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Project Title: Surveying the Total Microbiome as Trace Evidence for Forensic Identification

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ABSTRACT

The human skin microbiome can potentially be utilized as trace evidence for forensic applications because microbiome is variable within and between persons. During the project period (Jan. 1, 2017-Jun. 30, 2022), we have examined different human touched trace objects and disclosed which objects are more suitable for the microbiome-based forensic applications and which microbiome is more applicable for forensic identification (Objective 1). In addition, we developed the reverse lifting method as a non-invasive fingerprint lifting method to identify human touched objects suitable for both fingerprint and microbiome-based analysis. Identifying the significant taxa from microbial signatures that can be used for distinguishing individuals is crucial for any possible forensic investigation. we recruited and collected a total of 450 swab samples from 40 subjects belonging to Category 1, 2, and 3 (Objective 2). DNA was isolated from swab samples, quantified using a spectrophotometer, amplified using modified 515(Parada)-806R(Apprill) and ITS1F-ITS2R primers, and sent for amplicon sequencing. Due to the low yield of DNA, a whole genome amplification was employed for whole shotgun metagenome sequencing. The sequencing results were analyzed using next-generation sequencing (NGS) data analysis apps in the BaseSpace Sequence Hub (Illumina) and the pipelines of Nephele. In addition, we collected a total of 54 swab samples from the post-contact intervals (PCI) study (Objective 3). DNA was extracted, quantified, and amplified for amplicon sequencing and quantitative PCR assay. In this study, we developed the reverse lifting method of fingerprints that may provide an alternate way to collect and analyze evidences at a crime scene since this method is less invasive and compatible with other forensic applications like microbiome-based analysis on the same evidence. In addition, this study underlined the use of the total human skin microbiome, including bacteriome, archaeome, fungiome, and virome to provide fundamental information on the application of the microbiome as trace evidence. We explored the applicability of the microbiome retrieved from human-touched objects as a forensic tool by employing cutting-edge NGS technologies, which provides a practical insight into the microbiome-based forensic identification. This microbiome-based forensic identification may provide alternative method to identify individuals associated with a crime scene.

In addition, we have recruited and trained nine undergraduate students as research assistants and provided them a total of 96 months of research experience throughout the project period.

TABLE OF CONTENTS

ACCOMPLISHMENTS

1. Project Goals

The human body is covered with various microorganisms that can be transferrable to where humans interact. Therefore, microbial signatures on the objects that are routinely touched by humans may have forensic implications and thus can be used as forensic trace evidence. Human skin microbiota that is microbial population or community found on humans are known to have high inter-individual variability, and thus may lead to the identification of individuals who touched specific objects. In addition to bacterial community, the human body also contains eukaryotic microorganisms including fungi. Moreover, those bacteria and fungi have their own viruses. Therefore, the profiles of human fungal and viral communities as well as bacterial (and archaeal) communities may serve as trace evidence (microbial fingerprints) for forensic identification. In this study, we propose to determine the total human microbiota including bacteria (and archaea), fungi, and viruses found on human touched objects to see whether it can serve as trace evidence for forensic (human) identification. We also proposed the changes in the microbiome left on touched objects could be used for determining post-touch intervals.

Objective 1. To identify trace evidence suitable for the microbiome-based forensic application.

Hypothesis: The objects that humans touch on a daily basis can be classified or categorized based on the transferability of the microbiome from human skin.

Rationale: Trace evidence analysis includes the identification and comparison of transferred materials, which is a core of forensic science and have played a crucial role in forensic investigations (Roux et al., 2014). Since trace evidence refers to the materials transferred between people, objects or the environment during a crime (De Forest, 2001), literally any materials can be trace evidence at a crime scene. The identification and comparison of these materials can often associate a suspect to a crime scene or with another individual. Physical anthropology (skeletal remains) examinations are also performed. These examinations are conducted to assist in the identification of human remains. Recent applications of the microbiome in forensic sciences raised questions on which material is more suitable for the microbiome-based analysis and to which materials the microbiome is transferred and is more persistent. Identifying and investigating appropriate trace evidence is the fundamental step in forensic investigations. Since spatial factor may play an important role in sharing the microbiome between people (Hauther et al., 2015), we propose to survey trace evidence in three offices in two different locations. We also propose to isolate DNA from trace evidence and measure the quantity and quality of DNA to determine which material is suitable for the microbiome-based analysis.

Task 1.1. *Collect trace evidence in three different offices in two different locations.*

Task 1.2. *Perform standard forensic procedures using human touched objects as trace evidence.*

Task 1.3. *Identify and classify the objects which can be readily applied for microbiome-based forensic identification.*

Objective 2. To determine the total microbiome as trace evidence left on human touched objects.

Hypothesis: The structure and composition of the total microbiome including bacteriome, archaeome, fungiome, and virome from touched objects may have high inter-individual variability and can be used to distinguish individuals and, in turn, identify human identity.

Rationale: Our body harbors at least 100 trillion microbial cells that are 10 times more abundant than human cells (Turnbaugh et al., 2007; Whitman et al., 1998). In general, microorganisms and prokaryotes, especially bacteria are often used interchangeably. As such, most microbiome-based studies focus on the diversity of bacteria and archaea. However, microorganisms found in and on our body include eukaryotic microorganisms such as fungi and protists and viruses as well. Skin is the largest organ of our body, which is a complex ecosystem harboring a variety of microbial populations (Castelino et al., 2014). Although skin contains less abundant microbial biomass compared to the gut, it provides a niche to fungi and viruses as well as bacteria and archaea. Recent advances in metagenomics technologies enable us to study the skin microbiota and identify novel microorganisms (Hannigan et al., 2015). Since less than 1% of bacteria and archaea have been identified, more bacteria and archaea may be identified by metagenomics approaches and thus provide a better picture in terms of bacterial and archaeal diversity. Besides bacteria and archaea, other members of the skin microbiome have not been unequivocally addressed. Recent culture-independent molecular surveys using 16S ribosomal RNA gene sequences have revealed immense microbial diversity and variability of microbial populations in various parts of our body, including skin, oral cavity, and gastrointestinal and urogenital tracts (Costello et al., 2009; Eckburg et al., 2005; Grice et al., 2009; Ravel et al., 2011). These surveys showed that inter- and intra-individual variation of the skin bacteriome and archaeome (Castelino et al., 2014). While intra- and inter-individual variability was high within the same habitat, temporal variability of the microbiome was minimal within an individual (Costello et al., 2009; Franzosa et al., 2015; Human Microbiome Project Consortium, 2012), which indicates that the human microbiome can be used to distinguish and identify individuals. Fungi are also a prominent member of the skin microbiome. However, only a handful of studies have been studied on the skin fungiome. Those fungiome studies surveyed a phylogenetic marker, a small subunit ribosomal RNA gene (18S rRNA gene) to disclose fungal diversity in various habitats (Paulino et al., 2006). Findley et al. (2013) recently reported that the fungal diversity is rather dependent on body site than on individuals, showing higher intra-individual variability. However, only a limited number of studies are available on the fungal diversity in human. The most neglected and less characterized member of the skin microbiome is viruses largely due to the lack of a common molecular marker gene (Grice, 2015). Skin virome is the least addressed, and thus very little is known about the composition of viral community on our

skin. However, Hannigan et al. (2015) recently surveyed the skin virome and reported that more than 90% of retrieved viral sequences were not taxonomically classified yet. Changes in the composition of bacterial, archaeal, fungal, and viral communities found in and on our body often show intra- and inter-individual variability. Thus, the total human microbiome, including bacteriome, archaeome, fungiome, and virome recovered from human touched objects can distinguish individuals and, in turn, serve as trace evidence for forensic (human) identification.

Task 2.1. *Collect samples for the microbiome-based analysis.*

Task 2.2. *Determine the structure and composition of bacterial, archaeal, fungal, and viral signatures from human touched objects.*

Task 2.3. *Conduct post-sequencing analyses.*

Objective 3. To identify core/variable/transient microbiome associated with different post-contact intervals.

Hypothesis: The structure and composition of the microbiome left on the objects may be changed over time after contact, which may serve as a tool for determining time elapsed after contact, post-contact intervals (PCI).

Rationale: Human skin harbors core (resident), variable, or transient members of microorganisms (Foulongne et al., 2012). For example, a few dominant (core) bacterial taxa are present on human skin, including Propionibacterium, Corynebacterium, and Staphylococcus with some less abundant (variable) taxa (Costello et al., 2009; Grice et al., 2009; Oh et al., 2014). Fungi are also a prominent member of the skin microbiota and the dominant taxa is Malassezia (Findley et al., 2013). The most neglected and less characterized member of the skin microbiome is viruses largely due to the lack of a common molecular marker gene (Grice, 2015). However, recent studies have identified common viral members, including Propionibacterium and Staphylococcus phages, beta and gamma human papillomaviruses (β- and γ–HPVs) and Merkel cell polyomaviruses (Foulongne et al., 2012; Oh et al., 2014; Wylie et al., 2014). Identifying the core/variable/transient microbiome is important for understanding the stable and consistent components across the complex skin microbial community. The diversity of the human skin microbiome is stable over time (Jalanka-Tuovinen et al., 2011), but will drastically change after human death (Hauther et al., 2015; Metcalf et al., 2013). Thus, the composition and abundance of the human skin microbiome left on touched surfaces will change over time, which may help determine time elapsed after contact, post-contact intervals (PCI), and in turn, provide forensic implications on when the person has been at a crime scene.

Task 3.1. *Identify the core (resident)/variable/transient microbiome associated with different post-contact intervals (PCI).*

Task 3.2. *Quantify the core (resident)/variable/transient microbiome associated with different post-contact intervals (PCI).*

2. Major Activities

Objective 1. To identify trace evidence suitable for the microbiome-based forensic application.

Task 1.1. *Collect trace evidence in three different offices in two different locations.*

Sample collection

The objects that humans touch on daily basis, such as mobile phones, computer keyboards and mice, cups (glass, ceramic), door knobs, steering wheels, clothes, pillows etc. may serve not only as indirect evidence for traditional forensic investigation but as direct evidence for microbiome-based forensic applications. Fifteen human-touched objects were identified in office and automobile environments, from which swab samples were collected. For the swab test study, four different commercial swabs were employed: cotton, rayon, HydraFlock, and polyester (Puritan). It has been reported that swabbing is suitable for the collection of skin microbes for analysis (Grice et al., 2008). A total of three samples per swab and kit type were collected per object. Sterilized swabs in both studies were pre-moistened using sterile ST solution (0.15 M NaCl with 0.1% Tween 20) before swabbing to increase sampling efficiency from a dry surface (Fierer et al., 2008; Lax et al., 2015; Paulino et al., 2006). Sampling was performed unidirectionally and horizontally for each object to maximize DNA recovery (Wood et al., 2017). Swab heads were aseptically severed into 2.0 ml PowerBead tubes containing 0.7 mm garnet beads (Qiagen).

Task 1.2. *Perform standard forensic procedures using human touched objects as trace evidence.*

We have 1) developed methods used for reverse lifting method and alternate light source, 2) compared the newly developed “Reverse Lifting” method with the traditional lifting method, and 3) identified objects that are feasible for the reverse lifting method. Traditionally, fingerprints are developed using various types of colored powder, fluorescent or magnetic powders (Fig. 1). Beside powder-based methods, various chemicals have also been used to reveal prints. However, traditional methods for lifting fingerprints are invasive, which may hinder other forensic applications such as microbiome-based analysis. Thus, in order to avoid invasiveness of employing powders or other chemicals in the fingerprint analysis, we developed and applied the reverse lifting method to 20 different objects to retrieve fingerprints and compared to traditional lifting methods.

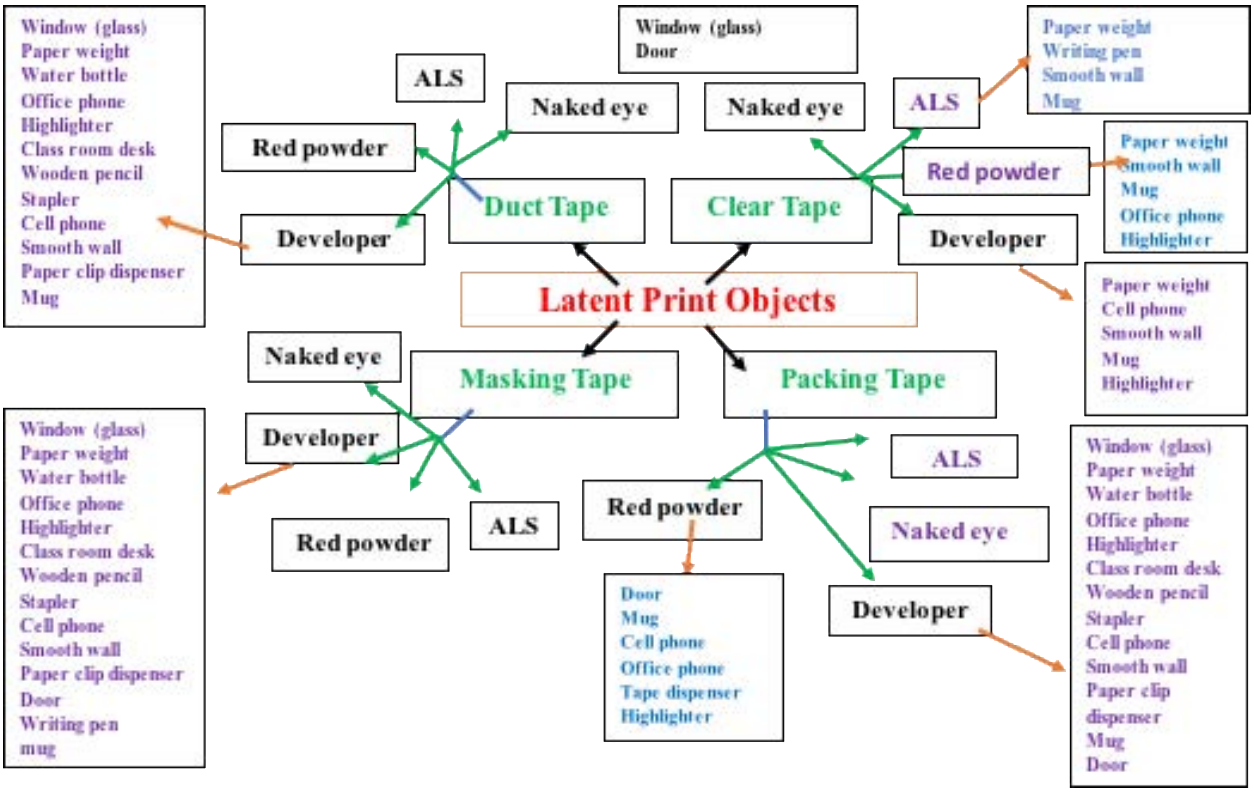
Objects

The method involves collection and processing, which means lifting the latent print from the source and then processing to develop the print. This method is just opposite of traditional invasive fingerprint development called reverse lifting. A total of 21 objects that are usually found in an office set up were selected to carry out the experiment, including computer screen, paper

weight, glass window, computer keyboard, computer mouse, cell phone, doorknob, door, writing pen, mug, paper clip holder, wall (smooth surface), wall (rough surface), water bottle, office phone, highlighter, table, wooden pencil, stapler, and cabinet handle. Red/black print powder, magnetic powder, dusting brush, illuminator, and adhesive-side developer were used to develop the latent prints from the above objects.

A forensic light source, Luma-Lite ^{R} 2000 A, (Dayton Scientific Inc.) at wavelengths 365, 450, 485, and 570 nm was used for enhancing the visualization. The four different types of tape that were used in this experiment were masking tape, packing tape, clear tape, and duct tape. Each tape produced different results. The objects and the developed prints from the tapes were photographed documented by a Canon EDS REBEL T5i.

Figure 1. Experimental scheme of reverse lifting of prints on 20 objects using different tapes, light source, powders, and developer.



Deposition of the Fingerprint

Intentional latent fingerprints were placed on each of the 21 objects by placing a finger onto the selected objects for 1-2 seconds with light pressure sufficient to ensure the contact between the finger and the object. Attention is made to keep the time and pressure same to all objects to maintain uniform deposition.

Lifting of the Fingerprint

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To detect and enhance the fingerprint on the surface, a forensic light source was used. After the fingerprint is illuminated, if possible, a photograph of the print is taken. Then the print was lifted with packing tape, duct tape, masking tape, and clear fingerprint lifting tape. The tapes were then examined under the alternative light source and photographed.

Developing the Fingerprint with Light Source

The lifted prints on the tapes, which are not visible to the naked eye, require treatment to make them visible. We used alternative light sources as some prints were visible under a forensic light source. Then we developed the latent print by the application of both powder and adhesive side developer. The tape was laid on a flat surface. The developer was swabbed over the surface while rotating the application.

Developing the Fingerprint with Powder Dusting

Fingerprint powder was applied to the surface of the tapes bearing the latent print with a fingerprint brush.

Developing the Fingerprint with Adhesive Side Developer

First, the brush was dipped gently into the developer solution and then slightly swirled over the latent print area in the tapes for 30 to 40 seconds. The adhesive side solution adhered to the area in the form of a print. The developer was washed away with water to reveal the print, and then a photograph was taken.

Traditional Method of Development

The fingerprint was first placed on one of the objects. The red powder was then dusted over the area where the print was presumed to be. Once the entire print was visible, the tape was pressed onto the print and pulled off carrying the print with it. The print was then placed on a transparent sheet which made them easier to view.

Storage of Fingerprints

Fingerprints developed using both methods were saved and analyzed on to different types of high definition microscopes; the FX-E HD Forensic Optical Comparator and DinoCapture 2.0.

Task 1.3. *Identify and classify the objects which can be readily applied for microbiome-based forensic identification.*

DNA preparation

Samples were isolated using DNeasy PowerSoil kit (Qiagen) to mitigate biases in DNA isolation. For the DNA kit test, three different commercial DNA isolation kits were employed: FastDNA SPIN Kit for Soil (MP Biomedical), DNeasy Blood & Tissue Kit (Qiagen), and DNeasy PowerSoil Kit (Qiagen). Sterile cotton swabs (Puritan) as used in the previous study were used in obtaining samples to mitigate biases in sample collection. Total genomic DNA was extracted according to the manufacturer's instructions. Quality and concentration of the extracted genomic DNA were assessed using a NanoDrop ND-2000 spectrophotometer (Thermo Fisher Scientific)

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and Qubit 3.0 fluorometer (Thermo Fisher Scientific). Swab samples were immediately stored at -20 °C until use. Both DNA quantity and quality are fundamental and the most critical in any DNA-based molecular analyses including microbiome-based analysis. Thus, we basically used DNA quantity and quality to identify which objects are suitable for microbiome-based forensic applications.

PCR amplification

Bacterial DNA was amplified using modified primer set (515F-806R) described in Parada et al. (2016) and Apprill et al. (2015) with overhang adapters (Table 1; Caporaso et al., 2011) targeting of the variable region 4 (V4) of the 16S rRNA gene. Fungal PCR primer sets ITS1F-ITS2R were used to amplify the internal transcribed spacer (ITS) (Table 1). PCRs were conducted in a final 25-µL volume containing nucleotide-free water (pH 8.0; Thermo Fisher Scientific), 12.5 µL of GoTaq G2 colorless Master Mix (Promega), 1 µL of each 10x primer set, and 10 ng/µL of isolated DNA. Thermocycling consisted of denaturation for 2 min at 95 °C, 33 cycles of denaturing for 30 s at 95 °C, 30 s of annealing at 62 °C, and 1 min of extension at 72 °C, followed by final extension for 7 min at 72 °C. PCR reactions were performed in a T100 Thermal Cycler (BioRad). PCR products were electrophoresed on 1% (wt/vol) Tris-acetate-EDTA-agarose gels containing ethidium bromide. Gels were visualized using UV light. Autoclaved nuclease-free water (Invitrogen) was used as the negative control in each run. To further optimize the PCR condition, we tested different concentrations of the primer set at 10 µM, 12 µM, 15 µM, 18 µM, and 20 µM. The thermal gradient (between 52 and 62 °C) was also used to optimize the PCR reaction conditions by identifying the best annealing temperature for the primer set.

Table 1. Primer sequences for PCR and qPCR amplification.

Primers	Sequences (5'-3')	Length (bp)
515F (Parada et al., 2016)	GTGYCAGCMGCCGCGGTAA	19
806R (Apprill et al., 2015)	GGACTACNVGGGTWTCTAAT	20
ITS1F	CTTGGTCATTTAGAGGAAGTAA	22
ITS2R	GCTGCGTTCTTCATCGATGC	20

Abbreviations: F, Forward primer; R, Reverse primer; W = A or T; M = A or C; N = any base; V = A or C or G.

