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Analysis and Interpretation of Short Tandem Repeat Microvariants and Three-Banded Allele Patterns Using Multiple Allele Detection Systems

ABSTRACT: The Palm Beach County Sheriffs Office (PBSO) Crime Laboratory and the Alabama Department of Forensic Sciences (ADFS) have validated and implemented analysis of short tandem repeat (STR) sequences on casework using silver staining kit and SYBR® Green I detection systems and are presently validating fluorescently tagged STR alleles using the Hitachi FMBIO 100 instrument. Concurrently, the Broward County Sheriff's Office (BSO) Crime Laboratory is validating the ABI Prism310 Genetic Analyzer capillary electrophoresis STR detection system (ABI CE310) from Perkin Elmer Applied BioSystems. During the course of analyzing over 10,000 individuals for the STR loci CSF1PO, TPOX and THO1(CTT) using silver staining for allele detection, 42 samples demonstrated alleles that were ''off ladder,'' contained three-banded patterns at a single locus, or exhibited an apparent THO1 ''9.3,10'' allele pattern. PBSO, ADFS and BSO Crime Laboratories have collaborated on the verification of the allele patterns observed in these 42 samples using the following allele detection systems: (1) manual silver staining, (2) SYBR® Green I staining, and/or (3) fluorescently tagged amplified products separated by polyacrylamide gel electrophoresis or capillary electrophoresis followed by laser detection. Regardless of the CTT allele detection system utilized, concordant results were obtained for 41 of the 42 samples. The only exception was a sample in which a wide band within the THO1 locus was identified as a THO1 ''9.3, 10'' genotype by silver staining kit and SYBR® Green I staining but was verified to be a THO1 ''9.3'' homozygote by all other allele detection systems. Manual allele detection could readily identify microvariants, as a visual assessment of stained gels clearly shows that alleles do not migrate coincident with well-characterized allele size standards. As would be predicted, however, the manual detection systems did not provide adequate resolution to approximate the basepair size for off-ladder variants. All fluorescent software program systems were consistent in designating alleles ''not in range'' or ''off ladder,'' thereby indicating true microvariants. All single-locus three-banded patterns were detected using all of the STR multiplex systems. In addition, individual locus-specific primers verified multiplexed amplified products were specific for the locus in question.

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Guidelines for the initiation of DNA protocols for forensic human identification include the validation of each polymorphic locus for relevant regional populations. Once validation protocols have been completed, DNA profiling may be conducted on convicted-offender databases and casework evidence. Over the past decade, generation of data regarding the feasibility of utilizing short tandem repeat (STR) sequences for forensic casework has been well documented with both the number of potential STR candidates and the types of STR allele detection systems under intense investigation (1–23). STR allele detection systems include manual detection of amplified products using silver staining kit, staining with fluorescent dyes such as SYBR® Green I or ethidium bromide, or detection of fluorescently tagged STR primers using laser technology. It is important that, regardless of the system utilized, the STR allele profile obtained for a sample be identical with regard to nominal allele calls. There have been well-documented reports in the literature identifying microvariants for specific STR loci with the most common being the THO1 9.3 allele (17) and the F13A 3.2 allele (23). In general, an amplified STR microvariant consists of an allele in which the repeat sequence deviates from the predicted canonical size.

The presence of a STR microvariant in a DNA sample becomes evident post-electrophoresis of the amplified STR product. Manual visual comparison of the microvariant allele adjacent to the allelic ladder shows an obvious migration of the allele above or below the allelic size standards. When using fluorescently-tagged primers detected by lasers, sophisticated STR allele analysis software evaluates the migration of an internal lane standard composed of well characterized DNA fragments. This analysis provides a natural logarithmic scale to extrapolate allele sizes (19,20). As a result, the software will assign a basepair size and nominal allele designation to the STR amplified products. The software will record a basepair size but will not designate a repeat size to an allele that migrates outside the default allele size ranges. It is possible to extrapolate the allele designation for a microvariant by simply determining the basepair difference between the well characterized canonical repeat size and the microvariant.

Although manual STR detection systems allow for visual detection of microvariant alleles, the resolving power of the staining techniques with regard to identification of the base pair size is difficult at best. Regardless of the STR allele detection system

used, single basepair STR differences such as a THO1 ''9.3,10'' genotype should be easily identified.

The purpose of the study presented herein was to determine if manual and automated STR allele detection systems employed by different laboratories could characterize apparent CTT microvariants, single-locus three-banded patterns and one basepair genotype differences from single-source DNA samples. Specifically, using the silver staining kit and SYBR® Green I staining allele detection systems, 42 samples were selected from the CTT screening of over 10 000 individuals in which allele designations required verification. The PBSO, ADFS and BSO laboratories utilized manual staining techniques and allele detection laser technology with FMBIO 100 and ABI CE310 instruments to verify the genotypes of these 42 samples. In addition, individual fluorescently tagged locus specific primer sets were used to verify the allele in question for each of the 42 microvariants. In summary, for each sample in this study, several different primer set sequences from several different manufacturers, both as multiplexed primers and individual primer sets, provided concordant allele profile results.

