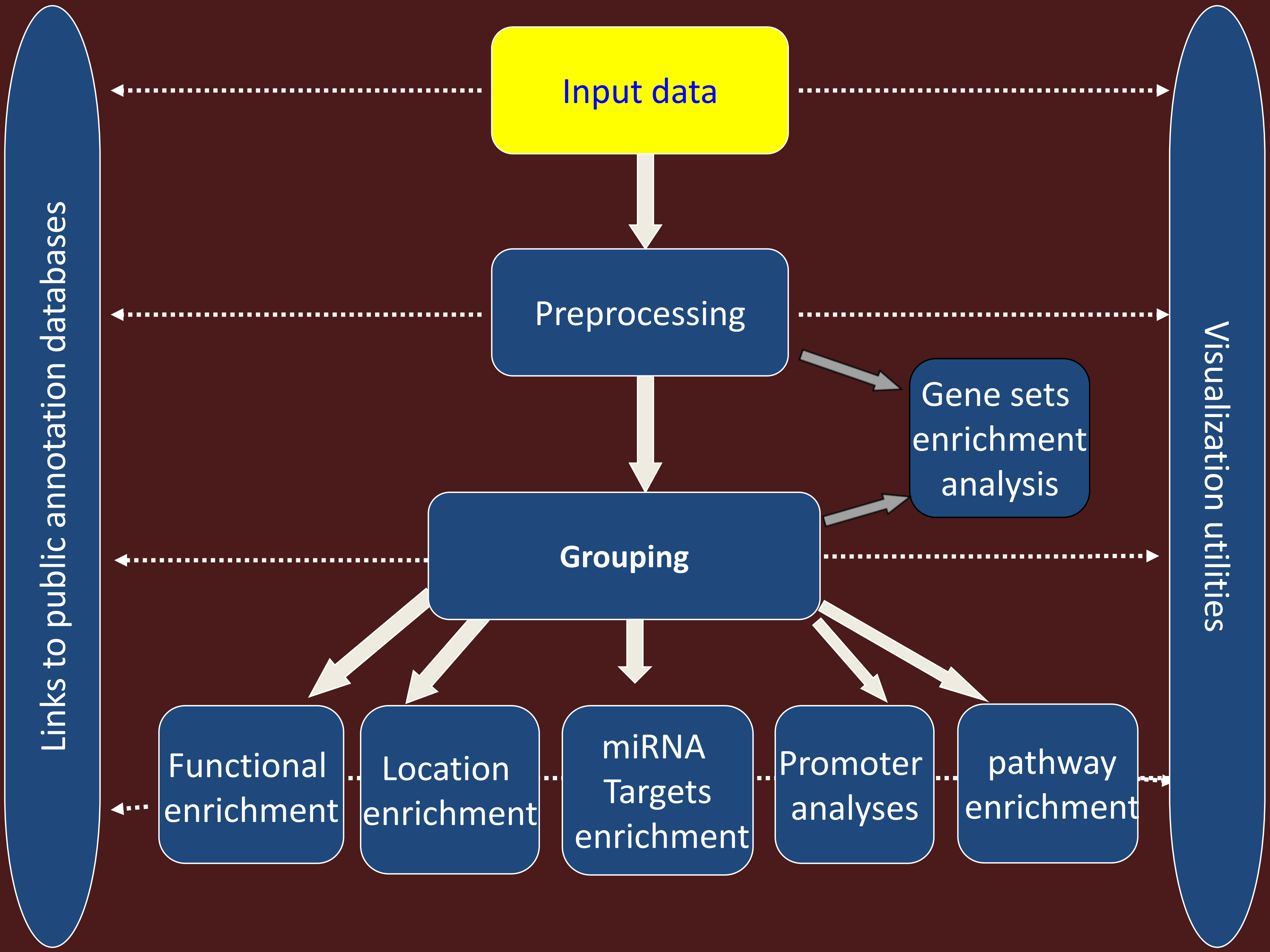
*Data from Rashi-Elkeles, Warnatz and Elkon et al, 2014, Science Signaling, DOI: 10.1126/scisignal.2005032

EXPANDER status

- 691 citations since 2003
- 67 citations since 2015
- 16,544 downloads since 2003
- 1427 since 2015

What can it do?

- ❑ **Low level analysis**
  - Data adjustments (missing values, merging, divide by base, log)
  - Normalization
  - Probes & condition filtering
- ❑ **High level analysis**
  - Group detection (supervised clustering, differential expression, clustering, bi-clustering, network based grouping).
  - Ascribing biological meaning to patterns via enrichment analysis



Links to public annotation databases

Input data

Preprocessing

Gene sets enrichment analysis

Grouping

Functional enrichment

Location enrichment

miRNA Targets enrichment

Promoter analyses

pathway enrichment

Visualization utilities

EXPANDER – Data

- Expression matrix (probe-row; condition-column)
  - One-channel data (e.g., Affymetrix)
  - Dual-channel data, in which data is log R/G (e.g. cDNA microarrays)
  - ‘.cel’ files
  - RNA-Seq counts OR absolute/relative intensities data
- ChIP-Seq data: **in BED or GFF3 formats**
- ID conversion file: maps probes to genes
- Gene groups data: defines gene groups
- Gene ranks data: defines gene ranking for GSEA
- Network information (e.g. PPI network) - .sif format

First steps with the data – load, define, preprocess

- **Load dialog box , “Data menu”, “Preprocessing menu”**
- Data definitions
    - Defining condition subsets
    - Data type & scale (log)
    - Define genes of interest

- Data Adjustments
    - Missing value estimation (KNN or arbitrary)
    - Flooring
    - Condition reordering
    - Merging conditions
    - Merging probes by gene IDs
    - Assigning genes to ChIP-Seq peaks
    - Divide by base
    - Log data (base 2)

Data preprocessing

- **Normalization=** removal of systematic biases
  - **Quantile** = equalizes distributions
  - **Lowess** (locally weighted scatter plot smoothing) = a non linear regression to a base array
- Visualizations to inspect normalization:
  - box plots
  - Scatter plots (simple and M vs. A)

$M=\log_2(A1/A2)$

$A = 0.5*\log_2(A1*A2)$

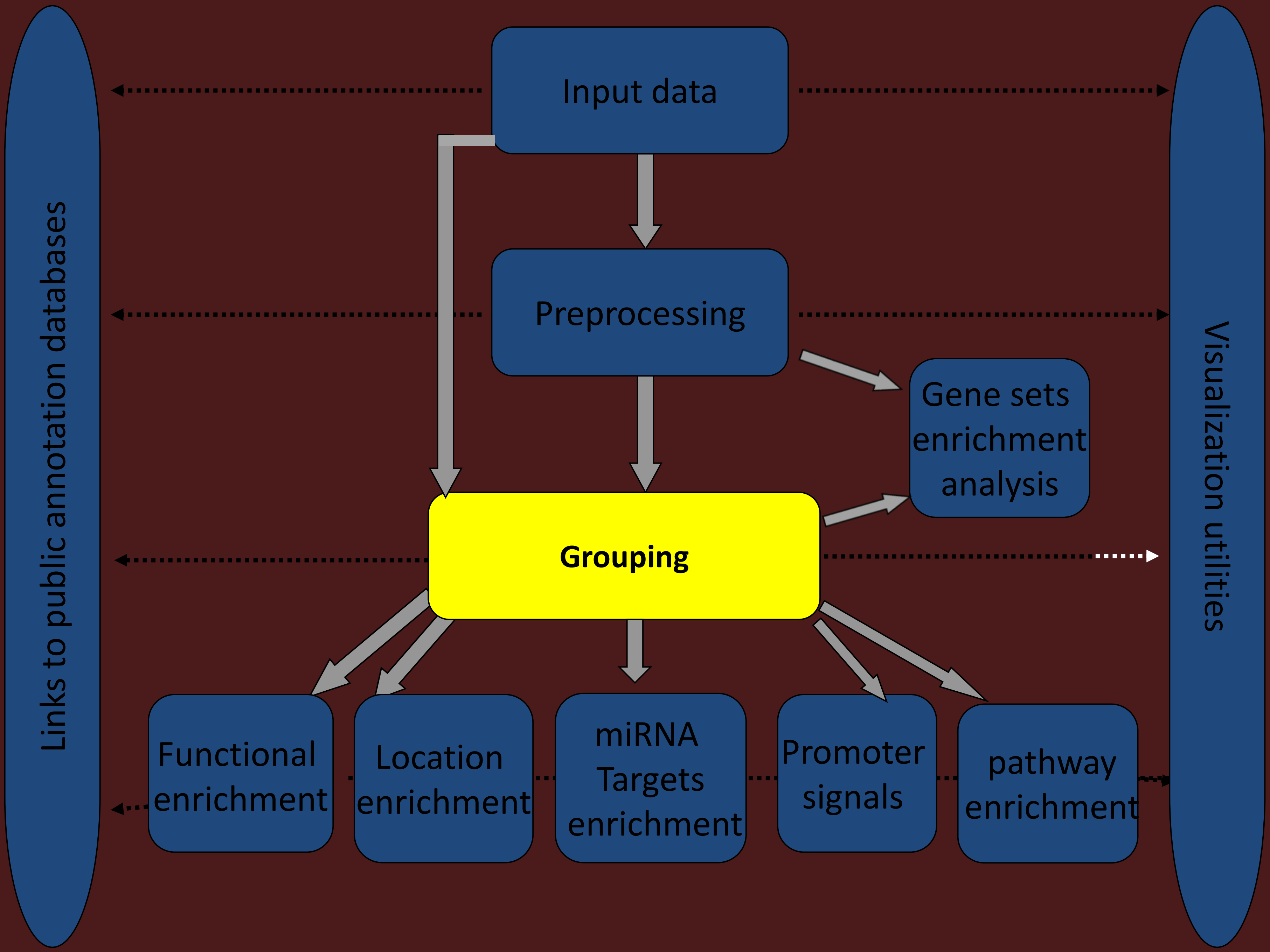
Data preprocessing

- Probe filtering

  Focus downstream analysis on the set of “responding genes”

  - Fold-Change
  - Variation
  - Statistical tests: T-test, SAM (Significance Analysis of Microarrays)
  - It is possible to define “VIP genes”.
- Standardization : Mean=0, STD=1 (visualization)
- Condition filtering
- Order of operations

Hands-on (1-2)



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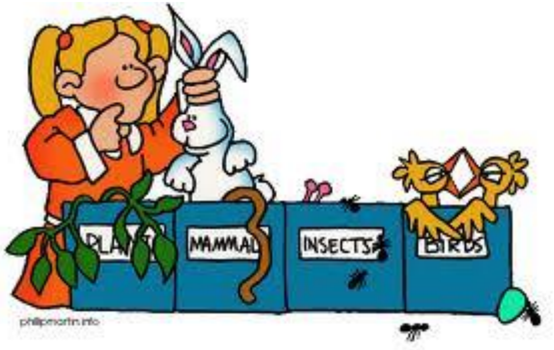
Supervised Grouping



- Differential expression:
  a) Under normality assumption: t-test, SAM
  b) No normality assumption (RNA Seq data): Wilcoxon rank sum test , Negative binomial (edgeR/DESeq2)

- Similarity group (correlation to a selected probe/gene)

- Rule based grouping (define a pattern)

Unsupervised grouping - cluster Analysis

Partition into distinct groups, each with a particular expression pattern

- *co-expression → co-function*
- *co-expression → co-regulation*

Partition the genes attempts to maximize:

- **Homogeneity** within clusters
- **Separation** between clusters

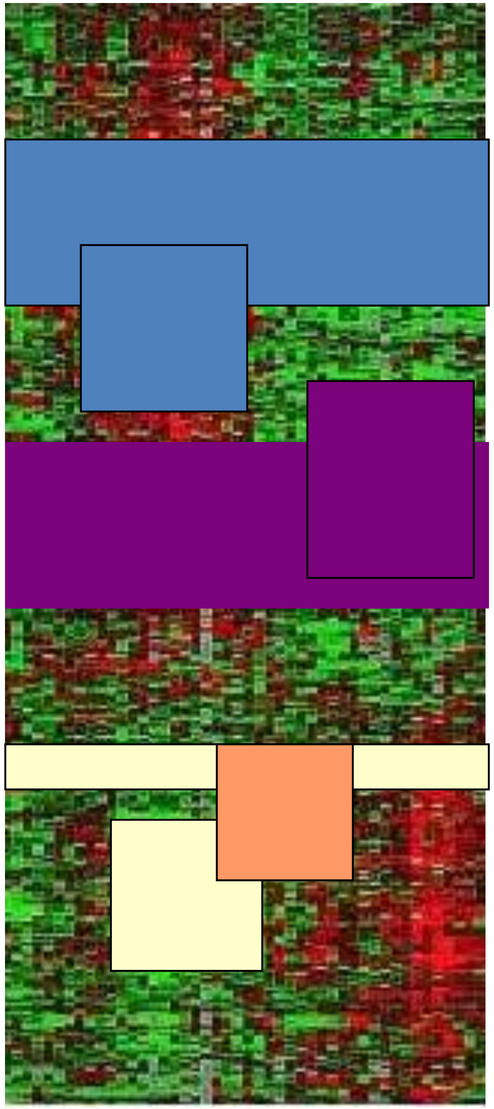
Cluster Analysis within Expander

- Implemented algorithms:
  - CLICK, K-means, SOM, Hierarchical
- Visualization:
  - Mean expression patterns
  - Heat-maps
  - Chromosomal positions
  - Network sub-graph **(Cytoscape integration)**
  - PCA
  - Clustered heat map

Biclustering

Clustering seeks global partition according to similarity across **ALL** conditions >> becomes too restrictive on large datasets.

