



## Rapid Monitoring of Microbiota Composition and Targeted Culturomics of Species of Interest using Polyclonal Antibodies in Combination with Flow Cytometry



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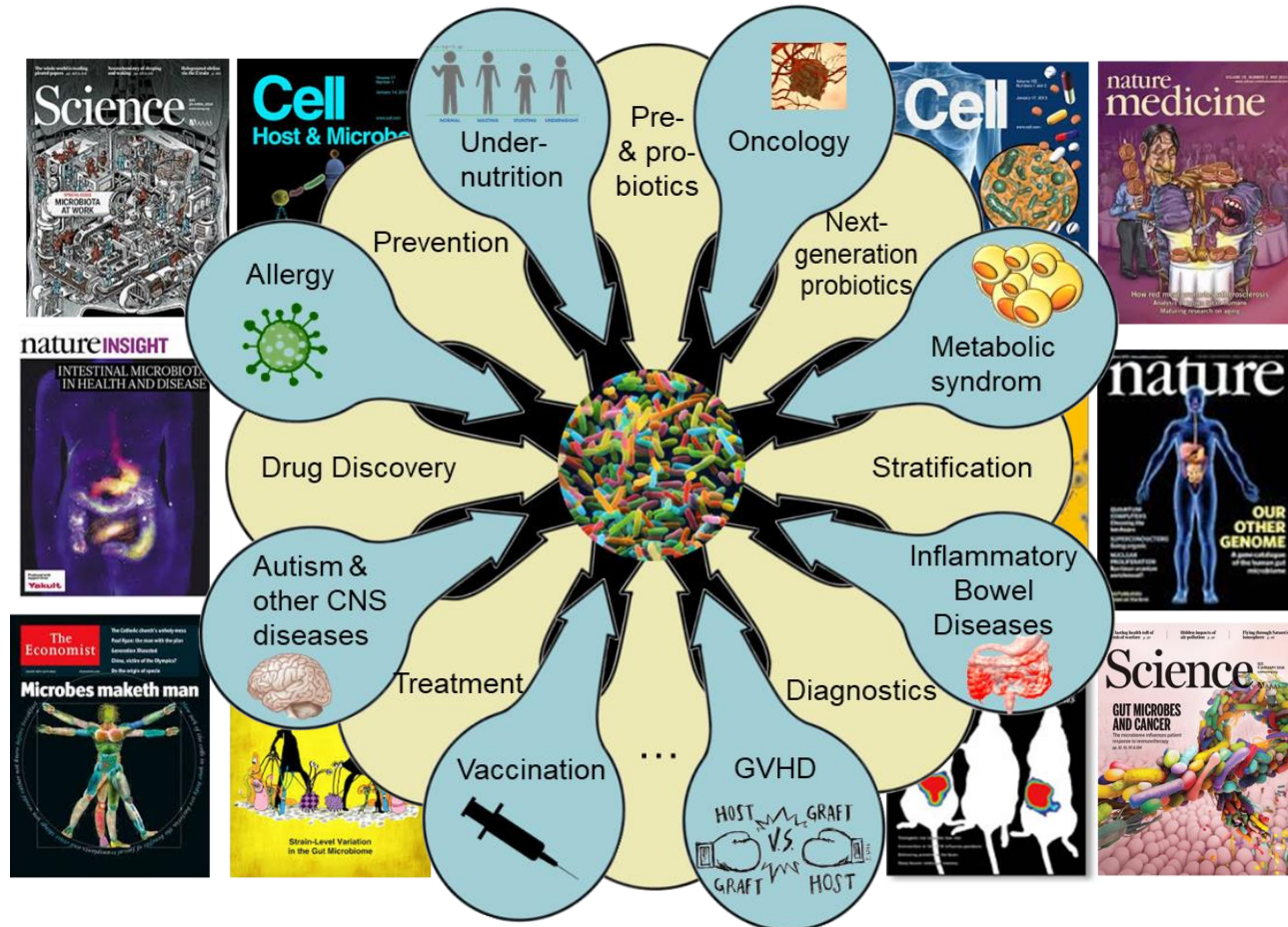
*USP Symposium: Emerging technologies in  
probiotic, live biotherapeutic products and  
microbiome analysis  
06 & 07 Oct 2022*



