

# The Influence of Saliva Preservatives and DNA Isolation Methods for Oral Microbiota Metagenomics

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Prior studies have shown that preservatives could minimize the variability of microbiota authenticity during transportation at ambient temperatures, therein improving nucleic acid (NA) quality and quantity for downstream applications. These preservatives have been beneficial for many collaborative research projects which were previously limited by sample transportation conditions and shipping costs, since no chilling equipment is required. A popular application of the preservative is in human oral microbiome studies, such as those of the oral cavity. Illumina sequencing has been a powerful tool in understanding the diversity of the oral microbiome, yet NGS sequencing often encounters biases introduced by sample variation, the method of DNA isolation and purification, and primer selection. Thus far, no study has elucidated the impact of preservatives and isolation methods in navigating these biases. The objective of the current study was to evaluate comparative 16S metagenomic data from the oral microbiome, obtained from combinations of two different preservatives and four DNA isolation methods. Results indicated that the preservative and DNA isolation method influenced the oral microbiota metagenomic profile, suggestive of differences in NA quality, quantity, and consistency depending on the methods applied. Irrespective of the isolation method, saliva DNA preserved using Norgen's preservative consistently yielded more enriched data (associated with higher readings) in metagenomics analysis. This may be a beneficial and a convenient method for handling samples, whilst maintaining a high degree of consistency and NA quality for human oral microbiome studies.

## Introduction

The oral cavity harbours one of the most diverse microbiomes in the human body, comprised of viruses, fungi, protozoa, archaea and bacteria. Recent studies have profiled oral microbiomes on a species level using NGS technologies; collectively, these studies offer key insights into host-microbiome interactions, with particular relevance for oral health and bacterial disease. However, the quality and interpretation of NGS data may be undermined at several steps – from sample collection, storage, and DNA extraction to PCR bias, sequencing errors, and statistical analyses. Nucleic acid fidelity and consistency is crucial to the internal validity of such studies, suggesting the importance of preservatives. The current study examined the oral microbiota from saliva DNA, which was preserved and isolated with different methods; measures such as reproducibility and quality of readings were evaluated in each case, with the goal of identifying optimal methodologies for future studies.

## Methods & Materials

Table 1. Summary of preservation and DNA isolation methods

Preservative	DNA Isolation Method			
	Alcohol precipitation (Norgen)	EtOH precipitation (Competitor)	Spin column (Norgen)	Magnetic bead (Norgen)
Norgen	1-2	5-6	9-10	13-14
Leading Competitor	3-4	7-8	11-12	15-16

- ❖ Saliva samples were collected from a healthy volunteer, and preserved using Norgen's preservative or the leading competitor's preservative.
- ❖ In duplicates, DNA was isolated using Norgen's Saliva DNA Isolation Kit (Cat # RU45400), with either the Alcohol Precipitation, Magnetic Bead, or Spin Column system; or the leading competitor's DNA Ethanol Precipitation kit.
- ❖ The default Norgen method (Norgen's preservative and Norgen's Alcohol isolation) was used in samples 1-2. The default competitor method (competitor's preservative and competitor's EtOH isolation) was used in samples 7-8.
- ❖ The bacterial composition of each sample was determined using bar-coded Illumina MiSeq sequencing of the bacterial hypervariable V4 region of 16S rDNA

