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THE USE OF SNP MICROARRAYS FOR BIODIVERSITY STUDIES OF SHEEP – A REVIEW*

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Abstract

The conservation of farm animal genetic resources and their protection against genetic erosion requires knowledge of biodiversity status. Genetic variation in populations can be estimated using both traditional pedigree-based methods and molecular techniques. SNP microarrays are a new generation of molecular genetic tools, which have found application in analysis of biodiversity in populations of domestic and wild sheep, in studies of resistance to intestinal parasites and foot rot, and in searching for markers associated with meat and milk yield, or colour inheritance traits. The aim of the study is the review of recent literature on the biodiversity and the use of molecular markers for population genetics in different breeds and populations of sheep.

Key words: sheep, diversity, molecular methods, microarray, SNP

The state of the world's sheep genetic resources

The domestic sheep (*Ovis aries*) is among the most numerous and first species of animals to have been domesticated. From the earliest days of domestication, which took place approximately 11,000–9,000 years B.P. in central or south-western Asia (Zeder, 2008), sheep have accompanied humans on their travels and in the settlement of new areas. Sheep constantly gained in importance because of their many uses. The migrating animals adapted to new environments, giving rise to different types (differing in anatomy and physiology) and consequently breeds, which were later improved through conscious breeding efforts. Sheep are a species with the greatest variety of breeds (1,400) according to the World Watch List (Scherf, 2000). The DAD-IS database (Figure 1) shows that Europe and Caucasus is the richest region with over half of all known sheep breeds (1271 breeds), whereas North America is the least varied (78 breeds of sheep).

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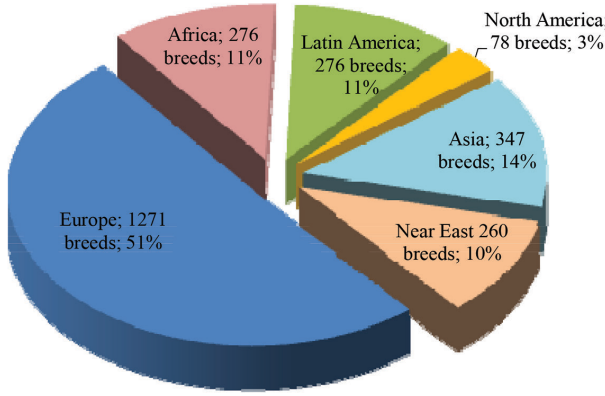

Figure 1. Diversity of sheep breeds in the world (DAD-IS, 2015)

Local breeds, i.e. those that occur only in one country, account for 80% of all breeds. The other breeds are transboundary breeds (those that occur in more than one country), regional transboundary breeds (those occurring in one geographical region), and international transboundary breeds (breeds that occur in more than one region) (Figure 2). According to the DAD-IS database, over 200 breeds of sheep are considered transboundary. For example, Suffolk meat sheep are found in as many as 46 countries, Texel in 32, Karakuls in 25, Awassi dairy sheep and East Friesian sheep in 20 countries each.

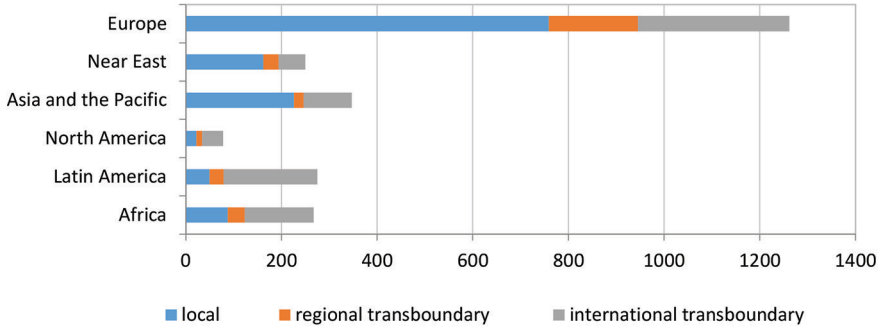

Figure 2. Distribution of local and transboundary breeds of sheep in different regions of the world (DAD-IS, 2015)

Farm animal biodiversity is threatened by many factors. The development of intensive livestock breeding is paralleled by ongoing genetic erosion, which is associated with the globalization of breeding and the increasing contribution of industrial livestock breeding. This model marginalizes local breeds and the related production

systems through gradual replacement of local breeds with high-yielding breeds, popularization of biotechnology (e.g. artificial insemination, embryo transfer), natural disasters, diseases or climatic changes.

