A review: Forensic DNA technology to meet the stock theft challenges in South Africa

Zwane A.A.[1], E. van Marle-Köster[2], B.J. Greyling[3] & N. Mapholi[4]

[1,3 & 4]Department of Animal Breeding and Genetics, Agricultural Research Council-Animal Production Institute, Private Bag X2, Irene, 0062, South Africa; [2]Department of Animal and Wildlife Sciences, University of Pretoria, Pretoria, 0002, South Africa.

Abstract

Stock theft, one of South Africa's most persistent crimes, is one of the factors that pose a serious threat to food security and biosecurity. It impacts negatively on animal production in South Africa and represents a risk to animal health programs, affecting both the commercial sector and emerging farmers. Rural farmers, with no or limited resources, are often more severely affected due to the impact it has on their livelihood. Nationally, 377 114 animals (cattle, goats and sheep) to the value of more than R1 billion were reported stolen between 2006 and 2010. A major obstacle, central to the prosecution of stock thieves, is the positive identification and proof of ownership of livestock. Conventional identification methods such as brand-marking and ear-tagging, although serving as a deterrent, can easily be altered and are also often not individual specific. DNA, however, is an irrefutable means of identification of an individual, and can be used to trace the lawful owner of an animal in the presence of a reference sample. DNA profiling of exhibits that originate from forensic stock theft cases is routinely used to link suspects to either a crime scene or the crime itself. A huge challenge, however in animal forensics is the nature of samples submitted for DNA analysis. Samples are often aged or degraded, resulting in a compromised efficiency of using DNA profiling in forensics cases. This paper is aimed at reviewing the use of DNA technology as a tool address the challenge of stock theft and the constraints associated with the forensic analyses of DNA samples. Short tandem repeat (STR) markers are commonly used in forensic DNA analysis. Developments in molecular genetics suggest that single nucleotide polymorphisms (SNPs) are potential markers to use in forensics.

Keywords: stock theft, forensics, DNA technology, DNA markers
[#]Corresponding author: ZwaneA@arc.agric.za

Introduction

The livestock industry contributes 49% to South African agricultural production and 3% to the Gross Domestic Product (GDP) (Department of Agriculture, Forestry and Fisheries, Pocket Guide to South Africa 2011/2012; Agriculture, South African Government online, 2013). Livestock are a major source of protein for the growing South African population and cattle, sheep and goats play an integral role in the livelihoods of rural communities in particular. These animals are also kept as a source of investment, insurance against disaster and also for cultural purposes (Kunene and Fossey, 2006).

Stock theft dates back at least to the early 18th century, when Xhosa livestock farmers crossed the Kei River, after the war with the British army, following the invasion of their land by the European settlers in Great Fish River region in Eastern Cape, in 1779. As more Cape migrants arrived from Britain, the population pressure increased and competition over land, cattle and good grazing intensified. Cattle-raiding was common on all sides. These raids and other confrontations sparked more serious conflict and occasionally flared into wars between the Xhosa and the British (Walker and Wyndham, 1941; Pieres, 1976). After many centuries of livestock farming in South Africa stock theft remains a common and persistent crime (Johnson, 2010), adversely affecting the sustainability of especially rural farming practices by restricting their production capacity. Today stock theft remains a contributing factor affecting food security (Dzimba and Matooane, 2005).

Stock theft has recently become more violent and organized. Studies have shown that syndicates operate across national borders, transporting animals to neighboring countries such as Lesotho, Swaziland and Botswana (Dzimba and Matooane, 2005). Animals are exchanged for cash, drugs such as *Cannabis Sativa* and weapons, making stock theft a lucrative business (Kynoch and Ulicki, 2001; Dzimba and Matooane, 2005). Cross-border stock theft has further implications with regard to veterinary and health

issues as it increases the country's vulnerability to outbreaks of contagious diseases, e.g. foot and mouth disease (South African Police Services (SAPS) Research Unit, 2011; Public Eye Online, 2012).

