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### Authentication of *Rusa timorensis* DNA using Sequencing Assay with Modified Extraction Method

Nooratiny Ishak<sup>\*a</sup>, Azwan Mat Lazim<sup>b</sup>

<sup>a</sup>Halal Analysis Division, Industry and Custom Tariff Analysis Centre, Department of Chemistry, Sultan Road, 46661, Petaling Jaya, Selangor.

<sup>b</sup>Department of Chemical Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor  
Konsortium Institut Halal IPT Malaysia, Ministry of Higher Education, Block E8, Complex E, Federal Government Administrative Centre, 62604 Putrajaya.

\*Corresponding author: E-mail address: [noratiny@kimia.gov.my](mailto:noratiny@kimia.gov.my)

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#### Abstract

A technique to isolate DNA from deer meat was established to authenticate the deer species and was successfully extracted from deer meat. The method was based on the Epicentre MasterPure™ Complete DNA and RNA Purification Kit extraction kit with slight modification by increasing the volume of Proteinase K and RNase from 5 µL to 20 µL each. The recovery of deer DNA was then analysed by Polymerase Chain Reaction using cytochrome b gene oligonucleotide primers for the deer family, which targeted the mitochondrial DNA. The increasing volume of Proteinase K and RNase resulted in clean DNA and no smearing after performing Polymerase Chain Reaction. Furthermore, the amplified DNA product was extended to sequence assay to authenticate deer species above 95% maximum identification. The result showed that the increasing volume of Proteinase K and RNase offered a high yield of DNA recovery, which provided more than 100 ng per µL and was useful in validating animal-based sources, especially in deer products.

#### 1. Introduction

*Rusa timorensis* are the largest deer species, and they are medium-sized deer, rough-coated deer, and anthropogenic activities of *R. timorensis* are influenced by habitat, human hunting activities, and translocation. The criteria that affect the antropogenic activities of this deer species lead to a substantial impact on genetic diversity and the long-term conservation of the local population of this species.

The breeder also faces a problem sustaining the population of this species because the supply and demand are too high. In order to overcome this situation, efficient management of wild or captive deer population to maintain the deer population viable and enhance the potential breeding performance of farmed population requires the development of molecular genetic tools (Bonnet *et al.*, 2002; Zein & Maharadatunkamsi, 2003).

The approaches of mtDNA known as specific, reproducible, sensitive, rapid processing time, and low costs have been adapted in the molecular technique to obtain the DNA sequence and construct the intra-specific relationship for these six deer. Specifically, mitochondrial DNA has proven to be an ideal sequence for phylogeographic analyses due to its high rate of sequence evolution, uniparental inheritance, lack of recombination, and variable marker for phylogenetic genetic diversity purposes (Avisé 2000; Lowe *et al.* 2004).

The major problem in DNA extraction from meat is the high fat content, which contributes to inhibiting PCR analysis, and these inhibitors are difficult to remove during the DNA extraction process and remain until the final DNA preparations (Di Pinto *et al.*, 2007). Thus, DNA purity and reliability of the DNA extraction technique are crucial to yielding as much DNA as possible from the sample, and the technique must be improved in recovering nucleic acid and removing inhibitors prior to PCR analysis.

The characterisation of the structural integrity of DNA (plasmid and genomic) is essential in a wide variety of biological applications. Therefore, a simple protocol to assess DNA quality, such as this one, may have critical clinical and molecular research applications. For example, it can assess the DNA samples before analysing tissue specimens for various meat samples, as DNA quality is frequently poor due to tissue fixation and processing.

