Current Research in Food Science 7 (2023) 100609

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Design and development of a rapid meat detection system based on RPA-CRISPR/Cas12a-LFD

Yaqun Liu [a,b,1], Liyun Lin [a,b,1], Huagui Wei [c], Qiulan Luo [a,b], Peikui Yang [a,b], Mouquan Liu [a,b], Zhonghe Wang [a,b], Xianghui Zou [a,b], Hui Zhu [a,b], Guangcai Zha [a,b], Junjun Sun [a,b], Yuzhong Zheng [a,b,c,*], Min Lin [a,b,c,**]

[a] School of Food Engineering and Biotechnology, Hanshan Normal University, Chaozhou, Guangdong, PR China
[b] Guangdong Provincial Key Laboratory of Functional Substances in Medicinal Edible Resources and Healthcare Products, Chaozhou, Guangdong, PR China
[c] Shool of Laboratory Medicine, Youjiang Medical University for Nationalities, Baise, Guangxi, PR China

A R T I C L E I N F O

Keywords:
Meat identification
RPA
CRISPR/Cas12a
LFD

A B S T R A C T

In recent years, meat adulteration safety incidents have occurred frequently, triggering widespread attention and discussion. Although there are a variety of meat quality identification methods, conventional assays require high standards for personnel and experimental conditions and are not suitable for on-site testing. Therefore, there is an urgent need for a rapid, sensitive, high specificity and high sensitivity on-site meat detection method. This study is the first to apply RPA combined with CRISPR/Cas12a technology to the field of multiple meat identification. The system developed by parameter optimization can achieve specific detection of chicken, duck, beef, pork and lamb with a minimum target sequence copy number as low as 1×10^0 copies/μL for 60 min at a constant temperature. LFD test results can be directly observed with the naked eye, with the characteristics of fast, portable and simple operation, which is extremely in line with current needs. In conclusion, the meat identification RPA-CRISPR/Cas12a-LFD system established in this study has shown promising applications in the field of meat detection, with a profound impact on meat quality, and provides a model for other food safety control programs.

1. Introduction

Food quality and safety are relevant to everyone, and with the pursuit of healthy food, nutritional quality has become the direction of personalized consumption in modern life. Meat has become a daily consumer product, and the demand for quantity as well as quality is increasing, so the raising of prices of high-quality meat such as beef and lamb in the market is obvious (Scollan et al., 2017; Vidal et al., 2022). To enhance the profit space, there are cases where poor-quality meat is used as high-quality meat (Li et al., 2020). Among them, low-priced chicken, duck, pork, etc., pretending to be beef and lamb to become the main meat adulteration method (Siddiqui et al., 2021). The deception of consumers through meat adulteration has spread without borders around the world and is becoming increasingly sophisticated and hidden. For example, the horsemeat scandal in Europe (Brooks et al., 2017), counterfeit beef in the Turkey and Egyptian markets (Galal-Khallaf, 2021; Alamprese et al., 2016), unidentified animal meat mixed in meat and milk products in Bangladesh (Afifa et al., 2021), and various meat products adulterated in China (Liu et al., 2023), Korea (Ha et al., 2017) and Poland (Kowalczyk, 2021) have also been reported. The proliferation of meat adulteration not only terferes with the market discipline and causes serious loss of interest to consumers but also poses significant food safety risks. Therefore, the development and innovation of meat identification technology is a strong guarantee and urgent need to combat the problem of adulterated meat.

There are many methods to identify meat adulteration, including electronic nose and electronic tongue (Zaukuu et al., 2021), immunoassay (Mandli et al., 2018), spectroscopy (Fengou et al., 2021), DNA molecular hybridization technology (Demarquoy, 2013), etc. With the use of electronic noses and electronic tongues to identify samples, it is

* Corresponding author. School of Food Engineering and Biotechnology, Hanshan Normal University, Chaozhou, Guangdong, PR China.
** Corresponding author. School of Food Engineering and Biotechnology, Hanshan Normal University, Chaozhou, Guangdong, PR China.
E-mail addresses: zhengyuzhon@hstc.edu.cn (Y. Zheng), 2641@hstc.edu.cn (M. Lin).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.crfs.2023.100609
Received 15 May 2023; Received in revised form 12 September 2023; Accepted 2 October 2023
Available online 5 October 2023
2665-9271/© 2023 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

difficult to accurately quantify each component of the sample due to the different doping ratios. Although methods such as immunoassay possess the advantages of specificity and sensitivity, they also carry significant limitations, including requirements for sample structure, low reproducibility, and high false positive rates. The main meat identification techniques developed based on nucleic acid detection are polymerase chain reaction (PCR) and its derivatives. Although PCR technology is very accurate, it is time consuming, costly, and requires a high level of expertise. To better identify the quality of meat, a rapid, sensitive, low-cost, portable, and easily operated detection technology is needed.

In recent years, emerging isothermal amplification methods, such as loop-mediated isothermal amplification (LAMP) (Yan et al., 2022), cross-primer amplification (CPA) (Zheng et al., 2020) and recombinase polymerase amplification (RPA) systems (Lin et al., 2021), have been applied to meat identification. RPA, the most widely used system, is a rapid, simple and highly discriminatory DNA amplification technique. Only one pair of specific primers can amplify target fragments to detectable levels in a short period of time at a constant temperature of 37–42 °C without the need for expensive instrumentation (Lin et al., 2021). Clustered regularly interspaced short palindromic repeats/CRISPR-associated12a (CRISPR/Cas12a) is an effector protein of the type V CRISPR/Cas system, which relies on small CRISPR-derived RNAs (crRNAs) to guide specialized nucleases to foreign nucleic acids. Coupled with a fluorescent probe at the same time, the color change of the reaction system can be directly observed by the naked eye (Paul et al., 2020). Since the optimal temperature for Cas12a to function as a nuclease is similar to that of RPA (both at approximately 37 °C), the two can be used in combination. In addition, lateral flow dipstick (LFD) is a common way to present qualitative visualization data, allowing for direct and rapid determination of assay results (Lin et al., 2021). The reporter probes in the reaction system can display labeled signals on the LFD, enhancing and ensuring visualization.

Currently, CRISPR/Cas12a combined with the RPA detection system has been successfully applied in the fields of medical inspection and clinical diagnosis (Jirawannaporn et al., 2022; Zheng et al., 2022) and has been widely explored in food quality and safety, including the detection of foodborne pathogens such as *Burkholderia gladioli* and *Venturia carpophila* (Zheng et al., 2023; Hu et al., 2023), the detection of genetically modified crops (32,711,280), and the detection of food adulteration (Zhao et al., 2022; Huang et al., 2023). However, the actual application and promotion of RPA-CRISPR/Cas12a in the food field is still in the developmental stage, and actively exploring the application of this technology in food quality and safety can effectively enhance the effectiveness of food safety assurance. Therefore, this study will combine RPA amplification, CRISPR/Cas12a and LFD to establish a rapid visualization molecular technology for meat quality assessment. By optimizing the detection conditions, a rapid, sensitive and specific identification platform for on-site detection of meat quality in molecular biology was established with the aim of providing information for meat identification studies and reliable technical support for immediate detection of meat.