## Results & Discussion

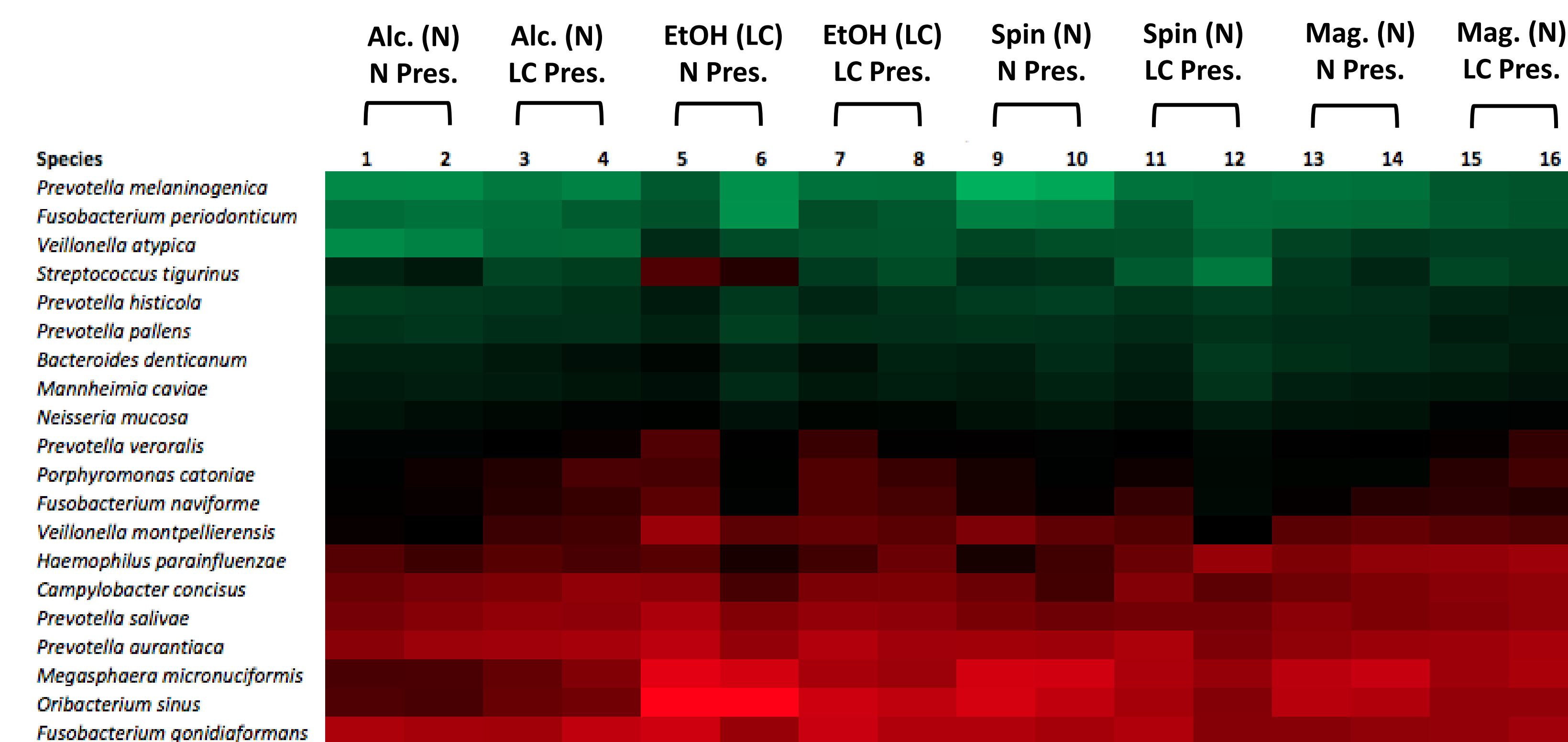


Figure 1. Heat map of relative frequencies of bacterial species identified in differentially processed saliva samples. "N" = Norgen; "LC" = leading competitor; "Pres." = preservative; "EtOH" = ethanol precipitation method; "Alc." = alcohol precipitation method; "Spin" = spin column method; "Mag." = magnetic bead method

- ❖ Greater consistency between duplicates was observed in samples processed with Norgen's preservative
- ❖ Norgen's default method (1-2) yielded more enriched readings than competitor's default method (7-8)
- ❖ The greatest reproducibility was observed using Norgen's Spin Column method (9-12), irrespective of preservative

