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Use of molecular biology techniques for animal identification and traceability

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ABSTRACT

Animal husbandry is considered a substantial part of agriculture which is important for the economic development of a country. The local potential for the livestock sector has remarkably developed in the past few decades with the introduction of technological advancements. Identification and traceability of livestock populations evolved as critical points in the production chain. Molecular marker-based genetic studies have addressed the preliminary complications of the livestock field. The intended objective of genetic studies is to select and maintain superior traits which have a direct impact on economic value. In that regard, the application of genetic markers has a significant role. RFLP, SCAR, AFLP, SSR, ISSR, EST, RAPD, STR, SCoT, and STS markers are prominently used despite animal identification and traceability, determination of genetic distance, sex determination, parentage determination, genetic conservation, gene mapping approaches in the livestock field. The present review is a critical study of current molecular biological techniques and their versatile applications in animal identification and traceability approaches and possible improvements of the available techniques to address the major concerns in the field.

KEYWORDS:

Livestock, animal identification, traceability, molecular markers, ISSR, RAPD, SNP, Genetic diversity

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Introduction

The process of critical identification and registration of a particular animal individually within its epidemiological unit or group using a unique identifier or distinct characteristics is Animal Identification (ID) (Greene, 2010). This enables "record-keeping", which assists with the tracking of an animal throughout their lifetime (Hoffmann et al., n.d.). Parallelly, the process of record-keeping and studying of a particular animal within its whole lifetime and reversely, to the point of origin, is commonly known as animal traceability which plays a major role in the animal production and livestock section. The preliminary objective of animal traceability and identification is to maintain the unique profile of a particular animal throughout its lifetime (Caja & Hernández-Jover, 2004). In earlier times, the main objective of animal identification was to indicate ownership as well as to prevent thievery. Today, animal identification has expanded to include information on the animal's origins including birthplace, pedigree, parentage, sex, breed, genetics as well as traceability, as a result of the complexation of human requirements compatible with technological development (Ha, 2012). Starting from the neolithic era to today, animal husbandry has become a prominent industry in most of the countries in the world (Greene, 2010). The livestock populations provide both products and services such as food sources including meat, milk, eggs, fiber, draught powder, etc (Caporale et al., 2001).

Historical shreds of evidence prove that Egyptians, Greeks, Romans, nomadic people of Scandinavia, Asia, and Africa, and pre-Hispanic Americans used different methods for distinguishing the particular animal from its group, to use them for different purposes (Greene, 2010). Though animal identification methods are varied, they can be summarized under several classifications for ease of study as natural or artificial, based on the nature of the characters used; and permanent or temporary, based on the permanence of the character and on the animal natural characters, including coat color, horns, hair curls, fingerprinting (Grossman, 2006).

The authors have come up with a large amount of historical data which emphasize the idea of the variability of animal identification systems (Caporale et al., 2001). In 1981, Sánchez Belda presented his data on animal identification techniques. According to most reviewers, there is a considerable effect of the cultural and economic conditions of human societies on the variability of the identification systems (Hoffmann et al., n.d.). However current objectives of animal identification and traceability contain slight deviations compared to the past, including indicating property ownership through registered brands, identifying individual animals for individual performance recording which mainly focused on improved breeding and management systems, and especially on the mechanism for disease and residue traceback to the property of origin (Grossman, 2006).

The traditional animal identification and traceability techniques include branding, ear notching, tattooing, ear tagging, etc. Branding techniques vary depending on the source of branding (Adcock et al., 2018). In hot iron branding, the scar is made on the skin using hot iron whereas in caustic branding, caustic chemicals like corrosive acids, caustic soda paste, caustic potash are used to made scars (It, 2011). Branding destroying melanocytes, which are the cells that produce the pigment in the skin and hair, is known as freeze branding (Hutu,

2020). The paint branding is done with pigments (Department of Agriculture Republic of South Africa, 2008). In-ear notching, small cuttings are made by knife cutting or by using special cutting pliers. Ear tattooing is one of the best conventional methods of permanently identifying animals. Apart from the symbols, permanent number codes are also used for tattooing (Sharp et al., 2007). Tattoos are usually applied on left or right ears (all species), lip (horses), groin (pets), and under the tail (sheep and goat). Ear tagging is a slightly advanced animal identification approach compared to tattooing and punching. (Sharp et al., 2007). Electronic ear tags are used combinedly with the RFID method (Wyld, 2009) ; Ccia & Ear, 2002). Injectable transponders and electronic rumen boluses, nose prints, DNA profiling, iris scanning, and retinal scanning are currently being used as non-invasive method for herd animals, especially cattle and goat identification (Ccia & Ear, 2002). These are long-lasting identification methods that allow reliable identification (>98%) in cattle, horses, camels, and goats.

