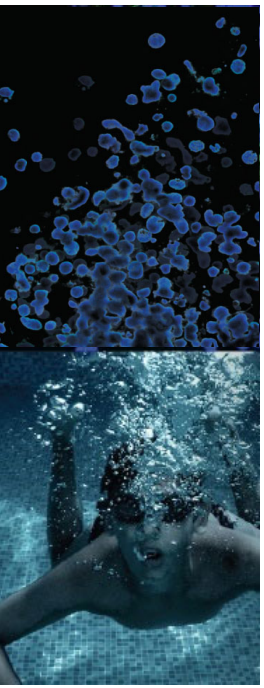
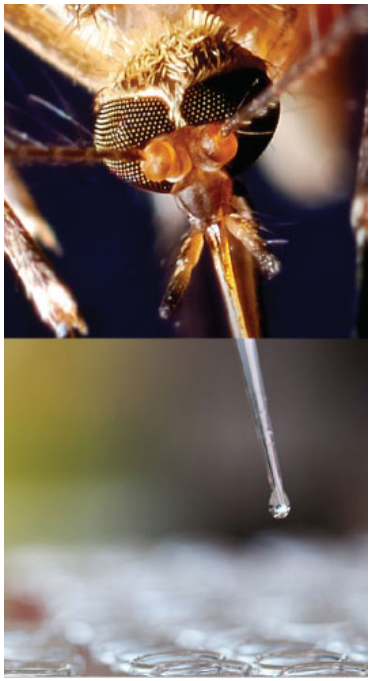
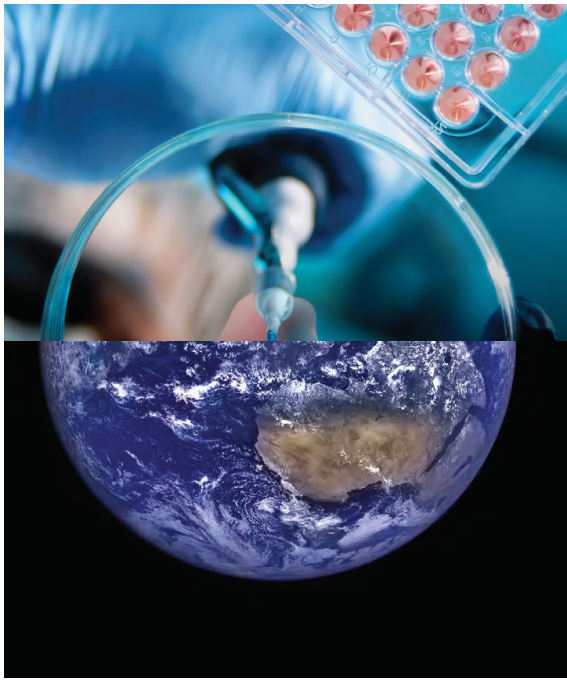
Skin Microbiome: Considerations, Applications, and Future Directions

Tasha M. Santiago-Rodriguez, PhD
Data Scientist III, Diversigen, Inc.

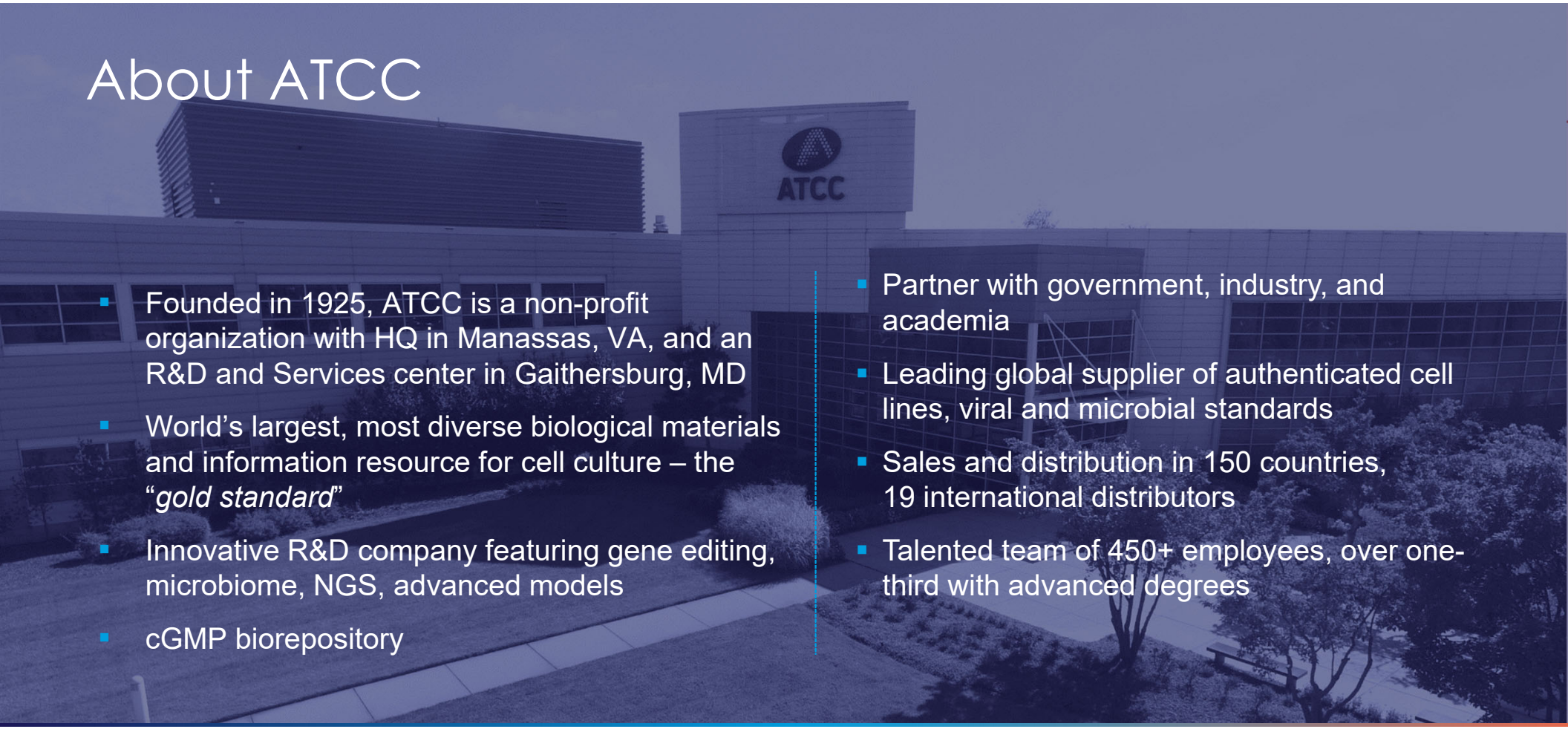
Credible Leads to Incredible™







About ATCC



- Founded in 1925, ATCC is a non-profit organization with HQ in Manassas, VA, and an R&D and Services center in Gaithersburg, MD
- World's largest, most diverse biological materials and information resource for cell culture – the "*gold standard*"
- Innovative R&D company featuring gene editing, microbiome, NGS, advanced models
- cGMP biorepository
- Partner with government, industry, and academia
- Leading global supplier of authenticated cell lines, viral and microbial standards
- Sales and distribution in 150 countries, 19 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

ATCC®

The skin microbiome: Considerations, applications and future directions

Tasha M. Santiago-Rodriguez, Ph.D.
Diversigen, Houston, TX
R&D Data Scientist III

Agenda

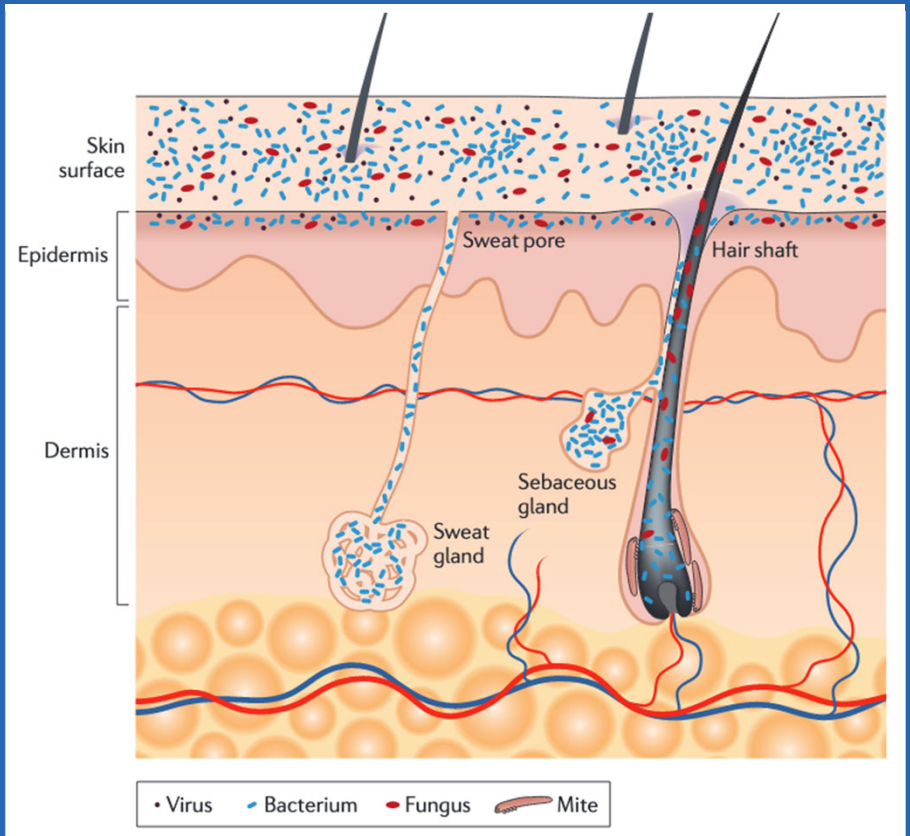
- Introduction to the skin microbiome
- Biases in skin microbiome research
  - Collection
  - Extraction and amplification
  - Database and annotation
- Future directions in skin microbiome research

