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PORTECT: Porcine detection kit using CRISPR-CAS method as new innovation for rapid and accurate halal detection

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A B S T R A C T

The halal industry is growing rapidly, increasing the demand for reliable methods to verify product compliance with Islamic laws. Traditional verification processes are often time consuming and prone to fraudulent practices, emphasizing the need for more efficient solutions. Therefore, this study proposed the development of the PORTECT Kit, a CRISPR-Cas-based tool designed to detect porcine DNA in food products. The method included designing specific guide Ribonucleic Acid (gRNA) sequences targeting porcine DNA, producing the Cas12 protein through gene expression in *Escherichia coli*, and forming a ribonucleoprotein (RNP) complex. When applied to a detection strip, the complex produced a colorimetric response upon cleavage of the reporter compound, confirming the presence of porcine DNA. The results showed that the PORTECT Kit offered a highly efficient, accurate, and rapid method for halal food verification, with the ability to provide outcomes in minutes without requiring complex laboratory equipment. Furthermore, it had significant potential for widespread adoption in both consumer and production settings. In conclusion, the PORTECT Kit addresses critical challenges in the halal industry by offering a cost-effective and user-friendly solution for ensuring halal certification. Future study should focus on expanding the detection capabilities to include other harmful substances.

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1 Introduction

The concept of halal is growing significantly due to the increasing Muslim population and the rising interest in halal products. Halal refers to the requirements for Muslims to consume or use products that are permissible and acceptable, according to Islamic Law. This concept is rooted in the teachings of the Al-Quran and Sunnah. According to Islamic scholars, halal can be interpreted in various aspects of life, such as food and beverages, finance, tourism, cosmetics, services, and pharmaceuticals. The certification also plays a crucial role in ensuring that the products used or consumed are in accordance with Islamic laws. This allows consumers to securely make informed purchasing decisions and ensure high quality hygiene and sanitation measures. For Muslim consumers, halal certification assures that the products comply with Islamic Law. Meanwhile, for non-Muslims, it serves as an assurance of quality, reflecting adherence to good manufacturing practices (GMP) and hazard analysis and critical control point (HACCP) guidelines (Khan & Haleem 2016). The increasing demand and awareness of halal products have driven technological advancement within the industry.

Despite rapid growth, the halal industry faces several challenges. Contamination, even with trace amounts of non-halal substances, renders a product harmful. Fraudulent practices such as mislabeling or intentional contamination, erode consumer trust. Traditional halal verification methods, while effective, are often time-consuming and labor-intensive, leading to delays in certification processes. The rigorous and lengthy certification process can also be a barrier for producers who may struggle with associated costs and time commitments. Furthermore, the halal industry is faced with the complexities of global supply chains and diverse cultural practices. Ensuring compliance with halal standards across different regions can be challenging, particularly when dealing with ingredients from various countries. The industry also needs to adapt to changing consumer preferences and emerging trends, such as the demand for organic, sustainable, and ethically produced halal products. To address these issues, new technologies such as CRISPR offer innovative solutions. CRISPR is a powerful gene-editing tool that can be used to detect specific DNA sequences associated with non-halal substances. By precisely identifying these sequences, the technology provides a highly accurate and efficient method for food verification, improving the reliability of certification and making it more accessible to producers.

Ahmed *et al.* (2020) outlined the significant potential of CRISPR systems in food authentication, particularly in identifying the origin and composition of food products. The results showed that the application of this cutting-edge technology could profoundly impact the industry by providing a highly advanced and precise method for ensuring that food products adhere to halal standards. This capability enhance consumer trust by detecting even trace amounts of non-halal substances with significant accuracy. While the benefits of CRISPR technology are evident, it is essential to consider potential limitations, such as the current reliance on controlled laboratory settings, need for skilled personnel, as well as costs associated with developing and implementing the technology at scale.

Wu *et al.* (2021) developed an amplification-free, mix-to-read CRISPR-Cas12 assay for detecting pork DNA by targeting the cytochrome b (Cytb) gene. This allows for the rapid and specific identification of pork DNA in various processed halal meat products, showing promise as a quick and reliable detection method. However, the reliance on laboratory conditions highlights a key challenge in adapting the method for field deployment or routine inspections in real-world settings. While the technique is faster and more specific than some traditional methods, it may face challenges in terms of scalability, cost-effectiveness, and regulatory acceptance.

