Ribosomal binding protein L8 homologue detected in the gills of longhorn sculpin, *Myoxocephalus octodecimspinosus*

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Analysis of gene expression using differential detection methods is a very valuable tool in molecular biology. Once the gene of interest is characterized, the varying expression levels of mRNAs must be confirmed. In order to confirm the identified transcript expression levels, a standard gene that does not change in expression must be utilized to accurately determine differential expression patterns. Choe et al. [2] showed that the highly conserved ribosomal binding protein L8 mRNA expression remains constant during salinity and acid-base changes in the Atlantic stingray (*Dasyatis Sabina*). The L8 protein complex consisting of L7/L12 and L10 in ribosomes is assembled on the conserved region of 23 S rRNA and is termed the GTPase-associated domain [1] and is highly conserved. Here we show the partial cDNA sequence detection of the ribosomal binding protein L8 gene in longhorn sculpin (*Myoxocephalus octodecimspinosus*) for future use in differential gene expression analysis.

Sculpin gill total RNA isolation was performed using Tri Reagent (MRC, Inc.) according to the company protocol. Reverse transcription using oligo dT primers was used to synthesis sculpin cDNA (Invitrogen). Specific primers for ribosomal binding sequences in mammals were generated (L8F1 sense and L8R2 antisense) [2]: 5′-AAGAAGGCTGAGTTGAACATTGGA-3′ in combination with 5′-TGTACTTGTGATAAGCCGAGCAG-3′ . Actin and NHE3 primers[3] were used as controls for the PCR reaction. The PCR reaction cycle parameters were performed in 50 µl volumes: initial denaturation at 95 °C for 5 min., 25 cycle denaturation at 95 °C for 1 min., cycle annealing at 55 °C for 1 min, cycle extension at 72 °C for 1 min., and a final extension at 72 °C for 10 min. A PCR product of approximately 400 bp was detected by gel electrophoresis and cloned into a Topo plasmid vector system (Invitrogen) according to manufacturers protocol for sequencing at the DNA Sequence facility Mount Desert Island Biological Laboratory.

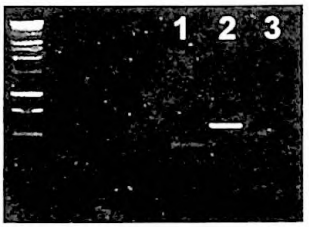

Fig. 1: PCR product analysis by 1% agarose gel electrophoresis. DNA marker shown at left. Lane #1 shows band of interest of 400 bp. NHE3 and actin positive controls shown in lanes 2 & 3 respectively.

Genebank sequence analysis of the 400 bp PCR product shown in Figure 1 shows a high homology to other known ribosomal binding L8 cDNAs. Future work will use the longhorn sculpin L8 mRNA expression as a control for standardizing differential expression of longhorn sculpin NHE3 previously characterized by Lanier and Claiborne [3].

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3. **Lanier, Curtis E. and James B. Claiborne**. Analysis of branchial longhorn sculpin (*Myoxocephalus octodecimspinosus)* Na^+/H^+ exchanger isoform 3 using Northern hybridization. *Bull. Mt. Desert Isl. Biol. Lab.* 43: 107, 2004.