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STR typing from human faeces: a modified DNA extraction method

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1. Introduction

Human faeces can be found in forensic cases in a small but significant proportion of crime cases in Italy. This type of biological sample is a complex mixture of micro-organisms, digested and undigested food residues, mucus, soluble and insoluble products of the gastrointestinal tract and degradative enzymes derived from cells, food and bacteria (Iyengar et al. [1]). Although an estimated 10 billion cells are lost per day from the gastrointestinal tract (Sidransky et al. [2]), routine STR analysis was not possible following DNA extraction by current extraction methodology. It is known that faecal constituents such as bilirubin and bile salts inhibit PCR even when present at low concentrations (Widjojoatmodjo et al. [3]). Therefore for PCR analysis, DNA purification is required prior to amplification; alternatively, inhibitors can be reduced by dilution of the extract, but this is accompanied by a loss in sensitivity proportional to the dilution factor. The success of STR analysis depends on a minimum amount of DNA, on the absence of PCR inhibition factors and on the DNA degradation level. In faeces samples, all of these factors are present and the success depends on the extraction methodology applied. We have tested different extraction protocols such as Chelex-100®, phenol-chloroform and Qiagen extraction. None of these allowed us to obtain positive results.

In this report, we present a modified methodology that uses a phenol-chloroform based extraction method followed by a Sephadex and a Microcon®100 purification step. This modified method allowed us to obtain full STR profiles from faeces samples.

2. Materials and methods

Faeces samples from the crime scene were collected, air-dried and stored at +4 °C until analysis. DNA extraction was carried out essentially as described in Sambrook et al.

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[4]. The samples were digested at 37 °C overnight in 500 μl extraction buffer (10 mM Tris–HCl pH 8, 100 mM NaCl, 10 mM EDTA, 2% SDS, 39 mM DTT) in a single tube with 15 μl of proteinase K (20 mg/ml). The DNA was phenol-chloroform extracted and ethanol precipitated. Finally, the samples were resuspended in 50 μl of sterile deionized water. The extract was first purified on Sephadex® column G-200 (Pharmacia Biotech) following the manufacturer's recommendations with an additional washing step before DNA elution. Subsequently, the DNA was repurified with Microcon®100 (Millipore) in alkaline buffer. Quantitation of the human genomic DNA extracted was conducted using the ACES 2.0 Human DNA quantitation system (GIBCO BRL). A small amount (5 μl) of the extract was quantified by direct comparison to DNA standards of known concentration, through the use of a biotinylated human-specific DNA probe from the alpha satellite locus D17Z1, and ECL detection as described by Walsh et al. [5]. Simultaneous amplification of the 13 CODIS STR loci was performed by use of AmpFlSTR® Profiler Plus (Applied Biosystems) [6] and Promega GenePrint® PowerPlex 1.2 systems [7]. STR amplification as well as the gender determination marker, Amelogenin, was conducted in a 12.5 μl final reaction volume containing 0.5 ng of genomic DNA, PCR Reaction Mix, Primer Set Solution, 0.5 μl of AmpliTaqGold® DNA polymerase (5 U/μl stock) and 0.5 μl BSA (25 mg/ml stock). PCR was conducted, following standard parameters, in a Perkin Elmer GeneAmp PCR System 9600 thermal cycler using thin-walled 0.2 ml MicroAmp reaction tubes. Analysis of the amplified products was performed as follows: an aliquot of 1 μl of each PCR reaction was mixed with 0.3 μl of ABI Gene Scan-500 Internal Lane Size Standard (labelled with 6-carboxy-X-rhodamine and 2.7 μl of denaturing loading buffer). Following denaturation at 95 °C for 2–3 min, samples were snap-cooled in ice and 1 μl aliquots were loaded on a 6.75% (19:1) acrylamide:bisacrylamide gel containing 6M urea (12 cm well-to-read glass plate format, Lago et al. [8]), which had been pre-run at constant voltage for 30 min and equilibrated to 51 °C. Electrophoresis was conducted for 2.25 h at constant voltage (650 V, 50 mA) 1 × TBE using an ABI PRISM 377 DNA Sequencer with the laser set at 40 mW. Allele sizes were determined using Gene Scan Analysis v.2.1

