J Forensic Sci, July 2005, Vol. 50, No. 4
Paper ID JFS2004481
Available online at: www.astm.org

TECHNICAL NOTE

John M. Butler,[1] *Ph.D.; Amy E. Decker,*[1] *B.S.; Margaret C. Kline,*[1] *M.S.; and Peter M. Vallone,*[1] *Ph.D.*

Chromosomal Duplications Along the Y-Chromosome and Their Potential Impact on Y-STR Interpretation*

ABSTRACT: Y-chromosome short tandem repeat (Y-STR) markers are being used as potential tools for distinguishing low levels of male DNA in the presence of excess female DNA as is present in many sexual assault samples. Usually single copy Y-STR loci produce a single amplicon in single source samples, and thus the observation of multiple peaks at such a locus could suggest to an analyst that a mixture of more than one male contributor is present in the tested sample. However, many regions of the Y-chromosome are duplicated or even triplicated in some individuals and this fact can thus complicate potential mixture interpretation. Reasons for the presence of duplications at multiple loci within a single sample are explored in the context of Y-STR marker location along the chromosome. True male-male mixtures commonly exhibit more than one locus-specific PCR product across multiple Y-STR loci that are not adjacent to one another on the Y-chromosome. In addition, duplicated loci typically possess alleles that differ by only a single repeat unit and possess similar peak heights.

KEYWORDS: forensic science, DNA typing, Y-chromosome, short tandem repeat, DNA mixtures, DNA interpretation

The Y-chromosome is specific to males and therefore useful in many sexual assault case situations where the perpetrator's DNA needs to be identified in the presence of the female victim's DNA (1–3). A number of short tandem repeat (STR) markers have been identified on the Y-chromosome and developed into multiplexed polymerase chain reaction (PCR) assays that can help differentiate between unrelated males (4,5).

One of the potential uses of Y-STR testing is to aid in determining the number of contributors to an evidentiary sample in gang rape situations (2). These situations often result in mixed DNA profiles from the multiple gang rape assailants that need to be interpreted by examining the number of peaks observed per locus and relative peak heights if more than one peak is present. Most of the commonly used Y-STR markers are single-copy loci, with the notable exception of the polymorphic locus DYS385 (4). Thus, due to the fact that the Y-chromosome is without a fully homologous chromosome typically only a single PCR product is observed when amplifying a Y-STR marker with a single PCR primer pair. However, in conducting a recent population study (6) we found that over 1% of the samples tested contained multiple alleles with loci that were previously considered to be "single-copy" markers. In addition, we observed simultaneous duplication in up to four different "single-copy" Y-STR loci in samples originating from a single individual. This brief report addresses the impact of duplicated (and even triplicated) regions of the Y-chromosome and how they might relate to assessment of whether or not a mixture of multiple male DNA is present in an evidentiary sample.

[1] Biotechnology Division, National Institute of Standards and Technology, Gaithersburg, MD 20899.

 Sources of Support: National Institute of Justice (NIJ) funded this work in part through an interagency agreement with the NIST Office of Law Enforcement Standards.

Received 13 Nov. 2004; and in revised form 1 Feb. 2005; accepted 5 Feb. 2005; published 25 May 2005.

Materials and Methods

DNA Samples

Anonymous liquid blood samples with self-identified ethnicities were purchased from Interstate Blood Bank (Memphis, TN) and Millennium Biotech, Inc. (Ft. Lauderdale, FL) in accordance with approval for use by the human subjects internal review board at the National Institute of Standards and Technology (NIST). These samples were previously extracted, quantified, and typed with 15 autosomal STRs using the AmpFlSTR® Identifiler™ kit (Applied Biosystems, Foster City, CA) to demonstrate that all samples are unique and single-source (7).

Y-STR Typing Reagents

The PowerPlex® Y kit from Promega Corporation (Madison, WI) was used to amplify the Y-STR loci DYS19, DYS385 a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, and DYS439. PCR amplification was performed according to the manufacturer instructions with 30 cycles and 1–2 ng of DNA template. Some testing was also performed with the NIST 20plex and 11plex assays as previously described (6).

Detection and Analysis of PCR Products

Separation and detection of Y-STR PCR products were accomplished with either the ABI Prism® 310 or the ABI Prism® 3100 Genetic Analyzer 16-capillary array system (Applied Biosystems) following manufacturer's protocols as previously described (6). Following data collection, samples were analyzed with Genescan® 3.7 (Applied Biosystems) and allele designations were made in Genotyper 3.7 (Applied Biosystems).

