Saudi Journal of Biological Sciences 30 (2023) 103791

HOSTED BY

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Two-step method for rapid isolation of genomic DNA and validation of R81T insecticide resistance mutation in *Myzus persicae*

Muhammad Umair Sial [a,b,*], Tahir Farooq [c,d], Luaay Kahtan Khalaf [e], Saqib Rahman [b], Muhammad Asad [f], Bilal Ahamad Paray [g]

[a] Department of Entomology, University of Agriculture, Faisalabad 38000, Pakistan
[b] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, 100193, PR China
[c] Plant Protection Research Institute, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, PR China
[d] Guangdong Provincial Key Laboratory of High Technology for Plant Protection, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, PR China
[e] Department of Plant Protection, College of Agricultural Engineering Science, University of Baghdad, Baghdad, Iraq
[f] College of Life Science, Fujian Agriculture and Forestry University, Fuzhou 350002, PR China
[g] Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 12 June 2023
Revised 14 August 2023
Accepted 27 August 2023
Available online 29 August 2023

Keywords:
DNA
Phenol:chloroform
Salt out
Yield
Purity
Myzus persicae
R81T

ABSTRACT

Isolation and amplification of nucleic acid (DNA) is considered a vital and potent instrument in molecular biological research. However, its functioning outside of a laboratory setting is difficult because of complex procedures that demand expert personnel and expensive equipment in addition to the fulfillment of several additional requirements. DNA isolation from minute insects is sometimes difficult, making diagnostic and genotyping procedures problematic. Thus, the current work offers a high-throughput, cost-effective, straightforward, and faster approach for isolating DNA from the aphid *Myzus persicae*. Intriguingly, two-step DNA extraction process yielded a high yield of extremely pure genomic DNA and required only 10 s to complete. PCR investigation aiming at amplifying the non-synonymous R81T region on the loop D site of the nAChR gene of *M. persicae* was subsequently utilized to successfully validate the recovered DNA. Moreover, the proposed method was compared in terms of yield and purity with conventionally used DNA isolation methods including, phenol:chloroform, salt out, and commercially available kits. In conclusion, this newly developed method would enable researchers to quickly process many biological samples used to analyze genetic diversity, mutant screening, and large spectrum diagnosis both in laboratory and field conditions.

1. Introduction

The present era is characterized by the continuous development of new technologies to broaden the knowledge of molecular biology. Detection and amplification of specific genomic DNA (gDNA) sequence is a key tool routinely employed with different objectives like clinical diagnostics, mutant screening, and selection of qualitative trait loci (QTL). Since the first discovery of DNA by Friedrich Miescher in the 1860 s, efforts have been made for the isolation of this novel molecule with variable results (Faraj et al., 2019; Dahm, 2005; Holmes, 2001; Meselson and Stahl, 1958). Though DNA-based microarrays have many advantages over conventionally used enzyme or antibody-based assays which have been modified to meet new challenges, the major obstacle to the widespread diagnostic applications outside sophisticated laboratories is the requirement to isolate or purify DNA from hundreds of samples at once, that is quite a tough task and traditionally requires trained subject specialist and involves many liquid steps to handle (Allen et al., 2006; Sambrook and Russell, 2001; Tan and Yiap, 2009).

Extracting DNA from minute individuals is complex and challenging. Successful application of molecular techniques is affected greatly by its reliance on efficient cell lysis and the recovered DNA quality (Ariefdjohan et al., 2010; De Lipthay et al., 2004; McOrist

* Corresponding author at: Department of Entomology, University of Agriculture, Faisalabad 38000, Pakistan.
E-mail addresses: umair.sial@uaf.edu.pk (M.U. Sial), tfarooq@gdppri.com (T. Farooq), luaay.k@coagri.uobaghdad.edu.iq (L.K. Khalaf), 2017y90100004@caas.cn (S. Rahman), axadch@fafu.edu.cn (M. Asad), bparay@ksu.edu.sa (B. Ahamad Paray).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2023.103791
1319-562X/

et al., 2002; Moore et al., 2008). Successful DNA extraction involves efficient cell disruption, protein denaturation, enzyme inactivation, and DNA recovery. Low protein contaminants, lipids, carbohydrates, and RNAs are requisites to quality DNA. The selection of extraction methods relies upon the target DNA molecular weight, required quantity, extraction time, purity, and cost. Nucleic acid quality and integrity directly affect the results of experimental work (Ariefdjohan et al., 2010).

Nucleic acid isolation has become an indispensable technique in insect pest research including taxonomic classification, population genetics, vector diagnostics, evolutionary studies, and insecticide-resistant mutant allele detection (Aguirre et al., 2019; Altameme and Ibraheam, 2019; Al-Saad and Aletby, 2018). A wide range of such studies require enormous samples, hundreds or even thousands at once, and DNA extraction then becomes a seizing step in research in terms of cost, time, and sophisticated equipment access. Conventionally used single tube extraction methods are often cost-effective and accessible but could be laborious, time taking, and used hazardous chemicals (Thomsen et al., 2009). A single tube extraction method (example Chelex resin extraction) is cheaper, time-saving, and even less hazardous but not suited for long-term DNA storage and could inhibit polymerase chain reaction (PCR) in samples used (Walsh et al., 1991; Hoy, 2003). While, commercially available kits differ in their usage, offer high cost, and often require specialists along with expensive laboratory equipment to perform (Ball and Armstrong, 2008). Many publications have been reported recently on the purification or extraction of DNA, these different types of methods have simplified the extraction methods by eliminating the nucleic acid elution step (AlShabar et al., 2021). This is an advantage over many conventional solid-phase extraction techniques that inhibit DNA amplification frequently (Kim et al., 2010; Shoffner et al., 1996). Although, despite excluding this step, all the methods require complex experimental setups, electrical equipment, and multiple pipetting, which again, seize their useful application to field-based assays.