Quantitative PCR assay

SYBR Green-based qPCR assay was performed using the standard curve method. Triplicate reactions were prepared for each sample. qPCRs were conducted in a final 20-µL volume containing nucleotide-free water (pH 8.0; Thermo Fisher Scientific), 10 µL SYBR-Green Master Mix (Thermo Fisher Scientific), 1 µL of each 10x primer set, and 10 ng/µL of isolated DNA. Thermocycling consisted of 2 min at 55 °C, denaturation for 2 min at 95 °C, 33 cycles of

denaturing for 15 s at 95 °C, 15 s of annealing at 55 °C (for 515F-806R primers) or 62 °C (for ITS1F-ITS2R primers), and 1 min of extension at 72 °C, followed by a dissociation step (15 s of 95 °C, 1 min of 60 °C, 15 s of 95 °C, and 1 min of 60 °C). Data collection was performed at the extension stage. qPCR reactions were performed in a 7300 Real Time PCR System (Applied Biosystems). Autoclaved nuclease-free water (Invitrogen) was used as the negative control in each run. Isolated DNAs of *Escherichia coli* and *Saccharomyces cerevisiae* were used to generate the standard curve.

Amplicon sequencing

Amplified DNAs were sent off for amplicon sequencing at the Georgia Genomics and Bioinformatics Core (GGBC) of the University of Georgia (UGA). Resulting sequences were pre-processed. FASTQ Toolkit was used to trim adaptor sequences, and 16S Metagenomics app was used to perform taxonomic classification of 16S rRNA targeted amplicon reads in BaseSpace (Illumina, San Diego, CA). Resulting paired sequence reads were merged, filtered, aligned using reference alignment in BaseSpace. The reads were further analyzed using BaseSpace 16S Metagenomics application to get OTU (Operational Taxonomic Unit) generation and taxonomic classification (Wang et al., 2007). QIIME2 was also used for amplicon sequencing analyses at Nephele (Weber et al., 2018). The RDP classification was made using the RefSeq RDP Database v3 (May 2018) in BaseSpace. Shannon's diversity index (SHDI) and number of identified bacterial, archaeal, fungal, and viral species (i.e., richness) were calculated to represent microbial diversity and richness.

Statistical analysis

One-way analysis of variance (ANOVA) and Tamhane's T2 post-hoc multiple comparison test was applied for comparison between groups. Pearson's correlation matrix was employed to assess relationships between groups. Statistical analysis was performed with qPCR and sequencing data using IBM SPSS Statistics 21 software.

Objective 2. To determine the total microbiome as trace evidence left on human touched objects.

Task 2.1. *Collect samples for the microbiome-based analysis.*

Sample collections

The composition and structure of microbiome can be influenced distally by host-environmental factors; we included the context of one's host-environmental construct. Thus, we have recruited volunteers 1) who work (study) and live on campus at Albany State University, 2) who work on campus but live off campus, and 3) who work and live off campus. Since the skin microbiota is affected by many host-environmental factors, including temporal and individual variability (i.e. age, gender, race/ethnicity, and season), we recruited volunteers aged 18 to 29 to

minimize the age factor, but later extended the age range to 39 because of the limited number of subjects available in the research sites. We have recruited and collected 387 swab samples from 34 subjects belonging to Categories 1 and 2 during the project period (Table 1). Swab samples collected were immediately placed on ice, transferred to the laboratory, and stored at −20°C until processed.

Samples were taken from the subjects using sterile cotton swabs (Puritan) pre-moistened with sterile ST solution from subjects' hands as well as human-touched objects in their offices. Four samples were obtained from subjects' hands: left fingers (LF), right fingers (RF), left palm (LP), and right palm (RP). Human-touched objects sampled from subjects include keyboard (laptop) (LKB), trackpad (laptop) (LTP), cellphone (CP), doorknob (DK), pen (PEN), closet door handle (DH), and stapler (STP). Samples collected were immediately placed on ice, transferred to the laboratory, and stored at -20°C until processed.

Table 2. A total of 34 subjects (Categories 1 and 2) from whom we collected swab samples during the project period.

No. of Subjects	Category	Gender	Age Range	Location of Office
1	1	Female	18-29	East Campus
2	1	Female	18-29	East Campus
3	2	Female	18-29	East Campus
4	2	Female	18-29	East Campus
5	1	Female	18-29	East Campus
6	1	Female	18-29	East Campus
7	2	Male	18-29	East Campus
8	2	Male	18-29	East Campus
9	1	Female	18-39	East Campus
10	1	Female	18-39	East Campus
11	1	Female	18-29	East Campus
12	1	Female	18-39	East Campus
13	1	Female	18-29	East Campus
14	1	Female	18-29	East Campus
15	1	Female	18-29	East Campus
16	1	Male	18-29	West Campus
17	1	Male	18-29	West Campus
18	1	Male	18-29	West Campus
19	1	Female	18-29	East Campus
20	1	Female	18-29	East Campus
21	1	Female	18-29	East Campus
22	1	Female	18-29	East Campus
23	1	Female	18-29	East Campus
24	1	Female	18-29	East Campus

25	1	Male	18-29	East Campus
26	1	Male	18-29	East Campus
27	1	Female	18-29	East Campus
30	2	Male	18-29	West Campus
31	2	Male	18-29	West Campus
32	2	Male	18-29	West Campus
33	2	Male	18-29	West Campus
34	2	Female	18-29	East Campus
35	2	Male	18-29	East Campus
36	2	Female	18-29	West Campus

We have recruited and collected 63 swab samples from six subjects belonging to Category 3 during the project period (Table 2).

Table 3. A total of six subjects (Category 3) from whom we collected swab samples during the project period.

No. of Subjects	Category	Gender	Age Range	Location
41	3	Female	18-29	CSU campus
42	3	Female	18-29	CSU campus
43	3	Female	18-29	CSU campus
44	3	Female	18-29	CSU campus
45	3	Female	18-29	CSU campus
46	3	Female	18-29	CSU campus

Task 2.2. *Determine the structure and composition of bacterial, archaeal, fungal, and viral signatures from human touched objects.*

DNA extraction

DNA was isolated using the DNeasy PowerSoil Kit (Qiagen). Isolated DNA was measured its quantity and quality using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific) and a Qubit 3.0 fluorometer with a Qubit dsDNA HS Assay kit (Thermo Scientific). DNA was stored at -20 °C until processed.

Amplicon Sequencing

To obtain bacteriome (and archaeome) profiles, community DNA was amplified using the modified primer set (515F-806R) described in Parada et al. (2016) and Apprill et al. (2015) with overhang adapters, targeting the variable 4 region of the 16S rRNA gene. For fungiome, a fungal primer set ITS1F-ITS2R was used to amplify the internal transcribed spacer (ITS) (Table 1). PCR

was conducted in a final 25-µL volume containing nucleotide-free water (pH 8.0; Thermo Fisher Scientific), 12.5 µL of GoTaq G2 colorless Master Mix (Promega), 1 µL of each 10x primer set, and 10 ng/µL of isolated DNA. The PCR condition consisted of denaturation for 2 min at 95 °C, 33 cycles of denaturing for 30 s at 95 °C, 30 s of annealing at 62 °C, and 1 min of extension at 72 °C, followed by final extension for 7 min at 72 °C. PCR reactions were performed in a T100 Thermal Cycler (BioRad). PCR products were electrophoresed on 1% (wt/vol) Tris-acetate-EDTA-agarose gels containing ethidium bromide. Gels were visualized using UV light. Autoclaved nuclease-free water (Invitrogen) was used as the negative control in each run. A total of 368 amplicons were sent off for amplicon (16S rRNA and ITS) sequencing at the Georgia Genomics and Bioinformatics Core (GGBC) of the University of Georgia (UGA) and 126 amplicons were at the Rhode Island Genomics and Sequence Center (RIGSC) of the University of Rhode Island.

Metagenome Sequencing

Due to the low yield of DNA, DNA was amplified using the whole genome amplification method with Qiagen's REPLI-g Mini kit. DNA was further purified using a DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's supplementary protocol. Purified DNA was sent for the fragment analysis to check up on both the quantity of DNA and the degree of DNA degradation before the library preparation using the Illumina Nextera XT library preparation kit (Hannigan et al., 2015). Whole shotgun metagenome sequencing was performed on a paired end Illumina HiSeq 2500 at the Georgia Genomics Facility of University of Georgia or the Nevada Genomics Center at the University of Nevada - Reno.

Task 2.3. *Conduct post-sequencing analyses.*

Sequence analysis

Raw sequences were pre-processed. FASTQ Toolkit was used to trim adaptor sequences. Resulting paired sequence reads were merged, filtered, aligned using reference alignment in BaseSpace. For amplicon sequences, the16S Metagenomics app was used to get OTU (Operational Taxonomic Unit) generation and taxonomic classification (Wang et al., 2007). The RDP classification was made using the RefSeq RDP Database v3 (May 2018) in BaseSpace. QIIME2 was also used for amplicon sequencing analyses at Nephele (Weber et al., 2018). Shannon's diversity index (SHDI) and number of identified bacterial, archaeal, fungal, and viral species (i.e., richness) were calculated to represent microbial diversity and richness.

For metagenome sequences, we used the DRAGEN Metagenomics app to perform taxonomic classification of reads and MetaPhlAn to profile the composition of microbial communities in the BaseSpace Sequence Hub (Illumina). In addition, we used other pipelines, WGSA and bioBakery available at Nephele (Weber et al., 2018) to perform de novo read assembly from the PE reads and to produce biologically informative metagenomic assemblies. We then used

MicrobiomeDB to analyze relative abundance, α-diversity, β-diversity, differential abundance, and correlation (Oliveira et al., 2018).

Task 3.1. *Identify the core (resident)/variable/transient microbiome associated with different post-contact intervals (PCI).*

Sample preparation and DNA extraction

Samples were collected from three human-touched glass windows across post-contact intervals (PCI) for two weeks. Each glass window was touched by a human volunteer with their hands pre-moistened with ST solution, and the glass window was inverted to ensure a homogenous application. Swabbing was performed diagonally, horizontally, and vertically within a 4x4 cm area on the glass window pane. Sampling was performed using cotton swabs pre-moistened using sterile ST solution on both sides of the three glass windows. A total of 54 samples were collected (Table 2). DNAs were isolated using the DNeasy PowerSoil Kit (Qiagen) and quantified spectrophotometrically.

Amplicon sequencing

Isolated DNAs were amplified using 515F(Parada)-806R(Apprill) primer set, and PCR products were sent off for amplicon (16S rRNA) sequencing at the Rhode Island Genomics and Sequence Center (RIGSC) of the University of Rhode Island.

Task 3.2. *Quantify the core (resident)/variable/transient microbiome associated with different post-contact intervals (PCI).*

Quantitative PCR assay and statistical analysis

SYBR Green-based qPCR assay was employed using 515F(Parada)-806R(Apprill) and ITS1F-ITS2R primer sets. Statistical analysis including MANOVA and Pearson's correlation matrix was performed using qPCR data.

3. Significant Results

Task 1.2. *Perform standard forensic procedures using human touched objects as trace evidence.*

Development of reverse lifting method

1) Objects lifted by tapes and developed by adhesive-side developer. All most all the fingerprints placed on the objects are invisible to the naked eye but some are visible under Alternate light Source (ALS). Prints reverse-lifted by using masking tape, duct tape, packing tape, and clear tape and then developed using chemical developer (Figs 2 to 4). Table 4 shows the results of the reverse lifting done on objects and developed by adhesive side developer.

Table 4. Fingerprints lifted by tapes using the reverse lifting method and developed by adhesive side developer

No	Object	Masking Tape	Duct Tape	Packing Tape	Clear Tape
1	Paper Weight	L	L	L	L
2	Highlighter	L	L	L	L
3	Window (Frame)	L	L	L	NL
4	Water Bottle (Plastic)	L	L	L	NL
5	Office Phone	L	L	L	NL
6	Class room table	L	L	L	NL
7	Cell Phone	NL	L	L	L
8	Smooth Wall	L	NL	L	L
9	Mug (ceramic)	L	NL	L	L
10	Pencil (wooden)	L	NL	L	NL
11	Stapler	L	NL	L	NL
12	Paper clip holder	L	NL	L	NL
13	Cello tape Dispenser	L	NL	L	NL
14	Door	L	NL	NL	NL
15	Writing pen	L	NL	NL	NL
16	Mouse	NL	NL	L	NL
17	Keyboard	NL	NL	L	NL
18	Door knob	NL	NL	L	NL
19	Computer Screen	NL	NL	L	NL
20	Rough wall	NL	NL	NL	NL

L= Lifted; NL=Not lifted

Figure 2. Fingerprint reverse-lifted from a mug using masking tape with adhesive-side developer.

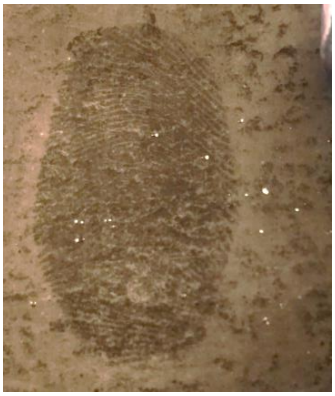

Figure 3. Fingerprint reverse-lifted from a mug using packing tape with adhesive-side developer.

Figure 4. Fingerprint lifted from a stapler using packing tape with adhesive-side developer.

2) Objects lifted by tapes and developed by red powder. Prints reverse-lifted by using masking tape, duct tape, packing tape, and clear tape with red powder (Figs 5 and 6). Table 5 shows the results of the reverse lifting done on objects and developed by red powder.

Table 5. Objects lifted by tapes and developed by dusting red powder.

No	Object	Masking Tape	Duct Tape	Packing Tape	Clear Tape
1	Paper Weight	NL	NL	NL	L
2	Highlighter	NL	NL	L	NL
3	Window Glass	NL	NL	NL	NL
4	Water Bottle Plastic	NL	NL	NL	NL
5	Office Phone	NL	NL	L	NL
6	Class room desk	NL	NL	NL	NL
7	Cell Phone	NL	NL	L	NL
8	Smooth Wall	NL	NL	NL	L
9	Mug	NL	NL	L	NL

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10	Wooden pencil	NL	NL	NL	NL
11	Stapler	NL	NL	NL	NL
12	Paperclip dispenser	NL	NL	L	NL
13	Cello tape Dispenser	NL	NL	L	NL
14	Door	NL	NL	L	NL
15	Writing pen	NL	NL	NL	L
16	Mouse	NL	NL	L	NL
17	Keyboard	NL	NL	L	NL
18	Door knob	NL	NL	NL	NL
19	Computer screen	NL	NL	L	L
20	Rough Wall	NL	NL	NL	NL

L= Lifted; NL=Not lifted

Figure 5. Fingerprint reverse-lifted from a computer mouse using packing tape with red powder.

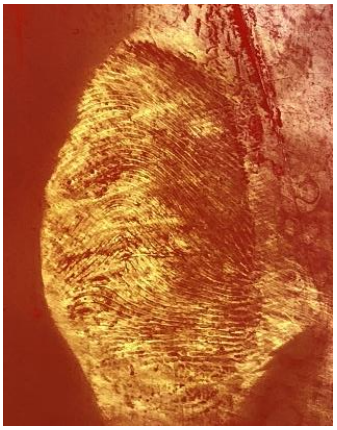
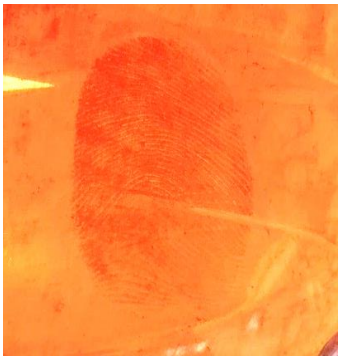


Figure 6. Fingerprint reverse-lifted from an office phone using packing tape with red powder.



3) Comparison of traditional and reverse lifting methods. Fingerprints were lifted by using traditional and reverse lifting methods with masking tape, duct tape, packing tape, and clear tape

(Table 6). The reverse lifting methods for fingerprint development has been successfully performed in at least seven out of 21 objects selected for this study.

Table 6. Comparison of traditional and reverse lifting methods

No	Object	Masking Tape	Duct Tape	Packing Tape	Clear Tape
1	Paperweight	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print, while reverse lifting displayed partial print.	Traditional lifting displayed print **(Fig. 7)**, while reverse lifting displayed partial print.
2	Highlighter	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print **(Fig. 8)**, while reverse lifting displayed partial print.	Traditional lifting displayed print, while reverse lifting displayed partial print.
3	Office Phone	Traditional lifting displayed print, while reverse lifting didn't display print.	Both traditional and reverse lifting displayed partial prints.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 9)**, while reverse lifting displayed partial print.
4	Smooth Wall	Traditional lifting displayed partial print, while reverse lifting displayed print.	Both traditional and reverse lifting displayed visible prints.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 10)**, while reverse lifting didn't display print.
5	Mug (Ceramic)	Both traditional and reverse lifting didn't display visible prints.	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print, while reverse lifting displayed partial print.	Both traditional **(Fig. 11)** and reverse lifting displayed visible prints.
6	Water Bottle	Traditional lifting didn't display print, while reverse lifting displayed print.	Traditional lifting didn't display print, while reverse lifting displayed print.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 12)**, while reverse lifting didn't display print.
7	Table	Traditional lifting didn't display print, while	Traditional lifting didn't display print, while	Traditional lifting displayed partial print **(Fig. 13)**,	Traditional lifting displayed print shape, while

		reverse lifting displayed print.	reverse lifting displayed print.	while reverse lifting displayed print.	reverse lifting didn't display print.
8	Cellphone	Both traditional and reverse lifting displayed visible prints.	Both traditional and reverse lifting didn't display visible prints.	Both traditional and reverse lifting displayed visible prints.	Both traditional **(Fig. 14)** and reverse lifting displayed visible prints.
9	Pencil (Wooden)	Both traditional and reverse lifting didn't display visible prints.	Both traditional and reverse lifting didn't display visible prints.	Both traditional and reverse lifting didn't display visible prints.	Both traditional **(Fig. 15)** and reverse lifting displayed visible prints.
10	Stapler	Both traditional and reverse lifting didn't display visible prints.	Traditional lifting displayed partial print, while reverse lifting didn't display print.	Traditional lifting displayed partial print, while reverse lifting displayed print.	Traditional lifting displayed print **(Fig. 16)**, while reverse lifting displayed partial print.
11	Paper Clip Holder	Traditional lifting didn't display print, while reverse lifting displayed print.	Both traditional and reverse lifting didn't display visible prints.	Both traditional and reverse lifting displayed partial prints.	Both traditional **(Fig. 17)** and reverse lifting displayed partial prints.
12	Window (Glass)	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print, while reverse lifting didn't display print.	Both traditional and reverse lifting displayed prints.	Traditional lifting displayed print **(Fig. 18)**, while reverse lifting didn't display print.
13	Writing Pen	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional lifting displayed print **(Fig. 19)**, while reverse lifting displayed partial print.	Traditional lifting displayed print, while reverse lifting displayed partial print.
14	Wooden Door	Traditional lifting displayed print, while reverse lifting didn't display print.	Traditional and reverse lifting both displayed visible prints.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 20)**, while reverse lifting didn't display print.
15	Cello Tape Dispenser	Both traditional and reverse lifting	Traditional lifting displayed partial	Both traditional and reverse lifting	Traditional lifting displayed print,

		didn't display visible prints.	print, while reverse lifting didn't display print.	didn't display visible prints.	while reverse lifting didn't display print.
16	Mouse	Both traditional and reverse lifting displayed visible prints.	Both traditional and reverse lifting displayed visible prints.	Both traditional and reverse lifting displayed visible prints.	Both traditional **(Fig. 21)** and reverse lifting displayed visible prints.
17	Keyboard	Both traditional and reverse lifting displayed visible prints.	Both traditional and reverse lifting displayed partial prints.	Traditional lifting displayed print, while reverse lifting displayed partial print.	Traditional lifting displayed print **(Fig. 22)**, while reverse lifting didn't display print.
18	Door Knob	Traditional lifting displayed print, while reverse lifting didn't display print.	Both traditional and reverse lifting displayed partial prints.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 23)**, while reverse lifting displayed partial print.
19	Computer screen	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed partial print, while reverse lifting displayed print.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 24)**, while reverse lifting didn't display print.
20	Rough Wall	Both traditional **(Fig. 25)** and reverse lifting displayed partial prints.	Both traditional and reverse lifting displayed partial prints.	Traditional lifting displayed partial print, while reverse lifting displayed print shape.	Traditional lifting displayed partial print, while reverse lifting didn't display print.
21	Cabinet Handle	Traditional lifting displayed print, while reverse lifting displayed partial print.	Both traditional and reverse lifting displayed prints.	Both traditional and reverse lifting displayed visible prints.	Traditional lifting displayed print **(Fig. 26)**, while reverse lifting didn't display print.

Figure 7. Fingerprint lifted by the traditional method from a paperweight using clear tape with red powder.

