THE USE OF CYTOCHROME B GENE AS A MARKER FOR MEAT RABBIT (*Oryctolagus* spp.) AUTHENTICATION

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How to cite this paper
Nuraini H, Brahmantiyo B., Sumantri C, Irine E.A., 2013. The use of cytochrome b gene as a marker for meat rabbit (*Oryctolagus* spp.) authentication. *3rd Conference of the Asian Rabbit Production Association, 27-29 August 2013, Bali, Indonesia, 185-189 + poster*

The Use of Cytochrome B Gene as A Marker for Meat Rabbit (*Oryctolagus* spp.) Authentication

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ABSTRACT

Identification of origin species as meat source is very important to ensure the food safety. A suitable technique to identify the source of species was using multiplex PCR, with more than one primer used together for amplification of multiple target regions. This study was to develop specific primer derived from cytochrome b sequences in the Lagomorphs ordo, *Oryctolagus* spp. in order to be used as marker for authentication material. Sources of DNA used in this study were blood samples from cat (*Felis catus*), meat derived from rabbit (*Oryctolagus cuniculus*) and chickens (*Gallus gallus*), and the variety of meat products *i.e.* meatball, corned meat and sausage. DNA was extracted from meat samples using phenol-chloroform method. The detection of authentication through multiplex PCR approach using specific primer. Amplification of cyt b gene in three species of animals with a length of the different fragments indicated the specificity of cyt b gene sequences of each species. Amplified fragment length for rabbit, chickens and cat were 537, 227 and 568 bp, respectively. Test mixtures of DNA were prepared by adding 0.1, 1 and 5% levels of rabbit to chickens meat. The results indicated that the species of meat in various combinations can be accurately determined by PCR. It is concluded that primers from cyt b gene using multiplex PCR can be useful for fast, easy and reliable control of food safety and violation of labeling requirements

Key Words: Cytochrome B Gene, *Oryctolagus cuniculus*, Meat and Meat Products

INTRODUCTION

Increasing meat demand should be followed by a diversified source of meat other than conventional livestock, including rabbit meat. Rabbit (*Oryctolagus cuniculus*) is one of the pet animals, easy to maintain, prolific, has soft fur and a delicious flavor of meat. Rabbit meat is well known throughout the world, and in Indonesia it was part of a government program that aims to improve nutrition as a substitute for beef and chicken. However, the program is slowly progressing because it still has many problems, *i.e.* management, feeding, meat processing and culture of society.

At this time, rabbit meat is gaining popularity again as the need for meat and as a source of healthy food. According to various sources, rabbit meat provides benefits such as: having a higher protein content than other types of meat, low levels of cholesterol, rabbit liver for cure of asthma, rabbit brain believed to increase fertility. This various benefits cause rabbit meat gaining popularity among various other types of meat. On the other hand the continuity of rabbit meat supply is very low so there may be a process of substitution with other meats such as chicken, rat and cavy.

DNA sequences through a process of multiplication technique Polymerase Chain Reaction (PCR) is an alternative in determining the authenticity of a product. This method can use a universal marker or a specific marker that is only found in animal species. In pigs (Sus scrofa) and their relatives it has been found a specific sequence in which these sequences are repetitive sequences and is called with Porcine Repetitive Element 1 (PRE-1) (Nuraini 2004).

Cytochrome b gene is a gene that is often used in phylogenetics to compare multiple species in the same genus or family. The diversity of the cytochrome b gene has been used to detect the source of milk that comes from cows (Bos), sheep (Ovis), goats (Capra) or buffalo (Bubalus) (Lanzilao et al. 2005). Likewise, Pfeiffer et al. (2004) identified the cytochrome b gene diversity in species of cattle (*Bos taurus*), sheep (*Ovis aries*), goat (*Capra hircus*), roe buck (*Capreolus capreolus*) and red deer (*Cervus elaphus*), moose (Wolf et al.

1999), rat (*Rattus norvegicus*) (Nuraini et al. 2012) and guinea pigs (*Cavia porcellus*) (Primasari et al. 2011). Specificity of the cytochrome b gene is expected to be used to identify the presence of rabbit meat mixing with other types of white meat like chicken and cat.

In the present study the aim was to create a specific primer derived from cytochrome b sequences in the Lagomorphs ordo that can be used as a marker for the presence of mixture between rabbit meat with the other meat. The results could be used to assist in the decision-making process related to food security and labelling especially of animal origin, and to participate in efforts to protect the Indonesian people as consumers.

