

MOLECULAR MARKERS IN ANIMAL GENOME ANALYSIS

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Review paper

Abstract: Over the last two decades advances in molecular genetics have introduced a new generation of molecular markers for the genetic improvement of livestock. This study is focused on the most advanced DNA-based molecular markers which have acted as versatile tools and have found their own position in various fields. Ever since their development, they are constantly being modified to enhance their utility and to bring about automation in the process of genome analysis. The discovery of PCR (polymerase chain reaction) was a milestone in this endeavour and proved to be an unique process that brought about a new class of DNA profiling markers. In this article DNA-based molecular markers developed during the past decades - restriction fragment length polymorphisms (RFLPs), microsatellites (SSRs), amplified fragment length polymorphism (AFLPs), single nucleotide polymorphisms (SNPs) for various applications in the area of animal genome analysis are reviewed.

Key words: animals, molecular markers, genome analysis, PCR, MAS, QTLs

Introduction

The discovery of the genetic code, first published by Watson and Crick in 1953, has showed how the genetic information was passed from generation to the next. In the past years the developments in understanding the genetic code of animal species have opened the possibility to evolve the genetic evaluation of all species of livestock.

Traditionally, the genetic improvement of livestock breeds has been based on phenotypic selection. The past century was characterized by the development of quantitative theory and methodology towards the accurate selection and prediction of genetic response (*Walsh, 2000*).

This resulted in the selection of a number of economically important genetic traits in cattle, sheep, pigs and poultry. The first work on detection of genome variation in animal livestock is based on morphological, chromosomal

and biochemical markers. Most of morphological markers are sex limited, age-dependent, and are significantly influenced by the environment. Biochemical markers show low degree of polymorphism. The various genotypic classes are indistinguishable at the phenotypic level because of the dominance effect of the marker and low genome coverage (*Montaldo and Meza-Herrera, 1998*).

The development of molecular biology during the past three decades created new means for studying livestock genetics and animal breeding. Selection according to genotype has become an important tool in the breeding of farm animals. The molecular markers, capable of detecting the genetic variation at the DNA sequence level, have removed the above mentioned limitations of morphological, chromosome and protein markers but also possess unique genetic properties that make them more useful than other genetic markers. They are numerous and distributed ubiquitously throughout the genome. These markers 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 are noninfluenced by the environmental factors, and generally do not have pleiotropic effects on quantitative trait loci (QTL).

Molecular markers, revealing the polymorphism at the DNA level are now key players in animal genetics. Molecular markers serve as an useful tool for animal identification and genetic distance estimation. 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%). These polymorphisms referred to as molecular markers can be used to build up genetic maps and to evaluate differences between markers in the expression of particular traits that might indicate a direct effect of these differences in terms of genetic determination on the trait (*Vignal et al., 2002*). Extensive genetic maps were prepared in the last few decades in a variety of animal species such as cattle, sheep, swine, used for marker assisted selection (MAS), QTL segregating analysis and for detection of major genes (*Kinghorn et al., 1993; Roher et al., 1994; Kinghorn et al., 1997; Kinghorn 1997; Vignal et al., 2002*).

The present review is a brief account of molecular markers and techniques and its various applications in animal genetic improvement.

DNA based markers

Various types of molecular markers are utilized to evaluate DNA polymorphism and are generally classified in three major groups:

- Hybridization-based DNA markers - Restriction Fragment Length Polymorphisms (RFLPs) and oligonucleotide fingerprinting;
- PCR-based DNA markers which involve *in vitro* amplification of particular DNA sequences or loci, with the help of specifically or arbitrarily chosen

oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme - Random amplified Length Polymorphic DNAs (RAPDs) Simple Sequence Repeats or microsatellites (SSRs), Amplified Length Polymorphism (AFLPs);
●DNA chip and sequencing-based DNA markers such as Single nucleotide Polymorphism (SNPs)

The properties of ideal DNA markers are:

- Highly polymorphic nature;
- Codominant inheritance (determination of homozygous and heterozygous status of diploid organism);
- Frequent occurrence in genome;
- Selective neutral behaviour (the DNA sequences of any organism are neutral to environmental conditions or management practices);
 - Easy access (availability);
- Easy and fast assay;
- High reproducibility;
- Easy exchange of data between laboratories.