A breed is considered endangered if the number of breeding females is less than or equal to 1 000, or the number of breeding males is less than or equal to 20, or the overall population size is less than or equal to 1 200 and decreasing and the percentage of females being bred is below 80% (FAO, 2007). Europe and Caucasus, the region with the greatest variety of sheep breeds, also has the largest number of breeds classified as endangered. Out of 180 extinct breeds (Figure 3), 148 are found in this region (FAO, 2007).

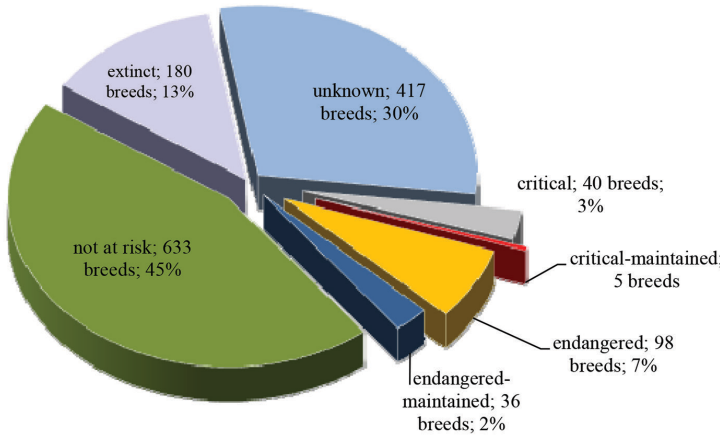

Figure 3. Risk status of the domestic sheep species (FAO, 2007)

The adoption of the Convention on Biological Diversity provided an incentive for the international community to take action on biodiversity conservation in agriculture, including the conservation of farm animal genetic resources. The Global Plan of Action for Animal Genetic Resources was aimed to characterize and monitor the world's farm animal genetic resources, to support measures to develop and implement endangered breeds conservation programmes, and to promote their wider use in production (Martyniuk et al., 2013).

The conservation of farm animal genetic resources and their protection against genetic erosion requires knowledge of biodiversity status. The estimation of diversity, description and analysis of the genetic structure of populations, as well as the inventory and periodic monitoring of trends using different methods and tools are critical for the conservation and management of farm animal genetic resources. Genetic variation in populations can be estimated using both traditional pedigree-based methods and molecular techniques.

Molecular markers may be useful for estimating the population's genetic variation and degree of inbreeding, for parentage testing, and for estimating genetic dis-

tance between breeds, populations or lines of animals. They also have application in phylogenetic studies. The most important tools include analysis of microsatellite sequences, haplotypes, mitochondrial DNA, AFLP technique, or the increasingly used single nucleotide polymorphism (SNP) (Lenstra et al., 2012).

The aim of the present article was to review current studies that use molecular techniques for estimating biodiversity in sheep, with particular consideration of SNP microarrays.

Molecular methods for biodiversity characterization

For many years, the genetic structure of sheep breeds and lines, and the differences within and between them have been investigated with class I genetic markers (**erythrocyte antigens**, **polymorphic plasma and erythrocytes proteins**), which are identified using serological methods. Studies using these markers were performed in many native Polish breeds (Rychlik and Krawczyk, 2007). The studied breeds differed in genetic variation of the markers, which was reflected in the number and frequency of blood group alleles and polymorphic blood proteins, in the effective number of alleles, and in the degree of heterozygosity. The observed differences might be due to differences in origin of the breeds or result from the breeding work. Studies performed by Rychlik et al. (2006) with Wrzosówka sheep over 15 years showed a decrease in genetic variation, as evidenced by reductions in the total number of alleles (from 0.62 to 0.42), effective number of alleles (from 4.48 to 2.9) and mean degree of heterozygosity (from 0.56 to 0.49).