Nationally, 377 114 animals (cattle, goats and sheep) to the value of more than R1 billion were reported stolen from 2006 to 2010. Only 30% of these animals were recovered (Mare, 2012). The Red Meat Industry (RMI) loses approximately R300 million per annum due to stock theft (South African Federation of Red Meat Producers, 2012). Stock theft not only threatens the sustainability of livestock industries and food security, but also destroys valuable and costly genetic resources (Markwick and Davies, 2006). Even though there is some recovery of stolen animals, it seems to be of little consolation to the owners. Most of the animals are found slaughtered at the crime scenes and some animals are transported across the borders. The owners either get the remains at the crime scene or don't find their animals at all. Poor animal recovery reduces the profitability of the livestock enterprises and also disrupts the government's land reform process and empowerment of emerging farmers. Among the main risks and contributing factors in relation to stock theft are unattended grazing, the practice of keeping unmarked livestock and the poor documentation of livestock movements (Department of Community Safety and Liaison, KwaZulu-Natal). The incidence of stock theft among smallholder farmers is three times higher than in emerging farmers. The emerging farmers generally have larger number of animals than smallholder farmers and they are also generally properly identified with brands and/or ear tags. Considering the resource limitations of smallholder farmers, and their small number of animals, they suffer a greater loss of stock (Macaskill, 2011).

According to SAPS statistics, the theft of cattle, goats and sheep has increased by more than 128% percent since 2011 (Duze, 2012). A major challenge that is central to stock theft is the problem of individual identification and traceability of an animal to its lawful owner. Conventional methods such as brand-marking and ear-tagging alone are not reliable or individual-specific because they can be tampered with (Evans and Van Eenennaam, 2005). On smallholder farms, very few animals are branded and ear-tagged because of lack of resources and also ignorance of the law regarding animal identification (Barclay, 2001).

Advancements in molecular genetics over the past three decades have led to the development of deoxy ribonucleic acid (DNA) based technology especially in forensic studies (Baldi and Hatfield, 2002; Beja-Pereira *et al*., 2009). The massive global investment in genomic research of livestock species holds significant benefits for livestock production, including new approaches for effective identification of animals. The use of DNA evidence in a court of law became popularized in the 1990s (Easteal & Easteal, 1990), and is currently used to prove guilt, exonerate the innocent and/or to determine the identity of an individual animal. However, these determinations are the result of a scientific process that is not easily understood by the public and legal advisors (Forrest & Woody, 2010).

The ultimate purpose of DNA typing in livestock forensics is to test the hypothesis that a particular animal is the source of an item of biological evidence. Analysis is undertaken to establish whether two samples, evidence and reference, originate from the same biological source (Meintjies-van Der Walt, 2008). The aim of this paper is to review DNA based animal forensic technology with reference to the constraints and prerequisites for application in the livestock industry.

DNA-based forensic technologies

DNA in forensic science is used on a regular basis in humans and also in animals (Jobling and Jill, 2004; Tamaki & Jeffreys, 2005). DNA profiling has become an established forensic tool for individual identification (Curran, 1997; Loftus, 2005; Girish *et al*., 2010). Forensic laboratories receive material recovered from the crime scenes together with reference samples from suspects and victims. Forensic genetics subsequently assist in the investigation process by comparing samples, resulting in a report that can be presented in court (Easteal & Easteal, 1990; Goodwin *et al*., 2007). Molecular techniques aimed at obtaining good quality DNA have successfully been developed to produce reliable and reproducible results for forensic analyses (Wong *et al*., 2012).

The key to the usefulness of the DNA typing procedure is the fact that each individual has a unique DNA pattern, with the exception of identical twins (Loftus, 2005). One of the most important and central issues associated with forensics is the individuality of a DNA fingerprint. Unique identification with DNA typing is therefore possible provided that enough variable sites on the DNA are examined. Using 16 or more microsatellite loci, a match between two DNA patterns can be considered strong evidence that the two samples came from the same source (Cummings, 2008). It is however important that the source of DNA is of good quality for reliable polymerase chain reaction (PCR) amplification.