Compared to traditional halal food authentication methods, such as Polymerase Chain Reaction (PCR)-based techniques, CRISPR offers advantages in speed and simplicity. However, PCR remains a widely used method due to its established protocols, lower costs, and broader acceptance in the industry. A comprehensive comparison of these methods, considering factors such as accessibility, infrastructure requirements, and long-term costs, provide a clear picture of the feasibility of CRISPR technology in commercial halal food authentication.

In Indonesia, the regulatory framework for food safety and biosafety could potentially support the adoption of CRISPR-Cas technology. Desoite the absence of specific regulations governing the technology, existing laws, such as Law Number 7 of 1996 concerning food and Law No. 21 of 2004 ratifying the Cartagena Protocol on Biosafety, provide a foundational

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framework. These laws emphasize food safety and biosafety, emphasizing the importance of evaluating new biotechnological advancements to ensure they do not pose risks to health or the environment. Furthermore, Government Regulations 28 of 2004 and 21 of 2005 address the safety, quality, and testing of genetically modified products, offering a pathway that could be extended to include CRISPR-modified food products.

The regulatory adoption of CRISPR technology would require addressing ethical, religious, and technical concerns specific to the halal industry. Additionally, efforts to streamline the integration of the technology into halal food testing benefits from collaboration among regulatory bodies, religious authorities, and technological innovators. The Regulation of the Head of the Indonesian Food and Drug Monitoring Agency could play a pivotal role by establishing technical guidelines for incorporating CRISPR into halal food safety assessments.

By considering these critical aspects, the integration of CRISPR-Cas technology into halal food authentication holds potential as a transformative solution. Future study should focus on overcoming practical challenges, evaluating cost-benefit dynamics, as well as addressing consumer and regulatory acceptance to maximize the impact of this innovative technology.

2 Proposed Ideas Portect Kit

An innovative technology, the PORTECT Kit, was proposed to detect porcine DNA and determine the halal status of products using the CRISPR-Cas method. CRISPR or Clustered Regularly Interspaced Short Palindromic Repeats, is a gene-editing technology guided by Ribonucleic Acid (RNA) (Alamillo *et al.* 2023). This technology facilitates changes, repairs, deletions, or insertions in an organism DNA (Zhu 2022). CRISPR consists of 2 main components, namely Cas and gRNA (Xu & Li 2020). The gRNA was used to identify a part of the DNA sequence (Liu *et al.* 2020). Before locating the targeted, the DNA in food samples was extracted using a lysis buffer. At the point where the gRNA identifies the targeted sequence, it alters the conformation of the Cas protein to become active and placed on the targeted DNA. Cas12 will be used as it does not only cleaves the targeted DNA, but also cuts single-stranded DNA within the vicinity (Chen *et al.* 2018). This characteristics is crucial for detecting halal food, as Cas12 cleaves the reporter compound that produces a visual color, signifying the presence of porcine DNA (Feng *et al.* 2023). A visual image of the PORTECT is shown in Figure 1.

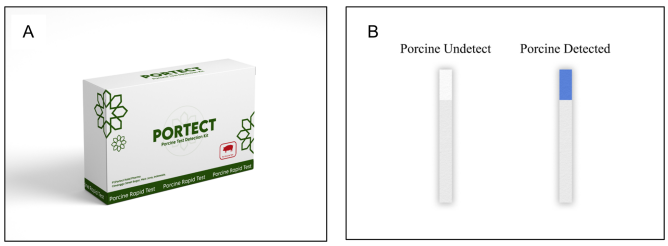

Figure 1: PORTECT packaging (a) and PORTECT strip visual (b)

2.1 PORTECT Production

PORTECT, a CRISPR-Cas-based porcine detection kit, exemplies the practical application of this technology. Its production begins with fhe synthesis and design of gRNA to specifically detect porcine DNA. The gRNA was designed using bioinformatics software such as the CRISPR RGEN Tools website to create a specific sequence of porcine DNA which was then synthesized. The Cas12 protein was produced through gene expression in *Escherichia coli*. First, a plasmid containing the Cas gene was inserted into *E. coli*, and isopropyl β-D-1-thiogalactopyranoside (IPTG) induced its expression. The Cas protein was purified using affinity chromatography (His-tag purification) to ensure high purity.

Cas protein and gRNA were combined and incubated to obtain stable and active RNP. To visualize detection, the complex was connected to Alkaline Phosphatase (AP) and p-Nitrophenyl Phosphate (pNPP). The RNP complex was applied to paper detection and immobilized with buffers containing glycerol and trehalose, which can maintain moisture and stability during storage.

