

## Genome scanning, parentage assignment

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### VALIDATION OF THE SNP PANEL FOR PARENTAGE ASSIGNMENT IN LOCAL RUSSIAN SHEEP BREEDS

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#### Abstract

Creating panels for parentage assignment based on the most informative SNPs (minor allele frequency,  $MAF \geq 0.3$ ) is an important problem of the modern sheep breeding. International Society of Animal Genetics (ISAG) recommends the parentage panel consisting of 88 autosomal SNPs, developed by the International Sheep Genomics Consortium. However, selection of SNPs, which were included into the panel, was performed on the base of DNA profiles of North American, Australian and New Zealand sheep. There were no Russian breeds in these researches, and the possibility of applying ISAG panel to parentage testing of these sheep must be studied. We have performed the whole genome SNP study in four local Russian sheep breeds — Romanov (ROM,  $n = 22$ ), Baikal's fine-fleeced (ZBL,  $n = 7$ ), Buryat sheep Buubey (BUB,  $n = 15$ ), and Tuvan short fat tailed (TUV,  $n = 16$ ) using Ovine SNP50k BeadChip. Data were processed for the total number of markers (54241 SNPs) and for 88 autosomal SNPs, recommended by ISAG. We estimated percentage of markers with  $MAF \geq 0.3$ , mean MAF value, probability of identity (PI) and probabilities exclusion ( $P_1$ ,  $P_2$ ,  $P_3$ ) to evaluate the power of parentage panel for each of single breed and for entire sample. The universality of the panel was evaluated by comparing the degree of genetic differentiation of breeds based on the study of the entire number of SNPs and panel ISAG. For this purpose, we took in account such criteria as pairwise values of  $F_{st}$  (AMOVA) and results of principal component analysis (PCA-plot). We did summary statistics in Plink 1.09 and GenAlEx 6.5. After the quality control of the entire sample, we selected 47385 SNPs with mean MAF of  $0.292 \pm 0.131$  for the further analysis. The mean MAF for 88 parentage SNPs was  $0.380 \pm 0.091$ . Analysis of the SNPs' distribution depending on their MAF showed that most of the SNPs (81.8 %) were informative ( $MAF \geq 0.3$ ). Proportion of informative SNPs differed between breeds and was 56.8 % in ROM, 63.4 % in ZBL, 71.6 % in BUB and 72.7 % in TUV. Twenty-one SNPs (23.9 %) were highly informative in all four breeds, while 37 SNPs (42.0 %), 17 SNPs (19.3 %) and 10 SNPs (11.4 %) were informative, respectively, in three, two or only one breed. Marker DU196132\_525.1 was monomorphic in TUV ( $MAF = 0$ ). Three SNPs with  $MAF < 0.3$  (DU232924\_365.1, DU501115\_497.1 and DU372582\_268.1) were not informative for all four breeds. Lower pairwise values of  $F_{st}$  based on 88 SNPs with the same character of genetic relations compared with those using whole genome SNP profiles shown high flexibility of ISAG panel. PCA confirmed the low breed's dependence of SNP panel by creating purely consolidated overlapping areas corresponding to different breeds. The probability of identity for 88 SNPs ranged from  $4.32 \times 10^{-33}$  in TUV to  $7.48 \times 10^{-33}$  in BUB. Probability of exclusion was  $P_1$  99.99 % for all four breeds. The value of  $P_2$  was the highest in TUV ( $P_2$  99.99 %) with  $P_2$  99.98 % for others. The value of  $P_3$  was 99.9 % for all breeds. Instead of some breed-dependent character of DNA profiles of 88 autosomal SNPs, our results confirmed the possibility of applying of ISAG panel for parentage testing in four local Russian sheep breeds.

Keywords: SNP genotyping, local sheep breeds, MAF, ISAG panel, parentage assign-

ment in sheep.

Genetic improvement of livestock breeds is based on the evaluation and selection of specimens that have the highest estimated breeding value (EBV) for important breeding traits. EBV reliability is directly related to the accuracy of pedigree records, so the errors in parental identification cause a decrease in genetic progress and, as a result, economic losses [1]. Pedigree information is necessary to control the degree of inbreeding being one of the most important elements of the animal genetic resources control [2].

Microsatellites were discovered in 1984 and are also known as short tandem repeats (STRs) or short sequence repeats (SSRs) [3]. Due to the high polymorphism, Mendelian type of inheritance and uniform distribution throughout the whole genome, they remained the most commonly used type of DNA markers for parentage assignment in various farm animal species for over 25 years [4-8].

