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Article

Amplification-Based CRISPR/Cas12a Biosensor Targeting the *COX1* Gene for Specific Detection of Porcine DNA

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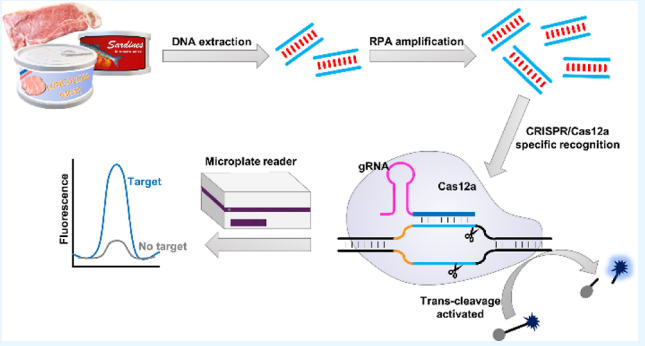
ABSTRACT: We propose a CRISPR/Cas12a-mediated recombinase polymerase amplification (RPA) detection method that combines RPA with Cas12a cleavage for the detection of halal food adulteration, which is of global concern, particularly for Muslim consumers. We optimized the reagent concentrations for the Cas12a cleavage steps and designed and screened gRNA targeting a conserved area of the mitochondrial cytochrome C oxidase subunit I (*COX1*) gene. This procedure successfully detected the presence of porcine components as low as 5 pg/μL in the linear range of 5−1000 pg/μL. The assay's detection limit was 500 times lower than CRISPR-based approaches that exclude a preamplification step, allowing the detection of trace porcine DNA in food samples. The assay additionally showed no cross-reaction with nontarget species. Therefore, this detection platform shows tremendous potential as a method for the quick, sensitive, and specific detection of porcine-derived components.



INTRODUCTION

Globalization and the exponential growth of food sciences and technologies have made it more feasible for individuals as well as products to traverse easily across borders. Sourcing raw materials for the food sector is therefore no longer restricted to regional suppliers but can be achieved quickly and easily from other nations as well.[1] Concerns about religious practices, health implications, vegetarianism, and the rampant issue of food counterfeiting have all contributed to widespread alarm about this change among people who are conscious of the source of animal-derived food products.[2,3] Halal standards stipulate that no food may include any kind of porcine or any of its byproducts.[4] A reliable meat identification tool can help the food industry comply with legal, ethical, and medical concerns worldwide and help consumers avoid inappropriate, restricted, or disagreeable items.[5] Extensive research has been conducted to investigate the protein content of porcine and develop methods for its detection.[6−8] Electrophoretic and immunological approaches, which use proteins, could pose challenges in differentiating closely related species.[9] Therefore, more accurate and sensitive detection technologies, especially those based on DNA analysis, have largely replaced these earlier procedures.[10] Species can be identified with better precision using DNA-based approaches because of their increased discriminatory power. These cutting-edge techniques have made important strides in the realm of meat species identification, bolstering confidence in food labels and quality assurance procedures.[9] DNA-based methods that are employed to determine porcine-derived components in meat products predominantly include conventional polymerase chain reaction (PCR),[11] PCR-restriction fragment length polymorphism (PCR-RFLP),[12] and DNA barcoding.[13] The limitations of the PCR have prompted the development of various complementary nucleic acid amplification techniques.[14] A single-heat-block isothermal DNA amplification method was developed as an alternative to PCR-based nucleic acid detection.[15] Recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) are two such isothermal DNA amplification techniques that have advantages over traditional amplification procedures since they do not require thermal cycling or any additional processing steps, such as electrophoresis or fluorescence labeling, after amplification.[16−18] A LAMP-based ECL sensor was reported

