

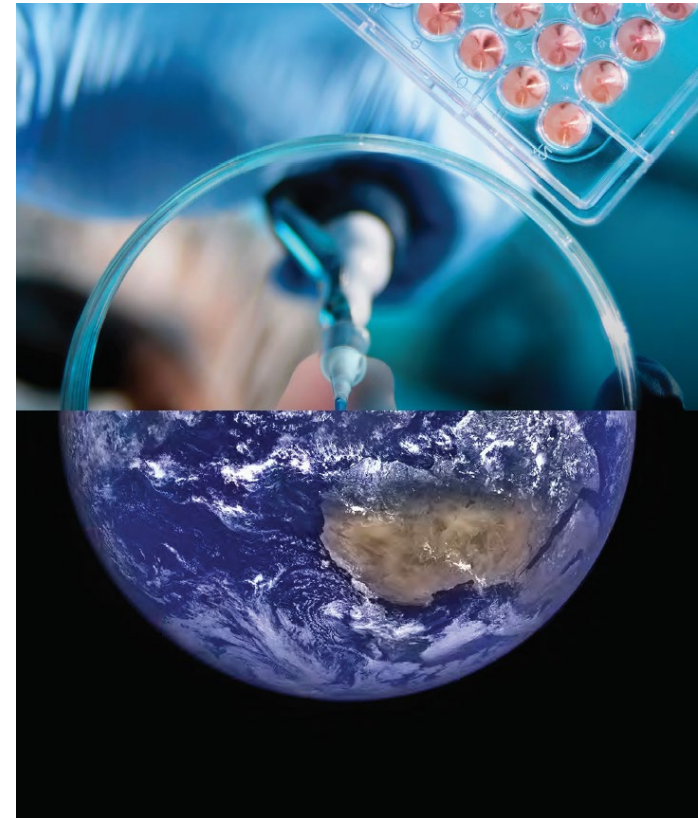
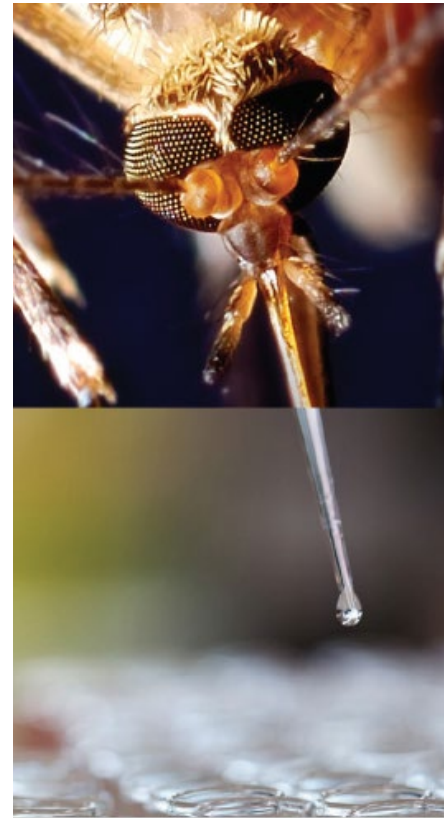
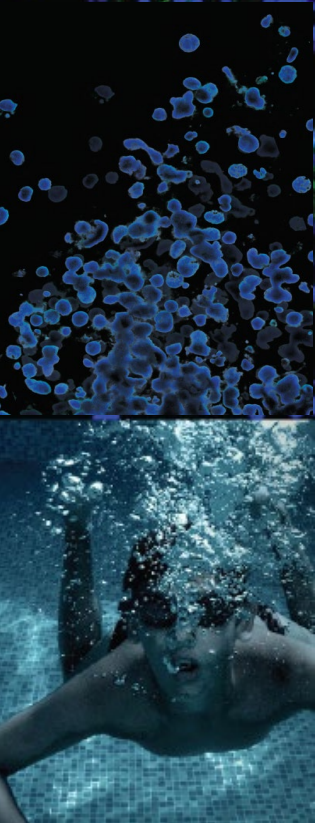