Results and Discussion

Duplications at Multiple Loci within the Same Sample

As part of a review of data collected from approximately 650 U.S. population samples (6), we observed several samples with duplications at multiple Y-STR loci. Some of these samples were subsequently retested with the PowerPlex Y kit to verify the presence of multiple alleles at the various Y-STR loci. Figure 1 shows the Genotyper results from one of these samples where multiple Y-STR alleles were observed at more than one locus. Note that two alleles are often observed with the DYS385 locus since two copies are commonly found on the Y-chromosome (4). Since the DYS389I, DYS439, DY389II, and DYS437 allele signals are practically equal within each locus, at first glance this observation might suggest the presence of two male components of similar concentrations. However, this particular sample, which was part of a previous study with autosomal STRs (7), when retested with 15 autosomal STR loci, was found to possess well balanced heterozygous alleles at 13 loci and was homozygous at only TPOX and D5S818 (data not shown). Due to the lack of more than two alleles at any one autosomal STR locus when this many polymorphic STRs were examined, it is extremely unlikely that a well-balanced mixture of two different males is present in this particular sample.

Since the loci DYS389I, DYS439, DYS389II, and DYS437 are typically considered single copy markers and therefore should only exhibit a single peak when amplified from a single male contributor, we decided to investigate this phenomenon of Y-STR marker duplication a little further and consider its potential impact on detection of male-male mixtures with Y-STR testing.

Literature Review of Reported Y-STR Duplications

In order to better understand the Y-STR loci previously observed to exhibit duplication, we reviewed almost 200 publications containing Y-STR haplotype population information. The papers examined are part of the comprehensive STR reference list found at http://www.cstl.nist.gov/biotech/strbase/str_ref.htm. Many of these population studies have been entered into the Y-STR Haplotype Reference Database (YHRD; http://www.yhrd.org; formerly http://www.ystr.org). Table 1 contains a summary of duplications or triplications reported in the literature organized by Y-STR locus. Literature references containing information that is now part of YHRD were not placed into Table 1 to avoid representing the same data twice. New information found as part of this study is also included in Table 1.

It is likely that the information in Table 1 is an underestimate of true occurrences for Y-STR duplications because a duplicated locus could have alleles of the same size and laboratories may not note that a particular allele is twice the height it would normally be as a "single copy" marker. Additionally, laboratories may not mention duplications or leave them out of data sets in population studies (8) possibly because they suspect "contamination" or do not want to analyze these variant alleles in the same context as regular samples (9).

Triplications

A sample containing a DYS19 tri-allelic pattern (6) due to triplication of the DYS19 locus was amplified with the PowerPlex Y kit (Figure 2). It is interesting to note that Santos et al. (10) observed this same DYS19 type although unfortunately they did not

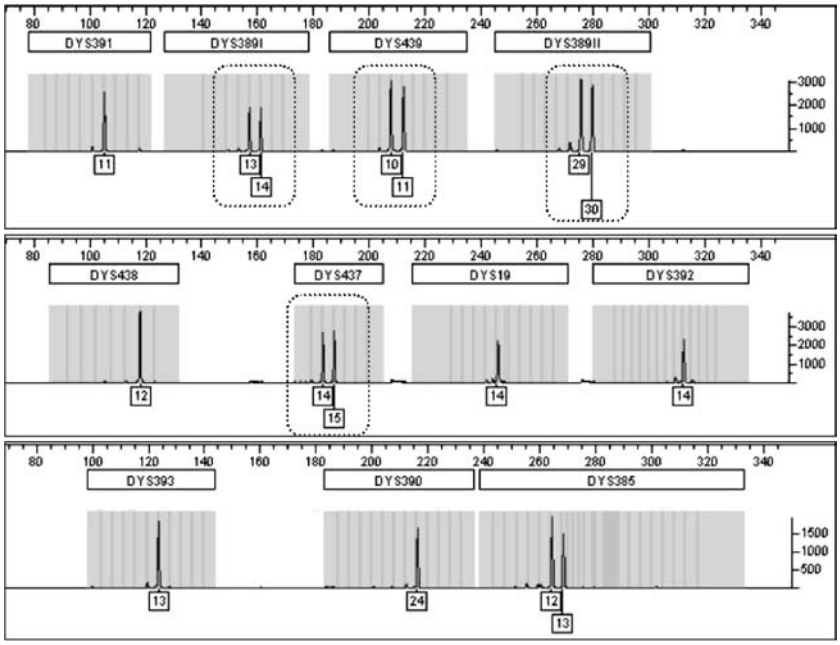

FIG. 1—*PowerPlex Y electropherogram for a single-source male DNA sample with multiple alleles at the "single copy" loci DYS389I, DYS389II, DYS439, and DYS437 (boxed). This sample was confirmed to be a single-source sample through testing with 15 autosomal STR loci using the Identifiler kit. Conditions as in the Materials and Methods section with 1 ng DNA and detection on the ABI 3100.*

