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Molecular Cloning and *In-Silico* Analysis of A WGS derived genomic contig of a putative Angiotensinogen from the Teleost *Sebastes Schlegelii*.

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Angiotensinogen (AGT) is the major substrate in the Renin-Angiotensinogen system (RAS), the primary hormonal signaling cascade ascribed primarily towards body fluid and blood pressure regulation, with peripheral albeit salient pro-inflammatory immune roles [1]. A WGS derived genomic DNA contig sequence with a presumed angiotensinogen gene (3802bp with a 1383bp, 6-exon coding region) was acquired from *Sebastes schlegelii* (Rock Fish) and subjected to extensive computer-assisted sequence analysis. The polypeptide derived via sequence based prediction tools defined a length of 460 amino acids, with a molecular mass of 51.3KDA. Furthermore, RFAgt revealed a signal peptide incorporating approximately 19-residues upstream the putative angiotensinogen I signature motif (^{20}NRVYVHPFYL^{29}), with the peptide cleavage site residing between ^{19}Ala- Asp^{20}, indicating its secretory nature. RFAgt also demonstrated a Serpin domain (between residues 9-458) with conserved sequence motif (^{431}LSINRPFFFSV^{441}), implicating a sequence-specific non-inhibitory role [1]. Sequence homology and genetic distance based phylogenetic analysis (augmented by 1000-iteration bootstrap analysis) revealed that RFAgt is evolutionary proximate to the AGT's of *Oplegnathus fasciatus, Larimichthys crocea* and *Rhabdosargus sarba*. Validation of the *In-silico* predicted ORF conducted via PCR amplification using sequence specific primers (F-$^{5'}$ATG CGG TCG CCT CTT CTA GC-$^{3'}$ and R-$^{5'}$- TTA CAG TGT AGG ATT GAT GAT CTT GCC-$^{3'}$), and subsequent visualization via Gel-electrophoresis revealed a concomitant band at 1383 bp Consecutively, upon purification, an attempt was made to ligate the product into a pGEM®-T Easy vector (size 3015bp). The experimental component will further expound on the Tissue-specific expression analysis with anticipated highest expression in the liver and a challenge (injury/infection) based expression study with a potential upregulation of RFAgt expression during physiological stress expected [1].

References

1. Griendling, K., Murphy, T. and Alexander, R. (1993). Molecular biology of the renin-angiotensin system. *Circulation*, 87(6), pp.1816-1828.