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Microsatellite panels for gene localization in red drum, *Sciaenops ocellatus*

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ABSTRACT

A mapping tool for the economically important, aquacultured species red drum (*Sciaenops ocellatus*) is described. The tool consists of multiplex panels, each of which contains 3–4 microsatellite markers that span individual linkage groups, with a spacing of ≤25 cM in either male- or female-specific maps or a combined map. A total of 27 newly-designed microsatellite markers were added to a previously generated map and then localized to specific linkage groups, using a *de novo* single-pair cross, in order to demonstrate the utility of the tool. All 27 markers were successfully localized when 100 progeny were genotyped; 26 of the markers were localized when 25, 50, or 75 progeny were genotyped. The tool will allow researchers without access to tissue from original crosses to localize genes of interest, including quantitative trait loci (QTL), to specific linkage groups (chromosomes), using a subset of microsatellite markers.

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1. Introduction

A central goal of aquaculture programs is to minimize mortality of fingerlings while increasing biomass of adult fish produced per unit time and effort. These goals can be accomplished in part through selective breeding programs aimed at increasing growth rates and/or decreasing susceptibility to stress such as disease or temperature change. However, these types of phenotypic traits are not easily or rapidly manipulated because they may be influenced by multiple genes or quantitative trait loci (QTL) that have small, often cumulative effects (Liu and Cordes, 2004). Consequently, it is often preferable to employ molecular techniques to develop a large number of DNA markers that can be used to identify chromosomal regions which have detectable effects on traits of interest. Identifying DNA markers in tight linkage association with QTLs then allows for marker-assisted selection (Ribaut and Hoisington, 1998), greatly expediting selective breeding for economically desirable or management-related traits (Lynch and Walsh, 1998). Using linkage relationships, researchers also can identify the location of known genes of interest that may have been previously characterized. To accomplish this it is necessary to first generate a genetic linkage map (Dekkers and Hospital, 2002), an undertaking that requires a large number of markers (Slate, 2005).

In this technical note, we describe a genetic mapping tool that can be used for gene localization and marker-assisted selection in red drum, *Sciaenops ocellatus*, a species that is the focus of both public and private aquaculture in the USA and elsewhere (Hong and Zhang, 2004; Lutz, 1999; Smith et al., 1997; Tringali et al., 2008). The purpose for developing the tool is that gene localization and marker-assisted selection can be problematic for species like red drum because most aquaculture facilities generally will not have access to the individuals and/or tissues from the original crosses that were used to generate the genetic maps, and consequently will need to analyze crosses of their own. The tool presented here provides researchers an efficient means for localizing genes of interest to a chromosomal region and a method to quickly screen for sections of chromosomes linked to QTLs. The tool developed here consists of 83 nuclear-encoded microsatellites divided into 25 multiplex panels. Each panel covers one of 25 linkage groups described previously by Portnoy et al. (2010), where a total of 237 microsatellites markers were mapped. The 83 microsatellites included in the panels were selected to maximize coverage across the 25 linkage groups. The utility of the tool was tested by localizing 27 newly mapped microsatellite markers in a mapping family that had not been genotyped previously.

2. Materials and methods

A total of 27 microsatellite markers were isolated from an enriched genomic library of red drum, *S. ocellatus* (Table 1). Full descriptions detailing generation of enriched microsatellite libraries in our laboratory may be found in Renshaw et al. (2010). Genomic DNA was extracted from fin clips using a modified Chelex extraction protocol (Estoup et al., 1996). After two-minute centrifugation at 16,000×g, 1 μl of supernatant was used directly as a template for all polymerase-chain-reaction (PCR) amplifications. Genotypes at 27 microsatellites were acquired for parents and progeny of three single-pair crosses using the 'tailed' methodology described in Karlsson et al. (2008). Two of the crosses, Family A ($n = 103$) and Family B ($n = 104$),

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Table 1
Summary data for 27 new microsatellites used for gene localization.