Due to the characteristic of the DNA itself and in consideration of the difficulties faced by the scientist in choosing the correct commercial DNA kit available widely in the market, Epicentre MasterPure™ Complete DNA and RNA Purification Kit extraction kit is chosen due to its high DNA purity and eliminates the inhibitor present in food samples. This kit includes a rapid desalting process to remove contaminating macromolecules and avoid toxic organic solvents (Miller *et al.*,

1988; Shimizu & Burns, 1995). In the present study, we report a protocol to extract deer DNA using Epicentre MasterPure™ Complete DNA and RNA Purification Kit and identify and evaluate its quality using PCR and sequencing analysis. The conventional extraction protocol was not chosen since the reagent itself, such as phenol or chloroform, could degrade the quality of DNA.

The molecular techniques offer advanced techniques to assist in the prevention of low-quality deer meat fraud. The low meat quality in the business has created serious medico-legal and toning Malaysia export activities. The molecular technique is a more reliable method to authenticate the deer DNA in fraud cases, which shows promising results compared to the traditional method. In new molecular techniques, a specific primer is used for the specific deer species (Hisho *et al.*, 1998; Zein *et al.*, 1998).

As know that DNA extraction and purification is a process of separating DNA from various other cell components where; this is an essential factor used as a basis for DNA or molecular-based research. The information about DNA concentration and purity is needed to analyse the degree of contamination and to find out whether the sample is in good condition or not to be used at a later stage (Yalcinkaya *et al.*, 2017).

The success of this approach is limited by the presence of inhibitors in the meat matrix. The recovery of high-quality DNA is vital in the PCR process to produce good sequencing results without any inhibitor. Without DNA or poor quality of DNA, the oligonucleotide primers are not able to amplify the targeted gene in the PCR assay, and the sequencing assay is not able to perform (Botteto *et al.*, 2002).

## 2. Materials and methods

### 2.1 Sampling

A total of four (n=4) individual genomic samples of *R. timorensis* were taken from a breeder in Perak (2 individuals of deer meat sample) and Pahang (2 individuals of deer meat sample) in February 2020. The meat sample was preferred to the blood sample since the blood sample contained much serum that would contribute to high inhibitor.

Approximately 250 g of meat samples were taken after the slaughtering was performed, and the meat samples were kept in sterile and tight containers to ensure there were no elements of cross-contamination introduced to the meat sample. Then the container was labelled accordingly based on the location of the meat sample taken.

During the slaughtering procedure, the breeder has been taught how to apply sterile steps and precautions to avoid cross-contamination to the meat sample since molecular techniques quickly detect small amounts of contamination.

### 2.2 DNA extraction

A total of 10 g of meat from 250 g of *R. timorensis* was sampled from each individual and frozen immediately before DNA extraction with MasterPure™ Complete DNA and RNA Purification Kit from Biosearch Technologies, United Kingdom. The extraction protocol was followed according to the manufacturer protocol with slight modification by increasing the volume of Proteinase K and RNase from 5 µL to 20 µL for each reagent. After the extraction procedure was

completed, the obtained DNA was kept in a 4°C refrigerator before performing an optical density reading using a spectrophotometer (Eppendorf, GmbH) to measure the purity and concentration of the obtained DNA from the extraction procedure.

### 2.3 Polymerase chain reaction (PCR) assay

The DNA extracted product was then used as a template to perform a Polymerase Chain Reaction (PCR). The final DNA concentration of the DNA extracted product used in the PCR product was 50 ng/µL and was conducted in the 25 µL PCR reaction mixture of: 12.5 µL of PCR master mix (1.5 unit Taq polymerase (Promega), 10 mM Tris-HCL, 50 mM KCL, 1.5 µM dNTPs, and 1.5 mM MgCl<sub>2</sub> Promega 10 X PCR reaction buffer), 2 µL of DNA, 8 µL of water, and 1 µL of 10 pM each of the forward and reverse primers (Helix BioTech). The vertebrate-specific primer pair (F – 5" AAACCAGAAGGAGACAAC 3") and (R-5" TCATCTAGGCATTTTCAGTGCC 3") were used to successfully amplify DNA for *R. timorensis* (Zein, 2007). The PCR amplification was carried out in a Biotherm (Analytik Jena, GmbH).

