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Molecular markers and their Potentials in Animal Breeding and Genetics

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Target Audience: Breeders, Government policy makers, academics

Abstract

Over the centuries, livestock improvements largely depend on the selective breeding of the individual animals with superior phenotype. The advent of DNA markers in recent years allowed for easy selection of a number of valuable traits more directly. Molecular markers have played a significant role in animal breeding and genetics by providing the opportunities in maximizing selection particularly for those traits that have low heritability or traits for which measurement of phenotype is difficult, expensive, or only possible in late life. Different types of molecular markers such as restriction fragment length polymorphisms (RFLPs), microsatellites (SSR), and single nucleotide polymorphisms (SNPs), have been widely used in molecular breeding as they can be amplified easily through polymerase chain reaction (PCR) and could be employed to estimate the genetic diversity within or between the breeding populations. These markers can be used simply as reference points in transgenic breeding to identify the animals with specific transgenes or to select the genes/genomic regions that affect economic traits through marker-assisted selection. Hence, the overall improvement in livestock species is greatly aided through the use of molecular markers.

Keywords: Molecular marker; livestock breeding; MAS; selection; Polymorphisms

Description of Problem

Animal breeding in its conventional form, largely depend on the phenotypic selection of an animal with superior trait within segregating populations derived from crosses. Other technologies, such as artificial insemination, multiple ovulation, and embryo transfer that aided reproduction have been employed and there has been a significant change in the productivity of animals from the selective breeding of animals(1, 2 and 3).However, in this practice, there are so many obstacles, especially in relation to genotype x environment (GE) interactions. Conventional breeding strategies in livestock production consume much of the times and do not consider all sources of genetic variability efficiently. Likewise, in those traits which are sex-limited, lowly heritable or late-expressed traits, the effect of conventional breeding is limited and in most cases, the techniques that were employed in the selection of phenotype

are usually expensive. In order to improve the animal breeding program, it is therefore important to balance molecular genetic techniques with conventional animal breeding techniques (4, 5 and 6). Recent advancement in the area of molecular biology and molecular technology has made it possible to disclose a large number of genetic polymorphisms at the DNA level. As such, scientists and researchers utilized them confidently as markers in order to determine the genetic basis for the observed phenotypic variability (7). The unique genetic attributes as well as methodological merit of genetic markers, make them essential and amenable, to a larger extent, for scientific research that is related to genetic when compared to other genetic markers (8-11). In term of the direct application, these DNA markers covered wide range of area some of which include: estimation of genetic distance, determination of twin zygosity and freemartinism, parentage determination, gene mapping, sexing of pre-implantation embryos and disease carrier identification (12). Molecular markers can be used simply as reference points in transgenic breeding and to point out the animals with specific transgenes. The overall improvement in livestock species is therefore achieved greatly by the application of molecular markers (3).

Genetic marker

Genetic marker is a general term used for any observable or assayable phenotype or the genetic basis for assessing of the detected phenotypic variability. Genetic markers are mainly classified based on physically evaluated traits (morphological and productive traits), or based on gene product (biochemical markers), and finally based on DNA analysis (molecular markers) (13).Molecular marker also known as DNA marker and is defined as a segment of DNA indicating mutations or variations, which can be employed to detect polymorphism (base deletion, insertion and substitution) between different genotypes or alleles of a gene for a particular sequence of DNA in a given population or gene pool.(3, 14).

They have characteristic biological attributes that can be determined and measured in various parts of the body such as the blood or tissue at any given stage of animal development (15, 16). and they are not influenced by environment, pleiotropic, or any epistatic effects (17).

For effective utilization in marker assisted breeding an ideal DNA marker according to (18) should meet the following criteria depending upon the utilization and species involved:

- High level of polymorphism (That is concurrent existence of a trait at the same population of two or more discontinues variants or genotypes.)
- Even distribution across the whole genome (not clustered in certain regions)
- Co-dominance in expression (Various form of marker must be identified in a diploid organism so that heterozygotes can be differentiated from homozygotes)
- Clear distinct allelic features (so that the different alleles can be easily identified)
- Single copy and no pleiotropic effect
- Low cost to use (or cost-efficient marker development and genotyping)
- Easy assay/detection and automation
- High availability (un-restricted use) and suitability to be duplicated/multiplexed (so that the data can be accumulated and shared between laboratories)
- Genome-specific in nature (especially with polyploids)
- There should be no any detrimental effect on phenotype

Classifications of Molecular Markers

Botstein et al (19), were the first to used DNA restriction fragment length polymorphism (RFLP) in human linkage mapping, since then significant progress has been made in the development and enhancement of molecular techniques that assist in finding the markers of interest simply on a large scale, this has resulted in extensive and successful utilization of DNA markers in human genetics, animal genetics and breeding, plant genetics breeding, as well as germplasm characterization and management. New powerful and easily available technique are recently introduced to discover more types of DNA markers. Different types of molecular markers used to identify the DNA polymorphism in livestock are commonly classified into three main groups base on the methods applied for their identification (14).

