Forensic Science International: Genetics 7 (2013) 428–433

Contents lists available at SciVerse ScienceDirect

Forensic Science International: Genetics

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

Short communication

# Extraction of nuclear DNA from rhinoceros horn and characterization of DNA profiling systems for white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhinoceros

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ARTICLE INFO

*Article history:*
Received 10 March 2013
Received in revised form 10 April 2013
Accepted 12 April 2013

*Keywords:*
African rhinoceros
Microsatellite
Genotyping
Short tandem repeat analysis

ABSTRACT

Rhinoceros horn is now worth more, per unit weight, than gold, diamonds, or cocaine. Rhinoceros horn has been used in traditional Asian medicine as a presumed cure for a wide range of ailments. Rhinoceros poaching in South Africa has, on average, more than doubled each year over the past 5 years with the rapid economic growth in east and southeast Asia being assumed to be the primary factor driving the increased demand for horn. Here we report on the characterization of methods for genomic DNA extraction from rhinoceros horn and on DNA profiling systems for white (*Ceratotherium simum*) and black (*Diceros bicornis*) rhinoceros. The DNA profiling system described includes 22 short tandem repeat (STR), or microsatellite, markers and a gender marker (ZF1), which have been used previously in various studies on rhinoceros. Using a θ value of 0.1, a conservative estimate of random match probability in 5 white rhinoceros ranged from 1:7.3 $\times$ $10^6$ to 1:3.0 $\times$ $10^8$. Given that the total population of white rhinoceros is approximately 20,000 such random match probabilities indicate that the genotyping system described provides data which can be used for evidentiary purposes. Furthermore, the methods are appropriate for use in investigations involving trace amounts of rhinoceros horn and the matching of profiles obtained from seized rhinoceros horn with material collected from live animals or poached carcasses.

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## 1. Introduction

The analytical techniques capable of providing DNA evidence to assist in conservation law enforcement have developed in parallel to human forensic genetics. Short tandem repeat (STR) markers are commonly used to establish a link between an evidence sample and an individual through a unique DNA profile consisting of a subset of these markers [1,2]. Such systems are used in human forensics and are being applied increasingly to criminal investigations involving domestic [3,4] and wild animals [1,2,5].

Illegal trade in rhinoceros horn poses a serious and increasing threat to the long-term survival of the rhinoceros [6]. Rhinoceros horn is used in traditional Asian medicine (TAM) in South-East Asia and as dagger handles in mainly Yemen [6]. The demand for horn has escalated as a result of the economic boom in South-East Asia and endemic poverty in the habitat of the rhinoceros [7]. The structure of rhinoceros horn has been described as an epidermal derivative, consisting of keratinized tubules of cells connected with a matrix of melanin and calcium [8]. It continues to grow at a rate of 5–6 cm/year and can be harvested from the live animal [9]. Techniques have been described for the extraction of mitochondrial DNA from rhinoceros horn which then allows for the subsequent confirmation of the species of origin [10]. To date, methods to extract genomic DNA from rhinoceros horn and marker systems for the individual identification of rhinoceros from their horns have not been described.

The objective of this study was to develop and characterize a method to extract nuclear DNA from rhinoceros horn of sufficient quality and quantity to allow the amplification of STRs producing a DNA profile capable of uniquely identifying an individual rhinoceros. This, in turn, could provide a mechanism for the matching of a DNA profile obtained from seized rhinoceros horn

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http://dx.doi.org/10.1016/j.fsigen.2013.04.003

with that obtained from other samples collected from the same animal when it was alive or when samples were collected following poaching.