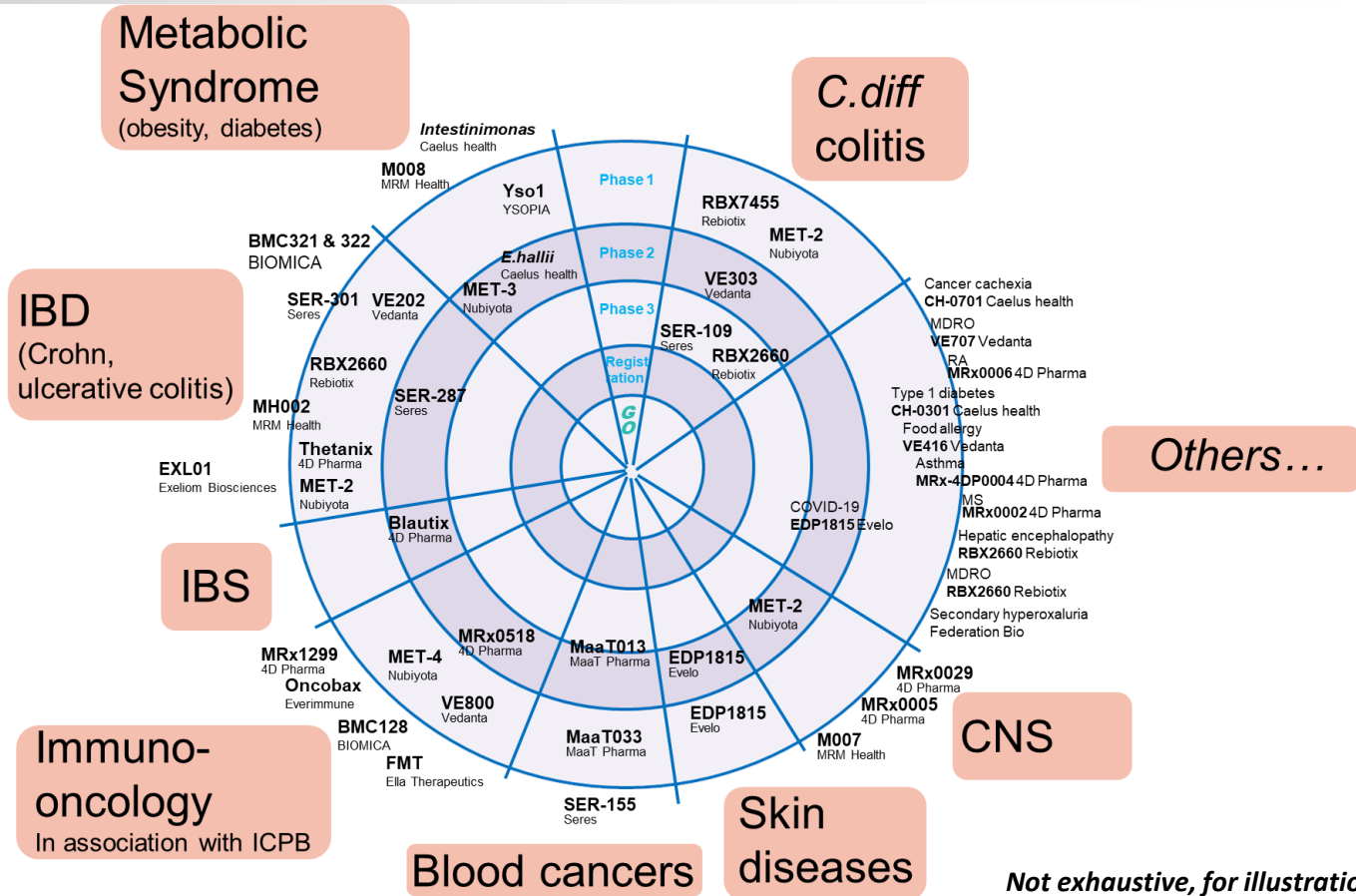
# CONTEXT

Commensal gut microbiome:  
the new Eldorado

# Host-associated microbiota: Increasing interest and many potential applications...



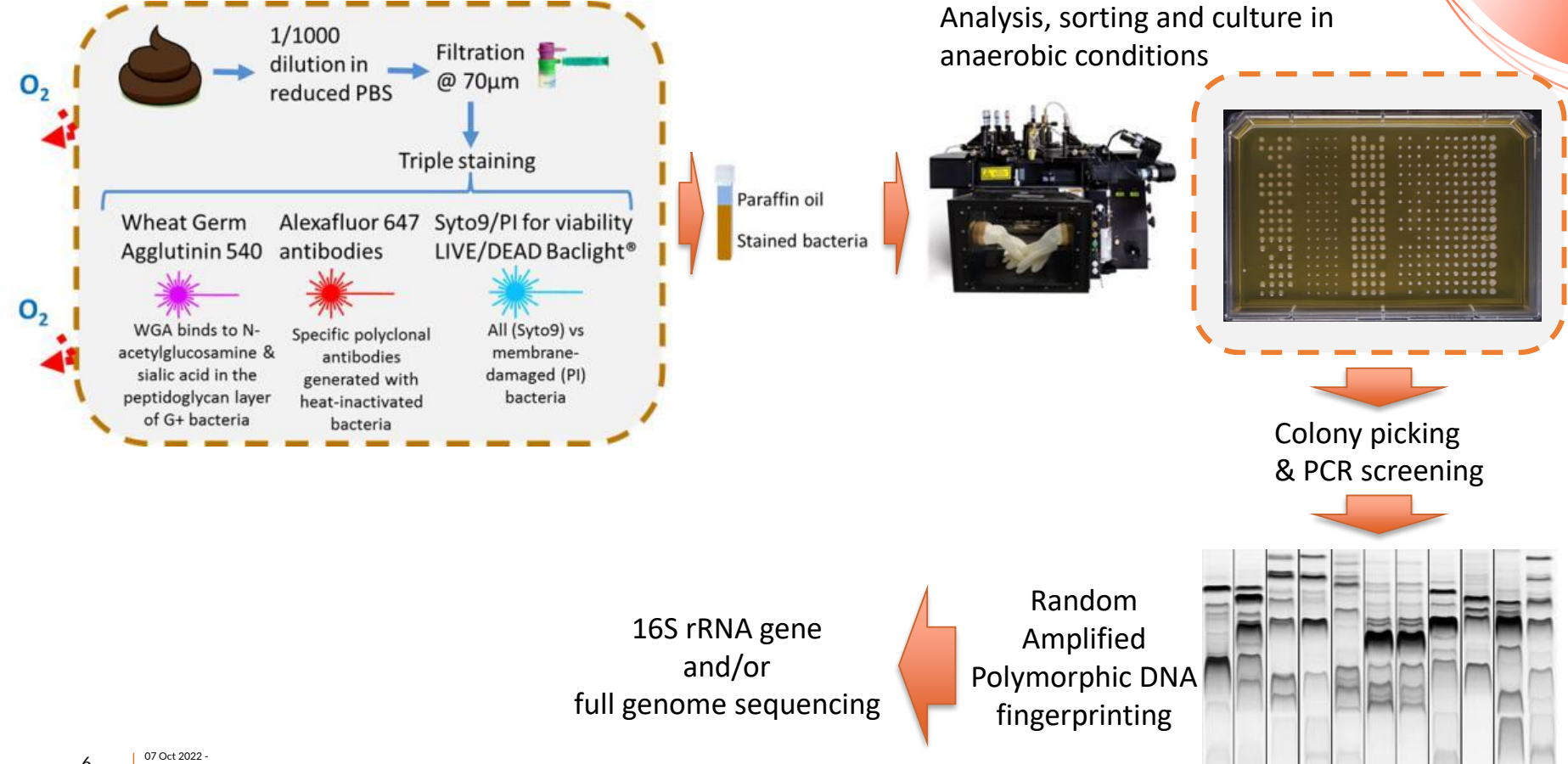
# Live Biotherapeutics Products & Fecal Microbial Transplantation: increasing interest and many potential applications...