TABLE 1—*Summary of duplications or triplications for common Y-STR loci. Information from the Y-STR Haplotype Reference Database (http://www.yhrd.org) is from "release 13" of July 1, 2004, which consists of 25,066 minimal haplotypes. Bolded values contain alleles separated by more than a single repeat unit. AMG represents a personal communication from Ann Marie Gross of the Minnesota Bureau of Criminal Apprehension Laboratory.* * *Listed DYS448 nomenclature follows Redd et al. (23).*

Locus	Alleles Observed	Reference
DYS19	13,14	http://www.yhrd.org (2 times); Kayser and Sajantila 2001 (11)
DYS19	**13,15**	http://www.yhrd.org (1 time)
DYS19	14,15	http://www.yhrd.org (1 time)
DYS19	15,16	Henke et al. 2001 (24); http://www.yhrd.org (11 times)
DYS19	**15,17**	http://www.yhrd.org (1 time)
DYS19	16,17	http://www.yhrd.org (3 times)
DYS19	14,15,17	Schoske et al. 2004 (6); Santos et al. 1996 (10)
DYS389I	12,13	http://www.yhrd.org (1 time)
DYS389I	13,14	This study (1 time); http://www.yhrd.org (2 times); AMG (1 time)
DYS389II	28,29	http://www.yhrd.org (1 time)
DYS389II	29,30	This study (2 times); http://www.yhrd.org (2 times)
DYS389II	**29,31**	AMG (1 time)
DYS389II	30,31	This study (1 time); http://www.yhrd.org (1 time)
DYS389II	**30,32**	http://www.yhrd.org (1 time)
DYS390	23,24	AMG (1 time)
DYS391	9,10	http://www.yhrd.org (1 time); Fernandes et al. 2001 (25)
DYS391	10,11	http://www.yhrd.org (1 time)
DYS393	12,13	http://www.yhrd.org (1 time)
DYS393	13,14	http://www.yhrd.org (1 time)
DYS393	**13,15**	http://www.yhrd.org (1 time)
DYS435	11,12	Johnson et al. 2003 (26) (1 time)
DYS437	14,15	This study (1 time)
DYS437	15,16	AMG (1 time)
DYS438	9,10	Pepinski et al. 2004 (27) (1 time)
DYS439	10,11	This study (1 time); AMG (1 time)
DYS439	11,12	This study (1 time)
DYS448*	19,20	Schoske et al. 2004 (6) (3 times)
DYS448*	**18,20**	Schoske et al. 2004 (6) (1 time)
DYS448*	20,21	Schoske et al. 2004 (6) (1 time)
DYS385a/b	9,13,14	Sahoo et al. 2003 (28) (1 time)
DYS385a/b	11,12,13	Kurihara et al. 2004 (13) (1 time)
DYS385a/b	11,12,14	http://www.yhrd.org (1 time)
DYS385a/b	11,14,15	http://www.yhrd.org (2 times)
DYS385a/b	12,13,18,19	http://www.yhrd.org (1 time)
DYS385a/b	12,13,19,20	http://www.yhrd.org (1 time)
DYS385a/b	12,16,17	Kayser and Sajantila 2001 (11) (1 time)
DYS385a/b	13,14,15	http://www.yhrd.org (3 times); AMG (1 time)
DYS385a/b	13,14,18	AMG (1 time)
DYS385a/b	13,18,19	http://www.yhrd.org (1 time)
DYS385a/b	13,19,20	http://www.yhrd.org (1 time)
DYS385a/b	14,16,17	http://www.yhrd.org (1 time)
DYS385a/b	15,16,17	Butler et al. 2002 (12) (1 time)

report any further loci that could have enabled a full haplotype comparison. Notice that the three alleles for DYS19 are all similar in peak height and that allele peak heights from other loci are all similar suggesting that each allele observed is present one time on the Y-chromosome.

