香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

Imaging techniques in host-microbiome interactions, early diagnosis, and monitoring of bacterial infections

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Joint Graduate Seminar
Department of Microbiology, Faculty of Medicine, CUHK
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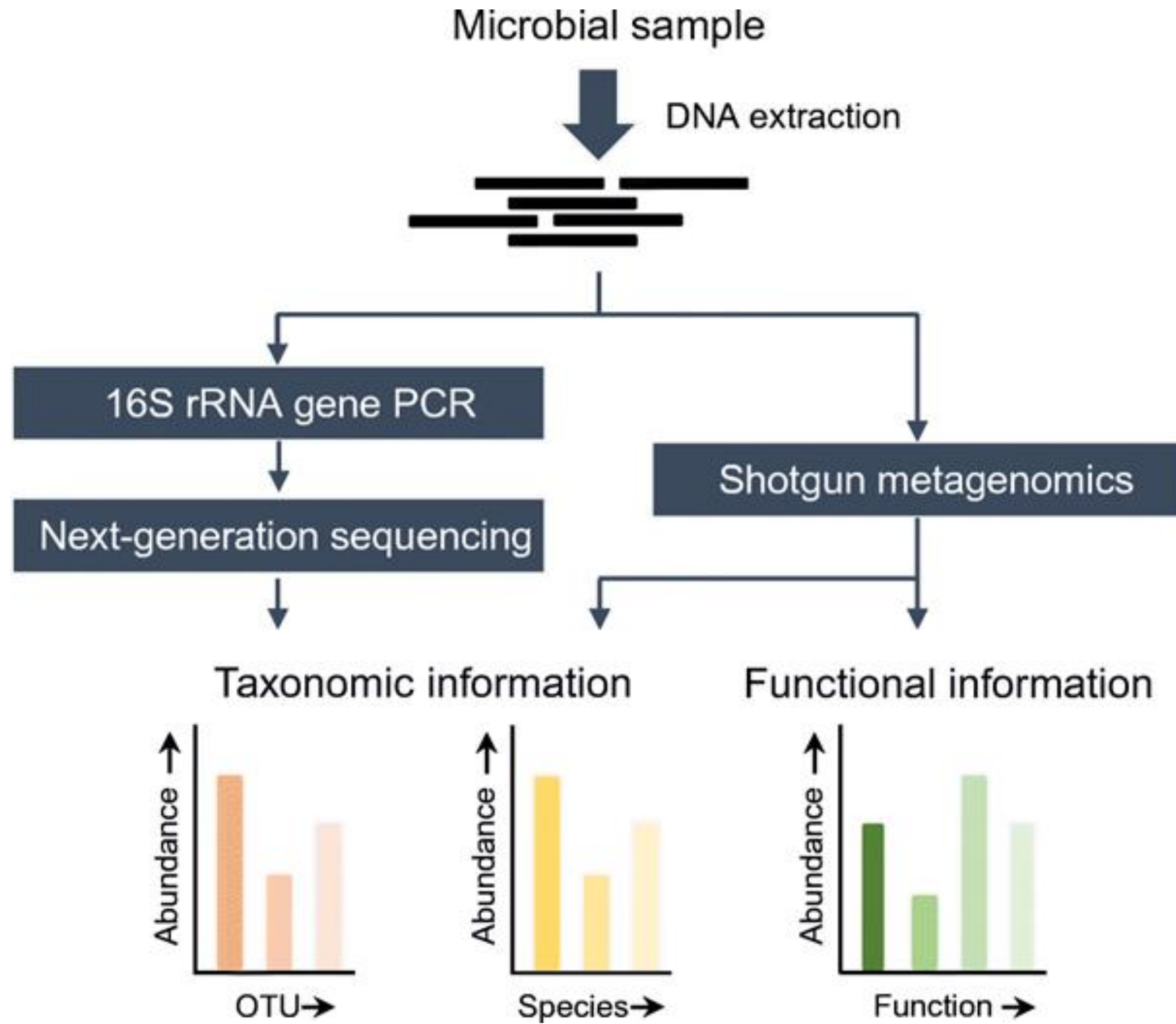
1. Single-Cell Strain Distinction *in vivo*
2. Modulating gene expression of gut commensals *in vivo*
3. Single cell fluorescence imaging of glycan uptake by intestinal bacteria
4. Molecular imaging of bacterial infections: Overcoming the barriers to clinical translation

