DNA from Nails for Genetic Analyses in Large-Scale Epidemiologic Studies

DNA from Nails for Genetic Analyses in Large-Scale Epidemiologic Studies

Janneke G.F. Hogervorst[1], Roger W.L. Godschalk[2], Piet A. van den Brandt[1], Matty P. Weijenberg[1], Bas A.J. Verhage[1], Leonie Jonkers[1,2], Joy Goessens[1], Colinda C.J.M. Simons[1], Joris R. Vermeesch[3], Frederik J. van Schooten[2], and Leo J. Schouten[1]

Abstract

Background: Nails contain genomic DNA that can be used for genetic analyses, which is attractive for large epidemiologic studies that have collected or are planning to collect nail clippings. Study participants will more readily participate in a study when asked to provide nail samples than when asked to provide a blood sample. In addition, nails are easy and cheap to obtain and store compared with other tissues.

Methods: We describe our findings on toenail DNA in terms of yield, quality, genotyping a limited set of SNPs with the Sequenom MassARRAY iPLEX platform and high-density genotyping with the Illumina HumanCytoSNP_FFPE-12 DNA array (>262,000 markers). We discuss our findings together with other studies on nail DNA and we compare nails and other frequently used tissue samples as DNA sources.

Results: Although nail DNA is considerably degraded, genotyping a limited set of SNPs with the Sequenom MassARRAY iPLEX platform (average sample call rate, 97.1%) and high-density genotyping with the Illumina HumanCytoSNP_FFPE chip (average sample call rate, 93.8%) were successful.

Conclusions: Nails are a suitable source of DNA for genotyping in large-scale epidemiologic studies, provided that methods are used that are suitable or optimized for degraded DNA. For genotyping through (next generation) sequencing where DNA degradation is less of an issue, nails may be an even more attractive DNA source, because it surpasses other sources in terms of ease and costs of obtaining and storing the samples.

Impact: It is worthwhile to consider nails as a source of DNA for genotyping in large-scale epidemiologic studies.

See all the articles in this *CEBP Focus* section, "Biomarkers, Biospecimens, and New Technologies in Molecular Epidemiology."

Cancer Epidemiol Biomarkers Prev; 23(12); 2703–12. ©2014 AACR.

Introduction

Nails contain genomic DNA that can be used for genetic analyses, which is important for large epidemiologic studies that have collected nail clippings at baseline and for future epidemiologic studies that consider collecting nails as a DNA source for genetic analyses. The DNA in nail clippings (keratinous tissue) originates from germinal matrix cells in the nail root. During nail growth, these cells differentiate into nail plate and are filled with keratin. In this keratinization phase, the cells undergo programmed cell death, which results in considerable DNA fragmentation (1). Once the nail root cells are keratinized (and the DNA fragmented) during nail growth, the keratin tissue probably protects the DNA from further damage because keratinous tissue makes the DNA less accessible to oxidants and does not contain water. Water in samples leads to DNA damage through hydrolytic deamination of cytosine (1).

Nails are easy and cheap to obtain and store compared with other tissues such as blood or saliva samples. Moreover, study participants are likely to be more willing to participate in a study in which they are asked to provide nail samples than when they are asked to provide a blood sample.

In a previous publication, we described the use of toenails as a source of genomic DNA for genotyping a limited set of 10 single-nucleotide polymorphisms (SNP; ref. 2). We concluded that DNA could be successfully isolated from 20-year-old toenail material from the Netherlands Cohort Study on diet and cancer (NLCS) at a higher success rate compared with buccal swabs, and that we could successfully genotype 10 SNPs simultaneously, by

[1]Department of Epidemiology, School for Oncology and Developmental Biology (GROW), Maastricht University, Maastricht, the Netherlands. [2]Department of Toxicology, School for Nutrition, Toxicology, and Metabolism (NUTRIM), Maastricht University, Maastricht, the Netherlands. [3]Laboratory for Cytogenetics and Genome Research, Centre for Human Genetics, University Hospital Leuven, KU Leuven, Leuven, Belgium.

Current address for J. Goessens: Department of Human Movement Sciences, School for Nutrition, Toxicology, and Metabolism (NUTRIM), Maastricht University, Maastricht, the Netherlands.

Corresponding Author: Janneke G.F. Hogervorst, Department of Epidemiology, School for Oncology & Developmental Biology (GROW), Maastricht University Medical Centre, PO Box 616, 6200 MD Maastricht, the Netherlands. Phone: 31-433882385; Fax: 31-433884128; E-mail: jgf.hogervorst@maastrichtuniversity.nl

doi: 10.1158/1055-9965.EPI-14-0552

means of multiplex PCR amplification and single base extension. Importantly, 20-year-old toenail DNA performed just as well as DNA from freshly clipped nails, as expected because of the keratinous tissue protecting the DNA from further damage.

Since our previous article on DNA from toenails, we have explored the further possibilities for using toenail DNA for other genetic analyses. In the era of genome-wide association studies, 10 SNPs are a limited number and it is important to know how well toenail DNA performs in arrays focused on determining many genetic markers at once.

In the present article, we report on the yield and purity of DNA from nails, the application of toenail-derived DNA in the Sequenom MassARRAY iPLEX for the genotyping of SNPs (3), and the application of toenail-derived DNA in the HumanCytoSNP_FFPE-12 DNA Array (>262,000 genetic markers) after restoration with the Infinium HD FFPE DNA Restore Kit (4). We discuss our findings together with findings from other studies on nail DNA and we compare nails as a source of DNA to other frequently used sources of DNA.

Materials and Methods

Toenails

The toenails used for the studies described in this article originate from NLCS. The NLCS is a prospective cohort study, which started in 1986 and includes 120,852 subjects ages 55 to 69 years (5). At baseline (1986), the cohort members completed a self-administered food-frequency questionnaire on dietary habits and other risk factors for cancer and approximately 90,000 (∼75%) participants provided toenail clippings in a small paper envelope, which were collected initially with the purpose of determining selenium status. The envelopes with the clippings have since been stored (>25 years) in a basement with no climate control, without any further treatment of the nails.

