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MICROSATELLITE PANEL FOR EVALUATING THE ACCURACY OF SHEEP ORIGIN

Abstract

The article presents the results of a study of the vegetation cover of the lambing site of the Ural population saigas in the areas of the two regions of the West Kazakhstan region, Kaztalov and Zhanibek.The species composition of the flora of the study area is represented by 75 species from 57 genera and 22 families.

Keywords: *saiga feeding, flora and vegetation cover, species, families, genus.*

The study of the genetic structure of farm animal species using the latest biotechnological methods is a fundamental factor in increasing the performance of pedigree work in the agricultural industry.

Earlier, genetic certification of agricultural animals in the world was carried out with the help of immunogenetic labels, but preference was given to them with the development of modern biotechnological methods that allowed to estimate the animal by origin with a high accuracy of 99,999%, and now testing with the help of immunogenetic markers is considered unreliable. One of the molecular genetic methods is the analysis of the animal on STR loci (microsatellites, short tandem repeats), which has the highest polymorphism, and also makes it possible to determine the purebreed and inbreeding degree during one analysis [1].

Several years ago, a multi-locus STR-panel for cattle of the Belarusian black-and-white breed was introduced on the territory of the Republic of Belarus, allowing to hold on pedigree work not only using traditional breeding methods, but also using modern molecular genetic methods [2]. These methods allow you to monitor the reliability of the origin of animals, as well as diagnose genetic hereditary anomalies, the presence of which does not allow the use of the animal in the future process of reproduction. Since cattle is not the only type of animal farming that are bred on the territory of the Republic of Belarus, it is necessary to conduct studies on the development of a multilocus panel to assess the authenticity of origin and other animals of high value, including sheep. Therefore, the purpose of these studies was to develop a multilocus STR panel to assess the reliability of the breed origin of sheep bred on the territory of the republic.

The research was carried out on the basis of the branch research laboratory of «DNA technologies» of the Grodno State Agrarian University. Samples of ear tissue of sheep were used as the biological material necessary for the isolation of nuclear DNA. DNA extraction was carried out by a perchlorate method with double purification (according to the method of Zinoviyeva). The degree of purification and the natality of the isolated DNA was checked with the Implen P330 spectrophotometer.

We selected 11 STR-loci, which has the greatest informative value and polymorphism in sheep, bred in SEC «Khvinevichi» of Grodno region of the Republic of Belarus.

The final volume of the reaction mixture for the PCR analysis is 14 μl and consists of the following components, manufactured by «PRIMTECH» (the Republic of Belarus): PCR buffer: 50 mM KCl, 10mMTris-HCl, 2,5 mM dNTPs; 1,5 mM $MgCl_2$, 20-50 pmol / μl of each primer and 0.1 μL of 5U Taq polymerase; we bring the deionized water to the final volume. In the following steps, 10-100 ng / μl of isolated DNA was added to the reaction mixture.

The PCR program developed by us consists of the following steps: initial denaturation, which took place at a temperature of 95^0C for 1 minute; 3 cycles: 95^0C - 45 seconds, 62^0C - 1 min; 3 cycles: 95^0C - 45 sec and 59^0C - 1 min; 3 cycles: 95^0C - 45 sec and 56^0C -1 min; 3 cycles: 95^0C - 45 sec and 53^0C - 1 min; 25 cycles: 95^0C - 45 sec and 50^0C - 1 min; 72^0C - 5 min.

The temperature regime for annealing of the primers was selected taking into account the melting parameters of single sequence of oligonucleotides. The copied fragments were examined using a genetic analyzer (sequencer) 3500, manufactured by Applied Biosystems (USA). With the help of specialized software, in particular GeneMapper, the allelic dimensions of each locus were determined.

There has been developed a system for assessing of the authenticity control of sheep origin of local breeds. The aim of the study was achieved by selecting STR-loci having the necessary characteristics, components of the reaction mixture, optimizing the protocols, as well as the conditions necessary for fragment and PCR analysis.

Taking into account the recommendations of the International Society for Animal Genetics (ISAG), we selected 11 loci with the greatest informativeness, as well as those mentioned in the largest number of scientific publications of scientists from all over the world [3]. From the published articles, we selected the main characteristics of the investigated STR loci, such as, chromosome localization, the number of alleles, the size of the repeats and, respectively, the sequence of oligonucleotides for each microsatellite [4].

One of the main tasks when optimizing the PCR protocol is to correctly select primers capable of annealing under the same conditions. At the same time, when forming a multilocus mixture, it is necessary to take into account all characteristics of the sequence of oligonucleotides, in order to avoid the formation of dimers, during the passage of PCR, which, when accumulated, can give false positive results.

The number of cycles also plays an important role, because when using a large number of cycles, all the components used, such as a mixture of dNTPs, a buffer, Taq polymerase and others, can be depleted, resulting in the possibility of accumulating nonspecific reaction products, therefore, in optimized PCR The program included only 35 cycles.

