

# RNA-Seq of Bacterial outer membrane vesicles and transcriptome identify sRNAs targeting immune relevant genes in rainbow trout susceptible or resistant to *Flavobacterium psychrophilum*

Pratima Chapagain<sup>1</sup>, Ali Ali<sup>2</sup>, Desta Kidane<sup>1</sup>, Mary Farone<sup>1</sup> and Mohamed Salem<sup>2\*</sup>

<sup>1</sup>Department of Biology and Molecular Biosciences Program, Middle Tennessee State University, Murfreesboro, TN, 37132, USA

<sup>2</sup>Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742-231, USA

## Abstract

The outer membrane vesicles (OMVs) of gram-negative bacteria contain sRNAs, toxins, and virulence factors that are released from the bacterial surface during host-pathogen interaction. In this study, OMV from *Flavobacterium Psychrophilum* (Fp), the etiological agent of Bacterial Cold-Water Disease (BCWD) in salmonids, was isolated and visualized by Transmission Electron Microscopy (TEM) as small spherical nano-shaped particle around 50-100nm. RNA-Seq identified 747 transcripts expressed in OMVs and 1,939 transcripts expressed in the whole transcriptome with 700 transcripts in common. Three transcripts associated with OMVs formation (2 OmpA family protein transcripts, and ompA family outer membrane protein) have been highly expressed in the transcriptome.

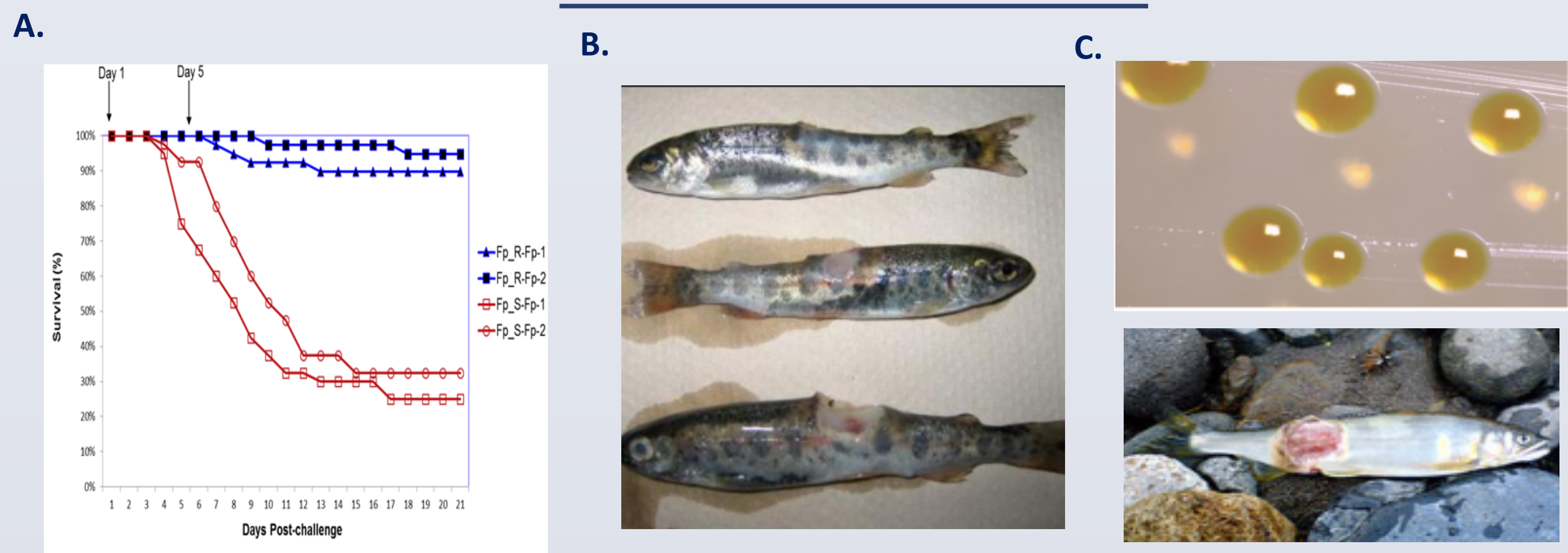
Out of 232 sRNAs computationally identified in the Fp genome, 5 sRNAs were expressed in the OMVs. The potential interaction of sRNAs-trout immune genes was investigated in two rainbow trout genetic lines, BCWD-resistant and susceptible, created by selective breeding. qPCR was used to determine the reciprocal expression of the sRNAs and their mRNA targets in whole-body lysates after 5 days of infection.

