Identifying \textit{FecB} genotypes in the muscle from sheep breeds indigenous to Xilingol, and establishment of a \textit{TaqMan} real-time PCR technique to distinguish \textit{FecB} alleles

Liang Guo | Chun-Dong Li | Guo-Qiang Liu | Jian-Xing Luo | Wei-Liang Xu | Yuan-Sheng Guo

\section*{1 | INTRODUCTION}

The Booroola locus (\textit{FecB}) carries a dominant autosomal mutation that is responsible for the hyperprolific characteristics of Booroola ewes, which was first discovered in the Australian Merino breed (Davis, 2004). The high fecundity of Booroola ewes results from the \textit{FecB} mutation in the \textit{bone morphogenetic protein receptor 1B} (\textit{BMPR-1B}) gene, which encodes a transforming growth factor \( \beta \) gene (\textit{TGF\( \beta \)})(Mulsant et al., 2001; Souza et al., 2001). The \textit{FecB} mutation leads to higher ovulation rate by affecting follicular fluid and ovarian vein serum (Guo et al., 2018). The point mutation (A turn to G) at base 746 of the coding region of \textit{BMPR-1B}, changing a glutamine to an arginine, is associated with the hyperprolific profile of Booroola ewes (Souza et al., 2001; Wilson et al., 2001). Thus, the \textit{FecB} mutation has become one of the candidates for breeding sheep for high prolificacy using marker-assisted selection (Chen et al., 2015; Hua & Yang, 2009).

The Xilingol grassland in China is a natural grazing area, it is known for its muscle and for its high-quality pollution-free and free-range sheep. The average annual output in Xilingol is more than 10 million sheep. The indigenous sheep breeds from Xilingol are Sunit sheep, Ujimuqin sheep, and Chahar sheep. Despite the thriving sheep farming in the Xilingol region, SNPs in the \textit{FecB} locus remain unknown in the above three breeds. The hyperprolific effect of the \textit{FecB} mutation can serve as a genetic marker in marker-assisted selection strategies to increase litter size of sheep (Chen et al., 2015; Wang et al., 2018). SNPs in the \textit{FecB} locus are usually identified using PCR-RFLP (Chu et al., 2007; Mahdavi et al., 2014), PCR-SSCP (Chu et al., 2011; Mulsant et al., 2001), pyrosequencing (Souza et al., 2001), ARMS PCR (Ahlawat et al., 2014), the KASPar method (Wang et al., 2018), and \textit{TaqMan}.

\textbf{Abstract}

The muscle from Xilingol indigenous sheep breeds are famous in China, and the \textit{FecB} genotype in this population remains uncharacterized. In this study, SNPs in the \textit{FecB} locus were investigated by pyrosequencing, and an optimized PCR-RFLP technique was generated to identify SNPs. In addition, an efficient technique for high-throughput identification of SNPs in \textit{FecB} was optimized using \textit{TaqMan} real-time PCR and breed-conservative primers and SNP-specific probes. By genotyping the \textit{FecB} locus in the muscle of Xilingol indigenous sheep breeds using a novel \textit{TaqMan} real-time PCR assay, our study has generated the groundwork for the authentication of Xilingol mutton based on the specific gene and the prolificacy-oriented breeding of Xilingol sheep using marker-assisted selection strategies in the future.

\textbf{KEYWORDS} \textit{FecB, PCR-RFLP, SNPs, TaqMan}
real-time PCR (Woodward, 2014). In general, real-time PCR using TaqMan probes is regarded as the most rapid and high-throughput assay when using probes that are specific for the breed of interest.

The objective of this study was to develop a novel TaqMan real-time PCR protocol that can be used to identify SNPs in the FecB locus in the muscle from Xilingol indigenous sheep breeds using SNP-specific probes targeting the FecB locus. The assay with different TaqMan probes can be further used for the authentication of Xilingol mutton based on the specific gene and genotyping sheep from the Xilingol region for marker-assisted selection.

