

## **Microsatellite markers – a tool for molecular characterization of cattle genetic resources**

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### **Abstract**

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Molecular markers are essential tool for determining the specific genetic makeup on individual and population level. They are valuable approach for the genetic improvement of farm animals. In cattle breeding their application is useful for improvement of breeding programs for desired traits, better productivity and high quality products. These markers provide more accurate genetic information and better knowledge of the animal genetic resources. This review gives a brief summary on the application of one of more advanced DNA-based molecular markers in cattle breeding – the microsatellites.

*Keywords:* cattle; molecular markers; microsatellites; genome; polymorphism; genetic diversity

### **Introduction**

The first molecular markers used in livestock were the protein polymorphisms. Later the proteins such as hemoglobin and transferrin were involved in all studies. Most of the conducted studies for genetic variation were based on allozyme protein markers. The level of polymorphism observed in proteins is often low which has reduced the general application of protein-typing in the studies of diversity.

In recent development, molecular biology created valuable new means for studying cattle livestock genetics and breeding techniques – the DNA based molecular markers that are based on the mutations of the nucleotide sequence within the individual's genome. They are the most informative markers available so far (Yang et al., 2013).

The simple technique discovered in 1993 by Kary Mullis that revolutionized the molecular biology was polymerase chain reaction (PCR) (Nicholas, 1996; Marle-Köster and Nel, 2003). PCR is a fast, sensitive and reliable method. It became an essential tool in molecular biology and plays a main role in “in vitro” techniques that are now applicable to the analysis of genomes. After discovery of this major scien-

tific development, blood group typing and protein biochemical proteins in animal populations were replaced by the use of molecular DNA markers.

By this review the attempt is to highlight the application of short tandem repeats (STR) or microsatellites to complement morphological and productive information about animal genetic resources, contributing to an increase in the efficiency of processes of genetic diversity analysis, to generate information for planning of crossings and selection of genotypes in genetic breeding programs for conservation and management.

#### ***Molecular markers***

Genetic markers are two types – protein and DNA (molecular) markers. Molecular markers can be categorized into two classes, nuclear DNA and mitochondrial DNA (mtDNA) markers, based on their transmission and evolutionary dynamics (Hanotte et al., 2003). Nuclear DNA markers are usually bi-parently inherited. Mitochondrial DNA markers are maternally inherited, express high rates of mutation, and are non-recombining such that they have one-quarter of the genetic effective population size ( $N_e$ ) of nuclear markers (Hanotte et al., 2003).

Molecular marker or genetic marker is a fragment of DNA sequence that is associated to a certain region of the genome (Wakchaure et al., 2015). Molecular markers are classified on the basis of techniques used for discovery of polymorphism. There are several types of markers used today: hybridization-based markers such as RFLP (Restriction Fragment Length Polymorphism) and PCR-based markers, e.g. Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Short Tandem Repeats (STR) or Microsatellites, Minisatellite, Single Nucleotide Polymorphism (SNP) (Marle-Köster and Nel, 2003).

In animal genetic studies, the molecular markers revealing polymorphism at the DNA level play an important role. The term “Smart Breeding” is used to describe marker supported breeding strategies (Al-Samarai and Al-Kazaz, 2015).

Among the most polymorphic DNA markers are the short tandem repeats (STR) or microsatellites (SSR) and sequence tagged microsatellite repeats (STMR) because of their high abundance in eukaryotic genomes. STR are di-, tri-, or tetra nucleotide tandem DNA repeats that are presented in variable copy numbers at each locus and throughout the genome (Ashley and Dow, 1994; Bruford et al., 1996; Ellegren et al., 1997; Montaldo and Meza-Herrera, 1998; Schlötterer, 1998; Schmid et al., 1999; Toth et al., 2000; Beuzen et al., 2000; Hancock, 1999; Gündüz et al., 2016). PCR-amplified microsatellite repeats in the alleles can be detected using various methods including Fragment Analysis.

STR are located predominantly in the noncoding intronic regions of the bovine genome. They are most valuable and informative markers for genetic studies in cattle parentage verifications, genetic variability, genome mapping, relationships of individuals and populations, evaluation of inbreeding levels ( $F_{IS}$ ), the genetic structure of subpopulations and populations, assessment of effective population size ( $N_e$ ) and the gene flow between populations. They are used as markers for certain cattle disease in cattle diagnosis because several microsatellite alleles are associated with mutations in coding regions of the DNA that can cause a variety of medical disorders and variation in productive traits (Selkoe and Toonen, 2006).

#### **The advantages of PCR- based microsatellite markers for cattle studies are as follows:**

- Locus-specific;
- Co-dominant (heterozygotes could be distinguished from homozygotes);
- Highly polymorphic („hypervariable“);
- Allow obtaining of rapid results in 48 hours or less;
- Useful at a range of scales from individual ID to fine-

scale phylogenies;

- Easy to standardize and automate, results are very reproducible.

