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Research Article

The Identification of Yak Meat Using Loop-Mediated Isothermal Amplification Method Coupled with Hydroxy Naphthol Blue for the Prevention of Food Fraud

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Objective. Yak is found in the Qinghai-Tibet Plateau and represents a meat of high nutritional value and good flavor. However, the production of yak is limited, and yak meat adulteration is a growing concern in the marketplace. To protect consumer rights and prevent unfair competition, it is necessary to use an efficient assay to identify the species of yak meat rapidly and accurately being sold. *Methods*. Loop-mediated isothermal amplification (LAMP) combined with hydroxy naphthol blue (HNB) was used to identify potential adulterants. The specificity and sensitivity tests of yak-derived components were carried out to achieve the monitoring of yak-derived components. *Results*. The optimal color development was achieved with an external primer-to-internal primer ratio of 400 nmol/L : 1200 nmol/L, 1.5 mmol/L dNTP, and 0.32 U/μL Bst DNA polymerase with 5 mmol/L $MgSO_4$ at 62°C amplification temperature. The detection sensitivity of LAMP-HNB for yak-derived DNA was up to 1 pg/μL. *Conclusion*. The LAMP-HNB assays provided a valuable tool for the identification of yak gene from adulterated meat. This further enabled the LAMP-HNB assay to be applicable in the identification of other meat products.

1. Introduction

Yak is a breed of livestock found in the Qinghai-Tibet Plateau, and yak meat has advantages including being high in protein and minerals and low in fat, making it highly valuable in terms of nutrition [1]. The cornerstones for the detection of adulteration in animal products require a tool for species identification to assess the food composition before it reaches to the consumer's table. Protection against species substitution and/or admixture in meat and their products is of significant importance in terms of religion, consumer's choice, public health concerns, and government regulations [2]. Illegitimate adulteration of yak meat is a common practice in China. Therefore, it is of great significance to establish a rapid detection method for yak-specific components to combat the adulteration of yak meat.

Various rapid detection methods for animal-specific components have been reported. PCR-based rapid identification assay is widely used due to the advantage of being fast, simple, and easy to operate [3]. However, both conventional PCR and real-time fluorescent PCR require complex operational steps, long reaction times, and expensive laboratory instruments. Loop-mediated isothermal amplification (LAMP) is a molecular diagnostic technique based on nucleic acid amplification [4]. It utilizes a strand displacement DNA polymerase, namely, Bst DNA polymerase and two pairs (or three pairs) of primers to amplify specific DNA [5]. Compared to other DNA polymerases, Bst DNA polymerase has strong heat stability, strand displacement activity, and polymerase activity, making it more suitable for isothermal amplification [6]. Due to the constant DNA denaturation and extension temperature, the nucleic acid amplification

reaction can be completed under isothermal conditions [7] (e.g., incubation at 63°C for 45-60 min). LAMP has relatively high sensitivity, allowing for DNA amplification of up to 10^9-10^{10}-fold, and it can greatly improve the detection rate of the target gene by designing specific primers [8]. Even if meat products undergo further processing that leads to DNA degradation, the amplification of the target gene will not be affected, enabling rapid batch testing suitable for large-scale meat source detection. Thus, the LAMP assay has gradually expanded to various fields, such as the detection of foodborne pathogens [9], foodborne viruses [10], and genetically modified foods and parasites [11].

The colorimetric-based LAMP detection method is commonly used to clarify reaction products. In this study, hydroxy naphthol blue (HNB) dye was employed to add to the LAMP reaction system, which is a widely used metal ion indicator that reacts with magnesium ions and exhibits a violet color [12]. After double-stranded DNA synthesis, the magnesium pyrophosphate ions precipitate from the dNTPs. By utilizing HNB in the LAMP method, the detection results become visually observable. The positive sample tube containing yak-derived components will appear sky blue, while the negative sample tube without yak-derived components will appear violet.

Relatively, few reports have been published on the application of LAMP technology in the detection of yak-derived ingredients in food. Therefore, in this study, the LAMP primers were designed according to the mitochondrial Cytb genes, and the LAMP detection assay for yak-derived components was established by optimizing several conditions during the LAMP reaction, aiming to provide technical support for the rapid identification of yak products.