## 2. Materials and methods

### 2.1. Sample materials

Matching blood and horn samples were obtained from 6 white rhinoceros during routine capture operations in the Kruger National Park as part of a project that was approved by the Animal Ethics Committees of SANParks and the University of Pretoria. Blood was collected into vacutainer tubes with EDTA (Ethylenediaminetetraacetic acid) (BD Vacutainer®) using 20 gauge needles from the ear vein. Horn samples were collected from the same animals from the tip, middle or base of the horn by excision of a piece of horn approximately 2 $cm^3$ using a saw. These pieces of horn weighed between 2.1 g and 4.8 g. In addition, 5 horn and hair samples collected from animals during routine identification and translocation procedures in Mpumalanga Province, South Africa and were submitted to the Veterinary Genetics Laboratory for routine genotyping and their DNA profiles were compared. Two horns, one from a black rhinoceros (*Diceros bicornis minor*) and one from a southern white rhinoceros (*Ceratotherium simum simum*), that were donated by the Ezemvelo KZN Wildlife from two rhinoceros of approximately the same age and size to the Forensic Science Laboratory of the South African Police Service were used to investigate the variation in DNA extracts from different parts of the horn. Samples submitted to the Veterinary Genetics Laboratory for routine genotyping were used for the further characterization of DNA profiling systems for white and black rhinoceros.

### 2.2. DNA extraction

Approximately 200 mg of rhinoceros horn was obtained by drilling into the horn with either a new drill bit or a drill bit decontaminated by washing with soap followed by soaking in an undiluted solution of commercial household bleach (Jik/Sodium Hypochlorite) and rinsed with deionised water and allowed to dry before using on a new sample and the drill shavings transferred to a labelled plastic tube (4 ml screw cap tube, J-Plast). The horn shavings were homogenized to a fine powder using a tissue homogenizer (Omni International TH). Approximately 20 mg of the powder was transferred to a labelled eppendorf tube. A total of 500 μl of Prepfiler™ lysis buffer (Life Technologies) and 5 μl of DTT (Dithiothreitol, Sigma) was added to each tube. The tubes were placed on a heated shaker (Vortemp 56, Labnet) for 1 h at 70 °C. Tubes were centrifuged (M-240 Boeco Germany) at 10,000 rpm for 2 min. A total of 300 μl of supernatant was transferred to individual wells in a Kingfisher 96 Magnetic Particle Processor (Thermo Scientific) deepwell plate and 15 μl of Prepfiler™ Magnetic Beads (Life Technologies) were added to each well. The plate was vortexed at 1000 rpm for 10 s on a shaker and 180 μl of Isopropanol (Sigma) was added to each well and vortexed again at 1000 rpm for 10 s. The DNA extraction was completed on a Kingfisher 96 Magnetic Particle Processor (Thermo Scientific) according to the Prepfiler™ V2 protocol (supplied by Applied Biosystems). Briefly, DNA binding was performed for 10 min followed by 3 washes using 300 μl Prepfiler™ Wash Solution per wash, 5 min drying at room temperature and elution into 75 μl of elution buffer performed at 70 °C. Blood was extracted using 50 μl of whole blood as described in the Prepfiler™ protocol (Life Technologies). Further processing was performed on the Kingfisher 96 Magnetic Particle Processor (Thermo Scientific) as described above. The hair was extracted using NaOH (sodium hydroxide) and heat as described previously [11]. The DNA concentration and quality of extracts were measured spectrophotometrically in triplicate using a Nanodrop™ 1000 spectrophotometer (Thermo Scientific).

### 2.3. DNA extraction from different positions in rhinoceros horn

Each horn was mounted in a drill press so that the median plane of the horn was horizontal and the drill press was set to stop at the median plane. Drilling was done from the side of the horn to the medial plane. Drillings were performed using a 7 mm drill bit at distances of approximately 10%, 25%, 50%, 75% and 90% from the base to the tip of the horn. Up to 3 separate samples representing drillings at different depths were collected into separate sample tubes and extracted individually. The depth of each drilling was recorded in millimetres from the scale on the drill press.

### 2.4. Comparison of DNA profiles obtained from horn and other samples of the same animal

The DNA profiles were obtained from the blood and horn samples collected from 6 white rhinoceros during routine capture operations in the Kruger National Park and the 5 horn and hair samples collected from animals during routine identification and translocation procedures in Mpumalanga Province and compared.