Table 1: Sequences of targeted DNA and CRISPR gRNA

Oligo name	Sequences (5'-3')
Targeted DNA	GCCTGCTTTCGTAGCACGTATTTA
gRNA	GCCUGCUUUCGUAGCACGUAUGUUUUAGAGCUAGA AAUAGCAAGUUAAAAUAAGGCUAGUCCGUUAUCAAC UUGAAAAAGUGGCACCGAGUCGGUGCUUUU

2.2 Design gRNA

CRISPR gRNA was designed using Cas-Designer on the CRISPR RGEN Tools website (http://www.rgenome.net) (Naito *et al.* 2015). This process begins by entering the pig gene cytochrome c oxidase subunit 1 (COX1) from GenBank (code MG725630.1). Subsequently, the system automatically evaluated the target sequences based on specificity. The results showed that the target sequence had the highest specificity, as presented in Table 1. The target RNA (gRNA) sequence was identified and prepared for gRNA synthesis.

2.3 Synthesis of gRNA

The synthesis of gRNA was performed using in vitro transcription (IVT) as described by He *et al.* in 2024. A linear DNA template containing the T7 promoter sequence was prepared, comprising a spacer complementary to the DNA target, and a scaffold for Cas protein binding. The IVT mixture included T7 RNA polymerase, NTP mix (ATP, GTP, CTP, and UTP), IVT buffer, RNase inhibitor, and DNA template. The reaction was incubated at 37℃ for 2-4 hours, then DNase I was added to degrade the remaining DNA template. The transcribed RNA was purified by RNA affinity chromatography.

2.4 Cas12 Production

Cas12a gene expression in *E. coli* was reported by Gomez-Quintero *et al.* in 2024. The process included media preparation, bacterial transformation, pre-inoculum culture, primary culture inoculation, induction of Cas12a expression, and protein purification. Media preparation started by dissolving 12.5 g of Lactose Broth (LB) media in 1 L of water and sterilizing using an autoclave. Competent *E. coli* was transformed with the Cas12a plasmid pDS015 using a thermal shock method (Mohanraju *et al.* 2018). This entailed mixing 2 µL of plasmid with competent cells, coolinf on ice for 5 min, heating at 42℃ for 30 s, and cooled again. Subsequently, 100 µL of LB medium was added, and the mixture was incubated at 37℃ for 1 h. The transformed cells were exposed to agar containing ampicillin (100 µg/mL) and incubated overnight. The colonies formed were grown in 5 mL of LB medium containing ampicillin. Approximately 1 mL of this culture was inoculated into 100 mL of LB or TB medium containing ampicillin and 1% glucose when necessary. Cultures were incubated at 37℃ until the OD reached 0.6–0.8, then the temperature was reduced to 12, 18, 30, or 37℃ according to the experimental requirements. After 30 min, expression was induced by addition of IPTG (0.3–1.6 mM) (Briand *et al.* 2016), and cultures were incubated for 4–16 h.

2.5 Ribonucleoprotein (RNP) Production

The formation of RNP followed the method described by Janudin *et al.* in 2023. The CRISPR gRNA and Cas12a served as target DNA detectors, while AP and pNPP enabled colorimetric detection (Xiang *et al.* 2024). The RNP complex was formed by mixing Cas12a protein and gRNA at a 1:1 molar ratio, with each component at a concentration of 20 nM. After RNP formation, ssDNA probe conjugated with AP using carbodiimide was added at a final concentration of 1 μM. These probes were designed for cleavage by Cas12a upon recognition of the DNA target. HEPES buffers containing MgCl2 enhanced RNP stability (Tyumentseva *et al.* 2021).

Stability of the RNP complex is crucial for kit's detection sensitivity and specificity. Therefore, special treatments should be applied to the PORTECT kit. CRISPR-CAS12 contained in the topl remains stable for up to 2 months when stored at 4℃ (Karlsson *et al.* 2005), necessitating refrigeration. The packaging requirements included a neutral pH, free of heavy metals, harsh detergents, and ultraviolet (UV) exposure to protect the components. The recommended packaging material is chemically resistant plastic with airtight seals to maintain optimal environmental conditions within the kit.

2.6 Kit Production

The Kit was produced by combining the RNP complex, ssDNA conjugated with AP, and pNPP as substrates. The steps include conjugating ssDNA with AP using a cross-linking reagent carbodiimide (Wickramathilaka & Tao 2019), then pNPP as a substrate was added to nitrocellulose paper. After the components were detected, the paper was dried using a flash freeze at -20℃, followed by lyophilization (freeze-drying) to remove air without destroying enzyme activity or substrate. The dried paper was then packaged in airtight container with a desiccant to maintain enzyme and substrate stability during storage, ensuring the efficacy of the kit for color change-based detection.

