



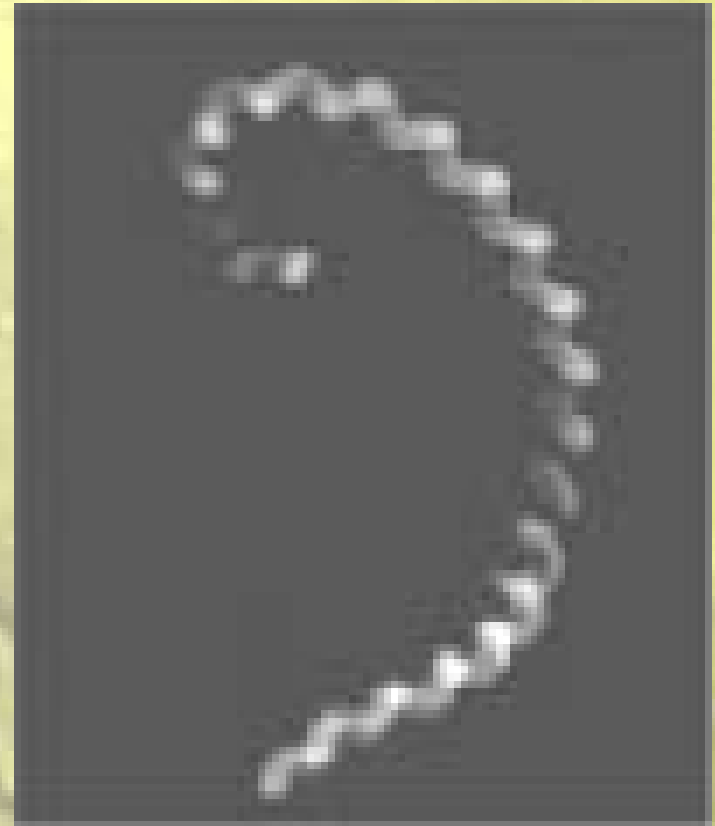
# Transcriptomic response to serum of *Leptospira interrogans* serovar Copenhageni

**Assist.Prof.Kanitha Patarakul, MD,PhD**

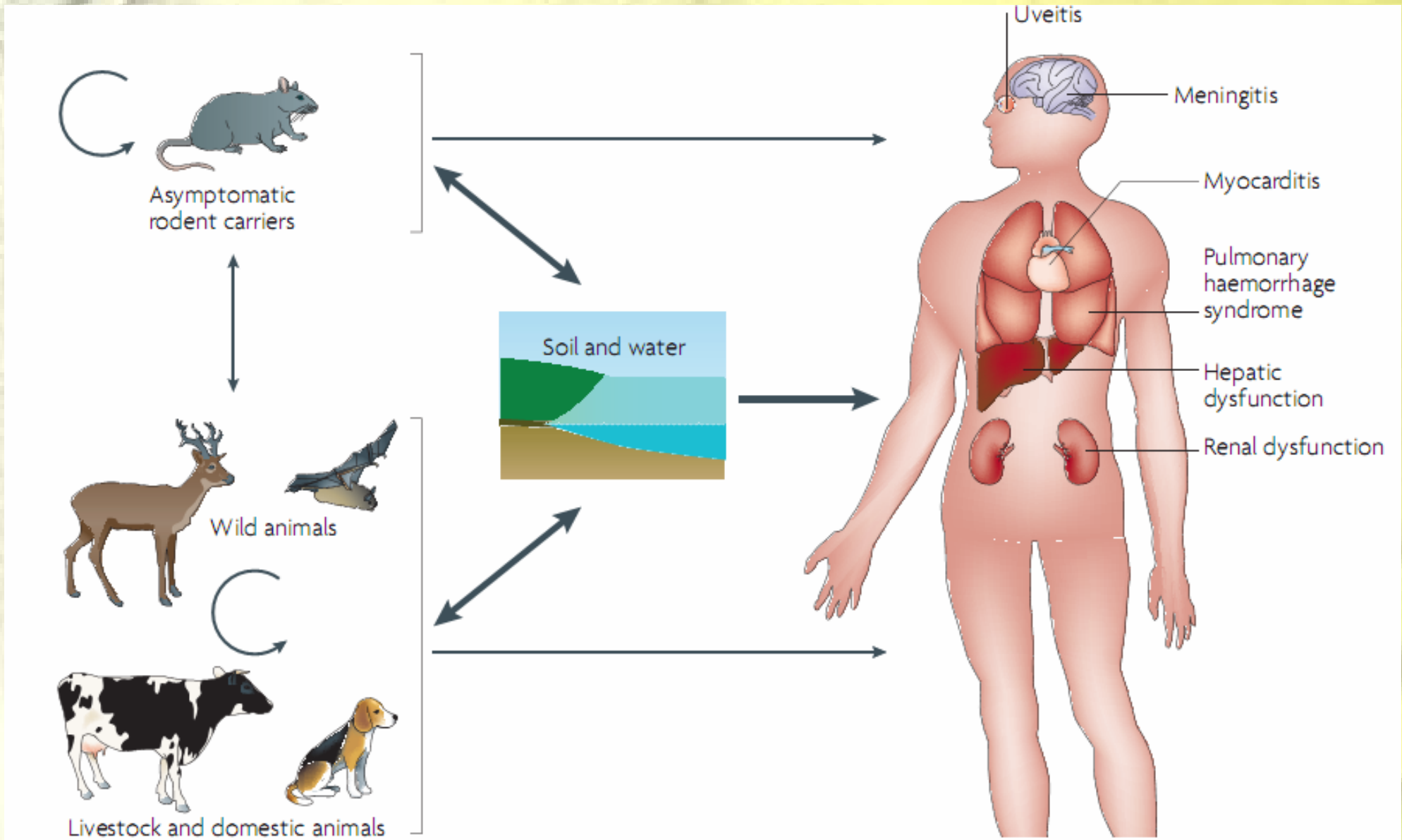
Department of Microbiology  
Faculty of Medicine  
Chulalongkorn University, Thailand

