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Jim;

We have analyzed the DNA extracted from fin clips from trout collected from the following locations:

Sample #	Water Name/Location/ Collection Date/ Collector	a N	b #Markers	c Taxa ID	d Power	e %	f # Fish
4859	Plimpton Creek 45.824074 -113.612932 7/28/2016 Jim Olsen	17 (103)	R19W20Y20	WCT	R99Y99		
4860	Twelvemile Creek 46.037493 -113.730633 7/20/2016 Jim Olsen	29(82)	R19W20Y20	WCT	R99Y99		
4861	Rabbia Creek 45.534157 -113.222586 9/20/2016 Jim Olsen	30(78)	R19W19Y20	WCT	R99Y99		
4862	Tendoy Lake 45.517422 -112.964894 9/16/2016 Jim Olsen	26	R19W20Y20	WCT WCT X YCT YCT			3 10 13

4864	North Gorge Lake	30	R19W20Y20	YCT X RBT	Y99.9R0.1	28
	45.494784 -112.966090			YCT X RBT	Y94R6	1
	8/16/2016			WCT		1
	Jim Olsen					
4865	South Gorge Lake	30	R19W20Y20	YCT X RBT	Y99.9R0.1	30
	45.489792 -112.964593					
	8/17/2016					
	Jim Olsen					

^aNumber of fish successfully analyzed. If combined with a previous sample, the number in parentheses indicates the combined sample size.

^bNumber of diagnostic loci analyzed for the taxon (R=rainbow trout *Oncorhynchus mykiss*, W=westslope cutthroat trout *O. clarkii lewisi*, Y=Yellowstone cutthroat trout *O. c. bouvieri*).

^cTaxa: WCT = westslope cutthroat trout; RBT = rainbow trout; YCT = Yellowstone cutthroat trout. Only one taxon code is listed if the sample was considered to contain only individuals from it. However, we cannot definitely rule out the possibility that some or all of the individuals are hybrids. We may not have detected any evidence of hybridization at the loci analyzed because of sampling error (see *d*). Taxa separated by "x" indicate hybridization between them was detected.

^dPower: the number corresponds to the percent chance we have to detect 0.5% introgression in a hybrid swarm (a random mating population in which taxa markers are randomly distributed among individuals such that essentially all of them in the population are of hybrid origin) given the number of individuals and diagnostic markers analyzed. For example, with 12 individuals we have better than a 95 % chance to detect as little as a 0.5% rainbow (39 diagnostic loci) or Yellowstone cutthroat trout (40 diagnostic loci) genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. Not reported when hybridization is detected. Taxa as in b.

^eIndicates the genetic contribution of the hybridizing taxa (amount of admixture) denoted as in b. This number is usually reported only if the sample appears to have come from a hybrid swarm.

^fIndicates the number of individuals with genetic characteristics corresponding to the taxa ID code column when the sample contains individuals from two or more genetically distinct groups.

Methods and Data Analysis

We developed a ‘chip’ specifically for analysis of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) populations. This chip allows us to simultaneously genotype up to 95 single nucleotide polymorphic loci (SNPs) in 91 trout using a Fluidigm EP1 Genotyping System. Each SNP locus has only two states (alleles). Thus, considering hybridization among rainbow (*O. mykiss*), westslope cutthroat, and Yellowstone cutthroat trout (*O. c. bouvieri*) a single locus can only distinguish one of the taxa from the other two. In order to address hybridization issues among these fishes, therefore, each chip contained 19 loci that differentiate rainbow from westslope cutthroat and Yellowstone cutthroat trout (rainbow markers), 20 loci that distinguish westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and 20 loci that distinguish Yellowstone cutthroat from westslope cutthroat and rainbow trout (Yellowstone markers, Table 1). We verified the diagnostic property of each marker by analyzing them in reference samples that had previously been determined to be non-hybridized westslope cutthroat, Yellowstone cutthroat, or rainbow trout by analysis of allozymes, paired interspersed nuclear elements (PINEs), a combination of insertion/deletion (indel loci) events and microsatellite loci, or two or all of these techniques (Table 2).