Diversigen®

Introduction to the skin microbiome

Skin is an ecosystem

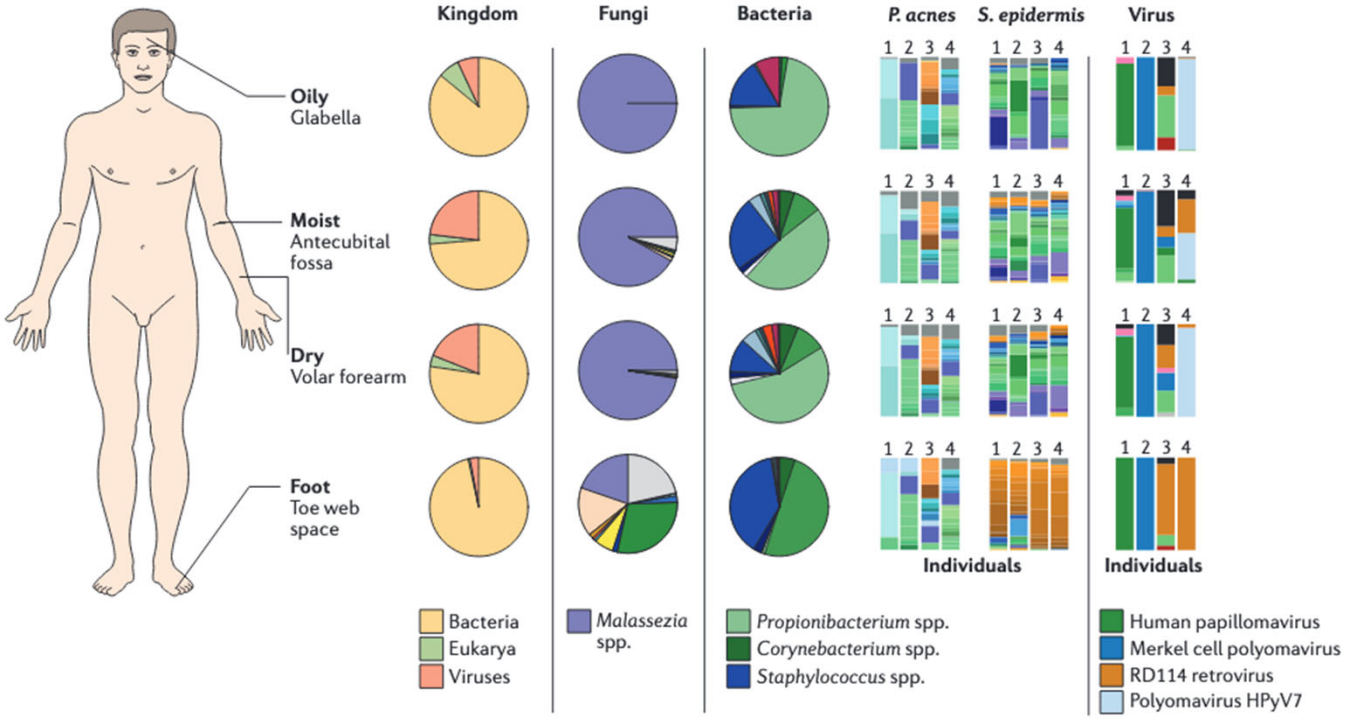
- As the largest human organ, skin serves as a physical barrier and acts as an ecosystem with diverse micro environments (pH, light exposure, moisture and oil content)
- Bacteria fungi and viruses inhabit skin
- Most of these microorganisms are harmless, providing protection and modulation of the immune system



Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature reviews microbiology*, *9*(4), 244-253.

Diversigen®

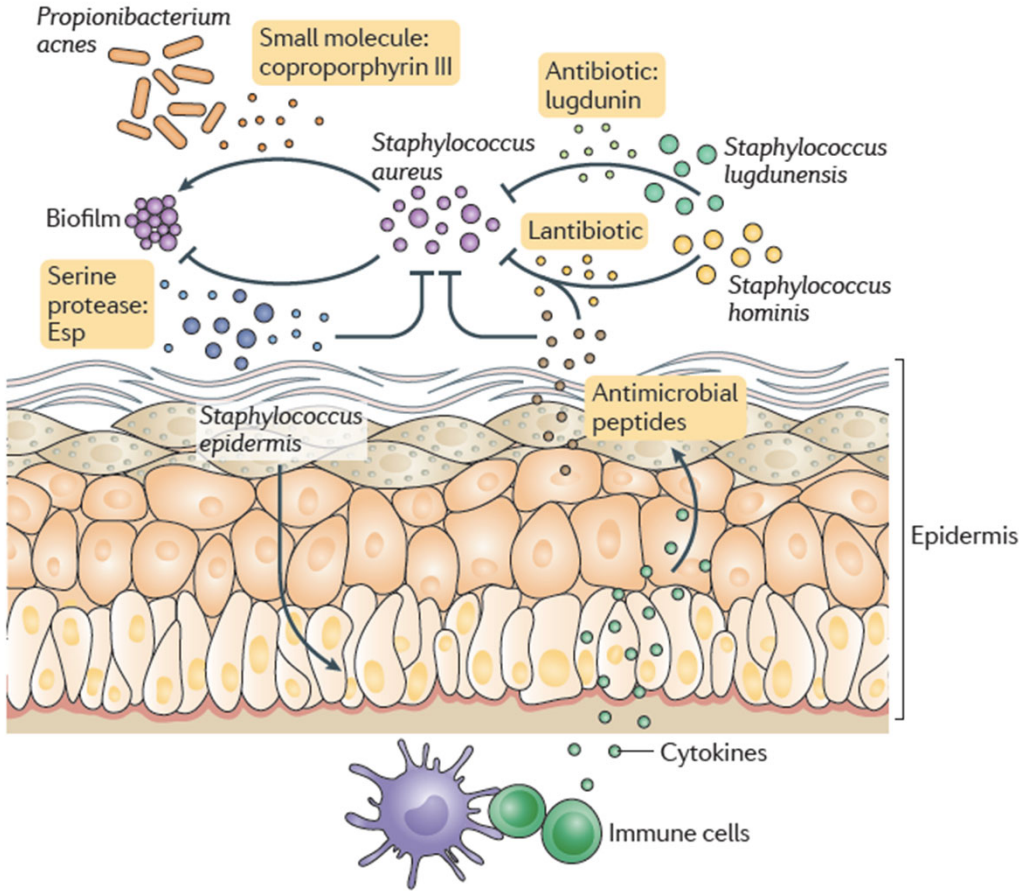
Skin contains diverse micro environments



Left: Eisenstein, M. (2020). The skin microbiome and its relationship with the human body explained. *Nature*, *588*(7838), S211-S211.
Right: Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, *16*(3), 143-155.

Diversigen®

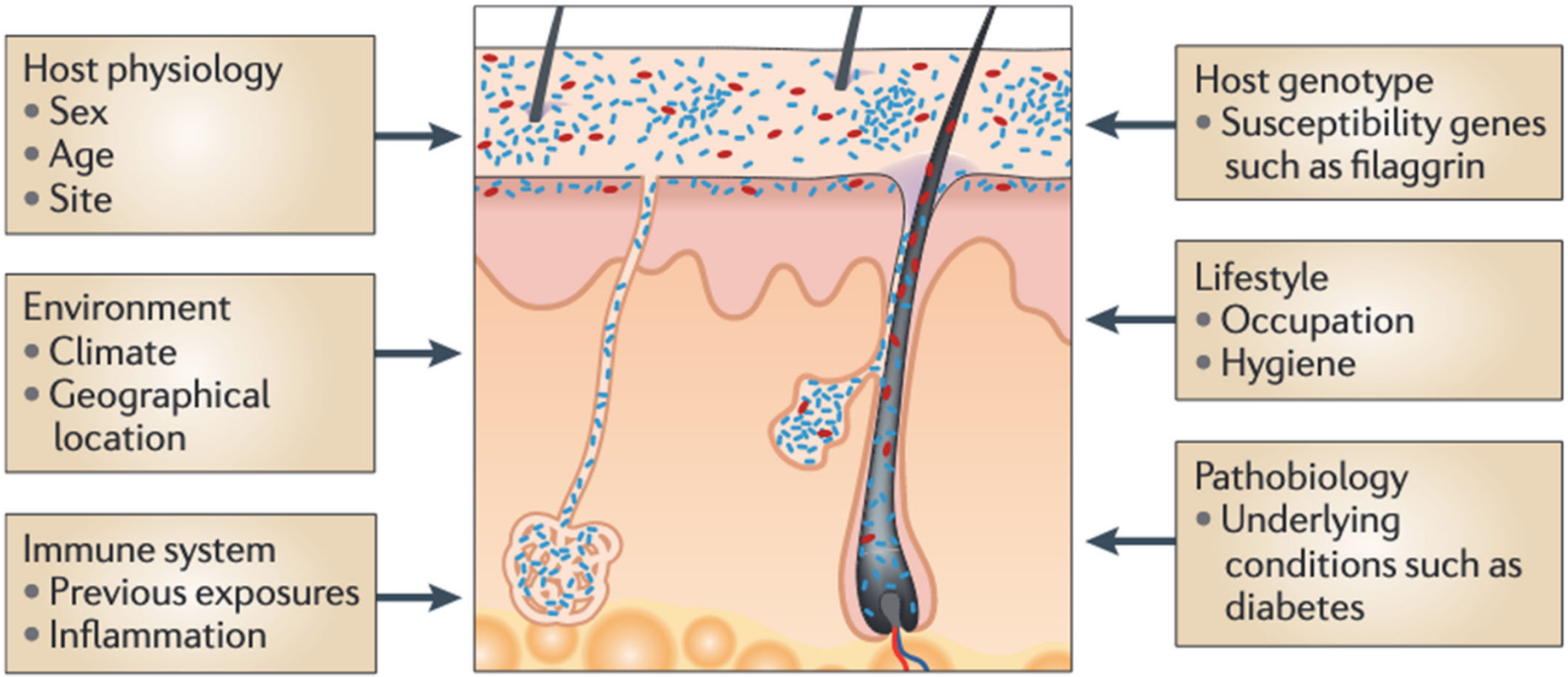
The skin microbiome protects us from pathogens and modulates the immune system



Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, *16*(3), 143-155.

Diversigen®

Different factors are associated with the skin microbiome



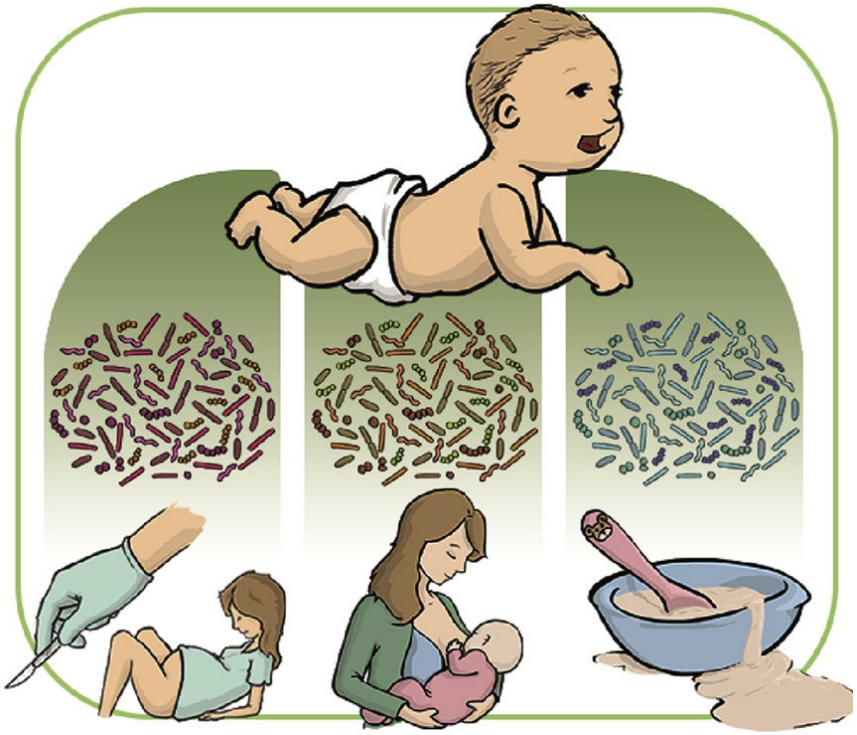
Grice, E. A., & Segre, J. A. (2011). The skin microbiome. *Nature reviews microbiology*, *9*(4), 244-253.

