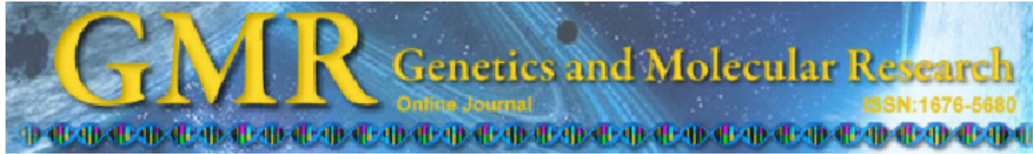

Development and characterization of 60 microsatellite markers in the abalone *Haliotis diversicolor*

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Genet. Mol. Res. 10 (2): 860-866 (2011)
Received November 29, 2010
Accepted February 15, 2011
Published May 9, 2011
DOI 10.4238/vol10-2gmr1182

ABSTRACT. The abalone, *Haliotis diversicolor*, is one of the most important mariculture species in southern China. We developed 60 new polymorphic microsatellite markers for *H. diversicolor* and characterized them in 30 individuals from a cultured population in Sanya, China. All 60 markers were found to be polymorphic. The number of alleles ranged from two to nine per locus, with an average of 4.12/locus. The expected and observed heterozygosities ranged from 0.10 to 0.88 and from 0.07 to 0.87, respectively. Forty-four loci were in Hardy-Weinberg equilibrium. These 44 microsatellite markers should be useful for genome mapping and population genetic studies.

Key words: Abalone; Microsatellite; Aquaculture; *Haliotis diversicolor*

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INTRODUCTION

Haliotis diversicolor, also called small abalone, is distributed throughout the coastal regions of southern China, Vietnam, Korea, and Japan, and is one of the most important commercial mariculture species (Rouvay, 2007). Selective breeding programs for economically important traits, such as disease-resistance and growth rate, have already been initiated (Ke et al., 2000; Shi et al., 2007). They will be greatly enhanced by identifying genetic markers associated with these traits. Genetic markers will facilitate pedigree reconstructions, the development of linkage maps, the identification of quantitative trait loci, and marker-assisted selection within these programs. Genetic markers could also be useful to study natural population structures and dispersal patterns.

Microsatellite markers are the markers of choice for many genetic studies (Schlotterer, 2004). To date, only 22 polymorphic microsatellite markers are available for *H. diversicolor* (Ren et al., 2008; Zhan et al., 2009). In this study, we report the development and characterization of 60 new microsatellite markers for *H. diversicolor*.

MATERIAL AND METHODS

Samples and DNA extraction

Thirty abalone samples were randomly collected from a cultured population in 2007 at Sanya Hongtang Abalone, Ltd., Hainan, China. The cultured population containing 50,000 individuals was produced by random mating.

DNA was extracted from the fresh gastropod muscles using the Cell/Tissue Genomic DNA Extraction kit (TianGen, Beijing, China).

Microsatellite-enriched library construction and sequencing

An enriched microsatellite DNA library was constructed using a selective hybridization and magnetic bead enrichment method (Wang et al., 2009), modified from Carleton et al. (2002). In brief, genomic DNA was digested using *Mbo*I (TaKaRa, Dalian, China). Fragments sized 300-1000 bp were purified from agarose gels using the Gel DNA Extraction kit (TianGen). Following ligation of the fragments to double-stranded *Mbo*I-adapters, the DNA-library was enriched for microsatellite-containing sequences by hybridization with biotin-labeled $(CA)_{12}$, $(GA)_{12}$, $(ACA)_8$, $(AGA)_8$, $(GACA)_6$, and $(GATA)_6$ probes (mixed in advance at a ratio of 3:1:1:1:2:2). Hybridized DNA fragments were captured using Streptavidin MagneSphere® Paramagnetic Particles (Promega, USA). The microsatellite repeat-enriched elute was amplified by the polymerase chain reaction (PCR) using one strand of the adapter. The amplification products were purified with an Ultrafree column (Pall, USA), and cloned using the T-Easy system (Promega). Clones containing potential microsatellite loci were selected and sequenced on ABI 3730xl DNA analyzers at Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China).

