Exploring Microbial Life with Minimal DNA input: Shotgun Metagenomics at Ultra-Low Biomass

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Commercialization Manager

PLANETARY PROTECTION METAGENOMICS IN SPACEFLIGHT WORKSHOP
November 21st, 2024

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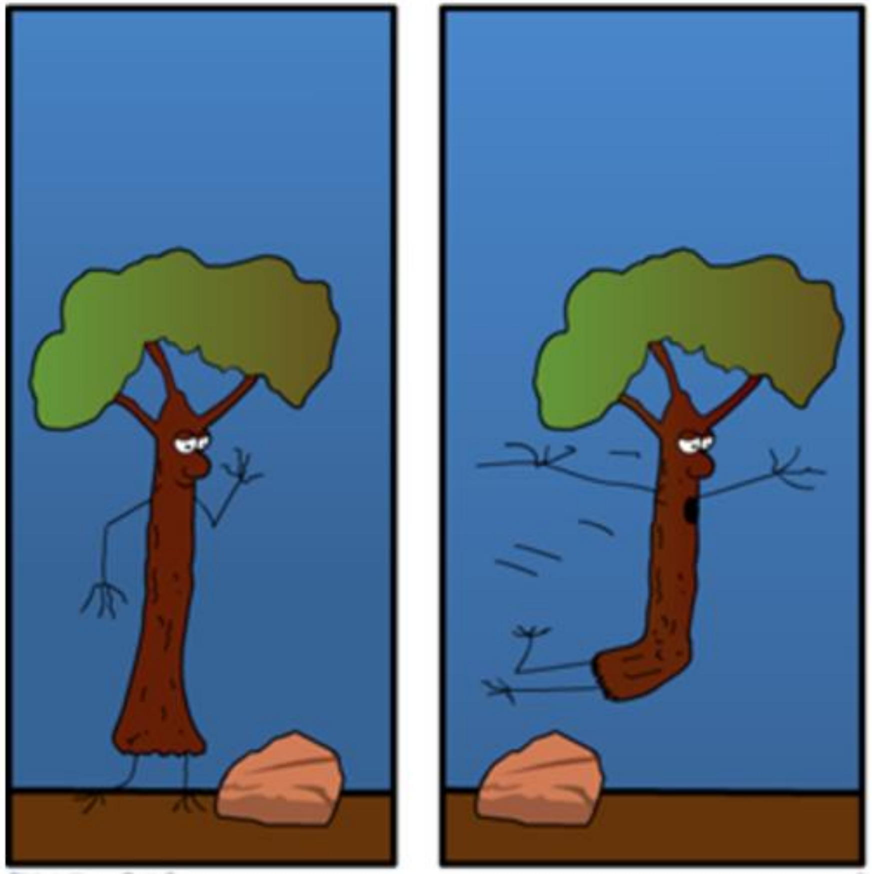
If a tree falls in a forest & no one is around to hear it, does it make a sound?



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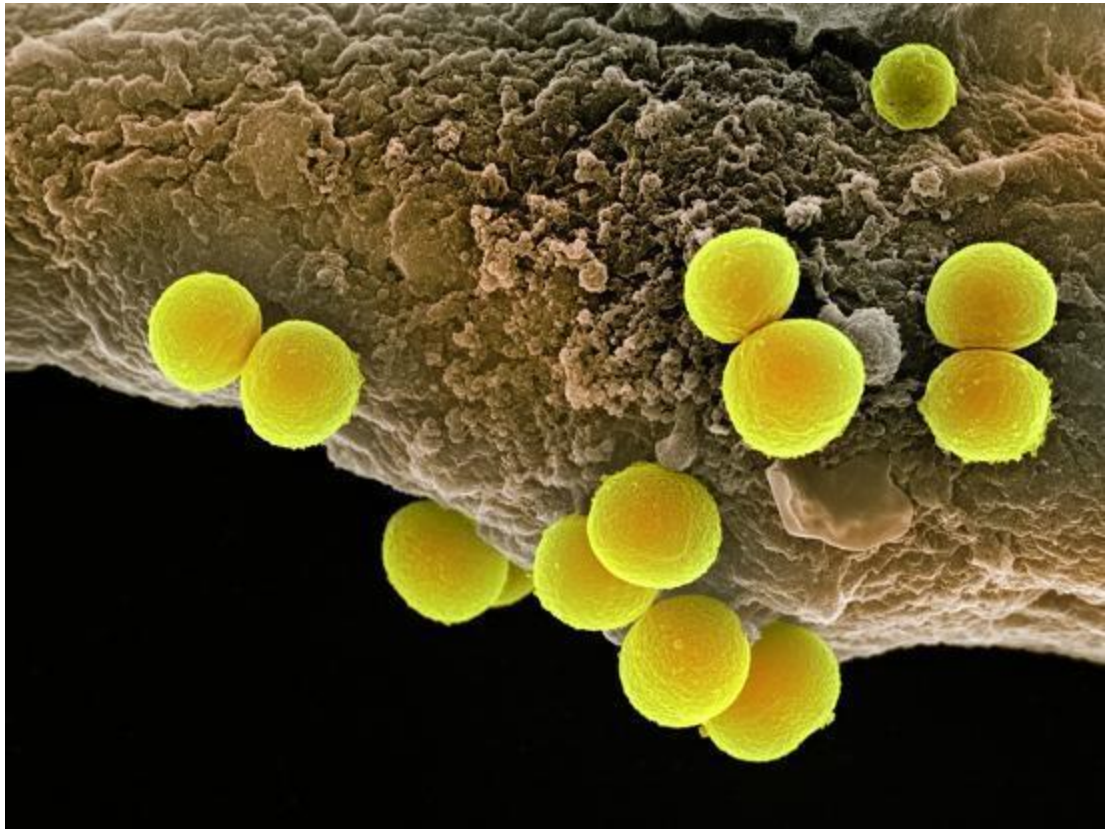
If microbes are too sparse to detect, are they truly there?



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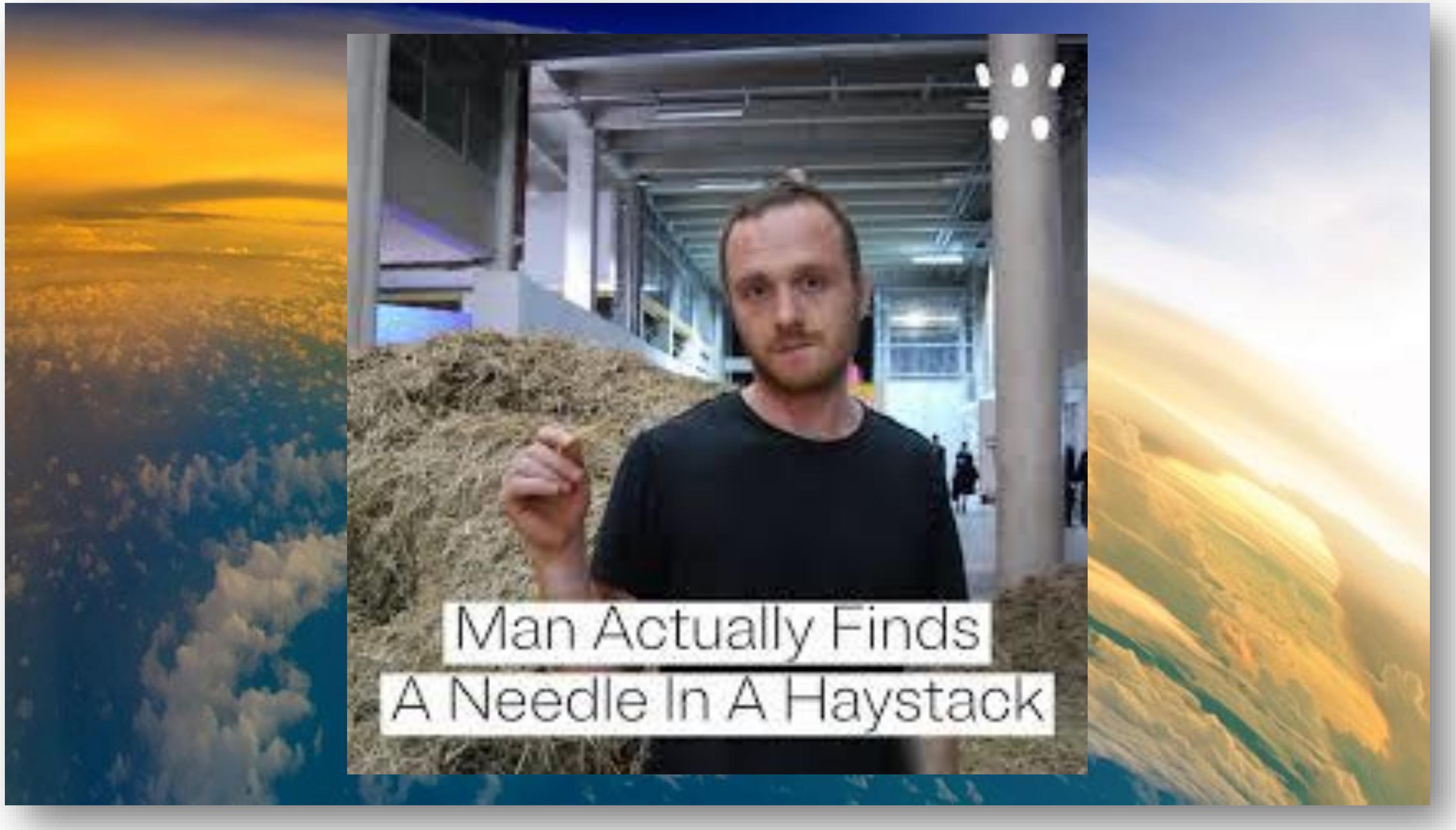
How do you find the needle when the haystack is invisible?



