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MYOSTATIN GENE ANALYSIS IN THE FIRST GENERATION OF THE BELGIAN BLUE CATTLE IN INDONESIA

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ABSTRAK

Telah dilakukan suatu penelitian untuk mengidentifikasi variasi gen myostatin dan mengevaluasi keberadaan marker genetik untuk sifat *double muscling* (delesi 11 basa pada ekson 3 gen myostatin) pada generasi pertama (F1) hasil persilangan dengan sapi Belgian Blue pertama di Indonesia. Sebanyak 8 sampel DNA sapi yang dipelihara di PT. Karya Anugerah Rumpin (KAR) digunakan dalam penelitian ini. Analisa *Single Strand Conformation Polymorphism* (SSCP) dan sekuensing digunakan untuk mengidentifikasi keragaman dari gen myostatin. Terdapat 3 tipe alel gen myostatin berdasarkan hasil analisa SSCP. Pejantan Belgian Blue memiliki tipe alel A. Sapi Simmental, Wagyu, SO x BX, Charolais, dan sapi PO memiliki tipe alel B. Sementara itu anak sapi hasil perkawinan Belgian Blue x FH dan Belgian Blue x SO memiliki tipe alel C (heterozigot). Berdasarkan hasil analisa sekuen gen myostatin, ditemukan adanya delesi 11 basa pada ekson 3 gen myostatin pejantan Belgian Blue. Hasil analisa sekuensing juga memberikan informasi bahwa generasi F1 (Belgian Blue x FH dan Belgian Blue x SO) memiliki gen myostatin yang berada dalam kondisi heterozigot. Hasil penelitian ini memberikan bukti ilmiah bahwa delesi 11 basa pada ekson 3 gen myostatin yang merupakan marker untuk sifat *double muscling* pada sapi Belgian Blue juga ada (diwariskan) pada anak-anak sapi generasi F1 (jantan dan betina).

Kata kunci: myostatin, Belgian Blue, generasi F1, Indonesia

ABSTRACT

A study was conducted to identify the variations of the myostatin and also to evaluate the existence of genetic marker for “double muscling” (11-bp deletion in the third exon of the myostatin gene) in the first generation of Belgian Blue cattle in Indonesia using the Single Strand Conformation Polymorphism (SSCP) and the sequencing analysis. A total of 8 DNA samples belonged to Karya Anugerah Rumpin (KAR) Farm were used in the Single Strand Conformation Polymorphism (SSCP) and the sequencing analysis. There were 3 allele types of myostatin gene based on the SSCP analysis. The Belgian Blue sire has type A allele. The Simmental, Wagyu, SO x BX, Charolais, and the PO cattle have the type B allele, while the Belgian Blue x FH and the Belgian Blue x SO have the type C allele (heterozygous). There are 11-bp deletion in the third exon myostatin gene for the Belgian Blue sire based on the sequencing analysis. The myostatin gene in the Belgian Blue F1 generation individual was heterozygous. This study provides scientific evidence that the 11-bp deletion in the third exon of myostatin gene in the Belgian Blue sire was inherited to its F1 generation (male and female).

Keywords: Myostatin, Belgian Blue, F1 generation, Indonesia

INTRODUCTION

One of the problems faced by the Indonesian beef cattle industry was the difficulty of increasing population and productivity. In order to increase the productivity, the crossing program between local cattle breed and European cattle breed is an option that can be applied in Indonesia. Since 2012, Research Center for Biotechnology-Indonesian Insitute of Sciences has worked together with *Karya Anugerah Rumpin* (KAR) Farm to initiate beef cattle breeding program based on an industrial approach. In 2013, a number of semen of the Belgian Blue cattle were reported introduced into Indonesia and used in the crossing program (Agung and Said, 2014).

The Belgian Blue cattle are original cattle breed from Belgium. From 1850 to 1890, they were developed into a dual purpose cattle. The Belgian Blue cattle have unique phenotype known as the "double muscling". In 1992, it was reported that double muscling in the Belgian Blue cattle can increase carcass percentage (Purchas *et al.*, 1992). Later, McPherron and Lee (1997) reported that deletion of 11 bases in the third exon of myostatin gene caused the double muscling phenomenon and made the myostatin gene as a major gene candidate for animal growth. This phenomenon makes the Belgian Blue highly preferred in the crossing program and successfully raised meat production in many countries (Domingo *et al.*, 2014; Keane, 2003; Keane and Drennan, 2008).