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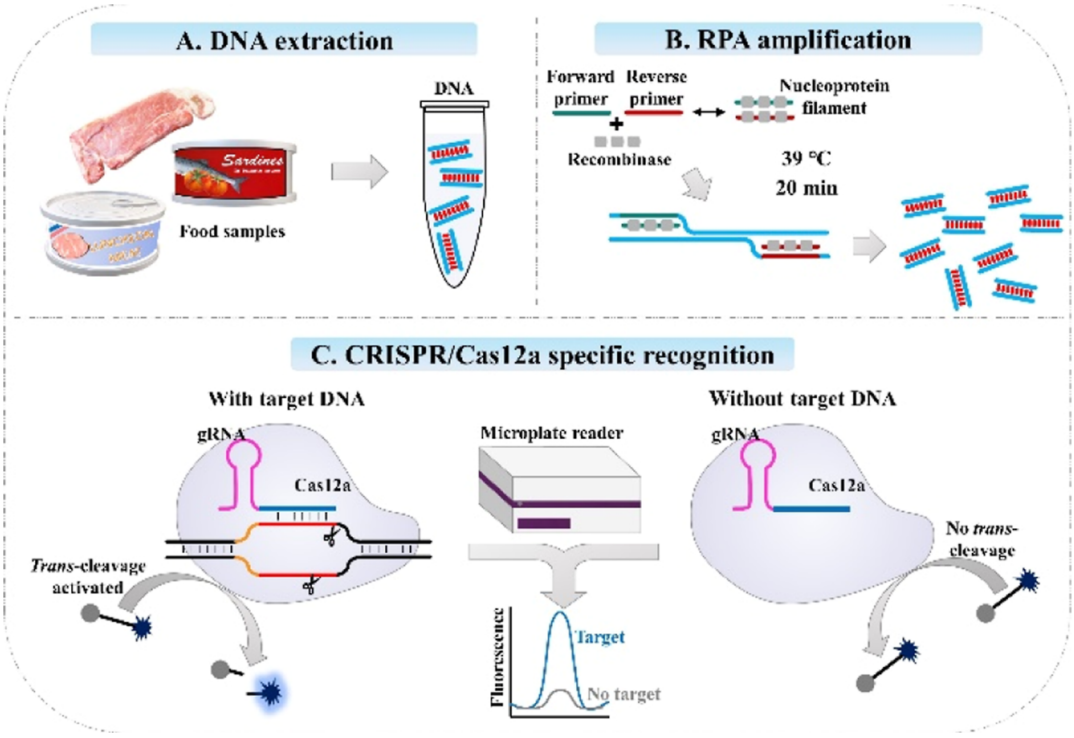

Figure 1. Schematic representation of the RPA-CRISPR/Cas12a fluorescence assay. (A) DNA was extracted from the food samples. (B) DNA was amplified by RPA, which involves a primer set that binds specifically to the target DNA with the aid of a recombinase protein. (C) In the presence of the target, the Cas12a/gRNA complex recognizes and binds to the target DNA, activating the *cis*-cleavage activity of the Cas12a protein. Subsequently, the quenched ssDNA probe was *trans*-cleaved, and the fluorescence of the ssDNA probe fluorophore was recovered. When the target DNA is absent, no *cis*- and *trans*-cleavage activities take place, which results in a weak fluorescence signal.

with luminol as the ECL redox molecule to detect porcine DNA. ECL was chosen to leverage luminol's ability to emit light, which varies in the presence and absence of target DNA.[19] RPA simplifies assay design and reduces the risk of primer interactions or cross-reactivity by employing a fewer number of primers in comparison to LAMP.[20] DNA can be amplified in 20 min at 37 to 42 °C with minimal effort and at room temperature using RPA.[21,22] Due to these advantages, RPA has been used in a wide variety of meat adulteration detection methods.[23−25] Nonetheless, the specificity of these approaches can be enhanced so that they can be applied in a wider variety of situations.

Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) is a specialized immune system that many bacteria and archaea have evolved to defend against foreign invaders.[26] Guide RNA (gRNA) and CRISPR-associated (Cas) genes are transcribed from short DNA repeats in CRISPR arrays and subsequently translated into Cas endonuclease, respectively.[27] The gene editing technology CRISPR/Cas has been receiving a lot of attention recently due to its promising future in fast diagnostics.[28] When the target DNA is present, the gRNA recognizes and binds to the target DNA, guiding the Cas protein to perform specific *cis*-cleavage activity on the target.[29] Since the discovery of the *trans*-cleavage activity possessed by several class II CRISPR/Cas systems (Cas12, Cas13, and Cas14), numerous CRISPR/Cas-based nucleic acid detection systems have been proposed and developed.[30−33] Combining the CRISPR/Cas system with RPA for nucleic acid detection provides an added level of precision due to the distinct sensitivity profiles of the two methods.[34−36]

This research is the first to report a novel method for detecting porcine DNA by targeting the porcine *COX1* gene using the RPA technique and the CRISPR/Cas12a system (Figure 1). The high copy number of mitochondrial genes (nearly 5 copies per mitochondrion)[37] ensures that sufficient portions of the genome are present after sample extraction to enable more precise detection. Mitochondrial DNA is less likely to recombine during evolution, which can lead to low levels of variation within a species but potentially high levels of variation between different species. Being a mitochondrial gene, the *COX1* gene has been conserved throughout evolution, although slight changes have been noted across species. Several detection technologies have found this gene to be a successful target due to its properties.[38−40] The remarkable specificity of the CRISPR/Cas system was combined with the great efficiency of RPA to enable the sensitive and accurate identification of porcine. The application of the approach to commercial food products and simulated meat mixes proved its practical applicability. The developed RPA-CRISPR/Cas12a assay has significant prospects to be utilized in food authentication.