In recent years, short tandem repeat (STR) microsatellite markers have become the most popular of the genetic markers. The microsatellite markers, which are used in many areas of genetics, are simple tandem repeats of two to four nucleotide motifs. They are characterized by large variation in the number of basic motifs in the repeated sequence, which determines microsatellite polymorphism. This characteristic, along with high frequency, repeatability and ease of identification, make these markers extremely useful for characterizing the genetic structure of populations and for estimating genetic variation in animals. Microsatellite markers are used to estimate the degree of population inbreeding, to test parentage (Radko and Rychlik, 2010; Souza et al., 2012), and to estimate genetic distance between breeds, populations or lines of animals (Kusza et al., 2009; Salamon et al., 2014). They also have application in phylogenetic studies (Tapio, 2010) and in estimating genetic variation in local breeds of sheep that are often threatened with extinction (Ferrando et al., 2014; Glowatzki-Mullis et al., 2012). These activities are also foreseen in the Global Plan of Action for Animal Genetic Resources. The Global Project for the Measurement of Domestic Animal Diversity (MoDAD) proposes a set of markers useful for studying the genetic structure of farm animals (FAO, 2004). These were used in the ECONOGENE project, which aimed to expand knowledge of sheep and goat biodiversity in Europe and the Middle East using molecular biology techniques, taking into account the socio-economic conditions where these animals are raised (www.econogene.eu; Peter et al., 2007). Many projects dealt with local populations and searched for the effect of other breeds in a historical context (Blackburn et al., 2011; Tapio et al., 2010).

Analysis of variation in mitochondrial DNA (mtDNA) has become widely used in various scientific fields (e.g. species identification of feed components, individual identification of female pedigree lines). It also proved very useful in phylogenetic studies (Koseniuk and Rychlik, 2013). Studies using mtDNA polymorphism as a marker showed the contribution of mouflon (*Ovis musimon*) to the *Ovis aries* species raised in Europe, Asia and North America. At the same time, the role of *O. vignei* and *O. ammon* in creating the sheep breeds raised today has been questioned. Based on the analysis of mtDNA sequences, Eurasian sheep were classified into 5 haplogroups. Haplogroup A is the lineage found in Asia, haplogroup B in Europe, and haplogroup C encompasses sheep originating from the Iberian Peninsula and Turkey. All of these haplogroups share a phylogenetic line with *O. musimon* and *O. orientalia*, but not with *O. vignei* and *O. ammon.* Haplogroup D shares a lineage with all of the ancestors studied. The fifth haplogroup (haplogroup E) combines haplogroups A and C (Meadows et al., 2007; Tapio et al., 2006). Pariset et al. (2011) used mtDNA (D-loop) and single nucleotide polymorphism (SNP) to study geographic distribution of diversity in sheep from Mediterranean countries and to search for information about their migration history. The study detected 154 unique haplotypes and 93 polymorphic sites. The greatest number of haplotypes was observed in sheep originating from Greece, and the highest single nucleotide polymorphism in Albanian sheep. Three haplogroups (A, B and C) were found in the studied population; haplogroup B was the most frequent (89%) and haplogroups A and C much less frequent (8% and 3%, respectively). All three haplogroups occurred in the Albanian and Greek breeds, and no haplogroup C was revealed in the Italian sheep. Haplotype diversity was high within breeds (95%) and much lower between regions (less than 1%).

In studies of sheep diversity, **amplified fragment length polymorphism (AFLP)** is used less often than the previous methods. This DNA fingerprinting technique detects fragments obtained after digestion of genomic DNA with restriction enzymes. The findings of Hoda et al. (2010) showed the usefulness of this marker for estimating genetic variation. The authors found high variation within Albanian breeds, as well as small genetic distance between the Albanian Shkodrane breed and the Bardhoka breed from Kosovo, which may be indicative of their common origin.

A new tool that has been increasingly used in animal breeding in recent years are **SNP microarrays**, which allow genotyping thousands of polymorphic sites simultaneously. **Single nucleotide polymorphism (SNP)** is a variation in DNA sequence, in which a single nucleotide is substituted in different regions of the nuclear genome. Single nucleotide substitutions are scattered throughout the genome. This natural phenomenon is the main source of genetic variation. SNP microarrays allow the simultaneous analysis of several dozen thousand such regions. Genome-wide scanning with a large SNP panel enables genetic variation to be analysed in the whole genome using the phenomenon of linkage disequilibrium.

