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A genome-wide SNPs searching using the Illumina BeadChip in Jalgin Merino sheep breed

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Abstract

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The massive implementation of genotyping by sequencing method (GBS) in Jalgin Merino sheep breed, requires the identification of loci in the genome with a sufficient frequency of occurrence in the population. To identify them, genotyping of Jalgin Merino sheep was carried out using Ovine Infinium HD BeadChip 600K. As a result of polymorphism evaluation of 606 000 loci, 729 SNPs were selected with a frequency of occurrence of homozygous variants in the range of 0.2850-0.3149. After excluding substitutions located closer than one centimorganide, a list of 468 polymorphisms was obtained. The selected substitutions were located on all 26 autosomes. The greatest number of polymorphisms were on the first three chromosomes. The least substitutions were found on chromosomes 24, 25, and 26. Only one substitution with the required frequency of occurrence was identified on the X chromosome. The average distance between SNPs was 3200 to 7000 kbp. The list of selected polymorphisms can be used to confirm the reliability of the origin in the molecular genetic examination of sheep of the Jalgin Merino breed.

Keywords: Jalgin merino sheep breed; SNP; genotyping by sequencing (GBS); NGS

Introduction

Genotyping of farm animals is a promising method for assessing and predicting breeding value. The use of methods such as marker-associated selection could significantly increase the productivity of many breeds of cattle (Mrode et al., 2019), pigs (Gao et al., 2021), sheep (Xu et al., 2021). Animal genotyping includes two main areas. This is the determination of breeding value by confirming the proving of parental origin (Al-Atiyat, 2015) and the identification of markers of productive qualities (de Camargo, 2019). For each of these areas, different methods of genome research are preferred.

To confirm the parental origin, the study of polymorphism of microsatellite loci is most often used. This method allows, at a relatively low cost, to reliably assess the contribution of each parent to the formation of the offspring genotype, but this is where its ability to study the inheritance of productivity parameters ends (Al-Atiyat, 2015). More promising is the use of methods for genotyping and confirmation of paternity based on the detection of single nucleotide polymorphisms (SNPs) in the genome. This can be done using an

instrumentation similar to that used to study microsatellite markers (Edea et al., 2017). However, in order to use the data obtained for serious population genetic studies, it is necessary to expand the list of studied SNPs to several hundred. In this case, detection requires either DNA biochips (Ciani et al., 2020) or next generation sequencing (NGS) technologies (Willis et al., 2018).

Nowadays, the study of SNP markers is a priority in the practical genotyping of animals, since they can also be used as molecular genetic markers for the productive qualities of animals (Rahman et al., 2021). Great effect was shown using of SNP-markers for increase meat productivity in sheep (Brito et al., 2017), milk and meat productivity in cattle (Gebrehiwot et al., 2021), meat productivity in pigs (Gao et al., 2021). At the same time, the ongoing study of the genome constantly offers new genetic markers, which should be promptly added to the studied group. The most flexible approach for genotyping is the use of next-generation sequencing, which makes it easy to change the list of studied loci and adapt to the specific requirements of certain breeds of animals. Sequencing technology also allows simultaneously identification of new SNPs, which cannot be done using DNA biochips (De Donato et al., 2013).

Sequencing is a promising technology that uses platforms (for example, AgriSeq from Thermofisher, USA) that provide a flexible tool for designing a panel of studied loci (Willis et al., 2018).

However, in order to develop such a panel, a number of preliminary studies are necessary. This would allow selecting the most appropriate loci for a particular breed with sufficient allele frequency and proven association with productive traits. That kind of study is most effectively performed using a genome-wide association search based on DNA biochips. From a large number of loci studied on the chip, which in sheep reaches 600 thousand, it is possible to select those passing for further use in genotyping by sequencing (Braz et al., 2021).

The aim of the present study was to perform the whole-genome sequencing of rams from Jalgin Merino sheep breed by using DNA biochips with a density of 600K and suggesting loci for further detection by the NGS method.

Materials and Methods

Ethics statement

The sample collection and study purpose were approved by the Institutional Animal Care and Use Committee (approval number 2022-0061, 11.02.2022) of the All-Russian Research Institute of Sheep and Goat Breeding, Stavropol, Russian Federation.

