

GEM2Net a module of CATdb from Gene Expression Modeling to Networks in Plants



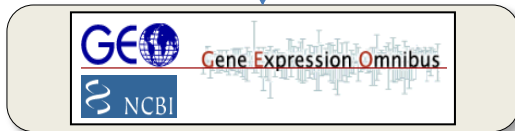
IPS2 Institute of Plant Science Paris Saclay
V. Brunaud
Team Genomic Networks (ML Martin-Magniette)



Bioinformatic and Transcriptomic PF



CATdb
a Complete Arabidopsis Transcriptome database



Biological question



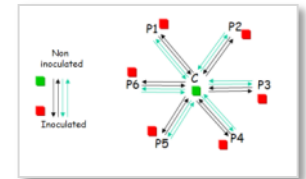
Experimental design



Libraries-Sequencing
Hybridizations



Bioinformatic and Statistical analyses



Extrait de résultats : cinétique de culture de protoplastes

Gene	Accession	Log2 (Inoculé/Non inoculé)	Log2 (P1/Non inoculé)	Log2 (P2/Non inoculé)	Log2 (P3/Non inoculé)	Log2 (P4/Non inoculé)	Log2 (P5/Non inoculé)	Log2 (P6/Non inoculé)
AT1G01010	AT1G01010	0	0	0	0	0	0	0
AT1G01020	AT1G01020	0	0	0	0	0	0	0
AT1G01030	AT1G01030	0	0	0	0	0	0	0
AT1G01040	AT1G01040	0	0	0	0	0	0	0
AT1G01050	AT1G01050	0	0	0	0	0	0	0
AT1G01060	AT1G01060	0	0	0	0	0	0	0
AT1G01070	AT1G01070	0	0	0	0	0	0	0
AT1G01080	AT1G01080	0	0	0	0	0	0	0
AT1G01090	AT1G01090	0	0	0	0	0	0	0
AT1G01100	AT1G01100	0	0	0	0	0	0	0
AT1G01110	AT1G01110	0	0	0	0	0	0	0
AT1G01120	AT1G01120	0	0	0	0	0	0	0
AT1G01130	AT1G01130	0	0	0	0	0	0	0
AT1G01140	AT1G01140	0	0	0	0	0	0	0
AT1G01150	AT1G01150	0	0	0	0	0	0	0
AT1G01160	AT1G01160	0	0	0	0	0	0	0
AT1G01170	AT1G01170	0	0	0	0	0	0	0
AT1G01180	AT1G01180	0	0	0	0	0	0	0
AT1G01190	AT1G01190	0	0	0	0	0	0	0
AT1G01200	AT1G01200	0	0	0	0	0	0	0
AT1G01210	AT1G01210	0	0	0	0	0	0	0
AT1G01220	AT1G01220	0	0	0	0	0	0	0
AT1G01230	AT1G01230	0	0	0	0	0	0	0
AT1G01240	AT1G01240	0	0	0	0	0	0	0
AT1G01250	AT1G01250	0	0	0	0	0	0	0
AT1G01260	AT1G01260	0	0	0	0	0	0	0
AT1G01270	AT1G01270	0	0	0	0	0	0	0
AT1G01280	AT1G01280	0	0	0	0	0	0	0
AT1G01290	AT1G01290	0	0	0	0	0	0	0
AT1G01300	AT1G01300	0	0	0	0	0	0	0
AT1G01310	AT1G01310	0	0	0	0	0	0	0
AT1G01320	AT1G01320	0	0	0	0	0	0	0
AT1G01330	AT1G01330	0	0	0	0	0	0	0
AT1G01340	AT1G01340	0	0	0	0	0	0	0
AT1G01350	AT1G01350	0	0	0	0	0	0	0
AT1G01360	AT1G01360	0	0	0	0	0	0	0
AT1G01370	AT1G01370	0	0	0	0	0	0	0
AT1G01380	AT1G01380	0	0	0	0	0	0	0
AT1G01390	AT1G01390	0	0	0	0	0	0	0
AT1G01400	AT1G01400	0	0	0	0	0	0	0
AT1G01410	AT1G01410	0	0	0	0	0	0	0
AT1G01420	AT1G01420	0	0	0	0	0	0	0
AT1G01430	AT1G01430	0	0	0	0	0	0	0
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AT1G01460	AT1G01460	0	0	0	0	0	0	0
AT1G01470	AT1G01470	0	0	0	0	0	0	0
AT1G01480	AT1G01480	0	0	0	0	0	0	0
AT1G01490	AT1G01490	0	0	0	0	0	0	0
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AT1G01590	AT1G01590	0	0	0	0	0	0	0
AT1G01600	AT1G01600	0	0	0	0	0	0	0
AT1G01610	AT1G01610	0	0	0	0	0	0	0
AT1G01620	AT1G01620	0	0	0	0	0	0	0
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AT1G01650	AT1G01650	0	0	0	0	0	0	0
AT1G01660	AT1G01660	0	0	0	0	0	0	0
AT1G01670	AT1G01670	0	0	0	0	0	0	0
AT1G01680	AT1G01680	0	0	0	0	0	0	0
AT1G01690	AT1G01690	0	0	0	0	0	0	0
AT1G01700	AT1G01700	0	0	0	0	0	0	0
AT1G01710	AT1G01710	0	0	0	0	0	0	0
AT1G01720	AT1G01720	0	0	0	0	0	0	0
AT1G01730	AT1G01730	0	0	0	0	0	0	0
AT1G01740	AT1G01740	0	0	0	0	0	0	0
AT1G01750	AT1G01750	0	0	0	0	0	0	0
AT1G01760	AT1G01760	0	0	0	0	0	0	0
AT1G01770	AT1G01770	0	0	0	0	0	0	0
AT1G01780	AT1G01780	0	0	0	0	0	0	0
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AT1G01860	AT1G01860	0	0	0	0	0	0	0
AT1G01870	AT1G01870	0	0	0	0	0	0	0
AT1G01880	AT1G01880	0	0	0	0	0	0	0
AT1G01890	AT1G01890	0	0	0	0	0	0	0
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AT1G01920	AT1G01920	0	0	0	0	0	0	0
AT1G01930	AT1G01930	0	0	0	0	0	0	0
AT1G01940	AT1G01940	0	0	0	0	0	0	0
AT1G01950	AT1G01950	0	0	0	0	0	0	0
AT1G01960	AT1G01960	0	0	0	0	0	0	0
AT1G01970	AT1G01970	0	0	0	0	0	0	0
AT1G01980	AT1G01980	0	0	0	0	0	0	0
AT1G01990	AT1G01990	0	0	0	0	0	0	0
AT1G02000	AT1G02000	0	0	0	0	0	0	0

