

In-Depth Analysis of the Microbial Diversity in Saliva Samples Preserved at Ambient Temperature for over 6 Years

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Abstract

Norgen's Saliva DNA Collection and Preservation Device was introduced several years ago to stabilize DNA in saliva samples during transportation at ambient temperature. Prior studies have also shown that the preservative suppress the viability of microorganisms during transportation which has benefits for many core facility lab workers dealing with potentially infectious samples daily. Furthermore, allowing ambient temperature sample shipping has been beneficial to many collaborative research projects which have been limited by sample transportation conditions and shipping cost. Here we present and discuss the microbial diversity analysis using an Illumina NGS platform (as a part of Norgen's 16s rRNA metagenomics sequencing service) for the preserved saliva samples for over 6 years at ambient temperature. Saliva DNA was isolated at various time points using different saliva DNA isolation methods. The complexity of the datasets was analyzed in phylogenetic trees, principal coordinate analysis (PCoA) and relative abundance of predominant phyla. The sets of data showed that a level of microbial diversity affected by the time of preservation and the method of DNA isolation.

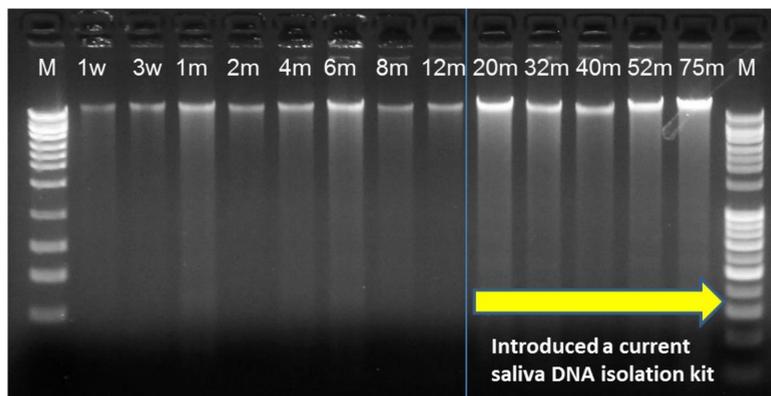
Methods & Materials



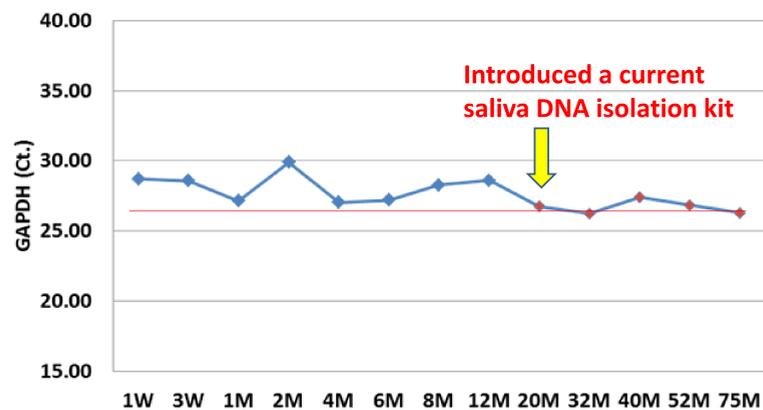
Pooled saliva from 6 healthy donors were preserved at room temperature using Norgen's Saliva DNA Collection and Preservation Devices (50) (Cat. RU49000) and the saliva DNA was isolated using Norgen's Saliva DNA Isolation Kit (Cat. RU45400) for various periods of time up to 6 years. 16S Metagenomic paired-end DNA libraries targeting the V3 and V4 region were prepared according to the manufacturer's (Illumina MiSeq system) instructions.

Result & Discussion

Stability and Quality of Preserved Saliva DNA at Room Temperature for up to 75 Months (6 years and 3 months).



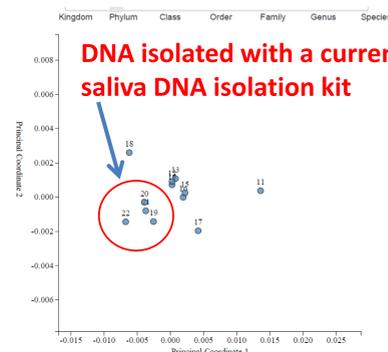
Saliva DNA isolated at various time points was visualized on an agarose 1x TAE gel and the result indicated an excellent DNA stability (>24 kb) up to 75 months at room temperature. Also it noticed that Norgen's current (improved) saliva DNA isolation kit (20m-75m) could provide consistently higher DNA yield than the previous DNA isolation method (1w-12m).



Detection of GAPDH using TaqMan real-time PCR reveals that the quality of the saliva DNA stored at room temperature for 75 months is suitable for any sensitive downstream applications.

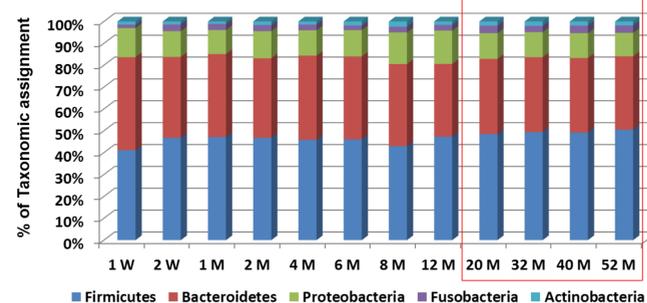
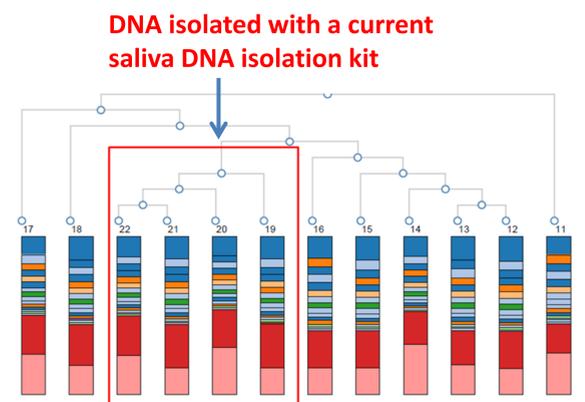
Furthermore switching to a current Saliva DNA Isolation Kit (Cat# RU45400) improved overall DNA yield reflected by the GAPDH detection sensitivity (lower Ct after 20m). This observation indicates that in addition to the DNA preservation, the DNA isolation method is also an important factor to downstream applications such as NGS.

Illumina MiSeq 16s rRNA metagenomic sequencing data



Principal Coordinate Analysis (PCoA) of 12 saliva microbiomes shows differences in the distribution of taxonomic classifications between samples up to Phylum level.

Hierarchical clustering of 12 saliva microbiomes based on genus-level classifications, including a bar chart showing the relative abundance of genus-level classifications for each sample in the dendrogram.



Percentage of Taxonomic assignment indicates that the room temperature preserved saliva could maintain the originality of the taxonomic assignment for over 6 years.

Moreover the current saliva DNA isolation kit demonstrated its reproducible performance showing the consistency of the NGS result.

Summary

1. Saliva samples preserved for over 6 years at room temperature using Norgen's Saliva DNA Collection and Preservation Devices (50) (Cat. RU49000) maintain the stability of saliva DNA without compromising the quality and yield of DNA which are suitable for Illumina MiSeq 16s rRNA metagenomic sequencing.
2. Improved (current) saliva DNA isolation kit demonstrated its reproducibility and reliability for NGS showing a consistency of the taxonomic assignment for over 6 years.