Comparison of Stool DNA Isolation Methods for Bacterial and Mammalian DNA Detection

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INTRODUCTION

Stool is considered an excellent sample source for the diagnosis of gastro-intestinal infections, and it is also a non-invasive sample source for the isolation and genetic testing of human genomic DNA. Despite the current battery of advanced and powerful molecular tools, the isolation of genetic materials from stool samples for medical and research purposes remains difficult. This is largely due to the PCR-inhibiting organic compounds that are difficult to separate from the genetic information of interest (Pontiroli et al., 2011, Braun and Methner, 2011, Gioffre et al, 2004).

In this application note, we compare two commercially available kits designed for the isolation of stool DNA – Norgen's Stool Nucleic Acid Isolation Kit and Competitor's DNA Stool Mini Kit. These kits were compared on the basis of DNA quality, DNA quantity and ease of use. To assess DNA quantity, samples were analyzed using gel electrophoresis as well as spectrophotometry. To assess DNA quality, samples were compared based on their A260/280 and A260/230 values, as well as their PCR performance at various sample input volumes.

METHODS AND MATERIALS

Stool Collection and Distribution

A single stool sample was collected from a healthy individual and homogenized by mixing with a spatula. The sample was then divided into four 250 mg samples, two of which were processed using Norgen's kit while the other two were processed using Competitor's kit. Both kits were used according to the manufacturer's protocols. A flowchart of Norgen's protocol is depicted in **Figure 1**.

DNA Gel Electrophoresis

The purified DNA was run on a 1X TAE 1.0% agarose gel containing ethidium bromide (5 µ L per 100 mL gel) for visual inspection. It should be noted that approximately 10% of eluted DNA was run on the gel for Norgen samples and 5% of eluted DNA was run on the gel for Competitor samples.

qPCR Assay

The eluted stool DNA was then used as the template for a two-step qPCR reaction involving primers specific for 16SrDNA (Bacterial DNA; detected using SYBR Green), 18S rDNA (Human; SYBR Green), and GAPDH (Human; TaqMan®) genes. Template inputs used in the reactions were 2 µL of a 1/10 diluted sample (0.2 µL), 2 µL, 4 µL, 6 µL and 8 µ L. SYBR Green reactions consisted of 20 µ L containing 2 µ L primer mix, 0.1 µ L 50x probe, 10 µ L Norgen's commercial 2x master mix, and water. TaqMan® reactions were 20 µL in volume, and contained: 0.4 µL primer mix, 0.2 µL probe, 10 µL Norgen's commercial 2x master mix, and water.

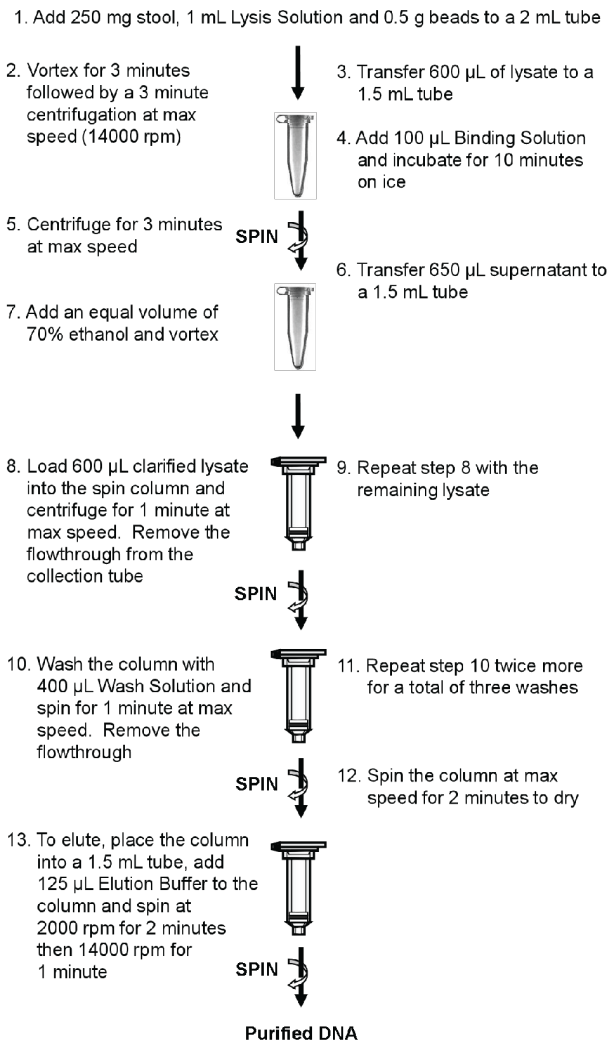

Figure 1. Flowchart for the Purification of Stool DNA using Norgen's Stool Nucleic Acid Isolation kit.

