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REVIEW ARTICLE

Detection of Mixed Profiling via Y-Filer Mode of Analyzation

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ABSTRACT

In this research study, we will look into the mixed profiling via Y marker analysis in a sexual assault case. Ascertaining the identification of the assailant or victim can be determined conclusively by DNA profiling of the samples found on the crime scene. There are two types of DNA's, nuclear DNA, and mitochondrial DNA. Generally, mitochondrial DNA is taken in cases where nuclear DNA cannot be extracted which are mostly present in hair and bone. Various types of DNA analysis, STR analysis, RFLP, mtDNA analysis, and Y-filer depending on the case involved. In the cases involving mixed profiling, it is Y-filer profiling which is used to find out the actual profile to give a report as conclusive evidence. In this research, we will look at the analysis of mixed profiling via Y-filer analysis in a sexual assault case. Problem in the cases involving mixed gene analyzer profiling that is two accused involved in a particular crime scene becomes a condition whose admissibility in the court comes under question. In such cases it is Y-chromosome present on "AMEL" that is the locus studying for the X- and Y-chromosome signifying the presence or absence of the criminal under question.

KEYWORDS | dna profiling, y-filer, mixed profiling, sexual assault

INTRODUCTION

DNA evidence can be used in various cases such as paternity testing, criminal identification, a study of evolution when considering the human population and inherited and autosomal disorder related study. In modern scenario, it has become a most common test in any criminal investigation, and is high in demand in the process of criminal examination. Due to the considerable authentic studies and challenges in courts, DNA is now regarded as 'gold standard' in forensic science.

The DNA test is just the first step while looking at DNA profiling. Matching DNA is the next step involving marker to analyze peaks, which is unique to each nucleotide when looking at an analysis, often known as the fingerprint of the individual. This leads to result in formation namely: inclusive, exclusive, or inconclusive in nature. In case that states the result to be inclusive involves the accused to be included in as a possible source of DNA considering it conclusive. In the result stating exclusion, the suspect's DNA is considered non-suspect and can be excluded from being a chance of a source of DNA origin. In the case that has the interpretation to be inconclusive, it is generally the result

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of DNA being degraded or in the case where the quantity is below 40 micro liters, which is the limit for analysis. The focus of this research is to detect individual DNA profiles in the cases involving mixed profiling via Y-filer mode in PCR.

For the purpose of this research, we have examined a collection of biological exhibits in the cases mostly involving rape preliminary cases of POCSO Act, Automation of exhibits in automator, PCR (Y-filer mode), Genetic Analyzer–ABI3130. The purpose is to study the analysis of mixed profiling with the use of Y marker analysis in sexual assault case.

THE PROCESS

After procuring the biological exhibits obtained at the crime scene, the exhibits were analyzed first to recognize the presence of semen, saliva, blood etc and, if present, on the sample using analytical techniques like chemicals (Sodium Acetate, Fast Blue B-salt, Glacial Acetic acid, Hydrogen peroxide solution, Sodium-1 Alpha naphthalene phosphate, Benzedrine Powder) and microscope. The second step involves the extraction of DNA using automater. The DNA thus extracted will be subjected to PCR loaded in a portable thermal cycler to magnify and amplify the targeted loci (sites). The amplified or magnified products are then filled within the capillaries of DNA Sequencing machine, which segregate and characterize the STR fragments.

MATERIALS & METHOD

Exhibits, chemicals (Sodium Acetate, Fast Blue B-salt, Glacial Acetic acid, Hydrogen peroxide solution, Sodium-1, Alpha Naphthalene Phosphate, Benzedrine powder), Distilled water Buffer-Prepfiler, DNA express, Forensic DNA Extraction kit, DDT, Master mix, Primer mix, Hydride, Allelic Ladder, Column, Pipette, Vortex, Sphinx, Automate Express, Genetic analyzer.

Instruments: Weighing Machine, Thermo mixture, Centrifuge Machine, Automate Express, PCR Machine, Genetic DNA Analyzer.

Preliminary and Confirmatory Test-Physical, Chemical and Microscopic Examination-

Biological examination for detection of Semen and Blood was done by preliminary test and confirmatory test and sent for DNA analysis and examination.