# ***Leptospira interrogans***

- **Spirochete**
- **Size 0.1-0.25 x 6-25 um**
- **Periplasmic flagella**
- **Double membrane**
- **Geographic distribution:**
  - Pathogenic *Leptospira***
  - > 250 serovars**
  - > 19 species**



# The cycle of leptospiral infection





# Pathogenesis

**Penetrate skin or mucosa**



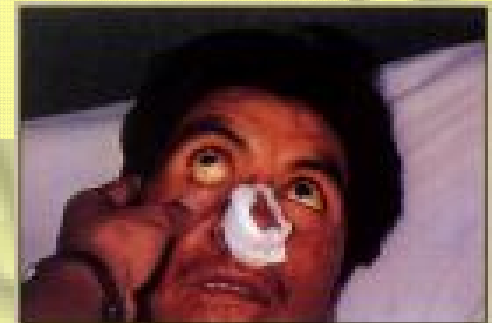
**Spread hematogenously**



**Localize in target organs**  
eg. kidney, liver, lung



**Target organ damage**





# Previous microarray studies

- Effects of temperature on gene expression patterns in *Leptospira interrogans* serovar Lai as assessed by whole-genome microarrays (Infect Immun 2006; 74(10): 5848–5859)
- Genome-wide transcriptional analysis of temperature shift in *L. interrogans* serovar lai strain 56601 (BMC Microbiology 2006; 6:51)
- Response of *Leptospira interrogans* to physiologic osmolarity: relevance in signaling the environment-to-host transition (Infect Immun 2007; 75(6): 2864–2874)



# Objectives

- **To compare genes expressed in pathogenic *Leptospira* at 2 different environments**
  - *In vivo* (serum) vs *in vitro* (EMJH media)
  - Transcription level at a global scale: microarray technology

# Serum killing assay

- Pathogenic serovars
  - Copenhageni, Lai, Manilae
- Serum killing assay
  - $2 \times 10^8$  cells/ml in EMJH
  - 50% normal guinea pig serum
  - **30 min**, 1 h, 2 h
  - Percentage of viability: compared to incubation with heat-inactivated serum
- Control
  - non-pathogenic serovar: Patoc (serum-sensitive)

# **Serum killing assay (37°C, 30 min)**

<b>Serovar</b>	<b>% Viability</b>
<b>Copenhageni</b>	<b>97.3 ± 2.2</b>
<b>Lai</b>	<b>10.6 ± 6.4</b>
<b>Manilae</b>	<b>99.5 ± 1.1</b>
<b>Patoc</b>	<b>9.0 ± 5.1</b>



# Microarray spotted oligonucleotides

- Based on *L. interrogans* serovar Lai strain 56601 genome
  - 3,626 ORFs, 36 tRNA genes, and 4 rRNA gene
  - 45 ORFs unique to serovar Copenhageni strain Fiocruz L1-130
- Oligonucleotides: 70 bases in length