I. Non PCR-based or Hybridization-based Molecular Markers. The most common example of this type of marker is Restriction Fragment Length Polymorphisms [RFLPs].
II. PCR-based DNA Markers these includes; Random Amplified Length Polymorphic DNAs [RAPDs], Simple Sequence Repeats or microsatellites [SSRs], Amplified Fragment Length Polymorphisms [AFLPs].
III. DNA Chip and Sequencing-based DNA Markers. Single nucleotide Polymorphisms [SNPs] is an example of these types of markers.

I. Hybridization-based Molecular Markers

In hybridization based molecular marker technologies, cDNA, cloned DNA elements, or synthetic oligonucleotides are used as probes, which are tagged with radioisotope or with conjugated enzymes that catalyze a coloured reaction, to hybridize DNA. The DNA is either cut with restriction enzymes or is PCR amplified (14).

Restriction Fragment Length Polymorphisms (RFLPs);

Restriction Fragment Length Polymorphisms (RFLPs) was the first form of DNA marker utilized to construct the first true genomic map (20).This hybridization based marker technology employed synthetic oligonucleotides as probes, which are labelled fluorescently to hybridize DNA (13). RFLP technology was first developed by Botstein and his co-workers since 1980. This technology used the restriction enzymes that cleave the DNA at distinct site to observe the differences at the level of DNA structure (21). Differences are marked by using RFLP when the length of DNA segments are different, this imply that the RE (restriction enzymes) cleave the DNA at specific locations. The change or polymorphism that take place as result of mutation indicate creation or removal of the RE site and produce new RE site. The variations are determined by using hybridization probe. In RFLP analysis, the choice of the DNA probe is very crucial. Gel electrophoresis is needed for the identification of RFLPs to separate the DNA fragments of various lengths and to transfer the fragments into a nylon membrane in which the radioactive labelled probe is applied to observe the segments of DNA exposed to an X-ray Film (22).This technique is normally employed in hybridization definition of nucleic acid, detection and diagnosis, description of polymorphisms on the gene construction of a genetic linkage map and recombinant DNA technology in livestock species (6).

Some of the advantages RPLPs markers include; Production of co-dominant markers which enable the separation of homozygotic and heterozygotic conditions in a diploid organism. Selective neutrality, stability and reproducibility are the other outstanding

qualities of these markers. However, Application of RPLPs required higher quality and larger quantities of the starting DNA which is not always available. Additionally, the technique involved, is labor intensive and time consuming. Moreover, RFLPs are limited with regard to identification of the whole genome variation in animals and due low variability found in farm animals by inbreeding, renders many RFLPs sites un useful (14).

II. PCR-based molecular markers

Following the emergence of AFLP, new advanced technologies which utilizes PCR also emerged in 1990 (23).

PCR is a laboratory technique that involves the synthesis of nucleic acid simply by replicating a specific region of the target DNA (24).The technique uses two oligonucleotide primers that flank the desire DNA segment which is amplified after a series of repeated cycles. During the cycling process, the DNA is denatured by high temperature and the primers anneals to their complementary sequences at a temperature lower than that of denaturation. A thermophilic DNA polymerase is then used to completed extension of the annealed primers. As the extension products themselves are also complementary to primers, successive cycles of amplification basically twofold the target amount of generated DNA in the preceding cycle and the result is an exponential accumulation of a particular target fragment.

Genomic DNA from two distinct individual frequently produces different amplification and a specific fragment produced from one individual but not for other represent DNA polymorphism and can be employed as genetic markers. The obtained pattern of replicated bands could be used for genomic fingerprint (25).