In the cases involving mixed gene analyzer profiling of the two accused involved in a particular crime scene becomes a condition whose admissibility in the court comes under question in such cases it is Y chromosome present on "AMEL" that is the locus studying for the X and Y chromosome signifying the presence or absence of the criminal under question.

This study was conducted in a case of mixed profiling in which a 12-year-old girl was raped by two men (suspects A and B) aged 22 and 47, respectively. Victim was found dead in B's house and was covered with a cloth. The victim had marks on her neck which seemed to be of a plastic rope which may be the cause of her death. Her hands and legs were tied with a plastic rope. Her mouth was taped shut with plastic tape. Both suspects were arrested by the police and their blood samples taken for examination. The exhibits received are listed below:

- A 5x11.5cm transparent tape stuck on the mouth of the deceased
- A mahanadi-colored underwear of the deceased (make-DIXCY 85cm).
- A white-colored banian of the accused A.
- A light blue underwear of the accused A.
- Pubic hair of the accused A.
- Nails of the accused A.
- Blood sample of the accused A in EDTA vial (reference DNA sample).
- Two vaginal slides of the deceased.
- Two cervix slides of the deceased.
- Cervix swab of the deceased.
- Nails of the deceased.
- Three vaginal swabs of the deceased.
- An anal swab of the deceased.
- Blood sample of the deceased in EDTA vial (reference DNA sample).
- A torn yellow and black colored full sleeves sweat shirt of the deceased.
- Blood sample of the accused B in EDTA vial (reference DNA sample).
- A dark brown-colored underwear of the accused B.
- 5 pieces of plastic rope tied around the legs of the deceased. (approximate sizes: 38.5cm, 49cm, 172cm, 31cm and 184cms).

Figure 1 Purple coloration signifies positive test

Figure 2 Green coloration signifies positive test

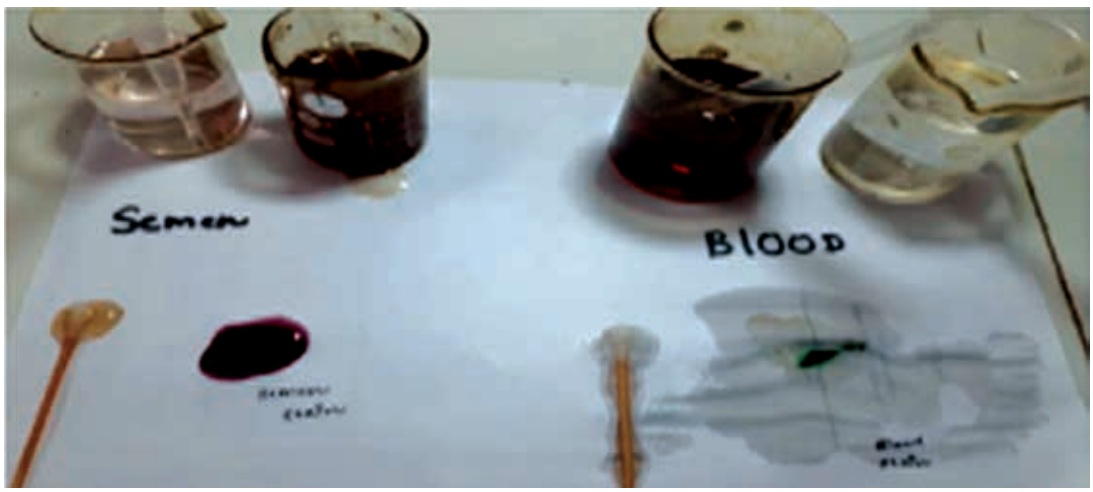


Figure 3 Purple coloration signifies positive test

Exhibit No.	Acid Phosphate Test	Barberious Crystal Test /Florence Crystal Test	Spermatozoa Detection
1	-ve	-ve	-ve
2	**Positive**	**Positive**	**Positive**
3	-ve	-ve	-ve
4	-ve	-ve	-ve
5	-ve	-ve	-ve
6	-ve	-ve	-ve
7	-ve	-ve	-ve
8	-ve	-ve	-ve
9	-ve	-ve	-ve
10	**Positive**	**Positive**	**Positive**
11	-ve	-ve	-ve
12	-ve	-ve	-ve
13	-ve	-ve	-ve
14	-ve	-ve	-ve
15	-ve	-ve	-ve
16	-ve	-ve	-ve
17	-ve	-ve	-ve
18	-ve	-ve	-ve
19	-ve	-ve	-ve
20	-ve	-ve	-ve
Control	**Positive**	**Positive**	**Positive**
U/S Control	-ve	-ve	-ve