The thermal cycler programme was conducted with an initial pre-denaturation step at 95°C for 5 minutes, followed by 40 cycles that consisted of a denaturing step at 95°C for 30 seconds, an annealing step at 60°C for 30 seconds, and an extension step at 72°C for 40 seconds. This step was followed by a final extension step for 10 minutes at 72°C and stored at a constant 4°C in the refrigerator. The PCR products were analysed by horizontal electrophoresis on a 1.5% (w/v) agarose gel in 1 X Tris Borate EDTA (TBE) Buffer at 120 volts for 60 minutes, and the amplified fragments were visualised with a UV transilluminator (Alpha Imager TM2200).

### 2.4 mtDNA cytochrome b (CYTB) gene sequencing assay

The amplicon of the Polymerase Chain Reaction product was cleaned using GeneAll Purification Kit (GeneAll Biotechnology Co, LTD, Kr). Using a Big Dye Terminator Cycle Sequencing Kit purchased from Applied Biosystem, USA, the fragment was sequenced automatically in both forward (F) and Reverse (R) primer. The cycle sequencing reaction was performed in a Thermal Cycler (Takara Bio USA, Inc) with the following parameters: 35 cycles of 10 seconds at 96°C, 5 seconds at 50°C, and 4 minutes at 60°C. The PCR cycle sequencing product was then purified using a DyeX Purification kit (QIAGEN, GmbH), and the fragment was then sequenced using an automatic sequencer ABI 3500 Genetic Analyzer (Applied Biosystem, USA).

### 2.5 Sequencing DNA analysis by MEGA and National Center for Biotechnology Information software

The DNA sequences were analysed using the software program Molecular Evolution Genetic Analysis (MEGA) version 7.0 (USA) (Kumar *et al.*, 2016) to compute and obtain the consensus sequence of *R. timorensis* meat DNA sequence by multiple alignments of the forward and reverse sequences. Then, the obtained DNA sequences of the *R. timorensis* were compared with databases using the BLAST programme from National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/>) to ascertain the identity of the *R. timorensis* based on the consensus of DNA sequences.

The genetic distance of this species was then computed using a p-distance calculation to obtain the genetic variation. To construct the intra-specific relationship between these two *R. timorensis* in different locations, the same formula of p-distance was applied in the MEGA software.

### 3. Results

The comparison of the efficiency of the DNA extraction treated with 5 µL and 20 µL, carried out by measuring the DNA concentration and suitability for PCR, gave the results reported in Table 1 and Figure 1 and 2.

Furthermore, the study showed that the primer used provides a more explicit opportunity to observe the population's structure. This is because DNA analysis is an accurate indicator of the population's dynamics and structure (Hishe *et al.*, 1998). The results of genetic variation in samples of *R. timorensis* collected from Perak and Pahang were obtained in this study.

#### 3.1 Assessment of DNA integrity and quantity by DNA gel electrophoresis

The integrity and relative quantity of extracted DNAs were examined by DNA gel electrophoresis. Based on the DNA gel electrophoresis image, DNA products treated with 20 µL of Proteinase K and RNase (Figure 1) gave higher DNA intensities than 5 µL of Proteinase K and RNase (Figure 2).

The deer samples were also extracted using similar methods but treated with 5 µL of Proteinase K and RNase, showing the low intensity of DNA and illustrated in Figure 2. Due to the low intensity and yield of DNA product treated with 5 µL, sequencing assay performed toward the 5 µL of DNA product showed below 94% of maximum identification of deer sequence DNA (Figure 3) while DNA product treated with 20 µL Proteinase K and RNase showed 99% of maximum identification (Figure 4). Figure 3 and 4, sequence DNA from Bilut, Pahang were used for nucleotide alignment pairwise for maximum identification purposes.

