MOLECULAR ECOLOGY
RESOURCES
Molecular Ecology Resources (2011) **11 (Suppl. 1),** 31–49 doi: 10.1111/j.1755-0998.2010.02971.x

SNP DISCOVERY: CHAIN TERMINATION SEQUENCING

Discovery and characterization of single-nucleotide polymorphisms in steelhead/rainbow trout, *Oncorhynchus mykiss*

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Abstract

Single-nucleotide polymorphisms (SNPs) have several advantages over other genetic markers, including lower mutation and genotyping error rates, ease of inter-laboratory standardization, and the prospect of high-throughput, low-cost genotyping. Nevertheless, their development and use has only recently moved beyond model organisms to groups such as salmonid fishes. *Oncorhynchus mykiss* is a salmonid native to the North Pacific rim that has now been introduced throughout the world for fisheries and aquaculture. The anadromous form of the species is known as steelhead. Native steelhead populations on the west coast of the United States have declined and many now have protected status. The nonanadromous, or resident, form of the species is termed rainbow, redband or golden trout. Additional life history and morphological variation, and interactions between the forms, make the species challenging to study, monitor and evaluate. Here, we describe the discovery, characterization and assay development for 139 SNP loci in steelhead/rainbow trout. We used EST sequences from existing genomic databases to design primers for 480 genes. Sanger-sequencing products from these genes provided 130 KB of consensus sequence in which variation was surveyed for 22 individuals from steelhead, rainbow and redband trout groups. The resulting TaqMan assays were surveyed in five steelhead populations and three rainbow trout stocks, where they had a mean minor allele frequency of 0.15–0.26 and observed heterozygosity of 0.18–0.35. Mean F_{ST} was 0.204. The development of SNPs for *O. mykiss* will help to provide highly informative genetic tools for individual and stock identification, pedigree reconstruction, phylogeography and ecological investigation.

Keywords: *Oncorhynchus mykiss*, rainbow trout, single-nucleotide polymorphism, steelhead

Received 24 July 2010; revision received 9 November 2010; accepted 17 November 2010

Introduction

The development of highly informative molecular markers is an important first step in the investigation of population, ecological, evolutionary and conservation genetic questions. Several types of molecular markers have been widely used since the development of the polymerase chain reaction (PCR), including randomly amplified polymorphic DNA, amplified fragment length polymorphisms, mitochondrial DNA sequences and variable number of tandem repeat markers, such as microsatellites and minisatellites. More recently, single nucleotide polymorphisms (SNPs) have begun to see use in population genetics, although primarily for model organisms. SNPs are nucleotide variants found at particular genomic locations and are normally bi-allelic (Vignal *et al.* 2002). SNPs have several advantages over other markers, including that they are the most abundant polymorphisms in eukaryotic genomes, with an approximate density of 10^{-3} SNPs per base pair (Wang *et al.* 1998; Smith *et al.* 2005), they are found in both coding and noncoding regions (Brumfield *et al.* 2003), and they have a lower mutation rate (Brumfield *et al.* 2003), which is an important source of error in many applications. The use of SNP markers with humans and other model organisms is extensive and has focused on genetic mapping, disease diagnosis, toxicology and pharmacogenomics (Wang *et al.* 1998; McCarthy & Hilfiker 2000; Sachidanandam *et al.* 2001). Conversely, in nonmodel organisms, such as salmonid fishes, the use of SNP markers is quite recent and has focused more on population identification and ecological genetic questions (Narum *et al.* 2008).

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Oncorhynchus mykiss is a salmonid species native to the North Pacific rim. Its current native distribution extends from the Kamchatka Peninsula in north-eastern Asia to northern Mexico in North America. However, it has been introduced throughout the world for recreational fisheries and aquaculture, and there are now naturalized populations of the species in the southern hemisphere (e.g. Pascual *et al.* 2001) and in Europe (Fausch 2007). Two widespread and phylogenetically distinct lineages *of O. mykiss* have been identified in North America, and they correspond approximately to inland and coastal groups separated by the Cascades mountain range (Burgner *et al.* 1992; Busby *et al.* 1996), although the full phylogenetic picture is more complicated (McCusker *et al.* 2000). In addition, many ecotypes and life history strategies are present in the species. Generally, the anadromous form of the species is termed steelhead and the nonanadromous freshwater form rainbow, golden or redband trout. Steelhead spend from 1 to 7 years in fresh water and then migrate to the ocean where they spend from 1 to 3 years before returning to fresh water to spawn. However, life history strategy in *O. mykiss* is governed by a complex mix of environmental and heritable factors, such that a single interbreeding population can contain individuals expressing nearly every possible combination of years in fresh and salt water (Shapovalov & Taft 1954). There are also several ecotypes of steelhead that can coexist as distinct temporal 'runs' or 'races' that are defined by the season (spring, summer, fall or winter) of peak river entry and associated reproductive maturity (Busby *et al.* 1996).

This life history complexity makes monitoring and evaluation of the species, and its multitude of managed populations and stocks, difficult. Such assessment has become increasingly important, because salmonid populations on the west coast of the United States have declined dramatically during the past few decades and many steelhead populations are now protected under the United States Endangered Species Act (ESA; NOAA 2006). The most important causes for this decline include habitat loss, habitat degradation, recreational harvest and hatchery operations. In addition, genetically depauperate hatchery rainbow trout have been stocked in great numbers in basins containing native steelhead. Introgression by these trout has been reported and may pose a substantial threat to at least some steelhead populations (Garza & Pearse 2008; Clemento *et al.* 2009).

One of the most important methods for monitoring the effects of such threats on fish populations, and for providing other types of biological inference about them, is the use of molecular population genetic analysis. Microsatellite loci have seen widespread use with *O. mykiss* and have proven powerful in studying population structure and interactions among different groups (Beacham *et al.* 2000; Narum *et al.* 2004; Aguilar & Garza 2006; Pearse *et al.* 2007; Clemento *et al.* 2009). Fortunately, due primarily to the importance of *O. mykiss* in aquaculture, many additional genomic resources have been developed for the species, including expressed sequence tag (EST) databases and linkage maps (Rexroad *et al.* 2008).

These resources are allowing more detailed analyses of ecological and conservation genetic questions than previously possible (e.g. Martínez *et al.* in press). They also allow the identification and development of SNP markers for salmonid species that can be surveyed on a large scale (Smith *et al.* 2005; Castaño-Sánchez *et al.* 2009). Such markers will allow large-scale monitoring and will further elucidate some of the pressing questions regarding *O. mykiss* ecology and life history evolution, through both traditional population genetic analyses and large-scale parentage inference (Anderson & Garza 2006), particularly with the advent of high-throughput genotyping methods.

In this study, we describe the discovery, characterization and development of assays for a large number (139) of SNP loci for steelhead/rainbow trout. We exploited EST databases to design nearly 500 primer sets for functional genome regions. PCR products resulting from these genes, which include both exonic and intronic regions, were then sequenced in an ascertainment panel of 22 fish designed to simultaneously represent some of the phylogenetic diversity of the species and to provide polymorphic markers for focal populations in California. Such 'balanced' ascertainment is intended to reduce the bias against polymorphism in other populations and lineages of a species when only particular groups are used in marker discovery (Clark *et al.* 2005). These SNP markers represent a valuable resource for studying ecological interactions, phylogeography and conservation status, as well as for pedigree reconstruction, individual and genetic stock identification and, eventually, for linkage mapping.

Methods

Ascertainment panel

Individuals from multiple populations and lineages of *O. mykiss* were chosen for the ascertainment panel. A total of 22 fish from five distinct steelhead populations or rainbow trout strains were included: 10 anadromous adult steelhead from Scott Creek, four anadromous adult steelhead from the Middle Fork Eel River summer run, two redband trout (*O. mykiss newberrii*) from the Upper Klamath River basin and six hatchery rainbow trout raised at Fillmore Hatchery on the Santa Clara River near Los Angeles, CA. Three of these trout were from either the Virginia or Wyoming strains, and three were from

the Mt. Whitney Strain (Busack & Gall 1980). In addition, two coastal cutthroat trout (*O. clarki clarki*) from Little River, Humbolt County, CA were included in the ascertainment panel, to detect and avoid designing assays for polymorphisms that might be because of past hybridization between steelhead and cutthroat trout (Young *et al.* 2001).

Genetic analysis

Tissue samples were digested with proteinase K, followed by DNA extraction with a semi-automated membrane-based system (DNeasy 96 Tissue Kit, QIAGEN Inc.) on a QIAGEN BioRobot 3000. All of these samples had been previously genotyped with microsatellites, so that DNA quality was known to be high. Purified DNA was diluted 1:20 in ddH_20 for PCR.

A total of 480 *O. mykiss* ESTs were selected using a random number generator from the rainbow trout 'Gene Index' online database hosted at the Dana-Farber Cancer Institute and Harvard School of Public Health (http://compbio.dfci.harvard.edu/tgi/; accessed on December 8, 2006). Primers were designed using the program *primer3* v. 0.4.0 (Rozen & Skaletsky 2000) for each of these loci. PCR amplifications were conducted using the following parameters: 0.041 U Ampli*Taq* DNA polymerase (Applied Biosystems Inc.), 1.5 μL PCR buffer (Applied Biosystems Inc.), 0.9 mM $MgCl_2$, 0.5 mM dNTPs, 5 μmol of each primer and 4 μL of DNA template. Thermal cycling conditions employed a 'touchdown' protocol and were as follows: an initial denaturation of 3 min at 94 °C, then 2 min at 63 °C and 1 min at 72 °C, followed by [94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min] × 12 (−1 °C/cycle), [94 °C for 30 s, 48 °C for 30 s, 72 °C for 1 min] × 11, [94 °C for 30 s, 48 °C for 30 s, 72 °C for 1 min (+10 s/cycle)] × 9 and finally 5 min at 72 °C. PCR products were surveyed by gel electrophoresis in 2% agarose. PCR products that exhibited a single robust band were purified using an Exo-SAP protocol (USB Inc): 5 μL of PCR product, 0.15 mL of Exonuclease I (20 U/mL), 1 μL of shrimp alkaline phosphatase (1 U/mL), 0.5 μL of 10× buffer and 3.36 μL of deionized water were incubated at 37 °C for 60 min and then 80 °C for 20 min with a cool down to 4 °C. Clean products were then Sanger sequenced on both the forward and reverse strands using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems Inc.). Sequencing reaction products were purified using 6% Sephadex columns and visualized by capillary electrophoresis on a 3730 DNA Analyzer (Applied Biosystems Inc.).

All sequences from each locus were aligned and assembled into contigs using Sequencher 4.9 (Gene Codes Corporation). Where the alignments indicated a polymorphism, the chromatograms were visually examined for verification. To consider a polymorphism for development as a SNP assay, we used the criterion that all three genotypes (the homozygotes for both alleles and the heterozygote) for that site must have been observed at least once in the ascertainment panel. No distinction was made with respect to the population or strain in which the genotypes were found. This ascertainment criterion was employed to reduce the identification of sequencing artefacts as SNPs and to select the nucleotide sites that had the highest probability of being sufficiently polymorphic for downstream applications. A BLAST search was also performed on each consensus sequence to determine whether the EST corresponded to an identified gene and to ensure that each SNP marker would represent a novel assay in an independent gene. We chose one potential SNP for each EST analysed to reduce the probability of markers in linkage disequilibrium. The site with the highest minor allele frequency in the ascertainment sample that also met the assay design criteria (e.g. more than 25 bp from the end of the sequence, no adjacent polymorphism) was chosen for assay design.

SNP assay development and validation

Consensus sequences, with the selected nucleotide sites indicated, were submitted for the design of 5′ nuclease allelic discrimination, or TaqMan, assays (Applied Biosystems Inc.). When it was not possible to design an assay for a selected site and another nucleotide in the consensus sequence met both the ascertainment and design criteria, a second attempt was made to design an assay for that locus.

Single-nucleotide polymorphism assays were validated by genotyping a total of 186 fish from the following eight steelhead populations or rainbow trout strains: Scott Creek (n = 46), Klamath River-Kelsey Creek (n = 23), Eel River-Middle Fork summer run (n = 24), Sacramento River-Battle Creek (n = 23), Columbia River-Willamette River (n = 23), Kamloops Strain-Hot Creek Hatchery (n = 15), Mount Whitney Strain-Fillmore Hatchery (n = 16) and Eagle Lake Strain-American River Hatchery (n = 16). SNP genotyping was carried out in 96.96 Dynamic Genotyping Arrays on an EP1 Genotyping System (Fluidigm Corporation), which uses nanofluidic circuitry to simultaneously interrogate up to 96 loci in 96 individuals.

