Published in final edited form as:

Am J Hum Biol. 2007 ; 19(3): 319–326. doi:10.1002/ajhb.20586.

New Saliva DNA Collection Method Compared to Buccal Cell Collection Techniques for Epidemiological Studies

NIKKI L. ROGERS^{1,*}, SHELLEY A. COLE², HAO-CHANG LAN², ALDO CROSSA¹, and ELLEN W. DEMERATH¹

¹Lifespan Health Research Center, Department of Community Health, Wright State University School of Medicine, Dayton, Ohio 45420

²Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, Texas 78245-0549

Abstract

Epidemiological studies may require noninvasive methods for off-site DNA collection. We compared the DNA yield and quality obtained using a whole-saliva collection device (Oragene™ DNA collection kit) to those from three established noninvasive methods (cytobrush, foam swab, and oral rinse). Each method was tested on 17 adult volunteers from our center, using a random crossover collection design and analyzed using repeated-measures statistics. DNA yield and quality were assessed via gel electrophoresis, spectrophotometry, and polymerase chain reaction (PCR) amplification rate. The whole-saliva method provided a significantly greater DNA yield (mean $\pm$ SD = $154.9 \pm 103.05 \mu\text{g}$, median = 181.88) than the other methods (oral rinse = $54.74 \pm 41.72 \mu\text{g}$, 36.56; swab = $11.44 \pm 7.39 \mu\text{g}$, 10.72; cytobrush = 12.66 ± 6.19 , 13.22 μg) (all pairwise $P < 0.05$). Oral-rinse and whole-saliva samples provided the best DNA quality, whereas cytobrush and swab samples provided poorer quality DNA, as shown by lower OD₂₆₀/OD₂₈₀ and OD₂₆₀/OD₂₃₀ ratios. We conclude that both a 10-ml oral-rinse sample and 2-ml whole-saliva sample provide sufficient DNA quantity and better quality DNA for genetic epidemiological studies than do the commonly used buccal swab and brush techniques.

Although DNA is easily obtained from blood, the desire for wider population sampling in large epidemiological studies sometimes necessitates home-based, noninvasive methods of DNA collection. The literature on noninvasive DNA methods to date has focused on the adequacy of DNA yields from buccal (cheek) cell and oral-rinse (mouthwash) samples for basic polymerase chain reaction (PCR) protocols and genotyping (e.g., Cozier et al., 2004; Garcia-Closas et al., 2001; Kozlowski et al., 2002; Lench et al., 1988). We focus here on obtaining adequate DNA yield and quality for high-throughput PCR for whole-genome marker mapping. In the only similar study we found to date, Meulenbelt et al. (1995) explored buccal cotton-swab sampling for an 800-marker whole-genome scan. They found that 20 swabs per individual were required to achieve their criterion of 40 μg DNA (mean) per subject.

The potential uses of human DNA for the study of disease risk are expanding rapidly beyond whole-genome linkage analysis to association studies using whole-genome amplification (WGA) (Carlson et al., 2004; Ehm et al., 2005), gene-expression analysis (Mak et al., 2004; Pravenec et al., 2003), and epigenetic studies (Egger et al., 2004; Jaenisch and Bird, 2003). Such methods are increasingly being used in large-scale epidemiological investigations. Using

collection methods that do not unduly burden study participants, while increasing DNA yield and quality at every collection opportunity, is therefore integral to improving participation rates for genetic epidemiologic studies. Further, genetic epidemiologists may wish to collect a DNA volume that is both adequate for immediate study needs and archiving for use in the future as new assays are developed (Holland et al., 2003).

Our goal in this analysis was to compare the DNA yield and quality of three established noninvasive DNA sampling methods (buccal cytobrush, buccal foam swab, and oral rinse) to a recently introduced whole-saliva collection kit (Oragene™ DNA collection kit, DNA Genotek, Inc., Ottawa, Ontario, Canada). Whole saliva has the potential advantage of providing large numbers of nucleated cells (e.g., epithelial cells, leukocytes) per sample. However, previous evaluations reported that yields from whole saliva were significantly (up to 50%) lower than yields from buccal cell and mouth-wash samples (van Schie and Wilson, 1997; Terasaki et al., 1998; Walsh et al., 1992). Whole-saliva collection and isolation using the Oragene™ DNA collection kit have yet to be independently evaluated.

