Discovery and characterization of novel genetic markers for coastal cutthroat trout (Oncorhynchus clarkii clarkii)

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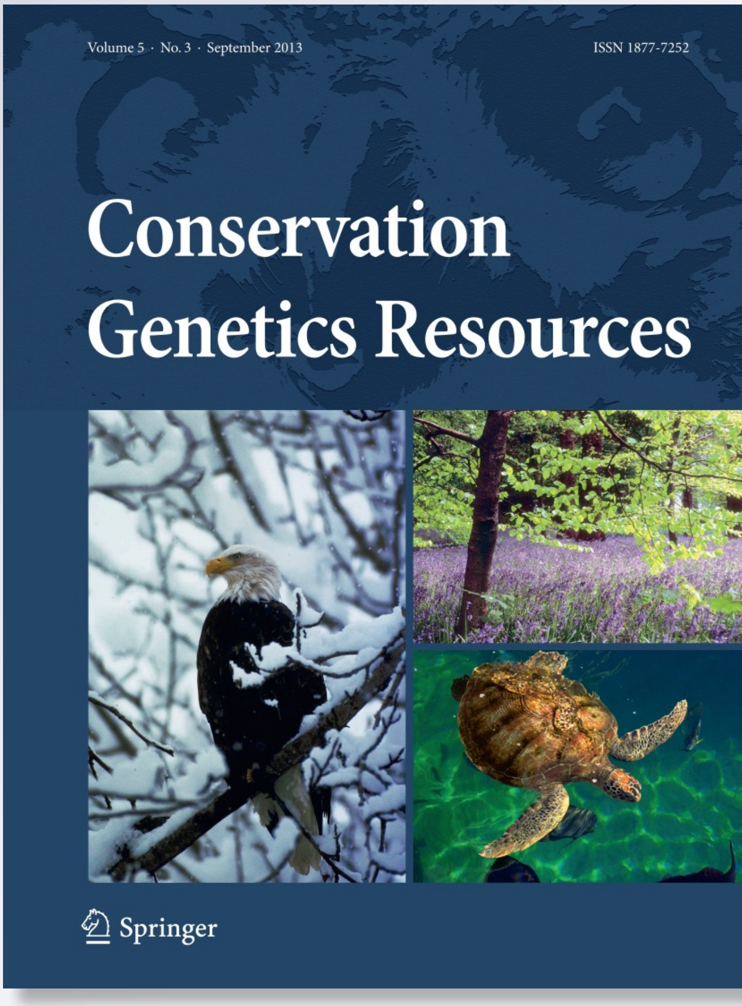

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TECHNICAL NOTE

Discovery and characterization of novel genetic markers for coastal cutthroat trout (*Oncorhynchus clarkii clarkii*)

Victoria L. Pritchard · John Carlos Garza

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Abstract Coastal cutthroat trout (*Oncorhynchus clarkii clarkii*), native to the west coast of North America, has declined over much of its range. Population genetic studies can aid conservation, but few suitable markers have been available. We describe 62 novel single nucleotide polymorphism (SNP) markers variable in coastal cutthroat trout. We additionally show that 22 SNPs previously identified in other taxa are also polymorphic in the subspecies. These 84 SNP assays are the first to be developed for coastal cutthroat trout and will be a useful tool in coastal cutthroat trout management.

Keywords Single nucleotide polymorphism · Coastal cutthroat trout · *Oncorhynchus clarkii*

The cutthroat trout (*Oncorhynchus clarkii*) of North America comprises nine extant subspecies (Behnke 2002). Coastal cutthroat trout (*O. c. clarkii*), native to Pacific drainages from northern California to Alaska, is the most widespread subspecies and the sole one with an anadromous form (Trotter 1989). It has declined throughout much of its range, primarily due to habitat loss (e.g. Wofford et al. 2005). Additionally, coastal cutthroat trout can hybridize with sympatric steelhead (*O. mykiss*), and habitat disturbance may break down barriers to gene flow (e.g. Bettles et al. 2005).

Population genetic studies are important for coastal cutthroat trout management. Genetic markers currently available are limited to allozymes and relatively few microsatellites, largely developed for other taxa (Wenburg et al. 1996; Condrey and Bentzen 1998). Markers that detect introgression of *O. mykiss* into cutthroat trout populations have recently been developed (e.g. Pritchard et al. 2012). Here, we describe a suite of 84 single nucleotide polymorphisms (SNPs) that are variable within multiple populations of coastal cutthroat trout.

Protocols for SNP discovery, TaqMan assay design, and SNP genotyping were as described in Pritchard et al. (2012 and 2013). The ascertainment panel (Pritchard et al. 2012) contained both *O. clarkii* and *O. mykiss* and included samples from four coastal cutthroat trout populations: Slippery Lake, Alexander Archipelago, Alaska (n = 1); Abernathy Creek, Columbia River, Washington (n = 1); Mill Creek, Columbia River, Washington (n = 1) and Little Creek, California (n = 2). SNPs containing an allele rare in *O. c. clarkii* but common in *O. mykiss* were considered indicative of recent introgression and excluded from assay design. Assays were validated using 184 coastal cutthroat trout from 8 populations across the subspecies' range (Table 1).

Seven SNPs diagnostic between *O. mykiss* and other cutthroat subspecies but known to be polymorphic in coastal cutthroat trout were included in the validation step (Pritchard et al. 2012). Additionally, assays interrogating SNP sites within *O. mykiss* (n = 136, Abadía-Cardoso et al. 2011; Aguilar and Garza 2008), and other *O. clarkii* subspecies (n = 179, Campbell et al. 2012; Pritchard et al. 2013), were screened with 14–23 coastal cutthroat trout

Electronic supplementary material The online version of this article (doi:10.1007/s12686-013-9863-2) contains supplementary material, which is available to authorized users.