Differential Gene expression

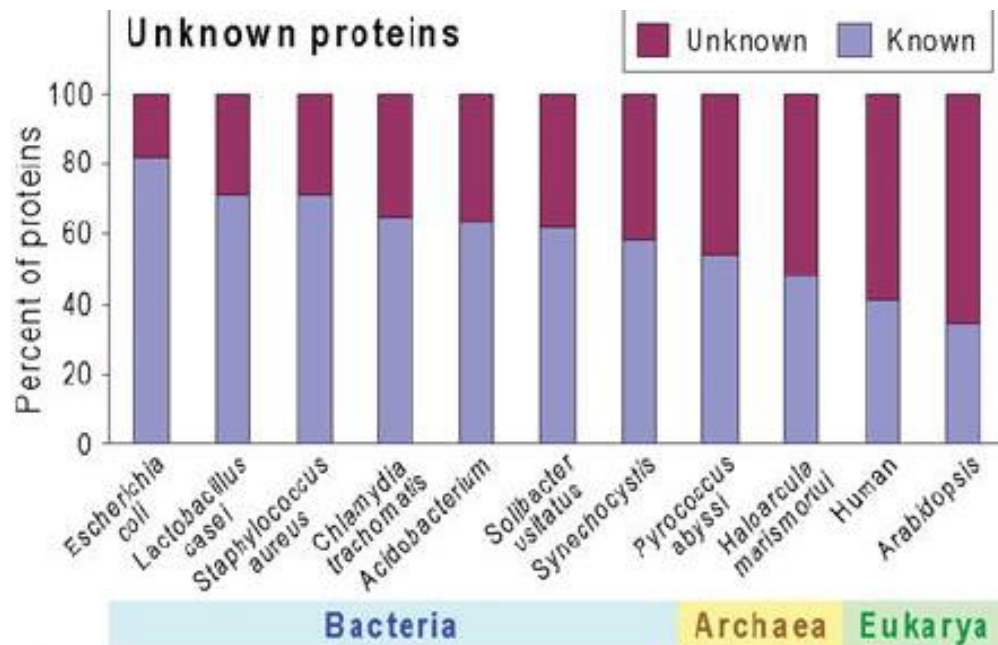


**Which gene is regulated?
Information on gene function**

Functional Annotation

A huge “orphan of function” gene space

- orphan genes = genes without homologs with a known function (Fukushi et al., 2003).
- 20% to 40% of the predicted genes for completely sequenced eukaryotic organisms have no assigned function (Hanson et al., 2010).



For Arabidopsis genome

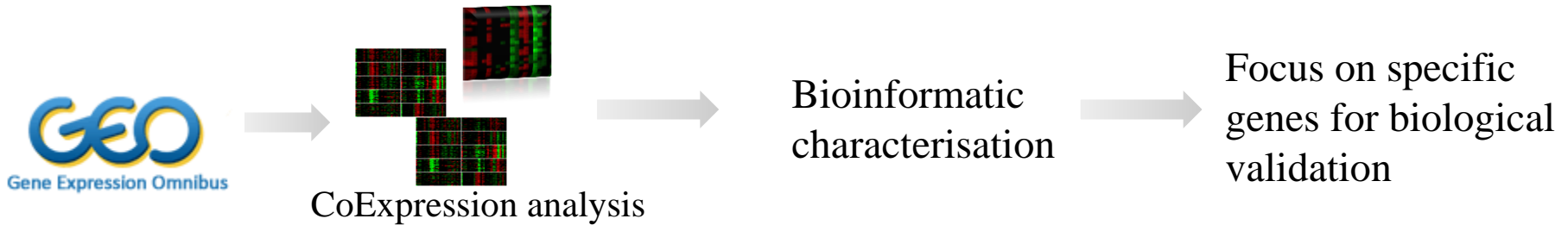
- **Only 16% of genes have a validated function**
- **More than 5000 Arabidopsis genes are still orphans (TAIRv10)**

Classical analyses by sequence similarity

Based on a comparison of protein sequences to identify structural similarities

- Some sequences with a low similarity may share a same function (Galperin et al, 1998)
- A high similarity does not guarantee a functional similarity (Tian et al, 2003)
- Protein sequence comparison gives information about the biochemical function (Nehrt et al, 2011) → common proteic motif/domain

Classical analyses of co-expression



Transcriptomic studies seem relevant approaches since co-expressed genes are often involved in a same biological process

- Data are generally extracted from international repositories
- With heterogeneous data in terms of acquisition and preprocessing

Co-expression usually measure a correlation value

- Comparison by Pearson correlation based on “gene pairs”
- Difficult to interpret since the number of gene pairs is large

Local point of view of a complex question since genes work in modules

Integration of omics data

Integrating various resources of omics data improves the success of prediction (Radiovojac et al, 2013) ‘Guilt by association’

But various sources of heterogeneity exist

- Data are qualitative or quantitative
- Available information describes the biological entities or their relationships
- Observations are obtained with various techniques / workflows

Not obvious links between these various data

e.g. Protein Interactome (PPI) by Arabidopsis Interactome Mapping consortium (2011)

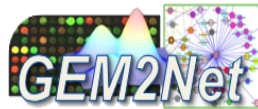
- only 10% have been studied
- high rate of false positive

GEM2Net: Goals

Goals: from Gene Expression Modeling TO gene Network to investigate Arabidopsis thaliana stress responses

→ Agronomic challenge to characterize genetic variants resistant to biotic/abiotic attacks

CATdb



GEM2Net: Goals & methods

Goals: From gene expression modeling to gene network to investigate *Arabidopsis thaliana* stress response



Methods: original features

- homogeneous and dedicated transcriptomic data in CATdb
- classification of several CATdb projects in biotic/abiotic stress
- method of clustering: model based method
- integration of various resources to improve the functional inference



<http://tools.ips2.u-psud.fr/CATdb>

4613 diff. samples on 25 species
 → 1657 treated (183 exp. Factors)
 → 32 organs, 132 development stages


Explore the 247 Arabidopsis projects in public access

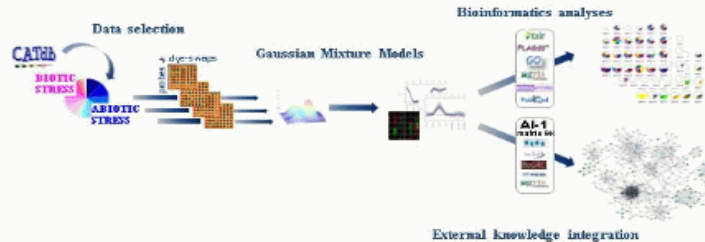
CATdb provides access to a large collection of transcriptomic data for *Arabidopsis thaliana*, mainly hybridized with the 2-colors CATMA (Complete Arabidopsis Transcriptome Micro-Array, Crow et al. NAR 2003). The CATMA design has been updated to include almost all the annotated genes in Arabidopsis (TAIR + EuGene predictions)

Number of Arabidopsis microarrays per array type	146 CATMA_2	352 CATMA_2.1	1394 CATMA_2.2	868 CATMA_2.3	1819 CATMA_5	98 CATMA6-HD12	294 CATMA6.2-HD12	24 CATMA6.1-HD12
Number of gene loci covered per array design	22486	22679	22702	23001	28230	35656	35656	35656

NEW GEM2Net a module of CATdb to explore Arabidopsis Stress Response

GEM2Net is a new module of CATdb which the main objective is to provide a global overview of gene response of Arabidopsis under stress stimuli. This approach is based on a clustering of a subset of CATdb data and explores various resources to annotate clusters in order to get insights into gene functions.

In CATdb, projects selected for GEM2Net are indicated by this icon  ... click on it to access data & analyses.



Explore the 44 Other Species projects in public access

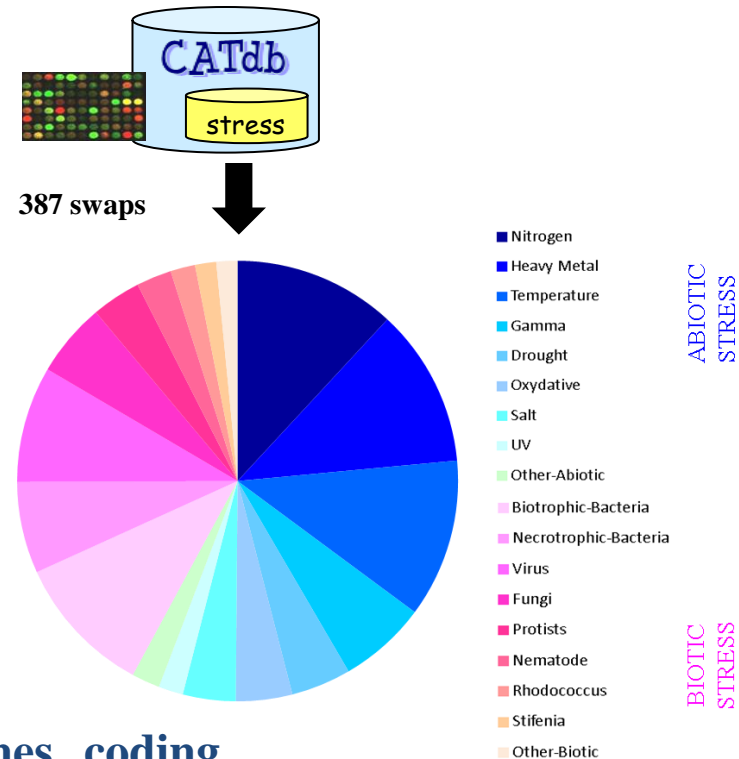
CATdb stores data of 11 array types to study transcriptome of various species (mainly Plants). These array designs are produced with different technologies (Affymetrix, NimbleGen, Agilent)

