FISHERY RESEARCH

PARENTAGE BASED TAGGING OF SNAKE RIVER HATCHERY STEELHEAD AND CHINOOK SALMON

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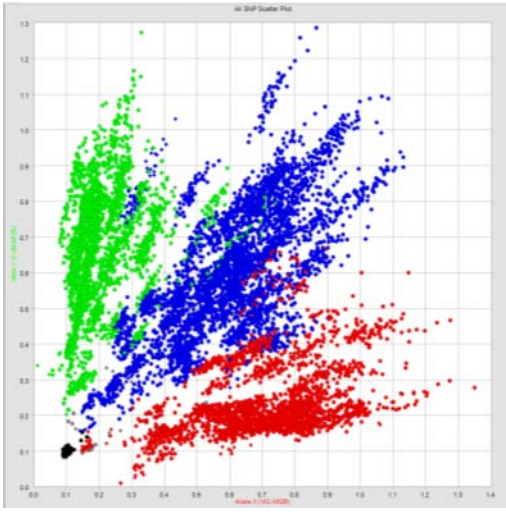

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PARENTAGE BASED TAGGING OF SNAKE RIVER HATCHERY STEELHEAD AND CHINOOK SALMON

Project Progress Report

2010 Annual Report

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ABSTRACT

This report summarizes the progress in development and evaluation of a new, dynamic genetic technology called Parentage Based Tagging (PBT) that can serve as a versatile tool for mass marking of steelhead and Chinook salmon in the Snake River basin. While PBT is potentially a more economic and efficient technique for marking fish than coded-wire tags (CWT), it also has the capability to address aspects of hatchery reform, salmonid life history, harvest patterns, and trait heritability. This project addresses five objectives focused on the development and demonstration of the feasibility of PBT in the Snake River basin: Objective 1) annual sampling of hatchery broodstock, Objective 2) identification of SNP loci and assessment of their assignment power, Objective 3) standardization of loci for PBT and integration with loci for Genetic Stock Identification (GSI), Objective 4) creation of parental genetic databases and, Objective 5) utilization of PBT methods to provide parentage assignments. This project has sampled and inventoried nearly 100% of hatchery broodstock (Objective 1) for steelhead (~16,000 total individuals) and Chinook salmon (~27,000 total individuals) from spawn years 2008, 2009, and 2010. Collection of samples for spawn year 2011 is underway. In close collaboration with the Columbia River Inter-Tribal Fisheries Commission (CRITFC), we identified and developed informative Single Nucleotide Polymorphism (SNP) loci for steelhead and Chinook salmon (Objective 2). We standardized a set of 95 SNP loci for use in PBT of hatchery stocks within the Snake River basin that can also be used in conjunction with an additional set of SNP loci identified for GSI methods in the Columbia River basin (Objective 3). Using the PBT SNPs identified for each species, we have genotyped nearly 100% of the sampled steelhead and Chinook salmon broodstock from spawn years 2008 and 2009 (Objective 4). The broodstock baselines will be used to provide parentage assignments for hatchery strays, CWT fish, and origin of hatchery kelts. Results indicate that annual sampling, inventorying, and genotyping of all steelhead and Chinook salmon broodstock in Idaho is feasible and that the SNP set identified for PBT is sufficient for accurate assignment of offspring to brood year, hatchery stock, and even individual parents; thereby allowing an unprecedented ability to mark millions of Snake River smolts and an opportunity to address future objectives of parentage-based research.

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INTRODUCTION

For over 40 years, researchers and managers in the Columbia River basin have used coded-wire tags (CWTs) to monitor and assess the harvest patterns and survival rates of salmon and steelhead in the Columbia River basin (Johnson 2004). Recovery of CWTs are one of the primary tools used by managers in Oregon, Washington, and Idaho to estimate the number of hatchery Chinook salmon *Oncorhynchus tshawytscha* and steelhead *O. mykiss* contributing to in-state and out-of-state fisheries and to estimate harvest of individual hatchery stocks.

Despite the predominance of CWT technology in addressing management concerns, it has several limitations. The process of physically tagging tens of thousands of juveniles from different hatchery stocks is logistically difficult, labor intensive, extremely costly, and potentially increases physiological stress to the juveniles just prior to their release for downstream migration. All of these restrictions ultimately limit the total number of juveniles that get tagged each year, which in turn limits the number of CWT recoveries. The resulting small sample sizes greatly reduce statistical power to estimate stock contributions because the precision of these estimates are directly related to the number of CWTs recovered in fisheries or escapements (Hankin et al. 2009).

Parentage-based genetic tagging (described in Anderson and Garza [2005]), a technological alternative to CWT, management would eliminate the problem of small sample sizes. Parentage-based tagging (PBT) involves annual sampling and genotyping of hatchery broodstock, creating a parental genotype database. Progeny from any of these parents (either collected as juveniles or adults), if genotyped, could be assigned back to their parents, thus identifying the hatchery they originated from and their exact brood year. The exceptional advantage PBT has over CWT technology is increased sample size. By genotyping all parental broodstock, every juvenile is genetically "tagged."

While theoretically appealing (Anderson and Garza 2005; 2006), PBT technology still needs to be empirically tested and validated. Over the last several years, several committees and science review groups have recommended that two or more large-scale evaluations of the technology be performed (PFMC 2008; PSC 2008; ISAB/ISRP 2009).

Given these recent advancements, this project constructs the first PBT genetic baselines for steelhead and Chinook salmon hatcheries in the Snake River basin. This project addresses current and future objectives in creating PBT baselines within the Snake River basin that can be used for not only monitoring harvest of hatchery stocks but also for addressing additional issues, such as the origin of hatchery strays and steelhead kelts and effectiveness of hatchery integration programs.

OBJECTIVES

The Snake River PBT project includes five primary objectives as follows:

Objective 1: Genetic sampling of Chinook salmon and steelhead broodstock

This objective demonstrates the feasibility of sampling, inventorying, and genotyping all hatchery broodstock each year for steelhead and Chinook salmon and recording accurate biological information for every fish.

Objective 2: Identify a sufficient number of SNP loci

We have identified a set of Single Nucleotide Polymorphism (SNP) markers that can achieve high parental assignment accuracy even when parentage involves hatchery populations that are very similar genetically due to shared ancestry or in situations where the broodstock contains closely related individuals (ISRP/ISAB 2009).

Objective 3: SNP standardization and integration

A set of 95 SNP loci has been identified that can be used either independently for performing PBT on hatchery steelhead and Chinook salmon originating from the Snake River basin or in conjunction with an additional SNP set for performing Genetic Stock Identification (GSI) across the Columbia River basin.

Objective 4: Create parental databases for Snake River hatcheries

Parental genotype databases were created for spawn years 2008 and 2009 for Chinook salmon and steelhead.

Objective 5: Utilization of PBT methods to provide accurate parental assignments

We intend to demonstrate the applications of this technology through “backend” sampling/genotype projects that will demonstrate the versatility of PBT. In future years (starting with 2011 adult returns) this will include: 1) an assessment of the stock composition of hatchery Snake River salmon and steelhead passing Bonneville Dam and harvested in main-stem Columbia River fisheries; 2) run reconstruction of hatchery steelhead at Lower Granite Dam, 3) identification of hatchery-origin steelhead kelts out-migrating past Lower Granite Dam, 4) a paired CWT and PBT recovery experiment as part of existing Lower Snake River Compensation Plan (LSRCP) hatchery evaluations in Idaho, and 5) an assessment of the origin of straying hatchery steelhead in the Salmon River basin.

REPORT STRUCTURE

This report is divided into five sections, one for each of the objectives of the study. The first section reports on sampling efforts and the second section on SNP discovery in Chinook salmon and steelhead. The third section summarizes the standardization of markers between Idaho Fish and Game (IDFG) and Columbia River Inter-Tribal Fisheries Commission (CRITFC) labs and designation of SNP markers for PBT. The fourth section includes genotyping of broodstock with the SNP markers to create parental genetic baselines. The fifth section provides an overview of future applications of PBT.

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SECTION 1: ANNUAL SAMPLING OF TISSUE FROM 100% OF HATCHERY CHINOOK SALMON AND STEELHEAD BROODSTOCK IN THE SNAKE RIVER BASIN

Introduction

The implementation of PBT methods requires a complete sampling of broodstock from all hatcheries contributing to the production of steelhead and Chinook (Figure 1). This objective address the ability to annually sample tissue from 100% of the hatchery Chinook and steelhead broodstock in the Snake River basin.

Methods

The overall goal was to obtain high quality tissue samples and accurate biological data from every adult that contributes to spawning. Biological data includes species, sex, hatchery/stock, date sampled/spawned, tag information, and markings; most hatcheries also record length and cross information. Tissues samples are collected in the form of fin tissue or operculum punches, stored in 2 ml vials of 100% non-denatured ethanol, and shipped to the IDFG genetics lab in Eagle, Idaho. Care is taken to avoid contamination during sampling by rinsing scissors or hole-punch tools in water or ethanol and wiping with a paper towel in between each tissue sample.

Each sample is labeled with a field ID#, which is used to track the samples until they arrive at the lab, at which time they are given a standardized lab database code. The associated data is carefully scrutinized at the lab to ensure that accurate data was recorded for every fish sampled. Any discrepancies that are discovered are solved via correspondence with the hatchery employee in charge of recording data.

Once the samples are extracted and genotyped, genetic data are recorded into a Progeny database and stored with collection and individual fish data. Due to the scope of this project, this database was recently created to manage, organize, and track physical tissue samples along with their associated DNA extractions and genotypes. Progeny allows genetic data to be exported along with individual fish data in a variety of formats, which has proven to be essential for the transfer of data between the IDFG and CRITFC laboratories.

Results

We have collected and properly inventoried approximately 10,900 genetic samples from the steelhead broodstock (Table 1) and approximately 19,500 samples (Table 2) from Chinook salmon broodstock spawned in the Snake River basin during SY2008 and SY2009. Most hatcheries provided biological information on all fish sampled (sex and length). Some hatcheries provided individual cross information.

Discussion

We have demonstrated the feasibility of sampling, inventorying, and genotyping thousands of broodstock fish each year. The completion of this objective lays the foundation for continuation of PBT sampling in the Snake River basins.

SECTION 2: IDENTIFICATION OF A SUFFICIENT NUMBER OF SNP LOCI

Introduction

Parentage based tagging (PBT) and genetic stock identification (GSI) require the use of an informative suite of genetic markers for individual assignment of samples to parents and stock of origin, respectively. Single nucleotide polymorphisms (SNPs) are ideal markers for large-scale parentage and GSI studies because of the speed of high-throughput screening, transferability between laboratories, and low genotyping error rates (Anderson and Garza 2006). This section describes the selection and evaluation of the most informative sets of markers to meet PBT and GSI objectives in two species of Pacific salmonids: steelhead and Chinook salmon.

For PBT to work efficiently and accurately, it is necessary to have a sufficient number of markers that provide resolution to accurately assign offspring back to the correct hatchery stock, cohort, and parents. Two kinds of assignment errors are possible with PBT: false negatives, in which offspring with parents in the genetic database fail to assign to those same parents, and false positives, in which offspring with no parents in the database incorrectly assign to a parental pair in the database. Ensuring that molecular markers with high resolving power are used for PBT can reduce both types of error. This objective identifies a sufficient number of SNP loci for both Chinook salmon and steelhead while minimizing both false positive and false negative rates.

In addition, we also recognize that although modeling indicates as few as 60–100 SNPs may allow for accurate pedigree reconstruction (Anderson and Garza 2006), this number has been met with skepticism (PSC 2008) and has not been demonstrated empirically. We assess the power and assignment accuracy for various numbers of discovered SNPs. We also compare the assignment ability of SNPs to that of microsatellites by analyzing a dataset for parentage assignment using a panel of 95 SNPs and a panel of 13 GAPS-standardized microsatellites (Seeb et al. 2007) plus an additional four microsatellites developed for rainbow trout and steelhead.

Methods

SNP marker selection

To select the most informative subset of markers for PBT and GSI applications, a total of 395 and 245 SNP markers were evaluated for steelhead and Chinook salmon, respectively. The total number of markers available for this evaluation included published, unpublished, and newly designed SNP markers for both species. All available SNP markers for steelhead were used to genotype 288 individuals representing seven collections from the lower, middle, and upper Columbia River; Snake River; SE Alaska; McCloud Hatchery; a mixture from Bonneville Dam; and six Snake River hatchery stocks (Dworshak, East Fork Salmon, Grande Ronde, Lyon's Ferry, Pahsimeroi, and Sawtooth). The 245 available SNP markers for Chinook salmon were used to genotype 16 individuals from each of the following Snake River hatchery stocks: Sawtooth, Pahsimeroi, South Fork Salmon, Lostine, South Fork Clearwater, and Rapid River. Genotypes were collected using Fluidigm 96.96 chips and scored using the Fluidigm SNP genotyping software. Collection level minor allele frequency (MAF) and proportion of individuals genotyped per collection were calculated for each marker and each group of samples. Deviation from Hardy-Weinberg (HWE) and linkage equilibrium (LD) were tested using GENEPOP (Raymond and Rousset 1995).

A modified sex-specific assay for both steelhead (*IDFG-OMY-SEX*) and Chinook salmon (*IDFG-OTS-SEX*) was developed in order to differentiate sex in these species. We used published (Brunelli et al. 2008) and unpublished primers (Joseph Brunelli, personal communication) to sequence a Y-chromosome-specific region (*OmyY1*; EU081756) and develop primer extension assays that amplify a Y-specific product along with an autosomal product to act as a control. These products were interrogated using standard fluorogenic probes (TaqMan® chemistry; Applied Biosystems, Foster City, CA). Primer and probe sequences of the sex assay for each species are summarized in Table 3 and Table 4. Sex identification with these assays is accomplished through analysis of allelic discrimination plots of end-point fluorescent. The FAM fluorophore is associated with the probe for the Y-specific product (males), while the VIC fluorophore labels the autosomal product. Samples that exhibit VIC fluorescence but not FAM are identified as females. Accuracy of genetic sex typing was determined by comparing genotypes to phenotypic sex.

SNP evaluation

After the first round of screening and ranking the SNP markers according to overall MAF and proportion genotyped, subsets of loci for each species were further evaluated using 15 steelhead collections (n = 1,039) and nine Chinook salmon collections (n = 888) throughout the Columbia and Snake River basins (Tables 5a and 5b). Markers were evaluated for plot quality, and again ranked by MAF. Scoring concordance was evaluated by repeat genotyping a subset of samples, and scores were compared between IDFG and CRITFC labs for steelhead and between rerun samples within a lab for Chinook salmon. Conformation to HWE was again assessed for all collections, and linkage equilibrium was again evaluated via pairwise comparisons of all markers for six collections of steelhead and all Chinook salmon collections using GENEPOP. Significance values for each collection and pairwise marker comparisons were examined using Bonferroni corrected critical values (p = 0.00625 for Chinook salmon and p = 0.00333 for steelhead). A SNP marker was determined to be problematic if it was found to deviate from HWE in at least one collection, or if it was shown to be significantly linked to another locus in at least two collections (i.e., tests of LD).

