Exymes

Extraction of DNA from cigarette butts using *forensic*GEM Universal

Charis Shepherd[1], Sallyann Harbison[2] and David J. Saul[1]

1 Exymes NZ Ltd, Dunedin, Otago, New Zealand

2 Forensic Division, Institute of Environmental Science and Research Ltd, Mt Albert Science Centre, Auckland, New Zealand.

Introduction

Cigarette butts are a common trace sample at crime scenes and obtaining DNA profiles from this evidence is an important capability in the forensic science repertoire. DNA extraction is a critical step in forensic analysis because the quality of DNA directly affects the ability to obtain a good quality forensic profile. Cigarette butts are notably difficult samples in that they produce DNA that is contaminated with inhibitors of the polymerase chain reaction (PCR) [1]. Typically, these substances are tars and phenolics from the smoke, paper additives and flavour additives (around 200 different additives are approved [2]). The range of additives may lead to different levels of inhibition from different brands, and to complicate matters, the brand is not always easily determined. Most methods in current usage solve the problem by adding a series of post-extraction steps to selectively remove inhibitors. These steps make the method complex, non-automatable, susceptible to contamination, and the many steps reduce DNA yields – a critical factor with trace samples [1, 3].

The thermophilic enzyme EA1 proteinase [4, 5] has been validated for a range of samples of forensic significance [6]. This enzyme is now available and has been formulated for cigarette butts as *forensic* GEM Universal. Instead of aggressively extracting all the organic compounds, *forensic*GEM Universal uses a gentle method minimising the release of inhibitors into the extracted DNA solution. The method can be used in a 96-well format or for any number of samples using PCR tubes and a thermal cycler. Furthermore, the procedure is closed-tube thereby minimising the risk of extraneous, or cross-contamination.

Method

Preparation of samples

1. All preparation was performed in a clean-room or PCR hood and all plastic-ware was irradiated with UV for 5 minutes prior to extraction.
2. A single volunteer smoked two each of 21 different cigarette brands.
3. 1 cm of the circumference of the cigarette butt paper was removed using sterile scissors and forceps. This paper was cut into quarters and one quarter was used in each extraction. Each quarter was again cut into four and the paper added to a well of a 96 well tray or a 0.2 ml PCR tube. Two extractions were performed for each cigarette to cover within and between cigarette sample variations.

Extraction

1. 89 µl water, 10 µl Exymes 10x Buffer **BLUE** and 1 µl of *forensic*GEM were added to each sample.
2. The samples were heated to 75°C for 15 minutes then 95°C for 5 minutes in a thermal cycler.
3. The paper was removed to prevent slow leaching of inhibitors into the extract.

Amplification

1. Extracts were amplified using primers for the glyceraldehyde phosphate dehydrogenase (GAPDH) gene; (Forward: TTCACCACCATGGAGAAGGCTGGG and Reverse GTCCACCACCCTGTTGCTGTAGCC). 5 µl of extract was added to a 25 µl PCR.
2. Amplification products were visualised by using agarose gel electrophoresis (Figure 1). Four replicates were amplified for each cigarette type: two samples from two different cigarette butts for each cigarette brand.

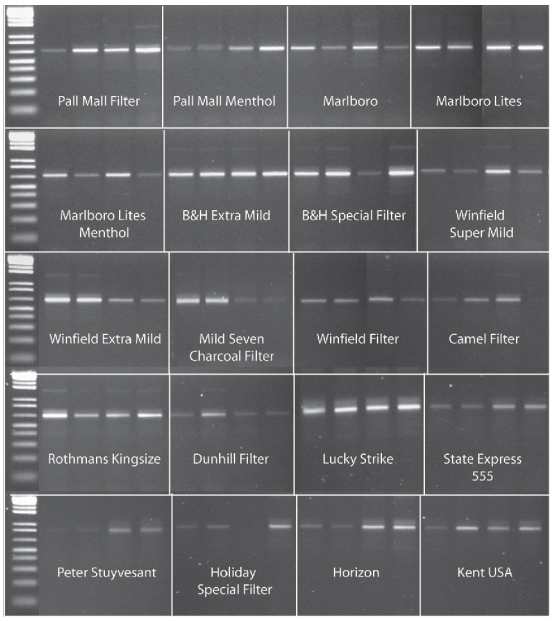

Figure 1. GAPDH PCR products obtained from cigarette butt extractions performed using *forensic*GEM. A composite gel has been constructed showing amplified products from a PCR using 5 µl of an extraction derived from one 0.5 x 1 cm piece of cigarette butt paper from a range of cigarette types. Four replicates were amplified for each cigarette type (2 samples from 2 different cigarette butts for each sample type). The amplified product is approximately 850 bp.

