

## **MEF2B GENE SNP MARKERS OF MEAT PRODUCTIVITY IN SEVEROKAVKAZSKAYA SHEEP BREED**

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One of the new promising candidate genes defining productive qualities of sheep is MEF2B. Protein from the MEF2 group encoded by it affects the production of myostatin and the expression of the genes responsible for the growth of skeletal muscle fibers. Thus, the knowledge of the MEF2B gene structure is important for genomic selection. We have studied the structure of the MEF2B gene at sheep of Severokavkazskaya breed bred in Russia. To detect alleles we use NimbleGen sequencing technology by Roche (USA). As a result, it was revealed 14 single nucleotide polymorphisms (SNP) at the given breed. The discovered SNPs are located in non coding areas. From them 7 polymorphisms are in the area of 5' upstream gene in loci: c.-1713, c.-1319, c.-839, c.-321, c.-246, c.-161, c.-3; 6 polymorphisms are in introns, loci: c.55-51, c.258+312, c.258+380, c.259-52, c.452+95,

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c.452+103, 1 SNP is in 3' downstream gene, c.\*252. Two of the identified SNPs are significantly connected with high indices of meat productivity: c.55-51 and c.259-52. At the same time it was not possible to find out the impact on productivity of c.-1713 polymorphism. Our investigation is a base of next research of affection of different MEF2B gene alleles on meat quality and can be used to prepare PCR test-system for genomic selection.

*Key words:* genomic election, MEF2B, sequence, sheep, SNP

## INTRODUCTION

Genomic selection is a modern and promising method for an accurate estimation and prediction of breeding and productive qualities of animals, which allows us to expedite the selection work and to reduce the financial expenses connected with it. Very convenient and perspective genetic markers are single nucleotide polymorphisms (SNPs). Their presence in the regulatory and structural areas of genes can influence the expression of a gene or sequence of amino acids in protein that is reflected in the meat quality.

During the search of candidate genes associated with productivity, Chinese scientists have conducted genotyping of 319 sheep. A single nucleotide polymorphism of s58995.1 (position 3858663 in contig) located in the regulatory area of myocyte enhancer factor-2 (MEF2B) has appeared as one of making the greatest impact on the meat quality (ZHANG *et al.*, 2013).

MEF2B gene encodes protein from the MEF2 family. Interaction of proteins from MEF2 family with promoter of myostatin gene in sheep has a stimulating effect on the expression of myostatin, protein that limits muscle growth in mammals (DU *et al.*, 2007). Thus, mutations in the MEF2B gene can make a significant impact on the sheep meat productivity through the change of myostatin production. This was confirmed in bucks, whose expression level of MEF2B in the study positively correlated with the diameter of muscle fibers (CHEN *et al.*, 2015).

The database dbSNPNCBI contains information on 106 single nucleotide polymorphisms in the area of MEF2B gene in sheep (<http://www.ncbi.nlm.nih.gov/snp/?term=MEF2B+Ovis+aries>). But the information on frequency of polymorphism occurrence in different breeds at the moment is not present. This refers also to sheep breeds developed in Russia.

Severokavkazskaya breed of sheep has half-fine wool and meat-wool directions. It is developed in Russia by crossing of the Stavropol breed ewes with rams of Romney Marsh and Lincoln. Sheep of this breed successfully combine high wool yield and live weight with precocity and adaptive qualities ( *et al.*, 2007).

Therefore, revealing of SNPs in the MEF2B gene structure and definition of their relationship with productive indices of Severokavkazskaya sheep breed became the purpose of our work.

## MATERIALS AND METHODS

All work was provided in the Genetic Laboratory of Science-Diagnostic and Veterinary Care Center (Stavropol State Agrarian University, Russian Federation). We have investigated 19 rams (n = 19) at the age of one year of Severokavkazskaya breed, from livestock breeding farm of Stavropol Krai, Russian Federation. In order to obtain data about the maximum number of MEF2B gene alleles we selected for the research 13 animals with maximum height and weight, and 6

animals of the same population with a minimum height and weight. All animals were healthy, kept in optimal conditions and fed with a full ration.

#### **DNA collection**

Genomic DNA was extracted from blood samples obtained from the jugular vein under aseptic conditions. Blood samples were collected in Vacutainer® vials with stabilizer EDTA (Becton Dickinson and Company, USA) and were transported to the laboratory at +4 C within 6 hours. DNA was extracted from 0.2 ml of blood using a kit PureLinkGenomic DNA MiniKit (Invitrogen, USA).