2. Materials and Methods

2.1. Materials. Four meat species including chicken (*Gallus gallus*), duck (*Anas platyrhynchos*), sheep (*Ovis aries*), and cattle (*Bos taurus*) were purchased from local grocery markets (Guangzhou, China). The yak meat was purchased from the natural grazing and breeding areas and yak meat market in Tibet. Each of the meat samples (20 mg) was used for DNA extraction using the animal-derived genomic DNA extraction kit (centrifugal column, Aodong Inspection & Testing Technology Co. Ltd, Shenzhen, China) according to the manufacturer's instructions. The DNA from the yak meat has already been extracted and obtained from Guangzhou Pharmaceutical University. All DNA samples were then stored at 4°C for further analysis. Bst DNA polymerase (containing 8 U/μL reaction buffer) was bought from Magigene Technology Co. Ltd (Guangzhou, China). dNTP was purchased from Fei Peng Biological Co. Ltd (Shenzhen, China). Hydroxy naphthol blue (HNB) was purchased from Jianyang Biomedical Technology Co. Ltd (Shenzhen, China), and RNase-free water was purchased from Biosharp (Langeke Technology Co. Ltd, Shenzhen, China).

2.2. Methods

2.2.1. Strategy for LAMP Primer Design and Synthesis. The mitochondrial gene sequences of yak (KM280688.1), cattle (MH714783.1), and buffalo (MT182644.1) were obtained from the GenBank database. The yak LAMP amplification primers were designed using the LAMP primer online design software PrimerExplorer version 5 (Figure 1), among which two outer primers, F3 and B3, and two inner primers, FIP (F1c+F2) and BIP (B1c+B2) were included. The primers were synthesized by Hunan Akerui Biological Co. Ltd (Changsha, China) and purified by PAGE. Primer sequences for yak-derived components are shown in Table 1. The sequence alignment of the amplified fragment was compared with the sequences of cattle and buffalo using the BLAST online alignment software. The results showed that the designed primer sequence had several base site differences, indicating that the amplified fragment had good specificity. The information for the sequence alignment is shown in Figure 2.

2.2.2. DNA Extraction. Each sample (0.1 g) was mixed with 800 μL of TE solution for 15 min, and then, 200 μL of homogenate was aspirated to mix with 800 μL of animal lysate (containing 5 mol/L guanidine isothiocyanate, 0.05 mol/L Tris-HCl (pH = 6.4), 0.02 mol/L EDTA (pH = 8.0), and 1.3% Triton X-100). The mixture was put in a 65°C water bath for 2 h. After cooling, 5 μL of 20 mg/mL RNase was added to the mixture to react at room temperature for 5 min. Thereafter, 1 mL of phenol-chloroform-isoamyl alcohol (25 : 24 : 1, $V/V/V$) was added. The mixture was centrifuged at 12,000 g for 15 min. The supernatant was collected to mix with the same volume of chloroform-isoamyl alcohol (24 : 1, V/V). The mixture was then centrifuged at 12,000 g for 15 min. The supernatant was collected, followed by adding 1/10 volume of sodium acetate (pH = 5.2) and equal volume of isopropanol to precipitate at -20°C for 30 min. After centrifugation at 12,000 g for 15 min, the supernatant was discarded and 500 μL of 70% ethanol was added and centrifuged again at 12,000 g for 1 min (repeat twice). The supernatant was discarded. Afterwards, 100 μL of sterilized deionized water was added into the tube and stored at -20°C. DNA concentration was determined using a UV-vis spectrophotometer.

2.2.3. Determination of the Validity of LAMP Primer. To determine whether the designed yak-derived LAMP primers could be amplified in the reaction system, a yak-derived LAMP fluorescent dye reaction was performed. EvaGreen fluorescent dye was added to the LAMP reaction system to confirm the validity of LAMP primers. The total volume of the reaction system was 25 μL. The final reactant contained 2.5 μL of PCR buffer (10x), 1.0 mmol/L of dNTP, 0.8 μmol/L of FIP and BIP, 0.4 μmol/L of F3 and B3, 5 mmol/L of $MgSO_4$, 8 U/L of Bst DNA polymerase, 1.25 μL of EvaGreen fluorescent dye, and 5 μL of yak nucleic acid. The nuclease-free water was set as a negative control. The system was supplemented with ddH_2O to 25 μL. The fluorescent PCR was 40 cycles in total, and the procedure was 60°C for 30 s, followed by 60°C for 60 s. The fluorescence was collected at 60°C for 60 s.

