HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2017 June 13.

Published in final edited form as:

J Invest Dermatol. 2017 March ; 137(3): 561–568. doi:10.1016/j.jid.2016.10.033.

Performing skin microbiome research: A method to the madness

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Abstract

Growing interest in microbial contributions to human health and disease has increasingly led investigators to examine the microbiome in both healthy skin and cutaneous disorders, including acne, psoriasis and atopic dermatitis. The need for common language, effective study design, and validated methods are critical for high-quality, standardized research. Features, unique to skin, pose particular challenges when conducting microbiome research. This review discusses microbiome research standards and highlights important factors to consider, including clinical study design, skin sampling, sample processing, DNA sequencing, control inclusion, and data analysis.

Introduction

The relationship between host and cutaneous microbes has been of great clinical scientific interest, often studied with traditional cultivation methods and focused on a single/few bacteria (Evans *et al.*, 1950; Kligman *et al.*, 1976; Lai *et al.*, 2010; Marples, 1965; Nizet *et al.*, 2001). Reduced costs and increased access to high-throughput sequencing have enabled global examination of the skin microbiome, broadly defined as skin microbiota with their genomes and surrounding environmental conditions (Marchesi and Ravel, 2015).

Early skin microbiome studies described healthy human skin microbial communities as more diverse than previously recognized through cultivation methods (Dekio *et al.*, 2005;

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Conflict of Interest

The authors state no conflict of interest.

Gao *et al.*, 2007; Grice *et al.*, 2008a) and unique to skin (Costello *et al.*, 2009; Human Microbiome Project, 2012a, b). Several reviews (Clavel *et al.*, 2016; Goodrich *et al.*, 2014; Huttenhower *et al.*, 2014) have outlined important elements of high-quality microbiome studies. The unique aspects of skin, including low microbial biomass, high contamination risk (Salter *et al.*, 2014), accessibility and diversity of cutaneous habitats, site-specific microbiota, and a distinct immune system (Naik *et al.*, 2012; Watanabe *et al.*, 2015) necessitate important considerations for conducting skin microbiome studies (Figure 1). Several reviews have summarized skin microbiome literature (Edmonds-Wilson *et al.*, 2015; Jo *et al.*, 2016b; Schommer and Gallo, 2013; Zeeuwen *et al.*, 2013).

In emerging fields, studies to identify optimal methodologies are often performed, and several include elements related to the skin microbiome (Human Microbiome Project, 2012a, b). Study design for skin microbiome research is multifaceted and integral to all downstream steps. Published studies examined skin sampling methods (Chng *et al.*, 2016; Grice *et al.*, 2008a), sample storage (Lauber *et al.*, 2010), controls and contamination sources (Salter *et al.*, 2014), sequencing biases (Meisel *et al.*, 2016), and possible quantitation (Gao *et al.*, 2010). The current review integrates this combined expertise and focuses on methodology and challenges of factors important for skin microbiome research to promote reliability and comparability (Figure 1). Of note, we primarily discuss 16S rRNA gene amplicon sequencing, as the most widely used method.

Potential Pitfalls

Similar to other interdisciplinary fields, multiple factors are important in conducting or assessing a skin microbiome study.

- *Study Design*: Consistent metadata collection; considering potential confounding factors
- *Skin Sample Collection/Storage*: Standardized collection/handling of samples
- *Sample Processing/Sequencing*: DNA extraction; PCR conditions, Primer selection
- *Process Controls*: Negative/blank controls; mock community comparison
- *Analysis Methods*: Pipeline description; sequencing data availability with associated metadata

Study design

Since the skin microbiome is comprised of different microbes including bacteria, fungi, and viruses, whether the scientific focus is on one particular kingdom or all microbes will influence study design, sequencing, costs, and analysis. Bacteria have been the main focus, but several have used sequencing methods to examine skin fungal (Findley *et al.*, 2013; Jo *et al.*, 2016a; Paulino *et al.*, 2006; Zhang *et al.*, 2011), viral (Foulongne *et al.*, 2012; Hannigan *et al.*, 2015; Oh *et al.*, 2014; Oh *et al.*, 2016; Wylie *et al.*, 2014) and archaeal communities (Probst *et al.*, 2013).

