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TECHNICAL NOTE

Ten novel tetranucleotide microsatellite DNA markers from Asiatic black bear, *Ursus thibetanus*

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Abstract Ten polymorphic microsatellite markers were developed for the endangered Formosan black bear (*Ursus thibetanus formosanus*) from a partial genomic library enriched for GAAA repeat. Polymorphism of these loci was evaluated in 27 Formosan black bear specimens of unknown relationship. The number of alleles per locus ranged from 5 to 15 and the observed heterozygosity of each locus ranged from 0.556 to 0.889. These loci should provide useful molecular tools to study conservation genetics of the Formosan black bear and other Asiatic black bears.

Keywords Tetranucleotide microsatellite · Tailed primers · *Ursus thibetanus* · Formosan black bear

The Formosan black bear (*Ursus thibetanus formosanus*) is an endemic subspecies of Asiatic black bear inhabiting Taiwan (Wozencraft 2005). Similar to all other Asiatic black bears, degradation and fragmentation of habitat as well as poaching have caused a decrease in population and distribution of the Formosan black bear (Wang 1999; Hwang and Wang 2006). To formulate proper conservation strategies, it is important to understand the genetic diversity and genetic structure within and among populations of this subspecies.

In this study, we reported ten novel easy-scored polymorphic tetranucleotide repeat (GAAA) microsatellite loci from the Formosan black bear. We followed the protocol developed by Hsu et al. (2003) to enrich microsatellite-contained fragment in a partial genomic library. The library was constructed from genomic DNA which extracted from tissue sample of a Formosan black bear individual using the proteinase *K*-chloroform method (Sambrook et al. 1989). Microsatellite-enriched PCR (polymerase chain reaction) library was ligated into pGEM-T Easy vector (Promega) and transformed into *Escherichia coli* DH5α. A total of 880 clones were lifted to Hybond-N + membranes (Amersham Pharmacia Biotech) and hybridized with [γ^{32}P] ATP end-labelled $(GAAA)_{10}$ oligonucleotides, then 56 hybridized clones were sequenced using DYEnamic ET Dye Terminator Cycle Sequencing Kit for MegaBACE (Amersham Bioscience) on a MegaBACE 1000 autosequencer (Amersham Bioscience). Sequences were proofread using software SEQUENCER 4.2 (Gene Codes). We found 47 clones with microsatellite motif, of which 33 loci containing more than 10 units of GAAA motif were chosen to design the PCR primers.

All forward primers were 5′-tailed with an M13-tail (5′-GGAAACAGCTATGACCAT-3′) or a CAG-tag (5′-CAG TCGGGCGTCATCA-3′) (Schuelke 2000; Boutin-Ganache et al. 2001). DNA extracted from tissue samples of 17 Formosan black bears and from faecal samples of ten Formosan black bears with unknown relationship were used to characterize these 33 loci. PCRs were set up in

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