

ECOLOGY OF UPPER KLAMATH LAKE SHORTNOSE AND LOST RIVER SUCKERS

5. Molecular evolution and ecology of Klamath Basin suckers

A. Use of anonymous nuclear loci as species markers in Klamath Basin suckers (Catostomidae).

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U. S. Biological Resources Division
US Geological Survey
104 Nash Hall
Oregon State University
Corvallis, Oregon 97331-3803

&
Klamath Project
U. S. Bureau of Reclamation
6600 Washburn Way
Klamath Falls, OR 97603

By
D. Wolfe Wagman & Douglas F. Markle
Oregon Cooperative Research Unit
104 Nash Hall
Department of Fisheries and Wildlife
Oregon State University
Corvallis, Oregon 97331-3803

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ABSTRACT

We used low copy number anonymous nuclear loci to search for species markers in four Klamath Basin and four outgroup suckers. We recognize four classes of markers: fixed species differences, strictly diagnostic markers, operationally diagnostic markers, and frequency dependent markers. Only the first two can be used independently to identify individuals to species.

We examined 28 randomly chosen loci, sequenced 10,421 base pairs, and found no fixed differences in four Klamath Basin species. Genotype 6 of locus 142 was a strictly diagnostic marker for shortnose suckers, but occurred in only 16% of specimens. The homozygous BB genotype of Collagen 1 was an operationally diagnostic marker for Klamath smallscale suckers, but corroboration is currently lacking. The homozygous BB genotype of Aldehyde dehydrogenase was a frequency dependent marker for Klamath smallscale suckers, but, again, corroboration is lacking.

Some of the loci, such as Ankyrin_G, were much better markers for outgroup species than for Klamath Basin suckers and suggest that the technique has power and that the genetic similarity detected in Klamath Basin suckers is not an artifact. The management implications of this and related studies are not yet clear. Because genetic similarity might be a result of hybridization and because hybridization could be a natural and necessary source of genetic variation, it is not currently advisable to make management decisions detrimental to hybrids.

INTRODUCTION

The goals of this study were: 1) to isolate and characterize genetic species markers for each species of Klamath Basin sucker (shortnose sucker, (-SNS), *Chasmistes brevirostris*; Lost River sucker, (LRS), *Deltistes luxatus*; Klamath largescale sucker (KLS), *Catostomus snyderi*; and Klamath smallscale sucker , (KSS), *Ca. rimiculus*); 2) to investigate the potential for hybridization; and 3) to examine population structure or relatedness between sucker populations.

Unique molecular markers would allow species and hybrid identification for all ontogenetic stages. Various techniques were available for this goal but most had drawbacks. Allozymes have been used to distinguish sucker species (Buth 1978; Buth et al. 1987, Crabtree and Buth 1987) and to investigate catostomid phylogeny (Ferris and Whitt 1978). This method requires fresh frozen tissues from several organ systems, multiple developmental stages and is difficult to use on larvae. Mitochondrial DNA (mt DNA) analysis could have used the universal primers of Kocher et al. (1989) but because it is maternally inherited it does not, by itself, address the issue of hybridization. Instead, we used low copy number anonymous nuclear loci, a technique previously used to distinguish populations of green turtles (Karl et al. 1992) and oysters (Karl and Avise, 1993). The technique begins with a genomic library that is randomly sampled for clones. Clones are amplified for each species using polymerase chain reaction (PCR), sequenced and aligned to determine variation.

METHODS

Fish samples

Fish used for this study are listed in Appendix 1. Adults from Klamath and Rogue river basins were collected by U. S. Bureau of Reclamation (BOR) in 1993 and were meant to be representative samples of each species and each known spawning group. BOR collected 333 adult fish and 296 tissue samples were available for this study.

Library Construction

DNA preparation for cloning

A genomic library was constructed from a SNS, AR-041 (OS 015963-B), captured in the lower Williamson River. DNA was isolated from muscle tissue following the procedure of Taggard et al. (1992) with two modifications. DNA was precipitated overnight at -20 °C followed by a 4 °C centrifugation for 20 minutes at 13,000 X g and centrifuged again after washing with 70% ethanol. DNA was quantified by spectrophotometry. Total DNA (10 ug) was restriction digested with Sau 3AI (Promega, React 4, 10 X buffer) following the manufacturer's directions. Restricted DNA was size fractionated on a 0.8% agarose gel next to size ladder made from Hind III-restricted Lambda Phi X 174 (Sigma). The electrophoretic agarose gel was visualized on an ultraviolet (UV) light source and the region between 300-600 bp was excised. DNA was eluted from using BIO 101 Geneclean protocol and 2.1 ug of restricted DNA was recovered.

Vector DNA preparation

Plasmid pUC18 (Promega) was restriction digested with Bam HI (Promega, React 3, 10 X Buffer). BamH1 and Sau3A1 have similar restriction sequences and are complimentary. Restricted DNA was extracted with tris saturated phenol (pH 7.6) and chloroform (24:1 isopropanol), centrifuged, the aqueous phase collected and precipitated with 1/10th volume of 3M sodium acetate and 2.2 volumes of 99% ethanol for 3 hours at -73 °C. DNA was centrifuged for 20 min at 12,000 X g, aspirated and re-suspended in 17 ul water. The vector was de-phosphorylated to prevent recircularization to its self. Dephosphorylation occurred with 2 ul 10 X Calf intestinal phosphatase (CIP) buffer (Promega) and 1 ul CIP at 37 °C for 48 hours. Reaction was precipitated as before and stored at -73 °C.

Ligation Reaction

Ligation of sucker restricted DNA to dephosphorylated pUC 18 vector was accomplished using protocols in Sambrooke et al. (1989). Two ligation protocols were used, 1:1 and 1:3 ratios of vector arms to insert DNA arms. Each 20-ul reaction contained 25ng or 75 ng of insert DNA, respectively. The reaction also

contained 50 ng of dephosphorylated, BamH1 restriction digested pUC 18 DNA, 4 ul of 5 X Ligation Buffer (Promega), 1 unit of T4 Ligase (Promega), and water. pUC 18 DNA and sucker restricted DNA (insert DNA) were combined, incubated for 3.5 minutes at 48 °C, and placed on ice. Buffer and enzyme were then added and incubated for 3.5 hours at room temperature (R.T.). The ligation reaction was diluted with 30 ul of water and mixed with 200 ul of competent DH5 α *E. coli*. Transformation was accomplished by incubating the mixture for 30 minutes on ice, followed by a 3 minute 43 °C heat shock. Luria Broth (LB) (1 ml) was added and the mixture incubated for 1 hr at 37 °C with shaking at 200 rpm. LB ampicillin (100mm) plates were prepared with 4 ul IPTG (200 mg/ ml) and 40 ul X-Gal (20 mg/ ml) for blue-white color determination. The two ligation libraries were then plated in densities of 10, 25 and 100 ul per plate. Plates were inverted and incubated at 37 °C overnight. Each ligation library (500 ul) was mixed with an equal volume of sterile glycerol and frozen at –73 °C. The 1:1 and 1:3 libraries were approximately 50 % recombinant and contained an estimated 412 and 2012 recombinant clones, respectively. Clones were haphazardly chosen and grown for DNA isolation.

Clone DNA isolation

We picked 202 recombinant clones and each was grown in 4.5 ml of ampicillin inoculated LB at 37°C overnight at 250 rpm. In the morning, 500 ul of each culture was aliquoted and mixed with an equal volume of sterile glycerol and stored at –73 °C for archival purposes. Cultures were centrifuged at 12,000 X g for 5 minutes at 4 °C. Pellets were re-suspended in 100 ul STET buffer per ml of culture (Sambrooke et al., 1989), vortexed to fully re-suspend the pellet, and 10 ul of fresh lysozyme (10 mg/ml) per 100ul STET buffer added and incubated at 95 °C for 70 seconds. Suspensions were centrifuged for 15 min at 12,000 X g at R.T. Cellular debris was removed using a sterile toothpick. DNA was precipitated with 110 ul of 100% isopropanol and incubated at –20 °C. DNA was pelleted by centrifugation for 30 minutes at 12,000 X g, the aqueous phase aspirated, and the pellet air dried and re-suspended in 150-ul sterile water. All clone DNA preparations were quantified by spectrophotometry.

Clone DNA amplification

Insert DNA was amplified by PCR and visualized on ethidium bromide containing (2ul/ 100 ml (10mg/ml) 1.1% agarose (BRL) gels. PCR reactions were optimized using master mix solutions set for a reaction volume of 25ul and a DNA volume of 6 ul. Four master mix concentrations of MgCl₂ were used (1, 2, 3, 4 mM MgCl₂). Master mix solutions contained 10 X polymerase buffer (Promega), 5 mM dNTP (BRL), the appropriate amount of 25mM MgCl₂ and water for 50 reactions. The appropriate volume of master mix solution (16.75 ul / 25 ul reaction) was aliquoted into an iced sterile tube. Forward and reverse primers were added (1 ul 20pM-primer solution / 25 ul PCR reaction). TAQ polymerase (Promega) (1-1.25 units per reaction) were added to the master mix and aliquoted (19 ul/ reaction) on top of the previously added template DNA. A three-step PCR method was used: an initial 3 minute de-naturation at 94 °C for one cycle; and a three-step program of 45 sec de-naturation at 93 °C, 45 second annealing, and 60 second extension at 72 °C (annealing temperatures varied with each clone and ranged between 45 and 58 °C). The PCR reaction was optimized for each clone by varying the MgCl₂ concentration and annealing temperatures experimentally.

Surveying copy number

Amplified clones were sized on 1.5% agarose gels using Hind III-restricted Lambda Phi X 174 size markers. Only clones in a size range of 300-700 bp were used for further analysis. This size range corresponds with a single sequencing run on the ABI automated sequencer at Oregon State University. Based on insert size and measured concentration of the preparation, the volume that would contain 1 ug of insert DNA was calculated and this amount used to qualitatively examine copy number of each insert from the SNS genome (Kafatos, et al., 1979). Using a vacuum slot blotting system, 1 ug of insert DNA was adjusted to 21 ul with water and the DNA denatured with 7 ul of 1M NaOH (final concentration of 0.2 M). The solution was incubated 5 min at 37 °C, placed on ice and 60 ul of 10 X SSC (Sambrooke et al. 1989) added, resulting in a final volume of 90 ul. Using MSI nitroPure nitrocellulose as the matrix, the DNA

solution was applied to each well. All liquid was passed through the transfer membrane under vacuum. Each well was washed twice with 90 ul 10X SSC with the vacuum applied. Blots were removed and placed on wet 3mm Whatman filter paper. The DNA was linked to the membrane by an Ultra Lum ultraviolet crosslinker (UVC 515) at 1200 X 100 microjoules. Blots were air dried and stored at – 20⁰C . Each blot contained a positive control of total sucker DNA and a negative control of pUC 18 DNA.

Probe preparation

Probe preparation involved 2 ug of total Sau 3A1 restricted DNA (Promega following the manufacturer's protocol). Digested DNA (315 ng) was labeled using alpha –³² P –dCTP (NEN) and the Multiprime system from Amersham. Unincorporated label and dNTP were eluted from the labeled probe DNA using Elutip-d (Schleicher and Schuell) following the manufactures procedures. The resulting probe had a radiation level of 162,970 cpm/ul and a specific activity of 92.7 uCi.

Individual blots were layered between fine meshed sheets, placed into a hybridization bottle and pre-hybridized 1 hr at 65 ⁰C in 200ml 5X SSC, 1% sarcosyl in a Biometra hybridization oven. Probe DNA was denatured for 5 min at 95⁰C and placed on ice. Pre-hybridization solution was replaced with fresh 5X SSC and 1% sarcocyl and contained 3 X 10⁶ cpm labeled probe. Hybridization occurred overnight at 65 ⁰C. In the morning, hybridization solution was removed and blots washed twice for 30 min each in 200 ml of 3 X SSC, 0.5% sarcosyl at 65 ⁰C. The blots were further washed twice in 200 ml of 3 X SSC for 30 minutes. Blots were air dried for 60 minutes and placed on Kodak X-Omat Ar film at –73⁰C overnight. Films were developed using an automated X-Omat developer.

Low copy number clones were identified by their weak signal on the film. Sequences with low copy number have less hybridization to the probe and have a weak photographic signal. Low copy number clones were 95% of all clones. Once low copy number clones were identified, some were randomly chosen for sequencing and primer selection. Clones were grown in 100ml cultures of ampicillin inoculated LB medium overnight at 37⁰C at 250 rpm. DNA was

isolated using Fisher's Wizard Midiprep DNA isolation kit. DNA was quantified by spectrophotometry and diluted appropriately for the automated sequencer depending on insert size. Sequence primers used were standard forward and reverse primers for the pUC18 plasmid. Primer sites were approximately 50 bp from the start and end of the insert DNA. Raw sequence data was aligned by eye using the program SeqEd. Primer selection was performed by the program Oligo (Version 4.0, National Biosciences Inc.) using the basic default parameters. Primers were synthesized at the Center for Gene Research (Oregon State University) yielding 25-40 nmoles of each primer. Primer stocks were prepared by diluting to 50 pM in water. PCR primer working stocks were prepared by diluting the stocks to 20pM.

PCR optimization

One individual from each of the four Klamath Basin suckers was used to find fixed differences between the species: *D. luxatus* AR-002 (OS 015922) from Upper Klamath Lake, *C. brevirostris* AR-041 (OS 015963-B) from the lower Williamson River, *C. snyderi* AR-57 (OS 015900-F) from the upper Williamson River, and *C. rimiculus* AR-105 (OS 015908-B) from Topsy Reservoir. Each fish was amplified with a particular set of primers. Total PCR product was visualized on 2 % agarose gels containing ethidium bromide (2.5ul/100ml gel 10mg/ml). Clean PCR products were cut from the gel and eluted using 0.45 μ m Ultrafree-MC filter units (Millipore) following the manufacturer's protocol. Eluted DNA was precipitated with 1/10th of a volume of 3M sodium acetate and 2.2 volumes of 95% ethanol (Sambrooke, et al., 1989) and re-suspended in 25 ul water. Re-suspended DNA (5 ul) was used on a mini-2% agarose gel and quantified by comparison to a Hind III-restricted lambda PhiX 174 ladder. DNA was sequenced in both directions using the PCR primers and the sequences were aligned using SeqEd (version 1.0.1. Applied Biosystems, Inc.).

Once variation was detected either de-naturing polyacrylamide gel electrophoresis (PAGE) or single stranded conformational polymorphism (SSCP) analysis was used for population wide surveys (Marklund, et al, 1995). PAGE was used for size variation detection. Gels were made of Long Ranger

acrylamide (FMC) following the manufactures' directions and a square tooth 55-sample comb. Gels were allowed to polymerize overnight. Gels were pre-warmed to 40-50 °C. Individuals were amplified and 7 ul of amplified product were mixed with 5 ul of 100% formamide. Samples were denatured at 83°C for 3 minutes and placed on ice. Five ul was loaded into each well and the gel was run for 2-3 hours at constant power of 50 watts at R.T. Gels were disassembled, overlaid with a gel staining gasket (Wagman, in preparation) with the gasket secured by binder clamps. The gel was placed in the dark and stained with Sybr Gold (Molecular probes) (50ul/ 500ml of 0.6x TBE pH 8.0) for 30 minutes. Gels were inverted and illuminated on an UV light source and photographed with a Kodak MP-3 system using Polaroid 667 film. SSCP gels were made from MDE acrylamide (FMC) following the manufactures' protocol. Gels polymerized overnight with a square tooth 55-sample comb. Gels were set up on the bench at room temperature. Samples were prepared as for PAGE, 5ul were loaded into each lane and the gel was run for 5 minutes at 50 watts to allow the samples to fully penetrate the gel matrix. The gel was disconnected from its power source and moved into a 4 °C cabinet. Gels ran for 20-24 hours at 4 watts per gel. Gels were stained and photographed in a similar manner as PAGE.

Basic population statistics were generated using web based Genepop program (Raymond and Rousset, 1995). Statgraphics plus (version 3.0, 1997) was also used for statistical comparisons.

RESULTS

Forty-four clones had their DNA inserts sequenced for primer selection (Appendix 2). Twenty-eight DNA inserts were successfully amplified and sequenced in each of the four species (Table 1). The remaining 16 inserts were either difficult to optimize in all species, were duplicates of another insert, or were not used for primer selection. Appendix 2 contains the sequence, PCR primers, PCR conditions, and the protein and nucleic acid sequence homologies for each insert as determined in Genbank.

In the following, we refer to each of these inserts as a genetic locus, identified by an arbitrary numbering system or by the name of the coded protein. Each locus successfully amplified different numbers of individuals.

For the 28 randomly chosen loci, we sequenced and aligned 10,421 base pairs. Twenty-one of 28 loci had open reading frames and were homologous to proteins found in Genbank (overall 70.5% (31/44) of the loci in this study contained open reading frames (ORF)). We found no fixed sequence differences between Klamath Basin sucker species, but suckers outside Klamath Basin (outgroups) exhibited unique sequence variation for at least two loci (4 and 184). Eight loci were polymorphic with two or more alleles, four had rare variants and four had common variants.

Rare variants

The rare variant group contained loci 117, 119, 126 and 146. Locus 117 had no open reading frame (ORF) and weak homology (21 nucleotides (nt)) to a human sequence on chromosome 22q12. The two alleles were the result of a position 123 transition (C→T) but the rare allele was only found in two of 150 individuals (both KSS). Locus 119 also had no ORF and weak homology (19nt) to a human sequence on chromosome 12p13. The two alleles were the result of a position 361 transversion (G→ T) but the rare allele was again only found in two of 150 individuals (both KSS). Locus 126 contained an ORF that had 61% identity and 72% overall homology (19/26 amino acids (aa)) to a highly specific repeat in ORF1 found in *Batrachocottus baicalensis* (Kholodilov,N.G., unpublished). The two alleles were the result of a position 143 transition (G→A)

but the rare allele was only found in 2 of 48 individuals (SNS and LRS). Locus 146 had an ORF and was moderately homologous to a protein in the nematode *Caenorhabditis elegans* (23/33 aa) and had strong homology to a 26 nt long *Danio rerio* mRNA sequence. Locus 146 had 2 alleles caused by a position 249 transversion (G→A) but the rare allele was only found in 2 of 48 individuals (both KSS).

Common variants

The common variant group contained loci 4 and 120 (size deletion alleles) and 142 and 184 (sequence variant alleles).

Locus 4, Collagen 1

Locus 4 had 59% identity (73% overall homology, 20/27 aa) to Collagen 1 gene from *Ephydatia muelleri*. Locus 4 had two alleles caused by a 10-bp deletion between positions 260-270. (Appendix 2) and exhibited allelic frequency differences between the four Klamath Basin species (Table 2). LRS and SNS had very high frequencies of allele A (95 and 99% respectively), KLS intermediate (81%), and KSS low frequency (18%). The frequencies were significantly different (ANOVA, p= 0.00001). A Fisher's multiple range test identified three groups, SNS plus LRS, SNS plus KLS, and KLS plus KSS.

In the Rogue River, all KSS were fixed homozygotes, BB, and in Upper Klamath Lake all species were fixed homozygotes, AA (Figure 1). If Collagen 1 is a species marker, all KSS are BB, and all SNS, KLS, and LRS are AA. All heterozygotes (AB) would be F1 hybrids or F2+ backcrosses and some F2+ backcrosses could show a parent genotype. However, the B allele is widespread in the Lost River and Williamson River subbasins of the Upper Klamath Basin and only one KSS has ever been documented in the upper basin (Figure 1). Therefore, if Collagen 1 is a species marker, hybrids must be viable to account for the frequencies in the upper basin and hybridization is either on-going or ancient with the B allele maintained by random mating.

Collagen 1 also showed interesting differences between outgroup and Klamath Basin species (data not shown). A coastal *C. macrocheilus* had a single base pair insert. A nominal *C. macrocheilus* from Hood River had a large (ca. 20

bp) deletion. *C. occidentalis* had an 11 or 12-bp deletion. Other *C. macrochielus*, *C. sp.* (Wall Canyon), and *X. texanus* were similar in PAGE profile to Klamath Basin suckers.

Locus 120, Aldehyde dehydrogenase

Locus 120 had 45% identity (69% overall homology, 26/37 aa) to a tumor associated aldehyde dehydrogenase from *Ratus norvegicus* (Appendix 2). The aldehyde dehydrogenase locus exhibited allelic frequency differences between the four Klamath Basin species (Table 3) based on a four bp deletion. SNS and KLS had high frequencies of allele A (86-87%), LRS was intermediate (76%) and KSS had the lowest frequency (26%). The frequencies were significantly different (ANOVA, $p= 0.00001$). Fisher's multiple range test identified three groups, SNS plus KLS, LRS, and KSS.