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Table 1 Minor allele frequency (MAF), expected (H_e) and observed heterozygosity (H_o) of **(a)** novel coastal cutthroat trout SNPs and **(b)** additional SNPs within validation populations

	Gines Creek, Alaska (n = 13–19)			Alexander Archipelago, Alaska (n = 15–20)			San Josef Creek, British Columbia (n = 12–19)			Abernathy Creek, Washington (n = 33–41)			Chinook River, Washington (n = 28)			Cummins Creek, Oregon (n = 14)			Little Creek, California (n = 15–22)			Ryan Creek, California (n = 21)		
	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}
a																								
Ocl_94903c	0.00	–	–	0.50	0.51	0.50	0.40	0.49	0.37	0.12	0.22	0.24	0.32	0.44	0.50	0.04	0.07	0.07	0.00	–	–	0.00	–	–
Ocl_95769c	0.69	0.44	0.13	0.35	0.48	0.30	0.53	0.51	0.47	0.22	0.35	0.39	0.40	0.49	0.30	0.10	0.19	0.20	0.02	0.05	0.05	0.53	0.51	0.72
Ocl_96127c	0.00	–	–	0.38	0.48	0.35	0.00	–	–	0.06	0.12	0.12	0.20	0.32	0.32	0.00	–	–	0.00	–	–	0.00	–	–
Ocl_96500c	0.63	0.48	0.53	0.25	0.39	0.20	0.03	0.05	0.05	0.42	0.50	0.53	0.30	0.43	0.30	0.46	0.52	0.64	0.43	0.51	0.43	0.05	0.09	0.10
Ocl_97077c	0.11	0.19	0.21	0.55	0.51	0.40	0.11	0.19	0.21	0.42	0.49	0.49	0.21	0.34	0.36	0.21	0.35	0.14	0.00	–	–	0.05	0.09	0.10
Ocl_97865c	0.61	0.49	0.68	0.30	0.43	0.40	0.90	0.19	0.21	0.53	0.51	0.52	0.38	0.48	0.54	0.64	0.48	0.43	0.00	–	–	0.21	0.35	0.33
Ocl_98188c	0.00	–	–	0.03	0.05	0.05	0.03	0.05	0.05	0.11	0.20	0.17	0.04	0.07	0.07	0.54	0.52	0.21	0.61	0.49	0.50	0.55	0.51	0.71
Ocl_98409c	0.03	0.05	0.05	0.00	–	–	0.24	0.37	0.47	0.26	0.39	0.32	0.20	0.32	0.18	0.18	0.30	0.36	0.61	0.49	0.23	0.55	0.51	0.52
Ocl_101704c	0.32	0.44	0.53	0.20	0.33	0.40	0.11	0.19	0.21	0.11	0.20	0.17	0.07	0.14	0.14	0.00	–	–	0.16	0.27	0.32	0.52	0.51	0.19
Ocl_102420c	0.00	–	–	0.00	–	–	0.00	–	–	0.05	0.09	0.09	0.05	0.10	0.11	0.11	0.20	0.21	0.33	0.46	0.67	0.07	0.14	0.14
Ocl_102510c	0.00	–	–	0.00	–	–	0.05	0.10	0.11	0.46	0.50	0.54	0.27	0.40	0.32	0.46	0.52	0.36	0.89	0.21	0.23	0.67	0.46	0.29
Ocl_103122c	0.29	0.42	0.58	0.18	0.31	0.37	0.08	0.15	0.16	0.20	0.32	0.29	0.15	0.26	0.30	0.04	0.07	0.07	0.11	0.21	0.23	0.07	0.14	0.14
Ocl_104216c	0.00	–	–	0.00	–	–	0.00	–	–	0.04	0.07	0.07	0.14	0.25	0.14	0.18	0.30	0.36	0.52	0.51	0.41	0.12	0.22	0.24
Ocl_105385c	0.34	0.46	0.47	0.00	–	–	0.26	0.40	0.42	0.29	0.42	0.43	0.30	0.43	0.37	0.36	0.48	0.43	0.43	0.50	0.41	0.60	0.49	0.33
Ocl_105407c	0.74	0.40	0.32	0.30	0.43	0.50	0.26	0.40	0.42	0.59	0.49	0.39	0.30	0.43	0.39	0.21	0.35	0.43	0.77	0.36	0.18	0.45	0.51	0.43
Ocl_105768c	0.00	–	–	0.57	0.51	0.20	0.17	0.29	0.33	0.03	0.06	0.06	0.27	0.40	0.46	0.18	0.30	0.36	0.33	0.46	0.27	0.21	0.35	0.33
