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TECHNICAL NOTE

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A Y-chromosome STR Marker Should Be Added to Commercial Multiplex STR Kits

ABSTRACT: Autosomal short tandem repeat (STR) analysis has become highly relevant in the identification of victims from mass disasters and terrorist attacks. In such events, gender misidentification can be of grave consequences, yet the list reporting amelogenin amplification failure using STR multiplex kits continues to grow. Presented here are three such examples. In the first case, we present two male suspects who demonstrated amelogenin Y-deficient results using two commercial kit procedures. The presence of their Y chromosomes was proven by obtaining a Y-haplotype. The second case demonstrated a profile from a third male suspect where only the Y homolog of the XY pair was amplified. In events such as mass disasters or terrorist attacks, timely and reliable high throughput DNA typing results are essential. As the number of reported cases of amplification failure at the amelogenin gene continues to grow, we suggest that the incorporation of a better gender identification tool in commercial kits is crucial.

KEYWORDS: forensic science, amelogenin, gender typing, STR multiplex kits, mutation, X chromosome, Y chromosome, Y marker

Homologs of the amelogenin gene, which codes for a protein of dental enamel, are found on both the X and Y chromosomes. The forensic community utilizes a 6 base-pair difference in the nucleotide length of the X and Y homologs of this gene to define the gender of the donor of unknown stains on items of evidence (1). Primers specific for polymerase chain reaction (PCR) amplification of the amelogenin sequence are incorporated into commercial multiplex short tandem repeat (STR) kits, which are mainly used to analyze forensic samples and database reference samples.

While the incorporation of these primers into multiplex STR typing kits provides an efficient and effective sex identification tool for forensic samples, an alarming number of cases have been reported where mutations or deletions in the primer binding site failed to demonstrate the Y-specific homolog (2–5). Cases have also been reported where the X-specific allele was not amplified because of primer site mutations (6,7).

Gender misidentification may occur in biological samples carrying such mutations. When large numbers of samples are typed simultaneously, as in the event of a mass disaster, erroneous conclusions may be reached regarding such samples. In database profiling, these mutations may be more easily identified if the sample has been received from a phenotypically presenting male. However, as in our database, where demographic information is separated from profiles prior to data entry, apparent gender may be in error.

We present three examples, from routine database typing, where PCR failure occurred at the amelogenin gene. In the first case, two samples were received by the database laboratory from suspects of Bedouin origin, nomadic Arabs from the Negev who live in large family or tribal groups. Although both samples had the same last name, and both had been collected and sent from the same geographical region, no familial connection between the two suspects was specifically brought to our attention. Upon typing, the samples provided AMEL Y-deficient genotypes using both SGM Plus™ (Applied Biosystems, Foster City, CA) and PowerPlex® 16 (Promega Corporation, Madison, WI) STR multiplex kits. The presence of their Y chromosome was consequently demonstrated by obtaining complete Y-STR profiles from both samples using the Yfiler™ kit (Applied Biosystems).

In the second case, we obtained a profile from a phenotypically male suspect's sample using the SGM Plus™ kit, in which the X chromosome specific AMEL allele failed to amplify but the Y chromosome allele was observed.

Materials and Methods

Buccal cells, collected on FTA cards from the two Bedouin suspects and a third male suspect were received in the Israel Police DNA Database Laboratory for processing and profiling.

Sample Preparation, Amplification and Analysis Using Applied Biosystems AmpFlSTR® SGM Plus™ Kit

Pre-PCR preparation was carried out on single 1.2 mm punches using an in-house developed method employing H_2O rinses, which does not necessitate quantification before PCR. Amplification was carried out using the AmpFlSTR SGM Plus™ system kit for 27 cycles in a final volume of 15 μL. These amplified products were separated and detected by capillary electrophoresis on the ABI Prism 3100 Genetic Analyzer using GeneScan® and GeneMapper® ID software (Applied Biosystems) for analysis.

Sample Preparation, Amplification and Analysis Using the Promega PowerPlex® 16 Kit

For amplification with PowerPlex® 16, DNA was extracted from the two Bedouin reference samples using a Chelex extraction (8). The extracted DNA was quantified using the Quantifiler™ Human

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DNA Quantification Kit (Applied Biosystems) according to the manufacturer's instructions and then amplified using the PowerPlex® 16 kit, in accordance with the manufacturer's recommendations. The amplified products were separated and detected by capillary electrophoresis as described above using the manufacturer's recommended run conditions. GeneScan® and the PowerTyper16 MacroV2® were used to collect and size the PCR products generated using the PowerPlex® 16 kit.