A “study population” may refer to individuals with/without a particular disease, in a specific age range (Capone *et al.*, 2011; Costello *et al.*, 2013; Dominguez-Bello *et al.*, 2010; Oh *et al.*, 2012; Ying *et al.*, 2015), or in a geographic region (Blaser *et al.*, 2013; Clemente *et al.*, 2015). Studies have demonstrated some interindividual differences in the skin microbiome even when matched for body site and sexual maturity, highlighting the need for the study design (e.g. sample size) to account for a certain degree of heterogeneity in the skin microbiome of a target study population; however, many features of the skin microbiome can be commonly observed (i.e. sebaceous sites hosting lipophilic bacteria). Skin bacterial communities in neonates, infants, and young children are notably distinct from those in sexually mature children and adults, particularly at certain skin sites (Capone *et al.*, 2011; Costello *et al.*, 2013; Dominguez-Bello *et al.*, 2010; Jo *et al.*, 2016a; Oh *et al.*, 2012; Ying *et al.*, 2015). The skin microbiomes in patients with different cutaneous and general medical conditions show distinctive patterns of skin microbiomes, but heterogeneity in the experimental study designs highlights the challenges in comparing results between studies and emphasizes a need for minimal standards.

Screening subjects involves collecting demographic data, obtaining detailed history on prior and/or current medical conditions and topical/systemic medications, performing clinical examinations, and considering diagnostic criteria. Explicit criteria for defining healthy individuals are important (Aagaard *et al.*, 2013). Disease phenotyping (validated diagnostic criteria, severity scoring, and clinical photography) enables more accurate comparison of subpopulations within a particular disorder.

A typical exclusion criterion for healthy individuals is prior systemic antibiotic usage, based on antibiotic use within the last 12 months (Grice *et al.*, 2009a), 6 months (Costello *et al.*, 2009; Findley *et al.*, 2013; Human Microbiome Project, 2012b), or 1 month (Gao *et al.*, 2007). For individuals with skin disorders, prior usage of topical and systemic medications can be used as exclusion criteria or defining metadata which can affect analyses (Kong *et al.*, 2012). Other medications may influence the skin microbiome, and collecting a complete medication history is desirable.

Clinical metadata documentation is critical for downstream analyses and may help explain differences within/between studies. Commonly collected metadata include age, sex, antibiotic use, and sampling sites. Some factors such as pet ownershp (Song *et al.*, 2013), deodorant usage (Callewaert *et al.*, 2013), physical activities (Meadow *et al.*, 2013), season, time of day, country of birth, race/ethnicity, mode of delivery and diet may influence the skin microbiome.

Calculating sample sizes for skin microbiome studies can be difficult without pre-existing data for estimating effect sizes. A few methods have been proposed to calculate sample sizes (Kelly *et al.*, 2015; La Rosa *et al.*, 2012), including a web-based tool called Evident (https://github.com/biocore/Evident). With growing numbers of skin microbiome studies, pre-existing data for estimating potential effect sizes are increasingly available for use in designing well-powered studies.

Skin preparation

Questions often arise regarding factors to control in skin microbiome studies. Standardizing controllable factors reduces confounders, maximizing ability to determine the experimental variable responsible for any observed difference. Factors such as washing can alter the bacterial communities present on hands (Fierer *et al.*, 2008) and after experimental exposure to non-skin bacteria (Costello *et al.*, 2009; Two *et al.*, 2016). Due to potential fluctuations in skin microbial communities in the hours after bathing/showering, several studies have utilized a minimum number of hours after baths/showers before sampling; the minimum time since last washing has ranged from 12 hours (Human Microbiome Project, 2012a, b) to 24 hours (Grice *et al.*, 2009a; Oh *et al.*, 2016; Oh *et al.*, 2012). Skin hygiene (e.g. soap and shampoo practices) can also alter the skin microbial communities in some circumstances (Perez Perez *et al.*, 2016) but not in others (Two *et al.*, 2016). Use of emollients can influence the skin microbiome (Seite *et al.*, 2014); therefore, the restrictions on emollient use in studies has varied between 1 to 7 days of avoidance prior to sampling (Human Microbiome Project, 2012a, b; Kong *et al.*, 2012). The effects of topical medications, e.g. steroids, on the skin microbiome can persist for up to 7 days (Kong *et al.*, 2012).

