

Win Chaeychomsri 2008: Development of Molecular Markers for Identification of Thai Crocodiles. Doctor of Philosophy (Agricultural Biotechnology), Major Field: Agricultural Biotechnology, Interdisciplinary Graduate Program. Thesis Advisor: Associate Professor Voravit Siripholvat, Ph.D. 74 pages.

Microsatellite DNA marker was developed from a wild caught *Crocodylus siamensis*. Genomic DNA was extracted from whole blood cells and digested with *Taq* I, and DNA fragments in the range of 500 to 1000 bp were ligated into pBluescript II KS+ phagemids and transformed into fresh competent XL1-Blue *E. coli* host cells by heat shocking. Positive clones were transferred to Hybond N membranes and hybridized with synthetic oligonucleotide microsatellite probe d (AC)₁₅. The ligated target-plasmid DNA was isolated and recombinants that containing microsatellite repeats were sequenced automatically. Primers were designed for microsatellite loci containing dinucleotide repeats. Sequences of 22 positive clones were submitted in GenBank (accession numbers EF413033-EF413054). Twenty microsatellite loci were evaluated in 5 captive breeding crocodile farm for species testing. Ten (50 % of total microsatellite primers) of the 20 microsatellite loci were polymorphic. These primers were successful across eighteen loci for the 40 crocodiles of 29 *C. siamensis*, 4 *C. porosus*, 5 *Caiman crocodilus crocodilus* and 2 hybrids. Fourteen microsatellite loci showed specific allele were considered as specific markers for identify species of crocodiles. These markers composed of CS-25 for identify *C. siamensis*, CS-4, CS-26 and CS-30 for identify *C. porosus*, CS-2, CS-7, CS-10, CS-12, CS-14, CS-17, CS-22, CS-24 and CS-35 for identify *C. c. crocodilus* and CS-20 that can identify all species. Twelve microsatellite loci showed polymorphic alleles in the same species that were considered as intra-specific markers. These markers composed of CS-4, CS-5, CS-10, CS-12, CS-17, CS-18, CS-21, CS-24, CS-26, CS-30, CS-32, and CS-35. Seven microsatellite loci showed absent of allele in *C. c. crocodilus* that were considered as genus-specific markers. These markers composed of CS-4, CS-5, CS-18, CS-21, CS-26, CS-30, and CS-32. These microsatellite loci can be used for species testing and also evaluated for assigning parentage in situations where maternity, paternity, or both may not be known. It is therefore recommended that more microsatellite markers be used in these situations. The use of these microsatellite markers will broaden the scope of a breeding program, allowing progeny to be tested from adults maintained in large breeding lagoons for selection as future breeding animals.



Student's signature

 1, 1 May, 2008

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