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Identification and characterization of microsatellites for striped bass from repeat-enriched libraries

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Abstract

Striped bass (*Morone saxatilis*) is economically important in the US due to its value as an aquaculture species and in supporting commercial and recreational fisheries, especially those off the Atlantic coast and in the Gulf of Mexico. Modern strategies for managing fishery populations and aquaculture broodstocks employ the use of molecular genetic markers to identify individuals, assign parentage, and characterize population genetic structure and levels of inbreeding and migration. As part of a collaborative effort to utilize molecular genetic technologies in striped bass breeding programs we generated microsatellite markers for use in population genetic studies, broodstock selection and management strategies, and the construction of a genetic map. We developed 345 new microsatellite markers for striped bass, a subset ($n=71$) of which was characterized by genotyping samples from two striped bass broodstock populations to evaluate marker polymorphism, percent heterozygosity, Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD) and utility for population genetic studies.

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Four repeat-enriched libraries were constructed by Genetic Identification Services (GIS; Chatsworth, CA, USA) to identify AAG, ATG, TACA,

and TAGA repeats in striped bass. The libraries were constructed from genomic DNA using protocols which enrich for fragments containing a motif of interest (Peacock et al. 2002). Single pass sequencing using the ABI Prism Big Dye Terminator cycle sequencing kit (ABI, Foster City, CA, USA) with a universal M13 forward primer was conducted on 408 clones from each of the four libraries. Sequences were trimmed for quality and vector sequences removed using Phred and Crossmatch (Ewing et al. 1998). All sequences in the data set and all other *Morone saxatilis* microsatellites from GenBank were analyzed for redundancy using Sequencher (Gene Codes Corporation, Ann Arbor, Michigan). Novel unique sequences were candidates for primer design using Oligo 6.0 (Rychlik and Rhoads 1989). An M13 tail was added to each forward primer for genotyping (Boutin-Ganache et al. 2001). Primer pairs were optimized for PCR by varying annealing temperatures and $MgCl_2$ concentrations. Optimizations were conducted in a 12 μl reaction volume containing 12.5 ng DNA, 1.5–2.5 mM $MgCl_2$, 1.0 μM of each primer, 200 μM of dNTPs, 1× manufacturer's reaction buffer, and 0.5 Units *Taq* DNA polymerase. Thermal cycling parameters consisted of an initial denaturation at 95 °C for 15 min followed by 30 cycles of 95 °C for 1 min, annealing temperature for 45 s, 72 °C extension for 45 s and a final extension at 72 °C for 10 min. PCR products were visualized on agarose gels after staining with ethidium bromide. Primers were designed for 398 microsatellites, 345 of which were successfully optimized for PCR (see Appendix). Marker development efficiency was 16.9% for AAG, 18.6% for ATG, 12.5% for TACA, and 22.5% for TAGA. Specific marker development efficiency (markers containing the enriched-repeat) was 8% for AAG, 15.9% for ATG, 7% for TACA, and 20.3% for TAGA (see Table 1).

A subset of 71 microsatellites was randomly chosen for further characterization by genotyping striped bass from University of Maryland broodstock (UMD, $n = 14$) and from North Carolina State University broodstock (NCSU, $n = 10$). DNA was isolated from fin clips using the phenol–chloroform method (Sambrook and Russell 2001). Optimized PCR reaction conditions for each marker were used with the addition of 0.1 μM of fluorescently labeled (FAM™, HEX™, or NED™) M13 primer. Samples were electrophoresed on an ABI Prism® 3730 DNA Analyzer and output files were analyzed using GeneMapper® software version 3.7.

Of the 71 microsatellite markers genotyped, 13 were monomorphic in both broodstock populations and an additional five were monomorphic in the NCSU population. Five markers consistently produced more than two fragments, suggesting the presence of PCR artifacts or duplication of the locus within the genome (MSM1373, MSM1374, MSM1382, MSM1402, and MSM1447). Of these, amplification of MSM1402 produced two sets of fragments separated by ≥125 base pairs in all individuals and therefore was treated as two separate loci for population analyses (MSM1402a and MSM1402b). Locus name, GenBank accession

Table 1. Discovery of microsatellites from repeat-enriched libraries

Library	# Clones sequenced	# Markers developed	# Matched[a]	# Perfect repeats[b]	Unmatched[c]			
					Di	Tri	Tetra	Other
AAG	408	69	33	6	26	7	2	1
ATG	408	76	65	18	8	1	2	
TACA	408	51	29	11	21		1	
TAGA	408	92	83	50	6	2	1	
GIS[d]		57		12	12	16	29	
Totals	1632	345	210	97	73	26	35	1

[a]Number of markers whose repeat matched the enriched repeat for that library.
[b]Number of markers whose repeat matched the enriched repeat and are perfect.
[c]Number of markers whose repeat did not match the enriched repeat. (Di, Tri and Tetra designations represent di-, tri- or tetranucleotide repeat types).
[d]Number of markers identified from two or more of the GIS libraries.

Table 2. Summary data for 51 microsatellite loci for striped bass

Locus *GenBank*	Repeat	Forward primer, Reverse primer	T_A[e]	$MgCl_2$	Size range	# Alleles	% Het	*P*-value[c]
MSM1334[a]	$(ATAG)_{24}$	F-aaaagttctgcacatggatcg	62	2.0	301–365	10	69	0.00025[d]
BV678387		R-gtccttgccctccagttacag						
MSM1335[a]	$(CAT)_7(CAC)_7$	F-gcctgtttgccctcatatagc	58	2.0	238–248	4	52	0.00221[d]
BV678388		R-gatgtttccagccttttacgc						
MSM1355[a]	$(CA)_{20}$	F-atcctgactgcatccactagccta	62	2.0	304–353	10	91	0.57967
BV678319		R-tgtgtggcccaatactagactgac						
MSM1357[a]	$(GATA)_{21}$	F-gtacctgacacgcataatg	58	2.0	242–282	11	91	0.00147[d]
BV678321		R-ccaagcaaaccgtttagtg						
MSM1359	$(ATCT)_{17}$	F-accatgcaccttaggacacta	62	2.0	361–381	6	81	0.04037[d]
BV678323		R-ttcatgcccatttagacacca						
MSM1362[a]	$(ATCT)_{23}$	F-atccactttgctgctgttaca	62	2.0	343–396	11	75	0.06761
BV678325		R-gcagcacgtacgcagtacaca						
MSM1375[a]	$(CT)_{14}$	F-gtgtcggatgatgactctt	58	2.0	206–267	7	69	0.33174
BV678410		R-ccactacacccctcacgtt						
MSM1394	$(GAA)_{12}$	F-cagtgtaggcaatcggagtt	58	2.0	188–206	4	36	0.03061[d]
BV678429		R-tctccctctttgcatgtcca						
MSM1402a	$(CTT)_{12}$	F-cgcagctagctaacgagtgtatc	62	2.0	194–200	10.00	69	0.00256[d]
BV678437		R-ggtcgttcaaggaaaaaggttgg						
MSM1402b[a]					325–373	3.00	61	0.38964
MSM1424[a]	$(AAG)_{13}$[b]	F-tagtcagccaagggagacgag	62	2.0	151–157	2	16	0.03442[d]
BV678456		R-ctccgcttcctcacgcatcat						
MSM1427[a]	$(GGT)_8(TCT)_{14}$[b]	F-cttacctggttcattgtc	62	2.0	341–381	7	58	0.00009[d]
BV678459		R-cagaaaggaggcgataac						
MSM1429[a]	$(GAA)_{40}$[b]	F-ctacaactttgttggacga	62	2.0	247–359	17	82	0.00095[d]
BV678461		R-gcagtgggaagtggacttt						
MSM1437[a]	$(CTT)_8$[b]	F-tcgacagatctgcgctcta	62	2.0	180–196	4	29	0[d]
BV678469		R-gtgaaggtgcggttggaca						
MSM1442[a]	$(GGAT)_8$[b]	F-gagaatgaaaggagggtggatta	62	2.0	320–336	2	41	0.01658[d]
BV678474		R-ctttctcctgcctgccagttgta						
MSM1443[a]	$(TA)_8(TG)_{13}$	F-ccagagtgaagaccaggtagt	62	2.0	288–303	4	21	0[d]
BV678475		R-agagggaagaagtggaacgtg						
MSM1445[a]	$(GAT)_9$	F-gagagagctggatgcagaatgat	58	2.0	136–140	3	33	0[d]
BV678477		R-ctgacgcgacagcttgtgtatga						
MSM1451	$(GAT)_8(GT)_{21}$[b]	F-gattagccatgtgcttag	58	2.0	182–184	2	100	0.00002[d]
BV678483		R-cctctcctttacgtggtg						
MSM1453[a]	$(GAT)_{12}$[b]	F-gcttgttctgtgccctatctc	58	2.0	183–202	5	75	0.00044[d]
BV678484		R-gcaaagggacggcaaatttag						
MSM1491[a]	$(TGA)_9$	F-gcatgcggatgaataactgtc	58	2.0	196–208	5	66	0.00039[d]
BV678519		R-ttcgagggcatctgaaccttg						
MSM1501[a]	$(CAT)_{11}$	F-gtcagagggaataataacatgacc	62	2.0	179–182	2	25	0.00003[d]
BV678529		R-agttcagttcgagagtaatgcacg						
MSM1520[a]	$(CA)_{31}$	F-tctgcgtctcccatactgt	62	2.0	172–222	15	100	0.00943[d]
BV678547		R-ttcaagcgttccggttctc						
MSM1524[a]	$(TACA)_{13}$	F-agcaccttcatggtacccaag	62	2.0	337–345	3	75	0.92657
BV678550		R-cgaacgcaaccgcagtaatgt						
MSM1526[a]	$(GT)_{26}$	F-gactctgttgcatttaggt	58	2.0	159–176	8	91	0.00039[d]
BV678552		R-ggctgtattccatttaggc						
MSM1536[a]	$(GT)_{29}$	F-ccatatgagccaggtaaagta	62	2.0	254–296	10	91	0.00767[d]
BV678561		R-gaaacacaaacacgcacatct						
MSM1547[a]	$(TATG)_{14}$	F-atgcaagcttaaccctgtt	58	2.0	280–353	15	78	0[d]
BV678569		R-ccctctgctggctgacata						
MSM1555[a]	$(TATC)_{16}$	F-cctgtttacctgctagtccac	62	2.0	323–360	6	29	0.4197
BV678575		R-agtgaagttagcccggtctga						