EXPERIMENTAL SECTION

Materials and Reagents. Fresh meat and processed food products, including food seasonings and meat products, were acquired from local supermarkets. Porcine-chicken meat models with varying porcine percentages (10 to 0.0001%) were prepared. The raw pork and chicken samples were homogenized individually by using a blender. These samples were then mixed to a final weight of 50 g, according to Zhao et al.,[8] with a spatula. All animal genomic DNA used for

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Table 1. Sequences of Novel RPA Primers, PCR Primers, gRNA, and ssDNA Probe Used in This Work

oligo name	sequences (5′−3′)
RPA-S3	F: TGGACACCCGAGCATACTTTAC
	R: ATAGGAAGATGAAGCCCAGAGC
PCR	F: CCGCAATGTCTCAATACCAAAC
	R: GTTGCGGTCTGTCAGTAGTATAG
gRNA	AAUUUCUACUGUUGUAGAUAUAUUGCCACCGUGCAGGGU
ssDNA probe	CY3-TTTTTTTT-BHQ2

specificity analysis was purchased from Zyagen (California, USA). The designed primers, CRISPR gRNA, and ssDNA probe were synthesized by SBS Genetech Co., Ltd. (Beijing, China). EnGen Lba Cas12a and its corresponding NEBuffer r2.1 were procured from New England Biolabs (Ipswich, Massachusetts, USA), and the RPA Kit used in this study was procured from TwistDx Ltd. (Cambridge, UK). The SYBR Select Master Mix was acquired from Applied Biosystems (Waltham, Massachusetts, USA).

Extraction and Purification of DNA. The NucleoSpin Food Kit (Macherey-Nagel, Düren, Germany) was used to extract the DNA from the food samples. The extraction was carried out according to the guidelines provided by the manufacturer in the user manual. In brief, lysis buffer and Proteinase K were added to the homogenized food sample (200 mg) for cell lysis. After incubation at 65 °C for 30 min, the lysed cells were centrifuged at 10,000*g* for 10 min. An equal volume of binding buffer and ethanol was added to the supernatant. The mixture was then transferred into a NucleoSpin Food Column and centrifuged. Next, the food column was washed with wash buffers three times and incubated with elution buffer at room temperature for 5 min to elute the DNA. NanoPhotometer P-Class (Implen GmbH, Munich, Germany) was used to estimate the concentration and purity of the extracted DNA samples. The DNA was diluted to 50 pg/μL and stored at −80 °C until further use.

Designing and Screening of RPA Primer Sets. Mitochondrial DNA (mtDNA) was the focus of the detection in this investigation. Four distinct regions of mtDNA (*D-loop*, *COX1*, *Cyt b*, and *ND2*) were investigated to identify the most promising gene location. Using DNAMAN software (Lynnon Biosoft, California, USA) and PrimerQuest Tool (Integrated DNA Technologies Inc., Iowa, USA), six RPA primer sets were designed based on these genes (GenBank accession numbers: AM040615.1, MG725630.1). To preliminarily evaluate the specificity of the primers, the UCSC genome browser in silico PCR tool (https://genome.ucsc.edu/cgi-bin/hgPcr) was used. Details of the designed primers are listed in Table S1 (Supporting Information). Based on gel electrophoresis results, the primer set targeting the *COX1* gene (Table 1), which does not exhibit primer-dimer formation and is highly specific toward porcine, was chosen.

Optimization of the CRISPR/Cas12a System. The CRISPR gRNA (Table 1) was designed using Cas-Designer at the CRISPR RGEN Tools Web site (http://www.rgenome.net/). It is composed of a constant repeat-derived sequence followed by a spacer sequence, which is complementary to the target sequence, also known as the protospacer located in the positive RPA amplicons.

To optimize the CRISPR/Cas12a cleavage reaction system, the ratio of gRNA and ssDNA probe to the Cas12a protein, which can greatly influence the fluorescence intensity of the system, was determined. The concentration of Cas12a was first set to 20 nM. The different Cas12a/gRNA molar ratios tested were 1:1, 1:2, 1:3, 1:4, and 1:5. The evaluated ssDNA probe concentrations were 100, 250, 500, 750, and 1000 nM. The fluorescence intensities were measured using the FLUOstar Omega plate reader (BMG LABTECH, Offenburg, Germany). The excitation wavelength used was 544 nm, and the emission wavelength was 590 nm.