Although triplications are more rare, they have been observed previously in DYS19 (10) and DYS385 (11,12; Table 1). Triplications at DYS385 can arise through a duplication of one of the already duplicated DYS385 alleles or a duplication of the entire Y-chromosome region containing both the "a" and "b" alleles. Often the sum of two alleles is equal to the other allele with DYS385 triplications (13).

A Model for Y-STR Locus Duplication and Divergence

An analysis of the finished sequence of the euchromatic region of the Y-chromosome noted that much of the Y-chromosome sequence occurs more than once and often in palindromic patterns (14,15). Since few genes seem to be present on the Y-chromosome, less constraint exists to maintain a particular sequence context and therefore insertion polymorphisms of large regions of the Y-chromosome are possible. With no recombination between other chromosomes, the Y-chromosome is likely to accumulate these insertion events leading to multiple copies and hence primer binding sites for loci that were originally single-copy markers. An example of this phenomenon was recently reported for the flanking regions of DYS19 (16). Upon PCR amplification, two copies of the locus would be amplified instead of one if two primer-binding sites exist along the Y-chromosome. Duplications can also complicate Y-chromosome single nucleotide polymorphism (Y-SNP) interpretations with loci such as P25 and 92R7 (17,18) making possible mixture interpretation with these loci that occur multiple times on the Y-chromosome more challenging.

Figure 3 illustrates how this Y-STR marker duplication might occur giving rise to first a duplicated region (a′) somewhere else on the Y-chromosome, which could then independently mutate over time at a rate of approximately 10^{-3} changes per generation in a single step fashion (19) to produce an allele "b" that would most likely be divergent from the original by a single repeat unit.

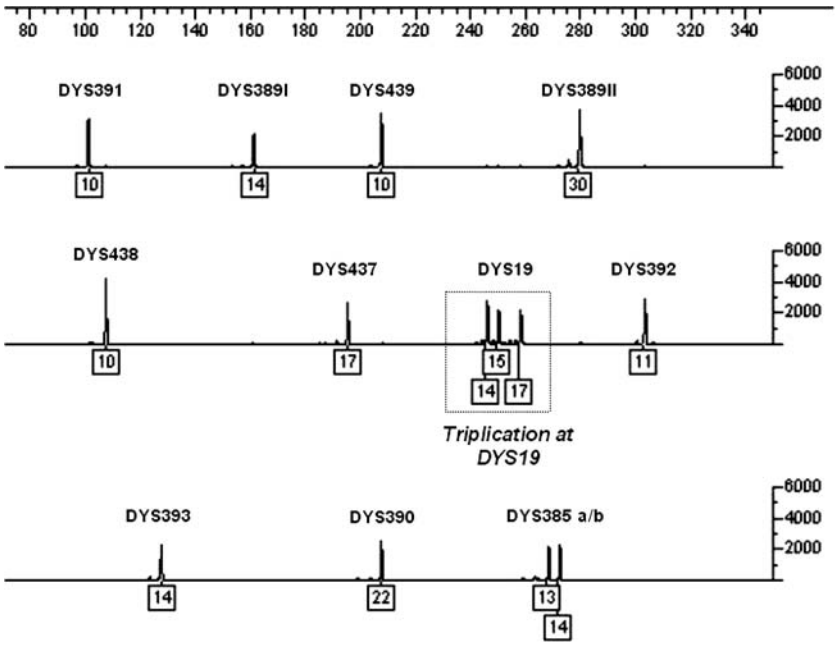

FIG. 2—*PowerPlex Y electrophoregram of a single-source male DNA sample possessing three alleles at DYS19. Conditions as in the Materials and Methods section with 1 ng DNA and detection on the ABI 3100.*