Primer design and genotyping

After removing vector and adaptor sequences, DNA sequences were aligned with each other and with 22 *H. diversicolor* microsatellite sequences from GenBank (accessed October

11, 2010) to check for duplications, using Vector NTI Advance 11.0.0 (http://www.invitrogen.com). The MISA software (http://pgrc.ipk-gatersleben.de/misa/) was used to identify sequences containing at least six di-, five tri-, five tetra-, four penta-, and three hexa-, hepta- and octa-nucleotide repeats. PCR primers were designed from unique flanking sequences using Primer 3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi).

PCR was conducted in a final volume of 15 µL and contained ~30 ng template DNA, 1X reaction buffer [20 mM Tris-HCl, pH 8.4, 20 mM KCl and 10 mM $(NH_4)_2SO_4$; TianGen], 1.5-2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 1.0 pmol of each primer pair, 0.4 U Taq polymerase. PCR was performed in an Eppendorf Master Cycler Gradient (Germany) with the following steps: 94°C for 4 min followed by 38 cycles of 94°C for 30 s, annealing (temperatures indicated in Table 1) for 30 s and 45 s at 72°C, followed by a final extension at 72°C for 5 min. Products were separated on 12% polyacrylamide gels and visualized with silver staining. Sizes of alleles were scored by reference to a 50-bp standard base pair ladder (TianGen).

Statistical analysis

GENEPOP on the web (http://genepop.curtin.edu.au/) was used to test for deviations from Hardy-Weinberg equilibrium (HWE) for each locus as well as for linkage disequilibrium (LD) between all pairs of loci (exact tests, 1000 iterations). The ARLEQUIN 3.0 software (Excoffier et al., 2005) was used to calculate observed (H_O) and expected (H_E) heterozygosities. All tests were corrected for multiple comparisons by Bonferroni's correction (Rice, 1989).

RESULTS

One hundred and ninety-five clones were sequenced, of which 114 contained microsatellite repeat tracts. This corresponded to an enrichment efficiency of 58.5%. The most frequent microsatellite type was dinucleotide repeats (75.2%), followed by tetranucleotide repeats (12.6%) and trinucleotide repeats (5.3%). Eighty-four primer pairs were designed. Sixty of 84 (Table 1) primer pairs successfully amplified PCR products. All 60 microsatellites were polymorphic (mean number of alleles = 4.12 ± 1.48, ranging from 2-9 per locus).

The H_E and H_O heterozygosities ranged from 0.10 to 0.88 (mean 0.47 ± 0.20) and from 0.07 to 0.87 (mean 0.40 ± 0.22), respectively (Table 1). No LD was detected between any two loci ($P > 0.05$ after Bonferroni's correction). Sixteen loci (Table 1) showed significant deviation from HWE ($P < 0.05$ after Bonferroni's correction).

DISCUSSION

In this study, we identified 60 polymorphic microsatellites. After characterization of the 60 microsatellites, we identified 44 polymorphic ones that showed HWE. These 44 microsatellite markers will be useful in characterizing wild populations of *H. diversicolor*, assisting the ongoing selective breeding programs of *H. diversicolor* and linkage mapping.

We noticed that 16 of 60 microsatellite loci (26.7%) deviated significantly from HWE, which may be due to the presence of null alleles. This finding suggests a pervasive occurrence of null alleles at microsatellites of this species, which is consistent with the findings in other mollusks including pacific oyster (Hedgecock et al., 2004) and various abalone (Cruz et al.,

Table 1. Primer sequences and characteristics of 60 *Haliotis diversicolor* microsatellite loci.

