PRIMER NOTE

Microsatellite DNA loci from the Diamondback terrapin (*Malaclemys terrapin*)

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Abstract

We describe polymerase chain recation (PCR) primers and conditions to amplify one dinucleotide and five tetranucleotide microsatellite DNA loci isolated from the Diamondback terrapin (*Malaclemys terrapin*). The PCR primers were tested on 21 terrapins from Cape Romain, SC, USA. The microsatellite primers developed yielded a high number of alleles (8–14) and high observed heterozygosities (0.57–1.0).

Keywords: dinucleotide repeats, *Malaclemys terrapin*, microsatellites, primer, SSR, tetranucleotide repeats

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The Diamondback terrapin is the only strictly estuarine species of turtle in North America and is distributed along the Atlantic and Gulf Coast of the United States. Mark–recapture data have indicated that terrapins have extraordinarily high site fidelity to specific tidal creeks and sections of creeks (Gibbons *et al*. 2001; Tucker *et al*. 2001) or sections of a river (Roosenburg *et al*. 1999), and female terrapins have also been found to return to the same nesting beaches (Roosenburg 1996). We developed microsatellite markers to study multigenerational site fidelity and population genetic structure in Diamondback terrapins from South Carolina.

DNA was extracted from terrapin blood or tail-tip tissue using diatomaceous earth as described by Davis *et al*. (2001), or standard phenol/chloroform extraction (Sambrook *et al*. 1989). Extracted DNA was enriched for $(AC)_{13}$, $(AG)_{12}$, $(AAAG)_6$, $(ACAG)_6$, $(AGAT)_8$, $(ATCC)_5$ and $(ACAT)_8$ following a protocol modified from Hamilton *et al*. (1999). The detailed protocol is available from TCG (glenn@srel.edu). In brief, the DNA was digested with *Rsa*I, ligated to SuperSNX linkers, hybridized to biotinylated microsatellite oligonucleotides, captured on Dynabeads (Dynal Biotech Inc.), unwanted DNA was washed away, captured DNA was recovered by polymerase chain reactions (PCR) with SuperSNX-f (5′-GTTTAAGGCCTAGCTAGCAGAATC-3′) and cloned using the TOPO TA Cloning System (2.1) (Invitrogen). White colonies were amplified using M13 forward and reverse primers. PCR products of 500–1000 bp were sequenced using BigDye version 2.0 (Applied Biosystems) chemistry on an ABI 377 sequencer. Sequences from both strands were assembled and edited in Sequencher 4.1.2 (Genecodes) and exported to Ephemeris 1.0 (available at http://www.uga.edu/srel/DNA_Lab/programs.htm) to automatically search sequences for microsatellite repeats. PCR primers were developed, and an M13Reverse (5′-GGAAACAGCTATGACCAT-3′) or CAG tag (5′-CAGTCGGGCGTCATCA-3′) was added to the 5′ end of one of each primer pair (Table 1) using Oligo 6.67 (Molecular Biology Insights) to determine which tag would produce the least secondary structures. Inclusion of the 5′-tag allows use of a third primer in the PCR (M13R or CAG) that is fluorescently labelled for detection on the ABI 377 (cf. Boutin-Ganache *et al*. 2001).

Primers were optimized using eight Diamondback terrapin DNA samples from a South Carolina terrapin population from Cape Romain. PCR amplifications were performed in a 25 µL volume using a GeneAmp PCR System 2700 Thermocycler (Applied Biosystems). PCR final concentrations for optimizing reactions were 10 mM Tris pH 8.4, 50 mM KCl, 25.0 µg/mL BSA, 0.2 µM unlabelled primer, 0.02 µM tag labelled primer, 0.18 µM universal dye labelled primer, 1.5 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 units *Taq* DNA Polymerase and 40 ng DNA template. M13R and CAG universal primers were labelled with a Fam, Hex or Ned fluorescent dye. Primers were tested using touchdown thermal cycling programs (Don *et al*. 1991)

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Table 1 Characterization of six primer pairs that amplify microsatellites from *Malaclemys terrapin*. Sequences used to introduce sites for universal fluorescent primers are in italics. Bases shared between the terrapin sequence and the universal fluorescent primers are underlined. Touchdown temperature refers to the initial annealing temperature of the touchdown protocol used. N refers to sample size. Size range refers to the observed distribution of alleles at each locus. *Primer TerpSH 3L was designed with a run of three cytosines; however, further investigation of the original clone sequences indicate that there are only two cytosines at this location. This primer works well despite the additional base