DNA isolation

For the isolation of the DNA used in all the pilot studies described in this article, the DNA extraction protocol of Cline and colleagues (6) was chosen as the standard protocol, with some adjustments (2). To remove possible nail polish, nails (15 mg) were twice soaked in acetone for 10 minutes and dried. Exogenous material was removed by incubating the nails in 200 μL 1% SDS/25 mmol/L EDTA and 1 μL 20 mg/mL proteinase K for 1 hour. The nails were then rinsed three times in MilliQ and incubated in 200 μL of 2 mol/L NaOH on an automated vortex overnight to dissolve the nail material. The solution was neutralized by adding 100 μL of 200 mmol/L Tris (pH, 7–8) and 34.5 μL of 11.6 mol/L HCl. Diluted NaOH was added to redissolve the precipitates. Next, an equal volume of phenol/chloroform was added to the neutralized sample, mixed for 10 minutes by inversion, and centrifuged at 19,000 $\times g$ for 15 minutes at 4°C. The aqueous top layer was transferred to a clean tube and DNA was precipitated by adding 1/10 volume (35 μL) of 3 mol/L sodium acetate (pH, 5.2), 2 volumes (700 μL) of 95% ethanol and 1 μL 20 mg/mL glycogen overnight at −20°C. DNA was pelleted by centrifugation at 19,000 $\times g$ for 30 minutes at 4°C, washed once in 700 μL 75% ethanol, centrifuged at 19,000 $\times g$ for 15 minutes at 4°C, after which the supernatant was removed and the pellet dried and resuspended in 100 μL 10 mmol/L Tris–HCl (pH, 8).

Currently, we have isolated DNA from toenail clippings of more than 21,000 NLCS study participants, among which are samples of controls ($n = 3{,}856$) and of various cancer case groups with 20.3 years of follow-up: head and neck ($n = 507$), esophageal ($n = 359$), stomach ($n = 795$), colorectal ($n = 3{,}560$), pancreatic ($n = 544$), lung ($n = 2{,}593$), melanoma (isolation in progress), breast (n women $= 2{,}512$; n men $= 31$), endometrial ($n = 434$), ovarian ($n = 400$), advanced prostate ($n = 1{,}016$), invasive bladder ($n = 845$), renal cell ($n = 585$), and brain (isolation in progress) cancers, and lymphatic malignancies (isolation in progress).

Results

DNA yield and purity

DNA yield and purity of the toenail samples was assessed using the NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific). The average yield of DNA obtained from the toenails using the protocol described above was 393 ± 873 ng/mg toenail (average ± SD). It should be noted that the spectrophotometrically assessed amount of DNA is not only human DNA, because bacterial and fungal DNA may be present in the nail material. In addition, phenol used for isolation of the DNA has its absorption peak at 270 nm, which, to some extent, carries over to absorbance at 260 nm, and also the absorption peak of proteins at 280 nm overlaps with absorption at 260 nm. Thus, the amount of true human DNA in the toenail DNA samples may be overestimated to varying degrees when assessed with the NanoDrop.

The average A260/A280 ratio of the toenail DNA samples was 1.5 ± 0.2, which is lower than the ratio of pure double-stranded DNA (i.e., ∼1.8). This could be due to contamination of the samples with proteins (e.g., keratin) and phenol.

The average A260/A230 ratio of the samples was 0.7 ± 0.5, substantially lower than the ratio of pure double-stranded DNA, which is ∼2.0. This could indicate that the toenail DNA samples are contaminated with organic compounds and salts used for DNA isolation such as EDTA, sodium acetate, ethanol, and glycogen. We have tried to purify the isolated toenail DNA samples using GlycoBlue Coprecipitant, but this led to considerable losses of DNA. As shown later in this article, toenail DNA rendered good results in the genotyping assays that we have applied so far and, therefore, we have not yet studied other ways to purify the toenail DNA with high DNA recovery.

Other studies have also determined DNA yields from nails, but their protocols for DNA extraction and techniques to quantify DNA yield were different from those that we used. Klassen and colleagues (7) obtained a yield of 9.9 ± 1.7 ng/mg fingernail DNA, using a fluorescence method to quantify DNA, which specifically assesses double-stranded DNA. Using a commercial DNA extraction kit, Park and colleagues (8) obtained a yield of 68.7 ng/mg finger or toenail using NanoDrop to quantify DNA, but only 2.9 ng/mg nail when using PicoGreen. When they used NanoDrop, fingernails had almost two times more DNA yield than toenails, but with PicoGreen, the yield of toenails was slightly higher than that of fingernails. Also using a commercial DNA isolation kit, Tanigawara and colleagues (9) obtained 25 to 50 ng/mg fingernail as assessed spectrophotometrically. Using a protease derived from musk melons, Yoshida-Yamamoto and colleagues (10) achieved yields up to 250 ng/mg fingernail using phenol–chloroform extraction and NanoDrop to quantify DNA, which is comparable with what we observed. The average A260/A280 ratio of their melon-based protease-treated DNA samples was slightly better than the ratio of our samples, namely 1.7. This can be explained by the fact that the protease from the musk melon has a higher keratinolytic activity than proteinase K (10). When these authors used proteinase K to digest protein, they achieved a DNA yield of 60 ng/mg nail, which corresponds exactly to the yield that Kaneshige and colleagues (11) had for fingernails also using proteinase K digestion. Nakashima and colleagues (12) obtained 100 ng DNA per mg of nail using crushed fingernail clippings. Matsuzawa and colleagues (13) obtained enough DNA from a 1 × 10 mm piece of fingernail to do at least 500 PCR reactions requiring 5 ng each using finely crushed fingernail clippings and a commercial DNA extraction kit.

In conclusion, the yield of DNA from nails seems to vary according to the protocol by which DNA was isolated. The method that we have used, as described in this article, seems to have the highest DNA yield. In addition, DNA yield varies according to the method by which it was quantified. Most studies have used NanoDrop and the yield across the studies varied from 60 to 393 ng/mg nail. PicoGreen only measures double-stranded DNA, whereas single-stranded DNA is also suitable for PCR and genotyping. Both NanoDrop and PicoGreen are not specific for human DNA, and NanoDrop in addition measures RNA, nucleotides, and protein at 260 nm.