METHODS

Specific primers

Primers used for amplification of specific DNA fragments of chickens were designed as described by Matsunaga et al. (1999). Forward primer used for all 3 types of animals (rabbits, chickens and cats), the 5'-AGCT GACCT CCATCAAACATCT CATCTTG ATGAAA CCC-3 '. Reverse primers for amplification of specific fragments of rabbits were prepared using primer designing software tool (http://www.ncbi.nlm.nih.gov/tools/primerblast /index.cgi). Reverse primer sequences used are presented in Table 1.

Source of DNA

DNA was extracted from blood samples of cats and meat from rabbits and chickens. Meat products used were meatballs, sausage and corned meat coming from rabbits. DNA extraction process was performed using phenol-chloroform method (Sambrook & Russell 2001).

Amplification of specific DNA fragments

Amplification of specific DNA fragments was made by PCR (polymerase chain reaction). Reaction components used were as much as 25 mL, consisting of 35 pmol forward primer, reverse primer @ 5 pmol, 200 lm dNTP mix, 1 mM MgCl2, and 0.5 units of taq polymerase and bufer. Amplification process was run on a GeneAmp ® PCR System 9700 (Applied Biosystems ™) with the conditions of initial denaturation at 95°C for 5 min, 30 cycles consisting of denaturation at 95°C for 10 seconds, annealing at 60°C for 20 sec and elongation of new DNA at 72°C for 30 sec, and final elongation at a temperature of 72°C for 5 minutes

The interpretation results visualization and amplification

Visualization of the results of amplification performed on 2% agarose gel (v/w) were stained with EtBr (ethidium bromide) at UV transiluminator. Specific DNA fragments of chicken, rabbit and cat were demonstrated by DNA bands along the 227, 537 and 568 bp. Visualization results with two or more bands indicated the presence of a mixture of rabbit meat with other meat.

Table 1. PCR oligonucleotide primers

Species	Reverse primer (5'-3')	PCR products	Reference
Rabbit	GAG GAG GTG AAT TAA GAC TAA AGT	537 bp	
Chicken	AAG ATA CAG ATG AAG AAG AAT GAG GCG	227 bp	Matsunaga et al. (1999)
Cat	TGA AAC AGG ATC TAA CAA CCC CT	568 bp	

RESULTS AND DISCUSSION

The degree of similarity between specific primers

The degree of similarity, or often called the homology percentage, was carried out to determine the level of specificity of the DNA sequences. Testing the degree of similarity in specific primer sequences used in this study are presented in Table 2. Based on the testing, the forward primer has a relatively high degree of similarity to the sequences of chicken, rabbits and cats, ranging from 84.211 to 89.474% of the sequences of 38 bases. Reverse primer sequences have a high degree of homology to a certain animal species, so that it can be said that the reverse primer is specific for rabbit.

Amplification of specific fragments of cyt b gene on chicken, rabbit and cat

Successfully cytochrome b gene was amplified using primers for the three species of animals, chickens, rabbits and cats with fragment length of 227, 537 and 568 bp respectively. DNA samples derived from rabbit were successfully amplified using primers that were prepared using primer designing software tools based on the cytochrome b gene sequences in *Oryctolagus cuniculus*.

Visualization of specific DNA fragments of the cytochrome b gene amplification results in this study are presented in Figure 1. Column two and three were chicken and rabbit DNA derived from meat and the fourth column was the cat DNA bands derived from blood, three were positive control. Mixture of DNA to the three species were shown in columns 5 and 6. DNA derived from rabbit and cat were amplified in a separate tube because both have relatively adjacent fragments (537 and 568 bp).

Amplification of specific fragments of DNA cytochrome b at meat product and the DNA mixture

Processed meat products such as meatballs, sausages and corned meat are meat products that are well known by the public. Primary testing was conducted on these meat processed products. The results showed the success rate of rabbit meat and chickens specific primers for the cytochrome b gene amplified sequence on species-specific regions. The results of amplification of specific DNA fragments of cytochrome b in meat rabbit product samples are presented in Figure 1. In addition, the sensitivity of primers to identify particular DNA mixtures was determined by mixing rabbit DNA at the level of 0,1; 1 and 5%. The results of the cyt b gene amplification in the DNA mixture of rabbit DNA are presented in Figure 2. These results might be useful for effective control of authentication and violation of labeling requirements for meat products.

