Naif Arab University for Security Sciences
Arab Journal of Forensic Sciences and Forensic Medicine
المجلة العربية لعلوم الأدلة الجنائية والطب الشرعي
https://journals.nauss.edu.sa/index.php/AJFSFM

A Forensic DNA Investigation of Incest and Murder Using GlobalFiler™ and YFiler Plus™ Kits

تحقيق جنائي في قضية مواقعة غير مشروعة وقتل باستخدام أطقم فحص DNA GlobalFiler™ و YFiler Plus™

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Received 30 Mar. 2026; Accepted 5 Apr. 2026; Available Online 10 Sep. 2026.

Abstract

Incest is a sexual activity between close biological relatives prohibited from getting married due to customs or law. This study examines a forensic case involving a brother and sister, where specialized forensic procedures were applied. DNA samples play a vital role in forensic investigations, where they can provide critical evidence for identifying perpetrators. Biological samples were taken from the victim, the suspect, and crime scene evidence (a pair of male pants and a female scarf). DNA extraction was performed using EZ1&2® DNA Investigator Kits, and quantified by the Quantifiler Trio DNA Quantification Kit. Amplification was performed using both the GlobalFilerTM PCR Amplification Kit and the YFiler plusTM PCR Amplification Kit. The PCR products were analysed using a SeqStudio Genetic Analyser, and the data were interpreted with GeneMapper ID-X software version 1.3.

DNA profiles were successfully obtained from the reference samples (victim and suspect) and from bloodstains on a pair of male pants, and the profiles matched the victim's profile using the GlobalFiler™ kit. The profile from the headscarf yielded a mixed profile which made it difficult to compare the suspect's profile. Therefore YFiler Plus™ kit was used to profile both the sample from the head scarf and suspect which yielded completely matching profiles.

Trace samples recovered from crime scenes are critical for resolving forensic cases.

Keywords: forensic sciences, forensic DNA, incest, GlobalFiler™ kit, YFiler Plus™ kit, SeqStudio Genetic Analyzer

المستخلص

تُعرف المواقعة غير المشروعة بأنها نشاط جنسي بين أقارب بيولوجيين مقربين يُمنعون من الزواج بموجب الأعراف أو القانون. وتتناول هذه الدراسة حالة جنائية تضمنت شقيقاً وشقيقة، حيث أُجريت التحليلات اللازمة ضمن سياق التحقيق الجنائي الفني. تلعب عينات الحمض النووي (DNA) دوراً حيوياً في التحقيقات الجنائية، حيث يمكنها تقديم أدلة حاسمة لتحديد الجناة.

تم أخذ عينات بيولوجية من الضحية، والمشتبه به، ومن أدلة مسرح الجريمة (سروال رجالي ووشاح نسائي). استُخلص الحمض النووي باستخدام أطقم (EZ1&2® DNA Investigator)، وتم تقدير كميته بواسطة طقم (Quantifiler Trio). كما أُجريت عملية التضخيم باستخدام طقم (Global-Filer™ PCR) وطقم (YFiler plus™ PCR). وحُللت نواتج تفاعل البلمرة المتسلسل (PCR) باستخدام جهاز التحليل الجيني (SeqStudio)، كما فُسرت البيانات باستخدام برنامج (GeneMapper ID-X) الإصدار 1.3.

تم الحصول بنجاح على البصمات الوراثية (DNA profiles) من العينات المرجعية (الضحية والمشتبه به) ومن بقع الدم الموجودة على السروال الرجالي، وقد تطابقت هذه البصمات مع بصمة الضحية باستخدام طقم (GlobalFil-er™). أما البصمة الوراثية المستخلصة من الوشاح (غطاء الرأس) فقد أعطت نمطاً مختلطاً (Mixed profile)، مما جعل من الصعب مقارنتها ببصمة المشتبه به؛ ولذلك استُخدم طقم (YFiler Plus™) لتحليل عينة الوشاح وعينة المشتبه به، مما أدى إلى الحصول على بصمات متطابقة تماماً.

تعد عينات الآثار (Trace samples) التي يتم رفعها من مسرح الجريمة حاسمة في حل القضايا الجنائية.

الكلمات المفتاحية: علوم الأدلة الجنائية، الحمض النووي الجنائي، المواقعة غير المشروعة، طقم GlobalFiler™، طقم YFiler Plus™، جهاز التحليل الجيني SeqStudio

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doi: 10.26735/DSVU7549

1. Introduction

Incest is usually defined as sexual activity between close family members within a family unit, which usually leads to legal and social consequences [1, 2]. Recovery of trace-level biological samples from the crime scene is critical, as this can be used for DNA-based human identification, which can lead to the identification of the suspect [3].

