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Comparison of Two Different DNA Isolation Methods (Quick & Dirty vs. Commercial Kit) in Small Insects

Ferhat Kiremit[1,a,*], Fulya Kaya Apak[2,b], Hayrettin İlker Özdemir[2,c], Gül Ayar[3,d]

[1]*Ziraat Fakültesi, Tarımsal Biyoteknoloji Bölümü, Aydın Adnan Menderes Üniversitesi, Aydın, Türkiye*
[2]*Koçarlı Meslek Yüksekokulu, Kimya ve Kimyasal İşleme Teknolojileri Bölümü, Aydın Adnan Menderes Üniversitesi, Aydın, Türkiye*
[3]*Fen Bilimleri Enstitüsü, Tarımsal Biyoteknoloji Ana Bilim Dalı, Aydın Adnan Menderes Üniversitesi, Aydın, Türkiye*
[*]*Corresponding author*

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ABSTRACT

The COI gene region is the most commonly employed method for species identification in insects, as in all animals. The importance of DNA isolation methods is increasing, and they are of great importance for identifying insects. Unlike other large animals, DNA isolation from insects, especially from agricultural pests with very small structures, is possible with expensive commercial kits. In this study, an expensive commercial kit and a fast and economical method were compared in terms of time, cost, DNA quantity and quality. It was found that the success of commercial kits in terms of DNA quality and quantity is unquestionable. However, it was determined that the modified 'quick and dirty' method can be successfully used to extract DNA from insects and insect parts of all sizes, especially in studies that do not require long-term storage of DNA. Average isolation time for Q&D was 45 minutes, compared to 4 hours or more for commercial kits. Finally, cost is the most important factor in today's global economy. The unit cost of DNA isolation using the Q&D method is approximately 88% lower.

[a] *fkiremit@gmail.com* *https://orcid.org/0000-0003-4729-7676*
[b] *fulya.apak@adu.edu.tr* *https://orcid.org/0000-0001-9226-6583*
[c] *ozdrog@gmail.com* *https://orcid.org/0000-0002-5480-7227*
[d] *gulayar26@gmail.com* *https://orcid.org/0009-0008-8841-4262*

Introduction

The DNA barcoding method is based on using a standard region of mitochondrial DNA, which is 600-700 base pairs long, to swiftly and accurately identify species (Hebert & Gregory, 2005). By utilizing a small section of about 655 base pairs of the mitochondrial cytochrome c oxidase subunit I (COI) gene, accurate identification at 98-100% levels has been achieved successfully in numerous species, including those within complex groups such as birds, fish, and butterflies. In addition to rapid and reliable species identification through barcoding, phylogenetic relationships between different groups can also be more easily resolved (Aravind et al., 2007). Morphological analysis do not provide sufficient opportunity to identify difficult-to-distinguishable species, larvae, nymphs and body parts at the species level. However, the current method used in molecular identification is very expensive and requires 50 mg of tissue, making it difficult to identify insect species. Because insects of some species are quite small, and insect eggs and larvae, for which morphological species determination is difficult, cannot reach this weight. It is clear that many of the methods previously described for DNA extraction from arthropods are flawed. They require the use of multiple steps, toxic or corrosive chemicals, or expensive components (Favret, 2005; Gilbert et al., 2007; Hunter et al., 2008; Pons, 2006; Rowley et al., 2007). However, in this method, which we call quick and dirty DNA extraction, we can easily and accurately obtain results from the desired part of the insect, regardless of its weight. This can be a single leg, a head or any other part of insect. This method is more economical and timesaving compared to the method using the standard kit, and it does not require the insect samples to be of a certain weight. For these reasons, the Q&D method provides the acquisition of DNA in the quality and quantity required for accurate identification of the species in a short time, using less chemicals, cheaply, quickly and accurately.