Therefore, we recently tested the amplification of a 596-bp sequence in the *cytochrome P4501A2* (*CYP1A2*) gene and a 197-bp sequence in the *myeloperoxidase* (*MPO*) gene in a qPCR analysis in 30 toenail samples. Input toenail DNA was 2 ng as determined by NanoDrop. Human high-molecular weight DNA was obtained from A549 cells and a calibration curve was obtained by 10-fold dilutions of 2 ng to 0.002 ng DNA. We determined threshold cycle numbers of the toenail DNA samples, and the amount of amplifiable DNA in toenail DNA was then determined by using the calibration curve. The starting toenail DNA amount for the PCR of *MPO* as derived from the calibration curve was only 0.021 ± 0.006 ng, which equals approximately 1% of the spectrophotometrically determined input DNA. Because toenail DNA is highly fragmented, we expected the production of the *CYP1A2* amplicon to be less effective compared with the *MPO* amplicon. Indeed, amplification of the 600-bp product resulted in 0.006 ± 0.001 ng input DNA, which is only approximately 0.3% of the amount that was expected spectrophotometrically. These results are in line with data from Park and colleagues (8), who measured only a fraction (~3%) of the DNA yield that they measured with NanoDrop when they used PicoGreen. Thus, when using nail DNA for genetic analyses, it is important to take into account that NanoDrop overestimates the amount of amplifiable DNA (>200 bp) in the samples and that more DNA should be used than what is required for a certain assay when DNA concentration is quantified with NanoDrop. However, using the PicoGreen method to assess the amount of sample needed to meet input DNA requirements for a genotyping assay probably leads to an overestimation of the amount of sample needed because single-stranded DNA is also useful for genotyping. Thus, when setting up a study using nails as DNA source for genotyping, it is best to first test what the optimal amount of input nail DNA for that specific assay is.

Length of fragments and PCR success rate of toenail DNA

Toenail DNA is highly fragmented. We investigated DNA fragment length of our toenail samples with agarose gel electrophoresis. Most of the fragments appeared to be <200 bp, as shown in Fig. 1, but larger fragments were present.

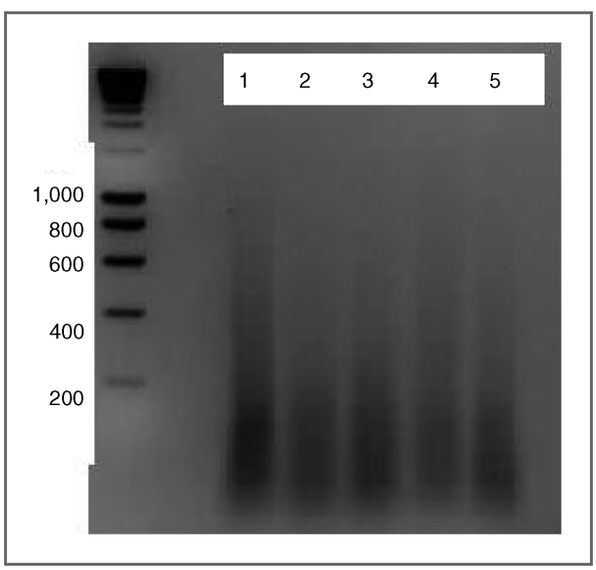

Figure 1. Five random toenail DNA samples on agarose gel (1.5%), 60 minutes, 100 V.

Table 1. Call rates in each quadruplicate analysis and overall (*n* samples = 82)

SNPs	Call rate quadruplo 1 (%)	Call rate quadruplo 2 (%)	Call rate quadruplo 3 (%)	Call rate quadruplo 4 (%)	Average call rate (%)
Sample call rate, average (range)	98.7 (63.3–100)	97.7 (0[a]–100)	99.5 (80–100)	98.8 (76.7–100)	98.7 (73.3–100)
rs1695	98.8	98.8	98.8	100	99.1
rs6670	98.8	95.1	98.8	97.6	97.6
rs727479	97.6	97.6	100	97.6	98.2
rs743572	96.3	93.9	97.6	96.3	96.0
rs822396	97.6	98.8	98.8	98.8	98.5
rs1019731	98.8	96.3	98.8	98.8	98.2
rs1056836	98.8	97.6	98.8	98.8	98.5
rs1062935	98.8	97.6	98.8	98.8	98.5
rs1076160	98.8	97.6	100	98.8	98.8
rs1501299	98.8	97.6	98.8	97.6	98.2
rs1799941	100	97.6	100	100	99.4
rs1801278	100	98.8	100	100	99.7
rs1801282	100	98.8	100	100	99.7
rs1805097	95.1	95.1	98.8	95.1	96.0
rs1877926	98.8	97.6	98.8	100	98.8
rs2132571	98.8	97.6	100	100	99.1
rs2132572	100	98.8	100	97.6	99.1
rs2241766	98.8	97.6	100	100	99.1
rs2672890	100	98.8	100	100	99.7
rs2854744	96.3	98.8	100	98.8	98.5
rs2994329	100	97.6	100	100	99.4
rs4684847	98.8	98.8	98.8	100	99.1
rs5742678	97.6	98.8	100	97.6	98.5
rs6444175	98.8	98.8	98.8	96.3	98.2
rs7014346	100	97.6	100	98.8	99.1
rs7208536	97.6	96.3	100	98.8	98.2
rs7874234	100	98.8	100	100	99.7
rs10505477	100	98.8	100	98.8	99.7
rs12584136	98.8	98.8	100	100	99.4
rs9890502	100	97.6	100	98.8	99.1
rs2471551	Failed				
rs2854746	Failed				
rs8063461	Failed				

[a]In one sample in this quadruplicate analysis, none of the 30 SNPs were called, probably explained by absence of DNA in the well.