### 2.5. Sensitivity of DNA extraction method

The sensitivity of the DNA extraction method was tested using variable amounts of horn powder in the extraction protocol. A single piece of rhinoceros horn was used that was obtained from the tip of a horn that was part of a horn stockpile. The piece of horn was drilled using a new 4 mm drill bit. Shavings from this piece of horn were collected into a plastic tube and homogenized to a fine powder using a tissue homogenizer. The powder was weighed and an amount of 0.1 mg, 1 mg, 2 mg, 5 mg, 10 mg, 15 mg, 20 mg, 25 mg and 35 mg was placed into separate labelled Eppendorf tubes and processed as described above.

### 2.6. Marker selection and PCR amplification

Analysis was performed using 22 dinucleotide STR markers published previously [12–17]. Details of the markers and multiplexes are provided in Table 1. The zinc finger (ZF) locus [18] was used to determine the gender of the animal from which the sample originated. STR analysis was performed using 4 multiplex reactions with between 5 and 8 markers included in each multiplex (see Table 1). Extracted DNA (1 μl diluted to approximately 30 ng/μl or undiluted at less than 30 ng/μl) was added to a PCR mastermix consisting of 5 μl of KAPA2G Fast Multiplex PCR Kit (Kapa Biosystems) and 4 μl of primer mix in a 10 μl reaction volume. PCR was performed using a thermal cycler (GeneAmp® PCR System 9700, Life Technologies) with cycling conditions standardized as follows: 3 min at 95 °C, 30 cycles of 95 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s followed by an extension step at 72 °C for 10 min.

### 2.7. Capillary electrophoresis and genotyping

PCR product (0.5 μl) was loaded with 10 μl Hi-Di™ formamide and 0.25 μl GeneScan™ 500 LIZ® size standard (Life Technologies) and run on an 3130xl Genetic Analyzer (Applied Biosystems) and data transferred to a personal computer and analyzed using STRand software (University of California, Davis) [19]. A set of bins for each locus within the four different panels were set up in STRand using fixed bin sizes to determine and standardize the allele calls between samples. Known control samples for both

**Table 1**
Summary of the forward and reverse primers, repeat motifs, GenBank accession numbers, reference, dye label, size range and multiplex in which the loci used for genotyping of white and black rhinoceros were included.

