Forensic Science International 369 (2025) 112417

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

# Exploring the techniques and challenges for recovering human touch DNA from white rhino (*Ceratotherium simum*) to combat poaching

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ARTICLE INFO

*Keywords:*
DNA profiling
Touch DNA
Rhino poaching
Wildlife Forensics
Swabbing
Tape Lifting

ABSTRACT

The trade in white rhinoceros (*Ceratotherium simum*) horns poses a significant threat to the survival of this species and additional investigative tools for rhino poaching cases are essentially required to address this challenge. This study explored additional techniques and challenges for recovering human touch DNA from rhino. Experiments depositing touch DNA on rhino during dehorning projects were carried out. Fifty-five human touch DNA samples were gathered from target regions on 15 rhinos (ears, head, legs, horn and back). Recovery of touch DNA using swabs with different tips and compositions as well as a tape lifting method were tested. DNA profiling was performed using the PowerPlex$^{R}$ ESI 16 kit (Promega). From the readable profiles (n = 35), 34 % partial and 3 % full profiles were reportable and thus it would have been possible to use these DNA profiles to link suspects to criminal activities. The study demonstrated that it is possible to obtain reportable human DNA profiles from rhinos and that the technique should be considered as an additional forensic investigative tool to be used in wildlife crimes.

## 1. Introduction

Wildlife and human forensics share a common objective to establish a triangular connection between the suspect, "victim," and the crime scene. This connection relies on analysing physical evidence recovered from the crime scene. The progress in human forensic technology serves as an excellent foundation to advance wildlife investigation [1].

Rhinoceros were once abundant across Africa and Asia, but despite intensive conservation efforts, the poaching of this iconic species is escalating significantly, bringing the remaining rhino populations dangerously close to extinction. This dire situation led to the declaration of the Western black rhino's extinction by the International Union for Conservation of Nature (IUCN) in 2011. Poaching was identified as the primary cause. Currently, there are five surviving rhino species: two African species, *Ceratotherium simum* (white rhino) and *Diceros bicornis* (black rhino), and three Asian species, *Rhinoceros unicornis* (Indian rhino), *Rhinoceros sondaicus* (Javan rhino), and *Dicerorhinus sumatrenis* (Sumatran rhino) [2].

Lunstrum *et al.* (2020) [3] conducted a field study in the Mozambican borderlands, where many poacher recruits originate, to explore the motivations behind individuals choosing to engage in poaching. This overview of drivers highlighted the multi-layered nature of the supply-side factors behind rhino poaching. There is a complex network of actors, encompassing government and park corruption, the absence of political will, and the greediness of criminal syndicates orchestrating the trade. A lucrative black market exists stemming from the rising demand from Southeast Asian countries, particularly Vietnam and China. Rhino horns are continuously smuggled in and out of the continent and some of the proceeds of wildlife crimes are used to fund rebel wars [4].

Poaching in South Africa showed a clear peak during 2013–2017 with an average of 1000 rhinos killed annually [5]. In 2021, the South African Department of Forest Fisheries and Environmental Affairs (DFFEA) released official poaching statistics and noted a decline in the number of poached rhinos compared to pre-COVID in 2019. Four-hundred and fifty-one rhinos were poached in 2021, with 327 poached on government reserves, predominantly in Kruger National Park [5–7]. Despite the majority of poached rhinos being on government-owned land, there is also a noticeable rise in poaching on private reserves [5–7]. The DFFEA reported a decrease in rhino poaching of 394 and 451 during 2020 and 2021, respectively. Rhino deaths amounted to 229 during January – June 2024 [4]. Due to various interventions by the South African government, there has been a slight decline in the number of poaching cases. However, the numbers remain high with an average of more than 600 rhinos lost to illegal hunting per

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https://doi.org/10.1016/j.forsciint.2025.112417
Received 20 November 2024; Received in revised form 16 January 2025; Accepted 23 February 2025
Available online 25 February 2025

year since 2007.

