

GeneFix™ Saliva DNA Collectors & Kits for Human 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.

Human saliva, as well as containing host DNA, also carries DNA originating from the microflora in the oral cavity. Isohelix GeneFix™ collectors and isolation kits generate DNA of high yield and purity from human saliva samples, and provide long-term sample stability. Isohelix has recently adapted these technologies for use in 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.

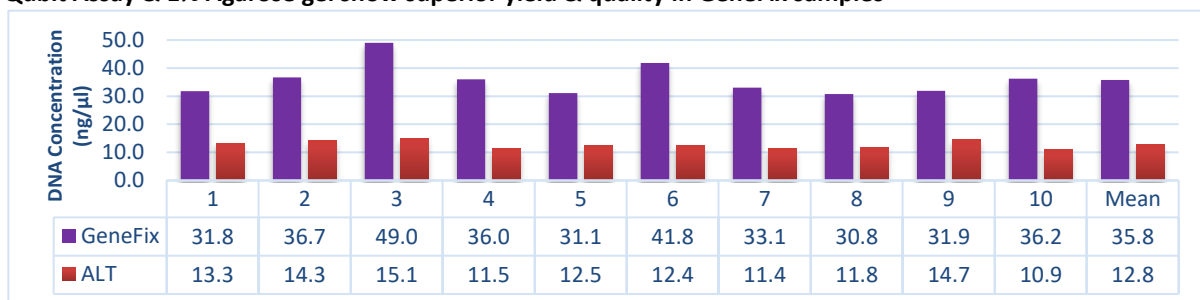
Methods & Materials

Saliva samples were collected from seven healthy adult volunteers, which were pooled together and homogenized. Twenty aliquots from this were divided equally to be isolated by GeneFix™ and an alternative kit commonly used in microbiome analysis following the instructions in each kit's 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 accelerated ageing, with 500µl aliquots taken at each time point. Samples were analysed for yield and quality by Qubit assay & 1% agarose gel.

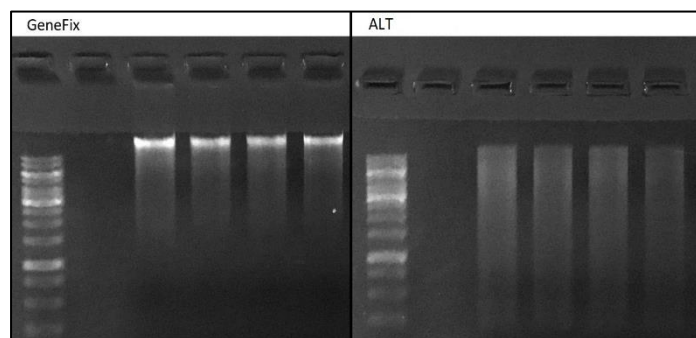
qPCR reactions were prepared for each sample isolated in the comparison & stability studies, alongside no-template controls & *E.coli* gDNA standard. 10ng of sample DNA was used per reaction. The PCR primers chosen were from a conserved region of the 16S gene that cover a broad range of bacterial genera.

Results

Qubit Assay & 1% Agarose gel show superior yield & quality in GeneFix samples

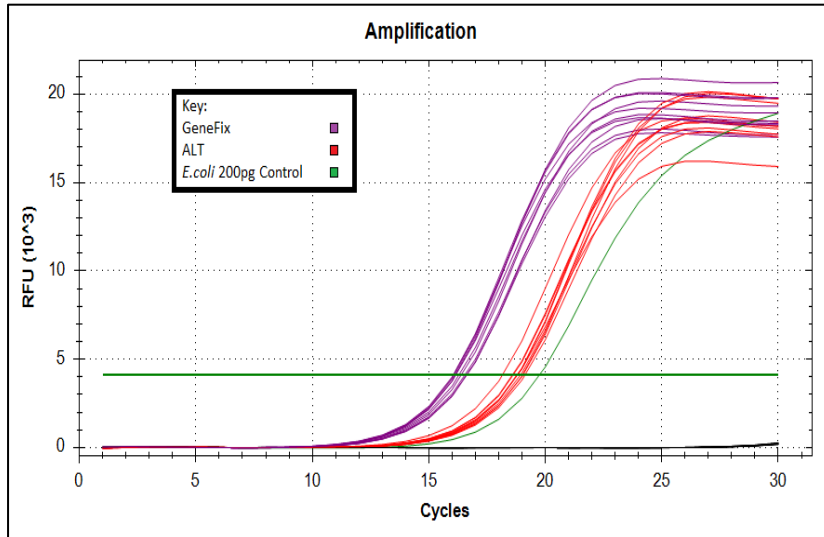


- Concentrations of DNA were on average 2.8 times higher in aliquots isolated by GeneFix compared to the Alternate kit.
- Agarose gel analysis of samples showed high quality DNA present in GeneFix samples, whereas some degradation was present in the Alternate kit samples.



16S rRNA qPCR displays abundance of microbial DNA present in GeneFix samples

Cq	1	2	3	4	5	6	7	8	9	10	Mean
GeneFix	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



- qPCR data shows that GeneFix is capable of isolating high quality, amplifiable DNA from microbial samples, suitable for downstream application such as sequencing.
- A higher fraction of DNA isolated in a 10ng sample of GeneFix is microbial compared to the Alternative, as evidenced by lower Cq thresholds indicating higher abundance.

Stability Data of GeneFix Microbial samples incubated for 12 months

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

- Cq thresholds of all GeneFix microbiome samples, which are indicators of starting quantities of microbial DNA in a sample, remain stable from Day 1 up to 12 Months. This demonstrates stability of microbial DNA in GeneFix.

Conclusions

- IsoheliX GeneFix collectors can collect and store microbial DNA from saliva, suitable for downstream applications such as microbiome genotyping & sequencing.
- GeneFix provides superior yields and quality when compared to other kits commonly used for oral microbiome analysis.
- GeneFix collectors provide long-term stability of microbiome samples, keeping them stable for up to a year at room temperature.

References:

1. Dewhirst, F *et al.* The Human Oral Microbiome. *J Bacteriol.* 2010 Oct; 192(19): 5002–5017.
2. 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.