

A ball-lock type separation system has been attached to the bottom plate of the satellite in order to eject the satellite with a separation velocity of approximately 1 m/s. The unit with a pitch circle diameter of 23 cm is supplied by Antrix/ISRO.

PSLV injected DLR-TUBSAT, after the separation of both IRS-P4 and KITSAT-3, about 127 s after cut-off the 4th stage.

During the launch phase however, the NiH_2 batteries were discharged for safety reasons. After separation from the launcher, as per the plan, the solar panels are used to recharge the batteries within the following 7 h. However, contrary to the planned schedule, the DLR-TUBSAT was tracked on the 2nd orbit itself and the telemetry was received over the university ground station in Berlin. Since then, the satellite has been functioning very well and good pictures are being received from the on-board cameras.

Conclusion

With the demonstration of the fourth consecutive successful launch, PSLV has established its versatility. It

can provide multiple launches to Low Earth Orbit (LEO), polar sun-synchronous missions and also for Geo-synchronous Transfer Orbit (GTO) missions. The payload capability is more than 3000 kg in 400 km LEO, up to 1200 kg polar at 800 km altitude and 850 kg in GTO. Further planned improvements of PSLV are:

- A high performance upper stage by this year end which would increase the payload capability further in all the above orbits.
- A single engine version of the 4th liquid stage to increase the payload volume inside the heat shield by about 25%.
- A dual launch adapter to utilize the above-increased payload volume to enable the launch of a mix of payloads

With the above and the PSLV-C2 mission success, PSLV has opened the way for dedicated commercial launch services for bigger and heavier satellites.

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Molecular markers and their applications in livestock improvement

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Recent developments in DNA technologies have made it possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use them as markers for evaluation of the genetic basis for the observed phenotypic variability. The markers revealing variations at DNA level are referred to as the molecular markers. Based on techniques used for detection, these markers are classified into two major categories: Hybridization-based markers and PCR-based markers. The molecular markers possess unique genetic properties and methodological advantages that make them more useful and amenable for genetic analysis compared to other genetic markers. The possible applications of molecular markers in livestock improvement have been reviewed with reference to conventional and transgenic breeding strategies. In conventional breeding strategies, molecular markers have several short-range or immediate applications and long range applications. In transgenic breeding, molecular markers can be used as reference points for identification, isolation and manipulation of the relevant genes, and for identification of the animals carrying the transgenes. The progress in development of molecular markers suggests their potential use for genetic improvement in livestock species.

THE progress in recombinant DNA technology and gene cloning during the last two decades has brought in

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revolutionary changes in the field of basic as well as of applied genetics by providing several new approaches for genome analysis with greater genetic resolution. It is now possible to uncover a large number of genetic polymorphisms at the DNA sequence level, and to use

them as markers for evaluation of the genetic basis for the observed phenotypic variability. Though theoretically, DNA sequencing is the direct approach to reveal such DNA polymorphism, it has two practical limitations: (i) sequencing needs initial cloning of the gene or DNA fragment at which allelic variation of interest exists, and (ii) it requires suitable and cost-effective method for scoring DNA sequence variation. However, the indirect approach for uncovering of genetic variation at the DNA level using molecular or DNA markers obviates the above limitations.

Since the first demonstration of DNA-level polymorphism, known as the restriction fragment length polymorphism (RFLP)¹, an almost unlimited number of molecular markers have accumulated. Currently, more powerful and less laborious techniques to uncover new types of DNA markers are steadily being introduced. The introduction of polymerase chain reaction (PCR)² in conjunction with the constantly increasing DNA sequence data also represents a milestone in this endeavour. The present review is a brief account of molecular markers, and their various applications in livestock improvement.

Molecular markers and their different types

A marker is usually considered as a constituent that determines the function of a construction (Webster's Dictionary). Genetic marker can be defined as any stable and inherited variation that can be measured or detected by a suitable method, and can be used subsequently to detect the presence of a specific genotype or phenotype other than itself, which otherwise is nonmeasurable or very difficult to detect³. Such variations occurring at different levels, i.e. at the morphological, chromosomal, biochemical or DNA level can serve as the genetic markers. The markers revealing variations at the DNA level are referred to as the molecular markers, and on the basis of techniques used for their detection, these have been classified into two major categories: Hybridization-based markers and PCR-based markers.

