MARSHALL FORENSIC SCIENCE

# An Improved Method for Extraction of DNA from Envelopes

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AFDIL Armed Forces DNA Identification Laboratory

Armed Forces DNA Identification Laboratory

## Abstract

At the Armed Forces DNA Identification Laboratory (AFDIL) envelopes can be used as alternative reference samples to help in the human identification process. Currently at AFDIL, envelope DNA extractions are completed by using a steam bath to open the envelope seal, swabbing a dime-sized section and extracting the swab using an organic extraction. Due to the sensitive nature of mtDNA analysis, the steam bath method can yield inconclusive results and introduce contamination from individuals who have previously handled the envelope.

Here, a new method was developed that maximizes the quantity of authentic DNA recovered from envelopes. To mimic casework conditions, mock envelopes containing a known mtDNA sequence in the seal of an envelope and a different known mtDNA sequence on the outside were created for experimentation. The mock envelopes were used to evaluate how variations to the original process can lead to higher quality results.

Results indicate that increasing the volume of extraction buffer from 500 µL to 1250 µL and proteinase K from 20 µL to 50 µL increases the quantity of DNA obtained. Secondly, a cutting of the seal, rather than a swabbing, yields higher quantities of both nuclear and mtDNA after an organic extraction and an automated extraction did not yield more favorable results. Although mock envelopes were purposefully created to contain a mixture, none were observed. This is likely due to a non-shedder being used for contamination and the more recent deposition of buccal swabs in the seal of the mock envelopes compared to historic envelopes.

## Introduction

Currently at AFDIL, the envelope extraction protocol uses a steam bath and tweezers to open the seal of an envelope followed by a dime-sized swabbing and organic extraction. The leading problem with this approach is that the steam bath saturates the paper of the envelope long before the seal of the envelope is softened, causing the paper to rip apart during the seal separation process. Additionally, it is believed that the steam bath in combination with tweezers is a source of the false-positive mixtures that are observed in mtDNA sequence data. Because the envelope is saturated with water, the envelope contributor's DNA on the outside of the envelope might be mixed with the authentic DNA within the seal from capillary action, and if the tweezers are not used in the same orientation at all times, the DNA can be transferred from the outside of the envelope to the seal on the inside. Another issue with the current protocol is the low quantity of DNA recovered when the steam bath is successful in separating the two sides of the envelope seal and has been reported earlier and attributed to the multiple centrifugation and tube transfer steps in the organic extraction procedure. More often than not, the quantity of amplified DNA obtained from envelopes at AFDIL is too low to be used in further mtDNA analysis.

In order to improve the currently validated envelope DNA extraction protocol, several modifications were evaluated. These include an increase in volume of both extraction buffer and proteinase K during the incubation period, which has been shown to increase total DNA extracted from a substrate; changing the sampling to a cutting and investigating how large a sample size is needed; using UV irradiation to limit the chance of contaminating a sample with DNA from the envelope contributor; and the use of the QIAamp DNA Investigator kit™ to allow for an automated DNA extraction on the QIAcube™. The evaluation of the automated DNA extraction reflects the needs of the forensics community in shifting more and more toward automation in all aspects of DNA analysis.

## Method

Mock envelopes were created by having Individual 1 lick and seal the envelope to leave their DNA within the seal, Individual 2 handling the envelope to leave their DNA on the outside of the seal, and Individual 3 extracting the DNA. The known mtDNA profile of each individual was used to determine which regions of mtDNA would be examined to assess the results. Individual 1 has a T to C polymorphism at position 16126 and a G to A polymorphism at position 16390, while Individual 2 has an A to G polymorphism at position 16240, and Individual 3 has a C to T polymorphism at position 16069. DNA was extracted from each envelope using an Organic extraction protocol (Phenol, Chloroform, Isoamyl Alcohol) or the QIAamp DNA Investigator kit ™.

