Application of mathematical expectation (ME) strategy for detecting low frequency mutations: An example for evaluating 14-bp insertion/deletion (indel) within the bovine PRNP gene

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\textbf{ABSTRACT}. The detection method based on the mathematical expectation (ME) strategy is fast and accuracy for low frequency mutation screening in large samples. Previous studies have found that the 14-bp insertion/deletion (indel) variants of the 3' untranslated region (3' UTR) within bovine PRNP gene have been characterized with low frequency (\leq 5\%) in global breeds outside China, which has not been determined in Chinese cattle breeds yet. Therefore, this study aimed to identify the 14-bp indel within PRNP gene in 5 major Chinese indigenous cattle breeds and to evaluate its associations with phenotypic traits. It was the first time to use ME strategy to detect low frequency indel
polymorphisms and found that minor allele frequency was 0.038 (Qinchuan), 0.033 (Xianan), 0.013 (Nanyang), 0.003 (Jiaxian), and zero (Ji’an), respectively. Compared to the traditional detection method by which the sample was screened one by one, the reaction time by using the ME method was decreased 62.5%, 64.9%, 77.6%, 88.9% and 66.4%, respectively. In addition, the 14-bp indel was significantly associated with the growth traits in 2 cattle breeds, with the body length of Qinchuan cattle as well as the body weight and waistline of Xianan cattle. Our results have uncovered that the method based on ME strategy is rapid, reliable, and cost-effective for detecting the low frequency mutation as well as our findings provide a potential valuable theoretical basis for the marker-assisted selection (MAS) in beef cattle.

KEYWORDS. association, bovine, growth traits, insertion/deletion (indel), low frequency, prion protein (PRNP) gene

INTRODUCTION

Chinese indigenous cattle breeds are characterized with many potential advantages (e.g. strong disease resistance, good adaptability), however, they also demonstrate several serious shortcomings, e.g., slow growth, serious varieties degradation, so these obvious disadvantages need to be rapidly and accurately improved using marker-assisted selection (MAS). Molecular markers includes the single nucleotide polymorphism (SNP) and the insertion/deletion (indel). Compared with the SNPs, indel variants have the advantage of convenient detection and remarkable effects as well as they also are characterized with low minor allele frequency (<10%) across bovine genome, such as mutations in the bovine ALX4 gene, lymphotoxin A gene and DGAT1 gene. For the minor allele frequency detection, the traditional detection methods by which the sample was screened one by one are very time-consuming and high-cost, therefore, a quick and accurate method is required for application on detecting the low frequency mutation of potential critical candidate genes, which would improve crucial flaws in beef cattle industry using the MAS strategy.

To date, a method based on the theory of mathematical expectation (ME) has been widely applied to detect low frequency events in epidemiology, population genetics, quantitative genetics, molecular evolution, e.g. the general screening of parasites, tuberculosis, leukemia in large samples. In details, principal of the ME method is described as follows: all detected individuals are randomly divided into several mixed groups, and each mixed groups is genotyped; if a mixed group shows the single specific genotype, then all mixed individuals are regarded that possess the same genotype; while if one mixed group demonstrates different genotypes, then the genotype of each individual is required to be identified again. So, this ME method can quickly and accurately detect the genotypes of the individuals, which would save time and decrease expenses.

Prion protein (PrP) encoded by the PRNP gene plays a critical role in bovine spongiform encephalopathy (BSE) in cattle and buffalo, and scrapie in goat and sheep. Furthermore, numerous polymorphisms of PRNP gene have been shown to influence the susceptibility of these diseases, and they significantly decreased the production, milk traits in several animal breeds. To date, several polymorphisms were reported in the cattle PRNP gene, including a 23-bp indel, a 12-bp indel, a 14-bp indel, and the variable number of octapeptide repeats in the open reading frame. According to the previous reports, the 14-bp indel in 3' UTR have been characterized with low frequency (≤5%) in breeds outside China. For instance, the minor allele frequency of the 14-bp indel was less than 0.01 in cows and artificial insemination sires of the USA. However, the frequency of this indel locus has not been determined in Chinese cattle. Therefore, the 14-bp indel was genotyped in healthy animals of Chinese indigenous cattle breeds.

