

REPORT

Identification of Fish Species from DNA Samples

Prepared by:

Genomics and Proteomics Facility, CREAT Network
Memorial University of Newfoundland

14July09

The Genomics and Proteomics Facility, CREAT Network, received 23 samples of extracted fish DNA, plus two samples of Arctic Charr DNA controls. The purpose of this study was to identify the species of fish from which these samples originated.

Methods

Approximately 100 ng of sample DNA was used for PCR amplification of the ND1 locus (NADH dehydrogenase subunit 1, mtDNA). The PCR reaction included 1XPCR Master Mix (containing *Taq* polymerase, 1.5 mM MgCl₂ and dNTPs; Promega Inc., Maddison, WI, USA), and 0.8 uM each primer (SfoND1F- AAAGTGGCAGAGCCCGTAATTG and SfoND1R- AATGGTCCTCCAGCGTATTCTAC). Samples were amplified in a GeneAmp PCR 9600 (Applied Biosystems, Foster City, CA, USA) with the following profile: 94°C for 3min, followed by 35 cycles of 94°C for 30sec, 55°C for 30sec, and 72°C for 2min, followed by a final extension at 72°C for 7min. PCR products were purified using a 100K AcroPrep MWCF Plate (Pall Life Sciences, Ann Arbor, MI, USA) on a vacuum manifold according to the manufacturer's protocol. Cleaned ND1 amplicons were resuspended in 20 uL ddH₂O.

The ND1 PCR products were cycle sequenced using BigDye Terminator v3.1 chemistry (Applied Biosystems, Foster City, CA, USA), in a reaction containing 3.2 pmol forward or reverse primer, and approximately 65 ng PCR product in a final volume of 10 µL. The cycle sequencing hot start profile was 98°C for 5min, followed by 25 cycles of 96°C for 10sec, 50°C for 30sec, and 60°C for 4min, in a GeneAmp PCR 9600 (Applied Biosystems, Foster City, CA, USA). Cycle sequencing products were cleaned by ethanol precipitation and 70% ethanol washes. Cleaned cycle sequencing products were analyzed with an ABI 3730 DNA Analyzer.

Sequence data were edited by eye and used to search nucleotide databases with BLAST on the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between nucleotide sequences generated for samples to sequence databases and calculates the statistical significance of matches.

Results

Of the 23 test samples, 16 provided sequence in both directions, and 4 resulted in sequence in one direction . Three samples (23, 24, and 25) provided no sequence in either direction.

BLAST search results are presented in Table 1. The two control samples gave excellent sequence which aligned to Arctic Charr sequence in Genbank. Nineteen sample sequences were successfully aligned, 15 samples were homologous with Arctic Charr (*Salvelinus alpinus*) and four were homologous with Brook Trout (*Salvelinus fontinalis*).

Acknowledgements

Please request that the end user acknowledges that results were generated and interpreted by the Genomics and Proteomics Facility of the CREAT Network at Memorial University of Newfoundland. Thank you.

Table 1: Results of BLAST comparisons of sample sequences to sequence databases. Associated statistics include proportion of nucleotide similarity between samples and reference sequences (% Match) and proportion of each sample sequence that aligned with reference sequence (% Query).

Sample	# Base pairs	Species Match	% Match	% Query	Genbank Reference Sequence Accession #
1	Forward only - 670 (unedited)	<i>Salvelinus alpinus</i>	97%	99%	AF154851.1
2	656	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
3	678	<i>Salvelinus fontinalis</i>	99%	100%	AF154850.1
4	633	<i>Salvelinus alpinus</i>	98%	100%	AF154851.1
5	634	<i>Salvelinus fontinalis</i>	100%	100%	AF154850.1
6	676	<i>Salvelinus alpinus</i>	99%	99%	AF154851.1
7	588	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
8	R – 403 (unedited)	<i>Salvelinus alpinus</i>	94%	93%	AF154851.1
9	670	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
10	651	<i>Salvelinus fontinalis</i>	99%	100%	AF154850.1

11	660	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
12	507	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
13	654	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
14	Forward only – 676 (unedited)	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
15	675	<i>Salvelinus alpinus</i>	99%	99%	AF154851.1
16	677	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
17	638	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
18 (Control)	648	<i>Salvelinus alpinus</i>	99%	100%	AF154851
19 (Control)	649	<i>Salvelinus alpinus</i>	99%	100%	AF154851
20	626	<i>Salvelinus fontinalis</i>	100%	100%	AF154850.1
21	Reverse only – 259 (unedited)	<i>Insufficient sequence</i>			
22	625	<i>Salvelinus alpinus</i>	99%	100%	AF154851.1
23		<i>No Sequence</i>			
24		<i>No Sequence</i>			
25		<i>No Sequence</i>			