| Locus | Forward primer (5′-3′) | Reverse primer (5′-3′) | Repeat motif | Accession | Reference | Dye label | Fragment size | Multiplex |
|---|---|---|---|---|---|---|---|---|
| BlRh1B | GATCAGTAACACCAAAGTCC | AGTGAAGACAGAAGGATCAC | $(GT)_{13}GCA(TG)_3$ | AY606078 | [17] | NED | 230–250 | 3 |
| BlRh1C | AGATTCTTGGAAAGGTCACT | AACATTGGGTTTCACCTC | $(AC)_{17}G(CA)_4$ | AY606079 | [17] | NED | 120–160 | 2 |
| BlRh37D | ACATGTGTAAACTTGGGAAC | TGGTTCATTGATCTCTTCTC | $(TG)_6(AG)_{11}GA(AG)_5$ | AY606083 | [17] | NED | 200–250 | 1 |
| BR6 | TCATTTCTTTGTTCCCCATAGCAC | AGCAATATCCACGATATGTGAAGG | $(CA)_{15}$ | | [13] | PET | 150–165 | 3 |
| DB1 | TAAGTCACAGGGACTAATCTG | GAGGGTTTATTGTGAATGAG | $(CA)_{14}$ | AF129724 | [14,17] | VIC | 230–250 | 3 |
| DB23 | ATCTTCCTCAGCAATAAGG | ATCATCAGAGTTTCCAGTTC | $(CA)_{12}$ | AF129734 | [14,17] | FAM | 180–214 | 4 |
| DB44 | AGGGTGGAATGTCAAGTAG | CTTCTAGAGGGAGACTAGGAG | $(TG)_4C\ (GT)_{16}$ | AF129730 | [14,17] | VIC | 200–230 | 3 |
| DB52 | CATGTGAAATGGACCGTCAGG | ATTTCTGGGAAGGGGCAGG | $(CA)_{21}$ | AF129732 | [14,17] | PET | 110–140 | 1 |
| DB66 | CCAGGTGAAGGGTCTTATTATTAGC | GGATTGGCATGGATGTTACC | $(CA)_7TA(CA)_{16}$ | AF129733 | [14] | PET | 210–230 | 3 |
| IR10 | CAGTGAGGAAGATTGGTTGC | CCTGACTCACACATCACCAG | $(CA)_{22}$ | | [12] | NED | 120–140 | 4 |
| IR12 | GAATGCTGATCATTTAGTGAC | GGGTCCAGTTGAGATATCAC | $(CA)_{18}$ | | [12] | PET | 170–200 | 4 |
| IR22 | ATGGTGGAAGAAGTGCAGCC | ACTTCTGTGTCTCTAGCGCC | $(CA)_{22}$ | | [12] | VIC | 200–230 | 2 |
| SR63 | CTTGAGCAGAGTAGAATTTGG | CTCTGTATCCACCTCATTCC | $(AC)_{19}$ | AY427965 | [16] | FAM | 180–210 | 4 |
| SR74 | CAGCACAATGTTTGGCACTTG | TTGGAGTCTTATGTCACCACC | $(CA)_{19}$ | AY427967 | [16] | NED | 160–180 | 2 |
| SR262 | CTGCCTTAACAACTGAACTGC | TGGAGGTTATCTCATGCCAC | $(TG)_{28}$ | AY606077 | [12] | FAM | 80–110 | 3 |
| SR268 | GTTTATACTATGCCCTGCAC | GGATGCTACCGAATAGATTG | $(CA)_{25}$ | AY427972 | [12] | VIC | 170–200 | 4 |
| SR281 | AGGTGATTAGGGAATTGCTGG | TTCTTCTGTCCTGGCATTGC | $(GT)_{23}$ | AY427974 | [16] | FAM | 220–250 | 2 |
| 7B | AACCAACTTGTAATGAGAGG | AATGAACAGGAAGGAAGAC | $(TG)_{16}A(GT)_5$ | AY138544 | [15,17] | PET | 220–230 | 3 |
| 7 C | GTCAGTTCAAGTTTTTGCTC | CTCATCCATGCTTCTTCTAC | $(CT)_{14}(AT)_{11}$ | AY138543 | [15,17] | FAM | 130–170 | 3 |
| 12F | ACAGCTAGAATCACCAAAAC | TCCTGCTGCATAAATCTC | $(TA)_8(AA)_4$ | AY138545 | [15,17] | VIC | 220–240 | 1 |
| 32A | CTAGCAAAATCTCAAAGAGG | TTACTAAGGGAATCACCAAG | $(AC)_6\ldots(AC)_{15}$ | AY138541 | [15,17] | FAM | 190–210 | 1 |
| 32F | GGCAAAACTAAGAGAACTTG | GATACCAAACTGGAAATGG | $(AC)_{18}$ | AY138542 | [15,17] | VIC | 170–240 | 1 |
| ZF1 | GATTTGGAASCTAGGCATTTCC | GCCATGATACTCATGAATGACA | | | [18] | FAM | 95–105 | 4 |

black and white rhinoceros were included with each sample set that was run to ensure the accuracy of allele calls between runs.

### 2.8. Population genetic analysis

The genotypes from a total of 367 samples from southern white rhinoceros (*C.s. simum*) and 33 samples from black rhinoceros of 3 subspecies (*Diceros bicornis bicornis* ($n$ = 5), *Diceros bicornis minor* ($n$ = 25) and *Diceros bicornis michaeli* ($n$ = 3)) submitted to the Veterinary Genetics Laboratory for routine genotyping were used and genotyped using the procedures described above. Allele frequencies, observed ($H_{Obs}$) and expected ($H_{Exp}$) heterozygosities were calculated using Cervus V3.03 [20]. *F* statistics were calculated using FSTAT [21] and GENEPOP [22] for the white and black rhinoceros populations without population subdivision to calculate a $F_{is}$ value for each population. Probability of identity (PI) for each locus, and over all loci, for the white and black rhinoceros populations was calculated using GenAlEx [23].