Though animal identification methods have gradually developed with time and technology, they have not met traceability requirements to an acceptable level. Specifically for animal production and health as well as in animal forensic studies, it requires a molecular level traceability approach to have a reliable result (Singh, Deb, Alyethodi, et al., 2014a). To achieve this, animal traceability and identification methods should be developed using molecular biological techniques through further studies.

Molecular biological techniques for animal identification and traceability

Ever since Watson and Cricks' great discovery of the DNA structure in the 1950s, it has been the center of numerous studies to determine the secrets of life (Naqvi, 2007). A sub-disciplinary field of biology that mainly focuses on understanding the molecular basis of the biological activity of a particular cell can be defined as molecular biology (Naqvi, 2007). Molecular-level studies are based on the genetic materials DNA and RNA along with their interrelationships, protein synthesis, and their regulation. The involvement of DNA variations in the determination of different characteristics in livestock animals has a positive impact on both animal production and health. As a result of research and development efforts, the application of molecular techniques for quantitative genetic studies of livestock animals has obtained a remarkable increment in the 20th century.

As the livestock industry has spread to many areas throughout the world, there is a huge diversity among the livestock animals within inter and intra populations. Remarkable variations of both genotypic and phenotypic characteristics have been observed among the same species (Caporale et al., 2001). Due to the variability of genomic profiles of the dominant and recessive characters, performance and adaptations vary within the same species and different species as well (Petrovic et al., 2018). However, the genetic variation within the animal population enables evolution through natural selection. Changing conditions like climatic deviations, diseases, competition, and pressure for food reservoirs and manipulated genetic improvements to obtain higher production rates have a direct effect here (Yaro et al., 2017).

The intended outcome of an industry is obtaining a higher profit with the least amount of resources. The livestock industry follows the same. Animal genetics is considered a preliminary approach for increasing profit as it enables trait identification, traceability, trait improvement and conservation (Petrovic et al., 2018).

Genetic characterization involves the individual identification of livestock animals within populations as well as their production environments utilizing a genetic improvement approach. Genetic improvement is dependent on gene function and regulation (Zenke et al., 2022). The addition and removal of characteristics using gene manipulation, which refers to alteration of existing genes or enhancement of characters by introducing new genes, is the preliminary objective in gene editing. Apart from that, the approaches for the conservation of genomic data in livestock have improved in the last few decades to avoid the extinction of animal breeds (Zenke et al., 2022). Conservation involves both live population conservation and cryo-conservation by semen or embryos. Both of these approaches are not cost-effective and need special requirements in order to be successful. Maintaining genetic data sets in in-silico resources has addressed these major concerns (Kyselová et al., 2021). The critical identification and traceability of a particular animal within its population through molecular markers is a prominent requirement to have a proper data set of a targeted animal population. Specifically in animal husbandry, the conservation of genomic data has a significant effect on the development of novel breeding programs (Petrovic et al., 2018).

A molecular marker is basically a gene or short DNA fragment in the genome which exists in a known location in the chromosome and is closely related to the desired gene or a trait. Sometimes mutation or polymorphisms are also identified as molecular markers (Polegri & Negri, 2010). These markers allow for the identification of a targeted DNA sequence within a gene pool by representing the heritable differences in homologous DNA sequences. The variations and mutations occur due to the deviations in base pairs, Indels-Insertions or Deletions, variations in tandem repeat numbers, and rearrangements etc. (Kumar & Sheep, 2015). The concept of molecular markers evolved in the 19 th century (Singh, Deb, Alyethodi, et al., 2014a). From then to today, these markers are utilized in numerous approaches in genetic studies. The prominent objection in molecular genetics is the identification of markers compatible with gene function for desired traits (Polegri & Negri, 2010). Apart from breeding, conservation, biodiversity studies, and selection of traits, the molecular markers can be involuntarily used in critical animal identification and traceability (Yaro et al., 2017).

Application of molecular marker-based approaches, in livestock animal identification, traceability, and breeding have advantageous effects over the conventional methods of breeding and identification (Erhardt & Weimann, 2007). It makes it easy to develop particular test and detection techniques with molecular markers as they are ubiquitous. These markers are stably inherited from the parental population to progeny. Each marker is represented by multiple alleles and these markers are free from pleiotropic effects. The specificity of the molecular markers is represented in almost all the cells and tissues in

all stages of life hence it is deliberately a sustainable approach for developing animal identification and traceability approach (Ebegbulem & Ozung, 2013).

Conventional animal identification and traceability vs Molecular marker-based identification and traceability

Table 1- Conventional identification and traceability vs Molecular marker- based identification and traceability

Conventional identification and breeding	**Livestock animal identification and breeding with molecular markers**
Generally morphological markers and manual markers like tags, branding techniques are used	Molecular markers are used
Use simple laboratory techniques if required	Require sophisticated laboratory conditions
Have a direct effect on environmental factors	No direct effect on environmental factors
Relatively low accuracy	High accuracy
Require 10-15 years to obtain a new variety	Take only 3-4 years for the whole process
Should consider gene interaction	No effect of gene interactions
Animal screening at the seedling stage for economic traits is not possible	Animal screening for economic traits is possible in the seedling stage

The molecular markers are categorized into two types based on the detection method as molecular markers based on hybridization and molecular markers based on PCR.

