

## 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.