MATERIALS AND METHODS

Adult volunteers (N = 17) aged 24–56 years were solicited from the staff and faculty of the Lifespan Health Research Center (Wright State University Boonshoft School of Medicine, Dayton, OH). Participants gave written informed consent and provided samples via each collection method to control for intra-individual variation in collection quality and amount. Donors refrained from eating or drinking for at least 60 min prior to each collection. One sample of whole saliva was collected from each participant using the Oragene™ DNA collection kit. Two buccal and mouthwash samples were collected via the swab, cytobrush, and oral rinse, so that one DNA sample could be isolated using a protocol typically used for that method (see below), and another sample could be processed and extracted using the Oragene™ solutions.

A randomized crossover design was used in which half of the participants were randomly selected to provide saliva samples on the first collection day, and the other half provided oral-rinse samples on the first collection day. Treatment groups were reversed the following day. A second crossover experiment was performed at least 1 week later, in which buccal cells were collected with cytobrush collection as treatment 1, and swab collection as treatment 2. Treatment groups were again reversed the following day.

Cytobrush and swab

Each participant was asked to 1) vigorously rinse his/her mouth with water for about 15 sec to remove food particles, and 2) rub one cytobrush (CytoSoft™ brush, Medical Packaging Corp., Camarillo, CA) or one foam swab (Puritan Medical Products Co., Guilford, ME) against his/her right cheek for 45 sec. Donors were reminded to turn the swabs to utilize both sides of the swab. In order to maximize buccal cell yield, cytobrushes and swabs were immediately frozen at –80°C and shipped to the laboratory (Southwest Foundation for Biomedical Research, San Antonio, TX) as frozen specimens.

Oral-rinse and whole-saliva collection

Participants were asked to rub their tongues around the inside of their mouths for about 15 sec and to swish vigorously with 10 ml Scope® Original Mint mouthwash (Proctor & Gamble, Cincinnati, OH) for 45 sec (see Heath et al., 2001). Participants were directed to expectorate the mouthwash into a clean specimen cup (120 cc, nonsterile). The cup was closed, sealed with tape, and packed in individual plastic bags. Samples were immediately frozen at –80°C and shipped to the laboratory as frozen specimens.

Whole saliva was collected using the Oragene™ DNA self-collection kit following the manufacturer's instructions (DNA Genotek, Inc., 2004b, 2006). Participants were asked to rub their tongues around the inside of their mouths for about 15 sec and then deposit approximately 2 ml saliva into the collection cup. When an adequate sample was collected, the cap was placed on the vial and closed firmly. The collection cup is designed so that solution from the vial's lower compartment is released and mixes with the saliva when the cap is securely fastened. This starts the initial phase of DNA isolation, and stabilizes the saliva sample for long-term storage at room temperature or in low-temperature freezers (Birnboim, 2004a; DNA Genotek, Inc., 2004a). Whole-saliva samples were stored and shipped at room temperature.

DNA isolation

DNA samples were isolated using standard methods designed to optimize DNA yield for each method. Chelex 100 Resin (Bio-Rad, Hercules, CA) was used to isolate DNA from the cytobrush and swab samples according to Walsh et al. (1991) by first following the protocol for handling the samples as oral swabs, and then performing the Chelex DNA extraction for cells in culture. A forensic phenol-chloroform method was used to isolate DNA from the oral-rinse samples (Lum and Le Marchand, 1998; Walsh et al., 1992). Whole-saliva samples were isolated according to the manufacturer's instructions (Chartier and Birnboim, 2004; DNA Genotek, Inc., 2006). In addition, the second set of samples from cyto-brush, swab, and oral rinse were isolated using the Oragene™ solutions as an experimental control. For this purpose, frozen brush and swab tips were clipped from the handles and immersed in Oragene™ saliva collection cups. The tops were sealed, allowing the isolation solution to enter the chamber, and the samples were inverted several times. The isolations were then continued following the manufacturer's instructions for buccal brushes and swabs (DNA Genotek, Inc., 2005). The oral-rinse samples were thawed and spun in a centrifuge to concentrate the epithelial cells. The cells were collected, resuspended in distilled water, and transferred to an Oragene™ saliva collection kit. The isolation was then conducted per the manufacturer's instructions.

