Multiplex PCR assay for species identification of meat and dairy products from buffalo (*Bubalus bubalis*), cattle (*Bos indicus* and *Bos taurus*), goat (*Capra hircus*), and sheep (*Ovis aries*)

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Abstract

Cases of fraudulent meat and dairy products have increased worldwide, especially in developing countries. To determine the misrepresented animal species, appropriate tools in routine monitoring should be available for food inspections. In the present work, a multiplex polymerase chain reaction assay for species identification of products from ruminants including buffalo, cattle, goat, and sheep was developed. The primer set KUMUT_cFarmSp1 was composed of five species-specific primers and a pair of positive-control primers. The primer set amplified 106-, 163-, 232-, and 308-bp specific fragments from the cytochrome *b* (*cyt b*) gene of buffalo, cattle, goat, and sheep, respectively, and 370-bp positive-control fragment from 16S ribosomal RNA (16S rRNA). The detection limit of this PCR assay is 0.1 ng of DNA template. The developed primer set exhibited strong specificity, sensitivity, robustness, and simplicity for food verification, thus indicating its usefulness for species verification in food quality control and law enforcement.

Keywords

consumer protection, DNA marker, food surveillance, PCR-based detection, species-specific markers

Introduction

The most important domesticated animals in livestock agriculture are in the family Bovidae, specifically buffalo, cattle, goat, and sheep (Craine et al., 2017). Several million tons of the meat and dairy products from these species are supplied to global markets, and employed as important food ingredients for many consumers worldwide (FAOSTAT, 2017). While the demand and market value of the meat and dairy products from ruminants are continuously increasing (Sans and Combris, 2015; IFCN, 2018), the incidents of food fraud have skyrocketed (Ballin, 2010; Handford et al., 2016). The majority of food fraud reports regarding these ruminants’ products have involved substitutions of products from less expensive species with / in those of more expensive species (Romero et al., 1996; Song et al., 2011; Abdul-Hassan and Tauma, 2014; Pinto et al., 2015; 2017; Chuah et al., 2016; Esteki et al., 2019). Fraudulent meat and milk products represent global threats in many aspects such as public health, fair trade, law enforcement, as well as in religious, cultural, and ethnic considerations (Spink and Moyer, 2011; Alamprese et al., 2013; Curll, 2015; Kamruzzaman, 2016; Cartin-Rojas, 2017).

To detect the cases of fraud in the meat and dairy industry, it is necessary to develop an effective investigation tool for species identification of raw materials in food products. Previously, a number of species-specific analytical techniques have been reported; these techniques employ four major types of analytical techniques namely chromatography, immunology, spectroscopy, and electrophoresis (Bargen et al., 2014; Rodriguez et al., 2010; Zelenakova et al., 2010; Zhou et al., 2016). Although all these techniques are reliable for species identification, their advantages and disadvantages vary depending on the user’s objectives and applications, with the disadvantages of these methods are preventing them from being used routinely (Esteki et al., 2019). In brief, protein-based techniques such as immunological and electrophoretic methods are less sensitive to heat-treated meat and dairy products due to the protein denaturation and high complexity of milk proteins. Additionally, these procedures are generally time-consuming, labour-intensive, and expensive (Murugaiah et al., 2009; De et al., 2011; Poonia et al., 2016). Similarly, DNA-based techniques for species identification of food products such as PCR-RFLP, real-time PCR, DNA sequencing, and next-generation sequencing cannot be applied routinely simply because they are labour-intensive,
time-consuming, and have a high cost per sample due to the requirements of expensive reagents and equipment (Kumar et al., 2015; Reuter et al., 2015; Rahmati et al., 2016).

Recently, fraudulent meat and milk products cases have increased significantly, especially in the developing and underdeveloped countries (Azad and Ahmed, 2016; Tibola et al., 2018). Therefore, appropriate monitoring tools that can be easily expedited and overcome the challenges faced by previously utilised techniques should be developed to detect fraudulent meat and dairy products. In the present work, a new primer set for multiplex end-point PCR and optimal PCR conditions were developed and validated for both meat and milk products. The target gene employed for the identification of each target species was *cytochrome b* (*cyt b*), which was previously applied to identify animal species in various taxa. Multiplex end-point PCR was designed for simultaneous amplification of species-specific fragments which may be detected by a simple DNA sizing method with agarose gel electrophoresis. Therefore, this DNA-based marker may provide a more robust species identification tool that can resolve future cases of fraudulent food, thus benefiting public health and fair trade worldwide.