In the Rogue River, 72% of KSS were fixed homozygotes, BB, and in Upper Klamath Basin 71% of all species were fixed homozygotes, AA (Figure 2). No case can be made that any of the four species is fixed for either allele. Both alleles must pre-date differentiation of the species or indicate ancient hybridization prior to Klamath KSS dispersal into the Rogue River, or separation from the Klamath Basin.

Locus 142

Locus 142 did not have an ORF and had weak sequence homology (21 nt.) to a human chromosome 5 sequence. Locus 142 has at least 12 alleles in 7 genotypes based on SSCP analysis. The locus has not been fully investigated at this time. Locus 142 was polyploid because individuals had up to three different alleles. Because catostomids are allotetraploids (Ferris and Whitt 1978, Buth 1979) we assume this is a tetraploid locus. Twelve alleles have been found, but only eight have been sequenced. Alleles B, C, D, E, migrate very closely to the A allele and therefore isolation has been difficult. The A allele is identical to the clone sequence.

Locus 142 showed allelic segregation because all alleles were not equally available to all genotypes (Figure 3). Assuming that B, C, D, and E alleles have similar sequences to A, genotype groups appear to have closely related alleles

(Figure 3A). Genotypes 1-4 only have A-E alleles, genotype 6 only has F-H alleles, genotype 7 only has I and J alleles and genotype 8 only has K and L alleles (Figure 3B). The F, G, and H alleles share unique changes at positions 40 and 350 and the I and J alleles share a unique change at position 59 (Figure 3). Only genotype 8 alleles appear to deviate from this pattern. Alleles G and H had the greatest amount of changes from A, with 2 base pair plus an 80 bp repeat (G) and a 70 bp deletion (H).

The most common genotype group contains the A allele and was found in all four Klamath Basin suckers, but with different frequencies (Table 4). Genotype 1 occurred in 50% of SNS, 46% of LRS, 34% of the KLS and 4% of KSS. Genotype 3 was the dominant genotype in KSS (70%). Genotype 6 occurred in 21 specimens of SNS from Upper Klamath Lake, Sprague River and Clear Lake. Genotype 7 was found in all species and accounted for 9% of SNS, 15% of KLS, 20% of KSS and 37% of LRS. Major genotypes per species were: SNS, genotypes 1, 2 and 6 (81 % of specimens); KLS, genotypes 1, 2, 3, and 7 (93%); KSS, genotypes 3 and 7 (90%); and LRS, genotypes 1 and 7 (83%).

Locus 184, Ankyrin_G

Locus 184 had 88% identity (99% overall homology, 18/18 aa) to human Ankyrin_G (Kordeli, et al 1995). Locus 184 had two alleles caused by a position 353 transversion (C→A) and exhibited allelic frequency differences between the four Klamath Basin species (Table 5). Frequencies of allele A were significantly different between the four species (ANOVA, p= 0.0133). Fisher's multiple range test identified three groups, SNS plus LRS, SNS plus KLS, and KSS plus KLS. Throughout the basin, Ankyrin_G genotype frequencies were similar and most fish (89%) were heterozygous. No fish were homozygous BB.

Ankyrin_G SSCP variation in outgroups (Figure 4) was considerable and showed all species possessed the common A allele plus a unique secondary allele. All outgroups were heterozygous. The first 100 bp of sequence data were unclear for *Ca. occidentalis* and one *Ca. macrocheilus* from Hood River (Table 6). Excluding these bases, the Klamath/Rogue allele B was most like the A allele, differing only at the position 353 transversion. All outgroup secondary Ankyrin_G

alleles shared a substitution at position 140. Among outgroups, the secondary allele in *Ca. occidentalis* was the most similar to allele B, differing only at positions 140 and 209. Considering the lack of variation in the secondary allele in the four Klamath Basin species, the variation in five specimens of the nominal *Ca. macrocheilus* was huge. The secondary alleles in two specimens collected about a month apart in Hood River (OS 15885 and OS 15886) differed at 19 positions. One of these (Hood A) was more like coastal *Ca. macrocheilus* from Woahink Lake (Siuslaw drainage) than the other Hood River fish, yet also differed from coastal *Ca. macrocheilus* at 15 positions.

DISCUSSION

Our selection of low copy number anonymous nuclear loci resulted in a large number of structural genes (70.5% ORF). Surprisingly, among the four species and three genera of Klamath Basin suckers, there were no fixed differences in 10,421 bp from 28 randomly selected loci. Only eight loci were polymorphic and only four of these had common variants.

Among the common variant loci, we found three types of species markers: strictly diagnostic, operationally diagnostic and frequency dependent. We define a strictly diagnostic species marker as an allele or genotype that is only found in one species and indicates that an individual can be identified to species. An operationally diagnostic species marker is an allele or genotype fixed for one population (drainage or sub basin) of a species and may, with corroborative information, indicate that an individual can be identified to species. A frequency dependent species marker is an allele or genotype showing a statistically significant difference in frequency between species and may, with corroborative information, indicate that a population can be identified to species.

In the Klamath/Rogue basins among the common variant loci, genotype 6 of locus 142 was strictly diagnostic. Genotype 6 was only found in SNS, but not in all SNS (Table 4). The genotype was not geographically restricted and appears to be a good, but limited, species marker for SNS. The homozygous BB genotype of Collagen 1 was operationally diagnostic for KSS in the Rogue River (Figure 1). The Rogue River sample was limited to one site on a single day and

may represent a single spawning group or sampling artifact. The B allele of Aldehyde dehydrogenase was a frequency-dependent marker for KSS (Figure 2). Again, 72% of the Rogue River fish were homozygous, BB, for this locus and sampling artifact can not be dismissed.

The analysis indicated that the catostomid genomes of Klamath/Rogue basins were conserved and highly related. The sequence similarity of random loci and low levels of polymorphism of those loci across the four species does not support their current classification in three genera. Further, most differences followed geographic, rather than taxonomic, patterns with KSS from the lower Klamath Basin and Rogue River more often the “distinct” entity while species from three genera in Upper Klamath Basin (SNS, LRS, and KLS) were more similar (Figures 1 and 2).

The alleles for locus 142 did not exhibit random assortment. If they did, alleles of all types should be found together, such as A with F, G, H, I, J, K, or L. This has not been found (Table 4) and suggests either assortative mating through mate recognition or selection against particular allele combinations.

Ankyring illustrated that low copy number anonymous nuclear loci can provide strictly diagnostic species markers in other catostomids. The *Ca. macrocheilus* group was especially variable. One form differed by 19 and 15 positions from two other forms of nominal *Ca. macrocheilus*. In the four Klamath/Rogue species there were no differences, again suggesting genetic relatedness of suckers within the basin.

These preliminary data need to be integrated with other molecular and morphological data to better understand their meaning. We believe there are at least four biological species of suckers in the Klamath/Rogue basins. Our data indicate they may be hybridizing, but that they also may show assortative mating (locus 142, especially genotype 6). At this point, it is not clear if hybridization is a natural and necessary source of genetic variation, as would be expected from a syngameon, or a waste of each species’ reproductive potential. Consequently, it is not currently advisable to make management decisions detrimental to hybrids.

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Table 1. List of 28 anonymous nuclear loci used in this study. Search type refers to either a protein (aa) search using the blastn program or a nucleic acid search using the blastx program.

Locus #		PCR size	search type	residues	Homology
1	2	501	protein	20/22 aa	unknown protein [<i>Mycoplasma genitalium</i>]
2	4	454	protein	16/27 aa	collagen COLF1 - freshwater sponge [<i>Ephydatia muelleri</i>]
3	8	543	protein	14/36 aa	NADH dehydrogenases subunit 8 [<i>Crithidia oncopelti</i>]
4	9	348	protein	22/74 aa	13KD protein [<i>Saccharomyces cerevisiae</i>]
	9		nucleic acid	39/41 nt	diphtheria toxin repressor (dtxR) gene [<i>Corynebacterium diphtheriae</i>]
5	13	320	protein	17/32 aa	probable membrane protein [<i>Saccharomyces cerevisiae</i>]
6	19	421	protein	40/125 aa	serine/threonine kinase, [<i>Homo sapiens</i>]
7	39	338	nucleic acid	32/36 nt	ependymin (sh) precursor gene [<i>Notropis chrysoleucas</i>]
8	54	286	protein	13/39 aa	hypothetical protein HI1195 [<i>Haemophilus influenzae</i>]
9	61	215	protein	28/30 aa	myelin basic protein kinase-like protein [<i>Xenopus laevis</i>]
	61		nucleic acid	75/88 nt	ERK2, exon 1 [<i>Mus musculus</i>]
10	63	278	nucleic acid	23/24 nt	cosmid T10B9, [<i>Caenorhabditis elegans</i>]
11	67	305	protein	17/49 aa	potassium channel [<i>Rattus norvegicus</i>]
12	76	190	protein	21/34 aa	KIAA0335, [<i>Homo sapiens</i>]
13	79	249	protein	16/40 aa	pyruvate,orthophosphate dikinase [<i>Thermotoga maritima</i>]
14	81	261	nucleic acid	20/20 nt	cadherin-7 (CDH7) mRNA [<i>Homo sapiens</i>]
	81		nucleic acid	20/20 nt	mRNA for guanylyl cyclase C [<i>Xenopus laevis</i>]
15	82	262	nucleic acid	34/34 nt	Rat transferrin receptor mRNA, 3' end.
16	88	482	protein	19/28 aa	Hypothetical protein Rv2035 [<i>Mycobacterium tuberculosis</i>]
17	94	372	protein	23/64 aa	AbcA [<i>Dictyostelium discoideum</i>]
			nucleic acid	147/156nt	transposon Tsn1-3 transposase pseudogene [<i>Salvelinus namaycush</i>]
18	107	543	protein	23/85 aa	Hypothetical protein, len: 1676 aa [<i>Plasmodium falciparum</i>]
19	117	498	nucleic acid	21/21nt	Human DNA sequence from PAC 272J12 on chromosome 22q12-qter

20	119	460	nucleic acid	19/19 nt	12p13 BAC RPCI11-436I9 [<i>Homo sapiens</i>]
21	120	430	protein	17/34 aa	tumor-associated aldehyde dehydrogenase [<i>Rattus norvegicus</i>]
22	126	306	protein	16/26 aa	orf2 [<i>Batrachocottus baikalensis</i>]
23	142	361	nucleic acid	21/21 nt	chromosome 5, Pac clone 162o17 (LBNL H147 [<i>Homo sapiens</i>])
24	146	381	protein	12/33 aa	No definition line found [<i>Caenorhabditis elegans</i>]
	146		nucleic acid	26/26 nt	no arches (nar) mRNA [<i>Danio rerio</i>]
25	176	401	protein	11/23 aa	hypothetical protein HI1418 [<i>Haemophilus influenzae Rd</i>]
26	182	373	protein	63/109 aa	brain-specific angiogenesis inhibitor 1 precursor [<i>Homo sapiens</i>]
27	184	434	protein	16/18 aa	ankyrin G [<i>Homo sapiens</i>]
28	187	382	protein	35/122 aa	envelope glycoprotein [Human immunodeficiency virus type 1]
Total:		10421			

Table 2: Genotypic and allelic frequencies (Freq) for Collagen 1 for all species and sites. n = sample size.

Species	Site	n	AA	AB	BB	Freq A	Freq B
SNS	ALL	133	0.9	0.08	0.02	0.94	0.06
KLS		63	0.71	0.19	0.1	0.81	0.19
KSS		52	0.13	0.06	0.81	0.16	0.84
LRS		41	0.95	0.025	0.025	0.96	0.04
		289					
SNS	Sprague	5	1	0	0	1	0
KLS		25	0.76	0.08	0.16	0.8	0.2
LRS		5	0.8	0.2	0	0.9	0.1
KLS	Sycan	7	0.285	0.428	0.285	0.5	0.5
KLS	Rocky Ford	27	0.74	0.22	0.04	0.85	0.15
SNS	Lower Williamson	13	1	0	0	1	0
KLS		1	1	0	0	1	0
LRS		2	1	0	0	1	0
SNS	UKL	13	1	0	0	1	0
KSS		1	1	0	0	1	0
LRS		16	1	0	0	1	0
SNS	Gerber	20	0.78	0.22	0	0.89	0.11
KLS		2	1	0	0	1	0
SNS	Lost River	1	1	0	0	1	0
LRS		4	1	0	0	1	0
SNS	Clear Lake	71	0.9	0.07	0.03	0.94	0.06
LRS		14	0.93	0	0.7	0.93	0.07
SNS	Topsy	4	1	0	0	1	0
KLS		1	0	1	0	0.5	0.5
KSS		22	0.27	0.14	0.59	0.34	0.66
SNS	Copco	5	0.8	0.2	0	0.9	0.1
KSS	Rogue River	29	0	0	1	0	1
SNS	Hatchery	1	1	0	0	1	0
TOTAL		289					

Table 3: Genotypic and allelic frequencies (Freq) for aldehyde dehydrogenase for all species and sites. n = sample size.

Species	Site	n	AA	AB	BB	Freq A	Freq B
SNS	ALL	128	0.73	0.24	0.02	0.86	0.14
KLS		54	0.74	0.26	0	0.87	0.13
KSS		51	0.02	0.49	0.49	0.26	0.74
LRS		37	0.62	0.27	0.11	0.76	0.24
		270					
SNS	Sprague	5	0.6	0.2	0.2	0.7	0.3
KLS		20	0.65	0.35	0	0.82	0.18
LRS		5	0.4	0.4	0.2	0.6	0.4
KLS	Sycan	7	0.43	0.57	0	0.71	0.29
KLS	Rocky Ford	24	0.88	0.12	0	0.95	0.05
SNS	Lower Williamson	13	0.84	0.16	0	0.92	0.08
KLS		1	1	0	0	1	0
LRS		2	1	0	0	1	0
SNS	UKL	11	0.73	0.27	0	0.86	0.14
KSS		1	0	0	1	0	1
LRS		14	0.79	0.14	0.07	0.86	0.14
SNS	Gerber	20	0.7	0.3	0	0.85	0.15
KLS		1	1	0	0	1	0
SNS	Lost River	1	0	1	0	0.5	0.5
LRS		3	0.33	0.67	0	0.67	0.33
SNS	Clear Lake	68	0.78	0.19	0.03	0.89	0.11
LRS		13	0.54	0.31	0.15	0.56	0.44
SNS	Topsy	4	0.5	0.5	0	0.67	0.33
KLS		1	1	0	0	1	0
KSS		21	0.05	0.81	0.14	0.45	0.55
SNS	Copco	5	0.4	0.6	0	0.7	0.3
KSS	Rogue River	29	0	0.28	0.72	0.14	0.86
SNS	Hatchery	1	1	0	0	1	0
TOTAL		270					

Table 4: Genotypic (GT) frequencies for Locus 142 for all species and sites. n = sample size.

Species	Site	n	GT1	GT2	GT3	GT4	GT6	GT7	GT8
SNS	ALL	129	0.5	0.15	0.05	0.02	0.16	0.09	0.03
KLS	ALL	61	0.34	0.18	0.262	0	0	0.15	0.07
KSS	ALL	45	0.04	0.07	0.7	0	0	0.2	0
LRS	ALL	35	0.46	0.06	0.09	0.03	0	0.37	0
		270							
SNS	Sprague	5	0	0.4	0.2	0	0	0	0
KLS		23	0.26	0.22	0.04	0	0	0.3	0.18
LRS		4	0.5	0.25	0	0	0	0.25	0
KLS	Sycan	7	0.29	0.29	0.42	0	0	0	0
KLS	Rocky Ford	27	0.41	0.11	0.44	0	0	0.04	0
SNS	Lower Williamson	13	0.46	0.23	0.15	0	0.08	0	0.08
KLS		1	0	0	0	0	0	1	0
LRS		2	1	0	0	0	0	0	0
SNS	UKL	12	0.581	0	0.083	0.083	0.17	0	0.083
KSS		1	1	0	0	0	0	0	0
LRS		15	0.27	0	0.13	0	0	0.6	0
SNS	Gerber	22	0.5	0.045	0	0	0.14	0.27	0.045
KLS		1	0	1	0	0	0	0	0
SNS	Lost River	1	0	0	0	1	0	0	0
LRS		4	0.33	0	0	0.33	0	0.33	0
SNS	Clear Lake	66	0.56	0.11	0.03	0	0.2	0.09	0.01
LRS		10	0.6	0.1	0.1	0	0	0.2	0
SNS	Topsy	4	0.5	0.5	0	0	0	0	0
KLS		1	1	0	0	0	0	0	0
KSS		20	0.05	0.05	0.8	0	0	0.1	0
SNS	Copco	5	0.4	0.6	0	0	0	0	0
KSS	Rogue River	25	0	0.08	0.64	0	0	0.28	0
SNS	Hatchery	1	failed						
		270							

Table 5: Ankyrin_G genotypic and allelic frequencies by species and site.

Species	site	n	AA	AB	BB	Freq A	Freq B
SNS	All	124	0.13	0.87	0	0.56	0.44
KLS		48	0.04	0.96	0	0.52	0.48
KSS		48	0.02	0.98	0	0.51	0.49
LRS		33	0.21	0.79	0	0.61	0.39
		253					
SNS	Sprague	5	0.2	0.8	0	0.6	0.4
KLS		17	0	1	0	0.5	0.5
LRS		5	0.2	0.8	0	0.6	0.4
KLS	Sycan	7	0.14	0.86	0	0.57	0.43
KLS	Rocky Ford	21	0.04	0.96	0	0.52	0.48
SNS	Lower Williamson	14	0.07	0.93	0	0.54	0.46
KLS		1	0	1	0	0.5	0.5
LRS		2	0	1	0	0.5	0.5
SNS	UKL	11	0	1	0	0.5	0.5
KSS		1	0	1	0	0.5	0.5
LRS		15	0.27	0.73	0	0.63	0.37
SNS	Gerber	21	0.14	0.86	0	0.57	0.43
KLS		1	0	1	0	0.5	0.5
SNS	Lost River	1	0	1	0	0.5	0.5
LRS		3	0	1	0	0.5	0.5
SNS	Clear Lake	63	0.17	0.83	0	0.59	0.41
LRS		8	0.25	0.75	0	0.63	0.37
SNS	Topsy	3	0	1	0	0.5	0.5
KLS		1	0	1	0	0.5	0.5
KSS		19	0.05	0.95	0	0.53	0.47
SNS	Copco	5	0	1	0	0.5	0.5
KSS	Rogue River	28	0	1	0	0.5	0.5
SNS	Hatchery	1	0	1	0	0.5	0.5
Total		253					

Table 6: Ankyring sequences for alleles A and B, *Catostomus occidentalis*, *Catostomus wallcanyon*, *Xyraunchan texanus*, *Catostomus macrocheilus* A and B from Hood River and *Catostomus macrocheilus* from the coast.

