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PRIMER NOTE

Microsatellite DNA markers for kinship analysis and genetic mapping in red drum, *Sciaenops ocellatus* (Sciaenidae, Teleostei)

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Abstract

Thirty-eight nuclear-encoded microsatellites were isolated from the marine fish *Sciaenops ocellatus* (red drum). The species is of economic importance in the southeastern United States, and declines in abundance have led to augmentation of the 'wild' fishery with hatchery-raised fingerlings. The microsatellites will be useful for studies designed to assess larval/juvenile recruitment of hatchery-raised individuals at varying spatial and temporal scales and for assessment of genetic components contributing to variation in performance and survival of hatchery-produced fingerlings in the wild. The microsatellites also will prove useful as 'anchor' loci in constructing a genetic map.

Keywords: genome library, marine fish, microsatellites, PCR primers, Sciaenidae, red drum

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The red drum, *Sciaenops ocellatus* (Sciaenidae), is an economically important, estuarine-dependent marine fish found in the western Atlantic Ocean, primarily in the northern Gulf of Mexico (Gulf) and along the east (Atlantic) coast of the United States (Pattillo *et al*. 1997). Declining abundance of red drum (Matlock 1984; Goodyear 1989) prompted long-term genetic efforts to identify potential geographical boundaries of discrete subpopulations in US waters (Gold *et al*. 2001). Knowledge of geographical boundaries is necessary for critical assessment and allocation of fishery resources (Hilborn 1985; Sinclair *et al*. 1985) and for appropriate design, execution and monitoring of stock-enhancement programmes (Shaklee & Bentzen 1998). With respect to the latter, a large-scale stock-enhancement for red drum has been ongoing in Texas waters since the 1980s (McEachron *et al*. 1995).

Turner *et al*. (1998) developed several microsatellites from red drum, and a limited number of these were employed by Gold & Turner (2002) to assess population structure of red drum in the northern Gulf. However, additional polymorphic microsatellites are required to carry out large-scale, family printing for use in studies designed to assess larval/juvenile recruitment of hatchery-raised individuals at varying spatial and temporal scales. Large-scale family printing also will permit direct assessment of genetic components contributing to variation in performance and survival of hatchery-produced fingerlings in the wild. Finally, additional polymorphic microsatellites will prove useful as 'anchor' loci in constructing a genetic map for red drum. In this note, we describe development of 38 'new' microsatellites isolated from a red drum genomic library.

Red drum genomic DNA, extracted using a standard phenol–chloroform procedure, was digested with *Sau*3A1 (Promega) and size-selected in the range of 500–1200 base pairs (bp). Gel-purified fragments (QIAEX II gel extraction system, Qiagen) were ligated into a *Bam*HI (Promega)-digested and dephosphorylated (Shrimp Alkaline Phosphatase, Promega) pBluescript vector and transformed into DH10-Beta electro-competent cells via the Gibco BRL™ Cell-Porator® Electroporation System I. The transformation inoculation included 1 mL SOC medium (2% Bacto tryptone, 0.5% Bacto yeast, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose, pH 7.0) incubated at 37 °C for 45 min. Transformed cells were

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plated on LB agar with 50 μg/mL of ampicillin and grown overnight at 37 °C. White (recombinant) colonies were picked using a Genetix QBOT and inoculated into 384-well plates containing 50 μL LB freezing media (36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH4)_2SO_4$, 4.4% (v/v) glycerol, 50 μg/mL Amp, LB) and incubated at 37 °C overnight before freezing at –80 °C.

A total of 13 800 clones (36 × 384-well plates) from the red drum library were spotted onto 22.5 cm × 22.5 cm membranes (Amersham). The membrane contained 13 800 clones in a 4 × 4 array, with each clone spotted twice to rule out false positives on X-ray film (Kodak BioMax). Membranes were placed on LB agar plates with 50 μg/mL of ampicillin and grown at 37 °C until the diameter of each colony reached 1–2 mm (18–24 h). Colonies were fixed to membranes by placing the filters on chromatography paper (3M) soaked in the following solutions: 10% SDS for 3 min, denaturing solution (1.5 mM NaCl, 1.5 M NaOH) for 5 min, neutralizing solution (1.5 mM NaCl, 1.5 Tris) for 5 min, and 2× SSC for 5 min. Filters were incubated for 5 h at 65 °C.

