OMNIgene®•SKIN

OMNIgene®•SKIN (OMR-140): an optimized collection device for the capture and stabilization of the human skin microbiome

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Introduction

The human skin is a vast and diverse environment that harbours hundreds of bacterial species as well as fungal and viral taxa in lower relative abundance. The discrete regions of the human skin can be divided into three main subgroups: sebaceous, wet and dry, all of which exhibit different physical and chemical properties and tend to harbour different microbial populations. As the skin surface is generally poor in nutrients, has exposure to external environments, and lacks a mucosal surface, it cannot sustain microbial biomass associated with more commonly studied internal body sites, such as the gut or oral cavity. Low average microbial abundance and significant variability between potential collection sites make skin microbiome sampling notoriously difficult, and warrant the development of a standardized and fit-for-purpose collection method. To this end, DNA Genotek™ has developed OMNIgene®•SKIN (OMR-140), a swab-based skin microbiome collection device that effectively captures and stabilizes the human skin microbiome across all major sampling sites.

Material and methods

Sample collection

Healthy donors self-collected microbial samples from a variety of skin sites using OMNIgene•SKIN following the device Instructions For Use (IFU). Briefly, the swab was immersed in the provided wetting solution and used to collect microbial cells from the skin surface of interest: face, scalp, toe web, axilla or forearm. An area approximately 2.5 in × 2.5 in (6.35 cm × 6.35 cm) (if applicable) was rubbed for a total of 60 seconds. The swab was then stored in the stabilization solution (OMNIgene® collection tube) until extraction.

For skin collection performance experiments, paired samples were collected using two devices: eNAT™ specimen collection device (Copan Diagnostics, Murrieta, CA, USA) and the Swab collection and DNA preservation system (Norgen Biotek Corp., Thorold, ON, Canada). Collection instructions from the Human Microbiome Project (HMP)[1] were used for both these devices as IFUs for the collection of skin samples were not provided by the manufacturers.

For simulated collections, 2N puncture resistant artificial skin (SynDaver®, Tampa, FL, USA) was used. To assess collection performance, decreasing amounts of an overnight culture of *Francisella philomiragia* (ATCC 25017) were spread on a 2.5 in × 2.5 in (6.35 cm × 6.35 cm) piece of artificial human skin and allowed to dry for 5 minutes. Samples were then collected using OMNIgene•SKIN devices as per the IFU.

Extractions and qPCR

OMNIgene•SKIN-collected samples were extracted using QIAamp® PowerFecal® Pro DNA Kit (QIAGEN®, Germantown, MD, USA) or our optimized extraction workflow, an in-house protocol specifically developed for low biomass samples. A high-throughput version of this optimized extraction method is also available for Diversigen® customers. DNA was quantified using PicoGreen™ and used as a template in qPCR assays and/or next generation sequencing (NGS) applications. Samples collected with Copan's eNAT device were extracted with PowerFecal Pro DNA Kit while samples collected using the Norgen device were extracted using Norgen's saliva DNA isolation kit. Recovery of *F. philomiragia* from artificial skin was determined by qPCR using primers targeting iglC3 (Fwd 5'-AGCCAACAGAACTTATGGGTGT-3' and Rev 5'-ACTTGCAGCACCGCATACA-3'). Serial dilutions of *F. philomiragia* gDNA were used as a standard curve.

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16S copy number in extracted DNA was also determined by qPCR using universal bacterial primers (Fwd 5'-ATTACCGCGGCTGCTGG-3'; Rev 5'- CCTACGGGAGGCAGCAG-3'). Serial dilutions of *E. coli* gDNA were used as a standard curve.

