An NGS Workflow Designed/Optimized for Sterility Testing in Gene and Cell Therapy Products

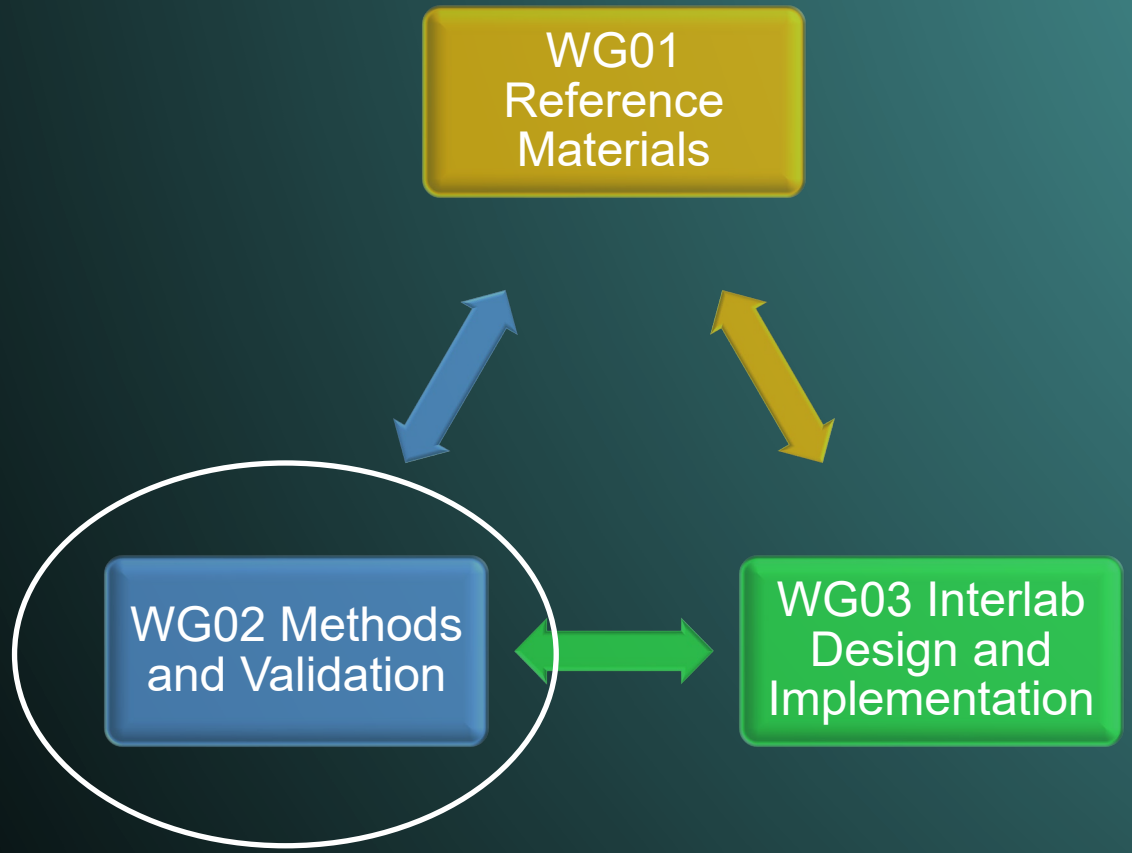
The NIST Rapid Microbial Testing Methods (RMTM) Consortium

April 8th, 2025
Scott Jackson
Tyler Laird

National Institute of Standards and Technology
U.S. Department of Commerce

Graphic Credit: Natasha Hanacek

RMTM CONSORTIUM WORKING GROUPS



Next Generation Sequencing (NGS)-Based Sterility Testing

- In 2023-ish, the RMTM WG02 committed to the development of NGS-based tools for microbial sterility testing in the biomanufacturing environment.

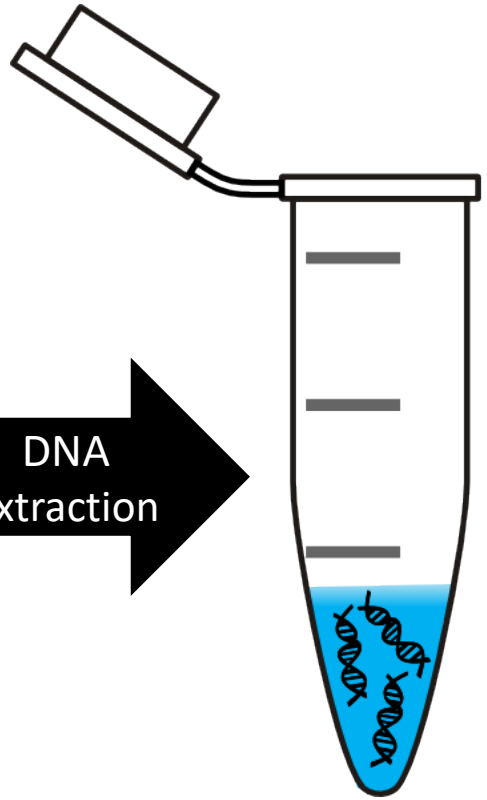
NGS-Based Metagenomics as an RMTM

- Untargeted microbial detection – “see everything”
- “Agnostic Diagnostics”
- Being adopted across various fields/disciplines
  - Infectious Disease Diagnostics
  - Epidemiology
  - Environmental Biosurveillance
  - Biothreat Detection
  - Biomanufacturing QC – Adventitious Agent Detection
- NIST has been developing standards for NGS-based microbial detection and identification for ~ 10 years

Metagenomics 101

A Complex Sample

DNA Extraction



Genomic DNA

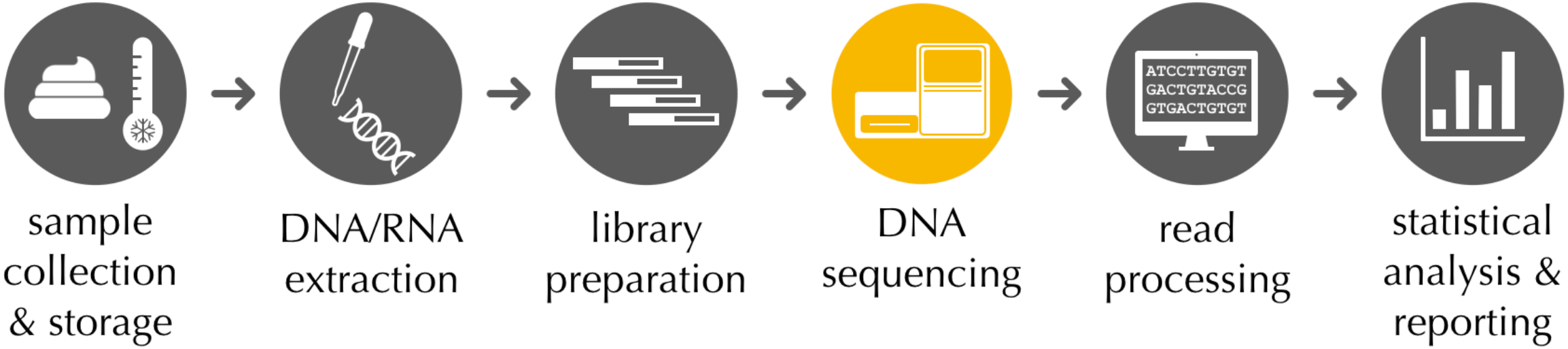
DNA Sequencing

Culture Independent!

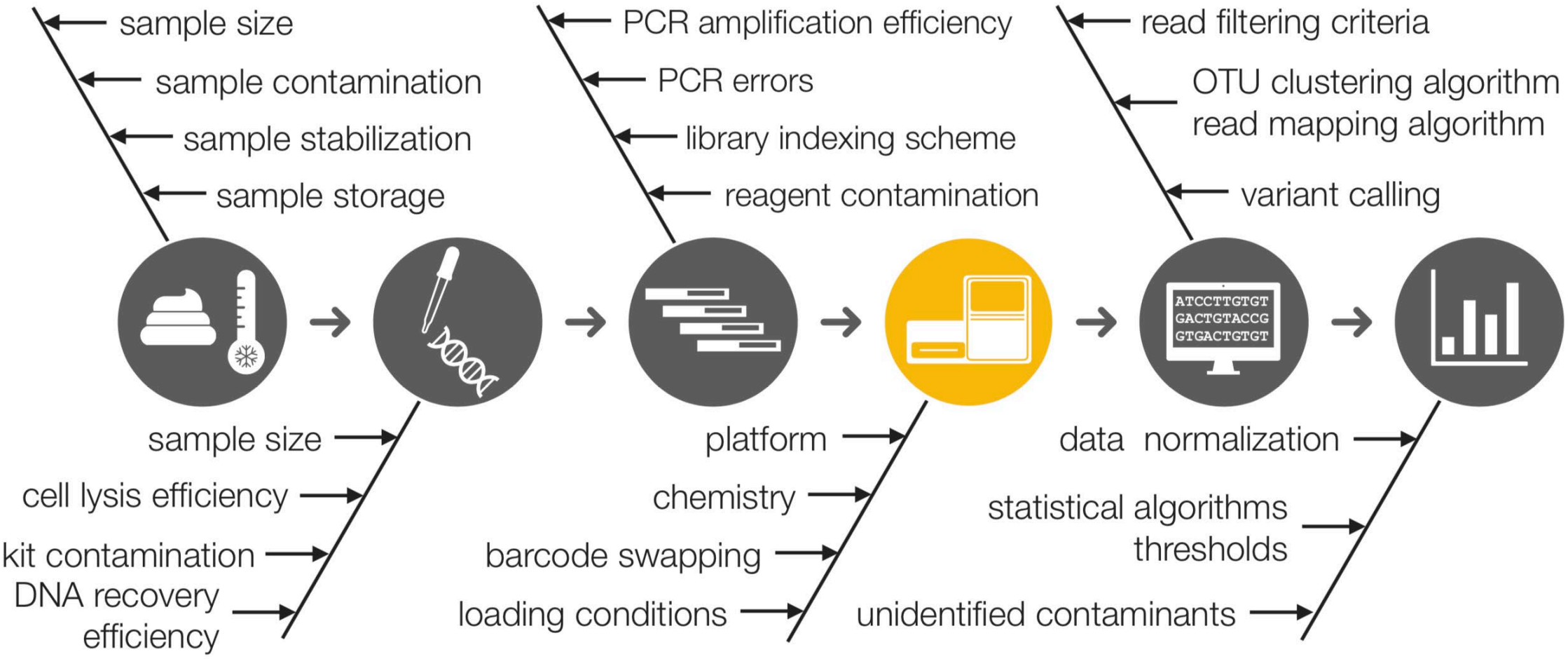
TCACTGGTTAATCAGGGATGTCTTCATAAGATTATACTTG
GGACTTCAGATACGCTAGAATTTAAAGGGTCTCTTACACC
CTACTTGTCTCAGTCTAATAACGCGCGAAGCCGTGGGGCA
GGACGCTAATATGGGTGAATAGAGACTTATATCATCAGGG

DNA Sequence Data

NGS-Based Metagenomics
A Multitude of Methodological Variables



A Multitude of Methodological Variables



An International Interlaboratory Study: Assessing the Impact of Metagenomic Methodological Variability

scientific reports

Check for updates

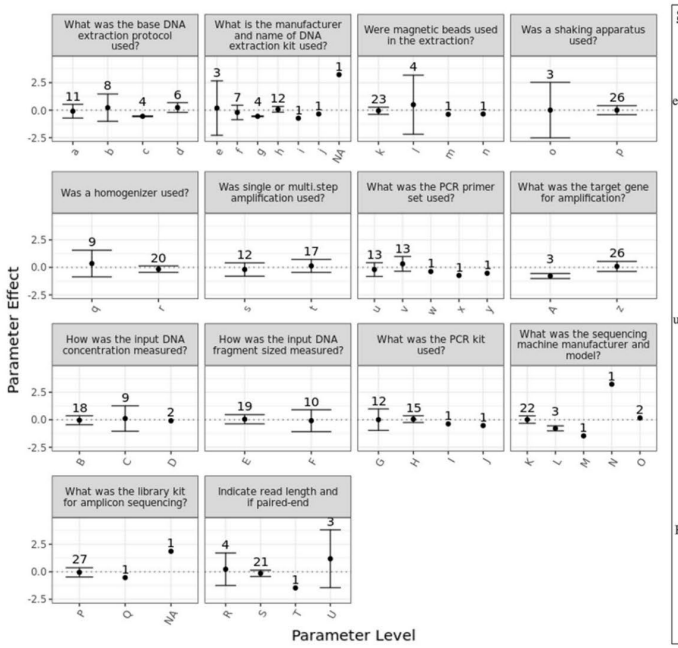
OPEN

Variability and bias in microbiome metagenomic sequencing: an interlaboratory study comparing experimental protocols

