

## PERMANENT GENETIC RESOURCES

# PCR primers for 100 microsatellites in red drum (*Sciaenops ocellatus*)

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## Abstract

One hundred nuclear-encoded microsatellites from a genomic library of red drum (*Sciaenops ocellatus*) were isolated and characterized. Eight microsatellites had tetranucleotide motifs; 92 had dinucleotide motifs. The average number of alleles per microsatellite (sample of 22–24 fish) was 17.7 (range = 2–30); gene diversity averaged 0.796 (range = 0.227–1.000). Following Bonferroni correction, genotype frequencies at 90 microsatellites did not deviate significantly from Hardy–Weinberg equilibrium expectations. Occurrence of null alleles was inferred at 15 microsatellites; alleles differing by only a single base were observed at 11 microsatellites. The microsatellites developed should prove useful for population-genetic studies of ‘wild’ red drum and in construction of a genetic map.

*Keywords:* Microsatellites, red drum, *Sciaenops ocellatus*

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Nuclear-encoded microsatellites are useful for a variety of studies, including (i) assessment of population structure and genetic effective size (Saillant & Gold 2006), (ii) parentage assignment and identification of quantitative trait loci (QTLs) (Liu & Cordes 2004), and (iii) ‘anchor’ loci in construction of genetic maps (Silver *et al.* 1991). Added to the 68 already developed primer pairs for red drum microsatellites (Saillant *et al.* 2004), the 100 developed here should provide a firm basis for a red drum genetic map that should prove useful in future studies of red drum.

Whole genomic DNA was extracted from ethanol-preserved fin clips, using a DNeasy gel extraction kit (QIAGEN). DNA was digested with *DpnII* (NEB), and 500–1500 base-pair (bp) fragments were dissected from an agarose gel, purified with a DNA gel extraction kit, and ligated in a P-Bluescript II KS-plus plasmid. Transformation with XL-Gold Ultracompetent cells (Stratagene) and screening of transformants were performed as described in Renshaw *et al.* (2006). A total of 48 000 clones were spotted in a 4 × 4 array onto two identical Hybond H + nylon membranes (22.5 × 22.5 cm, Amersham), using a Q-bot (Genetix). DNA from each clone was fixed to the membranes as outlined in Renshaw *et al.* (2006) and each clone was spotted twice to

minimize the possibility of false positives. One of the membranes was hybridized with <sup>32</sup>P-labelled tri- and tetranucleotide probes [(CAA)<sub>8</sub>(GAA)<sub>8</sub>(TAA)<sub>13</sub>(GATA)<sub>9</sub>(CATA)<sub>8</sub> and (GACA)<sub>8</sub>], while the other was hybridized with <sup>32</sup>P-labelled dinucleotide probes [(CA)<sub>13</sub> and (GA)<sub>13</sub>]. A total of 104 positive clones were identified on the membrane probed with tri- and tetranucleotide repeats; 459 positive clones were identified on the membrane probed with dinucleotide repeats.

A total of 523 positive clones (64 tetra-/trinucleotide and 459 dinucleotide repeats) were inoculated into frozen glycerol stocks arrayed in 96-well plates with 1 mL of Luria Broth selective media (ampicillin) and incubated over night at 37 °C. Plasmid DNA was isolated with a BioRobot 8000 (QIAGEN). Miniprep DNA was quantified and normalized, and both strands were sequenced using primers 555 and 837 (Makova & Patton 1998) and ABI PRISM BigDye Terminator version 3.1 (Applied Biosystems). Sequencing products were separated and visualized on an ABI 3100 (Applied Biosystems). A total of 197 sequences were edited, the vector sequence was removed, and repeats were identified using Sequencher 3.0 (Gene Codes Corporation); seven of the sequenced clones (197) contained no repeats. Primer design was performed by using PRIMER 3 vs. 0.3.0 (Rozen & Skaletsky 2000). Primers generated by PRIMER 3 were assessed further using NETPRIMER

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(<http://www.premierbiosoft.com/netprimer>). Primers were imported to AMPLIFX 1.4.0 (available at <http://ifjr.nord.univ-mrs.fr/AmplifX-Home-page?lang=en>), where simulations of PCR amplification of designed primer pairs to the imported target sequence were performed.