Sequencing and analysis

Library preparation and sequencing were conducted by amplification of the 16s rRNA gene V3-V4 hypervariable regions (bacterial) and the internal transcribed spacer 2 (ITS2) (fungal). Paired-end sequencing was performed using PE-300 V3 kit (2x300) on an Illumina MiSeq® platform. Raw sequence data were processed using a DADA2 (v 1.14.1) workflow[2]. Briefly, primers were removed (Cutadapt 2.1)[3], and reads were quality filtered and trimmed. Using DADA2's error estimation model, reads were dereplicated, merged into full-length amplicons, and chimeras were removed before generating the amplicon sequence variants (ASVs) used for downstream analyses. ASVs were assigned taxonomy via DADA2 using the SILVA v132 database[4] (for 16S amplicons) or the UNITE (10.10.2017) database[5] (for ITS2 amplicons). Only ASVs having at least 100 reads total, and at least 1% abundance in any one sample were retained for downstream analyses. The filtered 16S dataset had a median of 91780 reads per sample (IQR: 41574, 107120) and for ITS2 a median of 37811 reads per sample (IQR: 17511, 68479). For whole genome sequencing (WGS), samples were sequenced using BoosterShot™ methodology (Diversigen, New Brighton, MN, USA) on Illumina's NovaSeq™ platform, and downstream sequence processing and taxonomic annotation were performed within Diversigen's CoreAnalysis™ pipeline. The feature counts table was filtered to keep taxa that were present in at least 2 samples and with a total of 10 reads or more, resulting in a median of 3412298 reads per sample (IQR: 1436762, 6552988). Further data analyses and raw figure generation were performed in R[6] using the ggplot2[7] and MicrobeR[8] packages. For statistical analysis, taxonomic table were filtered to an appropriate level for each analysis, and transformed to centre log-ratios (CLR). Changes in the total microbial profile between pairs of samples were measured with Aitchison's distance, and by differential analysis of each taxonomic component using ALDEx2[9]; differences were only reported if statistically significant. Taxonomic barplots are represented as percent abundance per taxonomic group out of total filtered reads.

Results

An optimized workflow to maximize yields from OMNIgene•SKIN collected samples

Skin samples tend to yield particularly low amounts of microbial DNA which can lead to significant issues in downstream processing such as poor or lack of amplification in PCR assays or amplification of background DNA contamination stemming from processing steps or inappropriate collection means. To maximize yields from OMNIgene•SKIN devices, we developed two optimized extraction workflows where the entire sample can be processed in a single extraction. Using both approaches, presented in Figure 1A, we were consistently able to recover

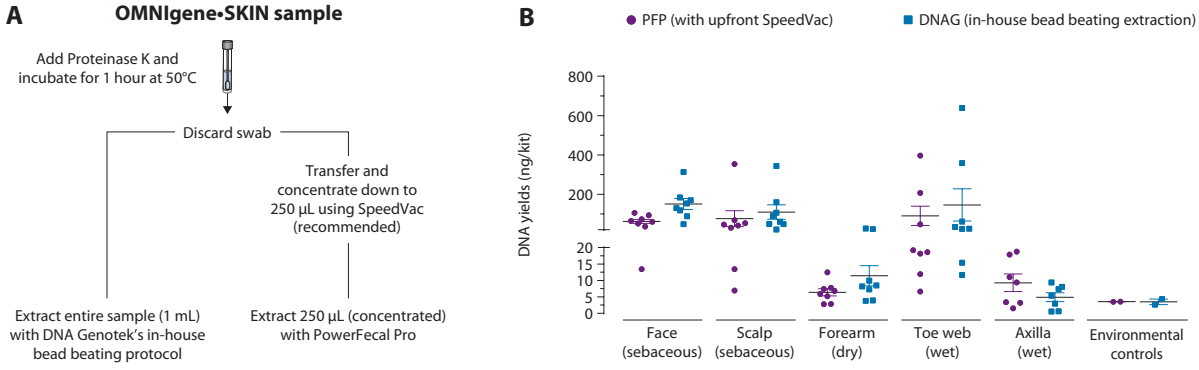

Figure 1: Processing steps for optimal DNA recovery from OMNIgene•SKIN samples. (A) *Schematic of the optimized workflows allowing processing of the entire sample: PowerFecal Pro DNA Kit (PFP) vs. DNA Genotek's in-house bead beating extraction protocol (DNAG).* ***(B)*** *Total DNA yields (ng) extracted from samples collected in OMNIgene•SKIN from the 5 commonly studied sites which together represent three major types of skin sites (sebaceous, dry and wet). Paired samples collected by 8 donors were extracted using either PFP or DNAG protocol and quantified using the PicoGreen assay. Mean DNA yield from each site is presented here.*

detectable levels of DNA from all skin sites sampled with OMNIgene•SKIN, including particularly low biomass regions such as forearm (dry skin). As expected, yields were highest for sebaceous skin (151 ng/kit ± 79 ng for face and 110 ng/kit ± 104 ng for scalp), lowest for dry skin (11.4 ng/kit ± 8.6 ng for forearm), while wet skin sites were highly variable (146 ng/kit ± 231 ng for toe webs and 4.9 ng/kit ± 3.6 ng for scalp) (Figure 1B). Importantly, the amount of DNA extracted from environmental control samples was quite low and represented on average less than 5% of the total DNA extracted from toe web, scalp and face samples and approximately 30% of the total DNA extracted from forearm samples. This is indicative of an overall low bioburden in the OMNIgene•SKIN device.