Table 2. Homology percentage of spesific reverse primers

Specific primer	*Gallus gallus* (227 bp)	*Oryctolagus cuniculus* (537 bp)	*Felis catus* (568 bp)
Forward (38 nt)	89.474	86.842	84.211
Chicken (27 nt)	100.000	62.963	62.963
Rabbit (24 nt)	50.000	100.000	58.333
Cat (23 nt)	78.261	78.261	100.000

Table 3. Sensitivity primers of Oryctolagus *cyt b* gene

Sample	Rabbit	Chickens
Sensitivity 5% (chickens 95% : rabbit 5%) (1 µl)	+++	+++
Sensitivity 5% (chickens 95% : rabbit 5%) (2 µl)	+++	+++
Sensitivity 1% (chickens 99% : rabbit 1%) (1 µl)	++	+++
Sensitivity 1% (chickens 99% : rabbit 1%) (2 µl)	++	+++
Sensitivity 0.1% (chickens 99.9% : rabbit 0.1%) (1 µl)	+	+++
Sensitivity 0.1% (chickens 99.9% : rabbit 0.1%) (2 µl)	+	+++

+: indicates the intensity of the thickness of the PCR product

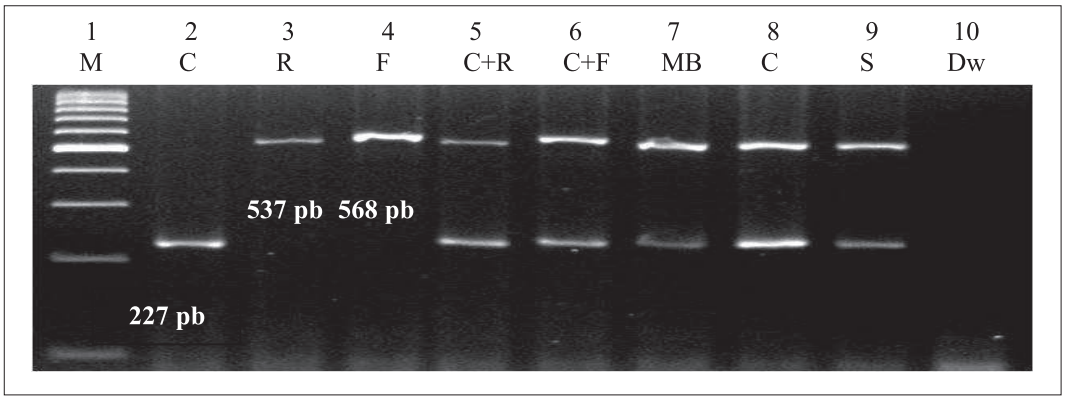

Figure 1. PCR product amplified with specific. M: marker 100 pb; C: chicken, R: rabbit; F: cat; C+R: chicken + rabbit; C+F: chicken + cat; MB: meatball; C: corned; S: sausage; Dw: water (negative control)

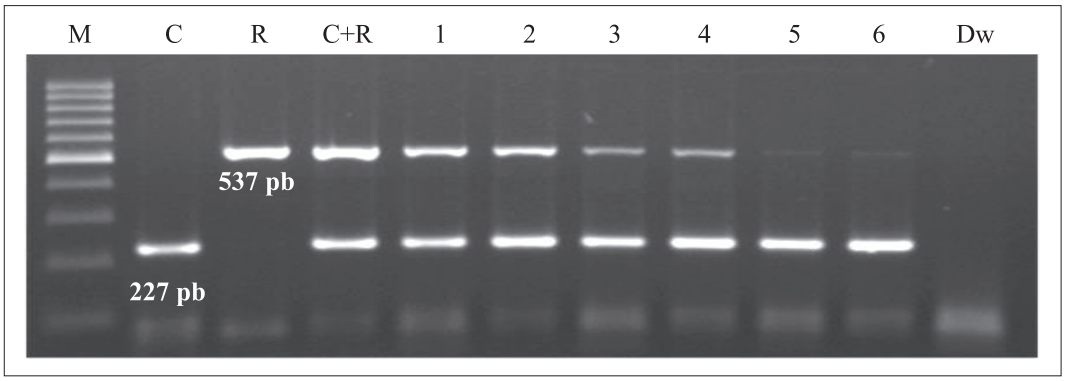

Figure 2. PCR products from mixtures of chicken and rabbit meat with rabbit-specific primer. M: marker 100 bp; C: chicken; R: rabbit; (1); 5% rabbit meat (1 µl); (2) 5% rabbit meat (2 µl); (3) 1% rabbit meat (1 µl); (4) 1% rabbit meat (2 µl); (5) 0.1% rabbit meat (1 µl); (6) 0,1% rabbit meat (2 µl); Dw: water (negative control)

CONCLUSION

The authentication of meat's origin is important for the protection of consumers, for this reason primer from cyt b gene using multiplex PCR can be useful as fast, easy and reliable method.