The advantage of using SNPs in large-scale studies results from the availability of high numbers of annotated markers, low-scoring error rates, relative ease of calibration among laboratories compared to fragment-size based markers (like microsatellites) and the related ability to accumulate data sets from different laboratories. Furthermore, the potential for high-throughput genotyping improved genotypes

reliability for lower quality samples (such as historical, noninvasive or degraded samples) (Morin and McCarthy, 2007; Smith et al., 2011). A simple mutation model, and the ability to examine both neutral variation as well as regions under selection offers unparalleled scope for extensive screening of genomes and large sample sizes from various populations. Although several early studies questioned the advantage of SNPs over microsatellite markers (Rosenberg et al., 2003), more recent studies have shown that SNPs are also showing high informativity, as many studies based on large numbers of SNPs have shown that even a small fraction of the SNPs may have a very high information content for population structure analysis (Lao et al., 2006; Gurgul et al., 2013), outperforming microsatellites (Liu et al., 2005). Although microsatellites typically display far greater allelic diversity per locus, individual SNPs often segregate among populations (Karlsson et al., 2011).

Establishing a set of SNP markers, which can be used to assess regions of the genome involved in local adaptation and in speciation is important to understand fundamental similarities and differences between populations and breeds of farm animals. Once a highly polymorphic markers panel is established it can be further used to search for signatures of natural or artificial selection. A number of studies have established genetic differences between populations of livestock mainly with respect to differential or production traits-directed selection (Gurgul et al., 2015).

SNPs have been used to establish differences between individuals (Gill, 2001), populations (Paschou et al., 2007; Yamaguchi-Kabata et al., 2008) and species (Kong et al., 2008). They also are a useful tool for analysing susceptibility to disease (Johnson et al., 2007), disease states (Poehlmann et al., 2007), and evidence of the genetic basis of adaptation to environmental conditions (Namroud et al., 2008).

SNP analysis has found application in the study of population structure and in the determination of differences between single-species populations (Wojciechowska and Olech, 2013). Kijas et al. (2009) investigated 28 populations of domestic sheep from Africa, Australia, New Zealand, Asia, North America, Europe as well as wild populations using a microarray with 1536 SNPs. The indicators of genetic diversity were higher for *O. aries* than for wild sheep. Among domestic sheep, the genetic diversity indicators were highest for the Australian Merino population and lowest for the African Namaqua Afrikaner breed. The study showed that the African and Asian populations developed separately from the European ancestor and from the European, Australian and American populations.

Today, BeadArray (BeadChip) technology-based microarrays are widely used for different animal species, in which probes for different SNP markers are attached to 3-micron silica beads assembled in microwells on microarray surface. Amplified and fragmented DNA is dispensed on the array surface. Following DNA hybridization with probes on the microarray surface, fluorescently labelled nucleotides are incorporated to enable identification of an animal's SNP genotype. Illumina's BeadChip microarrays include panels of markers developed for cattle (BovineSNP50), pigs (PorcineSNP60) and dogs (CanineSNP20). The sheep genome is analysed using the OvineSNP50 BeadChip, which has been developed for domestic sheep (www.illumina.com) in collaboration with the International Sheep Genomics Consortium (ISGC). The microarray allows the analysis of 12 samples simultaneously (Fi-

gure 4). Each microarray area is designed to analyse one sample and features 54 241 probes. These microarrays are medium-density arrays because the average gap between adjacent SNPs in the genome is 50.9 kb. This microarray design (high density of probes) makes it a very powerful, efficient and economical tool with a broad range of uses in genetic analysis of most sheep breeds in the world. When designing the OvineSNP50 BeadChip, the scientists from Illumina and ISGC genotyped over 3000 samples collected from 75 economically important breeds of domestic sheep (*Ovis aries*) (Table 1). The tests also used wild species of the *Ovis* genus: mouflons (*Ovis musimon*), North American bighorn (*O. canadensis*) and thinhorn sheep (*O. dalli*), or Asiatic urials (*O. vignei*) and argali (*O. ammon*). The designed microarray is applicable to a variety of genetic analyses: genomic selection, identification of quantitative trait loci (QTL), study on linkage disequilibrium, comparative genetic studies, and characterization of population genetic structure for evaluation of genetic diversity.

