J Forensic Sci, Sept. 2002, Vol. 47, No. 5
Paper ID JFS2001263_475
Available online at: www.astm.org

TECHNICAL NOTE

Nicholas Vandenberg,[1] *and Roland A. H. van Oorschot,* [1] *Ph.D.*

Extraction of Human Nuclear DNA from Feces Samples Using the QIAamp DNA Stool Mini Kit

ABSTRACT: The use of a QIAamp DNA Stool Mini Kit (QIAGEN) for extracting human nuclear DNA from feces samples is reported. This method employs a stool lysis buffer and a unique matrix (InhibitEX tablet) to remove PCR inhibitory substances specific to feces samples. DNA extracted from various amounts of stool and from stool samples exposed to different environmental impacts was successfully amplified and typed using the Profiler Plus Amplification Kit and ABI PRISM 310 Genetic Analyser.

KEYWORDS: forensic science, DNA extraction, feces sample, genotyping, polymerase chain reaction inhibitors, environmental study

Fecal material is occasionally found in casework as smears on clothing or bedding, on items related to sexual assaults, or as a deposit at a crime scene. The ability to generate genetic profiles using DNA extracted from fecal material would be advantageous in a forensic context, and a quick and easy method for purifying nuclear DNA for use in PCR/genotyping is desirable.

However, the successful isolation and typing of human nuclear DNA from fecal material is often difficult to achieve due to: (a) the limited amount of human nuclear DNA found in feces, presumably derived from exfoliated epithelial cells, (b) a high background of microbial DNA, and (c) inhibitors of PCR such as complex polysaccharides, bilirubin, and bile salts, which co-extract with human DNA (1,2). In addition, it has been suggested that the nuclear DNA in feces may degrade rapidly over time, also as a result of the presence of bile acids (2).

Anecdotal evidence from casework samples analyzed in our laboratory, and our own tests on fecal material using routinely employed DNA extraction methods such as organic extraction or Chelex extraction (3–5), suggest that current extraction methods are inadequate for feces samples. A previous study has also reported poor results from adult fecal specimens using various DNA extraction methods, including the use of phenol-chloroform (1).

Our own previous investigations revealed that DNA quantitation results of feces samples extracted using an organic method only occasionally showed human DNA was present. Comparisons of different *Taq* polymerases (*Taq* Gold vs. AmpliTaq) (Applied Biosystems) indicated that the use of *Taq* Gold polymerase can increase the specificity of PCR assays in order to compensate for the low ratio of target human DNA to background bacterial DNA. However, we found that even when using *Taq* Gold, DNA extracted from feces samples using an organic method does not readily amplify. Further, a test for inhibition using a known amount of amplifiable template DNA and extract from an organic extraction of a feces sample confirmed the presence of inhibitory substances in the extract, as the DNA failed to amplify when the samples were mixed (6).

In the search for an extraction method that is better able to remove the inhibitors found in feces samples, and thereby provide reliable genotyping, we decided to test the QIAamp DNA Stool Mini Kit (QIAGEN). The QIAamp stool kit differs from other DNA extraction techniques in that it has been specifically designed for feces samples. The method utilizes a proprietary stool lysis buffer and a unique matrix (Inhibit EX tablet) to remove inhibitory substances specific to this type of sample. The DNA is then selectively bound to a silica gel membrane, in the presence of chaotrophic salts, and, after washing, elution of DNA takes place under low-salt conditions (7).

The aim of this study was to determine if various amounts of human feces, and human feces samples exposed to different environmental impacts, could provide sufficent human nuclear DNA for genotyping, subsequent to extraction of DNA using the QIAamp stool kit. Variation in results between individual people, for the same amounts of starting material, was also examined.

Materials and Methods

General

All stool samples were obtained from healthy adult subjects and used for research purposes only. Consent was obtained from participants in this study, and individual genetic profiles were not retained.

