Development of major histocompatibility (MH)-associated microsatellite markers and characterization of linkage disequilibrium patterns in Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*).

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Running title: New MH microsatellites in salmon and trout

Abstract

Genes of major histocompatibility complex play a major role in self recognition, immune response and disease resistance in vertebrates. Here, we describe the development of 22 new highly polymorphic microsatellite markers in both classical and nonclassical major histocompatibility (MH) regions of Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). These newly developed microsatellite loci allow detailed characterization of the diversity, differentiation, level of linkage disequilibrium and haplotype structure that enables to further understand the relationships between MH variability and disease resistance in these ecologically and commercially important species.

Major histocompatibility gene complex (MHC) is a multigene family that plays a significant role in self recognition, the initiation of immune response and disease resistance in vertebrates. These genes are typically highly variable and are believed to be maintained by balancing selection (Garrigan & Hedrick 2003). In particular, MHC class I and class II genes encoding the receptor glycoproteins which present antigenic peptides to the T cells are regarded as one of the most polymorphic genes described to date. In teleosts, MHC class I and class II genes have evolved independently and therefore often referred as MH genes (Grimholt *et al.* 2002). MH class I and class II genes in salmonids reside in different chromosomes (Sato *et al*. 2000; Phillips et al. 2003). In Atlantic salmon, the classical MH class IA region (*Sasa UBA*) is located in linkage group 15 (LG15) while classical MH class II (*Sasa DAA, Sasa DAB*) genes reside in LG6 (Harstad *et al.* 2008). Atlantic salmon also possesses several additional classical and nonclassical MH genes that in general show lower levels of variability and are involved in a variety of specific immune functions (Lukacs *et al,* 2007, 2010; Harstad *et al.* 2008).

To date, most population genetic studies focusing on MH genes in salmonids have used traditional sequencing of limited number of exonic regions in classical MHC class I and II genes (e.g. Landry *et al,* 2001). However, screening of large number of individuals by Sanger sequencing is both time consuming and expensive (but see also Pavey *et al.* 2011). As an alternative strategy, several studies have demonstrated that screening microsatellite loci tightly linked to MH genes can be used as a proxy for functionally important variation (e.g. Grimholt *et al.* 2002; Vasemägi *et al*. 2005; Tonteri *et al.* 2010). Here, we describe the development of 22 new polymorphic MH associated microsatellite markers located in classical and nonclassical MH class I and class II regions in Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). To demonstrate the usefulness of the novel markers we

characterize linkage disequilibrium patterns in multiple Atlantic salmon and brown trout populations.

Methods

Microsatellite identification

The least overlapping BAC clone sequences were chosen for the development of the microsatellite markers that contained additional MH class I genes (*UCA, UDA, SAA, UHA1, UHA2, UGA*) and nonclassical MHC IIα and IIß (*Sasa DBA* and *Sasa DBB*) genes in Atlantic salmon (Harstad *et al.* 2008; Lukacs *et al.* 2010). Altogether, nine BAC clones from LG15 [Genbank: EF427381, EF210363, GQ505858], LG3-1 [Genbank: GQ505859, GQ505860], LG3-2 [Genbank: FJ969490], LG-10 [Genbank: FJ969488], LG-14 [Genbank: FJ969489] and LG5 [Genbank, EU008541] were selected.

Primer design & sample information

A total of 48 primer pairs were designed from the selected sequences using MsatCommander software (Faircloth, 2008). A M13-tail (CACGACGTTGTAAAACGAC) was added to the 5' end of the forward primer. To enhance 3'adenylation a GTTT sequence was added to the 5′ end of each reverse primer (Browstein *et al.* 1996). The initial amplification success of the microsatellite loci were tested in a set of sixteen individuals comprising eight Atlantic salmon and eight brown trout specimens from different populations (*S. salar*: River Teno, Finland; River Selja, Estonia; River Varzuga, Russia; River Sella, Spain; Saint John River, Canada; *S. trutta*: Lake Inari, Finland; River Danilovka, Russia; River Mustoja, Estonia). For characterization of the variability and linkage disequilibrium (LD) patterns four Atlantic

salmon populations (landlocked population from River Kamennaja, Russia; anadromous populations from River Ponoi, River Pulonga, White Sea, Russia; River Narva, Baltic Sea, Estonia), and four brown trout populations (upstream and downstream of River Mustoja and Pada, Estonia) were selected for genotyping (48 individuals per population). Total DNA was extracted by salt extraction method (Aljanabi *et al,* 1997) and was diluted in 10Mm TE buffer.