RPA-CRISPR/Cas12a Fluorescence Assay Establishment. The TwistAmp Basic Kit was used to perform all of the RPA amplification. The lyophilized enzyme pellet was first dissolved in 29.5 μL of primer-free rehydration buffer and 13 μL of ultrapure water. The mixture was then aliquoted so that each RPA reaction had a total volume of 10 μL, including 400 nM forward and reverse primers (0.4 μL of 10 μM primers), 14 mM magnesium acetate [$Mg(CH_3COO)_2$] [0.5 μL of 280 mM $Mg(CH_3COO)_2$], and 0.2 μL of target DNA. The reactions were incubated at 39 °C for 20 min in a mini-dry bath and cleaned at 65 °C for 10 min. Around 4 min into the incubation period, the reaction tubes were withdrawn from the dry bath, vortexed, and spun before the incubation was continued. Following the completion of the RPA reaction, 2 μL of the RPA amplicon was used as the target in the CRISPR/Cas12a fluorescence assay. The reaction mixture was then transferred into a 96-well black microplate and incubated at 37 °C for 30 min in a plate reader before the measurement was taken.

Evaluation of the RPA-CRISPR/Cas12a Assay Specificity and Detectability. The specificity of the proposed RPA-CRISPR/Cas12a method was investigated using 50 pg/μL genomic DNA of porcine, sheep, chicken, rat, rabbit, and buffalo as the target. Porcine genomic DNA was serially diluted to 1000, 100, 10, 1, and 0.1 pg/μL to determine the detectability of the assay. The analysis of the RPA amplicons using the CRISPR/Cas12a system was carried out in triplicate.

Application of the RPA-CRISPR/Cas12a Assay in Food Samples. To validate the reliability and applicability of the assay in actual situations, porcine-chicken binary mixtures and 25 processed food products composed of porcine-free and porcine products were tested for porcine DNA.

Assay Validation of RPA-CRISPR/Cas12a. Conventional PCR was used to verify the accuracy of the designed RPA-CRISPR/Cas12a assay. A PCR primer set which targets the *COX1* gene (GenBank accession number MG725630.1) was designed with the help of the PrimerQuest Tool (Integrated DNA Technologies Inc., Iowa, USA). The Applied Biosystems Veriti 96-Well Thermal Cycler (Applied Biosystems, Waltham, Massachusetts, USA) was employed to conduct all conventional PCR amplifications. A DNA template (4 μL) was added to a 16 μL PCR system containing 10 μL of 2× SYBR Select Master Mix, 0.5 μL of 10 μM forward and reverse primers, and 5 μL of ultrapure water. The PCR protocol was carried out as follows: 95 °C initial denaturation for 2 min, 35 cycles of 15 s denaturation (95 °C), 15 s annealing (52 °C), and 1 min

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extension (72 °C) before final extension at 72 °C for 5 min and 4 °C infinite hold. To visualize the PCR amplicon, 5 μL of the amplicon was loaded onto a 2% agarose gel and electrophoresed at 80 V.

Statistical Analysis. GraphPad Prism software 9 (GraphPad Software Inc., Chicago, USA) was used to perform statistical analyses. The significant differences between the data sets were evaluated via the analysis of variance test, whereby a *P* value lower than 0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

Working Principle of the RPA-CRISPR/Cas12a Fluorescence Assay. Figure 1 depicts the principle of the RPA-CRISPR/Cas12a fluorescence assay. DNA from the food samples was extracted, and the targeted DNA sequence, the porcine *COX1* gene, was amplified by RPA. Optimization of the RPA reaction volumes to 10 μL results in an 80% reduction of the total cost of the experiment (the recommended capacity of the RPA reaction was 50 μL, according to the manufacturer's instructions). The CRISPR/Cas12a system was then employed to detect the RPA amplicons. In fluorescence-based CRISPR/Cas systems, the Cas12a endonuclease, gRNA, and ssDNA probe are the key components.[41] The interaction between the Cas12a and the gRNA (Figure 2A) alters the Cas12a conformation, exposing the protein's catalytic domain.[42] If the RPA amplicon, containing a T-rich protospacer adjacent motif sequence, binds to the Cas12a/gRNA complex, it will be cleaved by the Cas12a.[42,43] Subsequently, Cas12a can indiscriminately cleave the ssDNA probes that are nearby, inducing fluorescence that can be measured using a microplate reader.[42] If the targeted DNA is absent, the *trans*-cleavage activity of the Cas12a will not be activated, and the fluorophore of the ssDNA probe remains quenched, generating low fluorescence intensity.[44]