Table 1 Semen Analysis

- 3 pieces of plastic rope tied around hands (approx sizes: 38cm, 37cm and 8cm) of the deceased.
- 5 pieces of plastic rope tied around neck (approx sizes: 48.5cm, 51.5cm, 29cm, 52cm and 39cm), of the deceased.

Material Method:

Biological examination for detection of semen and blood was done through preliminary test, and confirmatory test and then isolation from the exhibits was carried out by using Prep Filer Express™ DNA Extraction Kit. The quantified DNA was subjected to multiplex PCR reaction for fifteen Autosomal STR Loci and one amelogenin loci using commercially available AmpFISTR® Identifiler® plus kit and AmpFISTER® Y-Filer kit. The capillary electrophoresis of amplified products was done on automated DNA Sequencer and analysis was carried out using Gene Mapper IDX® software.

RESULTS

Result from examination of detection of semen and blood:

1. Semen was detected on exhibits of 2 and 10
2. Semen could not be detected on exhibits of 1, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20
3. Human blood was detected on exhibits of 2, 4, 8, 9, 10, 12, 13 and 15
4. Human blood was not found on exhibits of 1, 3, 5, 6, 7, 11, 14, 16,17,18,19 and 20.

Results of DNA Examination

1. The DNA test was performed on exhibits 1-20
2. The alleles were amplified at each loci to obtain the DNA Profiles of the sources of the exhibits-1, 2, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19 and 20.
3. The DNA Profiles obtained from the exhibits-1, 4, 8, 9, 11, 12 and 13 (transparent tape, underwear of accused A, vaginal smear slide of deceased, cervix slide of deceased, nails of deceased, vaginal swab of deceased, and anal swab of deceased) belong to a single female human resource and matched with the DNA profile taken from exhibit-14(blood sample of deceased).
4. The mix DNA profiles collected from exhibits-2,10,18,19 and 20 (underwear of deceased, cervix swab of deceased, plastic rope tied around leg of deceased, plastic rope tied around hand of deceased and plastic rope tied around neck of deceased) were also matched with each of the DNA profiles taken from the

Exhibit No.	Benz./TMB Test	Takayama Test	Anti Human
1	-ve	-ve	-ve
2	Positive	Positive	Positive
3	-ve	-ve	-ve
4	Positive	Positive	Positive
5	-ve	-ve	-ve
6	-ve	-ve	-ve
7	-ve	-ve	-ve
8	Positive	Positive	Positive
9	Positive	Positive	Positive
10	Positive	Positive	Positive
11	-ve	-ve	-ve
12	Positive	Positive	Positive
13	Positive	Positive	Positive
14	-ve	-ve	-ve
15	Positive	Positive	Positive
16	-ve	-ve	-ve
17	-ve	-ve	-ve
18	-ve	-ve	-ve
19	-ve	-ve	-ve
20	-ve	-ve	-ve
Control	Positive	Positive	Positive
U/S Control	-ve	-ve	-ve

Table 2 Blood Analysis. **Method of Isolation:** Automate Express

Sample No.	Area/Volume of Sample used	Final Volume of Isolated DNA in L	Kit/Chemical Used
1	Tape	50µL	Prepfiler Express Forensic DNA Extraction Kit (500L Lysis Buffer + 5L DDT)
2	5*5mm^2	50µL	—Do—
4	5*5mm^2	50µL	—Do—
5	Pubic hair	50µL	—Do—
6	Nail	50µL	—Do—
7	40µL	40µL	—Do—
8	Slides	50µL	—Do—
9	Slides	50µL	—Do—
10	Swab	50µL	—Do—
11	Nails	50µL	—Do—
12	Swab	50µL	—Do—
13	Swab	50µL	—Do—
14	40µL	50µL	—Do—
15	5*5mm^2	50µL	—Do—
16	40µL	50µL	—Do—
17	5*5mm^2	50µL	—Do—
18	Rope	50µL	—Do—
19	Rope	50µL	—Do—
20	Rope	50µL	—Do—

Table 3 DNA Case Worksheet: DNA Isolation. **Method of Isolation:** Automate Express

exhibit-7 and 14 (blood sample of accused A and blood sample of deceased) and not matching with the DNA profile taken from the exhibit 16 (blood sample of accused B).