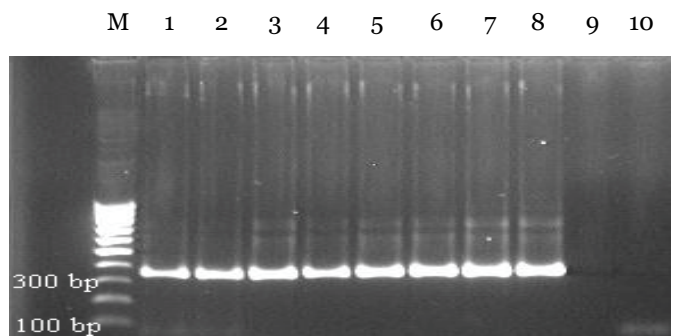


Figure 1: Amplicon of *Rusa timorensis* DNA isolated from deer meat treated with 20 µL of Proteinase K and RNase. Lane M:100 bp ladder; Lane 1; Deer (1st Individu: Tanjung Malim, Perak); Lane 2: Deer (2nd Individu: Tanjung Malim, Perak); Lane 3: Deer (1st Individu: Bilut, Pahang), Lane 4: Deer (2nd Individu: Bilut, Pahang); Lane 5: Extraction Control (Buffalo); Lane 6-7: PCR Control (Chicken); Lane 8: Deer CRM Control; Lane 9: Blank; Lane 10: Water

By performing improvisation on Proteinase K and RNase, the DNA showed a sharp band on 1.5% (w/v) agarose gel, which

explained that the quality of extracted DNA was good by increasing the volume of K and RNase from 5 µL to 20 µL (Figure 1). Then all deer samples were positive with deer DNA, indicated a single band with a molecular weight of 358 base pairs, proceeded to sequencing assay, and were successfully amplified and identified as *R. timorensis* at 95% of maximum identification. The finding confirmed that the DNA extracted from deer meat was a deer DNA using oligonucleotide primers as described by Zein, 2007 based on the BLAST result obtained.

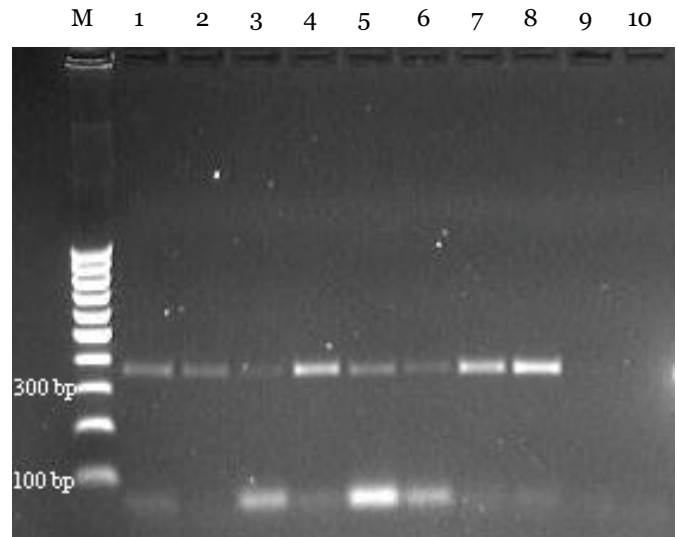


Figure 2: Amplicon of *Rusa timorensis* DNA isolated from deer meat treated with 5 µL of Proteinase K and RNase. Lane M:100 bp ladder; Lane 1; Deer (1st Individu: Tanjung Malim, Perak); Lane 2: Deer (2nd Individu: Tanjung Malim, Perak); Lane 3: Deer (1st Individu: Bilut, Pahang), Lane 4: Deer (2nd Individu: Bilut, Pahang); Lane 5: Extraction Control (Buffalo); Lane 6-7: PCR Control (Chicken); Lane 8: Deer CRM Control; Lane 9: Blank; Lane 10: Water