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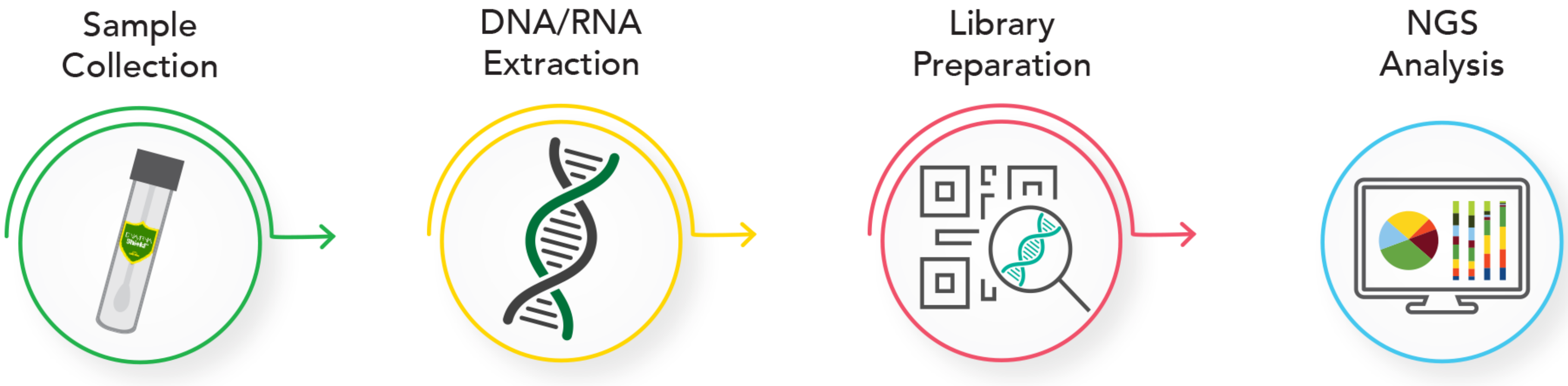
Design an experiment to show that a sample is sterile…



Sample Collection

DNA/RNA Extraction

Library Preparation

NGS Analysis

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Design an experiment to show that a sample is sterile…

Sample Collection

Collect swab sample

Microbiome Standards

Perform serial dilutions on ZymoBIOMICS Spike-in Control II log-distributed cells (10^3 to 10^5 cells)

Spike desired dilution at a consistent concentration into collected samples.

DNA/RNA Extraction

Perform mechanical (unbiased) lysis

Library Preparation

Desired library prep & sequencing to detect microbial composition & confirm presence/absence of spiked-in control.

NGS Analysis

Evaluate if the spike-in microbes are detectable to verify the workflow sensitivity.

Determine microbial load levels & check for potential contamination.

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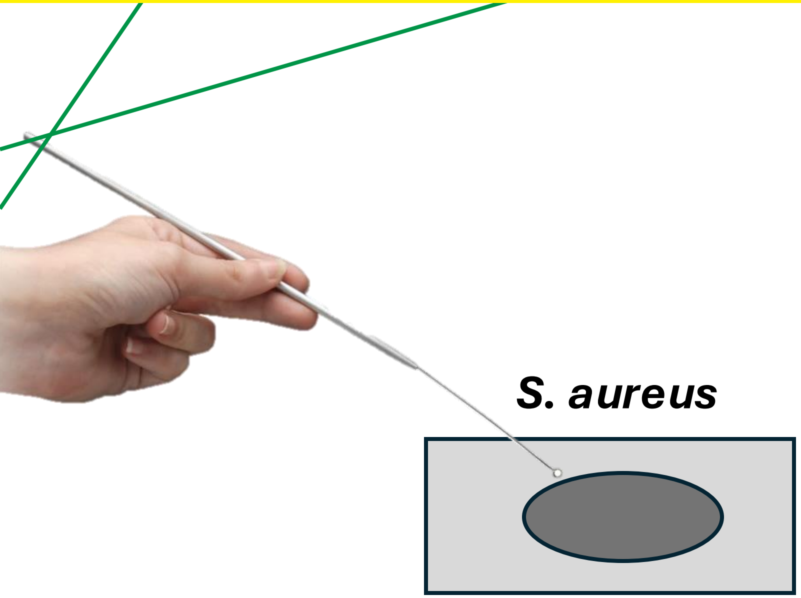
RNA Extraction Optimization for Space Flight

- Dr. Camillia Urbaniak from NASA-JPL (Pasadena) approached us with the problem of separating a *Staphylococcus aureus* biofilm from a plastic coupon and recovering RNA from the biofilm to assess affects of gravity on *S. aureus* growth…

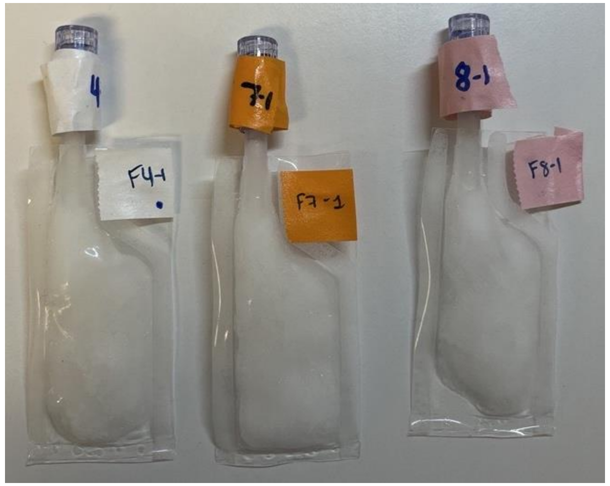
Experimental Design

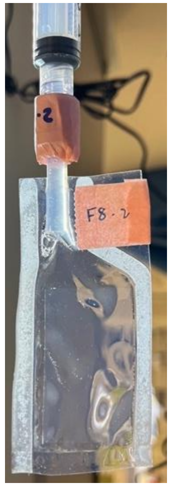


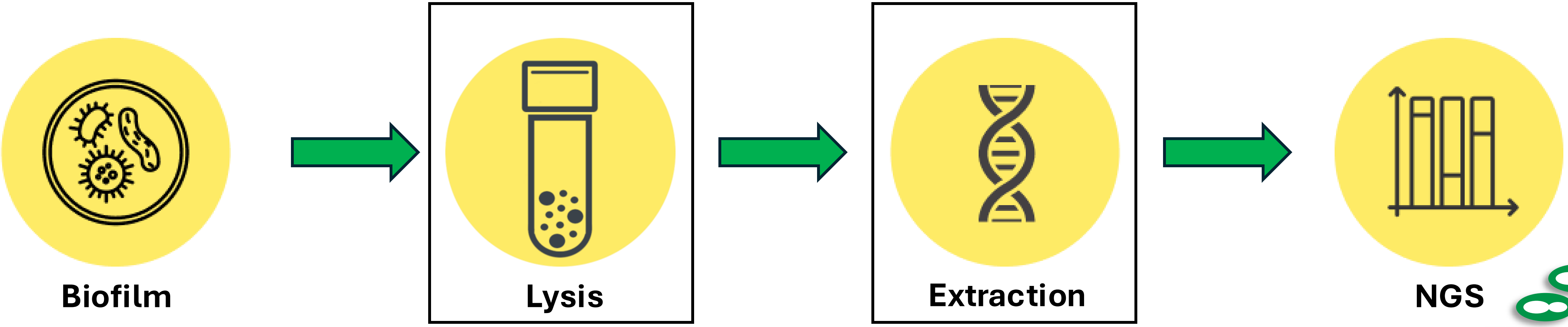
S. aureus

52
Hours

+ DNA/RNA
Shield







Biofilm

Lysis

Extraction

NGS

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Optimizing RNA Extraction

RNA Quantification Before Optimization (NASA JPL)

Nanodrop (ng/ul)	A260/280
4.4	2.2
7.4	1.97
5	1.85
Average 5.6 ng/ul	

RNA Quantification After Optimization (Zymo Research)

Nanodrop (ng/ul)	A260/280	A260/230
137.5	2.13	2.06
140.5	2.12	2.3
183.7	2.13	2.37
Average = 153.9 ng/ul		

- Focusing on optimizing lysis and purification of RNA allowed us to increase yields by over 2000% (27.5-fold)!
- Multiple flight verifications have already taken place, and samples will be placed on a space flight in 2025!
- More results to follow!

Research conducted by Clay Villars (Zymo Research), Brett Farthing (Zymo Research), Dr. Camilla Urbaniak (JPL)

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Commonly Studied Low-Biomass Environments

- Human skin (specific areas)
- Clean rooms & controlled laboratory environments
- Spacecraft & space station surfaces
- Polar & permafrost soils
- Deserts & arid environments
- Deep-ocean & subsurface sediments
- Atmosphere & high-altitude environments
- Glacial & ice sheet ecosystems
- Oligotrophic lakes & deep-sea brines
- Hydraulic fracturing fluids & oil reservoirs
- Hospital & sterile surgical environments
- Deep groundwater aquifers
- Mars analog sites

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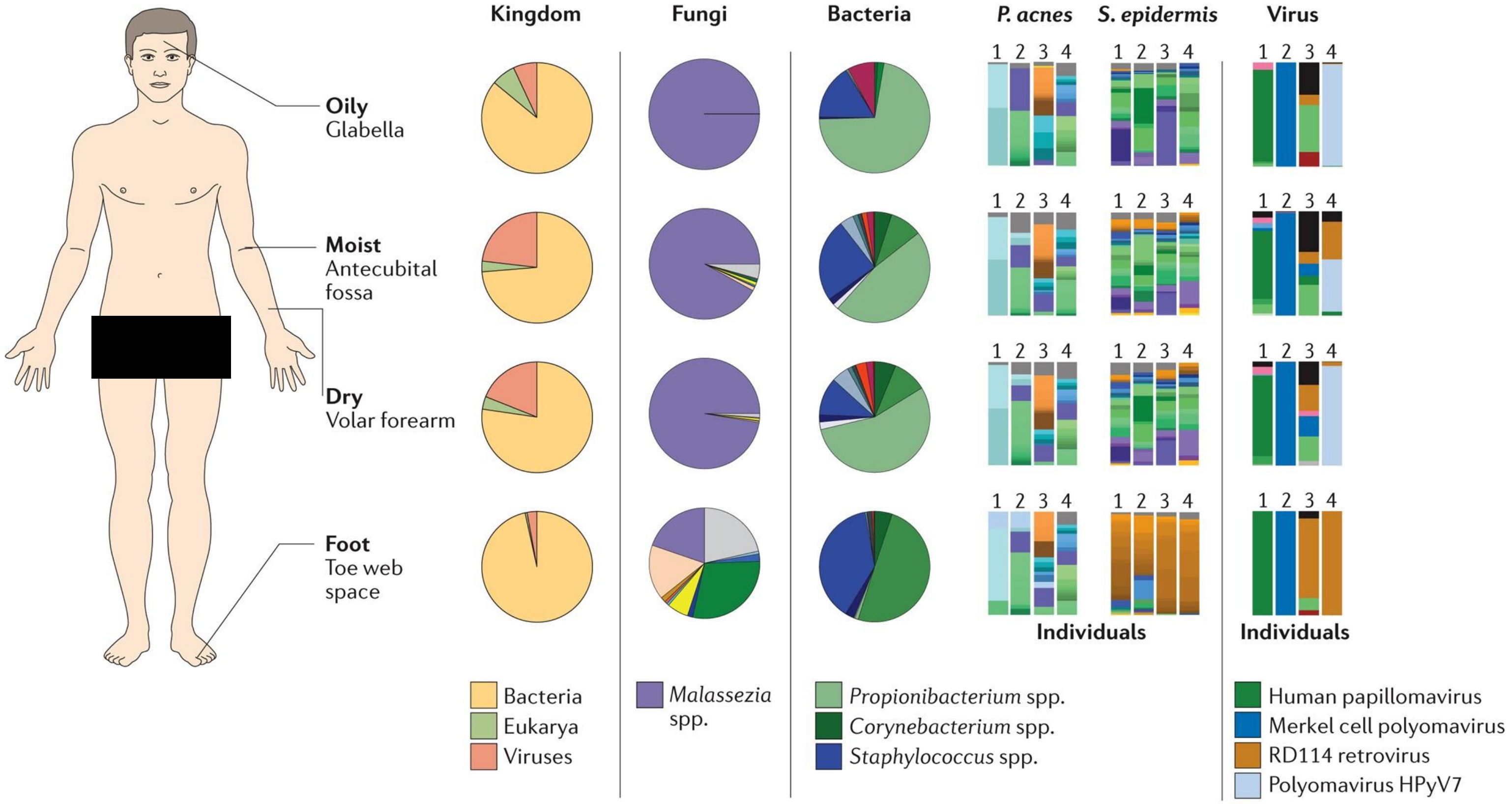
Skin Microbiome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Nature Reviews | Microbiology

