Nucleic acid isolation

Fecal microbiome diversity: family, diet, probiotics, and pregnancy as associated factors

Summary

- The Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit compares favorably to kits from other suppliers
- The MagMAX Microbiome Ultra Nucleic Acid Isolation Kit enables effective isolation of high-quality nucleic acid from human feces
- The isolated nucleic acid can be used to analyze aspects of the gut microbiome including:
  - Microbial community diversity among members of a family
  - Changes associated with diet
  - Changes associated with probiotic usage
  - Changes associated with pregnancy

Introduction

The human gastrointestinal tract contains the most diverse microbial community in the body. Factors that can affect the composition of the gut microbiota include the external environment, genetics, lifestyle, nutrition, health status, and age. Although gut microbiomes differ in diversity across individuals, family members living in the same household are often observed to have more similarities between their microbiota than unrelated individuals [1]. Familial similarities are usually attributed to shared environmental influences such as dietary preference, a powerful shaper of microbiome composition [2,3]. There is growing evidence that close social relationships correlate with the composition of the human gut microbiota [4]. Furthermore, the gut microbiome is impacted by diet [3] and the use of probiotics [5]. Pregnancy has also been shown to affect the microbiota of both mother and child in diverse ways. The microbiota governs the infant's immune, allergic, and metabolic responses—even into adulthood. Delivery method, exposure to antibiotics, and breastfeeding have a significant impact on the gut microbiome and on the overall health of the infant [6]. Here we investigate four case studies that focus on different factors affecting the composition of the gut microbiome.

The use of a consistent methodology is an important component in researching the role of gut microbiota in health and disease. Reliable and reproducible extraction of nucleic acids from the gut microbiome is essential to avoid the introduction of biases during sample preparation. Here we report on the utility of the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit for studying the gut microbiome using total nucleic acids extracted from fecal samples. Microbial diversity was evaluated by 16S rRNA sequencing using the Ion GeneStudio™ S5 System. We highlight the use of the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit in the effective analysis of the microbial community from a single family (father, mother, and son); the microbial community changes associated with diet and probiotics usage; and the distinct changes in microbial profiles that occur throughout pregnancy and the postpartum period. Furthermore, we demonstrate that various workflow options of the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit show equivalent performance when compared to other similar kits available on the current market.

Materials and methods

Total nucleic acid extraction using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit

Human fecal samples were obtained from donors, and isolations of total nucleic acid were performed in duplicate using the standard protocol for the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. For benchmarking experiments, fecal samples were collected from two donors and processed using various workflow options from the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit with the KingFisher Flex Purification System. These options included bead-beating in tubes for 10 minutes, plate bead-beating for 2 minutes, plate bead-beating for 20 minutes, and tube bead-beating for 10 minutes and performing a manual nucleic acid extraction. Similar kits from QIAGEN included spin column and magnetic bead–based options (DNeasy™ PowerSoil™ Kit and MagAttract™ PowerSoil™ DNA Kit, respectively), and isolations were performed following each kit's standard protocol. For evaluating the microbial profiles of individual family members, fecal samples were collected from a father, mother, and son living in the same household but following different diets.

Probiotics case study: Fecal samples were collected from an individual 1 week before probiotic usage to create a baseline. Additional fecal samples were collected every week during probiotic usage for 8 weeks and at week 12, which marked the end of probiotic consumption.

Diet case study: Samples were collected from an individual before following the diet schedule to create a baseline and at 16 weeks after following the vegetarian dietary changes recommended by a nutritionist.

Pregnancy and postpartum case study: Samples were collected from the pregnant donor at 5 timepoints: at 23 weeks gestation, at 33 weeks gestation, at 37 weeks gestation, at 3 weeks postpartum, and at 8 weeks postpartum.

