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## Mobile element-based assay for human gender determination

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Notes & Tips

## Mobile element-based assay for human gender determination

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Determination of gender from human DNA samples is a common problem in forensic laboratories. While several PCR-based assays are currently available for human sex typing, each of the current approaches has limitations. Methods based on male-specific amplification, such as the amplification of the SRY locus [1], lack an internal positive control to discriminate between female DNA and male DNA which has failed to amplify for technical reasons. Restriction fragment length polymorphism assays based on sex-specific mutations at the ZFX/ZFY locus [2] require a second enzyme digestion or hybridization step following the initial PCR amplification. A recent method proposed by Cali et al. [3] based on a single adenine insertion within a tandem repeat array at the DXYS156 locus requires access to allele detection equipment potentially unavailable to forensic labs with limited resources. The most widely used approach is based on the *Amelogenin* locus, which yields different-sized PCR amplicons for the X and Y chromosome versions of the *Amelogenin* gene [4]. However, this method misidentifies males as females in some cases due to a deletion in the *AMEL Y* region [5–7]. This deletion has previously been reported to be present at a frequency of 0.018% in Caucasian males, 1.85% among Indians, and as high as 8% in Sri Lankans [5–7]. While the frequency of the deletion is relatively low, the crucial nature of forensic test results in circumstances such as rape and prenatal gender determination where there is risk for male-specific inherited disorders makes any source of error a legitimate cause for concern. This has led several researchers to recommend that *Amelogenin* should not be relied upon as the sole determinant of gender [5–8]. Here, we present an

alternative PCR method of human gender identification based on the presence/absence of *Alu* sequences.

*Alu* elements are transposable elements which have amplified throughout primate evolution and comprise roughly 10% of the human genome [9]. *Alu* insertions are generally considered to be homoplasmy free with respect to human population genetics, as the probability of two *Alu* elements independently inserting in the same genomic location is extremely small [9]. The insertion of an *Alu* element into a nonrecombining X–Y homologous region creates a way of differentiating between inserted and noninserted chromosomes based on PCR amplicon size. While some recently integrated *Alu* insertions remain polymorphic in the human population, many ultimately reach fixation for the presence of the *Alu* insertion [9]. Fixed insertions on either the X or the Y chromosome provide a way of identifying the respective chromosome, as the inserted chromosome yields a larger fragment when the homologous region is amplified with PCR (Fig. 1). By screening X–Y homologous *Alu* insertions for levels of insertion polymorphism, we identified two monomorphic *Alu* insertions that meet the necessary criteria for a gender determination assay, one fixed on the X chromosome, *AluSTXa*, and one fixed on the Y chromosome, *AluSTYa*. Both of the *Alu* elements presumably inserted and reached fixation in the human lineage prior to the radiation of modern humans from Africa. Amplification of DNA samples from 778 diverse (African-American, European-American, and Hispanic-American) individuals of defined sex from paternity/identity cases for both the *AluSTYa* and the *AluSTXa* loci showed 100% accuracy in gender identification. The DNA samples used in the study consisted of 389 females (278 African-American, 102 European-American, and 9 Hispanic-American) and 389 males (288 African-American, 90 European-American, and 11 Hispanic-American).