DNA quantification and quality determinations

We estimated that a yield of $\geq 6 \mu\text{g}$ is required, based on our desire to perform 10-cM whole-genome scans with 400 microsatellite markers (assuming 15 ng of DNA per PCR reaction). DNA integrity was determined by gel electrophoresis, and DNA yield and quality were assessed by spectrophotometric analysis using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE). Absorbance of ultraviolet light at wavelengths of 230, 260, and 280 nanometers was used to calculate the $\text{OD}_{260}/\text{OD}_{280}$ and $\text{OD}_{260}/\text{OD}_{230}$ ratios to compare the ratio of nucleic acid concentration in the sample (OD_{260}) to that of protein and organics (OD_{280}), and salt and alcohol (OD_{230}) contaminants. A ratio of 1.7–1.8 is generally preferred for the $\text{OD}_{260}/\text{OD}_{280}$ ratio (indicating limited protein and organic contamination), and higher values are preferred for the $\text{OD}_{260}/\text{OD}_{230}$ ratio (indicating limited salt and alcohol contamination).

The isolated DNA was amplified by PCR to confirm its utility for genetic epidemiological studies. DNA samples (15 ng per reaction) were amplified with 14 primer pairs from the ABI PRISM Linkage Mapping Set, version 2.5, panel 14 (Applied Biosystems, Foster City, CA), designed to amplify microsatellite markers (with product size ranges of approximately 100–340 base pairs) for use in genetic linkage studies. The number and percentage of successful PCR amplifications were recorded for each set of samples.

Statistical analysis

Analyses were performed using SAS 9.1 for Windows (SAS Institute, Inc., Cary, NC). We first analyzed observations of DNA from each collection method isolated via protocols optimized for each method. PROC MIXED was used to test method differences in yield and

spectrophotometric absorbance ratios while accounting for the correlated measures data from each subject. "Subject" was therefore assigned as the repeated term, collection method was treated as a fixed effect/treatment, and a compound symmetry model was chosen as best-fitting for the covariance structure of the data. Comparisons were made using one-way, repeated-measures ANOVA with Bonferroni adjustment to maintain an overall alpha level of 0.05. Experiment-wise PCR amplification rates were compared by collection method, using Friedman's nonparametric statistic. Pairwise differences were assessed using the Wilcoxon signed rank test. Analyses were then repeated on cytobrush, swab, and oral-rinse samples isolated using the Oragene™ kit solutions, to determine if yield differences were due primarily to type of sample collection (whole saliva vs. buccal cells) or to the isolation protocols (Chelex and phenol-chloroform vs. Oragene™ solutions). These comparisons were conducted using PROC MIXED to obtain a two-way, repeated-measures ANOVA (two treatments: collection and isolation), again with Bonferroni adjustment.

RESULTS

Whole-saliva collection provided an average DNA yield that was significantly greater than all other methods (all $P < 0.05$). Median yield (182 μg per subject) was approximately three times the median yield of the oral rinse, and more than 12 times the median yields for the buccal swab and brush methods (Table 1). Both the oral-rinse and whole-saliva methods provided adequate DNA yield ($\geq 6 \mu\text{g}$) for a 400-microsatellite marker whole-genome scan for all participants. A majority of cytobrush samples met this criterion, but three samples did not (yields were 3.88–4.23 μg). Similarly, most of the samples collected by swabs provided adequate DNA yield, but five did not (yields were 2.37–5.04 μg). Given these results, one would need to collect at least 2 cytobrushes and 3 cotton swabs per subject to assure sufficient DNA yield to meet our minimum criterion of 6 μg .

DNA quality can be affected by collection method (primarily integrity and protein contamination) and by isolation method (integrity and protein, organic, salt, and alcohol contamination). As shown in Table 2, mean and median OD₂₆₀/OD₂₈₀ ratios for both the oral-rinse method and whole-saliva method fell within the criterion range (1.7–1.8), indicating acceptably low protein and organic contamination of their DNA products. The whole-saliva method had somewhat greater overall salt and alcohol contamination (range, 0.66–1.53; median, 1.12) than the oral-rinse method (range, 1.28–2.26; median, 2.05). Cytobrush and swab methods produced DNA products that had significantly greater protein/organic and salt/alcohol contamination (Table 2).

