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CASE REPORT

A SINGLE-CENTRE EXPERIENCE IN THE APPLICATION OF Y-STR ANALYSIS IN CHALLENGING FORENSIC CASEWORK: A CASE SERIES

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ABSTRACT

Short Tandem Repeats (STRs) are the gold standard for human identification. They are widely used in crime scene investigations, kinship testing, and disaster victim identification due to their high polymorphism, sensitivity, and discrimination power. Combining autosomal STRs and sex-specific STRs (Y-STRs and X-STRs) across multiple tissues yields highly informative genetic profiles. Y-STRs, which target Y-chromosome DNA inherited through the paternal line, are particularly useful when autosomal STR profiling is inconclusive. They enhance the reliability of the results in establishing patrilineal relationships of male offspring, paternal kinship testing, and missing persons and disaster victim identification, as well as in sexual assault cases when male DNA is masked by female DNA. In complex mixtures involving male victims or multiple male contributors, autosomal DNA profiling is challenging. Even with advanced probabilistic genotyping software, separating these mixtures can still be limited due to issues like template imbalance, low quantity DNA, or degraded DNA. In such cases, Y-STR analysis serves as a valuable complementary tool. However, Y-STRs cannot distinguish between closely related males within the same paternal lineage, limiting their individualising power. Their evidentiary value also depends on robust population-specific databases for accurate statistical interpretation. This review outlines the practical uses and limitations of Y-STR analysis, emphasising the importance of population databases such as the Y Chromosome Haplotype Reference Database in evaluating evidentiary weight.

Based on these considerations, this study highlights the effectiveness of the PowerPlex® Y23 system, as a valuable complementary tool to autosomal STRs, particularly in challenging cases where autosomal results alone are insufficient.

Keywords: *Autosomal STR; PowerPlex® Y23 System; sex-specific STR; short tandem repeats*

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INTRODUCTION

DNA fragment analysis is an essential tool in forensic investigations and plays a critical role in supporting the judicial system, contributing to both civil and criminal proceedings. Various technological approaches to DNA fragment analysis are employed for different forensic applications. Short Tandem Repeats (STRs) are considered the gold standard in human identification due to their high level of polymorphism, sensitivity, speed, and strong discriminatory capability. STR markers are repeating sequences of nucleotides located within the non-coding regions of the human

genome. STR analysis has proven to be extremely valuable in various applications, such as parentage testing, kinship determination, locating missing persons, and identifying disaster victims. It also plays a crucial role in forensic investigations, such as analysing DNA from biological evidence found at crime scenes, on related items, or on victims' bodies[1].

The combination of autosomal STRs with sex-specific STR genetic markers, across various evidentiary items and bodily fluids, provides highly informative genetic information. Autosomes are inherited from both parents by all offspring through the process of recombination. Daughters inherit one X chromosome from each parent, allowing recombination between them, whereas sons inherit their single X chromosome from the mother and a Y chromosome from the father, so no X recombination occurs in sons[2]. Y-STR in the male-specific region of the human Y chromosome is commonly used in forensic DNA analysis, especially in cases where conventional autosomal DNA profiling is not informative. These Y-STR haplotypes are inherited as a unit from father to son, and this distinct pattern of inheritance makes them useful for tracing paternal lineages across generations and for male-specific DNA analysis[3].

The Y-STR analysis is used in resolving paternity disputes involving male offspring, as well as in other paternal kinship testing and in the identification of missing persons and disaster victims. Y-STR haplotyping, when used in crime scene investigations, can eliminate male suspects, establish the paternal lineage of male offenders, reveal multiple male contributors within a DNA sample, and provide valuable leads for identifying unknown males involved in a crime[4].

In conventional STR testing in sexual assault cases, the presence of excess female DNA can mask male DNA, potentially leading to incomplete or undetectable male STR profiles. Y-STR genotyping effectively isolates the male component in such complex female–male DNA mixtures. Additionally, in mixtures involving multiple unrelated male contributors, such as in gang-related cases, Y-STR analysis can separate the male lineages, as each contributor has a unique Y-STR pattern. This is useful when autosomal STRs may overlap, and individual contributor profiles are difficult to resolve. However, since Y-STRs do not undergo recombination and are passed from father to son, they cannot distinguish between paternally related males, such as brothers or father and son, who will share identical Y-STR profiles[5,6,7].