Hybridization based techniques

This method include RFLP- Restriction Fragment Length Polymorphisms, and minisatellites based analysis.

RFLP- Restriction Fragment Length Polymorphisms

As the name implies, these markers are identified by the resulting DNA fragments with size variations after digestion with restriction endonucleases. These markers are categorized under both PCR-based and hybridization-based techniques (Djønne et al., 2005). In hybridization combined RFLP marker technique, blotting techniques are used to interpret results. In the test, the DNA is extracted from animal populations and DNA intergrity is tested. Then, the DNA is digested with a suitable restriction enzyme (Djønne et al., 2005). This may be one or a combination of two to three enzymes. The restricted DNA is subjected to gel electrophoresis and the southern blotting technique is performed with

the resulting gel. Here, the hybridization is done with radioactive probes. Non-radioactive probes are also applicable (Djønne et al., 2005).

In the PCR-based RFLP marker techniques, detection is performed by PCR with relevant primers. After the DNA extraction, the PCR amplification is performed with required conditions depending on the primers used. Most of the time, these markers are used in animal traceability. Apart from that, these markers play a major role in livestock products traceability (Sharma et al., 2006).

The use of RFLP with hybridization technique is limited due to the requirement of radioisotopes. These markers are co-dominant markers and species-specific. So, in the hybridization method, polymorphic probes should be developed. As a result, this technique costs more (Tabit, 2016). The RFLP markers-based systems are mainly applicable in genetic studies in closely related taxonomic groups. Here, the detection and results interpretation is based on the DNA band pattern diversity. The genetic distance of the livestock population can be determined through these markers (Hashim & Al-Shuhaib, 2019). This character is used by many researchers for DNA fingerprinting. Apart from these, genetic mapping and disease prediction can also be performed with these marker-based techniques (Hashim & Al-Shuhaib, 2019).

Variable Number Tandem Repeat (VNTR)

The Variable Number Tandem Repeat-VNTR is a nucleotide sequence with average size of 20-100 bp which shows variability of its copy number or organized as tandem repeats (Marwal et al., 2013).These sequences mainly include microsatalites and minisatallites. Early DNA fingerprinting techniques included hybridization of labelled minisatellite probes to the restriction enzyme digested DNA which was detected by autoradiography (Chambers et al., 2014).

Minisatellites

Minisatellites are a type of tandem repeats and exist as motifs with a size of approximately 0.5 kb (Vergnaud & Denoeud, 2000) These regions are rich in GC nucleotides which are arranged asymmetrically and the number of repeats vary within the genome. The tendency of these microsatellites sequences to hybridize with other similar sequences within the genome result in the motifs' structure and it enables the DNA fingerprinting and unique identification of individuals. The detection is mainly based on hybridization. No requirement of specific primers and hypervariability make these markers more special. Other than identification and traceability approaches, these exit as highly polymorphic multiallelic markers that enable the genetic diversity and relationship studies. Barriers to the use of PCR technique and a lack of locus specificity for multilocus markers are a few drawbacks of these markers (Dalvit et al., 2007).

PCR based techniques

In PCR based techniques, the targeted sequences of the genome are amplified with the designed primers and the amplified products are analysed with gel electrophoresis(Petrovic et al., 2018). The amplification patterns are almost unique to each individual and this enables the proper identification and traceability approach. SCAR (Sequence Characterized Amplified regions), AFLP (Amplified Fragment Length Polymorphism), SSR (Single Sequence Repeats), ISSR (Inter Simple Sequence Repeats), EST (Expressed Sequence Tags), RAPD (Random Amplified Polymorphic DNA), STR (Short Tandem Repeats), SCoT (Start Codon Targeted Markers), and STS (Sequence Tagged Sites) are categorized under markers based on the PCR technique (Ebegbulem & Ozung, 2013).

