

PRIMER NOTE

Characterization of 26 new microsatellite loci in the dugong (*Dugong dugon*)

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Abstract

Twenty-six microsatellite loci have been isolated from a dugong (*Dugong dugon*). The average heterozygosity was 0.52 with two to 10 alleles per locus surveyed from 50 individuals. The markers are suitable for genetic mark–recapture ($P_{ID} = 5 \times 10^{-16}$) in dugongs and they could also be used to quantify physical tag loss, estimate relatedness, assign paternity, elucidate population structure and identify migrants. The loci also amplified in Florida manatees (22/26) and Asian elephants (6/26).

Keywords: DNA, *Dugong*, genetic mark–recapture, microsatellite, probability of identity

Received 9 February 2007; revision accepted 27 April 2007

Dugongs are large (up to 400 kg), long-lived (up to 70 years) herbivorous marine mammals found throughout Indo-Pacific waters (Marsh *et al.* 2002) that are listed as vulnerable (IUCN 2006). In Australia, dugongs co-occur with seagrass beds in shallow habitat, often in coastal embayments. Our goal was to develop a genetic mark–recapture program for a population of dugongs in Moreton Bay in southeast Queensland (Australia) that is adjacent to an urban settlement of 1.5 million.

Microsatellite loci for dugongs were prepared using the method of Fischer & Bachman (1998) with some modifications. RNA-free high molecular weight DNA was extracted from 0.3 g of freshly frozen skin tissue ground in liquid nitrogen using a standard phenol–chloroform method (Sambrook *et al.* 1989). The purified DNA was digested with *RsaI* (New England Biolabs Inc.) and size fractionated to 500–1000 bp on a 1% LMT agarose gel. Adapter primers were ligated to DNA fragments and then hybridized to 10 μ M of each biotin-labelled oligo in separate tubes [(TAGA)₁₀ (GACA)₁₀ (AAG)₁₀ and (ATG)₁₀]. The hybrid complexes were bound to streptavidin-coated magnetic beads (Dynabeads; Dynal GmbH) and washed twice at low-stringency conditions (25 °C in 2 \times SSC, 0.1% SDS, 5 min each) and four times at high-stringency conditions

(twice in 1 \times SSC at 25 °C and twice in 1 \times SSC at 5 °C less than the T_M of each oligo for 2 min and 5 min, respectively) on a slowly rotating wheel. The enriched DNA was eluted into ultrapure Milli-Q water and polymerase chain reaction (PCR) amplified prior to cloning using Lucigen's PCR-Smart blunt end cloning kit with their plasmid vector and E. coli 10G electrocompetent cells. Colonies were picked and grown overnight in terrific broth; DNA was purified using the alkaline lysis miniprep (Engebrecht *et al.* 1991) and Lucigen's SL1 and SR2 primers were used to sequence bidirectionally with ABI BigDye terminator chemistry and gel separated on an ABI PRISM 3130XL sequencer. Inserts from 102 clones were sequenced totalling 64 933 bp (GenBank Accession nos EF078590–691). REPEATMASKER (Smit *et al.* 2006) was used to identify microsatellite repeats and areas of low complexity DNA (including repeated elements), which represented 6.67% and 22.76% of the total sequence surveyed, respectively.

Primer pairs from 48 sequences were designed with PRIMER 3 on the WWW (Rozen & Skaletsky 2000) to amplify microsatellites under uniform conditions. Cycling conditions were an initial denaturation at 94 °C for 15 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 45 s and extension at 72 °C for 90 s, followed by a final extension at 72 °C for 45 min in a 9700 PerkinElmer thermocycler. Ten-microlitre PCR reactions contained 20 ng of genomic DNA, 10 pmol of each primer,

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Table 1 Characteristics of 26 microsatellite loci developed for dugong: locus name, GenBank Accession no., number of genotypes per locus (n), number of alleles (N_a), observed heterozygosity (H_O), expected heterozygosity (H_E), uncorrected P value for departure from Hardy–Weinberg equilibrium [$P(HWE)$], forward primer, reverse primer, repeat motif (* denotes interrupted repeat), product range in dugongs, genotypes of Florida manatee (*Trichechus manatus latirostris*) and Asian elephant (*Elephas maximus*) where x denotes amplification failure