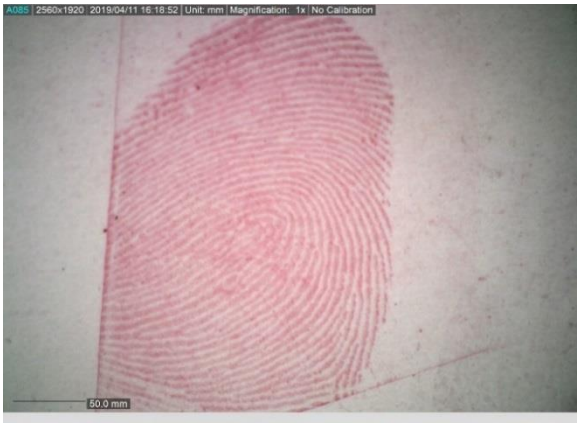


Figure 8. Fingerprint lifted by the traditional method from a highlighter using packing tape with red powder.

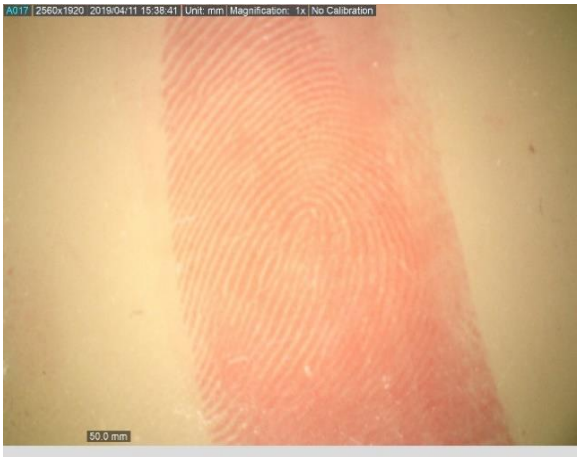


Figure 9. Fingerprint lifted by the traditional method from an office phone using clear tape with red powder.

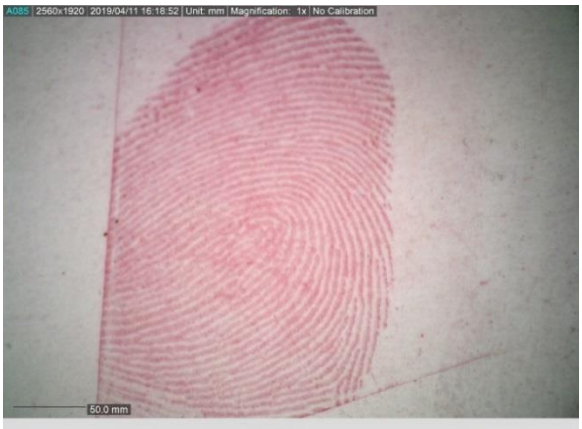


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Figure 10. Fingerprint lifted by the traditional method from a smooth wall using clear tape with red powder.

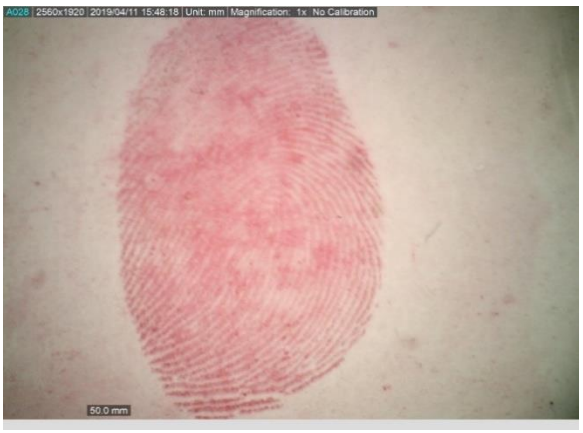

Figure 11. Fingerprint lifted by the traditional method from a mug using clear tape with red powder.

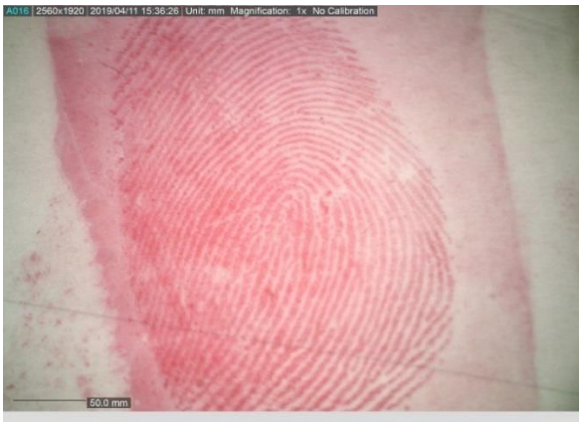

Figure 12. Fingerprint lifted by the traditional method from a water bottle using clear tape with red powder.

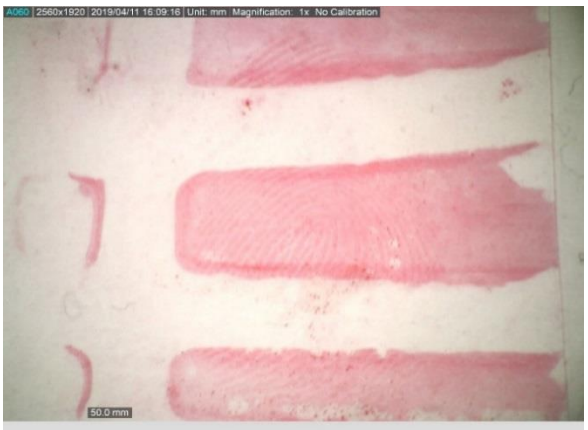

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Figure 13. Fingerprint lifted by the traditional method from a table using packing tape with red powder.

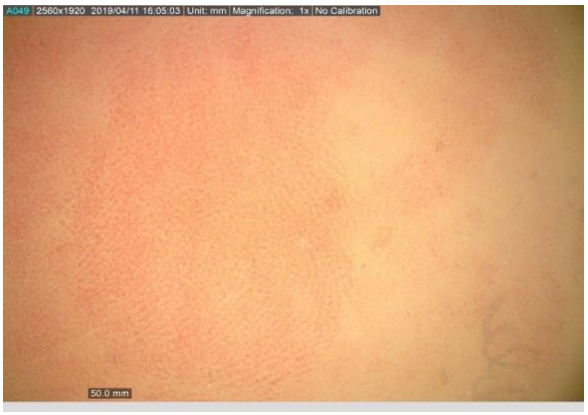

Figure 14. Fingerprint lifted by the traditional method from a cellphone using clear tape with red powder.

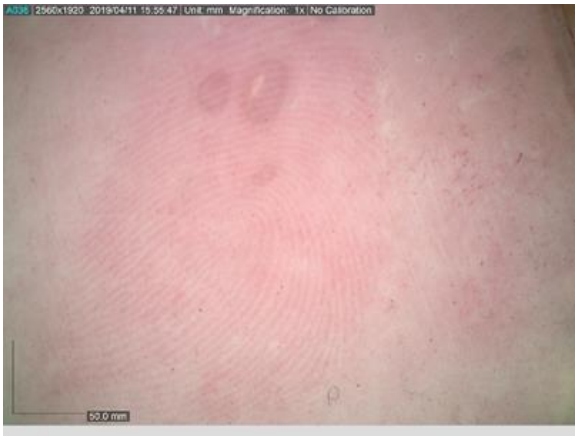

Figure 15. Fingerprint lifted by the traditional method from a wooden pencil using clear tape with red powder.

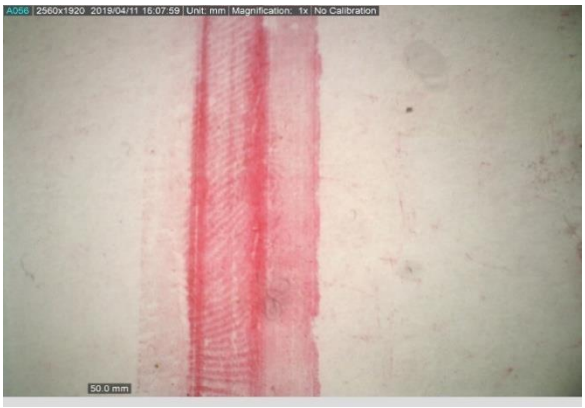

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Figure 16. Fingerprint lifted by the traditional method from a stapler using clear tape with red powder.

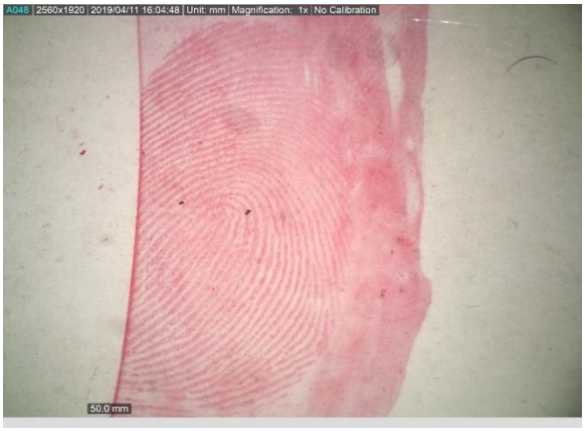

Figure 17. Fingerprint lifted by the traditional method from a paper clip holder using clear tape with red powder.

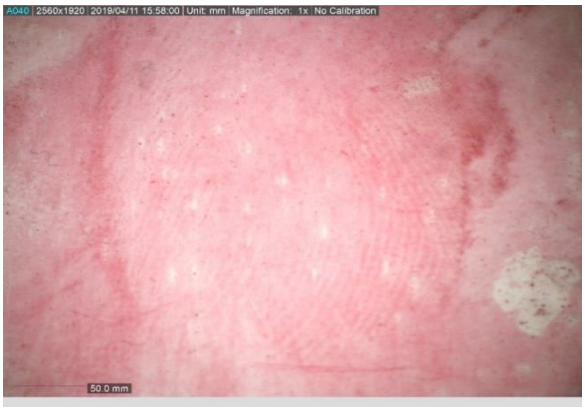

Figure 18. Fingerprint lifted by the traditional method from a window using clear tape with red powder.

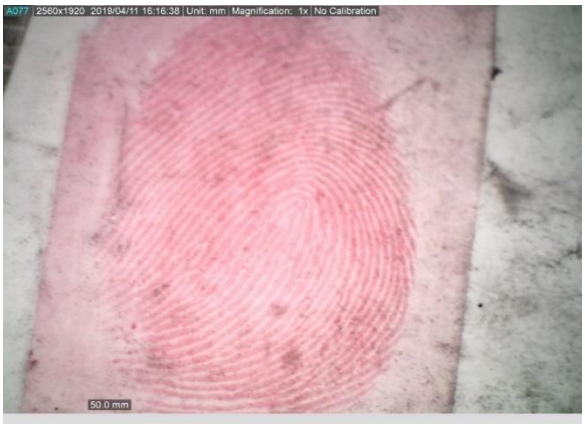

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Figure 19. Fingerprint lifted by the traditional method from a pen using packing tape with red powder.

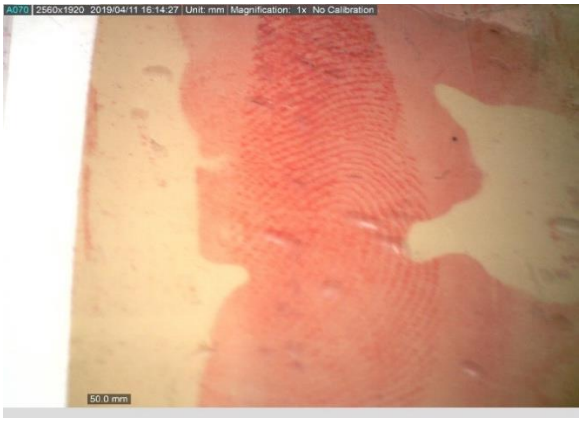


Figure 20. Fingerprint lifted by the traditional method from a door using clear tape with red powder.

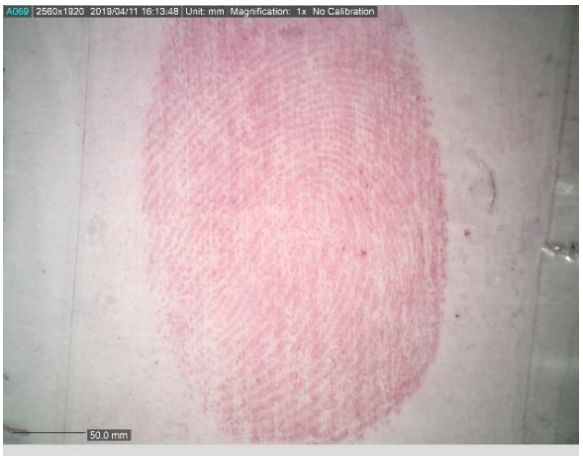


Figure 21. Fingerprint lifted by the traditional method from a mouse using clear tape with red powder.

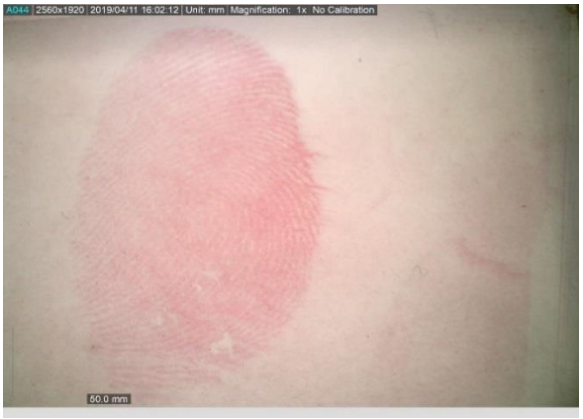


Figure 22. Fingerprint lifted by the traditional method from a keyboard using clear tape with red powder.

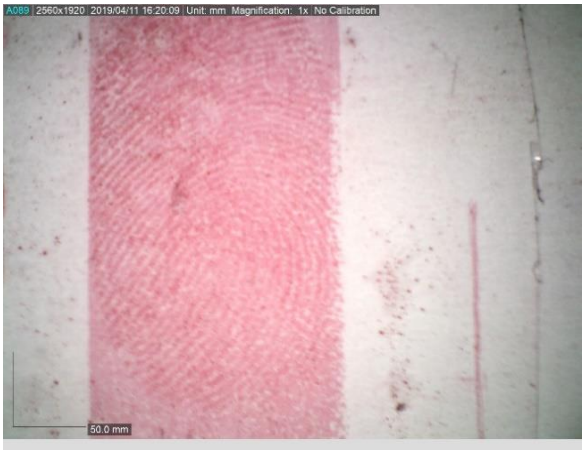


Figure 23. Fingerprint lifted by the traditional method from a doorknob using clear tape with red powder.

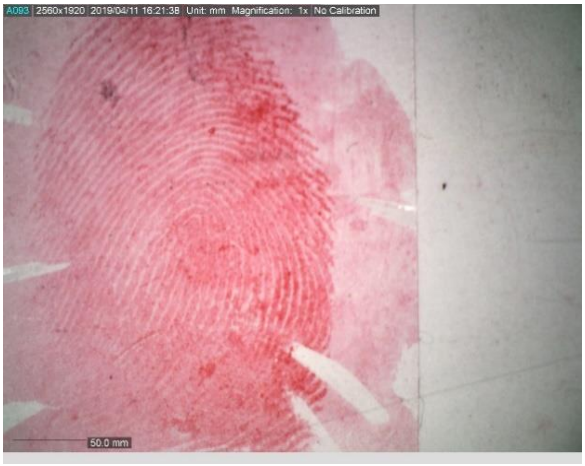


Figure 24. Fingerprint lifted by the traditional method from a computer screen using clear tape with red powder.

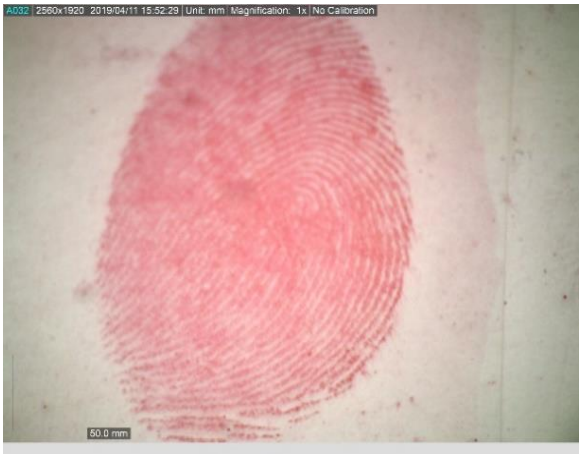


Figure 25. Fingerprint lifted by the traditional method from a rough wall using masking tape with red powder.

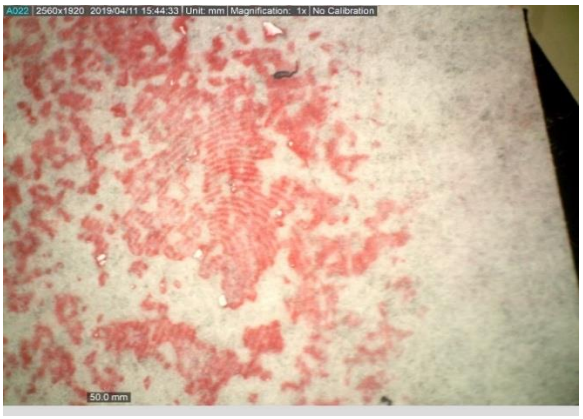
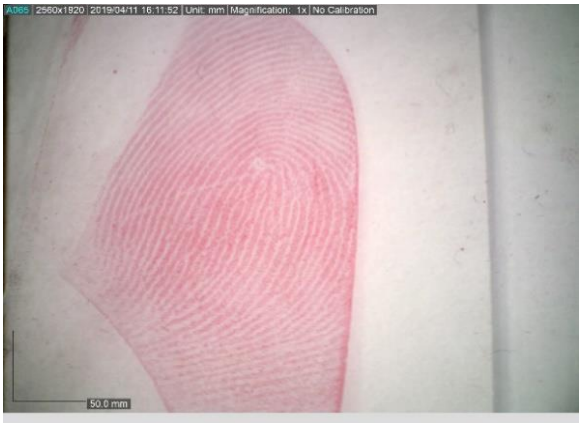


Figure 26. Fingerprint lifted by the traditional method from a cabinet handle using clear tape with red powder.



The results have shown that the ridge details developed in the new methods are comparable to the conventional methods of fingerprint development. The objects selected for this study from an office setting can be very well used for other forensic evidence examination as the method does not contaminate or destroy other forensic evidences like human microbiome. Cell phone, office phone, mouse, mug, plastic water bottle, paperclip holder etc. are some of the objects successfully tested for the reverse lifting. Packing tape and masking tape were able to lift the invisible prints more successfully compared to duct tape and clear tape. Developing solution worked well with those objects compared to dusting powders. Other chemical methods of fingerprint development are under consideration for future studies. In this paper, the quality of fingerprints of traditional and reverse lifting methods were compared. Traditional methods have been proven cumbersome when implemented in an office setting. They destroy the chances of obtaining a microbiome and

the possibility for the evidence to be used in any future testing. Therefore, the reverse lifting method might provide a better option to retrieve more trace evidences in a crime scene.

Task 1.3. *Identify and classify the objects that can be readily applied for microbiome-based forensic identification.*

Comparison of swabs

Sample collection using different swabs may also affect DNA quantity and quality, so we tested and evaluated five different swabs (Fig. 27; Table 7). There are significant differences observed among different swabs, showing cotton swab performed better in retrieving DNA from objects, followed by HydraFlock, rayon, and polyester. We also identified and classified objects that are readily applied for a microbiome analysis. However, most DNA extracted showed low yields, mostly ranged from N/D (not detectable) to 11.58±0.91. Thus, due to the low DNA yield, PCR or whole genome amplification was employed for downstream applications.

Figure 27. Comparison of DNA yields from eight objects using five different swabs.

