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# Essence of Short Tandem Repeat (STR) as Potential Markers for DNA Profiling Paving a Way for Forensic Investigations- An Update

**Sharma Loveleen[1], Sharma Varruchi[2], Malik Rupal[1], Sharma Anupam[3], Ramniwas Seema[4] and Sharma Anil K.[5]***

[1]Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala 133207 (Haryana), India
[2]Department of Biotechnology and Bioinformatics, Sri Guru Gobind Singh College Sector-26, Chandigarh 160019 (UT) India
[3]MMIS, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala 133207 (Haryana), India
[4]University Centre for Research and Development, University Institute of Biotechnology, Chandigarh University, Gharuan, Mohali, India
[5]Department of Biotechnology, Sector-82-A, IT City Road, Amity University, Mohali 140306, Punjab, India

**Corresponding Author*

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**Abstract:** As we know that wildlife is one of the most important treasures of the planet earth, as it makes our nature more and more powerful in one way or another, and helps us build ownselves. But with increasing needs of humans and multiple necessities, it is very much getting affected one way or the other and also being constantly under threat of degradation. Do we have appropriate and foolproof measures to preserve the deterioration stature of wild-life? The answer is still negative. Thus, highly essential steps are needed to preserve them from being poached and to protect them. Poaching these days is getting to newer heights because of the need for fetching more powers. Such species are being targeted and it is becoming very difficult to catch the culprits and to identify them even at a smaller scale. To do such forensic identification for wildlife, one has to come forward with newer research and ideas so as to minimise the rate of degradation for each species, making each species important and full of quality, and to be saved. Thus, like a human DNA database, a database for wildlife should be prepared on the basis of species identification, and this can be done by making the Short Tandem Repeats (STR) marker, the main component of the database and registering the species. With all this data, each species' DNA is required to be saved with all its morphological and non-morphological characters, including phenotypic and genotypic characters as well.

**Keywords:** DNA database, Short Tandem Repeats, Markers, DNA profiling, Phenotypic, Genotypic

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## Introduction

Wildlife is one of the most important treasures of the planet earth, as it makes our nature more and more powerful in one way or another, and helps us build our own selves (Coetzer *et al.*, 2017). But with increasing needs of humans and multiple necessities, it is very much getting affected one way or the other and also being constantly under threat of degradation. Do we have appropriate and foolproof measures to preserve the deterioration stature of wild-life? The answer is still negative. Thus, highly essential steps are needed to preserve them from being poached and to protect them (Coetzer *et al.*, 2017). Poaching these days is getting to newer heights because of the need for fetching more powers. Such species are being targeted and it is becoming very difficult to catch the culprits and to identify them even at a smaller scale. To do such forensic identification for wildlife, one has to come forward with newer research and ideas so as to minimise the rate of degradation for each species, making each species important and full of quality, and to be saved. Thus, like a human DNA database, a database for wildlife should be prepared on the basis of species identification, and this can be done by making the Short Tandem Repeats (STR) marker, the main component of the database and registering the species. With all this data, each species' DNA is required to be saved with all its morphological and non-morphological characters, including phenotypic and genotypic characters as well (Johnson *et al.*, 2014). These characters of the species are needed to be preserved with the said data so as to have precise identification and comparison. This data will also help us to know the essential measures for their main identification and preservation.

### *DNA Analysis*:

In spite of morphological studies, DNA analysis also plays a very important role in species individualization (Trail, 2021) ; therefore, to study the genome of a species, it is not necessary to study the whole DNA, as 99.9% of the DNA is identical and only 0.1% shows variation. This 0.1% of DNA is the one that has all variations and includes both coding and non-coding regions. These regions are to be analysed for any variation or recombination (Spruijtenburg *et al.*, 2023). The non -coding region has genes having multiple copies for short repeating sequences. These sequences are around 2-7 bp long which are forming series of around 100 nucleotides, termed as Short Tandem Repeats. STR are repeated sequences, also known as micro or simple sequence repeats (SSR), which are termed as the DNA backbone as they contain maximum number of variations in a DNA (Ruitberg *et al.*, 2001). These STR sequences that account for around 3% of repeated sequences, representing the non-coding region are also termed as Junk DNA (Fagundes *et al.*, 2022). Figure 1 highlights the stepwise outline of the process of data collection till the final confirmation of the result.

