

**“A preliminary gene expression study on reference genes in Pikeperch (*Sander lucioperca*)”**

The proposed Short-Term Scientific Mission project was “A preliminary gene expression study on reference genes in pikeperch (*Sander lucioperca*)”. To perform gene expression studies, it is necessary to use genes to be expressed constantly and independently from the state of the cell. These kind of genes are the so called reference genes. Therefore, an identification and characterization of reference genes in pikeperch such as tubulin- $\alpha$ ,  $\alpha$ -tubulin-1C and SAC-1 was carried out (Suppressor of actin mutations). The techniques used for the observation of the expression of these genes in *S.lucioperca* were:

- *in situ* hybridization (ISH) in tissues
- and whole mount *in situ* hybridization (WISH) in embryos

The selection of reference genes which were evaluated in this study is been performed by alignment of the nucleotides and degenerated primers design. From pikeperch tissues the total RNA was obtained and subsequently the mRNA was isolated. Reverse transcription of this mRNA sequence in cDNA was performed. The cDNA sequence was cloned and sequenced, later on followed by linearization of the plasmid to facilitate the labeling of our probe. These probes were already prepared to be used *in situ* hybridization techniques.



**Figure 1. Gene expression tubulin- $\alpha$  in 50 somite stage.** a) Sense and b) antisense probes. Significant differences between sense and antisense probes can be observed in this picture. The embryo of antisense show high expression to be observed in his head.

The results of gene expression were:

- These reference genes were characterized.
- The  $\alpha$ -tubulin gene was not expressed in early stages of the embryo until reaching somitogenesis.
- The expression of this gene was gradually increasing in larva-state, being higher in the head, notochord and veins.
- The  $\alpha$ -tubulin-1C gene is not expressed as strongly as tubulin- $\alpha$  gene. The SAC-1 gene is expressed in the muscle.

It was achieved to establish WISH technique in pikeperch, but ISH technique must still be improved and adjusted to the appropriate conditions.



Host: Martin Pšenička, PhD

Laboratory of Germs Cells.

Faculty of Fisheries and Protection of Waters in Vodňany (Czech Republic)

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