Table 2. (Continued)

Locus *GenBank*	Repeat	Forward primer, Reverse primer	T_A[e]	$MgCl_2$	Size range	# Alleles	% Het	*P*-value[c]
MSM1556[a]	$(AGAT)_{26}$	F-taccagaagaacaccctagac	62	2.0	211–293	18	73	0[d]
BV678576		R-tatgtcctgctactgcgattc						
MSM1557[a]	$(AGAT)_{11}$	F-gtggatcagtgagcgactatgtat	62	2.0	356–404	10	75	0.03645[d]
BV678577		R-tgcactgcttactgtagcttccgt						
MSM1558[a]	$(TAGA)_{22}$	F-gggagttctttggtcaagg	62	2.0	325–373	12	75	0.00488[d]
BV678578		R-tttctcgatcaccgccact						
MSM1559[a]	$(AGAT)_{27}$	F-agctgacctcctgtgctatct	62	2.0	320–376	11	91	0.02089[d]
BV678579		R-agaagcgagagcgtcaagtga						
MSM1560[a]	$(AGAT)_{19}$	F-ttagagggctaatgagtacag	62	2.0	333–364	8	73	0.44623
BV678580		R-cacagacccaaactgatactg						
MSM1577[a]	$(CTAT)_{18}$	F-gaaagagggcatcataatacagca	52	2.5	335–380	9	83	0.00225[d]
BV678596		R-caaaacaaagagagcgcggtctaa						
MSM1578[a]	$(AGAT)_{12}$	F-gggcaagttgacaggtagaca	62	2.0	263–289	8	7	0.00115[d]
BV678597		R-gttagcctcattcgccccaat						
MSM1579[a]	$(ATCT)_{11}$	F-atacagtgtcacccggtactat	62	2.0	269–286	5	87	0.01464[d]
BV678598		R-ggacagttatccctgcactatg						
MSM1584	$(ATCT)_{19}$	F-ctctaaggctaatggggttac	62	2.0	251–303	11	95	0.02886[d]
BV678601		R-ctgttctgcgttttagttgga						
MSM1587	$(ATCT)_{19}$	F-gaggggtcaagacatttaaccat	52	2.5	300–353	9	1	0.00451[d]
BV678604		R-tgtgtcgatgtccttgtcctacg						
MSM1589	$(TAGA)_{22}$	F-tgaataccttcctgtagcct	52	2.5	236–309	10	73	0[d]
BV678606		R-catccatttgcaccgtttac						
MSM1591[a]	$(TAGA)_{28}$	F-gatggatggctttcctaccta	62	2.0	293–361	11	78	0.00091[d]
BV678608		R-agccacacgattatgaccacc						
MSM1592	$(TAGA)_{33}$	F-cggcactggataaagttac	62	2.0	172–237	12	81	0[d]
BV678609		R-tacaatttccctcgggatg						
MSM1598	$(AGAT)_{20}$	F-gacaaatcctctgatgaac	52	2.5	356–388	9	93	0.07568
BV678613		R-ctcagcgtctgttaccgta						
MSM1602	$(CTAT)_{23}$	F-gggaatgtgctaaacttggaatag	52	2.5	214–267	11	72	0[d]
BV678616		R-tgggattactgggcagatagttga						
MSM1604	$(ATCT)_{32}$	F-attagtctgtggataccgctgga	62	2.0	188–246	10	94	0[d]
BV678618		R-acagggctgcagtggaaggtaag						
MSM1617	$(ATCT)_{21}$	F-agagtgggggaagaagtcgttt	62	2.0	361–403	14	9	0[d]
BV678627		R-gctgaactctggtccaaggcaa						
MSM1625	$(ATCT)_{20}$	F-aagcttccatatagtgcaccc	62	2.0	197–258	10	66	0.00029[d]
BV678633		R-tgtccgagtttgcctgatctc						
MSM1626[a]	$(TAGA)_{34}$	F-cgcaactacgtttcgttaccata	62	2.0	280–353	15	78	0[d]
BV678634		R-cagaagccaggcagactgcatac						
MSM1628[a]	$(TAGA)_{16}$	F-aatcccacatggagttgtag	62	2.0	172–221	11	83	0[d]
BV678636		R-ccagaccaataaaacgtccc						
MSM1634	$(CTAT)_{12}$	F-gagtgtaatgctgctgctacaact	62	2.0	168–205	8	65	0[d]
BV678641		R-tgatttctaacaacagccaacgtg						
MSM1638	$(ATAG)_{28}$	F-acagtgacacacgcttatatgc	62	2.0	278–327	10	8	0.00012[d]
BV678644		R-cgttgcccatttcctcttacat						
MSM1645[a]	$(CTAT)_{16}$	F-caatgcaccactcttatac	62	2.0	226–259	8	7	0[d]
BV678651		R-ggaacacagccatcattag						

[a]Marker was used for F_{ST} determination.
[b]Imperfect repeat.
[c]Exact *P*-value using a Markov chain method (50 batches with 1000 iterations per batch).
[d]Loci are statistically significant for differentiating two populations.
[e]T_A is the PCR annealing temperature in degree Celsius.

number, PCR annealing temperatures and $MgCl_2$ concentrations, observed allele size ranges, number of alleles and percent heterozygosity are reported in Table 2.

Arlequin version 2.000 software (Schneider et al. 2000) was used to estimate Wright's *F*-statistics (F_{ST}) and to evaluate HWE and LD for the marker loci for each population. Thirty-five loci (missing genotype data <0.05) were used to estimate genetic distance, F_{ST}, between the UMD and NCSU broodstock. F_{ST} was 0.18233 for these broodstock groups with an exact *P*-value = 0.000001 (100,000 simulations), suggesting a high level of genetic differentiation between these two broodstock populations. Significant departures from HWE were identified for one marker in only the UMD group (MSM1335), one marker in both broodstock populations (MSM1402a), and nine markers only in the NCSU population (MSM1362, MSM1375, MSM1536, MSM1559, MSM1579, MSM1584, MSM1598, MSM1604, and MSM1617). Tests for pairwise LD revealed 92 pairs (7%) of loci for NCSU and 17 pairs (1%) of loci for UMD with significant LD. However, only the loci pair MSM1603-MSM1612 was in LD in both populations.

GENEPOP version 3.4 (Raymond and Rousset 1995) Option 3 sub-option 2 was used to test for genetic differentiation to characterize the utility of each marker for distinguishing the two populations. Exact *P*-values for these tests using a Markov chain method are reported in Table 2. Allele frequencies were statistically different for 42 markers between the pair of populations. These markers likely will be very useful for applications in conservation and selective breeding of striped bass.