2. Materials and methods

2.1. Sampling and genomic DNA extraction

Meat samples (chicken, duck, beef, pork and lamb) were purchased from local supermarkets and markets in Chaozhou City, Guangdong Province, China. Chicken, duck, beef, pork and lamb were each weighed to 5 mg and ground to powder in liquid nitrogen using a mortar and pestle. The total genomic DNA of each sample was extracted using a Tissue Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. The quantity and quality of DNA were verified by 1% agarose gel electrophoresis and measuring their absorbance at 260 and 280 nm (NanoDrop ND-2000, Thermo-Fisher Scientific Inc., Waltham, MA, USA). All DNA samples were stored at −20 °C for further amplification.

2.2. Design of RPA primers for meat

The meat mitochondrial genomic sequences used in this study were obtained from the National Center for Biotechnology Information website (https://www.ncbi.nlm.nih.gov): chicken (*Gallus*, NC_040902.1), duck (*Anas platyrhynchos*, NC_009684.1), pork (*Sus scrofa*, NC_000845.1), beef (*Bos taurus*, NC_006853.1) and lamb (*Ovis aries*, NC_001941.1). Three pairs of RPA primers were designed in specific gene regions of each meat according to the RPA design principles proposed in the TwistAmp DNA amplification kit combination instructions (TwistDx, Cambridge, U.K.) (Table 1), followed by primer specificity verification by primer-BLAST. Design of meat target-specific CrRNAs based on CRISPR/Cas12a recognition of specific target sequences via the CRISPR-DT online website (http://bioinfolab.miamioh.edu/CRISPR-DT/) (Table 2). The primers and CrRNAs were synthesized by Genewiz Biotech Co. (Jiangsu, China).

2.3. Optimization of the RPA-CRISPR/Cas12a-LFD system

The RPA amplification system was performed using the TwistAmpTM Basic kit (TALQBAS01, TwistDx Limited, UK), and the reaction conditions were A buffer (29.4 μL), MgAc (2.5 μL), primer pairs (10 nM, 2 μL for each) and DNA template (2 μL). Then, nuclease-free water up to 50 μL was incubated at 39 °C for 15–25 min. Successful RPA amplification was verified by 2% agarose gel electrophoresis. Subsequent RPA-CRISPR/Cas12a reaction conditions were as follows: RPA product (5 μL), buffer (10X, 2 μL), CrRNA (1 μM), Cas12a (Tolo Biotech, China, 0.35 μM), ssDNA fluorescent probe (0.5 μM) and nuclease-free water (3 μL). The above system was mixed and incubated at 42 °C in a fluorescence reader (Biolifesci Co., Ltd., Guangzhou, China) for 30 min, and fluorescent signals were collected every 15 s (ssDNA FQ substrate = λex: 485 nm; λem: 535 nm) (Wei et al., 2023). The same RPA-CRISPR/Cas12a system was incubated for 30 min at 39 °C. The product was added to 50 μL of nuclease-free water and transferred into a commercial lateral flow test strip (No. JY0301, Tiosbio Biotechnology Co., Ltd., Beijing, China). The control line (C line) and the test line (T line) were observed within 10 min, with negative showing only the C line and positive showing both lines.

In the above RPA-CRISPR/Cas12a-LFD system, the other parameters were kept unchanged, and the RPA primers (three pairs of primers randomly paired for each meat), MgAc additions (set to 2.0, 2.3, 2.5, 2.8 and 3.0 μL), RPA temperature (set to 37, 39, 42, 45 and 48 °C), RPA time (set to 10, 15, 20, 25, 30 min) and Cas temperature (set to 37, 42, 48, 52 and 60 °C) were adjusted to select the unchanged reaction parameters based on the band brightness and fluorescence intensity.

2.4. Specificity verification

On each established optimized meat detection platform, the corresponding meat was used as a control to test and observe other meats. For example, in the RPA-CRISPR/Cas12a-LFD system for chicken, the genomes of chicken, duck, pork, beef and lamb were assayed (chicken genome as positive control), and the fluorescence signal and LFD results were captured. Similarly, in the RPA-CRISPR/Cas12a-LFD assay system for duck, pork, beef and lamb, the individual genomes were used as controls for other meats to distinguish the specificity of each system. Samples of commercially available meat products were also tested to verify the feasibility of the system.

2.5. Sensitivity verification

The plasmids containing the target DNA fragments were constructed by Hangzhou QingkeZixi Biotechnology Co., Ltd. The recombinant plasmids of five meats were quantified and diluted from 1×10 to 10^6

Table 1
Primer sequences used in the RPA assay for meat.

Meat	F[a]	Primer sequences	R[a]	Primer sequences
Chicken	F1	CCAGCAAATTATAGACCCAC	R1	GATTGTTGGTAGGATGATCT
	F2	TCGACCCAGCAAATTATAGACCCAC	R2	AGTATGATTGTTGGTAGGATGATCT
	F3	TGGTTTCGACCCAGCAAATTATAGACCCAC	**R3**	GCAAGAGTATGATTGTTGGTAGGATGATCT
Duck	F1	TCCTTCCCACAGTATCAATC	R1	CGGCTAGCAGGATAGATGAGTT
	F2	GCCCATCCTTCCCACAGTATCAATC	**R2**	CGGCGGCTAGCAGGATAGATGAGTT
	F3	CTCATGCCCATCCTTCCCACAGTATCAATC	R3	AGGCCGGCGGCTAGCAGGATAGATGAGTT
Beef	**F1**	ACATCGCCTAGCTCCATACA	R1	AGTGAAGAGGCCATAGGGA
	F2	ATTATACATCGCCTAGCTCCATACA	R2	TCTTTAGTGAAGAGGCCATAGGGA
	F3	CCACAATTATACATCGCCTAGCTCCATACA	**R3**	GGTTCTTTAGTGAAGAGGCCATAGGGA
Pork	**F1**	CACTCGCATTAACAATCACC	R1	GGTGTATTTTGGTAGCACGGA
	F2	AAACACTCGCATTAACAATCACCTT	R2	AATGTGGTGTATTTTGGTAGCACGGA
	F3	AAAACACTCGCATTAACAATCACCTTC	**R3**	TGTAGAATGTGGTGTATTTTGGTAGCACGGA
Lamb	**F1**	GCCTCTCCAGTATTAAACTTGC	R1	CGTTTGCATGTTTAAGACAGAC
	F2	AAAGAGCCTCTCCAGTATTAAACTTGC	**R2**	GTACTCGTTTGCATGTTTAAGACAGAC
	F3	AATATAAAGAGCCTCTCCAGTATTAAACTTGC	R3	ACTATGTACTCGTTTGCATGTTTAAGACAGAC

[a] F: forward primer; R: reverse primer. The optimal RPA primer pairs are bolded.