Thus, herein we compared different techniques that could isolate quality DNA from soft-bodied insect pest, Aphid, *M. persicae* (Fig. 1), in only a few seconds (10 s and 2.5 min). Aphids are minute, soft-bodied insects with intricate life cycles include parthenogenetic generations that alternate with sexual generations. They inflict damage through direct feeding and by vectoring plant viruses (Blackman and Eastop, 2000; Morrison and Peairs, 1998). Control has chiefly relied on insecticides application that directly results in chemical resistance involving genetic mechanisms. Therefore, from diagnostics to genotyping, techniques that are relatively cheaper, time-saving, eliminating laboratory equipment special expertise are much needed to isolate hundreds and thousands of samples at once. Recently, Zou et al., 2017, reported nucleic acid purification from a range of biological samples in under 30 s. This is the first report of rapid DNA isolation from arthropods through modification of a previously described method.

2. Material and methods

2.1. Specimen collection

Aphids, *M. persicae* were collected from the open field and greenhouse crops from different counties of China, including Lang fang, Fujian, and Beijing (Sial et al., 2018, 2020). The collected populations were reared on Chinese cabbage leaves (Brassica napus L

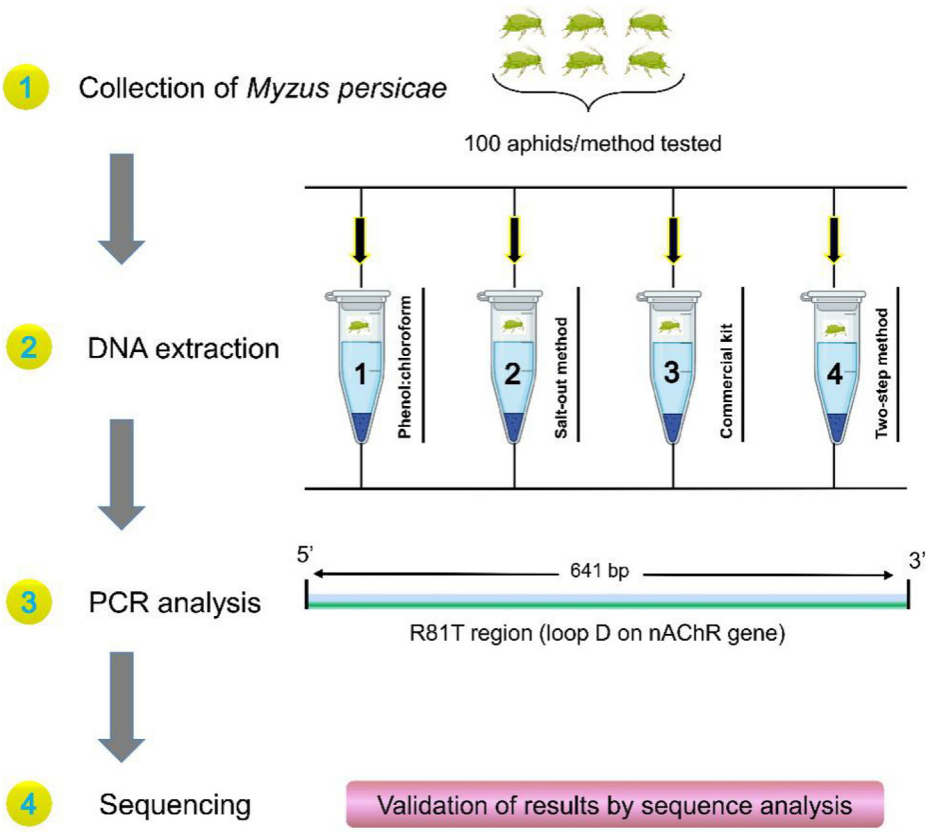

Fig. 1. A schematic representation of the experimental workflow. One hundred aphids (*M. persicae*) were used for DNA extraction by four different protocols (phenol: chloroform, salt-out, commercial kit and two-step method). Subsequently, PCR analysis was performed to amplify a 641 bp long R81T region on loop D of nAChR gene to evaluate the quality of extracted DNA. The amplified PCR products were sequenced, and BLAST searched for validation of results.

var chinensis) and maintained under the climatic chamber at 20–22 °C having light–dark photoperiod of 16:8h and 65 ± 5 RH. Before nucleic acid isolation, individual aphids were frozen by liquid nitrogen in a 1.5 ml Eppendorf tube and then subjected to analysis.

2.2. Phenol chloroform isolation protocol

Isolation of gDNA was done from a single aphid by Phenol: Chloroform protocol (Sambrook et al., 1989). A total of 100 aphids were selected randomly from the collected population maintained in the laboratory. Step by step extraction protocol is described here *viz.,* 1, A single aphid was homogenized in 1.5 ml micro-centrifuge tube containing liquid nitrogen for 30 s. 2, Ice cold 0.5 ml TNE buffer was added to the tube and mixed well, samples were kept on ice till this step. 3, Subsequent steps were performed at room temperature and 25 µl of 20% SDS was added and mixed well. 4, 10 µl of proteinase K (20 mg/ml) was added and mixed well. 5, Homogenate was heated at 50 °C overnight. 6, Equal volume Phenol: Chloroform (250 µl) was added and incubated on a seesaw shaker for 30 m, later centrifuged (CF) at 13 k rpm for 1 m and transferred the aqueous solution into a new tube. 7, Repeated steps 6. 8, 250 µl Chloroform was added and incubated on a seesaw shaker for 30 m, later CF for 1 m, and transferred the transparent solution into a new tube. 9, 1.2 µl RNase A (20 mg/ml) was added and well mixed, later incubated at 37 °C for 60 m. 10, 5 µl proteinase K was added and well mixed, later subjected to 120 m incubation at 50 °C but gently vortexed after every 30 m. 11, Steps 6–8 were repeated then. 12, 10 µl 5 M NaCl were added and well mixed and then 1 ml EtOH was gently added and mixed. 13, Samples were kept at −80 °C for 20 m and later CF at 13 k rpm for 10 m. 14, Supernatant was then discarded carefully and pellets were rinsed with cold 70% EtOH. 15, Solution was removed and pellets were air-dried for 5–10 m and later pellets were dissolved in 30 µl TE buffer.