Materials and Methods

Eight Mediterranean fruit flies [*Ceratitis capitata* Wied. (Diptera: Tephritidae)] of similar size were used in this study. Only the heads of fruit flies were used for DNA isolation. The aim of the study was to compare our rapid isolation method with an expensive commercial kit in terms of time, cost and yield. Four samples were taken for DNA isolation using the commercial kit and four samples were taken for DNA isolation using the method we call "quick and dirty". This method will be referred to as Q&D throughout the following text.

All samples were dried in an incubator at 37°C for 15 minutes to completely remove the alcohol from the tube and sample. In the samples tested with the kit, the isolation procedure was carried out according to the manufacturer's instructions, but in general the 30 mg starting tissue amount of commercial kits was not provided for insects. Total DNA was isolated using the Q&D DNA extraction method, which is not suitable for long-term storage of DNA, but is effective and fast in small insects. To begin the extraction process, add 100 µL of 1X extraction buffer (Extraction Buffer (10X) 200 mM Tris-HCl (pH 8.4), 500 mM (KCl) and 1 µL (10 mM) Proteinase K to the sample in a 1.5 mL microcentrifuge tube. Crush the sample using a sterile glass crusher. This lysate was kept in a 100°C water bath for 15 minutes and then the tubes were centrifuged at 12000g for 3 minutes to precipitate the residues. After centrifugation, the supernatant containing crude DNA was carefully transferred to a new tube. The amount of dsDNA and dsDNA purity in ng/µL were checked by measuring the absorbance of DNA obtained by both methods at 260 and 280 nm in Thermo Nanodrop 2000 (Figure 1). The measurements were repeated on 16th February 2024, 1st March 2024, 15th March 2024, and 29th March 2024 in DNA samples stored at +4°C in the fridge, and the change in the amount of double-stranded DNA was closely monitored (Figure 2).

The Polymerase Chain Reaction (PCR) was performed using 2µL of template DNA and 12.5µL of PCR master mix (Ampliqon Taq DNA polymerase 2x Master Mix RED) with the universal COI primer pair LCO1490 (5'-GGTCAACAAATCATAAAGATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). PCR was performed in a total volume of 25 µL. PCR thermal conditions involve an initial denaturation of 5 minutes at 95°C (1 cycle), followed by denaturation at 95°C for 30 seconds, primer annealing at 55°C for 40 seconds, and extension at 72°C for 40 seconds (35 cycles). The final extension is carried out at 72°C for 5 minutes (1 cycle). PCR products obtained by both methods were run in 1.5% agarose gel at 60V for 1 hour (Figure 3). Sequence analyses were conducted in both directions using the same primers employed in the polymerase chain reaction (BM Labosis, ABI 3730XL DNA sequencer, Applied Biosystems) (Figure 4).

Results

DNA was isolated from four randomly selected samples (FD1, FD2, FD3, and FD4) out of a total of eight samples using the Q&D method. Spectrophotometry measurements of the DNA samples showed a concentration ranging from 13.68 ng/µL to 33.71 ng/µL, a 260/280 ratio between 1.23 and 1.79, and a 260/230 ratio between 1.62 and 2.12 (Figure 1). The remaining four samples (CK1, CK2, CK3, and CK4) were isolated using a commercial DNA isolation kit. The DNA samples were confidently analysed using spectrophotometry, revealing a range of DNA quantities from 38.87 ng/µL to 42.54 ng/µL. The 260/280 ratio ranged from 1.76 to 1.90, and the 260/230 ratio ranged from 1.98 to 2.18 (Figure 1). Samples isolated using the Q&D method showed a 52.26% decrease in DNA concentration from 84.03 ng/µL to 40.12 ng/µL. In contrast, samples isolated using the commercial kit showed only a 10.90% decrease in DNA concentration from 162.72 ng/µL to 145.02 ng/µL. These results highlight the superiority of the commercial kit isolation method in maintaining DNA concentration over time.

