Better safe than sorry; RNA extraction method selection for salivary metatranscriptomics

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BACKGROUND AND AIM

WP2 aims to longitudinally characterise the microbiome in stool, blood, gastrointestinal biopsies and saliva using cohorts of diseased and healthy individuals. Part of this endeavor is conducting salivary metatranscriptomics. Metatranscriptomic analysis enables understanding of microbiomes their actual functional activities and has the potential to better associate host-microbiome interactions than sole taxonomic analysis. However, the approach is highly challenging due to numerous technical obstacles, including the short half-live of mRNA, presence of inhibitory substances, ineffective cell lysis and/or ineffective enrichment of bacterial mRNA. The choice of RNA extraction method can, as such, determine the success of the metatranscriptomics approach.

In order to make a well-considered decision regarding the RNA extraction approach for salivary metatranscriptomics, we conducted a pilot study to compare the performance of three commercially available RNA extraction kits.

METHODS

RNA was extracted from 200ul of three fresh saliva samples and three saliva samples from the StoolPredict cohort using three commercially available RNA extraction methods; 1) RNeasy Powermicrobiome kit from Qiagen (RNeasy), 2) Quick-RNA miniprep kit from ZymoResearch (Quick-RNA), 3) ZymoBIOMICS DNA/RNA Miniprep Kit from ZymoResearch (DNA/RNA). Due to limited materials, only one fresh sample was extracted with the DNA/RNA method.

Quality control, rRNA depletion, library preparation and RNA sequencing were performed by BaseClear B.V. (Leiden, The Netherlands). In short, rRNA depletion was performed using the Ribo-Zero Plus rRNA Depletion Kit (Illumina), followed by library preparation using the TruSeq RNA Library Prep Kit (Illumina). Remaining RNA was sequenced (Illimina NovaSeq6000 System; 150bp paired-end). A schematic representation of the workflow is shown in figure 1.

Reads representing ribosomal RNA (rRNA) which escaped the biochemical depletion step were identified by SortMeRNA, which filters reads with a database of known rRNA sequences.

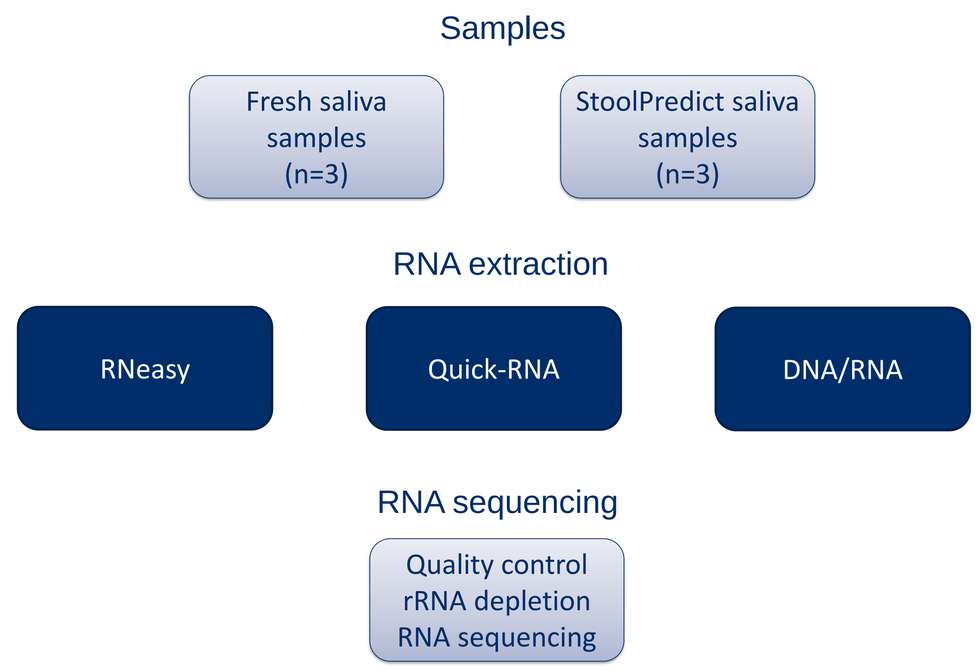

Figure 1 | A schematic overview of the RNA extraction and sequencing workflow.

RESULTS

The Quick-RNA miniprep kit (ZymoResearch) resulted in highest RNA concentrations for fresh and StoolPredict saliva samples, followed by the RNeasy Powermicrobiome kit (Qiagen) and the ZymoBIOMICS DNA/RNA Miniprep Kit (ZymoResearch) [Table 1].

Table 1 | RNA concentrations (ng/ul) per extraction method for fresh and StoolPredict saliva samples.

	Method	Sample 1	Sample 2	Sample 3	Average
Fresh	RNeasy	112,0	33,0	56,0	67,0
	Quick-RNA	140,0	130,0	132,0	134,0
	DNA/RNA		31,8		31,8
StoolPredict	RNeasy	42,2	54,0	7,1	34,4
	Quick-RNA	146,0	89,0	21,5	85,5
	DNA/RNA	42,5	20,3	6,2	23,0

Sequencing of obtained RNA resulted in high quality sequencing reads, of which on average ~16-17% was rRNA, independent of RNA extraction method [Table 2]. It should be noted, however, that two RNeasy-derived RNA samples did not pass quality control and were therefore not sequenced.

Table 2 | RNA sequencing quality determinants per extraction method for fresh and StoolPredict saliva samples. Averages are shown.

	Method	Read pairs	Quality	% rRNA
Fresh	RNeasy	4169999	36,0	17,2
	Quick-RNA	6543005	36,0	17,0
	DNA/RNA*	6492046	35,9	16,8
StoolPredict	RNeasy*	4177349	35,6	27,9
	Quick-RNA	5800311	36,1	16,0
	DNA/RNA	4662326	35,9	16,7

* Based on one sample.

CONCLUSION

- Choice of RNA extraction method affects RNA concentration and quality.
- Choice of RNA extraction method does not affect quality of sequencing data and the percentage of rRNA reads.

Based on these results, it was decided to use the Quick-RNA miniprep kit for salivary metratranscriptomics.

APPLICATION

The Quick-RNA miniprep kit was used to extract RNA from 299 StoolPredict saliva samples.

Sufficient RNA concentration (>1ng/ul) was obtained from 272 samples and these were submitted to BaseClear B.V. for rRNA depletion and sequencing.

We are currently awaiting the results from quality control and rRNA depletion.

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