**Rusa timorensis mitochondrion, partial genome**  
 Sequence ID: [KY117575.1](#) Length: 16422 Number of Matches: 1

Score	Expect	Identities	Gaps	Strand
486 bits(263)	4e-133	300/318(94%)	2/318(0%)	Plus/Plus
Query 9	ACTAGG-ATCTG-CTTATCCTACAATCCTAACAGGCCATTTCCTAGCAATACATTATAC	66		
Sbjct 14255	ACTAGGAATTGTCTAATCCTACAATCCTAACAGGCCATTTCCTAGCAATACATTATAC	14314		
Query 67	ATCCGACACAATAACAGCATTTCCTCCGTCACACATATTTGTTGACATGTCAATTATGG	126		
Sbjct 14315	ATCCGATACAATAACAGCATTTCCTCCTGTCACACATATCTGTCGAGATGTTAATTATGG	14374		
Query 127	CTGAATCATTTCGATATACACAGCAGAGCGGGCATCAATATTCCTCATCFGCTAATTAT	186		
Sbjct 14375	CTGAATCATTTCGATATATACACGAAACGGGGCATCAATATTCCTCATCTGCTAATTAT	14434		
Query 187	ACATGTAGGACGAGGACTGTACTACGGATCTTATACTTTTTACAAAACATGAACATCGG	246		
Sbjct 14435	ACATGTAGGACGAGGCTGTACTACGGATCTTATACTTTTTAGAAAACATGAACATCGG	14494		
Query 247	AGTAGTCTCCTGTTTACCGTTATAGCCACCGCTTCGTAGGATAAGTCTACCATGAGG	306		
Sbjct 14495	AGTAGTCTCCTGTTTACAGTTATAGCCACAGCGTTTCGTAGGATAAGTCTACCATGAGG	14554		
Query 307	ACAAATATCATTCTGAGG	324		
Sbjct 14555	ACAAATATCATTCTGAGG	14572		

Figure 3: Pairwise DNA Sequence alignment of Bilut deer treated with 5 µL to conform to the *R. timorensis* with 94% of maximum identification

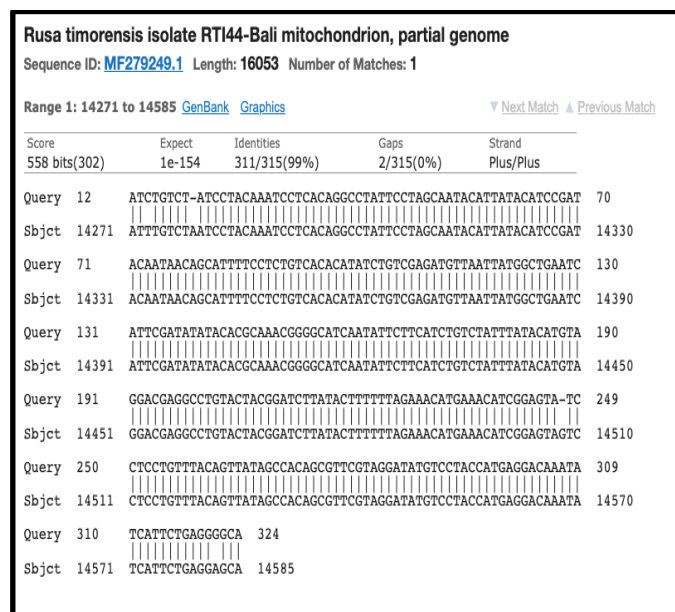


Figure 4: Pairwise DNA sequence DNA alignment of Bilut deer treated with 20 µL to conform the *R. timorensis* with 99% of maximum identification

### 3.2 Determination of DNA quantity by absorbance and fluorescence spectrophotometer

The result showed that the DNA concentration of meat sample treated with 20 µL Proteinase K and RNase was highest at 2706 ng/µL and lowest at 1791 ng/µL. Modification in increasing the volume of Proteinase K and RNase before isolation and to the stage of DNA isolation can affect the DNA results. The addition of 20 µL of Proteinase K and RNase was quite influential in increasing the DNA concentration, and this was possible because of the effect of the enzymes at optimal work to remove the possible introduced inhibitor from the sample or from the reagent itself.