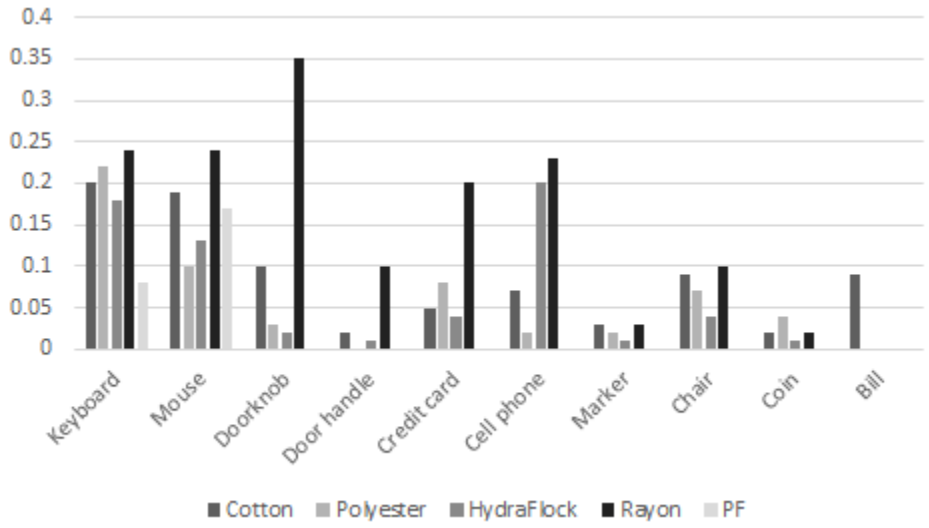


Table 7. Comparison of DNA yields from eight objects using five different swabs

	DNA yield (µg/µl)				
	Cotton	HydraFlock	Polyester	Rayon	PurFlock
Cell phone	0.0752	0.2145	0.0243	0.247	ND[a]
Coin	0.0209	0.0122	0.0159	0.0245	ND
Credit card	0.0576	0.0388	0.0522	0.2043	ND
Doorknob	0.1032	0.0245	0.03	0.2955	ND

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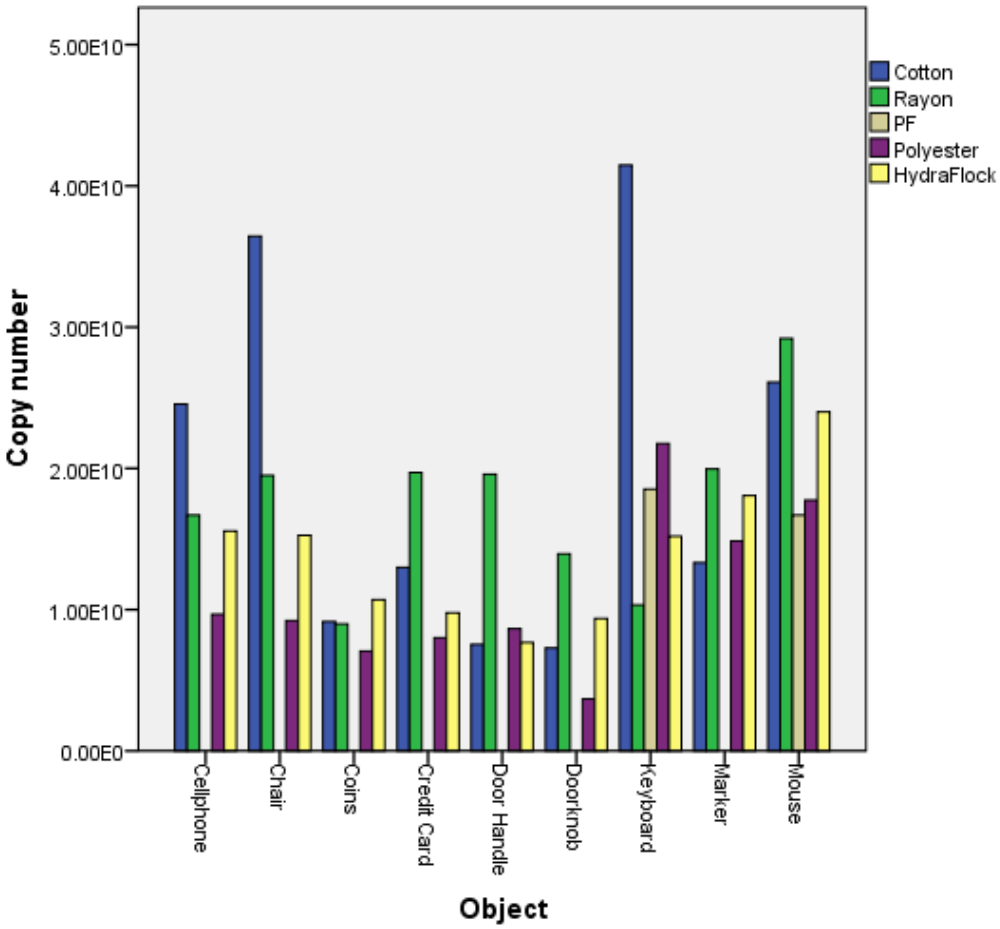
Door handle	0.0203	0.0164	OOR[b]	0.0567	ND
Keyboard	0.2133	0.0961	0.229	0.248	0.0805
Marker	0.0303	0.0151	0.0179	0.0302	ND
Mouse	0.197	0.1315	0.1093	0.239	0.1718

[a]ND, not determined.

[b]OOR, out of detection range.

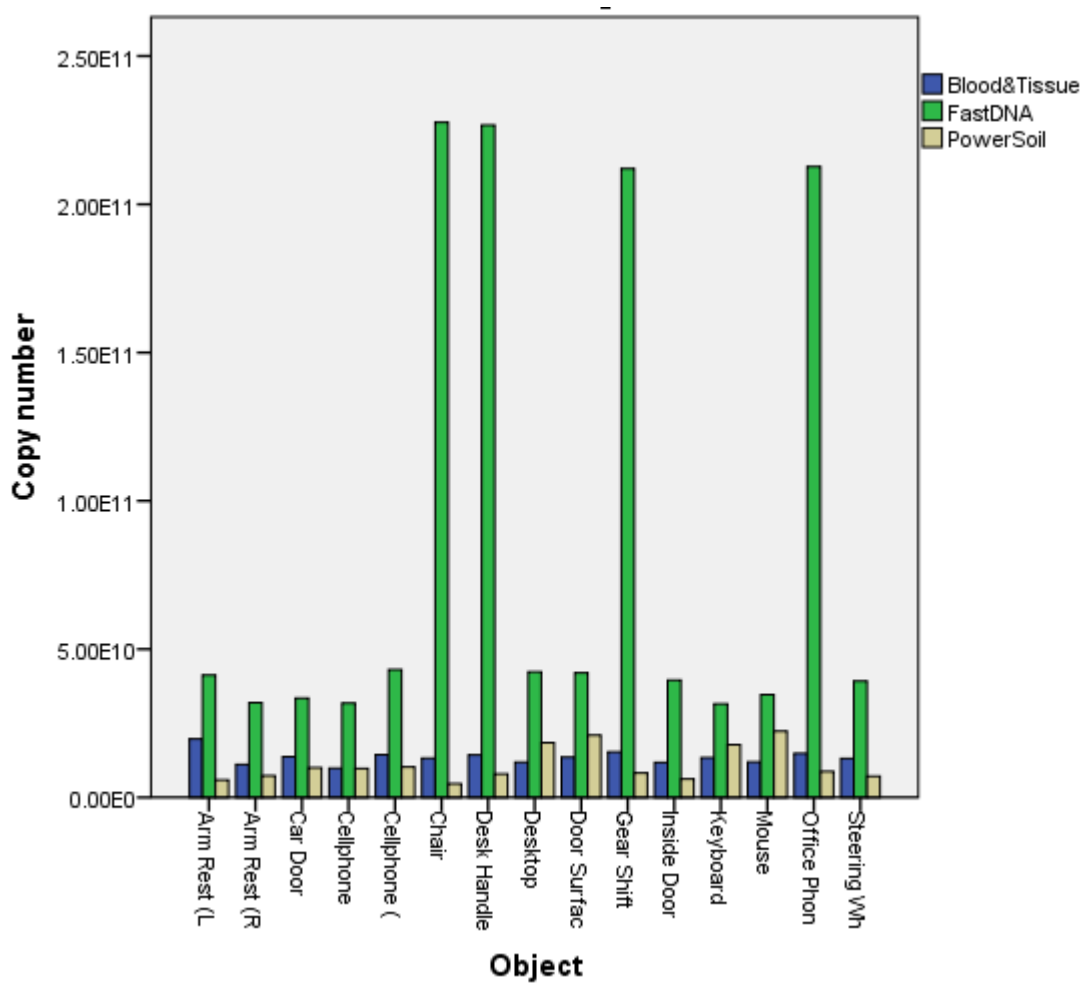
Using data obtained from SYBR-Green based qPCR assays and subsequent statistical analyses, significance was found at the 0.05 level for objects ($F = 3.71$, $P = 0.001$) and swabs ($F = 3.148$, $P = 0.019$); significance was identified at the 0.01 level for commercial DNA isolation kits ($F = 1285.708$, $P < 0.01$). Among the 14 human-touched objects from both an automobile and office environments, keyboard and mouse showed the highest DNA yield. Among five swabs tested for their efficiency in DNA extraction, cotton and rayon swabs showed higher yields (Fig. 28).

Figure 28. Comparison of mean bacterial 16S rRNA V4 copy numbers between various commercial swabs. PF swabs were only used for two objects: keyboard and mouse.



Cotton swabs performed significantly better in retrieving DNA, followed by HydraFlock and rayon. However, rayon swab provided more consistent results. Of the objects tested with the different swabs, coins provided the lowest yields while both mouse and keyboard provided the highest.

Figure 29. Comparison of mean bacterial 16S rRNA V4 copy numbers between various commercial kits.



There are significant differences observed among different swabs, showing cotton swab performed better in retrieving DNA from objects, followed by HydraFlock, rayon, and polyester. Based on the qPCR assay, it shows that kits employing the bead-beating method may perform slightly better. Among three commercial DNA extraction kits, the FastDNA SPIN Kit for Soil (MP Biomedicals) showed the highest yield, particularly for chair, desk handle, gear shift, and office phone (Fig. 29), followed by DNeasy Blood & Tissue Kits (Qiagen) and DNeasy PowerSoil Kit (Qiagen). DNeasy PowerSoil Kit performed most optimally with desktop, door surface, keyboard, and mouse. From the office objects, keyboards and computer mice provided the highest DNA yields.

Optimization of DNA extraction for microbiome-based forensic identification

We extracted DNA from swab samples taken from the petri dishes with different dilution factors (10^{-7}, 10^{-8}, and 10^{-9}) at different time points (day 0, day 1, and day 2). We tested three different commercial DNA extraction kits to determine which kit is more suitable for microbiome study based on the quantity and quality of microbial DNA extracted from objects: A) DNeasy Blood & Tissue Kit, B) DNeasy PowerSoil Kit, and C) FastDNA SPIN Kit for Soil (Table 8).

Table 8. A comparison of DNA yield and purity generated by three commercial DNA extraction kits: A) DNeasy Blood & Tissue Kit, B) DNeasy PowerSoil Kit, and C) FastDNA SPIN Kit for Soil.

Dilutions	Day	DNA yield (ng/µl)[a]			DNA purity (Abs. 260/280)[a]		
		A	B	C	A	B	C
-7	0	2.16±1.82	7.36±2.55	11.65±0.11	1.55±0.20	1.25±0.07	1.75±0.03
	1	3.26±1.39	14.14±12.46	9.50±1.43	3.86±2.44	1.86±0.30	2.03±0.30
	2	2.86±0.31	3.36±0.08	11.62±0.45	1.38±0.09	1.22±0.08	1.80±0.08
-8	0	4.66±0.96	18.87±11.74	12.21±0.59	1.67±0.18	1.36±0.04	1.70±0.06
	1	1.9±0.25	3.08±0.70	10.01±0.71	1.26±0.17	1.79±0.25	1.58±0.06
	2	2.71±0.61	3.10±0.31	10.34±0.50	1.67±0.11	1.23±0.12	1.73±0.08
-9	0	1.17±0.04	18.12±11.57	13.55±2.56	1.56±0.27	1.34±0.10	1.47±0.03
	1	2.59±0.23	11.42±6.15	10.04±1.51	1.71±0.27	1.53±0.10	2.03±0.18
	2	1.82±0.58	4.07±0.04	9.89±0.15	1.10±0.23	1.31±0.04	1.75±0.10

[a] Values are means ± standard errors (SE).

[b] Negative values were removed from the calculation.

We collected swab samples from 14 different objects from an office setting and a vehicle setting and extracted DNA from samples using three different commercial DNA extraction kits: A) DNeasy Blood & Tissue Kit, B) DNeasy PowerSoil Kit, and C) FastDNA SPIN Kit for Soil. DNA was then further analyzed using the Qubit 3.0 fluorometer (Thermo Scientific) to identify which DNA extraction kit yields large quantity and high quality DNA (Table 9).

Table 9. Comparison of DNA yield from 14 objects using three commercial DNA extraction kits: A) DNeasy PowerSoil Kit; Tissue Kit, B) FastDNA SPIN Kit for Soil, and C) DNeasy Blood &Tissue Kit.

	DNA yield (µg/µl)[a]		
	A	**B**	**C**
Cell Phone	0.0107±0.0005	0.0044±0.026	0.0206±0.0025
Chair	6.26E-02±0.025	0.0303±0.010	0.19±0.062
Desk Handle	0.0032±0.0104	0.0075±0.0003	0.087±0.0093
Desktop Monitor	0.038±0.019	OOR	0.0411±0.0108
Door Surface	0.048±0.013	OOR	0.045±0.00403

Keyboard	0.047±0.0047	0.104±0.0072	0.064±0.0037
Mouse	0.039±0.0045	0.068 ±0.0087	0.047±0.0074
Office Phone	0.012±0.0015	0.011±0.0015	0.068±0.012
Armrest	0.0208±0.0035	0.0911±0.0355	0.035±0.007
Car Door	2.91E-02±0.008	0.07±0.0204	OOR
Gear Shifter	0.035±0.0091	0.0504±0.0343	0.10±0.014
Inside Car Door	0.0132±0.0014	0.026±0.00505	0.0133±0.011
Left Armrest	0.033±0.01	0.063±0.013	0.007±0.016
Steering Wheel	0.048±0.0085	0.18±0.046	0.096±0.042

[a]Values are means ± standard error.

[b]Negative values were removed from the calculation.

[c]OOR, out of detection range.

Because DNA yields were low, we used a PCR assay with the universal primer set for bacterial 16S rRNA gene to confirm DNA isolated from swab samples (Table 10).

Table 10. Comparison of PCR amplicons with DNAs of 14 objects isolated using three commercial DNA extraction kits: A) DNeasy PowerSoil Kit; Tissue Kit, B) FastDNA SPIN Kit for Soil, and C) DNeasy Blood &Tissue Kit

	Level of PCR amplification		
	A	**B**	**C**
Cell Phone	++	++	++
Chair	++	++	+
Desk Handle	++	+	++
Desktop Monitor	++	+	++
Door Surface	+	-	-
Keyboard	++	+	+
Mouse	++	+	++
Office Phone	++	++	++
Armrest	+	+	+
Car Door	+	+	-
Gear Shifter	++	++	+
Inside Car Door	+	-	+
Left Armrest	+	-	+
Steering Wheel	-	++	+

*, + means positive PCR product with weak intensity.

**, ++ means positive PCR product with high intensity.

***, – means have no PCR product.

Based on the DNA quantity (Table 9), the agarose gel electrophoresis analysis (data not shown), and the band intensity (Table 10), there is no significant differences existed among the commercial DNA extraction kits. We chose to use DNeasy PowerSoil Kit for further analyses because it gives more consistent, takes less time and steps, and is easy and straightforward to process swab samples. In addition, more suitable objects for the microbiome-based analysis were identified, including cell phones, keyboard, mouse, and office phone in an office setting. A higher DNA quantity in a vehicle setting includes inside car door handle, armrest, and steering wheel yielded.

Optimization of PCR amplification for microbiome-based forensic identification

We identified and classified objects that are readily applied for a microbiome analysis. However, most DNA extracted showed low yields, mostly ranged from N/D (not detectable) to 11.58±0.91. Thus, due to the low DNA yield, we employed either PCR or whole genome amplification for downstream analyses. We amplified DNA using 12 different sets of primer combinations for small subunit ribosomal RNA gene for an optimum amplification of the V4 region of 16S rRNA gene (Table 11).

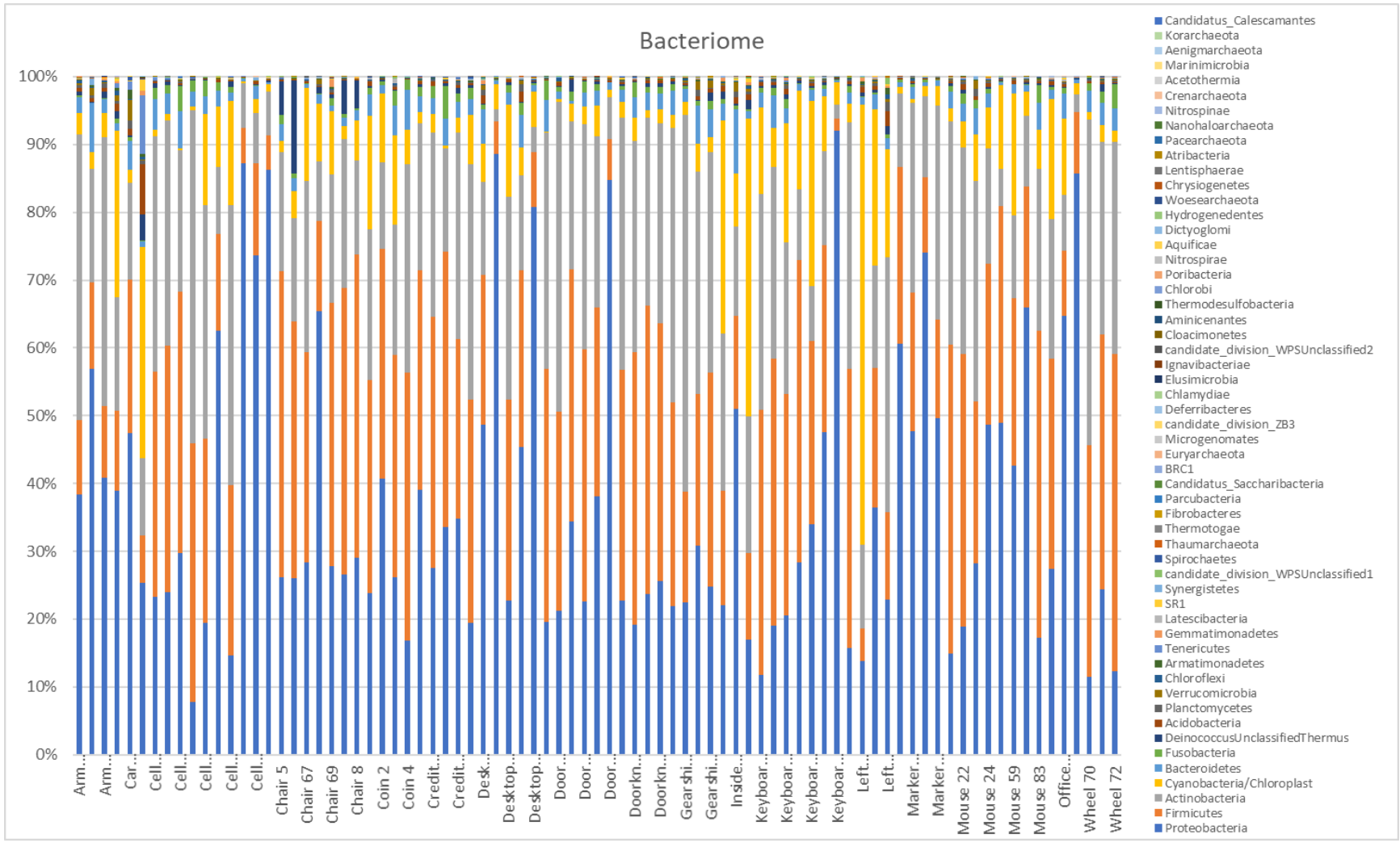
Table 11. Combinations of primer sets used in this study

Combinations	Forward	Reverse
1	356F	806R
2	356F	806R(April)
3	515F	806R
4	515F	806R(April)
5	515F(Parada)	806R
6	515F(Parada)	806R(April)
7	356F	926R
8	356F	1064R
9	515F	926R
10	515F	1064R
11	515F(Parada)	926R
12	515F(Parada)	1064R

We performed gradient PCR using the primer combinations 1-12. The optimal annealing temperatures for each combination determined were: 53 °C for primer combinations 1, 2, and 7, 58 °C for combinations 3, 4, 5, and 6, and 62 °C for combinations 8, 10, 11, 12. Based on the results of PCR amplification and agarose gel electrophoresis (data not shown), both 515F–1064R and 515F(Parada)–1064R combinations performed the best out of all other primer combinations, with an optimal annealing temperature of 62°C. Primer combinations 27F–1510R, 8F–1510R, 8/27F–1492(Caporaso), and 8/27F–1510R were found to be unsuitable for retrieving microbial signatures form human-touched objects as they did not produce distinct, individual bands.

Amplicon sequencing analysis

Figure 30. Proportions of bacterial phyla in the bacterial 16S rRNA gene amplicon libraries (n = 83) from 15 different human touched objects.



We have amplified 83 DNAs extracted from 14 human-touched objects using the modified primers 515F(Parada)-806R(Apprill) and sent for amplicon (16S rRNA) sequencing. In addition, we used DNAs from two subjects, six samples each, and amplified with various numbers of cycles, ranging from 20 to 40 cycles to test the effects of different number of PCR cycles on the diversity of bacteriome. These additional 38 amplified PCR products were also sent for amplicon sequencing.

We analyzed the amplicon sequencing results of 83 PCR products (amplified with Caporaso 16S V4 primer set). Amplicon sequencing results showed 32,245K identified reads (PF) were generated. The resulting paired sequence reads were merged, filtered, aligned using reference alignment in BaseSpace (Illumina). The reads were further analyzed using BaseSpace 16S Metagenomics application to get OTU (Operational Taxonomic Unit) generation and taxonomic classification (Wang et al., 2007). The RDP classification was made using the RefSeq RDP Database v3 (May 2018) in BaseSpace.