Development of high-performance methods of genome analysis has led to the creation and use of the panels based on single nucleotide polymorphism (SNP) in parentage assignment [9-13]. Although SNP markers are less polymorphic compared to STRs (most SNPs are biallelic) this deficiency is completely leveled with a possibility to conduct simultaneous analysis of several tens or even hundreds of SNPs at relatively low cost. The advantages of SNP analysis are the absence of specific requirements for DNA quality (SNP analysis is usually performed by obtaining short amplicons of less than 100 bp), automation of genotyping process, ease of result interpreting, and the possibility of direct comparison of data between laboratories.

Providing high reliability of SNP based parentage assignment control requires a greater number of loci compared to the STRs. Studies in humans [14, 15], pigs [16], meat and dairy cattle [17, 18] demonstrated that to achieve similar informativeness, 3-6 SNPs are required per 1 STR. The authors suggest that the increase in SNP panel informativeness can be reached by increasing the number of investigated polymorphisms and approaching the average minor allele frequency (MAF) to 0.5. According to K.G. Dodds et al. [19], to achieve the information content similar to that in commonly used STR-based panels, 3-4 times more SNPs are required, while in the case of panels based on dominant markers (e.g., ISSR and AFLP), 17 times more SNPs are necessary.

Paternity exclusion is one of the most commonly used approaches for parentage assignment by DNA markers (single exception, categorization and factions, genotype reconstruction) [20, 21]. It is based on the following principle: the offspring have only parental alleles in each locus, and the probability of exclusion (PE) is the probability of the claimed individual exclusion as a parent [22]. This method requires high accuracy of genotyping (> 99 %) and  $MAF \geq 0.3$ . SNP selection is the key factor which determines the effectiveness of the parentage assignment control system.

Development of SNP marker panel for analyzing sheep parentage was started after the creation of the Ovine SNP50K BeadChip medium density chip which included 54241 SNPs, by International Sheep Genomics Consortium (ISGS) [23]. Currently, there are six sheep panels with different SNP sets and numbers. ISAG Consortium has developed the parentage panel consisting of 88 SNPs [24], CSIRO (Commonwealth Scientific and Industrial Research Organization, Australia) and SheepCRC (Sheep Cooperative Research Centre, Australia) recommend 382 SNPs [25], AgResearch Institute (New Zealand) offered 84 and 300 SNPs [12]. M.P. Heaton et al. [13] developed the SNP panel for the assessment of global species diversity which includes 163 SNPs, and allocated a set of

109 SNPs for the use in North American sheep breeds.

International Society of Animal Genetics (ISAG) recommends the parentage panel consisting of 88 autosomal SNPs, developed by ISG Consortium supplemented by one Y chromosomal SNP [26]. Selection of SNPs which were included into the panel was performed based on results of testing 22 sheep breeds from Africa, Asia, and Europe using the Golden Gate technique [10]. Selected SNPs were assessed in an extended sample of 74 breeds using the Infinium technique [23]. Later, a possibility of the analysis with this panel using the Fluidigm and Sequenom techniques was demonstrated [27].

It should be noted that the above SNP panels have not been applied for parentage testing in Russian sheep breeds. Among the 74 breeds used to assess the informativity of the official ISAG panel, there were the sheep of the North American population of the native Russian Romanov breed. However, the limited sample size and its parentage uncertainty do not make it possible to discuss the representativeness of the breed as a whole.

The purpose of this study was to evaluate the informativeness of the ISAG panel of 88 autosomal markers adopted as the official panel for parentage testing in domestic sheep, under the control of Russian local breed parentage assignment.

*Technique.* Study sample consisted of four local Russian sheep breeds, i.e. Romanov (ROM,  $n = 22$ ), Baikal's fine-fleeced (ZBL,  $n = 7$ ), Buryat sheep Buubey (BUB,  $n = 15$ ), and Tuvan short fat tailed (TUV,  $n = 16$ ).

Genomic DNA was isolated using Nextec columns (Nextec™ Biotechnology GmbH, Germany) according to manufacturer's recommendations. SNP screening was performed using the Ovine SNP50K BeadChip chip (Illumina Inc., USA). Data were processed for both the total number of markers (54241 SNPs) and for 88 autosomal SNPs, recommended by ISAG for sheep parental assignment (ISAG panel, or parental panel).  $MAF \geq 0.3$  was selected as the threshold criterion for determining alleles as informative ones.

