GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

# Characterization of 10 novel microsatellite loci for the brown marbled grouper, *Epinephelus fuscoguttatus* (Serranidae)

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Genet. Mol. Res. 10 (2): 885-888 (2011)
Received October 21, 2010
Accepted December 15, 2010
Published May 17, 2011
DOI 10.4238/vol10-2gmr1117

**ABSTRACT.** *Epinephelus fuscoguttatus* is a commercially important marine fish species in southeast Asia. Due to overfishing and water pollution, this species has been declared as near-threatened. Thus, to provide information to help maintain and preserve the species, microsatellites were developed, using an enriched genomic library method. Thirty individuals were collected from the hatchery of the Fishery Research Institute, Terengganu, Malaysia. These individuals, from four to six years old, originated from Sabah and are maintained in captive culture as broodstock. Genomic DNA was extracted from the fins of selected individuals that weighed 3-8 kg. Ten microsatellite loci were found to be polymorphic in this population, with 5 to 21 alleles per locus. Observed and expected heterozygosities ranged from 0.53 to 0.97 and 0.59 to 0.95, respectively. Only one locus deviated significantly from Hardy-Weinberg equilibrium and no significant linkage disequilibrium was found among the pairs of loci. These

 

polymorphic microsatellite loci will be used by the Malaysian Fishery Research Institute for investigating genetic diversity and for developing breeding strategies.

**Key words:** *Epinephelus fuscoguttatus*; Enriched genomic library; Microsatellite; Grouper

## INTRODUCTION

Grouper is a member of the large fish family Serranidae, which is divided into 5 sub-families, i.e., Anthiinae, Epinephelinae, Grammistinae, Liopropomatinae, and Serraninae. Most commercial groupers are found in the Epinephelinae sub-family (Heemstra and Randall, 1993).

The fish is an important marine-cultured species in Malaysia, Brunei, China, Korea, Kuwait, Philippines, Hong Kong, Taiwan, Indonesia, Thailand, Singapore, and UAE (FAO, 2010). However, there is a decrease in resources as *Epinephelus fuscoguttatus* (or brown marble grouper) faces many threats, particularly due to the effects of overfishing and water pollution. Its status has been proclaimed as "near threatened" by the International Union for Conservation of Nature and Natural Resources (IUCN). To date, there is still limited number of publications focusing on the genetics of these species.

Several studies have been conducted using different molecular markers, such as microsatellites for broodstock management (Lo and Yue, 2007) and hybrid identification (GenBank, accession No. GQ912319-328); cytochrome b for population studies (Ding et al., 2006); cytochrome oxidase 1 for DNA barcoding (GenBank, accession No. EU600139-140), 16S and 12S markers for revising classification of the Epinephelini (Craig and Hastings, 2007), and control region for the study of genetic variation (GenBank, accession No. EU518640-647). Microsatellite markers exhibit high levels of polymorphism and abundance, and they are co-dominantly inherited; these features make them suitable for assessing genetic variability. To date, there are 218 microsatellite sequences available in GenBank for *E. fuscoguttatus*. In order to retain exploitation and conservation of *E. fuscoguttatus*, adequate polymorphic microsatellite markers are required. Therefore, we developed and characterized novel microsatellite markers for *E. fuscoguttatus*.

## MATERIAL AND METHODS

Thirty fish samples of tiger grouper were obtained from the *ex situ* Fishery Research Institute (FRI) site, located at Besut Terengganu, Malaysia. These samples originated from Sabah, Malaysia, and have been maintained by captive breeding by FRI. DNA was isolated using the MasterPure™ Complete DNA and RNA Purification kit (Epicentre, USA). Polymerase chain reaction (PCR) was then performed using primers that were designed from our previous study, which are now available in the GenBank database (data not shown). Microsatellites were isolated using the method of Glenn and Schable (2005) by employing an oligonucleotide repeat probe label with biotin. Approximately, 43 primer pairs were initially designed from these sequences using the Primer Premier 5.0 software. Screening for polymorphisms was then carried out in the 30 individuals using PCR conditions that were optimized for each primer pair. Amplifications were performed using a thermal cycler (MyCycler™, BioRad, USA) in a 10-µL

 

volume containing 1X Green GoTaq PCR Buffer (Promega), 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each primer, 0.5 U *Taq* DNA polymerase and approximately 100 ng genomic DNA. The amplifications were programmed using the following conditions: initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, at primer-specific annealing temperature for 30 s (Table 1), at 72°C for 90 s extension, with a final extension step at 72°C for 5 min. Amplified PCR products were mixed with Hi-Di formamide and a 500 LIZ size standard, and the mixture was then denatured for 5 min at 95°C. The sample was then loaded into an ABI PRISM® 3130 DNA Sequencer and subjected to the fragment analysis protocol. Allele scoring was done using the GeneMapper 4.0 software (Applied Biosystems). Expected and observed heterozygosities ($H_O$ and $H_E$) and deviation from Hardy-Weinberg equilibrium (HWE) were determined using POPGENE 1.32 (Yeh et al., 1999). The MICRO-CHECKER 2.23 software (Van Oosterhout et al., 2004) was employed to detect the most probable technical cause of departure from HWE, including null alleles and miss-scoring due to stuttering. Multiple test results were adjusted using Bonferroni's correction (Rice, 1989).