- Relevant knowledge can be revealed by identifying genes with common pattern across a subset of the conditions
- Novel algorithmic approach is needed: *Biclustering*



Biclustering II

* *Bicluster* = subset of genes with similar behavior under a subset of conditions

Computationally challenging: has to consider many combinations

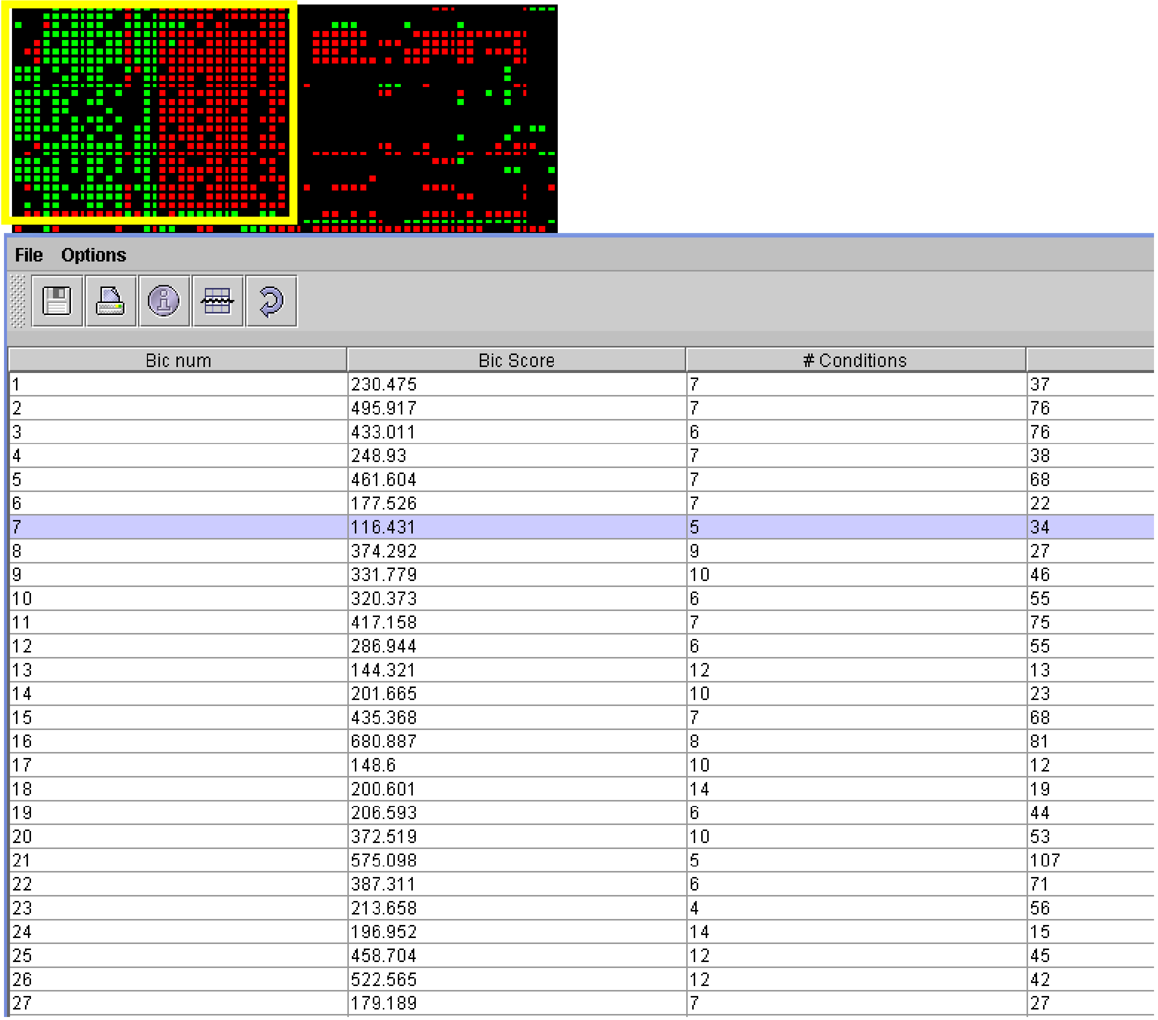
Biclustering methods in EXPANDER:
ISA (Iterative Signature Algorithm) - Ihmels et.al Nat Genet 2002

SAMBA = Statistical Algorithmic Method for Bicluster Analysis (A. Tanay, R. Sharan, R. Shamir *RECOMB 02)*

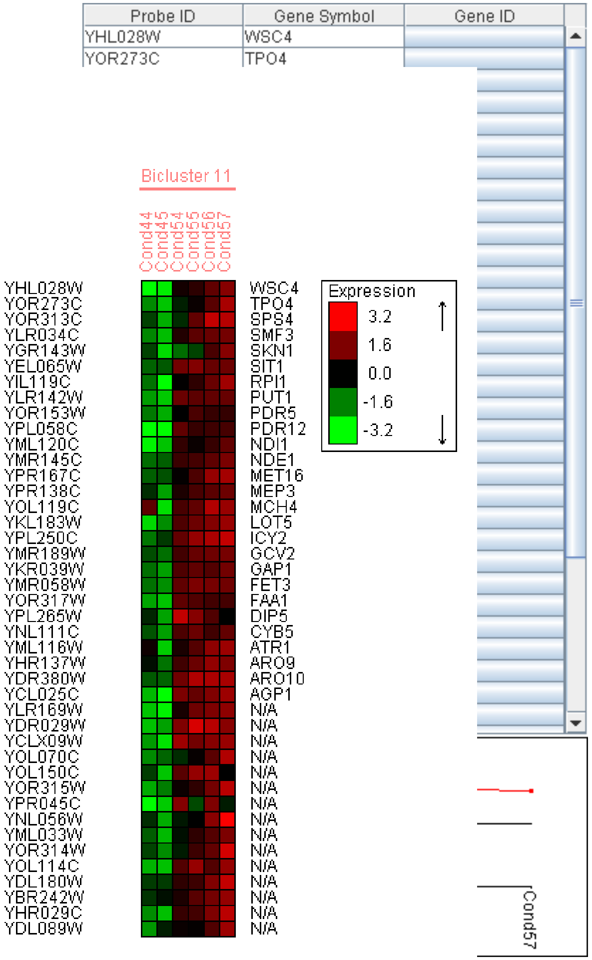
Drawbacks/ limitations:

- Useful only for over 20 conditions
- Parameters
- How to asses the quality of Bi-clusters

Biclustering Visualization



Bic num	Bic Score	# Conditions	
1	230.475	7	37
2	495.917	7	76
3	433.011	6	76
4	248.93	7	38
5	461.604	7	68
6	177.526	7	22
7	116.431	5	34
8	374.292	9	27
9	331.779	10	46
10	320.373	6	55
11	417.158	7	75
12	286.944	6	55
13	144.321	12	13
14	201.665	10	23
15	435.368	7	68
16	680.887	8	81
17	148.6	10	12
18	200.601	14	19
19	206.593	6	44
20	372.519	10	53
21	575.098	5	107
22	387.311	6	71
23	213.658	4	56
24	196.952	14	15
25	458.704	12	45
26	522.565	12	42
27	179.189	7	27



Probe ID	Gene Symbol	Gene ID
YHL028W	WSC4	
YOR273C	TPO4	

YHL028W	WSC4
YOR273C	TPO4
YOR313C	SPS4
YLR034C	SMF3
YGR143W	SKN1
YEL065W	SIT1
YIL119C	RPI1
YLR142W	PUT1
YOR153W	PDR5
YPL058C	PDR12
YML120C	NDI1
YMR145C	NDE1
YPR167C	MET16
YPR138C	MEP3
YOL119C	MCH4
YKL183W	LOT5
YPL250C	ICY2
YMR189W	GCV2
YKR039W	GAP1
YMR058W	FET3
YOR317W	FAA1
YPL265W	DIP5
YNL111C	CYB5
YML116W	ATR1
YHR137W	ARO9
YDR380W	ARO10
YCL025C	AGP1
YLR169W	N/A
YDR029W	N/A
YCLX09W	N/A
YOL070C	N/A
YOL150C	N/A
YOR315W	N/A
YPR045C	N/A
YNL056W	N/A
YML033W	N/A
YOR314W	N/A
YOL114C	N/A
YDL180W	N/A
YBR242W	N/A
YHR029C	N/A
YDL089W	N/A

Network based grouping

Goal: to identify modules using gene expression data and interaction networks

GE data + Interactions file (.sif)

MATISSE (**M**odule **A**nalysis via **T**opology of **I**nteractions and **S**imilarity **SE**ts)
I. Ulitsky and R. Shamir. *BMC Systems Biology* 2007)

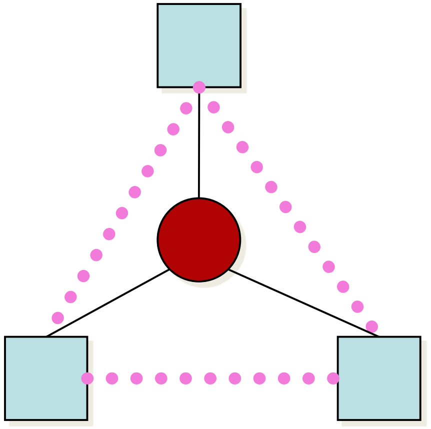
DEGAS (**D**ysr**E**gulated **G**ene set **A**nalysis via **S**ubnetworks)
I. Ulitsky et. Al. Plos One 2010

Motivation

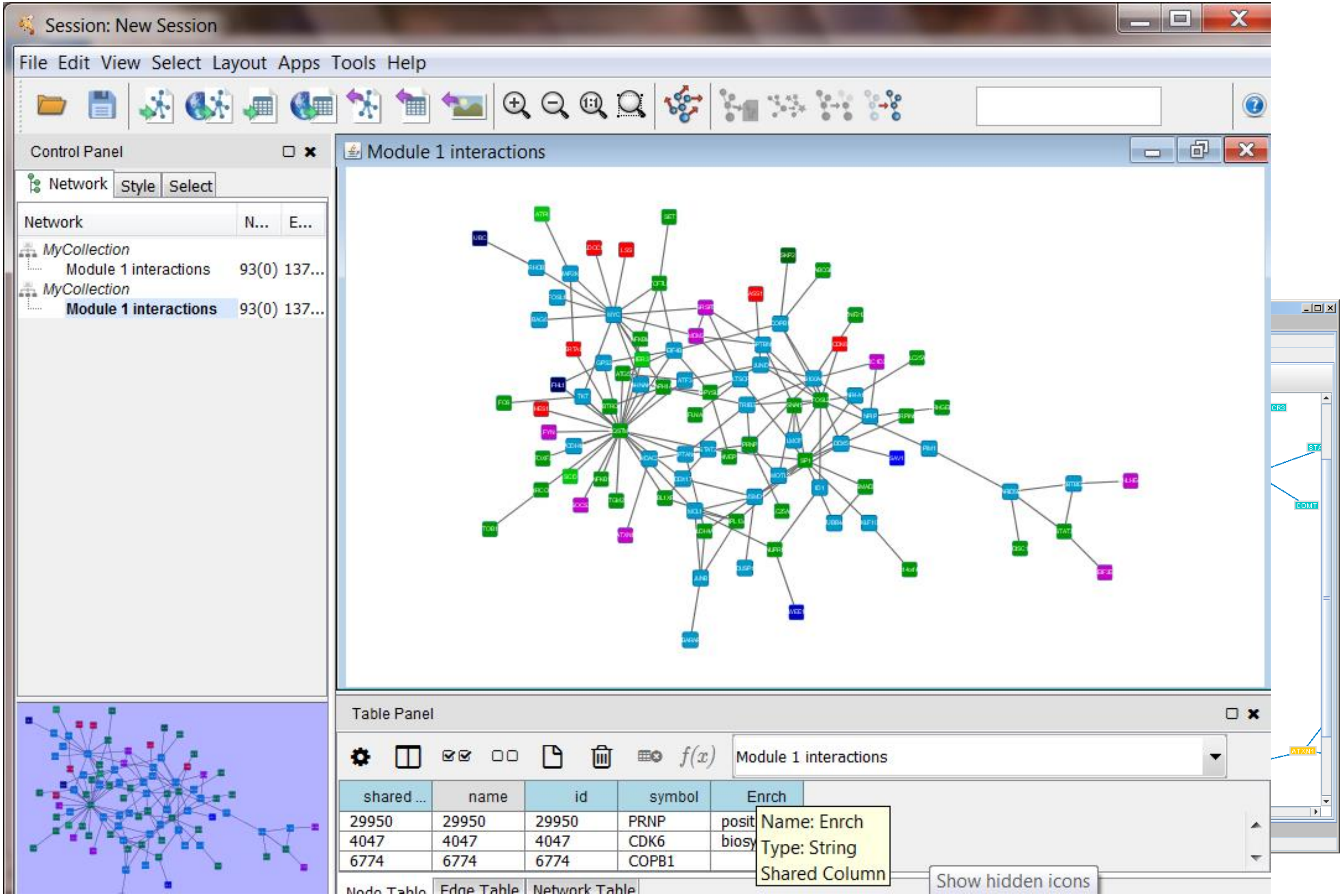
- Detect **functional modules**, i.e. groups of
  - interacting proteins
  - co-expressed genes
- Integrative analysis - can identify weaker signals
- Identifies a group of genes as well as the connections between them