Microsatellites

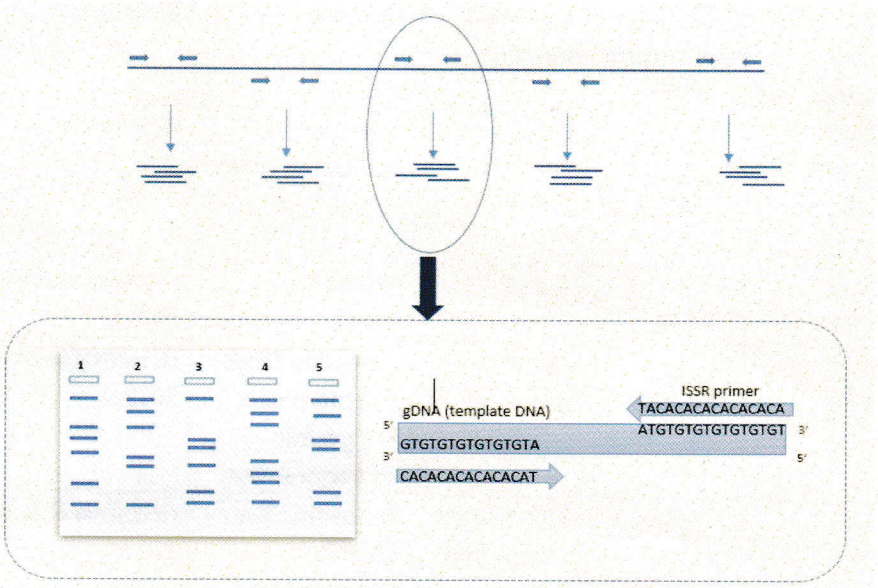
Microsatellites are the tandemly repeated motif sequences consisting of nucleotides ranging from one to six as mononucleotides, dinucleotides, trinucleotides, tetra nucleotides, pentanucleotides, and hexa-nucleotides (Napolitano et al., 2004). Sometimes the SSR- Simple Sequence Repeats and STR- Short Tandem Repeats and sequence-tagged microsatellite repeat (STMR) are often refered to as microsatellites (Hashim & Al-Shuhaib, 2019). As these markers are co-dominant, it bears high polymorphic levels of expression. This feature enables the study of genetic diversity among closely related breeds. It is easy to detect the results by PCR rather than by hybridization techniques as a large number of varients are available.

The reaction can be performed at a low cost due to the ability to apply multiplex PCR. However, these microsatellites are not abundant in chromosomal DNA and there is a difficulty of initial identification of these markers (Hashim & Al-Shuhaib, 2019)

ISSR-Inter Simple Sequence Repeats

The ISSR marker belongs to genome regions that exist in between the microsatellite loci (Kostova et al., 2017). The variations are detected through PCR with designed primers. These are a type of multilocus dominant markers that don't allow a clear distinction between homozygotes and heterozygotes. Normally, these markers amplify DNA fragments within a single reaction resulting from a huge number of loci through the genome of the targeted species (Ng & Tan, 2015). The specificity of the ISSR technique is that there is no requirement of sequence information for the test. As ISSR markers are dominant markers, they can be used to formulate co-dominant markers like SCAR and microsatellite markers. These ISSR markers are universal, quick, easy to apply, highly reproducible, and polymorphous. The ISSR method has been used in genetic diversity studies in several species such as cattle, goat,sheep, and other livestock animals (Kostova & Bojinov, 2018b).

Figure 1- ISSR PCR Amplification and result interpretation



ISSR markers are considered as an ideal genetic marker due to the higher variability, reproducibility, polymorphism, and ability to generate multilocus data from the genome. These markers are applicable in most genetic studies including genetic diversity, DNA fingerprinting, and phylogenetic studies due to the specificity (Kostova & Bojinov, 2018b). Most researchers have stated that using ISSR markers for livestock animal genetic studies has huge potential over other markers due to the higher efficiency. As these techniques result from a unique band pattern for each animal within the population, this can be successfully applied to make unique identification and traceability system (Kostova et al., 2017).

