

### Short Communication

## Feasibility of Collecting Buccal Cell DNA by Mail in a Cohort Study<sup>1</sup>

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### Abstract

This study assessed the feasibility of obtaining buccal cell DNA by mail from participants in a large, community-based cohort study in Hawaii. Mouthwash collection kits were sent to a total of 355 randomly selected Japanese, Caucasian, and Hawaiian cohort members. Subjects were requested to swish 10 ml of mouthwash in their mouth for 60 s and expel it into a collection cup, which they mailed back to our laboratory. Half of the subjects were requested to collect a second sample. After up to two mailings and two reminder phone calls, two-thirds of the subjects returned a sample. The participation rate was lower for Hawaiians (59.0%) than for Caucasians (68.1%) and Japanese (76.3%). Participation was not affected by requesting two specimens. Participants did not differ from the total sample in terms of education and smoking status. The mean DNA yield was lower in females (41.7  $\mu\text{g}$ ) than males (53.4  $\mu\text{g}$ ) and in Japanese (37.8  $\mu\text{g}$ ) as compared with Hawaiians (51.9  $\mu\text{g}$ ) and Caucasians (54.5  $\mu\text{g}$ ). For subjects who returned two samples, the DNA yields were similar when both specimens were extracted in the same batch. All samples were successfully genotyped for polymorphisms in the *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, and *NQO1* genes by PCR-RFLP. From these and previous data, we conclude that, in situations where blood samples cannot be obtained, mail collection of mouthwash samples should be considered because it yields substantial amounts of high-quality genomic DNA for large numbers of study subjects.

### Introduction

Blood remains the specimen of choice for obtaining genomic DNA for epidemiological studies. However, alternate sources are needed, especially when subjects are reluctant to provide a blood sample or when they are geographically dispersed. To date, alternative methods have focused on collecting oral epithelial cells, either through swabs, brushes, or rinses (reviewed

in Refs. 1–3). We recently proposed a self-administered, non-invasive, and relatively inexpensive buccal cell collection procedure in which a small bottle of commercial mouthwash is mailed to subjects (1). Participants swish 10 ml of mouthwash in their mouth for 60 s and expel it into a collection cup, which is mailed back to the study center. The brand of mouthwash used contains alcohol to minimize bacterial growth during transportation. We showed that samples collected in this manner yield DNA of high molecular weight that can be used for a variety of PCR-based assays (1). The quantity and quality of the human DNA obtained by this protocol have now been further characterized by Garcia-Closas *et al.* (4). The method appears promising for large-scale studies because the samples have been shown to remain stable for several days in conditions mimicking shipment at room temperature and for at least a year during storage at  $-80^{\circ}\text{C}$  before DNA extraction (1, 4). We tested the feasibility of using the mouthwash method to collect genomic DNA by mail among the participants of a large, community-based, cohort study in Hawaii (the Multiethnic Cohort Study; Ref. 5). We focused on assessing the practicality and subject acceptability of this approach, as well as the likelihood of increasing the amount of DNA collected by obtaining two samples instead of one.

### Materials and Methods

The human subject protocol for the study was reviewed and approved by the Committee on Human Studies of the University of Hawaii. In the fall of 1998, we mailed a letter of invitation and a mouthwash collection kit to 355 subjects of either Japanese, Caucasian, or Hawaiian ancestry (about 60 in each ethnic group and sex group), randomly selected among Multiethnic Cohort Study participants residing in Hawaii who had mailed back a 26-page, baseline questionnaire in 1993–1996 (5). In this pilot study, up to two mailings and two reminder phone calls were made to each subject. The collection kit included a sealed 1.5-ounce bottle of a widely used mouthwash product (Scope), a plastic collection cup, an instruction sheet, a consent form, and a prepaid return envelope. To mimic the conditions of a full-fledged study, the consent form stated the purpose of the sample collection and the type of genotyping (*i.e.*, for genetic variants with low to moderate penetrance) that would be performed on the subjects' stored DNA as part of future studies. Subjects were randomized into two equal-sized groups within each ethnic group and sex group. One group was requested to collect two mouthwash samples, the first at night and the second the following morning, after brushing their teeth, two situations in which we thought subjects would be comfortable using mouthwash and likely to remember collecting the samples. The other group was asked to collect the morning sample only. All subjects, were instructed to mail back their sample(s) within 24 h of collection. The collection protocol was essentially unchanged from our previous report (1), except for switching to a mouthwash brand (Scope) with a lower alcohol content (14.3% alcohol). This was done to address reports by some subjects of a burning sensation with

