Chasing the human skin microbiome: developing an efficient and versatile device to collect microbial samples from a broad range of skin sites

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Abstract

The human skin is a vast and diverse environment that harbours a rich microbiota composed of hundreds of bacterial species as well as fungal and viral taxa in lower relative abundance. The discrete regions of the human skin can be grouped into three main groups based on their physico-chemical properties: sebaceous, wet and dry. Previous studies have indicated that each of these sites tend to support different microbial species; for instance *Cutibacterium acnes* is primarily found on lipid-rich sebaceous sites. The skin surface is generally poor in nutrients and therefore it is only able to sustain a much lower microbial biomass than the gut or the oral cavity. Very low microbial abundance combined with a wide variety of collection sites makes skin microbiome sampling notoriously difficult, and no standardized reliable collection methods are available. To this end, we developed a swab-based skin microbiome collection and stabilization device (P-189), and demonstrated that this prototype can consistently collect microbiome samples from sebaceous, wet and dry skin sites. Given the critical importance of DNA yields in skin microbiome studies, we developed an optimized processing pipeline for P-189 collected samples, and show that our collection device is able to reliably yield DNA in sufficient quantities for downstream analysis, including from traditionally very low yield dry skin sites. Taxonomic analysis of P-189 collected skin microbiome samples using 16S/ITS and shotgun sequencing revealed microbial profiles consistent with published data, indicating efficient capture of site-specific microbial taxa, with minimum background contamination. Lastly, we also tested the quantitative performance of our device, and determined that the P-189 prototype can successfully collect as little as 104 bacterial cells spiked on artificial skin. Taken together, our data indicates that the P-189 is a versatile collection device optimized for skin microbiome studies.

P-189: an optimized method for skin microbiome collections

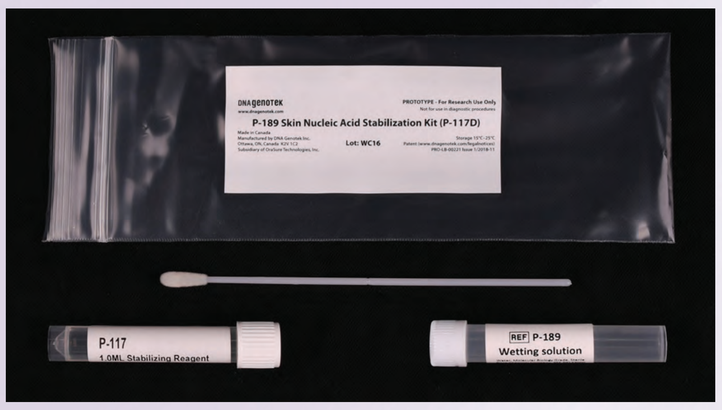

***Figure 1**: P-189 kit contents. The kit is optimized for self-collection of skin microbiome samples using a flocked swab and a wetting reagent. Collected sample on the swab is shipped while stored in DNA Genotek's stabilizing solution.*

Materials and methods

Samples

Skin microbial samples were collected by 8 donors using the P-189 prototype from dry (forearm), sebaceous (face and scalp) as well as wet (toe web) skin sites. Collected samples were processed with our optimized pipelines and extracted using PowerFecal® Pro (PF-Pro) or bead beating, an in-house protocol specifically developed for low biomass samples. DNA was quantified using PicoGreen® and used as a template for 16S and ITS sequencing. Skin intact cell mock community (MSA-2005) was purchased from ATCC and either spiked directly in the extraction tubes or collected using the P-189 swab.

Sequencing and analysis

Library preparation, sequencing and bioinformatics were conducted using 16S V3-V4 hypervariable regions (bacterial) and ITS 2 (fungal) paired-end amplicon sequencing, with PE-300 V3 kit on an Illumina® MiSeq platform. Raw sequence data were processed using the Dada2 (v 1.10.1) pipeline. Briefly, primers were removed (cutadapt 2.1), 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 (16S amplicons) or the UNITE (10.10.2017) database (for ITS2 amplicons). Only ASVs having at least 1% abundance in any one sample were retained for downstream analyses. For metagenomics sequencing, samples were processed using CoreBiome's proprietary BoosterShot™ on a NovaSeq platform.