The working principle of the assay was verified by investigating the influence of critical variables on the CRISPR/Cas detection system. As the fluorescence of the Cy3 fluorophore was quenched by the BHQ-2 quencher, the probe gave a low fluorescence. Without Cas12a, gRNA, or the target RPA amplicon, the detected fluorescence intensity is still low, demonstrating that the cleavage activity was not activated in the absence of the three variables. Adding the target to the Cas12a/gRNA complex in the presence of the ssDNA probe led to a significant increase in fluorescence intensity, as depicted in Figure 2B. This illustrates that the fluorescence response is linked to the RPA amplicons, which act as an activator for the Cas12a protein to perform *cis*- and *trans*-cleavage activity. Collectively, the results showed the feasibility of the CRISPR/Cas12a system to detect porcine DNA.

Optimization of the CRISPR/Cas12a System. The optimal reaction conditions were determined to enhance the performance of the CRISPR detection system. Five ratios of Cas12a to gRNA were compared, and it was observed that 1:1 was the optimal ratio with the highest fluorescence intensity and signal-to-background noise (S/B) ratio. As illustrated in Figure 3A, the fluorescence intensity decreases as the Cas12a/

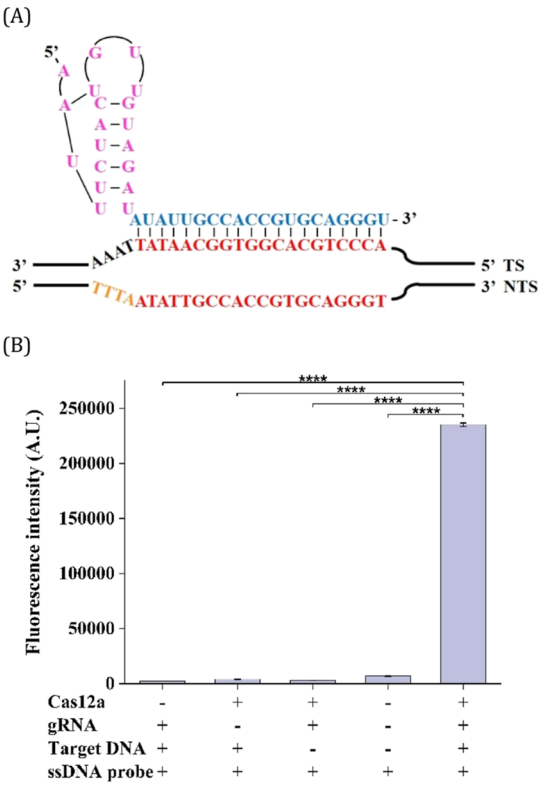

Figure 2. (A) Detailed schematic of the target DNA and gRNA used in this research. The constant repeat-derived sequence, spacer, and target sequence are highlighted in pink, blue, and red, respectively. (B) Fluorescence analysis of the working principle of the CRISPR/Cas12a system. Error bars represent the standard deviation (n = 3); *P < 0.05; **P < 0.01; ***P < 0.001; and ****P < 0.0001; ns, not significant.

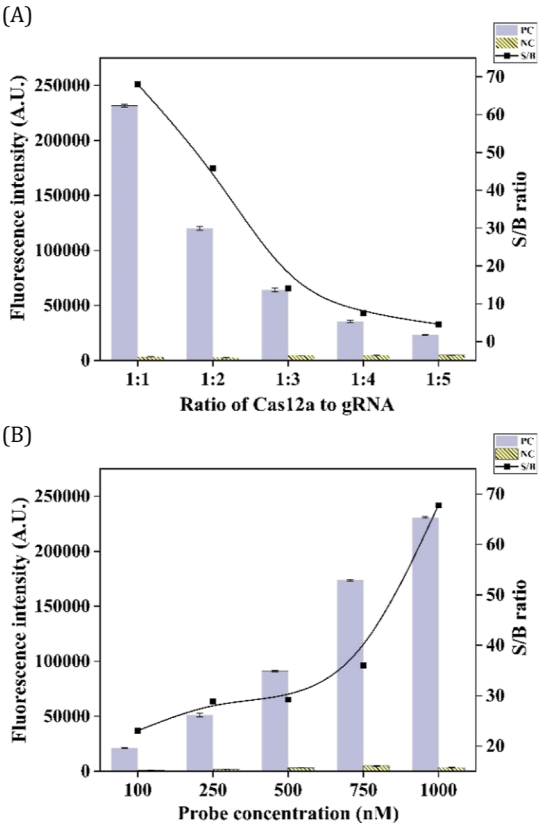

Figure 3. Optimization of the CRISPR/Cas12a system reaction conditions. Effects of different (A) Cas12a/gRNA molar ratios and (B) probe concentrations on the fluorescence response and S/B ratio. Error bars represent the standard deviation (n = 3).