2.2.4. Optimization of the LAMP Reaction System. Based on the primer design, the conditions of the LAMP reaction

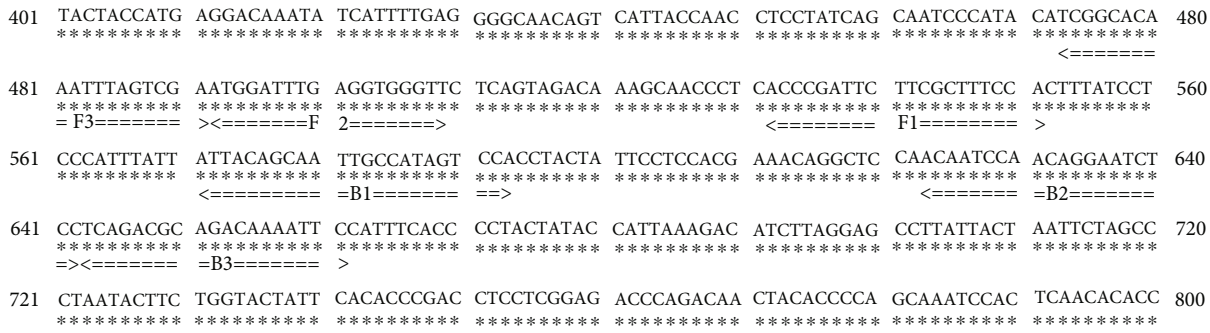


Figure 1: Strategy for yak-derived components LAMP primer design.

Table 1: LAMP primer sequences for yak-derived components.

Primers	Primer sequences (5′-3′)
Bg F3	TCGGCACAAATTTAGTCGA
Bg B3	GAATTTTGTCTGCGTCTGA
Bg FIP	TGGAAAGCGAAGAATCGGGTATGGATTTGAG GTGGGTT
Bg BIP	ATTACAGCAATTGCCATAGTCCAGGAGATTC CTGTTGGATTGT

system were optimized using the pMD-cytb recombinant plasmid as the amplification template. The concentration of each component was preset as follows: 1 μL of Bst DNA polymerase (8 U/μL), 2.5 μL of buffer containing 2 mmol/L $MgSO_4$ (10x), 3 μL of 25 mmol/L Mg^{2+}, 2.5 μL of 10 mmol/L dNTP, 2 μL of 10 μmol/L FIP/BIP(each), 2 μL of 10 μmol/L F3/B3, 1 μL of 2.5 mmol/L HNB, and 5 μL of DNA template. The LAMP reaction volume was supplemented to 25 μL by sterile ultrapure water. The procedure was set at 60°C for 60 min. If the final LAMP amplification product was blue, the test specimen was positive, while it would be negative if the amplification product was purplish red.

(1) Refinement of the Ratio of LAMP Inner and Outer Primers. The concentration of outer primer pair F3/B3 in the reaction system was fixed at 0.4 μmol/L, while the concentration of inner primer pair FIP/BIP was set at 0.4, 0.8, 1.2, and 1.6 μmol/L separately to explore the optimized concentration of FIP/BIP.

(2) Optimization of Mg^{2+} Concentration. In the reaction system, the concentration of Mg^{2+} was sequentially set as 2, 3, 4, 5, and 6 mmol/L to select the optimal Mg^{2+} concentration.

(3) Optimization of dNTP Concentration. The concentration of dNTP was set as 0.2, 0.4, 0.6, 0.8, 1.2, and 1.4 mmol/L separately to select the optimal dNTP concentration.

(4) Optimization of Hydroxy Naphthol Blue Concentration. HNB was used as a color indicator in LAMP reactions. The concentration of HNB was sequentially set as 50, 100, 150, 200, and 250 μmol/L to explore the optimal HNB concentration [12].

(5) Optimization of Bst DNA Polymerase Concentration. The concentration of Bst DNA polymerase was sequentially set as 0.256, 0.32, and 0.384 U/μL to select the optimal concentration of Bst DNA polymerase.

(6) Optimization of LAMP Reaction Temperature. The optimal temperature for product amplification in the reaction was optimized by setting the reaction temperatures as 59, 60, 61, 62, 63, 64, and 65°C separately.