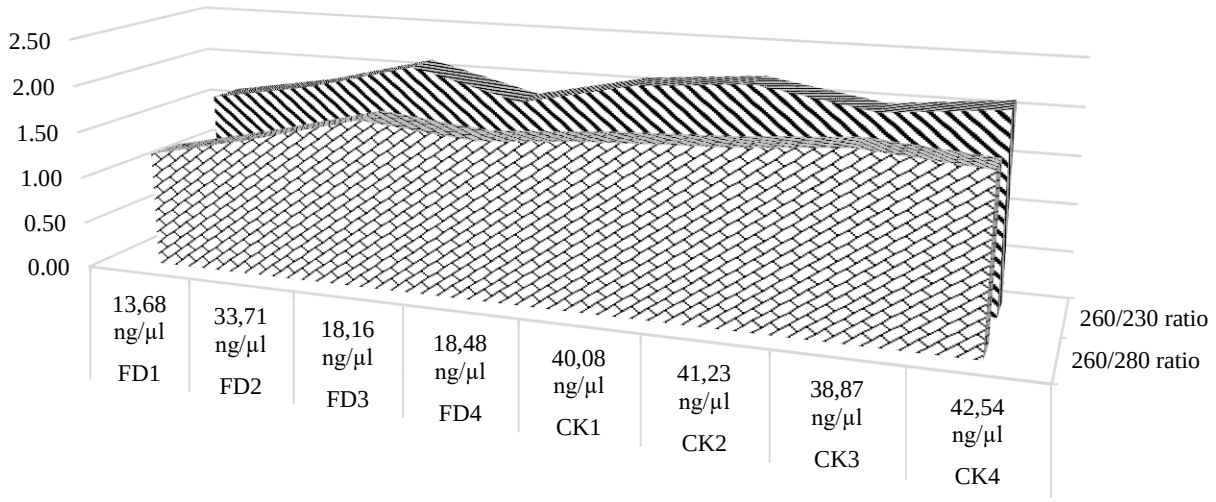

Figure 1. Graph showing the absorbance ratios of the samples at 260/280 and 260/230 nm and the calculated nucleotide amounts. FD1-FD4: Q&D method, CK1-CK4 commercial kit method.

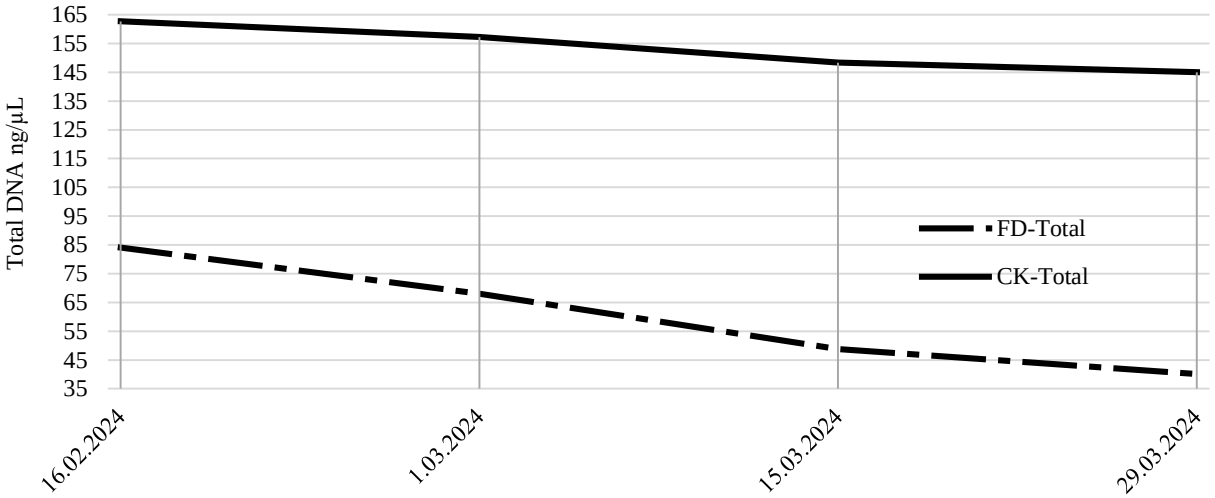

Figure 2. The graph clearly demonstrates the difference in DNA quantities between the Q&D method (FD1+FD2+FD3+FD4) and Commercial Kit Method (CK1+CK2+CK3+CK4) samples during storage at +4°C.