Biological evidence

In stock theft and poaching cases, the main samples found on crime scenes are blood and blood stains, hair, faeces, tissue, skin, bones, urine etc. The suspects can also leave materials at the scene such as clothes, hair, cigarette butts and saliva. In most cases the investigating officers will trace suspects to their houses to collect additional evidence such as meat and blood-stained clothes found in their possession. However, the issue of contamination needs to be considered when collecting samples from both the crime scene and suspect(s) alike. Full protective clothes must be worn to avoid contact between evidentiary material and that of the crime investigator. The evidence from the crime scene and suspect(s) will then be sent to the forensic lab for DNA comparision (Nelson 2009; Norrgard 2008).

Biological samples collected from crime scenes are often exposed to harsh environmental conditions such as heat, direct sunlight and water (Nelson, 2009; Misner et al., 2009). Environmental exposure damages DNA by breaking the molecules into smaller pieces. This occurs as a result of endonuclease activity and spontaneous depurination. Damaged and fragmented DNA blocks the extension step in PCR. To recover those fragments can be difficult (Sikorsky *et al*., 2004; Deagle *et al*., 2006). In case of advanced level of degradation more cellular material is required to produce a useable DNA profile. If the material is highly degraded, even the highly sensitive DNA profiling may not be able to generate a usable high quality DNA profile (Goodwin *et al*., 2007). MacCord *et al*. (2011) investigated the effect of DNA degradation and subsequent inhibition on PCR amplification of forensic samples. They indicated the quality of PCR product decreases as the template DNA fragment sizes becomes smaller, and found that environmental damage to DNA in tissue samples occurs rapidly and reaches a state where DNA becomes nearly unrecoverable. The template in such samples breaks down to small pieces in as little as 3 weeks. In addition, poor specificity can result in numerous artifacts which interfere with the interpretation of experimental results (Wang *et al*., 2004; Foran, 2006)

DNA damage is an alteration in the chemical structure of DNA, a break in a DNA strand, a missing base from the backbone of DNA or a chemically changed base (De Bont & van Larebeke, 2004; Deagle *et al*., 2006). DNA damage is a challenge in forensic analysis, resulting in a compromised ability to successfully obtain a good quality DNA profile. The latter can easily lead to inconclusive results, no results or false positive results (Yang & Speller, 2006; Barbisin *et al*., 2011). DNA damage can be due to exposure to acid, alkylating agents, heat, light, phenol/chloroform extraction, and reactive oxygen species or simply by time (Curran, 1997; Nelson, 2009). Over time, unless frozen, dried or preserved, DNA in a cell will degrade and even PCR-based DNA testing methods will no longer be successful. The inefficient amplification of DNA sequence can increases the chance of getting the nonspecific DNA profiles (Butler *et al*., 2003).

In a study done by Nemakonde (2012), the effect of sample degradation and efficiency of different enzymes used in PCR were investigated. Bovine tissue samples were subjected to degradation under different environmental conditions to mimic the degradation of samples under real crime scene conditions. Tissue samples were degraded for 15 consecutive days to obtain complete degradation. Degradation was also repeated during winter and summer. Samples were subjected to DNA extraction, PCR and DNA profiling using a panel of 16 selected bovine microsatellite markers. PCR was undertaken using three different polymerase enzymes, the normal Taq polymerase and two high proof reading enzymes. Results indicated differences in the level of sample degradation in response to different seasonal conditions. With regard to producing reliable DNA profiles, high proof reading enzymes were capable of restoring damaged DNA and yielded conclusive DNA profiles.

Samples collected from a crime scene must be stored under conditions that slow the rate of DNA degradation e.g. low temperatures should be used. A cool dry environment limits the action of bacteria and fungi that find biological materials as a rich source of nutrients and can rapidly degrade them. Hair samples, buccal swabs and blood collected in 'Fast technology for analysis' (FTA) paper can be stored at room temperatures for many years and allows for the collection, transport, and stable storage of nucleic acid samples from a wide variety of sample types (Turner *et al*., 2002; Lee *et al*., 2010). A study conducted by Tak *et al*. (2005) used FTA samples stored by the Federal Bureau of Investigation (FBI) at room temperature. The high DNA yield from FTA was enough for the PCR-based method analysis to deliver acceptable results.

