# Beatriz Novoa & Antonio Figueras

## Immunology & Genomics Group

## Marine Research Institute (IIM-CSIC), Vigo, Spain

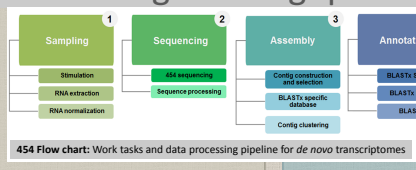

## Bivalves

## Fish

### Transcriptomics: High-throughput sequence analysis

**454 –** *R. philippinarum* Hemocytes

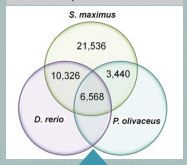
| | |
|---|---|
| Number of high quality reads | 974,976 |
| N50 read length | 338 |
| Average read length | 256.78 |
| Number of reads assembled | 842,957 |
| Number of contigs | 51,265 |
| Average contig length | 582.4 |
| N50 contig length | 677 |
| Number of contigs >500 pb | 26,675 |
| Number of clusters | 29,679 |
| Percentage of contigs annotated | 44.7 |



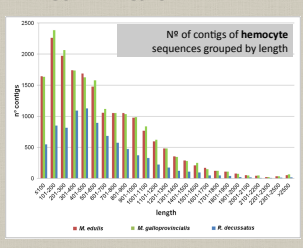

**454 Flow chart:** Work tasks and data processing pipeline for *de novo* transcriptomes

| | |
|---|---|
| Number of high quality reads | 915,256 |
| N50 read length | 381 |
| Average read length | 297.14 |
| Number of reads assembled | 733,411 |
| Number of contigs | 55,404 |
| Average contig length | 671.3 |
| N50 contig length | 756 |
| Number of contigs >500 pb | 31,764 |
| Number of clusters | 41,870 |
| Percentage of contigs annotated | 44.4 |

**Common and exclusive sequences of turbot** obtained with the **454** approach, compared to zebrafish and Japanese flounder.




**REPROSEED PROJECT:**

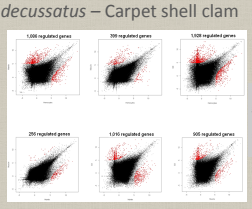



**- 454 :**

*M. galloprovincialis* – Mediterranean mussel

*M. edulis* – Blue mussel

*R. decussatus* – Carpet shell clam



**NOISeq expression graphs:** in **red** the **regulated sequences** in the indicated tissues.

**- RNAseq (millions of sequences):**

*M. galloprovincialis*

**AQUAGENOMICS-CONSOLIDER project:**

**- 454 sequencing :**

*S. maximus* - Turbot

*D. labrax* - Sea bass

*S. aurata* - Sea bream

- Thousands of sequences included in public databases
- Characterization and analysis of processes such as: feeding, metabolism, growth, behavior, immune response against pathogens…
- Development of tools such as **microarrays**

### Microarrays

- *R. philippinarum* challenged with *V. alginolyticus* (*Moreira et al.* 2013)

3h → Signaling Transcription Apoptosis → 8h → Immunity and defense → 24h → Homeostasis → 72h → Return to basal conditions

Differences between the transcriptomes of control and challenged clams against bacteria (*V. alginolyticus*) and parasites (*Perkinsus*).

-*Turbot* challenged with VHSV (*Díaz-Rosales et al.*, 2012): : Study of gene expresion differences between resistant and susceptible turbot families against viral challenges.

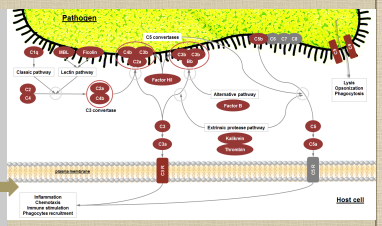
- Changes in the transcriptome profile after vaccination and after viral infection with /without previous vaccination: which are the changes associated to protection in fish?

- Zebrafish as a model for obesity and response to bacterial and viral stimuli.