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gRNA molar ratio increases. This could be attributable to the steric hindrance effect, which hinders the gRNA from binding to the Cas12a protein when the gRNA concentration is high.[45] Besides the Cas12a/gRNA ratio, the probe concentration was also optimized. When the probe concentration was increased, the fluorescence response increased, and a maximum S/B ratio was detected at 1 μM probe concentration (Figure 3B). Hence, the optimum reaction conditions for 50 μL of the Cas12a reaction system were 20 nM Lba Cas12a, 20 nM gRNA, 1 μM ssDNA probe, 5 μL of 10× NEBuffer r2.1, 36.5 μL of ultrapure water, and 2 μL of the RPA amplicon.

Specificity of the RPA-CRISPR/Cas12a Fluorescence Assay. At the optimum reaction conditions, the fluorescence response of the RPA-CRISPR/Cas12a assay was monitored under different animal species. Based on the detection results (Figure 4A), only porcine DNA activates the Cas12a protein to cleave the probe and yield a dramatic fluorescence signal. The addition of other animal species (chicken, sheep, buffalo, rabbit, and rat) to the system gave a fluorescence response close to that of the negative control. The results obtained were consistent with the conventional PCR results (Figure S1A, Supporting Information), demonstrating that the method has high specificity. Combining RPA with the CRISPR/Cas12a system enables the target DNA and RPA amplicons to be recognized by the RPA primers and the Cas12a system, respectively. This dual recognition process eliminates the possibility of false positives and enhances the specificity of the developed method.

Detectability of the RPA-CRISPR/Cas12a Fluorescence Assay. Serially diluted porcine DNA ranging from 0.1 to 1000 pg/μL was used to assess the detectability of the method. With a lower concentration of porcine DNA, less RPA amplicon was generated. Consequently, fewer Cas12a proteins are activated, leading to a decrease in fluorescence intensity (Figure 4B). The detection limit of the method was determined to be the lowest porcine DNA concentration that yielded a significant result from the negative control in all repeats. In this work, the value was found to be 5 pg/μL as no significant difference was observed between the negative control and lower porcine DNA concentrations. Conventional PCR analysis of the same dilution series verified the results obtained by the RPA-CRISPR/Cas12a assay (Figure S1B, Supporting Information).

The plot of the fluorescence intensities against the porcine DNA concentration displayed in Figure 4C exhibited a good linear correlation over the range of 5 to 1000 pg/μL with a coefficient of determination (R^2) of 0.9921. The RPA-CRISPR/Cas12a fluorescence assay provides a significantly lower detection limit than previously reported isothermal amplification methods.[46,47] A similar detection limit has also been described in a qPCR.[48] When compared to other reported amplification-based CRISPR/Cas detection approaches, the proposed assay provides a notable detection limit (Table S2, Supporting Information). Additionally, the assay obtained a detection limit approximately 500-fold lower,[44] enabling the identification of trace amounts of porcine DNA in food samples compared to CRISPR-based approaches that did not include a preamplification phase. Target amplification time has been reduced by over 70% when using RPA rapid isothermal amplification technology (20 min) compared to conventional PCR (60 to 90 min).

In the case of the linear range, the proposed assay provides the advantage of a broader range. The assay has a wider range