The genotyping of microsatellite markers is performed automatically and with a low cost due to the use of multiplex technique, that allows the analysis of more microsatellites in one reaction.

Autosomal microsatellite loci in cattle are often used for genetic identification of individual and parentage analysis for the successful implementation and monitoring of ex-situ conservation programs, population diversity, differentiation of populations, genetic distances and genetic relationships. Microsatellite loci are highly sensitive to genetic bottlenecks and they are commonly used for inbreeding determination in cattle populations (Hanotte and Jianlin, 2005). They are still the “gold standard” for many genetic population and identification purposes (Brenig and Schütz, 2016).

#### **Genetic diversity analysis**

In 1994, MacHugh et al. reported the use of 12 microsatellite markers in 6 European cattle (*Bos taurus*) breeds (MacHugh, 1994). The observed heterozygosity ranged from 0.00 to 0.91. A lot of the studies based on microsatellites have been performed to estimate both the relationships among the breeds and the genetic diversity within and between populations (Ashwell et al., 2004; Sun et al., 2007). Microsatellite markers were considered as a marker of choice for diversity assessment in breeds (FAO, 2004). A list of microsatellite markers for genetic characterization of cattle breeds have been approved by FAO (Navani et al., 2002). The 12 selected markers (BM1814, BM1818, BM2113, ETH3, ETH10, ETH225, INRA023, SPS115, TGLA53, TGLA122, TGLA126, TGLA227) were included in an International comparison test of International Society of Animal Genetics (ISAG). The inbreeding process and various crossbreeding systems may lead to the loss of genetic variation within breeds. In this reason a lot of breeds may become extinct. The scientific community alarmed the necessity for the conservation of livestock resources. In 1992 Food and Agricultural Organization (FAO) launched a program for the Global Management of Farm Animal Genetic Resources, with the main objective being to identify conservation activities and create an awareness of possible losses of genetic resources on an international basis (Gandini and Oldenbroek, 1999).

A global program was initiated directed towards genetic characterization of all farm animal species using DNA markers (Groeneveld et al., 2010). Microsatellite markers have been widely used for studying the genetic diversity in cattle (MacHugh et al., 1997). Genetic variability within and among populations is of high importance and may contrib-

ute to the selection and preservation of genetic resources (Groeneveld et al., 2010). Genotyping data of 30 microsatellite loci in 69 European breeds were used to outline the main criteria for the conservation of breeds (European Cattle Genetic Diversity Consortium, 2006). These breeds showed high degree of genetic diversity, that is an apparent reason for their conservation. The Busa and Anatolian breeds were considered to be valuable genetic resources on the basis of their high level of genetic diversity (Medugorac et al., 2009). Conservation priorities of Nordic cattle based on genetic diversity were outlined by Bennewitz et al. (2006) and Tapio et al. (2006).

The use of common microsatellite markers to assess genetic diversity within breeds and the inbreeding levels was used in many cattle breeds (Teneva et al., 2005, 2007; Garcia et al., 2006; Tapio et al., 2006; Ginja et al., 2009; Li and Kantanen, 2010; Qi et al., 2009). Several studies have been conducted in European and Eurasian cattle (*Bos taurus*), in which microsatellites were used to assess genetic variability and differentiation (Canon et al., 2001; European Cattle Genetic Diversity Consortium, 2006; Tapio et al., 2006; Li and Kantanen, 2010). For Creole breeds, several microsatellite-based studies were reported (Martinez et al., 2005; Armstrong et al., 2006; Quiroz-Valiente et al., 2006; Aquino et al., 2008; Ulloa-Arvizu et al., 2008; Martinez-Correal et al., 2009).

Genetic diversity and relationships among 26 Creole cattle breeds from 10 American countries were assessed using 19 microsatellites, representing North, Central, South America and the Caribbean Islands (Delgado et al., 2011). Creole cattle populations showed high level of genetic diversity comparing with breeds that are under intensive breeding. Regardless of the detected high genetic diversity, significant inbreeding was also detected on the base of microsatellites. Creole cattle breeds represent great reservoirs of cattle genetic diversity but measures to avoid inbreeding and uncontrolled crossbreeding is highly necessitated (Delgado et al., 2011).

A number of fluorescent labeled microsatellite markers have been used to characterize the Kenkatha and Gaolao breeds, indicating a little genetic differentiation between the two breeds (Alex et al., 2013). Several microsatellite markers have also been used for conservation studies concerning certain other important cattle breeds (Frankham et al., 2002; Navani et al., 2002).

The mixed origin of Indonesian zebu was confirmed in the microsatellites study for diversity by Mohamad et al. (2009). In contradiction, the microsatellite analysis and indicated that the Indonesian Bali cattle is a pure breed (*Bos javanicus*) (Groeneveld et al., 2010).

