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PERMANENT GENETIC RESOURCES NOTE

# Subspecies-informative SNP assays for evaluating introgression between native golden trout and introduced rainbow trout

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## Abstract

**We characterize 20 single nucleotide polymorphism assays for evaluating hybridization between native golden trout subspecies (*Oncorhynchus mykiss aguabonita* and *O. m. whitei*) and introduced rainbow trout strains. These assays utilize the 5′-nuclease reaction, facilitating high-throughput genotyping of many individuals and making them useful in quantifying and monitoring introgression and potentially applicable to studies of other *O. mykiss* groups. Minor allele frequency differentials (δq) among native and introduced rainbow groups ranged from 0 to 1, with an average differential of 0.75 for both subspecies *aguabonita* and *whitei* relative to the hatchery rainbow trout strain.**

*Keywords*: ancestry-informative markers, hybridization, *Oncorhynchus mykiss*, single nucleotide polymorphism, SNP

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Hybridization is an increasingly common threat to native species (Moyle & Light 1996; Levin 2002) and a documented conservation concern in a wide variety of taxa. It is particularly prevalent and well-studied in native North American trout of the genus *Oncorhynchus*, which are frequently threatened within their native ranges by hybridization with closely related introduced species (Cordes *et al*. 2006; Gunnell *et al*. 2007; Pritchard *et al*. 2007).

The difficulties associated with quantifying and monitoring introgression at lower taxonomic levels can be significant. Diagnostic loci may be unavailable, particularly in comparisons between closely related subspecies. Long-term genetic monitoring of focal species requires standardization of data over time and often across research laboratories (Welsh & May 2006; Seeb *et al*. 2007). Such coordination efforts can be expensive but necessary to characterize the threat of increasing introgression over time or under particular environmental conditions.

Single nucleotide polymorphism (SNP) markers have several advantages over other marker types, chiefly the SNPs' reproducibility within and across hardware platforms, capacity for high throughput genotyping (Melton 2003), and sequence-based data that do not require subsequent standardization to compare data across studies or laboratories. Recent SNP marker development efforts for selected rainbow trout subspecies found highly informative SNP loci for the detection of introgression between small numbers of individuals from hatchery and wild rainbow trout (*O. mykiss* sspp., and *O. m. irideus*, respectively) when compared to several native *O. mykiss* subspecies (Sprowles *et al*. 2006); here we describe the development of SNP and insertion-deletion (indel) marker assays for 20 hybridization-informative loci for native golden trout.

Fin clip samples were collected from populations representing the subspecies/groups of interest: Volcano Creek (VC) California golden trout (*O. m. aguabonita*), Upper Soda Spring Creek (USSC) Little Kern golden trout (*O. m. whitei*), Mount Shasta Strain (MSS) hatchery rainbow trout (*O. mykiss* sspp.) and North Fork American River (NFAR) wild rainbow trout (*O. m. irideus*). Samples were preserved in DMSO storage buffer (20% DMSO, 0.25 M EDTA, NaCl to saturation, pH 7.8), in 95% ethanol, or as dry fin clips. Whole genomic DNA was extracted using the QIAGEN DNeasy Tissue Kit. Extracted DNA samples were stored at –30 °C.

In addition to the SNP markers from efforts described above (Sprowles *et al*. 2006), three SNP loci were adapted from previous research (Bagley & Gall 1998), including two from the mitochondrial DNA control region (locus RTDL