All samples were extracted using the QIAamp DNA Stool Mini Kit supplied by QIAGEN Australia (Cat. No. 51304), following the manufacturer's guidelines. Extracted DNA was concentrated using YMC-100 Microcon devices (Amicon). Selected samples extracted from known amounts of fecal material were quantitated using the Quantiblot method (Perkin Elmer) in order to determine the average amount and range of DNA present. To determine if a ge-

[1] Victoria Forensic Science Centre, Victoria Police, Macleod 3085,Victoria, Australia.

Received 1 Aug. 2001; and in revised form 14 Nov. 2001 and 18 Feb. 2002; accepted 18 Feb. 2002; published 17 July 2002.

netic profile could be generated, amplification of extracted DNA was performed using the AmpF/STR Profiler Plus Amplification Kit (Applied Biosystems) on a PE 9600 under standard conditions (8). A known percentage of the total extracted DNA for a given sample was used in each amplification. Amplified samples (1 mL) were combined with an internal size standard (ROX 400-HD) and resolved on an ABI PRISM 310 Genetic Analyser. The injection time for samples was 10 s. PCR fragment sizes were determined using GeneScan Analysis 3.1 software. Genescan analysis parameters were set with the peak amplitude threshold at 100 RFUs. Allelic assignment was made by reference to allelic ladders, employing Genotyper version 2.5 software.

Sample Amounts and Individual Variation

Samples of 50, 200, and 500 mg amounts of feces were taken from both the edges of a single fresh stool sample and then again from the same stool sample once it had been mixed with a spatula (i.e., inside region mixed with outside area). A 200 mg sample was also taken from the tail end (last section out) of the stool sample before it was mixed. Each sample was placed in a 2 mL Eppendorf tube. All the samples were analyzed on the same day as they were collected.

Differences between individuals were studied by determining the ability to generate a genetic profile using Profiler Plus from a standard sample of 200 mg of mixed feces material obtained from ten individuals of known sex and age. Samples were stored for a period of 1 to 3 weeks at–20°C prior to analysis.

Environmental Impact Study

A fresh stool sample was mixed and then 50 and 200 mg samples were prepared by weighing out the desired amount in a 2 mL Eppendorf tube. Smears (~2 × 5 cm) of 200 mg were also prepared on sterile cotton cloth and toilet paper (1 ply), using the same mixed stool sample, and placed into Petri dishes.

Immediately, 50 and 200 mg amounts, and a 200 mg smear on both cloth and paper, were placed in the freezer for storage at–20°C prior to extraction as controls. The remaining samples were left with their lids off under natural light at fluctuating room temperature (RT) during the months of September to November (~15 to 25°C). These samples were recovered after various intervals of time (see Table 2) and also placed in a freezer at–20°C prior to extraction.

The cloth and paper samples were extracted as half-size samples (~1 × 2.5 cm), cut into pieces, where all the pieces from one half were added to a single extraction tube, and the pieces of the other half added to a second tube. Each tube was subsequently extracted in full volumes of buffers/reagents. The extracted DNA from both tubes (i.e., each half of the same sample) was then pooled and concentrated using a Microcon device (YMC-100).

Results

Sample Amounts and Individual Variation

Table 1 shows that complete Profiler Plus genotypes were obtained from all samples when 90% of the DNA extract from a sample was used in a single Profiler Plus amplification. This was true both for the different volumes tested (50 to 500 mg) and for the various regions sampled (e.g., edges, mixed). However, when just 10% of the extracted DNA was used for amplification, the mixed samples of 50 and 200 mg amounts gave only partial profiles. The 200 mg sample from the tail gave a result comparable with that of a 200 mg sample taken from the edges of a stool sample.

Quantitation of DNA from a stool sample used to compare Profiler Plus typing results for different amounts of feces yielded 1 ng per 200 mg sample. By comparison, quantitation of DNA extracted from 200 mg amounts of feces from ten other individuals produced an average yield of 2.6 ng, with the lowest and highest amounts of DNA recovered from a single 200 mg sample being 0.6 and 8.0 ng, respectively. Complete Profiler Plus genotypes were obtained from 8 of the 200 mg samples from ten different individuals. The remaining two samples gave partial profiles.

