



In Silico Design and Bioinformatic Validation of LAMP Primers Targeting Porcine Cytochrome b for Halal Authentication

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Abstract. This study aimed to design and validate qPCR and LAMP primers targeting the porcine mitochondrial cytochrome b gene (GenBank: AF034253.1) for rapid and specific halal authentication in processed meat products. Using Primer3Plus and the NEB LAMP Primer Design Tool, optimized primers were developed based on parameters including primer length (18–25 nt), melting temperature (55–65 °C), GC content (36–60%), and minimal secondary structure. Structural analysis of the Cyt b protein using AlphaFold 3 confirmed its suitability as a specific biomarker. In silico PCR validation using UCSC tools showed that both qPCR and LAMP primers produced single, specific amplicons in Pig without cross-reactivity to Cow, Sheep and Chicken. The LAMP method, which operates under isothermal conditions and uses 5 primers targeting five distinct regions, demonstrated high sensitivity and specificity, making it ideal for point-of-need applications. These findings indicate that the designed primers are reliable molecular tools for ensuring the integrity of halal-certified food products.

Keywords: Halal authentication, LAMP, Porcine cytochrome b.

1 Introduction

The growing global halal food market is estimated to be valued at approximately USD 2 trillion, emphasizing the need for ensuring food authenticity, particularly in processed meat products. Consumer confidence, especially among Muslim populations, is increasingly threatened by instances of pork adulteration, which have been reported to affect a significant percentage of various meat products globally [1, 2]. In Indonesia, for example, there has been a notable increase in the consumption of processed meat items such as sausages, meatballs, and nuggets over the past decade. This trend raises concerns due to the lack of sufficient resources for swift, reliable, and cost-effective halal verification methods—an issue that needs addressing to meet rising consumer demand for food integrity [3–5].

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Traditional DNA-based authentication methods, including Polymerase Chain Reaction (PCR) and real-time PCR, are recognized for their high accuracy in species identification but are often impractical for field applications because they rely on thermal cyclers and controlled lab environments, with processing times extending up to several hours [2, 6]. In contrast, Loop-Mediated Isothermal Amplification (LAMP) provides a compelling alternative. It enables precise amplification of DNA targets under isothermal conditions (60-65°C) within just 30-60 minutes using specially designed primers and Bst DNA polymerase [7, 8]. Furthermore, LAMP methods can be combined with simplified "boil-and-spin" DNA extraction techniques, reducing reliance on costly extraction kits and enhancing usability and portability in diverse field scenarios [9].

For species-specific detection, the mitochondrial Cytochrome b gene is identified as an optimal candidate due to its high copy number per cell and conserved regions, making it suitable for primer design. This gene's sensitivity, down to femtogram levels, positions it as a viable target for LAMP-based halal authenticity detection [9]. However, implementing this method effectively necessitates meticulous design and validation of specific primers for LAMP through rigorous in silico analysis, ensuring they do not cross-react with DNA from other common meat sources, such as beef, goat, and poultry [10].

This study, therefore, focuses explicitly on the in silico design and validation of LAMP primers targeting the Cytochrome b gene of pork. The aim is to develop a reliable, rapid, and field-applicable detection mechanism for halal authentication in processed meat products. The successful development and validation of these primers will be a pivotal step towards establishing a robust molecular tool that supports national halal food security and consumer confidence. Such methods promise to mitigate the risk of oversights in meat identification and enhance the integrity of halal-certified products [11].

2 Method

2.1 Data Collection

The study required an extensive search for the genome of Porcine (*Sus scrofa*) as markers of pork contamination in processed meat food products. This research focuses on *Sus scrofa*, an animal that has meat that is forbidden for Muslim consumption. The Cytochrome b gene in *Sus scrofa* was chosen because it has a very specific sequence in the species. These targeted genome sequences were taken from the GenBank sequence database by the National Center for Biotechnology Information (NCBI) with the accession number code AF034253.1[12].

2.2 Primer Design

Primer design was conducted using the complete sequence of the mitochondrial cytochrome b (cyt b) gene from porcine origin (GenBank accession number AF034253.1).