Statistical analysis

Deviations from Hardy–Weinberg and gametic phase (linkage) equilibrium were evaluated with GENEPOP 4.0 (Rousset 2008). Observed and expected heterozygosity (Nei 1978), the fixation index F_{ST} (Weir & Cockerham

1984) and allele frequencies were estimated using GENETIX 4.05 (Belkhir *et al.* 1996–2004).

Results

Of the 480 primer pairs designed from *O. mykiss* ESTs, 264 produced a single-sized PCR product in most or all fish in the ascertainment panel. Of these 264 ESTs, 236 yielded sequence at one or more individuals. All PCR products were subjected to sequencing, even if a band was not visible for every individual on an agarose gel. A mean of 18 (range 1–22) individuals produced sequence for each locus, and most of these resulted in broadly or completely overlapping forward and reverse sequences. Because EST sequences are derived from mRNA and therefore lack intronic regions, many of the PCR products were larger than the predicted size and several of them did not have overlapping forward and reverse strand sequences. None of the ESTs were identified as coming from the same gene in a BLAST search (Appendix S1), nor did they match any published SNP assays for *O. mykiss*.

More than 2.3 MB of genomic sequence was produced and aligned (Table 1), or 4.6 MB when both strands were considered separately, and a composite consensus sequence of 130 KB (mean 551 bp/locus) was used for discovery and the determination of density. To account for the lack of sequence for all individuals in all sequences and the consequent decrease in probability of finding variability, we calculated a consensus length weighted by the number of individuals for which sequence was obtained. The weighted consensus sequence was 120 KB (mean 513 bp/locus). In other words, 92.3% (120 KB/130 KB) of the entire consensus sequence from these 236 loci was obtained for all 22 individuals in the ascertainment panel. The density of all nucleotide sites with apparent substitutions was 0.0111, or one every 111 bp. When weighted by the number of fish for which sequenced was obtained, the density of substitutions was 0.0122 or one every 122 bp.

A total of 175 sequences were submitted for assay design. In addition, one sequence (GHPROM1) with a SNP identified in a previous effort (Aguilar & Garza 2008) was submitted for design. Of those, 167 yielded designs suitable for assay manufacture. From these 167, we then eliminated 28 because of problems with genotype calling or because the assay was not interrogating a single Mendelian locus (all apparent homozygotes or heterozygotes).

This elimination process left 139 SNP assays for further validation and characterization. A list of these assays, with primer/probe information and with the variable base indicated, is found in Table 2. To evaluate the utility of these loci in different parts of the species' geographic range and for both natural populations and hatchery/aquaculture rainbow trout, we genotyped all 139 loci in eight steelhead populations or rainbow trout strains (Table 3). Several loci were not in Hardy–Weinberg equilibrium for some populations or strains, but only four loci deviated from equilibrium in more than one group and no locus deviated in more than three populations or strains. Very little linkage disequilibrium between markers was found. Three markers (Omy_114448-87, Omy_121006-131 and Omy_127236-583) were in complete disequilibrium, in spite of the fact that they were designed from unique ESTs, but aside from those three, only eight pairs of markers (out of a total of 9005 pairs), were in significant linkage disequilibrium ($P < 0.001$; 53 more pairs if $P < 0.01$), which is similar to the number expected by chance alone.

Table 1 Summary of EST sequencing effort

	Total	Mean [Range] per locus
EST loci sequenced	236	
Base pairs sequenced	2 322 269	
Length of consensus sequence (base pairs)	130 025	550.95 [109–1417]
Weighted consensus (base pairs)	119 969	512.69
Number of observed substitutions	1366	5.84 [0–21]
Number of SNPs (all three genotypes observed)	506	2.16 [0–10]
Loci with no variable sites	10	
Insertions/deletions (indels)	182	
Transitions (A-G or C-T)	676	
Transversions (A-C or G-C or A-T or G-T)	681	
Possible duplicated genes	14	
Sites with 3 nucleotides observed	9	
Total number of substitutions + indels	1548	
Density of substitutions in consensus sequences		0.0111
Density of substitutions in weighted consensus sequences		0.0122

Table 2 SNP type, forward and reverse primers (5′-3′), TaqMan probes and dye, length of consensus sequences, GenBank accession numbers and dbSNPaccession numbers for the 139 SNP loci

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_95318-147	C/T	F: CGTGTTCTATTTGAAGGCTGTTAAAGG R: TCCTGAACTTAAACCTGCCGTTTT	VIC: TTGGTTCTGGATATTAT FAM: CTTGGTTCTGAATATTAT	377	HR504810	275517390
Omy_95442-108	G/A	F: CCCTGATTATATAGGGAGCTTTCCACTT R: CTTCGGCTCTCGCCAAGT	VIC: TTTCCCACCCAGCATT FAM: CTTTCCCACTCAGCATT	255	HR504811	275517391
Omy_95489-423	T/C	F: TGAGTCCAGTAAAATCCAATCAATAATCATGT R: ACTAGAGCACTGATAGGCTGTCA	VIC: CTGCCACTAACATAC FAM: CTGCCACTGACATAC	595	HR504812	275517392
Omy_96158-277	T/G	F: TTGTGACGGGATCCTTCATTGAG R: CACCTCGATCTCCTTTCGGTAAAA	VIC: AAATACGACCCAACAATA FAM: AAATACGACCCCACAATA	336	HR504813	275517393
Omy_96222-125	T/C	F: GTAAGGAACTAATTGGCGCAACATT R: CAGTTTGTCTAACACCCAGGCATAT	VIC: AACTACAACTGTAGCTAATT FAM: CAACTGTGGCTAATT	615	HR504814	275517394
Omy_96529-231	C/T	F: GCGGTCCACAACCTTCTATCC R: GCCACGGCAAGGTTAAGG	VIC: ATTTCACATAGTGGGTCTG FAM: ATTTCACATAATGGGTCTG	305	HR504815	275517395
Omy_96899-148	T/G	F: CCGGACTACAGGGCTCTGA R: GTGACCCTCCCAGCTTCTG	VIC: CAGGCCTTAGTGCAGC FAM: AGGCCTTCGTGCAGC	568	HR504816	275517396
Omy_97077-73	T/A	F: GTGTAAACAAAATGACTCTGGGATTCAG R: AGAAGTGGCAATGGTGTGAAGTAT	VIC: TGGTGCAATAGAAATA FAM: CATGGTGCAATAGTAATA	295	HR504817	275517397
Omy_97660-230	C/G	F: TCAGTTATGTGTAATCTCATTACCTCTCCAA R: AACAGAAAAGGTCTCAATGTATTTTTTGCA	VIC: ACGTAACTTGTAGCGTTTT FAM: ACGTAACTTGTACCGTTTT	461	HR504818	275517398
Omy_97865-196	A/G	F: TCCAGACTTCTGGTTTGTTCCATT R: CCAGCCCCTATATTCACAATTAAGTGT	VIC: ATGAGCTTGTTAATTAAT FAM: AGCTTGTCAATTAAT	299	HR504819	275517399
Omy_97954-618	C/T	F: GCTCTGCTTCCTCGGCAAATA R: CACAATTGGTTTTTGCACAAAAGTAAAGTATT	VIC: CAACGCTTACCGGTGTGT FAM: CAACGCTTACCAGTGTGT	871	HR504820	275517400
Omy_98188-405	T/C	F: CACAGTTGCAAGGTAGAGGCTTATA R: GCTGAAAGATTAATCCCAGACTGTAGATT	VIC: CTCTCATAAGTCTATCCTCC FAM: CTCATAAGTCTGTCCTCC	425	HR504821	275517401
Omy_98409-549	A/G	F: CGCCTTCCTCAGTATGACATATGTA R: AGGATTTCAGGAAAACCAGGGAATT	VIC: ATTTTGCAACTCTACTTTC FAM: TTGCAACCCTACTTTC	1077	HR504822	275517402
Omy_98683-165	A/C	F: GCCATTGCCAGAGAATTTGGTTAA R: AACACACGCACCATCTTAAAGC	VIC: AGCCAGATACATATTTGT FAM: CCAGATACAGATTTGT	897	HR504823	275517403
Omy_99300-202	T/A	F: CAGTTTGACCCGATGGTGTGA R: GATTATGGCGTGGCCTTTTGG	VIC: TCAGGCATGAGAGAAA FAM: ATCAGGCATGTGAGAAA	386	HR504824	275517404
Omy_100771-63	T/A	F: CATTTAAGGAGGTGGGTTTGTGAAA R: AGTTTGCTGCCACTTGACAGTATT	VIC: AAAGAGCTAGAAATACTG FAM: AAAGAGCTAGAATTACTG	399	HR504825	275517405
Omy_100974-386	T/C	F: ACATGCAAATTAAGTGTTTGTTTTTAAAAATCGAA R: CGACTTCATCCTTTTCATGTAGTGAGT	VIC: CACAGTATTATCAAGATTTT FAM: CAGTATTATCGAGATTTT	471	HR504826	275517406
Omy_101119-554	A/G	F: GGTGGCTTGTTTCTCCCTGTTT R: GCTCCTACCAGCTGAACAGA	VIC: CATGGACATGATGTTACC FAM: ATGGACATGACGTTACC	1110	HR504827	275517407
Omy_101341-188	T/C	F: CTGGAGATAGAAAATATCACACAGAACAGT R: CCTCATTTGATGCATGATTCTGTTGT	VIC: TGATATACTGCAGGTTCC FAM: ATATACTGCGGGTTCC	668	HR504828	275517408
Omy_101554-306	T/C	F: GCCTGTATTTCTCCTGTATGTGCAT R: TCAACTTTTGCAAACTTTTTTATTCTTTGTCATTT	VIC: TGCTTCTCACATTTTTA FAM: TGCTTCTCACGTTTTTA	411	HR504829	275517409

Table 2 Continued

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_101704-329	A/C	F: TGTGTGTTTAACTGACAGAGATGCT R: GGAGCAGGAGCTCAAGGA	VIC: CACCTCCTCTCGGCTGT FAM: CTCCTCGCGGCTGT	591	HR504830	275517410
Omy_101770-410	T/C	F: GTTTTCTATGAGCAGGAGAGGGTTAA R: CTTAGAAAGTACTTCTTTAAATATCAAATGCATTCAGT	VIC: CCTGTCTTTCAAAACTAA FAM: CTGTCTTTCAGAACTAA	795	HR504831	275517411
Omy_101832-195	A/C	F: TGGCTCTGGACCTGTTGAGA R: CGTCACAGCTATTTTAGGCGTAGT	VIC: TGTAGTCTTTCAGAGTAGTATG FAM: TAGTCTTTCAGAGGAGTATG	611	HR504832	275517412
Omy_101993-189	A/T	F: ACAAAACACAGTGGAATTACAATTAACGTT R: GGAAGTTAAATTTCGCTTCGTCAGAA	VIC: CTTGATTTGCAGCTTGTCAA FAM: TGATTTGCAGCATGTCAA	782	HR504833	275517413
Omy_102213-204	T/G	F: AGATGTTAACTACATTCCATGACAATGATTGA R: GAGTATCTCATTCGCAACACTATGGT	VIC: CTAAAAACCCATTAATTCAAT FAM: AAAACCCATTCATTCAAT	640	HR504834	275517414
Omy_102420-634	T/G	F: GGTCGTAGTACACACCTGAGTAAAT R: CACGACACATGCCAGTAGACT	VIC: CCTAAAGCGCTTATCTTAA FAM: CTAAAGCGCTTCTCTTAA	732	HR504835	275517415
Omy_102457-423	T/G	F: CGATGAGTCAAGATAGTCGCTACT R: GGCGTATGGAATTTAGTAGACTAGATTTTCA	VIC: CCCCCAAAAATGTC FAM: CCCCCAAAATGTC	584	HR504836	275517416
Omy_102505-102	A/G	F: CTGCAAACTGACATGGTAGCAAAA R: TGCTTGCTTTTTAAAAACAATCTCCCA	VIC: AACAGGATGTTTTTGC FAM: CAGGATGCTTTTGC	150	HR504837	275517417
Omy_102510-682	T/G	F: AAGATCAGTGTGGCATCAATGTCA R: TCGTGCCTGGATGTAAGTTAACTG	VIC: TTGTCCTCAATATTCAC FAM: TTGTCCTCACTATTCAC	732	HR504838	275517418
Omy_102867-443	T/G	F: CATTTGTTTAATTTGATTTGGCACAACTTCA R: CCCTAGTTCTGTAACACAAGACGTAA	VIC: TTTGGGTACATAATTTTT FAM: TGGGTACATCATTTTT	443	HR504839	275517419
Omy_103350-395	A/C	F: CGCGTGTTGAACCTAGAATGAC R: GGAAAATTCCTGCCAATGACACATG	VIC: AGAACCAGGAAATTAACTAC FAM: CCAGGAAATGAACTAC	471	HR504840	275517420
Omy_103577-379	T/A	F: GGAGTGATCCAAGGTTATGTACCAA R: CCAGCAATTTCCTTTCGAATCATTGA	VIC: AAGTGTGCACACGTTCA FAM: AAGTGTGCACTCGTTCA	759	HR504841	275517421
Omy_103705-558	T/C	F: CTCCAATCGCAAATACCCAGACT R: CGCAGGAGACGGATGCC	VIC: AGACTTACCCAGAGTGAGAG FAM: ACTTACCCAGGGTGAGAG	658	HR504842	275517422
Omy_103713-53	T/G	F: TCATGAGTGAAGCGCACAGAA R: CTTTAGTAGGAGGTTGTAACCAAGTCA	VIC: AGGTTACTGGAGAAATCT FAM: ACTGGCGAAATCT	423	HR504843	275517423
Omy_104519-624	T/C	F: CGTGTGAGTTTGCGGTAAAGAC R: TGACGAGTCCGTCTTATCATCCT	VIC: CAGCAGGATACATCCGACT FAM: AGCAGGATACGTCCGACT	1061	HR504844	275517424
Omy_104569-114	A/C	F: CCGAGGCCGACGTGATC R: GCGCCTCGCTCATCATCA	VIC: CGCCACTCCGACGCC FAM: CCACGCCGACGCC	565	HR504845	275517425
Omy_105075-162	T/G	F: GGAGAAGGACAAGGACATTGGTAAT R: AAAGCAGACCACACCATACTTCTC	VIC: CTTTCTCTCCTACTTTCC FAM: CTTTCTCTCCTCCTTTCC	443	HR504846	275517426
Omy_105105-448	C/T	F: CAATTTGCAAGCAGGGAAAGGTTAT R: GTGATGGGCTGCAATTGCTT	VIC: AAGGAGAATGCATAATC FAM: TGAAAGGAGAATACATAATC	810	HR504847	275517427
Omy_105115-367	C/G	F: GCTCCCTCCGAAGAAATCTCA R: CATACTCGTCAATCACCCAAGCT	VIC: CATGCTGGAGCGCAAT FAM: CATGCTGGACCGCAAT	401	HR504848	275517428
Omy_105235-713	C/T	F: AGGCCATAAAATCAGGCATTAGGAT R: TGGGCTCTGCAAAGACAAGA	VIC: AGAGAGTCAATCGTTGCAAA FAM: AGAGAGTCAATCATTGCAAA	788	HR504849	275517429