Skin Sampling Methods

Multiple methodologies have been documented and validated for skin microbiome sampling, including swabs, biopsies, surface scrapes, cup scrubs and tape strips (Chng *et al.*, 2016; Costello *et al.*, 2009; Findley *et al.*, 2013; Gao *et al.*, 2007; Grice *et al.*, 2008b; Nakatsuji *et al.*, 2013; Oh *et al.*, 2014; Zeeuwen *et al.*, 2012). Each method varies in biomass yield, human DNA contribution, sampling depth and discomfort level. Method should be based on scientific question, study design, study population, and sampling sites. The skin microbiome composition at the skin surface can differ from deeper layers of the skin (Grice *et al.*, 2008b; Nakatsuji *et al.*, 2013; Zeeuwen *et al.*, 2012) and between skin sub-compartments and structures. Thus, consistency is key for skin microbiome sampling methods.

The most established collection method is premoistened swabbing (Aagaard *et al.*, 2013; Human Microbiome Project, 2012a, b; Paulino *et al.*, 2006). Dry swabbing (Schowalter *et al.*, 2010) has not been as widely utilized, due to reduced biomass collection. Tape stripping provides a reproducibly high amount of biomass (Chng *et al.*, 2016) with stratum corneum depth analysis in a defined collection area (Zeeuwen *et al.*, 2012). Tape stripping is not suitable for all body sites because of its dimensions and sample acquisition time. Scrapes increase biomass collected and could be useful for studies of low abundance microbes, e.g. fungi (Findley *et al.*, 2013; Grice *et al.*, 2008b). Cup scrubbing was pioneered for culture-based studies (Williamson and Kligman, 1965) and can also be used for microbiome studies (Chng *et al.*, 2016). Skin punch biopsies enable analysis of microbial DNA potentially deeper in skin. In addition to tissue homogenization for total DNA collection, a portion of skin biopsies can be processed for other analyses, including laser capture microscopy (Grice *et al.*, 2008b; Nakatsuji *et al.*, 2013). However, biopsies are invasive with reduced ability to sample multiple sites in patients.

Most major bacterial taxa are similarly identified in swabs, scrapes, and biopsies, but a tree-based analysis could segregate sampling methods (Grice *et al.*, 2008b). Comparing swabs,

tape strips, and cup scrubs for 16S rRNA gene or shotgun metagenomic sequencing provided high concordance when analyzing relative abundance (Chng *et al.*, 2016). While highlighting that multiple methods can be used, it is important to maintain a consistent standardized approach for sample collection throughout a study.

Other aspects of sample collection have been emphasized in prior studies, i.e. location, sampling frequency, and use of controls. Skin microbial communities exhibit striking site-specific differences and notable topographical diversity over the human skin surface, even within the same individual. Yet, regions of skin with common physiological characteristics - sebaceous, moist, dry - share some similarities in the composition of skin microbial communities (Costello *et al.*, 2009; Grice *et al.*, 2009a; Oh *et al.*, 2014; Oh *et al.*, 2012). Consistent sampling of the same anatomic area in the entire study cohort reduces confounders and maximizes the ability to identify microbiome differences (Costello *et al.*, 2009; Grice *et al.*, 2009a). Sampling body sites with bilateral symmetry has also been used to confirm that consistency is observed with low intra-individual variability (Chng *et al.*, 2016; Grice *et al.*, 2009a). Frequency of sampling has varied in different studies from a single timepoint to repeated sampling. Longitudinal sampling of skin has demonstrated that skin microbiomes are highly individualized (Flores *et al.*, 2014; Oh *et al.*, 2016), which suggests that repeated sampling can provide an internal control, and help to increase the statistical power for analyzing changes in a chronic skin disorder (Kong *et al.*, 2012).

Sample storage

For sample handling, most studies have used the standard method of immediate freezing after sample collection, followed by storage at −80°C (Blaser *et al.*, 2013; Costello *et al.*, 2009; Grice *et al.*, 2009a). No major differences in the bacterial communities were found in skin and fecal samples collected from 2 individuals and stored at different temperatures for two weeks (Lauber *et al.*, 2010). However, storage at −80°C is generally advised (Goodrich *et al.*, 2014). Studies have demonstrated that freeze-thaw cycles can alter the microbial composition observed in samples and should be avoided (Cuthbertson *et al.*, 2015; Sergeant *et al.*, 2012).