The quantitative PCR (qPCR) primers were designed by using the web-based software Primer3Plus, available at <https://www.primer3plus.com/index.html> for positive control standard operating procedure for molecule detection. Simultaneously, primers specific for loop-mediated isothermal amplification (LAMP) were developed using the NEB LAMP Primer Design Tool (<https://lamp.neb.com/#/>), which automatically generated sets of primers (F3/B3, FIP/BIP, and loop primers) targeting conserved regions within the *cyt b* gene to ensure high specificity and sensitivity for halal authentication [13–15].

2.3 In Silico PCR Validation

The optimized primer sets for qPCR and LAMP targeting the porcine cytochrome b (*cyt b*) gene were subjected to UCSC in silico PCR validation using the virtual UCSC PCR tool available at <https://genome.ucsc.edu/cgi-bin/hgPcr>. The primer specificity was critically evaluated to ensure high specificity to porcine DNA and minimal potential for off-target amplification. For the specificity analysis, virtual PCR was performed against genomic sequences representing common livestock and meat-producing species, including Cow, Sheep and chicken confirming the exclusivity of primer binding to porcine genomic sequences [16–18].

2.4 Data Analysis and Bioinformatic Validation

The nucleotide and protein sequences of the complete porcine mitochondrial cytochrome b (*cyt b*) gene (NCBI accession number AF034253.1) were retrieved in FASTA format. The protein sequence (GenBank: AAD34197.1) was structurally assessed and visualized using Alphafold 3 (<https://alphafoldserver.com/>). Primer sets designed for both qPCR (using Primer3Plus at <https://www.primer3plus.com/index.html>) and LAMP (using NEB LAMP Primer Design Tool at <https://lamp.neb.com/#/>) were analyzed based on several stringent parameters including primer length (18–25 nucleotides), melting temperature (T_m : 58–62°C), and GC content (40–60%).

The specificity of both optimized primer sets (qPCR and LAMP) was verified using in silico PCR at <https://genome.ucsc.edu/cgi-bin/hgPcr> against a database containing genomic sequences of livestock species commonly found in meat products, including Cow, Sheep and Chicken. Specificity was indicated by the generation of a single, distinct PCR amplicon exclusively matching the targeted porcine *cyt b* gene, with no cross-amplification observed for non-porcine species.

Additionally, the precise position and alignment of primers within the porcine *cyt b* sequence were confirmed using Sequence Manipulation Suite (SMS2): Primer Map online tool (https://www.bioinformatics.org/sms2/primer_map.html), ensuring robust specificity and suitability of the designed primers for halal authentication assays [12, 15, 18–20].

3 Results and Discussion

3.1 The Feature of Cytochrome b in *Sus scrofa*

The bioinformatic analysis successfully designed and validated loop-mediated isothermal amplification (LAMP) primers targeting the mitochondrial cytochrome b (cyt b) gene from porcine origin (GenBank accession number AF034253.1) Table 1 shows the amino acid sequence of the Cyt B protein. Optimal primer sets were generated with precise characteristics, including ideal primer length (18–25 nucleotides), melting temperatures (55–65°C), and GC content (36–60%), ensuring efficient isothermal amplification and reliability for halal authentication. Structural validation using Alphafold 3 revealed a stable 3D configuration of the porcine cyt b protein (Figure 1) [15, 21].

Specificity was rigorously confirmed via in silico PCR against genomic sequences of several food-producing animals, including Cow, Sheep and chicken. Results demonstrated that the primers exclusively amplified the porcine cyt b sequence without any cross-reactivity. Additionally, primer-target sequence alignment and structural analyses using bioinformatics tools confirmed precise hybridization, providing confidence in their specificity, accuracy, and suitability as robust molecular biomarkers for halal authentication of food products.

Table 1. Protein sequence of cytochrome b in *Sus scrofa* (GenBank: AAD34197.1).