Phenotypic data

The objects of the study were rams of the Jalgin Merino breed at the age of 12 months from breeding farms in the Stavropol Territory (Russian Federation). Total of 50 individuals with the most prominent breed characteristics were randomly selected for genotyping. They included the length, density and fineness of the wool, the quality of the fat, the parameters of the exterior, live weight. Wool parameters were assessed according to the recommendations of Holman & Malau-Aduli (2012). The exterior was assessed by measurements using a measuring tape and a pelvimeter. Weighing was performed using a balance with an accuracy of 10 g. The live weight was 75.6±0.8 kg, the height at the withers was 76.3±0.5 cm, the length of the wool fibers was 11.4±0.5 cm, the fineness of the wool was 18.3±0.3 μm. All animals were clinically healthy, kept under optimal conditions and received a balanced diet.

Genotyping

Genomic DNA was isolated from whole blood samples taken under aseptic conditions from the jugular vein using the Pure Link Genomic DNA MiniKit (Invitrogen Life Technologies, USA) in accordance with the manufacturer's protocol. Animal genotyping was performed using Ovine Infinium HD BeadChip 600K (Illumina Inc. CA, USA) according to the manufacturer's protocol. Initial processing of the genotyping results was performed using the Genome Studio 2.0 software (Illumina Inc. CA, USA).

Quality control of genotyping

Quality control of genotyping was carried out using PLINK V.1.07 software (Purcell et al., 2007). The data processing included samples with call rate of detected SNPs more than 0.95. Substitutions with a minor allele frequency (MAF) of less than 0.05 and a missing genotype of more than 0.1 were also excluded. The value $p = 0.0001$ was used as the threshold according to the Hardy-Weinberg equilibrium criterion. With a positive result, all 50 samples of studied animals underwent genotyping quality control.

Genetic and statistical analysis

The analysis of genotyping results, the study of the frequency of occurrence of polymorphisms, and the assessment of the distribution of SNPs by genome regions were performed using Genome Studio 2.0 software (Illumina Inc. CA, USA). Descriptive statistics for distances between SNPs were calculated using Excel for Windows (Microsoft, USA). For SNP mapping, the Ovis_Aries_3.1 genome assembly was used.

Results

Genotyping of Jalgin Merino sheep using Ovine Infinium HD BeadChip 600K revealed a large number of SNPs with a high frequency of both wild and mutant alleles. For genotyping by sequencing, was aimed to be selected at least 400 SNPs with a frequency of wild homozygous genotypes, mutant homozygous genotypes, and heterozygotes of about 0.3. To do this, the frequency range for wild homozygous genotypes was first established, among which mutant homozygotes were then selected in the same frequency range. Next, the total number of selected SNPs corresponding to both criteria was estimated. The results were presented in Table 1. The frequency range of 0.2950-0.3049 was insufficient, as 57 polymorphisms corresponded to it. Also, the intervals slightly increased to 0.2900-0.3139 did not meet the required criteria (№ 2-4, Table 1). Wider intervals (№ 6,7, Table 1) also failed the criteria, as the number of polymorphisms included in them exceeded 1000.

For further study were selected SNPs whose frequency ranged of wild and mutant homozygous genotypes ranged from 0.2850 to 0.3149. 729 polymorphisms met this criterion, exceeding the initial requirement of 400 SNPs. But this allowed for further selection by excluding closely spaced and jointly inherited substitutions. An analysis of the frequency of occurrence of heterozygous genotypes for the selected SNPs showed that they were also quite frequent in the population and were in the range of 0.3569–0.4296 with an average value of 0.394. During the study of the location of SNPs on chromosomes 2 SNPs without physical localization were excluded (OAR3_57752691.1 and s24661.1), using 727 polymorphisms for the next stage of research.

At the next stage, the number of selected SNPs was estimated depending on the frequency of occurrence in the studied group of animals, individuals or rams of the Jalgin Merino breed. The largest number of wild homozygous variants of these polymorphisms were substituted by a frequency near the lower limit of the selected range of 0.2850-0.3149 (Figure 1A). The distribution of the frequency of occurrence of wild homozygous variants of the studied SNPs depended on the localization on the chromosomes and showed that the average frequency of occurrence on the chromosomes was in the range of 0.285-0.300 (Figure 1B). The exception was the X chromosome, which contained only one of the chosen substitutions, and therefore the average frequency is not calculated. The maximum values of the frequency of occurrence of SNPs on some chromosomes exceeded 0.308, but for thirteen chromosomes they were lower, within 0.305. The minimum frequencies of almost all chromosomes were close and were in the range of 0.285–0.290.