Front vs Back nodes

- Only variant genes (front nodes) have meaningful similarity values
- These can be linked by non regulated genes (back nodes).
- Back nodes correspond to:
  - Post-translational regulation
  - Partially regulated pathways
  - Unmeasured transcripts



Network based clustering visualization



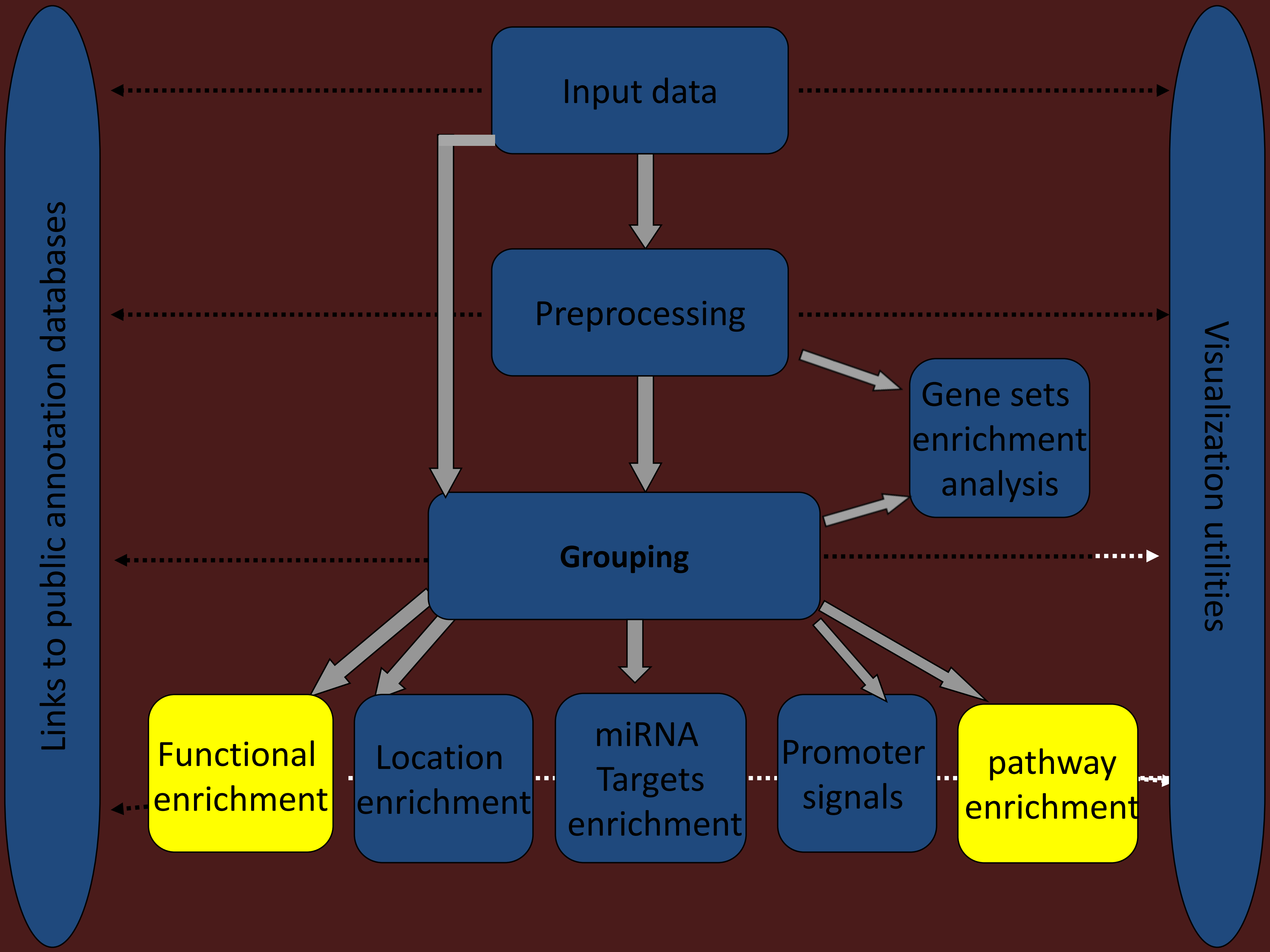
Network	N...	E...
MyCollection		
Module 1 interactions	93(0)	137...
MyCollection		
Module 1 interactions	93(0)	137...

Module 1 interactions

shared ...	name	id	symbol	Enrch
29950	29950	29950	PRNP	posit
4047	4047	4047	CDK6	biosy
6774	6774	6774	COPB1	

Name: Enrch
Type: String
Shared Column

Hands-on (3-5)



Links to public annotation databases

Input data

Preprocessing

Gene sets enrichment analysis

Grouping

Functional enrichment

Location enrichment

miRNA Targets enrichment

Promoter signals

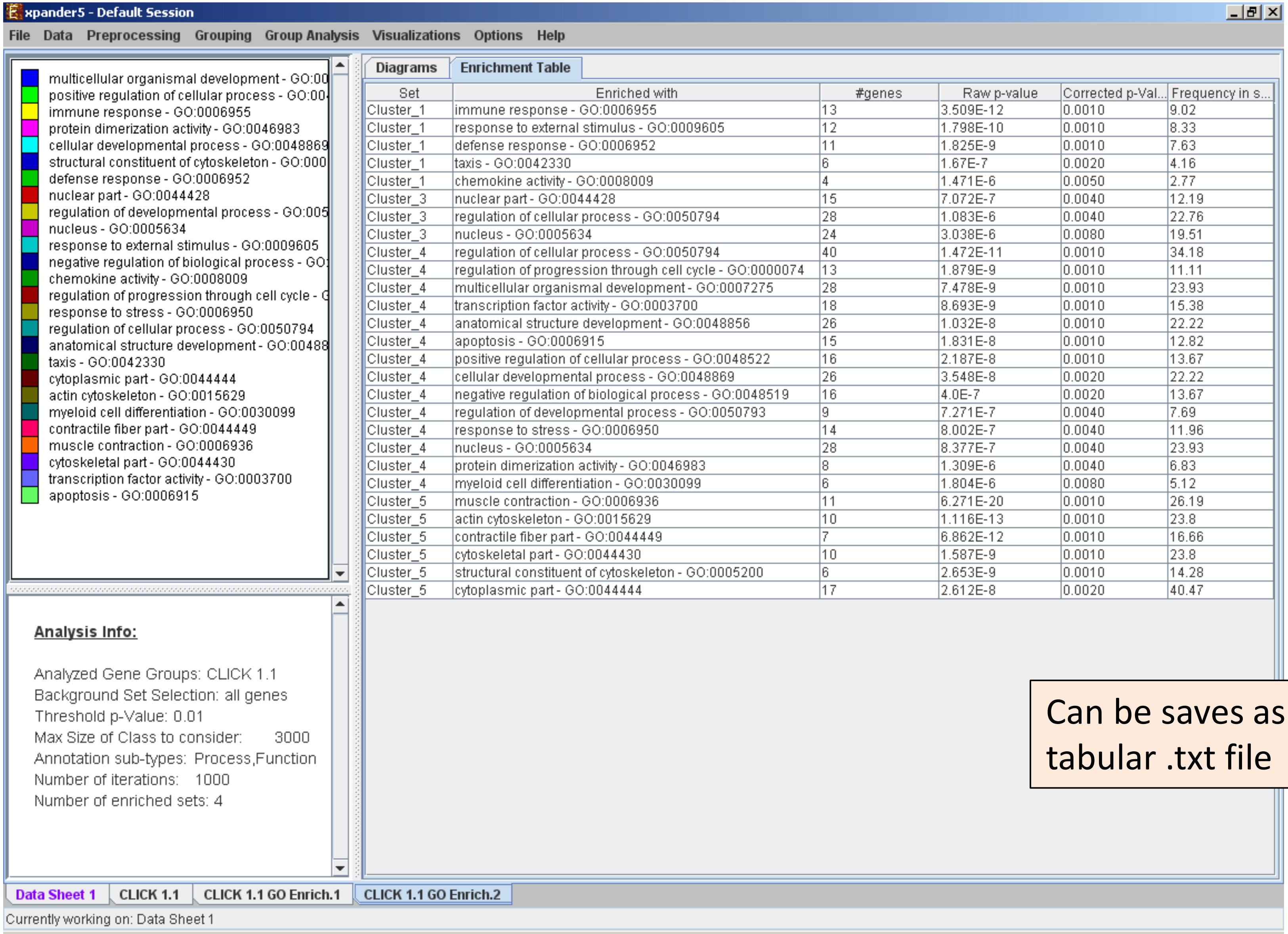
pathway enrichment

Visualization utilities

Functional enrichment analysis - Ascribing functional meaning to gene groups

- Gene Ontology (GO) annotations for all supported organisms
- TANGO: Apply statistical tests that seek over-represented GO functional categories in the groups

Functional Enrichment - Visualization



■ multicellular organismal development - GO:00
■ positive regulation of cellular process - GO:00
■ immune response - GO:0006955
■ protein dimerization activity - GO:0046983
■ cellular developmental process - GO:0048869
■ structural constituent of cytoskeleton - GO:000
■ defense response - GO:0006952
■ nuclear part - GO:0044428
■ regulation of developmental process - GO:005
■ nucleus - GO:0005634
■ response to external stimulus - GO:0009605
■ negative regulation of biological process - GO:
■ chemokine activity - GO:0008009
■ regulation of progression through cell cycle - G
■ response to stress - GO:0006950
■ regulation of cellular process - GO:0050794
■ anatomical structure development - GO:00488
■ taxis - GO:0042330
■ cytoplasmic part - GO:0044444
■ actin cytoskeleton - GO:0015629
■ myeloid cell differentiation - GO:0030099
■ contractile fiber part - GO:0044449
■ muscle contraction - GO:0006936
■ cytoskeletal part - GO:0044430
■ transcription factor activity - GO:0003700
■ apoptosis - GO:0006915

Analysis Info:

Analyzed Gene Groups: CLICK 1.1
Background Set Selection: all genes
Threshold p-Value: 0.01
Max Size of Class to consider: 3000
Annotation sub-types: Process,Function
Number of iterations: 1000
Number of enriched sets: 4