If a sample possessed alleles characteristic of only westslope cutthroat trout at all westslope markers and had no alleles characteristic of rainbow trout at the rainbow markers or Yellowstone cutthroat trout at the Yellowstone markers, then it was considered to contain non-hybridized westslope cutthroat trout. Evidence for potential hybridization between rainbow and westslope cutthroat trout was generally considered to be present when three criteria were met. First, the sample had to contain alleles characteristic of rainbow trout

at, at least, some of the rainbow markers. Next, at least some of the westslope markers also had to be genetically variable (polymorphic). Finally, no Yellowstone cutthroat trout alleles were detected at the Yellowstone markers. In this situation, the alleles at the rainbow markers shared between westslope cutthroat and Yellowstone cutthroat trout can confidently be assigned to having originated from westslope cutthroat trout and the alleles shared between rainbow and Yellowstone cutthroat trout at the westslope markers can confidently be assigned to having originated from rainbow trout. Thus, in terms of hybridization between westslope cutthroat and rainbow trout the data set contains information from 39 diagnostic loci. Likewise, when evidence of hybridization was detected only between westslope and Yellowstone cutthroat trout (no rainbow alleles at rainbow markers, at least some westslope markers polymorphic, and Yellowstone cutthroat trout alleles present at, at least, some Yellowstone markers) the data set contains information from 40 diagnostic loci. When all three sets of markers were polymorphic, this generally indicates hybridization among all three taxa. In this situation, the rainbow markers (19) provide information about rainbow trout hybridization and the Yellowstone markers (20) provide information about Yellowstone cutthroat trout hybridization.

An important aspect of SNPs is that they demonstrate a codominant mode of inheritance. That is, all genotypes are readily distinguishable from each other. Thus, at marker loci the genotype of individuals in a sample can directly be determined. From these data, the proportion of alleles from different taxa in the population sampled can be directly estimated at each marker locus analyzed. These values averaged over all marker loci yields an estimate of the proportion of alleles in the population that can be attributed to one or more taxa (proportion of admixture). In samples showing evidence of hybridization among all three taxa, we estimated the amount of rainbow trout admixture using only the 19 rainbow markers and the amount of Yellowstone cutthroat trout admixture using only the 20 Yellowstone markers. The amount of westslope cutthroat trout admixture was then estimated by subtracting the sum of the former two values from one. We used this procedure so the estimates would sum to one. Because of sampling error, it is unlikely that all three estimates from the marker loci would sum to one.

When evidence of hybridization is detected, the next issue to address is whether or not the sample appears to have come from a hybrid swarm. That is, a random mating population in which the alleles of the hybridizing taxa are randomly distributed among individuals such that essentially all of them are of hybrid origin.

A common, but not absolute, attribute of hybrid swarms is that allele frequencies at marker loci are similar among them because their presence can all be traced to a common origin or origins. Thus, one criterion we used for the assessment of whether or not a sample appeared to have come from a hybrid swarm was whether or not the allele frequencies among diagnostic loci reasonably conformed to homogeneity using contingency table chi-square analysis.

In order to determine whether or not alleles at the marker loci were randomly distributed among the fish in a sample showing evidence of hybridization, we calculated a hybrid index for each fish in the sample. The hybrid index for an individual was calculated as follows. At each marker locus, an allele characteristic of the native taxon was given a value of zero and an allele characteristic of the non-native taxon a value of one. Thus, at a single diagnostic locus the hybrid index for an individual could have a value of zero (only native alleles present, homozygous), one (both native and non-native alleles present, heterozygous), or two (only non-native alleles present, homozygous). These values summed over all diagnostic loci analyzed yields an individual's hybrid index. Considering westslope cutthroat and rainbow trout, therefore, non-hybridized westslope cutthroat trout would have a hybrid index of zero, non-hybridized rainbow trout a hybrid index of 78, F_1 (first generation) hybrids a hybrid index of 39, and post F_1 hybrids could have values ranging from zero to 78. The distribution of hybrid indices among the fish in a sample was statistically compared to the expected random binomial distribution based on the proportion of admixture estimated from the allele frequencies at the diagnostic loci. If the allele frequencies appeared to be statistically homogeneous among

the marker loci and the observed distribution of hybrid indices reasonably conformed to the expected random distribution, then the sample was considered to have come from a hybrid swarm.