This article presents three cases that had analytical challenges due to complex DNA mixtures and the lack of one biological parent for comparison, highlighting the importance of including additional autosomal and sex-determining markers to enhance the reliability of the results.

CASE HISTORIES

Case 1

A case involving the sexual assault of a mentally disabled girl was reported, with the identity of the perpetrator(s) unknown. Two garments worn by the victim at the time of the incident, along with blood samples from the victim and a suspect, were submitted to the Government Analyst's Department's DNA laboratory through a court order.

Case 2

A case of sexual assault of a female by an unidentified individual was reported. A vaginal swab and blood samples from the victim and the suspect were submitted through a court order.

Case 3

A severely decomposed body was found hanging under suspicious circumstances in a jungle area near the deceased's residence. An individual had identified the body as his son's based on personal belongings and accessories found with the remains. No other close relatives were available to confirm the relationship. A piece of the humerus bone from the deceased and a blood sample from the alleged father were submitted through a court order for identification.

DNA extraction and analysis were carried out on all submitted samples following standard guidelines and operational procedures (Fig. 1).

For the interpretation of the obtained DNA profiles, the laboratory followed established guidelines to assess whether the DNA typing results were suitable for comparison.

Y-STR analysis gives lineage-specific information, and its interpretation is based on haplotype frequency estimates derived from population databases such as the Y Chromosome Haplotype Reference Database (YHRD). However, YHRD statistics are not presently incorporated into the reports.

