NIFES National Institute of Nutrition and Seafood Research

Selection of reference genes for qRT-PCR examination of wild populations of Atlantic cod *Gadus morhua*

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Summary

The stability of 10 potential reference genes was examined in six tissues of Atlantic cod obtained from four populations. Relative transcription levels of genes encoding β-actin (ACTB), elongation factor 1A (EF1A), actin-related protein-2 (ARP-2), glyceraldehyde-3P-dehydrogenase (GAPDH), ubiquitin (Ubi), acidic ribosomal protein (ARP), ribosomal protein S9 (S9), ribosomal protein L4 (RPL4), RPL22 and RPL37 were quantified in gills, brain, liver, head kidney, muscle and middle intestine in six juvenile fish from three wild populations and from farmed fish. Reference gene stability was investigated using the *geNorm* and *NormFinder* tools. Overall, this work suggests that the Ubi and ARP can be useful as reference genes in qRT-PCR examination of gene expression studying wild populations of Atlantic cod.

Background:
Extensive sequencing efforts have been taking place for the Atlantic cod (*Gadus morhua*) in recent years, the number of ESTs in the Genbank has reached more than 180.000. Despite its importance in North Atlantic fisheries and potential use in aquaculture, relatively few gene expression examinations exist for this species.

The aim of this work was to evaluate the usefulness of 10 potential reference genes for qRT-PCR in the Atlantic cod. Genes encoding 5 commonly used housekeeping proteins plus 5 ribosomal proteins were selected for examination. RNA from six tissues (gill, brain, liver, head kidney, muscle and intestine) of six adult male cod from four populations were subjected to qRT-PCR analysis. Two of the populations were sampled from heavily contaminated recipients, one control from an unpolluted fjord locality and one from an aquaculture facility.

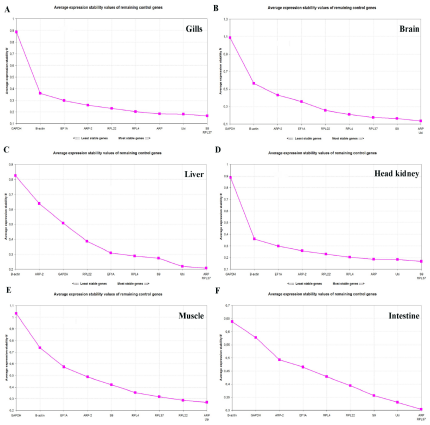

Figure 1
Ranking of the reference genes according to the *geNorm* software. A) Gills, B) Brain, C) Liver, D) Head kidney, E) Muscle and F) Intestine.

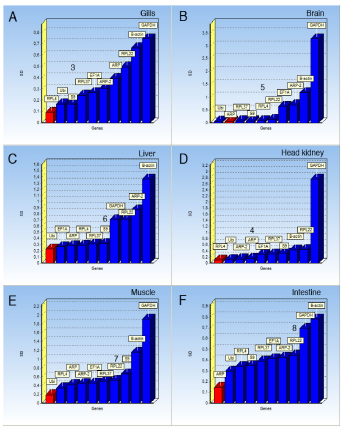

Figure 2
NormFinder ranking. A) Gills, B) Brain, C) Liver, D) Head kidney, E) Muscle and F) Intestine. The optimal number of genes suggested used for normalization by *NormFinder* is shown for each tissue.

Results:
Relative transcription levels of 10 potential reference genes for qRT-PCR analysis were quantified in six different tissues in six juvenile fish from three wild populations and from farmed Atlantic cod. Based on calculations performed with *geNorm*, ARP, Ubi, S9 and RPL37 were among the most stable genes in all tissues (Fig. 1). When the same calculations were done with *NormFinder*, the same genes plus RPL4 and EF1A were ranked as the preferable genes (Fig. 2). Principle component analysis was used to search for patterns in gene expression. In liver, the 5 ribosomal genes grouped together (Fig. 3), whereas farmed fish grouped together for intestinal tissue (Fig. 4). In conclusion, Ubi and ARP appear to be promising reference genes in qRT-PCR examination of gene expression studying wild populations of Atlantic cod.

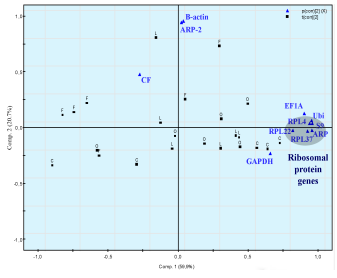

Figure 3.
Population-specific principle component analysis (PCA) of gene expression in **liver**. **Ribosomal genes grouped together.** Gene names, populations (C = Control, F = Farmed fish, L = Store Lungegårdsvann and O = Odda/Sørfjorden) and CF = Condition Factor are presented in the figure. PCA applied to the entire data set (component 1 and 2) model explaining r^2=0.81 and predicting Q^2=64% of the data variation. 95% confidence intervals.

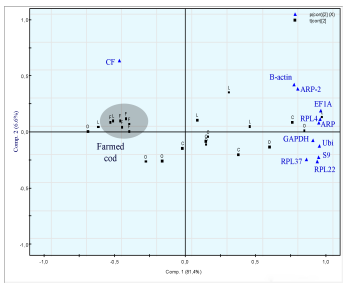

Figure 4.
Population-specific PCA of gene expression in **intestinal tissue** in order to check if fish from the contaminated sites group together. **Farmed cod grouped together.** Gene names, populations (C = Control, F = Farmed fish, L = Store Lungegårdsvann and O = Odda/Sørfjorden) and CF = Condition Factor are presented in the figure. PCA applied to the entire data set (component 1 and 2) model explaining r^2=0.88 and predicting Q^2=74% of the data variation. 95% confidence intervals.

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