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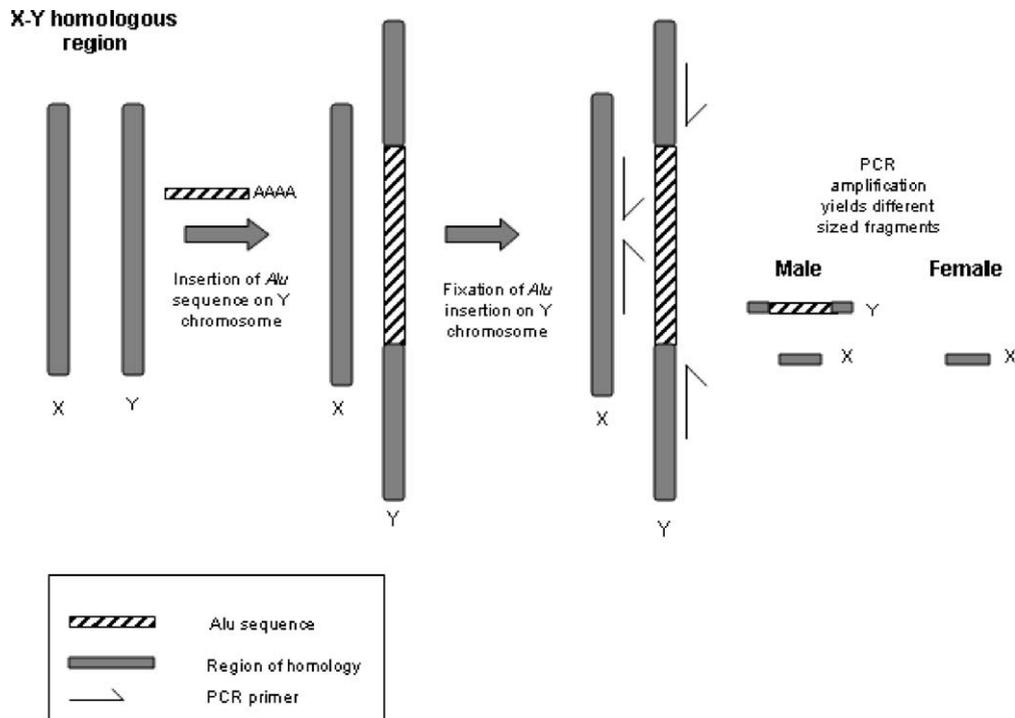


Fig. 1. Schematic diagram of mobile element-based gender determination. In the diagram an *Alu* insertion has occurred on the Y chromosome within an X–Y homologous region. Once fixed in the population, the *Alu* insertion sequence results in a larger amplicon on the Y chromosome, allowing for the differentiation of the sex chromosomes via PCR amplification. X chromosome-specific insertions function in the same manner.