Improved processing pipeline for P-189 for optimal DNA yields from the three major types of skin sites

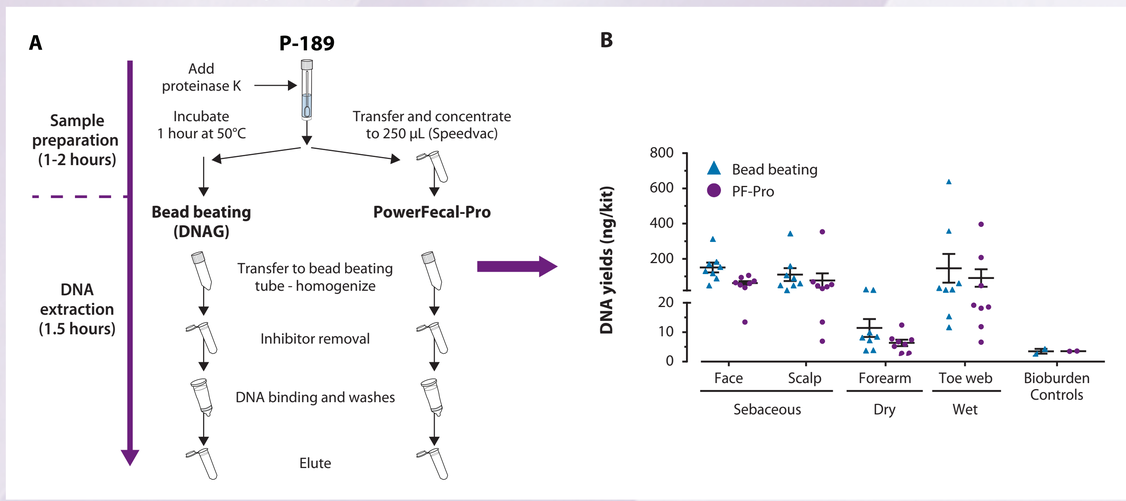

***Figure 2:** Processing strategies for P-189 samples for optimal DNA recovery. (A) Schematic of the optimized pipelines used to process entire P-189 samples in a single extraction. Performance of a commercially available extraction kit (Qiagen's PowerFecal-Pro) or an in-house developed extraction (bead beating) were tested and compared. (B) Total DNA yields extracted from samples collected from the three major types of skin sites (sebaceous, dry and wet) using P-189 prototype. Duplicate samples collected by 8 donors were extracted using either PowerFecal-Pro or bead beating and quantified using the Picogreen assay.*

Optimized processing of P-189 samples efficiently captures microbial profiles from mock communities and donor skin sites

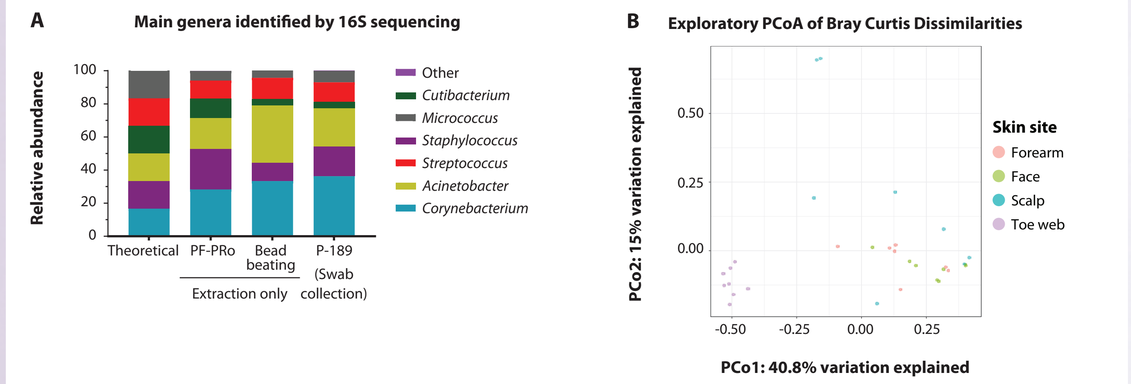

***Figure 3**: P-189 offers efficient capture and recovery of microbial profiles from mock community and major types of skin sites. (A) Taxonomic profiles of ATCC intact cell skin mock community (MSA-2005) extracted with PowerFecal-Pro, bead beating or collected using the P-189 prototype and extracted using bead beating. (B) Principal coordinate analysis (PCoA) plot of samples collected from the three major types of skin sites (sebaceous, dry and wet) using P-189 prototype. Samples were collected by 8 donors and extracted using bead beating. For taxonomic profiling, the V3-V4 region of the 16S gene was amplified. Plots were generated from the filtered ASV data, rarefied to 5000 reads, and generating Bray-Curtis dissimilarities between samples.*