Locus	Repeat motif	Primer sequences (5'-3')	Ta (°C)	a	as	H_E	H_O	P_{HWE}	GenBank accession No.
HUHD01	$(TG)_n(TGTC)_n$...$(TG)_n(TGTC)_n$	F: GTTAGAAGCTGCACCAAAACG R: TTCATACATGGCCCAAAGAAC	60	9	271-383	0.66	0.57	0.20	FJ607683
HUHD02	$(CA)_n$...$(AAAAAC)_n$	F: TACCCAAAACAGGCATACACG R: TCAGGGAACAGAGAAATGGTG	60	6	334-429	0.76	0.30	0.00*	FJ607684
HUHD03	$(TCAC)_n$	F: AATGTGCGAAGACGATTTCTG R: TTACATCTACCGCGAGCAAAC	60	4	306-402	0.55	0.67	0.12	FJ607685
HUHD04	$(ACTC)_n$	F: CAAATTGCTAATGGCTGAAGG R: TCACGGATATAACATCTAATGGTAAC	60	5	363-403	0.60	0.33	0.00*	FJ607686
HUHD05	$(TGT)_n$	F: ACATAGGCGACAGGTGGTTC R: ACAACAGCACCCTTTCACATC	60	3	158-167	0.46	0.30	0.05	FJ607687
HUHD06	$(ACTC)_n$... $(ACTC)_n$	F: ACCAGTATTCTCACCCGTTCC R: AGTGAATGGTTCAGGGAGGTG	60	3	265-289	0.48	0.77	0.01	FJ607688
HUHD07	$(CTCA)_n$	F: AGCCTCGTTAACTTCCGTGTC R: TGACATCTGTCAACCAGCAAG	60	3	270-298	0.39	0.30	0.18	FJ607689
HUHD08	$(GTGA)_n$... $(GTGA)_n$	F: GCTGGAGTGGCACAGAGTTAC R: TGCTAAAAGCAGAACCAAACC	60	5	250-298	0.41	0.43	0.12	FJ607690
HUHD09	$(TGAG)_n$... $(TGAG)_n$	F: CGTCCTTTACCATCGTTTCC R: ATTTTCACCCCTGGCTGTTC	60	5	261-297	0.45	0.40	1.00	FJ607691
HUHD10	$(GCAC)_n(CA)_n$...$(AAAC)_n$...$(AAAC)_n$	F: CCGATTTAGTAATATTTTACACGCAAG R: TGTCATATCCCGATTTTCTCG	60	5	320-380	0.59	0.50	0.03	FJ607692
HUHD11	$(AACA)_n(AG)_n$	F: TTACAGACGGCTGCCATATAAC R: AGGCGAACTTCATTGTCATTC	60	5	235-325	0.74	0.40	0.00*	FJ607693
HUHD12	$(TCAC)_n$... $(TCAC)_n$	F: GGCCACTGACTGAGAAACATC R: ACAGTTTGACGACACTGCTTG	60	2	210-218	0.16	0.07	0.10	FJ607694
HUHD13	$(TCTG)_n$	F: CGAGCCACTTTGATGTTTTG R: CACCTAGAAAAATGCTTAGTCTCTG	60	5	483-539	0.44	0.63	0.03	FJ607695
HUHD14	$(CTGT)_n(ATGT)_n$	F: GACATCGGTGAATGAAAGTGG R: CTAACAGACCCACGCACAAAC	60	4	223-259	0.39	0.40	0.18	FJ607696
HUHD15	$(GA)_n(GT)_n$	F: TGTCACACGGTTCTGTTTCAG R: AGGACTGTGTTTAACATTATATCTTCG	60	3	188-276	0.70	0.87	0.00*	FJ607697
HUHD16	$(AC)_n(TC)_n(GT)_n(ACTG)_n$	F: TTCGTAGAAATACCTAGGACACAAG R: GTAAAAAGCGGCGTAAAACC	62	3	198-234	0.68	0.27	0.00*	FJ607698
HUHD18	$(TGAG)_n(GT)_n$	F: CCATATCCGCATAGGTCATTC R: GTCAGTGATTTTGCTGCATTG	60	7	193-273	0.70	0.77	0.02	FJ607700
HUHD19	$(CACACG)_n$	F: TGACCAGGTATAACGGCACAG R: GCGTTCGAGGATATTTCATGC	60	5	200-254	0.45	0.47	0.91	FJ607701
HUHD20	$(TGT)_n$	F: CCAAACCTTCATTCTGTTCTTG R: TTCAATAGCACCCACTGACAC	60	4	169-187	0.38	0.17	0.08	FJ607702
HUHD21	$(GA)_n$... $(GA)_n$	F: TTTATTCCACCACACGTCTTTG R: GGTGTACTTTTCCGCATATTCAC	60	4	219-277	0.63	0.27	0.00*	FJ607703
HUHD23	$(AC)_n$	F: CCTTTGTTTTCTTCTGTCATGG R: GAACTGGCGGAAACAAACTC	50	4	230-298	0.43	0.40	1.00	FJ607705

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Table 1. Continued.