The heterozygous variants of the studied polymorphisms with the highest numbers had frequencies of occurrence between 0.3569-0.4296 (Figure 1). The distribution of the frequency of occurrence of heterozygous genotypes by chromosomes differed significantly from wild homozygous genotypes (Figure 1D). The average frequency of occurrence varied from 0.400 to 0.410. The same applies to the difference in the minimum values of the frequency of occurrence of heterozygous SNP variants. Their values ranged from 0.375 on chromosomes 8 and 15 to 0.396 on chromosome 7 and 0.4 on chromosome 26. The scatter interval of the maximum frequency values was larger. It ranged from 0.408 (on four chromosomes 4, 10, 23, and 25) to 0.428 on fourteen chromosomes.

The frequency distribution of mutant homozygous variants of the selected SNPs in the Jalgin Merino was similar to the distribution of wild homozygous genotypes (Figure 1E). The largest number had a frequency of about 0.285 (more than 270 polymorphisms) and much less in the upper part of the range, closer to 0.3149. The frequency distribution of the selected SNPs over chromosomes showed that the minimum frequency of 0.285 was found on almost all chromosomes (chromosome X was excluded from the analysis due to the presence of only one SNP). On eleven chromosomes, the maximum frequency of occurrence reached 0.313. The average frequency of occurrence on chromosomes fluctuated in the range of 0.285-0.300.

Table 1. Numbers of SNP's in different frequency ranges in Jalgin Merino rams

№ of selection	WH frequency range (min-max)	WH SNP's (n)	MH frequency range (min-max)	WH+MH SNP's (n)	Heterozygotes frequency (M±m)
1.	0.2950-0.3049	569	0.2950-0.3049	57	0.381±0.017
2.	0.2950-0.3099	10407	0.2950-0.3099	145	0.386±0.019
3.	0.2930-0.3129	13236	0.2930-0.3129	225	0.391±0.011
4.	0.2900-0.3139	16221	0.2900-0.3139	355	0.403±0.012
5.	0.2850-0.3149	20435	0.2850-0.3149	729	0.394±0.009
6.	0.2800-0.3299	42883	0.2800-0.3299	1173	0.379±0.004
7.	0.2500-0.3549	83381	0.2500-0.3549	11387	0.383±0.003