Diversigen®

The skin microbiome is acquired at birth

Skin microbiome composition at birth and early on in life is influenced by:

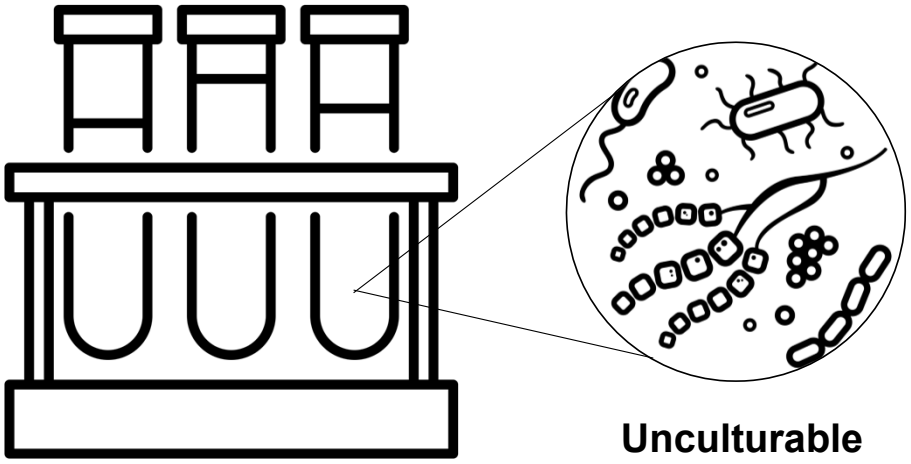
- Delivery mode
- Maternal microbiota
- Antibiotic use
- Soaps and detergents
- Nutritional factors
- Housing
- Animal contact
- Outdoor play



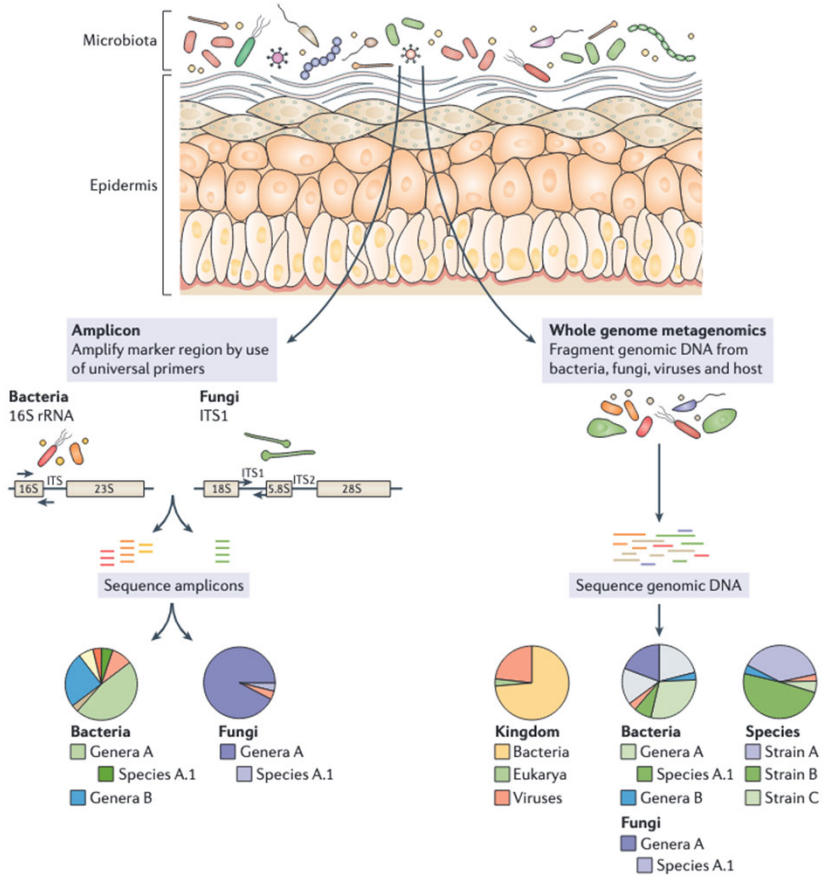
Galazzo, G., van Best, N., Bervoets, L., Dapaah, I. O., Savelkoul, P. H., Hornef, M. W., ... & Penders, J. (2020). Development of the microbiota and associations with birth mode, diet, and atopic disorders in a longitudinal analysis of stool samples, collected from infancy through early childhood. *Gastroenterology*, *158*(6), 1584-1596.

Diversigen®

Culture-independent methods for studying the skin microbiome



Unculturable microorganisms

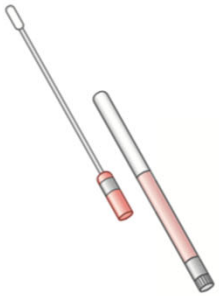


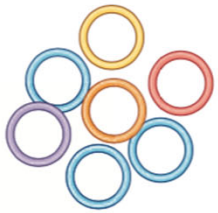
Left: http://teachthemicrobiome.weebly.com/uploads/2/6/4/3/26438968/1392600741.jpg
Right: Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, *16*(3), 143-155.

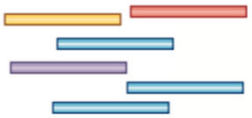
Diversigen®

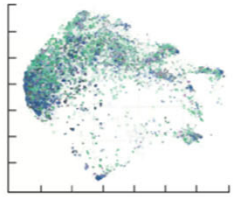
Skin microbiome pipeline: from sample collection to analysis

Sample processing steps







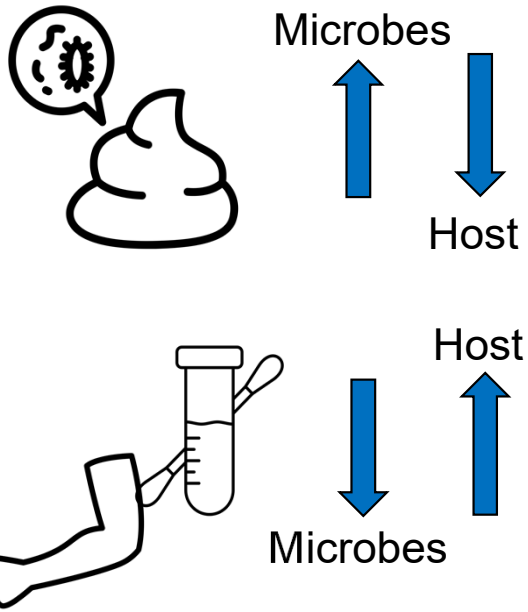


Sample collection	Sample storage	DNA extraction	Sequencing library preparation	DNA sequencing	Computational analysis

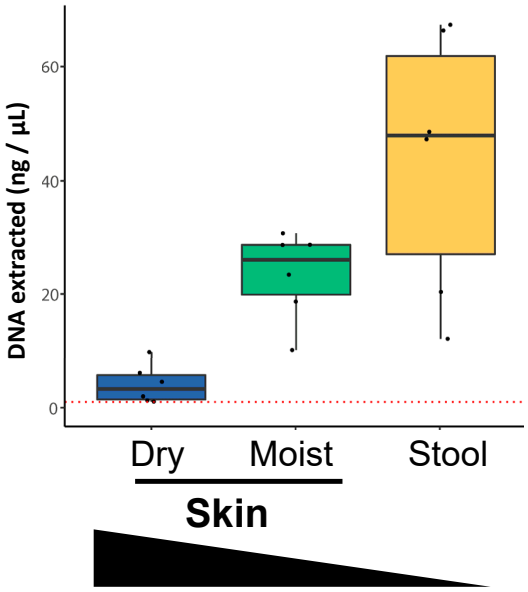
Diversigen®

Considerations for studying the skin microbiome

1. Microbial recovery



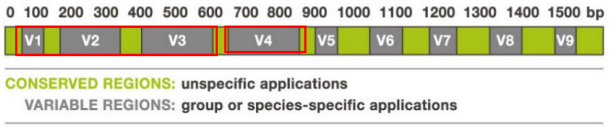
2. Microbial content



3. Sequencing and analysis

Amplicon sequencing

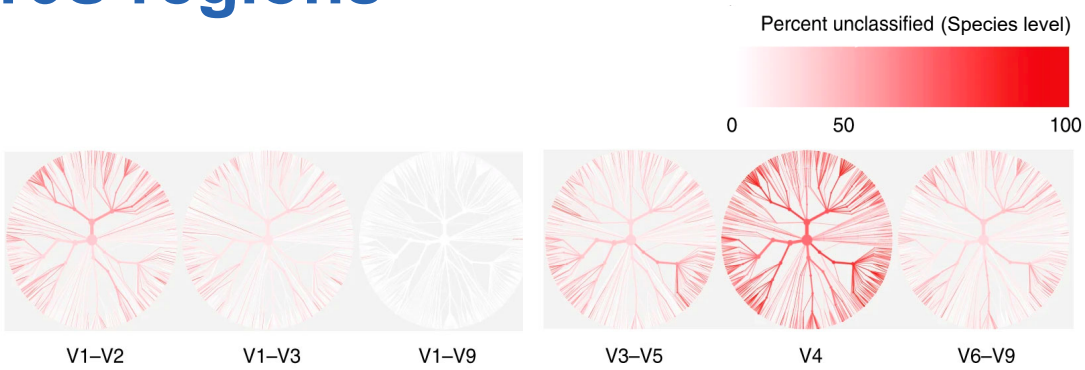
16S V4: gut microbiome

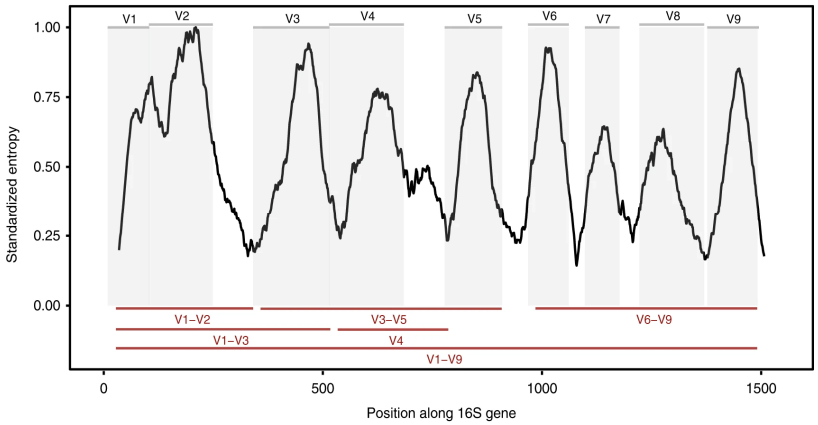


16S V1-V3: skin microbiome

Diversigen®

Trade-offs between primers targeting V4 and V1-V3 16S regions





V4 16S region
- Inability to capture certain species without primer editing
- Ability to compare to a broad literature and across body sites
- Improved sequence quality

V1-V3 16S region
- Challenges in bioinformatic analysis due to long amplicon
- Improved species-level resolution for skin samples
- Better correlation with whole genome sequencing results

Johnson, J. S., Spakowicz, D. J., Hong, B. Y., Petersen, L. M., Demkowicz, P., Chen, L., ... & Weinstock, G. M. (2019). Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature communications*, *10*(1), 1-11.