# Development of Fungal Mock Community Standards for Mycobiome Studies

Juan Lopera, PhD  
Scientist, ATCC

ASM Microbe 2019  
San Francisco, CA  
June 21, 2019

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 premier biological materials resource and standards development organization*

- 5,000 cell lines
- 80,000 microorganisms
- Genomic & synthetic nucleic acids
- Media/reagents

- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Growing portfolio of products and services
- Sales and distribution in 150 countries, 17 international distributors
- Talented team of 450+ employees, over one-third with advanced degrees

# An Emerging Need For Reference Materials in Mycobiome Research

frontiers in  
**MICROBIOLOGY**

**REVIEW ARTICLE**  
published: 13 February 2015  
doi: 10.3389/fmicb.2015.00089



**The lung mycobiome: an emerging field of the human respiratory microbiome**

Linh D. N. Nguyen<sup>1</sup>, Eric Viscogliosi<sup>1</sup> and Laurence Delhaes<sup>1,2\*</sup>

Cui et al. *Genome Medicine* 2013, 5:63  
<http://genomemedicine.com/content/5/7/63>



**REVIEW**

**The human mycobiome in health and disease**

Lijia Cui<sup>1</sup>, Alison Morris<sup>2</sup> and Elodie Ghedin<sup>1,3\*</sup>

**Review Article**

**The mycobiome of the human urinary tract: potential roles for fungi in urology**

A. Lenore Ackerman<sup>1</sup>, David M. Underhill<sup>2</sup>

**Cell** Article

**Temporal Stability of the Human Skin Microbiome**

Graphical Abstract

Authors  
Julia Oh, Allyson L. Byrd, Morgan Park, NISC Comparative Sequencing Program, Heidi H. Kong, Julia A. Segre




International Journal of  
*Molecular Sciences*

Review

**The Fungal Mycobiome and Its Interaction with Gut Bacteria in the Host**

Qi Hui Sam<sup>1</sup>, Matthew Wook Chang<sup>2,3</sup> and Louis Yi Ann Chai<sup>1,4,\*</sup>