### *STR as Forensic Tool*:

If we have to study DNA of an individual, STR are termed to be the best forensic tool for the comparable analysis because of the variable nature of its region showing polymorphism. STR are classified on the basis of the length of the major repeated units as mono, di, tri, tetra, penta, and hexanucleotide repeats, and each STR has multiple alleles found to be present separately on variable chromosomes (Dinh *et al.*, 2023). These positions of the STR, which are defined by the number of repeats as described, help in knowing the genetic makeup of a particular species. These sequences are further surrounded by non-variable segments of DNA called "flanking regions." These flanking regions are found to be present upstream or downstream, i.e., from 5’ to 3’, further helping with transcription. These flanking regions also help in knowing the STR profiles with the help of techniques like polymerase chain reactions (PCR) and RT-PCR (reverse transcriptase polymerase chain reaction). It is also seen that each STR segment found in the DNA has a certain type of

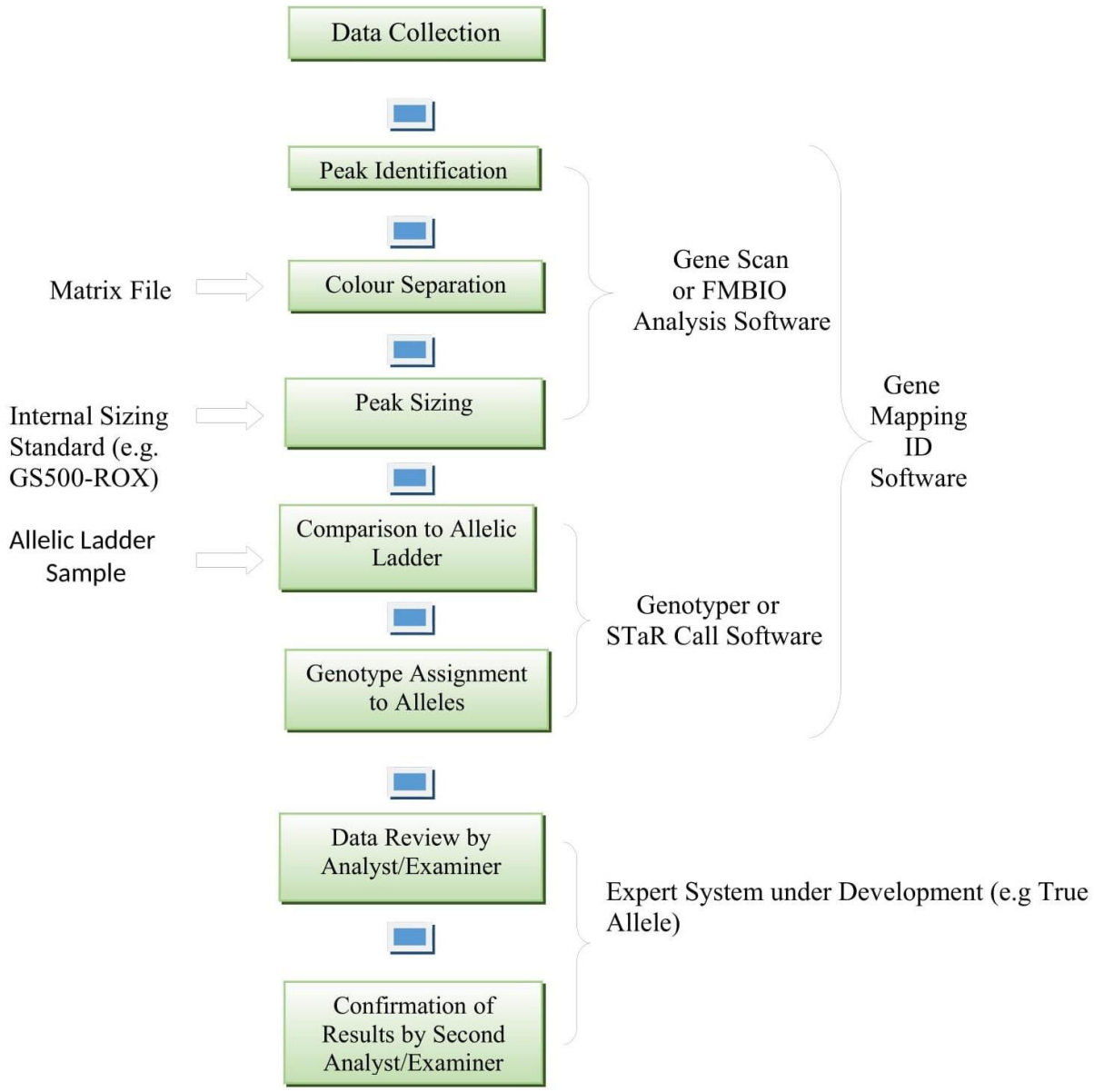


Fig. 1: Stepwise outline of the process of Data collection till the final confirmation of the result.

repeating unit, and these repeating units are termed as alleles. These alleles help making the STR segments to be unique as they help in solving paternity and maternity deputes along with species identification (Sahajpal andAmbers, 2023). It is because of their extraordinary nature of being transferred from both mother and father. Also, these alleles help in giving a clear-cut picture of an individual, whether it is found to be heterozygous or homozygous. Thus, STR play a very important role in forensic identification by calculating the allelic frequencies. Because of this unique characterization of the STR, these were selected by the Combined DNA Index System (CODIS). 13 of these CODIS loci were established and marked of outmost importance by the Federal Bureau of Investigations in 1988 (Kevin *et al.*, 2011). These days, these loci are further used for PCR multiplexes, and these STR profiles help in updating of data along with their use in forensic casework. In 1944 by FSS (Forensic Science Services) in United Kingdom, the quadruplex amplification system consisting of four tetranucleotides STR– TH01, vWA, FES/FPS and