A total of 122 primer pairs were designed and used in genotype assays of 24 red drum individuals obtained from Galveston Bay, Texas. Total genomic DNA was extracted from ethanol-preserved fin clips. PCR amplifications were performed with a PTC-200 thermocycler (MJ Research) or a MyCycler thermocycler (Bio-Rad), using unlabelled 5' (forward) and 3' (reverse) primers and a fluorescently labelled, 21 bp 5'-tail-sequence primer (as described in Boutin-Ganache *et al.* 2001). The 5'-tail-sequence primer was arbitrarily chosen from the microsatellite primer sequence CATR8 used to amplify DNA from the Madagascar periwinkle *Catharanthus roseus* (Shokeen *et al.* 2007). Because only the 5'-tail-sequence primer needs to be labelled, the approach significantly reduces cost when evaluating multiple primer pairs. The unlabelled 5' forward and 3' reverse primers and the 5'-tail-sequence primer were purchased from Invitrogen. The 5'-tail-sequence primer was labelled with either with NED (Applied Biosystems) or with FAM (Invitrogen). PCR amplifications were performed in a 10 µL reaction volume containing ~100 ng DNA, 1× reaction buffer (50 mM KCl, 10 mM Tris, 1% Triton-X 100), 0.5 µM of each primer (tailed forward, reverse, and 5'-tail-sequence), 2 mM MgCl<sub>2</sub>, 200 µM of each dNTP, and 0.1 U *Taq* DNA polymerase (Invitrogen). All microsatellite amplifications were run under the same PCR conditions, using a PCR protocol with a three-step decrease in annealing temperature (with the same denatur-

ation and extension conditions). The PCR protocol consisted of initial denaturation at 95 °C for 3 min, 10 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 45 s, extension at 72 °C for 1 min; 10 cycles of annealing (45 s each) at 56 °C, 15 cycles of annealing (45 s each) at 52 °C, and final extension for 10 min at 72 °C. PCR products were separated and visualized on an ABI 377 automatic sequencer. Alleles were sized using the GeneScan® 400 ROX-Size Standard (Applied Biosystems) and GeneScan 3.1.2® (Applied Biosystems); allele-calling was performed using GENOTYPER® software, version 2.5 (Applied Biosystems).

One hundred microsatellites (eight with tetra-nucleotide motifs and 92 with dinucleotide motifs) were amplified successfully and reliably scored for genotypes. The remaining 22 primer pairs designed failed to amplify product or product could not be scored reliably. *Soc500* and *Soc506* were designed from the same clone, while the remaining primer pairs were designed from different clones. Searches in GenBank, NCBI (nucleotide blast) did not reveal any matches with target sequences of previously designed primer pairs for red drum. Number of alleles, observed and expected heterozygosities, tests for conformity to Hardy-Weinberg equilibrium expectations, and tests for linkage equilibrium were performed using GENEPOP (Raymond & Rousset 1995). Evidence for occurrences of null alleles and/or large allele drop-out was explored using MICRO-CHECKER (Van Oosterhout *et al.* 2004).

Primer sequences and summary genotypic data are presented in Table 1. The number of alleles detected per microsatellite varied from two to 30; the average gene diversity varied between 0.227 and 1.000. Significant deviation from