Identifiler

1. 2 µl of a DNA extract from each brand was amplified using the AmpFlSTR® Identifiler® PCR Amplification Kit system (Applied Biosystems).
2. Resolution of STR amplicons was performed using an ABI 3130 Genetic Analyser and analysis undertaken using the GeneScan™ Analysis, Genotyper® and GeneMapper® software packages (Applied Biosystems). Results from profiling with Identifiler are listed in Table 1 and typical profiles are shown in Figure 2.

Results

It can be seen from comparison of band brightness in Figure 1 that both within-, and between-sample variation occurs. This observation is consistent with the results of others [7]. Only two extracts failed to amplify. With Identifiler (Figure 2 and Table 1) sixteen of the twenty-one extracts gave full and usable profiles using standard forensic allele-calling criteria.

Cigarette Type	Number of valid STR loci
Pall Mall Filter	7/15
Pall Mall Menthol	15/15
Marlboro	14.5/15
Marlboro Lites	15/15
Marlboro Lites Menthol	10/15
B&H Extra Mild	15/15
B&H Special Filter	15/15
Winfield Super Mild	15/15
Winfield Extra Mild	15/15
Winfield Menthol	15/15
Winfield Filter	15/15
Camel Filters Generous Flavor	15/15
Rothmans King Size Filter Tipped	15/15
Dunhill Filter	15/15
Lucky Strike Original Red	15/15
State Express 555 Filter Kings	15/15
Peter Stuyvesant Filter King Size	15/15
Holiday Special Filter	14.5/15
Horizon King Size	15/15
Kent USA Charcoal Filter	13.5/15
Mild Seven Charcoal Filter	15/15

Table 1. AmpFlSTR® Identifiler® (Applied Biosystems) results for cigarette butt extractions.

These results are significant because current procedures for DNA extraction from cigarette butts are not readily amenable to automation or high-throughput processing. This extraction method provides an opportunity to improve the processing of an evidence type typically associated with volume crime cases such as burglaries or car thefts. The results demonstrate that the *forensic*GEM cigarette method is a quick and easy method for processing cigarette butts. While being substantially faster than current organic extraction methods, it is also capable of producing full profiles using AmpFlSTR® Identifiler® – a multiplex fingerprinting system that we have found is particularly sensitive to inhibition.

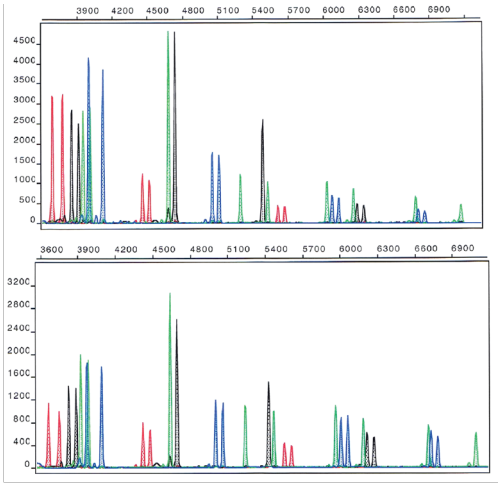

Figure 2. Typical AmpFlSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) profiles of two of the cigarette types. 2ul of extract was used in a standard Identifiler PCR. Top: Rothmans King Size Filter Tip. Bottom: Winfield Extra Mild.

References

[1] Gunther, S., Herold, J., Patzelt, D. (1995) Extraction of high quality DNA from bloodstains using diatoms. Int J Legal Medicine 108:154-156

[2] Permitted Additives to Tobacco Products in the United Kingdom, Department of Health. March 2000

[3] Watanabe, Y., Takayama, T., Hirata, K., Yamada, S., Nagai, A., Nakamura, I., Bunai, Y., Ohya, I. (2003). DNA typing from cigarette butts. Legal Med Tokyo 5:177–1799

[4] Coolbear, T., Whittaker, J.M., Daniel, R.M., Morgan, H.W. (1992). The effect of metal ions on the activity and thermostability of the extra cellular proteinase from a thermophilic Bacillus strain EA1. Biochem J 286:367–374.

[5] Saul, D.J., Williams, L.C., Toogood, H.S., Daniel, R.M., Bergquist, P.L. 1996 Sequence of the gene encoding a highly thermostable neutral proteinase from Bacillus sp. strain EA1: expression in Escherichia coli and characterisation. Biochim Biophys Acta1308:74–80.

[6] Moss, D., Harbison, S-A., Saul, D.J. (2003). An easily automated, closed tube forensic DNA extraction procedure using a thermostable proteinase. Int J Leg Med 117: 340-349.

[7] Hochmeister, M.N., Budowle, B., Jung, J., Borer, U.V., Comey, C.T., Dirnhofer, R. (1991). PCR-based typing of DNA extracted from cigarette butts. Int J Leg Med 104:229-233

For more information visit: exymesplc.com
or email: info@exymesplc.com