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PRIMER NOTE

Isolation and characterization of microsatellite DNA loci from Russell's snapper (*Lutjanus russellii*)

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Abstract

A total of 37 microsatellite loci from the Russell's snapper, *Lutjanus russellii*, were successfully isolated and characterized. Thirty-four loci were polymorphic in *L. russellii* samples. Twenty of the 37 markers did not differ significantly from Hardy–Weinberg expected genotype proportions. Significant linkage disequilibrium was detected in one pairwise comparison. The numbers of alleles and observed heterozygosities in polymorphic loci ranged from two to 16 and from 0.41 to 0.95, respectively. These markers will be useful for studying the population genetic structure of this species.

Keywords: isolation, *Lutjanus russellii*, microsatellite, molecular markers, Russell's snapper

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Russell's snapper *Lutjanus russellii* is a tropical fish distributed throughout the Indo-West Pacific. The artificial propagation and breeding of Russell's snapper have made it an important fishery resource in China since the 1990s (Hong & Zhang 2002). Although microsatellites have been isolated for other *Lutjanus* species, including *L. campechanus* (Gold *et al*. 2001), *L. erythropterus* (LO *et al*. 2006) and *L. argentimaculatus* (Zhang *et al*. 2006), less genetic work has been conducted on *L. russellii*. In this study, we successfully isolated and characterized microsatellite markers by developing and screening a genomic library for Russell's snapper.

Genomic DNA was extracted from the blood of a Russell's snapper captured in Zhanjiang Harbor of the South China Sea, using a standard phenol–chloroform procedure (Sambrook *et al*. 1989). Following digestion with *Hae*III and *Dra*I, almost all DNA fragments were distributed between 200 bp and 1500 bp (Luchezar *et al*. 1993). Digestive fragments were ligated to oligonucleotide A using dATP and inserted into the pUCm-T vector (Bio Basic Inc.), then transformed into DH5α competent cell of *Escherichia coli*. The presence of genomic DNA inserts was confirmed by polymerase chain reaction (PCR) amplification using the M13 universal primers and $(CA)_{15}$ (Lunt *et al*. 1999). A total of 2318 colonies were screened for microsatellites, and 115 positive clones with repeat motifs were isolated and sequenced using an Applied Biosystems 377 automated DNA sequencer from either end or both ends by using standard M13 sequencing primers. Primers for 37 microsatellite loci were designed using the OLIGO 6 software package (Molecular Biology Insights, Inc.) and were consistently amplifiable and scorable with optimized PCR condition.

To assess the variability of the microsatellites, genomic DNA was isolated from the blood of 20 individuals captured in Zhanjiang harbour of the South China Sea (Sambrook *et al*. 1989). PCRs were performed in a 10-μL cocktail containing 10 ng of genomic DNA, 0.5 μM of each primer, 1 μL 10× PCR buffers (Bio Basic Inc., 10 mM Tris-HCl (pH 9.0)), 10 mM KCl, 10 mM ($(NH_4)_2SO_4$, 2 mM $MgSO_4$), 0.2 mM each dNTPs and 1 U of *Taq* plus polymerase. PCR runs began with an initial denaturation step at 94 °C for 3 min followed by 30 cycles at 94 °C for 30 s, 30 s at the annealing temperature (T_a), 72 °C for 45 s, and a final extension step at 72 °C for 5 min. Amplified fragments were size fractionated on 8% polyacrilamide gels and visualized using silver staining. Heterozygosity values, tests of Hardy–Weinberg and genotypic equilibrium were calculated with the package GENEPOP 3.4 (10 000 dememorizations, 100 batches, 5000 iterations per batch) (Raymond & Rousset 1995). Three loci, Lru006, Lru033 and Lru037 were monomorphic. Descriptive statistics for the polymorphic microsatellites were given in Table 1. The numbers of alleles and observed heterozygosity in polymorphic loci ranged from two to 16

