Molecular Ecology Notes (2003) **3**, 616–618 doi: 10.1046/j.1471-8286.2003.00530.x

PRIMER NOTE

Characterization of microsatellite DNA loci for the southern flying squirrel (*Glaucomys volans*)

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Abstract

Polymerase chain reaction primers for microsatellite DNA loci (one dinucleotide, four tetranucleotide and two compound) and the conditions necessary to amplify each are described for the southern flying squirrel (*Glaucomys volans*). These primers were tested on 22 or more individuals from a population at the Savannah River Site in South Carolina. These microsatellite primers yielded a high allelic diversity (6–22 alleles/locus), and moderate to high observed heterozygosities (0.318–0.826). Primers developed for the northern flying squirrel (*Glaucomys sabrinus*) were also tested for use on *G. volans*, with only two successful cross amplifications from the seven loci.

Keywords: dinucleotide repeats, *Glaucomys volans*, microsatellite, polymerase chain reaction, primer, tetranucleotide repeats

Received 15 May 2003; revision received 3 July 2003; accepted 30 August 2003

The southern flying squirrel (*Glaucomys volans*) is a common pine-hardwood forest inhabitant with a continuous distribution across the eastern half of the United States and northward into southern portions of Ontario and Quebec (Dolan & Carter 1977). Additionally, a few small disjunct populations are found in subtemperate montane habitats in Mexico, Guatemala, and Honduras (Diersing 1980). The widespread distribution of this species and its local abundance in areas of optimal habitat makes the southern flying squirrel a useful model for genetic-based studies of behavioural ecology, in particular, mating systems. In addition, this species inhabits areas that have experienced considerable levels of habitat fragmentation, and the limited mobility of this species in nonforested environments makes it a suitable model for studies of gene flow and genetic diversity. This paper describes seven polymorphic microsatellite loci isolated from *Glaucomys volans* and also tests the applicability of loci developed for the sister species, the northern flying squirrel (*Glaucomys sabrinus*) by Zittlau *et al*. (2000).

DNA was extracted from southern flying squirrel ear tissue using Qiagen DNeasy Kits (catalogue no. 69506). Extracted DNA was enriched for $(TG)_{12}$, $(AG)_{12}$, $(AAG)_8$, $(ATC)_8$, $(AAC)_8$, $(AAT)_{12}$, $(ACT)_{12}$, $(AAAC)_8$, $(AAAG)_6$, $(AATC)_6$, $(AATG)_6$, $(ACCT)_6$, $(ACAG)_6$, $(ACTC)_6$, and $(ACTG)_6$ following a protocol modified from Hamilton *et al*. (1999). The detailed protocol is available from TCG. In brief, the DNA was digested with *Rsa*I (New England Biolabs), ligated to SuperSNX linkers (SuperSNX24 Forward 5′-GTTTAAGGCCTAGCTAGCAGCAGAATC-3′ and SuperSNX24 Reverse 5′-GATTCTGCTAGCTAG-GCCTTAAACAAAA-3′; modified from the SNX linkers of Hamilton *et al*. (1999)), hybridized to biotinylated microsatellite oligonucleotides and captured on streptavidin-coated paramagnetic beads (Dynal). Unwanted DNA was washed away and 'captured' DNA was recovered via polymerase chain reaction (PCR) using SuperSNX Forward primer. The product was ligated into a PCR®2.1 Vector and inserted into Top 10 Chemically Competent *Escherichia coli* (Invitrogen). The bacterial clones were screened for inserts using the β-galactosidase gene. Using M13 forward and reverse primers, 192 positive colonies were amplified and 96 PCR products of 500–1000 base pairs (bp) were sequenced using Big Dye 3.0 (Applied Biosystems) chemistry and an ABI 377-96 sequencer. Sequences from both strands were assembled and edited in SEQUENCHER 4.1 (Genecodes) and exported to EPHEMERIS 1.0 (available at http://www.uga.edu/