However, the meat sample treated with 5 ul of Proteinase K and RNase showed the highest DNA concentration at 665 ng/µL and the lowest at 186 ng/µL. The absorbance of the isolate is affected by the structural property of nucleic acid called the hypochromic effect. Low DNA concentrations can be influenced by several factors, such as dissociation and DNA precipitation from cells that are less than optimal, so the DNA extraction results are negligible.

The concentration of the DNA samples showed that optimisation of Proteinase K and RNase toward the meat sample is needed before amplification is carried out to ensure clean DNA is obtained and no interruption occurred during the sequencing assay. This will result in the highest maximum identification of deer meat samples to authenticate the species accurately.

According to spectrophotometer measurements, the highest DNA concentration contributed by meat sample treated with 20 uL of Proteinase K and RNase compared to 5 µL of Proteinase K, and RNase showed a low yield of DNA concentration. The DNA yield of treated Proteinase K and RNase is shown in Table 1. The data indicated that the meat sample treated with 20 µL of Proteinase K and RNase gave a

good yield of DNA results, as reflected in Figure 1. Compared to the meat sample treated with 5 µL of Proteinase K and RNase, it shows a low yield of DNA concentration in Figure 2 which the intensity brightness of the band appeared very faint and not sharp, as in Figure 1.

Table 1: Results of Measurement of DNA Concentrations using Spectrophotometer for meat sample treated with 5 µL and 20 µL Proteinase K and RNase for two (2) individuals of *Rusa timorensis* from Tanjung Malim, Perak and Bilut, Pahang

No.	Sample Name	Proteinase K & RNase	Yield (ng/µ L)	Proteinase K & RNase	Yield (ng/µ L)
1	<i>R.Timor-1-Bilut-Pahang</i>	5 µL	654	20 µL	1791
2	<i>R.Timor-2-Bilut-Pahang</i>	5 µL	186	20 µL	2817
3	<i>R.Timor-1-Tanjung-Malim</i>	5 µL	351	20 µL	2193
4	<i>R.Timor-2-Tanjung-Malim</i>	5 µL	665	20 µL	2706

### 3.3 Intraspecific relationship within *Rusa timorensis* Perak and Pahang

Based on the *CYTB* gene partial sequence of mitochondrial DNA, the genetic distance among deer from Bilut, Pahang, and Tanjung Malim, Perak was determined, ranging from 0.252 to 0.788 (Table 2). Due to the partial region targetting gene, the low variation in the genetic distance occurred between the deer sample taken from a different locations.

The *R. timorensis* samples collected from a Pahang display a fairly high degree of variation (0.252) in terms of an intraspecific relationship. However, *R. timorensis* from Perak surprisingly showed less variation (0.696) compared to this single *R. timorensis* from Pahang. Thus, individuals of *R. timorensis* from Pahang are more genetically varied than those of *R. timorensis* from Perak.

The study showed a low intra-specific relationship between these species even though they were from a different locality. In addition, small populations tend to occur in inbreeding as well. These can adversely affect the survival of existing deer populations. The indication is that the decrease in genetic variety in the population can reduce individual capacity in the face of natural selection pressure, especially due to environmental changes (Hedrick, 2000; Hartl, 2000; Lande & Barrowclough, 1987).



