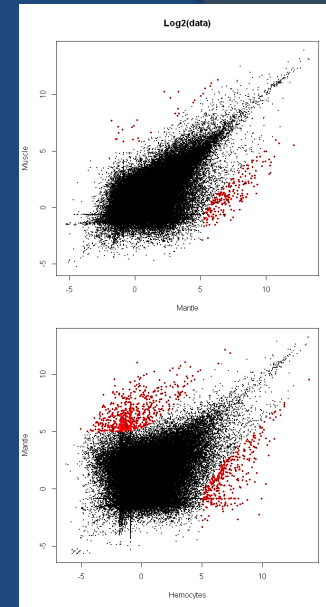
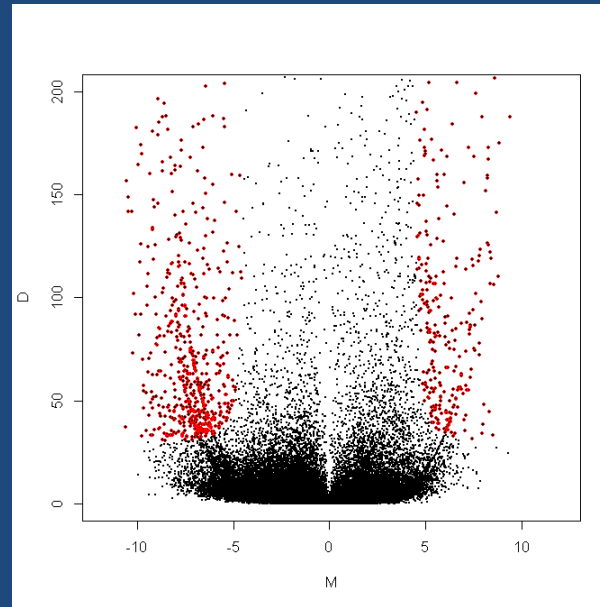


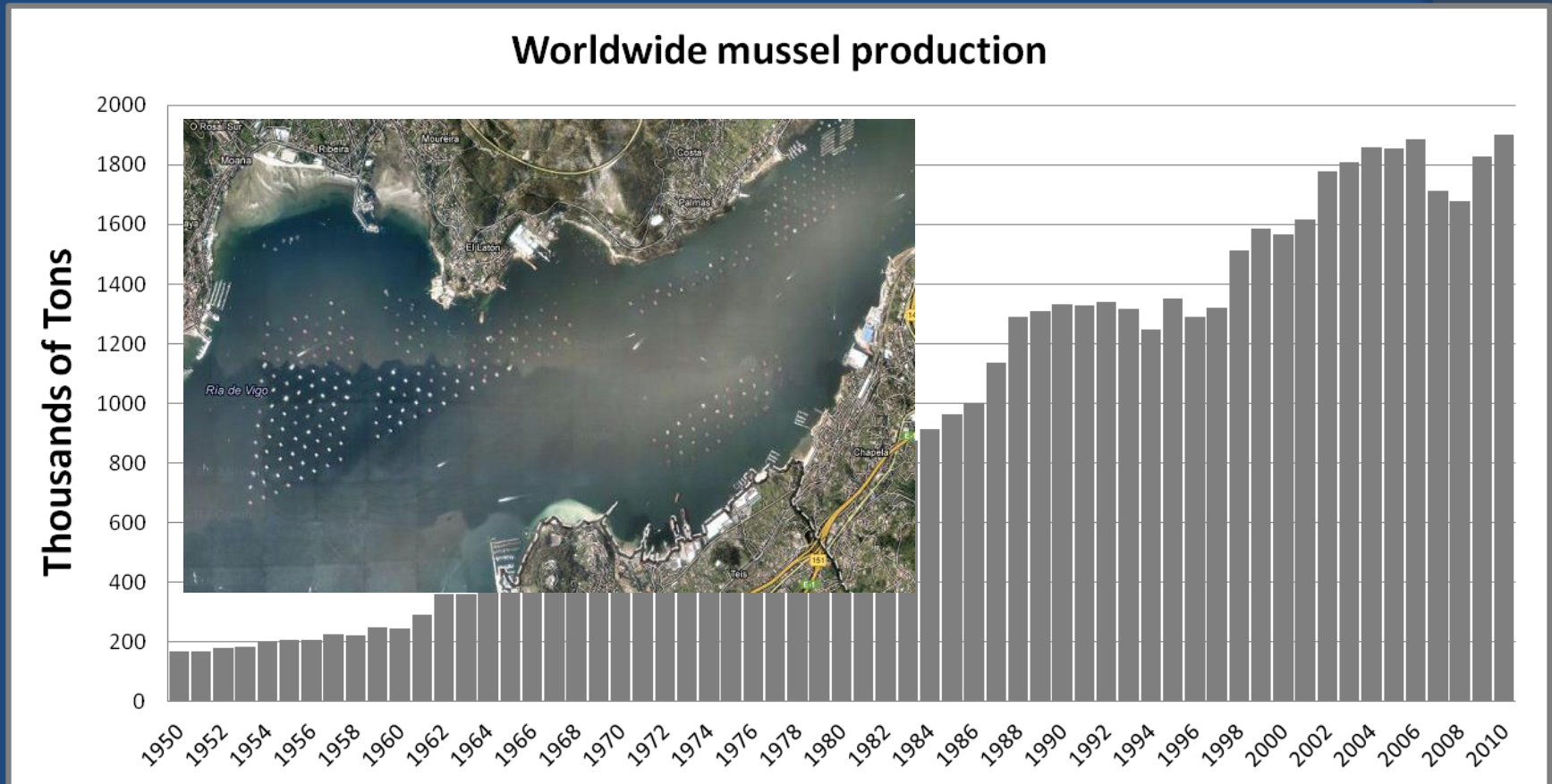
Tissue-specific transcripomes of *Mytilus galloprovincialis* reveal new functions