Table 2 Continued

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_105385-406	T/C	F: ACCTACCCTCACCTGAACTTCA R: CGCTCTTCTGGGCGTATCG	VIC: CTTGGAACCATTGCTAC FAM: TTGGAACCGTTGCTAC	691	HR504850	275517430
Omy_105386-347	A/C	F: CCAGGAAATCGTCAGCTCTATTTAATACAT R: GAAACCTCCTTCAACCTCTGGATAA	VIC: ACATTTCAACTCAATTAATAATTA FAM: TACATTTCAACTCAATGAATAATTA	438	HR504851	275517431
Omy_105401-363	A/G	F: GGCACCCTCATTCACACATACTAT R: GTCTTCTCAAATAACCCCTGTGGAT	VIC: CCAAGTACCCTAGGTTGG FAM: CAAGTACCCCAGGTTGG	419	HR504852	275517432
Omy_105407-74	T/G	F: GGATGGCTTGGAATGTGCAA R: GCGGATGTACACAAAATACACTCAA	VIC: CTCTTTGCGTTTAGTCCTA FAM: TCTTTGCGTTTCGTCCTA	472	HR504853	275517433
Omy_105714-265	C/T	F: CCACTCAGTGCAAGCATGGA R: GCTTTCAATCCTTGGCTCCAATATC	VIC: CTGTTGTTTGAGGTTCAG FAM: TGTTGTTTGAGATTCAG	476	HR504854	275517434
Omy_105897-101	T/A	F: GAACCAATACACAATGCCAAGGATT R: GCTAGGGCTGCTATCTTTGTGATG	VIC: TCTCTCCACAGTTCTC FAM: TCTCTCCACTGTTCTC	382	HR504855	275517435
Omy_106172-332	T/G	F: CCACTTTGTTACTAAATGTTCCCATGAC R: ACATTCCAAAGACTGTCACATTCCA	VIC: ATGAACAGAATGTAATCTAG FAM: TGAACAGAATGTCATCTAG	467	HR504856	275517436
Omy_106313-445	T/G	F: CCAACTGTTGTGTCTTGATTTGTGA R: GTTCTGTGTCTGAAGTCCATTGGT	VIC: TTGATTTTTCCAAACCATGTGTG FAM: TTGATTTTTCCAAACCCTGTGTG	729	HR504857	275517437
Omy_106560-58	C/T	F: CCACCCAGCCATCAACGA R: CGTTCTTTCCCAGCGAGTGA	VIC: CTCAGAGCGCAGGCC FAM: CTCAGAGCACAGGCC	387	HR504858	275517438
Omy_106747-707	A/G	F: CCGTTAAGAAAGGGTGACATCATGT R: AGATCCATGGCCCCAGTCT	VIC: CGATACTCACACTGGCCTG FAM: ATACTCACACCGGCCTG	753	HR504859	275517439
Omy_107031-704	C/T	F: GGCTTTCGGATACTGAGCAACAA R: TGAACTCACTGTTGGTATGGACTAGA	VIC: TGGACATGATTGCATAGAC FAM: CTGGACATGATTACATAGAC	798	HR504860	275517440
Omy_107074-217	A/G	F: CCGGGCTGTCATGTGACT R: CTGCTGACAGGCCTGAGA	VIC: CCCTGGTCTTGACCC FAM: CCTGGCCTTGACCC	397	HR504861	275517441
Omy_107285-69	C/G	F: GCCCTTGTGACAATGCACTGTTATA R: AGGTCTAGACAGTGTGCCATTTG	VIC: ATACGTTACTTTTGACCTTGT FAM: ACGTTACTTTTCACCTTGT	704	HR504862	275517442
Omy_107336-170	C/G	F: GCCCTCTCACTCATGACATCAAC R: GCTCCAGCCACTCGCA	VIC: CACTCCTGGGTGCAGAA FAM: ACTCCTGCGTGCAGAA	471	HR504863	275517443
Omy_107607-137	T/G	F: TGAGACAACCCAAAGCTTTAAGGAA R: CAACGCACACTATCAGATCACATC	VIC: ATGTTCCGACAATAAAT FAM: TGTTCCGACCATAAAT	517	HR504864	275517444
Omy_107786-314	G/A	F: TGGTTGTCCAAAGCTTTCTTCAGAA R: GCTGATACTACAGCATCCAAGGT	VIC: CACCTCACCCTCCTCC FAM: ACCTCACCTTCCTCC	635	HR504865	275517445
Omy_107786-584	T/G	F: GGACACAAGTGGGTACTATTCCATT R: AGTCAGTCAAGCTCTCTGGAGATAG	VIC: CAATGGTAAGATTTTG FAM: CAATGGTACGATTTTG	635	HR504866	275517446
Omy_107806-34	C/T	F: TCTTTGTCCATGCACATTGATATT R: AGCACATTTAGTTAGCAGTGATGGA	VIC: ATTGGATGTCAGTGTCATT FAM: ATTGGATGTCAATGTCATT	983	HR504867	275517447
Omy_108007-193	A/G	F: GTGAATACCACCCAGGCTTGT R: GTCCCTTCCCCAGTTTCACTTAATT	VIC: ATGTTTTCTCCCTACTTAAC FAM: TTTTCTCCCCACTTAAC	441	HR504868	275517448
Omy_108735-311	C/T	F: GTTTAATCCTGACTTTTCACTTTTGTCATCT R: GCGTGCCCTCAATTCCATT	VIC: AACGCCTCGTGACAAT FAM: AACGCCTCATGACAAT	428	HR504869	275517449

Table 2 Continued

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_108820-85	T/G	F: CACCAACAACGTGTAGATTTCCTTAAAATATT R: TTTGGTTGGTTGTTTTTATCATTGATACAGTT	VIC: TTGATATGTGAATTTTG FAM: TTGATATGTGCATTTTG	397	HR504870	275517450
Omy_109243-222	A/C	F: ATGTGCACCTCTTAAATTGTAAGTAAAATGT R: ACCCTATATTCAGTGGCAAGATTGC	VIC: TGTTCATTAAATTGACTTTTT FAM: TTCATTAAATGGACTTTTT	521	HR504871	275517451
Omy_109390-341	C/T	F: ATTACAAACACAAGTCCTCATACAAGTGA R: TGTAGGCAACGTTGGTTTATGGT	VIC: CATTTTGGCGGTCCAGAA FAM: CATTTTGGCGATCCAGAA	426	HR504872	275517452
Omy_109525-403	A/G	F: CCTCATTCTCATTGGTGAGTTGTCT R: TGTAAGATCTGACCACATGAGTATAACCA	VIC: CCTACACCTCTTTTTTCCACA FAM: CCTACACCTCTTTTCTCCACA	1045	HR504873	275517453
Omy_109651-445	C/T	F: CCTGATTTTGCCCACATTTCAAGAA R: GCTGTTGTCATATCATCCCGTTAAC	VIC: CATATGTTAACGTGGGCTAT FAM: CATATGTTAACATGGGCTAT	615	HR504874	275517454
Omy_109693-461	T/A	F: GCCTCACCTGATGCCCATT R: TGGAGGATTCAGCATTTGGATACC	VIC: ACGACAGCCACACACAG FAM: ACGACAGCCTCACACAG	474	HR504875	275517455
Omy_109874-148	A/G	F: GTATGTGTGAGTATGTAATGACTGTATTTAGGA R: CTCCTCCCTCAGTGCATTACATTTT	VIC: ACAGCATTGATTTTGTCACC FAM: CAGCATTGATTTCGTCACC	392	HR504876	275517456
Omy_109894-185	T/C	F: CGGTGTCATTATGGTTGTCATTGTG R: GGGAGGAATTGGAATGACAGATTAAC	VIC: CTCCCTGATCCCCC FAM: CTCCCTGGTCCCCC	581	HR504877	275517457
Omy_109944-74	T/G	F: CCGGGACCAATTGAGAAATCGATAA R: GGGTTCAAGAGTACACGCCAA	VIC: ACGTGACTGTATAGAGACT FAM: ACGTGACTGTATCGAGACT	116	HR504878	275517458
Omy_110064-419	T/G	F: GTGCAAGGGACCTAGCTAATCC R: TCTGAACTGACACTGAAGAACAAAGAA	VIC: ACGTTAGCTTTTAATTTC FAM: AACGTTAGCTTTTCATTTC	798	HR504879	275517459
Omy_110078-294	A/G	F: GCAGTAAATCAGCAGAGACCTACA R: CCTTAAGCTCAGATTTAAACGATCAAAACA	VIC: TGTCTACGGATGACTTC FAM: TCTACGGACGACTTC	478	HR504880	275517460
Omy_110201-359	T/G	F: GGTAAGGCCTGTCTGACTATTTTGA R: AGAGGTCAATGGATGCCAGTTT	VIC: TTTGGCTATTGAAATTATACATT FAM: TTGGCTATTGAAATTCTACATT	588	HR504881	275517461
Omy_110362-585	G/A	F: GCAGCCAAGATGAACGAAAACTTC R: CCGGCCTGGGTCTCAATG	VIC: CACCGCCCTGCCCGT FAM: CACCGCCTTGCCCGT	653	HR504882	275517462
Omy_110571-386	C/T	F: CACTTGGCTCTGCACTAGCA R: GGGTTGTTAAGAGTCCATTAGAAAGAAC	VIC: CTGTGTAAAATCCATGTCAACA FAM: TGTGTAAAATCCATATCAACA	479	HR504883	275517463
Omy_110689-148	A/C	F: GTGTGTGGCAGAGAACTAACTGAT R: GGTTAAGACATTAACATAACACTGGACTCT	VIC: CAAATGAACACATTATTTATC FAM: ATGAACACATGATTTATC	379	HR504884	275517464
Omy_111005-159	C/T	F: ATCTGTCAGACAGTTGTGGATAATGTC R: TCGATGACCAACATTGTAGTGTTAAATACA	VIC: AGTCAAAAGGGCACAAAA FAM: AAGTCAAAAGAGCACAAAA	463	HR504885	275517465
Omy_111084-526	A/C	F: CACCACACCAAGCAACTATTTCATT R: ACCCAACTACTGTCCCATTTTTCAT	VIC: CCAGTGAAATTTATTTTT FAM: CAGTGAAATGTATTTTT	709	HR504886	275517466
Omy_111383-51	C/T	F: CACGCGCAATCTCTCGTTTTAC R: TCTTTAGGCAACAAGCGTGTCA	VIC: ACCTAGTGCGCTTGCT FAM: ACCTAGTGCACTTGCT	495	HR504887	275517467
Omy_111666-301	T/A	F: GGGTGAAAAGAGTGGGACATTTACA R: GTCAATTTCAAGGCACCAGACAAT	VIC: AGTATAACACAGTAAGACAAT FAM: AGTATAACACAGTTAGACAAT	639	HR504888	275517468
Omy_111681-432	C/T	F: GGCGGTTTAAGCAGCAGAAATAC R: GTGGATCATGCTCGCTAGGT	VIC: TCCCTCTCGGGTGCTG FAM: CCCTCTCAGGTGCTG	693	HR504889	275517469