#### **Targeted enrichment and NextGeneration sequencing.**

In order to detect mutations in the genes there were performed target enrichment and subsequent sequencing of the investigated DNA fragments. For enrichment of target regions we used the NimbleGen technology (ROCHE NIMBLEGEN, 2015). Probes for target regions were developed in cooperation with the firm Roche NimbleGen (USA). Libraries of DNA fragments of investigated animals, were prepared in accordance with the protocol Rapid Library Preparation Method Manual (Standard protocol GS Junior system manual, 2015) undergo the procedure of enrichment using NimbleGenSeqCap EZ Developer Libraries (Roche NimbleGen, USA) in accordance with the protocol NimbleGenSeqCap EZ Library LRUser'sGuideVersion 2.0 (2015).

Monoclonal amplification procedure of finished enriched target regions of DNA was carried out according to standard protocol emPCR Amplification Method Manual, Lib-L (Standard protocol GS Junior system manual, 2015).

Sequencing was performed using a genomic sequencer GS Junior (Roche, USA). The resulting sequencing fragments mapped to the reference genome assembly OvisariesoviAri3 (The National Center for Biotechnology Information, Genome. (2012) Ovisaries (sheep), 2015) by software GS Reference Mapper v2.9 (Roche, USA).

To describe an SNP we use HGVS nomenclature (The recommended nucleotide numbering nomenclature, 2015).

#### **Statistical analysis**

Phylogenetic analysis was performed using the software Unipro UGENE 1.15.1 (Unipro, Russia).

For statistical analysis used Student's t-test in Excel for Windows statistical plugin. Significant difference detected if  $p < 0.05$ .

## **RESULTS**

As a result of MEF2B gene sequencing, we found 14 single nucleotide polymorphisms (Table 1). In the database dbSNPNCBI are included 13 of them. In the same way we have been revealed the new polymorphism earlier not described, in a locus c.258+380.

The predominant percentage of the point mutations accounts for transition – 79%. Discovered SNPs are located in not coding regions. In introns are replaced 6 polymorphisms, 7 polymorphisms are in the region of 5' upstream gene, 1 SNP is in 3' downstream gene (Table 2).

It has been revealed SNP of c.452+95C>G in the homozygous variant at all examined animals.

With frequency above 0.85 in the investigated group it was revealed 3 mutant alleles in loci: c.259-52, c.452+95 and c.452+103.

The lowest number of mutant alleles is noted in loci c.-839, c.-321, c.-246 and c.258+380, where it does not exceed 0.1.

*Table 1. The frequency of polymorphic alleles and variants of genotype in Severokavkazskayasheep breed*

	Name of SNP in HGVS nomenclature	Identifier in the NCBI database	Position in contig	Allele		Genotype		
1	c.-1713	rs420767326	3858663	G	A	GG	GA	AA
				0.58	0.42	0.37	0.42	0.21
2	c.-1319	rs421508023	3859057	A	G	AA	AG	GG
				0.55	0.45	0.32	0.47	0.21
3	c.-839	rs424153320	3859537	T	C	TT	TC	CC
				0.90	0.10	0.79	0.21	0.00
4	c.-321	rs600185996	3860055	C	T	CC	CT	TT
				0.90	0.10	0.79	0.21	0
5	c.-246	rs408899236	3860130	A	G	AA	AG	GG
				0.90	0.10	0.79	0.21	0.00
6	c.-161	rs416020496	3860215	T	G	TT	TG	GG
				0.66	0.34	0.42	0.47	0.1
7	c.-3	rs160009954	3860373	G	A	GG	GA	AA
				0.50	0.50	0.32	0.36	0.32
8	c.55-51	rs413905991	3860890	C	T	CC	CT	TT
				0.87	0.13	0.74	0.26	0.00
9	c.258+312	rs410366832	3861456	A	G	AA	AG	GG
				0.66	0.34	0.53	0.26	0.21
10	c.258+380	Not in base	3861524	C	T	CC	CT	TT
				0.92	0.08	0.84	0.16	0
11	c.259-52	rs401426310	3862115	G	A	GG	GA	AA
				0.13	0.87	0	0.26	0.74
12	c.452+95	rs412071743	3863894	C	G	CC	CG	GG
				0.00	1.00	0	0	1.00
13	c.452+103	rs423157022	3863902	A	C	AA	AC	CC
				0.13	0.87	0	0.10	0.90
14	c.*252	rs428633663	3864577	T	C	TT	TC	CC
				0.40	0.60	0.26	0.26	0.47

Table 2. MEF2B gene Genotypes identified in Severokavkazskaya sheep breed.