Table 2: Genetic distance table computed using the p-distance formula for four (4) individuals of deer from Tanjung Malim, Perak, and Bilut, Pahang

	R.Timor-1-Bilut-Pahang	R.Timor-2-Bilut-Pahang	R.Timor-1-Tanjung-Malim	R.Timor-2-Tanjung-Malim
R.Timor-1-Bilut-Pahang				
R.Timor-2-Bilut-Pahang	0.252			
R.Timor-1-Tanjung-Malim	0.776	0.788		
R.Timor-2-Tanjung-Malim	0.752	0.712	0.696	

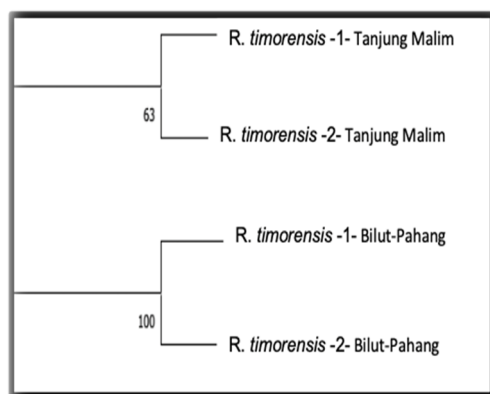


Figure 5: Phylogenetic tree of relationship among four (4) individuals of *R. timorensis* collected from Tanjung Malim, Perak and Bilut, Pahang

The results of this study show that the genetic variation between this species is low even if it is obtained from a different locality. This means the differences in genetic variation among this species show a low intra-specific relationship, which might be due to the low genetic distance comparison (Table 2). The relationship between local abundance and geographical distribution is one of the best documented statistical patterns observed in nature to predict the state of deer populations in breeding and conservation areas (Gaston *et al.*, 1997).

Due to the value of 100%, the intraspecific relationship is shown by Pahang deer and can be used as an effective tool for accurately examining population processes like dispersal patterns and population structuring in *R. timorensis* species in order to solve the population bottleneck (Webley *et al.*, 2004) in contrast with *R. timorensis* (Perak) which showed low genetic variability at 63% of intraspecific-relation.

#### 4. Discussion

DNA extraction, purification, and concentration constitute the first critical stage in molecular analytical techniques. They need procedures capable of eliminating the various inhibitor compounds of the amplification reaction. As a result, by

increasing the volume of Proteinase K and RNase from 5  $\mu$ L to 20  $\mu$ L, the extracted deer meat was successfully modified, yielding a high yield of DNA purity (Wilson, 1997; Zimmermann *et al.*, 1998).

Moreover, four indispensable steps are generally required for successful nucleic acid purification require the cell lysis through disruption of the cellular membranes, cyst wall or egg wall, dehydration and precipitation of the cellular proteins (protein denaturation), and separation of cellular proteins and other cellular components out of the nucleic acid (Tataurov & Owaczary, 2008).

Additionally, during the process of extraction (lysis step), DNA molecules are under triple protection by EDTA or NaCl, which need to be removed, and the role of Proteinase K and RNase is essential here as a remover for any possible substance contributed by EDTA or NaCl. These introduced substances are potential inhibitors that can interfere with the extraction reagent and affect the yield of DNA.

Therefore, the optimisation volume of Proteinase K and RNase in the extraction procedure is essential to ensure the extraction method can remove introduced inhibitors such as food residue, additives, and preservatives, which might interfere with downstream applications. Thus, modifying Proteinase K and RNase from 5  $\mu$ L to 20  $\mu$ L can give optimal quality DNA yield.

DNA is essential for any polymerase chain reaction (PCR) analysis, and without DNA, the PCR process is incomplete and sequencing as well (Di Pinto *et al.*, 2007). DNA quality is essential prior to PCR analysis, and raw meat will provide a high yield of DNA concentration, and the volume of Proteinase K and RNase is indispensable during extraction to avoid smearing occurring in the DNA extracted product. The increasing volume of Proteinase K and RNase reagent that was applied in the extraction of DNA was necessary to eliminate possible unnecessary contaminants from the deer meat, resulting in a good yield of DNA.

The method developed with the increasing volume of Proteinase K and RNase is desirable to prevent adulteration of meat and meat products, especially in the deer industry is essential not only for economical, religious, and health reasons but also crucial for fair trade practices. The result shown in this study indicates that the increase of Proteinase K and RNase to 20  $\mu$ L contributes to the high recovery of DNA yield (Figure 1).