Interestingly, immune-related genes, mitogen-activated protein kinase-7 (MAPK), and Macrophage mannose receptor-1, c-c motif chemokine 21-like were downregulated in the susceptible compared to resistant fish whereas sRNAs (expressed in OMVs) targeting those genes were showing the reciprocal expression. Further research is suggested to explain the mechanism of sRNAs entry through vesicles into host cells.

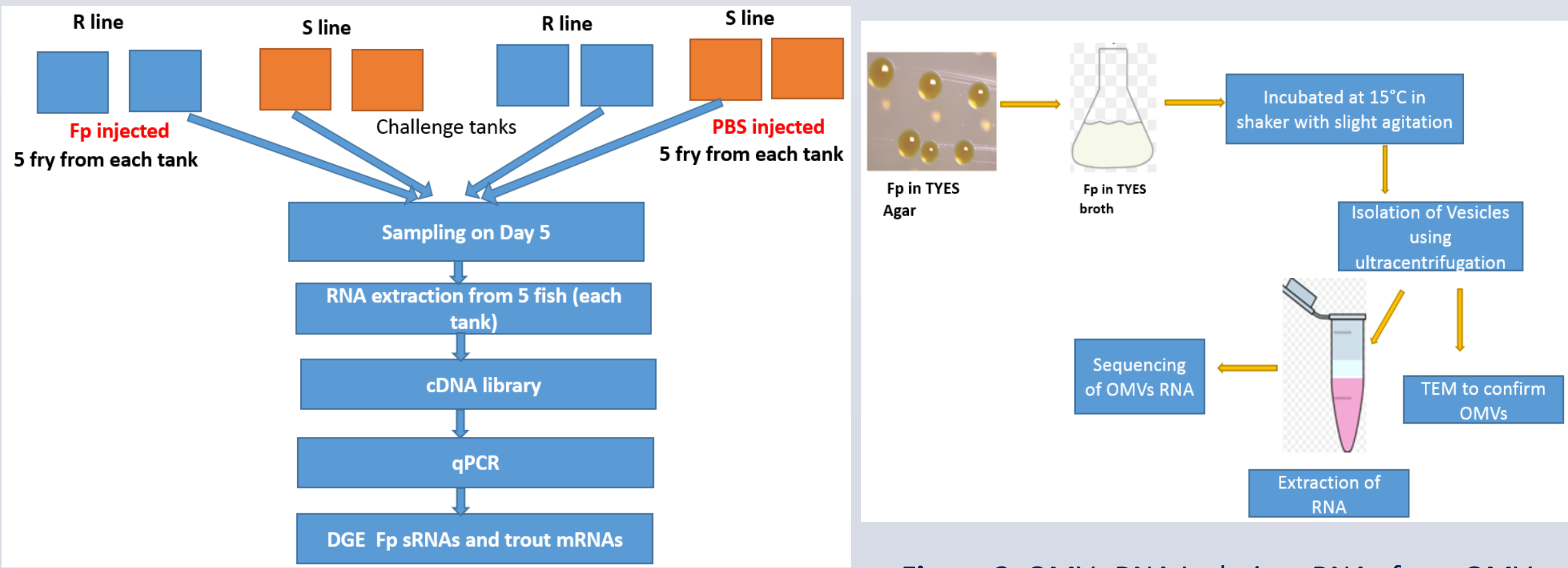
## Objectives

- To identify sRNAs and determine the expression of sRNAs in OMVs and the whole transcriptome of *Flavobacterium psychrophilum*
- To identify immune related genes in rainbow trout targeted by Fp sRNAs
- To quantify the reciprocal expression of highly expressed sRNAs in OMVs and their immune-related gene targets in ARS-Fp-R (resistant) and ARS-Fp-S (susceptible) genetic lines of rainbow trout after 5 days Fp challenge