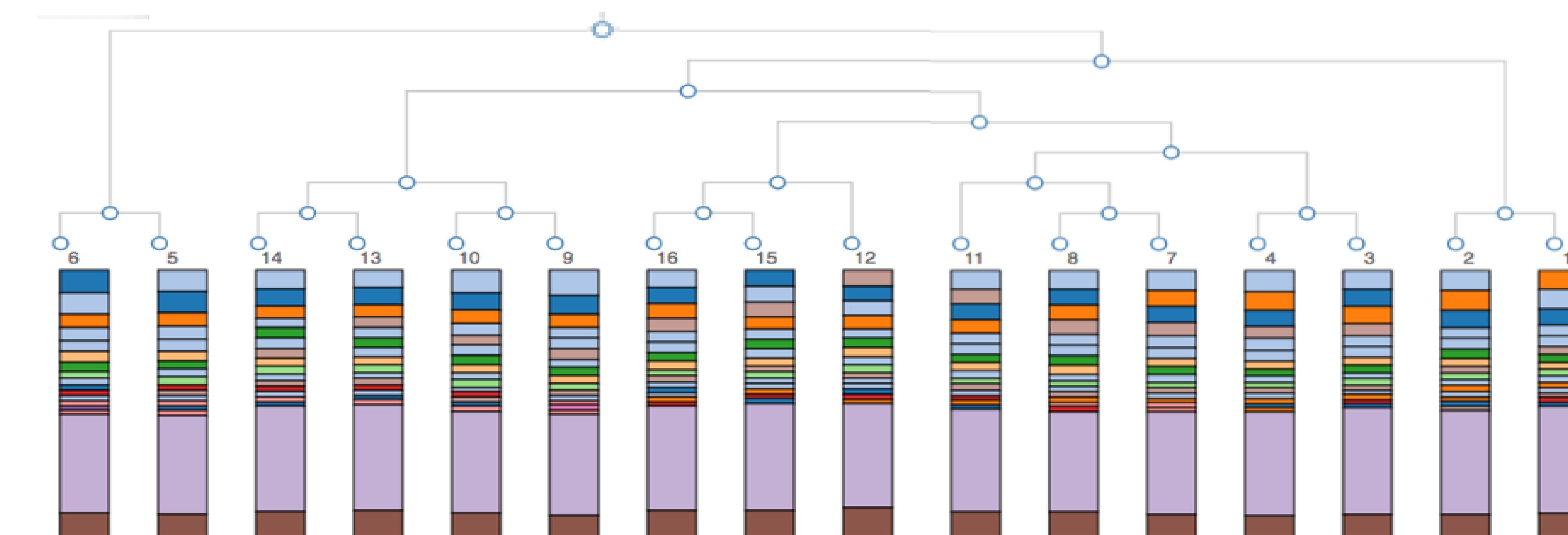


Figure 2. Dendrogram of hierarchical clustering of saliva samples based on genus-level classifications. The barchart beneath each sample show the relative abundance of its genus-level classifications.

- ❖ Consistently tight coupling was observed between duplicates of Norgen-preserved samples (1-2, 5-6, 9-10, 13-14) including those processed using the leading competitor's EtOH method (5-6)
- ❖ Lower reproducibility was observed in competitor-preserved samples processed with Norgen's Spin Column method, leading to greater branch separation of duplicates (11-12)