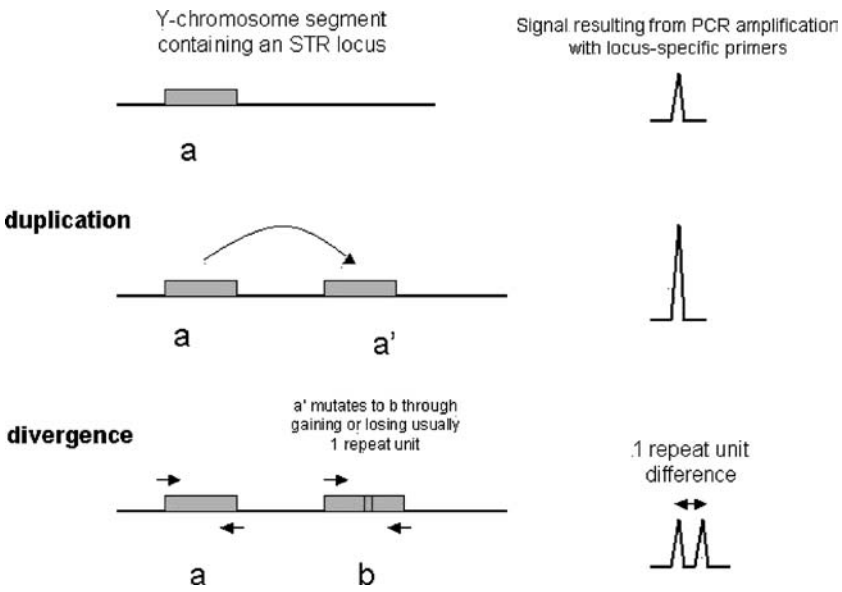

FIG. 3—*Schematic of duplication and divergence processes that can give rise to multiple alleles on the Y-chromosome when Y-STR locus-specific primers are used. Section (a) is first duplicated to form section (a′), which then may acquire a mutation at a specific Y-STR locus over time through the gain or loss of a repeat unit to form an allele that is now designated (b). Note that most observable multiple alleles should be a single repeat unit apart because single step mutations are most common for STR markers.*

Entire sections of the Y-chromosome spanning hundreds of kilobases (kb) could potentially be duplicated along the Y-chromosome (14,15). Thus, Y-STR markers that are close to one another physically along the Y-chromosome may be duplicated and exhibit divergent locus-specific alleles in the same DNA sample. Notably samples possessing specific duplications, such as DYS19 alleles 15 and 16, may all belong to the same haplogroup (8). Table 2 contains a summary of Y-STR marker positions for the most commonly used loci to-date. Note that DYS437, DYS439, and DYS389I/II, which are duplicated in the sample displayed in Figure 1, all occur within 150 kb of one another and therefore more likely to be duplicated together. This AZFa region of the Y-chromosome was also noted by Bosch and Jobling (29) to be duplicated in some individuals.

An Example Male-Male Mixture

In order to see if the Y-STR locus duplication observed with Fig. 1 could be differentiated from a true male-male mixture, a sample

TABLE 2—*Physical location of 50 Y-STR markers on the Y-chromosome based on a sequence search using the July 2003 Human Genome sequence assembly (http://genome.ucsc.edu). Megabase (Mb) positions are rounded to the nearest thousand base pair. The bolded loci are the ones examined in this study. The boxed region shows the close proximity of the duplicated loci observed in Fig. 1 (DYS437, DYS439, DYS389I, DYS389II) suggesting that this entire region has been duplicated followed by STR allele divergence over time.*

p-arm		q-arm	
Y STR Marker	Position (Mb)	Y STR Marker	Position (Mb)
DYS453	2.730	**DYS391**	13.413
DYS393	3.039	DYS635 (C4)	13.690
DYS446	3.039	DYS434	13.777
DXYS156Y	3.060	**DYS437**	13.778
DYS456	4.236	DYS435	13.807
DYS455	6.877	**DYS439**	13.826
DYS463	7.609	**DYS389 I/II**	13.923
DYS458	7.833	**DYS388**	14.057
DYS450	8.092	DYS442	14.071
DYS449	8.183	**DYS438**	14.248
DYS454	8.190	DYS441	14.292
DYS19	9.437	DYS436	14.514
Centromere		**DYS447**	14.589
		YCAIII a	15.409
		YCAIII b	15.477
		DYS390	16.521
		Y-GATA-A10	17.965
		Y-GATA-H4	17.990
		DYS426	18.381
		YCAII a	18.869
		YCAII b	19.754
		DYS385 a	19.998
		DYS385 b	20.039
		DYS461 (A7.2)	20.247
		DYS460 (A7.1)	20.247
		DYS462	20.514
		DYS452	20.817
		DYS392	21.780
		DYS448	23.511
		DYS464 a	24.388
		DYS464 b	24.619
		DYS459 a	25.225
		DYS425 a	25.326
		DYS464 c	26.021
		DYS464 d	26.245
		DYS425 b	26.940
		DYS459 b	27.041

containing two different males was tested with the PowerPlex Y kit. This particular mixture was well studied previously as "sample X" in NIST Mixed Stain Study #3 as it contains an excess of female DNA with two male DNA components (20). Individual components with autosomal STR allele calls can be challenging to decipher when DNA from more than one contributor is present in the sample (21). As noted by Prinz (2), determination of the number of semen contributors in a sexual assault is normally much simpler with Y-STRs because the female DNA component is not detected.