### 2.9. Match probability

Five DNA profiles from white rhinoceros were selected and the random match probabilities calculated using the formula of Balding and Nichols [24] at different values of theta for each locus and the multilocus match probability was calculated as the product of the locus specific match probabilities.

## 3. Results

Fig. 1 shows the positions of the holes drilled in the horns from the black and white rhinoceros. Table 2 summarizes the DNA concentrations and number (and percentage) of alleles that amplified in extracts from powdered horn obtained from different locations from the base to the tip of the horns and at different depths from the median of the horn. The DNA concentration in extracts from powdered horn obtained from incurred horn samples that were compared with the DNA profiles of blood ($n$ = 6) and plucked hairs ($n$ = 5) of the same animal ranged from 14.8 to 149.5 ng/μl and all horn, blood and plucked hair samples provided full DNA profiles that matched in the same animal. An example of the DNA profile obtained from a horn and matching blood sample is provided in Fig. 2. The DNA concentration in extracts from between 0.1 and 35 mg of horn powder varied from 0.5 to 20.8 ng/μl and all extracts gave full DNA profiles except that from 0.1 mg of horn powder which gave a profile with 21 of the possible 23 loci amplifying successfully with a single locus, DB1 showing non-amplification of the second allele of a heterozygous pair.

The allele frequencies for each locus are summarized using a standardized nomenclature system [25] as supplementary data in Table S1 for the white ($n$ = 367) and black rhinoceros ($n$ = 33). The number of alleles ($N_a$), observed ($H_{Obs}$) and expected ($H_{Exp}$) heterozygosities, polymorphic information content (PIC), inter-individual inbreeding coefficient ($F_{is}$) and probability of identity for individuals (PI) and siblings ($PI_{Sibs}$) for each locus and the population means are provided in supplementary data Table S2. The calculated random match probabilities for 5 individual white

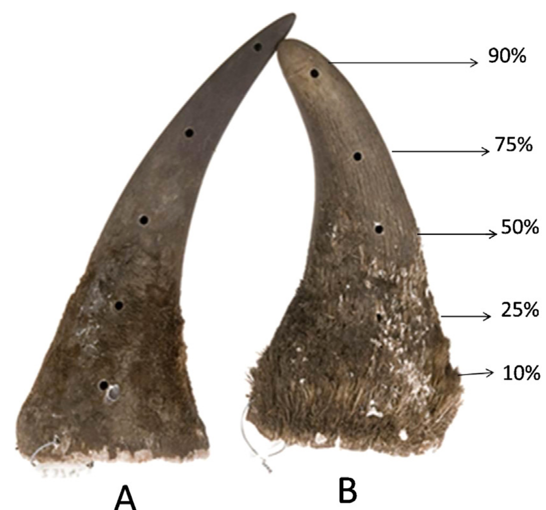


**Fig. 1.** Two horns, one from a black rhinoceros (A) and one from a white rhinoceros (B) indicating the position of drillings taken for DNA analysis along the length of the horn.

**Table 2**
Summary of the drill depths from the median (mm) at five different levels from the base (10%) to the tip (90%) of the horn with the DNA concentration (in ng/μl), the number of alleles that amplified and the percentage of alleles that amplified for the horns from the white rhinoceros and the black rhinoceros.