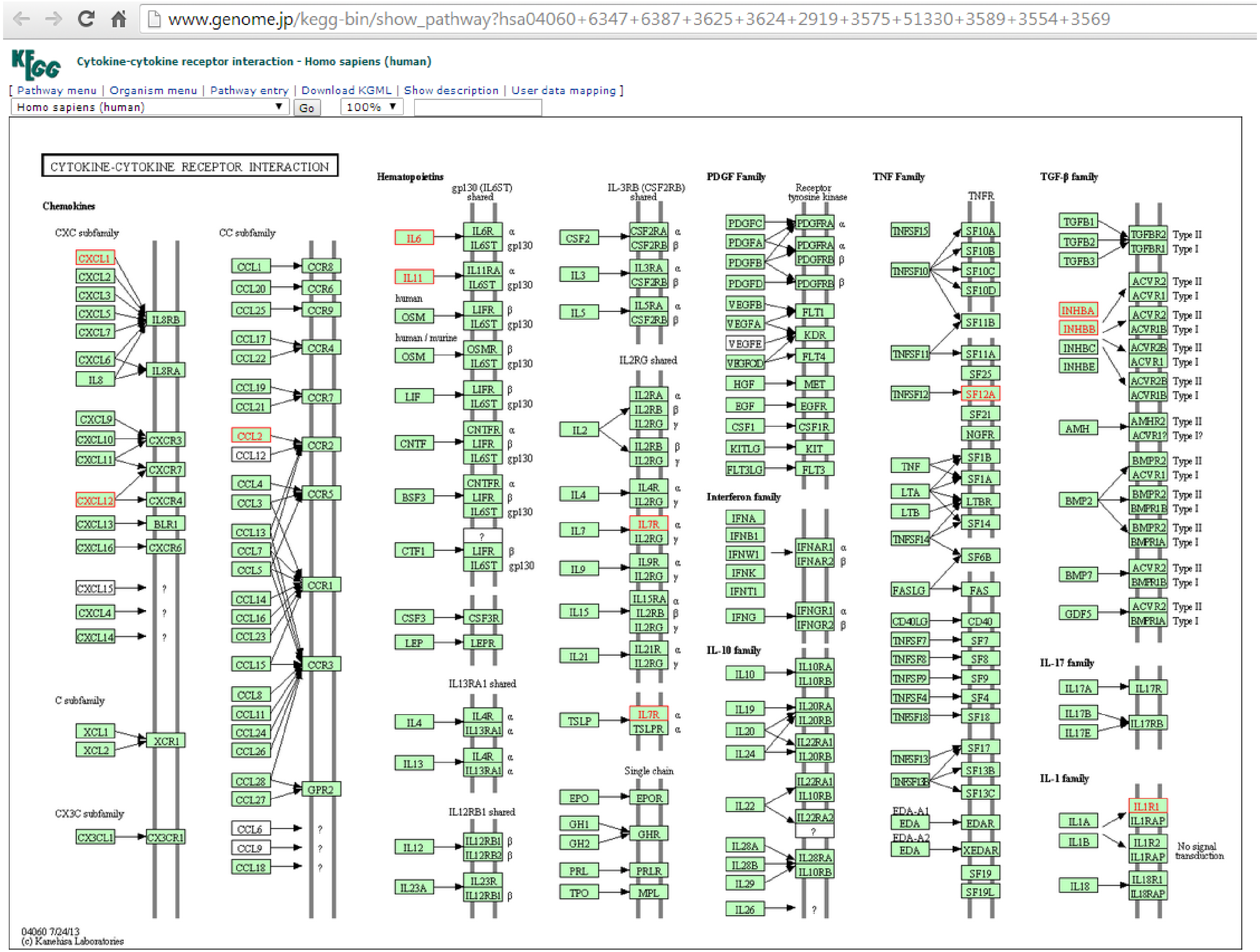
Set	Enriched with	#genes	Raw p-value	Corrected p-Val...	Frequency in s...
Cluster_1	immune response - GO:0006955	13	3.509E-12	0.0010	9.02
Cluster_1	response to external stimulus - GO:0009605	12	1.798E-10	0.0010	8.33
Cluster_1	defense response - GO:0006952	11	1.825E-9	0.0010	7.63
Cluster_1	taxis - GO:0042330	6	1.67E-7	0.0020	4.16
Cluster_1	chemokine activity - GO:0008009	4	1.471E-6	0.0050	2.77
Cluster_3	nuclear part - GO:0044428	15	7.072E-7	0.0040	12.19
Cluster_3	regulation of cellular process - GO:0050794	28	1.083E-6	0.0040	22.76
Cluster_3	nucleus - GO:0005634	24	3.038E-6	0.0080	19.51
Cluster_4	regulation of cellular process - GO:0050794	40	1.472E-11	0.0010	34.18
Cluster_4	regulation of progression through cell cycle - GO:0000074	13	1.879E-9	0.0010	11.11
Cluster_4	multicellular organismal development - GO:0007275	28	7.478E-9	0.0010	23.93
Cluster_4	transcription factor activity - GO:0003700	18	8.693E-9	0.0010	15.38
Cluster_4	anatomical structure development - GO:0048856	26	1.032E-8	0.0010	22.22
Cluster_4	apoptosis - GO:0006915	15	1.831E-8	0.0010	12.82
Cluster_4	positive regulation of cellular process - GO:0048522	16	2.187E-8	0.0010	13.67
Cluster_4	cellular developmental process - GO:0048869	26	3.548E-8	0.0020	22.22
Cluster_4	negative regulation of biological process - GO:0048519	16	4.0E-7	0.0020	13.67
Cluster_4	regulation of developmental process - GO:0050793	9	7.271E-7	0.0040	7.69
Cluster_4	response to stress - GO:0006950	14	8.002E-7	0.0040	11.96
Cluster_4	nucleus - GO:0005634	28	8.377E-7	0.0040	23.93
Cluster_4	protein dimerization activity - GO:0046983	8	1.309E-6	0.0040	6.83
Cluster_4	myeloid cell differentiation - GO:0030099	6	1.804E-6	0.0080	5.12
Cluster_5	muscle contraction - GO:0006936	11	6.271E-20	0.0010	26.19
Cluster_5	actin cytoskeleton - GO:0015629	10	1.116E-13	0.0010	23.8
Cluster_5	contractile fiber part - GO:0044449	7	6.862E-12	0.0010	16.66
Cluster_5	cytoskeletal part - GO:0044430	10	1.587E-9	0.0010	23.8
Cluster_5	structural constituent of cytoskeleton - GO:0005200	6	2.653E-9	0.0010	14.28
Cluster_5	cytoplasmic part - GO:0044444	17	2.612E-8	0.0020	40.47

Currently working on: Data Sheet 1

Can be saves as a tabular .txt file

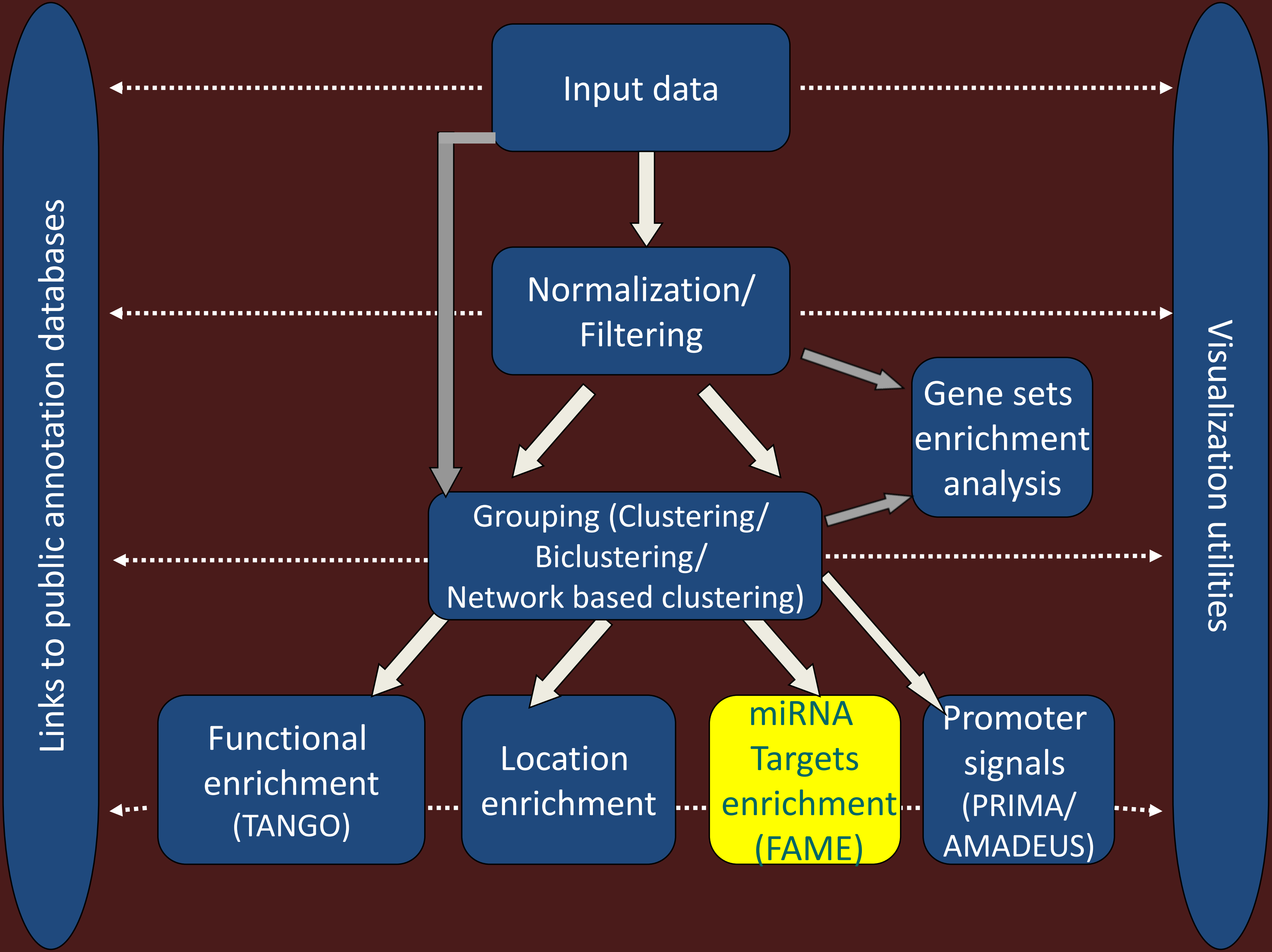
Pathway analysis

- Searches for biological pathways that are over-represented in gene groups
- **KEGG: Kyoto Encyclopedia of Genes and Genomes** (mainly metabolic),all 18 orgs
- **WikiPathways** – various biological pathways(~20 species, 1765 pathways) – open resource
- Statistical hyper-geometric (HG) cumulative distribution score + multiple testing correction



www.genome.jp/kegg-bin/show_pathway?hsa04060+6347+6387+3625+3624+2919+3575+51330+3589+3554+3569

Cytokine-cytokine receptor interaction - Homo sapiens (human)



Links to public annotation databases

Input data

Normalization/ Filtering

Gene sets enrichment analysis

Grouping (Clustering/ Biclustering/ Network based clustering)

Functional enrichment (TANGO)

Location enrichment

miRNA Targets enrichment (FAME)

