



# Use of 23-Y STR Loci in Paternity Testing: A Case Study

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## Abstract:

Y-chromosome DNA testing is potentially useful in forensic analysis. The male specific amplification can enable examination of a male perpetrator's profile even in mixtures with high levels of female DNA in sexual assault cases. This case study presents the application of Y-23 STR loci for identification of perpetrator in the rape case of a poor mentally retarded girl. The crime was revealed when her pregnancy became apparent. The victim was unable to identify the accused and also could not recollect the incident. Throughout the investigation, the investigating authorities submitted blood samples of 22 suspected individuals, along with blood samples of the victim and her male baby for DNA analysis. All the submitted samples were analysed using autosomal and Y chromosome STR DNA analysis.

## Keywords:

Forensic Casework

Y-Chromosome

Exclusion

Paternity Testing

STR

## 1. Introduction:

The Y-chromosome DNA analysis is important in various useful applications of human genetics including forensic evidence examination, paternity testing, historical investigations, studying human migration patterns throughout history and genealogical research. There are huge numbers of crimes, where DNA evidence is helpful, particularly sexual assaults, where males are involved as the perpetrator. The ChrYSTR test designed to only examine the male portion can be valuable. With this analysis, interpretable results can be obtained in some cases where autosomal tests are limited by the evidence, such as high level of female DNA in the presence of minor amounts of male DNA. The number of individuals involved in a “gang rape” may be easier to decipher with Y-chromosome results than with complicated autosomal STR mixtures. A series of validation experiments and casework application of a Y chromosome specific STR multiplex have been reported in literature. [1] The chances of detecting of low levels of the perpetrator’s DNA in high background of a female DNA can be improved using ChrY-specific PCR primers. [2] Y-chromosome analysis have also been used to verify amelogenin Y-deficient males. [3] The Y-chromosome has also become a popular tool for tracing historical human migration patterns through male lineages. [4] Jobling et al. [5] describes the use of Y-chromosomes in forensic analysis and paternity testing in detail.

We describe here a case in which identification of actual perpetrator was quite difficult, since the victim girl is mentally retarded and her parents were physically challenged. The crime was revealed when her pregnancy became apparent. 23 Y-STR loci help here to identify paternal progeny of perpetrator; and then individual paternity was proved using Autosomal STR analysis.

## 2. Case History

A mentally challenged 27 year old unmarried girl was raped. The crime against unknown perpetrator was registered when her pregnancy became apparent. The victim was unable to identify the accused and also was unable to recollect the incident. Initially the blood samples of victim and her baby boy were submitted for DNA analysis. Throughout the investigation, the investigating authorities submitted blood samples of 22 suspected individuals. All the submitted samples were analysed using STR DNA analysis.

## 3. Material and methods

### 3.1 Extraction:

DNA from submitted blood samples was extracted with PrepFiler Express Forensic DNA Extraction Kit (Applied Biosystems, US), with an automated DNA extractor machine Automate Express Nucleic Acid Extraction System (Applied Biosystems, US).

### 3.2 Quantitation of Extracted DNA:

Extracted DNA of all the 24 samples was quantified by Quantifiler Human DNA Quantitation Kit (Applied Biosystems, US), using 7500 Fast Real-Time PCR System (Applied Biosystems, US). The samples were diluted to 1 ng/ $\mu$ L of input DNA for each sample for further PCR analysis.

### 3.3 PCR Protocol:

Three PCR Kits were used throughout the case, as and when required. All the extracted DNA samples were analysed at 15 Autosomal STR markers and Amelogenin marker using AmpFISTRIdentifiler PCR Amplification Kit (Applied Biosystems, US). Y-chromosome STR DNA Analysis was undertaken

using PowerPlex Y23 PCR Amplification Kit (Promega Corporation, Wisconsin, US). To further confirm paternity, samples were also analysed with 24-locus autosomal marker GlobalFiler PCR Amplification Kit (Applied Biosystems, US). Veriti Thermal Cycler (Applied Biosystems, US) was used for amplifying the target STR Loci.