Materials and Methods

Sample Source, DNA Extraction and Quantitation

Blood samples were obtained from individuals in the Alabama CODIS database as per standard operating procedures. PBSO population database blood or buccal swab samples were collected from area hospitals and Sheriff's Office personnel. PBSO DNA extraction was done using Chelex or the one-step organic extraction method as previously described (24,25). The ADFS extraction protocol utilized the organic extraction (25). All DNA samples were quantitated using the QuantiBlot system as per manufacturer with the exception that in addition to the recommended DNA template concentrations, an additional standard at 0.075 ng/μL was blotted on the nylon filter (Perkin Elmer, Foster City, CA).

Amplification and Allele Detection Systems

Approximately 2 ng of template DNA was amplified for each sample using the GeneAmp®PCR System 9600 (Perkin Elmer Applied BioSystems, Foster City, CA). All amplification reactions were as per manufacturer (Promega, Madison, WI and Perkin Elmer, Foster City, CA). Promega STR multiplex kits used for these studies included the triplex CSF1PO/TPOX/THO1 (CTT), quadraplex CSF1PO/TPOX/THO1/vWA (CTTv), and the megaplex CSF1PO/TPOX/THO1/vWA/D16S539/D7S820/D13S317/D5S818 (PowerPlex). The Perkin Elmer Applied BioSystems' AmpFlSTR Green I kit includes the loci CSF1PO/TPOX/THO1 and amelogenin.

Prior to polyacrylamide gel or capillary electrophoresis, all amplified products were visualized on a horizontal 3% agarose gel (Life Technologies Incorporated, Gaithersburg, MD). FMBIO 100 and Perkin Elmer Applied Biosystems ABI CE310 allele detection software was utilized to identify the presence of microvariants, three-banded patterns and single base pair differences in single source THO1 ''9.3,10'' genotypes.

The following protocols were used for the STR amplified products that were obtained from Promega multiplex systems: (1) CTT products were electrophoresed on 0.4 mm 4% polyacrylamide gels on a SA32 apparatus (Life Technologies Incorporated, Gaithersburg, MD) for 60 min and 40 W. The gels were stained with either silver staining kit (Promega, Madison WI) then developed using Kodak EDF film (VWR, Sawanee, GA) or the gels were stained with a 1 : 10,000 dilution of SYBR® Green I (Molecular Probes, Eugene, OR) and then scanned using the Hitachi FMBIO 100. Fluorescently tagged CTTv and PowerPlex systems were electrophoresed on 4.5% polyacrylamide gels using Hitachi R³ disposable gels (Hitachi, San Francisco, CA) for 90 min at a constant 30 W. Fluorescent allele detection for the CTTv and PowerPlex systems was done on the Hitachi FMBIO 100 instrument. The Promega Fluorescent Ladder, a CXR-labeled (carboxy-X-tetramethylrhodamine) internal lane standard, was used with each sample as well as allelic ladders.

Samples were also amplified using the Perkin Elmer Applied BioSystems AmpFlSTR Green I multiplex primer system. AmpFlSTR Green I amplified products were detected using the ABI CE310 instrument. Data were evaluated with the GenoTyper 2.0 software that assigned appropriate nominal alleles and base pair sizes.

FMBIO 100 and ABI CE310 allele detection software was utilized to visually identify the presence of microvariants, three-banded patterns and single basepair differences in single source THO1 ''9.3,10'' genotypes. Additionally, individual fluorescently tagged primer sets for CSF1PO, TPOX and THO1 were used as per manufacturer's protocol (graciously provided by Promega Corporation) in order to verify that three-banded STR allele patterns were amplified from specific locus sequences.

Results

CODIS databasing and population frequency studies for the CSF1PO/TPOX/THO1 loci revealed the presence of alleles that were determined to be atypical, that is, more than two bands at a single locus or alleles that migrated outside of well characterized allele default windows. Table 1 summarizes the categories of alleles analyzed for this study, the number of occurrences, and the allele detection systems used to verify original allele designations. This table indicates the total number of samples analyzed by each of the allele detection systems. There was not always enough DNA to test all samples using all of the instruments. However, all samples were tested by at least three allele detection systems.

Three-banded Patterns

Three-banded patterns were observed for 19 samples including 18 at the TPOX locus and one at the CSF1PO. The 18 samples exhibiting the three-banded pattern at the TPOX locus were confirmed using silver staining kit and SYBR® Green I staining, and the CTTv and PowerPlex multiplex systems. ABI CE310 analysis of the AmpFlSTR Green STR CTT multiplex verified 15 of the 18 samples. There was not enough DNA in three of the samples to conduct further analysis. Figure 1 shows the results of amplifying 18 DNA extracts using fluorescently-tagged TPOX-specific primers followed by FMBIO 100 analysis. All 18 samples exhibited the same three-banded patterns with each allele exhibiting approximately the same signal intensity regardless of the TPOX primer source, i.e., using primer sequences from different multiplexed systems or individual primer sets. Figure 2 shows the results of six of the three-banded TPOX genotype samples (compare to Fig. 1, samples 7, 16, 18, 12, 8 and 5, respectively) using the ABI CE310 STR allele detection system. The peak heights for each of the three alleles in each sample are similar, thereby supporting FMBIO 100 analysis that they are most likely present in equal copy number. This observation is in contrast to what others have reported (personal communication, Cydne Holt, Perkin-Elmer).