Sample Processing

DNA extraction

During early steps of sample preparation, bias can easily be introduced (Brooks *et al.*, 2015; Yuan *et al.*, 2012), especially during the lysis of microbial cells (Yuan *et al.*, 2012). Mechanical lysis (with or without enzymatic treatment) provides more comprehensive profiles of Gram-positive bacterial populations and fungi than enzymatic treatment alone, and is thus strongly advisable (Albertsen *et al.*, 2015; Findley *et al.*, 2013; Santiago *et al.*, 2014; Sergeant *et al.*, 2012; Walker *et al.*, 2015; Yuan *et al.*, 2012). After lysis, DNA can be purified following different approaches, e.g. by alcohol precipitation or via binding to affinity columns. Downstream applications will dictate genomic DNA requirements: higher standards in terms of fragment size, amount, and purity of target DNA are often required for some sequencing methods. Therefore, consistent documentation of the quality and quantity of DNA obtained (e.g. fluorometry, UV spectroscopy) is important (Olson and Morrow,

2012) and performing gel electrophoresis on high yield samples will help to characterize fragment size distribution.

Library construction: Amplicons

PCR amplification introduces biases in datasets. Use of PCR replicates, controls, appropriate PCR primers and PCR cycle conditions help control library quality. PCR replicates (at least duplicates; if possible triplicates) that are pooled in downstream steps (e.g. during library purification) can be prepared to limit effects of early amplification bias (Acinas *et al.*, 2005).

Primer choice significantly influences microbial profiles as PCR efficiency can vary between organisms (Hiergeist *et al.*, 2016; Meisel *et al.*, 2016; Walker *et al.*, 2015). The most commonly used gene in amplicon metagenomics studies is the 16S rRNA gene. Several sets of 16S rRNA gene-specific primers have been designed, targeting different variable (V) regions and amplicon sizes (Klindworth *et al.*, 2013). Multiple studies have evaluated primer combinations and compared the utility with different sample types. Primer region selection depends on the scientific question and potential bacterial taxa of interest (Human Microbiome Project, 2012a). *Staphylococcus* and *Streptococcus spp.* are major skin bacteria best distinguished using V1 and V2 regions (Conlan *et al.*, 2012). A recent study showed that products spanning V1–3 were more accurate in classifying common skin bacteria and were more similar to results obtained using shotgun metagenomic sequencing of a mock community, as compared to V4 (Meisel *et al.*, 2016). The currently most used region for analysis of skin bacterial communities is V1–3. Consistent use of a single target region facilitates comparison between studies yet may limit differentiation of bacteria optimally sequenced with other primer regions. Fungal sequencing studies have targeted a few different regions associated with the 5.8S, 18S, 28S, and ITS1/2 ribosomal genes (Findley *et al.*, 2013; Paulino *et al.*, 2006; Wang *et al.*, 2015; Zhang *et al.*, 2011).

A key PCR parameter is the number of cycles used for amplification. An increase in the number of amplification cycles can result in lower diversity, and can skew bacterial profiles towards detection of low abundant taxa (Acinas *et al.*, 2005; Bonnet *et al.*, 2002). Hence, it is advisable that PCR cycles are kept to the lowest number that still delivers reproducible DNA amounts across a wide range of samples while also avoiding overamplification and ensuring that negative controls do not yield a product. Several groups have used ~30 cycles of PCR (Dekio *et al.*, 2005; Gao *et al.*, 2007; Grice *et al.*, 2009a; Human Microbiome Project, 2012a, b; Smeekens *et al.*, 2013). The quality of the amplicon libraries produced should be documented, e.g. by controlling for the presence of unspecific bands using high-sensitivity electrophoresis.

Sequencing

Different sequencing platforms are available with various chemistries and technologies, and selection of the platform will be dependent on sequencer availability, amplicon size, sequencing depth, sequencing accuracy, and/or budget. With increased sequencing capacity, it has become feasible to routinely carry out shotgun metagenomic sequencing from human skin samples (Chng *et al.*, 2016; Kang *et al.*, 2015; Oh *et al.*, 2014; Oh *et al.*, 2016). Some advantages of this strategy include reduced amplification bias, generation of multikingdom

genetic information, strain identification, and detailed genomic coverage for prediction of functional capacity (Chng *et al.*, 2016; Human Microbiome Project, 2012b; Oh *et al.*, 2014; Oh *et al.*, 2016). Challenges with whole metagenomic sequencing are associated with data analysis complexity and low biomass from human skin samples, typically in the pg to ng range, which can make sequencing library construction difficult and increases sensitivity to contamination with mammalian DNA and microorganisms from the environment and laboratory reagents.

