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Қазақстан Республикасы Ұлттық ғылым академиясы "ҚР ҰҒА Хабарлары. Геология және техникалық ғылымдар сериясы" ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Webof Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Геология және техникалық ғылымдар сериясы Emerging Sources Citation Index-ке енуі біздің қоғамдастық үшін ең өзекті және беделді геология және техникалық ғылымдар бойынша контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Известия НАН РК. Серия геологии и технических наук» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science. Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Social Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество и глубину контента для исследователей, авторов, издателей и учреждений. Включение Известия НАН РК. Серия геологии и технических наук в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному контенту по геологии и техническим наукам для нашего сообщества.

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APPROBATION OF PCR-RFLP AND AS-PCR METHODS FOR GENOTYPING CATTLE BY THE DGAT1 GENE

Abstract. The DGAT1 gene Bos taurus is an economically valuable lipid metabolism gene in cattle, affecting upon milk production and milk quality, the allele polymorphism assessment of which is diagnostically significant. The purpose of this work was to test a number of methods for carrying out PCR-RFLP and AS-PCR for genotyping cattle using allelic variants of the DGAT1 gene and then evaluating allelic polymorphism in the studied animal sample in the context of consistency of the used molecular genetic approaches. Studies have been conducted on a sample of mixed and pure bred Holstein cattle. DNA Extraction from samples of whole canned blood of cattle carried out by the combined alkaline method. Proven methods of genotyping effectively identified the analyzed genotypes, showing consistent with each other reliable results, in accordance with the calculated data of alignments analysis, restriction mapping and simulations of generated PCR and RFLP profiles.

Keywords: Bos taurus, DGAT1, allele: genotype, genotyping, PCR, RFLP.

Introduction. PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) – combined molecular genetic method for genotyping animals [1], plants [2], bacteria [3] and viruses [4], where the length of the generated amplicons and restricts is analyzed for single nucleotide polymorphism (SNP) and allelic polymorphism in eukaryotes, in particular, with the subsequent conclusion about the homozygous or heterozygous state of a certain gene of the individual being studied.

Various modifications of PCR-RFLP analysis [5-7] have found practical application in evaluating the allelic polymorphism of *DGAT1* (diacylglycerol o-acyltransferase 1) *Bos taurus*, an economic-valuable lipid metabolism gene in cattle [8, 9], affecting on milk production and milk quality in the context of the content and yield of milk fat [10-12], as well as fat-acid, protein and mineral composition of milk [9], which is especially important in the production of functional and gerodietic dairy products [13-15, 21].

Along with this, other of SNP detection methods, such as AS-PCR (Allele-Specific PCR) [16], direct sequencing of the amplified gene locus, HRM analysis (High Resolution Melting) [17] and Real-time PCR [16, 18].

The goal of the study was to test a number of PCR-RFLP and AS-PCR methods for genotyping cattle using allelic variants of the *DGAT1* gene and then evaluating allelic polymorphism in the studied animal sample in the context of consistency of the molecular used genetic approaches.

Materials and Methods. Studies were conducted on a sample of mixed and pure bred Holstein cattle of one of the breeding enterprises of the Russian Federation.

Extraction of nucleic acid from samples of whole blood of cattle, canned 10 mm EDTA-Na2, carried out by the combined alkaline method [19].

Tested methods of PCR-RFLP and AS-PCR for cattle genotyping for alleles *A* and *K* of the *DGAT1* gene were performed on a "Tertsik" amplifier ("DNA -Technology, Russia) in volumes of 20 μl,

containing standard buffer (60 mM Tris -HCl (pH 8.5); 1.5 mM MgCl2; 25 mM KCl; 10 mM 2-mercaptoethanol; 0.1% Triton X-100), 0.25 mM dNTP, 1 U Taq DNA polymerase, 0.25 mM of direct standard (DGAT1-F [19]) and allele-specific primers (DGAT1-1 + DGAT1-2 [7] and DGAT1A + + DGAT1K [16]), 0.5 μM of reverse common primers (DGAT1-R [19], DGAT1-3 [7] and DGAT1R [16]), 1 μl of the DNA sample in the following thermal cycling modes:

PCR with primers DGAT1-F + DGAT1-R:

×1: 94°C – 4 min; ×35: 94°C – 60 sec., 59°C – 30 sec., 72°C –30 sec.; ×1: 72°C – 10 min; storage: 4°C.