Randomly Amplified Polymorphic DNA (RAPD):

Random Amplified Polymorphic DNA also called arbitrarily primed PCR (AP-PCR), or as a DNA amplification fingerprinting technique (DAF) and was introduced in 1990. This technology utilizes an *in-vitro* amplification to randomly amplify the unknown loci of nuclear DNA with a matching pair of short oligo-primers, (8-10 base pairs) in length (25, 26). Multiple primers in the range of (5 to 21) nucleotides are mostly used and has proven to be successful when detection is combined with polyacrylamide gel electrophoresis. The amplified products range from less than 10 to over a 100 depending upon the ratio and primer/template combination (14).RAPD technology had been used to estimates the genetic differences within or between the certain taxa of interest by evaluating the occurrence or lack of each product, which is directed by modification in the DNA sequence at each locus. Scientist have also reported the application RAPD markers for stock identification of *Nemipterus japonicas(27).*

The simple procedure of RAPD involves;

- DNA extraction (highly quality DNA)
- Addition of single random primer,
- Amplification by (PCR,
- Separation DNA fragments via electrophoresis for
- Ethidium bromide staining and visualization of RAPD PCR fragments under UV light
- Determination of different fragment size by comparing with known molecular marker using gel analysis software.

As compared to RFLP, RAPD technique offers a quick, simple, cheap but an efficient technique of producing molecular information. Being highly polymorphic, only very small quantity of DNA is needed to be amplified by PCR technique in the absence of DNA

sequence information. This is the major reason why RAPD technique has been used successfully in various phylogenetic and taxonomic researches (14).

However, one major disadvantage of RAPD technique is that the RAPD primers are very sensitive to PCR conditions and this may lead to poor reproducibility with respect to other methods. Additionally, the result of amplification profile by each primer frequently covers numerous different loci within the genome, which essentially is unable to distinguish between heterozygous and homozygous individuals (28).

Amplified Fragment Length Polymorphism (AFLP);

AFLP method is a simple and inexpensive finger printing technique which provide more valuable information by producing multi-locus and consistent genomic fingerprints (29). the basic idea behind AFLP polymorphism was the insertion and deletion or substitution of nucleotides between and at restriction sites. The base substitutions are normally done at primer binding sites during PCR as in the case of RAPD. This technique is distinctive, as it enables the binding of adaptors of known sequences to DNA segments that are produced through the complete digestion of genomic DNA. This ensure easy separation of the generated DNA fragments following amplification the subset of entire fragments. Though, the main goal of AFLP is the same as that of RFLP that is polymorphism, however as an alternative method of analyzing one locus at a time, it permits concurrent study of many loci (30, 31).

The major steps involve in AFLP techniques are;

- ❖ Extraction of highly quality DNA,
- ❖ Digestion of DNA using restriction enzymes (enzyme mixture, commonly Eco RI + MseI),
- ❖ Adapters ligation (enzyme adapters),
- ❖ Pre-PCR amplification of the digested fragments; pre-selective amplification using EcoRI primer + A and MseI primer + C
- ❖ Selective PCR by labeled pair of primer (primer + three base pairs; for used labeled, reverse unlabeled)
- ❖ Analysis of fragment using automated sequencing machine following gel electrophoresis. A programs such as Gene Mapper (AFLP,2005) can be used for the analysis of the electrophoretograms (11).

AFLP technique offer an effective, fast and cost-effective means for detecting a large number of polymorphic genetic markers which are very consistent and reproducible, and are capable of being genotyped automatically. The technique is considered as the most effective method for molecular epidemiological studies of pathogenic microorganisms and it is also used extensively in forensic science.

The AFLP technology has been widely utilized in identification of genetic polymorphisms, evaluating and characterizing breed resources, measuring the correlation among breeds, constructing genetic maps and identifying genes in the main species farm animals (30-35). Apart from microsatellites, AFLP technology remained the best molecular system for population genetics and genome typing (14). Despites the outstanding features of AFLP, yet the technique has some limitations some of which include; demanding of more DNA (300-1000ng per reaction) and is technically more difficult as compared to RAPD. however, the recent availability of kits and automation will possibly bring the technique into a higher level of applicability (36).