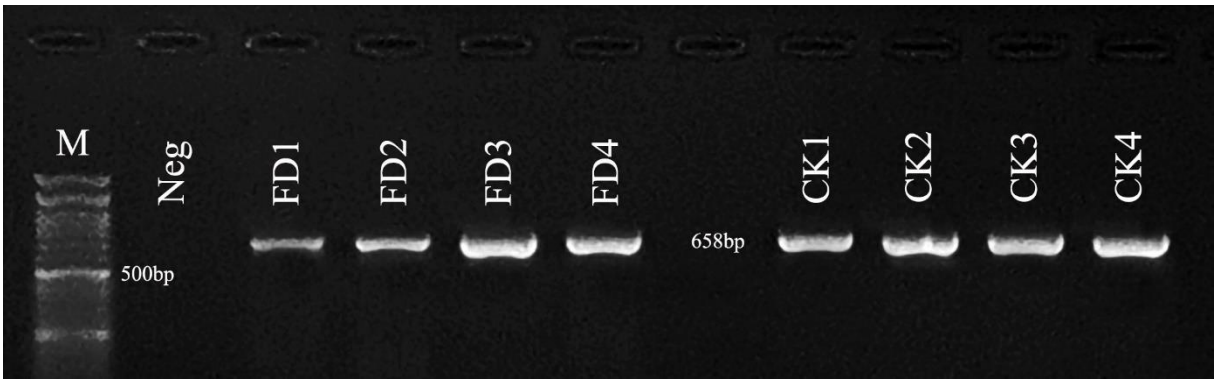

Figure 3. PCR products run at 60V for 1 hour on 1.5% agarose gel, 100bp DNA Ladder (M), negative control (Neg), samples extracted by the Q&D method (FD1-FD4), and samples extracted with a commercial kit (CK1-CK4).

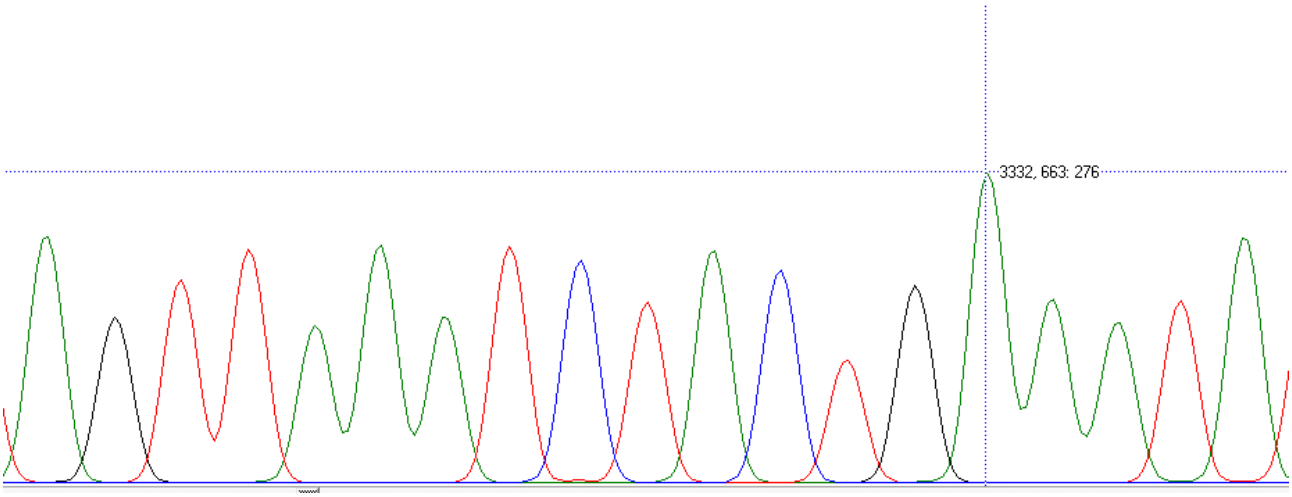

Figure 4. Section from the sequencing chromatogram of the Q&D method weak link FD1 sample.