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**Table 1** Comparison of subjects who returned a mouthwash specimen to the total study sample

	Participants ( <i>N</i> = 239)	Total sample ( <i>N</i> = 355)
Age (yrs)	62.7	63.0
Males (%)	50.6	49.7
Race		
Japanese (%)	37.7	33.3
Caucasians (%)	33.5	33.9
Hawaiian (%)	28.9	33.1
Education (yrs)	12.5	12.4
Smoking status (% ever smoker)	55.7	55.4

FreshBurst Listerine (21.6% alcohol), the mouthwash product used in the original study (1), and after verifying that total DNA yield, sample stability, and ability to PCR-amplify were similar for samples obtained with the two brands.<sup>3</sup> On arrival at our laboratory, samples were frozen at  $-20^{\circ}\text{C}$  until processing, which took place within 1 year of collection (within 4 months for the paired samples). Samples were extracted over a 13-month period with a DNA extraction kit (catalogue number 9304 until January 1999, when it was discontinued and replaced by catalogue number 51306; Qiagen, Valencia, CA). Total DNA concentrations were determined spectrophotometrically using a GeneQuant II RNA/DNA Calculator (catalogue number 80-2105-98; Pharmacia Biotech, Cambridge, United Kingdom). A detailed description of the collection kit, collection protocol, and DNA extraction method can be found on the World Wide Web.<sup>4</sup> Finally, DNA samples were assayed by the PCR-RFLP method for the same polymorphisms in the *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, and *NQO1* genes and using the same methods as described in our previous report (1). Mean DNA yields were compared using the unpaired or paired Student's *t* test after appropriate transformation of the variables.

## Results

A total of 239 subjects (121 males and 118 females) returned a mouthwash sample with a signed consent form. These included 90 Japanese, 80 Caucasian, and 69 Hawaiian subjects (age, 49–80 years; mean age, 63.4 years). The cumulative sample return rate was 34.6% after the first mailing, 53.5% after the second mailing, and 67.3% after up to two phone reminder calls. These figures were similar regardless of whether one or two samples were requested (one sample, 33.3%, 53.9%, and 70.0%; two samples, 36.0%, 53.1%, and 64.6%). In contrast, the overall participation rate was somewhat lower for Hawaiians (59.0%) than for Caucasians (68.1%) or Japanese (76.3%). An additional 5.5% of the subjects stated that they would participate but were too busy to do so before the end of the study period. Other reasons for nonparticipation were definite refusal (11.5%), death (3%), illness (0.6%), and inability to locate (12.1%). Table 1 compares selected characteristics for subjects who returned a mouthwash specimen with those for the entire random sample of the cohort. No differences were found between the two groups with regard to age, education, and smoking status (Table 1).

Only one sample was lost during shipping, apparently due to leakage, leaving a total of 238 subjects for analysis. The total

**Table 2** Mean DNA yield ( $\mu\text{g}$ ) from mouthwash samples

	<i>n</i>	Mean	SD	<i>P</i> <sup>a</sup>
All	238	47.7	$\pm 40.5$	
Sex				<0.02
Male	121	53.4	$\pm 49.8$	
Female	117	42.7	$\pm 26.9$	
Race				0.02
Japanese	89	38.2	$\pm 27.9$	
Caucasian	80	54.5	$\pm 48.3$	
Hawaiians	69	51.9	$\pm 42.6$	
Sample pairs	107			0.01
Sample 1		42.7	$\pm 41.3$	
Sample 2		33.8	$\pm 32.2$	
Sample pairs <sup>b</sup>	40			0.81
Sample 1		54.3	$\pm 59.7$	
Sample 2		52.6	$\pm 41.8$	

<sup>a</sup> *P* for differences between means.

<sup>b</sup> Samples from the same subjects extracted in the same batch.