An input of 100 mg of feces in 400 µL of lysis buffer was used for all extractions, for a total extraction volume of 400 µL. Extractions were done in duplicate, and the final samples were eluted in 200 µL of elution solution. Total nucleic acid was isolated in an automated fashion using the Thermo Scientific™ KingFisher™ Flex Magnetic Particle Processor with 96 Deep-well Head. 16S rRNA sequencing was performed on total nucleic acid using the Ion GeneStudio S5 System as shown in Figure 1. The RStudio™ program was used to generate heat maps.

Results

Comparison of other kits to the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit using different workflow options

The MagMAX Microbiome Ultra Nucleic Acid Isolation Kit can be used to efficiently isolate total nucleic acid from human fecal samples using its various recommended workflows. We compared the microbiome profiles generated from total nucleic acid isolated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit with other kits available on the market (Figure 2). Although the total microbiome profiles remained consistent across all extraction types, there was a greater relative percent abundance of microbial diversity detected by workflows from the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit compared to the spin-column and magnetic bead–based kits (DNeasy PowerSoil Kit and MagAttract PowerSoil DNA Kit, respectively) from QIAGEN. For instance, a whole metagenome profile showed a greater abundance of *Prevotellaceae* in donor 1 using any of the four workflows tested for the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. The spin-column extraction using the DNeasy PowerSoil Kit showed relatively low abundance for *Prevotallaceae,* while there was no detection of this family with the MagAttract PowerSoil DNA Kit. *Bacteroidaceae* was predominantly higher in donor 2 with very little to no detection of *Prevotellaceae* using any of the compared extraction workflows, suggesting that there is variation between donors for this family of bacteria.

Comparison of the microbiome profiles between family members: a case study

Fecal samples were collected from a single family living in one household in which individuals had different dietary habits. Total nucleic acid was isolated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. Members of the family—father, mother, and son—showed strikingly similar microbial diversity for a few species of *Bacteroides*, *Eubacterium*, and *Ruminococcus* (Figure 3). However, significant differences were apparent between individuals. *Prevotella* strains are associated with plant-rich diets [7], suggesting that the significantly higher abundance of *Prevotella copri* from donor 1 (the father) is attributable to his vegetarianism.

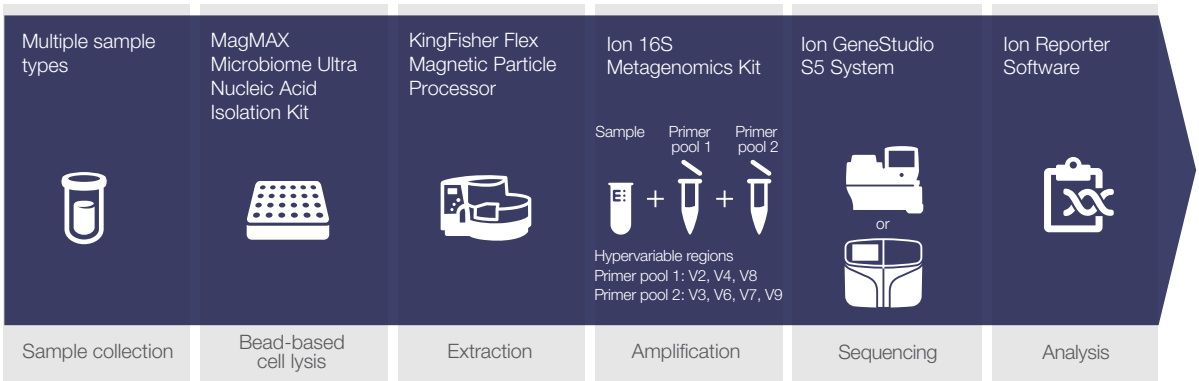

Figure 1. End-to-end workflow for 16S rRNA sequencing. The Ion 16S™ Metagenomics Kit and the Ion Plus Fragment Library Kit were used to synthesize 16S rRNA libraries. The barcoded libraries were pooled and templated on the Ion Chef™ Instrument followed by sequencing on the Ion GeneStudio S5 System. Automated analysis, annotation, and taxonomic assignments were performed using Ion Reporter™ Software.