2 | MATERIALS AND METHODS

2.1 | Muscle DNA extraction

A total of 150 muscle samples belonging to five pure breeds of sheep (Sunit sheep, Ujimuqin sheep, Chahar sheep, Hu sheep, and Small Tail Han sheep) were analyzed. Sheep breeds indigenous to the Xilingol region are Sunit sheep, Ujimuqin sheep, and Chahar sheep, and the muscle samples for these breeds were collected from the conservation farm of Xilingol Animal Improving Station in China. Muscle for Hu sheep and Small Tail Han sheep were obtained from the conservation farms of Linan city and Heze city in China, respectively. Genomic DNA was extracted using the Genomic DNA Kit (Tansgen, Beijing, China), and the concentration and integrity of the DNA samples were assessed using spectrophotometer and agarose gel electrophoresis analyses, and only samples that met the requirements (DNA concentration ≥100 ng/μl and no degradation of DNA) were used. Muscle DNA was extracted using the Genomic DNA Kit (TaKaRa, Dalian, China), and sequenced by Funglyn Biotech Inc., Ontario, ON, Canada).

2.2 | Pyrosequencing and PCR-RFLP

The primers (LP1 and RP1) for pyrosequencing and PCR-RFLP were generated by Ruibiotech Company (Beijing, China) using the sequences described by Wilson et al. (2001) (Table 1). Moreover, optimized primers (LP2 and RP2) were developed to identify PCR-RFLP results easier (Table 1). Conventional PCR reaction mixtures (20 μl) contained 10 μl PCR SuperMix (Tansgen), 1 μl LP (10 μmol/l), 1 μl RP (10 μmol/l), 1 μl template (100 ng/μl), and 7 μl ddH2O. The PCR reactions were performed under the following program: 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at corresponding annealing temperature (Table 1) and 60 s at 72°C, and 10 min at 72°C (ABI2720; Applied Biosystems, Waltham, MA, USA). The amplified PCR products were gel purified using the MiniBEST Agarose Gel DNA Extraction Kit (TaKaRa, Dalian, China), and sequenced by Ruibiotech Company (Beijing, China). The purified DNA fragments were digested using AvaII for 4.5 h at 37°C following the manufacturer’s protocol (New England Biolabs, Ipswich, MA, USA).

2.3 | TaqMan Real-time PCR

TaqMan real-time PCRs were carried out using primers (LP3 and RP3) and probes (Probe-A and Probe-G) (Table 1) designed to identify SNPs in the FecB locus. The primers and probes were synthesized by Ruibiotech (Beijing, China). TaqMan real-time PCR reaction mixtures (20 μl) contained 10 μl Probe qPCR SuperMix (Tansgen), 1 μl LP3 (10 μmol/l), 1 μl RP3 (10 μmol/l), 1 μl Probe-A (10 μmol/l), 1 μl Probe-G (10 μmol/l), 1 μl template (100 ng/μl), and 5 μl ddH2O. The real-time PCR reactions were performed under the following program: 30 s at 94°C, 40 cycles of 5 s at 94°C and 31 s at 60°C (FTC-3000P; Funglyn Biotech Inc., Ontario, ON, Canada).

3 | RESULTS AND DISCUSSION

3.1 | Amplification and sequencing of the FecB locus in the muscle from Mongolian sheep breeds

The FecB locus has two alleles in mutton, where A is the wild-type nucleotide and G is the mutant nucleotide. For the FecB locus, the G variant leads to a change in amino acid from glutamine to arginine (Souza et al., 2001). The presence of the FecB mutation has been investigated in a few prolific breeds, such as Booroola Merino (Mulsant et al., 2001), Indian Bonpala sheep (Roy et al., 2011).

| Primers Probes | Sequence (5′–3′) | Annealing Temp. |
|----------------|------------------|-----------------|
| LP1            | CCAGAGGCAATAGCAAAGCAA | 58°C |
| RP1            | CAAGATGTCTTCATGCCTCATCAAGGTC | |
| LP2            | TTTAACAGGTTCAGAGGACATAGCAAAGCAA | 61°C |
| RP2            | AATACACAGTTATATACTACCCCAAGATGTTTTTCATGCCTCATCAACAGGTC | |
| LP3            | AGCTGTGGAATGTTCTTCA | 60°C |
| RP3            | GATGTTTTTCATGCCTCATCA | |
| Probe-A        | FAM-CACCGTCTGATATATTTCAGCTGTCCTCGG-TAMRA | |
| Probe-G        | HEX-CACCGTCTGATATATTTCAGCTGTCCTCGG-TAMRA | |
Iranian Kalehkoohi sheep (Mahdavi et al., 2014), Chinese Hu sheep (Chu et al., 2011; Guan et al., 2007), Chinese Merino prolific meat strain (Guan et al., 2007), and Chinese Small Tail Han Sheep (Chu et al., 2004, 2007). Nevertheless, the mutations in the $FecB$ gene remain unknown in the muscle from three Xilingol indigenous sheep breeds. We analyzed the nucleotide sequences of the $FecB$ locus by pyrosequencing from 30 animals each of the three Xilingol indigenous sheep breeds. After amplifying the $FecB$ region using conventional PCR, the nucleotide sequences of the $FecB$ locus were sequenced. Sunit sheep, Ujimuqin sheep, and Chahar sheep all had the A variant at base 746 of the coding region of $BMPR-1B$ (Figure 1a). Interestingly, all Hu sheep individuals had the G variant in the $FecB$ locus (Figure 1a). Of the 30 individuals that were sampled from the Small Tail Han sheep, 25 had the G variant, three had the A variant, and two had the A/G heterozygous variant (Figure 1a). Therefore, the breeds indigenous to Xilingol had the wild-type A variant of the $FecB$ locus, while Hu and Small Tail Han sheep had the mutant G nucleotide.