Figure 2- ISSR gel image obtained from cattle DNA

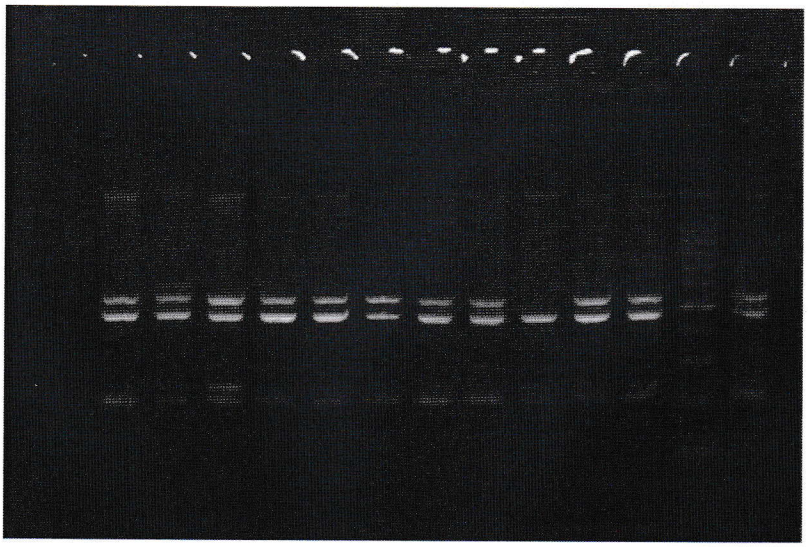


Table 2-Application of ISSR markers in animal genetic studies

ISSR Primer sequence	Annealing temperature	Livestock animals	purpose	References
AG(8)CTG AG(8)YT GA(8)YC CT(8)RG	72	Goat	Detection of genetic variation	(Kostova et al., 2017)
(AG)9C (GA)9C	55	sheep	Detection of genetic variation	(Mohammadabadi, 2017)
(AG)9C (GA)9C	55	Cattle, goat, and sheep	Assessing DNA polymorphism and genetic characterization	(Kostova & Bojinov, 2018a)
(GA)9C (AG)9C	55	Holstein and Jersey cattle	Determination of genetic polymorphism	(Kostova & Bojinov, 2018a)
(AC)9T (CA)9T (TG)9C (GT)9C	55	Iranian mohair goat	Analysis of genetic diversity	(Science, 2014).
AG(8)CTG AG(8)YT GA(8)YC CT(8)RG	72	Goat	Genetic variation	(Kostova et al., 2017)
(GA)9C (CA)9G	72	Horse	Traceability	(Sheikh et al., 2018)

Requirement of a small amount of DNA, the effect of DNA purity is less for the test reliability, efficient detection due to the even distribution of markers in the genome, simple result interpretation, the applicability of multiplex PCR, better analytical resolution, and high reproducibility and high reliability of dominant markers can be identified as the strengths of these markers. Apart from genetic diversity studies and population genetic studies, these markers are prominently used for individual genotyping, parentage detection, genome mapping, and evolutionary studies (Labastida et al., 2015).

The important components of the ISSR experiment include genomic DNA extraction and its integrity, ISSR primer detection, PCR amplification, gel electrophoresis, and data analysis. In the data analysis, the bands are scored based on the presence and absence in the resulted gel. Theis scoring data is then subjected to computer software, a dendrogram is developed and the genetic similarity and dissimilarity is commented on (Labastida et al., 2015). However, ISSR is a multilocus technique. This results in the non-homology of the fragments of the same size, and this is a disadvantage of these markers in animal identification and traceability approaches.

RAPD-Randomly Amplified Polymorphic DNA

Randomly amplified polymorphic DNA markers are dominant markers resulting from genomic sequences recognized by arbitrary primers (Tang et al., 2019). These can be identified as DNA segments with size variations amplified by the same loci (Huang et al., 2003a). The detection is completely based on the PCR technique. Due to the use of short primers, the annealing temperature should be reduced to 30-40. With the use of low temperatures, nonspecific amplification can be observed. The polymorphism is detected by gel electrophoresis and by scoring the bands based on their presence or absence (Mhuka et al., 2017a).

However, there are some advantageous effects of RAPD markers over other techniques. A large number of markers can be generated within a short period using these markers. So, these are considered efficient and cost-effective techniques. Depending on the ability to use these markers as genetic markers, they can be readily applied in genetic studies of closely related taxonomic groups, gene mapping due to high abundancy, population genetics, molecular evolutionary studies, livestock animal breeding, and animal identification and traceability approaches (Huang et al., 2003c). Additionally, this technique can be precisely applied in sex determination. RAPD markers have been also used for characterization, estimation of genetic relatedness, and determination of genetic diversity in a large number of studies. These markers can be used to distinguish between genotypes but are limited to comparisons of populations from a few sources. RAPD markers are a more suitable technique for cloned organisms (Huang et al., 2003c).

This technique is a highly sensitive technique that requires sophisticated laboratory conditions. A single mismatch of the primer and template leads to the total absence or decreased amount of the PCR product in the gel. So, it is difficult to interpret results easily. Additionally, this requires high-quality DNA and optimum PCR conditions to have a reliable result.

In Zimbabwe, the RAPD-PCR technique has been used to genetically identify cattle breeds in which the DNA samples have been examined with several RAPD primers. In a study, the primers were prominently used to study the genetic variations of crossbred animals (Mhuka et al., 2017b). Additionally, in Taiwan, DNA fingerprinting attempts that were taken with the DNA samples of local chickens, Aboracres broilers, Leghorn chickens, quails, doves, emus, Beltsville small white turkeys, pheasants, Chinese geese, mule ducks, Holstein cattle, and Landrace pigs, were amplified with random primers by RAPD-PCR (Huang et al., 2003a). In this study, it was concluded that the type of primer and animal species have a significant effect on reproducibility of results (citation required). The main objective of this study was to determine the meat traceability of cattle and ostrich with RAPD-PCR technique (Huang et al., 2003b).

AFLP-Amplified Fragment Length Polymorphism

This is also a PCR-based technique in which unique fragments of DNA are generated by the digestion of DNA and selective amplification of the resulting DNA fragments of genomes of interest (Marchi et al., 2003). AFLP markers are biallelic markers which are

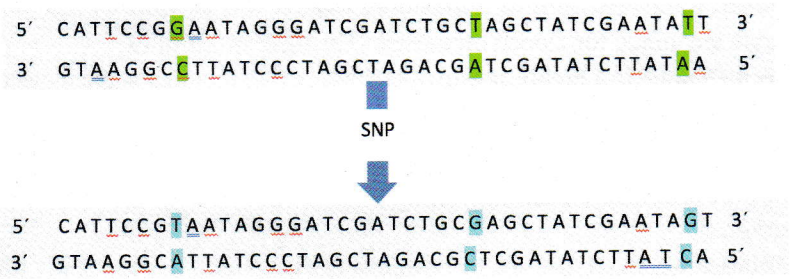
dominant (Marchi et al., 2003). These markers bear significant variability in majority of loci. This feature enables the simultaneous detection of frequent variations of a single nucleotide concealed genomic regions (Zhao et al., 2018).