Name	GenBank	Primer sequences	Repeat	Clone size	Size range
Soc 744	JF509110	F: TGTCTTCAGATGGACGCAGA R: CAGAGAGGGCTTGTTTGAGG	(CA)4-4bp-(CA)11	236	271–291
Soc 753	JF509111	F: TCCAGCCTGCTCAGATTTTT R: AAAGCAGGATGCAGTTCACTC	(CA)26	155	164–170
Soc 758	JF509112	F: CCAGGATGCCAAGGATACAA R: TGCCTTACACAATGCTGGAG	(CA)6-2bp-(CA)5-2bp-(CA)3-4bp-(CA)15	289	311–385
Soc 759	JF509113	F: GCAGAAAAGCCCTGTTTCAA R: TGCATGCCAATCTCATCATT	(GT)20	210	224–234
Soc 761	JF509114	F: TGCAAACGTTCTGTGAGACC R: CTCCCTTGTCTCTGGGATCA	CA)7	205	224–226
Soc 762	JF509115	F: AAGCAGGCTCAGTCTTCAGC R: CCCCCAAAATTACCAAATCTC	(CA)9	286	303–313
Soc 770	JF509116	F: AGAGCATGGGGGAGTCAGAT R: ACAGACACGCAGGAACATAAT	(GT)8	142	163–169
Soc 773	JF509117	F: CCGCTTCCTGCTGAAAATTA R: TTCGTGCCTAAACCTAACCAA	(TC)21	291	293–323
Soc 778	JF509118	F: ATACACGTAAGCGCACCTGA R: ACGGCCAGACATACAAGGAC	(TG)19	222	223–245
Soc 781	JF509119	F: TCGATCGAGCCACCTAATCT R: AGCGAGCGCTAATATCGTGT	(GT)11	164	185–189
Soc 783	JF509120	F: ATTCCCTGCTCACATCCAAC R: TCCTTCACTGGACACACCAA	(CT)24	106	124–148
Soc 785	JF509121	F: CCTGACAATAGACACAAACACATACA R: CCTTCACTGCAGGTGCTACA	(CA)17	281	295–321
Soc 786	JF509122	F: TCTCTCCCACTTTTATTCTCTTTCTC R: GGAGTGAGAAAGTCCCACGA	(CT)38	179	166–190
Soc 792	JF509123	F: GCACCATAACCTCCCATCAC R: GCCCCGCTTTAAATAACCAT	(GT)9-2bp-(GT)16	200	208–250
Soc 796	JF509124	F: GTTGAGGAGGTCATCGTCGT R: TCACCCTCCTGTCCACTCAT	(CA)17	238	243–261
Soc 800	JF509125	F: AGTTGGTGTGGCTGTTCTGA R: TGCACCACTGACAGACAAAAG	(TG)15	183	197–207
Soc 804	JF509126	F: GTGCTCGATCTCTCCGTCTC R: CCGTTGTCTGGTCCCTCTAA	(CT)9(CA)12	248	266–268
Soc 807	JF509127	F: ACCATTCCTCCGGATCATAA R: GCTGTGCCCAGATTTTCACT	(CA)14	141	158–207
Soc 810	JF509128	F: AACACGCACTTGCTCTCTCA R: AATAAATCGGGGGAAACTGG	(CT)6(CA)10	150	170–174
Soc 812	JF509129	F: AAGGCATCACTTCCAACATTT R: CATGGAGACATCACCGTTTG	(GT)27	143	137–149
Soc 814	JF509130	F: CCTCCCCATAATTGTGCCTA R: TGATATGTGGGGAACTGTGTG	(CA)12	123	139–143
Soc 819	JF509131	F: CATGTGATCCGCTCAATGAC R: CGTGTCACCTGTGGAAACTG	(CA)20-2bp-(CA)5	274	292–306
Soc 825	JF509132	F: CATGCAACATTAGCCCAGTG R: TGTTGATGAGCAGCCTTACG	(GT)12	204	226–234
Soc 826	JF509133	F: GGCAGGATTTAGGCAATTCA R: ACACACTCCTGTGTGCAACC	(GTGA)11	180	183–207
Soc 834	JF509134	F: TGAGAACAGCTCTGCCTCCT R: TCATTCCGTCAATGTTCAGG	(CA)19	248	279–305
Soc 835	JF509135	F: CCTGTGCTCATATGAACAAGA R: CACACAGAATCTTTCAGGGATG	(GT)23	112	114–168
Soc 837	JF509136	F: CAGATGAAGGGAGGGAACAA R: CACACAAACATGCACAAGCA	(TG)14	168	181–207