Our results are consistent with previous studies that have investigated the $FecB$ locus in Hu and Small Tail Han sheep (Hua & Yang, 2009). Moreover, the G mutant nucleotide in the $FecB$ locus was directly linked to the high prolificacy in Hu and Small Tail Han sheep breeds (Chu et al., 2004, 2011; Guo et al., 2018; Wang et al., 2018). The National Commission of Animal Genetic Resources of China reported an annual breeding rate of 113% for Sunit sheep, 113% for Ujimuqin sheep, 126.4% for Chahar sheep, 277.4% for Hu sheep, and 267.1% for Small Tail Han sheep (China National Commission of Animal Genetic Resources, 2011; Xu et al., 2016). We had hypothesized that the A nucleotide in the $FecB$ locus in the Xilingol indigenous sheep breeds promoted...
their fecundity to be lower compared to Hu and Small Tail Han sheep. Furthermore, the genetic relationships of Sunit sheep, Ujimuqin sheep, Hu sheep, and Small Tail Han sheep suggest that they evolved from a Mongolian breed of sheep (China National Commission of Animal Genetic Resources, 2011; Xu et al., 2016). We speculated that the grazing regime of Mongolian sheep in the Xilingol prairie in Northern China was selected for its low prolificacy, allowing these breeds to survive the winter, and farmers in the Shandong and Zhejiang provinces in Western China reared sheep in barns and artificially selected sheep for their high prolificacy. Eventually, the Mongolian sheep with low prolificacy in the Xilingol prairie gradually evolved to form Sunit sheep in the East and West Sunit banner, and Ujimuqin sheep breed in East and West Ujimuqin banner, and these with high prolificacy formed Hu sheep breed in Zhejiang province, and Small Tail Han sheep breed in Shandong province.

3.2 Optimization of PCR-RFLP to identify FecB alleles

In order to differentiate between the A and G variants of FecB, we used a published pair of primers (LP1 and RP1) to amplify a 190-bp fragment containing the FecB locus (Davis et al., 2002; Wilson et al., 2001). A restriction enzyme site was introduced into the RP1 primer, which led to the amplification of PCR fragments containing the AvaII restriction site of the FecB locus carrying the G variant (G|GACC). Sequences from four sheep with the A nucleotide, four sheep with the G nucleotide, and four sheep with the A/G nucleotide were amplified and restriction digested using AvaII to verify the introduction of the cut site (Figure 2a). Individuals carrying the A or G nucleotide had a 190-bp or 160-bp band, respectively (Figure 2a). Fragments from the heterozygous individuals were digested into both 190- and 160-bp bands (Figure 2a). Taken together, the new primers were effective in distinguishing SNPs in the FecB locus after restriction enzyme digestion.

PCR-RFLP is regarded as a simple method for identifying SNPs. The above PCR-RFLP approach using the AvaII restriction site has been used previously to genotype prolific sheep (Chu et al., 2007; El-Seedy et al., 2017; Ganai et al., 2012; Gootwine et al., 2008; Kumar et al., 2008; Mahdavi et al., 2014; Xu et al., 2010). Nevertheless, improvements were needed to better screen for prolific sheep. First, conventional DNA ladders cannot discriminate the 190- and 160-bp bands, which can be difficult to distinguish in the agarose gel. Second, the 190- and 160-bp bands can sometimes appear as one band in gels with low concentration of agarose. Thus, we developed optimized primers (LP2 and RP2) for PCR-RFLP to identify the FecB alleles (A, G, or A/G). RP2 was designed to introduce a point mutation that results in a 220-bp fragment containing the AvaII restriction site (G|GACC) if the fragment contains the G nucleotide. DNA from four individuals each carrying the A, G, and A/G variant in the FecB locus were used to test the effectiveness of the LP2 and RP2 primers (Figure 2b). PCR products that were restriction digested using AvaII yielded 220- and 170-bp bands for individuals carrying the A or G nucleotides, respectively (Figure 2b). Individuals that were heterozygous yielded both 220- and 170-bp bands (Figure 2b). These results validate the new primer pair, which allowed for easier differentiation of the two genotypes, as it is easier to separate 220- and 170-bp bands, compared to 190 and 160 bp.