The temperature regime during the annealing phase was selected using special software Oligo 7, which takes into account the annealing temperature for each sequence of synthetic oligonucleotides separately. However, during the experiment this temperature regime was corrected. At the elongation stage, the temperature was selected experimentally, as a result, the selected temperature regime has the maximum polymerase activity.

Determining the concentration of DNA using the Implen P330 spectrophotometer at a length of 260 nm plays a significant role in the PCR setting, so in the studies the optimal DNA concentration did not exceed 100 ng / μl. The amount and concentration of all the other components of the reaction mixture was chosen empirically.

The sequence chosen for us, as well as the fluorescent labels and the length of the fragments, are presented in Table 1.

Table 1 - Characterization of STR loci selected to establish of sheep origin

Locus	Primer structure	Length of a fragment	Primer label
McM042	CATCTTTCAAAAGAACTCCGAAAGTG – for CTTGGAATCCTTCCTAACTTTCGG - rev	86-109	FAM
OarVH72	GGCCTCTCAAGGGGCAAGAGCAGG - for CTCTAGAGGATCTGGAATGCAAAGCTC - rev	121-145	FAM
McM527	GTCCATTGCCTCAAATCAATTC – for AAACCACTTGACTACTCCCCAA - rev	155-195	FAM
CSRD247	GGACTTGCCAGAACTCTGCAAT – for CACTGTGGTTTGTATTAGTCAGG - rev	213-219	FAM
OarFCB20	GGAAAACCCCCATATATACCTATAC – for AAATGTGTTTAAGATTCCATACATGTG - rev	94-118	TMR
INRA006	AGGAATATCTGTATCAACCGCAGTC – for CTGAGCTGGGGTGGGAGCTATAAATA - rev	101-141	TMR
INRA063	GACCACAAAGGGATTTGCACAAGC – for AAACCACAGAAATGCTTGGAAG - rev	157-195	TMR
MAF065	AAAGGCCAGAGTATGCAATTAGGAG – for CCACTCCTCCTGAGAATATAACATG - rev	110-144	JOE
ETH152	TACTCGTAGGGCAGGCTGCCTG – for GAGACCTCAGGGTTGGTGATCAG - rev	157-169	By-5
MAF214	AATGCAGGAGATCTGAGGCAGGGACG – for GGGTGATCTTAGGGAGGTTTTGGAGG - rev	175-205	By-5
INRA023	GAGTAGAGCTACAAGATAAACTTC – for TAACTACAGGGTGTTAGATGAACTC - rev	160-195	JOE

Before placing the amplified fragments in the genetic analyzer 3500, they must be subjected to a denaturation process for 3 minutes at 95^0C using a TDB-120 thermostat, Biosan (Latvia), after this time, the sample must be quickly transferred to ice and left for a short time . After cooling, the samples were loaded directly into the sequencer, always following all instructions from the device manufacturer. The results obtained were analyzed using the specialized software GeneMapper Software 5.0.

A multiplex panel of DNA microsatellite has been developed to assess the reliability of the origin of sheep, which allows to achieve control with the highest accuracy (99,999%) when confirming the origin of breeding animals. However, for further use of this animal in reproduction, one should not focus only on genetic testing of animals using modern biotechnological methods, it is also necessary to take into account both the conditions of the content, the balanced diet and other criteria that in the complex most favorably affect the animal grown to produce high-quality products.

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ТҮЙІН

Қазіргі кезде, қазіргі заманғы ғылымның арқасында, биотехнологиялық әдістерді қолданатын жануарлардың шығу тегі түпнұсқалығын бақылауға болады. STR талдау - жануарлардың шығу тегі сенімділігін бағалауда жоғары дәлдікке (99,999%) қол жеткізуге мүмкіндік беретін әдістердің бірі. Беларусь Республикасының аумағында бұл технология малдың шығу тегі сенімділігі үшін енгізілді. Бірақ, бұл әдістер қой шаруашылығында пайдаланылмайды, сондықтан қойлардың шығу тегі сенімділігін бақылау үшін микросателлитналық талдау әдісі әзірленді, оның ішінде STR loci және primers таңдау және талдау үшін жағдайды оңтайландыру.

РЕЗЮМЕ

В настоящее время благодаря современной науке, можно проводить контроль достоверности происхождения животных с помощью биотехнологических методов. STR анализ - один из методов, позволяющий достичь высокой точности (99,999%) в оценке достоверности происхождения животных. На территории Республики Беларусь внедрена эта технология для достоверности происхождения крупного рогатого скота. Но данные методы не используются при селекции овец, именно поэтому была разработана методика микросателлитного анализа для контроля достоверности происхождения овец, включающая подбор STR-локусов и праймеров, а также оптимизацию условий для проведения анализа.