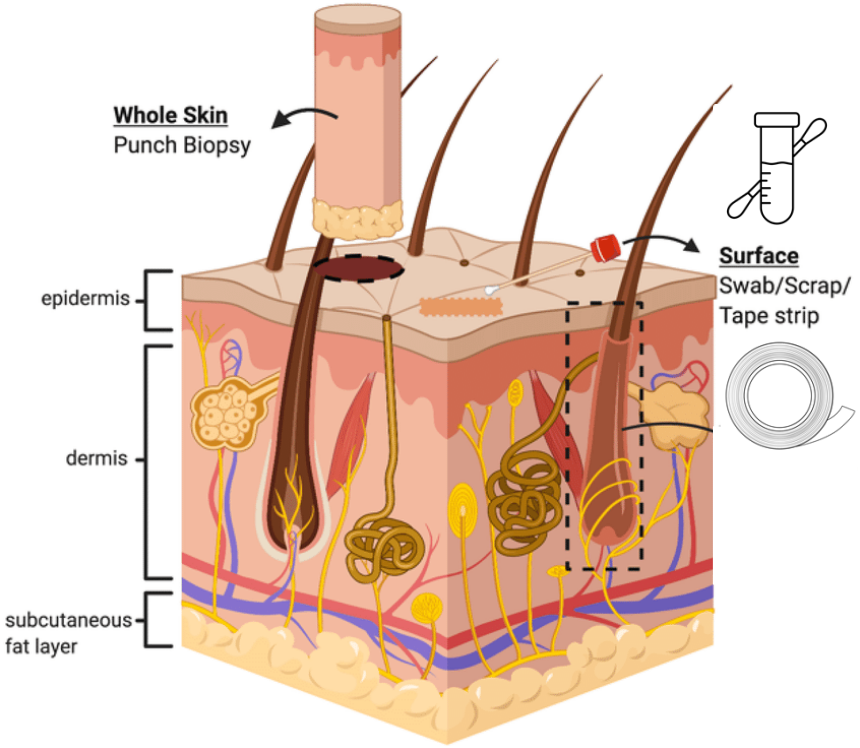
Diversigen®

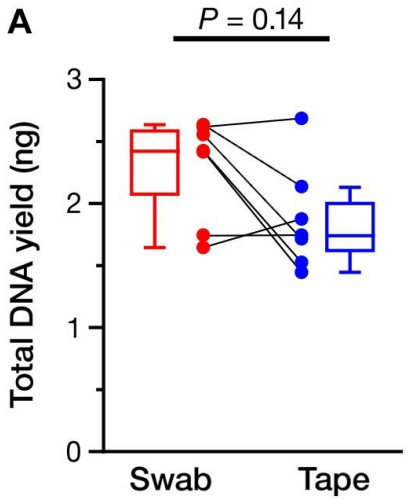
Biases in ski
microbiome r

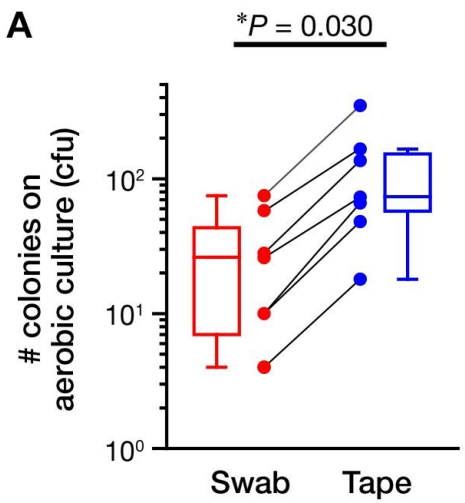
Sample collection

Diversigen®

Human skin microbiome collection methods







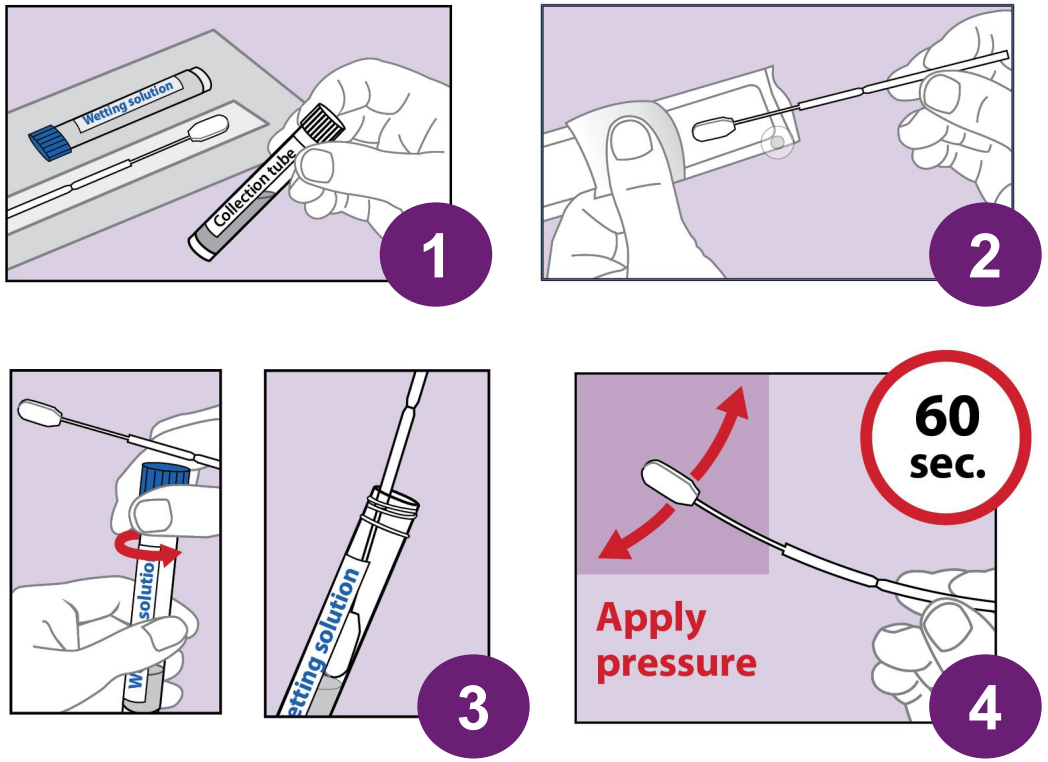
Left: McLoughlin, I. J., Wright, E. M., Tagg, J. R., Jain, R., & Hale, J. D. (2021). Skin Microbiome—The Next Frontier for Probiotic Intervention. *Probiotics and Antimicrobial Proteins*, 1-18.
Right: Ogai, K., Nagase, S., Mukai, K., Iuchi, T., Mori, Y., Matsue, M., ... & Okamoto, S. (2018). A comparison of techniques for collecting skin microbiome samples: swabbing versus tape-stripping. *Frontiers in microbiology*, 2362.

Diversigen®

OMNIgene®·SKIN

A swab-based microbiome collection device

Optimized Instructions for Use (IFU)