CellPress

**Through the Scope Darkly:  
The Gut Mycobiome Comes into Focus**

Nydiaris Hernández-Santos<sup>1</sup> and Bruce S. Klein<sup>1,2,3,\*</sup>

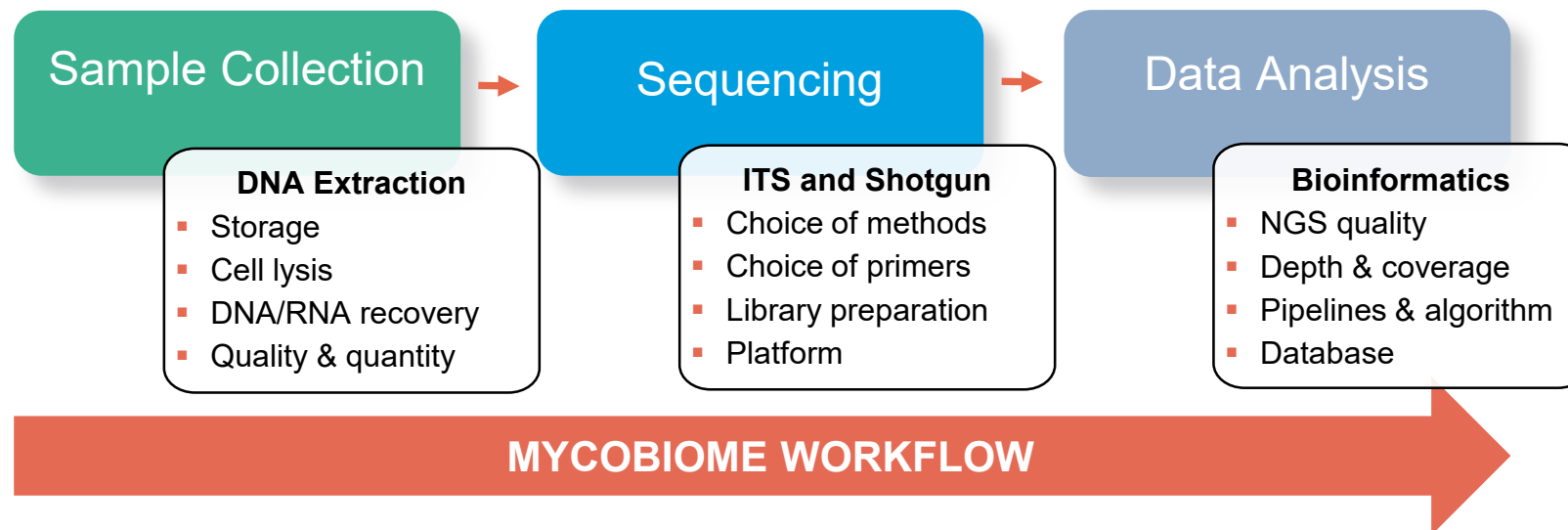
Cell Host & Microbe  
**Previews**

# ATCC® Mycobiome Standards

Product description, research uses, and applications



Standard	ATCC® Catalog No.	Preparation	Description
Mycobiome	MSA-2010™	Whole Cell	Even mixture of whole cells comprising 10 fungal strains (2 x 10 <sup>6</sup> cells of each organism)
	MSA-1010™	Genomic DNA	Even mixture of genomic DNA comprising 10 fungal strains (2 x 10 <sup>6</sup> genome copies of each organism)



# Mycobiome Composition

Fungal strain selection, attributes, and clinical relevance

ATCC® No.	Species Name	Genome Size (Mb)	Relevancy
MYA-4609™	<i>Aspergillus fumigatus</i>	28.8	Opportunistic, airborne pathogen that is responsible for fungal infections in immunocompromised patients.
10231™	<i>Candida albicans</i>	17.1	Commensal fungus of the oral cavity that can form biofilms on denture surfaces, leading to mucosal infections.
2001™	<i>Candida glabrata</i>	12.3	Commensal fungus of the oral cavity and human gut that can acquire resistance to azole antifungals, leading to infection.
208821™	<i>Cryptococcus neoformans</i> var. <i>grubii</i>	18.9	Responsible for cryptococcal meningitis in immunosuppressed patients.
MYA-4612™	<i>Malassezia globosa</i>	9.0	Part of the normal skin flora, but can be responsible for skin diseases such as dandruff, dermatitis, and folliculitis.
201390™	<i>Saccharomyces cerevisiae</i>	12.2	Bakers' and brewers' yeast originating in food. Emerging pathogen in immunocompromised patients.
9533™	<i>Trichophyton interdigitale</i>	21.9	Can infect skin and nails to cause chronic infections such as athlete's foot and ringworm.
204094™	<i>Cutaneotrichosporon dermatis</i> ( <i>Trichosporon dermatis</i> )	23.3	Emerging opportunistic agent of invasive fungal infections, particularly in severely immunocompromised patients.
10106™	<i>Penicillium chrysogenum</i>	32.5	Spore-former, less prevalent, but can be responsible for intestinal infection in immunosuppressed patients.
36031™	<i>Fusarium keratoplasticum</i> ( <i>F. solani</i> complex)	48.6	Filamentous, opportunistic pathogen that causes fungal keratitis.