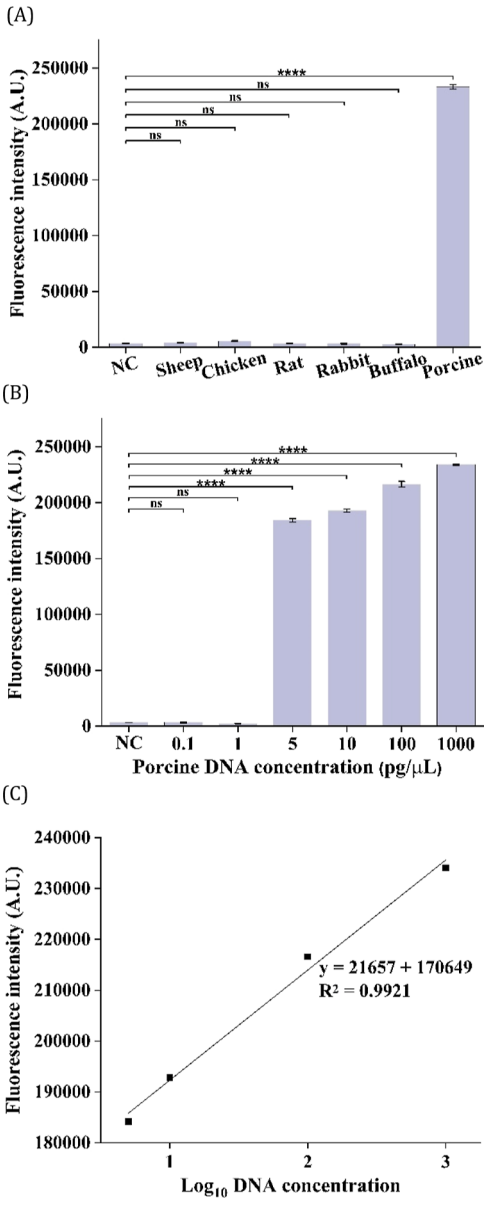

Figure 4. Evaluation of the specificity and detectability of the RPA-CRISPR/Cas12a fluorescence assay. The fluorescence response of the assay to different (A) animal species and (B) porcine genomic DNA concentrations. (C) Plot of fluorescence intensities against the porcine DNA concentration. Error bars represent the standard deviation (n = 3); NC, negative (no-template) control; *P < 0.05; **P < 0.01; ***P < 0.001; and ****P < 0.0001; ns, not significant.

of 5 to 1000 pg/μL, whereas a previous work by Wu et al.[44] indicated a reasonable linear range between 1 and 10 ng/μL. Therefore, the RPA-CRISPR/Cas12a fluorescence assay offers better accuracy and detection range.

Quantitative Analysis of Porcine DNA. To demonstrate the ability of the assay for quantitative detection, three concentrations of porcine DNA were amplified and evaluated by the CRISPR/Cas12a system at 5 min intervals of up to 20 min. The quantitative analysis was carried out following a procedure previously described.[49] The gradual increase and difference in fluorescence intensities illustrate that the RPA amplicon quantity and amplification rate vary depending on the initial amount of DNA template (Figure 5A). A high initial DNA template concentration showed a faster amplification

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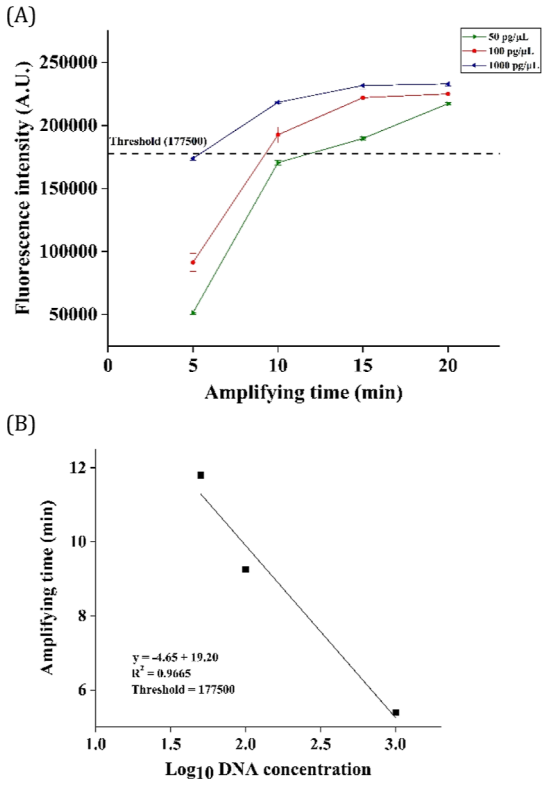

Figure 5. (A) Fluorescence for different concentrations of porcine DNA at 5 min intervals up to 20 min. (B) Log-linear regression plot determined from (A) at a threshold value of 177,500. The equation, slope, and R^2 of the standard curve are shown. Error bars represent the standard deviation (n = 3).

rate. To generate three data points, the threshold intensity was set to 177,500. At this threshold value, the intensities for 50, 100, and 1000 pg/μL of porcine DNA template intersect the threshold at 5.4, 9.25, and 11.8 min, respectively. The log-linear regression curve plotted using the amplifying time and DNA concentration indicates a slope of −4.65 and an R^2 of 0.9665 (Figure 5B), depicting the suitability of the RPA-CRISPR/Cas12a assay for quantitative DNA detection.