## Method Variations

| | Sample Collection | Sample Size | Buffer Volume | UV Irradiation | Extraction Type |
|---|---|---|---|---|---|
| Envelope 1 | Steam bath and Swabbing | Dime-sized swabbing, tip of swab | 500 µL Extraction buffer, 20 µL Proteinase K | None | Organic |
| Envelope 2 | Steam bath and Swabbing | Dime-sized swabbing, tip of swab | 1250 µL Extraction buffer, 50 µL Proteinase K | None | Organic |
| Envelope 3/4 | Cutting | 1 cm2 | 1250 µL Extraction buffer, 50 µL Proteinase K | 5 minutes | Organic |
| Envelope 5 | Cutting | 1 cm2 | 1250 µL Extraction buffer, 50 µL Proteinase K | 0-9 minutes | Organic |
| Envelope 6 | Cutting | 1 cm2 | 1250 µL QIAamp Digestion buffer | None | QIAamp DNA Investigator kit |
| Envelope 7 | Cutting and Punching | 6 sizes ranging $1 cm^2$ to $0.08 cm^2$ | 1250 µL Extraction buffer, 50 µL Proteinase K | None | Organic |

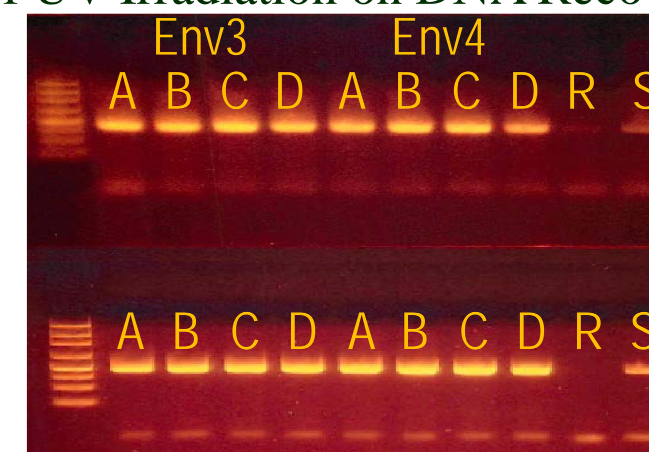
After extraction, the DNA concentration was determined using the Quantifiler ® Duo kit and then amplified using AFDIL's mini primer sets for the 15989-16158 and 16222-16410 regions. Yield gels were obtained for each sample, followed by purification with ExoSAP-IT ®, Sanger Sequencing, and additional purification of the product with the EDGE Biosystems Performa® DTR 96-well Ultra Gel Plate Kit. Finally, all samples were run on a 3130*xl* and analyzed using Sequencher, comparing the results of each sample with the Revised Cambridge Sequence.

## Results

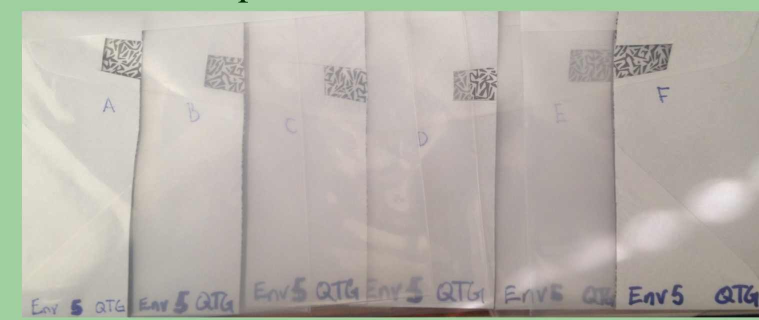
Average Concentration of DNA Recovered from Each Variation

| | DNA (pg/µL) |
|---|---|
| Envelope 1 | 2.5 |
| Envelope 2 | 3.75 |
| Envelope 3/4 No UV | 60.6 |
| Envelope 3/4 UV | 64.25 |
| Envelope 5 | 99.3 |
| Envelope 6 | 68.0 |