Success rates for PCR amplification of 14 genetic markers from the ABI Prism Linkage Mapping set (version 2.5) are compared across the four methods in Table 2. The mean amplification success rate was 99% for DNA isolated from whole saliva, and 98% for the oral-rinse samples. Mean PCR amplification rates for cytobrush (84%) and swab DNA (75%) were not significantly different from each other ($P = 0.13$), and were significantly lower than for oral-rinse DNA and whole-saliva DNA (largest $P < 0.0012$), and had lower PCR success ratios for the longer products. These results were consistent with the comparison of DNA integrity across methods using gel electrophoresis. High molecular weight DNA was obtained using all collection techniques and all isolation techniques, except in the case of the Chelex method, when it was used to isolate the cytobrush and swab DNA (data not shown).

To determine if the greater DNA yield and purity for the whole-saliva samples were due to the isolation protocol or mode of sample collection (whole saliva), the Oragene™ isolation solutions were used to isolate DNA for the three traditional methods. Mean DNA yield for cytobrush samples was significantly lower using the Oragene™ isolation protocol compared to the standard protocol, while mean yields for swab and oral-rinse samples were not

significantly affected by the isolation protocol (Fig. 1). These results indicate that the higher DNA yield observed for whole saliva was due to the large volume of nucleated cells from the 2-ml sample rather than to properties of the isolation protocol. DNA purity improved somewhat for the cytobrush (OD_{260}/OD_{280} ratio = 1.82 ± 0.11 vs. 1.10 ± 0.07 mean $\pm$ SD) and swab (OD_{260}/OD_{280} ratio = 1.50 ± 0.18 vs. 1.13 ± 0.07 mean $\pm$ SD), but decreased for the oral-rinse method (OD_{260}/OD_{280} ratio = 1.68 ± 0.08 vs. 1.78 ± 0.03 mean $\pm$ SD) when using the Oragene™ solutions for isolation. Similar trends were seen for the OD_{260}/OD_{230} ratio (data not shown), but the increase in the OD_{260}/OD_{230} ratio for the cytobrush and swab using the Oragene™ isolation protocol did not reach the level of purity observed for the whole-saliva or oral-rinse samples.

DISCUSSION

The Oragene™ saliva collection kit provided DNA yield for all participants (median, 181.88 μ g per subject; minimum, 16 μ g per subject) that greatly exceeded our baseline criteria required for a 10-cM, 400-microsatellite marker whole-genome scan (≥ 6 μ g). Oral-rinse samples also provided greater-than-adequate yields for all individuals (median, 36.56 μ g; minimum, 14 μ g). While the median DNA yield for the buccal cytobrush (median, 13 μ g) and swab (median, 11 μ g) collection methods exceeded our minimum criterion, 18% of cytobrush samples and 24% of swab samples fell below this criterion. Our yield results for these two buccal scraping methods agree with those reported in previous studies, which suggests that at least two (Cozier et al., 2004; Freeman et al., 1997; King et al., 2002; Neuhaus et al., 2004; Saftlas et al., 2004) and as many as seven (e.g., Tanigawara et al., 2001) swabs or brushes per individual would be required to meet this criterion.

The yield we observed for whole saliva is far greater than those reported by most previous studies of noninvasive DNA methods using cytobrush (Garcia-Closas et al., 2001; Saftlas et al., 2004), buccal swabs (Freeman et al., 1997; King et al., 2002; Meulenbelt et al., 1995), and oral rinse (Andrisin et al., 2002; Bauer et al., 2004; Cao et al., 2003; Cozier et al., 2004; Garcia-Closas et al., 2001; King et al., 2002; Le Marchand et al., 2001; Neuhaus et al., 2004; Tobal et al., 1989). Previous studies of DNA yield from whole saliva collected and processed by other methods (Harty et al., 2000; Ng et al., 2004; Terasaki et al., 1998; van Schie and Wilson 1997; Walsh et al., 1992) reported much lower yields than we observed using the Oragene™ kit (range, 8–30 μ g), despite similar amounts of collected saliva (1–2 ml). These earlier whole-saliva collection methods did not involve mixture of the sample with a buffered stabilizing solution immediately after collection, which may be the central advantage of the new kit. Our results for whole saliva using the Oragene™ kit were also higher than those reported by the manufacturer (median, 110 μ g) (Birnbom, 2004b).

