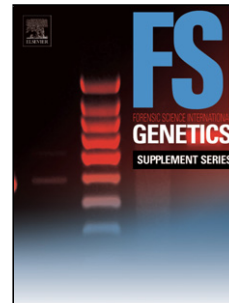


# Journal Pre-proof

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## Application of 13 loci STR multiplex for cannabis sativa genotyping

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### Keywords

Cannabis sativa, plant DNA, forensic botany

### Abstract

Our work describes an application of a STR multiplex systems for 13 loci, previously developed and validated in our laboratory following international guidelines, on 4 cannabis specimens seized by Anti-Narcotic Italian Police.

STR loci were chosen from the set of publicly available STRs for cannabis sativa and among the highly polymorphic available in literature.

Gas chromatography with a flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS) analysis revealed and confirmed four comparable molecular spectrums, approximatively descriptive of the same plant/seizure origin. Due to these uncertain results, investigative authorities approved the genetic analysis. DNA profiling allowed to identify the same genetic profile and consequently the same origin and individualization of the specimens analysed.

### Introduction

Forensic genetics laboratories usually performed DNA typing on human DNA samples collected from crime scenes [1,2]. Analysis of these evidences is crucial to establish a strong link between a suspect and a victim or between a suspect and a specific crime scene.

Like human DNA, also plant DNA represents a useful genetic evidence tool in forensic analysis. A reliable example relating to this aspect is represented by forensic botany [3].

A wide range of botanical evidence is being increasingly used in forensic investigations, and molecular characterization of plant DNA is extensively being adopted for the identification of species from botanical evidence.

The most common system used for discrimination between individuals in a human forensic investigation, short tandem repeat (STR) markers, have been developed for Cannabis Sativa.

In 2003, first STR markers were published for Cannabis Sativa and research has shown the usefulness of these loci in individualizing Cannabis samples [4].

As in the other country of the world, in Italy marijuana represent the most commonly used illicit substance become a highly trafficked drug to and within Italy by organized crime.

Consequently, a novel method is required for individualization and origin determination of cannabis samples, in order to establish a certain link between cannabis cases.

This paper describes an application of a 13-STRs markers set, previously developed and validated in our laboratory following SWGDAM guidelines, on four cannabis samples derived from a casework. Samples were chosen mainly due to positive toxicological analysis, carried out using gas chromatography with a flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Toxicological analysis revealed and confirmed four similar and comparable molecular spectrums, approximatively descriptive of the same plant/seizure origin. Molecular analysis of these samples, using the method proposed, allowed to identify the same genetic profile and consequently the same origin and individualization of the specimens analyzed.

### **Materials and methods**

Cannabis samples were collected from two police seizures, carried out in province of Ferrara. A set of two specimens were randomly collected from each case set (4 samples), consisting in flower fragments derived from plants grown using “pot” method.

DNA extraction was carried out using 5% chelating resin Chelex 100 (BIORAD®).

In a 1,5mL Eppendorf tube, 10-15mg of flower fragments were homogenized with a pestle for 1-2 minutes in 250-300µl of resin. Samples were vortexed 10 seconds, soaked in boiling water for 5 minutes, vortexed again for 10 seconds and, finally, centrifuged at 13500rpm for 2 minutes.

An aliquot (1-2µl) of supernatant was used as template for PCR reaction.

Multiplex reaction consisted in 13 STRs markers previously described and published [5,6].

Thermal-cycling steps were accomplished in a Veriti 96-Well Fast thermal cycler (Thermoscientific®).

PCR reaction mix was run in a final volume of 12µl.

PCR cycling conditions were as follows: 10 min at 95°C for the activation of AmpliTaq Gold polymerase, followed by 10 cycles of 30 sec at 95°C, 30 sec at 66°C (reducing by 3°C every second cycle down to 54°C – touchdown protocol), 45 sec at 72°C, followed by 30 cycles of 30 sec at 95°C, 30 sec at 50°C, 45 sec at 72°C, and a final extension held at 72°C for 30 min.

Detection of amplified fragments were accomplished on an ABI PRISM 310 HID Genetic Analyzer (Thermoscientific®). Genotyping of PCR products was performed on GeneMapper ID-X v.1.0. software (Thermoscientific®), using an homemade bin/panel set.

### **Results and discussion**

As illustrated in figure 1, all samples tested and originated from two independent replicate sampling were successfully amplified under our reaction conditions.

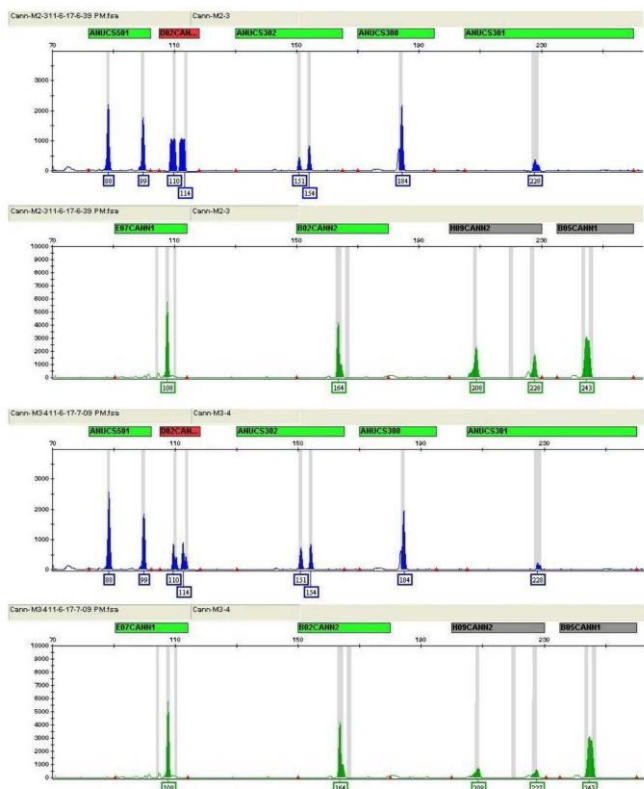


Figure 1. STR profiles achieved from the analysis of the two police seizures

From each specimen tested (two samples collected from each seizure), DNA was analyzed and the same genetic profile were achieved from all the samples sequenced.

The sampling system adopted in the present work permitted to test also the reproducibility of results. As observed during the replicate evaluation, there were no drop-outs or drop-in using the chosen DNA amount, approximately equal to 30-50ng.

The protocol previously developed and validated proved the chance to using a molecular analysis to identify different cannabis seizures within one PCR reaction.

These findings also confirmed the results previously achieved using current toxicological methods mainly used to supplimentify origin and individualization of cannabis.

## Conclusions

STRs typing proves itself as a valid and more robust strategy to identify a particular plant or seizure. As demonstrated by the casework described in this paper, the assay developed and applied helped police trace back dealers with maximized genetic information.

Also, DNA-based method allowed to improve the toxicological analysis and results.

This protocol will be also applied to link Cannabis plants or inflorescence seizures to a specific crime scene.

## Conflict of interest

None

## Acknowledgments

None

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