Not exhaustive, for illustration purposes only

# FLOW CYTOMETRY FOR TARGETED ENRICHMENT AND CULTUROMICS

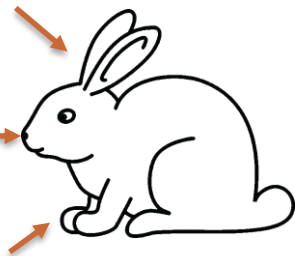
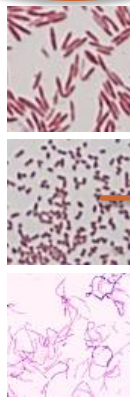
## Pipeline for « targeted culturomics »



## Polyclonal antibodies for the detection of specific commensal species

### «Beneficial» gut commensal species

Heat-inactivated bacteria



*Akkermansia muciniphila*

- Beneficial properties on adiposity, insulin resistance and glucose tolerance
- Presence associated with enhanced response to immune checkpoint inhibitors



*Faecalibacterium prausnitzii*  
(phylogroups IIa & IIb)

- Highly abundant species that is reduced in IBD, IBS, CRC, obesity...
- Anti-nociceptive



*Faecalibacterium 'moorei'*  
(phylogroup I)

- Presence associated with enhanced response to immune checkpoint inhibitors



*Eubacterium hallii*

- Has been associated with increased insulin sensitivity



*Christensenella minuta*

- Highly heritable, inversely related to host body mass index (BMI) and related to healthy status

