Short Communication

Sexing of Human and Other Primate DNA

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We present a rapid and simple method for the sexing of human and other primate DNA. Homologous introns from the X- and Y-linked zinc finger protein genes (ZFX and ZFY) are PCR amplified with one primer pair. An Alu element insertion in the ZFX intron results in a size difference in the amplicons which is resolved by agarose gel electrophoresis.

Key words: PCR / ZFX / ZFY.

Sexing of DNA samples is desirable in many fields from forensics and archaeology to molecular ecology. The simplest techniques rely on the amplification of fragments from the mammalian X- and Y-chromosomes. However, in many cases separate PCRs and subsequent restriction digestion are required in order to determine the sex (e.g. Reynolds and Varlari, 1996; Stacks and Witte, 1996).

The most frequently used human DNA sexing technique is the amelogenin system (Sullivan et al., 1993). Fragments differing in size by 6 bp are amplified from homologous genes on the sex chromosomes. The amplification products are routinely fluorescently or radioactively labelled for subsequent separation by polyacrylamide gel electrophoresis. However, due to a deletion polymorphism, the Y-specific band has recently been found to be absent in 0.6% of a world-wide sample of male individuals (Santos et al., 1998).

Here, we describe a simple, fast, and cheap protocol which allows the sex determination in primates by a single PCR reaction followed by agarose gel electrophoresis. ZFX and ZFY are homologous genes on the non-pseudoautosomal parts of the sex chromosomes. An Alu element inserted into the last intron of ZFX before the old world monkey-new world monkey split (Shimmin et al., 1993). When PCR is performed with non-degenerate primers which amplify this intron from both ZFX and ZFY, the resulting size difference (422 bp) is readily resolved by agarose gel electrophoresis. The presence of an X-derived fragment (1151 bp) serves as a positive control for the amplification reaction and indicates that the sample is of female origin, while the presence of both this and the shorter (729 bp) Y-derived fragment is diagnostic for males. It is advantageous that the X-specific control band for the PCR reaction represents the longer amplicon, because the Y-specific sex-determining shorter band will then always, if present, amplify more strongly. This system allows sexing of humans, apes, old and new world monkeys (Figure 1).

It is unlikely that the amelogenin sexing system (Sullivan et al., 1993) will be useful in non-human primates as there are several priming site mismatches in the orang-utan and squirrel monkey, while the Y-linked amelogenin gene is absent in baboons (Nakahori et al., 1991; Huang et al., 1997). In contrast, the last intron of ZFX and ZFY is conserved among primates (Shimmin et al., 1993; Burrows and Ryder, 1997) and it is unlikely that the Alu element would be precisely deleted in any species. Indeed, sexing was successful in a world-wide sample of 129 humans as well as in six chimpanzees, six orangutans and three orang-utans (data not shown). Furthermore, analyses of the ZFY intron in 38 men (Donit et al., 1995) and the ZFX intron in 29 men (Huang et al., 1998) showed no evidence of internal deletion.

Fig. 1 Sexing by PCR of Male and Female Primate DNAs.

Electrophoresis was carried out in a 0.8% agarose gel (Bioco BRL) with TAE buffer, and the bands were visualised under ultraviolet transillumination after ethidium bromide staining. Lanes: 1, 1 kb ladder (Gibco BRL); 2, male human; 3, female human; 4, male chimpanzee; 5, female chimpanzee; 6, male gorilla; 7, female gorilla; 8, male orangutan; 9, female orangutan; 10, male gibbon; 11, female gibbon; 12, male baboon; 13, male marmoset; 14, 1 kb ladder.

PCR (Mullis and Faloona, 1987) was performed in a total volume of 25 μl with 10–100 ng of genomic DNA in 1.5mM MgCl₂, 0.5μM of each primer (Rik6 5' ATTCCAGCGACTAAACG 3' and Jim9 5' GOATCGAGCCATAATTAG 3') (synthesised by MWG Biotech, Ebersberg, Germany), 0.25 mM dNTPs (Pharmacia), with 2.5 μl 10 X reaction buffer II (Perkin-Elmer) and 1 unit AmpliTaq DNA polymerase (Perkin-Elmer). Cycling conditions (on an MJ PTC-200) were 94°C for 2 min, and 30 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 1 min 30 s. The whole procedure takes less than three hours.

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References


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