OMNIgene•SKIN accurately captures site-specific bacterial and fungal profiles

We next assessed the performance of DNA samples extracted from OMNIgene•SKIN samples in high-throughput sequencing assays. Samples collected from all skin sites showed robust amplification in 16S and ITS2 amplicon sequencing applications. Final library concentrations from collected samples were at least 5× higher than those of corresponding environmental controls, which confirms the low overall bioburden of the device.

16S taxonomic profiles revealed that OMNIgene•SKIN captures bacterial taxa known to be associated with discrete skin sites (Figure 2A). For instance, *Cutibacterium* (formally known

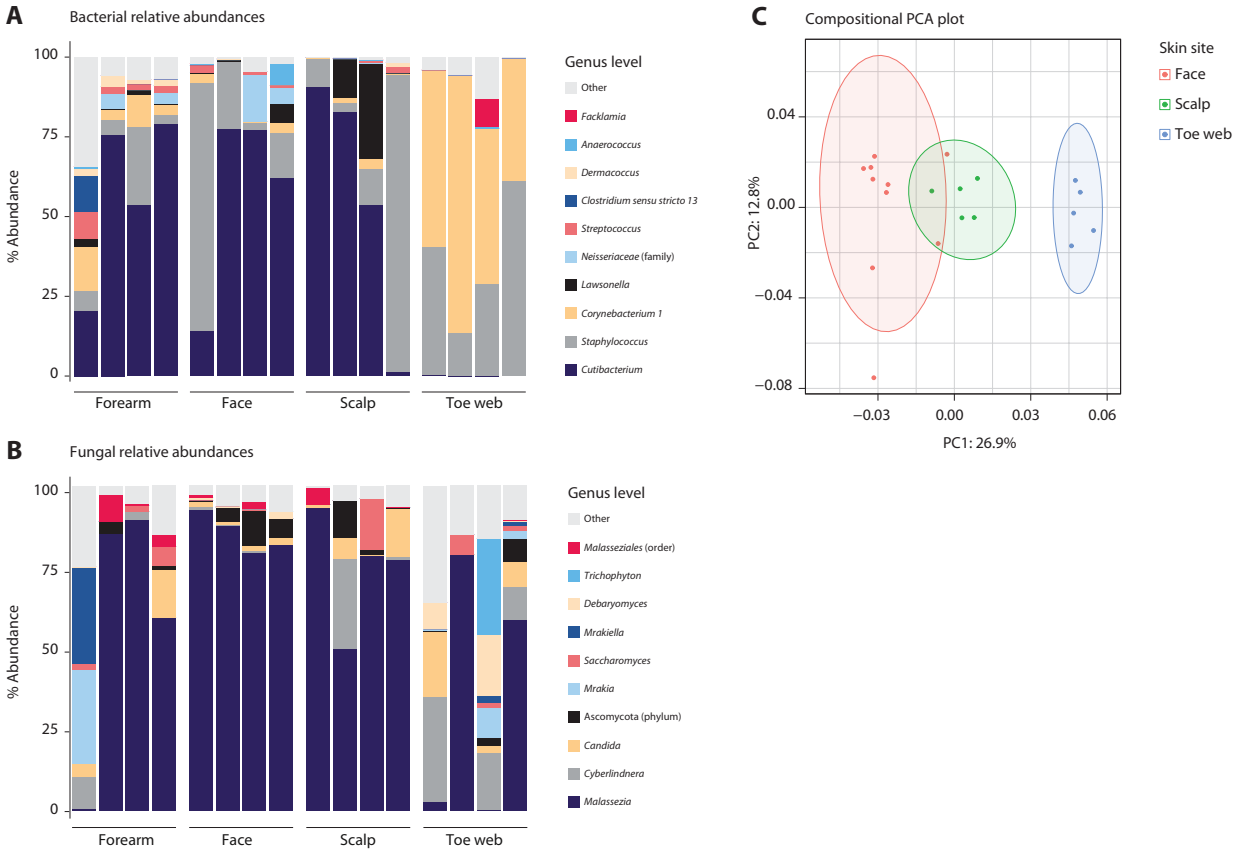

Figure 2: Bacterial and fungal taxonomic profiles of skin microbial samples collected from the forearm (dry), face and scalp (sebaceous) and toe webs (wet) using OMNIgene•SKIN. (A) *16S rRNA gene was amplified using primers targeting V3-V4. Relative abundance plot was generated from the filtered amplicon sequence variant (ASV) data classified using the SILVA database and aggregated to genus-level, or lowest assignable taxonomic level as denoted in brackets.* ***(B)*** *For fungal analysis the ITS2 region was amplified by PCR and the relative abundance plot was generated from the filtered ASV data classified using the UNITE database and aggregated to genus-level, or lowest assignable taxonomic level as denoted in brackets. In both (A) and (B), the top 10 most abundant taxa from 4 representative donors are shown with the remaining reads grouped as "other".* ***(C)*** *Principal component analysis (PCA) plot of samples collected from the scalp, face and toe web using OMNIgene•SKIN. Samples were sequenced by WGS and taxonomic assignment was performed within Diversigen's BoosterShot and CoreAnalysis workflows. Aitchison distance was calculated between samples, and the first two components explaining the most variation between samples (PC1: 26.9% and PC2: 12.8%) are shown. Ellipses represent the 95% confidence interval for each sample group (face, scalp, and toe web).*

as *Propionibacterium*) was primarily associated with sebaceous skin sites (face and scalp) while *Corynebacterium* and *Staphylococcus* were the two dominant genera on toe webs (wet skin site), as previously reported[10]. Similarly, ITS2 taxonomic profiles showed that *Malassezia* was found at high relative abundance on sebaceous and dry skin of the majority of donors while a greater diversity of fungal genera was detected on toe webs (Figure 2B).