Moreira R, Canchaya C, Novoa B, Posada D, Figueras A



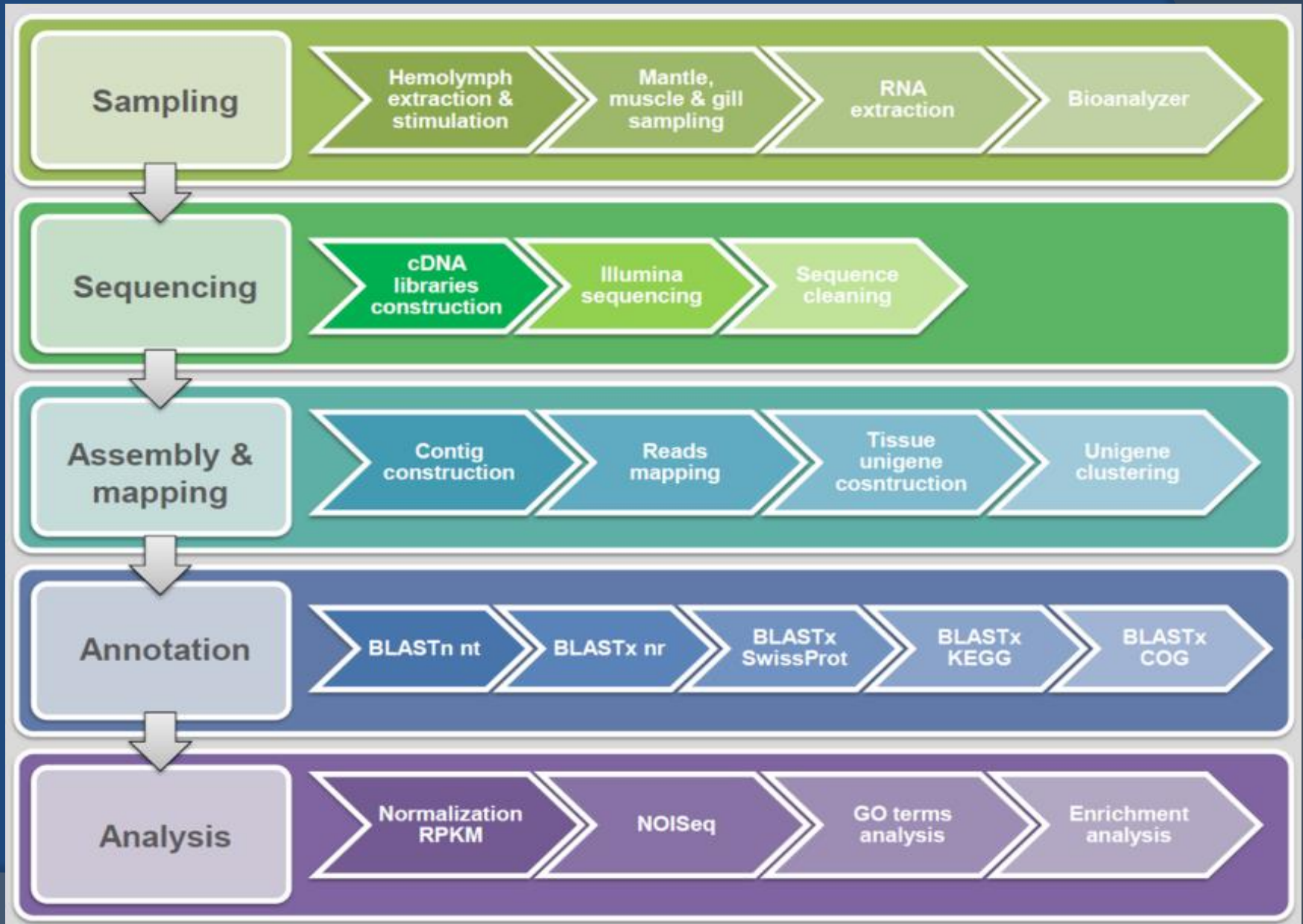
Economic importance

Worldwide mussel production (FAO):



- n°1 China > 60%
- n°2 Spain >20%
- Galicia ≈ 100%

Objectives



Sampling

Basal

Mantle (2)

Gill (1)

Muscle (2)



Sample recovery and centrifugation



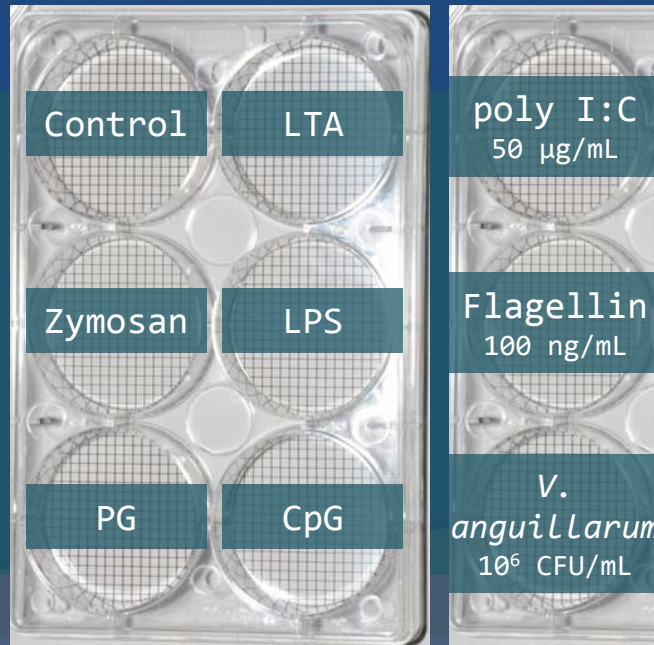
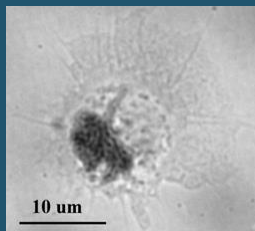
RNA extraction

Bioanalyzer

RNAstable

Stimulated

Hemocytes (2)