**Log-phase *L. interrogans*  
serovar Copenhageni**

**Serum incubation  
(37°C, 30 min)**

**Total RNA extraction**

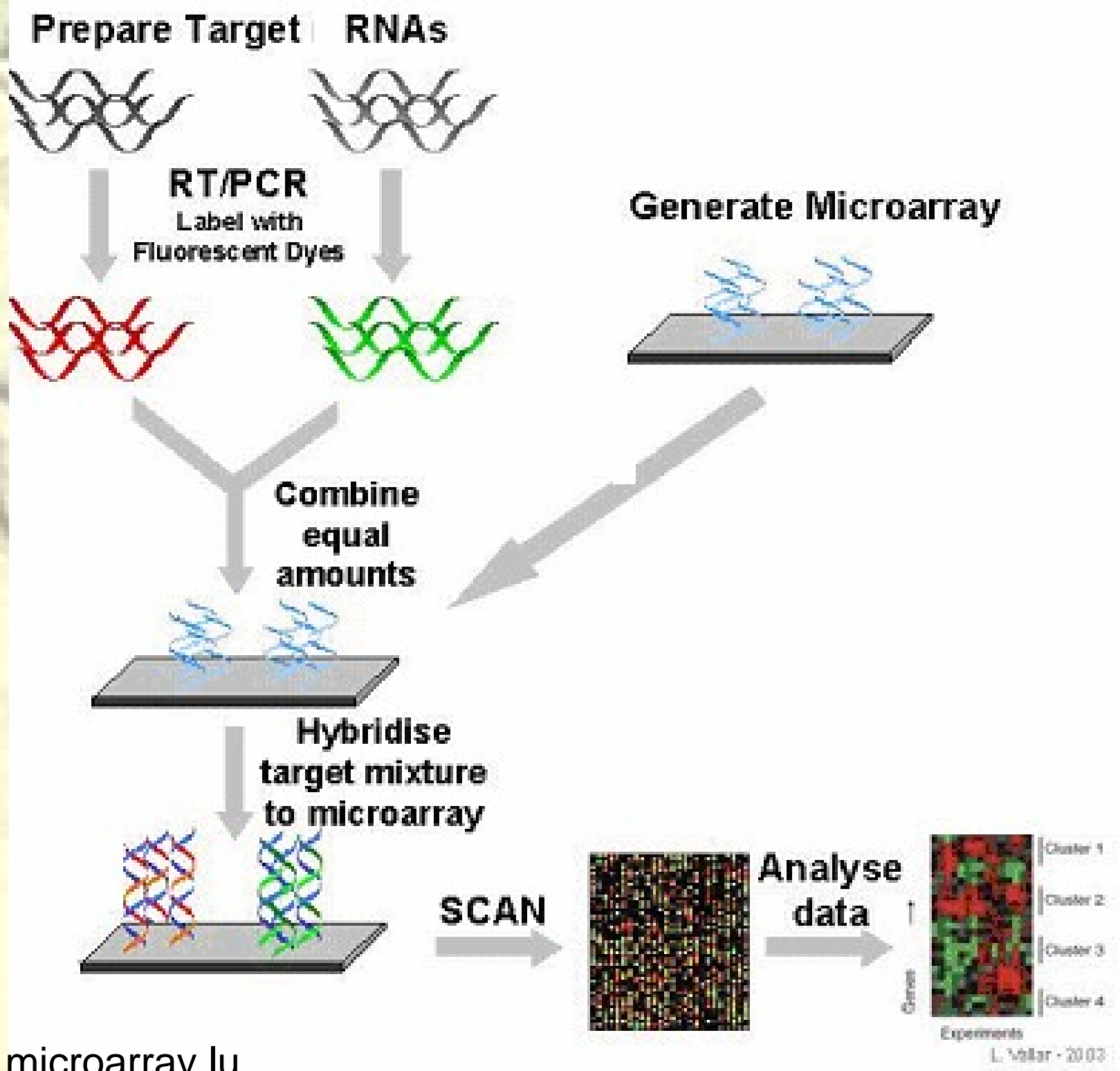
**cDNA synthesis and labeling  
(Cy3, Cy5)**

**EMJH media incubation  
(37°C, 30 min)**

**Total RNA extraction**

**cDNA synthesis and labeling  
(Cy5, Cy3)**





# Microarray sample replicates

- 3 biological replicates
- Dye swap/biological replicate
- 4 spot replicates on the same slide



# Differentially expressed genes NGS vs EMJH medium

- **BASE (BioArray Software Environment)**
- **Statistical significance**
  - **Fold ratio  $\geq 1.5$  (up or down-regulation)**
  - **Adjusted *P* value  $< 0.01$**

# Results





# Number of genes differentially expressed in serum

Genes	No. of genes (%*)		
	Up-regulated	Down-regulated	Total
Known or predicted function	30 (54.5)	51 (45.1)	81
Unknown function	25 (45.5)	62 (54.9)	87
Total	55	113	168

\* % percentage of genes per total number of genes in up-regulated or down-regulated group

**168 genes (4.5% of whole genome)**

# Cluster of Orthologous Groups (COGs)

## **INFORMATION STORAGE AND PROCESSING**

- [J] Translation, ribosomal structure and biogenesis
- [A] RNA processing and modification
- [K] Transcription
- [L] Replication, recombination and repair
- [B] Chromatin structure and dynamics

## **CELLULAR PROCESSES AND SIGNALING**

- [D] Cell cycle control, cell division, chromosome partitioning
- [Y] Nuclear structure
- [V] Defense mechanisms
- [T] Signal transduction mechanisms
- [M] Cell wall/membrane/envelope biogenesis
- [N] Cell motility
- [Z] Cytoskeleton
- [W] Extracellular structures
- [U] Intracellular trafficking, secretion, and vesicular transport
- [O] Posttranslational modification, protein turnover, chaperones