Environmental Impact Study

Table 2 shows that successful Profiler Plus typing of feces samples were obtained across all the time intervals examined. Provided 90% or more of the extract was used in a single amplification, profiles were obtained from stool samples left exposed at RT for time intervals ranging from 1 to 91 days (see Table 2).

At each time interval, all of the neat 50 and 200 mg samples gave complete profiles. There was no significant reduction in peak heights over time for the 50 mg samples. The peak heights for the 200 mg samples were also not reduced over time. At each time interval the peak heights were approximately three-fold smaller for the 50 mg samples when compared to the peak heights of the 200 mg samples.

Of the seven stool samples smeared onto toilet paper, four gave negative results, one gave a partial profile, and only two gave a full profile. By comparison, of the seven cloth samples, all but one gave a full profile. The remaining sample gave a partial profile (see Table 2). Profiles generated from feces samples exhibited complete

TABLE 1—*Ability to generate a Profiler Plus genotype from different amounts and regions of a feces sample.*

Extraction		Profiler Plus Amp (% extract)	Profiler Plus Typing Result*
Amount, mg	Description		
50	Mixed	10	½
		90	F
	Edges	10	F
		90	F
200	Mixed	10	½
		90	F
	Edges	10	F
		90	F
	Tail	10	F
500	Mixed	10	F
	Edges	10	F

* F = Full profile, ½ = partial profile (four or more loci).

TABLE 2—*Ability to generate a Profiler Plus genotype from feces sample presented on different substrates and left at room temperature for increasing lengths of time.*

	Substrate*			
Time (days)	50 mg	200 mg	Paper	Cloth
0	F	F	_	F
1	F	F	_	½
4	F	F	_	F
7	F	F	_	F
21	F	F	½	F
42	F	F	F	F
63	F	F	F	F
91	F	F	–	–

* F = Full profile, ½ = Partial profile (4 or more loci), _ = negative, – = not tested.

concordance with those of standard reference material from the same individuals, and no stochastic effects were observed.

Discussion

Our preliminary results suggest that successful typing can be achieved by sampling from the edges of a stool sample. Ideally, 200 mg is the preferred sample amount to work with, with respect to the DNA isolation protocol provided with the QIAamp DNA stool kit. However, we have also shown that amounts ranging from 50 to 500 mg can be accommodated without requiring any changes to the extraction protocol.

For both the mixed and unmixed larger feces samples of 500 mg, it is possible to generate a genetic profile using only 10% of the total extract. This enables the greater portion of the sample extract to be set aside for further analysis/re-analysis. However, for smaller volumes of mixed feces samples (e.g., 50 mg, 200 mg) it may be necessary to use the whole sample extract to obtain a result by concentrating the extract down with a Microcon and using the entire volume in a single amplification.

DNA was successfully isolated from the ten individual feces samples of 200 mg; however, two samples gave only partial profiles. Feces samples from different individuals, and even from the same individual on different days, may fluctuate in the amount of bile salt they contain (9). As a result, PCR inhibition observed for these two samples may be due to higher concentrations of bile salts than in the other samples, though further analysis would be required to confirm this.

Results from our experiment with stool samples left in opened containers at RT showed complete Profiler Plus genotypes could be readily obtained from samples up to 91 days old when using the QIAamp stool kit to recover human DNA. Stool samples, however, smeared on varying substrates such as toilet paper or cotton cloth did not always give complete profiles. Table 2 indicates that for feces smeared on paper and cloth, there is no uniform reduction in the quality of the typings over time. (For example, 200 mg smears of feces on paper gave a negative result at 4 and 7 days, but full profiles were obtained at 42 and 63 days). This may be an effect of inhibitors in the sample itself, or of the substrate. In particular, there may be substances present in the paper that are co-extracted and inhibit amplification. Alternatively, the substrate may simply be physically inhibiting the extraction process. Though the amount used in the smears was the same as for the larger amount of neat sample, it is possible that, in part, the poorer results from smears are an effect of a greater surface area of the feces sample being exposed to the environment. However, feces smeared on cloth in the same manner as for paper gave much better results ($^{6}/_{7}$ full profiles vs. $^{2}/_{7}$ for paper).