5. The DNA profiles obtained from exhibit- 6 (nails of accused A) is from a single male human source and matching with the DNA profile obtained from exhibit-7 (blood sample of accused A)
6. The mix DNA profiles collected from exhibits-15 (sweatshirt of the deceased) was matched with the DNA profiles taken from the exhibit-14 (blood sample of the deceased) but not matched with the DNA profile of the exhibit-7 and 16 (blood sample of accused A and blood sample of accused B.)

DISCUSSION

In a majority of the sexual assaults, blood and semen samples, which are left after the crime over the body of the victim, clothes as well as on the location of the crime generally plays a significant role during the investigation. A major challenge to the investigators is to properly extract the swab specimens from the crime scene, which are commonly found on those surfaces like the victim's body, the parts of the body of the victim with which the culprit came into contact. The priority was to collect the assailant's DNA as early as possible to ensure the chances of accurate recognition of the person who committed the crime.

Blood and semen can be exposed by preliminary or chemical test followed by confirmatory or microscopic test and DNA analysis. A DNA test will not set out to verify if the biological exhibit provided contains semen but rather its aim is to extract a DNA profile of the individual to whom the exhibit belongs using the semen and blood sample provided.

Microscopic tests of blood and semen will not help to recognize the criminal. In order to do this, examiners need a DNA profile and a finalized DNA profile can help precisely distinguish different people. No two individuals can have the same DNA profile (with the exception of monozygotic twins). Once the DNA profile has been made, it can be collated to the DNA profiles of any questionable person to look for a match. In cases where no match occurs, examiners and police might run a search in a DNA database. A DNA database will carry the DNA profiles of offender. However, a match between two DNA profiles does not surely confirm the person is culpable of the crime and is the true culprit. Other evidences will need to be provided in that case.

DNA is relatively long lasting. It is most likely that forensic samples assembled from a rape victim will yield results. However, time factors, chemical factors like washing using soaps and detergents, external factors such as temperature and humidity and internal factors like body fluids may damage the effectiveness of a sample. The earlier the samples are assembled and tested, the higher the chance of yielding solid, reliable results.

CONCLUSION

The DNA tests performed on the exhibits pro-

Exhibit No	Volume of DNA(L)	Primer Set(L)	PCR Reaction(L)	Additional Component	Total Volume(L)
1	10	10	5	– Nil –	25
2	10	10	5	– Nil –	25
3	10	10	5	– Nil –	25
4	10	10	5	– Nil –	25
5	10	10	5	– Nil –	25
6	10	10	5	– Nil –	25
7	10	10	5	– Nil –	25
8	10	10	5	– Nil –	25
9	10	10	5	– Nil –	25
10	10	10	5	– Nil –	25
11	10	10	5	– Nil –	25
12	10	10	5	– Nil –	25
13	10	10	5	– Nil –	25
14	10	10	5	– Nil –	25
15	10	10	5	– Nil –	25
16	10	10	5	– Nil –	25
17	10	10	5	– Nil –	25
18	10	10	5	– Nil –	25
19	10	10	5	– Nil –	25
20	10	10	5	– Nil –	25
Positive control	10	10	5	– Nil –	25
Negative control	10	10	5	– Nil –	25

Table 4: **DNA Case Worksheet:** DNA Amplification **Name of Kit:** ID

Notes: *Preheating: 95°C for 11 mins, Denaturation: 94°C for 20 mins. Annealing: 59°C for 3 mins, Extension: 60°C for 10 mins. Total Cycle: 29*