Table 2 Continued

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_112208-328	T/C	F: GTCAACAGTTGGACGTAGATGCT R: CCTTCAGCTTGATCACCTCATAGG	VIC: CTGACAGTGATTATTTTGT FAM: TGACAGTGATTGTTTTGT	904	HR504890	275517470
Omy_112301-202	T/G	F: GTAAACCCTGCCCACATAATTAGGT R: CTGAGACACTGCTCCAAGGT	VIC: AATGCGAAGACAAACT FAM: AATGCGAAGCCAAACT	1146	HR504891	275517471
Omy_112820-82	G/A	F: CCTTTCCTTTTGCATTTCCTCTACTTATTTATTT R: AAATGAACTCACGTTGACCTCTGA	VIC: CGCCGCCAAGTTA FAM: CGCCGCTAAGTTA	393	HR504892	275517472
Omy_112876-45	T/C	F: GGACTACATGAAGGCGTGAGT R: ATCAGTCCTAGCCCAAACACATG	VIC: TTTTAGTGACGAGTGTCTG FAM: TAGTGACGGGTGTCTG	805	HR504893	275517473
Omy_113109-205	T/G	F: GTGGGCACTGTTACACAAAGTTC R: CCAGTCAACTTACAAACAAGCCATT	VIC: CGTCATCTTAAATTATCTTTG FAM: CGTCATCTTAAATTCTCTTTG	416	HR504894	275517474
Omy_113128-73	C/G	F: CCTCCTACTCTGATCTAAAGATTACAGAA R: TTCTCTGCCCTCTCGATTTTGG	VIC: TGGCAGGGTTTCCGG FAM: TGGCAGGCTTTCCGG	374	HR504895	275517475
Omy_113242-163	T/C	F: TGGTGGACTGATCTGATGATGAAAG R: CCTCGTCCATATTTTCCTCCTCAA	VIC: TCTGAGACAACACGCTAT FAM: CTGAGACAACGCGCTAT	389	HR504896	275517476
Omy_113490-159	C/T	F: CATAGTACATTTACAGATAATGTTTTAAAGTGCATGT R: CGAGATACCAAAATGCCACAGTTACAT	VIC: CATCTGTTTTGGTTTAGC FAM: CATCTGTTTTAGTTTAGC	288	HR504897	275517477
Omy_114315-438	T/G	F: CCTCACCGATCTAGTCAACTTCATC R: AGGAGGCTGAGGGAGATTCTAG	VIC: TTATGGGCTTAAGGGTC FAM: TTATGGGCTTACGGGTC	555	HR504898	275517478
Omy_114448-87	C/T	F: GCCGAAAGGTAAAATCCACAAATCC R: GGACTAGGCTAACAGGAGAAGCT	VIC: TGGTTGATCGAACATTT FAM: TGGTTGATCAAACATTT	530	HR504899	275517479
Omy_114587-480	T/G	F: CAGATTACGTTATTACGTTTGGGAAATTTTTAAGT R: GTGAAAGAGTGGGAAATATAATTATAAGGTCAGA	VIC: CCTGTCCAAAATTGT FAM: CCTGTCCACAATTGT	1266	HR504900	275517480
Omy_114976-223	T/G	F: GACAAACAGCACTTCATTGCAGTAA R: GTTGCTCCAGCACCAGGT	VIC: ACCGATGGAACAATC FAM: CCGATGGCACAATC	735	HR504901	275517481
Omy_115987-812	C/T	F: GAGCTCCTGAAGACCTATAAGAATGTT R: GGTCGAGGAAGAGCTCAATGC	VIC: CTGAAAAGACTGCTCCAC FAM: CTGAAAAAACTGCTCCAC	1166	HR504902	275517482
Omy_116104-229	T/C	F: GCTAGAAGATAACAGGCCACACT R: ATGGTATTCAATGGCATTTTCAGTTTCAAA	VIC: TGACAAGTTTAAGCTTG FAM: TGACAAGTTTAGGCTTG	513	HR504903	275517483
Omy_116362-467	T/G	F: CTGGATCCAAGAGGCTGTTCT R: TGCCTGCTATAGTTCCATGTCAAAA	VIC: CTCACCTGAATCCAG FAM: CTCACCTGCATCCAG	508	HR504904	275517484
Omy_116733-349	C/T	F: GAAATGGACATGCCTACAAATTGCT R: GATGTGATCAGTTTAGGCAAGGC	VIC: AGAGAATCTGATAGTATTTC FAM: AGAGAATCTGATAATATTTC	641	HR504905	275517485
Omy_116938-264	A/G	F: GTTCATTCATGTTGAAGTGCGACAT R: CTCTGCATGCTCCCATCCT	VIC: CCTTGTCTCAATTTTTCCTCT FAM: CTTGTCTCAATTTCTCCTCT	530	HR504906	275517486
Omy_117242-419	G/A	F: GTCTTCTCTCTTTCTCTCCCTCTCT R: CCACTGGCCTTCAATTGTAACAG	VIC: CCTCCCTGCCTCCCT FAM: CCTCCCTGTCTCCCT	479	HR504907	275517487
Omy_117259-96	T/C	F: CAAGGGAAGAGCTCTGAGATGAG R: GGGATCAGTGGCAGGTAGAG	VIC: CGTCATGCCATCATGT FAM: CGTCATGCCGTCATGT	409	HR504908	275517488
Omy_117286-374	A/T	F: TGATGTGTTGTTCCTCATGGCTTA R: CTGTGCATTTATTCTTGTGATGCTAGG	VIC: CTTTCCTCATCATACTCTATGG FAM: TCCTCATCATACACTATGG	453	HR504909	275517489

Table 2 Continued

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_117370-400	A/G	F: TGCAAACACAGAGGAAAGGGATTT R: GGCTTATTTGTTCCGTACTTGCATT	VIC: CAACTCCAATGAATTAA FAM: AACTCCAACGAATTAA	596	HR504910	275517490
Omy_117432-190	C/T	F: GGAGAACGCCTTGAGGTTGT R: TGCCTCATCCTTGGGACTGAT	VIC: TCATGGTGGATCCTGG FAM: TCATGGTGAATCCTGG	441	HR504911	275517491
Omy_117540-259	T/G	F: GGCAGGTTAACACAGTCATCTACTATAAA R: CAGCATGTTGCTTTAATCCTTCACA	VIC: TGTCACTTCAAAGTTTG FAM: TGTCACTTCAACGTTTG	575	HR504912	275517492
Omy_117549-316	A/G	F: CCAGTACCCTTACATCTGAGAACCA R: GGCCTTGGTTGTAGTTGTCACT	VIC: CTGCCCTTGCTGGC FAM: TGCCCCTGCTGGC	425	HR504913	275517493
Omy_117743-127	C/T	F: ACCTGCACCTTGTAAATAATTTATATAGTAG CTAAATAATT R: GCCTGCCTGTGAACAACAC	VIC: ACATACAGAACGTTCACTG FAM: ACATACAGAACATTCACTG	477	HR504914	275517494
Omy_117815-81	C/T	F: CTGCTTTATGCACACCACATTGT R: GCTCTTTCTGGAGAACAAGGTACTG	VIC: CTATACGGAGACCAGC FAM: CTATACGGAAACCAGC	402	HR504915	275517495
Omy_118175-396	T/A	F: AGGCTTCACACACACATGCA R: GACGCGCAACCTCTAGATTATACTT	VIC: CTCTTGCAGACATACCCGTA FAM: CTCTTGCAGACATTCCCGTA	463	HR504916	275517496
Omy_118205-116	A/G	F: CTGCGGTGGGCTACACA R: CGCAGCTGCGGATGAG	VIC: CTACTGAGGCTGAGTGCT FAM: TACTGAGGCCGAGTGCT	485	HR504917	275517497
Omy_118654-91	A/G	F: CAGCGTAGACCGTTTCCTCATTAT R: GCGCCGATGAGCAGCTT	VIC: TCAGCTTGTCTTGCCGC FAM: CAGCTTGTCCTGCCGC	454	HR504918	275517498
Omy_118938-341	A/T	F: GAGGGACAGACTTCAAGATTTCATGA R: AGTCATCATAAAGACTTGTTCATTAAGGAAGG	VIC: TGTTGTTCAGATTGTAAAAA FAM: TGTTGTTCAGATAGTAAAAA	625	HR504919	275517499
Omy_119108-357	T/C	F: GGTAGAAGCAGCCCATGCA R: TGTGGCAAGGACATGTGTGA	VIC: CGCGTCCAAGCAG FAM: CGCGTCCAGGCAG	949	HR504920	275517500
Omy_119892-365	T/G	F: GGTTATAGGTTCGTCACCATCCAAA R: TTGTCTGTGGTGTTATGTCTAATTTCAAG	VIC: AATTCTACCTACAGCTAACA FAM: ATTCTACCTACCGCTAACA	755	HR504921	275517501
Omy_120255-332	A/T	F: GGCTACAGGGACTTTACAATGGG R: GCTAGCTAACATTGAAGGGTGGAAT	VIC: ACTATGCCATGAAGTTA FAM: ACTATGCCAAGAAGTTA	601	HR504922	275517502
Omy_120950-569	T/G	F: TCACACTCAGATTATTGTGGCGATT R: GCTGACTCATAAAAATGTTGGTAATGCT	VIC: ATTGTTTAACCTAAAAGCTT FAM: TGTTTAACCTACAAGCTT	759	HR504923	275517503
Omy_121006-131	T/G	F: ACAGTGAATCAGCGGAGAAACA R: AGTCCGTTTCCTGTTAGTGTAAGC	VIC: TTCGTACGAGACCAAAG FAM: TCGTACGAGCCCAAAG	505	HR504924	275517504
Omy_121713-115	T/A	F: TGTGACAGAGCCAAGGAAAACC R: TGGGCTAGTGAGGGAGTGA	VIC: TCAGGTTGAGTATTGC FAM: TCAGGTTGTGTATTGC	501	HR504925	275517505
Omy_123044-128	C/T	F: CTGGGTGAGTGAGTTGACTATACAC R: CGGGTGTGCATGAGAAAATGAC	VIC: ATTTCTGGCGGTCCGG FAM: ATTTCTGGCAGTCCGG	784	HR504926	275517506
Omy_123048-119	C/T	F: ATGTATCTGGTGCATTGGGATGATT R: ACAGCCACATGTACAGGGAAAAA	VIC: ACTTGCCCGATACTT FAM: ACTTGCCCAATACTT	797	HR504927	275517507
Omy_123921-144	T/C	F: AACTCTGAAGTGGGATGTGATGTTC R: GGATGATGTTACAAAAGGAGAGCATGT	VIC: CTAAGGTTCAGGACTTGGA FAM: AAGGTTCAGGGCTTGGA	1045	HR504928	275517508

