ORAgene®•RNA

Stability and yield of RNA collected from saliva using Oragene®•RNA for expression analysis

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Introduction

Oragene®•RNA for expression analyis is the first available self-collection kit that is non-invasive and easy-to-use. To date, the most common sources for collecting human RNA are blood (white blood cells), biopsy and surgically resected samples. These traditional sources are invasive and present multiple challenges in sample collection, storage and transport.

In contrast, Oragene•RNA uses saliva as the sample source. The kit is easy-to-use, non-invasive and facilitates sample transport. After a donor spits 2 mL of saliva into the Oragene•RNA vial and the cap is tightened, RNA is released from cells and stabilized at room temperature. Sample collection methods and techniques that are non-invasive are increasingly popular, enable increased donor compliance rates, and reduce overall costs.

This white paper describes the quality, yield, and stability of saliva RNA samples collected using Oragene•RNA and stored at room temperature up to 8 weeks.

Materials and methods

Saliva samples were collected and purified according to protocols supplied with the Oragene•RNA kits. In brief, after collection[1] the samples were vigorously mixed and 250 µL aliquots were removed at pre-determined time points ranging from 0 days to 8 weeks. The remainder of each sample was stored at room temperature (~22-24°C). At each time point, the 250 µL aliquot was purified according to protocol[2]. A fraction of the purified RNA was run on a 1% agarose gel to examine the integrity of ribosomal RNA (rRNA) bands. A small fraction of the sample was used to measure total RNA yield and purity as assessed by absorbance at 260 nm, 280 nm, and 320 nm. Absorbance values were corrected for sample turbidity by subtracting the A_{320} value from A_{260} and A_{280} values. Finally, 6 µL of the purified sample was used to prepare cDNA according the instructions shipped with the Invitrogen M-MLV Reverse Transcriptase (Cat. No. 28025-013) using Invitrogen Random Primers (Cat. No. 48190-011). Real-time PCR assays were performed on a Corbett Rotorgene RG-3000A instrument using 1/100th of the cDNA per 25 µL PCR reaction.

Results

The average total RNA yield per 2 mL of saliva collected in Oragene•RNA was 23.4 µg with an average corrected A_{260}/A_{280} ratio of 1.9.

Donor	Total RNA µg/ 2 mL saliva	A_{260}/A_{280}	Donor	Total RNA µg/ 2 mL saliva	A_{260}/A_{280}
A	32.8	1.8	I	31.4	2.0
B	19.2	1.8	J	15.4	2.0
C	16.8	1.7	K	25.4	1.9
D	14.2	1.8	L	10.0	1.9
E	41.6	1.9	M	13.6	1.7
F	13.0	1.7	N	32.6	1.9
G	24.4	2.1	O	18.4	1.7
H	12.6	2.1	P	53.0	1.9
***Average**: 23.4 µg total RNA/2 mL saliva A_{260}/A_{280} = 1.9*					

***Table 1**: Yield and quality of RNA from saliva collected using Oragene•RNA.*

Saliva samples collected using Oragene•RNA were stored at room temperature for a period of 8 weeks. The stability of the RNA purified from these samples was assessed by examining the integrity of rRNA on an agarose gel and by real-time PCR probing for human mRNA (ribosomal 18S, β-actin, β-2-microglobulin, Interleukin-8, and Histatin-3). The agarose gel demonstrated that the rRNA remained intact at room temperature for at least 8 weeks.

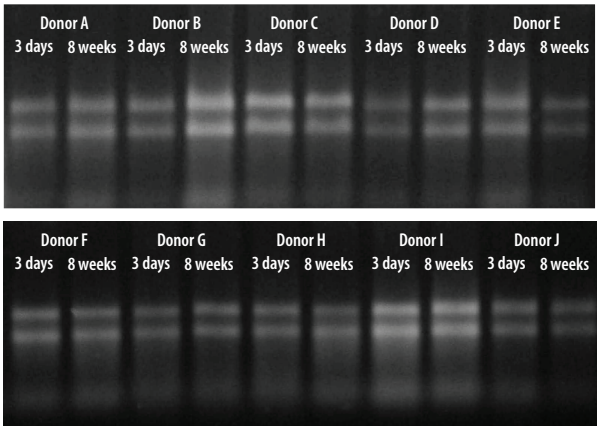

***Figure 1**: Agarose gel electrophoresis of rRNA extracted from Oragene•RNA/saliva samples stored at room temperature for 3 days and 8 weeks.*

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A real-time PCR assay was used to demonstrate stability of human RNA in Oragene•RNA/saliva samples stored at room temperature (Figure 2). The data shows that the Oragene•RNA solution stabilized RNA messages for 8 weeks as demonstrated by the low variability in Ct values. Real-time PCR signal for each of the 5 specific RNA messages did not deviate more than 2 Ct values (Figure 3). Results summarize the data for 5 gene products of interest: human 18S rRNA, β-actin, β-2-microglobulin, Interleukin-8, and Histatin-3.