In addition to the TPOX three-banded patterns, a three-banded pattern was also observed at the CSF1PO locus in one of the sam-

TABLE 1—*Collaborative study summary.*

Observation	Locus	No. Observed Silver and SYBR Green	FMBIO CTTv†	FMBIO Ind. Primers†	FMBIO Power Plex†	PE CE310†
Three-banded patterns	CSF1PO	1	1	1	N/D§	N/D
	TPOX	18	18	18	18	15
	THO1	1	1	1	1	1
THO1 ''9.3,10'' genotype	THO1	11‡	9	10	10	9
THO1 ''10,10'' genotype	THO1	2	2	2	2	2
Alleles outside bounds of allelic ladder	CSF1PO	2	2	2	1	1
	TPOX	1	1	1	1	1
	THO1	4	4	4	4	2
Alleles off-ladder but within allelic	CSF1PO	1	1	1	1	N/D
ladder standards	THO1	1	1	1	1	1

* = Numbers reflect total number of samples analyzed and confirmed for each detection method.
† = Fluorescently-tagged primers.
‡ = One of these ''9.3,10'' genotypes was confirmed to be a ''9.3'' homozygote by all fluorescent systems.
§ N/D = Not done due to lack of DNA sample.

FIG. 1—*Polyacrylamide gel electrophoresis of 18 samples (A1–A18) amplified with Promega's fluorescently tagged TPOX specific primers. Amplified products were detected using the Hitachi FMBIO 100 instrument. Unlabeled lanes contain the TPOX allelic ladder.*

ples that was confirmed using the CTTv multiplex system and the CSF1PO individual primer set (data not shown).

Single-Basepair Differences Between Alleles

Initial silver staining and SYBR® Green I staining of the database and population samples detected 11 samples which may contain a THO1 ''9.3,10'' genotype and two samples which may have a THO1 ''10,10'' genotype. The fluorescently tagged systems readily detected the one basepair difference confirming the presence of a THO1 ''9.3,10'' genotype in 10 of the 11 samples. One of the DNA extracts appeared to contain a THO1 ''9.3,10'' genotype by silver staining and SYBR® Green I staining but using fluorescently-tagged systems demonstrated a discrete ''9.3'' allele homozygote (data not shown). The initial genotype call of a THO1 ''9.3,10'' heterozygote could be the result of the staining process whereby amplified products may produce a wider band pattern due to the staining of both strands of the DNA fragment. The advantage of fluorescently-tagged PCR primers is that only one amplified strand is detected, thus providing more discrete bands. Two samples amplified at the CTT loci were subsequently analyzed by gel electrophoresis and visualized with SYBR® Green I and the silver staining kit were interpreted as a ''10'' homozygote, both of which were verified using all fluorescent allele detection systems (data not shown).

Examples of THO1 ''9.3,10'' genotypes are shown in Figs. 3a and 3b. The ABI CE310 GenoTyper2.0 software (Fig. 3a, panels a through e) provide discrete peaks representing the one basepair allele difference in these samples at the THO1 locus. An example of a ''9.3,10'' THO1 genotype amplified with PowerPlex THO1 primers is shown in Fig. 3b. The gel file from the FMBIO 100

analysis (Fig. 3b—top, lane 3) clearly shows migration of a THO1 double band in the 9.3 and 10 allele position. The ''9.3'' standard from the Promega CTT kit was electrophoresed alone in lane 4 in order to aid in the visualization of the ''9.3'' migration pattern. The doublet is also evident as two peaks representing the ''9.3,10'' genotype in the FMBIO 100 electropherogram (Fig. 3b—bottom). The FMBIO 100 STaRCALL2.0 and ABI CE310 GenoTyper2.0 software basepair designations for the ''9.3,10'' genotypes indicated there was approximately a one basepair difference between the two alleles.