(7) Determination of Specificity and Sensitivity of LAMP Detection Assay. Amplification was performed using DNA templates from sheep, chicken, pig, and buffalo separately based on the LAMP system established according to the above assays. The products were identified through HNB coloration to assess the specificity of the LAMP assay. Regarding the sensitivity verification, 5 μL of nucleic acid DNA derived from yak with each concentration of 1 ng/μL, 100, 10, and 1 pg/μL (diluted from 10 ng/μL stocking solution of yak DNA) was used as templates separately for the reaction. In addition, referring to the standard of ISO 20813:2019, nucleic acid samples in nucleic acid libraries of different meat products were added for specificity verification.

3. Results

3.1. Confirmation of the Efficiency of LAMP Detection Primers. According to the results of the fluorescence PCR, as shown in Figure 3, the reaction wells containing yak-derived DNA exhibited clear fluorescence signal accumulation, while the negative control showed no fluorescence accumulation. This indicated that the designed yak-specific LAMP detection primers could effectively amplify yak-derived DNA without causing false-positive results due to nonspecific amplification between primers.

3.2. Conditions of the LAMP Detection Method. Table 2 illustrates that the optimal ratio of inner and outer primers was 1 : 3, with a concentration of 0.4 μmol/L for the inner primers and 1.2 μmol/L for the outer primers. The optimal concentration of $MgSO_4$ and dNTP was 5 and 1 mmol/L, respectively. The final determined reaction system for LAMP was 25 μL, consisting of 2.5 μL of 10x PCR buffer, 3 μL of 5 mmol/L $MgSO_4$, and 1 mmol/L dNTP. The final

Species (Abbrv)	
1. Bos_mutus_	TCGGCACAAATTTAGTCGAATGGATTTGAGGTGGGTTCTCAGTAGACAAAGCAACCCTCACCCGA
2. Bos_taurus	TCGGCACAAATTTAGTCGAATGAATCTGAGGCGGATTCTCAGTAGACAAAGCAACCCTTACCCGA
3. Bubalus_bubalis	TTGGTACAAGTCTGGTTGAGTGAATTTGAGGGGGATTCTCAGTAGACAAAGCAACCCTCACCCGA

Species (Abbrv)	
1. Bos_mutus_	TTCTTCGCTTTCCACTTTATCCTCCGATTTATTATTACAGCAATTGCCATAGTCCACCTACTATT
2. Bos_taurus	TTCTTCGCTTTCCATTTTATCCTTCCATTTATCATCATAGCAATTGCCATAGTCCACCTATTATT
3. Bubalus_bubalis	TTCTTCGCATTTCACTTCATCCTCCCATTCATTATCGGAGCACTTGCAATAGTCCACCTATTATT

Species (Abbrv)	
1. Bos_mutus_	CCTCCACGAAACAGGCTCCAACAATCCAACAGGAATCTCCTCAGACGCAGACAAAATTC
2. Bos_taurus	CCTCCACGAAACAGGCTCCAACAATCCAACAGGAATCTCCTCAGACGTAGACAAAATCC
3. Bubalus_bubalis	TCTCCACGAAACAGGATCCAACAACCCAACAGGAATCTCATCAGACACAGACAAAATCC

Figure 2: Cytb gene comparison among yaks, scalpers, and buffaloes.

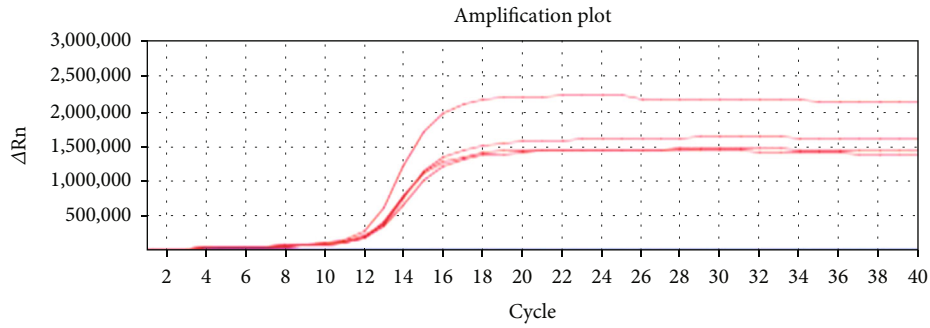


Figure 3: Confirmation of the yak-derived LAMP primers. Red curve represents the sample, while blue curve depicts the negative control.

concentration of FIP and BIP was 1.2 μmol/L, while the final concentration of F3 and B3 was 0.4 μmol/L. Bst DNA polymerase was used at 8 U/L, and 5 μL of yak-derived DNA was added. The reaction was carried out at a temperature of 62°C for 60 min.

