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# The sequence study and expression profiles of *ACSL1, ME1,* and *ELOVL6* genes in Kielecka, Landes and White Koluda® geese livers

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**Health recommendations for human diet include introducing products with a high content of mono- and poly-unsaturated fatty acids (MUFA and PUFA, respectively). Omega 3 and 6, essential fatty acids, are not synthesized de novo and must be supplied to the body with food. The research carried out so far has shown that a high content of polyunsaturated fatty acids characterizes goose meat. Therefore, our study focused identifying the molecular background for the differences in gene polymorphism and gene expression between three Polish breeds. Based on the literature, we chose the ACSL1, ME1, and ELOVL6 genes, which have a proven effect on the formation of unsaturated fatty acids in goose liver.**

**We conducted our research on samples from the Kielecka, White Koluda® and Landes breeds. The real time PCR based on Taqman probes was conducted on RNA isolated from livers from 15 samples, 5 samples per breed. A sequencing proceeded on the DNA isolated from feathers collected from 26 individuals from each breed (total number of samples n = 78). In the White Koluda®, we**

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**identified two SNPs in ACSL1 gene g. 4 542 604 G>A and g. 4 542 623 C>A, in the Kielecka only one SNP Chr1.g. 4 542 604 G>A. The Landes breed remained monomorphic for all studied genes. The significant differences in gene expression between all the examined breeds were observed for ACSL1 gene and ME1. We assume these two genes can potentially reveal the genetic background for differences in fatty acid accumulation capacity and polyunsaturated fatty acid biosynthesis in the livers of White Koludzka®, Kielecka, and Landes geese.**

**KEY WORDS: fatty acids / goose / gene expression / Sanger sequencing**

In highly developed societies, there is an epidemic of diseases related to improper nutrition. Excessive consumption of saturated fats is responsible for the concentration of cholesterol in the blood and, as a long-term effect, causes strokesheart attacks, etc. Moreover, the most frequently mentioned diseases associated with the excessive consumption of saturated fats include type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) - Perry *et al.* [2014]. Health recommendations advise limiting the consumption of the saturated fatty acids (SFA) contained in red meat (including pork and beef) in the diet, and introducing products with a high content of mono- and poly-unsaturated fatty acids (MUFA and PUFA, respectively). Omega 3 and 6 acids are essential fatty acids, so they are not synthesized *de novo* and must be supplied to the body with food. The research carried out so far on native gees breeds bred in Poland had shown that goose meat is characterized by a high content of polyunsaturated fatty acids, including n-6: C 18:2 (linoleic), C 20:4 n-6 (arachidonic), C 22:4 at the level of 13.20 – 16.20%, 3.61 – 7.79%, 0.55-0.88% of the total amount of fatty acids, respectively; n-3: C 18:3 (linolenic), C 20:5 (EPA) and C 22:6 (DHA) at the level of 0.87 - 1.63%, 0.58 - 1.51%, 0, respectively 34 - 0.44% of total fatty acids (Haraf et al., 2018). For the optimal functioning of the body, the ratio of n-6 to n-3 fatty acids is important and should be close to 1:1 [Simopoulos 2008]. Studies conducted on Polish geese breeds indicate that the n-6:n-3 ratio and the composition of fatty acids depend on the way the birds are kept, including the type of food they eat and the genotype [Haraf *et al*. 2014, Orkusz *et al.* 2015].

Over 98% of the geese population in Poland is the White Kołuda® goose. The Kołuda Wielka Experimental Station keeps two parent strains on the farm, W33 (paternal) and W11 (maternal). The interbred hybrid (W31) resulting from this cross is the basis for producing slaughter geese using various technologies, including the White Kołuda Oats Goose listed on the Polish List of Traditional Products in the category fresh meat and meat products. The White Kołuda® goose is famous for its high body weight before slaughter, good use of farm feed, bird health and adaptation to various environmental conditions. In comparison, the Kielecka breed is much smaller than the White Koluda@. It has been included in the Genetic Resources Program to prevent the Kielecka from extinction. Finally, the Landes breed has a foreign origin and is characterized by good meatiness, much higher than the Kielecka but notably lower than White Koluda® goose [Calik *et al*. 2023]. Moreover, according to the literature , the French Landes has a genetic predisposition for efficient accumulation of fatty acids in the liver [Fournier *et al.* 1997].