DNA isolation

The most important factor to consider when extracting DNA from the samples collected from the crime scene is efficiency, meaning extracting sufficient quantity and good quality DNA. This mainly depends on the nature and type of the evidence samples obtained from the crime scene. In most cases genetic profiling is based only on a few cells extracted from trace material found on a crime scene as all of these potentially carry trace amounts of biological material (Dieltjes, 2011). There are different methods used to extract DNA from forensic biomaterials, such as the Chelex method (Phillips *et al*., 2012), silica based DNA extraction (Baker *et al*., 2001), phenol-chloroform (Kochl *et al*., 2005) and also using commercial kits. The Chelex DNA extraction method is a fast, cheap, and effective method compared to other methods such as phenol/chloroform/isoamyl extraction. Alternately phenol/chloroform extraction yields high molecular weight, double stranded DNA and is a preferred method of extraction for samples that are old and/or degraded because it consistently yields higher quality DNA (Phillips, 2009).

Silica-based and phenol-chloroform DNA extraction methods have a high binding capacity for DNA molecules, and have been used mainly for DNA isolation, especially from old and degraded samples. These organic extraction methods are commonly used in isolation of DNA for STR-based analysis and have been used widely with high yield of DNA (Goodwin *et al*., 2007; Phillips 2009). Commercial DNA extraction kits provide pure and high quality DNA compared to organic methods. They are easy to use and more reliable though they are more expensive (Shams *et al*., 2011).

Genetic markers

Genetic markers have been identified throughout human and animal genomes. These markers refer to a known DNA sequence by which their inheritance can be tracked and can thus be used for individual identification. Several markers are used for DNA genotyping. Markers such as Restriction Fragment Length Polymorphisms (RFLPs), Mitochodrial DNA (mtDNA), Short Tandem Repeats (STRs) and single nucleotide polymorphism (SNPs) markers are often used in forensics analysis.

RFLPs were one of the first markers to be applied in forensic investigations. These markers are used to analyse the variable lengths of DNA fragments. A DNA sample is digested with a special enzyme called restriction endonuclease, which cuts the DNA at a specific site called a restriction endonuclease recognition site. The resulting restriction fragments are separated according to their lengths by gel electrophoresis (Easteal & Easteal, 1990). Due to the development of newer, more efficient DNA-analysis techniques, RFLP is seldom used because it requires large amounts of DNA. RFLP typing is furthermore not very successful when working with samples degraded by environmental factors, such as dirt or mould (Marks, 2009).

Mitochondrial DNA analysis can be used to examine the DNA from samples that cannot be analyzed by RFLP or STR (Yamada *et al*., 2002). Older biological samples that lack nucleated cellular material, such as hair, bones, and teeth cannot be analyzed using STRs and RFLP, but they can be analyzed using mtDNA. Mitochondrial DNA is more valuable in the investigation of cases that have not been solved for many years (Feulner *et al*., 2007). Analysis of genetic markers on the Y-chromosome is especially useful for tracing individual relationships among populations, i.e. if a calf is stolen, mtDNA can be used to link it back to the dam or half-sibs if DNA from those animals is available (Wallner *et al*., 2013); and also for analyzing biological evidence (Girish *et al*., 2010). However, the maternal inheritance of mtDNA restricts it to explore events at the maternal angle. Also, mtDNA is of little use in investigating recent loss of genetic variation and any individual-level events such as identity, individual dispersal, and mating systems (Wan *et al*., 2004).