''Off-Ladder/Not in Range'' Alleles

Interpretation of the laser-scanned amplified products by instrument software is straightforward unless a microvariant is detected. In these cases, the microvariant is not assigned a nominal allele designation. In this study, amplified STR products that migrated between, above, or below the allelic ladder standards were detected in ten samples. In order to discern the difference between an allele that migrates between the default alleles versus an allele that migrates outside the allelic ladder default range, PBSO and ADFS protocols have assigned the following nomenclature for such microvariants. A rare microvariant migrating between two alleles within an allelic ladder is recorded as the lower molecular weight allele designation followed by a ''.x''. An example would be if an allele migrated between the 10 and 11 allele of the CSF1PO allelic ladder, the allele would be designated a ''10.x''. Further, if an allele migrates above or below the defined allelic ladder, the allele is described as ''>'' or ''<'' than the nearest allele. An example of this would be an allele which migrates above the upper
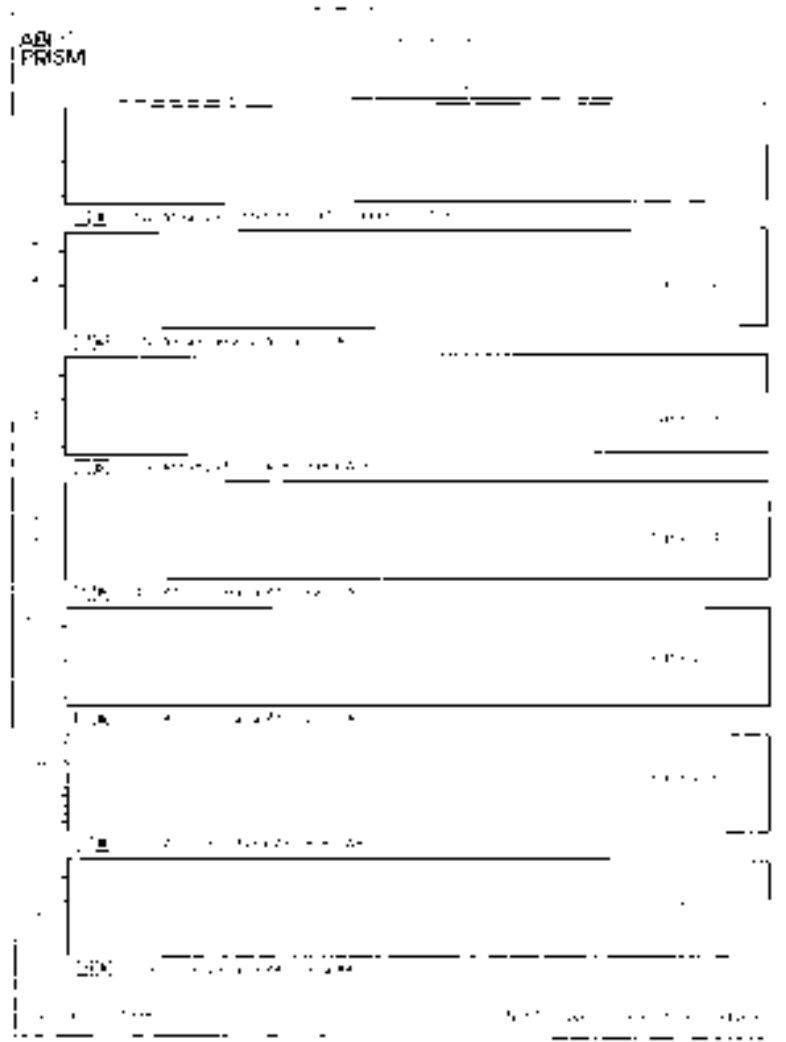
FIG. 2—*Capillary electrophoresis of six fluorescently-tagged TPOX three-banded patterns (compare Fig. 1 lanes 7, 16, 18, 12, 8 and 5 with panels 2 through 7, respectively). AmpFlSTR Green I multiplex was used for amplification and the ABI CE310 for allele detection. The TPOX allelic ladder is shown in panel 1.*

bounds of the CSF1PO allelic ladder. In this case, the allele would be assigned as a ''>15'' allele.

Seven DNA samples exhibited amplified alleles which migrated above (>) or below (<) the designated allelic ladder size range (Table 1). Two samples were observed for the CSF1PO locus, one at the TPOX locus and four at the THO1 locus. Typical examples of alleles migrating outside an allelic ladder range are shown in Fig. 4. Using the ABI CE310 GenoTyper2.0 software, Fig. 4, panel 2 shows the results from a sample with a THO1 allele migrating below the THO1 allelic ladder (arrow) indicating an ''off-ladder'' allele. As per PBSO and ADFS nomenclature, this THO1 band was designated as a ''<5'' allele. This ''<5'' THO1 allele was found to have a basepair size of 162.8 that would indicate a fragment one repeat size smaller than the THO1 ''5'' allele. A TPOX amplified allele migrating off-ladder is shown in Fig. 4, panel 4 (arrow). The genotype of this sample was recorded as ''11,>13''. The higher molecular weight allele had a basepair size of 234.65 (>13) and the ''11'' allele 304.51 bp. The difference is 16 bp or 4 repeat arrays making the ''>13'' allele most likely a ''15'' repeat allele based on current nomenclature. FMBIO 100 STaRCALL2.0 analysis of all the off-ladder variants were in concordance with

ABI CE310 allele calls, that is, basepair sizing verified the number of repeat differences between the microvariant and the common alleles identified in standard allelic ladders.