PCR optimization & population screening

Out of 48 loci, twenty two markers were successfully amplified (17 and 15 in Atlantic salmon and brown trout, respectively; electrophoregrams available at http://users.utu.fi/antvas/Electrophoregrams/Table1.htm). Twenty six loci were discarded from subsequent analyses because of lack of amplification, multiple PCR products and lack of polymorphism or peaks difficult to interpret (Table S1, Supplementary information). For subsequent screening of different Atlantic salmon populations, the loci were divided according to their size ranges into four multiplex and five single PCRs (Table S2, Supporting information). Selected loci were amplified in 6.1 µL total reaction volume that consisted of 20-50 ng of DNA, 0.6 µM of forward, 1.2 µM of reverse and 17 µM of M13 primer labeled with one of the four fluorescence dye (FAM, VIC, PET or NED) and 1x QIAGEN multiplex PCR mastermix. The PCR program consisted of initial activation step of 95°C for 15 min followed by 36 cycles of denaturation in 94°C for 30 s (s), annealing temperature 58°C (14 cycles) followed by 52°C (25 cycles) for 1 min 30 s (s), extension reaction at 72°C for 1 min and final extension at 72°C for 10 min (s). For brown trout, fifteen loci were divided according to their size ranges into three multiplex and five single PCRs (Table S2, Supplementary information) using the same PCR conditions and amplification profile as in Atlantic salmon. A previously published microsatellite locus at 3'-UTR of *Sasa–UBA*

(classical MHCI region, Grimholt *et al.,* 2002) was used for comparison of the LD patterns along the linkage group 15. PCR products were pooled by adding 1.2 µL from each multiplex PCR product and 1 µL from each single PCR product to 100 µL of sterile water (Milli-Q). An aliquot of 2 µL of the pooled PCR product was added to 0.1 µL of GeneScan 600LIZ size standard (Applied Biosystems) and 9.95 µL of HiDi-formamide. The samples were electrophoresed on an ABI PRISM 3130xl (Applied Biosystem) instrument. The results were analyzed using GeneMarker V1.96 (Softgenetics). Population genetic analysis was carried out using FSTAT 2.9.3.2 (Goudet 2005), Genepop 1.2 (Raymond & Rousset 1995) and Power Marker 3.25 (Liu & Muse 2005) and Micro-Checker 2.2.3 (Van Oosterhout et al. 2004).

Results/Discussion

Atlantic salmon

The total number of alleles per locus and allelic richness in Atlantic salmon ranged from 3 to 32 and 2.15 to 16.32, respectively. The expected heterozygosity estimates in Atlantic salmon ranged from 0.44 to 0.87 (Table 1). Altogether, 9 loci out of 17 significantly deviated ($P<0.05$, before multiple correction) from the Hardy-Weinberg equilibrium expectations at least in one population (Table 2). Pair-wise linkage disequilibrium analysis showed significant linkage ($P<0.05$, before multiple correction) in 30 pair-wise comparisons out of 43 in LG15, 3 pair wise comparisons out of 6 in LG3, 2 pair wise comparisons out of 3 in LG5. The extent of linkage disequilibrium measured as *D'* along the LG15 (*Sasa-UBA* region) revealed considerable variation among Atlantic salmon populations (Figure 1a-d). As expected, landlocked Kammenaja population showed the highest levels of LD (Fig. 1a) while

the largest anadromous population (Ponoi) exhibited the lowest pair-wise *D'* estimates (Fig. 1c).

Brown trout

The total number of alleles per locus and allelic richness in brown trout ranged from 2 to 19 and 1.95 to 11.28, respectively. The expected heterozygosity estimates ranged from 0.13 to 0.88 (Table 1). Significant deviations from HW equilibrium (P<0.05, before multiple correction) were found in 9 out of 15 loci at least in one population (Table 2). Pair-wise linkage disequilibrium analysis showed significant linkage (P<0.05, before multiple correction) in 20 pair wise comparisons out of 21 in LG15, 7 pair wise comparisons out of 10 in LG3. Similar to Atlantic salmon, considerable variation in LD at classical MH class I region were observed among different brown trout populations (Fig. 1e-h). Moreover, large differences in LD were observed within the same river system (Fig. 1g, h; River Pada upstream vs. downstream).