Figure 4. OvineSNP50 BeadChip Illumina® (www.illumina.com)

Miller et al. (2011) used the OvineSNP50 BeadChip array (49 034 probes) to show differences between the populations of wild bighorn (*O. canadensis*) and thinhorn sheep (*O. dalli*). The bighorn sheep population accounted for animals originating from two subpopulations: Ram Mountain and Wyoming. Genotypes were determined for over 90% of the markers, but the number of polymorphic SNPs was very low (less than 2%). Based on the polymorphic markers, differences at the molecular level were found between these species, as well as differentiation within the *O. canadensis* population, in which family groups occurred. Despite the small number of polymorphic markers, the study showed that the microarray developed for domestic sheep is useful for genetic analyses of wild species, for analyses of domes-

tication consequences, and for finding the relationships between domestic sheep and their wild counterparts.

Table 1. Number of polymorphic SNP loci and diversity indices of selected European breeds of sheep

Breed	SNP[1]	P_n [2]	H_e [2]	F[2]	A_r [2]	pA_r [2]
Southern and Western Europe						
Altamurana	46 656	0.94	0.36	0.10	1.97	0.01
Castellana	46 970	0.94	0.37	0.07	1.97	0.01
Chios	43 360	0.91	0.32	0.18	1.93	0.00
Churra	47 048	0.96	0.36	0.11	1.99	0.00
Comisana	46 747	0.94	0.36	0.06	1.97	0.00
Leccese	46 852	0.95	0.37	0.12	1.97	0.01
Merinolandschaf	45 531	0.93	0.35	0.11	1.96	0.00
Lacaune	47 840	0.96	0.36	0.11	1.99	0.00
Rambouillet	46 670	0.96	0.36	0.14	1.99	0.00
Rasa Aragonesa	48 676	0.95	0.38	0.04	1.98	0.01
Sardinian Ancestral Black	45 451	0.92	0.33	0.11	1.94	0.02
Central Europe						
Black Headed Mutton	44 296	0.92	0.33	0.18	1.95	0.01
East Friesian Brown	41 295	0.90	0.30	0.26	1.92	0.00
East Friesian White	41 800	0.80	0.29	0.22	1.82	0.00
Swiss Black-Brown Mountain	44 961	0.93	0.35	0.11	1.95	0.00
Swiss Mirror	46 122	0.92	0.35	0.11	1.95	0.00
Swiss White Alpine	44 986	0.93	0.35	0.11	1.96	0.01
Valais Black Nose	41 547	0.87	0.31	0.24	1.90	0.00
Valais Red	40 855	0.86	0.31	0.18	1.89	0.00
Northern Europe						
Dorset Horn	39 029	0.87	0.29	0.20	1.89	0.00
Finnsheep	46 566	0.95	0.36	0.14	1.98	0.01
Galway	44 443	0.93	0.33	0.15	1.95	0.00
German Texel	45 987	0.95	0.35	0.13	1.97	0.00
Irish Suffolk	43 627	0.93	0.33	0.22	1.95	0.02
Old Norwegian spaelsau	45 479	0.90	0.35	0.17	1.94	0.00
Scottish Blackface	44 521	0.95	0.36	0.11	1.98	0.01
Scottish Texel	44 521	0.94	0.33	0.13	1.97	0.01
Soay	36 728	0.78	0.26	0.33	1.82	0.00
Spael Coloured	35 619	0.67	0.28	0.18	1.71	0.00

Source: [1]http://www.illumina.com; [2]Kijas et al., 2012.

Legend: P_n – proportion of SNP displaying polymorphism; H_e – expected heterozygosity; F – inbreeding coefficient; A_r – allelic richness; pA_r – private allelic richness.

Ciani et al. (2014), who used the OvineSNP50 BeadChip to analyse the genetic structure of 19 Italian breeds, found that Sardinian breeds diverged from other breeds as a result of the combined effect of ancient sporadic introgression of feral mouflon and long-lasting genetic isolation from continental sheep populations. The authors

