

Preservation Solution Stability Assay

Summary

In order to test the preservation capacity of Massive Bioinformatics' own preservation solution, this experiment was designed to test this solution in comparison to other commercially available solutions for a duration of four weeks. Two donors were asked to donate a fecal sample in 19 different tubes each. Five different solutions were tested for four weeks in terms of the preservation capabilities of each different solution and their impact on the bacterial abundances. We have demonstrated that our preservation solution has the capacity to preserve fecal samples in room temperature for up to four weeks.

Study Details

MassBiome is a project designed to analyze the human gut microbiota using stool samples. Its purpose is to provide information about the types of microorganisms residing in a person's gut, offer personalized guidance on clients' overall health, and make recommendations for dietary habits. MassBiome analysis requires a spoonful of fecal sample, stored and transported in conditions that enable the preservation of the microbial community. In this study, the aim is to test the capacities of several preservation solutions for four weeks.

For this experiment, 38 healthy human faecal samples obtained from 2 healthy individuals, preserved in different conditions were received to examine their bacterial distribution and to determine the appropriate storage solution for the MassBiome project. A sampling plan was illustrated below in Figure 1.

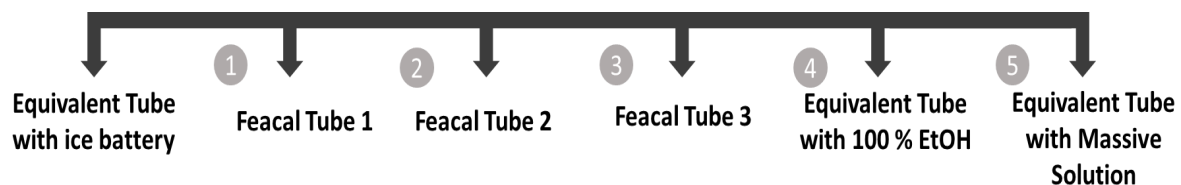


Figure 1: fecal samples from donors were tested for 5 different conditions.

Firstly, DNA isolation was performed on the day of arrival from two fecal samples preserved on ice. These two samples, one from each donor, represents the bacterial diversity truth. Then, for 4 weeks, DNA isolation from samples in different tubes was carried out. Fecal DNA was isolated each week from tubes 1,2,4 and Fecal DNA from Tube 3 was tested twice with 2-week intervals. Experiment plan was shown below in Figure 2.

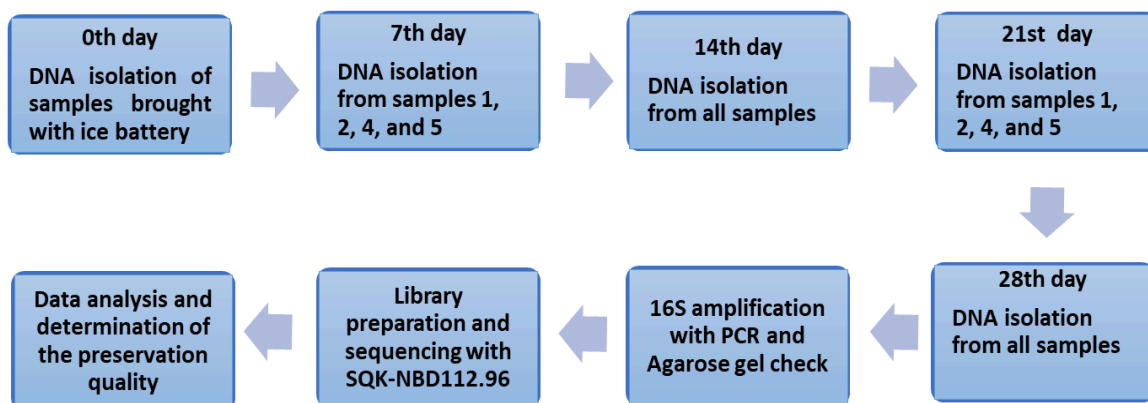


Figure 2: Stability Assay Experiment Plan

Following the plan, DNA isolation was repeated with 1-week intervals to determine the preservative capabilities of different solutions within 4 weeks. DNA isolation was performed using Zymo Research's Quick-DNA Fecal/Soil Microbe Miniprep Kit (D6010), following the Massbiome guide. After DNA isolation, DNA concentrations were measured using a Qubit fluorimeter. Then, PCR was set up with 16S primers amplifying the V1V9 region, as described in the Massbiome user guide. Amplicons obtained were run on agarose gel electrophoresis to