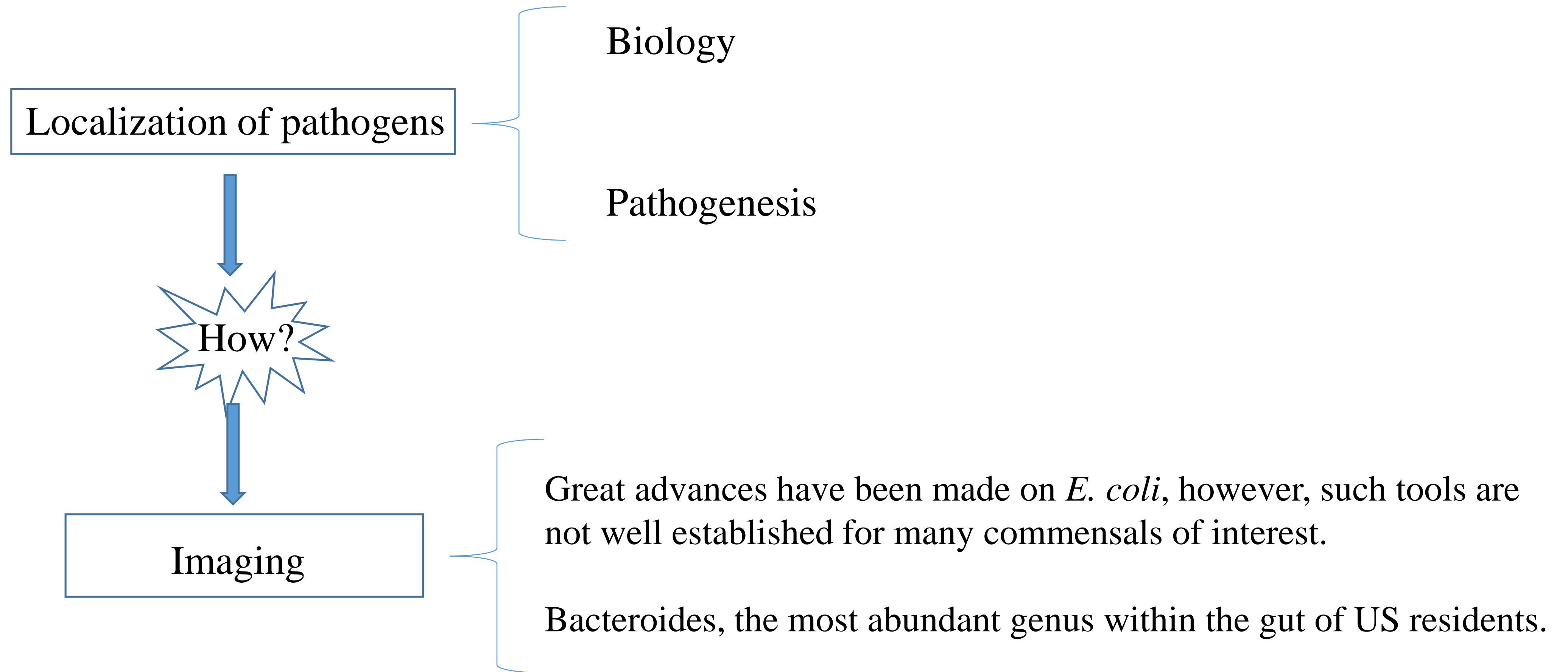
Introduction



Drawback: No biogeographic information of microbiota

