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Title: Validation of splice altering morpholino knockdown of anoctamin 1

Morpholino oligonucleotides (MO) are commonly used to reduce gene expression. MO selectivity is determined by selection of a unique oligonucleotide sequence in the target of interest Suitable targets for splice altering MO are at an intron-exon junction in pre messenger RNA. The MO is predicted to anneal to the complimentary sequence and to inhibit intron-exon splicing. However, it is possible that an MO may anneal to an un-identified target(s) resulting in a phenotype that is unrelated to the gene of interest. Therefore it is necessary to validate MO efficacy on the targeted gene. This objective for this project is to validate anoctamin 1 splice-altering MO efficacy in zebrafish. Quantitative polymerase chain reaction (Q-PCR) will be used to compare anoctamin 1 expression levels in non-injected control embryos and in MO-injected embryos at 2 and 5 days post fertilization. Anoctamin 1 splice altering MO was designed to excise exon 4. We predict that MO knockdown will be greater at 2 dpf compared to 5 dpf. Data will be presented validating primer design and efficacy. This work will contribute to a better understanding of the general MO mechanism of action and will also validate an important technique to study anoctamin 1 function in zebrafish.

Keywords: zebrafish, morpholino, q-PCR, quantitative PCR