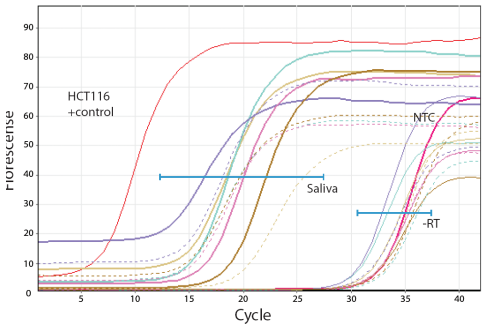

***Figure 2**: Real-time PCR data for 5 Oragene•RNA/saliva samples stored at room temperature for 1 (solid lines) and 8 weeks (dashed lines) before being purified and probed using primers for human 18S rRNA. RNA from cultured HCT116 cells was used as a positive control.*

Donor	Time (weeks)	Ct value for human 18S rRNA	-RT Ct value for human 18S rRNA	CT value for human ß-actin	-RT Ct value for human ß-actin
A	1	12.6	26.7	23.6	29.8
	8	13.0	29.1	23.2	30.2
B	1	13.7	27.9	23.2	32.6
	8	16.5	28.6	27.0	29.6
C	1	13.9	26.5	22.5	30.6
	8	13.8	27.8	23.0	31.9
D	1	13.7	28.4	26.2	31.8
	8	13.3	28.5	25.3	30.1
E	1	14.9	28.6	25.6	30.8
	8	13.6	29.1	22.7	28.1

***Table 2**: Summary of Ct values from real-time PCR data for human 18S rRNA and ß-actin mRNA.*

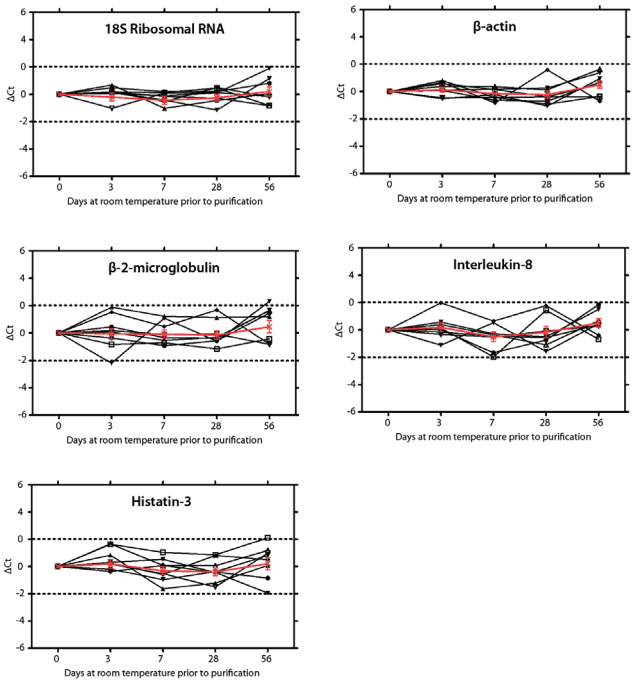

***Figure 3**: Summary of Ct values for 5 genes. RNA from saliva from 8 donors collected and stored up to 8 weeks was purified at given time points. Real-time PCR was used to detect the 5 genes of interest. Ct values at each time point were compared to Ct values obtained from samples purified immediately after collection. The red curve represents the average Ct for all donors.*

Conclusions

Oragene•RNA is a non-invasive RNA self-collection kit that can be used by untrained subjects to collect high quality RNA. The RNA collected and purified from saliva using Oragene•RNA is high quality, with an average corrected A_{260}/A_{280} of 1.9. Oragene•RNA can stabilize total RNA at room temperature for up to 8 weeks as demonstrated by agarose gel electrophoresis. Specifically, 5 human specific genes were demonstrated by real-time PCR to be stable at room temperature for 8 weeks.

References

1 *Oragene•RNA (RE-100) collection instructions. DNA Genotek. PD-PR-020.*
2 Oragene•RNA purification protocol using the Qiagen RNeasy Micro Kit for volumes up to 1,000 µL. DNA Genotek. PD-PR-021.