Array design for species	Cotton	Gallus gallus	Human GenomeUL33A 2.0	Medicago	SUNFLOWER	Vitis	maize	poplar	rice	rose	wheat
Number of microarrays per array type	4	14	18	190	24	12	38	116	86	50	16

Transcriptomic Dataset

CATdb : experiments implicated into stresses

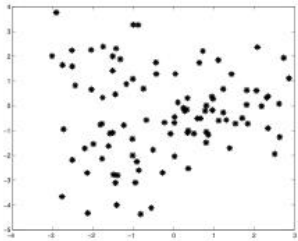
- ❑ Large and homogeneous transcriptome resource generated by the POPS platform and available in CATdb (Gagnot et al., NAR 2008).
- ❑ ~ 400 conditions dedicated to stresses
9 biotic and 9 abiotic stress categories



- Based on differential analyses, **60% of the genes coding proteins** have their transcription impacted directly or not by a stress
- Large overlap of impacted genes between biotic and abiotic stresses

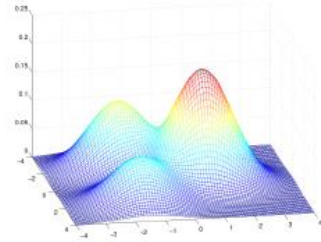
Co-expression analysis by mixture models

what we observe

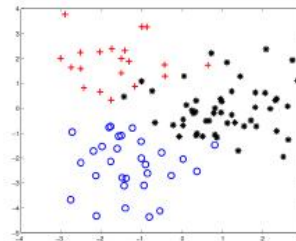


$Z = ?$

the model



the expected results



$Z : 1 = \circ, 2 = +, 3 = *$

Data-driven method

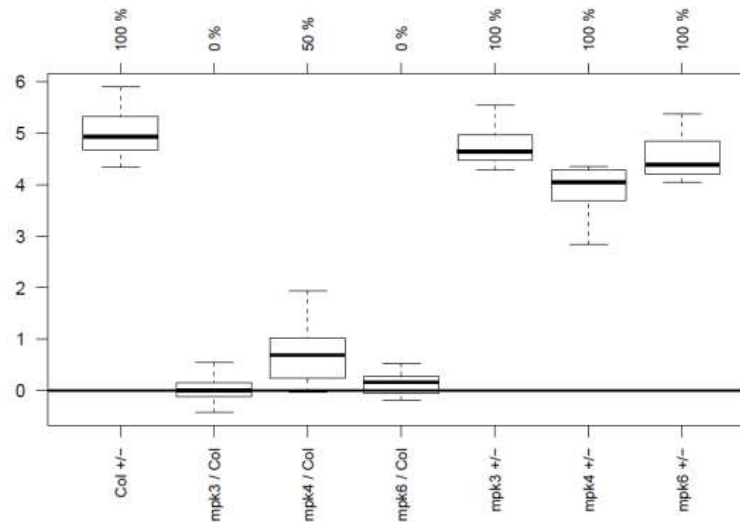
- number of cluster chosen by BIC
Bayesian Information Criterion
- gene classification based on the
conditional probabilities

Matrix by stress
{ genes x log-ratios}

Gaussian mixture

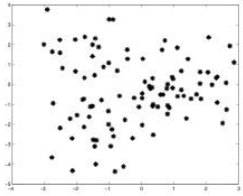


clusters of
co-expressed genes
(groups of genes with same
profile)



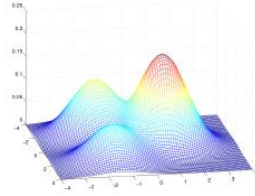
Co-expression analysis by mixture models