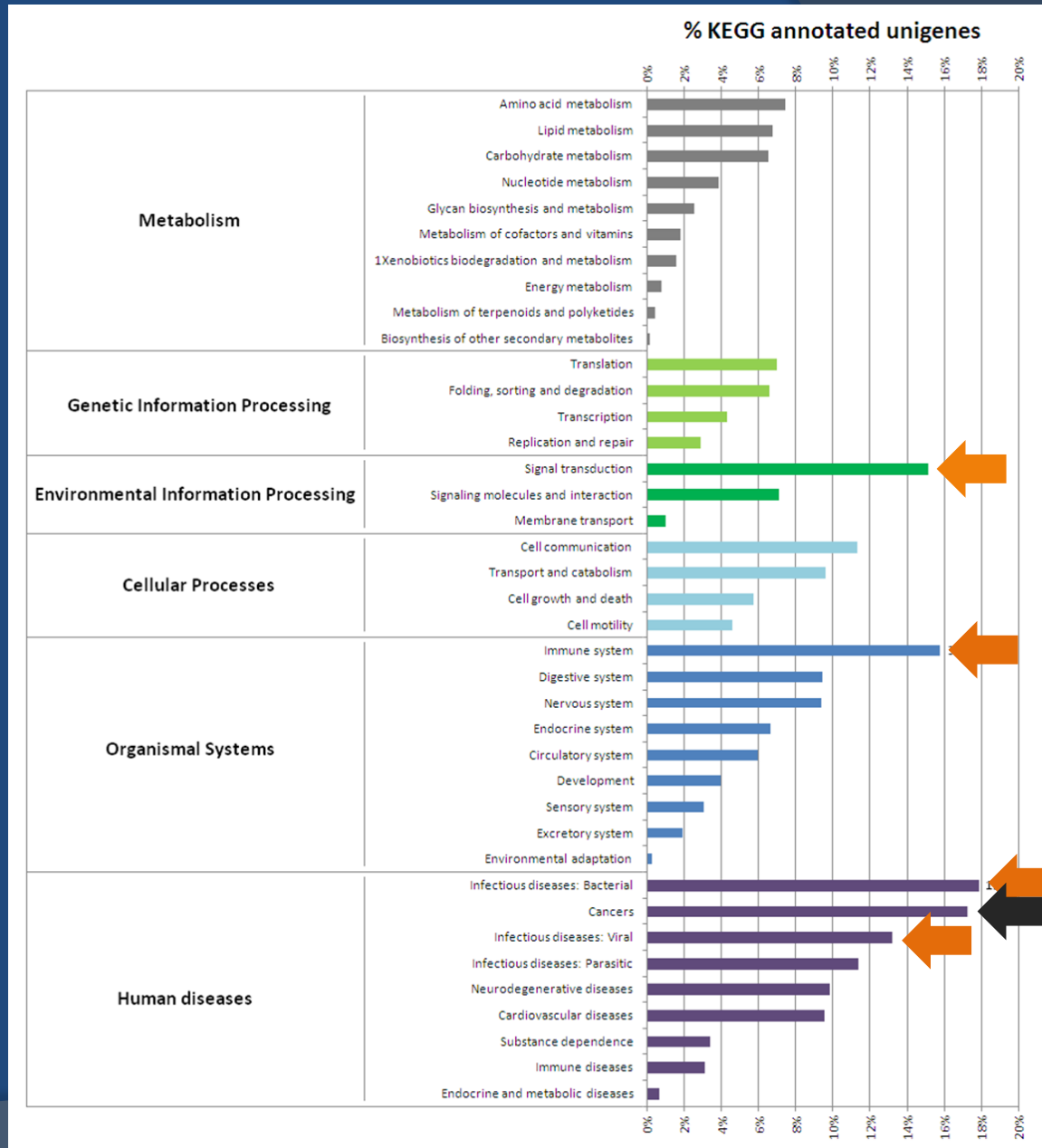
Assembly and annotation statistics

	Reads statistics	All	Hemocytes	Mantle	Muscle	Gill
Millions of raw reads			112.706	111.322	113.045	56.244
Millions of clean reads			107.386	106.060	107.127	53.335
Total Megabases			9,665	9,545	9,641	4,800
% GC content			38.99%	38.32%	38.28%	37.54%
Assembly statistics						
Number of contigs			261,332	428,939	313,554	238,650
Number of unigenes		151,320	107,045	131,935	120,572	120,254
Average unigene length		570				
N50 unigene length		774				
Range unigene length		200 - 17,690				
Number of unigenes <500pb		104,757				
Number of unigenes >500pb		46,563				
	Annotation statistics	Unigenes	Percentage			
Annotated unigenes by nt		14,207	9.4%			
Annotated unigenes by nr		45,182	29.8%			
Annotated unigenes by SwissProt		36,656	24.2%			
Annotated unigenes by KEGG		31,144	20.6%			
Annotated unigenes by COG		14,503	9.6%			
TOTAL annotated unigenes		50,998	33.7%			
Unigenes with GO terms		18,899	12.5%			