| Position (%) | White rhinoceros | | | | Black rhinoceros | | | |
|---|---|---|---|---|---|---|---|---|
| | From median | [DNA] ng/μl | Alleles | % amplified | From median | [DNA] ng/μl | Alleles | % amplified |
| 90 | 0–8 | 89.9 | 46 | 100 | 0–7 | 170.9 | 46 | 100 |
| | 8–13 | 60.2 | 46 | 100 | 7–12 | 217.4 | 46 | 100 |
| 75 | 0–8 | 139.8 | 46 | 100 | 0–5 | 302.1 | 46 | 100 |
| | 8–13 | 31.2 | 46 | 100 | 5–12 | 179.8 | 46 | 100 |
| | 13–18 | 21.9 | 32 | 70 | 12–17 | 43.5 | 40 | 87 |
| 50 | 0–8 | 226.9 | 46 | 100 | 0–8 | 68.3 | 46 | 100 |
| | 8–16 | 20.5 | 36 | 78 | 8–15 | 22.4 | 32 | 70 |
| | 16–21 | 17.8 | 11 | 24 | 15–20 | 20.3 | 32 | 70 |
| 25 | 0–14 | 247.7 | 46 | 100 | 0–12 | 135.1 | 46 | 100 |
| | 14–25 | 99.7 | 46 | 100 | 12–19 | 40.8 | 46 | 100 |
| | 25–30 | 48.3 | 46 | 100 | 19–24 | 13.7 | 12 | 26 |
| 10 | 0–20 | 217.5 | 46 | 100 | 0–18 | 261.2 | 46 | 100 |
| | 20–45 | 104.5 | 46 | 100 | 18–30 | 241.9 | 46 | 100 |
| | 45–50 | 30.0 | 46 | 100 | 30–35 | 31.9 | 40 | 87 |