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Appendix

Appendix. PCR optimization information for 345 microsatellites for striped bass (*Morone saxatilis*)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1248	BV678310	$(TTC)_{16}$	Y	ctccacttttacctccacctc	ctcttcgtccactgatgactc	62	1.5
MSM1249	BV678311	$(GAA)_{14}$	Y	cctgaatatcagaagggattg	ctcccagtttacacctgagtaa	58	2.0
MSM1255	BV678312	$(GAT)_{11}$	N	caggaaatacaggaaaacagg	taaaggcgtcgtaatgagg	58	2.0
MSM1252	BV678313	$(CTT)_9$	N	cgcttttagatgaagtctgg	ggaggagaaggtgtgtgag	62	1.5
MSM1256	BV678314	$(TCA)_7$	N	acccacatctaccctcagagac	cctgctgtcagaaatagtccct	58	2.0
MSM1257	BV678315	$(CAT)_9$	N	gccgataacgggttcatag	ggtaggaggaggctgtgtg	62	1.5
MSM1258	BV678316	$(TGA)_{22}$	Y	acaaacttgtctcatcacattg	gccgcatgttacagtcat	58	2.0
MSM1254	BV678317	$(TTC)_{20}$	Y	caacattttgtatggctctga	agctccaagcactcatattaaa	58	2.0
MSM1259	BV678318	$(ATG)_{14}$	N	catccaaaccccagaagtg	aacgcaatcccaaacaaag	58	2.0
MSM1355	BV678319	$(CA)_{20}$	N	atcctgactgcatccactagccta	tgtgtggcccaatactagactgac	62	2.0
MSM1356	BV678320	$(TACA)_9(CA)_{16}$	Y	gccatagcacgctgata	ttgcgtgggtggtctgt	62	2.0
MSM1357	BV678321	$(GATA)_{21}$	N	gtacctgacacgcataatg	ccaagcaaaccgtttagtg	58	2.0
MSM1358	BV678322	$(TAGA)_{32}$	Y	cctacgccacatcaatcgata	tcaccacagcagctttgaccc	62	2.0
MSM1359	BV678323	$(ATCT)_{17}$	N	accatgcaccttaggacacta	ttcatgcccatttagacacca	62	2.0
MSM1360	BV678324	$(TCTA)_{36}$	Y	ctgctggaaactgtgaat	tggtgagtcacggcttct	62	2.0
MSM1362	BV678325	$(ATCT)_{23}$	N	atccactttgctgctgttaca	gcagcacgtacgcagtacaca	62	2.0
MSM1363	BV678326	$(TA)_8(CT)_{20}$	Y	gggatttgctaacatgctaac	taaagcaccatgcgtaacacc	54	2.0
MSM1364	BV678327	$(TAGA)_{25}$	Y	ggtgctttaagatgttcac	tataccacctgacgcagtg	54	2.0
MSM1260	BV678328	$(GAT)_{11}$	Y	aggcagaacaacagtcccat	tgaagaccgcaaagcattag	58	2.0
MSM1261	BV678329	$(TGA)_7$	N	ccacagtgtcaaaatgctaagg	ctggagaaagaagaacaccaca	58	2.0

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1262	BV678330	$(TCA)_9$	N	cgccctcaatctgctgta	tctgaagacccgcatctg	58	2.0
MSM1263	BV678331	$(TGA)_9$	N	tggtttccctggttactaataa	caatcaatcaacactgaatctg	58	2.0
MSM1264	BV678332	$(GAT)_{10}$	Y	ccgaccactgagcttattg	aatttccccagagtcacatc	56	1.5
MSM1265	BV678333	$(CAT)_{12}$	N	cagttggtacatcagcagtgt	gcacaattttagggtgtatttg	58	2.0
MSM1266	BV678334	$(TCA)_6$	N	cattaactccgactgtcagttg	gctttaagcagcgtgagg	58	2.0
MSM1269	BV678335	$(ATG)_{21}$	Y	tgcaagacagtgattcaga	ctgcaactcttattctttagga	58	2.0
MSM1273	BV678336	$(ATGT)_{18}$	N	ctgagaaacacatcaacacat	ccacataccaggtgaggt	58	2.0
MSM1270	BV678337	$(ATG)_{10}$	N	tactgctgctcaaccaactc	actcaggctgcgtgttatc	58	2.0
MSM1281	BV678338	$(AGAT)_{18}(ACAG)_6$	N	gttaggctgtccaatgattatc	caagagaaattgcagttgtca	58	2.0
MSM1271	BV678339	$(TCA)_9$	N	cagtgcaaaaacacaccac	gccaaaggtaagctgagc	58	2.0
MSM1282	BV678340	$(TAGA)_{19}$	N	ctttcctggactttctgattt	agcccattactgaaaaaagag	58	2.0
MSM1274	BV678341	$(TACA)_5$	N	tgcactaattcactccatagca	tgcgtgtgtatgtgtttctttc	58	2.0
MSM1283	BV678342	$(ATCT)_{13}$	N	ctcaacattctctcggagttac	gtgacccttttaacagttagcac	58	2.0
MSM1285	BV678343	$(GATA)_{28}$	N	ggactgactgactggtttgtt	ccttggggatgaataaagtatc	58	2.0
MSM1275	BV678344	$(CATA)_{12}$	N	tccatccgacccaactaac	ttgtctggttttccctctga	58	2.0
MSM1286	BV678345	$(GATA)_{12}$	N	tggaggagaagagtggaca	cccttggggatgaataaag	58	2.0
MSM1276	BV678346	$(ATAC)_{10}$	N	atccacgaacattcataggag	gttggcctaatatcttccctt	58	2.0
MSM1277	BV678347	$(ATAC)_{23}$	Y	tcattcaaagcaccacatatc	caatgtatcggtcgatcact	58	2.0
MSM1278	BV678348	$(CATA)_{14}$	Y	cttttctcttttcaggtgtcac	gcttcaaggtcagcaactt	58	2.0
MSM1279	BV678349	$(TATG)_{10}$	N	caaggctttctgtttggatta	cgtctgtcaacaaatcactga	58	2.0
MSM1287	BV678350	$(GATA)_{15}$	N	ttatggtcccactggtagttgt	catgctcgttatttggctattt	58	2.0
MSM1289	BV678351	$(TCTA)_{20}$	N	tttagactcgcagaggtgatg	ccccaacagtgtcagtgg	58	2.0
MSM1290	BV678352	$(TCTA)_7$	N	agttgtccctgtccaatgataa	ggcctgtttaagctctgtatga	58	2.0
MSM1291	BV678353	$(TCTA)_{12}(ATCC)_8$	N	cttggataggttcttttgctgt	acggtggtgtcatcttctaaat	58	2.0
MSM1292	BV678354	$(TAGA)_{23}$	N	agcaccacagaagttcagtg	acggaagacatccaggaa	58	2.0
MSM1293	BV678355	$(AGAT)_{13}$	Y	ggcttccttgtcctcaga	gctgctactggatgcttg	58	2.0
MSM1294	BV678356	$(CTAT)_{20}$	N	gcagcagccattgttattt	gcatcctgttccagttttg	56	1.5
MSM1296	BV678357	$(ATAG)_{14}$	N	atctgtgggtcatttgtgtta	aattccttagcacttcacga	56	1.5
MSM1297	BV678358	$(AGAC)_{12}(AGAT)_{24}$	N	cgtagataaaccccatcaact	tcctctgtctgttccaactatt	56	1.5
MSM1268	BV678359	$(TCA)_{12}$	Y	aactcctgctgagctgacag	ggatcaaacctgtggaaaca	56	1.5
MSM1299	BV678360	$(CAT)_{17}$	Y	tctccaccaccatgacgac	aagctaccggcaaatcgtg	58	2.0
MSM1302	BV678361	$(CTAT)_{27}$	N	gcacacttcacttacgagg	atgacagggtgctgataaa	65	2.0
MSM1303	BV678362	$(CTAT)_{19}$	Y	cacctgaggctgaatgtcaag	ccgaccacagactaaaccaga	65	2.0
MSM1305	BV678363	$(GAA)_{13}$	Y	tgaggatggaggggtagaagc	cctcttcaccataacgcagca	65	2.0
MSM1306	BV678364	$(GGAT)_{14}(AGAT)_{24}$	N	aggtcctggaagattgtgg	tgtacctgggctgctatga	65	1.5
MSM1307	BV678365	$(GATA)_{18}(GATT)_17$	Y	tggcgtttcataagcgtcatc	gccacagaggtaatgcccttg	65	2.0
MSM1308	BV678366	$(CT)_{19}$	Y	cttctcccatcccctcttact	aagaaatcacagcgagtcaca	65	2.0
MSM1309	BV678367	$(GAA)_{31}$	Y	tcctacttctgggtggacaat	tcatgagtgcaggagattagc	62	2.0
MSM1311	BV678368	$(GAT)_{10}(GA)_{10}$	Y	cagaaggggtgtgggggtaat	gagcggggttagaccaatcaa	65	2.0
MSM1312	BV678369	$(CTAT)_{18}$	N	gcagggacaaataacagcttg	cagagacactgaacgcagaca	65	1.5
MSM1313	BV678370	$(CTAT)_{25}$	Y	ccttgagcgaggcaacctgat	cacagggctgatttggcaaca	65	1.5
MSM1314	BV678371	$(TA)_{31}$	Y	aggtccacgatgctgattgtgta	tgaatgtcccgtcgtcctattct	65	2.0
MSM1315	BV678372	$(GT)_8(GAT)_{12}$	Y	ttgttgattggagcaggttggtg	gatctgagagccgaactccgtca	65	1.5
MSM1316	BV678373	$(CCT)_{14}$	Y	aaggtcacactcagaacaccc	gaagacgataacagctgcgat	65	1.5
MSM1317	BV678374	$(CTAT)_{30}$	Y	gagagaggaacagttagaggacat	tgtttactggctgacaatagagtc	65	2.0
MSM1318	BV678375	$(AAG)_{15}$	Y	tttccccctgctaatctactg	taccggcgttatccatattgt	56	1.5
MSM1319	BV678376	$(GT)_{21}$	Y	acgtgttctgcagccatattc	gaggcaaggataatcgggtag	65	2.0
MSM1320	BV678377	$(GAA)_{11}$	Y	gttcacgagaaaaacaagcgag	ctggctcttctcaggttactgc	65	2.0
MSM1321	BV678378	$(CTAT)_{23}$	N	ctgggatagctgtttcatacc	ggggaaatgtactgaatcctt	65	2.0
MSM1322	BV678379	$(GTAT)_8$	Y	tgcttcatgtgcgagtgag	caaatgggcaaggactgac	65	2.0
MSM1323	BV678380	$(CA)_{30}(CATA)_{15}$	Y	aggagtgaggaggacaacaca	ttaacccgagtgaccttgttc	65	2.0

