STR-TYPING OF NUCLEAR DNA FROM HUMAN FECAL MATTER USING THE QIAGEN QIAAMP® STOOL MINI KIT

Liane R. Martin
California State University, Los Angeles, Department of Criminal Justice, Los Angeles, CA

Human fecal matter is routinely encountered in forensic casework—identifying the source of this material could link a suspect or victim to a crime scene. Previous methods used to identify the source of the fecal matter have primarily involved microscopic comparisons. While these methods can be useful, two sources of fecal matter are required, and typically the analysis cannot individualize the sample. In addition, the results cannot be compared to that of other biological material.

An estimated 10 billion cells are shed from the epithelial lining of the human intestine daily; some of these cells survive the digestive processes to be excreted intact into the feces. The presence of these nucleated cells offers another approach to individualization—DNA analysis. Previous advances made in fecal typing have involved mitochondrial DNA, however this type of DNA analysis is not routinely performed in forensic laboratories. Attempts to type nuclear DNA have been problematic due to low DNA yields, and the presence of high amounts of bacteria and PCR amplification inhibitors. Recently, QIAGEN, a biotechnical company, developed a kit for the extraction of nuclear DNA from fecal matter using a relatively rapid, silica-based extraction procedure. Included in this kit are reagents, which reportedly remove the PCR inhibitors innate to fecal matter, and increase DNA yields by preferentially lysing the human cells over the bacterial cells.

In this study, nuclear DNA was extracted from human fecal matter using the QIAGEN QIAamp® Stool Mini Kit, and evaluated against a standard phenol-chloroform procedure. The QIAamp® kit resulted in varying DNA yields, whereas no DNA was obtained from the phenol-chloroform procedure. QIAGEN yields from fresh fecal samples ranged from 2.14 pg DNA/mg stool to 169.01 pg DNA/mg stool. The extracted DNA was then amplified and typed using the PE Applied Biosystems AmpF/STR Profiler Plus™ and COfiler™ kits, and the ABI PRISM® 310 Genetic Analyzer. The Profiler Plus™ and COfiler™ kits require a sample volume of 20 *µl* with the recommended range of input DNA of 14.0 - 2.5ng. The typical volume of the QIAGEN eluate was 200 *µl*. Some eluates contained low levels of DNA, and therefore required a concentration step; a Microcon® 100 step was added at the end of the QIAGEN extraction procedure. The initial typing results of concentrated (undiluted) DNA extracts demonstrated the presence of a PCR inhibitor; this inhibition was eliminated with the progressive dilution of the eluate. The high concentration of EDTA, 0.5mol, in the QIAGEN elution buffer was suspected to be the source of the inhibition; the substitution of 0.1 mmol EDTA/Tris for the QIAGEN elution buffer removed most, if not all, inhibition and resulted in overall successful STR-typing.

Using this modified extraction procedure, DNA was extracted, amplified, and STR-typed from stool samples subjected to various environmental conditions and sampling methods. The specimens were either processed immediately as fresh samples, or under the following environmental conditions: water immersion for two hours; air dried for one week; frozen for one week and processed with or without thawing. Two sampling methods were evaluated: swabbing versus excision. The results of this study showed that DNA can be successfully extracted from all test samples. With approximately 100-200 grams of feces eliminated daily, a theoretical yield of 3.0 $x10^5$ –6.0 $x10^5$ pg DNA/mg stool can be estimated. These values stand in sharp contrast to those values actually obtained. It is apparent that the cells and DNA are subjected to considerable amounts of digestion/degradation. Partial or complete STR-typing results were obtained from all samples extracted using the QIAGEN kit. There were no unexpected or inexplicable results with any of the samples. All alleles detected in the fecal samples matched that the subject's reference sample. No extraneous peaks were noted; therefore, there is no direct evidence of foreign DNA from plant or animal sources being typed. This study also demonstrated that the quantity and quality of DNA obtained not only varies between test conditions, but also within stool samples, between bowel movements, and among individuals. Therefore, it is difficult to evaluate the extent to which the test conditions affected the DNA obtained. However, samples dried for one week did consistently produce the lowest yields of DNA as well as displaying the highest degree of degradation. Furthermore, insufficient amounts of DNA may be obtained by sampling a stool using a single swab, so this sampling method is not recommended.

Importantly, this study demonstrates that successful STR-typing results are possible with this modified extraction procedure if the sample permits.