2.3. Salt out isolation protocol

A total of 100 individual aphids were used in the salting-out isolation method as described by Sunnucks and Hales (1996) with few modifications. Briefly, 1, single aphid was collected in a 1.5 ml eppendorf tube and killed by liquid nitrogen. 2, 10 µl of proteinase K (20 mg/ml) was included followed by grinding of aphid. 3, 300 µl TNES buffer was added on pestle to wash all the grinded aphid into the tube. 4, Mixing and incubation of tube at 55 °C for a minimum of 4 h or overnight was done. 5, 85 µl of 5 M NaCl was added and quickly shaken for 15 s until proteins became precipitated. 6, CF at maximum speed for 15 min was done till proteins became pelleted. 7, The supernatant was carefully removed and transferred into a new tube, followed by the addition of 400 µl cold absolute EtOH, and tubes were slowly inverted a couple of times until DNA was precipitated. 8, CF at maximum speed for a minimum of 5 min was done to pellet DNA. 9, All the EtOH was carefully removed. 10, Pellets were rinsed with 70% 500 µl EtOH and CF for 5 min at 13 k rpm. 11, Pellets were then air-dried by covering tubes with clean tissue paper to avoid any contamination, kept in hot block at 37 °C for 1 h until the pellets were completely dried. 12, Finally, the DNA was resuspended in 30 µl of TE buffer and stored at 4 °C (Sunnucks and Hales, 1996).

2.4. DNA extraction in 2.5 min using a commercial kit

A Chinese commercially available kit named, Rapid DNA Extraction Assay kit (KG203)/ 快速DNA提取检测试剂盒 (KG203) was purchased from Tiangen Biotech (Beijing) Co., Ltd. and used for the extraction of DNA. The kit contained two buffers B1, B2, and the methodology was modified other than the company's description. In short, a total of 100 individual aphids were chosen randomly for DNA isolation purposes. 1, Single aphid was either freshly used or collected in 1.5 ml of eppendorf tube and killed using liquid nitrogen. 2, Then in 20 µl of buffer B1 grinding of aphid was done for 10–12 s. 3, Vortex was done for 5–7 s. 4, By adding 20 µl of B2 buffer, the solution was mixed for 10–12 s. followed by vortex for 5–7 s. 5, Finally, CF for 3 min at 12 k rpm was done and the liquid was either directly used as a template for PCR or stored at 4 °C.

2.5. DNA extraction in 10–15 s

To avoid tedious and time-consuming methods for DNA isolation, a modified method was employed for surprisingly rapid isolation of good quality DNA. To achieve this, 100 individual aphids were collected in a 1.5 ml eppendorf tube and freshly used/killed by liquid nitrogen. Then aphids were ground with a pestle for 5–7 s followed by addition of 40–50 µl EB to wash the pestle for 2–3 s. DNA at this stage was ready to use (total 10 s). Alternatively, 40–50 µl EB was added and aphid was ground with pestle for 5–7 s. Then, vortexed for 2–3 s followed by CF 2–3 s (total 15 s). Afterward, DNA was used for PCR amplification or stored at 4 °C. By this method, hundreds and thousands of samples can be extracted at once, even in countries deprived of basic laboratory facilities, and can be done on-site field.

2.6. Quantitative and qualitative analysis of DNA

The yield and purity of extracted DNA from all protocols were exposed to Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE) to find out the absorbance ratios; A260/280 nm, by doing 1 µl sample. DNA absorbance ratios: A260/280 nm, were measured for protein contamination. DNA can absorb light at 260 nm and A260/280 nm ratios; 1.6–1.8, indicating the samples with good purity and no or low contamination (Vesty et al., 2017). Likewise, the quality of DNA was also verified by operating an aliquot of 2 µl on 1% agarose gel.

2.7. PCR amplification

To confirm the working operation of extracted DNA from all the protocols, the DNA was used to amplify *M. persicae*, nicotinic acetylcholine receptor (nAChR) channel, containing the R81T non-synonymous mutation at loop D region. Gene-specific primers targeting R81T nAChR gene (Accession# LOC111030190) mutation site was designed using Primer Premier 5.0 software and were synthesized by Beijing Genomics Institute (BGI). Sequence for forward and reverse primers (5́-3́) were, F: GTTACAGAATCTTATTTCATGAGGTTT and R: CGTTTTCAAAATGATCTGCTG with an amplicon size of 641 bp. The PCR amplification reaction was conducted in 200 µl tubes. Each reaction consisted of the following components: 10 µl of 5X PrimeStar gxl buffer (Mg2 + plus), 4 µl of dNTP mixture, 0.5 µl eacg forward and reverse primers (10 µM concentration), 1 µl of template, 1 µl of PrimeStar gxl DNA polymerase (Clontech Takara, China), and 33 µl of double-distilled water (ddH_2O) to achieve a total reaction volume of 50 µl. PCR cycling conditions involved a three-step process. The reaction mixture was initially heated to 98 °C for 10 s, followed by 35 cycles of denaturation at 54 °C for 15 s and extension at 68 °C for 60 min. After amplification, the PCR products were analyzed by subjecting them to a 1% agarose gel and visualizing under UV light. Subsequently, the amplicons were purified using the QIAquick® Gel Extraction Kit (Qiagen, ON, Canada) and sent to the Beijing Genomics institute for sequencing.

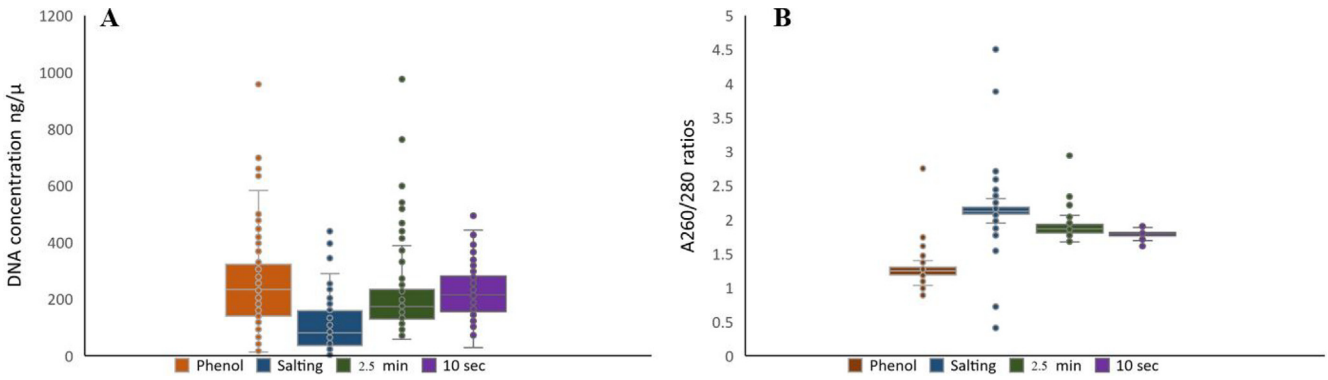

Fig. 2. Box and whisker plot is used to display the distribution of two variables: A, DNA concentration, and B, UV absorbance 260/280 ratios. The data includes measurements from different DNA isolation methods, with a sample size of 100 for each method. The height of the box corresponds to the interquartile range (IQR), which is the range between the 25th and 75th percentiles. The whiskers extend from the box to show the 10th and 90th percentiles, indicating the spread of the data. Any extreme outliers in the data, which are values significantly different from the rest, are represented by dots (•) and indicate the maximum and minimum values observed. The middle line within the box represents the median, which is the midpoint of the data set.