16S and ITS sequencing reveals differences across P-189 sampled skin sites consistent with literature data

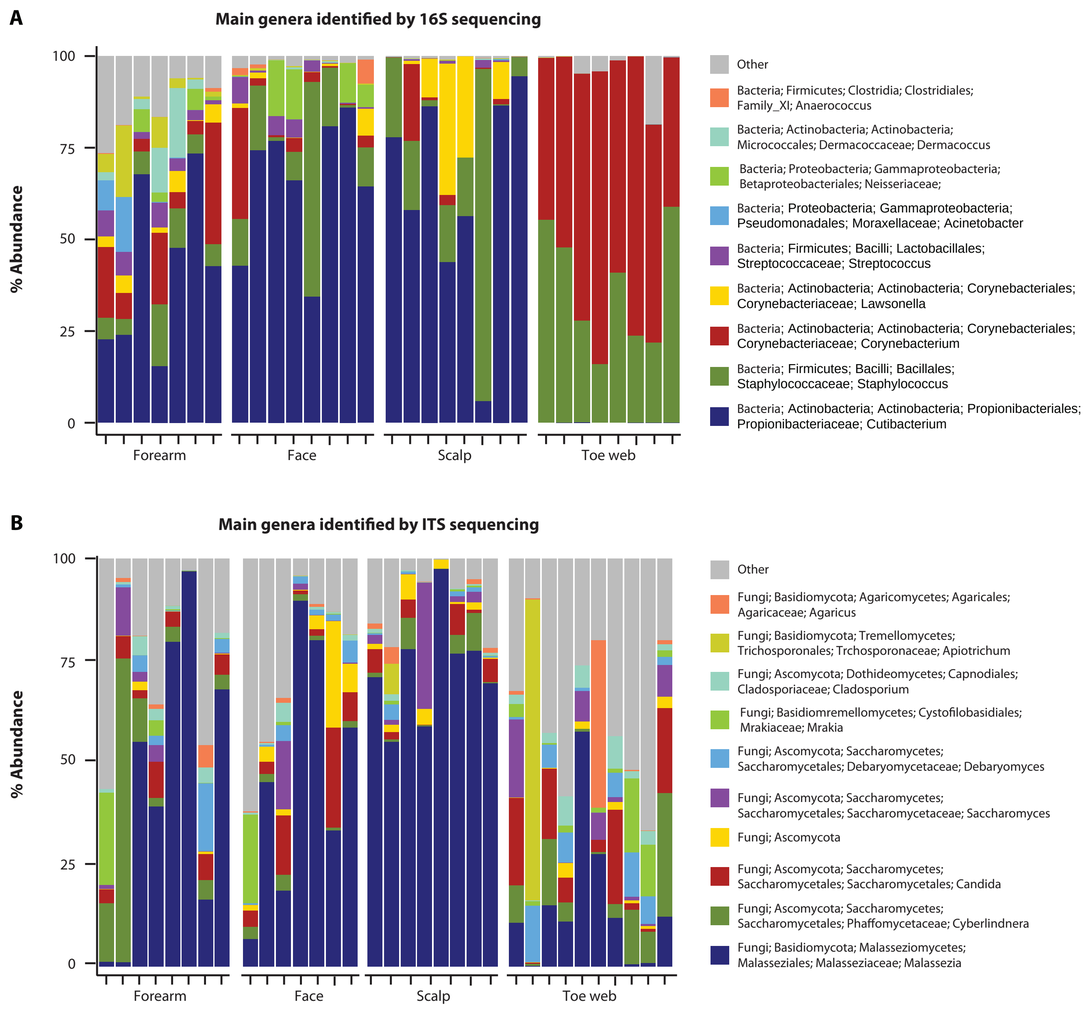

Figure 4: Bacterial and fungal taxonomic profiles of skin microbial samples collected from the forearm (dry), face and scalp (sebaceous) and toe webs (wet) using the P-189 prototype. Samples were collected form a total of 8 donors and extracted using bead beating. (A) 16S rRNA gene was amplified using primers targeting V3-V4. Relative abundance plot was generated from the filtered ASV data classified using the Silva database. (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.