Evaluation of Parental Assignment

Once candidate SNPs were evaluated for each species and specific loci were designated for PBT, the ability of these SNPs to accurately identify parentage was evaluated by screening a portion of parents from each Snake River hatchery stock with these SNPs along with samples of known-parentage offspring. Markers were evaluated for parentage assignment accuracy using the strict exclusion based method in CERVUS (Marshall et al. 1998; Kalinowski et al 2007).

Chinook Salmon SNPs—For Chinook salmon, parentage assignment accuracy was determined using 125 juveniles with known parentage based on previous analysis with microsatellite DNA markers. Multiple brood years of Chinook salmon at Johnson Creek (tributary to S. Fork of Salmon R.) were sampled to represent both parents and their offspring and used for parentage analysis (Table 6). The program CERVUS was used to examine mismatches of SNP genotypes between parents and progeny. Markers with low parentage assignment accuracy (i.e., 1 or more mismatching markers between a known parent-offspring pair) and/or the highest number of problems with LD and HWE were considered for replacement.

Steelhead SNPs—During screening and selection of steelhead SNPs, parental assignment accuracy was first evaluated using 90 juveniles with known parents based on hatchery broodstock records. To further evaluate the assignment ability of SNPs and empirically test the predictions of Anderson and Garza (2006) we ranked all 188 discovered steelhead SNPs by their minor allele frequency in a portion of the broodstock samples. We then used the top tiers of the ranked SNPs to assign 651 known-parentage offspring from six stocks back to a parental pair (thereby estimating a false negative rate) and tried to assign offspring known to not have parents in the database back to a parental pair (thereby estimating a false positive rate). Genotypes of parents and offspring were analyzed with SNPPIT, a software program specifically developed for large-scale PBT projects using SNP markers (Anderson 2010). This approach allowed us to estimate both the false negative and false positive rates using different numbers of SNPs and thereby determine a threshold at which these error rates are minimized. Based on results (below) a finalized set of 95 SNPs, comprising mainly of the top ranked loci with high assignment ability, was designated for PBT of hatchery stocks within the Snake River Basin. The assignment ability of this finalized set of SNPs was then compared to the assignment ability of a panel of 17 microsatellite loci.

Results

SNP marker selection

Markers with poor performance (i.e., poor plot quality), no variation, and/or violated HWE were not considered for further evaluation. In cases where two markers were found to be significantly linked, the marker with the lower MAF was discarded. The remaining markers were sorted by MAF within the Snake River hatchery samples, and the top 95 markers (MAF >0.10) were selected for the PBT panels for each species (Chinook salmon, Table 7; steelhead, Table 8). The GSI panel for steelhead included the next most variable markers, which do not overlap with those used for the PBT panel.

SNP evaluation

Chinook salmon—Eleven pairs and six individual loci showed linkage disequilibrium and deviations from HWE, respectively. However, only one pair of markers (Ots_OTSTF1-SNP1 and Ots_Tnsf) was significantly linked in seven out of the nine collections. Repeat genotyping yielded no discrepancies between scores. Two out of ten markers with poor plot quality were determined problematic. Eight markers with severe problems for a single analysis or moderate problems for multiple analyses were replaced (Table 9).

Steelhead—Two of the 95 markers showed significant deviations from HWE, but no pairs of markers showed significant linkage disequilibrium. Concordance of genotypes between IDFG and CRITFC labs was >99%. Three of the 21 markers with poor plot quality were determined to be problematic. Of the 90 offspring with known parentage, 35 were exact matches with their parents at all markers. The remaining 55 samples had single locus mismatches. These 55 samples represented a total of 31 crosses, and mismatches at 24 markers. Four markers with mismatches involved multiple crosses and were determined problematic. Eleven markers were exchanged between the PBT and GSI panels, and six markers with severe problems for a single analysis or moderate problems for multiple analyses were replaced (Table 10).

Assessment of Parental Assignment

Chinook salmon—A panel of 95 SNPs suitable for PBT were identified (Table 7). Of 125 progeny with known parentage (based on previous microsatellite analysis), 111 matched the parents at all loci. The remaining 14 samples had single locus mismatches. These 14 samples represented a total of nine crosses, and mismatches at four loci (Table 11). Of particular note, Ots_IsoT had seven mismatches across six known crosses.

Steelhead—Assignment accuracy using the top tiers of ranked SNPs was consistently high except when a small number of SNP loci were used (36 and 48 SNPs) (Figure 2, Table 12). The false negative rate never exceeded 10% except for the two smallest panels and the false positive rate never exceeded 1% except in the case of the 48 SNP panel (Table 12). As few as 72 SNPs produced greater than 95% accuracy and less than 5% false negatives, with only slight improvement when additional markers were evaluated (96, 120,144, 168, and 188; Figure 2, Table 12).

The assignment accuracy of this 95-SNP panel was then compared to that of a panel of 17 microsatellite loci. Assignment of offspring back to correct hatchery stock and to the same parents identified in the cross records was generally high for both microsatellites and SNPs (Table 13). Microsatellites assigned 98.6% of genotyped offspring and SNPs assigned 97.8%. In every case when a fish was assigned back to a stock it was the correct stock regardless if SNPs or microsatellites were used. None of the Lyons Ferry offspring, which purposefully had no parents in the dataset, assigned to any stock for either microsatellites or SNPs. Both kinds of molecular markers assigned the same offspring back to the same parents identified in the cross records. SNPs performed marginally better than microsatellites by assigning 91 offspring from the Grande Ronde hatchery stock to the same parents identified in the cross records while microsatellites assigned just 90 offspring to the same parents identified in the cross records.

Discussion

Initial screening of SNP markers was used to choose three panels of 96 markers (1 set for each species for PBT, and another set for steelhead GSI) in Chinook salmon and steelhead. These 96 marker subsets were then further evaluated by genotyping a larger number of collections representing the Columbia River and several Snake River hatcheries. This second-round evaluation of markers identified the most appropriate subsets of markers for PBT based on high MAF, minimal deviations from HWE and LD, high parentage accuracy, concordance of repeat genotyping, and scorability of plots. The remaining high-ranking markers were used to populate the GSI panel for steelhead. The GSI panel for Chinook salmon had been determined prior to the PBT panel selection and will remain in its original form for the current year, despite overlap of 50 markers with the PBT panel. Future modification to the Chinook salmon GSI panel is planned.

Chinook salmon SNPs

Low levels of mismatches between adult Chinook salmon and their offspring suggests several tendencies. First, genotyping of SNPs is consistent across generations. Second, low genotyping errors will translate to accurate assignment of offspring back to parents. Genotyping error is present in almost all genetic data, and can result in failing to assign a true parent-offspring trio due to mismatching loci. Such errors can be accommodated for in the software program SNPPIT, which allows for mismatches between pairs. Ultimately, the ability to empirically demonstrate the accuracy of parentage for offspring produced from multiple cohorts,

representing tens of thousands of potential parents, will have to be demonstrated after generational hatchery broodstock baselines have been constructed.

Steelhead SNPs

From the 188 designated SNPs, 95 were selected for use as a PBT SNP panel. These 95 SNPs represent loci that produced consistently clean genotype plots and have high minor allele frequencies in Snake River hatchery broodstock. Results of the iterative analysis with the top tiers of ranked SNPs demonstrate that the size of this final set of PBT SNPs is sufficient in power to conduct PBT in the Snake River basin.

Patterns in parentage assignment (Table 12, Figure 2) suggest ~72 SNPs is the minimum number needed for an acceptable level of assignment accuracy that also maintains an acceptable level of false negative and false positive rates. A false negative rate is meant to indicate the proportion of offspring that do not assign because of limitations in PBT or limitations in the resolution of the SNP markers used. The false negative values in this study are low, but are also likely inflated because some parents were either not sampled or had enough missing data to exclude them from assignment. This suggests that the ability of PBT to assign parentage using SNPs will not be limited by either the methodology or the resolving power of the selected molecular markers, but rather by the ability to sufficiently genotype all broodstock.

SNPs performed as well as microsatellites when comparing the assignment accuracy of the two kinds of markers. Both molecular markers assigned >97% of genotyped fish and both assigned the same offspring back the same parents identified in the cross records. These results demonstrate that the panel of 95 SNPs selected for PBT is just as powerful as a panel of 17 microsatellites in resolving parentage.

Regardless of the molecular marker used, some offspring did not assign to a hatchery stock. There was not 100% assignment back to stock for several reasons. In the case of SNPs, every instance of non-assignment was associated with one of two factors: 1.) large amount of missing genotype data for a parent or offspring, or 2.) one or both of the parents were not sampled. In the case of the first reason, large amounts of missing data prevent assignments from being made due to inconclusiveness. In the second, assignment is simply precluded because, even though we tried to sample every spawned adult, there were some cases we know some parents were missed, and offspring cannot be assigned to a parent that is not included in the dataset.

In some cases, offspring assigned to a parental pair but the parents did not match those identified in the cross records. All parentage assignments made by SNPs matched those made with microsatellites except for one offspring from the Grande Ronde hatchery stock that assigned to parents identified in the cross records using SNPs, but did not assign to those same parents using microsatellites. Because both markers showed discordance between the cross records and the parent assignments for the same offspring, this suggests that cross information is probably incorrect and the genetic markers caught the error.

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SECTION 3: INTEGRATION OF SNP MARKER SETS

This objective describes how the discovered SNPs were integrated such that, for each species, one panel of 95 loci plus a sex-specific marker can be used either independently for PBT of Snake River basin hatcheries or in conjunction with an additional panel of 93 loci plus 3 diagnostic rainbow/cutthroat-hybrid loci (steelhead) or 96 loci (Chinook salmon) for GSI within the Columbia River basin.

Introduction

A parental PBT genotype database could be incorporated into a genetic stock identification (GSI) database that shares molecular markers. This would allow for both wild and hatchery fish to be sampled and genotyped from the same fishery/migration with wild fish identified to stock using GSI, and hatchery fish identified to hatchery stock and cohort using PBT (Anderson and Garza 2005).

The Columbia River Intertribal Fish Commission's BPA Accord project (#2008-907-00) is a well-designed study to develop SNP baselines for steelhead and Chinook salmon in the Columbia River basin for the purposes of GSI (http://www.nwppc.org/fw/projectselection/accord/200890700.pdf). Through close collaboration with CRITFC, we identified a set of SNPs that can be used for both PBT and GSI. This will allow the integration of sampling programs at Bonneville and Lower Granite dam and Columbia River and Snake River main-stem fisheries such that any sampled wild adult could be included in mixture analyses to determine stock composition and any sampled Snake River hatchery adult could be assigned to a hatchery stock and cohort.

Methods

Descriptions of the process and results for SNP discovery, screening, and designation are covered in Section 2. Comparisons of SNP marker sets to meet PBT and GSI goals and objectives were conducted by IDFG and CRITFC labs.

Results

For Chinook salmon, a panel of 95 SNPs was designated for PBT of Snake River hatchery stocks (Table 7). This panel, when used in conjunction with an additional SNP panel, provides GSI in the Columbia River basin.

For steelhead, a panel comprising 95 SNPs that showed the most variation within Idaho hatchery populations was also designated for PBT (Table 8). A second panel comprising an additional 93 SNPs that showed high variability across the distribution of wild populations was designated for GSI. As demonstrated in Section 2, the PBT SNP panel is well-suited for accurate assignment of offspring back to hatchery stock while minimizing false positive and false negative error rates.

Discussion

For both species, one 95-SNP marker set has been identified for PBT purposes (Tables 7 and 8). SNPs that were screened, but not included in the PBT marker set, were effective for use in GSI. This integration of SNPs allows stock composition of wild fish sampled in the

Columbia River to be determined as well as stock composition and cohort of any sampled Snake River hatchery fish.

The PBT panel used in conjunction with the GSI panel provides the power needed for fine-scale resolution of reporting groups needed for GSI analyses. This creates an elegant and efficient solution for establishing a standardized set of SNPs that can be used for PBT and GSI projects.

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SECTION 4: CREATE PARENTAL GENETIC DATABASES FOR SNAKE RIVER HATCHERY CHINOOK SALMON AND STEELHEAD

This section presents summary statistics of genotypes for steelhead broodstocks in 2008 and 2009. Chinook salmon broodstocks from 2008 and 2009 have been genotyped, and analyses will be presented in the 2011 annual report.

Introduction

Once a set of PBT SNPs was identified for steelhead and Chinook salmon (Tables 7 and 8) and it was demonstrated that the selected SNPs would provide sufficient resolving power (Tables 11, 12, and 13), we began genotyping samples collected in 2008 and 2009 (Table 1 and 2).

During the first year of this project (FY2010), IDFG and CRITFC labs extracted and genotyped all sampled broodstock for steelhead and Chinook salmon (~15,000 IDFG, ~15,000 CRITFC = ~30,000 total samples). In the next contract year (FY2011), all broodstock samples collected in 2010 and 2011 will be genotyped by IDFG and CRITFC labs. By FY2012, all previous year's samples will have been genotyped and only samples collected in 2012 will need to be run.

Creation of these parental genetic databases establishes an unprecedented ability to mark millions of Snake River smolts and an opportunity to address a variety of parentage-based research objectives.

Methods

Laboratory Protocol

Genomic DNA extraction and amplification and SNP genotyping using multiplex 5'-nuclease reactions followed the methods described in Matala et al. (*In Press*). DNA was extracted using the Nexttec Genomic DNA Isolation Kit from XpressBio (Thurmont, MD) or Qiagen DNeasy (Valencia, CA). Prior to DNA amplification of SNP loci using primer-probe sets (fluorescent tags), an initial polymerase chain reaction (PCR) "pre-amp" was implemented using whole genomic DNA to jumpstart SNP amplification via increased copy number of target DNA regions. The PCR conditions for the pre-amp step were as follows: an initial mixing step of 95°C for 15 min, followed by 14 cycles of 95°C for 15 seconds and 60°C for four minutes, ending with a final 4°C dissociation step. For steelhead, all individuals were genotyped at 95 SNPs and a Y-specific allelic discrimination assay that differentiates sex. For Chinook salmon, all individuals were genotyped at 95 SNPs (including one mtDNA SNP) and a Y-specific allelic discrimination assay that differentiates sex. Genotyping was performed using Fluidigm 96.96 Dynamic Array IFCs (chips). For each genotyping run, 96 samples (including an extraction negative control, a PCR negative control, and a PCR positive control) and 96 TaqMan SNP assays were either hand-pipetted or auto-pipetted onto the 96.96 chips. Sample cocktail and SNP assay cocktail recipes are available by request from mike.ackerman@idfg.idaho.gov. Each 96.96 chip was pressurized to load the DNA and SNP assays into the array using a Fluidigm IFC Controller HX. SNP amplification on the 96.96 chips were performed using either an Epperndorf Stand-Alone Thermal Cycler (protocol: thermal mixing step of 50°C for 2 min, 70°C for 30 min, and 25°C for 10 min, a hot-start step of 50°C for 2 min and 95°C for 10 min, followed by 50 cycles of 95°C for 15 sec and 60°C for 60 sec, and a final cool down step of 25°C for 10 min) or a Fluidigm FC1 Fast-cycler (protocol: thermal mixing step of 70°C for 30 min and 25°C for 10 min, a hot-start step of 95°C for 60 sec, followed by 50 cycles of 95°C for 5 sec and 25°C for 25 sec, and a final

cool down step of 25°C for 10 min). Chips were imaged on a Fluidigm EP1 system and analyzed and scored using the Fluidigm SNP Genotyping Analysis Software version 3.1.1.