Size ranges represent results obtained from three mapping families. F is the forward primer, R is the reverse primer, and clone size is the size of the originally isolated clone.

were used previously to create a linkage map (Portnoy et al., 2010) and as such had been genotyped for 237 microsatellite markers. Family C ($n=100$) was generated *de novo* at the Texas Parks and Wildlife Marine Development Center (MDC) in Corpus Christi, Texas, specifically for this project.

A total of 25 multiplex panels were optimized following protocols described in Renshaw et al. (2006). Markers for each panel were selected to optimize coverage across 25 individual linkage groups; the current sex specific genetic maps generated from Family A and B, which now cumulatively include 264 microsatellite markers, may be accessed at <http:wfsc.tamu.edu/doc> under the file name 'Red drum, *Sciaenops ocellatus*, linkage map.' The general strategy was to use as few markers per panel as possible to span a linkage group while leaving gaps no longer than 25 cM between markers in each linkage group. All multiplex reactions were 10 µl, with a final concentration of 1× reaction buffer, 2 mM $MgCl_2$, 0.25 mM each dNTPs, 0.1 U/µl *Taq* polymerase and 1 µl of template, with differing concentrations of primers. Cycling conditions consisted of a denaturation of 95 °C for 5 min, followed by a 'touchdown' protocol where annealing temperatures dropped from 62 °C to a final annealing temperature between 48 °C and 54 °C. For each annealing temperature, there were two cycles of 95 °C for 1 min, annealing for 1 min, and extension at 72 °C for 1 min. For the final annealing temperature, there were 28–32 cycles of 95 °C for 1 min, annealing for 0.5 min, and extension at 72 °C for 1 min. The final cycle was always an extension of 72 °C for 10 min. Descriptions of primers, primer concentrations, dye labels, and details of annealing temperatures used in each 'touchdown' protocol are given in Table 2. The panels were used to acquire genotypes for 83 microsatellites from parents and progeny ($n=100$) of Family C.

PCR amplicons were electrophoresed on 6% polyacrylamide gels and visualized using an ABI Prism 377 sequencer (Applied Biosystems®). Scoring was conducted manually with the aid of GENESCAN 3.1.2 (Applied Biosystems®) and GENOTYPER 2.5 (Perkin Elmer®). Analyses were conducted using LINKMFEX v. 2.1 (R. Danzmann, University of Guelph, http://www.uoguelph.ca/~rdanzman/software/LINKMFEX). Pairwise recombination fractions (θ) and logarithm-of-odds (LOD) ratios were computed for each individual in mapping families A and B. The 27 new microsatellite markers were then assigned to linkage groups in each of the two families, using LOD scores of ≥5.9 in order to exclude microsatellite pairs with θ >0.25 (Danzmann and Gharbi, 2007). A second analysis, using LOD scores ≥4.0, was then undertaken to allow more microsatellites to be incorporated into each linkage group; results of the two assignments were compared for consistency. Finally, all microsatellites that appeared in a given linkage group in any individual in the initial analyses were reanalyzed separately without using LOD criteria. Construction of the map in this hierarchal manner allowed for inclusion of the maximum number of microsatellite markers per individual and linkage group, while ensuring correct groupings and consistent marker order. Finally, the female maps and male maps from mapping families A and B were merged to produce sex-specific linkage maps using the MERGE module in LINKMFEX.

For Family C, a series of analyses were conducted with LINKMFEX using LOD scores ≥3.0. These analyses were conducted first using all 100 progeny, and then using random subsamples of 25, 50, and 75 progeny. Results were compared to the maps produced from crosses involving Families A and B. For each analysis, the number of markers successfully localized (LOD ≥3) was recorded for males and females separately and for both sexes combined. Observed recombination fractions between panel markers and new microsatellites were recorded.