Most studies on geese compare liver gene expression between normally fed and overfed individuals to induce steatosis. (Mourot et al., 2000; Zhu et al., 2011). However, several studies on Polish goose breeds reveal an excellent composition of unsaturated fatty acids in tissues. Therefore, in our study, we focused on searching for molecular background for the differences in gene polymorphism and gene expression between three breeds used in Poland. Concerning the fact that the Landes breed accumulates hepatic triglycerides efficiently, we assumed that this breed would genetically significantly differ from the Kielecka and White Koluda®.

Based on the literature, we chose ACSL1, ME1 and ELOVL6 genes as they have a proven effect on the formation of unsaturated fatty acids in goose liver [Zhu *et al.* 2011].

ACSL1 is believed to activate long-chain fatty acids by converting them into fatty acyl-CoA, a known precursor for elongation and desaturation [Ellis *et al.* 2010]. Then Elovl-6 extends these fatty acids, preferably those with 12 to 16 carbon atoms. As a result, very long-chain fatty acids, which are components of unsaturated fatty acids, are formulated. NADP+-dependent malic enzyme generates NADPH, a critical cofactor for the desaturation process, where enzymes like stearoyl-CoA desaturase (SCD) introduce double bonds into the elongated fatty acids, thus creating unsaturated fatty acids [Zhu *et al.* 2011, Lu *et al.* 2015]. This pathway is essential for synthesizing crucial lipids involved in various physiological processes in geese, including energy storage, membrane fluidity, and signaling [Zhu *et al*. 2011].

## Material and methods

### Material for gene expression study

The material for the gene expression study was fragments of liver tissue from three geese breeds: Kielecka (Ki), White Koluda® strain W33 (WK), and Landes (LsD); 5 samples from each breed. All the samples were taken from males. Gene expression analysis was performed on the liver. After being transcribed into cDNA, the remaining isolates were banked at -20°C.

The birds were kept on straw litter in the same environmental conditions. Up to 3 weeks of age they were kept in a windowless confinement facility, and from 4 weeks of age they were housed with outdoor access. The birds were fed with the same complete KB-1 (0-3 weeks of age), KB-2 (4-8 weeks of age), and KB-3 (9-16 weeks of age) feed mixtures containing 20.0, 18.5, and 14.5% crude protein; 3.0, 2.3 and 2.3% crude fat; 11.72, 11.50 and 11.00 MJ/kg EM; 1.03, 1.50 and 0.64% Ca; and 0.43, 1.00, and 0.33% available P, respectively, following the breeding goose nutrition program. At 16 weeks of age, following the 12-hour fasting, the geese were slaughtered, and the liver samples were taken from the right liver lobe no longer than 10 minutes *postmortem*. The tissues were collected into the described cryo-tubes and immediately placed in liquid nitrogen.

**Material for DNA sequence analysis**

At the Kołuda Wielka Waterfowl Experimental Station, feathers were collected from the geese of the following breeds: Ki (n= 26), WK (n= 26), and LsD (n= 26). The sex of the birds was unknown. The samples were collected with careful attention to minimizing genetic relatedness, based on the breeding documentation.

The samples were obtained during commercial slaughter at the Kołuda Wielka Waterfowl Experimental Station.. Therefore, the consent of the Local Ethical Committee was not required, and no such ethical commission permission is needed regarding feather samples.

**RNA isolation and reverse transcription**

RNA from the liver was isolated using Total RNA mini kit (A&A Biotechnology, Poland). Isolation was carried out according to the instructions provided by the reagent manufacturers. RNA quality was checked in a 2% agarose gel, RNA concentration and purity were measured using a Qubit (ThermoFisher, USA). Reverse transcription was performed using the TranScriba Kit reagents (A&A Biotechnology, Poland), using the thermal profile recommended by the reagent manufacturer.