The hybridization-based markers

These include the traditional RFLP analysis⁴, where appropriately labeled probes for genes of importance (e.g. cDNA or genomic DNA sequences) are hybridized onto filter membranes containing restriction enzyme (RE)-digested DNA, separated by gel electrophoresis and subsequently transferred onto these filters by Southern blotting. The polymorphisms are then visualized as hybridization bands. The individuals carrying different allelic variants for a locus will show different banding patterns. Hybridization can also be carried out with the probes (e.g. genomic or synthetic oligonucleotide) for

the different families of hypervariable repetitive DNA sequences namely, minisatellite⁵, simple repeats⁶, variable number of tandem repeats (VNTR)⁷, and microsatellite⁸ to reveal highly polymorphic DNA fingerprinting patterns (DFP).

The PCR-based markers

These have, however, removed the necessity of probe-hybridization step, and have led to the discovery of several useful and easy-to-screen methods. Depending on the type of primers (i.e. primers of specific sequences targeted to a particular region of a genome or primers of arbitrary sequences) used for PCR, these markers can be further sub-divided into the following two groups:

(i) The sequence-targeted PCR assays: In this assay system, a particular fragment of interest is amplified using a pair of sequence-specific primers. In this category, PCR-RFLP or cleaved amplified polymorphic sequence (CAPS) analysis is a useful technique for screening of sequence variations that give rise to the polymorphic RE sites (Figure 1). Such analysis involves amplification of a specific region of DNA encompassing the polymorphic RE site, and digestion of the amplified DNA fragment with the respective RE. However, for the screening of the sequence variations that do not lead to creation or abolition of restriction sites, other approaches namely allele specific PCR (AS-PCR)⁹, PCR amplification of specific alleles (PASA)¹⁰, allele specific oligonucleotide (ASO) hybridization assay¹¹, amplification refractory mutation system (ARMS)¹², and oligonucleotide ligation assay (OLA)¹³ are used. These assays are based on the principle of high specificity of



Figure 1. PCR-RFLP of bovine kappa-casein (CSN3) locus. Using sequence-specific primer pair (K1 and K2) a 379 bp fragment (encompassing exon IV and a part of intron V) of CSN3 gene containing a polymorphic *Hind*III site was amplified. The amplified fragment was digested with *Hind*III, electrophoresed in 3% Nusieve agarose gel and visualized under UV light after staining with ethidium bromide. When the *Hind*III site is present, as in case of CSN3 B allele, the amplified fragment is cleaved into two fragments of 156 and 223 bp. In case of CSN3 A, the amplicon remained undigested indicating the absence of *Hind*III site. Genotypes of the sample are shown above the lanes. (Source: ref. 83)