Exhibit No	Volume of DNA(L)	Primer Set(L)	PCR Reaction(L)	Additional Component	Total Volume(L)
2Y	10	0.5	9.5	0.5	25
7Y	10	0.5	9.5	0.5	25
10Y	10	0.5	9.5	0.5	25
15Y	10	0.5	9.5	0.5	25
16Y	10	0.5	9.5	0.5	25
17Y	10	0.5	9.5	0.5	25
18Y	10	0.5	9.5	0.5	25
19Y	10	0.5	9.5	0.5	25
20Y	10	0.5	9.5	0.5	25
Positive Control	10	0.5	9.5	0.5	25
Negative Control	10	0.5	9.5	0.5	25

Table 5: **DNA Case Worksheet:** DNA Amplification **Name of Kit: Y-Filer**

Notes: *Preheating: 95°C for 11 mins, Denaturation: 94°C for 01 min. Annealing: 61°C for 01 mins, Extension: 72°C for 01 mins. Total Cycle: 30*

Exhibit No	Amplified Product Allelic Ladder (L)	Size Standard (L)	Hi-Di Formamide (L)	Total Volume(L)
1	01	0.3	8.7	10
2	01	0.3	8.7	10
3	01	0.3	8.7	10
4	01	0.3	8.7	10
5	01	0.3	8.7	10
6	01	0.3	8.7	10
7	01	0.3	8.7	10
8	01	0.3	8.7	10
9	01	0.3	8.7	10
10	01	0.3	8.7	10
11	01	0.3	8.7	10
12	01	0.3	8.7	10
13	01	0.3	8.7	10
14	01	0.3	8.7	10
15	01	0.3	8.7	10
16	01	0.3	8.7	10
17	01	0.3	8.7	10
18	01	0.3	8.7	10
19	01	0.3	8.7	10
20	01	0.3	8.7	10
Negative Cuntrol	01	0.3	8.7	10
Positive Control	01	0.3	8.7	10
AL. ID	01	0.3	8.7	10

Table 6 **DNA Case Worksheet**: Genotyping. Sample Preparation and Electrophoresis

Notes: *Instrument: ABI 3130 Genetie Analyzer. Capillary Length: 36cms.*
Module: Genemapper 1D-X. Duration: 95°C for 3 mins. Snap Cooling in ice for 03 mins.

vided is sufficient to conclude that:

1. The DNA profiles obtained from the exhibits of transparent tape, underwear of the accused A, vaginal smear slide, cervix slide, nails, vaginal swab, and anal swab of the deceased belong to a single female human and matched with the DNA profile taken blood sample—exhibit-14—of the deceased).
2. The mix DNA profiles collected from the exhibits—underwear of the deceased, cervix swab, plastic rope tied around the leg of the deceased, plastic rope tied around hand of the deceased and plastic rope tied around neck of the deceased—were also matched with each of the DNA profiles taken from the exhibit-7 and 14 (blood sample of the accused A and blood sample of deceased) and not matching with the DNA profile taken from the exhibit 16 (blood sample of accused B).
3. The DNA profiles obtained from exhibit- 6 (nails of the accused A) is from a single male human source and matching with the DNA profile obtained from exhibit-7(blood sample of accused A).
4. The mix DNA profiles collected from exhibits-15 (sweatshirt of the deceased) was matched with the DNA profiles taken from the exhibit-14 (blood sample of deceased) but not

EXHIBIT 01

EXHIBIT 02

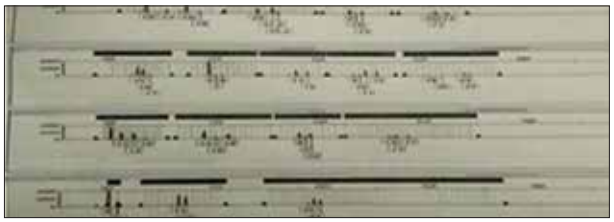
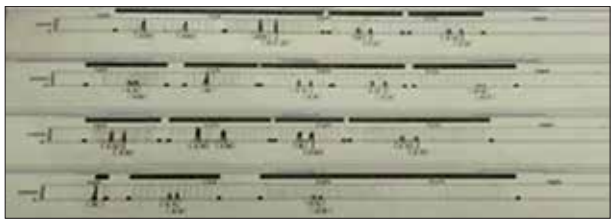