Use of Blank and Control Samples

For the low biomass of skin samples, blank and control samples must be collected and run in parallel to target samples (Costello *et al.*, 2009; Grice *et al.*, 2009b; Paulino *et al.*, 2006). Blank samples are controls intended to contain no biological starting material of interest (e.g. only collection/storage buffers), including mock samples collected during patient sampling (e.g. premoistened swab fanned in the air) and DNA extraction processes, and PCR/sequencing blanks (containing only purified PCR-grade water as template). Blanks are processed with target samples to identify extraction contaminants and amplification artefacts, cross-contamination during library preparation, and contaminants entering during sequencing.

Mock communities (artificial mixtures of known target microorganisms) and reference samples of known composition are critical for benchmarking sample processing and sequence analysis (D'Amore *et al.*, 2016; Jumpstart Consortium Human Microbiome Project Data Generation Working, 2012; Yuan *et al.*, 2012). A mock community used with each sequencing run or across sequencing centers can promote standardization (Sinha *et al.*, 2015) and should include multiple taxa important to the ecosystem of interest. A widely available synthetic DNA mock community (BEI Resources HM-276D) has been developed (Jumpstart Consortium Human Microbiome Project Data Generation Working, 2012); this includes bacterial species important to skin (*P. acnes, S. aureus,* and *S. epidermidis*).

Additionally, when studying a patient cohort, it would be ideal to leverage data generated from healthy volunteers in previous small and larger studies. While in principle this increases the power of the study, collection and analysis of data from some healthy volunteers alongside the patient population of interest would control for factors specific to the study.

Analysis Methods

The quality of analysis is dependent on consistent and thorough documentation of clinical metadata, sample collection/storage, DNA processing of target samples, negative controls, mock community positive controls, and sequencing methods (Goodrich *et al.*, 2014). The metadata allow researchers to delineate potential causes/associations with sequencing results. Analysis pipelines, e.g. QIIME (Caporaso *et al.*, 2010) mothur (Schloss *et al.*, 2009), and IMNGS (Lagkouvardos *et al.*, 2016), have tutorials and best practices with parameters that should be evaluated for appropriate use with a particular dataset. There are many

approaches to analyzing 16S rRNA gene sequencing data and the following overview briefly highlights most commonly used methods.

The 16S rRNA gene sequence analysis pipeline is comprised of three main components: pre-processing sequences, constructing OTU (Operational Taxonomic Units, similar to bacterial taxa or species) tables, and annotating OTU tables. Sequence pre-processing is used to remove low quality sequences prior to OTU table construction. Chimeric sequences generated during the PCR amplification process also need to be identified and removed from the dataset (Haas *et al.*, 2011). The appropriate analysis methods and parameters are dependent on the sequencing method and amplicon region (D'Amore *et al.*, 2016).

After pre-processing, the OTU table is constructed by clustering (grouping) similar sequences based on a defined similarity threshold. There are several approaches to clustering (Chen *et al.*, 2013). The reference clusters used and the similarity threshold significantly affect the clustering results (He *et al.*, 2015; Kopylova *et al.*, 2016; Schloss, 2016). Normalization or rarefaction is used to address issues due to sequencing depth differences (McMurdie and Holmes, 2014).

The resulting OTU table is annotated based on representative OTU taxonomic and phylogenetic relatedness, using specific databases (Conlan *et al.*, 2012; Schloss, 2010). A number of different methods are used to classify 16S rRNA gene sequences, e.g. reference-based clustering, sequence similarity (e.g. BLAST), K-mer based methods (e.g. RDP), and phylogenetic placement (Nguyen *et al.*, 2016). Phylogenetic trees are commonly used in diversity metrics (e.g. UniFrac) or for data visualization.

Analysis of shotgun metagenomic data is challenging due to the orders of magnitude larger amounts of data generated and requires filtering high percentages of human sequences (Chng *et al.*, 2016; Oh *et al.*, 2014; Oh *et al.*, 2016). Limitations of microbial sequencing analyses relate to the need for more reference genomes and for fundamental research on the function of genes identified in metagenomic sequencing. Analytical methods will continue to evolve to utilize both reference-based and reference-free mapping strategies (Human Microbiome Project, 2012b; Ma *et al.*, 2014; Oh *et al.*, 2014; Wylie *et al.*, 2014). For microbiome and other genomic data, depositing data with relevant metadata in public repositories is a standard requirement for many funding agencies and allows acceleration of research through data sharing, re-analysis, validation, and compilation.

