Genetic non-invasive sampling (gNIS) as a cost-effective tool for monitoring elusive small mammals

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Online Resource 2 – Characteristics of the 20 microsatellite loci and nuclear introns typed using tissue (TS) and faecal (FC) samples of *Microtus cabrerae*.

Locus	Repeat motif	Multiplex TS	Multiplex FC	Primer sequence (5' – 3')	Primer TS (µM)[a]	Primer FC (µM)[b]	Dye[c]	Multiplex amplification success (%)	Individual amplification success (%)
Ma30[d]	(TC)(TG)	Mix A		F: TAGAACAAAGCTTGGACCC R: CCTGGCTTAGACAGAGTGG	F: 0.16 R: 1.60		VIC	95.00	100.00
MAG18[e]	AC	Mix B		F: GTATGCCTGCTATTGTGAAGAC R: GTTTGCTCTTTTGCTCCCAGTAACTCAC	F: 0.40 R: 4.00		NED	100.00	100.00
MAG25[e]	CA	Mix A		F: TGGGATAGCCTAGCAGCAAGA R: GTTTGTAGGGTTAGGTTCTCAGTTG	F: 0.32 R: 3.20		PET	90.00	50.00
Mc07	TCTT	Mix C		F: ATTCCCCTGCTATTCCCATT R: CAGGCTCCAAAGCTACAGAGA	F: 0.16 R: 1.60		VIC	58.23	88.89
Mc12	GA	Mix D		F: ACATGATCACCAATTGAGGAAA R: TCAGCCTGAACTACTGGATGAG	F: 0.12 R: 1.20		VIC	97.44	100.00
Mc14	ATCT	Mix C		F: TCAAACCACTACAGCTGGTCA R: GGCTTTGGGCACCTTATAGA	F: 0.16 R: 1.60		PET	54.43	100.00
Mc27	AC	Mix D		F: CAAGGAGACCCCTTTTCAGA R: GCACGCACATACGTGAACTC	F: 0.16 R: 1.60		FAM	95.00	100.00
Mc34	CA	Mix C		F: TCCACTCAGTCTCCTTTCTGG R: GCCAGCCTAAACATCCACAG	F: 0.16 R: 1.60		FAM	68.35	80.00
Mc37	CTAT	Mix C		F: CTTGGTAGCTGATCGATTTGA R: TGCTAGAAATGACTGGAAATACAGA	F: 0.24 R: 2.40		FAM	21.52	90.63
MSMM-3[f]	CA	Mix B	Mix 3[h]	F: TACGCCCTTCAAACTCATGTG R: TCCTTTATCTTAGGTGATGGAG	F: 0.14 R: 1.40	F: 0.04 R: 0.35	NED	100.00	100.00
Ma25[d]	(GT)(GA)	Mix B	Mix 3[h]	F: GATGAACAAAGGTTAATTCCAG R: ATCAGGCAGCTCACAGCTC	F: 0.24 R: 2.40	F: 0.06 R: 0.60	VIC	100.00	100.00
Mar03[g]	TG	Mix A	Mix 2[i]	F: GGAGATACAAGGCCCAAACA R: TGGCATTAGATGACCTGTGG	F: 0.24 R: 2.40	F: 0.06 R: 0.60	VIC	94.00	100.00