RESULTS AND DISCUSSION

DNA Quantity

DNA quantity was assessed using both gel electrophoresis **(Figure 2)** and spectrophotometry **(Table 1)**. It should be noted that approximately 10% of the total elution volume was run on the gel for Norgen samples and 5% of the total elution volume was run on gel for Competitor samples. Even when one takes this into consideration, it is still evident from Figure 2 that the Norgen samples contain a greater concentration of DNA than the Competitor samples. The identity of the heavily stained low molecular weight material present in the Norgen samples has been shown to be RNA in other experiments (data not shown).

DNA Quality

DNA quality was assessed both spectrophotometrically **(Figure 3)** and by PCR **(Table 2 and Figure 4)**. Spectrophotometry provides scientists with excellent data for evaluating the purity of a DNA sample. Two absorbance ratios, A260/A280 and A260/A230, provide information on the amount of contaminating protein and organic compounds respectively that are present in a sample. Samples with an A260/A280 outside of the ideal range of 1.8-2.0 have a significant amount of protein contaminating their sample and may have issues with PCR amplification. Samples with a low A260/230 (below 2.0) have a significant amount of organic contaminants that will also interfere with downstream processes, including PCR. In Figure 3 the superior quality of Norgen's DNA samples is evident. The Norgen samples have an ideal A260/280 and A260/230 ratios (both around 2.0) whereas Competitor's samples fall well short of both of these values. Based on Competitor's low ratios, one would expect PCR inhibition as well as inhibition of other downstream applications. To verify the spectrophotometric results concerning sample purity, a series of qPCR reactions were carried out for three different target genes.

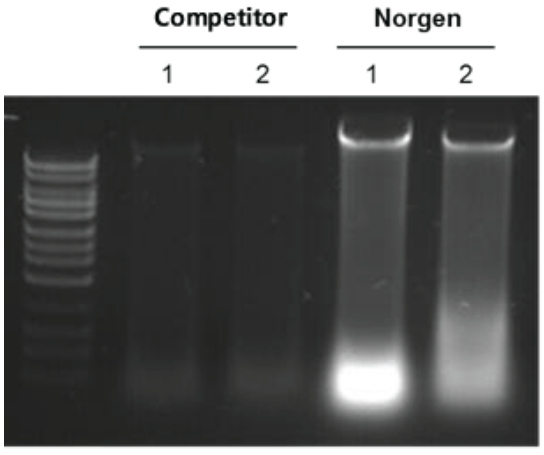

Figure 2. DNA quantification by gel electrophoresis on a 1.0% agarose gel. Ten percent of the eluted volume was loaded for Norgen samples and 5% of eluted volume was loaded for Competitor samples.

The first target gene tested was 16S rDNA and was chosen to test for successful isolation of prokaryotic DNA (for purposes of pathogen detection). Norgen's kit was able to detect the target gene with an excellent Ct up to 8 µL of input volume **(Figure 4A)**. The Competitor kit was only able to detect the target gene when 0.2 µL of template was used in the reaction, and Competitor's Ct values were still approximately 6 cycles higher than Norgen's sample at this input volume **(Table 2)**.

Kit	Concentration (ng/uL)	
	Individual	Average
Norgen	562.0 482.5	522.25
Competitor	25.5 36.0	30.75

Table 1. Spectrophotometric quantitation of samples prepared using Norgen and Competitor kits.

The second target gene tested was 18S rDNA, which was chosen to test for successful isolation of human DNA. Once again, Norgen had no issues detecting the target, achieving a low Ct value (22.1) even when inputting 8 µL of sample (3.9 µg DNA) into the reaction **(Table 2)**. Again, the gene was only detectable in the Competitor's samples when 0.2 µL template was used and the Ct value was much higher than the Norgen's sample of the same input, indicating a high concentration of inhibitors **(Figure 4B)**.