Table 2 Continued

Assay name	Assay target	Primers (5′-3′)	Probes (5′-3′)	Cons. Length	GenBank No.	DBSNP No.
Omy_124774-530	A/T	F: AGTACCACCGCCGTCTGATATAT R: CCAGAGCAAAGCATGTCCTCAAATA	VIC: CAAATAAAAGGCTAAATAAA FAM: AAATAAAAGGCAAAATAAA	705	HR504929	275517509
Omy_125998-61	T/G	F: GGTGTCCAGCCACAGTACAG R: TGTTCCTTTATTGGGCCTGCATA	VIC: TGACCTCCATCCCCC FAM: ATGACCTCCCTCCCCC	459	HR504930	275517510
Omy_126160-242	T/G	F: CAAGGGAGTGACCGGAATGTTATAT R: GCCCAGACATTTACAGCAGTATCA	VIC: CAATCATGTGTTAACACTAA FAM: ATCATGTGTTCACACTAA	648	HR504931	275517511
Omy_127236-583	C/G	F: TGGATCAAGACAGATTTCCCCTACA R: GCCACCAGTGAGATGTCTTTGAAA	VIC: ATTGTGAAACGGCCCCT FAM: ATTGTGAAACCGCCCCT	685	HR504932	275517512
Omy_127510-920	C/T	F: GTGTTATGCCAACAAGGCTTGT R: TTTGACAATATCAATATCATGAAAATGTTTGTGAGT	VIC: AACAAATAACAGACGACATTA FAM: ACAAATAACAGACAACATTA	1182	HR504933	275517513
Omy_127645-308	A/T	F: ACACTGATATTAACATGGCACAAGTCA R: CAGGGCCGGTCGTAGATTTT	VIC: AAGTTTGTTACATATTTTG FAM: TTTGTTACAAATTTTG	401	HR504934	275517514
Omy_127760-385	A/T	F: CGGCTATTCTCGCGTAAAAGCT R: AAATGCAACCAGAAACGGAATGTC	VIC: TCCTTATCCAAAATTATTGTGC FAM: CTTATCCAAAATAATTGTGC	756	HR504935	275517515
Omy_128302-430	C/T	F: GTATGGCATTTTTGTTCCCAAGGT R: CATGTGGTTGCCCTCCTTATAGAG	VIC: CATCATCGTAAATCAG FAM: CATCATCATAAATCAG	1025	HR504936	275517516
Omy_128693-755	A/C	F: GATACACTCTACTGACTAGTCCATCCA R: GTCCTGAAAGAGAGAAACACAGACA	VIC: CTCTGACCATTATTTTGTC FAM: CTGACCATTAGTTTGTC	869	HR504937	275517517
Omy_128851-273	T/A	F: GTACAGATGAATGTGTTTTATTTGGCATTG R: CTGCCCATCAAGGTCTTCATCTTAT	VIC: CCTGTCAAATAAAG FAM: CCCTGTCTAATAAAG	348	HR504938	275517518
Omy_128923-433	T/C	F: ACGTTTCTTTGGGCTGAGACTTATT R: CTATGTCCTTGGCAGAAGTCTACA	VIC: CTTCATTTTCATTCACTGTTTT FAM: CATTTTCATTCGCTGTTTT	505	HR504939	275517519
Omy_128996-481	T/G	F: CTCATCCACACTGTACAGTACAAGT R: CATGCCTTCGTCTCATCAATAACAC	VIC: CTTGTGGTTGAGGTTTG FAM: TTGTGGTTGCGGTTTG	515	HR504940	275517520
Omy_129170-794	T/G	F: GTTAGAAACCATGACTCACCATCCA R: CTGTAGCAGTGATGCTATGGAATAGG	VIC: CCCTGTGGAGTGTCAG FAM: CCTGTGGCGTGTCAG	830	HR504941	275517521
Omy_129870-756	C/T	F: TCGTTATTTTGCCTCGCGGTA R: TCCCATGAAGATGTATACATGTTTTGTGA	VIC: ACAGGTATTTCGTGAAATG FAM: CAGGTATTTCATGAAATG	965	HR504942	275517522
Omy_130295-98	A/C	F: GGGACCACAGAATATTTTTCTTGTTCAT R: TGGACAGAATGTTCTACAAGTTGCA	VIC: CTTATGCCTTTTCTAATTCTGTA FAM: TTATGCCTTTTCTAAGTCTGTA	583	HR504943	275517523
Omy_130524-160	C/G	F: CGAAGGTAGCGATTGGTCGTT R: TGTCTGTTCTGCTGTGTGCTT	VIC: ATGGCTTGATCCTCA FAM: ATGGCTTCATCCTCA	388	HR504944	275517524
Omy_130720-100	C/T	F: CGGTCATTGTAAATGTCAACGGTTT R: TGCTTGCATGTTCTTGGTGTAGTA	VIC: ACCTGTCCCGTTCCCA FAM: CCTGTCCCATTCCCA	547	HR504945	275517525
Omy_131460-646	C/T	F: GTGAAAAGGAATGGAGGAGTACAGT R: TGCTAGGACAGGAAGATCATTTGTG	VIC: AATAAAGCAGAATTTGTTACTG FAM: AAAGCAGAATTTATTACTG	1276	HR504946	275517526
Omy_131965-120	C/T	F: AGAGATACATTAAAGCTGTGCTCATTCA R: GCAGAGTTGCTTCAAAACTGTTAGT	VIC: CATTGTAAACGACCATTTT FAM: CATTGTAAACAACCATTTT	240	HR504947	275517527
Omy_GH1-PROM1-1	A/T	F: TCAAACTGCATTTGATGGAAACAAACAT R: AGGACAATTCTAAGTGACCTCAAACTG	VIC: TAGTGTTCACTGACTTCA FAM: TAGTGTACACTGACTTCA	n/a	J03797	n/a

Table 3 Summary statistics of 139 SNP assays in 5 steelhead populations and 3 hatchery rainbow trout strains. The allele frequency reported for all groups is the minor allele ($P \leq 0.5$) in Scott Creek. He is expected heterozygosity, and Ho is the proportion of observed heterozygotes

	Scott Creek			Klamath River-Kelsey Creek			Eel River-Middle Fork (summer)			Sacramento River-Battle Creek			Columbia River-Willamette River			Kamloops Strain-Hot Creek Hatchery			Mount Whitney Strain-Fillmore Hatchery			Eagle Lake Strain-American River Hatchery			
	N = 46			*N* = 23			*N* = 24			*N* = 23			*N* = 23			*N* = 15			*N* = 16			*N* = 16			
Assay Name	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	F_{ST}
Omy_95318-147	0.500	0.51	0.49	0.087	0.16	0.17	0.063	0.12	0.13	0.146	0.25	0.29	0.587	0.50	0.65	0.800	0.33	0.40	0.469	0.51	0.56	0.133	0.24	0.27	0.277
Omy_95442-108	0.174	0.29	0.26	0.043	0.09	0.09	0.021	0.04	0.04	0.152	0.26	0.30	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.031	0.06	0.06	0.073
Omy_95489-423	0.337	0.45	0.37	0.976	0.05	0.05	0.438	0.50	0.46	0.609	0.49	0.43	1.000	0.00	0.00	0.867	0.24	0.27	0.125	0.23	0.25	0.906	0.18	0.19	0.404
Omy_96158-277	0.455	0.50	0.55	0.182	0.30	0.36	0.021	0.04	0.04	0.348	0.46	0.61	0.000	0.00	0.00	0.133	0.24	0.27	0.250	0.39	0.50	0.344	0.47	0.56	0.161
Omy_96222-125	0.256	0.38	0.29	0.595	0.49	0.62	0.167	0.28	0.33	0.109	0.20	0.13	0.091	0.17	0.09	0.067	0.13	0.13	0.031	0.06	0.06	0.000	0.00	0.00	0.195
Omy_96529-231	0.111	0.20	0.18	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.077
Omy_96899-148	0.389	0.48	0.56	0.913	0.16	0.17	0.667	0.45	0.42	0.545	0.51	0.64	0.848	0.26	0.30	0.567	0.51	0.47	0.313	0.44	0.63	0.375	0.48	0.50	0.182
Omy_97077-73	0.250	0.38	0.45	0.043	0.09	0.09	0.500	0.51	0.58	0.283	0.41	0.39	0.130	0.23	0.26	0.000	0.00	0.00	0.313	0.44	0.50	0.000	0.00	0.00	0.153
Omy_97660-230	0.058	0.11	0.12	0.000	0.00	0.00	0.271	0.40	0.29	0.174	0.29	0.26	0.364	0.47	0.45	0.700	0.43	0.47	0.188	0.31	0.25	0.094	0.18	0.19	0.231
Omy_97865-196	0.057	0.11	0.11	0.000	0.00	0.00	0.000	0.00	0.00	0.087	0.16	0.17	0.000	0.00	0.00	0.000	0.00	0.00	0.267	0.40	0.13	0.375	0.48	0.25	0.185
Omy_97954-618	0.360	0.47	0.35	0.023	0.05	0.05	0.500	0.51	0.50	0.717	0.41	0.30	0.000	0.00	0.00	0.367	0.48	0.33	0.719	0.42	0.56	0.156	0.27	0.31	0.294
Omy_98188-405	0.378	0.48	0.58	0.045	0.09	0.09	0.063	0.12	0.13	0.196	0.32	0.39	0.217	0.35	0.35	0.300	0.43	0.20	0.063	0.12	0.13	0.188	0.31	0.13	0.090
Omy_98409-549	0.489	0.51	0.50	0.261	0.39	0.35	0.333	0.45	0.58	0.310	0.44	0.43	0.087	0.16	0.17	0.000	0.00	0.00	0.000	0.00	0.00	0.250	0.39	0.38	0.152
Omy_98683-165	0.337	0.45	0.50	1.000	0.00	0.00	0.478	0.51	0.52	0.609	0.49	0.43	0.957	0.09	0.09	0.867	0.24	0.27	0.594	0.50	0.44	0.750	0.39	0.38	0.270
Omy_99300-202	0.171	0.29	0.34	0.022	0.04	0.04	0.000	0.00	0.00	0.217	0.35	0.43	0.174	0.29	0.26	0.500	0.52	0.47	0.313	0.44	0.38	0.156	0.27	0.31	0.122
Omy_100771-63	0.250	0.38	0.27	0.000	0.00	0.00	0.833	0.28	0.33	0.565	0.50	0.26	0.114	0.21	0.23	0.067	0.13	0.13	0.000	0.00	0.00	0.625	0.48	0.38	0.409
Omy_100974-386	0.167	0.28	0.24	0.190	0.32	0.29	0.188	0.31	0.29	0.391	0.49	0.43	0.023	0.05	0.05	0.100	0.19	0.20	0.469	0.51	0.44	0.219	0.35	0.44	0.089
Omy_101119-554	0.000	0.00	0.00	0.068	0.13	0.14	0.000	0.00	0.00	0.326	0.45	0.30	0.000	0.00	0.00	0.067	0.13	0.13	0.063	0.12	0.13	0.281	0.42	0.44	0.191
Omy_101341-188	0.239	0.37	0.35	0.000	0.00	0.00	0.271	0.40	0.38	0.043	0.09	0.09	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.158
Omy_101554-306	0.478	0.50	0.47	0.048	0.09	0.10	0.354	0.47	0.46	0.217	0.35	0.35	0.022	0.04	0.04	0.267	0.40	0.40	0.563	0.51	0.75	0.688	0.44	0.25	0.209
Omy_101704-329	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.304	0.43	0.43	0.000	0.00	0.00	0.133	0.24	0.13	0.469	0.51	0.44	0.063	0.12	0.13	0.287
Omy_101770-410	0.211	0.34	0.33	0.182	0.30	0.36	0.208	0.34	0.33	0.217	0.35	0.35	0.130	0.23	0.26	0.233	0.37	0.20	0.125	0.23	0.25	0.375	0.48	0.50	0.006
Omy_101832-195	0.433	0.50	0.51	0.870	0.23	0.26	0.708	0.42	0.42	0.717	0.41	0.39	0.196	0.32	0.22	0.567	0.51	0.60	0.969	0.06	0.06	0.906	0.18	0.19	0.265
Omy_101993-189	0.478	0.50	0.48	0.977	0.05	0.05	0.958	0.08	0.08	0.417	0.50	0.42	0.023	0.05	0.05	0.700	0.43	0.47	0.125	0.23	0.13	0.406	0.50	0.44	0.418
Omy_102213-204	0.125	0.22	0.20	0.972	0.06	0.06	0.125	0.22	0.25	0.304	0.43	0.26	0.022	0.04	0.04	0.033	0.07	0.07	0.313	0.44	0.50	0.767	0.37	0.33	0.480
Omy_102420-634	0.341	0.45	0.45	0.696	0.43	0.26	0.438	0.50	0.54	0.761	0.37	0.39	0.848	0.26	0.22	0.533	0.51	0.53	0.719	0.42	0.44	0.938	0.12	0.13	0.186
Omy_102457-423	0.211	0.34	0.11	0.425	0.50	0.35	0.636	0.47	0.27	0.717	0.41	0.30	0.909	0.17	0.09	0.767	0.37	0.33	0.577	0.51	0.23	0.438	0.51	0.38	0.222
Omy_102505-102	0.228	0.36	0.37	0.130	0.23	0.17	0.229	0.36	0.29	0.022	0.04	0.04	0.250	0.38	0.41	0.067	0.13	0.13	0.156	0.27	0.31	0.000	0.00	0.00	0.051
Omy_102510-682	0.067	0.13	0.13	0.130	0.23	0.26	0.125	0.22	0.25	0.217	0.35	0.43	0.043	0.09	0.09	0.000	0.00	0.00	0.313	0.44	0.38	0.063	0.12	0.13	0.064
Omy_102867-443	0.273	0.40	0.45	1.000	0.00	0.00	0.708	0.42	0.42	0.783	0.35	0.35	1.000	0.00	0.00	0.967	0.07	0.07	0.219	0.35	0.44	1.000	0.00	0.00	0.498
Omy_103350-395	0.283	0.41	0.35	0.182	0.30	0.27	0.625	0.48	0.50	0.652	0.46	0.43	0.087	0.16	0.17	0.167	0.29	0.20	0.406	0.50	0.81	0.767	0.37	0.33	0.227
Omy_103577-379	0.291	0.42	0.26	0.045	0.09	0.09	0.083	0.16	0.17	0.065	0.12	0.13	0.184	0.31	0.26	0.000	0.00	0.00	0.313	0.44	0.50	0.031	0.06	0.06	0.098
Omy_103705-558	0.337	0.45	0.40	0.152	0.26	0.30	0.250	0.38	0.42	0.130	0.23	0.26	0.239	0.37	0.30	0.200	0.33	0.40	0.000	0.00	0.00	0.094	0.18	0.06	0.052