Nowadays the most commonly used molecular markers are: RFLP, AFLP, RAPD, SSR, SNP. These markers differ in genetic information, interpretation and standardization of results, potential for automation and animal genetic improvement. The properties of different molecular markers are listed in Table 1 (*Mburu and Hanotte, 2005*).

Restriction fragment length polymorphisms (RFLPs)

Hybridisation – based marker technologies use cDNA, cloned DNA elements, or synthetic oligonucleotides as probes, which are labeled with radioisotope or with conjugated enzymes that catalyse a coloured reaction, to hybridise DNA. The DNA is either cleaved with restriction enzymes or amplified by PCR. RFLP is representative of this type of technology and was first developed in 1980 (*Botstein et al., 1980; Schimenti, 1998*) to visualize the differences of the DNA structure based on the use of bacterial restriction enzymes that cut the DNA at sites with specific nucleotide sequences (*Mburu and Hanotte, 2005*). RFLPs are based on the analysis of patterns derived from a DNA sequence digested with known restriction enzymes. Differences are evident when the length of fragments are different, implying that the restriction enzyme cut the DNA at unrelated locations. Restriction polymorphism occurs when mutations remove an existing restriction site or create a new restriction site. The alterations are detected by using a hybridization probe. The choice of the DNA probe is crucial in RFLP analysis. The identification of RFLPs requires the use of gel electrophoresis to separate the DNA fragments of differing sizes followed by transfer of the fragments to a nylon membrane (Southern blot) and visualization of specific DNA sequences using

radioactive or chemiluminescent probes exposed to an X-ray film (*Drinkwater and Hetzel, 1991; Smith and Smith, 1993; Albert et al., 1994; Lewin, 1994; Bishop et al., 1995; Stein et al., 1996*). RFLP is a result of:

- point mutation creating or destroying a restriction site;
- insertions/deletions (Indels) altering the size of a given restriction fragment.

The main advantages of RFLP markers are:

1. Produces co-dominant (also known as semi-dominant) markers - this allows discrimination of homo- and heterozygotic states in diploid organisms;
2. Stable and reproducible - gives constant results over time, and location;
3. Selective neutrality.

The disadvantages are the following:

1. Long methodology.
2. Labour intensive.
3. Requires high quality and large quantities of DNA.
4. RFLPs limited the identification of the whole genome variation in animals.
5. The reduced variability observed in domestic animals by inbreeding makes many RFLPs sites non-informative.

These disadvantages replace RFLP markers with new type of markers - PCR based microsatellite markers (*Drinkwater and Hazel, 1991; Vignal et al., 2002*). RFLP markers are most widely applied in genome mapping, marker – aided breeding, systematic and evolution studies.

Polymerase Chain Reaction (PCR)

The process known as **polymerase chain reaction** or **PCR** is developed by Kary Mullis in 1983 (*Mullis et al., 1986*). The PCR is a biochemistry and molecular technique for exponentially amplifying a fragment of DNA, via enzymatic replication, without using a living organism (*Pavlov et al., 2004*). PCR can be used for amplification of a single or few copies of a piece of DNA – single gene, part of a gene, or a non-coding sequence. As PCR is an *in vitro* technique, it can be performed without restrictions on the form of DNA, and it can be extensively modified to perform a wide array of genetic manipulation (*Altshuler, 2006; Deden et al., 2006*).

PCR is closely patterned after the natural DNA replication. Two oligonucleotide primers flank and define the target sequence to be amplified. These primers hybridize to opposite strands of the DNA to serve as an initiation points for

the synthesis of new DNA strands. A thermostable DNA polymerase, such as a Taq DNA Polymerase catalyzes this synthesis. This enzyme reads the original template in the 3'-5' direction and synthesizes a new complementary template in the 5'-3' direction, using free deoxynucleotide triphosphates (dNTP's) as building blocks. A PCR thus consists of a number of cycles of denaturation, annealing and extension. The product synthesized in one cycle serves as a template in the next, so that the original DNA is doubled in every cycle and the target DNA molecules are typically increased by several millionfold. This is only in 20 cycles PCR provides about million (2^{20}) copies of the target (*Mullis and Faloona, 1987; Buratowski, 1994; Koleske and Young, 1995; Stein et al., 1996; Aoyagi, 2001*).