Standardized parental genotypes were stored on a Progeny database server housed at EFGL. Progeny software (http://www.progenygenetics.com/) is already used by the majority of GAPS labs throughout the Pacific Northwest: Idaho Department of Fish and Game, University of Washington, NOAA-Northwest Fisheries Science Center, Washington Department of Fish and Wildlife, Columbia River Intertribal Fish Commission, and U.S. Fish and Wildlife Service.

Data quality was inferred from estimates of completion rate, missing data, poor performing loci, and error rates. The program ML-NULLFREQ (Kalinowski and Taper 2006) was used to identify loci with null alleles and estimate the proportion of null alleles per locus. Basic diversity indices were calculated for the 2008 and 2009 steelhead databases. This included estimates of average heterozygosity (observed Ho and expected He) using ARLEQUIN (Excoffier and Lischer 2010), genetic structure (Fst and assignment tests) using GENEPOP (Rousset 2008) and ONCOR (Kalinowski et al. 2007), and effective population size (Ne) using LDNE (Waples and Do 2008). Genotyping of 2008 and 2009 Chinook salmon database was recently completed and calculation of diversity indices is underway and will be reported in the 2011 annual report.

Results

Completion Rate and Missing Data

If a sample failed to genotype at 10 or more SNPs it was re-extracted and regenotyped. If that sample failed a second time at 10 or more SNPs, it will be automatically precluded from future PBT analyses because the excess missing data prevents accurate parentage assignment. Samples with missing data for more than 10 SNPs were not excluded from the summary statistics presented in this report because useful information can still be gleaned from such samples.

For 2008 steelhead, all 5,151 samples were extracted and genotyped with 94 PBT SNPs and a sex-identification SNP. Because original primers and probes for locus Omy_Ogo4-212 failed to produce consistent genotypes, it was removed from the 2008 dataset and analyses proceeded with 94 SNPs. Modified primers and probes were created for the locus and it was included in the 2009 PBT SNP panel. Of the 5,151 samples, 5,070 (98.4%) were genotyped with an acceptable level of missing data (Table 14). In the final 2008 PBT baseline comprising the remaining 5,070 samples, there were only 1,980 missing genotypes out of 476,580 due to failure to genotype at particular SNPs, which accounted for 0.4% of the genotypes.

For 2009 steelhead, 5,636 of the 5,761 samples (97.8%) were genotyped with an acceptable level of missing data (Table 14). In the final 2009 PBT baseline comprising the remaining 5,636 samples, there were only 2,632 missing genotypes out of 529,784 (0.05%) due to failure to genotype at particular SNPs.

Poor performing loci

For 2008 steelhead, two loci failed to genotype at >3% of samples. Locus Omy_114315-438 failed to genotype for 300 of the samples (5.8%) and locus Omy_rapd-167 failed to genotype at 179 of the samples (3.5%). All remaining loci failed at <3% of samples.

For 2009 steelhead, eight loci failed to genotype >3% of samples. These loci failed at the following number of samples: locus Omy_Ogo4-212 at 464 (8.1%), Omy_114315-438 at 277 (4.8%), Omy_Il1b-198 260 (4.5%), OMS00179 failed to genotyped at 183 (3.2%), OMS00039 failed to genotyped at 182 (3.2%), OMS00118 failed to genotype at 180 (3.1%), Omy_114587-480 at 179 (3.1%), Omy_hsf2-146, At 179 (3.1%).

Error rate (Quality Control)

For 2008 steelhead a subset of 142 samples representing all 96-well extraction plates were regenotyped with the 94 SNPs and checked for discrepancies with original genotypes in order to estimate genotyping error rates. This resulted in 13,348 rerun genotypes being compared to the original genotypes. Of these genotypes, 258 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 13,090 genotypes with 57 discrepancies between the original and samples and a genotyping error rate of 0.44%.

For 2009 steelhead a subset of 252 samples representing all 96-well extraction plates were rerun with all 95 SNPs and checked for discrepancies. This resulted in 23,940 rerun genotypes being compared to the original genotypes. Of these genotypes, 1,638 had a SNP failure either in the original genotype or the rerun genotype and could not be used in error estimation. This resulted in 22,302 genotypes with 224 discrepancies between the original and samples and a genotyping error rate of 1.00%.

Null alleles

For 2008 steelhead, 58 of the 94 PBT loci were found to have a frequency of null alleles greater than zero, but only four loci had frequencies greater than 5% (Table 15). For 2009 steelhead, 72 loci of the 94 PBT loci were found to have a frequency of null alleles greater than zero, but only four loci had frequencies greater than 5% (Table 16). Frequency of null alleles ranged from <1% to 9.2% in 2008 and from <1% to 7.7% in 2009.

Sex markers

The sex-specific assay for steelhead matched phenotypic sex in 98.4% of the samples (Table 17). For instances in which genetically-determined sex did not correspond to the phenotypic sex, all were phenotypic males that were misidentified by genotype as females except for one case in which a phenotypic female was misidentified as a male. The sex assay either failed to genotype or provided ambiguous results for 2.3% of the samples.

The sex-specific assay for Chinook salmon matched phenotypic sex in 96.1% (Table 18) of samples. All the discrepancies between phenotypic and genotypic sex involved phenotypic males being misidentified by genotypes as females. The assay produced ambiguous results or failed to genotype for 17.9% of samples.

Average He

Levels of observed heterozygosity were approximately 0.4 for all hatcheries and were similar among hatchery broodstocks and between years (Table 19).

Population Structure (Fst)

Pairwise Fst was calculated for 2008 and 2009 hatchery stocks (Tables 20 and 21). Values ranged from a low of 0.006 between Oxbow and Sawtooth in 2008 and a low of 0.005 between Oxbow and Pahsimeroi in 2009 to a high of 0.05 between Dworshak and East Fk. Salmon River in 2008 to a high of 0.069 between Touchet and Squaw Crk. All Fst values among stocks were significant within each year.

Inferences about population structure were also examined by conducting a leave-one-out test conducted in ONCOR (Kalinowski et al. 2007). This test determines what proportion of fish in a population can be assigned back to their population of origin and identifies the most common population that fish are misassigned to. A high proportion of individuals from both the 2008 and 2009 broodstocks assigned back to their population of origin (Tables 22 and 23). Populations that fish most often misassigned to were stocks with shared population histories.

Effective Population Size (Ne)

Effective population size (Ne) and 95% CI for each hatchery stock in 2008 and 2009 was calculated (Table 24). Estimates of effective population size ranged from a low of 18 for East Fk. Salmon R. in 2008 and a low of 19 for Squaw Cr. in 2009 to a high of 226 for Dworshak in 2008 and a high of 261 for Pahsimeroi in 2009.

Discussion

Completion Rate and Missing Data

The high rate of sample completeness and low rate of missing data demonstrates the feasibility of collecting high quality data for nearly all Snake River basin broodstock.

Poor loci

Locus Omy_rapd-167, which was identified as a poor performing locus in 2008, failed to genotype 3.5% of samples in 2008 also failed at 2.5% of samples for 2009 samples. Locus Omy_114315-438 failed to genotype 5.8% of samples in 2008 and 4.8% of samples in 2009. Locus Omy_Ogo4-212 was removed from the 2008 parental database because of problems with consistent genotyping and included again for the 2009 dataset after modified primers and probes were created. However, even after modification this locus still failed to genotype 8.1% of samples in 2009. This locus also has the highest frequency of null alleles (Table 16). This locus may need to be replaced or further modified to ensure consistent genotypes. All other loci were retained with an acceptable level of missing data.

Error rate (Quality Control*)*

To minimize false negatives in parentage assignments, genetic markers need to exhibit low genotyping error rates and researchers should accommodate estimated error rates during data analysis (Kalinowski et al. 2007). Genotyping error rates for microsatellite markers are variable, but have often been reported between 1-2% (Pearse et al. 2009; Hauser et al. 2011). For the parentage software programs CERVUS and SNPPIT, the default error rate used is 1%. In our study, we observed error rates ≤1% for both years.

Null alleles

Three loci consistently had the highest estimated levels of null alleles between years. These three loci had frequency estimates of >5% for both years: Omy_113490-159, Omy_vatf-406, OMS00118. These three loci may have population specific problems with amplifying heterozygous individuals and will be analyzed with caution in future samples. Null alleles at all other loci were minor.

Sex Markers

Sex-specific assays for both species performed very well with the genotypically determined sex matching phenotypic sex in 98.4% of steelhead samples and in 96.1% of Chinook salmon samples. The steelhead sex assay also had a high level of genotyping completeness and failed to produce genotypes for just 2.3% of samples. However, the Chinook salmon sex assay had a much higher failure rate of 17.9% and additional modification of the Chinook salmon sex assay may be needed to reduce its failure rate. Overall, the results are generally encouraging in that these modified assays can provide an accurate and nonlethal method of sex determination for these species.

Average He

The average expected heterozygosity was high and uniform across hatchery stocks (39.5%-43.0%), demonstrating that this set of SNP loci should be useful for parentage tagging throughout the Snake River basin.

Population Structure (Fst)

For both 2008 and 2009, the highest Fst values were seen between Dworshak Hatchery and other locations, except with the Squaw Creek source, which is derived from Dworshak stock. The lowest Fst values were seen among Oxbow, Sawtooth, and Pahsimeroi stock (Tables 20 and 21). Low divergence among Oxbow, Sawtooth, and Pahsimeroi reflect their shared history of being recently derived from stocks whose brood source came from wild adult steelhead trapped at the Hells Canyon Dam on the Snake River in the late 1960s (Nielsen et al. 2009). The larger degree of divergence between Dworshak and the other stocks reflects the distinctness of Clearwater origin fish to those in the Salmon and Snake rivers.

Patterns from the leave-one-out-test also reflect the population history of the hatchery stocks (Table 22). The large amount of misassignment (45.5% in 2008 and 18.2% in 2009) of Squaw Crk. stock to Dworshak stock reiterates the close relatedness of the two stocks. Similarly, the relatively high degree of misassignment of Pahsimeroi and Sawtooth stocks (respectively, 15.8% and 20.9% in 2008 and 14.6% and 13.3% in 2009) to the Oxbow stock also reflects the shared history of these stocks. This further emphasizes the need for a PBT approach to determine the origin of these hatchery stocks as GSI approaches would be unable to distinguish them.

Effective Population Size (Ne)

Effective population sizes generally corresponded to size of broodstock. Larger hatchery programs (e.g. Dworshak, Oxbow, Pahsimeroi, and Sawtooth) tended to have larger Ne, while programs with smaller broodstocks (Squaw Crk, EFSR, and Grande Ronde) had smaller Ne. Estimates of Ne for Touchet yielded negative values and both Touchet and Tucannon exhibited infinitely large CI intervals, indicating that there was no evidence of linkage disequilibrium for these populations.

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SECTION 5: UTILIZE PBT METHODS TO PROVIDE ACCURATE PARENTAL ASSIGNMENTS

Once initial baselines are completed for each species, work will begin on assessing the accuracy and utility of PBT for addressing a multitude of conservation and management questions involving hatchery stocks. This includes questions associated with hatchery and harvest management, understanding and quantifying hatchery straying and natural spawning stock composition, understanding the origin of hatchery kelt steelhead out-migrating in the Snake River, and understanding mechanisms that affect age at maturity in hatchery salmon and steelhead. Upcoming projects that will utilize the PBT datasets include identifying origin of hatchery strays, identification of hatchery kelts, composition of Snake River hatchery fish passing Bonneville Dam and harvested in mainstem Columbia, and comparisons of stock and cohort assignments between CWT and PBT.

Progress reports on these ongoing projects will be presented in the next annual report.

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TABLES

Table 1. Total steelhead hatchery broodstock genetically sampled in 2008 and 2009 in the Snake River basin. *Early January egg takes missed. **No hatchery broodstock samples were collected in 2008. The remaining hatcheries/years had 100% of the broodstock sampled.

Snake River Hatchery	2008 Sampled	2009 Sampled
LSRCP/IDFG Sawtooth (IDFG & SBT)	639	979
LSRCP/IDFG Sawtooth (EFSR)	119	132
LSRCP/IDFG Sawtooth (USB/Squaw)	35	53
Idaho Power/IDFG, Oxbow F.H.	927	592
Idaho Power/IDFG, Pahsimeroi F.H.	1531	968
Idaho Power/IDFG, Pahsimeroi F.H (SBT)	312	336
LSRCP/IDFG/USFWS Dworshak/C.W.	1419*	1880
LSRCP/ODFW-Wallowa F.H.	0**	460
LSRCP/WDFW-Lyons Ferry	0*	215
LSRCP/WDFW-L.F. (Tucannon)	0*	25
LSRCP/WDFW-L.F. (Touchet)	0*	26
LSRCP/WDFW-L.F. (G.R. cottonwood)	169	95
Total	5151	5761

Table 2. Total Chinook salmon hatchery broodstock sampled in 2008 and 2009 in the Snake River basin. *Broodstock collected directly from a trap near mouth of creek.

Snake River Hatchery	2008 Sampled	2009 Sampled
Idaho Power/IDFG, Rapid River	2866	2098
LSRCP/USFWS, Dworshak	1216	1180
LSRCP/IDFG, Clearwater (Powell)	902	860
LSRCP/IDFG, Clearwater (SF)	1029	861
LSRCP/IDFG, Sawtooth	1188	1010
Idaho Power/IDFG, Pahsimeroi River	713	628
LSRCP/WDFW-L.F. (Tucannon)	131	175
LSRCP/IDFG, McCall (SFSR)	1920	946
LSRCP/ODFW, Imnaha	247	227
LSRCP/ODFW/NPT, Lostine	109	106
LSRCP/ODFW, Catherine Crk.	57	80
LSRCP/ODFW, Grande Ronde	27	112
LSRCP/ODFW, Lookingglass Crk.	150	65
Nez Perce Tribal Hatchery (NPTH)	193	428
Johnson Crk. (EFSF Salmon River)*	63	54
Total	10748	8776

Table 3. Primer and probe sequences for modified Y-specific assay (IDFG-OMY-SEX) that differentiates sex in steelhead.

Primer/Probe Name	Primer/Probe Sequence
OmyY1 Probe e2	6FAM-CCT ACC AAG TAC AGC CCC AA
OmyA Probe e500 (autosomal)	VIC-GAG GGG TAG TCG TTT GTT CG
OmyY1.4 F primer	5'- CAC AAC ATG AGC TCA TGG G -3'
OmyY1.4 R primer	5'- CGA TTA GAA AGG CCT GCT TG -3'
OmyA F primer (autosomal)	5'- GCC TGC TTG CAG AAG TTT TT -3'
OmyA R primer (autosomal)	5'- CTT GAC TGT GTC CAG CTT GC -3'

Table 4. Primer and probe sequences for modified Y-specific assay (IDFG-OTS-SEX) that differentiates sex in Chinook salmon.