WH – Wild homozygous genotype; MH – Mutant homozygous genotype

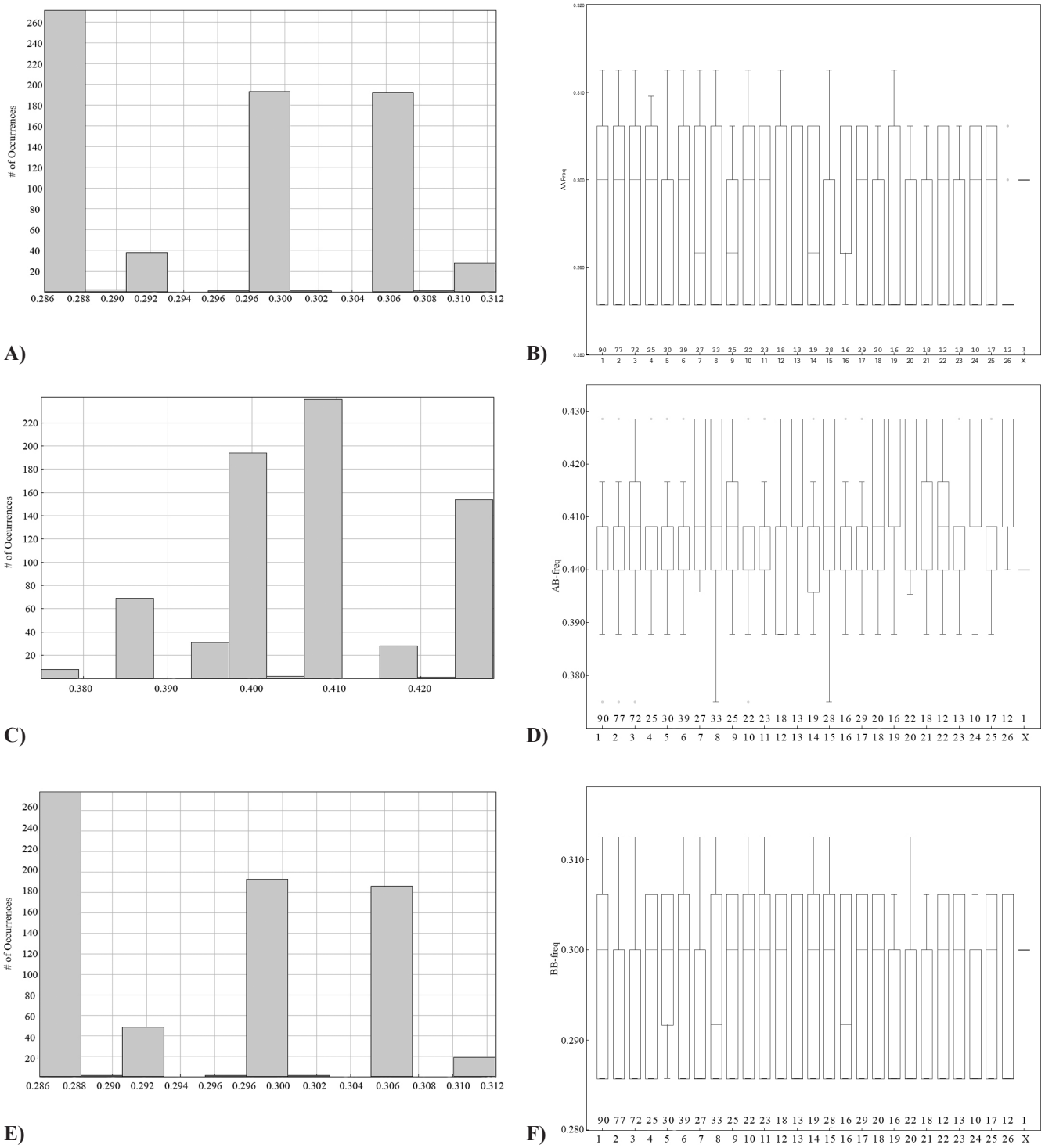

Fig. 1. The number of SNPs with different frequency of occurrence and the distribution of the frequency of occurrence of polymorphisms on individual chromosomes in rams of the Jalgin Merino breed: A, B – wild homozygous genotypes; C, D – heterozygous genotypes; E, F – mutant homozygous genotypes

The distribution of the number of SNPs was studied and they were selected depending on the location in the genome loci corresponding to the polymorphism indices on Illumina Beadchip Ovine 600K, from 1 to 606.000 (Figure 2). Polymorphism indices were associated with their location on chromosomes and allowed the estimation of the number of SNPs on a single chromosome. The study showed that there were three regions in the genome with the largest number of polymorphisms (35 or more). These regions were located on chromosomes 1, 21, and 2. In four regions of the genome, it was found a relatively low number of SNPs (from 3 to 12). These regions were located on chromosomes 18, 20, X, and 4. In other regions of the genome, the number of polymorphisms ranged from 13 up to 30 for every 2000 index units. In general, the chosen substitutions were presented in all regions of the genome covered by the studied loci on Illumina Beadchip Ovine 600K.

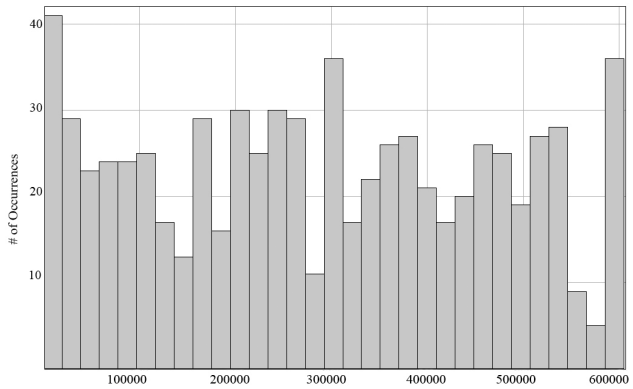

Fig. 2. Distribution of SNPs selected for genotyping by regions of the genome in accordance with the Illumina BeadChip Ovine 600K index from 1 to 606 000 in Jalgin Merino sheep

At the next stage of the following research, it was estimated the distance in thousands of nucleotide pairs (kb) between individual SNPs on each chromosome. Substitutions located at a distance of less than one centimorganide (1000 kb) and inherited together with a probability of more than 99% were excluded from the list recommended for genotyping by sequencing. As a result, 468 substitutions were left for the final set of genotyped loci.