Most of the microsatellite data indicate a separate position of Mediterranean cattle, but divide the Transalpine cattle into two different clusters of breeds: Central-European and Northern European (Lenstra et al., 2006). Conservation priorities for Nordic cattle were reported by Bennewitz et al. (2006) and Tapio et al. (2006).

Using 25 microsatellite markers, Chaudhari et al. (2009) estimated 21.21% and 22.48% heterozygotes in Gaolao and Kenkatha populations, respectively. Numerous factors such as inbreeding, genetic hitchhiking, null alleles (non-amplified alleles) and occurrence of population substructures have been established as reasons of heterozygote deficit in the studied populations.

Jersey is a common and unique cattle breed originating from the UK Channel Island of Jersey. Jersey Island cattle is isolated from other UK and European cattle populations for approximately 50 generations. The genetic diversity of this breed is described for the first time by Chikhi et al. (2004) using 12 microsatellite markers – HAUT27, HEL5, BM1314, BM1818, BM2113, INRA005, INRA063, ILSTS006, ETH10, ETH225, TGLA122, and TGLA227. This study showed that the average number of alleles per locus and expected heterozygosity were comparatively high with respect to that observed in a number of continental breeds. The authors suggested reported for absence of a loss of genetic diversity and inbreeding and concluded that it is unnecessary to import unrelated animals for management purposes despite of the fact that no imports have taken place to the Island since 1789.

Egito et al. (2007) also reported a significant amount of genetic variation in Brazilian local cattle populations on the base of observed microsatellite variation in 22 STR loci. These data showed that Brazilian Creole breeds constitute an important and diverse source of genetic diversity for bovine breeding and conservation.

Recently, Sharma et al. (2015) investigated genetic diversity and relationship among 11 Indian cattle breeds using 21 microsatellite markers, and concluded that the Southern breed 'Ongole' is distinct from breeds of Northern/Central India. The results provide basic information about the genetic diversity and structure of Indian cattle which should have implications in the management and conservation of indicine cattle diversity.

Several studies have been conducted in European and Eurasian cattle (*Bos taurus*), in which microsatellites were used to assess genetic diversity and differentiation (Canon et al., 2001; Tapio et al., 2006; European Cattle Genetic Diversity Consortium, 2006; Li and Kantanen, 2010).

The 16 microsatellite loci were studied for genetic diversity analyses: CSSM 66, ETH 10, ETH 152, ETH 225,

ETH 3, HEL 1, HEL 5, HEL 9, ILSTS 005, INRA 023, INRA 032, INRA 035, INRA 037, INRA 005, INRA 063, and TGLA 44 in 10 Spanish, 5 Portuguese and 3 French cattle breeds (Canon et al., 2001). A total of 173 alleles were detected across the 16 loci analysed. Observed and expected heterozygosities per breed ranged from 0.54 to 0.72. Levels of breed differentiation were considerable with multilocus  $F_{ST}$  values indicating that 93% corresponded to differences among individuals and the remaining 7% of the total genetic variation corresponded to differences between breeds. The authors concluded that the microsatellites provides reasonable statistical power for breed assignment and allow future management of the breeds to be based on greater knowledge of their genetic structuring and the relationships between their populations based on STR markers.

In Romania the genetic diversity among Romanian Grey, Brown, Spotted and Black and White cattle breeds has been evaluated with focus on the endangered Romanian Grey breed on the base of 11 microsatellite loci (Ilie et al., 2015). Authors established a high level of genetic diversity in the endangered Romanian Grey cattle population. The results confirmed that the breed's genetic diversity is preserved correctly on the current conservation program in order to protect from genetic loss.

In Indian zebu cattle (*Bos indicus*) Chaudhari et al. (2009) reported the data of 145 purebred, unrelated cattle Kenkatha and Gaolao breeds which showed 25 microsatellite loci with a high PIC value ( $> 0.5$ ), indicating that these markers are highly informative for characterization of both cattle populations. Genetic markers with PIC values higher than 0.5 are normally considered as informative in a population (Botstein et al., 1980). Higher PIC values were also observed in the taurine and indicus breeds with microsatellite markers (Bradley et al., 1994; Canon et al., 2001; Maudet et al., 2002; Kumar et al., 2003; Metta et al., 2004; Mukesh et al., 2004; Pandey et al., 2006; Sodhi et al., 2006).