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316 and RTDL695) and one anonymous single copy nuclear DNA locus (A1A8_94). The 18 nuclear SNPs occur in 15 loci among which the degree of physical linkage is unknown, except in instances in which SNPs reside within the same locus (recombination activating gene loci R0917 230, R1175 137, and R1564 272; RAPD intergenic sequence loci RAPD 132 and RAPD 167). One TaqMan assay was originally presented in a previous study (SNP locus LDH_156, Sprowles *et al*. 2006). A total of 20 TaqMan assays (Table 1) consisting of forward and reverse primers and VIC- and FAM-labelled allele-specific probes were developed for each locus using either Applied Biosystems, Inc. Assays by Design or PrimerSelect software for use in the 5′-nuclease reaction (Holland *et al*. 1991). Each probe bore a minor groove binder and nonfluorescent quencher on the 3′ end. Assay reactions were optimized on the individuals used in SNP marker discovery (Sprowles *et al*. 2006), including individuals of known genotype based on sequencing data. Known homozygotes, heterozygotes, and 'composite' heterozygotes (generated by combining DNA from known homozygotes in ratios of 3:1, 1:1, and 1:3) were included as positive controls on every plate of samples analysed, along with one no-template negative control. Reactions were carried out in 96-well microplates at a 5 μL volume. The majority of assays utilized 2× TaqMan Universal Master Mix (Applied Biosystems), 540 nM each primer, 120 nM each probe, and 10–20 ng template DNA. Promega reagents were used for selected loci (Table 1) at the following concentrations: 20 units/mL *Taq* Polymerase, 0.2 mM each dNTP, 5 mM MgCl, 50 mM KCl, 10 mM Tris-HCl, 0.1% Triton X-100, and concentrations of primers, probes, and template as given above. Reactions were performed using the Chromo4 Real-Time PCR Detector (MJ Research/Bio-Rad Laboratories, Inc.) and the following general thermal cycling protocol: initial denaturation of 94 °C for 5 min, followed by 40 cycles of 92 °C for 15 s and an annealing temperature ranging from 55 to 63.5 °C for 1 min. Genotypes were scored using MJ Opticon Monitor analysis software (version 3.1, Bio-Rad Laboratories, Inc.) to visualize plots of endpoint fluorescence, and identify clusters of fluorescence corresponding with each probe. Genotyping results were confirmed for consistency with positive controls.

Allele frequencies and observed and unbiased expected heterozygosities (Nei 1978) were calculated for each locus-

**Table 1** SNP assays developed for detecting hybridization between native and non-native trout. Information on TaqMan assay name (locus identifier and nucleotide position) and reference GenBank dbSNP Accession number from Sprowles *et al*. (2006) is provided, along with oligonucleotide sequences for unlabeled primers (forward and reverse) and fluorescently labelled (VIC* or FAM*) probes for each allele, and PCR annealing/extension temperature ($T_a$). Delta values calculated between native golden trout populations (VC, Volcano Creek, California golden trout; USSC, Upper Soda Spring Creek, Little Kern golden trout) vs. Mount Shasta Strain (MSS) rainbow trout and North Fork American River (NFAR) rainbow trout are also shown

| Assay name | dbSNP | Oligonucleotide sequences (5′–3′) for: forward primer/reverse primer/ VIC* probe/FAM* probe | $T_a$ (°C) | *O. m. aguabonita* | | *O. m. whitei* | |
|---|---|---|---|---|---|---|---|
| | | | | $\delta q_{VC\text{-}MSS}$ | $\delta q_{VC\text{-}NFAR}$ | $\delta q_{USSC\text{-}MSS}$ | $\delta q_{USSC\text{-}NFAR}$ |
| B9_164 | ss52084306 | F:GCACAGAACACAGCCAATATTAACA<br>R:GCCTTGACTCTCCCTTCATGAC<br>VIC-CCTACAACTTGATCTAACGTG<br>FAM-CCTACAACTTGATCTACGTG | 63.5 | 0.05 | 0.05 | 1.00 | 1.00 |
| ID1c_77-83 | ss46566540 | F:CAGGCTTTTTTTTCTATCAGAATTAAGTC<br>R:TGTATGCTAACTTGTAATTTGCTGTTGT<br>VIC-AGTTAACAGTTAATGAGT<br>FAM-AGGCAGTTAACGAGTC | 58 | 0.98 | 1.00 | 0.86 | 0.88 |
| LDH_156*† | ss46565746 | F:GTTTTGAAACCAGTTTAAGGTTGATTGC<br>R:ACGGCATAGTCTGGACAGAGAT<br>VIC-CCATTTAGACGTTTTTT<br>FAM-CCATTTAGATGTTTTTT | 62 | 0.88 | 0.14 | 0.88 | 0.14 |
| Omy_f1_259-260 | ss46566536 | F:CCACACACACAAACACACATACAC<br>R:CAAGCATTCTTCTGTTAAATGTGGTCTA<br>VIC-CACACACAAACAGCA<br>FAM-ACACACACACACAGCA | 60 | 0.50 | 0.85 | 0.05 | 0.40 |
| RAPD_132 | ss38508087 | F:ATCATTACCACGCCCAACGTTA<br>R:AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT<br>VIC-CATGTTGGGATATATGA<br>FAM-ATGTTGGGAAATATGA | 60 | 0.92 | 0.05 | 0.92 | 0.05 |
| RTDL_695‡ | — | F:AAGCCGGGCGTTCTCTTATATG<br>R:GTTAGACTTCTTTGCTTGCACTTGT<br>VIC-CATAGGGTTCTCTTTTTT<br>FAM-ATAGGGTTCTCCTTTTT | 62 | 0.87 | 0.87 | 1.00 | 1.00 |