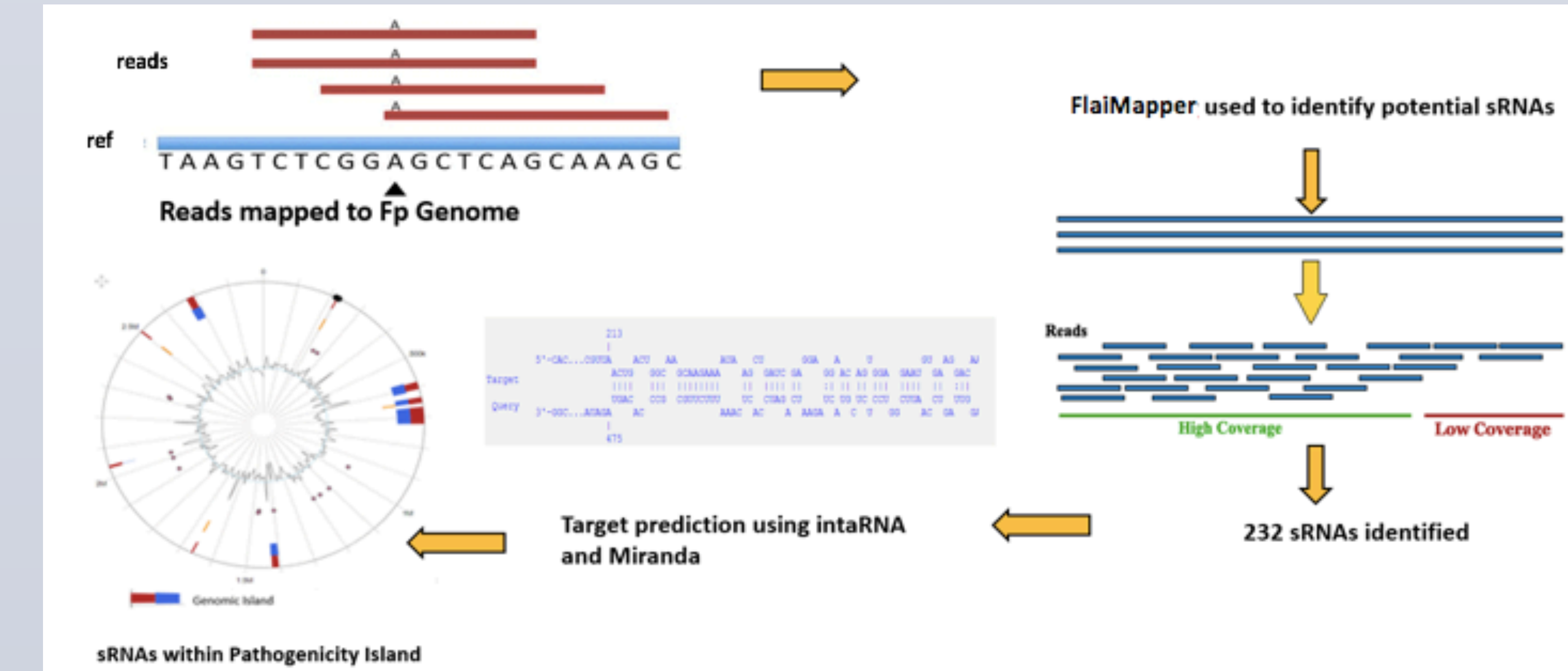
## Materials and Methods



**Figure. 1 A)** Post-challenge survival of resistant (blue line), and susceptible (red line) fish. Survival differences were significant between genetic lines ( $p < 0.001$ ). **B)** Fish infected with BCWD. **C)** Colonies of Fp bacteria causing caudal muscle degradation in rainbow trout

