

[HFT#114]

Extraction Methods Comparison in Determine Deer DNA

Nooratiny Ishak

Halal Analysis Division, Industry and Custom Tariff Analysis Centre, Department of Chemistry, Sultan Road, 46661, Petaling Jaya, Selangor

* Correspondence: nooratiny@kimia.gov.my

ABSTRACT

The rapid development of food technology is a major challenge to DNA technology where DNA technology must be in line with current developments in the meat-based food technology industry to detect any fraud that occurs. The study to detect fraud in food products starts with the extraction process, where the DNA extraction process is the most critical process because it has a great impact on the extraction results and subsequent test methods. Therefore, this study aims to describe and compare the most commonly used extraction methods for Deer DNA detection. In this study, two (2) different extraction methods, the Epicenter MasterPure™ Complete DNA Purification Kit and the DNeasy Blood & Tissue Kit were examined to determine their relative effectiveness for extracting DNA from deer samples. Deer meat samples were taken from Village Dusun and Batas Ubi Rusa Farm located in Kedah, North Malaysia. The results show that the DNeasy Blood & Tissue Kit provides good DNA quality resulting in 95% maximum deer species identification compared to Epicenter's MasterPure™ Complete DNA Purification Kit, where the maximum deer species identification is below 95%. Therefore, this method is proposed as an alternative method for the isolation of DNA from meat and meat by-products.

Keywords: Deer, Polymerase Chains Reaction (PCR), sequencing assay

1 Introduction

The quantity and quality of DNA obtained from the extraction procedure have a major impact on the success of downstream applications such as polymerase chain reaction (PCR), sequencing, and genotyping. Therefore, this study has taken the initiative to compare these two (2) extraction kits, namely the Epicenter MasterPure™ Complete DNA Purification Kit and the DNeasy Blood & Tissue Kit, both of which are DNA extraction methods that have been widely used in molecular biology and genetics research.

There have been many experimental studies conducted comparing different extraction methods, and the findings vary depending on the types of samples and the specific methods being compared, but some general trends have emerged. For example, some studies that have been conducted by [1], have shown that bead-beating methods tend to yield higher DNA than column-based methods, while others have found that column-based methods are more effective at removing contaminants. Ultimately, the best DNA extraction method will depend on the specific research question, the type of sample being analyzed, and other factors such as cost and ease of use. There are many academic research articles on DNA extraction method comparison that have been conducted by researchers on various sample types and applications [2-3]

The choice between the two methods depends on the specific needs of the experiments, such as the type of tissue or organism being studied, how it is used downstream, and the availability of resources. Each method has its own advantages and disadvantages in terms of yield and purity. Therefore, these



methods should be thoroughly evaluated and compared to select the most appropriate one for a particular experiment.

2 Materials and Methods

Sampling involved collecting ten (n=10) individual genomic samples of *R. timorensis* from two (2) locations in 2022 which are in Kampung Dusun (5 individuals of deer meat sample) and Pahang (5 individuals of deer meat sample). Meat samples were chosen over blood due to lower inhibitor levels. About 250 g of meat per individual was collected post-slaughter and stored in the contamination-free container, then labeled based on location. For DNA extraction, 10g of meat was used from each individual, extracted using Epicenter MasterPure™ Complete DNA Purification Kit and the DNeasy Blood & Tissue Kit. Extracted DNA's purity and concentration were assessed with Nanodrop. Polymerase Chain Reaction assay used 50 ng of extracted DNA in a 25uL of reaction mix with vertebrate-specific primers [4], conducted using Biometra Tone thermal cycler. The mtDNA cytochrome b gene sequencing employed cycle sequencing in a thermal cycler, followed by purification using DyeX Purification Kit and the DNA fragment was sequenced using an automatic sequencer SeqStudio Genetic Analyzer.

3 Results

The study found that the DNeasy Blood & Tissue Kit extracts meat samples with the highest DNA concentration (913 ng/μL) and lowest (413 ng/μL), while the Epicenter MasterPure™ Complete DNA Purification Kit extracts the lowest at 24 ng/μL and the highest was at 86 ng/μL (Table 1). High yield and purity are crucial for amplification to ensure clean DNA without interruptions during sequencing. This results in accurate identification of deer meat samples, as shown in Table 2.

Table 1: Measurement of DNA Concentrations for meat sample treated Epicenter MasterPure™ Complete DNA Purification Kit and the DNeasy Blood & Tissue Kit for a total of ten (10) individuals of *Rusa timorensis* from Kampung Dusun, Kulim and Batas Ubi Farm, Yan, Kedah.