	Genotype	SNP						
		upstream gene						
		.-1713	.-1319	.-839	.-321	.-246	.-161	.-3
1	A							
2	B	1						
3		2						
4		3						
5		4						
6		5						
7		6						
8		7						
9	C	1						
10		2						
11		3						
12		4						
13		5						
14		6						
15		7						
16	D	1						
17		2						
18		3						
	Genotype	SNP						
		Intron 1-2	Intron 2-3			Intron 4-5		downstream gene
		.55-	c.258+		c.259-	c.452+		c.*252
		51	312	380	52	95	103	
19	A							
20	B	1						
21		2						
22		3						
23		4						
24		5						
25		6						
26		7						
27	C	1						
28		2						
29		3						
30		4						
31		5						
32		6						
33		7						
34	D	1						
35		2						
36		3						

Cell shaded in black indicate homozygous mutant allele:  
gray - heterozygous,  
white - homozygous wild-type allele.

Being based on 14 found out SNPs by means of UGENE software test animals have been divided into basic groups and subgroups (total of 18 genotypes) (Table 2, Figure 1). It is not revealed individuals without SNP in the MEF2B gene.

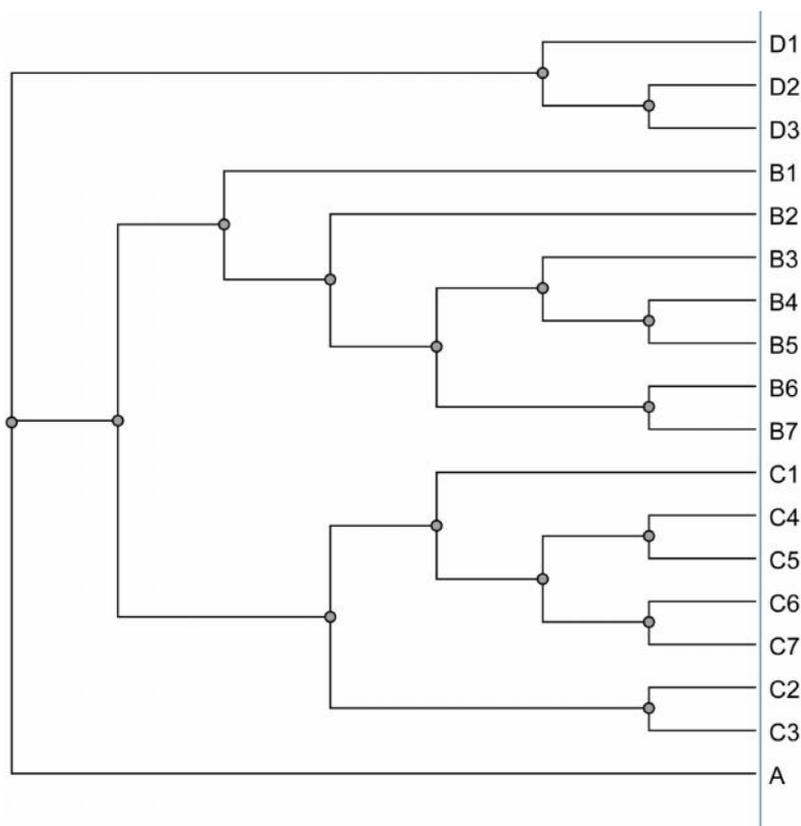


Figure 1. Phylogenetic tree of detected genotypes

A-genotype is characterized by the presence of 9 single nucleotide polymorphisms, 5% of the investigated animals have it.

The group of B-genotypes is presented at 37% of examined animals, it includes 7 subgroups. The difference between genotypes makes 1-4 mutations. The average number of substitutions per genotype is 8.

C-group is the most numerous and makes 42% from total number of the samples investigated, has 7 subgroups. The average number of SNPs at the animals in a group equals 7.

D-group includes 3 subgroups making in general of 16% from studied young rams. Subgroups differ among themselves on 1-3 mutations and have by 9-10 SNPs.

The average number of SNPs in the MEF2B gene for sheep of the Severokavkazskaya breed makes 7.7. The animals with B6, D1, D2 genotypes have the maximum number of polymorphisms.