Locus	Repeat motif	Primer sequences (5'-3')	Ta (°C)	a	as	H_E	H_O	P_{HWE}	GenBank accession No.
HUHD24	$(TG)_n$	F: TACAGAAAGCCAAGGCAGAAG R: CACCCGTTTAGGTAACAAGAGG	60	2	228-246	0.48	0.47	0.15	FJ607706
HUHD25	$(TATG)_n$...$(TGTGTG\ TT)_n$...$(TG)_n$	F: GCCAGATGTCCGAGTAACAAC R: AGTCTTAAACCTTGCCCCTTG	60	6	242-314	0.70	0.87	0.01	FJ607707
HUHD26	$(GA)_n$	F: ATAAATGCCACGTTTGACAGC R: ATACGAGCGGTTCCTAAGGTG	60	2	250-256	0.10	0.07	1.00	FJ607708
HUHD27	$(GT)_n(GC)_n$	F: CACTTGCACTTGAACATTTGG R: CAGTACTACCGCATAAACAAAACC	60	2	276-330	0.30	0.37	0.55	FJ607709
HUHD28	$(GT)_n$	F: ACAGATTACAAAGACCCAACAGC R: ACATGCATCGTGCATACATTG	55	5	174-196	0.55	0.63	0.85	FJ607710
HUHD29	$(AGTG)_n$	F: TCTCAGCAGGAGTGGACCTAC R: ATTAAATGTTGACGGCGACTG	60	2	185-189	0.47	0.67	0.01	FJ607711
HUHD30	$(CA)_n$	F: CGATTAAAACAGTGGAAAGTGG R: GTTGTATCGTCGAGTCGAGTG	60	4	210-272	0.46	0.53	0.77	FJ607712
HUHD31	$(ACAG)_n$	F: GCTTCAAATGGCTATTGTTGG R: GCTTCAAATGGCTATTGTTGG	55	5	191-211	0.44	0.47	0.30	FJ607713
HUHD33	$(CA)_n$	F: CGTGGTGCTTCTTTTGTTTTC R: AACCTTCAATACCTGCAATCG	60	6	233-269	0.64	0.23	0.00*	FJ607715
HUHD34	$(ACA)_n$	F: CGCCATTTTGGACAGTATTTG R: TGGTTGTAGCCTAAACATCAACTG	60	3	333-354	0.37	0.40	1.00	FJ607716
HUHD35	$(CATA)_n(AC)_n$	F: ATGCTCAGGGCTCTTTTGTG R: CCTCACTCGGAAGAAGCAAC	60	3	472-508	0.59	0.37	0.00*	FJ607717
HUHD36	$(ACGC)_n(CA)_n$	F: GCCAACATTTATGGCTAGGTG R: GAGTCAGGATTGCGGAGTATG	55	5	370-418	0.38	0.33	0.90	FJ607718
HUHD37	$(TG)_n$... $(TG)_n$	F: CCACTTGTCATTGGAGATAACG R: TCATCACACCCTTCTGAATTTG	60	4	198-236	0.67	0.57	0.10	FJ607719
HUHD38	$(AC)_n$	F: CACATTGAAAGTACCAGGAACG R: TTGTTTCACTCTGATTCCCAAG	60	3	190-258	0.72	0.97	0.00*	FJ607720
HUHD39	$(CT)_n$	F: GCCTACTTGGAAATGAAAGCAC R: CGCATGTGGACAACAAATTAAC	60	4	208-226	0.13	0.10	1.00	FJ607721
HUHD40	$(ATAC)_n(ATGC)_n(ATGA)_n(GA)_n$	F: TCATTATTTCCACGCATCTGG R: TCGCCAAGGTTTAGACACATC	60	2	226-250	0.33	0.37	0.55	FJ607722
HUHD41	$(TG)_n$... $(TG)_n$	F: TCGCTGGTGTAATTGATGTTG R: CGGCTTAGATTCAGTGCAGAC	60	4	229-257	0.46	0.47	0.02	FJ607723
HUHD42	$(AC)_n(GC)_n$... $(AC)_n$	F: TCTCGGTGACTCAAAATCTGG R: AAAATAGGGGAGGGGAGAGTG	60	3	196-236	0.65	0.43	0.02	FJ607724
HUHD43	$(GA)_n$	F: TTGGGTCAGAGTGCCATAAAC R: TGACGTGCAGTGTAGTGTGAAC	60	5	207-265	0.65	0.70	0.20	FJ607725
HUHD44	$(CT)_n$	F: AACAGACCGCCTCCATATAGC R: ACGGAGTGACAGACAGATAATGAC	62	4	387-493	0.22	0.07	0.00*	FJ607726
HUHD45	$(ACTC)_n$	F: CAAAGACCCAGGTTCAATTCC R: TCCCATTGAGTGCTGGTTTAG	60	5	194-226	0.50	0.43	0.13	FJ607727

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Table 1. Continued.