Qinchuan, Nanyang, Jiaxian and Ji’an cattle are indigenous beef cattle breeds in China, whereas Xianan cattle are Chinese first cross-breeding beef breed. However, the frequency of
the 14-bp indel polymorphism of PRNP as well as whether this indel affects growth traits in these breeds have not been elucidated until now. Therefore, the aim of the present study was to use ME strategy to determine the 14-bp indel polymorphism in Chinese cattle breeds and to analyze the relationship between this indel and growth traits in healthy cattle. The results may provide the basis for MAS of beef cattle.

MATERIAL AND METHODS

All animal experiments were implemented in accordance with the relevant laws and institutional guidelines and approved by the Institutional Animal Care and Use Committee of Northwest A&F University (NWSUAF).

Animals and Data Collection

A total of 1005 Chinese cattle from 5 cattle breeds, Qinchuan (n = 160), Xianan (n = 214), Jiaxian (n = 199), Nanyang (n = 197), and Ji’an (n = 235) were randomly sampled. These five groups are the main Chinese cattle breeds distributed in the provinces of Shaanxi (Qinchuan), Henan (Xianan, Jiaxian, Nanyang), and Jiangxi (Ji’an). Growth traits and physical parameters in unrelated Nanyang cattle were measured at birth and after 6, 12, 18 and 24 months, which included body weight (kg), body height (cm), body length (cm), heart girth (cm), hucklebone width (cm), and the average daily weight gain (kg).24,25

Genomic DNA Isolation and DNA Pool Construction

Genomic DNA was isolated from the leukocyte fraction of the blood or the ear tissue following the procedures described by Lan et al.26 and Pan et al.24 The genomic DNA concentration was quantified, and the working solution of each DNA sample was brought to 50 ng/µL.26 In each breed, a total of 50 DNA samples were randomly selected to construct genomic DNA pools, respectively.26 The genomic DNA pools were used as a template for PCR amplification of the 14-bp indel within PRNP gene.

PCR Amplification and Genotyping of the 14-bp Indel of PRNP

Based on bovine PRNP gene sequence (AC_000170.1), a pair of primers (F: 5'-TGGCT TGCACTTTGTGGTAT-3'; R: 5'-CCCACGTCTCCTTAGTACCTT-3') was designed to amplify DNA pools within region covering the PRNP 3'UTR indel.11,15 The PCR was performed in 25 µL reaction volume containing 1.0 µL genomic DNA (constructed from 10 different individuals), 0.5 µL each primer (forward and reverse primer), 12.5 µL 2× Eco Taq PCR Super mix (+dye) and 10.5 µL ddH₂O. Touch Down PCR reaction was carried out as follows: initial denaturation for 4 min at 95°C, followed by 18 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 68°C (with a decrease of 1°C per cycle), and extension for 1000bp/min at 72°C, another 23 cycles of 30 s at 94°C, 30 s at 50°C, and 45 s at 72°C, and a final extension of 10 min at 72°C. The samples were then cooled to 4°C. The PCR products specificity was confirmed by sequencing.11 The 14-bp indel of PRNP was detected in 5 Chinese indigenous cattle breeds by electrophoresis using 3.5% agarose gel stained with ethidium bromide.
The Theory of ME on Low Frequency Mutations

The ME, also known as the mean value, is very important digital feature in the theory of possibility. It represents the overall average value of random variables. The model of ME can be used to analyze abstract methods in practical application.4-6 Given the previous reported data, the frequencies of the 14-bp indel within PRNP were predicted to be low in the tested Chinese cattle breeds.23 Therefore, initial randomly selected 50 animals were genotyped separately (not pooled) to establish the allele frequencies in a population. The experimental results confirmed the low frequency of the 14-bp indel in PRNP gene in the studied Chinese cattle breeds. So, the theory of ME has value of application.4-6

Then, 3 individuals pooled in mixed groups were established by an ME equitation. Subsequently, all animals were divided into pools and were genotyped. If two bands were observed in a pool, the animals were
genotyped separately to specifically assign the genotype. In this experiment, the low mutation frequency of 14-bp indel was identified in every cattle breed (Fig. 1).

Therefore, given the low frequency events, according to the theory of ME, the best method is to use the following equation.\(^4\)–\(^6\):

\[
\begin{align*}
n &= N \times \left( \frac{1}{a} \times (1-p)^a + \left(1 + \frac{1}{a}\right) \times (1 - \left(1-p\right)^a) \right)
\end{align*}
\]

where \(N\) is the sample size, \(a\) is the sum of a group, \(p\) is the probability of the low frequency event (http://www.msrrcall.com/DALMcall.aspx).