It is detected least of all substitutions in animals with C4 and C5 genotypes. They have only by 2 SNPs upstream of the gene and 3 SNPs are in introns. All polymorphisms are shown in homozygous variant with the exception of c.-1713G>A in the C4 genotype.

The most common of the detected SNPs are the SNPs located in the introns: c.259-52G>A, c.452+95C>G and c.452+103A>C. They are identified at 100% of the investigated animals, and in most cases in a homozygous variant.

Table 3. Association between the MEF2B genotypes and body measurements.

Trait	Genotype				
	c.-1713, -3		c.55-51, c.259-52		
	+/, M±m (n=7)	+/M, M±m (n=8)	M/M, M±m (n=4)	+/M, M±m (n=5)	M/M, M±m (n=14)
1. Liveweight (kg)	65.03±1.12	<b>59.08±2.07*</b>	<b>68.27±2.14#</b>	59.08±2.07	<b>65.60±1.14#</b>
2. Heightatwithers (cm)	70.71±1.17	72.75±2.18	73.06±1.22	72.75±2.18	71.10±0.97
3. Heightatcroup (cm)	71.89±1.12	74.25±2.23	74.67±1.63	74.25±2.23	73.30±0.89
4. Widthatcroup (cm)	20.86±0.28	20.04±0.47	20.33±0.41	20.06±0.47	20.70±0.22
5. Lengthofcroup (cm)	25.57±0.62	24.75±0.55	25.02±0.71	24.75±0.55	25.40±0.45
6. Carcasslength (cm)	87.86±0.60	87.25±1.19	87.35±0.41	87.25±1.19	87.70±0.42
7. Chestwidth (cm)	29.14±0.50	<b>26.50±1.02*</b>	28.31±0.82	26.50±1.05	<b>28.90±0.40#</b>
8. Chestdepth (cm)	33.86±0.50	33.25±1.66	32.34±2.04	33.25±1.66	33.40±0.61
9. Chest girth (cm)	103.57±1.27	100.50±1.04	102.06±3.24	100.50±1.04	103.10±1.14
10. Metacarpalgirth (cm)	8.86±0.44	10.05±1.01	9.04±1.23	10.03±0.67	<b>8.90±0.41#</b>
11. Metacarpallength (cm)	15.43±0.70	17.11±1.70	16.07±1.41	17.12±1.71	15.60±0.57
12. Metatarsuslength (cm)	17.71±0.51	18.75±1.28	18.12±0.70	18.75±1.28	17.80±0.38
13. Loinwidth (cm)	19.02±0.47	<b>17.04±0.82*</b>	<b>19.44±0.71#</b>	17.14±0.84	<b>19.04±0.35#</b>
14. Widthofback (cm)	28.57±0.52	27.11±1.83	29.11±0.77	27.03±1.84	28.70±0.39
15. Half girth of back (cm)	74.86±2.61	73.09±3.74	<b>79.67±1.08*#</b>	73.10±2.08	76.30±1.92

n – number of animals.

Significantly differ with wild type homozygotes: \* - p<0.05

Significantly differ with heterozygotes: # - p<0.05

Studying of sheep genotype variants depending on combinations of present SNPs has allowed us to single out two of the most common combinations of substitutions located in functionally significant regions of a gene (Table 2).

The first pair of substitutions, c.-1713 and c.-3, is met in common practically at all studied animals, with the exception of B1-genotype, in which the substitution of c.-3 is present in heterozygous variant, and in the position of c.-1713 are located only the wild alleles. In two genotypes, C4 and C6, this substitution is represented in heterozygous variant, and the mutant homozygotes correspond to it on c.-3.

The second pair of substitutions, c.55-51 and c.259-52, at the majority of animals is present in the form of wild homozygote in the position of c.55-51 and mutant homozygote in the region of c.259-52. Discrepancies have been observed only in the genotypes of C3 and D3, where there is the presence of heterozygous alleles. Individuals with genotypes of B4-B7 significantly differ from the other animals, since both of these SNPs are presented in the form of heterozygotes.

Therefore, we studied the relationship between the presences of these two SNP pairs with lifetime indices of sheep productivity.

The results of body measurements showed that there are significant differences in parameters of the animals that depend on whether they have specific alleles of MEF2B gene (Table 3).