Sequence	0	10	20	30	40	50	60
Allele A:	AGGGCCTGAG	AGCATAAGAT	GAAAAA-CTAT	GATG-AATAT	TTTAGTACAT	ATTTTTT CAG	
Allele B:	AGGGCCTGAG	AGCATAAGAT	GAAAAA-CTAT	GATG-AATAT	TTTAGTACAT	ATTTTTT CAG	
Ca. occidentalis	AGGGCCTGAG	AGCATAAGAT	GAAAAA-CTAT	GATG-AATAT	TTTAGTACAT	ATTTTTT CAG	AA GTG
Ca. wallcanyon	AGGGCCTGAG	AGCATAAGAT	GAAAACCTAT	GA-G -AATAT	TTTAGTACAT	ATTTTTT CAG	
X. texanus	AGGGCCTGAG	AGCATAAGAT	GAAAACCTAT	GA-G -AATAT	TTTAGTACAT	ATTTTTT CAG	
Ca. macrocheilus Hood A	AGGGCCTGAG	AGCATAAGAT	GGATA--CTAT	GA-GAAATAT	TGTAGTACAT	ATTTTTT CAG	
Ca. macrocheilus Hood B	AGGGCCTGAG	AGCATAAGAT			GATA-G	TGTGATGATG	
Ca. macrocheilus Coastal	AGGGCCTGAG	AGCATAAGAT	GGAAA-CTAT	GA-GCAATAT	TGTAGTACAT	ATTTTTT CAG	
	70	80	90	100	110	120	
Allele A:	AGAACGTTAGA	A AACTAATGC	AGC-AGATTG	---CCCTTT	TCTGGTCAAG	CAAAGATAAT	
Allele B:	AGAACGTTAGA	A AACTAATGC	AGC-AGATTG	---CCCTTT	TCTGGTCAAG	CAAAGATAAT	
Ca. occidentalis	TATAGAGAAC	TAAGTGCTGC	AGGATCTGCG	----CCTTT	TCTGGTCAAG	CAAAGATAAT	
Ca. wallcanyon	AGAACGTTAGA	AAACTAATGC	AGC-AGATTG	---CCCTTT	TCTGGTCAAG	CAAAGATAAT	
X. texanus	AGAACGTTAGA	AAACTAATGC	AGC-AGATTG	---CCCTTT	TCTGGTCAAG	CAAAGATAAT	
Ca. macrocheilus Hood A	AGAACGTTAGA	AAACTAATGC	AGC-AGATTG	---CCCTTT	TCTGGTCAAG	CAAAGATAAT	
Ca. macrocheilus Hood B	GATCTCCATG	---GCACGTCG	AG-- AG---TG	TCGCTCCCTT	TCTGGTCAAG	CAAAGATAAT	
Ca. macrocheilus Coastal	AGAACGTTAGA	AAACTAATGC	ACGCAGATTG	--CCCTTT	TCTGGTCAAG	CAAAGATAAT	
	130	140	150	160	170	180	
Allele A:	TAAGGTTTCA	GAGAGATTTG	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
Allele B:	TAAGGTTTCA	GAGAGATTTG	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
Ca. occidentalis	TAAGGTTTCA	GAGAGATTTT	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
Ca. wallcanyon	TAAGGTTTCA	GAGAGATTTT	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
X. texanus	TAAGGTTTCA	GAGAGATTTT	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
Ca. macrocheilus Hood A	TAAGGTTTCA	GAGAGATTTT	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
Ca. macrocheilus Hood B	TAAGGTTTCA	GAGAGATTTT	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	
Ca. macrocheilus Coastal	TAAGGTTTCA	GAGAGATTTT	TGCATTGAAC	TGTGCAAACG	TTTCGGCAC	AATAATGTG	

	190	200	210	220	230	240
Allele A:	TCAATG TTTC	ACATTTCTCT	TAAACTGGTT	ATTTTATATC	TTCATCTTAA	GTGTGTCAGT
Allele B:	TCAATG TTTC	ACATTTCTCT	TAAACTGGTT	ATTTTATATC	TTCATCTTAA	GTGTGTCAGT
Ca. occidentalis	TCAATGTTTC	ACATTTCTCT	TAAACTGGCT	ATTTTATATC	TTCATCTTAA	GTGTGTCAGT
Ca. wallcanyon	TCAATGTTTC	ACATTTCTCT	TAAACTGGTT	ATTTTATATC	TTCATCTTAA	GTGTGTCAGT
X. texanus	TCAATGTTTC	ACATTTCTCT	TAAATTGGTT	ATTTTATATC	TTCATCTTAA	GTGTGTCAGT
Ca. macrocheilus Hood A	TCAATGTTTC	ATATTTCTCT	TAAACTGGTT	ATTTTATATC	TTCAGCTTAA	GTGTGTCAGA
Ca. macrocheilus Hood B	TCAATGTTTC	ATATTTCTAT	TAAACTGGTT	ATTTTATATC	TTCAGCTTAA	GTGTGTCAGA
Ca. macrocheilus Coastal	TCAATGTTTC	ATATTTCTCT	TAAACTGGTT	ATTTTATATC	TTCAGCTTAA	GTGTGTCAGA

	250	260	270	280	290	300
Allele A:	GGGAAGTATC	GATTTTAGAT	TTCCCCTGTC	ACAAAAATCT	AATGAAAAAA	AAAGAAAGT
Allele B:	GGGAAGTATC	GATTTTAGAT	TTCCCCTGTC	ACAAAAATCT	AATGAAAAAA	AAAAGAAAGT
Ca. occidentalis	GGGAAGTATC	GATTTTAGAT	TTCCCCTGTC	ACAAAAATCT	AATGAAAAAA	AAAAGAAAGT
Ca. wallcanyon	GGGAAGTATC	GATTTTAGAT	TTCCCCTGTC	ACAAAAATCA	AATGAAAAAA	AAAAGAAAGT
X. texanus	GGGAAGTATC	GATTTTAGAT	TTCCCCTGTC	ACAAAAATCT	AATGAAAAAA	AAAAGAAAGT
Ca. macrocheilus Hood A	GGGAAGTATC	GA-----TAGAT	TTCCCCTGTC	ACGAAAATCA	AATGAAAAAA	AAAAGAAAGT
Ca. macrocheilus Hood B	GGGAAGTATC	GA-----TAGAT	TTCCCCTGTC	ACGAAAATCA	AATGAAAAAA	AAAAGAAAGT
Ca. macrocheilus Coastal	GGGAAGTATC	GA-----TAGAT	TTCCCCTGTC	ACGAAAATCA	AATGAAAAAA	AAAAGAAAGT

	310	320	330	340	350	360
Allele A:	TGGATAAAAG	GAGTGAGGAA	TATTTTTCT	TTTGTAATTG	ATAAAGACTC	ATGGATTATT
Allele B:	TGGATAAAAG	GAGTGAGGAA	TATTTTTCT	TTTGTAATTG	ATAAAGACTC	ATTGATTATT
Ca. occidentalis	TGGATAAAAG	GAGTGAGGAA	TATTTTTCT	TTTGTAATTG	ATAAAGACTC	ATGGATTATT
Ca. wallcanyon	TGGATAAAAG	GAATGAGGAA	TATTTTTCT	TTTGTAATTG	ATAAAGACTC	ATGGATTATT
X. texanus	TGGATAAAAG	GAGTGAGGAA	TATTTTTCT	TTTGTAATTG	ATAAAGACTC	ATGGATTATT
Ca. macrocheilus Hood A	TGGATAAAAG	GGGGGGGGAA	ATTTTTTTT	TTTGGATTG	GAAAAACCCC	AAGGGTTTTT
Ca. macrocheilus Hood B	TGGGTAAAAG	GGGGGGGGGA	AATTTTTTT	TTT TTAATTG	AGAAAAACTC	AGGGGTTTTT
Ca. macrocheilus Coastal	TGGATAAAAG	GGGGGGGGAA	ATTTTTTTT	TTT TGAAATT	GAAAAAGCCC	CA-GGGATT

	370	380	390	400	410	420
Allele A:	TATTTTTCAT	CCAAACATTC	CTTGTGCAA	TAGAATAGAA	TAGAAGAAGT	CCTGCAACAT
Allele B:	TATTTTTCAT	CCAAACATTC	CTTGTGCAA	TAGAATAGAA	TAGAAGAAGT	CCTGCAACAT
Ca. occidentalis	TATTTTTCAT	CCAAACATTC	CTTGGGCAA	TAGAATAGAA	TAGAAGAAGT	CCTGCAACAT
Ca. wallcanyon	TATTTTTCAT	CCAAACATTC	CTTGTGCCA	TAGAATAGAA	GCTA-G-----T	CC-GGAACAT
X. texanus	TATTTTTCAT	CCAAACATTC	CTTGAGGAAA	TAGAGTAGAA	TAGAAG-----T	CCTGCAACAT
Ca. macrocheilus Hood A	TTTTTTTTAA	GCCA-ACCTT	TC-TTGGCA	AAAAAAA	AAAAAAAGCC	CCCCCCCATA
Ca. macrocheilus Hood B	TTTTTTTTTT	GCACACATT	TTTTGCAA	AAAAAAA	AAAAAAAGC	CCTCCACCAT
Ca. macrocheilus Coastal	TTTTTTTTTC	ATCCAAACAT	TTCC-TTGGC	AAAAAAA	AAAAAAAGC	CCCCCGCAT
	430	439				
Allele A:	ATGGTATGGT	CCCAGGACT				
Allele B:	ATGGTATGGT	CCCAGGACT				
Ca. occidentalis	ATGGTATGGT	CCCAGGACT				
Ca. wallcanyon	ATGGTATGGT	CCCAGGACT				
X. texanus	ATGGTATGGT	CCCAGGACT				
Ca. macrocheilus Hood A	ATGGTATGGT	CCCAGGACT				
Ca. macrocheilus Hood B	ATGGTATGGT	CCCAGGACT				
Ca. macrocheilus Coastal	ATGGTATGGT	CCCAGGACT				

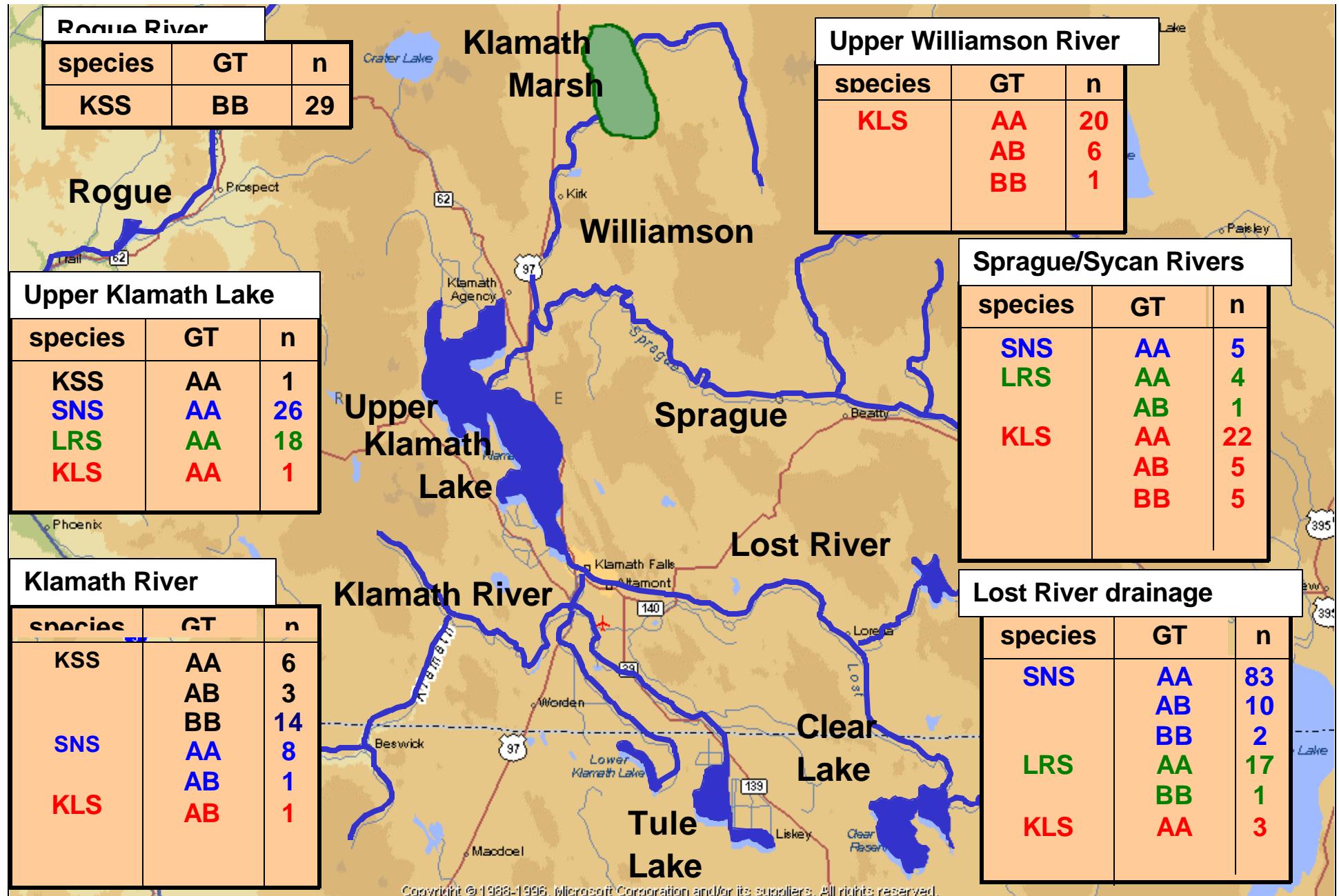


Figure 1: Distribution and number (n) for Collagen I genotypes for all species in each sub-basin n=292.

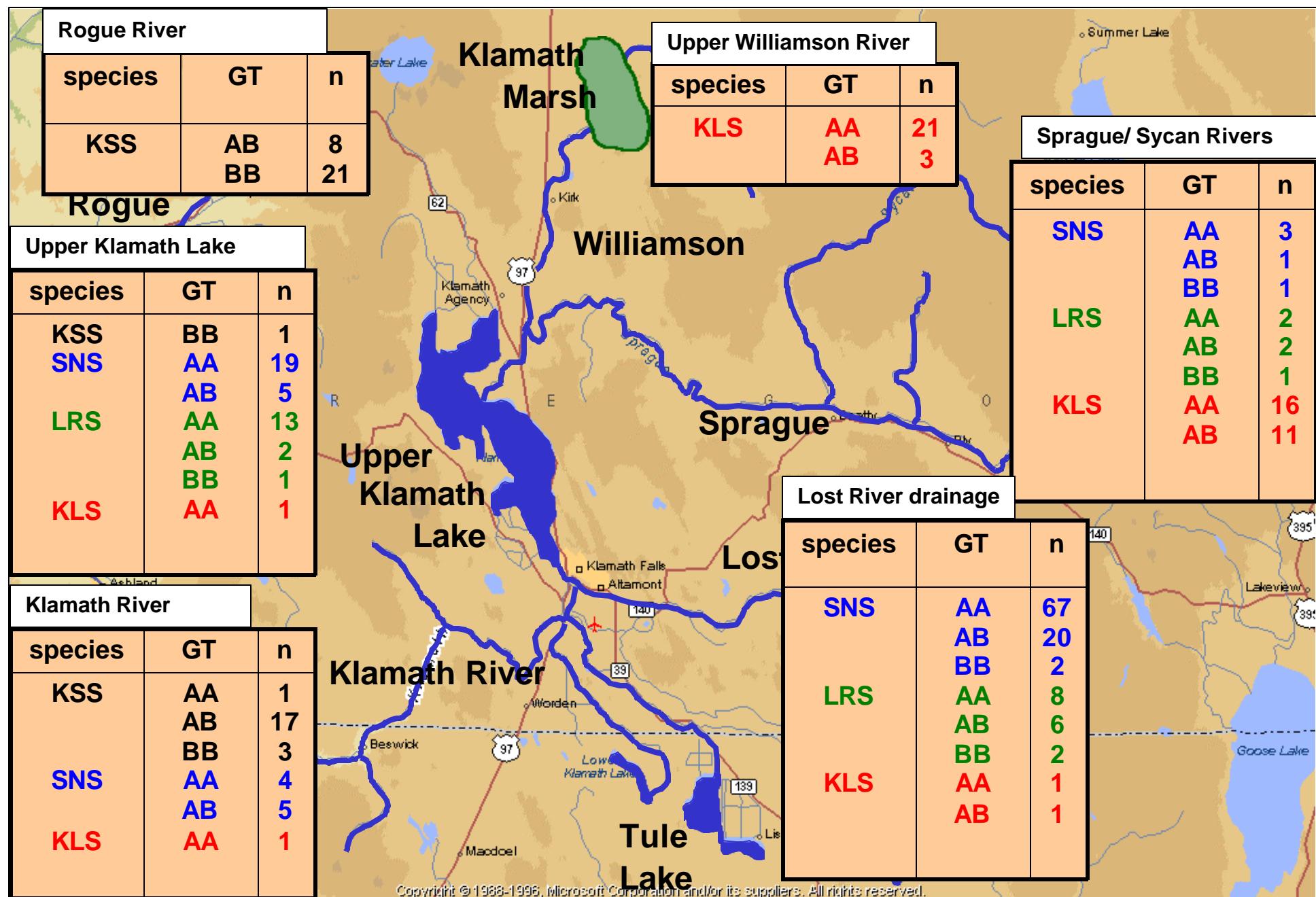
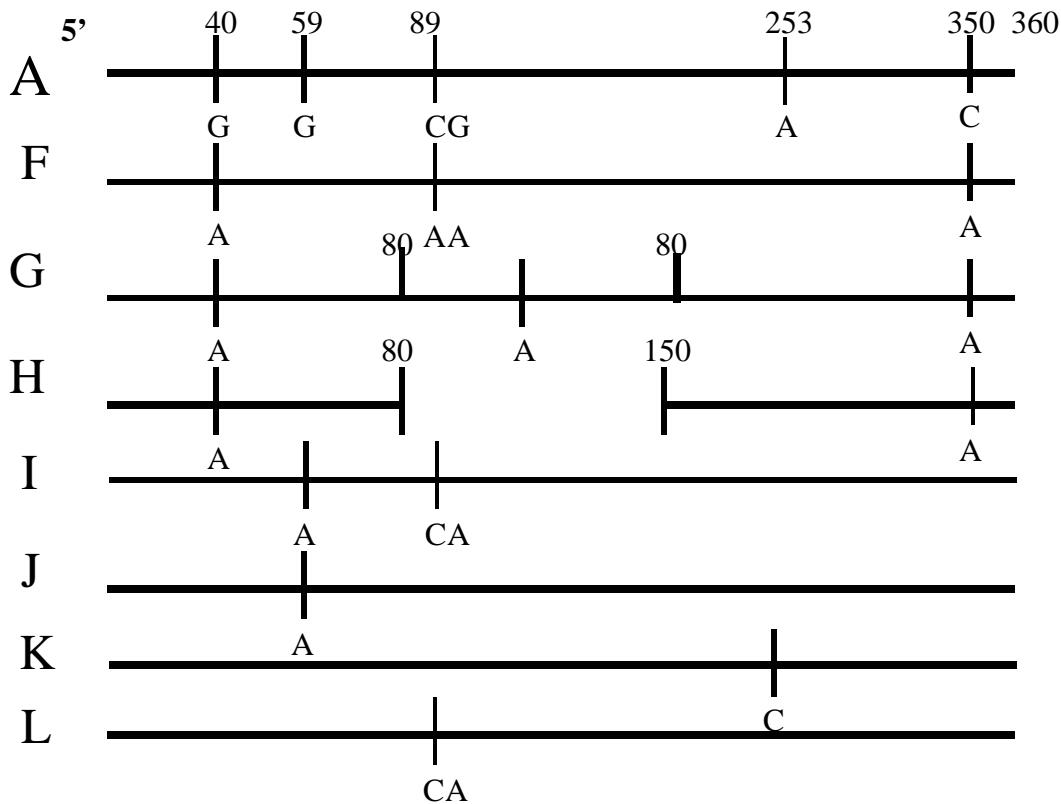


Figure 2: Distributions and numbers (n) of Aldehyde dehydrogenase genotypes for all species in each subbasin n=270.

A-



B- Locus 142

GENOTYPE #	ALLELLES
1	A ₄
2	A ₂ B ₂
3	A ₂ C ₂
4	A ₂ D ₁ E ₁
6	F ₂ G ₁ H ₁
7	I ₂ J ₂
8	K ₂ L ₂

Figure 3: **A-** Line diagrams representing 8 of the 12 alleles for Locus 142. "A" allele is identical to the clone sequence. Sequence that deviates from the A allele is represented by a vertical bar with the corresponding base under it. **B-** Genotype number and alleles found in that genotype.

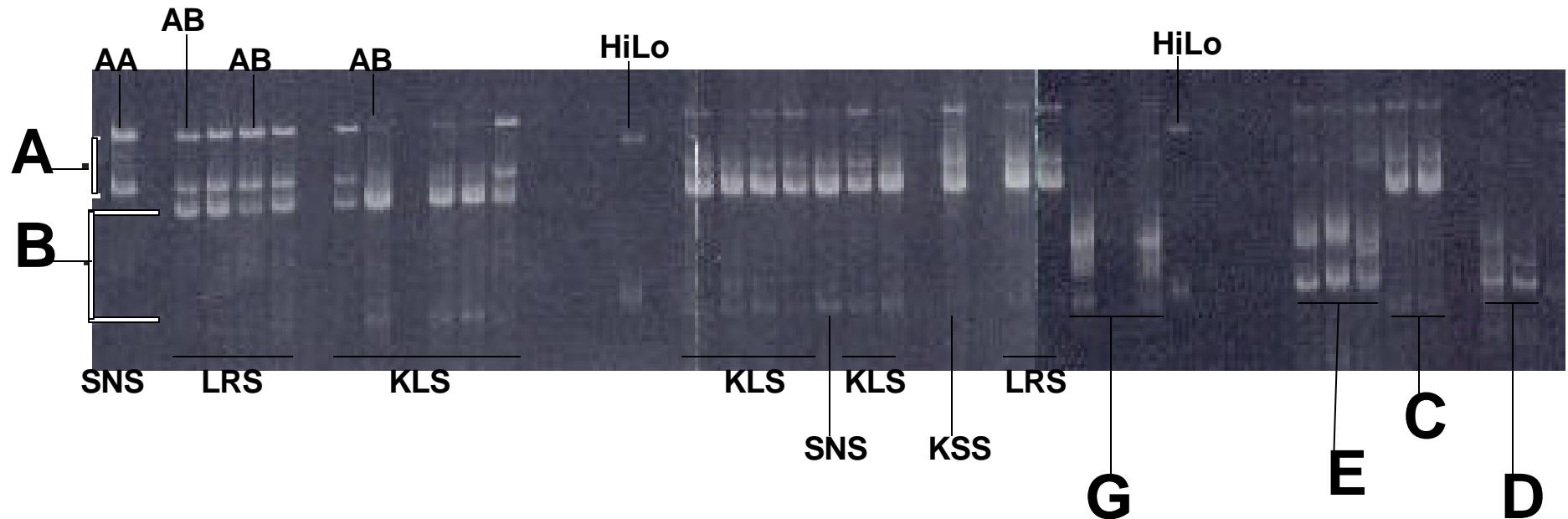


Figure 4: SSCP genotypes of Klamath basin and outgroup catostomids. Allele A is found in all suckers; Allele B is found in Klamath Basin suckers; Allele C is found in *C. occidentalis*; Allele D is found in *C. macrocheilus* (Columbia); Allele E is found in *X. texanus*; Allele F (not shown) is found in *C. sp.* (wall canyon); Allele G is found in *C. macrocheilus/ tslitcoosa* (Coastal)

Appendix 1: Klamath basin adults collected by BOR by location. Fish are listed by OS catalogue number, alpha, sample number, tag number, Genus, species, and sample status. Sample status lists the Loci number(s) each fish was used for. "Complete" means a fish was amplified in loci 4, 120, 142, 184. "No" means a sample was not available.