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Table 1 Microsatellite primer pairs for Russell's snapper *Lutjanus russellii*. Reported are locus name; sequences for forward (F) and reverse (R) primers; allele size (bp); optimal annealing temperature (T_a); repeat motif; number of alleles (N_a); observed heterozygosity (H_O); expected heterozygosity (H_E); and probability of Hardy–Weinberg equilibrium (HWE *P*). An asterisk denotes a significant deviation from Hardy–Weinberg equilibrium including a Bonferroni correction. GenBank Accession nos EF070346–EF070392

Locus	Primer sequence (5′–3′)	Size (bp)	T_a (°C)	Repeat motif	N_a	HWE *P*	H_E	H_O
Lru001	F: TCCCTCTGTTGTTGAAAG R: CCTGATCTCGATAGTGCC	149	58	$(CA)_{30}$	14	0.083	0.95	0.93
Lru002	F: AGGTCTCCCCTGCAACAG R: CACAACCCCACTTCAAAA	196	58	$(AC)_{22}$	10	0.509	0.90	0.89
Lru003	F: GCATCTGCCTGGGAACTT R: GCAAGAGGCTGTCGGTGT	158	60	$(AC)_{43}$	13	0.196	0.95	0.93
Lru004	F: GATGGCAATGGAAGGCACA R: CTGGGATCTATGAAAGCAAGAG	100	58	$(CA)_{14}$	5	0.068	0.70	0.74
Lru005	F: AACAGGCACATTTTCACA R: GAAGGAGCAGTACCAAGA	216	58	$(AC)_{58}$	12	0.000*	0.70	0.93
Lru008	F: CAGTCTTCCACTTTCATT R: TTGCTACAGTTTCAACCC	130	50	$(CA)_{15}$	4	0.000*	0.25	0.70
Lru010	F: GCAAACGGAGGAAACAAA R: CTGAAGCTCGGATGAGGA	153	60	$(CA)_{28}$	14	0.075	1.00	0.94
Lru011	F: TGTGCTGCTGAGGACTGA R: CACCCTGCGTGCGTAAGT	165	60	$(AC)_{18}$	14	0.992	1.00	0.93
Lru012	F: ATGTTGGCTGAATCGTAG R: GACCAGGTCTCCTTGAGGTT	250	52	$(AC)_{32}$	12	0.059	0.90	0.92
Lru013	F: CATCGGGTATTTAGACAA R: AGTGCCAACTACTGCTTT	212	55	$(CA)_{23}$	6	0.001*	0.55	0.74
Lru014	F: TGGAGGAAAATCTGTCTA R: AGAGTAGCAGGTTTGATG	216	56	$(AC)_{19}$	16	0.498	1.00	0.95
Lru019	F: AAATGCGACATCACCAAC R: TTAGCGTAACCTCAAACTCC	120	56	$(AC)_8$	6	0.000*	1.00	0.64
Lru020	F: CACCCAAGTACACTCATG R: GTGCAGCTTTCTCCGTAT	190	60	$(CA)_{10}$	6	0.060	0.79	0.66
Lru021	F: TGAGGGCATACCGATTTT R: GACCAGGTCTCCCACAGC	126	54	$(CTC)_{12}$	3	0.076	0.29	0.67
Lru022	F: TTGGGGACGGCAGATACA R: GAGGTGGAGTGAAAGAAGATAA	113	57	$(CA)_{10}$	6	0.000*	1.00	0.72
Lru023	F: ACACCACCAGTAAACACCG R: GCTGCTAACACGCTAACC	154	56	$(AC)_8(CA)_2(CA)_5(CA)_4$	6	0.002	0.41	0.53
Lru024	F: CACCCGTTGCGTCAGATT R: TCGTGCAGCGTGGTTTGG	127	57	$(AC)_8$	4	0.003	1.00	0.68
Lru025	F: ACCACGCTGCACGAGATT R: GGCTTATACCGACCCACC	137	66	$(AC)_5(AC)_2(AC)_{10}(AC)_7$	10	0.111	0.79	0.84
Lru026	F: GACCAGGTCTCCGAACGC R: TCGCCTCAGTGAATCCGT	120	57	$(CA)_9(CA)_6$	4	0.000*	0.06	0.61
Lru027	F: GGCTACAGCAGGAAGACT R: TTGGAGGTTGTTGAGGAC	204	56	$(AC)_9$	2	0.256	0.56	0.41
Lru028	F: AGAACCAAACCGACCTGA R: ACCTGTGCCTGTGCTTAC	198	57	$(CA)_8(CA)_5(CA)_5(CA)_2$	7	0.000*	0.84	0.78
Lru029	F: CCGTTACGAAATCATCAG R: TGCCTCCAGACTCAAATA	193	56	$(AC)_9$	4	0.000*	1.00	0.55
Lru030	F: TGCCATTCAGTCCCATTA R: CCAGTCCACAGTTCACCC	239	62	$(CT)_{12}(CA)_7(CA)_{27}(AATA)_7$	13	0.239	1.00	0.91
Lru031	F: TGTCACTTCACCCATTCC R: TTCGCTTTGATATTCACG	181	56	$(CA)_9(AC)_4$	5	0.001*	0.63	0.72
Lru032	F: AGACAGGCTGGAATAACA R: TACTGAATTGAGGACTTT	213	56	$(CA)_3(CA)_4(AC)_{10}(CA)_2$	4	0.066	0.35	0.58
Lru034	F: ACCACCAAAAGTCACAGA R: ATACACCCCTCACGCATC	154	56	$(AACA)_5$	4	0.026	0.40	0.57
Lru035	F: ACCAGGTCTCCTTCATCC R: CTCCAGTCTTCCCTACAT	231	48	$(CA)_8$	2	0.280	0.30	0.43