Given the relatively high concentrations of DNA recovered from several skin sites, we also performed WGS on OMNIgene•SKIN samples collected from the face, scalp and toe webs. Samples collected from the face and scalp consistently yielded high enough concentrations of DNA for WGS, compared to 40% of samples collected from the toe web (Table 1). In WGS, the proportion of reads mapping to bacterial genomes was highly site and donor dependent, with sebaceous skin sites averaging 30% bacterial reads. Overall, taxonomic profiles were similar to the ones observed with 16S amplicon sequencing (data not shown). WGS increased our ability to detect the discrete taxonomic profile differences between the three different sites (Figure 2C), especially between toe web samples (wet) and sebaceous skin sites (face/scalp). Moreover, we were able to detect up to 20 unique *Cutibacterium acnes* strains in our WGS dataset, with face and scalp samples having the highest prevalence of detectible *C. acnes*, and toe web having little to none (Table 1).

	Proportion of samples compatible with WGS ([DNA] > 0.5 ng/µL)	Average proportion of bacterial reads (Range)	Number of *Cutibacterium acnes* strain detected
Face (sebaceous)	100%	27% (8 to 63%)	20
Scalp (sebaceous)	100%	38% (6 to 63%)	20
Forearm (dry)	0%	N/A	N/A
Toe web (wet)	40%	96% (93 to 99%)	N/A

Table 1: Overview of WGS outcome for OMNIgene•SKIN samples. *The proportion of samples meeting library prep input requirement ([DNA]>0.5 ng/µL) as well as the average proportion of reads mapping to bacterial genomes are shown and expressed as percentages. The number of unique Cutibacterium acnes strains detected following WGS is also shown for the two sebaceous sites sampled in our study (face and scalp).*

OMNIgene•SKIN stabilizes the skin microbial profile at room temperature and during transport and storage without introducing a bias

To assess stability of the skin microbial samples collected in OMNIgene•SKIN, paired half-face samples (left vs. right) were collected from 20 donors. One sample from each pair was extracted at baseline while the matching sample was stored at room temperature (20°C to 26°C / 68°F to 79°F) for up to 30 days then extracted. Bacterial profiles were then generated using 16S rRNA gene (V3-V4) amplicon sequencing and analyzed for each sample pair. The difference in total microbial profile following storage at room temperature for 30 days was not significantly different from normal biological sampling variability seen between replicate samples (left vs. right) extracted at baseline (control group), and much lower than the differences observed between donors (Figure 3A).