Online resource 2 – *(cont)*

Locus	Repeat motif	Multiplex TS	Multiplex FC	Primer sequence (5' – 3')	Primer TS (µM)[a]	Primer FC (µM)[b]	Dye[c]	Multiplex amplification success (%)	Individual amplification success (%)
Mar16[g]	GA(AA)GA	Mix A	Mix 3[h]	F: CATCATCTTCTGGGGCACTG R: ACGGTCTGTGCAAACCACTT	F: 0.16 R: 1.60	F: 0.05 R: 0.50	FAM	95.00	83.33
Mar76[g]	AC	Mix A	Mix 3[h]	F: TCACCAGGACCTACTGAGCA R: GCCAGCTTCATTTCAAGAGG	F: 0.16 R: 1.60	F: 0.04 R: 0.40	PET	97.00	100.00
Mar113[g]	AC	Mix A	Mix 2[i]	F: AAGAGCCTGCTGTGGTTTGT R: TCAGCTGGGAATCAGGTCTT	F: 0.20 R: 2.00	F: 0.05 R: 0.50	NED*	96.00	80.00
Mc02	AC	Mix C	Mix 2[i]	F: ACCCTGCCAATGTAGAAGGA R: ATGGACAGCTGTTGACTGGT	F: 0.16 R: 1.60	F: 0.04 R: 0.40	NED	77.22	100.00
Mc18	CTAT	Mix D	Mix 1	F: GGCACTGCGATTAAAGGTGT R: TGCTACAATTCCCAAGCATGT	F: 0.16 R: 1.60	F: 0.04 R: 0.40	PET	95.00	100.00
Mc23	GT	Mix D	Mix 2[i]	F: CCGTTGTGCTCCACCTTATT R: GCAAACACTGACAGGCACAT	F: 0.28 R: 2.80	F: 0.07 R: 0.70	NED	92.50	100.00
Mc24	AC	Mix D	Mix 1	F: ATAATTCCCCTTAGATAATGGTACG R: GACGATGTTGAGGTTTGGAG	F: 0.20 R: 2.00	F: 0.05 R: 0.50	NED	95.00	100.00
Mc30	AC	Mix C	Mix 1	F: AAATCCTGGCCAGACATGTAA R: TGTTGGAGACTGGACTGAGG	F: 0.36 R: 3.60	F: 0.07 R: 0.70	NED	53.16	81.25
Sexing									
DBY7-S		None[j]	Mix 2[i]	F: GTGCTTGGCTCCTCACAAAT R: TGTCCTGTGTGAGTACCTGATGTCT	F: 0.04 R: 0.40	F: 0.04 R: 0.40	FAM		
DBX5-S		None[j]	Mix 2[i]	F: CATTAGTTGAATGTAGTTGCCATTG R: TGTTATCTGAAGGCACCCTGT	F: 0.07 R: 0.70	F: 0.07 R: 0.70	FAM		

TS, tissue samples; FC, faecal samples; F, Forward primer; R, Reverse primer.

* For non-invasive amplification, the dye of the locus Mar113 was changed to FAM.

[a] Concentrations of the primers for a multiplex mix with a final volume of 25µl. A volume of 0.5µl of the mix of primers was added to a reaction final volume of 5µl, with the addition of 2.5µl of Qiagen© Multiplex PCR Kit MasterMix (Qiagen, Hilden, Germany) and 0.5µl DNA. For individual amplification, we used 0.2µM of each primer, in a final volume of 5µl, with the addition of 2µl of Qiagen© Multiplex PCR Kit MasterMix (Qiagen, Hilden, Germany) and 0.5µl of DNA.

[b] Concentrations for a PCR final volume of 10µl, with the addition of 4µl of Qiagen© Multiplex PCR Kit MasterMix (Qiagen, Hilden, Germany) and 2µl of DNA.

[c] A M13(-21) tail was added to the 5' end of each forward primer to allow for fluorescent labelling with 1 out of 4 fluorescent dies (NED, PET, VIC, 6-FAM) according to Schuelke 2000.

[d] Primers originally optimised for *Microtus arvalis* samples (Gauffre et al. 2007).

[e] Primers originally optimised for *Microtus agrestis* samples (Jaarola et al. 2007).

[f] Primers originally optimised for *Microtus montebelli* samples (Ishibashi et al. 1999).

[g] Primers originally optimised for *Microtus arvalis* samples and showing cross-amplification in other *Microtus* (Walser and Heckel 2007).

[h] The multiplex is amplified in two different PCR reactions: Mar16+Mar76 and Ma25+MSMM-3. After amplification, the PCR results are pooled using 2µl of each sample.

[i] The multiplex is amplified in three different PCR reactions: Mc23+Mc02; Mar03+Mar113 and DBY7-S+DBX5-S. After amplification, the PCR results are pooled using 2µl of each sample.

[j] The introns used for sexing were amplified and scored separately from any multiplex mix in the tissue samples.

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