A total of 48 different bacterial phyla were detected (Fig. 30). The most abundant bacterial phylum detected was Proteobacteria followed by Firmicutes, Actinobacteria, Cyanobacteria, and Bacteroidetes. Among objects, credit card, keyboard, and mouse showed higher number of reads. A total of 8 archaeal phyla were also detected. Thaumarchaeota was the most abundant, followed by Euryarchaeota, Woesearchaeota, Pacearchaeota, Nanohaloarchaeota, Crenarchaeota, Aenigmarchaeota, and Korarchaeota (Fig. 30).

QIIME2 was also used for amplicon sequencing analyses at Nephele (Weber et al., 2018). The analysis showed 9,884 sequence counts with 42 minimum, 312 maximum, and 291 mean length. Total frequency of 83 samples was 4,886,113 with 12,579 minimum, 161,355 maximum, and 55,483 median frequency.

One-way ANOVA was performed with Tukey's HSD post-hoc multiple comparison test for statistical analysis (Table 1). No significance was identified when testing the effects of different DNA extraction kits (Shannon: $P = 0.894$; No. of Species: 0.081) and swabs (Shannon: $P = 0.886$; No. of Species: 0.968). When comparing means alone, FastDNA was the highest for both Shannon (1.61) and no. of species (1059.93) for kits. For swabs, cotton (1.82) and HydraFlock (1.83) were highest for Shannon while rayon (1,064.11) and cotton (1,055.56) were for no. of species. However, there was significance found between different human touched objects (Shannon: $P = 0.002$; No. of Species: $P < 0.001$). Among the objects, the highest mean value for Shannon index was observed from door knob (2.30), followed by left arm rest (2.23) and gear shift (2.01). For no. of species, credit card (1360) showed the highest mean value, followed by wheel (1336.33) and arm rest (1257.67).

Table 12. One-way ANOVA showing variation between sample means for Shannon's index and number of bacterial species identified with amplicon sequencing performed using MiSeq PE300 (Illumina) for 83 swab samples.

Groups	Source of Variation	SS	df	Mean Square	F	*P*-value
Shannon	Between Groups	9.36	18	0.52	2.70	0.002
	Within Groups	12.31	64	0.19		
	Total	21.67	82			
No. of Species	Between Groups	4334053.98	18	240780.78	3.82	<0.001
	Within Groups	4036080.94	64	63063.77		
	Total	8370134.92	82			

Objective 2. To determine the total microbiome as trace evidence left on human touched objects.

Task 2.1. *Collect samples for the microbiome-based analysis.*

A total of 450 swab samples were collected from 40 subjects and their offices/rooms. Please see Tables 2 and 3.

Task 2.2. *Determine the structure and composition of bacterial, archaeal, fungal, and viral signatures from human touched objects.*

DNA extraction

A total of 106 swab samples were collected from nine subjects belonging to Category 1 (Table 13) and processed for DNA extraction using a DNeasy PowerSoil kit. DNA quantity was measured by using a NanoDrop spectrophotometer (data not shown) and a Qubit 3.0 fluorometer (Table 13).

Table 13. The quantity of DNA extracted from six subjects (#19, 20, 21, 22, 23, 24, 25, 26, and 27) and the objects in their dormitory rooms, measured by a Qubit 3.0 fluorometer.

	DNA Yield (ng/µl)								
	19	20	21	22	23	24	25	26	27
Cell phone	0.014	0.049	0.019	0.050	0.023	0.500	0.240	0.404	0.228
Desk handle	0.047	NC	0.082	0.106	0.031	0.390	0.662	2.640	0.306
Doorknob	0.242	0.016	0.054	0.037	0.064	0.124	0.145	2.320	0.103
Door lock	0.019	0.025	0.041	NC	0.100	0.134	0.274	4.160	0.152
Keyboard	0.038	0.254	0.112	0.272	0.132	2.320	NC	NC	0.446
Left finger	0.087	0.109	0.031	0.708	0.198	0.125	0.097	OR	1.090
Left palm	0.092	0.096	0.053	0.212	0.224	0.020	0.470	0.151	0.043
Pen	0.011	0.160	0.017	NC	NC	0.526	NC	0.108	0.192

Right fingers	0.082	0.109	0.038	0.414	0.302	0.149	0.086	0.051	0.664
Right palm	0.097	0.093	0.039	0.168	0.346	0.054	0.220	0.466	0.610
Sink handle	2.300	NC	0.068	NC	0.368	0.266	0.091	0.620	0.222
Stapler	NC	0.022	0.020	0.126	NC	NC	NC	NC	NC
Tape dispenser	NC	0.022	NC	NC	NC	NC	NC	NC	NC
Tooth brush	0.033	0.041	0.604	0.860	0.500	0.057	0.065	0.107	0.098
Track pad	0.049	0.115	0.076	0.340	1.360	0.070	NC	NC	0.350
TV remote	0.044	0.338	0.081	0.107	NC	0.070	NC	NC	NC

OR, out of range; NC, not collected

Based on the DNA quantity measured by using Nanodrop and Qubit 3.0, we have low DNA yield from the swab samples of nine subjects (Category 1) and their human touched objects. Among the objects, keyboard and office phone showed higher DNA yields than other objects found in an office setting, while desk handle, keyboard, sink handle, and tooth brush showed higher DNA yields in a (dormitory) room setting.

A total of 89 swab samples were collected from seven subjects belonging to Category 2 (Table 14) and processed for DNA extraction using a DNeasy PowerSoil kit. DNA quantity was measured by using a Nanodrop spectrophotometer (data not shown) and a Qubit 3.0 fluorometer (Table 14).

Table 14. The quantity of DNA extracted from seven subjects (#30, 31, 32, 33, 34, 35, and 36) and the objects in their offices, measured by a Qubit 3.0 fluorometer

	DNA Yield (ng/µl)						
	30	31	32	33	34	35	36
Cell phone	0.888	0.196	0.076	0.0981	0.187	0.0488	0.0415
Desk handle	0.019	0.025	0.041	0.141	0.058	0.0883	2.0933
Doorknob	0.070	0.034	0.103	0.0758	0.068	0.0684	0.0399
Keyboard	0.266	0.164	0.158	0.224	0.148	0.202	0.163
Left finger	2.660	3.260	0.195	0.31	0.469	0.552	0.43
Left palm	0.165	0.434	0.176	0.164	0.14	0.336	0.37
Office phone	0.324	0.141	0.148	0.0348	0.14	0.195	0.282
Mouse	NC[a]	NC	0.082	0.171	0.094	NC	0.0969
Pen	0.012	0.906	0.052	0.0364	NC	0.051	0.049
Right fingers	0.486	0.764	0.170	0.246	0.327	0.314	0.312
Right palm	0.183	0.170	0.145	0.154	0.162	0.462	0.316
Stapler	0.308	NC	0.055	0.0352	0.052	0.0266	0.203
Tape dispenser	0.047	0.071	NC	0.0262	NC	0.0498	0.0235
Track pad	0.059	0.131	0.294	0.16	NC	0.646	NC

[a]NC, not collected.

Based on the DNA quantity measured by using Nanodrop and Qubit 3.0, we had low DNA yield from the swab samples collected from seven subjects (Category 2) and their human touched objects. Among the objects, keyboard and office phone showed higher DNA yields than other objects found in an office setting (Table 14).

A total of 63 swab samples were collected from six subjects belonging to Category 3 (Table 15), and DNA was extracted using a DNeasy PowerSoil kit. DNA quantity was measured by using a Nanodrop spectrophotometer (Table 15) and a Qubit 3.0 fluorometer (data not shown).

Table 15. The quantity of DNA extracted from six subjects (# 41, 42, 43, 44, 45, and 46) and the objects in their offices, measured by a NanoDrop spectrophotometer (ND-1000)

	DNA Yield (ng/µl)					
	41	42	43	44	45	46
Cell phone	5.37	6.56	5.67	5.73	10.49	4.31
Door handle	5.31	NC[a]	2.80	NC[a]	NC[a]	2.80
Doorknob	2.97	1.78	3.72	4.87	6.37	6.52
Keyboard (laptop)	6.95	2.68	8.47	3.59	3.57	3.00
Trackpad	6.69	4.67	6.81	4.97	1.78	8.51
Pen	2.24	3.39	4.74	4.35	7.79	4.12
Tumbler	9.27	1.24	8.67	1.35	1.66	6.30
Left fingers	2.94	2.30	4.34	9.97	8.42	4.51
Left palm	7.17	13.33	3.55	10.42	12.26	7.40
Right fingers	9.86	3.68	4.50	5.67	2.4	3.63
Right palm	6.83	2.76	10.42	8.99	2.51	4.34

[a]NC, not collected.

Based on the DNA quantity measured by using Nanodrop, we had low DNA yield from the swab samples collected from six subjects (Category 3) and their human touched objects. Among the objects, cell phone and trackpad showed higher DNA yields than other objects found in an office setting (Table 15). However, individual variability of DNA yield was also observed. Due to the low DNA yield, we employed whole genome amplification prior to the metagenome sequencing to obtain profiles of bacteriome, archaeome, fungiome, and virome.

Whole genome amplification

DNA extracted from 20 subjects and their office objects were chosen to determine the total microbiome using metagenome sequencing analysis. Due to low yield of DNA, we amplified DNA using a REPLI-g Mini kit (Qiagen). The quantity of amplified DNA was measured by both a NanoDrop ND-1000 spectrophotometer and a Qubit 3.0 fluorometer (Tables 16 and 17). DNAs were further purified using a DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's supplementary protocol and sent for whole shotgun metagenome sequencing.

Table 16. The quantity of DNA from seven subjects (#19, 20, 21, 22, 23, 24, and 27) and the objects in their offices amplified using a REPLI-g Mini kit and measured by Qubit 3.0 fluorometer

	DNA Yield (ng/µl)						
	19	20	21	22	23	24	27
Cell phone	10.8	10.4	9.74	9.74	8.8	5.78	2.52
Keyboard	9.52	8.64	10.2	10.2	10.01	11	2.12
Left finger	9.78	9.9	9.9	6.72	2.34	10.2	9.92
Left palm	3.72	3.84	10.8	6.48	0.038	9.36	4.78
Toothbrush	NC[a]	10.2	10.2	7.94	6.06	10.8	9.58
Right fingers	10.2	10.4	10.2	6.46	2.36	4.14	9.84
Right palm	10.8	11	6.1	6.24	5.28	2.78	11.8
Track pad	3.8	6.86	7.56	7.8	7.22	8.14	3.98

[a]NC, not collected.

Table 17. The quantity of DNA from seven subjects (#30, 31, 32, 33, 34, 35, and 36) and the objects in their offices amplified using a REPLI-g Mini kit and measured by Qubit 3.0 fluorometer

	DNA Yield (ng/µl)						
	30	31	32	33	34	35	36
Cell phone	12	10	6.24	10	8.64	5.36	8.66
Keyboard	5.64	0.091	8.94	9.34	8.72	8.88	10
Left finger	5.52	OOR[b]	10.4	5.36	6.9	9.06	10.4
Left palm	11	OOR	9.74	1.77	8.62	9.78	10
Office phone	12	3.52	8.18	8.18	8.78	9.2	10.2
Mouse	NC[a]	NC	3.2	6.48	1.05	NC	1.01
Right fingers	4.6	OOR	9.32	9.36	8.86	8.98	9.82
Right palm	11.6	OOR	7.12	0.8	8.94	9.22	10.2
Track pad	1.89	1.5	NC	NC	NC	6.28	NC

[a]NC, not collected.
[b]OOR, out of range.

Based on the DNA quantity measured by using Nanodrop and Qubit 3.0, we increased DNA quantity using the whole genome amplification method and conducted amplicon sequencing and whole shotgun metagenome sequencing to obtain profiles of bacteriome, archaeome, fungiome, and virome.

Task 2.3. *Conduct post-sequencing analyses.*

Amplicon sequencing analysis

We have received and analyzed the amplicon (V4 region of 16S rRNA) sequencing results of 63 DNAs extracted from six Category 3 subjects. Amplicon sequencing results showed 15,579K identified reads (PF) were generated. The resulting paired sequence reads were merged, filtered, aligned using reference alignment in BaseSpace (Illumina). The reads were further analyzed using the QIIME2 and DADA2 pipelines at Nephele (Weber et al., 2018) to get OTU (Operational Taxonomic Unit) generation and taxonomic classification (Wang et al., 2007). The 16S amplicon sequencing analysis showed a total of 412,058 OTUs with 0 minimum, 15,007 maximum, and 6,541 mean OTUs. We further used MicrobiomeDB to analyze relative abundance, alpha diversity, beta diversity, differential abundance, and correlation (Oliveira et al., 2018).

The alpha diversity of OTUs in Category 3 samples ($P = 0.001$) was found to be significant (Tables 18 and 19). Standard deviation index (SDI) differed heavily between Category 3 subjects, with subjects 43 and 45 having a significantly lower SDI compared to 41, 42, 44, and 46 (Fig. 31).

Table 18. Descriptive statistics for the quantity of DNA from Category 3 subjects. Data was utilized in statistical analysis.

					Interval for Mean*			
Day	N	Mean	Std. Dev.	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
41	11	3.4363	.68692	.20711	2.9748	3.8978	1.96	4.03
42	10	3.2497	.47641	.15065	2.9089	3.5906	2.29	3.99
43	11	2.4587	1.16436	.35107	1.6765	3.2409	.55	3.96
44	10	3.2524	.64980	.20548	2.7875	3.7172	2.16	4.05
45	10	2.4071	.68654	.21710	1.9159	2.8982	.75	3.15
46	11	3.4130	.24630	.07426	3.2476	3.5785	2.85	3.70
Total	63	3.0394	.81161	.10225	2.8350	3.2438	.55	4.05

*95% Confidence

Table 19. Analysis of variance (ANOVA) for Category 3 alpha diversity obtained from amplicon sequencing. Comparison was significant at $P = 0.001$. Tukey's LSD was utilized for post-hoc comparisons.

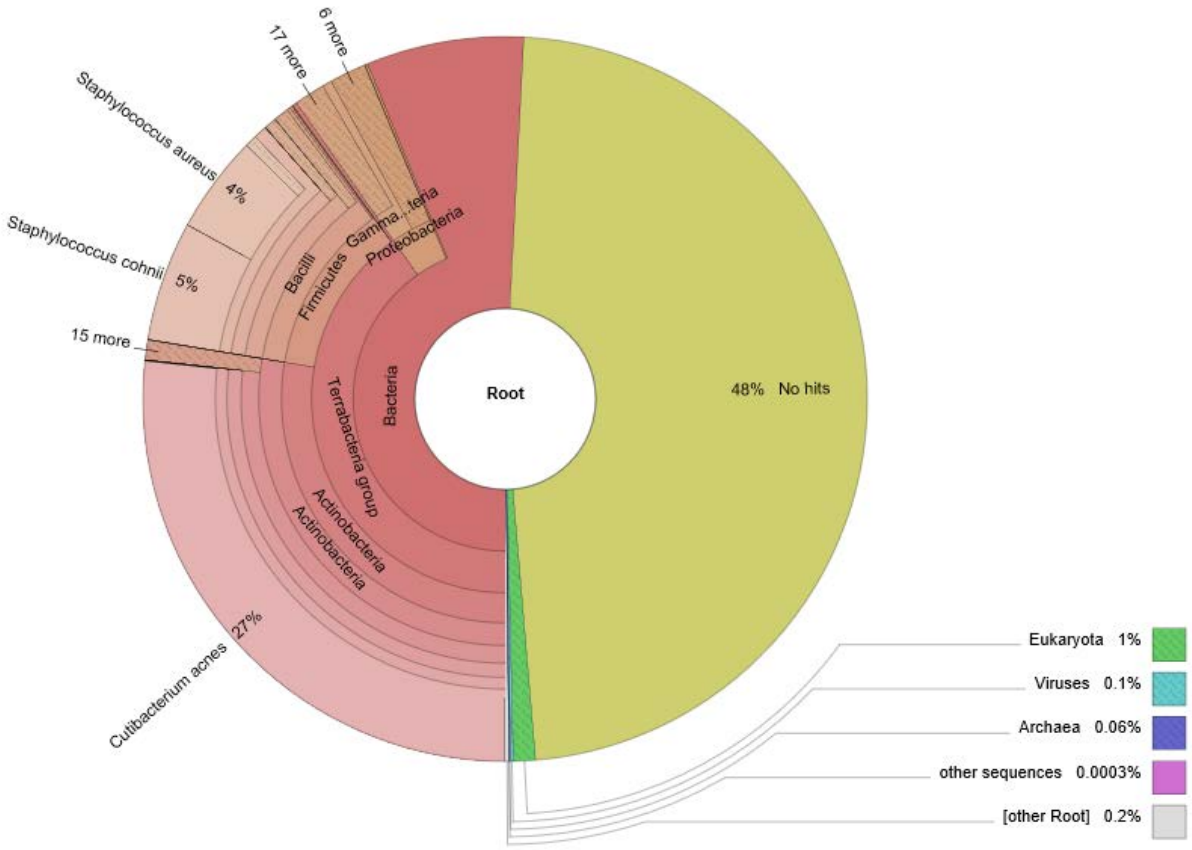
	Sum of Squares	*df*	Mean Square	*F*	Sig.
Between Groups	11.872	5	2.374	4.672	.001

Within Groups	28.967	57	.508
Total	40.840	62	

Whole shotgun metagenome sequencing analysis

Whole genome amplified DNAs from 14 subjects from Category 1 and 2 were sent for the fragment analysis, and then a total of 103 samples were selected and proceeded for whole metagenome sequencing using the Illumina Nextera XT library preparation kit (Hannigan et al., 2015). A snapshot of taxonomic profile of sample 10A showed that bacteria were the most dominant microbiome (Fig. 32). Among them, *Cutibacterium acnes*, *Staphylococcus cohnii*, and *S. aureus* were the three major bacterial species, which coincides with the previous studies on human skin microbiome.

Figure 32. A snapshot of taxonomic profiles of sample 10A (Subject #34; cellphone) identified by the DRAGEN Metagenomics pipeline of BaseSpace after shotgun metagenome sequencing.



The data obtained by whole metagenome sequencing consisted of 1,217,044,974 identified reads (91.6% PF) with an average number of reads of 5,516,933 (Table 20). Based on the reads identified by DRAGEN Metagenomics, bacteria were dominant, followed by eukarya, viruses, and archaea. Interestingly, viral reads were more abundant than eukarya and archaea in some samples (Table 20), suggesting that a viral profile could be used as a tool in microbial forensic identification.

Table 20. Identified reads after shotgun metagenome sequencing of 103 whole genome amplified DNA samples from Categories 1 and 2 subjects and the objects in their dorms/offices.