Locus	Repeat motif	Primer sequences (5'-3')	Ta (°C)	a	as	H_E	H_O	P_{HWE}	GenBank accession No.
HUHD46	$(GTGA)_n(TG)_n$	F: TGCTGTGCAAATAATCCTTGAC R: CCGGATGTTTCAACGTGTTAC	55	7	333-465	0.72	0.20	0.00*	FJ607728
HUHD47	$(GA)_n$... $(GA)_n$	F: TCAATGTTACCAGCAGAAGCAG R: ACTGAGGATAACGTGCAATCG	60	6	190-244	0.64	0.50	0.00*	FJ607729
HUHD48	$(AC)_n$	F: AACACCCGAATCATACAGACAC R: CCTTGTTTCCAATGTTGATGTC	55	5	300-348	0.33	0.37	0.55	FJ607730
HUHD50	$(AAG)_n$	F: ACATCATTCAGGGTGGTGTTC R: TCTTGTACGATGGGTCGAAAG	60	3	122-131	0.38	0.10	0.00*	FJ607732
HUHD51	$(CA)_n(CGCA)_n$	F: ATATTAATACTTGGCCGTGCTG R: CATGGGGTTGGTACATCTCTG	50	2	191-199	0.22	0.13	1.00	FJ607733
HUHD52	$(TC)_n$	F: ATTTCAGGCGATGGAATGTG R: CGTACTTGGCGTGGAATTG	60	4	265-291	0.64	0.63	0.00*	FJ607734
HUHD53	$(CA)_n(CACT)_n$... $(CACT)_n$	F: TCATGCTGAGGATTTTGGTTC R: GGCATTGTTTGAGTGGTTGAG	50	7	128-164	0.88	0.70	0.01	FJ607735
HUHD54	$(GTT)_n$	F: ATGCAAGATGTGGCTTGATTC R: GGGTTAGCAGCATACAACAGG	60	5	175-205	0.77	0.80	0.00*	FJ607736
HUHD55	$(ACTC)_n$	F: ACGTTATGGCTGAAACACTGC R: GGGACAGCTGGAAAAGAAAAC	62	2	198-206	0.19	0.10	0.17	FJ607737
HUHD56	$(ACAA)_n$	F: GAGGAGTAAGCGTGGTTGATG R: ACTTGGGTGAAAGTGTATGTGC	50	2	182-194	0.10	0.07	1.00	FJ607738
HUHD57	$(TCAG)_n(ACTC)_n$	F: TGTGAAGCCATTTCTAGTGTCC R: CAAACACGCTGCTTCCATAC	60	3	256-300	0.30	0.30	1.00	FJ607739
HUHD58	$(GA)_n$	F: CCAGCGATACTATGACCCAAG R: GATTGCCCGAAATGAAAACTC	62	3	159-192	0.60	0.07	0.00*	FJ607740
HUHD59	$(TGT)_n(ATG)_n$	F: GCGTATTGGAAGAGGGATAAAG R: CCCCCTTCTATCTGAATTGTTG	60	3	226-250	0.22	0.23	1.00	FJ607741
HUHD60	$(CT)_n$	F: CTTAGGAAATGGCAGCAAGC R: GATCACTGCAAGTCTTAACATCC	60	6	143-173	0.52	0.33	0.03	FJ607742
HUHD61	$(AC)_n$	F: ACAAAGTCATCGCAAACAAGG R: ACGGGCAAGTTCACTGATATG	60	4	200-212	0.25	0.23	1.00	FJ607743
HUHD62	$(GACA)_n$	F: TATGTTCCGGACAGAGTACCG R: CGAGGCTCTTTTTGATGTGAG	60	3	205-213	0.53	0.53	0.22	FJ607744
HUHD63	$(AAAT)_n$	F: AGGGTACACATTTGGTGACGTAG R: AATCCGGAAAATACCGTCTTG	60	2	218-226	0.16	0.13	1.00	FJ607745
HUHD64	$(GTCCGCA)_n$	F: ATTCAACGGTACGTTTTCACG R: CCACATGAAACTCTGGAGCAG	60	3	226-254	0.22	0.20	1.00	FJ607746

Ta = annealing temperature; a = number of alleles; as = allele size range in bp; H_E = expected heterozygosity; H_O = observed heterozygosity; P_{HWE} = probability of Hardy-Weinberg equilibrium. *Significant deviation from HWE ($P < 0.05$ after Bonferroni's correction).