The analysis of the distribution of the selected SNPs showed that the average distance between them on most chromosomes was from 3200 to 7000 kb. (Figure 3). The greatest distance was about 12 000 kbp and it was observed between substitutions on chromosome 24. At the same time, the smallest number of substitutions (4 SNPs) was detected on chromosome 24 as well. Only one substitution was chosen on the X chromosome, the average distance between SNPs was not calculated. It was found large average distances between SNPs, exceeding 7000 kb, on chromosomes 23 and 26. The maximum number of polymorphisms from the list for genotyping was noted on the first three chromosomes. It was 59, 46 and 43, respectively.

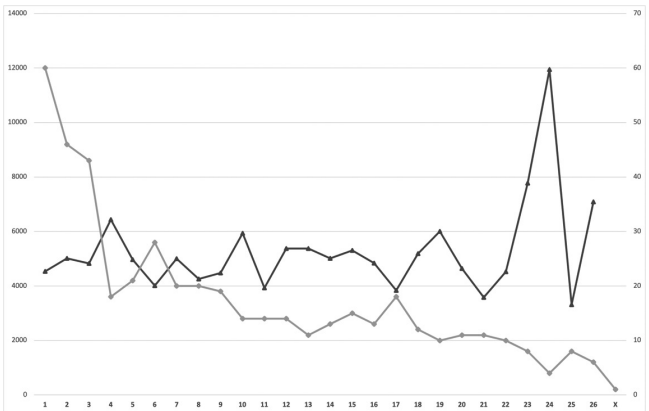

Fig. 3. The number and average distance between 468 SNPs selected for genotyping on different chromosomes in sheep of the Jalgin Merino breed. Dark grey, left y-axis – Average distance between SNPs, kbp; Light grey, right y-axis – Number of SNPs; X-axis – chromosomes

As a result of the study on whole genome genotyping of Jalgin Merino sheep using the Illumina Beadchip Ovine 600K consortium chip, it was obtained data on the presence of polymorphisms in 606 000 genome loci. Subsequent analysis of the frequency of occurrence of wild and mutant variants of the detected SNPs made it possible to select the most suitable ones for use in genotyping by sequencing, with a sufficient number of both homozygous and heterozygous variants in the studied population. Based on this, as well as the estimation of the distances between individual SNPs, were selected 468 substitutions that met the established criteria. They can be further used in the development of a custom DNA biochip or for genotyping by sequencing Jalgin Merino in the course of breeding work.

Discussion

As the main criterion, it was used the frequency of occurrence of both wild and mutant homozygous variants of substitutions. Using sorting by these parameters in Genome Studio 2.0 several frequency intervals around frequency 0.3 were examined. In a study of Tortereau et al. (2017), it was considered

a frequency of 0.3 to be the minimal limit for selecting polymorphisms. But their goal was to select a total of 249 substitutions. Therefore, in the present study the minimal frequency selection limit was decreased to 0.285. The use of narrow intervals did not give a sufficient initial number of substitutions. According to this criteria, they should have exceeded 600-700 units for the subsequent exclusion of SNP. A wider interval gave a list of too many substitutions. As a result, the following study settled on the frequency range of occurrence, giving 729 loci. They have same frequencies of both homo- and heterozygous genotypes in the studied population. This is a close number to those proposed for bovine genotyping by McClure et al. (2018). They suggested a list of 800 polymorphisms. It is clear that the approach we used is individual for the breed under study. However, the principle of SNP selection can also be used in the genotyping of other sheep breed.