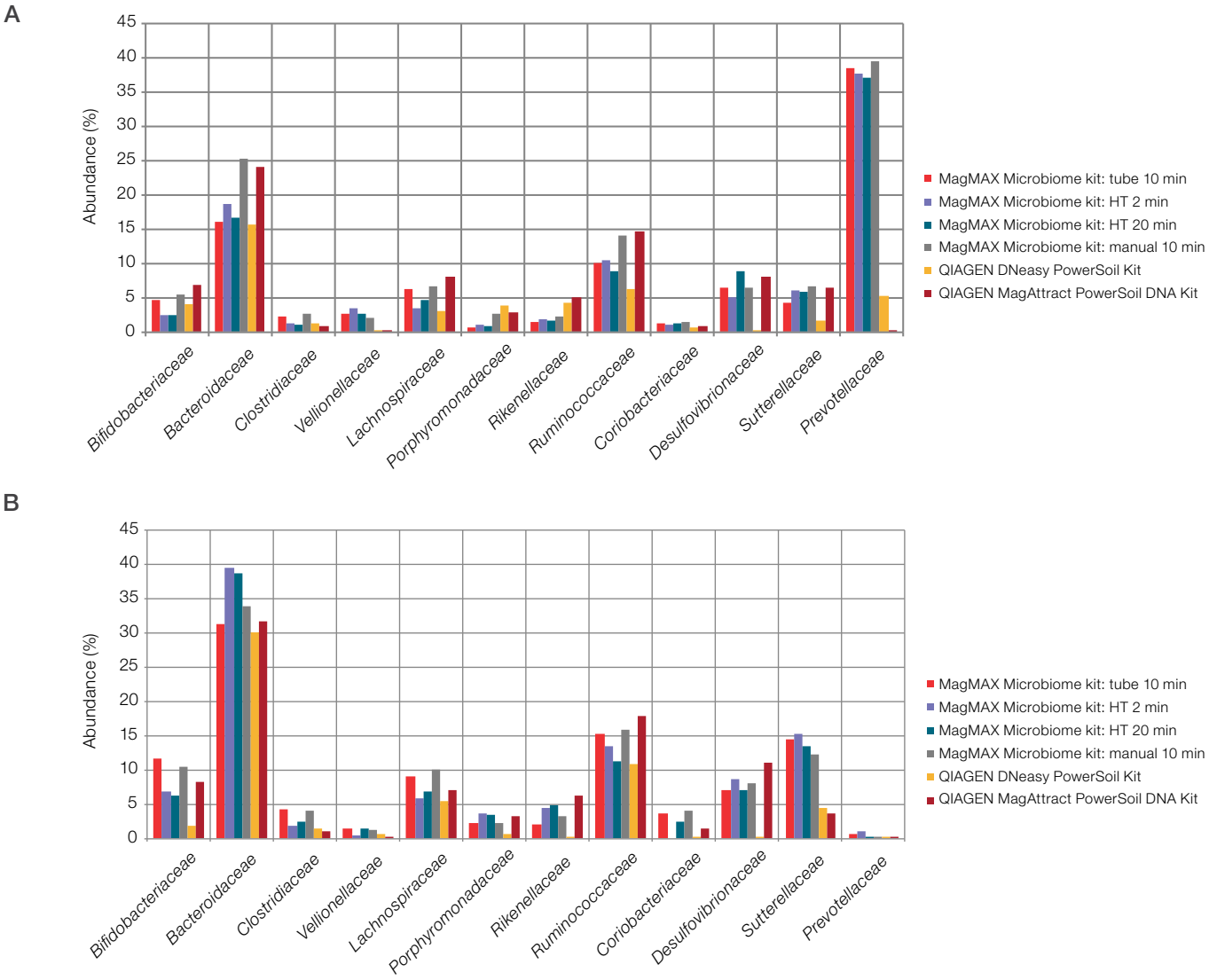

Figure 2. Benchmarking microbiome kit workflows with fecal samples from two donors (A and B). MagMAX Microbiome Ultra Nucleic Acid Isolation Kit workflows show superior performance in terms of relative abundance of diverse bacterial families compared to kits from QIAGEN.

