Single nucleotide polymorphisms associated with allozyme differences between inland and coastal rainbow trout

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Abstract. Native inland populations of rainbow trout, *Oncorhynchus mykiss gairdneri*, particularly resident populations, often hybridize with introduced populations of the widely-cultured coastal, *O. m. irideus*, form of the species. The inland and coastal subspecies genetically differ from each other by allozyme polymorphisms at lactate dehdyrogenase (*LDH-B2**) and superoxide dismutase (*sSOD-1**) loci which can be detected using protein electrophoresis. Fewer laboratories, however, are now using allozyme technology and most genetic studies from wild organisms are now being conducted using DNA rather than protein analyses. We have identified the single nucleotide polymorphism (SNP) differences responsible for the protein variations by sequencing the cDNAs for the *LDH-B2** and *sSOD-1** genes in a large number of individuals whose genotypes were also determined by protein electrophoresis. The genetic differences causing the allozyme polymorphisms have been converted into SNP allelic discrimination assays. This should allow simple, efficient tests to be used in a large number of laboratories as aids in assessing levels of hybridization between inland and coastal rainbow trout. It should also allow DNA studies to be more directly related to previous allozyme studies. High variability was also found at other sites in the superoxide dismutase gene.

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Introduction

Although not formally recognized, there are two subspecies of rainbow trout *Oncorhynchus mykiss* commonly described with a relatively broad natural distribution in western North America. Coastal rainbow trout, *O. m. irideus*, are native to waters ranging from northern Mexico northwards to Alaska and then westwards to Kamchatka (Behnke 1992, 2002). In the Columbia River drainage, inland rainbow trout, *O. m. gairdneri*, reproduce in freshwater systems east of the Cascade crest (Behnke, 1992, 2002). Both subspecies exhibit life history variation with resident, migratory and anadromous individuals being common.

Native inland rainbow trout populations, particularly resident populations, have often hybridized with introduced populations of the widely-cultured coastal rainbow trout (e.g. Behnke 1992; Currens et al. 1997; Small et al. 2007). This is a concern because introduced fish may lack the local adaptations of the native population. For example, there is some evidence indicating that inland rainbow trout may be more resistant to local diseases than coastal rainbow trout and hybrids (Currens et al. 1997). Recent practices are more conservative regarding introductions of coastal rainbow trout within the historic range of inland rainbow trout, and in some cases stocking coastal rainbow trout is limited to the use of sterile individuals (Dillon et al. 2000). However, changes in stocking practices have only occurred relatively recently and the genetic impacts of past stocking are unknown in many areas. Thus, it is important to identify and conserve the remaining native inland populations and in some cases to replace hybridized populations with inland populations.

The inland and coastal forms of rainbow trout genetically differ from each by allozyme polymorphisms at lactate dehdyrogenase (*LDH-B2*) and superoxide dismutase (*sSOD-1*) loci which are detectable using protein electrophoresis. These differences were first demonstrated by Allendorf and Utter (1979) and have subsequently been found to be relatively consistent among rainbow trout

populations in the Columbia River basin (Utter 2001; summary data presented by Knudsen et al. 2002). Inland populations usually possess *LDH-B2**76 at frequencies greater than 0.250 (often much greater) while coastal rainbow trout populations usually have frequencies of less than 0.100. Similarly, inland populations usually possess *sSOD-1**152 at frequencies less than 0.100 and coastal populations usually have frequencies of greater than 0.150. These *LDH-B2** and *sSOD-1** allele frequency differences have been successfully used to differentiate native and hybridized rainbow trout populations throughout the Columbia River basin, including the Yakima (Campton and Johnston 1985), Kootenai (Allendorf et al. 1980; Knudsen et al. 2002) and Deschutes (Williams et al. 1997; Currens et al. 1997) drainages. In some cases such as the Yakima River it is unclear whether the hybridization represents a recent or ancient event (Utter 1998).