The distribution of the number of selected SNPs depending on the frequency of occurrence of the genotype differed for homozygous and heterozygous variants. Homozygotes were found in greater numbers near the minimal limit of the selected frequency range. This indicated that expanding the selection interval to a higher frequency range would be inappropriate. It is due to the decreasing number of substitutions that have the needed parameters. The study of the frequency of selected SNPs on individual chromosomes showed a uniform distribution of substitutions. This indicates the similar distribution of substitutions within the chromosomes and confirmed the expediency of the approach used in the selection of SNPs. The largest number of heterozygous substitution variants has average values over selected interval. Based on this, it was considered that the selected frequency interval was sufficiently optimal for choosing the number of SNPs required for genotyping by sequencing.

To assess the efficiency of genome coverage by the selected substitutions, the distribution according to their individual Illumina index were used. Not all chromosomes in it were indexed in order, but individual SNP indices characterized the location of substitutions on the DNA chain. The distribution by indices made it possible to conclude that the set of selected substitutions adequately characterized all areas represented on the chip. In the study of Hulsegge et al. (2019) was announced for uneven distribution of SNPs suitable for genotyping across chromosomes. The available regions with a small number of SNPs were less informative in terms of genotyping. Authors noted, this decrease in information content was not critical for confirming the reliability of the origin and identification of animals.

One of the tasks of genotyping was the maximum possible coverage of the genome so it was recommended to use polymorphisms that were sufficiently distant from each other on chromosome. An increase in the distance between the detected SNPs reduces the likelihood of their linked inheritance. It makes possible to use predominantly independent substitutions with little correlation with each other in the study. In this case, the set of loci for genotyping can be used for a longer period of time for more generations without the significant changes. In our study, the distance between the majority of SNPs from the final list was within 3200-7000 kb. We consider quite sufficient for optimal genotyping. In the present study the authors suggested the use of a panel of 468 SNPs, the average distance between polymorphisms will be less than in research by Tortereau et al. (2017). They recommend to use almost half of the substitutions for genotyping. Large distances on chromosomes 23, 24, and 26 were associated with a small number of polymorphisms that meet our requirements on these chromosomes. It is also worth noting that the X chromosome has only one polymorphism with sufficient frequencies of wild and mutant variants. But we consider it necessary to include it in the list for genotyping. Since it minimally, but still marks this chromosome in the process of genome evaluation. In the future, it will be necessary to re-search for polymorphisms on the X chromosome. In the next generations of the animals from Jalgin Merino sheep breed it will be searched new SNPs with a sufficient frequency of occurrence. They could appear as a result of the selection process and genetic drift during sexual reproduction. The number of polymorphisms on the remaining chromosomes generally correlates with the size of the chromosomes. The largest of them had the maximum of the substitutions selected in the present study.

The selection of loci set for genotyping depends on the breeders needs and the peculiarities of the animal breed. The most common task is to assess the reliability of the origin of animals. It is also solved using SNP genotyping. In this case, the number of loci should be at least 100. With an increase in the number of studied polymorphisms, the reliability of the study increases, but its cost also increases (McClure et al., 2018). We set the task of choosing 400 or more substitutions with suitable parameters. It will allow us to use all of them for genotyping by sequencing in the future. Also, we can select the required number of SNPs taking into account the economic feasibility of using this method in breeding.

Conclusion

The whole genome sequencing of Jalgin Merino rams using Illumina BeadChip Ovine 600K allowed the identification of sufficiently large number of single nucleotide polymorphisms suitable for genotyping with a smaller number of polymorphisms. SNPs were identified with a high frequency

of occurrence (0.2850-0.3149) of both allelic variants in the homozygous form. The frequency of most homozygous variants was near 0.2850 limit of chosen interval, heterozygotes predominantly had a frequency in the middle of the range 0.3569-0.4296. A total of 729 SNPs matching the selected criteria were found. They were located within the entire length of the genome genotyped by the chip. The exclusion of substitutions that do not had physical localization, as well as those located closer than one centimorganide from each other, allowed the selection of 468 SNPs for mass genotyping by sequencing of Jalgin Merino. This number of loci was quite sufficient to solve the problems of confirming the proving of parental origin, identifying animals in linear crossing, and controlling the level of heterozygosity in the population. The selected SNPs can be used both for the formation of custom SNP arrays and for genotyping using next generation sequencing.

Data availability

Data are available in supplement file.

Competing interests

The authors declare that they have no conflict of interest.

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