**Materials and methods**

**Samples**

Three different groups of samples were collected between October 2018 and May 2019. The first group included blood samples obtained from buffalo (*Bubalus bubalis*), cattle (*Bos indicus*), goat (*Capra hircus*), and sheep (*Ovis aries*). These samples were used as DNA sources for primer testing, optimisation of PCR conditions, and positive controls. The second group contained blood samples of eight animal species including the four target species in group 1 along with pig (*Sus scrofa domesticus*), dog (*Canis lupus familiaris*), cat (*Felis catus*), and horse (*Equus ferus caballus*). Meat samples of six animal species were also obtained and analysed namely chicken (*Gallus gallus domesticus*), duck (*Anas platyrhynchos domestica*), goose (*Anser cygnoides domesticus*), East Asian bullfrog (*Hoplobatrachus rugulosus*), Nile tilapia fish (*Oreochromis niloticus*), and blue swimming crab (*Portunus pelagicus*). These samples were utilised to extract DNA for the primer set specificity test. The third group consisted of food product samples, as described in Table 1. All blood samples were collected from the Faculty of Veterinary Medicine, Mahanakorn University of Technology, Bangkok, Thailand, while meat and food product samples were purchased from local open markets and supermarkets in Bangkok, Thailand.

**DNA extraction**

The total genomic DNA extraction from blood and meat samples was performed using the FavorPrep™ Blood Genomic DNA Extraction Mini Kit and the FavorPrep™ Tissue Genomic DNA Extraction Mini Kit, respectively, following the manufacturers’ protocols (Favorgen Biotech Corp., Taiwan).

DNA extraction from variety of meat and milk products was performed using a modified procedure that combined the Tris-EDTA method and salting-out method (Yalcinkaya et al., 2017). All extractions from meat in processed foods were performed in 1.5-mL microcentrifuge tubes. Meat samples (30 - 50 mg) were added to 400 µL of

<table>
<thead>
<tr>
<th>No.</th>
<th>Meat product</th>
<th>Labelled/expected species</th>
<th>Detected species</th>
<th>No.</th>
<th>Dairy product</th>
<th>Labelled/expected species</th>
<th>Detected species</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stir-fried buffalo and basil</td>
<td>buffalo</td>
<td>buffalo</td>
<td>15.</td>
<td>Raw buffalo milk</td>
<td>buffalo</td>
<td>buffalo</td>
</tr>
<tr>
<td>2.</td>
<td>Dry buffalo leather</td>
<td>buffalo</td>
<td>buffalo</td>
<td>16.</td>
<td>Raw cow milk</td>
<td>cattle</td>
<td>cattle</td>
</tr>
<tr>
<td>3.</td>
<td>Dry cow leather</td>
<td>cattle*</td>
<td>cattle</td>
<td>17.</td>
<td>Raw goat milk</td>
<td>goat</td>
<td>goat</td>
</tr>
<tr>
<td>4.</td>
<td>Roast beef</td>
<td>cattle</td>
<td>cattle</td>
<td>18.</td>
<td>Cow milk pasteurized</td>
<td>cattle</td>
<td>cattle</td>
</tr>
<tr>
<td>5.</td>
<td>Pastrami beef</td>
<td>cattle</td>
<td>cattle</td>
<td>19.</td>
<td>Goat milk UHT</td>
<td>goat</td>
<td>goat</td>
</tr>
<tr>
<td>6.</td>
<td>Meatball</td>
<td>cattle</td>
<td>cattle</td>
<td>20.</td>
<td>Cow milk powder</td>
<td>cattle</td>
<td>cattle</td>
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<tr>
<td>8.</td>
<td>Beef biryani</td>
<td>cattle</td>
<td>cattle</td>
<td>22.</td>
<td>Cow-milk mozzarella</td>
<td>cattle</td>
<td>cattle</td>
</tr>
<tr>
<td>9.</td>
<td>Herby goat stew</td>
<td>goat</td>
<td>goat</td>
<td>23.</td>
<td>Buffalo-milk mozzarella</td>
<td>buffalo</td>
<td>buffalo</td>
</tr>
<tr>
<td>10.</td>
<td>Fried goat with garlic</td>
<td>goat</td>
<td>goat</td>
<td>24.</td>
<td>Cheddar cheese</td>
<td>cattle</td>
<td>cattle</td>
</tr>
<tr>
<td>11.</td>
<td>Lamb moussaka</td>
<td>lamb</td>
<td>lamb</td>
<td>25.</td>
<td>Goat cheese</td>
<td>goat</td>
<td>goat</td>
</tr>
<tr>
<td>13.</td>
<td>Meat dog food (animal feed)</td>
<td>cattle</td>
<td>cattle</td>
<td>27.</td>
<td>Yoghurt</td>
<td>cattle</td>
<td>cattle</td>
</tr>
<tr>
<td>14.</td>
<td>Lamb dog food (animal feed)</td>
<td>lamb</td>
<td>lamb</td>
<td>28.</td>
<td>Butter</td>
<td>cattle</td>
<td>cattle</td>
</tr>
</tbody>
</table>

