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# An Automated DNA Extraction and PCR Analysis as A Rapid Method for Identification of Meat Species in Halal-Meat Products

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**ABSTRACT**

Rising awareness of halal food quality and safety has increased the need for halal food authentication, which includes the identification of undeclared components and species substitution in meat products. Adulteration and fraud have become common in the meat processing industry as a result of technological advancements. Thus, there is a need for extensive research into analytical methods for determining whether materials such as processed meat ingredients are safe to consume and free of food-borne zoonotic threats. In this study, as a preliminary study, a fully automated DNA extraction system was used to isolate the DNA of several fresh meat samples. The extracted DNA was used as templates to detect the DNA of pork, bovine, and chicken, by designing various PCR species-specific primers targeting the mitochondrial cytochrome b (Cyt b), Cytochrome Oxidase Subunit I (Cox1), and NADH dehydrogenase subunit 5 (ND5) coding genes. The use of a fully automated DNA extraction system and primer-specific PCR assays to detect different species of meat in meat products will improve accuracy, speed, and reliability. This analytical technique has the potential to authenticate commercial meat products on the market with rapid detection and high accuracy.

**Keywords:** Automated DNA extraction; PCR; Identification; Meat; Halal

## 1 Introduction

The halal industry has become the world's fastest-growing market, covering one-third of the world market [1]. During the recent COVID-19 crisis, the global market for halal food reached $1400 billion in 2020 and is anticipated to increase to $ 15,000 billion by 2050 [1], which indicates that halal products have gained global acceptance. This development, however, also increased the number of manufacturers who produced adulterated food or food contaminated with non-halal or low-quality meat substances to increase profit and reduce production costs. Meat and animal-based foods are a major subject of discussion in this issue. Counterfeit meat products are a serious problem for public health, food safety and for consumers who have religious restrictions on eating non-halal meats. In order to mitigate the risk of transmissible disease from the adulteration and substitution of meat products and to determine the


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authenticity of meat products that might be contaminated with low-quality meat (such as rat or dog) or non-halal meat source, there is a significant need to develop analytical method for detecting the presence of non-halal meat.

DNA-based methods using polymerase chain reaction (PCR) has become one of the powerful tools for species identification of non-halal meat, such as pork meat in various processed meat. This method provides fast, sensitive, highly specific, and cheaper alternatives for identifying meat species, even in complex processed foods [2,3]. High-quality DNA is required for PCR-based food authentication. DNA, as a biological marker, is a suitable target for detection due to its abundant presence in cells and stability at high temperatures. However, DNA extraction protocols are frequently laborious and time-consuming, which often require an overnight digestion of proteinase K [4]. Besides, the presence of inhibitors might inhibit DNA polymerase activity in PCR or in downstream applications. Thus, improvements in the DNA extraction method platforms that automate and simplify the process are needed to generate a faster turnaround and to process a large number of biospecimens for the initial step of food identification. In this study, a rapid detection method is developed using an automated DNA extractor and various species-specific primers were designed for PCR assays to identify the meat species.

## 2 Materials and Methods

### 2.1 Sample preparation

Several types of meat (pork, bovine, and chicken) were used as DNA template sources. All meat samples were transported under ice-chilled condition (4 °C) and stored at around -20 °C until they were processed for DNA extraction.

### 2.2 Extraction of DNA

DNA of fresh meat samples was extracted by first pre-treating the meat sample by cutting and grinding the meat sample into small pieces and homogenized. For rapid, low-cost, and high-quality genomic DNA recovery, the genomic DNA of each meat sample was extracted using an automatic nucleic acid extractor (MagLEAD 12GC, PSS Co., Chiba, Japan) and using the reagents cartridge set (MagDEA Dx SV 200). The extracted DNA was stored in Tris-HCl buffer (pH 8.0) at −20 °C for PCR assay. The extracted DNA genome was visualised using 2 % agarose gel electrophoresis.

### 2.3 PCR assay conditions

The results of DNA extraction were amplified using PCR in a 50 µl tube using KOD FX Neo DNA polymerase (Toyobo, Osaka, Japan). Amplification was performed with 1 µl of genomic DNA as the template, 5 µl of 10× Buffer, 5 µL of 1× 2mM dNTPs, 2 µL of 25 mM $MgSO_4$, 3 µL primer mix of the targetted species, 1 µL of the KOD Plus Neo (1U/µL), and distilled water. The PCR program used was: 94 °C/2 min × 1 cycle, (94 °C/15 s, 50 °C/30 s) × 30 cycles, 68 °C/30

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s × 1 cycle, and 4 °C hold. The amplified PCR product was run on 2 % agarose gel and stained in ethidium bromide. Species-specific primers for all the species of interest are constructed by targetting the mitochondrial cytochrome b (Cyt b), Cytochrome Oxidase Subunit I (Cox1), and NADH dehydrogenase subunit 5 (ND5) coding genes.