Primer/Probe Name	Primer/Probe Sequence
OtsY1 Probe e2	6FAM-CCT ACC AAG TAC AAC CCC AA
Ots474 Probe (autosomal)	VIC-CAG AAT TAG CTT TGG ACA TT
OmyY1.4 F primer	5'-CAC AAC ATG AGC TCA TGG G -3'
OmyY1.4 R primer	5'-CGA TTA GAA AGG CCT GCT TG -3'
Ots474 F primer (autosomal)	5'-TCC TTG TGT CTA AAG GGC TTT GAG -3'
Ots474 R primer (autosomal)	5'-GGG CTT GCT AGT CCT AAA CAG ATC -3'

Table 5. Collection sites and sample numbers for Chinook salmon (a) and steelhead (b) used for evaluation of the initial panels of SNP markers.

a.)

***O. mykiss* collections**	**n**
Dworshak Hatchery *	92
East Fork Salmon River	90
Grande Ronde River *	48
Hood River	94
John Day River	94
Kalama River	94
Loon Creek	45
Methow River *	93
Pahsimeroi Hatchery	96
Pelton Hatchery *	49
Sawtooth Hatchery *	43
Shitike Creek *	32
Squaw Creek	34
Tucannon	45
Upper Salmon B stock	90

(b.)

***O. tshawytscha* collections**	**n**
Cowlitz hatchery	93
Johnson Creek	184
Lostine River	90
Pahsimeroi River	92
Sawtooth Hatchery	94
Shitike Creek	92
South Fork Clearwater River	88
South Fork Salmon River	94
Tucannon Creek	61

* Populations analyzed to evaluate LD

Table 6. Samples from multiple brood years at Johnson Creek to test assignment ability of Chinook salmon SNPs.

Population	**Stage**	**n**
Johnson Creek 08	Adult	83
Johnson Creek 05	Adult	54
Johnson Creek 98	Adult	4
Johnson Creek 99	Adult	29
Johnson Creek 01	Adult	14

Table 7. Primers and probe information for the Chinook salmon PBT SNP panel.

SNP Name	Forward (F) and Reverse (R) Primers	Fluorescently Labeled Probes
Ots_SEXY1	F - CACAACATGAGCTCATGGG	
	R - CGATTAGAAAGGCCTGCTTG	6FAM - CCTACCAAGTACAGCCCCAA
Ots474 (autosomal control)	F - TCCTTGTGTCTAAAGGGCTTTGAG	VIC - CAGAATTAGCTTTGGACATT
	R - GGGCTTGCTAGTCCTAAACAGATC	
Ots_128757-61R	F - CGTGTCCGGCTTCTTTTATTTCATT	VIC - TTGTGCATTTTCCCC
	R - GATGGGTATGTTAATCATATTACCAGCGTAA	6FAM - TGTGCATTTCCCCC
Ots_Ikaros-250	F - GAGGCTGACTTGGACTTTGC	VIC - ACAGAAGATTTTCGGCTGC
	R - GGCCTGTCAGCCAAGGA	6FAM - ACAGAAGATTTTCGACTGC
Ots_nkef-192	F - CATTTAGCAGACACTCTTATCTTAGTGTCA	VIC - AATAGGCCGACATCAA
	R - CGAATGTCCACCTCAGATGTTACAA	6FAM - AAATAGGCCAACATCAA
Ots_u07-07.161	F - GTCAACAAATGCAGGTAACATAAATGGT	VIC - ATCAGTGACATAAGTTGTCCA
	R - GATGCAAACACCTGTGAAATTGTGA	6FAM - TCAGTGACATAAATTGTCCA
Ots_u6-75	F - GAAAAAGTAAAGTAAAAGTAAAGTATTATACCACTAAAGACAAT	VIC - TTAGTCAACTGTTGTTTTT
	R - GATCCACACTGTTGGTCTACTACAA	6FAM - TTAGTCAACTGTTATTTTT
Ots_113242-216	F - GAGGCCTAATGTCTCTTGTGACT	VIC - ATTACCAACGGAGAACC
	R - GACATCTTCAACAAGTGTTCATTCACC	6FAM - TTACCAACAGAGAACC
Ots_CD59-2	F - TGTTTATCTCTGAGTGAAAAAGGTGTGT	VIC - CTAAAATGTCATGTAAATAT
	R - CATGTTACCCAGCTAAAAGTCTATAGCA	6FAM - ACTAAAATGTCATATAAATAT
Ots_GDH-81x	F - CTTTTCTGAATTAGTGCTGTGCTTGT	VIC - TGTTACGGGACATACT
	R - CCAACTTCTTCAACTCTGTCAGTGA	6FAM - TCTGTTACGGACATACT
Ots_IL8R_C8	F - CGTGGTGTTCGCCTTCCT	VIC - CTGGACGCCGTTACA
	R - TGTCGGCCATCACTGTCATG	6FAM - TGGACGCCATTACA
Ots_NOD1	F - GTGCTGCAGGAACCATGTG	VIC - CCAACGGCGACTTG
	R - CTGTGTGGACTGCTGTCTAAGG	6FAM - CCAACGCCGACTTG
Ots_SWS1op-182	F - TCAAAGACATCGAACACAAGAACGA	VIC - ATGTACTTTAACGATTCATTT
	R - GCAGGTAAATTCAAACGTCATCATAAGAA	6FAM - ATGTACTTTAACGTTTCATTT
Ots_u07-17.135	F - CTCGCCTCTGTCATTGTATTACCTT	VIC - AAAATGTACCACATACTTGT
	R - TGACACACGAGCCATTTTGATGAT	6FAM - AAATGTACCACATACTCGT
Ots_unk526	F - TCAAGACTGTGCTGTAGTTGTCTAC	VIC - CAACATTCCAGTCTGAAAC
	R - CCTCCCCCTTTTCCACATCAG	6FAM - CATTCCAGCCTGAAAC
Ots_105105-613	F - AGTACAAGTGCAGAGAATGACATCATG	VIC - CCGAGCTTGAGTTAGGA
	R - GGTGTTTTATTTTCCCATATATCTTTTAACTTTAAGCT	6FAM - CCGAGCTTGACTTAGGA
Ots_94857-232R	F - GGCACTCTCCCTGGCTAGA	VIC - CAGGATAATAACAAACAAG
	R - CCCCATCACTTCTCTGGCTTTAAAT	6FAM - CAGGATAATAACGAACAAG
Ots_GPH-318	F - GGTGATAACAGGTGTTGCACCAA	VIC - ATCAAGCTGACGAACCA
	R - TCAGGTGGTGGTGGACAAC	6FAM - CAAGCTGACAAACCA
Ots_mapK-3'-309	F - CGTGACCCTTGTAACTGAAAAGC	VIC - ATGCTATTAAATGAATATTC
	R - GGCCACTGTCATAGAATTAGGCATT	6FAM - ATGCTATTAAATGACTATTC
Ots_TAPBP	F - TTTCTCATCCTTCTCTCTTCCAGTCT	VIC - CTGGACAGCTGGTCC
	R - GGACAAACCAGCACTCCAGAA	6FAM - CTGGACAACTGGTCC

Table 7. Continued.

SNP Name	Forward (F) and Reverse (R) Primers	Fluorescently Labeled Probes
Ots_u07-18.378	F - GGAAACCAGCTAGGATTCAGGAA	VIC - ATATGGTATGTAGAGGCTAGTTA
	R - CGTTATATGGTTTGCTTGTTTGCGATA	6FAM - TATGTAGAGGCAAGTTA
Ots_94903-99R	F - CCGTCTGAGTAGGAGGATCAATACA	VIC - CAAACCAGCAAACAT
	R - TTTGGATCCAGCTCTCCGTATAGA	6FAM - ACAAACCAGAAAACAT
Ots_cox1-241	F - CACTGAACTGTAAGCCATTGTGATT	VIC - CACTACGGTAAGACCAT
	R - GTAAATGTAGTATACAGTATAGGCATCGTAGGT	6FAM - CACTACAGTAAGACCAT
Ots_GTH2B-550	F - TGACTACCCGTTGTACCAATGAAC	VIC - TTAATGCTGCAGATGTTAT
	R - CACAGGAAGGACGTGTTTTGATG	6FAM - ATGCTGCACATGTTAT
Ots_mapKpr-151	F - TGTTGTCTCGGACTGCATGAC	VIC - CATGCATTGCACATAC
	R - GAAGGCACAGAGATGAAGGACAT	6FAM - CATGCAATGCACATAC
Ots_Prl2	F - CCTGGTCTGTTTGTGATCAAGATG	VIC - ATGTATTGTTCATTTAATG
	R - GGTTAACTCAAATAGAACATACTCTGACACA	6FAM - TGTATTGTTCGTTTAATG
Ots_TGFB	F - GCCTCACATTTTACTGATGTCACTTC	VIC - CTTCCGAGAGCTAGGCT
	R - GAGCAGATCTCTTCAGTAGTGGTTT	6FAM - CTTCCGAGAACTAGGCT
Ots_u07-25.325	F - AGACAATCATGGTGTTTTGAGTCTTTCT	VIC - CCGCTTGAAAGTTTGA
	R - GCCTAGGCTTGATGGAGTCA	6FAM - CGCTTGAAGGTTTGA
Ots_96500-180	F - GATCATGTCAGATAGGATGCTGAAAGT	VIC - AAAACAAATCATTTTTCG
	R - CAGGTCTGGTCTACATCGAACAC	6FAM - AAAAACAAATAATTTTTCG
Ots_E2-275	F - GGTGCCACTTTAGTATAGCTGCTTA	VIC - CCCCCATATTGCTG
	R - CCCTACCCCCTGTGTTCCA	6FAM - CCCCACATTGCTG
Ots_MHC1	F - GTCCACATTCTCCAGTACATGTATGG	VIC - CATCATCCCGTGAGCAG
	R - CAAACCCCTCTGTCTGTTCAGT	6FAM - TCATCATCCCATGAGCAG
Ots_RAG3	F - CATTTCCACGAAAAGCCAGATGAC	VIC - CTCTACAGTATGAACTATG
	R - ACAGAATAAAGTATCTTCCTCTTACATCACTACTAAT	6FAM - CTCTACAATATGAACTATG
Ots_u07-49.290	F - GCTGAGGAAGGATTCTGTATTTGCT	VIC - CTTTCCCCGTGTTGGT
	R - TCGGACAGAGCGCATCC	6FAM - ACTTTCCCTGTGTTGGT
Ots_96899-357R	F - TCTCCTGAACTAATTTAGACCTCTGAATGT	VIC - CTGAATGTTTTTTTTAATCTTT
	R - CCTCATATTGCTTTCATCTGAAGAGAGA	6FAM - CTGAATGTTTTTTTTTATCTTT
Ots_hsc71-3'-488	F - TGCATCCATTCATACCTGACCAATT	VIC - TTTCCAATGGTATAGATATGA
	R - TTTGGTTAGGCACACGATAATTTGC	6FAM - TTTCCAATGATATAGATATGA
Ots_MHC2	F - GTCCTCAGCTGGGTCAAGAG	VIC - CTGGAGCGTTTCTGTA
	R - GTAGTGGAGAGCAGCGTTAGG	6FAM - CTGGAGCGTGTCTGTA
Ots_TLR3	F - TGCACCTGCGAGAGCAT	VIC - CTGTGGTTTGTGGCGTG
	R - CTGGCGTTTGTTCCGTTCAG	6FAM - CTGTGGTTTGTAGCGTG
Ots_102414-395	F - GCCTACTGATAAATGTATGACAGTAATGGA	VIC - CACATAGTGTAGCTTTACTAC
	R - CAATAACAAACAAGCTAGGAACAAAAGTGT	6FAM - CACATAGTGTAGCTCTACTAC
Ots_ARNT	F - CCACTGGCTGTGGAGCTT	VIC - TACAGATGTCATTTTAC
	R - GGGTTCAGTGATAGTTGGGCAAAT	6FAM - CTACAGATGTAATTTTAC
Ots_ETIF1A	F - TCTGAACTCACCAAAGGAACACTTG	VIC - CAACTGAAGAAAATAATATG
	R - GAGAGAAAAGGAGAAATGATTGCCATT	6FAM - CTGAAGAAAAGAATATG
Ots_HSP90B-100	F - CACCTTAGTTCCACGCAACATG	VIC - TCTATGGTGTGATTCATT
	R - CTGCGTGTATTGTAGTGGTGACA	6FAM - TTCTATGGTGTAATTCATT

Table 7. Continued.

SNP Name	Forward (F) and Reverse (R) Primers	Fluorescently Labeled Probes
Ots_mybp-85	F - CAAGGGATGTGACAAATTAATCAAACACATAA	VIC - AGAGCATGTAGTTTTG
	R - AAGAGGTCTAATAAATCTCCAATGTAAAAACGT	6FAM - AGCATGTAATTTTG
Ots_P53	F - GGAACTTCCTCTCCCGTTCTG	VIC - CTGGGTCGGCGCT
	R - GCACACACACGCACCTCAA	6FAM - TGGGTCGACGCTC
Ots_S7-1	F - TGCCATCATAAACAACCTAACAAGTAACT	VIC - TACAGGAGATAAGGTCGCA
	R - CCTGGTTTAAAAACGGCCAACTG	6FAM - CAGGAGATAGGGTCGCA
Ots_u211-85	F - TGGTGAGAGCAGCTTTAAATGTCTT	VIC - TCCCAAAGTCGAGTGTG
	R - ACCCATTCTTCTGTCTGGTTTAAGC	6FAM - CCCAAAGTCAAGTGTG
Ots_AsnRS-60	F - CCGACGCCTCACTGAGT	VIC - TGAGTCCCTGACCAGC
	R - TGGTTTTTCAGGTCATGGTTTCCA	6FAM - AGTCCCCGACCAGC
Ots_FGF6B_1	F - GAGACAAAGGTTTGCAGGTTCATG	VIC - CCTGTTATCAGACCCAAAT
	R - GGGAGCCATGCACTAATATATTGGA	6FAM - CTGTTATCAGCCCCAAAT
Ots_IGF-I.1-76	F - GGTAGGCCGTCAGTGTAAAATAAGT	VIC - CTGCCTAGTTAAATAAAATA
	R - GATGGAGGCCACTGTGTTCTTA	6FAM - CTGCCTAGTTAAATTAAATA
Ots_SClkF2R2-135	F - CCAAATACAGACCAGCTACTTGTGT	VIC - ATTCAAAGTCAAATTTT
	R - CTTCAAGTCCCTGAATAATGGTACGT	6FAM - ATTCAAAGTCTAATTTT
Ots_u4-92	F - ATCCAAGGAGCCCCATTAAAGATTT	VIC - CTGTGTTGAATTTAACATAAT
	R - CGTACCAGAGTTGTAGAAGCATCT	6FAM - TCTGTGTTGAATTTAACGTAAT
Ots_110064-383	F - AACAAAGAATGTTAAACACCAAACAGGAA	VIC - CTACGTAATGAACGTTAGCT
	R - GTGCAAGGGACCTAGCTAATCC	6FAM - ACGTAATGAACATTAGCT
Ots_100884-287	F - CGGAAGACCAGATTCTCCAAGAGTA	VIC - ATAGAACTACAATTCACATATAT
	R - CGACCAAGTAGCGGCACTT	6FAM - AACTACAATTCGCATATAT
Ots_101554-407	F - TGAAAGATATCAATTGTAGTAGTGGTGGTG	VIC - ATGGAGGATTGTGGTTGT
	R - ACACGCCAGTCCACAAGT	6FAM - ATGGAGGATTCTGGTTGT
Ots_101704-143	F - ACTTCTTGAGCCAATCGGATGATG	VIC - CTTAGACGTCAGAGGTC
	R - CCAGAGATAAACTAGTGGAGGAGATCA	6FAM - CTTAGACGTCCGAGGTC
Ots_102801-308	F - TGGGACAGAGGTGGGAATTGA	VIC - AGGGACAGTTTCGCAGACG
	R - CCCAAAGATGCTTAACTGAAGATGTG	6FAM - AAGGGACAGTTTCTCAGACG
Ots_103122-180	F - CAAACGCGCACTCACACA	VIC - CATCAACACAATCTGC
	R - TCACAATGGTACGATTTTACGACTCAA	6FAM - CATCAACACGATCTGC
Ots_104415-88	F - CCTGAGCATCCCAGTTGAACT	VIC - TCCTGAAAAACGACATCC
	R - TGTTTTCAATACACTGCAATTTAGTTTTGGT	6FAM - CTGAAAAACAACATCC
Ots_105132-200	F - CGATGTACTGAGGGCAGTGT	VIC - CAAGAGTGGCATAAAA
	R - GAGTGGAGTTCCTTAATAATCATTGACCTT	6FAM - CAAGAGTGGAATAAAA
Ots_105385-421	F - GACTGTCTTGGAACCGTTGCTA	VIC - CCTCCTGGGTATATCG
	R - TCCCGGAACACACCAATGTC	6FAM - CTCCTGGGCATATCG
Ots_105407-117	F - TGTGTACATCCGCGTAAATATTGAAGATAA	VIC - CAGGTTAGGAATGGTTG
	R - CTGTGAGCTGCTGCAAACC	6FAM - CAGGTTAGGATTGGTTG
Ots_108820-336	F - TGAAATAAATTGTTCTGTTGATATGTGAATTTTGGA	VIC - ATTGCCCATCTCAGAATA
	R - CAACGACACACCAACAACGT	6FAM - AATTGCCCATCTTAGAATA
Ots_109525-816	F - GCCAGATAGTAGCGTACATCATGAG	VIC - CATGAGGCGTTCGGC
	R - CTCCCCATGTCCCTGAGTCT	6FAM - ATGAGGCATTCGGC