Ocl_105897c	0.11	0.19	0.21	0.06	0.11	0.00	0.13	0.24	0.26	0.15	0.25	0.29	0.00	–	–	0.21	0.35	0.43	0.57	0.50	0.57	0.15	0.26	0.20
Ocl_106172c	0.00	–	–	0.16	0.27	0.21	0.03	0.05	0.05	0.15	0.25	0.29	0.30	0.43	0.52	0.32	0.45	0.36	0.43	0.50	0.23	0.26	0.40	0.43
Ocl_106419c	0.53	0.51	0.42	0.13	0.22	0.15	0.13	0.24	0.16	0.18	0.29	0.35	0.04	0.07	0.07	0.14	0.25	0.14	0.18	0.30	0.36	0.02	0.05	0.05
Ocl_106747c	0.00	–	–	0.05	0.10	0.10	0.18	0.31	0.26	0.39	0.48	0.34	0.16	0.28	0.32	0.39	0.49	0.15	0.64	0.47	0.36	0.14	0.25	0.29
Ocl_107074c	0.00	–	–	0.15	0.26	0.20	0.00	–	–	0.35	0.46	0.45	0.29	0.42	0.43	0.29	0.42	0.57	0.71	0.43	0.23	0.31	0.44	0.33
Ocl_107607c	0.24	0.37	0.47	0.20	0.33	0.40	0.34	0.46	0.58	0.07	0.14	0.15	0.09	0.17	0.18	0.14	0.25	0.14	0.23	0.36	0.18	0.07	0.14	0.14
Ocl_108007c	0.00	–	–	0.20	0.33	0.30	0.29	0.42	0.47	0.27	0.40	0.39	0.23	0.36	0.18	0.11	0.20	0.21	0.50	0.51	0.36	0.26	0.40	0.42
Ocl_109243c	0.00	–	–	0.05	0.10	0.10	0.00	–	–	0.20	0.32	0.39	0.20	0.32	0.32	0.25	0.39	0.36	0.82	0.31	0.37	0.57	0.50	0.38
Ocl_109894c	0.00	–	–	0.10	0.19	0.10	0.03	0.05	0.05	0.29	0.42	0.49	0.23	0.36	0.46	0.00	–	–	0.25	0.38	0.23	0.33	0.46	0.57
Ocl_110064c	0.00	–	–	0.11	0.19	0.21	0.24	0.37	0.47	0.34	0.46	0.29	0.32	0.44	0.43	0.21	0.35	0.43	0.73	0.41	0.55	0.71	0.42	0.38
Ocl_110495c	0.37	0.48	0.63	0.03	0.05	0.05	0.63	0.48	0.32	0.52	0.51	0.46	0.62	0.48	0.46	0.32	0.45	0.36	0.59	0.50	0.55	0.20	0.33	0.40
Ocl_111084c	0.00	–	–	0.00	–	–	0.00	–	–	0.13	0.24	0.17	0.07	0.14	0.14	0.00	–	–	0.00	–	–	0.00	–	–
Ocl_111312c	0.34	0.46	0.26	0.43	0.50	0.15	0.18	0.31	0.26	0.29	0.42	0.49	0.43	0.50	0.64	0.07	0.14	0.14	0.00	–	–	0.00	–	–
Ocl_111383c	0.00	–	–	0.30	0.43	0.50	0.00	–	–	0.04	0.07	0.07	0.04	0.07	0.07	0.14	0.25	0.29	0.00	–	–	0.00	–	–
Ocl_112419c	0.40	0.49	0.37	0.08	0.14	0.05	0.16	0.27	0.32	0.49	0.51	0.34	0.27	0.40	0.39	0.18	0.30	0.21	0.82	0.30	0.27	0.79	0.35	0.33
Ocl_113109c	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.32	0.44	0.09*	0.00	–	–
Ocl_113128c	0.00	–	–	0.00	–	–	0.05	0.10	0.11	0.06	0.12	0.07	0.11	0.20	0.14	0.04	0.07	0.07	0.18	0.30	0.27	0.05	0.09	0.10
Ocl_113600c	0.13	0.24	0.26	0.00	–	–	0.03	0.05	0.05	0.20	0.32	0.34	0.20	0.32	0.32	0.31	0.44	0.46	0.57	0.50	0.50	0.24	0.37	0.48
Ocl_114315c	1.00	–	–	0.45	0.51	0.60	0.45	0.51	0.47	0.37	0.47	0.39	0.32	0.44	0.43	0.39	0.50	0.50	0.05	0.09	0.09	0.24	0.37	0.38
Ocl_114336c	0.29	0.42	0.37	0.38	0.48	0.45	0.53	0.51	0.74	0.09	0.16	0.12	0.05	0.10	0.11	0.00	–	–	0.11	0.21	0.23	0.26	0.40	0.43
Ocl_114448c	0.00	–	–	0.15	0.26	0.20	0.16	0.27	0.21	0.35	0.46	0.42	0.41	0.49	0.32	0.79	0.35	0.43	0.30	0.43	0.50	0.57	0.50	0.57