Database and Accession Number	Sequence (in FASTA Format)	Size
Protein and AAD34197.1	MTNIRKSHPLMKIINNAFIDLPAAPSNISS WWNFGSLLGICLILQILTGLFLAMHYTS DTTTAFSSVTHICRDVNYGWVIRYLHA NGASMFFICLFIVGRGLYYGSYMFLET WNIGVLLFTVMATAFMGYVLPWGQM SFWGATVITNLLSAIPYIGTDLVEWIWG GFSVDKATLTRFFAFHFILPFIITALAAV HLLFLHETGSNNPTGISSDMDKIPFHPYY TIKDILGALFMMLILLILVLFSPDLLGDP DNYTPANPLNTPPHIKPEWYFLFAYAIL RSIPNKLGGVLALVASILILMLPMLHTS KQRSMMFRPLSQCLFWMLVADLITLTW IGGQPVEHPFIIIGQLASILYFLIILVLMPI TSHIENLLKW	42,812 kDa



Fig. 1. The cytochrome b in *Sus scrofa* in 3D Structure

3.2 Optimization of Primer Pair Design

The complete sequence of the porcine mitochondrial cytochrome b gene (GenBank AF034253.1) was used as the only template for oligonucleotide construction (Table 2), and one complete package of primers was obtained using Primer3Plus (for qPCR) and NEB LAMP Designer (five primers), after successive screening based on length (18–25 nt), melting temperature T_m (55–65 °C), GC content (36–60%), and ΔG threshold that eliminates hairpins or dimers (≤ -4 kcal mol⁻¹). The primers were blast tested against the genomes of non-pig food animals and validated again with virtual PCR on the genomes of Cow, Sheep and chicken; one qPCR pair and a complete set of LAMPs that meet all the criteria and produce one specific amplicon in silico, thus being designated as an optimized primary array for rapid halal detection [15, 21].

Table 2. Gene sequence of cytochrome b in *Sus scrofa* (GenBank: AF034253.1).

Database and Acces- sion Number	Sequence (in FASTA Format)	Size
Nucleotide and AF034253.1	ATGACCAACATCCGAAAATCACACCC ACTAATAAAAATTATCAACAACGCAT TCATTGACCTCCCAGCCCCCTCAAAC ATCTCATCATGATGAAACTTCGGTTCC CTCTTAGGCATCTGCCTAATCTTGCAA ATCCTAACAGGCCTGTTCTTAGCAATA CATTACACATCAGACACAACAACAGC TTTCTCATCAGTTACACACATTTGTCG AGACGTAAATTACGGATGAGTTATTC GCTATCTACATGCAAACGGAGCATCC ATATTTCTTTATTTGCCTATTCATCCAC GTAGGCCGAGGTCTATACTACGGATC	1,143 bp

CTATATATTCCTAGAAACATGAAACAT
 TGGAGTAGTCCTACTATTTACCGTTAT
 AGCAACAGCCTTCATAGGCTACGTCCT
 GCCCTGAGGACAAATATCATTCTGAG
 GAGCTACGGTCATCACAAATCTACTAT
 CAGCTATCCCTTATATCGGAACAGACC
 TCGTAGAATGAATCTGAGGGGGCTTTT
 CCGTCGACAAAGCAACCCTCACACGA
 TTCTTCGCCTTCCACTTTATCCTGCCAT
 TCATCATTACCGCCCTCGCAGCCGTAC
 ATCTCCTATTCCTGCACGAAACCGGAT
 CCAACAACCTACCGGAATCTCATCA
 GACATAGACAAAATTCCATTTACCC
 ATACTACACTATTAAGACATTCTAGG
 AGCCTTATTTATAATACTAATCCTACT
 AATCCTTGACTATTCTCACCAGACCT
 ACTAGGAGACCCAGACAACCTACACCC
 CAGCAAACCCACTAAACACCCACCC
 CATATTAACCCAGAATGATATTTCTTA
 TTCGCCTACGCTATTCTACGTTCAATT
 CCTAATAAACTAGGTGGAGTGTGGC
 CCCTAGTAGCCTCCATCCTAATCCTAA
 TTTAATGCCATACTACACACATCCA
 AACAACGAAGCATAATATTTGACCA
 CTAAGTCAATGCCTATTCTGAATACTA
 GTAGCAGACCTCATTACACTAACATG
 AATTGGAGGACAACCCGTAGAACC
 CGTTCATCATCATCGGCCAACTAGCCT
 CCATCTTATACTTCCTAATCATTCTAG
 TATTGATACCAATCACTAGCATCATCG
 AAAACAACCTATTAATGAAGA

All oligonucleotides selected for the porcine cytochrome b target (Table 2) met the established physical criteria length 18–25 nt, T_m 55–65 °C, and GC content 36–60 % and showed no ΔG dimer or hairpin values below -4 kcal mol⁻¹. Among the five LAMP primers, it was confirmed that only F3 and B3 were directly attached to the target gene and no loop formation occurred, this primer resulted in a target band of 145 bp whereas the qPCR pair had a target band of 575 bp. Then the order of oligonucleotides of all the selected primers is presented in table 3.