**Reverse transcription and real-time PCR**

TaqMan MGB probes (Invitrogen, USA) were designed based on the Pink-footed goose genome (ASM259213v1) available in the Ensemble database. The TaqMan assay was implemented with the Probes listed in Table S1 (Supplemental material) and the endogenous control RPL4 (NXHY01000053.1) and for the tested genes: ACSL1, ME1, ELOVL6; a real-time PCR was conducted using the TaqMan™ Fast Advanced Master Mix reagent kit (Applied Biosystems™) and performed on Step-one Real time device (Applied Biosystems, USA).

**Statistical analysis of gene expression**

The expression results were analyzed using the one-way analysis of variance. The Student's test was used to evaluate statistical differences between the means of the breeds. Moreover, a post hoc Bonferroni correction was applied. The p-values reflect unadjusted p-values from the Student's test, conducted between groups. The Bonferroni correction was then applied as a post hoc adjustment to control for multiple comparisons. The analysis of the data was conducted using the text package (v0.9.10, Kjell and Schwartz, n.d.).

**Genomic DNA isolation and DNA sequencing, identification of polymorphisms**

DNA from feather shafts was isolated using the Sherlock AX kit (A&A Biotechnology, Poland). The concentration, quality and purity of the isolates were verified using a NanoDrop fluorometer (ThermoFisher, USA).

All genes were amplified in a 25 µL reaction volume. The reaction mixture included 2 µL of genomic DNA (30-50 ng), 1 unit of HotStarTaq DNA Polymerase

(Qiagen, Cat. No. 203205, Germany), 1x PCR buffer, 0.3 mM $MgCl_2$, 1x Q-Solution, 0.4 μM dNTPs (10 mM; Thermo, USA), and 0.2 μM each of forward and reverse primers. PCR and sequencing primers and the thermal program are listed in Table S2 and S3 (Supplemental material).

The sequencing was performed using the BigDye™ Terminator v3.1 Cycle Sequencing Kit reagents (Applied Biosystems™, USA) and the same primers as for PCR. PCR products underwent capillary electrophoresis on a 3500xl sequencer (Applied Biosystems™)

The sequence analysis was performed using the BioEdit program [Hall 1999] and the Clustar W application [Thomson *et al*. 1994.

#### Statistical analysis of gene polymorphism

Based on sequencing results, we calculated population genetic variation using Fixation Index (FST) statistics via the web version of Genepop [Raymond and Rousset 1995].

## Results and discussion

#### RNA – concentration and quality measurement results and concentration normalization

RNA with a concentration above 100ng/ul and an absorbance ratio of 260nm/280nm of approximately 2.00 was used for further studies. The degree of degradation was assessed based on the result of electrophoretic separation - two clear bands visible in the gel indicate the lack of degradation.

#### Real-time PCR

**Gene expression of *ACSL1, ME1 and ELOVL6.*** Table 1 and Supplemental material Figure 1 show the mean relative quantification (RQ) results for ACSL1, ME1, and ELOVL6 gene expression in the livers of White Koluda®, Kielecka, and Landes breeds.

ACSL catalyzes the ATP-dependent acylation of fatty acids (FA) into long-chain acyl-CoA (LCA-CoA). This is the first step in lipid metabolism after fatty acids enter the cell [Mashek *et al*. 2007]. LCA-CoA can then be oxidized to obtain energy or further esterified to phospholipids, cholesteryl esters and triglycerides [Ellis *et al.* 2010]. To date, five ACSL isoforms have been identified: ACSL1, ACSL3, ACSL4, ACSL5, and ACSL6 (formerly known as ACS2) - Mashek *et al.* 2004]. These isoforms have been identified in various tissues and have varying affinities for different fatty acid subtypes. ACSL1, ACSL4, and ACSL5 are expressed in the liver and adipocytes, while ACSL3 and ACSL6 are expressed in the brain. Expression of ACSL4 is highest in steroidogenic tissues and ACSL5 in the intestine.