Locus name	GenBank no.	N	N_a	H_O	H_E	$P(HWE)$	Forward primer	Reverse primer	Repeat motif	Product range	Manatee	Elephant
DduA01	EF078590	50	3	0.40	0.43	0.780	TTTAAAGGCCAAAACAGTATGTC	CAAAACGGGCCCTAACCTACA	(TG) ₂₂	192–200	200/200	x
DduA07	EF078594	50	3	0.36	0.43	0.182	AGACCCAGGCAGAGTTGAGA	CCCAAACCTCTAGGGACCACA	(TC) ₁₃	218–222	333/333	x
DduA12	EF078598	50	4	0.46	0.48	0.626	ACAGAAGGCTCAAAGGCTGA	CAGCACTTCTCTCCTCCAAGG	(CA) ₁₆	219–237	223/225	x
DduB01	EF078599	50	8	0.80	0.78	0.551	CACTGTGGTGTAAAAGGGACA	TTATTTGGCTTGGGACTTGG	(TG) ₃₃	375–397	349/349	x
DduB02	EF078600	50	8	0.84	0.80	0.140	AAACCCAAAATCGGATCATGT	GCTGGGTTTTCCATTCTCAT	(CA) ₂₆	222–252	224/224	x
DduB05	EF078602	50	3	0.58	0.52	0.525	AAGCCTCACAGGAGACCTCA	TGTAACCTGCTGGAATCACC	(CA) ₁₇	328–334	343/343	324/324
DduC03	EF078611	50	4	0.60	0.58	0.542	ACGGCCTAGAATCACATTTGG	CTTTGCAATGCCCTCACTC	(TG) ₂₀ *	385–391	x	x
DduC05	EF078613	50	8	0.80	0.79	0.186	CCATTGGCATTTACATTCGTG	TGTTGTTCCCTTCTGAAGCA	(CA) ₂₇	242–256	227/227	x
DduC09	EF078614	50	6	0.52	0.46	0.985	GCTTCTCTTTTGGGGTAGGC	GGCATGGGGTCATTAGAAGA	(CA) ₃₄ *	318–330	288/288	267/288
DduC11	EF078616	50	3	0.34	0.35	0.358	GAAAGGCTTGGCACAATCAC	GGATTTGCCAAAAGCAGAC	(TG) ₁₃	213–241	210/210	x
DduD08	EF078625	50	6	0.54	0.75	0.042	TGCATGTTCCTCTTTTGAATGG	TCCGTCTCATGCTACCTCAA	(CA) ₂₀	291–313	284/297	291/291
DduD11	EF078628	50	4	0.34	0.39	0.090	CTGAAAGGCACAGGAGAAGG	CAGGCTGTCCAGGGAAAATA	(TG) ₃₄ *	246–252	x	x
DduE03	EF078630	50	2	0.22	0.20	1.000	TTTAGGGGCTCCAAAGGAAT	CTGCCATGCAAGTGACCTTA	(TG) ₁₅ *	340–342	344/344	x
DduE04	EF078631	50	7	0.76	0.77	0.562	TATCACAAACCCCATTTCCA	CTGTCCAGAGGGAAAAGGTCA	(CA) ₂₈	324–338	330/336	x
DduE08	EF078635	50	3	0.52	0.50	0.394	GGGAAAAGGGGTAGGGAGTT	TGAAGTGCCAAATGTTTTGTT	(TG) ₁₇	198–202	210/210	166/166
DduE09	EF078636	50	6	0.72	0.67	0.348	CCTGCCTGCTTCAGAGAATC	CAGGAGCCAAACAGTGTCAA	(TG) ₂₈	376–388	364/364	358/358
DduE11	EF078637	50	3	0.54	0.45	0.525	CCCAGCCAACTGATACAGA	CCATTCTAAGGGTCAGCAA	(TCTA) ₁₇ *	318–326	290/312	x
DduF06	EF078641	50	2	0.20	0.18	1.000	CAGAGGTGGCTTGAAGAAA	GGTAGATCAGTCCGGTCTCT	(TG) ₁₉ *	220–222	234/234	320/320
DduF07	EF078642	50	4	0.30	0.37	0.351	TCCAGGGGAAGATTGATGAG	GACCTATGCCAGGCTGTTA	(CA) ₂₂	276–292	273/322	351/353
DduF11	EF078646	50	4	0.18	0.17	1.000	CCCCCAAATTTCCCTAAATCA	GCAGGTGGCTCTCCATTTTA	(TG) ₁₉ *	260–268	248/248	270/272
DduG10	EF078657	50	2	0.12	0.11	1.000	AGTCCCGGTCCCAGGGC	CATGTCTGTGTTCCCCACTG	(TG) ₂₇ *	373–377	x	x
DduG11	EF078658	50	7	0.74	0.74	0.672	GGAGGCAAAAGGAAAAAGC	GCCTTTTCTCACTCTGTGG	(TG) ₁₈	351–384	x	x
DduG12	EF078659	50	10	0.72	0.64	0.635	TGGCACTTCTGAAACTTTGC	TCTTCTCCAGCTTTGCCAAT	(TG) ₂₇	378–406	366/366	x
DduH02	EF078661	50	3	0.70	0.54	0.022	GGAAATCAACTCAACGGCAAT	CCCTTCTACCAGACCCTTCC	(TG) ₁₇	208–212	180/180	x
DduH04	EF078663	50	5	0.76	0.65	0.357	CTGAATGCCCTCACATCTT	TATGCCCTTAGATGCCTTGG	(CA) ₂₂	355–365	359/359	x
DduH09	EF078668	50	3	0.34	0.42	0.070	GGAAGCCCTATGAAGCACAG	TGGACGGGTATCGTATGTCA	(TG) ₁₉	214–220	206/206	x