No.	Sample Name	Extraction Method	Yield (ng/μL)	Extraction Method	Yield (ng/μL)
1	KD- Ind1	Epicenter MasterPure™ Complete DNA Purification Kit	54	DNeasy Blood & Tissue Kit	791
2	KD- Ind2		86		817
3	KD- Ind3		51		913
4	KD- Ind4		65		706
5	KD- Ind5		45		845
6	BU – Ind1		24		614
7	BU – Ind2		60		764
8	BU – Ind3		35		535
9	BU – Ind4		54		413
10	BU – Ind5		55		755

Table 2: Maximum identification (%) of ten (10) individuals of *Rusa timorensis* from Kampung Dusun, Kulim, and Batas Ubi Farm, Yan, Kedah treated Epicenter MasterPure™ Complete DNA Purification Kit and the DNeasy Blood & Tissue Kit.

Department	Matrice	Sample	Maximum Identification	Cervidae sp
Kampung Dusun	Raw meat	KD- Ind1	94.42%	<i>R. timorensis</i>
		KD- Ind2		
		KD- Ind3		
		KD- Ind4		
		KD- Ind5		
Batas Ubi Farm	Raw meat	BU – Ind1	98.84%	<i>R. timorensis</i>
		BU – Ind2		
		BU – Ind3		
		BU – Ind4		
		BU – Ind5		

4 Discussion

There are several academic research studies that have been conducted to compare the performance of different DNA extraction methods, including Epicenter MasterPure™ Complete DNA Purification Kit to compare the efficacy and cost-effectiveness of five different DNA extraction methods for the isolation of high-quality and high-quantity DNA from small animals [5].

The results of the study showed that the DNeasy Blood & Tissue Kit methods produced high-quality DNA, with significant differences in DNA yield or purity compared to Epicenter MasterPure™ Complete DNA Purification Kit. A similar finding was discovered by [6-7], which compares the performance of the DNeasy Blood & Tissue Kit with two other commercially available kits, such as the QIAamp DNA Micro Kit and the Promega Wizard SV 96 Genomic DNA Purification System.

Another study demonstrated by [8] also found the DNeasy Blood & Tissue Kit was the most efficient method for DNA extraction from environmental samples which this extraction method produces higher quality of DNA yield rather than phenol-based extraction, salting-out extraction, and manual CF11 cellulose extraction method. The DNeasy Blood & Tissue Kit has been shown to be reliable and efficient methods for DNA extraction in various research studies. Thus, these studies indicate that DNeasy Blood & Tissue Kit is efficient for the extraction of high-quality DNA across various sample types.

5 Conclusions

The studies reveal DNA extraction method selection depends on sample type, downstream evaluation, and research desires, emphasizing systematically comparing methods for accurate results.

References

1. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander S. K. & Schlossa, P. D. Development of a Dual-Index Sequencing Strategy and Curation Pipeline for Analyzing Amplicon Sequence Data on the MiSeq Illumina Sequencing Platform. *Applied and Environmental Microbiology* **2013**; 79; 5112–5120.
2. Querci, M., Savini, C., Kagkli, D., Jacchia, S. & Lievens, A. Measuring Digital PCR Quality: Performance Parameters and Their Optimization. *PLoS ONE* **2016**, 11(5).

3. Yuan, J., Li, M. & Senjie, L. An Improved DNA Extraction Method for Efficient and Quantitative Recovery of Phytoplankton Diversity in Natural Assemblages. *PLoS ONE* **2015**; 10(7).
4. Zein, M.S.A. & Maharadatunkamsi, (2003). *Analisis Gen 12SrRNA dari DNA Mitokondria Kelelawar Pemakan Buah Chironax melanocephalus (Chiroptera: Pteropodidae) di Taman Nasional Gunung Halimun*. *Biota VIII* (1): 17-26.
5. Zhang, J., Zheng, W., Huang, K., Liao, S., Li, Y., & Chen, L. Comparison of five DNA extraction methods for molecular analysis in small animals. *Journal of Genetics and Genomics* **2018**, 45(2), 61-69.
6. Hishe, S., Westerman, M. & Schmitt, L.H. Biogeography of the Indonesian archipelago: mitochondrial DNA variation in the fruit bat. *Eonycteris Spelaea*. *J. Linn. Soc.* **1998**, 65: 329-345.
7. Kudirkiene E., Cohn M. T., Stabler R. A., Strong P. C. R., Serniene L., Wren B. W., et al. Phenotypic and genotypic characterizations of *Campylobacter jejuni* isolated from the broiler meat production process. *Curr. Microbiol.* **2012**, 65, 398–406.
8. Boers, S. A., Hays, J. P., Flick, A. J., Zapata, A., & Paquin, B. A comparison of DNA extraction methods for environmental DNA surveillance in an operating setting. *Journal of Forensic Sciences* **2018**, 63(5), 1353-1358.