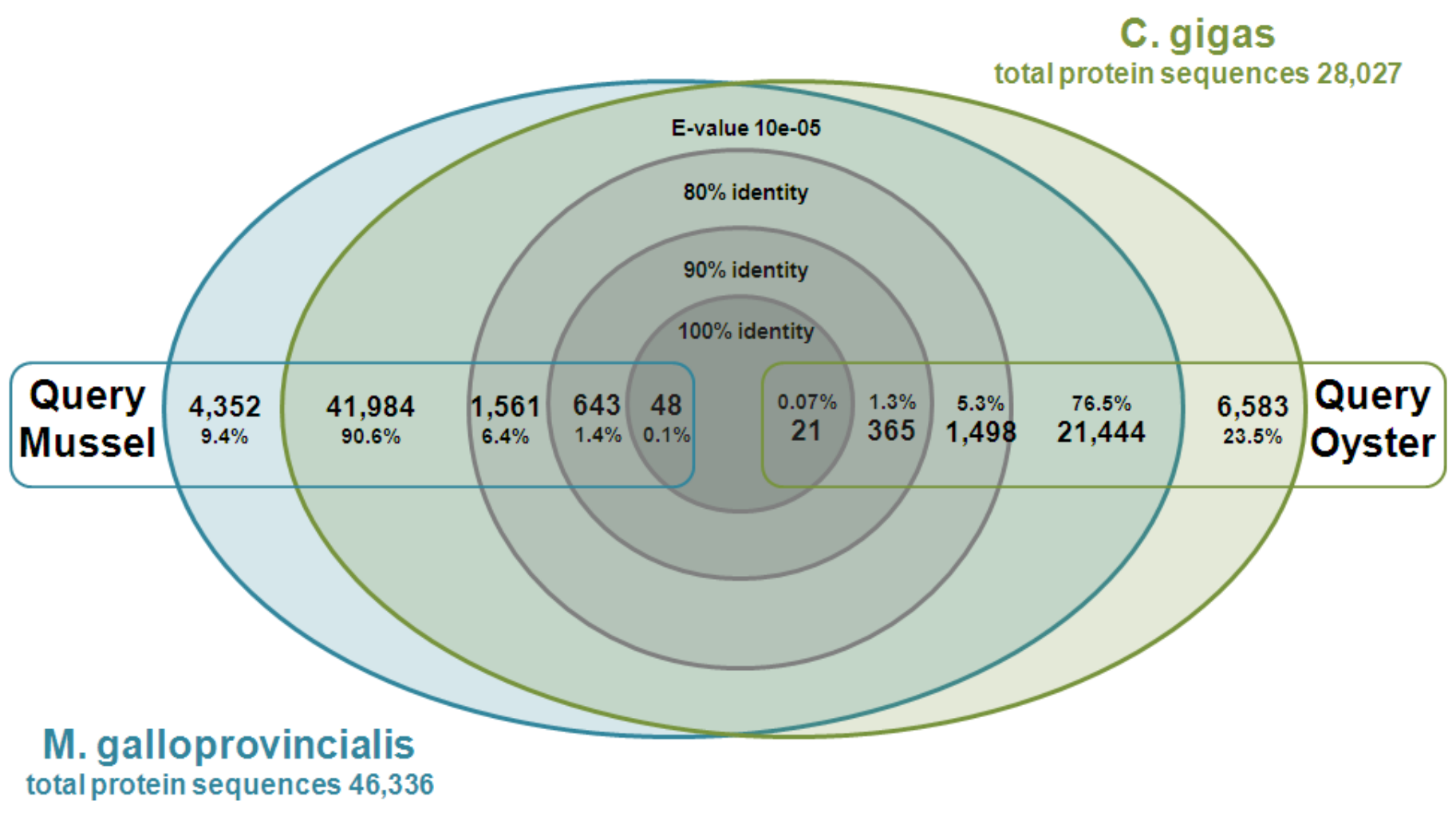
KEGG pathways

256 pathways

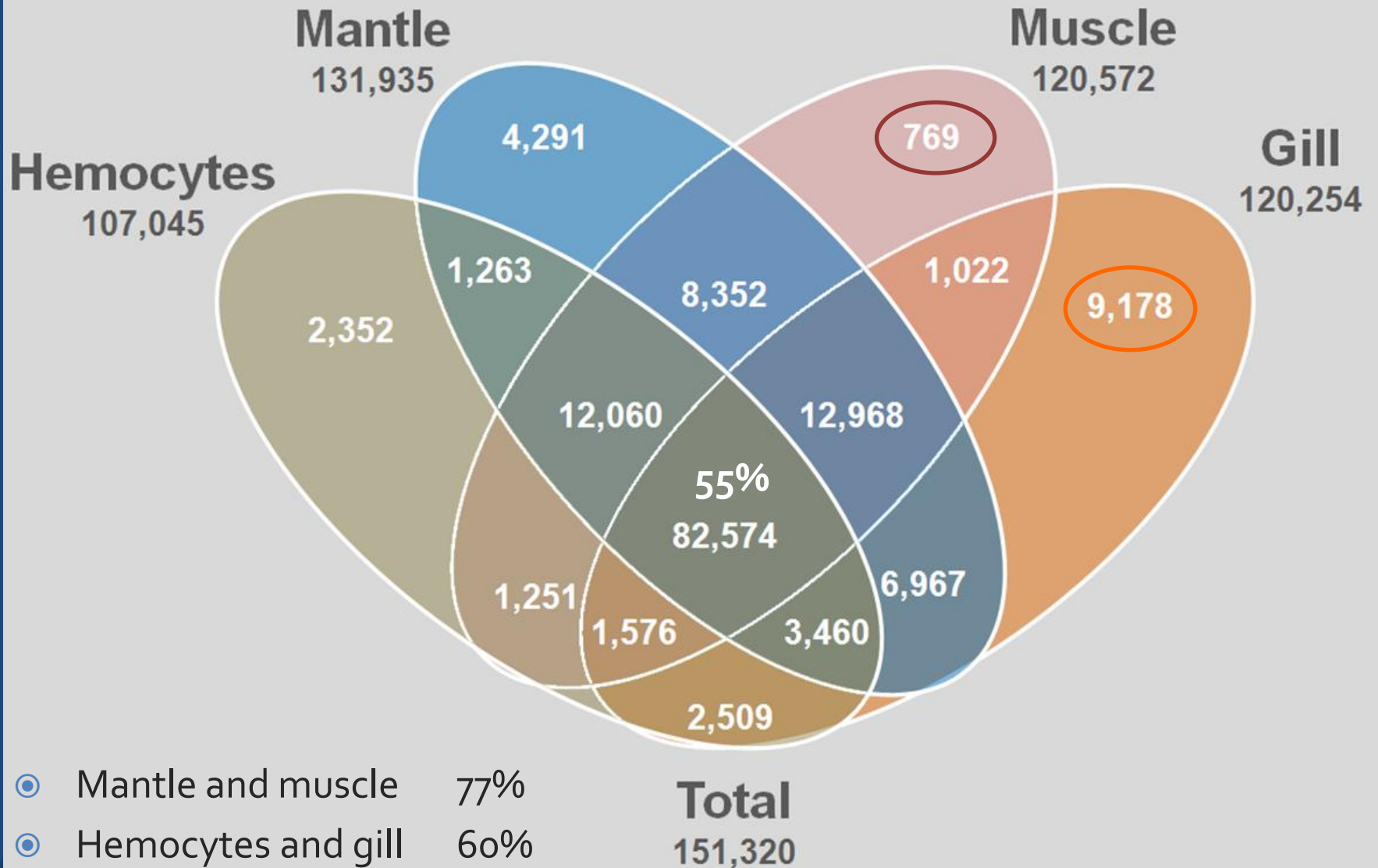
- Defense
- Signal transduction
- Cancer



Mussel vs oyster



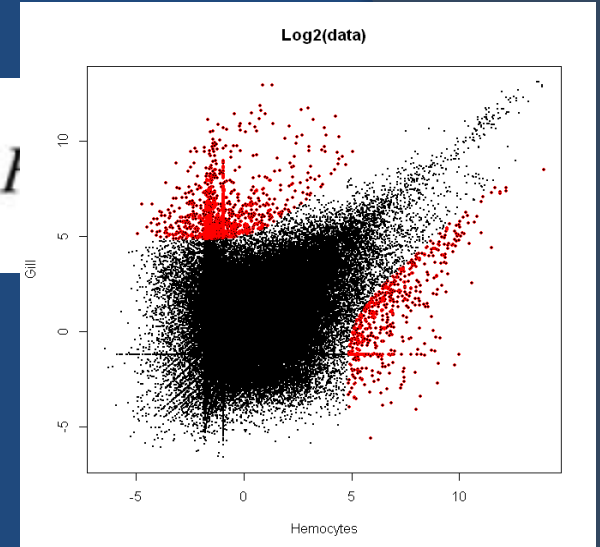
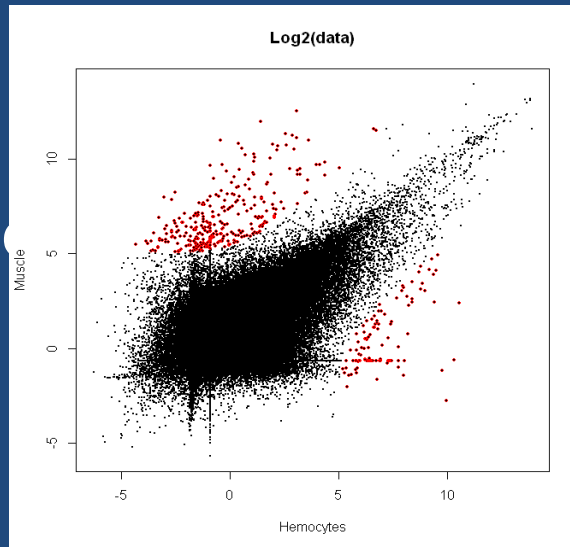
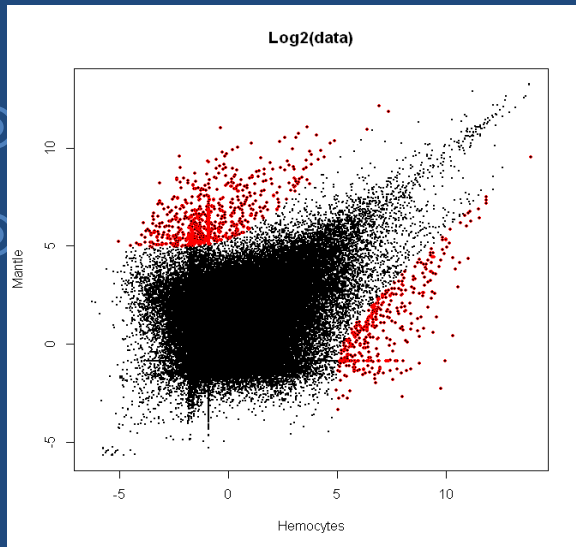
Transcriptomes general description