The amplicon or PCR-product can then be visualized on an agarose or polyacrylamide gel (*Erlich, 1991; Nicholas, 1996; Turner et al., 1998*). PCR has the advantage of being a relatively fast, sensitive and reliable method. Not only can it amplify very small amounts of DNA, but can also amplify degraded or poor sources of DNA (*Erlich, 1991; Strachen and Read, 1999*). PCR has become an essential tool in molecular biology and plays a leading role in all techniques that are presently applicable to the analysis and characterization of genomes. PCR is applied to amplify known sequences of a sample of DNA or for arbitrary priming of variable regions of the genome. The technique is suitable for use with various DNA markers such as Variable Number Tandem Repeats (VNTR's), Randomly Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Restricted Fragment Length Polymorphism (RFLP) (*Erlich, 1991; Sunnucks, 2001; Freeman et al., 2006*).

Randomly amplified polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA is also known as arbitrarily primed PCR (AP-PCR), or as a DNA amplification fingerprinting technique (DAF) and was described in 1990 (*Williams et al., 1990*). This technique is based on the use of short, arbitrary primers in PCR reaction and can be used to produce relatively detailed and complex DNA profiles for detecting amplified fragment length polymorphisms between organisms. In the simplest format, only one short oligonucleotide, usually eight to 10 nucleotides in length, is used. Multiple primers are usually applied and a range of five to 21 nucleotides has proven successful if detection is coupled with polyacrylamide gel electrophoresis. Depending on the primer/template combination and ratios, amplified products range from less than 10 to over a 100. In this way, a spectrum of products characteristic for each template and primer combination is typically obtained and these can be adequately resolved and visualized using polyacrylamide gel electrophoresis and silver staining (*Caetano-Anolles and Gresshoff, 1998*).

Certain primers will produce unrelated patterns between unrelated animals and identical ones for very closely related animals. Presumably primer sites are randomly distributed along the target genome, and flank both conserved and highly variable regions. Wide variation in band intensity can be shown to be reproducible between experiments, which could be the result of multiple copies of the amplified regions in the template or the efficiency with which particular regions are amplified. The polymorphic bands obtained from a RAPD can also be cloned for further analysis. The polymorphisms are detected as the presence or absence of bands of a specific size.

According *Vignal et al. (2002)* and *Mburu and Hanotte (2005)* the advantages of RAPD markers are following: cost effective; simple and quick, large number of bands are produced, no prior sequence knowledge is necessary, the required samples are very small because DNA will be amplified by PCR technique.

There are also some disadvantages with respect to other methods (*Dodgson et al., 1997; Atienzar et al., 2000*):

- detection of polymorphism is limited, reproducibility of results maybe be inconsistent e.g. low annealing temperature may cause;
- some unspecific non reproducible binding of primers;
- dominant markers (homozygote cannot be discerned from heterozygote so allele frequencies cannot be estimated);
- the RAPD primers are very sensitive to PCR conditions and this may lead to poor reproducibility.

It had also applications for the conservation of endangered species in avian species (*Nusser et al., 1996; Tansley and Brown, 2000; Ambady et al. 1996*).

Amplified fragment length polymorphism (AFLP)

AFLP is a common combination of RFLP and PCR techniques (*Zabeau and Vos, 1993; Vos et al., 1995*). This DNA fingerprinting technique detects DNA restriction fragments by means of PCR amplification. Genomic DNA is first digested by appropriate restriction enzymes. A subset of resultant fragments representing many loci is then ligated to synthetic adaptors and amplified with specified primers which are complementary to a selective sequence on the adaptors. Subsequent separation of the resultant fragments is performed on a highly resolving sequencing and visualized using autoradiography. Where radiolabelled nucleotides are not used in the PCR step, fluorescence or silver staining technique can be used to visualise the application products. AFLP is similar to RAPD assay in that no prior knowledge of the sequence is required. AFLP detects a greater number of loci than RAPD does.

