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Low-DNA-Input Shotgun Metagenomic Sequencing and Its Application to Skin Microbiome Characterization

Introduction

Low-DNA-input shotgun metagenomic sequencing focuses on analyzing genetic material from microbial communities in samples with limited DNA, a challenge commonly encountered in clinical and environmental settings where biomass is scarce. Commercial shotgun library prep kits, such as the Illumina DNA Prep Kit and NEBNext® Ultra™ DNA Library Prep Kit, are designed to streamline workflows but typically require more than 1 ng of DNA input. To address this challenge, researchers often must collect samples from multiple sites then combine the extracted DNA to bring up the concentration, resulting in complication in sample collection and "un-realistic" microbial presentation. Others opt for whole genome amplification (WGA), such as Multiple Displacement Amplification, to increase DNA quantity before shotgun library preparation. However, WGA can introduce bias by unevenly amplifying DNA fragments, leading to significant distortions in microbial community structure [1]. For example, *Enterobacteriaceae*, a key indicator of fecal contamination in water, has been unpredictably affected by WGA [2]. Additionally, WGA kits may introduce contaminating DNA, which is particularly problematic in diagnostic settings [3]. In this study, we present a low-DNA-input shotgun metagenomic sequencing workflow provided by Zymo Research's Microbiome Sequencing Service. This workflow allows for the successful analysis of samples with DNA inputs as low as 100 femtograms with minimal bias, as demonstrated using ZymoBIOMICS microbiome standards. When applied to skin samples, this workflow enabled us to analyze the distribution of *Cutibacterium acnes* at the strain level and capture the abundance of both bacteria and fungi in a single measurement, thus paving the way for comprehensive characterization of the skin microbiome even with limited DNA input.

Results

Firstly, we applied our new workflow to sequence our Microbial Community DNA Standard across a range of biomass inputs, from 10 nanograms down to 100 femtograms.

We observed that the microbial composition profiles closely matched the theoretical composition profile at all input levels, including the lowest input of 100 femtograms (Figure 1). This consistency shows that our workflow introduces minimal bias to microbial composition profiles, making it highly suitable for characterizing samples with low biomass inputs.

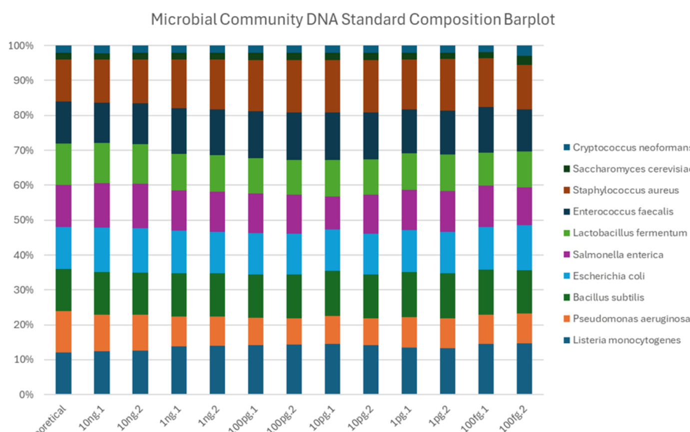

Figure 1. Species composition of Microbial Community DNA Standard at varying DNA input for our shotgun metagenomic workflow. The theoretical composition of the standard is shown on the leftmost side. Two replicates were used for each biomass input amount.

Next, we applied our workflow to human skin samples, which present a particular challenge for shotgun metagenomic sequencing due to their low microbial biomass [4]. Despite this challenge, we successfully characterized the microbial profiles of these skin samples (Figure 2), identifying various bacterial species, including *Cutibacterium acnes*, one of the most abundant bacteria on human skin and a key contributor to skin health [5]. Additionally, we were able to identify fungal species, such as *Malassezia restricta* (Figure 2).

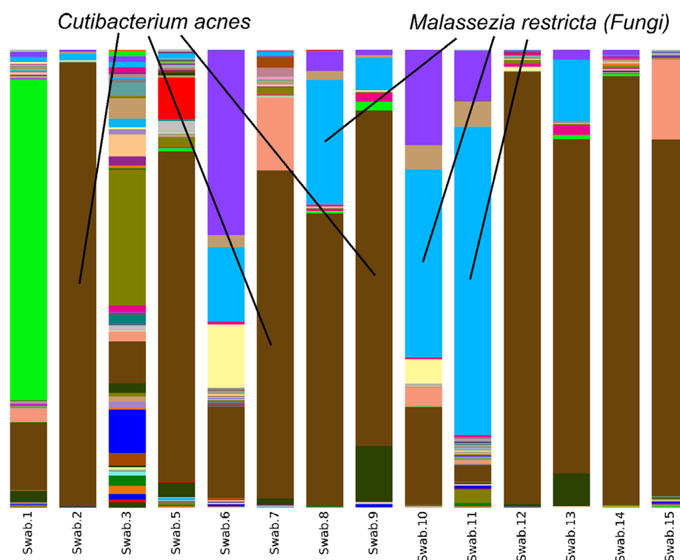

Figure 2. Species composition of skin samples analyzed with our shotgun metagenomic workflow.