Samuel P. Forry[1]✉, Stephanie L. Servetas[1], Jason G. Kralj[1], Keng Soh[2], Michalis Hadjithomas[3], Raul Cano[4], Martha Carlin[4], Maria G. de Amorim[5], Benjamin Auch[6], Matthew G. Bakker[7], Thais F. Bartelli[5], Juan P. Bustamante[10,8,9], Ignacio Cassol[8], Mauricio Chalita[11], Emmanuel Dias-Neto[5], Aaron Del Duca[12], Daryl M. Gohl[13,6], Jekaterina Kazantseva[14], Muyideen T. Haruna[15], Peter Menzel[16], Bruno S. Moda[17,5], Lorieza Neuberger-Castillo[18], Diana N. Nunes[5], Isha R. Patel[19], Rodrigo D. Peralta[10,8], Adrien Saliou[20], Rolf Schwarzer[16], Samantha Sevilla[21,22], Isabella K. T. M. Takenaka[5], Jeremy R. Wang[23], Rob Knight[24], Dirk Gevers[25] & Scott A. Jackson[1]

Several studies have documented the significant impact of methodological choices in microbiome analyses. The myriad of methodological options available complicate the replication of results and generally limit the comparability of findings between independent studies that use differing techniques and measurement pipelines. Here we describe the Mosaic Standards Challenge (MSC), an international interlaboratory study designed to assess the impact of methodological variables on the results. The MSC did not prescribe methods but rather asked participating labs to analyze 7 shared reference samples (5 × human stool samples and 2 × mock communities) using their standard laboratory methods. To capture the array of methodological variables, each participating lab completed a metadata reporting sheet that included 100 different questions regarding the details of their protocol. The goal of this study was to survey the methodological landscape for microbiome metagenomic sequencing (MGS) analyses and the impact of methodological decisions on

www.nature.com/scientificreports/



Symbols	Parameter	Option
a, b,c,c	What was the base DNA extraction protocol used?	(a) Internal, (b) Others, (c) HMP, (d) IHMS
e, f, g, h, i, j	What is the manufacturer and name of DNA extraction kit used?	(e) In-house protocol, (f) No-kit Other method, (g) Mo Bio DNeasy PowerSoil Kit, (h) QIAGEN QilAmpStoolMinikit, (i) Chemagic DNA Blood Kit, (j) Promega Maxwell
k, l, m, n	Were magnetic beads used in the extraction?	(k) glass_beads_0,1mm, (l) No, (m) silica_beads_0,1mm, (n) ceramic_beads_>1mm
o, p	Was a shaking apparatus used?	(o) No, (p) Yes
q, r	Was a homogenizer used?	(q) No, (r) Yes
s, t	Was single or multi.step amplification used?	(s) Single, (t) Multi
u, v, w, x, y	What was the PCR primer set used?	(u) NA, (v) V4 515F/ 806R, (w) Schloss 2013, (x) 318F/806R, (y) EMP V4 515F/806R
z, A	What was the target gene for amplification?	(z) 16S rRNA, (A) Other
B, C, D	How was the input DNA concentration measured?	(B) PicoGreen, (c) Qubit, (D) Other
E, F	How was the input DNA fragment sized measured?	(E) Agilent Bioanalyzer, (F) Other
G, H, I, J	What was the PCR kit used?	(G) Other, (H) Kapa HI Fi Polymerase, (I) NEB Q5 Polymerase, (J) AccuPrime Taq Polymerase High Fidelity
K, L, M, N, O	What was the sequencing machine manufacturer and model?	(K) Illumina MiSeq, (L) Illumina NextSeq 500, (M) Thermofisher Ion Torrent S5, (N) Other, (O) Illumina MiniSeq
P, Q	What was the library kit for amplicon sequencing?	(P) Other, (Q) QIAseq 1-Step Amplicon Library Kit
R, S, T, U	Indicate read length and if paired-end	(R) 100 PE, (S) 150 PE, (T) 100 SE, (U) 250 PE

Figure 7. Within labs performing 16S amplicon sequencing, the parameter effect on the Firmicutes:Bacteroidetes ratio was calculated as described in Fig. 6 for each relevant metadata parameter. Shown here from just one stool sample, results from other stool samples were similar and are provided in Fig. SI-6.

An Intralaboratory Assessment of Methodological Variables Associated with Metagenomic Measurements

AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Spectrum

RESEARCH ARTICLE
February 2025 Volume 13 Issue 2 e00696-24
https://doi.org/10.1128/spectrum.00696-24

A sensitivity analysis of methodological variables associated with microbiome measurements

Samuel P. Forry [1], Stephanie L. Servetas[1], Jennifer N. Dootz[1], Monique E. Hunter[1], Jason G. Kralj [1], James J. Filliben[2], Scott A. Jackson[1]

[1]Complex Microbial Systems Group, National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA
[2]Multimodal Information Group, National Institute of Standards and Technology (NIST), Gaithersburg, Maryland, USA

The experimental methods employed during metagenomic sequencing analyses of microbiome samples significantly impact the resulting data and typically vary substantially between laboratories. In this study, a full factorial experimental design was used to compare the effects of a select set of methodological choices (sample, operator, lot, extraction kit, variable region, and reference database) on the analysis of biologically diverse stool samples. For each parameter investigated, a main effect was calculated that allowed direct comparison both between methodological choices (bias effects) and between samples (real biological differences). Overall, methodological bias was found to be similar in magnitude to real biological differences while also exhibiting significant variations between individual taxa, even between closely related genera. The quantified method biases were then used to computationally improve the comparability of data sets collected under substantially different protocols. This investigation demonstrates a framework for quantitatively assessing methodological choices that could be routinely performed by individual laboratories to better understand their metagenomic sequencing workflows and to improve the scope of the datasets they produce.

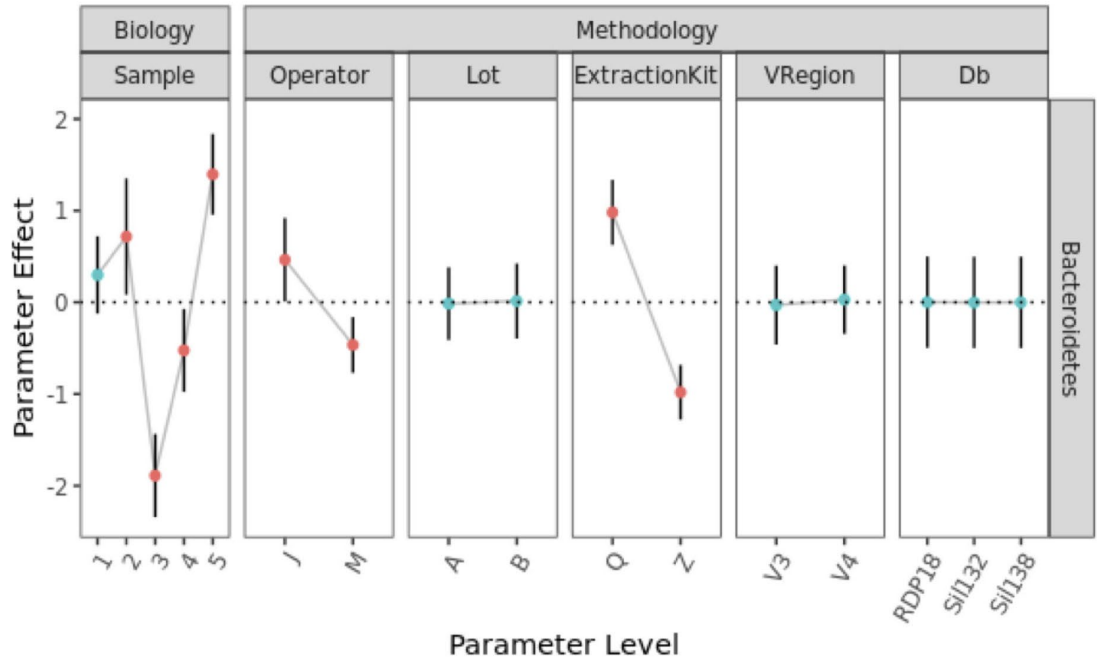


FIG 6 Quantitative comparison between methodological parameters. The Bacteroidetes:*Leifsonia* ratio was calculated for each data set to enable comparison between protocols. The effect of each parameter was calculated by dividing the average ratio for all data sets at the denoted parameter level by the average ratio across all parameter levels, as shown in equation 1. This parameter effect was plotted as a fold change on a log2 scale, such that the horizontal line at 0 denotes the null hypothesis of no effect. The magnitude of the effect of protocol choices (e.g., extraction kit) can be directly compared between parameters and parameter levels. Data error bars showed 99% confidence intervals, and the points that are statistically significantly different from the mean ($P < 0.01$) were colored red.

Tools Needed for mNGS as an RMTM

- Custom Reference Genome Database with ‘Relevant’ Organisms
- Custom Bioinformatic Analysis Tools - Sequence Alignment
- All analysis tools should be open-source
  - But proprietary (commercial) tools are welcome (cloud analytics)
- Spike-Ins and analysis framework for data normalization
- Benchmarking Studies – Require Benchmarking Materials
  - In silico datasets - metagenomes

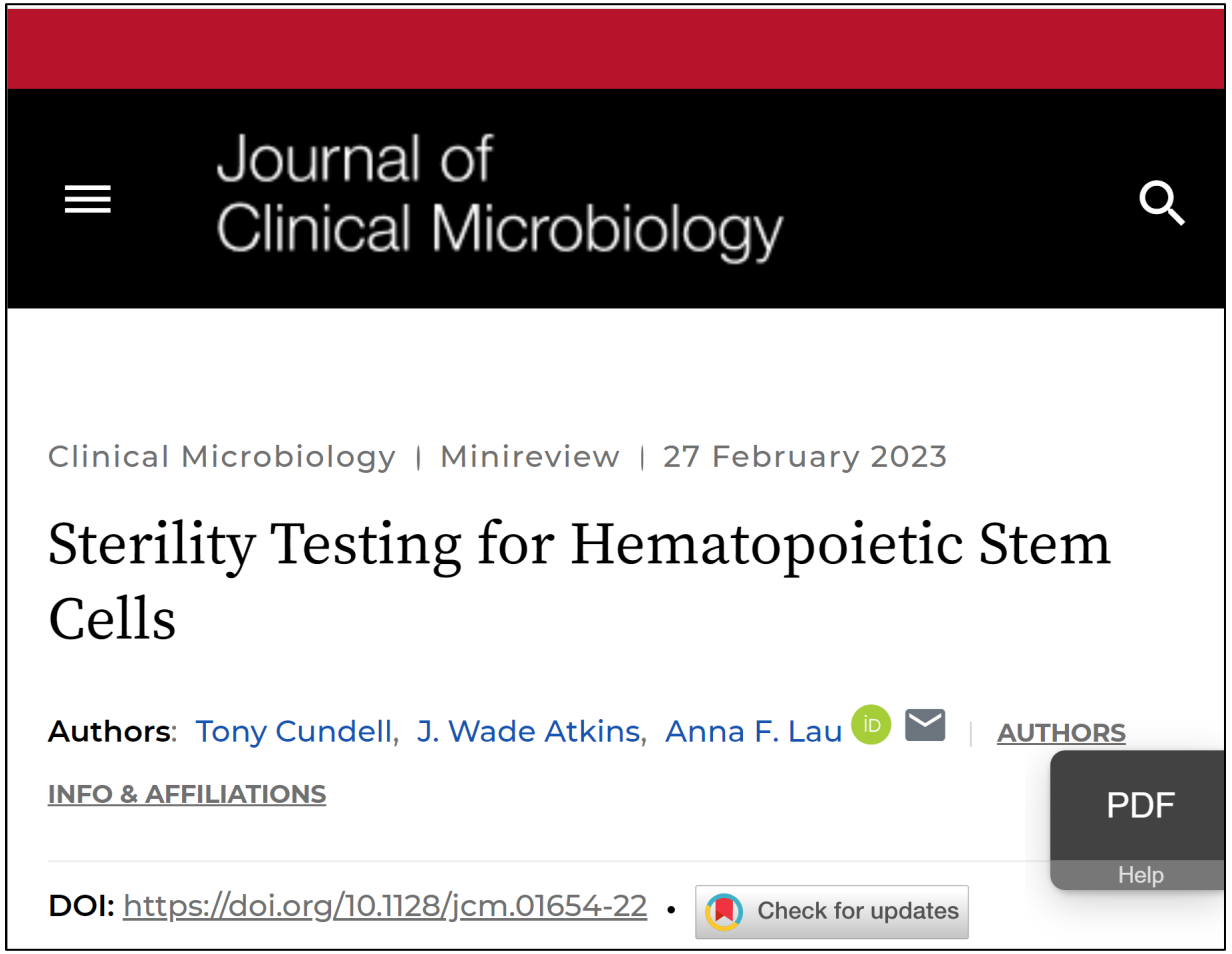
The Reference Genome Database

What do we include?
What not to include?

