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TECHNICAL NOTE

Development and characterization of microsatellite markers for the Pacific Oyster *Crassostrea gigas*

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Abstract Thirty-three polymorphic microsatellite DNA markers were developed and characterized for the Pacific Oyster *Crassostrea gigas*, a widespread oyster species with great economic and ecological importance, by the end-sequencing of an in-house fosmid library. The polymorphism of each locus was analyzed by screening 30 Pacific oysters from a natural population. The size of repeat motif ranged from 2 to 6 and the number of alleles per locus ranged from 3 to 9. The observed and expected heterozygosity ranged from 0.080 to 0.880 and from 0.174 to 0.876, respectively. These microsatellite markers will contribute to the increasing genetic studies in *C. gigas*.

Keywords Pacific Oyster · *Crassostrea gigas* · Microsatellite

The Pacific Oyster *Crassostrea gigas*, natively distributed around coast of Asia is now one of the most important economic bivalves produced throughout the world. The large demand and exploitation of this marine mollusk accelerated the development and implementation of genetic management practices and selection breeding programmes.

Microsatellites, also known as simple sequence repeats or SSRs, are a small array of tandemly arranged bases (one to six) spread throughout the genomes, which as DNA markers have been useful tools for many studies in the species (Sekino et al. 2003; Li et al. 2003; Hubert and Hedgecock 2004; Li and Kijima 2006; Wang et al. 2008) as they exhibit high polymorphism and are inherited co-dominantly in a Mendelian fashion. Much more novel and useful DNA markers are stilled needed for the increasing genetic studies. Here we report our work on exploitation of microsatellite markers for *C. gigas*.

All microsatellite loci were identified and isolated by the MISA software (http://pgrc.ipk-gatersleben.de/misa/) from end sequences of an in-house fosmid library and all microsatellite primers were designed using PRIMER3 software (Rozen and Skaletsky 2000). Thirty wild Pacific oysters were used for polymorphism analysis and the extraction of genomic DNA was performed by a standard phenol–chloroform protocol (Sambrook et al. 1989). PCR was carried out in a volume of 15 μl containing 40 ng of template DNA, 0.5 U of *Taq* polymerase, 10 mM Tris–HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTP and 0.3 μM each primer. The thermal cycling conditions were: 95°C for 2 min, then 30 cycles of denaturation at 94°C for 30 s, T_a of each primer pair (shown in Table 1) for 30 s and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. The PCR products were separated by 12% non-denaturing polyacrylamide, stained by ethidium bromide and then photoed by Image-Master VDS (Pharmacia Biotech).

The data including number of alleles, heterozygosity, tests of Hardy–Weinberg equilibrium (HWE) and pairwise check of linkage disequilibrium (LD) were analyzed using the software POPGENE version 1.32 (Yeh et al. 2000). As was shown in Table 1, the size of repeat motif ranged from 2 to 6 and the number of alleles per locus ranged from 3 to 9. The observed and expected heterozygosity values varied from 0.080 to 0.880 and from 0.174 to 0.876 respectively. Twelve loci did not conform to HWE due to the limited

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Table 1 Characterization of 33 novel polymorphic microsatellite loci for the Pacific Oyster *Crassostrea gigas*