### 3.4 Detection of PCR Products:

The detection of PCR products was done using capillary electrophoresis. 1µL of PCR product from each kit was mixed with 10µL HiDi-Formamide and size standards i.e GeneScan 600 Liz Size Standard (Applied Biosystems, US) and WEN ILS 500 Y23 (Promega Corporation, US), respectively. The PCR products were then denatured and analysed using 3500 Genetic Analyser (Applied Biosystems, US). Appropriate Allelic Ladders were used. The electrophoresis data was analysed on computer using GeneMapper v 1.2 (Applied Biosystems, US).

## 4. Results and Discussion

In the said case, initially blood samples of victim and her baby boy were submitted for analysis. After around 4 months blood sample of one suspected accused 1 was submitted. The results thus obtained were compared. It was observed that accused 1 was excluded to be the biological father of baby boy. After 14 months, the investigation agency submitted 12 blood samples of suspected individuals, age ranging from 16 to 75 years. All the suspects lived near home of the victim. Autosomal analysis at 15 STR loci along with gender specific Amelogenin marker was performed. All these suspected individuals were excluded and did not match at various STR loci as shown in following table. (Table 1)

Suspected Accused Number	Number of autosomal STR Loci failed to match with baby at 15 STR Loci
1	13
2	9
3	10
4	12
5	7
6	7
7	4
8	11
9	11
10	7
11	7
12	8
13	10

**Table 1:** The number of autosomal STR loci of suspected individuals failed to match with baby.

As discussed earlier Y-chromosome analysis plays a major role in proving the paternity of alleged son in question. Comparison of Y chromosomes is be a simple way of excluding an alleged father. Since the baby of the victim was male, Y-chromosome STR DNA Analysis was done, along with all accused. We were able to narrow down to the main accused using Y-chromosome STR Analysis. One of the Y23 DNA profile from suspected individuals (Accused 8) was a complete match with that of the baby boy (Table 2). Yet the autosomal STR profile of the individual was not a match for paternity. Matching of Y-STR profile became the key evidence as to prove that the victim was raped by one of the men in this family, yet not the one whose sample was submitted.



Y-STR Locus	Baby boy	Suspect 1	Suspect 2	Suspect 3	Suspect 4	Suspect5	Suspect 6	Suspect 7	Suspect 8	Suspect 9	Suspect 10	Suspect 11	Suspect 12	Suspect 13
DYS576	18	17	19	18	19	19	18	18	18	17	17	17	17	19
DYS389 I	12	13	13	12	12	12	13	13	12	13	12	13	14	12
DYS448	19	19	20	19	21	21	19	19	19	18	18	19	20	21
DYS389 II	28	31	30	28	29	29	29	29	28	30	27	29	30	29
DYS19	14	14	15	15	16	16	16	16	14	14	15	15	15	16
DYS391	10	10	11	10	11	11	10	10	10	10	10	10	11	11
DYS481	23	22	23	24	22	22	22	22	23	25	23	21	24	22
DYS549	12	13	12	12	13	13	12	12	12	12	13	13	13	13
DYS533	12	12	12	12	9	9	12	12	12	12	10	12	11	9
DYS438	10	9	11	9	10	10	11	11	10	11	9	8	9	10
DYS437	15	15	14	14	16	16	14	14	15	15	15	14	15	16
DYS570	14	15	18	19	18	18	19	19	14	16	18	19	18	19
DYS635	23	21	23	20	21	21	23	23	23	23	20	20	22	21
DYS390	22	23	24	22	22	22	26	26	22	25	24	24	27	22
DYS439	12	11	10	11	11	11	10	10	12	12	13	11	11	11
DYS392	14	11	11	11	11	11	11	11	14	13	11	11	11	11
DYS643	10	10	10	10	13	13	10	10	10	11	9	9	11	13
DYS393	11	13	13	12	13	13	13	13	11	13	12	12	13	13
DYS458	16	16	17	18	16	16	17	17	16	16	16	18	18	16
DYS385	13, 17	13.2, 20	11, 14	15, 16	15, 15	15, 15	11, 11	11, 11	13, 17	13, 17	14, 16	15, 16	13, 15	15, 15
DYS456	15	16	15	16	16	16	16	16	15	15	13	15	15	16
YGATAH4	11	12	14	12	12	12	13	13	11	12	11	11	12	12

Table 2: Y23 DNA profiles of suspected individuals (1 to 13) and baby boy.