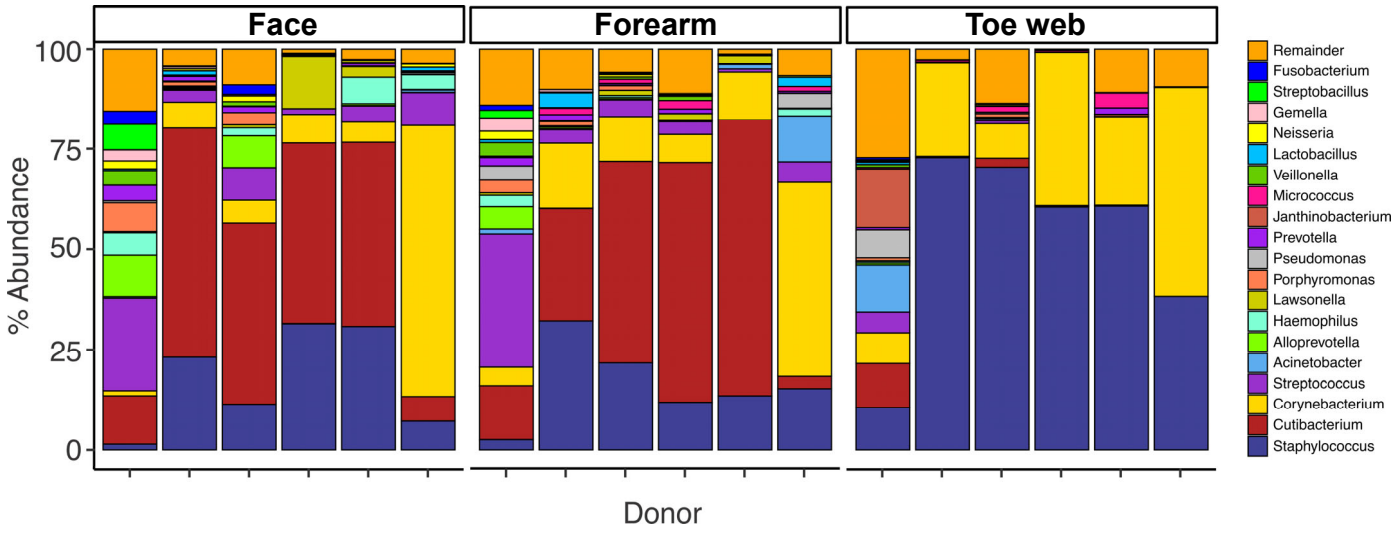


Diversigen®

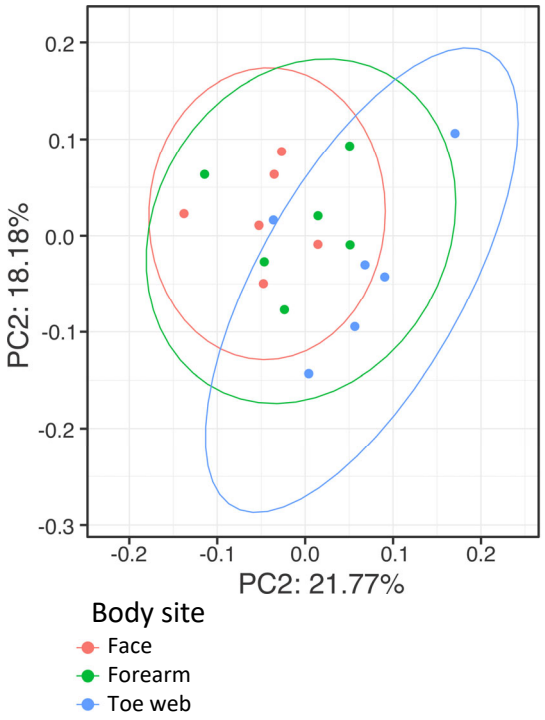
OMNIgene·SKIN

Capture site-specific bacterial profiles

V1-V3 16S taxonomic profiles across skin sites



PCoA plot of skin microbiome body site profiles

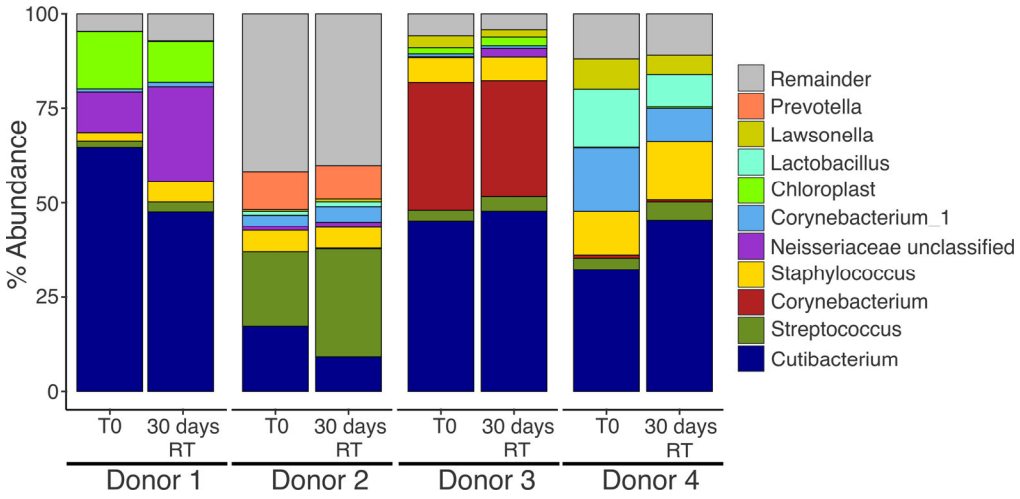


Diversigen

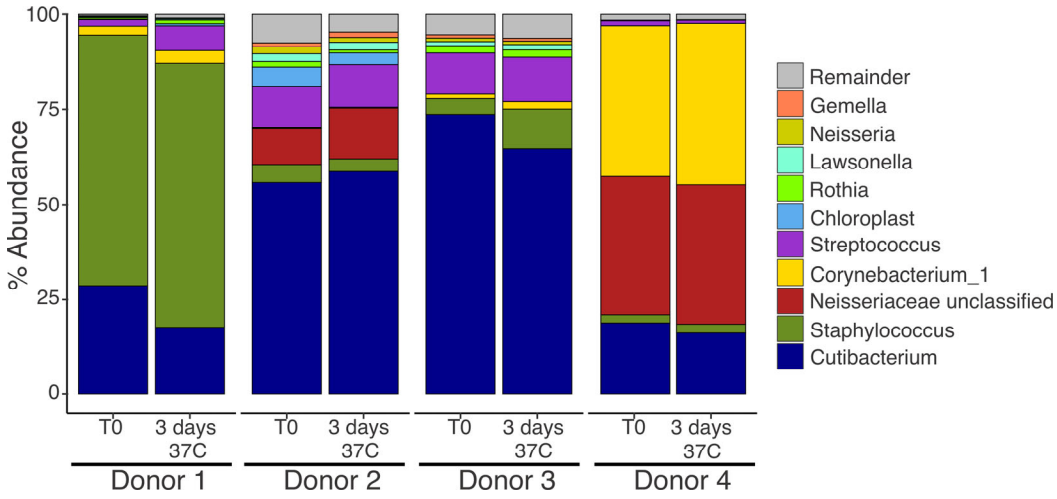
OMNIgene®·SKIN

Stabilize the skin microbial profile during shipping and storage

Room temperature, 30 days



37°C, 3 days



Diversigen®

Considerations for selecting a skin microbiome collection device

- Validated across various skin sites (i.e., dry, oily and moist)
- Optimized IFU for improved collection/sample performance
- Low bioburden
- Captures and stabilizes the skin microbiome during storage or shipping at ambient temperatures
- Consistent performance

Diversigen®

Biases in ski
microbiome r

Extraction and amplification

Diversigen®

DNA extraction can add biases to skin microbiome studies

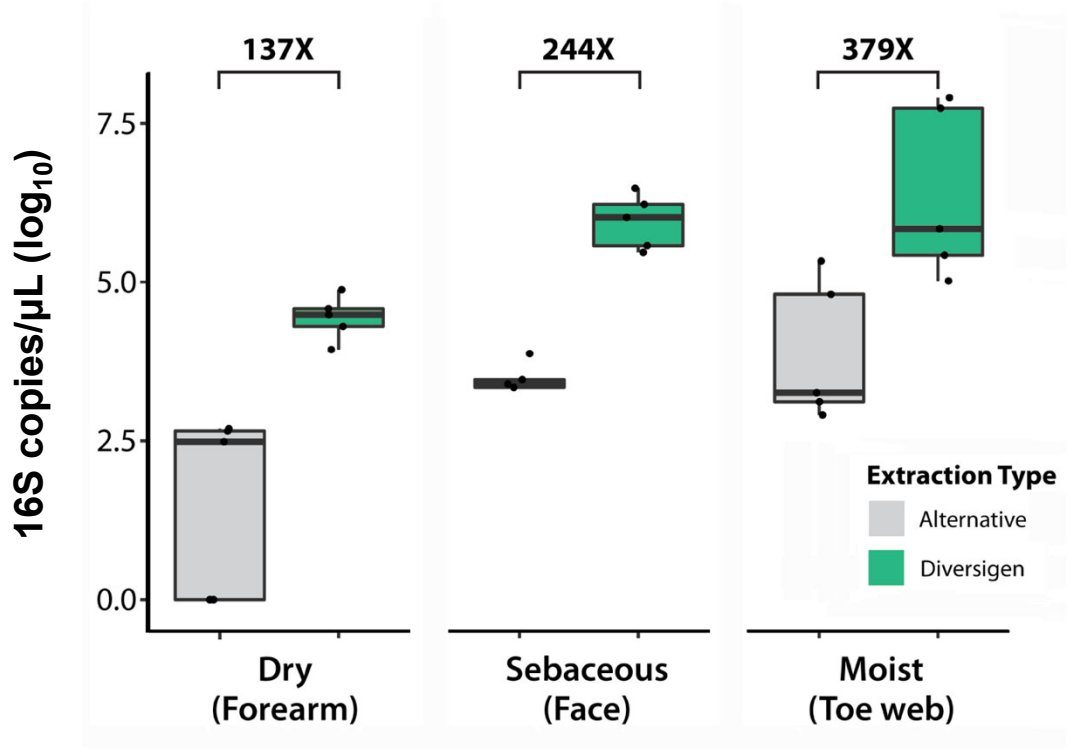
- Commercial DNA extraction kits are the most common for skin microbiome analyses
- Different approaches exist, including enzymatic treatment and/or mechanical lysis
- DNA concentrations and success of library preparation are associated with extraction method

	Kit number	DNA concentration (ng/µl) Skin samples average
	1	0.05
	2	0.02
	3	0.01
Mechanical + enzymatic treatment (high salt)	4	0.00
	5	0.00
	6	0.03
	7	0.02
Mechanical + enzymatic treatment	8	0.99
	9	0.01
	10	0.03
	11	0.02
	12	0.04

Modified from Bjerre, R. D., Hugerth, L. W., Boulund, F., Seifert, M., Johansen, J. D., & Engstrand, L. (2019). Effects of sampling strategy and DNA extraction on human skin microbiome investigations. *Scientific reports*, *9*(1), 1-11.

Diversigen®

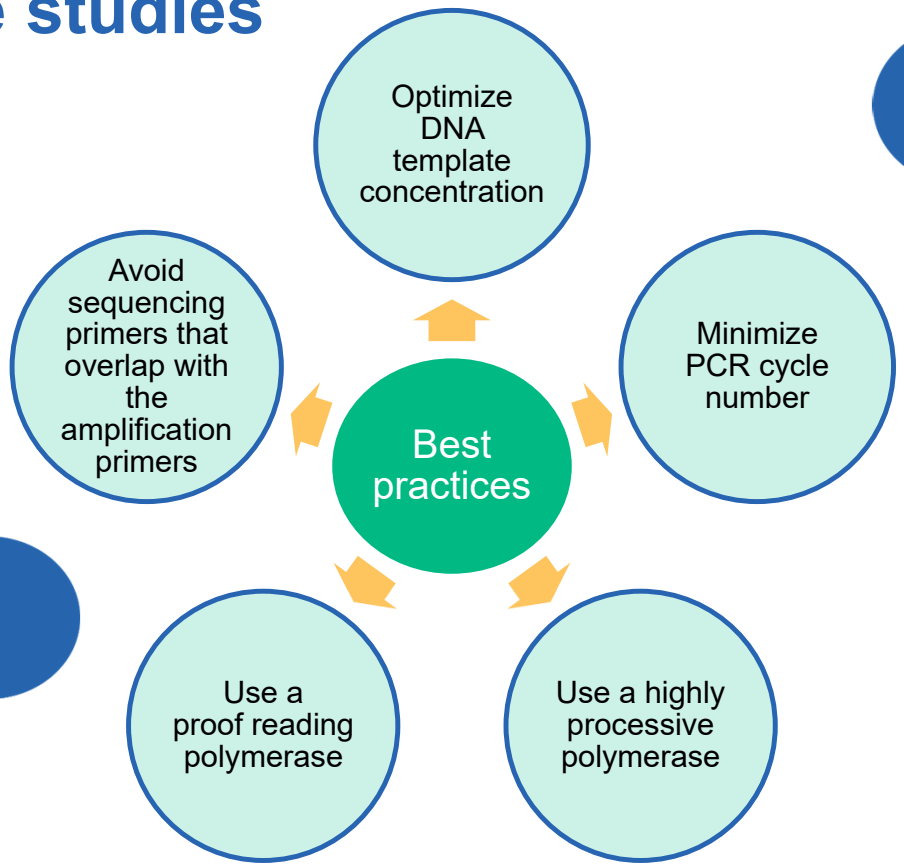
DNA extraction optimization is essential for skin microbiome studies



- Optimized collection kits and extraction workflows maximize DNA yields from low biomass sites
- Increases rate of successful collection (fewer sample dropouts)
- Ensured success of downstream applications, including amplicon generation