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2005; Baranski et al., 2006; Ruhan et al., 2008). Null alleles tend to underestimate heterozygotes in natural populations and cause deviations from HWE. The occurrence of null alleles can pose a problem for the use of relevant microsatellite markers in population studies, although null alleles may not be a problem in mapping studies (Wang and Guo, 2007), especially for organisms with large family sizes (e.g., marine mollusks) (McGoldrick et al., 2000). Furthermore, the presence of null alleles can cause an overestimation of F_{ST} and some reduction in the percentage of correctly assigned individuals in parentage assignment (Carlsson, 2008). Therefore, caution must to be taken when these markers are used.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#40866003 and #31060354), the Special Program of the National Basic Research Program of China (#2009CB126005), and Agricultural S&T Commercialization Fund of Ministry of Science and Technology of China (#376).

REFERENCES

Baranski M, Rourke M, Loughnan S, Austin C, et al. (2006). Isolation and characterization of 125 microsatellite DNA markers in the blacklip abalone, *Haliotis rubra*. *Mol. Ecol. Notes* 6: 740-746.

Carleton KL, Streelman JT, Lee BY, Garnhart N, et al. (2002). Rapid isolation of CA microsatellites from the tilapia genome. *Anim. Genet.* 33: 140-144.

Carlsson J (2008). Effects of microsatellite null alleles on assignment testing. *J. Hered.* 99: 616-623.

Cruz P, Ibarra AM, Fiore-Amaral G, Galindo-Sánchez CE, et al. (2005). Isolation of microsatellite loci in green abalone (*Haliotis fulgens*) and cross-species amplification in two other North American red (*Haliotis rufescens*) and pink (*Haliotis corrugata*) abalones. *Mol. Ecol. Notes* 5: 857-859.

Excoffier L, Laval G and Schneider S (2005). Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* 1: 47-50.

Hedgecock D, Li G, Hubert S, Bucklin K, et al. (2004). Widespread null alleles and poor cross-species amplification of microsatellite DNA loci cloned from the Pacific oyster, *Crassostrea gigas*. *J. Shellfish Res.* 23: 379-385.

Ke C, Tian Y, Zhou S and Li H (2000). Preliminary studies on hybridization of three species of abalone. *Mar. Sci.* 24: 39-41.

McGoldrick DJ, Hedgecock D, English LJ, Baoprasertkul P, et al. (2000). The transmission of microsatellite alleles in Australian and North American stocks of the Pacific oyster (*Crassostrea gigas*): selection and null alleles. *J. Shellfish Res.* 19: 779-788.

Ren P, Wang Z, Yao C, Liu Y, et al. (2008). Development of 11 polymorphic microsatellite loci in the small abalone (*Haliotis diversicolor* Reeve). *Mol. Ecol. Res.* 8: 1390-1392.

Rice WR (1989). Analyzing tables of statistical tests. *Evolution* 43: 223-225.

Rouvay RW (2007). Abalone ranching: a review on genetic considerations. *Aquacul. Res.* 38: 1229-1241.

Ruhan S, Nicola RR, Nicol CVDB, Darrell LL, et al. (2008). Isolation and characterization of 63 microsatellite loci for the abalone, *Haliotis midae*. *J. World Aquacult. Soc.* 39: 429-435.

Schlotterer C (2004). The evolution of molecular markers-just a matter of fashion? *Nat. Rev. Genet.* 5: 63-69.

Shi Y, Wang Y, Qu Y, Ye H, et al. (2007). RAPD analysis on the first generation from inbreeding and crosses of wild and cultured abalone, *Haliotis diversicolor* Reeve. *Mar. Fish. Res.* 28: 47-53.

Wang Y and Guo X (2007). Development and characterization of EST-SSR markers in the eastern oyster *Crassostrea virginica*. *Mar. Biotechnol.* 9: 500-511.

Wang Y, Wang A and Guo X (2009). Development and characterization of 30 polymorphic microsatellite markers for the Atlantic surfclam, *Spisula solidissima* (Dillwyn, 1817). *Mol. Ecol. Res.* 9: 1264-1267.

Zhan X, Hu HY, Ke CH, Hu SN, et al. (2009). Isolation and characterization of eleven microsatellite loci in small abalone, *Haliotis diversicolor* Reeve. *Conserv. Genet.* 10: 1185-1187.

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