Catalog, Alpha, Number, Tag number, Genus species, Sample status

Sprague River:

OS 015895,A,200,BR00962,*Catostomus snyderi*, "4, 184, 142"
OS 015895,B,201,BR00966,*Catostomus snyderi*,complete
OS 015895,C,202,BR00968,*Catostomus snyderi*, "4, 120, 142"
OS 015895,D,203,BR00965,*Catostomus snyderi*, "4, 142"
OS 015895,E,204,BR00963,*Catostomus snyderi*,complete
OS 015895,F,205,BR00964,*Catostomus snyderi*,complete
OS 015895,G,206,BR00967,*Catostomus snyderi*, "4, 120, 142"
OS 015893,A,308,BR00413,*Catostomus snyderi*,complete
OS 015893,B,309,BR00414,*Catostomus snyderi*,complete
OS 015893,C,310,BR00415,*Catostomus snyderi*, "4, 142"
OS 015893,D,311,BR00416,*Catostomus snyderi*,complete
OS 015893,E,312,BR00418,*Catostomus snyderi*,complete
OS 015893,F,313,BR00419,*Catostomus snyderi*,no
OS 015893,G,314,BR00420,*Catostomus snyderi*,complete
OS 015893,J,315,BR00421,*Catostomus snyderi*, "4, 120, 184"
OS 015893,H,316,BR00422,*Catostomus snyderi*,no
OS 015893,I,317,BR00423,*Catostomus snyderi*,4
OS 015894,A,318,BR00424,*Catostomus snyderi*,complete
OS 015894,B,319,BR00425,*Catostomus snyderi*, "4, 120, 142"
OS 015894,C,320,BR00576,*Catostomus snyderi*,complete
OS 015894,D,321,BR00577,*Catostomus snyderi*,no
OS 015894,E,322,BR00579,*Catostomus snyderi*,complete
OS 015894,F,323,BR00580,*Catostomus snyderi*,no
OS 015894,G,324,BR00581,*Catostomus snyderi*,complete
OS 015894,H,325,BR00582,*Catostomus snyderi*,complete
OS 015894,I,326,BR00583,*Catostomus snyderi*,no
OS 015894,J,327,BR00584,*Catostomus snyderi*,complete
OS 015894,K,328,BR00585,*Catostomus snyderi*,no
OS 015894,L,329,BR00586,*Catostomus snyderi*,no
OS 015894,M,330,BR00587,*Catostomus snyderi*,complete
OS 015894,N,331,BR00588,*Catostomus snyderi*,no
OS 015894,O,332,BR00589,*Catostomus snyderi*,complete
OS 015894,P,333,BR00590,*Catostomus snyderi*,no
OS 015956,A,016,G01549,*Chasmistes brevirostris*,complete
OS 015956,B,018,G01601,*Chasmistes brevirostris*,complete
OS 015956,C,019,G01602,*Chasmistes brevirostris*,complete
OS 015924,C,023,G01606,*Chasmistes brevirostris*,no
OS 015957,A,027,BR00244,*Chasmistes brevirostris*,complete

OS 015957,B,030,BR00242,*Chasmistes brevirostris*,complete
OS 015923,B,017,G01600,*Deltistes luxatus*, "4, 120, 184"
OS 015923,A,020,G01603,*Deltistes luxatus*,complete
OS 015924,A,021,G01604,*Deltistes luxatus*,complete
OS 015924,B,022,G01605,*Deltistes luxatus*,complete
OS 015924,D,024,G01607,*Deltistes luxatus*,no
OS 015925,B,028,BR00240,*Deltistes luxatus*,no
OS 015925,A,029,BR00241,*Deltistes luxatus*,complete

Sycan River:

OS 015897,A,077,GF01630,*Catostomus snyderi*,complete
OS 015897,B,078,GF01629,*Catostomus snyderi*,complete
nonlethal,,083,NA,*Catostomus snyderi*,no
nonlethal,,084,NA,*Catostomus snyderi*,no
OS 015898,A,120,G01792,*Catostomus snyderi*,complete
OS 015898,B,121,G01790,*Catostomus snyderi*,complete
OS 015898,C,122,G01789,*Catostomus snyderi*,complete
OS 015898,D,123,G01788,*Catostomus snyderi*,complete
OS 015968,,124,G01787,*Catostomus snyderi*,complete

Upper Williamson River: Rocky Ford

OS 015903,A,025,GF01608,*Catostomus snyderi*,complete
OS 015903,B,026,BR00243,*Catostomus snyderi*,complete
OS 015903,C,033,BR00245,*Catostomus snyderi*,complete
OS 015903,D,034,BR00246,*Catostomus snyderi*,complete
OS 015904,A,049,G01616,*Catostomus snyderi*,complete
OS 015904,B,050,NA,*Catostomus snyderi*,no
OS 015900,A,051,G01617,*Catostomus snyderi*, "120, 184"
OS 015900,B,052,G01618,*Catostomus snyderi*,complete
OS 015900,C,053,G01619,*Catostomus snyderi*, "4, 120, 142"
OS 015900,D,054,G01620,*Catostomus snyderi*,142
OS 015900,E,055,G01621,*Catostomus snyderi*,complete
OS 015899,,056,GF01622,*Catostomus snyderi*,complete
OS 015900,F,057,G01623,*Catostomus snyderi*,complete
OS 015900,G,058,G01649,*Catostomus snyderi*, "4, 120, 142"
OS 015900,H,059,G01648,*Catostomus snyderi*,complete
OS 015900,I,060,G01647,*Catostomus snyderi*,complete
OS 015900,J,061,G01646,*Catostomus snyderi*, "4, 142"
OS 015901,A,062,G01645,*Catostomus snyderi*,1
OS 015901,B,063,G01644,*Catostomus snyderi*, "4, 120, 142"
OS 015901,C,064,G01643,*Catostomus snyderi*, "4, 184, 142"
OS 015901,D,065,G01642,*Catostomus snyderi*,complete
OS 015901,E,066,G01641,*Catostomus snyderi*, "4, 120, 142"
OS 015901,F,067,G01640,*Catostomus snyderi*,complete
OS 015901,G,068,G01639,*Catostomus snyderi*, "4, 142"
OS 015901,H,069,G01638,*Catostomus snyderi*,complete
OS 015901,I,070,G01637,*Catostomus snyderi*,complete
OS 015901,J,071,G01636,*Catostomus snyderi*,no

OS 015901,K,072,G01635,*Catostomus snyderi*,complete
OS 015901,L,073,G01632,*Catostomus snyderi*,complete
OS 015901,M,074,G01626,*Catostomus snyderi*,complete
OS 015901,N,075,GF01650,*Catostomus snyderi*,complete

Lower Williamson River:

OS 015896,,082,G01631,*Catostomus snyderi*,complete
OS 015965,A,031,G01612,*Chasmistes brevirostris*,complete
OS 015965,B,032,G01613,*Chasmistes brevirostris*,complete
OS 015959,A,035,GF01609,*Chasmistes brevirostris*,complete
OS 015959,B,036,GF01611,*Chasmistes brevirostris*,complete
OS 015965,C,037,GF01614,*Chasmistes brevirostris*,complete
OS 015959,C,038,G01610,*Chasmistes brevirostris*, "4, 184"
OS 015963,A,040,BR00247,*Chasmistes brevirostris*,120184142
OS 015963,B,041,BR00248,*Chasmistes brevirostris*,complete
OS 015963,C,042,BR00249,*Chasmistes brevirostris*,complete
OS 015963,D,043,BR00250,*Chasmistes brevirostris*,complete
OS 015964,A,044,01825,*Chasmistes brevirostris*,complete
OS 015964,B,045,?01826,*Chasmistes brevirostris*,complete
OS 015964,C,046,?01827,*Chasmistes brevirostris*,complete
OS 017480,,047,?01828,*Chasmistes brevirostris*,complete
OS 015930,,039,GF01615,*Deltistes luxatus*,complete
OS 015931,B,048,?01829,*Deltistes luxatus*,complete

Upper Klamath Lake:

OS 017490,,289,BR00708,*Catostomus rimiculus*,complete
OS 015954,A,001,GF01540,*Chasmistes brevirostris*,complete
OS 015954,B,004,GF01543,*Chasmistes brevirostris*, "4, 120"
OS 015960,,005,GF01544,*Chasmistes brevirostris*,complete
OS 015952,,013,G01546,*Chasmistes brevirostris*,complete
OS 015953,A,014,G01547,*Chasmistes brevirostris*,complete
OS 015953,B,015,G01548,*Chasmistes brevirostris*,complete
OS 015961,A,287,BR00704,*Chasmistes brevirostris*,complete
OS 015961,B,288,BR00706,*Chasmistes brevirostris*,complete
OS 015961,D,290,BR00711,*Chasmistes brevirostris*,complete
OS 017479,,295,BR00712,*Chasmistes brevirostris*,complete
OS 015961,F,296,BR00713,*Chasmistes brevirostris*, "4, 184, 142"
OS 015961,G,300,BR00715,*Chasmistes brevirostris*,complete
OS 015962,,307,BR00719,*Chasmistes brevirostris*, "4, 142"
OS 015922,,002,GF01541,*Deltistes luxatus*,complete
OS 015927,,003,GF01542,*Deltistes luxatus*,complete
OS 015926,,006,GF01545,*Deltistes luxatus*,complete
OS 015928,A,291,BR00702,*Deltistes luxatus*,4
OS 015928,B,292,BR00703,*Deltistes luxatus*,complete
OS 015928,C,293,BR00705,*Deltistes luxatus*,complete
OS 015928,D,294,BR00707,*Deltistes luxatus*,complete
OS 015928,F,297,BR00709,*Deltistes luxatus*,complete
OS 015928,G,298,BR00710,*Deltistes luxatus*,complete

OS 015928,H,299,BR00714,*Deltistes luxatus*,complete
OS 017491,,301,BR00716,*Deltistes luxatus*,complete
OS 015929,A,302,BR00720,*Deltistes luxatus*,"4, 184, 142"
OS 015929,B,303,BR00721,*Deltistes luxatus*,complete
OS 015929,C,304,BR00722,*Deltistes luxatus*,complete
OS 015929,D,305,BR00724,*Deltistes luxatus*,complete
OS 015929,E,306,BR00725,*Deltistes luxatus*,complete

Lost River:

nonlethal,,008,NA,*Chasmistes brevirostris*,complete
nonlethal,,007,NA,*Deltistes luxatus*,complete
nonlethal,,009,GF5595,*Deltistes luxatus*,complete
nonlethal,,010,NA,*Deltistes luxatus*,complete
nonlethal,,011,7F7B1C713F,*Deltistes luxatus*,no
OS 017478,,012,7F7B1F3269,*Deltistes luxatus*,"4, 142"

Gerber Reservoir:

OS 015892,A,079,NA,*Catostomus snyderi*,"4, 142"
OS 015892,B,080,GF01627,*Catostomus snyderi*,complete
nonlethal,,085,NA,*Catostomus snyderi*,no
OS 015943,A,187,BR00952,*Chasmistes brevirostris*,no
OS 015943,B,188,BR00953,*Chasmistes brevirostris*,"4, 142"
OS 015949,A,189,BR00954,*Chasmistes brevirostris*,complete
OS 015949,B,190,BR00955,*Chasmistes brevirostris*,no
OS 015949,C,191,BR00956,*Chasmistes brevirostris*,complete
OS 015949,D,192,BR00957,*Chasmistes brevirostris*,complete
OS 015949,E,193,BR00958,*Chasmistes brevirostris*,complete
OS 015949,F,194,BR00959,*Chasmistes brevirostris*,complete
`OS 015949,G,195,BR00960,*Chasmistes brevirostris*,complete
OS 015949,H,196,BR00961,*Chasmistes brevirostris*,complete
OS 015946,A,197,BR00969,*Chasmistes brevirostris*,complete
OS 015946,B,198,BR00970,*Chasmistes brevirostris*,complete
OS 015946,C,199,BR00971,*Chasmistes brevirostris*,complete
OS 015947,A,207,BR00972,*Chasmistes brevirostris*,complete
OS 015947,B,208,BR00973,*Chasmistes brevirostris*,complete
OS 015947,C,209,BR00974,*Chasmistes brevirostris*,"4, 184, 142"
OS 015947,D,210,BR00975,*Chasmistes brevirostris*,"4, 142"
OS 015947,E,211,GF01800,*Chasmistes brevirostris*,complete
OS 015947,F,212,GF01801,*Chasmistes brevirostris*,complete
OS 015947,G,213,GF01802,*Chasmistes brevirostris*,complete
OS 015947,H,214,GF01803,*Chasmistes brevirostris*,complete
OS 015947,I,215,GF01804,*Chasmistes brevirostris*,complete
OS 015947,J,216,GF01805,*Chasmistes brevirostris*,complete
OS 015948,A,217,GF01806,*Chasmistes brevirostris*,complete
OS 015948,B,218,GF01807,*Chasmistes brevirostris*,complete
OS 015944,A,231,BR00734,*Chasmistes brevirostris*,no
OS 015944,B,232,BR00735,*Chasmistes brevirostris*,no
OS 015944,C,233,BR00736,*Chasmistes brevirostris*,no

OS 015944,D,234,BR00737,*Chasmistes brevirostris*,no

OS 015944,E,235,BR00738,*Chasmistes brevirostris*,no

Clear Lake Reservoir:

OS 015966,A,086,GF01652,*Chasmistes brevirostris*,complete
OS 015966,B,087,GF01653,*Chasmistes brevirostris*,complete
OS 015966,C,088,GF01654,*Chasmistes brevirostris*,complete
OS 015966,D,089,GF01655,*Chasmistes brevirostris*,complete
OS 015966,E,090,GF01656,*Chasmistes brevirostris*,complete
OS 015966,F,091,GF01657,*Chasmistes brevirostris*,complete
OS 015966,G,092,GF01658,*Chasmistes brevirostris*,complete
OS 015966,H,093,G01659,*Chasmistes brevirostris*,complete
OS 015966,I,094,GF01660,*Chasmistes brevirostris*,complete
OS 015966,J,095,GF01661,*Chasmistes brevirostris*,complete
OS 015966,K,096,GF01662,*Chasmistes brevirostris*,complete
OS 015966,L,097,GF01663,*Chasmistes brevirostris*,complete
OS 015966,M,098,GF01664,*Chasmistes brevirostris*,complete
OS 015966,N,099,GF01665,*Chasmistes brevirostris*,complete
OS 015969,A,100,GF01667,*Chasmistes brevirostris*,complete
OS 015969,B,101,GF01668,*Chasmistes brevirostris*,complete
OS 015969,C,102,GF01669,*Chasmistes brevirostris*,complete
OS 015969,D,103,GF01670,*Chasmistes brevirostris*,complete
OS 015969,E,107,GF01673,*Chasmistes brevirostris*,complete
OS 015969,F,108,GF01674,*Chasmistes brevirostris*, "4, 120"
OS 015969,G,109,GF01676,*Chasmistes brevirostris*,complete
OS 015969,H,110,G01677,*Chasmistes brevirostris*,complete
OS 015969,I,111,GF01678,*Chasmistes brevirostris*,complete
OS 015969,J,112,GF01679,*Chasmistes brevirostris*,complete
OS 015969,K,113,GF01680,*Chasmistes brevirostris*,complete
OS 015967,A,114,GF01794,*Chasmistes brevirostris*,complete
OS 015967,B,115,GF01799,*Chasmistes brevirostris*,complete
OS 015967,C,116,GF01798,*Chasmistes brevirostris*,complete
OS 015967,D,117,GF01797,*Chasmistes brevirostris*,complete
OS 015967,E,118,GF01796,*Chasmistes brevirostris*, "4, 120, 184"
OS 015967,F,119,GF01795,*Chasmistes brevirostris*,complete
OS 015934,A,125,GR01775,*Chasmistes brevirostris*,complete
OS 015934,B,126,GF01776,*Chasmistes brevirostris*,complete
OS 015934,C,127,GF01777,*Chasmistes brevirostris*,complete
OS 015934,D,128,GF01778,*Chasmistes brevirostris*,complete
OS 015934,E,129,GF01779,*Chasmistes brevirostris*, "4, 120"
OS 015934,F,130,GF01780,*Chasmistes brevirostris*,complete
OS 015934,G,131,GF01781,*Chasmistes brevirostris*,complete
OS 015934,H,132,GF01782,*Chasmistes brevirostris*,complete
OS 015934,I,133,GF01783,*Chasmistes brevirostris*,complete
OS 015934,K,135,GF01785,*Chasmistes brevirostris*,complete
OS 015934,L,136,GF01786,*Chasmistes brevirostris*,complete
OS 015934,M,137,GF01875,*Chasmistes brevirostris*,no

OS 017477,,139,GF01877,*Chasmistes brevirostris*,142
OS 015934,N,163,BR01000,*Chasmistes brevirostris*,"4, 120, 184"
OS 015934,O,164,BR00999,*Chasmistes brevirostris*,complete
OS 015934,P,165,BR00998,*Chasmistes brevirostris*,complete
OS 015934,Q,166,BR00997,*Chasmistes brevirostris*,complete
OS 015934,R,167,BR00996,*Chasmistes brevirostris*,complete
OS 015934,S,168,BR00995,*Chasmistes brevirostris*,"4, 142"
OS 015934,T,169,BR00994,*Chasmistes brevirostris*,complete
OS 015934,U,170,BR00993,*Chasmistes brevirostris*,complete
OS 015934,V,171,BR00991,*Chasmistes brevirostris*,no
OS 015934,W,172,BR00990,*Chasmistes brevirostris*,complete
OS 015939,A,242,BR00746,*Chasmistes brevirostris*,complete
OS 015939,B,243,BR00752,*Chasmistes brevirostris*,complete
OS 015939,C,244,BR00753,*Chasmistes brevirostris*,"4, 120, 142"
OS 015939,D,245,BR00754,*Chasmistes brevirostris*,"4, 120"
OS 015939,E,246,BR00755,*Chasmistes brevirostris*,complete
OS 015937,A,250,BR00759,*Chasmistes brevirostris*,no
OS 015937,B,251,BR00760,*Chasmistes brevirostris*,complete
OS 015937,C,252,BR00761,*Chasmistes brevirostris*,complete
OS 015937,D,253,BR00762,*Chasmistes brevirostris*,complete
OS 015937,E,254,BR00763,*Chasmistes brevirostris*,complete
OS 015933,A,258,BR00767,*Chasmistes brevirostris*,complete
OS 015933,B,259,BR00768,*Chasmistes brevirostris*,"4, 142"
OS 015933,C,260,BR00769,*Chasmistes brevirostris*,complete
OS 015933,D,261,BR00770,*Chasmistes brevirostris*,4
OS 015933,E,262,BR00771,*Chasmistes brevirostris*,complete
OS 015936,A,263,BR00773,*Chasmistes brevirostris*,complete
OS 015936,B,264,BR00774,*Chasmistes brevirostris*,"4, 120"
OS 015936,C,265,GF07924,*Chasmistes brevirostris*,complete
OS 015936,D,268,GF01922,*Chasmistes brevirostris*,complete
OS 015938,,269,GF01921,*Chasmistes brevirostris*,complete
OS 017489,,134,GF01784,*Deltistes luxatus*,complete
OS 015916,A,138,GF01876,*Deltistes luxatus*,complete
OS 015916,C,140,GF01878,*Deltistes luxatus*,"4, 120, 142"
OS 015916,D,141,GF01879,*Deltistes luxatus*,complete
OS 015916,E,142,GF01880,*Deltistes luxatus*,"4, 120"
OS 015916,F,174,BR00989,*Deltistes luxatus*,4
OS 015916,G,175,BR00988,*Deltistes luxatus*,complete
OS 015916,H,176,BR00987,*Deltistes luxatus*,complete
OS 015916,I,177,BR00986,*Deltistes luxatus*,no
OS 015915,A,247,BR00756,*Deltistes luxatus*,complete
OS 015915,B,248,BR00757,*Deltistes luxatus*,complete
OS 015915,C,249,BR00758,*Deltistes luxatus*,"4, 120"
OS 015920,A,255,BR00764,*Deltistes luxatus*,complete
OS 015920,B,256,BR00765,*Deltistes luxatus*,"4, 120, 184"
OS 015920,C,257,BR00766,*Deltistes luxatus*,complete