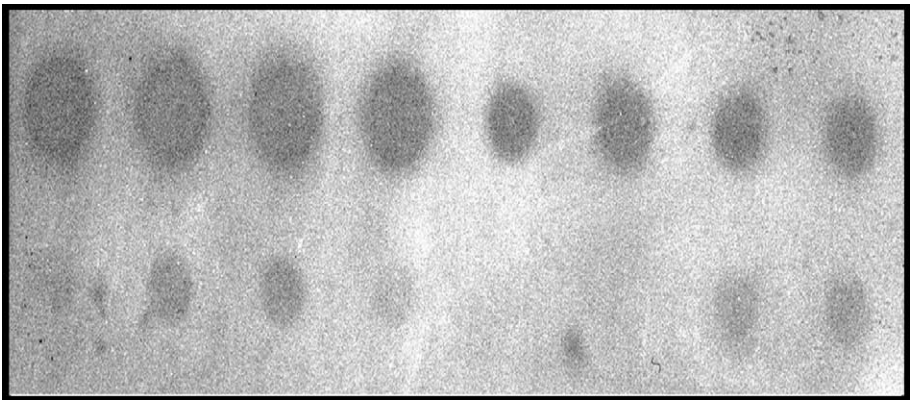

Fig. 1. Slot blot analysis.

software. Automatic allele designation was achieved by use of Genotyper v.2.1 software (Applied Biosystems) [9].

3. Results and discussion

STR analysis from human faeces is not routinely carried out. Faeces are a very complex mixture of biological and non-biological elements that can significantly inhibit PCR reaction and typing. We tested different DNA extraction protocols such as Chelex-100®, and phenol-chloroform but none of these gave satisfying results in PCR amplification even when a considerable amount of human DNA, confirmed by slot blot analysis, was obtained (Fig. 1). We decided to modify the single tube extraction protocol by an additional

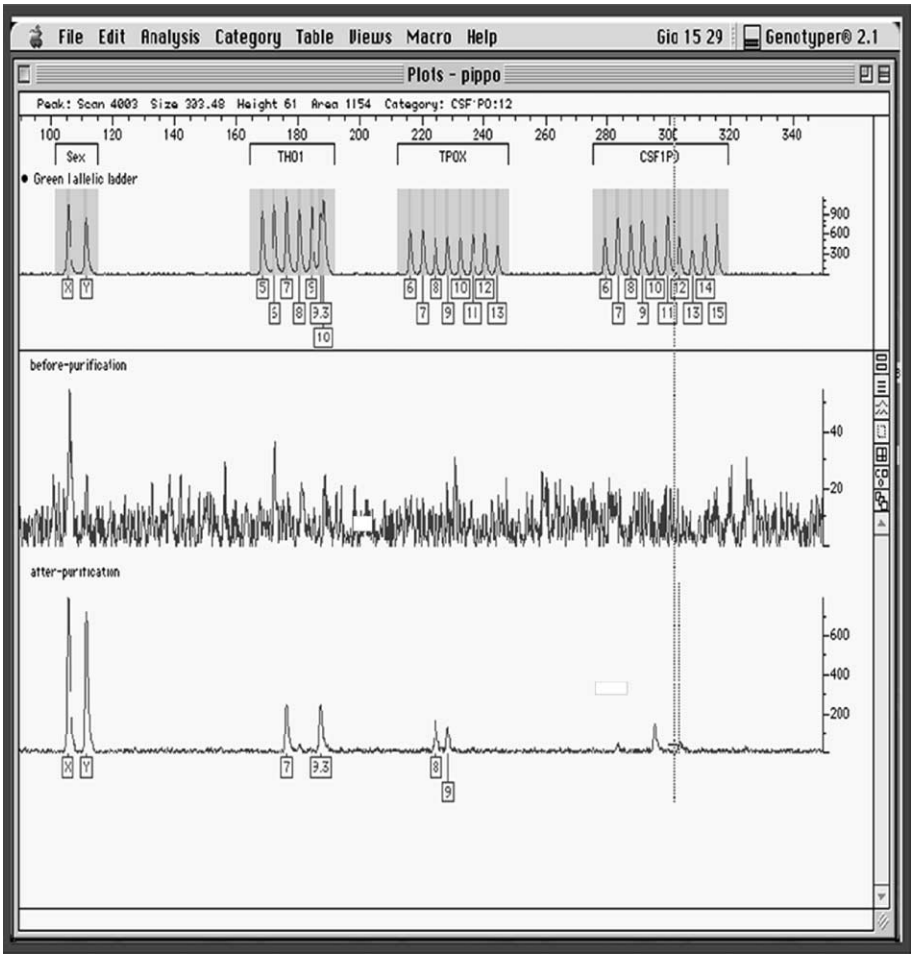

Fig. 2. STR analysis on ABI PRISM 377 DNA Sequencer. Lower lane shows typing after purifications.

Sephadex® column and a Microcon® 100 purification step. The Sephadex column is based on an exclusion chromatography technique that allows us to separate physically low molecular weight molecules from heavy ones. Microcon® 100 is a filter membrane used to purify or concentrate DNA/RNA preparations. The low absorption characteristic of the membrane allows us to recover up to 95% of the sample. These two combined steps enabled us to eliminate soluble inhibitors from the sample. STR analysis, carried out on an ABI 377, confirmed our idea by showing a full profile of the DNA sample (Fig. 2).We are confident that this methodology can be routinely applied in forensic cases where faeces are collected as specimens.

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