3. Results and discussion

All 27 microsatellite markers were successfully mapped to 19 linkage groups in Family A and/or Family B. With the addition of new markers, two previously identified linkage groups, 24 and 25, were resolved as a single linkage group. This reduced the number of linkage groups to 24, consistent with the previously described, haploid chromosome number in red drum (Gold et al., 1988). In addition the sex-averaged length of the map increased from 1196.9 cM (Portnoy et al., 2010) to 1306.0 cM. Both developments are important as they have increased the coverage and resolution of the red drum linkage map. In addition, this

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Table 2
Twenty-five multiplex panels that comprise the red drum mapping tool developed from 83 markers spread across 24 chromosomes.

Linkage group	Microsatellite	Pmol	Color	TD	Size range
1	*Soc* 11	0.6	6-FAM	62–52	217–240
	Soc 400	1	6-FAM	52, 32 cycles	248–290
	Soc 735	3	HEX		145–223
	Soc 725	4	HEX		80–104
2	*Soc* 416	2	NED	62–52	153–183
	Soc 636	4	6-FAM	49, 30 cycles	110–196
	Soc 243	0.75	6-FAM		91–105
3	*Soc* 504	5	6-FAM	62–52	155–187
	Soc 432	2.5	HEX	52, 32 cycles	98–118
	Soc 83	1	HEX		114–142
4	*Soc* 553	1	6-FAM	62–52	229–249
	Soc 444	2	NED	52, 32 cycles	161–165
	Soc 723	8	HEX		161–224
5	*Soc* 434	3	NED	62–52	170–220
	Soc 625	1.25	HEX	50, 30 cycles	97–112
	Soc 696	6	6-FAM		171–233
	Soc 572	4	6-FAM		113–133
6	*Soc* 412	4	HEX	62–52	104–150
	Soc 691	2	6-FAM	49, 30 cycles	91–137
	Soc 201	3	HEX		224–243
7	*Soc* 419	5	6-FAM	62–52	240–256
	Soc 405	10	6-FAM	49, 32 cycles	187–215
	Soc 621	5	HEX		149–173
	Soc 726	8.5	6-FAM		120–164
8	*Soc* 580	5	HEX	62–52	238–290
	Soc 639	3.5	6-FAM	49, 30 cycles	128–148
	Soc 631	7.5	HEX		168–220
	Soc 638	3.5	6-FAM		215–229
9	*Soc* 623	5	6-FAM	62–52	125–149
	Soc 409	9	HEX	49, 32 cycles	326–370
	Soc 558	5	HEX		166–196
	Soc 711	3	6-FAM		241–267
10	*Soc* 50	13	HEX	62–52	169–191
	Soc 686	4	HEX	49, 32 cycles	211–243
	Soc 423	4	6-FAM		174–200
11	*Soc* 19	4	6-FAM	62–52	195–267
	Soc 573	1.5	HEX	52, 30 cycles	245–307
	Soc 648	1	HEX		158–204
	Soc 688	4	NED		198–242
12	*Soc* 592	2.5	6-FAM	62–54	106–128
	Soc 538	5	HEX	50, 29 cycles	176–198
	Soc 718	0.8	NED		177–203
	Soc 49	5.5	6-FAM		230–238
13	*Soc* 507	1.25	6-FAM	62–52	271–377
	Soc 683	0.75	6-FAM	50, 30 cycles	170–210
	Soc 85	1	6-FAM		80–122
	Soc 716	1	HEX		125–163
14	*Soc* 651	6	6-FAM	62–52	172–242
	Soc 699	5	HEX	50, 29 cycles	147–205
	Soc 715	0.9	6-FAM		92–102
15	*Soc* 508	3.5	HEX	62–52	116–152
	Soc 672	2	HEX	50, 29 cycles	178–222
	Soc 601	3	6-FAM		144–170
16	*Soc* 550	0.7	HEX	62–52	182–216
	Soc 697	0.8	6-FAM	50 29 X	163–179
	Soc 533	2	HEX		81–109
17	*Soc* 731	2	6-FAM	62–52	199–252
	Soc 681	2	HEX	50, 29 cycles	102–132
	Soc 618	2	6-FAM		104–124
18	*Soc* 668	4	HEX	62–52	86–142
	Soc 667	1.5	6-FAM	50, 30 cycles	224–240
	Soc 629	6	6-FAM		126–156
	Soc 424	3	HEX		202–228
19	*Soc* 532	3	6-FAM	62–52	108–140
	Soc 684	5	HEX	50, 30 cycles	164–212
	Soc 547	6	6-FAM		188–204
20	*Soc* 622	20	HEX	62–52	224–256
	Soc 630	2.5	HEX	50, 30 cycles	134–154
	Soc 613	26	6-FAM		189–223
21	*Soc* 680	4	HEX	62–52	85–131
	Soc 687	5	6-FAM	50, 30 cycles	151–185
	Soc 578	7.5	6-FAM		285–323
	Soc 635	7	HEX		229–249