Table 1. Comparison of commonly used genetic markers by *Mburu and Hanotte (2005)*

Feature	RFLP	SSR	RAPD	AFLP	Isozymes	STS/EST
Origin	Anonymous/ Genic	Anonymous	Anonymous	Anonymous	Genic	Genic
Maximum theoretical number of possible loci in analysis	Limited by the restriction site (nucleotide) polymorphism (tens of thousands)	Limited by the size of genome and number of simple repeats in a genome (tens of thousands)	Limited by the size of genome, and by nucleotide polymorphism (tens of thousands)	Limited by the restriction site (nucleotide) polymorphism (tens of thousands)	Limited by the number of enzyme genes and histochemical enzyme assays available (30-50)	Limited by the number of expressed genes (10,000-30,000)
Dominance	Codominant	Codominant	Dominant	Dominant	Codominant	Codominant
Null alleles	Rare to extremely rare	Occasional to common	Not applicable (presence/absence type of detection)	Not applicable (presence/absence type of detection)	Rare	Rare
Transferability Across related species	Across genera	Within genus or species	Within species	Within species	Across families and genera	Across related species
Reproducibility	High to very high	Medium to high	Low to medium	Medium to high	Very high	High
Amount of sample required per sample	2-10 mg DNA	10-20 ng DNA	2-10 ng DNA	0.2-1 µg DNA	Several mg of tissue	10-20 ng DNA
Ease of development	Difficult	Difficult	Easy	Moderate	Moderate	Moderate
Ease of assay	Difficult	Easy to moderate	Easy to moderate	Moderate to difficult	Easy to moderate	Easy to moderate
Automation / multiplexing	Difficult	Possible	Possible	Possible	Difficult	Possible
Genome and QTL mapping potential	Good	Good	Very good	Very good	Limited	Good
Comparative mapping potential	Good	Limited	Very limited	Very limited	Excellent	Good to very good
Candidate gene mapping potential	Limited	Useless	Useless	Useless	Limited	Excellent
Potential for studying adaptive genetic variation	Limited	Limited	Limited	Limited	Good	Limited
Development	Moderate	Expensive	Inexpensive	Moderate	Inexpensive	Expensive
Assay	Moderate	Moderate	Inexpensive	Moderate to expensive	Inexpensive	Moderate
Equipment	Moderate	Moderate to expensive	Moderate	Moderate to expensive	Inexpensive	Moderate to expensive

AFLPs are dominant biallelic markers (*Vos et al., 1995*). Variations at many loci can be arrayed simultaneously to detect single nucleotide variations of unknown genomic regions, in which a given mutation may be frequently present in undetermined functional genes (*Young et al., 1999*). AFLP provides an effective, rapid and economical tool for detecting a large number of polymorphic genetic markers that are highly reliable and reproducible, and are able to be genotyped automatically. AFLP is considered as the "gold standard" for molecular epidemiological studies of pathogenic microorganisms and it is also widely used in forensic science. The AFLP technique has been used extensively to detect genetic polymorphisms, evaluate and characterize breed resources, assess the relationship between breeds, construct genetic maps and identify genes in the main livestock species - cattle, sheep, pigs (*Ajmone-Marsan et al., 2002; Negrini et al., 2006, 2007; De Marchi et al., 2006; Sancristobal et al., 2006; Buntjer et al., 2002; Jun et al., 2004; Foulley et al., 2006; Marsan et al., 2007*). AFLP is the ideal molecular approach for population genetics and genome typing except microsatellites (*Luikart et al., 2003*).

Mburu and Hanotte (2005) have shown the most useful advantages and disadvantages of AFLPs: high sensitive method, large number of polymorphisms is generated, selective neutrality, highly reproducible, no prior sequence information or probe generation is needed, expensive technique, dominant markers, technically demanding.

Microsatellites

Microsatellites consist of a stretch of DNA a few nucleotides long – 2 to 6 base pairs (bp) repeated several times in tandem (CACACACACACACA) (*Litt and Luty, 1989; Tautz, 1989*). Microsatellites loci are also known as simple sequence repeats (*SSR's*), short tandem repeats (*STR's*), simple sequence tandem repeats (*SSTR*), variable number tandem repeats (*VNTR*), simple sequence length polymorphisms (*SSLP*), simple sequence repeats (*SSR*), sequence tagged microsatellites (*STMS*).