what we observe

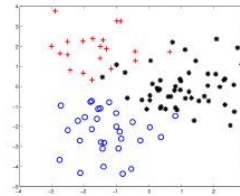


$Z = ?$

the model



the expected results



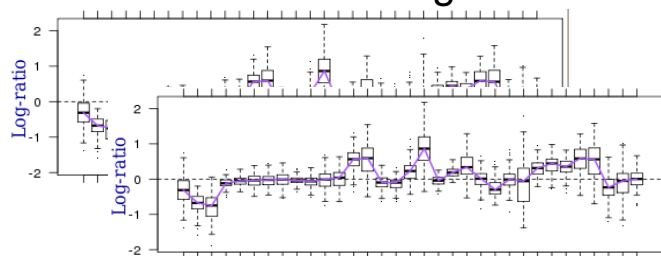
$Z : 1 = \circ, 2 = +, 3 = *$

Matrix by stress
{ genes x log-ratios}

Gaussian mixture



~ 700 clusters of genes

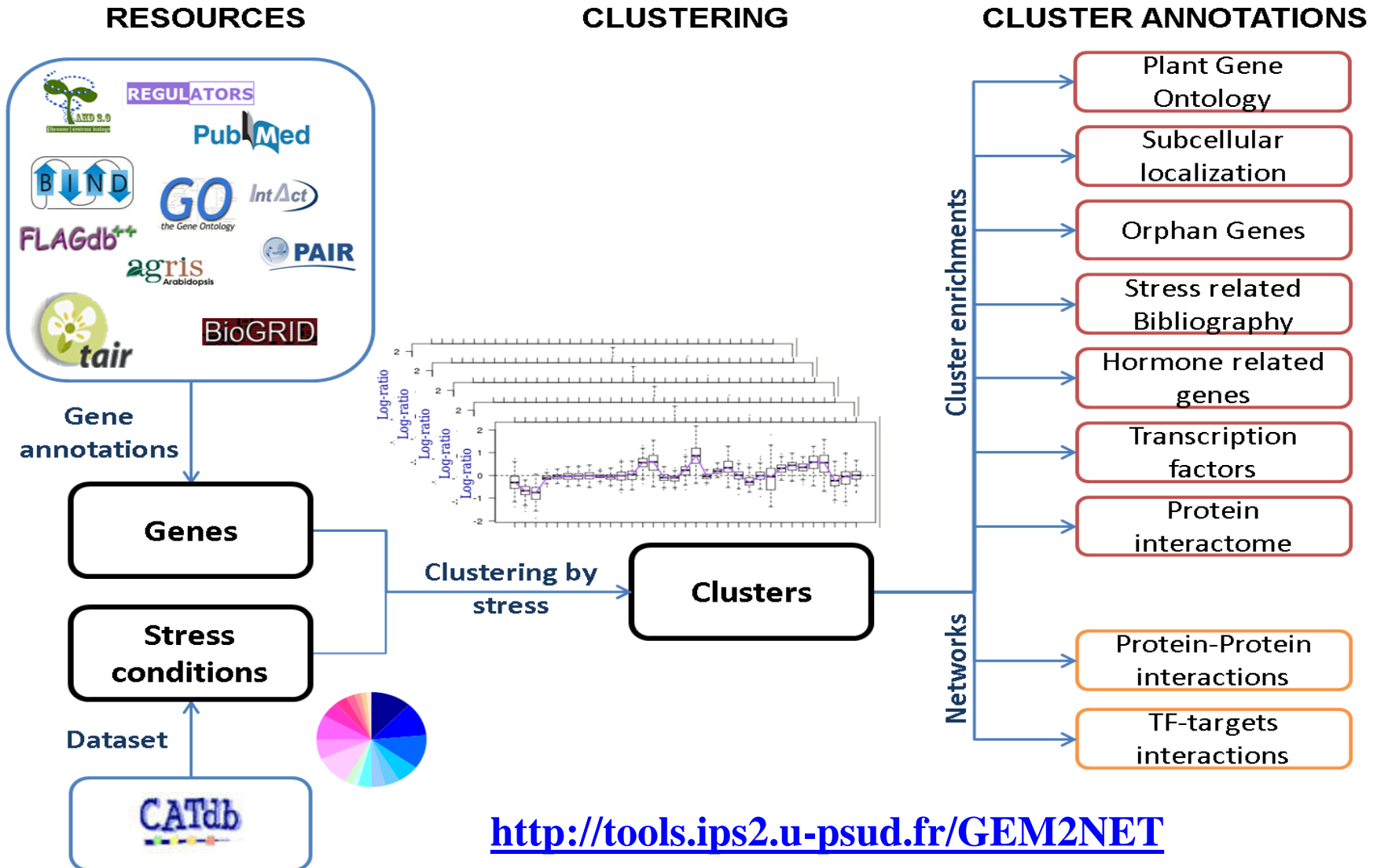


Stress category	Gene_nb	Cluster_nb
Nitrogen	13 495	59
Temperature	11 365	34
Drought	8 143	34
Salt	5 729	30
Heavy metal	10 617	57
UV	7 894	37
Gamma	5 350	32
Oxydative stress	10 127	52
Nectrophic bacteria	11 220	50
Biotrophic bacteria	12 023	56
Fungi	9 773	51
Rhodococcus	1 900	13
Oomycete	5 508	31
Nematode	7 413	27
Stifenia	1 525	17
Virus	11 832	54

18 stress categories

GEM2Net Flowchart

This project has been implemented as a new CATdb module: GEM2Net associated with a user-friendly Interface (Zaag et al., 2015)



<http://tools.ips2.u-psud.fr/GEM2NET>

Cluster Annotations

681 coexpression clusters covering 17264 genes

- 98% of clusters have a functional bias in a term of GO
- 80% of clusters are associated to a Stress term

GEM2Net gene set

	Total	Orphan	BP stress	Bibliostress	TF	Hormone
Arabidopsis Reference	34042	5105 (15%)	5106 (15%)	2580 (7.5%)	2260 (6.5%)	695 (2%)
GEM2Net dataset	17264	2165 (13%)	4003 (23%)	2064 (12%)	1578 (9%)	487 (3%)

GEM2Net Snapshot:



from Gene Expression Modeling To Networks in Plants

< back to Stress categories & Search

Stress category: VIRUS

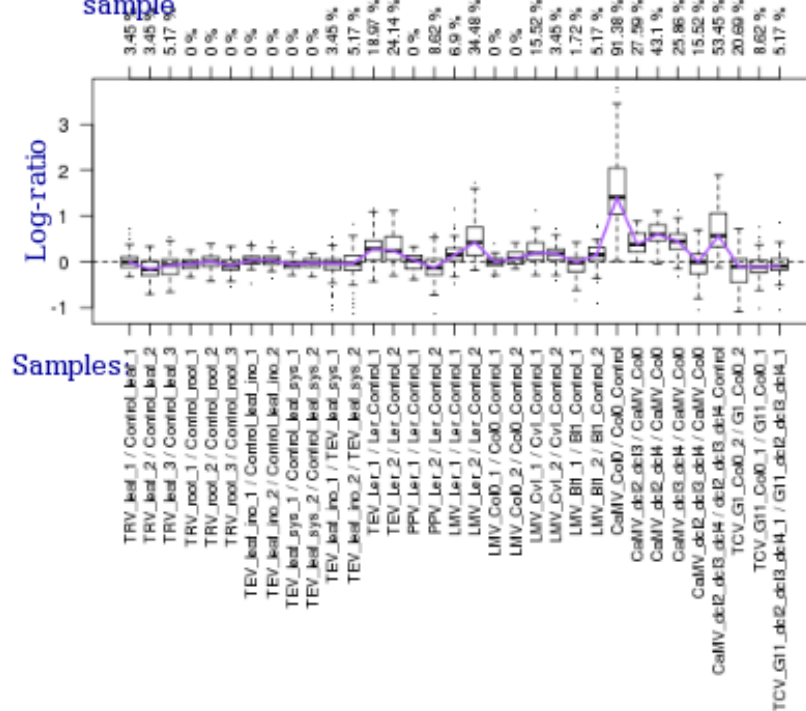
Total genes: 11685 # Clusters: 54 Classification rule: MFDR # Classified genes: 6053 # CATAB projects: 5 >>

Clustering **Biological process** Cellular component Molecular function Subcell Bibliostress Orphan Transcription factor Hormone Interactome Networks

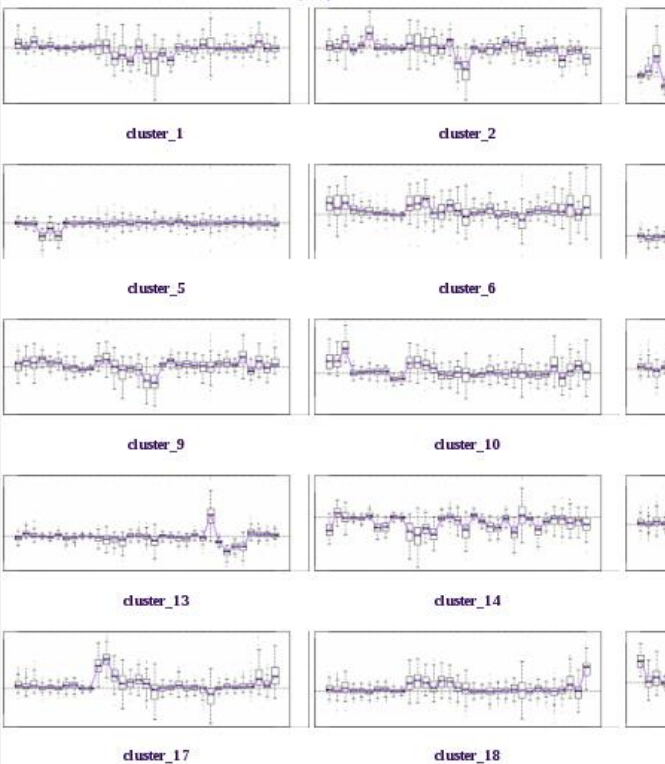
The box-plot charts displayed below result from the model-based clustering method performed on expression data for the stress category VIRUS. Then, the MFDR classification rule was applied to assign genes in clusters with high confidence. While the mouse hovers over a graph, the total number of genes in cluster appears in a popup and in the cluster are summarized as blue dots to give a synoptic view.