The most marked differences in animals with single nucleotide polymorphisms of c.-1713 and c.-3 in MEF2B promoter were observed in the measurement of live weight. Live weight in individuals heterozygous for these mutations was significantly lower at 9.1% compared with homozygotes for the wild type. Higher indices of live weight are found out in carriers of the mutant allele in the homozygous variant. Live weight which they had was on 15.7% more than that of heterozygotes, and it was not significantly different from wild type homozygotes.

Also, significant differences have been revealed at measurement of loin width. The given index was lower in the group of heterozygotes by 11.4% than that of the homozygotes for the wild type. The width of the loin was significantly greater in mutant homozygotes, on 14.4%, compared with heterozygotes. Animals with homozygous wild and homozygous mutant genotypes were not significantly different among themselves on these indices.

At mutant homozygotes the index of half girth of back was significantly higher on 9.1% than at heterozygotes. In homozygotes for the wild type the index of half girth of back did not differ significantly from heterozygotes.

Other parameters in sheep with the presence of c.-1713 and c.-3 substitutions given in Table 3 were not significantly different among them and were not dependent on the presence of MEF2B gene allele.

Analysis of differences in lifetime indices of productivity in sheep depending on the presence of mutations in the regions of c.55-51 and c.259-52 showed that they have a significantly greater impact on the studied parameters than substitutions previously described.

Live weight in animals with mutation in the form of homozygous alleles was significantly more by 11.2% than in heterozygotes.

Presence of mutations in a homozygous variant has affected the chest width in the form of significant increase of this index by 11.1% in comparison with the heterozygotes.

Metacarpal length study has revealed a significant reduction in the size of homozygous animals by 11% compared with the heterozygotes.

In animals homozygous on investigated substitutions the loin width was significantly more on 12% than in carriers of heterozygous genotype.

It was not possible to us to reveal mutation influence in positions of c.55-51 and c.259-52 on other indices.

#### DISCUSSION

The family of transcription MEF2 factors plays a key part in the morphogenesis of skeletal, cardiac and plain musculature (JUSZCZUK-KUBIAK *et al.*, 2011; KNAPP *et al.*, 2006). In vertebrates, the family is represented by 4 proteins: MEF2A, MEF2B, MEF2C, MEF2D (MORISAKI *et al.*, 1997). MEF2 proteins link up with A/T-rich regions of DNA, which comprise the majority of promoters and enhancers, genes responsible for the growth of skeletal muscle fibers (GOSSETT *et al.*, 1989). Proteins of the MEF2 family are required for transcription of muscle-specific genes and differentiation of myoblasts during the myogenesis (LYONSET *et al.*, 1995; OLSON *et al.*, 1995; MOLKENTIN *et al.*, 1995, 1996; AKKILA *et al.*, 1997; DU *et al.*, 2007). Moreover, MEF2 proteins are located at the center of the Ca<sup>2+</sup> signaling pathway, which plays a role in hypertrophic growth and the remodeling of skeletal muscle (OLSON and WILLIAMS, 2000).

According to our researches, MEF2B gene in sheep has many variations. Genotypes differ sharply. Even within a single breed of sheep it is difficult to find the animals with identical structure of a gene. The study of MEF2B gene structure has revealed a number of DNA chain regions differed in variability of single nucleotide polymorphisms located in them. The most detected by us SNPs are found without apparent regularity being present in various combinations with other single nucleotide polymorphisms. Unfortunately, it is not possible to carry out the comparative analysis of genotypes with other breeds at this moment. This is due to the fact that there is no information on the structural changes of a gene as a set of SNPs for other breeds of sheep. In spite of the fact that the substitutions found out by us are deposited in dbSNP database they are marked as “verified by cluster”, and the date about their prevalence among sheep is not available.

The analysis of genotypes made by us from the available data of sequencing has revealed a number of SNP combinations occurring in a complex and allowing us to divide animals into groups according to their presence or absence. In addition, it was found out, in what regions (coding or regulatory) the revealed SNPs are located. So, c.-1713 and c.-3 SNPs are located in the promoter region, and c.-3 is located directly ahead of the start codon at a distance of only three nucleotides. Substitutions of c.55-51 and c.259-52 are located in intron region. However, as our researches have shown, we can assume their relationship with productive indices of sheep either of regulatory character, or as markers of genotypes.