They are spread over the eukaryote genome and have a sufficiently high mutation rate and were suggested in order to overcome the limitations associated with RFLP and RAPD. Microsatellites are of a small size. They easily amplified using PCR from DNA extracted from a variety of sources including blood, hair, skin or faeces. Polymorphisms can be visualized on a sequencing gel, and the availability of automatic DNA sequencers allows high-throughput analysis of a large number of samples (*Goldstein et al., 1995; Jarne and Lagoda, 1996; Goldstein and Schlotterer, 1999*). *Vos et co-workers (1995)* are stating that the heterozygosity of SSRs is seven to ten times higher than that of RFLPs. The AFLP technique is more laborious and time consuming than RAPD methods but is also

more reliable, AFLP being able to detect a large number of polymorphic bands in a single lane rather than high levels of polymorphism at each *locus* such as is the case for SSR methods. Although this lower sensibility in detecting informative genotypic classes might be associated with the inability to distinguish heterozygotes from homozygotes because of binary scored AFLPs, *Gerber et al. (2000)* suggested that the high numbers of polymorphic loci revealed by AFLP methods counterweigh the loss of information resulting from dominance, while *Garcia-Mas et al. (2000)* showed that AFLPs had higher efficiency in detecting polymorphism than either RAPD or RFLP markers. It is also known that the AFLP technique has lower initial costs and is more transferable across species than SSR methods.

If cloned and sequenced microsatellite loci can be subjected to PCR amplification and such microsatellite loci can be recovered by PCR, such loci are termed as sequence tagged microsatellite site (STMS) 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 map.

In the recent years, microsatellites are the most popular markers – marker of choice in livestock genetic characterization studies (*Sunnucks, 2001; Civanova et al., 2006*). Microsatellites have been proven to be useful markers for a variety of purposes such as identification of animals, evaluation of genetic resources, parentage determination, disease research, determination of genetic variation within and among breeds, determining population substructure, reconstruction of phylogenetic relationships among populations and historical studies of domestication and migration of breeds because their high abundance in the genome, extremely high degree of polymorphism and easy detection (*Albert et al., 1994; Lewin, 1994; MacHugh et al., 1994; Bishop et al., 1995; Smith and Smith, 1993; Stein et al., 1996; Ritz et al., 2000; Naicy and Anilkumar 2008*).

Until recently, micro-satellites were the markers used for mapping quantitative trait loci for production and functional traits in farm animals (*Hiendleder et al., 2003; Kühn et al., 2003*) and tightly linked markers are used for marker assisted selection in practice. They are also the prerequisite for the identification of positional and functional candidate genes responsible for quantitative traits. Since the discovery of dinucleotide repeats in a variety of eucaryotic species (*Tautz, 1989*), PCR based microsatellites assays have revolutionized the construction of genetic maps in most livestock species. In cattle microsatellite sequences were first described in 1990 and until year 2000 over than 2000 microsatellite sequences have been identified and mapped in this species (*Radko and Dunec, 2002*). Various commercial kits are available for animal genotyping and animal parentage verifications for example StockMarks for Cattle®

and StockMarks for Horse® parentage typing (<http://www.appliedbiosystems.com>).

Many authors show the advantages and disadvantages of SSR markers (*Bishop et al., 1994; Bishop et al., 1995; Baron et al., 2002; Mburu and Hanotte, 2005; Erhardt and Weimann, 2007*).

Advantages: low quantities of template DNA required (10-100 ng), high genomic abundance, random distribution throughout the genome, high level of polymorphism, band profiles can be interpreted in terms of loci and alleles, co-dominant markers, allele sizes can be determined with high accuracy, comparison across different gels possible using size standard, high reproducibility, different microsatellites may be multiplexed in PCR, wide range of applications, amenable to automation.

Disadvantages: initial high development costs, heterozygotes may be misclassified as homozygotes when null-alleles occur due to mutation in the primer annealing sites, stutter bands may complicate accurate scoring of polymorphisms, underlying mutation model (infinite alleles model or stepwise mutation model) largely unknown, homoplasy due to different forward and backward mutations may underestimate genetic divergence, time-consuming and expensive to develop, microsatellite markers help to identify neutral biodiversity but do not provide information on functional traits biodiversity.

Single Nucleotide Polymorphisms (SNP)

Recently, DNA sequencing has allowed the discovery of single nucleotide polymorphism (SNPs). They represent one of the more interesting approach in genotypization, because they are abundant in the genome, genetically stable and amenable to high-throughput automated analysis (*Vignal et al., 2002*). Single nucleotide polymorphisms (also referred to as “snip”) is the most recent contribution to studying DNA sequence variation. A SNP is found where different nucleotides occur at the same position in the DNA sequence. These markers are abundant in the genome, genetically stable and amenable to high-throughput automated analysis. They are found in both coding and non-coding regions of the genome and are present at one SNP in every 1000 bp (*Stoneking, 2001; Vignal et al., 2002*).