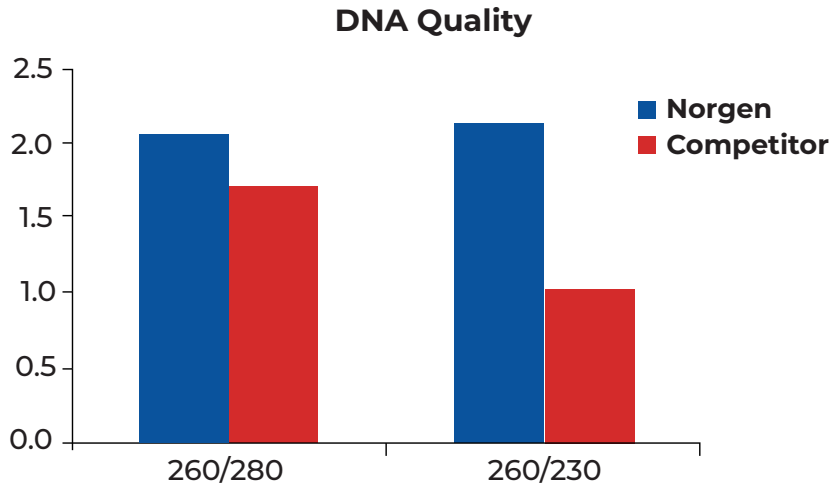

Figure 3. Comparison of DNA quality as assessed by spectrophotometric ratios. DNA purity was determined by analyzing 3 µL of each sample using a NanoVue spectrophotometer. Values shown are averages calculated for duplicate samples.

The last target gene tested was the gene coding for GAPDH. It was important to test for this low copy number gene because its detection often requires a large input of DNA. Similarly to the previous two target genes, Norgen's samples exhibited PCR amplification and had Ct values lower than the negative control at all input volumes except 0.2 µL **(Figure 4C)**. It should be noted that the gene was also not detected in the Competitor sample at this 0.2 µL input volume. The GAPDH gene was detected in both the Norgen and Competitor sample at an input volume of 2 µL, however detection occurred in the Norgen sample 0.5 cycles earlier than in the Competitor sample **(Table 2)**.

Collectively, the Ct data in Table 2 suggests that Norgen samples are much lower in PCR-inhibiting organic compounds than Competitor samples. This is based on the observation that when comparing Ct values of Norgen samples and Competitor samples of the same input, Norgen samples consistently have a lower Ct value, and therefore perform better in PCRs. This is important for the purposes of detecting low copy number genes (such as the GAPDH gene) because it allows for the maximum amount of DNA to be used in the reaction without PCR inhibition.

PCR input (µL)	Template Amount (ng) in PCR reaction		16S rDNA (SYBR Green)		18S rDNA (SYBR Green)		GAPDH (TaqMan)	
	Competitor	**Norgen**	Competitor	**Norgen**	Competitor	**Norgen**	Competitor	**Norgen**
0.2	3.6	**48.25**	23.4	**17.7**	28.8	**23.3**	39.1	**N/A**
2.0	72	**965**	29.9	**16.0**	34.7	**21.7**	37.2	**36.7**
4.0	144	**1930**	N/A	**16.5**	36.9	**22.0**	42.9	**35.3**
6.0	216	**2895**	N/A	**18.3**	39.9	**21.8**	N/A	**35.2**
8.0	288	**3860**	N/A	**22.9**	N/A	**22.1**	N/A	**34.9**
NTC	0	**0**	30.5	**29.2**	29.8	**29.7**	N/A	**39.4**

Table 2. Comparison of bacterial and mammalian DNA detection by qPCR analysis of human stool DNA samples obtained from Norgen's Stool Nucleic Acid Isolation Kit and Competitor's DNA Stool Mini Kit.

Ease of Use

It was found that while both kits were simple enough in terms of their protocols, Norgen's kit took approximately 30 minutes to extract DNA, whereas Competitor's kit took slightly longer (around 40 minutes).