DNA-based approaches to polymerase chain reaction (PCR) techniques are known for a specific, reproducible, sensitive, rapid processing time, and low costs. However, the success of this approach is limited by the presence of inhibitors in the food matrix. The recovery of good-quality DNA is vital in the PCR process. Without DNA or poor quality, the oligonucleotide primers cannot amplify the targeted gene in the PCR assay. In meat specification, either nuclear or mitochondrial DNA (nDNA and mtDNA) genes have been targeted in PCR analysis.

The mtDNA gene is a common target region used for species identification in meat speciation analysis (Meyer *et al.*, 1996; Matsunaga *et al.*, 1999; Che Man *et al.*, 2007; Sahilah *et al.*, 2011), especially *cytochrome b* (*CYTb*) gene. A high copy number of mtDNA is found in the cells. It remains intact during food processing, minimising DNA degradation and not including any introns (Unselde *et al.*, 1995).

Biomolecule extraction, such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from a variety of starting biological materials to be used in downstream applications and other analytical or preparative purposes, is the most critical first step in the molecular biology (William *et al.*, 2016). Widely employed nucleic acid isolation methods can be divided into organic extraction method (phenol/chloroform), inorganic extraction method (salting out), and solid-phase extraction method (solid matrix); moreover, four indispensable steps are generally required for successful nucleic acid purification. The combination between Proteinase K and RNase and the increasing volume of each reagent produces a high yield and purity of the DNA extracted product.

Further, proteinase K works incredibly well along with several other enzymes such as RNase, and the present study performed to determine the quality and the quantity of DNA extracted from deer meat samples worked well with 20  $\mu$ L of Proteinase K and RNase instead of 5  $\mu$ L.

In nucleic acids extraction, yield and quality are essential fundamental features and ultimate goals for any researcher, and here, a simplified, semi-unified, effective, and toxic material free protocol for extracting DNA from different prokaryotic and eukaryotic sources exploiting the physical and chemical properties of nucleic acids (Saeed *et al.*, 2016; Wallach *et al.*, 1984; Wallach *et al.*, 1989; Penney *et al.*, 1998).

The molecular techniques offer advanced techniques to assist in the prevention of low-quality deer meat fraud. The low meat quality in the business has created serious legal medico-legal and toning Malaysia export activities. The molecular technique is a more reliable method to authenticate the deer DNA in fraud cases which shows promising results compared to the traditional method; in a new molecular technique, a specific primer is used for the specific deer species (Ogunkanmi *et al.*, 2008).

This study will require a sequence of nucleotide sequence fragments in the mitochondrial DNA control area to provide a clear picture of the diversification of deer populations in the Malaysian region.

DNA-based sequencing assay approaches are explicit, reproducible, sensitive, have rapid processing time, and have low costs. However, the success of this approach is limited by the presence of inhibitors in the meat matrix. The recovery of good quality DNA is vital in the PCR process to produce reliable sequencing results without any inhibitor (Sharma & Singh, 2005). Without DNA or poor quality DNA, the oligonucleotide primers cannot amplify the targeted gene in the PCR assay and the sequencing assay is unable to perform. In recent years, the development of molecular techniques has created a need to establish simple and efficient novel DNA and RNA extraction methods for PCR amplification and other related techniques (Di Pinto *et al.*, 2007).

## 5. Conclusion

Considering the results obtained, it is possible to conclude that the increase of Proteinase K and RNase in the DNA isolation successfully resulted in high DNA concentration, indicating the removal of inhibitors and the clean sequence of deer DNA. Thus useful for animal DNA species identification. Furthermore, a MasterPure™ Complete DNA and RNA Purification Kit were described as a simplified, semi-unified, effective, and toxic

material free protocol for extracting DNA and RNA utilising the physicochemical properties of nucleic acids has been described, and optimisation of DNA samples is needed before amplification is carried out.

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