Tetranucleotide microsatellites from the loggerhead sea turtle (*Caretta caretta*)

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Abstract

We describe primers and polymerase chain reaction conditions to amplify 15 tetranucleotide microsatellite loci from the loggerhead sea turtle (*Caretta caretta*). The primers were tested on 30 individuals that nested along the Georgia, USA coast. The primer pairs developed in this study yielded an average of 13.9 alleles per locus (range of 10–21), an average observed heterozygosity of 0.91 (range 0.79–1.00), and an average polymorphic information content of 0.88 (range 0.84–0.92).

Keywords: *Caretta caretta*, loggerhead turtle, microsatellites, primers, sea turtle, SSRs, tetranucleotide repeats

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The threatened loggerhead sea turtle (*Caretta caretta*) occurs globally in warm temperate and tropical ocean basins. Although extensive mitochondrial analyses aimed at characterizing phylogeography and the resolution of management units within this species have been conducted (Bowen et al. 1993, 1994; Encalada et al. 1998), complementary microsatellite analyses have received comparatively less attention until recently (Bowen et al. 2005). Despite demonstrated conservation of several microsatellite loci across all genera of extant cheloniid turtles (Fitzsimmons et al. 1993), there remains a need for additional, highly polymorphic loci to address questions of population genetics. We describe the development of a panel of species-specific, tetranucleotide microsatellite markers that will facilitate, in conjunction with previously developed dinucleotide microsatellite markers, the study of the relationship between kinship and spatial distribution in the loggerhead sea turtle.

We extracted DNA from blood obtained from two nesting (female) loggerheads using the DNAzol reagent (Invitrogen) followed by a phenol–chloroform extraction to remove remaining impurities. We digested DNA with *Rsa*I and *Bst*UI (New England Biolabs) and double-enriched for (AAAG)$_6$ (ACCT)$_6$ (ACTC)$_6$ (AATC)$_6$ (ACAG)$_6$ (ACTG)$_6$ (AAAC)$_6$ (AATG)$_6$ (AAGT)$_6$ and (ACAT)$_6$ (Glenn & Schable 2005). We ligated enriched product for tetranucleotide repeats into (polymerase chain reaction) PCR 2.1-TOPO vector, which was used to transform OneShot Top10 Chemically Competent *Escherichia coli* cells (TOPO TA cloning kit, Invitrogen). We screened 672 colonies for inserts using the β-galactosidase gene and sequenced 672 positive (white) colony PCR products using BigDye (version 3.1, PE Applied Biosystems) chemistry and an ABI 3730 sequencer. Sequences were assembled and edited in sequencher 4.2 (Gene Codes) and exported to ephemeris 1.0 (available at http://www.uga.edu/srel/DNA_Laboratory/dnacomputer_programs.htm) to search for microsatellite repeats. We designed 90 primer pairs for 200 contigs containing unique microsatellite repeats and added an M13-reverse or CAG tag to the 5′ end of each primer pair using oligo 4.0 (Molecular Biology Insights) to facilitate fluorescent size detection using ABI sequencers (Boutin-Ganache et al. 2001; Schable et al. 2002). Either a CAG or an M13-reverse tag was chosen for either the forward or reverse primer on the basis of minimizing self-complementarity, pair complementarity, and secondary structure of each primer or primer pair. We added GTTT ‘pigtales’ to the 5′ end of primers lacking either CAG or M13-reverse tag to facilitate the nontemplated addition of adenosine by *Taq* polymerase (Brownstein et al. 1996). We selected 48 primer pairs for testing based

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on microsatellite repeat number and primer characteristics used in choosing tags.

We optimized primer pairs using DNA samples obtained from loggerheads nesting on several Georgia barrier islands. DNA was extracted from samples using the DNeasy Kit (QIAGEN). PCR amplifications were performed in 10 µL volumes using GeneAmp PCR System 9700 thermal cyclers (PE Applied Biosystems). Final concentrations for optimizing reactions were 10 mM Tris pH 8.4, 50 mM KCl, 0.5 µM ‘pigtailed’ primer, 0.05 µM CAG or M13-reverse tagged primer (CAG or M13-reverse + primer), 0.45 µM dye-labelled tag (HEX or FAM + CAG or M13-reverse), 1.5 mM MgCl₂, 0.5 mM dNTPs, 0.5 U Taq DNA polymerase, and 50 ng DNA. M13 and CAG universal primers were labelled with FAM or HEX fluorescent dyes. Reactions were optimized with six individuals using two touchdown thermal cycling programs (Don et al. 1991), each encompassing a 10.5 °C span of annealing temperatures (ranges: 60–49.5 °C, 65.0–54.5 °C). Cycling parameters were: 21 cycles of 95 °C for 20 s; highest annealing temperature for 20 s at −0.5 °C per annealing cycle; and 72 °C for 30 s, followed by 14 cycles of 95 °C for 20 s; 49.5 °C or 54.5 °C, respectively, for 30 s; 72 °C for 30 s; and a final extension period of 10 min at 72 °C.

PCR products were checked for amplification and scored using an ABI 3730 sequencer with genescan Rox500 fluorescent size standard (PE Applied Biosystems). Results were analysed using genemapper software (PE Applied Biosystems) and optimal touchdown cycling schemes were identified. Following optimization, 24 additional individuals were genotyped. We calculated observed and expected heterozygosities and polymorphic information content for each locus using cervus 2.0 (Marshall et al. 1998). genepop 3.4 (Raymond & Rousset 1995) was used to test for Hardy–Weinberg equilibrium and genotypic linkage disequilibrium. We conducted a posteriori sequential Bonferroni correction (Rice 1989).

Table 1 summarizes the characteristics of 15 primer pairs developed from the loggerhead sea turtle. The number of alleles per locus ranges from 10 to 21, averaging 13.9. Total exclusionary power with both parents unknown is 1.000000. No deviations from Hardy–Weinberg equilibrium or significant linkage disequilibrium were detected after sequential Bonferroni correction.

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Sequences used to introduce sites for the universal primer are in bold italics.
Underlined bases indicate sharing of nucleotides between CAG (5′-CAGCTGGGCCCTCATCA-3′) tag, M13R (5′-GGAAACAGCTATGACCAT-3′) tag, or GTTT ‘pigtail’ and the locus-specific primer binding site.
Clone size refers to the predicted size of the PCR product amplified from the clone used to develop each locus; N, number of individuals genotyped at each locus; A, number of alleles; $H_O$, observed heterozygosity; $H_E$, expected heterozygosity; $P_{HW}$, probability that genotype proportions conform to Hardy–Weinberg equilibrium; and PIC, polymorphic information content.
References