Table 3. The Selected qPCR and LAMP primers of the Cyt B gene of *Sus scrofa*.

Primer and Amplicon Size	Sequence	Feature
qPCR and 575 bp	Forward (Start=491) AATCTGAGGGGGCTTTTCCG	Length=20 bp, Tm=60.0C, GC=55.0%
qPCR and 575 bp	Reverse (Start=1065) GGCTAGTTGGCCGATGATGA	Length=20 bp, Tm=59.9C, GC=55.0%
LAMP and 145 bp	F3 (Start=437) CATCACAAATCTACTATCAGCT	Length=22 bp, Tm=55.04, GC=36%
LAMP and 145 bp	B3 (Start=606) GTTGTTGGATCCGGTTTC	Length=22 bp, Tm=55.04, GC=36%
LAMP and 145 bp	FIP CTTTGTCGACGGAAAAGCCCTCCCTTA TATCGGAACAGAC	Length=40 bp
LAMP and 145 bp	BIP ACCCTCACACGATTCTTCGCGAATAGG AGATGTACGGCT	Length=39 bp
LAMP and 145 bp	LB (Start=557)GCCATTCATCATTACCGCCC TC	Length=22 bp, Tm=65.51, GC=55%

The accuracy of primary attachment positions for qPCR and LAMP targeting mitochondrial cytochrome b (cyt b) genes *Sus scrofa* (GenBank: AF034253.1) has been verified using SMS2 Primary Map Online Analysis. This validation step ensures that the entire primer adheres appropriately to the predefined region in the target gene sequence. Although modern primary designing software can produce sequences efficiently, the accuracy of the nucleotide attachment position still needs to be verified. Therefore, the primary attachment location was reconfirmed and showed that the qPCR included nucleotide positions 491 to 1065, fully in accordance with the initial design results. Similarly, all primaries in LAMP were also mapped in regions 437 to 606, ensuring correct orientation and specificity of the target (Figure 2). This verification reinforces the reliability of a primer designed for accurate detection of porcine DNA in halal testing.

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Primer Map results
Results for linear 1143 residue sequence "sample sequence" starting "atgacccaaca"