2.8. Statistical analysis

DNA yield (ng/µl) and purity by UV absorption 260/280 by all methods 100 samples/method were subjected to one-way analysis of variance (ANOVA) to check the level of significance. Box-whisker graphs were plotted in Microsoft office 16, to determine the maximum, minimum, median and outlier ranges in data. All the mean significance analysis was analyzed in SPSS Statistics 22.0 (SPSS, Chicago, Illinois, USA).

3. Results

3.1. Quantity and quality of DNA

Quantitative (concentration ng/µL) and qualitative (A260/A280) data of 100 samples of DNA extraction by all four methods are given by a box-whisker plotting analysis. DNA concentrations of all samples varied greatly; the extreme concentration of DNA was distinguished 957.1, 439, 974.8, and 493.7 ng/µL, whilst, least concentration observed as 13.7, 0.7, 57.1 and 29.3 ng/µL by phenol: chloroform, salt out, 2.5 min and 10 s methods, respectively (Fig. 2, A). Extreme high and low outliers dots showed maximum and minimum observations. All the median values were in the range of 81–233 ng/µL and indicated by horizontal lines in the box. DNA isolated by 10 s method were symmetric in concentration and lied in box and slightly near towards up whisker (Fig. 2, A). Moreover, significantly ($p < 0.0001$) the maximum gDNA concentration were achieved by phenol:chloroform (259.85 ± 16.85), 15 s (226.35 ± 8.93) and 2.5 min (224.54 ± 15.63) while the least was through salt out method i.e., 104.69 ± 8.97 (Table 1, 2).

In case of purity or UV absorption of 260/280 ratios of all extracted DNA samples are given in Fig. 2, B, displaying the obvious strong higher 2.75, 2.74, 2.94 and lower outliers 0.89, 0.41, 1.67 values by phenol:chloroform, salt out and 2.5 min methods. All the extreme values were considered void as were supposed to contain higher proteins and phenols concentration. The values of the UV absorbance 260/280 ratios were consistently within a narrow range with maximum value of 1.97 and a minimum value of 1.61. These values were well-contained within the box (Fig. 2, B). The values of medians were in the range of 1.79–2.13. Anyhow, significantly best absorbance A260/A280 ratio was achieved by 10 s protocol i.e., 1.79 ± 0.01 with $p < 0.0001$ which identifies sample purity with least or no impurities (Table 1, 2).

Table 1
Qualitative and quantitative analysis of gDNA through UV absorption at 260 and 280 nm (mean ± SE, n = 100/each method).

Isolation Protocol	DNA (ng/µl) ± SE	Absorbance 260/280 ± SE
Phenol:Chloroform	259.85 ± 16.85a	1.27 ± 0.02
Salt out	104.69 ± 8.97b	2.15 ± 0.04
2.5 min	224.54 ± 15.63a	1.88 ± 0.02
10 s	226.35 ± 8.93a	1.79 ± 0.01*

Means followed by different letters are significantly different, while, * indicated the best ratio statistically among all DNA isolated protocols ($\alpha = 0.05$).

Table 2
One-way analysis of variance (ANOVA) indicating the significant levels among all methods of DNA isolation.

Parameters	*df**	*df***	*df****	*p*	*F*
DNA concentration	3	399	396	<0.0001	26.9
A/260/A280	3	399	396	<0.0001	235

3.2. Gel agarose qualitative determination

All the protocols described for DNA isolation were found to be appropriate for the extraction of gDNA especially from a single aphid, *M. persicae*. Although, the isolated DNA was subjected to UV absorbance for purity assessments but also subjected to 1% agarose gel for visual determination. The method of Phenol:chloroform DNA extraction was so tedious and time-consuming. Moreover, at the final step of DNA extraction, in many of the samples, no pellet formation was observed, indicating that DNA extraction had failed. Likewise, some successfully extracted samples failed during gel electrophoresis and no bands were shown. In Fig. 3A; the extracted DNA from phenol methods is given after gel electrophoresis. DNA isolation through the salt-out method was quite simple and less time-taking but with this method, the pellet formation was observed to be difficult, as pellets were hard to see due to minute in size and many times they were discarded. Nonetheless, gel electrophoresis analysis for quality DNA is shown in Fig. 3B. The protocol in 2.5 min time, of DNA isolation, through Chinese commercial kit was found to be best suitable for extracting a mass quantity of samples in a short period. The quality determination of 2.5 min DNA extraction protocol is shown in Fig. 3C. Notably, the finest and new approach of DNA isolation in under 10 s is reported in the presented study that not only saves the time but also, works efficiently even with hundreds and thousands of samples at once, eliminating the need for specialized laboratory equipment and can be operated on-site field. This method only