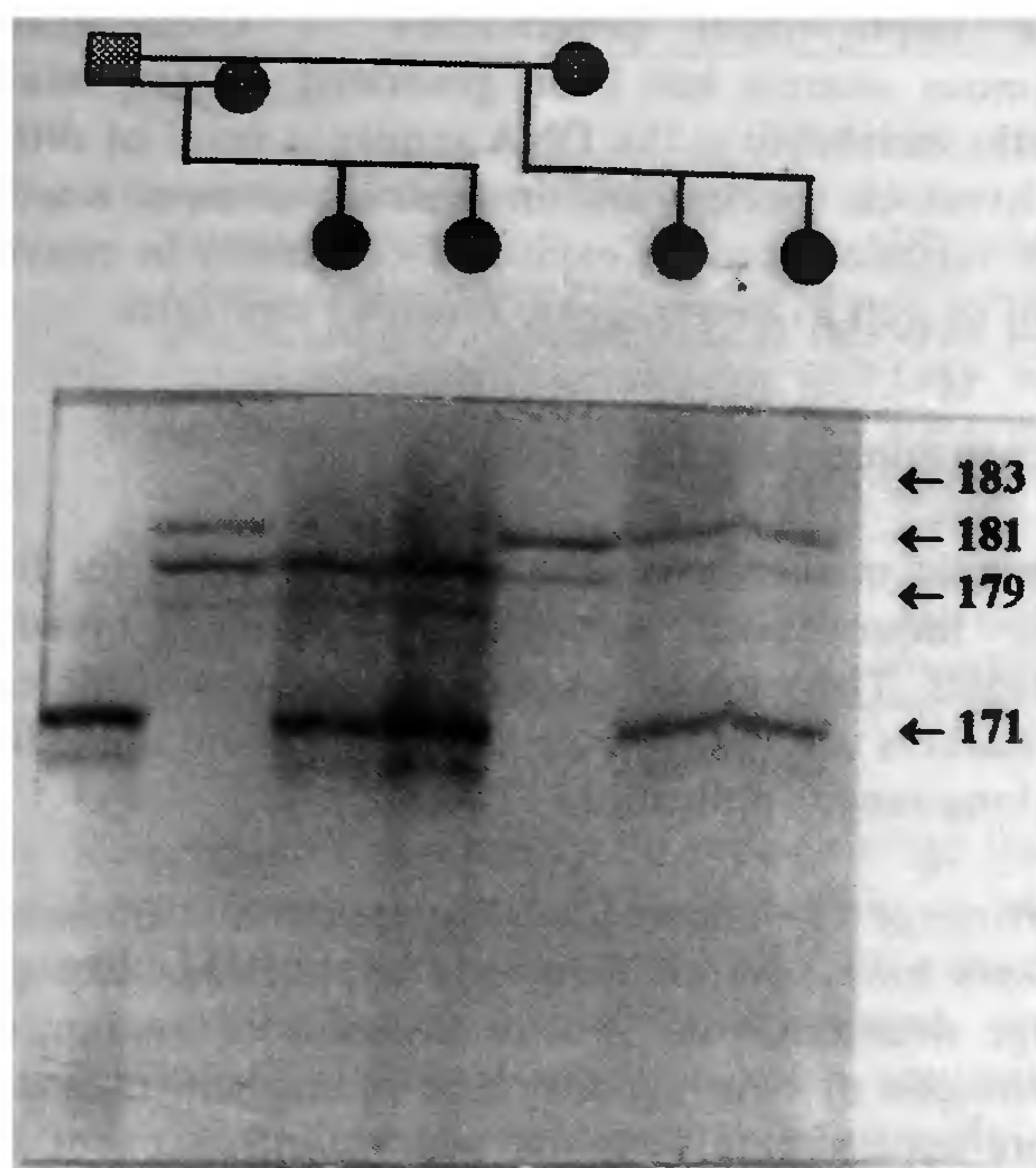


Figure 2. Autoradiograph of gel showing di-nucleotide repeat microsatellite polymorphism in goat. Genomic DNA samples were amplified using bovine BM7160 primer pair. α - ^{32}P dCTP was added in reaction mixture. Amplified products were resolved by denaturing acrylamide gel (7%) electrophoresis and visualized by autoradiography. (Source: ref. 84)

PCR to selectively amplify specific alleles using primers that match the nucleotide sequence of one, but mismatch the sequence of other allele. The sequence-targeted PCR approach is also employed to reveal simple sequence length polymorphism (SSLP), using a pair of primers that flank the simple sequence repeat (SSR) motifs (Figure 2). If cloned and sequenced microsatellite loci can be subjected to PCR amplification and such microsatellite loci recovered by PCR, they are termed as sequence-tagged microsatellite site (STMS)¹⁴ markers. Microsatellite markers in STMS format can be completely described as information in databases that can serve as common reference points and will allow the incorporation of any type of physical mapping data into the evolving map¹⁵.

(ii) The arbitrary PCR assays: In this assay system, however unlike the standard PCR protocol, randomly designed single primer is used to amplify a set of anonymous polymorphic DNA fragments. It is based on the principle that when the primer is short (usually 8 to 10 mer), there is a high probability that priming may take place at several sites in the genome that are located within amplifiable distance and are in inverted orientation. Polymorphism detected using this method is called randomly amplified polymorphic DNA (RAPD)¹⁶. Based on this principle, several techniques, which do not require any prior sequence knowledge, have been devel-

oped. However, they differ in number and length of primers used, stringency of PCR conditions, and the method of fragment separation and detection. In arbitrary primed PCR (AP-PCR)¹⁷, slightly longer primer is used (e.g. universal M13 primer) and amplification products are detected by radioactive or nonradioactive method following polyacrylamide gel electrophoresis. In DNA amplification fingerprinting (DAF)¹⁸ analysis, shorter primer is used (5 to 8 mer) which reveals relatively greater number of amplification fragments by polyacrylamide gel electrophoresis and silver staining. All these techniques having similar features can be described by a common term multiple arbitrary amplicon profiling (MAAP)¹⁹. Besides these, a number of modifications of the basic MAAP assays (namely, template endonuclease cleavage MAAP and RAPD-RFLP) have been developed as well.