Other genetic markers to date have shown limited capacity for distinguishing between inland and coastal rainbow trout. Chromosome polymorphisms have been detected among rainbow trout populations (Thorgaard 1983; Ostberg and Thorgaard 1999), but they do not consistently differentiate inland and coastal populations. Mitochondrial DNA polymorphisms in conjunction with nuclear data have been locally useful in assessing hybridization between inland and coastal rainbow trout (Williams et al. 1996, 1997). These differences, however, have not been demonstrated to be consistent over broader geographic areas (McCusker et al. 2000). Microsatellites show high levels of variation and can be locally useful for assessing hybridization. However, these markers also do not consistently differentiate inland and coastal populations over broad geographic areas (e.g., Knudsen et al. 2002; Narum et al. 2004; Small et al. 2007).

The use of allozymes for differentiating the populations, however, has several disadvantages. Many laboratories are no longer using this technology, which limits the options available for having the analyses performed. Samples for DNA analysis are easier to collect and store because they can be collected as fin clips and stored in ethanol. In contrast, fish for allozyme analyses must normally be sacrificed and frozen. Lethal sampling is often a concern when small, sometimes threatened, populations are studied. Most of the genetic studies on rainbow trout in the Columbia River drainage are now

focusing on applying DNA technologies, but unfortunately some of the most useful markers for differentiating inland and coastal rainbow trout populations are only currently detectable using allozyme analysis.

Studies relating allozyme variation to DNA sequence variation within coding regions have demonstrated that considerable hidden variation is present at the DNA level (e.g., Kreitman 1983). Where allozymes have been demonstrated to usefully differentiate populations, conversion of allozyme types to DNA-based markers can facilitate population genetic studies. McMeel et al. (2001) successfully converted an allozyme test for two *LDH-C1* alleles in brown trout (*Salmo trutta*) into an RFLP-based DNA test.

We have successfully identified the single nucleotide polymorphisms (SNPs) responsible for the allozyme variation at *LDH-B2** and *sSOD-1** by sequencing the complementary DNA (cDNA) for these genes in a number of individuals. We also converted these genetic polymorphisms into PCR-based SNP assays. Our study demonstrated the feasibility of using these SNP assays by comparing genotypes from the SNP assays to those obtained by allozyme electrophoresis. Sequence comparisons also revealed surprisingly high sequence variation in the *sSOD-1** coding region in some populations.

Methods

Sample collection and processing:

Tissue samples were collected from rainbow trout from two hatchery populations. Previous allozyme analyses of these populations indicated that together they would assure us of obtaining numerous individuals of the three genotypes at both *LDH-B2** and *sSOD-1** (Knudsen et al. 2002). One hundred inland rainbow trout from the anadromous (steelhead) population at the Clearwater Hatchery (Idaho Department of Fish and Game) on the Clearwater River, Idaho, and one hundred rainbow trout of predominantly coastal origin (Williams et al. 1996) from the Hayspur Hatchery (Idaho Department of Fish and Game) were sampled. Samples were also collected from the Fisher River between Miller Creek

and Wolf Creek (n=30) and Basin Creek (n=15) in the Kootenai River drainage, Montana by Montana Department of Fish, Wildlife & Parks personnel to further test the reliability of the SNPs to accurately detect the polymorphisms at *LDH-B2** and *sSOD-1**. Basin Creek was considered a likely source of native trout (Knudsen et al. 2002) and Fisher River a potentially hybridized population, based on historic stocking practices. Liver samples were flash-frozen on dry ice and stored at -80°C for subsequent allozyme analyses and RNA isolation. Fin clips were also taken from each fish and stored in 95% ethanol for subsequent DNA extraction. Genomic DNA samples were purified by proteinase-K digestion followed by phenol/chloroform extraction. Extracted DNA was then precipitated with one volume of isopropyl alcohol, followed by a 70% EtOH wash, dissolved in TE buffer and quantified (Sambrook et al. 1989). Allozyme analyses of liver samples for *LDH-B2* and *sSOD-1* were conducted as previously described by Allendorf et al. (1977), Allendorf and Utter (1979), and Leary and Booke (1990).