To conclude, we developed a set of new highly polymorphic microsatellite markers linked to classical and nonclassical MH regions in Atlantic salmon and brown trout that enable detailed characterization of the diversity, differentiation and level of linkage disequilibrium. This, in turn, helps to further understand the role of natural and artificial selection affecting different MH loci in these ecologically and commercially important species.

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Figure 1. Linkage disequilibrium (LD) patterns among newly developed microsatellite loci in LG15 measured as *D'* in four populations of Atlantic salmon: (a) Kammenaja (b) Narva (c) Ponoi (d) Pulonga; and brown trout: (e) Mustoja upstream (f) Mustoja downstream (g) Pada upstream (h) Pada downstream. The genes in LG15 are denoted as white boxes whereas the locations of polymorphic microsatellite loci are marked as black bars. Box with cross mark indicates monomorphic marker in the particular population (Kammenaja).

Table 1. Characteristics of 22 polymorphic MH-associated microsatellite loci in *Salmo salar* and *Salmo trutta*.

Locus	BAC clone	Primer Positions*		Relative Location (distance from the MH gene)	Motif	*Salmo salar*					*Salmo trutta*					primer sequence 5'-3'	
	Genbank accession number	Start	End	(bp)		Size range (bp)	Ho	He	*A*	*Ar*	Size range (bp)	Ho	He	*A*	*Ar*	Forward	Reverse
LG15_1	EF427381	12,197	12,379	UBA (-)205351	(TG)18	212-236	0.67	0.78	11	6.86	201-213	0.57	0.79	9	7.19	TCTGTACCGTGTCAGGCATC	TACAGCCTGCTCCTCCAGTT
LG15_3	EF427381	53,090	53,493	UBA (-)164457	(GA)26	_	_	_	_	_	403-415	0.47	0.61	4	3.86	CCACTGTGAGATGCTGCTGT	CATTCTCTCCTCGGTTCTGC
LG15_7	EF427381	202,218	202,611	UBA (-) 15329	(AC)29(N)11(CT)12	395-404	0.39	0.68	10	7.00	_	_	_	_	_	TATTGCGATTCGATGTTCCA	GTGTCGGCCATCTTCTGATT
LG15_11	EF210363	227,845	228,313	UBA (+) 1029	(ATG)6	497-552	0.25	0.66	5	3.37	_	_	_	_	_	GGGACGGTAGAGAGAAGCAG	GCTGTTCGACTGACACAGGA
LG15_13	EF210363	316,687	316,940	UBA (+) 99141	(AT)10T(AT)4	278-285	0.27	0.45	5	3.16	285-319	0.82	0.88	19	11.28	CAATGCAAACTACCGCTCAA	TCACCATGATTCCGTTTCAA
LG15_14	EF210363	347,258	347,693	UBA (+) 129712	(CAGA)32	445-588	0.39	0.95	32	16.32	_	_	_	_	_	GCTCCGTTACACTGGGGTTA	TCCGCTCACAACAACACATT
LG15_15	EF210363	379,513	379,762	UBA (+) 161967	(CT)22	284-302	0.43	0.49	5	3.66	_	_	_	_	_	GAAAAACAGAGAGCGGGACA	CCTCCAGTCTGAGCTGAACA
LG15_17	EF210363	412,879	413,056	UBA (+) 195333	(AT)15	196-238	0.72	0.87	17	10.35	_	_	_	_	_	TGACAACAAGGTGGGGTTTT	GGAAAACACAGGGGTTAGCA
LG15_18	EF210363	453,489	453,823	UBA (+) 235943	(GA)12	372-374	0.41	0.44	3	2.15	376-378	0.54	0.61	6	4.92	CGCAGCCACTTATTCCATTT	CTGCTCTTGCATCCTTCTCC
LG15_20	GQ505858	492,458	492,912	UBA (+) 274912	(CTTT)16	462-568	0.43	0.91	22	13.24	427-432	0.55	0.51	4	2.41	CTAAGACTGTGCCCGTTTCC	GGAACGTGTTTTCGGTCAGT
LG15_24	GQ505858	630,405	630,853	UBA (+) 412859	(AG)3AA(AG)2GG(AG)2G(AG)3	_	_	_	_	_	480-486	0.14	0.13	2	1.95	GAAAACAGATGCACCCACAA	CAAAGCTCCCTCTCACTGCT
LG3_1_4	GQ505859	11,334	11,463	UGA (+) 87434	(AC)14	163-173	0.53	0.64	4	3.84	169-190	0.82	0.83	10	7.73	CAATCTCAGAGGGCACTTCA	AGCCCATTCTTGGTCATTGT
LG3_1_6	GQ505860	85,173	85,514	UGA (+) 152273	(TAA)10	364-382	0.40	0.62	5	3.87	384-398	0.71	0.72	5	4.81	GGACCATCAGGTGAGGAAGA	CAAGACAGTGAGGCGACAAA
LG3_1_9	GQ505860	247,559	247,664	UGA(+) 314659	(TG)12	_	_	_	_	_	153-173	0.34	0.36	4	3.77	TGTGGTGGAGAAACCATTCA	GATTCAGCACACACCCCTTC
LG3_2_7	FJ969490	160,575	160,773	UCA (+) 10138	(CA)16	221-241	0.55	0.80	12	7.05	216-221	0.08	0.50	2	2	ATGACCACAAAGGCCAGAAC	AGTAGGAGACGCCAGCATGT
LG3_2_9	FJ969490	209,525	210,017	UCA (+) 1050334	(CTGT)13	515-531	0.48	0.57	11	5.57	506-518	0.78	0.71	5	4.48	GATGCACGCATAATCCTGAA	AGCTGTGGGAATGAAAATGG
LG10_2	FJ969488	20,335	20,630	SAA (-) 39935	(GA)24	_	_	_	_	_	303-307	0.54	0.57	3	2.96	CTTGGGGAACTCTGGGTACA	TCATTTTGTGGGAAGTGTGC
LG10_6	FJ969488	50,571	50,672	SAA (+) 10932	(TG)12	_	_	_	_	_	136-184	0.70	0.65	13	7.70	GGGGTATAGAGGTGGGCAGT	GGGGACAGGAGAAGAGAGGT