F13A1, was established and was used for wildlife species identification.

But this quadruplex system did not give a vast option for examination because of lesser allelic frequency. Subsequently, FBI nominated 13 autosomal STR loci for human population. Many studies have also shown that 16 loci of full panel have been found to have a full discriminatory power to be used in parentage analysis and in the wildlife identification similar to humans, as wildlife also has the same formulation for life. These loci have also helped in determining if any bird or animal is in captivity or has been found to be in illegal trade or not. The 16 loci panel found for wildlife also helps in discriminating between wild and domestic animals and helps in gender identification with an additional amelogenin locus for sex identification. These 16 loci are BM1818, BM1824, (BM2113, CSRM60, CSSM66, ETH3, ETH10, ETH225, HAUT27, ILSTS006, INRA023, SPS115, TGLA53, TGLA122, TGLA126 and TGLA227) (Van De Goor *et al.*, 2009). Additionally 22 microsatellites (D8UIA2, DoUIA2, PG6,PG5, FH2361, FH2100, GCT025, LEI0049, HMS6, HMS3, BT165, BT150, D3S3045, MAF33, MCM164, SW742 and EF046 loci) (Van De Goor *et al.*, 2009), 31 microsatellites and 28 microsatellites loci were identified which were found to be useful in sheep, cat and lion genotyping and sexing (Hirak *et al.*, 2022). These loci were having 219 alleles out of which 15 alleles were found to be the highest of all with one zinc finger allele used for sexing a marker respectively, out of which 22 markers helped in showing genetic sources of origin and their heterozygosity was also specified in lions with F41 locus having 101 alleles, thus is considered to be the main part for lion species identification. Thus, for the STR markers to be used in a database, the development of full genetic profile is required, which can be achieved from a variety of samples, thus including samples like tissue, blood samples, bone samples, and various body secretions.