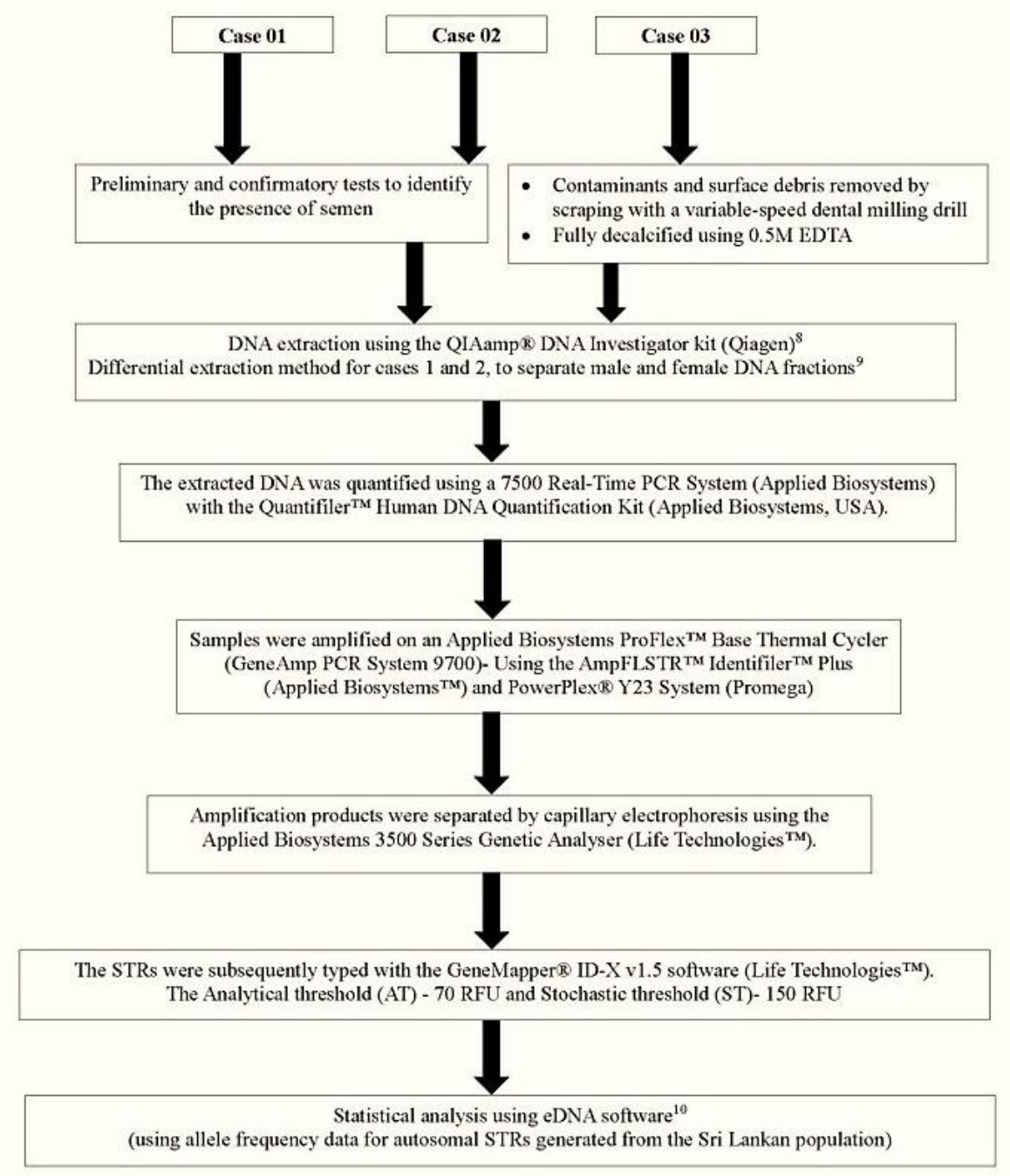

Figure 1: Flow chart showing the process of DNA analysis in the submitted samples

RESULTS INTERPRETATION

Preliminary and confirmatory tests were performed on samples from cases 1 and 2 before DNA extraction, to identify the presence of semen, which were positive in only garment-2 and vaginal swabs. The following DNA profiles were generated for autosomal STR amplification on garment-2 and the two blood samples from case 1 (Table 1). In the male fraction of garment-2, the bolded alleles matched the suspect's bolded alleles at each locus.

Table 1: Autosomal DNA profiles for garment-2 and blood samples of the victim and suspect, generated using Identifiler™ Plus

STR Locus	Garment-2 (Male fraction)	Garment-2 (Female fraction)	Blood sample (Victim)	Blood sample (Suspect)
D8S1179	**10,**11,**16**	10,11	10,11	**10,16**
D21S11	29,30,**32.2,33.2**	29,30	29,30	**32.2,33.2**
D7S820	11,**12**	11,12	11,12	**12,12**
CSF1PO	**11,12**	11,11	11,11	**11,12**
D3S1358	**15,16**,18	15,18	15,18	**15,16**
TH01	**9**	9,9	9,9	**9,9**
D13S317	8,**11**,14	8,14	8,14	**11,11**
D16S539	**10**,11,12,**14**	11,12	11,12	**10,14**
D2S1338	**18**,23,24,**26**	23,24	23,24	**18,26**
D19S433	**13,13.2,**14	13,14	13,14	**13,13.2**
vWA	**14,**17,**19**	17,19	17,19	**14,19**
TPOX	8,**9,11**	8,8	8,8	**9,11**
D18S51	**14,16**,19	14,19	14,19	**14,16**
D5S818	**10,12,**13	13,13	13,13	**10,12**
FGA	**22,23**,24	24,24	24,24	**22,23**
Amell	XY	X	X	XY

Interpretation: *STR profiles of the male and female fractions of Garment-2 were compared with blood samples from the victim and suspect. The male fraction showed alleles from both individuals, indicating a DNA mixture, while the female fraction matched the victim.*

Given the peak imbalance in the initial mixture, Y-STR analysis was also performed to provide additional support (Table 2).

Table 2: Y-STR profiles for garment-2 and suspect's blood sample, generated using PowerPlex® Y23

STR Locus	Garment-2	Blood sample (Suspect)
DYS576	18	18
DYS389 I	12	12
DYS448	20	20
DYS389 II	28	28
DYS19	16	16
DYS391	10	10
DYS481	26	26
DYS549	12	12
DYS533	10	10
DYS438	11	11
DYS437	16	16
DYS570	20	20
DYS635	22	22
DYS390	26	26
DYS439	13	13
DYS392	13	13
DYS643	11	11
DYS393	12	12
DYS458	18	18
DYS385	14,19	14,19
DYS456	15	15
YGATAH4	11	11

Interpretation: *The Y-STR profile from Garment-2 and the suspect's blood were identical, indicating the male DNA on the garment is consistent with originating from the suspect or a paternal relative.*

In Case study 2, the vaginal swab collected from the victim produced a mixed DNA profile in the autosomal STR analysis. Table 3 summarises the DNA profiles obtained from the vaginal swab and the blood samples from both the victim and the suspect, using autosomal STR amplification. In the female fraction of the vaginal swab, the bolded alleles matched the victim's bolded alleles at each locus.