# Cluster of Orthologous Groups (COGs)

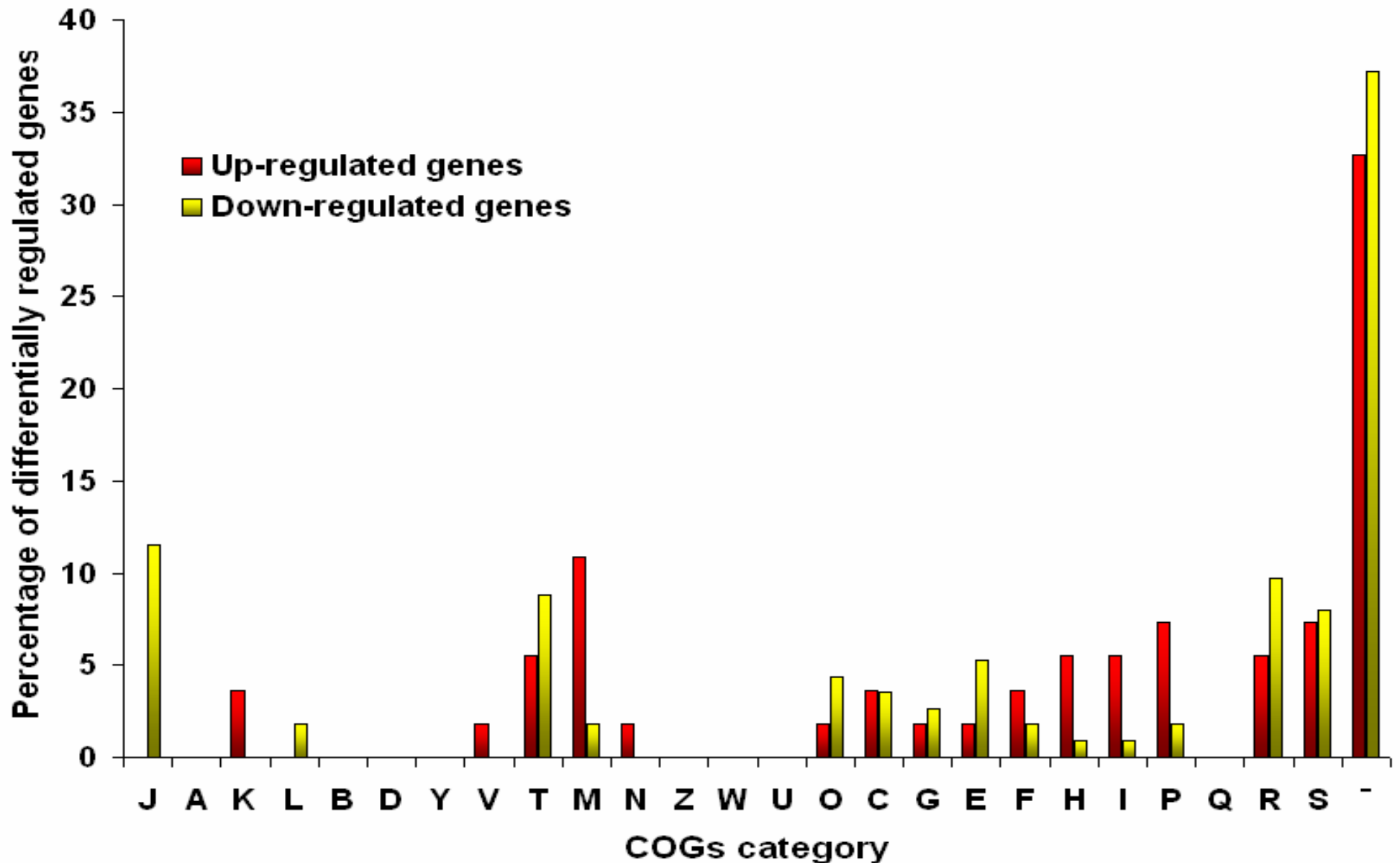
## **METABOLISM**

- [C] Energy production and conversion**
- [G] Carbohydrate transport and metabolism**
- [E] Amino acid transport and metabolism**
- [F] Nucleotide transport and metabolism**
- [H] Coenzyme transport and metabolism**
- [I] Lipid transport and metabolism**
- [P] Inorganic ion transport and metabolism**
- [Q] Secondary metabolites biosynthesis, transport and catabolism**

## **POORLY CHARACTERIZED**

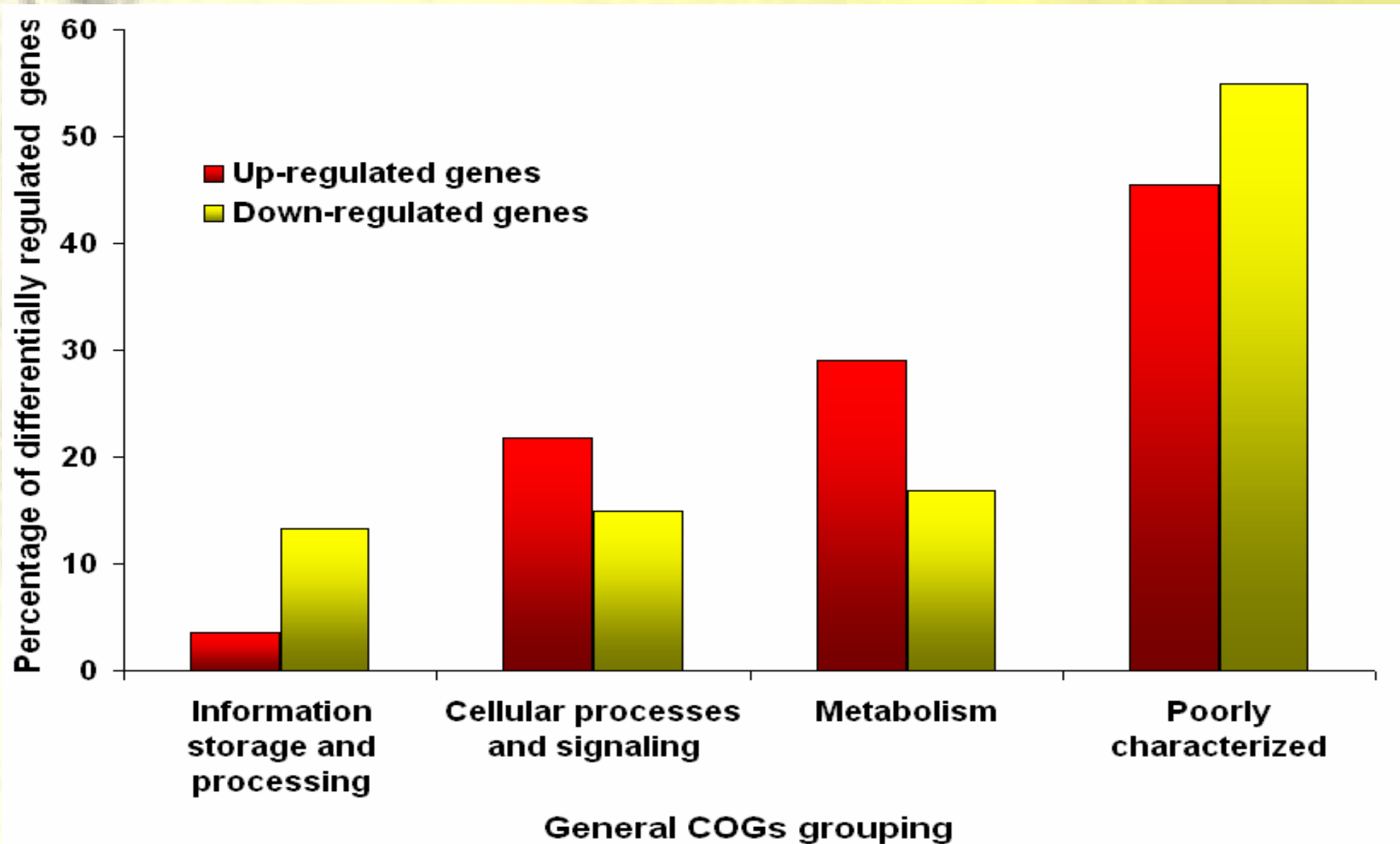
- [R] General function prediction only**
- [S] Function unknown**
- [-] Not in COGs**