It is known that untreated fecal homogenate will totally inhibit PCR due to the presence of co-purified excremental substances (9). PCR of DNA samples purified in the absence of any adsorption matrix to remove these substances is completely inhibited (2). Extraction of human nuclear DNA with the QIAamp stool kit involves the use of an inhibitEX tablet, unique to this method, as the absorption matrix. This may help in part to explain the success of this particular method, especially on neat stool samples, where the matrix acts to remove many of the inhibitors found in fecal material, like bile salts. In addition, the use of an effective lysis buffer, designed to enhance the selective binding of DNA to the silica gel-based membrane, also contributes to the success of this method (7).

Nuclear DNA suitable for genetic analysis has previously been recovered from fecal material of animals such as languars, brown bears, lions, and tigers (10–12). Microbial DNA and human mtDNA have also successfully been isolated from human feces (9,13,14). However, prior to the development of the QIAamp DNA stool kit, an easy and reliable method of extracting human nuclear DNA from feces has not been readily available. This preliminary study has shown that for differing amounts and regions of feces samples, for feces samples from a number of individuals, and for feces samples exposed to different environmental impacts, human nuclear DNA suitable for genotyping can be reliably extracted using the QIAamp DNA stool kit.

Acknowledgments

The authors would like to thank Nicole Holding for her assistance in acquiring some of the feces samples used in this study.

References

1. Monteiro L, Bonnemaison D, Vekris A, Petry KG, Bonnet J, et al. Complex polysaccharides as PCR inhibitors in feces. J Clin Microbiol 1997:995–8.
2. Deuter R, Pietsch S, Hertel S, Muller O. A method for preparation of fecal DNA suitable for PCR. Nucleic Acids Res 1995;23(18):3800–1.
3. Victoria Forensic Science Centre. The preparation of DNA for amplification by the polymerase chain reaction. VFSC internal methods manual 2000.
4. Hochmeister M, Budowle B, Borer U, Eggmann U, Comey C, et al. Typing of deoxyribonucleic acid (DNA) extracted from compact bone from human remains. J Forensic Sci 1991;36:1649–61.
5. Walsh P, Metzger D, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques 1991;10:506–13.
6. Surace D. Retrieval of nuclear DNA from human feces: Implications and techniques in forensic science [hons. thesis]. La Trobe University 1999.
7. QIAGEN. QIAamp DNA Stool Mini Kit handbook 2000:6.
8. PE Applied Biosystems. AmpFlSTR Profiler Plus PCR Amplification kit. Users manual 1997.
9. Lantz P-G, Matsson M, Wadstrom T, Radstrom P. Removal of PCR inhibitors from human fecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR. J Micro Methods 1997;28:159–67.
10. Launhardt K, Epplen C, Epplen J, Winkler P. Amplification of microsatellites adapted from human systems in fecal DNA of wild Hanuman langurs (Presbytis entellus). Electrophoresis 1998;19:1356–61.
11. Taberlet P, Camarra J-J, Griffin S, Uhres E, Hanotte O, et al. Noninvasive genetic tracking of the endangered Pyrenean brown bear population. Mol Ecol 1997;6:869–76.
12. Shankaranarayanan P, Singh L. A rapid and simplified procedure for isolating DNA from scat samples. Curr Sci 1998;75:883–4.
13. Clement B, Kitts C. Isolating PCR-quality DNA from human feces with a soil DNA kit. BioTechniques 2000;28:640–6.
14. Hopwood A, Mannucci A, Sullivan K. DNA typing from human feces. Int J Leg Med 1996;108:237–43.

Additional information and reprint requests:
Nicholas Vandenberg
Victoria Forensic Science Centre
Forensic Drive, Macleod 3085, Victoria, Australia
Tel: +613 9450 3444
Fax: +613 9459 0477