DNA genotek

Human genomic DNA content of saliva samples collected with the Oragene® self-collection kit†

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Estimating the amount of human genomic DNA in oral samples is important because normal oral samples (as compared to blood samples) contain variable amounts of bacterial DNA. We determined that the median bacterial DNA content in a series of 50 Oragene®/saliva samples was 11.8% of the total DNA, suggesting that 88.2% was of human origin. As an independent measure of the human DNA content, the TaqMan® RNase P assay was used. When corrected for efficiency using bacteria-free blood DNA from the same donors, the human DNA content was determined to be 80%. Thus, two complementary methods allow the same conclusion, that the vast majority of DNA in Oragene/saliva samples is of human origin.

Introduction

For any type of genetic analysis (e.g., clinical genetic testing, paternity testing, pharmacogenomic testing, population studies), non-invasive methods and self-collection techniques are preferred. For this reason, Oragene/saliva samples are often used as a convenient source of high quality DNA. Though Oragene/saliva samples contain a small amount of bacterial DNA in addition to human genomic DNA, this bacterial DNA has minimal practical significance. Numerous studies have shown that DNA from Oragene/saliva samples gives equivalent results to DNA from blood for applications such as PCR, SNP genotyping, microarrays and next-generation sequencing[1, 2, 3]. Furthermore, of all oral collection methods (e.g., swabs, mouthwash, cytobrush), the Oragene kit provides the highest yield and highest quality of human genomic DNA[4], with the lowest bacterial DNA content. This technical bulletin provides quantitative information about the amount of human genomic DNA in Oragene/saliva samples, as estimated by two different methods.

Materials and methods

Sample collection

Paired blood and saliva samples were collected from 50 donors. After providing informed consent, each donor was first asked to deliver 2 mL of saliva into an Oragene kit according to the instructions for use included with the kit. The saliva collection was unsupervised. Shortly after collection, the Oragene/saliva samples were mixed by shaking for 15 seconds and then stored at room temperature. A phlebotomist then drew 8 mL of venous blood from each donor into a BD Vacutainer EDTA tube (BD Cat. No. 36643). The blood samples were immediately placed onto a rocker and allowed to mix for >10 minutes to avoid the formation of microclots.

DNA purification

Saliva: several days after collection, a 500 µL aliquot of saliva was purified according to the optimized Oragene purification protocol using prepIT™•L2P (DNA Genotek) for 0.5 mL[5].

Blood: immediately after collection, the buffy coat was prepared by spinning the collected samples at 2500 × *g* for 10 minutes; plasma was discarded and the buffy coat was transferred to a microcentrifuge tube. DNA was purified from the buffy coat using Qiagen's QIAamp blood mini kit (Cat. No. 51106) according to the manufacturer's instructions.

Purified DNA from both sources was stored at -20°C.

DNA quantification

Aliquots of purified DNA from both blood and saliva were thawed at room temperature and then incubated at 50°C for 30 minutes to ensure that the high molecular weight DNA was fully dissolved.

† Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

The total DNA concentration was determined by fluorescence using SYBR® Green I dye (Invitrogen, Cat. No. S-7563) and a Rotor-Gene™ 6000 real-time thermal cycler (Corbett Life Science) according to the DNA Genotek DNA Quantification assay protocol[6].

The DNA was then diluted in TE to 7.5 ng/µL and the final concentration was verified using the DNA Quantification assay.

TaqMan RNase P assay to measure human genomic DNA: a Standard Curve was prepared using Human Genomic DNA supplied with the TaqMan RNase P Detection Reagents. A standard curve was prepared by serial dilution (5 ng to 30 ng/reaction).

Real-time PCR: the human content of the blood and saliva DNA samples was measured directly using the TaqMan™ RNase P Detection reagents (FAM) (ABI, Cat. No. 4316831) and the TaqMan Universal PCR mastermix no AMPErase UNG (ABI, Cat. No. 4324018). Real-time PCR was done according to the manufacturer's specifications. 15 ng (2 µL of 7.5 ng/µL) of total DNA was added to each reaction and the percent of human content was determined by dividing the amount of human DNA (as determined by the TaqMan assay) by the total amount of DNA added to the reaction (as determined by the DNA Quantification assay).

Bacterial DNA assay

The bacterial DNA assay developed by DNA Genotek was employed. The detailed protocol is available upon request.

Standard Curve: purified E. coli control DNA was obtained from Sigma (E. coli, strain B, Cat. No. D4889). A standard curve was prepared using a serial dilution (0.31 ng to 20 ng/reaction).