LG14_1	FJ969489	24,889	25,138	UHA1 (-) 72423	(CATT)7	285-301	0.60	0.76	6	4.40	281-285	0.50	0.59	5	4.36	GCCAGGCTTGTCTACAAAGG	GGTGGTGAAGCTGAAGGGTA
LG5_II_1	EU008541	7,997	8,531	DBA (-) 10379	(CA)18	557-582	0.62	0.85	17	9.45	_	_	_	_	_	GGAGGAAGGGGAGTGTGTTT	ACCTGCGTCATTCATGTTCA
LG5_II_2	EU008541	25,102	25,640	DBA (-) 86688	(CGA)15	568-582	0.57	0.70	8	5.20	_	_	_	_	_	CGTTGTCATGTTGGAAGTGG	TCAGGATCGAGGGAAAAATG
LG5_II_6	EU008541	147,593	147,724	DBA (+) 35804	(AG)13AA(AG)7	156-234	0.54	0.71	8	5.14	140-148	0.31	0.33	6	3.14	GCATACCGGGGAAGTGACTA	CCACCAGCCTCCTCTGTTT

*Start of the forward primer and the reverse primer from the BAC sequence; Relative location: distance of the microsatellite from the MH gene; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *A*, total number of alleles; *Ar*, allelic richness; _ , lack of amplification.

Table 2. Significance values from the Hardy Weinberg exact test and estimated null allele frequencies.