Over the course of DNA isolation of the >21,000 NLCS toenail samples, we have performed a PCR analysis on 618 random samples (1–2 samples per 48 isolated samples to monitor isolation quality) to determine the presence of the earlier mentioned 596-bp and 197-bp CYP1A2 and MPO amplicons. Amplification of the 596-bp fragment was successful for 85% of the samples and amplification of the 197-bp fragment for 80% of the samples.

There are few observations from other studies on DNA fragment sizes in nails. Park and colleagues (8) were able to produce amplicons of 100-, 200- and 400-bp sequences in the nuclear *b-actin* gene for 62.5%, 22.2%, and 16.7% of the samples, respectively. These seem low percentages compared with our results. This difference may be explained by differences in washing and extraction methods used, resulting perhaps in increased DNA fragmentation or increased presence of PCR inhibitors in the DNA samples of Park and colleagues, or by the fact that analyses were done on different DNA sequences. Klassen and colleagues were able to generate a 456-bp amplicon of *RYR2* gene exon 97 in 66% of the fingernail DNA samples, more than twice as often as for formalin-fixed paraffin-embedded (FFPE) tissue DNA samples. Amplification of 911- and 969-bp PCR products in fingernail DNA samples was unsuccessful. In the majority of fingernail samples they tested, Yoshida-Yamamoto and colleagues (10) were able to amplify a 286-bp fragment of the *ESRX* gene.

In conclusion, our study and other studies have shown that toenail DNA is considerably fragmented to sizes generally <200 bp, but larger fragments are present and can be amplified in part of the samples, probably depending on the protocols used to isolate the DNA from the nails and the specific genomic regions of interest to be amplified.

Toenail DNA for genotyping

Study on Sequenom MassARRAY iPLEX SNP genotyping platform. For this pilot study on using Sequenom MassARRAY iPLEX technology for SNP genotyping, 82 DNA samples from toenails of prostate cancer cases from the NLCS were used. Thirty-three SNPs (as listed in Table 1) in the insulin-like growth factor pathway genes were determined. SNP genotyping analysis on 100 ng of toenail DNA (as measured with NanoDrop) was carried out by Sequenom in Hamburg using the MassARRAY iPLEX SNP genotyping platform (Sequenom; ref. 3). Four aliquots from each of the 82 DNA samples (100 ng each, as measured by NanoDrop) were taken and genotyped.

Genotyping of two SNPs (rs2471551 and rs2854746) failed because primers were not adequate. In addition, we excluded the results of one additional SNP (rs8063461) because this SNP was frequently called in water samples, which was probably caused by self-extension due to formation of a hairpin or extension due to a primer-dimer formation with another primer (please note that the analysis is based on a multiplex PCR reaction).

Table 1 shows the average and range of the sample call rates for each quadruplicate analysis and the average call rate across the quadruplicate analyses. The sample call rate was high, with averages ranging from 97.7% to 99.5%.

SNP call rates fluctuated slightly across quadruplicate analyses and ranged from 93.9% to 100% (Table 1).

Table 2 shows the reproducibility of the SNP call rates across the four repeated analyses, which ranged from 98.8% to 100%. On 11 occasions, there were differences in alleles (heterozygotes called as homozygotes) between quadruplicate analyses residing in three samples in total. After exclusion of samples below a sample call rate threshold of 95%, only two discrepancies (each in a different sample) remained out of a total of 9,643 calls that were made (~0.02%).

In conclusion, DNA from toenails enables genotyping using Sequenom's MassARRAY technique with good sensitivity and reproducibility.

Further experience with the Sequenom MassARRAY iPLEX SNP genotyping platform. The Sequenom MassARRAY iPLEX platform has so far been used in three large NLCS genotyping projects on gene–environment interactions (containing sets of 24, 20, and 30 SNPs (all with minor allele frequency > 10%) and 6,230, 2,872, and 4,238 samples, respectively) and three more such genotyping projects are ongoing. For these three projects, Sequenom genotyped 7,635 unique toenail DNA samples on the MassARRAY iPLEX platform. Articles describing the results are published (14) and in preparation.

Table 2. Reproducibility of SNP genotyping across quadruplicate analyses (*n* samples = 82)

	Samples with calls in at least 2 quadruplicate analyses (*n*)	Samples with the same genotyping result (*n*)	Samples with the same genotyping result (%)
rs1695	81	80	98.8
rs6670	81	80	98.8
rs727479	81	81	100
rs743572	80	80	100
rs822396	81	81	100
rs1019731	81	81	100
rs1056836	81	81	100
rs1062935	81	81	100
rs1076160	82	82	100
rs1501299	81	81	100
rs1799941	82	82	100
rs1801278	82	82	100
rs1801282	82	82	100
rs1805097	81	81	100
rs1877926	81	81	100
rs2132571	82	82	100
rs2132572	82	82	100
rs2241766	82	82	100
rs2672890	82	82	100
rs2854744	82	81	98.8
rs2994329	82	82	100
rs4684847	82	82	100
rs5742678	82	81	98.8
rs6444175	82	82	100
rs7014346	82	82	100
rs7208536	81	81	100
rs7874234	82	82	100
rs9890502	82	82	100
rs10505477	82	82	100
rs12584136	82	82	100

In each of these projects, the genotyping was successful: average sample call rates were higher than 95% (overall, 97.1%). Of note, 95.5% of the samples had a call rate >90% and 92.3% had a call rate >95%. Seventy-one of the 74 SNPs that were determined across the three projects adhered to Hardy–Weinberg equilibrium. In one of the projects, 23 samples of toenail DNA were compared with 23 samples of paraffin-embedded normal tissue DNA samples from the same NLCS participants. The genotype concordance (of 30 SNPs) between the two types of samples was 99.1%. Using data from the three projects, we have investigated whether the A280/A260 and A230/A260 ratios affected the sample call rates. Table 3 illustrates the average call rates for strata of the A260/A280 and A260/A230 ratios and the percentage of samples that passed sample call rate thresholds of 95% and 90%. Average sample call rates and the percentage of samples passing sample call rate thresholds were slightly lower for lower 260/A280 and A260/