Stress category VIRUS cluster_7 60 genes

Percentage of differentially expressed genes in each sample



click on a Cluster name to see all Functional Analyses for the cluster



GEM2Net Snapshot:



from Gene Expression Modeling To Networks in Plants

< back to Stress categories & Search

Stress category: VIRUS

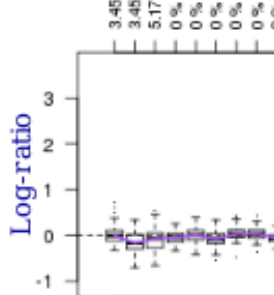
Total genes # Clusters Classification rule # Classified genes # CATdb projects
 11685 54 MFDR 6053 5 >>

Clustering Biological process Cellular component Molecu

The box-plot charts displayed below result from the meta-hooc to assign genes. While the meta-hooc cluster are s

Stress category

Percentage of d sample



Samples

TRV_leaf_1 / Control_leaf_1
 TRV_leaf_2 / Control_leaf_2
 TRV_leaf_3 / Control_leaf_3
 TRV_root_1 / Control_root_1
 TRV_root_2 / Control_root_2
 TRV_root_3 / Control_root_3
 TEV_leaf_ino_1 / Control_leaf_ino_1
 TEV_leaf_ino_2 / Control_leaf_ino_2

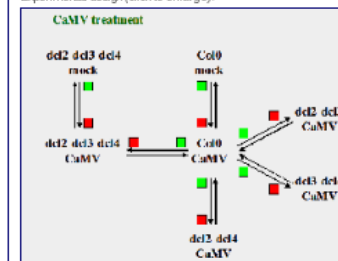


CATdb a Complete Arabidopsis Transcriptome database

~ Project: RS06-06-miRNA_CaMV_treatment ~ Experiment: CaMV treatment ~

Project name	RS06-06-miRNA_CaMV_treatment
Project title	Gene profiling during CaMV infection.
Biological interest	Which genes (including miRNA genes) are differentially regulated by CaMV? Requirement of 21nt, 22nt, 24nt virus-derived siRNAs in this differential gene regulation?
Experiment name	CaMV treatment
Experiment type	gene knock out, normal vs disease comparison
Experiment factors	cell line
Type of array	Arrays type CATMA
Overall design	7 dye-swap (technical replicates)
Contributor	Olivier, Voinnet
Contributor	Ludvine, Taconat
Contributor	Jean-Pierre, Renou
Contributor	Marie-Laure, Martin-Magniette
Experiment description	4/5 weeks old seedlings (Col-0, dd2dd3, dd2dd4, dd3dd4, dd2dd3dd4) infected with CaMV and harvested 21 days post infection.
Public repository	GEO accession# GSE36457

Experimental design (click to enlarge):



Access normalized data for this experiment

Swap: CaMV_Col0 / Col0_Control

Swap name	CaMV_Col0 / Col0_Control
Array type	CATMA_2_3
Channel	Cy5
Sample name	Col0 CaMV
organism	Arabidopsis thaliana (columbia)
organ	rosette
characteristics	ecotype: columbia - genotype: col-0 - age:
mutation	none
treatment protocol	Name: CaMV Infection - pathogen - pathogen infection: camv time 21 day - Infection of 4/5 week old plant using CaMV SAP (mechanical infection of virions upon 2 leaves). Mock inoculation using H2O as control.
growth protocol	rosette - Media=Soil Temperature=22°C Light=short day (8h) After treatment : Media=Soil Temperature=24°C Light=long day (12h)
molecule type	total RNA
extract protocol	Col0 CaMV: 10 ug -
labelling protocol	labelling Cy3 and Cy5 direct, amplify=yes, aRNA 5ug
Channel	Cy3
Sample name	Col0 Mock
organism	Arabidopsis thaliana (columbia)
organ	rosette
characteristics	ecotype: columbia - genotype: col-0 - age:
mutation	none
treatment protocol	Name: Mock Treatment - control buffer - control buffer: water time 21 day - Infection of 4/5 week old plant using CaMV SAP (mechanical infection of virions upon 2 leaves). Mock inoculation using H2O as control.
growth protocol	rosette - Media=Soil Temperature=22°C Light=short day (8h) After treatment : Media=Soil Temperature=24°C Light=long day (12h)
molecule type	total RNA
extract protocol	Col0 Mock: 10 ug -
labelling protocol	labelling Cy3 and Cy5 direct, amplify=yes, aRNA 5ug

GEM2Net Snapshot:

Stress category: **VIRUS**

Total genes 11685 # Clusters 54 Classification rule MFDR # Classified genes 6046 # CATdb projects 5 >>

Clustering **Biological process** Cellular component Molecular function Subcell Bibliostress Orphan Transcription factor Hormone Interactome Networks

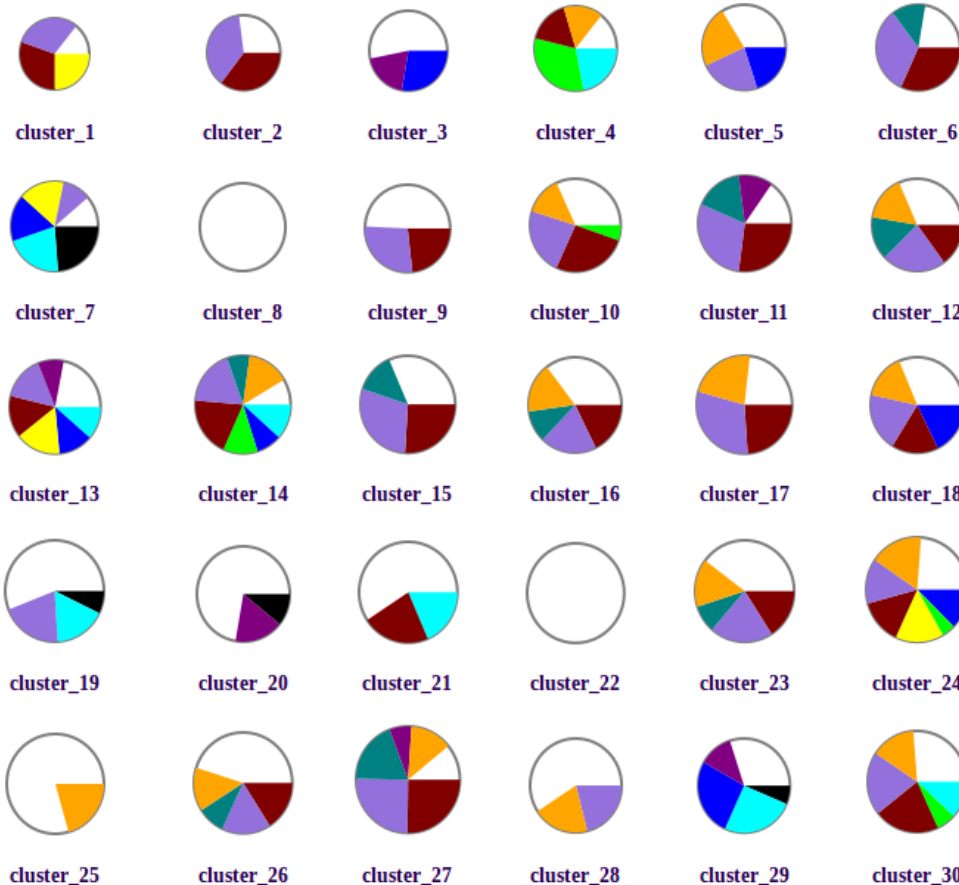
The GO Biological process was used to characterize the clusters for the stress category VIRUS. Results of gene set enrichment analyses are displayed as one pie chart per cluster, its size reflecting the total number of genes in the cluster.

While the mouse hovers over a pie chart, the total number of genes in cluster appears in a popup and in the 'Biological process' frame on the right side. As well, the number of genes annotated with a GO term is displayed and the hypergeometric test p-value is mentioned when statistical significance is achieved.