They are bi-allelic markers, indicating a specific polymorphism in two alleles only of a population. SNP in coding regions can be directly associated with the protein function and as the inheritance pattern is more stable, they are more suitable markers for selection over time (*Beuzen et al., 2000*). Most SNPs, actually about two of every three SNPs, involve the replacement of cytosine (C) with thymine (T). Many SNPs have no effect on cell function, but it is believed that they could predispose an organism to disease or influence their response to a drug.

SNP have become the preferred markers in genetic disease studies for various livestock species, as researches direct their attention towards functional genomics (*Heaton, 2000; White et al., 2001*). SNPs are becoming especially important as markers because they are very stable, i.e. have very low mutation rates and can be amplified with PCR for testing. Single nucleotide polymorphisms can be detected using Single Stranded Conformation Polymorphism (SSCP), Allele specific oligonucleotides (ASO), Single nucleotide polymorphic discrimination by an electronic dot blot assay, (ASO) on semiconductor microchips, Reverse dot blot on DNA chips, Dynamic allele specific hybridisation (DASH), Allele-specific PCR (amplification refractory mutation system or ARMS test), Mutation detection the ARMS test in combination with the TaqmanTM 5'exonuclease assay (exploiting the 5'→3' exonuclease activity of Taq DNA polymerase), Minisequencing and analysis of the extension products by PAGE, Minisequencing and analysis of the extension products on DNA chips, Minisequencing and analysis of the extension products using matrix-assisted, laser desorption/ionization time-of-flight mass spectrometry (MALDITOF), Pyrosequencing, OLA (*Mburu and Hanotte, 2005*). There are over two million of SNPs identified in cattle to date (more will be discovered) (*Simianer, 2007*).

One disadvantage of these markers is the lower informational content compared with that of a highly polymorphic microsatellite, but it can be compensated by the use of a higher number of markers (*Werner et al. 2002, 2004*).

Conclusion

The genetic improvement of animals is a fundamental, incessant, and complex process. In recent years many methods have been developed and tested. The genetic polymorphism at the DNA sequence level has provided a large number of markers and revealed potential utility of application in animal breeding. Since the first demonstration of DNA-level polymorphism, known as the restriction fragment length polymorphism (RFLP) (*Botstein et al., 1980*), an almost unlimited number of molecular markers have accumulated (*Mburu and Hanotte, 2005; Guimaraes, 2007*). The invention of polymerase chain reaction (PCR) in accordance with the constantly increasing DNA sequence data also represents a milestone in this endeavour. The putting into practice 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 are influenced by certain factors - the degree of polymorphism (PIC), the automation of the analysis, radioisotopes used, reproducibility of the technique, and the cost involved. Presently, the huge development of molecular markers will continue in the near future. It is expected that molecular markers will serve as an underlying

tool to geneticists and breeders to create animals as desired and needed by the society.

Molekularni markeri u analizi genoma životinja

A. Teneva

Rezime

U poslednje dve decenije progres u molekularnoj genetici je uključio novu generaciju molekularnih markera u cilju genetskog unapređenja stočarstva. Ovo istraživanje je usmereno na napredne molekularne markere koji se zasnivaju na DNK i koji postaju svestrano oruđe i pronašli su svoje mesto u mnogim oblastima. Od samog početka njihovog razvoja stalno se modifikuju kako bi se povećala njihova korisnost i mogućnost upotrebe i automatizovali u procesu analize genoma. Otkriće PCR-a (lančana reakcija polimeraze -polymerase chain reaction) predstavlja prekretnicu u ovom poduhvatu i pokazalo se kao jedinstven proces koji je uveo u praksu novu klasu markera za određivanje DNK profila. U ovom radu je dat pregled molekularnih markera na bazi DNK koji su razvijeni poslednjih decenija – polimorfizam dužine restriktivnih fragmenata (RFLP), mikro sateliti (SSRs), polimorfizam dužine amplificiranih fragmenata (AFLP), pojedinačni nukleotidni polimorfizam (SNP) za različite primene u oblasti analize genoma životinje.

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