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**Table 1** *Continued*

| Assay name | dbSNP | Oligonucleotide sequences (5'–3') for: forward primer/reverse primer/ VIC* probe/FAM* probe | $T_a$ (°C) | *O. m. aguabonita* $\delta q_{VC\text{-}MSS}$ | *O. m. aguabonita* $\delta q_{VC\text{-}NFAR}$ | *O. m. whitei* $\delta q_{USSC\text{-}MSS}$ | *O. m. whitei* $\delta q_{USSC\text{-}NFAR}$ |
|---|---|---|---|---|---|---|---|
| A1A8_94‡ | — | F:GTGTTTTCACATGCAGAAGTGATTACT<br>R:GGCCCTTCTCAATTGGAACAGTA<br>VIC-CTATATCTACCTTCCTAATGAA<br>FAM-CTATATCTACCTTCCTATATCTACCTTCTTAATGAATTAATGAA | 60 | 1.00 | 1.00 | — | — |
| B1_266 | ss52084317 | F:TCATGTGAACTTTAATTGACTAGGAAGTCG<br>R:GATATGAAAATATCTGAAGAGTTATATTTGGGAAATTGAC<br>VIC-TCTATAAACAACATTTTTC<br>FAM-TCTATAAACAAAATTTTTC | 62 | 0.69 | 0.52 | — | — |
| B9_388† | ss52084313 | F:CTCTCTTCTCCTCGTATGGTGACT<br>R:GCACCTGGTCTGCACCT<br>VIC-CCCCCATGGATGTGTAT<br>FAM-CCCCATGGACGTGTAT | 60 | 0.95 | 0.95 | — | — |
| Omy_g1_103 | ss38350764 | F:CTCAGCAAAAAAGAAACGTCCCTTT<br>R:AGTCGTGACAATGAGAAACAGTGTT<br>VIC-CCTTTTACAATGAAGATC<br>FAM-CTTTTACAGTGAAGATC | 61.5 | 0.86 | 0.86 | — | — |
| R0917_230 | ss52049619 | F:CGAGTAAACAGGGAAGCAAGTGA<br>R:ACAACTCAAAATGGTGTCATCAGAGA<br>VIC-AATAAACTATCAAATCATTTCAG<br>FAM-ATAAACTATCAAATAATTTCAG | 60 | 0.95 | 0.98 | — | — |
| R1175_137 | ss52049646 | F:ACTGTCATGACTTTAACCTGATGATGTAC<br>R:ACATACCGTCATGTAAAACGTGATGT<br>VIC-AGATTTTCATAATGTATAATATT<br>FAM-ATTTTCATAATGTGTAATATT | 57 | 0.95 | 0.98 | — | — |
| R1564_272 | ss52049625 | F:GGTTTATGTTATTACACCTGTGTGAACTG<br>R:ACTGGCACAACTGTATGTAAACCT<br>VIC-ATATGTTATGATAAAAAAAATTACA<br>FAM-ATGTTATGATAAAAAAATTACA | 60 | 0.97 | 0.98 | — | — |
| RAPD_167 | ss38508088 | F:CCCAACATGCTCTATTGCAGCTA<br>R:AGTTGCATAAGATGAATCAATAAATTAAAAACACAGAT<br>VIC-ATTAAAACAATCCCCCCCAAAA<br>FAM-TTAAAACAATCCCACCCAAAA | 55 | 0.00 | 0.55 | — | — |
| RTDL_316‡ | — | F:AACATACGGTGATTTTAACCCCTCAT<br>R:GTAAAGACGGAGCCCGTGTTA<br>VIC-CTTGGATTTGTGCTGATGT<br>FAM-CTTGGATTTGTACTGATGT | 60 | 1.00 | 1.00 | — | — |
| URO_373 | ss38509092 | F:ACATCTGTAAACAGATGCTGCTGAA<br>R:GCCAGAGTTTAAGTAAATCTGCAAGGA<br>VIC-TTATTGCCTATTGACATATAA<br>FAM-TTGCCTATTGAAATATAA | 60 | 0.45 | 0.45 | — | — |
| CHIT_80 | ss38350751 | F: GGCCTTATCAATTATGTCACGTGGAT<br>R: CCCTTTTCTCTCACAGTAACTTTCCA<br>FAM-CACCCTTCAATAACA<br>VIC-CACCCTTGAATAACA | 60 | — | — | 0.72 | 0.63 |
| CRB2677_117 | ss46566267 | F: TCTGCCAAATTCACATGACAAAAGAC<br>R: ATTACAATGAAAGTACTTGAGTGTTTATGCAAA<br>FAM-TGCAACAGAGGGTTG<br>VIC-CATTGCAACATAGGGTTG | 60 | — | — | 0.66 | 0.29 |
| F5_306 | ss52084339 | F: GAACACTTGGTGTGATTTGCATCAT<br>R: GCTGAGGAGAGAAAAGGAGAAATGA<br>FAM-CATTACACATTATTTTCT<br>VIC-CCATTACACATTCTTTTCT | 60 | — | — | 0.69 | 0.44 |
| OMYP9_180 | ss52049649 | F: CTGGATGTGTAGTATCGGTGGAAAA<br>R: CACTGGGCACCTCTGATCTC<br>FAM-CTGTAGTAGTCCCCATTGT<br>VIC-CTGTAGTAGTCCGCATTGT | 62 | — | — | 0.72 | 0.68 |