Legends

DNA_or_RNA_metabolism	●
cell_organization_and_biogenesis	●
developmental_processes	●
electron_transport_or_energy_pathways	●
protein_metabolism	●
response_to_abiotic_or_biotic_stimulus	●
response_to_stress	●
signal_transduction	●
transcription_DNA_dependent	●
transport	●

click on a Cluster name to see all Functional Analyses for the cluster // click inside a circle to see the Clustered Gene list



Biological process

31 genes in cluster_2

term name	nb genes	p-value	Ref nb
response_to_abiotic_or_biotic_stimulus	17	3.64e-9	3758
response_to_stress	18	1.57e-9	4117
protein_metabolism	1		
cell_organization_and_biogenesis	2		
electron_transport_or_energy_pathways	2		
developmental_processes	4		
signal_transduction	4		
transport	6		
unknown_biological_processes	6		
other_metabolic_processes	15		
other_cellular_processes	16		
other_biological_processes	20		

Ref nb: number of genes annotated with the term in the reference set
(see documentation)

GEM2Net Snapshot:

Stress category: VIRUS					cluster_49	
# Total genes	# Clusters	Classification rule	# Classified genes	# CATdb projects	# Protein-protein interactions	# TF-target interactions
11685	54	MFDR	6046	5 >>	42	0

- Clustering
- Biological process
- Cellular component
- Molecular function
- Subcell
- Bibliostress
- Orphan
- Transcription factor
- Hormone
- Interactome
- Networks**

Networks of Protein-protein interactions or Target genes of Transcription factors (TFs) are shown for a selected cluster. By default, all protein interactions (experimental and predicted interactomes), as well as confirmed links of TFs to their targets are displayed for gene accessions inside the selected cluster. Out-cluster interactions can be seen on option. Functional annotation is available to characterize nodes. On the right frame, Filters are provided to view only nodes of the selected term(s). Additional information is available on the bottom side by clicking on a node or an edge.

Notice that this is a beta-test version

Select a cluster: cluster_49

FUNCTIONAL ANNOTATION: Transcription Factor Hormone All Hormone Orphan

PROTEIN INTERACTOMES **TARGETS of TRANSCRIPTION FACTORS**

in-Cluster interactions: All interactions Confirmed interactions *in-Cluster interactions:* Confirmed interactions

options: Self interactions out-Cluster interactions (confirmed) *option:* out-Cluster interactions (confirmed)

Filter **Search** **Save**

Use filters to view nodes of the selected item(s)

Filter by GO terms

BIOLOGICAL PROCESS

Terms	Pvalue
response_to_stress	
other_cellular_processes	
other_metabolic_processes	
protein_metabolism	
response_to_abiotic_or_biotic_stimulus	
unknown_biological_processes	
cell_organization_and_biogenesis	
transcription_DNA_dependent	
developmental_processes	
electron_transport_or_energy_pathways	
other_biological_processes	
DNA_or_RNA_metabolism	
transport	

CELLULAR COMPONENT

MOLECULAR FUNCTION

↶ ↷ ↵ ↶ +

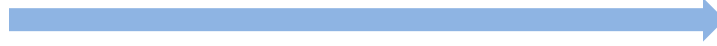
Layout: Circle Node Labels

From co-expression to co-regulation

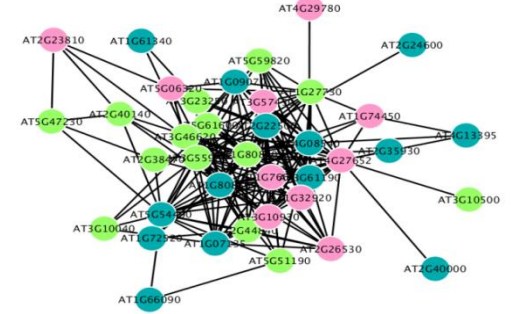


Clusters of co-expression
By stress

Horizontal integration

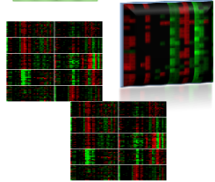
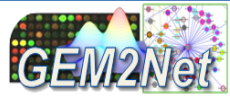


across the 18 stresses



Co-regulation networks

From co-expression to co-regulation

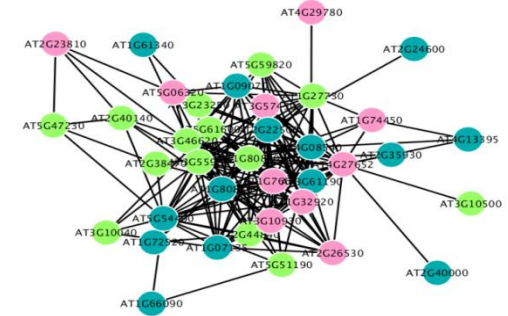


Clusters of co-expression
By stress

Horizontal integration



across the 18 stresses



Co-regulation networks

- Method : Identify pairs of genes co-expressed in different stress categories compared to random networks (resampling test)

#stress	2+	3+	4+	5+	6+	7+	8+	9+	10+	11+	12+	13+	14+
#paires	295105	57833	19087	7997	3782	1908	903	395	190	83	36	9	2
# Paires aléatoires	68338	733	5	~0	0	0	0	0	0	0	0	0	0
Taux d'erreur	23,15%	1,2%	0,026 %	0	0	0	0	0	0	0	0	0	0

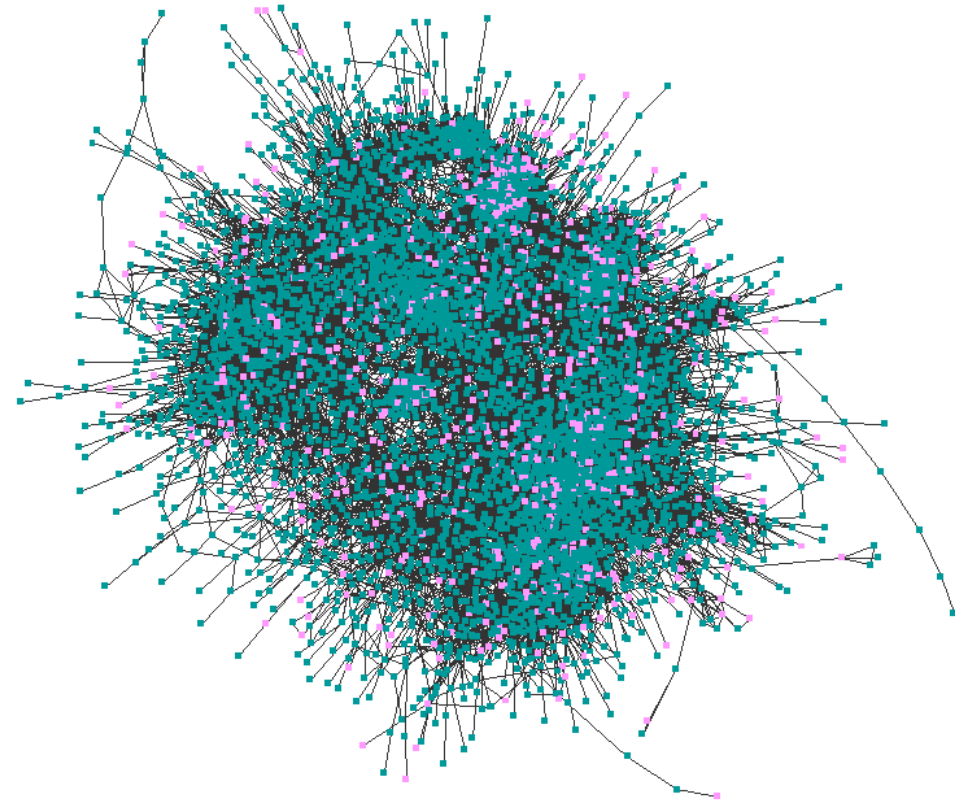
- a pair observed more than 3 times is statistically significant

Co-Regulation (R. Zaag PhD)