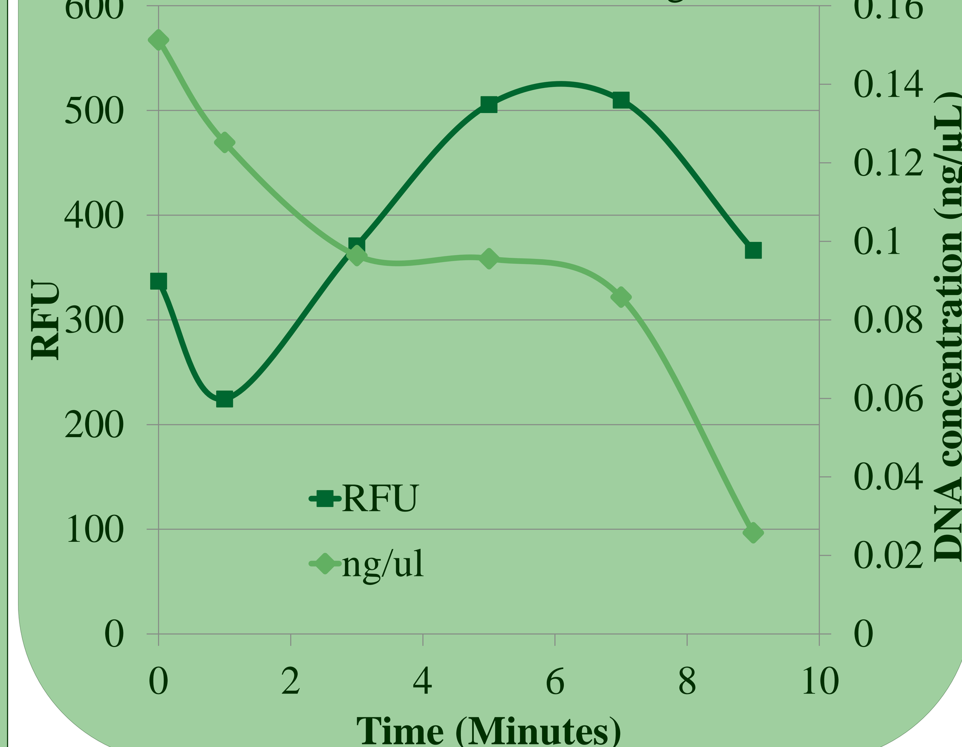
Yield Gel Results Observing Effects of UV Irradiation on DNA Recovery