The South African government combats rhino poaching strategically. The Minister of DFFEA initiated collaboration with key entities, including the South African Police Service (SAPS), the South African National Defence Force (SANDF), and the South African National Parks (SANParks). The SAPS has declared the illegal killing of rhinos and the illicit trade in rhino horn a priority crime, receiving increased attention. Positive outcomes have been achieved from the National Environmental Compliance and Enforcement Report 2019–2022 on the number of arrests over recent years [8].

Poaching has continued in such a way that wildlife forensic DNA techniques should be tailored to focus on rhinos cases. Application of DNA-based techniques involves both the poached animal and its associated products, and the poacher. For the first of these focus areas, the Rhino DNA Index System (RhODIS®) project was created by researchers at the Veterinary Genetics Laboratory of the University of Pretoria to track confiscated rhino products. The project has two components: firstly, owners of rhinos across the country regularly contribute samples of their animals for genotyping using a set of microsatellite loci. The unique DNA profile of an individual rhino, with information on its origin, is then added to the database. Then, when a rhino product is confiscated, the sample can be genotyped, and the result compared to the database. If the rhino in question has previously been genotyped, the animal profile will be on the database and a match can be made. A RhODIS® Kit for sampling has also been developed in partnership with the Forensics Science Laboratory of the South African Police Services, to aid with correct sample collection.

In terms of human DNA, updated techniques make human DNA profiles more readily obtainable from compromised samples. Profiling in cases involving wildlife has, however, not benefited from the recent advances in human DNA profiling technology because of a lack of collaboration between the two disciplines. The use of molecular techniques has become vital in the investigation of wildlife cases. Due to the remoteness of many wildlife crimes, there is often a lack of other evidence such as eyewitnesses [9,10] and thus biological evidence is even more important under these circumstances. The improvement of the recovery and profiling of human touch DNA from wildlife crime scenes will add a new approach to the fight against poaching. In the context of wildlife forensics the use of DNA from rhinos is well established whereas the human DNA is not.

The collection of biological samples from wildlife crime scenes through various methods, including traditional forensic approaches to collecting human touch DNA, has become crucial. Touch DNA can be left on surfaces through contact, and despite low concentrations, it can still provide individual short tandem repeat genetic profiles. These profiles connect suspects to crime scenes and aid investigations. The number of human forensic cases that resolved solely on touch DNA evidence has experienced a substantial and remarkable increase, ranging from property crimes, murder cases and business robberies [11]. Recently, McLeish et al. [14] conducted a study on the recovery of human touch DNA from outdoor environments. Understanding the source of touch DNA will increase the weight of DNA evidence obtained in such circumstances [12]. The Locard exchange principle holds that with contact between two items, there will be an exchange of material. This constitutes the realm of touch DNA science [13]. While Locard principle forms the foundation of forensic science, it is worth acknowledging that factors such as environmental conditions, the nature of interaction, the surface, and the duration of contact can affect the transfer and persistence of evidence. The method and samples used along with the software applied in human genetics demonstrate the robustness and reliability of results when using the correct forensic procedures. Therefore, this study explored an alternative approach to investigating rhino poaching cases by retrieving human touch DNA from the carcasses of poached rhinos. Additionally, potential locations on body of the rhino where poachers could have left touch DNA were pinpointed and evaluated as a source for human touch DNA.

## 2. Materials and methods

### 2.1. DNA transfer

After receiving ethical approval and signed consent from the participants, samples were collected during a normal dehorning process at two game reserves. During the process, veterinarians and game rangers were present to attend to the health and safety of the animal. The veterinarians and game rangers touched the rhinos as expected a poacher would have during an illegal poaching incident (see Fig. 1), in the process of removing the horns. Observation of where the veterinarians and game rangers touched/grabbed the rhino was made and these areas were considered the target regions for collection. The target regions from which the rhinos sampled potential human touch DNA included both ears, head, legs, horn, and back. Except for the smooth horn, the surface from all other target regions of the rhino is dense and thick with no hair and in some areas, it is covered with mud.