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1324	BV678381	$(GTAT)_{16}$	Y	agaaggagggggtcataactc	ccactataaccaccaactggc	65	2.0
MSM1326	BV678664	$(CA)_{38}$	Y	acctgcttaaagccagtacag	gcaccagaacgatgaatatct	65	2.0
MSM1327	BV678382	$(CTAT)_{14}$	Y	gagcaaacagcggatttac	ctgcattgagtgtgacagc	65	2.0
MSM1328	BV678383	$(GAT)_{17}$	Y	ccggctctgttattgtgagat	aagcggaagacagataaggtg	62	2.0
MSM1331	BV678384	$(GAA)_{11}$	Y	tgcagggactgagaggttact	gactttcaaatttccgacagc	62	2.0
MSM1332	BV678385	$(GTAT)_{25}$	Y	cggatgtagtcttcaccaatc	gaaagctaccacagcaatcag	62	1.5
MSM1333	BV678386	$(CA)_{29}$	Y	ggcatgaactaagcattgag	attcttgagggcttgacatc	62	1.5
MSM1334	BV678387	$(ATAG)_{24}$	N	aaaagttctgcacatggatcg	gtccttgccctccagttacag	62	2.0
MSM1335	BV678388	$(CAT)_7(CAC)_7$	N	gcctgtttgccctcatatagc	gatgtttccagccttttacgc	58	2.0
MSM1337	BV678389	$(CA)_9(TACA)_6(CA)_9$	Y	agatccctcagagagataggc	cacatacacagcaggatagca	62	2.0
MSM1338	BV678390	$(GT)_{22}$	Y	aagtgctgagttacagttgagacg	tcgaagtcctatagagcacagaac	58	2.0
MSM1339	BV678391	$(GATA)_{10}$	N	taacccagtttccccagtcagt	gtagagcgggtcgtccactaag	58	2.0
MSM1341	BV678392	$(CTGT)_9$	Y	aggtcttctgttcagtgtcggtc	cttaccctctgatctccaacgtg	62	2.0
MSM1342	BV678393	$(GAT)_{24}$	Y	tgcatcctgggagtttag	gaagtgatgtcgccagtt	62	2.0
MSM1343	BV678394	$(CTAT)_{20}$	Y	aacagtgctctgagtgtg	gataaagccgttagagga	62	2.0
MSM1344	BV678395	$(GT)_{17}$	Y	aagggcagaattaaccac	taataggggtcaatgctg	62	1.5
MSM1345	BV678396	$(TACA)_9$	Y	gtagaaaagttttgctggctcgta	ctgggagtgtgttgtacaatgatt	62	1.5
MSM1346	BV678397	$(GTAT)_{30}(CCAT)_{10}$	Y	agcagcatgcatacagc	tgaagccacataccagg	52	1.5
MSM1347	BV678398	$(GAT)_{15}(GAG)_7$	Y	tctgggaggagtgcatgag	tgcgggaagaggtttaagc	62	2.0
MSM1348	BV678665	$(AGAT)_{32}$	Y	ctgtgggtgtacaactggc	ttctccttccccataatgc	54	2.0
MSM1350	BV678399	$(TACA)_{19}$	Y	gccagcacacgctttatac	ctggctttcggttagtttg	58	2.0
MSM1351	BV678400	$(GACA)_{11}(GATA)_{16}$	Y	cacaggagacagagtgtt	gttagagacgtgcacatt	58	2.0
MSM1352	BV678401	$(GAA)_{13}$	N	acagcccagatttcctcataccac	tggagggtaaagacaacacgtgaa	58	2.0
MSM1353	BV678402	$(GT)_{24}$	Y	caaatgggaggggcaaggta	tcacccgccacaacctatca	62	2.0
MSM1367	BV678403	$(TAT)_{14}(CAT)_{22}$	Y	acttcttcccggagttc	tgtctgcctcgacctca	62	2.0
MSM1368	BV678404	$(CA)_{12}$	Y	gttgcacacccttaaacc	cgccaagcttaaccatga	52	1.5
MSM1369	BV678405	$(CTT)_{13}(CCT)_{10}$	Y	gtcagtgctctcggaggtaat	aaccctgcaatttgtcaagcc	62	2.0
MSM1371	BV678406	$(GAA)_{33}$	Y	gagaacaatggcctgatt	ccaggccattgatcgtag	62	2.0
MSM1372	BV678407	$(GA)_{18}$	Y	ggaaggacagaaagatctgag	gcagagaaggaggtgatgctt	62	2.0
MSM1373	BV678408	$(GT)_{17}$	N	gaggaggtagcctacagatta	gatgagttccagcacctagag	62	2.0
MSM1374	BV678409	$(GAA)_{12}$	N	aggtgctggaactcat	gccgtcagaatcgaca	62	2.0
MSM1375	BV678410	$(CT)_{14}$	N	gtgtcggatgatgactctt	ccactacacccctcacgtt	58	2.0
MSM1376	BV678411	$(GT)_{15}$	Y	acagcaagtcccagaatagaa	gaaacacgggggagaaactaa	62	2.0
MSM1377	BV678412	$(GA)_{18}$	Y	gggtcagtcactgaggataca	ctctttcaaaggcccacgtct	58	2.0
MSM1378	BV678413	$(GA)_{22}$	Y	gtttagcattcggaaca	tgatccagggcctaaca	62	2.0
MSM1379	BV678414	$(GAA)_{10}$	Y	ggaataaccaaaacgcaacac	cccgagggtcaaaattatcca	62	2.0
MSM1380	BV678415	$(CCT)_{46}$	Y	gcatgcaagcttctcgt	tccttcagccgcgagat	54	2.0
MSM1381	BV678416	$(CCT)_{13}$	Y	acagttaatcaggcgtctg	acggggagctacaatgtga	62	2.0
MSM1382	BV678417	$(CTT)_6(CT)_7$	N	ccagtttcacctcctgtacacta	actttggtgatcccttggcttac	62	2.0
MSM1383	BV678418	$(GAA)_{25}$	Y	gtattcagagcagacagcgtg	aaagaaccagggcttccgact	62	2.0
MSM1384	BV678419	$(CTT)_{15}$	Y	aaagcagagcctgatag	taggtggcggtaatgag	62	1.5
MSM1385	BV678420	$(GAA)_{56}$	N	taaacggaccttacattgttggac	tcgctgcctgaagatgtgtatgat	62	1.5
MSM1386	BV678421	$(GAA)_{10}$	Y	gattcttcgcctcctgttagacta	accttcgggacctacgctacctac	62	1.5
MSM1387	BV678422	$(CTT)_9$	Y	gctgcgtacagtgttaatgcc	acacgaatgtcgccgatgttt	62	1.5
MSM1388	BV678423	$(AAG)_{27}$	Y	gatttgcaggaggtggatttaga	gctgacacaccattgaggaccat	62	1.5
MSM1389	BV678424	$(AAG)_{12}$	Y	ggaacttgaggagctagagga	tgctgctgtcaccatgtgata	54	2.0
MSM1390	BV678425	$(TA)_{11}$	Y	agttcttatggtgcatgct	tggcctgtcactattcgtc	58	2.0
MSM1391	BV678426	$(GA)_{14}$	Y	cactctctccgattgtatcat	tgaatgtctggacgcttacag	58	2.0
MSM1392	BV678427	$(GT)_{18}$	Y	aatagacctcttggctatgtc	aacagataaagtcggactccc	62	2.0
MSM1393	BV678428	$(GA)_{31}$	Y	gaggagaaggggttagattga	tttgagtgtgcccatacgtgt	58	2.0
MSM1394	BV678429	$(GAA)_{12}$	N	cagtgtaggcaatcggagtt	tctccctctttgcatgtcca	58	2.0
MSM1395	BV678430	$(GAA)_{13}$	Y	aggttcaaaataggcccaagtgt	taccacagaaacttccccgtgct	62	2.0

