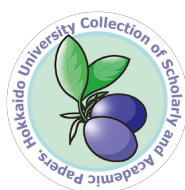




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A new primer set for sex identification in the genus *Sorex* (Soricidae, Insectivora)

K. Matsubara,* Y. Ishibashi,† S. Ohdachi[§] and Y. Matsuda*†

5 *Laboratory of Cytogenetics, Graduate School of Environmental Earth Science,
Hokkaido University, Sapporo 060-0810, Japan, †Chromosome Research Unit, Faculty
of Science, Hokkaido University, Sapporo 060-0810, Japan and [§]Institute of Low
Temperature Science, Hokkaido University, Sapporo 060-0819, Japan.

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

Correspondence: Satoshi Ohdachi. Address: Institute of Low Temperature Science,
Hokkaido University, Sapporo 060-0819, Japan; Fax: (81) 11 706 7142; E-mail:

15 ohd@pop.lowtem.hokudai.ac.jp

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
5 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-Michielsen 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'-CGGGTATTTCTCTTTGTGCA-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'-CATGGTGTGGGCTCGCAATC-3' and R1, 5'-CTGCCTGTAGTCTCTGTGCC-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.

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References

- Churchfield S (1990) *The Natural History of Shrews*. A & C Black, London.
- Crowcroft P (1957) *The Life of the Shrew*. Max Reinhardt. London.
- Crowcroft P (1964) Note on the sexual maturation of shrews (*Sorex araneus* Linnaeus, 1758) in captivity. *Acta Theriologica* 8, 89-94.
- Croin-Michielsen N (1966) Intraspecific and interspecific competition in the shrews *Sorex araneus* L. and *S. minutus* L. *Archives Néerlandaises de Zoologie* 18, 73-174.
- Griffiths R (2000) Sex identification using DNA markers. In: *Molecular Methods in Ecology* (ed. Baker AJ), pp. 295-321. Blackwell Science, Oxford.
- Inoue T (1988) A new method for sexing young of the big-clawed shrew, *Sorex unguiculatus* (Dobson). *Journal of the Mammalogical Society of Japan* 13, 143-145.

Sánchez A, Bullejos M, Burgos M, Hera C, Jiménez R, Díaz de la Guardia R (1996)
High sequence identity between the SRY HMG box from humans and insectivores.
Mammalian Genome 7, 536-538.

- Sinclair AH, Berta P, Palmer MS, Hawkins JR, Griffiths BL, Smith MJ, Foster JW,
5 Frischauf A-M, Lovell-Badge R, Goodfellow PN (1990) A gene from the human sex-
determining region encodes a protein with homology to a conserved DNA-binding
motif. *Nature* 346, 240-244.

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 Φ X174, M; Male, F; Female, SUN; *S. unguiculatus*, SCA; *S. caecutiens*, SGR; *S. gracillimus*, SHO; *S. hosonoi*, SIS; *S. isodon*, SMI; *S. minutissimus*.

CATGGTGTGGGCTCGCAATCAAAGACGTAAGATAGCTCTAGAAAATC
CTAAAATGCAAAACCTCAGAGATCAGCAAACAGCTGGGATACCAGTGG
AAAATGCTTACAGAAGATGAAAAATTGCCATTCTTTCAGGAGGCACA
GAGACTACAGGCAG

Fig.1 Matsubara *et al.*

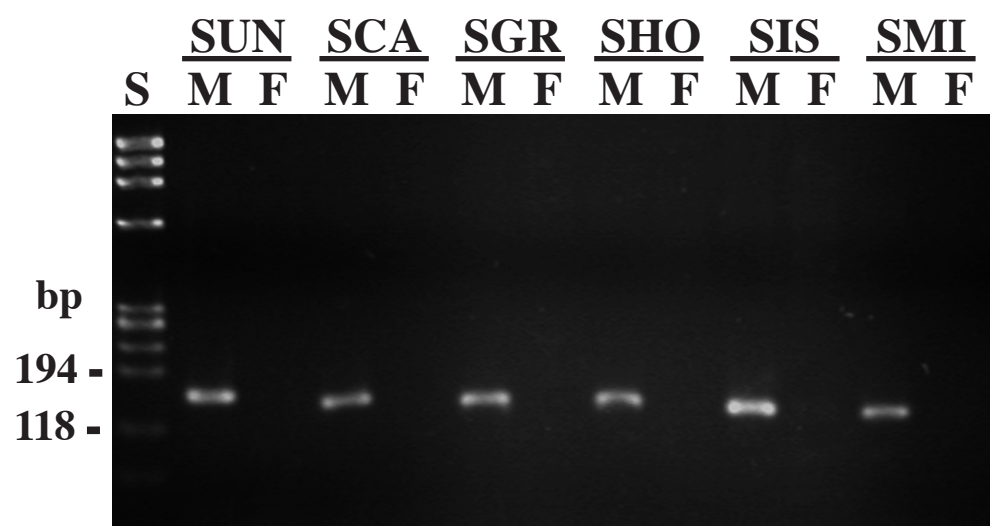


Fig.2 Matsubara *et al.*