Microsatellite marker/ single sequence repeat (SSR)

Microsatellites are polymorphic loci present in DNA and contain 1-6-nucleotide

repeats which are arranged head to tail without interruption by any other base or motif and are dispersed throughout the genome (37). or they are DNA segments characterized by a different number of copies (usually 5-50) of sequence motifs of around two to five (2-5) bases (known as repeat unit) (38). They are highly polymorphic and plentiful, usually found in non-coding regions of genes (39-41). The most common dinucleotide motif in mammals is (CA)*n*, where *n* is the number of repeats (42). In avian species, the frequency of $(CA)_{\geq 10}$ is evaluated at once every 140 to 180 kb, and that of $(CA)_{\geq 14}$ is one every 350 to 450 kb(43). Microsatellites loci are also termed as short tandem repeats (*STR's*), simple sequence repeats (*SSR's*) and simple sequence tandem repeats (*SSTR*). Scientists have also suggested the terms minisatellites and macro satellites to define the regions with increasing larger repeat units and overall lengths. The microsatellites and minisatellites altogether make up the variable number of tandem repeats (VNTRs). The lengths of a specific microsatellite sequences tend to be highly variable among individuals. These variations make up the molecular alleles (14). Microsatellite-derived markers exhibit a powerful way of mapping genes controlling more valuable traits. As soon as the simple repeat region is identified, by sequencing its immediate flanking regions, specific primers can be designed for PCR and for genotyping. Normally, the size of a microsatellite PCR product is obtained by electrophoresis in a denaturing polyacrylamide gel. One of the two primers employed in the PCR is often labelled with a fluorescent or radioactive tag. This technique of detection usually works better but suffers from an inherent weakness in determining the size of DNA accurately. More recently, there is an alternative to the gel-based approach to determine the size of DNA products; there are a series of technological advancement based on mass spectrometry. One new trend is the application of MALDITOF (Matrix Assisted Laser Desorption Ionisation Time of Flight) mass spectrometry, as a swift and promising way of differentiating polymorphic DNA fragments (44). Their co-dominant nature coupled with high mutation rate make them the most precious marker in the estimation of genetic diversity within and between breed (45).

Recently microsatellites markers are the most valuable genetic markers in livestock genetic characterization studies (46, 47). These markers offers a series of benefits over other types of markers, specifically, that multiple SSR alleles can be identified at a single locus by utilizing simple PCR based screen, very less amounts of DNA are essential for screening, and analysis is amenable to automated allele identification and sizing (48). Greater understanding of genomes and genomics has been achieved through the studies of the potential biological function and evolutionary relevance of microsatellites (49). Microsatellites markers were mainly considered to be evolutionally neutral (39). More recent data assumes that they microsatellites can possibly play a significant role in genome evolution (41) and provide points of recombination. They are presumed to take part in gene expression, regulation and function (50, 51) and have been found to hybridize nuclear proteins and function as transcriptional activating elements (52), scilicet they suggested to have an important functional role. The option of microsatellites marker in livestock improvement than RFLP marker, is due the large variety of its molecular applications which includes, genetic characterization studies, analysis of population structure (53), estimation of genetic variability and inbreeding (54),determination of paternity (55), phylogenetic relationships among populations (56), disease diagnostics, forensic analysis, development of genetic map (quantitative trait loci) and Marker assisted

breeding among others (9, 27, 57-59). However, one of the major drawback of this technique is that it is very expensive and time-consuming. Moreover, Heterozygotes can mistakenly be classifying as homozygote when there is occurrence of null-alleles as result of mutations in the primer annealing sites. The accurate scoring of polymorphisms can also be complicated due to shutter bands. microsatellite markers are helpful in identifying neutral biodiversity however, they cannot tell about the information concerning the functional traits biodiversity (14).

III. DNA chip and sequencing-based molecular Markers

Single nucleotide polymorphisms (SNPs

Single nucleotide polymorphism is a variation in DNA sequence which occur due to a substitution in the nucleotide at a particular location in the genome. (38) In other words, SNP marker is just a one base replacement in a sequence of DNA (6). SNPs comprise more than 90% of all variances between the individuals; thus, they are the excellent genetic variation resource for population studies and genome mapping (60). Genomic selection by means of the SNP markers is a powerful novel tool for genetic selection (61). These type of marker are becoming highly attractive in molecular marker development due to their abundance in the genome of any organism (coding and non-coding regions), and ability to identifying hidden polymorphism which is not commonly recognized by other genetic markers and techniques (62). Currently, there have been a series of progress in complete genome sequencing, in the development of next generation sequencing technologies and high throughput genotyping platform. Improvement in these technologies has resulted in the development of the high-density single nucleotide polymorphism (HD-SNP) arrays as an up-to-the-minute implement for the genetic and genomic analysis of farm animals (63).