STRs are the most widely used DNA markers for forensic purposes. These markers are used to evaluate specific regions (loci) within nuclear DNA (Samuels & Asplen, 2000). They are highly polymorphic, easily amplified using PCR and require small amounts of DNA (Tautz, 1989; Ellegren *et al*., 1997). Variability in STR regions can be used to distinguish between animals' DNA profiles (Chistiakov *et al*., 2006; Selkoe & Toenen, 2006). They are in most cases selectively neutral and codominant and it is possible to distinguish between homozygous and the heterozygous individuals (Wright & Bentzen, 1994; Chistiakov *et al*., 2006). STRs arise predominantly through slippage synthesis during DNA replication and are mostly found in the non-coding part of the genome (Curran, 1997). These features provide the foundation for their successful application in a wide range of fundamental and applied fields of biology and medicine, including forensics, molecular epidemiology, parasitology, population and conservation genetics, genetic

mapping and genetic classification of complex traits (Anderson, 2001; Szibor *et al*., 2003; Chistiakov *et al*., 2006; Greenhouse *et al*., 2006).

Different STR marker panels are designed for different animal species (van de Goor *et al*., 2009). Many cases have been solved through STR analysis nationally and internationally. In South Africa, the Agricultural Research Council - Animal Production Institute (ARC-API) is one of the institutes that provide an animal forensic DNA analysis service to Sub-Saharan countries. The aim of the service is to combat stock theft through DNA technology. They receive about 500 stock theft cases per year (Mnisi *et al*., 2012). This includes cases to prove ownership through parentage analysis, to link samples found at the crime scene with the suspect and species identification. Analyses are done using STR markers and DNA profiles are analyzed on an ABI 3130XL genetic analyzer. The panel of STR markers and the PCR methods used vary according to the species, which includes livestock and wildlife.

An example of a case received dates back to 2011, where three sheep were stolen from a farm in the Amersfoort area in Mpumalanga. Following police investigation three suspects were arrested and police found three sheep heads in their possession. Three sheep skins were also found in the nearby forest where the crime was suspected to have taken place. DNA analysis was requested to determine whether the skin samples found in the forest were from the same animals of which the heads were found in the suspect's possession. DNA was extracted, subjected to PCR and genotyped using standardized sheep STR markers. DNA profiles were analyzed and indicated that two of the samples found at the crime scene matched the heads. The suspects were found guilty in the court of law.

The recent development of high density SNPs panels has drawn attention to both human and animal forensics (Amorim & Pereira, 2005; Schneider, 2012). A SNP is a site in the genome where individuals differ in their DNA sequence by a single base (Collins *et al*., 2003). SNPs are usually bi-allelic. Therefore, the probability of two independent base changes occurring at the single position is very low. SNPs are bias in mutations (Vignal *et al*., 2002). They are simplest and most common type of genetic variation and comprise around 90% of genetic variation in humans (Sensen, 2008). They occur during meiosis when DNA is replicated. SNPs are genetically stable in mammals and amenable to high-throughput automated analysis. SNP panels have been developed for animal genotyping, mainly in cattle, although the present cost of SNP genotyping is typically high (Matukumalli *et al*., 2009). A prerequisite for using cattle SNPs in animal identification and paternity analysis is the description of a minimal set of SNPs with sufficient power to uniquely identify individuals. The potential use of these markers has been estimated, and the polymorphisms in the DNA surrounding the target SNPs has been identified to facilitate the design of accurate, low-cost SNP assays on variety of high-throughput genotyping platforms (Heaton *et al*., 2002).

Karniol et al. (2009) developed a 25-plex SNP assay for traceability in cattle. They used SNaPshot to multiplex 25 SNPs that have been previously validated as useful for identity verification. They estimated the allele frequencies for different cattle breeds and significant positive Pearson-correlation coefficients were obtained among the breeds. The probability that two randomly unrelated individuals would share identical genotypes for all 25 loci varied from $10^{(-8)}$) to $10^{(-10)}$ for these breeds. For parentage verification, the exclusion power was found to be 99.9% when the genotypes of both putative parents were known. A traceability test of duplicated samples indicated a high genotyping precision of >0.998. Karniol *et al*. (2009) further verified this process through analysis of 60 cases of parent-sib pairs and trio families. The 25-plex SNaPshot assay was adapted for low- and high-throughput capacity and thus presents an alternative method for DNA-based traceability in cattle.