The research showed that the expression of the *ACSL1* gene differs statistically between Landes, White Koluda®, and Kielecka, reaching the highest values in Landes. The study conducted by Fournier *et al.* [1997] indicates French Landes grey goose for

**Table 1**. Mean relative quantification of ACSL1, ME1 and ELOVL6 gene expression in the liver of White Koluda®, Kielecka and Landes

| Item | ACSL1 | | | ME1 | | | ELOVL6 | | |
|---|---|---|---|---|---|---|---|---|---|
| | mean | SD | p-value | mean | SD | p-value | mean | SD | p-value |
| Ki | 1.0788[a] | 0.1232 | 0.0096 | 0.9440[a] | 0.0653 | 0.0007 | 1.1850 | 0.1224 | 0.0570 |
| LsD | 1.3697[ab] | 0.1240 | 0.0383 | 0.6310[a] | 0.0997 | 0.0743 | 0.9118 | 0.1811 | 0.9989 |
| WK | 0.8346[b] | 0.8346 | 0.0004 | 0.7689 | 0.1607 | 0.1889 | 1.8541 | 0.3812 | 0.3252 |

SD – standard deviation.
[ab]The same letter means the significant differences of mean RQ comparison between breeds, at p<0,01 Bonferroni Correction.
The p-values reflect unadjusted p-values from the Student's test, conducted between groups.
The Bonferroni correction was applied as a post hoc adjustment.

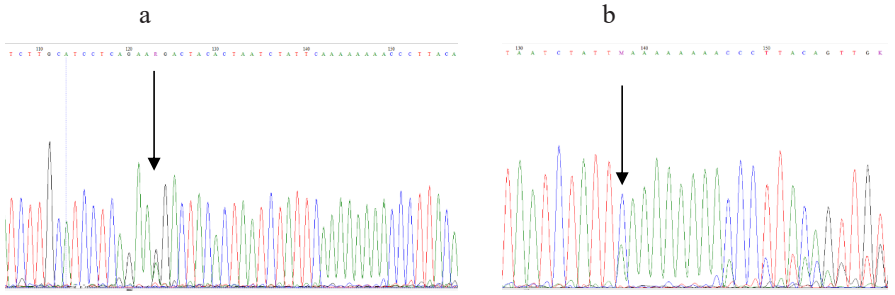


Fig. 1. The chromatograms depicting *ACSL1* two polymorphic sites identified in White Koluda® and Kielecka; a) g. 4 542 604 A>G in White Koluda®; b) g. 4 542 623 A>C in Kielecka.

high susceptibility to fatty liver production by accumulating of triglycerides in the hepatocytes. The same authors noted that the Polish white goose (without specifying the exact breed or line) showed a lower tendency toward fat accumulation.

Polymorphism in the malic enzyme (ME) in geese is particularly interesting due to its role in lipogenesis, a critical metabolic pathway for fat deposition. In geese, variations in the malic enzyme gene can affect the enzyme's activity and efficiency in producing NADPH, which is crucial for fatty acid synthesis [Zhu *et al*. 2011]. Studies have identified several polymorphic sites in the ME gene that correlate with differences in fat deposition traits among different goose breeds. These genetic variations can influence the rate of lipogenesis, impacting the economic traits of geese, such as meat quality and fat content [Zhu *et al*. 2011].

French Landes Grey geese are known for 'Foie gras', a delicate premium product from geese livers. In Poland, the Landes geese are descended from a French lineage but are not bred for fatty liver production. Instead, they are maintained within the Polish Genetic Resource Protection Program, where they are notable for their relatively high body weight compared to other goose breeds in the program. This adaptation reflects a focus on preserving their genetic traits, particularly those enhancing overall body mass and meat yield [Calik *et al.* 2023]. Studies comparing French Landes and Polish white goose showed that they differ in the level of expression of the ME1 gene.