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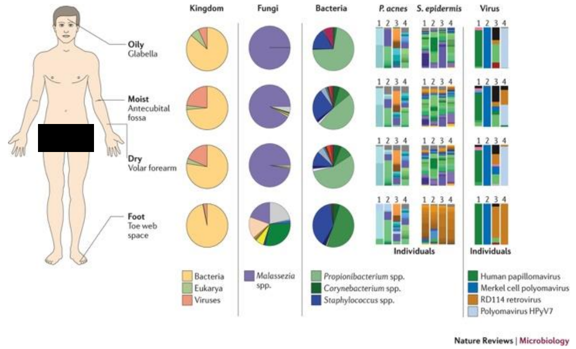
Microbiome Analysis Approaches: Pros & Cons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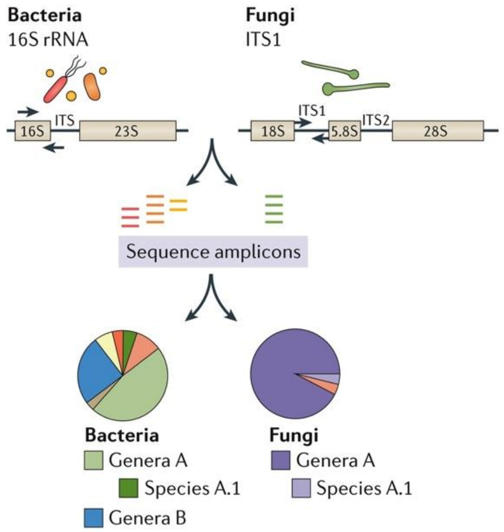
Pro:

- Target specific.
- Minimal input.

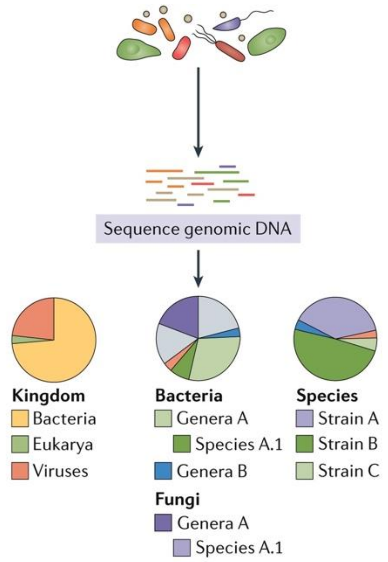
Cons:

- Miss out information (strain, functional, AMR)
- Can’t compare cross-kingdom abundance.

16S/ITS Amplicon



Shotgun Metagenomics



Pro:

- Multi-Kingdom Strain ID.
- Functional pathway information.
- Virulence factor/ AMR.

Cons:

- Need substantial DNA input.

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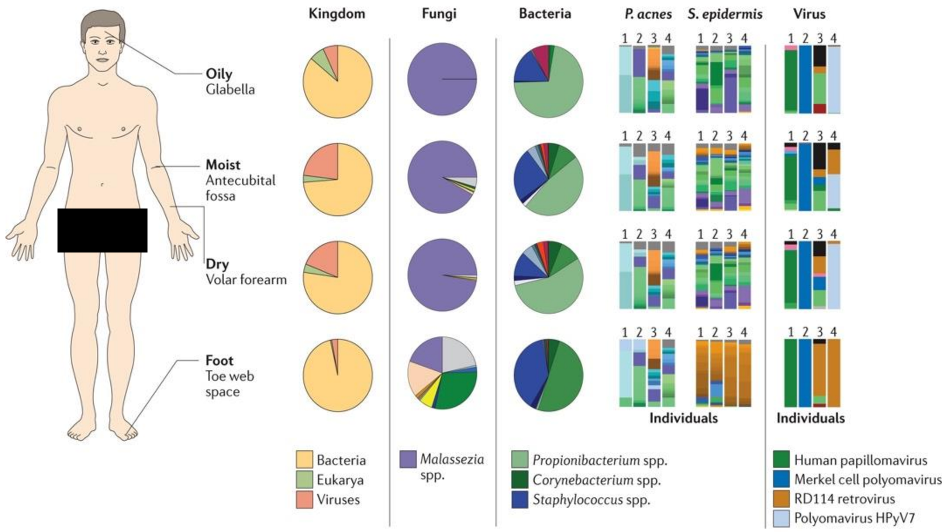
Challenges of Low-biomass Samples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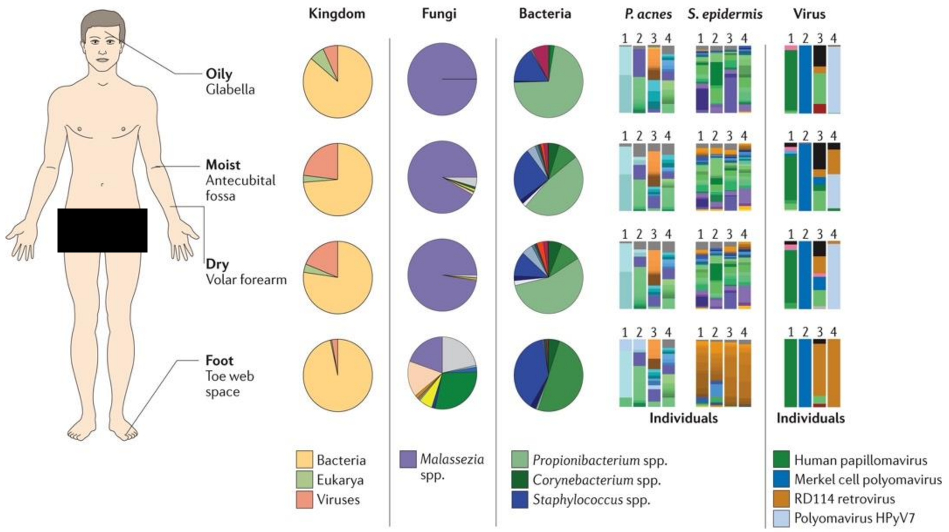
Challenges of Low-biomass Samples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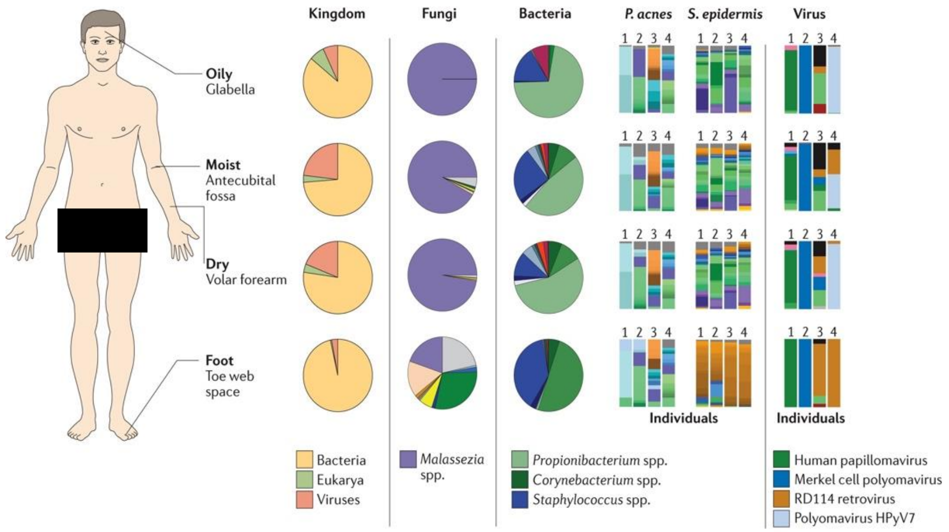
Incompatible with Shotgun Metagenomic Sequencing

- Miss out important information
- Miss out holistic view of entire microbiome profile
- Introduce potential bias/contamination

Challenges of Low-biomass Samples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Incompatible with Shotgun Metagenomic Sequencing

Perform multiple collections

- Increase cost
- Complicated logistic
- Introduce potential bias/contamination

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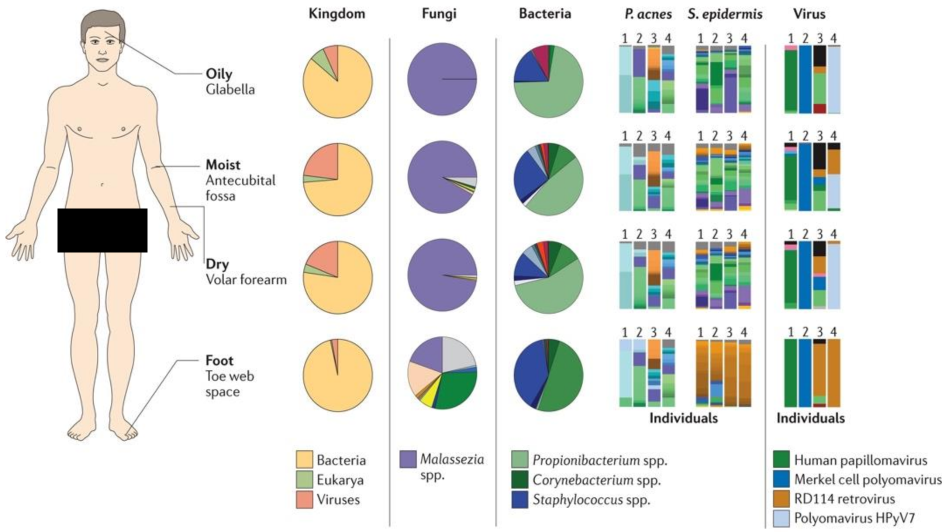
Challenges of Low-biomass Samples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Incompatible with Shotgun Metagenomic Sequencing

Multiple collections

Whole Genome Amplification

- Significant increase cost
- Introduce potential bias/contamination

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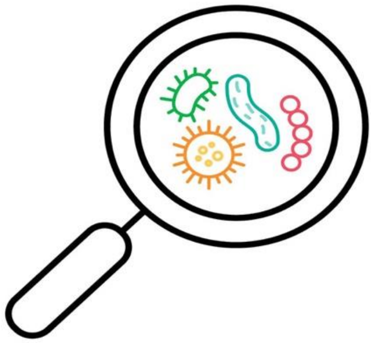
Zymo Research's
Microbiome Sequencing Services



Shotgun Metagenomics Sequencing Service

Compatible with Ultra-low DNA input

Only 100 femtograms!

