Study in The Sequence of *Bovine* ASCL2 Gene as a Potential Candidate Gene for Reproductive Traits of Cows

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Abstract

Bovine Achaete-scute like-2 (*b*ASCL2) gene is a maternally expressed gene that important to pre-natal and post-natal development in mammals. This study was aimed to analyse the *b*ASCL2 gene with reference sequences. The *b*ASCL gene sequence that using in this study based on GenBank ID: NM_001040607.1 (*Bos taurus*) and XM_027532629.1 (*Bos indicus* × *Bos taurus*). The BioEdit software was used in this study to detect single nucleotide polymorphism (SNP) in *b*ASCL gene. Hence, the Primer3Plus software (online) was used in this study to obtain the target sequence for PCR-RFLP analysis. Research showed that two missense mutation of SNP c.331W (Ser/Cys) and SNP c.731R (Gly/Glu) were occured in *b*ASCL2 gene. In addition, the primer pairs of Forward: 5'- AAG TGG ATG CAG ACG CGA TG -3' and Reverse: 5'- CTG GAG AAG TCG AGC AGC TC -3' can be used to obtain the target sequence (exon 1) along 580 bp. The SNP c.331W can be detected with *Sfc*I and *Pst*1 restriction enzyme. Meanwhile, SNP c.731R can be detected with *Bp1*I restriction enzyme. However, the PCR-RFLP analysis based on these results is important to confirm the missense mutation in *b*ASCL gene as the potential candidate gene for reproductive trait of cows.

Keywords: *bASCL2 gene, Bioinformatic, GenBank, Missense mutation, SNP*

Introduction

The *bovine* Achaete-scute like-2 (*b*ASCL2) gene has been identified as a critical gene for normal placenta development and successful pregnancies in mammalas (Arnold et al. 2006). Moreover, this gene is a maternally expressed gene that encodes a lineage-specific transcription factor that is essential for neurectoderm and trophectoderm development and is implicated in pre-natal and post-natal development in mammals (Bogutz et al. 2018). The ASCL2 protein is a member of the basic helix-loop-helix (bHLH) family of transcription factors that are involved in chromosomal segregation and nervous system development in mammals (Rebhan et al. 1997)

Recently, study of molecular selection based on ASCL2 gene in cattle is not reported. Previous studies have been worked with PCR-RFLP method for detecting single nucleotide polymorphism (SNP) of ASCL2 gene in pig (Cheng et al. 2007) and human (Miyamoto et al. 1998). This study was aimed to perform bioinformatic analysis in *b*ASCL2 gene based on the reference sequence from GenBank. The results in this study can be used as the early information to obtain the genetic marker of reproductive trait in cattle through ASCL2 gene polymorphism.

Materials and Methods

The DNA sequence of *b*ASCL2 gene was obtained from GenBank (http://www.ncbi.nlm.nih.gov). Two different of *b*ASCL2 gene sequences of *Bos taurus* (GenBank: NM_001040607.1) and *Bos indicus* × *Bos taurus* (GenBank: XM_027532629.1) were used in this study. The aligment analysis was performed in both different sequences with BioEdit software for detection of mutation points. Thus the restriction map analysis was performed in this study to obtain the suitable restriction enzyme to detect mutation points of *b*ASCL2 gene. Moreover, the online Primer3Plus software (https://www.primer3plus.com) was performed to obtain the target sequence of *b*ASCL2 gene.

Conclusion

According to the *b*ASCL2 gene from GenBank sequences, this gene was showed polymorphic and needed to try with DNA sample in the laboratory. Further research to detect the genetic diversity in this gene is important to obtain the genetic marker for reproductive traits of cow

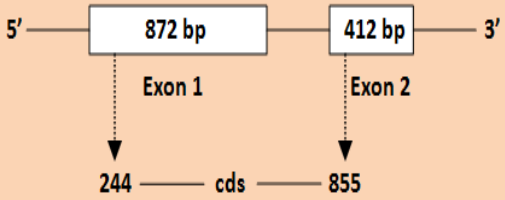

Fig. 1. The structure of *b*ASCL2 gene (GenBank: NM_ 001040607.1)

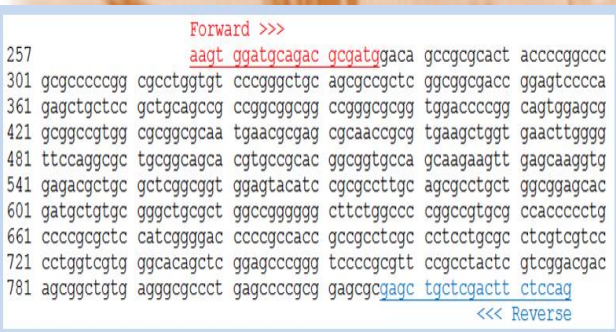

Fig. 2. The primer position (underline) in the exon 1 region (580 bp) of *b*ASCL2 gene (GenBank: NM_001040607.1)

Table 1. The primer design to amplify the target sequence of *b*ASCL2 gene according to Primer3 software

Primer	Tm (°C)	G/C (%)
ASCL2-F: 5'- AAGTGGATGCAGACGCGATG -3'	61.1	50.0
ASCL2-R: 5'- CTGGAGAAGTCGAGCAGCTC -3'	60.2	60.0

Table 2. Detection SNP's in the *b*ASCL2 gene

SNP	Region	*Bos taurus* (NM_001040607.1)	Taurindus (XM_027532629.1)	Aminp acid change
g.137M	Exon 1	A	C	-
c.331W	Exon 1*	A	T	Serine / Cysteine
g.615Y	Exon 1*	T	C	Synonymous
c.731R	Exon 1*	G	A	Glycine / Glutamic acid
g.804Y	Exon 1*	C	T	Synonymous
g.1047R	Exon 2	G	A	-
g.1176indel.T	Exon 2	-	T	-

*Coding region

Table 3. The predicted DNA fragment of *b*ASCL2 gene from PCR-RFLP analysis with selected restriction enzyme

SNP / Enzyme	Restriction site	Length of DNA fragment (bp)		
		AA genotype	BB genotype	AB genotype
c.331W				
*Sfc*I	C*TRYAG	45; 72; 463	117; 463	45; 72; 117; 463
*Pst*I	CTGCA*G	45; 76; 459	121; 459	45; 76; 121; 459
c.771R				
*Bp*1I	GAAGAC(N)$_2$*	580	32; 82; 466	32; 82; 466; 580

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