A COMPARISON OF TWO METHODS OF ELUTING INSECT DNA FROM FLINDERS TECHNOLOGY ASSOCIATES CARDS

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Flinders Technology Associates (FTA™) cards contain chemicals that lyse cells and protect DNA from degradation for room-temperature storage (Whatman™, 2009). Consequently, DNA extraction takes less time and storage requires less energy and space than traditional methods requiring lysis, purification, and -20 °C storage in tubes. These cards are also easy to transport and face fewer shipping restrictions and less opportunity for damage than mounted specimens or those stored in alcohol. DNA stored on FTA™ cards produces a full allele compliment during genotyping after 11 yr (Kline, 2010) and the manufacturer reports successful PCR with FTA™ stored DNA for 17 yr and counting (Whatman™, 2009). Despite these benefits, this technology has been utilized infrequently with insects and other arthropods (Miller et al. 2012). Desloire et al. (2006) reported mixed results obtaining successful PCR from mite DNA and Bujang et al. (2011) were unable to obtain successful PCR reactions from termite DNA when using the manufacturer's protocol. This lead to the development of an alternative method of preparing an FTA™archived sample for successful PCR (Bujang et al. 2011). There may be several hurdles impeding broader use of the FTA™ technology by arthropod researchers. First, the DNA quality and quantity of the washed, single-use, FTA™ disc is untestable leading to uncertainty about the cause when PCR fails and second, the wash reagents are relatively expensive ($0.23-$0.36 for two 200μL FTA™ purification reagent washes). Initial attempts to PCR amplify FTA™ archived whitefly DNA using the manufacturer's protocol and the methods of Bujang et al. (2011) yielded mixed results but PCR was successful when discs were boiled for 5 min in lysis buffer. This study compares the new "quick boil" method of eluting total genomic DNA from FTA™ cards to the method used successfully by Bujang et al. (2011).

DNA yield, quality, and purity were assessed for 2 inexpensive elution protocols using DNA from adult female sweet potato whiteflies *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodoidae). Individual whiteflies were smashed onto FTA™ Plant Card matrix (Whatman™, Florham Park, New Jersey) and stored at room temperature in a sealed plastic bag containing desiccant for 19 d. A 2.0 mm diam disc containing a single whitefly was removed with a Harris micro-punch and cutting mat (Whatman™, Florham Park, New Jersey) for each of 10 replicates in two treatments. To elute the DNA, discs were either incubated in 20 μL of 1 × TE buffer (10 mM Tris-HCl pH 8.4, 1 mM EDTA pH 8.0) at 4 °C for 1 hr (cold recovery) or lysis buffer (50 mM KCl, 50 mM Tris-HCl pH 8.4, 0.45% Tween 20, 0.45% NP40) at 95 °C for 5 min (quick boil). Subsequently, discs were pressed with a sterile micropestle to remove as much DNA as possible and discarded. For the cold recovery protocol, the TE buffer was slightly more alkaline than that used by Bujang et al. (2011) (pH 8.4 Tris-HCl instead of pH 8.0). Hill et al. (2008) also used a similar protocol but with a 30 min incubation. The crude samples were then cleaned using a DNeasy™ Blood and Tissue kit (Qiagen™, Valencia, CA), minus the initial lysis buffer and proteinase K incubation. Three 200 μL elutions of the filter were combined and each sample was concentrated to a volume of ~10 μL in a SpeedVac™ DNA 110 Concentrator (Savant, Farmingdale, New York). As a control, DNA was extracted from fresh adults using the DNeasy kit according to manufacturer's instructions and concentrated as above. Yield and purity of each sample were measured by averaging duplicate readings of concentration and 260/280 nm optical density respectively from a ND-1000 spectrophotometer (NanoDrop™, Wilmington, Delaware). Yield was then calculated by multiplying concentration by the sample volume measured in an air displacement pipette (Rainin, Oakland, California). The mean yield ± 95% confidence interval from the quick boil (987 ± 138 ng) and cold recovery (906 ± 124 ng) treatments did not differ from the control (914 ± 87 ng) (P = 0.40 and 0.92 respectively, unpaired t-tests Fig. 1a). The purity of the cold recovery (1.71 ± 0.06) treatment did not differ from the control (1.70 ± 0.02) (P = 0.73, unpaired t-test) but the purity of the quick boil (1.63 ± 0.04) treatment was significantly different from the control (P = 0.02, unpaired t-test Fig. 1b). The DNA quality of each sample was assessed by electrophoresis on a 0.7% agarose gel with a 10 Kb DirectLoad™ Wide Range Marker (Sigma-Aldrich®, St. Louis, Missouri). High molecular weight genomic DNA (> 10 Kb) was recovered from the control samples while both the control and cold recovery samples had a distinct DNA band at ~8 Kb. The quick boil samples showed neither band suggesting the DNA had been sheared in this treatment (Fig. 1c).

