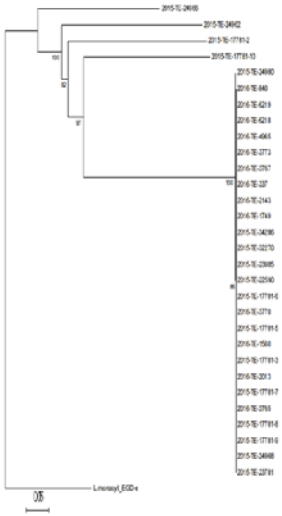
Lm 2015TE24968 multi-system response to Salt-pH-Temp stress

V. Di Lollo, M.Orsini

Teramo, Lm Workshop 4th, July 2019

Lm 2015TE24968, a success history?

- >38 infected people
- Growth conditions
- Spreading
- Re-emerging (2018)



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Genome Sequences

Whole-Genome Sequence of a Reemerging *Listeria monocytogenes* Serovar 1/2a Strain in Central Italy

Massimiliano Orsini, Marina Torresi, Claudio Patavino, Patrizia Centorame, Antonio Rinaldi, Vicdalia Aniela Acciari, Gabriella Centorotola, Maurilia Marcacci, Massimo Ancora, Marco Di Domenico, Giuliana Blasi, Anna Duranti, Samuel Perticara, Adriano Di Pasquale, Cesare Cammà, Francesco Pomilio, Giacomo Migliorati

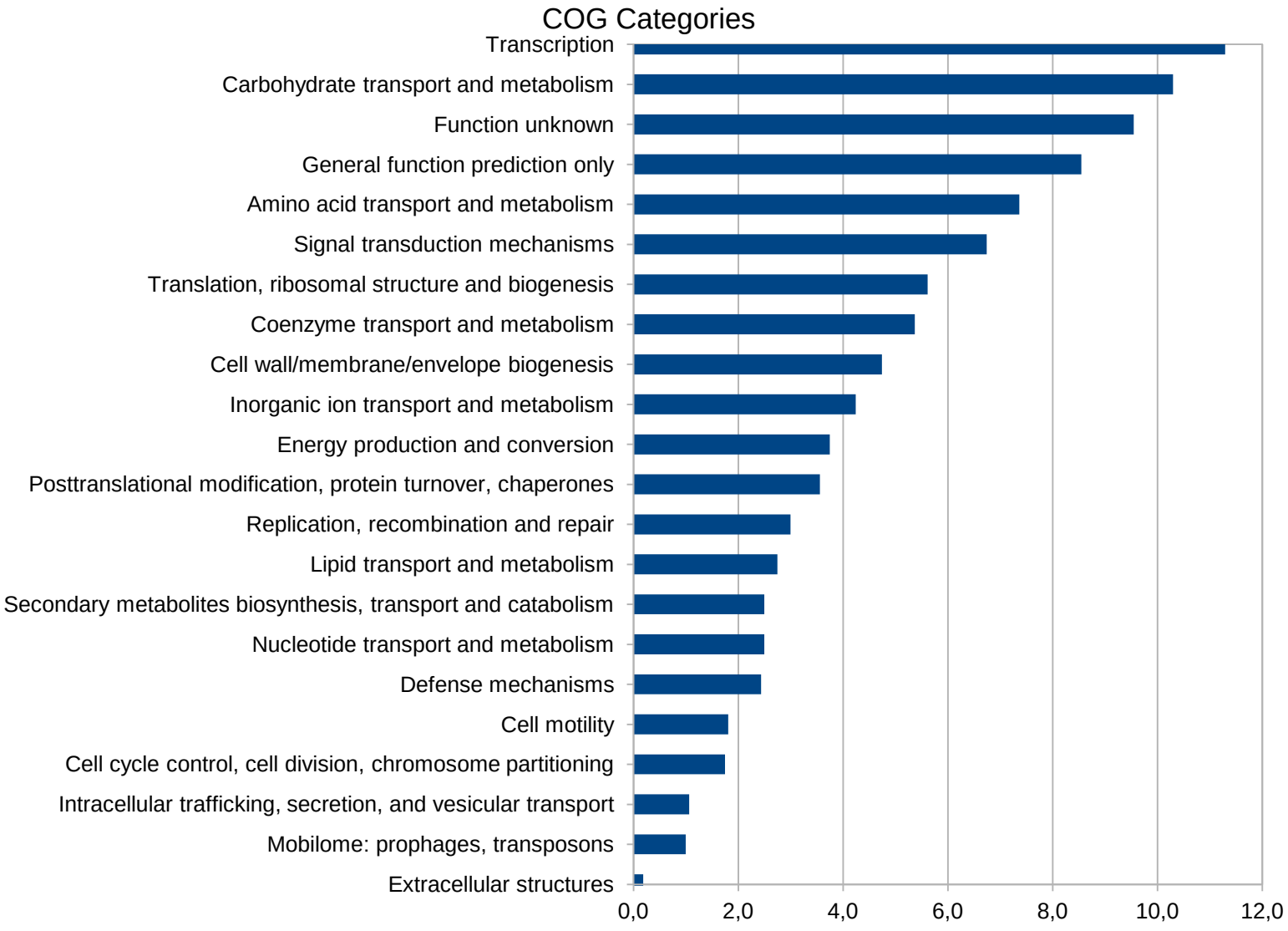
Aim and Design

To identify functional elements of such a fitness by integrating Systems Biology and Differential Expression Analysis