In old or hybrid swarms with small effective population size, allele frequencies at marker loci can randomly diverge from homogeneity over time because of genetic drift. In this case, however, the observed distribution of hybrid indices is still expected to reasonably conform to the expected random distribution. Thus, if the allele frequencies were statistically heterogeneous among the marker loci in a sample but, the observed distribution of hybrid indices reasonably conformed to the expected random distribution the sample was also considered to have come from a hybrid swarm.

The strongest evidence that a sample showing evidence of hybridization did not come from a hybrid swarm is failure of the observed distribution of hybrid indices to reasonably conform to the expected random distribution. The most likely reasons for this are that the population has only recently become hybridized or the sample contains individuals from two or more populations with different amounts of admixture. At times, previous samples and the distribution of genotypes at marker loci and the observed distribution of hybrid indices can provide insight into which of the latter two factors appears mainly responsible for the nonrandom distribution of the alleles from the hybridizing taxa among individuals in the sample. At other times, the distribution of genotypes at marker loci and the observed distribution of hybrid indices may provide little or no insight into the cause of the nonrandom distribution of alleles among individuals. The latter situation is expected to be fairly common as the two factors usually responsible for the nonrandom distribution of alleles are not necessarily mutually exclusive. Regardless of the cause, when alleles at the marker loci do not appear to be randomly distributed among individuals in a sample, estimating the amount of admixture has little if any biological meaning and, therefore, is generally not reported.

Failure to detect evidence of hybridization in a sample does not necessarily mean the population is non-hybridized because there is always the possibility that we would not detect evidence of hybridization because of sampling error. When no evidence of hybridization was detected in a sample, we assessed the likelihood the population is non-hybridized by determining the chances of not detecting as little as a 0.5 percent genetic contribution of a non-native taxon to a hybrid swarm. This is simply 0.995^{2NX} where N is the number of fish in the sample and X is the number of marker loci analyzed.

The chip also contained 34 loci that are generally polymorphic within westslope cutthroat trout populations west of the Continental Divide. Information from these loci can be used to address issues concerning the relative amount of genetic variation within and divergence among westslope cutthroat trout populations. Because of the reduced amount of genetic at these loci in populations east of the Continental Divide, results of such comparisons should be treated with caution.

Finally, the chip contained two mitochondrial DNA (mtDNA) loci that differentiate cutthroat and rainbow trout. Data from these loci were used only if an individual appeared to be an F_1 hybrid. Because mtDNA is inherited only from females (maternal inheritance), in this situation we can determine the taxon of the female, and by default the taxon of the male, that produced the hybrid.

When two or more samples were collected from the same area of a water body in different years or different reaches of a stream in the same year, we used the log likelihood G test of Goudet et al. (1996) in GENEPOP version 4.2 (Rousset 2008) to test for genetic differences among the samples. In instances where multiple loci were compared among samples and some demonstrated significant differences, significance was determined using Rice's (1989) method for correcting for multiple comparisons (modified level of significance). When no differences were detected at the modified level, any observed differences were considered to most likely represent chance departures from homogeneity and the samples were usually combined for further analysis. When evidence of genetic differences was detected between samples they were generally kept separate for analysis and the relative amount of divergence between them was estimated

as F_{ST} using the method of Weir and Cockerham (1984) available in GENEPOP version 4.2. We used F_{ST} as this estimate provides some information about the degree of reproductive isolation (i.e. the average number of migrants per generation) among samples.