The Prostrate Specific Antigen (PSA)-Seratec SemiQuant and Seratec HemDirect tests, are chromatographic immunoassays which are presumptive tests to verify the widely used in forensic cases [5]. Although PSA is a major protein in seminal fluid, PSA is not exclusive to the prostate and can be detected in other female biological fluids, including serum, urine, amniotic fluid, and secretions from periurethral glands (Skene's glands), the breast, the ovaries, and the endometrium [6]. However, PSA demonstrates high sensitivity and is routinely used to detect semen in forensic casework [7, 8].

DNA-based methods have revolutionized forensic sciences and are highly sensitive. However, there are substantive issues regarding trace sample DNA profiling and mixture analysis, which can become complex and difficult to interpret [9]. Short Tandem Repeat (STR) markers have long been regarded as the gold standard for human identification in forensic genetics because they can be efficiently amplified by polymerase chain reaction (PCR) without significant differential amplification issues [10]. Multiple STR amplification kits, including GlobalFiler and Yfiler Plus, were employed in this study.

1.1 Case Study

This case study is based on crime scene examination of a teenage girl. It was reported in the police station located in Erbil, KRG, Iraq. At first, the case was reported by her family as the suicide of a teenage girl. A team of forensic experts was called for a crime scene examination. After examination, it was observed that the body had multiple injuries. The place of murder, i.e., the house, has been examined by the expert team and found a female black scarf and pair of male beige pants. The findings implicated the victim's brother as the primary suspect, confirming that the victim had been sexually assaulted and subsequently murdered.

2. Methodology

2.1. Samples collection

- The victim (a sexually abused and murdered)/ female/ blood.
- The suspect (perpetrator)/ Male/ Buccal swab.
- Pair of beige Pants (bloodstain)/ for male.
- Black Scarf (white stain)/ for female.

The blood sample was collected from victim at Erbil Medico-Legal Institute, while a buccal swab was collected from the suspect at the Criminal Evidence Directorate/ DNA laboratory. The evidence samples (pants and scarf) are first sent to the serology laboratory for identification (presumptive and confirmatory tests for blood and semen) and then to the DNA laboratory for advanced processing (DNA extraction, DNA concentration using real-time PCR, DNA amplification PCR, genetic analysis and DNA profile).

2.2. Serology Test

The evidence samples (pants and scarf) were screened by Crime-lite ML Pro (Foster+Freeman). Later, serological tests were done for both evidence samples using the Seratec Kit (PSA SemiQuant for human semen and HaemDirect test for human blood).

2.3. Extraction

DNA was extracted from the suspect's saliva, victim's blood, blood on pants and semen on scarf by EZ1&2® DNA investigator kit using two different protocols according to the manufacturer's instruction [11] and run by EZ2 connect Fx automated system.

Table 1- *DNA concentrations for pants and scarf*

Evidence	Serological Test	DNA Concentration	Y-Target
Pants	ve blood+	ng/ µL 0.19	0.0
Scarf	ve semen-	ng/ µL 5.67	ng/ µL 0.81

Table 2- *Genotypes of the victim, suspect, pants, and scarf using the GlobalFiler STR Kit.*

Loci	Victim	Suspect	Pants	Scarf
D3S1358	15,17	17,17	15,16,17	15,17
vWA	16,19	16,19	16,19	16,17,19
D16S539	8,9	8,9	8,9	8,9
CSF1PO	12,12	11,12	12,13	11,12,13
TPOX	8,11	11,11	8,11	8,11
Yindel	-	2	-	2
AMEL	XX	XY	XX	XX, XY
D8S1179	12,14	12,13	11,12,13,14	12,13,14
D21S11	30,32.2	30,30	33.2 ,30,31,32.2	30,32.2
D18S51	17,18	14,18	17,18,19	14,17,18
DYS391	-	10	-	10
D2S441	14,14	14,14	13,14	11,14
D19S433	14,15.2	14,14	14,15.2	14,15.2
TH01	6,9	6,7	6,7,9	6,7,9
FGA	23,23	23,23	22,23,24	23,23
D22S1045	15,16	15,15	15,16	15,16
D5S818	12,12	11,12	12,13	11,12
D13S317	10,12	11,12	10,12	10,11,12
D7S820	9,12	9,12	9,12	9,12
SE33	26,30.2	28.2,29.2	30.2,31.2 ,25.2,26,29.2	30.2 ,28.2,29.2 ,26 ,25.2

2.4. Quantification

Extracted DNA quantified by Quantifiler Trio DNA Quantification Kit (Applied Biosystems, 2018) with HID Real-Time PCR Analysis Software v1.3 employed by 7500 Real-Time PCR System according to the manufacturer's recommendations [12].