*assay originally presented in Sprowles *et al*. 2006.
†assay used Promega reagents as described in text.
‡Bagley & Gall 1998.

**Table 2** Frequency (*P*) of SNP alleles (alternative allele given parenthetically) for 21 SNP loci genotyped in populations representing four groups: Volcano Creek (VC; California golden trout, *O. m. aguabonita*), Upper Soda Spring Creek (USSC; Little Kern golden trout, *O. m. whitei*), Mount Shasta Strain hatchery rainbow trout (MSS; *O. m.* sspp.), and North Fork American River wild rainbow trout (NFAR; coastal rainbow trout, *O. m. irideus*). Number of individuals genotyped (*N*) and observed and expected heterozygosities ($H_O$ and $H_E$) are reported for each locus

| | | VC (California golden trout) | | | | USSC (Little Kern golden trout) | | | | MSS (Mount Shasta hatchery) | | | | NFAR (wild rainbow trout) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Locus | Allele | *N* | *P* | $H_E$ | $H_O$ | *N* | *P* | $H_E$ | $H_O$ | *N* | *P* | $H_E$ | $H_O$ | N | *P* | $H_E$ | $H_O$ |
| B9_164 | A(:)* | 38 | 0.95 | 0.10 | 0.11 | 20 | 0.00 | 0.00 | 0.00 | 30 | 1.00 | 0.00 | 0.00 | 20 | 1.00 | 0.00 | 0.00 |
| ID1c_77–83 | AGTTAAT(:::::::)† | 37 | 0.00 | 0.00 | 0.00 | 19 | 0.61 | 0.49 | 0.37 | 28 | 0.98 | 0.04 | 0.04 | 20 | 1.00 | 0.00 | 0.00 |
| LDH_156‡ | T(C) | 36 | 0.00 | 0.00 | 0.00 | 24 | 0.00 | 0.00 | 0.00 | 30 | 0.88 | 0.21 | 0.23 | 18 | 0.14 | 0.25 | 0.28 |
| Omy_f1_259–260 | ::(AA)§ | 39 | 0.00 | 0.00 | 0.00 | 24 | 0.25 | 0.38 | 0.25 | 31 | 0.50 | 0.51 | 0.48 | 20 | 0.85 | 0.26 | 0.20 |
| RAPD_132 | A(T) | 39 | 0.00 | 0.00 | 0.00 | 26 | 0.00 | 0.00 | 0.00 | 30 | 0.92 | 0.16 | 0.10 | 20 | 0.05 | 0.10 | 0.00 |
| RTDL_695 | T(C) | 39 | 0.13 | n/a | n/a | 28 | 0.00 | n/a | n/a | 30 | 1.00 | n/a | n/a | 18 | 1.00 | n/a | n/a |
| A1A8_94 | C(T) | 38 | 0.00 | 0.00 | 0.00 | — | — | — | — | 30 | 1.00 | 0.00 | 0.00 | 20 | 1.00 | 0.00 | 0.00 |
| B1_266 | G(T) | 39 | 0.31 | 0.43 | 0.46 | — | — | — | — | 30 | 1.00 | 0.00 | 0.00 | 20 | 0.83 | 0.30 | 0.25 |
| B9_388 | A(G) | 38 | 0.05 | 0.10 | 0.11 | — | — | — | — | 30 | 1.00 | 0.00 | 0.00 | 20 | 1.00 | 0.00 | 0.00 |
| Omy_g1_103 | T(C) | 39 | 0.14 | 0.25 | 0.28 | — | — | — | — | 30 | 1.00 | 0.00 | 0.00 | 20 | 1.00 | 0.00 | 0.00 |
| R0917_230 | G(T) | 39 | 0.00 | 0.00 | 0.00 | — | — | — | — | 31 | 0.95 | 0.09 | 0.10 | 20 | 0.98 | 0.05 | 0.05 |