Table 1 *Continued*

Locus	Primer sequence (5′–3′)	Size (bp)	T_a (°C)	Repeat motif	N_a	HWE *P*	H_E	H_O
Lru036	F: AGGTGCCAGATGAGGTAG R: GGTTGGTAGGTGGAAGAG	155	56	$(AC)_{11}$	9	0.076	0.75	0.85
Lru038	F: AGAACCAAACCGACCTGA R: ACCTGTGCCTGTGCTTAC	198	58	$(CA)_8(CA)_5(CA)_5(CA)_2$	11	0.053	1.00	0.82
Lru039	F: AGTTCTTGTTAGCACCTTT R: TTGTAGTTTCACGAGCAG	221	56	$(AC)_{11}$	7	0.001*	0.90	0.80
Lru040	F: CAATCACCCATGAACACG R: ACATCAGGCTGCAGACGA	223	62	$(AC)_{12}(TA)_3(AT)_9$	7	0.002	1.00	0.86
Lru041	F: CACTTTGCTCTTCTCCCTG R: CTCCTCCATCATTCATTCTC	191	58	$(ATTTT)_3$	0.3	1.000	0.50	0.54
Lru042	F: TTGGGGACGGCAGATACA R: GAGGTGGAGTGAAAGAAGATAA	113	56	$(CA)_{10}$	5	0.182	0.70	0.63
Lru043	F: CACAAATGGGCACAATAA R: GGCAACATGGACGTGTAA	258	56	$(CACT)_{13}$	10	0.273	0.85	0.88

and from 0.41 to 0.95, respectively. Ten microsatellite loci deviated significantly from Hardy–Weinberg expectations including a Bonferroni correction (Bonferroni 1935), indicating either that null alleles are present or that the sample size was too small. Significant genotypic disequilibrium within samples was found in one pairwise comparison (Lru003-Lru004, $P = 0.000$). Collectively, these results indicated that genotypes at pairs of microsatellites which appeared randomly associated and suggested that most microsatellites were inherited in a Mendelian fashion.

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