The whole-saliva kit and the oral-rinse method provided high-quality DNA products. Both were characterized by acceptably low protein and organic contamination (OD_{260}/OD_{280} ratios within the criterion range of 1.7–1.8), and both had relatively low alcohol and salt contamination (OD_{260}/OD_{230} = 1.94 ± 0.32 for oral rinse and 1.12 ± 0.2 for whole saliva). In contrast, the buccal brush and swab methods provided DNA products of significantly lower quality. Our PCR amplification success rates are consistent with these DNA quality estimates, as the whole-saliva kit and oral-rinse methods showed greater rates of successful PCR amplification than the other two methods.

Our finding of excellent yields for both the oral-rinse and whole-saliva collection methods makes them suitable for genetic epidemiological studies, and we encourage direct comparison of the long-term stability of these two methods over various isolation and storage conditions. The Oragene™ saliva collection kit is reported to be stable at room temperature for a period of up to 12 months from time of collection, and may be stored for longer periods of time at –

20°C to prevent sample evaporation (Birboim, 2004a; DNA Genotek, Inc., 2004a). We did not test these claims in this study, but, if accurate, this would be a key advantage in the shipping and storage of DNA samples for large-scale epidemiological studies with off-site collection. Oral-rinse samples were reported to be stable sources of DNA for 1–2 weeks at room temperature (Hayney et al., 1996; Heath et al., 2001; Lum and Le Marchand 1998), though Feigelson et al. (2001) reported significant reductions in DNA yield after 10 days. At least one study (Garcia-Closas et al., 2001) reported no significant reduction of DNA yield following 1-year storage at –80°C. These issues are important to fully understand the benefits and caveats for implementing each method in large epidemiological studies.

Basic PCR amplification is typically used to test DNA integrity (Andrisin et al., 2002; Cozier et al., 2004; Garcia-Closas et al., 2001; Heath et al., 2001; King et al., 2002; Le Marchand et al., 2001), and we therefore used this benchmark for comparison. Our results and other initial reports indicate that DNA products from whole saliva collected using the Oragene™ kit may be of sufficient quality to use with specific SNP and WGA technologies (Chartier, 2005; Chartier and Birboim, 2005; Chartier and Pinard, 2005a,b), but we encourage independent investigators to examine these applications.

The primary strengths of the current study include using the same 17 individuals in a randomized crossover comparison, which allowed us to use statistical methods for repeated measures to control for interindividual factors that may have confounded other studies. To date, this design has only been used in two similar studies of noninvasive DNA collection methods (King et al., 2002; Saftlas et al., 2004). Further, the fact that we used the Oragene™ kit solutions to isolate samples from each of the three other collection methods allowed us to confirm that the whole-saliva collection method itself, and not its associated isolation solutions, was primarily responsible for the higher DNA yields.

As the first test of a new whole-saliva collection method, this study has limitations. First, the small sample size did not permit us to examine ethnic and age differences that were suggested to affect the yield of DNA collection methods (Garcia-Closas et al., 2001; Le Marchand et al., 2001; van Schie and Wilson, 1997). Second, because all samples were collected on-site from staff at our research center, we cannot measure possible differential participation and return rates for the different specimens, which is an important aspect of judging the feasibility of a new data-collection method for off-site epidemiological studies. Third, the spectrophotometry method that we used to estimate amount of DNA does not differentiate between human and nonhuman DNA sources. To minimize the effect of contaminating DNA, we had participants refrain from eating and rinse their mouths out with water prior to sample collections. We also immediately froze the oral-rinse and buccal collection samples to avoid bacterial growth. The manufacturer states that the median bacterial content of samples collected through the Oragene™ DNA collection kit is 6.8% (Chartier and Birboim, 2004), but this may vary based on the relative amounts of cell constituents in saliva (e.g., viruses, fungi, bacteria, food residues) (Edgar and O'Mullane, 1996; van Schie and Wilson, 1997). This compares favorably to the medians for bacterial DNA reported in mouthwash samples (34%, Feigelson et al., 2001; 49.5%, Garcia-Closas et al., 2001) and cytobrush (11.5%, Garcia-Closas et al., 2001). Nevertheless, even if the nonhuman DNA content was higher for the whole-saliva samples than reported, the greater overall yield would still provide more usable DNA than the other three methods.