Diversigen®

Best practices for generating amplicons for microbiome studies

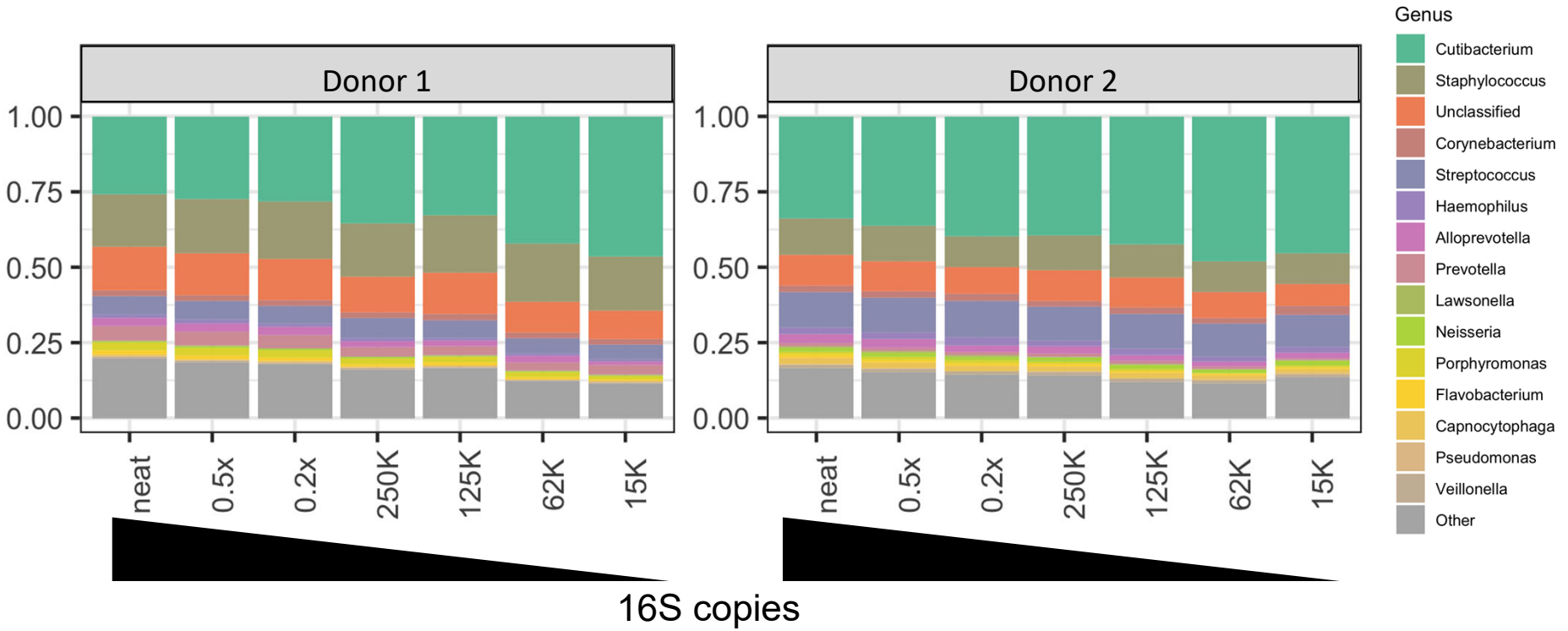


Improved quantitative accuracy

Improved qualitative accuracy

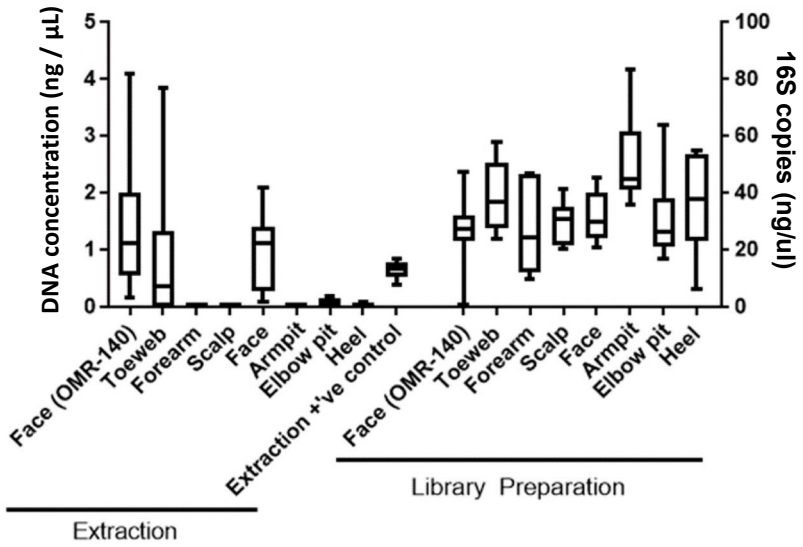
Diversigen®

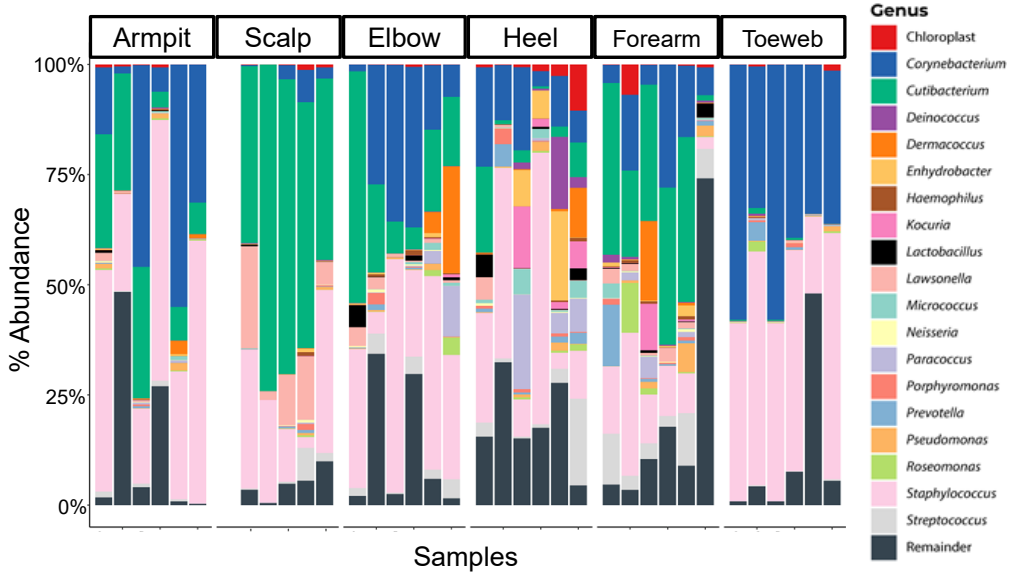
Reproducible skin profiles were generated, regardless of sample concentration



Diversigen®

Library preparation provides consistent DNA concentrations for sequencing and bioinformatic analyses





- Sufficient library yields are achieved, regardless of extracted biomass
- Unique microbial profiles can be generated across various skin sites

Diversigen®

Considerations for skin sample processing

- Validated across various skin sites (i.e., dry, oily and moist)
- Optimized to maximize DNA yields and 16S rRNA gene copy number
- Capture expected taxonomic profiles of the skin microbiome across a range of DNA concentrations
- Provides consistent performance, which will depend on the number of PCR cycles, polymerases, as well as amplification and sequencing primers

Diversigen®

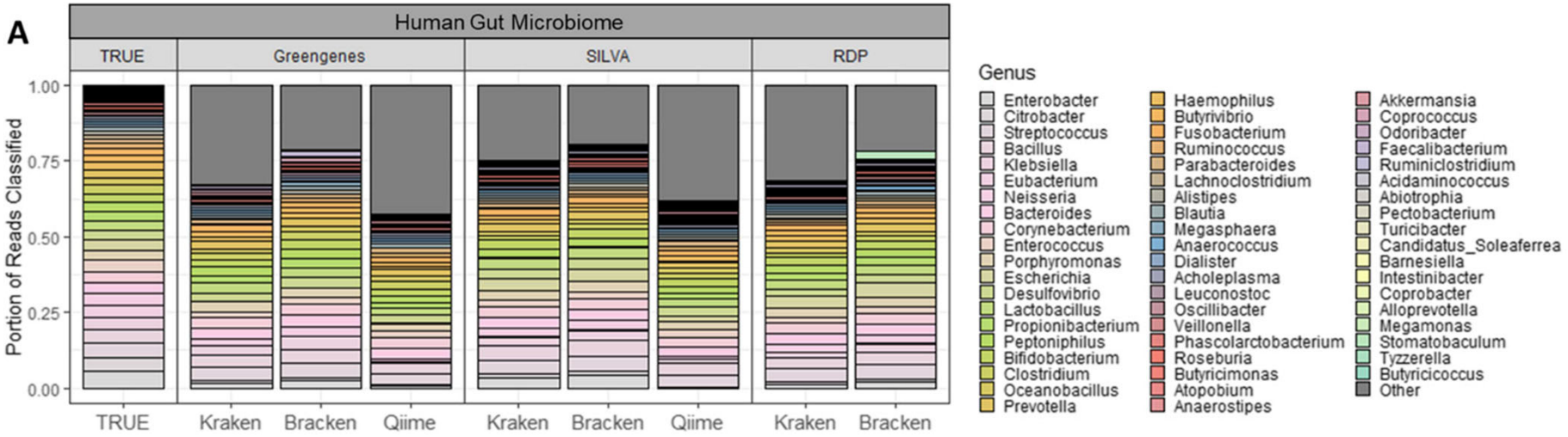
Biases in ski
microbiome r

Database and annotation

Diversigen®

Effects of different databases and taxonomic annotations on microbiome

- Annotation tools are known to affect the taxonomic composition of the human microbiome



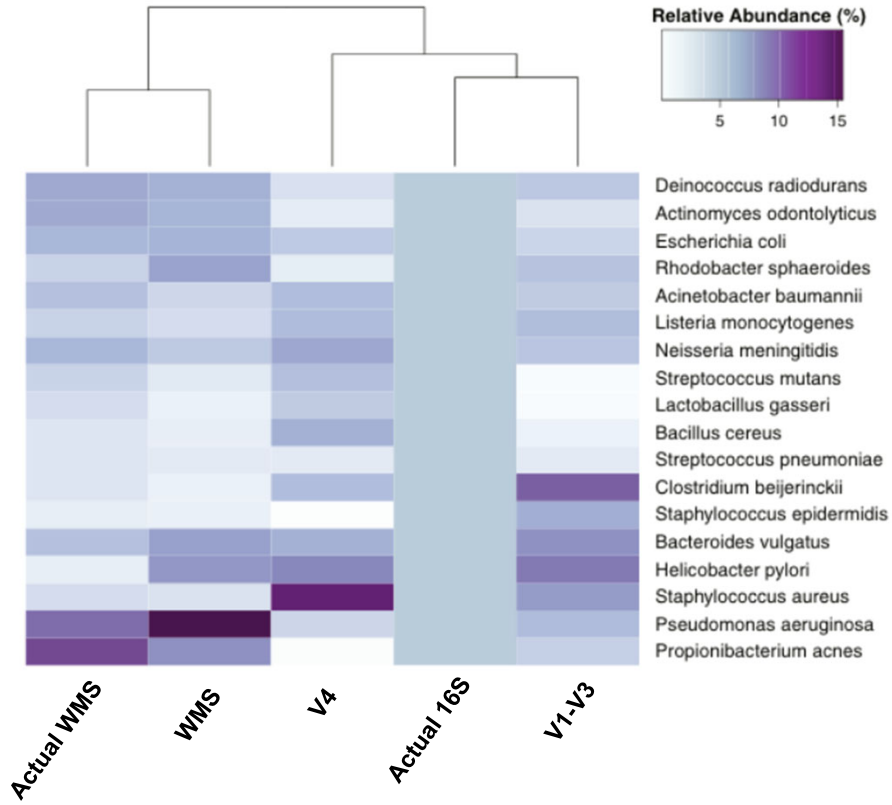
- Limited literature on the effect of different annotation tools on the skin microbiota composition

Lu, J., & Salzberg, S. L. (2020). Ultrafast and accurate 16S rRNA microbial community analysis using Kraken 2. *Microbiome*, *8*(1), 1-11.

Diversigen®

Amplification of 16S rRNA gene V4 vs. V1-V3 regions affects taxonomic classification

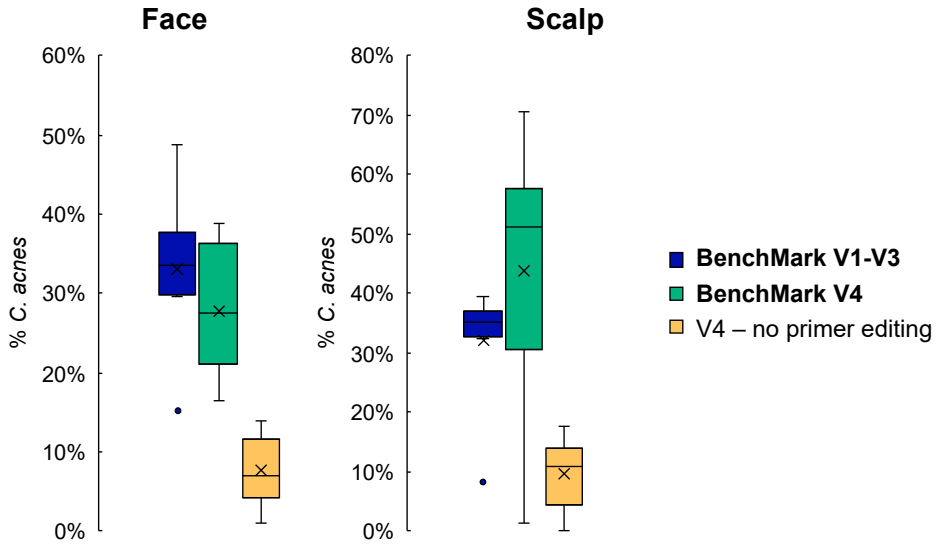
- Amplification of 16S V4 region resulted in an over representation of *Staphylococcus aureus* and an under-representation of *Cutibacterium acnes* and *Staphylococcus epidermidis*
- Amplification of the 16S V1-V3 region better recapitulated the expected proportions of mock communities