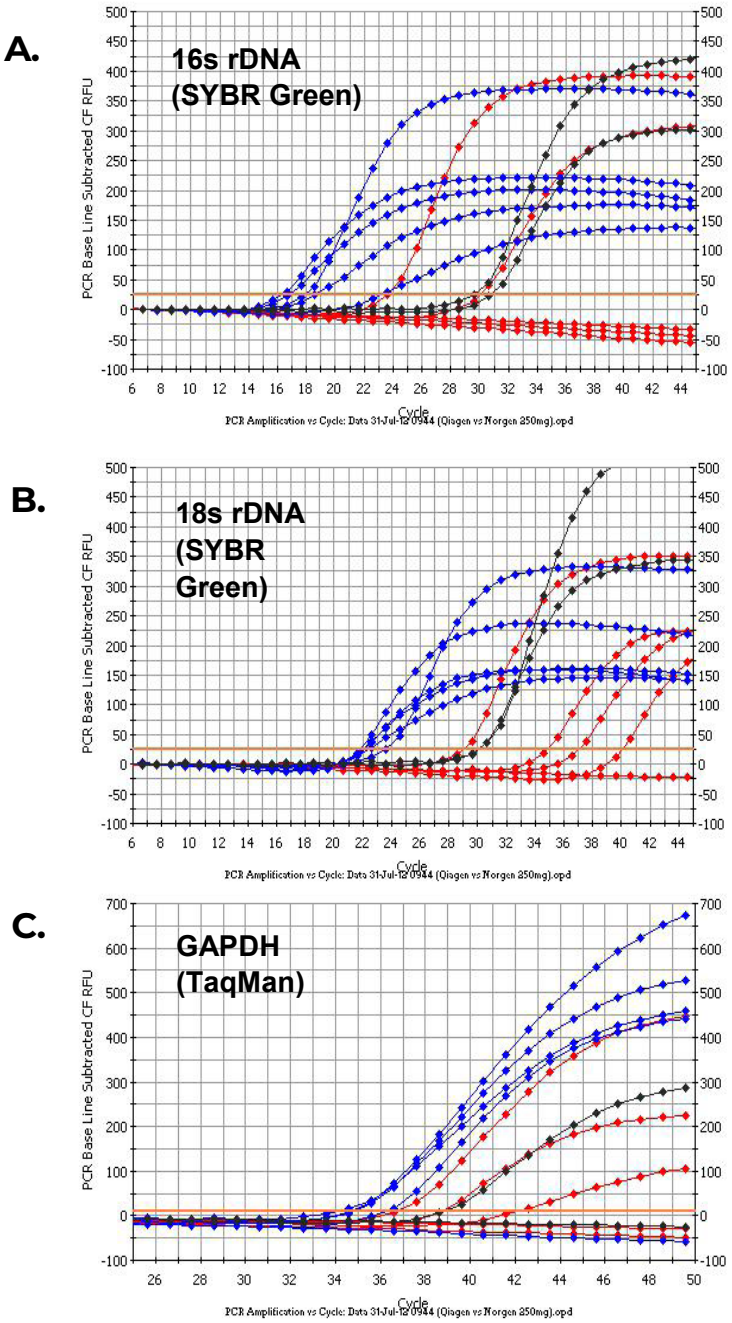

Figure 4. Detection of bacterial and human genes from stool using qPCR. DNA was isolated from 250 mg samples of stool using Norgen's Stool Nucleic Acid Isolation kit (Blue) and the Stool DNA kit (Red). Three different genes were targeted to assess sample purity: 16S rDNA (A), 18S rDNA (B) and GAPDH (C).

CONCLUSIONS

Based on the data presented here (and other data not shown), it can be concluded that:

1. Norgen's kit isolates a greater quantity of DNA, a result supported by gel electrophoresis and spectrophotometry.
2. Norgen's kit isolates DNA of a higher quality, efficiently separating the PCR inhibiting organic compounds from the genetic material of interest. Furthermore, Norgen's kit isolates DNA that is pure enough for use in large enough volumes to detect low copy number genes.
3. While both kits are customer-friendly, Norgen's kit has a shorter processing time, allowing researchers to process more samples in a shorter period of time.

Overall, it is clear that based on the parameters tested – DNA quantity, DNA quality and ease of kit use – Norgen's Stool Nucleic Acid Isolation Kit is superior to Competitor's DNA Stool Mini Kit.

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