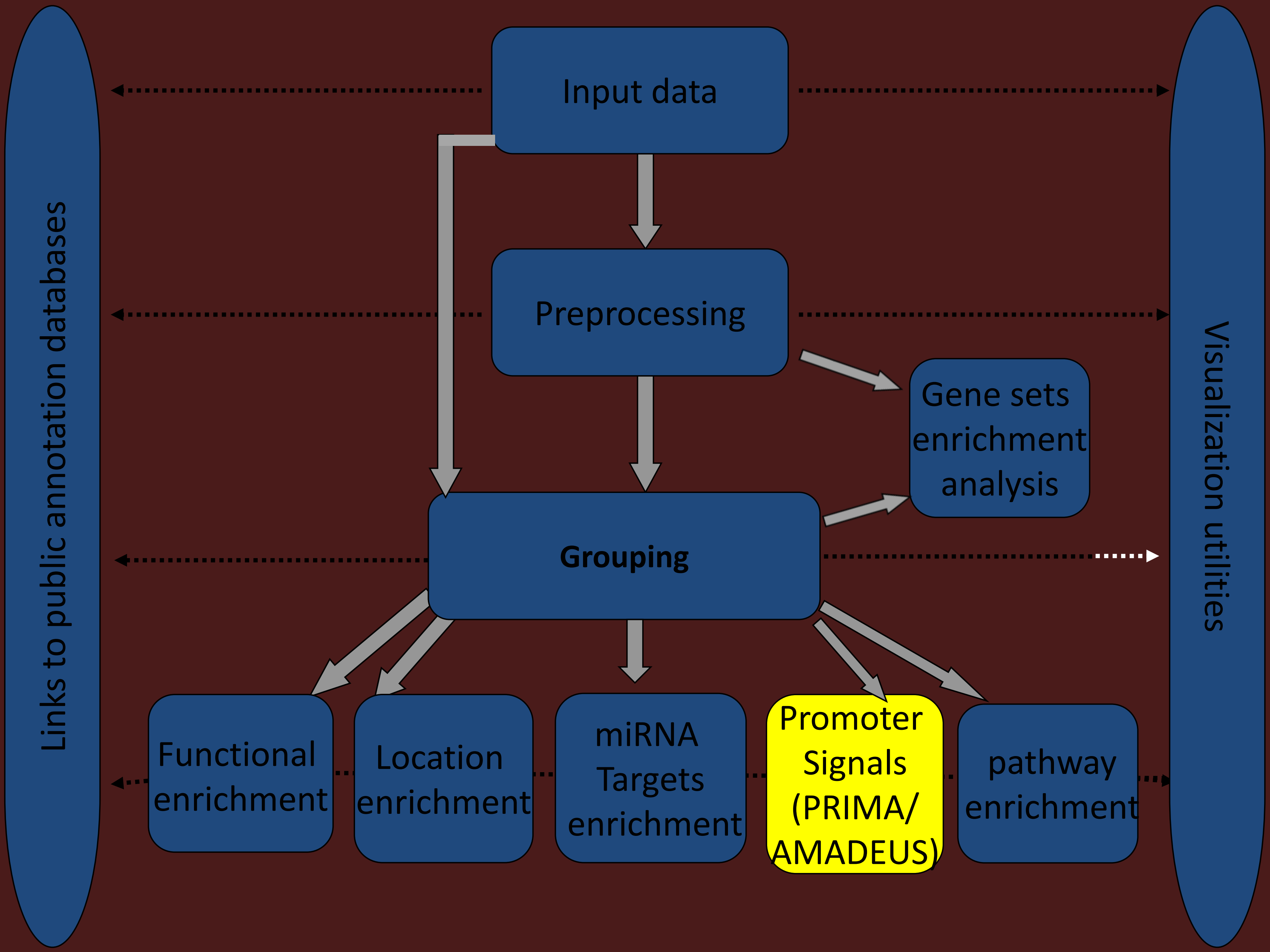
Promoter signals (PRIMA/ AMADEUS)

Visualization utilities

miRNA Enrichment Analysis

- **Goal**: to predict micorRNAs (miRNAs) regulation by detecting miRNAs whose binding sites are over/under represented in the 3' UTRs of gene groups.

- **FAME** = **F**unctional **A**ssignment of **M**iRNAs via **E**nrichment



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Inferring regulatory mechanisms from gene expression data

Assumption:

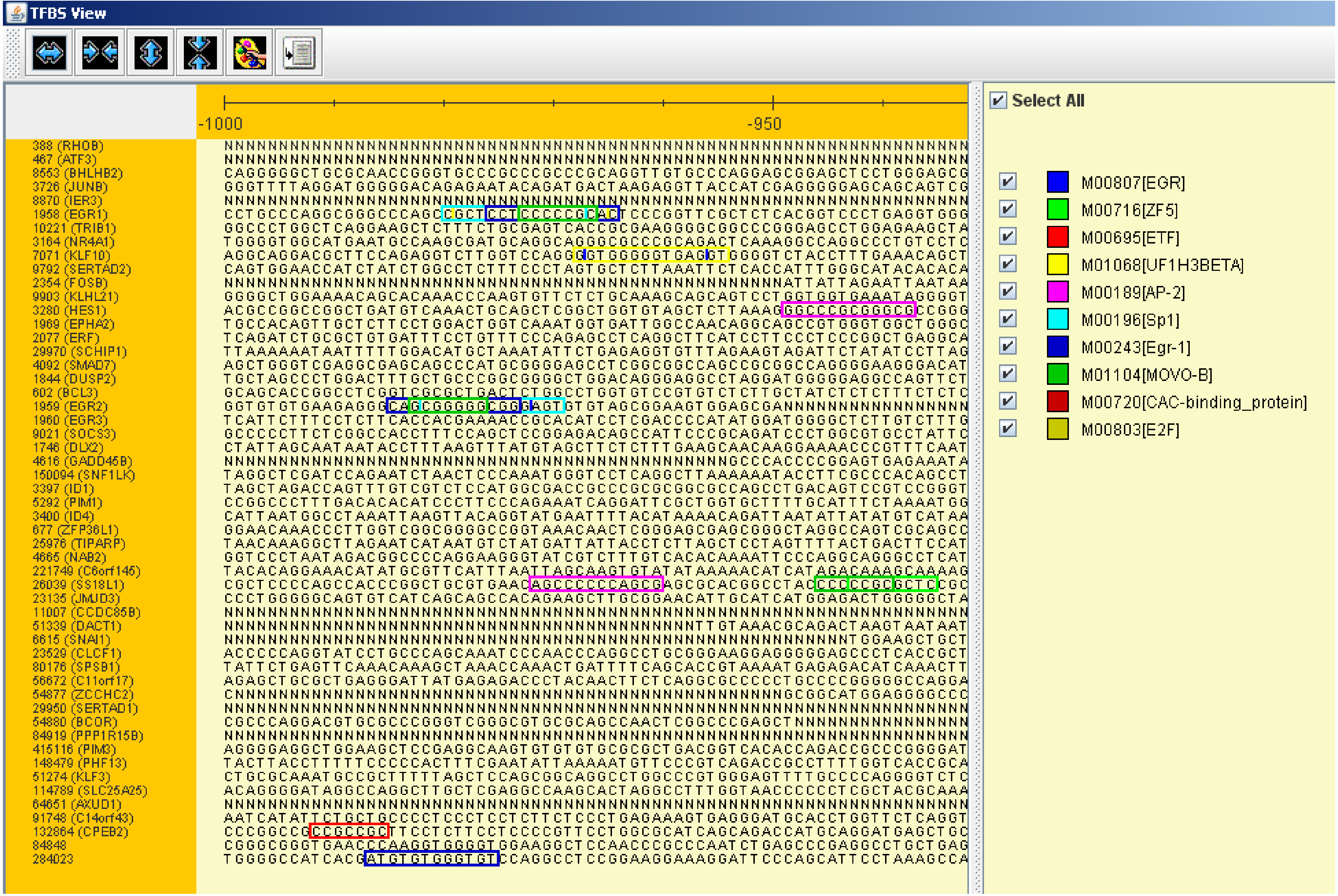
co-expression → transcriptional co-regulation → *common cis-regulatory promoter elements*

- Computational identification of *cis*-regulatory elements over-represention
- **PRIMA** - **PR**omoter **I**ntegration in **M**icroarray **A**nalysis (Elkon, et. Al, Genome Research , 2003)
- **AMADEUS** – novel motif enrichment analysis

PRIMA – general description

- *Input:*
  - *Target set* (e.g. co-expressed genes)
  - *Background set* (e.g. all genes on the chip)
- *Analysis:* Detects TFs with high target set prevalence
- TF binding site models – TRANSFAC DB
- Default: From -1000 bp to 200 bp relative the TSS

Promoter Analysis - Visualization



```
388 (RHOB)        NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
467 (ATF3)        NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
8553 (BHLHB2)     CAGGGGGCTGCGCAACCGGGTGCCCGCCCGCCCGCAGGTTGTGCCCAGGAGCGGAGCTCCTGGGAGCG
3726 (JUNB)       GGGTTTTAGGATGGGGACAGAGAATACAGATGACTAAGAGGTTACCATCGAGGGGGAGCAGCAGTCG
8870 (IER3)       NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
1958 (EGR1)       CCTGCCCAGGCGGGCCCAGCCGCTCCTCCCCCGCACTCCCGGTTCGCTCTCACGGTCCCTGAGGTGGG
10221 (TRIB1)     GGCCCTGGCTCAGGAAGCTCTTTCTGCGAGTCACCGCGAAGGGGCGGCCCGGGAGCCTGGAGAAGCTA
3164 (NR4A1)      TGGGGTGGCATGAATGCCAAGCGATGCAGGCAGGGCGCCCGCAGACTCAAAGGCCAGGCCCTGTCCTC
7071 (KLF10)      AGGCAGGACGCTTCCAGAGGTCTTGGTCCAGGGGTGGGGGTGAGGTGGGGTCTACCTTTGAAACAGCT
9792 (SERTAD2)    CAGTGGAACCATCTATCTGGCCTCTTTCCCTAGTGCTCTTAAATTCTCACCATTTGGGCATACACACA
2354 (FOSB)       NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNATTATTAGAATTAATAA
9903 (KLHL21)     GGGGCTGGAAAACAGCACAAACCCAAGTGTTCTCTGCAAAGCAGCAGTCCTGGTGGTGAAATAGGGGT
3280 (HES1)       ACGCCGGCCGGCTGATGTCAAACTGCAGCTCGGCTGGTGTAGCTCTTAAAGGGCCCGCGGGCGCCGGG
1969 (EPHA2)      TGCCACAGTTGCTCTTCCTGGACTGGTCAAATGGTGATTGGCCAACAGGCAGCCGTGGGTGGCTGGGC
2077 (ERF)        TCAGATCTGCGCTGTGATTTCCTGTTTCCCAGAGCCTCAGGCTTCATCCTTCCCTCCCGGCTGAGGCA
29970 (SCHIP1)    TTAAAAAATAATTTTTGGACATGCTAAATATTCTGAGAGGTGTTTAGAAGTAGATTCTATATCCTTAG
4092 (SMAD7)      AGCTGGGTCGAGGCGAGCAGCCCATGCGGGGAGCCTCGGCGGCCAGCCGCGGCCAGGGGAAGGGACAT
1844 (DUSP2)      TGCTAGCCCTGGACTTTGCTGCCCGGCGGGGCTGGACAGGGAGGCCTAGGATGGGGGAGGCCAGTTCT
602 (BCL3)       GCAGCACCGGCCTCGGTCGCGCTGACTCTGGCCTGGTGTCCGTGTCTCTTGCTATCTCTCTTTCTCTC
1959 (EGR2)       GGTGTGTGAAGAGGGCAGCGGGGGCGGGAGTGTGTAGCGGAAGTGGAGCGANNNNNNNNNNNNNNNNN
1960 (EGR3)       TCATTCTTTCCTCTTCACCACGAAAACCGCACATCCTCGACCCCATATGGATGGGGCTCTTGTCTTTG
9021 (SOCS3)      GCCCCCTTCTCGGCCACCTTTCCAGCTCCGGAGACAGCCATTCCCGCAGATCCCTGGCGTGCCTATTC
1746 (DLX2)       CTATTAGCAATAATACCTTTAAGTTTATGTAGCTTCTCTTTGAAGCAACAAGGAAAACCCGTTTCAAT
4616 (GADD45B)    NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGCCCACCCCGGAGTGAGAAATA
150094 (SNF1LK)   TAGGCTCGATCCAGAATCTAACTCCCAAATGGGTCCTCAGGCTTAAAAAATACCTTCGCCCACAGCCT
3397 (ID1)        TAGCTAGACCAGTTTGTCGTCTCCATGGCGACCGCCCGCGCGGCGCCAGCCTGACAGTCCGTCCGGGT
5292 (PIM1)       CCGGCCCTTTGACACACATCCCTTCCCAGAAATCAGGATTCGCTGGTGCTTTTGCATTTCTAAAATGG
3400 (ID4)        CATTAATGGCCTAAATTAAGTTACAGGTATGAATTTTACATAAAACAGATTAATATTATATGTCATAA
677 (ZFP36L1)    GGAACAAACCCTTGGTCGGCGGGGCCGGTAAACAACTCGGGAGCGAGCGGGCTAGGCCAGTCGCAGCC
25976 (TIPARP)    TAACAAAGGCTTAGAATCATAATGTCTATGATTATTACCTCTTAGCTCCTAGTTTTACTGACTTCCAT
4665 (NAB2)       GGTCCCTAATAGACGGCCCCAGGAAGGGTATCGTCTTTGTCACACAAAATTCCCAGGCAGGGCCTCAT
221749 (C6orf145) TACACAGGAAACATATGCGTTCATTTAATTAGCAAGTGTATATAAAAACATCATAGACAAAGCAAAAG
26039 (SS18L1)    CGCTCCCCAGCCACCCGGCTGCGTGAACAGCCCCCAGCGAGCGCACGGCCTACCCCCGCGCTCCGC
23135 (JMJD3)     CCCTGGGGGCAGTGTCATCAGCAGCCACAGAAGCTTGCGGAACATTGCATCATGGAGACTGGGGGCTA
11007 (CCDC85B)   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
51339 (DACT1)     NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTTGTAAACGCAGACTAAGTAATAAT
6615 (SNAI1)      NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNTGGAAGCTGCT
23529 (CLCF1)     ACCCCCAGGTATCCTGCCCAGCAAATCCCAACCCAGGCCTGCGGGAAGGAGGGGGAGCCCTCACCGCT
80176 (SPSB1)     TATTCTGAGTTCAAACAAAGCTAAACCAAACTGATTTTCAGCACCGTAAAATGAGAGACATCAAACTT
56672 (C11orf17)  AGAGCTGCGCTGAGGGATTATGAGAGACCCTACAACTTCTCAGGCGCCCCCTGCCCCGGGGGCCAGGA
54877 (ZCCHC2)    CNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNGCGGCATGGAGGGGCCC
29950 (SERTAD1)   NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
54880 (BCOR)      CGCCCAGGACGTGCGCCCGGGTCGGGCGTGCGCAGCCAACTCGGCCCGAGCTNNNNNNNNNNNNNNNN
84919 (PPP1R15B)  NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
415116 (PIM3)     AGGGGAGGCTGGAAGCTCCGAGGCAAGTGTGTGTGCGCGCTGACGGTCACACCAGACCGCCCGGGGAT
148479 (PHF13)    TACTTACCTTTTTCCCCCACTTTCGAATATTAAAAATGTTCCCGTCAGACCGCCTTTTGGTCACCGCA
51274 (KLF3)      CTGCGCAAATGCCGCTTTTTAGCTCCAGCGGCAGGCCTGGCCCGTGGGAGTTTTGCCCCAGGGGTCTC
114789 (SLC25A25) ACAGGGGATAGGCCAGGCTTGCTCGAGGCCAAGCACTAGGCCTTTGGTAACCCCCCTCGCTACGCAAA
64651 (AXUD1)     NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
91748 (C14orf43)  AATCATATTCTGCTGCCCCTCCCTCCTCTTCTCCCTGAGAAAGTGAGGGATGCACCTGGTTCTCAGGT
132864 (CPEB2)    CCCGGCCGCCGCCGCTTCCTCTTCCTCCCCGTTCCTGGCGCATCAGCAGACCATGCAGGATGAGCTGC
84848             CGGGCGGGTGAACCCAAGGTGGGGTGGAAGGCTCCAACCCGCCCAATCTGAGCCCGAGGCCTGCTGAG
284023            TGGGGCCATCACGATGTGTGGGTGTCCAGGCCTCCGGAAGGAAAGGATTCCCAGCATTCCTAAAGCCA
```