The deletion of 11 bases in the third exon of myostatin gene in the Belgian Blue cattle semen that introduced into Indonesia has been confirmed (Agung and Said, 2014). Recently, the first F1 generation of the Belgian Blue in Indonesia can be found in KAR Farm, thus allowing analysis of the inheritance of the genetic marker for the "double muscling" phenonemon. The objectives of this study was to identify the variations of the myostatin gene and also to evaluate evaluate the existence of genetic marker for "double muscling" (11-bp deletion in the third exon of the myostatin gene) in the first F1 generation of the Belgian Blue cattle in Indonesia.

MATERIALS AND METHODS

Samples and DNA Collection

The samples from the Belgian Blue sire (Figure 1.A) were collected as semen samples. A total of 125 μL Belgian Blue semen samples was used for DNA extraction using the Qiamp DNA Mini Kit (Qiagen, Germany). The samples of the Belgian Blue F1 generation (Figure 1.B and 1.C) were collected as blood samples. Blood samples (3-5 ml) were taken from the cattle coccigea vein using Venoject and collected in Vaccutainer tubes containing anticoagulant. The blood samples were used for obtaining DNA samples through DNA extraction process using DNeasy® Blood and Tissue Kit (Qiagen, Germany) following the producer's method. For comparative analysis, DNA samples from Simmental, Wagyu, Charolais, Ongole Grade (known as *Peranakan Ongole* [PO]), and Sumba Ongole (SO) x Brahman Cross were used in this study. All individual cattle samples belonged to Karya Anugerah Rumpin (KAR) Farm, West Java. A total of 8 DNA samples were categorized based on breed (Table 1).

Primers and DNA Amplification

A pair of primer was designed from the available cattle sequence (Acc. No: AF320998) to amplify the third exon of the myostatin gene. The sequence of the primer is as follows: forward 5'-ggaagaatcaagcctagtgt-3' and reverse 5'-gcttgtgcttaagtgactgt-3'. Using this primer, the expected PCR product size was 660 base pairs (bp) approximately. The PCR reagents composition were as follows: KAPA2G Robust HotStart Ready Mix PCR Kit (Kapa Biosystems, South Africa) (18 μL), forward and reverse primers (200 ng/μL), nuclease free water, and DNA samples (5-30 ng/μL). The PCR program is set as follows : denaturation at 94°C for 5 min; followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s, extension at 72°C for 30 s; with a final extension at 72°C for 5 min on Mastercycler® gradient (Eppendorf, Germany).

Polymorphism and Data Analysis

The Single Strand Conformation Polymorphism (SSCP) and the sequencing analysis were used to identify the variations of myostatin gene in all samples. The SSCP analysis was conducted in the Bogor Agricultural University Laboratory, Indonesia and the sequencing analysis was conducted in the 1st BASE Laboratory, Malaysia. Results of the DNA sequencing was analyzed using MEGA ver. 6.0 (Tamura *et al.*, 2013).

Figure 1.The Belgian Blue cattle used in the study. The Belgian Blue sire (A); The F1 generation [The Belgian Blue x FH] (B); and the F1 generation [The Belgian Blue x SO] (C). Picture A was taken from individual semen catalog; Picture B and C was private collection from individual cattle in KAR Farm, West Java.

Table 1. Samples Used in the Study

Breed	Sex	Sample Material	n
Belgian Blue	Male	Semen	1
Belgian Blue x FH	Male	Blood	1
Belgian Blue x SO	Female	Blood	1
Simmental	Male	Blood	1
Wagyu	Male	Blood	1
Sumba Ongole (SO) x Brahman Cross (BX)	Male	Blood	1
Charolais	Male	Blood	1
Ongole Grade (PO)	Male	Blood	1
Total			8

RESULTS AND DISCUSSION

Amplification of Myostatin Gene

The myostatin gene was successfully amplified in the PCR process using a pair of primer for the third exon of the myostatin gene. The PCR products were visualized with 1% agarose gel. The results showed that amplification fragment has a good specificity, which could directly proceed to SSCP and sequencing analysis. Visualization of PCR products is shown in Figure 2.