EXHIBIT 04



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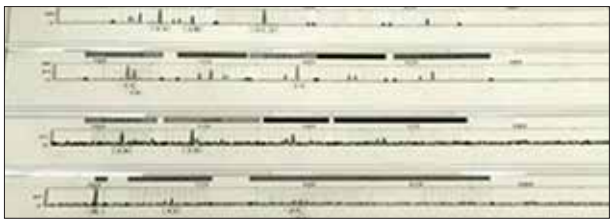

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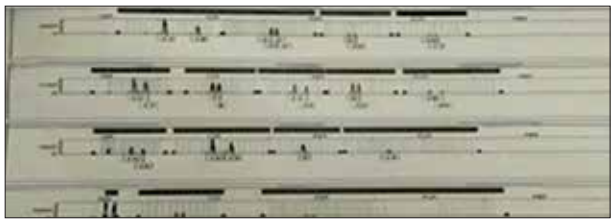

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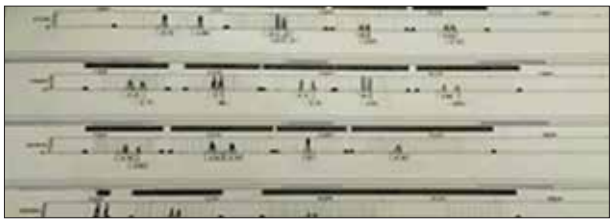

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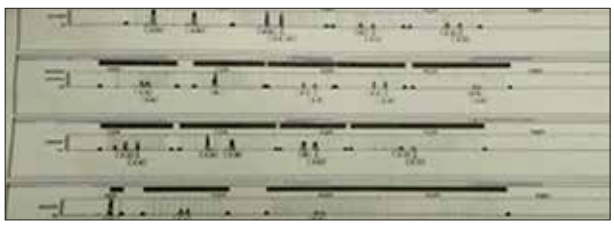

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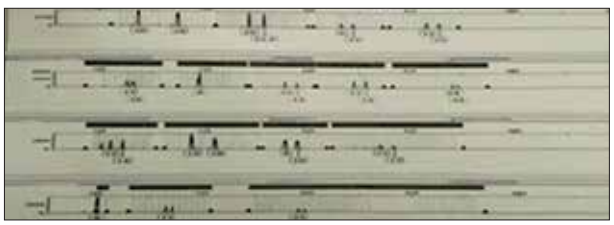

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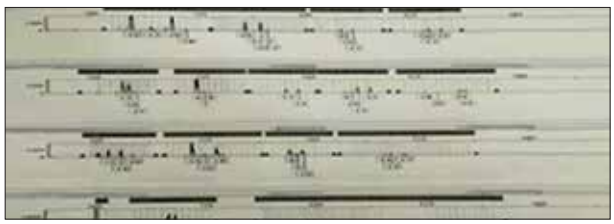

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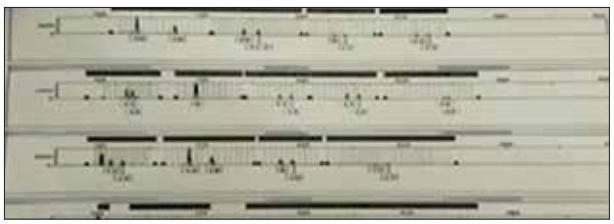

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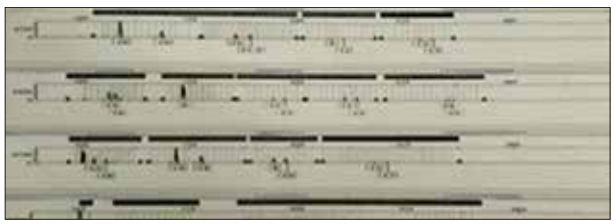