# Differentially expressed genes in response to serum





# Differentially expressed genes in response to serum



# Up-regulated genes in response to serum

- **Ankyrin repeat protein (LIC13291)**
  - Highest fold ratio (+11.52 fold)

# Up-regulated genes of known or predicted function in serum

Locus	Gene Name	COG category	Fold ratio	Temperature effect	Osmolarity effect
LIC11149	Metallopeptidase	M	2.75		
LIC20052	Fatty acid desaturase	I	2.59		
LIC13053	Fatty acid desaturase	I	2.59		
LIC12979	Signal transduction protein	T	2.49		
LIC20148	Heme oxygenase	P	2.47		↑
LIC12151	Nucleoside-diphosphate sugar epimerase	M	2.45		
LIC12629	Enoyl-CoA hydratase	I	2.42		
LIC10200	Glycosyltransferase	M	2.17		
LIC13289	Sensor histidine kinase of a two-component response regulator	T	2.17		↑
LIC10587	Glycosyltransferase	M	2.07		
LIC13433	Dihydroorotate dehydrogenase	F	2.01		
LIC11728	Amidase	M	2.01		↑
LIC11657	Endoflagellar biosynthesis	O	1.98		
LIC10464	LigB lipoprotein	N	1.89	↑	↑
LIC13470	Reductase	P	1.82		

