GENETIC EDITING FOR VIRAL RESISTANCE IN AQUACULTURE FISH.

Martínez P[a]; Arana A[a]; Cuesta A[b]; Sánchez L[a]; Robledo D[a].

a. University of Santiago de Compostela, Lugo, Lugo, Spain
b. University of Murcia, Murcia, Spain

INTRODUCTION

Dicentrarchus labrax

- **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

Sparus aurata

Ifi27l2a* - 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 *ifi27l2a* 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.

RESULTS

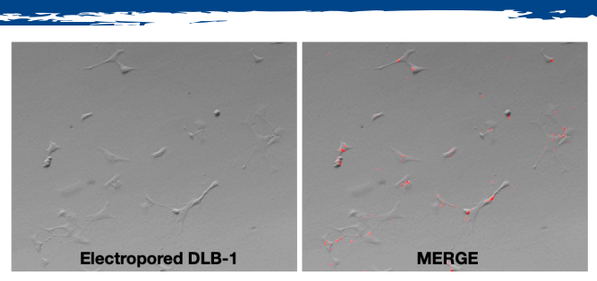

Figure 1: Electroporated DLB-1 gilt-head bream brain cell line with Cas9 tagged with Cy3. The left panel shows the cells under brightfield microscopy, while the right panel (MERGE) displays the co-localization of Cas9 (red fluorescence) in the cells, confirming successful electroporation.

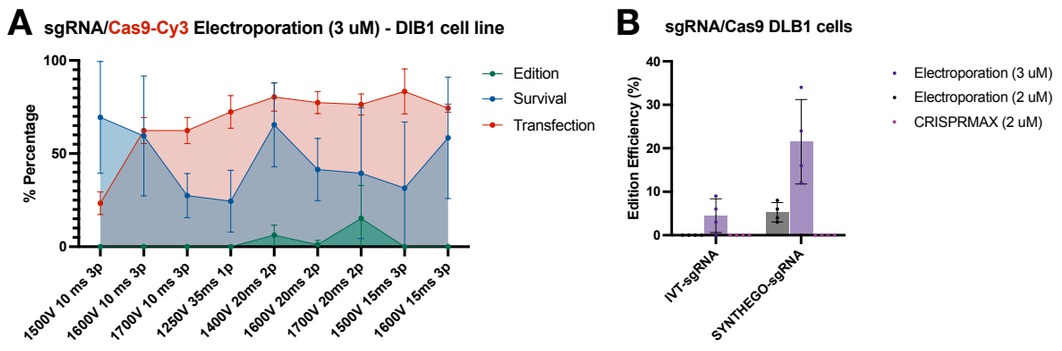

Figure 2: A. Cas9-Cy3 delivery in sea bass brain cell lines (DLB1) via electroporation, showing survival, transfection, and editing efficiency across various concentrations. **B**. Editing efficiency comparison between IVT-sgRNA, SYNTHEGO-sgRNA, and Lipofectamine CRISPRMAX in DLB1 cells. Error bars represent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

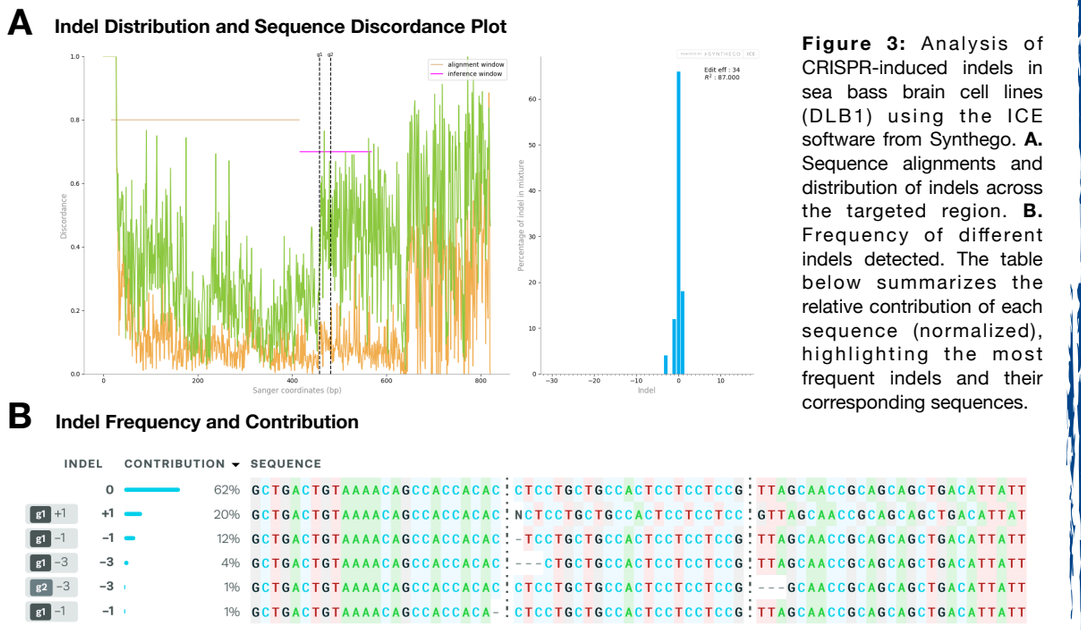

Figure 3: Analysis of CRISPR-induced indels in sea bass brain cell lines (DLB1) using the ICE software from Synthego. **A.** Sequence alignments and distribution of indels across the targeted region. **B.** Frequency of different indels detected. The table below summarizes the relative contribution of each sequence (normalized), highlighting the most frequent indels and their corresponding sequences.