(continued on next page)

Table 2 (*continued*)

Linkage group	Microsatellite	Pmol	Color	TD	Size range
22	*Soc* 656	1	6-FAM	62–52	200–258
	Soc 657	2	HEX	52, 30 cycles	189–247
	Soc 505	2	6-FAM		93–157
23	*Soc* 500	6	6-FAM	62–52	136–174
	Soc 588	1.5/3	HEX	50, 30 cycles	162–200
	Soc 590	4/6	6-FAM		177–213
24a	*Soc* 526	1	6-FAM	62–52	208–252
	Soc 566	1	NED	52, 30 cycles	106-142
24b	*Soc* 664	1.5	6-FAM	62–52	117–149
	Soc 719	1	HEX	50, 30 cycles	169–209

Linkage group(s) are those described in the linkage map available at <http:wfsc.tamu.edu/doc> under the file name 'Red drum, *Sciaenops ocellatus*, linkage map'; Pmol is primer quantity in picomoles; color is fluorescent label used for the forward primer; and TD is the annealing temperatures and number of cycles used in the 'touchdown' protocol. Size ranges were determined previously and can be found at <http:wfsc.tamu.edu/doc> under the file name 'PCR primers for red drum (*Scianenops ocellatus*) microsatellites.'

demonstrates that the addition of a modest number of new markers to a moderately saturated map can provide important information.

Of the 27 new microsatellites mapped, 20 were informative in the male from Family C and 25 were informative in the female of Family C. When the multiplex tool was applied to all 100 progeny from Family C, 19 of 20 microsatellites were localized correctly in the male, while 23 of 25 microsatellites were localized correctly in the female. Cumulatively, all 27 microsatellites were assigned correctly to the linkage groups indicated in the full map. When the multiplex tool was applied to fewer progeny (25, 50, and 75), the fraction of correct assignment decreased for both female and male maps. However, the overall fraction of correct assignment in the combined map was never fewer than 26 of the 27 microsatellites.

None of the microsatellites were assigned to the wrong linkage group in any of the analyses. Observed recombination fractions tended to show a downward bias with smaller progeny sample sizes when pairs of markers had relatively low observed recombination fractions; whereas observed recombination fractions tended to show an upward bias with smaller progeny sample sizes when pairs of markers had higher observed recombination fractions. This suggests that researchers using the tool may want to begin with a limited number of progeny to localize genes or traits of interest to a linkage group, and then increase the number of progeny and use all the markers on a given linkage group for more accurate localization. Performance of the mapping tool is summarized in Table 3.

4. Conclusions

The multiplex tool generated will reduce greatly time, effort, and cost for researchers interested in gene localization and/or marker-assisted selection in red drum. The screening using the 27 newly mapped markers in a *de novo* cross demonstrated that the tool is accurate and fairly precise even when relatively few progeny are screened. The approach employed also can be applied to generate mapping tools for other aquacultured species where genetic linkage maps are available.

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Table 3
Performance of multiplex mapping tool in localizing 27 new microsatellite markers in Family C.

