In Silico PCR Study Amplifying Mitochondrial *ND5* Gene to Detect Food Adulteration

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Abstract. The design of the primers affects the PCR's specificity. A mitochondrial gene called *Cyt-b* is frequently used in PCR to detect the presence of pork, but because the sequence is nearly identical to that of bovine *Cyt-b*, false positive results frequently occur in lab tests. Therefore, the purpose of this study is to create primers that amplify *NADH dehydrogenase subunit 5 (ND5)*, another mitochondrial gene, and to carry out in silico PCR experiments utilizing bioinformatics tools. Using Geneious Prime bioinformatic software, the mitochondrial *ND5* sequences of *Sus scrofa* and *Bos taurus* were pairwise matched after being retrieved from NCBI. For both pigs and cows, primers were created using a species-specific sequence. Using Primer BLAST tools in NCBI, the specificity of the primers was examined. The outcome of this research was two sets of primers that amplified a 467 bp fragment of *ND5* that was unique to pork and 206 bp fragment of *ND5* specific to bovine.

Keywords: in silico PCR · food adulteration · NADH dehydrogenase subnunit 5 (ND5) · pork

1 Introduction

Muslims have provisions to follow, one of which is that every Muslim is ordered to eat halal and good food. According to Islamic law, Muslims are not allowed to consume pork or products made from ringed animals like dogs, cats, monkeys, or rats [1]. The commitment of the Indonesian government to safeguarding Muslim consumers' right to consume halal products has been codified in Law No. 33 of 2014, also known as the Halal Product Guarantee (Jaminan Produk Halal/JPH), which mandates that all goods entering, circulating, and traded within Indonesian territory must be halal-certified [2, 3]. Nevertheless, people's worries about consuming foods containing non-halal content is a concern. In the community there were irresponsible people who commit fraud products that concern the status of halal. A non-halal component often found in various food products is pork derivatives. The majority of meat-based food items still include pork when processed, with processed beef being the most prevalent.

The use of molecular technology, notably Polymerase Chain Reaction (PCR), which can swiftly and specifically amplify non-halal DNA sequences from small samples, is an alternative method that can be used to accurately detect the amount of pig in food

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M. Fadilah et al. (Eds.): IcoBioSE 2021, ABSR 32, pp. 538–543, 2023.
https://doi.org/10.2991/978-94-6463-166-1_64

products. Research on the detection of non-halal gene content using PCR techniques is considerable [4–8]. However, in general the target gene used for detection is the cyt-b gene found in mitochondrial DNA. Cyt-b genes in porks and bovine have a high sequence similarity, so sometimes this method gave false positive results in the laboratory practice.

Efficiency and specificity of PCR depends on primer designing. Primers designed without following the guideline, only matters about the sequence, will produce little to no PCR product due to primer dimerization and/or non-specific amplification, which prevents the primer from annealing to the target gene [9]. The primer needs to meet the ideal requirements, which include having a length between 18 and 30 mer, a GC composition of 40 to 60%, a Tm differential between the forward and reverse primer of no more than 2–3 °C, and not producing a secondary structure (hairpin or self-dimer) [10]. In order to get specific primer, therefore the objective of this study is to create primers that amplify NADH dehydrogenase subunit 5 (ND5) [11], another mitochondrial gene, and carry out in silico PCR experiments utilizing bioinformatics tools.

2 Material and Methods

2.1 Primer Design

Mitochondrial *ND5* reference sequence of *Sus scrofa* (NC_000845.1: 12935-14755) and *Bos taurus* (NC_006853.1: 12109-13929) were downloaded from NCBI. Both sequences were pairwise-aligned using Geneious Prime bioinformatic software (https://www.geneious.com). Primers were designed on species-specific sequence for *Sus scrofa* and *Bos taurus*. The primers specificity was checked using Primer BLAST tools in NCBI.

2.2 In Silico PCR

Genome Compiler bioinformatic software (https://designer.genomecompiler.com/app) was used to perform in silico PCR. The software's tools for creating PCR products were fed the designed primer sequences. Gel electrophoresis simulations are also carried out to make sure the amplicon size produced is adequate.