NCBI Currently has >1 million microbial reference genomes

USP Compendial Organisms
7-8 organisms

Bacterial and Fungal Contaminants in Cell Therapy Products



Journal of Clinical Microbiology

Clinical Microbiology | Minireview | 27 February 2023

Sterility Testing for Hematopoietic Stem Cells

Authors: Tony Cundell, J. Wade Atkins, Anna F. Lau

AUTHORS

INFO & AFFILIATIONS

DOI: https://doi.org/10.1128/jcm.01654-22 •

PDF

Help

Check for updates

TABLE 4 Frequency of bacterial and fungal HSC contaminants and typical source ranked by frequency of organism occurrence based on literature reports spanning from 2003 to 2021

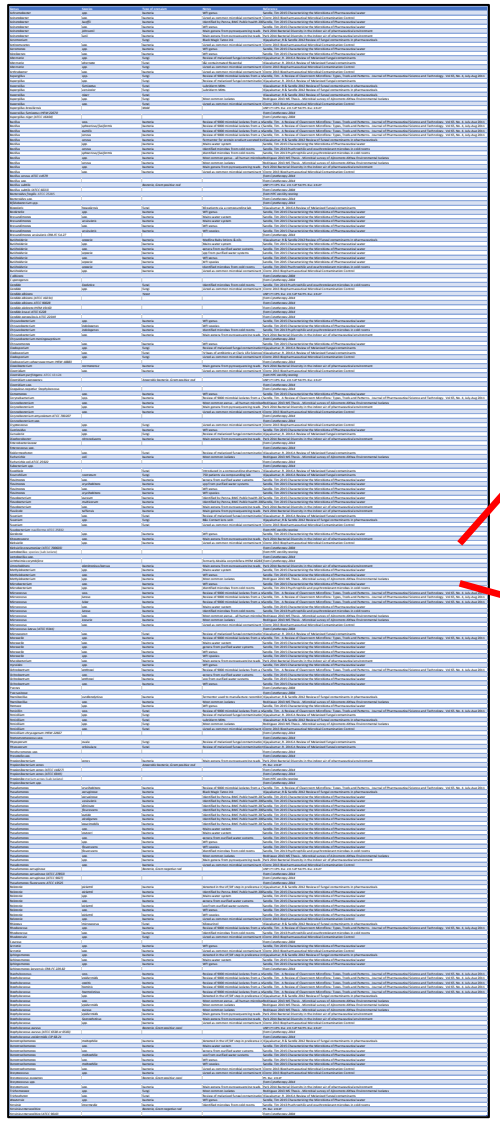
Organism	Typical source	Frequency reported	References
Coagulase-negative *Staphylococcus* spp.	Skin	17	9–11, 19, 24–26, 29–32, 36, 38, 39, 42, 47, 49
Staphylococcus aureus	Skin	9	11, 24, 27, 30–32, 38, 42, 47
Bacillus spp.	Environment	7	24, 25, 29, 32, 36, 47, 49
Corynebacterium spp.	Skin	7	11, 19, 25, 39, 42, 47, 49
Cutibacterium acnes	Skin	7	10, 11, 19, 24, 36, 42, 49
Escherichia coli	Enteric	7	9, 19, 25, 26, 30, 31, 42
Streptococcus spp.	Oral	7	24, 25, 30–32, 42, 49
Pseudomonas aeruginosa	Environment	6	9, 19, 29–31, 42
Ralstonia pickettii	Water	5	10, 24, 31, 39, 42
Enterococcus faecalis	Enteric	4	26, 31, 42, 49
Bacteroides spp.	Enteric	3	25, 27, 49
Clostridium spp.	Enteric	3	9, 10, 25
Enterobacter spp.	Enteric	3	11, 42, 47
Enterococcus faecium	Enteric	3	26, 27, 30
Klebsiella pneumoniae	Enteric	3	25, 26, 30
Sphingomonas paucimobilis	Water	3	9, 38, 39
Acinetobacter spp.	Environment	2	24, 30, 42
Aspergillus spp.	Environment	2	25, 32
Candida parapsilosis	Environment	2	25, 39
Chryseobacterium spp.	Environment	2	10, 42
Enterococcus spp.	Enteric	2	25, 49
Lactobacillus spp.	Enteric	2	26, 29
Micrococcus spp.	Skin	2	32, 42
Mold, no further identification	Environment	2	32, 42
Penicillium spp.	Environment	2	10, 32
Peptostreptococcus spp.	Enteric	2	25, 49
Proteus mirabilis	Enteric	2	38, 49
Pseudomonas fluorescens/Pseudomonas putida	Environment	2	42, 49
Stenotrophomonas maltophilia	Environment	2	42, 49
Actinomyces spp.	Oral	1	49
Aerococcus spp.	Environment	1	25
Aeromonas hydrophila	Water	1	31
Bifidobacterium spp.	Enteric	1	25
Bipolaris spicifera	Environment	1	11
Burkholderia cepacia	Environment	1	25
Candida albicans	Oral, enteric	1	10
Chaetomium spp.	Environment	1	42
Citrobacter spp.	Enteric	1	47
Enteric Gram-negative rod, no further identification	Enteric	1	39
Histoplasma capsulatum	Environment	1	9
Kocuria spp.	Skin	1	32
Methylobacterium spp.	Environment, water	1	42
Moraxella spp.	Oral	1	42
Rhodotorula spp.	Environment	1	32
Roseomonas spp.	Water	1	26
Rothia spp.	Skin, oral	1	49
Salmonella enterica	Enteric	1	40
Shigella spp.	Enteric	1	38
Ustilago spp.	Environmental	1	10
Veillonella spp.	Oral	1	24

“Minimal List” of Microbial Strains for NGS-Metagenomics Database Developed via SCB RMM Consortium

Genus	spp.	Type
Aspergillus	*fumigatus*	Mold
Aspergillus	*versicolor*	Mold
Aspergillus	*niger*	Mold
Bacillus	*cereus*	*Bacteria, Gram positive rod*
Bacillus	*subtilis*	*Bacteria, Gram positive rod*
Brevundimonas	*vesicularis*	*Bacteria, Gram negative rod*
Burkholderia	*cepacia*	*Bacteria, Gram negative rod*
Candida	*albicans*	Yeast
Candida	*glabrata*	Yeast
Candida	*krusei*	Yeast
Chryseobacterium	*indologenes*	*Bacteria, Gram negative rod*
Cladosporium	*sphaerospermum*	Mold
Clostridium	*perfringens*	*Anaerobic bacteria, Gram positive rod*
Clostridium	*sporogenes*	*Anaerobic bacteria, Gram positive rod*
Corynebacterium	*spp.*	Bacteria, Gram-positive rod
Fusarium	*spp.*	Mold
Klebsiella	*pneumoniae*	*Bacteria, Gram negative rod*
Lactobacillus	*spp.*	*Anaerobic bacteria, Gram positive rod*
Micrococcus	*luteus*	*Bacteria, Gram positive cocci*
Penicillium	*spp.*	Mold
Propionibacterium	*acnes*	*Anaerobic bacteria, Gram positive rod*
Pseudomonas	*aeruginosa*	*Bacteria, Gram negative rod*
Pseudomonas	*flourescens*	*Bacteria, Gram negative rod*
Ralstonia	*pickettii*	*Bacteria, Gram negative rod*
Serratia	*marcesens*	*Bacteria, Gram negative rod*
Sphingomonas	*spp.*	*Bacteria, Gram negative rod*
Staphylococcus	*epidermidis*	*Bacteria, Gram positive cocci*
Staphylococcus	*aureus*	*Bacteria, Gram positive cocci*
Stenotrophomonas	*maltophilia*	*Bacteria, Gram negative rod*
Streptococcus	*pyogenes*	*Bacteria, Gram positive cocci*
Yersinia	*enterocolitica*	*Bacteria, Gram negative rod*

https://drive.google.com/drive/folders/13XAfcyMnZC95EmyxjzJ_99BcV8Z3aaYT?usp=drive_link

Expanded List of 245 Microbial Strains for NGS-Metagenomics Database



Cloacibacterium	*normanense*	bacteria	Main genera from pyrosequencing reads	Park 2014 Bacterial Diversity in the indoor air of pharmace
Clostridium	*spp.*	bacteria	Listed as common microbial contaminant in biopharm	Clontz 2013 Biopharmaceutical Microbial Contamination C
Clostridium perfringens ATCC 13 124				*from HPC sterility testing*
Clostridium sporogenes		*Anaerobic bacteria,*		USP<71>/Ph. Eur. 2.6.1/JP 54/ Ph. Eur. 2.6.27
Clostridium spp.				*from Cytotherapy 2014*
Coagulase-negative Staphylococcus				*from Cytotherapy 2014*
Comamonas	*spp.*	bacteria	WFI genus	Sandle, Tim 2015 Characterizing the Microbiota of Pharma
Corynebacterium	spp.	bacteria	Review of 9000 microbial isolates from a range of differer	Sandle, Tim. A Review of Cleanroom Microflora: Types, Tr
Corynebacterium	*spp.*	bacteria	Most common genus - all human microbes	Rodriguez 2013 MS Thesis - Microbial survey of Ajinomoto

245 Environmental Isolates

https://drive.google.com/drive/folders/13XAfcyMnZC95EmyxjzJ_99BcV8Z3aaYT?usp=drive_link

But Wait! There's More!

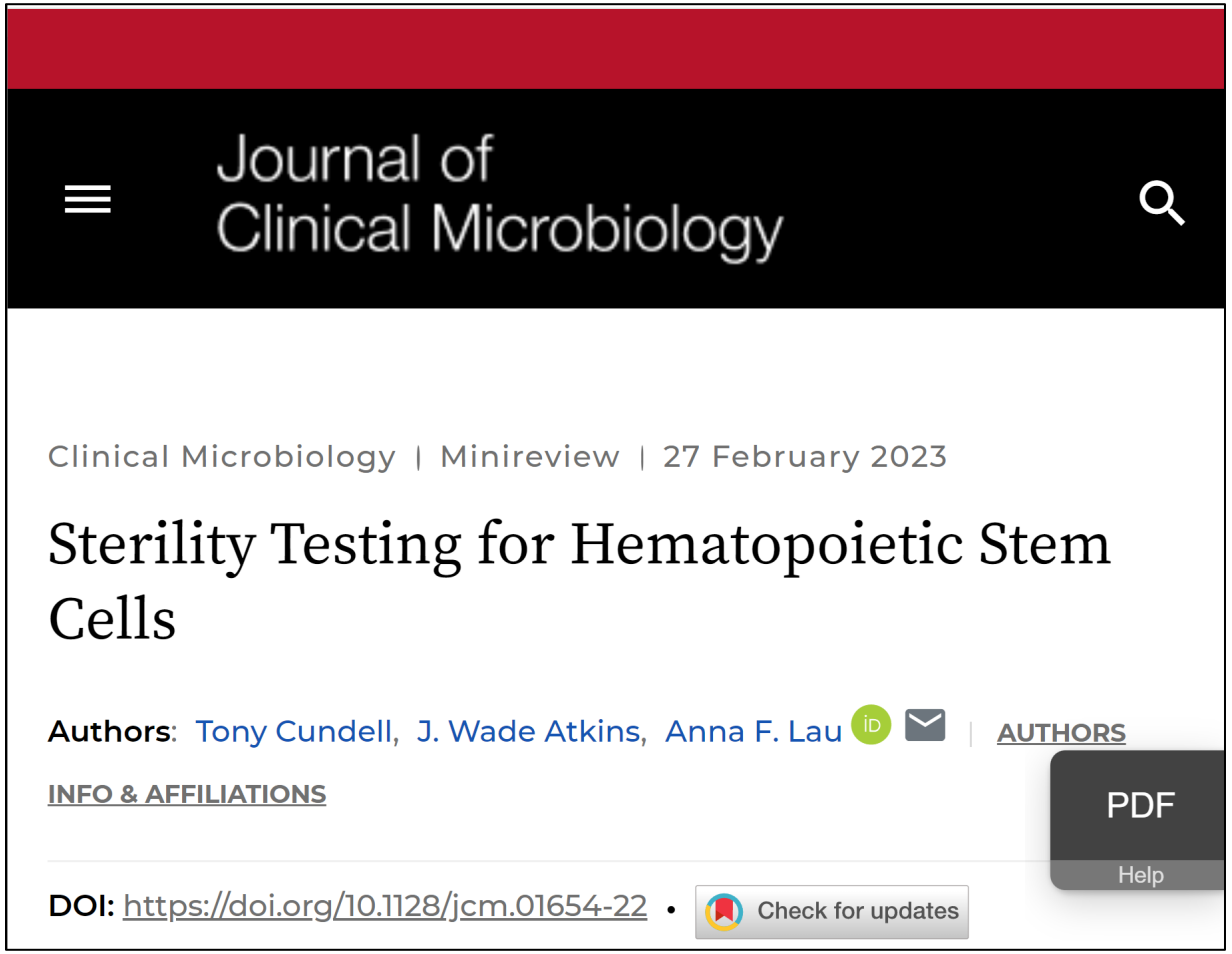
Genus	spp.	Type
Aspergillus	fumigatus	Mold
Aspergillus	versicolor	Mold
Aspergillus	niger	Mold
Bacillus	cereus	Bacteria, Gram positive rod
Bacillus	subtilis	Bacteria, Gram positive rod
Brevundimonas	vesicularis	Bacteria, Gram negative rod
Burkholderia	cepacia	Bacteria, Gram negative rod
Candida	albicans	Yeast
Candida	glabrata	Yeast
Candida	krusei	Yeast
Chryseobacterium	indologenes	Bacteria, Gram negative rod
Cladosporium	sphaerospermum	Mold
Clostridium	perfringens	Anaerobic bacteria, Gram positive rod
Clostridium	sporogenes	Anaerobic bacteria, Gram positive rod
Corynebacterium	spp.	Bacteria, Gram-positive rod
Fusarium	spp.	Mold
Klebsiella	pneumoniae	Bacteria, Gram negative rod
Lactobacillus	spp.	Anaerobic bacteria, Gram positive rod
Micrococcus	luteus	Bacteria, Gram positive cocci
Penicillium	spp.	Mold
Propionibacterium	acnes	Anaerobic bacteria, Gram positive rod
Pseudomonas	aeruginosa	Bacteria, Gram negative rod
Pseudomonas	flourescens	Bacteria, Gram negative rod
Ralstonia	pickettii	Bacteria, Gram negative rod
Serratia	marcesens	Bacteria, Gram negative rod
Sphingomonas	spp.	Bacteria, Gram negative rod
Staphylococcus	epidermidis	Bacteria, Gram positive cocci
Staphylococcus	aureus	Bacteria, Gram positive cocci
Stenotrophomonas	maltophilia	Bacteria, Gram negative rod
Streptococcus	pyogenes	Bacteria, Gram positive cocci
Yersinia	enterocolitica	Bacteria, Gram negative rod