The resulting 13 800 colonies were probed with three separate cocktails of [gamma] ^{32}P-labelled oligonucleotides: (i) dinucleotides $[GT]_{15}$, and $[CT]_{15}$; (ii) trinucleotides $[GTT]_{10}$ and $[AAT]_7$; and (iii) tetranucleotides $[TCTG]_8$ and $[GATA]_8$. A total of 93 positive clones were amplified by PCR by using M13 primers. PCR products were cleaned with QIAquick PCR purification Kit (Qiagen) and sequenced in both directions using ABI PRISM BigDye™ Terminator v3.0 Cycle Sequencing Ready Reaction Kit with Ampli*Taq* DNA Polymerase, FS. Sequences were visualized on an ABI 377 DNA sequencer. A total of 31 complete sequences containing microsatellite arrays were obtained from the 93 positive clones. All 31 sequences have been accessioned in GenBank (Table 1). Six of the 31 clones contained two or more repeat (microsatellite) sequences from which we were able to design multiple primer pairs. A total of 38 primer sets (Table 1) specific for 38 red drum microsatellites were designed using the software OLIGO® 4.0 (National Biosciences, Inc.). Almost all (37 of 38) of the microsatellites were 'perfect' dinucleotide repeats; one microsatellite was a 'perfect' trinucleotide repeat (Table 1). Of the dinucleotide repeats, 21 were $[TG]_n$, 15 were $[CA]_n$ and one was $[GA]_n$. The trinucleotide repeat was $[TCC]_n$.

Unlabelled PCR primers, purchased from Invitrogen (Carlsbad, CA), were tested for reliable amplification by screening a panel of DNA isolated from different red drum individuals. PCR amplifications were performed in a 10-μL reaction volume containing 1 μL (100 ng) DNA, 1 μL 10× reaction buffer (500 mM KCl, 200 mM Tris-HCl [pH 8.4]), 1.5 mM $MgCl_2$, 2.5 mM of each dNTP, 5 pmol of each primer and 0.5 units *Taq* DNA polymerase (Gibco-BRL). Polymerase chain reaction (PCR) was performed in an Omne-E thermal cycler (Hybaid) and consisted of an initial denaturation at 95 °C for 5 min, followed by 30

Table 1 Summary data for microsatellites developed from red drum (*Sciaenops ocellatus*). PCR primer sequences are forward (top) and reverse (bottom). Primers developed from a single clone, and presumably amplifying tightly linked microsatellites, are designated with the same letter subscripts. The fluorescently labelled primer is in bold type. Repeat sequence indicates the repeat motif. *N* is number of individuals assayed; N_A is number of alleles detected. Size range refers to alleles thus far uncovered. H_E and H_O are expected and observed heterozygosity, respectively. Sequences of the clones are listed in GenBank under Accession nos AY161010–AY1040

Microsatellite	PCR primer sequences (5′ → 3′)	Repeat sequence	Annealing temperature	N/N_A	Size range (bp)	H_E/H_O
Soc 400	**TGCCATTGTCATTCTACAGAGC** TTATAGTGGGGTGAGTGTTTGA	$[CA]_{19}$	52	8/7	248–290	0.828/1.000
Soc 401	**ACGTCTTAATCGGTCTCTGTCC** ATCTCTGTGTGAAAGGAAAACA	$[TG]_{14}$	52	7/6	174–202	0.724/1.000
Soc 402	**CATATTTAACGAGCGACATAGC** AAACAGATGAAGCACCTGGACT	$[CA]_{20}$	52	8/5	138–152	0.773/1.000
Soc 403_A	**AGGGAAATGGTTGGTGAAGTAG** GTCTGGACCTGTTTGTTGAGAG	$[TG]_{36}$	58	6/11	273–311	0.903/1.000
Soc 404_A	**AGACCCTTTTGTTGATTTCATA** ATGACTGCACCATTTCAAAAAG	$[TG]_{23}$	52	8/9	159–205	0.859/1.000
Soc 405	**CTTAGCCTTTTGTTTAGTTTCC** CACACTCATGGTCACTCCTCTC	$[CA]_{12}$	56	8/5	188–216	0.656/0.250
Soc 406	**TAGGGGGTAAGGTAGGATGATG** GAAGAGCAGTGACGCTATCAAT	$[TG]_{10}$	52	8/1	167	0.000/0.000
Soc 407	**AAAGTCTGCCTCTTACAGCTTC** GAGTTAAAGCGTGTGCTAGTCC	$[CA]_{13}$	56	7/6	142–158	0.755/1.000
Soc 409_B	**TTTATCTGCTCTGTGTGGAAGT** ATCTATTTGTCGGTTTCTCTGC	$[TG]_{11}$	52	8/7	326–370	0.844/1.000
Soc 410_B	**GTACCAAGTCAGCCAGTGTCAG** TCTCTGTGTCCCTCTGTGTTTG	$[TG]_{17}$	56	8/7	316–332	0.828/1.000