## 3 Results and Discussion

### 3.1 DNA Extraction

The isolation of DNA aimed at getting a DNA template to be amplified using PCR. DNA was isolated from raw meats, comprises of pig, bovine, and chicken, by first himogenizing it and the DNA was extracted using the automatic nucleic acid extractor (MagLEAD 12GC, PSS Co., Chiba, Japan) and using the reagents cartridge set (MagDEA Dx SV 200). The concentration of the extracted DNA was evaluated, with 121.2 ng/μL from the pig sample, 92.3 ng/μL from the bovine sample, and 342.9 ng/μL. With the automated DNA extractor, parallel processing of multiple sample types can be performed with single reagent. In comparison to the conventional DNA extraction using the kit, automatic DNA extractor can provide faster, cleaner and more efficient magnetic bead extractions for high-purity nucleic acid recovery. The results demonstrated that all the DNA products were acceptable and sufficient to be used as the template for PCR amplification for the detection of DNA of various meat samples.

### 3.2 Polymerase Chain Reaction (PCR) amplification

In this study, various species-specific primers were designed to target Cyt b, Cox1, and ND5 coding genes for the detection of pork, bovine, and chicken meat samples. These primer sets were selected as they are found in multiple copies per cell and fully protected by the mitochondrial membrane. Besides, due to its moderate and distict evolutionary rates and patterns, cytb is the ideal gene for assessing the phylogenetic evolution at the species levels [5]. Thus, these primer sets were tested to evaluate their specificity to identify the DNA of all meat types. The PCR amplification products are demonstrated in Figure 1.

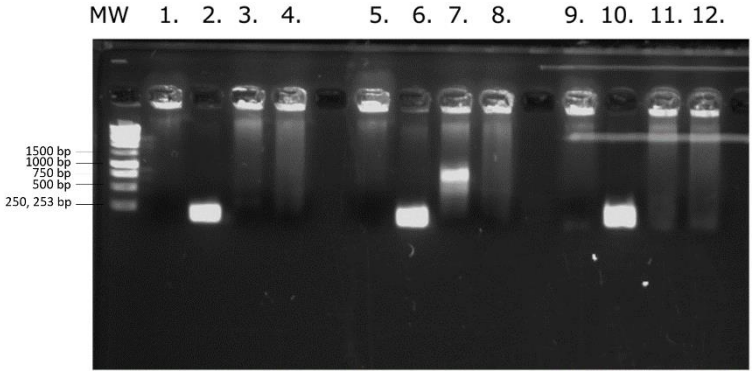


**Figure 1:** *Agarose gel electrophoresis of PCR products targeting Cox1 genes (band 1: negative control; band 2: pig; band 3: bovine; band 4: chicken), Cytb genes (band 5: negative control; band 6: pig; band 7: bovine; band 8: chicken), and ND5 genes (band 9: negative control; band 10: pig; band 11: bovine; band 12: chicken)*

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Based on the agarose gel electrophoresis of PCR products, the initial screening for the detection of pork revealed that all the designed primers set targeting Cox1, Cytb, and ND5 genes can successfully detect the presence of pork. This can be observed by the presence of a specific single band at band 2, band 6, and band 10. However, the Cytb primer set targetted for the detection of bovine DNA generated false band. The designed primers used for the detection of bovine and chicken were unable to detect the presence of bovine and chicken DNA samples. Thus, other targetted genes might need to be evaluated to detect the DNA of bovine and chicken samples. Previously, 12SrRNA and 16SrRNA gene regions were used for the detection of cattle and chickens, respectively [6].

## 4 Conclusions

Animal detection and identification techniques, particularly in food products, are critical because they are linked to health, economy, and religion. Thus, using the automated DNA extractor coupled with a PCR system can provide a solution to facilitate rapid detection with high accuracy.

## References


1. Ng P C, Ahmad Ruslan N A S, Chin L X, Ahmad M, Abu Hanifah S, Abdullah Z and Khor S M 2022 Recent advances in halal food authentication: Challenges and strategies *J. Food Sci.* **2022,** 87, 8–35
2. Sajali N, Wong S C, Hanapi U K, Abu Bakar @ Jamaluddin S, Tasrip N A and Mohd Desa M N 2018 The Challenges of DNA Extraction in Different Assorted Food Matrices: A Review *J. Food Sci.* **2018,** 83, 2409–14
3. Vaithiyanathan S, Vishnuraj M R, Narender Reddy G and Srinivas C 2021 Authentication of camel meat using species-specific PCR and PCR-RFLP *J. Food Sci. Technol.* **2021**, 58, 3882–9
4. Yalçınkaya B, Yumbul E, Mozioğlu E and Akgoz M. Comparison of DNA extraction methods for meat analysis *Food Chem.* **2017**, 221, 1253–7
5. Sultana S, Hossain M A M, Zaidul I S M and Ali M E 2018 Multiplex PCR to discriminate bovine, porcine, and fish DNA in gelatin and confectionery products *LWT.* **2018**, 92, 169–76
6. Natonek-Wiśniewska, M., Krzyścin, P., & Piestrzyńska-Kajtoch, A. The species identification of bovine, porcine, ovine and chicken components in animal meals, feeds and their ingredients, based on COX I analysis and ribosomal DNA sequences. *Food Control* **2013**, *34*(1), 69-78.


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