### Applications

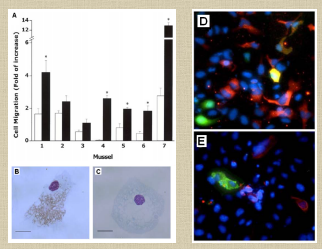
**- Identification of bioactive molecules:**

- Caspases and apoptotic genes in mussel (Romero *et al.*, 2011): **possible biomarkers for aquatic pollution**
- Pore-forming molecules in mussel (Estévez-Calvar *et al.*, 2011)
- Immunue pathways inferred from the Manila clam 454 results (Moreira *et al.*, 2012): putative "immune moleecules"
  - **TLR signaling pathway**
  - **Apoptosis**
  - **Complement cascade**




**-The antimicrobial peptides: highly expressed genes in bivalves:**

**Identification of high variable molecules in mussel: *Myticin C***: an antimicrobial peptide with chemotactic, antiviral and immunoregulatory properties (Vera *et al.*, 2011; Balseiro *et al.*, 2012)

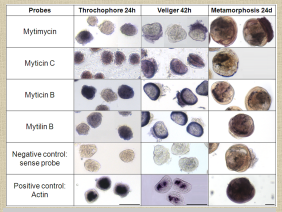



**Chemotactic assay** (A) of mussel hemocytes. Hemocyte **immunocytochemistry** after migrating to the chamber with Myticin C containing plasmid (B) and after migrating to the control chamber (C).
**Antiviral properties of Myticin C:** CHSE cells transfected with empty plasmid (D) or Myticin C containing plasmid (E)**. Cells expressing myticins are not infected with the virus.**
Blue: DNA staining
Green: transfected cells
Red: VHSV

**- Expression studies:**

Immunocompetence in mussel larvae

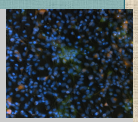
(Balseiro et al., 2012)



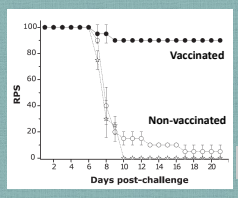

***In situ* hybridization** of mussel larvae for different antimicrobial peptides.

**-Identification of bioactive molecules:**

- Hepcidin (Pereiro et al., 2012)
- WAP65
- **NK lysin immunofluorescence assay:**
  NK lysin expressing cells do not become infected
  **Green:** SVCV infected EPC cells
  **Orange:** Nk lysin expressing cells



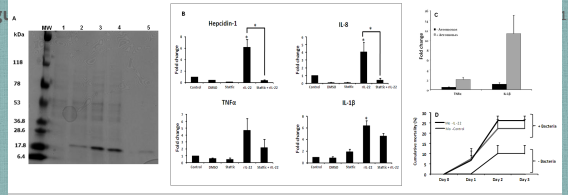
- Immune pathways inferred from the turbot 454 results (Pereiro et al., 2012):
  **TLR signaling pathway | B-cell & T-cell signaling pathway | Apoptosis | Complement cascade**




Survival (RPS) of turbots infected with VHSV .

**Use of Immune genes to produce a higher protection induced by DNA vaccine against VHSV in turbot:**

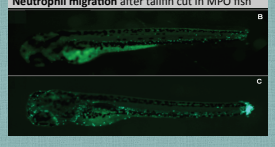
**Use of immune genes as immunostimulants: IL-22, a bioactive molecule and key regu… 13)**




**Recombinant turbot IL-22** (A). The **IL-22 induced inflammatory proteins through STAT3 pathway** (B). In zebrafish, the blocking of the **IL-22 with morpholinos** after an *in vivo A. hydrophila* infection induced higher inflammation and mortality (C, D).

The **zebrafish as a model** for the study of i… ocesses:

**Neutrophil migration** after tailfin cut in MPO fish



**3D reconstruction** of the effect of the **SVCV infection in zebrafish** embryos after 24 hours. The virus induces cell death by pyroptosis and apoptosis mechanisms.
Blue: pyroptosis
Purple: virus
Green: apoptosis