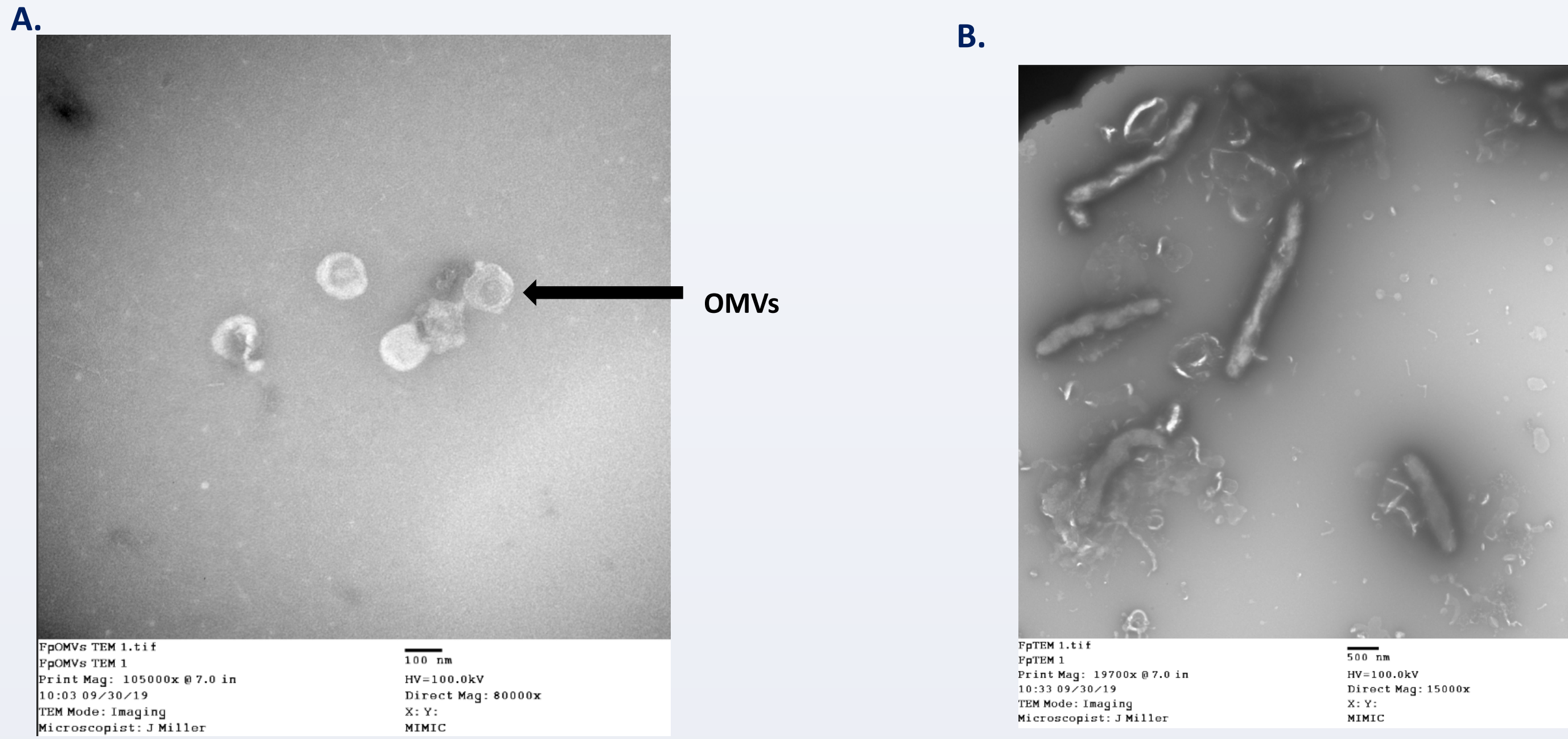


**Figure 2.** Overall project design, sample collection and analysis of DGE



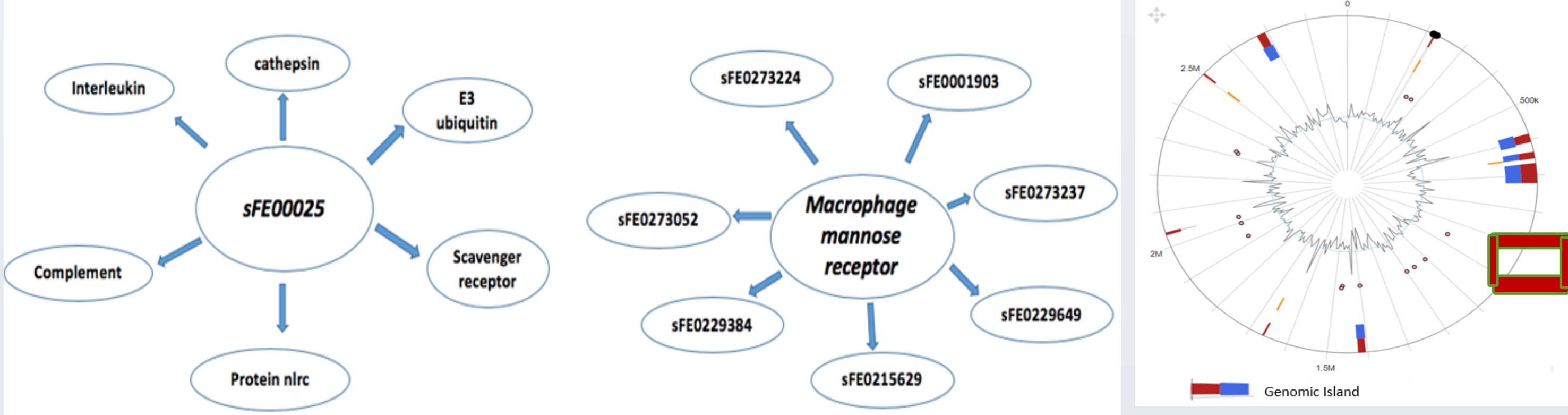
**Figure 4.** Workflow of genome-wide prediction of sRNAs. RNA-seq reads were mapped to Fp Genome; 232 sRNAs were identified which were then subjected to target prediction and PI.