With identical feed programs, the French Landes were characterized by a significant increase in ME1 in the liver compared to the Polish white goose [Fournier et al. 1997]. The reason for these differences may be a variable lipogenesis in the liver. In birds, this process occurs via ME-dependent NADPH, unlike in mammals where it is mediated by glucose-6-phosphate dehydrogenase (G6PD) - Zhu *et al*. [2011]. ME activity is the best indicator of the lipogenic capacity of the liver in various bird species [Hermier 1997]. Notably, in our research, *ME1* gene expression statistically differed between the Landes and Kielecka breeds. The higher level of *ME1* gene expression in the Kielecka may indicate a higher lipogenic process in the liver in this breed.

ELOVL6 has acetyl-CoA elongase activity specific for long chains (C22 to C24 and C26 saturated and monounsaturated fatty acids) and its activity significantly affects the composition of fatty acids in tissues [Lu et al. 2015]. The expression of genes involved in UFA biosynthesis, such as ELOVL6, was higher in breeds with higher body and liver weights in Zhu *et al.* [2011]. However, the research results presented here do not confirm these observations. In our tests on the White Koluda®, Kielecka and Landes breeds, no differences in *ELOVL6* expression in the liver were found. This discrepancy prompts further investigation into possible regulatory mechanisms that may uniquely modulate ELOVL6 expression in the White Kołuda® geese, perhaps related to breed-specific metabolic or genetic factors that deviate from established patterns.

**DNA sequencing, identification of polymorphisms**. Sequences of selected genes (*ELOVL6, ME, ACSL1*) were obtained for all breeds studied. The analyzed sequences showed no polymorphisms except the ACSL1 gene (Fig.1ab).

*ACSL1* is a gene consisting of 21 exons located on Chr 1. We have chosen exons 1 and 21 for sequencing as they cover the 5' and 3'UTR (untranslated region) sequences. We identified two SNPs in *ACSL1* exon 21 along the 3'UTR: g. 4 542 604 A>G and g. 4 542 623 A>C (respectively g.547A>G and g. 575A>C of the *ACSL1* sequence). The 3'UTR plays a crucial role in post-transcriptional regulation by influencing mRNA stability, translation, and localization. Its regulatory capacity allows cells to adjust protein production dynamically, which is essential for cellular responses and adaptation to environmental changes [Huang and Teeling 2017]. Both polymorphisms have a putative effect on translation and protein synthesis. Further studies, however, should be conducted to investigate this issue in detail. The g. 4 542 604 A>G polymorphism was found in the Kielecka breed only, whereas the g. 4 542 623 A>C polymorphism was detected only in two samples of the White Koluda® breed. Samples form the Landes breed were monomorphic for all three genes studied.

Analyzing $F_{ST}$ values by SNP across multiple populations can offer insights into genetic differentiation, potential selective pressures, and evolutionary relationships among the populations. $F_{ST}$ values calculated for all three breeds were 0.5647 for the g.547A>G SNP and 0.0166 for the g.575A>C SNP. Interpreting the $F_{ST}$ results separately for each SNP may lead to inconsistent conclusions. This is because the $F_{ST}$ calculated for the g.547A>G SNP indicates that populations share little genetic similarity. On the contrary, the $F_{ST}$ for g.575A>C SNP indicates moderate genetic

differentiation. For both SNPs combined, the overall $F_{ST}$ values were 0.5262, and indicate very high genetic differentiation, suggesting that these populations are nearly entirely isolated from one another. This is likely a result of the genetic resource conservation programs for the Kielecka and Landes breeds, which aim to preserve their unique genotypes. They involve maintaining consistent genetic balance while preserving the distinct phenotypic characteristics of both male and female birds. Additionally, the programs aim to sustain essential production traits, such as meat flavor, dietary quality, and disease resistance, alongside behavioral traits unique to each population, ensuring these qualities are retained at optimal levels [Calik *et al.* 2023]. The White Koluda®, on the other hand, is a breed with a strong selection for economical traits like meatiness and plumage. Its rearing system includes the mating of specific genetic lines only [Gumułka and Połtowicz 2012].