In Busha cattle in Serbia, Stevanov-Pavlović et al. (2015) evaluated 12 microsatellite markers (TGLA227, BM2113, TGLA53, ETH10, SPS115, TGLA126, TGLA122, INRA23, BM1818, ETH3, ETH225, BM1824), recommended by International Society of Animal Genetics (ISAG) for paternity testing. The authors found high PIC (Polymorphism Information Content) values ranging from 0.513 to 0.905. The results showed that the 12 marker's set recommended by ISAG can be used with high confidence for forensic purposes in Busha cattle. Molecular characterization of Indian breed Hallikar, the native cattle breed of Karnataka was performed using 19 cattle specific, highly polymorphic microsatellite markers recommended by FAO. The study proved that the cattle specific microsatellite markers used were highly poly-

morphic and highly informative for genetic characterization of cattle breeds (Kumar et al., 2006).

In comparison with other European and Balcan countries, in Bulgaria there is a big gap in cattle genetic molecular characterization. Teneva et al. (2005, 2007) studied local cattle breeds using microsatellite markers. They established a high PIC value ( $>0.5$ ) and high heterozygosity based on 11 STR.

Neov et al. (2013) genotyped local Bulgarian Grey cattle in CSN1S1 and CSN3 genes. The results showed that C allele of CSN1S1 is predominantly found in the population with frequency of 57.4%, while in CSN3 gene B allele was predominant, with a frequency of 54%. These results show that this ancient cow population may be genetically similar to other cattle population in Southern Europe.

#### **Parentage control and cattle identification**

In 1993, with the development of a high density map of the bovine genome, many microsatellites became available (Fries et al., 1993; Steffen et al., 1993). In that year initial steps in using microsatellites in cattle identification and parentage control were performed (Trommelen et al., 1993a, b). Parentage testing using DNA based markers yields much higher exclusion probability ( $> 90\%$ ) than the testing with blood groups (70–90%) or other biochemical markers (40–60%) (Wakchaure et al., 2015).

Further studies were performed to establish an internationally comparable panel of molecular markers (Glowatzki-Mullis et al., 1995; Heyen et al., 1997; Kemp et al., 1995; Peelman et al., 1998; Kantanen et al., 2000; Canon et al., 2001; Hanotte et al., 2003; Gargani et al., 2015). In many investigations FAO list of microsatellites in large number of cattle breeds were implemented (Ajmone-Marsan, 2010).

Microsatellite markers were widely used in cattle paternity analysis studied in different continents (Bruford et al., 1996; Montaldo and Meza-Herrera, 1998; Beuzen et al., 2000; Schlötterer, 2004; Visscher et al., 2002; Hansen et al., 2002; Ibeagha-Awemu and Erhardt, 2005).

#### **Statistical methods used in microsatellite analysis**

The average number of alleles ( $MNA$ ), observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity and estimation of polymorphism information content (PIC), are the most commonly calculated population genetic parameters for assessing the diversity within cattle breeds (Hanotte and Jianlin, 2005; Mburu and Hanotte, 2005). PIC values indicate the informativeness of the studied microsatellite loci. Hardy-Weinberg equilibrium test is always used to predict whether the population is stable or not. The observed genotypes are compared with the expected genotypes in a  $\chi^2$ -test for likeness of fit.

The high heterozygosity values observed in the studies indicate the presence of large number of polymorphic loci. The most simple parameters for evaluating the distribution of diversity between breeds using genetic markers are the genetic differentiation or fixation indices, e.g.  $F_{st}$ ,  $G_{st}$ ,  $R_{st}$ . They reveal the variation among populations. The most widely used is  $F_{st}$ , which measures the degree of genetic variation between subpopulations through the calculation of the standardized variances of allele frequencies amongst populations (Weir and Basten, 1990; Mburu et al., 2003). Genetic distances can also be analyzed in terms of genetic diversity and individual breed contributions to total diversity of breeds.

The main cattle microsatellite genetic parameters like observed number of alleles, allele frequency,  $F_{IS}$ , observed and expected heterozygosity, the presence of null alleles, the neutrality of the microsatellites, genetic distances, analysis of molecular variance (AMOVA) usually are analyzed by a number of commonly used population genetic computer programs for genetic microsatellite statistical analysis: GENPOP, ARLEQUIN, POPGENE, MICROSAT, PHYLIP, STRUCTURE MICROSATELLITE ANALYZER (MSA), Microchecker (Mburu and Hanotte, 2005).

## Conclusion

The development of polymorphic microsatellite markers in advanced genetics and biotechnology gives the opportunity for the selection, improvement of cattle health and production. The PCR-based microsatellite technology with its advantages and disadvantages has a huge variety of applications in cattle breeds. Microsatellite markers for improving milk production and other main productive traits as well as their association with disease in cattle breeds are useful for breeders. They may also be efficiently applied in conservation decisions. The employment of microsatellite markers in determining the resistance to economically important diseases such as mastitis and other cattle diseases is helpful to test the leak of animals and their productivity. Consequently, this genomic technology provides valuable information for cattle genetics and breeding today and in the future.

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