The P-189 prototype demonstrates efficient capture/release of skin-associated bacterial cells

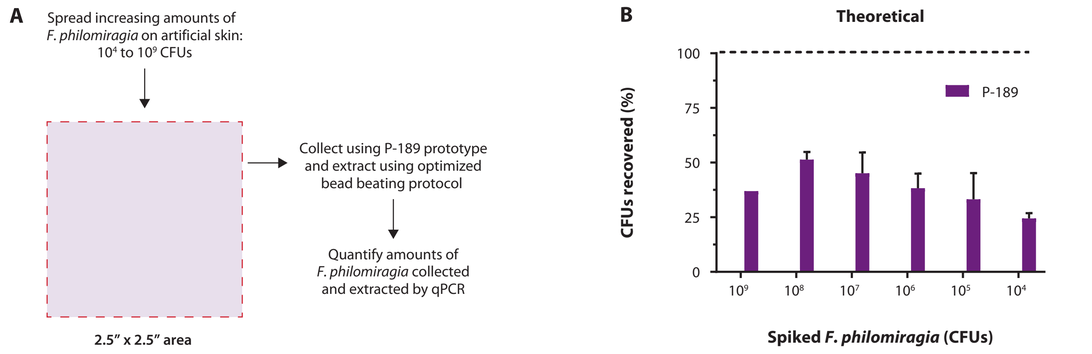

***Figure 5**: Quantitative performance of the P-189 prototype using an artificial skin model. (A) Increasing amounts of F. philomiragia were spread on approximately a 6 square inches piece of artificial skin (SynDaver) and allowed to dry. The spiked bacteria were collected using P-189 devices following our standard IFUs and processed using bead beating. (B) Recovery of F. philomiragia was assessed using a qPCR assay targeting the iglC3 gene. Recovery efficiency was calculated as a percentage of the total CFUs spiked on the artificial skin surface prior to collection and averaged 38% across the range of spiked amounts.*

The P-189 prototype yields high quality DNA in high enough concentrations for shotgun sequencing using BoosterShot

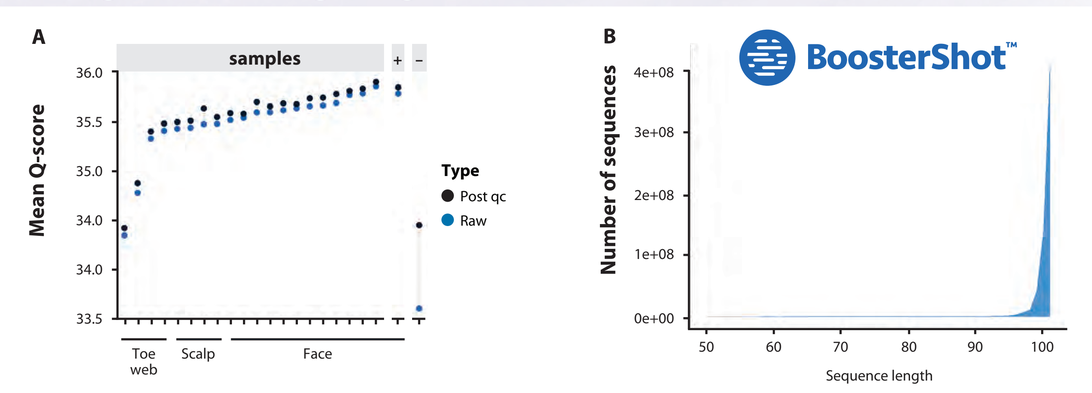

***Figure 6**: Quality metrics of skin samples library prepped and sequenced using CoreBiome's BoosterShot. (A) Mean Q-scores of skin samples collected from the toe web, scalp and face using the P-189 devices. Post-QC: post filtering reads such as read length filtering and chimera removal. (B) Sequence length distribution of the post-qc sequencing reads from P-189 skin samples.*

Conclusions

- P-189 skin collection prototypes can be used to collect human skin microbial samples from a wide variety of sites. Our device is compatible with the three major types of skin sites (sebaceous, dry and wet).
- Using our optimized pipelines, we are able to process the entire collected sample in a single extraction, maximizing DNA yields from a wide range of skin sites, including forearm (a particularly low biomass dry site).
- Taxonomic analysis of bacterial and fungal profiles using 16S and ITS2 demonstrate that the P-189 device can accurately capture the unique microbial features from each sampled site.
- The quantitative performance testing of our swab-based kit shows that capture and recovery of bacterial cells from skin is efficient (38% recovery on average). As little as 104 CFUs can be recovered/detected when using our collection kit, confirming the performance observed with dry skin sampling.
- P-189 collected samples processed using our pipelines yields high quality DNA in high enough concentrations for shotgun metagenomics sequencing allowing for greater insights into the human skin microbiome.

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