Analysis of the relationship of genotypes identified in SNP pairs with lifetime indices of sheep meat productivity has shown that animals with substitutions in promoter (c.-1713 and c.-3) do not differ practically on most indices from allele carriers of wild type in a homozygous variant. Thus, studying of one of the most important lifetime indices of the meat productivity as live weight has allowed us to reveal an interesting regularity. The animals having a heterozygous genotype on SNPs studied had a significantly lower weight than homozygotes with wild allele. In young rams having in genotype two mutant alleles live weight was not significant different from homozygotes without detected SNPs. Similar character of changes allows to make a conclusion that lifetime parameters of animals are influenced not by a genotype with c.-1713 and c.-3 substitutions, but any another, and at a part of animals it contains both SNPs, c.-1713 and c.-3.

Perhaps, this is due to the fact that in the work of LI ZHANG (2013) the dependence of the productive qualities on c.-1713 SNP has been still revealed. Apparently, MEF2B gene in sheep of Severokavkazskaya breed differs in structure from the animals studied by authors in China.

This hypothesis was confirmed by research of the productive parameters relationship with the presence in the sheep genotype of c.55-51 and c.259-52 SNPs. Apparently from Table 2, these substitutions in a heterozygous variant are found out at a part of sheep with genotypes, homozygous on the wild allele in the positions of c.-1713 and c.-3. In this case, some animals with wild alleles of c.-1713 and c.-3 in the positions of c.55-51 and c.259-52 have homozygous variant of SNP instead of heterozygous one.

As a result of comparison of productivity lifetime indices significant distinctions in several parameters have been found out in animals with a heterozygous variant of c.55-51 and c.259-52 SNP carriage with the data at homozygotes on the mutant type in the position of c.259-52 and homozygotes on the wild type of c.55-51. From this we can conclude that differences of sheep genotypes on these SNPs are just the reliable marker, the use of which is applicable in the genomic selection of Severokavkazskaya sheep breed.

#### CONCLUSION

The study indicates highly conserved exon gene of MEF2B and significant variability of not coding regions. From 14 identified SNPs 13 are included into NCBI database. We have found also the new previously not described c.258+380C>T substitution. During researches were found out two SNPs associated with high meat productivity. In our study it is not established significant effect on productivity of c.-1713 substitution earlier described as positively affecting the meat parameters. Based on these data it is possible to correct the breeding program on improvement of Severokavkazskaya breed by consolidation of positive alleles in population.

#### ACKNOWLEDGEMENT

This project was funded by Ministry of Agriculture of the Russian Federation (agreement on the procedure and conditions for granting subsidies to financial security the state order for the provision of public services (works) by December 30, 2013 3119/13).

Received July 09<sup>th</sup>, 2015

Accepted January 25<sup>th</sup>, 2016

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## MEF2B GEN SNP MARKERA ZA PRODUKCIJU MESA KOD SEVEROKAVKAZSKE RASE OVACA

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### Izvod

Jedan od novih perspektivnih gena kandidata koji definišu produktivne osobine ovaca je MEF2B. Protein iz grupe MEF2 koji utiče na proizvodnju miostatina i ekspresije gena odgovornih za rast vlakana skeletnih mišića. Između ostalog, poznavanje strukture MEF2B gena je važno za genomsku selekciju. Mi smo proučavali strukturu MEF2B gena na ovcama Severokavkazske rase u Rusiji. Za detekciju alela koristili smo NimbleGen tehnologiju sekvenciranja - Roche (SAD). Kao rezultat toga, otkriveno je 14 singl nukleotidnih polimorfizama (SNP). Otkriveni SNP-ovi nalaze se u ne kodirajućim zonama. Od njih 7 polimorfizama su na području 5' u gornjoj regiji gena, lokusi: c.-1713, c.-1319, c.-839, c.-321, c.-246, c.-161, c.-3 ; 6 polimorfizama su u intronima, lokusi: c.55-51, c.258 + 312, c.258 + 380, c.259-52, c.452 + 95, + 103 c.452, 1 SNP je u 3' donjoj regiji gena, c.\* 252. Dva identifikovana SNP značajno su povezana sa visokim indeksima produktivnosti mesa: c.55-51 i c.259-52. Istovremeno, nije bilo moguće saznati uticaj polimorfizma c.-1713 na produktivnost. Naša istraživanja su osnova budućih istraživanja o uticaju različitih genskih alela MEF2B na kvalitet mesa i mogu se koristiti za pripremu PCR test-sistema za genomsku selekciju.

Primljeno 09. VII. 2015.

Odobreno 25.I.2016.