

April 28, 2005 Lee Nelson Genetics Contact, Region 3 Mt. Dept. of Fish, Wildlife, and Parks **415 South Front Street** Townsend, Montana 59644

Lee:

We have completed the protein electrophoretic analysis of the following samples presumed to be westslope cutthroat trout, Oncorhynchus clarki lewisi:

Fax (406)243-4184

Summary of results.

a Sample # Water Name/Location/Collection Date/	Ν	b # markers	c <b>Species ID</b>	d Power (%)	e % WCT	f Individuals	
3036	Stone Creek	30	R6Y11	WCT	R99.9Y99.9	100	xx
	4/5/2005 Lee Nelson						

<sup>a</sup>Number of fish successfully analyzed. If combined with a previous sample (Indicated in "Location" column), the number indicates the combined sample size. If present, the number in () is the average number of individuals successfully analyzed per locus (some individuals are not scoreable at all loci).

<sup>b</sup>Number of markers analyzed that are diagnostic for the non-native species (R=rainbow trout, W=westslope cutthroat trout, Y=Yellowstone cutthroat trout).

<sup>c</sup>Codes: WCT = westslope cutthroat trout (Oncorhynchus clarki lewisi); RBT = rainbow trout (O. mykiss); YCT = Yellowstone cutthroat trout (O. clarki bouvieri). Only one species code is listed when the entire sample possessed alleles from that species only. However, it must be noted that we cannot definitively rule out the possibility that some or all of the individuals are hybrids. We may not have detected any non-native alleles at the loci examined because of sampling error (see Power %). Species codes separated by "x" indicate hybridization between those species. <sup>d</sup>Number corresponds to the percent chance we have to detect 1% hybridization given the number of individuals successfully analyzed and the number of diagnostic markers used. For example, 25 individuals are required to yield a 95% chance to detect 1% hybridization with rainbow or a 99% chance to detect 1% hybridization with Yellowstone cutthroat trout into what once was a westslope cutthroat trout population. Not reported when hybridization is detected.

<sup>e</sup>Indicates the genetic contribution of the hybridizing taxa in the order listed under c to the sample assuming Hardy-Weinburg proportions. This number is reported if the sample appears to have come from a hybrid swarm. That is, a random mating population in which species markers are randomly distributed among individuals.

<sup>f</sup>Indicates number of individuals with genetic characteristics corresponding to the species code column when the sample can be analyzed on the individual level. This occurs when marker alleles are not randomly distributed among individuals and hybridization appears to be recent and/or if the sample appears to consist of a mixture of populations.

### **Methods and Analysis**

Horizontal starch gel electrophoresis was used to determine each fishes genetic characteristics (genotype) at 46 loci (genes) coding for proteins present in eye, liver, or muscle tissue (Table1). At some of these loci, the westslope cutthroat trout and rainbow trout, O. mykiss, rarely share alleles (form of a gene) in common (Table 2). This situation also pertains to a comparison of westslope and Yellowstone cutthroat trout, O. c. bouvieri (Table 2). Loci at which such fixed genetic differences exist between taxa are commonly termed

diagnostic loci because the alleles detected at them can be used to help determine whether a sample came from a non-hybridized population of one of these fishes or a population in which hybridization between two or all three of them has or is occurring.

### **Results and Discussion**

## Stone Creek 3036

This sample is composed of fish collected from five different locations in the Stone Creek drainage: A(N=6) Stone Creek, B(N=6) Stone Creek, C(N=6) Stone Creek, D(N=6) Middle Fork Stone Creek, and E(N=6) Left Fork of Stone Creek. The samples are listed in a downstream to upstream direction.

This sample is a follow up to the PINE analysis of fin clips collected October 1, 2004 (sample # 2976). The PINE results suggested this to be a non-hybridized westslope cutthroat trout population. There was some question, however, about the validity of this interpretation because a PINE fragment usually characteristic of Yellowstone cutthroat trout was detected in the sample. The relatively high frequency (0.077) of this fragment and the absence of other fragments characteristic of Yellowstone cutthroat trout suggested this was simply westslope cutthroat trout genetic variation but, the possibility this population was an old hybrid swarm between westslope and Yellowstone cutthroat trout could not reasonably be excluded. Since this population was being considered as a source of fish or gametes for broodstock or transfer purposes, it was considered necessary to obtain a clearer understanding of its genetic status using protein electrophoresis.

Considering all five locations, evidence of genetic variation was detected at five loci. There was no evidence of genetic differences (contingency table chi-square; P>0.05) among the fish collected from the different locations. Thus, all the locations were combined into a single sample for subsequent analysis.

