

# Benchmarks

## Isolating PCR-Quality DNA from Human Feces with a Soil DNA Kit

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Gastrointestinal (GI) microbial community dynamics influence host physiology and disease resistance. Assessing species presence and abundance over time is important for understanding GI community response to pathogens, diet and chronic disease (10). Recent advances have allowed researchers to examine the GI community using PCR-based methods (11,12,13). These and newer methods such as terminal restriction fragment patterns (TRFP or T-RFLP) (3,8) share a need for relatively clean DNA that reflects the community structure of the original sample.

Fecal samples are a convenient means of studying the GI community, but they present problems in terms of DNA solution quality. Direct addition of fecal suspensions will inhibit PCR (1), and researchers have addressed this problem by separating cells and other fecal debris by dilution and centrifugation (11,12,13). These techniques may eliminate cells attached to debris and bias any subsequent assay. To isolate community DNA without this bias, recent studies of different environments have used modified versions of the method of Boom et al. (2,3,7). Cell lysis (chemical and/or mechanical) is combined with protein denaturation and followed by purification of the DNA while bound to silica. Such methods are rapid and can produce DNA suitable for PCR directly from feces.

For our purposes, we desired a simple, commercially available kit for purifying microgram quantities of PCR-quality DNA from human fecal samples as part of a 135-sample TRFP study. We adapted the UltraClean Soil DNA Isolation Kit™ (Mo Bio Laboratories, Solana Beach, CA, USA) for use with feces. The kit proceeds like Boom method derivatives with a notable exception: DNA purification and recovery is performed using a matrix immobilized on a small filter in a 2 mL centrifuge tube. These tubes are compatible with microcentrifuges for rapid, thorough washing and

**Table 1. Normal Yield Protocol for Isolating DNA from Human Feces**

Exceptions to the manufacturer's protocol are underlined.

1. Add 90–130 mg feces to a pre-weighed lysis tube containing only beads. (Dry lysis tubes allow the adjustment of sample weight without dilution.)
2. Add 500  $\mu$ L "Bead Solution" and 60  $\mu$ L solution "S1", process for 10 at 4 m/s on a FastPrep™ instrument (Bio 101, Vista, CA, USA) (lyses cells and denatures proteins).
3. Centrifuge for 5 min at 10 000 $\times$  *g* and transfer 450  $\mu$ L supernatant to a clean tube.
4. Add 250  $\mu$ L solution "S2", mix thoroughly and incubate at 4°C for 5 min.
5. Centrifuge for 1 min at 10 000 $\times$  *g* and transfer 450  $\mu$ L supernatant to a clean tube (precipitates and pellets protein and other debris).
6. Add 900  $\mu$ L solution "S3", mix thoroughly and transfer 675  $\mu$ L into a "spin filter" tube (selectively binds DNA to the matrix immobilized on the filter).
7. Centrifuge the spin filter for 1 min at 10 000 $\times$  *g* and discard eluate.
8. Repeat steps 6 and 7, then centrifuge for 30 s at 10 000 $\times$  *g* to dry the filter and discard any eluate. (The second wash step significantly clarifies the final DNA solution.)
9. Add 300  $\mu$ L solution "S4" to the filter, centrifuge for 30 s at 10 000 $\times$  *g*, discard eluate and repeat once (washes matrix-bound DNA to remove salts and other soluble compounds).
10. Centrifuge for 30 s at 10 000 $\times$  *g* to dry the filter (assures that ethanol/salt wash solutions will not contaminate final DNA solution).
11. Transfer filter unit to a clean tube and add 50  $\mu$ L solution "S5" directly onto the matrix.
12. Centrifuge for 30 s at 10 000 $\times$  *g* to elute DNA solution, remove filter unit and store as desired.

drying during DNA purification.

As part of our ongoing study, we determined maximum yield, average yield and efficiency of the DNA isolation kit. We also created TRFPs with spiked samples to determine the quantitative potential of the DNA isolation and subsequent PCR. Fecal samples were collected by Leatherhead (London, England, UK) and kept frozen at -80°C. Two sample sets were used, maximum yield and normal yield. The maximum yield set consisted of four DNA isolation replicates from one sample and was used to determine efficiency and maximum possible DNA yield. The normal yield set consisted of all 135 fecal samples from which we determined PCR success and DNA yield for the normal protocol.

To isolate DNA, we adjusted the manufacturer's standard protocol (Table 1). For the maximum yield samples, the entire supernatant was recovered after lysis, and subsequent reagent vol-

umes were adjusted accordingly. DNA was recovered from all of the maximum yield samples and 134 of the 135 normal yield samples. The protocol was completed on average in approximately 2 h with 24 samples and produced DNAs approximately 12 kbp in length (Figure 1).