Locus ID	GenBank ID	Repeat motif	Primer sequences (5′-3′)	T_a (°C)	Size range (bp)	N_A	H_O	H_E	*P* value
otgfa0_0007_B07	GQ869866	$(TA)_{10}$	F: TATCATCGCGGCAATTCGTG	55	279–295	4	0.467	0.673	0.016
			R: GCAACTTAGCTGGTCGTTCC						
otgfa0_0129_E11	GQ869867	$(CA)_{10}$	F: TGACTGTTCTTCGTACCCATCA	56	155–165	4	0.500	0.705	0.147
			R: AGGTGGAACGAGATTGCCTTT						
otgfa0_0139_G12	GQ869868	$(TA)_6$	F: GTGCTTCAGGGTATCTCTTTCC	56	169–173	3	0.172	0.612	0.000
			R: AGCTACTGCATGGACACGATT						
otgfa0_0142_G12	GQ869869	$(AG)_{10}$	F: CGCAGTAGTCGTGCAATGAG	56	161–167	3	0.174	0.527	0.000
			R: TGCATACTTCCACTCGTCTTTC						
otgfa0_023816	GQ869870	$(TGAAT)_5$	F: ACGACGCCCATTGCTACTTA	58	206–216	3	0.233	0.269	0.410
			R: GGACCGACCAATCAACAGACA						
otgfa0_0732_B03	GQ869871	$(AGCAT)_6$	F: TCTTCTGTGTATGGAGGCGATT	58	204–234	5	0.348	0.764	0.000
			R: TGTGAGCAAGCTACTGTCAATTC						
otgfa0_0764_A03	GQ869872	$(TAAAC)_7$	F: GTAGTTGCCACGAGAACTTCAG	56	162–182	5	0.577	0.762	0.059
			R: CAGCCACGTACATTCCTTTCG						
otgfa0_0691_E12	GQ869873	$(AATTTC)_5$	F: AGAGTGACATAGAATGGGTGAA	57	175–199	4	0.367	0.460	0.826
			R: GGCCAAGTAGTTCTGGAGTAG						
otgfa0_408293	GQ869874	$(CT)_6$	F: ACCCTGGTTTGATCTGAGAAATG	56	118–122	3	0.621	0.673	0.131
			R: TCTAAGGAGTGTTGAGTGTTAGTAG						
otgfa0_0479_H01	GQ869875	$(TGA)_6$	F: CCAACCATGAGAGGGACTTCTA	57	137–146	3	0.586	0.491	0.650
			R: CTTGCAGGCATCTGACATTGAT						
rotgfa0_3707_G06	GQ869876	$(TTC)_7$	F: GGCAAGGTCATTTCCTCTTCTAA	58	121–133	4	0.556	0.721	0.036
			R: GTATTTAAGTTACGGCCTGGACAA						
otgfa0_2462_D04	GQ869877	$(AG)_8$	F: TGGTCATGAATATCACTCTCTGG	56	120–126	3	0.080	0.538	0.000
			R: TGTCCATTGAAGAACTAACAGACC						
otgfa0_0104_H07	GQ869878	$(GA)_{17}$	F: GAACCGTCAAGTACACTGCAATAC	57	180–202	4	0.458	0.702	0.150
			R: GGTCCTATCGCATGAATATGGTTT						
otgfa0_018662	GQ869879	$(TG)_{14}$	F: TCTGTTGAAATTGACTGATGGTCTC	59	176–196	6	0.571	0.750	0.087
			R: TTTGTGGTTGTCAGCTTTCTAGG						
otgfa0_018860	GQ869880	$(TG)_{16}$	F: GAAACAGACACGTAGCATTAGACC	56	171–201	6	0.310	0.845	0.000
			R: ATTATGATTTGTTCGGGCACGTAG						
otgfa0_0277_A01	GQ869881	$(TA)_{10}$	F: GGTTCAATCGCAGGCCCTATC	59	177–183	3	0.481	0.635	0.120
			R: ACATTAGGAGATATAAGCTGGGTGTC						
otgfa0_0303_D07	GQ869882	$(AT)_{10}$	F: CCTGTTTGGGAGGGTATTAACTG	55	157–161	3	0.280	0.589	0.002
			R: CACTACCAGAACTTCACATAGCAT						
otgfa0_0468_E01	GQ869883	$(TTC)_9$	F: GAATGTCAAGGGAATTTGCCCAGT	56	169–199	9	0.815	0.876	0.059
			R: GAACACAGTCCACCGTTTGGT						
otgfa0_106148	GQ869884	$(TAT)_7$	F: GTTTCCAACGGGAAGACCTTATTG	59	166–172	3	0.423	0.567	0.285
			R: ACAAGGTTCAGACCAATACCTATCA						
otgfa0_3837_C08	GQ869885	$(TGA)_{10}$	F: AACTCACGACATGCTGTATCAATC	60	170–191	6	0.552	0.806	0.009
			R: ATGATAGAACCCTGGCTAGAAAGC						
rotgfa0_0011_F02	GQ869886	$(TGA)_{10}$	F: TATTACGACCTGCAATCTGCTATG	57	174–183	3	0.429	0.613	0.134
			R: CGCTGAATACACAATACAAAGACC						
rotgfa0_069213	GQ869887	$(TTA)_9$	F: CTTACTCCGACTTAACGGAAGACC	56	165–177	4	0.577	0.691	0.553
			R: AACGTCCCTAGCGTTTATCATTTC						
rotgfa0_1196_F01	GQ869888	$(TCA)_8$	F: CTTATGCAACATCATAGGGAGGTG	58	132–177	3	0.185	0.174	0.974
			R: GAGTTCGTCATCAATCTTGCTTGT						
otgfa0_2051_C02	GQ869889	$(TCTA)_7$	F: CCCGCTTGGAGTCACTATATCTTA	55	148–192	5	0.786	0.766	0.087
			R: CCTACCTCCTAAAGAAACAGACAT						

