

POPULATION-BASED GUT MICROBIOME PROFILING IN 5000 SAMPLES

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Introduction

Collection and storage procedures in human gut microbiome studies are essential factors for the identification of robust microbiome-based taxonomic biomarkers. Our group has been collecting stool samples in elderly from the Rotterdam Study (RS) and in children from the Generation R Study (GenR) cohorts. Participants produced their samples at home and sent them to the laboratory by regular mail. The samples were stored at -20°C upon arrival at the laboratory. We have validated this collection and storage approach using human-microbiota derived 16S rRNA gene-based deep-sequencing analysis.

Materials and methods

Stool samples from 4 volunteers were collected under the following conditions: at room temperature (RT), at room temperature while homogenized in stabilizing buffer (RT+St), and immediately frozen at -80°C (IF) as golden standard. In addition samples from the same volunteers were stored at -20°C and at -80°C for 0, 3, 6, and 12 months to study the effect of storage temperature and time. Genomic DNA was extracted using an automated stool DNA extraction kit, which included a “bead beating” step. The 16S rRNA gene, regions V3-V4, were amplified and sequenced on the Illumina MiSeq platform. Bacterial 16S, V3-V4, profiles were generated using UPARSE and Qiime packages and the profiles were compared by means of correlation analysis.

Results

Correlation analysis of the collecting study showed R values > 0.9 for the RT samples for up to 3 days compared to IF samples. However, the correlation drops to 0.8 after 5 and 7 days. Adding stabilizer resulted in a higher correlation (R > 0.87). Bacterial diversity did not change substantially over time. Correlation analysis of the storage study showed R values > 0.9 for samples stored up to 9 months and drop to 0.7 in the 12th month regardless of storage temperature.

Conclusions

Storage temperature and duration did not affect the phylogenetic structure and bacterial diversity significantly. Leaving samples at room temperature (for up to 3 days) does not significantly change the bacterial composition and diversity.