SNP quality control and statistical analysis, including estimation of MAF, calculation of pairwise values of  $F_{st}$  according to B.S Weir et al. [28], and the principal component analysis (PCA) were performed using the Plink 1.07 software [29]. For the analysis, SNPs localized on autosomal chromosomes were selected and quality-controlled for the following parameters: for GenCall which indicates the accuracy of the results of genotyping ( $GC > 0.5$ ), for minor allele frequency ( $MAF > 0.01$ ), for the Hardy-Weinberg equilibrium test ( $HWE > 1e^{-6}$ ) and for the genotyping level ( $GENO > 0.01$ ).

Data visualization, including construction of PCA plots and the graphs of SNP marker grouping distribution on MAF was performed using the R programming language [30].

Probability of genotype identity (PI) was calculated for each locus by the following formula [31]:

$$PI = 2 \times (\sum p_i^2)^2 - \sum p_i^4,$$

where  $p_i$  is frequency of the  $i$ -th allele in the locus.

PI values for the number of unlinked markers  $k$  was defined as the product of individual PIs for each marker.

Probability of exclusion (PE) for each locus was determined for three particular cases.

1. Exclusion of one parent if the genotypes of both parents are known (P1) [32]:

$$P1 = 1 - 2 \times \sum p_i^2 + \sum p_i^3 + 2 \times \sum p_i^4 - 3 \times \sum p_i^5 - 2 \times (\sum p_i^2)^2 + 3 \times \sum p_i^2 \times \sum p_i^3.$$

2. Exclusion of a parent if the genotypes of one parent and the descen-

dant are known (P2) [33]:

$$P2 = 1 - 4 \times \sum p_i^2 + 2 \times (\sum p_i^2)^2 + 4 \times \sum p_i^3 - 3 \times \sum p_i^4.$$

3. Exclusion of both parents if the genotypes of the parents and the descendant are known (P3) [33]:

$$P3 = 1 + 4 \times \sum p_i^4 - 4 \times \sum p_i^5 - 3 \times \sum p_i^6 - 8 \times (\sum p_i^2)^2 + 8 \times (\sum p_i^2) \times (\sum p_i^3) + 2 \times (\sum p_i^3)^2.$$

P1, P2, P3 (P) values for the  $k$ -th number of unlinked markers was determined using the formula:

$$P = 1 - (1 - P_1) \times (1 - P_2) \times (1 - P_3) \dots \times (1 - P_k).$$

To calculate PI, P1, P2, and P3, the GenAlEx 6.5 software was used [34].

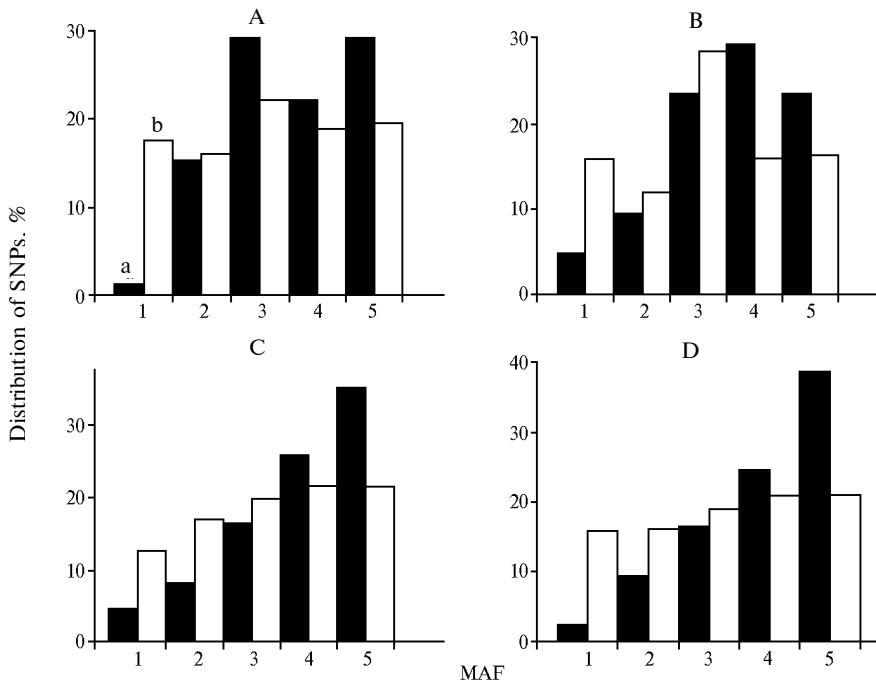
**Results.** The effectiveness of whole genome SNP genotyping (call rate) varied in breeds from 91.5 % (BUB and TUV) to 91.8 % (ROM) and 91.9 % (ZBL), and amounted 91.7 % in the total sample. All the animals studied were controlled for compliance with the animal call rate control criterion  $\geq 90$  %. As the studies included the analysis of autosomal markers only, 1828 SNPs localized on the sex chromosomes were excluded from the analysis. Besides, 1371, 1469, 1665, and 1636 SNPs in ROM, ZBL, BUB, and TUV, respectively, did not pass the control for the reading quality criteria (GC Score  $\geq 0.5$ ) and clustering degree (GC Score  $\geq 0.3$ ). Then, 3479, 3663, 3743 and 3786 polymorphisms, respectively, were excluded in the breeds studied, as the ones not relevant with the SNP call rate criterion  $\geq 90$  %. All of the remaining markers matched the  $\chi^2$  criterion for the population Hardy-Weinberg equilibrium, and 47563 (87.7 %) SNPs in ROM, 47281 (87.2 %) SNPs in ZBL, 47005 (86.7 %) SNPs in BUB, and 46991 (86.6 %) SNPs in TUV were selected for further analysis. Finally, 47385 polymorphisms were selected for the total sample analysis (87.4 % of polymorphisms studied).