Figure 4 shows that in this particular 2-male mixture most of the single copy Y-STR loci have more than one peak and the highly polymorphic locus DYS385 contains four peaks rather than the typical two. In addition, the peak height/area for DYS392 is approximately twice that of the other Y-STR peaks in same dye color suggesting that two copies of allele 11 are present—one from each male contributor. Also notice that in this particular mixture the DYS439 locus possesses alleles 10 and 13, which are three repeats apart. Likewise DYS437 contains alleles 14 and 16, which are two repeat units apart.

In this example, where the apparent ratio of peak heights/areas between the contributors is close to 1:1, it is not possible to confidently assign alleles to the "major" and "minor" male contributors. (It is important to note that most mixtures encountered in casework will probably not be 1:1). Instead of assigning alleles to specific contributors to form Y-STR haplotypes that could be searched against a reference database, it is more likely that a suspect would be either included or excluded from matching the evidentiary mixture profile in a case such as illustrated in Fig. 4. An assignment of alleles to a particular contributor to form a composite Y-STR haplotype would most likely be easier with an imbalanced mixture (e.g., 3:1 instead of 1:1).

Deciphering between a Mixture or a Locus Duplication

To help in differentiating possible locus duplication from a potential mixture of two male contributors when performing a Y-STR test, a few ad hoc rules are presented here. First, note the number of loci possessing multiple alleles. The greater the number of loci having more than one allele, besides the DYS385a/b system, the more likely it is that a mixture of multiple males is present in the sample. Second, if more than one locus has multiple alleles, determine if these loci are located near one another on the Y-chromosome using relative Y-STR position information such as found in Table 2. If the putative duplicated loci are close to one another (e.g., <1 Mb), then the entire section of the Y-chromosome may have been duplicated at some time in the past and now possess divergent alleles. The further the putative duplicated loci are apart on the Y-chromosome (e.g., DYS19 and DYS438 which are on different arms of the Y-chromosome), the more likely a sample containing multiple alleles at multiple loci is a mixture. Third, examine the repeat unit spread in the multiple alleles amplified with locus-specific primers to see if any loci possess alleles with greater than a single repeat unit difference. Duplications will usually have a single repeat unit spread with alleles exhibiting similar PCR product yields (Fig. 1; e.g., DYS437 alleles 14 and 15) while mixtures will likely have one or more loci with a greater than one repeat unit spread (Fig. 4; e.g., DYS437 alleles 14 and 16). A review of the allele spreads for the duplicated loci observed in Table 1 finds that most of the alleles are separated by a single repeat unit. Finally, examine DYS385 and see if this highly polymorphic locus possesses more than two alleles. If it does and the other criteria match that of a mixture, then a mixture is more likely than a simple locus duplication.

Of course, if an evidentiary sample contains a single source sample that exhibits duplication, then the true perpetrator should also display this same duplication. Therefore, duplication events such as described in this report should not be cause for concern in forensic investigations when using Y-STR assays. The purpose of this report is to illustrate that multiple duplications are possible in the same single-source sample and thus analysts should not draw premature conclusions regarding the number of contributors when more than one equal intensity allele is observed at a "single copy" locus.

Locus duplication along the Y-chromosome is in many ways analogous to heteroplasmy in mitochondrial DNA (22), which depending on the circumstances can provide greater strength to a match between two samples. The phenomenon of locus duplication should not prevent the reliable interpretation of mixtures although it may complicate the explanation of results in court. The male-specific Y-chromosome is a useful tool to aid human identity applications even though it can be more complex than perhaps previously perceived.