Single Strand Conformation Polymorphism (SSCP)

The SSCP analysis was conducted to find variation in the third exon myostatin gene based on its single strand DNA conformation. SSCP analysis was used in this study because it was simple and easy to detect the presence of diversity (Bastos *et al.*, 2001; Orita *et al.*, 1989), reliable, efficient and highly sensitive in detecting the presence of mutations in DNA fragments (Barroso *et al.*, 1999; Nataraj *et al.*, 1999; Prizenberg *et al.*, 2005). A remarkable advantage of SSCP analysis is that it can be used to detect mutations at various positions in a fragment (Orita *et al.*, 1989). The assumption of the SSCP analysis, i.e. the changes or differences (even only one nucleotide) in the DNA fragments will affect the shape (conformation) of the single strand of DNA (Bastos *et al.*, 2001). Visualization of the SSCP analysis result was shown in Figure 3, Figure 4, and Figure 5.

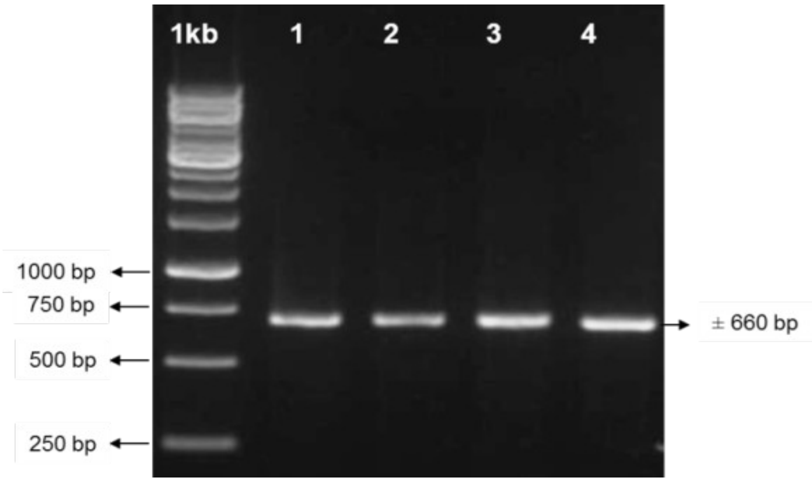

Figure 2. Visualisation of the PCR Products (1kb: DNA ladder; 1-4: samples).

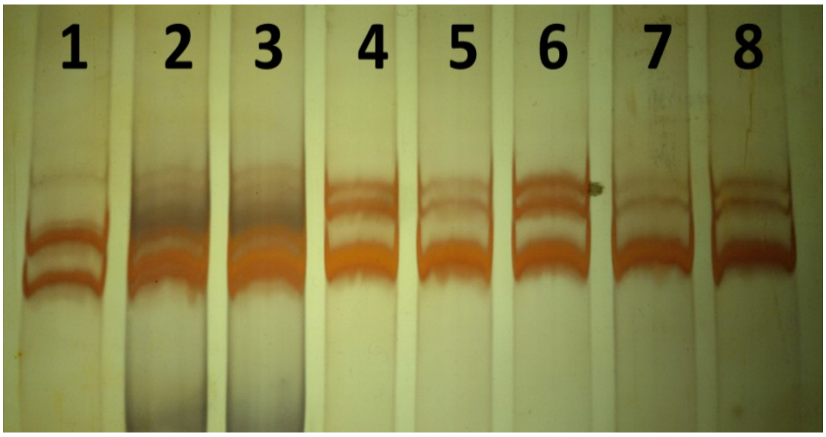

Figure 3. Visualisation of the SSCP Analysis Result (1: Belgian Blue sire; 2: Belgian Blue *x* FH; 3: Belgian Blue *x* Sumba Ongole (SO); 4: Simmental; 5: Wagyu; 6: Sumba Ongole (SO) *X* Brahman cross; 7: Charolais; 8: Ongole Grade (PO)).

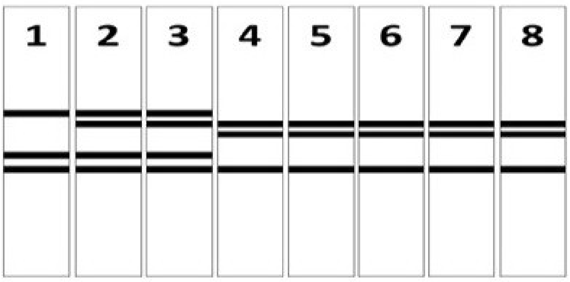

Figure 4. Representation of the SSCP Analysis Result (1: Belgian Blue sire; 2: Belgian Blue *x* FH; 3: Belgian Blue *x* Sumba Ongole (SO); 4: Simmental; 5: Wagyu; 6: Sumba Ongole (SO) *x* Brahman cross; 7: Charolais; 8: Ongole Grade (PO)).

