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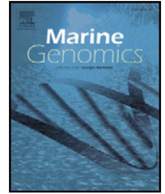

Method paper

Sequencing and characterization of a multi-organ Arctic charr transcriptome: A toolbox for investigating polymorphism and seasonal life in a high Arctic fish

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ABSTRACT

The Arctic charr (*Salvelinus alpinus* L.) inhabits fresh water ecosystems of the high North. The species has developed a strong phenotypic plasticity and variability in life history characteristics which has made this species an attractive model for investigations on phenotype plasticity, morph formation and ecological speciation. Further, the extreme seasonal variations in environmental conditions (e.g. food availability) in the high North induce seasonal changes in phenotype, which require precise timing mechanisms and physiological preparations. Individual gating of life-history strategies (e.g. formation of resident and sea-migrating morphs) and transitions (e.g. maturation) depends on conditional traits (size/energy status) at specific assessment time windows, and complex neuroendocrine regulation, which so far is poorly understood. In the absence of a reference genome, and in order to facilitate the investigation of the complex biological mechanisms of this unique fish model, the present study reveals a reference transcriptome for the Arctic charr. Using Roche 454 GS FLX+, we targeted various organs being either at the crossroads of many key pathways (neuroendocrine, metabolic, behavioral), of different ontological origins or displaying complementary physiological functions. The assemblage yielded 34,690 contigs greater than 1000 bp with an average length (1690 bp) and annotation rate (52%) within the range, or even higher, than what has been previously obtained with other teleost de novo transcriptomes. We dramatically improve the publically available transcript data on this species that may indeed be useful for various disciplines, from basic research to applied aspects related to conservation issues and aquaculture.

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1. Introduction

The Arctic charr (*Salvelinus alpinus* L.) is the world's northernmost fresh water fish and is considered the most cold-adapted species within the salmonid family (Johnson, 1980). For long a commercial fishery for Arctic charr has been active in Canada (Dempson and Shears, 1998) and the Arctic charr has lately become an important aquaculture species with a global production ranging between 6000 and 10,000 metric tons per year (Sæther et al., 2013). This species is also a highly attractive sport fish, and is an important household and cultural species for indigenous people in the North (Boivin et al., 1989).

Further, the Arctic charr represents a fascinating case study of intraspecific polymorphism expressing a diversity of ecologically specialized phenotypes and morphs. This might be the result of the austere habitat conditions of the high North, including extreme seasonal changes in the external environment (light, temperature, food availability). For example, "landlocked" populations (with no access to the sea) exhibit several morphs, including specialized zoobenthos and plankton feeders, as well as a piscivorous form (Jonsson and Jonsson, 2001; Klemetsen et al., 2003). In watercourses with access to the sea, one part of the population, the "residents", complete its entire life cycle in freshwater, while another part, the "anadromous", establishes a migratory life strategy, as they migrate from the fresh to the seawater environment in a seasonal manner. The migratory life-strategy is accompanied by a seasonal phenotype transition which implies precise timing mechanisms and long-term physiological preparations (Jørgensen and Johnsen, 2014). It is now well documented that the polymorphism in the Arctic charr is rooted in both genotypic differences and phenotypic plasticity (Klemetsen et al., 2003). The latter is evidenced by the fact that different sympatric morphs of Arctic charr may exhibit a high degree of interbreeding (Jonsson and Jonsson, 2001), and that offspring from anadromous and resident Arctic charr phenotypes segregate into either phenotype, irrespective of the parental one (Nordeng, 1983). Such life-history (phenotype) transitions (fresh water to seawater; immature to mature) are initiated by environmental cues (photoperiod), operating in concert with innate occurring "assessment windows" during

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which developmental thresholds in e.g. energetic status are assessed, and neuroendocrine mechanisms that regulate and control the transition. These complex mechanisms are poorly understood.

In summary, morph determination and phenotype transitions involve a wide range of physiological responses to developmental rates and environmental cues. There is a strong need for more knowledge on the proximate mechanisms initiating and regulating phenotype formation and transitions, but their complexity and the high number of actors interacting make it difficult to undertake comprehensive studies with traditional methods (e.g. RT-qPCR). Therefore, a reference genome sequence would facilitate the development of molecular technologies that utilize high throughput characterizations of gene expression and genetic variation in the Arctic charr. In the absence of this reference genome, the alternative lies in the sequencing of the transcriptome, a rapid and efficient means to discover genes and develop genetic markers (Qian et al., 2014). The present study takes advantage of a powerful pyrosequencing method on the 454 GS FLX+ platform to reveal molecular tools for studying biological mechanisms in a unique fish model. We targeted various organs, which either are at the crossroads of many key neuroendocrine, metabolic and behavioral regulations, or possess complementary physiological functions or different ontological origins. This selection is aimed at obtaining the widest variety of reconstructed transcripts. Immature and mature male and female fish from two geographical origins, and possessing different body conditions and age, were included in this analysis in order to capture transcripts expressed before and after morph transition, but also to encompass more thoroughly Arctic charr polymorphism.