☑ Select All

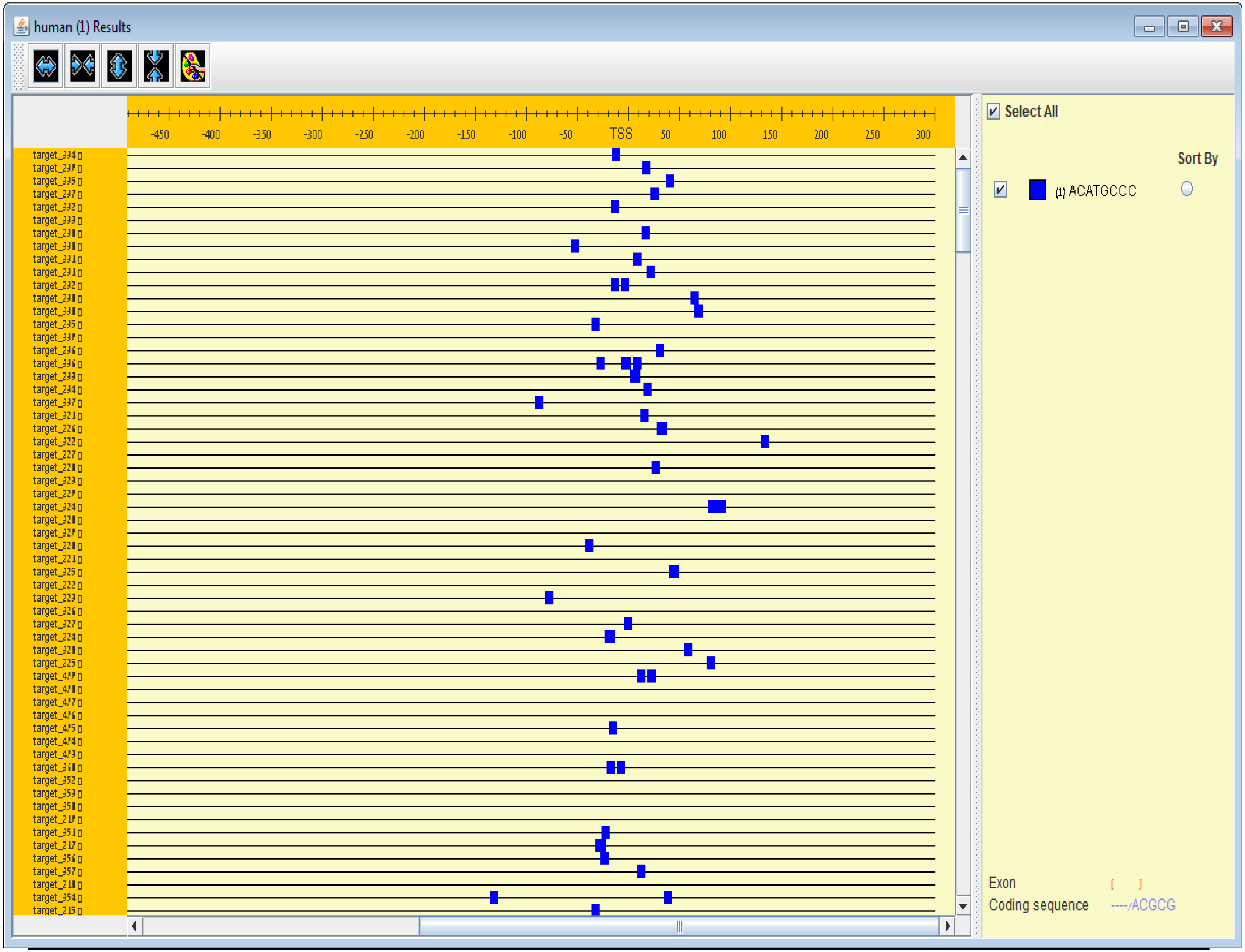
- ☑ 🟦 M00807[EGR]
- ☑ 🟩 M00716[ZF5]
- ☑ 🟥 M00695[ETF]
- ☑ 🟨 M01068[UF1H3BETA]
- ☑ 🟪 M00189[AP-2]
- ☑ 🟦 M00196[Sp1]
- ☑ 🟦 M00243[Egr-1]
- ☑ 🟩 M01104[MOVO-B]
- ☑ 🟥 M00720[CAC-binding_protein]
- ☑ 🟨 M00803[E2F]

Amadeus

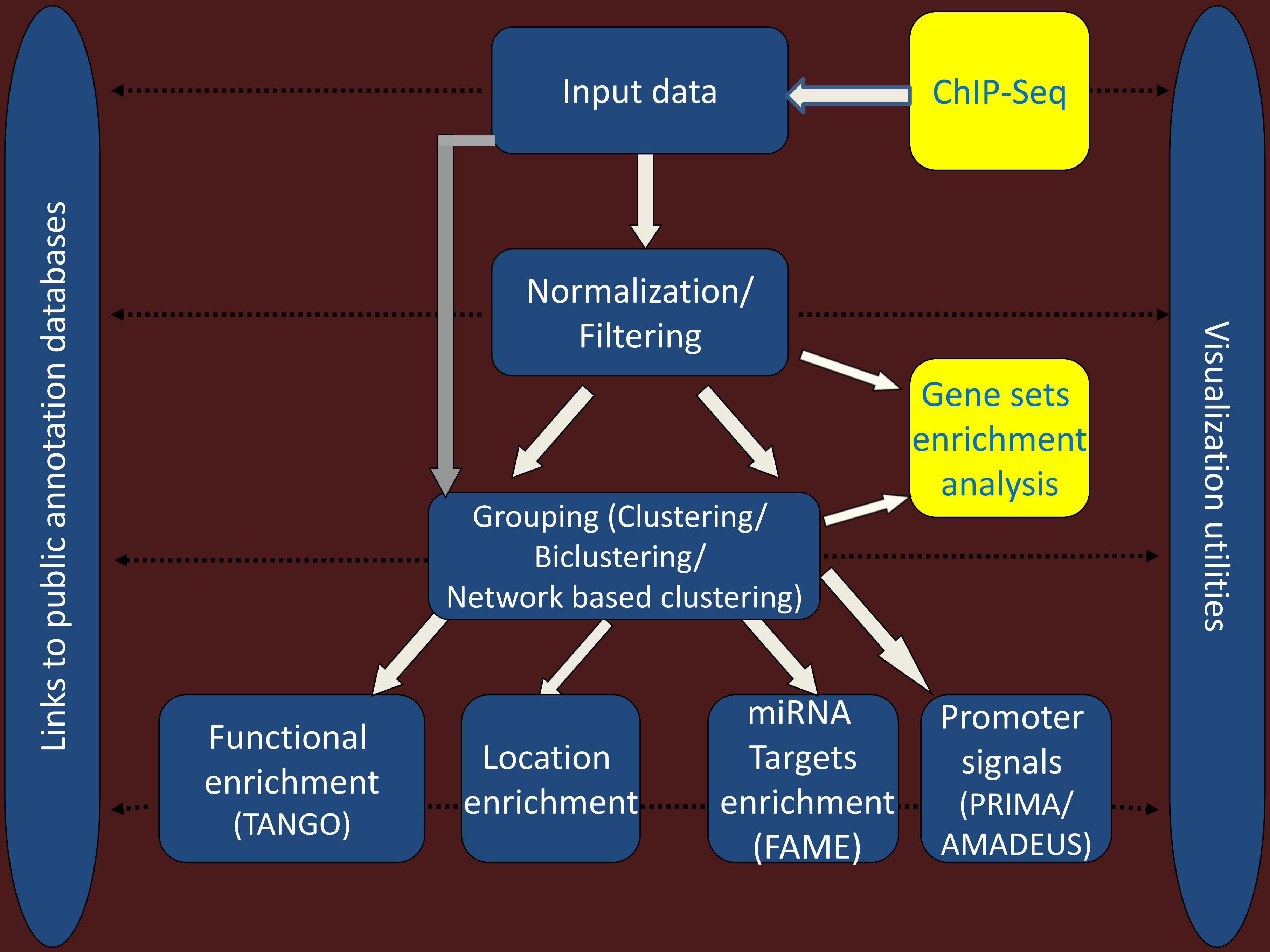
A Motif Algorithm for Detecting Enrichment in multiple Species

- **Supports diverse motif discovery tasks:**
  1. Finding **over-represented** motifs in one or more given **sets of genes**.
  2. Identifying motifs with **global spatial features** given **only** the genomic **sequences**.
- **Possible Gene-sets:**
  1. Identified gene sets clusters vs. all genes promoters.
  2. ChIP-Seq peaks sequences using Expander built-in FASTA sequences generation.