Detection of Food Samples. Processed food products, including porcine-free and porcine products, were subjected to porcine DNA detection using the RPA-CRISPR/Cas12a fluorescence assay. All five porcine products showed a significant enhancement in fluorescence signal, illustrating the successful detection of porcine DNA (Table S3, Supporting Information). On the other hand, the porcine-free labeled products yield a fluorescent response close to that of the negative control, demonstrating that the food products were not mislabeled. In addition, the different proportions of porcine-chicken meat admixture prepared to mimic adulterated meat products that may be sold on the market were also analyzed (Figure 6). Fluorescence response can be observed for as low as 0.001% porcine. This limit was comparable to or lower than the previously described values (Table S2, Supporting Information).

Most of the other approaches utilized for detecting porcine-derived components, such as mass spectrometry,[50] spectroscopy,[51] chromatography,[52] and immunoassays,[11] necessitate either costly, bulky apparatus or difficult, expert-level analyses. Unlike these detection methods, the developed assay does not rely on high-cost equipment and time-consuming procedures,

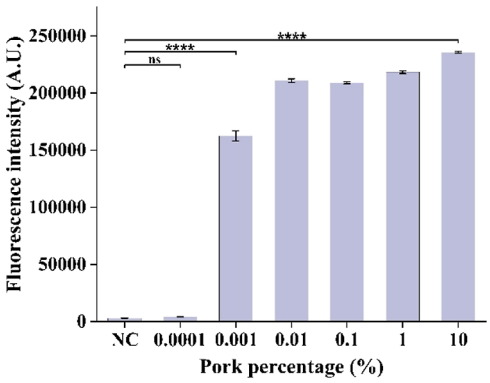

Figure 6. Detection of different porcine percentages in a simulated porcine/chicken mixture using the RPA-CRISPR/Cas12a fluorescence assay. Error bars represent the standard deviation (n = 3); NC, negative (no-template) control; *P < 0.05; **P < 0.01; ***P < 0.001; and ****P < 0.0001; ns, not significant.

which makes the assay ideal in settings with scarce resources. The assay would only require a portable dry heating bath and fluorescence reader for on-site detection. The RPA-CRISPR/Cas assay could easily be operated by individuals who received minimal training, offering a more efficient and practical alternative to existing porcine detection methods. Hence, the assay has the potential to be applied for the inspection and monitoring of adulteration in foods.

Validation of the RPA-CRISPR/Cas12a Assay. A total of 25 processed food samples were used to evaluate the accuracy of the RPA-CRISPR/Cas12a method. There were a total of 5 samples that tested positive for porcine-derived components. The RPA-CRISPR/Cas12a assay identified the same number of positive samples that the conventional PCR did, demonstrating the RPA-CRISPR/Cas12a method's complete accuracy for the porcine-derived component detection (Table S3, Supporting Information). These results validate the applicability and reliability of the assay to detect porcine adulteration in food products.

CONCLUSIONS

In this study, a new RPA-CRISPR/Cas12a fluorescence assay targeting the porcine *COX1* gene was successfully constructed. Specificity analysis of the method revealed no cross-reactivity with other animal species. Owing to the isothermal RPA technique, the assay attained a detection limit of 5 pg/μL porcine DNA. The developed assay's applicability was demonstrated by analyzing various processed food products and simulated adulterated meat samples for porcine DNA. RPA amplification has reduced the target amplification time by nearly 70%. In contrast to the standard PCR process, which may require up to 90 min, this method simply needs a tiny dry bath to amplify the target DNA. In addition, by replacing the bulky fluorescence microplate reader with a portable one, detection of porcine DNA can be carried out on-site. Thus, the RPA-CRISPR/Cas12a assay can serve as a beneficial food authenticity monitoring tool.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04473.

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Details of the RPA and PCR primer sets; comparison of existing detection methods for porcine DNA; result of conventional PCR amplicons visualized by 2% agarose gel electrophoresis; and detection of 25 processed food products using the proposed assay and conventional PCR assay (PDF)

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A.A.S.J.: design and investigation, data curation, and original draft writing. C.P.K.: original draft writing. L.Y.C. and N.F.M.-N.: editing and initial draft preparation and funding acquisition. M.U.A.: conceptualization, editing, supervision, project administration, funding acquisition, and writing−review and editing. All authors contributed and have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

RPA, recombinase polymerase amplification; *COX1*, cytochrome C oxidase subunit I; PCR, polymerase chain reaction; PCR-RFLP, PCR-restriction fragment length polymorphism; LAMP, loop-mediated isothermal amplification; CRISPR/Cas, clustered regularly interspaced short palindromic repeats-associated protein

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