Table 7. Continued.

SNP Name	Forward (F) and Reverse (R) Primers	Fluorescently Labeled Probes

Ots_110201-363	F - GTTTGGCTATTGAAATTATACATTAAAACATGTAGCT	VIC - TGGATGCCAGTTTTAAAA
	R - CCATGGCATCCTGTAAAGAACAACA	6FAM - TGGATGCCAGTTTAAAAA
Ots_110495-380	F - GCCTAGGTATGTACGAAACTTCACA	VIC - ATGGCCCCTGTCTATG
	R - AGGCTTTTTCAGATGGTCGTATGA	6FAM - ATGGCCCCTGTGTATG
Ots_110551-64	F - GAGTGGTCAAGGTTTCAGTTTCTG	VIC - ACGCTCGGAACATT
	R - GAAATGGACAGACACAAGGTCAAAC	6FAM - ACGCTCTGAACATT
Ots_110689-218	F - GTATAAACTAGAGTCCAGTGTTATGTTAATGTCTT	VIC - CACCAATCAATTAATTATT
	R - CATGGCAGACAACAGTAGAGAATATGA	6FAM - ACCAATCAATTCATTATT
Ots_112301-43	F - GCATGGCTGCCCTAGAACA	VIC - CGTCGCATTCAGC
	R - TCAGAACATTTCCTTCAGCTTCGT	6FAM - CGTCGCGTTCAGC
Ots_112419-131	F - GTGGGTAATCGATGCCAAAGAGAT	VIC - AAGCGACTTGATTATC
	R - TGGCAGTGTTTTCAACTAGCTTTG	6FAM - AGCGACATGATTATC
Ots_112820-284	F - CATAGATGTTTATATGAAAAACCTCCCACTGT	VIC - ACTCACACTCGAGTGACT
	R - GCATCCAAAAAGACGTGTGTGTTT	6FAM - ACTCACACTCAAGTGACT
Ots_112876-371	F - GCCTACAGCAAATTCAGCTACACAT	VIC - CATCACAACGATGTGTG
	R - TGGACCTTCAATCATCACAGCTT	6FAM - CACATCACAACTATGTGTG
Ots_115987-325	F - GGAGGTGTAGTGAAATGGGAAGAT	VIC - ATGCATAAAAGGTAATTGTG
	R - GCATTCAGTGAACCAGTAGTGCTAT	6FAM - ATGCATAAAAGGTCATTGTG
Ots_117432-409	F - TCATCAAAACATGCCTCTTCTGTGT	VIC - TTTAGACTTTGCTCTATAACAG
	R - TGTTGAACCTGTCACTCTGTCTTC	6FAM - ACTTTGCTCCATAACAG
Ots_118205-61	F - CCATACAGCCAGTCCAGGTG	VIC - TAGTAGCCCCTACACCTC
	R - ACTGGACAGGGCTGGGT	6FAM - TAGCCCCTGCACCTC
Ots_118938-325	F - ATTTTCAAACAGGCATTTATCATTGGTGAA	VIC - AGAGATGCAAAGTGGAGTT
	R - GGTCTGTCCCTCATTCTTTGCA	6FAM - AGAGATGCAAAATGGAGTT
Ots_123921-111	F - TCGCTAGGCAGAAATATAGGGTTCT	VIC - TGCTAAATGGCATATATTAT
	R - GAGCATGGCGCTTGCA	6FAM - CTAAATGGCACATATTAT
Ots_124774-477	F - AGTTGTTCTTTTTATATTGTGTTTTTATTCCATTCCA	VIC - CCACCGCCATCTGATA
	R - GCCAAATAAAAACAAAGCATGAACACA	6FAM - CACCGCCGTCTGATA
Ots_129458-451	F - TGGGACCCACATAAAGCAACTG	VIC - CATCTGGCAATGCCTT
	R - GACATAAGACCCATTTAGCCCCTTTT	6FAM - CATCTGGCAGTGCCTT
Ots_brp16-64	F - ACTCTGGGTCCAGGAGGTTTT	VIC - AAGTCAGCATCTTTCA
	R - CTGACGAGACCATGCACCAA	6FAM - AGTCAGCGTCTTTCA
Ots_CirpA	F - GCTGTGATTGTGCTCTAAAGACATG	VIC - AATGCATTACAGAACTGA
	R - CTCCCACTTAGCATTCCTACCTT	6FAM - AATGCATTACAAAACTGA
Ots_Est740	F - GGACTCGTGCTTGAGGAAGATG	VIC - TCTGGATGGAACCGTTAG
	R - TGCATGGCTCCAACTCCTT	6FAM - CTGGATGGAGCCGTTAG
Ots_GCSH	F - GTTCTTTTTAATGATGACTACAGGTCTTTCAC	VIC - TATCTGGGCGGGCTG
	R - GCTACTTTACATAATACCATTTGAGCTGAGA	6FAM - CTATCTGGACGGGCTG
Ots_HMGB1-73	F - TGCTTCAGTGAAAATAAGCGTGAGA	VIC - ACTGTATATGTTACGTTTTC
	R - GTCGAGCGGTATGAATACTTTCTGA	6FAM - ACTGTATATGTTAAGTTTTC
Ots_NFYB-147	F - CCGTCCACAGCACAAGACTATAATA	VIC - TGTTCCAATGTAAAATGTATGC
	R - CAGATGATAGCTTCAGTAAGTGGTTCA	6FAM - TTCCAATGTAAAATATATGC

Table 7. Continued.

SNP Name	Forward (F) and Reverse (R) Primers	Fluorescently Labeled Probes
Ots_ntl-255	F - TGCAGTTACAAGCCTAAGACAATCT	VIC - TTGTAGAGGAAGAATATTC

	R - CAACTAAAGTAACACACCAGCAACTG	6FAM - TTGTAGAGGAAGTATATTC
Ots_OTALDBINT1-SNP1	F - CGCTGGGCATGGATGAGT	VIC - CTACTGTTGTATTTTCTC
	R - GGCCAACACTGCTACTTCCT	6FAM - CTGTTGTGTTTTCTC
Ots_OTDESMIN19-SNP1	F - GGTCTGTCTGTCTGTCTATCTGTCA	VIC - CCAGTCATGGGTCATT
	R - TGTGTGTCTTTGTTCATTCCTACCA	6FAM - TCCAGTCATTGGTCATT
Ots_OTSTF1-SNP1	F - CGGACAAAGAGCTACAGAAATGC	VIC - CCGCCACCTTGGCT
	R - CGTCCCTCTTCACGCATGA	6FAM - CGCCACATTGGCT
Ots_parp3-286	F - AGTCAGTGTTGGTGTAGTGAAGAGA	VIC - AGTTACAAGTGGTGTTTCA
	R - CATTTGTGGAGTGTTTATTGAACAGTAACA	6FAM - ACAAGTGGCGTTTCA
Ots_pigh-105	F - GTTTGGAATGTTTCTCTGATTGTGTTAACAA	VIC - TGACCTGAAAATATATATTTTT
	R - GCATTACTAAAAACTGGTGTGTGGAA	6FAM - ACCTGAAAATATATTTTTTT
Ots_pop5-96	F - CTCTTGCTACTTGCAGTGTATCTCA	VIC - TTCTGTTACTGGACTGATG
	R - AGTTTGAGGGCTCTATTCTGTCATG	6FAM - CTGTTACTGGGCTGATG
Ots_ppie-245	F - TGTTTTTGGTCATGTATTTTCTCTGCTATTTTT	VIC - ATGTCTGAAATGAAAGCC
	R - GGACTGGAGCTGCTGAACATA	6FAM - AATGTCTGAAATTAAAGCC
Ots_redd1-187	F - TTCTGGGTTGCCATACTCTTTCAAT	VIC - ATTCTGACAGCTGTTTTG
	R - AGTTGAGACCTTCAGTTCTTAGGGTAT	6FAM - CTGACAGCCGTTTTG
Ots_Thio	F - TTTTAAAAATGGAGATAAACTCCTGACCTGAA	VIC - CAGTGTATTAGTCATTCTTA
	R - AATACCAAACCATGCCACTAATACCT	6FAM - CAGTGTATTAGTCGTTCTTA
Ots_tpx2-125	F - TGTTGTAATCTTTCTGAATATTTGCTTGCTT	VIC - CAGGCGGTTCTCC
	R - TCTTCCAAATTGAGCACAAAAGCAT	6FAM - CAGGCAGTTCTCC
Ots_txnip-321	F - CCTTCAAACTAACACATCATAGACATGCTT	VIC - TCTGGCGGATTTACA
	R - TTATCAAACTGAAGGCGGATTTACTGA	6FAM - CTGGCGGGTTTACA
Ots_u1002-75	F - CCGCCTTTCCCACCTTCTC	VIC - ATGGCCCTTACACTATC
	R - TCAAACGAGAACACACTAAGGTTGT	6FAM - TGGCCCTTACGCTATC
Ots_vatf-251	F - CTTTTCGGGTTATTCATGCTGTTGT	VIC - AGACCACAAGATACAGTACC
	R - GCAAGCATTTGAAAAACAGACTGGAT	6FAM - AGACCACAAGATA--GTACC

Table 8. Primers and probe information for the steelhead PBT SNP panel.

SNP Name	Primers	Probes
Omy_SEXY1	F - CACAACATGAGCTCATGGG	
	R - CGATTAGAAAGGCCTGCTTG	6FAM - CCTACCAAGTACAGCCCCAA
Autosomal control	F - GCCTGCTTGCAGAAGTTTTT	VIC - GAGGGGTAGTCGTTTGTTCG
	R - CTTGACTGTGTCCAGCTTGC	
M09AAD.076	F - ACTGTTACCACTCTCTCATCAACCT	VIC - CACCAACCACTGGTGAA
	R - GGGTCCAGGAGGTTTTTAAACAACAT	6FAM - CCAACCGCTGGTGAA
M09AAJ.163	F - TCCCATGGCCCTTACTCTATCAA	VIC - AACAAAGTGAAAGTGTCCTTA
	R - TTGAGGTGTATGTTGAAAAGTAAACTT	6FAM - CAAAGTGAAAGTGTCTTTA
M09AAE.082	F - CTATGTGCAGTGCCCTTCTCA	VIC - AGGTTGTTTTACAAATTTAA
	R - GGCTTACAAGTATGCATGACTAGCT	6FAM - AGGTTGTTTTACACATTTAA
OMS00002	F - TTTGATTTGATTTGTATCTGCTTCTT	VIC - TGTTTTGCAGCGCTC
	R - CCAACATGCCTCACACAAAA	6FAM - TGTTTGGCAGCGCT
OMS00006	F - TCCACGTAGGACATAGTTTGAGCTA	VIC - CACTTACAAATACAAAATT
	R - TGTGGTGTCATGTTTGCCCTAC	6FAM - CTTACAAATGCAAAATT
OMS00024	F - CACATACAACCATCACCCTTCCTAA	VIC - AAAAACCCAAATTTTAC
	R - AGCATTGAGCGAAATTACCAAGAGT	6FAM - AACCCCAATTTTAC
OMS00039	F - GTCAGTACTGTGTGTGTCTGTGT	VIC - CAGAGACACGTACGCACA
	R - CCATCTACATTGTCAGCAGTGTGA	6FAM - AGACACGCACGCACA
OMS00053	F - GGAGCCAGGTCAAGGTGATC	VIC - TGTGTGATTGATACATATAAAT
	R - GGATGTCTGGTGTGGCTGTAAA	6FAM - TGTGATTGATACGTATAAAT
OMS00057	F - GAGAAAGGGAGCATGAGACAGA	VIC - CTCCACAGAACCTTG
	R - GTTGGGCTCCGGTACGAT	6FAM - CTCCACAGCACCTTG
OMS00058	F - GTGACATTTGGAGCCACTGC	VIC - CAACACTTTGTACCCCTC
	R - GCTAGGAGACAGAGGGTGAAAG	6FAM - CACTTTGCACCCCTC
OMS00062	F - ACCCTGGGAAGGCTACTGTAC	VIC - TTGACCAGCAGATGGTGTA
	R - TGAACAGAGATCTGGAGAGTTGGAT	6FAM - ACCAGCAGGTGGTGTA
OMS00064	F - GTGGATATGTAGTTCGATGGAACAGT	VIC - CAGGCAACATTTTATATAACTA
	R - TTTACAACAATCTTCTTTTAATAAAAATATAGCCACTTAT	6FAM - CAGGCAACATTTTATCTAACTA
OMS00068	F - GCACTAACTGGACAACATTTTTAAGAATGA	VIC - AATATGCCTCCTTCGTCTC
	R - GGCAGTTGAGCATTTTGGGATATT	6FAM - TATGCCTCCTCCGTCTC
OMS00070	F - CGTTCCTGCGGGACAGT	VIC - CAAAATACGGAAATGCAG
	R - GTTTCTCTCACGTCCACAGATCT	6FAM - AAATACGGGAATGCAG
OMS00071	F - CCGGAGTGACCTCACATTTGG	VIC - CTTGTTTGAGCTTTTTCT
	R - GCATCGTACAGTTCACCTACCT	6FAM - TTGTTTGAGCCTTTTCT
OMS00072	F - GTGGGAGAGCTCGTCTATGG	VIC - TAGAAGGTCCATGTATCTC
	R - ACAACAGGTCATTGGATGTGATCAG	6FAM - AAGGTCCATGCATCTC
OMS00074	F - CCTGTTTATTCATCTAAACCAGTTCTTTAAAAT	VIC - TGAAACAAAACAAATGTTCC
	R - AACTTAATTTAGCAAACAAATGTCTGAACAGAA	6FAM - AAACAAAACACATGTTCC
OMS00077	F - AATACCATCTTGAGCTCATTAGTAATTATTCAA	VIC - TTCCGGTGGTGAAGTT
	R - CCAGACTTTACACACTCTTGACTGA	6FAM - CCGGTGCTGAAGTT