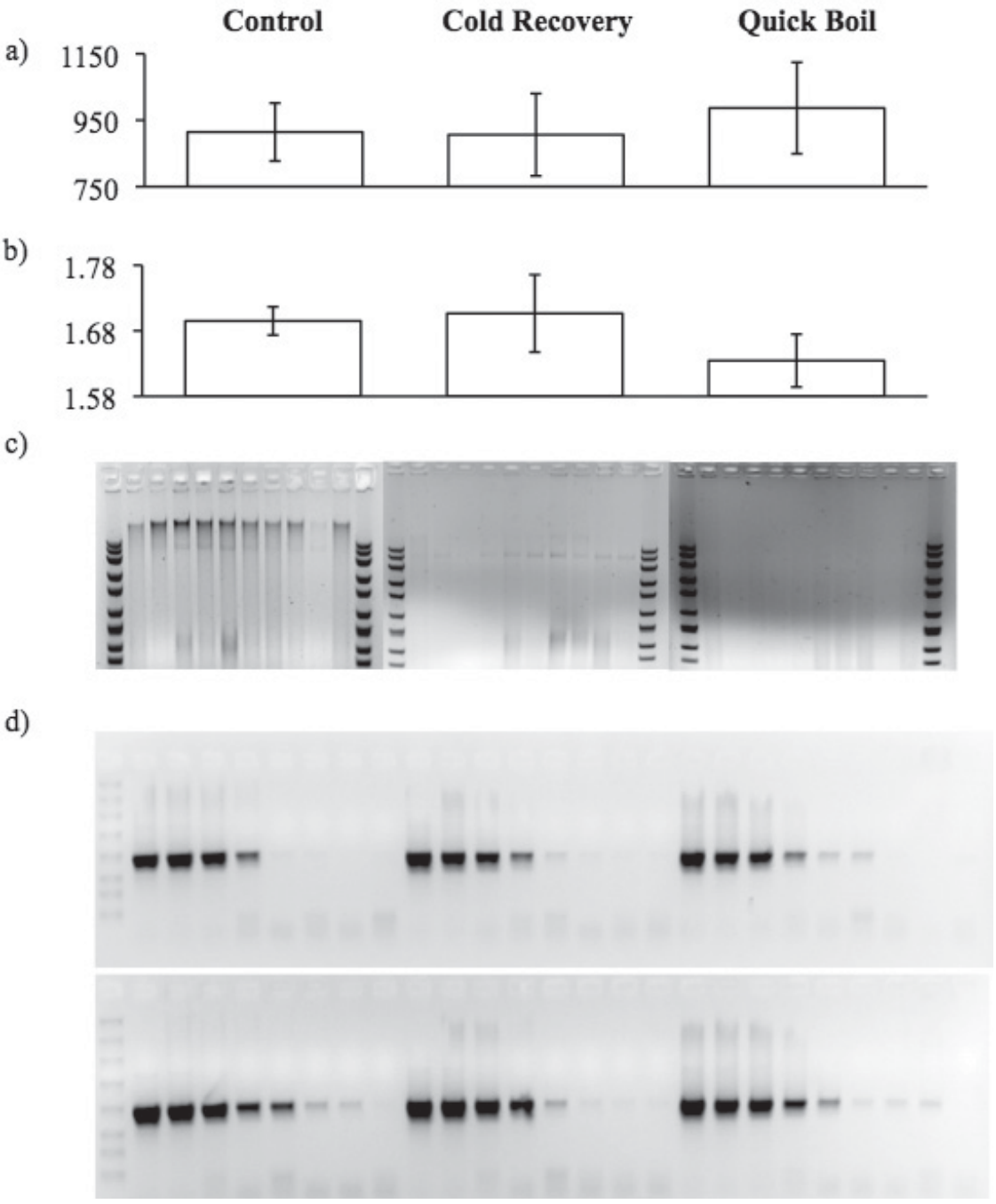

Fig. 1. Two methods of eluting whitefly DNA stored on FTA™ cards compared with DNA extracted from fresh whiteflies. a) Yield (ng), b) Purity (260/280 optical density ratio), c) Quality (0.7% agarose gel electrophoresis), and d) Downstream performance (PCR amplification of a 478 bp fragment of the mitochondrial COI gene with 10 × serially diluted DNA in duplicate). Error bars denote 95% confidence intervals. DNA yield from FTA™ cards was not different from the control and purity was only lower in the quick boil treatment. Genomic DNA (>10 Kb) is only present in the control while a large ~8 Kb band is present in both the control and cold recovery replicates. The quick boil treatment contained only sheared DNA. The PCR assay was sensitive to at least a 10^{-4} dilution for both elution treatments suggesting comparable downstream performance to the control.

In a second experiment, duplicate samples of each elution treatment were prepared as above but were not cleaned up. These were used in 10^{-1} × serial dilution PCRs with 2 µL template and 1 × GoTaq® Green Master Mix (Promega, Madison, Wisconsin) to amplify a 478 base pair fragment of the mitochondrial cytochrome oxidase I gene (Shatters et al. 2009) and electrophoresed on a 2% agarose gel with a 4 Kb FlashGel Marker (Lonza, Rockland, Maine). Refer to Shatters et al. (2009) for primers and thermocycling protocol for the PCR. Duplicate controls contained DNA extracted from a fresh adult by incubating in 100µL lysis buffer at 95 °C for 5 min. The PCR assay was sensitive to at least a 10^{-4} dilution of crude DNA eluted from FTA™ matrix using either method with weak bands and mixed results for subsequent dilutions (Fig. 1d). There was no evidence of PCR inhibition at high concentrations of FTA™ eluate in the cold recovery treatment, which contrasted with Hill et al. (2008) and the results of a previous experiment using 4 µL template (data not shown).

While not found in this experiment, the possibility of PCR inhibition with the cold recovery treatment should be noted and the source of and limits to any inhibition should be investigated. Both DNA recovery protocols yielded comparable quantities of DNA to a fresh extraction in contrast with Lall et al. (2010) who found a 3-fold reduction in tsetse fly DNA yield from FTA™ cards after 2 mo when DNA was recovered using cetyltrimethylammonium bromide (CTAB) extraction. The DNA purity is lower in the quick boil treatment and DNA quality is progressively lower for the cold recovery and quick boil elution treatments relative to the control. Both DNA recovery methods are easy and inexpensive (<$0.01 per sample for incubation chemicals compared with $0.23-$0.36 for two 200 uL FTA™ purification reagent washes) and unlike washed FTA™ discs, eluted DNA samples can be quantitatively and qualitatively assessed and are easily diluted for PCR or other downstream applications. Both elution methods can be incorporated easily with non-destructive DNA extraction methods that preserve whole-body slide mounted voucher specimens of soft-bodied arthropods like whiteflies (Miller et al. 2012).

Neither Lall et al. (2010), using CTAB extraction, nor this study succeeded in recovering arthropod genomic DNA from the FTA™ matrix. The cold recovery method did, however, recover higher quality DNA than the quick boil method based on the presence of a large ~8 Kb DNA fragment (Fig. 1c). This feature may make the cold recovery method especially useful to arthropod researchers amplifying larger nuclear genes (Bujang et al. 2011).

According to the manufacturer's protocol, FTA™ discs undergo 4 separate five-min reagent washes (20 min) and are subsequently dried completely (10-30 min) prior to PCR. In contrast, the quick boil method is complete in 5 min and the eluate is ready immediately for PCR. Whitefly DNA eluted with this method performed comparably to control and cold recovery samples in amplifying a portion of the mitochondrial COI gene (Fig. 1d). Though not required for successful PCR in this study (Fig. 1d), DNA recovered by either method can be further cleaned if researchers wish to assess the quantity and quality of their starting material for a downstream reaction.

Summary

Flinders Technology Associates (FTA™) technology lyses cells and stabilizes DNA for room-temperature storage in a single step but it has been infrequently used with arthropods. One possible reason is the paucity of quick and inexpensive protocols to subsequently elute the DNA from the card matrix. This report compares 2 such protocols for eluting *B. tabaci* DNA from FTA™ cards including a new quick boil method. The quick boil method elutes DNA from the FTA™ card in 5 min while the cold elution method recovers higher quality DNA.

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