	Salmo salar								*Salmo trutta*							
	Kamennaja		Narva		Ponoi		Pulonga		Mustoja Upper		Mustoja Lower		Pada Upper		Pada Lower	
Locus	P-value	Null allele freq.*	P-value	Null allele freq.*	P-value	Null allele freq.*	P-value	Null allele freq.*	P-value	Null allele freq.*	P-value	Null allele freq.*	P-value	Null allele freq.*	P-value	Null allele freq.*
LG15_1	ns		ns		ns		ns		0.0001	0.220	0.012	0.128	ns		0.0001	0.143
LG15_3	_		_		_		_		ns		0.003	0.219	ns		ns	
LG15_7	ns		0.0001	0.230	0.0001	0.153	0.001	0.195	_		_		_		_	
LG15_11	ns		0.047		0.0001	0.387	0.017		_		_		_		_	
LG15_13	ns		ns		0.032		0.01	0.176	0.044		ns		ns		0.036	
LG15_14	0.0001	0.306	0.0001	0.247	0.0001	0.377	0.0001	0.175	_		_		_		_	
LG15_15	ns		ns		ns		ns		_		_		_		_	
LG15_17	ns		ns		ns		ns		_		_		_		_	
LG15_18	na		ns		ns		ns		ns		ns		ns		0.041	
LG15_20	ns		0.0001	0.295	0.0001	0.295	0.003	0.086	ns		0.011		ns		ns	
LG15_24	_		_		_		_		ns		ns		ns		ns	
LG3_1_4	ns		ns		ns		ns		0.017		ns		ns		ns	
LG3_1_6	ns		ns		ns		ns		0.041		0.025		ns		ns	
