Author/s:
Soanes, K;Banks, SC;van der Ree, R

Title:
Development of nine polymorphic microsatellite loci in the squirrel glider (Petaurus norfolcensis)

Date:
2014-01-01

Citation:
Soanes, K., Banks, S. C. & van der Ree, R. (2014). Development of nine polymorphic microsatellite loci in the squirrel glider (Petaurus norfolcensis). Conservation Genetics Resources, 6 (3), pp.785-786. https://doi.org/10.1007/s12686-014-0219-3.

Persistent Link:
https://hdl.handle.net/11343/283153

**Development of nine polymorphic microsatellite loci in the squirrel glider (*Petaurus norfolcensis*)**

K. Soanes[ab*], S.C. Banks[c] and R. van der Ree[a]

[a]Australian Research Centre for Urban Ecology, Royal Botanic Gardens, Melbourne, VIC 3010, Australia

[b] School of Botany, University of Melbourne, VIC 3010, Australia

[c]Fenner School for Environment and Society, Australian National University, ACT, 0200, Australia

*corresponding author: Phone +61 (03) 8344 0146, Fax +61 (03) 9347 9123, e-mail: k.soanes@pgrad.unimelb.edu.au (K. Soanes)

**Keywords:** squirrel glider, microsatellite, fragmentation, *Petaurus norfolcensis*

**Abstract**

We designed nine polymorphic markers for the squirrel glider (*Petaurus norfolcensis*), an arboreal marsupial in eastern Australia. These markers will assist in the management of isolated populations and the evaluation of wildlife corridors.

**Main text**

The squirrel glider is a small gliding marsupial in Australia. Squirrel gliders are threatened with extinction in the south-eastern parts of their range due to ongoing habitat loss and fragmentation. Much of the species' habitat in south-eastern Australia is in the form of fragmented linear roadside strips and small patches, and enhancing connectivity by creating corridors or road crossing structures is a key conservation management aim. Molecular genetic techniques are necessary to evaluate the long-term effectiveness of these measures. However, only five microsatellite markers are available for the species (Brown et al. 2004; Mills 2000), three of which are difficult to score reliably.

To develop new microsatellites for *P. norfolcensis*, we extracted DNA from the ear tissue of a single individual (Miller et al. 1988) and sequenced a genomic library prepared from this sample on a Roche Genome Sequencer FLX 454 (half a sequencing plate shared with two other samples) using Roche XL+ sequencing chemistry. We assembled the reads (de novo) with CLC Genomic Workbench 5.1 and searched the resulting contigs for microsatellite motifs between 2 and 6 bp (with a minimum repeat length of 8) in MSATCOMMANDER v. 0.8.2 (Faircloth 2008). We identified 1495 contigs containing microsatellite repeats, for which we designed primers for 60 for trial at the Australian Genome Research Facility. Fourteen of the loci were amplified successfully and nine were polymorphic in 20 test samples (additional data provided as Electronic Supplementary Material 1). The polymorphic markers were screened on an additional 29 squirrel gliders (8 male, 21 female) from a single population in south-east Australia (Table 1). Markers were amplified in 6 µl reaction volumes containing 15 ng gDNA, 0.03 uM of fluorescently labelled M13 primer (FAM, NED, PET or VIC), 0.01 uM of M13-labelled forward primer, 0.02 uM of reverse primer, PCR buffer, Immolase DNA polymerase (Bioline), 250 uM dNTPs and 1.5 mM MgCl2. PCR amplification was performed with a 5 minute, 94°C, denaturation step, followed by 41 cycles of 94°C for 30 sec, annealing for 45 sec (dropping 0.5°C per cycle from 60°C to 55°C), and 72°C for 45 sec, with a final extension of 72°C for 10 min. Allele sizes of the pooled PCR products were scored on an Applied Biosystems 3730 DNA Analyzer and genotying was conducted with GeneMapper v. 4.0.

The new loci had a mean expected heterozygosity of 0.47 and allelic diversity of 4.3 (Table 1). No locus showed evidence of null alleles, large allele drop out or stuttering (van Oosterhout et al. 2004). There were no deviations from Hardy-Weinberg equilibrium or evidence of linkage disequilibrium (GENEPOP v. 3.4). In combination with existing markers, these nine loci will enable research into the fine-scale effects of habitat fragmentation on squirrel glider populations and help guide and evaluate restoration efforts.

**Table 1** Characteristics of polymorphic microsatellite markers for squirrel gliders as estimated in GenAlEx v. 6.5.

| Locus | Primer sequence | Repeat | Size (bp) | Na | $H_o$ | $H_e$ |
|---|---|---|---|---|---|---|
| *Pno5* | L: TCATGAGCACAAGTCCCTAC<br>R: TTCCCAGCTGTCAGTGTTC | $(AGAT)_{17}$ | 272-284 | 4 | 0.59 | 0.61 |
| *Pno7* | L: GGGTACCTTGGAGCCTAGC<br>R: GGATATCGGCAATTCCGGC | $(GT)_{20}$ | 354-379 | 5 | 0.45 | 0.40 |
| *Pno12* | L: AGCAGCTGAGCCACTTAGG<br>R: GGGCGTTTCTGCAGTTATC | $(AC)_{16}$ | 191-193 | 2 | 0.38 | 0.31 |
| *Pno18* | L: AGTCACTTAAACTCTGCTTGCC<br>R: TGAGGAAACTGAGGCTGAC | $(AGAT)_{13}$ | 281-293 | 3 | 0.59 | 0.61 |
| *Pno31* | L: TCCTGAGTTAGGGCATGAGC<br>R: ACAGGAAGTAGGTAACCGTG | $(CTT)_{20}$ | 259-317 | 6 | 0.72 | 0.62 |
| *Pno40* | L: TGGATCGCTTAACCTCTCGG<br>R: TCATCCCAACACTGGAGCC | $(ATCT)_{13}$ | 284-313 | 5 | 0.59 | 0.61 |
| *Pno44* | L: GTGTGACCAGGGACATGTTG<br>R: TCCACTCACGGTTCTTCCC | $(GTT)_{13}$ | 235-257 | 3 | 0.17 | 0.16 |
| *Pno47* | L: AACTGGATTACCATCTTCAGACC<br>R: ACACACACTTATGAATTGGAGG | $(AC)_{10}$ | 166-169 | 2 | 0.07 | 0.07 |
| *Pno56* | L: AGCCTAAGGTGGTAGATTACAGG<br>R: CTGTTTGCATTTCCCTATATGTTG | $(ATCT)_{14}$ | 477-515 | 9 | 0.86 | 0.84 |

**Acknowledgements**

We thank the Baker Foundation, the Roads and Maritime Service New South Wales, the Australian Research Council Discovery Grant (DP0984876) and the Holsworth Wildlife Research Endowment for funding this research. Thanks to Andrea Taylor, Paul Sunnucks and Melinda Ziino (Australian Genome Research Facility) for advice during this project.

**References**

Brown M, Kendal TA, Cooksley H, Saint KM, Taylor AC, Carthew, SM, Cooper SJB (2004). Polymorphic microsatellite markers for the gliding marsupials *Petaurus australis* and *Petaurus breviceps*. Mol Ecol Notes 4: 704-706.

Faircloth BC (2008) msatcommander: detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol Ecol Resour 8:92–94.

Miller SA, Dykes DB, Polesky HF. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:12215.

Millis AL (2000) Isolation and characterization of microsatellite loci in the marsupial gliders, *Petaurus norfolcensis*, *P. breviceps* and *P. gracilis* Mol Ecol 9:1661–1686.

van Oosterhout C, Hutchinson WF, Wills DP, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538