Table 1 continued

	Gines Creek, Alaska (n = 13–19)			Alexander Archipelago, Alaska (n = 15–20)			San Josef Creek, British Columbia (n = 12–19)			Abernathy Creek, Washington (n = 33–41)			Chinook River, Washington (n = 28)			Cummins Creek, Oregon (n = 14)			Little Creek, California (n = 15–22)			Ryan Creek, California (n = 21)		
	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}
Ocl_115987c	0.05	0.10	0.11	0.00	0.00	0.00	0.26	0.40	0.32	0.18	0.30	0.32	0.23	0.36	0.25	0.57	0.51	0.43	0.76	0.37	0.38	0.57	0.50	0.48
Ocl_116865c	1.00	–	–	0.28	0.41	0.45	0.40	0.49	0.47	0.17	0.29	0.34	0.36	0.47	0.57	0.18	0.30	0.36	0.21	0.33	0.41	0.00	–	–
Ocl_116938c	0.11	0.19	0.11	0.05	0.10	0.00	0.45	0.51	0.47	0.27	0.40	0.29	0.27	0.40	0.32	0.14	0.25	0.14	0.21	0.33	0.41	0.55	0.51	0.43
Ocl_117144c	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.07	0.14	0.14	0.73	0.41	0.27	0.41	0.49	0.52
Ocl_117259c	0.00	–	–	0.00	–	–	0.00	–	–	0.08	0.14	0.15	0.02	0.04	0.04	0.04	0.07	0.07	0.40	0.49	0.47	0.26	0.40	0.43
Ocl_117370c	0.18	0.31	0.16	0.50	0.51	0.50	0.32	0.44	0.32	0.23	0.37	0.34	0.32	0.44	0.48	0.21	0.35	0.29	0.24	0.37	0.37	0.60	0.49	0.43
Ocl_117432c	0.00	–	–	0.00	–	–	0.00	–	–	0.30	0.43	0.49	0.05	0.10	0.11	0.00	–	–	0.23	0.36	0.25	0.00	–	–
Ocl_117540c	0.45	0.51	0.58	0.09	0.17	0.06	0.21	0.34	0.32	0.27	0.40	0.30	0.30	0.43	0.39	0.18	0.30	0.36	0.78	0.36	0.22	0.71	0.42	0.48
Ocl_118654c	0.00	–	–	0.00	–	–	0.05	0.10	0.11	0.17	0.28	0.27	0.32	0.44	0.36	0.14	0.25	0.14	0.26	0.40	0.32	0.52	0.51	0.48
Ocl_120255c	0.00	–	–	0.26	0.40	0.32	0.40	0.49	0.47	0.08	0.14	0.15	0.11	0.20	0.22	0.18	0.30	0.07	0.03	0.05	0.05	0.07	0.14	0.14
Ocl_120751c	0.67	0.46	0.22	0.73	0.41	0.27	0.25	0.39	0.33	0.18	0.30	0.36	0.17	0.28	0.26	0.46	0.52	0.42	0.75	0.39	0.17	0.39	0.50	0.33
Ocl_123048c	0.63	0.48	0.42	0.50	0.51	0.40	0.66	0.46	0.58	0.41	0.49	0.52	0.21	0.34	0.36	0.32	0.45	0.36	0.42	0.50	0.53	0.43	0.50	0.38
Ocl_123205c	0.00	–	–	0.18	0.30	0.25	0.21	0.34	0.32	0.41	0.49	0.46	0.25	0.38	0.36	0.50	0.52	0.71	0.78	0.36	0.35	0.81	0.32	0.29
Ocl_124454c	0.18	0.31	0.16	0.33	0.45	0.45	0.11	0.19	0.21	0.49	0.51	0.42	0.29	0.42	0.50	0.61	0.50	0.50	0.18	0.30	0.35	0.24	0.37	0.29
Ocl_125998c	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.07	0.14	0.14	0.45	0.51	0.50	0.45	0.51	0.33
Ocl_128302c	0.33	0.46	0.44	0.68	0.45	0.53	0.13	0.24	0.26	0.58	0.50	0.39	0.48	0.51	0.80	0.00	–	–	0.39	0.49	0.31	0.58	0.50	0.39
Ocl_128757c	0.00	–	–	0.20	0.33	0.20	0.13	0.24	0.16	0.26	0.39	0.52	0.20	0.32	0.39	0.58	0.51	0.33	0.95	0.10	0.11	0.62	0.48	0.48
Ocl_128923c	0.76	0.37	0.47	0.40	0.49	0.30	0.74	0.40	0.42	0.39	0.49	0.49	0.63	0.48	0.44	0.43	0.51	0.43	0.23	0.36	0.15	0.10	0.18	0.19
Ocl_128996c	0.00	–	–	0.00	–	–	0.00	–	–	0.59	0.49	0.46	0.56	0.50	0.44	0.14	0.25	0.29	0.32	0.44	0.42	0.16	0.27	0.21
Ocl_129144c	0.00	–	–	0.38	0.48	0.25	0.55	0.51	0.47	0.61	0.49	0.61	0.48	0.51	0.61	0.39	0.50	0.50	0.32	0.44	0.21	0.48	0.51	0.57
Ocl_129170c	0.24	0.37	0.47	0.30	0.43	0.60	0.34	0.46	0.68	0.45	0.50	0.70	0.54	0.51	0.43	0.57	0.51	0.71	0.35	0.47	0.40	0.17	0.29	0.24
Ocl_130524c	0.55	0.51	0.47	0.50	0.51	0.50	0.18	0.31	0.26	0.57	0.50	0.40	0.54	0.51	0.57	0.23	0.37	0.31	0.36	0.48	0.28	0.60	0.49	0.33
Ocl_131460c	0.00	–	–	0.23	0.36	0.25	0.08	0.15	0.16	0.12	0.22	0.18	0.21	0.34	0.21	0.32	0.45	0.36	0.55	0.51	0.70	0.60	0.49	0.52
Ocl_131785c	0.53	0.51	0.74	0.58	0.50	0.45	0.45	0.51	0.47	0.26	0.39	0.33	0.00	–	–	0.29	0.42	0.43	0.08	0.14	0.15	0.07	0.14	0.14
Ocl_131802c	0.00	–	–	0.00	–	–	0.00	–	–	0.24	0.37	0.42	0.05	0.10	0.11	0.04	0.08	0.08	0.08	0.14	0.15	0.00	–	–
Overall H_e		0.17			0.25			0.24			0.32			0.31			0.28			0.32			0.31	

	Gines (n = 13–19)			Alexander (n = 15–20)			San Josef (n = 12–19)			Abernathy (n = 10–41)			Chinook (n = 28)			Cummins (n = 14)			Little (n = 7–22)			Ryan (n = 21)		
	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}
b																								
Ocl_100884D[a]	0.50	0.51	1.00*	0.30	0.43	0.60	0.13	0.24	0.26	0.38	0.48	0.56	0.43	0.50	0.50	0.46	0.52	0.36	0.11	0.21	0.14	0.10	0.18	0.19
Ocl_106457D[a]	0.16	0.27	0.32	0.28	0.41	0.35	0.76	0.37	0.37	0.26	0.39	0.42	0.45	0.50	0.54	0.18	0.30	0.36	0.39	0.49	0.41	0.31	0.44	0.43
Ocl_109568D[a]	0.42	0.50	0.53	0.35	0.47	0.50	0.32	0.44	0.42	0.05	0.09	0.09	0.21	0.34	0.36	0.36	0.48	0.57	0.38	0.48	0.35	0.29	0.42	0.57
Ocl_112876D[a]	0.90	0.19	0.21	0.13	0.22	0.25	0.45	0.51	0.58	0.30	0.42	0.38	0.29	0.42	0.43	0.36	0.48	0.43	0.45	0.51	0.60	0.69	0.44	0.33
Ocl_128693D[a]	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	0.13	0.22	0.25	0.00	–	–	0.35	0.47	0.40	0.48	0.51	0.57
Ocl_129870D[a]	0.56	0.51	0.33	0.65	0.47	0.35	0.97	0.06	0.06	0.29	0.42	0.47	0.29	0.42	0.36	0.42	0.51	0.54	0.37	0.48	0.42	0.30	0.43	0.50
Ocl_130295D[a]	0.50	0.51	0.47	0.75	0.39	0.30	0.68	0.44	0.53	0.47	0.51	0.52	0.41	0.49	0.46	0.11	0.20	0.21	0.38	0.48	0.45	0.50	0.51	0.43