Both bacteria and fungi play essential roles in the skin microbiome. While targeted amplicon sequencing would require separate 16S and ITS sequencing runs to capture both bacterial and fungal components, our shotgun sequencing approach enables us to capture and compare both in a single measurement, providing insights into their relative abundances. The full sample report of this skin microbiome study is available here.

When examining the read coverage of the *C. acnes* genome, we observed an even distribution of reads across the entire genome using our workflow (Figure 3A and 3B). This contrasts with the uneven coverage produced by a shotgun sequencing workflow that uses WGA to boost DNA inputs on the same sample (Figure 3C and 3D), highlighting that our workflow avoids the bias typically introduced by WGA and provides more accurate genomic coverage.

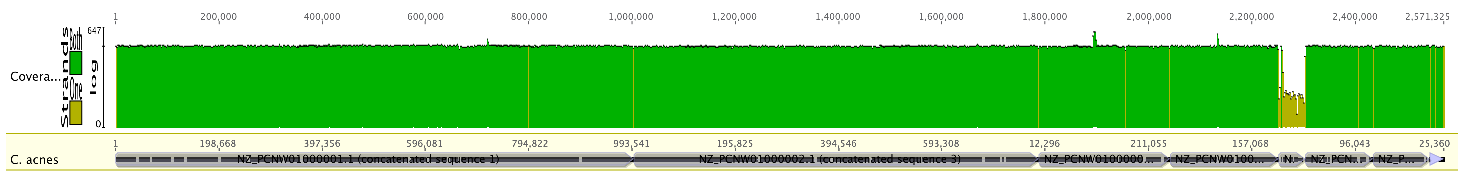

Figure 3A. Overall read coverage from a sample processed using our workflow.