3.2.1. Optimization of the Ratio between Inner and Outer Primers in LAMP Reaction. Generally, the concentration of the outer primer pair F3/B3 had no influence on the amplification of the LAMP reaction. LAMP amplification could not proceed if the concentration of the inner primer pair was too low, while increasing the concentration of the inner primer pair could improve the amplification efficiency of LAMP. According to Figure 4(a), in the 25 μL of the LAMP reaction system, different concentrations of outer primers (B3/F3) and inner primers (BIP/FIP) were added at a ratio of 400 nM : 400 nM, 400 nM : 800 nM, 400 nM : 1200 nM, and 400 nM : 1600 nM separately, totally using 10 μmol/L primers. The isothermal amplification reaction was performed, and the results were observed by the naked eye under natural light. The amplification results showed that the optimal color development was achieved when the ratio of outer primers (B3/F3) to inner primers (BIP/FIP) was 400 nM : 1200 nM. Other concentration ratios of the outer primers and inner primers resulted in relatively poor color development. When the concentration of the inner primers was lower, a higher likelihood of violet color development and false negatives was observed.

Table 2: Optimal system of 25 μL for LAMP detection assay.

Reagents	Concentration	Volume (μL)
10x buffer	—	2.5
DNTP	1 mmol/L	2.5
$MgSO_4$	5 mmol/L	3
FIP	1200 mmol/L	3
BIP	1200 mmol/L	3
F3	400 mmol/L	1
B3	400 mmol/L	1
Bst DNA polymerase	0.8 U/μL	1
HNB	0.15 mmol/L	1.5
ddH_2O	—	Up to 25
Sample DNA solution	—	5

3.2.2. Optimization of Mg^{2+} Concentration. The LAMP reaction is always accompanied by the generation of a large amount of precipitated magnesium pyrophosphate. The concentration of Mg^{2+} can directly affect the production of magnesium pyrophosphate and the change of pH value, which may lead to misinterpretation of the color reaction in LAMP, thus affecting the efficacy of this assay [13]. In 25 μL of the LAMP reaction system, different concentrations of $MgSO_4$ (2, 3, 4, 5, and 6 mmol/L) were added separately. The isothermal amplification reaction was performed, and the result was observed by the naked eye under natural light.

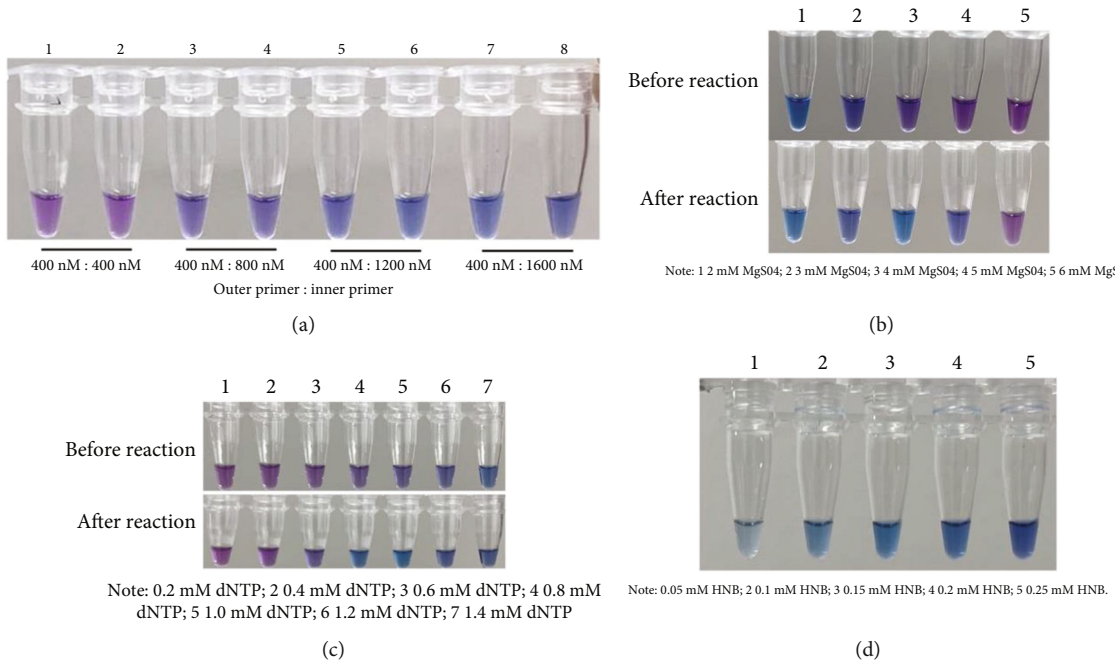


Figure 4: (a) The optimal concentration ratio of outer and primers. (b) The optimal concentration of Mg^{2+}. (c) The optimal concentration of dNTP. (d) The optimal concentration of HNB.