PCR with primers DGAT1-1 + DGAT1-2 + DGAT1-3:

×1: 94°C – 4 min; ×40: 94°C – 10 sec., 72°C – 10 sec.; ×1: 72°C – 5 min; storage: 4°C.

PCR with primers DGAT1A + DGAT1K + DGAT1R:

×1: 94°C – 4 min; ×40: 94°C – 20 sec., 65°C – 20 sec., 72°C – 20 sec; ×1: 72°C – 5 min; storage: 4°C.

The sequence of used oligonucleotide primers sets is presented in table 1.

Table 1 – Oligonucleotide Primers Sets for PCR-RFLP and AS-PCR

Oligonucleotide Primers Sets for PCR-RFLP and AS-PCR	Ref
DGAT1-F: 5/-GCACCATCCTCTTCCTCAAG-3/ DGAT1-R: 5/-GGAAGCGCTTTCGGATG-3/	[19]
DGAT1-1: 5/-CCGCTTGCTCGTAGCTTTCGAAGGTAACGC-3/ DGAT1-2: 5/-CCGCTTGCTCGTAGCTTTGGCAGGTAACAA-3/ DGAT1-3: 5/-AGGATCCTCACCGCGGTAGGTCAGG-3/	[7]
DGAT1A: 5/-CGTAGCTTTGGCAGGTAAAGC-3/ DGAT1K: 5/-CCGCTTGCTACTAGCTTTGGCAGGTAACAA-3/ DGAT1R: 5/-TCAGGTTGTCGGGGTAGCTC-3/	[16]

To determine the allelic polymorphism of the *DGAT1* gene with use of allelic variants *A* and *K*, 20 μl of PCR samples were treated with 10 U of *Aco*I restrictase in 1× buffer “G” at 37 °C (DGAT1-F + +DGAT1-R) and 20 U of *Taq*I restrictase in 1 × buffer "Y" (DGAT1-1 + DGAT1-2 + DGAT1-3) at 65 °C overnight.

The results of PCR-RFLP and AS-PCR were detected by horizontal electrophoresis in 3% agarose gel in TBE buffer (pH 8.0), containing ethidium bromide at a concentration of 0.5 μg/ml, followed by visualization of the amplified products in an ultraviolet transilluminator (λ = 310 nm).

We used reagents for molecular biological studies produced by SibEnzyme LLC (Russia) and DNA-Synthesis LLC (Russia).

$$p = n/N,$$

where p - genotypes frequency; n - number of animals having a certain genotype; N - total number of examined animal units.

The calculation of individual alleles frequency is determined by the formula:

$$p = (2N1+N2)/2n,$$

where $N1$ - homozygotes number for studied allele; $N2$ - heterozygotes number; n - sample size.

To compare the observed and expected frequency distribution of genotypes, the chi-square correspondence criterion is used (χ^2).

The expected genotypes frequencies in the studied sample are calculated according to the Hardy-Weinberg law.

The obtained results were processed by a biometric method using computer and Microsoft Excel software application. The level of their reliability is determined by the Student criterion.

Results and discussion. Calculated data for verification of PCR-RFLP and AS-PCR validated methods were obtained based on alignment analysis, *Aco*I and *Taq*I restriction mapping of amplified partial nucleotide sequences of *A* and *K* allelic variants DGAT1 *Bos taurus* gene with modeling of the generated PCR and RFLP profiles of the corresponding genotypes .

Thus, the well-known primers DGAT1-F and DGAT1-F [19] initiate amplification of the cattle DGAT1 gene locus with a length of 411 bp, and AcoI-RFLP analysis of the genotype-specific fragments generated (*AA* = 208/203 bp, *KK* = 411 bp and *AK* = 411/208/203 bp) provides the correct genotyping procedure (table 2).

Table 2 – Oligonucleotide Primers First Set for PCR-RFLP

Oligonucleotide Primers Set	PCR Product (bp)	*Aco*I-RFLP Fragments		
		DGATI genotypes		
		AA	*KK*	*AK*
DGAT1-F + DGAT1-F	411	208 203	411	411 208 203

Another tested primer set DGAT1-1 + DGAT1-2 + DGAT1-3 [7] triggers the amplification of DGAT1 *Bos taurus* gene locus with a length of 100 bp followed by the generation of DGAT1-PDLP-*Taq*I profiles (*AA* = 82/18 bp, *KK* = 100 bp and *AK* = 100/82/18 bp) (table 3).