Table 3. Average sample call rates and percentages of samples passing 95% and 90% sample call rate thresholds, stratified by different A260/A280 and A260/A230 ratio cutoffs

	Samples, *n* (%)	Average call rate, %	Percentage of samples with sample call rate ≥95%	Percentage of samples with sample call rate ≥90%
A260/A280				
<1.8	6,083 (96.7)	97.3	92.8	95.7
≥1.8	209 (3.3)	97.2	95.2	96.2
<1.5	3,134 (49.8)	96.9	91.6	95.0
≥1.5	3,158 (50.2)	97.6	94.2	96.4
A260/A230				
<2	6,276 (99.7)	97.3	92.9	95.7
≥2	16 (0.3)	88.0	87.5	87.5
<1.0	5,359 (85.2)	97.2	92.6	95.4
≥1.0	933 (14.8)	97.8	94.9	97.4
<0.75	4,110 (65.3)	97.0	92.1	95.0
≥0.75	2,182 (34.7)	97.9	94.5	97.0

A230 ratios. However, despite the relatively low purity ratios, toenail DNA renders good results with the Sequenom MassARRAY technique. For other genetic analyses, the purity of the toenail DNA may be more crucial. Therefore, it would be worthwhile to test DNA purification methods with a high DNA recovery.

Pilot study on Infinium HumanCytoSNP_FFPE-12 DNA chip. The Infinium HD FFPE DNA Restore Kit was originally developed to restore degraded DNA in FFPE tissues in preparation for use with the Infinium HD FFPE Assay (4). The Infinium HD FFPE Assay gives results for 262,739 genetic markers: selected tag SNPs that provide comprehensive coverage of the genome. For Infinium platforms, Illumina recommends fragment sizes of >2 kb. To test if samples are eligible for restoration, the Infinium HD FFPE Quality Control (QC) Kit is used. The QC Kit provides primers and DNA template for a real-time PCR using standard instrumentation and reagents. Extracted FFPE samples that pass the QC test (i.e., do not cross a certain PCR cycle threshold) are suitable for restoration using the restore kit.

We tested whether toenail DNA could be restored with this restore kit as well. We selected 29 prostate cancer samples from the NLCS study, from which 24 samples (83%) were eligible for restoration based on the QC test.

After restoring, we prepared the 24 toenail DNA samples for genotyping with the Illumina HumanCytoSNP_FFPE-12 DNA Analysis Kit, through which DNA is purified and whole-genome amplified. The chips were prepared and analyzed at the Genomics Core at Leuven University using the Illumina iScan reader and the iScan Array Scanner.

The average sample call rate of the 24 toenail DNA samples was 93.8%. Nineteen of the 24 samples (79%) had a sample call rate greater than 90%, whereas two other samples had a call rate very close to 90% (Fig. 2). This percentage is comparable with the percentage of FFPE samples that passes the threshold of >90% sample call rate, as reported in the Illumina product description leaflet of the HumanCytoSNP_FFPE DNA Restore Kit (4). The average SNP call rate was 93.8% across the 24 samples, with a SD of 9.8%. Of the total of 262,739 SNPs, 0.01% ($n = 28$) could not be called at all. Forty-two percent of the SNPs called 100%, and 69%, 82%, and 96% of the SNPs had a call rate greater than 95%, 90%, and 75%, respectively. It should be noted that we have overestimated the amount of input DNA that was used in this assay, because NanoDrop was used to quantify DNA and we did not yet have the results of the qPCR experiment that showed that only a fraction of spectrophotometrically determined DNA in toenails is amplifiable (>200 bp) human DNA. The results

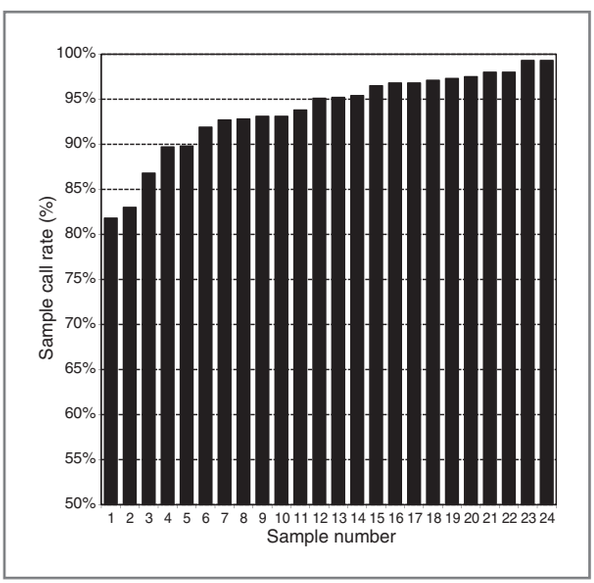

Figure 2. Sample call rates of 24 toenail DNA samples genotyped with the Illumina HumanCytoSNP_FFPE-12 DNA Array.

Table 4. Comparison of different frequently used sources of DNA in epidemiologic study settings