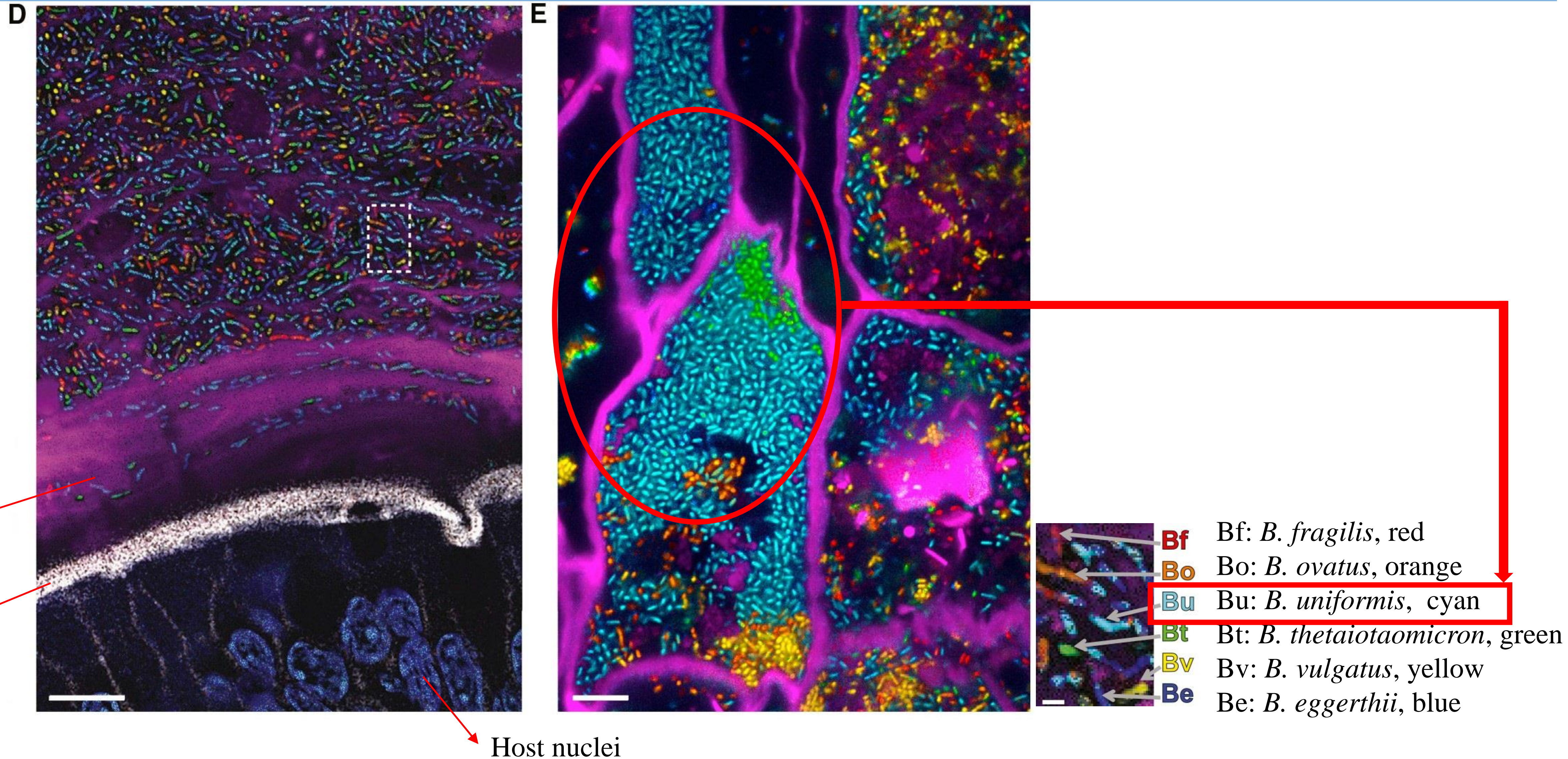
Boers *et al*., Eur J Clin Microbiol Infect Dis, 2019