3 Results and Discussion

3.1 Sus-Specific and Bos-Specific Primer Design

Geneious Prime was used to create primers specific to both cows (Bos taurus) and pork (Sus scrofa). The NADH dehydrogenase subunit 5 (ND5) gene in mitochondrial DNA was the target gene. The ND5 genes from S. scrofa and B. taurus were aligned using pairwise alignment on Geneious Prime to provide a particular primary sequence. The primer was created in regions where each species' nucleotide sequence is unique (Fig. 1).

The designed *Sus*-specific primer were Sus-F (5'-CCATTAATCGGCCTACTCC-3') and Sus-R (5'-GGTATTGCTTTATACAGTCCG-3') and *Bos*-specific primer were Bos-F (5'-ACCCTTGATTGGACTAGCAT-3') and Bos-R (5'-GGCG TAATTAATAGATTGGA TGTA-3'). These primers must meet the ideal criteria. Therefore, the primer

Fig. 1. Pork-specific primer designs (Sus-F and Sus-R) and bovine (Bos-F and Bos-R) on pairwise alignment results using Geneious Prime.

Fig. 2. Characteristics of Sus and Bos-specific primer design results.

characteristics were comprehensively checked using Geneious Prime, as described in Fig. 2. The primer pair of Sus-F and Sus-R will generate 467 bp PCR products, while the Bos-F and Bos-R primer pair will generate 206 bp PCR products.

Specificity of designed primers were rechecked with NCBI Primer BLAST with two steps. First, the primer sequences of Sus-F and Sus-R were checked for all nucleotide collections in the NCBI database and the results obtained was PCR products specific to *Sus scrofa*. Step two, the primers sequences of Sus-F and Sus-R were checked for the DNA template of *Bos taurus* and the result was obtained that the primer sequence could not amplify the genes found in *Bos taurus* (Fig. 3). These results suggest that the primer sequences of Sus-F and Sus-R are specific to porks and will not provide false positive results.

The same thing was also done for *Bos* primer. First the primer sequence of Bos-F and Bos-R checked for all nucleotide collections in the NCBI database and the results obtained was PCR products specific to *Bos taurus*. Step two, the primer sequences of

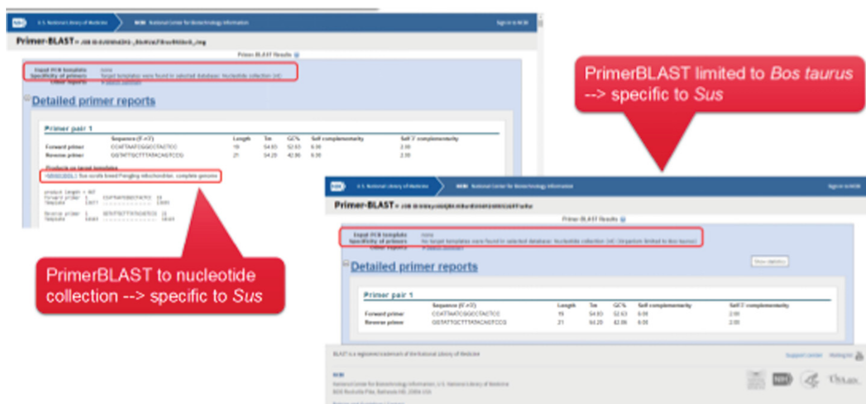

Fig. 3. Primer BLAST results of Sus-F and Sus-R.

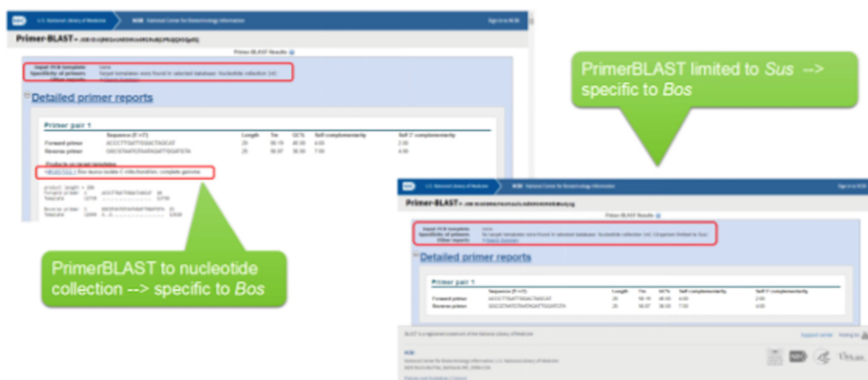

Fig. 4. Primer BLAST results of Bos-F and Bos-R.