Guidelines and Future Needs

Maintaining a standardized format for reporting sample handling and processing with a common scientific language (Marchesi and Ravel, 2015) is important to promote reproducibility and advance science (Ravel and Wommack, 2014). Minimal standards/guidelines are often developed to further these goals, e.g. MIQE for quantitative real-time PCR (Bustin *et al.*, 2009), MIAME for microarray studies (Knudsen *et al.*, 2005), CONSORT for clinical trials (Begg *et al.*, 1996), and MIMARKS and MIxS for genomics (Yilmaz *et al.*, 2011). Research utilizing large DNA sequence datasets requires significant data analysis with a unique set of criteria for reproducibility (Peng, 2011). Depositing

primary data into public databases enables independent cross-evaluation and comparison with previous studies, but requires access to raw data, metadata, software, and code used to process the data (Sandve *et al.*, 2013; Stodden *et al.*, 2014). The Genomic Standards Consortium has promoted minimum information standards and provides checklists for a broad range of genomic studies (Yilmaz *et al.*, 2011). Adapting existing guidelines to skin microbiome studies, key areas that are important to clearly describe in manuscripts and attach (as metadata) to sequencing data:

> **Study design**: Include clinical protocol information, study population, inclusion/exclusion criteria, medical and medication history, clinical phenotyping/validated tools for diagnosis and/or severity assessment, documented clinical metadata, and skin preparation regimen.
>
> **Sample collection/storage**: Include description of sampling methods, skin sites, sample storage, and controls.
>
> **Sample processing/sequencing**: Include detailed lysis methods, DNA purification, PCR primers, PCR conditions, processing controls, and sequencing.
>
> **Process controls:** Include negative controls during sample collection, processing, and sequencing and a mock community.
>
> **Analysis pipeline**: Include software (version), any specific commands, parameter settings, statistical tests, scripts for new pipelines (if applicable), and reference databases (version, access date, and modifications).
>
> **Data deposition**: Genomic data sharing is often required with publication in many journals. Note submission of raw sequences to a publicly available database should be accompanied by specific relevant metadata, e.g. collection date, skin site/location, clinical features (Yilmaz *et al.*, 2011).

In addition, several areas for growth could facilitate advancement towards mechanistic studies that better elucidate host-microbial interactions. Skin microbiome studies are an important window into our microbial communities and enable formulation of hypotheses, but cause-or-effect relationships can be difficult to untangle. To test functional hypotheses, conducting studies that progress beyond DNA sequence data to include clinical isolates is key. Genomic and biological differences are well-recognized at the microbial species and strain level, e.g. *Staphylococcus aureus* strains with or without methicillin-resistance (Greenblum *et al.*, 2015; Oh *et al.*, 2014). Thus, strains of a particular species available from biorepositories may or may not function in the same manner as the strains directly obtained in parallel from subjects studied as part of a microbiome analysis. Cultivating and curating skin-associated strains from human studies and making these available would improve sequencing analyses and provide live and highly relevant microbes for biological experiments. To isolate new reference strains, it is important to optimize methods for capturing a diverse set of skin microbes that reflects the complexity of the microbial communities as has been done for other body sites (Browne *et al.*, 2016). Single cell microbial sequencing could be a future option (Lasken and McLean, 2014), which would still benefit from reference genomes that take advantage of the relative ease of culturing most skin-associated bacterial strains.

Current methods to study the skin microbiome are based on relative abundance of microbes. Determination of bioburden and quantification of skin microbes is relevant to understanding the bioburden of microbes the skin typically harbors and whether it increases in certain diseases. One could perform quantitative PCR of the 16S rRNA gene to determine bacterial recovery in a given sample. A major issue is normalization; does one normalize to total DNA, which would include both human and microbial and could alter with disease state? Or does one normalize to surface area sampled, which might be affected by pressure applied and thus layers of skin released? In parallel with sequencing, developing microscopy methods to improve visualization of skin bacterial communities would greatly advance our ability to understand the structure and potential interactions within microbial communities.