			Buccal cells			
	Nails	**Blood**	**Mouth wash/saliva**	**Buccal swap/ cytobrush**	**Fast Technology for Analysis (FTA) card**	**Hair with follicle**
Typical collection amount (units)	80 mg[c]	3–15 mL	4–20 mL	1–3 swaps/brushes	1 card	3–6 hair with follicles
Mean DNA yield per unit[a]	0.03–0.4 μg/mg nail References:[c], (8–10, 12, 15)	20–60 μg/mL Reference: (17)	0.4–23 μg/mL References: (18–25)	1.3–32 μg/brush References: (19–21, 23–26)	3–36 μg/card References: (19, 20)	0.05–1.5 μg/hair with follicle Reference: (9)
Percentage human DNA (%)	?	94 Reference: (24)	34–68 Reference: (24)	11 Reference: (24)	?[e]	?
DNA purity						
A260/A280	1.5–1.7 Reference:[c], (10)	1.8 References: (19, 27)	1.5–1.9 References: (19–21, 23–25)	1.1–2.0 References: (19–21, 23–27)	0.9–1.1 References: (19, 20)	1.8 Reference: (27)
A260/A230	0.7 Reference:[c]	1.4 Reference: (19)	0.8–1.9 References: (19, 21)	0.2 References: (19, 21)	0.2 Reference: (19)	?
Majority of DNA fragment sizes	200 bp Reference:[c], (9)	>23 kb	>23 kb Reference: (20)	?	?	?
Success rate of approximately 200-bp fragment amplification (%)	80–100 Reference:[c], (11)	100 Reference: (19)	84–100 References: (19, 21, 23, 24, 28)	23–100 References: (19, 21, 23, 24, 29)	0–100 References: (19, 20, 29, 30)	[f] Reference: (31)
Contains high-molecular weight DNA (>23 kb) (−, +/−, or +)	− Reference:[c], (9)	+ Reference: (9)	+ References: (20, 32)	+/− References: (9, 20, 24)	− Reference: (20)	− Reference: (31)
Suitability for WGA and subsequent genotyping (− or +)	?	+	+ Reference: (33)	+ Reference: (34)	+ References: (30, 34)	+[b] References: (31, 35)
Suitability for pediatric epidemiologic studies (− −, −, +, or ++)	++	− −	− −	+	+	−
Ease (in terms of invasiveness) of collection (− −, −, +, or ++)	++	− −	+	+	+	−
Response rate for participation (%)	75 Reference: (2)	31 Reference: (19)	67–72 References: (19, 22)	80 Reference: (19)	76 Reference: (19)	?
Self-administered collection (yes or no)	Yes	No	Yes	Yes	Yes	Yes

(Continued on the following page)

Table 4. Comparison of different frequently used sources of DNA in epidemiologic study settings (Cont'd)

			Buccal cells			
	Nails	Blood	Mouth wash/saliva	Buccal swap/ cytobrush	Fast Technology for Analysis (FTA) card	Hair with follicle
Ease (in terms of costs of materials, postage) of obtaining sample (− −, −, +, or ++)	++	−−	−	+/−	+/−	++
Ease of tissue sample storage (− or +)	+	−	−[d]	−	+	−

[a]As measured with NanoDrop/spectrophotometrically.
[b]WGA worked with GenomePlex, not with MDA.
[c]Present results.
[d]Depends on collection method. Samples may be stable at room temperature for >1 year (Oragene; ref. 19).
[e]Because cytobrushes or swaps are used to collect the buccal cells that are then transferred to the FTA card, it can be assumed that the FTA card contains the same percentage of human DNA, although FTA cards are treated to prevent microbial growth. Thus, the percentage of human DNA on FTA cards may be higher than that obtained from buccal swaps or brushes.
[f]Only successful for freshly plucked hairs, not for hairs stored for 7–11 years.

on the Illumina HumanCytoSNP_FFPE-12 DNA chip might have been even better if we had used more input DNA.

This pilot study shows that the HumanCytoSNP_FFPE array works for toenail DNA samples. However, in a more elaborate study, the reproducibility and accuracy of the genotyping of nail samples through this array should be investigated. In this more elaborate study, more input DNA should be used and it may be preferable to call the samples based on internal clustering, so on the clusters of the nail samples. In the pilot described in this article, calling was done based on a standard clustering of FFPE samples provided by Illumina.

Toenails as a source of DNA as compared with other sources

For large-scale genetic epidemiologic studies, it is of interest to know how different tissues as a source of DNA compare with regard to various aspects. Not only the quantity and quality of DNA is important, but also the ease of obtaining and storing samples, and participation rates of study participants are variables to take into consideration when choosing one tissue or the other. Table 4 shows various characteristics of different sources of DNA. This table is not an exhaustive summary of the literature.

In terms of DNA quantity and quality, blood is the optimal source of DNA. However, drawing blood is invasive and cannot be performed by study participants themselves. Thus, asking to provide a blood sample will reduce participation rates and it will make large-scale studies, especially of a geographically disperse population, difficult, if not impossible, in terms of logistics and associated costs. DNA yields from non-blood sources of DNA (buccal cells, hair, and nail) vary greatly between sources, depending on collection methods, storage conditions before DNA isolation, and DNA extraction methods. Nevertheless, of the non-blood DNA sources, mouth wash or saliva samples probably render the highest yield of human DNA of the highest quality, both in terms of DNA integrity and purity. Disadvantages of mouth wash samples are the relatively high costs associated with obtaining the samples (transport costs) and the problem of obtaining mouth wash samples in pediatric populations. Compared with the other sources of DNA, obtaining nails is least invasive and most suitable for use in pediatric studies, and together with hair samples, nail samples entail the lowest costs for obtaining and storing. On the other hand, isolating DNA from nails is more laborious than isolating DNA from blood samples or buccal samples, due to the washing steps for removing possible nail polish and other exogenous material and the incubation step to dissolve the nail material.

Overall Discussion

The pilot studies described in this article add to our previous findings (2) that nail clippings are a good source

of DNA for genetic analyses, despite the fragmented nature of the DNA (mostly <200 bp) and the relatively low purity. Toenail DNA samples proved well suited for genotyping of a limited set of SNPs on the Sequenom MassARRAY iPLEX with good call rates and reproducibility. After restoration with the Infinium HD FFPE DNA Restore Kit, toenail DNA showed satisfactory call rates on the Illumina HumanCytoSNP_FFPE-12 DNA high-density array (>262,000 markers). Good results using nail DNA for high-density (HD) genotyping were also obtained by others. Nakashima and colleagues (12) have genotyped five fingernail samples on the Affymetrix GeneChip Human mapping 250K Array, obtaining an average sample call rate of 94.8%. When they compared nail samples with blood samples, the concordance rates for homozygotes and heterozygotes were 99.8% and 98.8%, respectively. They did not observe differences between fresh clippings and >5 year old clippings stored at room temperature, which once again shows that nail DNA does not degrade when nail samples are stored at room temperature for extended periods (2). Oikawa and colleagues (15) have genotyped 11 fingernail DNA samples with the Affymetrix GeneChip mapping 10K 2.0 array optimized for FFPE tissue samples. The average sample call rate was 92.5%, which was comparable with the average SNP call rate (93.8%) in our pilot study using the Illumina HumanCytoSNP_FFPE-12 DNA Array.

