High Throughput Solution for DNA Extraction from Stool Samples Using Magnetic Beads

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Introduction

Stool samples are often preferred clinical specimens for detection of multiple disease-causing microbes, biomarker detection of cancers of the digestive tract to analysis of gut microbiome and their impact on physiological health and disease. The sample collection is relatively easy, non-invasive, and amenable for large sample sizes. Stool, however, is a complex sample source due to the presence of polyphenols, humic acid, lipids, and other PCR-inhibiting compounds. With advances in molecular diagnostics and next-generation sequencing-based analyses, there is a need for extracting high quality DNA from stool free of PCR inhibitors in an automated, high throughput format. Omega Bio-tek's Mag-BIND® Stool DNA 96 Kit (M4016) addresses this need and is designed for rapid and reliable isolation of high quality host, as well as pathogenic genomic DNA, from frozen, fresh, or preserved stool samples. It follows a magnetic bead-based approach for purification and the salient features of this kit are presence of 1) 96-well disruptor plate pre-filled with glass beads for effective homogenization of the sample and to facilitate lysis of gram-negative, gram-positive, and other tough-to-lyse microorganisms and 2) uniquely formulated cHTR reagent for eliminating PCR-inhibiting compounds. In this application note, we provide the high throughput extraction solution on Thermo Fisher Scientific's KingFisher™ Flex purification system. We also discuss the performance of the kit in terms of DNA yield, quality, and amplification potential using real-time PCR.

Materials & Methods

Fresh stool was collected from healthy donors and was diluted 10% w/v in PBS. The sample was mixed thoroughly. DNA from 250 µL of this stool sample was extracted using Omega Bio-tek's Mag-BIND® Stool DNA 96 Kit on Thermo Fisher Scientific's KingFisher™ Flex purification system equipped with a 96-well magnetic head. The homogenization, lysis and binding steps were performed offline. Homogenization using the Disruptor Plate can be performed on a standard vortex mixer with a plate adaptor, but we recommend using commercial mixer mills, such as SPEX CertiPrep Geno/Grinder® 2010 or Omni's Bead Ruptor 96 for optimum yields. The subsequent wash and elution steps were automated on the KingFisher™ Flex. DNA was extracted from 8 independent samples and eluted in 100 µL volume. Using this workflow, ninety-six 250 µL samples can be isolated in ~70 minutes. DNA yield was determined using both Thermo Scientific's NanoDrop® 2000c and Promega's QuantiFluor® dsDNA systems on 10X and 100X dilutions of the purified DNA. Briefly, a qPCR reaction was set up to a total volume of 20 µL using Agilent's Brilliant III 2X SYBR® as the master mix and 2 µL of purified DNA at appropriate dilution as template with suitable primers following a standard amplification protocol on the ABI 7900.

Table 1. DNA was extracted from 250 µL of stool samples and eluted in 100 µL volume. The DNA yield was determined using NanoDrop® and PicoGreen® quantification methods.

Sample ID	DNA Yield in µg (NanoDrop®)	A_{260}/A_{280}	DNA Yield in µg (PicoGreen®)
1	3.61	1.87	3.20
2	3.71	1.87	4.20
3	2.73	1.90	2.00
4	3.03	1.83	2.70
5	1.76	1.83	1.90
6	3.53	1.81	3.70
7	3.60	1.86	3.30
8	1.76	1.83	2.00

Results & Discussion

The purified DNA yield from the stool samples determined using both the quantification techniques were as shown in Table 1. The DNA yield from the NanoDrop® correlated well with the PicoGreen® measurements suggesting intact dsDNA with little interference from RNA, ssDNA, and degraded DNA. The A260/A280 ratios were all close to 1.8 suggesting high quality (Figure 1).

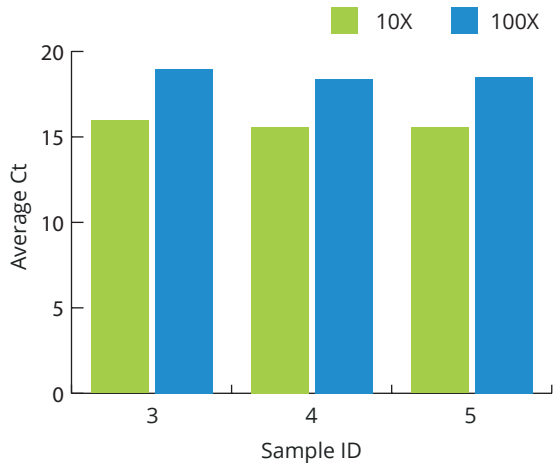

Figure 1. Average C_t values obtained using 16S bacterial specific primers on 10X and 100X diluted purified DNA as the template. Sample IDs 3, 4 and 5 are represented here.

The suitability of the purified DNA for downstream applications were determined by running a qPCR reaction. Figure 1 shows the average C_t values obtained on 10X and 100X dilutions of the purified DNA using 16S bacterial specifics primers. There was no detectable fluorescence in the no template control wells. The C_t values in Figure 1 indicate positive amplification at both the dilutions with an average ΔC_t value around ~3 indicating good PCR efficiency.

Conclusions

Omega Bio-tek's Mag-BIND® Stool DNA 96 Kit (M4016) offers an attractive high throughput solution for isolation of genomic host and pathogenic DNA from stool samples. Purified DNA is inhibitor-free and is suitable for various downstream applications such as PCR, restriction digestion, and NGS. The extraction system allows for automation after sample lysis via Hamilton Microlab® STAR™, Thermo KingFisher® Flex™, Applied Biosystems MagMAX™ 96, Qiagen BioSprint® 96, and other open-ended liquid handling instruments.

Product Information

Description	Product No.	Preps
Mag-BIND® Stool DNA 96 Kit	M4016-00	1 x 96
	M4016-01	4 x 96

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