There are four major reasons for the increasing interest in the utilization of SNPs as markers for genetic analysis. Initially, they are widespread and provide more potential markers near or in any locus of interest than other types of polymorphism such as microsatellites. For instance, in human genomic DNA there appears to be an SNP approximately every 1000 bases (64). Secondly, some SNPs are located within the coding regions and directly influence the protein function. These SNPs may be directly responsible for some of the differences among the individuals in economical traits. Thirdly, SNPs are inherited more stable than microsatellites, this attributes make them more promising as long-term selection markers. Lastly, SNPs are more reliable than microsatellites for high throughput genetic analysis, using DNA microarray technology (65). However, SNPs are generally biallelic systems, meaning that there are usually only two alleles in a population. As a result, the information content per SNP marker is less than multiallelic microsatellite markers.

SNPs can be detected by a series of techniques. The common gel-based approach employs the standard molecular techniques, i.e sequencing, PCR, restriction digests and different forms of gel electrophoresis, such as denaturing gradient gel electrophoresis (DGGE), single-strand conformation polymorphism (SSCP) and cleavage fragment length polymorphism (CFLP) (66-69),also introduced a well-designed method, cleaved amplified polymorphic sequence (CAPS) that utilized mismatch PCR primers to produce new restriction site on one of the alleles. Other technique includes the PCR-based TaqMan assay (64), the high-performance liquid chromatography-based WAVE DNA fragment analysis system(70), and MALDI-TOF mass spectrometry, including the utilization of

peptide nucleic acid probes (71). One mass spectrometry approach employs a distinct primer to extend into the SNP site using di-deoxynucleotides. The SNP is defined by the particular di-deoxynucleotide incorporated (72). All these methods require previous knowledge of the sequence of the polymorphic site.

One way to detect polymorphisms with a less mismatches of nucleotide, is the random amplified polymorphic DNA (RAPD) assay (73). This assay uses short oligonucleotide primers of arbitrary sequence to amplify distinct regions of the genome. Typically, only one 10-mer oligonucleotide of a particular arbitrary sequence is used in each PCR. RAPD polymorphisms are detected after gel electrophoresis as the presence or absence of particular fragments between the individual animals. The disappearance of a particular fragment is the result of sequence differences in one or both of the priming sites which keeps oligonucleotide from annealing and subsequent polymerization. A typical arbitrary oligonucleotide will produce up to a twelve or more discrete DNA bands.

The utilization of DNA microarray or 'DNA-on-Chip' technology to detect SNPs is a potentially strong tool for high throughput DNA screening (64). Through this technique, sequences up to 40000 can be screened on a single slide at one time. Study have also shown that, 2.3 Mb of genomic DNA scanned were successfully identified up to 3241 candidate SNPs, using more than 100 tiling microarrays.(65). In July 2010, Illumina released two new genotyping SNP chips including a low-density chip (Bovine3K) having 2,900 SNP (74) and a larger density chip (BovineHD) having up to 777,962 SNP and in January 2011, another a high density chip with 648,855 SNP was released (74). Even though, such chips can give the desire genotypes that improve the precision of genomic evaluation by better tracking of the loci responsible for genetic variation (75). SNP chips are recently available for human, ovine, bovine, canine, porcine and equine species (76). However, for the application of DNA chips technology into large scale genotyping of animal, there are series of major technical problems that need to be taken into account (77). These include the presence of secondary structures in the target and the difficulty that is associated with optimizing hybridization conditions over the complete array as result of differences in annealing temperature among oligonucleotides. The preparation of labelled genomic DNA segment with adequately high specific activity for hybridization to immobilized oligonucleotides can also be problematic, especially if a high number of loci are to be screened altogether. The major challenge, however, is to find out valuable SNPs that can estimate the breeding value of an animal. Recently, the association of ten pharmaceutical companies and The Welcome Trust have announced an initiative to produce a human SNP map. While detecting the presence of a known SNP is relatively simple, the major antagonist in this desirous attempt must hence be the identification of new SNPs at regular intervals throughout the human genome (78). It is therefore improbable that SNP mapping in farm animals will ever be carried out on this scale in the anticipated future. Nonetheless, DNA microarrays and SNP development are extremely applicable for future selection of livestock. In similar manner that microsatellites have substituted RFLPs, SNPs are anticipated to replace microsatellites as the technique of choice for detection of DNA polymorphisms.