**Table 1** Summary data for 100 microsatellites developed for red drum (*Sciaenops ocellatus*)

Microsatellite	GenBank	Primer sequence (5'-3')	Repeat sequence	Cloned allele	N	N <sub>A</sub>	Size range	H <sub>O</sub> /H <sub>E</sub>	P <sub>HW</sub>
<i>Soc500</i>	EF609016	GTCCGTGCGAGTGTGTATTAGA GCCAGAGAGAAAAACGAGAAAAG	(CA) <sub>27</sub>	162	24	16	157-195	0.833/0.905	0.180
<i>Soc501*</i>	EF609017	CTGTTGGTGTGTGTTGACTCTGA GCACACAAACTACAACCTGAATCTG	(GACA) <sub>7</sub>	211	22	8	203-239	0.227/0.823	<b>0.000</b>
<i>Soc504</i>	EF609018	CCAGTGTCTCCAGTGTACCAGT CCTGCTCTACACATCGGCTCGT	(GACA) <sub>6</sub> (GACA) <sub>8</sub>	160	23	8	176-208	0.956/0.849	0.639
<i>Soc505</i>	EF609019	ATGGCACTGGTATCAAGAAT GGAAATGGGAGCAAAAACA	(GATA) <sub>14</sub>	105	24	14	114-178	0.833/0.926	0.092
<i>Soc506</i>	EF609020	CCTCCTACAGCCTAGTTGTGTA GCTCTGCTTGGTATCCTTTGG	(AGCA) <sub>6</sub>	148	24	2	164-168	0.333/0.383	0.598
<i>Soc507</i>	EF609021	GCTGAGCAGAAAAGATGAGATAG CAGAGAGCCCAATGAAGGTC	(GATA) <sub>24</sub>	318	24	18	291-379	0.875/0.949	0.215
<i>Soc508</i>	EF609022	GCAGCACATTTTCAGCACAC TAATGCCCTGTATTCTATCTA	(GATA) <sub>18</sub>	148	24	9	136-172	0.917/0.868	0.597
<i>Soc509*</i>	EF609023	TTGACATCGCAGGAGTGAGA GGAGACAGACGGATAAGCATA	(GATA) <sub>11</sub>	192	24	8	206-238	0.542/0.756	0.029
<i>Soc510*</i>	EF609024	AGATGCCAACACCTCTAAAAC ACAGACCACTCCGACTCAAA	(GATA) <sub>8</sub>	256	24	7	269-297	0.458/0.637	0.110
<i>Soc512</i>	EF609025	CCATCCCTCGCTCTCTCTT GGCTCACCTAATGAATCCAG	(GA) <sub>17</sub>	104	24	9	122-138	0.750/0.831	0.222