Table 2
CrRNA sequences and target sequences used for meat.

Meat	crRNA sequences	Target sequences
Chicken	UAAUUUCUACUAAGUGUAGAUCGUCCUCCUCCUAAGCCUGU	ACAGGCTTAGGAGGAGGACG
Duck	UAAUUUCUACUAAGUGUAGAUCUACUGGUCAGAUUGAGAGG	CCTCTCAATCTGACCAGTAG
Beef	UAAUUUCUACUAAGUGUAGAUUAGAACAAUAGGACUCGAAC	GTTCGAGTCCTATTGTTCTA
Pork	UAAUUUCUACUAAGUGUAGAUUGAACGCUCAUGUCCGUGGG	CCCACGGACATGAGCGTTCA
Lamb	UAAUUUCUACUAAGUGUAGAUUAUAAUAACCACAACACCAA	TTGGTGTTGTGGTTATTATA

copies/μL according to the calculation formula proposed earlier (Lin et al., 2021), and the RPA-CRISPR/Cas12a-LFD reaction was performed under optimal conditions. The results were demonstrated by fluorescence signal and LFD.

2.6. Practical product validation

Commercially available products containing chicken, duck, beef, pork, and lamb (local supermarkets and online stores) and artificially adulterated samples (meat artificially mixed in different proportions) were blindly tested by RPA-CRISPR/Cas12a-LFD. The sample order was randomized and renumbered. Final validation was performed by PCR

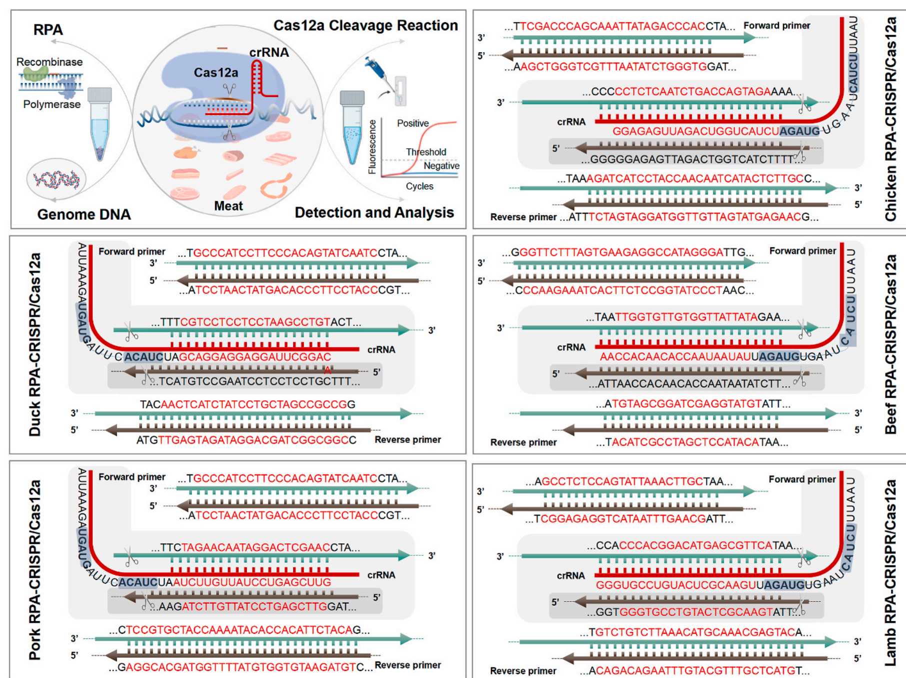

Fig. 1. Meat identification platform based on RPA-CRISPR/Cas12a and sequence-specific cleavage by crRNA. Top left: simulation principle diagram of RPA-CRISPR/Cas12a-LFD. Others: primer design and sequence-specific cleavage of chicken, duck, beef, pork and lamb.

(Lin et al., 2021).

3. Results

3.1. Construction scheme of RPA-CRISPR/Cas12a-LFD meat identification

The principle of the RPA-CRISPR/Cas12a-LFD meat identification platform established in this study is as follows: The target gene segment of the meat genome was amplified by RPA at 37 °C under isothermal conditions to enhance the signal. The Cas12a protein is then guided by crRNA to recognize specific nucleic acid sequences in isothermally amplified samples. In the presence of the target sequence in the test sample, crRNA binds with the target sequence to activate the non-specific cleavage activity of the Cas12a protein and cleaves the nucleic acid in solution. When a sample is irradiated with short-wave UV radiation, the positive sample fluoresces, while the negative sample does not, allowing for the identification of meat. In addition, we introduced LFD technology to visualize the amplification products, which provides a promising prospect for the application of rapid detection of nucleic acids in meat fields (Fig. 1).

3.2. Design and screening of RPA primers

To improve the accuracy of meat identification, preliminary screening of primers is needed. Three sets of primers were designed according to the conserved regions on the sequences of each of the five meats. The RPA primers were randomly combined to select primer pairs with high efficiency and non-specific amplification. The results showed that all the random primer pairs of meat exhibited a single band after RPA amplification. The RPA products corresponding to F1R2/F2R1/F2R3 for chicken, F2R2/F2R3/F3R2 for duck, F1R2/F3R3/F3R3 for beef, F1R1/F3R3/F3R1 for pork and F1R2/F2R2/F2R3 for lamb under the same conditions displayed brighter and clearer bands. Three preferred primer pairs for each meat were paired with CrRNA and further screened by Cas12a fluorescence assay, indicating that the optimal RPA primer pairs for chicken, duck, beef, pork and lamb were F2R3, F2R2, F1R3, F1R3 and F1R2, respectively (Fig. 2).

3.3. Optimization of the RPA-CRISPR/Cas12a-LFD meat identification platform

To obtain a more stable and reliable RPA-CRISPR/Cas12a-LFD platform for meat identification, comparative tests of fluorescence intensity induced by several major parameters were performed. The experimental results were compared, analyzed and evaluated to find the perfect choice of these main parameters. First, to screen the optimal MgAc concentration in the RPA reaction, five different MgAc additions (2.0, 2.3, 2.5, 2.8 and 3.0 μL) were set for RPA amplification, and the results showed that the optimal MgAc additions were 2.0 μL for both chicken and beef, 2.8 μL for duck and lamb, and 2.3 μL for pork. The second parameter is the RPA temperature (set to 37, 39, 42, 45 and 48 °C), with the optimal RPA temperature being 42 °C for chicken, beef and lamb and 37 °C for duck and pork. The third adjustment parameter was the RPA time (set to 10, 15, 20, 25, 30 min), and the optimal RPA reaction time was 20 min for chicken, lamb and pork, 25 min for duck and 15 min for beef. The last parameter is Cas temperature (set to 37, 42, 48, 52 and 60 °C); the optimal Cas temperature is 42 °C for chicken, beef and lamb and 37 °C for duck and pork, which is consistent with their own corresponding optimal RPA temperatures (Fig. 3).