100 femtograms (fg) is 1×10^{-13}grams

It is 10^{-4}parts of a nanogram

= divide a gram into a trillion parts, then take one of those parts and divide it into ten thousand—one of those pieces is 100 femtograms.

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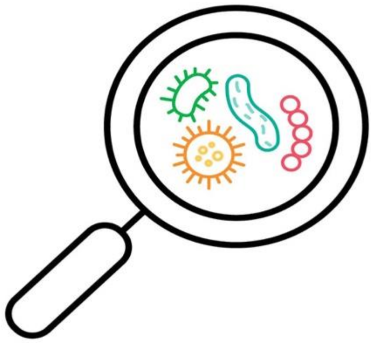
t is 10−410^{-4}10−4 parts of a nanogram

Zymo Research's
Microbiome Sequencing Services



Shotgun Metagenomics Sequencing Service

- Compatible with Ultra-low DNA input
- Strain-level identification
- Functional Genes Prediction
- Antibiotic Resistance Detection
- Cost effective

Only 100 femtograms!

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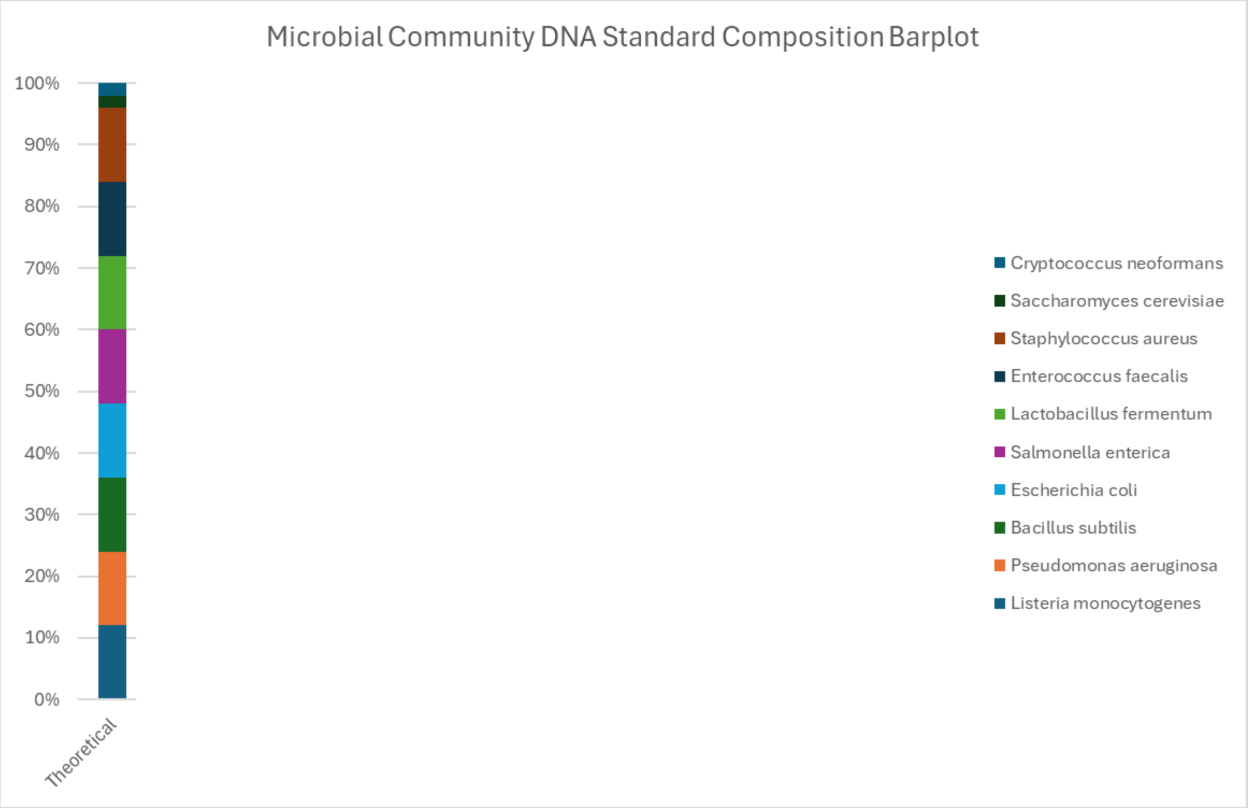
Consistent Microbial Profiling Across Different Input Levels



Benchmark with

ZymoBIOMICS Microbial Community Standard (D6306)

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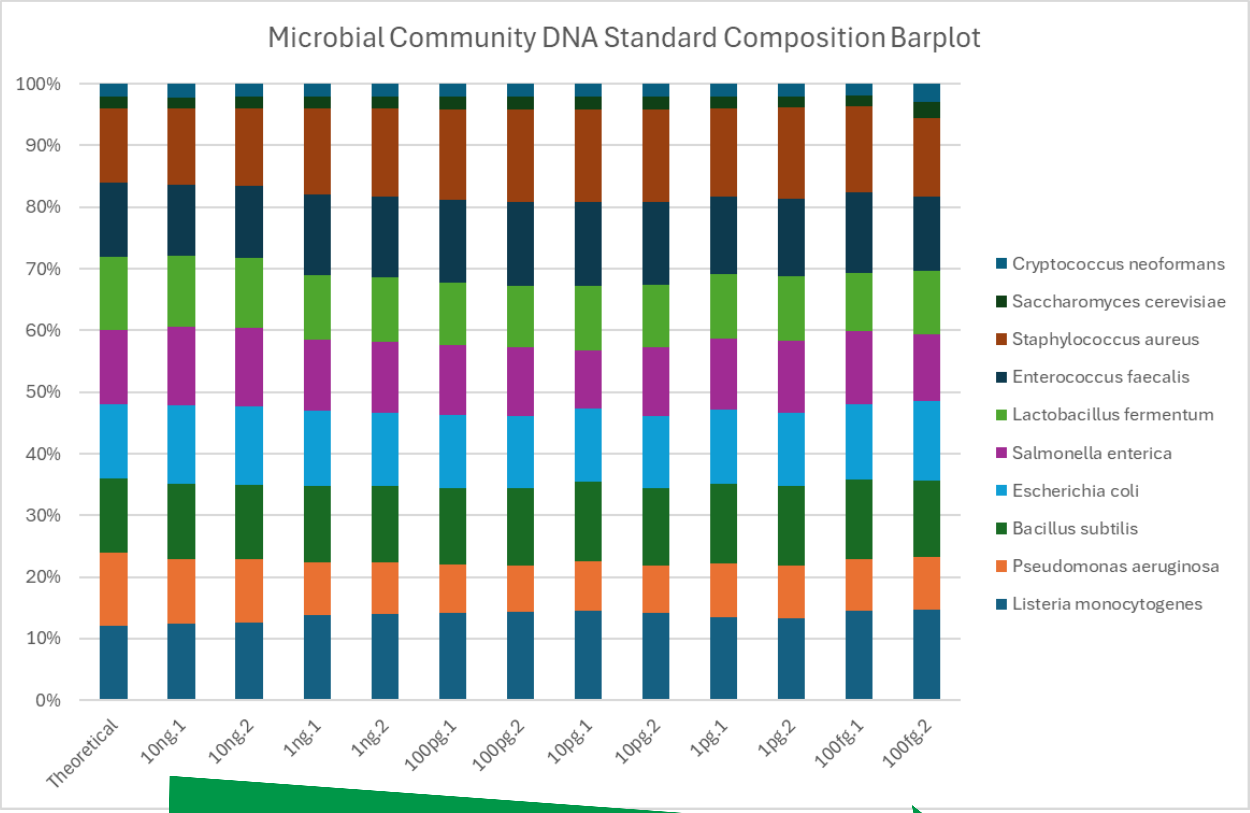
Consistent Microbial Profiling Across Different Input Levels



DNA Input concentration

Benchmark with
ZymoBIOMICS Microbial Community Standard (D6306)