The participants were asked to refrain from hand washing activities the entire morning before the start of the dehorning procedure. For the experiment to simulate a crime scene, No experiment was conducted to determine the participants' shedder status. Buccal swabs were collected as reference samples from veterinarians who were part of the dehorning process. The experiment was carried out during the winter season, with temperatures ranging from −2°C to 26°C. The interval between deposition and collection was 15–20 minutes, determined by the time required to complete the dehorning of the rhino. The whole rhino dehorning procedure, from when the dart goes in, to administering the reversal drug, takes approximately 30 minutes.

### 2.2. Sample collection

A total of 55 possible sources of human touch DNA samples from 10 participants were gathered from the target regions on 15 rhinos. This included five rhinos from a game reserve in Gqeberha (formerly Port Elizabeth) in the Eastern Cape and 10 rhinos from a game farm in the Bloemfontein area in the Free State.

Conventional tape lifts as well as Copan regular FLOQswab® (Copan Diagnostics, Inc. USA, supplied by Lasec®, South Africa) and Dacron polyester swabs (Thermo Fisher Scientific Inc. USA) were used to collect potential sources of human touch DNA. Samples collected using tape lifting method were modified on the rhinos in Bloemfontein by cutting them into small strips before collection. This was performed to reduce potential source of human touch DNA retention on the tape. Similarly, a proof-of-concept study by Mcleish *et al.* (2018) [14] suggested that the mini-taping method was better for DNA recovery than the double-swabbing method as higher quantities of DNA were recovered.

Sterile water was used to wet the swabs. Prescribed standard forensic regulations for the proper handling of samples were applied. Throughout the handling of the samples, appropriate protective equipment was worn, including a disposable jumpsuit, hair cover, shoe covers, a mask, and latex gloves. Collection and packaging were also handled following the procedures applied by the South African Police Service Forensic Science laboratory.

### 2.3. DNA processing

DNA was eluted from the swabs following the procedure described by Casework Direct Rapid Processing of Swabs protocol (Casework Direct Kit, Promega Corp. USA). DNA extraction was performed according to the manufacturer's instructions [15].

After extraction, the lysates originating from the legs and ears of the same rhino were combined to increase the DNA concentration prior to PCR [10]. The combination of the lysates resulted in a total sample size of 35. A quantification step was performed to verify the quantity and quality of the isolated DNA for downstream processes. The DNA concentration was determined using a NanoDrop Lite spectrophotometer

**Fig. 1.** Veterinary grabbing the neck and the forehead of a rhino during a dehorning procedure in the Gqeberha area.

**Fig. 2.** Forensic analyst swabbing the back of a rhino during a dehorning procedure in the Gqeberha area.

(Thermo Fisher Scientific, USA) by measuring absorbance at 260 nm.

Amplification of the human touch DNA samples was performed with a PowerPlex$^{R}$ ESI 16 kit supplied by Promega (Promega Corp. USA), The kit allowed co-amplification and detection of sixteen loci, which included D18S51, D21S11, TH01, D3S1358, Amelogenin, D16S539, D2S1338, D1S1656, D10S1248, FGA, D8S1179, vWA, D22S1045, D19S433, D12S391, and D2S441. Each tube contained 5.25 µl amplification grade water; 2.5 µl PowerPlex$^{R}$ ESI 5X Master Mix; 1.25 µl PowerPlex$^{R}$ ESI 16 10X Primer Pair Mix; X 2.5 µl AmpSolution™ reagent; and 2 µl of sample DNA extract to make a total volume of 13.5 µl. Samples were amplified on a GeneAmp$^{R}$ 9700 thermal cycler by (Applied Biosystems™) using the following conditions: 96 °C for 2 minutes, followed by 26 cycles of 94 °C for 30 seconds, 59 °C for 2 minutes, 72 °C for 90 seconds; and a final extension at 60 °C for 45 minutes. The standard protocol was modified by increasing the number of cycles to 30 cycles, to improve DNA detection sensitivity. This increase enabled DNA detection with samples containing less than 100 *pg* of DNA template. The samples obtained along with reference buccal swabs, underwent processing for DNA profiling on a 3500 Genetic Analyzer (Applied Biosystems™, USA) using the PowerPlex$^{R}$ ESI 16 kit supplied by Promega. Interpretation of the results was done using the Genemapper IDX 1.5 version (Applied Biosystems™, USA).