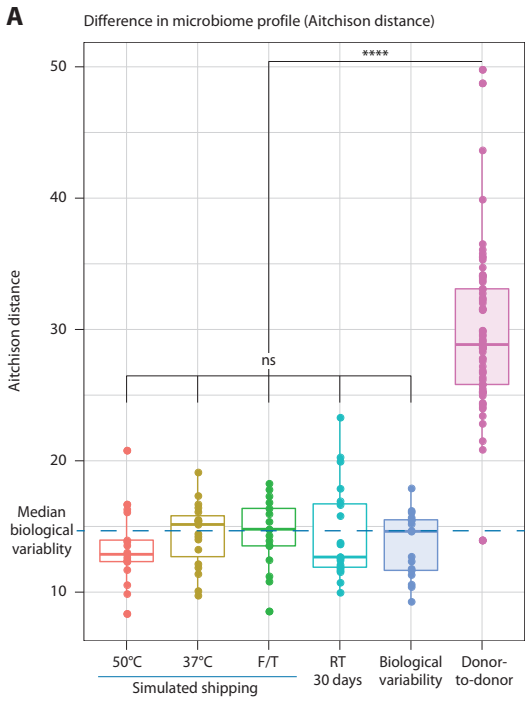

Figure 3A: Microbial profile stability in OMNIgene•SKIN samples. *Groupwise comparison of Aitchison distance as a measure of stability in microbial profile. Each point represents the difference between a baseline sample (T0) and a paired sample from the same donor extracted after simulated shipping (37°C for 3 days, 50°C for 1 day, or 3× cycles of freeze-thaw) or storage at room temperature for 30 days. Significance testing was performed against T0 replicate variability (T0 paired left and right sample replicates) and donor-to-donor variability. None of the condition tested exceeded the baseline (T0) variability, and therefore no significant changes were detected (Kruskal-Wallis non-parametric test, and two-group comparisons using a t-test).*

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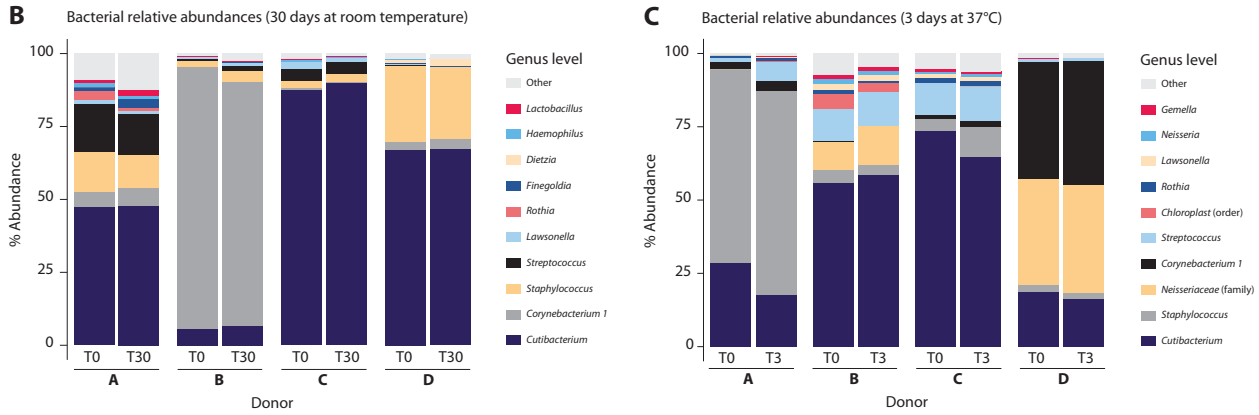