In addition to arbitrary primers, semi-arbitrary primers designed on the basis of RE sites or sequences that are interspersed in the genome such as repetitive sequence elements (*Alu* repeats or SINEs), microsatellites and transposable elements are also used. In the amplified fragment length polymorphism (AFLP) assay²⁰, template DNA is digested with two REs, and the resulting restriction fragments are then ligated with adapters and, subsequently, PCR amplification is carried out using specially designed primers which comprise (i) a unique part corresponding to selective bases; and (ii) a common part corresponding to the adapters and the RE site. Microsatellite-primed PCR (MAP-PCR) assay is carried out using microsatellite as the primer²¹.

Properties of molecular markers

In genetic analysis, various types of genetic markers such as morphological, chromosomal, biochemical and molecular markers are used. Morphological (e.g. pigmentation or other features) and chromosomal (e.g. structural or numerical variations) markers usually show low degree of polymorphism and, hence, are not very useful as genetic markers. Biochemical markers have been tried out extensively, but have not been found encouraging as they are often sex limited, age-dependent, and are significantly influenced by the environment. The various genotypic classes are indistinguishable at the phenotypic level owing to dominance effect. Furthermore, these markers reflect variability in their coding sequences that constitute less than 10 per cent of the total genome. The molecular markers, capable of detecting the genetic variation at the DNA sequence level, have not only removed these limitations but also possess unique genetic properties that make them more useful than other genetic markers. Moreover, they are numerous and distributed ubiquitously throughout the genome. These follow a typical Mendelian inheritance which

usually expresses in a co-dominant fashion, and are often multiallelic giving mean heterozygosity of more than 70 per cent. They remain unaffected by the environmental factors, and generally do not have pleiotropic effects on quantitative trait loci (QTL)²². Since gene expression is not a prerequisite, virtually the entire genome including the noncoding regions can be visualized.

For genetic analysis, molecular markers offer several methodological advantages that are both attractive as well as amenable. For example: (i) the DNA samples can not only be isolated very conveniently from blood of live individuals but can also be isolated from tissues like sperm, hair follicle, and even from archival preparations, (ii) the DNA samples can be stored for longer periods and can readily be exchanged between the laboratories, (iii) the analysis of DNA can be carried out at an early age or even at the embryonic stage, irrespective of the sex, (iv) once the DNA is transferred on to a solid support, such as filter membrane, it can be repeatedly hybridized with the different probes, and moreover, heterologous probe and *in vitro*-synthesized oligonucleotide probes can also be used, and (v) the PCR-based methods can be subjected to automation. The properties of different molecular markers are listed in Table 1.

Applications of molecular markers

Polymorphisms observed at the DNA sequence level have been playing a major role in human genetics for gene mapping, pre- and post-natal diagnosis of genetic diseases, and anthropological and molecular evolution studies. Similar approach for exploitation of DNA polymorphism as genetic markers in the field of animal genetics and breeding has opened many vistas in live-

stock improvement programmes^{23,24}. Consequently, enormous interest has been generated in determining genetic variability at the DNA sequence level of different livestock species, and in their assessment whether these variations can be exploited efficiently in conventional as well as in transgenic breeding strategies.

Conventional breeding strategies

Molecular markers can play an important role for livestock improvement through conventional breeding strategies. The various possible applications of molecular markers are short-range applications or immediate and long-range applications (Table 2).

Short-range or immediate applications: Molecular markers have several immediate applications like parentage determination, genetic distance estimation, determination of twin zygosity and freemartinism, sexing of pre-implantation embryos and identification of disease carrier. In the following subsections, each of these applications have been discussed briefly.