| R1175_137 | A(G) | 39 | 0.00 | 0.00 | 0.00 | — | — | — | — | 31 | 0.95 | 0.09 | 0.10 | 20 | 0.98 | 0.05 | 0.05 |
| R1564_272 | :(T)* | 39 | 0.00 | 0.00 | 0.00 | — | — | — | — | 30 | 0.97 | 0.07 | 0.07 | 20 | 0.97 | 0.05 | 0.05 |
| RAPD_167 | T(G) | 39 | 0.00 | 0.00 | 0.00 | — | — | — | — | 30 | 0.00 | 0.00 | 0.00 | 20 | 0.55 | 0.51 | 0.30 |
| RTDL_316 | C(T) | 39 | 0.00 | n/a | n/a | — | — | — | — | 30 | 1.00 | n/a | n/a | 20 | 1.00 | n/a | n/a |
| URO_373 | C(A) | 39 | 0.55 | 0.50 | 0.59 | — | — | — | — | 30 | 1.00 | 0.00 | 0.00 | 20 | 1.00 | 0.00 | 0.00 |
| CHIT_80 | C(G) | — | — | — | — | 28 | 0.02 | 0.04 | 0.04 | 30 | 0.72 | 0.41 | 0.37 | 20 | 0.63 | 0.48 | 0.55 |
| CRB2677_117 | T(G) | — | — | — | — | 22 | 0.00 | 0.00 | 0.00 | 28 | 0.66 | 0.46 | 0.11 | 19 | 0.29 | 0.42 | 0.16 |
| F5_306 | G(T) | — | — | — | — | 28 | 0.20 | 0.32 | 0.18 | 30 | 1.00 | 0.00 | 0.00 | 20 | 0.75 | 0.38 | 0.40 |
| OMYP9_180 | G(C) | — | — | — | — | 28 | 0.18 | 0.30 | 0.29 | 29 | 0.72 | 0.41 | 0.28 | 20 | 0.68 | 0.45 | 0.45 |

*Allele is a 1 base-pair insertion/deletion.
†Allele is a 7 base-pair insertion/deletion.
‡Frequencies are previously reported for a subset of individuals at this locus in Sprowles *et al*. (2006).
§Allele is a 2 base-pair insertion/deletion.
n/a, not applicable.

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population combination using Genetix 4.05 (Table 2; Belkhir *et al*. 1996–2004). Populations were tested for departure from Hardy–Weinberg equilibrium at each locus and for linkage disequilibrium between pairs of loci within each population using GDA (Genetic Data Analysis; Lewis & Zaykin 2001). All statistical significance values were computed using the Markov chain method to obtain unbiased estimates of Fisher's exact test based on 10 000 iterations (Guo & Thompson 1992) and corrected for type I error using Bonferroni correction for multiple simultaneous tests (Rice 1989). A single population exhibited significant departure from Hardy–Weinberg expectations (MSS population for locus CRB2677_117). Several loci, particularly SNPs within the same gene, exhibited some linkage disequilibrium, but only the MSS population showed significant linkage disequilibrium after Bonferroni correction for the following locus combinations: CRB2677_117/F5_306 and CRB2677_117/OMYP9_180.