Real-time PCR: PCR primers were chosen from a region of the 16S rRNA gene that is known to be conserved across a wide variety of microorganisms, and is not found in eukaryotes[7]. The blood and saliva DNA was tested for the presence of 16S rRNA gene by real-time quantitative PCR using a Rotor-Gene 6000 real-time thermal cycler. Each reaction used 15 ng (2 µL of 7.5 ng/µL) total DNA, and the efficiency of the reactions was determined by running a second 15 ng aliquot of sample spiked with 5 ng of E. coli control DNA in parallel. The percent bacterial DNA content was determined by dividing the corrected amount of bacterial DNA (as determined by the Bacterial DNA assay) by the total amount of DNA added to each reaction (as determined by the DNA Quantification assay).

Results

Comparison of DNA samples spiked and not spiked with E. coli control DNA showed that the real-time PCR reaction was not 100% efficient; without the correction, the true amount of bacterial DNA would be underestimated. The median percent of bacterial DNA content, as determined by the Bacterial DNA assay, of total DNA from 50 blood samples was 0.03% (range 0% to 0.48%). The median percent of bacterial content of total DNA purified from 50 saliva samples was 11.8% (range 2.0% to 39.9%) (Table 1, Figure 1).

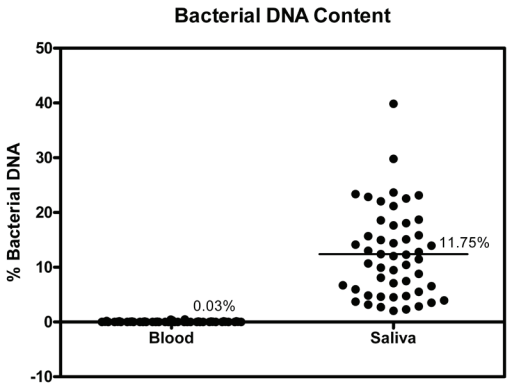

***Figure 1**: Bacterial DNA as a percentage of total DNA in 50 Oragene/saliva or blood samples. The horizontal line represents the median value.*

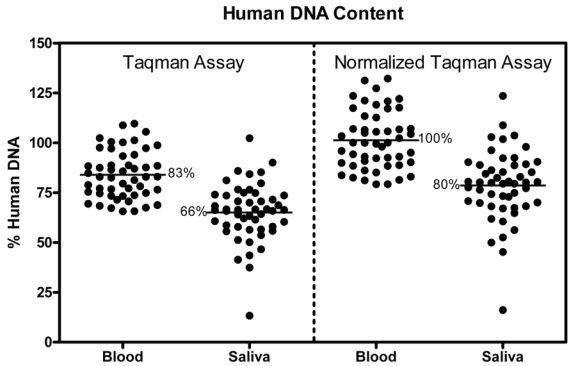

***Figure 2**: Human DNA as a percent of total DNA in 50 Oragene/saliva and blood samples. Left panel summarizes the TaqMan assay results while the right panel displays data normalized to 100% human DNA in blood samples. The horizontal lines represent the median value.*

	% Bacteria		% Human	
Donor	Blood	Saliva	Blood	Saliva
1	0.04%	22.04%	109.62%	61.48%
2	0.04%	5.53%	98.73%	70.69%
3	0.03%	11.47%	88.74%	71.51%
4	0.03%	9.47%	108.80%	85.86%
5	0.04%	13.01%	93.39%	66.25%
6	0.03%	3.16%	88.69%	85.24%
7	0.04%	21.16%	105.53%	66.44%
8	0.04%	10.70%	100.46%	102.31%
9	0.03%	14.39%	100.19%	76.49%
10	0.03%	2.83%	102.41%	84.32%
11	0.17%	4.52%	75.36%	64.28%
12	0.48%	9.91%	82.50%	56.43%
13	0.06%	2.29%	68.81%	55.55%
14	0.31%	14.12%	82.95%	41.39%
15	0.12%	15.84%	70.58%	50.15%
16	0.45%	15.10%	76.48%	46.57%
17	0.12%	23.36%	97.17%	63.32%
18	0.09%	12.03%	97.58%	81.16%
19	0.12%	18.70%	82.96%	65.84%
20	0.09%	18.04%	94.03%	74.82%
21	0.05%	29.79%	67.26%	60.34%
22	0.06%	17.63%	82.90%	73.98%
23	0.04%	6.72%	65.66%	62.07%
24	0.06%	7.46%	71.44%	60.71%
25	0.02%	10.44%	81.31%	79.72%
26	0.02%	12.77%	73.64%	63.99%
27	0.02%	3.94%	65.68%	69.95%
28	0.03%	8.79%	76.75%	57.81%
29	0.08%	3.52%	77.15%	13.33%
30	0.09%	22.84%	74.83%	53.67%
31	0.01%	12.41%	73.37%	68.72%
32	0.01%	8.08%	88.46%	73.60%
33	0.00%	4.60%	87.03%	73.48%
34	0.01%	39.86%	88.30%	51.27%
35	0.01%	12.30%	87.49%	66.36%
36	0.00%	2.04%	84.79%	68.31%
37	0.01%	2.72%	79.57%	66.76%
38	0.01%	23.66%	86.57%	55.85%
39	0.01%	23.13%	68.40%	58.03%
40	0.01%	14.98%	78.83%	66.69%
41	0.02%	18.58%	101.22%	74.99%
42	0.02%	7.09%	87.66%	56.54%
43	0.01%	6.54%	67.52%	65.83%
44	0.01%	3.72%	77.14%	90.11%
45	0.01%	13.94%	73.31%	70.60%
46	0.01%	15.66%	78.14%	66.59%
47	0.01%	4.74%	69.40%	76.59%
48	0.01%	22.55%	97.33%	58.62%
49	0.01%	4.84%	85.70%	37.44%
50	0.01%	5.98%	74.53%	43.53%
Median	0.03%	11.75%	82.92%	66.30%