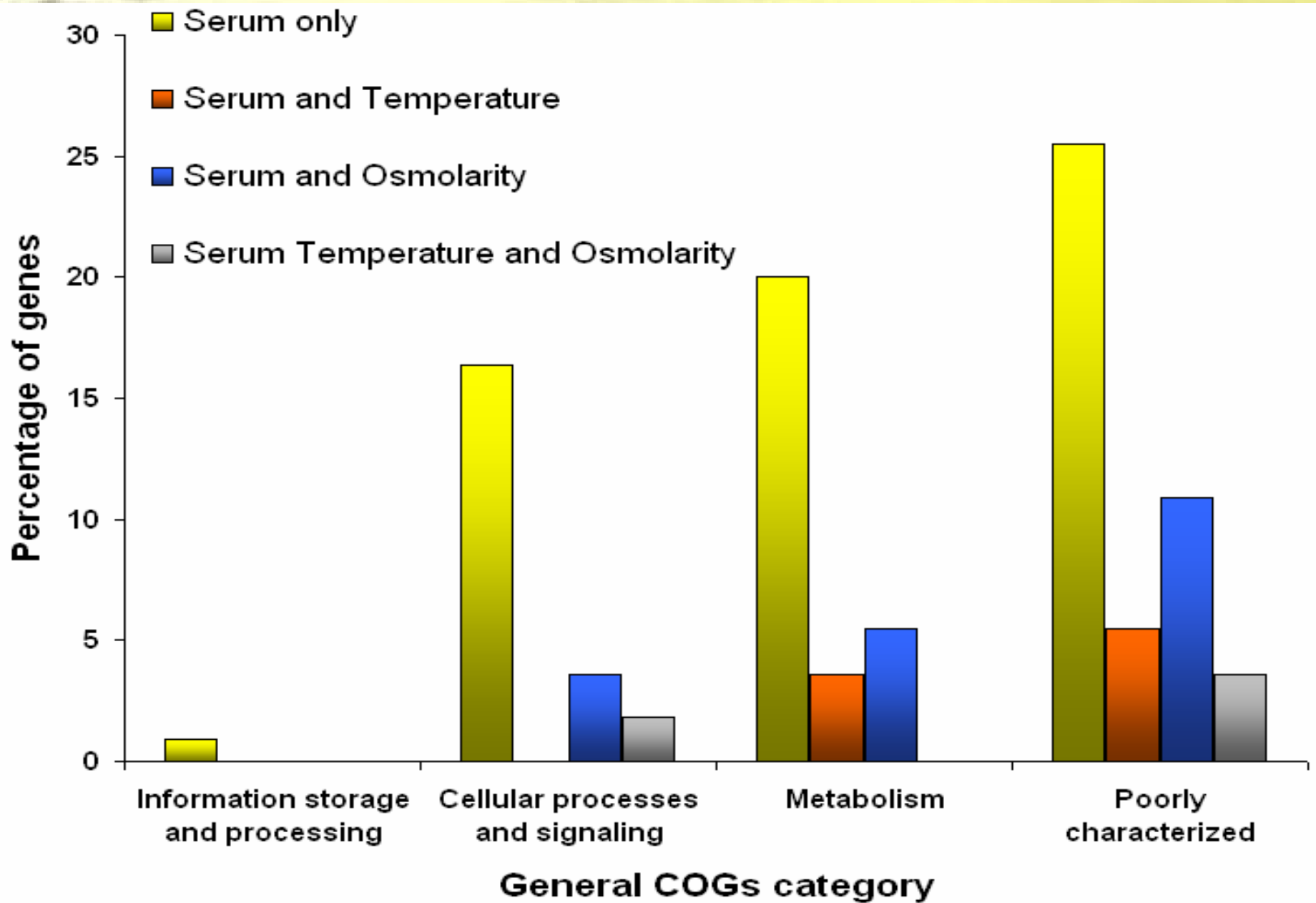
**LigB lipoprotein**



# Up-regulated genes of known or predicted function in serum

Locus	Gene Name	COG category	Fold ratio	Temperature effect	Osmolarity effect
LIC13208	Pyrimidine reductase	H	1.82		↑
LIC20149	Permease	G	1.77		↑
LIC10900	Adenylate/guanylate cyclase	T	1.72		
LIC11154	Transcriptional regulator	K	1.70		
LIC12184	Acetyltransferase	E	1.69	↑	
LIC12982	Cation transport ATPase	P	1.69	↑	
LIC13469	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	M	1.65		
LIC12182	ATP-binding protein of an ABC transporter complex	V	1.58		
LIC20082	Transcriptional regulator	H	1.58		
LIC13085	Dephospho-CoA kinase	H	1.55		
LIC11663	Deoxyguanosinetriphosphate triphosphohydrolase	F	1.54		
LIC10378	Transcriptional regulator	K	1.54		
LIC12992	Bifunctional protein	P	1.51		

# Up-regulated genes



# Down-regulated genes of known or predicted function in serum

Locus	Gene Name	COG category	Fold ratio	Temperature effect	Osmolarity effect
LIC10441	Ammonia permease	P	-3.10		
LIC20098	XerD related protein (integrase family)	L	-2.80		↓
LIC12111	30S Ribosomal protein S18	J	-2.64		
LIC20012	Sensor protein of a two component response regulator	T	-2.56		
LIC12017	Endopeptidase Clp	O	-2.48		
LIC10069	Dioxygenase superfamily protein	E	-2.17		
LIC10440	Nitrogen regulatory protein PII	E	-2.17		
LIC11201	Receiver component of a two-component response regulator	T	-2.16		
LIC10990	Potassium-transporting ATPase A chain	P	-2.09	↑	
LIC12762	Signal transduction protein	T	-1.97		↓
LIC12807	Receiver component of a two-component response regulator	T	-1.95	↑	
LIC12865	50S Ribosomal protein L29	J	-1.91		
LIC12765	Peroxiredoxin	O	-1.90	↓	↓
LIC12637	50S Ribosomal protein L31	J	-1.88		
LIC10344	AntiSigma factor antagonist	T	-1.88		
LIC10737	2-polyprenyl-3-methyl-5-hydroxy-6-methoxy-1	H	-1.86		