There are >160 species of *Corynebacterium*

Corynebacterium comprises the following species:[50]

- *C. accolens* Neubauer et al. 1991
- *C. afermentans* Riegel et al. 1993
- *C. alimapuense* Claverias et al. 2019
- "*C. alkanolyticum*" Lee and Reichenbach 2006
- *C. ammoniagenes* (Cooke and Keith 1927) Collins 1987
- *C. amycolatum* Collins et al. 1988
- *C. anserum* Liu et al. 2021
- *C. appendicis* Yassin et al. 2002
- *C. aquatimens* Aravena-Román et al. 2012
- *C. aquilae* Fernández-Garayzábal et al. 2003
- *C. argentoratense* Riegel et al. 1995
- "*C. asperum*" De Briel et al. 1992
- *C. atrinae* Kim et al. 2015
- *C. atypicum* Hall et al. 2003
- *C. aurimucosum* Yassin et al. 2002
- *C. auris* Funke et al. 1995
- *C. auriscanis* Collins et al. 2000
- *C. belfantii* Dazas et al. 2018
- *C. beticola* Abdou 1969 (Approved Lists 1980)
- "*C. bouchesdurhonense*" Ndongo et al. 2017
- "*C. bouchesdurhonense*" Lo et al. 2019
- *C. bovis* Bergey et al. 1923 (Approved Lists 1980)
- *C. callunae* (Lee and Good 1963) Yamada and Komagata 1972 (Approved Lists 1980)
- *C. camporealensis* Fernández-Garayzábal et al. 1998
- *C. canis* Funke et al. 2010
- *C. capitovis* Collins et al. 2001
- *C. casei* Brennan et al. 2001
- *C. caspium* Collins et al. 2004
- *C. choanae* Busse et al. 2019
- *C. ciconiae* Fernández-Garayzábal et al. 2004
- *C. comes* Schaffert et al. 2021
- *C. confusum* Funke et al. 1998
- *C. coyleae* Funke et al. 1997
- *C. crudilactis* Zimmermann et al. 2016
- *C. cystitidis* Yanagawa and Honda 1978 (Approved Lists 1980)
- "*C. defluvii*" Yu et al. 2017
- "*C. dentalis*" Benabdelkader et al. 2020
- *C. deserti* Zhou et al. 2012
- *C. diphtheriae* (Kruse 1886) Lehmann and Neumann 1896 (Approved Lists 1980)
- *C. doosanense* Lee et al. 2009
- *C. durum* Riegel et al. 1997
- *C. efficiens* Fudou et al. 2002
- *C. endometrii* Ballas et al. 2020
- *C. epidermidicanis* Frischmann et al. 2012
- *C. faecale* Chen et al. 2016
- *C. falsenii* Sjödén et al. 1998
- *C. felinum* Collins et al. 2001
- *C. flavescens* Barksdale et al. 1979 (Approved Lists 1980)
- *C. fournieri* corrig. Diop et al. 2018
- *C. frankenforstense* Wiertz et al. 2013
- *C. freiburgense* Funke et al. 2009
- *C. freneyi* Renaud et al. 2001
- *C. gerontici* Busse et al. 2019
- *C. glaucum* Yassin et al. 2003
- *C. glucuronolyticum* Funke et al. 1995
- *C. glutamicum* (Kinoshita et al. 1958) Abe et al. 1967 (Approved Lists 1980)
- *C. glycinophilum* (ex Kubota et al. 1972) Al-Dilaimi et al. 2015
- *C. gottingense* Atasayar et al. 2017
- *C. guangdongense* Li et al. 2016
- "*C. haemomassiliense*" Boxberger et al. 2020
- *C. halotolerans* Chen et al. 2004
- *C. hansenii* Renaud et al. 2007
- *C. heidelbergense* Braun et al. 2021
- *C. hindlerae* Bernard et al. 2021
- *C. humireducens* Wu et al. 2011
- "*C. ihumii*" Padmanabhan et al. 2014
- *C. ilicis* Mandel et al. 1961 (Approved Lists 1980)
- *C. imitans* Funke et al. 1997
- "*C. incognitum*" Boxberger et al. 2021
- *C. jeddahense* Edouard et al. 2017
- *C. jeikeium* Jackman et al. 1988
- *C. kalinowskii* Schaffert et al. 2021
- "*C. kefirresidentii*" Blasche et al. 2017
- *C. kroppenstedtii* Collins et al. 1998
- *C. kutscheri* (Migula 1900) Bergey et al. 1925 (Approved Lists 1980)
- *C. lactis* Wiertz et al. 2013
- "*C. lactofermentum*" Gubler et al. 1994
- *C. jeikliangguodongiiium* Zhu et al. 2020
- *C. lipophiloflavum* Funke et al. 1997
- *C. lizhenjunii* Zhou et al. 2021
- *C. lowii* Bernard et al. 2016
- *C. lubricantis* Kämpfer et al. 2009
- *C. lujinxingii* Zhang et al. 2021
- *C. macginleyi* Riegel et al. 1995
- *C. marinum* Du et al. 2010
- *C. maris* Ben-Dov et al. 2009
- *C. massiliense* Merhej et al. 2009
- *C. mastitidis* Fernandez-Garayzabal et al. 1997
- *C. matruchotii* (Mendel 1919) Collins 1983
- *C. minutissimum* (ex Sarkany et al. 1962) Collins and Jones 1983
- *C. mucifaciens* Funke et al. 1997
- *C. mustelae* Funke et al. 2010
- *C. mycetoides* (ex Castellani 1942) Collins 1983
- *C. nasicanis* Baumgardt et al. 2015
- "*C. neomassiliense*" Boxberger et al. 2020
- *C. nuruki* Shin et al. 2011
- *C. occultum* Schaffert et al. 2021
- *C. oculi* Bernard et al. 2016
- *C. otitidis* (Funke et al. 1994) Baek et al. 2018
- "*C. pacaense*" Bellali et al. 2019
- "*C. parakroppenstedtii*" Luo et al. 2022
- "*C. parvulum*" Nakamura et al. 1983
- *C. pelargi* Kämpfer et al. 2015
- *C. phocae* Pascual et al. 1998
- "*C. phoceense*" Cresci et al. 2016
- *C. pilbarense* Aravena-Roman et al. 2010
- *C. pilosum* Yanagawa and Honda 1978 (Approved Lists 1980)
- *C. pollutisoli* Negi et al. 2016
- *C. propinquum* Riegel et al. 1994
- "*C. provencense*" Ndongo et al. 2017
- "*C. provencense*" Lo et al. 2019
- *C. pseudodiphtheriticum* Lehmann and Neumann 1896 (Approved Lists 1980)
- "*C. pseudokroppenstedtii*" Luo et al. 2022
- *C. pseudopelargi* Busse et al. 2019
- *C. pseudotuberculosis* (Buchanan 1911) Eberson 1918 (Approved Lists 1980)
- *C. pyruviciproducens* Tong et al. 2010
- *C. qintianiae* Zhou et al. 2021
- *C. renale* (Migula 1900) Ernst 1906 (Approved Lists 1980)
- *C. resistens* Otsuka et al. 2005
- *C. riegelii* Funke et al. 1998
- *C. rouxii* Badell et al. 2020
- *C. sanguinis* Jaén-Luchoro et al. 2020
- "*C. segmentosum*" Collins et al. 1998
- "*C. senegalense*" Ndiaye et al. 2019
- *C. silvaticum* Dangel et al. 2020
- *C. simulans* Wattiau et al. 2000
- *C. singulare* Riegel et al. 1997
- *C. sphenisci* Goyache et al. 2003
- *C. spheniscorum* Goyache et al. 2003
- *C. sputi* Yassin and Siering 2008
- *C. stationis* (ZoBell and Upham 1944) Bernard et al. 2010
- *C. striatum* (Chester 1901) Eberson 1918 (Approved Lists 1980)
- *C. suicordis* Vela et al. 2003
- *C. sundsvallense* Collins et al. 1999
- *C. suranareeae* Nantapong et al. 2020
- *C. tapiri* Baumgardt et al. 2015
- *C. terpenotabidum* Takeuchi et al. 1999
- *C. testudinoris* Collins et al. 2001
- *C. thomssenii* Zimmermann et al. 1998
- *C. timonense* Merhej et al. 2009
- *C. trachiae* Kämpfer et al. 2015
- *C. tuberculostearicum* Feurer et al. 2004
- *C. tuscaniense* corrig. Riegel et al. 2006
- "*C. uberis*" Kittl et al. 2022
- *C. ulcerans* (ex Gilbert and Stewart 1927) Riegel et al. 1995
- *C. ulceribovis* Yassin 2009
- *C. urealyticum* Pitcher et al. 1992
- *C. ureicelerivorans* Yassin 2007
- "*C. urinapleomorphum*" Morand et al. 2017
- *C. urinipleomorphum* corrig. Niang et al. 2021
- *C. urogenitale* Ballas et al. 2020
- *C. uropygiale* Braun et al. 2016
- *C. uterequi* Hoyles et al. 2013
- *C. variabile* corrig. (Müller 1961) Collins 1987
- *C. vitaeruminis* corrig. (Bechdel et al. 1928) Lanéelle et al. 1980
- *C. wankanglinii* Zhang et al. 2021
- *C. xerosis* (Lehmann and Neumann 1896) Lehmann and Neumann 1899 (Approved Lists 1980)
- *C. yudongzhengii* Zhu et al. 2020
- *C. zhongnanshanii* Zhang et al. 2021

More Bacterial and Fungal Contaminants



Journal of Clinical Microbiology

Clinical Microbiology | Minireview | 27 February 2023

Sterility Testing for Hematopoietic Stem Cells

Authors: Tony Cundell, J. Wade Atkins, Anna F. Lau

AUTHORS

INFO & AFFILIATIONS

PDF

Help

DOI: https://doi.org/10.1128/jcm.01654-22 •

Check for updates

TABLE 4 Frequency of bacterial and fungal HSC contaminants and typical source ranked by frequency of organism occurrence based on literature reports spanning from 2003 to 2021