(i) Parentage determination: Since the breeding value of an animal is generally estimated using the information available from its relatives, the knowledge of correct parentage is therefore a prerequisite. Parentage testing using molecular markers yields much higher exclusion probability (> 90%) than the testing with blood groups (70–90%) or other biochemical markers (40–60%)²⁵. Highly polymorphic DNA fingerprinting markers²⁶ are quite useful for this purpose. Recently, DNA fingerprinting with oligoprobes (OAT18 and ONS1) has been successfully used for determining the parentage of IVF buffalo calf²⁷. With the advent of PCR-based microsatellite assays, a large number of microsatellite panels

Table 1. Properties of different molecular markers: A comparison

Features	Marker system				
	RFLP	CAPS (PCR-RFLP)	DFP	MAAP e.g. RAPD	Microsatellite
Detection method	Hybridization	PCR	Hybridization	PCR	PCR
Type of probe/primer used	Genomic DNA/cDNA sequence of structural genes	Sequence-specific primer	Minisatellite/synthetic oligos	Arbitrarily designed primer	Sequence-specific primer
Requirement of radioactivity	Yes	No/yes	Yes	Yes/no	Yes/no
Extent of genomic coverage	Limited (coding sequence)	Limited (coding sequence)	Extensive	Extensive	Extensive
Degree of polymorphism*	Low (SLDA)	Low (SLDA)	High (MLMA)	Medium to high (MLDA)	High (SLMA)
Phenotypic expression	Co-dominant	Co-dominant	Co-dominant/dominant	Dominant	Co-dominant
Possibility of automation	No	Yes	No	Yes	Yes

*SLDA = single locus di-allelic; MLDA = multi locus di-allelic; MLMA = multilocus multi-allelic.

Table 2. Molecular markers useful in conventional livestock breeding

Applications	Marker systems useful
Short-range/immediate applications	
Parentage determination	DFP, microsatellite
Genetic distance estimation	DFP, microsatellite, RAPD
Determination of zygosity/freemartinism	RFLP, PCR-RFLP, microsatellite
Sex determination	RFLP, PCR-RFLP, microsatellite, DFP
Identification of disease carrier	RFLP, CAPS, microsatellite
Long-range applications	
Gene mapping	Type II markers e.g. VNTR, minisatellite, microsatellite, RAPD
Marker-assisted selection	Any marker having direct or indirect association with the performance traits/QTL under question

have been reported that are useful for parentage testing in different livestock species. For example in cattle, Glowatzki-Mullis *et al.*²⁸ demonstrated that using two triplex microsatellite co-amplification systems, wrong parentage can be excluded with almost 99% accuracy. In addition, molecular markers also serve as a useful tool for animal identification, particularly for verification of the semen used for artificial insemination²⁵.

(ii) Genetic distance estimation: Genetic distance, a measure of overall evolutionary divergence, i.e. genetic similarities and dissimilarities between two populations (such as between species, breeds, strains), serves as a useful tool for authentication of the pedigree, for characterization of different breeds or strains within a species, and for evaluation of the change in variation in species over time. In principle, genetic distance can be measured on the basis of polymorphic characters occurring at the different levels, viz. morphological, biochemical, cellular and DNA level. Allelic frequencies of blood groups²⁹ as well as those of other biochemical loci, e.g. serum and milk proteins³⁰, have been used extensively for the estimation of genetic divergence of different livestock species. However, a great amount of genetic variations at protein loci remain undetected, since changes in the underlying nucleotide sequences may not necessarily lead to corresponding change in the amino acid sequences owing to degeneracy of the genetic code. Molecular markers capable of generating individual specific DFP patterns, useful for establishing familial relationships^{31,32}, can serve as an alternative. The similarities between the DFP patterns that are expressed by band-sharing values, provide a reliable method for evaluating genetic distance amongst populations^{33,34}. Presently, the PCR-based RAPD fingerprinting assays are being used for characterization of zebu cattle breeds³⁵, for detection of genetic variations in cattle and sheep³⁶, and characterization of highly inbred chicken lines in poultry³⁷.

(iii) Determination of twin zygosity and freemartinism: Correct knowledge of zygosity of twins, particularly in

monotocus animals, is very important. Monozygotic twins provide means for epidemiological as well as for genetical studies, and also help in transplant matching. Individual-specific DNA fingerprinting techniques have potential applications in determination of twin zygosity³⁸ and demonstration of spontaneous XX/XY chimaerism³⁹. Demonstration of XX/XY chimerism in heterosexual bovine twins, by PCR-RFLP assay using sex-chromosome-specific primers, has enabled the identification of freemartin animal^{40,41}.