In samples containing 10 or more individuals appearing to be non-hybridized westslope cutthroat trout, we compared the observed genotypic distributions at the polymorphic loci to expected random mating genotypic proportions (Hardy-Weinberg proportions) using the Markov Chain method of Guo and Thompson (1992) available in GENEPOP version 4.2. A deficit of observed heterozygotes can arise in a sample if it contains individuals from two or more genetically divergent populations or the fish in it are experiencing a fair to high amount of inbreeding at the population level. Conversely, fish produced from a very small number of parents may show an excess of heterozygotes compared to the expected random mating proportions (Pudovkin et al. 1996, 2010; Luikart and Cornuet 1999). Since multiple comparisons were performed in most cases, significance was again determined at the modified level. In samples showing significant departures of observed genotypic distributions from expected Hardy-Weinberg genotypic proportions at four or more loci (note four or more loci are required to establish a statistically significant difference with the statistical analysis used), we used chi-square analysis of only the loci demonstrating significant deviations to determine if there was a significant trend for these loci to show an excess or deficit of heterozygotes. In these samples, we also used all the polymorphic loci regardless of statistical significance to determine if there was a statistically significant trend over all the polymorphic loci for the sample to express a slight deficit or excess of heterozygotes compared to random mating proportions. Finally, we used the program ML-RELATE of Kalinowski et al. (2006) to estimate the degree of relationship among the fish in samples demonstrating a significant trend for there to be either a deficit or excess of heterozygotes compared to random mating expectations as this could possibly provide some insight into the cause of the trend.

Results and Discussion

Plimpton Creek 4859

In the sample from Plimpton Creek, no alleles characteristic of rainbow trout were detected at the rainbow markers, only alleles characteristic of westslope cutthroat trout were detected at the westslope markers, and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. These results are concordant with previous allozyme (#868, col.9/27/93, T1N R16W S20 SW1/4 NE1/4, N=1. #1192, col. 10/24/95, T11N R16W S17, N=10), SNP (#4336, col. 10/11/11, 45.84547 113.36825, N=53), and indel/microsatellite (#4371, col. 6/25/12, 45.81245-81619 113.60103-60360, N=26) analyses of trout sampled from Plimpton Creek. These analyses also indicated the fish to be non-hybridized westslope cutthroat trout. With the 6,268 diagnostic rainbow trout alleles and 5,740 Yellowstone cutthroat trout diagnostic alleles analyzed in the samples with the various techniques, we had better than a 99.99 percent chance of detecting as little as a 0.5% rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. We conclude, therefore, that the fish sampled from Plimpton Creek were very likely non-hybridized westslope cutthroat trout. From a genetics perspective, therefore, there is no reason not to use Plimpton Creek as a source of fish to transfer to Shultz Creek.

Twelvemile Creek 4860

No alleles characteristic of rainbow trout were detected at the rainbow markers, only alleles characteristic of westslope cutthroat trout were detected at the westslope markers, and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers in the sample from Twelvemile Creek. Previous PINE (#3249, col. 8/19/05, X=334689 Y=509361, N=17), indel/microsatellite (#4144, col. 7/29/10, 46.02372 113.1230, N=25), and SNP analyses (#4795, col. 8/9/15, 45.02509 113.13366, N=11) also detected no

evidence of hybridization in trout collected from Twelvemile Creek. With the 4,042 diagnostic rainbow trout alleles and 4,860 Yellowstone cutthroat trout diagnostic alleles analyzed in the samples with the various techniques, we had better than a 99.99 percent chance of detecting as little as a 0.5% rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. We conclude, therefore, that the fish sampled from Twelvemile Creek were almost certainly non-hybridized westslope cutthroat trout and the variation detected at *OclWD111312_Garza* in sample #4795 was a westslope cutthroat trout polymorphism. From a genetics perspective, therefore, there is no reason not to use fish from Twelvemile Creek for transfer or brood stock purposes.