OS 015917,,270,GF01923,i,"4, 120, 142"

Topsy Reservoir:

OS 015908,D,076,G01633,*Catostomus rimiculus*,complete
OS 015908,A,104,G01671,*Catostomus rimiculus*,complete
OS 015908,B,105,GF01672,*Catostomus rimiculus*,complete
OS 015908,C,106,G01675,*Catostomus rimiculus*,complete
OS 015906,A,236,BR00740,*Catostomus rimiculus*,no
OS 015906,B,237,BR00742,*Catostomus rimiculus*,complete
OS 015911,,266,BR00718.,*Catostomus rimiculus*, "4, 120, 184"
OS 015909,A,271,BR00311,*Catostomus rimiculus*,complete
OS 015909,B,272,BR00312,*Catostomus rimiculus*, "4, 120, 142"
OS 015909,C,273,BR00313,*Catostomus rimiculus*,complete
OS 015909,D,274,BR00314,*Catostomus rimiculus*, "4, 120, 142"
OS 015909,E,275,BR00315,*Catostomus rimiculus*,complete
OS 015909,F,276,BR00316,*Catostomus rimiculus*,complete
OS 015909,G,277,BR00317,*Catostomus rimiculus*,complete
OS 015909,H,278,BR00318,*Catostomus rimiculus*,complete
OS 015909,I,279,BR00319,*Catostomus rimiculus*,complete
OS 015909,J,280,BR00320,*Catostomus rimiculus*, "4, 120"
OS 015909,K,281,BR00321,*Catostomus rimiculus*, "4, 184, 142"
OS 015909,L,282,BR00322,*Catostomus rimiculus*,complete
OS 015909,M,283,BR00323,*Catostomus rimiculus*,complete
OS 015909,N,284,BR00324,*Catostomus rimiculus*,complete
OS 015909,O,285,BR00325,*Catostomus rimiculus*,complete
OS 015909,P,286,BR00701,*Catostomus rimiculus*,complete
OS 017476,A,267,BR00717.,*Catostomus snyderi*,complete
OS 017487,A,238,BR00743,*Chasmistes brevirostris*,complete
OS 017487,B,239,BR00741,*Chasmistes brevirostris*, "4, 120, 142"
OS 017487,C,240,BR00744,*Chasmistes brevirostris*,complete
OS 017487,D,241,BR00745,*Chasmistes brevirostris*,complete

Copco Reservoir:

OS 015905,,225,BR00728,*Catostomus rimiculus*,no
OS 015940,A,219,GF01808,*Chasmistes brevirostris*,complete
OS 015940,B,220,GF01809,*Chasmistes brevirostris*,complete
OS 015940,C,221,GF01810,*Chasmistes brevirostris*,complete
OS 015940,D,222,GF01811,*Chasmistes brevirostris*,complete
OS 015940,E,223,BR00727,*Chasmistes brevirostris*,complete
OS 015940,F,224,BR00726,*Chasmistes brevirostris*,no
OS 015940,G,226,BR00729,*Chasmistes brevirostris*,no
OS 015941,A,227,BR00730,*Chasmistes brevirostris*,no
OS 015941,B,228,BR00731,*Chasmistes brevirostris*,no
OS 015941,C,229,BR00732,*Chasmistes brevirostris*,no
OS 015941,D,230,BR00733,*Chasmistes brevirostris*,no

Rogue River:

OS 015913,A,143,GF01881,*Catostomus rimiculus*,complete
OS 015913,B,144,GF01882,*Catostomus rimiculus*,complete

OS 015913,C,145,GF01883,*Catostomus rimiculus*,complete
OS 015913,D,146,GF01884,*Catostomus rimiculus*,complete
OS 015913,E,147,GF01885,*Catostomus rimiculus*,complete
OS 015913,F,148,GF01886,*Catostomus rimiculus*,complete
OS 015913,G,149,GF01887,*Catostomus rimiculus*,complete
OS 015913,H,150,GF01888,*Catostomus rimiculus*, "4, 120, 184"
OS 015913,I,151,GF01889,*Catostomus rimiculus*, "4, 120, 185"
OS 015913,J,152,GF01892,*Catostomus rimiculus*, "4, 120, 186"
OS 015913,K,153,GF01893,*Catostomus rimiculus*,complete
OS 015913,L,154,GF01895,*Catostomus rimiculus*, "4, 120"
OS 015913,M,155,GF01890,*Catostomus rimiculus*,complete
OS 015913,N,156,GF01891,*Catostomus rimiculus*,complete
OS 015913,O,157,GF01894,*Catostomus rimiculus*,complete
OS 015913,P,158,GF01896,*Catostomus rimiculus*,complete
OS 015913,Q,159,GF01897,*Catostomus rimiculus*,complete
OS 015913,R,160,GF01899,*Catostomus rimiculus*,complete
OS 015913,S,161,GF01898,*Catostomus rimiculus*,complete
OS 015913,T,162,BR00976,*Catostomus rimiculus*,complete
OS 015913,U,173,BR00978,*Catostomus rimiculus*,complete
OS 015913,V,178,BR00979,*Catostomus rimiculus*,complete
OS 015913,W,179,BR00977,*Catostomus rimiculus*,complete
OS 015913,Z2,180,BR00980,*Catostomus rimiculus*,complete
OS 015913,X,181,BR00981,*Catostomus rimiculus*,complete
OS 015913,Y,182,BR00982,*Catostomus rimiculus*,no
OS 015913,Z,183,BR00983,*Catostomus rimiculus*,complete
OS 015913,Z1,184,BR00984,*Catostomus rimiculus*,complete
OS 015913,Z3,185,BR00985,*Catostomus rimiculus*,complete
OS 015913,Z4,186,BR00986,*Catostomus rimiculus*,complete

Hatchery:

OS 015950,,081,GF01651,*Chasmistes brevirostris*, "4, 120, 184"

Outgroups:

OS catalog number, OSU freezer number, Genus species, Location

OS 013656 A,1,*Catostomus macrochielus/tsiltcoosa*,Woahink Lake
OS 013656 B,2,*Catostomus macrochielus/tsiltcoosa*,Woahink Lake
OS 013656 C,3,*Catostomus macrochielus/tsiltcoosa*,Woahink Lake
OS 013908-1,61,*Catostomus wallcanyon*, "Wall Canyon Crk, Nv."
OS 013908-2,62,*Catostomus wallcanyon*, "Wall Canyon Crk, Nv."
OS 013908-3,63,*Catostomus wallcanyon*, "Wall Canyon Crk, Nv."
OS 015279,100,*Xyrauchen texanus*,Dexter Hatchery
OS 015279,101,*Xyrauchen texanus*,Dexter Hatchery
OS 015279,102,*Xyrauchen texanus*,Dexter Hatchery
OS 015623-1,224,*Catostomus occidentalis*,Larabee Crk.
OS 015623-2,225,*Catostomus occidentalis*,Larabee Crk.
OS 015623-3,226,*Catostomus occidentalis*,Larabee Crk.
OS 015885a,230,*Catostomus macrochielus/tsiltcoosa*,Hood R.
OS 015885b,231,*Catostomus macrochielus/tsiltcoosa*,Hood R.

Appendix 2: Each locus sequenced has an assigned locus number, statement about how complete the search was for a species marker, the original clone size (bp), Primers (+/-) designed for amplification, PCR product size (bp), the concentration of MgCl₂ in the PCR reaction and annealing temperature (°C) used. The clone sequence is reported with the primer sites underlined. The results of a Genbank blast “x” (amino acid homology) search and a blast “n” (nucleic acid homology) are reported.

Locus 001: PCR amplification in all Klamath species results in a strong single band. Sequence alignment showed that the first 70 bp were identical and then the sequences became confused as if there were another DNA type present. Possibly a tetraploid locus.

Clone size: 334 bp. PCR product size: 254 bp.

Primers: 1R+: 5' ACA ACA CTC CCA ATC CTT ATT CTT T 3'
1R-: 5' TCA CTG TAA ACT GAT AGC CCA AAC A 3'

Mg++ Concentration: 2mM Annealing Temp: 52 °C

Reverse Sequence:

AAGTATACTGTTCATTTAGAAGGGAAAGCAGATTAAAGATTACTGTAAACTG
ATAGCCCCAACAGGGACATCAACCCTGTACCCTCAGATTAAAAGTTTCATGC
GTTACTTGCTGAGCTACCCAGGCTCCTGAAAGCTTGACACCCCCGTGAAT
ATAAAGACAGTCCAGCCAATATGTGCACTTGACCTGTTGACTAAGTTAATT
CAAATCAAATCAAATCACTTATTGTCACACTACCATTTTAATTGCAATGA
ACTCTACAAAGAATAAGGATTGGGAGTGTGCTGCTTGGCAGGAAGG
GAATGCTAAAAAAAAGTGTGA

Blastx Search: No significant amino acid sequence similarity found in Genbank.

Blastn search: emb AL021808| HS24O18 Human DNA sequence from clone 24o18 on chromosome 6p21.31-22.2; zinc finger protein pseudogene. 60/70 bp (85% identity), 24% overall identity.

Locus 002: All Klamath catostomids species are identical at this locus.

Clone size: 619 bp. PCR Product size: 501 bp.

Primers: 2R+: 5' TTG TCG GAT GCA GTG AAA AGT CAG C 3'
2R-: 5' GAT TAAGTT GGG TAA CGC CAGGTT T 3'

Mg++ Concentration: 1mM Annealing Temp: 52 °C

forward Sequence:

GCTGACTTTCACTGCATCCGACAAAAACTGTCTTTGGTTGCCATGATATT
AATGCCTGTCATTATCCATGTTATCATTTCATAGTCATGGCAATTATTATTG
AGAAGTGAGCTGATTTCCCTCTGGTGAAGTTACATGGAGGCCACATAATT
GGAACATCCCATTACAAAAATATGGTTGTTCAAAAACCAAGTGAGCTGCT
TCCAGATAGACAGCTGCCTGTGGCAGTATCCTAATCATCATGGAACTTC
ATAAAATGACTGATTTAAACACTACCGTAAGCAGTAACTTGCCACACAAATAG
CTCTCCTCGTGTGCAGTGCACGGGAGACTCGCAGTTGATTGCGAGGTGCA
AGAGGATAGGATGTGATAATTCTCATAATTACTCAAGCTGACTTTCACT

GCATCCGACAAACAACGTGAGAATAGGCCAGAACATCAGTCATTTCAATGGAGA
NTTGGATGGGGACTGTGTTCCGACCATTGCCGATGTGGAATTT

Blastx search: [gb|AAD10590.1|](#) (U01771) unknown *Mycoplasma genitalium*
Identities = 20/22 (90%), Positives = 20/22 (90%)

Blastn search: [emb|X99258.1|](#) MTMOB *M.tuberculosis* genomic sequence
containing 4 ORF's Identities = 74/76 (97%)

Locus 003: PCR amplification in all Klamath species results in 3 product bands.
Attempts to optimize PCR reaction failed. Testing with this locus was discontinued.

Clone size: 580 bp. PCR Product size: 444 bp.

Primers: 3F+: 5' CCA AAG GTG CTT CAA CAA AGT ATT G 3'
3F-: 5' ATT TCA TTG CAG ATG TCA GGC AGA C 3'

Mg++ Concentration: 1mM Annealing Temp: 52 °C

Forward Sequence:

TGAAAATGGCTGTGTACCGACGCTCCCCATCCAACCTGATGGAGCTTGAGA
GGTCCTGCAAAGAAGAATGAGAGAAGCTGCCAAAAATAGGTGTGCCAAGC
TTGTAGCATCATACACAAGACTTGAGGCTGTAATTGGTGCAAAGGTGCTTC
AACAAAGTATTGAGCAAAGGCTGTGAATATTATGTACCTTTATATATAT
ATATATATAATGCATTGCAAAGATTCAAACAAACGTCTTCACGTTGTCA
TTATGGGTATTGTTGAGAATTAAATCCATTGTGGAATAAGGCTGTAACAT
GAAAAAAATTGGAAAAGTGAAGCACTGTGAATACTTCCGGATGCACTGGA
TATTGCAAAAGTATTGTTCTAGTGAAGGATCTAAACATTCTTA
AAATGTGATTGTTACTTGACAAACTGAAAAGTGGCATAAGANTGTAAGCT
TGTTTTAGAGAAATCTAATCAAATTATTGAACCTTAGACATNAAACAAAAAA
AAAACCAATCTGCCATGGGTAAACAACTGTCTGCCTGACATCTGCAATGAA
ATCCCCGGTACCGANCTCAAATCTC

Blastx search: [emb|CAB51372.1|](#) (AJ249085) transposase
Pleuronectes platessa Identities = 35/54 (64%), Positives = 40/54 (73%), Gaps = 1/54 (1%)

Blastn search: [gb|U51230.1|](#) DRU51230 *Danio rerio* transposon Tzf.49
Identities = 147/170 (86%), Gaps = 3/170 (1%)

Locus 004: Polymorphic locus, KSS are fixed for the B allele, all other species are homozygous AA or AB. Possible species marker.

Clone size: 540 bp. PCR Product: 454 bp.

Primers: 4F+: 5' GAGTCG CAA TCT GAC ACC TAC CTG T 3'
4F-: 5' CAC CAG CCT CTG AAA CCT GCC ATT T 3'

Mg++ Concentration: 1mM Annealing Temp: 52 °C

Forward Sequence: Bold nucleotide are the deleted bases.

ACCATAAAATGAATGAGGAGAGTCGCAATCTGACACCTACCTGTCGCAAC
ATTGTCAGCAGGGATTACCAACCAGGGCCAAAGGGGCCAGTGCCCAG

GGGCCCTTGAATCAGGGGCCCTTGACTGCCATCAACAACTTCAGTTGT
GTGAGTTGTGCTATATTCCAAGCCTCTGAAGTACACTGTGCTAATACAG
ATGCAACTCCATAAGAGTTGCAATTATGTCACACAGGATGCAAAATGTCA
CCTCAAAACTCATACACTAAAATGATAATTACTTATCAGAAAGAATTTC
ATATCAGAATTGTCAGATGAAAGAGTCTGTTCATTAACATAAATATTTCTC
ACTTAAGCTATAAGGCTGGAGAGTCTACATGGAGACTGTCGTATGTTGC
CATTGTGTATGAATATTCACATTGTGACAGTTGTAAATGGCAGGTTTCAGAG
GCTGGTGAGTGCTCACTGTTGTGGGCCAGGCAAAAAGAACAGTG
TTGTGTGCCAATCCCATCATG

Blastx search: [pir|S31521](#) collagen COLF1 - freshwater sponge *Ephydatia muelleri* >gi|9300|emb|CAA49472| (X69818) Emf1 alpha *Ephydatia muelleri* Identities = 16/27 (59%), Positives = 20/27 (73%), Gaps = 1/27 (3%)

Blastn search: [gb|AF222686.1|AF222686](#) *Homo sapiens* chromosome X PAC K6166 map Xp11.23, Identities = 20/20 (100%)

Locus 006: PCR difficulties, amplified in all Klamath species but was never a single band.

Clone size: 478 bp. PCR Product size: 415 bp.

Primers: 6F+: 5' TAG GCA GCT TTT TAG AGT CGT ATG T 3'

6F-: 5' GCT ATC TTC ACA ATA ATG ACA GTT C 3'

Mg++ Concentration: 2-3mM Annealing Temp: 48-49 °C

Forward Sequence:

ATGGCCAAAAAACACTTTTAATATTAGGCAGCTTTAGAGTCGTATGTCAC
CAAGATTAACACTTTCAATAAATATGACTTTCAAGTATTTCACATGAATG
CAGCTATGAAGTGCATGAGGGGACAAATGAATCCTGCAACATGTTGCAT
GCATTATGAGAGATGAAAGTCATTATAATGAAGAAATATGTGTTAAGAAA
GAAATGTGCAAACCTCTTAATACAGCTCTAAAGGCCAAATTCAACTTTTA
TTATTGGACTGCTGTTGGAGTCATATCTCATTAAATTATCAACTTTAAAAAA
AAAAGGGCATTAGTTAACATTAAAGTAGCTATGAAGTACATGAGAGTT
CAAAATGCATCATTACAACATGTGTCATGCGTTATAAGAGATGAACGTCA
TTATTGTGAAGATAGCAGTGTATTGTAAGAAATGTCAAACATCT

Blastx search: [emb|CAB12544.1|](#) (Z99107) alternate gene name: yfnJ; similar to cytochrome P450 /NADPH-cytochrome P450 reductase *Bacillus subtilis* >gi|2116974|dbj|BAA20123.1| (D87979) YfnJ *Bacillus subtilis* Identities = 19/61 (31%), Positives = 32/61 (52%), Gaps = 4/61 (6%)

Blastn search: [gb|AC006001.2|AC006001](#) *Homo sapiens* clone DJ0756H11, Identities = 21/21 (100%)

Locus 008: All Klamath catostomids species are identical at this locus. The primers for this locus amplify a region larger than the clone sequence.

Clone size: 341bp. PCR Product size: 543 bp

Primers: 008F+: 5'GCTGTCGTCGATAGAGGGTGGAGGAGTGG3'

008F-: 5' TGATTTGAAAAACAGTGTATCTTAGGAAG3'
Mg++ Concentration: 2mM Annealing Temp: 49 °C

Forward sequence:

CTGTCGTCCGATAGAGGGTGGAGGAGTGGCAGAGGAACAAGCTACGGCAT
ATTGGAGAACTGGCGAGTAACAGTTGTCTTCTGTTTAGTTATTATAA
AATATTATTATGTTGAAAAGCCGGTCTCACCTCCTTCCATTAACCTC
TTTACAGTGAGCCAACCATTGGACAATTCTGTGATTCACCATATAATT
TGTTGCACGTTACAAGCTAACAGATACATTGTTGCTAGTCAGATTGGTTTAAT
TTGTGGAGAGAAATAATTCAACAGAACACTAGACTCCTAACAGATAACACTGTTT
TCAAATCA

Blastx search: gi|3414805 (AF060882) NADH dehydrogenase subunit 8
Crithidia oncopelti 14/36 (38% identity)

Blastn search: gb|AC006971.2|AC006971 *Homo sapiens* PAC clone RP4-
791C19 from 7p11.2-q11.21 Identities = 29/31 (93%)

Locus 009: All Klamath catostomids species are identical at this locus.

Clone size: 528 bp. PCR Product size: 375bp.

Primers: 9F+: 5' TCC TGT AAA TTT GCT TAT TGG 3'
9F-: 5' ACA TAT ATG CCT GGT GAA AAA 3'

Mg++ Concentration: 2mM Annealing Temp: 49-51 °C

Forward sequence:

TTGCATGCCTGCAGGTCGACTCTAGAGATCAATTAAATGAGCTACCAAGAG
ACAAAAAACTTATGTTTGAGCTACATCAGACATCAGTATAACTGAAATATAC
TCAAATCATTGGAATTAAATTCCCTGAAATTGTCTATTGGGACGTTTATTG
TTTTGTCTATAAGTTTCTGCTTACAATATTACTAACATTACTTATTAGGT
AACACTCACATTGCTGAAAAGTGCATGTCAATTAACTTATTACAACGTGAGGGTT
GAAGCTTTATAACATCTACCAACTTGGTTAATTAAAGTCTTTAGGAAAA
ATAGGAGGTTAATTCTAAATTATAGCCTACAATTATTTCATTGGGTCTCT
GGGACTTAACACAGGTCTATATGTCAATACATGAGTGTAGAAATATGCGGGG
GTATTGGGACATGTCCCCCTCACTTTGATAAAACAAAGTCCGTCCACTT
CACTTTTCAACCAGGCATATATGTTTGTATATTCAGTGTATATGTGT
AAATAAAGATGTCAAAATTCTGAAACTGGATCCCCGGTACCGAACCTNAAT
TCGTAATCATGTCATA

Blastx search: sp|P40584|YIW0 Yeast Hypothetical 13.0 KD Protein in HYR 3'
region *Saccharomyces cerevisiae* 22/74 (29% identity), 31/74 (41% similar)

Blastn search: gb|M80338|CORDTXRAA *Corynebacterium diphtheriae*
diphtheria toxin repressor (dtxR) gene, Identities = 39/41 (95%)

Locus 010: PCR difficulties, amplified in all Klamath species but was never a strong single band. Clone size: 488 bp. PCR Product size: 421 bp.