Genemapper IDx 1.5 v Software allows an acceptance value of a minimum of 60 rfu for a heterozygous allele peak and one of a minimum value of 130 rfu for a homozygous allele peak. Data of the amplified loci were summarised as follows: full profile if all 16 loci were amplified; partial profile if any of the loci were absent or fell below the minimum values with a number of amplified profiles indicated; not enough DNA if only less than seven loci were above the cut-off value; mixture profile if there were more than two peaks per locus; and no DNA.

## 3. Results and discussion

All 35 samples yielded sufficient DNA quantity and quality. There were no notable differences in DNA quantity and quality regarding the sampling regions or collection methods. This suggested that the DNA extraction was successful across all samples, with some exhibiting exceptionally high concentrations. All 35 samples were used for further DNA processing.

Examples of electropherograms generated by GeneMapper IDX software data analysis are indicated in Figs. 3 and 4.

The quality and quantity of the peaks on the electropherograms are used to determine the quality of the DNA profile of a specific DNA sample, however for a peak to be seen as a true and reliable peak, it must meet certain minimum acceptance values.

Table 1 Potential sources of DNA collection areas, sampling methods and Promega ESI 16 profiles analysed on Genemapper IDX 1.5 v.

According to the National Forensic DNA Database (NFDD) policies of South Africa, once a profile has been generated, interpreted and reported, it is committed to the NFDD through software called STRlab™ [16]. Table 1 displays the amplified profiles, highlighting their potential in linking or excluding a suspect. Full profiles are amplified at all the targeted loci and have no contamination, which makes them better investigative leads. Negative controls that returned blank profiles with