Figure 3A and 3B: Microbial profile stability in OMNIgene•SKIN samples. (B, C) *Taxonomic profile stability in OMNIgene•SKIN samples stored at room temperature for 30 days* ***(B)*** *or 37°C for 3 days.* ***(C)*** *The 16S rRNA gene from T0 and T30 extracted samples was amplified using primers targeting V3-V4 and the relative abundance plot was generated from the filtered amplicon sequence variant (ASV) data classified using the SILVA database and aggregated to genus-level, or lowest assignable taxonomic level as denoted in brackets.*

A similar comparison was used to assess stability of samples during simulated shipping conditions (3× freeze/thaw cycles, 1 day at 50°C (122°F) or 3 days at 37°C (98.6°F)). Data showed that no significant changes in the overall variability were introduced by any of these conditions (Figure 3A). Importantly, no taxa were found to be differentially expressed in paired samples for any of the groups (ALDEx2: effect size <1, p-adj >0.05), and the taxonomic profiles from individual donors were maintained after storage at room temperature for 30 days (Figure 3B) or simulated shipping conditions such as incubation at 37°C for 3 days (Figure 3C), incubation at 50°C for 1 day or 3 cycles of freeze-thaw (data not shown). Fungal profile stability was also assessed, by spiking live *Malassezia globosa* cells in OMNIgene•SKIN samples collected from the face. qPCR analysis showed that fungal levels remained stable during simulated shipping and storage at room temperature for 30 days (data not shown). This demonstrates that OMNIgene•SKIN maintains the stability of the microbial profile from the point-of-collection to point-of-extraction even when subjected to harsh shipping conditions or storage at room temperature for up to 30 days.

We also assessed if collection of a sample in an OMNIgene•SKIN device could introduce a bias in the microbial profile. Paired samples from each side of a donor's face (left vs. right) were collected from 20 donors and placed in either OMNIgene•SKIN stabilization solution or PBS (phosphate-buffered saline) and extracted within 30 minutes of collection. Bacterial profiles were then generated using 16S amplicon sequencing and analyzed for each sample pair. Biological variability (left vs. right) was similar between OMNIgene•SKIN and PBS collected samples and again significantly lower than donor-to-donor variability (Figure 4). Similarly, no detectable changes in *M. globosa* levels (qPCR) were detected when comparing OMNIgene•SKIN and PBS collected samples (data not shown). Taken together, our data shows that sample collection with OMNIgene•SKIN captures the donor's unique microbiome signature at the point-of-collection without impacting its taxonomic composition.

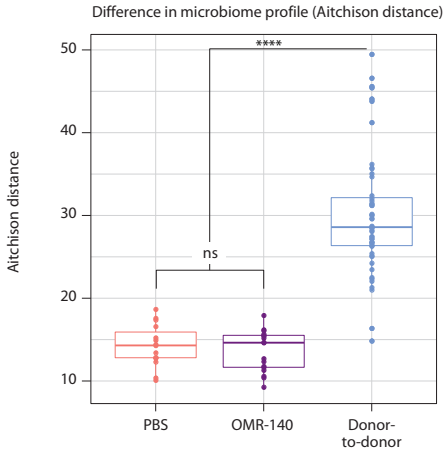

Figure 4: Sample collection in OMNIgene•SKIN is neutral and does not introduce a bias in the microbial profile. *Groupwise comparison of Aitchison distance as a measure of change in the microbial profile. Each point represents the difference in paired samples (left vs. right side of the face) collected either in PBS or OMNIgene•SKIN (OMR-140) and extracted at baseline (T0). There was no difference in sampling variability for either collection method at T0, and the differences between sample replicates in both PBS and OMNIgene•SKIN was significantly lower than the donor-to-donor differences. Significance tested with Kruskal-Wallis non-parametric test, and two-group comparisons using a t-test.*

OMNIgene•SKIN collects superior skin microbiome samples

We compared the collection performance of OMNIgene•SKIN to commonly used skin microbiome collection devices (Copan's eNAT specimen collection device and Norgen's swab collection and DNA preservation system) by collecting paired cheek (sebaceous skin) or forearm (dry skin) samples from healthy adults. Our data shows that DNA concentrations were consistently higher for OMNIgene•SKIN collected samples than samples collected with the other devices (Figure 5A). This was particularly pronounced for forearm samples where yields were 3 to 4-fold higher on average when collected with OMNIgene•SKIN. In fact, DNA concentrations collected from forearm were indistinguishable from environmental controls for >50% of donors when using the other swab-based devices.