*Steps for Sample Analysis*:

Thinking with a broader perspective for the STR analysis, the steps for sample' analysis should always be given a bird's eye view because of their specific qualities (Leclair *et al.*, 2004). Such samples can be analysed for the DNA extraction by various methods such as Phenol: Chloroform extraction, FTA card analysis, organic extraction and various kits made for STR analysis having the main 13CODIS STR protocol (Brookes *et al.*, 2012). The procedural extractions are followed for both mitochondrial and nuclear DNAs as the mitochondrial DNA being the shortest and most stable and being easily extracted from degraded samples and being transferred from only mother to child showing no paternal linkage, it helps in creating a perfect role in paternal human cases and also same is true for animal species, thus can be helpful in animal species identification and relation as well. As we know in human paternal linkage getting an intact sample from all the suspects is difficult, therefore in wildlife cases it is next to impossible to get intact samples (Singh *et al*., 2004). Thus, in such cases mitochondrial DNA helps in solving such issues as it is of small size, having 13 protein coding genes, 2rRNAs and 22tRNAs and being the most stable because of its highly rigid cell. membrane and high in protein content, making it stable in any tough condition, thus not degrading easily. The DNA extraction methods for mtDNA are to be performed very carefully under cold conditions and without much pressure to the samples and also with least contamination as mtDNA being very sensitive, need to be extracted and preserved with utmost care at -80°C. After the extraction, the DNA is subjected to PCR for further marker analysis with the help of genetic sequence analyser (Mitra *et al.*, 2018). These markers help in identifying the linkage for various cases. The markers that are established are from the mitochondrial components like cytochrome C oxidase subunit I (COI), 12S and 16S RNA segments and control region D loop (Panday *et al.*, 2014). Out of these mitochondrial components D loop region is the main region for any animal species identification and to create a co-relation between species. Having all such benefits with mitochondrial DNA,

sometimes mitochondrial DNA makes a difficult approach towards analysis as the samples recovered from the crime scene may be degraded or contaminated as well. Using such samples for analysis may result in wrong primer attachments, because the primer used for such samples will attach to more than one degraded DNA strands, and will become difficult to separate giving false results during analysis further giving non-matchable results for species identification. Therefore, in cases where the sample get degraded and contaminated, the nuclear DNA helps more deeply and broadly for such samples (Rebala *et al.*, 2016), as it is less likely to be contaminated or degraded. Because nuclear DNA is easy to preserve and easily extractable with less care and more volume, the nuclear markers have specific STR, used mainly for animal species identification. Out of these markers, more heterozygosity is found in ple46 marker (Bañuelos *et al.*, 2022). But the disadvantage of using the nuclear marker is that it is not available in degraded samples as compared to mitochondrial DNA (Perkins *et al.*, 2021).

Research findings indicate that most of the studied markers are based on the dinucleotides which do not play a wide role in forensic wildlife analysis other than tri and tetranucleotides (Miller *et al.*, 2014). Therefore, the tri- and tetranucleotides need to be identified more, and a wider attention is required. Before any STR analysis, a proper quality analysis and quality control check must be performed for species identification. The stutter products can create a great confusion in analysis and may result in fake STR peaks and also can make wrong species identification (Cui *et al.*, 2020). To exclude out such confusing results from wrong products, the samples should be properly analysed and the instruments used should be properly cleaned and calibrated so that there is no chance of contamination and hindrances in getting appropriate results. Such samples with stutter result should be subjected to re-analysis (Cui *et al.*, 2020) or should be considered non-suitable samples for analysis because it may interfere with mixture analysis (Sharma *et al.*, 2010).

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