# TECHNICAL FEASIBILITY OF GENOME EDITING FOR DEVELOPING GENETIC STRATEGIES TO CONTROL INVASIVE SEA LAMPREY

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**ABSTRACT:**

Genetics and molecular biology have not played major roles in either basic research or management strategies for sea lamprey because of the scarce availability of functional tools that could be adapted for research or management practice. While biochemical and histological approaches have long been used to understand aspects of lamprey biology, molecular approaches to management practices are recent, beginning with the isolation of larval and adult pheromones that show promise as new management tools for control. However, in the past two decades, researchers have begun to develop genetic tools that have advanced basic research and may also be applicable to biocontrol efforts. To address the feasibility of genome editing as a strategy for sea lamprey control, we developed and tested the genomic integration of DNA constructs expressed as transgene reporters (green fluorescent protein) and also for their ability to drive phenotypic changes in melanogenesis in larval lamprey (tyrosinase). We designed and built a recirculating system for long-term culture of genetically modified lampreys in the laboratory environment. Gibson Assembly was used to generate new KIL (Killing Invasive Lamprey) vectors for testing integration into the sea lamprey genome, mediated by either a homing endonuclease (I-SceI meganuclease) or by CRISPR/Cas9 gene editing protocols. Pressure injection was used to introduce integration constructs into zygote stage embryos, with embryos and larvae observed up to and beyond 30 days post fertilization. Larvae expressing an integrated GFP-Cas9 fusion protein and a guide RNA targeting the tyrosinase gene, introduced by I-SceI meganuclease, were found both to express GFP and also exhibited reduced pigmentation. Introduction of a GFP transgene into the genome under the control of a SoxE1 neural crest-specific enhancer yielded comparable results using either homing endonuclease or CRISPR/Cas9 mediated integration. Effects of genome modification were dependent on both the site of genome integration and target guide RNA sequence present on the transgene. Integration into the SoxE2 promoter of a transgene that perturbed tyrosinase expression reduced melanogenesis, while a vector containing a SoxE2 guide RNA sequence resulted in loss of pharyngeal arches, as observed in earlier studies, but with low survival of transgenic individuals. A smaller GFP reporter transgene was integrated into the genome adjacent to the SoxE1 enhancer using CRISPR/Cas9, resulting in high survival and GFP expression, with 1439 larvae examined for GFP expression and 96 sampled for presence of transgene integration. The 5.4 kb transgene integrated into 25% of larvae tested (24/96), and within those larvae testing positive for transgene integration, GFP was expressed in somitic mesodermal derivatives (83.3%) and branchial cartilage (50%), as well as in pharyngeal mesoderm, endostyle, and the neural tube. The same GFP reporter inserted into the SoxE2 promoter was expressed in a different set of derivatives, including epibranchial and trunk sensory neurons and ganglia, as

well as in branchial cartilage, indicating tissue-specificity of the reporter, dependent on its insertion location on the genome, either near the SoxE1 neural crest enhancer, or within the SoxE2 promoter region. Results are discussed in the context of how genetic control strategies will be able to leverage gene editing protocols developed in this project to target and perturb the activity of specific genes for potential management applications. Optimization of tools developed in this project will require addition research.