Molecular Analyses:

Total liver RNA was isolated from samples according to protocols outlined for TRIZOL RNA extraction (Invitrogen). Liver cDNA templates were synthesized by oligo-dT primed reverse transcription according to the protocol advised for SuperscriptIII Reverse Transcriptase (Invitrogen, Carlsbad, California). *LDH-B2* and *sSOD-1* gene sequences were derived by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) amplification of the respective target cDNA sequences from total liver cDNA template. Rainbow trout *LDH-B2** was identified from the TIGR database by homology to a lactate dehydrogenase cDNA (TC 78625; R. Drew, Washington State University, personal communcation). *LDH-B2* is the only lactate dehydrogenase locus expressed in rainbow trout liver (Utter and Hodgins 1972). The *sSOD-1** sequence was based on a cDNA isolation and GenBank deposition from the Washington State University laboratory (AF469663). RT-PCR amplifications were conducted in 20ul volumes, using 1 unit of Taq polymerase, in 1X Taq DNA polymerase PCR Buffer, 2 mM magnesium chloride, 1 ul of 10mM dNTP solution (Invitrogen), with 10pM of forward and reverse gene-specific primers (Table 1). PCR cycling parameters were 94°C for 120 sec pre-amplification dwell, followed by

30 cycles of 94°C for 45sec, 60°C for 50sec, 72°C for 60sec, and a 72°C, 120sec post-amplification dwell. *LDH-B2** cDNA RT-PCR amplification with *LDH-B2** (F1) and *LDH-B2** (R1) primers annealing in the 5' and 3' untranslated regions (UTRs) yielded a 1,192 bp product, including the coding region of 1,002 bp. *sSOD-1** cDNA RT-PCR amplification with *sSOD-1** (F1) and *sSOD-1** (R1) primers annealing in the 5' and 3' UTRs yielded a 596bp product, including the coding region of 462bp. Amplification products were evaluated by electrophoresis of 5ul of RT-PCR amplification product through 2% agarose gels and visualized by UV transillumination of ethidium bromide stained gels. 5ul of the remaining RT-PCR amplification product was then purified by ExoSAP-IT (US Biochemicals, Cleveland, Ohio) fragment purification to remove dNTPs and excess primers, yielding a template suitable for sequence analysis. The purified RT-PCR product was then directly PCR-sequenced by the Washington State University Biotechnology and Bioanalysis core facility using nested gene-specific sequencing primers. The nested sequencing primers *LDH-B2** (F2) and *LDH-B2** (R2) allowed complete one-pass sequencing of the coding region of the *LDH-B2** cDNA and the nested sequencing primers *sSOD-1** (F2) and *sSOD-1** (R2), allowed complete one-pass sequencing of the coding region of the *sSOD-1** cDNA (Table 1).

Identification of allozyme-defining SNPs:

Complete cDNA sequencing was performed on individual rainbow trout identified by allozyme electrophoresis to be homozygous for the sSOD-1*100, sSOD-1*152, LDH-B2*100, or LDH-B2*76 alleles. Sixteen Hayspur and 10 Clearwater individuals were sequenced for *sSOD-1**, and 8 Hayspur and 14 Clearwater individuals were sequenced for *LDH-B2**. These samples allowed the distinctive SNPs corresponding to the allozyme alleles to be identified. Our comparisons identified candidate SNPs responsible for allozyme differences by first distinguishing the nonsynonymous SNPs responsible for amino acid substitutions and then eliminating those substitutions which did not result in amino acid charge differences. The *sSOD-1** sequences showed both synonymous and nonsynonymous changes affecting the coding regions of these alleles, resulting in some amino acid alterations which were not responsible for allozyme differences. The *LDH-B2** sequences contained 3 SNPs, of which one at

nucleotide position 679 from the start codon resulted in the single nonsynonymous codon substitution responsible for the observed allozyme variation.