Sample ID	Cat.	Subjects	Objects	Reads	Shannon Index	No. of Species	Counts			
							Bacteria	Archaea	Eukarya	Viruses
3B	2	21	Keyboard	2169144	2.13	710	286978	1758	782	191
3C	2	21	Toothbrush	2324677	2.99	843	353534	1364	617	31
3D	2	21	Trackpad	3808414	2.26	938	854609	4607	616	525
3E	2	21	Left finger	2212291	1.62	971	407765	405	2106	6921
3F	2	21	Left palm	2840912	1.92	619	144527	346	5642	735
3G	2	21	Right finger	8870236	1.10	1007	2659919	17926	15462	155
3H	2	21	Right palm	2199887	2.11	424	442869	1618	513	205
4A	2	22	Cellphone	3086731	0.74	1765	935826	1078	2541	129
4B	2	22	Keyboard	11189470	0.93	2442	3604315	30879	2107	72
4C	2	22	Toothbrush	3718492	2.92	2752	1469507	3840	978	173
4D	2	22	Trackpad	3293199	1.36	2408	625538	2960	1081	229
4E	2	22	Left finger	8093204	1.99	1983	5285103	14178	6710	729
4F	2	22	Left palm	8461952	1.67	1285	6386870	10316	3483	121
4G	2	22	Right finger	9589840	2.20	2138	6471432	13531	4836	341
5A	2	24	Cellphone	10110269	1.86	1063	4133813	23918	6300	618
5B	2	24	Keyboard	3710290	2.01	1440	1186618	3503	14404	177
5C	2	24	Toothbrush	4052854	1.68	2101	1132121	2940	449	66
5D	2	24	Trackpad	3321654	2.57	713	518637	1016	2578	203
5E	2	24	Left finger	11006450	1.36	1282	5532346	23113	9275	1436
5F	2	24	Left palm	4074347	1.39	364	379529	2793	355	13
5G	2	24	Right finger	6643562	1.59	751	2969833	12987	1979	31
5H	2	24	Right palm	2562544	1.92	469	289128	1541	252	1782
6A	2	27	Cellphone	11079052	1.98	1401	4197526	9842	104938	1147
6B	2	27	Keyboard	9999580	1.62	1833	2897230	9348	53059	193
6C	2	27	Toothbrush	8204796	1.98	913	2850108	12547	20937	747
6D	2	27	Trackpad	10678558	1.56	1265	3406876	21141	54151	1102
6E	2	27	Left finger	8736793	1.98	1179	3410873	10105	49769	154
6F	2	27	Left palm	9245953	1.87	1301	2229830	14978	73409	716
6G	2	27	Right finger	11389747	1.85	1038	2945721	6462	74105	113
6H	2	27	Right palm	11628541	1.82	1301	2526941	8484	114953	309

7A	1	30	Cellphone	2289011	2.26	480	430748	897	1238	28
7C	1	30	Office phone	5024444	1.74	746	1303662	3474	3118	3656
7E	1	30	Left finger	3632420	2.53	1042	517968	1229	6914	548
7F	1	30	Left palm	4677776	2.66	1418	513145	2761	13938	3131
7G	1	30	Right finger	9306321	1.67	1537	4529668	10480	16784	1010
7H	1	30	Right palm	2327124	2.15	636	560036	532	2020	18638
8A	1	32	Cellphone	7970044	1.86	1271	4334876	12897	43973	13439
8B	1	32	Keyboard	9686541	1.85	1332	5498602	15523	21554	45900
8C	1	32	Office phone	8596168	1.85	1623	3920475	14813	31316	61011
8D	1	32	Mouse	11172271	1.80	1382	5293630	15530	30542	23069
8E	1	32	Left finger	10632194	1.84	1542	5349396	15484	37710	12313
8F	1	32	Left palm	12157255	1.70	1813	5129315	19980	41815	181204
8G	1	32	Right finger	11444113	1.86	1603	4646512	11900	68755	43875
8H	1	32	Right palm	4296709	2.34	1477	1197745	2838	8254	27695
9A	1	33	Cellphone	3665554	2.75	932	280456	1006	10371	526
9B	1	33	Keyboard	10536880	1.84	1322	4681475	11965	38195	1520
9D	1	33	Mouse	3448861	1.87	1233	1208896	1689	2473	960
9F	1	33	Left palm	11122533	1.65	1583	5126417	15157	33854	2452
9G	1	33	Right finger	10028380	1.73	1693	5105614	20449	23895	1955
9H	1	33	Right palm	3420227	2.60	966	1176223	1395	4505	548
10A	1	34	Cellphone	4023510	1.66	1775	843548	1048	16270	2073
10C	1	34	Office phone	4529038	2.19	1256	1119196	2019	11344	14774
10E	1	34	Left finger	4312252	2.97	1551	791306	1513	5939	1693
10G	1	34	Right finger	3165586	3.20	1979	419416	1035	4187	3618
10H	1	34	Right palm	11336410	1.60	2377	3168231	23362	62687	39690
11A	1	35	Cellphone	2995069	1.84	425	921843	1320	13945	768
11B	1	35	Keyboard	3896120	1.72	360	1370576	3802	9884	457
11C	1	35	Office phone	2478103	2.13	278	959745	827	7565	591
11D	1	35	Trackpad	10903894	1.81	1365	6478941	21308	11350	20079
11E	1	35	Left finger	1924310	1.78	331	502360	955	6956	21185
11F	1	35	Left palm	2424643	1.68	464	490840	1278	5683	1264
11G	1	35	Right finger	2440849	2.14	424	640158	807	5291	1463
11H	1	35	Right palm	2922635	1.49	471	998702	1314	11258	391
12A	1	36	Cellphone	3261819	1.83	796	1215516	1780	2517	217
12B	1	36	Keyboard	2414250	1.93	614	567328	766	1572	734
12C	1	36	Office phone	2112154	2.44	570	383967	619	1179	120
12D	1	36	Mouse	4567410	1.71	752	1910486	4856	2645	4229
12E	1	36	Left finger	3279870	2.61	761	712147	964	1411	835
12F	1	36	Left palm	4064468	2.27	1143	1111365	2498	6327	182
12G	1	36	Right finger	4369587	2.15	909	1061817	3735	4112	140
12H	1	36	Right palm	9905463	1.55	1273	4251241	15086	41544	301

23B	2	23	Keyboard	2753152	1.75	547	781543	1169	127	66
23C	2	23	Toothbrush	3488119	2.66	2194	2359833	1549	313	29
23D	2	23	Trackpad	3346384	1.79	524	1178664	2000	1410	380
23F	2	23	Left palm	5052224	2.47	1562	2701645	8936	3330	163
23G	2	23	Right finger	5290334	2.55	1676	2824434	6559	2517	173
23H	2	23	Right palm	10145570	1.89	1121	6216188	26935	9215	276
31A	1	31	Cellphone	2350466	2.84	648	508153	706	998	222
31C	1	31	Office phone	6299253	1.76	814	1852253	4577	42313	1845
31D	1	31	Trackpad	4577432	2.16	1171	1660009	4257	11011	534
31E	1	31	Left finger	2296192	2.90	722	186748	349	2936	221
31F	1	31	Left palm	2903574	2.79	1162	594003	1259	2357	8121
31G	1	31	Right finger	2463423	2.96	987	590048	616	1904	1796
31H	1	31	Right palm	3700529	2.51	764	1210908	2113	438	108
A1	2	19	Cellphone	3112256	2.22	367	620141	878	19164	605
A2	2	19	Keyboard	10790007	1.58	1214	6953112	12645	9740	1226
A3	2	19	Toothbrush	2197315	2.31	1117	900997	833	830	33953
A4	2	19	Trackpad	10096663	1.47	1111	3777699	18326	43658	23244
A5	2	19	Left finger	2590209	2.11	692	811350	703	632	3131
A6	2	19	Left palm	7560531	2.21	951	3296245	16702	2735	131297
A7	2	19	Right finger	2373787	2.37	749	754033	1031	2488	12652
A8	2	19	Right palm	2469975	2.33	738	539455	1079	1529	4985
B1	2	20	Cellphone	1634612	1.91	321	171385	431	2239	311
B2	2	20	Keyboard	3665647	1.19	552	2045012	1358	1618	7241
B3	2	20	Toothbrush	3742581	2.76	824	990372	3833	1025	943
B4	2	20	Trackpad	3674908	1.64	859	1475838	1965	962	9489
B5	2	20	Left finger	2434628	1.12	459	1430757	456	1688	13929
B6	2	20	Left palm	2622807	1.18	457	1611495	737	914	19068
B7	2	20	Right finger	3757247	1.31	510	1402223	1627	971	17115
B8	2	20	Right palm	2514672	1.13	381	1617145	392	1174	12102

Subsequent statistical analysis, one-way ANOVA was performed with Tamhane's T2 post-hoc multiple comparison test. Category-by-category analysis revealed that Category 2 (mean: 2.11) was significant ($P = 0.013$) with Shannon (Table 21).

Table 21. One-way ANOVA comparing Categories 1 (living on campus and working on campus) and 2 (living off campus and working on campus).

Groups	Source of Variation	SS	*df*	Mean Square	*F*	*P*-value
Reads	Between Groups	1.01E+12	1	1.01E+12	0.09	0.766
	Within Groups	1.11E+15	98	1.14E+13		

	Total	1.11E+15	99			
Shannon	Between Groups	1.48E+00	1	1.48E+00	6.41	0.013
	Within Groups	2.27E+01	98	2.31E-01		
	Total	2.42E+01	99			
No. of Species	Between Groups	2.18E+04	1	2.18E+04	0.07	0.791
	Within Groups	3.02E+07	98	3.08E+05		
	Total	3.02E+07	99			
Bacteria	Between Groups	8.44E+11	1	8.44E+11	0.24	0.624
	Within Groups	3.41E+14	98	3.48E+12		
	Total	3.42E+14	99			
Eukarya	Between Groups	3.50E+07	1	3.50E+07	0.07	0.794
	Within Groups	5.02E+10	98	5.12E+08		
	Total	5.02E+10	99			
Archaea	Between Groups	4.68E+07	1	4.68E+07	0.83	0.363
	Within Groups	5.49E+09	98	5.60E+07		
	Total	5.54E+09	99			
Viruses	Between Groups	8.70E+08	1	8.70E+08	1.50	0.224
	Within Groups	5.70E+10	98	5.82E+08		
	Total	5.79E+10	99			

For subjects, significance was found with reads ($P < 0.001$), Shannon ($P = 0.007$), no. of species ($P < 0.001$), bacteria ($P < 0.001$), eukarya ($P < 0.001$), archaea ($P = 0.013$), and viruses ($P < 0.001$) (Fig. 3; Table 4). No significance was found for objects.

Figure 33. Read counts of bacteria, archaea, eukarya, and viruses identified by DRAGEN Metagenomics after whole shotgun metagenome sequencing of 103 samples collected from 14 different subjects.

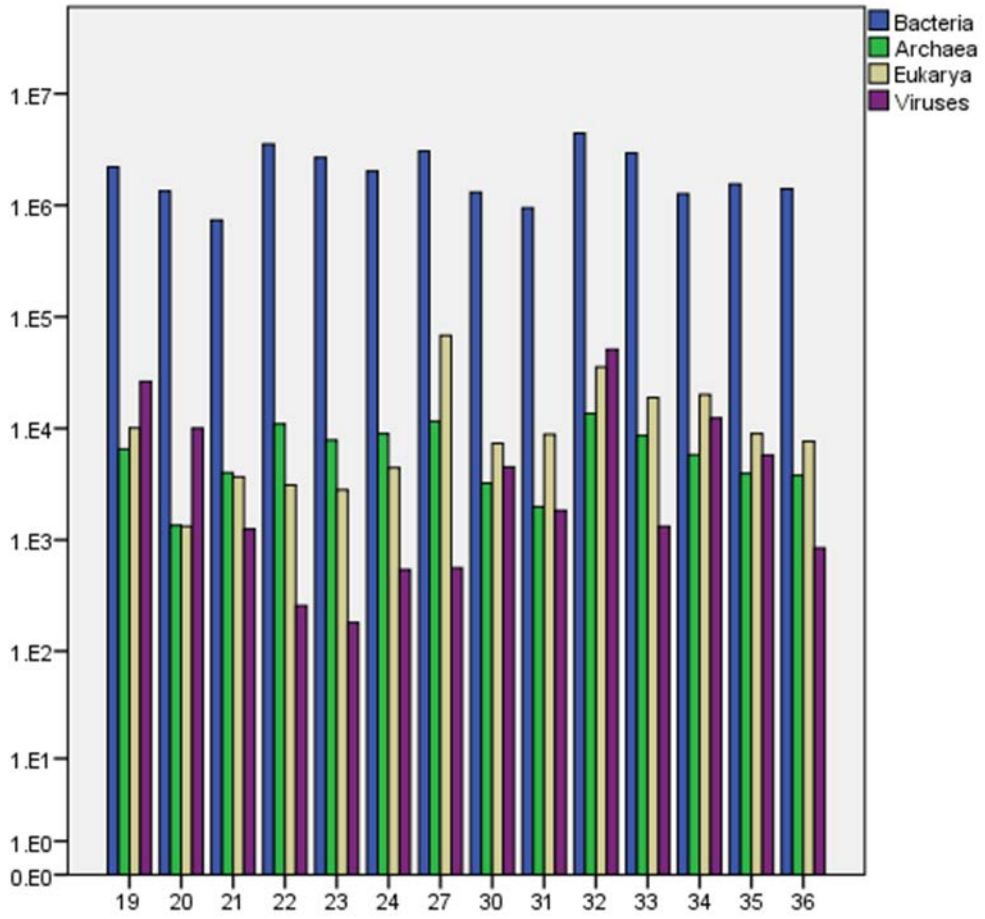


Table 22. One-way ANOVA comparing sequencing profiles from different subjects.

Groups	Source of Variation	SS	*df*	Mean Square	*F*	*P*-value
Reads	Between Groups	4.75E+14	13	3.65E+13	4.91	0.000
	Within Groups	6.40E+14	86	7.44E+12		
	Total	1.11E+15	99			
Shannon	Between Groups	6.52E+00	13	5.02E-01	2.45	0.007
	Within Groups	1.76E+01	86	2.05E-01		
	Total	2.42E+01	99			
No. of Species	Between Groups	1.87E+07	13	1.44E+06	10.77	0.000
	Within Groups	1.15E+07	86	1.34E+05		
	Total	3.02E+07	99			
Bacteria	Between Groups	1.12E+14	13	8.60E+12	3.21	0.001
	Within Groups	2.31E+14	86	2.68E+12		
	Total	3.42E+14	99			
Eukarya	Between Groups	3.29E+10	13	2.53E+09	12.54	0.000

	Within Groups	1.73E+10	86	2.02E+08		
	Total	5.02E+10	99			
Archaea	Between Groups	1.42E+09	13	1.09E+08	2.27	0.013
	Within Groups	4.12E+09	86	4.79E+07		
	Total	5.54E+09	99			
Viruses	Between Groups	2.06E+10	13	1.59E+09	3.67	0.000
	Within Groups	3.73E+10	86	4.33E+08		
	Total	5.79E+10	99			

In addition, we used WGSA and bioBakery pipelines at Nephele (Weber et al., 2018), and then used MicrobiomeDB to analyze relative abundance, alpha diversity, beta diversity, differential abundance, and correlation (data not shown).

Objective 3. To identify core/variable/transient microbiome associated with different post-contact intervals.

Task 3.1. *Identify the core (resident)/variable/transient microbiome associated with different post-contact intervals (PCI).*

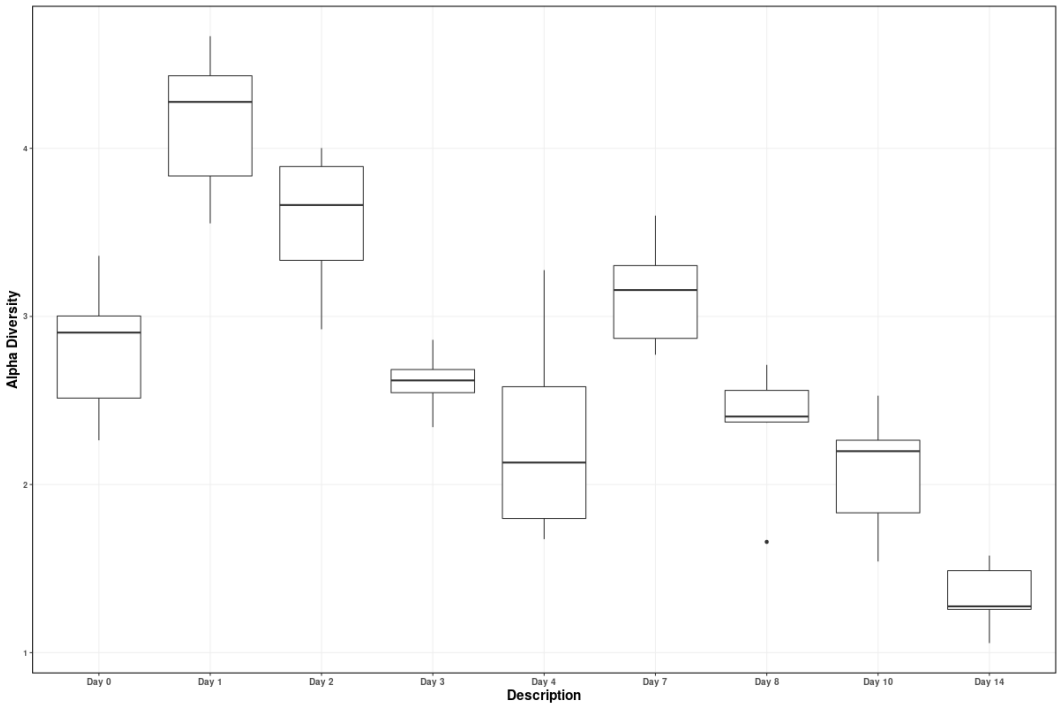
DNA concentration (ng/µL) and absorbance/quality (A260/280) of isolated DNAs from the Objective 3 longitudinal study were measured using a ND-1000 spectrophotometer (Table 23). Samples were obtained from three human-touched glass window panes designated "A," "B," and "C." Sampling was performed over a two-week period with swab collection and DNA extraction occurring within 24 hours of previous swab collection and extraction.

Table 23. DNA concentration (ng/µL) and absorbance/quality (A260/280) of isolated DNAs (Objective 3) measured spectrophotometrically using ND-1000 (Fisher).

	DNA Yield (ng/µl)					
	A		B		C	
Day	Front	Back	Front	Back	Front	Back
0	3.42	2.75	4.96	1.10	2.08	1.50
1	5.00	2.10	2.60	2.10	3.40	6.80
2	1.29	1.81	1.15	3.60	8.78	2.53
3	1.00	1.30	1.10	1.50	1.80	1.90
4	1.30	2.00	0.60	0.40	3.50	1.60
7	6.37	3.78	3.12	8.04	2.74	2.17
8	5.53	5.14	4.28	3.56	6.83	3.01
10	6.90	5.02	3.93	1.11	1.82	4.00
14	5.99	4.52	1.57	4.42	7.19	3.71

We have received and analyzed the amplicon (V4 region of 16S rRNA) sequencing results of 54 DNAs extracted from swab samples obtained from glass panes for PCI analysis. The sequencing reads were analyzed using the QIIME2 and DADA2 pipelines at Nephele (Weber et al., 2018) to get OTU (Operational Taxonomic Unit) generation and taxonomic classification (Wang et al., 2007). The 16S amplicon sequencing analysis showed a total of 470,027 OTUs with 0 minimum, 16,885 maximum, and 8,704 mean OTUs. The composition of bacterial community from the three glass windows changed as time elapsed after contact. Based on the sequencing analysis, Shannon's index and α-diversity (Pearson's correlation = -0.682; $P < 0.001$) decreased over time (Fig. 34).

Figure 34. Alpha diversity of OTUs was measured using Shannon index at MicrobiomeDB.



Alpha and beta diversity metrics calculated from the OUT table showed gradual turnover of microbial community over time (Figs. 34 and 35). Beta (β) diversity was significantly different ($P < 0.001$) according to PCIs (Fig. 35).

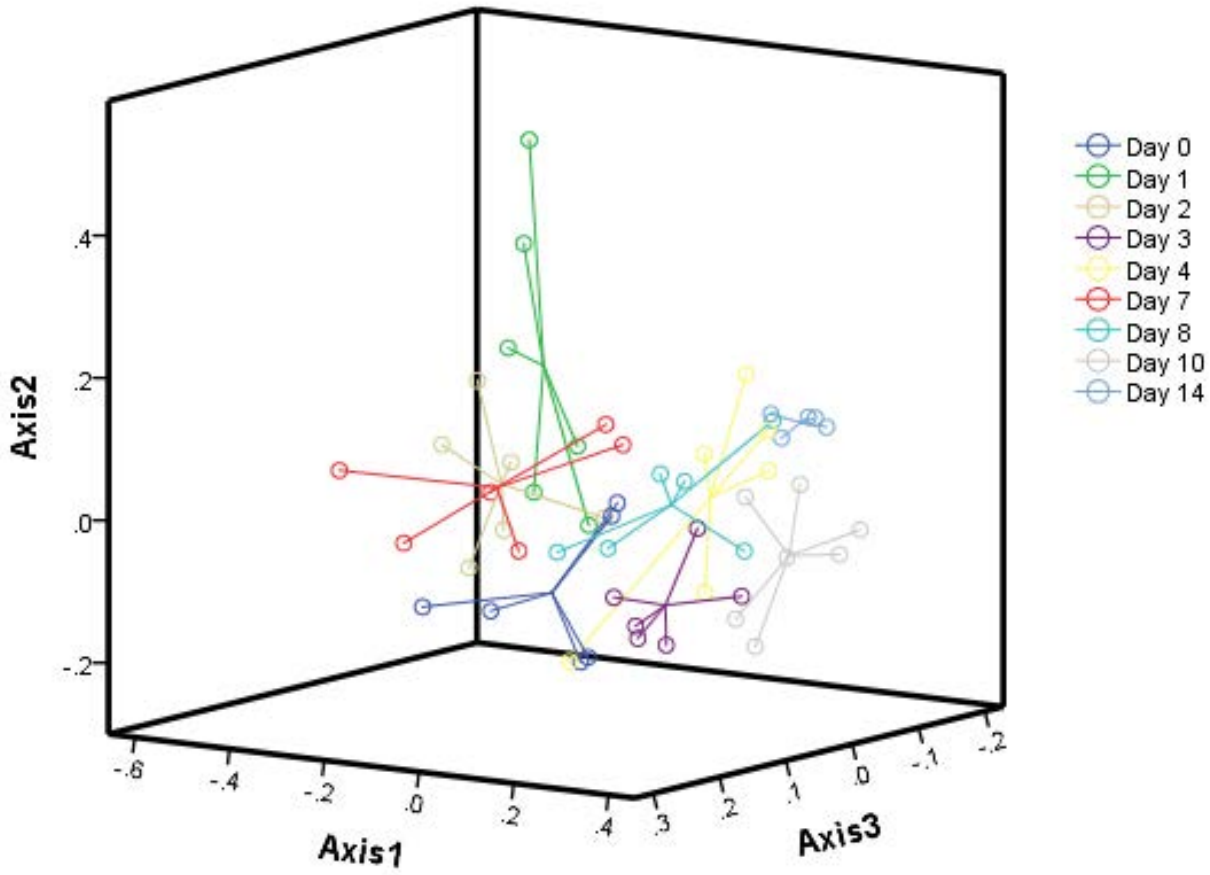
Using ANOVA and Tukey's LSD post-hoc multiple comparison test, alpha diversity ($P < 0.001$) was found to be significant (Table 24). Shannon diversity index (SDI) in the Objective 3 study decreased as the week progressed. SDI was negatively correlated (Pearson's correlation: -0.689; $P < 0.001$) with time elapsed, with SDI at 4.1572 within the first day and steadily decreasing to 1.3310 within the two week interval (data not shown).