Table 1 Continued

Microsatellite	GenBank	Primer sequence (5'-3')	Repeat sequence	Cloned allele	N	N <sub>A</sub>	Size range	H <sub>O</sub> /H <sub>E</sub>	P <sub>HW</sub>
Soc513†	EF609026	ATGACTCCCAGAGAGACAAAGA CCTCAGCACAAAGATAAAGACAA	(CA) <sub>34</sub>	287	24	12	291-311	0.667/0.811	<b>0.000</b>
Soc516	EF609027	GCAGACACAAAATGTTCAAAGCA GGAAGACTCACGGAGCAGGIT	(CA) <sub>11</sub>	157	24	5	169-189	0.750/0.566	0.058
Soc521	EF609028	CAGAAGGGCTACCAGGACC ACAAGGATTTTACTGTTGATACT	(GA) <sub>28</sub>	166	24	18	161-207	0.917/0.931	0.829
Soc522	EF609029	CTAGTGGATCTGCTTGATGCTTT GCACACGTCTGAGGAGTGAA	(CA) <sub>13</sub>	116	24	10	124-150	0.750/0.798	0.207
Soc523	EF609030	TGACACTTCAAAACATCCTCCTC GACCTCCCAAAGTCTGTAATCA	(CA) <sub>16</sub>	100	24	5	106-120	0.667/0.751	0.143
Soc524	EF609031	TGTTTCCCTGCTGCTGTAATG CCACACACCGACTACAATGAA	(CA) <sub>25</sub>	128	24	18	116-172	1.000/0.916	0.841
Soc525*	EF609032	CACGTGTTGGGACATTTAGAGG GGGTGTGACAAACTCAGGGAC	(CA) <sub>14</sub>	108	24	7	119-137	0.417/0.734	<b>0.000</b>
Soc526	EF609033	CTTCCTCCTCCACTTCAACA GTAGTGTTTGTGACAGCAGGAC	(CA) <sub>16</sub>	219	24	16	229-273	0.875/0.933	0.737
Soc527*	EF609034	CCAAATGTCAAGCCTGCTC GCTGTGAAAATCTCACCACACTT	(TG) <sub>16</sub>	193	24	13	204-236	0.708/0.908	0.045
Soc529	EF609035	ATGTGTGACAGACCCAGITTC CGTCAACAGGAAACACTAACAG	(CA) <sub>20</sub>	171	24	15	190-222	0.833/0.717	0.320
Soc530	EF609036	CTTGGACCTGTATCACAGATGGA GTTCTCCTTCTCATCTTCAGCAT	(CA) <sub>14</sub>	203	24	12	217-247	0.792/0.839	0.060
Soc532	EF609037	GAGTGCTCACAAAGTGCCAG GTGCCAGATAGATGCTGACC	(CA) <sub>14</sub>	124	24	12	130-162	0.833/0.857	0.030
Soc533	EF609038	CTGGACAGTGAAAACTTGAACA GTTAGGCTAATACACTCTTGGTG	(CA) <sub>25</sub>	101	24	13	102-130	0.708/0.838	0.073
Soc535	EF609039	CGAGCGTAAACAAAGCACCTA GACTCACTGTAAACACCACATCT	(CA) <sub>16</sub>	122	24	8	126-140	0.750/0.844	0.858
Soc536	EF609040	GCCACAAAAGTCACTACTGCTA GTGTGAAAACCTGTAAACTTGAATG	(CA) <sub>26</sub>	166	24	9	152-186	0.833/0.824	0.358