We also quantified the average number of 16S copies/µL recovered from cheek samples, and inferred that OMNIgene•SKIN collects more bacterial cells than other kits tested (Figure 5B). Moreover, the relatively high concentrations of 16S copies recovered from OMNIgene•SKIN samples is indicative of consistent performance across multiple donors.

Futhermore, we assessed the effectiveness of our IFUs for skin microbiome sampling. Paired skin face (left and right) samples were collected in our device using either our optimized IFUs or the standard skin collection instructions from the Human Microbiome Project (HMP protocol) and DNA was extracted with our optimized workflow. DNA yields for samples collected following our IFUs were significantly higher than for samples collected with HMP instructions (Figure 5C). This illustrates the effectiveness of our device's IFU in ensuring better collection performance which is paramount for successful sampling of low biomass sites.

Qualitative performance of the OMNIgene•SKIN device

The human skin has a highly variable microbial density[11,12] with dry skin harboring approximately 10^3 microbial cells/cm^2 of skin, while richer sites such as sebaceous skin can harbor as many as 10^9 cells/cm^2. To better assess the qualitative performance and collection efficiency of OMNIgene•SKIN devices, we designed an experiment where decreasing ($\log_{10}$) amounts of a bacterial culture were spiked on artificial skin and collected with OMNIgene•SKIN. Using a qPCR assay, we were able to demonstrate that the

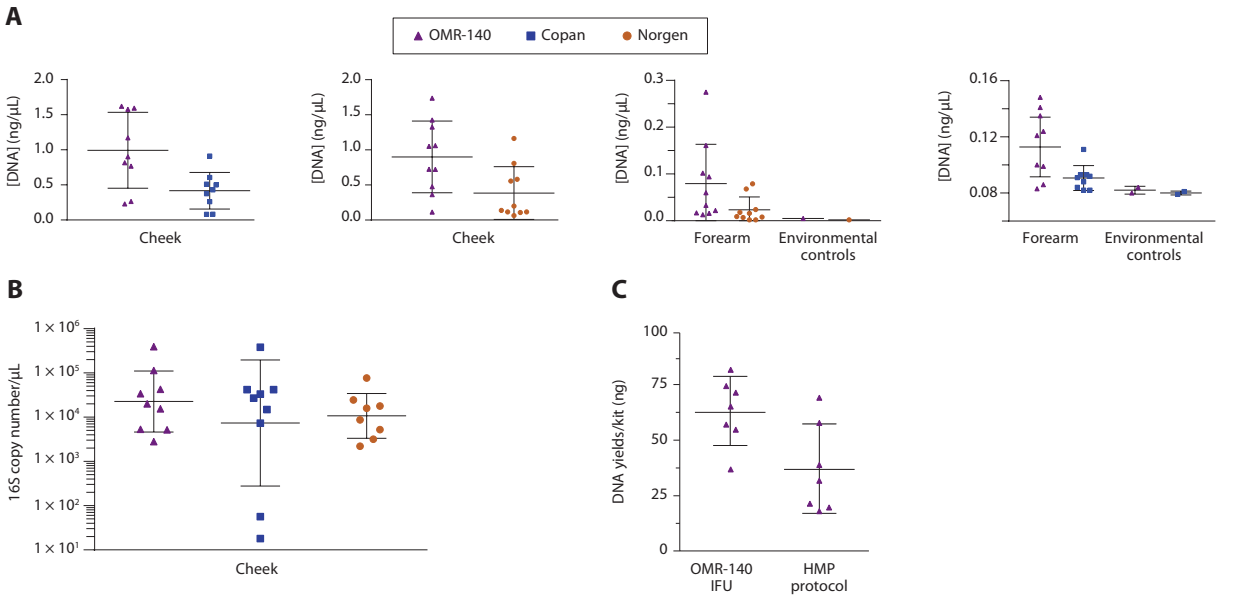

***Figure 5**: **OMNIgene•SKIN offers superior collection performance and improved usability. (A)** Paired cheek and forearm samples were collected using either OMNIgene•SKIN (OMR-140) or other swab-based collection devices (Norgen and Copan) from a total of 9 or 10 donors. DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (for OMR-140 vs. Copan) or Norgen Saliva DNA isolation kit (for Norgen vs. OMNIgene•SKIN) and quantified using PicoGreen. DNA concentration is shown for each donor and each sampled body site. **(B)** Average 16S copy number per µL of eluate (geometric mean ± geometric SD) in cheek samples collected with OMNIgene•SKIN vs. other swab-based collection device. 16S copy number was measured by qPCR using universal bacterial primers. **(C)** Impact of the end-user performance on OMNIgene•SKIN DNA yields. Paired face samples were collected using OMNIgene•SKIN following our optimized IFUs (OMR-140 IFU) or standard collection instructions from the Human Microbiome Project (HMP protocol).*