Table 1 *Continued*

Microsatellite	PCR primer sequences (5′ → 3′)	Repeat sequence	Annealing temperature	N/N_A	Size range (bp)	H_E/H_O
Soc 411	TCTGCCTCTTACAGCTTCAAGG **CTTGTTGAGTTAAAGCGTGTGC**	$[AC]_{13}$	54	7/6	147–163	0.765/1.000
Soc 412	**CACAGAAACTCAGCTCGAGACC** AGGAAGAATGTACAAGGTGTTC	$[AC]_{13}$	49	8/6	104–150	0.758/1.000
Soc 415_C	CTCAGCACCCTCAGACATATGG **CACAAGTTAAGTGGTATCGAGT**	$[TG]_{15}$	52	8/6	192–244	0.641/0.750
Soc 416_C	**CTCGATACCACTTAACTTGT** ATCGACATAATCTGGCACCA	$[GA]_{38}$	49	8/6	153–183	0.766/1.000
Soc 417_C	**CTTACGTGATAAAGTGTGGGTGA** ATATGCCAGTAATCCACCGAAG	$[AC]_{24}$	49	8/4	86–108	0.875/1.000
Soc 418	GTTTTTCTGGCATTTATGGATG **TGAGGTTATCAAACACCTGCCCACT**	$[TG]_{24}$	52	8/11	273–295	0.859/1.000
Soc 419	**ATTTAGCCAACTGCTCCGCTCA** GAGTGCGTGGTGTAGGGGGGTA	$[AC]_{20}$	56	8/6	240–254	0.820/1.000
Soc 421	CTCACTGCTCCCTCGTCACACG **CTGTGACAGGATGCGGCTTTTC**	$[TG]_{34}$	56	8/10	138–188	0.883/1.000
Soc 422	CTGAAGGGATGGCAATGTTGATTGG **ATTCTCTGGGTTTATGGGATGT**	$[TG]_{34}$	56	7/6	361–375	0.775/0.714
Soc 423	**GTCACCGCACCATGATGGAGAT** TACCACTTACACTCAGCAGGTG	$[CA]_{26}$	54	8/6	174–200	0.781/1.000
Soc 424	**CACTCTTCATCCCTCACTCGTC** TTCGATGGGTGACAGCGTCAGG	$[CA]_{15}$	56	7/9	202–228	0.816/0.857
Soc 425	**ACACCGCATTGCCCACCAGGAA** CGAGTTTATCCTTCACGCTTG	$[CA]_{14}$	54	8/2	149–150	0.219/0.000
Soc 426	**GAGAGGACGTGAGCTGCTGA** TGAGAAACAGAAACAGAAGGT	$[CA]_{11}$	52	8/4	139–153	0.484/0.625
Soc 428	GACATCGCATTTGTCTACAGAGTCG **AACTCCCAGTCATAATATCCCTTT**	$[TG]_{38}$	53	7/8	188–232	0.847/1.000
Soc 429	AAAAATTCTGCCTGCCTGTG **TTAAGAGCAACCTCCGTCTC**	$[TG]_{12}$	52	8/4	125–133	0.633/0.750
Soc 430	**TAACAGTCCCTAAACAGGTT** GTTTCTCCTCCCCTTTCCTC	$[TG]_{23}$	52	8/10	265–339	0.867/1.000
Soc 431	**GACACGCTGTGGTAGATGAAAACG** TGTATATTAGTTGGCAAGGCAGAG	$[TG]_{29}$	53	8/8	159–181	0.820/0.750
Soc 432_D	**TTTAGGCTACGTCTGGAGGCACA** GTGTGTTTGAGGGTCAGCGTAC	$[AC]_{16}$	52	8/5	98–118	0.703/0.875
Soc 433_D	**AGTACGCTGACCCTCAAACACA** TTCTCTTTGCCTCCTTTTTCCCTGA	$[TG]_{16}$	52	7/6	88–104	0.847/1.000
Soc 434	**GACACTCCCAGATATGCTGA** TCCTTGTTTATCTTGGTGCTGT	$[CA]_{23}$	52	7/7	170–220	0.786/1.000
Soc 435	**AACTGGAGCCTGACTCACTGC** GTGATAACTCTCTTTTCTTGTG	$[AC]_{22}$	49	8/1	173	0.000/0.000
Soc 437_E	**CTACTTTCTAGTCTTTGCTCCACT** GTCAAACGCTATTTTTTCCAGT	$[TG]_{36}$	54	5/7	299–333	0.820/1.000
Soc 438_E	**AATACAGCTAACTCGAAA** ACTGCACCATTTCAAAAACGCCTCT	$[TG]_{24}$	49	7/6	133–155	0.633/0.286
Soc 439	**ACTCTCGTCCCACTTACCACA** TATGTTTGCATATAAGCTCA	$[TG]_{17}$	49	6/4	92–106	0.514/0.667
Soc 442	**TTTGTTGGCAATAAACTGCGAGA** TTCTTAATACGTGCCCCGACT	$[TG]_{30}$	52	8/8	179–199	0.773/0.875
Soc 443	**CACAGGAGGAGTTTGTCCAAT** ATGTTTCGGTTTTCGTTTGCTC	$[TG]_{15}$	52	7/11	206–242	0.898/1.000
Soc 444_F	**TGAACTAATCCAGCCACAGATG** CACAGCCGATTAAAGAGAGGGAAT	$[TG]_{17}$	52	8/3	160–164	0.539/0.625
Soc 445_F	**ATACAAAGGGACTCTCATACTCTC** TTTTAATCCCATTACAGCTTT	$[TCC]_{10}$	52	8/7	135–163	0.781/0.625