Soc538	EF609041	CCAGCATATTTTGGAGCAGC TGAAGGTTTTTCCCGTAGT	(CA) <sub>17</sub>	182	24	11	196-218	0.875/0.847	0.631
Soc540	EF609042	GCTCGTATCTGTATCAAAGGIT CTTGAAGCAGCGTTTGGAT	(CA) <sub>13</sub>	154	24	6	174-184	0.667/0.720	0.723
Soc541	EF609043	CCGAATAGATAGACCCGTCACA GCTGCTGAGGTGTTGATGAAA	(CA) <sub>28</sub>	193	24	13	171-209	0.750/0.869	0.386
Soc542*	EF609044	CTGTTGGAAATACCAGGAAA CAGTAATAGACTCTGATTTGTGCTC	(CA) <sub>41</sub>	135	24	20	102-176	0.792/0.948	0.113
Soc543	EF609045	GAGCAITCAGGAGCCACAT CCATCTGTGCCTAACGACCA	(CA) <sub>16</sub>	189	24	16	206-282	0.833/0.837	0.762
Soc544*	EF609046	CAGACCTGCTGTGTCTTTTC TCTTGATTTGAGAGGGATAGTGC	(CA) <sub>26</sub>	121	24	25	127-259	0.792/0.969	0.010
Soc547	EF609047	AGTCACAAATACACAGGATGAAA AGGCATGGACAAAACCTGGTCA	(CA) <sub>16</sub> (TA) <sub>11</sub>	197	24	8	208-224	0.792/0.773	0.696
Soc548	EF609048	CGCACACACAAAAACAGTGAG CCATCGGTCCAGTATGAAGTC	(CA) <sub>13</sub>	160	24	9	170-186	0.958/0.874	0.849
Soc549*	EF609049	TGAGTGTCTAAAGTCCGATT CCATTTGAGTCCCGTAAC	(CA) <sub>29</sub>	236	24	22	219-283	0.792/0.949	<b>0.000</b>
Soc550	EF609050	CGCAGACAGACGCCTCTAT CATCCTCTCCTCAGTGTAGCC	(CA) <sub>26</sub>	216	24	11	202-236	0.958/0.886	0.724
Soc551	EF609051	TCAACCACACTCAGGTCCAG GCAACACAAAGCACTCACAAA	(CA) <sub>10</sub>	212	24	16	230-282	0.792/0.789	0.253
Soc552†	EF609052	ATCAGGAACCCAGCAATC TGGTGACAAGGTGGCAGAAT	(CA) <sub>26</sub>	153	24	12	153-185	0.875/0.885	0.255
Soc553†	EF609053	CAATCACACTCCGACACACTC GCATGGGTAGAGAGCTGGTG	(CA) <sub>13</sub>	241	24	12	250-270	0.708/0.864	0.012
Soc554	EF609054	GAAAGTAGTCCAACATCCAAGT AAATGCCAGTTTTCTCAGG	(CA) <sub>40</sub>	225	24	21	204-276	1.000/0.957	0.886
Soc555	EF609055	TACAAGCTGCCGAAACACAC AGAATGAGAGCCGACAGAGG	(CA) <sub>15</sub>	183	24	7	195-207	0.917/0.793	0.024
Soc556*	EF609056	CAGAACAGACACACCGACG CAAACACAGTTAGACAAACAGAGA	(CA) <sub>18</sub>	203	24	14	209-251	0.542/0.885	<b>0.000</b>
Soc557	EF609057	AGTCTCGTGTGTTTTGGTAT GCTGGTGGTTTGTGATTGAT	(CA) <sub>16</sub>	197	24	3	215-219	0.333/0.393	0.667