demonstrate the presence/amplification of the correct locations on the DNA. Subsequently, 200 fmol of each PCR product was used for library preparation according to the SQK-NBD112.96 native barcoding amplicons protocol. The final library was sequenced on Oxford Nanopore Technologies Mk1C device. Sequencing was stopped when a minimum of 10,000 reads were obtained from each sample, as predetermined. Data from each barcode was analyzed with default MassBiome analysis pipeline.

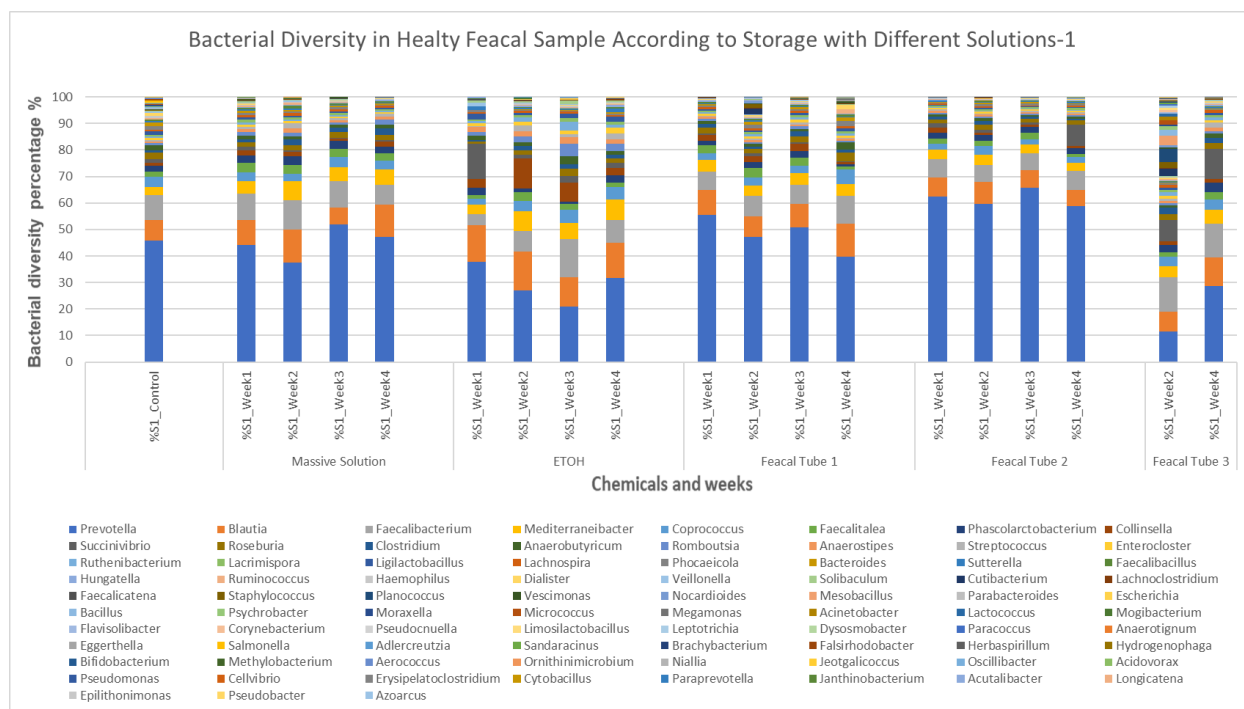


Figure 3: Bacterial distributions of donor #1's samples, stored in different chemicals throughout four weeks.

Figure 3 illustrates the bacterial distribution of the samples obtained from donor one throughout the experiment. Each bar demonstrates a different sample, and effects of storage for four weeks can be compared for each preservation solution. The most similar distribution to the control group was observed in the preservation solution developed by Massive Bioinformatics (referred to as Massive solution). When the sample was stored in pure ethanol (referred to as ETOH), it was observed that the bacterial distribution was different from the control; there was a decrease in the distribution of bacteria of the Prevotella genus, and the distribution of the Faecalibacterium genus changed significantly. Storing sample in faecal tube 1 significant changed the bacterial distribution at the end of the 4th week. When stored in Faecal tube 2, bacterial distribution was observed to be different from the control, and especially in the Prevotella genus, the distribution was higher than it expected. When stored in the Faecal tube 3, bacterial composition changed significantly.