Rabbia Creek 4861

In the sample from Rabbia Creek, no alleles characteristic of rainbow trout were detected at the rainbow markers and no alleles characteristic of Yellowstone cutthroat trout were detected at the Yellowstone markers. Only the westslope marker *OmyWD_RAD_54516_Hoh* was polymorphic (allele frequency = 0.033). This polymorphism could indicate a small amount of hybridization or it could simply be westslope cutthroat trout genetic variation. In this case we strongly favor the later interpretation as this locus has been observed to be the only locus polymorphic in many (N = 35) other samples that otherwise appeared to be non-hybridized westslope cutthroat trout (Table 3). Furthermore, previous samples from Rabbia Creek (#1137, col. 8/24/95, T13S R13W S4, allozyme, N=7, #2170, col. 8/2/01, T13S R13W S23, PINE, N=7, #2173, col. 8/1/01, T13S R13W S33 NW1/4, PINE, N=27, #4618, col. 7/9/14, 45.53448 113.22359, SNP, N=7) detected no evidence of hybridization. With the 3,318 diagnostic rainbow trout alleles and 3,312 Yellowstone cutthroat trout diagnostic alleles analyzed in the samples with the various techniques, we had better than a 99.99 percent chance of detecting as little as a 0.5% rainbow or Yellowstone cutthroat trout genetic contribution to a hybrid swarm that once was a non-hybridized westslope cutthroat trout population. We conclude, therefore, that the fish sampled from Rabbia Creek were almost certainly non-hybridized westslope cutthroat trout. From a genetics perspective, therefore, there is no reason not to use fish from Rabbia Creek for transfer or brood stock purposes.

Tendoy Lake 4862

Alleles characteristic of Yellowstone cutthroat trout were detected at all the Yellowstone markers, alleles characteristic of westslope cutthroat trout were detected at all the westslope markers, and no alleles characteristic of rainbow trout were detected at the rainbow markers in the sample from Tendoy Lake. The Yellowstone cutthroat trout alleles were clearly not randomly distributed among the fish in the sample. In contrast, the sample contained 13 Yellowstone cutthroat trout, three westslope cutthroat trout, and ten F₁ hybrids between these fishes. Of these, eight possessed mtDNA characteristic of westslope cutthroat trout and two characteristic of Yellowstone cutthroat trout ($X^2_1=3.60$, $P<0.05$). There was no statistical tendency, therefore, for there to be an excess of hybrid matings involving females from one of these fishes. Thus, the westslope cutthroat trout stocked prior to 2016 have clearly hybridized with the Yellowstone cutthroat trout introduced to the lake. Swamping, therefore, may be effective at replacing the Yellowstone cutthroat trout in the lake but, this will clearly require additional stocking of westslope cutthroat trout and will likely take more than 20 years. You, therefore might consider attempting to remove all the fish from the lake prior to the Willow Creek drainage restoration. If not, we are concerned about Yellowstone cutthroat trout and hybrids migrating down into the creek.

North Gorge Lake

In the sample from North Gorge Lake, alleles characteristic of Yellowstone cutthroat trout were detected at all the Yellowstone markers, alleles characteristic of westslope cutthroat trout were detected at all the westslope markers, and alleles characteristic of rainbow trout were detected at three of the rainbow markers. The rainbow trout alleles were not randomly distributed ($X^2_2=18.111$, $P<0.001$) among the fish in the sample.

Likewise, the westslope cutthroat trout alleles were detected in only one fish indicating it to be a non-hybridized westslope cutthroat trout. The nonrandom distribution of the rainbow trout alleles was solely due to one fish with a hybrid index of five. When this fish is removed from the sample, the rainbow trout alleles appear to be randomly distributed ($X^2_{1}=0.085$, $P>0.950$) among the remaining individuals. This sample, therefore, appears to have contained a single westslope cutthroat trout, 28 fish from a hybrid swarm between Yellowstone cutthroat and rainbow trout with a predominant (0.999) Yellowstone cutthroat trout genetic contribution, and one hybrid between Yellowstone cutthroat and rainbow trout with about a six percent Yellowstone cutthroat trout genetic contribution. Prior stocking of westslope cutthroat trout, therefore, has had little influence on the genetic characteristics of the fish in the lake. Thus, swamping, as practiced, appears to be an ineffective means of replacing the Yellowstone cutthroat and rainbow trout hybrids in the lake.