3.4. Experimental validation of the specificity of the RPA-CRISPR/Cas12a-LFD meat identification platform

Platform-specific identification with optimal primers and parameters in each meat RPA-CRISPR/Cas12a-LFD system using genomic DNA from chicken, duck, beef, pork and lamb as templates. Significantly strong fluorescent signals were observed on the corresponding meat RPA-CRISPR/Cas12a assay platform for each meat individually, and no detectable signals were detected in other meats and blank controls. That is, the RPA-CRISPR/Cas12a platform for chicken only works successfully in the presence of chicken, while duck, beef, pork and lamb do not pass. Similarly, the RPA-CRISPR/Cas12a platform for duck, beef, pork and lamb is only applicable to its own corresponding meat and cannot be applied to other meats. The final results were confirmed in both LFD strips and gel electrophoresis, where the meat genome fluoresced in the corresponding system and bands, then bands appeared in the electropherogram and were visualized with LFD (Fig. 4). Tests on commercially available meat samples also proved the feasibility of each system.

3.5. Experimental validation of the sensitivity of the RPA-CRISPR/Cas12a-LFD meat identification platform

Meat genome gradient dilution (concentration of 1×10^0–1×10^6 copies/μL) was used as a template for sensitivity experiments. The results showed that the RPA-CRISPR/Cas12a detection system for chicken, duck, beef, pork and lamb could detect different levels of fluorescence in the tubes, and the lower limit of detection was 1×10^0 copies/μL. The sensitivity trends were similar for chicken, beef and pork, with the lower limit of detection being more significant at higher concentrations (1×10^3–1×10^6 copies/μL). Duck and lamb were closer, with fluorescence detection results appearing strongly at a concentration of 1×10^0 copies/μL, especially for lamb. It is worth noting that the visualization results of the LFD can only be observed with the naked eye when the fluorescence is strong. This means that there is a lower limit of naked eye detection, which is 1×10^3 copies/μL for chicken, beef and pork and 1×10^0 copies/μL for duck and lamb (Fig. 5).

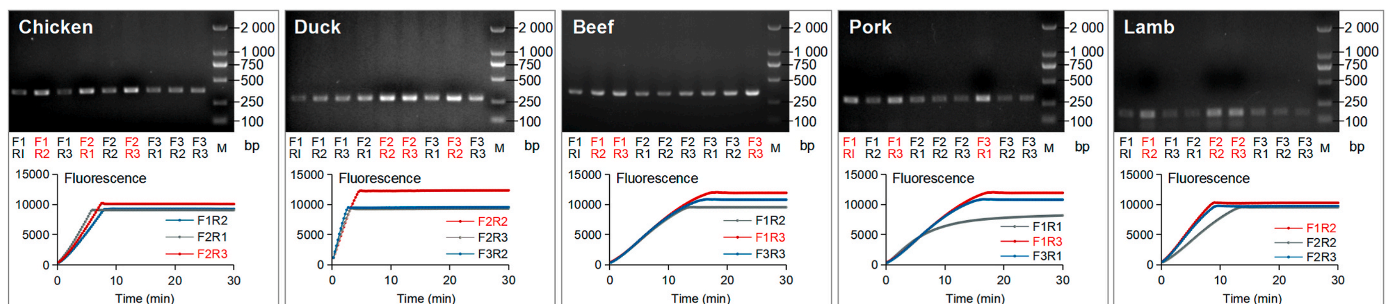

Fig. 2. RPA primer set screening. Above: gel electrophoresis of RPA products using random primer pairs. RPA primer pairs with bright and clear bands are labeled in red. Below: Fluorescence values of the RPA and CRISPR/Cas12a detection systems. RPA primer pairs with high fluorescence values are labeled in red. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

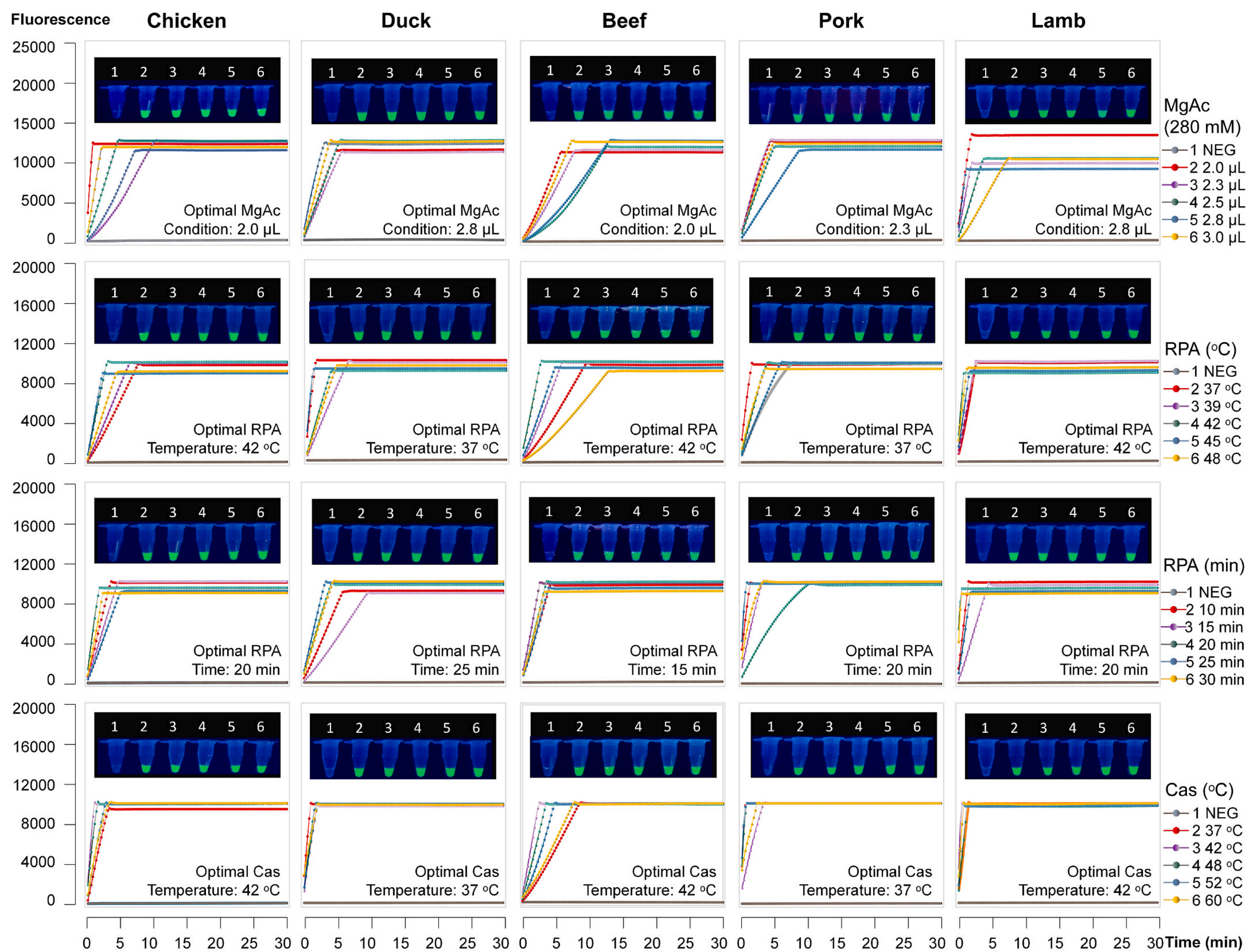

Fig. 3. Comparison of fluorescence detection results for RPA-CRISPR/Cas12a-LFD meat identification platform parameters. The parameters from top to bottom: MgAc additions, RPA temperature, RPA time and Cas temperature. Samples from left to right: chicken, duck, beef, pork and lamb. Optimal parameters are marked in the figure.