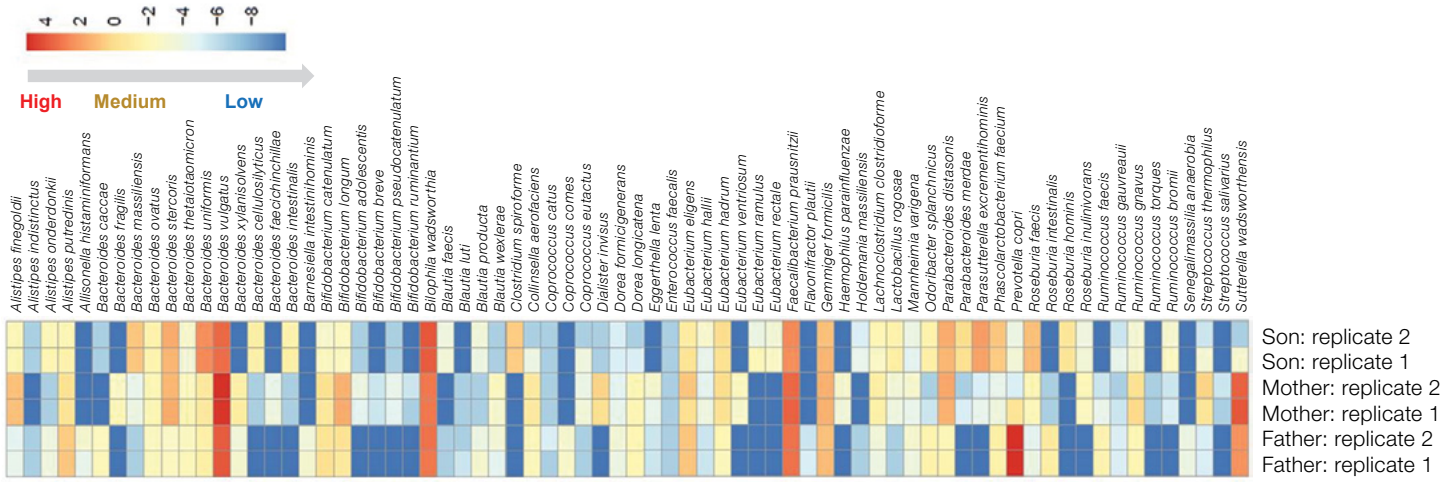

Figure 3. Microbiome profile within a single family. Fecal samples collected from individuals within a single family were processed using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. 16S rRNA sequencing was accomplished using the Ion Torrent platform. The DNA profile showed a unique microbiome from each individual at the species level.

Effects of probiotics on the gut microbiome: a case study

Probiotics intake for 12 weeks was associated with alteration of a donor's microbiome profile for *Firmicutes* and *Bacteroidetes*. Fecal samples were collected before starting the probiotic course, then at every week for 8 weeks and at week 12 of probiotics usage. Total nucleic acid was isolated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. 16S rRNA sequencing was performed to determine the microbiome diversity associated with probiotic usage (Figure 4). There was no significant difference over the course of the study in levels of *Proteobacteria*. Levels of *Firmicutes* were high at baseline but they decreased with usage of probiotics. *Bacteroidetes* were at medium levels before probiotics usage but were elevated after probiotics intake. The *Firmicutes/Bacteroidetes* (F/B) ratio is widely accepted to influence normal gut homeostasis. Increased or decreased F/B ratios are considered dysbiosis, which is associated with the development of obesity or inflammatory bowel disease (IBD), respectively [8]. In this case study, the consumption of probiotic preparations containing *Lactobacillus* in combination with *Bifidobacterium* led to a slight reduction in the F/B ratio as early as week 1. The early reduction in the F/B ratio suggests that the probiotic usage contributed to that reduction and to reversal of gut dysbiosis, which remained consistent throughout 12 weeks of probiotic consumption. However, the levels of *Actinobacteria* started at medium-to-low at baseline and decreased over time. Consistent with the case study, probiotic preparations containing *Bifidobacteria* are thought to contribute to a reduction in *Actinobacteria* that results in gut homeostasis; however, larger clinical studies are needed to confirm these encouraging results [9].