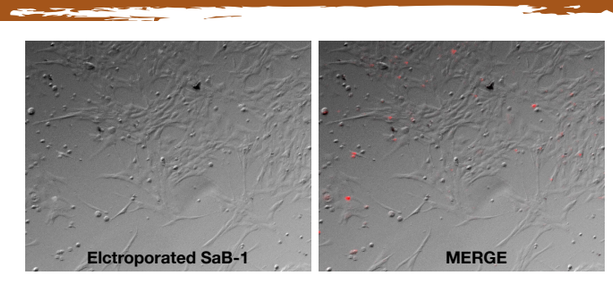

Figure 4: Electroporated SaB-1 seabass brain cell line with Cas9 tagged with Cy3. The left panel shows the cells under brightfield microscopy, while the right panel (MERGE) displays the co-localization of Cas9 (red fluorescence) in the cells, confirming successful electroporation.

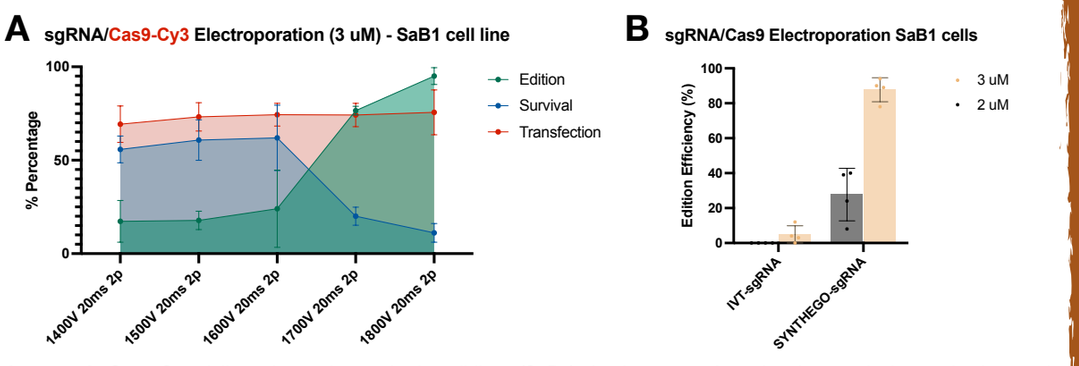

Figure 5: A. Cas9-Cy3 delivery in sea bream brain cell lines (SaB1) via electroporation, showing survival, transfection, and editing efficiency across various concentrations. **B**. Editing efficiency comparison between IVT-sgRNA, SYNTHEGO-sgRNA, and Lipofectamine CRISPRMAX in DLB1 cells. Error bars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

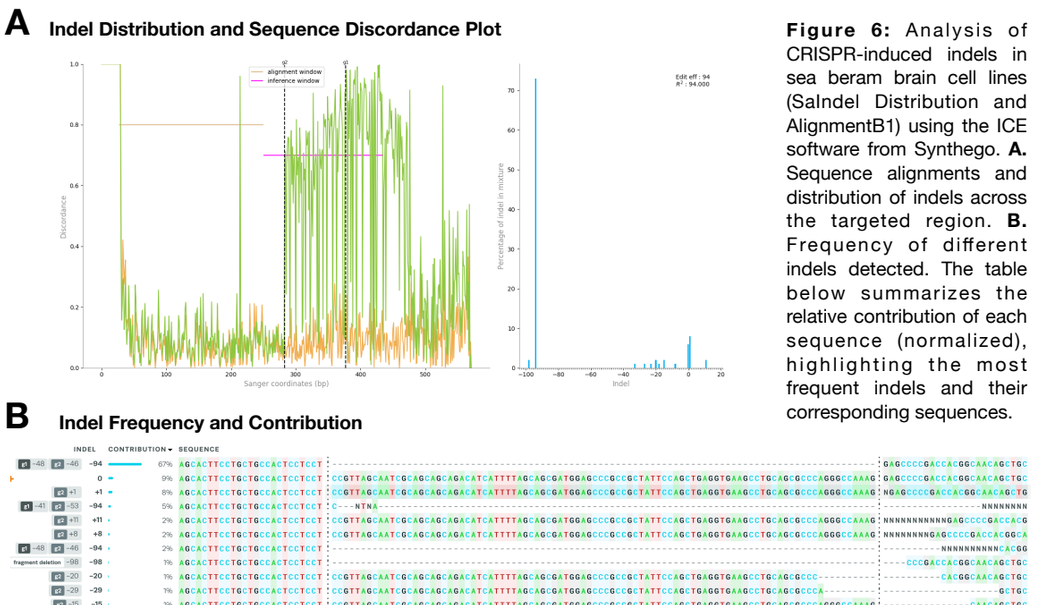

Figure 6: Analysis of CRISPR-induced indels in sea beram brain cell lines (SaIndel Distribution and AlignmentB1) using the ICE software from Synthego. **A.** Sequence alignments and distribution of indels across the targeted region. **B.** Frequency of different indels detected. The table below summarizes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

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:

- 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.