Table 3: Autosomal DNA profiles for vaginal swab and blood samples of the victim and suspect, generated using Identifiler™ Plus

STR Locus	Vaginal swab (Female fraction)	Blood sample (Victim)	Blood sample (Suspect)
D8S1179	10,**11,12**,14	**11,12**	11,15
D21S11	29,30,**31.2,32.2**	**31.2,32.2**	30,33.2
D7S820	**8,10**,11	**8,10**	10,11
CSF1PO	**12**	**12,12**	11,11
D3S1358	15,**16,17**	**16,17**	17,17
TH01	**7,9.3**,10	**7,9.3**	6,9.3
D13S317	**8,**10,**11**,12	**8,11**	9,11
D16S539	**9**,12,**13**	**9,13**	11,13
D2S1338	18,**22,24**	**22,24**	18,19
D19S433	12,13,**14,16**	**14,16**	14,15.2
vWA	14,16,**17,18**	**17,18**	18,19
TPOX	8,**11**	**11,11**	9,11
D18S51	**14,15**	**14,15**	15,17
D5S818	10,**11,13**	**11,13**	13,13
FGA	**20,22**,23,25	**20,22**	19,24
Amell	XY	X	XY

Interpretation: *The vaginal swab profile shows a mixture of alleles from at least two contributors. The victim's alleles are present, and other alleles not corresponding to the suspect suggest the presence of DNA from another individual.*

Table 4 presents the Y-STR DNA profile obtained from the vaginal swab and the suspect's blood sample.

Table 4: Y-STR profiles details for vaginal swab and suspect's blood sample, generated using PowerPlex® Y23

STR Locus	Vaginal swab	Blood sample (Suspect)
DYS576	18	17
DYS389 I	12	12
DYS448	19	19
DYS389 II	29	29
DYS19	14	15
DYS391	10	10
DYS481	23	26
DYS549	13	12
DYS533	13	12
DYS438	10	11
DYS437	15	14
DYS570	16	18
DYS635	24	22
DYS390	22	23
DYS439	12	12
DYS392	14	14
DYS643	10	11
DYS393	11	14
DYS458	15	16
DYS385	12,16	13,16
DYS456	15	15
YGATAH4	12	12

Interpretation: *The Y-STR profile obtained from the vaginal swab does not fully match the suspect's profile. Several loci show allele differences, indicating that the male DNA in the vaginal swab is not consistent with originating from the suspect or his paternal lineage.*

In Case study 3, autosomal STR DNA profiles were successfully obtained from both the bone and blood sample of the individual presented as the deceased's father. Table 5 displays the autosomal STR DNA profiles obtained from both samples. The bolded alleles show that the alleged father shares one matching allele with the bone sample at each locus.

Table 5: Autosomal DNA profiles for bone sample and blood sample of alleged father, generated using Identifiler™ Plus

STR Locus	Bone sample	Blood sample (Alleged father)
D8S1179	**9**,14	**9**,15
D21S11	**30**,32.2	29,**30**
D7S820	**11**,12	**11**,11
CSF1PO	**10,11**	**10,11**
D3S1358	16,**17**	**17**,17
TH01	7,**9.3**	6,**9.3**
D13S317	**8**,8	**8**,8
D16S539	**10**,11	**10,**12
D2S1338	**17**,18	**17**,26
D19S433	**13**,13	**13**,14
vWA	17,**18**	16,**18**
TPOX	**8**,11	**8**,10
D18S51	**11**,17	**11**,14
D5S818	**10,12**	**10,12**
FGA	**21**,23	**21**,27
Amel	XY	XY

Interpretation: *The bone sample shows a single-source autosomal STR profile, with one allele at each locus matching the alleged father's blood sample, consistent with a possible paternal relationship.*

According to our DNA laboratory's internal quality assurance procedures, Y-STR testing or Argus X-12 and PowerPlex Fusion analysis are employed when one parent is unavailable. In this case, PowerPlex Fusion analysis did not provide interpretable results. To address this limitation, Y-STR analysis was carried out to provide additional confirmation of the paternal lineage. The resulting Y-STR profile details are illustrated in Table 6.

