# **GENETIC EDITING FOR VIRAL RESISTANCE** IN AQUACULTURE FISH.

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## INTRODUCTION

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**DE COMPOSTELA** 

# Digadanaho lebrox

- VNN Resistance: QTL on chromosome 3 with multiple ifi27l2a copies linked to NNV resistance.
- Key SNP: Specific SNP in the promoter of one of the ifi27l2a copies, is strongly associated with resistance, explaining up to 38% of the genetic variation.
- Gene Expression: Higher ifi27l2a expression correlates with resistance, especially in Eastern Mediterranean populations.
- Conservation: ifi27l2a gene is highly conserved among sea bass, striped bass, and sea bream.

Nervous Necrosis Virus NNV

Ifi27I2a - Potential to improve disease resistance in aquaculture through marker-assisted selection.

- Antiviral Role: *ifi27l2a* is crucial in antiviral response, similar to sea bass.
- Differential Regulation: Differences in *ifi27l2a* regulation explain variability in NNV susceptibility between species.
- Conservation: *ifi27l2a* antiviral function is evolutionarily conserved.

## **OBJECTIVE & METHODS**

1. To explore the potential of CRISPR-based genetic editing to enhance disease resistance by knocking out the ifi2712a gene through electroporation, and to assess the efficiency of this gene-editing technique.

2. To perform a functional analysis of the *ifi27l2a* gene in sea bass (DLB1) and sea bream (SaB1) brain cell lines, investigating its role in resistance to Viral Nervous Necrosis (VNN), with the goal of translating these findings to improve resistance in the species in vivo.





**COMPLETED** X NOT COMPLETED

<u>OURA</u>





Figure 1: Electroporated DLB-1 gilt-head bream brain cell line with Cas9 tagged with Cy3. The left panel shows the cells under brightfield microscopy, while the right panel (MERGE) displays the co-localization of Cas9 (red fluorescence) in the cells, confirming successful electroporation.



Figure 2: A. Cas9-Cy3 delivery in sea bass brain cell lines (DLB1) via electroporation, showing survival, transfection, and editing efficiency across various concentrations. B. Editing efficiency comparison between IVT-sgRNA, SYNTHEGO-sgRNA, and Lipofectamine CRISPRMAX in DLB1 cells. Error bars represent standard deviations.

#### Α Indel Distribution and Sequence Discordance Plot



62% GCTGACTGTAAAACAGCCACCACCACCTCTGCCGCCACTCCTCCGCTGCAACCGCAGCAGCTGACATTATT g1 +1 +1 💻 **g1** –1 -1 12% GCTGACTGTAAAACAGCCACCACAC - TCCTGCTGCCACTCCTCCCCCCCCCCCACCCGCAGCAGCCGCAGCTGACATTATT g1 -3 -3 • 4% GCTGACTGTAAAAACAGCCACCACCACCACCAC---CTGCTGCCACTCCTCCCG!TTAGCAACCGCAGCAGCTGACATTATT **g2** -3 -3 **g1** –1 -1 1% GCTGACTGTAAAACAGCCACCACA- CTCCTGCTGCCACTCCTCCCG TTAGCAACCGCAGCAGCTGACATTATT





Figure 4: Electroporated SaB-1 seabass brain cell line with Cas9 tagged with Cy3. The left panel shows the cells under brightfield microscopy, while the right panel (MERGE) displays the colocalization of Cas9 (red fluorescence) in the cells, confirming successful electroporation.

Edit eff : 94 8<sup>2</sup> : 94.000

B sgRNA/Cas9 Electroporation SaB1 cells

3 uM

2 uM

### A sgRNA/Cas9-Cy3 Electroporation (3 uM) - SaB1 cell line



Figure 5: A. Cas9-Cy3 delivery in sea bream brain cell lines (SaB1) via electroporation, showing survival, transfection, and editing efficiency across various concentrations. B. Editing efficiency comparison between IVT-sgRNA, SYNTHEGO-sgRNA, and Lipofectamine CRISPRMAX in DLB1 cells. Error bars represent standard deviations.

#### A Indel Distribution and Sequence Discordance Plot



Figure 6: Analysis of CRISPR-induced indels in sea beram brain cell lines (Salndel Distribution and AlignmentB1) using the ICE software from Synthego. A. Sequence alignments and distribution of indels across the targeted region. B. Frequency of different indels detected. The table below summarizes the relative contribution of each sequence (normalized), highlighting the most frequent indels and their corresponding sequences.

#### В Indel Frequency and Contribution

g1 -48 g2 -46 AGACTTOCTGCTGCCACTGCTGCCACGGCCAAAG AGACTTOCTGCTGCCACTGCCACGGCCAAAG AGACTTOCTGCTGCCACTGCCACGGCCAAAG AGACTTOCTGCTGCCACTGCCACGGCCAAAG CACTTOCTGCCACTGCCCCCC C - TTAG **21** -41 **22** -53 AGCACITECTGCTGCCACTCCTCCT CCGTTAGCAATCGCAGCAGCAGCACATCATTTTAGCAGCGATGGAGCCCGCCGCTATTCCAGCTGAGGTGAAGCCTGCAGCGCCCAGGGCCAAAG AGCACTTCCTGCTGCCACTCCTCCT CCGTTAGCAATCGCAGCAGCAGACATCATTTTAGCAGCGATGGAGCCCGCCGCCGCTATTCCAGCTGAGGTGAAGCCCGCGCCCAGGGCCAAAG NNNNNNNNAGCCCCGACCACGGC g1 -48 g2 -46 AGCACTTCCTGCTGCCACTCCTCC CCGACCACGGCAACAGCTG g2 -20 AGCACTTCCTGCTGCCACTCCTCCT CCGTTAGCAATCGCAGCAGCAGCATCATTTTAGCAGCGATGGAGCCCGCCGCTATTCCAGCTGAGGTGAAGCCTGCAGCG - CACGGCAACAGCTG g2 -28 g**2** -15 g2 -8 AGCACTTCCTGCTGCCACTCCTCT

> Genetic editing in brain cell lines of fish

> > 1. Increase in survival

## Take-Home Message:

## Proof-of-Concept for Genetic Editing:

• Our research successfully demonstrates the potential and effectiveness of CRISPR-based genetic editing in fish brain cell lines, yielding promising results for studying gene function, improving survival rates, and enhancing genetic resistance to viral infections.

#### **Future In Vivo Applications:**

Genetic resistence identification Future in vivo applications 0°

• While current in vivo applications of genetic editing in aquaculture are focused on scientific research and validation, the results pave the way for future innovations. This approach could eventually lead to more efficient selection and breeding strategies in aquaculture, enhancing disease resistance across species.

**Towards Efficient Breeding:** 

 By confirming genetic resistance through in vivo studies, we can better understand the implications of genetic editing. This will allow for more targeted and efficient breeding programs, ultimately improving the resistance of aquaculture species to diseases.