Our results are consistent with other research on Polish geese breeds using microsatellite markers, which showed that the geese breed in Poland is genetically very uniform [Warzecha *et al.* 2019]. Moreover, according to the studies conducted by [Warzecha *et al*. 2019] the Landes breed proved to be less polymorphic among all 13 breeds studied. Only the whole genome scale technique, genotyping-by-sequencing (GBS), allowed the phylogenetic division of breeds [Grzegorczyk *et al*. 2021].

## Conclusion

According to *ACSL1* and *ME1* gene expression and sequencing results, the Landes breed diverges from other breeds studied. In the Kielecka and White Koluda® we identified two SNPs in the sequence for *ACSL1* gene. Both SNPs are located in the 3'UTR and therefore are likely to influence translation and protein synthesis. We assume these two genes can potentially find the genetic background for differences in fatty acids cumulation capacity and biosynthesis of polyunsaturated fatty acids in the White Koluda®, Kielecka and Landes goose livers. However, further research is required to determine whether the newly identified polymorphisms impact the levels and composition of polyunsaturated fatty acids in goose liver.

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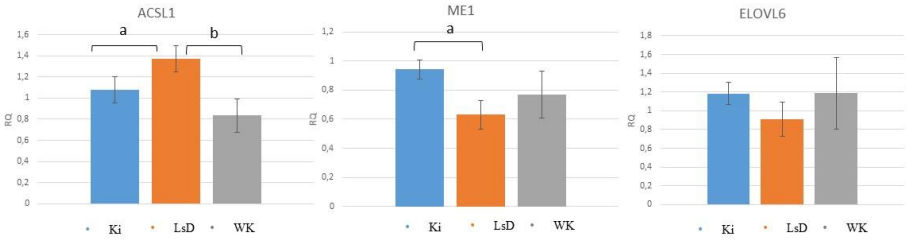
## Supplemental materials

**Table S1**. Taqman probes and primers sequences for ACSL1, ME1 and ELOVL6 genes expression study

| Gene name | F / R | Real-time PCR primers | Taqman probes |
|---|---|---|---|
| ACSL1 | F | TGATGGGTTTTGGTGCTTTTG | AGCTCTCACAACCTAC |
| | R | CTTTCGGTCTTGTCGCATACC | |
| ME1 | F | GGCATGGCTTTTACCTTGGAA | AGAGGCAACAATTGAAT |
| | R | CAAGGTGGCAGTAGTCCATGAA | |
| ELOVL6 | F | GGGCTGCTGGCTTCAGAGT | CACGCAAGTTTGCC |
| | R | TCTGCGACAAGGTGATGAACA | |
| PRL4 | F | TCGCCCTGATGTCGTGAA | TTCACACCAATTTGC |
| | R | AGGGCTGCCTGTTGTTCTTG | |




Supplemental Fig. 1. Mean relative quantification (RQ) of ACSL1, ME1, and ELOVL6 genes expression in the livers of White Koluda®, Kielecka and Landes; a, b - the same letter means the significant differences of mean RQ comparison between breeds, at $p<0,01$ Bonferroni correction; the error bars regard to SD.

**Table S3**. Thermal condition used for PCR of all genes studied

| PCR | | | |
|---|---|---|---|
| Reaction step | temp. | time | cycles |
| Denaturation | 95ºC | 15 min | |
| Annealing | 94ºC | 45 sec | 35 |
| | 60ºC | 50sec | |

