THE HURDLES OF DELIVERY CRISPR-Cas9 COMPONENTS FOR GENE EDITING IN PENAEID SHRIMPS

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CRISPR-Cas9 is often described as a revolutionary tool that unlocked the great potential of genetic edition due to the efficiency and feasibility of the method, allowing great advances in reverse and forward genetics studies. CRISPR-Cas platforms have expanded the toolbox in aquaculture, leading to great advances from the description of gene function to the establishment of new breeds with desirable phenotypes related to economic interest, and ultimately to food security and sustainability. Gene edition using the CRISPR-Cas9 system has been achieved in some crustacean species, however in Penaeid shrimps is particularly challenging, and several hurdles have halted the potential application of CRISPR-Cas9 technology in shrimp Aquaculture. In this work, the challenges to overcome during each step of the *in vivo* CRISPR-Cas gene edition process will be discussed.

A particular focus on experimental approaches based on microinjection-free protocols for delivery of CRISPR-Cas components will be presented. Physical (electroporation) and chemical (polyethylenimine and cationic lipids) transfection methods were applied in *P. vannamei* zygotes. Three different cargoes were prepared: DNA plasmid, mRNA, and a recombinant protein. Different ratios of sgRNA designed to recognize the PvCatL gene were used to prepare the CRISPR-Cas9 complexes. Treated shrimp zygotes were genotyped by HRM analysis and Sanger sequencing. Although high hatching rates were observed for most treatments, no irrefutable evidence of typical CRISPR-Cas9-induced gene edition was found; instead, an enrichment of gene variants was observed in treated organisms, which was detectable by HRM. The results are of interest to Aquaculture researchers working on this challenging topic, helping to improve their experimental design or as a reference to evaluate additional conditions to achieve the gene editing in Penaeid shrimps.

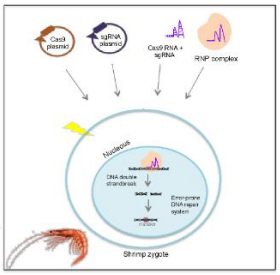

Figure 1. CRISPR-Cas9 gene edition system and its potential for Penaeid shrimp gene edition.