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PRIMER NOTE

Felid sex identification based on noninvasive genetic samples

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Abstract

We developed two tests for sex identification of felids using y-chromosome deletions in the zinc-finger and amelogenin regions. These tests provide positive results for both males and females, while reducing the need to co-amplify microsatellites to test for DNA quality in hair and scat samples. Furthermore, the y-chromosome deletions are absent in a wide-range of prey species; thus, when these tests are used on scat samples, potential contamination caused by prey DNA incidentally extracted, is minimized.

Keywords: felid, hair samples, lynx, noninvasive, scat, sex identification

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The most common DNA sex identification tests in mammals amplify the y-specific *SRY* locus (Woods *et al*. 1999; Dallas *et al*. 2000). The *SRY* tests can be problematic when used on poor-quality DNA samples obtained through noninvasive genetic sampling as *SRY* is specific to the y chromosome and females are identified by failure to amplify. Negative tests are suspect when analysing hair and scat samples where amplification success is limited. Therefore, the y-specific marker is often run in the same reaction as a microsatellite to serve as a control for DNA quality (Dallas *et al*. 2000). However, amplification at a particular microsatellite does not guarantee amplification success at *SRY*. Additionally, when working with scat samples, the generality of the *SRY* test can be problematic, as prey DNA in the scat can confound sex identification (Murphy *et al*. 2003).

We wanted a simple polymerase chain reaction (PCR)-based test for sex identification that would work on low-quantity DNA obtained from hair and scat samples, where sequencing is often difficult. We used sequences available for the zinc-finger region of the x and y-chromosomes (*Zfx* and *Zfy*) for domestic cat (*Felis silvestris*; GenBank accession AF253014, AF252989), bobcat (*Lynx rufus*; AF253002, AF252975), cougar (*Puma concolor*; AF253018, AF252988), and Eurasian lynx (*Lynx lynx*; AF253001, AF252974, Slattery *et al*. 2000) and designed primers from conserved regions in all four species. We chose these four species because of their presence in North America, and close shared ancestry with the Canada lynx (*Lynx canadensis*; Johnson & O'Brien 1997), which is our primary species of interest. In all these species, a portion of *Zfy* has a 3 bp deletion when compared to *Zfx*.

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We designed a second sex identification test for felids using the amelogenin region based on sequence data available for domestic cat (GenBank accession AF114709, AF197967). The amelogenin gene region is found on the sex chromosomes in many species of mammals (e.g. humans, mice, domestic goat, domestic cat, lemur; NCBI GenBank). In domestic cat, the y-chromosome copy (*AMELY*) has a 20 bp deletion when compared to the x-chromosome gene (*AMELX*). Both regions are found outside the pseudoautosomal region of the y-chromosome, and the deletions in both *Zfy* and *AMELY* provide sex identification with males and females both producing PCR (polymerase chain reaction) products.

We tested our protocol using DNA extracted from high-quality tissue from 20 known male and 20 known female lynx (Alaska, Montana), and 10 of each sex for bobcat (Florida, Minnesota, Montana, Oregon) domestic cat (Montana, Oregon), and cougar (Arizona, Montana, Washington, Wyoming). We also ran the tests on males and females of several nontarget species: human (*Homo sapiens*), Columbian ground squirrel (*Spermophilus columbianus*), red squirrel (*Tamiasciurus hudsonicus*), chipmunk (*Tamias ruficaudus*), snowshoe hare (*Lepus americanus*), white tail deer (*Odocoileus virginianus*), blacktail deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), moose (*Alces alces*), black bear (*Ursus americanus*), brown bear (*Ursus arctos*), skunk (*Mephitis mephitis*), marten (*Martes americana*), mink (*Mustela vison*),

Table 1 Primer sequences and features of sex identification loci used for lynx, bobcat, cougar, and domestic cat

Locus	Primer sequences (5′–3′)	Temp (°C)	Product size (bp)
Zn-finger	F: AAGTTTACACAACCACCTGG	56	Male: 163, 166
	R: CACAGAATTTACACTTGTGCA		Female: 166
Amelogenin	F: CGAGGTAATTTTTCTGTTTACT	51	Male: 194, 214
	R: GAAACTGAGTCAGAGAGGC		Female: 214

fisher (*Martes pennanti*), wolverine (*Gulo gulo*), coyote (*Canis latrans*) and domestic dog (*Canis familiaris*). Nontarget species samples were from Colorado, Idaho, Montana, Oregon, Wisconsin and Wyoming.

Genomic DNA from hair samples was extracted following the protocols in Mills *et al*. (2000); DNA from tissue samples was extracted with the DNeasy Tissue Kit (Qiagen Inc.). DNA from scat was extracted using the QIAamp DNA Stool Mini Kit (*Qiagen* Inc.). DNA was amplified with PCR using primers designed for the Zn-finger and amelogenin regions (Table 1). The reaction volume (10 μL) contained 1.0–3.0 μL DNA, 1 × reaction buffer (*Applied Biosystems*), 2.0 mM $MgCl_2$, 200 μM of each dNTP, 1 μM reverse primer, 1 μM dye-labelled forward primer, 1.5 mg/mL BSA, and 1 U *Taq* polymerase (Applied Biosystems). The PCR profile was 94 °C/5 min, [94 °C/1 min, × °C/1 min, 72 °C/30 s] × 29 cycles (Table 1) for tissue samples, and was increased to 45 cycles for use with hair samples. PCR products were run in a 6.5% acrylamide gel for 2 h on a *LI-COR DNA* analyser (LI-COR Biotechnology).

All four felid species successfully amplified at both regions. Males of all four felid species produced products of different length, whereas females produced products of the same length (Table 1). Felid hair samples containing quality DNA, based on microsatellite amplification (ability to amplify the same alleles using the multitube approach; Taberlet *et al*. 1996), also produced identical sex-specific allele patterns.

We tested our primers in other species to determine if sex-specific PCR products were produced. All nonfelid species produced PCR products of 166 bp at the Zn-finger region, but these products were not sex-specific. Chipmunk, snowshoe hare, Columbian ground squirrel, and red squirrel failed to amplify at the amelogenin region; whereas all others amplified, producing products of 214 bp, but these were also not sex-specific. Humans produced products of 218 bp which will be useful to detect human contamination.

Both the zinc-finger and amelogenin regions produced positive, sex specific tests for all of the targeted felids. The deletions in *Zfy* and *AMELY* are likely felid–specific, as males of other tested species lack this deletion. For non-invasive sex identification in felids, we therefore believe that this test represents an improvement over currently published methods, and to our knowledge, is the first published sex identification test that specifically targets felids.

While these tests are less sensitive to prey contamination when sexing scat samples, we recommend caution. Errors can occur if the felid DNA sample fails to amplify, prey DNA is present and quantity to amplify is not sufficient. In this case, a false positive for females could result. To avoid this error we recommend sexing those samples that have consistent amplification and no error at species-specific microsatellite loci, an indicator of quality nuclear DNA.

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