Using the above equation, we calculated the optimal number of individuals in a mixed group for each breed. We designed the most possible pooling strategy for each cattle breed, and the mixed group was genotyped. The results of individuals were same to the genotype of every individual. Simultaneously, the genotype of every individual was verified by the result obtained from mixed group of 3 individuals.

### Statistical Analysis

Genotypic and allelic frequency were calculated directly. The \(\chi^2\) test was carried out to test whether the polymorphism is in Hardy-Weinberg equilibrium (HWE). Polymorphism information content (PIC) was calculated by Nei’s method implemented in the GDiCall Online Calculator (http://www.msrrcall.com/GdiCall.aspx).\(^23\) Distribution differences for genotypic and allelic frequencies among/between different breeds were analyzed using the \(\chi^2\) test implemented in SPSS (Version 18.0) (IBM Corp., Armonk, NY, USA).\(^24\) The association of the 14-bp indel of \(PRNP\) with several growth traits (e.g., body length (cm)) in different breeds was tested using the analysis of variance (ANOVA) available in SPSS (Version 18.0). The data that did not follow normal distribution and homogeneity of variances were analyzed using the nonparametric (Kruskal-Wallis) test in SPSS (Version 18.0). The ANOVA applied the general linear model and the reduced linear model as follows: \(Y_{ijk} = \mu + \alpha_i + \beta_j + e_{ijk}\), where \(Y_{ijk}\) is the observation of the reproduction trait (testis weight, etc.) evaluated on the \(i^{th}\) level of the fixed factor age \((\alpha_i)\) and the \(j^{th}\) level of the fixed factor genotype \((\beta_j)\), \(\mu\) is the overall mean for each trait, and \(e_{ijk}\) is the random error for the \(ijk^{th}\) individual.\(^24\)

### RESULTS

Previous reports identified a 14-bp indel in the 3’ UTR of the bovine \(PRNP\) gene. The direct 3.5% agarose gel electrophoresis conducted herein detected one band (142-bp) for genotype insertion/insertion (II), one band (128-bp) for genotype deletion/deletion (DD), and 2 bands (142-bp, 128-bp) for genotype insertion/deletion (ID) (Fig. 2). These results were consistent with those previous reports.\(^23\)

Initially, all individuals were genotyped. The genotypic and allelic frequencies of the 14-bp indel of \(PRNP\) were evaluated in the Qinchuan, Xianan, Nanyang, and Jiaxian cattle breeds. The minor allele frequency was 0.038 (Qinchuan), 0.033 (Xianan), 0.013 (Nanyang), 0.003 (Jiaxian), and zero (Ji’an). The effective allele numbers were 1.078 (Qinchuan), 1.067 (Xianan), 1.026 (Nanyang), 1.008 (Jiaxian), and 1.000 (Ji’an). The polymorphism information content values of this indel were low for all analyzed breeds. Moreover, \(\chi^2\) tests showed that the 14-bp indel in the 3’ UTR of \(PRNP\) were in HWE in all analyzed breeds (\(P > 0.05\)), except in the Xianan breed.

The low frequency of 14-bp indel was characterized in \(PRNP\) for the 5 Chinese cattle breeds (Table 1). Using the ME method, individuals of each breed were assigned by order in groups (the least allowed number in a single group) to mixed groups. Hence, Qinchuan, Xianan, Nanyang, Jiaxian, and Ji’an breeds were divided into 54, 72, 66, 67, and 79 mixed groups (Table 2). Dependent on whether there was one single band (142-bp or 128-bp) in the mixed groups from different cattle breeds, the exact detection times were shown. Finally, a total of 90, 100, 81, 70, and 79 reactions times were carried out in Qinchuan, Xianan, Nanyang, Jiaxian, and Ji’an cattle, respectively. Simultaneously, the number of PCR reactions was decreased by 43.7%, 53.3%, 58.9%, 64.8%
and 66.4%, respectively, compared to those obtained by the traditional detection methods (Table 2).

In view of the minor allelic frequency of Ji’an cattle breed was 0.000, so, ME was not applied to this breed. Initial randomly selected 50 animals were genotyped separately (not pooled) to establish the allele frequencies in a population. Then, according to the ME evaluation equation, the optimal number of individuals in a single mixed group was 5 (Qinchuan), 6 (Xianan), 8 (Nanyang), and 16 (Jiaxian). Thereby, number of animals to be pooled in mixed groups was established by an ME equation. Subsequently, all animals were divided into 32, 36, 25, and 13 mixed pools and genotyped, respectively. If two bands were observed in a pool, the animals were genotyped separately to specifically assign the genotype. At last, the exact reaction times were shown. Finally, a total of 60, 75, 44, and 22 reaction times were carried out in Qinchuan, Xianan, Nanyang, and Jiaxian cattle, respectively. Simultaneously, the reaction times were decreased by 62.5%, 64.9%, 77.6%, and 88.9%, respectively, compared to those obtained by the traditional detection method. These results indicated that the ME detection method saves time greatly, which will ultimately reduce the expenses, compared to the traditional methods (Table 2).