AMADEUS on ChIP-Seq peaks



Hands-on (6-8)



Links to public annotation databases

Input data

ChIP-Seq

Normalization/ Filtering

Gene sets enrichment analysis

Grouping (Clustering/ Biclustering/ Network based clustering)

Functional enrichment (TANGO)

Location enrichment

miRNA Targets enrichment (FAME)

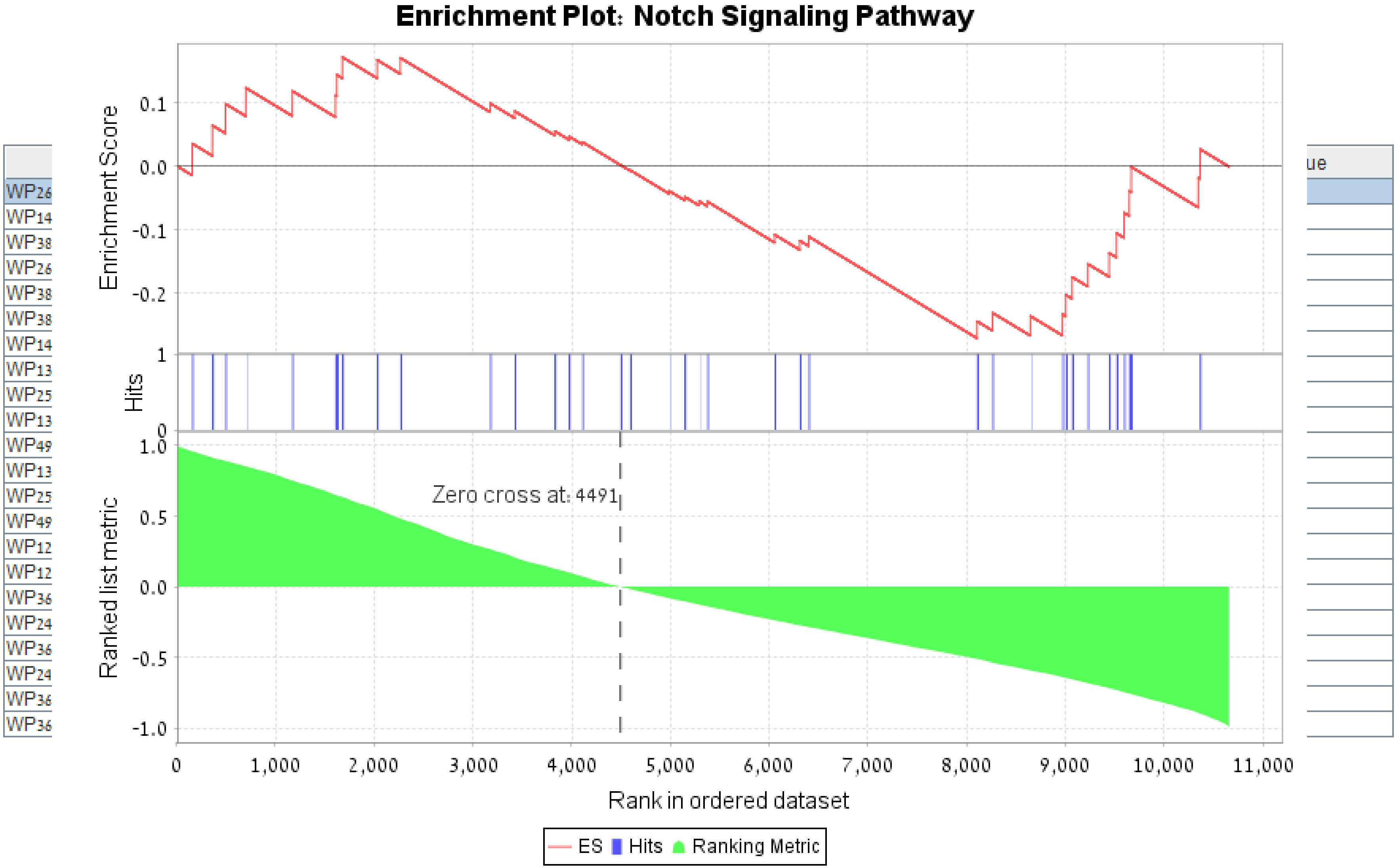
Promoter signals (PRIMA/ AMADEUS)

Visualization utilities

Gene Sets Enrichment analysis

- Goal: Determine whether an a priori defined set of genes shows concordance with a biological pattern (e.g. differences between two phenotypes)
- Gene set sources:
    - ✓ MSigDB (Broad molecular signature database)
    - ✓ KEGG
    - ✓ Wiki pathways
- Gene rank sources
    - ✓ Phenotype labels
    - ✓ Imported
    - ✓ Selected condition
- Significance estimated with permutations
- FDR correction for multiple comparisons

Gene Sets Enrichment visualization



WP26
WP14
WP38
WP26
WP38
WP38
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WP49
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WP36
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WP36
WP36

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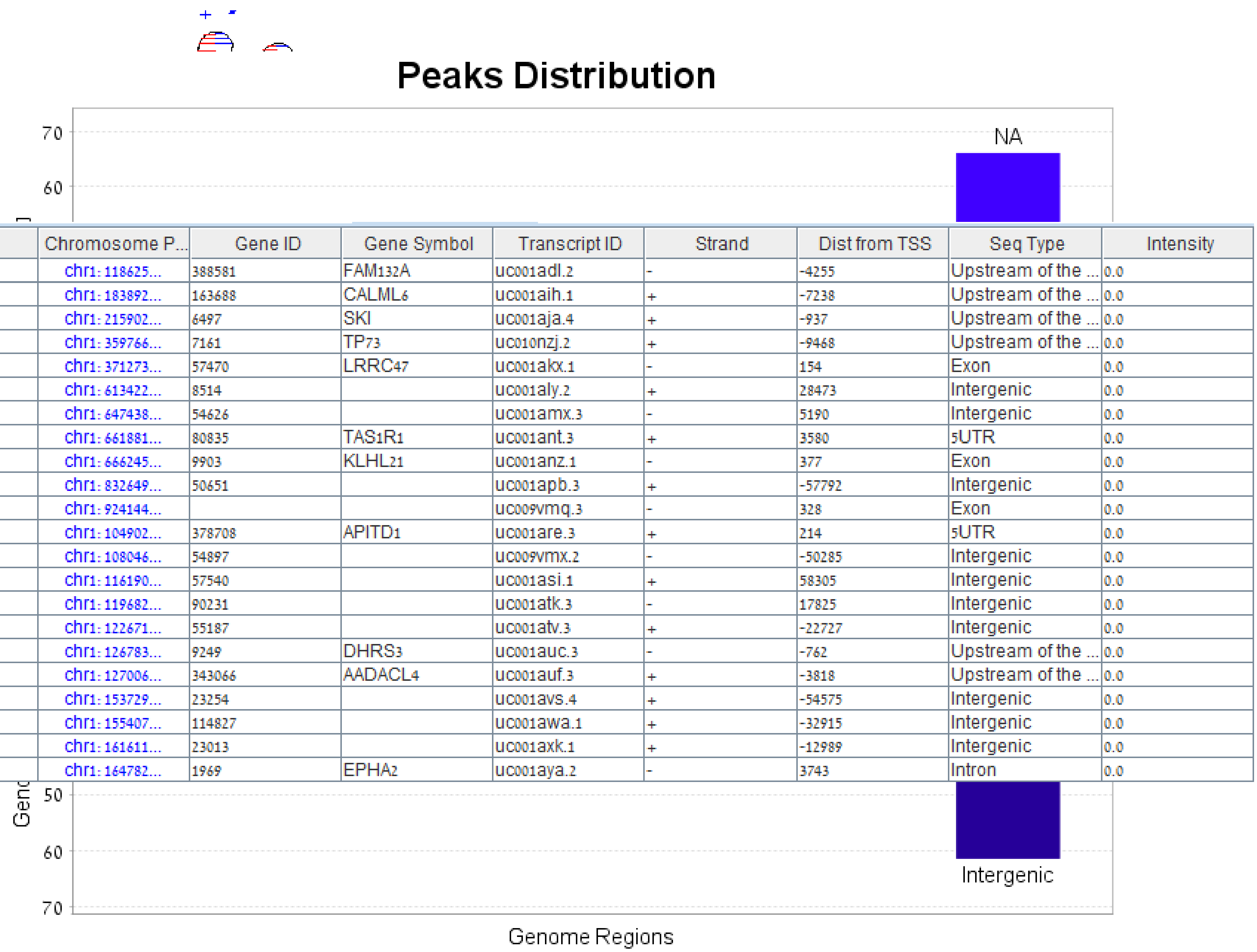
ChIP-Seq enrichment analysis

- Searches for over-representation of genes closest to ChIP-Seq data peaks
- Uses hyper geometric test
- Multiple testing correction (Bonferroni)
- Enrichment results visualization (same as other group analysis results)

ChIP-Seq visualization

- Peaks to genomic region distributions
- Closest gene to peak chromosome visualization
- Peaks enrichment in genomic regions
- Peaks annotation table including closest gene and genomic region (e.g., 5UTR, Exon etc)



Peak ID	Chromosome P...	Gene ID	Gene Symbol	Transcript ID	Strand	Dist from TSS	Seq Type	Intensity
1	chr1: 118625...	388581	FAM132A	uc001adl.2	-	-4255	Upstream of the ...	0.0
2	chr1: 183892...	163688	CALML6	uc001aih.1	+	-7238	Upstream of the ...	0.0
3	chr1: 215902...	6497	SKI	uc001aja.4	+	-937	Upstream of the ...	0.0
4	chr1: 359766...	7161	TP73	uc010nzj.2	+	-9468	Upstream of the ...	0.0
5	chr1: 371273...	57470	LRRC47	uc001akx.1	-	154	Exon	0.0
6	chr1: 613422...	8514		uc001aly.2	+	28473	Intergenic	0.0
7	chr1: 647438...	54626		uc001amx.3	-	5190	Intergenic	0.0
8	chr1: 661881...	80835	TAS1R1	uc001ant.3	+	3580	5UTR	0.0
9	chr1: 666245...	9903	KLHL21	uc001anz.1	-	377	Exon	0.0
10	chr1: 832649...	50651		uc001apb.3	+	-57792	Intergenic	0.0
11	chr1: 924144...			uc009vmq.3	-	328	Exon	0.0
12	chr1: 104902...	378708	APITD1	uc001are.3	+	214	5UTR	0.0
13	chr1: 108046...	54897		uc009vmx.2	-	-50285	Intergenic	0.0
14	chr1: 116190...	57540		uc001asi.1	+	58305	Intergenic	0.0
15	chr1: 119682...	90231		uc001atk.3	-	17825	Intergenic	0.0
16	chr1: 122671...	55187		uc001atv.3	+	-22727	Intergenic	0.0
17	chr1: 126783...	9249	DHRS3	uc001auc.3	-	-762	Upstream of the ...	0.0
18	chr1: 127006...	343066	AADACL4	uc001auf.3	+	-3818	Upstream of the ...	0.0
19	chr1: 153729...	23254		uc001avs.4	+	-54575	Intergenic	0.0
20	chr1: 155407...	114827		uc001awa.1	+	-32915	Intergenic	0.0
21	chr1: 161611...	23013		uc001axk.1	+	-12989	Intergenic	0.0
22	chr1: 164782...	1969	EPHA2	uc001aya.2	-	3743	Intron	0.0

Custom enrichment analysis

- Loads an annotation file supplied by the user
- Searches for annotations over-representation
- Uses hyper geometric test
- Multiple testing correction (Bonferroni)
- Enrichment results visualization (same as other group analysis results)

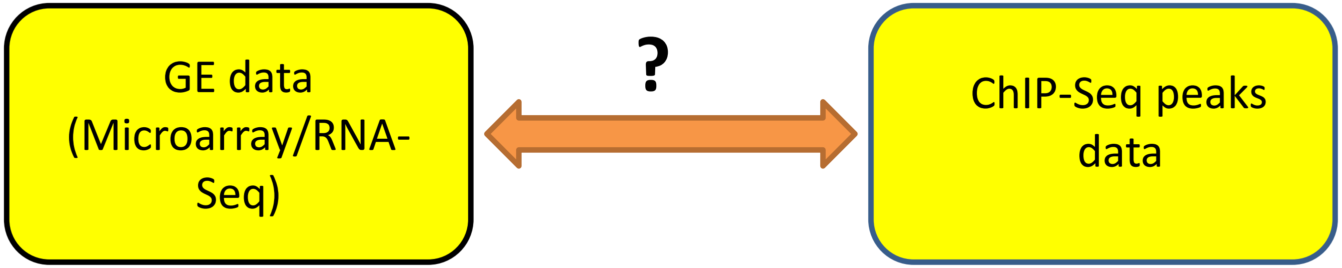
RNA-Seq edgeR/DESeq2

- RNA-Seq counts data
- Perform differential expression between two groups of conditions

Analysis wizard

- Allows performing multiple analysis steps at a push of a button
- Incorporates most of the tools available in EXPANDER
- All parameters are set in advance
- Standard default values are provided
- After performing analysis, all corresponding visualizations are automatically generated