*Cattle for meat and milk productions are most commonly *Bos indicus* and *Bos taurus*, respectively.*
extraction buffer [20 mM Tris-HCl and pH 8.0; 20 mM EDTA and pH 8.0; 2% (w/v) SDS; and 40 µL of 10 mg/mL proteinase K (Amresco, Solon, OH, USA)]. The mixture was vortexed, and incubated at 65°C for 3 h. Next, 200 µL of 5 M NaCl was added, and samples were incubated at 65°C for 2 min, followed by incubation on ice for 10 min. Following centrifugation at 15,000 g for 3 min, the supernatant was transferred to a new tube. Equal volumes of saturated phenol were added, and then the mixture was vortexed and centrifuged at 15,000 g for 3 min. The collected upper aqueous phase was subsequently purified using successive extractions with phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1). DNA was precipitated by the addition of an equal volume of isopropanol alcohol, subsequently mixed by inversion, and centrifuged at 15,000 g for 15 min. The pellet was washed with 70% (v/v) ethanol, air-dried, and resuspended in 100 µL TE buffer (10 mM Tris, 0.1 mM EDTA, and pH 8.0).

Most steps of the DNA extraction protocol for solid and soft-solid dairy products were the same as those employed for meat products, except for the sample weight, extraction buffer volume used, and volume of 5 M NaCl which were 1 g, 5 mL, and 1 mL, respectively. For liquid milk, 50 mL of milk was centrifuged at 15,000 g for 3 min to obtain the milk pellet, which was subsequently handled as a solid or soft-solid dairy product.

Primer design
A multiplex PCR primer set for species identification of buffalo, cattle, goat, and sheep was developed based on the cyt b and 16S rRNA genes. Reference nucleotide sequences of cyt b and 16S rRNA are available in GenBank including NC_006295 (Bubalus bubalis), NC_005971 (Bos indicus), NC_006853 (Bos taurus), NC_005044 (Capra hircus), and NC_001941 (Ovis aries). The nucleotide sequences were aligned using Clustal W2 software (Larkin et al., 2007). The primers were designed using the Primer-BLAST program (Ye et al., 2012). The designed primers were synthesised by Macrogen Incorporation, Seoul, South Korea.

Optimisation of multiplex PCR
The optimisation of multiplex PCR conditions for the designed primer set was undertaken in two main steps. In the first step, five primer combinations (four species-specific combinations and a positive-control combinations) were tested independently by monoplex PCR to identify a single suitable annealing temperature for all combinations.

In the second step, various multiplex PCR components which were determined according to the instructions of the Taq DNA polymerase manufacturer (Thermo Fisher Scientific, Lithuania, EU) were tested to increase their sensitivity while maintaining the specificity of the primer set.

The sizes of PCR products were determined using gel electrophoresis analysis on 2.5% (w/v) agarose for 70 min at 100 V in TBE buffer. The GeneRuler™ Low Range DNA Ladder of 25 - 700 bp (Thermo Fisher Scientific, Lithuania, EU) was employed for size estimation of PCR products. DNA bands on the gel were visualised by staining with ethidium bromide (0.5 µg/mL). Gel images were recorded by a UV transilluminator using a BioDoc-It™ Imaging System (UVP, Upland, California, USA).