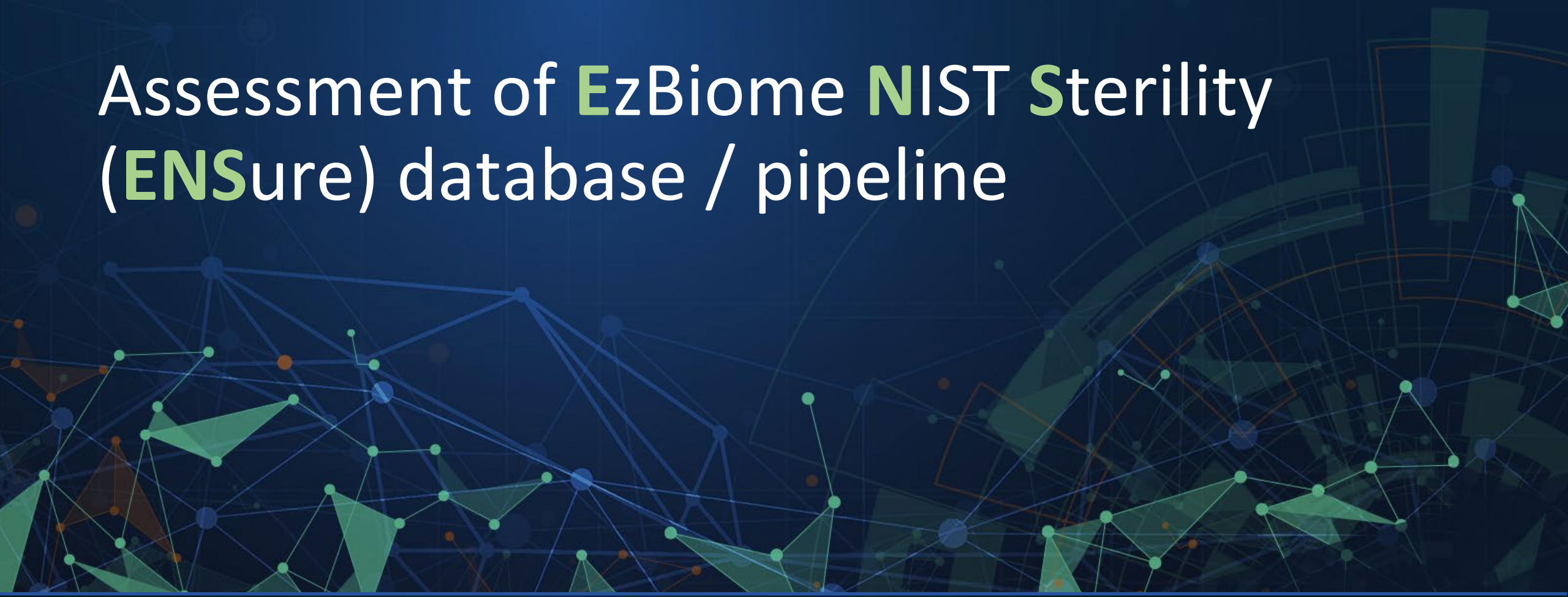
Organism	Typical source	Frequency reported	References
Coagulase-negative *Staphylococcus* spp.	Skin	17	9–11, 19, 24–26, 29–32, 36, 38, 39, 42, 47, 49
Staphylococcus aureus	Skin	9	11, 24, 27, 30–32, 38, 42, 47
Bacillus spp.	Environment	7	24, 25, 29, 32, 36, 47, 49
Corynebacterium spp.	Skin	7	11, 19, 25, 39, 42, 47, 49
Cutibacterium acnes	Skin	7	10, 11, 19, 24, 36, 42, 49
Escherichia coli	Enteric	7	9, 19, 25, 26, 30, 31, 42
Streptococcus spp.	Oral	7	24, 25, 30–32, 42, 49
Pseudomonas aeruginosa	Environment	6	9, 19, 29–31, 42
Ralstonia pickettii	Water	5	10, 24, 31, 39, 42
Enterococcus faecalis	Enteric	4	26, 31, 42, 49
Bacteroides spp.	Enteric	3	25, 27, 49
Clostridium spp.	Enteric	3	9, 10, 25
Enterobacter spp.	Enteric	3	11, 42, 47
Enterococcus faecium	Enteric	3	26, 27, 30
Klebsiella pneumoniae	Enteric	3	25, 26, 30
Sphingomonas paucimobilis	Water	3	9, 38, 39
Acinetobacter spp.	Environment	2	24, 30, 42
Aspergillus spp.	Environment	2	25, 32
Candida parapsilosis	Environment	2	25, 39
Chryseobacterium spp.	Environment	2	10, 42
Enterococcus spp.	Enteric	2	25, 49
Lactobacillus spp.	Enteric	2	26, 29
Micrococcus spp.	Skin	2	32, 42
Mold, no further identification	Environment	2	32, 42
Penicillium spp.	Environment	2	10, 32
Peptostreptococcus spp.	Enteric	2	25, 49
Proteus mirabilis	Enteric	2	38, 49
Pseudomonas fluorescens/Pseudomonas putida	Environment	2	42, 49
Stenotrophomonas maltophilia	Environment	2	42, 49
Actinomyces spp.	Oral	1	49
Aerococcus spp.	Environment	1	25
Aeromonas hydrophila	Water	1	31
Bifidobacterium spp.	Enteric	1	25
Bipolaris spicifera	Environment	1	11
Burkholderia cepacia	Environment	1	25
Candida albicans	Oral, enteric	1	10
Chaetomium spp.	Environment	1	42
Citrobacter spp.	Enteric	1	47
Enteric Gram-negative rod, no further identification	Enteric	1	39
Histoplasma capsulatum	Environment	1	9
Kocuria spp.	Skin	1	32
Methylobacterium spp.	Environment, water	1	42
Moraxella spp.	Oral	1	42
Rhodotorula spp.	Environment	1	32
Roseomonas spp.	Water	1	26
Rothia spp.	Skin, oral	1	49
Salmonella enterica	Enteric	1	40
Shigella spp.	Enteric	1	38
Ustilago spp.	Environmental	1	10
Veillonella spp.	Oral	1	24

Current Partner

EzBiome

- RMTM Consortium Member
- Expertise in microbial genomics, metagenomic analysis tool development, and building cloud analytic resources
- Curated database of 100’s of k’s of microbial genomes

Assessment of EzBiome NIST Sterility (ENSure) database / pipeline



National Institute of Standards and Technology
U.S. Department of Commerce

Tyler Laird

Custom Genome Database – Sterility Relevant Taxa

Minimal Target List

Minimal list	Type
Bacillus cereus	*Bacteria, Gram positive rod*
Bacillus subtilis	*Bacteria, Gram positive rod*
Brevundimonas vesicularis	*Bacteria, Gram negative rod*
Burkholderia cepacia	*Bacteria, Gram negative rod*
Chryseobacterium indologenes	*Bacteria, Gram negative rod*
Clostridium perfringens	*Anaerobic bacteria, Gram positive rod*
Clostridium sporogenes	*Anaerobic bacteria, Gram positive rod*
Corynebacterium spp.	Bacteria, Gram-positive rod
Klebsiella pneumoniae	*Bacteria, Gram negative rod*
Lactobacillus spp.	*Anaerobic bacteria, Gram positive rod*
Micrococcus luteus	*Bacteria, Gram positive cocci*
Propionibacterium acnes	*Anaerobic bacteria, Gram positive rod*
Pseudomonas aeruginosa	*Bacteria, Gram negative rod*
Pseudomonas flourescens	*Bacteria, Gram negative rod*
Ralstonia pickettii	*Bacteria, Gram negative rod*
Serratia marcesens	*Bacteria, Gram negative rod*
Sphingomonas spp.	*Bacteria, Gram negative rod*
Staphylococcus epidermidis	*Bacteria, Gram positive cocci*
Staphylococcus aureus	*Bacteria, Gram positive cocci*
Stenotrophomonas maltophilia	*Bacteria, Gram negative rod*
Streptococcus pyogenes	*Bacteria, Gram positive cocci*
Yersinia enterocolitica	*Bacteria, Gram negative rod*
Aspergillus fumigatus	Mold
Aspergillus versicolor	Mold
Aspergillus niger	*Mold*
Candida albicans	*Yeast*
Candida glabrata	*Yeast*
Candida krusei	*Yeast*
Cladosporium sphaerospermum	*Mold*
Fusarium spp.	Mold
Penicillium spp.	Mold

+

Environmental organism list

Genus	Species	Type of orgnaism
Achromobacter	*spp.*	bacteria
Acinetobacter	*spp.*	bacteria
Acinetobacter	*lwoffii*	bacteria
Acinetobacter	*spp.*	bacteria
Acinetobacter	*johnsonii*	bacteria
Acinetobacter	*junii*	bacteria
Acremonium		fungi
Actinomycetes	*spp.*	bacteria
Aeromonas	*spp.*	bacteria
Alcaligenes	*spp.*	bacteria
Alternaria	*spp.*	fungi
Alternaria	*alternata*	fungi
Alternaria	*spp.*	fungi
Arthrobacter	*spp.*	bacteria
Aspergillus	spp.	fungi
Aspergillus	*spp.*	fungi
Aspergillus	*fumigatus*	fungi
Aspergillus	*versicolor*	fungi
Aspergillus	*niger*	fungi
Aspergillus	*spp.*	fungi
Aspergillus	*spp.*	fungi
Aspergillus brasiliensis		*Mold*
Aspergillus fumigatus IHEM 22670		
Aspergillus niger (ATCC 16404)		
Bacillus	spp.	bacteria
Bacillus	*sphaericus/fusiformis*	bacteria
Bacillus	*pumilis*	bacteria
Bacillus	*cereus*	bacteria
Bacillus	*cereus*	bacteria

+

Spike-ins

Bacteria	species
1	*Brenneria nigrifluens*
2	*Delftia acidovorans*
3	*Deinococcus radiodurans*

Other-taxa

Bacteria	group	#spp.
1	*Actinomyces*	48
2	*Aerococcus*	12
3	*Citrobacter*	21
4	*Enterobacter*	25
5	*Enterococcus*	64
	Enterococcus faecalis	1
	Enterococcus faecium	1
6	*Roseomonas*	16
7	*Rothia*	13
8	*Veillonella*	20
1	*Proteus mirabilis*	1
2	*Salmonella enterica*	1

Fungi	group	#spp.
1	*Ustilago*	15
2	*Chaetomium*	4
	Histoplasma capsulatum	1

=

ENSure DB

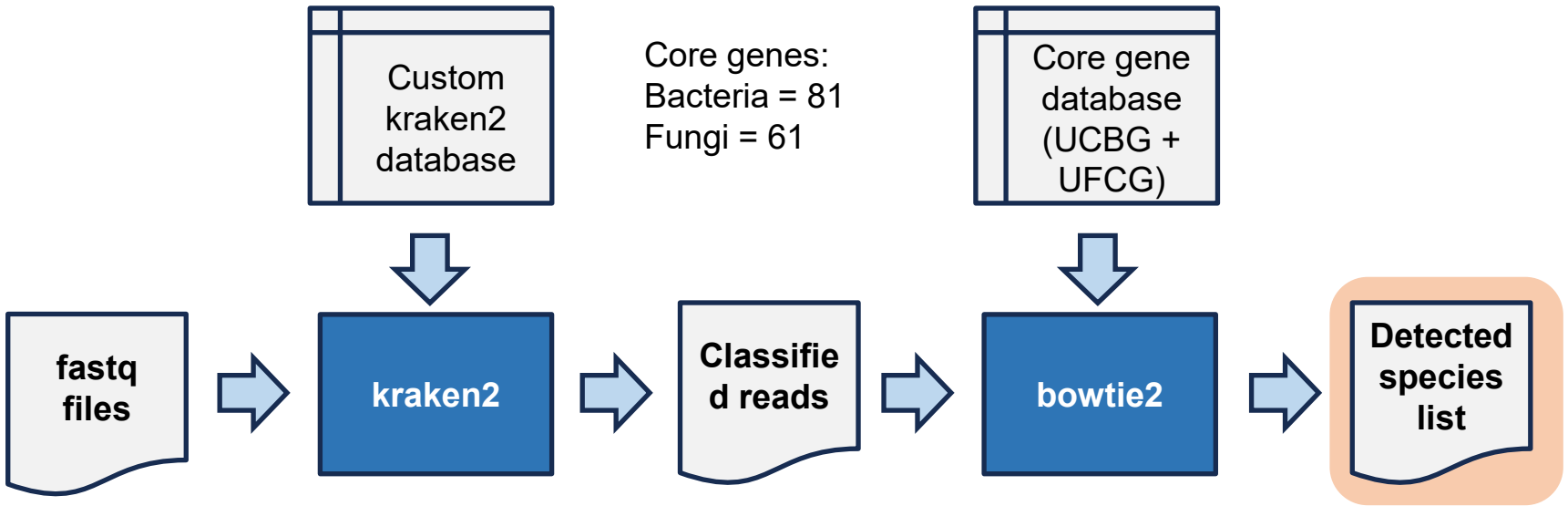
2543 Bacterial Genomes

654 Fungal Genomes

Target of 1 genome representative per species (based on OrthoANI calculations)

Work done by:
Hasan, Michael, Seon Young

EzBiome ENSure system bioinformatic pipeline



Classification of reads using core genes of custom sterility database

Alignment of reads to universal core genes from genomes in sterility database

Dependent on core gene threshold

Assessment of ENSure database / pipeline

Simulated Illumina Reads:

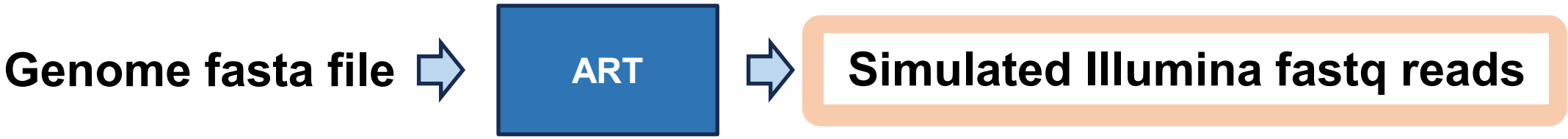
- In-silico read simulation of USP <71> genomes
- Comparison of ENSure with general purpose tools / databases
- Simulation with near-neighbor genome

Real Illumina Reads:

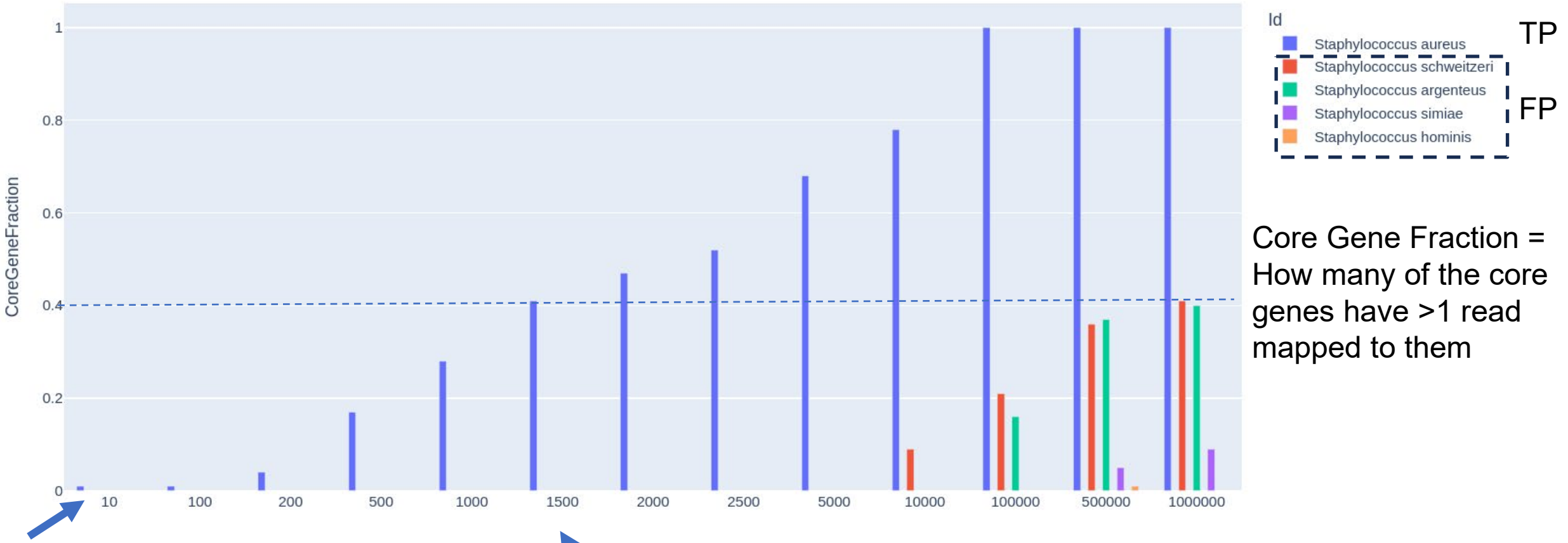
- Reads from USP <71> spike-in experiments
- Reads from USP <71> spike-in experiments (with pre-filtering)

In silico read simulation of USP <71> genomes

Genome_name
Staphylococcus aureus ATCC 6538
Pseudomonas paraeruginosa ATCC 9027
Bacillus spizizenii ATCC 6633 = JCM 2499
Clostridium sporogenes strain FDAARGOS_1470
Candida albicans ATCC 10231
Aspergillus brasiliensis CBS 101740



Staphylococcus aureus ATCC 6538 simulation



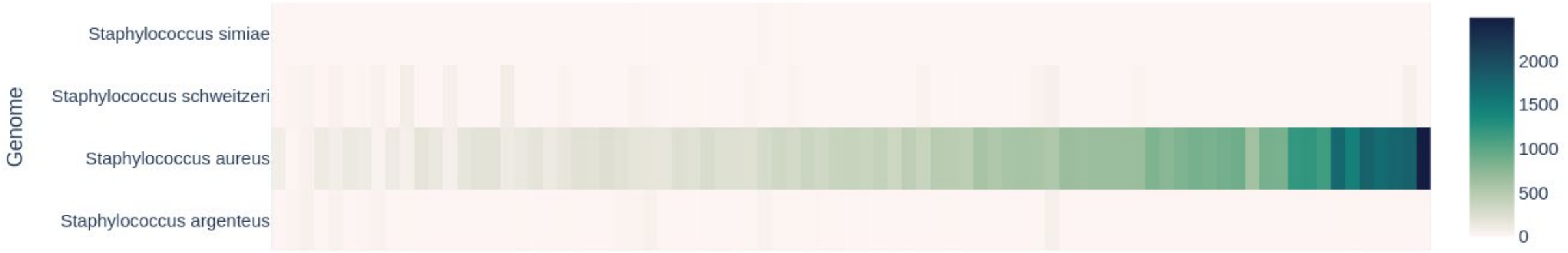
Core Gene Fraction = How many of the core genes have >1 read mapped to them

LOD = 10 reads total
where 1 read maps to dnaG core gene

Number of reads
Core gene threshold of 0.4 can be used if microbial reads are above 1500

Staphylococcus aureus ATCC 6538 simulation

Heatmap representing # of reads mapping to ENSure DB genes (1 million reads total)



False positives are in the same genus and have minimal hits

81 ENSure DB Core genes

Read abundance colorscale

In silico simulations

Summary metrics for all simulated USP <71> reads based on classification of reads at Species or Genus level

	Species Level		Genus Level		
Genome name	TPs	FPs	TPs	FPs	LOD
Staphylococcus aureus ATCC 6538	0.97	0.03	1.00	0.00	10 reads
Pseudomonas paraeriginosa ATCC 9027	0.95	0.05	1.00	0.00	100 reads
Bacillus spizizenii ATCC 6633 = JCM 2499	0.73	0.27	1.00	0.00	200 reads
Clostridium sporogenes strain FDAARGOS_1470	0.96	0.04	1.00	0.00	200 reads
Candida albicans ATCC 10231	0.61	0.39	1.00	0.00	500 reads
Aspergillus brasiliensis CBS 101740	0.85	0.15	0.99	0.01	200 reads

Other Fungi

- **Vast majority of reads are correctly identified at genus level**
- **Limit of detection is roughly associated with genome size**

Comparison with other tools / databases

Simulated Illumina fastq reads

Same USP <71> data as on previous slides

Taxonomic classification results

Tool : Database
Centrifuge: Refseq: bacteria & archaea compressed 2018.4.15 (6.2 GB)
Kraken2 – Bracken : plusPFP database (8 GB)
mOTUs : mOTU db v3.1.0 (3 GB)

Standardized results tables

Comparison with other tools / databases

	Precision (Genus Level)	Read Utilization
Centrifuge		
Staphylococcus	1.00	0.99
Pseudomonas	0.98	0.99
Bacillus	1.00	0.99
Clostridium	0.99	0.97
Candida	0.00	0.05
Aspergillus	0.00	0.02
kraken2-bracken		
Staphylococcus	1.00	0.73
Pseudomonas	1.00	0.73
Bacillus	1.00	0.72
Clostridium	0.99	0.65
Candida	0.59	0.59
Aspergillus	0.94	0.07
mOTUs		
Staphylococcus	1.00	0.0006
Pseudomonas	1.00	0.0003
Bacillus	1.00	0.0004
Clostridium	1.00	0.0004
Candida	0.00	0.00
Aspergillus	0.00	0.00
ENSure		
Staphylococcus	1.00	0.02
Pseudomonas	1.00	0.03
Bacillus	1.00	0.03
Clostridium	1.00	0.03
Candida	1.00	0.02
Aspergillus	0.99	0.01

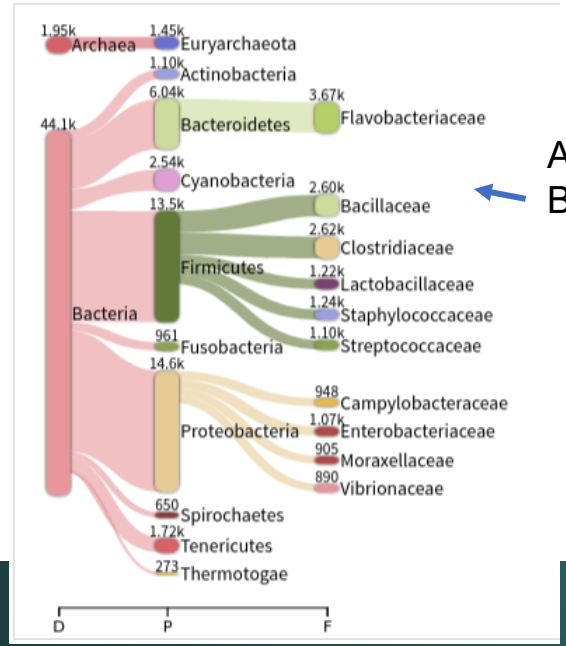
Not in DB

Precision = TP / (TP + FP) [Based on individual reads assigned @ genus level]
Read Utilization = # reads classified / total simulated

Benefits of ENSure

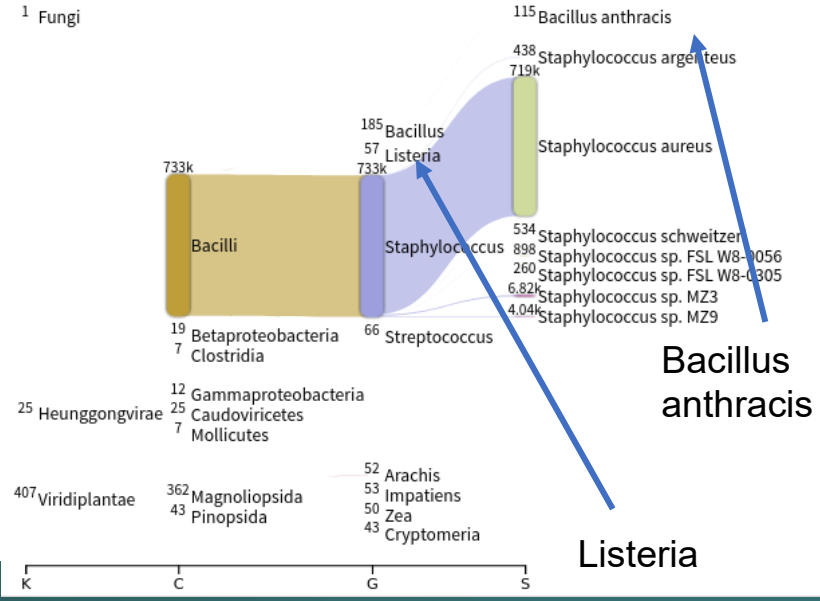
- Contains relevant taxa in DB
- ENSure v mOTUs
  - Both marker gene approaches but ENSure utilizes many more reads (i.e. lower LOD)
- Minimizes the false positive problem

***C. albicans* (Centrifuge)**



All sorts of Bacteria

***S. aureus* (kraken2-bracken)**



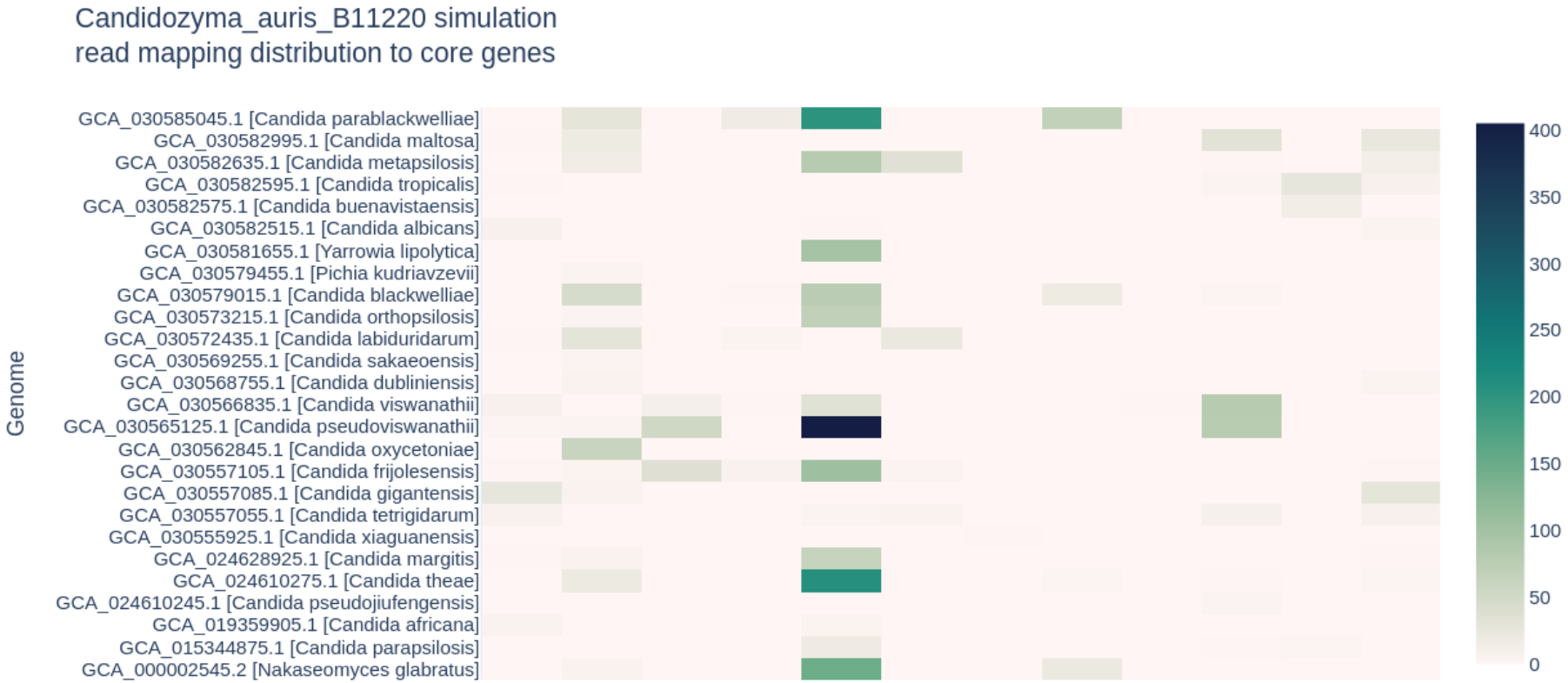
Bacillus anthracis

Listeria

Near neighbor taxa simulation

How does ENSure handle taxa that are not in the DB, but are somewhat related to a genome in the DB?