Table 8. Continued

SNP Name	Primers	Probes
OMS00078	F - GAGGGAAGCAGCCATAAACAGAATA	VIC - TTCACATGCATAAGAGTG
	R - GTCTCACTATGGTCCATATCTGTGTAGA	6FAM - TCACATGCATGAGAGTG
OMS00079	F - GTAACATTATGAATCTATCAGTTTCCCTAGCT	VIC - CTACTTTTCACAGTAACACAG
	R - ACCTGCAACGTTAGAGCTGTTTATT	6FAM - CTACTTTTCACAGTGACACAG
OMS00111	F - CATGCGGACCTGCATAGCT	VIC - CAACCAGACTACCATTC
	R - GCTTAGCCATTGACAGAGCATATCA	6FAM - AACCAGACTGCCATTC
OMS00089	F - GCACCATTTGAATAAAAAATCTGCTTTGT	VIC - ATGAATCCCAAATAAGAAC
	R - GCAACCCAATTCAATATTAAGCACATGAT	6FAM - AATCCCAAACAAGAAC
OMS00090	F - AGGGCACAACACCACTCTAAATT	VIC - ACAACCACACAAGATT
	R - TCGAAAAGCAACATCTGTCTCAGT	6FAM - AACCACGCAAGATT
OMS00101	F - GCGTGTCGTGGGTCAGTTAAATA	VIC - CTCTAGTAGCCTTATAGAAAG
	R - GTGCAATCCAACCTATTAGTAGATATGCT	6FAM - CTAGTAGCCTTACAGAAAG
OMS00105	F - ACATTTGAAGTCAGTATGGGTGTTGAG	VIC - CTGCTATTCAAATTGCT
	R - GAACCTCACCACAGTACTAAATGCA	6FAM - CTGCTATTCACATTGCT
OMS00106	F - CGTGTAGCATTCTTGAGGAAGCTT	VIC - TCTGATGGAAACTTTC
	R - TTTCCAACAGATGCCAGAATCCT	6FAM - TGATGGCAACTTTC
OMS00154	F - GATGTTGGCTGGAGGTGTAGT	VIC - ACAGGGCTTCTGATTGA
	R - TGGGAACACTTTGCCTACCC	6FAM - AGGGCTTCAGATTGA
OMS00112	F - TGGCAGCAAAAGGGATGCA	VIC - CCGGTTTCAAGTTTACTTGT
	R - TCCTGAGCAACCAGTCAACATT	6FAM - CGGTTTCAAGTATACTTGT
OMS00118	F - GCTTATTTAGAGTGCATGCCAGATG	VIC - AATGTGCACACCCCGC
	R - TGGAACCAATGGGACAGTCCTA	6FAM - AATGTGCACCCCCCGC
OMS00120	F - GGCAGAAGAGGAGAGAGATATGATTG	VIC - TCGCCCACTAAAAC
	R - CCTCAAATACCTCTGACATTGAAGGTT	6FAM - CGCCCACCAAAAC
OMS00121	F - GGAAGGAGGTCCAGTGTGAGT	VIC - ACAGCGTGATAAATT
	R - AAAATATGCAACACCACTAAAACTGGAAAA	6FAM - CAGCGTGGTAAATT
OMS00132	F - GTTTATGACTCCATTGCCGAAATGATT	VIC - CAGCAGTCCTCTGTGTGG
	R - ACGCGACCTGCAATTCATCAATA	6FAM - AGCAGTCCTCAGTGTGG
OMS00175	F - TTGCGATATGGGACTGTATACATTTATTCC	VIC - CATCACTAGTTCAAATACAA
	R - ACTACCTCCAGTTAAAATAGTGTGGGAAA	6FAM - CATCACTAGTTCAGATACAA
OMS00179	F - GTCATAACAAAATCAGGGCTTTCCAA	VIC - TGCCTCTTCTCTTTTCTCAT
	R - TGGGAGATTTGGGCTGCTTTAAA	6FAM - CCTCTTCTCTTGTCTCAT
OMS00180	F - GCGCCGAATGGCATTAGG	VIC - CTAAAAGTGCATTAAGCC
	R - CACATTGCTGTCGTTTAGTTTGACT	6FAM - CTAAAAGTGCCTTAAGCC
Omy_101832-195	F - TGGCTCTGGACCTGTTGAGA	VIC - TGTAGTCTTTCAGAGTAGTATG
	R - CGTCACAGCTATTTTAGGCGTAGT	6FAM - TAGTCTTTCAGAGGAGTATG
Omy_101993-189	F - ACAAAACACAGTGGAATTACAATTAACGTT	VIC - CTTGATTTGCAGCTTGTCAA
	R - GGAAGTTAAATTTCGCTTCGTCAGAA	6FAM - TGATTTGCAGCATGTCAA
Omy_102505-102	F - CTGCAAACTGACATGGTAGCAAAA	VIC - AACAGGATGTTTTTGC
	R - TGCTTGCTTTTTAAAAACAATCTCCCA	6FAM - CAGGATGCTTTTGC
Omy_104519-624	F - CGTGTGAGTTTGCGGTAAAGAC	VIC - CAGCAGGATACATCCGACT
	R - TGACGAGTCCGTCTTATCATCCT	6FAM - AGCAGGATACGTCCGACT

Table 8. Continued

SNP Name	Primers	Probes
Omy_105105-448	F - CAATTTGCAAGCAGGGAAAGGTTAT	VIC - AAGGAGAATGCATAATC
	R - GTGATGGGCTGCAATTGCTT	6FAM - TGAAAGGAGAATACATAATC
Omy_105385-406	F - ACCTACCCTCACCTGAACTTCA	VIC - CTTGGAACCATTGCTAC
	R - CGCTCTTCTGGGCGTATCG	6FAM - TTGGAACCGTTGCTAC
Omy_105714-265	F - CCACTCAGTGCAAGCATGGA	VIC - CTGTTGTTTGAGGTTCAG
	R - GCTTTCAATCCTTGGCTCCAATATC	6FAM - TGTTGTTTGAGATTCAG
Omy_107806-34	F - TCTTTGTCCATGCACATTGATATT	VIC - ATTGGATGTCAGTGTCATT
	R - AGCACATTTAGTTAGCAGTGATGGA	6FAM - ATTGGATGTCAATGTCATT
Omy_108007-193	F - GTGAATACCACCCAGGCTTGT	VIC - ATGTTTTCTCCCTACTTAAC
	R - GTCCCTTCCCCAGTTTCACTTAATT	6FAM - TTTTCTCCCCACTTAAC
Omy_109243-222	F - ATGTGCACCTCTTAAATTGTAAGTAAAATGT	VIC - TGTTCATTAAATTGACTTTTT
	R - ACCCTATATTCAGTGGCAAGATTGC	6FAM - TTCATTAAATGGACTTTTT
Omy_109894-185	F - CGGTGTCATTATGGTTGTCATTGTG	VIC - CTCCCTGATCCCCC
	R - GGGAGGAATTGGAATGACAGATTAAC	6FAM - CTCCCTGGTCCCCC
Omy_110064-419	F - GTGCAAGGGACCTAGCTAATCC	VIC - ACGTTAGCTTTTAATTTC
	R - TCTGAACTGACACTGAAGAACAAAGAA	6FAM - AACGTTAGCTTTTCATTTC
Omy_111383-51	F - CACGCGCAATCTCTCGTTTTAC	VIC - ACCTAGTGCGCTTGCT
	R - TCTTTAGGCAACAAGCGTGTCA	6FAM - ACCTAGTGCACTTGCT
Omy_113490-159	F - CATAGTACATTTACAGATAATGTTTTAAAGTGCATGT	VIC - CATCTGTTTTGGTTTAGC
	R - CGAGATACCAAAATGCCACAGTTACAT	6FAM - CATCTGTTTTAGTTTAGC
Omy_114315-438	F - CCTCACCGATCTAGTCAACTTCATC	VIC - TTATGGGCTTAAGGGTC
	R - AGGAGGCTGAGGGAGATTCTAG	6FAM - TTATGGGCTTACGGGTC
Omy_114587-480	F - CAGATTACGTTATTACGTTTGGGAAATTTTTAAGT	VIC - CCTGTCCAAAATTGT
	R - GTGAAAGAGTGGGAAATATAATTATAAGGTCAGA	6FAM - CCTGTCCACAATTGT
Omy_129870-756	F - TCGTTATTTTGCCTCGCGGTA	VIC - ACAGGTATTTCGTGAAATG
	R - TCCCATGAAGATGTATACATGTTTTGTGA	6FAM - CAGGTATTTCATGAAATG
Omy_116733-349	F - GAAATGGACATGCCTACAAATTGCT	VIC - AGAGAATCTGATAGTATTTC
	R - GATGTGATCAGTTTAGGCAAGGC	6FAM - AGAGAATCTGATAATATTTC
Omy_128923-433	F - ACGTTTCTTTGGGCTGAGACTTATT	VIC - CTTCATTTTCATTCACTGTTTT
	R - CTATGTCCTTGGCAGAAGTCTACA	6FAM - CATTTTCATTCGCTGTTTT
Omy_130524-160	F - CGAAGGTAGCGATTGGTCGTT	VIC - ATGGCTTGATCCTCA
	R - TGTCTGTTCTGCTGTGTGCTT	6FAM - ATGGCTTCATCCTCA
Omy_97660-230	F - TCAGTTATGTGTAATCTCATTACCTCTCCAA	VIC - ACGTAACTTGTAGCGTTTT
	R - AACAGAAAAGGTCTCAATGTATTTTTTGCA	6FAM - ACGTAACTTGTACCGTTTT
Omy_99300-202	F - CAGTTTGACCCGATGGTGTGA	VIC - TCAGGCATGAGAGAAA
	R - GATTATGGCGTGGCCTTTTGG	6FAM - ATCAGGCATGTGAGAAA
Omy_aldB-165	F - GGGTTAGGTGGATTTGAAGGAGTAA	VIC - ATGCTAAAATGAACTCCCCACCA
	R - AGGAAGGTGATGCCTGAGAGA	6FAM - CTAAAATGAACTCGCCACCA
Omy_anp-17	F - GGTAATGCCACATGCGGTAAATT	VIC - CTCTCATTGGTATAGTAACC
	R - GGCGAAATCTGAAAATGTGCTGTTA	6FAM - CTCATTGGTATATTAACC
Omy_arp-630	F - CTGCACAACTTGTTTCCTGCTATT	VIC - CCGCTCCGTCTGCT
	R - ACCAAGTGTCCCTGTAAGCC	6FAM - CCGCTCTGTCTGCT

Table 8. Continued

SNP Name	Primers	Probes
Omy_b1-266	F - TCATGTGAACTTTAATTGACTAGGAAGTCG	VIC - TCTATAAACAACATTTTTC
	R - GATATGAAAATATCTGAAGAGTTATATTTGGGAAATTGAC	6FAM - TCTATAAACAAAATTTTTC
Omy_BAC-B4-324	F - GCCTAATATTGGCCTAATGTCCTTCA	VIC - CATTGCCAAATACG
	R - CGTACTTTTCTTTTACAAAATTAAGTGGAGGAT	6FAM - TACATTGACAAATACG
Omy_ada10-71	F - TCTTTGAGCGACAAAGTCCTTGT	VIC - CTTCCTGCGTCCAATT
	R - ACCCACACATGAACGCAAAAG	6FAM - CTTCCTGCATCCAATT
Omy_redd1-410	F - GTACTCCCACTAACATACAGTAGACTCA	VIC - AAAATATCCTGCAAGGAAT
	R - GGCACCATTGTGTTTTAGGATGTAG	6FAM - AATATCCTGCAAGAAAT
Omy_cd59-206	F - CGATTGGCCCAGATGTTTCCAT	VIC - CAACAATCGAAGGTAAAT
	R - GCTCCGTTGCATAGGTGACT	6FAM - CAACAATCAAAGGTAAAT
Omy_colla1-525	F - CCTCGGCGTGACAACCT	VIC - CTGTTGGGAGAAGAG
	R - CCCAGAGAATGGTGCGATTAGG	6FAM - TGTTGGGAAAAGAG
Omy_cox1-221	F - CACTGAACTGTAAGCCATTGTGATT	VIC - CGGTAAGACCATTAAAA
	R - GCAACATGGGAATGATTCATAAATGCA	6FAM - CGGTAAGACCATTTAAA
Omy_crb-106	F - GCTCAAAAAGATTCTGCCAAATTCACA	VIC - TTGCAATGCGTCTTT
	R - ATTACAATGAAAGTACTTGAGTGTTTATGCAAA	6FAM - TTGCAATGAGTCTTT
Omy_g12-82	F - GATCAATTCGATCGCTCATGAAACTT	VIC - CAAACTCTCAGGATTAG
	R - CTTCTCTCGTTCTCATTGTGTCTCA	6FAM - AAACTCTCGGGATTAG
Omy_gluR-79	F - GACTGTCTATAGCTATTCTTCTCAAACTGT	VIC - CAAGTATTTTGCGTAGGAAT
	R - AGAAACTACCATTGTGATTAACAGATAGAAAATACAT	6FAM - CAAGTATTTTGCATAGGAAT
Omy_hsc715-80	F - CCGGTCTACCCTATAGCTGTTG	VIC - AACTGTATTTGGGAAAAT
	R - AGTCAGTCAATTAGTGGTTTGAAATACTATCA	6FAM - ATAAACTGTATTTGTGAAAAT
Omy_hsf2-146	F - GGAGCAGAAAAAGGATTGGACCTT	VIC - CAGCTGTTAGTAGATTAT
	R - CCAACAATTGCAGCCTCATCTTAAT	6FAM - ACAGCTGTTAGATTAT
Omy_IL17-185	F - CCACCACACTCTGCAGCTT	VIC - AAGAATCTCACCTGCCCAT
	R - TTGACGGGAATCCGAGACTTC	6FAM - AAGAATCTCACTTGCCCAT
Omy_Il-1b_.028	F - ACTGTCTGGCTAGAGCACATTG	VIC - CTGAGGCAACTTTTGT
	R - ATCTTCTACCACCGCACTGTTTTAA	6FAM - TGAGGCAGCTTTTGT
Omy_Il1b-198	F - TTTAATCTCGGTGCTGAGCTAGTG	VIC - ACCTTAGTTGTTGCTTCAT
	R - CAAGCAAAATTGACTCCAGCCATTA	6FAM - ACCTTAGTTGTAGCTTCAT
Omy_IL6-320	F - CTTGTTCCTCGTTGTCTTCCTTCTA	VIC - CTATAGGAGAGAGGACAACA
	R - CGACTGATCTCCTGCAGACATG	6FAM - ATAGGAGAGAAGACAACA
Omy_metA-161	F - CGCATGCACCAGTTGTAAGAAAG	VIC - CAAGTAAGTGGTTATATTCT
	R - AGTGCCACCAGCGATAAGAAAA	6FAM - CAAGTAAGTGGTTCTATTCT
Omy_NaKATPa3-50	F - GTTGAGCGTGTTATGGGAAAAGAG	VIC - CACTCTGTTTCCTTTCTTT
	R - TTGCATCGGCTTTCTGAAAACC	6FAM - TCTGTTTCCGTTCTTT
Omy_txnip-343	F - CCTTCAAACTAACGCATCATAGACATG	VIC - CCAACTGAAGAGATCTG
	R - GGTCACTTGGCTAATCCCCTTAT	6FAM - CAACTGAAGGGATCTG
Omy_nkef-241	F - AGTGTCATTGATGTCGGCCTATTTT	VIC - CTTCTGTATCATTTTTG
	R - AAACGAATGTCCACCTCAGATGTT	6FAM - TCTTCTGTATAATTTTTG
Omy_ntl-27	F - GGTGTGTTACTGTAGTTGTGTCCTT	VIC - CAGACAAGAGTACCCCAAGAC
	R - TGTGTAGCTAGTGATCCTGATTGTCT	6FAM - CAGACAAGAGTACTCCAAGAC

