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REPORT TITLE: Development of a Reverse Genetics System to Produce Live, Attenuated *Infectious Salmon Anemia Virus (ISAV)*

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ABSTRACT

Infectious Salmon Anemia (ISA), induced by the viral causative agent infectious salmon anemia Virus (ISAV), has had a large, negative economic impact on the salmon aquaculture industry in Maine, and has resulted in a considerable number of both direct and indirect job losses among communities near aquaculture operations. The goal of this project was to develop a reverse genetics system for ISAV to construct live viral particles from plasmid DNA molecules, with attenuated or reduced virulence that could induce a protective immune response in salmon and vaccinate against subsequent infection by ISAV in the environment. Although not complete, significant accomplishments towards this goal include: (i) Identification of errors in published ISAV genomic sequences. (ii) Development of procedures for high efficiency plasmid transfection into salmonid cells. (iii) Identification and cloning of a Salmonid Po1 I promoter. (iv) Construction of dual-functional plasmids capable of promoting both mRNA and protein expression, as well as expression of negative strand viral, non-mRNA molecules in salmonid cells. (v) Demonstration of protein, mRNA, and viral RNA production in salmonid cells and incorporation of a plasmid-encoded viral RNA into extracelluar viral particles.