## Results

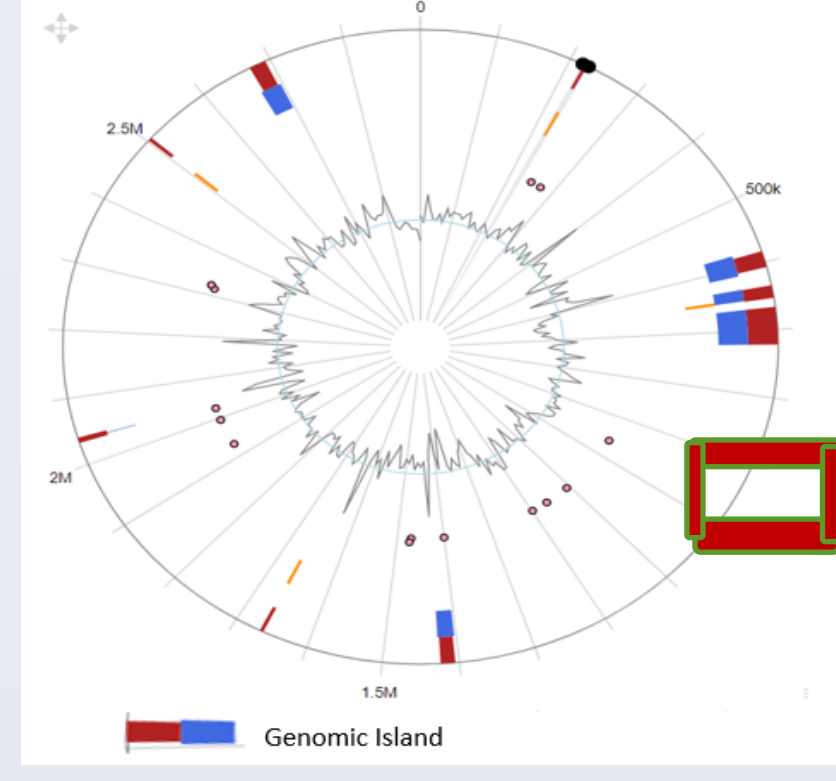


**Figure 5. A)** Transmission Electron Microscopy (TEM) of Fp OMVs. OMVs appeared as spherical, nano-shaped particle

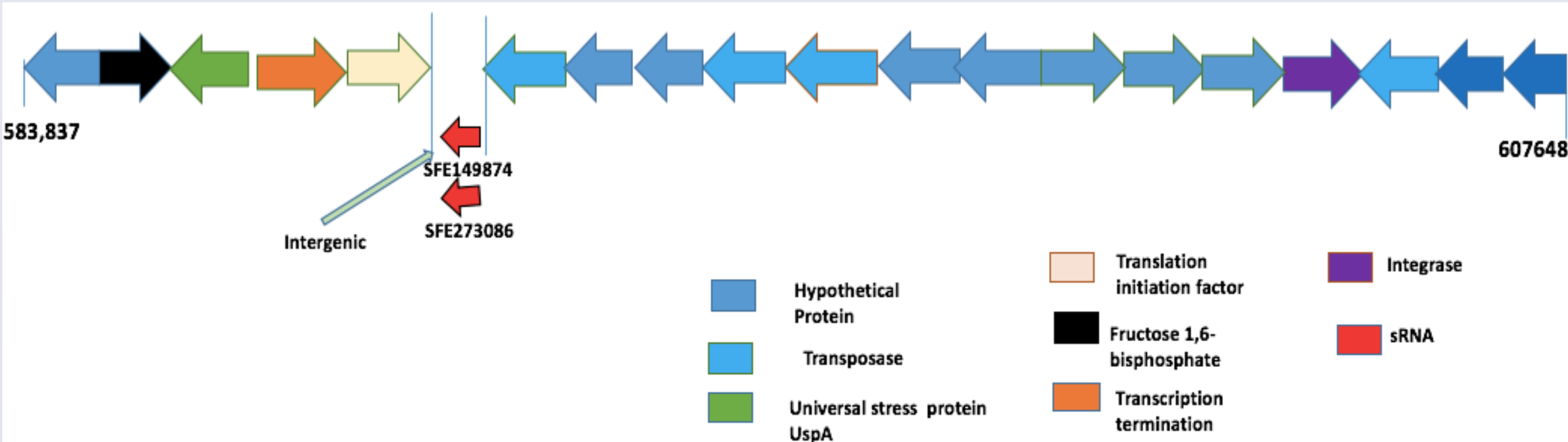
**Fig 5. B)** Transmission Electron Microscopy of Fp. It appeared as rod shaped structure



**Figure 6.** Target prediction showing single sRNA can have multiple targets and single gene can be targeted by multiple sRNAs