The associations between the 14-bp indel of the 3′ UTR and the bovine growth traits were evaluated in the tested Chinese cattle breeds (Table 3). Because there were less than 5 individuals with ID genotype in Nanyang, Jiaxian, and Ji’an breeds, the respective data were not included in the analysis. The 14-bp indel was significantly correlated to the body length in Qinchuan cattle as well as the body weight and waistline in Xianan cattle; the homozygous insertion genotype was predominant in these breeds, respectively.

**DISCUSSION**

This study is the first report of the 14-bp indel in the 3′ UTR of PRNP gene in 5 Chinese

### TABLE 1. Genotypic and allelic frequencies and population indexes for 14-bp indel in the bovine PRNP gene.

| Breeds/Loci   | Sizes | Genotypic frequencies | Allelic frequencies | HWE | Population parameters |
|---------------|-------|-----------------------|--------------------|-----|-----------------------|
|               | N     | II | ID | DD | I | D | P values | Ho | He | Ne | PIC | References |
| Qinchuan      | 160   | 0.925 | 0.075 | 0 | 0.962 | 0.038 | P > 0.05 | 0.928 | 0.072 | 1.078 | 0.160 | In this study |
| Nanyang       | 197   | 0.975 | 0.025 | 0 | 0.987 | 0.013 | P > 0.05 | 0.975 | 0.025 | 1.026 | 0.025 |
| Jiaxian       | 199   | 0.994 | 0.006 | 0 | 0.997 | 0.003 | P > 0.05 | 0.992 | 0.008 | 1.008 | 0.008 |
| Xianan        | 214   | 0.949 | 0.037 | 0.014 | 0.967 | 0.033 | P < 0.05 | 0.937 | 0.063 | 1.067 | 0.061 |
| Ji’an         | 235   | 1.000 | 0   | 0 | 1.000 | 0   | P > 0.05 | 1.000 | 0   | 1.000 | 0   |
| Healthy^a     | 48    | 0.900 | 0.100 | 0 | 0.950 | 0.050 | P > 0.05 | 0.901 | 0.099 | 1.110 | 0.094 | Seabury et al. (2004) |
| Affected      | 43    | 0.860 | 0.120 | 0.020 | 0.920 | 0.080 | P > 0.05 | 0.853 | 0.147 | 1.173 | 0.136 |
| U.S. sires    | 132   | 0.890 | 0.110 | 0 | 0.940 | 0.060 | P > 0.05 | 0.887 | 0.113 | 1.127 | 0.106 |
| Cows          | 234   | 0.987 | 0.013 | 0 | 0.994 | 0.006 | P > 0.05 | 0.988 | 0.012 | 1.012 | 0.011 | Czarnik et al. (2006) |
| Al sires      | 47    | 1.000 | 0   | 0 | 1.000 | 0   | P < 0.05 | 1.000 | 0   | 1.000 | 0   |
| Vietnam native| 100   | 0.680 | 0.280 | 0.040 | 0.820 | 0.180 | P > 0.05 | 0.705 | 0.295 | 1.419 | 0.252 | Shimogiri et al. (2010) |
| Laos native   | 72    | 0.597 | 0.375 | 0.028 | 0.780 | 0.220 | P > 0.05 | 0.657 | 0.343 | 1.523 | 0.284 |
| Myanmar native| 110   | 0.682 | 0.282 | 0.036 | 0.820 | 0.180 | P > 0.05 | 0.705 | 0.295 | 1.419 | 0.252 |
| Mongolia native| 44   | 0.614 | 0.318 | 0.068 | 0.770 | 0.230 | P > 0.05 | 0.646 | 0.354 | 1.548 | 0.291 |
| Japanese Hosen| 65    | 0.614 | 0.383 | 0.030 | 0.790 | 0.210 | P > 0.05 | 0.668 | 0.322 | 1.497 | 0.277 | Sander et al. (2004) |
| Japanese Short horn | 1 | 1.000 | 0 | 0 | 1.000 | 0 | P < 0.05 | 1.000 | 0 | 1.000 | 0 |
| Kuchinoshima  | 52    | 1.000 | 0   | 0 | 1.000 | 0   | P < 0.05 | 1.000 | 0   | 1.000 | 0   |
| Japanese Brown| 64    | 0.438 | 0.281 | 0.281 | 0.580 | 0.420 | P < 0.01 | 0.513 | 0.487 | 1.950 | 0.369 |
| BSE affected cattle | 43 | 0.880 | 0.110 | 0.010 | 0.930 | 0.070 | P < 0.05 | 0.870 | 0.130 | 1.150 | 0.122 |
| Mishima       | 2     | 0.500 | 0   | 0.500 | 0.500 | 0.500 | P < 0.05 | 0.500 | 0.500 | 2.000 | 0.375 |