Nakashima and colleagues and Oikawa and colleagues isolated DNA from frozen and crushed nails, whereas we lysed nails with NaOH before DNA isolation. We have compared DNA yield and fragment sizes of both preprocessing methods (10 samples) and we obtained better results for lysed nail method than for the freeze/crush method (results not shown). Using the latter method, we did not observe DNA fragments with a size of >200 bp, which we did observe for the lysed nail method, whereas the DNA yield was not meaningfully different between the two methods. Therefore, we speculate that nail DNA obtained through lysis of nails might lead to even better results using Affymetrix GeneChip mapping arrays than nail DNA obtained with the freeze/crush method.

Our toenail DNA was also tested on the Illumina VeraCode platform combined with Illumina GoldenGate genotyping, but the call rates were too low for meaningful analyses (results not shown). Possibly, toenail DNA does not contain enough DNA fragments >200 bp, as required for this technique, this technique is relatively sensitive to the low purity of the toenail DNA samples or not enough input DNA was used. Illumina advises to quantitate DNA by PicoGreen, but we used NanoDrop to quantify DNA.

In the near future, genotyping is likely to be performed through next-generation sequencing (NGS), and then toenails are expected to be a very suitable source of DNA for large-scale epidemiologic studies because NGS does not require high-integrity DNA, contrary to some HD genotyping platforms. NGS has been successfully applied in the field of paleontology, which deals with highly degraded ancient DNA with low quantity (16). We have genotyped 96 SNPs in two toenail DNA samples through the molecular inversion probe technique followed by NGS (Ion PGM sequencer) and this rendered equally good results as blood samples (preliminary results, not shown). Currently, we are performing a more elaborate study using these techniques on more toenail DNA samples.

In conclusion, nails are a cheap and easily obtainable and storable source of DNA suitable for genotyping of a limited set of candidate gene SNPs and HD genotyping in GWAS studies, despite its fragmented nature and relatively high pollution level. For HD genotyping, however, methods are needed (e.g., restore kit, PCR conditions) that are suitable or optimized for fragmented DNA.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J.G.F. Hogervorst, P.A. van den Brandt, M.P. Weijenberg, J.R. Vermeesch, F.J. van Schooten, L.J. Schouten
Development of methodology: R.W.L. Godschalk, P.A. van den Brandt, M.P. Weijenberg, J.R. Vermeesch, F.J. van Schooten, L.J. Schouten
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.W.L. Godschalk, P.A. van den Brandt, L. Jonkers, J. Goessens, J.R. Vermeesch, F.J. van Schooten, L.J. Schouten
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.G.F. Hogervorst, P.A. van den Brandt, L.J. Schouten
Writing, review, and/or revision of the manuscript: J.G.F. Hogervorst, R.W.L. Godschalk, P.A. van den Brandt, M.P. Weijenberg, B.A.J. Verhage, L. Jonkers, J. Goessens, C.C.J.M. Simons, F.J. van Schooten, L.J. Schouten
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): B.A.J. Verhage, L. Jonkers, J. Goessens
Study supervision: J.G.F. Hogervorst, P.A. van den Brandt, F.J. van Schooten, L.J. Schouten

Acknowledgments

The authors thank Simone van Breda, Stijn Lumeij, and Kristien Lemmens for their help with the pilot work on DNA isolation from toenails and the first phases of DNA isolation from the cohort toenail samples. Furthermore, the authors are indebted to Harry van Montfort for designing the databases used for storing and managing toenail DNA data; Sacha van de Crommert and Conny de Zwart for data management; Ivette Deckers and Milan Geybels for providing data; and Lara Oheimer for collecting toenail samples in the basement and weighing them. In addition, the authors thank the Biobank Maastricht UMC+ (Berry Soute and Claudia Bosma) for storing and retrieving our toenail DNA samples.

Received May 20, 2014; revised July 15, 2014; accepted July 16, 2014; published online December 3, 2014.

References

1. Bengtsson CF, Olsen ME, Brandt LO, Bertelsen MF, Willerslev E, Tobin DJ, et al. DNA from keratinous tissue. Part I: hair and nail. Ann Anat 2012;194:17–25.
2. van Breda SG, Hogervorst JG, Schouten LJ, Knaapen AM, van Delft JH, Goldbohm RA, et al. Toenails: an easily accessible and long-term stable source of DNA for genetic analyses in large-scale epidemiological studies. Clin Chem 2007;53:1168–70.
3. Gabriel S, Ziaugra L, Tabbaa D. SNP genotyping using the Sequenom MassARRAY iPLEX platform. Current Protocols in Human Genetics. 60:2.12:2.12.1 -2.12.16. 2009.