3 SWOT Analysis of Portect Kit

PORTECT could potentially be developed due to its broad market potential and high demand for halal product assurance. Moreover, the kit is integrated with biotechnology systems, making it easier for consumers to detect porcine DNA. Figure 2 shows the SWOT analysis of the PORTECT.

The advantage of using CRISPR as a halal kit is that it is able to detect porcine DNA compared to the traditional method in terms of speed, efficiency, and accuracy. Unlike PCR, which requires DNA amplification or Enzyme-Linked Immunosorbent Assay (ELISA) that deals with antigen-antibody reactions, CRISPR can detect DNA directly within minutes. CRISPR is a simple method that does not require complex laboratory equipment. CRISPR also has a lower operational cost because it does not require as much reagent as PCR or ELISA, although the initial cost of the CRISPR kit is high. CRISPR also has high accuracy because it can precisely detect the specific porcine DNA sequence. Unlike the commercial

kit that uses proteins as the target of detection, the result can be biased as there is a cross reaction from other animal proteins.

Figure 2: SWOT Analysis of PORTECT

Furthermore, PORTECT offers a simple kit that can detect porcine DNA efficiently, precisely, and accurately. It has also helped fulfill the market needs for halal foods and ensure compliance with halal product regulations. However, the initial cost of developing the PORTECT kit is high. The PORTECT kit is estimated to be approximately IDR 28,000 or USD 2 per kit for 1 test. Therefore, further development and mass production are required to reduce costs. Although the price may be slightly higher for a single test, it is still much cheaper and more efficient for individual instant use. Even so, PORTECT has huge opportunities in the near future, owing to the increase in demand and awareness of halal products. With support and collaboration from various parties, such as government authorities, which includes food safety and halal product assurance bodies, this idea can be easily developed.

Another challenge of PORTECT is that there are some similar halal test products available in the market, but generally most of the halal test products only detect halal through protein, which can cause cross-reactions with proteins from non-porcine sources, causing the results to be biased. In contrast to other innovations, our kit can precisely and accurately detect porcine DNA. Hopefully, in the future, this kit will be able to detect more harmful substances, such as dog-derived substances.

4 Conclusion and Limitation

In conclusion, the halalness of a product was considered essential in various aspects of daily life, requiring compliance with Islamic law. Industries faced challenges over time, particularly contamination with non-halal substances, which harm. CRISPR, a gene editing technology combined with RNA, was used to repair, delete, or insert parts of the DNA of an organism. An application of the CRISPR-Cas was detecting halal food to detect certain DNA sequences associated with non-halal substances.

PORTECT is produced using gRNA to specifically detect porcine DNA. The development of this tool enabled verification whether a meat product was halal or contained haram ingredients, such as pork. The tool offered high sensitivity, speed, efficiency, and reliable accuracy.

This study, based on a literature review, lacked laboratory validation and was limited to porcine detection. Future investigations were expected to include experimental methods to detect a broader range of prohibited substances, such as dog genes, for more comprehensive applications.

The primary limitation was the absence of laboratory-based experimental validation to assess the practical efficacy and reliability of the PROTECT kit. Additionally, the scope was restricted to the detection of porcine DNA, leaving other non-halal ingredients unaddressed. The technology was untested for real-world samples, and its scalability for mass production remains uncertain. As a result, the current application was limited to theoretical and laboratory-based study.

Future study should focus on enhancing the utilization of the PROTECT CRISPR-Cas-based detection kit. First, laboratory validation was crucial to confirm the effectiveness and accuracy in real-world applications by testing diverse food samples. Expanding detection beyond porcine DNA to include other haram substances, such as dog DNA, would increase its versatility for verifying the halal status of various products. Efforts should also focus on scalable, cost-effective methods for large-scale production to ensure broad accessiy while maintaining quality and ease of use. Establishing collaborations with halal certification bodies, food manufacturers, and regulatory agencies are crucial to facilitate the practical implementation of the technology. Engaging the stakeholders ensured that the kit was in line with industry standards and meets regulatory requirements. Addressing these study directions, positioned the PROTECT kit as a valuable tool for the halal food industry, supporting the integrity of the certification systems worldwide and fostering consumer trust in the food supply chain.

Conflict of Interest

The authors declare no conflict of interest.

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