Primers: 10R+: 5' TTT ACA CTA AAC ACA ACT AAT AA 3'
10R-: 5' AAG GCT AAT ATA CAT CAA TAG AT 3'

Mg++ Concentration: 8mM Annealing Temp: 57 °C

Reverse Sequence:

ATTTTACACTAAACACAACTAATAAATATAAATTAAATAAACTAGAAACACCC
TTATTTATACT?ATT?ATAGATAAAAAGTACTATATAAAAATAGTAATAAA
CAGCCACATTGAACAATATATTTGCTACAAAAAGATGTCAAATGTCAGTGAA
ACATTGTAACATCAATTATCTGACTTCCC~~AAA~~ACTAATTGAGGCAGGAC
AGTTTAGAACATTTCACATGTGTTTTGATTGTTGGGTCAAGCTCACTCAGCT
GTGTGTGCATAACAGCGTAACATTAAAACAGTGAAGTGTACATTCA
CCACTATTATTATGAAAATGAGCAAGTGTCAAGTTAGCAGCATTGCTA
GTTTAGTTAACCTCAATGATAGTTATTATCTATTGATGTATATTAGCCTTC
ATACTGCTGTATCACCTCTCAAATACTCCTCAAAACAGCTAATAGCCACTG
TTCTG

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|AC005039 CLONE NH0512E16 *Homo sapiens* 23/23
(100% identity) overall 4.7% identical

Locus 012: Amplified in all Klamath species, appeared to be a single band on agarose gel, but sequencing results were always confusing. They imply that another DNA was present in the sample. Clone size: 456 bp. PCR Product size: 456 bp.

Primers: 12F+: 5' TGA AAC TTC ACA GAC GCA TTC TGG A 3'
12F-: 5' AGG TCA TAC AGG GGT AAT TCT TAT T 3'

Mg++ Concentration: 2mM Annealing Temp: 51 °C

TGAAACTTCACAGACGCATTCTGGAGACACCTGAGACTTATTACATCTG
TGAAAAGGGCATAATAGGTCTCCTTAACGGTAACAAGATGATATTAAATGA
GCTTGACTCGGCACAGGTTTATCTAATGATTGCCAGACCTACTTACCTGCT
ACTAAAATGTNACGTCCTGACCATGCACATTAAAAGTGAACAGCATCATAT
CGAAATGCTTGATACTTAAAAGTAATGTAGTTGGGCTGACATGACTGTTAG
ACTCATGAGCTTTAAATTGGAAAATTGATAAAGCCCCAATGTTGAAGGTC
CCCATAGATTGAAACTTGGTCTGAACAAAGATGAGTGAATGATTATGAAGA
CATACAATGCAAATTCTGCACTTAATGATGCCGTGAATGAGTAATAAAATGT
NAATTGCCGTAAATAAGAATTACCCCTGTATGACCT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: emb|AL023878|HS618F1 sequence from clone 618f1
chromososome Xq25 *Homo sapiens*, Identities = 24/25 (96%)

Locus 013: All Klamath catostomids species are identical at this locus.

Clone size: 499 bp. PCR Product size: 320 bp.

Primers: 13F+: 5' AAT GTC ATT TAC TGG ACC ATA CT 3'
13F-: 5' TTA TTT AAA ATA CGA TTG TGA AT 3'

Mg++ Concentration: 1-2mM Annealing Temp: 53 °C

Forward Sequence:

AGTTAAGATACGGTATGATTCTGGACTGTACAGAGTATGTTGTCAATACGTG
ATACAGTAAGAATTGTGTAACAAATACAGTAATGTACACTGTAATGTCATT
CTGGACCATACTGTACCGTACTCTAACAGTACAGTATGAATGAACTAACAGT
TTACCTATTCAGATTGGGGTGAGAAATTACATTACTTTTGTCATTAGCAG
ACACTAATGGTAACCAATTGACTAAAAATGAGAGACTCCAAATTGTCA
CAGTCCTGTATCTGCATTACAGTGGTAAGCCTTGTATTGTAACACATG
GTGGACATTGACATTGATTGTGTACCTGCCACAGTTGCCTCGGCTGCTC
ACTGGGGTTATAAATACAATTATTAAATTACTTATTAAAACAATTCA
CAATCGTATTAAATTACACAATGATGACTCATAGACATTAGACATT
ACAGTTTATCTCTGTTAATGCCT

Blastx search: pir|S64826 probable membrane protein YLR004c
Saccharomyces cerevisiae 17/32 (53% identity), Positives = 21/32 (65%)

Blastn search: emb|X91894.1|MBMTBAGEN *M.barkeri* mtbA gene
Identities = 28/30 (93%)

Locus 019: SNS, LRS, KLS are identical at this locus, KSS never produced clean sequences.

Clone size: 526 bp. PCR Product size: 421 bp.

Primers: 19R+: 5' CGT CAA GCT AAC ACA GTG ATG TCC T 3'
19F-: 5' GTG GAG CGG CAA TGA AAG CAA GAG A 3'

Mg++ Concentration: 2mM Annealing Temp: 47 °C

Forward sequence:

AATTCTTACTGTTCCATAAAGCAAATTCGAAGGGCAACAGGAGAGGTGG
AGCGGCAATGAAAGCAAGAGAGAACACTCTCCATTACAAGTGTTCGTACA
GTAATTCTGTCCTCCCAGAGGAAAATTACAGCTCTTCCTGGCTCAGGAC
ACTTATGAAACTTTCCACTGCACGTTACAGTTGACTCGACTTGACACTGC
TCACTTTACTGTCTGAGCTGCTTCCAATGCAGGCTGATCGTCATAGTT
GTGCAGCCTCTACTGCTGTGACATCATTGTAACCGTAACACAAACACAAAC
AAAGGAGCAATGGAGGATTTCGAAGGAATGGTGTCTGATTCTCGGCGTG
TGGCTGTTTTCACAGCAAGACAAGACAAGACAATCTTGTTCAAAAGAGC
TGATGGCATCATACACCAAAAGCGCAGACAAGGACATCACTGTGTTAGCTTG
ACGACACATGGGTTAAGTTAACCTGCCATCTAGCTTATAGACGCTCCCTGA
CCAATCAGT

Blastx search: ref|NP_003150.1|PSTK9| serine/threonine kinase 9 protein *Homo sapiens*, Identities = 40/125 (32%), Positives = 56/125 (44%), Gaps = 21/125 (16%)

Blastn search: gb|AF037352.1|AF037352 *Mus musculus* T cell receptor gamma locus, TCR gamma 1 and gamma 3 gene clusters Identities = 21/21 (100%)

Locus 021: PCR amplification in all Klamath species but weakly, never sequenced in each species. Clone size: 462 bp. PCR Product size: 414bp.

Primers: 21F+: 5' TAA CAC AGC AGA ATG TCA GGG TAG C 3'

21F-: 5' ATA AAT AAA AGG TTT GGT AAC ACT T 3'

Mg++ Concentration: 1mM Annealing Temp: 49 °C

Forward Sequence:

CAAATATGTACTGTATAATGGGAGCTGGCACTTGTGAGTAACACAGCAGAAT
GTCAGGGTAGCCTACTGGAACATTCCGTAGTAACATGTCGACTTCCTGTGT
GAAAGATATGACTGCAGTCTTCATCTCATTTAGTTAATGCATAAGGTATCATT
AACAAACAATGGACAATATTTATTGCAGCATTAAAGCTTGTTACTGTT
AGCTAATAAAATTACAATCGTTAGTTAATGTTAGTTATATTGTTAG
TTTATAACTAATGTTAACATATACCACATTTAAAAATGTATTAGTTAATG
TTGTAATTAAACATTAACAAACATTAATAATGCTTAAAAGTATTGTTAATTG
TAGGTCACTGTTAACTAATGTTGATAACCAATGTTACAAATGGCACATGACT
GTAAAGTGTACCAACCTTTATTTATTTCAGAAAT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|U31357|NCU31357 *Notropis chrysoleucas* ependymin (sh)
precursor gene,
32/36 (88% Identity)

Locus 026: PCR amplification in all Klamath species but never as a single product. Not sequenced in each species. Clone size: 595 bp. PCR Product size: 493 bp.

Primers: 26R+: 5' GTC TCC CCA TGT CTA AGG AAG TGA C 3'

26F-: 5' CCA AAA ATG AAA AAT CTG TTA TCT T 3'

Mg++ Concentration: 1-4mM Annealing Temp: 48-53 °C

Reverse Sequence:

TTTACTCACATGACACATCCTTTGCTCATCTGTCTGTCTGGTCTCCCCA
TGTCTAAGGAAGTGACATAATAACCCAGCCATTCTGTCCCACAGATGCTGTC
TGCAACCGCCACGCTGGCTCTTATTGGCCTGCTGATGGGCCACAGTGGC
TCAACTCAGACCTCCATGTGCCGGAGGTTGTTGGGTTCAACTCATTCAAAAA
GATGCAAAGCCTAAGCAACAGTACACAACAAACAGAGAACAGTCAAACATT
AGGCCAGAAAACACACTCCTCAGATGGCCTAAAGAGATTGTTCCCCAAA
AAGGACAATTCTGTACATTACTCACTCTCATGTTGTCCTAAAGCTCTATGA
CTTCTTATATCGTGGAACACAAAAAGATATTAAGCTGTATGTTAATCTCAGT
CACCATTCACTTCAGAGCATATGAAAGTAAC TGACCCCTGTTAAAGTGCATA
AAAAGAAAATCACATTGATTGGAATAACTTAGGGTGAGTTAAAGATAACA
GATTTTCATTTGGCAAAACTGGTCCCTTAAAAAAAGTGGTGTACTTA
CAAAAAACACACTTATACAGTGG

Blastx search: gb|AAD38587.1|AF145612_1 (AF145612) BcDNA.GH02976

Drosophila melanogaster, Identities = 15/40 (37%), Positives = 22/40 (54%),
Gaps = 1/40 (2%)

Blastn search: dbj|AB009335|AB009335 *Carassius auratus* mRNA for brain aromatase

35/39 (89% Identity)

Locus 039: All Klamath catostomids species are identical at this locus.

Clone size: 459 bp. PCR Product size: 338 bp.

Primers: 39F+: 5' TCG ACT TCC TGT GTG AAA GAT 3'
39F-: 5' TAC AGT CAT GTG CCA TTT GTT 3'

Mg++ Concentration: 2mM Annealing Temp: 56°C

Forward Sequence:

AAATATGTACTGTATAATGGGAGCTGGCACTTGTGAGTAACACAGCAGAATG
TCAGGGTAGCCTACTGGAACATTCCGTAGTAACATGTCGACTTCCTGTGTGA
AAGATATGACTGCAGTCTTCATCTCATTAGTTAATGCATAAGGTATCATTAA
CAAACAATGGACAATATATTATTGCAGCATTTTAACGCTTGTACTGTTA
GCTAATAAAAATTACAATCGTTCATAGTTAGTTAATGTTAGTTATATTGTTAGT
TTATAACTAATGTTAACATATACCACTACATTAAAAATGTATTAGTTAATGTT
TGTAATTAACATTAACAAACATTAATAATGCTTAAAAGTATTGTTAATTGTT
AGGTCATGTTACTAATGTTGATAACCAATGTTACAAATGGCACATGACTG
TAAAGTGTACCAACCTTATTATTTCAGAAA

Blastx: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|AC004831|AC004831 *Homo sapiens* PAC clone DJ0538P11
from 7p11.2-p13,
23/24 (95% Identity)

Locus 054: All Klamath catostomids species are identical at this locus.

Clone size: 446 bp. PCR Product size: 286bp.

Primers: 54F+: 5' TAG GGA GAC ATG CTT TTG TTT 3'
54F-: 5' GCT ACT GGC ACC CAC ATC TTA 3'

Mg++ Concentration: 2mM Annealing Temp: 56°C

Forward sequence:

TAGGGAGACATGCTTTGTTGACATTGATAGTGGAAAGTTATCCACAAACAGT
GGGACCACATGCTTGTCAGCGAGGACTGACACAGCAAGTGGAAAGACCAC
ACTCAGGTCACTAAACACATCCAAGGGTTAGGCCTATGTAATTAGCTAC
TTTCAGTTGTCTAACACATCCAAGGGTTAGGCCTATGTAATTAGCTAC
CTCTCCTGCTAATTGCCATAATGTGACTGCTGCAATAACTATGTCACTTAAA
AATGAATCATCTGCTAAACATTAATTAAAGATGTGGGTGCCAGTAGC

Blastx search: pir||H64168 hypothetical protein HI1195 - *Haemophilus influenzae*
Identities = 13/39 (33%), Positives = 22/39 (56%)

Blastn search: gb|L14561|HUMCAATPX *Homo sapiens* plasma membrane
calcium ATPase isoform 1 (ATP2B1) gene, 20/20 (100% Identity)

Locus 061: All Klamath catostomids species are identical at this locus.

Clone size: 375 bp. PCR Product size: 215bp.

Primers: 61F+: 5' ACC TGT CGT ACA TCG GAG AGG 3'

61F-: 5' AAC CAT TAG GGA AAT ACT CCA 3'

Mg++ Concentration: 1-3mM

Annealing Temp: 48°C

Forward Sequence:

AAAAAGTACTTGAAAAATGTTAGACCCTGAATCAACTTGTCTATTATTGCCA
ATACACATTAAAACCATTAGGGAAATACTCCAAAGTCCACAGTACGCATTA
CAAGGAAAAGTCATGCAACTGGTTCAAGACAAGCAATAGTAGGCAAGAC
TAGGGCTGGTGACAGCTCTGTGATTGAATGAACAAACCGTAAGAGAAAA
GAAAACATTATTACAATTCTAGGAAAAACACGGAAACAATGCGGAAGCTGG
TGGTAAGTATAACAGCATCTAACCAACCATGCCGTATGCACCCTCTCCGAT
GTACGACAGGTTACTGTAGCGCGCCCCGACATCGAAAGCTTGACCACGGA
CCATCTCGGCACC

Blastx search: sp|P26696|ERK2_xenla mitogen-activated protein kinase (myelin xp42 protein kinase) *Xenopus laevis*, Identities = 28/30 (93%), Positives = 29/30 (96%)

Blastn search: dbj|D87264.1|D87264S1 *Mus musculus* DNA for ERK2, exon 1 75/88 (85% Identity)

Locus 063: All Klamath catostomids species are identical at this locus.

Clone size: 338bp. PCR Product size: 278 bp.

Primers: 63F+: 5' TTT CAA AGG GAG GGT CTC TGA TTT A 3'

63F-: 5' CCA AAG GGC ACT TTG AAG ATT GCT T 3'

Mg++ Concentration: 1-4mM

Annealing Temp: 53°C

Forward sequence:

TACAGTCTAAACTGGATAAATCGCACTCCAAAGGGCACTTGAAGATTGCTT
CAAACAGAATTCCATTAAAATTGAATTGTGAGTTCCGAATGACCATTATTTT
TGAGCCCCAAACCTTTAGCCCTCCAAAGTTCTCACAGTGGATGGTTAAGTC
AGTACACGTATGCTAATAAAATAGGCCTAAAAAGCACGCAATTGTAGCAC
CTTTGACTTTGCGCTTTATTATCATGCAGTAACACTTATGTAGTATTGA
TGTAGAGTATGTCGTAATTAATCAGAGACCCTCCTTGAAATCCAACAC
AAGTGGCCAGTAGAGAAGCATATTATGAT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: emb|Z48717|CET10B9 *Caenorhabditis elegans* cosmid T10B9 23/24 (95% Identity)

Locus 067: All Klamath catostomids are identical at this locus.

Clone size: 427 bp. PCR Product size: 305 bp.

Primers: 67F+: 5' TTG CAA TAA GTT ATG TGC TGT 3'

67F-: 5' TCT GCT AGA GAT GGA AAA ATA 3'

Mg++ Concentration: 0.5-2mM

Annealing Temp: 56°C

Forward Sequence:

TGCTCTTCTCAATGGTATTGGTCTATAATTGACATATGTAGCACTTATGA
ATTAGTTGTTGAAGCTGATTGCAATAAGTTATGTGCTGTATCTGTTCTTT

GCATCACAGATGATTCAAGGGAGGAGAGAGGATGTTGAGGATAGTACTAGAG
TGGAAAGATTAGGAACGTAGAACAGGCCAGGCCAGAGCAAAACTCAAAG
AATACCCTCTCACCAGCTTGGACTACAGGGAAAGTGGTTGCTAGTGTGAAA
ATAAAATGGTCTGGCTAACAGCCCCGGATGTCCTTCAATGCTGGAAACGCC
CTGCATCTGATGATAAATTACAATTAAAAGTGGATTGTTGTGCATATTTT
CCATCTCTAGCAGATGAGTGAGGTCTGACACAACACAAATCCAGTTAAGCC
CTATAGTCTAA

Blastx search: gi|2745729 (AF016192) potassium channel *Rattus norvegicus*
17/49 (34% Identity), Positives = 27/49 (54%), Gaps = 2/49 (4%)

Blastn search: gb|AC006067.3|AC006067 *Arabidopsis thaliana* chromosome
II section 83 of 255 of the complete sequence Identities = 22/22 (100%)

Locus 076: All Klamath catostomid species are identical at this locus.

Clone size: 260bp. PCR Product size: 190 bp.

Primers: 76F+: 5' TGA AGA GTA AAA TAC AAC CAG 3'

76F-: 5' TGT GGA CTC GTT TGA AAG ATA 3'

Mg++ Concentration: 1-3mM Annealing Temp: 48⁰C

Forward sequence:

TCTTCATCTGATTATCTTGTGTGGACTCGTTGAAAGATAATCACAGACTC
TAAATAATGATATGTGATATTATGTTCCGTTAATTGCATGAAGTTATTAG
CAAAAATGCGGCATATAAGCGACATCAACACATACTTGTCAAAAGACATATATT
AGCTGTTACTCGCTATATTTCATCTGTGAGTAAAACAAATAATCTGGTTGTA
TTTACTCTTCAAGAGATTCCAACACATATGACACATGACTATT

Blastx search: dbj|BAA20793.1| (AB002333) KIAA0335 *Homo sapiens*
Identities = 12/38 (31%), Positives = 21/38 (54%)

Blastn search: gb|AC005880|AC005880 *Homo sapiens* chromosome 10 clone
CIT987SK-1143A11 map 10q25 20/20 (100% Identity)

Locus 079: All Klamath catostomid species are identical at this locus.

Clone size: 328bp. PCR Product size: 249 bp.

Primers: 79R+: 5' CGT CCT CTT TCT GTC TTG TGA 3'

79R-: 5' TCC CAT TAA ATT GAT TGG AAT 3'

Mg++ Concentration: 0.5-2mM Annealing Temp: 56⁰C

Reverse Sequence:

TCAGCTCTGGACAGACTTTTGTGCTAAACCTCGTGTGCTTCTGCTTCTCC
AAGCCGCTCTTCTGTCTGTGAGTTCAAGAAAATCTGAGGAAGTCGAA
ATTGCGCGCGCAAATCCTTAAAGGGGTACGCACGAAATTTCGACATCCTC
CGATCTTAATGAAATTATACCATAGAAAGAGGAGGGCTGGAGATGAACAGA
AATACAAGTTCAGCTTCCAAAGTCTGTCTAGAGCTGAGATATGCACATCCC
AAAAATTAAATGTGGGTAGTCCCAATTCCAATCAATTAAATGGGACAAATTGT
ACATGGGATGTTCCA

Blastx search: gb|AAD35361.1|AE001709_7 (AE001709)
pyruvate,orthophosphate dikinase *Thermotoga maritima* Identities = 16/40
(40%), Positives = 26/40 (65%)

Blastn search: gb|AF086906.1|AF086906 *Arabidopsis thaliana* root gravitropism control protein (PIN2) mRNA, Identities = 21/21 (100%)

Locus 081: All Klamath catostomid species are identical at this locus.

Clone size: 402 bp. PCR Product size: 261 bp.

Primers: 081F+: 5' ACT ATG GGA AAC GTC TCT GTA 3'
081F-: 5' TGG GGA ACA CCG CAA GGT AAC 3'

Mg++ Concentration: 1mM Annealing Temp: 56°C

Forward sequence:

TCATTGAAATGATGTGACTGTGGTACAGTGGTTCTGTTCCAAACATTCTTA
GGAATTCTTCTCACTATGGGAAACGTCTGTACGAAGGGCAGACCAATTAC
AGCTTGTATAAGAGGCCGCCTCCTCCCTAAAATACCTGCTGTAATGCTCTG
AAAAGACACTTTCCCTCAGCACCAAGAACCTCACTATTAACTCTTGAGGC
GTCACCCAGCATAGTCACAGTATTACTCTGCTATTATTAAGCTGCTTGTCTG
CTTTCACTAAGCAGTGTGTTGTAACTGAAAATTGTTGGAGGTACCTTGC
GGTGTCCCCATGGCTTCGACAAAAGTTATCATAGACAAGAATAAAGATGCC
TCGTCTCACCTGTGTCGGCTGCCTGTGGATTAA

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|AF047826|AF047826 *Homo sapiens* cadherin-7 (CDH7)
mRNA, Identities = 20/20 (100%)

Locus 082: All Klamath catostomid species are identical for this locus.