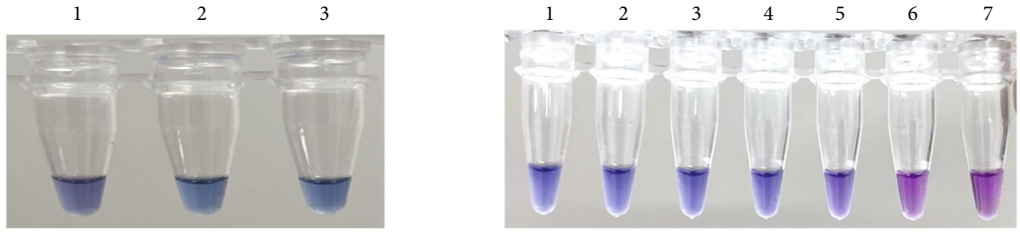
Referring to Figure 4(b), when 5 mmol/L of $MgSO_4$ was added, a more pronounced sky blue color was observed compared to other parallel groups. The color development was relatively poor with other concentrations of $MgSO_4$. As the concentration of $MgSO_4$ increased, there was a higher likelihood of violet color development and false negatives, which made it difficult to effectively identify the LAMP reaction based on color changes alone. Therefore, the optimal Mg^{2+} concentration selected in this study was 5 mmol/L.

3.2.3. Optimization of dNTP Concentration. A large number of nucleic acids will be produced during the LAMP reaction, during which the pyrophosphate ions released from dNTPs combine with Mg^{2+} to form a byproduct, namely, magnesium pyrophosphate precipitate [14]. The reduction of Mg^{2+} concentration during the LAMP reaction can lead to the change of pH value, resulting in a corresponding color change of the indicator HNB, allowing for rapid visual identification of the results. Therefore, in this study, the gradient of dNTP concentrations was performed. The results (as shown in Figure 4(c)) indicated that when the dNTP concentrations were lower than 0.6 mmol/L, there was no color change. As the dNTP concentration increased (0.8 and 1.0 mmol/L), the amplification efficiency gradually improved, and the color change became more pronounced. However, excessively high concentrations resulted in excessive binding of magnesium ions, leading to a sky blue color in the LAMP reaction system (>1.0 mmol/L), making it difficult to make judgments. Hence, 0.8-1.0 mmol/L dNTP was selected for the optimal concentration in this study.

3.2.4. Optimization of HNB Concentration. The result of LAMP reaction with different HNB concentrations is shown in Figure 4(d). According to the figure, the HNB concentration did not affect LAMP amplification. However, a significant color change was observed as the concentration increased. The most pronounced color change occurred when the HNB concentration in the LAMP system was 0.15 mmol/L. Therefore, the optimal HNB concentration in the LAMP system was selected as 0.15 mmol/L in this study.

3.2.5. Optimization of the Concentration of Bst DNA Polymerase. Bst DNA polymerase is one of the more expensive reagents in the LAMP reaction system. In order to reduce the detection cost, in this study, our objective was to screen the lowest concentration of Bst DNA polymerase that can enable LAMP reaction, thus achieving the goal of rapid detection. The optimization result is shown in Figure 5(a). At a concentration of 0.256 U/μL (6 U in 25 μL of reaction system), the color change was not obvious. At the concentration of 0.32 U/μL (8 U in 25 μL of reaction system), a noticeable color change was observed. Further increasing the concentration to 0.384 U/μL (6 U in 25 μL of reaction system) did not enhance the color difference, suggesting that the optimal concentration of Bst DNA polymerase should be 0.32 U/μL. Therefore, in a 25 μL LAMP detection system, the minimum concentration of Bst DNA polymerase required for the LAMP reaction was 8 U.