(iv) Sex determination: Sexing of pre-implantation embryos can serve as an important tool for improving herd for a desired purpose. A large number of invasive and noninvasive methods for sexing embryos are available. However, ideally the technique to be applied should not have any adverse effect on embryo survivability, its conception rate and subsequent development. Besides, the technique should be simple and easy to carry out, repeatable, and accurate and time saving. Embryos can be sexed by cytogenetical method⁴², which is quite accurate but invasive and needs a large piece of embryo. The molecular markers on the other hand, have potential application in determination of sex of pre-implantation embryos, since the embryos can be sexed using male-specific or Y-chromosome-specific DNA sequence as probes⁴³. However, this method is time consuming as well as tedious. Sexing of embryo using PCR-based approach^{44,45} involves amplification of male-specific DNA fragment and its visualization in ethidium-bromide-stained agarose gel following electrophoresis. The PCR-based method of sex determination offers several advantages over all the other methods: (i) It can be carried out in less than five hours with almost 100 per cent accuracy⁴⁴. (ii) It is less invasive and requires very small quantity (in nanograms) of DNA for PCR assay, which can be isolated from two to eight cells biopsied from the embryo⁴⁵. (iii) It can be done at an early stage of embryo, e.g. blastocyst stage (6 to 8 days) or even earlier at the 16–32 cell stage⁴⁶. (iv) The use of multiplex PCR allows simultaneous genotyping

for important loci like milk proteins, diseases carrier, etc.

(v) Identification of disease carrier: Many of the most serious incurable diseases result not from infections with bacteria or viruses but defects in genomes of the hosts. Certain allelic variations in the host genome lead to susceptibility or resistance to a particular disease. Kingsbury⁴⁷ reported that a particular RFLP in the Prion protein (Prn P) gene was responsible for the variation in host's response to the causative agent, and the incubation time of bovine spongiform encephalopathy (BSE). DNA polymorphism occurring within a gene helps to understand the molecular mechanism and genetic control of several genetic and metabolic disorders, and allows the identification of heterozygous carrier animals which are otherwise phenotypically indistinguishable from normal individuals. In case of genetic disorders caused by a single point mutation, for example citrulinaemia⁴⁸, bovine leukocyte adhesion deficiency (BLAD)⁴⁹, and deficiency of uridine monophosphate synthetase (DUMPS)⁵⁰ in cattle; hyperkalemic periodic paralysis in horses and malignant hyperthermia in pigs⁵¹, carrier animal possessing the defective recessive allele can be identified easily using PCR-RFLP assay. Using microsatellite (TGLA116) marker, Georges *et al.*⁵² demonstrated the identification of carrier animals of weaver disease in cattle.

Long-range applications: The foremost long-range application of molecular markers in conventional breeding includes mapping of the QTL by linkage. Such mapping information, if available, particularly, for those loci which affect the performance traits or disease resistance/susceptibility, can be used in breeding programmes by either within-breed manipulations, like marker-assisted selection of young sires, or between-breeds introgression programmes.

(i) Gene mapping: Molecular markers have three-fold applications in gene mapping: (i) A marker allows the direct identification of the gene of interest instead of the gene product and consequently, it serves as an useful tool for screening somatic cell hybrids. (ii) Use of several DNA probes and easy-to-screen techniques, a marker also helps in physical mapping of the genes using *in situ* hybridization. (iii) The molecular markers provide sufficient markers for construction of genetic maps using linkage analysis. Genetic maps are constructed on the basis of two classes of molecular markers⁵³: Type I markers, that represent the evolutionary conserved coding sequences (e.g. classical RFLPs and SSLPs), are useful in comparative mapping strategies where polymorphism is not an essential prerequisite. However, these are mostly single locus and di-allelic

(SLDA) and thus are not useful for linkage analysis. On the other hand, the type II markers (like microsatellites markers) have higher polymorphism information content (PIC, a measure of the usefulness of a marker for linkage studies⁴) than conventional RFLPs and can be generated very easily and rapidly. Therefore, major efforts are being made to produce gene maps based on the type II markers. Further utilization of molecular markers developed from DNA sequences information, namely ASO and STMS polymorphic markers are also helpful in rapid progress of gene mapping.