DNA isolation times were measured for both groups. The Q&D method had an average isolation time of 45 minutes, while the commercial kit protocol required 4 hours or more.

Finally, in terms of cost, which is the most important factor in today's global economy, the unit cost of DNA isolation using the Q&D method is approximately $0.80, while the unit cost of the same process using the commercial kit is $6.40.

Discussion and Conclusion

Spectrophotometers rely on the fact that certain molecules absorb specific wavelengths for absorbance measurements. RNA, ssDNA, and dsDNA are known to absorb wavelengths at 260 nm, with the amount of absorption being directly proportional to the quantity of the molecule present. The purity of RNA and DNA can be confidently determined by calculating the 260/280

absorbance ratio (Nelson & Cox, 2005). A 260/280 ratio of approximately 1.8 indicates pure DNA. A ratio of less than 1.8 indicates the presence of protein, phenolic compounds, and other contaminants in the medium that absorb the 280 nm wavelength (Nelson & Cox, 2005). The DNA samples isolated by the Q&D method, namely FD3 and FD4, have a 260/280 ratio of 1.79, and 1.67, respectively. Therefore, it can be confidently stated that the obtained DNA is pure. The 260/280 ratio of the FD1 and FD2 samples isolated by the Q&D method was 1.23, and 1.49, respectively. In samples CK1, CK2, CK3, and CK4 isolated with the commercial kit, the 260/280 ratio was 1.76, 1.81, 1.90, and 1.80, respectively. This level of purity is expected when using the commercial kit. Nanodrop spectrophotometric measurements may vary slightly compared to other spectrophotometric methods due to the nucleotide composition of the sample, the wavelength sensitivity of the spectrophotometer and the acidity of the sample. As a measurement is made without dilution of the samples tested in the nanodrop spectrophotometer, changes of ±0.2 in the 260/280 ratio may occur due to differences in sample acidity. The commercial kit had a final DNA buffer pH of 8.45, while the Q&D method had a final DNA buffer pH of 7,0. Small changes in the pH of the solution will cause the 260/280 to vary. Acidic solutions will under-represent the 260/280 ratio by 0.2-0.3, while a basic solution will over-represent the ratio by 0.2-0.3 (William et al., 1997). Nancy et al., (2010) directly compared the conventional CTAB and modified CTAB-PVP methods. They demonstrated that both methods yielded similar high-molecular-weight DNA (approximately 50 µg/100 mg fresh tissue). However, the A260/280 ratio for the CTAB method (1.21-1.32) and the CTAB-PVP modified method (1.69-1.76) clearly indicates a higher level of contamination in DNA isolated by the conventional CTAB method. Freitas et al., (2014) definitively stated that the average DNA concentrations obtained from mosquito eggs using Chelex® 100 resin ranged from 6.8 to 192.9 ng/µL.

The amount of DNA obtained by both methods was sufficient for PCR (Q&D range: 13.68 ng/µL to 33.71 ng/µL, commercial kit range: 38.87 ng/µL to 42.54 ng/µL). However, when DNA concentrations were measured at 15-day intervals in DNA samples stored at +4°C, it was observed that samples obtained using the Q&D method lost DNA faster than those obtained using the commercial kit.