Integration between different technologies



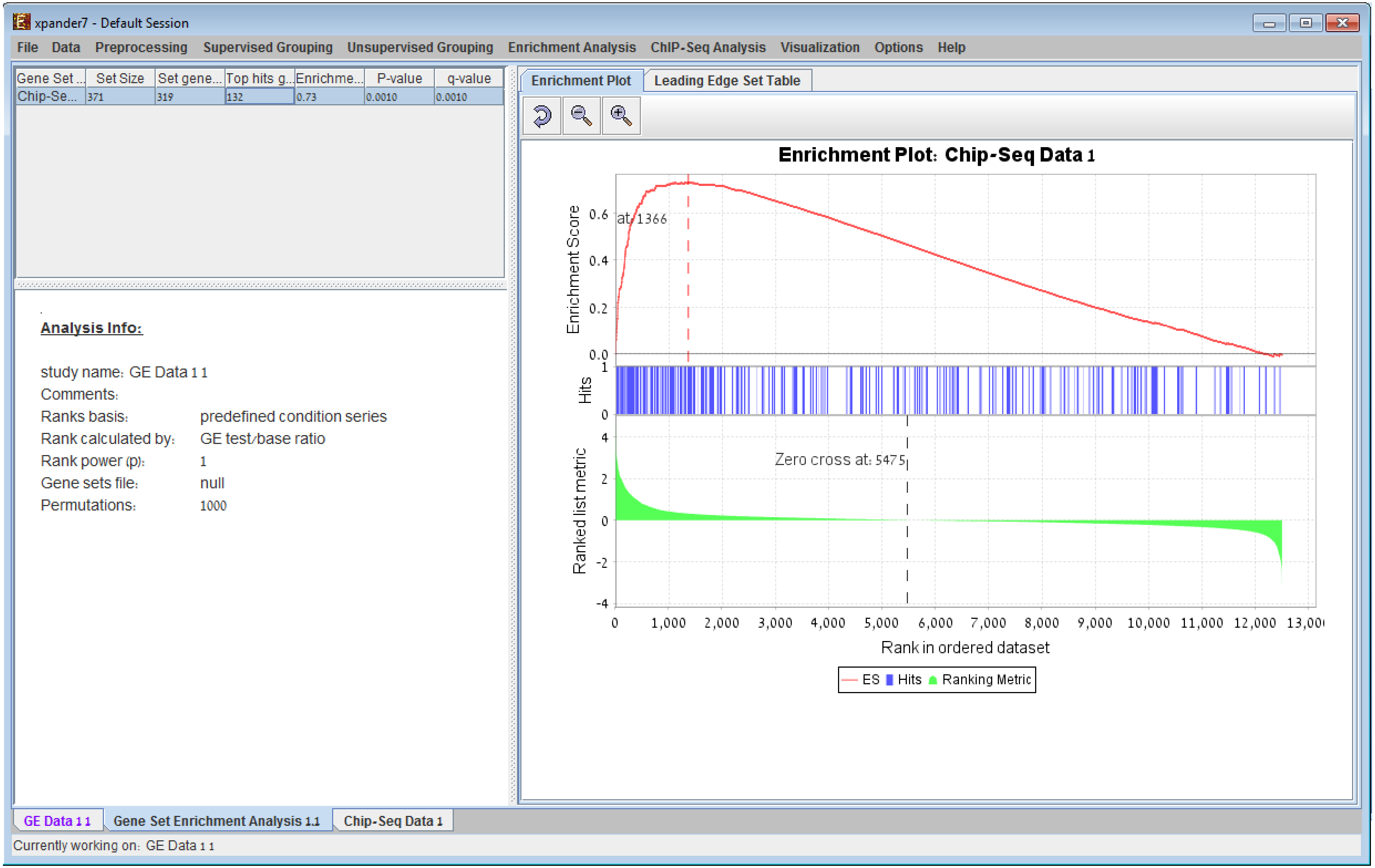
GE data (Microarray/RNA-Seq)

?

ChIP-Seq peaks data

- ChIP-Seq vs. GE analysis:
  - GSEA – ChIP-Seq target genes as a single set
  - ChIP-Seq enrichment of GE’s clusters
  - ChIP-Seq target genes distribution in GE
  - Expander enrichment tools (e.g., TANGO, PRIMA) – select ChIP-Seq target genes as a single cluster

GSEA – ChIP-Seq vs. GE



Gene Set ...	Set Size	Set gene...	Top hits g...	Enrichme...	P-value	q-value
Chip-Se...	371	319	132	0.73	0.0010	0.0010

Analysis Info:

study name: GE Data 1 1

Comments:

Ranks basis: predefined condition series

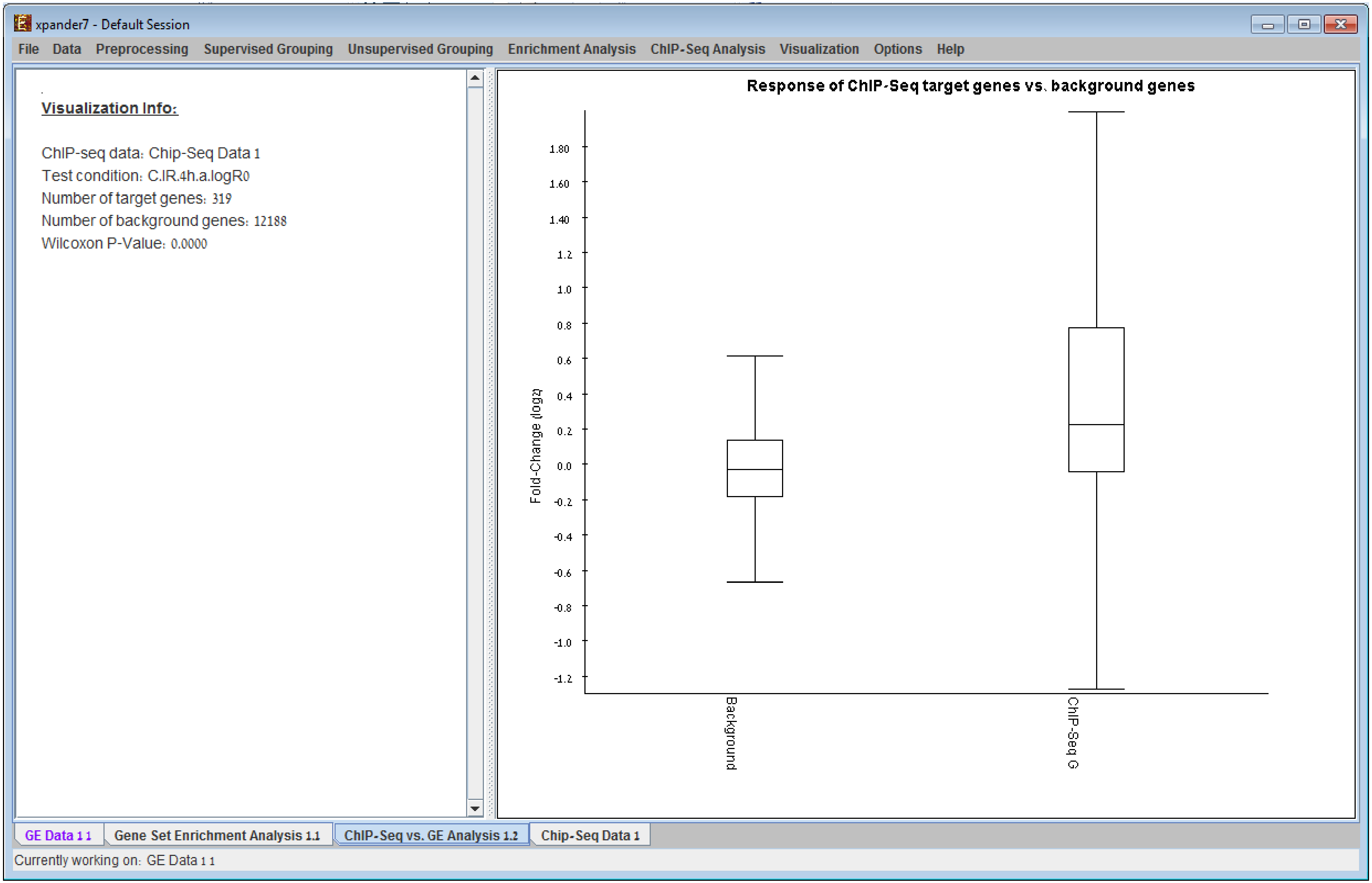
Rank calculated by: GE test/base ratio

Rank power (p): 1

Gene sets file: null

Permutations: 1000

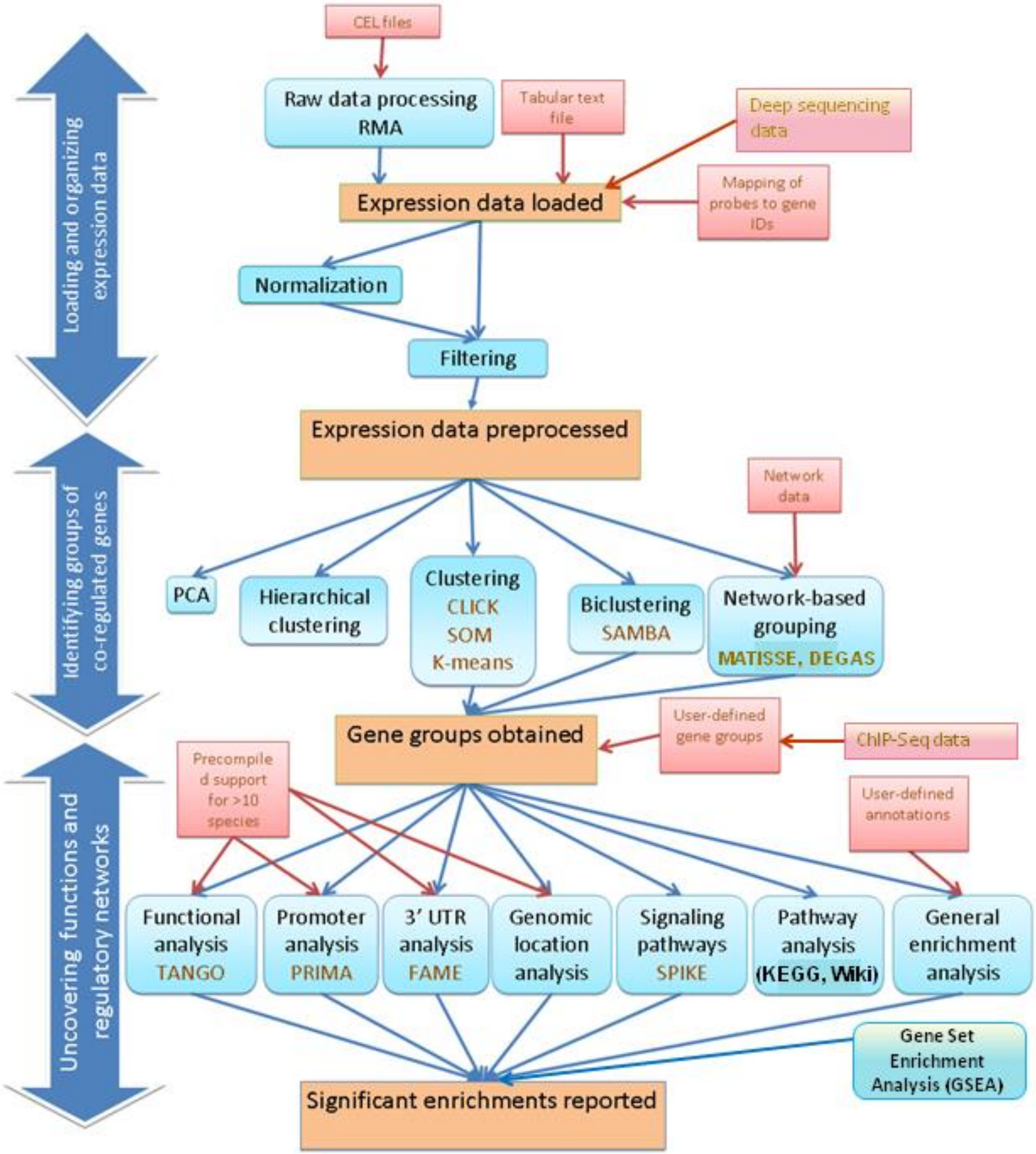
Rank distribution – ChIP-Seq vs. GE



Visualization Info:

ChIP-seq data: Chip-Seq Data 1
Test condition: C.IR.4h.a.logR0
Number of target genes: 319
Number of background genes: 12188
Wilcoxon P-Value: 0.0000

Currently working on: GE Data 1 1


Loading and organizing expression data
Identifying groups of co-regulated genes
Uncovering functions and regulatory networks
CEL files
Raw data processing RMA
Tabular text file
Deep sequencing data
Mapping of probes to gene IDs
Expression data loaded
Normalization
Filtering
Expression data preprocessed
Network data
PCA
Hierarchical clustering
Clustering CLICK SOM K-means
Biclustering SAMBA
Network-based grouping MATISSE, DEGAS
Gene groups obtained
User-defined gene groups
ChIP-Seq data
Precompiled support for >10 species
User-defined annotations
Functional analysis TANGO
Promoter analysis PRIMA
3' UTR analysis FAME
Genomic location analysis
Signaling pathways SPIKE
Pathway analysis (KEGG, Wiki)
General enrichment analysis
Gene Set Enrichment Analysis (GSEA)
Significant enrichments reported

Hands-on (New features)