Table 1 Continued

Microsatellite	GenBank	Primer sequence (5'-3')	Repeat sequence	Cloned allele	N	N <sub>A</sub>	Size range	H <sub>O</sub> /H <sub>E</sub>	P <sub>HW</sub>
Soc558	EF609058	CAGTGAACAGGAACGACTTTTAGA GACCCATCAACCTCCAGCA	(CA) <sub>18</sub>	168	24	12	187-217	0.958/0.913	0.472
Soc559	EF609059	TGTGGGACAAGATGTGAAAT TTACCTTGGAACGGTGTGA	(CA) <sub>31</sub>	221	24	26	233-311	0.958/0.964	0.911
Soc560	EF609060	ACGGCTCCCTACACATTACG GTCCTCGGCACAGAAAGTTG	(CA) <sub>15</sub>	176	24	6	196-206	0.625/0.729	0.089
Soc562†	EF609061	CTGAGGAAACACACACACGAA CACAAAATCAATGGCTGCTG	(CA) <sub>11</sub>	247	24	5	259-272	0.667/0.621	0.112
Soc563	EF609062	ATCCGTTATGTGGCTTCA CGCTTTCAAATGTGGTCTT	(CA) <sub>42</sub>	166	24	18	141-201	0.792/0.901	0.145
Soc564	EF609063	TGCCAGCATCCGTCTACTCA AAGAGCCGACCAATCAGAGAG	(CA) <sub>15</sub>	171	24	7	187-201	0.708/0.677	0.993
Soc565	EF609064	CCTCACCCACCCGATACAT CAGAATCAGAGAGAGAAATCAGGA	(CA) <sub>15</sub>	231	24	6	243-253	0.833/0.709	0.740
Soc566	EF609065	GGACAAGAGAAGCAGCAGACG TCATCACGTCCAAAGCTACG	(GA) <sub>13</sub>	111	24	10	127-163	0.917/0.855	0.187
Soc567*	EF609066	ACACACACTCCCACAGATGAAT CGCTGCCCCAAAATAGAAT	(CA) <sub>31</sub>	262	24	23	246-338	0.708/0.957	<b>0.000</b>
Soc568	EF609067	CGACTCTGATGGTGAATGTTGA GAGCGTTCCTGAAGAAGATG	(CA) <sub>17</sub>	197	24	12	196-228	0.875/0.885	0.817
Soc569	EF609068	CCCTGTGTGTCTGTTTCTTTG TGTGAGTGGTGGTGTGATGTT	(CA) <sub>13</sub>	196	24	6	212-222	0.708/0.683	0.787
Soc570*†	EF609069	GCGTCATCTTCTCCACCTCT ACGACTCCTCCATACTCTGTT	(CA) <sub>40</sub>	178	24	30	173-403	0.792/0.975	<b>0.000</b>
Soc571	EF609070	CGTGAATGAGCGGAGACA GGGAGTGTGTGAGAATGTGGA	(CA) <sub>20</sub>	241	24	20	247-289	1.000/0.953	0.922
Soc572	EF609071	AGCACTATGTCTCCAACT ATCACCTTCAAATCAGTCTCTT	(GA) <sub>32</sub>	130	24	10	134-154	0.875/0.872	0.752
Soc573	EF609072	AGAGCAGGAGATGTGACTTC CTTTCTGGGAGGTTGAGACA	(CA) <sub>23</sub>	263	24	27	267-329	0.917/0.971	0.541
Soc574	EF609073	TTTCTCATAATCTGCCATACG TTGGTGGAGTGTCTTTCT	(CA) <sub>21</sub>	211	24	8	220-242	0.750/0.760	0.949
Soc575	EF609074	GGACGCACCATCTCTCCATCT AGGCTTTGCTCTTTTCAGACG	(CA) <sub>11</sub>	229	24	6	245-255	0.875/0.778	0.750
Soc576	EF609075	CCTGTCCGTCAAGCGTCTGT ATCCAAGTCTGAACTGTCACTGTT	(CA) <sub>19</sub>	185	24	10	179-209	0.875/0.853	0.406
Soc577	EF609076	GGGATAGGCATCAGACACC CTTTGCGACAGCCCACTTC	(CA) <sub>14</sub>	244	24	8	256-274	0.750/0.777	0.142
Soc578	EF609077	GATGGGAGACTGTGCTGATGAA ATGTTGCTCTGAATGCCTGCT	(CA) <sub>20</sub>	296	24	14	306-344	0.958/0.894	0.824
Soc579	EF609078	GCTAACACACAGAAAAAAGATG GGAAAGCAAACGGAGAGAGA	(CA) <sub>32</sub>	295	24	17	275-321	0.750/0.886	0.026
Soc580	EF609079	CTGAGCCTGAACGCACATCAT GAACAACAACCTGTCACTGTCTG	(CA) <sub>25</sub>	275	24	18	259-311	1.000/0.939	0.983
Soc581*	EF609080	CGTTATTCTTTGGACATAGTAGGC CAGTGAGAGCGACAGTATCTGCT	(CA) <sub>2</sub> (N) <sub>2</sub> (CA) <sub>7</sub> (N) <sub>6</sub> (CA) <sub>24</sub>	210	24	19	221-275	0.792/0.933	0.012
Soc583	EF609081	CAAGGGAGGTGTGCTGATTA TGAGTGTGGTGGCTGATAGAT	(CA) <sub>12</sub>	111	24	3	125-129	0.417/0.370	0.143
Soc586	EF609082	GGGAAATGGACACAAAAGAAAT CACCTGGGACCTTAGTCACTT	(CA) <sub>35</sub>	180	24	22	162-228	0.833/0.917	0.146
Soc587†	EF609083	TGGAACATGAGGTGATAAGACG AGGGGATGTAAGCCTGAAT	(CA) <sub>23</sub>	249	24	17	242-307	0.875/0.912	0.101
Soc588	EF609084	TGAATGACTTGTCTTGTCTGAA AATAACCCCACTCTCCCT	(GA) <sub>22</sub>	191	24	12	182-220	0.917/0.878	0.520
Soc589	EF609085	ACGATGAGTGACGAGAATTTTT CTTGAGCCAATAGCAGGATGF	(CA) <sub>5</sub> (N) <sub>2</sub> (CA) <sub>4</sub>	137	24	3	149-159	0.458/0.414	1.000
Soc590	EF609086	CAATGGACAGTTTGAGAGTTC GAAACCCACACCAATCACT	(CA) <sub>18</sub>	190	24	12	199-235	0.875/0.878	0.412
Soc592	EF609087	AGAAGGAGGTGAGGAGCATT TCCCATTCAAAACACAAGCA	(CA) <sub>13</sub>	119	24	8	128-150	0.708/0.779	0.173
Soc593	EF609088	TTGTACCGAGGTTGTAGATG ACAGGAATGTGTCCAAAATG	(CA) <sub>12</sub>	94	24	4	106-112	0.667/0.630	0.614