1. Imaging technique for differentiation of bacterial strain *in vivo*



Whitaker *et al*., Cell, 2017

1.1. Imaging technique for Single-Cell Strain Distinction in the Gut Microbiome



Whitaker *et al*., Cell, 2017

Applications of the 1st Platform

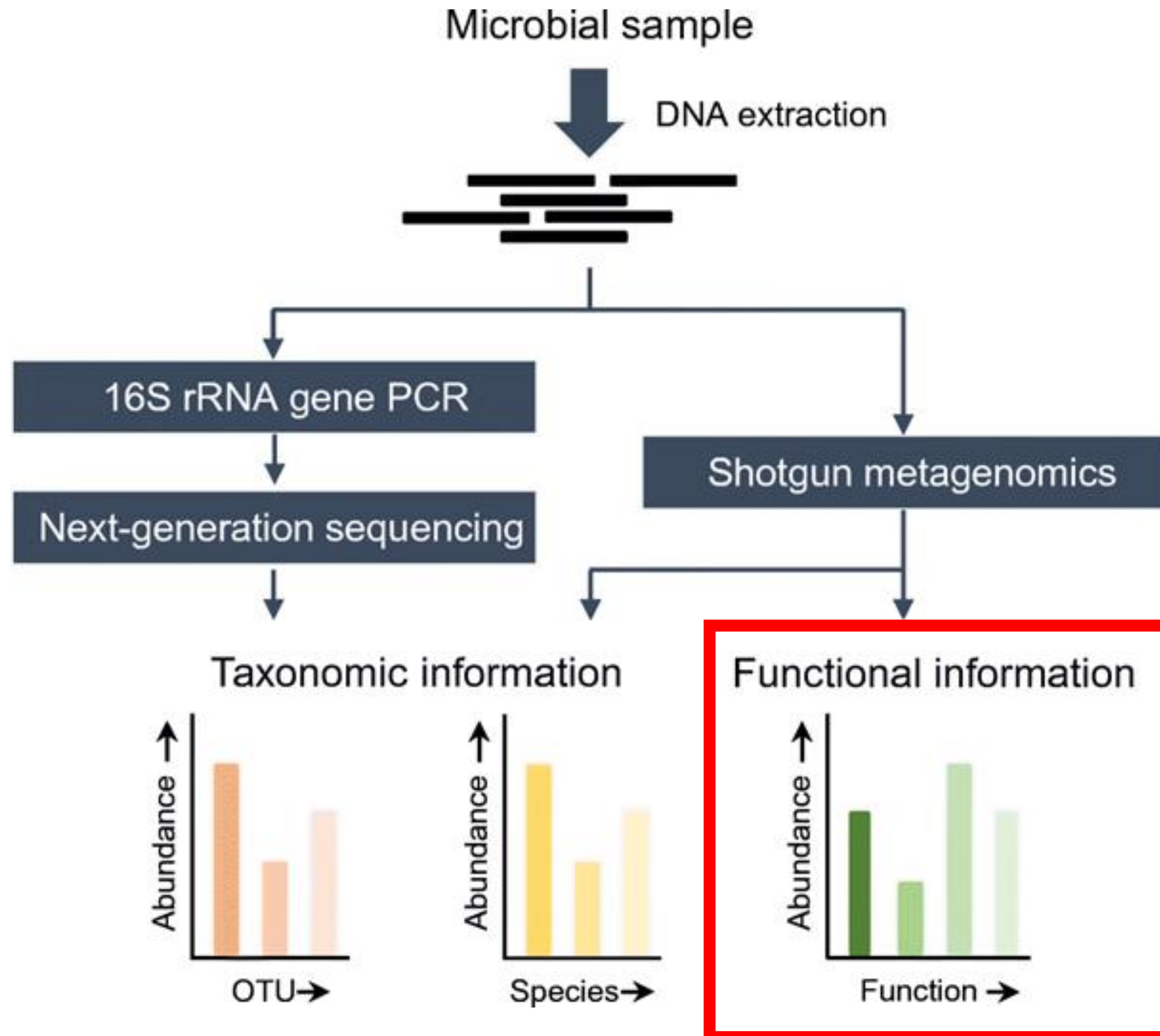
The major breakthrough

1. Imaging of fluorescently tagged Bacteroides strains at the single-cell level *in vivo* .

Study pathogenesis and microbiota-based therapies (probiotics)