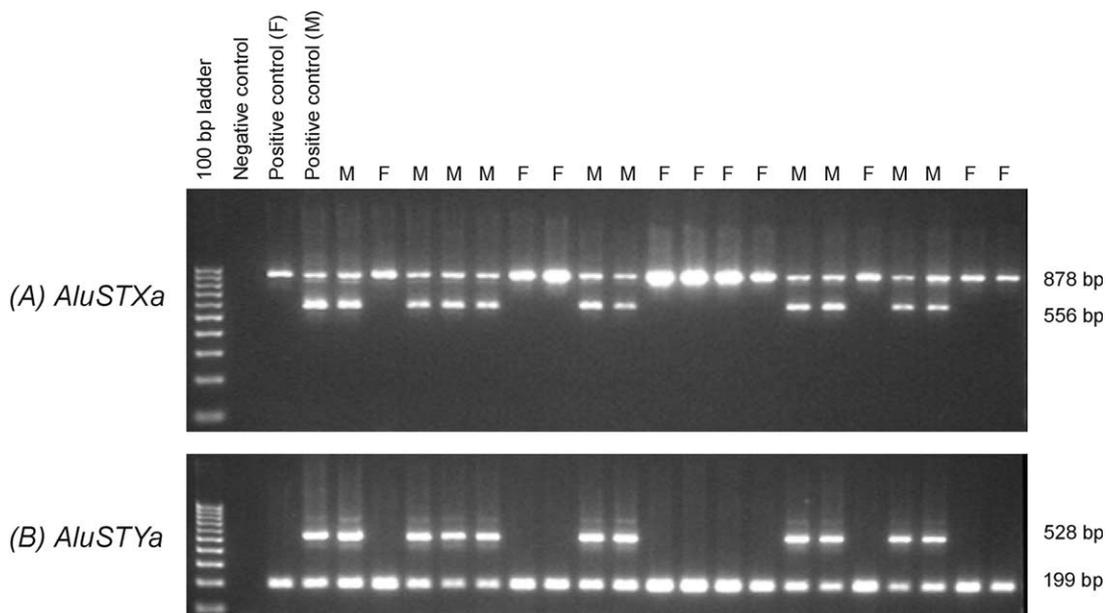


Fig. 2. Mobile element-based gender determination. An agarose gel chromatograph from the analysis of 22 individuals using the genetic systems (A) *AluSTXa* and (B) *AluSTYa* is shown. Males are distinguished by the presence of two DNA fragments, while females have a single amplicon. F (female) and M (male) above each sample indicate the known gender. Individual PCR amplifications were performed in 25- $\mu$ l reactions using 25 ng of template DNA, 0.2  $\mu$ M each oligonucleotide primer, 200  $\mu$ M deoxynucleotide-triphosphates, 1.5 mM  $MgCl_2$ , 10 mM Tris-HCl (pH 8.4), and *Taq* DNA polymerase (1 unit). Each sample was subjected to the same amplification cycle as follows: initial denaturation of 150 s at 94  $^{\circ}C$ , 32 cycles of 1 min of denaturation at 94  $^{\circ}C$ , 1 min at the specific annealing temperature (58  $^{\circ}C$  for *AluSTYa* and 60  $^{\circ}C$  for *AluSTXa*), 1 min of extension at 72  $^{\circ}C$ , followed by a final extension at 72  $^{\circ}C$  for 10 min. For analysis, 20  $\mu$ l of the PCR products were fractionated on a 2% agarose gel which contained 0.25  $\mu$ g/ml of ethidium bromide. PCR products were visualized using ultraviolet fluorescence.

Amplification of the loci was conducted via a PCR and fragments were resolved on a 2% agarose gel (Fig. 2). The primers used for the Y insertion, *AluSTYa*, were Forward 5'-CATGTATTTGATGGGGATAGAGG-3' and Reverse 5'-CCTTTTCATCCAACCTACCACTGA-3', yielding an *Alu*-filled site (Y chromosome) fragment of 528 bp and an empty site (X chromosome) fragment of 199 bp. Primers for the X insertion, *AluSTXa*, were Forward 5'-TGAAGAAATTCAGTTCATAGCTTGT-3' and Reverse 5'-CAGGAGATCCTGAGATTATGTGG-3', yielding an inserted (X chromosome) fragment of 878 bp and an empty site (Y chromosome) fragment of 556 bp. For both loci, males are distinguished as having two DNA amplicons present, while females have only a single amplicon (Fig. 2).

Combining these loci together for human gender identification will provide increased accuracy for sex typing since local deletions or other types of mutations that eliminate PCR would have to occur in at least two independent genomic locations. The speed and ease of agarose-based genotyping due to the ~300-bp difference between filled and empty alleles will also enhance the utility of the assay in forensic laboratories. This approach should also be amenable to fluorescence-based amplicon detection and quantitative PCR to resolve male and female contributions to sex-mixed samples. Furthermore, similar approaches based on repetitive element insertions located in homologous sex chromosome regions should be useful for gender determination in other taxa of heterogametic sex.

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### References

- [1] A.H. Sinclair, P. Berta, M.S. Palmer, J.R. Hawkins, B.L. Griffiths, M.J. Smith, J.W. Foster, A.M. Frischau, R. Lovell-Badge, P.N. Goodfellow, A gene from the human sex-determining region encodes a protein with homology to a conserved DNA-binding motif, *Nature* 346 (1990) 240–244.
- [2] R. Reynolds, J. Varlaro, Gender determination of forensic samples using PCR amplification of ZFX/ZFY gene sequences, *J. Forensic Sci.* 41 (1996) 279–286.
- [3] F. Cali, P. Forster, C. Kersting, M.G. Mirisola, R. D'Anna, G. De Leo, V. Romano, DXYS156: a multi-purpose short tandem repeat locus for determination of sex, paternal and maternal geographic origins and DNA fingerprinting, *Int. J. Legal Med.* 116 (2002) 133–138.
- [4] K.M. Sullivan, A. Mannucci, C.P. Kimpton, P. Gill, A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X–Y homologous gene amelogenin, *Biotechniques* 15 (1993) 636–638, 631–640.
- [5] F.R. Santos, A. Pandya, C. Tyler-Smith, Reliability of DNA-based sex tests, *Nat. Genet.* 18 (1998) 103.
- [6] M. Steinlechner, B. Berger, H. Niederstatter, W. Parson, Rare failures in the amelogenin sex test, *Int. J. Legal Med.* 116 (2002) 117–120.
- [7] K. Thangaraj, A.G. Reddy, L. Singh, Is the amelogenin gene reliable for gender identification in forensic casework and prenatal diagnosis?, *Int. J. Legal Med.* 116 (2002) 121–123.
- [8] B. Brinkmann, Is the amelogenin sex test valid?, *Int. J. Legal Med.* 116 (2002) 63.
- [9] M.A. Batzer, P.L. Deininger, Alu repeats and human genomic diversity, *Nat. Rev. Genet.* 3 (2002) 370–379.