DNA yield, as measured with the spectrophotometer, ranged from 2.2 to 362.8  $\mu\text{g}$  (median, 35.1  $\mu\text{g}$ ) and was comparable with that obtained in other studies (1, 4). The mean DNA yield (Table 2) was similar in Caucasians ( $54.5 \pm 48.3$   $\mu\text{g}$ ; median, 39.3  $\mu\text{g}$ ; range, 6.8–362.8  $\mu\text{g}$ ) and Hawaiians ( $51.9 \pm 42.6$   $\mu\text{g}$ ; median, 38.8  $\mu\text{g}$ ; range, 3.9–222.0  $\mu\text{g}$ ) but was lower in Japanese subjects [ $38.2 \pm 27.9$   $\mu\text{g}$ ; median, 30.1  $\mu\text{g}$ ; range, 2.2–177.1  $\mu\text{g}$  ( $P < 0.02$ )]. Samples from men yielded more DNA (mean,  $53.4 \pm 49.8$   $\mu\text{g}$ ; median, 35.0  $\mu\text{g}$ ; range, 3.9–362.8  $\mu\text{g}$ ) than did those from women [ $41.7 \pm 26.9$   $\mu\text{g}$ ; median, 35.2  $\mu\text{g}$ ; range, 2.2–150.8  $\mu\text{g}$  ( $P = 0.02$ )].

Based on previous data, we did not expect to find major differences in the DNA yields of repeat samples collected from the same individuals. However, among the subgroup of subjects who provided an evening sample and a morning sample in this study, the mean DNA yields obtained from the two samples were significantly different ( $42.7 \pm 41.3$  and  $33.8 \pm 32.2$   $\mu\text{g}$ ;  $P = 0.01$ ; Table 2). Nevertheless, in contrast to paired samples extracted on different days, no difference in DNA yield was found for the 40 pairs for which both samples were extracted together in the same batch ( $54.3 \pm 59.7$  and  $52.6 \pm 41.8$   $\mu\text{g}$ ;  $P = 0.81$ ), suggesting that the overall difference was due to laboratory drift. Only 10 subjects (9.3%) had DNA yields from both samples in the lowest quartile of the overall DNA yield distribution, indicating that collecting two samples markedly reduces the proportion of subjects with low amounts of DNA.

All samples obtained from the two groups of subjects were successfully amplified for the *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, and *NQO1* polymorphisms analyzed and yielded interpretable results on digestion and gel electrophoresis.

## Discussion

This study demonstrates under field conditions that it is possible, using a mail mouthwash protocol, to obtain genomic DNA adequate for PCR-RFLP-based genotyping from a large number of individuals participating in a multiethnic cohort study. It also shows that it is possible to almost double the amount of DNA collected by requesting two samples instead of one, without affecting the response rate. About two-thirds of the subjects returned a specimen, resulting in a study sample that appears to be comparable to the study population. We believe that this response rate is an underestimate because, due to time constraints, we were not able to follow-up on another 5.5% of the subjects who said that they would send their sample. We

<sup>3</sup> A. Lum-Jones and L. Le Marchand, unpublished data.

<sup>4</sup> <http://www.hawaii.edu/crch/CenPubsMouthwash.htm>.

also found that total DNA yield may be lower in some subgroups, namely, in women and in Japanese people. This may be the result of laboratory variation or may reflect less vigorous swishing among these groups or other unidentified factors.

The buccal cell DNA obtained with the mouthwash procedure is of sufficiently high quality to support PCR amplification for a variety of assays (1, 4). However, further work is needed to document the adequacy of mouthwash DNA for assays using emerging genotyping technologies. Using a similar collection protocol (which, however, did not include brushing teeth before sample collection), Garcia-Closas *et al.* (4) have recently estimated that about half of the total DNA extracted from mouthwash samples is human DNA. Thus, if this estimation is correct, the collection of two mouthwash samples should yield, on the average, about 50  $\mu\text{g}$  of human DNA, a quantity that would support a large number of PCR-based assays because PCR reactions usually require 50–100 ng of template DNA.

From the available data, we conclude that mail collection of mouthwash samples appears to offer a viable alternative to blood collection to obtain high-quality genomic DNA in large cohort studies. However, blood collection, when possible, remains the method of choice because it yields larger amounts of

high molecular weight DNA and provides other components (serum, plasma, and erythrocytes) that are useful to assess environmental exposures and phenotype/genotype relationships.

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