cycles consisting of 45 s at 95 °C, 45 s at the annealing temperature, 1 min at 72 °C, and a final extension of 10 min at 72 °C. The PCR product was electrophoresed in a 3% agarose gel, stained with ethidium bromide and photographed under ultraviolet light. Initially, the annealing temperature calculated by OLIGO® was used; amplification conditions, including annealing temperature, were then optimized based on results from agarose electrophoresis. Optimal annealing temperatures were similar across microsatellites, ranging from 49 to 58 °C (Table 1). One primer of each pair was then fluorescently labelled and the primer sets screened in a 6% denaturing polyacrylamide gel, using an ABI 377 DNA sequencer in GENESCAN® mode, version 3.1.2 (ABI 1996a).

Estimates of allele diversity for each of the 38 microsatellites were obtained by genotyping from five to eight red drum sampled off the Texas coast and currently used as broodstock by Texas Parks and Wildlife. Fragment analysis was conducted using the ABI 377, and allele scoring was performed with GENOTYPER® software, version 2.5 (ABI 1996b). Alleles were scored by length in base pairs. Thirty-six of the 38 microsatellites were polymorphic, with the number of identified alleles per (polymorphic) microsatellite ranging from two to 11 (Table 1).

The microsatellites developed in this work will prove useful for a number of studies in red drum, including estimating genetic variation/divergence on a fine geographical scale and kinship analysis. They should also prove useful as 'anchor' loci during construction of genetic maps of red drum and other, economically important sciaenids. Development of multiple primer pairs (microsatellites) from six of the clones should enhance pedigree analysis, as alleles at microsatellites developed from each clone are linked tightly and can be assayed as a single unique haplotype. Such an approach should prove extremely valuable, as it will permit alleles at these microsatellites to be evaluated in an identity-by-descent framework rather than solely by identity-in-state.

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References

ABI (Applied Biosystems Inc) (1996a) GENESCAN *672 Users Manual Revisions*. Perkin-Elmer Corp., Foster City, CA.

ABI (Applied Biosystems Inc) (1996b) GENOTYPER *2.0 Users Manual*. Perkin Elmer Corp., Foster City, CA.

Gold JR, Burridge CP, Turner TF (2001) A modified stepping-stone model of population structure in red drum, *Sciaenops ocellatus* (Sciaenidae) from the northern Gulf of Mexico. *Genetica*, **111**, 305–317.

Gold JR, Turner TF (2002) Population structure of red drum (*Sciaenops ocellatus*) in the northern Gulf of Mexico, as inferred from variation in nuclear-encoded microsatellites. *Marine Biology*, **140**, 249–265.

Goodyear CP (1989) *Status of the Red Drum Stocks of the Gulf of Mexico*. Contribution number CRD 88/89–14. Southeast Fisheries Center, Miami Laboratory, Coastal Resources Division, Miami, FL.

Hilborn R (1985) Apparent stock-recruitment relationships in mixed stock fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 718–723.

Matlock GC (1984) *A basis for the development of a management plan for red drum in Texas*. PhD Dissertation. Texas A & M University, College Station, TX.

McEachron LW, McCarty CE, Vega RR (1995) Beneficial uses of marine fish hatcheries: enhancement of red drum in Texas coastal waters. *American Fisheries Society Symposium*, **15**, 161–166.

Pattillo ME, Czapla TE, Nelson DM, Monaco ME (1997) *Distribution and Abundance of Fishes and Invertebrates in Gulf of Mexico Estuaries, II. Species Life History Summaries* (ELMR Report no. 11). NOAA/NOS Strategic Environmental Assessments Division, Silver Springs, MD.

Shaklee JB, Bentzen P (1998) Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science*, **62**, 589–621.

Sinclair M, Anthony VC, Iles TD, O'Boyle RN (1985) Stock assessment problems in Atlantic herring (*Clupea harengus*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences*, **42**, 888–898.

Turner TF, Richardson LR, Gold JR (1998) Polymorphic microsatellite DNA markers in red drum (*Sciaenops ocellatus*). *Molecular Ecology*, **7**, 1771–1773.