



STRs typing of DNA extracted from cigarette butts soaked in flammable liquids for several weeks

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Abstract. This paper refers to a case of arson, in which we analyzed three cigarette butts, apparently smoked, collected from a crime scene. They were soaked in a petroleum blend and used to ignite the fire. DNA extraction was carried out using QIAamp 96 DNA Swab BioRobot kit procedure. The amount of human DNA recovered was then quantified by slot-blot hybridization with the chemiluminescent signals recorded by GeneGnome CCD imaging system, whose values ranged from approximately 0.01 to 0.1 ng/µl. Two complete male profiles based on 17 STRs were obtained from two out of three cigarette butts, while a mixed profile compatible with the previous two individuals was obtained from the third butt. Chemical analyses suggested that the cigarette butts had been left soaking in a mixture of diesel and kerosene oils for at least 45 days before they were collected and sent to the lab. Due to these situations, we decided to carry out two experimental trials in order to establish the possibility of extracting and successfully typing DNA from cigarette butts, smoked by the same individual, under the same conditions as we faced with the evidence described above. The trials were carried out as follows:

- 1. The first set of three cigarettes was left soaking in three common flammable liquids (alcohol, gasoline and diesel oil) as well as in water for 12, 24 and 72 h;
- 2. The second set of three cigarettes was soaked in the same liquids, but for a longer period of time of 1, 2 and 3 months.

A total of 72 cigarette butts underwent STRs analyses. The results of the two trials were as follows: all samples were successfully typed, showing the great possibility of DNA analysis, even when exhibits are recovered from very critical situations and DNA is present in very low quantities. © 2005 Published by Elsevier B.V.

Keywords: Cigarette butts; Flammable liquids; DNA extraction

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1. Introduction

Three cigarette butts, apparently smoked, were soaked in a petroleum blend (almost one half gallon) and used to ignite the fire near a church, but the arson did not spread out, making it possible to collect them from the crime scene. Despite the long time elapsed, DNA isolation and STR typing was successfully obtained. Based on these results, we decided to carry out two experimental trials in order to establish the possibility of extracting and successfully typing DNA from cigarette butts, smoked by the same individual, under similar conditions as we faced with the evidence described above, with an high throughput DNA isolation method. The trials were carried out as follows:

- 1. The first set of three cigarettes was left soaking in three common flammable liquids (alcohol, gasoline and diesel oil) as well as in water for 12, 24 and 72 h;
- 2. The second set of three cigarettes was soaked in the same liquids, but for a longer period of time of 1, 2 and 3 months.

A total of 72 cigarette butts underwent STRs analyses, to evaluate the boundary conditions of successfully DNA extraction.

Factors considered important in the evaluation include DNA yield, the liquid capability to dilute cells from the butt surfaces, the time necessary for this process and the ability of butt filters to retain DNA from saliva.

2. Materials and methods

DNA was isolated from 72 cigarette butts, all smoked from the same individual, using a Qiagen automated extraction [1,2] and quantified by slot-blot hybridization with the chemiluminescent signals recorded by GeneGnome CCD imaging systems [3,4]. The experimental batch was achieved as described in Table 1.

For the amplification and typing, we used the AmpFISTR Identifiler PCR Amplification Kit (Applied BioSystems), following the manufacturer's recommendations. Electrophoresis was carried out on ABI Prism 310 Genetic Analyzer. Data were analyzed by Gene Scan v.3.1 analytical software and interpreted independently by two scientists.

3. Results and discussion

The amount of recovered human DNA from each sample collected from the scene of the arson case was quantified by slot-blot analysis and ranged from approximately 0.03 to 0.15 ng/µl DNA for the three cigarette butts, leading to two unknown STR profiles.

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Soaking liquids/ soaking time	12 h/0.5 day	24 h/1 day	72 h/3 days	720 h/1 month	1440 h/2 months	2160 h/3 months
Alcohol	3 butts	3 butts	3 butts	3 butts	3 butts	3 butts
Gasoline	3 butts	3 butts	3 butts	3 butts	3 butts	3 butts
Unleaded gas	3 butts	3 butts	3 butts	3 butts	3 butts	3 butts
DD water	3 butts	3 butts	3 butts	3 butts	3 butts	3 butts

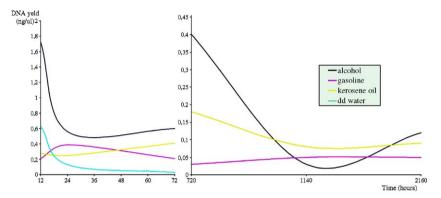


Fig. 1. Extracted DNA from cigarette butts soaked in different liquids at different time.

Alcohol and deionised water (DD $\rm H_2O$) gave an exponential loss of DNA recovery after the first 24 h, which is three times more efficient for $\rm H_2O$ (see the plotted graphs). Kerosene and gasoline showed a consistent DNA loss within 12 h, approximately equally to 50% if compared to the alcohol ones, then remaining constant within the first days and months.

From the results, we obtained it is amazing to note that is was possible to achieve suitable profiles even if the cigarette butts came from a long immersion in the liquids, especially in water. This remarkable result has underlined the outstanding role of the cigarette butts in trapping saliva cells and retain them despite their affinity for water solution.

Data show the great possibility of DNA analysis from cigarette butts related to arson cases, even when these exhibits are recovered from very critical situations and DNA is present in very low quantities (Fig. 1).

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