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1396	BV678431	$(GA)_{14}$	Y	cctctctgacacatctgaaccc	cttcttccagcccctgatcctc	62	2.0
MSM1397	BV678432	$(GA)_{35}$	Y	aaatactcccaggaccttg	agcggtgataacggtaacg	62	2.0
MSM1398	BV678433	$(GAA)_{12}$	Y	gctgtcgttaccgttatcacc	tgtgtagcaaaccgcagatcc	62	2.0
MSM1399	BV678434	$(CA)_9$	N	ccctagggggaaattgtttt	tgctcatggtgcgctggtaat	62	2.0
MSM1400	BV678435	$(GGA)_9$	Y	aaaagggagcttcacgtc	ggttgcgtcatcggattg	52	2.5
MSM1401	BV678436	$(TA)_{15}$	Y	aactccaggaagacctactgt	gccaagcttaacctgaagttg	52	2.5
MSM1402	BV678437	$(CTT)_{12}$	N	cgcagctagctaacgagtgtatc	ggtcgttcaaggaaaaaggttgg	62	2.0
MSM1405	BV678438	$(GA)_{16}$	Y	cagaagaggggaagtgactgata	tcctccaatgcctacttcatcgt	62	2.0
MSM1406	BV678439	$(GAA)_{16}$	Y	agggcagcagataaggc	cagcatgctcgttccca	62	2.0
MSM1407	BV678440	$(GA)_{20}$	Y	tggttggagaagcctacgaga	tcagcagtgggaatcgccttt	62	2.0
MSM1408	BV678441	$(CCT)_{10}$	Y	aactgctgccttattagtgac	acaggccacctcaacactatc	62	2.0
MSM1409	BV678442	$(CTT)_{10}$	Y	caacctaaccctgaaacttgctc	tcacctgtgaattgtacgtgcca	62	2.0
MSM1410	BV678443	$(CCT)_{19}$	Y	gtcgcttgacagcacaattac	gtgtgaacggaaaacgggatt	62	2.0
MSM1411	BV678444	$(CTT)_{18}$	Y	tttaccggcgttatccata	cacccaccatgcagtcatc	62	2.0
MSM1412	BV678445	$(GAA)_{11}$	Y	aggggtaagacactgtcattgcta	ggaagatgttgaagccgttaagcc	52	2.5
MSM1413	BV678446	$(CA)_{12}$	Y	ccaaaactgtgaaaccac	ttggctccatcaatcacc	58	2.0
MSM1414	BV678447	$(GAA)_{18}$	Y	cagtgacgtgcagtgatttagtg	agctatttcacaatggcgacagg	54	2.0
MSM1415	BV678448	$(GAA)_{17}$	Y	atcggtgtgggttcata	gaaggcaccatgagcgt	54	2.0
MSM1416	BV678449	$(GAA)_{16}(GGA)_{13}$	Y	gacgttaagaatgccaactgc	ccccccttcaacatctgactgc	54	2.0
MSM1417	BV678450	$(GAAA)_{11}$	Y	ctggggtttgtttactgtc	gcccattcattcagtcctt	62	2.0
MSM1419	BV678451	$(CTCTT)_8$	Y	ctcccttcttcaaagtactcta	ctcatacatgacactctgcatc	62	2.0
MSM1420	BV678452	$(GAA)_{17}$	Y	ctgtaggcgagaaggag	aaaggcgccatgtagga	62	2.0
MSM1421	BV678453	$(GA)_{25}$	Y	tctgagtccctgctttac	gcctctctgcccaatcta	62	2.0
MSM1422	BV678454	$(CTT)_8$	Y	tcacgcaggacttaatgt	tgcagtaggtggcggtat	62	2.5
MSM1423	BV678455	$(GGA)_{10}$	Y	ggcaaagaaggcaaaggtaga	caggaggcacagggcgttatt	62	2.0
MSM1424	BV678456	$(AAG)_{13}$	Y	tagtcagccaagggagacgag	ctccgcttcctcacgcatcat	62	2.0
MSM1425	BV678457	$(CA)_{14}$	Y	ctgataaagtagacgctaacacc	tctcttgcccttcctctaactct	62	2.0
MSM1426	BV678458	$(GA)_{25}$	Y	agctcttccccaacagtag	aaaaacagccaccacttgc	62	2.0
MSM1427	BV678459	$(GGT)_8(TCT)_{14}$	Y	cttacctggttcattgtc	cagaaaggaggcgataac	62	2.0
MSM1428	BV678460	$(GAA)_{13}$	Y	gagcagttagaagccatagtg	ttgtactaagtcatcggcagc	62	2.0
MSM1429	BV678461	$(GAA)_{40}$	Y	ctacaactttgttggacga	gcagtgggaagtggacttt	62	2.0
MSM1430	BV678462	$(CA)_{18}$	Y	catgatgtatgagccatgtcaca	cttcccgtctttctccaatgctg	62	2.0
MSM1431	BV678463	$(TA)_{12}$	Y	cagacagtctgacggactcactt	tgtttcagcttcaccctcgatca	62	2.0
MSM1432	BV678464	$(GA)_{21}$	Y	agaagagcctggtcaggtagaaa	tcagtggaagtgcagtgaagtgg	62	1.5
MSM1433	BV678465	$(GA)_{16}$	Y	agtctccacttcactgcacttcc	tcttcgcactcttgattggtccc	62	2.0
MSM1434	BV678466	$(GGAA)_{14}$	Y	ttcttacaggagacggcgttt	tgtcttgttctgctgcgtgct	62	2.0
MSM1435	BV678467	$(GA)_{17}$	Y	cagatggagaacgcataaa	acgctctgacacggacaat	62	2.0
MSM1436	BV678468	$(CA)_{19}$	Y	agcctacagtacatcacggtagt	tcattcctcctctcctctagcag	62	2.0
MSM1437	BV678469	$(CTT)_8$	Y	tcgacagatctgcgctcta	gtgaaggtgcggttggaca	62	2.0
MSM1438	BV678470	$(AAG)_{12}$	Y	agccaagaggaagagcaacc	tgaggcaaagcagcgaacag	62	1.5
MSM1439	BV678471	$(CAT)_{10}$	Y	aaccgcttaacgcaacag	tgcgggtgacgttatgga	62	2.0
MSM1440	BV678472	$(GAT)_{22}$	Y	tgcaactccctcactttacct	ctcgcttgactcccattgcta	62	2.0
MSM1441	BV678473	$(TGA)_9$	Y	aactgtgtgttcagggtgact	ctctggtctttcatcgcttcc	54	1.5
MSM1442	BV678474	$(GGAT)_8$	Y	gagaatgaaaggagggtggatta	ctttctcctgcctgccagttgta	62	2.0
MSM1443	BV678475	$(TA)_8(TG)_{13}$	N	ccagagtgaagaccaggtagt	agagggaagaagtggaacgtg	62	2.0
MSM1444	BV678476	$(GA)_{12}$	Y	gatgtgttagaccgaatac	tatcactggcgttaccttt	62	2.0
MSM1445	BV678477	$(GAT)_9$	N	gagagagctggatgcagaatgat	ctgacgcgacagcttgtgtatga	58	2.0
MSM1446	BV678478	$(GAT)_{12}$	Y	cagatcataggcagcatcgt	aactgtttgccgcgtcattg	54	1.5
MSM1447	BV678479	$(GT)_{14}$	N	gtacgccgtacatatcct	tggctgtgagagttcgat	58	2.0
MSM1448	BV678480	$(GTT)_{16}(GAT)_{14}$	Y	cttatttgcctagcctagt	gctgtctctgtacgctgtc	62	1.5
MSM1449	BV678481	$(ATG)_{12}$	Y	attgttgagcaggatgagcgt	tgtgaatgccacccaatgtga	62	1.5
MSM1450	BV678482	$(GAT)_{13}$	Y	agactcattgctaggatcggt	aaccctactcgcacagcttcc	58	2.0