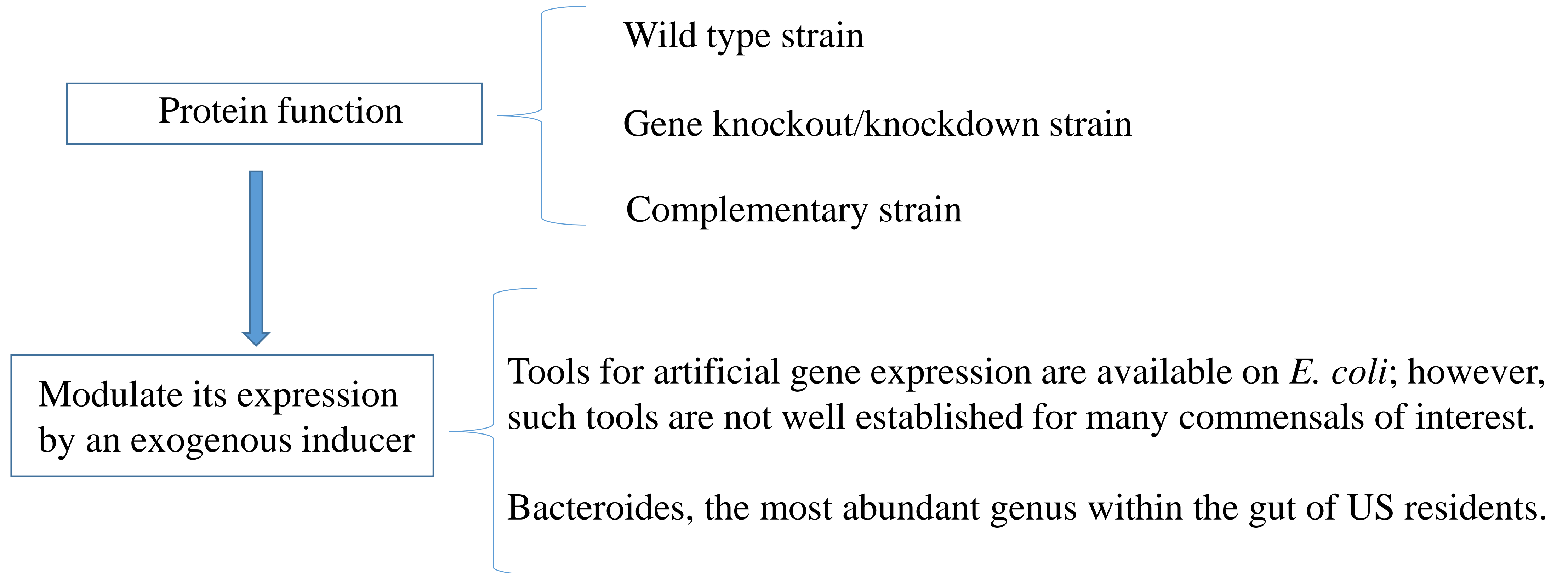
2. This platform opens the door for studying single-cell interactions and understanding spatial organization of the gut microbiota.