Utility evaluation of the species-specific multiplex PCR
The specificity of the novel primer set and its PCR conditions were evaluated on the DNA template of four species for target ruminants (buffalo, cattle, goat, and sheep), four species of other mammals (pig, dog, cat, and horse), three species of domestic avians (chicken, duck, and goose), and three species of other food animals (frog, fish, and crab).

To determine the DNA template concentration limit, the sensitivity of the multiplex PCR was tested using serial dilutions of DNA templates (10, 1, 0.1, and 0.01 ng/µL). The sensitivity tests were performed using both single and mixed DNA templates of target species.

The verification of the new primer set for species identification of commercial meat and milk products was performed on 28 samples, including 14 samples of each meat and milk product (Table 1). Intact or diluted (if necessary) DNA solutions with concentrations in the range of 1 - 100 ng/µL were employed for the multiplex PCR.

Results
Primer design
Independent alignment of full-length cyt b and 16S rRNA genes from buffalo, cattle, goat, and sheep yielded lengths of 1,140 for cyt b and 1,584 bp for 16S rRNA. A multiplex PCR primer set for identifying target ruminant species that consisted of species-specific and positive-control primers was successfully developed based on the obtained alignment results. Oligonucleotide primers for species-specific amplification consisted of four species-specific forward primers of buffalo, cattle,
goat, and sheep species and a common reverse primer, all of which were designed to target the \textit{cyt} \textit{b} gene (Figure 1a). Additionally, a pair of positive control primers specific to the conserved regions of the 16S rRNA gene were also designed (Figure 1b). The new primer set was named MUTKU\(_{\text{cFarmSp1}}\) (Table 2).

### Amplification of Multiplex PCR

The optimised PCR conditions employed with the MUTKU\(_{\text{cFarmSp1}}\) primer set were of 20-µL total reaction volume containing 2 µL 10X Taq Buffer, 1.25 mM MgCl\(_2\), 0.2 mM of each dNTP, 2 units Taq DNA polymerase (Thermo Fisher Scientific, Lithuania, EU), 0.250 - 20.000 µM of each primer (Table 2), and 1 µL of each DNA sample for both single and multiple template PCRs. The thermal cycler parameters were: initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, elongation at 72°C for 30 s, and final elongation at 72°C for 30 s. This PCR assay amplified 106-, 163-, 232-, and 308-bp specific fragments for buffalo, cattle, goat, and sheep, respectively (Figure 1). At the same time, the 370-bp positive-control fragment could be amplified alongside all species-specific fragments in the same PCR tube.

### Specificity

Verification of the MUTKU\(_{\text{cFarmSp1}}\) primer set was undertaken to assess the possibility of species misidentification. PCR amplification using DNA templates from the 14 animal species mentioned earlier showed simultaneous amplification of both species-specific and positive-control fragments of buffalo, cattle, goat, and sheep DNA templates. Only positive-control fragments were amplified when the template DNA consisted of pig, dog, cat, horse, goose, bullfrog, or Nile tilapia fish total DNA. However, the templates DNA from chicken, duck, and blue swimming crab yielded no amplification. Species-specific bands and positive-control bands could not be detected (Figure 2). Unexpected fragments or misidentified fragments were also not detected in any PCR products.

### Sensitivity

The sensitivity of the MUTKU\(_{\text{cFarmSp1}}\) primer set under its optimal PCR conditions was verified on serial dilutions of single and mixed DNA templates (Figure 3). The species-specific and control fragments could be detected by single-template multiplex PCR using 0.01 ng/µL of buffalo, cattle, and goat DNA samples, whereas 0.1 ng/µL was required for the sheep DNA sample. Therefore, the 0.1 ng/µL concentration of template DNA is considered to be the minimum DNA concentration that can be analysed by this primer set.

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### Table 2. Primer description, optimal concentration, and size of PCR products of the MUTKU\(_{\text{cFarmSp1}}\) primer set.