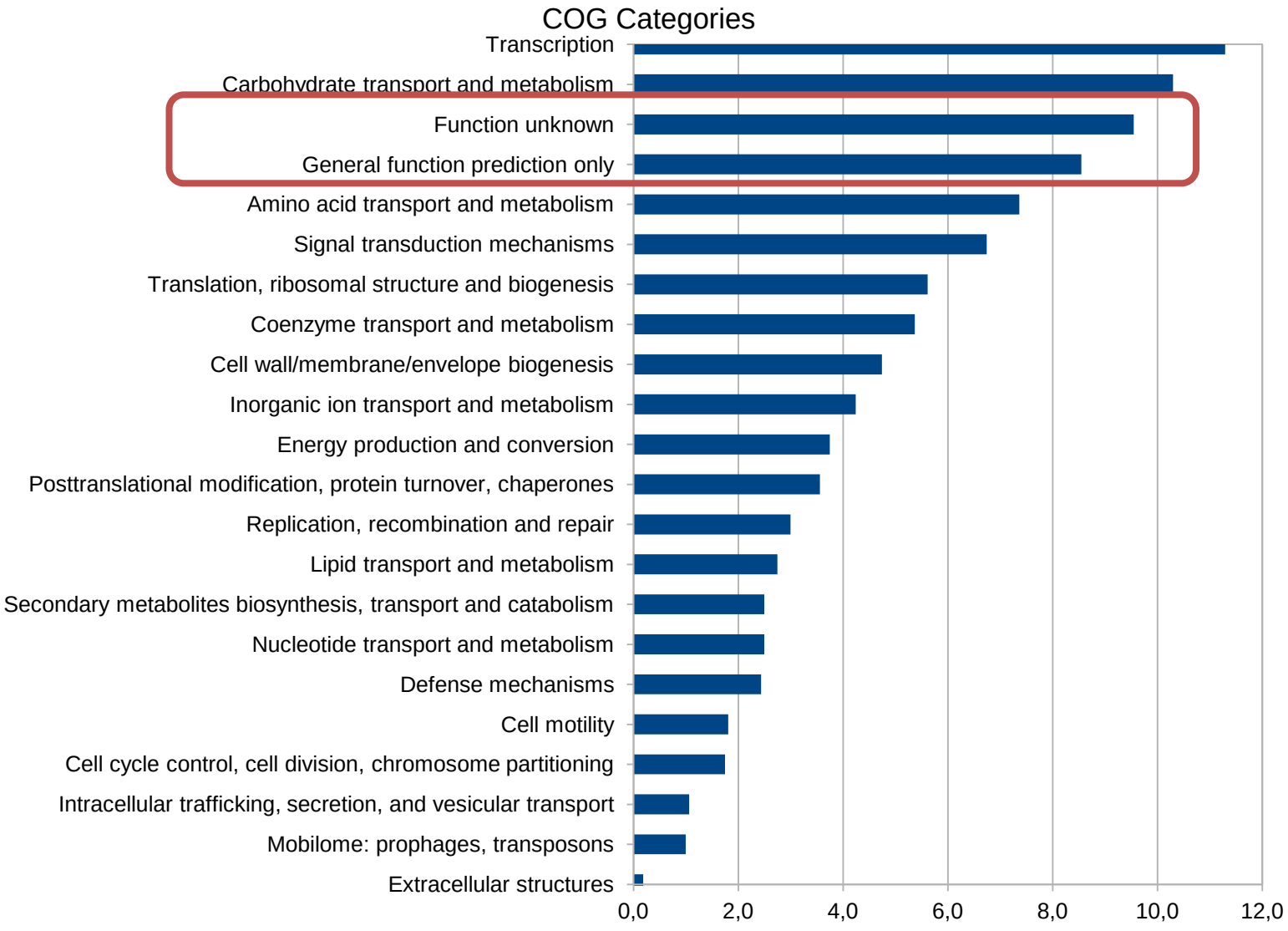
Lm 2015TE24968 proteome consists of:

- 2923 proteins
- 64 encoded by the Plasmid
- 2425 (82.9%) have a COG annotation
- 44 do not have a match in Uniprot
  - 25 of them are hypothetical
- 1078 (36.8%) have a GO term
- 397 hypothetical
  - 372 have a match in Uniprot
- 31 frameshifted

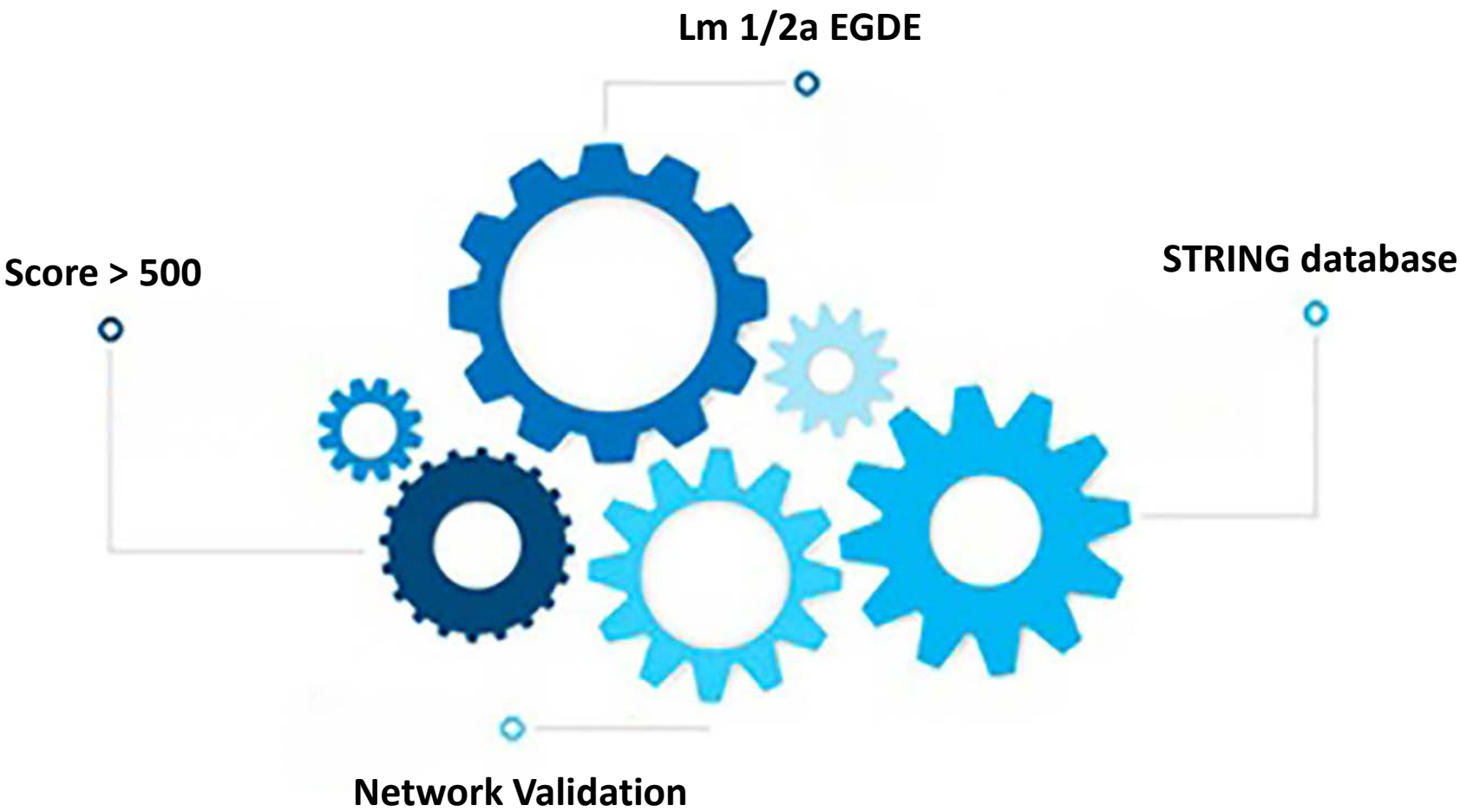
Lm 2015TE24968 proteome consists of:



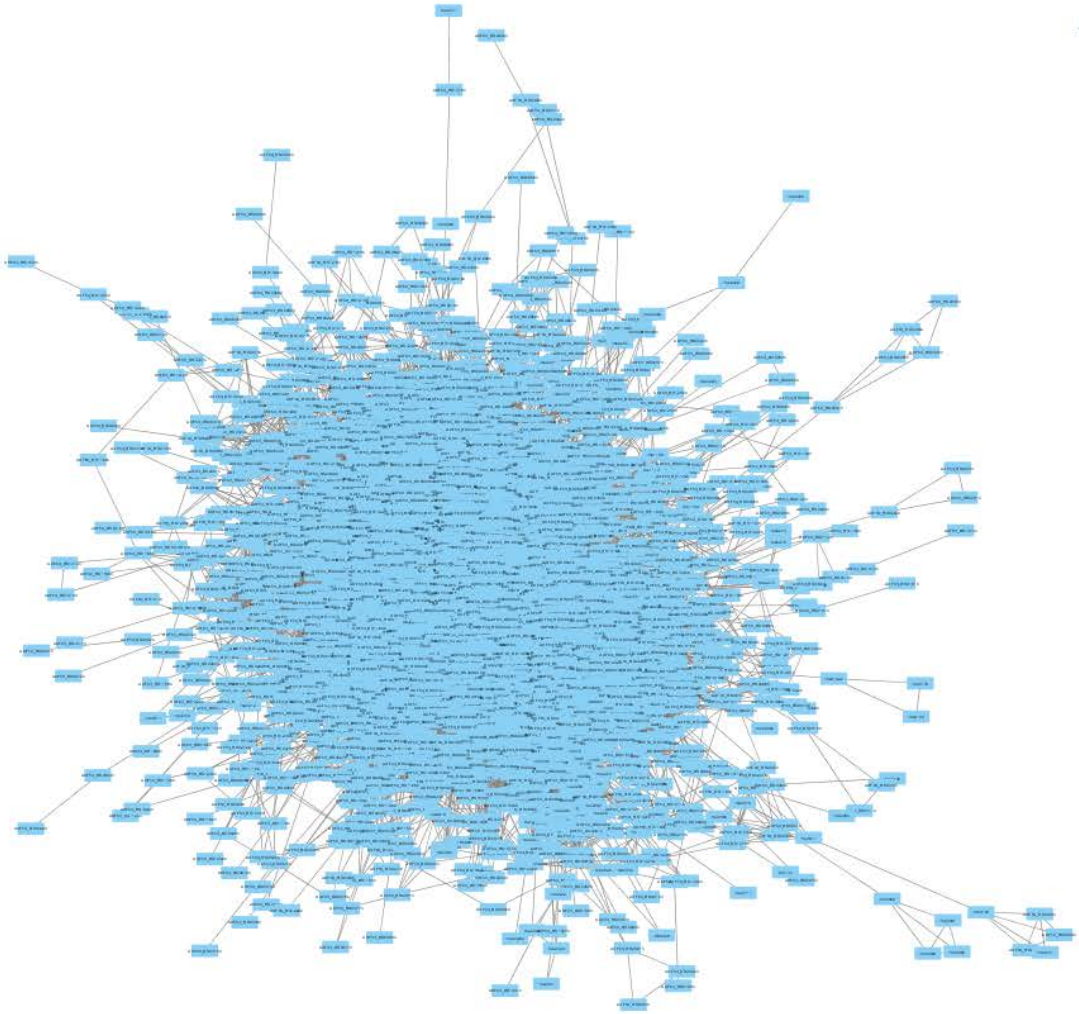
Lm 2015TE24968 proteome consists of:



Drawing the PPIN

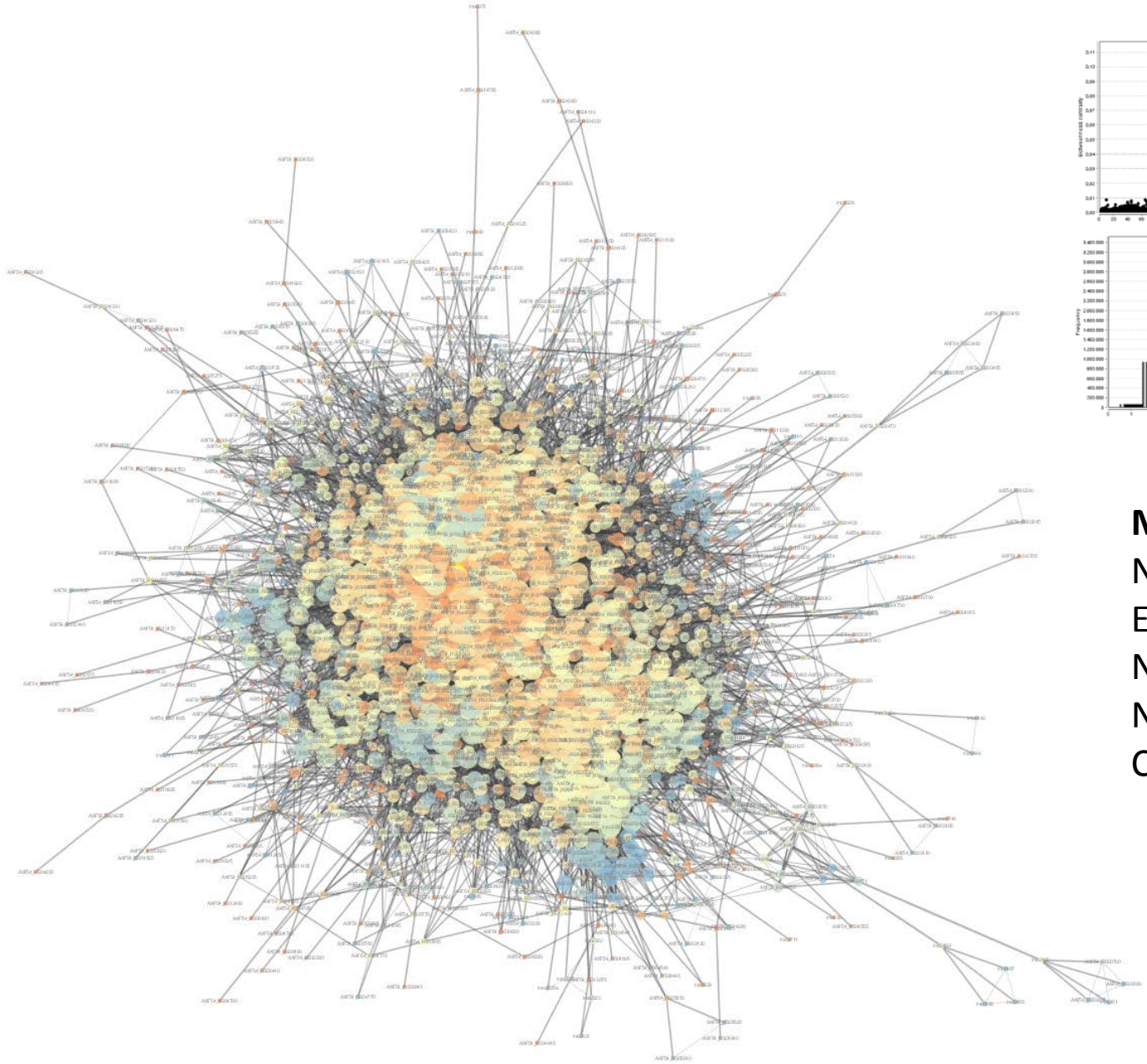


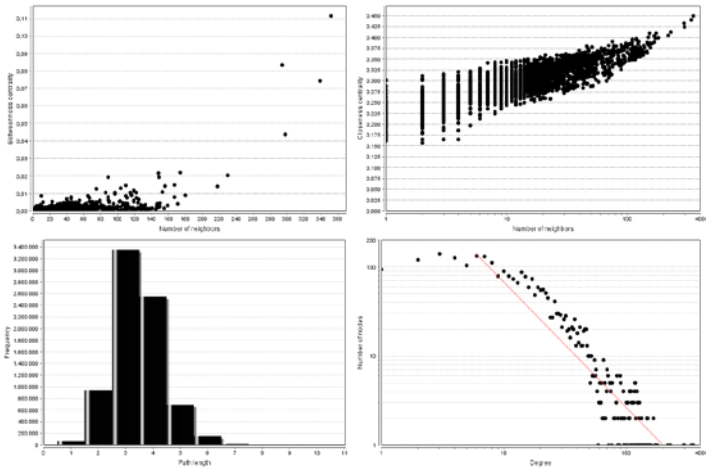
Lm 2015TE24968 PPIN



- **14** components
- **2827** nodes
- **32180** edges

Lm 2015TE24968 PPIN Topological Parameters



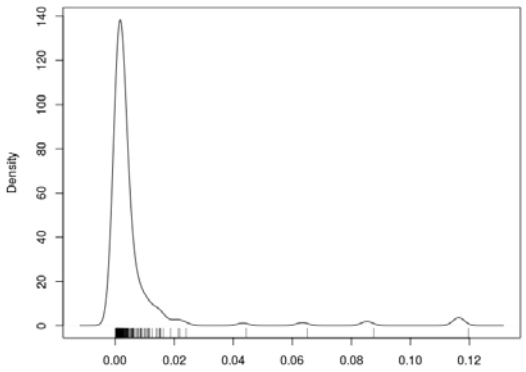


Main Component:

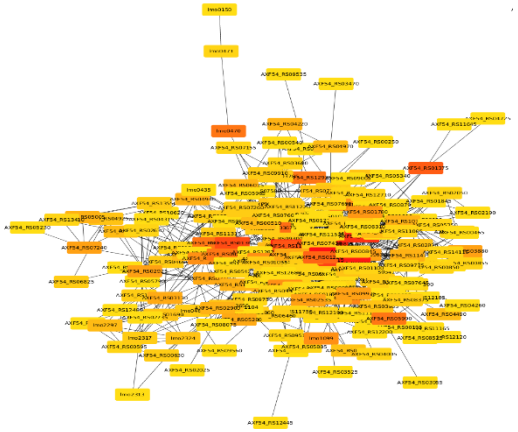
Nodes : 2788
Edges : 32135
Network diameter : 10
Network Centralization : 0.118
Characteristic Path Len : 3.440

Hubs, Bottlenecks, Modules

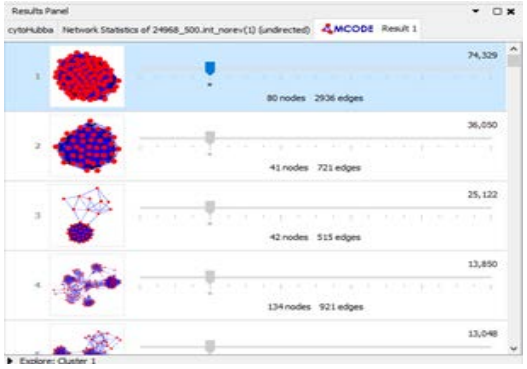
- Hubs (degree > avg_degree + stddev): **220**
  Date hub**: 7;** *Party* hub**: 213**



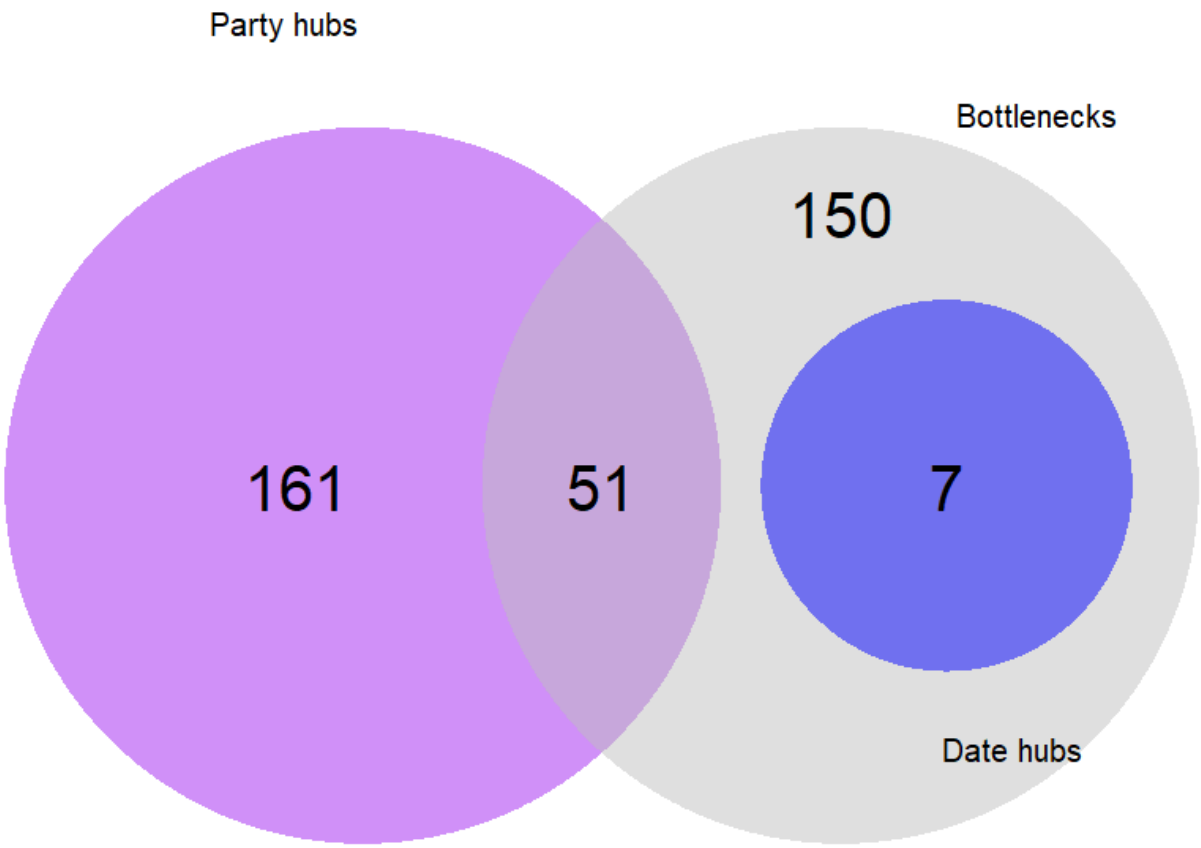
- Bottlenecks: arbitrarily fixed at: **220**
  higher betweenness