Introduction



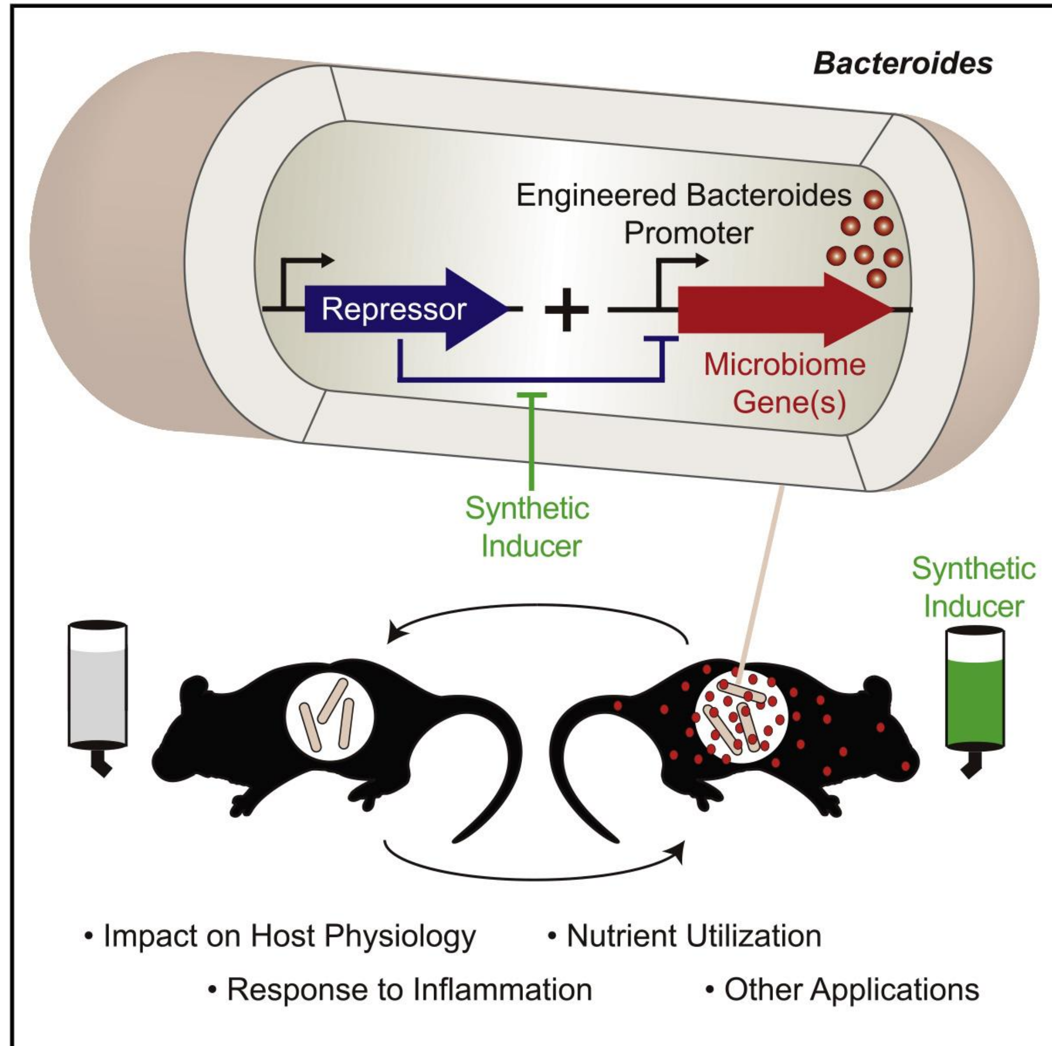
Boers *et al.*, Eur J Clin Microbiol Infect Dis, 2019

2. Modulating gene expression of gut commensals *in vivo*



Lim *et al*., Cell, 2017

2. Modulating gene expression of gut commensals *in vivo*



New platform enables modulating gene expression of Bacteroides inside the gut through introduction of a synthetic inducer in drinking water.