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1451	BV678483	$(GAT)_8(GT)_{21}$	Y	gattagccatgtgcttag	cctctcctttacgtggtg	58	2.0
MSM1453	BV678484	$(GAT)_{12}$	Y	gcttgttctgtgccctatctc	gcaaagggacggcaaatttag	58	2.0
MSM1454	BV678485	$(GAT)_7$	N	tcgggagcatttcatagttcag	caccaagctcctcttcagtcgg	62	1.5
MSM1455	BV678486	$(GAT)_8$	N	gacccgccttcactaatattgac	aatggctgccttggtaatgtgac	62	1.5
MSM1456	BV678487	$(CAT)_9$	Y	cttctgtcctgttgtagtaaacgc	aaagccagacagtggagatcaaat	62	1.5
MSM1457	BV678488	$(GAT)_{24}$	Y	cagagtggttaaaaaggcggataa	cctctgaacactttaatgcgagcg	62	1.5
MSM1458	BV678489	$(GAT)_{20}$	Y	atgtgctcgagcagactact	ccgacagcagaacaactcaa	62	1.5
MSM1459	BV678490	$(GGA)_{31}$	Y	tacgagacagacggaacc	aacaccacctcggcatct	54	1.5
MSM1461	BV678491	$(CAT)_{13}$	Y	caattagaagcgagcaa	aacatttcccgacaccc	62	1.5
MSM1462	BV678492	$(CAT)_{11}N_{32}(CT)_{14}$	Y	actgagagaggctgatgcta	accaccatgagcaagacctt	62	1.5
MSM1463	BV678493	$(CAT)_{19}$	Y	aaacgtgaagggtggtactga	taggagcctggttgggttgtg	62	1.5
MSM1550	BV678572	$(ATGT)_{13}$	N	ctacagcttctctgtgctaatca	gttcctgaaagcctgaagctaag	62	1.5
MSM1551	BV678666	$(CA)_{26}$	Y	ctacagcttctctgtgctaatca	gttcctgaaagcctgaagctaag	62	1.5
MSM1553	BV678573	$(GT)_{21}$	Y	agcagattgggggtgttgtt	acgccacagggaaacgctct	62	1.5
MSM1554	BV678574	$(TAGA)_{41}$	Y	aagatgaactgggacgtac	gctgctactggatgcttga	62	2.0
MSM1555	BV678575	$(TATC)_{16}$	N	cctgtttacctgctagtccac	agtgaagttagcccggtctga	62	2.0
MSM1556	BV678576	$(AGAT)_{26}$	N	taccagaagaacaccctagac	tatgtcctgctactgcgattc	62	2.0
MSM1557	BV678577	$(AGAT)_{11}$	N	gtggatcagtgagcgactatgtat	tgcactgcttactgtagcttccgt	62	2.0
MSM1558	BV678578	$(TAGA)_{22}$	N	gggagttctttggtcaagg	tttctcgatcaccgccact	62	2.0
MSM1559	BV678579	$(AGAT)_{27}$	N	agctgacctcctgtgctatct	agaagcgagagcgtcaagtga	62	2.0
MSM1560	BV678580	$(AGAT)_{19}$	N	ttagagggctaatgagtacag	cacagacccaaactgatactg	62	2.0
MSM1561	BV678581	$(ATCT)_{30}$	Y	gaggggtttgtattagtcctg	gcttaacagcaaaactgcgtc	62	2.0
MSM1562	BV678582	$(AGAT)_{19}$	Y	ggcggatataaataaccaag	tgtacctgctgtggattgtg	62	2.0
MSM1563	BV678583	$(TATC)_6$	N	aactataccacctgacgc	gtgtcacctttgccaatg	62	2.0
MSM1464	BV678494	$(CA)_{12}$	Y	attcctaatctcgctgtc	ccaccacggaaatagttg	58	2.0
MSM1465	BV678495	$(GAT)_{21}$	Y	tgataacagtgtgggtgcaaat	ttctccagcttctcagtgccag	62	1.5
MSM1466	BV678496	$(CA)_{15}$	Y	atgagctgattgcaggtaggt	cccttggcttttccttgtgtt	62	1.5
MSM1467	BV678497	$(CAT)_{12}$	Y	gtttgccctcacagtctat	ctcacatccatccgtcgta	62	1.5
MSM1468	BV678498	$(GAT)_{13}$	Y	gtcattaatggcacatcggt	gcgcagacagcagtaggtga	62	1.5
MSM1469	BV678499	$(CAT)_{19}$	Y	caatgaagtaagaggcggtgt	ggagccaatgaaaacgagagg	62	1.5
MSM1470	BV678500	$(GAT)_{18}$	Y	agagactgatctgtcggtggt	gtgaactgacttgtcgctggc	62	1.5
MSM1471	BV678501	$(GAT)_{14}$	Y	gccacgagttattagaccc	acaggcggtgttagtgcat	62	1.5
MSM1474	BV678502	$(CAT)_{14}$	Y	aagttaggtttccggttgtt	ctgggacaccaatttaccgt	62	2.0
MSM1475	BV678503	$(GAT)_{13}$	Y	agtttcctccacatagagtcc	gtaggccagttgaacgaattg	62	2.0
MSM1476	BV678504	$(TG)_{14}$	N	cttcatcactgtagccctca	ctcagtccctgccataacca	62	2.0
MSM1477	BV678505	$(CAT)_{14}$	Y	agtctgaagctctagggac	aggaggtgaaacactgacg	62	2.0
MSM1478	BV678506	$(CAT)_{16}$	Y	tctaaggaggaagagcatt	taaaggtggacgcacttct	62	2.0
MSM1479	BV678507	$(GAT)_{14}$	Y	cagattgcagggtgtcagg	acatcacacgccggtgtca	62	2.0
MSM1480	BV678508	$(CAT)_{10}$	Y	ccacacattcataggcgtgtt	tgagtccaggtcagacgcaca	62	1.5
MSM1481	BV678509	$(GAT)_{15}$	Y	gagtttctggctgcagtctac	aggaagacatccagggttgag	62	1.5
MSM1482	BV678510	$(CAT)_{12}$	Y	agcctcttgatgcactacgtt	catcactctcattgcgcaaca	62	1.5
MSM1483	BV678511	$(GT)_{28}$	Y	cgtctgtctgcgttcagt	catgctctctgcgttccc	62	1.5
MSM1484	BV678512	$(GAT)_{11}$	Y	gcagcactgttgtataagcct	aatgtttgaaagccgaagcag	62	1.5
MSM1485	BV678513	$(CAT)_{14}$	N	caccaggtttcacagaccatac	ctgcaaacaaacttcggatgct	62	1.5
MSM1486	BV678514	$(GAT)_{11}$	Y	acaatgacgcaactgat	ctgctggaaggcgttta	62	2.0
MSM1487	BV678515	$(GAT)_{10}$	Y	gagctcccactccatactatc	gttcacacaaagcacatcgaa	62	2.0
MSM1488	BV678516	$(GAT)_{14}$	Y	gttcatgtgttgggactactg	agcgctacaagagggacgtat	62	2.0
MSM1489	BV678517	$(GAT)_{12}$	Y	gaaaagaggtgggactaag	tgtttcctccgctgtaact	62	2.0
MSM1490	BV678518	$(CA)_{27}$	Y	gtctggtgaagtacgctgata	ctgctctctgattggtcgtct	62	2.0
MSM1491	BV678519	$(TGA)_9$	N	gcatgcggatgaataactgtc	ttcgagggcatctgaaccttg	58	2.0
MSM1492	BV678520	$(GAT)_{13}$	Y	agtgttttaccggaagt	agaggcctcctgttgtt	62	1.5
MSM1493	BV678521	$(GAT)_{14}$	Y	caatatcaggttaatgacgacggt	gcattaaatgatggcagcaaggac	62	1.5