3.6. RPA-CRISPR/Cas12a-LFD meat identification platform for detection in products

To observe the application of RPA-CRISPR/Cas12a-LFD in practice, we analyzed 23 different samples, including commercially available meat products, as well as different proportions of artificially mixed meat. The results showed that RPA-CRISPR/Cas12a-LFD clearly recognized all samples and clearly detected the source components in different products from chicken, duck, beef, pork and lamb. Pork-derived ingredients were detected in some duck and beef products, while chicken-derived ingredients were detected in pork products. After artificially mixing different meats in different proportions, RPA-CRISPR/Cas12a-LFD can also detect the corresponding sources. The accuracy of RPA-CRISPR/Cas12a-LFD was further validated by PCR (Fig. 6).

4. Discussion

Different detection techniques have been established to effectively evaluate and prevent hazards caused by meat safety. The combination of modern molecular methods and information technology has provided a broad platform for the identification of meat adulteration. The currently used techniques for meat identification in molecular biology are mainly common PCR techniques, which are not suitable for rapid detection on-site. The isothermal amplification technique can amplify rapidly at a constant temperature, which makes up for the shortage of ordinary PCR in the detection of meat product quality.

We have established an RPA detection method for chicken, duck, pork, beef and lamb (Lin et al., 2021). CRISPR/Cas12a can be used as a stand-alone assay system to directly detect the presence of target sequences in samples, or it can also be combined with gene amplification techniques such as PCR and RPA to improve the effect. The RPA-CRISPR/Cas12a system has been widely utilized for pathogen detection, such as SARS-CoV-2 (Sun et al., 2021), *Leptospira* (Jirawannaporn et al., 2022), and *Toxoplasma gondii* (Lei et al., 2022). Its application in food safety has also been reported (Li et al., 2022), such as RPA-Cas12a-μPAD for ultrasensitive SERS detection in foods (Zhuang et al., 2022). CRISPR/Cas12a was introduced in this study for meat identification on the basis of a previous study, and the optimization of the RPA-CRISPR/Cas12a system shows that the optimal temperature for both RPA and CRISPR/Cas12a reactions in the identification system for chicken is 42 °C. The same phenomenon is observed for duck, pork, beef and lamb, where the optimal reaction temperatures are consistently at 37 °C, 37 °C, 42 °C and 42 °C, respectively. The RPA and CRISPR/Cas12a optimal temperatures of these meats remained consistent, which contributed to the convenience of operation and stability of the

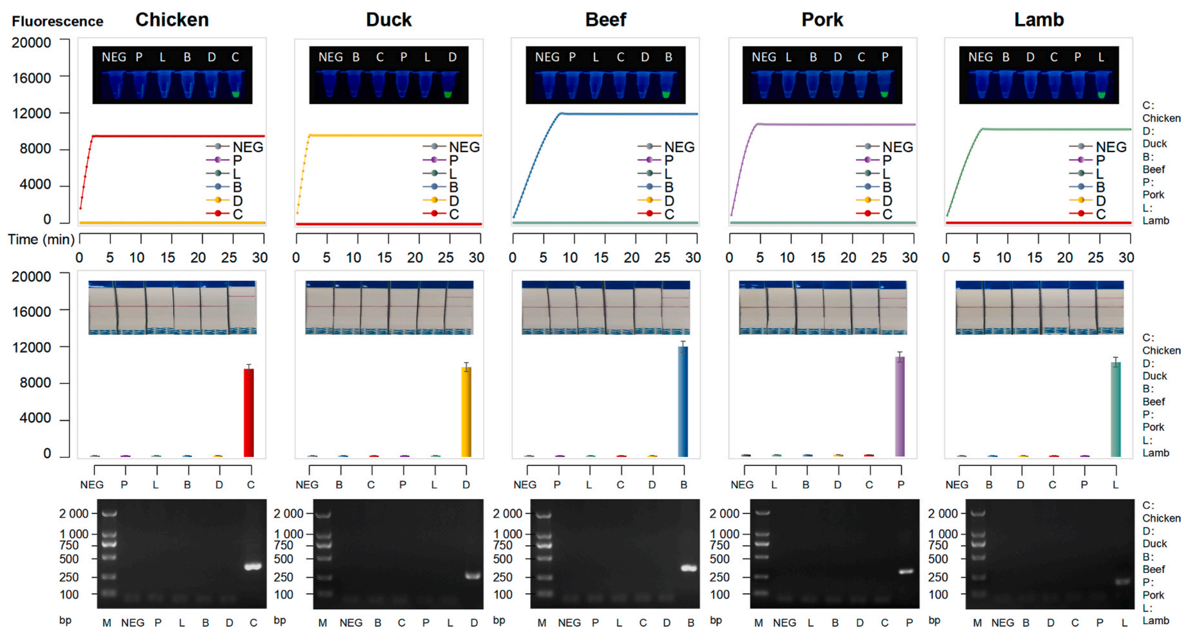

Fig. 4. Specificity of the RPA-CRISPR/Cas12a-LFD meat identification platform. From top to bottom: specific enrichment of fluorescent signals, LFD strips and agarose gel electrophoresis of the products.