An ABI CE310 electropherogram shows a sample (Fig. 4, panel 6, arrow) that contains a CSF1PO allele ultimately designated as a ''10.x'' as it has migrated between the 10 and 11 nominal alleles. The predicted size of this fragment was 298.1 bp which is two basepairs greater than the 10 allele, thus indicating this fragment is most likely a ''10.2'' allele. Figure 5A1 (top panels) shows this same sample amplified using primers from the CTT multiplex followed by SYBR® Green I staining (lane 2). The CSF1PO 12 allele clearly lines up with the 12 allele in the ladder whereas the faster migrating fragment does not align with any band in the CSF1PO allelic ladder agreeing with the films from silver staining and ABI CE310 ''10.x'' allele designation. This sample was also amplified using the fluorescently-tagged CTTv multiplex (Fig. 5A2, bottom panels). The amplified CSF1PO fragments from this sample were electrophoresed both diluted (lane 2) and neat (lane 3) to verify the results obtained from other allele detection systems. When the electropherogram from this sample is overlaid with the allelic ladder, the ''10.x'' allele (arrow) clearly has migrated
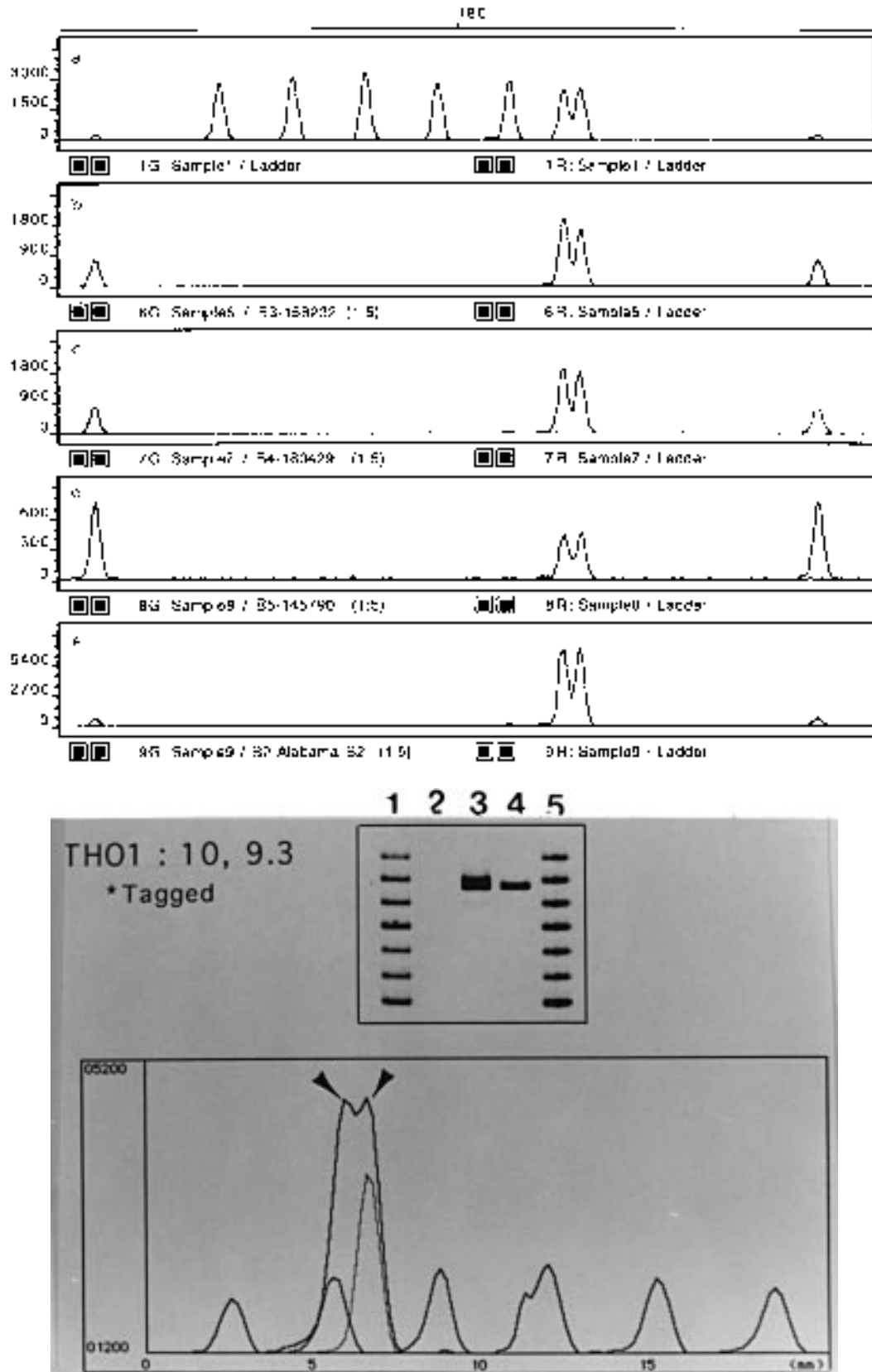
FIG. 3—Detection of THO1 ''9.3,10'' genotypes: Fig. 3A, panel ''a'' shows the electropherogram of the THO1 allelic ladder with the 9.3 and 10 allele standards using the ABI CE310 instrument. Panels b through e show the results of four THO1 ''9.3,10'' heterozygotes. Using PowerPlex primer sets and FMBIO 100 allele detection, Fig. 3B, top panel shows the resultant gel file of the THO1 allelic ladder (lanes 1 and 5), negative control (lane 2) an example of a THO1 ''9.3,10'' pattern (lane 3), and the THO1 ''9.3'' standard allele (lane 4). The resultant electropherogram is shown in the bottom panel. The arrows show the double peak indicating a heterozygote overlaid with the THO1 allelic ladder. The peak within the sample is the 9.3 standard allele.
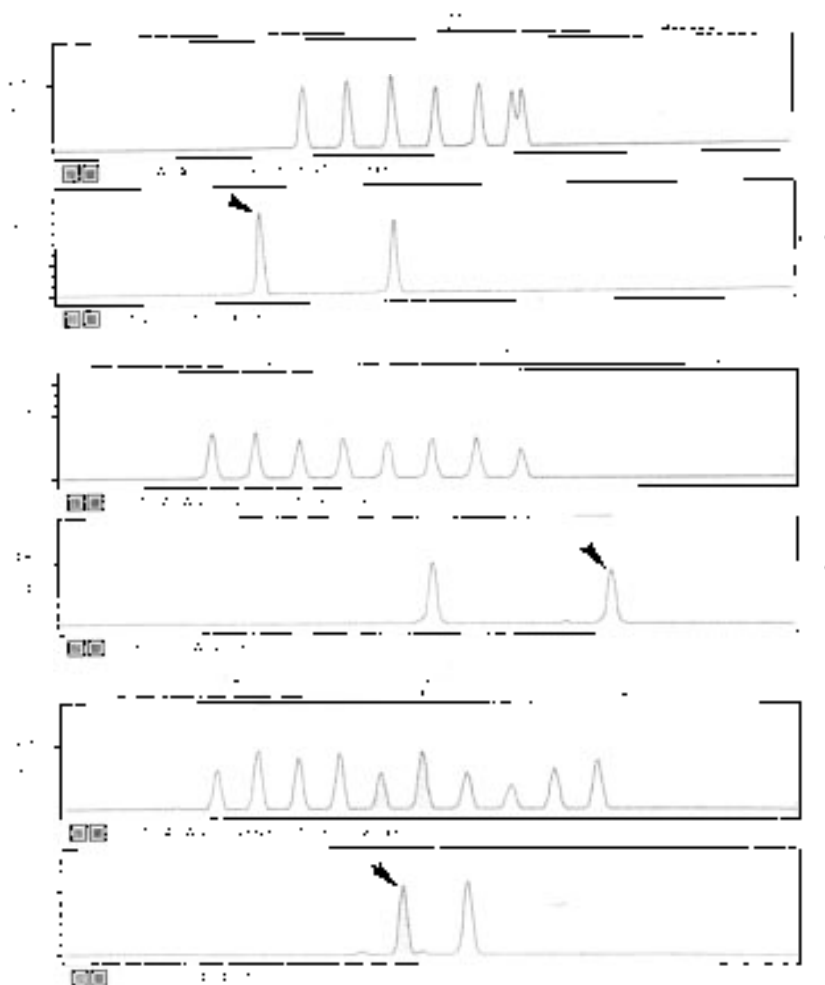
FIG. 4—*Capillary electrophoresis of samples exhibiting ''off ladder'' alleles: THO1 allelic ladder (panel 1), THO1 off ladder allele (panel 2, open arrow ''<5''); TPOX allelic ladder (panel 3), TPOX ''off ladder'' allele (panel 4, arrow ''>13''); CSF1PO allelic ladder (panel 5), CSF1PO off ladder allele (panel 6, arrow ''10.x'').*