Methods: Simulated reads for Candidozyma auris ("Candida auris") ---> ENSure workflow



ENSure can detect near neighbor "Candida" species even if not explicitly in the database with hits to 12 genes across 26 genomes

*ignoring a core gene threshold

Analysis of raw sequencing data from spike-in experiment

Sterility testing of USP <71> related spike-ins with Mesenchymal Stem Cell cultures (SRP161443)

A systematic sequencing-based approach for microbial contaminant detection and functional inference

Sung-Joon Park, Satoru Onizuka, Masahide Seki, Yutaka Suzuki, Takanori Iwata & Kenta Nakai

BMC Biology **17**, Article number: 72 (2019) | Cite this article

1100 CFU

DNA-seq of hPDL-MSCs (1100 CFU x6 microbe species with Antibiotics)

14. 1 ILLUMINA (Illumina HiSeq 3000) run: 364M spots, 72.8G bases, 27.7Gb downloads
Accession: SRX4668672

60 CFU

DNA-seq of hPDL-MSCs (60 CFU x6 microbe species with Antibiotics)

15. 1 ILLUMINA (Illumina HiSeq 3000) run: 365.5M spots, 73.1G bases, 27.5Gb downloads
Accession: SRX4668671

List of spike-in microbes

A. brasiliensis NCPF 2275

B. splizizeni NCTC 10400

C. albicans NCPF 3179

C. sporogenes NCTC 12935

P. paraeruginosa NCTC 12924

S. aureus NCTC 10788

From original study: "one microbial read will exist when 100 million host reads are sequenced"

Analysis of raw sequencing reads from spike-in experiments

1100 CFU spike in

Looking at taxa detected (i.e. a read mapping to any of the core genes counts that taxa as present)

Species Level
TPs: 5 / 6
FPs: 67

Genus Level
TPs: 5 / 6
FPs: 45

Aspergillus
Bacillus
Candida
Clostridium
Pseudomonas
Staphylococcus

A. brasiliensis NCPF 2275

B. splizizeni NCTC 10400

C. albicans NCPF 3179

C. sporogenes NCTC 12935

P. paraeruginosa NCTC 12924

S. aureus NCTC 10788

Many false positive detections (mainly of fungi) due to low complexity regions and host reads e.g. AAAAGGGGGGGAAAAAAA

60 CFU spike in

Species Level
TPs: 1 / 6
FPs: 58

Genus Level
TPs: 3 / 6
FPs: 42

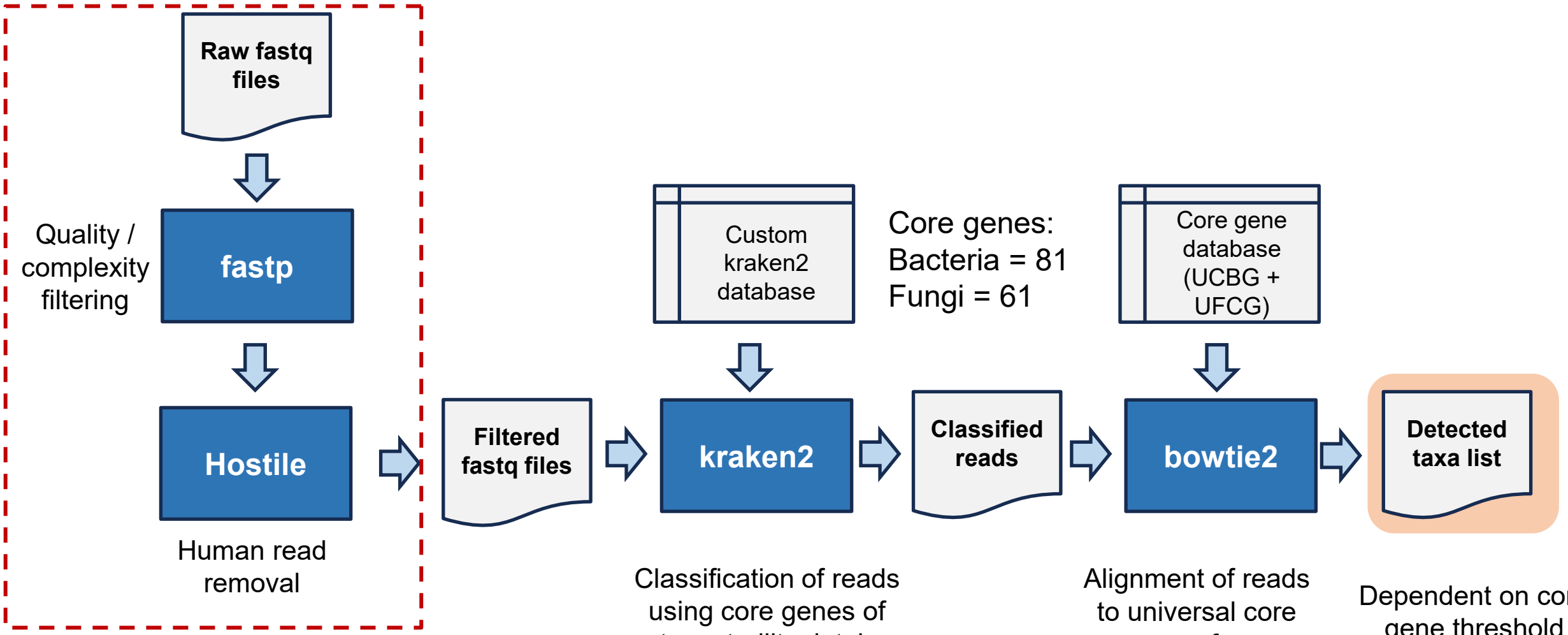
Aspergillus
Bacillus
Candida
Clostridium
Pseudomonas
Staphylococcus

Example low-complexity region in fungal marker gene

>ATP6

ATGCGTCATTTAGATTTTGTATTAAGTCCATTAgaccaatttgaagttagagatttattttctataaatgct
aacttattaggtaatttacacCTATCATTAACAaatataggtttatatttatcaattagtatttttttaatattaacatatagctt
attagctacaaataataataaaatagtaCCAAATAACTGATCAATAAGTCAAGAAAGTATATATGC
TACAGTACAcggtatagtagtaaatcaaattaaccCAAATAAAGGACAAATGTTCTTCCCTcttatgt
atgtattattcatatttatattagtaaataatttaataggtttagtacCATACAGTtttgcatcaacatcacattttatattaacat
tCTCAATTAGTTTTACTATTGTTTTAGGTGCAACAATATTAGGTTTCCAAAAACATGG
GTtaaaattcttctcattatttgtaCCTTCAGGTTGTCCATTAgcattattaccattattagtaataattgagTTTA
TTTCTTACTTATCTAGAAATGTTTCTTTAGGTTTAAGATTAGCTGCAAATATTTTAAG
TGGTCACATgcttttaagtatattaagtggatttacttataatataatgactagtggtataatattctttatattaggATTAA
TACCTTTAGCATTTATTATTGCATTCTCAGGTTTAGAGTTAGCAATAGCATTTATTCA
AGCACAAGTTTTCGTAGTTTTAGCTTgttcatatattaaagatggaTTAGATTTACACtaa

EzBiome ENSure system bioinformatic pipeline



Analysis of raw sequencing reads from spike-in experiments (with filtering)

1100 CFU spike in

Looking at taxa detected (i.e. a read mapping to any of the core genes counts that taxa as present)

Species Level

TPs: 5 / 6

FPs: ~~67~~ 11

Genus Level

TPs: 5 / 6

FPs: ~~45~~ 4

Aspergillus
Bacillus
Candida
Clostridium
Pseudomonas
Staphylococcus

A. brasiliensis NCPF 2275

B. splizizeni NCTC 10400

C. albicans NCPF 3179

C. sporogenes NCTC 12935

P. paraeruginosa NCTC 12924

S. aureus NCTC 10788

*still some false positives due to low complexity sequences e.g. AGAGAGAGAGAGAGA

Need to use additional tool bbduk since fastp does not account for all types of complexity

60 CFU spike in

Species Level

TPs: 1 / 6

FPs: ~~58~~ 6

Genus Level

TPs: 3 / 6

FPs: ~~42~~ 4

Aspergillus
Bacillus
Candida
Clostridium
Pseudomonas
Staphylococcus

Conclusions

For in Silico analysis

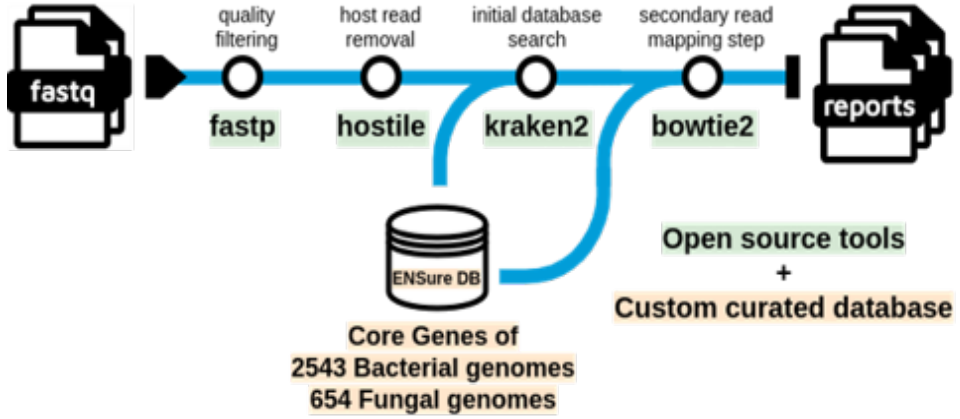
- TPs ranged from 0.61 to 0.97 (species level) and most FPs were in the same genus
- ENSure has high precision
- ENSure can detect near neighbors of database members

For Spike-in analysis:

- Analyzing real host data is important!
- Host read removal and filtering can remove many false positive hits

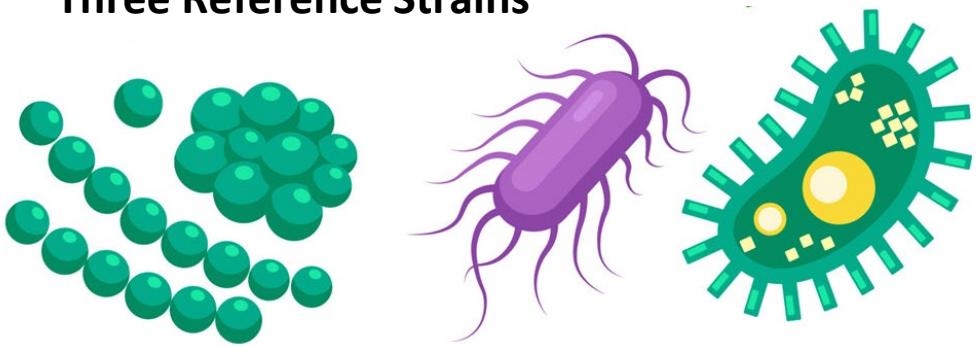
Challenges/Future work:

- Addressing the abundance of host reads in real samples
- What core gene threshold (if any) to use for low microbial abundance samples
- Adding optional mapping to whole genomes (not just core genes)
- Combining all tools used into a standardized reproducible Nextflow pipeline



Internal Standards – Spike-Ins

Three Reference Strains

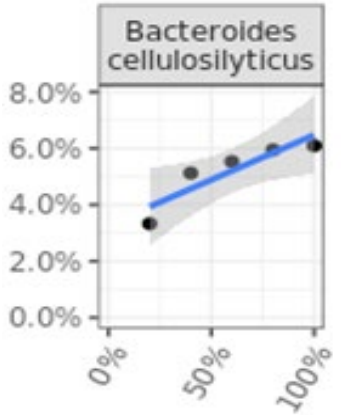


Manufacture Pellets

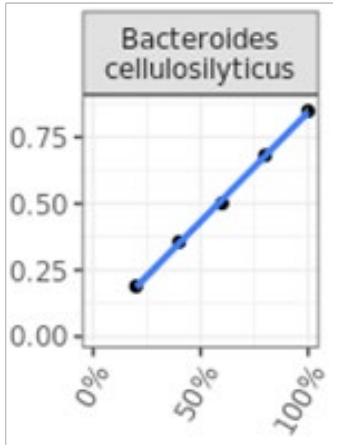
Spike Into Product (~1% each)