In assessing the results of genotyping SNPs included in the ISAG panel, quality control (SNP call rate = 99.2 %) was passed by 87 of the 88 loci except DU426312\_454.1 which was successfully genotyped in 55 % of the animals only. However, to have data comparable with those obtained in other studies, this SNP was included in further analysis.

In the studied sample, most of the sheep parentage markers (81.8 % of SNPs) were informative (MAF  $\geq 0.3$ ). Thus, 51.1 and 30.7 % of SNPs were found at the frequency rate of 0.4 to 0.5 and 0.3 to 0.4. Then, 1.1 % of SNPs with a frequency of 0 to 0.1 % were low informative. The grouping distribution for all the studied SNPs depending on MAF was more uniform: for MAF  $> 0.0$  -  $< 0.1$ ;  $\geq 0.1$  -  $< 0.2$ ;  $\geq 0.2$  -  $< 0.3$ ;  $\geq 0.3$  -  $< 0.4$  and  $\geq 0.4$  -  $\leq 0.5$ , proportions were 9.2; 17.8; 21.2; 24.6 and 26.2 %, respectively.

The proportions of informative SNPs were different in breeds and amounted 56.8 % in ROM, 63.4 % in ZBL, 71.6 % in BUB, and 72.7 % in TUV (Fig. 1). Polymorphisms with MAF  $< 0.1$  were observed in all the breeds studied, with the highest percentage of uninformative markers observed in ZBL and BUB (4.5 %), and the lowest percentage in ROM (1.1 %). Perhaps, this may be explained by the fact that there were 10 Romanov breed sheep among the animals involved in the testing of parentage panel [13, 26]. However, these sheep were presented by the North American population only, which can not characterize the breed in general. In TUV sheep, 2.3 % of markers had MAF  $< 0.1$ , with the monomorphic DU196132\_525.1 marker.

Average MAF values were  $0.332 \pm 0.110$  in ROM,  $0.335 \pm 0.118$  in ZBL,  $0.347 \pm 0.121$  in BUB,  $0.360 \pm 0.109$  in TUV, and  $0.380 \pm 0.091$  in the total sample. With the whole genome panel, average MAF values in ROM, ZBL, BUB, and TUV were  $0.261 \pm 0.146$ ;  $0.272 \pm 0.146$ ;  $0.269 \pm 0.143$ ,  $0.272 \pm 0.142$ , respectively, with  $0.292 \pm 0.131$  in the total sample.



**Fig. 1.** Distribution of studied SNPs (single nucleotide polymorphism) in Romanov (A), Baikal's fine-fleeced (B), Buubey (C) and Tuvan short fat tailed (D) sheep depending on the average minor allele frequency (MAF): 1 –  $0.0 < \text{MAF} < 0.1$ , 2 –  $0.1 \leq \text{MAF} < 0.2$ , 3 –  $0.2 \leq \text{MAF} < 0.3$ , 4 –  $0.3 \leq \text{MAF} < 0.4$ , 5 –  $0.4 \leq \text{MAF} \leq 0.5$ ; a – 88 SNPs included in the ISAG (International Sheep Genomics Consortium) panel, b – 47385 SNP, selected on breed control data.