This is widely used for the genetic relatedness of closely related animal groups and is effectively applied in the livestock industry for assessing the relationships between breeds as well as criminal studies and parentage determination. Genome mapping and QTL studies are possible with these markers due to the high reproducibility and sensitivity. These markers can amplify between 50bp and 100bp fragments which makes the technique more unique. There is no requirement of prior knowledge of sequence information to perform the test (Ajmone-Marsan et al., 2004).

Some studies revealed that these markers are not efficient as they are dominant markers which have significantly low functionality in breed diversity and inbreeding approaches. The analysis requires assumptions about heterozygote frequencies. At the same time, the sensitivity is higher. This is a relatively expensive technique. The test requires band quantitation as detection proceeds by comparison of pixel density in images from a gel scanner (Thurston et al., 2002).

Single Nucleotide Polymorphism (SNP)

Figure 4- Single Nucleotide Polymorphism



As the name implies, this involves detection of single nucleotide changes in selected regions of the genome (Para & Kumar Praveen, n.d.). The variation includes insertions or deletions. As the abundancy of SNPs is high in the genome microarray procedures have developed as automatically scoring large number SNP loci simultaneously at a low cost per sample (Heaton et al., 2002). SNP can frequently exist in both coding and non-coding regions of the DNA sequence. These SNPs are responsible for genetic variation in the trait of interest. These traits include inherited disorders, conformation, and production traits. The determination of genotype based on variability of SNPs loci can be done without a gel separation, as it is required for microsatellite and RFLP detection (Vignal et al., 2002).

Table 3-Molecular markers

Marker	Phenotype expression	procedure	Reproducibility	Source of variation
Amplified Fragment Length Polymorphism (AFLP)	Dominant	Genomic DNA is digested by restriction enzymes and adaptor sequences are ligated. The PCR is proceeded and analyzed with gel electrophoresis.	High	Restriction sites mutations Diversity of restricted fragments
Random Amplified Polymorphic DNA (RAPD)	Dominant/ co-dominant	DNA fragments are randomly amplified with designed random primers	Medium-low	Mutations in primer sites Diversity of amplified fragments
Inter-Simple Sequence Repeat (ISSR	Dominant	Random amplification of DNA fragments with primers with microsatellite sequences.	High	PCR primer site variations and Insertions, Deletions of amplified DNA.
Simple Sequence Repeats (SSR)	Co-dominant	Random amplification of DNA fragments with primers designed for conserved DNA sequences	Moderate	Variations of the number of repeats

Table 4- Molecular Markers

Marker	Phenotype expression	procedure	Reproducibility	Source of variation
Single Nucleotide Polymorphism (SNP)	Co-dominant	PCR amplification with primers designed for single nucleotide mutations	Moderate	Indels number variation

Restriction Fragment Length Polymorphism (RFLP)	Co-dominant	Restriction digestion of genomic DNA followed by PCR amplification with designed primers for restricted fragments.	High	Restriction site mutations Restriction fragments diversity (i.e. DNA fragments of variable length)
Short Tandem Repeats (STR)	Co-dominant	PCR amplification of fragments with primers designed for tandemly repeated sequence motifs.	Moderate	Short tandem repeats number variation

Molecular marker-based applications in livestock field

Estimation of genetic diversity

The diversity of almost all animal species is due to variations in genomic DNA. There is a remarkable effect of environmental factors too. Genetic diversity is the measurement of overall evolutionary deviations in between the animal species (Talle et al., 2005). The use of molecular techniques or molecular markers makes these studies more efficient and effective. Genetic diversity among inter and intra animal populations is possible with these markers due to the high variability. Genetic polymorphism is a measure based on polymorphic characters at morphological, biochemical, cellular, and DNA levels (Berthouly et al., 2008).

Parentage determination

This is related to both animal traceability and breeding approaches. To make this efficient, knowledge of the mating systems of the animal species is important. Research studies have shown that compared to testing with blood groups and other biochemical markers, DNA markers have a high impact on parentage determination attempts (Singh, Deb, Rahman, et al., 2014). However, the use of DNA markers to determine parentage is technically feasible when techniques become routine, and it is expected that costs will also decrease (Mrode et al., 2019).

Measure Genomic Response to Selection in Livestock

In these approaches, the genomic profiles of the selected breeds are monitored and the genomic response within large progeny groups is investigated (Singh, Deb, Rahman, et al., 2014). These applications are common among cattle and goat populations due to

the frequent use of artificial insemination in breeding approaches. Mainly, microsatellite markers are used for this purpose (Lenstra et al., 2012).