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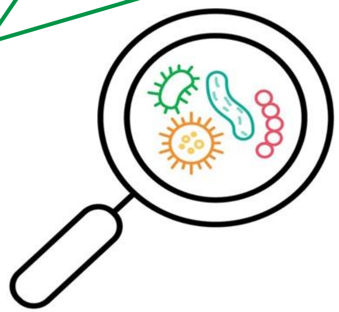
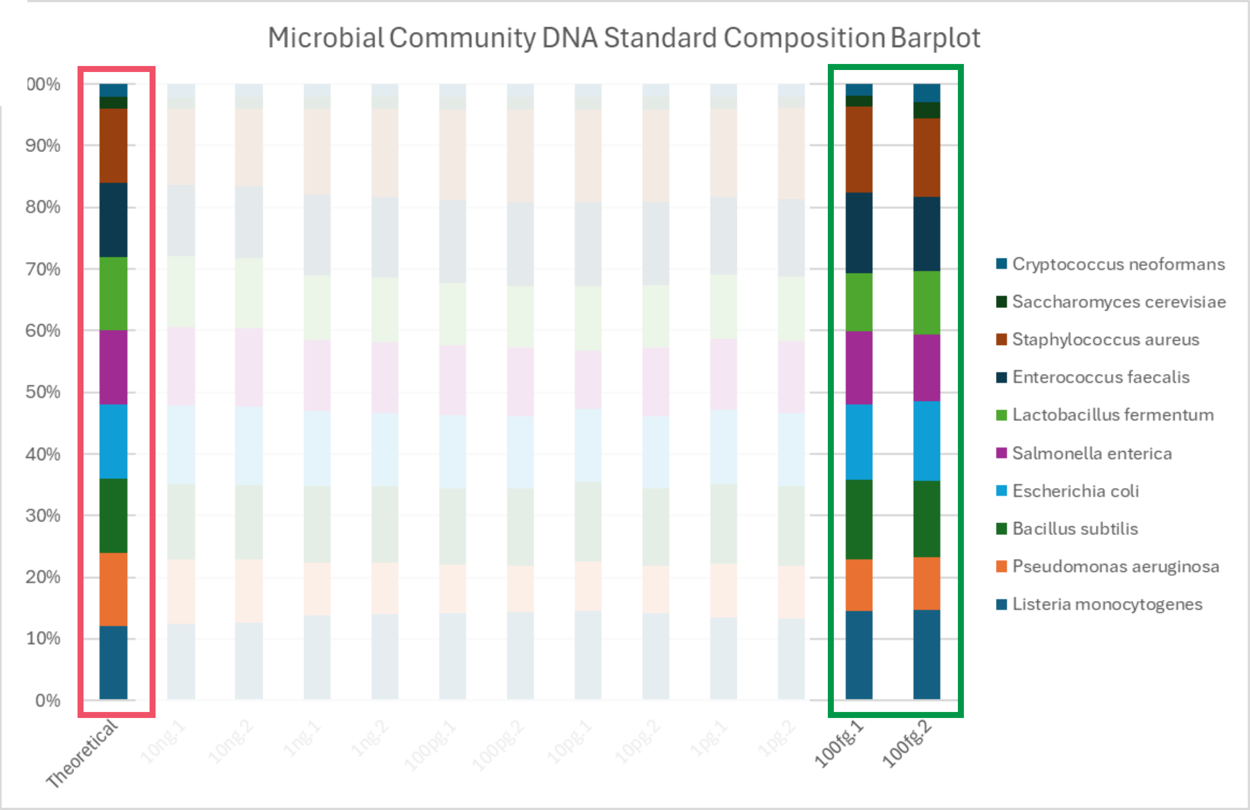
Consistent Microbial Profiling Even At 100 femtogram Input





Benchmark with
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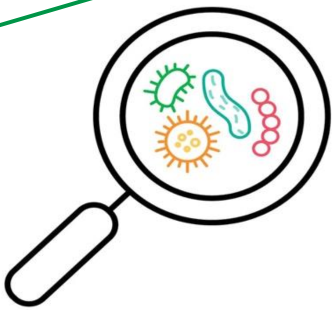
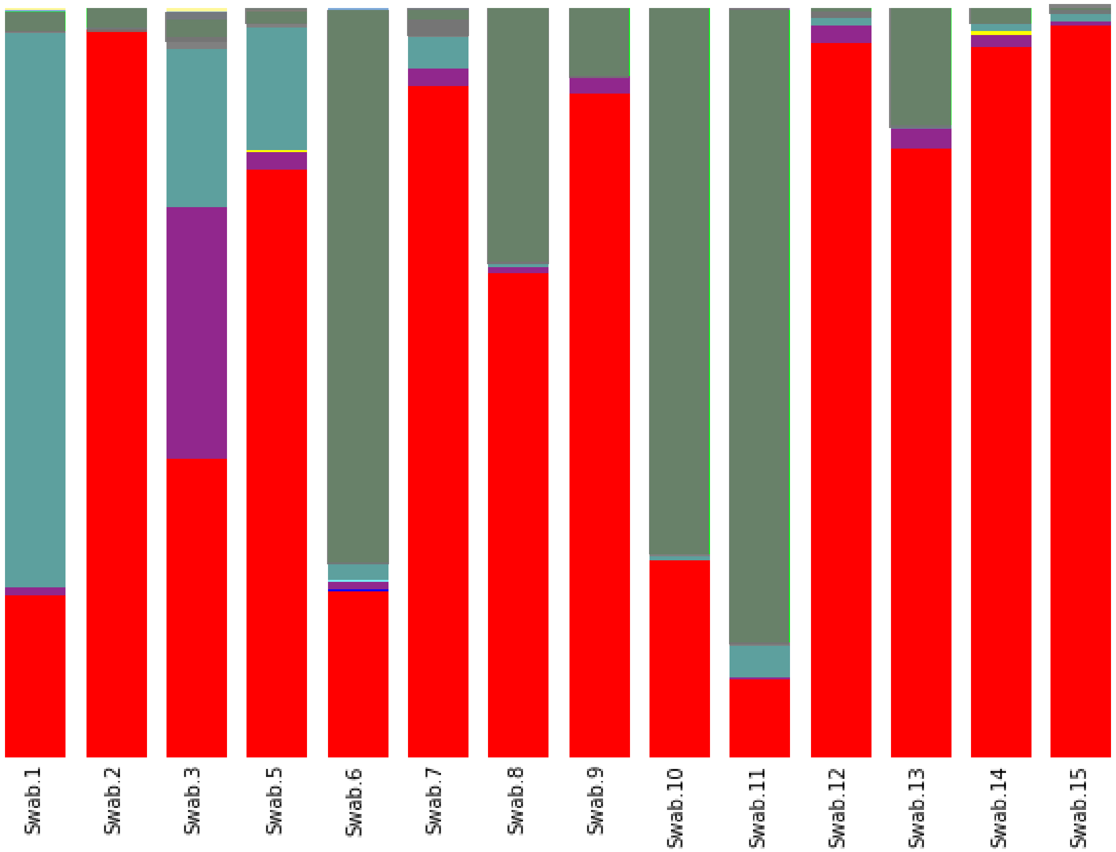
Cross-kingdom Relative Abundance



Prokaryotes



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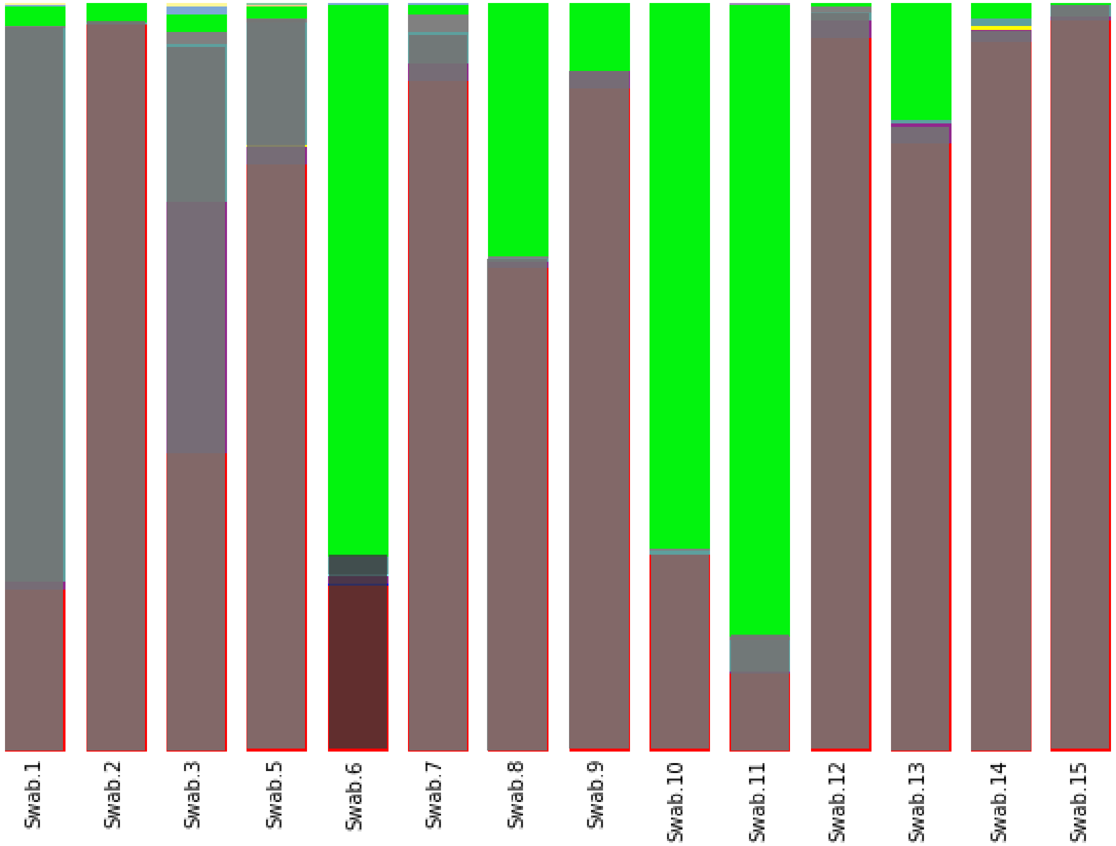
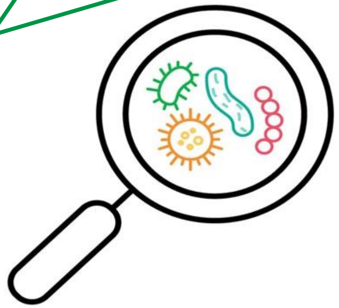
Cross-kingdom Relative Abundance