Table 24. Analysis of variance (ANOVA) for Objective 3 alpha diversity obtained from sequencing. Comparison was significant at $P < 0.001$. Tukey's LSD was utilized for post-hoc comparisons.

	Sum of Squares	*df*	Mean Square	*F*	Sig.
Between Groups	32.547	8	4.068	26.962	.000
Within Groups	6.790	45	.151		
Total	39.337	53			

For beta diversity, Axis 1 ($P < 0.001$), Axis 2 ($P = 0.019$), Axis 3 ($P = 0.037$), Axis 4 ($P = 0.001$), Axis 14 ($P = 0.005$), and Axis 15 ($P = 0.032$) were significant. Axis 1 (Pearson's correlation: 0.673; $P < 0.001$) positively correlated with time elapsed.

Figure 35. Beta diversity of OTUs was measured using the Bray-Curtis method at MicrobiomeDB.

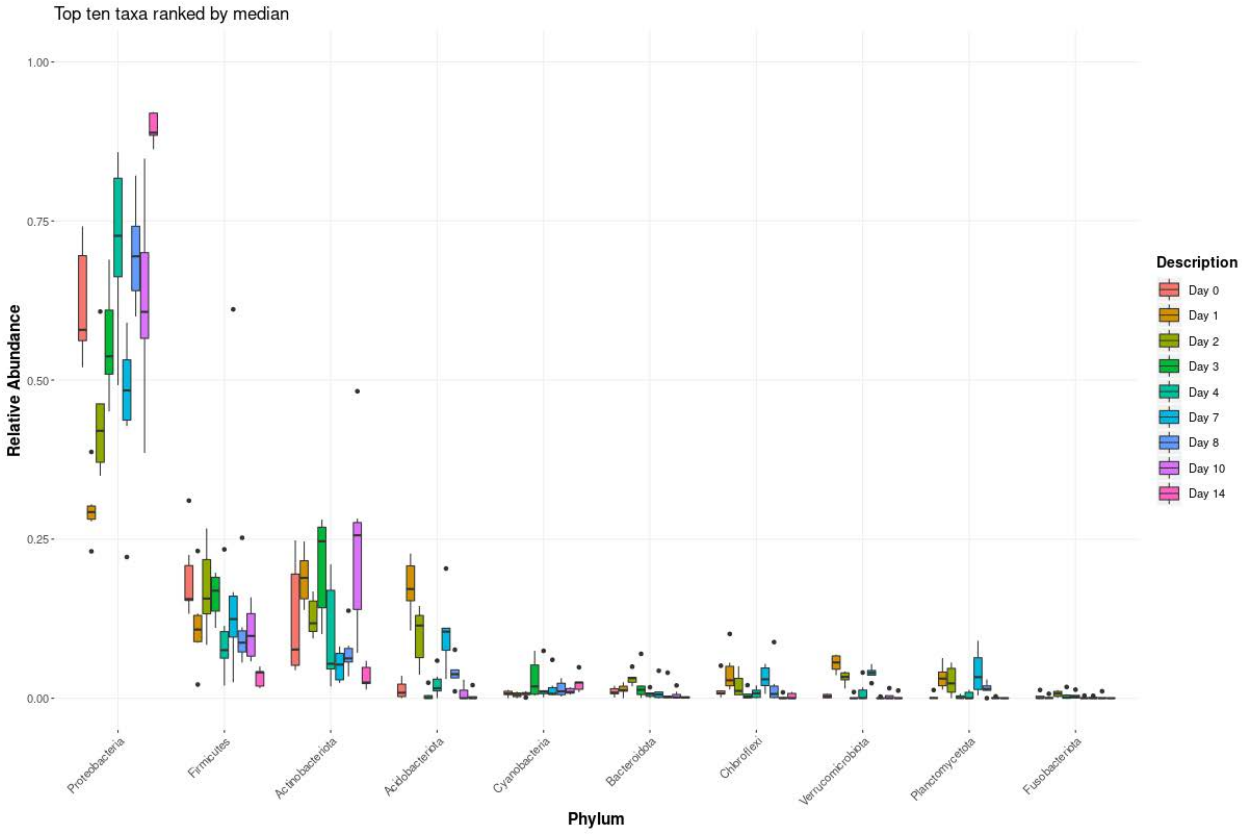


For relative diversity, Proteobacteria (0.584) was the most abundant microorganism, follow by Actinobacteriota (0.130) and Firmicutes (0.131) (Table 25). Abundance was positively correlated (Pearson's correlation: 0.129; P =0.003) with phyla (data not shown).

Table 25. Analysis of variance (ANOVA) for Objective 3 relative diversity obtained from amplicon sequencing, comparing phylum vs. abundance. Comparison was significant at $P < 0.001$. Tukey's LSD was utilized for post-hoc comparisons. Phylum utilized were *Fusobacteriota*, *Acidobaceriota*, *Bacteriodota*, *Planctomycetota*, *Verrucomicrobiota*, *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Chloroflexi*, and *Cyanobacteria*.

	Sum of Squares	*df*	Mean Square	*F*	Sig.
Between Groups	15.366	9	1.707	282.602	.000
Within Groups	3.202	530	.006		
Total	18.568	539			

Figure 36. A box plots for the top 10 taxa (by mean relative abundance) at the phylum level over time measured at MicrobiomeDB.



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The relative abundance of nearly all major bacterial phyla significantly decreased over a two-week period. The abundance of Acidobacteria (Pearson's correlation = -0.351; $P = 0.009$), Bacteroidetes (Pearson's correlation = -0.367; $P = 0.006$), Firmicutes (Pearson's correlation = -0.339; $P = 0.012$), Fusobacteria (Pearson's correlation = -0.279; $P = 0.041$), and Verrucomicrobiota (Pearson's correlation = -0.353; $P = 0.009$) negatively correlated with PCIs. However, the abundance of Proteobacteria (Pearson's correlation = 0.559; $P < 0.001$) was positively correlated with PCIs (Figs. 36-38).

Figure 37. A linear regression model of relative abundance of bacterial phyla with PCIs.

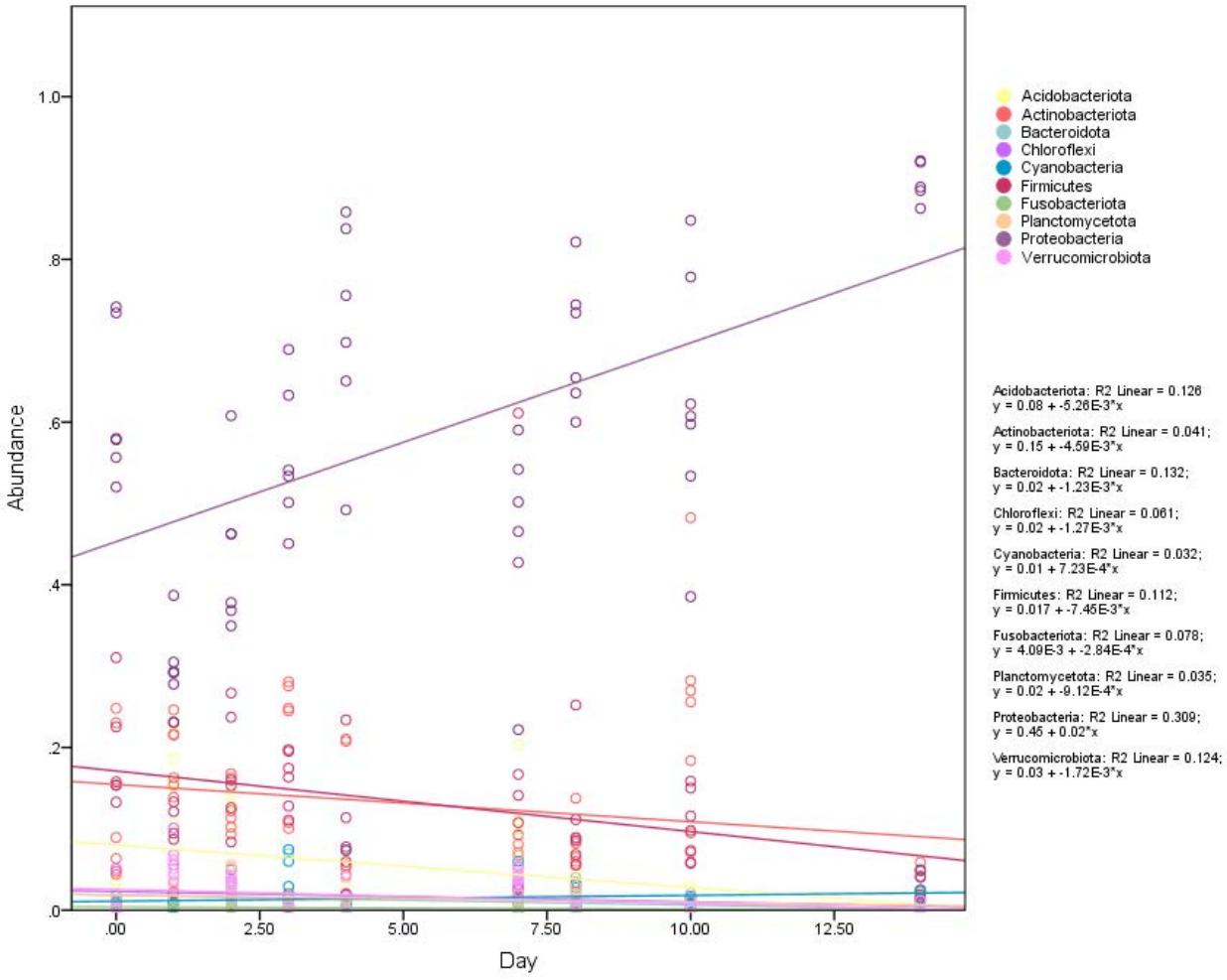

Figure 38. Relative abundance of bacterial phyla over a two-week period.

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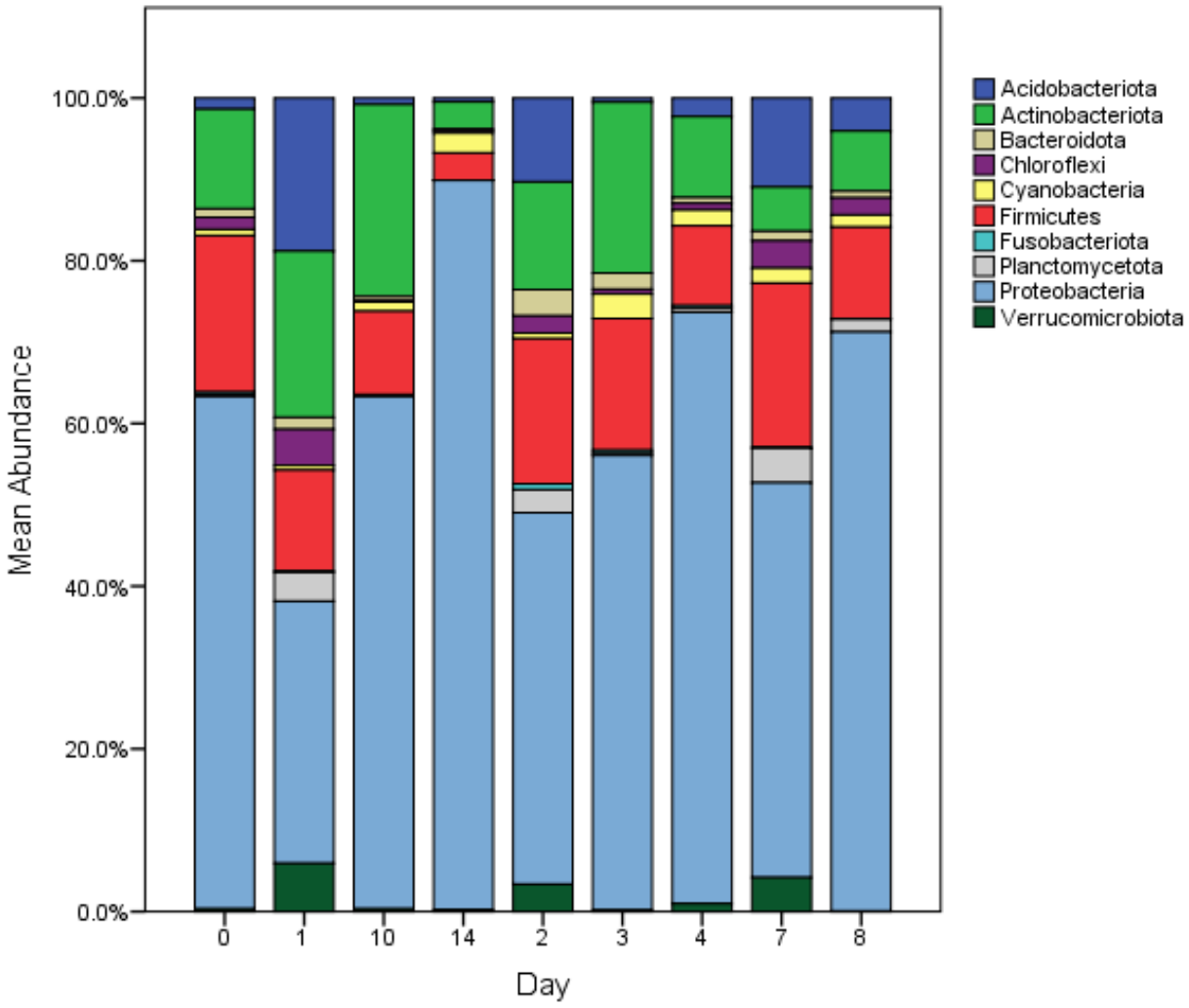

Major bacterial phyla found on human-touched objects, such as Proteobacteria, Actinobacteria, and Firmicutes may be used as indicators for determining PCI although more study is necessary.

Task 3.2. *Quantify the core (resident)/variable/transient microbiome associated with different post-contact intervals (PCI).*

Isolated DNAs from Objective 3 (Table 23) that were quantified using SYBR Green-based qPCR, amplified and sent for sequencing were received and analyzed. qPCR data were utilized in statistical analysis. No significant difference was found between glass panes sampled by the three volunteers (Table 26) or the front and back sides of the glass window panes (Table 27). Microbial abundance had slight variation, though no significant values, between glass windows, each of which were touched by a different volunteer.

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Table 26. Copy number by glass window pane in the Objective 3 longitudinal study calculated via qPCR.

Subject		Fungal	Bacterial
A	Mean	3.39E+09	7.18E+07
	Std. Deviation	4.56E+09	1.54E+07
B	Mean	1.99E+09	5.68E+07
	Std. Deviation	1.75E+09	2.72E+07
C	Mean	4.96E+09	
	Std. Deviation	8.54E+09	6.04E+07
Total	Mean	3.45E+09	6.62E+07
	Std. Deviation	5.56E+09	3.81E+07

No significant differences in glass window side were noted in statistical analysis. "Front" side was the side of the glass window directly facing the fluorescent lighting, whereas the "Back" side faced the interior of the laboratory cabinet.

Table 27. Copy number for glass window sides in the Objective 3 longitudinal study calculated via qPCR.

Side		Fungal	Bacterial
Front	Mean	3.32E+09	5.66E+07
	Std. Deviation	4.93E+09	2.58E+07
Back	Mean	3.58E+09	7.59E+07
	Std. Deviation	6.36E+09	4.66E+07
Total	Mean	3.45E+09	6.62E+07
	Std. Deviation	5.56E+09	3.81E+07

Multivariate analysis of variance (MANOVA) was utilized with qPCR data. Overall DNA yields were higher after initial contact ($P = 0.018$; η^2: 0.321) and positively correlated with time (Pearson's: $P = 0.011$; Spearman's: $P = 0.013$), suggesting a change in abundance in the transferred microbiome.

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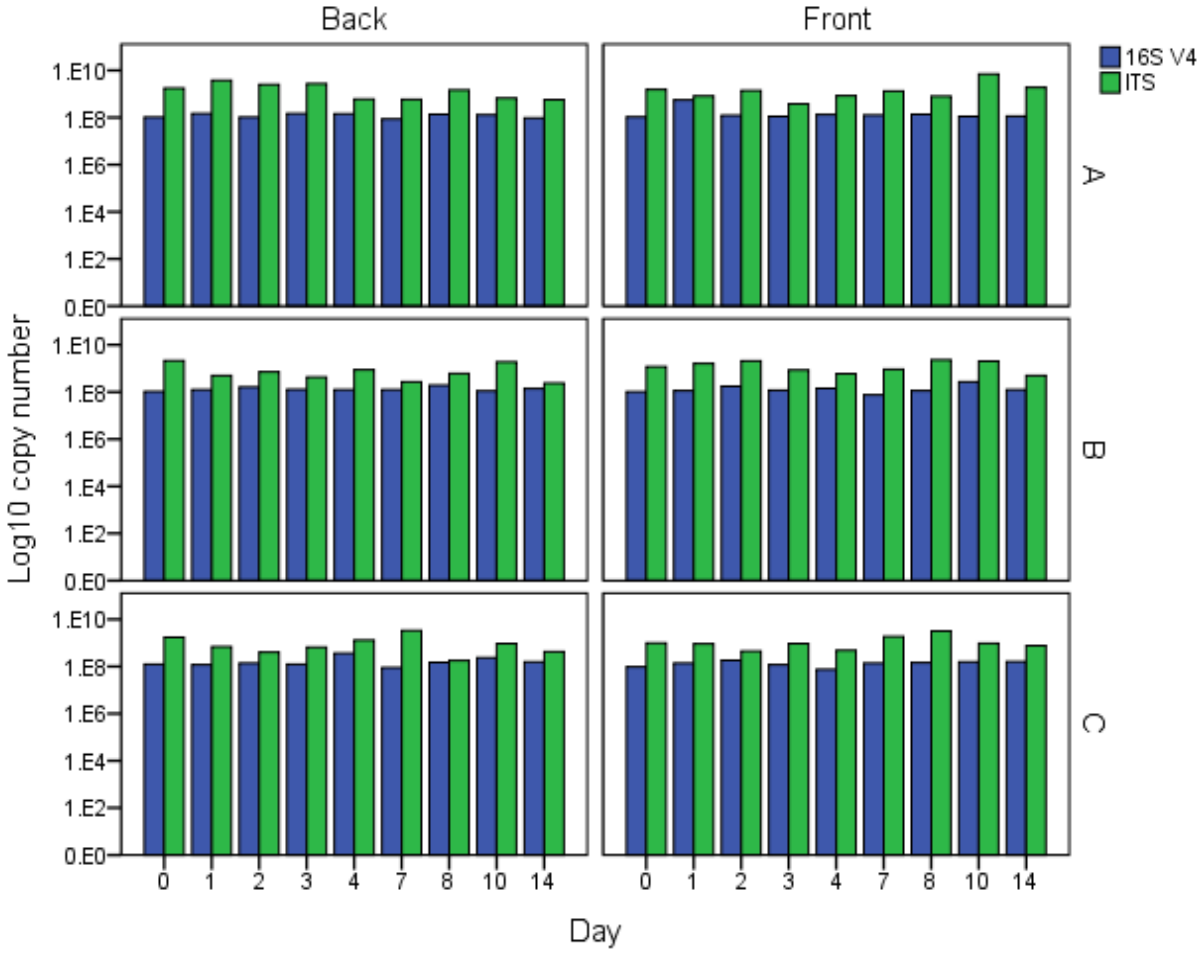
Based on the qPCR analysis, both 16S (Pearson's correlation = -0.031; $P = 0.826$) and ITS (Pearson's correlation = -0.005; $P = 0.974$) copy numbers did not have significant changes over time (Fig. 39).

Figure 39. Abundance of bacteria and fungi (16S and ITS copy numbers) associated with PCIs was identified in qPCR assays.



4. Training and professional development

A total of 30 students including six summer student assistants and three volunteer students have been trained in this research project during the project period. All students have finished the CITI training, students have been trained in 1) fingerprint analysis, comparison, and evaluation, 2) quantitative PCR analysis, and 3) collecting samples, processing samples, DNA extraction and quantification, PCR amplification, whole genome amplification, and quantitative PCR.

5. Dissemination of the results to communities of interest

Nothing to Report.

PRODUCTS

1. Publications, conference papers, and presentations

1) Journal publications.

- Johnson TC, Brown AS, Oommen Z, Okafor U, Y.-J. Lee. 2020. Development of Reverse Fingerprint Lifting Techniques for Forensic Applications. Journal of Forensic Investigation 8(1): 8.

2) Books, or other non-periodical, one-time publications.

3) Other publications, conference papers, and presentations.