Microsatellite	25		50		75		100	
	M	F	M	F	M	F	M	F
Soc 744	9 (0.00)	9 (0.04)	9 (0.00)	9 (0.08)	9 (0.01)	9 (0.09)	9 (0.01)	9 (0.10)
Soc 753	20 (0.00)	0	20 (0.00)	20 (0.14)	20 (0.00)	20 (0.12)	20 (0.00)	20 (0.09)
Soc 758	19 (0.12)	19 (0.04)	19 (0.16)	19 (0.02)	19 (0.16)	19 (0.03)	19 (0.15)	19 (0.02)
Soc 759	14 (0.08)	0	14 (0.08)	0	14 (0.08)	0	14 (0.08)	0
Soc 761	NA	21 (0.00)	NA	21 (0.00)	NA	21 (0.00)	NA	21 (0.02)
Soc 762	8 (0.08)	8 (0.08)	8 (0.08)	8 (0.12)	8 (0.07)	8 (0.12)	8 (0.10)	8 (0.12)
Soc 770	17 (0.00)	NA	17 (0.04)	NA	17 (0.05)	NA	17 (0.04)	NA
Soc 773	16 (0.04)	16 (0.08)	16 (0.02)	16 (0.16)	16 (0.03)	16 (0.12)	16 (0.06)	16 (0.13)
Soc 778	NA	11 (0.00)	NA	11 (0.00)	NA	11 (0.00)	NA	11 (0.00)
Soc 781	NA	22 (0.00)	NA	22 (0.00)	NA	22 (0.00)	NA	22 (0.00)
Soc 783	24 (0.00)	0	24 (0.04)	24 (0.22)	24 (0.03)	24 (0.21)	24 (0.04)	24 (0.22)
Soc 785	17 (0.00)	17 (0.04)	17 (0.02)	17 (0.10)	17 (0.03)	17 (0.11)	17 (0.02)	17 (0.09)
Soc 786	7 (0.00)	0	7 (0.00)	7 (0.16)	7 (0.00)	7 (0.21)	7 (0.00)	7 (0.26)
Soc 792	7 (0.00)	0	7 (0.00)	0	7 (0.00)	0	7 (0.03)	0
Soc 796	4 (0.04)	4 (0.00)	4 (0.02)	4 (0.00)	4 (0.04)	4 (0.01)	4 (0.03)	4 (0.01)
Soc 800	NA	8 (0.08)	NA	8 (0.14)	NA	8 (0.13)	NA	8 (0.13)
Soc 804	NA	0	NA	0	NA	0	NA	4 (0.25)
Soc 807	21 (0.04)	21 (0.04)	21 (0.02)	21 (0.06)	21 (0.07)	21 (0.05)	21 (0.06)	21 (0.07)
Soc 810	NA	17 (0.16)	NA	17 (0.12)	NA	17 (0.13)	NA	17 (0.10)
Soc 812	0	23 (0.08)	0	23 (0.13)	23 (0.21)	23 (0.12)	23 (0.17)	23 (0.13)
Soc 814	NA	6 (0.13)	NA	6 (0.16)	NA	6 (0.14)	NA	6 (0.12)
Soc 819	2 (0.04)	2 (0.00)	2 (0.10)	2 (0.00)	2 (0.09)	2 (0.00)	2 (0.13)	2 (0.00)
Soc 825	0	10 (0.00)	0	10 (0.00)	0	10 (0.00)	0	10 (0.00)
Soc 826	7 (0.00)	0	7 (0.00)	7 (0.16)	7 (0.00)	7 (0.23)	7 (0.00)	7 (0.28)
Soc 834	12 (0.04)	0	12 (0.04)	12 (0.20)	12 (0.04)	12 (0.17)	12 (0.05)	12 (0.18)
Soc 835	12 (0.00)	12 (0.04)	12 (0.02)	12 (0.02)	12 (0.01)	12 (0.01)	12 (0.02)	12 (0.06)
Soc 837	5 (0.00)	NA	5 (0.00)	NA	5 (0.00)	NA	5 (0.00)	NA

Microsatellites were localized for each sex using 25, 50, 75, and 100 progeny. M and F refer to males and females, respectively. Numbers outside the parentheses indicate the linkage group to which the microsatellite was localized. Numbers inside the parentheses indicate the observed recombination fraction between individual microsatellite and its closest panel marker. NA indicates that genotypes at a microsatellite were not informative; 0 indicates that the microsatellite could not be localized even though it was informative for a given sex.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.aquaculture.2011.06.006.

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