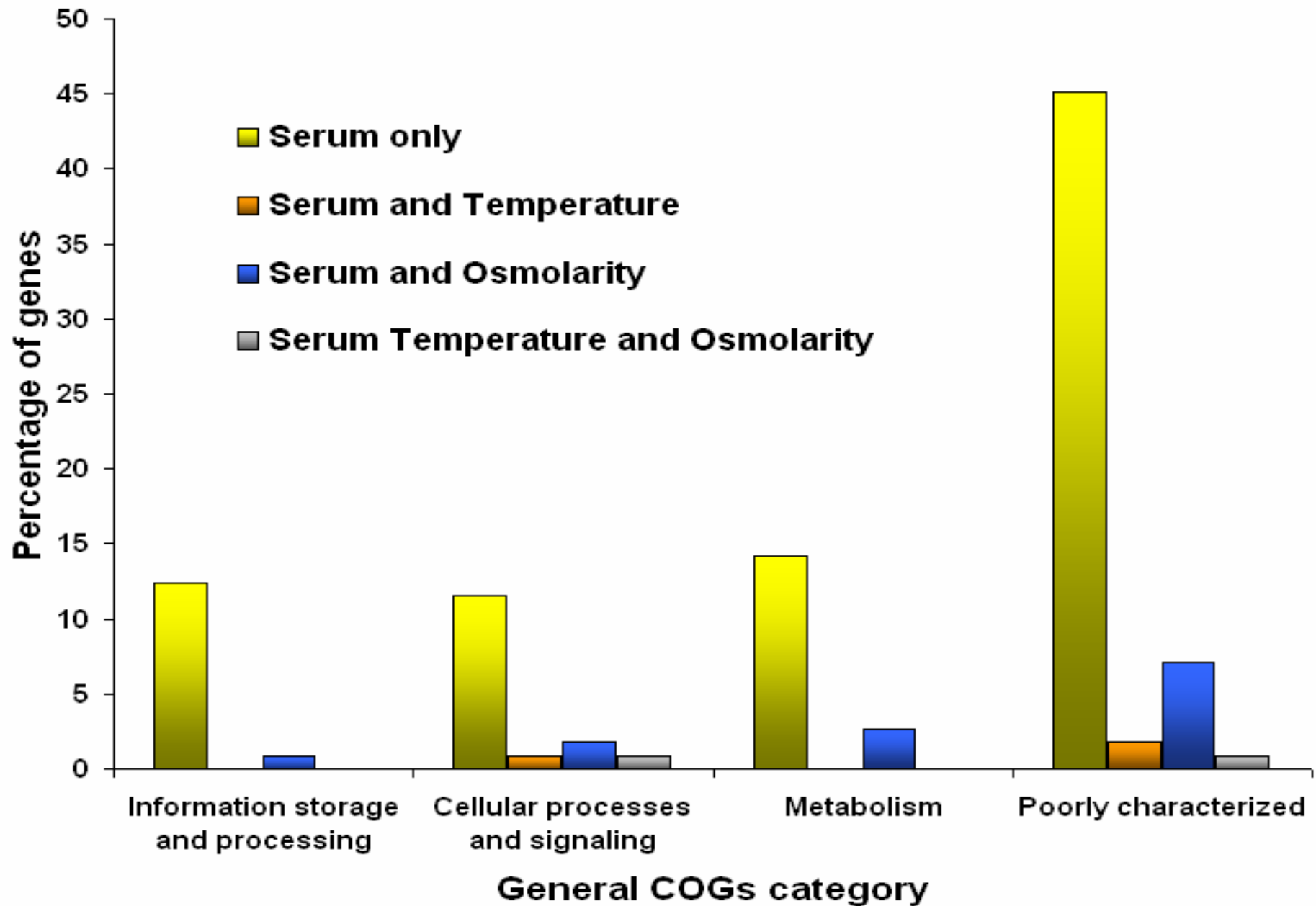
# Down-regulated genes of known or predicted function in serum

Locus	Gene Name	COG category	Fold ratio	Temperature effect	Osmolarity effect
LIC13344	Anti-sigma regulatory factor (Ser/Thr protein kinase)	T	-1.86		
LIC10750	50S Ribosomal protein L1	J	-1.82		
LIC12331	Phosphonomutase	G	-1.82		↓
LIC20108	Anti-sigma factor antagonist	T	-1.81	↓	
LIC20025	Cyclic nucleotide-binding protein	T	-1.77		
LIC10363	Isovaleryl-CoA dehydrogenase	I	-1.77		
LIC12862	50S Ribosomal protein L24	J	-1.75		
LIC12002	Succinate dehydrogenase/fumarate reductase subunit_A	C	-1.72		
LIC12733	Methylglyoxal synthase	G	-1.72		
LIC13442	Glutathione peroxidase	O	-1.70	↑	
LIC12112	Single-stranded DNA-binding protein	L	-1.70		
LIC12113	30S Ribosomal protein S6	J	-1.70		
LIC20044	HSP90	O	-1.68		
LIC10271	Metallopeptidase	M	-1.66	↑	
LIC13399	Purine-nucleoside phosphorylase	F	-1.65		
LIC12845	50S Ribosomal protein L17	J	-1.65		
LIC12476	Dihydrolipoyllysine-residue acetyltransferase and Dihydrolipoyllysine-residue succinyltransferase	C	-1.63		

# Down-regulated genes of known or predicted function in serum

Locus	Gene Name	COG category	Fold ratio	Temperature effect	Osmolarity effect
LIC12217	Plastocyanin_	C	-1.62		↓
LIC12774	50S Ribosomal protein L27	J	-1.61		
LIC20204	Selenocysteine lyase	E	-1.60		
LIC12860	30S Ribosomal protein S14	J	-1.59		
LIC12233	Aldolase	G	-1.58		↓
LIC11095	Adenylate/guanylate cyclase	T	-1.58		
LIC12852	Adenylate kinase	F	-1.55		
LIC12871	50S Ribosomal protein L23	J	-1.55		
LIC20093	Bacterioferritin comigratory protein	O	-1.54		
LIC10756	30S Ribosomal protein S7	J	-1.54		
LIC20239	Adenosymethionine decarboxylase	E	-1.54		
LIC10751	50S Ribosomal protein L10	J	-1.54		
LIC12829	Citrate (Si)-synthase	C	-1.53		
LIC12694	Glutamate synthase (NADH)	E	-1.53		
LIC12357	Membrane GTPase	T	-1.53		↓
LIC12855	50S Ribosomal protein L30	J	-1.52		
LIC10460	Lactoylgutathione or related lyase	E	-1.52		