**Table 1.** Characterization of 10 polymorphic microsatellite loci in *Epinephelus fuscoguttatus*.

| Locus | Repeat sequence | Primer sequence (5'- 3') | $T_a$ (ºC) | $N_a$ (size range, bp) | $H_O$ | $H_E$ | P | Accession No. |
|---|---|---|---|---|---|---|---|---|
| EF001 | $(TG)_{11}$ | F: ACAGCGAGGAGTCAAGC<br>R: CCCTGGCAAACTCTGATG | 56.0 | 8 (229-243) | 0.80 | 0.78 | 0.9337 | HM149347 |
| EF006 | $(AC)_{31}$ | F: CGTAACAAAGGACCAAC<br>R: GAAGCACAACATAGAGGG | 52.6 | 21 (162-218) | 0.97 | 0.95 | 1.0000 | GU799180 |
| EF008 | $(TTG)_{11}$ | F: CTGGGACTCAGGGATGAT<br>R:GATTTCCTGTTGTCAAGATC | 53.1 | 5 (170-182) | 0.57 | 0.70 | 0.0038* | GU799184 |
| EF011 | $(CA)_{19}$ | F: AGCAAGGCACCGAACCCC<br>R: CTAAAAGGAAAACGTCAG | 53.8 | 7 (176-208) | 0.63 | 0.66 | 0.4436 | GU799189 |
| EF013 | $(CA)_{23}$ | F: TTTTCCATGATCGCGTAA<br>R: GAAGCACAACATAGAGGG | 50.4 | 12 (170-234) | 0.70 | 0.59 | 1.0000 | GU799202 |
| EF014 | $(CA)_{36}$ | F: CACCCACAGGTAATAAG<br>R: AGCCAAAGGGATGTAATG | 50.8 | 12 (173-199) | 0.97 | 0.85 | 0.9945 | GU799203 |
| EF024 | $(CA)_{15}$ | F: CCCTTGCTCCACTTCTTC<br>R: CATCACTTCCTGGTCCCT2 | 55.2 | 19 (148-208) | 0.87 | 0.92 | 1.0000 | GU79913 |
| EF046 | $(GAAT)_{10}$ | F: AGGCGCTGTCTGTAATG<br>R: TAGGGCAGCACAGAAGAT | 52.6 | 8 (113-147) | 0.53 | 0.80 | 0.1947 | GU799310 |
| EF055 | $(GT)_{34}$ | F: AACCCAGCAGAGAACGGAG<br>R: CCTTTTTCTTTTCCCACCT | 54.1 | 18 (210-268) | 0.93 | 0.94 | 1.0000 | GU799170 |
| EF062 | $(TG)_{29}$ | F: TTTACTCGTGTCCAAAGTC<br>R: CTGAGTCAGTTGAGCCTAC | 56.2 | 16 (198-258) | 0.80 | 0.84 | 1.0000 | GU799261 |

Ta = annealing temperature; Na = observed number of alleles; HO = observed heterozygosity; HE = expected heterozygosity; P = probability; *indicates significant deviation from HWE after Bonferroni's correction ($P < 0.0051$).

## RESULTS AND DISCUSSION

In the present study, we developed and characterized 43 microsatellite markers based on the sequences available at GenBank (accession Nos. GU799132-GU799319 and HM149347). Ten loci were characterized, with the number of allele per locus ranging from 5 to 21, and $H_O$ and $H_E$ ranged from 0.53 to 0.97 and 0.59 to 0.95, respectively (Table 1). Twenty-five markers were monomorphic and were excluded from the analysis. Eight primer pairs amplified too many bands or produced nonspecific bands. These were also not included in the data analysis.

Only one locus (EF008) deviated from HWE in the population sampled after Bonferroni's correction (adjusted P = 0.0051), possibly due to the presence of a null allele or exis-

tence of a subpopulation (Xu et al., 2010); the remaining nine loci were in HWE. The presence of null alleles was confirmed by MICRO-CHECKER for locus EF008 and EF046; but there was no evidence of stuttering and allelic dropout in all loci. All pairwise tests for genotypic linkage disequilibrium between pairs of the 10 loci were non-significant. These indicate that none of the 10 locus studied derive from the same chromosome in the genome.

## CONCLUSION

These polymorphic microsatellite markers could be useful for studying population genetic structure and conservation of *E. fuscoguttatus*, besides designing broodstock breeding and management programs to meet commercial demand.

## ACKNOWLEDGMENTS

Research supported by the Ministry of Science, Technology and Innovation under grants of Agro-Biotechnology (ABI) Initiatives (project #07-05-ABI-AB005).

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