1 atgacccaacaatccgaaaaatcacaccccctaataaaaaatbatcaacaaacgcattccattgac
1 10 20 30 40 50
1 taactgggtgtaggcttttagtgggtgggtgattatttttaaagttgttgcgtaagtaactcg
61 ctcccagcccccccccaaacatctctcatgatgaaacttcggttccccctctaggcatctg
61 70 80 90 100 110
61 gaggggtcggggggagttttagagtagtactacttttgaagccaaaggggagaatcccgtagac
121 cctaaatcttggcaaatcctaaacagggcctgtcttagcaaatcaatcaacatcagacacaaac
121 130 140 150 160 170
121 ggtatagaaacggtttaggattgtccgggacaagaatcgtgtatgttaagtgtgtatgttggttg
181 aacagcctttctccacagtttaacacacatttctcgagacgtaaaatcagggataggttactcg
181 190 200 210 220 230
181 ttgtcgaaaagtagtcaatgtgtgttaaacagctctcgcattttaaagtcctcaacataaagc
241 ctatctcaatgcaaaacgggagcatccatattttcttttatttgcctattccatccacagtaggcc
241 250 260 270 280 290
241 gatagatgtacggtttgcctcgttaggtataaagaataaaacgggataagtagggtcattccgg
301 gagggctatctacagggatcctataatctccagaaacatgaaacacatggagtagcctctac
301 310 320 330 340 350
301 ctccagatgatgatgacctaggatataaaaggatcttctgtactcttgaacctcaagtagt
361 attttaccggttatagcaaacagcctctcataggctacgctcctgctgaggagcaaatatcat
361 370 380 390 400 410
361 ataaaatggcaaatatcgttctcggaaggtatcccgatgcaggaggggactcctgtttatagta
    >>>LAMP_F3>>> 437 to 458
421 tctgaggagctacgggtcaccacaaaatctcaatcaatcccttatatcccttataatcggaacagacc
421 430 440 450 460
421 agactcctctgactgcccagtagtggtttagatgatagtagtaggggaatataagccttgbctgg
    >>>qRT_Forward>>> 491 to 510
481 tctgtagaatgaaatctgaggggggcttttccgtctcgacaacaaagcaacccctcacaacgatctctg
481 490 500 510 520 530
481 agcatcttactctagactcccccgaaaaagggcagctgttctcgttggggagttgtctaaagaagc
541 ccttccactttatctcctccatctcatcattaccgcccctcgcagccctacatcctcccattctc
541 550 560 570 580 590
541 ggaaggtgaaataggcaggttaagttagtataaggcgggagcgtggccatgttagaggataaagg
    <<<LAMP_B3<<< 606 to 628
601 tgcacgaaacccggatcccaacaccccctaccggaaatctccatccagacatagacaaaatctccat
601 610 620 630 640 650
601 acgtgcttttggcctcctagggttcttggggatggcctctagagtagtctgtatctgttcttaaggta
661 ttcacccatactacaactatcaaacacattctcaggagccttattttataactaactaaatccctac
661 670 680 690 700 710
661 aagtgggtatgatgtgataatctctgtaaagatcctcggaaataaataatattttagttaggagtg
721 taatctccggtactatctcaccagacctacaggagaccagacaactacaccccagcaaa
721 730 740 750 760 770
721 attagggacaatgataaagtagtgggtctgggatgactcctctgggtctggttagtctggggctcgtt
781 acccactaaacacccccccccatattaaacccaagatgatatttcttattctcgcctaccgcta
781 790 800 810 820 830
781 tggggtagtttgggggtgggggtataatttggctcttactataaaaagaataaagcggatgctgat
841 tctctacgctcaatctccataaataactagggtggagtggttggcctccttagtagcctccactca
841 850 860 870 880 890
841 aagatgtcaagtttaaggattatcttgatccccctcacaacccggggatcattcctggaggtaggat
901 atctcaatctttaaagtcctcactacacacatcccaaacacaggaagcataatattcttcgacca
901 910 920 930 940 950
901 taggattaaaaatcaggggtatgatgtgtgttaggtttgttgcctctgattataaaagctgggt
961 ctaaagtcagtccctattctgaaatactagttagcagacctcattacacatcaacatgaattggga
961 970 980 990 1000 1010
961 gattcagttaccggataaagacttatgatcctcgtctggagtaaatgtgattgttacttaacct
    <<<qRT_Reverse<<< 1046 to 1068
1021 ggcacccccggtagaacaccgcttccatcctcctcagggccactcagcctccatcttactctc
1021 1030 1040 1050 1060 1070
1021 cctcttgggcctcttctggggcaagtagtagtagcgggttgatcggaggtgataaataatgaaag
1081 ctaatcctctcagatgatgtgacccaatcactagcattcatcgaaaacacccctataaaaatgga
1081 1090 1100 1110 1120 1130
1081 gatttagtaagatcataactatgggttagttagctagtagctcttcttgggtgataatcttact
1141 aga
1141 tct
    