In conclusion, both a 10-ml oral-rinse/mouthwash sample and a 2-ml whole-saliva sample provided adequate DNA yield and quality for a 10-cm, 400-microsatellite marker whole-genome scan. The commonly used buccal abrasion collection methods (cheek swabs and brushes) provided significantly lower DNA yield and relatively poor DNA quality relative to the whole-saliva and oral-rinse methods. With consideration of the logistic requirements, cost,

and results of previous studies, we suggest that either an oral-rinse/mouthwash sample or a whole-saliva sample suitably stabilized upon collection would be better suited than commonly used buccal abrasion methods for genetic epidemiological studies. The choice between these two methods depends on the expected time between collection and isolation, cost, labor requirements, and the DNA yield desired for both immediate use and archiving for future analyses.

Acknowledgments

We thank Dr. Abirami Chidambaram for expert technical advice, and our study volunteers for their participation.

Grant sponsor: NIH: NICHD, NIDDK, NHLBI; Grant numbers: HD12252, DK64870, DK64391, HL69995; Grant sponsor: National Center for Research Resources, NIH; Grant number: Research Facilities Improvement Program Grant 1 C06 RR13556-01.

LITERATURE CITED

- Andrisin TE, Humma LM, Johnson JA. Collection of genomic DNA by the noninvasive mouthwash method for use in pharmacogenetic studies. *Pharmacotherapy* 2002;22:954–960. [PubMed: 12173798]
- Bauer JE, Rezaishiraz H, Head K, Cowell J, Bepler G, Aiken M, Cummings KM, Hyland A. Obtaining DNA from a geographically dispersed cohort of current and former smokers: use of mail-based mouthwash collection and monetary incentives. *Nicotine Tobacco Res* 2004;6:439–446.
- Birnboim, HC. DNA stability with Oragene™. DNA Genotek, Inc.; Ottawa: 2004a.
- Birnboim, HC. DNA yield with Oragene™. DNA Genotek, Inc.; Ottawa: 2004b.
- Cao W, Hashibe M, Rao JY, Morgenstern H, Zhang ZF. Comparison of methods for DNA extraction from paraffin-embedded tissues and buccal cells. *Cancer Detect Prev* 2003;27:397–404. [PubMed: 14585327]
- Carlson CS, Eberle MA, Kruglyak L, Nickerson DA. Mapping complex disease loci in whole-genome association studies. *Nature* 2004;429:446–452. [PubMed: 15164069]
- Chartier, J. Oragene™ and whole genome amplification with GenomiPhi™. DNA Genotek, Inc.; Ottawa: 2005.
- Chartier, J.; Birnboim, H. Bacterial DNA content with Oragene™. DNA Genotek, Inc.; Ottawa: 2004.
- Chartier, J.; Birnboim, H. Oragene™ is compatible with TaqMan® SNP genotyping. DNA Genotek, Inc.; Ottawa: 2005.
- Chartier, J.; Pinard, M. Oragene™ and GenomePlex® whole genome amplification. DNA Genotek, Inc.; Ottawa: 2005a.
- Chartier, J.; Pinard, M. Oragene™ and REPLI-g® whole genome amplification. DNA Genotek, Inc.; Ottawa: 2005b.
- Cozier YC, Palmer JR, Rosenberg L. Comparison of methods for collection of DNA samples by mail in the Black Women's Health Study. *Ann Epidemiol* 2004;14:117–122. [PubMed: 15018884]
- DNA Genotek, Inc.. Long-term storage of Oragene™ samples. DNA Genotek, Inc.; Ottawa: 2004a.
- DNA Genotek, Inc.. Oragene™ DNA self-collection kit. DNA Genotek, Inc.; Ottawa: 2004b.
- DNA Genotek, Inc.. Saliva collection with cotton swabs or buccal brushes. DNA Genotek, Inc.; Ottawa: 2005.
- DNA Genotek, Inc.. Oragene™ product brochure. DNA Genotek, Inc.; Ottawa: 2006.
- Edgar, W.; O'Mullane, D., editors. Saliva and oral health. British Dental Association; London: 1996.
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;429:457–463. [PubMed: 15164071]
- Ehm MG, Nelson MR, Spurr NK. Guidelines for conducting and reporting whole genome/large-scale association studies. *Hum Mol Genet* 2005;14:2485–2488. [PubMed: 16037068]
- Feigelson HS, Rodriguez C, Robertson AS, Jacobs EJ, Calle EE, Reid YA, Thun MJ. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. *Cancer Epidemiol Biomarkers Prev* 2001;10:1005–1008. [PubMed: 11535555]

- Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R. DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behav Genet* 1997;27:251–257. [PubMed: 9210796]
- Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, Bender PK, Beck JC, Le Marchand L, Lum A, Alavanja M, Hayes RB, Rutter J, Buetow K, Brinton LA, Rothman N. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiol Biomarkers Prev* 2001;10:687–696. [PubMed: 11401920]
- Harty LC, Garcia-Closas M, Rothman N, Reid YA, Tucker MA, Hartge P. Collection of buccal cell DNA using treated cards. *Cancer Epidemiol Biomarkers Prev* 2000;9:501–506. [PubMed: 10815695]
- Hayney MS, Poland GA, Lipsky JJ. A noninvasive “swish and spit” method for collecting nucleated cells for HLA typing by PCR in population studies. *Hum Hered* 1996;46:108–111. [PubMed: 8666410]
- Heath EM, Morken NW, Campbell KA, Tkach D, Boyd EA, Strom DA. Use of buccal cells collected in mouthwash as a source of DNA for clinical testing. *Arch Pathol Lab Med* 2001;125:127–133. [PubMed: 11151066]
- Holland NT, Smith MT, Eskenazi B, Bastaki M. Biological sample collection and processing for molecular epidemiological studies. *Mutat Res* 2003;543:217–234. [PubMed: 12787814]
- Jaenisch R, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet [Suppl]* 2003;33:245–254.
- King IB, Satia-Abouta J, Thornquist MD, Bigler J, Patterson RE, Kristal AR, Shattuck AL, Potter JD, White E. Buccal cell DNA yield, quality, and collection costs: Comparison of methods for large-scale studies. *Cancer Epidemiol Biomarkers Prev* 2002;11:1130–1133. [PubMed: 12376522]
- Kozlowski LT, Vogler GP, Vandenberg DJ, Strasser AA, O'Connor RJ, Yost BA. Using a telephone survey to acquire genetic and behavioral data related to cigarette smoking in “made-anonymous” and “registry” samples. *Am J Epidemiol* 2002;156:68–77. [PubMed: 12076890]
- Le Marchand L, Lum-Jones A, Saltzman B, Visaya V, Nomura AM, Kolonel LN. Feasibility of collecting buccal cell DNA by mail in a cohort study. *Cancer Epidemiol Biomarkers Prev* 2001;10:701–703. [PubMed: 11401922]
- Lench N, Stanier P, Williamson R. Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 1988;1:1356–1358. [PubMed: 2898042]
- Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. *Cancer Epidemiol Biomarkers Prev* 1998;7:719–724. [PubMed: 9718225]
- Mak RH, Chang SL, Draksharapu A, Pak YK. Gene expression in uremic left ventricular hypertrophy: effects of hypertension and anemia. *Exp Mol Med* 2004;36:251–258. [PubMed: 15272237]
- Meulenbelt I, Droog S, Trommelen GJ, Boomsma DI, Slagboom PE. High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *Am J Hum Genet* 1995;57:1252–1254. [PubMed: 7485180]
- Neuhaus T, Geisen G, Bolt HM, Janzen V, Kraemer A, Vetter H, Ko Y. Reliability of non-invasively acquired human genomic DNA as a substrate for real-time PCR-assisted analysis of genetic polymorphism. *Arch Toxicol* 2004;78:390–396. [PubMed: 15007541]
- Ng D, Koh D, Choo S, Ng V, Fu Q. Effect of storage conditions on the extraction of PCR-quality genomic DNA from saliva. *Clin Chim Acta* 2004;343:191–194. [PubMed: 15115694]
- Pravenec M, Wallace C, Aitman TJ, Kurtz TW. Gene expression profiling in hypertension research: a critical perspective. *Hypertension* 2003;41:3–8. [PubMed: 12511522]
- Saftlas AF, Waldschmidt M, Logsden-Sackett N, Triche E, Field E. Optimizing buccal cell DNA yields in mothers and infants for human leukocyte antigen genotyping. *Am J Epidemiol* 2004;160:77–84. [PubMed: 15229120]
- Tanigawara Y, Kita T, Hirono M, Sakaeda T, Komada F, Okumura K. Identification of N-acetyltransferase 2 and CYP2C19 genotypes for hair, buccal cell swabs, or fingernails compared with blood. *Ther Drug Monit* 2001;23:341–346. [PubMed: 11477314]
- Terasaki P, Chia D, Sugich L. Saliva as DNA source for HLA typing. *Hum Immunol* 1998;59:597–598. [PubMed: 9757916]
- Tobal K, Layton DM, Mufti GJ. Non-invasive isolation of constitutional DNA for genetic analysis. *Lancet* 1989;2:1281–1282. [PubMed: 2573798]