**Figure 7.** Genomic islands of Fp. Red block indicates the pathogenicity island where sRNAs are located.



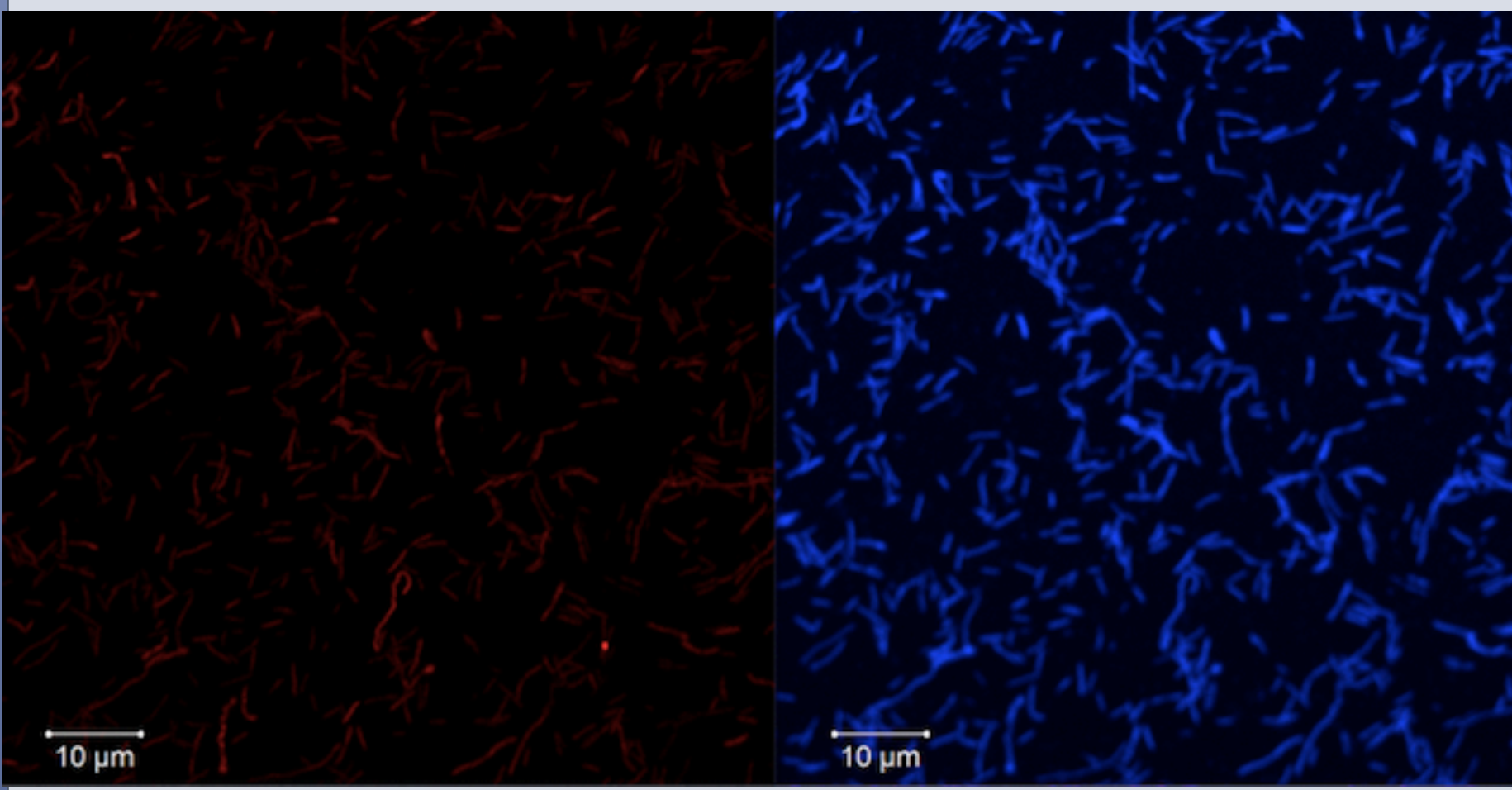
**Figure 8.** Location of OMVs expressed sRNAs within genomic island. Among 5 sRNAs, 2 sRNAs lies within pathogenicity island are intergenic and are antisense. Different color arrows indicate genes within that Pi whereas red arrow indicates sRNA.

