

DNA Isolation from Cigarette Butts

DNA extraction from cigarette butts using *forensicGEM*[™]

This method is recommended for DNA extraction directly from cigarette butts. Because the method is rapid and gentler than other methods, inhibitors in the paper are not released into the DNA solution. This removes the additional need to extract the inhibitors away from the DNA. The method can be used in a 96 well format or for any number of samples using PCR tubes and a thermal cycler.

Extraction Method

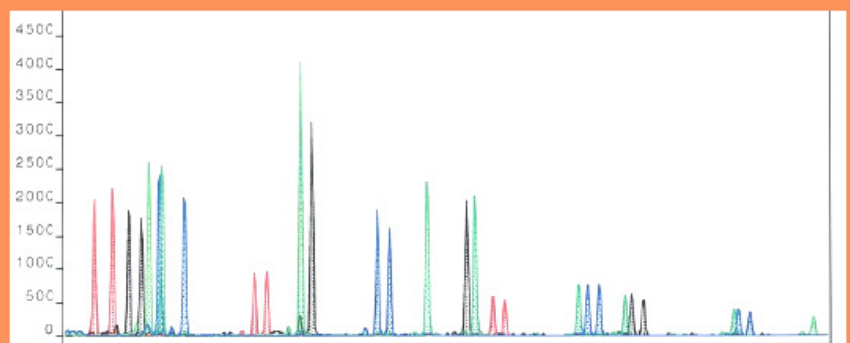
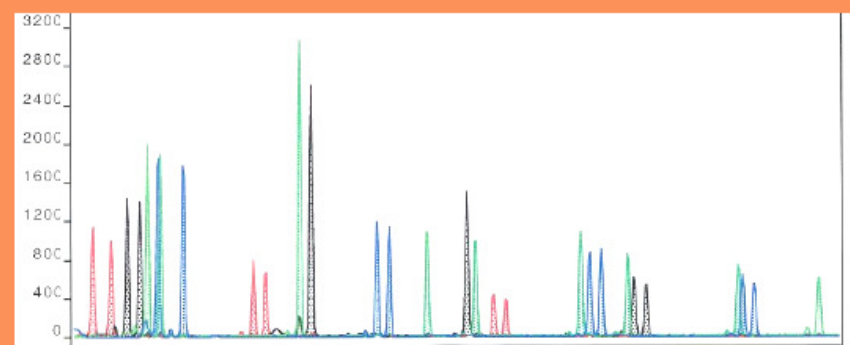
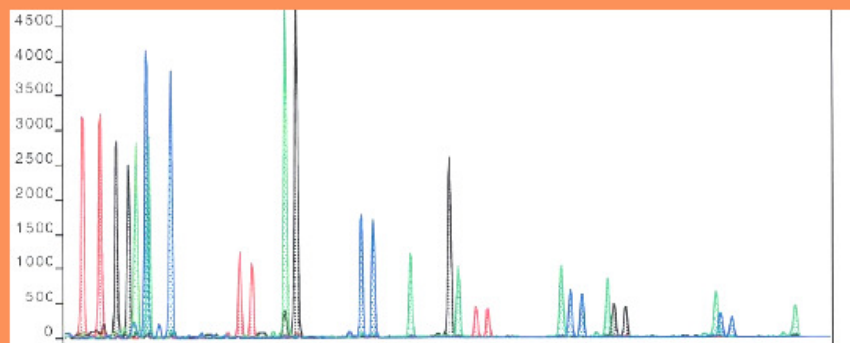
1. UV irradiate all plastic-ware prior to extraction for 5 minutes.
2. Cut approximately 0.5 x 1 cm of paper from the cigarette butt. Use the end of the butt closest to the mouth. Cut this paper into 4 smaller fragments.
3. Add the paper to a well of a microtitre dish or PCR tube.
4. Add 98 µl of *forensicGEM*[™] buffer 3, 1 µl of 5% TritonX-100 and 1 µl of *forensicGEM*[™] [NOTE1].
5. Incubate at 75 °C for 15 minutes.
6. Incubate at 95°C for 5 minutes [NOTE2].
7. Pipette the supernatant away from the cigarette paper [NOTE3].
8. Quantify the supernatant using Quantiblot or Quantifiler.