NGS Workflow

Before Normalization



After Normalization



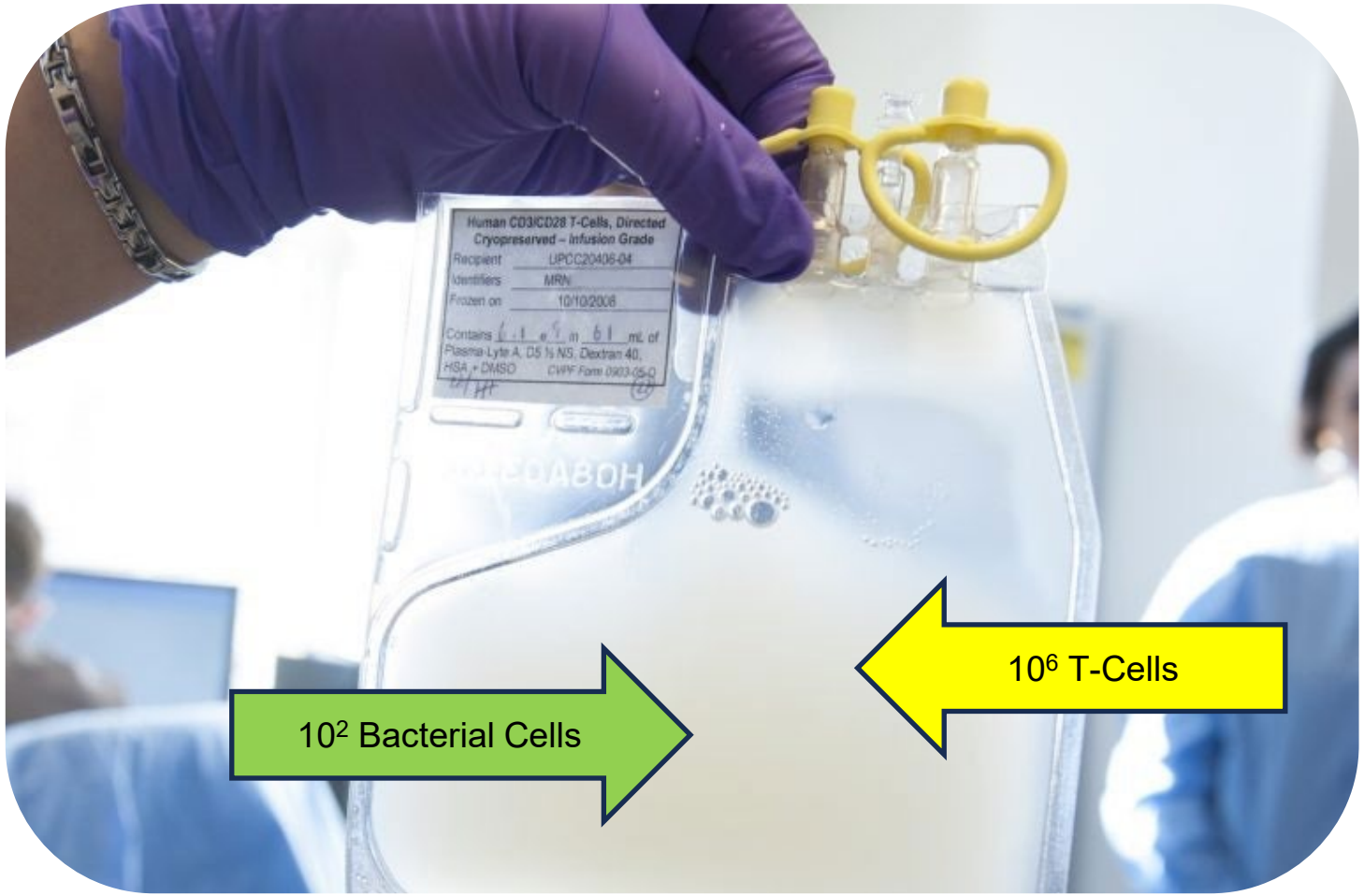
The Spike-In Species

1. *Deinococcus radiodurans*
2. *Delftia acidovorans*
3. *Brenneria nigrifluens*

Summary: Spike-Ins

- We are developing spike-in control materials to increase confidence in mNGS data.
- Manufacturing - Microbiologics Pellets (250x)
- One pellet will be sufficient to process ~100 samples

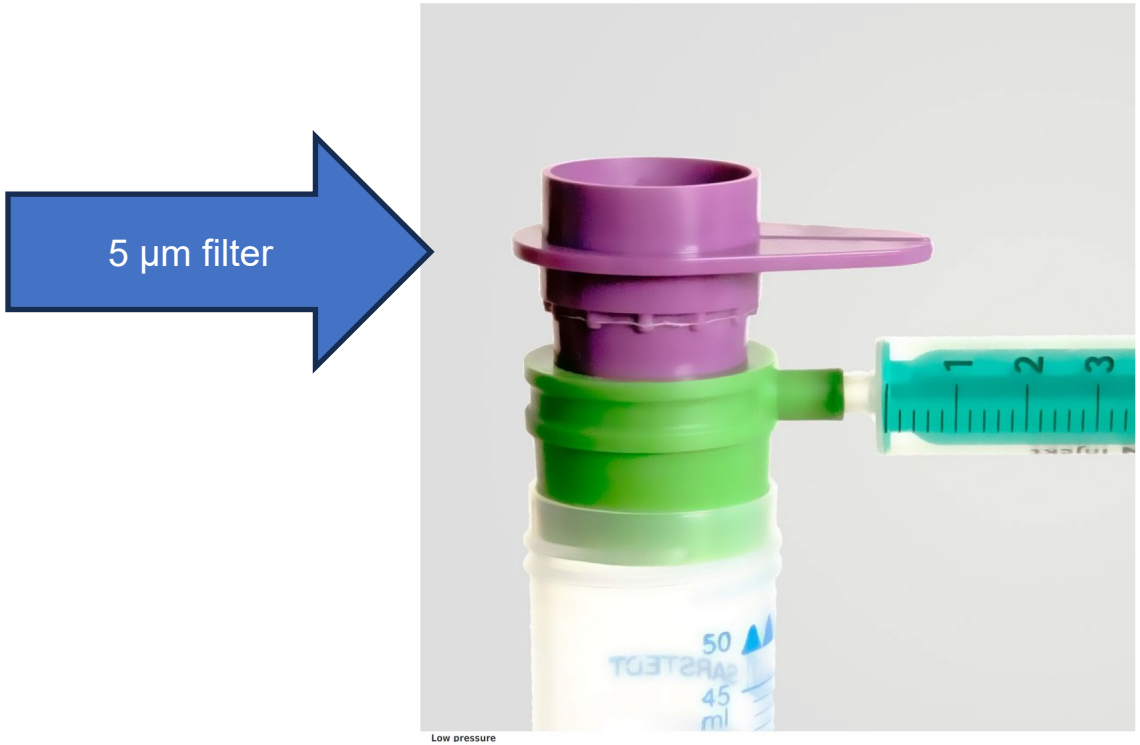
A Fundamental Problem with NGS-Based Approach



- In this scenario:
  - 99.99999% of gDNA is T-Cells
  - 0.000001% of gDNA is microbial
- Signal <<<<<<<< Noise
- Using untargeted shotgun NGS, cost per sample will be ~$1000 (because of signal:noise)

Enrichment Option

- Option 1 – Filtration – Using 5 µm Filter



Other Enrichment Options are Available

Micronbial DNA Enrichment Kit

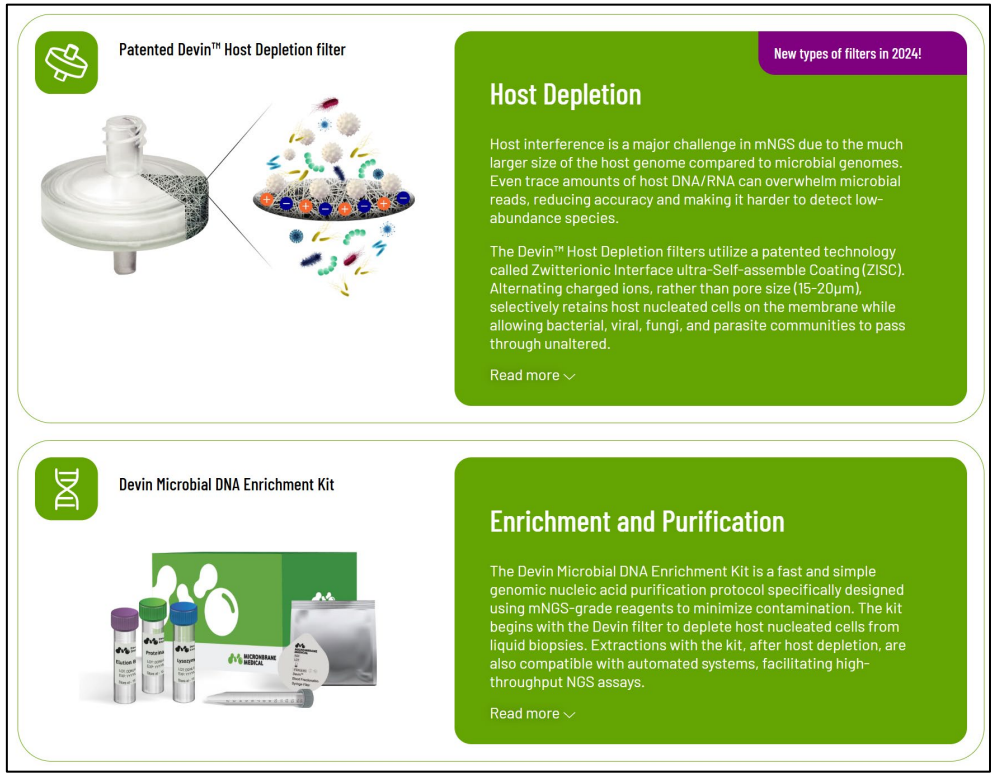
High Efficiency Microbial DNA Enrichment Kit (24 reactions)

・Include Devin® fractionation filters for host DNA depletion and much faster pathogen identification
・Fast and simple protocol (both manual and automated)
・Compatible with downstream applications including next generation sequencing on all platforms, qPCR and end point PCR
・With PaRTI-Seq® enables pathogen identification and reporting within less than 24 Hours

Fractionation Filter Devin®

Ready-to-Use Syringe-driven Filter for Wide Use in Microbiology Applications

・Depletes white blood cells within 5 minutes
・Provide higher percentage of sequencing reads in comparison with other depletion methods
・For filtering of whole blood, plasma and other body fluids
・100% integrity tested, individually packaged, and sterilized



Follow this preprint Previous

EVALUATION OF A METAGENOMIC NEXT-GENERATION SEQUENCING ASSAY WITH A NOVEL HOST DEPLETION METHOD FOR PATHOGEN IDENTIFICATION IN SEPTIC PATIENTS

Posted March 29, 2023.

Yen-Chia Chen, Po-Hsiang Liao, Yen-Wen Chen, Chia-Ming Chang, Maurice Chan, Deng Fong Chao, Yizhen Lin, Jiahao Chang, Hau Hung, Mengchu Wu, David Hung-Tsang Yen

doi: https://doi.org/10.1101/2023.03.28.23287867

Download PDF Email
Print/Save Options Share
Author Declarations Citation Tools
Data/Code Get QR code

Microbial Signal Enrichment: The Plan

- We'll continue making test materials (T-cells + microbes) to test and optimize enrichment protocols
- Or we might adopt an amplicon sequencing approach….
- We'll establish SOPs for at least 2 enrichment methods and demonstrate performance

Coordinating Upcoming mNGS Interlab Study

- What's the point of the NGS ILS?
  - Lower the barrier to entry (let's cross the bridge together)
  - Get this technology in the hands of stakeholders (biopharma sterility testing)
  - Demonstrate performance in real-world (sensitivity and specificity)
  - Assess variability/reproducibility across labs

  - Deploy ENSure NGS bioinformatic workflow
  - Demonstrate utility of spike-ins for data normalization

- Who are participants?
  - Biopharma – Cell Therapy Manufacturers
  - Academics
  - Biotech

Coordinating Upcoming mNGS Interlab Study

1. **Dates:** **Summer, 2025**
2. **Sample Type:** **T-cells spiked with microbes**
3. **Spike-In Materials:** **Provided by NIST**
4. **Host Depletion:** **TBD**
5. **DNA Extraction:** **TBD**
6. **NGS Technology:** **TBD**
7. **Bioinformatics:** **ENSure**

We will ask participants to define their methods in advance.

Two Flavors of ILS

Every Lab Runs Same Method:

- Assess reproducibility across labs
- Assess performance of specific method
- Validation of a method

Labs Run Different Methods:

- Assess impact of methodological variability
- Hard to compare reproducibility across labs
- Hard to assess performance in a rigorous way

Questions