To this end, we report here the release of an Arctic charr reference transcriptome, which represents a dramatic improvement of the publically available transcripts; there is currently no public assembled data available for this species. We demonstrate that our approach yielded a broad representation of the transcriptome that may indeed be useful not only for future investigations on morph characteristics and proximate mechanisms regulating phenotype transitions. It may also benefit various other disciplines, from basic research to applied aspects related to aquaculture and conservation issues.

2. Material and methods

2.1. Ethics statement

Fish handling and euthanasia (see below) were performed by a competent person and in accordance with the European Union regulations concerning the protection and welfare of experimental animals (European directive 91/492/CCE). The experiment was approved by the Norwegian Committee on Ethics in Animal Experimentation (ID 3630).

2.2. Biological samples and RNA preparation

Fish tissues were sampled on August 11, 2011, from 3 males and 3 females from each of a high-Arctic and a sub-Arctic population of Arctic charr, i.e. 12 individuals in total. The former were one year-old, immature offspring of wild, anadromous Arctic charr originating from Lake Vårflusjøen, Svalbard (79°72′N, 14°41′E), including both lean and fat individuals, and the latter three year-old mature offspring of charr originating from Lake Storvatnet, North-Norway (70°01′N, 22°55′E). All fish had been hatched and reared at the Aquaculture Research Station in Tromsø (ARST; 69°N) at natural light and temperature conditions (9 °C at the time of sampling) and fed a commercial Arctic charr feed (Skretting, Stavanger, Norway) until they were killed by a sharp blow to the head and decapitation, and sampled. In order to maximize the diversity of expressed transcripts, a variety of organs and tissues were sampled; the whole brain (including pituitary), gill and head kidney as well as pieces of the liver, gonad, abdominal fat and muscle. The organ and tissue sampled were stored in RNAlater (Ambion, Life Technologies, Carlsbad, CA, USA) at 4 °C overnight and from then on at −80 °C until use. All tissues were homogenized using a Qiagen TissueLyser Ⅱ (Qiagen, Hilden, Germany). Total RNA was extracted using the RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany). This kit includes an initial step of gDNA removal. RNA concentration and quality were checked by using NanoDrop (Fisher Scientific, Wilmington, DE, USA) and Bio Analyser (BioRad, Hercules, CA, USA). The RNA showed an A260/280 ratio between 1.9 and 2.1 (NanoDrop) and a RQI value of 9, and was consequently considered of prime quality. A total of 50 μg of RNA (containing equal amounts of RNA from each tissue/organ), dissolved in 20 μl of water, was sent to Eurofins on dry ice.

2.3. cDNA library construction and 454 sequencing

The normalized, random-primed cDNA library was prepared by Eurofins MWG Operon (http://www.eurofins.com). PolyA+ mRNA was isolated from total RNA. The cDNA preparation included a random-primed first strand synthesis followed by a second strand synthesis using specific primer ligated to free 3′-OH ends of first strand cDNA. The resulting non-cloned cDNA library was size fractioned to about 400–1200 bp, and normalized in order to reduce the copy-number variation. emPCR and sequencing in one and a half Roche GS FLX+ runs were performed using the GS FLX Titanium sequencing kit XL+ according to the manufacturer's protocols.

3. Data analyses

3.1. General statistics and data assembly

Data resulting from the full GS FLX+ run were checked for quality, filtered and assembled by Eurofins, using their routine procedure and the MIRA v2.9 assembler for transcriptomic data (Chevreux et al., 2004). This de novo assemblage generated 126,780 contigs which are now publically available (see below Data mining interface section). We focused our analysis on the 34,690 contigs with a size above 1000 bp. Their N50 reached 1,717, and N90 1,125 (Table 1). To assess the redundancy rate, reads were randomly picked in bam file to count the number of viewed contigs (Fig 1).

We validated the construction of our data set taking the advantage of Arctic charr public raw data. All reads from the gill transcriptome (SRA accession: SRX314607) were mapped to our set of contigs using BWA aligner (Li and Durbin, 2009). More than 85% of them got aligned on our Arctic charr dataset. A lower proportion (44 to 50%) of reads from Kapralova et al. (2014), SRA accession: SRP039492, mapped on our contigs. This lower match was explained by the fact that this public raw data constituted not only transcripts but also of miRNA, and the relative contribution of the two types of RNA was not made explicit. In addition, our approach deals with adult fish while that of Kapralova et al. (2014) targeted 4 early development stages (3 embryonic and one just before first feeding), and it is well known that quality and level of gene expression patterns change dramatically between early

Table 1
General statistics about the Artic charr multi organ transcriptome built using 454 GS FLX technology. Only contigs greater than 1000 bp were considered for annotation and genetic diversity investigation.

Feature	Number of features
Number of base pairs in the reads	896,221,168
Number of reads	2,092,607
Number of base pairs in the contigs (>1000 bp)	58,652,081
Number of contigs (>1000 bp)	34,690
N50	1,717
N90	1,125
Number of putative micro-satellites	17,236
Number of putative SNPs	62,515
Number of contigs including SNP	13,660

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