PCR Amplification and Analysis Using Applied Biosystems AmpFlSTR® Yfiler™ Kit

As described above, 1 ng of quantitated, Chelex-extracted DNA from each of the two Bedouin reference samples was amplified using AmpFlSTR® Yfiler™ Kit according to the manufacturer's instructions in a final volume of 25 μL. The amplified products were separated and detected by capillary electrophoresis on the ABI Prism 3100 Genetic Analyzer using GeneScan® and GeneMapper® ID software for analysis.

Results and Discussion

During the course of routine DNA database profiling using commercial STR kits, our database laboratory has come across, to date, (at time of article preparation approximately 10,000 samples typed), three separate instances of amplification failure at the amelogenin gene using commercial STR multiplex kits. In the first instance, two samples (M648, M650), received from phenotypically normal males of Bedouin origin, provided AMEL Y-deficient SGM Plus™ profiles (Fig. 1). In another instance, a male suspect (DB325) provided a profile where the X homolog of the XY pair failed to amplify (Fig. 2).

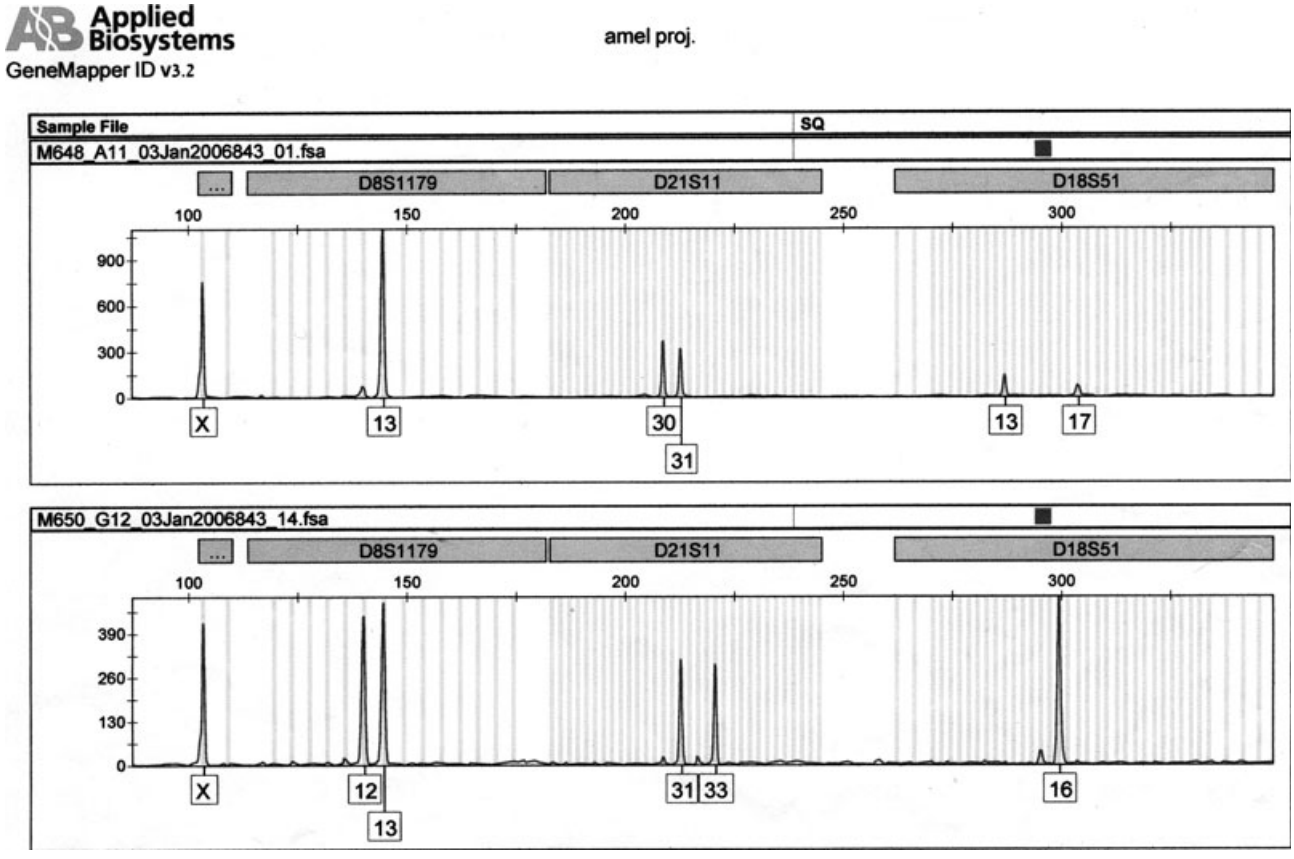

FIG. 1—*SGM Plus™ amelogenin Y-deficient results from two database samples received from two male suspects (M648, M650).*