South Gorge Lake

Alleles characteristic of Yellowstone cutthroat trout were detected at all the Yellowstone markers, no alleles characteristic of westslope cutthroat trout were detected at all the westslope markers, and alleles characteristic of rainbow trout were detected at one of the rainbow markers (*OmyRD_RAD_22111_Hoh*) in the sample from South Gorge Lake. The rainbow trout alleles appeared to be randomly distributed among the fish in the sample as three different individuals possessed a single copy of a rainbow trout allele. This sample, therefore, appears to have come from a hybrid swarm between Yellowstone cutthroat and rainbow trout with a predominant (0.999) Yellowstone cutthroat trout genetic contribution. This conclusion is debatable as with this small proportion of rainbow trout alleles in the sample it is statistically very difficult to determine if the distribution of them among the fish is nonrandom. Regardless, past stocking, as practiced, of westslope cutthroat trout into the lake seems to have had no influence on the genetic characteristics of the fish in it and swamping seems to be ineffective as a means of replacing hybrids between Yellowstone cutthroat and rainbow trout in this lake.

Robb Leary

Andrew Whiteley

Sally Painter

Angela Lodmell

Literature Cited

- Amish, S. J., P. A. Hohenlohe, S. Painter, R. F. Leary, C. Muhlfeld, F. W. Allendorf, and G. Luikart. 2012. RAD sequencing yields a high success rate for westslope cutthroat and rainbow trout species-diagnostic SNP assays. *Molecular Ecology Resources* 12:653-660.
- Campbell, N. R., S. J. Amish, V. L. Pritchard, K. S. McKelvey, M. K. Young, M. K. Schwartz, J. C. Garza, G. Luikart, and S. R. Narum. 2012. Development and evaluation of 200 novel SNP assays for population genetic studies of westslope cutthroat trout and genetic identification of related taxa. *Molecular Ecology Resources* 12:942-949.
- Finger, J. A., M. R. Stephens, N. W. Clipperton, and B. May. 2009. Six diagnostic single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trouts. *Molecular Ecology Resources* 9:759-763.
- Goudet, J., M. Raymond, T. deMeeus, and F. Rousset. 1996. Testing differentiation in diploid populations.

- Genetics 144:1933-1940.
- Guo, S. W. and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportions for multiple alleles. *Biometrics* 48:361-372.
- Harwood, A. S., and R. B. Phillips. 2011. A suite of twelve single nucleotide polymorphism markers for detecting introgression between cutthroat and rainbow trout. *Molecular Ecology Resources* 11:382-385.
- Kalinowski, S. T., B. J. Novak, D. P. Drinan, R. deM Jennings, and N. V. Vu. 2011. Diagnostic single nucleotide polymorphisms identifying westslope cutthroat trout (*Oncorhynchus clarkii lewisi*), Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and rainbow trout (*Oncorhynchus mykiss*). *Molecular Ecology Resources* 11:389-393.
- Kalinowski, S. T., A. P. Wagner, and M. L. Taper. 2006. ML-RELATE: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes* 6:576-579.
- Luikart, G. and J.-M. Cornuet. 1999. Estimating the effective number of breeders from heterozygote excess in progeny. *Genetics* 151:1211-1216.
- Pudovkin, A. I., O. L. Zhdanova, and D. Hedgecock. 1996. On the potential for estimating the effective number of breeders from heterozygote excess in progeny. *Genetics* 144:383-387.
- Pudovkin, A. I., O. L. Zhdanova, and D. Hedgecock. 2010. Sampling properties of the heterozygote-excess estimator of the effective number of breeders. *Conservation Genetics* 11:759-771.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223-225.
- Rousset, F. 2008. GENEPOP'007: a complete re-implementation of the GENEPOP software for Window and Linux. *Molecular Ecology Resources* 8:103-106.
- Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. *Evolution* 28:1358-1370.

Table1

SNP loci that differentiate rainbow from westslope and Yellowstone cutthroat trout (rainbow markers), westslope cutthroat from rainbow and Yellowstone cutthroat trout (westslope markers), and Yellowstone cutthroat trout from westslope cutthroat and rainbow trout (Yellowstone markers).