LG3_1_9	_		_		_		_		ns		ns		ns		ns	
LG3_2_7	0.0001	0.138	ns		ns		ns		0.0001	0.341	0.023	0.352	0.026	0.198	0.0001	0.397
LG3_2_9	ns		ns		ns		ns		ns		ns		ns		ns	
LG10_2	_		_		_		_		ns		0.0219		ns		ns	
LG10_6	_		_		_		_		ns		ns		ns		ns	
LG14_1	0.003		0.033		ns		ns		ns		ns		ns		ns	
LG5_II_1	ns		ns		0.0001	0.197	0.0001		_		_		_		_	
LG5_II_2	ns		0.022		0.017	0.160	ns		_		_		_		_	
LG5_II_6	ns		ns		ns		ns		ns		ns		ns		ns	

ns, non significant p value; _, lack of amplification; m, monomorphic; *Null allele frequency estimation based on method of van Oosterhout et al. (2006).

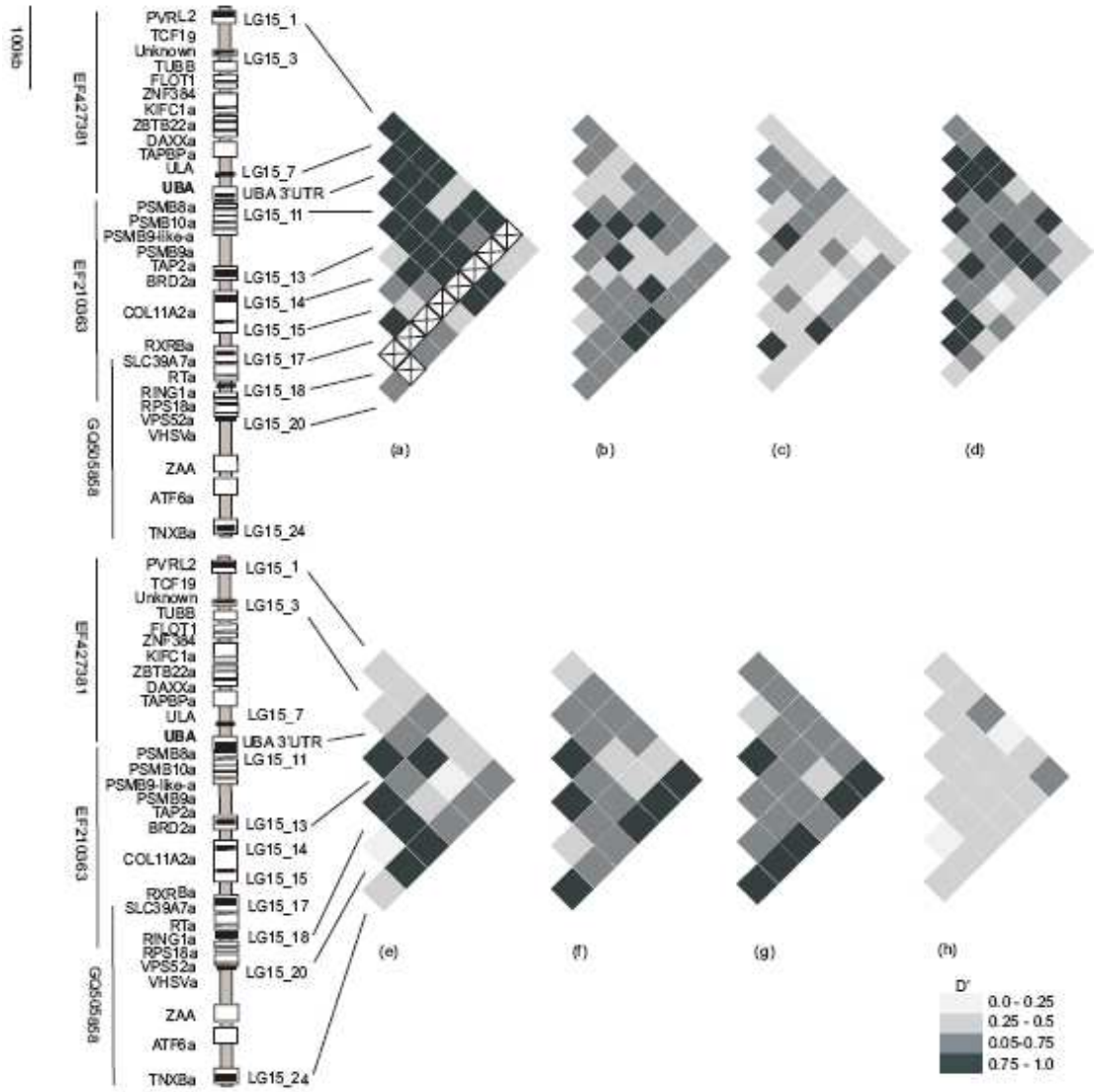
100kb
EF427381
EF210063
GQ505858
PVRL2
TCF19
Unknown
TUBB
FLOT1
ZNF384
KIFC1a
ZBTB22a
DAXXa
TAPBPa
ULA
UBA
PSMB8a
PSMB10a
PSMB9-like-a
PSMB9a
TAP2a
BRD2a
COL11A2a
RXRBa
SLC39A7a
RTa
RING1a
RPS18a
VPS52a
VHSVa
ZAA
ATF6a
TNXBa
LG15_1
LG15_3
LG15_7
UBA 3'UTR
LG15_11
LG15_13
LG15_14
LG15_15
LG15_17
LG15_18
LG15_20
LG15_24
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
D'
0.0 - 0.25
0.25 - 0.5
0.05-0.75
0.75 - 1.0