Hara *et al*. (2010) undertook a study on the development of SNP markers for individual identification and parentage test in a Japanese black cattle population. 29 unlinked SNPs were selected as diagnostic markers and, based on the frequency data, the estimated identity power of these markers was $2.73 \times 10^{(-12).}$ Parentage exclusion probabilities, when both putative parents' genotypes were known and when only one parent was known, were estimated as 0.96929 and 0.99693, respectively. Hara *et al*. (2010) also concluded that this panel of SNP markers was sufficient for individual identification and would be a powerful tool for a parentage testing.

Although STRs are still commonly used for animal forensic identity and parentage testing, there is an increasing interest in the forensic use of SNP genotyping (Van de Goor, 2011b). Much effort has been done to implement human SNPs for forensic parentage and identity testing, but further optimization is still required. In animals, studies are still underway to optimize the SNP panels for forensic purposes (Van de Goor, 2011a). Table 1 presents the summary of DNA markers and their use in livestock theft.

Table 1 DNA markers and their role in forensics

DNA Method	Purpose	Advantages	Disadvantages	Recommended use to reduce livestock theft
RFLPs – Restriction Fragment Length Polymorphisms	-Analyze the variable lengths of DNA fragments (Easteal & Easteal, 1990)	-Highly sensitive method for fingerprinting DNA of any origin and complexity (Bruns *et al*., 2007)	-Requires large amount of DNA (Marks, 2009) -Do not work on degraded samples (Bruns *et al*., 2007)	Yes
STRs – Short Tandem Repeats or microsatellites	-Evaluate specific regions (loci) within nuclear DNA (Samuels & Asplen, 2000).	-Highly polymorphic -Easily amplified using PCR -Require small amounts of DNA -Can be routinely amplified simultaneously in a single multiplex amplification reaction -Abundant throughout genome (Ellegren *et al*., 1997; Chistiakov *et al*., 2006)	-Occurrence of null alleles can lead to misinterpretation of results (Butler *et al*., 2007)	Highly recommended
mtDNA – mitochondrial DNA	-Useful for tracing individual relationships among populations (Wallner *et al*., 2013)	- Good for older biological samples that lack nucleated cellular material mtDNA is more stable over time/conditions (Feulner *et al*., 2007)	-Do not recombine -Cannot use the product rule when determine the probability of an ID match -Cannot separate direct relatives apart (Hammer & Redd 2006)	Yes
SNPs – Single Nucleotide Polymorphisms	-Analyze genetic polymorphisms between individual in non-coding parts of the genome (Williams *et al* 2010)	-Have lower mutation rate -More stable in terms of inheritance -Potential ability to work well on degraded DNA -Target small region of DNA -Amenable to automation (Vignal *et al*., 2002: Amorim & Pereira, 2005)	-Not as polymorphic as STRs -More SNPs are required to reach equivalent powers of discrimination -Have limited number of alleles -More expensive -SNP-panels not available for many species yet (Amorim & Pereira, 2005)	Yes

Constraints associated with DNA analyses

The main concerns in forensics are the difficulty in recovering the evidence from the crime scene and the suspect, extracting DNA from ancient or degraded samples, the quality of the DNA and the accuracy of the information it contains (Golenberg *et al*., 1996; Deagle *et al*., 2006). Additional concerns are whether the evidence contains enough biomaterial to be useful for DNA analysis and whether tissue samples of different ages, exposed to different conditions, will still provide positive results and reliable genetic information (Golenberg *et al*., 1996).

Depending on the degree of exposure to the environment and age of the DNA, some damaged DNA fragments can be repaired while others cannot. Samples exposed to different seasons (winter and summer) will have different degrees of degradation. Summer temperatures in South Africa are notoriously high, often reaching up to 40 °C. These conditions have a higher impact on the samples left at the crime scenes. Depending on the size of the sample, smaller samples such as blood, bones, saliva and tissue samples will dry up faster in higher temperatures, while larger samples or whole animals will be degraded. Therefore the maximum period that a sample can stay in the crime scene depends on the size, season and the location of the sample (Nemakonde, 2012).

