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A new primer set for sex identification in the genus *Sorex* (Soricidae, Insectivora)

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Keywords: PCR, sex identification, shrew, *Sorex*, Soricidae, SRY HMG box

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Running title: Sex identification in *Sorex*
Abstract

In order to develop a new accurate method for sexing in Sorex species (Soricidae, Insectivora), we synthesized a PCR primer set to amplify a part of Sry HMG box in the long-clawed shrew, Sorex unguiculatus. When the primers were applied to the samples of known sex, PCR products were successfully obtained for males as a clear, single band on 3 % agarose gels after electrophoresis in S. unguiculatus and other five Sorex species, but never for females of these six species. Thus, PCR amplification using the primer set may be applicable to discern sex in the six Sorex species.
In shrews (Soricidae, Insectivora), it is quite difficult to sex the young without checking internal genitals, because body size and external genitals are similar between sexes (e.g., Churchfield 1990). Some sexing techniques for the young have been proposed for the genus *Sorex* based on elaborate observations of external genitals (Crowcroft 1964; Inoue 1988) and nipples (Croin-Michelsen 1966), and hearing calling voices (Crowcroft 1957). These methods may, however, have the probability of misidentification, as they need the skill to perform. In addition, these methods may not be applicable to dead animals with damaged bodies. Thus, it is necessary to develop a new accurate method for sexing in shrews, especially when internal genitals can not be checked. Recently, PCR amplification of sex-linked genes such as *AMGY* (Amelogenin, Y), *ZFY* (Zinc Finger, Y) and *SRY* (Sex Determining Region, Y) is used to identify sex in some mammals (Griffiths 2000). Sánchez *et al.* (1996) developed a primer set for amplifying the *Sry* HMG box of some insectivores including three families, Talpidae, Soricidae and Erinaceidae. In our preliminary study, however, their primer set generated no clear band of expected size in the six *Sorex* species examined in this study, belonging to the family Soricidae under several PCR conditions. Here, we reported the sequences of a PCR primer set to amplify a part of *Sry* in the long-clawed shrew, *Sorex unguiculatus*, and its applicability to discern sex for several insectivoran species.

Two PCR primers were synthesized based on the sequence of the *SRY* HMG box, of which sequences were conserved between the human and the rabbit (Sinclair *et al.* 1990); Pri-1, 5'-AAGCGACCCATGAACGCATT-3' and Pri-3, 5'-CGGGTATTTCTTTGTGCA-3'. The primer set, however, provided a faint band of expected size (198 bp) with many bands of higher molecular weights even under a condition of high MgCl₂ concentration (3.0 mM) in *S. unguiculatus*. For direct cloning of PCR products, the faint band was excised from a 3 % agarose gel after
electrophoresis, and then ligated into the pCR2.1-TOPO vector (Invitrogen). Isolated
twelve clones were sequenced with the conventional dideoxy method using a Thermo
Sequenase pre-mixed cycle sequencing kit (Amersham) and a DNA Sequencer SQ5500-
L (Hitachi). Sequence analysis showed that four of the twelve clones were the part of
the Sry HMG box, although two of the four clones had a substitution in the nucleotide
sequences at different positions when compared with the others. Based on the consensus
sequence among them, two new primers were designed; F1, 5'-
CATGGTGTTGGCTCGCAATC-3' and R1, 5'-CTGCCCTGATGCTCTGTGCC-3' (Fig.
1).

Accuracy of the new primer set (F1 and R1) for sex identification was examined
in 47 individuals of S. unguiculatus from Bibai in Hokkaido, the northernmost island of
Japan (29 males and 18 females), whose sexes were anatomically identified. PCR
amplification was carried out using an ABI GeneAmp system 2400 (PE Biosystems) in
25 µl of reaction mixture containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.1 %
Triton X-100, 1.5 mM MgCl₂, 50 µM dNTP, 0.5 µM of each primer, 0.625 U Taq DNA
polymerase (Promega), and about 100 ng of genomic DNA. After denaturation at 93°C
for 2 min, cycling was performed for 30 cycles under the following condition: 15s at
93°C, 20s at 60°C, 20s at 72°C. PCR products were electrophoresed on 3% agarose gels
to examine whether the target gene site was amplified. We also tested the applicability
of the primers under the same PCR condition as described above in six shrew species
including Sorex caecutiens from Hokkaido (9 males and 15 females), S. gracillimus
from Hokkaido (6 males and 4 females), S. hosonoi from Honshu, the central island of
Japan (3 males and 5 females), S. isodon from various locations of northeastern Asia (4
males and 6 females), S. minutissimus from various locations of northeastern Asia (6
males and 4 females) and Crocidura suaveolens from various locations of Asia (5 males
and 2 females).

Our new primer set, F1 and R1, provided a clear, single band of expected size (155 bp) on an agarose gel in males but never in females of *S. unguiculatus* (Fig. 2). This indicated that the amplified sequence certainly is located on the Y chromosome, and implied that non-amplifying alleles due to nucleotide substitutions are rare in the shrew population. Additionally, the same results were obtained in other five *Sorex* species (Fig. 2), but this primer set provided a faint band of expected size and several bands of larger molecular size in males of *C. suaveolens*. Thus, these results suggest that the present primer set may be useful to discern sex in the six *Sorex* species.

**Acknowledgements**

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**References**


Figure legends

Fig. 1  The nucleotide sequence of part of the Sry HMG box in Sorex unguiculatus (155 bp; DDBJ accession #AB055219). The sequences of two primers, F1 and R1, used in this study are underlined.

Fig. 2  Sex identification in six Sorex species. PCR amplification using the Sry primers, F1 and R1, for Sorex unguiculatus (see Fig. 1 and text) generates products only in males. The size of bands is indicated in base-pairs (bp) on the left side of the lanes. S; Molecular size marker, Hae III-digested ΦX174, M; Male, F; Female, SUN; S. unguiculatus, SCA; S. caecutiens, SGR; S. gracillus, SHO; S. hosonoi, SIS; S. isodon, SMI; S. minutissimus.
Fig. 1 Matsubara et al.

CATGGTGTGGGCTCGCAATCAAAGACGTAAGATAGGCTCTAGAAATCCTAAAATGCAAAACTCAGAGATCAGCAAACAGCTGGGATACCAGTGGAAAATGCTTACAGAAGATGAAAAATTGCCATTCTTTTCAGGAGGCACAAGACTACAGGCAG
Fig. 2 Matsubara et al.