Table 8. Continued

SNP Name	Primers	Probes
Omy_Ogo4-212	F - TCCTCTCTCCCATTCAATCACTAATGA	VIC - CATTTGATGAGACATCTT
	R - AGACAGTAACAAAGCCTCAAACTTGA	6FAM - ATTTGATGAGGCATCTT
Omy_bcAKala-380rd	F - TTGCTCTCTTCTGGTTGCCTTA	VIC - CATACCCATCCTATGTCAG
	R - CTTCAGGAGAAAGCGCTACTGT	6FAM - CATACTCATCCTATGTCAG
Omy_Ots249-227	F - CCCCTAGATTAAACCTGTCCAGTCT	VIC - CCCTCTGAGAACTAC
	R - CTATCTATCTATCTATCTATCTATCTATCTATCTATCTACTTACTGAGA	6FAM - CCTCTGAAAACTAC
Omy_oxct-85	F - CGTCACTGAAACATTACTGTAACATCCA	VIC - CATCGCTTATTTATGC
	R - CATCATCACGCTGTTGGTTTCTTAA	6FAM - CATCGCTAATTTATGC
Omy_p53-262	F - CCCCAACATCCAGTATACAGTTTCA	VIC - CAAGTAGTATGGAGCTCTAT
	R - CCCAAATTGGCAATTTTAATAGGATTCAGA	6FAM - AAGTAGTATGGTGCTCTAT
Omy_rapd-167	F - CCCAACATGCTCTATTGCAGCTA	VIC - ATTAAAACAATCCCCCCCAAAA
	R - AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT	6FAM - TTAAAACAATCCCACCCAAAA
Omy_rbm4b-203	F - CTGAAATTTGATGAATGGAAGCTGCA	VIC - CACGTTATTATGAAAAGGATGT
	R - CGTATTCAAGTCGATATACAGTCACGAT	6FAM - ACGTTATTATGAAAAAGGATGT
Omy_srp09-37	F - TAGTTGTATTAACTCTTCTTTGAGTCTAGA	VIC - TTGTGCTATTGACGCCACAG
	R - TCATTCCAGCTCCGTTCTCTTC	6FAM - TTGTGCTATTGACACCACAG
Omy_stat3-273	F - CAGACCTCCTCTATCTCCCTATGAG	VIC - TTTTCCAGACTCCAGTTTG
	R - ACCTCCTTTAAATTGTGCCCAAGAA	6FAM - TTTTCCAGACTCAGTTTG
Omy_u09-53.469	F - ACAGCCTGAGCGTTTGCA	VIC - TTGCAGCCCTTATTGTG
	R - GGAAACTGGGAGAGATCAAAGGA	6FAM - TTGCAGCCCTTGTTGTG
Omy_u09-54-311	F - GTGGCTCCCCAGGAACAAG	VIC - TGGTAATTATTCAACAGATCAGT
	R - AAGTTTCATGTCACATTCCAGTTACCT	6FAM - TGGTAATTATTCAACAAATCAGT
Omy_U11_2b-154	F - GGGAAGCAGAAAAACTGGAAGTT	VIC - AATGATACTTTTCAGATTGTAAC
	R - CCCTCTGTGGGCTTGATATTCA	6FAM - TGATACTTTTCAGGTTGTAAC
Omy_vatf-406	F - TTGCTTCATTTTGTCATAACCTTGGG	VIC - TTGCAGATGACTATCCACA
	R - TGCATGCTCTGACAAATGTTACACT	6FAM - TGCAGATGACTGTCCACA
OMY1011SNP	F - GAGGCTGGTTTGGGATTCACT	VIC - CTTTACCTCGAAGACAAT
	R - CGCCAAACACTAACTCTCTGTCT	6FAM - ACTTTACCTCTAAGACAAT

Table 9. Chinook salmon SNP markers dropped from PBT panel and replacement loci.

Marker Name	Problems	Decision
Ots_IsoT	Plot, HWE, Parentage	Drop
Ots_103041-52	Plot, LDE, Parentage	Drop
Ots_106747-239	Plot, LDE, HWE	Drop
Ots_u1008-108	Plot, Parentage	Drop
Ots_nips-133	Plot, HWE	Drop
Ots_mtap-299	Plot	Drop
Ots_OTNAML12_1-SNP1	Plot	Drop
Ots_Tnsf	LDE	Drop
Ots_OTALDBINT1-SNP1	Plot	Replacement
Ots_MHC1	Minor HWE	Replacement
Ots_tpx2-125	Low MAF	Replacement
Ots_u4-92	Low MAF	Replacement
Ots_GCSH		Replacement
Ots_113242-216		Replacement
Ots_vatf-251		Replacement
Ots_Ikaros-250		Replacement

Table 10. Steelhead SNP markers dropped, exchanged between panels, and replacement loci.

Marker Name	Problems	Decision
S109	Parentage	Drop
114976-223	Parentage	Drop
121713-115	HWE, Parentage	Drop
Omy_123044-128	HWE, Plot	Drop
OMS00001	Parentage, Plot	Drop
Omy1005	Plot	Drop
Omy_129870-756		Move from GSI to PBT panel
Omy_ada10-71		Move from GSI to PBT panel
M09AAE.082		Move from GSI to PBT panel
OMS00154		Move from GSI to PBT panel
OMS00111		Move from GSI to PBT panel
Omy_redd1-410		Move from GSI to PBT panel
Omy_txnip-343		Move from GSI to PBT panel
Omy_ca050-64		Move from PBT to GSI panel
Omy_128996-481		Move from PBT to GSI panel
Omy_hsf1b-241		Move from PBT to GSI panel
Omy_ndk-152		Move from PBT to GSI panel
OMS00174		Replacement for GSI panel
M09AAC.055		Replacement for GSI panel
OMS00095		Replacement for GSI panel
OMS00087		Replacement for GSI panel
OMS00114		Replacement for GSI panel
OMS00143		Replacement for GSI panel

Table 11. Parent/Offspring Mismatches in Chinook salmon SNPs.

Locus	Locus Name	Mismatches	Crosses
PBT51	Ots_112301-43	1	1
PBT60	Ots_u1008-108	3	1
PBT61	Ots_103041-52	3	1
PBT79	Ots_IsoT	7	6

Table 12. Demonstration of assignment accuracy and error rates of top tiers of ranked steelhead SNPs.

No. of loci	Assign. Accuracy	False Neg.	False Pos.
36	38.5	53.6	0.0
48	87.6	10.2	1.1
72	95.7	4.3	0.0
96	96.1	3.9	0.0
120	96.2	3.8	0.0
144	97.3	2.7	0.0
168	97.1	2.9	0.0
188	97.1	2.9	0.0

Table 13. Comparison of parentage assignment between 17 microsatellites and the selected panel of 95 steelhead PBT SNPs.

	Sample Size		Juveniles Genotyped		Juveniles Assigned		No. assigned to correct stock		No. matching spawn records	
Hatchery Stock	**Adults**	**Juveniles**	**Micros**	**SNPs**	**Micros**	**SNPs**	**Micros**	**SNPs**	**Micros**	**SNPs**
Squaw Crk.	35	92	91	92	91	92	91	92	79	79
Sawtooth	234	93	93	93	91	91	91	91	82	82
Dworshak*	393	93	93	93	93	93	93	93	66*	66*
E. Fk. Salmon R.	119	94	94	94	88	88	88	88	77	77
Pahsimeroi**	255	93	93	93	93	88	93	88	---	---
Grande Ronde	169	93	91	93	91	91	91	91	90	91
Lyons Ferry***	---	93	93	93	0	0	---	---	---	---
Total	1205	651	648	651	547	543	547	543	394	395

* = cross information not available for parents of 27 offspring
** = cross information not provided
*** = fish with no parents in the database

Table 14. Samples and genotyping completion rate (those with less than 10% missing data) of 2008 and 2009 steelhead broodstock. Collections marked with an asterisk (*) indicate that genetic samples for early egg takes were missed in 2008.

	2008		2009	
Snake River Hatcheries	**Sampled**	**Genotyped (%)**	**Sampled**	**Genotyped**
LSRCP/IDFG Sawtooth (IDFG & SBT)	639	633 (99.1%)	979	977 (99.8%)
LSRCP/IDFG Sawtooth (EFSR)	119	118 (99.2%)	132	132 (100%)
LSRCP/IDFG Sawtooth (USB/Squaw)	35	35 (100%)	53	53 (100%)
Idaho Power/IDFG, Oxbow F.H.	927	895 (96.6%)	592	555 (93.8%)
Idaho Power/IDFG, Pahsimeroi F.H.	1531	1493 (97.5%)	968	944 (97.5%)
Idaho Power/IDFG, Pahsimeroi F.H (SBT)	312	311 (99.7%)	336	326 (97.0%)
LSRCP/IDFG/USFWS Dworshak/C.W.	1419*	1416 (99.8%)	1880	1856 (98.7%)
LSRCP/ODFW-Wallowa F.H.	0*	-	460	441 (95.7%)
LSRCP/WDFW-Lyons Ferry	0*	-	215	210 (97.7%)
LSRCP/WDFW-L.F. (Tucannon)	0*	-	25	23 (92.0%)
LSRCP/WDFW-L.F. (Touchet)	0*	-	26	25 (96.2%)
LSRCP/WDFW-L.F. (G.R. cottonwood)	169	169 (100%)	95	94 (99.0%)
Total	5151	5070 (98.4%)	5761	5636 (97.8%)

Table 15. Ranked estimates of null allele frequency for 58 loci from the steelhead 2008 PBT dataset.

Rank	SNP Name	Freq of null allele	Rank	SNP Name	Freq of null allele
1	OMS00058	0.004	30	OMS00024	0.016
2	OMS00077	0.004	31	OMS00089	0.016
3	OMS00068	0.006	32	Omy_txnip-343	0.016
4	Omy_Il1b-198	0.006	33	M09AAJ.163	0.018
5	OMY1011SNP	0.006	34	OMS00132	0.018
6	Omy_u09-53.469	0.007	35	OMS00180	0.019
7	M09AAE.082	0.009	36	Omy_cox1-221	0.020
8	OMS00039	0.009	37	Omy_99300-202	0.021
9	Omy_arp-630	0.009	38	OMS00070	0.024
10	Omy_oxct-85	0.009	39	OMS00179	0.024
11	OMS00053	0.010	40	Omy_97660-230	0.024
12	Omy_bcAKala-380rd	0.010	41	OMS00175	0.025
13	Omy_p53-262	0.010	42	Omy_104519-624	0.025
14	OMS00071	0.011	43	Omy_metA-161	0.025
15	OMS00101	0.011	44	Omy_101993-189	0.026
16	Omy_108007-193	0.011	45	Omy_crb-106	0.027
17	Omy_130524-160	0.011	46	Omy_hsf2-146	0.028
18	Omy_b1-266	0.011	47	OMS00074	0.029
19	Omy_ntl-27	0.011	48	Omy_105105-448	0.031
20	Omy_rbm4b-203	0.011	49	Omy_129870-756	0.031
21	OMS00111	0.012	50	OMS00072	0.032
22	Omy_107806-34	0.012	51	Omy_hsc715-80	0.037
23	Omy_nkef-241	0.012	52	Omy_anp-17	0.038
24	Omy_105714-265	0.013	53	OMS00064	0.043
25	Omy_109243-222	0.013	54	Omy_u09-54-311	0.047
26	Omy_NaKATPa3-50	0.013	55	Omy_113490-159	0.059
27	Omy_U11_2b-154	0.013	56	Omy_vatf-406	0.067
28	OMS00006	0.014	57	Omy_114315-438	0.077
29	OMS00106	0.014	58	OMS00118	0.092

Table 16. Ranked estimates of null allele frequency for 72 loci from the steelhead 2009 PBT dataset.

Rank	SNP Name	Freq of null allele	Rank	SNP Name	Freq of null allele
1	Omy_Ots249-227	0.001	37	Omy_gluR-79	0.016
2	OMS00106	0.002	38	Omy_ntl-27	0.016
3	OMS00057	0.003	39	OMS00024	0.017
4	OMS00071	0.003	40	OMS00175	0.017
5	Omy_107806-34	0.003	41	Omy_u09-54-311	0.017
6	Omy_rapd-167	0.003	42	OMS00111	0.018
7	OMS00006	0.004	43	OMS00089	0.019
8	OMS00101	0.004	44	Omy_110064-419	0.019
9	OMS00105	0.004	45	Omy_hsf2-146	0.019
10	Omy_BAC-B4-324	0.004	46	Omy_p53-262	0.019
11	Omy_g12-82	0.004	47	OMS00072	0.020
12	Omy_txnip-343	0.004	48	OMS00132	0.020
13	OMS00002	0.005	49	Omy_105105-448	0.021
14	OMS00090	0.005	50	Omy_bcAKala-380rd	0.021
15	OMS00121	0.005	51	Omy_129870-756	0.023
16	OMS00180	0.005	52	OMS00053	0.024
17	M09AAD.076	0.006	53	Omy_102505-102	0.024
18	OMS00062	0.006	54	Omy_Il1b-198	0.025
19	Omy_101832-195	0.006	55	Omy_99300-202	0.026
20	Omy_redd1-410	0.006	56	Omy_rbm4b-203	0.028
21	Omy_oxct-85	0.006	57	OMS00078	0.029
22	Omy_U11_2b-154	0.006	58	OMS00120	0.029
23	OMS00074	0.008	59	OMS00179	0.029
24	Omy_IL6-320	0.008	60	OMS00070	0.031
25	M09AAE.082	0.009	61	Omy_108007-193	0.031
26	Omy_116733-349	0.010	62	Omy_114315-438	0.032
27	Omy_aldB-165	0.010	63	Omy_97660-230	0.032
28	Omy_105714-265	0.011	64	Omy_metA-161	0.036
29	Omy_cd59-206	0.011	65	Omy_anp-17	0.037
30	Omy_Il-1b_.028	0.013	66	Omy_crb-106	0.040
31	Omy_NaKATPa3-50	0.013	67	M09AAJ.163	0.045
32	Omy_101993-189	0.014	68	OMS00064	0.047
33	Omy_cox1-221	0.014	69	Omy_113490-159	0.065
34	Omy_109243-222	0.015	70	Omy_vatf-406	0.072
35	Omy_130524-160	0.015	71	OMS00118	0.077
36	OMS00068	0.016	72	Omy_Ogo4-212	0.102

Table 17. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for steelhead (IDFG-OMY-SEX) from a subsample of 2008 broodstocks.