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Table 1 Description of seven primer pairs that successfully amplify microsatellites from the southern flying squirrel *Glaucomys volans* and two primers developed by Zittlau *et al*. (2000). Sequences used to introduce sites for universal fluorescent primers and 5′-GTTT tail additions are presented in italics. *N* refers to sample size. Size range refers to the observed range of allele lengths at each locus. Clone size refers to the size of the PCR product amplified from the clone used to develop each locus, which was identical to the size predicted from the DNA sequence. ND, not determined, NA, not available

Locus	Primer sequence (5′–3′)	Dye	GenBank Accession no.	Repeat sequence	*N*	No. of alleles	Size range (bp)	Clone size (bp)	H_O	H_E	PIC
SFS-02U	*CAGTCGGGCGTCATCA*ATGGAGTGTGGTGTATCT	6-FAM	AY268023	$(AAAG)_{11}(AG)_{15}(AAAG)_5$	24	22	166–280	272	0.792	0.961	0.938
SFS-02L	CTGGGTTCAATTCCTAATA										
SFS-03U	*GGAAACAGCTATGACCATG*CACTTGCTTAGTTTGTATG	NED	AY268024	$(AATG)_{12}$	22	6	235–255	263	0.682	0.653	0.571
SFS-03L	CAAGCTAGAGCCAATAACT										
SFS-04U	*GGAAACAGCTATGACCATG*TGAATCAAAATGCAGTCT	NED	AY268025	$(AAAG)_{15}$	23	10	155–175	171	0.739	0.890	0.857
SFS-04L	GTAAAAATAAAACCCAACTC										
SFS-05U	TTCGGGTCTGATTCTC	NED	AY268026	$(AAAC)_6$	22	9	292–312	300	0.318	0.771	0.720
SFS-05L	*GGAAACAGCTATGACCATG*CCTCGCCTTGAATCTC										
SFS-07U	AGGGCTCTGACGAAGT	6-FAM	AY268027	$(AATG)_{10}$	23	8	202–272	268	0.826	0.792	0.742
SFS-07L	*CAGTCGGGCGTCATCA*CCTGGCACCTGAACAT										
SFS-14U	CCCACATGCAGATCAC	NED	AY268028	$(AC)_8 \ldots (AC)_{12}$	24	7	167–179	ND	0.583	0.708	0.655
SFS-14L	*GGAAACAGCTATGACCATG*TAAACCGTCCTTGTATTC										
SFS-15U	*CAGTCGGGCGTCATCA*GGGAGAAAGGAACACA	6-FAM	AY268029	$(AC)_{14}$	24	7	117–131	ND	0.708	0.801	0.752
SFS-15L	CCAGAACCCAGCTAAT										
GS-08U	*CAGTCGGGCGTCATCA*TGCCATCTCCCCTCTC	6-FAM	AF220753	$(GT)_{20}$	24	8	219–247	NA	0.792	0.801	0.752
GS-08L	GCTGTGCTTCCAACCTGT										
GS-10U	*CAGTCGGGCGTCATCA*CTATGCTGAGGAGGAGTGGTG	6-FAM	AF220752	$(GT)_{18}$	21	8	221–235	NA	0.857	0.864	0.825
GS-10L	CGTTTATGTGAAGAGCCTTG										

srel/DNA_Lab/dnacomputer_programs.htm) to automatically search sequences for microsatellites. PCR primers were developed for 16 sequences and an M13Reverse (5′-GGAAACAGCTATGACCAT-3′) or CAG tag (5′-CAGTCGGGCGTCATCA-3′) was added to the 5′ end of one of each primer pair 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 (Boutin-Ganache *et al*. 2001).