Poster presentation

- Willis, L. J., V. B. Anagbo-Dowetin, Y. Lee. Quantitative Analysis of The Transferred Microbiome Associated with Different Post-contact Intervals (PCIs) on Human-Touched Objects. ASM Microbe 2022, Washington DC, 2022.
- Willis, L. J., C. Han, Y. Lee. Exploring the Total Microbiome on Human-Touched Objects as Trace Evidence for Forensic Identification. Pittcon 2022 Conference.
- Willis, L. J., C. Han, Y. Lee. Evaluating and Optimizing DNA Extraction and Amplification Protocols for Microbiome-Based Forensic Applications. 2021 National Institute of Justice Forensic Science Research and Development (R&D) Symposium.
- Han, C., L. J. Willis, C. J. Daniel, Y. Lee. Optimization of microbial DNA extraction from human touched objects. ASM Microbe 2020.
- Daniel, C. J., U. Okafor, Z. Oommen, Y. Lee. Retrieving Microbial DNA from Human Touched Objects for the Microbiome-Based Forensic Applications. ASM Microbe 2018, Atlanta, GA, USA, 2018.

2. Website(s), or other internet site(s)

3. Technologies or techniques

- Standard Operating Procedure 1. Traditional method of lifting fingerprint
- Standard Operating Procedure 2. DNA Extraction from Cotton Swabs Using DNeasy Blood & Tissue Kit
- Standard Operating Procedure 3. DNA Extraction from Cotton Swabs Using DNeasy PowerSoil Kit
- Standard Operating Procedure 4. DNA Extraction from Cotton Swabs Using MP Biomedicals' FastDNA Spin Kit for Soil

4. Inventions, patent applications, and/or licenses

5. Other products

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

1. Participants

Name: Yong Jin Lee
Project Role: PI
Nearest person month worked: 66 months
Contribution to Project: Overseeing and supervising all aspects of the project including project design, management, and performance, organizing project meeting, preparing IRB protocol and related documents, training undergraduate student assistants, recruiting human subjects, collecting samples, conducting microbiome-based analysis, analyzing data, writing report.

Name: Uzoma Okafor
Project Role: Co-PI
Nearest person month worked: 36 months

Contribution to Project: Project Design, preparation of IRB reviewed documents, literature review, undergraduate research students training and orientation, recruitment of human subjects, selection of objects, conducting quantitative PCR analysis.

Name: Zachariah Oommen
Project Role: Co-PI
Nearest person month worked: 36 months
Contribution to Project: Project Design, preparation of IRB reviewed documents, literature review, undergraduate research students training and orientation, recruitment of human subjects, selection of objects, fingerprint analyses and evaluations.

Name: Alese Brown
Project Role: Undergraduate assistant
Nearest person month worked: 9 month
Contribution to Project: Reviewing IRB protocol and related documents, collecting samples, conducting fingerprint analysis.

Name: Cecil Daniel
Project Role: Undergraduate assistant
Nearest person month worked: 10 months
Contribution to Project: Reviewing IRB protocol and related documents, collecting samples, conducting DNA isolation, determining DNA quantity and quality, preparing weekly report.

Name: Hailey Evans
Project Role: Undergraduate assistant
Nearest person month worked: 7 months
Contribution to Project: Reviewing IRB protocol and related documents, recruiting human subjects, collecting samples, conducting fingerprint analysis.

Name: Chowon Han
Project Role: Undergraduate assistant
Nearest person month worked: 21 months
Contribution to Project: Reviewing IRB protocol and related documents, recruiting human subjects, collecting samples, conducting DNA isolation, determining DNA quantity and quality, analyzing data.

Name: Tiffany Johnson

Project Role: Undergraduate assistant
Nearest person month worked: 9 months
Contribution to Project: Reviewing IRB protocol and related documents, recruiting human subjects, collecting samples, conducting fingerprint analysis.

Name: Morgan Josey
Project Role: Undergraduate assistant
Nearest person month worked: 3 months
Contribution to Project: Reviewing IRB protocol and related documents, recruiting human subjects, collecting samples, conducting fingerprint analysis.

Name: Alexis Taylor
Project Role: Undergraduate assistant
Nearest person month worked: 8 months
Contribution to Project: Reviewing IRB protocol and related documents, recruiting human subjects, collecting samples, conducting quantitative PCR analysis.

Name: Ashley Walker
Project Role: Undergraduate assistant
Nearest person month worked: 1 month
Contribution to Project: Reviewing IRB protocol and related documents, collecting samples, conducting fingerprint analysis.

Name: Logan Willis
Project Role: Research assistant
Nearest person month worked: 16 months + 12 voluntary months
Contribution to Project: Collecting samples, conducting DNA isolation, determining DNA quantity and quality, performing quantitative PCR assays, and analyzing data.

2. Other organizations involved as partners

Nothing to Report.

3. Other collaborators or contacts

Nothing to Report.

IMPACT

1. Impact on the development of the principal discipline(s) of the project

Reverse lifting method of fingerprints will provide an alternate way to collect and analyze evidences at a crime scene since this method is less invasive, which makes other forensic applications like microbiome-based analysis possible with the same evidence. In addition, standard operating procedures developed for microbiome-based analysis in this study will also provide a tool for efficient recovery of "microbial fingerprints" from the surface of human touched objects. This microbiome-based forensic identification will provide alternative method to identify individuals associated with a crime scene.

2. Impact on other disciplines

Nothing to Report.

3. Impact on the development of human resources

Nothing to Report.

4. Impact on physical, institutional, and information resources that form infrastructure

Nothing to Report.

5. Impact on technology transfer

Nothing to Report.

6. Impact on society beyond science and technology

Nothing to Report.

7. The award's budget spent in foreign country(ies)

Not applicable.

CHANGES/PROBLEMS

1. Changes in approach and reasons for change

1) Due to the low yield of DNA from swab samples and minimize the PCR biases, whole shotgun metagenome sequencing was employed to determine the structure and composition of the total microbiome, including bacteriome, archaeome, fungiome, and virome.

2) Due to the low yield of DNA, we employed a whole genome amplification method to obtain enough amount of DNA for downstream analyses.

2. Actual or anticipated problems or delays and actions or plans to resolve them

1) Recruiting human subjects is one of the most important aspects in this study. Since the skin microbiota is affected by many host-environmental factors, including temporal and individual variability (i.e. age, gender, race/ethnicity, and season), we recruited subjects aged 18 to 29 to minimize the age factor. However, it's difficult to find subjects within the age range, who belong to one of three categories. Thus, the change in the IRB protocol will be considered to broaden the age range from 18 to 29 to 18 to 39.

2) As expected, DNA quantity extracted from the swab samples were measured very low based on the NanoDrop readings. Thus, we will use PCR amplification of phylogenetic markers, nested PCR, or the whole genome amplification to obtain enough amount of DNA for the next generation sequencing analysis.

3) DNA quantity extracted from the swab samples were very low based on the NanoDrop readings. Thus, we will use PCR amplification of phylogenetic markers or the whole genome amplification to obtain enough amount of DNA for next generation sequencing analysis.

4) A shipping incident caused by USPS: As previously reported, DNA samples were shipped to the Georgia Genomics Facility at the University of Georgia on Feb. 27 for fragment analysis. However, the package was lost by the carrier. This incident caused a delay of the research since we had to recruit more subjects due to the departure of a few subjects previously recruited, collect, and process samples over again.

5) Due to the Covid-19 pandemic, ASU was closed for about three months April through June in 2020. Therefore, sample preparation and processing were delayed by the closing of ASU.

6) Recruiting and collecting samples from human subjects belonging to Category 3 (i.e., working and living off campus) delayed due to the COVID-19 pandemic.

7) There have been supply chain and vendor issues caused by the pandemic.

3. Changes that have a significant impact on expenditures

No Change.

4. Significant changes in use or care of human subjects, vertebrate animals, and/or biohazards

No Change.

5. Change of primary performance site location from that originally proposed

No Change.

BUDGETARY INFORMATION

Please see attached the SF 424 R&R.

REFERENCES

Apprill A, McNally S, Parsons R, Weber L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology, 75(2), 129–137.

Caporaso JG, Lauber CL, Walters WA, Berg-Lyons D, Lozupone CA, Turnbaugh PJ, et al. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 108: 4516–4522.

Castelino M, Eyre S, Upton M, Ho P, Barton A. (2014). The bacterial skin microbiome in psoriatic arthritis, an unexplored link in pathogenesis: challenges and opportunities offered by recent technological advances. Rheumatol 53: 777–784.

De Forest PR. (2001).What is trace evidence? In: Caddy B (ed.). Forensic examination of glass and paint – analysis and interpretation. Taylor & Francis, London, Philadelphia.

Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. (2005). Diversity of the human intestinal microbial flora. Science 308: 1635–1638.

Fierer N, Hamady M, Lauber CL, Knight R. (2008). The influence of sex, handedness, and washing on the diversity of hand surface bacteria. Proc Natl Acad Sci USA 105: 17994–17999.

Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, et al. (2013). Topographic diversity of fungal and bacterial communities in human skin. Nature 498: 367–370.

Foulongne V, Sauvage V, Hebert C, Dereure O, Cheval J, Meriadeg A, et al. (2012). Human skin microbiota: high diversity of DNA viruses identified on the human skin by high throughput sequencing. PLoS one 7(6): e38499.

Franzosa EA, Huang K, Meadow JF, Gevers D, Lemond KP, Bohannan BJM, Huttenhower C. (2015). Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci USA 112: E2930–E2938.

Grice EA, Kong HH, Conlan S, Deming CB, Davis J, Young AC, et al. (2009). Topographical and temporal diversity of the human skin microbiome. Science 324: 1190–1192.

Hannigan GD, Meisel JS, Tyldsley AS, Zheng Q, Hodkinson BP, SanMiguel AJ, et al. (2015). The human skin double-stranded DNA virome: topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome. mBio 6(5).

Hauther KA, Cobaugh KL, Jantz LM, Sparer TE, DeBruyn JM. (2015). Estimating time since death from postmortem human gut microbial communities. J Forensic Sci 60(5): 1234–1240.

Human Microbiome Project Consortium. (2012). Structure, function and diversity of the healthy human micro-biome. Nature 486: 207–214.

Jain A, Hong L, Pankanti S. (2000). Biometrics: promising frontiers for emerging identification market. Comm ACM 91–98

Lax S, Hampton-Marcell JT, Gibbons SM, Colares GB, Smith D, Eisen JA, Gilbert JA. (2015). Forensic analysis of the microbiome of phones and shoes. Microbiome 3: 21. doi:10.1186/s40168-015-0082-9.

Oliveira FS, Brestelli J, Cade S, Zheng J, Iodice J, Fischer S, Aurrecoechea C, Kissinger JC, Brunk BP, Stoeckert CJ Jr, Fernandes GR, Roos DS, Beiting DP. (2018). MicrobiomeDB: a systems biology platform for integrating, mining and analyzing microbiome experiments. Nucleic Acids Res 46(D1):D684-D691.

Parada AE, Needham DM, Fuhrman JA. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ Microbiol 18(5): 1403–1414.

Paulino LC, Tseng CH, Strober BE, Blaser MJ. (2006). Molecular analysis of fungal microbiota in samples from healthy human skin and psoriatic lesions. J Clin Microbiol 44: 2933–2941.

Ravel J, Gajer P, Abdo Z, Schneider GM, Koenig SS, McCulle SL, et al. (2011). Vaginal microbiome of reproductive-age women. Proc Natl Acad Sci USA 108(Suppl. 1): 4680–4687.

Ricci C, Bleay S, Kazarian SG. (2007). Spectroscopic imaging of latent fingermarks collected with the aid of a gelatin tape, Anal Chem 79:5771–5776.

Roux C, Beavis A, Benson S, Braybon E, Dawson M, Doble P, et al. (2014). Forensic science in the 21st century – will trace evidence ever reach the next level? (http://projects.nfstc.org/trace/docs/final/Roux_21st_century.pdf).

Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. (2007). The human microbiome project. Nature 449: 804–810.

Wang Q, GM Garrity, JM Tiedje, JR Cole. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 3: 5261–5267.

Weber N, Liou D, Dommer J, MacMenamin P, Quiñones M, Misner I, Oler AJ, Wan J, Kim L, McCarthy MC, Ezeji S, Noble K, Hurt DE. (2018). Nephele: a cloud platform for simplified, standardized and reproducible microbiome data analysis, Bioinformatics 34(8): 1411–1413.

Whitman WB, Coleman DC, Wiebe WJ. (1998). Prokaryotes: the unseen majority. Proc Natl Acad Sci USA 95: 6578–6583.

Wood I, Park S, Tooke J, Smith O, Morgan RM, Meakin GE. (2017). Efficiencies of recovery and extraction of trace DNA from non-porous surfaces. Forensic Science International: Genetics, 6, e153–e155.

Standard Operating Procedure 1. Traditional method of lifting fingerprint

1. Place the object on a flat surface in way so that the suspected print is easily accessible
2. Choose the dusting powder (black or gold or magnetic) based on the which powder will give best contrast to the color of the surface
3. Transfer a small amount of the powder to the lid of the box which contains the powder
4. Dip the bristles of the brush to the powder in the lid so that a small amount retain on the tip of the bristles
5. Sweep the brush circularly and twirling motion over the suspected area
6. Continue depositing powder until the latent print begins to appear, when the ridges begin to appear, change the direction of motion of the brush to follow the direction of the ridges
7. When the print is developed fully, stop brushing
8. Lift the print using an adhesive fingerprint tape and place it on a white paper

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Standard Operating Procedure 2. DNA Extraction from Cotton Swabs Using DNeasy Blood & Tissue Kit

1. Break off the cotton tip of a swab into a tube containing 1 ml of **PBS (pH 7.2; 50 mM potassium phosphate, 150 mM NaCl)**.
2. Vortex at maximum speed for 1 minute.
3. Centrifuge tubes at 4,000 × g for 15 minutes at 4°C.
4. Resuspend the pellet in 180 µl enzymatic lysis buffer. **Enzymatic lysis buffer:** 20 mM Tris·Cl (pH 8), 0 2 mM sodium EDTA, 1.2% Triton® x-100. Immediately before use, add lysozyme to 20 mg/ml.

※ For Gram negative bacteria, resuspend pellet in 180 µl Buffer ATL. Add 20 µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the tissue is completely lysed (the mixture inside the tube should be clear) (for 30 minutes to 1 hour). Vortex occasionally during incubation to disperse the sample. Vortex for 15 s. Add 200 µl Buffer AL to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing. Then proceed to **Step 10**.

5. Incubate for at least 30 minutes at 37°C. After incubation, heat the heating block to 56°C.
6. Add 25 µl proteinase K and 200 µl buffer AL (without ethanol). **Note:** do not add proteinase K directly to Buffer AL.
7. Mix by vortexing.
8. Incubate at 56°C for 30 minutes.
9. Add 200 µl ethanol (96-100%) to the sample, and mix thoroughly by vortexing.
10. Pipet the mixture from step 9 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at 6000 × g (8000 rpm) for 1 minute. Discard flow-through and collection tube.
11. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1, and centrifuge for 1 minute at 6000 × g (8000 rpm). Discard flow-through and collection tube.
12. Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2, and centrifuge for 3 minutes at 20,000 × g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube.

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13. Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 100 µl Buffer AE directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 minute at 6000 × g (8000 rpm) to elute.
14. For maximum DNA yield, add 100 µl of the flow through to the center of the DNeasy membrane. Allow the filter to set for 1 minute.
15. Centrifuge at room temperature for 1 minute at 6000 × g (8000 rpm) to elute again.
16. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application.
17. Measure DNA quantity and quality using a Nano-drop spectrophotometer (ND-1000).
18. Store DNA frozen (-20 to -80°C) until processed.

Standard Operating Procedure 3. DNA Extraction from Cotton Swabs Using DNeasy PowerSoil Kit

1. Add 60 µl of Solution C1 to a bead tube.

CAUTION: Check Solution C1 that contains SDS. If Solution C1 is precipitated, heat solution to 60°C until dissolved before use.

2. Break off the cotton tip of a swab into a bead tube containing 60 µl of Solution C1.
3. Incubate the tube at 65°C for 10 minutes.
4. Secure PowerBead Tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape.
5. Vortex at maximum speed for 10 minutes.
6. Centrifuge tubes at 10,000 × g for 30 seconds at room temperature. Make sure the PowerBead Tubes rotate freely in your centrifuge without rubbing.

CAUTION: Be sure not to exceed 10,000 × g or tubes may break.

7. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
8. Add 250 µl of Solution C2 and vortex for 5 seconds. Incubate at 4°C for 10 minutes.
9. Centrifuge the tubes at room temperature for 1 minute at 10,000 × g.
10. Avoid disturbing the pellet, transfer 400-450 (up to, but no more than, 600) µl of supernatant to a clean 2 ml Collection Tube (provided).
11. Add 200 µl of Solution C3 and vortex briefly. Incubate at 4°C for 5 minutes.
12. Centrifuge the tubes at room temperature for 1 minute at 10,000 × g.
13. Avoiding the pellet, transfer 600 (up to, but no more than, 750) µl of supernatant into a clean 2 ml Collection Tube (provided).
14. Shake to mix Solution C4 before use. Add 1,200 µl of Solution C4 to the supernatant and vortex for 5 seconds.
15. Load approximately 675 µl onto a Spin Filter and centrifuge at 10,000 × g for 1 minute at room temperature.
16. Discard the flow through and add an additional 675 µl of supernatant to the Spin Filter and centrifuge at 10,000 × g for 1 minute at room temperature.

17. Load the remaining supernatant onto the Spin Filter and centrifuge at 10,000 × g for 1 minute at room temperature.
18. Add 300 µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 × g. Add additional 300 µl of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 × g.
19. Discard the flow through.
20. Centrifuge again at room temperature for 1 minute at 10,000 × g.
21. Carefully place spin filter in a clean 2 ml Collection Tube (provided). Avoid splashing any Solution C5 onto the Spin Filter.
22. Add 50 µl of Solution C6 to the center of the white filter membrane. Allow the filter to set for 1 minute.

Alternatively, sterile DNA-Free PCR Grade Water may be used for elution from the silica Spin Filter membrane at this step.

23. Centrifuge at room temperature for 30 seconds at 10,000 × g.
24. Add 50 µl of the flow through to the center of the white filter membrane. Allow the filter to set for 1 minute.
25. Centrifuge at room temperature for 30 seconds at 10,000 × g.
26. Discard the Spin Filter. The DNA in the tube is now ready for any downstream application.
27. Measure DNA quantity and quality using a Nano-drop spectrophotometer (ND-1000).
28. Store DNA frozen (-20° to -80°C; Solution C6 contains no EDTA) until processed.

Standard Operating Procedure 4. DNA Extraction from Cotton Swabs Using MP Biomedicals' FastDNA Spin Kit for Soil

1. Break off the cotton tip of a swab into a Lysing Matrix E tube.
2. Add 978 µl of Sodium Phosphate Buffer to a Lysing Matrix E tube.
3. Add 122 µl of MT buffer.
4. Secure Lysing Matrix E tubes horizontally using the tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape.
5. Vortex at maximum speed for 10 minutes.
6. Centrifuge tubes at 14,000 × g for 5-10 minutes to pellet debris.
7. Transfer the supernatant to a clean 2 ml microcentrifuge tube.
8. Add 250 µl of PPS (Protein Precipitation Solution) and mix by inverting the tubes 10 times.
9. Centrifuge the tubes at 14,000 × g for 5 minute.
10. Transfer supernatant to a clean 15 ml tube. **Note:** Better mixing and DNA binding will occur in a larger tube.
11. Resuspend Binding Matrix suspension and add 1 ml to supernatant in 15 ml tube.
12. Invert by hand for 2 minutes to allow binding of DNA.
13. Place the tube in a rack for 3 minutes to allow settling of silica matrix.
14. Remove and discard 500 µl of supernatant being careful to avoid settled Binding Matrix.
15. Gently resuspend Binding Matrix in the remaining amount of supernatant.
16. Transfer approximately 600 µl of the mixture to a Spin Filter and centrifuge at 14,000 × g for 1 minute.
17. Empty the catch tube and add the remaining mixture to the Spin Filter and centrifuge as before. Empty the catch tube.
18. Add 500 µl prepared SEWS-M and gently resuspend the pellet by pipetting. **Note:** Add ethanol to the concentrated SEWS-M before this procedure.
19. Centrifuge at 14,000 × g for 1 minute. Empty the catch tube and replace.
20. Centrifuge at 14,000 × g for additional 2 minute to completely remove any residual wash solution.

21. Discard the catch tube and replace with a new, clean catch tube.
22. Air dry the Spin Filter for 5 minutes at room temperature.
23. Gently resuspend Binding Matrix (above the Spin Filter) in 50 µl of DES (dnASE/Pyrogen-Free Water).
24. (Optional) Incubate the tube for 5 minutes at 55°C in a heat block to increase DNA yields.
25. Centrifuge at 14,000 × g for 1 minute.
26. Discard the Spin Filter. The DNA in the tube is now ready for any downstream applications.
27. Measure DNA quantity and quality using a Nano-drop spectrophotometer (ND-1000).
28. Store DNA at -20°C until processed.