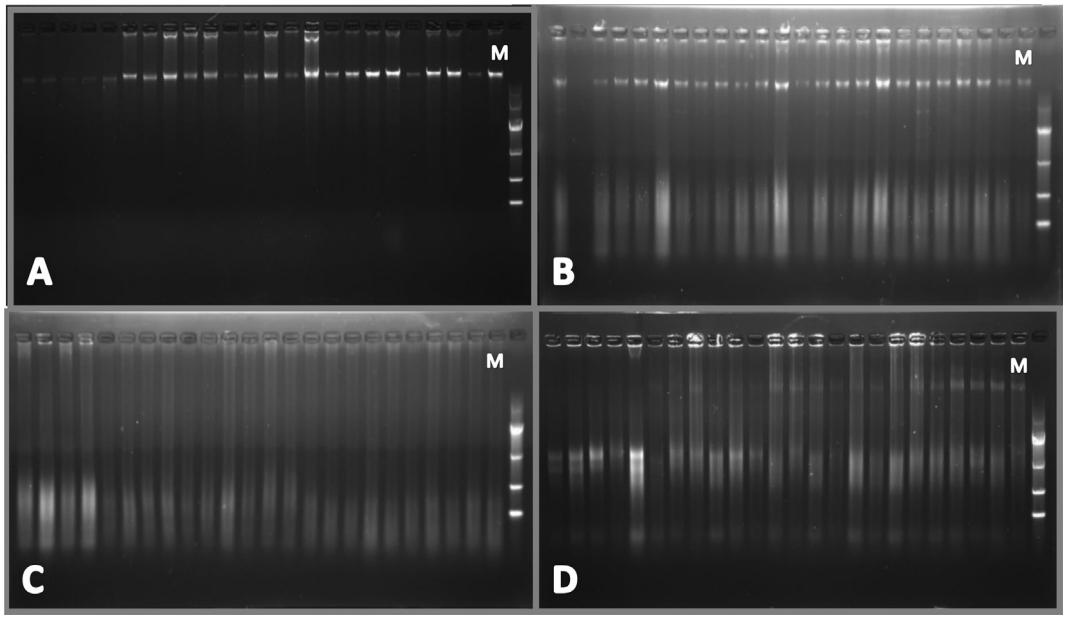

Fig. 3. Gel electrophoresis analysis of isolated DNA from *M. persicae,* using 2 µl template and 0.3 µl of 6x loading buffer; Lane M or 1 = Marker of 10 kb, Lanes 2–24 indicating the DNA bands. A, Phenol:Chloroform protocol; B, Salt out protocol; C, 2.5 min protocol; D, 15 s protocol.

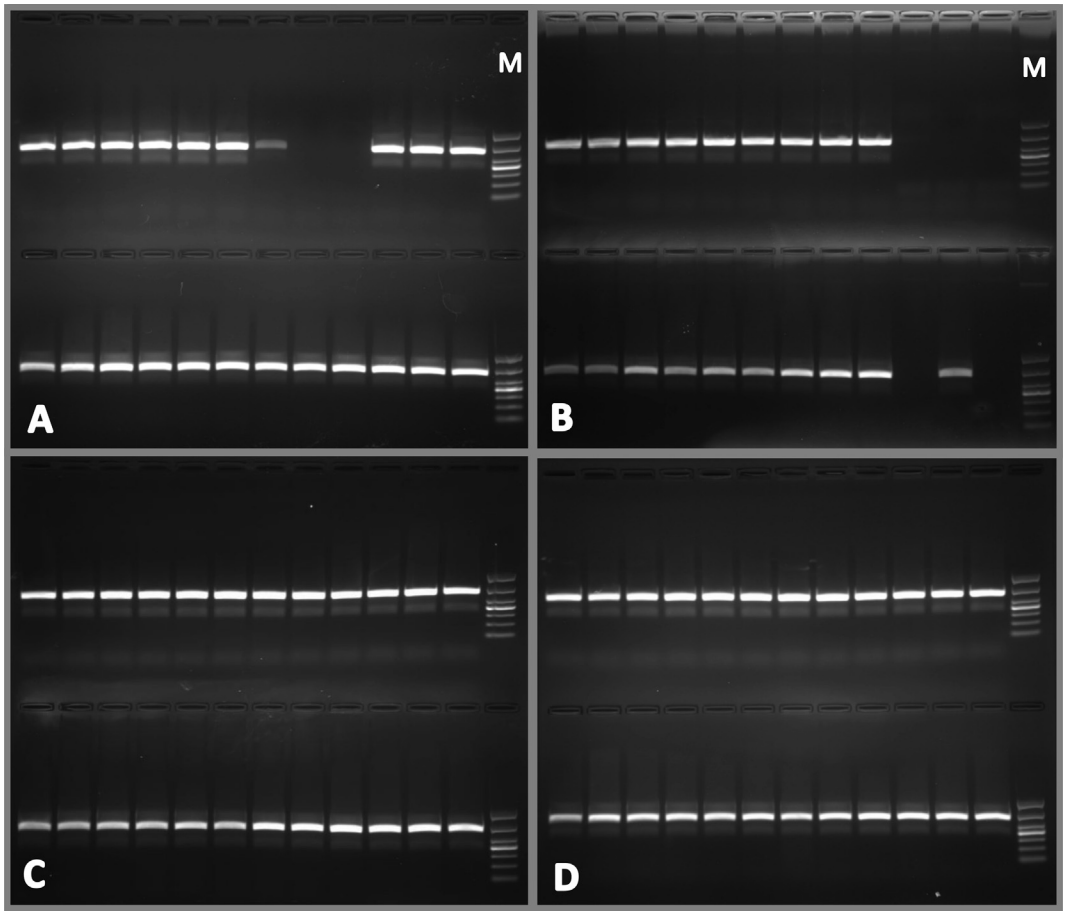

Fig. 4. Gel electrophoresis analysis of PCR amplification using 1 µl DNA template; Lane M or 1 = Marker of 1 kb, Lanes 2–13 indicating the *M. persicae*, nAChR gene encoding R81T mutation amplicon bands (641 bp). A, Phenol:Chloroform protocol; B, Salt out protocol; C, 2.5 min protocol; D, 15 s protocol.

required a 40–50 µl of EB solution (given in methodology section) in a microtube (1.5 ml) with a pestle to squish the aphid and subsequent few seconds vortex and CF, the DNA is then ready to run on gel agarose and further for PCR amplification. The extracted DNA by this method is shown in Fig. 3D.