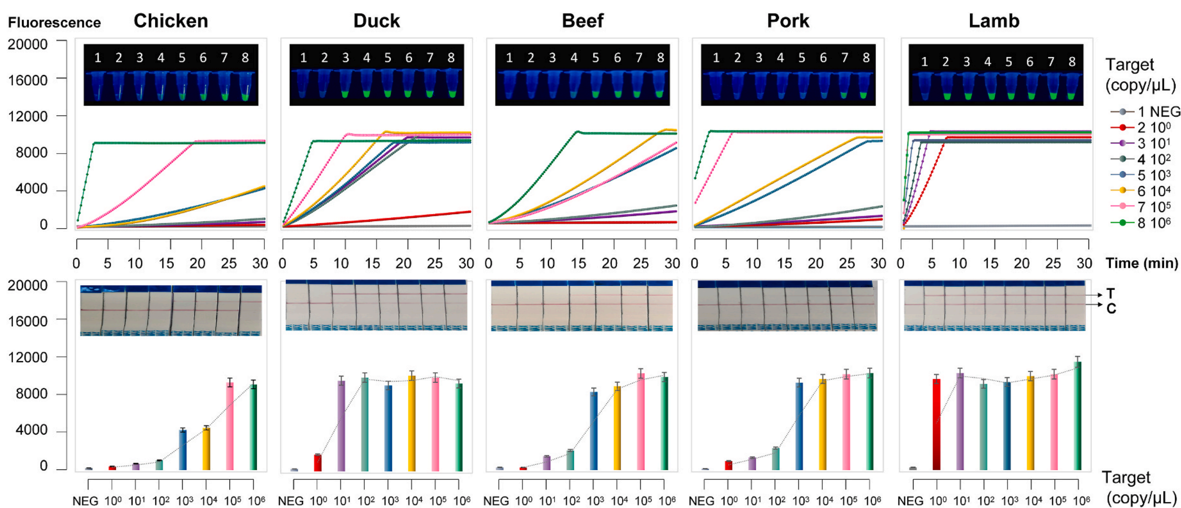

Fig. 5. Sensitivity of the RPA-CRISPR/Cas12a-LFD meat identification platform. From top to bottom: specific enrichment of fluorescent signals and visualization of LFD strips.

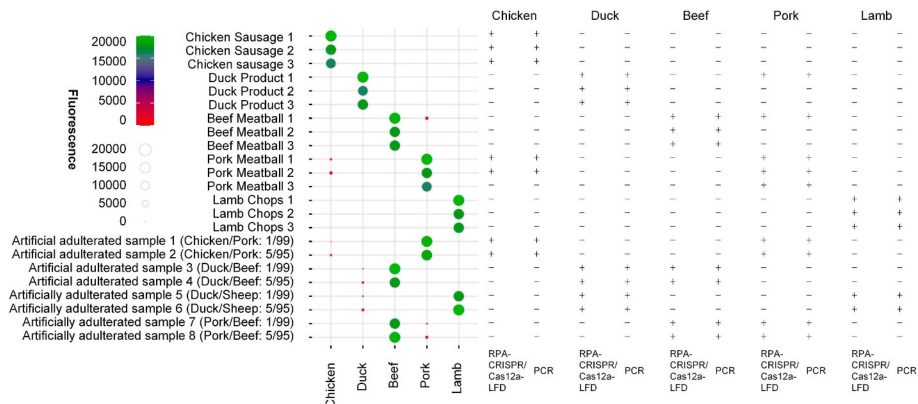

Fig. 6. Detection of practical products by RPA-LFD and PCR.

reaction system. The two are combined at the same or similar temperatures, with RPA responsible for thermostatically amplifying the copy number of the meat target, CRISPR/Cas12a in charge of specifically identifying the cleaved product, and visualizing the results relying on fluorescence signal and LFD. In addition to the reaction temperature, the reaction time in the RPA-CRISPR/Cas12a system is also a key influencing factor. The RPA reaction of each meat identification system reached stability within 20 min, and there was another 30 min in which CRISPR/Cas12a was also sufficient for detection, so the whole procedure took only 50 min. Moreover, the method provides the lowest detection limit of 1×10^0 copies/μL and a high specificity, which enables rapid and accurate detection of meat. Final assays of the product as well as artificially adulterated meat also demonstrated the feasibility of RPA-CRISPR/Cas12a-LFD. It can be observed that in some expensive meat products will be adulterated with cheaper meat, such as the detection of pork-derived ingredients in beef meatballs and chicken-derived ingredients in pork meatballs, which is consistent with the common adulteration phenomenon (Dobrovolny et al., 2019).

At the present stage, nucleic acid-based detection methods for meat are developing rapidly, with routine PCR assays for chicken, duck, pork, beef and lamb (Ulca et al., 2013; Dai et al., 2015), but direct PCR methods require complex manipulation and gel electrophoresis, which is time-consuming and labor-intensive. Multiplex-PCR(Uddin et al., 2021; Cheng et al., 2022), real-time fluorescence quantitative PCR (Liu et al., 2021; Li et al., 2021) and droplet digital PCR (Hu et al., 2021; Shehata et al., 2017) which produce higher specificity than direct PCR, have also been well developed in meat adulteration. Compared to direct PCR, the accuracy, sensitivity and detection efficiency of the later derived PCR techniques in meat adulteration detection are significantly enhanced. However, it is worth noting that they require high instrumentation and experimental conditions as well as specialized operators, making it difficult to achieve rapid on-site detection, which limits the application of such methods in industrial and commercial environments. To address these problems, temperature amplification techniques such as LAMP and RPA have rapidly emerged in meat quality identification (Xiao et al., 2023). With their short reaction time, high sensitivity, no need for PCR instruments and expensive reagents, etc., they effectively reduce the influence of the external environment on the test results and are very suitable for on-site rapid genetic testing, but their drawbacks, such as non-specific binding and false positive signals have also been a long-standing nuisance. The RPA-CRISPR/Cas12a method employed in this study is not only in line with the concept of rapid, sensitive, and portable POCT proposed in recent years, but also with the introduction of CRISPR/Cas12a, which is able to eliminate false-positive signals generated by non-specific amplification of RPA while realizing high specificity, high sensitivity recognition, and high efficiency of signal conversion of target gene sequences. The implementation of this method is an important approach in the detection of meat adulteration, which is of great significance for the protection of consumers' health and life safety.

Both RPA and CRISPR/Cas12a are performed at constant temperatures, and small portable thermostatic heaters or incubators can provide the required temperature conditions without the requirement for complex temperature control equipment. The LFD visualization approach does not require fluorescent equipment such as an excitation light source. Therefore, the proposed RPA-CRISPR/Cas12a-LFD meat identification platform is very appropriate for exploiting on-site large quantities of meat, which presents promising applications and provides new ideas for on-site rapid detection of other food qualities. It is worth noting that RPA-CRISPR/Cas12a-LFD suffers from limitations such as the difficulty of primer design and the artificial optimization of experimental conditions. This will require researchers working together to continuously improve the research system and continue to provide technical support for the field of rapid detection. In summary, combining RPA, CRISPR/Cas12a and LFD to form a complementary detection system for rapid screening and result confirmation is the future market demand and the development trend and direction of meat adulteration and meat quality identification technology, as well as a tool to maintain the economic interests of meat production enterprises and individual consumers, with important social and economic significance.

Funding source

This work was supported by the Guangdong Key Laboratory of Functional Substances in Medicinal Edible Resources and Healthcare Products (grant number 2021B1212040015); Scientific Projects of Key Disciplines in Guangdong Province (grant numbers 2021ZDJS042 and 2022ZDJS070); Special Research Projects of Hybribio (KP202304; KP202302 and KP202303); Guangxi Provincial Natural Science Foundation (grant numbers 2019JJD140052 and 2020JJA140656); Education Department Project of Guangdong Province (grant numbers 2019-GDXK-0032 and 2020KZDZX1146); and Eastern Guangdong Technological Engineering Research Center (grant number P19004).