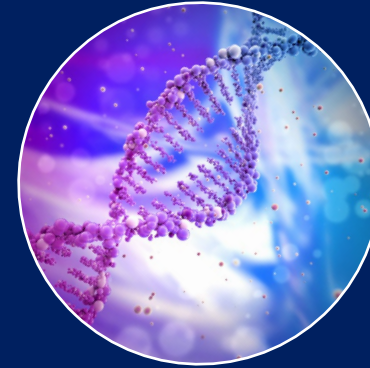
# Development of Mycobiome Standards

*Products specifications*



## Whole Cell Standards

- Authenticated ATCC cultures
- Growth and image cytometry cell counting
- Mixed in even proportion based on cell number
- Storage at 4°C



## Genomic DNA Standards

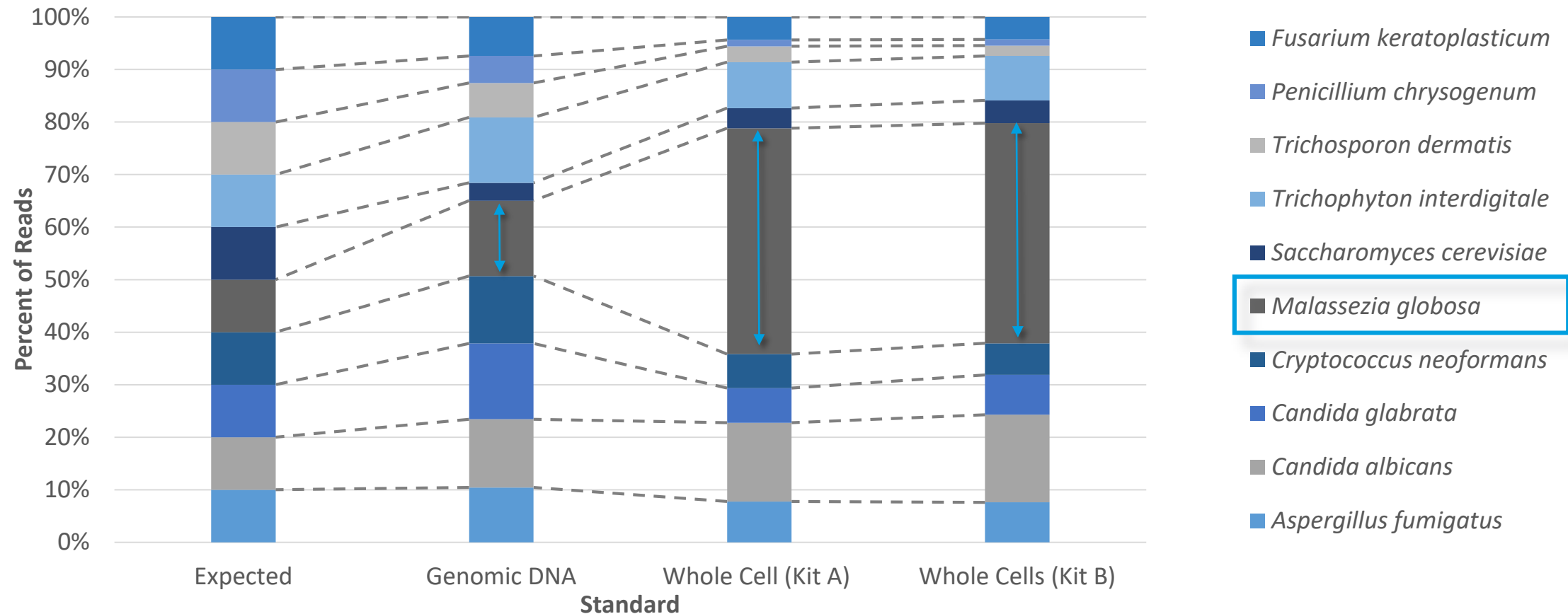
- Authenticated ATCC nucleic acids
- Fluorescent dye-based quantification
- Mixed in even proportions based on genome copy number
- Storage at -20°C

**Assay development, optimization, verification, and quality control**

# Evaluation of DNA Extraction Kits

Whole cell mock mycobiome communities can be used to evaluate the impact of DNA extraction on the mycobiome analysis