TaqMan (ABI) allelic discrimination assay:

The SNPs responsible for the homozygous allelic types *sSOD-1**100, *sSOD-1**152, *LDH-B2**100, or *LDH-B2**76, identified by correspondence of charge-altering nonsynonymous amino acid substitutions to the individual allozyme types, were converted into TaqMan allelic discrimination assays (Applied Biosystems, Foster City, California). TaqMan assays characterize SNP presence or absence by PCR amplification of a product from genomic DNA which contains the SNP (Livak 1999). Allelic discriminations are achieved by fluorescent emission produced by allele-specific probes. Effective conversion of SNPs identified in mRNA into SNP genotyping assays, however, requires characterizing intron positions in the target gene. The identification of intron location is necessary because the TaqMan products are being amplified from genomic DNA (containing introns) rather than cDNA. Intron positioning was not available in the TIGR rainbow trout or the NCBI Genbank databases. Intron positions, however, were correctly inferred by comparison with zebrafish genomic and cDNA Genbank depositions. In both the *sSOD-1** and the *LDH-B2** genes, intron positions disrupted the direct use of the SNP containing exon (cDNA) sequence for developing the allelic discrimination assays for the critical allozyme-defining SNP. This was indicated by SNP assay design failure when the SNP-containing exon sequence was evaluated using the Primer Express 3.0 program software for Real-Time PCR (Applied Biosystems).

Flanking intron sequence to the SNP-containing exon was amplified from trout genomic DNA by designing primers to the adjacent exon sequences for PCR amplification of the intervening intron. The amplified intron fragments were sequenced to provide the additional genomic sequence necessary for identifying effective primer annealing sites for the allelic discrimination assays and this was validated by SNP assay design success with the Primer Express 3.0 program. Primers and probes for the *LDH-B2** and *sSOD-1** SNP genotyping assays were successfully designed (Table 2). TaqMan SNP assay kits

were ordered from Applied Biosystems and all samples were typed for the *sSOD-1**100, *sSOD-1**152, *LDH-B2**100 and *LDH-B2**76 alleles using an Applied Biosystems 7300 Real-Time PCR System, according to manufacturer protocol. Genotypes obtained from the TaqMan assays were compared with allozyme genotypes for the Clearwater, Hayspur, Basin Creek and Fisher River samples to test for correspondence between the two types of analyses.

Results and Discussion

Development and validation of SNP assays for LDH-B2 and sSOD-1 alleles

Sequence analysis of mRNA from numerous individuals whose genotype at *sSOD-1** and *LDH-B2** had been determined by allozyme analyses allowed SNPs responsible for the allozyme variants to be identified (Table 3). These SNP differences did not correspond to any cut-site differences for restriction enzymes, precluding the use of restriction enzymes for their detection. Comparison of the *sSOD-1**100 and *sSOD-1**152 allozyme genotypes detected in the Hayspur (containing 34 *sSOD-1**100 homozygotes, 51 heterozygotes and 15 *sSOD-1**152 homozygotes) and the Clearwater population (all homozygous for *sSOD-1**100) with the TaqMan *sSOD-1** 100/152 SNP genotyping assay results revealed complete concordance between the two methods.

The Hayspur samples were all homozygous for the *LDH-B2**100 allele based on both the allozyme and SNP assays. Comparison of the *LDH-B2** TaqMan SNP genotyping assay with allozyme genotypes revealed two discrepancies between the assays in the Clearwater population samples (with 6 LDH-B2*100 homozygotes, 36 heterozygotes, and 57 LDH-B2*76 homozygotes). Both contradicting samples were then characterized by RT-PCR sequence analysis to determine genotypes independent of either the allozyme or SNP genotyping assay. The mRNA sequence analysis demonstrated that one reflected an allozyme scoring error and the other discrepancy represented a scoring error artifact in the TaqMan assay introduced during the automatic scoring for allele determination following real-time analysis. This error would have been correctly interpreted by visual evaluation of the real-time fluorescence plot of the TaqMan probes.

