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An investigation into methods to produce artificially degraded DNA

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Abstract. The aim of this study was to investigate different methods of artificially inducing DNA degradation with the purpose of producing a stock of compromised DNA with properties mimicking those found in crime scene samples. Blood samples, cigarette ends and chewing gum samples were collected for examination. UV light, humidity and temperature were investigated as degradation agents. A number of different extraction techniques were investigated. DNA profiles were generated using the AmpF/STR® SGM Plus® PCR Amplification Kit (Applied Biosystems). UV light caused a clear 'drop-out' of heavier alleles. This increased as exposure to UV light increased. Cigarette analysis yielded inconsistent results but partial profiles were produced that could assist in excluding a suspect. Chewing gum was an excellent material for obtaining profiles. Full profiles were obtained even after exposure to 30 h of sunlight. Humidity degradation experiments seemed to produce the most controlled method of degradation. The performance of different extraction techniques varied according to the extent of degradation. © 2006 Published by Elsevier B.V.

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1. Introduction

DNA samples recovered from a crime scene are often subjected to detrimental environmental conditions before they can be collected for analysis. Environmental sources of degradation, which can include heat, light and bacterial decomposition, are by their very nature random in the effect they have on the DNA deposited at the scene. These effects further test the scientist's ability to produce an evidentially valuable profile from a sample

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already compromised in terms of quantity. The facility to produce a 'stock' of degraded DNA on which to optimize existing protocols would go some way to help in the preparation of standard practices to follow when faced with an environmentally degraded sample. The knowledge gained from the preparation of such stocks has the potential to benefit those asked to give their Expert opinion in a court of law. Experience gained on the behaviour of DNA stored in a variety of hostile conditions (albeit in controlled environments) could help with the interpretation of results produced from degraded samples and may also be useful if asked to consider storage conditions of that sample prior to generating the resultant profile.

2. Materials and methods

2.1. Samples

Blood samples were collected from volunteers. The volume donated was large enough to enable multiple extractions to be performed for each degradation condition and to allow sample to sample comparisons to be made. Both these factors were considered important in determining whether the method was robust enough for consistent production of degraded DNA stock. Chewing gum and cigarette ends were also collected for investigation.

2.2. UV light

Blood smears were prepared using 5 μl blood and a clean glass slide. The smears were allowed to dry in the dark for 30 min before being exposed to short wave UV light. The exposure time varied from 10 to 120 s. Blood was removed using a wet swab, and DNA was extracted.

2.3. Temperature and humidity

Stains on 10 cm^2 cotton cloth squares were prepared using 1 ml of blood. Once dry, the cloth was hung in a humidity chamber (37 °C, >85% humidity). DNA was extracted daily during the first week and, subsequently, weekly. The prepared stains proved difficult to process in terms of laboratory space required for drying and maintaining sterility whilst monitoring degradation over an extended time frame. An alternative sampling source was found and subsequent experiments were performed using Salivette® (Sarstedt). Whole blood (2 ml) was applied to the Salivette® and distilled water placed in the lower chamber of the vessel. The sealed unit was incubated at 37 °C and 56 °C for up to 4 weeks. Small amounts of the cotton plug were removed daily and DNA extracted.

2.4. Cigarette ends and chewing gum

Samples were collected and degraded at room temperature, by sunlight exposure (cumulative exposure = 12–30 h) and in the humidity chamber (37 °C, >85% humidity).

2.5. DNA extraction, amplification and detection

Throughout the course of the work a number of different extraction techniques were investigated: Chelex® 100 (Sigma); Charge Switch™ (Invitrogen); Invisorb® Forensic Kit I (Invitek) and Qiagen. DNA profiles were generated using the AmpF*l*STR® SGM

Plus® PCR Amplification Kit (Applied Biosystems). Positive and negative controls were run in each experiment and used for comparison with profiles obtained from the degraded DNA. Extractions were performed in duplicate to check for reproducibility.

3. Results

3.1. UV light

UV light caused a clear 'drop-out' of heavier alleles which increased as exposure time increased. Although split peaks were prevalent, full profiles were obtained at all time intervals up to and including 60 s. No full profiles were observed following UV exposure for 120 s.

3.2. Temperature and humidity

With the cotton cloth experiments, PCR artefacts were apparent with 'drop-in' alleles regularly observed. Artefacts consistent with inhibition were also witnessed although attempts to compensate for this (such as dilution) failed to resolve the issue. The Salivettes® produced a more reliable DNA source in terms of sterility and consistency between sampling. Inconsistent results and PCR artefacts were obtained when incubated at 37 °C. Full profiles were obtained after 4 weeks incubation at 56 °C. Peak heights were greatly reduced but exceeded the 50 RFU threshold required for allelic designation.

3.3. Cigarette ends and chewing gum

The profiles obtained from the cigarette ends showed little reproducibility between samples. Full profiles were obtained from chewing gum following degradation at room temperature, incubation in the humidity chamber and after 30 h of sunlight exposure.

3.4. Extraction techniques

All extraction techniques used throughout this study had their benefits and drawbacks with regard to user-friendliness, DNA yield, cost and amenability to automation. Within the scope of this investigation, all were found to perform well. With the more degraded samples, Chelex® 100 would be the method of choice as more consistent profiles were observed with SGM Plus.

4. Summary

UV light caused a clear 'drop-out' of heavier alleles which increased with exposure time. Cigarette analysis yielded inconsistent results but partial profiles were produced that could assist in excluding a suspect. Chewing gum was an excellent material for obtaining profiles. Humidity degradation seems the most promising with regard to producing stocks of degraded DNA. The performance of different extraction techniques varied according to the extent of degradation.