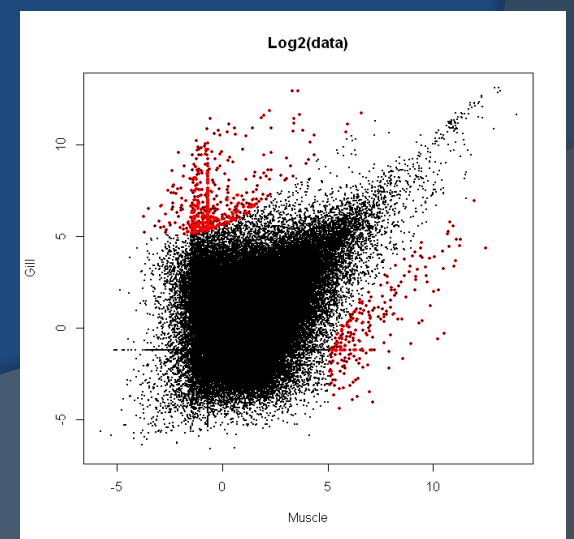
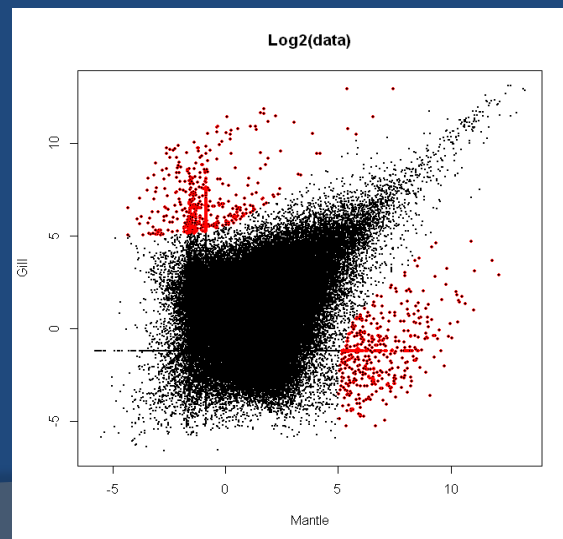
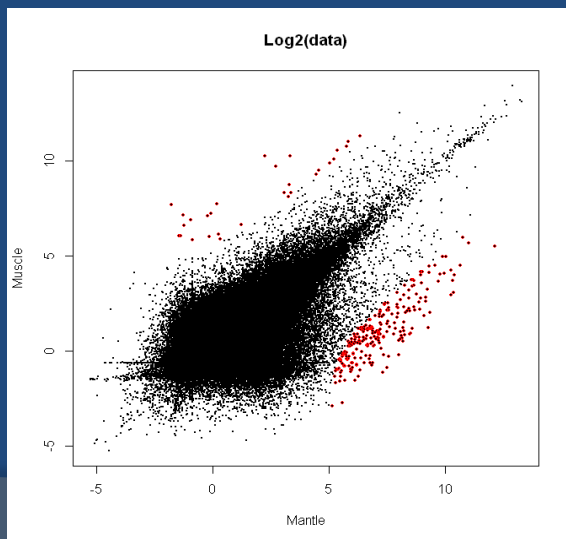
Exclusive transcriptomes

Reads	Hemocytes top exclusive	Reads	Mantle top exclusive
466	C1q domain containing protein MgC1q28	305	von Willebrand factor D and EGF domain-containing protein
300	defensin	293	Fibroin heavy chain
268	mytilin B	283.5	C1q domain containing protein MgC1q95
159	fibrinogen-related protein	245	nacrein B3
146	mytilin-6	215	ADAM family mig-17
141	Serine protease inhibitor Cvs1-2	165.5	fibrocystin L
112.5	complement component 4	160.5	Gigasin-6
73.5	C1q domain containing protein MgC1q56	152.5	C1q domain containing protein MgC1q69
69.5	myticin C gene	149	ATP-dependent RNA helicase A
56.5	fibrinogen-related protein (FREP_G1)	115.5	peroxidasin homolog (Drosophila)-like
Reads	Muscle top exclusive	Reads	Gill top exclusive
107	ribosomal RNA	6644	Perlucin-like protein
62	hsp90-2 gene for heat shock protein 90	3223	C1q domain containing protein MgC1q71
31	myticin C	997	Fucolectin
30.5	Angiopoietin-4	989	Collagen alpha-1(XII) chain
27	stress-70 protein, mitochondrial-like	916	C1q domain containing protein MgC1q17
25.5	ficolin-2-like, partial	897	C1q domain containing protein MgC1q52
12.5	C1q domain containing protein MgC1q22	889	C1q domain containing protein MgC1q36
11.5	Small heat shock protein hspl, mitochondrial	858	C1q domain containing protein MgC1q81
11	DnaJ homolog dnj-10	795	fibrinogen-related protein
9.5	Macrophage receptor MARCO	772	Fibrinogen C domain-containing protein 1