Locus	Primer Sequence 5′ → 3′	Genbank Accession Number	Touchdown Temperature	Repeat Sequence	N	Number of Alleles	Size Range (bp)	Observed Heterozyogsity	Expected Heterozygosity
TerpSH 1U	CCA CTG GGA TCT AAT CAC TT	AY 156709	65	$(AGAT)_{15}$	20	12	254–302	0.5714	0.9128
TerpSH 1L	*GGAAACAGCTATGACCATG* GG CAA CTT AGC AT								
TerpSH 2U	*GGAAACAGCTATGACCATG* GCC AGC AGG AGT AAT G	AY 156710	60	$(AGAT)_{12}$	21	12	171–227	1.0000	0.9024
TerpSH 2L	CTA TTA GGG CAG AGA CGA G								
TerpSH 3U	*GGAAACAGCTATGACCATG* TCC CCC AAT GCA CAC	AY 156711	65	$(CAAA)_{14}$	21	8	283–311	0.8571	0.8025
TerpSH 3L	CTG C*CCA ATC CAT TTA GA								
TerpSH 5U	TTG CTG CTA TAT GCT TAA T	AY156713	60	$(CTAT)_{12}$	21	8	157–189	0.9048	0.8246
TerpSH 5L	*GGAAACAGCTATGACCAT* CCT CCC TGC CTA TTG A								
TerpSH 7U	*CAGTCGGGCGTCATC* CAC ACA CAC TGT ATT TTG ATA	AY 156715	60	$(AGAT)_{13}$	18	10	97–137	0.7222	0.8722
TerpSH 7L	CTA TGC CCT TTC TAG TTT G								
TerpSH 8U	*GGAAACAGCTATGACCATG* CCA AAT TAA ATA TCT ACC	AY 156716	65	$(GA)_{19}$	19	14	193–221	0.8947	0.9132
TerpSH 8L	AGC CTT TCC AGT ATT CAG TA								

encompassing a 10 °C span of annealing temperatures ranging between 65 and 55 °C, 60–50 °C or 55–45 °C. Cycling parameters were: five cycles of 96 °C for 20 s, the highest annealing temperature for 30 s and 72 °C for 1 min; followed by 21 cycles of 96 °C for 30 s, the highest annealing temperature minus 0.5 °C per cycle for 30 s and 72 °C for 1 min; and finally 10 cycles of 96 °C for 30 s, annealing at the lowest temperature for 30 s and 72 °C for 1 min. PCR products were initially scored for amplification on agarose gels, and successful PCR products subsequently sized on an ABI 377XL sequencer using CXR ladder (Promega). Results were analysed using GENESCAN and GENOTYPER software (Applied Biosystems). Following optimization an additional 13 individuals from Cape Romain were genotyped (Table 1).

Table 1 summarizes the characteristics of the Diamondback terrapin primer pairs. We calculated observed and expected heterozygosity (H_O and H_E) using GENEPOP (Raymond & Rousset 1995). After Sequential Bonferroni adjustment of α for multiple comparisons, no loci deviated significantly from Hardy–Weinberg Equilibrium. The high numbers of alleles per locus within this terrapin population indicates the potential usefulness of these primers to characterize population genetic structure of Diamondback terrapins. Because these primers might also be useful for population genetic studies in other turtles, we tested them with DNA from seven other species (Table 2). Many of these primers produce amplification products of the expected size; however, we have not yet tested them for polymorphism.

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Table 2 PCR amplification of the six primer pairs in other turtle species. + Indicates amplification products of the expected size. – Indicates no amplification or multiple amplification products

Species	TerpSH 1	TerpSH 2	TerpSH 3	TerpSH 5	TerpSH 7	TerpSH 8
Chrysemys picta picta	+	+	+	+	+	+
Clemmys guttata	–	+	+	–	–	–
Terrapene carolina	–	+	+	+	+	+
Chelydra serpentina	+	+	–	–	–	–
Sternotherus odoratus	–	+	+	–	–	–
Caretta caretta	–	+	+	+	–	+
Dermochelys coriacea	+	+	–	+	–	+

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