Alleles characteristic of only westslope cutthroat trout were detected in the sample (Table 3). The failure to detect alleles characteristic of Yellowstone cutthroat at eleven additional diagnostic loci strongly indicates the PINE fragment previously detected simply is westslope cutthroat trout genetic variation. With the combined data sets, we have better than a 99.9% chance of detecting as little as a one percent rainbow or Yellowstone cutthroat trout genetic contribution to the population. The Stone Creek population, therefore, is undoubtedly non-hybridized westslope cutthroat trout and from a genetics perspective represents a suitable source of fish or gametes for broodstock or transfer purposes.

Sincerely,

Robb Leary

# Table 1

Enzymes and loci examined. Tissues: E=eye, L=liver, M=muscle.

Enzyme	Loci	Tissue	
Adenylate Kinase	AK-1*, AK-2*	М	
Alcohol Dehydrogenase	ADH*	L	
Aspartate Aminotransferase	sAAT-1*, sAAT-2* sAAT-3,4*	L M	
Creatine Kinase	CK-A1*, CK-A2* CK-B*, CK-C1*, CK-C2*	M E	
Dipeptidase	PEPA-1*, PEPA-2*	E	
N-acetyl-beta-Glucosaminidase	bGLUA*	L	
Glucose-6-phosphate Isomerase	<i>GPI-A*</i> <i>GPI-B1*, GPI-B2*</i>	E M	
Glyceraldehyde-3-phosphate Dehydroger	nase GAPDH-3*, GAPDH-4*	Е	
Glycerol-3-phosphate Dehydrogenase	G3PDH-1*, G3PDH-2*	L	
Iditol Dehydrogenase	IDDH*	L	
Isocitrate Dehydrogenase	mIDHP-1*, mIDHP-2* sIDHP-1*, sIDHP-2*	M L	
Lactate Dehydrogenase	LDH-A1*, LDH-A2* LDH-B1*, LDH-B2*, LDH-C*	M E	
Malate Dehydrogenase	sMDH-A1,2* sMDH-B1,2*	L M	
Malic Enzyme	mMEP-1*,mMEP-2*,sMEP-1* sMEP-2*	M L	
Phosphoglucomutase	PGM-1*, PGM-2*	М	

Table	1-continued
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Enzyme	Loci	Tissue
Phosphogluconate Dehydrogenase	PGDH*	М
Superoxide Dismutase	sSOD-1*	L
Tripeptide Aminopeptidase	PEPB*	Е
Xanthine Dehydrogenase-like	XDHl*	L

### Table 2

Alleles at the diagnostic loci that differentiate westslope cutthroat trout and rainbow trout, westslope and Yellowstone cutthroat trout, and rainbow and Yellowstone cutthroat trout. When more than one allele exists at a locus within a taxon, the most common allele is listed first.

Locus	Taxa and characteristic alleles		
	Westslope	Rainbow	
sAAT-1*	200,250	100	
CK-A2*	84	100	
GPI-A*	92,100	100	
IDDH*	40,100	100,200,40	
sIDHP-1*	86,71	100,114,71,40	
mMEP-1*	88	null	
	Westslope	Yellowstone	
sAAT-1*	200,250	165	
<i>CK-C1</i> *	100,38	38	
GPI-A*	92,100	100	
IDDH*	40,100	100	
mIDHP-1*	100	-75	
sIDHP-1*	86,71	71	
mMEP-1*	88	null	
sMEP-1*	100	90	
sMEP-2*	100	110	
PEPA-1*	100	101	
PEPB*	100	135	
PGM-1*	100,null	null	
	Rainbow	Yellowstone	
sAAT-1*	100	165	
CK-A2*	100	84	
<i>CK-C1</i> *	100,38,150	38	
mIDHP-1*	100	-75	
sIDHP-1*	100,114,71,40	71	
sMEP-1*	100	90	
sMEP-2*	100,75	110	
PEPA-1*	100,115	101	
PEPB*	100,120	135	
PGM-1*	100,null	null	

## Table 3

Allele frequencies at the loci showing evidence of genetic variation in a sample from a non-hybridized westslope cutthroat trout population in Stone Creek.

Locus	Alleles	Allele frequencies	
sAAT-1*	200	0.817	
	null	0.183	
CK-C1*	100	0.883	
	38	0.117	
bGLUA*	100	0.967	
	120	0.033	
sIDHP-2*	100	0.617	
	40	0.383	
PGM-2*	100	0.617	
	85	0.383	