Primer pairs for 16 loci were optimized using seven southern flying squirrel DNA samples collected from the Savannah River Site, South Carolina USA. PCR amplifications were done as 25-μL volume reactions using an Eppendorf Mastercycler Gradient thermal cycler. PCR final concentrations for optimizing reactions were 10 mM Tris pH 8.4, 50 mM KCl, 25.0 μg/mL bovine serum albumin, 0.4 μM unlabelled primer, 0.04 μM tag labelled primer, 0.36 μM universal dye labelled primer, 1.5 mM $MgCl_2$, 0.15 mM dNTPs, 0.5 units *Taq* DNA polymerase, and 50 ng DNA template. CAG and M13 universal primers were labelled with 6-FAM and NED fluorescent dyes, respectively. Primers were tested using touchdown thermal cycling programs (Don *et al*. 1991) with initial annealing temperatures of 65 °C, 60 °C, and 55 °C. Briefly, the cycling parameters were: 5 cycles of 96 °C for 20 s, the highest annealing temperature for 30 s, and 72 °C for 1 min; 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 lastly 10 cycles of 96 °C for 30 s, the lowest annealing temperature for 30 s, and 72 °C for 1 min. However, optimal yield of PCR products for all primers was obtained using the touchdown 60 °C protocol. PCR products were initially scored for amplification on 1.5% agarose gels, and successful PCR products were subsequently sized on an ABI 377-96 sequencer using Gensize ROX 500 ladder (Genpak). Results were analysed using Genescan and Genotyper software (PE Applied Biosystems). Following primer optimization, an additional 17 individuals were genotyped (Table 1).

A total of seven primers developed by Zittlau *et al*. (2000) were labelled with CAG-tags (5′-CAGTCGGGCGTCATCA-3′) at the 5′ end of one of each primer pair. These were then tested using the same touchdown thermal cycling program used for the *G. volans* primers and PCR products were similarly scored for amplification using 1.5% agarose gels, and those successfully amplified were subsequently sized on an ABI 377-96 sequencer.

Characteristics of all primer pairs are summarized in Table 1. Observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphism information content (PIC) were calculated using CERVUS 2.0 (Marshall *et al*. 1998). Locus SFS05 has a much smaller H_O than H_E (Table 1), suggesting null alleles. Indeed the predicted null allele frequency is 0.42 (calculated using Cervus 2.0; at least an order of magnitude greater than all other loci). This may limit the applicability of locus SFS05, at least with these primers.

Using GENEPOP 3.3 (Raymond & Rousset 1995), all loci were found to be in Hardy–Weinberg equilibrium, with the exception of SFS02 and SFS05 ($P < 0.001$), which may also indicate the presence of null alleles. In addition, no pairwise loci combinations were found to exhibit genotypic linkage disequilibrium. Despite the limitation of SFS02 and SFS05, these highly polymorphic loci were measured in only a single population, thus demonstrating a high potential for these primers to be used as tools in studies of geographical variation, habitat isolation and breeding structure.

Acknowledgements

Thanks to Julie Weston, Alessandra Seccomandi, and Olga Tsyusko for advice on data collection and analysis. This work was supported by contract DE-FC09–96SR18546 between the US Department of Energy and the University of Georgia's Savannah River Ecology Laboratory. TSR was supported by NSF grant DEB-9521013, National Environmental Research Park grant, and grants-in-aid from American Society of Mammalogists, American Museum of Natural History, Sigma Xi, and the ASM Shadle Fellowship.

References

Boutin-Ganache I, Raposo M, Raymond M, Deschepper CF (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *Biotechniques*, **31**, 24–28.

Diersing VE (1980) Systematics of flying squirrels, *Glaucomys volans* (Linnaeus), from Mexico, Guatemala, and Honduras. *Southwestern Naturalist*, **25**, 157–172.

Dolan PG, Carter DC (1977) Glaucomys volans. *Mammalian Species*, **78**, 1–6.

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. *Nucleic Acids Research*, **19**, 4008.

Hamilton MB, Pincus EL, Di Fiore A, Flesher RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. *Biotechniques*, **27**, 500–507.

Marshall TC, Slate J, Kruuk L, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology*, **7**, 639–655.

Raymond M, Rousset F (1995) GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Zittlau KA, Davis CS, Strobeck C (2000) Characterization of microsatellite loci in the northern flying squirrel (*Glaucomys sabrinus*). *Molecular Ecology*, **9**, 817–829.