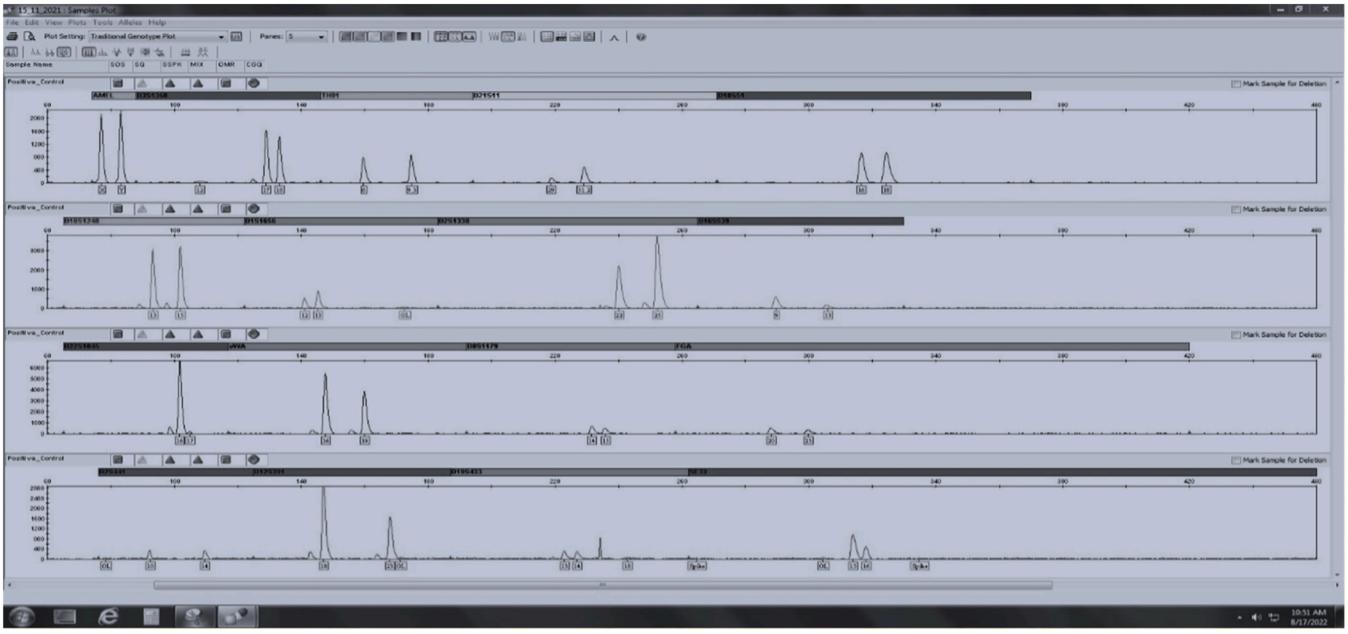

**Fig. 3.** An Electropherogram of Rhino 1, lifted from the horn using a Lasec Copan FLOQswab®. This profile is notable for being a full DNA profile.


**Fig. 4.** An Electropherogram of Rhino 13, lifted from the ear using a tape lift. This profile is notable for being a full DNA profile but a mixture of two or more persons.

no peaks, indicated that there was no contamination during the processing of the samples. One reference sample was contaminated. Since there was no duplication during the sampling of the references, the profiles could not be traced back to an individual. Due to the multitude of individuals participating in the rhino de-horning project, maintaining control over touch locations proved challenging. Consequently, full traceability of the other reference samples in comparison to the amplified sample was difficult, leading to sample exclusion.

Table 2 summarises the percentage of readable profiles from Gene Mapper 1.5 IDX software. The mixture of partial profiles yielded a small proportion of 1 out of 35 (3 %) of outcomes among the samples. Unfortunately, this outcome lacked interpretability and thus, is not suitable for use in a court of law to either incriminate or exonerate a suspect. However, 1 out of 35 (3 %) of full profiles was a positive outcome with significant implications because the profiles could be integrated into the Forensic National DNA Database. The integration of recovered DNA allows for automatic comparison against other profiles, potentially revealing whether the suspect has a history of previous crimes stored in the database. This approach is effective when criminals re-offend, providing valuable investigative leads for law enforcement.

Full profile with mixture comprised 3 out of 35 (9 %) of the samples. These profiles exhibited observations in all alleles and included mixtures. In touch DNA samples, it is a common occurrence to encounter mixtures, particularly when multiple individuals have touched the same area prior to sampling. The challenge arises when profiles from recent touch deposits mix with background profiles, leading to confounding interpretations. A similar study conducted by Tobe *et al.* [10] indicated that mixture profiles can be a limitation, but the suspect can still be identified. This complexity is heightened in situations where more than two suspects have touched the same area, resulting in a full profile mixture, which becomes especially difficult to interpret. A notable finding was that 5 out of 35 (14 %) of the DNA from the samples did not

**Table 1**
Potential sources of touch DNA collection areas, sampling methods and Promega ESI 16 profiles analysed on Genemapper IDX 1.5 v.