Of the 88 parentage markers, 21 SNPs (23.9 %) were highly informative in all the four breeds ( $\text{MAF} \geq 0.3$ ); 37 (42.0 %), 17 (19.3 %), and 10 SNPs (11.4 %) were informative in three, two or only one breed, respectively. Three SNPs were non-informative in all the four breeds (Table 1).

**1. Parentage of SNPs (single nucleotide polymorphism) and MAF (minor allele frequency) arithmetic means in four local Russian breeds**

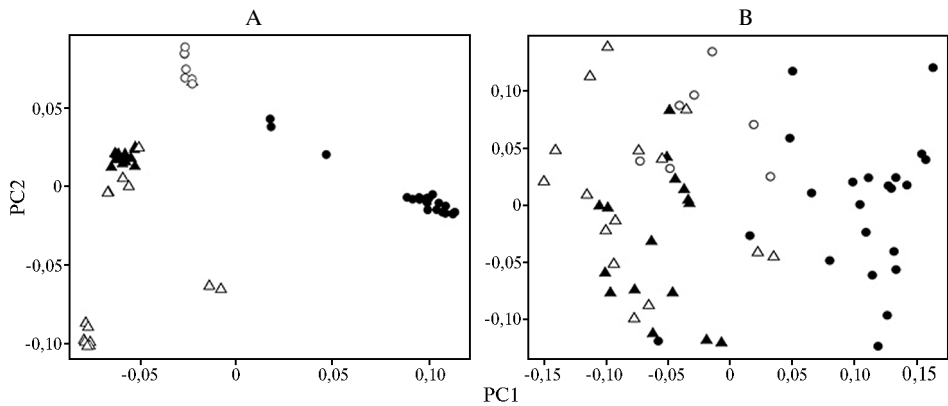
SNP	Chromosome	MAF				
		ROM	ZBL	BUB	TUV	all breeds*
SNP markers for the four local sheep breeds ( $\text{MAF} < 0.3$ )						
DU232924_365.1	1	0.068	0.071	0.067	0.156	0.092
DU501115_497.1	2	0.182	0.143	0.200	0.094	0.158
DU372582_268.1	9	0.159	0.071	0.067	0.188	0.133
SNP markers for the three local sheep breeds ( $\text{MAF} < 0.3$ )						
DU305004_417.1	4	0.250	0.125	0.393 <sup>i</sup>	0.156	0.242
DU453259_440.1	5	0.114	0.125	0.231	0.344 <sup>i</sup>	0.203
DU530067_219.1	7	0.114	0.375 <sup>i</sup>	0.250	0.125	0.183
DU213735_493.1	8	0.318 <sup>i</sup>	0.250	0.286	0.250	0.417
DU417675_79.1	11	0.386 <sup>i</sup>	0.250	0.179	0.219	0.358
DU275428_276.1	13	0.273	0.438 <sup>i</sup>	0.286	0.250	0.292
DU223894_556.1	14	0.364 <sup>i</sup>	0.250	0.286	0.281	0.408
DU264531_279.1	19	0.273	0.438 <sup>i</sup>	0.286	0.281	0.317
DU258053_237.1	19	0.295	0.563 <sup>i</sup>	0.250	0.219	0.300
DU380983_440.1	21	0.250	0.188	0.036	0.406 <sup>i</sup>	0.233

Note. ROM – Romanov, ZBL – Baikal's fine-fleeced, BUB – Buubey, TUV – Tuvan short fat tailed breeds. «i» marks informative SNPs, other SNPs are non-informative.

\* MAF is characterized not by arithmetic means, but by the values calculated at combining all the four breeds in a single pool.

Comparative analysis of PCA-plots constructed based on the results of studying 47385 SNPs and 88 SNPs (Fig. 2), demonstrated a fairly clear breed differentiation for the entire spectrum of markers, while low consolidated partially overlapping arrays were formed when the parentage panel was used. Analy-

sis of pairwise  $F_{st}$  values as a measure of genetic differences (Table 2) confirmed the expected decrease in the degree of differentiation using the parentage panel compared to genome-wide SNP profiles with maintaining the nature of the identified genetic interbreed relationships ( $r^2 = 0,95$ ). Therefore, although these data confirm the ISAG SNP panel dependence on breed, generally they indicate its relatively high versatility.