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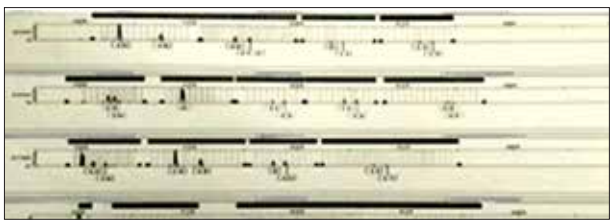
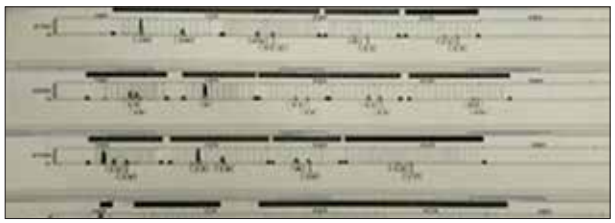


Table 7 DNA PEAKS. (Results: Identifiler Plus)

EXHIBIT 14



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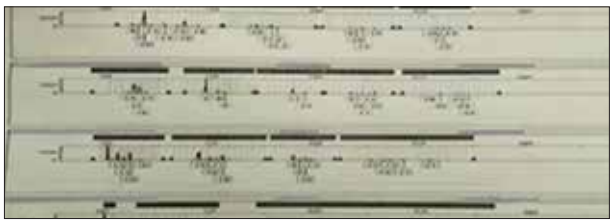

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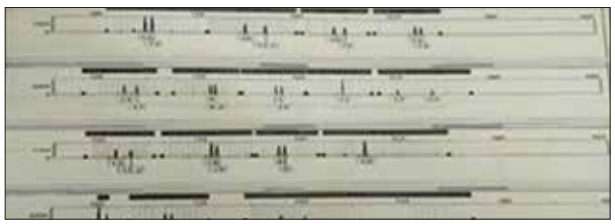

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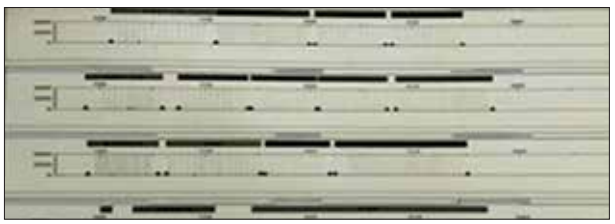

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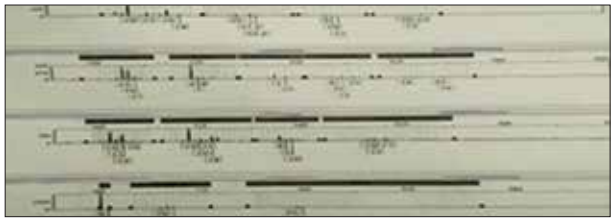

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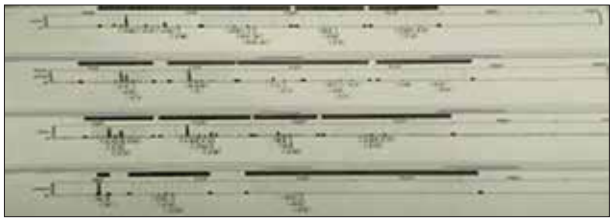

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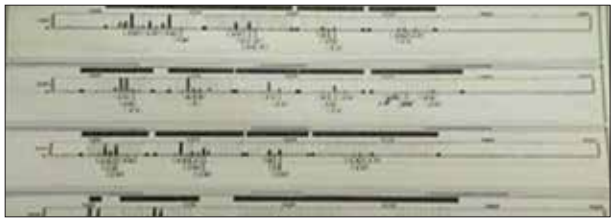
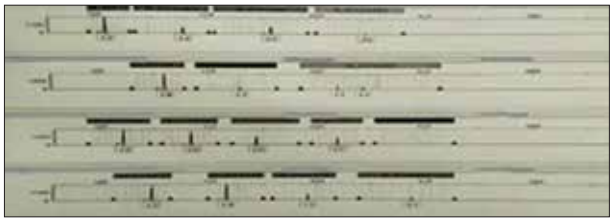


EXHIBIT 2Y



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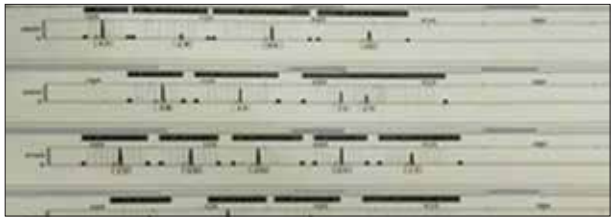