### «Detrimental» gut commensal species

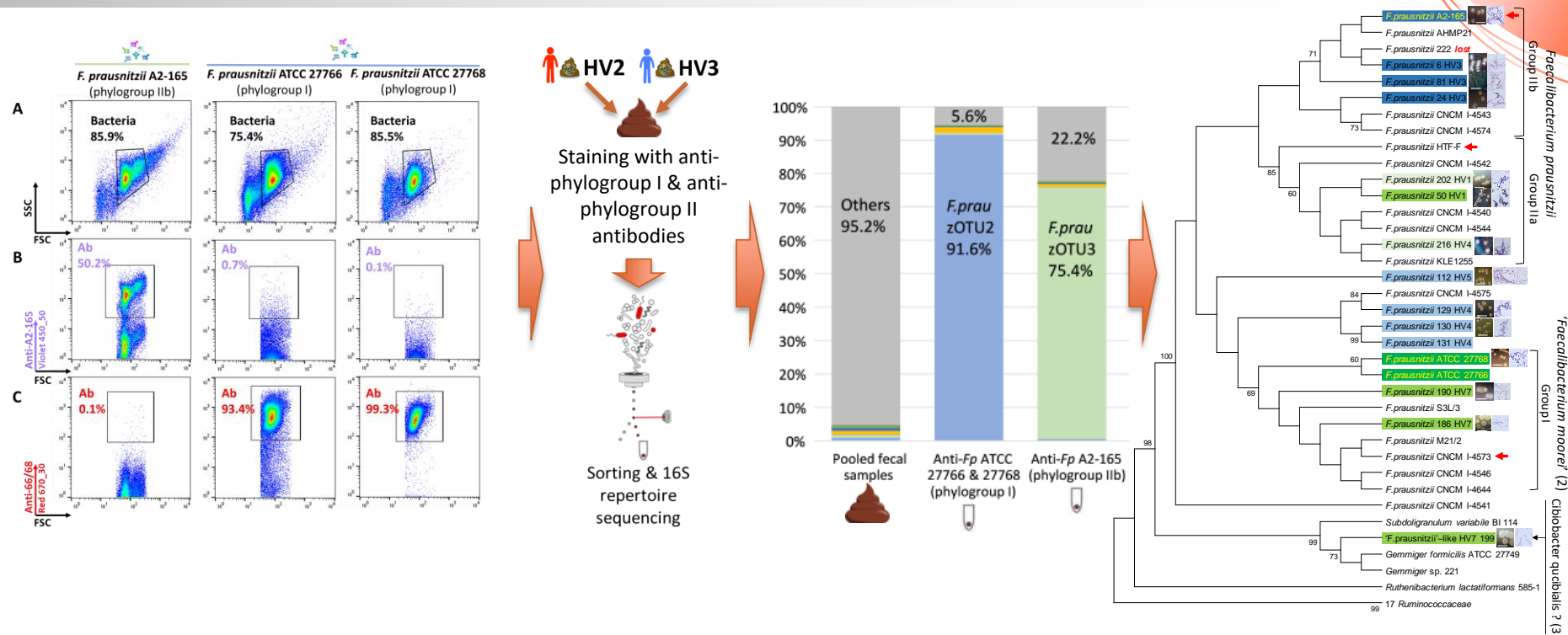


*Fusobacterium nucleatum*

- Has been associated with colorectal cancer

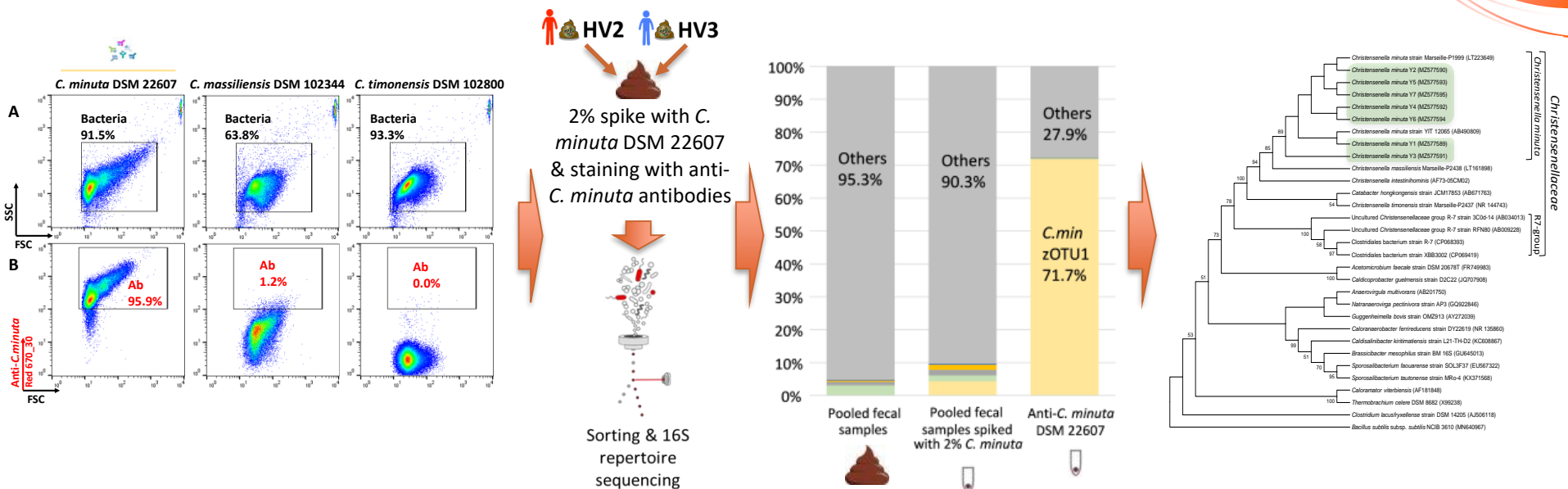
(not exhaustive: additional antibodies are available)

# POC study 1: sorting and cultivating **high-abundance** (5-10%) but **Extremely Oxygen Sensitive** species *Faecalibacterium prausnitzii*





# POC study 2: sorting and cultivating **very low-abundance** (approx. 0.02 to 0.2%) species *Christensenella minuta*



## Reverse Genomics: expand access to potential 'next generation probiotic' candidates

### Gut commensal bacteria



We know « who they are » and « what they do » thanks to shotgun sequencing, transcriptomics and metabolomics...



... but approx. 70% of the detected 'species' have never been cultivated so far!

Ongoing: reverse genomics to generate antibodies directed against uncultivated species of the gut microbiota (70% of the gut microbiota species!)

nature  
biotechnology

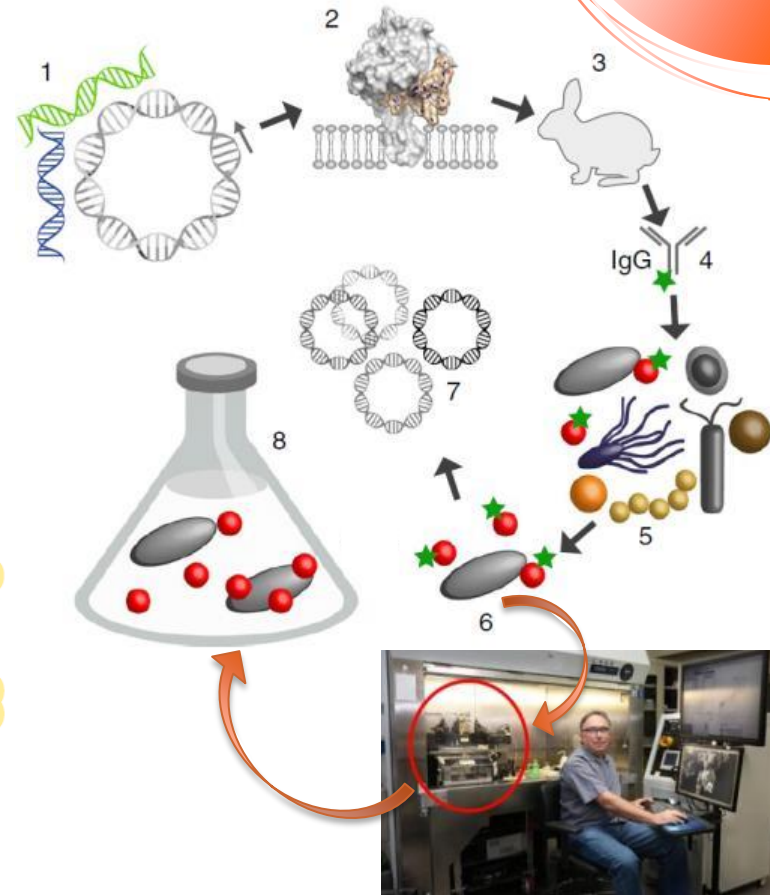
ARTICLES

<https://doi.org/10.1038/s41587-019-0260-6>

## Targeted isolation and cultivation of uncultivated bacteria by reverse genomics

Karissa L. Cross<sup>1,2,7</sup>, James H. Campbell<sup>1,6,7</sup>, Manasi Balachandran<sup>1</sup>, Alisha G. Campbell<sup>1,6,3</sup>, Sarah J. Cooper<sup>1,3</sup>, Ann Griffen<sup>4</sup>, Matthew Heaton<sup>5</sup>, Snehal Joshi<sup>1</sup>, Dawn Klingeman<sup>1</sup>, Eugene Leys<sup>4</sup>, Zamin Yang<sup>1</sup>, Jerry M. Parks<sup>1,3</sup> and Mircea Podar<sup>1,2,3\*</sup>