```

Primer:	Sequence:
qRT_Forward	5'-AATCTGAGGGGGCTTTTCCG-3'
qRT_Reverse	5'-GGCTAGTTGGCCGATGATGA-3'
LAMP_F3	5'-CATCACAAAATCTACTATCAGCT-3'
LAMP_B3	5'-GTTGTTGGATCCGGTTTC-3'

Fig. 2. The result of compatibility and primer position in the Cyt B *Sus scrofa* gene sequence

3.3 In-silico PCR performance

To ascertain the specificity of the primer designed against the porcine cytochrome b gene, in silico PCR analysis was performed using an online simulation device from UCSC In-Silico PCR (<https://genome.ucsc.edu/cgi-bin/hgPcr>). The platform supports simulation against the latest genomic databases curated from NCBI, including mitochondrial and plasmid genomes. This tool allows adjustment of primary inputs, mismatch tolerance (≤ 1 mismatch), and the maximum expected amplicon length (up to 10,000 bp). The qPCR and LAMP primers that have been designed are evaluated using

this system to predict their ability to selectively amplify the cyt b region of porcine as well as rule out possible amplification from non-target species. Degenerative bases were allowed to simulate extreme nucleotide variations, and plasmid sequences were also included to anticipate the possibility of false positive amplification in processed meat products.

Analysis was carried out on the mitochondrial genome of *Sus scrofa* and a comparison panel that included several other animal species commonly found in the processed meat food industry, including Cow, Sheep and chicken. The results of the PCR simulation in silico showed that both the qPCR primer and LAMP exclusively produced one amplicon sized according to the bp target in the pig genome. No amplification products were found in any non-pig species, which confirms a high level of specificity. These findings reinforce that the primer that has been designed is a reliable and discriminating molecular tool for halal authentication, and provides robust predictive validation prior to the implementation of laboratory tests [21, 22].

UCSC In-Silico PCR	
Genome: Cow	Assembly: Apr. 2018 (ARS-UCD1.2/bosTau9)
UCSC In-Silico PCR	
Genome: Sheep	Assembly: Nov. 2015 (Oar_v4.0/oviAri4)
UCSC In-Silico PCR	
Genome: Chicken	Assembly: Mar. 2018 (GRCg6a/galGal6)

Fig. 3. Data on non-target species that are often used as meat for food processing for testing to primary validity

PCR in silico amplification was then performed using qPCR and LAMP primers. The results of virtual amplification are shown in Figures 4 and 5. Based on the visualization, only sequences from porcine produced amplification targets according to the design, either by qPCR or LAMP primers. No amplification products were found in the genomes of other species in the test panel, including Cow, Sheep and Chicken. These results confirm the high specificity of the primer designed to recognize the pig cytochrome b gene without cross-reactivity against other species commonly used in processed meat food products. This confirms the strong potential of such primers in rapid molecular detection applications for halal authentication.

UCSC In-Silico PCR	
Genome: Cow	Assembly: Apr. 2018 (ARS-UCD1.2/bosTau9)
UCSC In-Silico PCR	
Genome: Sheep	Assembly: Nov. 2015 (Oar_v4.0/oviAri4)
UCSC In-Silico PCR	
Genome: Chicken	Assembly: Mar. 2018 (GRCg6a/galGal6)

Fig. 4. Primers qPCR Validation Results Obtained Target and Non-Target Species Using UCSC In-Silico PCR

UCSC In-Silico PCR	
Genome: Cow	Assembly: Apr. 2018 (ARS-UCD1.2/bosTau9)
UCSC In-Silico PCR	
Genome: Sheep	Assembly: Nov. 2015 (Oar_v4.0/oviAri4)