Table 3 Continued

	Scott Creek			Klamath River-Kelsey Creek			Eel River-Middle Fork (summer)			Sacramento River-Battle Creek			Columbia River-Willamette River			Kamloops Strain-Hot Creek Hatchery			Mount Whitney Strain-Fillmore Hatchery			Eagle Lake Strain-American River Hatchery			
	N = 46			*N* = 23			*N* = 24			*N* = 23			*N* = 23			*N* = 15			*N* = 16			*N* = 16			
Assay Name	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	F_{ST}
Omy_103713-53	0.464	0.50	0.50	0.022	0.04	0.04	0.229	0.36	0.29	0.542	0.51	0.42	0.000	0.00	0.00	0.179	0.30	0.21	0.250	0.39	0.38	0.156	0.27	0.31	0.199
Omy_104519-624	0.272	0.40	0.33	0.739	0.39	0.43	0.813	0.31	0.29	0.674	0.45	0.57	0.717	0.41	0.39	0.533	0.51	0.40	0.625	0.48	0.50	0.625	0.48	0.38	0.157
Omy_104569-114	0.152	0.26	0.26	0.523	0.51	0.50	0.292	0.42	0.33	0.217	0.35	0.43	0.000	0.00	0.00	0.036	0.07	0.07	0.133	0.24	0.27	0.000	0.00	0.00	0.173
Omy_105075-162	0.315	0.44	0.50	0.409	0.49	0.55	0.104	0.19	0.21	0.152	0.26	0.22	0.261	0.39	0.35	0.033	0.07	0.07	0.000	0.00	0.00	0.000	0.00	0.00	0.125
Omy_105105-448	0.078	0.15	0.07	0.705	0.43	0.41	0.396	0.49	0.46	0.239	0.37	0.39	0.652	0.46	0.17	0.567	0.51	0.47	0.469	0.51	0.69	0.063	0.12	0.13	0.267
Omy_105115-367	0.289	0.42	0.44	0.205	0.33	0.41	0.229	0.36	0.38	0.125	0.22	0.25	0.022	0.04	0.04	0.133	0.24	0.13	0.067	0.13	0.13	0.156	0.27	0.31	0.045
Omy_105235-713	0.238	0.37	0.38	0.000	0.00	0.00	0.208	0.34	0.33	0.065	0.12	0.13	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.135
Omy_105385-406	0.211	0.34	0.29	0.614	0.49	0.32	0.304	0.43	0.26	0.375	0.48	0.55	0.391	0.49	0.61	0.700	0.43	0.47	0.750	0.39	0.50	0.375	0.48	0.38	0.139
Omy_105386-347	0.333	0.45	0.44	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.065	0.12	0.13	0.000	0.00	0.00	0.000	0.00	0.00	0.656	0.47	0.19	0.369
Omy_105401-363	0.444	0.50	0.49	0.023	0.05	0.05	0.354	0.47	0.46	0.174	0.29	0.26	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.260
Omy_105407-74	0.359	0.47	0.33	0.571	0.50	0.48	0.583	0.50	0.58	0.609	0.49	0.78	1.000	0.00	0.00	1.000	0.00	0.00	0.500	0.52	0.50	0.375	0.48	0.50	0.230
Omy_105714-265	0.300	0.42	0.38	0.848	0.26	0.22	0.250	0.38	0.42	0.391	0.49	0.35	0.955	0.09	0.09	0.167	0.29	0.33	0.250	0.39	0.38	0.813	0.31	0.38	0.348
Omy_105897-101	0.011	0.02	0.02	0.022	0.04	0.04	0.000	0.00	0.00	0.208	0.34	0.33	0.000	0.00	0.00	0.100	0.19	0.20	0.094	0.18	0.19	0.094	0.18	0.19	0.085
Omy_106172-332	0.000	0.00	0.00	0.348	0.46	0.26	0.083	0.16	0.17	0.130	0.23	0.26	0.000	0.00	0.00	0.033	0.07	0.07	0.125	0.23	0.25	0.000	0.00	0.00	0.164
Omy_106313-445	0.395	0.48	0.51	0.652	0.46	0.52	0.833	0.28	0.33	0.370	0.48	0.39	1.000	0.00	0.00	0.867	0.24	0.13	0.406	0.50	0.44	0.969	0.06	0.06	0.291
Omy_106560-58	0.222	0.35	0.36	0.022	0.04	0.04	0.188	0.31	0.29	0.348	0.46	0.43	0.000	0.00	0.00	0.033	0.07	0.07	0.219	0.35	0.44	0.594	0.50	0.56	0.179
Omy_106747-707	0.318	0.44	0.45	0.522	0.51	0.52	0.542	0.51	0.75	0.386	0.49	0.32	0.478	0.51	0.61	0.767	0.37	0.47	0.563	0.51	0.38	0.438	0.51	0.38	0.051
Omy_107031-704	0.044	0.09	0.00	0.109	0.20	0.13	0.083	0.16	0.17	0.375	0.48	0.42	0.409	0.49	0.73	0.867	0.24	0.27	0.375	0.48	0.50	0.125	0.23	0.25	0.314
Omy_107074-217	0.109	0.20	0.13	0.978	0.04	0.04	0.458	0.51	0.58	0.848	0.26	0.30	1.000	0.00	0.00	1.000	0.00	0.00	0.344	0.47	0.56	0.938	0.12	0.13	0.606
Omy_107285-69	0.267	0.40	0.40	0.087	0.16	0.17	0.208	0.34	0.42	0.565	0.50	0.52	0.348	0.46	0.43	0.233	0.37	0.33	0.531	0.51	0.69	0.125	0.23	0.25	0.113
Omy_107336-170	0.000	0.00	0.00	0.109	0.20	0.22	0.000	0.00	0.00	0.239	0.37	0.48	0.045	0.09	0.09	0.267	0.40	0.27	0.000	0.00	0.00	0.281	0.42	0.44	0.149
Omy_107607-137	0.189	0.31	0.33	0.023	0.05	0.05	0.375	0.48	0.50	0.130	0.23	0.26	0.522	0.51	0.52	0.167	0.29	0.20	0.156	0.27	0.31	0.031	0.06	0.06	0.147
Omy_107786-314	0.120	0.21	0.15	0.109	0.20	0.22	0.104	0.19	0.21	0.717	0.41	0.39	0.913	0.16	0.17	0.900	0.19	0.07	0.406	0.50	0.56	0.300	0.43	0.33	0.479
Omy_107786-584	0.089	0.16	0.13	0.119	0.21	0.24	0.109	0.20	0.22	0.525	0.51	0.65	0.900	0.18	0.20	0.500	0.52	0.47	0.250	0.39	0.38	0.313	0.44	0.38	0.352
Omy_107806-34	0.081	0.15	0.02	0.568	0.50	0.59	0.021	0.04	0.04	0.391	0.49	0.43	0.400	0.49	0.30	0.500	0.52	0.47	0.844	0.27	0.19	0.875	0.23	0.25	0.381
Omy_108007-193	0.011	0.02	0.02	0.114	0.21	0.23	0.479	0.51	0.54	0.348	0.46	0.52	0.239	0.37	0.48	0.233	0.37	0.47	0.500	0.52	0.63	0.750	0.39	0.38	0.265
Omy_108735-311	0.198	0.32	0.26	0.395	0.49	0.47	0.375	0.48	0.42	0.478	0.51	0.70	0.587	0.50	0.48	0.833	0.29	0.33	0.406	0.50	0.44	0.750	0.39	0.25	0.161
Omy_108820-85	0.000	0.00	0.00	0.130	0.23	0.26	0.000	0.00	0.00	0.152	0.26	0.22	0.022	0.04	0.04	0.100	0.19	0.20	0.156	0.27	0.31	0.333	0.46	0.53	0.119
Omy_109243-222	0.122	0.22	0.24	0.395	0.49	0.26	0.125	0.22	0.25	0.478	0.51	0.43	0.087	0.16	0.09	0.067	0.13	0.13	0.531	0.51	0.44	0.813	0.31	0.25	0.283
Omy_109390-341	0.196	0.32	0.26	0.000	0.00	0.00	0.283	0.41	0.30	0.261	0.39	0.52	0.000	0.00	0.00	0.233	0.37	0.33	0.469	0.51	0.56	0.188	0.31	0.38	0.110
Omy_109525-403	0.378	0.48	0.49	0.350	0.47	0.40	0.271	0.40	0.38	0.739	0.39	0.43	0.477	0.51	0.50	0.500	0.52	0.60	0.563	0.51	0.50	0.906	0.18	0.19	0.142
Omy_109651-445	0.238	0.37	0.48	0.000	0.00	0.00	0.021	0.04	0.04	0.021	0.04	0.04	0.000	0.00	0.00	0.000	0.00	0.00	0.438	0.51	0.38	0.000	0.00	0.00	0.240
Omy_109693-461	0.405	0.49	0.48	0.913	0.16	0.17	0.479	0.51	0.38	0.913	0.16	0.17	0.978	0.04	0.04	1.000	0.00	0.00	0.563	0.51	0.38	0.625	0.48	0.63	0.286
Omy_109874-148	0.244	0.37	0.36	0.000	0.00	0.00	0.083	0.16	0.17	0.022	0.04	0.04	0.065	0.12	0.13	0.000	0.00	0.00	0.094	0.18	0.19	0.000	0.00	0.00	0.104