**Note:** HWE, Hardy-Weinberg equilibrium; Ho, homozygosity; He, heterozygosity; Ne, effective allele numbers; PIC, Polymorphism information content;
indigenous cattle breeds: Qinchuan, Xianan, Nanyang, Jiaxian, and Ji’an. Given the low frequency of the 14-bp indel within the 3' UTR in cows and artificial insemination sires of the USA (0.006 and zero, respectively), in Vietnamese native cattle (0.18) and Laos native populations of Asian origin (0.22) (Table 1), we hypothesized that the frequency of the 14-bp indel of the 3' UTR in PRNP gene may be low in Chinese cattle breeds. We used an ME strategy to detect the allele frequency in all individuals. Simultaneously, we verified experimentally that the frequency of the 14-bp indel in the 3' UTR was low in 5 cattle breeds. The results of our study were consistent with previous results. However, they did not corroborate the results presented by Shimogiri, who reported the frequency in Japanese Brown of more than 0.10. Those discrepancies might be due to the small sample size (n = 64). Overall, the 14-bp indel within the PRNP gene was characterized by low frequency in main Chinese cattle breeds.

Furthermore, our results confirmed that the 14-bp indel in the 3' UTR of PRNP gene was at HWE in populations of 5 Chinese indigenous cattle breeds except Xianan (P > 0.05), which consisted with the cultural background of different cattle breeds. Xianan cattle are the first crossbreeding beef cattle in China. Hence, crossbreeding might also be a cause of HWE deviation. According to the previous reported data, the 14-bp indel of the 3' UTR of PRNP was at HWE in most populations of breeds outside China, with the exception of the Japanese Brown, artificial insemination sires in USA, Japanese Short horn, and Kuchinoshima populations (P > 0.05), which might due to the small sample size of these breeds. There were no significant differences in the distribution of genotypic and allelic frequencies for the 14-bp indel among the Qinchuan, Xianan, Nanyang, Jiaxian, and Ji’an breeds (χ² test, P = 1.000). Moreover, we analyzed the reported data for foreign breeds and

### Table 2. All reaction times of different group in 5 cattle breeds.

| Breeds     | Qinchuan | Xianan | Nanyang | Jiaxian | Ji’an |
|------------|----------|--------|---------|---------|-------|
| Sizes      | 160      | 214    | 197     | 199     | 235   |
| Minor allelic frequencies (MAF) | 0.038 | 0.033 | 0.013 | 0.003 | 0.000 |
| Number of individuals in one reaction time (NR₁) | 1 | 1 | 1 | 1 | 1 |
| Reaction times (RT₁) | 160 | 214 | 197 | 199 | 235 |
| Number of individuals in one mixed group (NG₃) | 3 | 3 | 3 | 3 | 3 |
| Predicting reaction times by the formulate (pTT₃) | 71 | 92 | 73 | 68 | 79 |
| Reaction times (RT₃) | 90 | 100 | 81 | 70 | 79 |
| Reduction rate (RR) | 21.0% | 8.0% | 9.8% | 2.9% | 0 |
| The optimal number of individuals in one mixed group (NG₄) | 5 | 6 | 8 | 16 | — |
| Predicting reaction times by the formulate (pRT₄) | 93 | 90 | 52 | 29 | — |
| Reaction times (RT₄) | 60 | 75 | 44 | 22 | — |
| Reduction rate (RR) | 35.5% | 16.7% | 15.4% | 24.1% | — |

**Note:** MAF, minor allelic frequencies; NG, the optimal number of one mixed group; RT, reaction times; pRT, predicting reaction times by the formulate; RR, reduction rate.