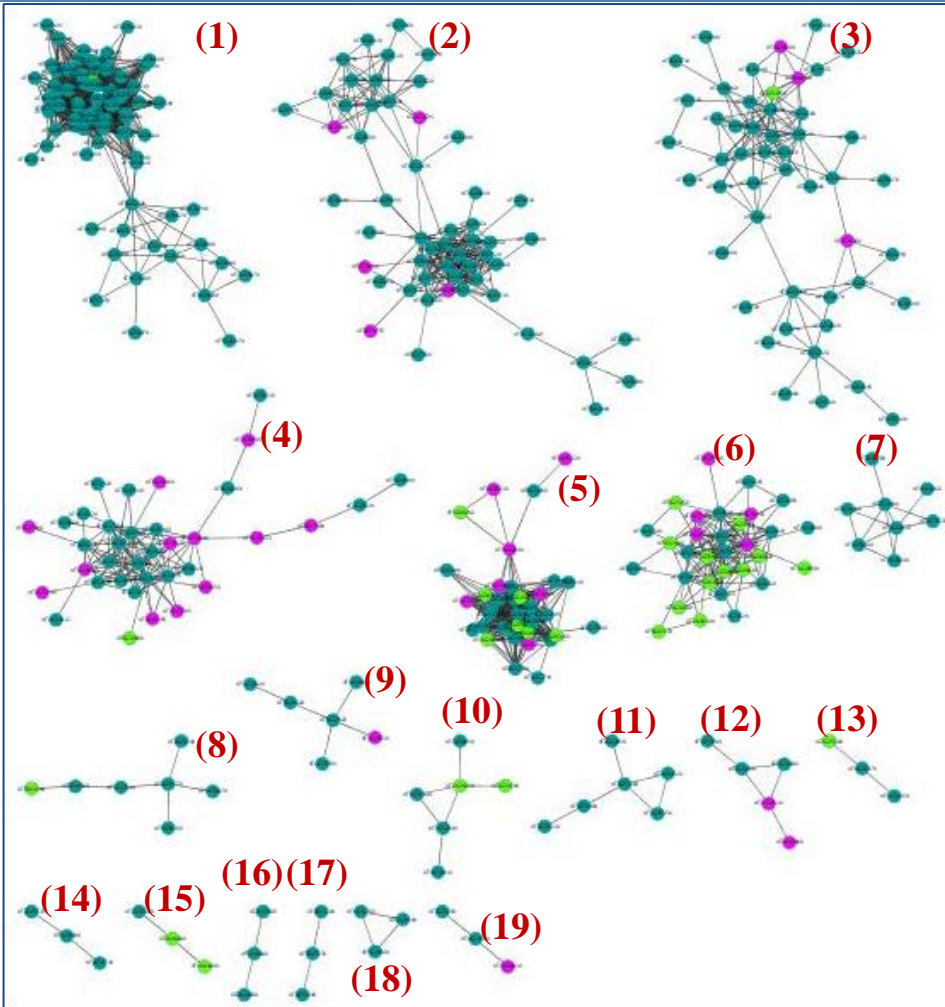
At Network « 3+ »
stresses scale



5626 nodes 57 833 edges/pairs
→ 713 orphans



Co-regulation Network (7 stresses)



The network with gene pairs conserved in at least 7 stresses is the first network showing connected components

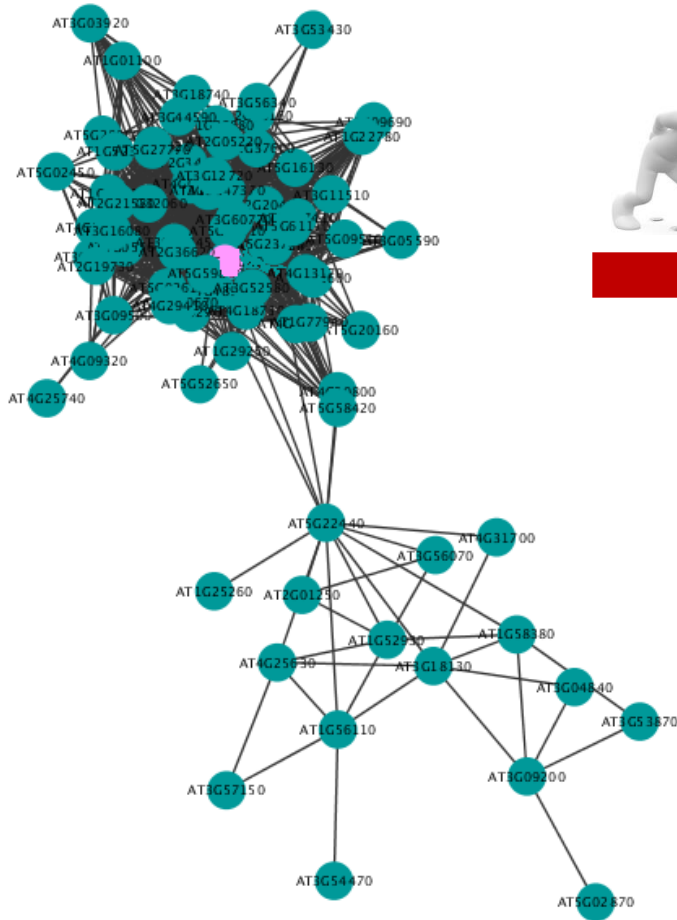
Legend

- Coregulated genes
- Orphan genes

415 genes with 41 orphan genes, 1908 interactions

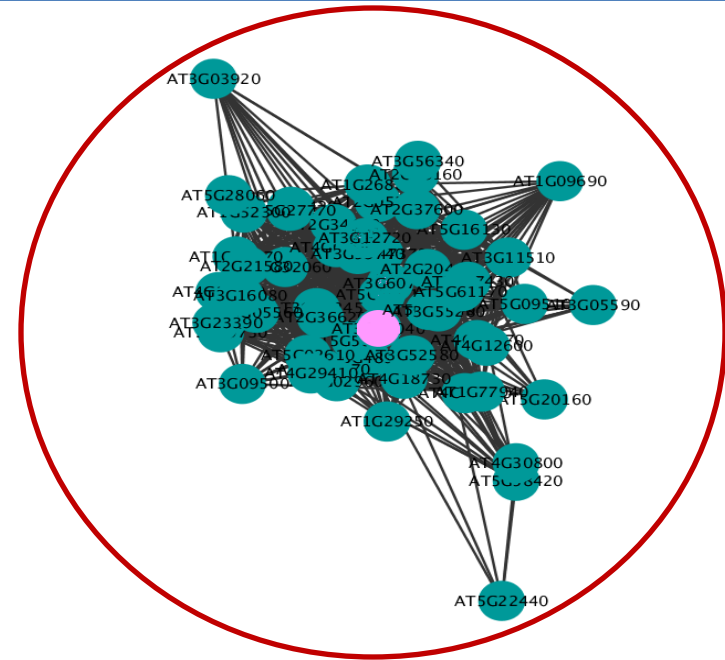
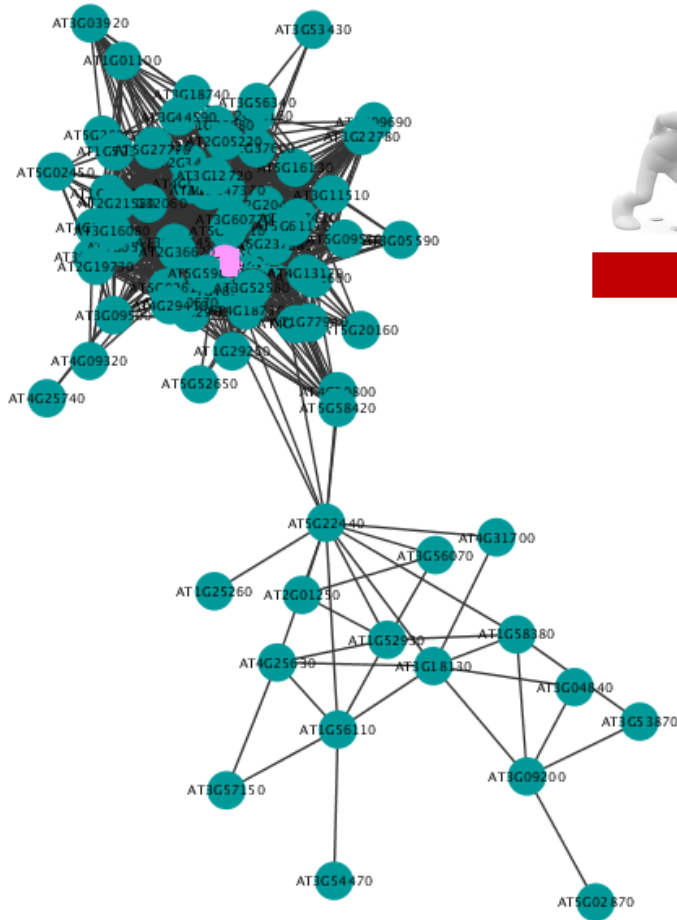
Example of an orphan identification within a module