3.3 Development and validation of a TaqMan real-time PCR technique to distinguish the FecB alleles

While PCR-RFLP can be carried out using basic laboratory equipment to relatively high levels of success, distinguishing the relative position of the amplified nucleotide sequence in an agarose gel can be prone to error. Error can be introduced through the difference in the quantity of DNA used for restriction digest, the digestion efficiency of the restriction enzyme, the length of digestion, the concentration of the agarose gel, and the volume of sample loaded during electrophoresis. TaqMan real-time PCR provides a more reliable alternative for genotyping SNPs (Gaedigk et al., 2015; Schleinitz et al., 2011; Woodward, 2014). In particular, real-time PCR results can be observed without electrophoresis, and can be used in a high-throughput manner.

For these reasons, we sought to develop an efficient technique for high-throughput identification of the FecB alleles using TaqMan real-time PCR with primers (LP3 and RP3) and SNP-specific probes (Probe-A and Probe-G) (Table 1). Samples from 10 individuals each carrying the A and G variant and eight sheep carrying the A/G variant were used to validate the probes (Figure 3). The A-specific TaqMan probe was labeled with FAM (fluorophore) and TAMRA (quencher), and the G-specific TaqMan probe was labeled with HEX (fluorophore) and TAMRA (quencher). This facilitated the simultaneous identification of the A and G variants by detecting the fluorophores FAM and HEX. Probe-A successfully amplified the A nucleotide, and Probe-G successfully amplified the G nucleotide using DNA from homozygous individuals (Figure 3a,b). In addition, Probe-A and Probe-G amplified fragments from the A/G heterozygous individuals (Figure 3c). Taken together, the duplex TaqMan real-time PCR was effective and robust for identifying SNPs in the FecB locus.

4 CONCLUSIONS

First, the FecB locus was genotyped in the muscle from three Xilingol indigenous sheep breeds. Sunit sheep, Ujimuqin sheep, and Chahar sheep all carried the wild-type variant of FecB. Then, the well-established PCR-RFLP method was used to detect SNPs in the FecB locus, and an optimized PCR-RFLP technique was developed. Lastly, we established an efficient technique for high-throughput identification of FecB alleles using TaqMan real-time PCR with SNP-specific probes. In conclusion, the FecB alleles of the muscle from sheep breeds indigenous to the Xilingol region were identified and a robust
FIGURE 3 TaqMan real-time PCR to identify the A variant (a), G variant (b), and heterozygous A/G variant (c) of the FecB locus. The homozygous variants were confirmed using 10 sheep with known genotypes. FAM or HEX were simultaneously identified in the amplification plots of eight heterozygous sheep in (c) and high-throughput TaqMan real-time PCR technique was established to authenticate the muscle from Xilingol sheep.

ACKNOWLEDGEMENTS
This work was supported by Xilingol Science and Technology Planning Project (202103), Inner Mongolia College Science Research Project (NJZY22711), and Xilingol Vocational College Key Research Project (ZD-2021-01 and YB-2022-13).

CONFLICT OF INTEREST
All authors declare no conflict of interest.