Table 3 – Oligonucleotide Primers Second Set for PCR-RFLP

Oligonucleotide Primers Set	PCR Product (bp)	*Taq*I-RFLP Fragments		
		DGATI genotypes		
		AA	*KK*	*AK*
DGAT1-1 + DGAT1-2 + DGAT1-3	411	82 18	100	100 82 18

The result of modeling the generated PCR and *Taq*I-RFLP profiles of the corresponding genotypes for the *DGAT1* gene is presented in figure 1.

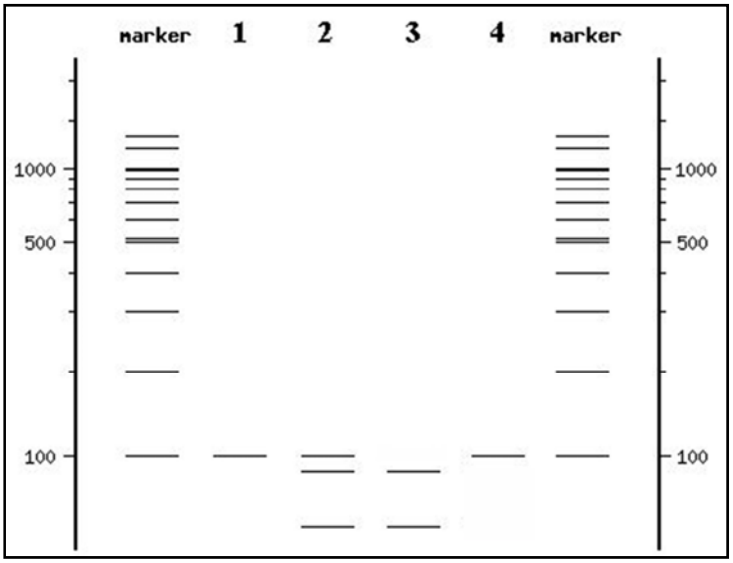

Figure 1 – DGAT1-PCR-RFLP-TaqI Profile of Bos taurus with Primers DGAT1-1 + DGAT1-2 + DGAT1-3.

Note: 1) PCR-product (100 bp); 2-4) RFLP-fragments: 2) genotype *AK* (100/82/18 bp); 3) genotype *AA* (82/18 bp); 4) genotype *KK* (100 bp).

An illustrative result of PCR-RFLP for genotyping cattle by alleles *A* and *K* of the *DGAT1* gene with the corresponding primers (DGAT1-1 + DGAT1-2 + DGAT1-3) and endonuclease digestion with the restriction enzyme *Taq*I is presented in figure 2

At the same time, a distinctive feature of this approach is that at the PCR stage, the “Single PCR” formulation uses three primers, one of which (DGAT1-3) is common for both alleles of the analyzed gene, and the other two (DGAT1-1 + DGAT1- 2) - allele-specific, but with a given restriction identification site for one of them (DGAT1-1), and artificially created, but not affecting the analyzed SNP itself, which is a competitive advantage when choosing the desired restriction enzyme (figure 3).

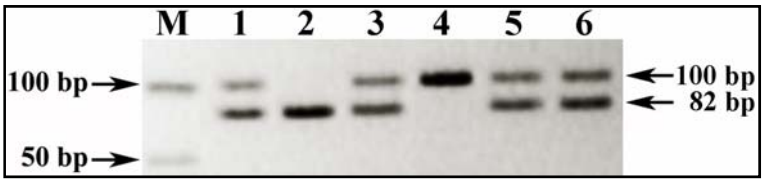

Fig. 2 Electrophoregram of the Result of a PCR-RFLP with the Primers DGAT1-1 + DGAT1-2 + DGAT1-3 and endonuclease digestion with the restriction enzyme TaqI for genotyping Bos taurus on the Allelic Variants A and K of the DGAT1 gene.