The first step in generating DNA barcodes is DNA extraction. This is often the rate-limiting step in very large barcoding studies. Therefore, the best method for high-throughput production of DNA barcodes is a DNA extraction method that is rapid, easy to use, cost-effective, and robust enough to cope with a range of tissue qualities and quantities. A simple and rapid method for isolating high molecular weight DNA from insects was also described by Henry et al., (1990). This method has no need for CsCl ultracentrifugation or extensive dialysis. High-molecular-weight DNA was obtained within 24 h. Since the whole insect was used for DNA isolation, a nuclear-enriched fraction was initially required. Organic phase separation (liquid/liquid extraction) was used to extract genomic DNA from lysed nuclei. The preparation of arthropods for morphological identification often damages or destroys the DNA within the specimen. Conversely, DNA extraction methods irrevocably damage the external physical characteristics essential for morphological identification. Castalanelli et al., (2010) have developed a rapid, simple and non-destructive DNA extraction technique for arthropod specimens. This technique was tested on four arthropod orders using specimens that were fresh, preserved by air drying, stored in ethanol, or collected with sticky or propylene glycol traps. It took 20 minutes for Coleoptera, Diptera and Hemiptera, and 2 minutes for the subclass Acarina. The specimens were not significantly distorted, discoloured or damaged. Ball & Armstrong (2008), tested the performance of a new commercial kit (prepGEM) that utilises a novel, streamlined approach to DNA extraction, and the results were clear. They compared it with two other commercial kits (ChargeSwitch and Aquapure) that differ in their DNA extraction methods. They reported that ChargeSwitch and prepGEM performed equally well and better than Aquapure. prepGEM was much faster, easier to use and cheaper than ChargeSwitch, but ChargeSwitch performed slightly better for older (> 5 years old) dried insect samples. PrepGEM is the clear winner overall, providing a highly streamlined DNA extraction method for fresh, ethanol-preserved and young, dried samples, especially when adapted for high throughput. All techniques for DNA extraction are equally important, but those techniques that require fewer reagents, such as Chelex, charge switching, DNAzol and prepGEM techniques, are more important. Because they require fewer reagents and less waste management, they are cost-effective. These four techniques are also rapid extraction techniques and we obtain DNA within 10-40 min (Chelex, Chargswitching, DNAzol and prepGEM; 37 min, 10 min, 10-30 min, 20 min, respectively).

Our Q&D method is faster, easier to use and cheaper, and it performs slightly better for fresh and dried insect samples. The Q&D method has an average isolation time of 45 minutes while the commercial kit protocol require 4 hours or more; and the unit cost of DNA isolation using the Q&D method is approximately $0.80, while the unit cost of the same process using the commercial kit is $6.40. In a study conducted by Chen et al., (2010) in medium-sized insects, 5 dna isolation methods (SDS method, CTAB method, DNAzol® reagent, Puregene® solutions and DNeasy®) were examined. In the results obtained with a mid-range dna isolation kit, the average isolation time was 1.3 hours and the average cost was determined as 2.72 USD.

Henry et al., (1990) also described a simple and rapid method for isolating high molecular weight DNA from insects. This method has no need for CsCl ultracentrifugation or extensive dialysis. High molecular weight DNA was obtained within 24 h. Since the whole insect was used to isolate DNA, a nuclear-enriched fraction was first required. Organic phase separation (liquid/liquid extraction) was used to extract genomic DNA from lysed nuclei.

The Chelex® 100 resin approach technique reliably extracts, amplifies and sequences a substantial amount of mitochondrial DNA from a single egg, which can then be used as a barcode region for species identification. STE is another standard technique used for that purpose and produces a good amount of DNA. However, the 260/280 optical density ratio clearly showed that the DNA was of

poor quality, which meant that it could not be amplified through PCR (Freitas et al., 2014).

This technique requires only three simple reagents and 37 minutes for complete extraction. The Chelex method is the ideal simple and sustainable approach in case of rate-limiting reagent supply. The analysis of DNA through PCR revealed 100% sensitivity and specificity (Musapa et al., 2013).

The Q&D method outperforms the other method in terms of DNA quantity and quality, DNA isolation time and cost; but performs worse of DNA degradation times. It is the method of choice for multi-sample PCR-based screening and DNA sequencing studies. The method has one disadvantage compared to commercial kits: the shelf life of the DNA obtained is limited to a few weeks. This method can be used successfully in all studies where long-term DNA storage is not required.

Declarations

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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