While skin microbiome studies may identify differences between affected and unaffected subjects, correlation-versus-causation questions remain. Longitudinal data across multiple timepoints can provide insights into the natural history of diseases and dynamics of ecological succession of the skin microbial communities. Another method to gather further evidence supporting causation is to identify colonization with a specific microbe or community of microbes prior to disease manifestation. Given the interest in microbial education of the immune system, birth cohort study designs provide an opportunity to obtain multiple skin samples prior to disease development, similar to published gut studies (Bokulich *et al.*, 2016; Vatanen *et al.*, 2016; Yassour *et al.*, 2016). Meta-transcriptomics would also offer valuable information regarding the expression of microbial genes during the natural course of disease.

Conclusion

Studies of skin microbiome research have the potential to improve our understanding of host-microbial interactions. A byproduct of the expansion in the number of published skin microbiome studies is the need to understand how studies interrelate. Several scientific communities have developed minimal standards to improve the overall quality of different fields of research (Yilmaz *et al.*, 2011). Minimal standards will contribute to the development of robust studies in skin microbiome research.

Acknowledgments

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. The authors thank Dr. Jay-Hyun Jo and Mr. Ethan Tyler for assistance with figures and Clay Deming for discussions. Certain commercial equipment, instruments, or materials are identified in this paper only to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the NIST, nor is it intended to imply that the materials or equipment are necessarily the best available for the purpose. Official contribution of NIST; not subject to copyrights in USA.

Abbreviations

rRNA	ribosomal RNA
ITS	internal transcribed spacer

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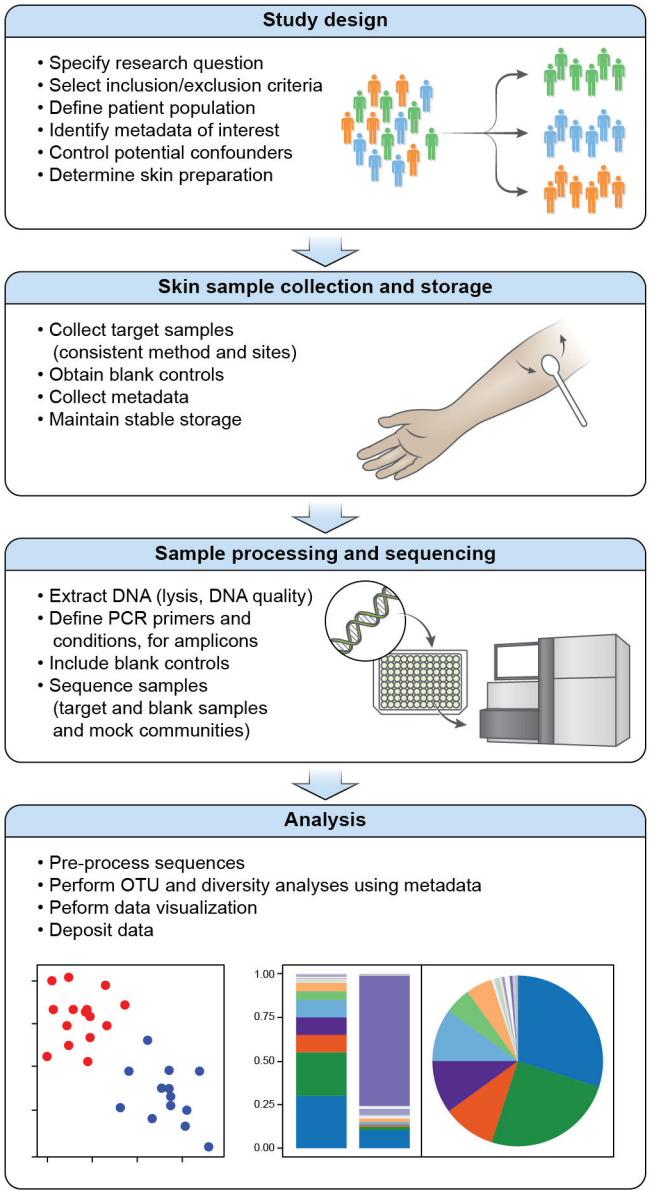

Figure 1.

Steps for Performing a Skin Microbiome Study. The multiple elements of a skin microbiome study begin with study design, followed by skin sample collection and storage, sample processing and sequencing, and analysis.