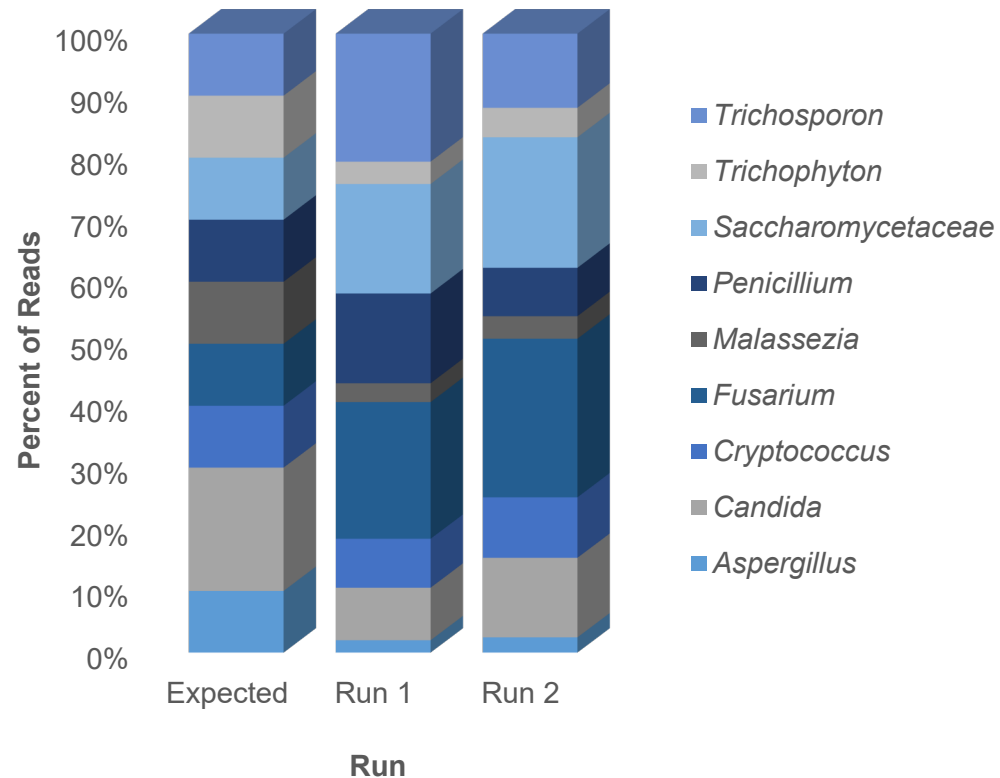
## Shotgun Metagenomic Analysis of Mycobiome Standards (ATCC® MSA-2010™ and MSA-1010™)