ACKNOWLEDGMENTS

The authors would like to thank the Head of the Laboratory of Animal Breeding and Genetics, Department of Animal Production and Technology, Faculty of Animal Science. Thanks are also extended to the Head of Halal Science Center, Bogor Agricultural University who has allowed to use the laboratory, the undergraduate and graduate students who helped in preparation of samples.

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THE USE OF *CYTOCHROME b* GENE AS A MARKER FOR MEAT RABBIT (*Oryctolagus spp.*) AUTHENTICATION

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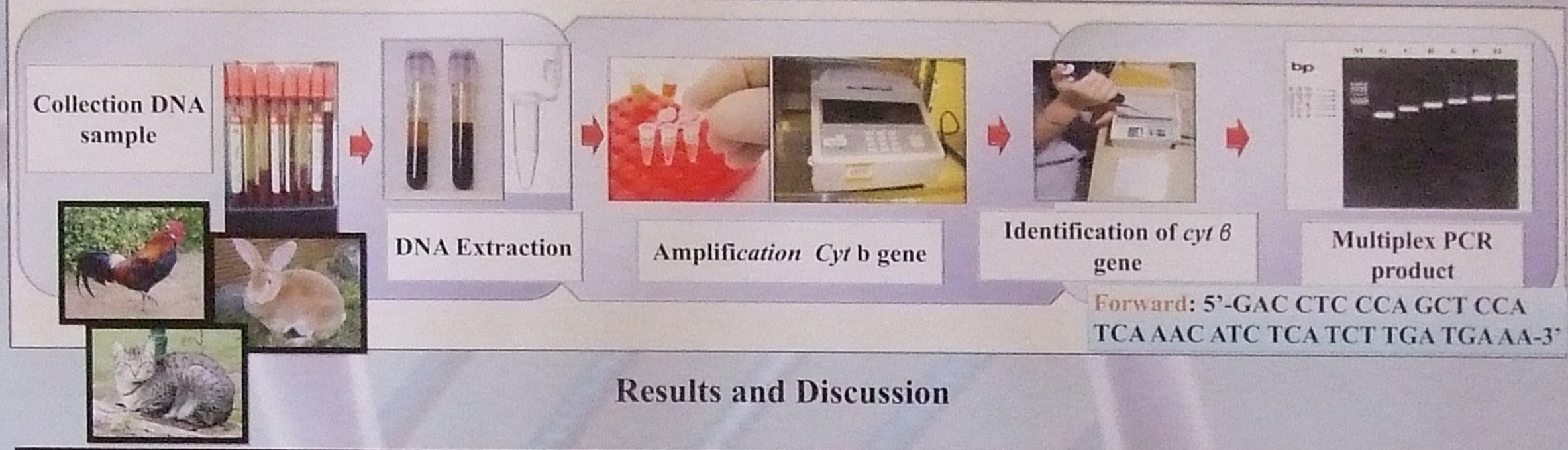
Introduction

Identification of origin species for meat source are very important to ensure the food safety. A suitable technique to identify the species source using multiplex PCR, which more than one primer used together for amplification multiple target regions. The objective of this research was to develop specific primer derived from *cytochrome b* (*cyt b*) sequences in the *Lagomorphs* ordo, *Oryctolagus spp* that used as a marker for authentication animal origin.