between the canonical 10 and 11 alleles. The ''10.x'' allele was designated as a 309.4 basepair fragment using FMBIO 100 STaR-CALL2.0 software that is two basepairs greater than the allelic ladder 10 allele.

There were also two THO1 microvariants detected during the course of this study (data not shown). One of the samples contained a THO1 ''8.x'' allele that migrated between the 8 and 9 nominal alleles when analyzed with all available detection systems. Analysis of this allele using GenoTyper2.0 and STaRCALL2.0 indicated the allele was a single basepair less than the 9 allele, that is, an ''8.3'' allele (data not shown). The other THO1 microvariant was determined to be a ''10.x''.

Amplification of all ''off-ladder'' microvariants with locus-specific primers confirmed not only that these alleles migrated outside the default allele parameters, but also that the alleles were specific for the locus to which their genotypes were assigned. As expected, all DNA samples in this study were identified as off-ladder, three-banded and were true single basepair difference genotypes or true homozygotes by all allele detection systems.

Discussion

Microvariants at the CSF1PO, TPOX and THO1 loci are relatively rare in the Caucasian, Hispanic, and African-American populations. DNA extracts from over 10,000 individuals were analyzed for CTT profiles for routine population databasing using the silver staining kit and SYBR® Green I staining technique for allele detection. As a result, over 60,000 allele calls were recorded from which 42 samples (approximately 0.42%) were selected for further analysis.