Table 1 continued

	Gines (n = 13–19)			Alexander (n = 15–20)			San Josef (n = 12–19)			Abernathy (n = 10–41)			Chinook (n = 28)			Cummins (n = 14)			Little (n = 7–22)			Ryan (n = 21)		
	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}	MAF	H_e	H_{obs}
Ocl_106560H[b]	0.00	–	–	0.45	0.51	0.40	0.18	0.31	0.26	0.40	0.49	0.55	0.29	0.42	0.36	0.54	0.52	0.62	0.71	0.43	0.41	0.41	0.49	0.62
Ocl_108210 h[b]	0.11	0.19	0.21	0.23	0.36	0.25	0.50	0.51	0.37	0.40	0.48	0.47	0.46	0.51	0.50	0.43	0.51	0.57	0.50	0.51	0.55	0.19	0.32	0.19
Ocl_120102Y[b]	0.00	–	–	0.10	0.19	0.20	0.00	–	–	0.25	0.38	0.40	0.05	0.10	0.11	0.10	0.19	0.20	0.05	0.09	0.10	0.13	0.24	0.26
Ocl_Nipsh[b]	nr	–	–	nr	–	–	nr	–	–	0.14	0.25	0.29	0.05	0.10	0.11	0.04	0.07	0.07	0.00	–	–	0.00	–	–
Ocl_ImPa1y[b]	nr	–	–	nr	–	–	nr	–	–	0.40	0.49	0.26	0.60	0.49	0.56	0.32	0.45	0.50	0.13	0.23	0.25	0.12	0.22	0.14
Ocl_gdh-33[c]	0.32	0.44	0.53	0.70	0.43	0.40	0.24	0.37	0.37	0.73	0.40	0.41	0.73	0.40	0.46	0.62	0.49	0.62	0.30	0.43	0.50	0.21	0.35	0.43
Ocl_myo1b-16[c]	0.13	0.24	0.26	0.33	0.45	0.45	0.29	0.42	0.47	0.44	0.50	0.58	0.25	0.38	0.36	0.68	0.45	0.21	0.30	0.43	0.60	0.31	0.44	0.52
Omy_97660-230[d]	0.32	0.44	0.11	0.27	0.40	0.53	0.22	0.36	0.33	0.48	0.51	0.39	0.72	0.41	0.41	0.29	0.42	0.43	0.34	0.46	0.47	0.33	0.46	0.57
Omy_97865-196[d]	0.18	0.31	0.37	0.10	0.19	0.20	0.05	0.10	0.11	0.06	0.12	0.12	0.00	–	–	0.18	0.30	0.36	0.41	0.50	0.35	0.25	0.39	0.28
Omy_105105-448[d]	0.00	–	–	0.00	–	–	0.00	–	–	0.00	–	–	nr	–	–	nr	–	–	0.21	0.36	0.43	nr	–	–
Omy_109894-184[d]	0.23	0.37	0.46	0.00	–	–	0.00	–	–	0.10	0.19	0.20	nr	–	–	nr	–	–	0.00	–	–	nr	–	–
Omy_113490-159[d]	0.00	–	–	0.00	–	–	0.00	–	–	0.11	0.19	0.21	0.02	0.04	0.04	0.19	0.32	0.23	0.05	0.10	0.10	0.02	0.05	0.05
Omy_117540-259[d]	0.22	0.35	0.44	0.00	–	–	0.21	0.34	0.21	0.15	0.26	0.06*	0.20	0.32	0.25	0.04	0.07	0.07	0.43	0.51	0.43	0.19	0.32	0.38
Omy_gh1prom1-1[d]	0.00	–	–	0.00	–	–	0.00	–	–	0.05	0.10	0.10	nr	–	–	nr	–	–	0.29	0.44	0.00	nr	–	–
Omy_AldoA[e]	0.50	0.51	0.47	0.14	0.25	0.06	0.32	0.44	0.63	0.29	0.42	0.33	0.05	0.10	0.11	0.14	0.25	0.14	0.04	0.07	0.07	0.36	0.47	0.52
Overall H_e		0.25			0.26			0.25			0.32			0.32			0.34			0.34			0.35	

* significant deviation from Hardy–Weinberg proportions; ‘nr’, SNP not genotyped within validation sample

[a] Pritchard et al. 2012; [b] Pritchard et al. 2013; [c] Campbell et al.2012; [d] Abadía-Cardoso et al. 2011; [e] Aguilar and Garza 2008

Table 2 Name, polymorphism, forward and reverse amplification primer and labeled TaqMan probe sequences for markers described