**Table S2**. PCR and sequencing primers; reference sequence No.: ELOVL6 ENSABRG00000018014, ACSL1 ENSABRG00000020240, ME1 ENSABRG00000019412.1

| Primer Name / Forward or Reverse | Primer sequence |
|---|---|
| ELOVL6_5_1F | CCACGTGCAGTCAAACCTC |
| ELOVL6_5_1R | AACTCTGGTGCCGTAACTCA |
| ELOVL6_5_2F | TTACTCAGTGTGGGAAGGAATC |
| ELOVL6_5_2R | TGGTTCCTCATCTGTCCCAG |
| ELOVL6_5_3F | GACCGTGTTGTGTTTGTGGA |
| ELOVL6_5_3R | TCCATTTGTAGTTTGCGTGGT |
| ELOVL6_5_4F | TCCTTGGCTTGCTGGATCAT |
| ELOVL6_5_4R | GCATGTCCTAGCTGAATGAGA |
| ELOVL6_5_5F | TGTTCTGTCACACTGGCTCA |
| ELOVL6_5_5R | GGTGAATCTCTCCCAGGCTG |
| ELOVL6_5_6F | TCTGCTTCTGAAGGCGAGAA |
| ELOVL6_5_6R | TGAAACGCCAGGAGACAGAT |
| ELOVL6_5_7F | AACCTGACGTGGAAAGTTGC |
| ELOVL6_5_7R | GATCTTGGCAAAAGCAAGGC |
| ELOVL6_5_8F | TGTAAGTATTGTGTCTGCCATGA |
| ELOVL6_5_8R | GGCACGTACATAGAACTGGC |
| ELOVL6_5_9F | CCTGTTGTGCACTGAGGTTC |
| ELOVL6_5_9R | CCCTGTGCTGACGTGAATTG |
| ELOVL6_5_10F | CAAGCCTAAACCACACACTCA |
| ELOVL6_5_10R | ACGTACTTTCAGGACCCATGT |
| ELOVL6_5_11F | AGAGCCTTGCCATGGAGATT |
| ELOVL6_5_11R | ACAGCAGAGCGAACGAAAAG |
| ELOVL6_5_12F | ACAGTGCTATGTAGGGTGAATT |
| ELOVL6_5_12R | TTTCTCCTCTTCCCCACCTG |
| ELOVL6_5_13F | TGAAATCTTCCTGCTCCGGA |
| ELOVL6_5_13R | TGTATCAAGATGGCAGAGTAACC |
| ELOVL6_5_14F | TTGGTTGGCTTGATTCTCCG |
| ELOVL6_5_14R | TGAAGGGCAGTGGTACTCAA |
| ELOVL6_5_15F | AATGCAGGGATGTGTGGGTA |
| ELOVL6_5_15R | TCAACCGTTCCCACTCATCA |
| ELOVL6_5_16F | ACAGTGTCCATTGATCCGGA |
| ELOVL6_5_16R | CGTGTGTGAGCAGAAGTTGT |
| ELOVL6_5_17F | TGTTCAGTTCAGTGTTGGGG |
| ELOVL6_5_18F | AGGGACATCATGGGACCAAG |
| ELOVL6_5_18R | CAACTCTTTGTGACAGGGGC |
| ELOVL6_5_19F | CCTGTGGTTGGCTCAATTATGT |
| ELOVL6_5_19R | ACTCTCTAAACCTTCAAGTCCCT |
| ELOVL6_5_20F | CTGGGCAGGGCAGTAAGTAA |
| ELOVL6_5_20R | CCGCTCGTAATGAAAAGTCGT |
| ELOVL6_5_21F | GTTGAGAAGCAGACAAGACCA |
| ELOVL6_5_21R | AGATCTTCTGGCTGCCGTT |
| ELOVL6_5_22F | TAGATGCTGTCCTTGTGCCA |
| ELOVL6_5_22R | TCTTCATCTGGCTGCAGTGA |
| ELOVL6_5_23F | CCTTTCCTTGTTTCGCACCA |