recovery rate of the spiked bacteria (*F. philomiragia*) with OMNIgene•SKIN was consistent across several logs, with an average recovery around 38% (Figure 6). Notably, we were able to efficiently recover and detect as low as 10^4 bacterial cells spread on a 6.25 in^2 surface (40 cm^2). This indicates that our collection methodology and processing workflow efficiently recover very low bioloads. This confirms the device's excellent performance with dry skin where total number of bacteria on a 6 in^2 surface is expected to be $<10^5$ cells.

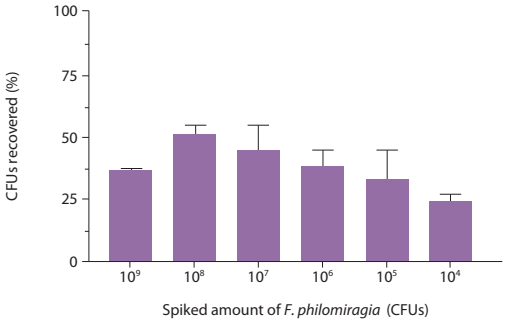

***Figure 6*: Collection performance of OMNIgene•SKIN.** *Increasing amounts of a F. philomiragia culture were spread on artificial skin (SynDaver) and allowed to dry. The spiked bacteria were then collected using our device following our standard IFUs and DNA was extracted using our optimized workflow (bead beating). Recovery of F. philomiragia was assessed using a qPCR assay targeting the iglC3 gene and was calculated as a percentage of the total CFUs spiked on the artificial skin surface.*

Conclusions

- OMNIgene•SKIN efficiently captures and stabilizes the microbiome profiles (bacterial and fungal) of commonly studied skin sites (sebaceous, wet and dry), for downstream high-throughput sequencing and qPCR applications.
- The collected skin microbial profile is stable during shipping at ambient temperature and up to 30 days during storage at room temperature.
- OMNIgene•SKIN's enhanced IFUs are optimized for collection of low biomass samples, ensuring superior performance.

References

1 Manual of Procedures for Human Microbiome Project Core Microbiome Sampling Protocol A, HMP Protocol # 07-001 Version Number 12.0, 29 Jul 2010.

2 Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581-583. doi:10.1038/nmeth.3869

3 Martin M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal*. 2011;17(1):10-12. doi:https://doi.org/10.14806/ej.17.1.200.

4 Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2013;41(Database issue):D590-D596. doi:10.1093/nar/gks1219

5 Nilsson RH, Larsson KH, Taylor AFS, et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Res*. 2019;47(D1):D259-D264. doi:10.1093/nar/gky1022

6 R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

7 Wickham H. Ggplot2: Elegant Graphics for Data Analysis. *Springer*. 2016. doi: 10.1007/978-0-387-98141-3

8 MicrobeR: Handy functions for microbiome analysis in R. R package version 0.3.2. URL https://github.com/jbisanz/MicrobeR

9 Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome*. 2014;2:15. Published 2014 May 5. doi:10.1186/2049-2618-2-15

10 Kong HH, Segre JA. Skin microbiome: looking back to move forward. *J Invest Dermatol*. 2012;132(3 Pt 2):933-939. doi:10.1038/jid.2011.417

11 Ross AA, Rodrigues Hoffmann A, Neufeld JD. The skin microbiome of vertebrates. *Microbiome*. 2019;7(1):79. Published 2019 May 23. doi:10.1186/s40168-019-0694-6

12 Leyden JJ, McGinley KJ, Nordstrom KM, Webster GF. Skin microflora. *J Invest Dermatol*. 1987;88(3 Suppl):65s-72s. doi:10.1111/1523-1747.ep12468965