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Twelve microsatellite loci for lake trout (*Salvelinus namaycush*)

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Abstract

We describe 12 microsatellite loci isolated from lake trout (*Salvelinus namaycush*). The number of alleles at these loci ranged from two to 11 with an average of 5.3 alleles per locus. The expected heterozygosity ranged from 0.29 to 0.76, with an average of 0.68. Accidental (or illegal) introductions of lake trout into watersheds are decimating native trout populations in the northern Rocky Mountains, and these loci will be useful for identifying the source of these introductions and for estimating the number of founding individuals.

Keywords: Lake trout, microsatellite, primer, *Salvelinus namaycush*

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Introduction

Lake trout (*Salvelinus namaycush*) are large, long-lived fish predators native to deep lakes of Canada and northern parts of the USA, including the Great Lakes (Benke 2002). During the late 19th and early 20th century, lake trout were extensively introduced into lakes outside their native ranges (Crossman 1995), and the species is currently expanding its range through dispersal into adjacent waters. Illegal (or accidental) introductions are also common. These introductions and population expansions have decimated native trout species, including populations of cutthroat trout in Yellowstone National Park (Ruzycki *et al*. 2003; Koel *et al*. 2005) and bull trout in Glacier National Park (Fredenberg 2002). Recently, lake trout have been discovered in Swan

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Table 1 Summary information for 12 microsatellite loci in lake trout genotyped in 96 individuals: locus names, primer sequences, repeat motifs, fragment size, annealing temperature (T_a), number of alleles (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), Hardy–Weinberg P value, and GenBank Accession number

Locus	Primer sequence (5′–3′)	Repeat motifs	Fragment size (bp)	T_a (°C)	N_a	H_O/H_E	P	GenBank Accession no.
SnaMSU01	F: 6FAM-TCACACACCCATTCGTTCAT R: AGCATGGGATAACCACAACC	$(GACA)_{28}$	246–282	57	6	0.68/0.75	0.42	EU331427
SnaMSU02	F: VIC-GCCCTTCATTGAGGAACAGA R: CTCACACACACGCACAACAA	$(GTCT)_2$, GTCAAT, $(GT)_5$, $(GTCT)_{11}$, GT, $(CCGT)_{15}$	221–257	57	4	0.67/0.75	0.63	EU331428
SnaMSU03	F: NED-TGGGCAAATTATTGAAGACAAA R: CAGTATACGTCTCTGCCTGTCTG	$(GACA)_3$, TATACATTCCCT $(GACA)_{22}$	197–256	57	4	0.77/0.75	0.66	EU331429
SnaMSU05	F: VIC-TTGAGGGCAAATTCACAATG R: GAGGCAGACAGTGGTAGCTTG	$(GT)_{23}$, $(GTCT)_8$	172–204	66	5	0.84/0.75	0.15	EU331431
SnaMSU06	F: 6FAM-GCTGGTGAGGGAGAGATGAC R: CAGCCATGAGAATGGGATTT	$(CA)_{12}$, $(GACA)_{22}$	263–292	60	7	0.88/0.74	0.06	EU331433
SnaMSU07	F: PET-TGCCTATTCCTATTCAATACAGG R: CCCCCACAGTCTGAATCAAT	$(GACA)_{33}$, $(GA)_{34}$, $(GACA)_{20}$	351–374	60	2	0.80/0.75	0.20	EU331435
SnaMSU08	F: NED-AGAGCAGTCGATTGCAGTAGC R: ACTGCCCTCACTGATGGTG	$(GTATGTCT)_{10}$, $(GTAT)_5$	157–177	66	11	0.29/0.29	1.00	EU331432
SnaMSU09	F: NED-TGATCAGAGATGGCAGTTTCA R: CATTATCCTGGCAACATGGA	$(GACA)_{25}$	251–329	66	7	0.83/0.75	0.10	EU331439
SnaMSU10	F: NED-GCACCTCACCACTCACCTTT R: TTATACAGCAGGGCTGAGCA	$(GTCT)_{36}$	196–243	63	4	0.51/0.60	0.19	EU331434
SnaMSU11	F: 6FAM-TGATGATGGAAAGGCAGAGG R: CCATTTGGGATGCACATACA	$(GACA)_{36}$	226–251	63	4	0.67/0.66	1.00	EU331436
SnaMSU12	F: VIC-ATTTTCCACATGCTGCGTCT R: TGAAATAGCTTGGAGCAGTAGC	$(GTCT)_{27}$	198–216	63	3	0.68/0.60	0.12	EU331437
SnaMSU13	F: PET-AGTTTCCAAGGCAGCACTGT R: TGCTACACAGCAAAATGTGTCA	$(GACA)_{36}$	237–256	63	6	0.80/0.76	0.65	EU331438

Lake, Montana, and the population has been growing rapidly. Neither the source of this introduction nor the number of founders is known. Microsatellite genetic variation could help answer these questions, but no microsatellite loci have been specifically developed for lake trout, and loci identified in other salmonids show modest levels of genetic variation (N. Vu, unpublished). In this study, we describe 12 microsatellite loci isolated from lake trout in Montana.