4. http://res.illumina.com/documents/products/datasheets/datasheet_ffpe_dna_restoration.pdf
5. van den Brandt PA, Goldbohm RA, van't Veer P, Volovics A, Hermus RJ, Sturmans F. A large-scale prospective cohort study on diet and cancer in The Netherlands. J Clin Epidemiol 1990;43:285–95.
6. Cline RE, Laurent NM, Foran DR. The fingernails of Mary Sullivan: developing reliable methods for selectively isolating endogenous and exogenous DNA from evidence. J Forensic Sci 2003;48:328–33.
7. Klassen TL, von Ruden EL, Drabek J, Noebels JL, Goldman AM. Comparative analytical utility of DNA derived from alternative human specimens for molecular autopsy and diagnostics. J Mol Diagn 2012;14:451–7.
8. Park J, Liang D, Kim JW, Luo Y, Huang T, Kim SY, et al. Nail DNA and possible biomarkers: a pilot study. J Prev Med Public Health 2012; 45:235–43.
9. 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;23:341–6.
10. Yoshida-Yamamoto S, Nishimura S, Okuno T, Rakuman M, Takii Y. Efficient DNA extraction from nail clippings using the protease solution from Cucumis melo. Mol Biotechnol 2010;46:41–8.
11. Kaneshige T, Takagi K, Nakamura S, Hirasawa T, Sada M, Uchida K. Genetic analysis using fingernail DNA. Nucleic Acids Res 1992;20: 5489–90.
12. Nakashima M, Tsuda M, Kinoshita A, Kishino T, Kondo S, Shimokawa O, et al. Precision of high-throughput single-nucleotide polymorphism genotyping with fingernail DNA: comparison with blood DNA. Clin Chem 2008;54:1746–8.
13. Matsuzawa N, Shimozato K, Natsume N, Niikawa N, Yoshiura K. A novel missense mutation in Van der Woude syndrome: usefulness of fingernail DNA for genetic analysis. J Dent Res 2006;85:1143–6.
14. Geybels MS, van den Brandt PA, Schouten LJ, van Schooten FJ, van Breda SG, Rayman MP, et al. Selenoprotein gene variants, toenail selenium levels, and risk for advanced prostate cancer. J Natl Cancer Inst 2014;106:dju003.
15. Oikawa M, Kuniba H, Kondoh T, Kinoshita A, Nagayasu T, Niikawa N, et al. Familial brain arteriovenous malformation maps to 5p13-q14, 15q11-q13 or 18p11: linkage analysis with clipped fingernail DNA on high-density SNP array. Eur J Med Genet 2010;53:244–9.
16. Rizzi E, Lari M, Gigli E, De Bellis G, Caramelli D. Ancient DNA studies: new perspectives on old samples. Genet Sel Evol 2012;44:21.
17. Caboux E, Lallemand C, Ferro G, Hemon B, Mendy M, Biessy C, et al. Sources of pre-analytical variations in yield of DNA extracted from blood samples: analysis of 50,000 DNA samples in EPIC. PLoS ONE 2012;7:e39821.
18. Philibert RA, Zadorozhnyaya O, Beach SR, Brody GH. Comparison of the genotyping results using DNA obtained from blood and saliva. Psychiatr Genet 2008;18:275–81.
19. Hansen TV, Simonsen MK, Nielsen FC, Hundrup YA. Collection of blood, saliva, and buccal cell samples in a pilot study on the Danish nurse cohort: comparison of the response rate and quality of genomic DNA. Cancer Epidemiol Biomarkers Prev 2007;16:2072–6.
20. Mulot C, Stucker I, Clavel J, Beaune P, Loriot MA. Collection of human genomic DNA from buccal cells for genetics studies: comparison between cytobrush, mouthwash, and treated card. J Biomed Biotechnol 2005;2005:291–6.
21. Rogers NL, Cole SA, Lan HC, Crossa A, Demerath EW. New saliva DNA collection method compared to buccal cell collection techniques for epidemiological studies. Am J Hum Biol 2007;19:319–26.
22. 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;10:701–3.
23. King IB, Satia-Abouta J, Thornquist MD, Bigler J, Patterson RE, Kristal AR, et al. Buccal cell DNA yield, quality, and collection costs: comparison of methods for large-scale studies. Cancer Epidemiol Biomarkers Prev 2002;11:1130–3.
24. Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, et al. Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. Cancer Epidemiol Biomarkers Prev 2001;10:687–96.
25. 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;14:117–22.
26. van Wieren-de Wijer DB, Maitland-van der Zee AH, de Boer A, Belitser SV, Kroon AA, de Leeuw PW, et al. Determinants of DNA yield and purity collected with buccal cell samples. Eur J Epidemiol 2009;24: 677–82.
27. Ghatak S, Muthukumaran RB, Nachimuthu SK. A simple method of genomic DNA extraction from human samples for PCR-RFLP analysis. J Biomol Tech 2013;24:224–31.
28. Zayats T, Young TL, Mackey DA, Malecaze F, Calvas P, Guggenheim JA. Quality of DNA extracted from mouthwashes. PLoS ONE 2009;4: e6165.
29. Milne E, van Bockxmeer FM, Robertson L, Brisbane JM, Ashton LJ, Scott RJ, et al. Buccal DNA collection: comparison of buccal swabs with FTA cards. Cancer Epidemiol Biomarkers Prev 2006;15:816–9.
30. He H, Argiro L, Dessein H, Chevillard C. Improved technique that allows the performance of large-scale SNP genotyping on DNA immobilized by FTA technology. Infect Genet Evol 2007;7:128–32.
31. Leanza SM, Burk RD, Rohan TE. Whole genome amplification of DNA extracted from hair samples: potential for use in molecular epidemiologic studies. Cancer Detect Prev 2007;31:480–8.
32. Feigelson HS, Rodriguez C, Robertson AS, Jacobs EJ, Calle EE, Reid YA, et al. Determinants of DNA yield and quality from buccal cell samples collected with mouthwash. Cancer Epidemiol Biomarkers Prev 2001;10:1005–8.
33. Rylander-Rudqvist T, Hakansson N, Tybring G, Wolk A. Quality and quantity of saliva DNA obtained from the self-administrated oragene method–a pilot study on the cohort of Swedish men. Cancer Epidemiol Biomarkers Prev 2006;15:1742–5.
34. Beckett SM, Laughton SJ, Pozza LD, McCowage GB, Marshall G, Cohn RJ, et al. Buccal swabs and treated cards: methodological considerations for molecular epidemiologic studies examining pediatric populations. Am J Epidemiol 2008;167:1260–7.
35. Agalliu I, Schweitzer PA, Leanza SM, Burk RD, Rohan TE. Illumina DNA test panel-based genotyping of whole genome amplified-DNA extracted from hair samples: performance and agreement with genotyping results from genomic DNA from buccal cells. Clin Chem Lab Med 2009;47:516–22.

DNA from Nails for Genetic Analyses in Large-Scale Epidemiologic Studies

Janneke G.F. Hogervorst, Roger W.L. Godschalk, Piet A. van den Brandt, et al.

Cancer Epidemiol Biomarkers Prev 2014;23:2703-2712.