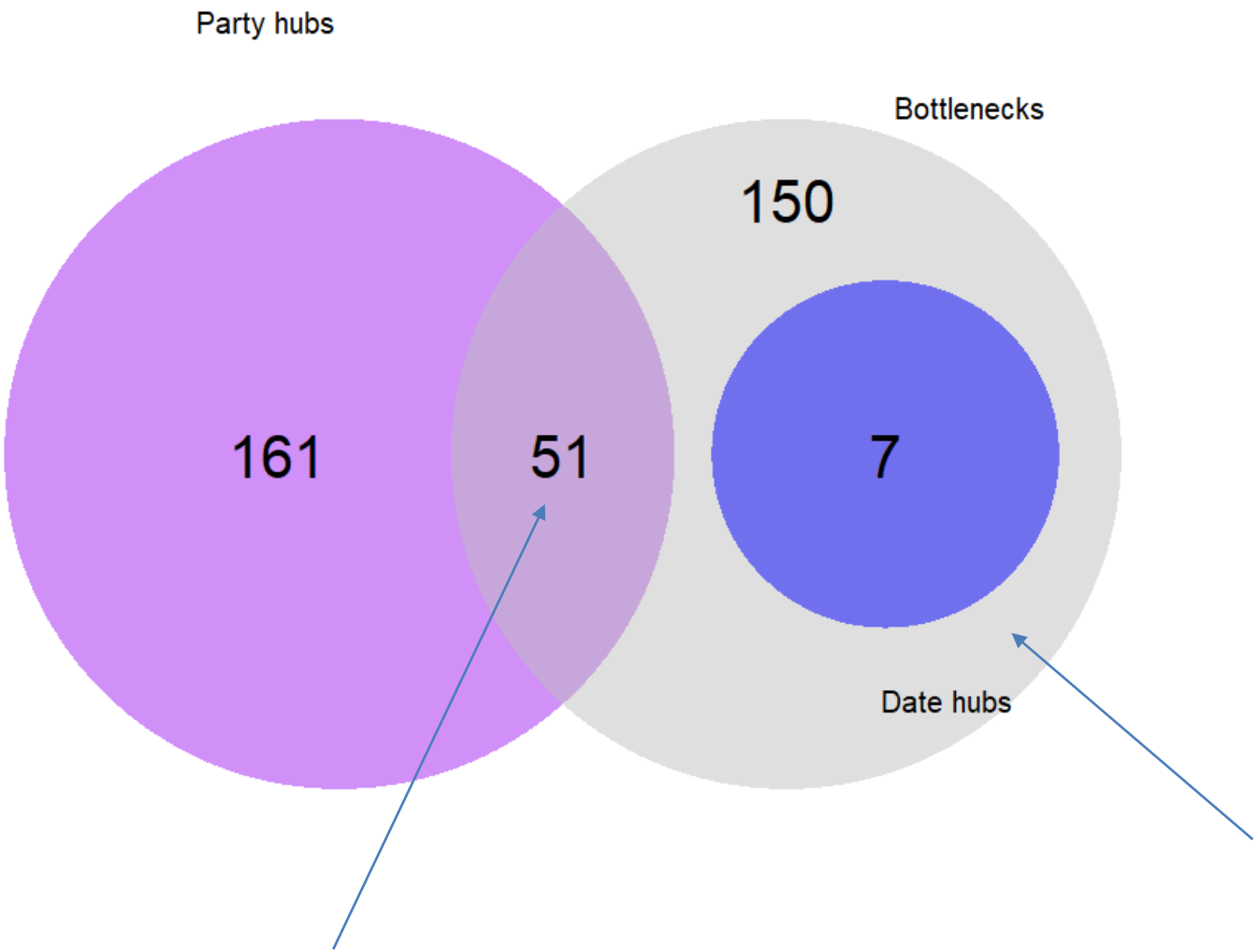
- Modules (subgraphs highly interconnected): **89**



Hubs, Bottlenecks, Modules



Hubs, Bottlenecks, Modules

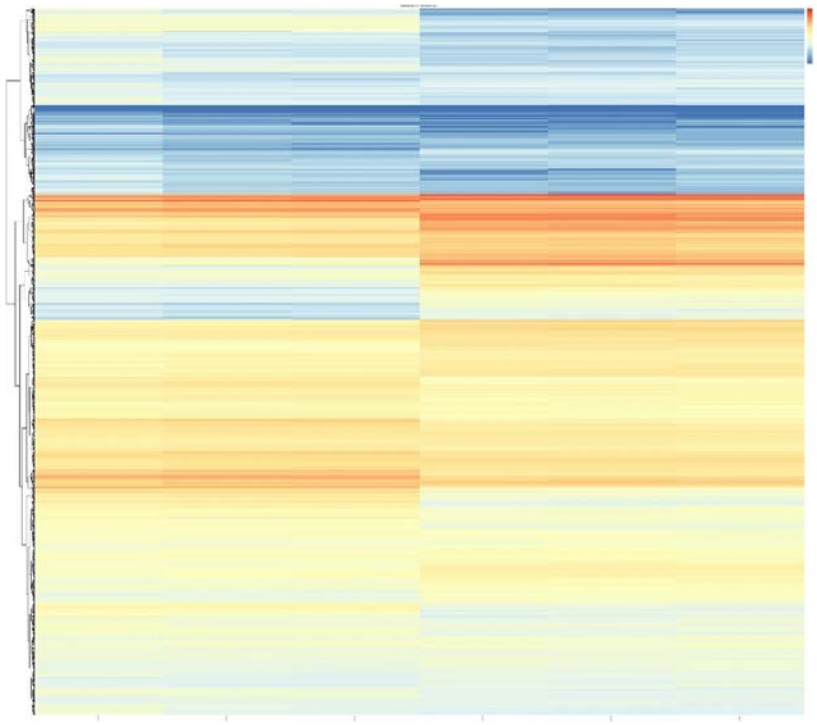


Take a breath….

Differential Expression Analysis

Reference
37°, pH 7.5

Stressed
12°, pH 5.5, NaCl 4.5%



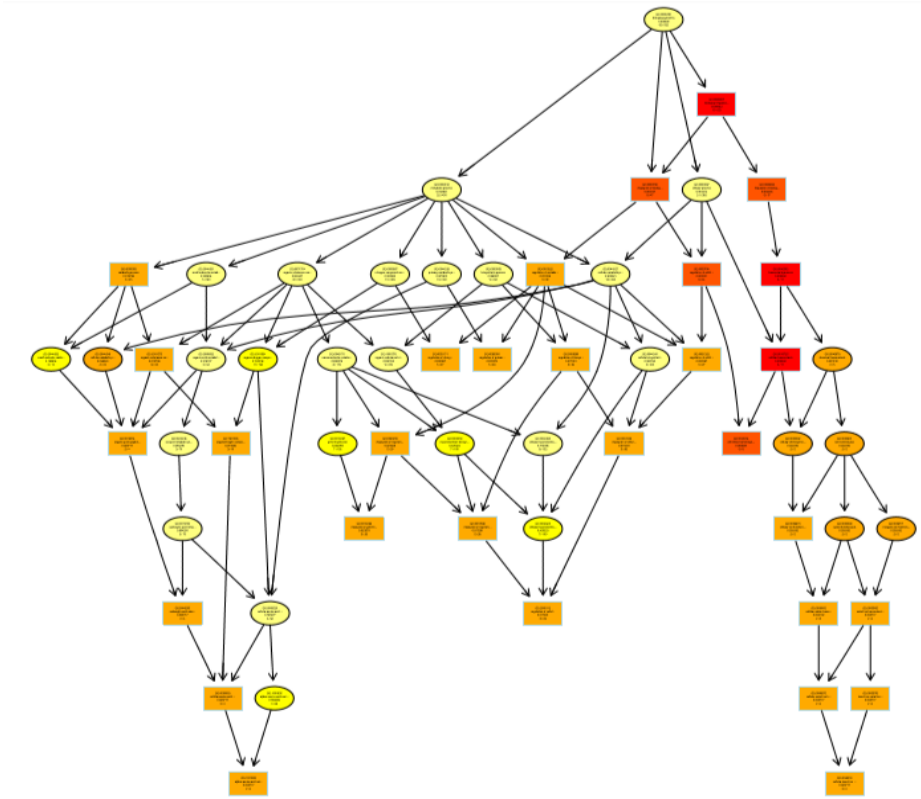
Expression Detected for **1850** loci,
636 Differentially Expressed, DEGs (qval < 0.05, |log(**FC**)| > 2)