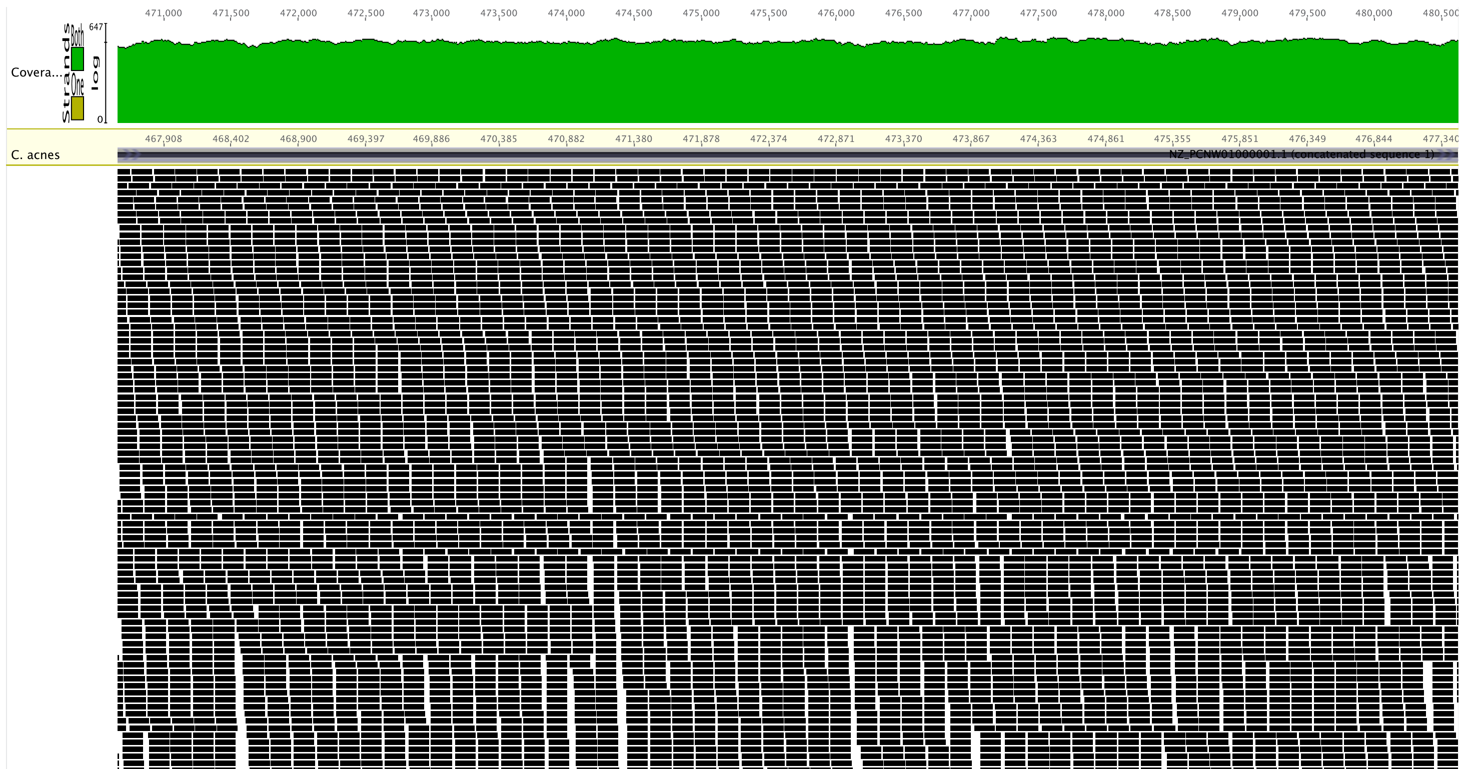

Figure 3B. Zoomed-in view of read coverage from a sample processed using our workflow.

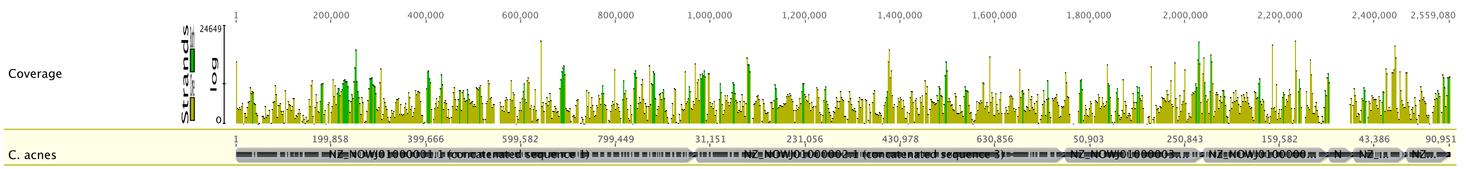

Figure 3C. Overall read coverage from a sample processed using whole genome amplification.