An independent assessment of the SNP assay was conducted by blind sampling of two populations in the Kootenai River basin provided by Montana Fish, Wildlife & Parks. Genotypes from these samples at *sSOD-1*** and *LDH-B2*** were determined using allozyme electrophoresis and the SNP allelic discrimination assay. The Basin Creek sample was believed to represent a non-hybridized, native inland rainbow trout population. In this sample, there were no *LDH-B2**100 homozygotes, 3 heterozygotes, and 12 *LDH-B2**76 homozygotes, and all fish were homozygous for the *sSOD-1**100 allele. Based on these data, the population strongly appears to be inland rainbow trout. The Fisher River sample was believed to potentially represent a hybridized population between inland and coastal rainbow trout. This sample had 14 *LDH-B2**100 homozygotes, 13 heterozygotes, 3 *LDH-B2**76 homozygotes, 3 *sSOD-1* heterozygotes and 27 *sSOD-1**100 homozygotes. The allele frequencies in this sample at *LDH-B2** and *sSOD-1** are quite characteristic of inland rainbow trout. We cannot definitively conclude that these fish are inland rainbow trout, however, because we do not know what the allele frequencies at these loci were in the fish in these locations prior to any possible hybridization with coastal rainbow trout. It is possible for what once was an inland rainbow trout population to now contain a substantial coastal rainbow trout genetic contribution but to still possess allele frequencies at *LDH-B2** and *sSOD-1** highly characteristic of inland rainbow trout. For example, consider an inland population with the frequency of *LDH-B2*76* initially being 0.500 and the frequency of *sSOD-1*152* being zero. We introduce coastal rainbow trout with the frequency of *LDH-B2*76* being zero and the frequency of *sSOD-1*152* being 0.200. If the latter fish had a 20 percent genetic contribution to the native inland population, then the allele frequencies in the hybridized population would be *LDH-B2*76* 0.400 and *sSOD-1*152* 0.040. Thus, whether or not the fish in these samples are non-hybridized is an open question. Regardless of whether or not these are hybridized populations, the SNP allelic discrimination assay showed 100% correspondence to the *sSOD-1** and *LDH-B2** allozyme-typing results for the Basin Creek sample and the *LDH-B2** results for the Fisher River sample. In the Fisher River sample, however, there were three discrepancies between the

allozyme and SNP analyses at *sSOD-1**. Of the three discrepancies, two were confirmed to be related to allozyme genotyping errors and one to errors in interpreting the TaqMan assay results.

Hypervariability at the superoxide dismutase locus

Sequence analysis of *sSOD-1** mRNA from numerous individuals of the Hayspur and Clearwater populations revealed striking within- and between-population diversity at this locus. In the mRNA sequence, 584bp of the transcript were aligned for comparison. Sequence comparison of 16 individuals from the Hayspur population and 10 individuals from the Clearwater population revealed 11 SNPs, resulting in four amino acid substitutions (Table 4). The *sSOD-1** locus of the Hayspur population has 17 times as many SNPs (10/584) as the *LDH-B2** locus derived from the same individuals (1/1002). The Hayspur population was reported by Williams et al. (1996) to have a mixed origin, which might have contributed to its high variability at the *sSOD-1** locus. Surprisingly, the Clearwater population contains only 3 SNPs in the *sSOD-1** locus (3/584), which reflects a closer correspondence to an expected 1 to 2 SNPs in a 500bp fragment (Kyle Martin, unpublished results from the Washington State University Laboratory) or to the SNP frequency seen in the *LDH-B2** locus from the same individuals (2/1002). Similar frequencies are well-established from studies in humans, other animals, and salmonid fishes (Gray et al. 2000; Vignal et al. 2002; Smith et al. 2005).