The existence and importance of repetitive sequences in eukaryotic genomes has been known for decades (26–28). The extraordinarily long tandem arrays stretching for greater than 100 megabases, such as those found in satellite DNA sequences, are too cumbersome for clinical or forensic use. The minisatellite, or variable number tandem repeat (VNTR) sequences have found considerable use in many scientific communities, including forensics. In the past decade, microsatellite DNA, commonly referred to as short tandem repeat arrays or STRs, have been found to be of significant importance for human identification (29). STRs are highly variable in their repeat size length and are ubiquitous in the human genome. What has become evident from reports in the literature is that there may be mutations at some STR loci that will change the length of the tandem array by a single basepair or an entire repeat. The molecular mechanisms involved in the dynamics of the polymorphic nature of STRs are thought to predominantly be the result of replication slippage or defective DNA replication repair (30,31). This may explain, for example, that the CSF1PO ''10.2'' microvariant was originally an ''11'' repeat size in which
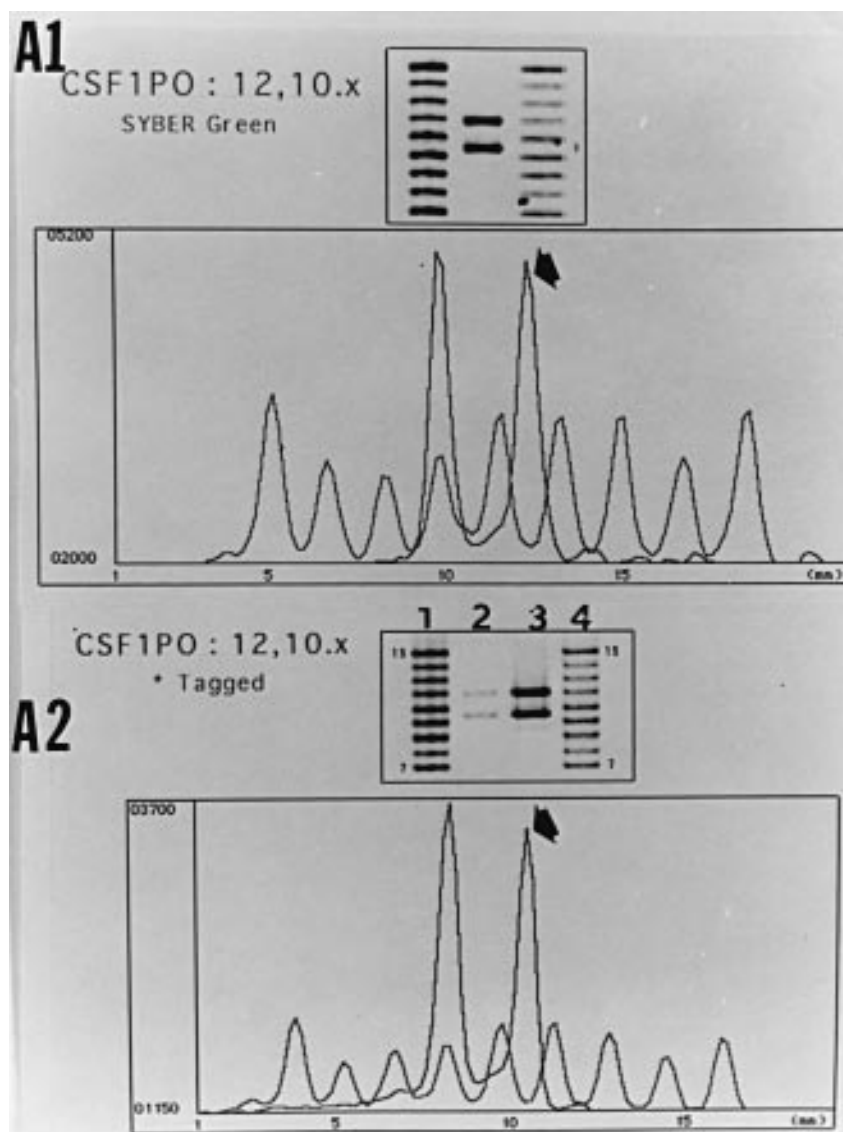
FIG. 5—*Hitachi FMBIO 100 analysis of a CSF1PO ''10.x'' allele: Amplification of a heterozygote ''12,10.x'' sample using CSF1PO specific primers followed by gel electrophoresis and SYBR® Green I staining. (A1 top panel, middle lane). Electropherogram of this sample is shown directly below the gel file overlaid with the CSF1PO allelic ladder. A2 shows this same sample amplified with the CTTv multiplex system. The amplified product was electrophoresed diluted 1:20 (lane 2) and neat (lane 3). The electropherogram of this sample is shown in the bottom panel of A2 overlaid with the CSF1PO ladder.*

two basepairs were deleted through replication slippage or faulty DNA repair. Although unequal DNA exchange, intrastrand exchange, rolling circle, or amplification of repeat sequences may also play a role in STR polymorphisms, these mechanisms would more likely explain mutations in larger repetitive sequence arrays such as VNTRs or satellite DNA.

The rate of replication slippage appears to be repeat sequence dependent. That is, a dinucleotide tandem array has a higher incidence of strand slippage than a trinucleotide array which in turn has a higher degree of slippage compared to tetranucleotide repeats and so on (30–32).

What is less clear is which genetic mechanism(s) is/are most likely responsible for the three-banded allele patterns observed at some loci for STRs. During the course of the present study this phenomenon was observed predominantly at the TPOX locus (18 times) and only one time at the CSF1PO locus. Individual TPOX or CSF1PO locus-specific primer sets confirmed the amplified bands

were specific for these loci. The fact that the amplified fragment signal intensities and fluorescent peak heights were uniform within a three-banded pattern strongly indicates these alleles occur in single copy number within the genome. An interesting question would be, ''Are these new mutations in the individuals or have these three-banded patterns been inherited with fidelity from generation to generation?'' Due to the confidentiality of the samples used in the study, this question cannot be answered.

The greater majority of forensic laboratories will most likely not have the facilities or funds to conduct DNA sequencing on microvariant alleles or to use a variety of allele detection systems to verify allele calls from evidentiary samples. It was because of this consideration that this paper addresses the feasibility of using locus-specific primers to verify the origin of the allele patterns. The locus-specific primers confirmed the allele designations for all samples analyzed in this study. When amplified products are recorded as ''off-ladder'' or ''not in range,'' amplification of the

DNA extract using locus-specific primers for allele source identification is a viable alternative for forensic laboratories.

In summary, when using STR detection methods which stain amplified alleles, such as silver staining kit or SYBR® Green I in which visual allele calls are necessary, samples should be run adjacent to allelic ladders. If fluorescently tagged primers are used to amplify STR loci followed by laser detection and allele calls are made using the appropriate software, "off-ladder" or "not in range" allele call results should be evaluated with caution. Allele patterns should be verified using locus-specific primers. Finally, regardless of the molecular mechanism responsible for the synthesis of a microvariant, what is important is that they do exist, albeit rarely, and all STR allele detection systems should readily identify these alleles in evidentiary samples. The present study clearly demonstrates this premise, as different CTT sequence primer sources and different allele detection systems provided concordant results for unusual CTT amplified band patterns.