To alleviate the problem of DNA damage, Kalmar *et al*. (2000) developed a simple and efficient method for PCR amplification of DNA extracted from ancient bones, using an ethanol precipitation-based method, a fast method that does not require hazardous chemicals or special devices. From 7^{th}-15^{th} century bone samples, they successfully amplified mtDNA fragments from the hypervariable region I. Their results showed that extraction of DNA from ancient bone samples using ethanol precipitation-based method increases the success rate of PCR amplification.

In another study, Western et al. (2009) used SNP markers to analyse mixed and degraded DNA samples. Since most SNPs are bi-allelic, they are ineffective in detecting mixtures, sometimes leading to incorrect genotyping. They then developed an algorithm to find non-binary SNPs in the National Center of Biotechnology Information (NCBI) dbSNP database and compared the genotyping efficiency of the tri-allelic SNP markers and STR markers. This was done by analyzing artificially degraded DNA, DNA from 500-year-old bone and molar samples. In both types of degraded DNA samples, the larger sized STR amplicons failed to amplify whereas the tri-allelic SNP markers still provided valuable information. They found that tri-allelic SNP markers are suited for the analysis of degraded DNA to enable the detection of a second DNA source in a sample.

To improve the quality of DNA found in degraded forensic samples, Battista (2012) developed a method to retrieve selected DNA fragments from complex mixtures using cell extracts isolated from repair proficient microorganisms. Since there is no way of knowing what type of DNA damage is present in biological evidence, intact cells provide an extensive array of DNA repair proteins that are capable of restoring damaged DNA. The study was aimed at isolating fragments including intact STR sequences from highly degraded samples. Samples were treated with extracts obtained from cultures of *D. radiodurans* exposed to 3000Gy dose of ionizing radiation. Initial trials suggested that extracts were repairing damaged DNA, but the controls indicated that undamaged DNA was adversely affected by the extract, lowering transformation efficiency. This indicated there could be more than one inhibitory factor present in the extract. Highly degraded samples are expected to have a range of fragment sizes. The methods used permit accurate amplification of STR loci in degraded DNA samples, prevent alteration of STR repeat length in the DNA sample to be analyzed and any additional degradation of, or damage to, the DNA sample to be analyzed.

All these methods are designed to maximize the quality of DNA and at the same time to minimize co-extraction of substances that inhibit PCR (Rohland & Hofreiter *et al*., 2007). Several substances, inherent to forensic samples, may also limit the success with which a DNA profile can be obtained. Inhibitors of the PCR, such as textile dyes, can also interfere with the ability to recover a full DNA profile from biological evidence (Misner *et al*., 2009). Other new DNA tests are being developed to recover information from smaller regions of DNA (MacCord *et al*., 2011) that are more likely to be intact following DNA damage. These methods will help to achieve high quality DNA yield to help solve stock theft forensic cases easily (Chung *et al*., 2004; Dixon *et al*., 2006).

Conclusion

From available statistics on reported stock theft cases, the incidence of both theft and recovery rates in respect of cattle have remained constant over the last four years. Therefore as a result of stock theft in our country, forensic DNA analysis can be an important tool in the conviction of stock thieves. DNA technology provides a tool for animal identification and prosecution of livestock thieves. DNA-based identification technologies have clear advantages over traditional identification methods such as branding, tagging, biometrics and blood typing. The development of DNA analysis for animal forensic purposes has led to new approaches for effective and irrefutable identification. Recent advances in DNA forensic analysis will allow predicting the loss of STR loci in the analysis of compromised or degraded samples. Nationally, animal forensic and dispute cases have been resolved through DNA typing and the results have been presented in South African courts. STRs are the current markers of choice for this purpose due to their high information content, the standardization of STR loci in the forensics and the commercial availability of STR kits. Recent research has raised the possibility that SNPs could replace the STRs in the near future. SNPs are mostly used in identification and kinship analysis in animal populations and are now applied for routine use in forensics analysis. However, considerable research is necessary to establish adequate scientific foundations for these applications. The use of SNPs in animal forensics and parentage analysis in South Africa is still under development. The cost of SNP genotyping is currently too expensive for the services or diagnostic labs. Studies are still underway to establish and deliver technology that will score SNPs at lower prices.

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