Based on the results of the SSCP analysis (Figure 3. and Figure 4.), there were 3 allele types of myostatin gene being observed (type A, B, and C). The allele type was identified by the number and mobility shift of the single strand DNA fragments (Figure 5.). Three bands were observed in the type A and B alleles with different mobility on gel electrophoresis (at position 1, 4, and 5 for the type A allele and at position 2, 3, and 5 for the type B allele). Meanwhile, four bands were observed in the type C allele with different mobility (at position 1, 2, 4, and 5). The Belgian Blue sire has type A allele, the Simmental, Wagyu, SO x BX, Charolais, and the PO cattle have the type B allele, while the Belgian Blue x

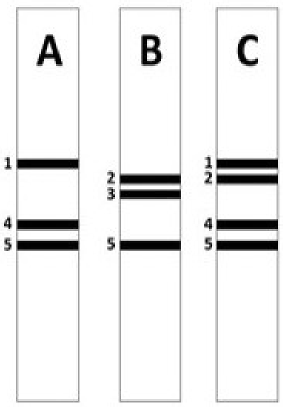

Figure 5. The Number of Band being Observed and Mobility Shift of the Single-stranded DNA Fragments due to its Conformation (A, B, and C; the allele types; 1-5: difference mobility position of the single strands on gel electrophoresis).

FH and the Belgian Blue x SO have the type C allele (heterozygous).

In SSCP analysis, a mutated sequence is detected as a change of mobility in polyacrylamide gel electrophoresis caused by its altered folded structure (Hayashi, 1991). A particular single-stranded DNA could take at least two different molecular shapes, depending on the condition of electrophoresis (Orita *et al*., 1989). In addition, Barroso *et al.* (1998) reported that usually the heterozygous individual will have four bands of the single strand DNA. The DNA polymorphisms at a variety of position in a fragment could cause a difference in its conformation and result in change in mobility of the single strand on gel electrophoresis (Orita *et al*., 1989). This study result demonstrates the possibility of inheritance of the myostatin gene from the Belgian Blue sire in the F1 generation individual. In order to confirm the SSCP results, the sequencing analysis was also conducted on the myostatin gene of the Belgian Blue sire and its F1 generation individual.

DNA Sequencing

DNA sequencing was performed on all PCR products. The PCR products from each cattle will be used as a template for sequencing reactions. Figure 6. shows the sequence of myostatin gene of the Belgian Blue sire compared to another breed including its F1 generation (Belgian Blue cross) and control sequence from GenBank (Acc. No: AF320998). Based on the sequencing result, there are 11-bp deletion in the third exon myostatin gene for the Belgian Blue sire. This mutation was not found in other breeds except the F1 generation of the Belgian Blue (Figure 6.). This result confirms the report from McPherron and Lee (1997) who found that the double muscling in the Belgian Blue cattle was caused by 11-bp deletion in the third exon of the myostatin gene.

Based on sequence analysis results, the F1 generation of the Belgian Blue does indeed hold the heterozygous myostatin gene. This provides scientific evidence that the 11-bp deletion in the third exon of myostatin gene in the Belgian Blue sire was inherited to its F1 generation (male and female). The myostatin gene sequence in the F1 generation of the Belgian Blue has two variants shown by the overlapping in the chromatogram peaks. This caused two possibility sequences of the myostatin gene in the F1 generation of the Belgian Blue. The possibility sequence of the myostatin gene in the F1 generation of the Belgian Blue is shown in Figure 7.

The sequencing analysis results were similar to the SSCP analysis results. This result confirms that the F1 generation of the Belgian Blue has the heterozygous myostatin gene. The F1 generation of the Belgian Blue not only has the normal DNA fragment but also has the double muscling marker DNA fragment (deletion of 11 bases in the third exon myostatin gene).