Note: M) DNA Molecular Weight Marker (100 bp + 50 bp ladder); 1, 3, 5, 6) genotype *AK* (100/82/18 bp); 2) genotype *AA* (82/18 bp); 4) genotype *KK* (100 bp).

```
                                  TaqI
                CCGCTTGCTC GTAGCTTTCG AAGGTAACGC = A-allele specific primer
DGAT1-gene      CCGCTTGCTC GTAGCTTTGG CAGGTAACAA = K-allele specific primer
Allele A   001 CCGCTTGCTC GTAGCTTTGG CAGGTAAGGC GGCCAACGGG GGAGCTGCCC
Allele K   001 .......... .......... ........AA .......... ..........

                                                          Common primer PCR
DGAT1-gene                                      CCTGA CCTACCGCGG TGAGGATCCT product
Allele A   051 AGCGCACCGT GAGCTACCCC GACAACCTGA CCTACCGCGG TGAGGATCCT 100 bp
Allele K   051 .......... .......... .......... .......... .......... 100 bp

DGAT1-gene Taq1-restriction mapping Taq1-PCR-RFLP-profile
Allele A   1-18/19-100              82/18 bp
Allele K   100                     100 bp
```

Figure 3 – Aligning and Taq-Restriction Mapping of the Nucleotide Sequence of DGAT1-gene locus of the Bos taurus Flanked with Primers DGAT1-1 + DGAT1-2 + DGAT1-3 (Alleles A and K)

The third tested primer set DGAT1A + DGAT1K + DGAT1R [16] also provides effective identification of the desired genotypes (*AA, KK, AK*) due to the correct interpretation of the generated 80-bp and/or 71 bp genotypes (table 4).

Table 4 – Oligonucleotide Primers Third Set for AS-PCR

Oligonucleotide Primers Set	PCR Product		
	DGATI genotypes		
	AA	*KK*	*AK*
DGAT1A + DGAT1K + DGAT1R	71	80	80 71

An illustrative electrophoretic picture of the AS-PCR result for the genotyping of cattle by alleles *A* and *K* of the *DGAT1* gene with a set of oligonucleotide primers DGAT1A + DGAT1K + DGAT1R is shown in figure 4.

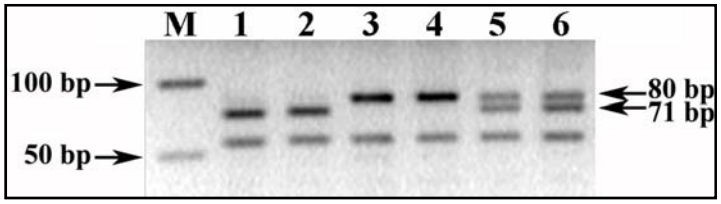

Figure 4 – Electrophoregram of the Result of a AS-PCR with the Primers DGAT1A + DGAT1K + DGAT1R for Genotyping Bos taurus on the Allelic Variants A and K of the DGAT1 gene.

Note: M) DNA Molecular Weight Marker (100 bp + 50 bp ladder); 1-2) genotype *AA* (71 bp); 3-4) genotype *KK* (80 bp); 5-6) genotype *AK* (80/71 bp).

This method of carrying out PCR for genotyping cattle in allelic variants A and K of the DGAT1 gene with detection in agarose gel electrophoresis is a type of allele-specific PCR (AS-PCR) [20].

It effectively discriminates against single nucleotide substitutions (SNPs) in the "Single PCR" formulation when using two forward allele-specific primers of different lengths with 3/- end bases complementary to the SNP site (DGAT1A + DGAT1K and one common reverse primer (DGAT1R), together initiating amplification of allele-specific PCR products of various lengths, separated in agarose gel electrophoresis (figure 5).

```
                          CGTAGCTTTGGCAGGTAAAGC = A-allele-specific primer
DGAT1-gene          CCGCTTGCTACTAGCTTTGGCAGGTAACAA = K-allele-specific primer
Allele A   01               CGTAGCTTTGGCAGGTAAGGCGGCCAACGGGGGAGCTGCCCAGC
Allele K   01 CCGCTTGCT...................AA.......................

                              Common primer PCR-
DGAT1-gene                 GAGCTACCCCGACAACCTGA product
Allele A   45 GCACCGTGAGCTACCCCGACAACCTGA 71 bp
Allele K   54 ............................ 80 bp
```

Figure 5 – Aligning of the Nucleotide Sequence of DGAT1 gene locus of the Bos taurus Flanked with Primers DGAT1A + DGAT1K + DGAT1R (Alleles A and K)

At the same time, to increase the specificity of the reaction, an unpaired nucleotide in the 3rd position from the 3/- end of oligonucleotides is introduced into the allele-specific primers, as well as two additional mismatch nucleotides in the 20th and 21st positions only in the DGATK primer.

The approved genotyping methods showed identical results of the evaluation of the allelic polymorphism of the *DGAT1* gene in the sample of manufacturing bulls, whose data are presented in table 5.

