The meta genomics performance of the NOPB-based MGIEasy Stool Sample Collection Kit

Highlights

Reduce data bias

- Reduce the sequencing bias result from microbe growth and nucleic acid degradation

Wide application

- Wide application, including 16S rRNA microbe analysis, meta genomics, Array, qPCR, etc

Room temperature preservation

- Store at room temperature, reduce the cost for shipping

Introduction

Recent advances in sequencing technics and bioinformatics, especially in the field of metagenomic sequencing, have increased our knowledge of the complex microbial communities in the gut of humans and animals. It is now well established that these microbes play important roles in relation to inflammation, metabolic disease, mental disorders, and several other diseases. Studies of the gut microbiota may help us to prevent, diagnose, and eventually cure or curb such diseases.

Fecal samples are widely used in metagenomic studies and are generally required to be immediately stored at 20 °C or below. However, this is difficult to achieve in many situations such as sampling in remote areas and may dramatically increase the costs of such studies. Few studies have in detail investigated the stability of fecal samples, and in many cases, samples have been stored at room temperature for several days prior to storage at 20 °C or below, To overcome the problems associated with sampling without possibilities for immediate storage at– 20 °C or below and transportation, and secure the generation of standardized and reproducible metagenomic sequencing data quality, we developed and tested a fecal sample preservation protocol using storage in a novel N-octylpyridinium bromide (NOPB)-based reagent.

Case

A novel affordable reagent for room temperature storage and transport of fecal samples for metagenomic analyses[1]

To evaluate the NOPB-based method, we collected ten fecal samples from eight healthy adult subjects and divided these samples into 110 aliquots. The aliquots were stored or transported at room temperature using different schemes before extraction. After storage and/or transportation, all aliquots were sequenced, and each was compared to the corresponding freshly extracted sample. Aliquots which have been stored without any additional reagents (termed "non-stabilized") were used for further comparison. Extracted DNA from all samples was sequenced on Illumina HiSeq 4000, and a subset was also sequenced on the DNBSEQ platform.

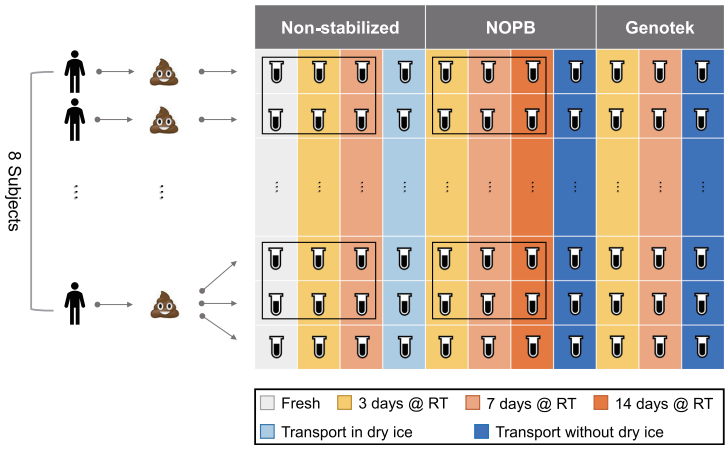

Figure 1. Layout of sampling and treatment. All aliquots except the "14 days @ RT" group were sequenced on the HiSeq 4000 platform, while the aliquots in the black frames were also sequenced on the DNBSEQ platform

Comparison analysis

The fresh sample as control, compare to the same kind product with differen stabilizer by gene relative abundance, species abundance, dissimilarity in relation to relative gene abundances and microbiome composition. The result indicate NOPB-based reagent data has no difference from the fresh sample.