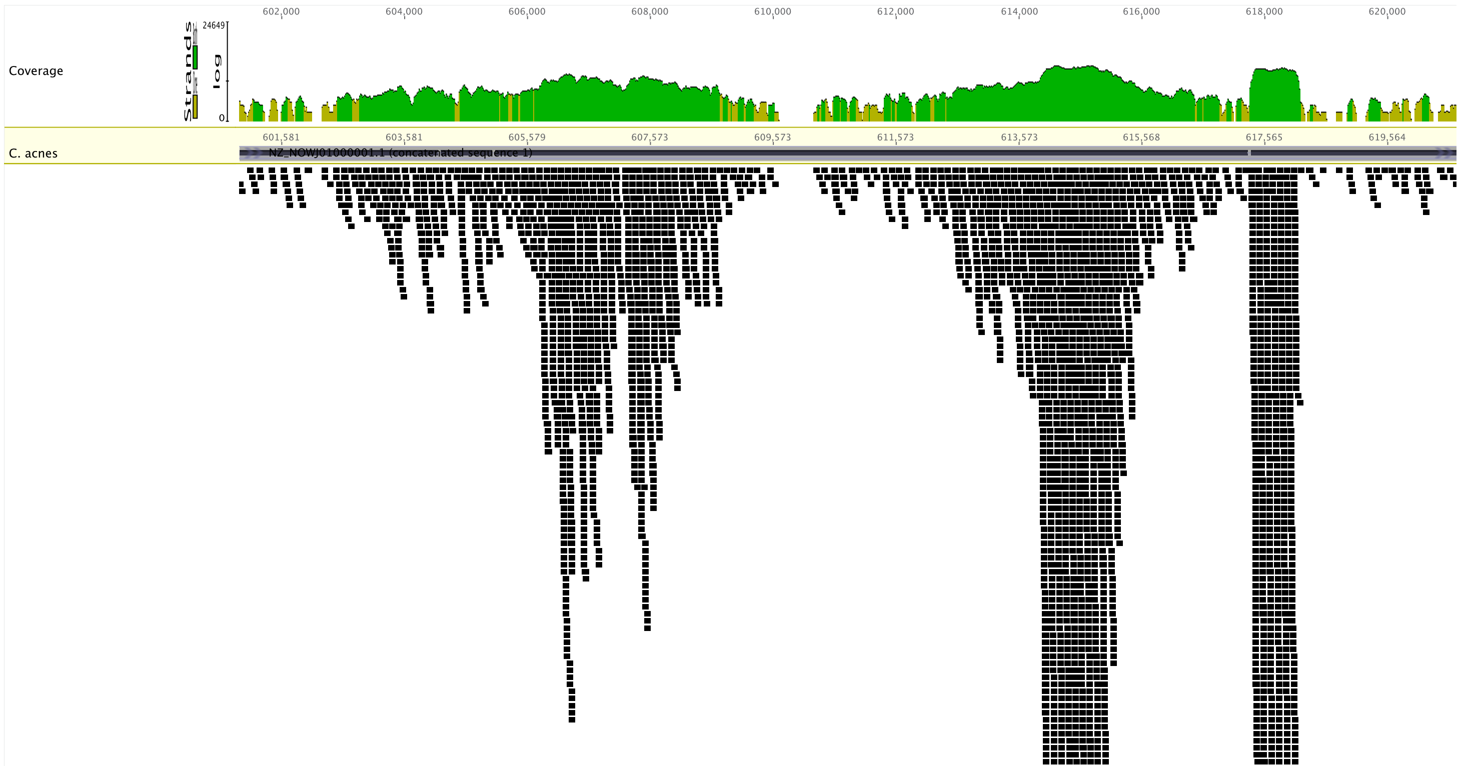

Figure 3D. Zoomed-in view of read coverage from a sample processed using whole genome amplification.

Furthermore, we examined the strain-level differences of *C. acnes* in the skin samples with StrainScan [6]. Among the 15 skin samples included in this analysis, we identified 16 distinct *C. acnes* strains. One strain, represented by the genome of GCA_003384705, was the most commonly detected and dominant across the samples. While some samples were dominated by a single strain, others exhibited multiple strains (Figure 4), highlighting the presence of *C. acnes* strain-level variation among individuals. Different microbial strains within the same species can have varying metabolic capabilities, which may influence health maintenance, disease risk, progression, and individual responses to diet and medications [7]. Therefore, exploring strain-level differences is crucial for understanding their biological significance. Our workflow offers a simple and effective method for conducting such strain-level analyses using a shotgun metagenomic approach, even with low biomass input.

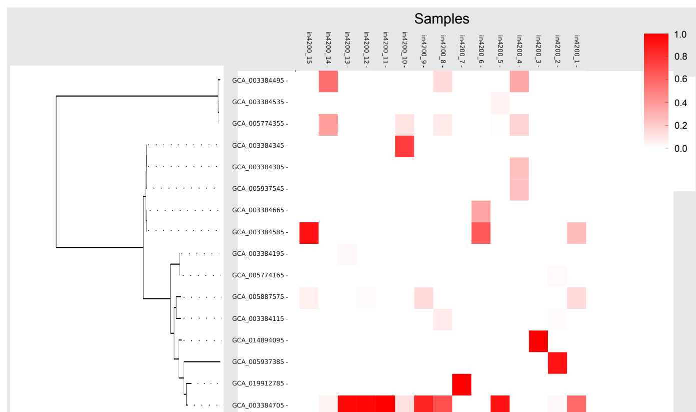

Figure 4. Strain-level identification of C. acnes in skin samples. Each column represents a sample, and each row represents a strain. The phylogeny of C. acnes genomes/strains is shown on the left side.

Methods

Skin samples were collected from 15 individuals, and DNA was extracted using the ZymoBIOMICS DNA Miniprep Kit (Cat No. D4300). Skin samples were collected by pressing a sterile collection swab against the forehead and rotating the swab for a total of 10 full rotations. For preservation, the swab tip was placed in a sterile 2 mL tube filled with 1.5 mL of DNA/RNA Shield (Cat No. R1100). For the DNA extraction, 1 mL of the DNA/RNA Shield including the swab tip was transferred into the lysis tube and bead beat at max speed for 40 minutes using a Vortex Genie with a Microtube Adaptor. DNA was extracted following the DNA Miniprep Kit protocol omitting the HRC filter step. Extracted DNA was eluted in 50 µl of DNase/RNase free water and stored at -20°C.

To assess the limit of detection of the modified Illumina DNA Prep protocol, 1:10 serial dilutions of ZymoBIOMICS Microbial Community DNA Standard (Cat No. D6306) was used as input for library pep.

To assess the bias and practicality of WGA in a low-biomass shotgun library prep workflow, a subset of skin samples was amplified using the REPLI-g kit (Qiagen) prior to library preparation.

Libraries were generated using the Illumina DNA Prep Kit (Illumina) with modifications. The quality of the libraries was assessed using the Qubit HS dsDNA assay (Invitrogen) and TapeStation (Agilent). For sequencing, the final library was diluted and denatured following the manufacturer's instructions and sequenced on an Illumina NovaSeq X instrument generating 2x150 bp paired-end reads.

Bacterial species-level identification was performed using an in-house pipeline offered by Zymo Research Microbiome Sequencing Service. This pipeline was built upon the interpretation of taxa identified using Sourmash [8]. Strain-level identification was performed using StrainScan [6] and our in-house *C. acnes* reference database curated from publicly available genomes.

References

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