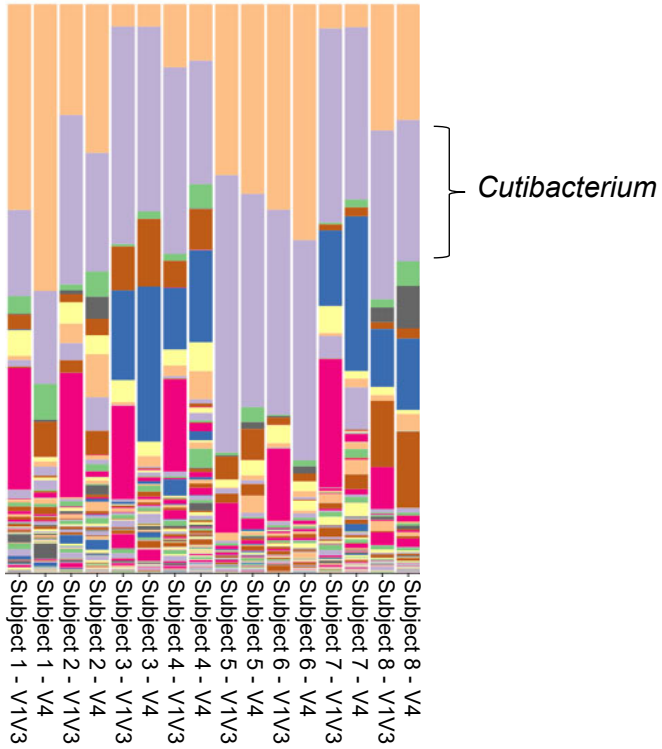


Meisel, J. S., Hannigan, G. D., Tyldsley, A. S., SanMiguel, A. J., Hodkinson, B. P., Zheng, Q., & Grice, E. A. (2016). Skin microbiome surveys are strongly influenced by experimental design. *Journal of Investigative Dermatology*, *136*(5), 947-956.

Diversigen®

Primer editing targeting the 16S rRNA V4 region can improve recovery of *Cutibacterium acnes*



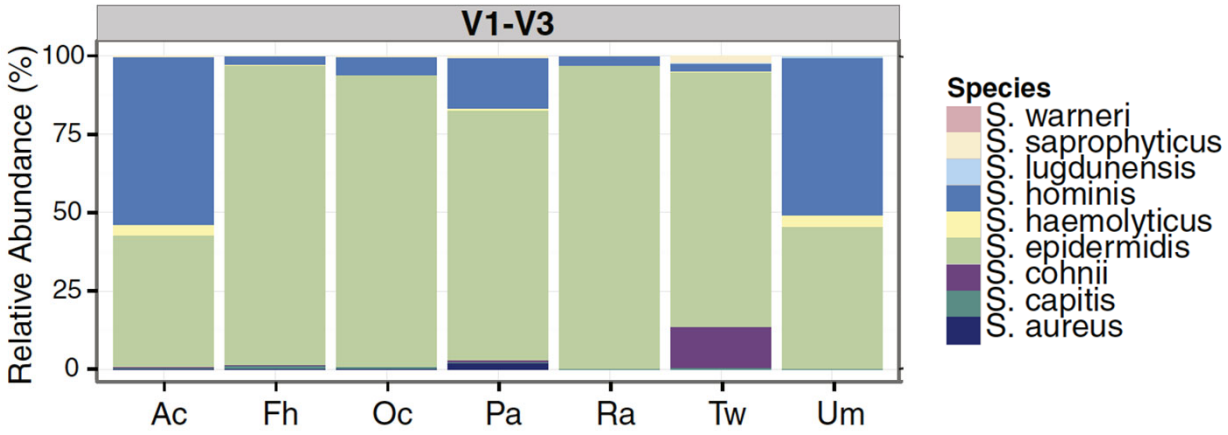


Gohl, D. M., Auch, B., Certano, A., LeFrançois, B., Bouevitch, A., Doukhanine, E., ... & Beckman, K. B. (2021). Dissecting and tuning primer editing by proofreading polymerases. *Nucleic acids research*, *49*(15), e87-e87.

Diversigen®

Limitations of 16S rRNA gene amplicon annotations to identify species

- Usually provide genus-level taxonomic resolution

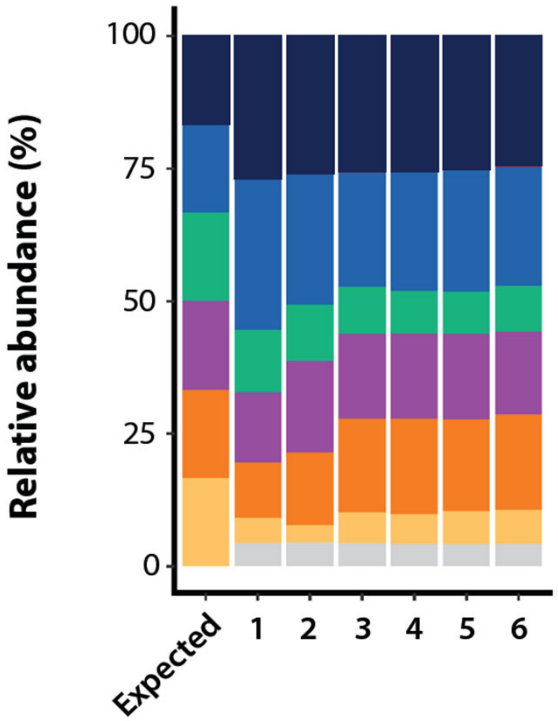


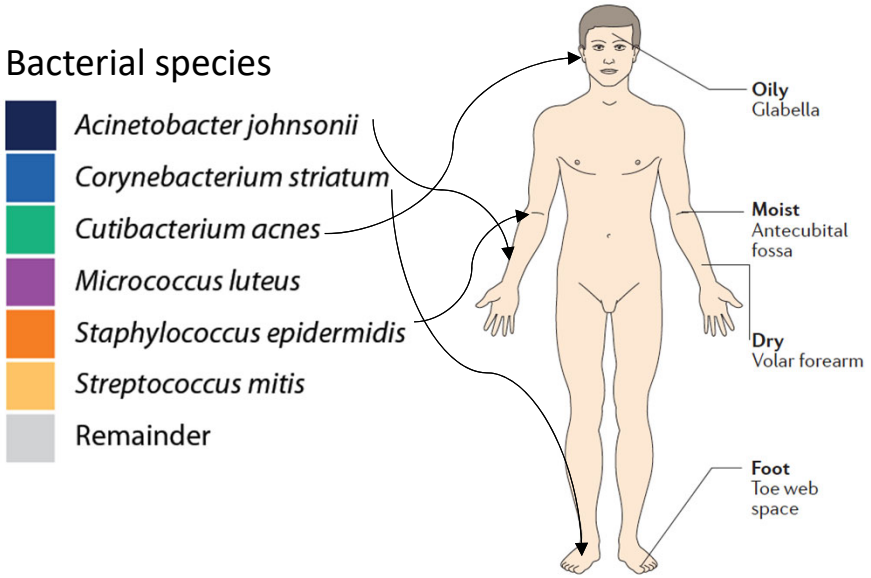
- Species-level resolution of skin microbiota is important
  - Pathogens vs. Commensals
- Phylogenetic placement algorithms have been employed with little success

Meisel, J. S., Hannigan, G. D., Tyldsley, A. S., SanMiguel, A. J., Hodkinson, B. P., Zheng, Q., & Grice, E. A. (2016). Skin microbiome surveys are strongly influenced by experimental design. *Journal of Investigative Dermatology*, *136*(5), 947-956.

Diversigen®

Leveraging 16S amplicon information to accurately identify species





- All bacterial species in ATCC® MSA-1005 skin mock community were detected across replicates

Diversigen®

Accurate species-level resolution among same genera

ATCC® MSA-1002

Expected (ATCC MSA1002)	Alternative	Diversigen
Streptococcus agalactiae	Genus-level identification	Species-level identification
Lactobacillus gasseri	Genus-level identification	Species-level identification
Cutibacterium acnes	Genus-level identification	Species-level identification
Staphylococcus epidermidis	Genus-level identification	Species-level identification
Streptococcus mutants	Species-level identification	Species-level identification
Staphylococcus aureus	Genus-level identification	Species-level identification
Acinetobacter baumannii	Genus-level identification	Species-level identification
Bacteroides vulgatus	Species-level identification	Species-level identification
Bifidobacterium adolescentis	Not identified	Not identified
Clostridium beijerinckii	Genus-level identification	Species-level identification
Deinococcus radiodurans	Genus-level identification	Species-level identification
Escherichia coli	Genus-level identification	Species-level identification
Neisseria meningitidis	Species-level identification	Species-level identification
Porphyromonas gingivalis	Species-level identification	Species-level identification
Pseudomonas aeruginosa	Genus-level identification	Species-level identification
Helicobacter pylori	Species-level identification	Species-level identification
Enterococcus faecalis	Genus-level identification	Species-level identification
Rhodobacter sphaeroides	Species-level identification	Species-level identification
Bacillus pacificus	Genus-level identification	Species-level identification
Schaalia odontolytica (former *Actinomyces*)	Genus-level identification	Genus-level identification

	Alternative	Diversigen
Classified species	7/20	18/20
Unclassified species	12/20	1/20
False negatives	1/20	1/20

Toe web

Alternative	Diversigen
Prevotella colorans	*Prevotella colorans*
Prevotella buccalis	*Prevotella buccalis*
***Cutibacterium* unclassified**	***Cutibacterium acnes***
***Corynebacterium* unclassified**	***Corynebacterium tuberculostearicum***
Methylobacterium-Methylorubrum	***Methylobacterium brachiatum***
***Staphylococcus* unclassified**	***Staphylococcus capitis***
***Staphylococcus* unclassified**	***Staphylococcus pettenkoferi***
***Staphylococcus* unclassified**	***Staphylococcus caprae***
***Staphylococcus* unclassified**	***Staphylococcus epidermidis***
***Staphylococcus* unclassified**	***Staphylococcus hominis***
***Staphylococcus* unclassified**	***Staphylococcus lugdunensis***

Diversigen®

Considerations for selection of databases and annotation tools for skin microbiome research