Prokaryotes

🟩 **Eukaryotes**



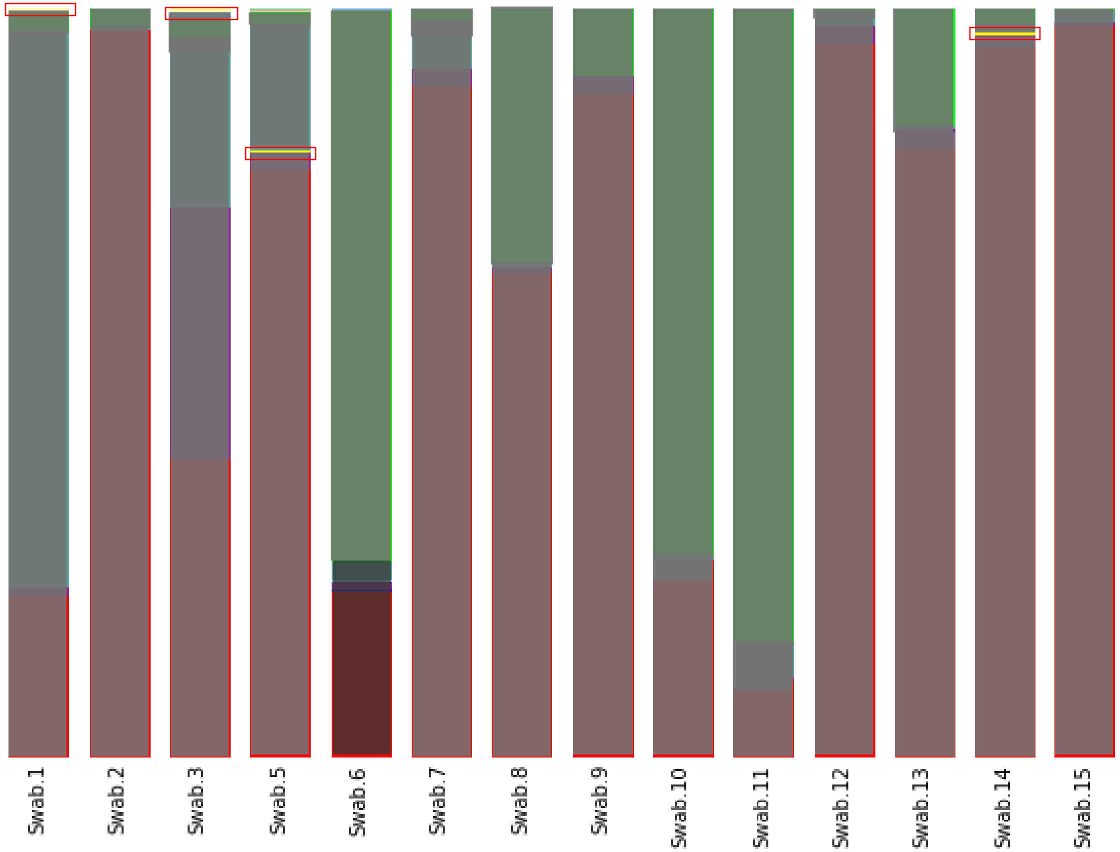
Cross-kingdom Relative Abundance



Prokaryotes

Eukaryotes

Viruses



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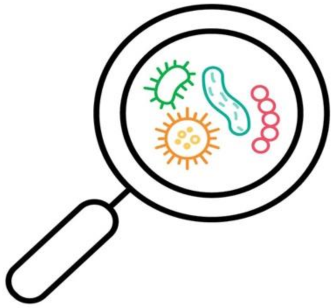
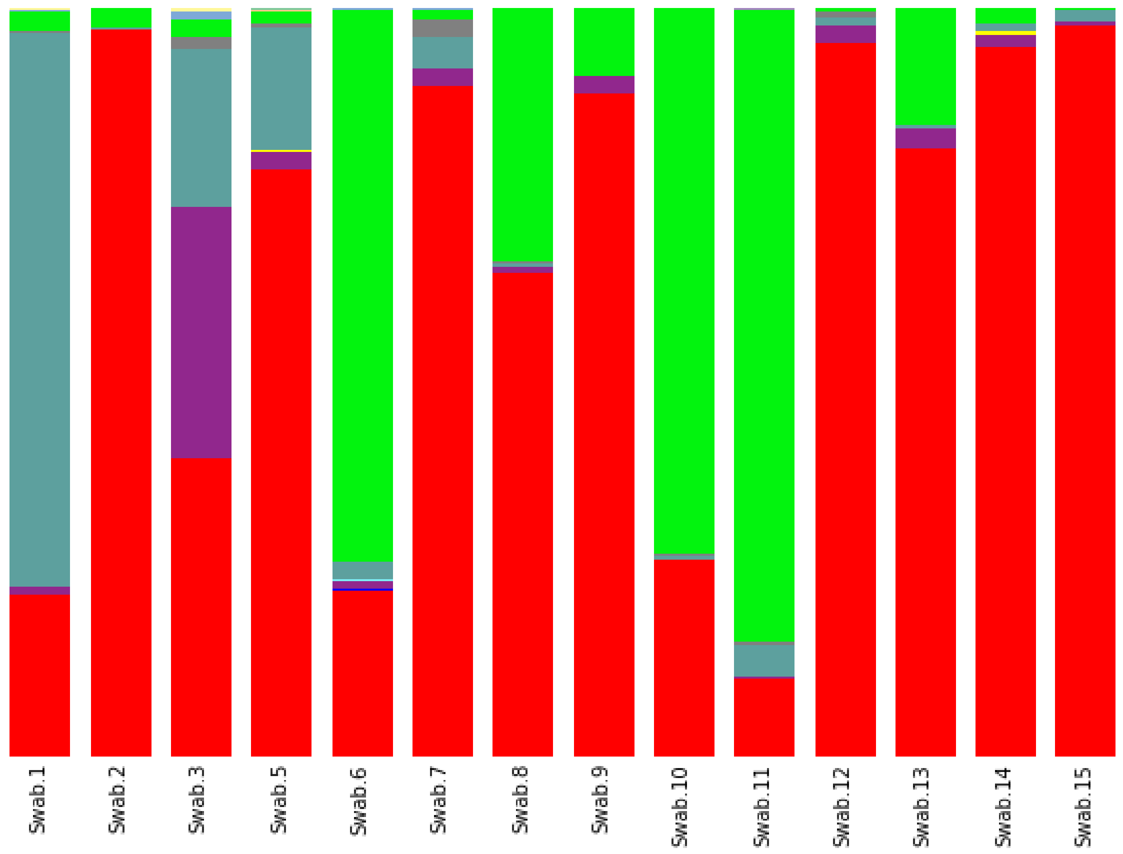


Cross-kingdom Relative Abundance

🟥 **Prokaryotes**

🟩 **Eukaryotes**

🟨 **Viruses**



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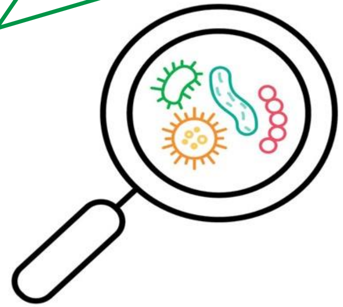
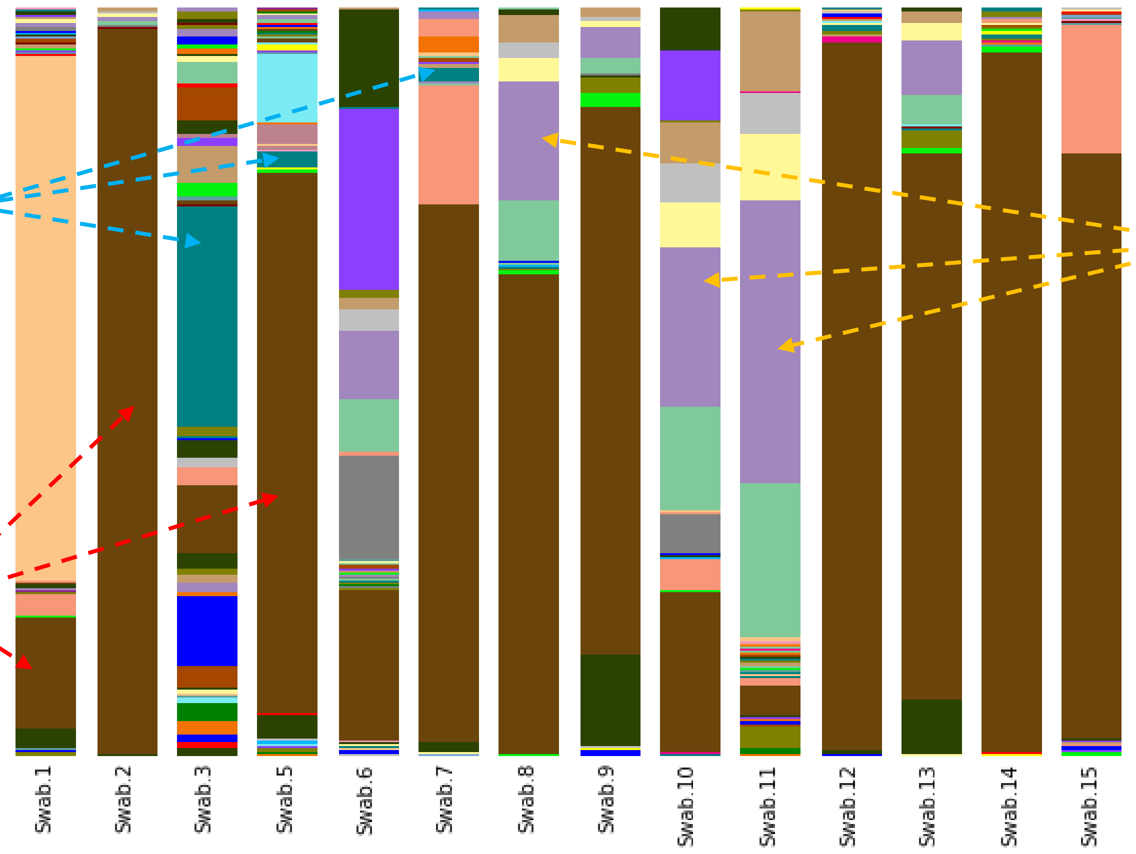
Accurate Strain-level Identification





Staphylococcus epidermidis
strain NBRC 100911

Malassezia restricta
strain KCTC 27527

Cutibacterium acnes
strain ACTCC 6919

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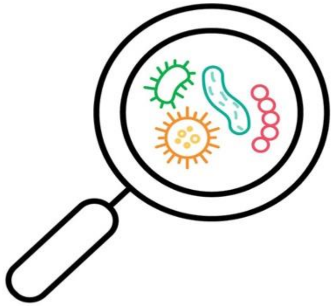
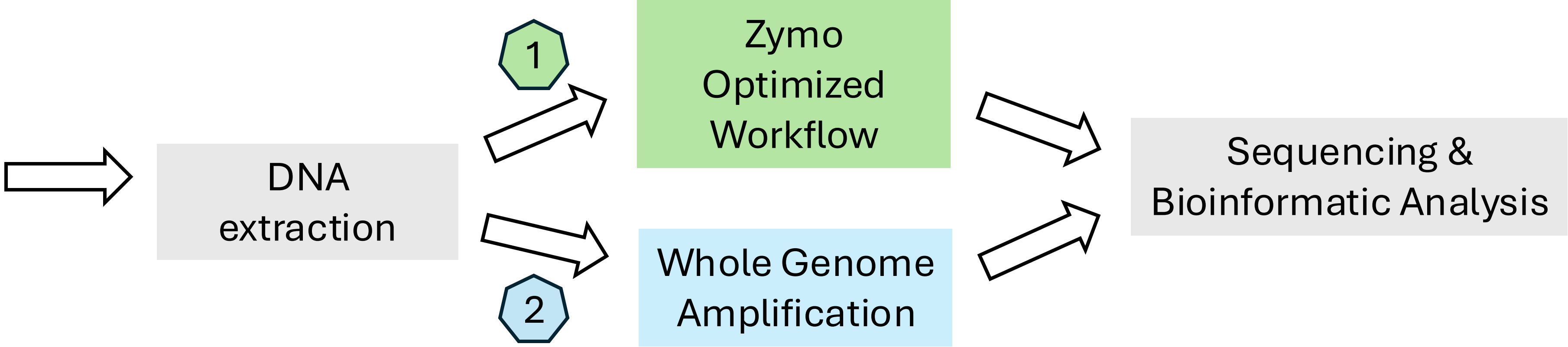