Most microorganisms from all taxonomic levels are uncultured. Single-cell genomes and metagenomes continue to increase the known diversity of Bacteria and Archaea; however, while 'omics can be used to infer physiological or ecological roles for species in a community, most of these hypothetical roles remain unvalidated. Here, we report an approach to capture specific microorganisms from complex communities into pure cultures using genome-informed antibody engineering. We apply our reverse genomics approach to isolate and sequence single cells and to cultivate three different species-level lineages of human oral Saccharibacteria (TM7). Using our pure cultures, we show that all three Saccharibacteria species are epibionts of diverse Actinobacteria. We also isolate and cultivate human oral SR1 bacteria, which are members of a lineage of previously uncultured bacteria. Reverse-genomics-enabled cultivation of microorganisms can be applied to any species from any environment and has the potential to unlock the isolation, cultivation and characterization of species from as-yet-uncultured branches of the microbial tree of life.



## Case study : fishing for *Oscillospira* species

### Forum

## *Oscillospira*: a Central, Enigmatic Component of the Human Gut Microbiota

Tom Konikoff<sup>1,2</sup> and Uri Gophna<sup>3,\*</sup>

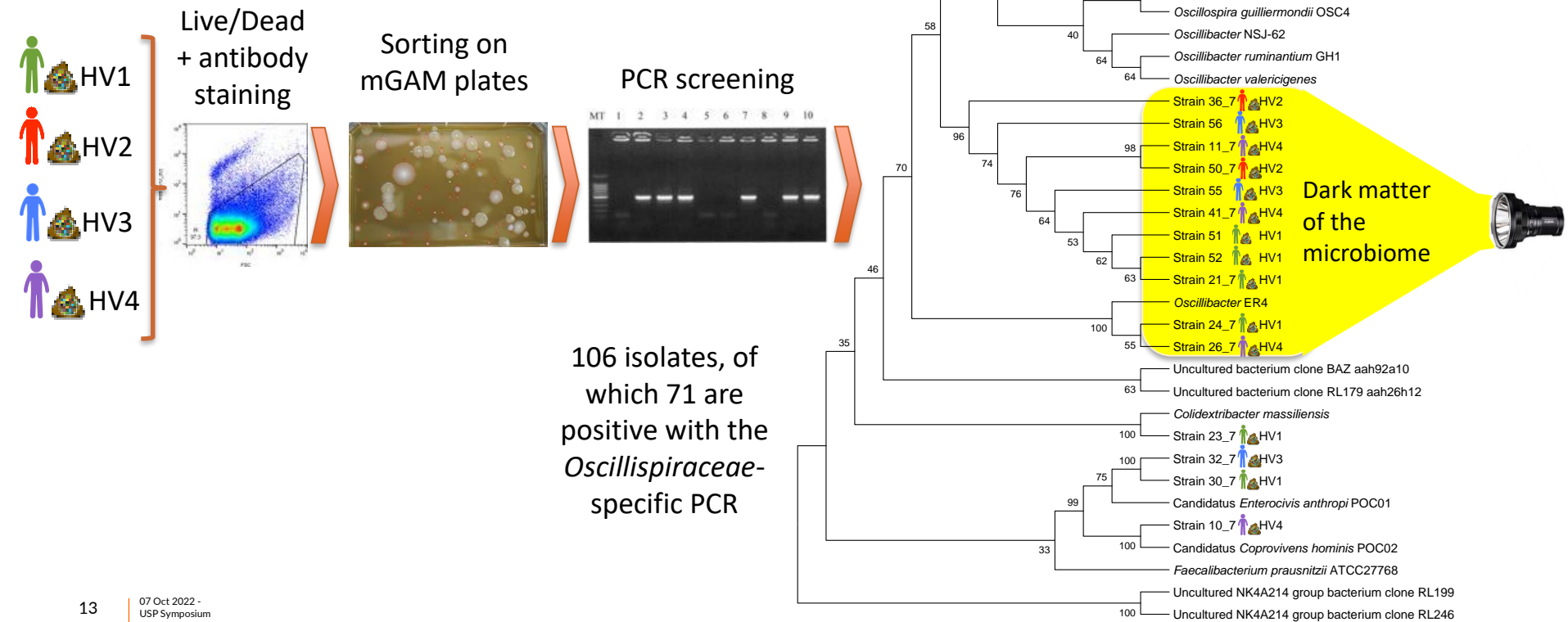
*Oscillospira* is an enigmatic bacterial genus that has never been cultured, but is constantly detected by 16S rRNA gene surveys of the human microbiome. Here we summarize recent evidence that *Oscillospira* is positively associated with leanness and health, speculate about its physiology, and argue its potential importance for human health.

logical properties in the intestinal tract. The large wealth of recent studies using 16S rRNA gene amplicon sequencing for the study of the human microbiota has indicated that *Oscillospira* is often an abundant component of the fecal microbiome of humans, sometimes exceeding 10% of 16S rRNA gene sequences in an individual