NOTE1: A 10X buffer is supplied.

NOTE2: 5 minutes is sufficient as long as ramping to temperature is rapid. For slow ramping, longer incubations can be used.

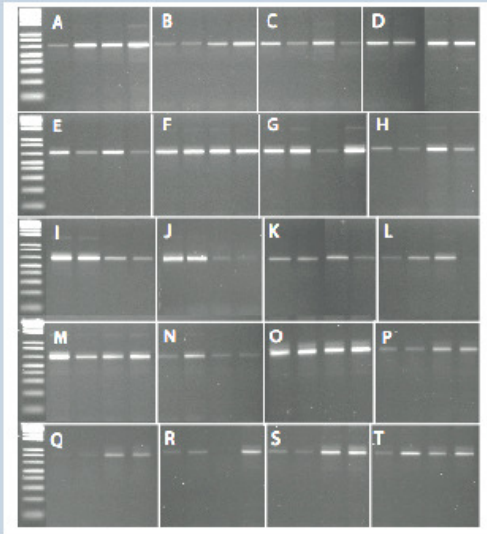
NOTE3: It is essential to remove the extracted DNA from the paper to prevent potential inhibitors leaching into the eluate.

CIGARETTE BUTTS



AmpFISTR[®] Identifiler[™] PCR Amplification Kit (Applied Biosystems) profiles of three of the cigarette types. 2µL of extract was used in a standard Identifiler PCR. Top: Rothmans King Size Filter Tip. Middle: Winfield Extra Mild. Bottom: Charcoal Filter.

CIGARETTES



- A. PALL MALL Filters
- B. PALL MALL menthol
- C. Marlboro
- D. Marlboro Lights
- E. Marlboro lights menthol
- F. B&H Extra mild
- G. B&H Special filter
- H. Winfield super mild
- I. Winfield extra mild
- J. Mild Seven charcoal filter
- K. Winfield filter
- L. Camel filters generous flavour
- M. Rothmans King Size filter tipped
- N. Dunhill filter
- O. Lucky strike original red
- P. state express 555 filter kings
- Q. Peter Stuyvesant filter king size
- R. holiday special filter
- S. horizon king size
- T. Kent USA charcoal filter

PCR Products A composite gel showing PCR amplified products from 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. 4 replicates were done for each cigarette type (2 samples from 2 different cigarette butts for each sample type).

Brand	Result	Alleles
PALL MALL Filter	Low level partial profile	7/15
PALL MALL Menthol		15/15
Marlboro	T _{po} x < 200 (8,0)	14.5/15
Marlboro Lights		15/15
Marlboro Lights Menthol	Low level, partial profile	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	Peak imbalance 58% @CSF	15/15
Camel filters generous flavour		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	D2(0,26)	14.5/15
Horizon king size	Peak imbalance 55%CSF 59%D1615/15	
Kent USA charcoal filter	Partial	13.5/15
Mild seven charcoal filter		15/15

Allele data from random chosen cigarettes, showing consistency and high incidence of full profiles across a variety of cigarette types.

A simple, rapid method for DNA extraction for all cigarette types

Cigarettes are common trace samples at crime scenes. DNA extracted from these samples invariably suffer from PCR inhibition caused by tars and phenolics from the smoke, paper additives and flavour additives - around 200 different additives are approved. A problem facing forensic scientists is that different additives have different effects and often the brand is not immediately obvious.

Most existing methods solve the problem by augmenting methods with a series of steps to selectively remove the inhibitors, but these steps make the method complex, non-automatable, susceptible to contamination and furthermore, the many steps reduce potential DNA yields - a critical factor with trace samples.

Here we present a very simple and rapid procedure for obtaining a high incidence of full genetic profiles from cigarette butts in as little as 20 - 30 minutes.