Clone size: 316 bp. PCR Product size: 262 bp.

Primers: 82F+: 5' TCC CAG TTG CAA CAT TTT GAG 3'
82F-: 5' ACT CTG TGC TCC CTC CCT CTC 3'

Mg++ Concentration: 2-3mM Annealing Temp: 53°C

Forward Sequence:

ACGGTGCATGCCTGCAGGTCGACTCTAGAGGATCCCATTAAATTGTTA
ATATTGCAAATAGCTTCCCAGTTGCAACATTTGAGCATTGGAGAGCAAT
AGGAAAAAAATAAGGAAATAAAAAAAAGTGAAAAGAAAGGAGAAACTTTTGCC
AACCTGCACAGTACAATCAGAGGTGTGCAGTCTGCTGGTTGCTTGACA
GCCTCTTCCCTGCCTCACTCTCCCTGACGTTCTGTCTCAATGAAAAC
CTCATGCATGTGTGCTCACTGCCGTCCCACTCTGAAAGAGAGAGCAAGAGA
GGGAGGGAGCACAGAGTAGGAGAGAGAATCACATCATCAGAG

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|M58040|RATTRFR Rat transferrin receptor mRNA, 3'
end. Identities = 34/34 (100%)

Locus 083: Klamath species are identical but differ from the clone sequence.

Clone size: 513 bp. PCR Product size: 451 bp.
Primers: 83F+: 5' CAT TTG TTC CCC ATT CTG ATG 3'
83F-: 5' CCA CAA CAT TAA AAC CAC CTG 3'
Mg++ Concentration: 2-4mM Annealing Temp: 53⁰C
Reverse Sequence:
AAAACAATGGAATATATAAATACATATATACATACATACACACACAAA
CTTATCAGCCACAACATTAAAACCACCTGCCTAATATTGTGTAGGTTCCCCTT
GTGCCACCAAAACAGCGCCAACCTGCATCTCAGAATAGCATTCTTCTTACCT
CAATTGTACAGAGTGGTTATCTGAGTTACGGTAGACTTGTCAAGTCGA
AGTCTGGCCATTCTCTTTGACCTCTCATCAACAAGACATTCCGTCCAC
AGAACTGATGCTCACTGGATGTTTTGTTGGCACCATTGGAGTAAAC
TCCTAGAGACTGTTGTGGGTGAAAATCCCAGGAGCAGTTACAGAAATACTC
AAACCAGCCCATCTGGCACCAACAATCATAAATGAGATTATCTAATCAGCCA
ATTGTGTGGCAGCAGTGCAGTGCATAAAATCATGCAGATGCGGTTGAAGA-
CTTCAGTTAATGTTCACATCAGCCATCAGAATGGGAACAAATGT

Blastx search: gi|1022896 (U37772) WI-1 adhesin *Ajellomyces dermatitidis*
Identities = 14/45 (31%), Positives = 22/45 (48%), Gaps = 1/45 (2%)

Blastn search: emb|AJ009633.1|DRAJ9633 Danio rerio spermine synthase gene
Identities = 70/81 (86%)

Locus 088: All Klamath catostomid species are identical for this locus.

Clone size: 538 bp PCR Product size: 482 bp
Primers: 88F +: 5'TGTGAAACTGTATGCCAACATTAA3'
88F- : 5'AGCTAGCTTGCTTCCCTCTGTGA3'
Mg++ Concentration: 1-4mM Annealing Temp: 53⁰C
Forward Sequence:
AATGGACTGTGAAACTGTATGCCAACATTAAAGACTGGTAGGCAGAGCCA
GGGCTAAAAACAGGTCCATGAAGAGCTAATTGGGCAAACACTGAGCAG
AGAGATTGTGACTCCACAAGAGGGCACTGCACTCATTGTTGTATGTGATG
AAATATTCTTAATTCTCTCCATTCTTCAATTCTCTCCCTCCCTCACA
CACACACACATTGACACCCACACCCACATAGGAACTGAATCAAACAGACAG
ACTGGTTGTCTACAGCATGTGGGAGGCCCTTCAACCAAGAGAACATCTCC
AGGTTAATCTAGCTCTAACTCTTCTATCTTATCTGTCAATTCAAACATCCCAC
TTAAGGGATTCTTGTAAACAGTCCAAATTCTAATTCTAAACAGA
AATTATCTTGCAGAATTGCATTCAACTAAATATTCAAGTGTATGTACACAGA
GAGGGAAGCAAGCTAGCTTGGCATTGCCTGCTAGAGTTAACACTGCATAG
CAACAGTCAGTGTATTA

Blastx search: emb|CAA17249.1| (AL021899) hypothetical protein Rv2035
Mycobacterium tuberculosis Identities = 11/28 (39%), Positives = 19/28 (67%)

Blastn search: gb|AC004866|AC004866 *Homo sapiens* PAC clone DJ0728H09
from 7q11.23-q21.1, complete sequence Identities = 22/22 (100%)

Locus 090: PCR difficulties, KSS amplified very weakly at different temperature but other species never amplified. Clone size: 517 bp PCR Product size: 455 bp

Primers: 90F+: 5'AGAACGGAAGAGTAAGACGAGGTCA3'
90F- : 5'CTCTACAGAGCCCTACCATCAATGA3'

Mg++ Concentration: 1-4mM Annealing Temp: 48-56°C

Forward Sequence:

CAAACCAAGAACTGTGAGCTGCAGCACAGCAGAGTCAGCTGATGCCCTG
GA~~T~~CCAGCAGAACGGAAAGAGTAAGACGAGGTCATTAACCTCTGTGACTAA
AATGCACTCAGATGGCTCCCTGGTCTGCAGGCTGATGACAAGCGTCTGTGT
TCTTTCAACAACTTGTGAGCTGTCCACCCCCACCTCTGAAACCAACAC
CACCTATCCCCATCTCAGGAATGTAACCCAAGCCCCTCTCCTCTCCAATCAC
TTTGAGAACAGCCCACTGAAGGTGTAAGACAGCTGTTGCTGTACTCTTCCT
TTTGTGCGTTATGTATTTCTCCACAACTGCCCTTGTATTCCCTGTGTTCA
CTCTCTCGCTGTGGCACTGCACAATAATTCATGATGCACGGACCTGAGTG
CAGTACAGTCGACTTGACTGACGGGAATCGAGCGCGTGTCTATAATGG
TGAAAGTGCTTGGTAATGGCTATGTCATTGATGGTAGGGCTCTGTAGAGG
GA

Blastx search: gi|203227| pir|B44173 calcitonin gene-related peptide alpha precursor *Rattus norvegicus* (M11597) alpha-type calcitonin gene-related peptide
Identities = 27/89 (30%), Positives = 37/89 (41%)

Blastn search: gi|3777528|gb|AF060228|AF060228 *Homo sapiens* retinoic acid receptor responder (tazarotene induced) Identities = 24/25 (96%)

Locus 094: All Klamath catostomids species are identical for this locus.

Clone size: 473 bp PCR Product size: 372 bp

Primers: 94F+ : 5'TATGACCTCACTATTATTCC3'
94F- : 5'CATTAAATCGTAGAAAACATT3'

Mg++ Concentration: 1-4mM Annealing Temp: 50°C

Forward Sequence:

CTTGTGTTATCACTGTGGTAAATATTATGAAATTTACATTATGACC
TCACTATTATCCCATCGTAAATTGTTATGTTGTTATGTTTAATTT
CTGTAACCTTGTAATTGTGCTGCCTGTCTGGCTAGGACGCTTGGA
AAATAGATTTTTAATCTCAATGAGTTTCTGGTAAATTAAAGGTAAAAAA
AAATTTAAATACAATTGACTCAAGTTAAACTATTAAAGGCAATGCCACC
AAATACTGACAAAGTGTATGAACTTCTGACCCACTGAGAATGTGATGAAA
GAAATAAAGCTGAAATAAATCATTCTCTACTATTATTCTGACATTCACAT
TCTAAAATAAAGCAAAGATGGGAATGTTTCTACGATTAAATGTCAGGAAT
TGTAAAAACTGAGTTTAATGTATTGGCTAAGGTGGTGTAAAGG

Blastx search: gi|1513298 (U66526) AbcA *Dictyostelium discoideum*
Identities = 23/64 (35%), Positives = 32/64 (49%), Gaps = 5/64 (7%)

Blastn search: gb|AF017232|AF017232 *Salvelinus namaycush* transposon Tsn1-3 transposase (Tsn1) pseudogene, Identities = 147/156 (94%)

Locus 107: All Klamath catostomids species are identical for this locus.

Clone size: 593 bp PCR Product size: 543 bp

Primers: 107R+ : 5'ACCCCATACTCAACACTCAATCA 3'
107R- : 5'TATCTTTGGCCTGCTGCTTCAG 3'

Mg++ Concentration: 1-2mM Annealing Temp: 55°C

Reverse Sequence:

TTGTTGTGGGAGAGCCCTTGAGGCTATCTTTGGCCTGCTGCTTCAGCTTCA
AACCCTTGCGAGTTGTATGCTCTACTGGTCAGCTCTAGCTTCTATGTTCT
GATTGCCAGTCTAAACCTTAGGCAGCTTTCAACTAAATTAAAGTCCCT
GCAGCTAAAACTGTCCGTCTTATTGGTTAGTAATGGTCTCTATGCCATTG
CTCACTGTGATTGGTAGATATTGATGCCAAGGCAGAATTGGTGTATTGTT
TTGGCGTGTCCCCAAGGC~~GGT~~GTTTGTTCTGATTGGATGGCCTAGGTAA
GGGCATGCACTGCCCTGGAGCATGTACACAGACCTTACACAACCCCCAAT
CGAATGACTGTATGAATAACATTACAAATCAAAAAAAGGATAGAAACACAAT
CATTGACAACTTTCTGATTAAACATTGGAACATTACGTGATAAAGAA
CTTCGTACAAAACTCCTCGAAATACAGCTGTCTCAATGTCTGATTGTAT
CTAGCATCGAAAACCTCTATATAGCATGATTGAGTGTGAGTATGGGTCTT
GCTTCATGCAGAAAGTCA

Blastx search: emb|CAB38989.1| (AL034558) predicted using hexExon;
MAL3P2.2 (PFC0165w), Hypothetical protein, len: 1676 aa *Plasmodium falciparum* Identities = 23/85 (27%), Positives = 44/85 (51%), Gaps = 1/85 (1%),
gb|AAD31534.1|AF148447_1 (AF148447) ubiquitin C-terminal hydrolase UCH37
Mus musculus Identities = 29/80 (36%), Positives = 42/80 (52%), Gaps = 6/80 (7%)

Blastn search: emb|Z97195|HS106H8 Human DNA sequence from PAC 106H8 on chromosome 1q24. Identities = 21/21 (100%)

Locus 117: All Klamath catostomids species are identical for this locus. This locus is polymorphic with a transversion of C to T at position 123 of the KSS sequence. This polymorphism was found in 2 of 150 (1.3%) individuals tested.

Clone size: 580 bp PCR Product size: 498 bp

Primers: 117R+ : 5'TTATTCGACTTATTGAACCATAGA3'
117R- : 5'TTAAACAAATCCCGCAACCAAAACA3'

Mg++ Concentration: 1-4mM Annealing Temp: 50°C

Reverse Sequence:

CGCTTTCTGCGGCCGTTGAACGGTGATTGCAAATTAAACTATGAT
GCTGGTACATACACTTAATTAAACAAATCCCGCAACCAAAACAATCCTATC
GTTTGCCCCGCGAAAGGATGTGCAGCTTGAACGACCTGTTCAAGGTAAAG
GCATTTTCAACTAAAAGAGTACCACAAACTAACAGTAGATGAACGCAACT
TTCAGTTATTTGAAGTAGTTCAAATGACCTGAAATGGTTTATGAACC
ATTATATTGATTAGCTAAAAATGACCTCTTTCTAAAATAAAAAAACCATCA
GTGAGGCACTTACAATGGAAGTGAATAAAAGCCTACTCACTGTTCAAAGC
ATAGCCACACACGTAAACAATGTGTAAACTAAAATTCCCGTGATTAT

AGCCAATATTACTCGCTGTCATTACGATGGAATGCCAACAAACCCTAAAAC
CACTTTTGCTTTGTTAAACCATTATAAAGCAACGTTCCACAACGTT
ATTTGAATTAAAGCAATATCTATGGTCAATAAGTCGAAATAATGTTATG
GT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: emb|Z82194|HS272J12 Human DNA sequence from PAC 272J12
on chromosome 22q12-qter contains ESTs Identities = 21/21 (100%)

Locus 119: All Klamath catostomids species are identical for this locus. This locus is polymorphic with a transversion of G to A at position 361 of the clone sequence. This polymorphism was found in 2 of 150 (1.3%) individuals tested.

Clone size: 524 bp PCR Product size: 460 bp

Primers: 119R+ : 5'TCTTCAGCTTCACTACTGGTA3'
119R- : 5'GTTCAATCTGCCGTAAACTG3'

Mg++ Concentration: 1-4 mM Annealing Temp: 51°C

Reverse Sequence:

TTGGGATACCATCTTATCAGTTCAATCTGCCGTAAACTGAGGGTGTGCAGT
AAAAAGTTTCACAAGATTTGTTGACAATGAACTTAAGTACTGTGTGTTT
GTAAATTAAAAATTGGAACAGAAAAAGGCATTCTGGTAAAACTGTATAGTTA
GAATGCATTCCATATTGAACAGTACTTCTGATATACAACATACCTGAATCC
AAAACATTATGGATTTGTCAGCTGGACGTGGTAGGCTGAATTATGTCTTAACACC
GAGATAAAGCGTTAAGGGTGCTTGCACCTGAGCTGAATTATGTCTTAACACC
TGTCTCTAATTCCATTGAGCATGGGGAGAGCAGCATATAAATGGTCATATCA
CAGCCAGACGAGAGAGAGAATGACACGAGTGACCAGTGACTGGTGTCTT
ATGTTATTGTGAAGCTGAAGACTCAGAGAAGTTATGTTGACAAGTACCAAGT
AGTGAAGCTGAAGACTCAGAAGCTGAGTTAAGTTGACAAAGTTGTGTTAC
ACT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|AC007436.1|AC007436 *Homo sapiens* 12p13 BAC RPCI11-
Identities = 19/19 (100%)

Locus 120: Polymorphic locus resulting from a 4 bp deletion at clone position 300. All KSS have the 4 bp deletion, most individuals of the 3 remain species do not have the deletion. This locus is a possible species marker. Other single bases appear to distinguish SNS (A at 319), LRS (T at 319, T at 341), KSS (G at 319).

Clone size: 511 bp PCR Product size: 430 bp

Primers: 120R+ : 5'GCGATTGTCTCTGTCTTCTTGA3'
120R- : 5'TACTGCCTTATGTGCTTTCTGAG3'

Mg++ Concentration: 2 mM Annealing Temp: 51°C

Reverse Sequence: Bold nucleotides are the site of the 4 bp deletion

CTTTTGAGACTTCAGCGATTGTCTGTCTTCTTGACAAAACGCAGCA
GGTTCTCAGCTGGTAATTCTGTACTCAAGTGATTGGTCCGACCTGTGAG
GAAGGCCTCCTGGCATGCTACTGCTTTGCTCACGAGACATACTGTGA
AAGAGGAAGTCATTCATTCACTCGGGTACACTTTGTACATTTACGTACA
TTTACCCATACATTATGAATGAGAGTCAAGTAGGGGGATTATAGTGGATT
GATATCTAACGCCCTACAGAGTGAATATTGAAATATTCCCCTATGAAAATTCT
CTCATCGTTCTCACCCCTCATATCATCACAGATGTTTACTTTTCCTCT
GCAGAACACAAAATTACGATTTAAGCTCTGTTAGTCCTCACATGCTAGTG
AATGGGTGCCAGAACCTTGAAGCTCAGAAAAGCACATAAAGGCAGTAAAAA
TAATTAATTAAGCAATATGATAGGTGTGGATGAGAAC

Blastx search: sp|P11883|DHAP_RAT aldehyde dehydrogenase, dimeric nadp-preferring (aldh class 3), tumor-associated aldehyde dehydrogenase (htc aldh) >gi|91936|pir|A30149 aldehyde dehydrogenase (NADP+) (EC1.2.1.4) 3, tumor-associated - rat>gi|202833|gb|AAA40713.1| (J03637) aldehyde dehydrogenase *Rattus norvegicus*, Identities = 17/37 (45%), Positives = 26/37 (69%)

Blastn search: gb|AF132287.1|AF132287 Cyprinus carpio pituitary specific transcription factor Pit-1 (PIT-1) gene, Identities = 23/24 (95%)

Locus 126: All Klamath catostomids species are identical for this locus. This locus does exhibit 4 single base pair positions that are heterozygous found in LRS and KSS.

Clone size: 453 bp PCR Product size: 306 bp

Primers: 126F + : 5'TCAGCGTGGCAGTTTGGAAAT3'

126F- : 5'GTTGAGGGAGAGGTTGTGCTC3'

Mg++ Concentration: 1 mM Annealing Temp: 51°C

Forward Sequence:

CGTCCATCCTTATCTCAAGCTTCTGTCCTAAGAAGTTAAAGACACTTGTG
ATGATTGGAAATTGTTAACGCCACAATGATATTAGTAGGAACATAATCAGCG
TGGCAGTTTGGAAATGATGGCAGGATTATCCGGCATTGGCCCTTGCCGT
ATACAGCACATTCTCCAGTAAGTGCCAGTCAGAGCTGGCACTAAGTGAA
GGCCAAATACCTAACGAAAGTATTACTCAAGTGAAAAAACATCTGTTTGTG
CTGAAATGTGAAAATGCTTTCGGTACTTAAACTCTCAACCTTGATTGTGTGA
CTAGGCAATATTCCATTACTGAAATAAAAACAGTGAATAAGTAGCAAGA
GAAATATCACCTGGTGTCAAGGAGCACAAACCTCTCCCTAACGTCAAGTAAAA
CCAAGGAGCTTGTGGACTTGAGGAAGAAAGACAGA

Blastx search: gi|1127551 (U18939) orf2 *Batrachocottus baikalensis* Identities = 16/26 (61%), Positives = 19/26 (72%)

Blastn search: dbj|D86995|D86995 Human (gene 1) DNA for phosphatase 2C motif, Identities = 20/20 (100%)

Locus 139: PCR amplifications were very weak and each species tended to work better in different Mg++, sequence was confusing, implying that more than one type of DNA was present.

Clone size: 518 bp PCR Product size: 431 bp

Primers: 139R+ : 5'AATTGAAACATAAAACATTCCACTA3'

139R- : 5'AGCCTACACTATTGGTTGAGTCAGT3'

Mg++ Concentration: 1,2,4 mM Annealing Temp: 56°C

Reverse Sequence:

TCTTGAAACACTCCCCTAGTCTTCCATTGACCACCCCAAACATAGCCTACAC
TATTGGTTGAGTCAGTGTGGCTGGTCAGTAGAGCTGGCGATATGGCTACA
AAAATTGTATCTGATATTTTCAGCTAATTGACGTTATTAATATATATCTTG
ATAATTATGTTCCCTCTGATTGACATAAAAGTTATTAGTTTGTTCAGAG
GAACCATTATCTCTGCTAAATAGACATAGAGCTCTGTTTCTTGAGTCAG
CAAAAAGTGCATTGAAAGTTGCTTAAAGCGAAATTGCTGTTTCTAAGAA
ATAGACATAGCTTAAACATCTCGTGCATGACCTGTTTCAGCATAGTG
CCTAAATTCAAGTCCAAACTCTGAAAATATAGTGGATGTTTATGTTCAATT
TAAAGTTCAAGTCCAAACTCTGAAAATATAGTGGATGTTTATGTTCAATT
ATTATTATTATTATTATGTTATTACAGTTGATTCT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|L10232|CHKMSLT *Gallus gallus* microsatellite DNA sequence.
Identities = 20/20 (100%)

Locus 140: Sequence over laps with Locus 139. No testing was done.