presented arguments that question, from a genomic point of view, the current breed classification of Bergamasca and Biellese into two separate breeds. Expected heterozygosity in Italian breeds ranged from 0.33 to 0.37. These values conform with those reported by Kijas et al. (2012) for south and west European sheep genotyped with the Illumina OvineSNP50 BeadChip (Table 1). The mentioned study provides an extensive description of the genetic structure of modern sheep breeds, which has been shaped through the processes of domestication and selection. The authors investigated DNA in sheep representing 74 breeds from all over the world. When evaluating the genotype of every animal, they analysed 49 034 SNP. The results showed that the sheep breeds exhibit a high level of genetic variation, unlike other domestic animals such as dogs. Different areas of the genome contain strong signs of selection pressure, e.g. directed towards the generation of hornless animals, which is a feature of many modern breeds. A genome scan revealed 31 regions encoding genes responsible for coat pigmentation, reproduction, and body size (Fariello et al., 2014). The usefulness of this tool in the study of species genetic variation, breed identification and detection of population structure was confirmed by Grasso et al. (2014), who investigated two breeds popular in Uruguay (Merino and Corriedale) and the endangered Creole breed. Merino and Corriedale were characterized by a high level of SNP polymorphism (89.4% and 86%, respectively) and intermediate differences between one another (FST = 0.08). Conversely, the Creole breed showed lower SNP polymorphism (69%) but a much higher fixation index (FST = 0.17). The authors attributed the differences in variation to the different selection processes in the breeds under study. An in-depth genome analysis of the Merino and the Merino-derived other breeds, used all over the world, was performed by Ciani et al. (2015) using the OvineSNP50 BeadChip. The estimated genetic diversity indices (ratio of polymorphic loci, heterozygosity, coefficient of inbreeding) for the Merinos were similar to those obtained for other modern breeds that had been formed without Merino contribution. The article cited above shows the history of "Merinization", with intensive gene flow, founder effect and geographic isolation as the main factors determining the current Merino population in the world. The genetic diversity of local Swiss breeds of sheep were analysed using 49 193 SNP by Burren et al. (2014), and the obtained results should support the conservation activities. A genome scan of 18 native Welsh breeds of sheep (Beynon et al., 2014) showed the existence of four subpopulations. Mountain breeds formed a relatively homogeneous group, standing in stark contrast to the Black Welsh Mountain sheep, selected over the years for dark wool colour, which led to a considerable reduction in the effective population size and increased the inbreeding. Comparison with the other breeds that had been used in the HapMap project (Kijas et al., 2012) showed that the European and Welsh breeds have a common ancestry.

In sheep breeding, SNP microarrays also found application in parentage verification. Heaton et al. (2014) analysed 47 693 SNP in 74 breeds of sheep and chose 163 SNP useful for parentage testing (MAF>0.3). From the viewpoint of the economics of sheep farming and the losses generated by diseases associated with grazing of sheep (helminthiasis, paronychia), it is important to find the relationships between genotype and resistance to these diseases. Riggio et al. (2014) analysed the genotype

of three breeds in association with the loci of resistance for gastrointestinal nematodes. In turn, Mucha et al. (2015), who used a 50K SNP array, searched the genome for regions involved in controlling resistance to foot rot in Texel sheep. Their findings revealed no major genes responsible for this trait, which indicates that it is polygenically determined. The large-scale use of SNP microarrays in sheep production is already the case on Australian and New Zealand farms (Auvray et al., 2014), but also on French farms (Baloche et al., 2014).

A 600 000 SNP chip is now also available (Ovine Infinium® HD SNP BeadChip; Anderson et al., 2014). Macedo et al. (2014) attempted to use this microarray as a tool in the genetic resources conservation programme for the Creole sheep. The study by Kijas et al. (2016) using ovine HD SNP BeadChip, showed the presence of loci associated with the unusual, four-horn phenotype in Navajo-Churro and Jacob sheep.

Conclusions

Microarray technology has generated huge quantities of data on animal genetic variation. These data are useful for fine-scaled inferences of livestock evolutionary history and, under some circumstances, the estimation of individual ancestry. In this context, the new data have contributed to a better understanding of the relationship between genetics and breeds formation. SNP microarrays are a new generation of molecular genetic tools, which could be useful in analysis of biodiversity in populations of farm and wild animals. It gives comprehensive view into their genomes and allows differentiation of even closely related breeds or populations. Genome analysis based on SNP markers allows for determining genetic variation in different species, breeds, and populations with unprecedented accuracy. The possibility of analysing several dozen thousand genetic markers simultaneously ensures the high efficiency and reliability of the analysis. In addition to analysing the genetic structure of sheep populations, SNP microarrays are used to search for genetic basis of sheep resistance to intestinal parasites and foot rot, as well as in searching for markers associated with meat and milk yield, or coat colour traits.

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