	Rainbow Markers		Reference
	Taxa and characteristic alleles		
	Rainbow	Westslope/Yellowstone	
OmyRD_RAD_29252_Hoh	1	2	Amish et al. 2012
OmyRD_RAD_30378_Hoh	1	2	Amish et al. 2012
OclRD_P53T7R1_Har	1	2	Amish et al. 2012
OmyRD_RAD_30423_Hoh	1	2	Harwood and Phillips 2011
OmyRD_RAD_59515_Hoh	1	2	Amish et al. 2012
OclRD_Thymo_320Kal	1	2	Amish et al. 2012
OmyRD_RAD_48301_Hoh	1	2	Kalinowski et al. 2011
OmyRD_RAD_49759_Hoh	1	2	Amish et al. 2012
OclRD_P53T7R2_Har	1	2	Amish et al. 2012
OmyRD_URO_302May	1	2	Harwood and Phillips 2011
OmyRD_RAD_20663_Hoh	1	2	Finger et al. 2009
OmyRD_RAD_51740_Hoh	2	1	Amish et al. 2012
OmyRD_RAD_22111_Hoh	1	2	Amish et al. 2012
OmyRD_RAD_55820_Hoh	2	1	Amish et al. 2012
OmyRD_RAD_5666_Hoh	2	1	Amish et al. 2012
OmyRD_F5_136May	1	2	Amish et al. 2012
OmyRD_RAD_42014_Hoh	2	1	Finger et al. 2009
OmyRD_RAD_54584_Hoh	2	1	Amish et al. 2012
OclRD_CLK3W5_Har	2	1	Amish et al. 2012

Table 1-continued

	Westslope Markers		Reference
	Taxa and characteristic alleles		
	Westslope	Rainbow/Yellowstone	
OclWD_CLK3W5_Har	2	1	Harwood and Phillips 2011
OclWD_CLK3W1_Har	2	1	Harwood and Phillips 2011
OclWD101119_Garza	2	1	Campbell et al. 2012
OmyWD_RAD_76689_Hoh	2	1	Amish et al. 2012
OclWD_114315L _Garza	2	1	Campbell et al. 2012
OclWD_Tnsf_387Kal	2	1	Kalinowski et al. 2011
OmyWD_RAD_55391_Hoh	2	1	Amish et al. 2012
OclWD_P53_307Kal	2	1	Kalinowski et al. 2011
OclWD111312_Garza	2	1	Campbell et al. 2012
OclWD_107031L _Garza	2	1	Campbell et al. 2012
OclWD_PrLcW1_Har	2	1	Harwood and Phillips 2011
OmyWD_RAD_54516_Hoh	2	1	Amish et al. 2012
OclWD_105075L_Garza	2	1	Campbell et al. 2012
OmyWD_RAD_52968_Hoh	2	1	Amish et al. 2012
OclWD_114336_Garza	1	2	Campbell et al. 2012
OclWD103713_Garza	2	1	Campbell et al. 2012
OclWD107074_Garza	2	1	Campbell et al. 2012
OclWD109651_Garza	2	1	Campbell et al. 2012
OclWD_129170L _Garza	1	2	Campbell et al. 2012
OclWD_ppie_32NC	1	2	Campbell et al. 2012

Table 1-continued

Yellowstone Markers			
	Taxa and characteristic alleles		Reference
	Yellowstone	Westslope/Rainbow	
OclYD_CLK3Y1_Har	2	1	Harwood and Phillips 2011
OclYGD100974_Garza	2	1	Campbell et al. 2012
OclYGD110571_Garza	2	1	Campbell et al. 2012
OclYSD117432_Garza	2	1	Campbell et al. 2012
OclYGD127236_Garza	2	1	Campbell et al. 2012
OclYGD112820_Garza	2	1	Campbell et al. 2012
OclYGD104216_Garza	2	1	Campbell et al. 2012
OclYGD113600_Garza	2	1	Campbell et al. 2012
OclYSD129870_Garza	2	1	Campbell et al. 2012
OclYGD104569_Garza	2	1	Campbell et al. 2012
OclYGD117286_Garza	2	1	Campbell et al. 2012
OclYGD117370_Garza	2	1	Campbell et al. 2012
OclYSD107607_Garza	2	1	Campbell et al. 2012
OclYGD106457_Garza	2	1	Campbell et al. 2012
OclYSD106367_Garza	1	2	Campbell et al. 2012
OclYGD107031_Garza	1	2	Campbell et al. 2012
OclYGD106419_Garza	1	2	Campbell et al. 2012
OclYSD123205_Garza	1	2	Campbell et al. 2012
OclYGD109525_Garza	1	2	Campbell et al. 2012
OclYSD113109_Garza	1	2	Campbell et al. 2012

Table 2

Reference samples used for the identification of marker SNPs among westslope cutthroat, rainbow, and Yellowstone cutthroat trout. Taxa: WCT=westslope cutthroat trout, YCT=Yellowstone cutthroat trout, IRT=redband trout, CRT=coastal rainbow trout. N=sample size.