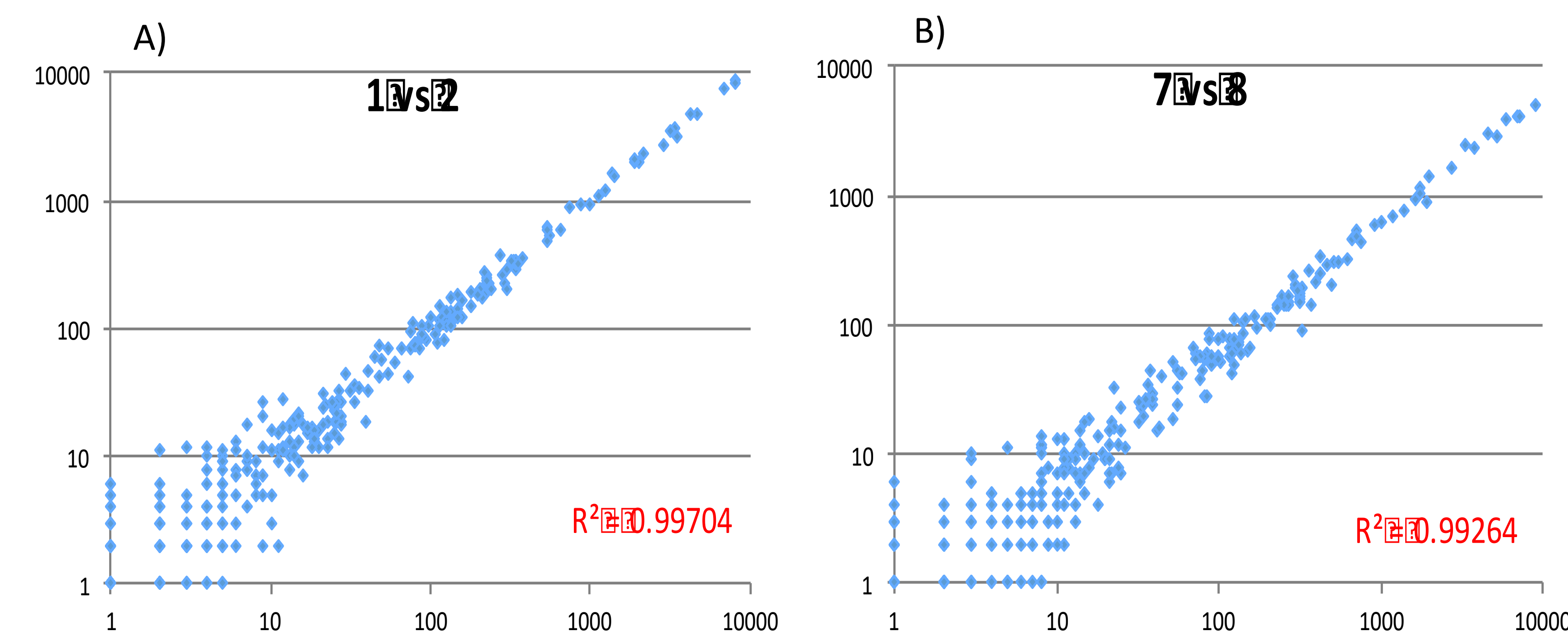


Figure 3. Correlation between duplicate reads using (A) Norgen's default preservative and isolation method, and (B) leading competitor's default preservative and isolation method

- ❖ A greater  $R^2$  was observed for Norgen's default preservation and isolation method, suggestive of higher consistency between duplicates and lesser variability
- ❖ For other sample pairs (not shown), the  $R^2$  ranged from 0.98728, obtained in samples 11-12 (Norgen's Spin Column with the leading competitor's preservative), to 0.99715, obtained in samples 5-6 (leading competitor's EtOH with Norgen's preservative). This suggests that the leading competitor's isolation method was more consistent when used with Norgen's preservative

## Conclusions

1. Norgen's saliva DNA preservative was able to consistently yield more enriched readings than the leading competitor's saliva DNA preservative for 16S rDNA oral microbiome metagenomics; the greatest reproducibility and read quality was achieved using Norgen's Spin Column method with Norgen's preservative, although the leading competitor's preservative performed similarly when used with Norgen's Spin Column method.
2. Norgen's default preservation and isolation method yielded greater consistency than the leading competitor's default preservation and isolation method; the leading competitor's isolation method performed better when used concomitantly with Norgen's preservative, as opposed to with the leading competitor's preservative
3. The benefit of this study may be found in the transportation and handling of human specimens for use in sensitive downstream applications such as NGS; preservatives and isolation methods play a crucial role in ensuring reliable, accurate data.