Future Crossing Program

Double muscling in the Belgian Blue cattle is one of the characteristics that serves as a major point in increasing Indonesian local cattle productivity. One of the advantages from the Belgian Blue cattle is the age of puberty that is reached within 48-49 weeks. This is much better compared to the Hereford, Angus, Brahman, Boran and Tuli sires (Freetly *et al.*, 2011). Regarding the quality of meat, Purchas *et al.* (1992) reported that the dressing-out percentage, total meat yield, and percentage of tenderloin for the Belgian Blue *X* Friesians are higher than Friesian. Due to the mutation in myostatin gene that caused double muscling phenomena in Belgian Blue cattle, using myostatin gene is fully potential to increase growth and quality of carcass in animal and also medical therapy (Dunner *et al.*, 2003).

The crossing program between the Belgian Blue sire and the Indonesian local cattle (e.g. the

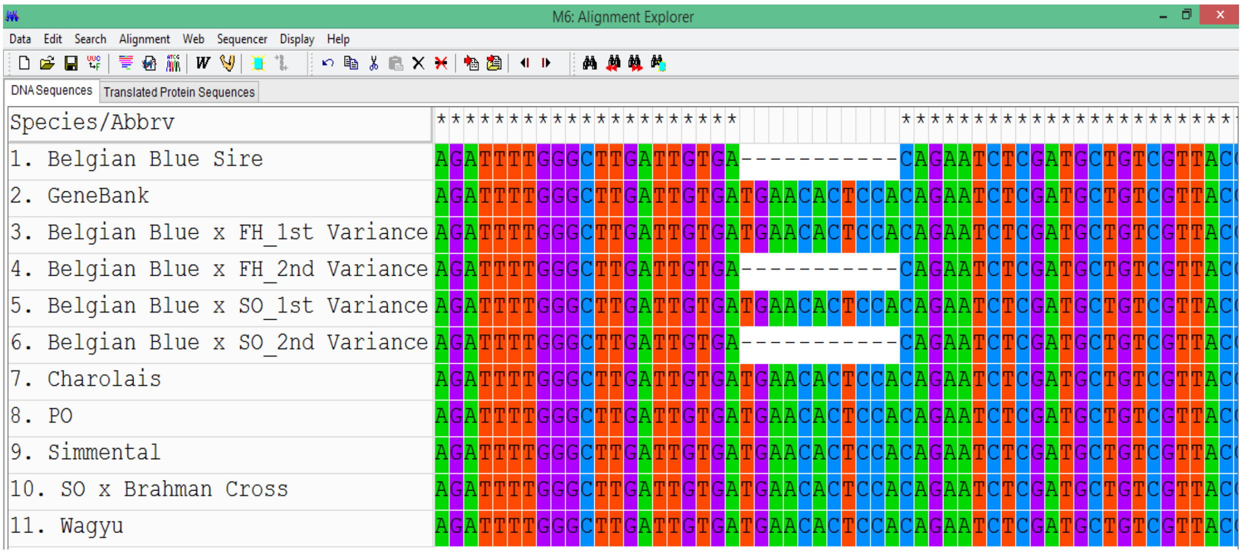

Figure 6. The Sequence of Myostatin Gene of the Belgian Blue Sire Compared to Another Breed Including its F1 Generation.