3.3. PCR amplification conformity

For the validation of isolated DNA from all protocols, DNAs were subjected to PCR amplification. For this purpose, nAChR receptor gene *β1* including the SNP site on loop D region, were chosen to confirm the amplicons by PCR. The amplification was consistent through all protocols even from the samples having yield and absorbance below the optimum level. The DNAs isolated by phenol:chloroform and salt-out methods produced no amplicons in 25–30% samples (Fig. 4A, B). Whilst all samples from 2.5 min and 10 s isolation protocols were amplified successfully (Fig. 4C, D). Here, the advantage was shifted towards the most suitable protocol through which isolation was done in 10 s only. The bands from all PCR amplification were settled and one, signifying that DNA isolation was reliable and enough for nAChR *β1* gene amplification. The amplicon size remained the same in all samples as expected. Moreover, products were later purified and sequenced by Beijing Genomics Institute in both directions and sequence was successful from both 2.5 min and 10 s except that few samples were void, the ones isolated from phenol:chloroform and salt out methods.

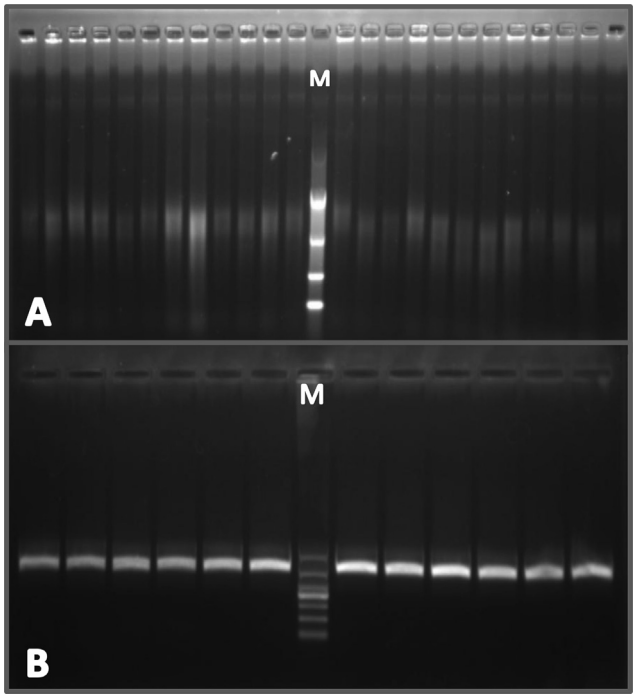

Fig. 5. A, Gel electrophoresis analysis of isolated DNA by 10 s method from *M. persicae*, using 2 µl template and 0.3 µl of 6x loading buffer; Lane M or 7 = Marker of 10 kb, Lanes 1–12 (right-left) indicating the DNA bands without vortex and CF, Lanes 13–24 (right-left) indicating the DNA bands with vortex and CF. B, Gel electrophoresis analysis of PCR amplification using 1 µl DNA template; Lane M or 7 = Marker of 1 kb, Lanes 1–6 (without vortex and CF) and 7–12 (with vortex and CF) (right-left) indicating the *M. persicae*, nAChR gene encoding R81T mutation amplicon bands (641 bp).

3.4. Comparison of new 10 s DNA isolation methods

Two different methods were adopted to check the influence of each on either purity or yield. One method required no vortexes and CF after squishing aphid in EB and performed in 10 s whilst other method required a few seconds of vortexes and CF and last for 15 s. There was no difference observed when isolated DNA was subjected to 1% agarose gel, the pattern was the same in each method (Fig. 5A), likewise, both methods gave nAChR *β1* amplicons (641 bp) encompassing R81T region (Fig. 5B) and later sequenced in both directions successfully. However, when DNA yields in each method were compared, a significant mean maximum concentration of DNA was observed by the samples subjected to vortexes and CF i.e., 192.17 ± 6.25 (Table 3). No difference was observed on UV absorbance 260/280 ratios in each method (Table 3). Thus, vortexes and CF were found to influence the increase in DNA yield only.

4. Discussion

Advancement in new molecular technologies towards the ease of diagnostic applications is exceeding and becoming a keyway in the scientific arena. Molecular assays have revolutionized the science technology in terms of early detection and as well as monitoring of diseases in all living organisms. Though the main obstacle in molecular assays is that, they rely on the successful isolation of nucleic acid that generally requires a long time, technician to operate and quite hectic procedures to adopt in a laboratory environment. More importantly, in the new era that is bounded with advanced technology requires on-site field assays, as well (Mumford et al., 2006; Rahman and Elaissari, 2012; Thatcher, 2015). Nucleic acid assays especially DNA-based, require specificity, sensitivity, and fast procedure compared to other technologies like lateral flow strips, enzyme-linked immunosorbent assay (ELISA) and cell culture/analysis (Ward et al., 2004; Dong et al., 2008; Liesenfeld et al., 2014).

In our study, proposed DNA isolation in 10 s can purify DNA with good yield and purity from a single aphid. Previously, this method was applied with extraction buffer (EB) and washing buffer and with the addition of cellulose-based disc in the PCR amplification tube that again requires specific paper as Whatman No.1 to bind the nucleic acid (Zou et al, 2017). In our study, we attained that squishing of individual aphid in the existence of 40–50 µl EB is enough to get good yield and purity that was determined by UV absorption of A260/A280 (Table 1). Squishing of aphid in EB and subsequent few seconds tapping by vortex and CF, eliminated the need for washing buffer and paper-based disc addition. In the validity test of this protocol, successful amplicons of *M. persicae* nAChR *β1* gene with expected size were obtained (Fig. 3 D) and later sequenced in both directions, successfully.

DNA extraction from insects in previously described methods offer the use of multiple steps including hazardous chemicals and expensive components (Favret, 2005; Pons, 2006; Gilbert

Table 3
Qualitative and quantitative analysis of gDNA by 10 s DNA isolation methods through UV absorption at 260 and 280 nm (mean ± SE, n = 50/each method).