Table 6: Y-STR profiles for the bone sample and the blood sample from the alleged father, generated using PowerPlex® Y23

STR Locus	Bone sample	Blood sample (Alleged father)
DYS576	17	17
DYS389 I	12	12
DYS448	18	18
DYS389 II	28	28
DYS19	15	15
DYS391	10	10
DYS481	21	21
DYS549	13	13
DYS533	12	12
DYS438	10	10
DYS437	16	16
DYS570	15	15
DYS635	23	23
DYS390	24	24
DYS439	10	10
DYS392	11	11
DYS643	8	8
DYS393	13	13
DYS458	16	16
DYS385	16,17	16,17
DYS456	16	16
YGATAH4	11	11

Interpretation: *The Y-STR profile from the bone sample matches the alleged father's blood sample across all tested loci. This indicates that the male DNA in the bone sample is consistent with the alleged father or a paternal relative.*

DISCUSSION

Y-STR analysis has become an essential tool in forensic science, with the development of high-resolution multiplex systems like Power Plex Y-23, which have enhanced discriminatory power. Although Y-STRs cannot replace autosomal STRs for individual identification, their uniparental inheritance and male specificity make them highly valuable in forensic investigations. They are particularly useful for detecting male contributors in complex DNA mixtures, including female-dominated samples or cases with allele dropout or peak imbalance. Additionally, Y-STRs are important for paternity testing without a

maternal reference and in complex kinship analysis where autosomal data alone may be insufficient.

According to international guidelines, when a mixture can be partially or fully deconvoluted, single-source statistical methods should be applied to the identifiable contributor(s). In contrast, when the mixture cannot be deconvoluted, and the contributors cannot be distinguished, a single statistical calculation should be performed for the entire mixed profile [11]. However, in complex mixtures involving multiple contributors with a high degree of allele overlapping, peak imbalance, low template, or degraded DNA, producing reliable statistics can be challenging. In such scenarios, probabilistic genotyping tools can assist in interpretation, but the results should be considered cautiously[12]. In our case, probabilistic genotyping tools are not presently incorporated. Instead, mixture deconvolution was performed using GeneMapper ID-X Software v1.5, the standard software supplied with the genetic analyser.

Differential extraction performed on garment-2 resulted in two DNA profiles; the female fraction matched the victim's profile, while the male fraction gave a mixed DNA profile for autosomal STR analysis. The DNA results for the male fraction from garment-2 showed the presence of multiple contributors, with at least two individuals involved. The mixture showed a significant peak imbalance between major and minor contributors. However, the analysis using GeneMapper ID-X Software v1.5, the standard software provided with the genetic analyser, revealed that the major component matched the suspect's DNA profile, while the minor component corresponded to the victim's profile.
The Y-STR analysis of garment-2 showed a single male DNA profile, as Y-STRs only detect male DNA and not female DNA. These findings suggest that the specific area of garment-2 contained DNA from both male and female contributors. While the presence of male DNA is initially indicated by amelogenin typing during autosomal STR analysis, Y-STR profiling added additional discriminatory power by generating a male-specific haplotype. This allowed for a direct comparison between the male component of the mixture with the suspect's reference profile, especially when the male DNA was present in very small amounts or mixed together with female DNA. It provides lineage-specific information that is critical in mixed or low-template samples where autosomal data may be insufficient for individualisation.

Differential extraction performed on the vaginal swab yielded no DNA profile in the male fraction, while the female fraction produced a mixed DNA profile for autosomal STR analysis. Similar to Case study 1, the DNA results indicated a mixed profile, involving at least two contributors with significant peak imbalances. Mixture deconvolution was performed using GeneMapper ID-X Software v1.5, and the major component matched the victim's profile, while the minor component was not consistent with the suspect's DNA profile. Although some autosomal STR loci like D8S1179, CSF1PO, and TH01 showed alleles inconsistent with the suspect's genotype, the overall autosomal profile represented a mixture containing alleles that could originate from multiple contributors. Because of the mixture's complexity and the possibility of allele dropout or peak imbalance, a definitive exclusion could not be made using autosomal data alone. Therefore, Y-STR analysis was performed to obtain a male-specific profile, enabling a more definitive comparison to the suspect. The Y-STR profile provided additional evidence for exclusion, showing mismatches at multiple Y-STR loci compared to the suspect's haplotype.