Table 1 Continued

Microsatellite	GenBank	Primer sequence (5'-3')	Repeat sequence	Cloned allele	N	N <sub>A</sub>	Size range	H <sub>O</sub> /H <sub>E</sub>	P <sub>HW</sub>
Soc594†	EF609089	TCGTGCTCTGTCTCCGTTTC TGAATAATTTTGTCTTTTACTTTC	(CA) <sub>13</sub>	175	24	5	193–232	0.333/0.543	0.045
Soc595	EF609090	TCATCATCTCTCTCTTTTCTCT CTTTACCCACCCAACTCCAT	(CA) <sub>23</sub>	184	24	13	188–218	0.958/0.867	0.587
Soc596	EF609091	TTTACGCTCTGAAAACCTCTA AGGGTCGCTTTGTGTATCCA	(TG) <sup>5</sup> (AG) <sub>14</sub>	163	24	9	175–191	0.917/0.856	0.171
Soc598	EF609092	CTGGCGTCTGAACACTGC GTGAATCCCTGTGGGTGCT	(CA) <sub>34</sub>	214	24	25	197–275	0.958/0.968	0.334
Soc600†	EF609093	CTAGTGGATCAGTTTGACTTCA ATGAGCCATTATACAGGAACC	(CA) <sub>11</sub>	201	24	9	211–236	0.750/0.834	0.446
Soc601	EF609094	CTTTGGGACAACAGAAATGC TCCAGCAAGCAGACAACAAT	(GA) <sub>22</sub>	156	23	11	165–191	0.913/0.858	0.644
Soc602	EF609095	AGCAACCATTTCTCCACAC CCATCACACACCAGGGTTAA	(CA) <sub>13</sub> (N) <sub>2</sub> (CA) <sub>5</sub>	118	24	6	125–143	0.708/0.702	0.854
Soc604	EF609096	TGGGGAAAATGGAGACAGG CGTGACAGCCAATGAAAAG	(CA) <sub>19</sub>	227	24	11	243–269	0.792/0.876	0.147
Soc607	EF609098	GGTGAAGGATGACAGGAGAG CATACCAGCGTGACAAAACA	(CA) <sub>27</sub>	234	24	18	238–278	1.00/0.944	0.967
Soc609	EF609099	CCCGCATTAGACAGAAAAC ATGGGTATGTGTGGCTTACAG	(CA) <sub>23</sub>	272	24	24	281–351	0.917/0.958	0.775
Soc610*†	EF609100	CCTGATGTGTTTACATGTGGTTA GAAGGGAGGTGTCTCTGTTG	(CA) <sub>32</sub>	163	24	25	134–209	0.667/0.969	<b>0.000</b>
Soc612	EF609101	TAATCCCTCTGTACGGTTCAT CTGTAACGATGCGGCTGAG	(CA) <sub>34</sub>	298	24	18	282–324	0.917/0.924	0.171
Soc613	EF609102	AGGGAGTGGCTCTTTGTCTGA AGGGGCATCCTGTCTGTTT	(CA) <sub>15</sub>	210	24	11	210–244	0.833/0.848	0.306
Soc615	EF609103	GATTACCAAGCACAAGGA GCTCATCAGAAGGCTAACAGA	(CA) <sub>26</sub>	97	24	25	80–150	0.917/0.965	0.016
Soc616	EF609104	TTCTCTCTCCGTGTTTGTGTT ACTGGGCAGGTTTCTTCTGAC	(CA) <sub>32</sub>	298	24	18	280–336	0.958/0.917	0.958
Soc617	EF609105	TGCCATGTTTGAACCTCAGA GAGGACAGAGGGTGTCACTCC	(CA) <sub>44</sub>	290	24	7	281–311	0.667/0.782	0.371
Soc618	EF609106	ACCCGATAAGGAACAAGCAGA CATCACCGTCCAACCAGAGAA	(GA) <sub>17</sub>	107	24	8	125–145	0.708/0.796	0.523
Soc619	EF609107	GCGTCTCTCTCTCGTGACAA GCTCTCCTGCGTCTCGTCTT	(CA) <sub>19</sub>	170	24	10	172–202	0.875/0.819	0.189
Soc620	EF609108	CGTGTGAGCCACAATCTCCA AGCCACGCCATAACCTCTCA	(CA) <sub>13</sub>	143	24	14	162–202	0.833/0.894	0.772
Soc621	EF609109	CACTCCTTCCACCTCCTCCT TGGTCTGCTGCTACTCTACTACTG	(CA) <sub>12</sub>	152	24	8	169–193	0.875/0.784	0.781
Soc622	EF609110	CTGCCTAACATCCTTCTTTCTACT CCATAAACGCCACCAAACA	(CA) <sub>29</sub>	251	24	14	244–276	0.875/0.864	0.439
Soc623	EF609111	CACTTTCACTTTCTGCCCCA TCTGGTTTCTGGCTTTCATACA	(CA) <sub>14</sub>	144	24	10	146–170	0.833/0.827	0.692
Soc624*†	EF609112	CACGCTGGTCTTTTCTCAA CTGGGGTTAATTTGTGTGTGC	(CA) <sub>28</sub> (N) <sub>2</sub> (CA) <sub>7</sub>	158	24	22	135–192	1.000/0.977	0.032
Soc625†	EF609113	TACCCCTCAGAAAATGGTCGCTT GAGAGTGTTTACCCCGTGTGC	(CA) <sub>12</sub>	109	24	11	118–133	1.000/0.919	<b>0.000</b>
Soc626	EF609114	ACTTTGAGCCAATGCTTTCC GTGAGCTGTGTATCCCTGTGC	(CT) <sub>6</sub> (CA) <sub>9</sub>	169	24	3	189–193	1.000/0.665	<b>0.000</b>
Soc627	EF609115	AGCAACGCATACAACCAGTG TCACGAGTAACGAAGCTGTGAT	(CA) <sub>27</sub>	190	24	15	184–236	1.000/0.935	0.072
Soc629	EF609097	CCATCTCATCTCACTCTCACTTG GGCGGGAGGAACAAAGAAT	(CT) <sub>38</sub> (N) <sub>5</sub> (CA) <sub>15</sub>	180	24	14	148–178	0.750/0.892	0.554