<table>
<thead>
<tr>
<th>No.</th>
<th>Primer name</th>
<th>Sequence (5’-3’)</th>
<th>Target gene</th>
<th>Description</th>
<th>Optimal final concentration (µM)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BbCB(_{\text{spH3}})</td>
<td>CATACATCCAAACAACGAAGTATG</td>
<td>\textit{cyt} \textit{b}</td>
<td>Buffalo specific forward primer</td>
<td>5.000</td>
<td>106</td>
</tr>
<tr>
<td>2.</td>
<td>BosCB(_{\text{spH2}})</td>
<td>TAGGAGGAGTACTAGCCTAGGCT</td>
<td>\textit{cyt} \textit{b}</td>
<td>Cattle specific forward primer</td>
<td>0.250</td>
<td>163</td>
</tr>
<tr>
<td>3.</td>
<td>ChCB(_{\text{spH1}})</td>
<td>CCTCACATTAAACCTGAGTGGTAT</td>
<td>\textit{cyt} \textit{b}</td>
<td>Goat specific forward primer</td>
<td>1.400</td>
<td>232</td>
</tr>
<tr>
<td>4.</td>
<td>OaCB(_{\text{spH4}})</td>
<td>CATGCTACTAGTACTATTTACGCT</td>
<td>\textit{cyt} \textit{b}</td>
<td>Sheep specific forward primer</td>
<td>20.000</td>
<td>308</td>
</tr>
<tr>
<td>5.</td>
<td>Runn(_{\text{uniL1}})</td>
<td>CTGGYGTKCCTCAATYCATG</td>
<td>\textit{cyt} \textit{b}</td>
<td>Common reverse primer</td>
<td>2.000</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>uFarm(_{\text{16sH11}})</td>
<td>AAGACGAGAAGACCTATGGGAC</td>
<td>16S rRNA</td>
<td>Positive control forward primer</td>
<td>1.000</td>
<td>370</td>
</tr>
<tr>
<td>7.</td>
<td>uFarm(_{\text{16sL12}})</td>
<td>GCTCCGGTCTGAACCTCGACG</td>
<td>16S rRNA</td>
<td>Positive control reverse primer</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>

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![Figure 2. Agarose gel electrophoresis of multiplex PCR using the MUTKU\(_{\text{cFarmSp1}}\) primer set. Lane M = Low Range GeneRuler\textsuperscript{TM}, 1 = negative control, 2 = buffalo, 3 = cattle, 4 = goat, 5 = sheep, 6 = pig, 7 = dog, 8 = cat, 9 = horse, 10 = chicken, 11 = duck, 12 = goose, 13 = East Asian bullfrog, 14 = Nile tilapia fish, and 15 = blue swimming crab.](image-url)
Assessment of commercial meat and dairy products

The primer set was tested on several types of commercial meat and milk food products. Gel images (Figure 4) showed specific fragments of meat or milk species in all food samples, while a positive control band (370 bp) could be detected in all food samples, except for dog meat. Fraudulent food was not detected in the food samples tested in the present work.

Discussion

In the present work, a multiplex PCR assay using the primer set MUTKU_cFarmSp1 (Table 2) was investigated as a species-specific DNA-based assay for the detection of any traces of farmed ruminants including buffalo, cattle, goat, and sheep in commercial food products (Figures 2 and 4); this assay can be used instead of a number of existing protein-based or DNA-based assays. The major disadvantages of the previous methods are expensive, time-consuming, labour-intensive, and produce results that are difficult to interpret. These drawbacks are the major obstacles facing the routine application of MUTKU_cFarmSp1 in basic laboratories of many.
developing countries where fraudulent meat and dairy products are often found (Handford et al., 2016). Therefore, MUTKU_cFarmSp1 was intentionally developed as a DNA marker that can be routinely employed for species detection with high specificity, sensitivity, robustness, and more importantly, simplicity. These properties are key characteristics that may enable MUTKU_cFarmSp1 to become a powerful detection instrument against cases of fraudulent food for the studied ruminants (buffalo, cattle, goat, and sheep) because this assay features a potent detection tool and accurate data for food components even when they are present only in trace amounts, and it can also identify down to the species level. The information obtained by this method may be a useful complement to the blockchain technology currently being employed in the food supply chain (Galvez et al., 2018).