We calculated the delta statistic (the absolute value of the allelic frequency difference between two populations; Smith *et al*. 2001) for each SNP locus as a means of assessing marker informativeness for detecting differences between golden and rainbow trout subspecies groups (Table 1). Delta values range from 0 to 1, with 1 indicating fixed (diagnostic) differences in allele frequencies between the populations being compared; a frequency differential between groups of 0.5 or more is generally informative for studies of admixture (Shriver *et al*. 1997). The value of $\delta$ was calculated between Upper Soda Spring Creek (2002) and Mount Shasta Strain ($\delta_{U\text{-}M}$; mean value = 0.75, range = 0.05–1.0) and North Fork American River ($\delta_{U\text{-}N}$; mean = 0.55, range = 0.05–1.0); likewise, the value of $\delta$ was calculated between Volcano Creek and Mount Shasta Strain ($\delta_{V\text{-}M}$; mean = 0.75, range = 0.00–1.0) and North Fork American River ($\delta_{V\text{-}N}$; mean = 0.70, range = 0.05–1.0). Application of selected SNPs has already revealed these markers to be powerful in detecting and quantifying rainbow trout introgression in native golden trout subspecies, yielding estimates comparable to microsatellite data for the same populations (M.R.S., unpublished data).

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## References

Bagley MJ, Gall GAE (1998) Mitochondrial and nuclear DNA sequence variability among populations of rainbow trout (*Oncorhynchus mykiss*). *Molecular Ecology*, **7**, 945–961.

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (1996–2004) *Genetix 4.05, Logiciel sous Windows pour la Génétique des Populations*. Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II, Montpellier, France.

Cordes JF, Stephens MR, Blumberg MA, May B (2006) Identifying introgressive hybridization in native populations of California golden trout based on molecular markers. *Transactions of the American Fisheries Society*, **135**, 110–128.

Gunnell K, Tada M, Hawthorne F, Keeley E, Ptacek M (2007) Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and introduced rainbow trout (*O. mykiss*) in the South Fork of the Snake River watershed, Idaho. *Conservation Genetics*, **9**, 49–64.

Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. *Biometrics*, **48**, 361–372.

Holland PM, Abramson RD, Watson R, Gelfand DH (1991) Detection of specific polymerase chain-reaction product by utilizing the 5′–3′ exonuclease activity of *Thermus aquaticus* DNA-Polymerase. *Proceedings of the National Academy of Sciences, USA*, **88**, 7276–7280.

Levin DA (2002) Hybridization and extinction: in protecting rare species, conservationists should consider the dangers of interbreeding, which compound the more well-known threats to wildlife. *American Scientist*, **90**, 254 (258).

Lewis PO, Zaykin D (2001) *Genetic Data Analysis: Computer Program for the Analysis of Allelic Data*. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

Melton L (2003) Pharmacogenetics and genotyping: on the trail of SNPs. *Nature*, **422**, 917–923.

Moyle PB, Light T (1996) Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation*, **78**, 149–161.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics*, **89**, 583–590.

Pritchard VL, Jones K, Cowley DE (2007) Estimation of introgression in cutthroat trout populations using microsatellites. *Conservation Genetics*, **8**, 1311–1329.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Seeb LW, Antonovich A, Banks AA *et al*. (2007) Development of a standardized DNA database for Chinook salmon. *Fisheries*, **32**, 540–552.

Shriver MD, Smith MW, Jin L *et al*. (1997) Ethnic-affiliation estimation by use of population specific DNA markers. *American Journal of Human Genetics*, **60**, 957–964.

Smith MW, Lautenberger JA, Shin HD *et al*. (2001) Markers for mapping by admixture linkage disequilibrium in African American and Hispanic populations. *American Journal of Human Genetics*, **69**, 1080–1094.

Sprowles AE, Stephens MR, Clipperton NW, May BP (2006) Fishing for SNPs: a targeted locus approach for single nucleotide polymorphism discovery in rainbow trout. *Transactions of the American Fisheries Society*, **135**, 1698–1721.

Welsh A, May B (2006) Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies. *Journal of Applied Ichthyology*, **22**, 337–344.

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