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1494	BV678522	$(TCA)_9$	Y	aaactgagcgggcttaaag	gccatcgttatcgcatcct	62	1.5
MSM1495	BV678523	$(GAAA)_{10}$	N	aacccaagacttagagcgaggtc	ccatgacttactcccacgcatca	62	1.5
MSM1496	BV678524	$(GAT)_{19}$	Y	ggccacgtgtgctatatgatta	cagatggttgttgccgagtctt	62	2.0
MSM1497	BV678525	$(TCA)_{14}$	Y	gccagtaacacacatacagtg	ggggatacatggctcaataga	62	2.0
MSM1498	BV678526	$(GAT)_{22}$	Y	agattggccctaatgag	tctgaggtgctcggagt	62	2.0
MSM1499	BV678527	$(GAT)_{23}$	Y	agactctgtgttgaagccattac	atatagtttgcccttccgctgtt	62	2.0
MSM1500	BV678528	$(GAT)_{17}$	N	gtcaaatgtctcaaactggcacta	cctgaggggactttattgcagtct	62	1.5
MSM1501	BV678529	$(CAT)_{11}$	N	gtcagagggaataataacatgacc	agttcagttcgagagtaatgcacg	62	2.0
MSM1502	BV678530	$(GAT)_{11}$	Y	accatcacgacgacgac	tgtgaccgccatcgttg	54	1.5
MSM1503	BV678531	$(AT)_{26}$	Y	caacagagctgaacgaagataga	aggaggagccacccatcactata	62	1.5
MSM1504	BV678532	$(GTAT)_{19}$	Y	gcagacagtgttgttgggtat	cctgccagtgtctgtgtcaaa	52	2.0
MSM1505	BV678533	$(GTAT)_{15}$	Y	gggcaaggctaatattgacac	catgcaacacaaatggcacat	62	1.5
MSM1506	BV678534	$(GTAT)_{10}$	Y	cagtacccgtcgtttca	ccctgcccataacccat	62	1.5
MSM1507	BV678535	$(GTAT)_{11}$	Y	cgtactgggtcaggagatata	ataccttgactccttgcgatg	62	1.5
MSM1508	BV678536	$(CT)_{14}(GT)_{28}$	Y	ggaataactgcccgaacta	cagtacccccaaaagcctt	62	2.0
MSM1509	BV678537	$(CAAA)_7$	Y	cagacaaaccctcactaac	cagtagtctgcggctaaca	62	2.0
MSM1510	BV678538	$(CA)_{15}$	Y	gaacaacagatgctgctgatgat	acacacataccagctgctccgat	62	2.0
MSM1511	BV678539	$(GA)_{32}(TACA)_6$	Y	ctcagaacagccttagcttag	gtcctctgctggaattgctac	62	2.0
MSM1512	BV678540	$(GT)_{13}$	N	tactgagctgcctaggcatgt	tatccacgcatccacaaccac	62	1.5
MSM1513	BV678541	$(GT)_9$	N	tatgtgactaagggtgactgt	ggtttctcaacaatacgacca	62	2.0
MSM1514	BV678542	$(GT)_{30}$	Y	gcagtaggttactttcgtcat	ggctgtattccatttaggctc	62	1.5
MSM1515	BV678543	$(CATA)_9$	N	gtgtgtgacctgctcttaagt	tcctgttccctcttatcacca	62	1.5
MSM1516	BV678544	$(CATA)_8$	N	gttgggatttctggttagt	tacttgcagtgtgtgaccg	62	1.5
MSM1518	BV678545	$(CATA)_8$	Y	ggttttagcagcaagtccatc	gggtaagggaagtttggggtc	62	1.5
MSM1519	BV678546	$(ACAT)_{15}$	Y	agaaccaccaggtaaacta	tgtgagaaccagggactta	62	1.5
MSM1520	BV678547	$(CA)_{31}$	N	tctgcgtctcccatactgt	ttcaagcgttccggttctc	62	2.0
MSM1521	BV678548	$(ATAC)_{13}$	Y	tctcttaggctagcagt	atgtcttttggtcggtc	58	2.0
MSM1522	BV678549	$(GT)_{18}$	Y	actgtgtacaactccagatcgct	atctccctccattcctcaacctg	62	2.0
MSM1524	BV678550	$(TACA)_{13}$	N	agcaccttcatggtacccaag	cgaacgcaaccgcagtaatgt	62	2.0
MSM1525	BV678551	$(TACA)_{12}$	Y	ttcaaacatggagcacatcatacc	agaggatttgctgcacccgtttag	62	2.0
MSM1526	BV678552	$(GT)_{26}$	N	gactctgttgcatttaggt	ggctgtattccatttaggc	58	2.0
MSM1527	BV678553	$(GTAT)_{10}(GT)_{17}$	Y	ccgctcagaccaaggtagagtt	ccatcatgttctgacgttccca	58	2.0
MSM1528	BV678554	$(TACA)_{11}$	Y	ccccagctaaattagtaga	tgccaagggttagattaga	62	2.0
MSM1529	BV678555	$(CA)_{22}$	Y	cccgttcttaccaatggtatg	tgcgtgtgtatgcgtaggtgt	62	2.0
MSM1530	BV678556	$(TACA)_8$	Y	gatggtgacagaaacagtaaggc	tggcgtcttcccctcttactatg	62	2.0
MSM1531	BV678557	$(TACA)_{12}$	Y	gtagggaaacacctctagcat	gtcactgcacctccagtcatt	62	2.0
MSM1533	BV678558	$(GA)_{14}$	Y	agctggagaaacggcgact	ttcagcatctcgcgggtga	62	2.0
MSM1534	BV678559	$(CATA)_{15}$	Y	ccattttgggccatagtgata	ggggacccagcaaggaatact	62	2.0
MSM1535	BV678560	$(GT)_{111}$	Y	gctaagccccactaactcact	tgttgaagaagcattcggtca	62	1.5
MSM1536	BV678561	$(GT)_{29}$	N	ccatatgagccaggtaaagta	gaaacacaaacacgcacatct	62	2.0
MSM1538	BV678562	$(GT)_{69}$	Y	acacgagcttccctaaccttt	cattgaagagacagcgaggca	54	1.5
MSM1541	BV678563	$(GT)_{12}$	Y	ggaggttatccagctgcttag	agtactgtgtggcccacaaca	62	2.0
MSM1542	BV678564	$(CA)_{22}$	Y	ctcaggtacttgcaggtgtcaat	gatggtgtgttcggtcttatgcc	62	2.0
MSM1543	BV678565	$(TACA)_{13}$	Y	tactgcagggtgtaggatgta	gtgtgtgagcgtagggttgtt	62	1.5
MSM1544	BV678566	$(CA)_{14}$	Y	ttaacgtctactcacacgcacag	cacgtgtgtagctgcagttgcat	62	2.0
MSM1545	BV678567	$(TACA)_7$	N	ctccgcactgtaagtcatt	gttgcctggatagcgtcat	62	2.0
MSM1546	BV678568	$(GT)_{25}$	Y	tgtgtgtctatactgccaactgc	gtctgaaacccgttgccatacat	52	2.0
MSM1547	BV678569	$(TATG)_{14}$	N	atgcaagcttaaccctgtt	ccctctgctggctgacata	58	2.0
MSM1548	BV678570	$(TACA)_{33}$	Y	cagcacacgctttatactgga	ttgggactggctttcggttag	62	2.0
MSM1549	BV678571	$(GT)_{20}$	N	atctccagcagttaaaccg	agcagcagcaaaaccacag	62	1.5
MSM1564	BV678584	$(TATC)_{19}$	N	agagacagcacaagagcaa	agcttttcgttccgctttc	62	2.0
MSM1565	BV678585	$(TAGA)_{20}$	Y	cgccaaatcgtaacaattacc	ctggctgctatgatgacccaa	62	2.0