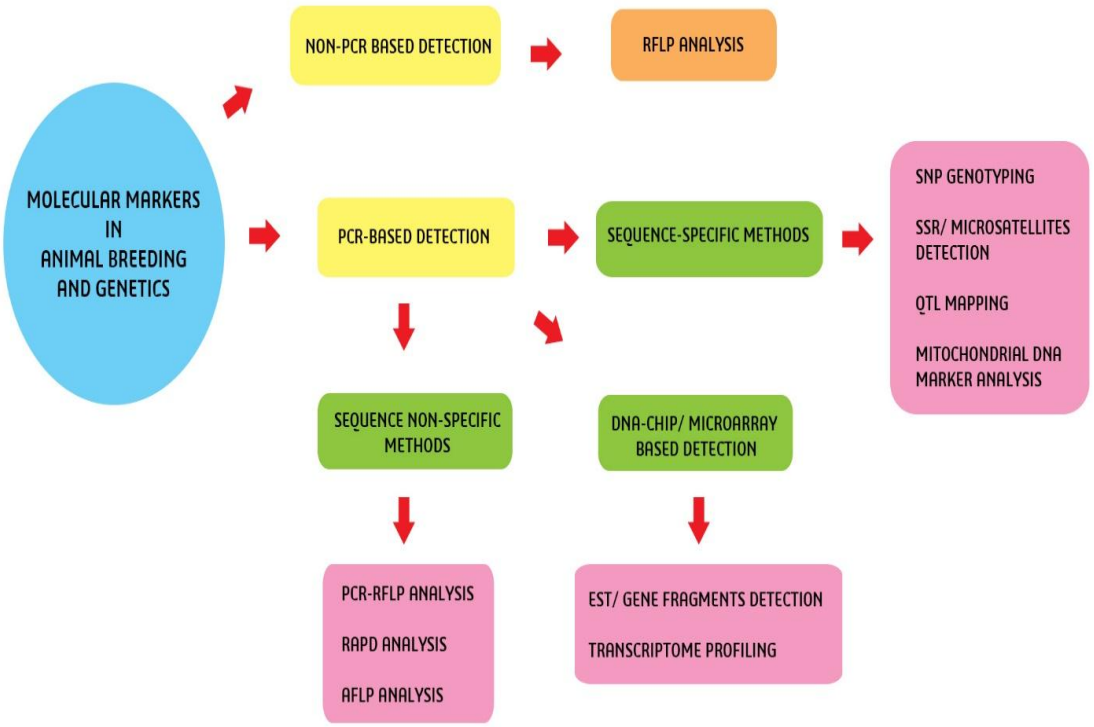

Figure 1. Summary of the various molecular markers technologies

Table 1.0 Common type of molecular markers and their important features

FEATURES	RFLP	RAPD	AFLP	SSR	SNP
DNA Require (µg)	10	0.02	0.5-1.0	0.05	0.05
PCR based	No	Yes	Yes	Yes	Yes
Number of Polymorph loci analysed	1-3	1.5-50	20-100	1-3	1
Type of polymorphism	Single base change, insertion, deletion	Single base change, insertion, deletion	Single base change, insertion, deletion	Change in repeat length	Single nucleotide change, insertion, deletion
Dominance	Co-dominant	Dominant	Dominant/Co-dominant	Co-dominant	Co-dominant
Reproducibility	High	Unreliable	High	High	High
Ease of use	Not easy	Easy	Easy	Easy	Easy
Automation	Low	Moderate	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low
Developmental cost	High	Low	Moderate	High	High
Need for sequence data	Yes	No	No	yes	Yes
Accuracy	Very High	Very Low	Medium	High	Very High
Radioactive detection	Usually yes	No	No	No	Yes
Genomic abundance	High	Very High	Very High	Medium	Medium
Part of genome surveyed	Low copy coding regions	Whole genome	Whole genome	Whole genome	Whole genome
Level of polymorphism [1]	Low	Low to moderate	Low to moderate	High	High
Effective multiplex ratio [2]	Low	Medium	High	Medium	Medium
Marker index [3]	Low	Medium	High	Medium	Medium
Inheritance	Co-dominant	Dominant	Dominant	Co-Dominant	Co-Dominant
Detection of alleles	Yes	No	No	Yes	Yes
Utility for genetic mapping	Species specific	Cross specific	Cross specific	Species specific	Species specific
Utility in Marker assisted selection	Moderate	Low to Moderate	Low to moderate	High	Low to moderate
Cost and labour involved in generation	High	Low to Moderate	Low to moderate	High	High

[1]Level of polymorphism (average heterozygosity) is an average of the probability that two alleles taken at random can be distinguished; [2]Effective multiplex ratios is the number of polymorphic loci analysed per experiment in the germplasm tested; [3]Marker index is the product of the average expected heterozygosity and the effective multiplex ratio. **Adapted from:** (1, 23, 79-82).