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ERRATUM

Erratum/Correction of Crouse CA, Rogers S, Amiott E, Gibson S, Masibay A. Analysis and interpretation of short tandem repeat microvariants and three-banded allele patterns using multiple allele detection systems. J Forensic Sci 1999 Jan;44(1):87–94.

Figure 2 in the above-referenced paper did not reproduce properly. A correct Figure 2 is published below, along with the original caption. The Journal regrets this error.
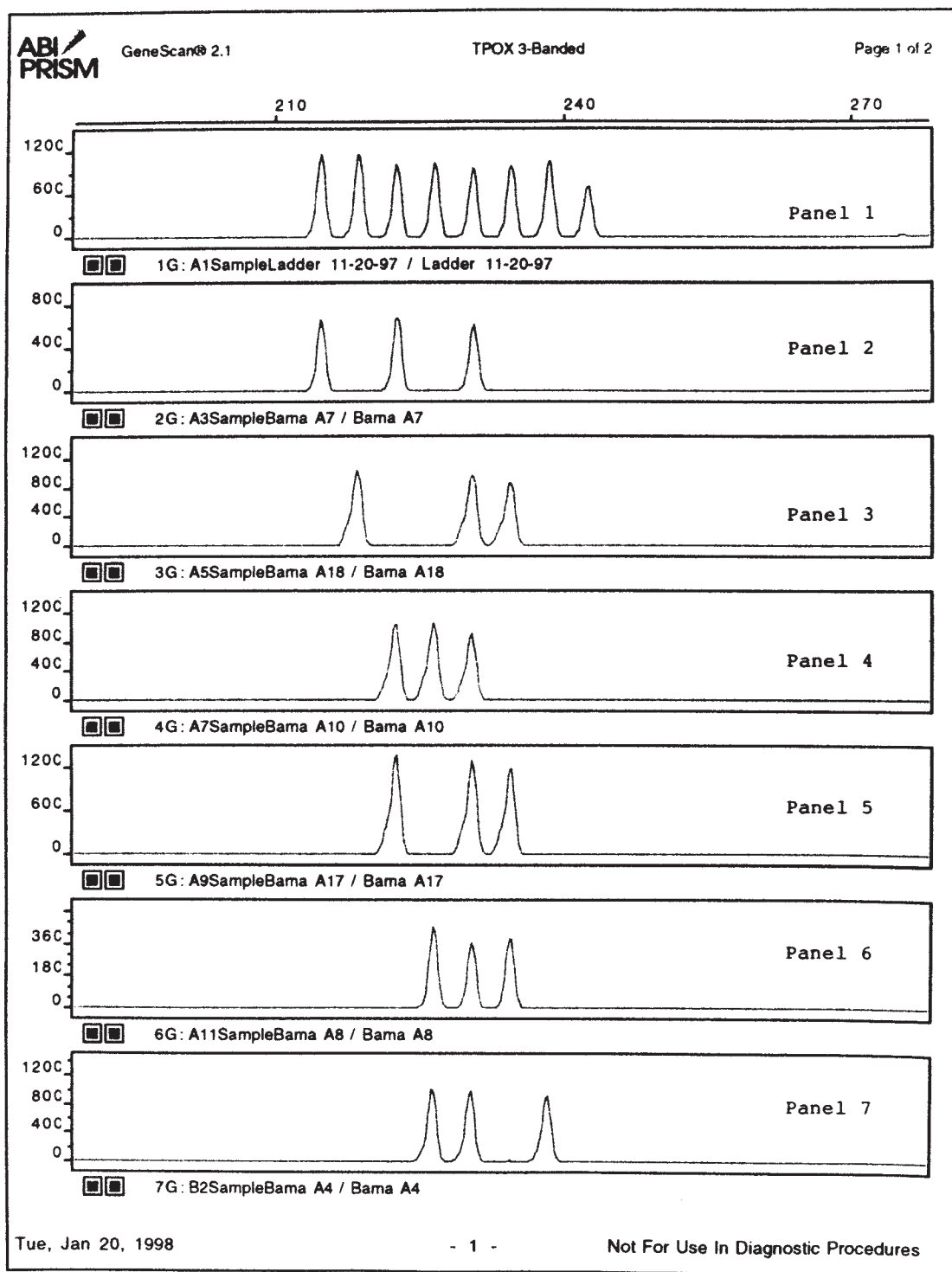

FIG. 2—*Capillary electrophoresis of six fluorescently-tagged TPOX three-banded patterns (Compare Fig. 1 lanes 7, 16, 18, 12, 8 and 5 with panels 2 through 7, respectively). AmpFISTR Green I multiplex was used for amplification and the ABI CE310 for allele detection. The TPOX allelic ladder is shown in Panel 1.*

Editor's Note: Any and all future citations of the above-referenced paper should read: Crouse CA, Rogers S, Amiott E, Gibson S, Masibay A. Analysis and interpretation of short tandem repeat microvariants and three-banded allele patterns using multiple allele detection systems. [published erratum appears in J Forensic Sci 1999 May;44(3)] Forensic Sci 1999;44(1, Jan):87–94.