3 mM MgCl₂, 0.5 µL of QIAGEN's Q-solution and 5 µL of QIAGEN's multiplex master mix (kit contains HotStarTaq DNA polymerase, MgCl₂, dNTPs and PCR buffers). Amplicons were diluted 150-fold with ultrapure Milli-Q water then gel separated by capillary electrophoresis on an ABI PRISM 3130XL sequencer. Alleles were sized against an internal size standard (GeneScan — 500 LIZ) and scored using GENEMAPPER software (version 3.7, Applied Biosystems).

Forty-five of the 48 loci amplified and 32 were polymorphic when screened against eight dugongs. Fifty adult dugongs from Moreton Bay, a Florida manatee (*Trichechus manatus latirostris*) and an Asian elephant (*Elephas maximus*) were genotyped at 26 selected loci (Table 1). The majority of loci amplified (22/26) in the Florida manatee and 6/26 loci amplified in the Asian elephant. For these species their utility needs to be carefully scrutinized with more samples as large differences in allele sizes compared to dugong were observed (loci DduA07, DduF06 and DduF07; Table 1).

The average heterozygosity among 50 dugongs was 0.52 with two to 10 alleles per locus (Table 1). After a Bonferroni correction for multiple comparisons, no loci departed from Hardy–Weinberg equilibrium. Two loci (DduA01 and DduH04) were linked when tested using GENEPOP-on-the-web (Raymond & Rousset 1995). We included these loci for genetic mark–recapture work as together they contributed 14 unique genotypes that will assist with the individual identification of dugongs.

At 26 loci, the probability of identity was low ($P_{ID} = 5 \times 10^{-16}$) indicating that this panel may be suitable for individual identification in inbred populations. This was confirmed in the simulation package SHADOWBOXER and LOCUSEATER (Hoyle *et al.* 2005) for sample sizes of up to 10 000. We are now using these markers to augment a conventional mark–recapture program (Lanyon *et al.* 2006).

Acknowledgements

Thanks to Helen Sneath, Erin Neal, John Kirkwood, Winifred Violet Scott Foundation, Consolidated Rutile P/L and Sea World

Research and Rescue Foundation. Dugong tissue samples were collected under Marine Parks permits # QS2004/CVL228 and # WISP01660304 and University of Queensland Animal Ethics # ZOO/ENT/344/04/NSF/CRL. Manatee tissue was provided by Robert Bonde under Federal Fish and Wildlife permit # MA791721-4, Cites Export Permit # 06US125187/9 and Cites Import permit # 2006-AU-443904. Elephant blood was provided by Caroline Lawrence, Perth Zoo.

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