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

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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-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’-AACCGACCCCATGAACGCATT-3’ and Pri-3, 5’-CGGTTATTCTTTTGTGCA-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
十二克隆被序列化，使用Thermo
Sequenase预先混合的循环测序试剂盒（Amersham）和DNA Sequencer SQ5500-
L（Hitachi）。序列分析表明，四十二克隆中
的Sry HMG盒，虽然其中两个克隆在核酸
序列未与其它克隆相比时发生突变。基于
的共识
序列中，两个新引物被设计；F1，5’-
CATGGTGTGGCTCGCAATC-3’ and R1, 5’-CTGCCCTGTACTCTGTGCC-3’ (Fig. 1).
准确度的新引物集（F1和R1）的性别识别
在47只来自Hokkaido的Bibai, 北方的岛屿
的人口（29名男性和18名女性），其中性别
的解剖学确认。PCR
的扩增在使用一个ABI GeneAmp系统2400（PE Biosystems）在
反应混合物中的25 μl, 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


High sequence identity between the SRY HMG box from humans and insectivores.
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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. gracilimus*, SHO; *S. hosonoi*, SIS; *S. isodon*, SMI; *S. minutissimus*. 
Fig. 1  Matsubara et al.
Fig. 2 Matsubara et al.