| Rhino and control reference numbers | Area of collection | Sampling method* | Profile |
|---|---|---|---|
| 1 | Ears (2) | Lasec (2 swabs combined) | PP8MX |
| | Head | Lasec | PP12 |
| | Legs (4) | Lasec (4 swabs combined) | NED |
| | Horn | Lasec | NED |
| | Back | Lasec | No DNA |
| 2 | Ears (2) | Lasec (2 swabs combined) | PP13 |
| | Head | Lasec | PP14 |
| | Legs (4) | Lasec (4 swabs combined) | PP6 |
| | Horn | Lasec | NED |
| | Back | Lasec | PP10 |
| 3 | Ears (2) | Lasec (2 swabs combined) | NED |
| 3 | Head | Lasec | PP7 |
| | Legs (4) | Lasec (4 swabs combined) | No DNA |
| | Horn | Lasec | NED |
| | Back | Lasec | PP8 |
| 4 | Ears (2) | Lasec (2 swabs combined) | No DNA |
| | Head | Lasec | NED |
| | Legs (4) | Lasec (4 swabs combined) | NED |
| | Horn | Lasec | PP6 |
| | Back | Lasec | No DNA |
| 5 | Ears (2) | Lasec (2 swabs combined) | PP7 |
| | Head | Lasec | NED |
| | Legs (4) | Lasec (4 swabs combined) | PP9 |
| | Horn | Lasec | NED |
| | Back | Lasec | PP13 |
| 6 | Ear (1) | Lasec | PP10 |
| 7 | Head | Lasec | NED |
| 8 | Leg (1) | Lasec | NED |
| 9 | Horn | Lasec | FP |
| 10 | Back | Lasec | NED |
| 11 | Back | Lasec | No DNA |
| 12 | Back | Lasec | NED |
| 13 | Ear (1) | Tape lift | FPMX |
| 14 | Ear (1) | Tape lift | FPMX |
| 15 | Back | Lasec | FPMX |
| Reference 1 | Buccal | Dacron | Contaminated MX |
| Reference 2 | Buccal | Dacron | FP |
| Reference 3 | Buccal | Dacron | PP15 |
| Reference 4 | Buccal | Dacron | FP-OL |

PP: partial profile; FP: full profile; MX: mixtures; NED: not enough DNA; OL: off ladder alleles; values indicate the numbers of loci; Lasec: Copan FLOQswab® (regular); Dacron: Dacron Polyester Swab

**Table 2**
Percentage readable profiles from Gene Mapper 1.5 IDX software.

| Profile names | Number of samples (n = 35) | Profiles (%) | Submittable for court proceedings |
|---|---|---|---|
| Partial profiles with mixtures (PPMX) | 1 | 3 | No |
| Full profile (FP) | 1 | 3 | Yes |
| Full profile with mixtures (FPMX) | 3 | 9 | Yes |
| No DNA | 5 | 14 | No |
| Not enough DNA (NED) | 13 | 37 | No |
| Partial profiles (PP) | 12 | 34 | Yes: all with 7 and more readable partial profiles |

register on the electropherogram, despite favourable outcomes during quantification. The absence of DNA during these runs was attributed to the low levels of DNA obtained during the sampling and processing of trace DNA. Insufficient DNA was observed in 13 out of 35 (37 %) of the samples. This challenge is inherent when sampling sources of touch DNA, where deposits typically contain smaller quantities compared to other biological materials. Compounding the issue is that sources of touch DNA are not visible to the naked eye, making target selection reliant on inference and past sampling experience with similar objects. The samples with inadequate DNA manifested as small peaks, registering less than 60 rfu's. While indicative of the presence of DNA, the quantity was insufficient to generate a readable profile, rendering these samples unsuitable for comparison with a suspect's DNA. A significant finding was the partial profiles in 12 out of 35 (34 %) of the entire sample set. These profiles, being readable and admissible in a court of law, have the potential to either link or exonerate individuals [17]. The range of 7–15 readable partial profiles is particularly promising in the field of wildlife crimes, given the challenges posed by the remote locations and harsh environmental conditions where rhinos are often found. When collected meticulously and processed with care, this minimal yet valuable evidence can effectively link perpetrators to wildlife crimes.