ETHICAL APPROVAL
This study does not involve any human or animal testing.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES
Ahlawat, S., Sharma, R., Maitra, A., Roy, M., & Tantia, M. S. (2014). Designing, optimization and validation of tetra-primer ARMS PCR protocol for genotyping mutations in caprine Fec genes. Meta Gene, 2, 439–449. https://doi.org/10.1016/j.mgene.2014.05.004
Chen, X., Sun, H., Tian, S., Xiang, H., Zhou, L., Dun, W., & Zhao, X. (2015). Increasing litter size in a sheep breed by marker-assisted selection of BMPR1B A746G mutation. Journal of Genetics, 94, 139–142. https://doi.org/10.1007/s12041-015-0470-8
China National Commission of Animal Genetic Resources. (2011). Animal genetic resources in China: Sheep and goats. China Agriculture Press.
Chu, M., Jia, L., Zhang, Y., Jin, M., Chen, H., Fang, L., Di, R., Cao, G., Feng, T., Tang, Q., Ma, Y., & Li, K. (2011). Polymorphisms of coding region of BMPR-IB gene and their relationship with litter size in sheep. Molecular Biology Reports, 38, 4071–4076. https://doi.org/10.1007/s11033-010-0526-z
Chu, M. X., Li, B. X., Wang, J. Y., Ye, S. C., & Fang, L. (2004). Association between PCR-SSCP of growth differentiation factor 9 gene and high prolificacy in Small Tail Han sheep. Animal Biotechnology, 15, 111–120. https://doi.org/10.1081/LABT-200032582
Chu, M. X., Liu, Z. H., Jiao, C. L., He, Y. Q., Fang, L., Ye, S. C., Chen, G. H., & Wang, J. Y. (2007). Mutations in BMPR-IB and BMP-15 genes are associated with litter size in Small Tailed Han sheep (Ovis aries). Journal of Animal Science, 85, 598–603. https://doi.org/10.2527/jas.2006-324
Davis, G. H. (2004). Fecundity genes in sheep. Animal Reproduction Science, 82–83, 247–253. https://doi.org/10.1016/j.anireprosci.2004.04.001
Davis, G. H., Galloway, S. M., Ross, I. K., Gregan, S. M., Ward, J., Nimbkar, B. V., Ghalsasi, P. M., Nimbkar, C., Gray, G. D., Subandriyo, Inouu, I., Tiesnamurti, B., Martyndti, E., Eythordsdttir, E., Mulsant, P., Lecerf, F., Hanraham, J. P., Bradford, G. E. & Wilson, T. (2002). DNA tests in prolific sheep from eight countries provide new evidence on origin of the Booroola (FecB) mutation. Biology of Reproduction, 66, 1869–1874. https://doi.org/10.1095/biolreprod.66.6.1869
El-Seedy, A. S., Hashem, N. M., El-Azrak, K. M., Nour El-Din, A., Ramadan, T. A., Taha, T. A., & Salem, M. H. (2017). Genetic screening of FecB, FecX(I) and FecXII mutations and their linkage with litter size in Barki and Rahmani sheep breeds. Reproduction in Domestic Animals, 52, 1133–1137. https://doi.org/10.1111/rda.13002
Gaedigk, A., Freeman, N., Hartschorn, J., Riffel, A. K., Irwin, D., Bishop, J. R., Stein, M. A., Newcorn, J. H., Jaime, L. K., Cherney, M., & Leeder, J. S. (2015). SNP genotyping using TaqMan technology: The CYP2D6*17 assay conundrum. Scientific Reports, 5, 9257. https://doi.org/10.1038/srep09257
Ganai, T. A., Misra, S. S., & Shabir, M. (2012). Polymorphism analysis of BMP1R gene by forced RFLP and PCR-SSCP techniques and expression of the mutation in introgressed sheep. Tropical Animal Health and Production, 44, 277–283. https://doi.org/10.1007/s11250-011-0015-y
Goottwine, E., Reicher, S., & Rozov, A. (2008). Prolificacy and lamb survival at birth in Awassi and Assaf sheep carrying the FecB (Booroola) mutation. Animal Reproduction Science, 108, 402–411. https://doi.org/10.1016/j.anireprosci.2007.09.009
Guan, F., Liu, S. R., Shi, G. Q., & Yang, L. G. (2007). Polymorphism of FecB gene in nine sheep breeds or strains and its effects on litter size, lamb growth and development. Animal Reproduction Science, 99, 44–52. https://doi.org/10.1016/j.anireprosci.2006.04.048
Guo, X., Wang, X., Di, R., Liu, Q., Hu, W., He, X., Yu, J., Zhang, X., Zhang, J., Broniowska, K., Chen, W., Wu, C., & Chu, M. (2018). Metabolic effects of FecB gene on follicular fluid and ovarian vein serum in

ORCID
Liang Guo https://orcid.org/0000-0002-1954-1179
sheep (Ovis aries). *International Journal of Molecular Sciences*, 19, 539. https://doi.org/10.3390/ijms19020539

Hua, G. H., & Yang, L. G. (2009). A review of research progress of *FecB* gene in Chinese breeds of sheep. *Animal Reproduction Science*, 116, 1-9. https://doi.org/10.1016/j.anireprosci.2009.01.001

