Nucleic acid isolation

Investigating microbiomes in various sample types with the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit

Summary

- The Applied Biosystems™ MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit enables efficient extraction of high-quality nucleic acid from skin, sweat, and milk.
- Up to 96 samples can be processed in 60–75 minutes for high-throughput workflows.
- The MagMAX Microbiome Ultra Nucleic Acid Isolation Kit enables reproducible recovery of nucleic acid from skin, sweat, and milk in high yields.

Introduction

A microbiome is a collection of genomes in a population of microbes [1]. Distinct populations of microbes reside in various parts of the human body, and knowing where specific types of microorganisms live can provide insight into their functions and the interactions they have with the body. For example, human skin supports a large and diverse population of microbes that can affect skin health [2,3]. Milk provides essential microbes to nursing infants, and human milk has been shown to have a diverse microbiome that is thought to be beneficial [4]. Nucleic acid can be efficiently extracted from soil and stool samples using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit (Cat. No. A42358). In this application note, we demonstrate that it is also effective for extracting microbial nucleic acid from skin swabs, armpit swabs, cow milk, and human milk.

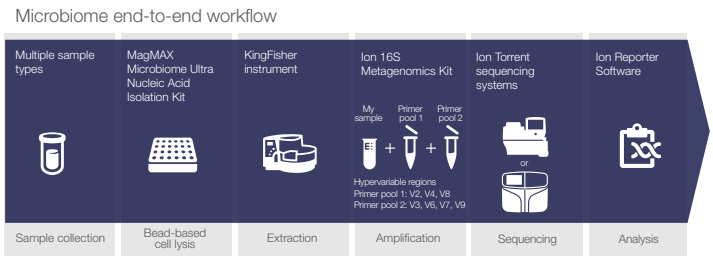

Figure 1. Sample collection, extraction, library creation, sequencing, and analysis workflow.

Materials and methods

Sample collection

Skin and armpit swabs were collected in duplicate by swabbing the surfaces of a human hand and armpit, respectively. The armpit swabs were obtained from a single donor who was not wearing deodorant. Human milk was obtained from a single donor 3 weeks, 7 weeks, and 19 weeks after giving birth. All of the human samples were processed in duplicate. USDA-certified organic and conventional whole cow milk samples were also collected and processed in duplicate.

Nucleic acid extraction and analysis

To prepare the milk samples for extraction, 400 µL of each sample was added to the bead beating plate and processed in 800 µL of lysis buffer. After removing the shafts from the hand and armpit swabs, each swab was also processed in 800 µL of lysis buffer in the bead beating plate. A Bead Ruptor™ 96 homogenizer was used for bead beating, and extraction was performed with the Thermo Scientific™ KingFisher™ Flex Purification System fitted with a 96 deep-well plate. After purification, each isolated nucleic acid sample (DNA and RNA) was eluted in 200 µL of elution solution.

Note: One duplicate set of hand swabs and one duplicate set of armpit swabs did not receive proteinase K treatment. This allowed changes in the abundance of gram-positive and gram-negative bacteria to be observed.

The Ion Plus™ Fragment Library Kit and the Ion 16S™ Metagenomics Kit were used to synthesize 16S rRNA gene libraries on the Ion Chef™ Instrument. The barcoded libraries were pooled and templated on the Ion Chef Instrument, then sequenced on the Ion GeneStudio™ S5 System. Automated analysis, annotation, and taxonomic assignment were performed using Ion Reporter™ Software. The RStudio™ program was used to generate heat maps. The nucleic acid extraction and analysis workflow is illustrated in Figure 1.

Results

We successfully extracted bacterial nucleic acid from skin swabs, sweat on armpit swabs, and milk samples using the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit. With the isolated nucleic acid, we were able to confirm the presence of various families of bacteria (Figure 2). We were also able to observe differences between bacterial abundance in various parts of the human body. Staphylococcaceae and Corynebacteriaceae are known to be abundant in moist areas of the human body. These families are less abundant in drier areas like the hands [5,6]. As shown in Figure 3, samples that were treated with proteinase K contained higher concentrations of nucleic acid from gram-positive bacteria, including Actinomycetaceae, Micrococcaceae, Propionibacteriaceae, and Streptomycetaceae [7-10]. Conversely, the concentrations of nucleic acid from gram-negative bacteria, such as Bacteroidaceae and Porphyromonadaceae, were higher in untreated samples [11,12].