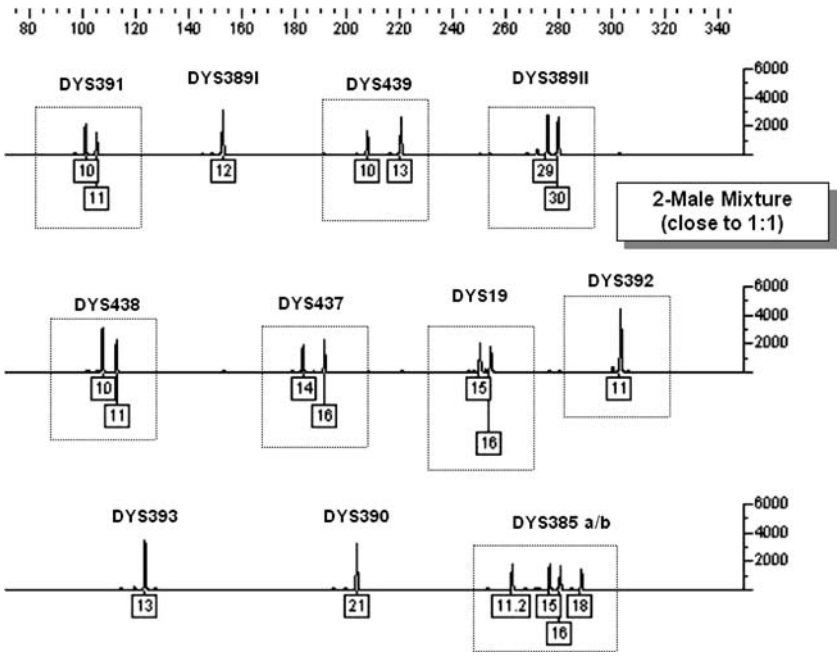

FIG. 4—*PowerPlex Y electropherogram for a 2-male mixture. The boxed loci exhibit multiple alleles many of which are separated by more than a single repeat unit. In addition, the highly polymorphic locus DYS385 has four alleles rather than its normal two. Conditions as in the Materials and Methods section with 1 ng DNA and detection on the ABI 3100.*

Acknowledgments

This study was supported by National Institute of Justice Grant Number 1999-IJ-R-A094, which is an interagency agreement between NIJ and the NIST Office of Law Enforcement Standards. Points of view in this document are those of the authors and do not necessarily represent the official position or policies of the US Department of Justice. Richard Schoske developed the NIST 20plex and 11plex assays as a graduate student in our laboratory and collected some preliminary data relative to this study.