(ii) Marker-assisted selection: The concept of marker-assisted selection (MAS), utilizing the information of polymorphic loci as an aid to selection, was introduced as early as in 1900s (ref. 54). However, its application in genetic improvement of livestock species has been limited due to lack of suitable genetic markers. The discovery of DNA-level polymorphisms in eighties and their subsequent use as molecular markers has renewed interest in the use of genetic markers in selection of breeding stocks. Implementation of MAS essentially involves two steps: Identification of the marker loci that is linked to QTL of economic importance, followed by the utilization of linkage association in genetic improvement programme. Once linkage between a QTL and a marker locus is established, it is possible to recognize the alternative QTL allele inherited by the individual. Such information can then be used for the selection of the breeding stock.

MAS is likely to complement rather than replace the conventional breeding systems leading to increased rate of genetic improvement through higher selection intensity, reduction of generation interval and increase in the accuracy of prediction. Furthermore, selection based on markers is possible in early life or in individuals of both sexes for sex-limited traits. However, there is a risk of reduced genetic response if the marker association information is inaccurate, as MAS is a form of indirect selection. The association between the markers and the QTL is a function of distance between the markers and target traits, type of linkage phase, and degree of linkage disequilibrium. Therefore, a high-density gene map with closer linkage is a prerequisite for successful implementation of MAS⁵⁵. It is estimated that an average marker density of 10 cM (5–20 cM), with about 200–250 markers, should be sufficient for the detection of marker-QTL association²³. Till recently, gene maps with average marker interval exceeding 5 cM in pigs⁵⁶, and 10 cM in cattle^{57,58}, sheep⁵⁹ and goat⁶⁰ were available. However, currently high resolution maps with 2.5 cM or even less marker density have been published^{61,62}.

One of the best examples of the application of MAS within population is the selection of young sires before their induction for actual progeny testing^{63,64}. Inclusion

of marker information for selection of young sires in progeny-testing programmes may lead to an increase of genetic gain by 15–30% (ref. 64), and an increase in the accuracy of prediction⁶⁵. MAS can be used efficiently across the population (between breeds) to incorporate the most desirable alleles into a group of selected individuals/strain/breeds, termed as introgression. Introgression is less common in domestic animals than in plant species because of limited fertility, longer generation interval and the greater expense of each individual⁶⁶; nevertheless, introgression can be applied in domestic animals for genes with major effects⁶⁷. Notable examples are the Booroola gene for increased fecundity⁶⁶, and gene for trypanosoma tolerance for N'Dama cattle⁶⁸.

Molecular markers are capable of unravelling genetic variations in both the coding and noncoding sequence regions. Based on this characteristic, Geldermann²⁵ suggested two approaches for the identification of markers that influence QTL, as given below.

(iia) Polymorphisms in the coding sequences: DNA polymorphisms that occur in and around the structural and/or regulatory sequences of a gene of physiological significance (e.g. hormone genes, milk protein genes, MHC) may directly affect gene expression (by changing the splicing of mRNA, stability of mRNA, rate of gene transcription or the sequence of gene product) and thereby contribute to the phenotypic variations among the individuals in terms of productivity and health (disease resistance/susceptibility). Consequently, such DNA polymorphisms, occurring in the genes which already have *a priori* possibility to be associated or closely linked with the performance trait of importance, can be selected as markers⁶⁷.

Various studies have shown that a number of single point mutations in structural genes that are inherited in a simple Mendelian manner are associated with quantitative traits of economic importance. For example, the milk protein polymorphisms have been found associated with the differences in composition and processing qualities of milk⁶⁹, and are linked to some of the production traits⁷⁰. Most reports^{70,71} suggest that α_{s1} -casein A, β -casein A2 and κ -casein B have desirable effects on first-lactation milk yield. Of the many variants of κ -casein, B variant has been found not only to possess some advantageous manufacturing properties but it also leads to a production of 8 to 10% more cheese^{60,70}. In comparison to exotic cattle breeds, the frequency of advantageous κ -casein B allele has been found to be low in Indian cattle breeds⁷². To increase the frequency of advantageous B allele, the progeny tested sires with favourable genotypes (BB and AB) can be used in AI programme. Mitra *et al.*⁷² reported a new genetic variant (ThrACC → IleATC at amino acid position 135) of κ -casein in buffalo. However, its associations with milk

production traits and processing qualities are yet to be ascertained.