Table 3 Continued

	Scott Creek			Klamath River-Kelsey Creek			Eel River-Middle Fork (summer)			Sacramento River-Battle Creek			Columbia River-Willamette River			Kamloops Strain-Hot Creek Hatchery			Mount Whitney Strain-Fillmore Hatchery			Eagle Lake Strain-American River Hatchery			
	N = 46			*N* = 23			*N* = 24			*N* = 23			*N* = 23			*N* = 15			*N* = 16			*N* = 16			
Assay Name	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	F_{ST}
Omy_109894-185	0.136	0.24	0.27	0.278	0.41	0.11	0.125	0.22	0.17	0.457	0.51	0.39	0.238	0.37	0.38	0.429	0.51	0.57	0.094	0.18	0.19	0.656	0.47	0.69	0.155
Omy_109944-74	0.033	0.07	0.07	0.065	0.12	0.13	0.167	0.28	0.25	0.409	0.49	0.36	0.000	0.00	0.00	0.000	0.00	0.00	0.156	0.27	0.31	0.844	0.27	0.31	0.430
Omy_110064-419	0.300	0.42	0.33	0.196	0.32	0.30	0.091	0.17	0.18	0.304	0.43	0.52	0.000	0.00	0.00	0.233	0.37	0.33	0.375	0.48	0.25	0.094	0.18	0.19	0.073
Omy_110078-294	0.315	0.44	0.37	0.783	0.35	0.35	0.104	0.19	0.21	0.457	0.51	0.48	0.891	0.20	0.22	0.800	0.33	0.40	0.300	0.43	0.33	0.063	0.12	0.13	0.355
Omy_110201-359	0.500	0.51	0.41	0.478	0.51	0.43	0.458	0.51	0.50	0.804	0.32	0.39	0.696	0.43	0.52	0.900	0.19	0.20	0.656	0.47	0.44	0.813	0.31	0.38	0.097
Omy_110362-585	0.326	0.44	0.48	0.043	0.09	0.09	0.438	0.50	0.46	0.391	0.49	0.61	0.109	0.20	0.04	0.200	0.33	0.40	0.000	0.00	0.00	0.125	0.23	0.25	0.128
Omy_110571-386	0.000	0.00	0.00	0.283	0.41	0.39	0.000	0.00	0.00	0.022	0.04	0.04	0.087	0.16	0.17	0.700	0.43	0.60	0.094	0.18	0.19	0.000	0.00	0.00	0.410
Omy_110689-148	0.489	0.51	0.53	0.477	0.51	0.41	0.458	0.51	0.42	0.391	0.49	0.52	0.500	0.51	0.39	0.167	0.29	0.33	0.563	0.51	0.50	0.313	0.44	0.50	0.026
Omy_111005-159	0.011	0.02	0.02	0.881	0.21	0.24	0.229	0.36	0.46	0.435	0.50	0.52	0.391	0.49	0.61	0.000	0.00	0.00	0.719	0.42	0.31	0.406	0.50	0.69	0.410
Omy_111084-526	0.109	0.20	0.22	0.043	0.09	0.09	0.021	0.04	0.04	0.152	0.26	0.22	0.130	0.23	0.26	0.100	0.19	0.20	0.000	0.00	0.00	0.375	0.48	0.38	0.084
Omy_111383-51	0.152	0.26	0.26	0.409	0.49	0.55	0.292	0.42	0.50	0.435	0.50	0.61	0.717	0.41	0.30	0.633	0.48	0.73	0.281	0.42	0.31	0.469	0.51	0.31	0.152
Omy_111666-301	0.456	0.50	0.56	0.283	0.41	0.30	0.583	0.50	0.50	0.543	0.51	0.48	0.022	0.04	0.04	0.233	0.37	0.47	0.438	0.51	0.25	0.500	0.52	0.50	0.125
Omy_111681-432	0.000	0.00	0.00	0.043	0.09	0.09	0.000	0.00	0.00	0.152	0.26	0.22	0.000	0.00	0.00	0.033	0.07	0.07	0.031	0.06	0.06	0.250	0.39	0.50	0.124
Omy_112208-328	0.341	0.45	0.64	0.543	0.51	0.48	0.271	0.40	0.46	0.696	0.43	0.52	0.306	0.44	0.39	0.200	0.33	0.40	0.250	0.39	0.38	0.813	0.31	0.25	0.161
Omy_112301-202	0.054	0.10	0.02	0.364	0.47	0.45	0.250	0.38	0.42	0.261	0.39	0.26	0.909	0.17	0.18	0.867	0.24	0.27	0.469	0.51	0.69	0.688	0.44	0.50	0.406
Omy_112820-82	0.178	0.30	0.27	0.286	0.42	0.48	0.146	0.25	0.29	0.413	0.50	0.39	0.957	0.09	0.09	0.933	0.13	0.13	0.250	0.39	0.38	0.167	0.29	0.20	0.409
Omy_112876-45	0.500	0.51	0.96	0.217	0.35	0.43	0.438	0.50	0.88	0.739	0.39	0.52	0.523	0.51	0.86	0.600	0.50	0.80	0.750	0.39	0.50	0.781	0.35	0.31	0.126
Omy_113109-205	0.300	0.42	0.47	0.000	0.00	0.00	0.042	0.08	0.08	0.326	0.45	0.39	0.000	0.00	0.00	0.100	0.19	0.20	0.563	0.51	0.50	0.250	0.39	0.21	0.199
Omy_113128-73	0.456	0.50	0.47	0.152	0.26	0.22	0.333	0.45	0.42	0.065	0.12	0.13	0.000	0.00	0.00	0.033	0.07	0.07	0.063	0.12	0.13	0.094	0.18	0.19	0.200
Omy_113242-163	0.044	0.09	0.00	0.065	0.12	0.13	0.000	0.00	0.00	0.087	0.16	0.17	0.000	0.00	0.00	0.033	0.07	0.07	0.000	0.00	0.00	0.406	0.50	0.31	0.187
Omy_113490-159	0.000	0.00	0.00	0.500	0.51	0.50	0.167	0.28	0.33	0.174	0.29	0.35	0.364	0.47	0.64	0.367	0.48	0.47	0.406	0.50	0.44	0.344	0.47	0.44	0.168
Omy_114315-438	0.411	0.49	0.47	0.152	0.26	0.30	0.125	0.22	0.25	0.348	0.46	0.52	0.043	0.09	0.09	0.133	0.24	0.27	0.156	0.27	0.31	0.063	0.12	0.00	0.115
Omy_114448-87	0.185	0.30	0.28	0.682	0.44	0.55	0.313	0.44	0.46	0.587	0.50	0.57	1.000	0.00	0.00	1.000	0.00	0.00	0.438	0.51	0.63	0.938	0.12	0.13	0.426
Omy_114587-480	0.228	0.36	0.33	0.068	0.13	0.14	0.000	0.00	0.00	0.022	0.04	0.04	0.174	0.29	0.26	0.033	0.07	0.07	0.000	0.00	0.00	0.063	0.12	0.13	0.087
Omy_114976-223	0.352	0.46	0.43	0.043	0.09	0.09	0.167	0.28	0.33	0.065	0.12	0.13	0.043	0.09	0.09	0.333	0.46	0.53	0.438	0.51	0.63	0.031	0.06	0.06	0.150
Omy_115987-812	0.380	0.48	0.37	0.205	0.33	0.41	0.271	0.40	0.54	0.375	0.48	0.67	0.065	0.12	0.13	0.067	0.13	0.13	0.094	0.18	0.19	0.031	0.06	0.06	0.107
Omy_116104-229	0.045	0.09	0.09	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.019
Omy_116362-467	0.000	0.00	0.00	0.022	0.04	0.04	0.000	0.00	0.00	0.239	0.37	0.39	0.000	0.00	0.00	0.033	0.07	0.07	0.688	0.44	0.50	0.375	0.48	0.50	0.425
Omy_116733-349	0.413	0.49	0.52	0.795	0.33	0.23	0.125	0.22	0.25	0.565	0.50	0.52	0.609	0.49	0.43	0.900	0.19	0.20	0.625	0.48	0.38	0.906	0.18	0.19	0.243
Omy_116938-264	0.000	0.00	0.00	0.065	0.12	0.13	0.000	0.00	0.00	0.261	0.39	0.43	0.022	0.04	0.04	0.100	0.19	0.20	0.688	0.44	0.38	0.219	0.35	0.44	0.359
Omy_117242-419	0.398	0.48	0.57	0.457	0.51	0.39	0.413	0.50	0.48	0.565	0.50	0.26	0.341	0.46	0.50	1.000	0.00	0.00	0.281	0.42	0.44	0.406	0.50	0.56	0.118
Omy_117259-96	0.307	0.43	0.48	0.087	0.16	0.17	0.229	0.36	0.38	0.065	0.12	0.13	0.261	0.39	0.43	0.000	0.00	0.00	0.063	0.12	0.13	0.000	0.00	0.00	0.101
Omy_117286-374	0.256	0.38	0.42	0.022	0.04	0.04	0.021	0.04	0.04	0.087	0.16	0.17	0.043	0.09	0.09	0.167	0.29	0.33	0.031	0.06	0.06	0.063	0.12	0.13	0.088
Omy_117370-400	0.466	0.50	0.30	0.348	0.46	0.43	0.333	0.45	0.33	0.435	0.50	0.43	0.370	0.48	0.39	0.667	0.46	0.53	0.469	0.51	0.44	0.750	0.39	0.38	0.047

Table 3 Continued

	Scott Creek			Klamath River-Kelsey Creek			Eel River-Middle Fork (summer)			Sacramento River-Battle Creek			Columbia River-Willamette River			Kamloops Strain-Hot Creek Hatchery			Mount Whitney Strain-Fillmore Hatchery			Eagle Lake Strain-American River Hatchery			
	N = 46			*N* = 23			*N* = 24			*N* = 23			*N* = 23			*N* = 15			*N* = 16			*N* = 16			
Assay Name	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	F_{ST}
Omy_117432-190	0.076	0.14	0.02	0.109	0.20	0.22	0.125	0.22	0.25	0.043	0.09	0.09	0.000	0.00	0.00	0.233	0.37	0.33	0.000	0.00	0.00	0.500	0.52	0.38	0.174
Omy_117540-259	0.411	0.49	0.56	0.786	0.34	0.33	0.565	0.50	0.43	0.717	0.41	0.39	0.978	0.04	0.04	0.700	0.43	0.07	0.844	0.27	0.31	0.906	0.18	0.19	0.188
Omy_117549-316	0.261	0.39	0.39	0.022	0.04	0.04	0.188	0.31	0.38	0.659	0.46	0.41	0.000	0.00	0.00	0.133	0.24	0.27	0.833	0.29	0.20	0.688	0.44	0.38	0.382
Omy_117743-127	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.130	0.23	0.26	0.000	0.00	0.00	1.000	0.00	0.00	1.000	0.00	0.00	1.000	0.00	0.00	0.124
Omy_117815-81	0.151	0.26	0.30	0.457	0.51	0.48	0.188	0.31	0.38	0.364	0.47	0.45	0.326	0.45	0.57	0.100	0.19	0.20	0.375	0.48	0.50	0.063	0.12	0.13	0.084
Omy_118175-396	0.196	0.32	0.30	0.130	0.23	0.17	0.354	0.47	0.54	0.125	0.22	0.25	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.094	0.18	0.06	0.104
Omy_118205-116	0.489	0.51	0.62	0.632	0.48	0.63	0.391	0.49	0.43	0.696	0.43	0.26	0.957	0.09	0.09	0.900	0.19	0.20	0.656	0.47	0.56	0.344	0.47	0.31	0.172
Omy_118654-91	0.267	0.40	0.40	0.870	0.23	0.26	0.708	0.42	0.50	0.478	0.51	0.43	0.543	0.51	0.57	0.867	0.24	0.27	0.500	0.52	0.38	0.438	0.51	0.38	0.190
Omy_118938-341	0.089	0.16	0.18	0.650	0.47	0.40	0.104	0.19	0.21	0.413	0.50	0.65	0.000	0.00	0.00	0.067	0.13	0.13	0.000	0.00	0.00	0.000	0.00	0.00	0.346
Omy_119108-357	0.322	0.44	0.47	0.065	0.12	0.13	0.104	0.19	0.13	0.239	0.37	0.39	0.000	0.00	0.00	0.000	0.00	0.00	0.500	0.52	0.50	0.219	0.35	0.31	0.152
Omy_119892-365	0.116	0.21	0.19	0.325	0.45	0.55	0.326	0.45	0.48	0.196	0.32	0.30	0.130	0.23	0.26	0.000	0.00	0.00	0.406	0.50	0.56	0.125	0.23	0.25	0.081
Omy_120255-332	0.268	0.40	0.34	0.075	0.14	0.15	0.188	0.31	0.21	0.130	0.23	0.17	0.196	0.32	0.39	0.000	0.00	0.00	0.156	0.27	0.19	0.313	0.44	0.38	0.036
Omy_120950-569	0.119	0.21	0.19	0.159	0.27	0.32	0.478	0.51	0.43	0.364	0.47	0.36	0.375	0.48	0.25	0.133	0.24	0.13	0.438	0.51	0.63	0.813	0.31	0.38	0.201
Omy_121006-131	0.217	0.34	0.30	0.717	0.41	0.48	0.375	0.48	0.42	0.609	0.49	0.43	1.000	0.00	0.00	1.000	0.00	0.00	0.375	0.48	0.63	0.938	0.12	0.13	0.399
Omy_121713-115	0.100	0.18	0.16	0.174	0.29	0.35	0.146	0.25	0.29	0.261	0.39	0.52	0.500	0.51	0.11	0.767	0.37	0.20	0.281	0.42	0.19	0.188	0.31	0.38	0.198
Omy_123044-128	0.457	0.50	0.43	0.833	0.28	0.33	0.708	0.42	0.42	0.717	0.41	0.48	1.000	0.00	0.00	1.000	0.00	0.00	0.688	0.44	0.38	0.844	0.27	0.31	0.191
Omy_123048-119	0.044	0.09	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.370	0.48	0.48	0.000	0.00	0.00	0.133	0.24	0.27	0.406	0.50	0.56	0.167	0.29	0.07	0.220
Omy_123921-144	0.193	0.32	0.25	0.318	0.44	0.55	0.271	0.40	0.54	0.152	0.26	0.30	0.413	0.50	0.65	0.333	0.46	0.27	0.031	0.06	0.06	0.031	0.06	0.06	0.075
Omy_124774-530	0.452	0.50	0.62	0.891	0.20	0.22	0.396	0.49	0.71	0.261	0.39	0.35	0.935	0.12	0.13	0.800	0.33	0.27	0.625	0.48	0.50	0.281	0.42	0.56	0.264
Omy_125998-61	0.304	0.43	0.30	0.957	0.09	0.09	0.438	0.50	0.46	0.739	0.39	0.35	1.000	0.00	0.00	0.767	0.37	0.47	0.500	0.52	0.38	0.781	0.35	0.44	0.308
Omy_126160-242	0.359	0.47	0.59	0.000	0.00	0.00	0.130	0.23	0.26	0.022	0.04	0.04	0.000	0.00	0.00	1.000	0.00	0.00	1.000	0.00	0.00	1.000	0.00	0.00	0.232
Omy_127236-583	0.244	0.37	0.36	0.696	0.43	0.52	0.396	0.49	0.46	0.773	0.36	0.36	1.000	0.00	0.00	1.000	0.00	0.00	0.844	0.27	0.31	0.969	0.06	0.06	0.415
Omy_127510-920	0.222	0.35	0.31	0.239	0.37	0.39	0.375	0.48	0.50	0.522	0.51	0.52	0.152	0.26	0.13	0.500	0.52	0.33	0.500	0.52	0.50	0.906	0.18	0.19	0.192
Omy_127645-308	0.012	0.02	0.02	0.000	0.00	0.00	0.000	0.00	0.00	0.304	0.43	0.43	0.000	0.00	0.00	0.033	0.07	0.07	0.000	0.00	0.00	0.719	0.42	0.44	0.506
Omy_127760-385	0.178	0.30	0.31	0.000	0.00	0.00	0.229	0.36	0.38	0.104	0.19	0.21	0.000	0.00	0.00	0.000	0.00	0.00	0.219	0.35	0.31	0.063	0.12	0.13	0.076
Omy_128302-430	0.000	0.00	0.00	0.000	0.00	0.00	0.104	0.19	0.21	0.000	0.00	0.00	0.043	0.09	0.09	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.060
Omy_128693-755	0.267	0.40	0.44	0.700	0.43	0.40	0.417	0.50	0.33	0.262	0.40	0.52	0.262	0.40	0.33	0.000	0.00	0.00	0.000	0.00	0.00	0.438	0.51	0.63	0.179
Omy_128851-273	0.217	0.34	0.09	0.000	0.00	0.00	0.250	0.38	0.00	0.109	0.20	0.22	0.000	0.00	0.00	0.000	0.00	0.00	0.344	0.47	0.31	0.000	0.00	0.00	0.118
Omy_128923-433	0.130	0.23	0.26	0.048	0.09	0.10	0.065	0.12	0.04	0.022	0.04	0.04	0.091	0.17	0.18	0.500	0.52	0.60	0.188	0.31	0.38	0.167	0.29	0.20	0.143
Omy_128996-481	0.186	0.31	0.33	0.500	0.51	0.52	0.125	0.22	0.25	0.500	0.51	0.39	0.478	0.51	0.70	0.000	0.00	0.00	0.125	0.23	0.25	0.281	0.42	0.31	0.149
Omy_129170-794	0.380	0.48	0.54	0.022	0.04	0.04	0.104	0.19	0.21	0.109	0.20	0.13	0.000	0.00	0.00	0.000	0.00	0.00	0.000	0.00	0.00	0.219	0.35	0.19	0.191
Omy_129870-756	0.244	0.37	0.44	0.614	0.49	0.68	0.417	0.50	0.67	0.239	0.37	0.39	0.652	0.46	0.35	0.833	0.29	0.20	0.250	0.39	0.38	0.563	0.51	0.50	0.171
Omy_130295-98	0.122	0.22	0.16	0.357	0.47	0.52	0.250	0.38	0.42	0.326	0.45	0.48	0.652	0.46	0.17	0.633	0.48	0.33	0.156	0.27	0.31	0.906	0.18	0.19	0.279
Omy_130524-160	0.466	0.50	0.43	0.591	0.49	0.55	0.521	0.51	0.54	0.333	0.45	0.42	0.391	0.49	0.78	0.367	0.48	0.47	0.750	0.39	0.38	0.719	0.42	0.31	0.060