3.2.6. Selection of the Optimal Reaction Temperature. The LAMP reaction temperature is normally determined by the optimized temperature of Bst DNA polymerase. The recommended optimum temperature for Bst DNA polymerase is 60-65°C, within which range the enzyme can exhibit great amplification efficiency [15]. Figure 5(b) shows that at temperatures ranging from 59 to 62°C, a significant color change



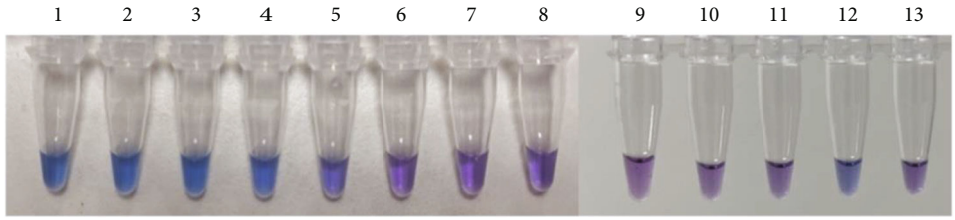
Note: 1. 6 U Bst polymerase; 2. 8 U Bst polymerase; 3. 10 U Bst polymerase

(a)

Note: 1. 59°C; 2. 60°C; 3. 61°C; 4. 62°C; 5. 63°C; 6. 64°C; 7. 65°C.

(b)

Figure 5: (a) The optimal concentration of Bst DNA polymerase. (b) The optimal temperature of LAMP reaction.



Note: 1-5 represents 10 ng/μL, 1 ng/μL, 100 pg/μL, 10 pg/μL and 1 pg/μL yak-derived DNA sample, Respectively. 6-11 represents DNA sample derived from cattle, cattle 2, pig, sheep, sleep 2 and chicken, Respectively. 12 depicts the positive control, while 13 is negative control.

Figure 6: Results of LAMP fluorescence chromogenic assay for sensitivity verification.

was observed, suggesting good amplification efficiency of Bst DNA polymerase. However, when the temperature exceeded 63°C, the color change became unconspicuous, revealing a significant decrease in amplification efficiency. Therefore, herein, the temperature for the rapid detection of yak-specific LAMP reaction was set at 62°C.

3.2.7. Specificity and Sensitivity of the LAMP Detection Assay. The result of the specificity and sensitivity of the LAMP detection assay is depicted in Figure 6. According to the result, the LAMP-HNB reaction system could only effectively amplify yak-derived DNA, resulting in a typical sky blue color, while it did not amplify DNA from other animal sources, which exhibited a violet color. This suggested that the LAMP-HNB colorimetric reaction system had good specificity for detecting yak-specific DNA. The result of the sensitivity test showed that when the concentration of yak-derived DNA was 10 pg/μL, 1 ng/μL, 100 pg/μL, and 10 pg/μL, sky blue color was observed, indicating a positive amplification. Even at a concentration of 1 pg/μL, a distinguishable sky blue color was still observed, differentiating it from the negative samples. Hence, the lowest detection limit of the LAMP-HNB method was 1 pg/μL, demonstrating its high sensitivity for detecting yak-specific DNA.

As the assay was designed to determine the authenticity of the meat species, the meat samples from various sources were also analyzed (Figure 7). According to the results, this established LAMP reaction system could only efficiently amplify yak, but not other species, indicating that the LAMP-HNB assay was highly efficient and accurate in identifying yak meat, with high specificity.

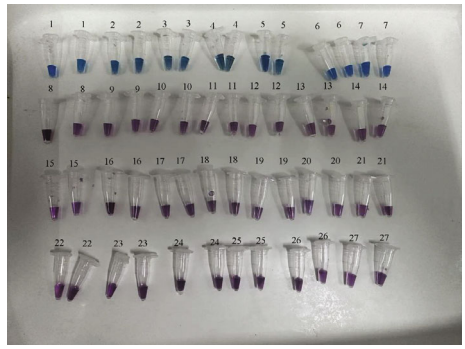


Figure 7: Results of different nucleic acid samples of different meat products using LAMP fluorescence chromogenic assay. Note: 1–7 show different breeds of yak samples; 8–12 are different breeds of buffalo samples; 13–17 represent different yellow beef samples; 18–27 depict mutton, duck, pork, horse, fox, goose, mouse, mule, artificial synthetic meat, and chicken, respectively.