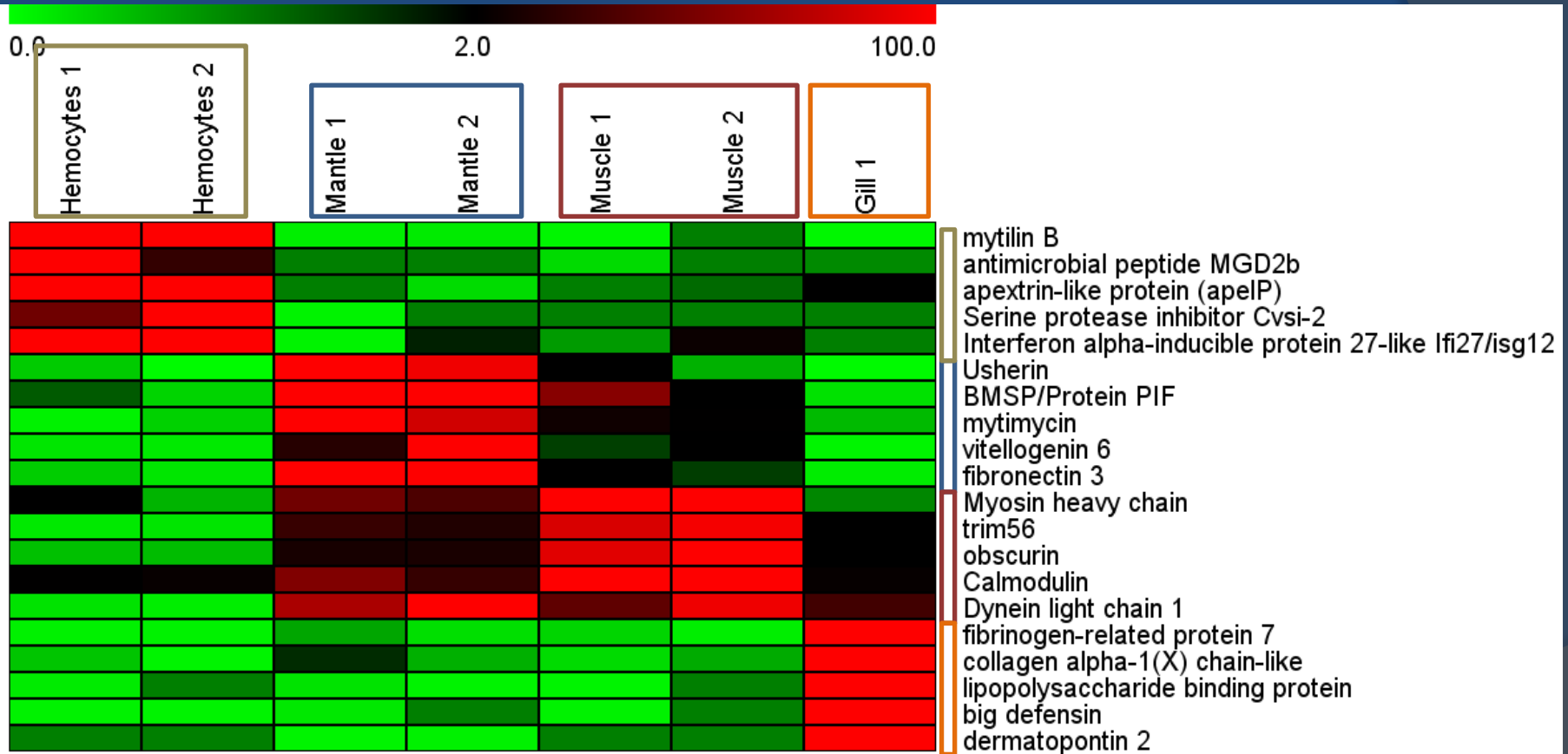
Expression analysis: NOIseq



Expression charts: in red the regulated sequences p-value 0.01



Tissues differential expression

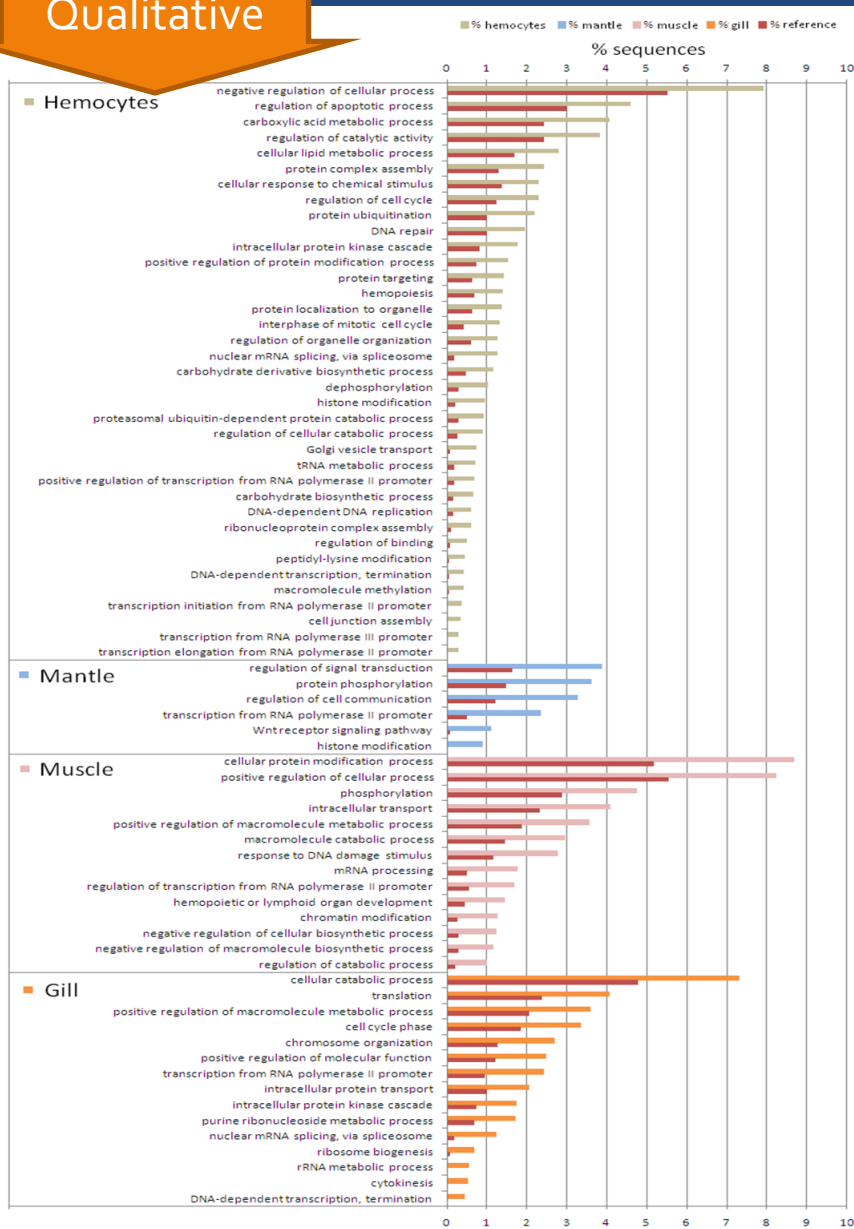


Top expressed sequences

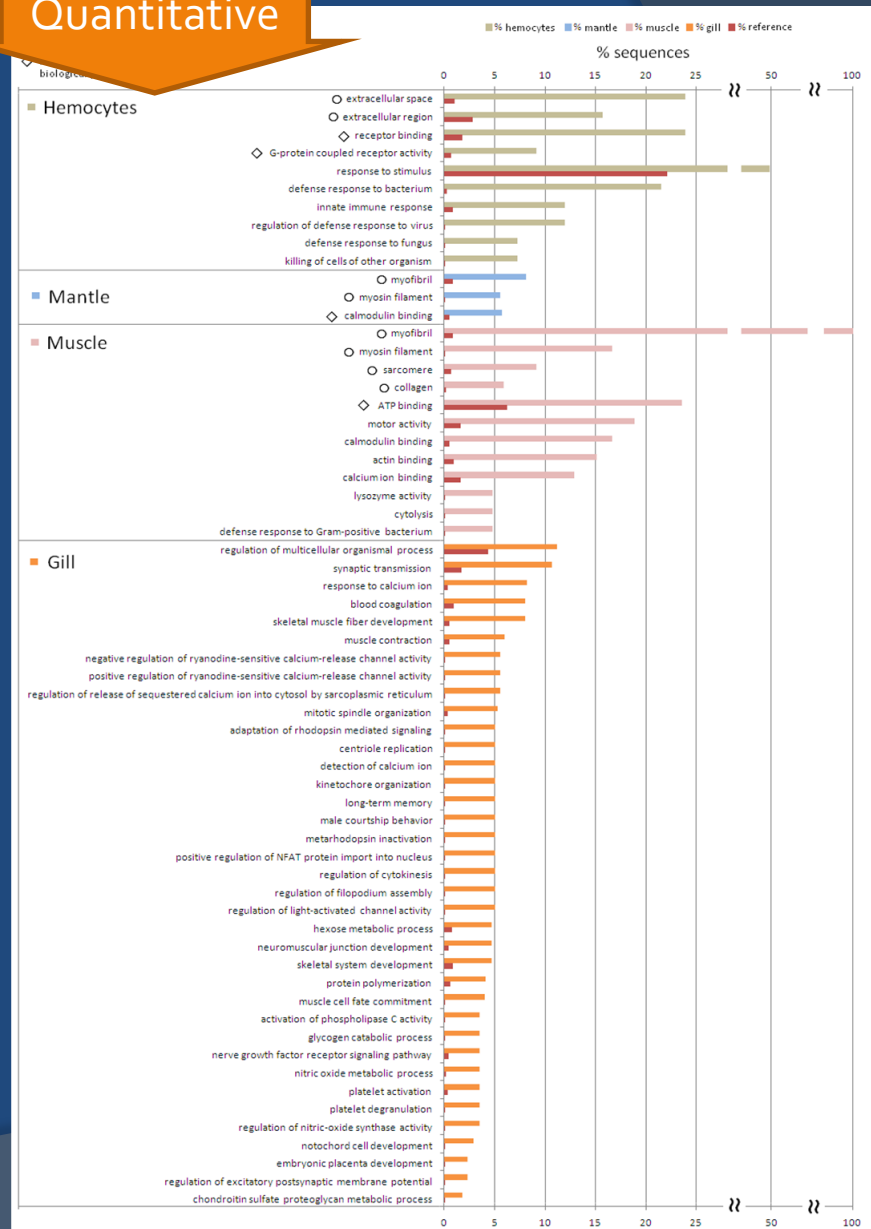
FC	Hemocytes top expressed	FC	Mantle top expressed
1,820	Mytilin B	5,008	Usherin
709	C-type lectin	2,521	BMSP / Protein PIF
478	Defensin 2b (MGD2b)	1,489	Mytimycin
465	C1q domain containing protein	1,409	L-rhamnose-binding lectin CSL3
428	Mytilin-6	1,314	Protein diaphanous
357	Apextrin-like protein (apelP)	1,314	Vitellogenin 6
260	Serine protease inhibitor Cvs.i-2	1,261	Fibronectin 3
258	MAC/perforin- and kringle-domain containing protein	1,218	Heat shock protein 70
246	Heat shock protein 70	1,184	Hornerin / filaggrin
241	Interferon alpha-inducible protein 27 2B (IFI27 / ISG12)	1,168	Chitinase 3
FC	Muscle top expressed	FC	Gill top expressed
1,226	Myosin heavy chain	2,837	BMSP / protein PIF
1,128	Heat shock protein 70	2,817	Perlucin
787	C1q domain containing protein	1,833	Fibrinogen-related protein 7
776	Tripartite motif-containing protein (TRIM56)	1,734	Collagen alpha-1(X) chain-like
481	Obscurin	1,479	Collagen triple helix repeat protein
471	Calmodulin	1,458	Lipopolysaccharide binding protein
471	Dynein light chain 1	1,269	Big defensin
458	Myosin light chain	1,261	Alpha 1 type V collagen
452	Calpain-5	1,243	Apextrin-like protein
440	Paramyosin	1,209	Dermatopontin 2