Table 3 Continued

	Scott Creek			Klamath River-Kelsey Creek			Eel River-Middle Fork (summer)			Sacramento River-Battle Creek			Columbia River-Willamette River			Kamloops Strain-Hot Creek Hatchery			Mount Whitney Strain-Fillmore Hatchery			Eagle Lake Strain-American River Hatchery		
	N = 46			*N* = 23			*N* = 24			*N* = 23			*N* = 23			*N* = 15			*N* = 16			*N* = 16		
Assay Name	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	Allele Freq	He	Ho	Allele Freq.	He	Ho	Allele Freq.	He	Ho	F_{ST}
Omy_130720-100	0.500	0.51	0.42	0.143	0.25	0.29	0.583	0.50	0.58	0.630	0.48	0.39	0.000	0.00	0.00	0.100	0.19	0.07	0.625	0.48	0.50	0.781	0.35	0.44	0.281
Omy_131460-646	0.380	0.48	0.54	0.022	0.04	0.04	0.542	0.51	0.50	0.087	0.16	0.17	0.022	0.04	0.04	0.133	0.24	0.27	0.031	0.06	0.06	0.188	0.31	0.25	0.224
Omy_131965-120	0.389	0.48	0.33	0.350	0.47	0.50	0.396	0.49	0.54	0.565	0.50	0.52	0.109	0.20	0.22	0.167	0.29	0.20	0.563	0.51	0.50	0.406	0.50	0.44	0.079
Omy_GH1PROM1-1	0.256	0.38	0.38	0.196	0.32	0.30	0.313	0.44	0.46	0.500	0.51	0.52	0.043	0.09	0.09	0.067	0.13	0.13	0.344	0.47	0.31	0.094	0.18	0.19	0.100
Mean	0.238	0.32	0.31	0.168	0.24	0.24	0.221	0.30	0.31	0.260	0.35	0.35	0.132	0.18	0.18	0.148	0.22	0.21	0.237	0.31	0.31	0.185	0.27	0.25	0.204
Polymorphic loci (%)	91.4			84.2			86.3			97.1			65.5			73.4			82.0			84.9		

Mean minor allele frequency averaged 0.199 over all loci, with a high of 0.260 in the Sacramento River-Battle Creek population and a low of 0.132 in the Columbia River-Willamette River populations. The proportion of polymorphic loci averaged 83.1% and varied from 97.1% in Battle Creek to 65.5% in the Willamette River. Expected and observed heterozygosity were generally very similar within each test sample, never differing more than 0.014 (i.e. 1.4%). Observed heterozygosity varied between 0.352 in Battle Creek and 0.182 in the Willamette River. Thus, all measures of genetic variability were consistent in identifying the Sacramento River-Battle Creek population as the most diverse and the Columbia River-Willamette River population as the least diverse. Mean F_{ST} was 0.204 and ranged from 0.006 to 0.606 at different loci.

Discussion

We report the discovery and development of assays for 139 novel single-nucleotide polymorphisms in the species *O. mykiss*, steelhead/rainbow trout, through sequence analysis of 236 ESTs with a total consensus length of 130 KB. We demonstrate how ESTs from existing public databases and directed Sanger sequencing of PCR products can be used to identify large numbers of SNPs in nonmodel organisms. In species and populations with large effective sizes, such sequencing from existing genomic information uncovers sufficient polymorphism that a preliminary screen of loci for potential polymorphism, using methods such as single-strand conformation polymorphism or high-resolution melt analyses, can be avoided, because nearly every locus will contain some variants.

The 139 SNP loci described here are broadly polymorphic in the species and should prove useful for a variety of applications, including phylogeography, genetic stock identification, individual identification, behavioural ecology and pedigree reconstruction. The availability of large numbers of SNPs known to be polymorphic in populations of steelhead and rainbow trout will allow the implementation of intergenerational genetic tagging through large-scale parentage inference, because this requires only about 100 SNP loci for sufficiently low tag recovery error rates (Garza & Anderson 2007). Such parentage-based tagging will allow an unprecedented level of monitoring and evaluation of natural and hatchery/aquaculture populations, including estimation of variance in reproductive success, migration rates, effective population sizes, life-stage-specific mortality rates and other population parameters. Parentage-based tagging is based on the principle that genotyping fish from the parental generation, either in a hatchery, an aquaculture operation or a natural population, provides intergenerational

genetic tags for their progeny that can be retrieved through large-scale parentage inference (Anderson & Garza 2006; Garza & Anderson 2007). Such pedigree reconstruction is greatly facilitated by the low genotyping error/mutation rates of SNP loci. In addition, as more SNP loci are described and more assays become available for the species, it will be possible to construct second-generation genetic linkage maps and high-density SNP genotyping microarrays. In conjunction with the pedigrees resulting from PBT, these will enable detailed understanding of the genetic architecture of phenotypic traits in the species. Because of its importance in recreational fisheries and in aquaculture, as well as the ESA protection of many populations, the species *O. mykiss* is among the most economically significant fishes in the world, and an increased understanding of its phenotypic variation is of great value.

During the past decade, microsatellite markers have dominated population genetic work in salmonids, because of their high variability and conservation among related species (Landry & Bernatchez 2001; Narum *et al.* 2004; Aguilar & Garza 2006; Clemento *et al.* 2009; Pearse *et al.* 2009). However, microsatellites have significant drawbacks, among them relatively high genotyping error/mutation rates, significant staff time necessary for data generation and allele calling and homoplasy. Moreover, the results obtained with microsatellites in one laboratory are not directly combinable with data generated in other laboratories, even when using the same instrumentation, because of subtle differences in electrophoretic conditions and consequent data output (Seeb *et al.* 2007). The requirement for a standardization process to be able to combine microsatellite data between laboratories adds significant time and expense to collaborative projects.

Conversely, data obtained from SNP loci are easily portable and combinable between laboratories, as long as the same primer/probe sequences and/or reporting conventions are used. This will allow large multilateral databases to be developed for applications in fishery management, ecological investigation and aquaculture/hatchery broodstock management using both standard (e.g. Seeb *et al.* 2007) and pedigree-based approaches (Anderson & Garza 2006). Moreover, the advent of new technologies, such as nanofluidic circuitry and spotted arrays, for thermal cycling and genotyping now allows the examination of a large number of SNPs in a large number of individuals in a short time period and at relatively low cost. This provides the prospect of SNP genotyping as a routine, and very valuable, tool for monitoring and evaluation of steelhead and rainbow trout populations throughout the world.

As SNP loci are typically bi-allelic, the amount of information per locus is more limited than for most multiallelic loci, such as microsatellites or amplified fragment length polymorphisms. In the future, however, analysis of haplotypes of tightly linked SNPs may provide additional information for many questions, including in phylogeography and pedigree resolution. Because we discovered many additional polymorphic sites in these genes, it would be possible to design additional assays for many of these sites and perform haplotype analyses. More complete analyses of this sequence variability will be reported elsewhere.

The number and density of substitutions and SNPs discovered here were consistent with what has been reported for other salmonids (e.g. Smith *et al.* 2005), but it is difficult to draw direct comparisons between different SNP discovery efforts, because the density of polymorphic sites uncovered depends critically on the number and phylogenetic diversity of the individuals in the ascertainment panel, the set of genes or genomic sequences interrogated for SNP discovery and accuracy of the sequencing method employed. Our ascertainment approach and stringent design criterion for SNP discovery were intended to fulfil several objectives. Included in the ascertainment panel were both representatives from populations in California where we are actively working and intend to apply the resulting markers, as well as from rainbow trout strains commonly used throughout the world for fishery stocking and/or aquaculture. By designing assays for variable sites only when all three genotypes were observed, and without regard to which individuals carried them, we selected both for markers with a higher mean minor allele frequency and markers that were more likely to be broadly useful in the species. This was intended to provide markers useful for study and management of both native steelhead populations and the millions of rainbow trout cultured for food and fisheries. However, it will also underrepresent rare variants, which could result in biases in phylogenetic and evolutionary applications of these markers. Still, it is important to point out that sets of microsatellite and other population genetic markers developed for salmonids and other nonmodel organisms suffer from the same biases. Therefore, applications of these SNP markers that depend upon a representative sampling of the site frequency spectrum in focal populations or lineages should ideally employ markers ascertained using diverse ascertainment populations and strategies.

Our ascertainment panel included fish from three coastal steelhead populations from several closely related lineages, a highly divergent population of redband trout and several rainbow trout strains domesticated from distinct lineages. This diverse ascertainment panel was intended to reduce ascertainment bias in populations in the southern part of the North American range. Nevertheless, because of the extensive phylogeographic

diversity in this species and the large amount of directed DNA sequencing involved in our discovery strategy, it was not possible to include a sufficient number and diversity of fish in our sequencing effort to completely eliminate ascertainment bias in this discovery. So additional effort will be necessary to identify additional SNPs for more phylogenetically distinct lineages, such as those in northern Mexico, interior Canada and Russia (McCusker *et al*. 2000; Hendrickson *et al*. 2002; McPhee *et al*. 2007).

Acknowledgements

We thank other members of the Molecular Ecology and Genetic Analysis Team of the Southwest Fisheries Science Center in Santa Cruz for their assistance, particularly Hilary Starks, Libby Gilbert-Horvath and Devon Pearse. Additional thanks to Andy Aguilar, Nate Campbell, Greg Charrier, Chris Donohoe, George Edwards, Heidi Fish, Sean Hayes, Andrew Matala and Paul Moran for help with protocol development, supplying samples or insightful discussions. Several anonymous reviewers and Lisa Seeb provided helpful comments that improved the manuscript.

Conflict of interest

The authors have no conflict of interest to declare and note that the sponsors of the issue had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1 Blast results for all consensus sequences used in SNP assay development.

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