### Table 3. Relationship between the 14-bp indel of PRNP gene and growth traits in Xianan and Qinchuan cattle breeds (LSM ± SE) (P < 0.05).

| Genotypes | Breeds     | Growth traits | II  | ID  | DD  | P values |
|-----------|------------|---------------|-----|-----|-----|----------|
| Xianan    | CC (cm)    | a161.77 ± b 202.29 ± c 201.33 ± 0.004 |
|           | BW (kg)    | a549.81 ± b557.11 ± c469.67 ± 0.040 |
| Qinchuan  | BL (cm)    | b140.76 ± a146.78 ± 0.046 |

**Note:** CC, chest circumference; BW, body weight; BL, body length. The values with different letters (a and b; a and c) within the same row differ significantly at P < 0.05 and P < 0.01, respectively.
obtained the results similar to those reported herein for the 5 Chinese native breeds. Thus, we verified that the distribution of genotypic and allelic frequencies in the 14-bp indel of \textit{PRNP} showed no significant differences among the different cattle breeds.

The low frequency of the 14-bp indel in the 3’ UTR of \textit{PRNP} in Chinese cattle breeds obtained experimentally confirmed that the ME method can be applied to detect low frequency mutations.\textsuperscript{4,6} This method could be used to calculate the optimal reaction time from the low frequency mutation and sample size. Using the GDiCall Online Calculator (http://www.msricall.com/Gdicall.aspx), we designed the best possible pooling strategy for 5 cattle

FIGURE 3. Reaction times about different sizes of one mixed group (different mutation frequency (1%–9%) when N = 1000 and N = 2000).
breeds, excluding Ji’an cattle breed which mutation frequency was zero. We found that the optimal number of individuals in a group was 5, 6, 8, and 16 in Qinchuan, Xianan, and Nanyang, Jiaxian, respectively, if all reaction time was least (Table 2). Moreover, we calculated other methods for division of individuals into groups with different low frequency mutations and sample size by using this equation and identified the best method among them (Fig. 3). Concurrently, using the results of the previous reports on low frequency mutations in breeds outside China, we proposed a better method, which was based on a new method we put forward (http://www.msrcall.com/Gdicall.aspx).12 Thus, for the experiments with U.S. Sires,30 each group should comprise 4 individuals, and any effect that may alter the accuracy of the experiment could be eliminated if the concentration of DNA in all individuals is the same. In other breeds, the ME method was also convenient for quick and accurate detection of the low frequency mutations.

Due to the low number of heterozygous animals (n < 5) in Jiaxian, Nanyang, and Ji’an cattle breeds, we only analyzed the association of the 14-bp indel with growth traits of Qinchuan and Xianan cattle breeds. The results of the association analysis suggested that the 14-bp indel in the 3’ UTR might affect body length of Qinchuan cattle and body weight and waistline of Xianan cattle. It could be concluded that the 14-bp indel in the 3’ UTR or the adjacent area might contain the binding sites of transcription factors of the genes affecting the growth traits. When the 14-bp indel in the 3’ UTR of PRNP was inserted or deleted, the structure and function of genes related to the growth traits would be affected by bonding with miRNA.31

In summary, an economic ME method was presented to quickly and accurately detect low frequency mutation, such as the 14-bp indel in the 3’ UTR of the PRNP gene, which would save time and reduce expenses. Besides, the detected 14-bp indel significantly affect growth traits, which might be a potential useful DNA marker for MAS in cattle.

**ABBREVIATIONS**

| Acronym | Description |
|---------|-------------|
| BSE | bovine spongiform encephalopathy |
| PrP | Prion protein (PrP) |
| PRNP | prion protein gene |
| 3’ UTR | 3’-untranslated region |
| Indel | insertion/deletion |
| SNPs | single nucleotide polymorphisms |
| SPSS | statistical product and service solutions |
| GLM | general linear model |
| bp | base pair |
| HWE | Hardy-Weinberg equilibrium |
| Ho | homozygosity |
| He | heterozygosity |
| Ne | effective allele numbers |
| PIC | Polymorphism information content |
| ME | mathematical expectation |
| PCR | polymerase chain reaction |
| ANOVA | analysis of variance |
| II | insertion/insertion |
| ID | insertion/deletion |
| DD | deletion/deletion |
| MAS | marker-assisted selection |

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

We confirm that this manuscript has not been published in whole or in part and is not being considered for publication elsewhere. The authors have no conflicts of interest to declare.

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