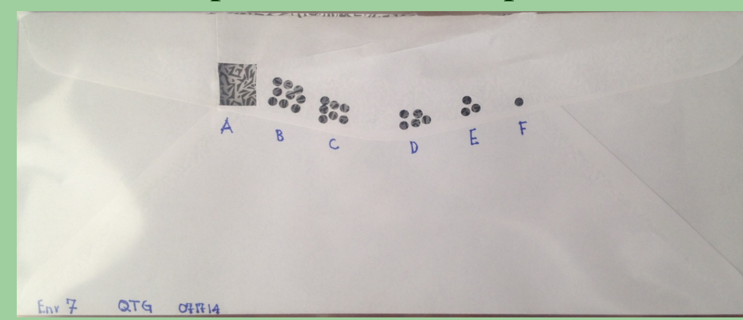

Envelope 5 – Varied UV Time



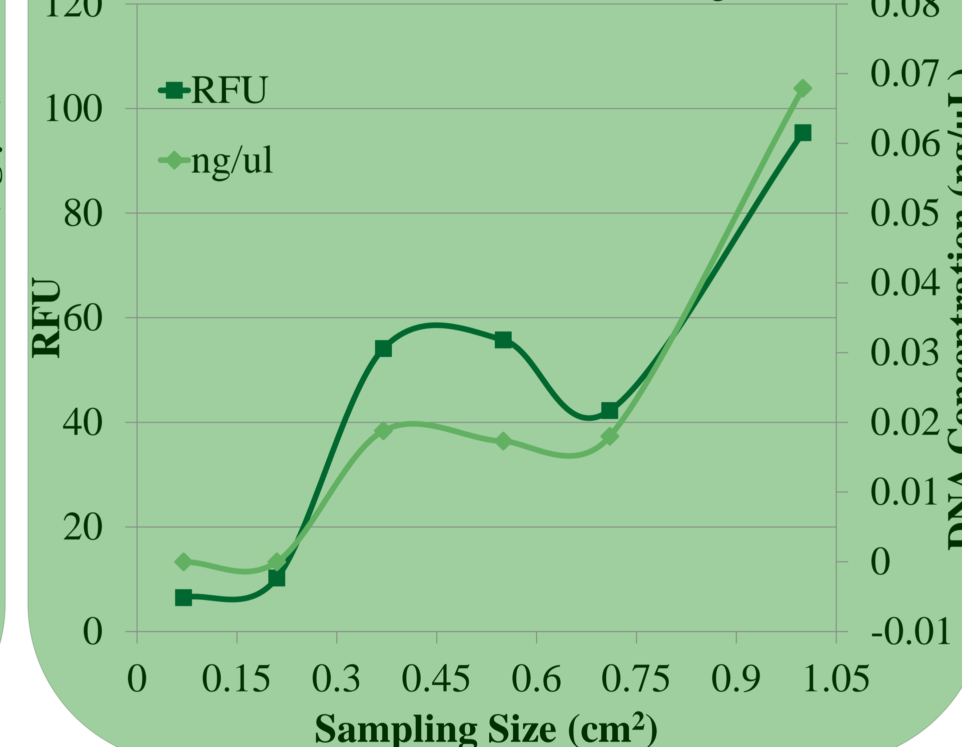

Envelope 5 - UV Irradiation effect on DNA Concentration and RFU Signal




Envelope 7 – Varied Sample Size




Envelope 7 - Sampling Size effect on DNA Concentration and RFU signal




## Discussion

The increased volumes of extraction buffer and proteinase K in Envelope 2 showed a small statistically insignificant increase in DNA recovered, however that procedure still suffered from the use of a steam bath and swabbing collection method. Envelopes 3 and 4, which were the first envelopes to use a $1 cm^2$ cutting rather than swabbing, showed vastly improved results. Envelopes 3 and 4 were also meant to demonstrate the use of UV irradiation for the reduction of mixtures, however no mixtures were ever found in either the non-UV treated or UV treated samples. It should be noted however, that the DNA within the seal of the envelope did not suffer greatly from the UV irradiation. As seen in Envelope 5, after seven minutes of UV irradiation noticeable amounts of DNA were still present in the seal of the envelope and successful amplification was achieved using the mini primer sets.

Automation of the overall process in order to save time and energy on behalf of the analyst, and to lower the chances of human error, did not match the results of the organic extraction. This is consistent with prior research into the use of the QIAcube™, and helped to determine that the organic extraction is the most efficient procedure for the extraction of DNA.

The final experiment demonstrated the need for an adequate sample size in the course of the extraction process. Although samples as low as $0.4 cm^2$ still showed adequate results, taking a $1 cm^2$ guaranteed the highest amount of DNA recovered. Although a least destructive form of sampling is desired throughout all of DNA analysis, it is still necessary to make sure that a sample is going to give the best results possible on the first try. Additionally, throughout all the envelopes, it was observed that the middle third of the seal consistently contained higher levels of DNA compared to the outer thirds of the envelope.

There was a statistically significant difference between groups as determined by one-way ANOVA ($F(5,26) = 3.766$, $p = .011$). Using a Q table for Tukey's HSD, the critical Q(.05) value, given 26 degrees of freedom and six treatments per group, is 4.35. A Tukey post-hoc test revealed that, the quantification results of Envelope 5 are statistically significantly different from Envelope 1, Envelope 2, and Envelope 7. This suggests that Envelope 5's larger sample size and increased buffer volumes lead to a definite increase in total DNA recovered from a sample.

## Conclusions

Overall, the process of DNA extraction from envelopes at AFDIL would greatly improve with just a few changes. First, the steam bath and swabbing should be abandoned in favor of using a 1cm2 cutting from the middle third of the envelope. Next, the volumes of extraction buffer and proteinase K should be increased to 1250 and 50 µL respectively. Automation of the process, as opposed to Organic extraction is not suitable at this point in time. Finally, if there is any suspicion of a mixture, the envelope should be placed in a cross linker for UV irradiation for no more than seven minutes. With these adjustments put in effect, there is no doubt that DNA analysis from envelopes for the identification of fallen service members would become a much more viable strategy.

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