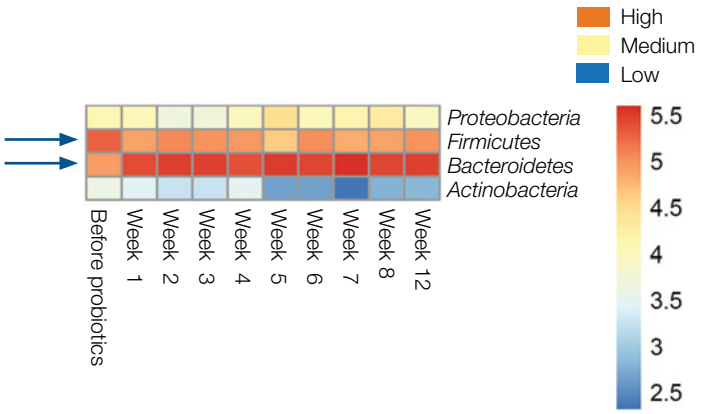

Figure 4. Effects of probiotics on the human gut microbiome. Fecal samples were collected from a donor before probiotic usage and for up to 12 weeks during probiotic consumption. Total nucleic acid was isolated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. 16S rRNA sequencing using the Ion GeneStudio S5 System showed a distinct microbiome profile shift for *Firmicutes* and *Bacteroidetes* levels over 12 weeks (blue arrows).

Effects of diet on gut microbiome: a case study

The MagMAX Microbiome Ultra Nucleic Acid Isolation Kit efficiently differentiated the intestinal microbiome to the species level for an individual who followed a strict vegetarian diet (Figure 3). Figure 5 shows that a vegetarian diet affects the relative abundance of certain species and significantly alters the gut microbial diversity relative to a non-vegetarian diet. This is consistent with studies that have shown that a vegetarian diet results in an increase in abundance of bacteria such as *Clostridium* that ferment dietary fiber [10-12], a reduction of the proportion of carbohydrate-metabolizing *Bacteroides* [13,14], and a decrease in the abundance of butyrate-producing *Bifidobacterium* [3]. Accordingly, several species belonging to the bile-tolerant *Alistipes*, which has previously been associated with a meat-rich diet [3], showed a lower abundance after the strict vegetarian diet was followed.