Touch DNA was obtained from external environments with swabs as well as the tape lifting method, with tape lifting giving the best results. Full profile mixtures were observed with the modified tape lifting technique (cutting tape into smaller pieces). The full profile mixtures were probably observed because the tapes were applied randomly in a certain area, which collected the underlying profiles as well. The swabbing collection method can also be used, giving most of the partial profiles, which can be interpreted and accepted in a court of law. The method is widely used by the forensic fraternity. The results align with studies by Tobe et al. (2013) and Mcleish et al. (2018), indicating that DNA recovery from animal carcasses and illegal traps is advisable when reporting the cases. The prerequisite procedures, encompassing collection, packaging, transportation, and analysis, along with the meticulous maintenance of the chain of custody and adherence to forensic regulation requirements of short tandem repeat profiles, are paramount in ensuring the credibility of profiles in legal proceedings [18].

## 4. Conclusion

The 34 % partial and 3 % of full profiles obtained were reportable and could potentially be used to link suspects to criminal activities. The technique to recover and profile human touch DNA from rhino carcasses was therefore practical to use in rhino poaching cases.

There are benefits to collect human touch DNA instead of rhino DNA to address wildlife crimes. No specialist protocols are required as the techniques are used daily at all crime scenes and the samples are analysed in human forensic science laboratories. The DNA profiles recovered can be cross-referenced with an established National Forensic DNA Database, aligning with global forensic standards and operating procedures. This scientifically sound approach is widely embraced in the legal community. It optimizes resources by relying on experienced forensic scientists who are already active in the field. Furthermore, the evidence recovery process can be entrusted to wildlife officers or veterinarians with proper training from forensic laboratory awareness trainers, the police, or non-governmental organisations. As specialist non-human DNA is not required, the cost is significantly reduced.

## 5. Recommendations

Further research is needed to establish the practicality of analysing evidence on poaching scenes based on the time elapsed since the crime. The degradation of touch DNA due to environmental conditions is a known factor, and Mcleish *et al.* (2018) found that the time of exposure for recoverable profiles varies. These authors reported that DNA can be recovered in at least 10 days for traps and 1 day for a bird carcass.

However, more investigation is necessary to pinpoint these durations accurately.

Given adequate resources, equipping crime scene investigators with light sources like Polyflaire flights to identify areas containing epithelial cells for swabbing or tape lifting during collection is recommended. It should be noted, though, that light sources may not be the most cost-effective option. Applying the Nucleic acid stain Diamond Dye to cellular material after collecting it on swabs and surfaces is a valuable technique. The use of Diamond Dye enables a focused approach to cell collection, ensuring that swabs undergoing DNA processing contain cellular material. This targeted method with DD ensures the collection of cells from areas where touch DNA still persists.

Optimizing the collection of touch DNA involves using various swabs tailored to different surfaces—considering factors such as porosity. While this approach incurs additional costs, it is particularly applicable to well-established laboratories. Further research is essential to comprehensively understand the interactions involved, to facilitate the development of an optimal recovery strategy.

To enhance the yield of DNA profiles, consider employing the direct PCR method. This approach has proven effective in amplifying DNA from various forensically relevant and challenging items, including touch DNA from fabrics and improvised explosive devices. Some laboratories have embraced direct PCR for sample amplification, acknowledging its utility in maximizing DNA profiles.

Providing wildlife rangers with training on securing crime scenes and collecting forensic evidence is crucial. This training should emphasize the importance of preserving the integrity of the scene without tampering and ensuring the proper chain of custody for the gathered evidence is maintained.

## CRediT authorship contribution statement

**M.M. Manqana**: Writing – original draft, **L. Wessels**: Supervision, **J. P. Grobler**: Supervision, **K. Ehlers**: Supervision.

## Ethical clearance

Ethical approval was obtained from the Animal Research Ethics Committee of the University of the Free State, Bloemfontein, South Africa, with reference number: **UFS-HSD2017/0978.**

## Declaration of Competing Interest

The authors declare that no conflict of interest regarding the publication of this manuscript. The study was partially funded by the University of the Free State, with selected consumables supplied by Promega.

## Acknowledgment

Our sincere thanks to private nature reserves that allowed sampling on the rhinos and to Stephan Kutranov (Promega, UK) for advice on sample collection and kit provision.

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