**Table 2.** Fp sRNAs expressed in Transcriptome and OMVs based on RNA-seq analysis

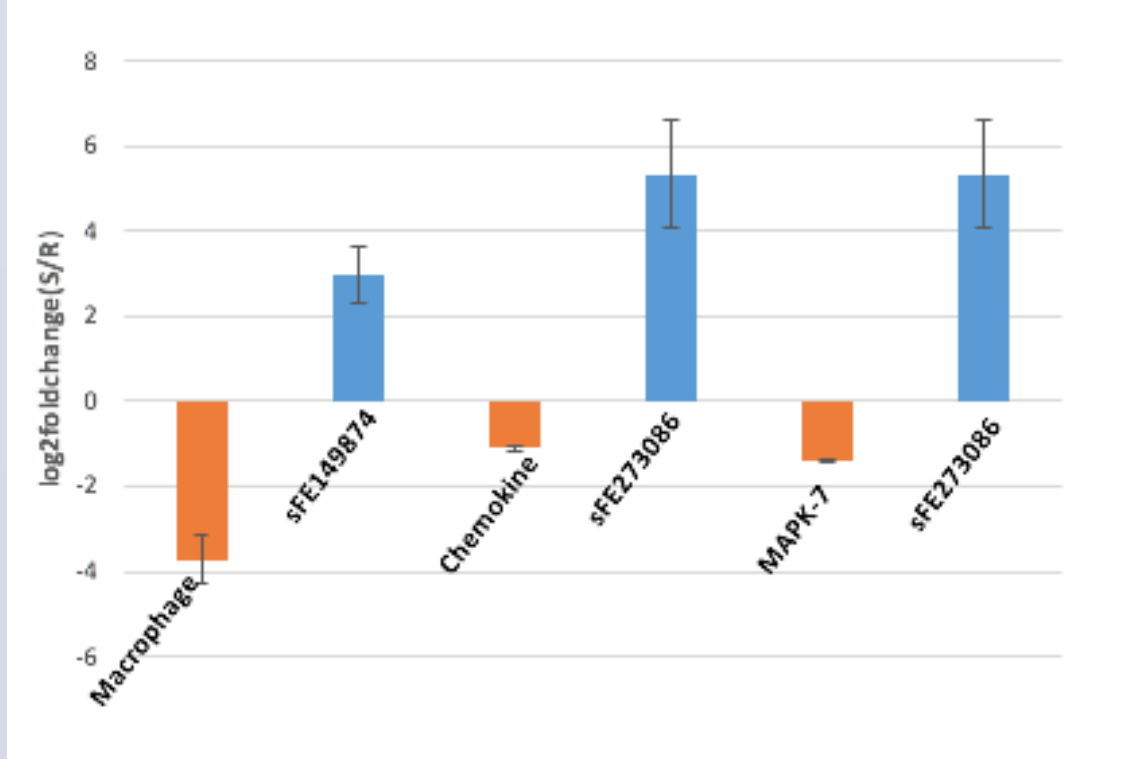
OMVs	
sRNAs	RPKM
sFE149874	115893
sFE273086	5318
sFE181757	2
sFE284886	0.9
sFE122037	0.8

**Table 3.** Transcripts associated with OMVs biogenesis and are expressed in transcriptome

Transcripts	Annotation
gene20	OmpA family protein
gene170	OmpA family protein
gene4	OmpH family outer membrane protein



**Figure 9 .** Confocal microscopy imaging of bacteria stained with DAPI and cy3- labeled sRNA probe. The bacteria having sRNA are stained and appeared as red fluorescence.



**Figure 10.** qPCR showing reciprocal relationship in expression, Fp sRNAs are upregulated in a susceptible fish whereas host mRNA targeted by that sRNA is downregulated in susceptible fish

## Conclusion

sRNAs may be involved in suppressing the host immunity and modulating the bacterial virulence during host pathogen interaction of Fp and rainbow trout. qPCR and confocal imaging determine the presence of sRNAs in Fp. However, the mechanism of sRNAs entry into host cells through OMVs should be fully understood and needs further research to study host pathogen interaction.

## Acknowledgements