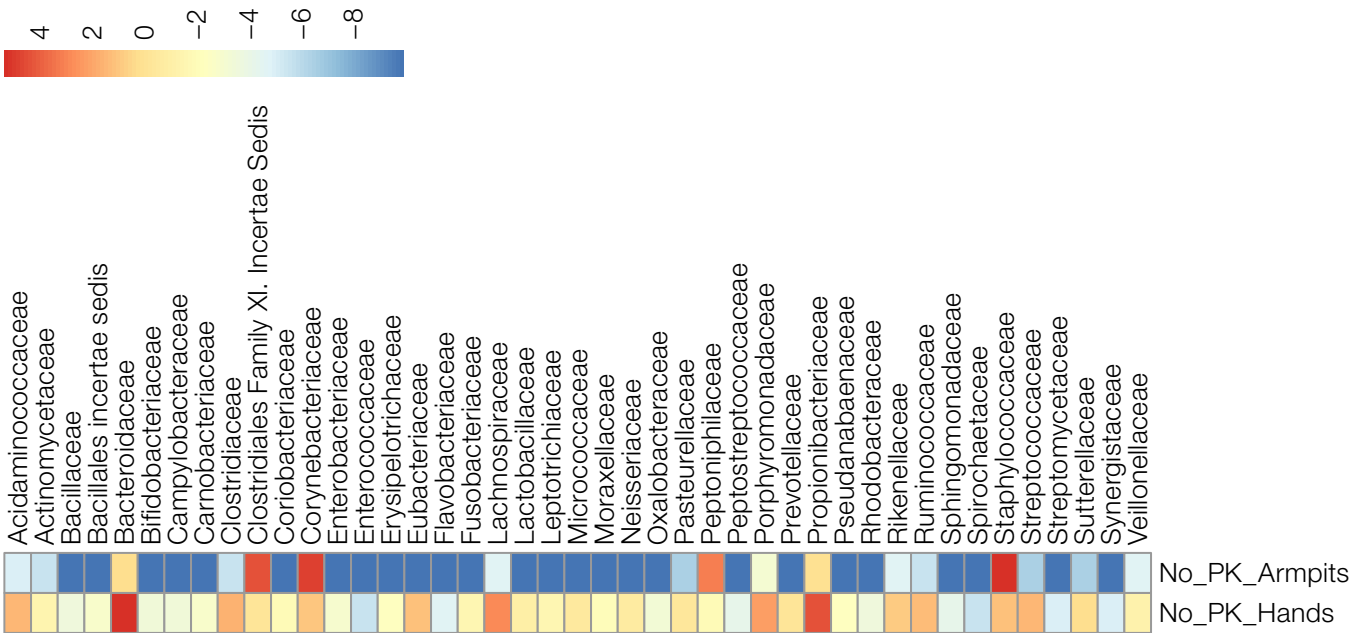

Figure 2. Abundance of bacteria on hand and armpit swabs without proteinase K (PK) treatment. Each data point is reported as the average of technical duplicates.

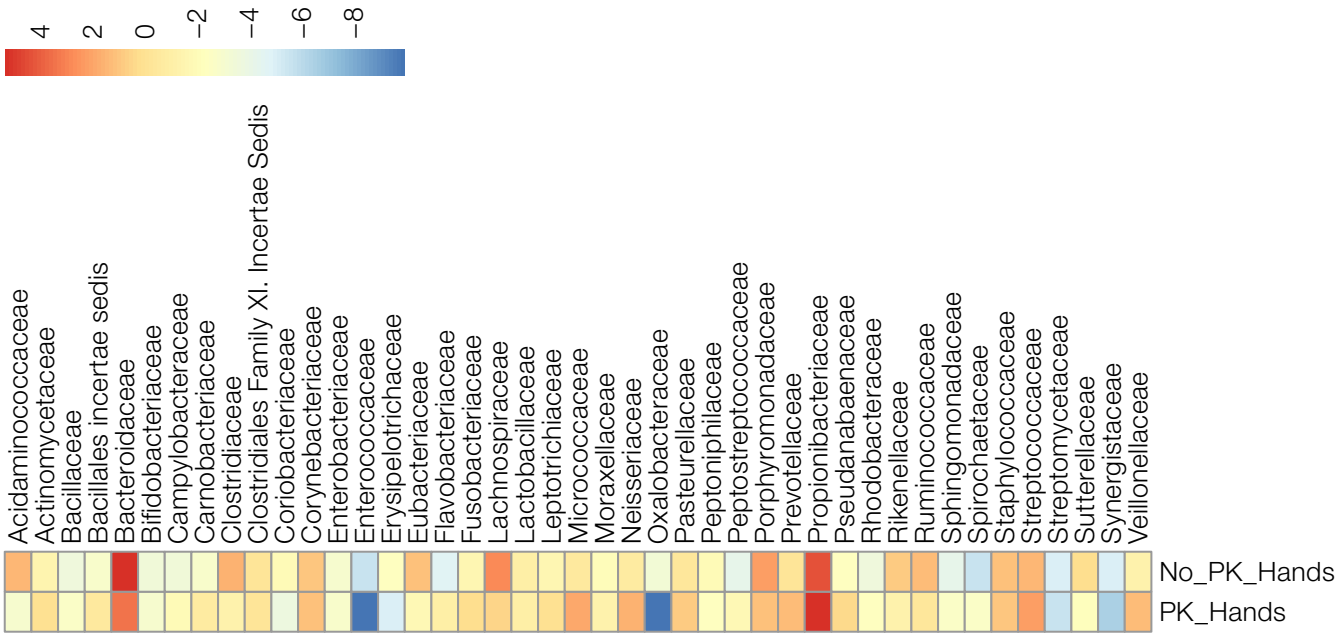

Figure 3. Abundance of bacteria on hand swabs with and without proteinase K (PK) treatment. Each data point is reported as the average of technical duplicates.