Assay	NCBI ss#	Target	Primers (5′–3′)	Probes (5′–3′)
Ocl_94903c	491232057	G/A	F: ATGCCGTCTGAGTAGGAGGAT	VIC: AAACATGCAGTATGTATTG
			R: CGGTTTGGATCCAGCTCTCC	FAM: ATGCAGCATGTATTG
Ocl_95769c	491232058	T/A	F: GGTTCACTTTGGGTCCGATT	VIC: CAGACTTGAGATGAGTAGACT
			R: GGGTCAAAACCCATTTCAGATCAAA	FAM: AGACTTGAGATGAGTAGTCT
Ocl_96127c	491232059	G/C	F: GACCTGGTGAGGATGATGTTCA	VIC: TGGTCCTCTAGTCCTCG
			R: GGACAGTGGGAAATGGAAGATGAC	FAM: TGGTCCTCTACTCCTCG
Ocl_96500c	491232060	G/A	F: TGACATCACGCCTGTGACAAAATAT	VIC: TTTTTCGACTCAATATACATTT
			R: CGATGTACAGAAAGATGTTTTTATGATGCT	FAM: TTTCGACTCAATATATATTT
Ocl_97077c	491232061	G/A	F: GTTCAGGTACCCATACATTTCCAAGA	VIC: TGGTTTGCAATCTTAC
			R: CAGGGCACAGGTAGGTTAAAAGAG	FAM: CTGGTTTGTAATCTTAC
Ocl_97865c	491232062	G/A	F: CTGGTTTGTTCCATTGGTTTTCTGA	VIC: CCACTGCATATGTTTTG
			R: GCCCCTATATTCACAATTAAGTGTTTTACCAT	FAM: CACTGCATACGTTTTG
Ocl_98188c	491232063	G/A	F: CGGTAGGCTTCGCGAATAATG	VIC: CAGTTACAGATATCCCC
			R: CCACAAGGCTGCATTATACAGAGA	FAM: AGTTACAGACATCCCC
Ocl_98409c	491232064	C/A	F: GCTCCCGAAGCATCAGCTT	VIC: TGCGTATCCGGTCCTAA
			R: CCATGAAAAGTGATGTGCGACAT	FAM: TGCGTATCCGTTCCTAA
Ocl_101704c	491232065	T/C	F: GTGTGGTCAGCGGTGAGA	VIC: TAGACGTCCAAGGTCC
			R: CCAAACTAGTGGAGGAGATCAAGAG	FAM: TAGACGTCCGAGGTCC
Ocl_102420c	491232066	T/G	F: ATCAGGTAACACGTTTCACACTGT	VIC: CCCTCTCTAAAAGCTGG
			R: CATCATGATGTAGCCCTGTTTGC	FAM: CCCTCTCTAACAGCTGG
Ocl_102510c	491232067	T/C	F: GATGTAAGTTAACTGCCAGTACTAGTGA	VIC: TGCCACACTAATCTT
			R: GGCTGCTTCACTCTAATTCATGTTT	FAM: TGCCACACTGATCTT
Ocl_103122c	491232068	T/C	F: GCTGTTTCTATCCTCATTACTTGGTACT	VIC: CTGTTTCTGCGTCCTTG
			R: ACAATCTGCAGAAGAATTGAGTCGTA	FAM: TGTTTCTGCATCCTTG
Ocl_104216c	491232069	G/A	F: CAGACCACAGGGACAAAAGGA	VIC: TGCTGCTACAGCTCTGAA
			R: GTCATTGTGTACGGAGGAGTGAT	FAM: TGCTGCTACAGCTCCGAA
Ocl_105385c	491232070	G/C	F: AGTGCCCCCCACTGTTAAAAAA	VIC: AACTGCAGATTTTG
			R: CCCTGCTCTAAAACACCTGATTCA	FAM: AACTGCACATTTTG
Ocl_105407c	491232071	G/C	F: TCCTATAGGCTGTCAGGACAGAATC	VIC: CGAACGGCGGACATA
			R: AAACGTCACATGCACCACAAC	FAM: CGAACGGCCGACATA
Ocl_105768c	491232072	T/C	F: AGCATTAAACTACACAACTACAGCTACTG	VIC: TAGCACCTTTGAGAGATT
			R: TTCAGGGAGAGGTAAAGGTCCTATC	FAM: CACCTTTGAGAAATT
Ocl_105897c	491232073	T/C	F: ATTGGCTTCAAATAACTACTGTTGTGA	VIC: TGGAGAGGTTCAGTTTAC
			R: CTAGGATTCTGTTCCTTTGCCTCAA	FAM: TGGAGAGATTCAGTTTAC
Ocl_106172c	491232074	T/A	F: GTCCCCGGCCTCTCC	VIC: CGTAGACGGGCCTCCAGG
			R: CATCAAGAATCCGCGCAGTAG	FAM: CGTAGACGGGCCACCAGG
Ocl_106419c	491232075	T/G	F: GTAATTGACCCCAACCCTGGTT	VIC: AGGTAGGGAGAAACATTTA
			R: ACGGCTGACGGACACTTC	FAM: AGGTAGGGAGAAACCTTTA
Ocl_106747c	491232076	T/G	F: GGTGGTGGGTCTAACTACAATGTAA	VIC: TAGAATGGCGTACAGATGT
			R: CCATCCACTTGACTCCTAACCA	FAM: AATGGCGTCCAGATGT
Ocl_107074c	491232077	C/A	F: GGCTACACTGCTTGATAGGCCTATA	VIC: CATCTTTTCCATGGCTGTG
			R: GTGCTGCTACGGTACAGTACA	FAM: CCATCTTTTCCATTGCTGTG
Ocl_107607c	491232078	T/C	F: CTACATCGCTGGAGAACATGGAA	VIC: TCGCTTTCTAGGAGATTT
			R: GTTCTGGATTCCATTGTGCAAAAGT	FAM: TCGCTTTCTAGAAGATTT
Ocl_108007c	491232079	G/C	F: TTCCGTTTGGTGCCTAGTGAAT	VIC: ACCACCCAGCCTTGTG
			R: GTCCCTTCCCCAGTTTCACTTAATT	FAM: CACCCAGGCTTGTG
Ocl_109243c	491232080	C/A	F: ATGTGCACCTCTTAAATTGTAAGTAAAATGT	VIC: ATTTGTTCATTAAATGGACTTT
			R: ACCCTATATTCAGTGGCAAGATTGC	FAM: ATTTGTTCATTAAATTGACTTT