Whole Genome Amplification vs. Zymo Service Workflow



DNA extraction

1 Zymo Optimized Workflow

2 Whole Genome Amplification

Sequencing & Bioinformatic Analysis

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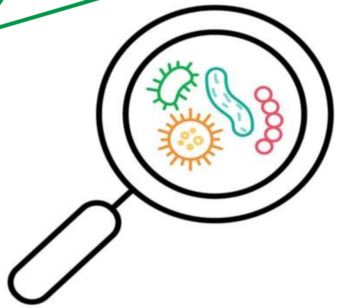
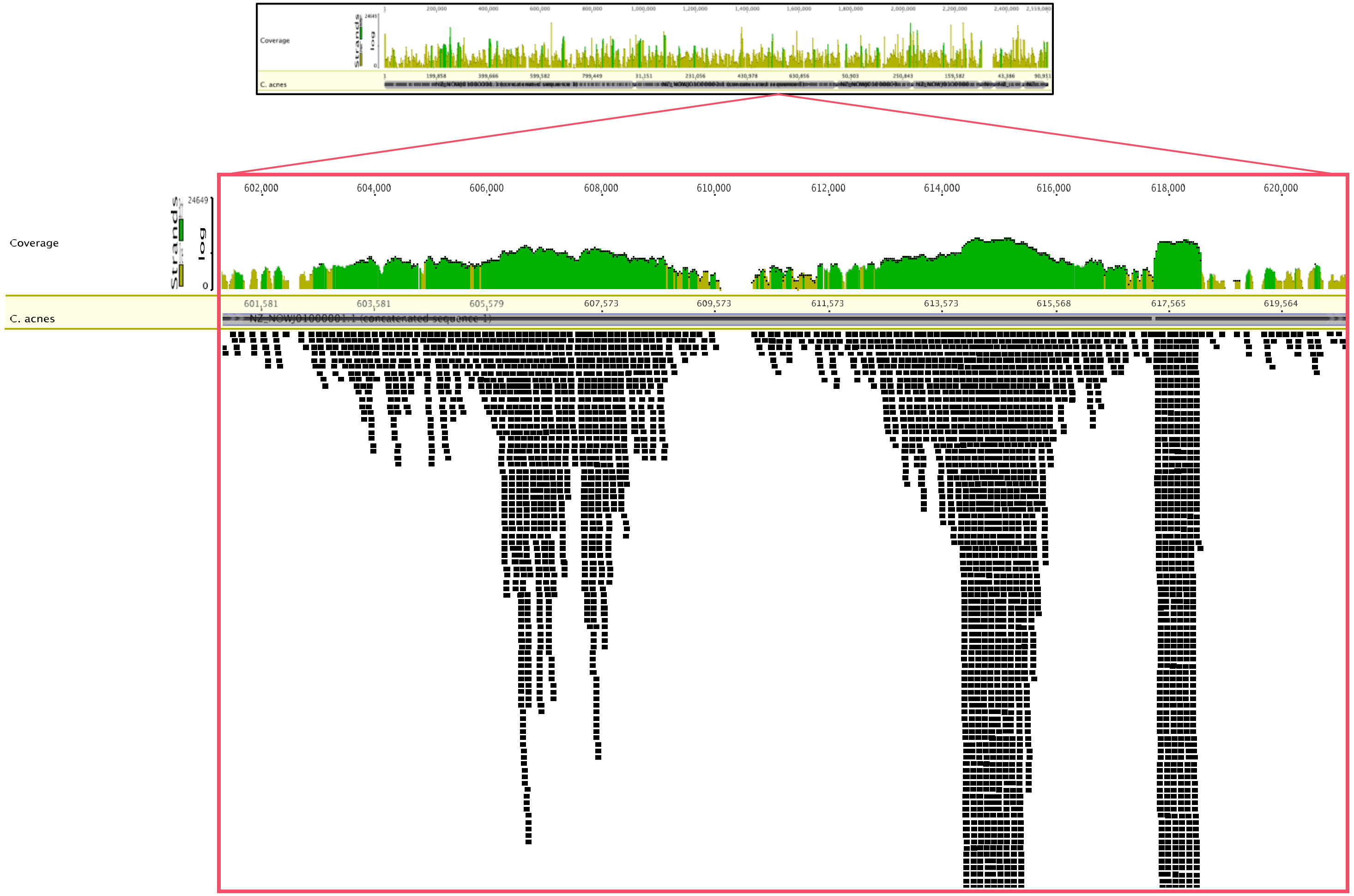
Incomplete Genome Coverage & Uneven Read Distribution



Whole Genome Amplification



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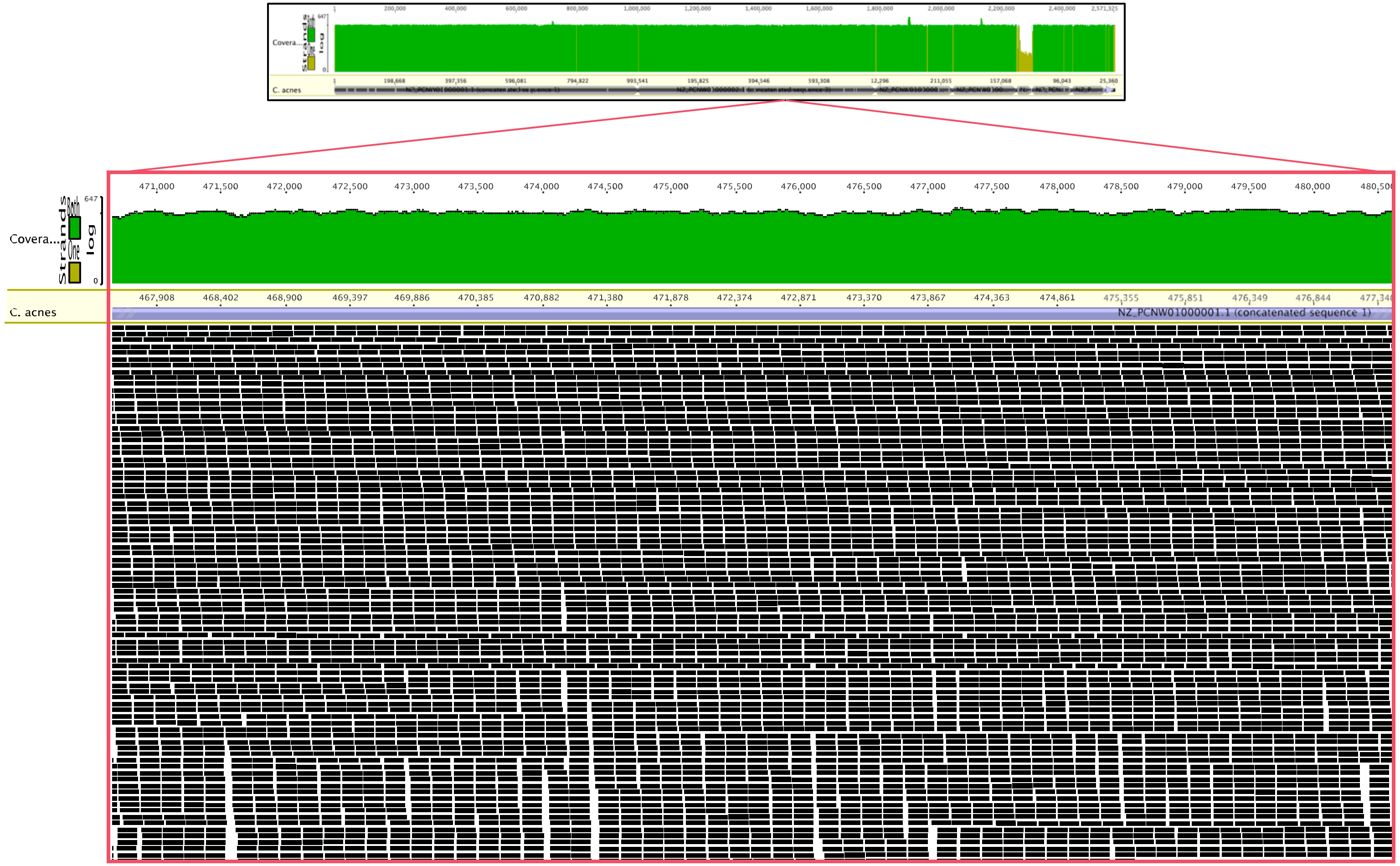
Accurate Genome Coverage & Even Read Distribution