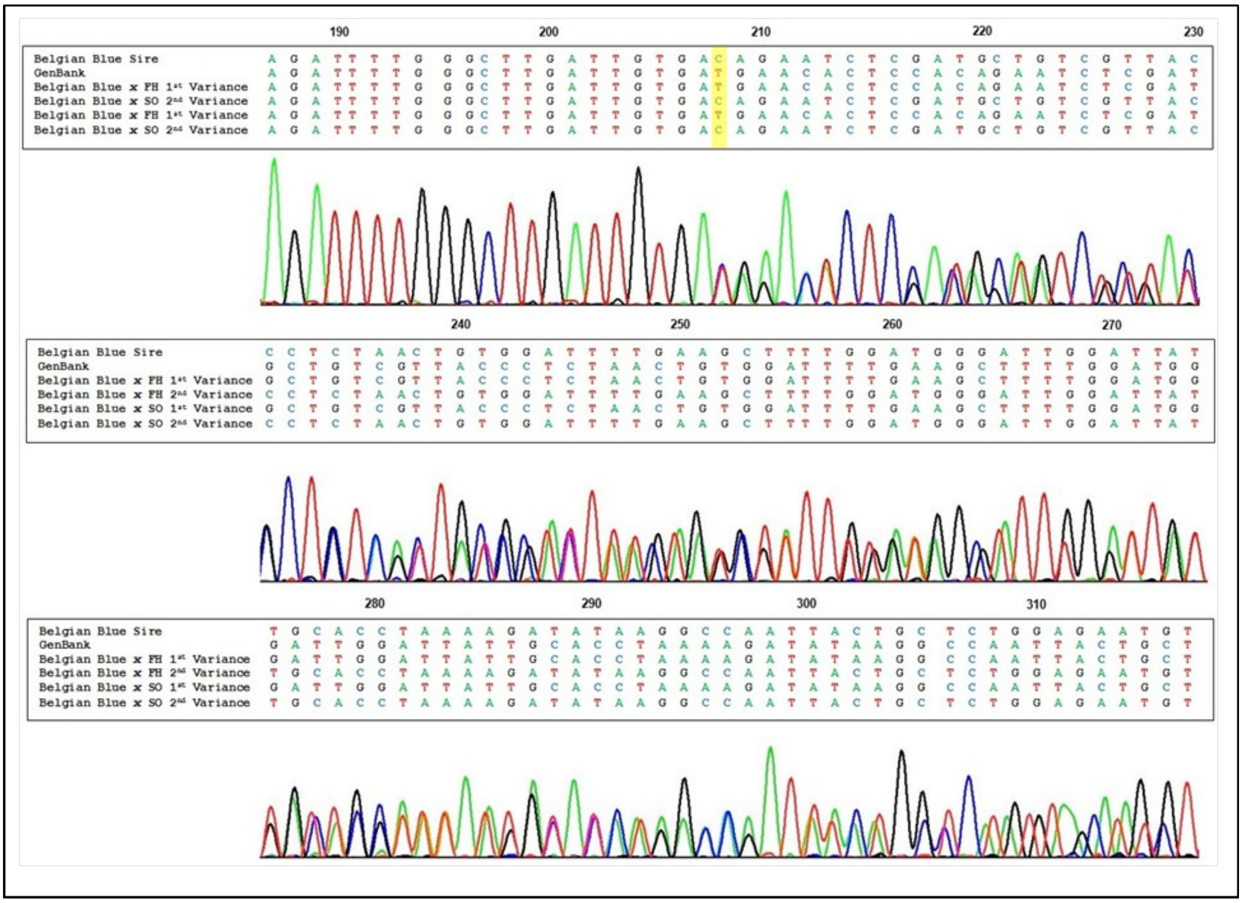

Figure 7. The Possibility Sequence of the Myostatin Gene in the F1 Generation of the Belgian Blue. Highlighted position is the initial position of the Variance.

SO cattle) was conducted to gain heterosis effect in the F1 generation. Domingo *et al.* (2014) reported a very good offspring resulting from the Belgian Blue and the FH cattle crossing program in Spain. The carcass weight was 218 kg approximately and the best conformation was found in the carcasses of Belgian Blue-White (BBW) crosses. Carcasses of BBW crosses were significantly thicker and more compact than Rubia Gallega and Limousine crosses. The Belgian Blue cattle has a shorter gestation length compared to the British, Brahman, Boran, and Tuli cattle. The birth weight is 43.9 kg for the Belgian Blue male cattle and 40.8 kg for the Belgian Blue female cattle. The 200 day weight is 237 kg and the average daily gain is 0.97 kg/day (Casas *et al.*, 2011).

The results in this study confirm that the F1 generation of the Belgian Blue does indeed hold the heterozygous myostatin gene. This information can be useful to investigate the economic traits of the F1 generation of the Belgian Blue and also to assess their individual performance. Based on the evaluation result of this research and the many advantages of the Belgian Blue characteristics, introducing the Belgian Blue cattle into Indonesia will give a chance to improve the Indonesian beef cattle productivity.

CONCLUSION

The results of the SSCP analysis showed that the Belgian Blue sire has type A allele, the Simmental, Wagyu, SO x BX, Charolais, and the PO cattle have the type B allele, while the Belgian Blue x FH and the Belgian Blue x SO have the type C allele (heterozygous). Based on the sequencing result, there are 11-bp deletion in the third exon myostatin gene for the Belgian Blue sire. This study provides scientific evidence that the 11-bp deletion in the third exon of myostatin gene in the Belgian Blue sire was inherited to its F1 generation.

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