10 s protocol	DNA (ng/µl) ± SE	Absorbance 260/280 ± SE	*df**	*df***	*df****	*p*	*F*
Without vortex, CF	129.47 ± 11.3b	1.79 ± 0.007^{ns}	1	99	98	<0.0001	23.7
With vortex, CF	192.17 ± 6.25a	1.78 ± 0.011^{ns}	1	99	98	0.2931	1.12

(*) degree of freedom treatment, (**) degree of freedom total, (***) degree of freedom error; (ns) non-significant.

et al., 2007; Rowley et al., 2007; Hunter et al., 2008) as in our study the expensive components were required through phenol:chloroform, salt-out and 2.5 min DNA isolation methods. Moreover isolated DNA from phenol:chloroform and salt out methods showed 25–30% void results and gave no amplicons (Fig. 3; A, B). On the other side, 2.5 min and 10 s protocols gave 100% amplicons (Fig. 3; C, D). Isolating high-quality DNA is the key and critical step in molecular research. However, time-efficient, and cost-effective DNA isolation methods often face many challenges, including, secondary chemical reaction reduction (that may lead to loss of DNA concentration), overnight samples, hazardous reagents, handling cost and use of proteinase K and RNase during extraction to remove RNA and protein contamination (Kotchoni and Gachomo, 2009). On the other side, none of the sophisticated equipment was required to isolate DNA from the new 10 s method, even, interestingly, a prodigious number of samples could be done at once with good yield and purity. The proposed DNA isolation method employs non-hazardous reagents and is inexpensive and, is perfect for routine use even on-site field-based assays.

The complete cost to perform nucleic acid (DNA) isolation by the proposed protocol is the key determinant for the adoption on a large-scale area and that is very affordable. Nonetheless, the overall cost was not determined but we observe that this method significantly reduces the cost compared to other used methods. This study was focused on proposing a simple, fast, free of expensive equipment and less hazardous reagents, DNA isolation method and subsequent amplification via PCR as well as genotyping assays. For amplification, a power thermocycler is used for most reactions that is not suitable and ideal for the laboratories lacking such thermocycler and even not possible to conduct research outside the laboratory or on-site field. Thus, our study in near future must also be coupled with isothermal based assays especially loop-mediated isothermal amplification (LAMP) (Notomi et al., 2000) that previously been successfully applied to a range of diagnostics application and for onsite field-based assays with few portable battery chargeable devices to perform LAMP (Curtis et al., 2012; Liu et al., 2011; Almassian et al., 2013; Myers et al., 2013). It is, therefore, would be interesting that a simple molecular diagnostics assay that requires simple and minimal equipment can be created with our 10 s DNA isolation protocol along with single tube reaction of LAMP for the amplification and detection in 45 m total.

5. Conclusion

In summary, we have presented here a simple, cost-efficient, fast and safe technique for DNA isolation, which is subsequently used for PCR amplification (M. persicae, nAChR β1) and later successfully genotyped, as well. Such a technique allows researchers to get nucleic acid with suitable yield and purity excluding contamination. In this study, we have omitted the use of equipment including pipette, vortex, CF, and hazardous reagents. Our technique consisted of two steps i.e., squishing and mixing in EB in just 10 s to perform. By the simplicity, rapidity and affordability among all methods, this isolation method would not only be suitable for field-based assays but would provide molecular-based diagnostics more easily and accessible to a larger spectrum area including, to those in academic and research institutions, farmers, and biosecurity environments.

Funding

Supported by the Deputyship for Research and Innovation, "Ministry of Education" in Saudi Arabia for funding this research (IFKSUORG-149-1).

Declaration of Competing Interest

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.

Acknowledgement

The authors extend their appreciation to the Deputyship for Research and Innovation, "Ministry of Education" in Saudi Arabia for funding this research (IFKSUORG-149-1).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103791.

References

Aguirre-Rojas, L.M., Khalaf, L.K., Smith, C.M., 2019. Barley varieties stoneham and sydney exhibit mild antibiosis and antixenosis resistance to the wheat curl mite, Aceria tosichella (Keifer). Agronomy 9, 748.
Allen, G., Flores-Vergara, M., Krasynanski, S., Kumar, S., Thompson, W.A., 2006. Modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat. Protocols. 1, 2320–2325.
Almassian, D.R., Cockrell, L.M., Nelson, W.M., 2013. Portable nucleic acid thermocyclers. Chem. Soc. Rev. 42, 8769–8798.
Al-Saad, L.A., Aletby, M.A., 2018. Molecular diagnosis and comparison study to the red palm weevil Rhyncophorous ferrugineus (Olivier, 1790) in Basrah province-Iraq. Iraqi J. Agric. Sci. 49, 228–234.
AlShabar, S.H., Timm, A., Khalaf, L., 2021. Population variation of *Polyphagotarsonemus latus* (Banks) in Baghdad province, central Iraq. In: 2021 Third International Sustainability and Resilience Conference: Climate Change. IEEE, pp. 138–141.
Altameme, H.J.M., Ibraheam, I.A., 2019. RAPD and ISSR analysis of the genetic relationship among some species in rutaceae in and apiceae in Iraq. Iraqi J. Agric. Sci. 50, 608–616.
Ariefdjohan, M.W., Savaiano, D.A., Nakatsu, C.H., 2010. Comparison of DNA extraction kits for PCR-DGGE analysis of human intestinal microbial communities from fecal specimens. Nutr. J. 9, 23.
Ball, S.L., Armstrong, K.F., 2008. Rapid, one-step DNA extraction for insect pest identification by using DNA barcodes. J. Econ. Entomol. 101, 523–532.
Blackman, R.L., Eastop, V.F., 2000. In: Aphids on the world's crops: an identification and information guide. Wiley, John & Sons, p. 476.
Curtis, K.A., Rudolph, D.L., Nejad, I., Singleton, J., Beddoe, A., Weigl, B., LaBarre, P., Owen, S.M., 2012. Isothermal amplification using a chemical heating device for point-of-care detection of HIV-1. PLoS One 7, e31432.
Dahm, R., 2005. Friedrich Miescher and the discovery of DNA. Dev. Biol. 278, 274–288.
De Lipthay, J.R., Enzinger, C., Johnsen, K., Aamand, J., Sørensen, S.J., 2004. Impact of DNA extraction method on bacterial community composition measured by denaturing gradient gel electrophoresis. Soil Biol. Biochem. 36, 1607–1614.
Dong, J., Olano, J.P., McBride, J.W., Walker, D.H., 2008. Emerging pathogens: challenges and successes of molecular diagnostics. J. Mol. Diagn. 10, 185–197.
Faraj, A.A., Hade, B.F., Al-Amery, A.M., 2019. Conventional and molecular study of Babesia spp. of natural infection in dragging horses at some areas of Baghdad city. Iraq. Iraqi J. Agric. Sci. 50, 909–915.
Favret, C., 2005. A new non-destructive DNA extraction and specimen clearing technique for aphids (Hemiptera). Proc. Enotomol. Soc. Wash. 107, 469–470.
Gilbert, M.T.P., Moore, W., Melchior, L., Worobey, M., 2007. DNA extraction from dry museum beetles without conferring external morphological damage. PLoS One 2, e272.
Holmes, F.L., 2001. Meselson, Stahl, and the Replication of DNA: A History of the Most Beautiful Experiment in Biology. Yale University Press, New Haven, CT.
Hoy, M.A., 2003. In: Insect Molecular Genetics: An Introduction to Principles and Applications. 2nd edn. Academic Press, San Diego, p. 544.
Hunter, S.J., Goodall, T.I., Walsh, K.A., Owen, R., Day, J.C., 2008. Nondestructive DNA extraction from blackflies (Diptera: Simuliidae): retaining voucher specimens for DNA barcoding projects. Mol. Ecol. Notes 8, 56–61.
Kim, J., Mauk, M., Chen, D., Qiu, X., Kim, J., Gale, B., et al., 2010. A PCR reactor with an integrated alumina membrane for nucleic acid isolation. Analyst 135, 2408–2414.
Kotchoni, S.O., Gachomo, E.W., 2009. A rapid and hazardous reagent free protocol for genomic DNA extraction suitable for genetic studies in plants. Mol. Biol. Rep. 36, 1633–1636.
Liesenfeld, O., Lehman, L., Hunfeld, K.P., Kost, G., 2014. Molecular diagnosis of sepsis: New aspects and recent developments. Eur. J. Microbiol. Immunol. (Bp) 4, 1–25.