For maximum DNA yields, a second protocol was used. The entire supernatant was recovered after step 3 (Table 1), its volume estimated visually and the other volumes adjusted accordingly. As determined by PicoGreen® analysis (manufacturer's protocol; Molecular Probes, Eugene, OR, USA), maximum yield samples were between 64.2 and 85.5  $\mu$ g/g. We elected to use the normal protocol because it was faster and produced more than enough DNA for our study.

DNA concentrations for the normal yield samples were between 1.0 and 37.0  $\mu$ g/g as determined by UV spectrophotometry. The differences in DNA

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content related to bacterial load were likely confounded by variation in sample water content. However, handling the samples “wet” allowed for rapid aliquoting and simple safety procedures.

To determine DNA recovery efficiency, direct epifluorescence microscopic counts were performed on sample 43A (the maximum yield sample source) following Kepner and Pratt (6). The extraction efficiency was greater than 100% (Table 2). However, the cellular DNA content estimate (5) that was used to calculate maximum possible yield ignores plasmid, viral and extracellular DNA as well as eukaryotic cells and pre-fission bacterial cells containing more than one genome. Also, debris and cellular aggregates, which can contribute to an underestimate of total cells (6), were observed on the fecal direct count slides.

To determine DNA solution quality, we performed PCRs using the normal yield samples. The PCR targeted an approximate 500 bp region of the 16S rRNA gene and was carried out in 50  $\mu$ L using 10, 50 or 500 ng of template DNA.

**Table 2. DNA Yields, Direct Count Results and DNA Recovery Efficiency**

Value	Mean (g feces <sup>-1</sup> )	Standard Deviation	Replicates
Normal Yield Protocol	13.9 $\mu$ g DNA	8.19	132 <sup>b</sup>
Maximum Yield Protocol	75.5 $\mu$ g DNA	9.64	4
Fecal Bacteria Direct Count	$1.81 \times 10^{10}$ cells	$8.39 \times 10^9$	3
Efficiency <sup>a</sup>	107%	13.7%	

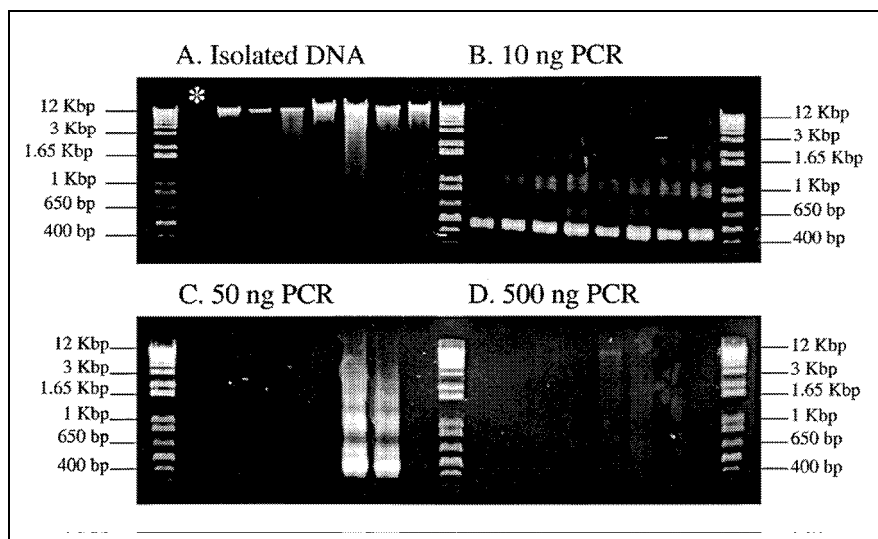
<sup>a</sup>Efficiency is the observed yield expressed as a percentage of expected DNA yield (70.4  $\mu$ g/g feces). Expected yield was the product of the mean fecal cell count and the mean prokaryotic cellular DNA content,  $3.89 \times 10^{-15}$ /g cell (3.6 Mbp/cell [5] multiplied by 650 g bp/mol). Standard deviation was estimated by using the standard deviations for yield and direct count.

<sup>b</sup>Out of the 135 samples, two sample weights were not recorded and one sample did not produce DNA.

All other PCR conditions were as previously described (3) except for the fluorescently labeled forward primer (Ba2f 5'-GCY TAA CAC ATG CAA GTC GA-3') and the 46.5°C annealing temperature. Reaction success was determined by agarose gel electrophoresis.

Previously, we determined that reliable community estimates could be made with 1 ng DNA per reaction in the PCR (data not shown), and our standard PCR for the TRFP method contains 10

ng DNA per reaction. All 134 normal yield samples were successfully amplified at this concentration without additional treatments. To test solution quality at higher DNA concentrations, eight normal yield samples were selected and used as PCR templates at 50 and 500 ng per reaction. At 50 ng per reaction, two samples amplified as expected. All eight samples failed to amplify at 500 ng per reaction (Figure 1). These data indicate that some inhibitors are still



**Figure 1. Electrophoresis of isolated DNA and subsequent PCR products (35 cycles).** All panels are (left to right) samples 9A, 14A, 47A, 35B, 7C, 48C, 51C and 62C. (A) Isolated DNA. (B, C and D) PCR products using 10, 50 and 500 ng, respectively, of template DNA. All samples (5  $\mu$ L) were analyzed with 1.5% agarose gels containing 0.5  $\mu$ g/mL ethidium bromide. An approximate 12 kbp band from sample 9A was visible to the naked eye, but did not photograph well.