Zymo Optimized Workflow



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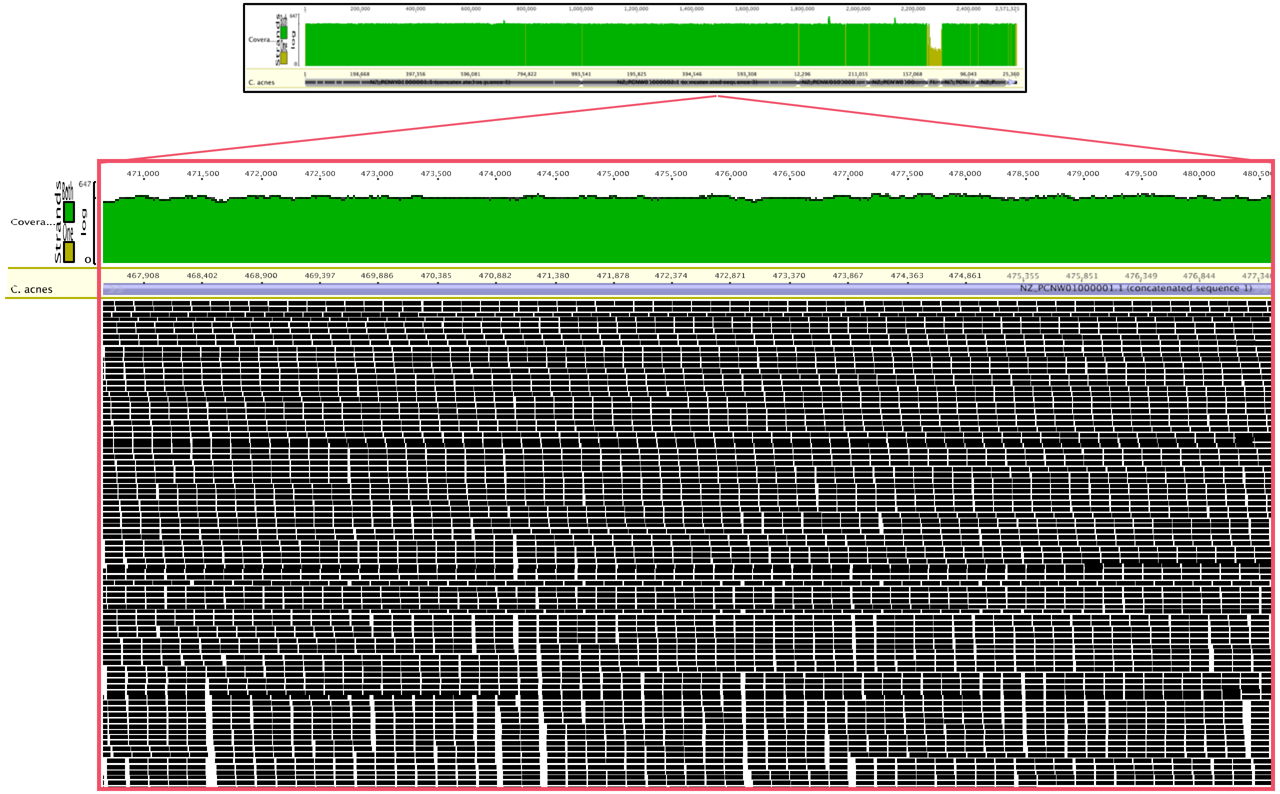
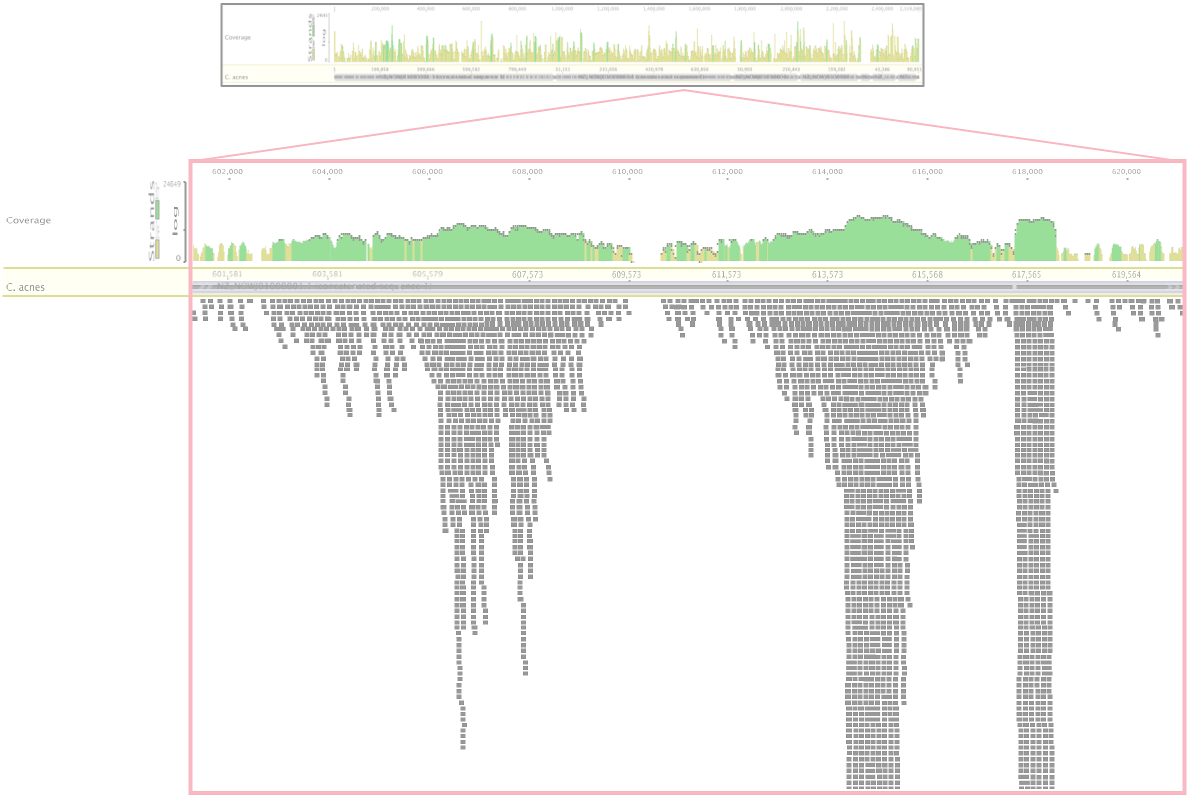
Unbiased & Better Accuracy Genome Coverage with Zymo Workflow



Zymo
Optimized
Workflow



Whole Genome
Amplification

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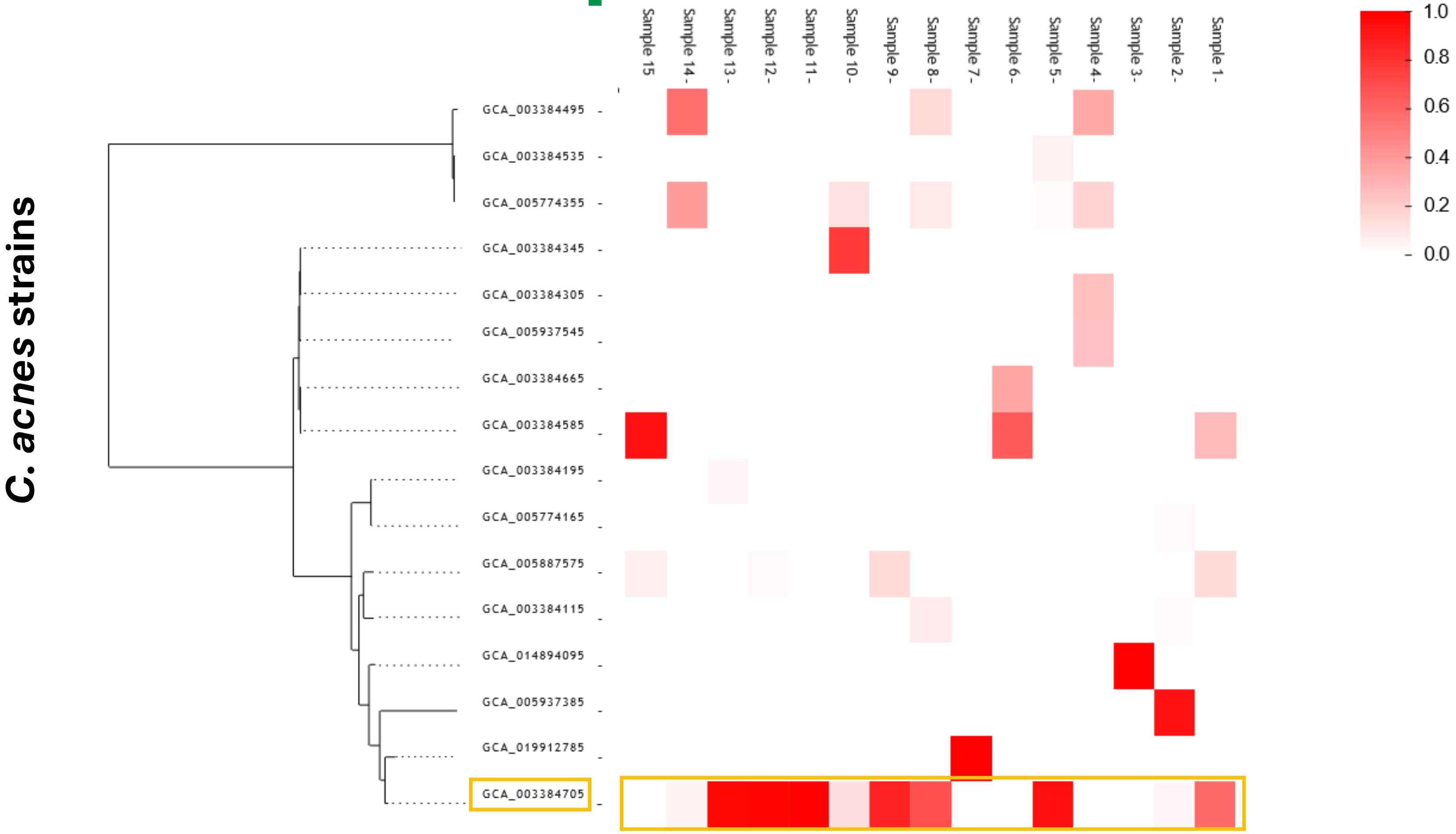
Highlight Strain-level Variation Across Samples



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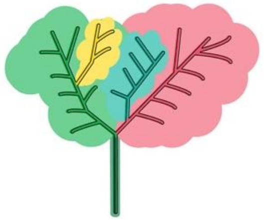
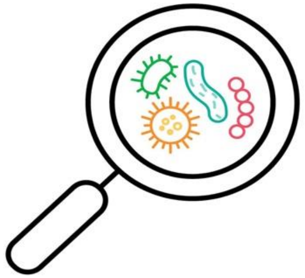
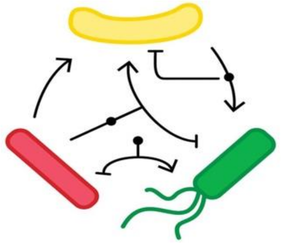
Sequencing Solutions for Any Microbiome Application



16/ITS Targeted Amplicon Sequencing



Shotgun Metagenomic Sequencing



Metatranscriptomic Sequencing

✓ **Free Personalized Consultation**

✓ **Complimentary Pilot Project**

Scan the QR code to explore our offering & review Sample Reports

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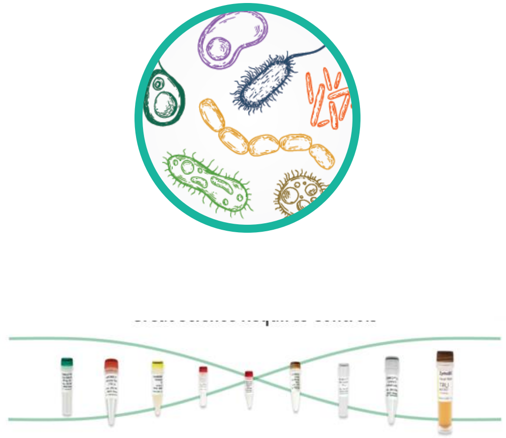
Ask about Zymo Research's Ultra-Low Biomass Microbiome Solutions

Microbiome Standards



Sample Collection

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NGS Analysis

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Acknowledgements

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- John Sherman

Thank you!

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