2016. Trends Microbiol 24:523-524.

Study	Main Findings	Study Size	Statistical Significance
Tims <i>et al.</i> [3]	Among monozygotic twins discordant for obesity, <i>Oscillospira</i> was significantly more abundant in the twin with the lower BMI	N (twin pairs) = 20	$P = 0.014$
Goodrich <i>et al.</i> [4]	<i>Oscillospira</i> was enriched in lean subjects; <i>Oscillospira</i> was strongly related ( $\rho = 0.71$ ) to <i>Christensenella minuta</i> that promoted leanness in inoculated mice	N = 1000 (including 416 twin pairs)	$P < 0.001$
Escobar <i>et al.</i> [10]	Lower <i>Oscillospira</i> relative abundance corresponded to higher BMI in 3 datasets from Colombia, USA, and Europe despite substantial differences between these datasets	N = 30 (Colombian dataset) N = 126 (all datasets)	$P < 0.03$
Verdam <i>et al.</i> [11]	<i>Oscillospira</i> relative abundance differed significantly between obese (lower levels) and non-obese (higher levels)	N = 28	$P < 0.05$

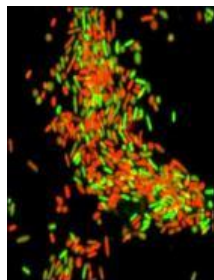
## Case study : fishing for *Oscillospira* species



# FLOW CYTOMETRY FOR MICROBIOME MONITORING

# Microbiota profiling using flow cytometry: Bacterial viability

Live/Dead staining



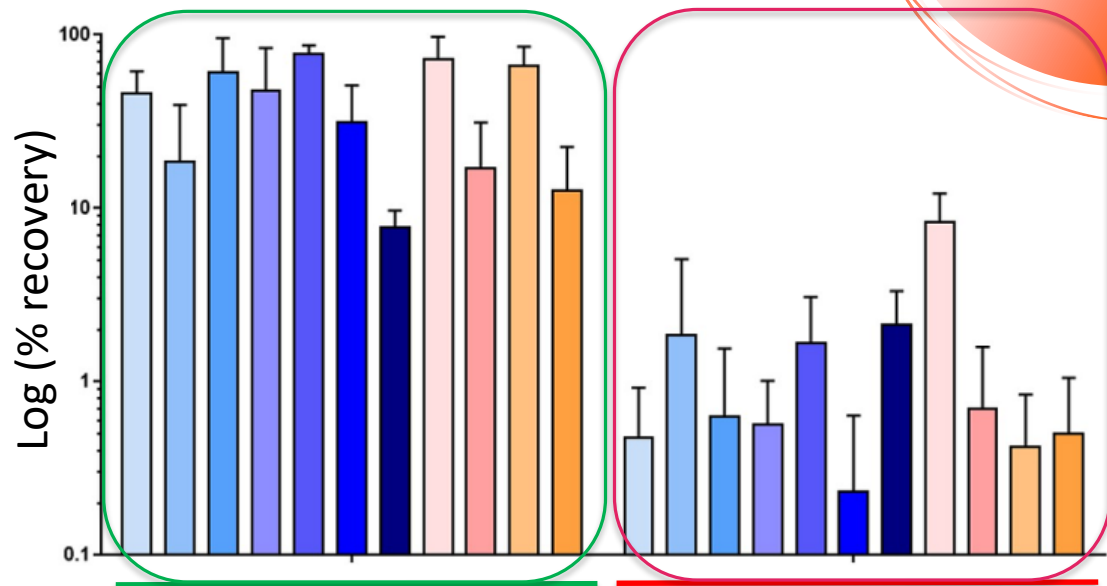
Sorting  
under  
anaerobic  
conditions