Material and Method



Results and Discussion

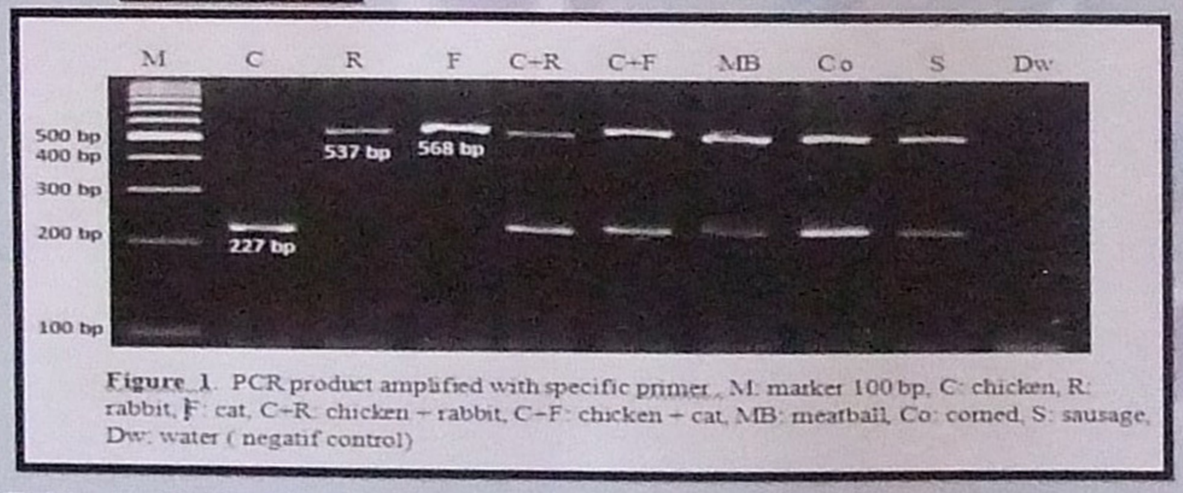


Figure 1. PCR product amplified with specific primer , M: marker 100 bp, C: chicken, R: rabbit, F: cat, C+R: chicken + rabbit, C+F: chicken + cat, MB: meatball, Co: corned, S: sausage, Dw: water (negatif control)

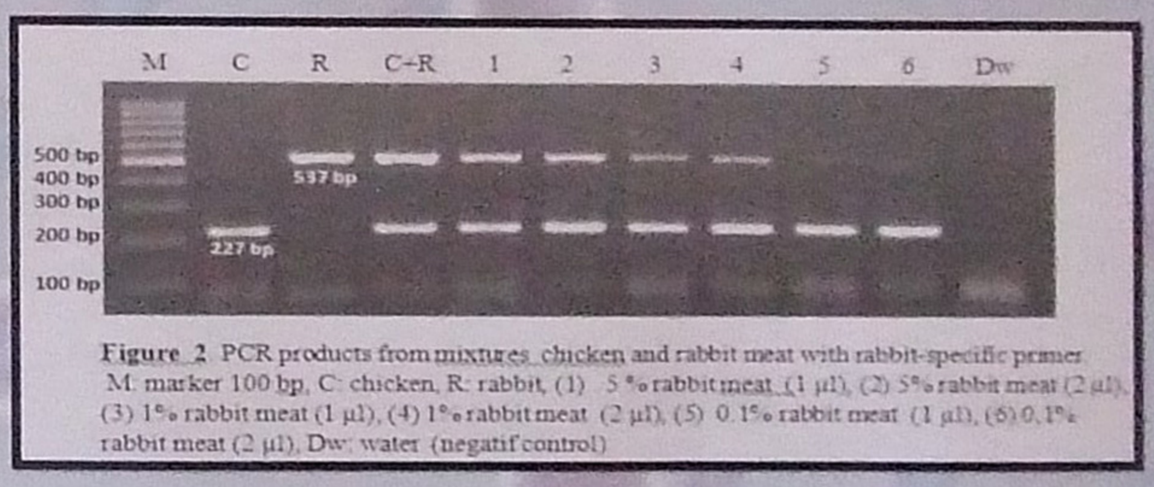


Figure 2 PCR products from mixtures chicken and rabbit meat with rabbit-specific primer. M: marker 100 bp, C: chicken, R: rabbit, (1) 5 % rabbit meat (1 µl), (2) 5% rabbit meat (2 µl), (3) 1% rabbit meat (1 µl), (4) 1% rabbit meat (2 µl), (5) 0.1% rabbit meat (1 µl), (6) 0.1% rabbit meat (2 µl), Dw: water (negatif control)

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Sensitivity 1% (chicken 99% : rabbit 1%) (1 µl)	++	+++
Sensitivity 1% (chicken 99% : rabbit 1%) (2 µl)	++	+++
Sensitivity 0.1% (chicken 99.9% : rabbit 0.1%) (1 µl)	+	+++
Sensitivity 0.1% (chicken 99.9% : rabbit 0.1%) (2 µl)	+	+++

Description: +: indicated thickness intensity of pcr product

- ✓ *Cyt b* gene amplification in chicken, rabbit, and cat with different fragments length indicated the specificity of *cyt b* gene.
- ✓ Amplified fragment length for rabbit, chicken and cat were 537 bp, 227 bp, and 568 bp, respectively.
- ✓ Rabbit primer sensitivity accurated to 0.1% DNA level in 99.9% chicken mixture

Conclusion

Specific primer designed from *cyt b* gene with multiplex PCR can be useful for fast, easy and reliable technique to control food safety and labeling fault.

Acknowledgments

- ✓ Indonesian Research Institute for Animal Production
- ✓ Head of the Livestock Breeding and Genetics, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University
- ✓ Halal Science Center, Bogor Agricultural University
- ✓ Undergraduate and graduate students

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