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Table S1. Information on 24 microsatellite markers that did not result in successful amplification in *Salmo salar* and *Salmo trutta* .

	BAC clone	Primer Position*		Relative Location (distance from the gene)		*S. salar*			*S. trutta*			Primer Sequence 5'-3'	
Locus	Genbank acc. No.	Start	End	(bp)	Motif	Quality	Size range (bp)	*A*	Quality	Size range (bp)	*A*	Forward	Reverse
LG15_5	EF42738	143469	143950	UBA (-) 74080	(CA)21	Unspecific	_	_	Unspecific	_	_	GGTTAGAGCGCAACTTACGG	GAATTTACGAACGCCCTGAG
LG15_10	EF210363	225716	226082	UBA(+) 8168	(GT)13	Unspecific	_	_	Unspecific	_	_	CAATGCCTGCAGAACACATC	GCATCGCTTATTGTTGCTGA
LG15_12	EF210363	238985	239170	UBA (+) 21436	(CA)6N3(CA)3TA(CA)2	Unspecific	_	_	Unspecific	_	_	CCTGGACAGCAAGACACAAA	GGGTGAGAGTGAGGAGTGGA
LG15_22	GQ505858	577303	577801	UBA (+)359753	(AT)21	Na	_	_	Na		_	AGCCAAGACAACGCAGAGAT	AGGCCTACAGTGCATTTGGA
LG3_1_1	GQ505859	19910	20229	UGA (-) 15006	(GA)5CA(GA)2GC(GA)3 GC (GA)7	Clear	354	1	Multiple	386-389	4	TGAAGCGAAATCACACAAGC	TTCTCTGCTCCTGCATCCTT
LG3_1_2	GQ505859	37403	37944	UGA (+) 2488	(AAC)7	Multiple	_	_	Too long	573-605	5	TCCTGCATTCCTACTGCTGA	TCCAGACCAACAGGGGTAAG
LG3_1_3	GQ505859	39067	39234	UGA (+) 14258	(CA)38	Unspecific	_	_	Unspecific	_	_	GACTCTAACCCCACACCCACT	GCAGCAGCGTGTGTAAAGAG
LG3_1_5	GQ505860	155459	155875	UGA (+)130648	(CA)34	Stutter	_	_	Unspecific	_	_	AATGCCAGCCGACTTCATAC	AAGCTGTTCTGGCTTGTGGT
LG3_1_7	GQ505860	243456	243552	UGA (+) 218643	(GA)11	Stutter	_	_	Stutter	_	_	CGAAGACCAACGAAACAACC	TCTGTTGGGGAGTTTTGTCC
LG3_1_8	GQ505860	268945	269163	UGA(+) 244130	(CA)22	Unspecific	_	_	Unspecific	_	_	TTTTCATTTATTGGTATTGGAACC	GTGCTACTGAGGCTTGCACA
LG3_2_1	FJ969490	10628	10962	UCA (-) 48566	(GAAA)2(GA)2(GAAA)2(GA)2(GAAA)2(GA)1(GA AA)1(GA)5	Multiple	_	_	Multiple	_	_	TAGGGCTCAGGTCTTCCAAA	TCAAGGCCATCAGACTGCTA
LG3_2_2	FJ969490	14682	15009	UCA (-) 44511	(AT)7A(AT)11GT(AT)6	Unspecific	_	_	Unspecific	_	_	TTTCGGTTACTAGGCCAACG	AACGAATGGTCGCCTAAATG
LG3_2_4	FJ969490	84416	84644	UCA (+) 25224	(GA)15	Multiple		_	Multiple		_	TTGTACCTGGGCGATACTTG	GAAACAGGGGGTGGACTACA
LG3_2_6	FJ969490	123541	123752	UCA (+)64348	(GT)17	Multiple	_	_	Multiple	_	_	TGGTTTGGAATGCTGCTACA	GCTCTGCAACCACATCTTCA
LG10_1	FJ969488	9372	9771	SAA (-) 50900	(GAGT)16	Unspecific	_	_	Unspecific	_	_	CCAGGAAATTGCTTTCACCT	GCTCTGGACAAAGGCATGAT
LG10_3	FJ969488	29188	29630	SAA (-) 31084	(TG)33	Unspecific	_	_	Unspecific	_	_	TGCATTCAACCCATTAACCA	GACCACCCGTCTATGTGTCC
LG10_4	FJ969488	52087	52201	SAA (-) 8184	(CA)11	Unspecific	_	_	Unspecific	_	_	TCTGGAGACACTGAGCATGG	CCCCATTCATTTTGATCCTG
LG10_8	FJ969488	112850	113030	SAA (+) 52580	(TA)10	Clear	220	1	Na	_	_	TCACAGCAAAAACTGGCAAA	CGCCTAAATGGAAAATGGAA
LG14_2	FJ969489	66875	67253	UHA1 (-) 30438	(CT)12	Clear	421	1	Stutter	538-674	_	AGCCAAGACAACGCAGAGAT	AGGCCTACAGTGCATTTGGA
LG14_4	FJ969489	10391	10557	UHA (+) 6604	(AT)5T(AT)11	Unspecific	_	_	Unspecific		_	ACGCTCCACATTTGTTTTCC	TTTCGAGGTTATTGCCCAAA
LG14_5	FJ969489	127530	127737	UHA1 (+) 30219	(TA)7(CA)6N5(TA)10	Unspecific	_	_	Unspecific	_	_	TGTGTGATTTTGTTGTCAGCAC	TTTTTGGTTGCAGCATGATT
LG14_7	FJ969489	151146	151586	UHA (+) 53835	(GT)29	Multiple	_	_	Multiple	_	_	AACAAAGGGCAGGAGACAGA	GGGCTGTGTGATGTTGTGAC
LG14_8	FJ969489	178029	178440	UHA (+) 80718	(TAA)8	Unspecific	_	_	Unspecific	_	_	CAGCCACTGCTACGACTTTG	GGGTACACCAGGGACACATC
LG5_II_3	EU008541	66984	67175	DBA (-) 44807	(CA)12	Unspecific	_	_	Unspecific	_	_	CTCAGGCACCAGTCCAGAAT	CCCATCATGTCAAGGGTCAT
LG5_II_4	EU008541	86589	87034	DBA (-) 25202	(CT)10	Clear	482	1	Clear	463	1	TTTCGCTCTATCACCAAGCA	TAGCACCTCAGGGGAAACAG
LG5_II_8	EU008541	191740	192269	DBA (+) 79950	(GT)10	Clear	561	1	Clear	561	1	TGTATGGCAGCAGGAAGATG	AAAGGCCGCACACCTTAATA

*Start of the forward primer and the reverse primer from the BAC sequence; Relative location: distance of the microsatellite from the MH gene; A, total number of alleles; Multiple, amplification of multiple bands; Inconsistent, inconsistent amplification; Clear, amplification of single clear band; Stutter, amplification of multiple stuttering bands; Na, lack of amplification

Table S2. Information about the PCR and multiplexing.

