



The Saliva RNA Interactome

The Salivary Transcriptome Revealed by Massively Parallel (RNA) Sequencing

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Introduction

In the past years, the nature, origin and characterization of salivary mRNA have been actively pursued ([Hu et al., 2007](#); [Li et al., 2004a](#); [Park et al., 2006](#); [Park et al., 2007](#); [Zhang et al., 2010](#)) and we have shown that saliva contains a transcriptome with discriminatory power for disease detection. Expanding upon those studies, we employ the first massively parallel (RNA) sequencing to compare relative abundance of annotated transcripts, non-annotated transcripts, non-coding transcripts and alternative transcript isoforms in human saliva.

Hypothesis

- Saliva contains more than just quantifiable messenger RNA.
- Saliva harbors a complex, dynamic transcriptomic network: the Saliva RNA Interactome.

Material & Methods

Normal subjects

Saliva samples were obtained from healthy individuals (age: 34 ±1.4 years) from the UCLA School of Dentistry, Dental Research Institute, University of California, Los Angeles (UCLA), CA, in accordance with a signed consent form approved by the UCLA Institutional Review Board.

Figure 1: Human saliva collection.



Unstimulated human whole saliva (WS) and cell-free saliva (CFS) samples were collected from healthy individuals between 9 AM and 10 AM (Figure 1).

Subjects were asked to refrain from eating, drinking, and oral hygiene procedures for at least 1 hour prior to saliva collection. Saliva samples were kept on ice during collection.

Salivary RNA extraction

Total RNA was isolated from WS, CFS and murine saliva as described in the user's manual using the mirVana PARIS Kit with an on-column DNase digestion for 15 minutes at room temperature.

Yield and quality of RNA samples

The integrity of total RNA was evaluated by the aid of capillary electrophoresis, RNase and DNase digestion were performed to confirm the nucleic acids were RNA.

SOLID™ Total RNA-Sequencing

Strand-specific cDNA libraries were constructed from 50ng salivary RNA using the SOLID™ Total RNA-Seq Kit (Applied Biosystems by Life technologies, SOLID™ Total RNA-Seq Kit, Cat# 4445374) and as described in the user's manual.

Due to the presence of limiting amounts of partially fragmented salivary RNA, the RNA fragmentation reaction had to be optimized for saliva. RNase III fragmentation was performed for 0, 3 and 10 minutes with the optimal digestion time of 3 minutes.

Gel size selection was performed to obtain cDNAs with inserts ranging from 100 to 200 base pairs (bp) ending in a final library size of 150-300 bp.

Read Alignments

Human Genome:

Whole transcriptome reads were aligned to the human reference genome (hg18).

Human Oral Microbiome:

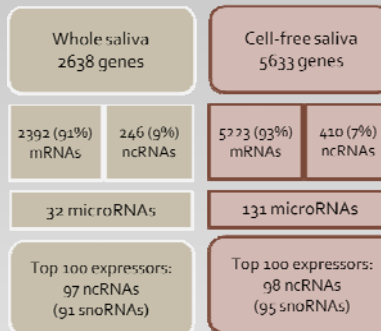
Whole transcriptome reads were also aligned to the Human Oral Microbiome Database and the HOMD 16S rRNA RefSeq database.

Results

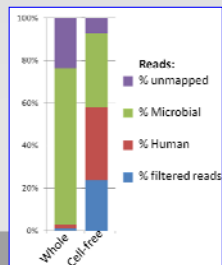
Saliva RNA amount (average yield)

Whole saliva: 30 ng/100 uL saliva
Cell-free saliva: 15 ng/100 uL saliva.

Transcription summary



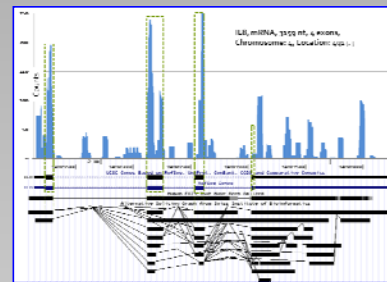
Alignment discrepancy



The majority (75%) of the reads in whole saliva are microbial RNAs. This microbial dominance is reducing the read sensitivity and thus less human transcripts have been detected.

Overall, we found around 600 different bacterial signatures in whole and around 250 in cell-free saliva.

Alternative splicing events

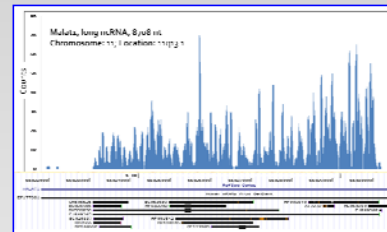


Exonic expression (green dotted bars) in Interleukin 8 (IL8) was present. Interestingly, we found additional expression pattern at the 5' and 3' ends and alignments to IL8 splice variants are suggesting alternative splicing events in this gene.

Furthermore, different expression profiles were found in the first intronic region. There are no annotations yet in this region and thus the expressions may imply unidentified exons, novel splice variants or snoRNAs.

These findings are unique, because so far, splice variants have not been investigated and described in human saliva.

Complete coverage across Malat1

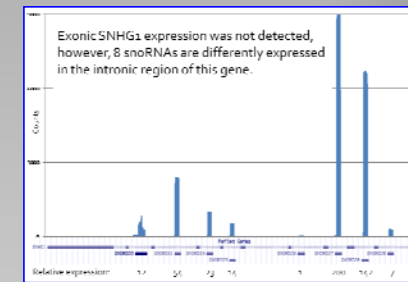


Salivary RNA was always found to be fragmented and we assumed that this is due to degradation processes.

Herewith, we get a new insight into the expression profile at each single nucleic acid position along transcripts and we find full coverage across Malat1, a long (8708 nt) non-coding RNA, in human saliva.

This finding is revolutionizing the transcriptomic picture of saliva and indicates that saliva has intact RNA with a presumably functional role.

Presence of snoRNA in saliva



To the best of our knowledge, this is the first evidence of small nucleolar RNA (snoRNA) in saliva.

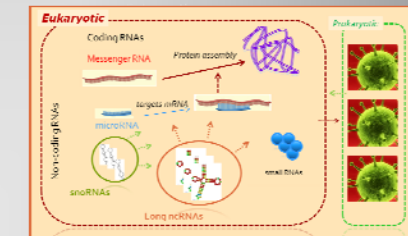
None of the SNHG1 exons is expressed, however, there are 8 snoRNA differently expressed within the intronic region of this gene.

Interestingly, even though two snoRNAs (SNORD26 and SNORD27) are direct genomic 'neighbors', their expression levels show a 200-fold difference which is similar to the findings in HeLa cells and blood.

Currently, snoRNAs are getting into the focus of interest because of their newly discovered role in gene expression.

Discussion

There is indeed more than quantifiable mRNA in saliva. We detected not only mRNAs and microRNAs but also snoRNAs and long ncRNAs in saliva.



These different RNA species have implicated roles in gene regulation based on cell models and the fact that they are all present in saliva is leading to the assumption that there is a saliva RNA interactome.

References

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