	Corresponding	Non-corresponding	Phenotypic females misidentified as male	Phenotypic males misidentified as female	Total phenotypic males:	Total phenotypic females:	Missing Data
Dworshak	380 (98.2%)	7 (1.8%)	0 (0.0%)	7 (1.8%)	209 (53.2%)	184 (46.8%)	6 (1.5%)
EFSR	114 (98.3%)	2 (1.7%)	0 (0.0%)	2 (1.7%)	36 (30.3%)	83 (69.8%)	3 (2.5%)
Squaw Cr.	32 (94.1%)	2 (5.9%)	1 (2.9%)	1 (2.9%)	20 (57.1%)	15 (42.9%)	1 (2.9%)
Pahsimeroi	239 (97.2%)	7 (2.9%)	0 (0.0%)	7 (2.9%)	134 (52.6%)	121 47.5%)	9 (3.5%)
Sawtooth	230 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	116 (49.6%)	118 (50.4%)	4 (1.7%)
Grande Ronde	163 (99.4%)	1 (0.6%)	0 (0.0%)	1 (0.6%)	84 (49.7%)	85 (50.3%)	5 (3.0%)
Total	1158 (98.4%)	19 (1.6%)	1 (0.08%)	18 (1.5%)	599 (49.7%)	606 (50.3%)	28 (2.3%)

Table 18. Results of comparisons between phenotypic sex and genetically determined sex using the modified sex-specific assay for Chinook salmon (IDFG-OTS-SEX) from a subsample of 2008 broodstocks.

	Corresponding	Non-Corresponding	Phenotypic females misidentified as male	Phenotypic males misidentified as females	Total phenotypic males:	Total phenotypic females:	Missing Data
Dworshak	319 (97.0%)	10 (3.0%)	0 (0.0%)	10 (3.0%)	166 (44.2%)	210 (55.9%)	47 (12.5%)
Rapid R.	52 (91.2%)	5 (8.8%)	0 (0.0%)	5 (8.8%)	90 (95.7%)	4 (4.3%)	37 (39.4%)
Totals	371 (96.1%)	15 (3.9%)	0 (0.0%)	15 (3.2%)	256 (54.5%)	214 (45.5%)	84 (17.9%)

Table 19. Average heterozygosity (Avg het), observed and expected, of hatchery stocks for 2008 and 2009.

	2008				2009			
	Avg het (Obs)	**SD**	**Avg het (Exp)**	**SD**	**Avg het (Obs)**	**SD**	**Avg het (Exp)**	**SD**
Dworshak	0.398	0.092	0.399	0.092	0.397	0.099	0.395	0.097
Lyons Ferry	---	---	---	---	0.418	0.097	0.421	0.082
Grande Ronde	0.424	0.082	0.428	0.072	0.442	0.107	0.430	0.079
Touchet	---	---	---	---	0.418	0.125	0.427	0.099
Tucannon	---	---	---	---	0.408	0.134	0.418	0.100
Oxbow	0.423	0.091	0.425	0.086	0.424	0.075	0.429	0.076
Pahsimeroi	0.435	0.077	0.431	0.07	0.435	0.081	0.429	0.074
Sawtooth	0.424	0.071	0.427	0.064	0.425	0.074	0.428	0.068
EFSR	0.400	0.11	0.404	0.094	0.414	0.120	0.409	0.097
Squaw Crk.	0.419	0.101	0.421	0.081	0.417	0.137	0.396	0.100
Wallowa	---	---	---	---	0.425	0.084	0.426	0.077

Table 20. Population structure (Fst) among steelhead hatchery stocks sampled in 2008. Asterisks (*) indicate that Fst values were significantly different from zero.

	Dworshak	**Grande Ronde**	**Oxbow**	**Pahsimeroi**	**Sawtooth**	**EFSR**	**Squaw Cr.**
Dworshak	---	*	*	*	*	*	*
Grande Ronde	0.042	---	*	*	*	*	*
Oxbow	0.049	0.025	---	*	*	*	*
Pahsimeroi	0.048	0.019	0.009	---	*	*	*
Sawtooth	0.045	0.020	0.006	0.007	---	*	*
EFSR	0.050	0.039	0.042	0.034	0.034	---	*
Squaw Cr.	0.016	0.028	0.029	0.033	0.027	0.033	---

Table 21. Population structure (Fst) among steelhead hatchery stocks sampled in 2009. Asterisks (*) indicate that Fst values were significantly different from zero (p <0.01).

	Dworshak	**Grande Ronde**	**Lyons Ferry**	**Touchet**	**Tucannon**	**Oxbow**	**Pahsimeroi**	**EFSR**	**Sawtooth**	**Squaw Cr.**	**Wallowa**
Dworshak	---	*	*	*	*	*	*	*	*	*	*
Grande Ronde	0.052	---	*	*	*	*	*	*	*	*	*
Lyons Ferry	0.051	0.025	---	*	*	*	*	*	*	*	*
Touchet	0.058	0.029	0.027	---	*	*	*	*	*	*	*
Tucannon	0.043	0.025	0.011	0.006	---	*	*	*	*	*	*
Oxbow	0.049	0.021	0.024	0.024	0.021	---	*	*	*	*	*
Pahsimeroi	0.053	0.022	0.026	0.024	0.020	0.005	---	*	*	*	*
EFSR.	0.055	0.040	0.047	0.049	0.046	0.033	0.029	---	*	*	*
Sawtooth	0.048	0.019	0.026	0.031	0.024	0.007	0.006	0.032	---	*	*
Squaw Cr.	0.032	0.052	0.057	0.069	0.049	0.045	0.048	0.060	0.043	---	*
Wallowa	0.047	0.006	0.023	0.020	0.014	0.018	0.018	0.038	0.018	0.046	---

Table 22. Proportion of individuals from the 2008 steelhead broodstocks that correctly assigned to their population of origin and the population to which the largest proportion misassigned.

	N	% Correct	Largest Misidentification and %	
Dworshak	1159	90.0%	Squaw Crk.	4.20%
Lyons Ferry- GR	125	80.8%	Pahsimeroi	8.80%
Oxbow	550	69.6%	Sawtooth	13.10%
Pahsimeroi	1338	60.7%	Oxbow	15.80%
Sawtooth	506	52.2%	Oxbow	20.90%
E. Fk. Salmon R	100	78.0%	Pahsimeroi	6.00%
Squaw Crk.	22	31.8%	Dworshak	45.50%

Table 23. Proportion of individuals from the 2009 steelhead broodstocks that correctly assigned to their population of origin and the population to which the largest proportion misassigned.

	N	% Correct	Largest Misidentification and %	
Dworshak	1417	92.80%	Squaw Crk.	3.00%
Lyons Ferry- GR	79	54.40%	Wallowa	25.30%
Lyons Ferry	150	70.00%	Tucannon	12.00%
Touchet	18	44.40%	Tucannon	27.80%
Tucannon	18	33.30%	Touchet	33.30%
Oxbow	385	46.80%	Pahsimeroi	17.90%
Pahsimeroi	843	53.70%	Oxbow	14.60%
E. Fk. Salmon R	130	70.80%	Pahsimeroi	6.90%
Sawtooth	723	55.70%	Oxbow	13.30%
Squaw Crk.	44	54.50%	Dworshak	18.20%
Wallowa	267	56.90%	Lyons Ferry- GR	18.40%

Table 24. Estimates of effective population size and 95% confidence intervals for steelhead hatchery stocks sampled in 2008 and 2009.

	2008		2009	
	Ne	95% CI	Ne	95% CI
Dworshak	226	211–242	249	235–265
Lyons Ferry-GR	60	55–66	70	61–81
Lyons Ferry	---	---	92	83–102
Touchet	---	---	-19310	156–Inf.
Tucannon	---	---	388	101–Inf.
Oxbow	110	103–117	195	179–213
Pahsimeroi	204	193–216	261	244–279
Sawtooth	170	157–184	176	165–188
Sawtooth-EFSR	18	17–19	26	24–28
Sawtooth-SQ	32	27–39	19	17–21
Wallowa	---	---	227	202–254

FIGURES

Figure 1. Location of sampled fish hatcheries in the Snake River basin.

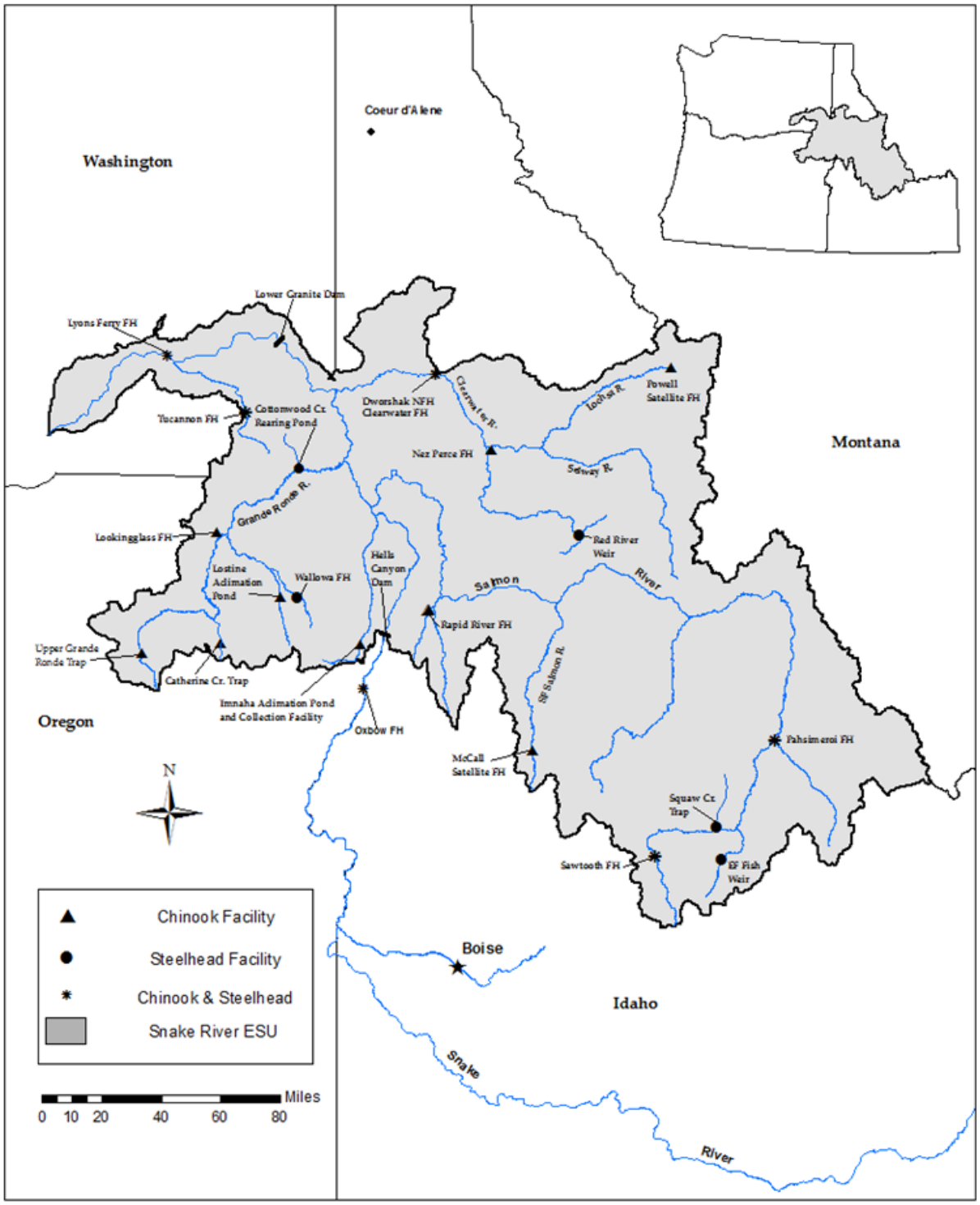
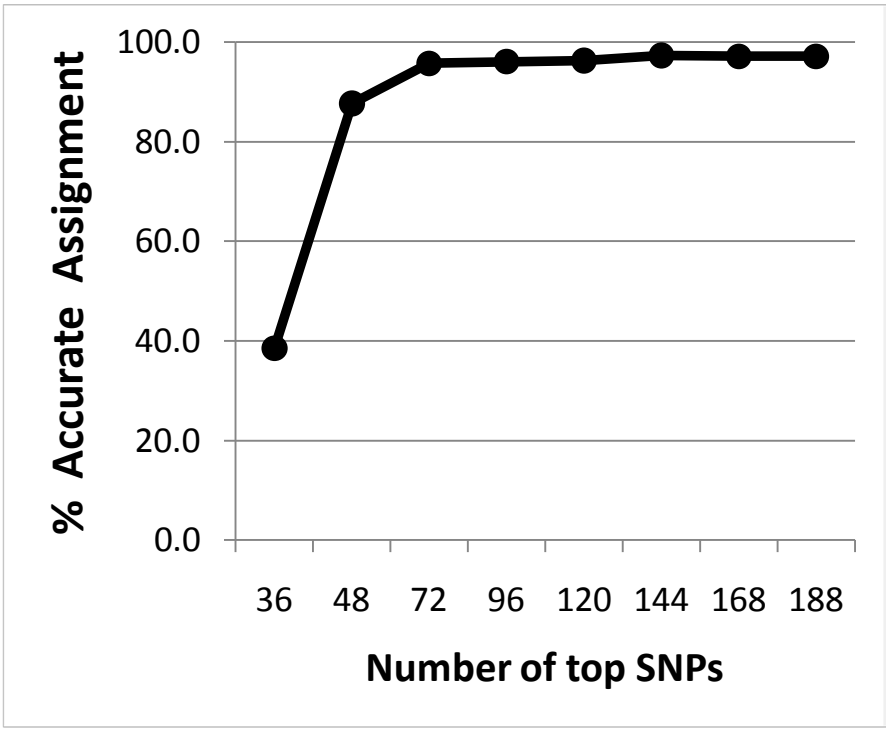


Figure 2. Assignment accuracy of 651 known-origin steelhead offspring to 1205 broodstock adults from six hatchery stocks in the Snake River basin using top tiers of SNPs ranked by adult minor allele frequency.



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