Allelic variations in either the structural or the regulatory sequences of growth hormone and prolactin genes have also been studied extensively for their possible direct or indirect effect on milk production and growth performance. With respect to the *Alu I* polymorphism in growth hormone gene (resulting in a leucine (L)\valine (V) substitution at amino acid position 127), the L allele has been reported to be more frequent among the dairy cattle breeds than the beef breeds⁷³. In Sahiwal cattle, one of the important milch breeds of India, the L allele was reported to be more frequent than the V allele⁷⁴. However according to Schlee *et al.*⁷⁵, this growth hormone genotype has no significant effect on the breeding values for dairy traits, but significantly influences breeding value for carcass traits. Beside these, Hoj *et al.*⁷⁶ reported association of *MspI* polymorphism at the third intron of growth hormone gene with the milk fat yield. However, further study is needed to obtain more definite conclusions about the effects of DNA polymorphism in growth hormone gene on milk traits.

(iib) Polymorphisms in the noncoding sequences: In this approach, the variations occurring in noncoding sequences (e.g. flanking regions or intergenic regions) are utilized indirectly as markers for linkage analysis^{23,24}. Microsatellite markers, which are often highly polymorphic, are presently being exploited to identify QTL for economically important traits. Ron *et al.*⁷⁷, using microsatellite markers, identified one marker (D21S4) associated with significant effects on milk and protein yields. Using 159 microsatellite markers in 14 US Holstein half-sib families, Georges *et al.*⁷⁸ demonstrated the presence of QTL for milk production on five chromosomes (namely chromosome no. 1, 6, 9, 10 and 20). In another study⁷⁹, significant association of microsatellite markers with somatic cell score (SCS, an indicator for susceptibility to mastitis), productive herd life and milk production traits were established. More recently, using microsatellite markers Ashwell *et al.*⁸⁰ identified potential QTL for SCS, fat yield, fat percentage, and protein yield and percentage. Characterization of QTL for economically important traits using microsatellite markers will help in formulating more efficient breeding programmes using MAS, especially for bulls prior to progeny testing.

Transgenic breeding strategies

The current livestock breeding strategies largely rely on the principle of selective breeding. In this method genetic improvement is brought about by increasing the frequency of advantageous alleles of many loci, though the actual loci are rarely identified. Moreover, in these

methods genes cannot be moved from distant sources like different species or genera due to reproductive barrier. The recent developments in molecular biology have given rise to a new technology called transgenesis, which has removed the breeding barrier between different species or genera. Transgenesis has opened up many vistas in understanding behaviour and expression of a gene. It has also made possible to alter the gene structure and modify its function⁸¹. Of the many applications of transgenesis, the most convincing one is the development of transgenic dairy animals for the production of pharmaceutical proteins in milk, and animals with altered milk composition⁸².

The starting point for this technology is the identification of the genes of interest. In this context, molecular markers can serve as reference points for mapping the relevant genes that would be the first step towards their identification, isolation, cloning (positional cloning), and their manipulation. After successful production of transgenic animals, appropriate breeding methods could be followed for multiplication of transgenic herd/flock. Molecular markers can also be used for identification of the animals carrying the transgenes. Though most of the QTL are polygenic in nature and in transgenesis presently single gene traits are being manipulated⁸², the technology nevertheless holds future promises in moving polygenic QTL across the breeding barriers of animals.

Conclusions

The genetic improvement of animals is a continuous and complex process. Ever since the domestication of animals by man, he has always remained busy in improving his animals. In this pursuit, many methods have been developed and tested. In recent years, the demonstration of genetic polymorphism at the DNA sequence level has provided a large number of marker techniques with a variety of applications. This has, in turn, prompted further consideration for the potential utility of these markers in animal breeding. However, utilization of marker-based information for genetic improvement depends on the choice of an appropriate marker system for a given application. Selection of markers for different applications is influenced by several factors, viz. the degree of polymorphism skill or expertise available, possibility of automation, radioisotopes used, reproducibility of the technique, and finally the cost involved. Presently, the pace of development of molecular markers is tremendous, and the trend suggests that explosion in marker development will continue in the near future. It is expected that molecular markers will serve as a potential tool to geneticists and breeders to evaluate the existing germplasm, and to manipulate it to create animals as desired and needed by the society.

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