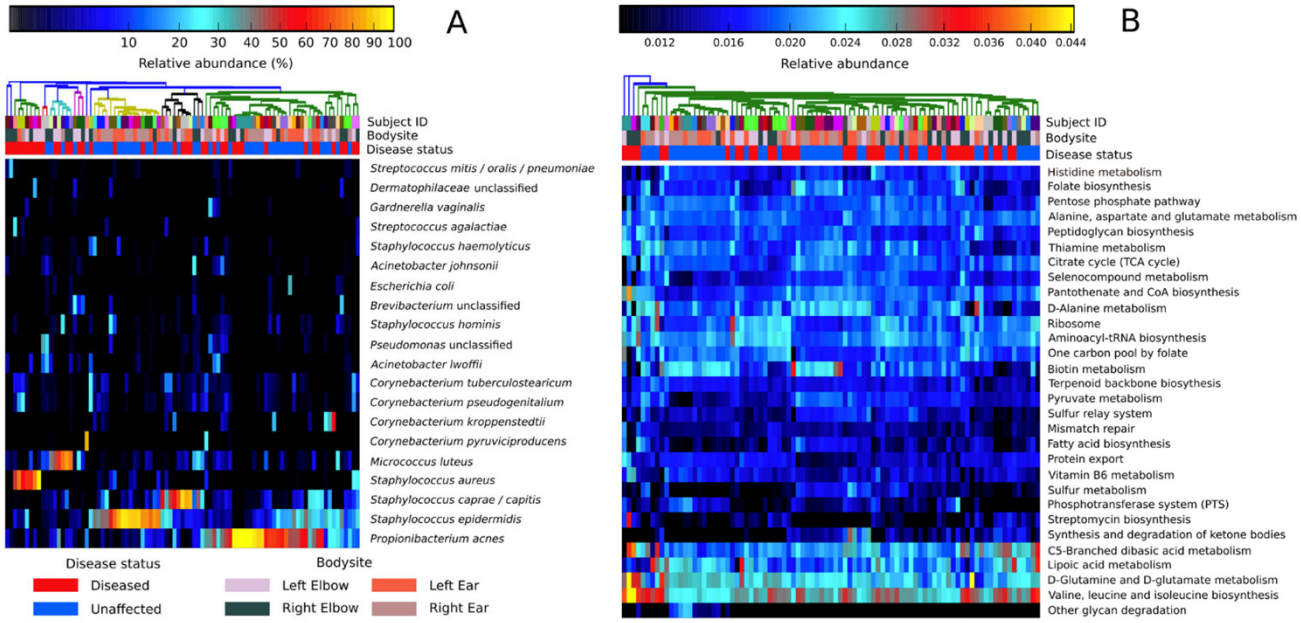
- Databases should be updated and curated on a regular basis
- Databases should provide reliable classifications at the genus and species level when possible
- Annotation tools should provide consistent results across skin sites and conditions
- Number of false positives, false negatives and ambiguous classifications should be minimized

Diversigen®

Next steps and future directions in skin microbiome research

Standardization of whole genome sequencing methods

- Enable bacterial strain identification and identification of other microbes (e.g., viruses, fungi)
- Identify genes with functions related to metabolism, antibiotic resistance and virulence

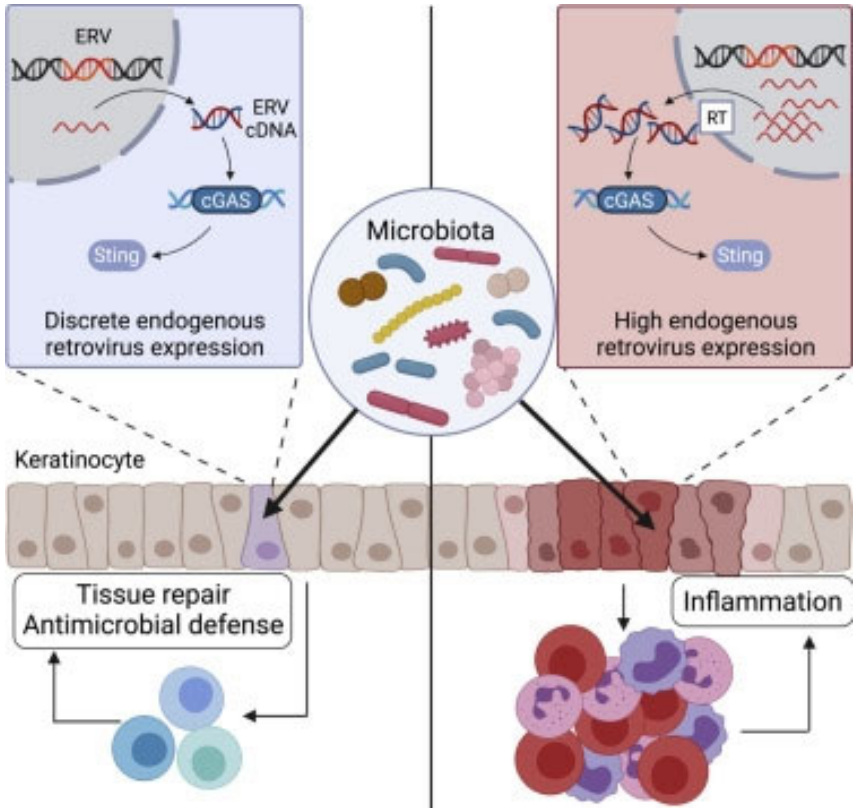


Tett, A., Pasolli, E., Farina, S., Truong, D. T., Asnicar, F., Zolfo, M., ... & Segata, N. (2017). Unexplored diversity and strain-level structure of the skin microbiome associated with psoriasis. *npj Biofilms and Microbiomes*, *3*(1), 1-12.

Diversigen®

Deciphering multi-kingdom interactions in skin microbiome

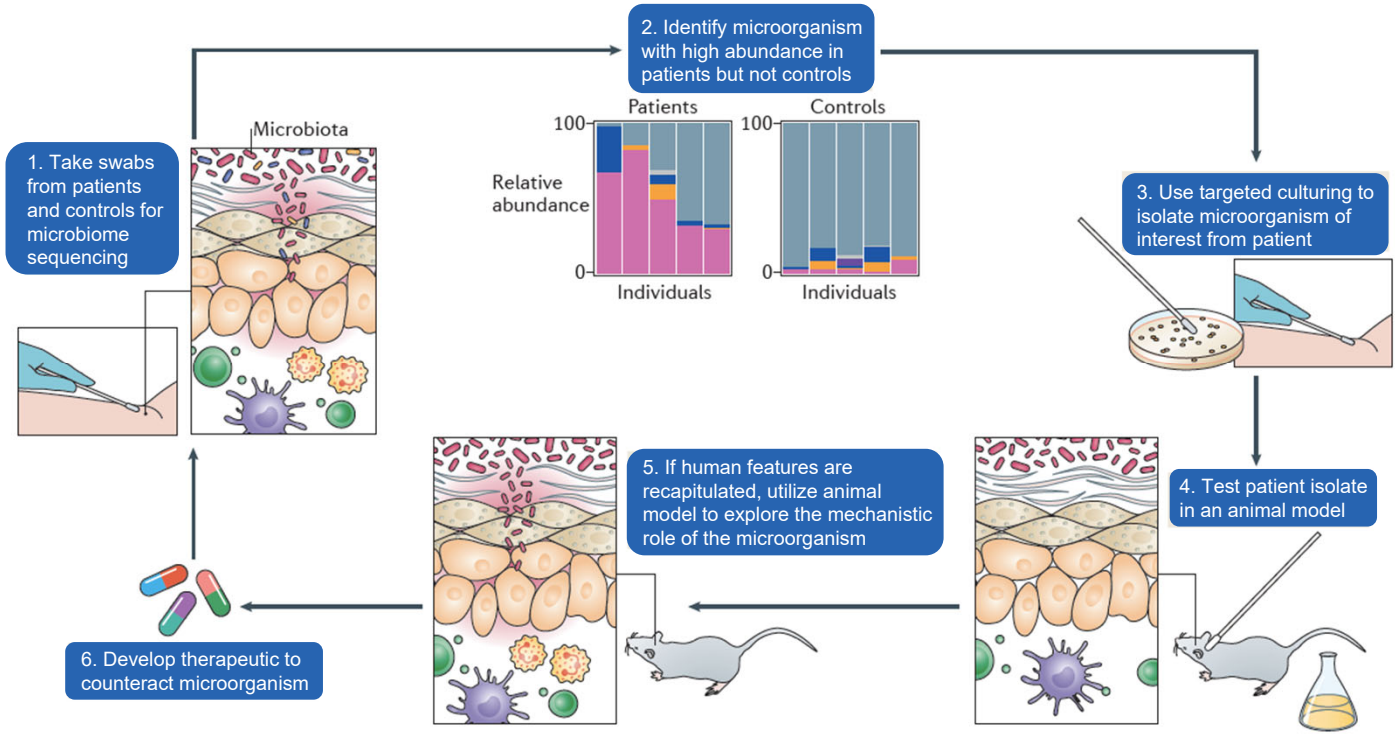
- Skin health and disease may be a function of interactions across organisms from different kingdoms, or multi-kingdom interactions
- Bacteria, fungi and viruses interact with the host and immune system to maintain health or promote disease



Lima-Junior, D. S., Krishnamurthy, S. R., Bouladoux, N., Collins, N., Han, S. J., Chen, E. Y., ... & Belkaid, Y. (2021). Endogenous retroviruses promote homeostatic and inflammatory responses to the microbiota. *Cell*, *184*(14), 3794-3811.

Diversigen®

Developing therapeutic approaches in skin microbiome



Byrd, A. L., Belkaid, Y., & Segre, J. A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, *16*(3), 143-155.

Diversigen®

Future directions in skin microbiome research

Expand understanding of the skin microbiome in health and disease

Continue improvements related to collection, stabilization, extraction and detection for skin microbiome assays

Layer information onto metagenomic, metatranscriptomic and metabolomic studies to provide new biological and clinical insights

Understand potential effects to skin conditions and the skin microbiome from novel treatments and microbial-based therapeutics

Diversigen®

Diversigen®

Questions?

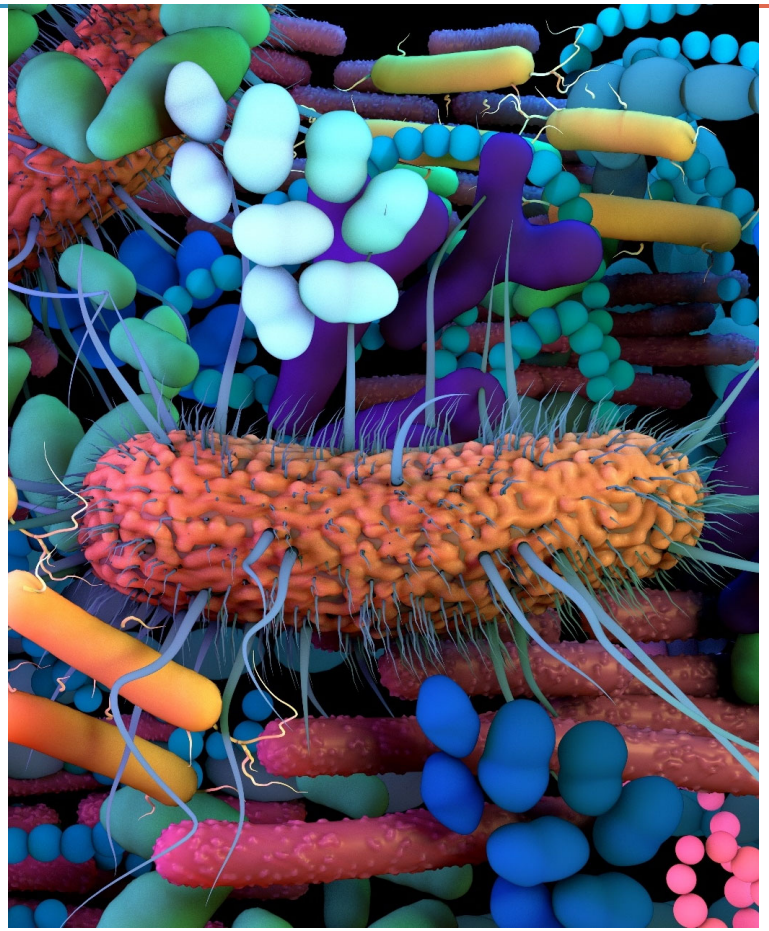
Thank you and questions

For more information about microbiome resources:

www.atcc.org/microbiome
info@diversigen.com
https://www.diversigen.com/services/skinmicrobiome/

Be sure to like and subscribe to ATCC's new podcast, *Behind the Biology*

ATCC®
Behind the Biology



ATCC®