Events  
/spot

Live

Dead

1  
1  
3  
10  
30  
100  
300  
1000



*A. finegoldii* DSM 17242

*A. muciniphila* DSM 22959

*B. fragilis* DSM 2151

*B. thetaiotaomicron* DSM 2079

*C. minuta* DSM 22607

*P. copri* DSM 18205

*S. variabile* DSM 15176

*C. scindens* DSMZ 5676

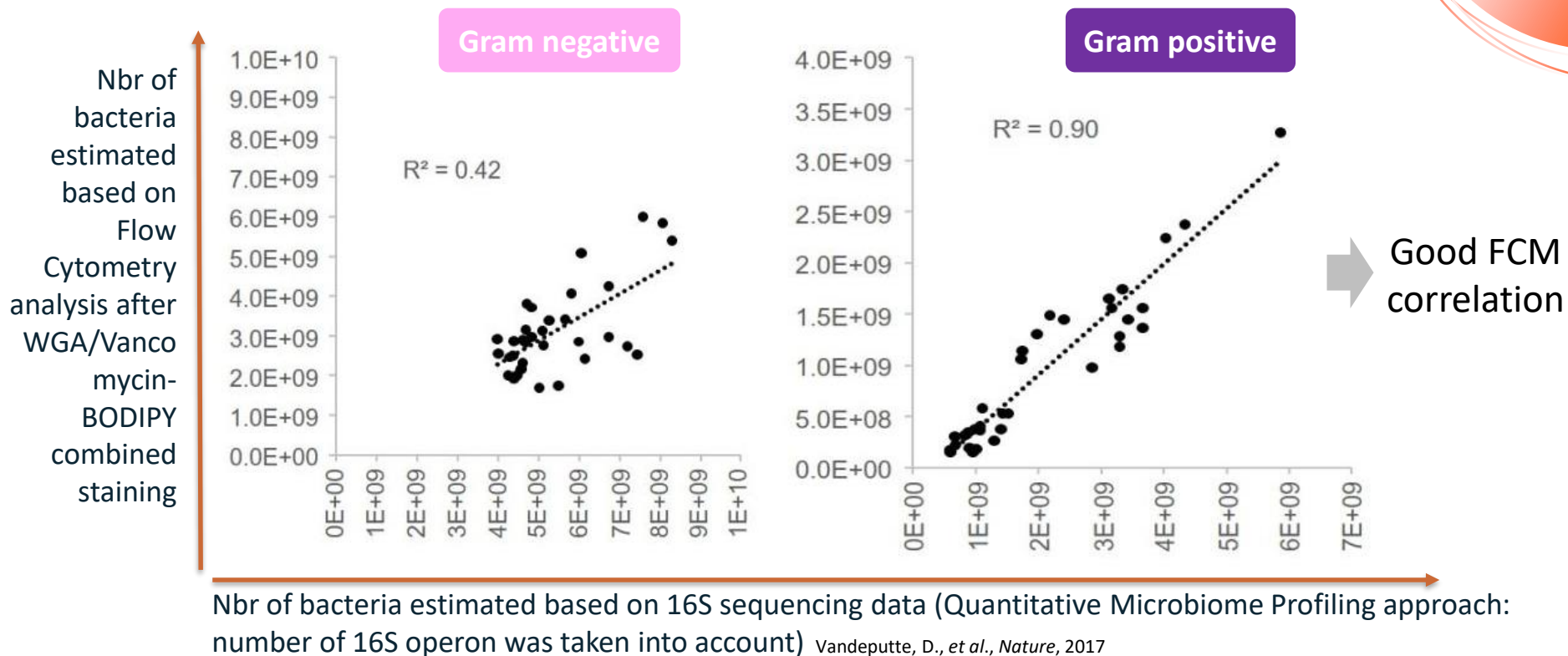
*E. halii* DSM 3353

*E. halii* DSM 17630

*R. intestinalis* DSM 14610

Duquenois A et al. 2020. Assessment of Gram- and Viability-Staining Methods for Quantifying Bacterial Community Dynamics Using Flow Cytometry. *Front Microbiol* 11:1469.

## Microbiota profiling using flow cytometry: Gram stain



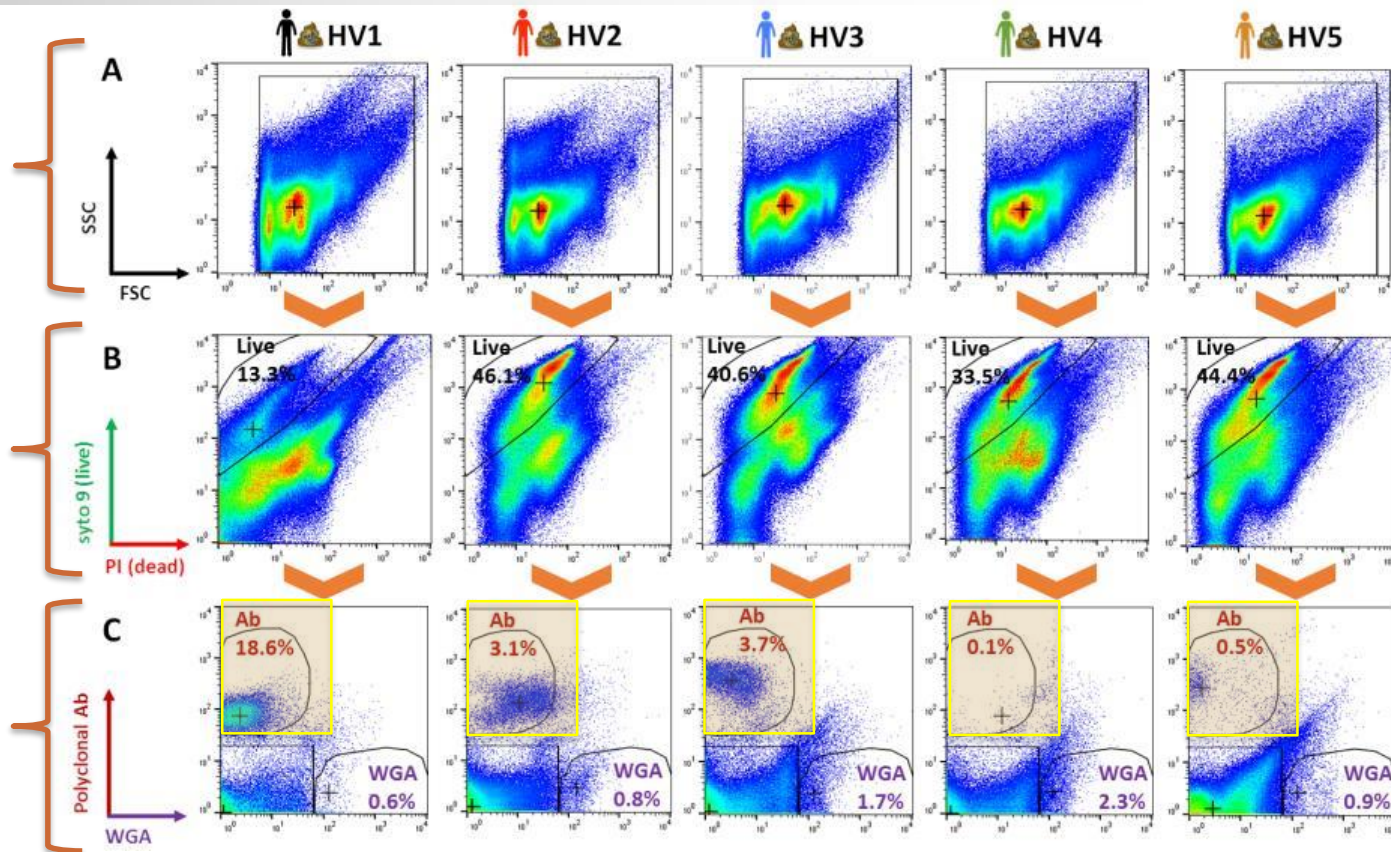


# Microbiota profiling using flow cytometry: antibodies for the detection of specific species

Physical parameters  
FSC/SSC

Live / Dead  
staining

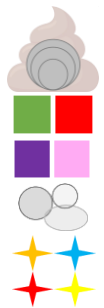
Polyclonal  
antibodies (Ab)  
directed against  
*F. prausnitzii*