Sex determination of pre-implantation embryos

The establishment of livestock breeding and management systems as well as parental diagnosis of cattle illnesses place a greater emphasis on this method (Singh, Deb, Alyethodi, et al., 2014a) Whether an X-bearing ovum is fertilized by a Y- or X-bearing spermatozoa determines an individual's genetic sex. Pre-implantation embryos' sex is determined by molecular markers, such as the SRY, ZFY, and TSPY genes, based on the location of the Y chromosome. According to the literature, TSPY was an effective male-specific marker whose utility was increased by the gene's high copy number (Singh et al., 2017).

Disease carrier identification

Here, detection of genetic disorders by vertical transmission of defective genes to offspring is done using molecular techniques. If the relevant allele is recessive, the carrier animals display normal morphological characters but can transfer the genetic defect to their offspring (Kyselová et al., 2021) These alleles carry mutations made by the non-functional proteins which result from both metabolic and developmental disorders that result in significant economic losses (Singh, Deb, Alyethodi, et al., 2014b).

Several molecular markers have been used for early identification of various diseases in livestock. According to research studies on Amplification Refractory Mutation System Polymerace Chain Reaction or ARMS PCR-based detection of signal transduction mechanism of foot-and-mouth disease, susceptibility can be identified by targeting single-nucleotide polymorphism in the 5' untranslated region of the bovine ITGB6 receptor gene in zebu cattle (Longjam et al., 2011). Studies on susceptibility to Paratuberculosis infection demonstrated that the TLR2-1903 T/C SNP is associated with resistance to MAP in Holstein Friesian cows (Koets et al., 2010). The study has mainly focused on the relationship of gene polymorphism and tuberculosis susceptibility. According to the results there was a direct impact of the polymorphism of the TLR1 gene for Bovine Tuberculosis- BTB infection status in Chinese Holstein cattle (Teneva, 2009)..

Genetic Conservation

Genetic conservation is a preliminary objective of developing animal traceability approaches. Molecular marker based identification and traceability enable the effective access of current germplasm and genetically modified profiles in conservation (Teneva, 2009). When choosing breeds for conservation, factors including the degree of endangerment; features with economic or scientific worth; and ecological, historical, and cultural aspects are taken into account (Hanotte & Jianlin, 2005). In assessing population genetic characteristics relevant to conservation biology, including as within-population heterozygosity, between-population gene flow, and the genetic uniqueness of taxonomic units, molecular markers are widely used.

Gene mapping

The use of molecular markers in gene mapping of livestock populations makes it more precise as it allows direct identification of the desired gene instead of a gene product and the identified gene can be efficiently used as a tool for somatic cell hybrid screening (Para & Kumar Praveen, n.d.) . Moreover, these markers enable the use of different DNA probes and the easy screen technique itself. In the physical mapping of genes, the molecular markers have a positive effect as they utilize in situ hybridization (Al-Samarai & Al-Kazaz, 2015). Markers such as RFLPs, which present the evolutionarily conserved cording sub-sequences are very essential in comparative mapping methods where polymorphism is not necessary. Conversely, markers such as microsatellites exhibit higher polymorphism information content than ordinary RFLPs and can be generated more quickly and easily. More efforts are taken in the generation of gene maps based on molecular markers. Additionally, ASO (Allele-specific oligonucleotide) and STMS (Sequence tagged microsatellite site) polymorphic markers are similarly useful in the rapid development of gene mapping (Naqvi, 2007).

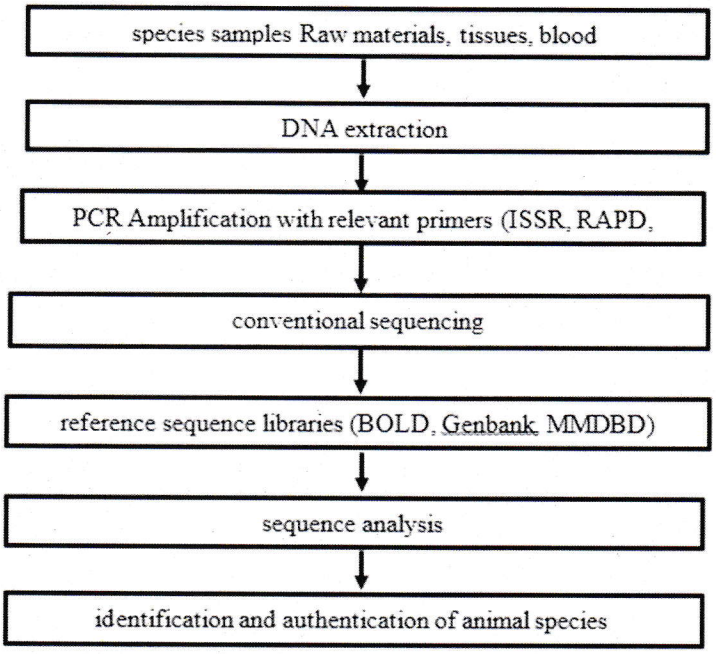
Future perspectives

The molecular biology field is updated yearly with a vast amount of literature data as a result of the tendency for research and development approaches in the field. However, the amount of literature data converted to practical aspects is limited due to several reasons like lack of funding, less knowledge on the field, decisions of the responsible parties, etc. The livestock field has a huge impact on the local economy. So, it is important to combine the research findings with practical aspects to reduce the knowledge gap while addressing the major concerns in the field.