Bos-F and Bos-R were checked for the DNA template of *Sus scrofa* and the result was obtained that the primer sequence could not amplify the genes in *Sus scrofa* (Fig. 4). These results suggest that the primer sequences of Bos-F and Bos-R are specific to cows and will not provide false negative results.

3.2 In Silico PCR

Using the software Genome Compiler, in silico PCR was carried out for each primer pairs individually, with results simulating electrophoresis gels (Fig. 5 and Fig. 6). The size of visualized DNA band on gel simulation were consistent with Primer BLAST result, 467 bp for *Sus scrofa* and 206 bp for *Bos taurus.*

In silico PCR is an important stage before starting experiments in laboratories whose reagent prices are quite expensive. Through in silico PCR we can find out the suitability of primary specificity that has been designed with target genes in DNA template sequence [12].

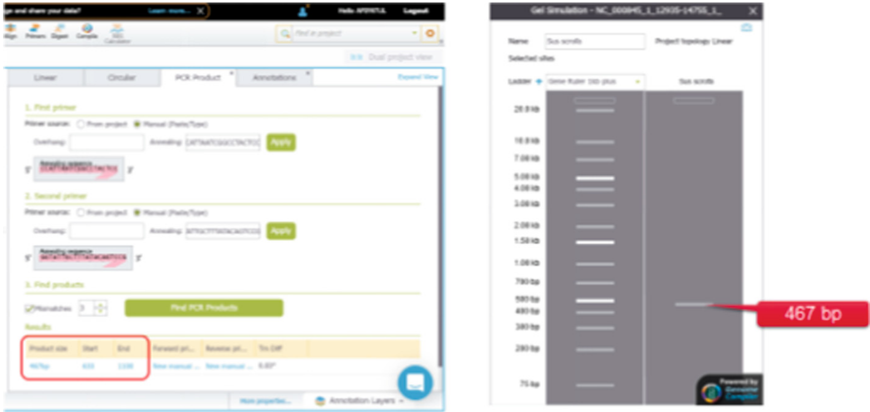

Fig. 5. In silico PCR and gel electrophoresis simulation result of Sus-F and Sus-R using Genome Compiler.

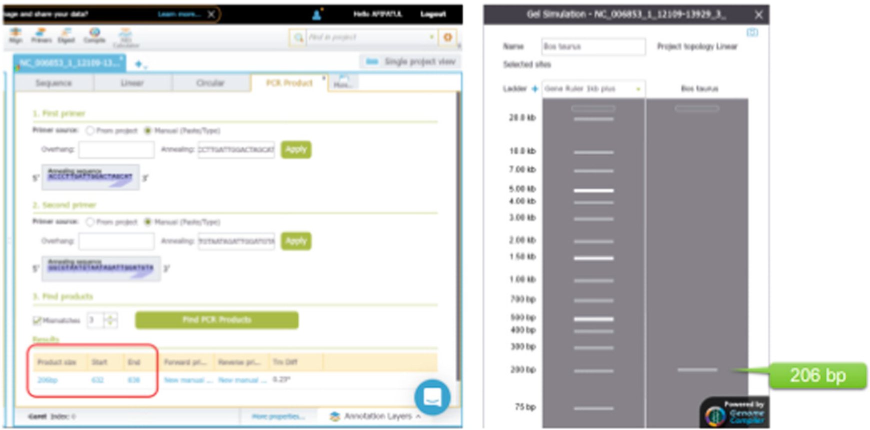

Fig. 6. In silico PCR and gel electrophoresis simulation result of Bos-F and Bos-R using Genome

4 Conclusion

Two pair of primers were generated from this study that amplify 467 bp fragment of *ND5* specific to pork and 206 bp fragment of *ND5* specific to bovine.

Acknowledgement. The Penelitian Dasar Grant from Universitas Negeri Padang, contract number 1562/UN35.13/LT/2020, is gratefully acknowledged by the authors.

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