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1566	BV678586	$(TAGA)_{29}$	Y	ctctagtggccgtagtaact	catggtttgggttgatacat	62	2.0
MSM1567	BV678587	$(TAGA)_{20}$	Y	tagagtggagggcaaattgta	acactagccccatgctcgtta	62	2.0
MSM1568	BV678588	$(CTAT)_{17}$	N	tctgtaatgctgctgctacac	ggtttcacccgcacataagtc	52	2.5
MSM1569	BV678589	$(ATAG)_{49}$	Y	aagtaaacagccgacta	ttggctggaagtacctt	62	2.0
MSM1570	BV678590	$(GGA)_{7}$	N	tgggctgtctcctgactgta	aaggttggaggcatcgtgct	62	2.0
MSM1571	BV678591	$(TAGA)_{26}$	Y	actgcagtacaaacatccacc	tgaaagccccaaacaactgtc	62	2.0
MSM1572	BV678592	$(ATAG)_{14}$	Y	cctctagtttatcctcagtttgc	tacgatgagtctccgatcagttc	58	2.0
MSM1573	BV678593	$(TAGA)_{33}$	Y	ctgcgaagttgtgcttaga	gggggtaaacaggttgcat	62	2.0
MSM1575	BV678594	$(TAGA)_{14}$	Y	gtcatggaaaacagagaaggctac	gcggaagactgattgtcattatgc	52	2.5
MSM1576	BV678595	$(AGAT)_{25}$	Y	atcgtaggaggatactcaacgga	aaatgggagagggcagggtaaac	52	2.5
MSM1577	BV678596	$(CTAT)_{18}$	N	gaaagagggcatcataatacagca	caaaacaaagagagcgcggtctaa	52	2.5
MSM1578	BV678597	$(AGAT)_{12}$	N	gggcaagttgacaggtagaca	gttagcctcattcgccccaat	62	2.0
MSM1579	BV678598	$(ATCT)_{11}$	N	atacagtgtcacccggtactat	ggacagttatccctgcactatg	62	2.0
MSM1582	BV678599	$(CTAT)_{16}$	Y	atgtcttgtatgctactgg	aagtgagtctgcgtttgtc	62	2.0
MSM1583	BV678600	$(GATA)_{10}$	N	tgaagcagtcgccaactac	gtggtttgctcagcatcgc	62	2.0
MSM1584	BV678601	$(ATCT)_{19}$	N	ctctaaggctaatggggttac	ctgttctgcgttttagttgga	62	2.0
MSM1585	BV678602	$(GATA)_{17}$	Y	gtccagcactgatacagat	agtaatggcctgttggttg	62	2.0
MSM1586	BV678603	$(CTAT)_{28}$	Y	gagagccctacctagaacta	cagccagaaacaggtcttat	52	2.5
MSM1587	BV678604	$(ATCT)_{19}$	N	gaggggtcaagacatttaaccat	tgtgtcgatgtccttgtcctacg	52	2.5
MSM1588	BV678605	$(ATCT)_{22}$	N	agcgtaagttgataccatga	ggtgtggaggcactatgaat	52	2.5
MSM1589	BV678606	$(TAGA)_{22}$	N	tgaataccttcctgtagcct	catccatttgcaccgtttac	52	2.5
MSM1590	BV678607	$(GATA)_{23}(GA)_{27}$	Y	gagaggcagataagaacagcgat	agcagcactgccagttgagtcat	62	2.0
MSM1591	BV678608	$(TAGA)_{28}$	N	gatggatggctttcctaccta	agccacacgattatgaccacc	62	2.0
MSM1592	BV678609	$(TAGA)_{33}$	N	cggcactggataaagttac	tacaatttccctcgggatg	62	2.0
MSM1593	BV678610	$(ATAG)_{15}$	N	tggctgagtcttcaagtac	aaacttatcacccggtgtg	62	2.0
MSM1595	BV678611	$(ATCT)_{17}$	Y	caagcttcttcagagtactg	gaagttggctgtaattggtc	58	2.0
MSM1597	BV678612	$(TCTA)_{9}$	N	gctgctatgacgacccaattt	ctctttcacagcggcgagatg	62	2.0
MSM1598	BV678613	$(AGAT)_{20}$	N	gacaaatcctctgatgaac	ctcagcgtctgttaccgta	52	2.5
MSM1599	BV678614	$(GA)_{27}$	Y	ccgctccctaatgtaggt	agggcagcaaacggtgta	52	2.5
MSM1600	BV678615	$(CA)_{26}$	Y	aaaatacacaacctgacgcac	tacagctaaaaggccacggtg	52	2.5
MSM1602	BV678616	$(CTAT)_{23}$	N	gggaatgtgctaaacttggaatag	tgggattactgggcagatagttga	52	2.5
MSM1603	BV678617	$(CTAT)_{19}$	N	gagaaggcagtgtgactat	caatacagcccttgttgtc	52	2.5
MSM1604	BV678618	$(ATCT)_{32}$	N	attagtctgtggataccgctgga	acagggctgcagtggaaggtaag	62	2.0
MSM1606	BV678619	$(ATCT)_{14}$	Y	gacacccaaacttgactgac	catttggtcctaacgcctgt	62	2.0
MSM1607	BV678620	$(CTAT)_{21}$	Y	gacacccaaacttgactgacttc	gccctaacgcctctgtaatttga	62	2.0
MSM1608	BV678621	$(ATCT)_{20}$	N	gatcctctgaggcgttatgta	gctgcttcacgtctttaaccc	52	2.5
MSM1609	BV678622	$(TAGA)_{19}$	Y	ttatgcaaacgcctgactagac	gtgttcatgattcctgcgtgtg	52	2.5
MSM1610	BV678623	$(ATAG)_{11}$	Y	cttcctgtcggatagttag	acagacacacgcctacaac	52	2.5
MSM1612	BV678624	$(ATAG)_{9}$	N	atcgataccctacctttc	ctcgctacgtgtgctaag	52	2.5
MSM1613	BV678625	$(AGAT)_{24}$	Y	tttgccaagggtcggatttat	aaggcagggaggcacgaaact	52	2.5
MSM1616	BV678626	$(AGAT)_{33}$	Y	atccagggaagagcgtacata	tgacgccctgggaataagact	62	2.0
MSM1617	BV678627	$(ATCT)_{21}$	N	agagtgggggaagaagtcgttt	gctgaactctggtccaaggcaa	62	2.0
MSM1618	BV678628	$(AGAT)_{18}$	Y	cagacctccacctactct	gtcagcccacaataatgc	62	2.0
MSM1619	BV678629	$(GT)_{24}$	Y	attgtgggctgactgacac	tttttcaccgcgagcagat	62	2.0
MSM1620	BV678630	$(ATCT)_{10}$	N	cagaaacactccgacgac	gctgctgacgggatcatt	62	2.0
MSM1622	BV678631	$(AGAT)_{23}$	Y	agttttacggtccaacatcgag	ggcccattaatgagcgagatca	62	2.0
MSM1624	BV678632	$(TAGA)_{15}$	N	ctgcgaggttgtgcttagata	gtgggggtaaacaggttgcat	62	2.0
MSM1625	BV678633	$(ATCT)_{20}$	N	aagcttccatatagtgcaccc	tgtccgagtttgcctgatctc	62	2.0
MSM1626	BV678634	$(TAGA)_{34}$	N	cgcaactacgtttcgttaccata	cagaagccaggcagactgcatac	62	2.0
MSM1627	BV678635	$(TAGA)_{28}$	Y	ttattgatggcagggctaga	aaacaaggcatcccggaaac	62	2.0
MSM1628	BV678636	$(TAGA)_{16}$	N	aatcccacatggagttgtag	ccagaccaataaaacgtccc	62	2.0

Appendix. (Continued)

Locus	GenBank #	Repeat	Imperfect?	f Primer sequence	r Primer sequence	T_A	MgCl2
MSM1629	BV678637	$(ATCT)_{11}$	Y	gcgcactatccaactcta	acacctcagatcccgtca	62	2.0
MSM1630	BV678638	$(GATA)_{25}$	Y	actgtcatggaggcaataatg	cacagagacaacaggcaagca	62	2.0
MSM1632	BV678639	$(GTT)_{11}$	Y	tgtctgccctgtatcaaa	cccaaggggaaattcaca	62	2.0
MSM1633	BV678640	$(GTAT)_{32}$	Y	agtctgtcgatactgct	ttgaagaactccattgc	62	2.0
MSM1634	BV678641	$(CTAT)_{12}$	N	gagtgtaatgctgctgctacaact	tgatttctaacaacagccaacgtg	62	2.0
MSM1636	BV678642	$(GATA)_{26}$	Y	caacatcctgctatgtgt	tcccaaggtctctgagtg	62	2.0
MSM1637	BV678643	$(CA)_{14}$	Y	ctagtgagcatatgagcc	tggaagcagcctaatgtt	62	2.0
MSM1638	BV678644	$(ATAG)_{28}$	N	acagtgacacacgcttatatgc	cgttgcccatttcctcttacat	62	2.0
MSM1639	BV678645	$(GATA)_{15}(TAGA)_{17}$	Y	gaagaaggctgtttaagatg	tgctcctccagaaatactga	62	2.0
MSM1640	BV678646	$(CTAT)_{24}$	Y	cttgaatttctctcgggata	caaagcagcactggtcctta	52	2.5
MSM1641	BV678647	$(ATAG)_{18}$	N	acctgtaggtggtgctagaag	gtatgtttcagggtcggctct	62	1.5
MSM1642	BV678648	$(GT)_{22}$	Y	gtgtgagggcttgtcctaaat	tgtccgccctatcctgttaca	62	2.0
MSM1643	BV678649	$(CTAT)_{11}$	Y	ctccttgggggatcaataa	atcagtgcggctatcggtc	52	2.5
MSM1644	BV678650	$(GT)_{12}$	Y	tcaacaagccatctctagttc	cacccaccacaaagtacatca	62	2.0
MSM1645	BV678651	$(CTAT)_{16}$	N	caatgcaccactcttatac	ggaacacagccatcattag	62	2.0

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