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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.

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 $\theta > 0.25$ (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