In contrast to the abundance of SNPs in *sSOD-1**, the *LDH-B2** sequences from the same individuals revealed only three SNPs among the Hayspur and Clearwater individuals, with the SNP at nucleotide position 679 from the start codon resulting in the nonsynonymous codon change reflecting the allozyme difference. A second SNP at position 456 substituted T in three Hayspur individuals for the otherwise common C. A third SNP at position 708 showed a correspondence with the SNP at position 679. With the exception of one Clearwater individual which had a G (100 allele) at position 679 and a C at position 708, all five Clearwater and eight Hayspur individuals with G at position 679 (100 allele homozygotes) had a T at position 708, and all eight Clearwater individuals with an A at position 679 (76 allele homozygotes) had a C at position 708.

Hypervariability affecting the *sSOD** locus has been observed before in populations of other species, and in some situations has been attributed to selective sweeps (Saez et al., 2003, Santovito et al., 2006). In the coding region of rainbow trout *sSOD-1** mRNA sequences we examined, a total of four amino acid substitutions were observed (Table 4). Sequence differences were also detected between each of eight clonal lines of rainbow trout (Young et al. 1996) being propagated at Washington State University (Table 4).

Conclusions

Our results demonstrate that the SNP genotyping tests we have developed are able to provide the same quality of data as the previously widely used allozyme analyses for assessment of potential hybridization between inland and coastal rainbow trout. Though the TaqMan SNP genotyping allelic discrimination assays provided through Applied Biosystems require the use of specific technologies, the assay are readily standardized for wide use. By using this SNP genotyping assay, populations can be easily sampled by fin-clips for future DNA analysis and many assays can be conducted upon the archived DNA samples because each specific SNP assay utilizes comparatively little DNA. While these assays alone may not be able to assess hybridization levels in all cases, as shown with our Fisher River example, they represent valuable tools for addressing this problem and can also allow current DNA studies to be directly compared to previous allozyme studies. The high variability in the superoxide dismutase gene detected in this study suggests the potential for interesting future investigations, given the central role of this gene in responses to oxidative stress (Hansen et al. 2006).

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Table 1. Primers for amplifying and sequencing the rainbow trout *LDH-B2** and *sSOD-1** genes from cDNA sequences. Primers designated by "F" and "R" represent forward and reverse primers, oriented to 5' mRNA sequence deposition. Primer designations of "1" and "2" represent primary amplification primers and secondary nested sequencing primers, respectively.

*LDH-B2** (F1)	5'CCATAAGTGAAGTCATCACG
*LDH-B2**(R1)	5' ACGGTTGGAACTAGGTCTG
*LDH-B2**(F2)	5'TCACTACCTGACTGTCGAC
*LDH-B2**(R2)	5'GTCTGTGTTTGACTGCATCG
*sSOD-1** (F1)	5' GGGTGTTGGTATTCGGGAC
*sSOD-1** (R1)	5' GTCTGCCGTAGCTACAGTG
*sSOD-1** (F2)	5' CGGGACTTGAGATCTTTAG
*sSOD-1** (R2)	5' GTCTTCAAGCAGAGGCTAG

Table 2. Primers for distinguishing single nucleotide polymorphisms at the *LDH-B2** and *sSOD-1** loci in rainbow trout using the custom TaqMan SNP Genotyping Assay.

*LDH-B2** Primer and Probe label	Nucleotide sequence
*LDH-B2**76/100F (primer)	5'ACCCAGAGTTTGGCCTTGAC
*LDH-B2**76/100R (primer)	5'CCTGTCGACCACTTCCTTATGG
*LDH-B2**76VIC (*76 probe)	5'CCTTCCAGT**T**CTCCTTG
*LDH-B2**100FAM (*100 probe)	5'CCTTCCAGT**C**CTCCTTG

*sSOD-1** Primer and Probe label	Nucleotide sequence
*sSOD-1**100/152-F (primer)	5'GCCGGACCCCACTTCAA
*sSOD-1**100/152-R (primer)	5'CAGACTAACCGAACAGCATCAGT
*sSOD-1**100VIC (*100 probe)	5'CCACAAC**A**AGACCC
*sSOD-1**152FAM (*152 probe)	5'CCACAAC**C**AGACCC

Table 3. Allozyme-defining single nucleotide polymorphisms for rainbow trout identified in the Clearwater (*LDH-B2**) and Hayspur (*sSOD-1**) populations.

