Selection of reference genes in real-time RT-PCR studies of Atlantic salmon *Salmo salar*

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Summary

Salmonids are among the most studied fish species, but so far only limited information is available on the selection of reference genes in qRT-PCR studies. In order to be able to use housekeeping genes for normalization, one needs to know their stability under various experimental conditions. In this study, we examined the stability of six potential reference genes in eight tissues of Atlantic salmon, to determine the most suitable genes to be used in quantitative real-time RT-PCR analyses. The results suggest that elongation factor genes have a stable expression in most Atlantic salmon tissues, and that these genes should be considered used for normalization in future examinations.

Methods

The stability of six potential reference genes was examined in eight tissues of Atlantic salmon (*Salmo salar* L.), to determine the most suitable genes to be used for normalization in quantitative real-time RT-PCR analyses. The relative transcription levels of genes encoding 18S rRNA, S20 ribosomal protein, β-actin, glyceraldehyde-3P-dehydrogenase (GAPDH), and two paralog genes encoding elongation factor 1A (EF1Aβ and EF1Aδ) were quantified in gills, liver, head kidney, spleen, thymus, brain, muscle, and posterior intestine in six untreated adult individuals. In addition, the stability of the same genes were examined in smolifying salmon, in order to study how physiological stress affects the transcription. Fish were sampled prior to smoltification (presmolt), during smoltification (smolt) and in fish after smoltification; smoltilified in seawater (smoltilified SW) or desmoltilified in freshwater (desmoltil FW). The Microsoft Excel applet geNorm, developed by Vandesompele et al. (2002), was used to assess the transcription stability of the genes.

Results

Based on calculations performed with the geNorm VBA applet, which determines the most stable genes from a set of tested genes in a given cDNA sample, the ranking of the examined genes in adult Atlantic salmon was EF1Aδ > EF1Aγ > β-actin > 18S rRNA > S20 > GAPDH (Fig. 1).

When the same calculations were done on a total of 24 individuals from four stages in the smotification process, the gene ranking was EF1Aδ > EF1Aγ > S20 > β-actin > 18S rRNA > GAPDH (Fig. 2) (Olsvik et al. 2005).

We are further evaluating the usefulness of elongation factor paralog genes as potential reference genes in Atlantic salmon. In current examinations, two new paralogs, EF1Aα and EF1Aη, are also studied, and the stability of these forms compared to EF1Aδ and EF1Aγ.

Figure 1


Figure 2

Stability of six reference genes during smoltification in Atlantic salmon. n=6 in each group.

References


Conclusions

Overall, this work suggests that the EF1Aδ and EF1Aγ genes can be useful as reference genes in qRT-PCR examination of gene expression in the Atlantic salmon.