**Fig. 2.** Analysis of principal components based on SNP (single nucleotide polymorphism) in Romanov (●), Baikal's fine-fleeced (○), Buubey (△) Tuvan short fat tailed sheep (▲): A — PCA plot for 47385 detected OvineSNP50K BeadChip markers, B — PCA plot for 88 included in the ISAG panel (International Sheep Genomics Consortium).

Calculation of the probability of genotype identity (PI) for 88 SNPs demonstrated highly informativeness of the panel: in ROM, ZBL, BUB, and TUV, PI values were  $5.86 \times 10^{-33}$ ,  $5.24 \times 10^{-33}$ ,  $7.48 \times 10^{-33}$  and  $4,32 \times 10^{-33}$ , respectively. Probability of exclusion of P1 as a parent was  $\geq 99.99\%$  for all the four breeds. P2 value was the highest in TUV ( $P2 \geq 99.99\%$ ), in the other three breeds P2 was  $\geq 99.98\%$ . P3 value in all breeds was  $\geq 99.99\%$ .

## 2. Pairwise $F_{st}$ for SNP parentage assignment panel and genome-wide SNP profiles in compared breeds

Breed	ROM	ZBL	BUB	TUV
ROM		0.0544	0.0578	0.0493
ZBL	0.0797		0.0437	0.0418
BUB	0.0834	0.0537		0.0261
TUV	0.0820	0.0542	0.0318	

Note. The values calculated based on the analysis of 88 SNP (single nucleotide polymorphism) markers included in the ISAG panel (International Sheep Genomics Consortium) are shown above the diagonal; the values calculated according to 47385 SNP markers selected for the results of the breed quality control are shown below the diagonal.

Creation of parentage assignment panels based on most informative SNPs is an actual problem of sheep breeding. For this purpose, M.P. Heaton et al. [13] used 2915 sheep belonging to 74 breed groups provided by the ISG Consortium, analyzed 47693 autosomal SNPs using multiple criteria and selected 163 SNPs with desirable properties for parentage assignment. On average, every selected SNP was highly informative ( $MAF \geq 0.3$ ) in  $48 \pm 5$  breed groups. Of 163 SNPs, 109 SNPs were selected to create a parentage assignment panel for North American sheep breeds. Scanning efficacy and accuracy for these 109 SNPs was more than 99 %.

The number of required SNPs depends on MAF and the marker panel used. Through mathematical modeling, E. Baruch and J.I. Weller [35] found that 15 to 54 SNPs are required to achieve the probability of exclusion of 99 %. The number of SNPs required increases with the restriction of the amount of information (if the genotype of only one probable parent is known) and with  $MAF < 0.1$ . When the international parentage panel of 88 autosomal markers was tested in Russian breeds, the proportion of highly informative SNPs was 82 % on average for the total sample. It should be noted that this value is formed not

only from specific SNPs that are highly informative in all breeds, but also due to the markers that are informative in two or three breeds, which is demonstrated by the interbreed differences for  $MAF \geq 0.3$  ranged from 50.0 to 61.4 %.

According to J.W. Kijas et al. [27], SNP selection for creating parentage assignment panels should be performed with a shift to high MAF values (0.3–0.5) to unify it, i.e. to provide a possibility to use a panel in a wide range of breeds. Our research has generally confirmed this rule for Russian local breeds: MAF was more than or equal to the threshold value of 0.3 in 56.8; 63.4; 71.6, and 72.7 % of ROM, ZBL, BUB, and TUV sheep, respectively.

Estimation of the number of SNPs required to obtain a high probability of parentage exclusion is one of the panel informativeness criteria. We have demonstrated that if the data of the both parents' genotypes were available, the minimum number of markers required for their exclusion as parents with a probability of more than 99.99 % (P3 criterion) was 27 for each breed. To achieve the same probability of exclusion of one of the parents if the information about both parents was available (P1), not less than 43 SNPs were required. If the genotype of one of the parents was unknown (P2), the minimum required marker number increased to 66.

Thus, based on a set of criteria including the genotype identity probability and the probability of parentage exclusion, we can conclude that the ISAG panel (International Sheep Genomics Consortium) which includes autosomal markers may be applied for parentage testing in four local Russian sheep breeds (Romanov, Baikal's fine-fleeced, Buubey, and Tuvan short fat tailed). To create the most informative panel to assess the origin of the whole spectrum of Russian breeds and their role in ensuring global domestic sheep biodiversity in the future, it is necessary to be focused on increasing the number of breeds studied, as well as on the analysis of other proposed SNP panels.

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