Since the Y-STR profile was a match, it became a clear indicator that the actual perpetrator belonged to the same paternal lineage, i.e from the same family. The investigating authorities were then directed to submit reference blood samples of males from the same family. After few days police investigating agency submitted reference blood samples of 8 more male suspects, from the family of interest and samples were subsequently analysed. The results obtained were interpreted. Seven male suspects were excluded; these suspected individuals did not match STR loci of the baby as shown in Table 3, yet there Y23 STR profiles were a complete match except suspects 22. (Table 4)

Suspected Accused Number	Number of autosomal STR Loci failed to match with baby at 15 STR Loci
14	10
15	7
16	8
17	9
18	5
19	1
20	0
21	5
22	6

Table 3: The number of autosomal STR loci of suspected individuals failed to match with baby boy.

Y-STR Locus	Baby Boy	Suspect 14	Suspect 15	Suspect 16	Suspect 17	Suspect 18	Suspect 19	Suspect 20	Suspect 21	Suspect 22
DYS576	18	18	18	18	18	18	18	18	18	18
DYS389 I	12	12	12	12	12	12	12	12	12	13
DYS448	19	19	19	19	19	19	19	19	19	20
DYS389 II	28	28	28	28	28	28	28	28	28	30
DYS19	14	14	14	14	14	14	14	14	14	16
DYS391	10	10	10	10	10	10	10	10	10	10
DYS481	23	23	23	23	23	23	23	23	23	24
DYS549	12	12	12	12	12	12	12	12	12	12
DYS533	12	12	12	12	12	12	12	12	12	12
DYS438	10	10	10	10	10	10	10	10	10	11
DYS437	15	15	15	15	15	15	15	15	15	15
DYS570	14	14	14	14	14	14	14	14	14	19
DYS635	23	23	23	23	23	23	23	23	23	23
DYS390	22	22	22	22	22	22	22	22	22	25
DYS439	12	12	12	12	12	12	12	12	12	10
DYS392	14	14	14	14	14	14	14	14	14	11
DYS643	10	10	10	10	10	10	10	10	10	10
DYS393	11	11	11	11	11	11	11	11	11	13
DYS458	16	16	16	16	16	16	16	16	16	14
DYS385	13, 17	13, 17	13, 17	13, 17	13, 17	13, 17	13, 17	13, 17	13, 17	11, 14
DYS456	15	15	15	15	15	15	15	15	15	16
YGATAH4	11	11	11	11	11	11	11	11	11	13

Table 4: Y23 DNA profiles of suspected individuals (14 to 22) and baby boy.

The autosomal STR DNA analysis was conducted alongside Y-Chr DNA. It was observed that one male suspect was excluded at 1 autosomal STR Locus(Accused 19) and Y23 STR Profile was a complete match. To further conclude the exclusion at 1 STR profile, Global Filer kit was used, which showed exclusion at 2 STR Loci. One male suspect (Accused 20) showed inclusion at all STR Loci. (With IdentiFiler; 15 STR Loci and Global Filer; 24 STR Loci)The Y23 STR profile was a match with that of the baby. Suspected accused 20 was concluded to be the biological father of the male baby of victim.

## 6. Conclusion

As shown in our casework example, exclusion of the suspects are unambiguous and straightforward using Y STRs. Y STR analysis does not only, exclude a single suspect, but also all of his paternal progeny and is a valuable investigative tool. This case is an example of the importance of scientific DNA analysis in crime investigation. Y-Chr STR DNA analysis of suspected individuals and baby boy of victim helped us to scientifically and investigatively confirm the actual culprit and since the victim is mentally retarded justice was guaranteed. Y STR analysis helped here to reduce the number of samples to be submitted for autosomal STR analysis. Otherwise sample size would have been greater, if only Autosomal STR analysis was relied upon. This case study is one of best example where Y- chromosomal DNA analysis can be done in combination with autosomal markers, and thus provide useful tool both in forensic studies and paternity testing.

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## 8. Conflict of interest

Authors declare no conflict of interest.

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