EXHIBIT 10Y

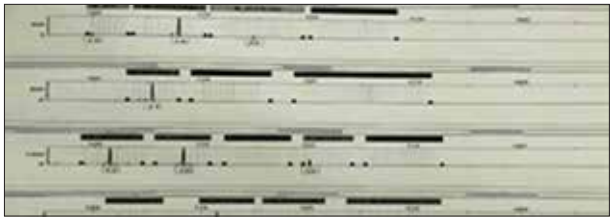


EXHIBIT 15Y

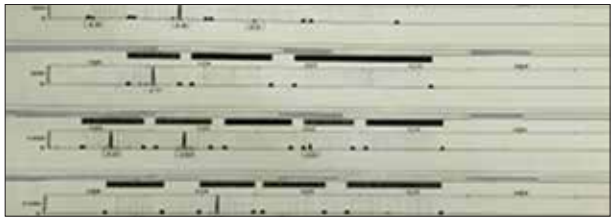
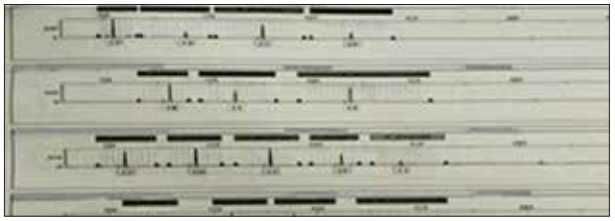


EXHIBIT 16Y



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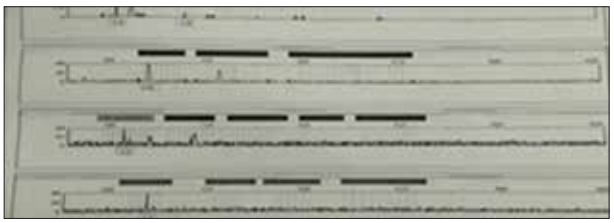

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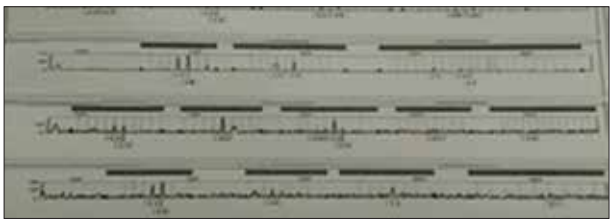


EXHIBIT 19Y

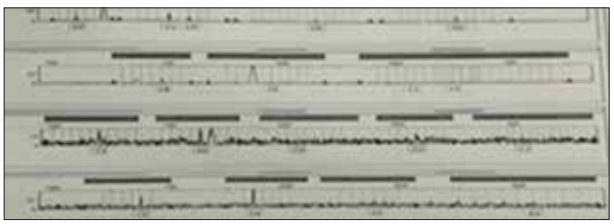
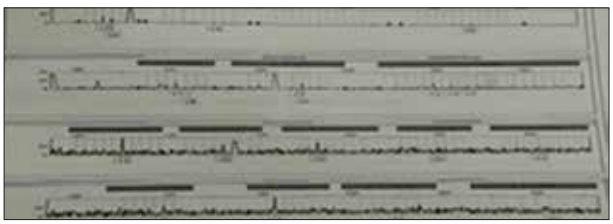


EXHIBIT 20Y



matched with the DNA profile of the exhibit- 7 and 16 (blood sample of accused A and blood sample of accused B.)

The DNA test performed on the exhibits provided is sufficient to conclude that the victim's DNA profile (of the deceased) matched with DNA profile of Suspect A and not with DNA profile of suspect B. So, this confirms that A is the accused.

Differentiation of Y gene via y-filer analysis in the case involving mixed profile. It would help solve the case, so as to match up to the profile of the accused. It would allow us to segregate from the mixed profile that occurs in the cases involving either sample degradation or the cases which involve two or more sample result crossover. IJFMP

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Conflict of Interest:

The author declares there is no conflict of interest in this project.

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