Liu, C.C., Mauk, M.G., Hart, R., Qiu, X.B., Bau, H.H., 2011. A self-heating cartridge for molecular diagnostics. Lab Chip 11, 2686–2692.
McOrist, A.L., Jackson, M., Bird, A.R., 2002. A comparison of fve methods for extraction of bacterial DNA from human faecal samples. J. Microbiol. Methods 50, 131–139.
Meselson, M., Stahl, F.W., 1958. The replication of DNA in Escherichia coli. PNAS 44, 671–682.
Moore, E., Arnscheidt, A., KrÜger, A., StrÖmpl, C., Mau, M., 2008. In: Section 1 update: simplifed protocols for the preparation of genomic DNA from bacterial cultures molecular microbial ecology manual. Springer, Netherlands, pp. 1905–1919.
Morrison, W.P., Peairs, F.B., 1998. Response model concept and economic impact. In: Quisenberry, S.S., Peairs, F.B. (Eds.), Response Model for an Introduced Pest-the Russian Wheat Aphid. Entomological Society of America, Lanham, MD.
Mumford, R., Boonham, N., Tomlinson, J., Barker, I., 2006. Advances in molecular phytodiagnostics—new solutions for old problems. Eur. J. Plant Pathol. 116, 1–19.
Myers, F.B., Henrikson, R.H., Bone, J.M., Bone, J., Lee, L.P., 2013. A handheld point-of-care genomic diagnostic system. PLoS One 8, e70266.
Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28, 12.
Pons, J., 2006. DNA-based identification of preys from non-destructive, total DNA extractions of predators using arthropod universal primers. Mol. Ecol. Notes 6, 623–626.
Rahman, M.M., Elaissari, A., 2012. Nucleic acid sample preparation for in vitro molecular diagnosis: from conventional techniques to biotechnology. Drug Discov. Today 17, 1199–1207.
Rowley, D.L., Coddington, J.A., Gates, M.W., Norrbom, A.L., Ochoa, R.A., Vandenberg, N.J., Greenstone, M.H., 2007. Vouchering DNA-barcoded specimens: test of a nondestructive extraction protocol for terrestrial arthropods. Mol. Ecol. Notes 7, 915–924.
Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. In: Molecular Cloning: A Laboratory Manual. Cold Spring Harbor laboratory press, New York, pp. 931–957.
Sambrook, J., Russell, D., 2001. Molecular Cloning: A Laboratory Manual. Cold Springs Laboratory Press, Cold Spring Harbor, New York.
Shoffner, M.A., Cheng, J., Hvichia, G.E., Kricka, L.J., Wilding, P., 1996. Chip PCR. I. Surface passivation of microfabricated silicon-glass chips for PCR. Nucleic Acids Res. 24, 375–379.
Sial, M.U., Zhao, Z., Zhang, L., Zhang, Y., Mao, L., Jiang, H., 2018. Evaluation of insecticides induced hormesis on the demographic parameters of *Myzus persicae* and expression changes of metabolic resistance detoxification genes. Sci. Rep. 8, 1–8.
Sial, M.U., Zhao, Z., Zhang, L., Zhang, Y., Mao, L., Jiang, H., 2020. Loop-mediated isothermal amplification for the detection of R81T mutation in nAChR with crude genomic DNA extracted from individual *Myzus persicae*. J. Pest. Sci. 93, 531–541.
Sunnucks, P., Hales, D.F., 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Mol. Biol. Evol. 13, 510–524.
Tan, S.C., Yiap, B.C., 2009. DNA, RNA, and protein extraction: the past and the present. BioMed Res. Int.
Thatcher, S.A., 2015. DNA/RNA Preparation for Molecular Detection. Clin. Chem. 61, 89–99.
Thomsen, P.F., Elias, S., Gilbert, M.T.P., Haile, J., Munch, K., Kuzmina, S., Froese, D.G., Sher, A., Holdaway, R.N., Willerslev, E., 2009. Non-destructive sampling of ancient insect DNA. PLoS One 4, e5048.
Vesty, A., Biswas, K., Taylor, M.W., Gear, K., Douglas, R.G., 2017. Evaluating the impact of DNA extraction method on the representation of human oral bacterial and fungal communities. PLoS One 12, e0169877.
Walsh, P.S., Metzger, D.A., Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10, 506–513.
Ward, E., Foster, S.J., Fraaije, B.A., McCartney, H.A., 2004. Plant pathogen diagnostics: immunological and nucleic acid-based approaches. Ann. Appl. Biol. 145, 1–16.
Zou, Y., Mason, M.G., Wang, Y., Wee, E., Turni, C., Blackall, P.J., et al., 2017. Nucleic acid purification from plants, animals and microbes in under 30 seconds. PLoS Biol. 15, e2003916.