Potentials applications of Molecular Markers in Live Stock Improvement

Through conventional breeding techniques, molecular markers can play a very significant role for the improvement of livestock. These roles could be categorized as immediate (short range) or long term applications. (10).

Immediate applications:

Molecular markers have various immediate applications some of which includes: Estimation of genetic distance, parentage determination, determination of twin zygosity and freemartinism, sexing of pre-implantation embryos, disease carrier identification, and gene mapping as well as marker-assisted selection (12). The short range applications of molecular markers were briefly descried in the following sub-suctions below:

These short range application can be describing briefly in the following sub-suctions below:

Estimation of genetic Diversity:

Diversity among organisms is as a result of variations in DNA sequences and environmental effects. Genetic distance a measure of overall evolutionary divergence between species, breeds, strains. Among various markers, microsatellites are the most popular markers in livestock genetic characterization studies (47). Due to their high mutation rate and codominant nature that allow the estimation of within and between breed genetic diversity. The relationships between populations and individuals are estimated through the genetic distances (83-87). Genetic distance is measure based on polymorphic characters at the different levels (morphological, biochemical, cellular and DNA level). Currently single nucleotide polymorphism (SNPs) is used to determine the genetic variation and relationships within and between populations and make it possible to genetically examine differences and determine special genomic attributes of indigenous livestock populations (88-90).

Parentage determination

The knowledge on mating system of species is important for successful breeding management because breeding value is usually estimated from off-springs and relatives. To determine genetic relatedness among individuals and assess kinship molecular marker are used as tools for investigating issues such as identifying the paternity of animals generated through multiple-sire pasture mating. five microsatellite loci were used to verify the genetic information of parentage in an ex situ population of marabou storks and interpret behavior during the breeding season using genetic pedigree (91). Scientist have proven the feasibility of using parentage tests to correctly identify animals generated by multiple sire mating (92, 93).

Sexing of pre-implantation embryos

Determination of the sex of the pre-implanted embryos is important for the management and breeding of livestock as well as for the prenatal diagnosis of livestock disorders. The genetic sex of an individual depends on whether the X-bearing ovum is fertilized by a Y or X- bearing spermatozoa. Among several established protocols for sexing farm animals, Molecular markers are used to determine sex of pre-implantation embryos, based on the identification of the Y chromosome, such as SRY, ZFY and TSPY genes. Study have showed that TSPY was a good male-specific marker, the usefulness of which was enhanced by the high copy number of the gene (94). Scientist have reported the simultaneous amplification of sequences corresponding to both X- and Y- amylogenic gene to establish a reliable, reproducible and efficient PCR-based goat sexing system (95).

Disease carrier identification

Genetic diseases resulted from vertical transmission of defective genes to the offspring, when this allele is recessive, heterozygotic (carrier) animals have a normal phenotype but can pass the genetic defect to their off-springs, the defective allele carries a mutation that results in the synthesis of a non-functional protein variant, leading to developmental or metabolic disorders which can lead to significant losses in agricultural yield during animal husbandry. Several molecular markers have been used for early identification and linked to various disease in livestock due to DNA polymorphism that occurs within a gene. It aids the understanding of the molecular mechanism and genetic control of several genetic and metabolic disorders (96-98). Likewise, allow the identification of heterozygous carrier animals which are otherwise phenotypically indistinguishable from normal individual. Studies on ARMS PCR-based assay for detection of a novel single-nucleotide polymorphism in the 5' untranslated region of the bovine ITGB6 receptor gene associated with foot-and-mouth disease susceptibility concluded that SNP G29A mutation in the 5 UTR of the ITGB6 gene (chromosome 2) associated with resistance to FMD infection in the zebu cattle (96). Studies on susceptibility to Para tuberculosis infection demonstrated that the TLR2-1903 T/C SNP was significantly associated with resistance to MAP in Holstein-Friesian cows (97).A correlation study of CARD15 gene style polymorphism and susceptibility of tuberculosis detected G1596A polymorphism in the TLR1 gene and found it to be associated with BTB infection status in Chinese Holstein cattle (98).