Kumar, S., Mishra, A. K., Kolte, A. P., Arora, A. L., Singh, D., & Singh, V. K. (2008). Effects of the Booroola (*FecB*) genotypes on growth performance, ewe's productivity efficiency and litter size in Garole x Malpura sheep. *Animal Reproduction Science*, 105, 319-331. https://doi.org/10.1016/j.anireprosci.2007.03.012

Mahdavi, M., Nanekarani, S., & Hosseini, S. D. (2014). Mutation in *BMPR-IB* gene is associated with litter size in Iranian Kalehkoohi sheep. *Animal Reproduction Science*, 147, 93-98. https://doi.org/10.1016/j.anireprosci.2014.04.003

Mulsant, P., Lecerf, F., Fabre, S., Schibler, L., Monget, P., Lanneluc, I., Pisselet, C., Riquet, J., Monniaux, D., Callebaut, I., Cribiu, E., Thimonier, J., Teyssier, J., Bodin, L., Cogne, Y., Chitour, N., & Elsen, J. M. (2001). Mutation in bone morphogenetic protein receptor-IB is associated with increased ovulation rate in Booroola Merino ewes. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 5104-5109. https://doi.org/10.1073/pnas.011577598

Roy, J., Polley, S., De, S., Mukherjee, A., Babatyal, S., Pan, S., Brahma, B., Datta, T. K., & Goswami, S. L. (2011). Polymorphism of fecundity genes (*FecB*, *FecX*, and *FecG*) in the Indian Bonapala sheep. *Animal Biotechnology*, 22, 151-162. https://doi.org/10.1080/10495398.2011.589239

Schleinitz, D., Distefano, J. K., & Kovacs, P. (2011). Targeted SNP genotyping using the TaqMan(R) assay. *Methods in Molecular Biology*, 700, 77-87. https://doi.org/10.1007/978-1-61737-954-3_6

Souza, C. J., MacDougall, C., MacDougall, C., Campbell, B. K., McNeilly, A. S., & Baird, D. T. (2001). The Booroola (*FecB*) phenotype is associated with a mutation in the bone morphogenetic receptor type 1B (*BMPR1B*) gene. *Journal of Endocrinology*, 169, R1-R6. https://doi.org/10.1677/joe.0.169r001

Wang, W., La, Y., Zhou, X., Zhang, X., Li, F., & Liu, B. (2018). The genetic polymorphisms of TGFβ superfamily genes are associated with litter size in a Chinese indigenous sheep breed (Hu sheep). *Animal Reproduction Science*, 189, 19-29. https://doi.org/10.1016/j.anireprosci.2017.12.003

Wilson, T., Wu, X. Y., Juengel, J. L., Ross, I. K., Lumsden, J. M., Lord, E. A., Dodds, K. G., Walling, G. A., McEwan, J. C., O’Connell, A. R., McNatty, K. P., & Montgomery, G. W. (2001). Highly prolific Booroola sheep have a mutation in the intracellular kinase domain of bone morphogenetic protein IB receptor (ALK-6) that is expressed in both oocytes and granulosa cells. *Biology of Reproduction*, 64, 1225-1235. https://doi.org/10.1095/biolreprod64.4.1225

Woodward, J. (2014). Bi-allelic SNP genotyping using the TaqMan(R) assay. *Methods in Molecular Biology*, 1145, 67-74. https://doi.org/10.1007/978-1-4939-0446-4_6

Xu, R. G., Li, B. L., Rong, W. H., Diao, Q. Y., & Liu, X. T. (2016). The dominant varieties of sheep for meat in China and its application prospect. China Agricultural Science and Technology Press.

Xu, Y., Li, E., Han, Y., Chen, L., & Xie, Z. (2010). Differential expression of mRNAs encoding BMP/Smad pathway molecules in antral follicles of high- and low-fecundity Hu sheep. *Animal Reproduction Science*, 120, 47-55. https://doi.org/10.1016/j.anireprosci.2010.02.009

How to cite this article: Guo, L., Li, C.-D., Liu, G.-Q., Luo, J.-X., Xu, W.-L., & Guo, Y.-S. (2022). Identifying *FecB* genotypes in the muscle from sheep breeds indigenous to Xilingol, and establishment of a TaqMan real-time PCR technique to distinguish *FecB* alleles. *Food Science & Nutrition*, 10, 2470-2475. https://doi.org/10.1002/fsn3.2853