Table 5 –Allelic polymorphism of the DGAT1 gene in the studied sample of servicing bulls

N=70	Occurrence Frequency								$\chi 2$
	genotypes						*alleles*		
	AA		*AK*		*KK*		*A*	*K*	
	n	*%*	*n*	*%*	*n*	*%*			
O	38	54.3	28	40.0	4	5.7	0.74	0.26	0.24
E	38	54.3	27	38.6	5	7.1			
Note: O – actually observed indicator, E – theoretically expected indicator.									

So, bulls, which are mixed and pure bred Holstein cattle, the frequency of allele *A* was 0.74, allele *B* - 0.26, and genotype distribution was as follows: *AA* - 38 heads (54.3%), *AK* - 28 heads (40.0%), *KK* - 4 heads (5.7%).

The observed frequency distribution of genotypes in the studied sample corresponds to the theoretically expected Hardy-Weinberg equilibrium distribution.

Conclusion. PCR-RFLP and AS-PCR methods for genotyping cattle using allelic variants *A* and *K* of the *DGAT1* gene effectively identified the analyzed genotypes, showing reliable results consistent with each other, in accordance with the calculated data of alignment analysis, restriction mappings and modeling of the generated profiles, comparable with the results of a previous study on the same sample of manufacturing bulls [7].

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DGAT1 ГЕНІ БОЙЫНША ІРІ ҚАРА ЖАНУАРЛАРЫН ГЕНОТИПТЕНДІРУ ҮШІН PCR-RFLP ЖӘНЕ AS-PCR ӘДІСТЕРІН СЫНАҚТАН ӨТКІЗУ

Аннотация. *DGAT1 Bos taurus* – бұл малдың сүттілігіне және сапасына әсер ететін, аллельдік полиморфизмді бағалауда диагностикалық мәні бар ірі қара жануарының май алмасуына қатысатын маңызды ген. Осы жұмыстың мақсаты – DGAT1 генінің аллельді нұсқаларын қолдану арқылы PCR-RFLP және AS-PCR операцияларын жүргізудің бірқатар әдістерін сынақтан өткізу, содан кейін зерттелген жануарлар үлгісінде қолданылатын молекулалық генетикалық тәсілдердің үйлесімділігі тұрғысында аллельді полиморфизмді бағалау. Зерттеулер жергілікті және таза тұқым голштин ірі қара малы бойынша жүргізілді. Біріктірілген сілтілі әдіспен жүргізілген малдың толық консервіленген қанының үлгілерінен ДНҚ алынды. Генотиптіліктің дәлелденген әдістері есептелген деректерді талдауға сәйкес, PCR және RFLP профилдерімен генерациялайтын шектеуді картаға түсіру және модельдеуге сәйкес, бір-біріне сенімді нәтижелерге сәйкес келетін талданатын генотиптер тиімді түрде анықталды.

Түйін сөздер: Bos taurus, DGAT1, аллель: генотип, генотиптеу, ТР, RFLP.

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АПРОБАЦИЯ СПОСОБОВ ПРОВЕДЕНИЯ PCR-RFLP И AS-PCR ДЛЯ ГЕНОТИПИРОВАНИЯ КРУПНОГО РОГАТОГО СКОТА ПО ГЕНУ DGAT1

Аннотация. Ген *DGAT1 Bos taurus* – хозяйственно-ценный ген липидного обмена у крупного рогатого скота, влияющий на молочную продуктивность и качество молока, оценка аллельного полиморфизма которого диагностически значима. Целью настоящей работы являлась апробация ряда способов проведения PCR-RFLP и AS-PCR для генотипирования крупного рогатого скота по аллельным вариантам гена *DGAT1* с последующей оценкой аллельного полиморфизма у исследуемой выборки животных в контексте согласованности использованных молекулярно-генетических подходов. Исследования проведены на выборке помесного и чистопородного голштинского скота. Экстракция ДНК из образцов цельной консервированной крови крупного рогатого скота осуществлена комбинированным щелочным способом. Апробированные способы генотипирования эффективно идентифицировали анализируемые генотипы, показав согласованные друг с другом достоверные результаты, в соответствии с расчетными данными анализа выравниваний, рестрикционных картирований и моделирований генерируемых PCR и RFLP профилей.

Ключевые слова: Bos taurus, DGAT1, аллель: генотип, генотипиование, ПЦР, RFLP.

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