- van Schie RC, Wilson ME. Saliva: a convenient source of DNA for analysis of bi-allelic polymorphisms of Fc gamma receptor IIA (CD32) and Fc gamma receptor IIIB (CD16). *J Immunol Methods* 1997;208:91–101. [PubMed: 9433465]
- Walsh DJ, Corey AC, Cotton RW, Forman L, Herrin GL, Word CJ, Garner DD. Isolation of deoxyribonucleic acid (DNA) from saliva and forensic samples containing saliva. *J Forensic Sci* 1992;37:387–392. [PubMed: 1500889]
- Walsh P, Metzger D, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 1991;10:506–513. [PubMed: 1867860]

Fig. 1.

Comparison of mean DNA yields ($\pm$ SD) by collection method: effect of using Oragene™ isolation protocol vs. standard isolation protocol for each method. ^aSignificantly different from whole saliva. ^bSignificantly different from oral rinse. ^cSignificantly different from cytobrush. ^dSignificantly different from swab. *Mean DNA yield significantly lower (paired *t*-test, $P < 0.05$) using Oragene™ isolation protocol rather than standard isolation protocol. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE 1Comparison of DNA yield (μg) by collection method¹

	Cytobrush	Swab	Oral rinse	Whole saliva
Isolation protocol	Chelex	Chelex	Phenol-chloroform	Oragene™ solutions
N	17	17	17	17
Mean $\pm$ SD	12.66 $\pm$ 6.19 ²	11.44 $\pm$ 7.39 ²	54.74 $\pm$ 41.72 ²	154.9 $\pm$ 103.05 ³⁴⁵
Median	13.22	10.72	36.56	181.88
Minimum	3.88	2.37	14.23	16.07
Maximum	22.19	29.07	140.72	314.38

¹ Mean DNA yields were compared using differences in least square means. Bonferroni adjustment was done to control for type I error rate for multiple comparisons.

² Significantly different from whole saliva.

³ Significantly different from oral rinse.

⁴ Significantly different from cytobrush.

⁵ Significantly different from swab.

TABLE 2Comparison of DNA quality by collection method¹

Collection method	Cytobrush	Swab	Oral rinse	Whole saliva
Isolation method	Chelex	Chelex	Phenol-chloroform	Oragene™ solutions
N	17	17	17	17
OD ₂₆₀ /OD ₂₈₀				
Mean ± SD	1.1 ± 0.07 ^{2,3}	1.13 ± 0.07 ^{2,3}	1.78 ± 0.03 ^{4,5}	1.74 ± 0.07 ^{4,5}
Median	1.10	1.13	1.77	1.76
Minimum	0.96	1.02	1.70	1.55
Maximum	1.23	1.29	1.83	1.81
OD ₂₆₀ /OD ₂₃₀				
Mean ± SD	0.22 ± 0.03 ^{2,3}	0.22 ± 0.03 ^{2,3}	1.94 ± 0.32 ^{2,4,5}	1.12 ± 0.2 ^{3,4,5}
Median	0.22	0.22	2.05	1.12
Minimum	0.19	0.18	1.28	0.66
Maximum	0.28	0.29	2.26	1.53
PCR amplification (percent amplified)				
Mean ± SD	84 ± 14% ^{2,3}	75 ± 19% ^{2,3}	98 ± 5% ^{4,5}	99 ± 4% ^{4,5}
Median	86%	86%	100%	100%
Minimum	50%	36%	86%	86%
Maximum	100%	100%	100%	100%

¹ OD₂₆₀/OD₂₈₀, ratio of optical density at 260 nm to optical density at 280 nm, a measure of protein and organic contamination; OD₂₆₀/OD₂₃₀, ratio of optical density at 260 nm to optical density at 230 nm, a measure of salt and alcohol contamination Mean DNA yields were compared using differences in least square means. Bonferroni adjustment was done to control for type I error rate for multiple comparisons.

² Significantly different from whole saliva.

³ Significantly different from oral rinse.

⁴ Significantly different from cytobrush.

⁵ Significantly different from swab.