Sample	Taxa	N	Location
Washoe Park State Trout			
Hatchery	WCT	12	Anaconda, Montana
Big Foot Creek	WCT	2	Upper Kootenai River, Montana
Runt Creek	WCT	3	Yaak River, Montana
Hawk Creek	WCT	2	North Fork Flathead River, Montana
Werner Creek	WCT	3	North Fork Flathead River, Montana
Morrison Creek	WCT	3	Middle Fork Flathead River, Montana
Sixmile Creek	WCT	3	Swan River, Montana
South Fork Jocko River	WCT	3	Lower Flathead River, Montana
Cottonwood Creek	WCT	3	Upper Clark Fork River, Montana
Copper Creek	WCT	2	Flint-Rock Creek, Montana
Gillispie Creek	WCT	3	Flint-Rock Creek, Montana
Davis Creek	WCT	4	Bitterroot River, Montana
Humbug Creek	WCT	2	Blackfoot River, Montana
Ringeye Creek	WCT	2	Blackfoot River, Montana
Flat Creek	WCT	3	Middle Clark Fork River, Montana
McGinnis Creek	WCT	3	Lower Clark Fork River, Montana
Bear Creek	WCT	1	Red Rock River, Montana
McVey Creek	WCT	1	Big Hole River, Montana
McClellan Creek	WCT	1	Upper Missouri River, Montana
Yellowstone River State Trout			
Hatchery-Goose Lake	YCT	6	Big Timber, Montana
Slough Creek	YCT	4	Yellowstone River, Montana
Lake Koocanusa	IRT	4	Upper Kootenai River, Montana
North Fork Yahk River	IRT	5	Yahk River, British Columbia
Jocko River State Trout Hatchery	CRT	7	Arlee, Montana
Arlee Rainbow			

Table 3

Frequency of the allele usually characteristic of rainbow and Yellowstone cutthroat trout at the westslope marker *OmyWD_RAD_54516_Hoh* in samples otherwise appearing to have come from non-hybridized westslope cutthroat trout populations or populations with a slight amount of admixture. Number in parentheses represents sample number.

Stream and sample number	Allele frequency
Blum (#4349)	0.341
Crevise (#4350)	0.310
Spotted Dog (#4351)	0.364
L Morse (#4390)	0.667
U Morse (#4391)	0.615
L Bear (#4357)	0.025
L Twin Lake (#4281)	0.071
Blue Joint (#4283)	0.067
WF Bitterroot (#4286)	0.067
Sidney (#4384)	0.051
S. F. L. Willow (#4430)	0.040
Threemile (#4428)	0.029
WF Bitterroot (#4480)	0.029
Deer (#4484)	0.118
Little Boulder (#4485)	0.100
Overwhich (#4486)	0.079
WF West (#4487)	0.200
Threemile (#4495)	0.062
S. F. L. Willow (#4496)	0.035

Table 3-continued

Stream and sample number	Allele frequency
L. Hughes (#4549)	0.192
U. Hughes (#4550)	0.167
Beaver (#4551)	0.063
Slate (#4552)	0.056
Deer (#4553)	0.100
Coal (#4554)	0.023
Lyman (#4671)	0.030
Burnt Fork (#4672)	0.043
WF Warm Springs (#4674)	0.400
Richardson (#4692)	0.167
Orifino (#4749)	0.775
Chicken (#4759)	0.060
Johnson (#4760)	0.120
WF Bitterroot (#4761)	0.080
Woods (#4548)	0.125
Rabbia (#4861)	0.033
Sheepshead (#4540)	0.050