

GeneFix™ Saliva Collectors & Kits for Human DNA Oral Microbiome Analysis

Introduction

The human oral cavity plays host to an abundance of bacteria and other microorganisms, colloquially known as the oral microbiome¹. These organisms are important in maintaining homeostasis of the mouth, and are of great interest to the scientific community due to their roles in various disease states such as oral cancers and periodontal disease².

Isohelix's current GeneFix™ collectors and isolation kits produce Human DNA of high yield and purity from saliva samples, and provide long-term sample stability. Isohelix has now developed a new chemistry designed to maximise the collection and preservation of microbial DNA from human saliva samples. This study aims to demonstrate, by use of 16S rRNA quantitative PCR (qPCR), that GeneFix™ collectors & kits can isolate high quality microbial DNA from saliva samples, ideal for use in microbiome analysis, and provide long-term stability of DNA from a broad range of microbial species.

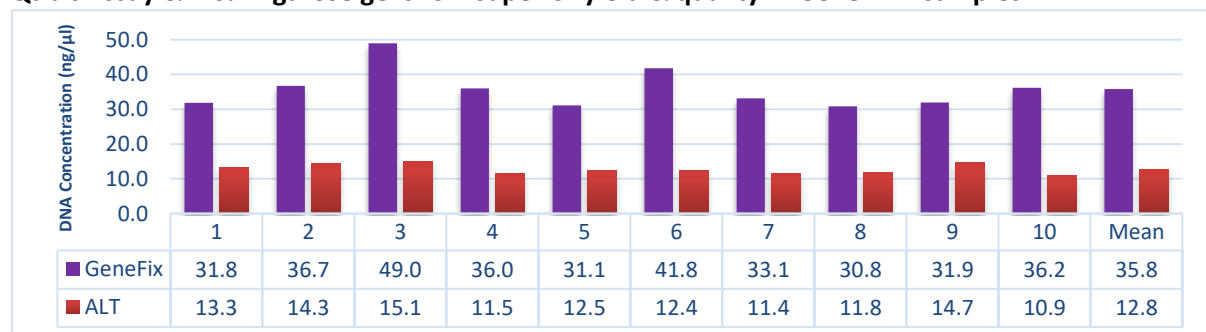
Methods & Materials

Saliva samples (using GeneFix™ MFX-01) were collected from seven healthy adult volunteers. These were pooled together and homogenized. Twenty aliquots from this were divided equally and isolated using GeneFix™ GSPN and compared against an alternative kit (ALT) commonly used in microbiome analysis, following the manufacturer's instructions for each respective protocol. For stability testing, three 2ml GeneFix™ samples were collected and incubated at 50°C for the equivalent of 3 months, 6 months & 12 months by accelerated ageing, with 500µl aliquots taken at each time point. Samples were analysed for yield and quality by Qubit assay & 1.0% agarose gel.

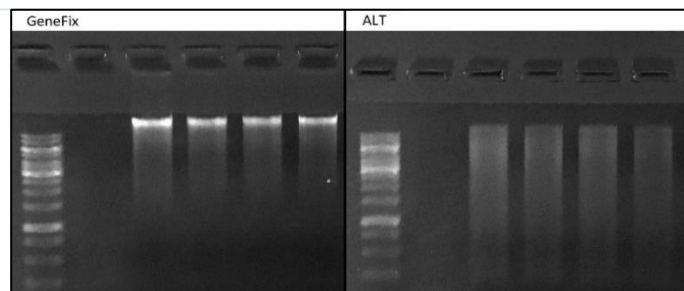
qPCR reactions were prepared for each sample isolated in the comparison & stability studies, alongside no-template controls & *E.coli* gDNA standard. 10ng of sample DNA was used per reaction. The PCR primers chosen were from a conserved region of the 16S gene that cover a broad range of bacterial genera.

Results

Qubit Assay & 1.0% Agarose gel show superior yield & quality in GeneFix™ samples:



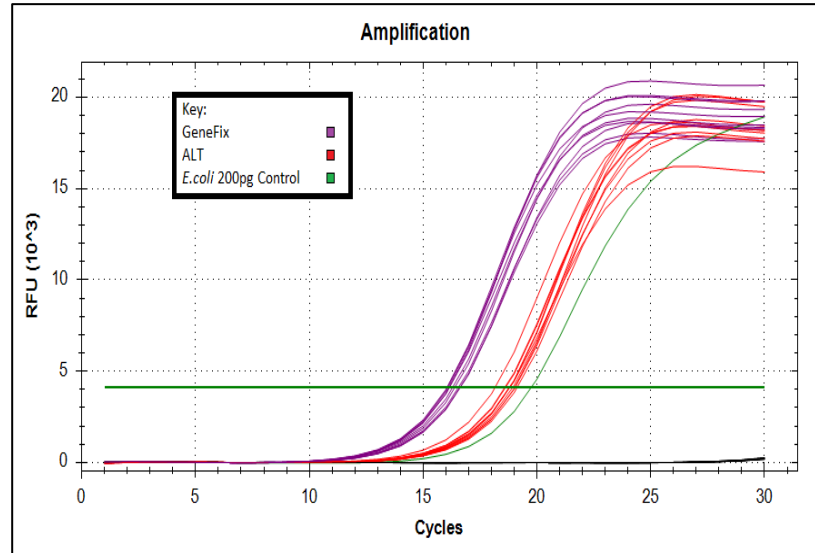
- Concentrations of DNA were on average 2.8 times higher in aliquots isolated by GeneFix™ compared to kit ALT.
- Agarose gel analysis of samples showed high quality DNA present in GeneFix™ samples, whereas some degradation was evident in samples purified by ALT.



16S rRNA qPCR Data of isolated Microbial DNA:

Cq	1	2	3	4	5	6	7	8	9	10	Mean
GeneFix™ MFX	16.38	16.54	16.63	16.29	16.05	16.15	16.10	16.06	16.14	16.61	16.30
ALT	18.58	19.04	18.80	18.61	18.78	18.93	19.02	18.80	19.13	18.15	18.78

- The qPCR data demonstrates that GeneFix™ MFX is capable of isolating high quality, amplifiable microbial DNA from samples, suitable for downstream applications such as sequencing.
- A higher fraction of DNA isolated in a 10ng sample of GeneFix™ MFX is microbial compared to kit ALT, as evidenced by lower Cq thresholds.



Stability Data of GeneFix™ Microbial samples incubated for 12 months:

- Cq thresholds of all GeneFix™ MFX microbiome samples (A, B & C), that indicate starting quantities of microbial DNA in a sample, remain stable from Day 1 up to 12 Months. This demonstrates the stability of microbial DNA in GeneFix™ MFX collectors.

Cq	Day 1	3 Months	6 Months	12 Months
A	15.2	14.7	14.5	14.4
B	15.7	14.6	14.1	14.1
C	15.5	15.2	14.6	14.9

Conclusions

- Isohelix GeneFix™ MFX microbiome collectors & kits are ideal for collection, storage & purification of microbial DNA from human saliva, suitable for downstream applications for the analysis of the human oral microbiome.**
- Fluorometric analysis & gel documentation demonstrate that GeneFix™ provides superior yield & quality of samples.**
- GeneFix™ collectors provide long-term stability of microbiome samples, keeping DNA stable for at least a year at room temperature.**

References:

- Dewhirst, F *et al.* The Human Oral Microbiome. *J Bacteriol.* 2010 Oct; 192(19): 5002–5017.
- Fan, X *et al.* Drinking alcohol is associated with variation in the human oral microbiome in a large study of American adults. *Microbiome.* 2018 Apr 24;6(1):59.