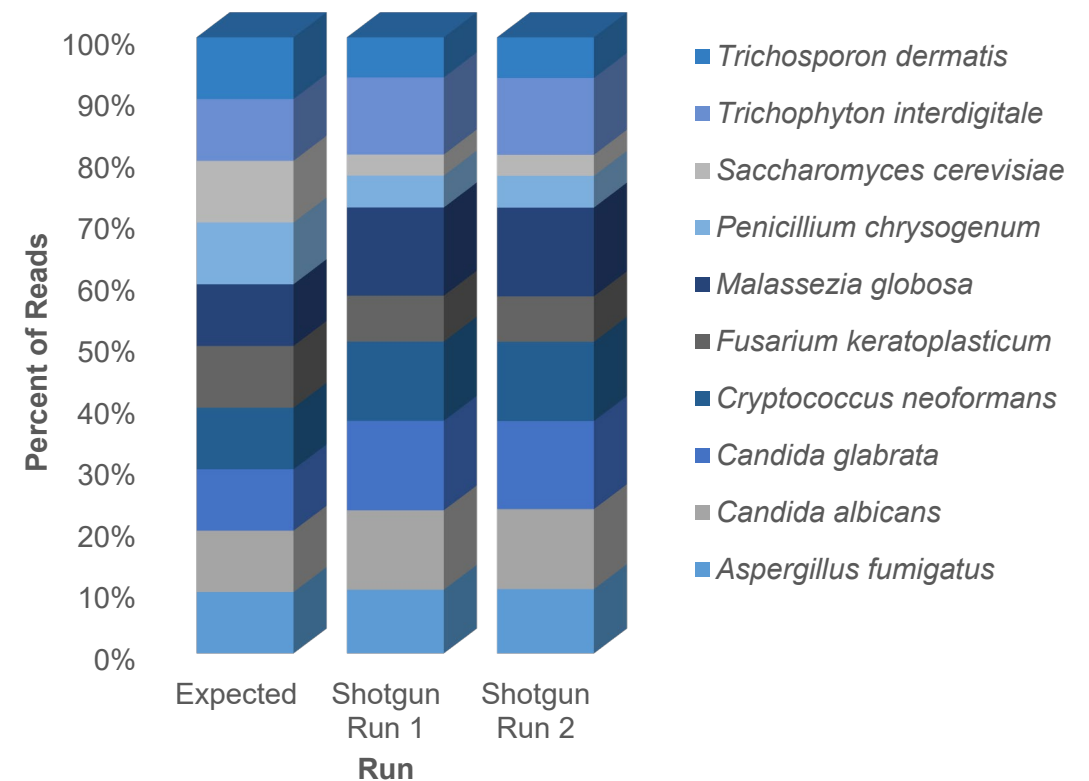
# Evaluation of ITS and Shotgun Analyses

*ITS analysis can resolve up to the genus level whereas shotgun metagenomics can identify to the species level*

