Development of new microsatellite genetic markers for white sturgeon and continued genetic monitoring of the KTOI broodstock.

Deliverable 1. Development of four DNA libraries enriched for tetranucleotide microsatellite motifs and the sequencing of 200 clones

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Introduction

White sturgeon (*Acipenser transmontanus*) from the Kootenai River were listed as endangered under the Endangered Species Act in 1994 (USFWS 1994). This population was listed due to its small size, lack of fish from smaller size classes indicating low reproduction, and geographic isolation (Duke et al. 1999, USFWS 1999); these factors have also reduced the genetic diversity in the population relative to other white sturgeon populations (Rodzen et al. 2004).

The recovery plan for this population emphasizes the protection of remaining genetic diversity in the conservation aquaculture program (USFWS 1999), which requires that hatchery broodstock genetics reflect the wild population genetics (Anders 1988, Ireland et al. 2002). In 2004, Rodzen et al. completed a population genetic analysis of the Kootenai system sturgeon and of the Kootenai broodstock using nuclear (microsatellite) DNA markers. The eight microsatellite loci used to analyze variation in the broodstock did not allow sufficient differentiation among fish for accurate parentage assignment. In order to improve parentage assignment in the Kootenai Hatchery progeny, the Kootenai Tribe of Idaho contracted with the UC Davis Genomic Variation Laboratory to develop new microsatellite genetic loci. This report covers the 2005 deliverable under that contract.

Deliverable 1 Results

Under the contract with the Kootenai Tribe of Idaho, the UC Davis Genomic Variation Laboratory has developed DNA libraries enriched for tetranucleotide microsatellites. See Figure 1 for a representative sequence containing a $(CAGA)_n$ microsatellite motif.

Sturgeon G7 Sequence:
CTTGCTGAATGATATTCCATGGCACTGC
AGTGGCAATGCAGACAGACAGACCGAC
AGACAGCAGCACTACAGTGGCAATGCA
GACAGACAGACAGACAGACAGACAGC
ACTACAGTGGCAATGCAGACAGACAGA
CAGACAGACAGACAGACAGCACTACAG
TGGCAATGCAGACAGACAGACAGACAG
ACAGCAGCACTACAGTGGCAATGCAGA
CAGACACGAGAATGCTTTTATCAATTTA
AGAAACGAAAAGCACCCATTGGGGCTC
CCCAGAGCTCTTAAACAGATCTTCTCTC
TAGTTTAGT

Figure 1. Representative white sturgeon DNA sequence showing a $CAGA_n$ microsatellite motif.

An initial screen of 35 sequenced clones

from four libraries [$(AAAC)_n$, $(CATC)_n$, $(TACA)_n$, and $(TAGA)_n$] yielded 16 sequences with microsatellite motifs. A total of 116 additional clones from the two most successful libraries [$(AAAC)_n$ and $(TAGA)_n$] were then sequenced and screened, yielding 97 additional sequences with microsatellite motifs. Thereafter, thirty-three sequenced clones from four new libraries [$(AAAG)_n$, $(AAAT)_n$, $(CAGA)_n$, and $(GTCA)_n$] were screened, yielding 13 new sequences with a microsatellite motif. Again, 112 additional clones from the two most successful libraries [$(AAAT)_n$, $(CAGA)_n$] were then sequenced and screened, yielding 64 sequences with microsatellite motifs. In total, the eight libraries yielded 190 sequences with microsatellite motifs. These sequences were checked for duplication, reducing the total to 138 sequences available for further analysis (Table 1).

Library	Sequences containing microsatellites		Total clones sequenced	Percentage containing microsatellites
AAAG	3	out of	9	33.3%
AAAT	26	out of	63	41.3%
CAGA	48	out of	64	75.0%
GTCA	0	out of	9	0.0%
AAAC	47	out of	72	65.3%
CATC	1	out of	8	12.5%
TACA	3	out of	9	33.3%
TAGA	62	out of	72	86.1%
Total	190	out of	306	43.4%

Table 1. Microsatellite motif yields by library.

Next Steps

These 138 sequences provide a large pool of potential microsatellites for the development of novel tetranucleotide microsatellite loci for white sturgeon. Pending funding for 2006, the GVL will amplify candidate loci and down select to the most

promising loci for assigning parentage in the broodstock. The GVL will develop a protocol for each new locus, including PCR and electrophoretic conditions, to amplify, resolve, and score each locus. The GVL will also use the novel loci to assess variability and assign parentage in the broodstock population. Finally, GVL will provide a written report in 2006 describing the results from the deliverables above.

Budget (2006)

Budget Item	Cost
Marker screening and primer development	$8,300
Genotyping of past and current broodstock (est. 200 fish @ $80 each)	$16,000
Genotyping of full sib families for validation (est. 240 fish @ $80 each)	$19,200
Data analysis and reporting	$5,000
Administration of contract	$5,000
Travel	$3,000
Subtotal	$56,500
Indirect (15% of subtotal)	$8,475
Total	$64,975

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