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carried over to the final solution, but that most PCR analyses can proceed. Studies requiring more template DNA for PCR could incorporate additional washes to further purify the sample DNA (step 6; Table 1). Alternatively, the manufacturer is now marketing an inhibitor removal solution with the kit, which may increase purity.

Another concern in any community analysis is proportional cell lysis and DNA recovery. Ideally, the abundance of a particular organism's DNA in the final solution will match that in the original sample. However, complex samples with free and attached cells at different levels of structural integrity present a challenge to even mechanical lysis methods. Furthermore, PCR is thought to skew product abundance relative to template abundance in multi-template reactions (i.e., community analyses) (4,9). Thus, many researchers analyze community data on a presence/absence basis. For our purposes, estimating abundance after PCR is of interest, and our data indicate that, with this protocol, abundance in the original sample may be calculated after DNA isolation and PCR. Briefly, we performed a spike experiment in which known quantities (by direct count) of the Gram positive bacterium *Lactobacillus acidophilus* were added to

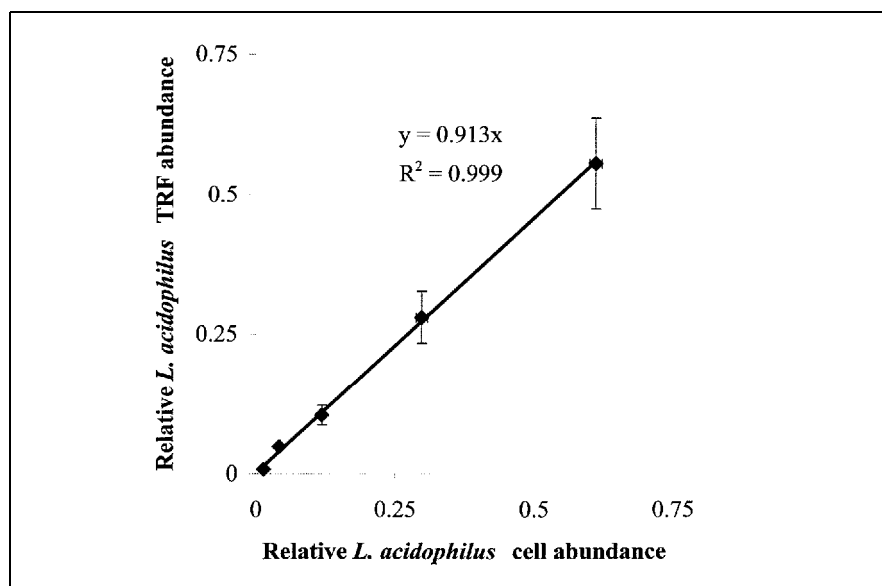
aliquots of sample 43A. We performed TRFP analysis on the spiked samples and determined the relative abundance of *L. acidophilus* DNA fragments. When compared to abundance in the original sample, a strong linear relationship close to 1:1 ( $R^2 = 0.999$ , slope = 0.91; Figure 2) was observed. The addition of *L. acidophilus* did not obscure other community members from analysis at any level up to approximately 60%, and its abundance could be assessed at about 1% of the total cells. While this is not conclusive proof of proportional lysis and DNA recovery, it indicates that quantitative analysis of difficult-to-lyse (i.e., Gram positive) cell types is possible using this method.

In summary, the UltraClean Soil DNA Kit produces PCR-quality DNA from human feces quickly and efficiently and appears to maintain original species abundance in the final DNA solution. Small modifications for fecal samples (dry lysis tubes and a second DNA wash step) were easy to incorporate. When optimized to recover the maximum DNA possible, the yield of this method was at or near 100%. When optimized for speed and convenience, the method recovered enough DNA for 100–3700 PCR analyses from each sample. PCR was 100% successful using 10 ng DNA, and the resulting prod-

ucts reflected the initial abundance of cells in each sample. This DNA isolation method, when paired with the PCR, can serve as a foundation for cloning, temperature/denaturing gradient gel electrophoresis (T/DGGE), TRFP or other studies of the human fecal microbial community.

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**Figure 2. Post-PCR abundance correlates with cell abundance.** Observed *L. acidophilus* TRF abundance correlated with *L. acidophilus* cell abundance in spiked fecal samples before DNA isolation. Each data point is the mean of four replicates. X-axis error bars indicate direct count variation; Y-axis error bars indicate TRF area variation. Error bars are  $\pm 1$  standard deviation.

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