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 need 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* (*EF1A*$_A$ and *EF1A*$_B$) 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 smoltifying 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; smoltified in seawater (smoltified SW) or desmoltified in freshwater (desmolt FW). The Microsoft Excel applet *geNorm*, developed by Vandesomple *et al.* (2002), was used to assess the transcription stability of the genes.

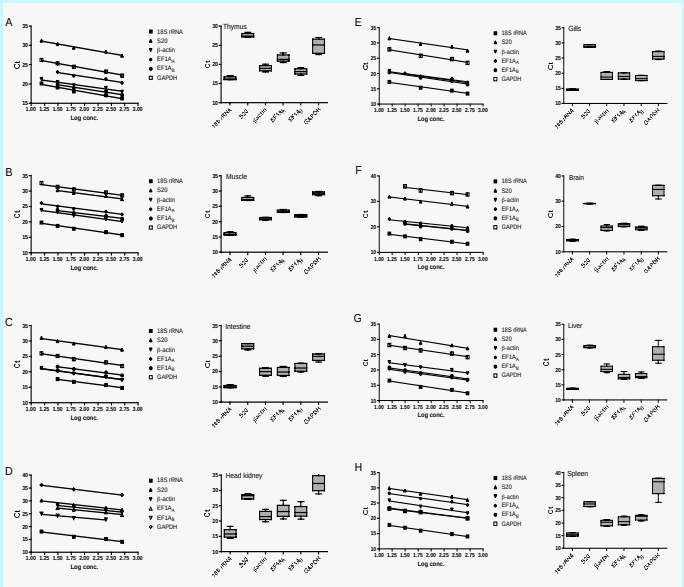

Figure 1

Stability of six potential reference genes in eight tissues of Atlantic salmon. A) Thymus, B) Muscle, C) Intestine, D) Head kidney, E) Gills, F) Brain, G) Liver and H) Spleen. n=15.

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 *EF1A*$_B$>*EF1A*$_A$>*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 smoltification process, the gene ranking was *EF1A*$_B$>*EF1A*$_A$>*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, *EF1A*$_C$ and *EF1A*$_D$, are also studied, and the stability of these forms compared to *EF1A*$_A$ and *EF1A*$_B$.

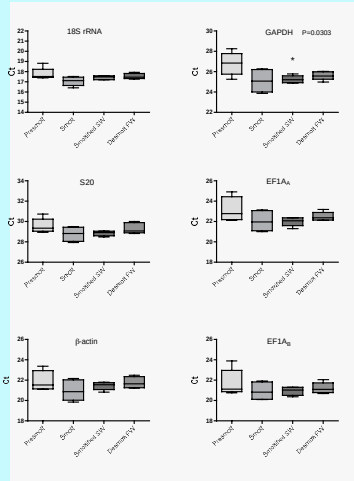

Figure 2

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

References

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Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A. and Speleman, F. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3, RESEARCH0034.

Conclusions

Overall, this work suggests that the *EF1A*$_A$ and *EF1A*$_B$ genes can be useful as reference genes in qRT-PCR examination of gene expression in the Atlantic salmon.

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