Table 2 continued

Assay	NCBI ss#	Target	Primers (5′–3′)	Probes (5′–3′)
Ocl_109894c	491232081	G/A	F: CCCACAGCACCGTCACA	VIC: ACGCTCTACCTCCCCGTACA
			R: TGCTGCGAGTGCCCATAC	FAM: ACGCTCTACCTCTCCGTACA
Ocl_110064c	491232082	T/C	F: GTGCAAAGCAAACCAATAGTCTAAAATAGG	VIC: TCAAACTGGTCCGTCCAGA
			R: TTGACACACTTGGCTTGAGACA	FAM: CAAACTGGTCCATCCAGA
Ocl_110495c	491232083	G/A	F: CGATGTTTACTCAAAACGTCAGGGA	VIC: CAAATTAACATGAACACCTTAT
			R: CGACCATCTGAAAAAGCCTGAGAAA	FAM: AATTAACATGAACACTTTAT
Ocl_111084c	491232084	G/A	F: CCACGTCCTGGGAACCAA	VIC: CACCCTGGTCATGCTG
			R: CTGAGCGACGTCTCGGA	FAM: CACCCTGGTTATGCTG
Ocl_111312c	491232085	T/A	F: GGAGGGCTAAAAATACAGACCAAGT	VIC: ACTGCTTTGTGTTTTAGATT
			R: GGGTCCTATTGCTACTGTATTCAACA	FAM: CTGCTTTGTGTATTAGATT
Ocl_111383c	491232086	G/A	F: CCGATGGGCTGCATGGATT	VIC: CATCCACCATTGATTGG
			R: CATGGGAAGGTCGCAACCA	FAM: ATCCACCATCGATTGG
Ocl_112419c	491232087	C/A	F: CATGAATTAACTCATGCAGACTTTCGA	VIC: CAGTTGGAAAAACG
			R: GAAAACACTGCCAGAGGTGACT	FAM: ACAGTTGTAAAAACG
Ocl_113109c	491232088	A/C	F: CATTCATTCAACATGGGACTCAAACT	VIC: AACATTTTAGAAAACAAGAGAC
			R: CAACCGGTAATGCATTTCCTGAAA	FAM: CATTTTAGAAAACAAGATAC
Ocl_113128c	491232089	T/C	F: CCTCCTACTCTGATCTAAAGATTACAGAA	VIC: CGCTGTCATACCAAC
			R: TTCTCTGCCCTCTCGATTTTGG	FAM: CGCTGTCGTACCAAC
Ocl_113600c	491232090	G/A	F: GTCATCAAGGTGAGATGCTTCTCT	VIC: AGGTTCATAGTCTTAATATGGTTC
			R: GCCAGGACAAACAGGCATGT	FAM: TTCATAGTCTTAACATGGTTC
Ocl_114315c	491232091	G/C	F: CCTCACCGATCTAGTCAACTTCATC	VIC: CATAACTCGCGAGGCAC
			R: GGCTGAGGGAGATTCTAGATCGA	FAM: CATAACTCCCGAGGCAC
Ocl_114336c	491232092	T/G	F: TCCCCATCCTAACAAGGCCTTATTA	VIC: TTAGGTCTGAATCATGCATC
			R: CTCCATACAATTCAATGTAACCTGAAAGC	FAM: TTAGGTCTGAATCCTGCATC
Ocl_114448c	491232093	T/C	F: GGTGTACCCTCTATTGGTGTGTAAT	VIC: TTGCTTTTGACTCGCCACAG
			R: GCATCCAGAGATTTCACAGATAAGC	FAM: TTGCTTTTGACTCACCACAG
Ocl_115987c	491232094	T/G	F: GAACTCAAGGTGTTTATGGCATTCA	VIC: ATGCATCTCTTATATTCCCA
			R: GGTCACTGTTTTGGAGGAGTAGT	FAM: ATGCATCTCTTATCTTCCCA
Ocl_116865c	491232095	T/A	F: AGCTATTTTATACAGTTGAGTCATCAAACCA	VIC: CAGCTGTGGTGGACAT
			R: AAAGTAGGTCCATAGAAACCAAATAAAATCCA	FAM: AGCTGTGGAGGACAT
Ocl_116938c	491232096	T/C	F: GTGGTGAGTGAGTATGTGTGTTCAT	VIC: AAATTGAGACAAGGAAAT
			R: CCCATTCATCCCAATCGAAAGCT	FAM: AATTGGGACAAGGAAAT
Ocl_117144c	491232097	T/C	F: GGAGTGAGTTTGAACAGTACCTGAA	VIC: TTGGCCTCGTGTGCC
			R: GCTCTCAGGTCCCTGCAT	FAM: TGGCCTCATGTGCC
Ocl_117259c	491232098	G/A	F: GCCAAACCAGGGACTTTCCT	VIC: CTTGTTTCCTTCACTGCC
			R: TCCGAGCCCCCAGATAAGAG	FAM: TTGTTTCCTCCACTGCC
Ocl_117370c	491232099	T/G	F: GAACAAGCCCCACCAAACATG	VIC: CGCCCAGACCTAC
			R: GTAGCATTAGTACATGTAGCCTTTGGA	FAM: CGCCCAGCCCTAC
Ocl_117432c	491232100	G/A	F: ACTCAACGCTGTGATCAACGA	VIC: CCTGGACAATCTCAAG
			R: CTGATGGGCCTGTCATGGT	FAM: CCTGGACAACCTCAAG
Ocl_117540c	491232101	G/A	F: AATGGCATGACCTTAAATTAGTCAAGGA	VIC: CACTGGGAGAAAGACGA
			R: CCACCATACCAGGCACGAAA	FAM: ACTGGGAGAAAGATGA
Ocl_118654c	491232102	T/C	F: GGCCGACCGCTGCTA	VIC: TGCCAGCAGCACGTG
			R: CCGAACGGCACAAAGAATACAG	FAM: TGCCAGCAACACGTG
Ocl_120255c	491232103	T/C	F: CTCAAATGACTAAAACGTCAGTTACTGG	VIC: TCATCCTAAAAGAAATGAGAG
			R: AGAATGCAGTCCAGCAGACA	FAM: CATCCTAAAAGAAATAAGAG