81 genes, 945 pairs, 1 orphan



Example of an orphan identification within a module

81 genes, 945 pairs, 1 orphan



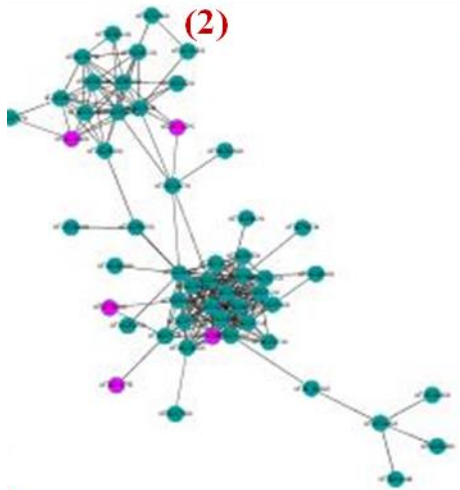
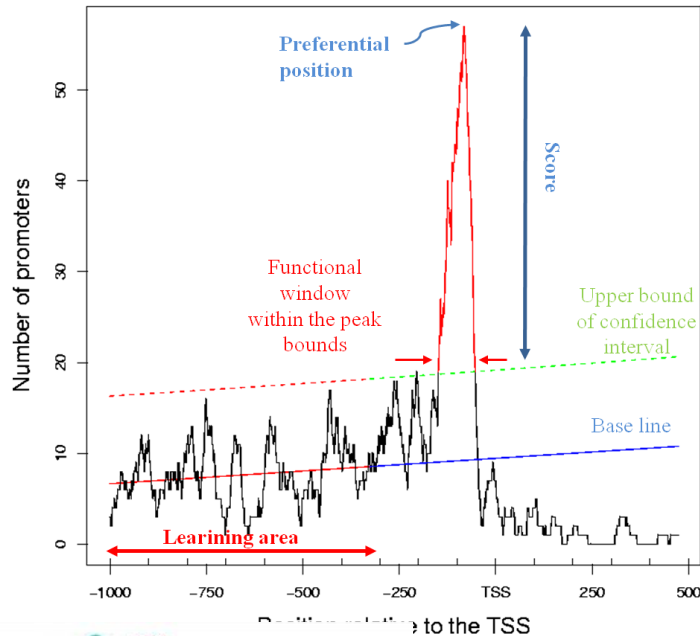
First Neighbors of the orphan gene

48 of the 55 neighbors are annotated as « Structural constituent of ribosome »

This orphan gene most likely codes for a ribosomal protein

Cis-regulatory motifs enrichment

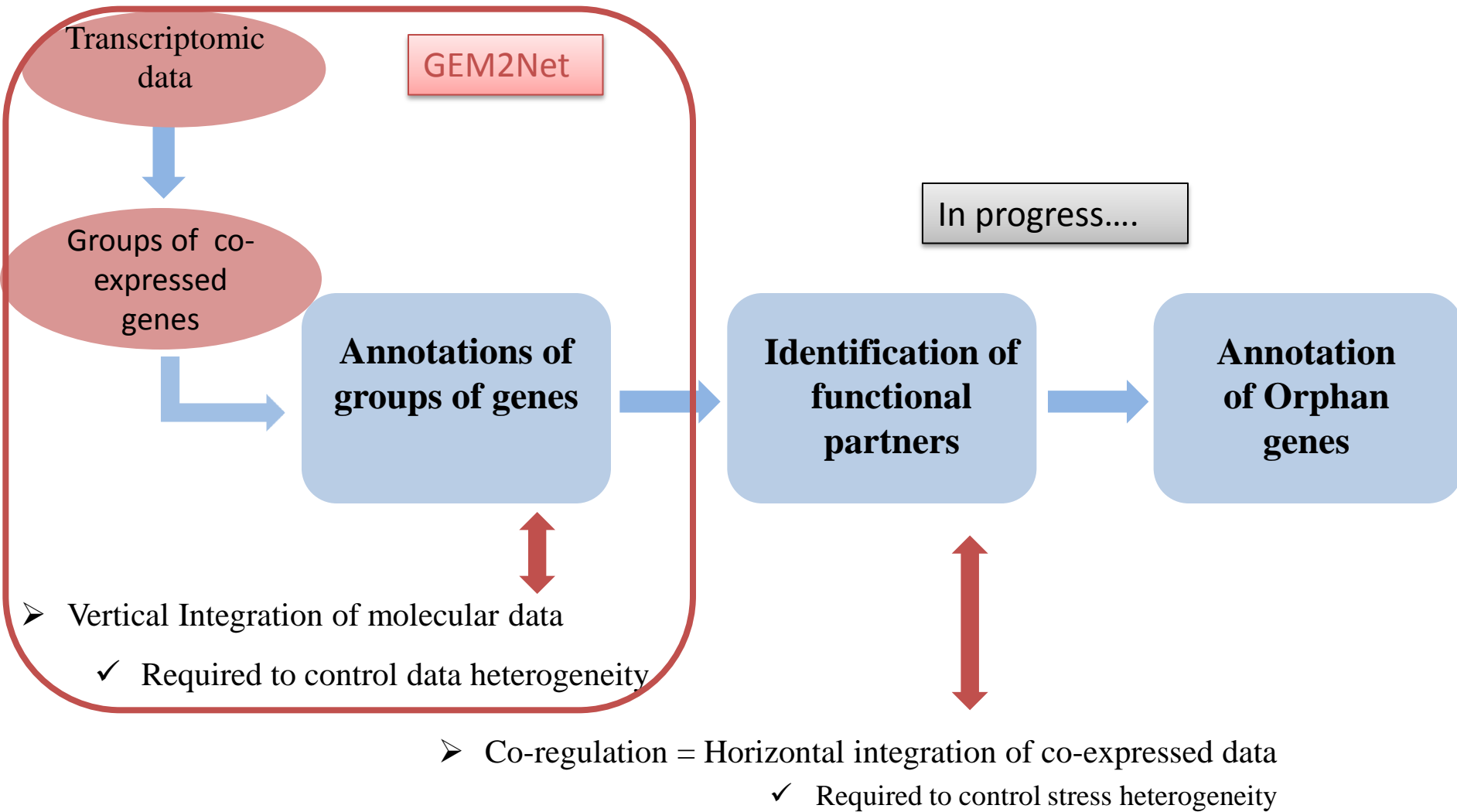
Distribution of CACGTG relative to the TSS



cis-regulatory motifs found by PLMdetect (Bernard et.,2010)

- 10 components are enriched in cis-regulatory motifs
- For 4 of them, the motif is present in over 80% of the gene promoters
- Component 2 has 5 motifs related to the light regulation, present at most in 50% of gene promoters

From Gene Expression Modeling to gene Network



Actors in GEM2Net project

Bioinformatics

ML Martin-Magniette (team GNet)

R. Zaag (PhD)

V. Brunaud

J.-P. Tamby

C. Guichard

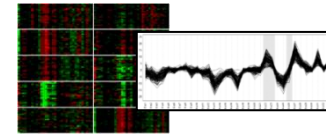
Z. Tariq

G. Rigail

S. Aubourg



Statistics



G. Celeux

C. Maugis

T. Mary-Huard



Biology



E. Delannoy

J-P Renou

S. Balzergue



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