Long term application of molecular markers;

The most common long term application of molecular markers in livestock improvements includes; Quantitative trait loci (QTL) mapping through linkage. Such mapping information if obtain, especially for those loci that influence the trait performance or vulnerability/tolerant to disease, can be employed in breeding programmes either by manipulations within the breed E.g. Marker assisted selection of young sires, or between the breed introgression programmes (99).

Gene mapping

The three major applications of molecular markers have been identified in relation to gene mapping. Firstly, molecular marker allows for direct identification of a desire gene instead of gene product and the identified gene can be efficiently used as a tool for somatic cell hybrid screening. Secondly, through the use of different DNA probes and easy-to-screen techniques, a molecular marker can be helpful in physical mapping of genes utilizing an in situ hybridization. Finally, molecular markers offer a sufficient markers system for construction of genetic map by applying linkage analysis (100). Markers such as RFLPs, which present the evolutionary conserved cording subsequences are very essentials in comparative mapping methods where polymorphism is not necessary. But these are usually one locus and di-allelic and are therefore not essential for linkage analysis. Conversely, markers such as microsatellite which exhibits higher polymorphism information content than ordinary RFLPs and can be generated more quickly and easily. As such, more efforts were put generate gene maps on the basis of such type of markers. Additional application of molecular markers obtained via DNA sequences data namely ASO (Allele specific oligonucleotide) and STMS (Sequence tagged microsatellite site) polymorphic markers are similarly useful in rapid development of gene mapping (99).

Marker assisted selection (MAS)

The recent advancement of molecular techniques has opened up the introduction of genes to animal breeding which were not available before through conventional breeding, creating a lot of interest about MAS (Marker assisted selection). The recent application of molecular genetic technologies increasingly presents the way to choose the breeding animals at an early stage (even embryo); to select for a superior variety of traits (99). Marker assisted selection (MAS) is a novel technique that complement traditional breeding methods in which the relative breeding value of a parent is predicted using genotypes of markers associated with the trait for rapid genetic gains towards achieving long term animal improvement. It depends on identifying association between genetic marker and linked Quantitative traits loci (QTL) based on the distance between marker and target traits. Selection for recessive genes and mutants is faster because an individual's phenotype can be predicted at a very early stage. Sex-limited traits (milk yield, egg production), low heritability traits, traits lacking selection response and genetic gain in conventional selection are easily predicted with Marker assisted selection (MAS). High linkage disequilibrium, a large population a large number of markers per chromosome, genomes containing a large number of chromosomes high heterozygote frequencies at the relevant genes and markers and markers that are located close to quantitative trait loci (QTLs) with large effects are expected have higher response to MAS. QTL involved in behavior of diary and udder conformation was identifies on chromosome 6 that could form the basis of QTL for clinical mastitis (101). Body conformation traits such as stature and body depth affect feed intake and thus milk production, while udder traits correlate with the incidence of clinical mastitis and the length of productive life. By hybridization between half-sib families, significant QTL for birth weight were identified in the centromeric region on specific bovine chromosome (102, 103). Marker-assisted selection based on a multi-trait economic index in chicken was also reported by (104). Studies on genetic relationships between economic traits and genetic markers were conducted in 147 goats demonstrated superior RAPD markers of body weight and Cashmere yield and superior one of body weight and Cashmere fineness (105).

Conclusions and applications

1. Molecular markers have revolutionized the agricultural science including animal breeding and genetics specifically.
2. Genetic polymorphism of DNA has led to the discovery of various marker techniques with a number of applications in the applied livestock breeding research. However, utilization of these markers for livestock genetic research largely relies on the optimal selection of a suitable marker technique for a specified application.
3. Molecular markers when compared with the conventional animal breeding techniques, provide more accurate genetic information and better knowledge of the animal genetic resources.
4. These markers hold great potentials for the Nigerian livestock breeding programmes. However, various bottlenecks such as poor infrastructure, inadequate capacity and operational support, lack of an enabling policy, the statutory and regulatory framework at the country level, which in turn affects research institutions could be the major reasons that will impede the proper adoption of these techniques.

5. In the near future, it is anticipated that the development of molecular marker will enormously continue in the developing countries like Nigeria, so as to serve as an underlying tool for geneticists and breeders that will be useful in the production of animals with desirable traits for human use.

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