Clone size: 518 bp PCR Product size: bp

Primers: 140R+ : 5'ATTGAAACATAAAACATTCCACTAT3'

140R- : 5'AGCCTACACTATTGGTTGAGTCAGT3'

Mg++ Concentration: 1-4mM Annealing Temp: 50.4°C

Reverse Sequence:

TTGAACACTCCCCTAGTCTTCCATTGACCACCCCAAACATAGCCTACACTA
TTGGTTGAGTCAGTGTGGCTGGTCAGTAGAGCTGGCGATATGGCTACAA
AATTGTATCTGATATTTTCAGCTAATTGACGTTATTAATATATCTTGAT
AATTATGTTCCCTCTGATTGACATAAAAGTTATTAGTTTGTATCAGAGGA
ACCATTATCTCTGCTAAATAGACATAGAGCTCTGTTTCTTGAGTCAGCAA
AAAGTGCAATTGAAAGTTGCTTAAAGCGAAATTGCTGTTTCTAAGAAATA
GACATAGCTTAACACATCTCGTGCACATGACCTGTTTCAGGATAGTGCC
TAAATTCAAGTCCGAACACTCTGAAAATATAGTGGATGTTTATGTTCAATT
AAGTTCAAGTCCGAACACTCTGAAAATATAGTGGATGTTTATGTTCAATT
TATTATTATTATTATGTTATTACAGTTGATTCT

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: emb|Y15279.1|GFP450III *Gibberella fujikuroi* P450III gene
Identities = 23/24 (95%)

Locus 142: Tetraploid locus containing 11 alleles and 8 genotypes throughout the Klamath basin. Possibly species maker locus for 21 SNS.

Clone size: 513 bp PCR Product size: 361 bp

Primers: 142R+ : 5'TCTCATTATGGAAAAGAGC3'
142R- : 5'TTTGTCTAGTCGGTAATCTT3'

Mg++ Concentration: 1-4mM Annealing Temp: 50°C

Reverse Sequence:

TGTAAATAAACTGTCATTTAAAAAGAACATTATTAACTTTTTTTTT
TTTGTCTAGTCGGTAATCTTAGGAAAGGAGGCTGCAGAACTTCTAAACCG
GAAAAAAAAGTTTACAGTTTGCTCAGAACTAACCGAAATTAAAAACA
TTTGTTTAAGCTTTCTAGCATTCAAATGTCAACTGGCTCAGACTTCT
GATGCAGGATTACAGGTTATGGCTAAACTACAGTTGTTATTATGGATA
TTGGAAGCCTAATGATTAAAGGGAGTATTCACCCAAACATTTACTCGCCCT
CATGTTGTTCAAACATATATGCCATATTCTCCAGTTAACACAAAATGAGA
TGTTAGCAGAATTCCATGCTGCTCTTCCATATAATGAGAGTAAATAAGG
GACCAGGGCCGGATTCAATTCTTAAGAAACAACTTCATCTACCATT
TTTCTTATTATTTCAAACCTTAAGAATATTTAGAA

Blastx search: No significant amino acid sequence similarity found in Genbank.

Blastn search: gb|AC003954|AC003954 *Homo sapiens* chromosome 5, Pac clone 162o17 (LBNL H147) Identities = 21/21 (100%)

Locus 146: All Klamath catostomids species are identical for this locus.

Clone size: 528 bp PCR Product size: 381 bp

Primers: 146R+ : 5'AGACATGGTGACGGCTTGATTCAT3'
146R- : 5'ATACTGACCTATGGGATTTGGAG3'

Mg++ Concentration: 1mM Annealing Temp: 53°C

Reverse Sequence:

TGGGTTCTACCCAAACCTATCAAATCACTCTGAAGACATTGATTAACC
GCTGGAGTCATATGGACCACTTTACTGACCTATGGATTTGGAGCTT
CAAAATTGGCAACCATTCACTTGCATTTATGGACCAACAGAGTTGAAATA
TTCTTCTAAATCTTATTGTGTTCTGCTGAAGAAAGAAAGTTATATACATCTG
GGATGGCATAAGTGTAGTAAATGATGATAATTTGGGTGAACATCCCTT
AAGTGTATAGAAGCACCACAAATGACAAGATTCCTCAAACTATTGGTG
AAATTAGTTGACCTGAAAGAAAACCTTACAGTAGAAAAACAGTTGAAAAA
ATTCCAGCTACAGTTTATTATAGCATTGTGACTGTGCTGTCCCGTAAATG
TTGTTTGCCCATGAAATCAAGCCGTACCATGTCTAGGTTAGTAAGTCTGC
AGCACTGGCTTAATAAGACATCTCAGTGTACTCTGAGAGCAAAGACATT
CA

Blastx search: gi|3790700 (AF099914) No definition line found

Caenorhabditis elegans Identities = 12/33 (36%), Positives = 23/33 (69%)

Blastn search: gb|U70479|DRU70479 Danio rerio no arches (nar)
mRNA, Identities = 26/26 (100%)

Locus 166: PCR amplifications were very weak and involved several other bands. No further testing was done. Clone size: 529 bp PCR Product size: 475 bp

Primers: 166R+ : 5'GAAACGTTATCTTTTGTATTGTT3'
166R- : 5'TTTTATGCTGCCTTATGTGCTTT3'

Mg++ Concentration: 1-4 mM Annealing Temp: 40°C

Reverse Sequence:

AAACCACGTAAAGTCGTGGATTACTTTATGCTGCCCTTATGTGCTTTGG
AGCTTCAAAGTTCTGCCACCATTCACTTGCAATTATGGACCTACAGAGCT
GAGATATTCTACTAAAAATCTCATTGTGTCCCGCAGAAGAAATAAGTCATA
CACATCTGGATGGCAGGAGGGTGAGTAATGATGAAAGAATTACATTTG
GGGTGAATTATTCTTTTAATAAGGTGGTGTAACCAAAATGCTGGTNGCTGT
TTTGTATACGTGAAGTAAAAAAAAAGGTGAAATTGAAATTGTGAAAGGC
ACATTGTTCAAGTTAAATGGACAAACTCTAATTGTGATTGCATTCAAAGAA
AAATATGTTAACGCTTTAAACTTGATGTTACACCATTGACATTAAAG
TCATTAAGTAAATTGTTGTAGGAAATGCTATAAAAGTGAAATTTCAAA
CAAAATAACAAAAAGATAACGTTCAACAAACTGACACGTGGTTTAAACC
A

Blastx search: emb|CAA19433| (AL023816) T05G11.6 *Caenorhabditis elegans*
Identities = 18/73 (24%), Positives = 31/73 (41%)

Blastn search: emb|X60419|CIGH *C.idellus* gene encoding growth hormone
Identities = 41/46 (89%)

Locus 169 : PCR amplifications were very weak and involved several other bands. No further testing was done. Clone size: 504 bp PCR Product size: 413 bp

Primers: 169R+ : 5'TCAGAGTTGGGTGTTCCCTCTAA3'
169R- : 5'ATATACTCCAGAATAAGGGCAAGAT3'

Mg++ Concentration: 2-3mM Annealing Temp: 40°C

Reverse Sequence:

CTGAATGACTCCACTGCTGCCACAGTGCTCATTAGAATGGTAGGGTAGTC
AGAGTTGGGTGTTCCCTCTAAAGTCCACAATCATCTCCATAGTTTGAGCA
TGTAAAGCATGTTAACGATGTTAACGCTCCAGGTTGCTCCAGGTTGTTGAC
TGCACCAGACAGCCAGCTGTTAACCTCCCTCTGTATGCAGACTCATCGTT
ATCTCGGATGAGGCCAATGACAGTGGTGTATCTGAAAAATTAGGAGCTT
GACAGAGGTGTCCTGGTGTAGCAGGCATTGGTAGAGGAAAGAAGACTAG
TGGGGAGAGCACAACCTCCCCTGGGGGACACCAGTTGATTGTACAGGT
GCTGGAATTGAATNTCCCCTGTCTCACAGCTGCTGCCTGTAATCCACTGAC
AGATAGATGTTGGGACAGAGAGTTGATCTGCCATTCTGGAGTATATC
TGGGATTATGGTGTGAAAGCCAACTGAAGTCCACATAAA

Blastx search: gi|1127550 (U18939) orf1 *Batrachocottus baikalensis*
Identities = 53/111 (47%), Positives = 65/111 (57%)

Blastn search: gb|U18939.1|BBU18939 *Batrachocottus baikalensis* orf1 and
orf2 genes, Identities = 33/36 (91%)

Locus 172: PCR amplified very well in all Klamath species, samples are
purified, but never sequenced. Clone size: 464 bp PCR Product size:
400 bp

Primers: 172R+ : 5'TGCTCCTATCGACACAGCCCTCAA3'
172R- : 5'CAGTCCCCCTTCGTCAGGTGGT3'

Mg++ Concentration: 1-4mM Annealing Temp: 50°C

Reverse Sequence:

CTCCGCCATACCTTACAGTCCCCCTTCGTCAGGTGGTGCCTGTTGGA
GACATCATCGGACGACAATCATCACAATGGCGGATGGCAGGTGAATCAGGA
GGGTGTTGAGTAGGTGGTTATTGGACGGAGGGCTCAATTGATGAGGTT
CAAAGTGGTCTCTGTTGGTCCCCTTTCCGGGGCTCCGTCGGAAACC
TACGGACATGGCTTCGAGCTGGCTGTTGATGTCATCTCGTTGAG
CCTCCTGTACAGGATGAAGGCCAGGCCTAGTGTCAAGGAAGTGGCTGATTCC
CTTAGAGATAACAGCGCAGTGTCCGCTGTCAGGCTCGAACACAG
GTGAGGTGGTCGAGATGGCAATCGAGACAAGGCTGGAGGGCTGTGTCG
ATAGGAGCAATGTCAATTAACTCGGAGCCCTCTTCGATCCATAGTTCCA
GTTCTG

Blastx search: gi|1078547|pir||S55100 hypothetical protein YMR218c yeast
Saccharomyces cerevisiae and gi|854470|emb|CAA89933| (Z49809) unknown
Saccharomyces cerevisiae Identities = 20/48 (41%), Positives = 26/48 (53%),
Gaps = 1/48 (2%)

Blastn search: gb|L78073.1|L78073 *Homo sapiens* clone 16513.1 HLA-G
cell surface glycoprotein (MHC-G)gene, Identities = 22/23 (95%)

Locus 176 : Amplified well in all Klamath species, one individual from each
species run on SSCP gel and showed no variation between them.

Clone size: 467 bp PCR Product size: 401 bp

Primers: 176F+: 5' TAACATCAACTCCCCTTCAT 3'
176F-: 5' TTAACCTCAACCCAAGTGGAA 3'

Mg++ Concentration: 3 mM Annealing Temp: 50°C

Forward Sequence:

ATGATTTCATATCATAATAACCATCACATCACGTTAATAACATCAACTCCCC
TTTCATGTTCTCTCTTTTGAGAAAGCTTGAGTAATGTTATTTACGACCC
TATCAGAGCAGAGCTGATAAGCAAGATTCTTATCTTATTTGAAAAATGTAT
GGCATTCAAGTTATTTAAGCACAAGATTGGTATGAAACATAGAATTGAAAG
TAACATCATACAAACATTTGATGCAATAACAATCTGCCATATTGAAAGC
ATAAGAACAAATATGCATGTATTGCAAGATTCTGCAAATACTGCCATATGA
GATACTGTACTATACTGACTGTACTCAGTGCAGGCACAGGCAATTGTGTAG

GAGTGTAGCCGGAAAATTACAACCTGGCACACTCTGCCAATGGGACTTTTC
CACTTGGGTTGAGGTTAACGAAGCTTAAGAAACTTTATTTTATAA

Blastx search: gi|1074769|pir||A64029 hypothetical protein HI1418 -
Haemophilus influenzae (strain Rd KW20) >gi|1574254 (U32821) *H. influenzae*
predicted coding region HI1418 [*Haemophilus influenzae* Rd] Identities = 11/23
(47%), Positives = 15/23 (64%)

Blastn search: emb|Z69718.1|CEW06D11 *Caenorhabditis elegans* cosmid
W06D11, Identities = 24/24 (100%)

Locus 181: PCR amplifications were weak and included many other band. No

further testing done. Clone size: 525 bp PCR Product size: 346 bp

Primers: 181F+ : 5'TTCACACTAATGCTAATCCAG3'

181F- : 5'AATATAAAAGGAGACTTCAGG3'

Mg++ Concentration: 1-4mM Annealing Temp: 47°C

Forward Sequence:

TTTCTAGAAGAAAAATGCATTTAGTCAAAAGCCATTTAGTGTGAATGTCGA
CCATCTTGTATACATTCACTGTCATTTTTTTACTTAATCTTCACACT
AATGCTAATCCAGTCAAACTAACGGGTACAAACAAAATTATTAAACCTCTGC
ATCAGTATGAATCTTACAGTATGAAGGATAACACCGTTTTGTGCTTAAATAT
CTTGTACATAATTAAAGGGATAGTTAACGCCACAAATAAAATTGTCTACAT
GTTTCACCTATATGTTGTTCCAAAAATGTATGACTTATTTTGCTGTGGAAC
ATAAAAGCTGATGTTAGGCAGAATGTTAGTCTCCGTGACCATTCAATTCTT
GTATGGAAAAAAAGATGCAGTGAAACTGAATGGTACTGAGGCTAACATTCT
GCCTGAAGTCTCCTTTATATTCCACATAAGTCAGTCATACGTGTTGGAGC
AACATGAGAAAATTATGACAGATGCTCAAATTAGATGAACATCATTT

Blastx search: gb|AAC61662.1| (U67083) KRAB-zinc finger protein KZF-2
Rattus norvegicus Identities = 14/40 (35%), Positives = 20/40 (50%)

Blastn search: emb|Z35595.1|CEC01G6 *Caenorhabditis elegans* cosmid
C01G6, Identities = 21/21 (100%)

Locus 182 : Amplified well in all Klamath species, one individual from each
species run on SSCP gel and showed no variation between them.

Clone size: 489 bp PCR Product size: 337 bp

Primers: 182F+ : 5'GGAACTGTTCGTCATTGT3'

182F- : 5'AGCTTCACTACCTCGTCAA3'

Mg++ Concentration: 1mM Annealing Temp: 50°C

Forward Sequence:

AACACAAGAAAAGGTGCAAAAGGAGAAATAATTCACTCTCCCTGTT
CTGCACATACTTCTGCCATTACAGCCATGATATCAAATTCTACGGAACT
GTTCGTCATTGTCCTGCTGTTCCCTTCTCCAATTACTGCATTGGAAATG
TTCAGTGCTGCCCTCTGGCCCAGAGTCTGACACTTGCTCCACCCTGGTC
CAGAGTCGTTCTTGTTCTCATCCTCTGTATTGCCAGCACACC

TTGCACCTGGACCCTGCAGAACCTGACCCACGGCGTACACCATCTTCAT
TAAGGTACAAAGCCAATCAGAGACTGCATTCCCCGACAGCACCGGACCTT
CCAGTTCGACTCCTCCTGGAGACAACACGAACCTCCTTGGATGGAGAG
CTTGACGAGGTAGTGAAGCTTGTATGCCTCCACACATGTTGCCCTCCTG
GAGGCAGGGAAACAATTCTGCA

Blastx search: ref|NP_001693.1|| brain-specific angiogenesis inhibitor 1 precursor>gi|2653432|dbj|BAA23647.1| (AB005297) BAI 1 *Homo sapiens*

Identities = 63/109 (57%), Positives = 88/109 (79%), Gaps = 3/109 (2%)

Blastn search: ref|NM_001702.1|| *Homo sapiens* brain-specific angiogenesis inhibitor 1 (BAI1) mRNA Identities = 57/68 (83%)

Locus 184 : Two alleles found in Klamath catostomids, present in all species.

AnkyrinG sequence. Clone size: 530 bp PCR Product size: 434 bp

Primers: 184R+ : 5'GAGGGCCTGAGAGCATAAGAT3'

184R- : 5'CAGTCCTGGGACCATAACCATA3'

Mg++ Concentration: 1,3 mM Annealing Temp: 50°C

Reverse Sequence:

AGCCCCAAAGTGCCTCAGCCCACCGGGAAAATGCCGGTATGCCAGATTAC
CTGTCCAGTCCTGGGACCATACCATATGTTGCAGGACTTCTTCTATTCTATT
CTATTGCACAAGGAATGTTGGATGAAAAATAAATAATCCATGAGTCTTTAT
CAATTACAAAAGAAAAAATATTCCCTACTCCTTTATCCAACCTTCTTTTTTT
TTCATTAGATTTTGTGACAGGGGAAATCTAAATCGATACTTCCCAGTGACA
CACTTAAGATGAAGATATAAAATAACCAGTTAAGAGAAATGTGAAACATTGA
CACATTATTGTGCCGAAACGTTGCACAGTTCAATGCACAAATCTCTCTG
AAACCTTAATTATCTTGCTTGACCAGAAAAGGGCAATCTGCTGCATTAGTTT
TCTAACTTCTCTGAAAAAATGTACTAAATATTCTCATAGTTTCCATCTTA
TGCTCTCAGGCCCTCAAAGTCCCTGTGAAAGAACAGACCTCGAATGGCTA
TTGTGGCA

Blastx search: pir||A55575|| ankyrin 3, long form - human >gi|608025
(U13616) ankyrin G *Homo sapiens*, Identities = 16/18 (88%), Positives = 18/18 (99%)

Blastn search: gb|AC004834.2|AC004834|| *Homo sapiens* clone DJ0550A13,
Identities = 20/20 (100%)

Locus 187: Amplified well in all Klamath species, one individual from each species run on SSCP gel and showed 2 allele types. LRS and SNS were fixed for one allele while KLS and KSS were heterozygous for both alleles. No other testing done.

Clone size: 482 bp PCR Product size: 382 bp

Primers: 187F +: 5'TGACAATTAAAGAAAGTTGACAAGC 3'

187F-: 5'TGGAAAATGTGGATAATCTCGTGAA 3'

Mg++ Concentration: 1 mM

Annealing Temp: 47°C

Forward Sequence:

ATTTTAAATGAAATGGCATACTAACATTAAATTGTAAAAGAATCTTACATGTTCCGTT
TAAAGTCCTTCACAAATGACAATTAAAGAAAGTTGACAAGCCCCCTGGT
GGGTTGGGTTAATCCCACGGTCAAGATTATTCACAACATCTTAGGAAGT
ATGTGTGTTTAAAGTATTATTAACACATTACCTGTAGCGCTGGCAAATG
TCTTAAAATGTCTTGAAACATTGAAAATGTGTTAAACAAAATGTTCAA
GTTGGCAAACACAACAAAGTCACTCTAAAATTATAAAAAACTTGACAACAT
TCACTAGTTTGTGTTGAGTTATTAACACTCGATAAAACATTCAACG
TAATTTAGATGTATTTAACACCTGTTGATTGCATTTTCAGTTCAAAGTC
CTTCACGAGATTATCCACATTTCCAAAAAGACTTTAAAGCGATAAGGTATT
TTT

Blastx search: gi|424235 (L21255) envelope glycoprotein [Human immunodeficiency virus type 1] Identities = 35/122 (28%), Positives = 50/122 (40%), dbj|BAA00448| (D00570) open reading frame (196 AA) *Mus musculus*, Identities = 15/49 (30%), Positives = 26/49 (52%)

Blastn search: gb|AC005410.1|AC005410 *Homo sapiens* chromosome 17, clone hRPK.1096_G_20, Identities = 23/23 (100%)

Locus 190: Primers were never made for this locus, not tested.

Clone size: 526 bp PCR Product size: 350 bp

Primers Designed: 190F+ : 5'CTCGGGCAGTAAAACAAATGT3'
190F- : 5'TTATGAATTGGACAAGAGAGC3'

Mg++ Concentration: mM Annealing Temp: 51°C

Forward Sequence:

TAGAGCATCCACATAACTGTCCCGAAGGTGTGTTCCACAAAACAAGCTC
CAGACACTAGCGGAACCAGCAAAACAAATTCAGTTCTCGGGCAGTAAA
ACAAATGTTCAGTGAGTCGGCTGTAATATGAGGGCAGCAGATGACCTAATC
ACCCCAATGTTTGCAAGAAATATTCCACAAAACAACTCCAGCCTCTATAC
GGAACCAACACAAAAAGAAACAAAAAGGCCTCAGTTCCCTCGGACAGTCA
AGTGAATGTTCAGTCAGTGAGTCAGGCGCACTCGGATGTTACATGAGTGCAACAA
ATTCCCAAATCACTCCAATGTTTGCAAGAAATATTCCACTAAACAAATTCCA
ACCAAAAGGAGGCATAAGCAAAATAACGTGCTTATTGATTGCCTGATTAT
TTGATAGCTCTTGTCCAATTCATAAGTCTGGTAACAAGAAAATGCTGTG
GTTCTCCAAGAAAGAGTCCTTACTCCTGCCTGTAAACATGAGATCTT
TATCGGAT

Blastx search: gb|AAC17659.1| (AF067943) F59B1.8 gene product *Caenorhabditis elegans* Identities = 17/41 (41%), Positives = 20/41 (48%)

Blastn search: emb|AL110485.1|CEY46G5A *Caenorhabditis elegans* cosmid Y46G5A, Identities = 21/21 (100%)