DEGs: Gene Ontology

BP	Term	Annotated	Significant	classicFisher
GO:0001539	cilium or flagellum-dependent cell motil...	5	5	2,20E-06
GO:0006928	movement of cell or subcellular componen...	5	5	2,20E-06
GO:0048870	cell motility	5	5	2,20E-06
GO:0051674	localization of cell	5	5	2,20E-06
GO:0071973	bacterial-type flagellum-dependent cell ...	5	5	2,20E-06
GO:0097588	archaeal or bacterial-type flagellum-dep...	5	5	2,20E-06
GO:0040011	locomotion	6	5	1,20E-05
GO:0051179	localization	98	15	0,0033
GO:0006935	chemotaxis	3	2	0,0166
GO:0042330	taxis	3	2	0,0166
GO:0071702	organic substance transport	52	8	0,0361
GO:0009056	catabolic process	35	6	0,0432
GO:1901575	organic substance catabolic process	35	6	0,0432

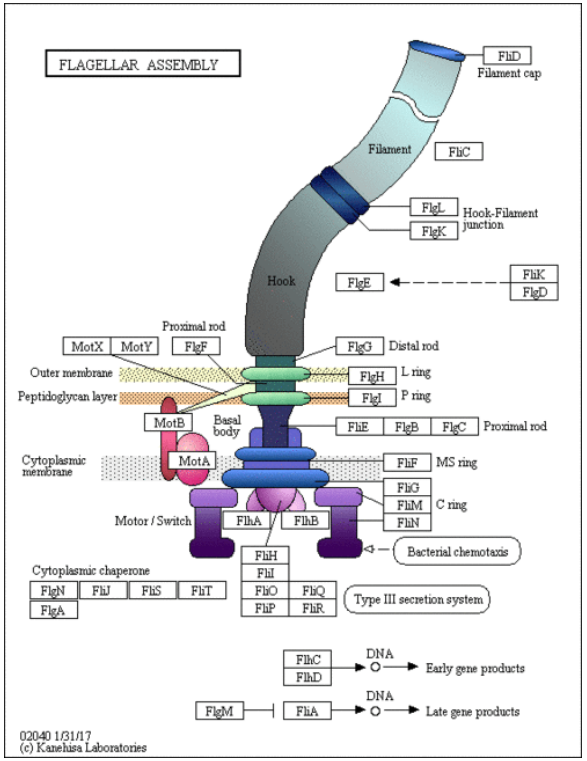
CC	Term	Annotated	Significant	classicFisher
GO:0009288	bacterial-type flagellum	6	6	2,00E-06
GO:0042995	cell projection	6	6	2,00E-06
GO:0044461	bacterial-type flagellum part	6	6	2,00E-06
GO:0044463	cell projection part	6	6	2,00E-06
GO:0009425	bacterial-type flagellum basal body	5	5	1,80E-05
GO:0044422	organelle part	16	7	0,001
GO:0016021	integral component of membrane	288	44	0,0025
GO:0031224	intrinsic component of membrane	288	44	0,0025

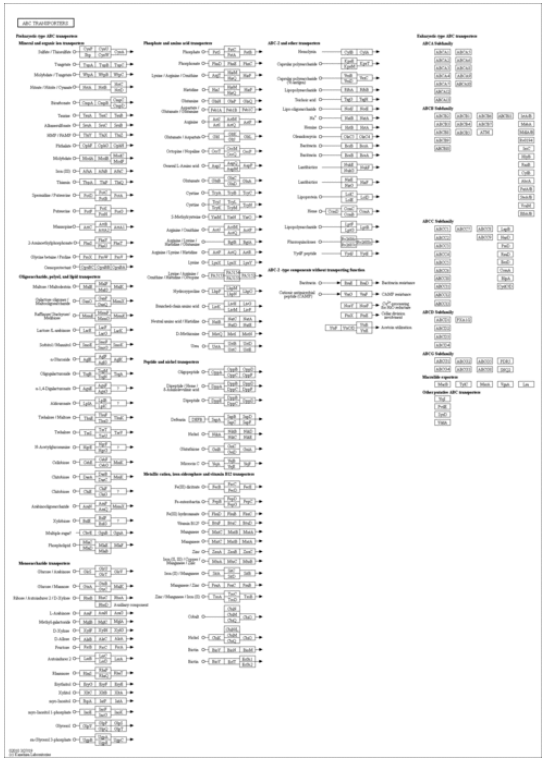
MF	Term	Annotated	Significant	classicFisher
GO:0003774	motor activity	3	3	0,00032
GO:0016810	hydrolase activity, acting on carbon-nit...	26	6	0,00657
GO:0004497	monooxygenase activity	3	2	0,01348
GO:0016813	hydrolase activity, acting on carbon-nit...	3	2	0,01348
GO:0016209	antioxidant activity	4	2	0,02577
GO:0019213	deacetylase activity	4	2	0,02577
GO:0015144	carbohydrate transmembrane transporter a...	36	6	0,03217
GO:0016787	hydrolase activity	231	22	0,04996