The Y-STR results from the vaginal swab showed a single male contributor, whereas the autosomal STR profile was a mixture, indicating DNA from both male and female contributors. Furthermore, the Y-STR profile from the vaginal swab did not match the Y-STR profile of the suspect. The Y-STR results obtained using the PowerPlex® Y23 System further confirmed the absence of the suspect's male DNA in the vaginal swab, thereby supporting the exclusion of the suspect from DNA analysis.

For case 3, autosomal STR DNA profiles were successfully obtained from both the bone sample and the blood sample of the deceased's father. A trio test, which includes DNA analysis of the father, mother, and child, was the ideal approach for paternity testing. However, the mother of the

deceased was not available for testing, and the only close relative present was his father. The autosomal STR analysis showed that half of the alleles at each STR locus in the deceased matched those of the alleged father. A statistical analysis to evaluate the probability of a paternal relationship was conducted using the eDNA 2.3 software package. The analysis produced a Combined Relationship Index (CRI) of 99.999%, indicating that the alleged father has a 99.999% likelihood of being the biological father of the deceased.

However, in rare cases, by coincidence, an unrelated random person could also share one allele at each locus with the child. In this case, the analysis was performed without the mother's sample; the possibility of a coincidental match cannot be entirely ruled out.

The Y-chromosome haplotypes of the deceased's bone sample and the blood sample of the father matched across all tested Y-STR loci, strongly suggesting that they share a common paternal lineage. Therefore, the autosomal STR results have a high statistical probability, providing strong evidence that the tested male is the biological father of the missing individual. In addition, the Y-STR analysis offered complementary confirmation of the paternal lineage.

However, the routine use of Y-STRs in casework remains limited due to their inability to identify a person rather than the paternal lineage and provide the same degree of confidence in identifying a specific individual as autosomal DNA. These limitations include the inability to distinguish between close paternal relatives, lower discriminatory power compared to autosomal STRs, and difficulties in interpreting mixed male profiles when contributors share the same Y-haplotype.

Although Y-STRs have been well established in the international literature, published data documenting their forensic applications are limited in this region. This study adds contextual novelty by demonstrating how internationally recognised forensic tools can be effectively applied in local casework, particularly in challenging scenarios such as complex male DNA mixtures and kinship testing in the absence of maternal references.

The absence of population frequency data for the observed Y-haplotypes may limit the ability to assign strong statistical weight to the findings. Nevertheless, for future consideration, the inclusion of YHRD statistics for male-specific markers should be accompanied by proper understanding and awareness within the judiciary system. This would help clarify how the combination of Y-STR haplotype with autosomal STRs can provide stronger and more comprehensive interpretation in forensic investigations.

Y-STR analysis provides information specific to paternal lineage, with interpretation relying on haplotype frequency estimates obtained from population databases like YHRD. When applying YHRD, the level of discrimination is not as strong as that provided by autosomal STR analysis[13]. Legal professionals may sometimes interpret these results incorrectly due to limited familiarity with the scientific differences. Therefore, YHRD statistics are not presently incorporated into the reports. In the future, the application of Y-STR statistics will be considered following thorough discussions with forensic science experts and legal professionals.

CONCLUSION

The outcomes of these three case studies highlight the importance of using Y-chromosome lineage markers as a supportive tool for establishing paternal relationships, particularly in cases where autosomal STRs provide inconclusive results.

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None.

CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

ETHICAL ISSUES

All DNA samples and associated data were anonymized prior to analysis, meaning that any personal identifiers were removed or replaced with unique codes to ensure that individuals could not be directly or indirectly identified.

SOURCES OF SUPPORT

None

AUTHOR CONTRIBUTIONS

RWRKR: Design of the work; the acquisition; analysis and interpretation of data for the work; Drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **EM:** Analysis and interpretation of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **LRTP:** Analysis and interpretation of data for the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. **PGS:** Design of the work; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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