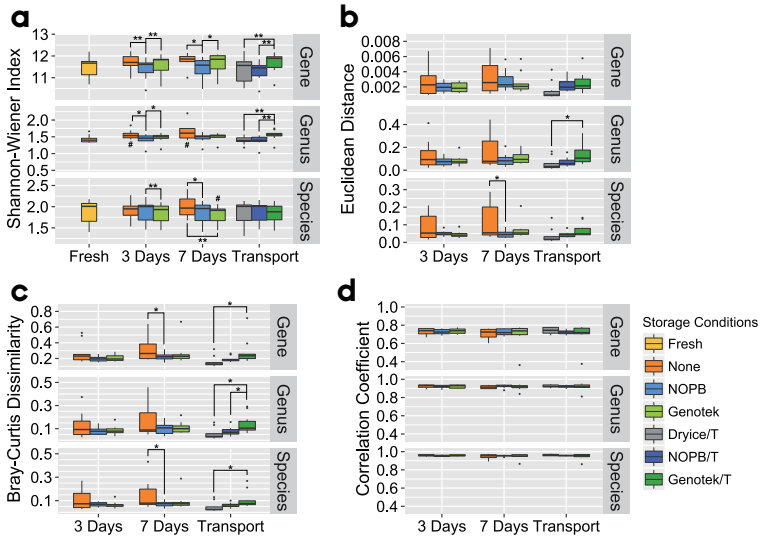

Figure 2. **Correlation, dissimilarity, and change in α-diversity between stored or transported and fresh fecal samples. Sequencing data (dataset A)used to plot this figure was generated by using the Illumina HiSeq 4000 platform. "/T" represents transportation groups. a The α-diversity of the freshly extracted samples and corresponding stored samples. b–d The Euclidean distance, Bray-Curtis dissimilarity, and Spearman correlation coefficient between the stored samples and corresponding freshly extracted ones. One asterisk indicates significant difference (p < 0.05, paired t-test), and two asterisks indicate highly significant difference (p < 0.01, paired t test). Number sign in d indicates significant difference in α-diversity in comparison to the corresponding fresh aliquots (p < 0.05, paired t test) (n = 10)**

The slope of the trendline and the R-square value of each storage condition were used to estimate the bias and variation in comparison to the original relative abundances at the gene, genus, and species level. The results indicate that samples stored in the NOPBbased reagent exhibited a slightly better performance in relation to recovery of low abundance species and exhibited a lower variation than observed for samples stored using the Chelating agent-based kits, especially when samples had been transported.

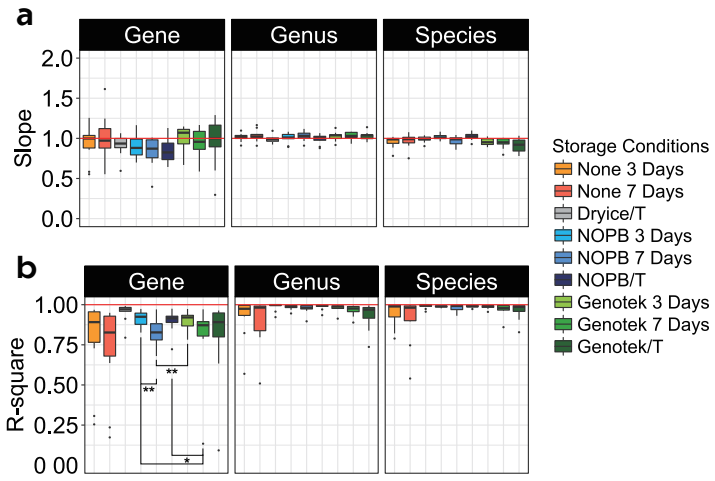

Figure 2. **Bias and variation of different storage conditions related to relative abundance. a The slopes of the linear trendlines in the q-q plot. b The R-square values. One asterisk indicates significant difference (p < 0.05, Wilcoxon test), and two asterisks indicate highly significant difference (p <0.01, Wilcoxon test)**

Conclusion

A reliable and cost-efficient method which maintains relative gene abundances and microbiota composition of fecal samples during storage and/or transportation at room temperature is highly desirable for large-scale metagenomic studies. The NOPB-based method represents such a reliable choice.

To solve the problem of preservation and transportation of fecal sample at room temperature, we developed MGIEasy Stool Sample Collection Kit based on NOPB, The results show that the method can be used for easy collection and storage of fecal samples for 7 days or even longer at room temperature, even when the samples need to be transported using normal commercial routes. This product will improve the reliability and reproducibility of metagenomic studies using fecal samples collected in remote areas and developing countries and reduce the costs of such studies.

Ordering information

Product	Specification	Item number
MGIEasy Stool Sample Collection Kit	1 dose	1000003702

Reference

1. Han et al. A novel affordable reagent for room temperature storage and transport of fecal samples for metagenomic analyses. Microbiome (2018) 6:43

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