Atlantic salmon

Primer conc. Is 10 pmol/ul
Fluorescence conc. is 50 pmol/ul

Single PCR	SasaUBA		
		Reaction	
		1X	98X
	forw	0.20	19.60
	rev	0.20	19.60
	DNA	1.00	1.00
	QMP	2X	3.00
	H2O	1.70	166.60
	Total vol.	6.10	

Single PCR	(LG3_1_6, LG15_4, LG5_II_6, LG5_II_1)	
	Reaction	
	1X ul	98X
forw	0.06	5.88
rev	0.12	11.76
Fluorescence	0.05	4.70
DNA	1.00	1.00
QMP	3.00	294.00
H2O	1.87	183.40
Total vol.		6.10

Multiplex 1			
		Reaction	
		1X	98X
LG15_1	forw	0.06	5.88
	rev	0.12	11.76
LG15_15	forw	0.06	5.88
	rev	0.12	11.76
LG15_18	forw	0.06	5.88
	rev	0.12	11.76
LG15_11	forw	0.06	5.88
	rev	0.12	11.76
LG5_II_2	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescer	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	0.86	84.28
	Total vol.	6.10	

Multiplex 2			
		Reaction	
		1X	98X
LG15_13	forw	0.06	5.88
	rev	0.12	11.76
LG15_7	forw	0.06	5.88
	rev	0.12	11.76
LG15_20	forw	0.06	5.88
	rev	0.12	11.76
LG3_1_2	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescence.	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	1.04	102.00
	Total vol.	6.10	

Multiplex 3		Reaction	
		1X	98X
LG10_6	forw	0.06	5.88
	rev	0.12	11.76
LG3_1_4	forw	0.06	5.88
	rev	0.12	11.76
LG3_2_7	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescer	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	1.22	119.95
	Total vol.	6.10	

Multiplex 4		Reaction	
		1X	98X
LG14_1	forw	0.06	5.88
	rev	0.12	11.76
LG15_3	forw	0.06	5.88
	rev	0.12	11.76
LG3_2_9	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescence.	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	1.22	119.95
	Total vol.	6.10	

Brown trout

Primer conc. Is 10 pmol/ul
Fluorescence conc. is 50 pmol/ul

Single PCR	SasaUBA	Reaction	
		1X	98X
	forw	0.20	19.60
	rev	0.20	19.60
	DNA	1.00	1.00
	QMP	2X	3.00
	H2O	1.70	166.60
	Total vol.	6.10	

Single PCR	(LG15_12, LG15_13, LG15_20, LG3_1_6, LG3_2_7)	Reaction	
		1X ul	98X
forw		0.06	5.88
rev		0.12	11.76
Fluorescence		0.05	4.70
DNA		1.00	1.00
QMP		3.00	294.00
H2O		1.87	183.40
Total vol.			6.10

Multiplex 1

		Reaction	
		1X	98X
LG10_6	forw	0.06	5.88
	rev	0.12	11.76
LG10_2	forw	0.06	5.88
	rev	0.12	11.76
LG15_18	forw	0.06	5.88
	rev	0.12	11.76
LG15_3	forw	0.06	5.88
	rev	0.12	11.76
LG3_2_9	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescer	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	0.86	84.28
	Total vol.	6.10	

Multiplex 2

		Reaction	
		1X	98X
LG3_1_9	forw	0.06	5.88
	rev	0.12	11.76
LG15_1	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescence	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	1.40	137.20
	Total vol.	6.10	

Multiplex 3

		Reaction	
		1X	98X
LG5_II_6	forw	0.06	5.88
	rev	0.12	11.76
LG3_1_4	forw	0.06	5.88
	rev	0.12	11.76
LG14_1	forw	0.06	5.88
	rev	0.12	11.76
LG15_24	forw	0.06	5.88
	rev	0.12	11.76
	Fluorescer	0.34	33.32
	QMP	3.00	294.00
	DNA	1.00	1.00
	H2O	1.04	102.00
	Total vol.	6.10	