4. Discussion

There are several rapid diagnostic assays currently available for the detection of yak-derived components [16]. However, most of these assays are complicated, costly, and time-consuming, making them difficult to implement at the grass-roots level and unsuitable for on-site rapid testing. In contrast, the LAMP technique has been widely used in the rapid detection of various animal-derived components due to its high sensitivity and specificity [17]. LAMP is advantageous, owing to its low cost and ease of operation, which makes it more suitable for on-site rapid testing. Previous

study [18] also revealed that the LAMP assay was simple, rapid, accurate, and sensitive for discrimination of several meat species and could be used to identify meat species that combat meat source fraud and adulteration. However, its widespread adoption has been hindered by the interpretation of LAMP results.

The determination of LAMP results typically involves turbidity detection or agarose gel electrophoresis [19, 20], which require specialized facilities and equipment that are always unavailable in most grassroots settings due to their limited resources [21, 22]. The detection of LAMP results relies on color changes resulting from the introduction of dyes into the reaction system [23]. Dye addition can occur before or after the amplification reaction [24]. If the dye is added after the reaction, the opening of the reaction system may lead to aerosol contamination, resulting in false-positive results and inaccurate outcomes. In comparison, the color change caused by HNB is less prone to visual interference [25]. However, without optimization of relevant factors, even with color development, it may be difficult to discern color changes, making result interpretation challenging. Therefore, it is crucial to optimize the LAMP reaction conditions, ensuring that each factor is at its optimal concentration, making this assay easily differentiable, simplified, cost-effective, and suitable for grassroots implementation.

HNB has also been reported to be useful as a colorimetric indicator for LAMP reaction by monitoring the Mg^{2+} concentration change as the large fragment of DNA was synthesized by Bst DNA polymerase under alkaline conditions [12]. The HNB-based LAMP assay is superior to the existing dye-based assays for LAMP to reduce the risk of contamination and helpful in high-throughput DNA and RNA detection. Herein, we used the HNB-based colorimetric assay for determining the LAMP product absorbance as described in previous study [12]. Several factors within the LAMP reaction system were explored and optimized. Satisfactory outcomes were achieved. The results suggested that the optimal color development was achieved with an external primer-to-internal primer ratio of 400 : 1200 nmol/L, 1 mmol/L dNTP, and 8 U/μL Bst DNA polymerase with 5 mmol/L $MgSO_4$. In addition, the HNB concentration and isothermal amplification temperature were also key factors for successful color development. If the reaction temperature was too high, the color tended to shift towards violet, resulting in an increased false-negative rate. Herein, the optimal reaction temperature was determined to be 62°C, and the HNB concentration was determined to be 0.15 mmol/L, which met the requirements of the LAMP detection system and enabled clear differentiation of samples containing yak-derived components. Furthermore, the LAMP-HNB assay employed in this study demonstrated high sensitivity and specificity, detecting yak-derived DNA samples as low as 10 pg/μL, which could ensure a high detection rate for positive samples. Other LAMP techniques based on HNB assay have determined similar values of analytical sensitivity. Minor differences between LAMP sensitivities could be explained by the higher number of replicates that we used, in comparison to the number of replicates considered in the referenced studies (<3). By contrast, another published work established about 300 pg/μL for a specific LAMP test based on SYBR Green fluorophore and using a portable tube scanner.

The LAMP-HNB assay for yak-derived components had minimal requirements for experimental conditions and equipment. In addition, when it is combined with the HNB colorimetric indicator, it would become both rapid and user-friendly. It is highly suitable for implementation in grassroots laboratories, breeding farms, and other settings where technical capabilities and resources are relatively limited, providing technical support for the dissemination of rapid diagnostic methods for yak-derived components.

5. Conclusion

In this study, the developed LAMP assay was yak-specific, sensitive, and visual, which facilitated the rapid detection of yak-derived DNA. This could be the first report, if any, on the development of a LAMP assay to detect yak meat. Due to the simplicity and specificity, the developed LAMP test can be easily adapted in any laboratory for rapid detection of yak species identification in livestock by-products.

Data Availability

Data is available at the corresponding author's side on request.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Zhina Yin provided substantial contributions to the writing—review and editing, writing—original draft, visualization, validation, software, resources, project administration, methodology, investigation, formal analysis, data curation, and conceptualization. Wenwei Zhao and Yufan Tan contributed to the writing—review and editing and writing—original draft. Shanshan Wang helped with the investigation, formal analysis, and data curation. Xiaoxiang Zhu, Jingyu Jiang, Hongbo Zhu, Peihan Zhu, and Wenyi Chen were involved in the writing—review and editing and methodology. Charles Brennan was in charge of writing—review and editing, supervision, project administration, and funding acquisition.

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