Application of Next Generation Sequencing to predict the effects of climate change in the Arctic

G Olafsdottir[1], K Olafsson[1], S Magnusdottir[1], LA Gudmundsson[2] and S Gudjonsson[2]

[1] Matís, Reykjavík, Iceland, [2] Institute of Freshwater Fisheries, Reykjavík, Iceland

Introduction

Climate change has been recognized as one of the most important threats to biodiversity, and the effects are already being felt in the Polar Regions. A greater understanding of how species are adapted to their environment is a central element in anticipating how species will adjust in the face of a changing climate.

The Arctic char (*Salvelinus alpinus*) has a circumpolar distribution in the Arctic and sub-Arctic regions and is well suited as a model species for predicting the effects of climate change in the Arctic.

Mitochondrial DNA (mtDNA) contains genes related to basic metabolic pathways which are temperature dependent.

The aim of this research is to identify genetic variation (single nucleotide polymorphisms; SNPs) in genes controlling basic temperature dependent metabolic pathways within the mtDNA, and to determine how they vary across the diverse ecological habitats in which Arctic char occurs.

The Materials and methods

A total of **1728** samples of the genus Salvelinus (93% *Salvinius alpinus*) were included in this study and next generation sequencing was applied to simultaneously analyse the whole mtDNA genome for 128 individuals, and key identified regions (amplicons) of mtDNA from a further 1600 individuals.

Phase I

During the first round of sequencing, a total of **128** samples from across the species range were selected for **whole genome sequencing** of the mtDNA using a Roche GS FLX genome sequencer.

Primers for **28 amplicons** were designed from the reference sequence NC_000861 (16.659 base pairs) to cover the complete mtDNA. The samples were divided into 2 groups, each with 64 individuals. DNA from each individual had Multiplex identifying sequences (MIDs) added before sequencing to allow assignment of the resulting DNA sequences back to the individuals.

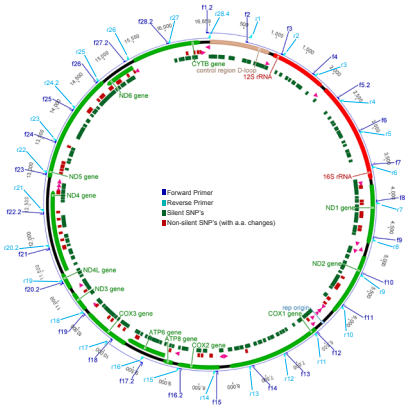

Figure 1. Phase I: Arctic Char mtDNA displaying the 28 amplicons and the SNPs.

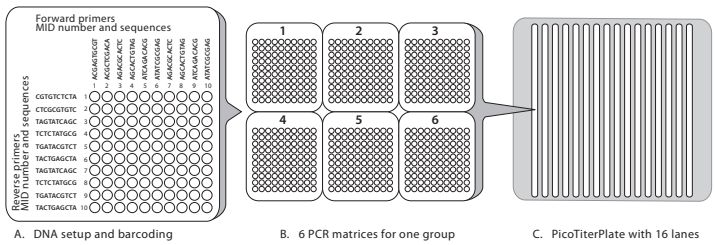

Figure 2. The workflow setup for Phase II.

Phase II

Six regions of the Arctic char mitochondrial genome (D-loop and parts of four coding genes; ND1, ND2, ND5 and ND6) were identified during the first stage of sequencing. These regions were selected for their high levels of polymorphism and the presence of informative, functional SNPs (i.e. variation changing the amino acid of the produced protein).

A total of **1600** individuals were sequenced for these six key amplicons. The samples were divided into 16 groups, each with 100 samples. MIDs were applied, allowing a unique identifier for all samples in a single sequence group.

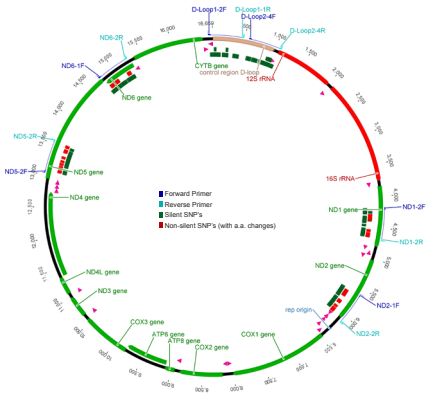

Figure 3. Phase II: Arctic Char mtDNA displaying the 6 amplicons and the SNPs.

Results

In Phase I, 16.659 bp were sequenced for each of the 128 samples and 468 SNPs were identified.

In Phase II, 3.071 bp were sequenced for each of the 1.600 samples and 546 SNPs were identified (254 SNP's identified in Arctic char).

Conclusions

In both phases of our experiment the pyrosequencing yielded ~ 210 million passed filter base pairs and generated a large mitochondrial SNP set. By using a genetic approach it's possible to estimate and map evolution and the divergence of the Arctic char. The results are being used to understand and further investigate both historical and contemporary elements of the phylogeographic structure of the species. Furthermore, this information can be used to forecast the development of the species associated with climate warming.

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Contact person: Gudbjorg Olafsdottir (gudbjorg@matis.is; http://www.matis.is; +354 4225051)