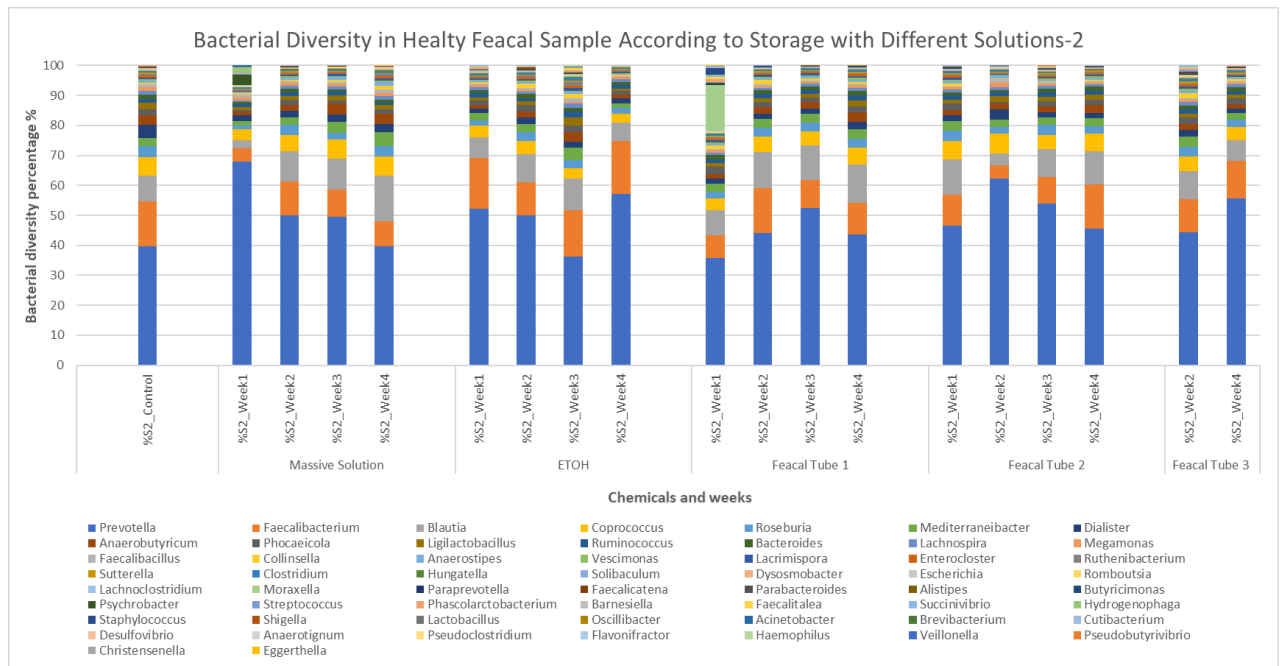


Figure 4: Bacterial distributions of donor #2's samples, stored in different chemicals throughout four weeks.

Figure 4 shows the bacterial distribution of the samples obtained from donor two throughout the experiment. Similar to Figure 3, each bar demonstrates a different sample, and effects of storage for four weeks can be compared for each preservation solution. The most similar distribution to the control group was observed in the preservation solution developed by Massive Bioinformatics (referred to as Massive solution), especially when stored for four weeks. For the sample obtained from donor 2, storing the sample in pure ethanol impacted the bacterial distribution less. However, with donor 1, EtOH was found the affect the bacterial abundances immensely. Therefore in terms of reproducibility, this chemical was found not to be a great preservative for all samples. Although the distributions for Faecal Tube 1, Faecal Tube 2, and Faecal Tube 3 were closer to the control compared to the previous study, it was observed that they were not reliable in terms of repeatability, considering the differences between the two studies. Additionally, when examining Faecal Tube 2, the distribution of the *Prevotella* genus was higher than expected, impacting the percentage distribution of other bacteria. For Faecal Tube 3, the results for week 2 were similar to the control, but by the end of the 4th week, the distribution had changed and differed from the control group.