Table 1 continued

Locus ID	GenBank ID	Repeat motif	Primer sequences (5′-3′)	T_a (°C)	Size range (bp)	N_A	H_O	H_E	P value
otgfa0_2417_C01	GQ869890	$(TATC)_{14}$	F: CTTTGACTTCGCAGGAACAGAAG	59	128–216	6	0.583	0.810	0.009
			R: GGAGGATATAACAATCTGTGGAGGT						
otgfa0_326555	GQ869891	$(TCCG)_7$	F: CACTTACTACTCCAGCTCGGTTT	58	145–153	3	0.304	0.565	0.047
			R: AGATGCAAGAGCAGTTTGAGAAAG						
rotgfa0_152941	GQ869892	$(AAAC)_7$	F: CACCATTGTCTTCTGGTGCAT	55	175–219	8	0.304	0.850	0.000
			R: TTAAGGTCGATGTTGGATTGCT						
rotgfa0_1610_B08	GQ869893	$(TTCA)_{11}$	F: GTCATTTATGGCGGGATTTATGGT	58	153–197	6	0.583	0.767	0.104
			R: ATTACCTGATGACACCCTGCACTA						
rotgfa0_199966	GQ869894	$(GATG)_8$	F: AGTAGCCTAATCAGCGAAGAGAAT	58	111–143	6	0.880	0.812	0.142
			R: TTTAATTCCACGCGCACTTGTC						
rotgfa0_2687_C04	GQ869895	$(CTGT)_7$	F: TTCCCTCCTTGTATGTCTGTGTTA	57	149–161	3	0.400	0.432	0.338
			R: GCAAGGTCAGAATGAATCGTAGAG						
rotgfa0_2769_C07	GQ869896	$(TATC)_9$	F: GACCACTTGACCAGTATAATAGGA	55	175–211	5	0.565	0.763	0.413
			R: GAGAAGGGTCTCCCAATTTCA						
rotgfa0_3663_G02	GQ869897	$(TCTT)_7$	F: AGTTGCGGGTATTGAAAGTTTGTC	56	136–152	4	0.633	0.685	0.143
			R: GATCAGTGTTTGGATTGTGGATCA						
otgfa0_357583	GQ869898	$(CTATC)_5$	F: GGATGCACGATACAGGATAGAACT	59	137–147	3	0.381	0.563	0.160
			R: ATTGTTGAGCCAATGTGCGTTTC						

T_a annealing temperature; N_A number of alleles; H_O observed heterozygosity; H_E expected heterozygosity; significant deviation from HWE ($P < 0.05$)

sample size and/or the possible presence of null alleles ($P < 0.05$). LD was detected for two pairs of loci, i.e. *otgfa0_0732_B03* & *rotgfa0_069213* and *otgfa0_018860* & *rotgfa0_1610_B08* ($P < 0.05$). All the 33 loci were compared with the 407 microsatellite sequences for *C. gigas* in public database (up to September 3, 2009, NCBI) and no homologies were found, indicating the loci reported here are new microsatellite DNA markers.

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