

Increasing DNA yield by collecting more saliva

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Abstract

The Oragene•DNA Self-Collection Kit instructs donors to collect 2 mL of saliva. If more DNA is desired, up to 4 mL of saliva can be collected for a proportional increase in DNA yield.

Introduction

The Oragene•DNA Self-Collection Kit collects a median of 110 µg of DNA from 2 mL of saliva (ref. 1). In the Oragene•DNA vial, the donor's saliva is mixed with 2 mL of Oragene•DNA fluid, which preserves the DNA in saliva at room temperature (ref. 2). For applications such as molecular epidemiology, some scientists would prefer to obtain even greater amounts of DNA. One way to increase the yield might be to collect an increased volume of saliva. The purpose of this study was to determine if the DNA yield from 4 mL of saliva was greater than from 2 mL, and whether the DNA stability was affected by changing the ratio of Oragene•DNA fluid to sample volume.

Materials and Methods

Saliva collection

Ten donors were enrolled in this study. Each donor was supplied with two Oragene•DNA vials—one standard vial with a fill-indicator at the 2 mL level, and a second vial with a modified fill-indicator at the 4 mL level. Donors were instructed to alternate spitting between the two vials, until they were filled to the 2 and 4 mL levels, respectively.

DNA purification and analysis

From each vial, a 100-µL aliquot was removed and purified according to the standard Oragene•DNA Purification Protocol (ref. 3). The DNA yield was determined using the Fluorescence/DNase (F/D) Assay (ref. 4), a fluorescent quantification assay using SYBR® Green (Molecular Probes). Agarose gel electrophoresis was used to assess the molecular weight of DNA purified from the Oragene•DNA/saliva samples stored at room temperature for 98 days. An equal amount of DNA (150 ng) was applied to each well.

Results

The DNA yields and actual saliva volumes for the 2 and 4 mL collections are shown in Table 1. The DNA yields for the 2 and 4 mL samples were extrapolated from the amount of DNA in the purified 100-µL aliquots. The average DNA yield was 46.9 and 83.3 µg for the 2 mL and 4 mL collections, respectively. An average of 2.4 mL of saliva was collected in the vials with the 2 mL fill-indicator, compared to 4.5 mL of saliva in the vials with the 4 mL fill-indicator.

Figure 1 shows the molecular weights of DNA purified from the 2 and 4 mL saliva samples after storage at room temperature for 98 days.

Donor	DNA yield for 2mL collection (µg)	DNA yield for 4mL collection (µg)	Actual volume of saliva for 2 mL collection (mL)	Actual volume of saliva for 4 mL collection (mL)
1	6.0	13.6	2.4	4.6
2	45.5	54.4	2.4	5.2
3	25.2	37.4	2.6	3.7
4	62.9	171.1	2.3	3.9
5	71.3	114.2	3.3	5.0
6	51.9	81.4	2.2	4.2
7	10.2	11.4	1.9	4.3
8	33.6	68.8	2.4	4.2
9	110.6	188.3	2.2	4.1
10	51.7	92.9	2.7	5.3
Average	46.9	83.3	2.4	4.5

Table 1. DNA yields and actual saliva volumes from the collection of 2 and 4 mL of saliva.

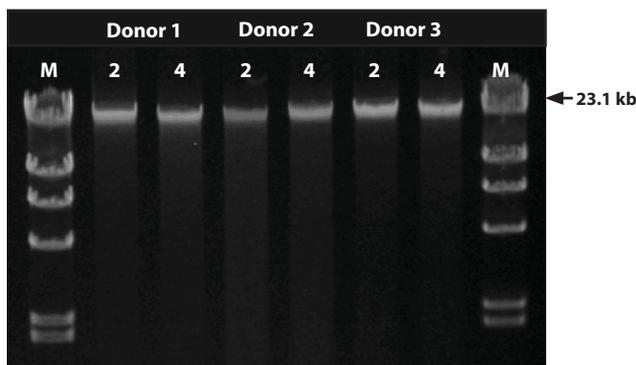


Figure 1. Agarose gel of DNA from Donors #1, 2, and 3. The DNA was purified from the 2 and 4 mL saliva samples after storage at room temperature for 98 days. A Lambda-Hind III digest was used as the marker.

Discussion and Conclusions

The DNA yield was increased proportionally to the saliva volume, with the 4-mL saliva samples yielding an average of 175% more DNA than the 2-mL samples. In a previous study with 208 donors (ref. 1), the median DNA yield was 110 ug from 2 mL of saliva. This is higher than the yield in this study and likely reflects the variability in DNA yield among different donors. In terms of DNA preservation, there was no observable difference in molecular weight between DNA purified from the 2 or 4 mL saliva samples after storage at room temperature for more than three months. In conclusion, the Oragene•DNA Self-Collection Kit can be used to collect and preserve DNA from 4 mL of saliva, with a proportionately higher yield than 2 mL of saliva.

References

1. Birnboim, H.C. (2004). DNA Yield with Oragene™•DNA DNA Genotek. PD-WP-001, April 05.
2. Birnboim, H.C. (2004). DNA Stability with Oragene™. DNA Genotek. PD-WP-005, Dec 04.
3. Laboratory Protocol for Manual Purification of DNA from 0.5mL of Oragene•DNA/ Saliva PD-PR-006
4. DNA Quantification Using the Fluorescence/DNase (F/D) Assay. (2004). DNA Genotek.