UCSC In-Silico PCR	
Genome: Chicken	Assembly: Mar. 2018 (GRCg6a/galGal6)

Fig. 5. Primers LAMP Validation Results Obtained Target and Non-Target Species Using UCSC In-Silico PCR

3.4 Advantages of using LAMP primer in the detection of Porcine Cytochrome b for Halal Authentication

The Loop-mediated Isothermal Amplification (LAMP) method offers several advantages for halal authentication, particularly in detecting porcine cytochrome b DNA. Unlike conventional PCR, LAMP operates under a constant temperature (typically 60–65 °C), eliminating the need for thermal cycling equipment. This single-temperature requirement makes LAMP highly suitable for point-of-need testing, portable diagnostics, or resource-limited settings. As illustrated in the provided schematic, the mechanism initiates with the annealing of outer primers (F3 and B3) and inner primers (FIP and BIP), followed by strand displacement and loop formation that enables continuous and exponential amplification. This self-priming capability accelerates reaction kinetics, typically producing detectable results within 30–60 minutes.

Moreover, LAMP's primer design enhances specificity by employing 4 to 6 primers that recognize up to 6 distinct regions on the target DNA: two outer primers, two inner primers (each comprising F1c+F2 and B1c+B2), and optional loop primers (LF and LB) (Figure 6). This multi-site targeting approach drastically reduces the risk of false positives and non-specific amplification, as successful amplification only occurs if all primers correctly hybridize to their respective regions. In the context of halal authentication, this means the presence of porcine DNA can be confirmed with high confidence, as the assay requires simultaneous alignment of multiple primer sets. This robust design makes LAMP a powerful molecular tool for reliable, rapid, and field-adaptable detection of non-halal contaminants [15, 21, 23].

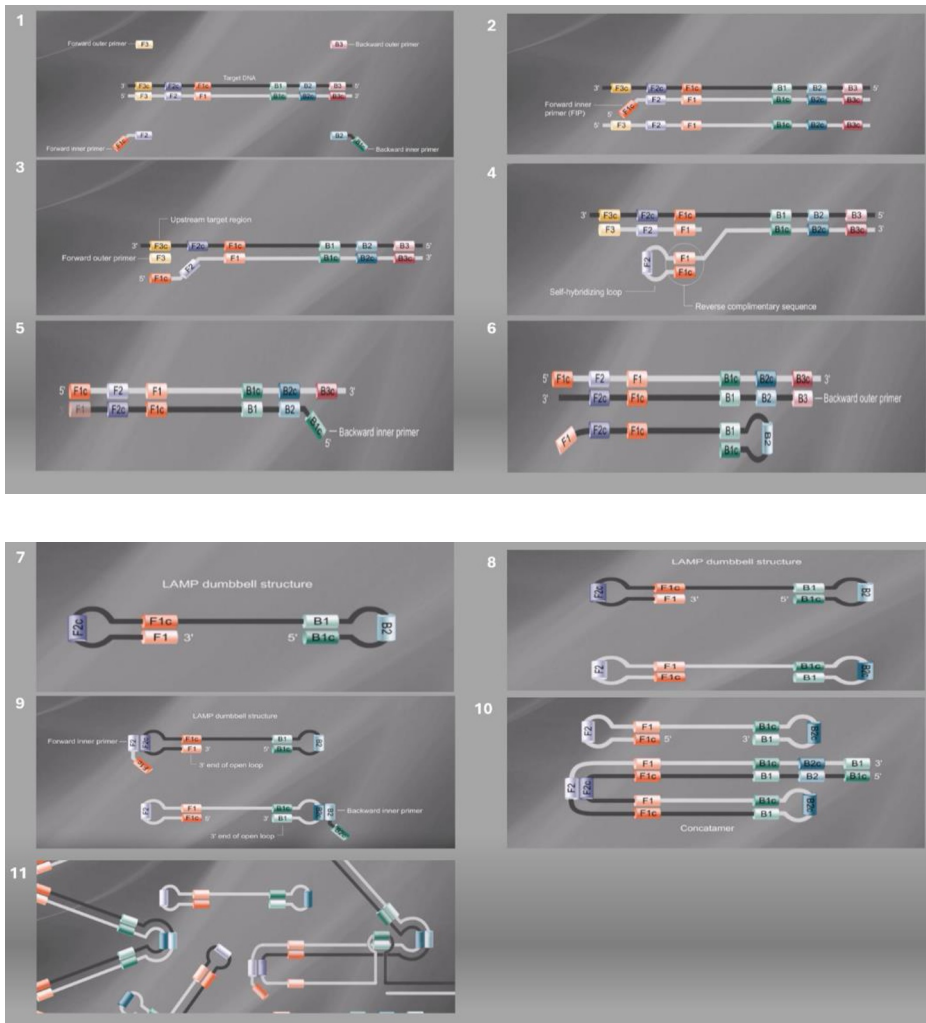


Fig. 6. Stages from the beginning (1) to the last (10) amplification process using LAMP Primer (Source: New England Biolabs)

4 Conclusion

This study successfully demonstrated the in silico design and bioinformatic validation of qPCR and LAMP primers targeting the porcine mitochondrial cytochrome b gene for halal authentication. The selected primers met all essential criteria including optimal length, melting temperature, GC content, and minimal secondary structure formation. Both qPCR and LAMP primers showed high specificity by producing a single, expected-size amplicon exclusively in *Sus scrofa* sequences, while no amplification was observed in non-target species such as *Bos taurus*, *Ovis aries*, and *Gallus gallus*. These findings confirm that the designed primer sets are highly discriminatory and effective for species-specific detection of porcine DNA.

Moreover, the application of LAMP offers a practical advantage due to its isothermal nature, requiring only a constant temperature and minimal instrumentation, making it suitable for rapid on-site testing. The use of multiple primers recognizing six distinct regions of the target gene significantly enhances the specificity of LAMP, reducing the possibility of false positives. Therefore, both qPCR and LAMP primers developed in this study are reliable molecular tools for halal authentication and can serve as a foundational method in food quality control and religious compliance monitoring.

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