\*Microsatellites where occurrence of null alleles was indicated by analysis using MICRO-CHECKER.

†Microsatellites with observed alleles differing by only a single base.

GenBank Accession numbers for clone sequences; primer sequences are forward (top) and reverse (bottom); repeat sequence indicates repeat motif; cloned allele is size (in base pairs) of sequence in GenBank; N is the number of individuals assayed; N<sub>A</sub> is the number of alleles detected; Size range refers to sizes (in base pairs) of alleles thus far uncovered — size includes the 21 base pair (bp) 5'-tail-sequence primer used for PCR amplification; H<sub>O</sub>/H<sub>E</sub> is observed and expected heterozygosities, respectively; P<sub>HW</sub> represents the probability of deviation from Hardy–Weinberg expectations — significant P-values following Bonferroni correction are in bold.

Hardy–Weinberg equilibrium expectations, following Bonferroni correction (Rice 1989), was observed at 10 microsatellites (Table 1). None of the pairwise comparisons of microsatellites indicated significant genotypic disequilibrium. Evidence of null alleles was detected at 15 microsatellites; single base-pair shifts (i.e. alleles differing by only a single base pair) were observed at 11 of the microsatellites. The 77 microsatellites whose genotypic proportions did not deviate from Hardy–Weinberg expectations and where null alleles and one base-pair shifts were not detected should be suitable for population-genetic studies. Almost all of the microsatellites should be suitable for constructing a genetic linkage map.

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