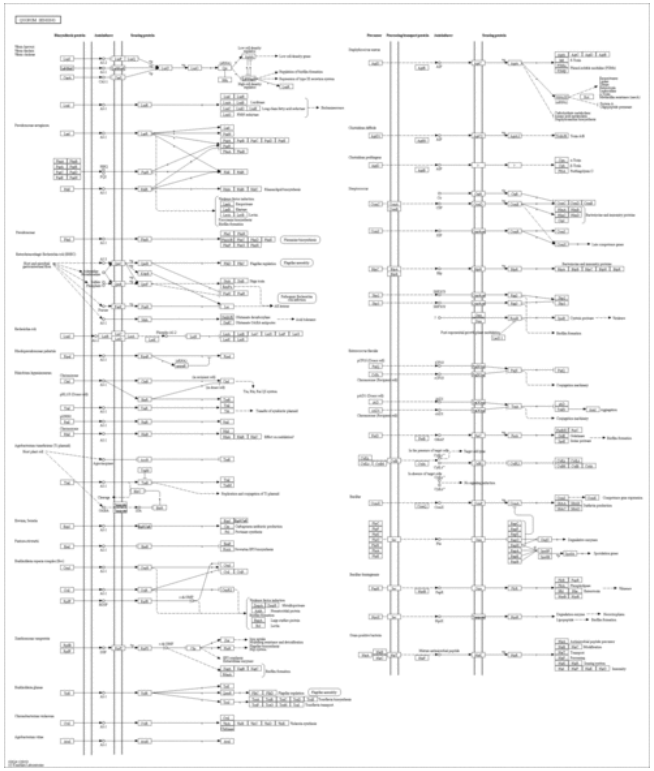


DEGs: Biochemichal Pathways

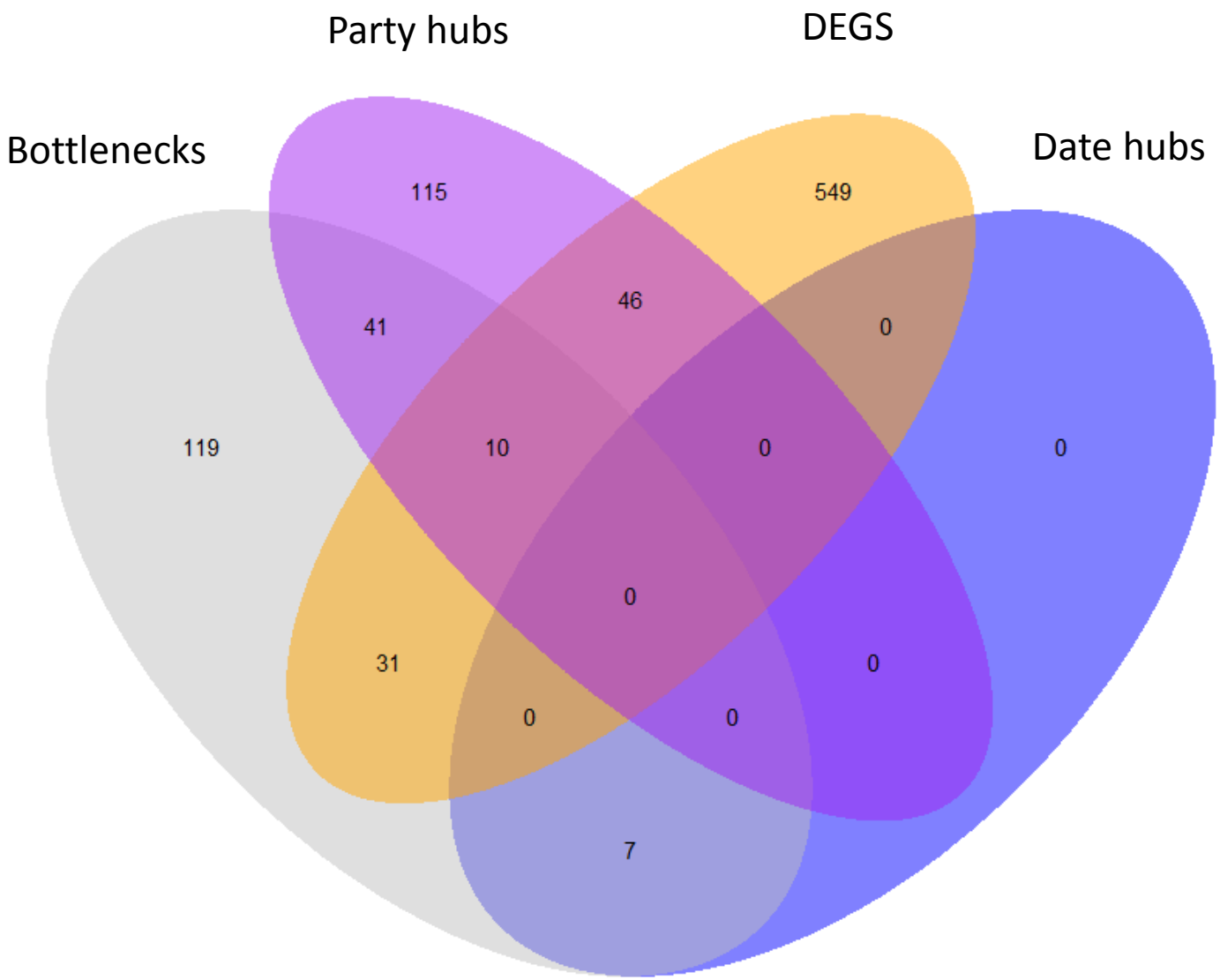
	Pathway	N	DE	P.DE
path:lmo02040	Flagellar assembly	26	24	1,69E-15
path:lmo03010	Ribosome	57	33	1,44E-10
path:lmo02030	Bacterial chemotaxis	12	11	1,58E-07
path:lmo02010	ABC transporters	78	28	5,22E-04
path:lmo02024	Quorum sensing	43	14	3,12E-02
path:lmo00121	Secondary bile acid biosynthesis	2	2	3,87E-02







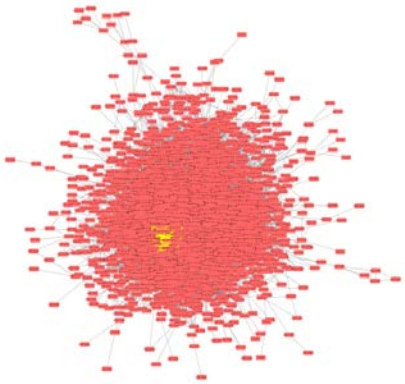
Integrating DEGs and topological analysis



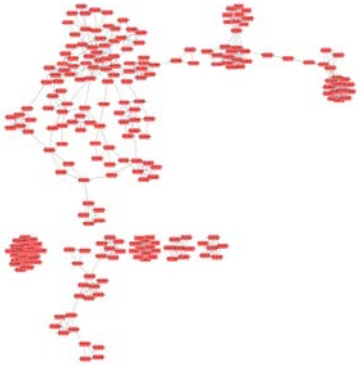
Integrating DEGs and Modules

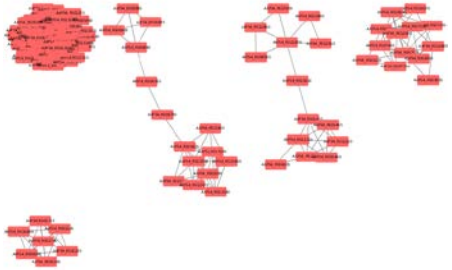
Pathway	Protein Modules	Genes	DEGs
Flagellar assembly	2	45	38
Ribosome	1	80	65
Bacterial chemotaxis	2	45	38
ABC transporters	2,17,9,14,6,25,26	220	160
Quorum sensing	1,20,38,22,14	371	245
Secondary bile acid biosynthesis	38	95	60

MOD1: 80,5,60

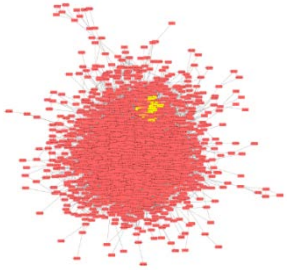


ABC-related: 220,62,98

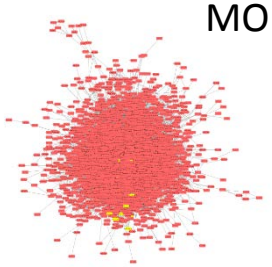




QS-related: 371,80,165



MOD38:95,32,28



MOD2: 45,6,32

In summary

Differential Expression analysis identified 5 major modulated pathways:

- Flagellar
- Chemotaxis
- Ribosome
- Quorum sensing
- Transporters

Systems Biology (modules) tells us that:

- **Flagellar and Chemotaxis** are linked
- **Ribosome and Quorum Sensing** are linked
- Transporters are a heterogeneous family

Date Hubs are:

related to AA metabolism and slightly over-expressed

Party Hubs are:

down-expressed: related to translation

over-expressed: involved in carbohydrate, transport and transcription

In summary

Lm 2015TE2496 responds to Temp/pH/Salt by:

- regulating homeostasis and cell cycle
- regulating chemotaxis and motility
- modulating Transporters toolkit

Next…

- To investigate protein modules independently
- To integrate protein modules and topological analysis (hubs and bottlnecks)
- To integrate protein modules and Differentially Expression
- To explore Transporters Proteins
- To identify Key Genes

Key Genes (topological)

Gene	name	GO	COG	FC
AXF54_RS09255	tRNA (guanosine(37)-N1)-methyltransferase TrmD	NOGO	J	2,649
AXF54_RS07430	RNA polymerase sigma factor RpoD	GO:0001123; GO:0003677; GO:0003700; GO:0005737; GO:0016987	K	1,664
AXF54_RS11410	uroporphyrinogen decarboxylase	GO:0004853; GO:0005737; GO:0006782	H	1,543
AXF54_RS12415	triose-phosphate isomerase	NOGO	G	1,464
AXF54_RS07145	competence/damage-inducible protein A	NOGO	H	1,433
AXF54_RS08340	bifunctional acetaldehyde-CoA/alcohol dehydrogenase	GO:0004022; GO:0006066; GO:0008774; GO:0015976; GO:0046872	C	1,396
AXF54_RS01010	L-lactate dehydrogenase	NOGO	C	1,296
AXF54_RS12405	phosphopyruvate hydratase	GO:0000015; GO:0000287; GO:0004634; GO:0005576; GO:0006096; GO:0009986	G	1,295
AXF54_RS10635	chaperonin GroEL	NOGO	O	1,166
AXF54_RS10730	helix-turn-helix transcriptional regulator	GO:0003677	K	-1,331
AXF54_RS01230	cysteine--tRNA ligase	GO:0004817; GO:0005524; GO:0005737; GO:0006423; GO:0008270	J	-1,384
AXF54_RS07435	DNA primase	NOGO	L	-1,601
AXF54_RS12940	arginine--tRNA ligase	GO:0004814; GO:0005524; GO:0005737; GO:0006420	J	-1,604
AXF54_RS00845	methionine--tRNA ligase	NOGO	J	-1,804
AXF54_RS06780	YlxQ family RNA-binding protein	GO:0003723; GO:0005840	J	-1,93
AXF54_RS14130	PTS beta-glucoside transporter subunit IIBCA	NOGO	G	-2,041
AXF54_RS01825	flavocytochrome c	NOGO	C	-2,078
AXF54_RS01690	hypothetical protein	NOGO	K	-2,09
AXF54_RS13995	IMP dehydrogenase	GO:0000166; GO:0003938; GO:0006177; GO:0046872	T	-2,457
AXF54_RS03675	chemotaxis protein CheA	GO:0005524; GO:0016301	NT	-2,471
AXF54_RS13470	30S ribosomal protein S7	GO:0000049; GO:0003735; GO:0006412; GO:0015935; GO:0019843	J	-2,703
AXF54_RS03660	chemotaxis protein CheV	NOGO	T	-2,733
AXF54_RS03655	glycosyltransferase	GO:0016740	M	-3,389
AXF54_RS07580	competence protein ComEA	GO:0003677; GO:0006281; GO:0016021	L	-3,64
AXF54_RS05715	glutamine-hydrolyzing GMP synthase	GO:0003922; GO:0005524; GO:0006541; GO:0016740	F	-1,434

Key Genes (differential expression)

locus	ProductName	FC		locus	ProductName	FC
AXF54_RS11400	hypothetical protein	7,270		AXF54_RS03330	phage gp6-like head-tail connector protein	-8,054
AXF54_RS07240	VanZ family protein	6,799		AXF54_RS03315	phage portal protein	-6,877
AXF54_RS04795	NAD-dependent succinate-semialdehyde dehydrogenase	6,763		AXF54_RS03345	hypothetical protein	-6,357
AXF54_RS13680	dihydroxyacetone kinase subunit DhaK	6,627		AXF54_RS03205	helix-turn-helix transcriptional regulator	-6,175
AXF54_RS01345	ArgE/DapE family deacylase	6,606		AXF54_RS11720	DeoR/GlpR transcriptional regulator	-6,082
AXF54_RS00095	hypothetical protein	6,396		AXF54_RS03230	hypothetical protein	-5,998
AXF54_RS15000	hypothetical protein	6,208		AXF54_RS11715	1-phosphofructokinase	-5,614
AXF54_RS13685	dihydroxyacetone kinase subunit L	6,140		AXF54_RS03335	head-tail adaptor protein	-5,506
AXF54_RS00650	hypothetical protein	6,116		AXF54_RS03290	hypothetical protein	-5,468
AXF54_RS13945	general stress protein	5,997		AXF54_RS03320	HK97 family phage prohead protease	-5,420
AXF54_RS11140	CsbD family protein	5,965		AXF54_RS14625	hypothetical protein	-5,254
AXF54_RS11695	IDEAL domain-containing protein	5,922		AXF54_RS10375	cold-shock protein	-5,212
AXF54_RS10630	choloylglycine hydrolase family protein	5,905		AXF54_RS03865	DUF4064 domain-containing protein	-5,075
AXF54_RS13690	PTS-dependent dihydroxyacetone kinase phosphotransferase DhaM	5,838		AXF54_RS11785	GNAT family N-acetyltransferase	-5,031
AXF54_RS08735	metal-dependent hydrolase	5,723		AXF54_RS03310	DUF1056 family protein	-5,013
AXF54_RS06625	glycerol-3-phosphate dehydrogenase/oxidase	5,660		AXF54_RS03210	DUF771 domain-containing protein	-4,945
AXF54_RS14895	hypothetical protein	5,490		AXF54_RS03695	flagellar basal body rod modification protein FlgD	-4,906
AXF54_RS09700	30S ribosomal protein S14	5,468		AXF54_RS03355	hypothetical protein	-4,853
AXF54_RS01660	GAP family protein	5,342		AXF54_RS11770	amino acid ABC transporter permease	-4,842
AXF54_RS12080	SDR family oxidoreductase	5,333		AXF54_RS03250	hypothetical protein	-4,815
AXF54_RS05845	hypothetical protein	5,307		AXF54_RS03325	phage major capsid protein	-4,775
AXF54_RS11500	low molecular weight phosphatase family protein	5,264		AXF54_RS00725	peptide ABC transporter substrate-binding protein	-4,658
AXF54_RS00655	N-acetyltransferase	5,215		AXF54_RS03295	HNH endonuclease	-4,594
AXF54_RS03565	SDR family oxidoreductase	5,211		AXF54_RS03640	flagellar motor stator protein MotA	-4,467