CRediT authorship contribution statement

Yaqun Liu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft. **Liyun Lin:** Conceptualization, Formal analysis, Investigation, Resources. **Huagui Wei:** Methodology, Formal analysis, Investigation, Data curation. **Qiulan Luo:** Validation, Resources. **Peikui Yang:** Validation, Resources. **Mouquan Liu:** Investigation, Data curation. **Zhonghe Wang:** Validation, Resources. **Xianghui Zou:** Resources, Data curation. **Hui Zhu:** Investigation. **Guangcai Zha:** Validation. **Junjun Sun:** Investigation. **Yuzhong Zheng:** Conceptualization, Writing – review & editing, Supervision, Proiect administration, Funding acquisition. **Min Lin:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Afifa, Khatun M., Hossain, A., Hossain, M.S., Kamruzzaman Munshi, M., Huque, R., 2021. Detection of species adulteration in meat products and Mozzarella-type cheeses using duplex PCR of mitochondrial *cyt b* gene: a food safety concern in Bangladesh. Food Chem. 2, 100017 https://doi.org/10.1016/j.fochms.2021.100017.

Alamprese, C., Amigo, J.M., Casiraghi, E., Engelsen, S.B., 2016. Identification and quantification of Turkey meat adulteration in fresh, frozen-thawed and cooked minced beef by FT-NIR spectroscopy and chemometrics. Meat Sci. 121, 175–181. https://doi.org/10.1016/j.meatsci.2016.06.018.

Brooks, S., Elliott, C.T., Spence, M., Walsh, C., Dean, M., 2017. Four years post-horsegate: an update of measures and actions put in place following the horsemeat incident of 2013. NPJ Sci. Food 1, 5. https://doi.org/10.1038/s41538-017-0007-z.

Cheng, Y., Wang, S., Ju, S., Zhou, S., Zeng, X., Wu, Z., Pan, D., Zhong, G., Cai, Z., 2022. Heat-treated meat origin tracing and authenticity through a practical multiplex polymerase chain reaction approach. Nutrients 14 (22), 4727. https://doi.org/10.3390/nu14224727.

Dai, Z., Qiao, J., Yang, S., Hu, S., Zuo, J., Zhu, W., Huang, C., 2015. Species authentication of common meat based on PCR analysis of the mitochondrial COI gene. Appl. Biochem. Biotechnol. 176 (6), 1770–1780. https://doi.org/10.1007/s12010-015-1715-y.

Demarquoy, J., 2013. Meat adulteration: the use of PCR. Meat Sci. 94 (2), 164. https://doi.org/10.1016/j.meatsci.2013.01.019.

Dobrovolny, S., Blaschitz, M., Weinmaier, T., Pechatschek, J., Cichna-Markl, M., Indra, A., Hufnagl, P., Hochegger, R., 2019. Development of a DNA metabarcoding method for the identification of fifteen mammalian and six poultry species in food. Food Chem. 272, 354–361. https://doi.org/10.1016/j.foodchem.2018.08.032.

Fengou, L.C., Lianou, A., Tsakanikas, P., Mohareb, F., Nychas, G.E., 2021. Detection of meat adulteration using spectroscopy-based sensors. Foods 10 (4), 861. https://doi.org/10.3390/foods10040861.

Galal-Khallaf, A., 2021. Multiplex PCR and 12S rRNA gene sequencing for detection of meat adulteration: a case study in the Egyptian markets. Gene 764, 145062. https://doi.org/10.1016/j.gene.2020.145062.

Ha, J., Kim, S., Lee, J., Lee, S., Lee, H., Choi, Y., Oh, H., Yoon, Y., 2017. Identification of pork adulteration in processed meat products using the developed mitochondrial DNA-based primers. Korean J. Food Sci. Anim Resour. 37 (3), 464–468. https://doi.org/10.5851/kosfa.2017.37.3.464.

Hu, H., Cheng, J., Wei, C., Li, S., Yu, C., Meng, X., Li, J., 2021. Pre-degassed microfluidic chamber-based digital PCR device for meat authentication applications. Micromachines 12 (6), 694. https://doi.org/10.3390/mi12060694.

Hu, J.J., Liu, D., Cai, M.Z., Zhou, Y., Yin, W.X., Luo, C.X., 2023. One-pot assay for rapid detection of benzimidazole resistance in Venturia carpophila by combining RPA and CRISPR/Cas12a. J. Agric. Food Chem. 71 (3), 1381–1390. https://doi.org/10.1021/acs.jafc.2c06549.

Huang, S., Liu, Y., Zhang, X., Gai, Z., Lei, H., Shen, X., 2023. A rapid RPA-CRISPR/Cas12a detection method for adulteration of goat milk. Powder. Foods. 12 (8), 1569. https://doi.org/10.3390/foods12081569.

Jirawannaporn, S., Limothai, U., Tachaboon, S., Dinhuzen, J., Kiatamornrak, P., Chaisuriyong, W., Bhumitrakul, J., Mayuramart, O., Payungporn, S., Srisawat, N., 2022. Rapid and sensitive point-of-care detection of Leptospira by RPA-CRISPR/Cas12a targeting lipL32. PLoS Neglected Trop. Dis. 16 (1), e0010112 https://doi.org/10.1371/journal.pntd.0010112.

Kowalczyk, S., 2021. Quality and methods of adulteration of meat and fish products on the Polish market in 2010-2020. Rocz. Panstw. Zakl. Hig. 72 (4), 361–372. https://doi.org/10.32394/rpzh.2021.0183.

Lei, R., Li, L., Wu, P., Fei, X., Zhang, Y., Wang, J., Zhang, D., Zhang, Q., Yang, N., Wang, X., 2022. RPA/CRISPR/Cas12a-Based on-site and rapid nucleic acid detection of *Toxoplasma gondii* in the environment. ACS Synth. Biol. 11 (5), 1772–1781. https://doi.org/10.1021/acssynbio.1c00620.

Li, Y.C., Liu, S.Y., Meng, F.B., Liu, D.Y., Zhang, Y., Wang, W., Zhang, J.M., 2020. Comparative review and the recent progress in detection technologies of meat product adulteration. Compr. Rev. Food Sci. Food Saf. 19 (4), 2256–2296. https://doi.org/10.1111/1541-4337.12579.

Li, T., Wang, J., Wang, Z., Qiao, L., Liu, R., Li, S., Chen, A., 2021. Quantitative determination of mutton adulteration with single-copy nuclear genes by real-time PCR. Food Chem. 344, 128622 https://doi.org/10.1016/j.foodchem.2020.128622.

Li, Y., Man, S., Ye, S., Liu, G., Ma, L., 2022. CRISPR-Cas-based detection for food safety problems: current status, challenges, and opportunities. Compr. Rev. Food Sci. Food Saf. 21 (4), 3770–3798. https://doi.org/10.1111/1541-4337.13000.