The sensitivity of the species-specific simplex and multiplex PCRs has typically been reported as a minimum requirement of DNA template per reaction for successful amplification of target DNA fragments. Recently, the sensitivity of species-specific PCRs has been reported through two different parameters based on different detection methods. For the first parameter, total genomic DNA, including both nuclear and organelle DNA in the PCR, is used. The range of detection sensitivity based on the amount of total genomic DNA was reported to be 5 - 0.001 ng (Zha et al., 2010; Dai et al., 2015), which included 0.1 ng sensitivity of MUTKU_cFarmSp1 (Figure 3). For the second parameter, the mtDNA copy number is utilised to set a sensitivity limit (Tobe and Linacre, 2008). For a direct-multiplex PCR assay...
for meat identification, the sensitivity limit was reported to be 12,500 mitochondrial copies, which was equivalent to 7 fg (Kitpipit et al., 2014). To obtain the mtDNA copy number, a complex direct evaluation of total non-human mammalian mtDNA must be performed. However, it has been commonly recognised that sensitivity limits reported by both parameters provide good references of minimal DNA amount required for PCR-based detection methods; as a result, the total genomic DNA remains the preferred parameter for sensitivity limit due to its simplicity of detection.

According to the robustness testing of the MUTKU_cFarmSp1 primer set on real samples, the primer set was able to identify its target species from various types of commercial meat and milk products (Table 1). Typically, the robustness of species-specific multiplex PCR for food product identification is primarily determined by the lengths of PCR products. The optimal lengths of the target fragments in the present work ranged from 100 to 400 bp (Table 2). Long PCR products (> 500 bp) would be adversely affected by DNA fragmentation, which can be caused by both food processing steps or particular ingredients in the food (Pereira et al., 2008; Piskata et al., 2017); while short products (< 100 bp) are difficult to analyse by agarose gel electrophoresis but can be easily managed using polyacrylamide gel electrophoresis (Pereira et al., 2008; Barril and Nates, 2012). Moreover, the MUTKU_cFarmSp1 primer set not only comprises species-specific primers but also includes a pair of positive control primers, which can notably simplify the interpretation of the results by providing clear verification of the presence of all PCR components, the absence of PCR inhibitors, and normal operation of the thermal cycler. Furthermore, the position of the positive control PCR products on a gel image could be used as a secondary size reference of species-specific bands in addition to the primary size reference provided by the commercial DNA ladders (Figure 2 and 4). Despite the advantages of having the positive control primers in species identification using multiplex primer sets, there have been only a small number of reports that included them in the identification of food products (Catanese et al., 2010; Hellberg et al., 2010; Sangthong et al., 2014; Suwannarat et al., 2017), and in some meat and dairy products from farmed ruminants (Xue et al., 2017).

Finally, there are two considerations to be contemplated when using the MUTKU_cFarmSp1 primer set. First, the time requirement for species detection is approximately 8 h, including 4 h for DNA extraction, 2 h for PCR, and 2 h for gel electrophoresis. This time period is longer than those of several previously reported methods, such as real-time PCR (Sawyer et al., 2003; Sakalar and Kaynak, 2016), the Lab-on-a-Chip electrophoretic system (Ali et al., 2015), and the direct multiplex PCR assay (Kitpipit et al., 2014; Thanakiatkrai et al., 2019). Although a rapid protocol is undoubtedly an advantage, if one takes into account that the main application of this primer set provides a simple and reliable method for accurate species identification that might be used for law enforcement and for quality control of meat and dairy products, then a small delay of a few hours is insignificant, as the time frame of inspection would still be within day(s) or week(s), similar to the old methods (FAO, 1993). Second, the MUTKU_cFarmSp1 primer set can easily and economically be upgraded to become high throughput by utilising an automated separation system of capillary electrophoresis. For this upgrading, only two primers (first: Rumn_uniL1 and second: uFarm_16sH11 or uFarm_16sL12) need to be labelled by fluorescent dyes, such as 6-carboxyfluorescein (6-FAM). The automated separation for the species identification of food products is suitable for large-scale service laboratories.

Conclusion

In the present work, a multiplex PCR primer set named MUTKU_cFarmSp1, and its optimised PCR conditions for species identification of buffalo, cattle, goat, and sheep were developed. This assay was designed to be a simple protocol with high specificity, high sensitivity, and strong robustness in the detection of target species. The primer set can be effectively tested not only on various meat and dairy products, but also on animal feed and leather products. This assay can be a useful application in routine food surveillance performed by both the private companies and government agencies.

Acknowledgement

The present work was financially supported by Mahanakorn University of Technology, Kasetsart University Research and Development Institute (KURDI), and the Graduate School of Kasetsart University.

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Sangthong, D., et al./IFRJ 28(4) : 716 - 725

724


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