2.5. DNA Amplification

GlobalFilerTM PCR Amplification kit for (suspect's saliva, victim's blood, blood on pants and semen on scarf) and YFiler plus TM PCR Amplification kit for (semen on scarf and suspect's saliva) were used in accordance with the manufacture's instruction

[13, 14], and PCR was done on the Veriti™ 96 Well Thermal Cycler in 0.2 ml PCR tubes. The thermal cycle was set up according to the instructions provided by the kit.

2.6. Capillary Electrophoresis

The amplification products were prepared in 9.6 μL Hi-Di Formamide and 0.4 μL GeneScanTM 600 LIZ TM Size Standard v2.0 (Applied Biosystems, Foster City, CA, USA). The electrophoretic separation of the amplified PCR product was performed on the SeqStudio™ Genetic Analyser with SeqStudio™ Data Collection Software v1.2.5 following manufacturer's protocols [15]. GeneMapperTM ID-X software v1.3, is used to analyze and interpret data [16].

3. Result and Discussion

A small red stain was observed on the inner back of the male's pants, and a white stainwas found on the female's scarf. The HaemDirect test, performed using the Seratec Kit, produced a positive result for human blood on the red stain from the male pants. The PSA-SemiQuant test yielded a negative result for the white stain on the female scarf. Quantitative analysis determined a bloodstain concentration of 0.19 ng/μL with no detectable Y-target (0.0), indicating an absence of male DNA in the bloodstain sample. In contrast, the scarf sample exhibited a total DNA concentration of 5.67 ng/μL, exceeding Seratec's internal standard threshold of 4 ng/μL [5], and a Y-target value of 0.81 ng/μL, indicating the presence of male-specific DNA. Similar findings were reported by Hochmeister et al. 1999, These results indicated that the scarf sample likely contained male biological material, consistent with semen [Table 1].

DNA profiles were generated for all extracted samples using the GlobalFiler Kit, revealing mixed DNA profiles for both evidence samples. These profiles were subsequently compared. Table 2 presents a comparison of allelic numbers among the victim, the suspect, the male pants, and the female scarf using the GlobalFiler Kit, with the following results:

- DNA profiles were obtained for the reference samples.

Table 3- *Genotypes of the suspect and scarf using the Yfiler Plus loci STR Kit.*

Loci	Suspect	Scarf
DYS576	16	16
DYS389I	12	12
DYS635	22	22
DYS389II	28	28,27
DYS627	21	21
DYS460	11	11
DYS458	16	16
DYS19	15	15
YGATAH4	11	11,12
DYS448	21	21
DYS91	10	10
DYS456	16	16
DYS390	23	23
DYS438	10	10
DYS392	11	11
DYS518	36	36
DYS570	17	17
DYS437	15	15
DYS385	12,15	12,15
DYS449	34	34
DYS393	14	14
DYS439	11	11
DYS481	21	21
DYF387S1	37,40	27,40
DYS533	9	9

- Mixed DNA profiles were obtained for the male pants and female scarf, containing genetic markers from both the victim and the suspect.
- The bloodstain on the male's pants yielded a mixed DNA profile that matches the victim's DNA profile.
- A mixed DNA profile was obtained for the white stain on the female scarf, complicating the comparison between the DNA profiles of the victim, the suspect, and the evidence sample.
- The white stain on the female scarf yielded a mixed DNA profile that matched suspect's DNA profile.

Additionally, the scarf and the suspect's buccal swab were analysed using the Yfiler Plus Kit to assess potential involvement. The comparison of allelic numbers is presented in Table 3.

4. Conclusion

DNA evidence is a powerful tool in forensic investigations. This is especially useful when crime scene samples contain genetic material from multiple individuals. It is essential to analyze these mixed DNA profiles to identify the contributors. Quantifiler Trio Kit strongly supports the presence of seminal fluid from a male contributor, even if Seratec gave a false negative. In forensic reporting, this would justify further STR profiling and inclusion in evidentiary analysis. Short Tandem Repeat (STR) analysis is particularly effective for matching DNA profiles. STR plays a crucial role in solving criminal cases.

Source of Funding

The author received no financial support for the research, authorship or publication of this paper.

Conflicts of Interest

The authors declare no conflicts of interest.

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