Lin, L., Zheng, Y., Huang, H., Zhuang, F., Chen, H., Zha, G., Yang, P., Wang, Z., Kong, M., Wei, H., Zou, X., Lin, M., 2021. A visual method to detect meat adulteration by recombinase polymerase amplification combined with lateral flow dipstick. Food Chem. 354, 129526 https://doi.org/10.1016/j.foodchem.2021.129526.

Liu, G.Q., Luo, J.X., Xu, W.L., Li, C.D., Guo, Y.S., Guo, L., 2021. Improved triplex real-time PCR with endogenous control for synchronous identification of DNA from chicken, duck, and goose meat. Food Sci. Nutr. 9 (6), 3130–3141. https://doi.org/10.1002/fsn3.2272.

Liu, D., Ma, Y., Yu, S., Zhang, C., 2023. Image based beef and lamb slice authentication using convolutional neural networks. Meat Sci. 195, 108997 https://doi.org/10.1016/j.meatsci.2022.108997.

Mandli, J., El Fatimi, I., Seddaoui, N., Amine, A., 2018. Enzyme immunoassay (ELISA/immunosensor) for a sensitive detection of pork adulteration in meat. Food Chem. 255, 380–389. https://doi.org/10.1016/j.foodchem.2018.01.184.

Paul, B., Montoya, G., Crispr-Cas12a, 2020. Functional overview and applications. Biomed. J. 43 (1), 8–17. https://doi.org/10.1016/j.bj.2019.10.005.

Scollan, N.D., Price, E.M., Morgan, S.A., Huws, S.A., Shingfield, K.J., 2017. Can we improve the nutritional quality of meat? Proc. Nutr. Soc. 76 (4), 603–618. https://doi.org/10.1017/S0029665117001112.

Shehata, H.R., Li, J., Chen, S., Redda, H., Cheng, S., Tabujara, N., Li, H., Warriner, K., Hanner, R., 2017. Droplet digital polymerase chain reaction (ddPCR) assays integrated with an internal control for quantification of bovine, porcine, chicken and Turkey species in food and feed. PLoS One 12 (8), e0182872. https://doi.org/10.1371/journal.pone.0182872.

Siddiqui, M.A., Khir, M.H.M., Witjaksono, G., Ghumman, A.S.M., Junaid, M., Magsi, S.A., Saboor, A., 2021. Multivariate analysis coupled with M-SVM classification for lard adulteration detection in meat mixtures of beef, lamb, and chicken using FTIR spectroscopy. Foods 10 (10), 2405. https://doi.org/10.3390/foods10102405.

Sun, Y., Yu, L., Liu, C., Ye, S., Chen, W., Li, D., Huang, W., 2021. One-tube SARS-CoV-2 detection platform based on RT-RPA and CRISPR/Cas12a. J. Transl. Med. 19 (1), 74. https://doi.org/10.1186/s12967-021-02741-5.

Uddin, S.M.K., Hossain, M.A.M., Chowdhury, Z.Z., Johan, M.R.B., 2021. Short targeting multiplex PCR assay to detect and discriminate beef, buffalo, chicken, duck, goat, sheep and pork DNA in food products. Food Addit. Contam. Part A Chem Anal Control Expo Risk Assess 38 (8), 1273–1288. https://doi.org/10.1080/19440049.2021.1925748.

Ulca, P., Balta, H., Çağın, I., Senyuva, H.Z., 2013. Meat species identification and Halal authentication using PCR analysis of raw and cooked traditional Turkish foods. Meat Sci. 94 (3), 280–284. https://doi.org/10.1016/j.meatsci.2013.03.008.

Vidal, P.O., de Cassia Vieira Cardoso, R., Nunes, I.L., Da Silva Lima, W.K., 2022. Quality and safety of fresh beef in retail: a review. J. Food Protect. 85 (3), 435–447. https://doi.org/10.4315/JFP-21-294.

Wei, H., Li, J., Liu, Y., Cheng, W., Huang, H., Liang, X., Huang, W., Lin, L., Zheng, Y., Chen, W., Wang, C., Chen, W., Xu, G., Wei, W., Chen, L., Zeng, Y., Lu, Z., Li, S., Lin, Z., Wang, J., Lin, M., 2023. Rapid and ultrasensitive detection of plasmodium spp. parasites via the RPA-CRISPR/Cas12a platform. ACS Infect. Dis. 9 (8), 1534–1545. https://doi.org/10.1021/acsinfecdis.3c00087.

Xiao, B., Zhao, R., Wang, N., Zhang, J., Sun, X., Huang, F., Chen, A., 2023. Integrating microneedle DNA extraction to hand-held microfluidic colorimetric LAMP chip system for meat adulteration detection. Food Chem. 411, 135508 https://doi.org/10.1016/j.foodchem.2023.135508.

Yan, S., Lan, H., Wu, Z., Sun, Y., Tu, M., Pan, D., 2022. Cleavable molecular beacon-based loop-mediated isothermal amplification assay for the detection of adulterated chicken in meat. Anal. Bioanal. Chem. 414 (28), 8081–8091. https://doi.org/10.1007/s00216-022-04342-7.

Zaukuu, J.Z., Gillay, Z., Kovacs, Z., 2021. Standardized extraction techniques for meat analysis with the electronic tongue: a case study of poultry and red meat adulteration. Sensors 21 (2), 481. https://doi.org/10.3390/s21020481.

Zhao, G., Wang, J., Yao, C., Xie, P., Li, X., Xu, Z., Xian, Y., Lei, H., Shen, X., 2022. Alkaline lysis-recombinase polymerase amplification combined with CRISPR/Cas12a assay for the ultrafast visual identification of pork in meat products. Food Chem. 383, 132318 https://doi.org/10.1016/j.foodchem.2022.132318.

Zheng, F., Li, S., Wang, S., Feng, T., Jiang, Z., Pan, J., 2020. Cross-priming isothermal amplification combined with nucleic acid test strips for detection of meat species. Anal. Biochem. 597, 113672 https://doi.org/10.1016/j.ab.2020.113672.

Zheng, S.Y., Ma, L.L., Wang, X.L., Lu, L.X., Ma, S.T., Xu, B., Ouyang, W., Rpa-Cas12aDS, 2022. A visual and fast molecular diagnostics platform based on RPA-CRISPR-Cas12a method for infectious bursal disease virus detection. J. Virol. Methods 304, 114523. https://doi.org/10.1016/j.jviromet.2022.

Zheng, J., Liu, L., Li, X., Xu, Z., Gai, Z., Zhang, X., Lei, H., Shen, X., 2023. Rapid and simple detection of Burkholderia gladioli in food matrices using RPA-CRISPR/Cas12a method. Foods 12 (9), 1760. https://doi.org/10.3390/foods12091760.

Zhuang, J., Zhao, Z., Lian, K., Yin, L., Wang, J., Man, S., Liu, G., Ma, L., 2022. SERS-based CRISPR/Cas assay on microfluidic paper analytical devices for supersensitive detection of pathogenic bacteria in foods. Biosens. Bioelectron. 207, 114167 https://doi.org/10.1016/j.bios.2022.114167.