# Down-regulated genes

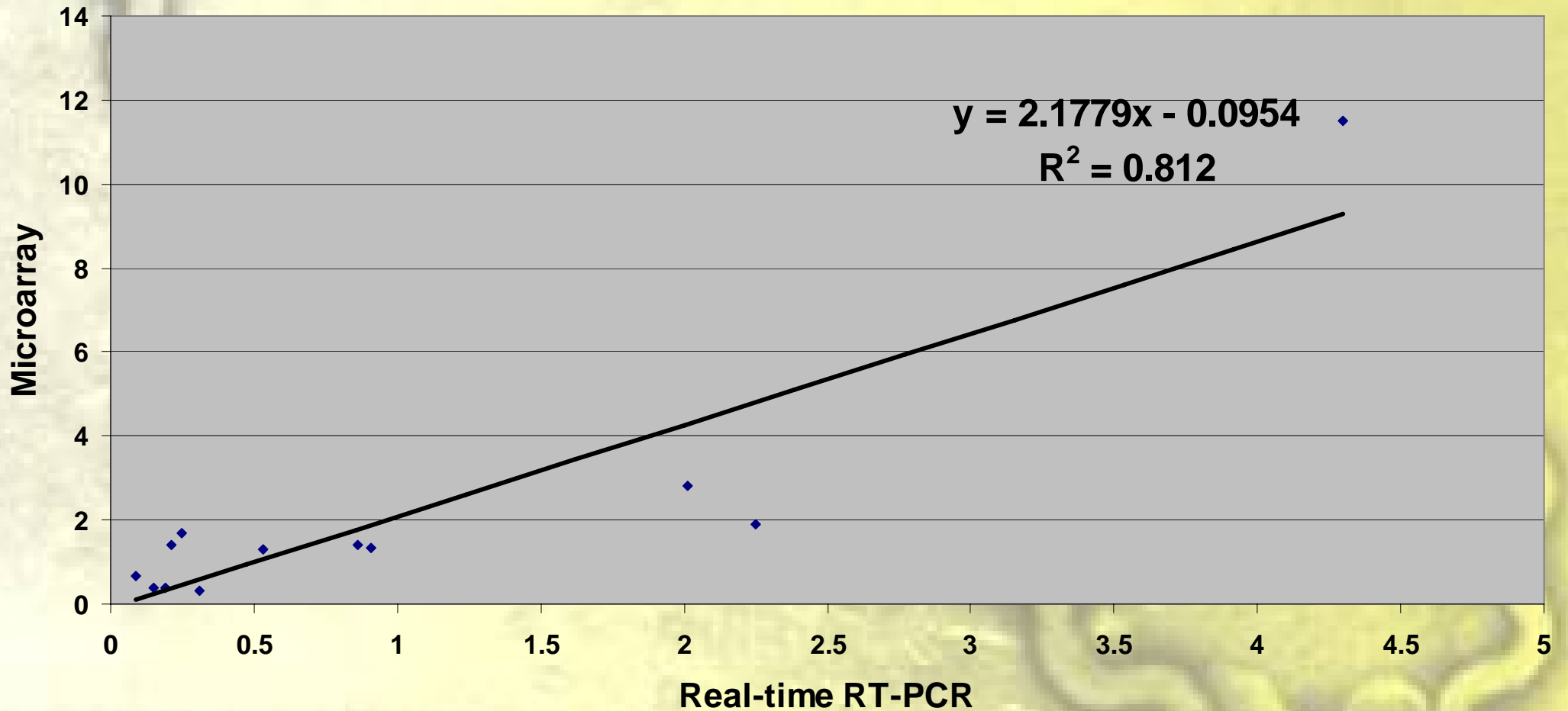




# Validation with real-time RT-PCR: 13 randomly selected genes

GeneID_Lai	Target gene	Microarray (fold ratio/p value)	Real-time RT-PCR (fold ratio)
LA4129	Ankyrin repeat protein	11.52/1.38e-12	3.6, 5.0
LA1402	Conserved hypothetical protein	2.81/0.00023	2.01
LA3778	LigB	1.89/0.00013	2.25
LA0594	Cation transport ATPase, possibly copper	1.69/0.003	0.15, 0.35
LA3806	Ammonia permease	0.32/0.00042	0.31
LB122	XerD related protein (integrase family)	0.37/3.61E-07	0.19
LA1879	Endopeptidase_Clp	0.40/2.5e-05	0.15
LA2024	LipL46	0.66/0.009	0.09
LA0286	Unique hypothetical protein	1.31/0.008	0.53
LA3532	Conserved hypothetical protein	1.34/0.006	0.91
LA1704	GAPDH	1.39/0.002	0.86
LA1859	Catalase	1.42/0.004	0.15, 0.27
LA0695	LfhA	0.86/0.81	0.12

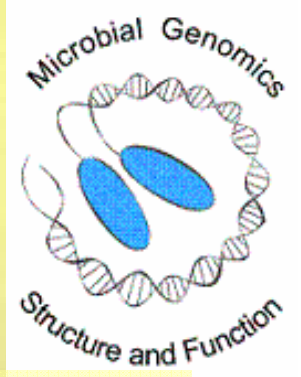
# Microarray-Real-time RT-PCR Data Correlation



# Summary

- Genes differentially expressed in serum
  - 4.5% of total ORFs
- Up-regulated genes (total 55 genes)
  - 54.5% known or predicted function
    - metabolism; cellular processes and signaling
  - 56.4% serum-specific effect
- Down-regulated genes (total 113 genes)
  - 45.1% known or predicted function
    - information storage and processing; cellular processes and signaling; and metabolism
  - 86.7% serum-specific effect





# Acknowledgements

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- Prof. Ben Adler, Dr. Miranda Lo, Adler leptospirosis group
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  - Department of Microbiology, School of Biomedical Sciences, Monash University, Australia

**BMC Microbiol. 2010 29;10:31.**