We also compared the abundance of microbiota in human breast milk, organic cow milk, and conventional whole cow milk (Figure 4). The microbiomes of human and cow milk differed significantly, and there also were differences between the microbiomes of organic and conventional whole milk. Hyphomicrobiaceae were not present in either organic or conventional cow milk, but they were both present in human milk 7 and 19 weeks after delivery. Ruminococcaceae were less abundant in cow milk as well as human milk during the early postpartum period. However, they were highly abundant in human milk during the later postpartum period. More research is required to understand why the microbiome of human milk changes over time.

Conclusion

We have demonstrated the versatility of the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit by extracting nucleic acid from gram-negative and gram-positive bacteria in cow milk, human milk, and human hand and armpit swabs. We found that proteinase K treatment could alter the yields of nucleic acid from gram-positive and gram-negative bacteria. We could efficiently obtain high yields of nucleic acid from both high- and low-abundance species. The versatility and robustness of the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit will make it a welcome tool in any laboratory that performs microbiome research.

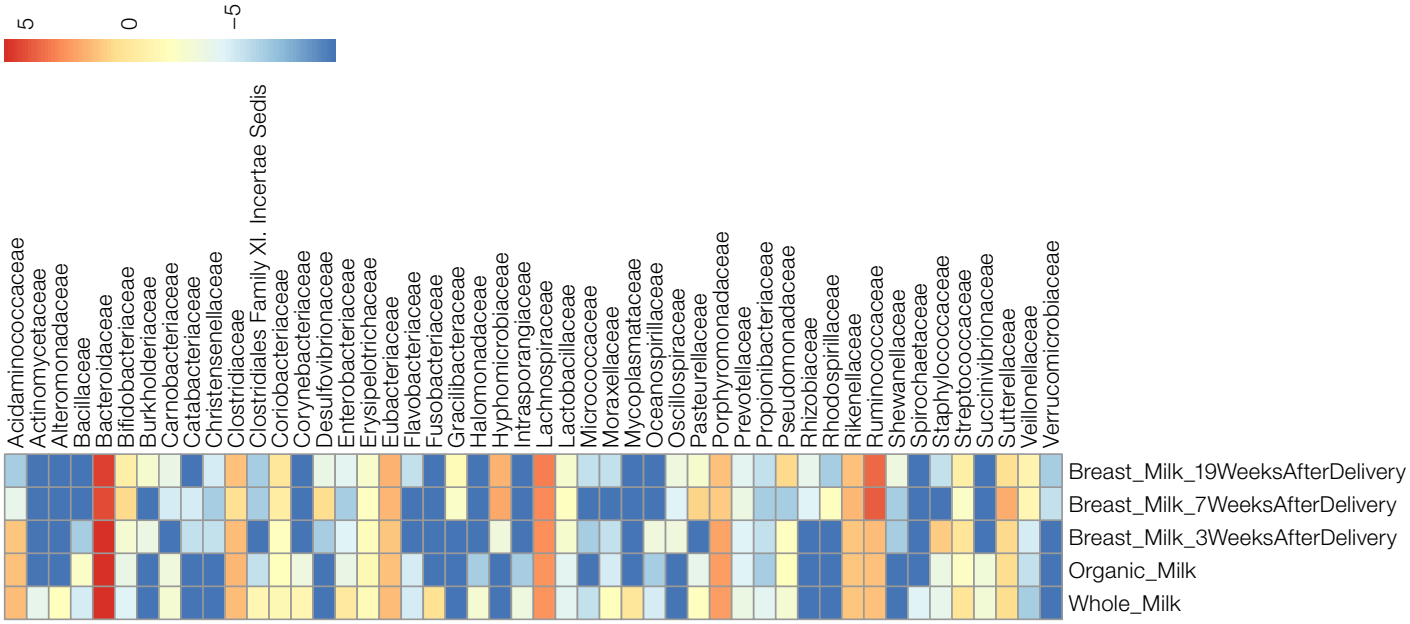

Figure 4. Heat maps of bacteria in organic and conventional whole cow milk and human breast milk collected at 3 different time points.

Ordering information

Description	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
Ion Plus Fragment Library Kit	10 reactions	4471252
Ion 16S Metagenomics Kit	100 reactions	A26216
KingFisher Flex Purification System with 96 Deep-Well Head	1 system	A32681
Ion Chef Instrument	1 system	4484177
Ion GeneStudio S5 System	1 system	A38194

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