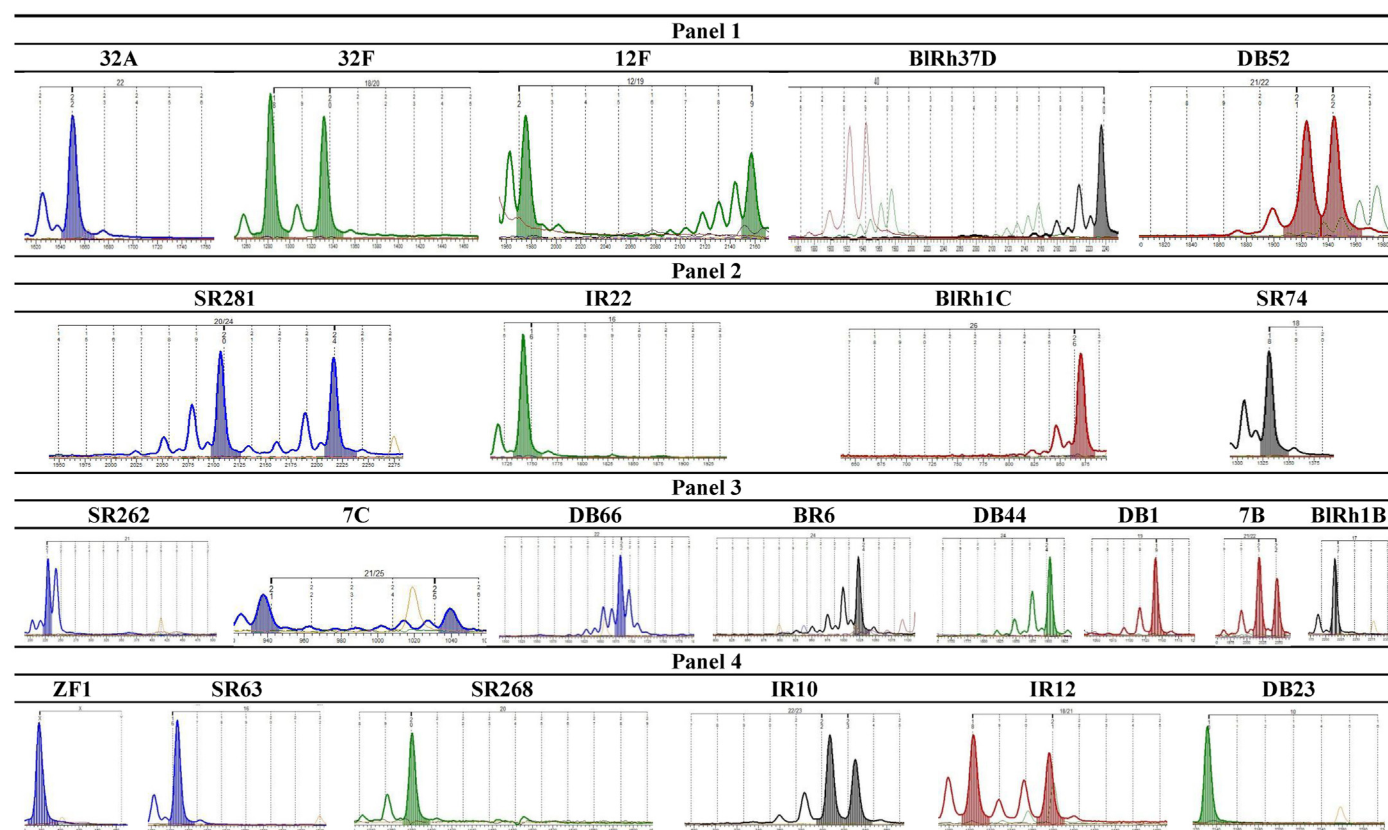


**Fig. 2.** DNA profile of a white rhinoceros determined from an extract from the horn. The profile comprises 4 separate panels consisting of 22 STR markers and a gender marker.

rhinoceros varied between $1.6 \times 10^8$ and $2.1 \times 10^{11}$ when $\theta$ was set at 0, between $7.3 \times 10^6$ and $3.0 \times 10^8$ when $\theta$ was set at 0.1 and between $1.7 \times 10^5$ and $6.0 \times 10^6$ when $\theta$ was set at 0.3.

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2013.04.003.

## 4. Discussion

Extraction of DNA from powdered horn of white and black rhinoceros using the Prepfiler kit on a Kingfisher Magnetic Particle Processor produced DNA extracts with DNA concentrations often in excess of 200 ng/ul. Samples collected from the centre of the horn anywhere from the base to the tip of the horn consistently produced DNA extracts with the highest concentration. Extractions further from the centre of the horn were less efficient and extractions closest to the outside surface of the horn sometimes resulted in incomplete DNA profiles. When collecting a sample from a detached horn it is recommended that one collects the sample by drilling into the dark area (increased melanisation) in the centre of the horn base to a depth of approximately 50 mm. When collecting horn samples from live rhinoceros by drilling into horn from the outside only the drillings from deeper in the horn

should be collected. These are easy to identify as they have are a darker brown to black colour when compared to the white material from the periphery of the horn. DNA extracts from horn samples collected in the field and powdered in the laboratory resulted in extracts with concentrations between approximately 15 and 150 ng/μl. The DNA profiles obtained from these samples matched the profiles obtained from the blood and hair samples collected simultaneously from the same animal on all 23 loci in all cases. Whilst previous studies have documented the extraction of mitochondrial DNA form rhinoceros horn which was subsequently used to identify the species of origin of the horn [10], and we have shown previously that nuclear DNA extracted from rhinoceros horn can be used to identify the gender of the animal of origin [18]. This paper provides the first description of a technique which can extract nuclear DNA from rhinoceros horn which is of adequate quantity and quality to allow STR analysis to be applied to generate profiles to individually identify the animal of origin.

The Prepfiler kit recommends that one should extract DNA from sample material weighing approximately 20 mg. However, pieces of material resembling rhinoceros horn may be substantially smaller than this and in an attempt to investigate the smallest sample size from which a DNA profile can be obtained we used the kit to extract DNA from 0.1 to 35 mg of powdered horn. Full DNA profiles were obtained from extracts of samples ranging from 1 to 35 mg and a partial profile which included 41 of the possible 46 alleles was obtained from a sample of 0.1 mg. These results show that one can generate complete DNA profiles from extremely small amounts of rhinoceros horn which may be of great value in matching a horn, or part of a horn, back to the animal from which it originated.