We used a variation of the method of Hamilton *et al.* (1999) to develop a lake trout microsatellite library in which the restriction of genomic DNA and the ligation of a universal DNA linker were combined into one reaction (DIG/LIG). The DIG/LIG reaction consisted of 2.0 μM double-stranded SNX linker, 6 μg Bovine Serum Albumin, 20 U restriction enzyme, 40 U *Xmn*I, 800 U T4 DNA Ligase with rATP, 1× NEBuffer2, 250 ng genomic DNA, in a total volume of 60 μL (New England BioLabs). To maximize diversity of DNA fragments, we performed two separate DIG/LIG reactions, each with a different restriction enzyme (*Hinc*II and *Pvu*II). The thermal profile for the DIG/LIG reaction was 22 cycles of (37 °C for 10 min and 16 °C for 30 min), followed by a 20-min enzyme denaturation step at 65 °C. Products were visualized on an agarose gel stained with ethidium bromide, and resulting genomic DNA fragments ranged in size from 300 to 1500 base pairs. We used tetra-oligonucleotide probes $(GATA)_8$ and $(GACA)_4$ for the subtractive hybridization procedure; both were performed at 48 °C. To isolate DNA fragments from the enriched libraries, we used the TOPO TA cloning kit (Invitrogen). From the two libraries, we purified and amplified a total of 1344 clones via a Templiphi reaction (GE Healthcare). Cloned DNA fragments from the Templiphi reaction were ready for DNA sequencing without any modification. We used BigDye Terminator version 3.1 kit, and for sequencing cleanup, we used the Ethanol/EDTA/Sodium Acetate Precipitation method (Applied Biosystems). Sequencing products were visualized with a 3100-*Avant* Genetic Analyser (Applied Biosystems).

We cloned and isolated 1344 DNA fragments from *Hinc*II and *Pvu*II libraries, and of these, 711 DNA fragments contained microsatellite repeat motifs, a 53% enrichment efficiency. We selected 128 DNA fragments that contained more than 12 microsatellite repeat motifs for primer design and subsequent screening (Rozen & Skaletsky 2000). To screen for potentially informative loci, we performed

polymerase chain reaction (PCR) and visualized the PCR products using agarose gel-electrophoresis. For loci that amplified and appeared variable, we fluorescently labelled the left primer at the 5′ end, and preformed PCR on a larger sample size. A typical PCR volume consisted of 1 μM of each primer, 5 μL 2× QIAGEN Multiplex PCR Kit, 50 ng DNA, and enough water for a final volume of 10 μL. The thermo profile consisted of one activation step at 95 °C for 15 min followed by 30 cycles (94 °C for 30 s, 60 °C for 90 s and 72 °C for 30 s), with a final extension step at 72 °C for 30 min (QIAGEN, catalogue no. 206143). For all PCR thermocycling, we used the DNA Engine DYAD thermocycler (Bio-Rad Laboratories). Fluorescently labelled PCR products were visualized on a 3100-*Avant* Genetic Analyser. From the screening processes, 12 loci were shown to be polymorphic. For each of these loci, we genotyped 96 individuals to screen for variability and assess Hardy-Weinberg (HW) equilibrium.

We summarized the genetic variation at these l2 loci with a few summary statistics and statistical tests (Table 1). The number of alleles ranged from two to 11, with an average of 5.3 alleles per locus. The expected heterozygosity for each locus was calculated using HW-QuickCheck (Kalinowski 2006) and ranged from 0.29 to 0.76, with an average of 0.68. Deviations from HW expectations were tested using the exact probability test of Guo & Thompson (1992), as implemented by the web version of GenePop (http://genepop.curtin.edu.au/; Raymond & Rousset 1995). None of the loci showed deviations from HW expectations at the 0.05 level of significance. We tested for genotypic equilibrium between all pairs of loci using a Markov chain version of Fisher's exact test as implemented by the web version of GenePop (http://genepop.curtin.edu.au/; Raymond & Rousset 1995). Two pairs of loci showed significant association of genotypes at the 0.001 level of significance: Sna_MSU02 and Sna_MSU10, and Sna_MSU07 and Sna_MSU12. These results show that the microsatellite loci here posses high levels of polymorphism and should be useful for identifying the source of lake trout introductions and for estimating the number of founders.

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