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|---|---|
| ELOVL6_5_23R | CCCTGTTTGCTTCTTGTGCT |
| ELOVL6_5_24F | GCTGGCACCATGTTTCATGA |
| ELOVL6_5_24R | GCTGAAGAATGCGGTGCTTT |
| ELOVL6_5_25F | TAGCAGCAGAAGGGGAACTC |
| ELOVL6_5_25R | CCTTTCGCTCAGGTTTGTGT |
| ELOVL6_5_26F | TCTGCAGAGACAAGGCCG |
| ELOVL6_5_26R | TGTGAGGTATTGAAATGAACTGC |
| ELOVL6_5_27F | TCTGATCAGTCTCTCTAGTTGCA |
| ELOVL6_5_27R | ACAACTGCAGAGCTCTCCTT |
| ELOVL6_5_28F | GCCACCGTGCCATGAAATTA |
| ELOVL6_5_28R | GTTTTCCCAGGAGCTGCATT |
| ELOVL6_5_29F | CCAATCTTCCTCCACCCAGT |
| ELOVL6_5_29R | CTTTGCTTGCTCCTGTCCAC |
| ELOVL6_5_30F | CACTTCTCCCCATGCACAAC |
| ELOVL6_5_30R | CAAAAGACATCCAGCTGACGT |
| ELOVL6_5_31F | AGGGACGTATGATATGGGCA |
| ELOVL6_5_31R | CAGTTTTCAGCACAGTCGGA |
| ELOVL6_5_32F | TGTAGTCTTCCAGCCACCAG |
| ELOVL6_5_32R | ACCAACTGAGGGGCTTTACG |
| ELOVL6_5_33F | CTGCCTCTTTCAGTCCTCCT |
| ELOVL6_5_33R | GGTGGTGAATTCCTGGCAAA |
| ELOVL6_5_34F | TGGCTCAAGGGTATTTGGCT |
| ELOVL6_5_34R | ACATCACACTGGAATGGGGA |
| ELOVL6_5_35F | ACAGTAACGTGCTTCCTCCC |
| ELOVL6_5_35R | GATGGTTGGAACTGAAACTGC |
| ELOVL6_5_36F | GCTAGAACTGCACGTCAACA |
| ELOVL6_5_36R | ACCACCTCAGCTATTGACCT |
| ELOVL6_5_37F | GGGGATACTCTGGTTTGGCA |
| ELOVL6_5_37R | TTGCTTTTGTCGTTTTGCCG |
| ACSL_EXON2F | GGGACAACTGCACTGAACTAC |
| ACSL_E2R | ACTTCCACAGACTGCATTGC |
| ACSL_E4_1.0 F | TGAAAATGGCCTGCTGACAC |
| ACSL_E4_1.0R | TCAGACCTGCCAAATCCCTT |
| ACSL_E4_2.0F | ATTTAAGACTTCATCGGCCAAAA |
| ACSL_E4_2.0R | TGTCAGCATAGCCTAGCCTC |
| ACSL_E4_3.0F | TAGAAGCCAGAACTCCACCC |
| ACSL_E4_3.0R | ACGAATCTTAGTGCAAACCTTGT |
| ACSL_E4_4.0F | AGGTATTTGTTCAGGGATGAGAA |
| ACSL_E4_4.0R | TGGTTTCCTTCATTGGTTTTGAA |
| ACSL_E5_1.0F | TGGATTGAGGAAGGTGTCACA |
| ACSL_E5_1.0R | ACTCAGATACCTTGCGCAAA |
| ACSL_E5_2.0F | AGGGATTTGGCAGGTCTGAA |
| ACSL_E5_2.0R | AGTGGCTAGTACAGGTGCAA |
| ACSL_E5_3.0F | AGTGGTGCCAGTCCTGTAAG |
| ACSL_E5_3.0R | GGCTTATTTCTGCTGATTTCTGA |
| ACSL_E5_4.0F | TCAGATATGCCGACAGAACTG |
| ACSL_E5_4.0R | TGGTTTCCTTCATTGGTTTTGAA |
| ACSL_ E6F | AGAGGAGGCTGAAACCCATG |
| ACSL_ E6R | GTTCAGTGCAGTTGTCCCAG |
| ME1_F | CGGGGTAGGAAAACACAGGT |
| ME1_R | TCCAAGACAGTCCACAGCTT |