**ITS Analysis – Mycobiome Standards (ATCC® MSA-1010™)**



**Shotgun Analysis – Mycobiome Standards (ATCC® MSA-1010™)**

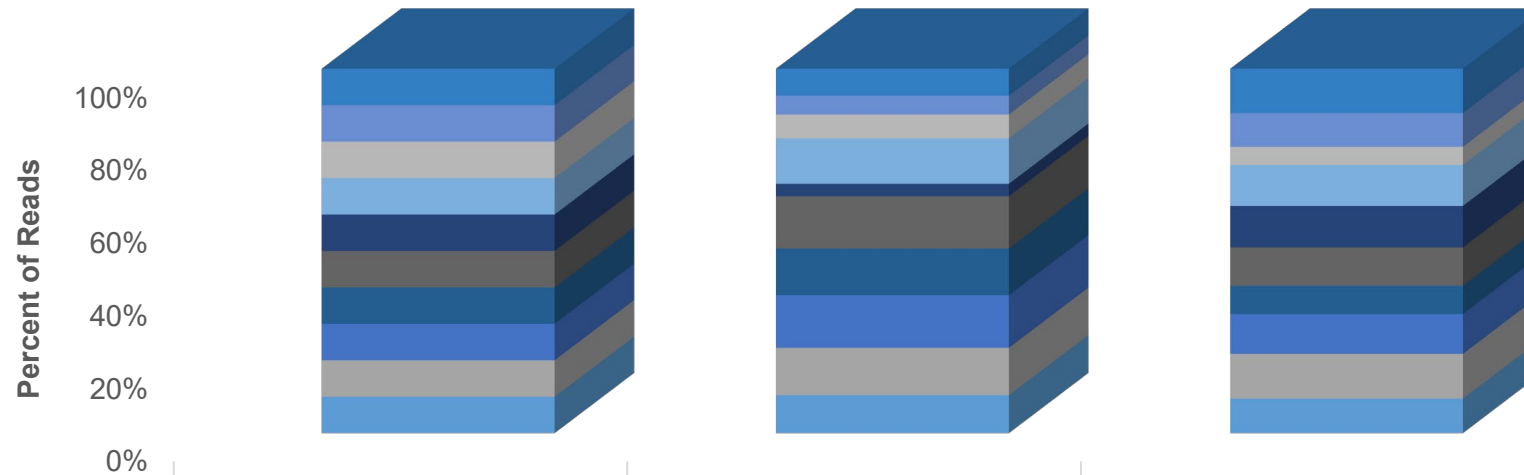




# Evaluation of Short- and Long-Read Technologies

ATCC Mycobiome Standards are platform agnostic

## Genomic DNA Standard (ATCC® MSA-1010™): Short- (Illumina) vs Long-Read (ONT) Sequencing

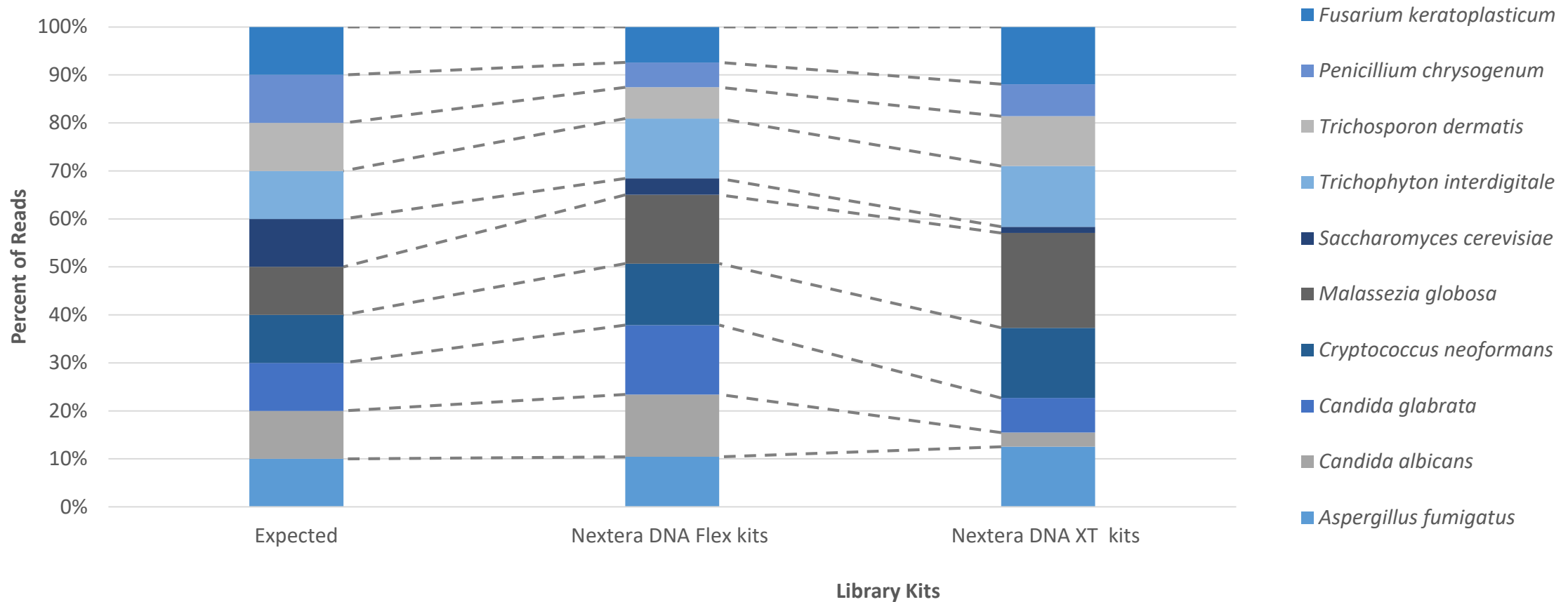


	Expected	Short-Read (Illumina)	Long-Read (ONT)
■ <i>Fusarium keratoplasticum</i>	10%	7.4%	12.2%
■ <i>Penicillium chrysogenum</i>	10%	5.2%	9.3%
■ <i>Trichosporon dermatis</i>	10%	6.5%	5.0%
■ <i>Trichophyton interdigitale</i>	10%	12.5%	11.2%
■ <i>Saccharomyces cerevisiae</i>	10%	3.4%	11.4%
■ <i>Malassezia globosa</i>	10%	14.3%	10.5%
■ <i>Cryptococcus neoformans</i>	10%	12.8%	7.7%
■ <i>Candida glabrata</i>	10%	14.4%	11.0%
■ <i>Candida albicans</i>	10%	13.0%	12.3%
■ <i>Aspergillus fumigatus</i>	10%	10.4%	9.5%