***Table 1**: Detailed results for each donor showing the calculated bacterial or human DNA as a percent of total DNA from Oragene/saliva or blood samples.*

Discussion

Some previous studies have reported that the amount of human genomic DNA in oral samples is only a small percentage of the total DNA due to the presence of a large amount of bacterial DNA. For example, Feigelson et al. (2001) found that the median percentage of bacterial DNA in mouthwash samples was 66% of the total DNA.

Similarly, Garcia-Closas et al. (2001) found that the median percentage of bacterial DNA was 50.5% in mouthwash samples and 88.5% in cytobrush samples (Table 2).

Collection method	Median percentage of bacterial DNA	References
Mouthwash	66.0%	Feigelson et al. (2001)
Mouthwash	50.5%	Garcia-Closas et al. (2001)
Cytobrush	88.5%	Garcia-Closas et al. (2001)
Oragene/saliva	11.8%	

***Table 2**: Comparison of the median percentage of bacterial DNA obtained using various oral collection techniques.*

In contrast to these reports, we report that the vast majority of DNA from Oragene/saliva samples is of human origin, with a median bacterial content of only 11.8%. The difference between our results and reports using other collection methods can be explained by the fact that the Oragene kit contains

antibacterial agents, which prevent the growth of bacteria between the time of collection and the time of DNA purification.

In this report, we have used two complementary methods to assess the amount of human DNA present in the total DNA purified from saliva samples. First, we used a Bacterial DNA assay developed by DNA Genotek, which directly measures bacterial DNA content using real-time PCR targeting a highly conserved region of bacterial 16S rRNA gene. Our assay includes an internal standard to more accurately calculate the amount of bacterial DNA in a sample. PCR efficiency was decreased by an average of 25.9% when analyzing both blood and saliva samples, suggesting it was independent of the purification method. After correction for PCR efficiency, the Bacterial assay indicated that DNA from blood had a median bacterial content of 0.03%, and DNA from saliva had a median bacterial content of 11.8%. This low amount in blood is expected since blood from healthy individuals contains no bacteria. In contrast, there are bacteria in the oral cavity and therefore saliva can be expected to contain bacterial DNA. Notably, bacterial DNA in saliva samples collected in the Oragene kit is appreciably lower than oral samples collected by other methods (Table 2). The results of the Bacterial DNA assay indicate that a median of ~12% of oral DNA collected with Oragene kit is bacterial and suggest that a median of 88% is of human origin.

To complement the results of the Bacterial DNA assay, we used a second method (the TaqMan RNase P Detection assay) to estimate the content of human DNA in saliva. As a control, the same assay was used to measure the human DNA content of blood, which is 100% human in origin. Presumably due to incomplete removal of all inhibitors during routine isolation of DNA from blood, the human DNA content was only 82.9% (range 65.7% to 109.6%) (Figure 2 and Table 1). If one were to assume that the remainder was bacterial DNA, one would incorrectly conclude that the bacterial content was 17.1%, something that clearly cannot be the case for a blood sample. Similarly, the percentage of human DNA from saliva is underestimated to be 66.3% (Figure 2 and Table 1), an amount that corresponds to previously published results using this assay[8]. By normalizing the 82.9% value obtained for blood DNA to 100%, then the true value for human DNA in saliva can be estimated to be 80%, a value in good agreement with the Bacterial DNA assay results.

Conclusion

In summary, our Bacterial DNA assay (when used with an internal standard) is as accurate a method as currently available for estimating the amount of bacterial DNA in a sample. This method estimates the human DNA in saliva samples, collected with the Oragene kit, to be about 88%. An independent method using the TaqMan RNase P assay produced similar results. We, therefore, conclude that the Oragene saliva sample kit is a reliable method for obtaining large amounts of human genomic DNA containing significantly less bacterial contamination than other oral collection methods.

References

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All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.