The STR markers investigated in this study included loci originally identified in white, black, Indian (*Rhinoceros unicornis*) and Sumatran (*Dicerorhinus sumatrensis*) rhinoceros [12–16]. Eighteen of the 22 markers investigated were polymorphic STR markers with between 2 and 4 alleles observed in the white rhinoceros. The remaining 4 markers (BlRh37D, DB23, IR22 and SR74) were monomorphic in the white rhinoceros but were polymorphic in the black rhinoceros. These four markers were all originally isolated from the black (BlRh37D, DB23), Indian (IR22) and Sumatran (SR74) rhinoceros. The marker 32F originally isolated from the white rhinoceros was polymorphic with 4 alleles in the white rhinoceros but was monomorphic in the black rhinoceros. In the case of SR74 the monomorphic allele in the white rhinoceros was of similar size to one of the 3 alleles observed (18) in the black rhinoceros whereas for all other monomorphic loci the size of the monomorphic allele was unique in the species in which it was monomorphic providing a mechanism for confirming the species of origin for the sample investigated. The marker DB66 was highly polymorphic in the black rhinoceros with 8 different alleles observed in this study and a PIC value of 0.658 indicating that this is a highly informative marker in the black rhinoceros. In the white rhinoceros this marker provided 4 alleles but two of these differed from the other two alleles by a single base pair. The mechanism for this observed difference warrants further investigation. The marker 7B originally isolated from the white rhinoceros was polymorphic with 3 alleles in the white and black rhinoceros. However 29 of the 33 black rhinoceros included in this study were homozygous for the 21 allele. The 20 allele only occurred in the 3 *D.b. michaeli* and all were homozygous for this allele. This locus may have specific alleles fixed within the black rhinoceros subspecies, but a larger number of individuals from each subspecies will need to be investigated to confirm this. A single *D.b. minor* from the Kruger National Park had the 19 allele.

The southern white rhinoceros population was reduced to between 20 and 40 animals in the early 1900s with all these animals being confined to the Hluhluwe/iMfoloza area within the KwaZulu-Natal Province of South Africa [26]. The current southern white rhinoceros population in Africa is just over 20,000 and all are descended from this single founder population. The low genetic diversity observed in our study (mean $N_a = 2.722$ and mean PIC = 0.329) is similar to that reported previously [15] and is a direct result of this bottleneck. In contrast, the genetic diversity was higher in the black rhinoceros (mean $N_a = 4.857$ and mean PIC = 0.456). Due to this bottleneck, the discriminatory power of the marker set used in this study was considerably higher when applied to black rhinoceros.

The random match probability calculations were only performed for the white rhinoceros and were calculated using 17 polymorphic markers (the 4 monomorphic markers and the marker DB66 were excluded from the calculations). Using these data, the random match probability calculated for of five white rhinoceros ranged from $1:1.56 \times 10^8$ to $1:2.1 \times 10^{11}$ without any correction for inbreeding and from $1:1.7 \times 10^5$ to $6.0 \times 10^6$ using a $\theta$ value of 0.3 to correct for significant inbreeding [27]. With $\theta$ set at 0.1 in the five animals investigated the estimated random match probability ranged from $1:7.3 \times 10^6$ to $1:3.0 \times 10^8$. Given that the total population of white rhinoceros in the world is approximately 20,000 [6] such random match probabilities indicate that the genotyping system described provides data which can be used for evidentiary purposes. Until such time that reliable estimates of Fst are obtained for the white and black rhinoceros, taking into account sub-structuring within the black rhinoceros population, we recommend that random match probabilities are calculated with $\theta$ set at 0.1 and 0.3 for the white and black rhinoceros, respectively.

The observed heterozygosity was lower than the expected heterozygosity in the black rhinoceros indicating an excess of homozygote loci. The inter-individual inbreeding coefficient was higher in the black (0.2879) than in the white (0.0760) rhinoceros population. The data from the 33 black rhinoceros included animals from all 3 sub-species of black rhinoceros and may indicate that there is significant sub-structuring within the black rhinoceros which could not be investigated in this study but warrants further study.

The DNA extraction and genotyping system described produces highly repeatable results even with small amounts of sample material. These data show that these methods are appropriate for use in investigations involving trace amounts of rhinoceros horn and the matching of profiles obtained from seized rhinoceros horn with material collected from live animals or poached carcasses.

## Acknowledgements

We would like to thank SANParks (South African National Parks), Ezemvelo KZN Wildlife, Mpumalanga Tourism and Parks Authority and the private rhino owners for samples and information supplied and the Forensic Science Laboratory in Silverton Pretoria, for assistance with the preparation of samples included in this study.

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