# Evaluation of Library Preparation Kits

Comparison of different library preparation kits with the genomic DNA Mycobiome Standards

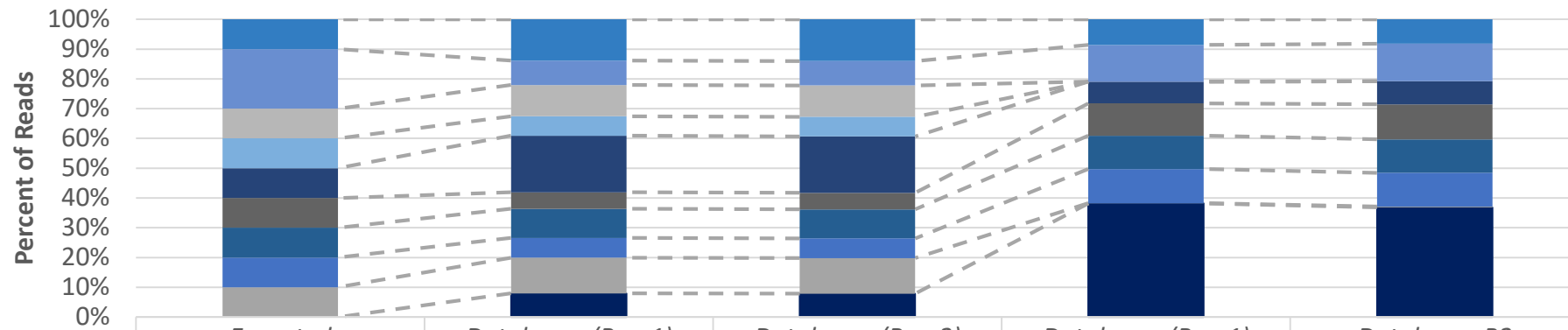
## Shotgun Analysis of Genomic DNA Standards (ATCC® MSA-1010™ )



# Evaluation of Analysis Platforms and Genome Databases

Data analysis platform impacts strain identification and taxonomic resolution

## Shotgun Analysis of Genomic DNA Standards (ATCC® MSA-1010™ )



	Expected	Databases (Run 1)	Databases (Run 2)	Databases (Run 1)	Databases B2
Aspergillus	10%	13.88%	13.99%	8.60	8.16
Candida	20%	8.17%	8.22%	12.31	12.61
Cryptococcus	10%	10.52%	10.51%	0.00	0.00
Cutaneotrichosporon	10%	6.53%	6.61%	0.00	0.00
Fusarium	10%	19.00%	18.94%	7.32	7.78
Malassezia	10%	5.53%	5.55%	10.86	11.74
Penicillium	10%	9.81%	9.76%	11.17	11.25
Saccharomyces	10%	6.63%	6.62%	11.48	11.35
Trichophyton	10%	12.01%	11.98%	0.05	0.29
Unclassified	0%	7.93%	7.82%	38.21	36.82

# Acknowledgements

Monique Hunter, MS

Kendra Grosso, BS

Pushpa Gujjari, MS

Sung-Oui Suh, PhD

Anna McCluskey, BS

Stephen King, MS

Briana Benton, BS

Dev Mittar, PhD

Jonathan Hetzel, PhD, Illumina®

Nick Greenfield, MA, One Codex

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# Thank you!

# Questions?

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