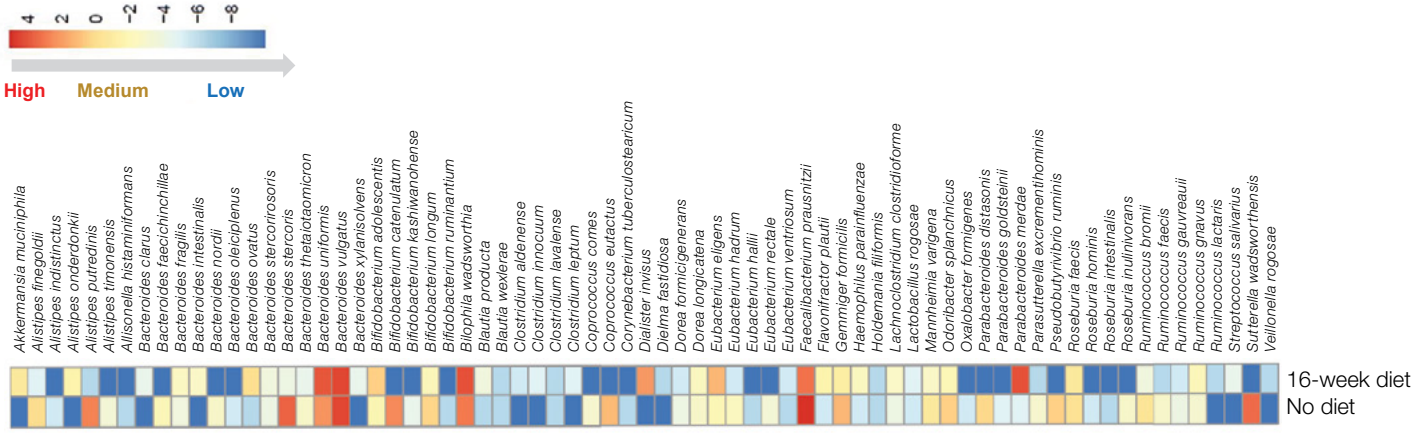

Figure 5. Effects of diet on the human gut microbiome. 16S rRNA sequencing results from DNA extracted from fecal samples using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit were analyzed to compare changes in an individual's microbiome profile following dietary changes that occurred over 16 weeks.

Changes in microbiota profile during pregnancy: a case study

Fecal samples were collected from the pregnant donor at 5 timepoints: at 23 weeks gestation, at 33 weeks gestation, at 37 weeks gestation, at 3 weeks postpartum, and at 8 weeks postpartum. Figure 6 shows that some bacterial families changed dramatically over the course of pregnancy and some stayed very similar. More research needs to be done to fully understand the cause and effect of gut microbiome changes over time in a pregnancy. Since microbiomes differ significantly in individuals, this study was meant to look at the profile of temporal concentration changes.

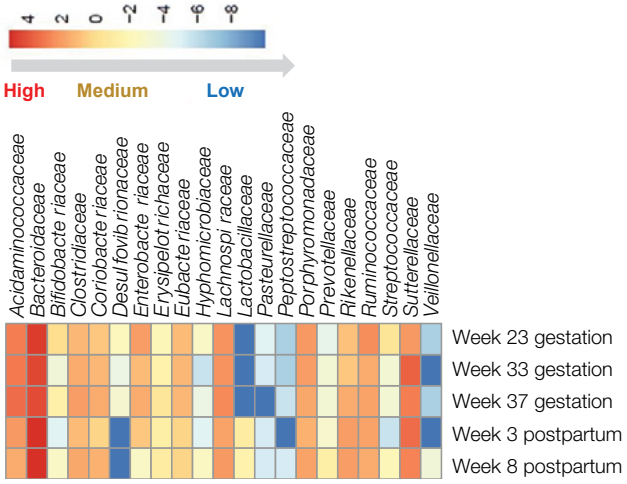

Figure 6. Comparison of the microbiome profile during pregnancy (at 23, 33, and 37 weeks of gestation) and postpartum (at 3 and 8 weeks).

Conclusion

Total nucleic acid from fecal samples can be efficiently isolated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit for downstream applications. Notably, DNA and RNA can be isolated for comprehensive analyses of the human gut microbiome. In addition, the workflow comparison with other suppliers shows better performance of the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit in terms of the relative abundance of different microbial families and comparable results in terms of distinguishing microbial profiles. This study showed the equivalent performance of all workflow options explored with the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. This consistent performance enables a researcher to study the microbiome profiles from fecal samples without any isolation biases. In addition, the changes in microbiome profiles between individuals living in the same household who have different dietary habits can be efficiently studied. The relative changes in microbiome profiles following diet and probiotics usage can be easily differentiated using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. Finally, the changes in microbiota profiles observed during pregnancy and postpartum can be analyzed following nucleic acid isolation with the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit.

Ordering information

Product	Quantity	Cat. No.
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead plate	100 preps	A42357
MagMAX Microbiome Ultra Nucleic Acid Isolation Kit, with bead tubes	100 preps	A42358
KingFisher Flex Purification System, KingFisher with 24 Deep-well Head	1 instrument	5400640

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