GO enrichment analyses

Qualitative



Quantitative



Hemocytes GO analysis

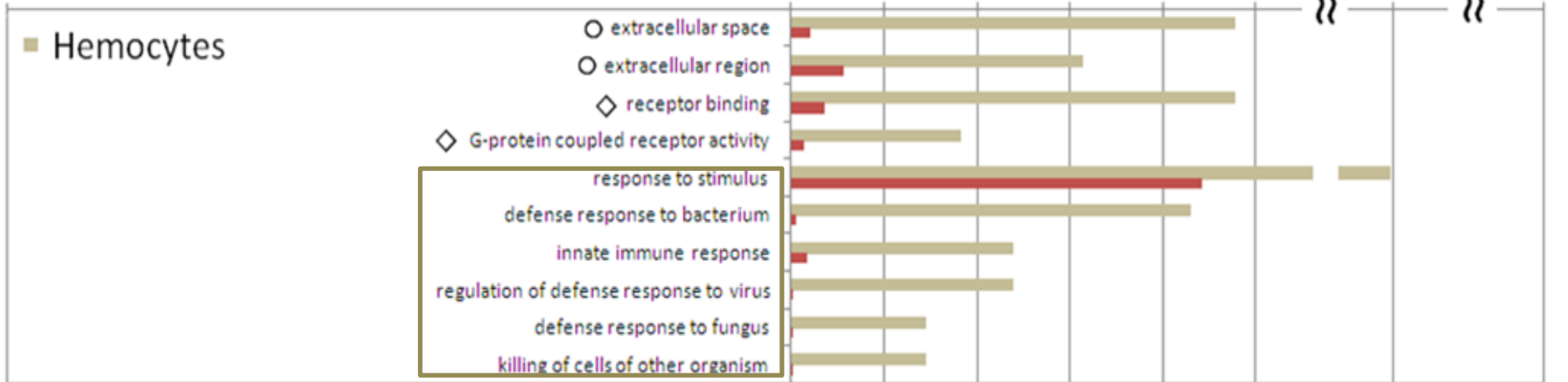
Quantitative

Differentially expressed genes

- cellular component
- ◇ molecular function
- biological process

■ % hemocytes ■ % mantle ■ % muscle ■ % gill ■ % reference

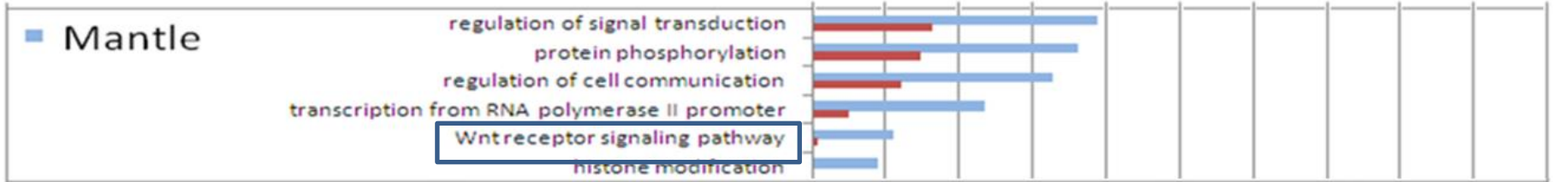
% sequences



Mantle and muscle GO analysis

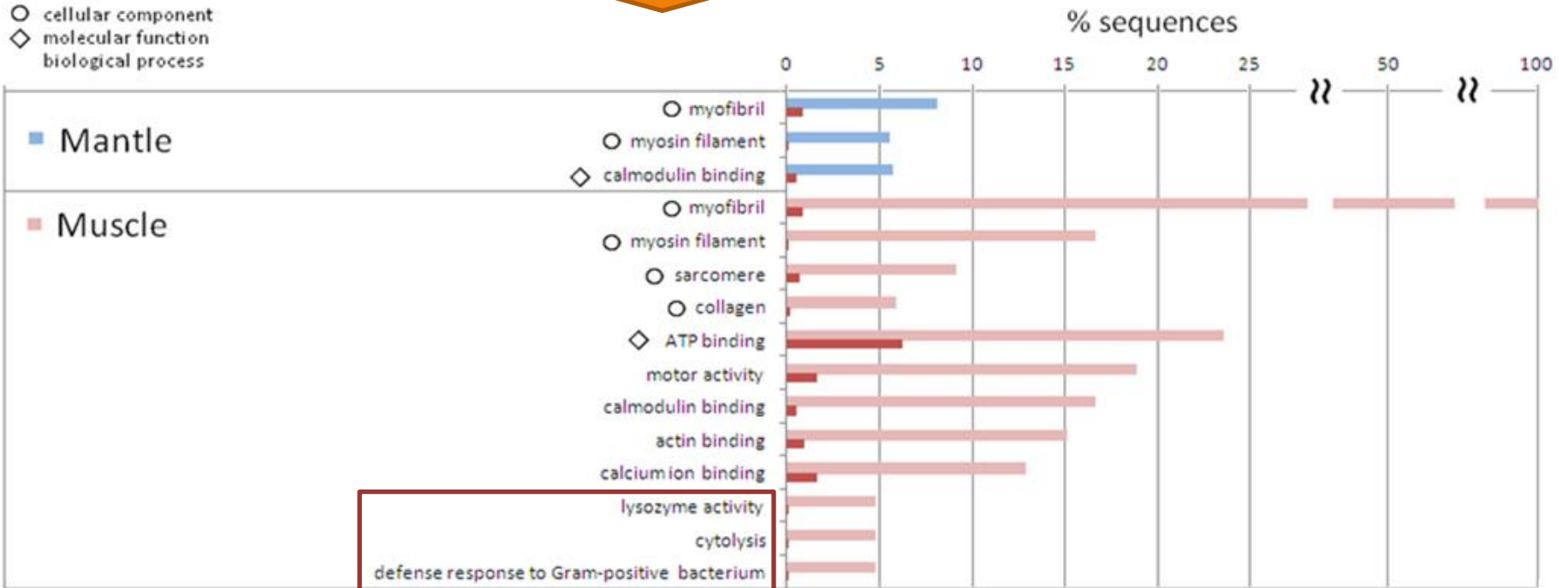
Qualitative

Complete transcriptomes



Quantitative

Differentially expressed genes



Gill GO analysis

Quantitative

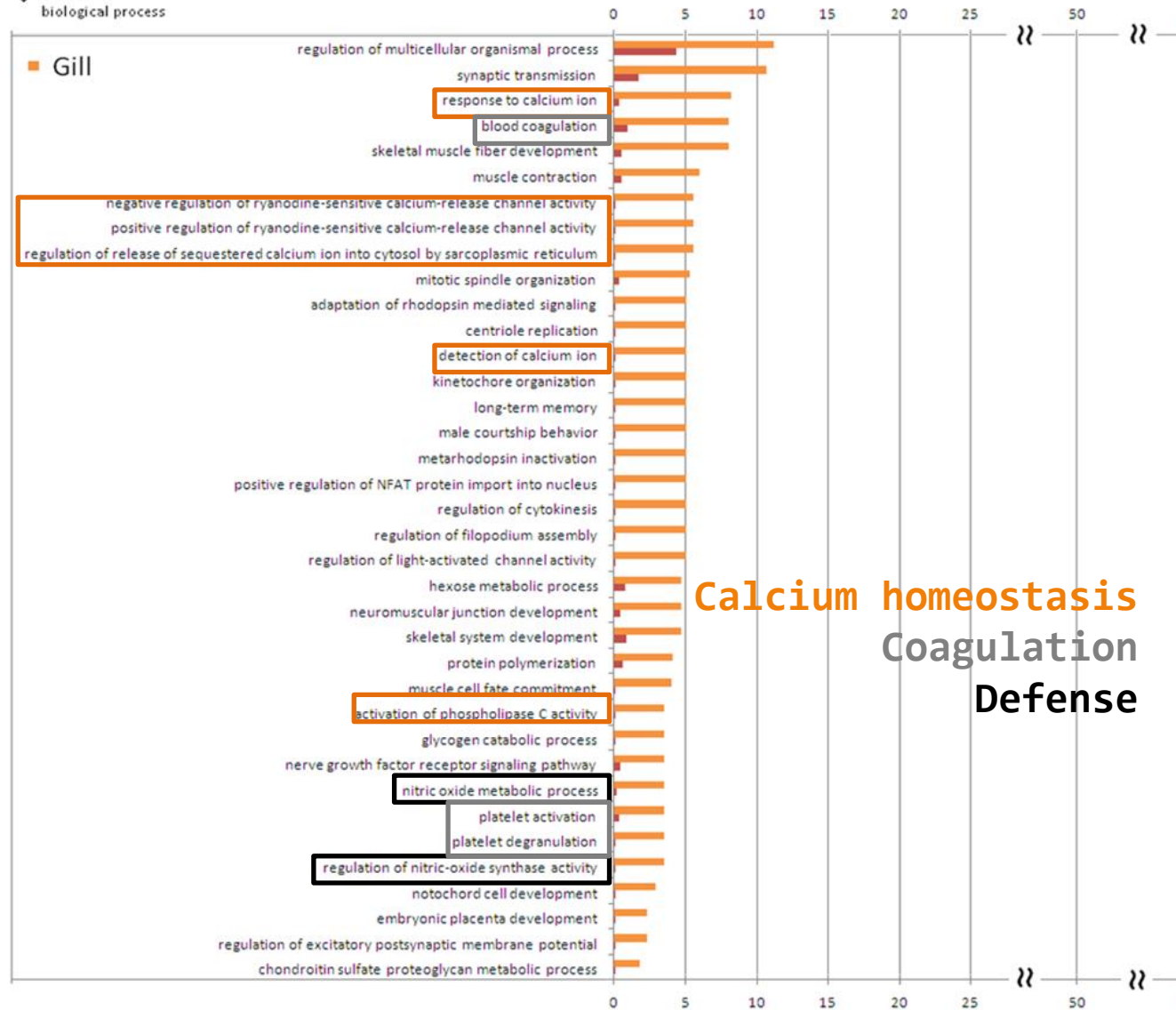
Differentially expressed genes

- cellular component
- ◇ molecular function
- biological process

■ Gill

■ % hemocytes ■ % mantle ■ % muscle ■ % gill ■ % reference

% sequences



Calcium homeostasis
Coagulation
Defense

Conclusions:

- ⦿ Almost 400 million reads obtained.
- ⦿ *M. galloprovincialis* hemocytes, mantle, muscle and gill transcriptomes and expression profile was established.
- ⦿ Important known and new functions were found:
 - **Hemocytes:** antimicrobial and defense proteins.
 - **Mantle:** hematopoietic, sensorial and antifungal function.
 - **Gills:** recognition molecules and homeostatic functions.
 - **Muscle:** stress and defense proteins.
- ⦿ The immune system of these animals seems to be a key regulator of many processes not only triggered by pathogens.

Acknowledgments:

- Ministerio de Ciencia e Innovación



- Mussel genome project





Thanks you for your attention!