References

1. Kayser M, Caglia A, Corach D, Fretwell N, Gehrig C, Graziosi G, et al. Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med [PubMed] 1997;110:125–33.
2. Prinz M. Advantages and disadvantages of Y-short tandem repeat testing in forensic casework. Forensic Sci Rev 2003;15:189–96.
3. Sinha SK, Budowle B, Chakraborty R, Paunovic A, DeVille Guidry R, Larsen C, et al. Utility of the Y-STR typing systems Y-PLEX™ 6 and Y-PLEX™ 5 in forensic casework and 11 Y-STR haplotype database for three major population groups in the United States. J Forensic Sci [PubMed] 2004;49(4):691–700.
4. Butler JM. Recent developments in Y-short tandem repeat and Y-single nucleotide polymorphism analysis. Forensic Sci Rev 2003;15:91–111.
5. Jobling MA, Tyler-Smith C. The human Y chromosome: an evolutionary marker comes of age. Nature Rev Genet 2003;4(8):598–612.
6. Schoske R, Vallone PM, Kline MC, Redman JW, Butler JM. High-throughput Y-STR typing of U.S. populations with 27 regions of the Y chromosome using two multiplex PCR assays. Forensic Sci Int [PubMed] 2004;139:107–21.
7. Butler JM, Schoske R, Vallone PM, Redman JW, Kline MC. Allele frequencies for 15 autosomal STR loci on U.S. Caucasian, African American, and Hispanic populations. J Forensic Sci 2003;48:908–11. [PubMed]
8. Zerjal T, Wells RS, Yuldasheva N, Ruzibakiev R, Tyler-Smith C. A genetic landscape reshaped by recent events: Y-chromosomal insights into central Asia. Am J Hum Genet 2002;71:466–82. [PubMed]
9. Coyle HM, Budowle B, Bourke MT, Carita E, Hintz JL, Ladd C, et al. Population data for seven Y-chromosome STR loci from three different [PubMed] population groups residing in Connecticut. J Forensic Sci 2003;48:435–7.
10. Santos FR, Gerelsaikhan T, Munkhtuja B, Oyunsuren T, Epplen JT, Pena SDJ. Geographic differences in the allele frequencies of the human Y-linked tetranucleotide polymorphism DYS19. Hum Genet 1996;97:309–13. [PubMed]
11. Kayser M, Sajantila A. Mutations at Y-STR loci: implications for paternity testing and forensic analysis. Forensic Sci Int 2001;118:116–21. [PubMed]
12. Butler JM, Schoske R, Vallone PM, Kline MC, Redd AJ, Hammer MF. A novel multiplex for simultaneous amplification of 20 Y chromosome STR markers. Forensic Sci Int 2002;129:10–24. [PubMed]
13. Kurihara R, Yamamoto T, Uchihi R, Li SL, Yoshimoto T, Ohtaki H, Kamiyama K, Katsumata Y. Mutations in 14 Y-STR loci among Japanese father-son haplotypes. Int J Legal Med 2004;118:125–31. [PubMed]
14. Skaletsky H, Kuroda-Kawaguchi T, Minx PJ, Cordum HS, Hillier L, Brown LG, et al. The male-specific region of the human Y chromosome is a mosaic of discrete sequence classes. Nature 2003;423:825–37. [PubMed]
15. Rozen S, Skaletsky H, Marszalek JD, Minx PJ, Cordum HS, Waterston RH, et al. Abundant gene conversion between arms of palindromes in human and ape Y chromosomes. Nature 2003;423:873–6. [PubMed]
16. Butler JM, Schoske R. Duplication of DYS19 flanking regions in other parts of the Y chromosome. Int J Legal Med 2004;118:178–83. [PubMed]
17. Sanchez JJ, Brion M, Parson W, Blanco-Verea AJ, Borsting C, Lareu M, et al. Duplications of the Y-chromosome specific loci P25 and 92R7 and forensic implications. Forensic Sci Int 2004;140:241–50. [PubMed]
18. Vallone PM, Butler JM. Y-SNP typing in U.S. African American and Caucasian samples using allele-specific hybridization and primer extension. J Forensic Sci 2004;49(4):723–32. [PubMed]
19. Dupuy BM, Stenersen M, Egeland T, Olaisen B. Y-chromosomal microsatellite mutation rates: differences in mutation rate between and within loci. Hum Mutat 2004;23:117–24. [PubMed]
20. Kline MC, Duewer DL, Redman JW, Butler JM. NIST Mixed Stain Study 3: DNA quantitation accuracy and its influence on short tandem repeat multiplex signal intensity. Anal Chem 2003;75:2463–9. [PubMed]
21. Clayton TM, Whitaker JP, Sparkes R, Gill P. Analysis and interpretation of mixed forensic stains using DNA STR profiling. Forensic Sci Int 1998;91:55–70. [PubMed]
22. Melton T. Mitochondrial DNA heteroplasmy. Forensic Sci Rev 2004;16(1):1–20.
23. Redd AJ, Agellon AB, Kearney VA, Contreras VA, Karafet T, Park H, et al. Forensic value of 14 novel STRs on the human Y chromosome. Forensic Sci Int 2002;130:97–111. [PubMed]

24. Henke J, Henke L, Chatthopadhyay P, Kayser M, Dulmer M, Cleef S, et al. Application of Y-chromosomal STR haplotypes to forensic genetics. [PubMed] Croat Med J 2001;42:292–7.
25. Fernandes AT, Brehm A, Gusmao L, Amorim A. Y-chromosome STR haplotypes in the Madeira archipelago population. Forensic Sci Int [PubMed] 2001;122:178–80.
26. Johnson CL, Warren JH, Giles RC, Staub RW. Validation and uses of a Y-chromosome STR 10-plex for forensic and paternity laboratories. [PubMed] J Forensic Sci 2003;48:1260–8.
27. Pepinski W, Niemcunowicz-Janica A, Ptaszynska-Sarosiek I, Skawronska M, Koc-Zorawska E, Janica J, et al. Population genetics of Y-chromosome STRs in a population of Podlasie, northeastern Poland. [PubMed] Forensic Sci Int 2004;144:77–82.
28. Sahoo S, Chainy GB, Kashyap VK. Allele frequency of eight Y-chromosome STR loci in Oriya population of India. J Forensic Sci 2003;48:245–52. [PubMed]
29. Bosch E, Jobling MA. Duplications of the AZFa region of the human Y chromosome are mediated by homologous recombination between HERVs and are compatible with male fertility. Hum Mol Genet 2003;12:341–7. [PubMed]

Additional information and reprint requests:
John M. Butler, Ph.D.
Research Chemist
National Institute of Standards and Technology
100 Bureau Dr., Mail Stop 8311
Gaithersburg, MD 20899-8311
Tel: 301-975-4049
Fax: 301-975-8505
E-mail: john.butler@nist.gov