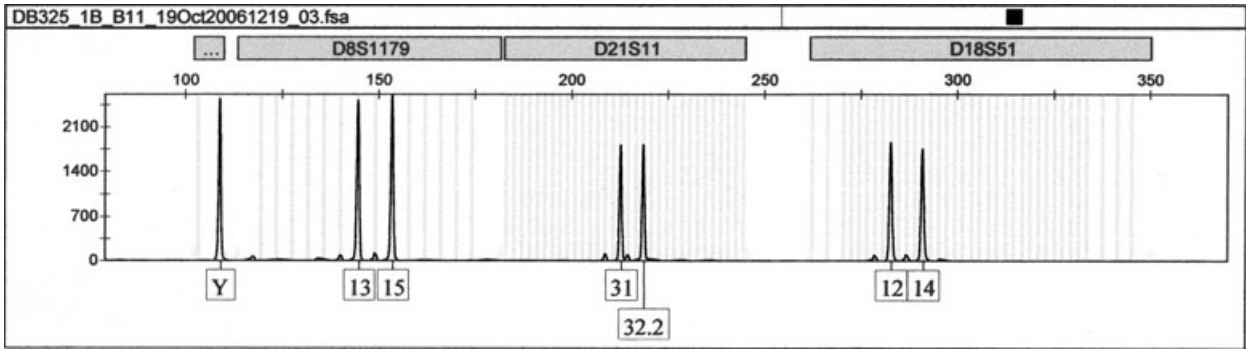

FIG. 2—*SGM Plus™ amelogenin X-deficient results from a database sample (DB 325) received from a male suspect.*

TABLE 1—*Y haplotypes obtained from two samples (M648, M650) in case 1 using the Yfiler™ Kit (17 Y-STR markers).*

	DYS456	DYS389 I	DYS390	DYS389 II	DYS458	DYS19	DYS385 a	DYS385 b	DYS393	DYS391	DYS439	DYS635	DYS392	Y GATA H4	DYS437	DYS438	DYS448
M648	13	13	22	29	21.2	14	13	17	12	11	11	22	11	11	14	10	20
M650	13	13	22	29	21.2	14	13	17	12	11	11	22	11	11	14	10	20

If a nucleotide change is present in a particular primer-binding site, an allele may fail to amplify when using a particular multiplex kit. Sequence variations in primer pairs originating from different commercial STR kits occasionally can be the source of "null" alleles. The Promega PowerPlex® 16 AMEL-F primer sequence differs from its Applied Biosystems counterparts (9,10).

In the first case cited, after obtaining AMEL Y-deficient results using SGM Plus™, the two Bedouin samples were additionally amplified using the PowerPlex® 16 STR kit, which also failed to provide a normal XY genotype at the amelogenin site (results not shown). To verify the existence of the Y chromosome in these samples, Y-STR typing was also carried out using the AmpFlSTR® Yfiler™ Kit. In both samples, identical Y haplotypes were obtained, demonstrating the presence of the Y chromosome (Table 1). When this haplotype was searched in the Applied Biosystems Yfiler Haplotype Database (which numbered at the time of the search 3561 haplotypes) no such haplotype was observed (11). In addition, this haplotype has not been seen within our laboratory's database of more than 250 male samples. Without complete demographic information regarding these two suspect samples, we were unable to determine their familial relationship. From our results, and from the fact that they share a common surname, we hypothesize that they share the same paternal lineage.

The third example (a profile lacking the allele representing the X homolog from a male sample) would not have resulted in gender misidentification, but it suggests that the frequency of such occurrences may be greater than realized.

For forensic and database use, the necessity for a more reliable sex determination assay than that found in the present commercial autosomal STR multiplex kits has previously been addressed (3,12). In the case of a mass disaster or large-scale terrorist attack, where detached and isolated body parts need to be typed and identified, a failure to amplify at the amelogenin gene may lead to an erroneous sex determination of the victim. This possible error could be avoided if amelogenin results would not be included and the comparison of the profiles from victims and relatives would be carried out only according to the STR loci. This conservative approach may also be appropriate when uploading profiles onto an offender or crime scene database. Sex determination errors can also be avoided if Y-STR alleles are included in results. We suggest, though, that it would not be economically practical to automatically test all samples with multiple autosomal and Y-STR kits, on the chance that a primer site mutation may be present.

The majority of casework from crime scenes provides male profiles. Where a female profile is acquired and there is no suspect for comparison, a possible approach to verify this result could be to perform an additional Y-specific test, such as Y-STR typing or Y-chromosome quantification. Although options to resolve ambiguous results exist, in high throughput and casework laboratories, an arsenal of such validated systems may not be readily available.

We are aware that kit modifications are complex (13), but in light of the continuing emergence of additional reports of failures at the amelogenin locus, and the necessity for timely and reliable typing results, we feel that the introduction of an additional Y-chromosome marker in commercial autosomal STR multiplex kits should be considered.

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