DNA barcoding

This is a technique used by developed countries for the unique identification and traceability of livestock animals. Apart from living animal identification, this method is successfully applied in the traceability of animal products in the market especially, meat. Locally, the industry faces many challenges with animal products in the market, mainly with regard to their quality and safety (Yang et al., 2018). These problems can be overcome through the DNA barcoding method. A DNA barcode is a standardized sequence of the genome with less than 1000 bp. It can be achieved through the universal primer utilization. This feature allows for easy amplification from diverse samples. DNA barcoding can be used on both fresh and raw materials for species authentication and species delimitation. The frequently used barcode marker is mitochondrial cytochrome c oxidase subunit I (COI), which is highly conserved across species (Carvalho et al., 2011).

Figure 5- methodology for developing animal traceability system



Animal identification and traceability systems

The molecular approaches established with molecular markers is applicable in developing reliable animal identification and traceability system. This is developed with computational bioinformatics software (Caporale et al., 2001). The preliminary requirement is the unique identification of targeted animals within its population. The genetic similarity and the dissimilarity measures obtained with computational software and taxonomic relationships are encountered. The same procedures can proceed with large populations including the inter and intra generations. Based on the interpreted results, a reliable data set is developed within-silico tools which enable remote access to any individual in clarification of the problems in the industry (Zhao et al., 2020).

Marker-Assisted Selection (MAS)

This is an indirect livestock selection method that reduces time consumption and cost of selection. Marker-assisted selection offers the advantage of reducing the number of years it takes to introduce genetic improvements into a livestock species. As the markers and genes are correlated on the chromosome, they tend to have genetic linkage. This linkage helps scientists to predict whether an animal will have the desired gene. With this method, it is possible to breed animals in the early stages of their lives, even in the embryo stage, targeting superior traits. This technique is a novel and an advanced technique in which the relative breeding value of a parent is predicted using genotypes of markers associated with the trait. The principle behind this is the identification of genetic linkage between markers

and linked Quantitative traits loci (QTL) based on the distance between marker and target traits (Sobiech et al., 2022). The economic traits that regard production yield, hereditary traits, and other genetic traits are easily predicted using MAS (Kyselová et al., 2021).

Marker-Assisted Introgression

The use of molecular marker techniques in breeding increases the cost-effectiveness of the approach by reducing the lower reproductive rates and higher rearing costs (Causse & Charcosset, 2002). Through the use of marker technology, the genes are transferred and the desired phenotypes between the breeds, such as disease resistance genes, are controlled (Causse & Charcosset, 2002) The genes for specific traits can be transferred from indigenous breeds to improved breeds as animals carrying the favourable alleles would be selected for breeding and backcrossed again to the improved breed. By repeated backcrossing and selective breeding from the animals carrying the favourable disease resistance alleles, it is possible to recover the majority of the genome from the improved breed while maintaining the disease resistance that originated from the indigenous breed (Ajmone-Marsan et al., 2004).

Conclusion

The combination of molecular biology and the livestock industry has revolutionized agricultural science. The literature provides that the molecular based applications have created novel possibilities in animal identification and traceability approaches in the livestock industry. Though most of the literature provides information on molecular approaches, the practical implementation data is limited for animal identification and traceability systems. However, compared to the global level, local exposure to these techniques is low as a limited number of studies have been conducted. However, the utilization of molecular markers for livestock genetic research largely relies on the optimal selection of a suitable marker technique for a specified application (Teneva, 2009). The marker selection is species dependent. By means of ethical considerations molecular based identification and traceability is more effective than conventional methods of identification and traceability. The molecular marker-based identification enables the study of genetic profiles of a particular animal in sequence level. Within all the markers, the ISSR, RAPD, AFLP, and SSR have been used frequently in many studies due to their highly polymorphic, and hence, informative nature. Most of the studies stated the feasibility of the use of these markers due to their complex and varied mutational

patterns. According to many authors, ISSR and RAPD markers are a more accurate means of assessing overall genomic diversity in cattle and goat populations. Though there is a huge gap between literal data and practical aspects based on the findings of research communities, a reliable approaches can be implement locally (Yaro et al., 2017).

As a developing country which has a livestock industry with great potential, local exposure to the application of research and development strategies could result in remarkable and sustainable development and improvement in this sector.

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