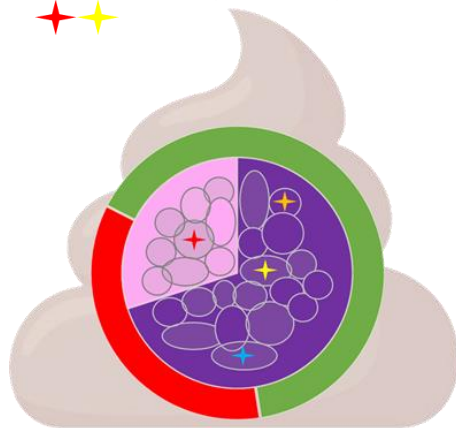


**Conclusion:** flow cytometry has the potential to become a fast and cost-effective tool for microbiota profiling

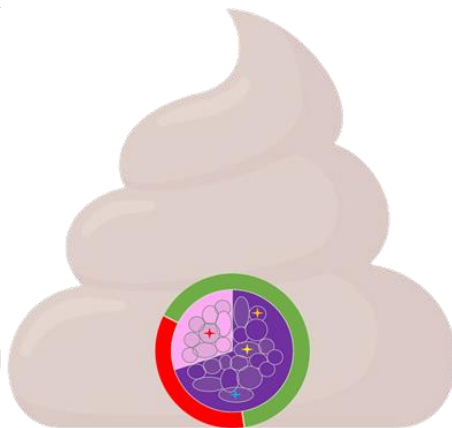
BIOASTER has developed and validated a series of staining methods that are compatible with flow cytometry analysis to explore fecal microbiota composition at various levels of precision:



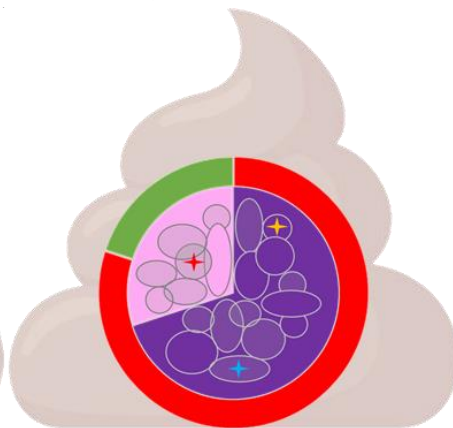
- **Bacterial load** = number of bacteria per gram of fecal material
- proportions of **Live** vs **Dead** bacteria,
- proportions of **Gram positive** vs **Gram negative** bacteria,
- bacterial **population complexity** based on simple physical parameters (FSC & SSC) and DNA staining
- Specific species of interest: polyclonal antibodies that target commensal bacterial species of interest



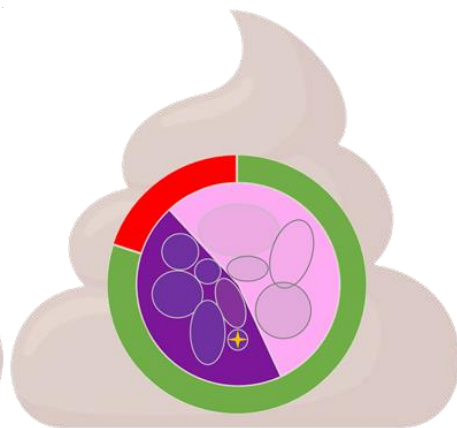
Reference



Decreased bacterial load



High proportion of dead bacteria



Decreased diversity  
& loss of species of interest

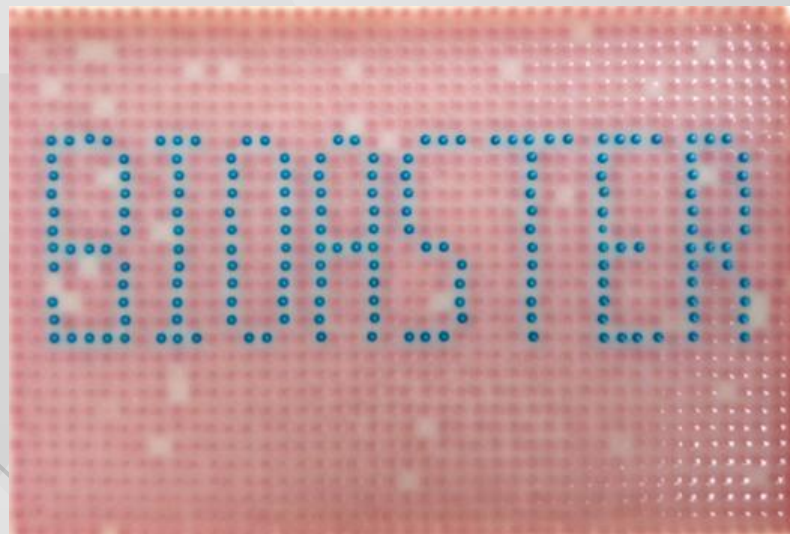
- Flow cytometry is a versatile tool with many potential applications in the field of microbiome research, from host-associated to environmental microbiomes
- BIOASTER is a private, non-for profit research organization: we are interested in collaborations to work on complex microbiota analysis using FCM as well as selection of specific microbial targets from any complex ecosystem
- Contact us!

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Mélanie NEHLICH  
Maryne ANIA  
Ilia BELOTSEKOVSKY



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# Thank you!



Florian PLAZA ONATE  
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fât  
Aurore DUQUENOY  
Carole SCHWINTNER



Wilfrid MAZIER  
Sandrine CLAUS



Nicole Simone TREICHEL  
Thomas CLAVEL



Jan BAIJER  
Ger van den ENGH