*LDH-B2**76	Amino acid 227	**N** (Asparagine = amide)	Codon: **A**AC
*LDH-B2**100	Amino acid 227	**D** (Aspartic acid = acidic)	Codon: **G**AC
*sSOD-1**152	Amino acid 70	**Q** (glutamine=amide)	Codon: **C**AG
*sSOD-1**100	Amino acid 70	**K** (lysine=basic)	Codon: **A**AG

Table 4. Variability in *sSOD-1** cDNA sequences from rainbow trout. Nucleotide alternatives are indicated by the specific base-pair number with the first nucleotide of the start codon **A**TG representing base-pair position 1. Allelic variants found for each *sSOD-1** allozyme type (four for 152, fifteen for 100) are numbered. Populations from which representative individuals were sequenced included the Hayspur (Hy) hatchery rainbow trout and the Clearwater hatchery (Cw), Idaho strain of steelhead. Numbers of individuals representing each allelic type are indicated. Sequences from selected homozygous clonal rainbow trout lines were all mutually distinct and are represented by the following acronyms: Arlee: Arl, Oregon State University: OSU, Swanson: Sw, Hot Creek: HC, Skookumchuck: Sko, Whale Rock: WR, Skamania: Ska, Klamath: Klm. SNP scoring for many individuals revealed two alleles in the same RT-PCR sequence reaction. Position 208 represents the SNP associated with the (Glutamine : Lysine Q:K) allozyme difference discussed in this paper. Other nucleotide positions associated with amino acid substitutions are 98 **(**Threonine : Isoleucine, T:I), 122 (Leucine : Proline, L:P), and 399 (Aspartic Acid : Glutamic Acid, D:E). The final SNP at position 497 is in the 3' untranslated region.

	***sSOD-1* nucleotide position and SNP alternatives**										
sSOD-1	98	122	**208**	240	243	258	330	399	444	447	497
allozyme type	C/T	C/T	**A/C**	C/G	C/T	C/T	C/T	G/T	C/A	G/T	A/T
allelic variant	**T:I**	**L:P**	**Q:K**					**D:E**			
152-1 (Hy-1)	T	C	**C**	G	C	C	C/T	G/T	A	G	T
152-2 (Hy-2)	T	C	**C**	G	C	C	C/T	G/T	A	G	A/T
152-3 (HC, Hy-5)	T	C	**C**	G	C	C	C	G	A	G	T
152-4 (Sw)	T	C	**C**	G	C	C	C	T	A	G	A
100-1 (Hy-1)	C	C	**A**	G	C	C	C	G	A	G	T
100-2 (Hy-2)	C	C	**A**	G	T	T	C	G	A	G	T
100-3 (Hy-1)	C/T	C	**A**	C/G	C	C	C	T	A	G/T	A
100-4 (Hy-1)	C	T	**A**	C	C	C	T	G	A	G	A
100-5 (Hy-1)	C	C	**A**	C	C	C	C	G	A	G	A
100-6 (Hy-1)	C	C	**A**	C/G	C	C	C/T	G	A	G	A/T
100-7 (Hy-1)	C/T	C	**A**	G	C	C	C	T	A	G	A
100-8 (Cw-8)	C	C	**A**	C	C	C	T	G	A	G	A
100-9 (Cw-2)	C	C	**A**	C/G	C	C	C/T	G/T	A	G	A
100-10 (Arl)	T	C	**A**	C	C	C	T	G	A	G	A
100-11 (Sko)	T	C	**A**	C	C	C	C	G	A	T	A
100-12 (OSU)	C	C	**A**	C	C	C	T	G	A	G	A
100-13 (WR)	C	C	**A**	G	C	C	T	T	A	G	T
100-14 (Ska)	C	C	**A**	C	C	C	T	G	C	G	A
100-15 (Klm)	C	C	**A**	C	C	C	C	G	A	T	A