Table 2 continued

Assay	NCBI ss#	Target	Primers (5′–3′)	Probes (5′–3′)
Ocl_120751c	491232104	G/A	F: TGCTCAGCTCCACGAAAGAG R: GAAGGTTGACGACAAGTTTTATAATTTGC	VIC: CTGTGATGTCTCCTCTCC FAM: TGTGATGTCCCCTCTCC
Ocl_123048c	491232105	T/C	F: CTCAACAGTGCACCTCCCTT R: TCAATGAGTTTAGTTTGTCAATTGACTAGCT	VIC: AATTTAACTTGACATTCTTGGGC FAM: ATTTAACTTGACATTCTTAGGC
Ocl_123205c	491232106	T/C	F: CCTACAACACCATGGCTGTGA R: TCATTGGCATGGATGGCACAT	VIC: CCCTTCTGGACACCAC FAM: CCTTCTGGGCACCAC
Ocl_124454c	491232107	G/A	F: AGCACCAAGGCCGTGATC R: GGAACAGTCCGGCACCAT	VIC: AGAGGGTTGAAACTT FAM: AGGGTCGAAACTT
Ocl_125998c	491232108	T/C	F: GAGGTCATTATGCAGGCCCAATA R: GGTTTGCGGTGAGTTAACA	VIC: CCCTCAGTATATTTGGTC FAM: CCTCAGTATGTTTGGTC
Ocl_128302c	491232109	C/A	F: GGAGGGCAACCACATGTAATCAG R: CAGAGGGTTTACAGTAAAGTACATACAATCA	VIC: TTCACTGAAAATGTTATATATT FAM: ATTCACTGAAAATTTTATATATT
Ocl_128757c	491232110	T/C	F: AGAGACAGGGTGCAACACAAAA R: CTTCGGAGATACTCGTGTGGATTTTA	VIC: AACGTTATTGTACGATGACC FAM: CGTTATTGTACAATGACC
Ocl_128923c	491232111	C/A	F: TGTCTTCTTGAACAGTCCATTTTCCT R: AGTACCTTTGACATTTAAAAGTGACGGA	VIC: TGGCTCTGGTGCCTTGT FAM: TTGGCTCTGGTTCCTTGT
Ocl_128996c	491232112	C/A	F: CTCAGGGAATCTAAGCAGGCAAA R: ACAGTACAGAAATACAACCACCGTTT	VIC: CAGTATTGTCTAGTGGTCTGT FAM: CAGTATTGTCTAGTTGTCTGT
Ocl_129144c	491232113	G/C	F: AGCCGGGACTAAACCTGTCT R: CTCAGTCATCCATTCAGTGAAGGT	VIC: CCTCCTTAGGGCTTCTA FAM: CCTCCTTAGGCCTTCTA
Ocl_129170c	491232114	T/C	F: AGCGCTGAACTTTCCAGAGTAAAA R: TCTTTCCGACACCCCCTAGAG	VIC: ATCTGGTCCTCATATCCACCG FAM: TATCTGGTCCTCATGTCCACCG
Ocl_130524c	491232115	T/C	F: TGTCTGTTCTGCTGTGTGCTT R: CTGCCAAGGTAACAATGGTATGACT	VIC: TCGTTGGCATGGCTT FAM: TCGTTGGCATGACTT
Ocl_131460c	491232116	G/C	F: CCGAGTATTGACTATTTCACAGCACTT R: GGTTTTCTAAAGTTATACATCACAATACGACACA	VIC: ATTTCTGAGGGATAACAC FAM: TTCTGAGGCATAACAC
Ocl_131785c	491232117	T/C	F: ATTAAGGGATGTGCAACAATCTGAGA R: ACATATTTAGGTGAGTGCGGGTTTT	VIC: AGTTGGTGAATATTCA FAM: AGTTGGTGAATGTTCA
Ocl_131802c	491232118	T/A	F: GCTTAGAGCCTCTTTAGCACAACTC R: ACTGAACAAAACATGGCTCCAACTA	VIC: AAGCAGACGTCATACAT FAM: AGCAGACGACATACAT
Ocl_impa1y[a]	491232119	T/A	F: AGAAGGGTTGTTGCTGCAAATAAC R: CTGTCTCTGTAGACGCATCATCTC	VIC: AGGATTGTCAAAGAGATT FAM: AGAGGATTGTCAATGAGATT

[a] SNP discovery described in Pritchard et al. (2013)

from California and Washington. Polymorphic SNPs were subsequently genotyped over the wider validation panel.

Observed (H_o) and unbiased expected heterozygosity (H_e) were calculated using Genetix (Belkhir et al. 2001). Deviations from Hardy–Weinberg and linkage equilibria were investigated using exact tests in Genepop (Raymond and Rousset 1995), applying the FDR correction (Benjamini and Hochberg 1995).

Of 242 primer pairs, 171 produced high-quality sequence for coastal cutthroat trout, generating 90,730 consensus base-pairs for a mean of 4.4 individuals per locus. There were 414 polymorphic sites observed, distributed over 130 loci and including 44 indels. Assays were successfully designed for a SNP in 71 of these loci (Table 2). Nine assays were rejected following validation due to lack of polymorphism or problems calling genotypes.

Twelve SNPs previously developed for *O. mykiss* (8.8 % of those tested), six SNPs developed for *O. c. henshawi* (7.7 %) and three SNPs from other cutthroat trout subspecies were variable in initial screening samples and included in validation. Six were subsequently rejected due to calling problems. Two of the 15 retained assays (Table 1b) were physically linked to SNPs developed for coastal cutthroat trout: Omy_117540-259, 158 bp from Ocl_117540c; and Omy_97865-196, 107 bp from Ocl_97865c.

Following FDR correction, we observed only three significant deviations from Hardy–Weinberg equilibrium amongst the final set of 84 assays, at three different loci (Table 1). We observed one significant deviation from linkage equilibrium: Ocl_117370c–Ocl_124454c within Gines Creek.

The 62 novel SNP assays described here are the first to be developed for coastal cutthroat trout. Together with the seven species-diagnostic SNPs known a priori to be variable in coastal cutthroat trout, and the 15 SNPs developed for other taxa, they will be useful for population genetic studies throughout the subspecies' range. Blast searches showed 70 of these 84 SNPs to be in a locus with a putative function, and seven are non-synonymous substitutions (Supplementary Table 1).

Validation of the novel SNPs revealed comparable genetic diversity in Washington, Oregon and California populations, but reduced diversity in British Columbia and Alaska. Only one Alaskan individual was in our ascertainment panel; thus, reduced diversity in northern populations may represent ascertainment bias. However, it may also reflect recent colonization of previously glaciated areas. Supporting this, the 22 SNPs not identified from our ascertainment panel also had reduced diversity at higher latitudes. A similar latitudinal trend has been documented in *O. mykiss* (McCusker et al. 2000).

Approximately 6 % of the *O. mykiss* SNPs screened were polymorphic and reliably scored in coastal cutthroat trout. The relatively high minor allele frequencies for these SNPs indicate that they represent shared polymorphisms rather than *O. mykiss* polymorphisms introduced by hybridization. A similar proportion of SNPs variable in Lahontan cutthroat trout were polymorphic in coastal cutthroat trout (5.7 %, Pritchard et al. 2013). Thus, polymorphic sites are only rarely shared between species and subspecies.

Only one significant deviation from linkage equilibrium was observed in our dataset, despite the inclusion of two physically linked SNP pairs, which may reflect the relatively low power of the corrected test. Current efforts to map loci containing these SNPs will provide further information on physical linkage.

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