Constitutively expressed repressor and inducible promoter: Four to five orders of magnitude

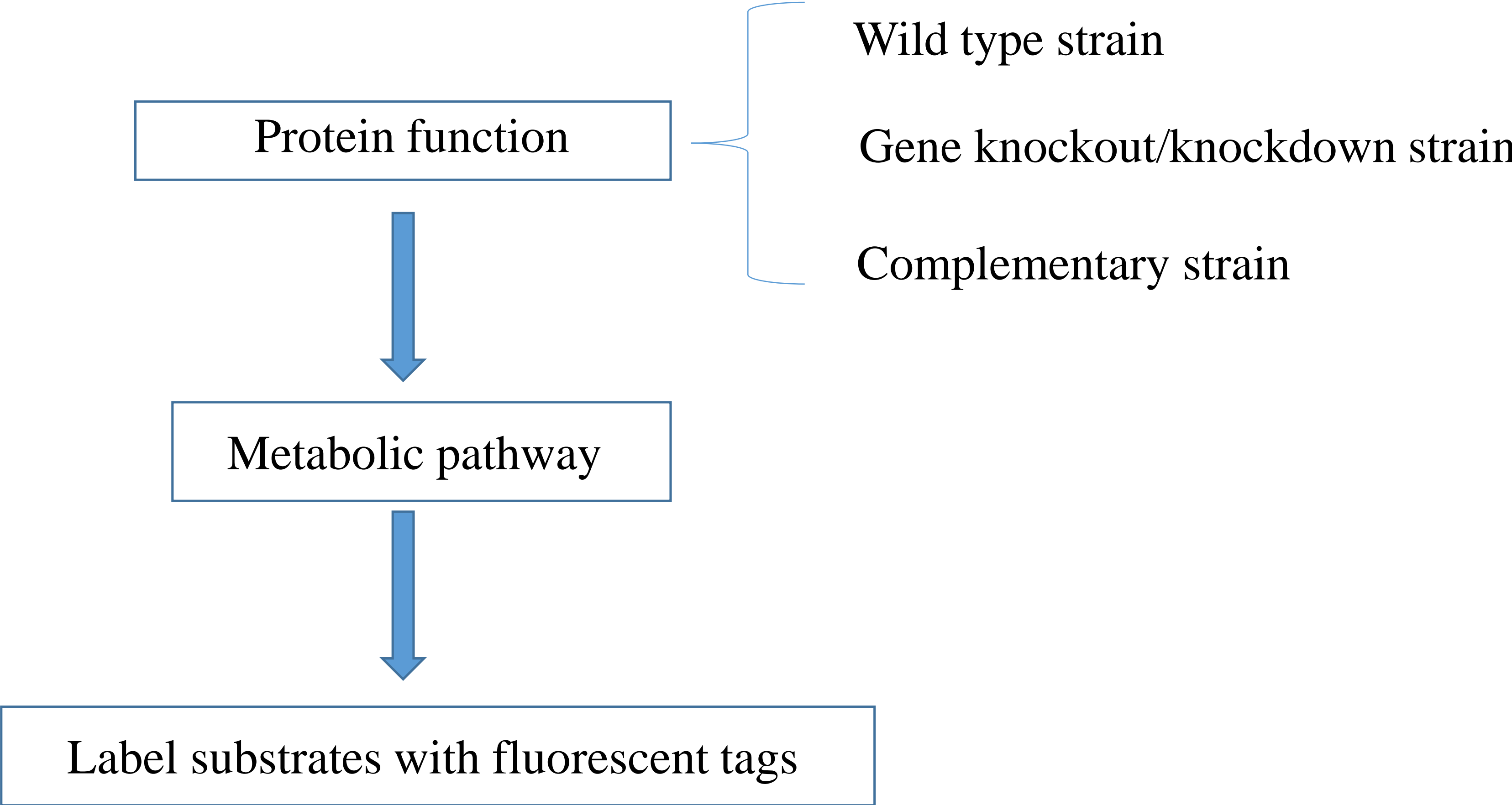
Lim *et al*., Cell, 2017

Applications of the 2nd Platform

1. Gene expression can be modulated with time in the same strain or experiment, permitting kinetic studies of the bacterial or host response to production, depletion, or repeated exposures of a gene product.

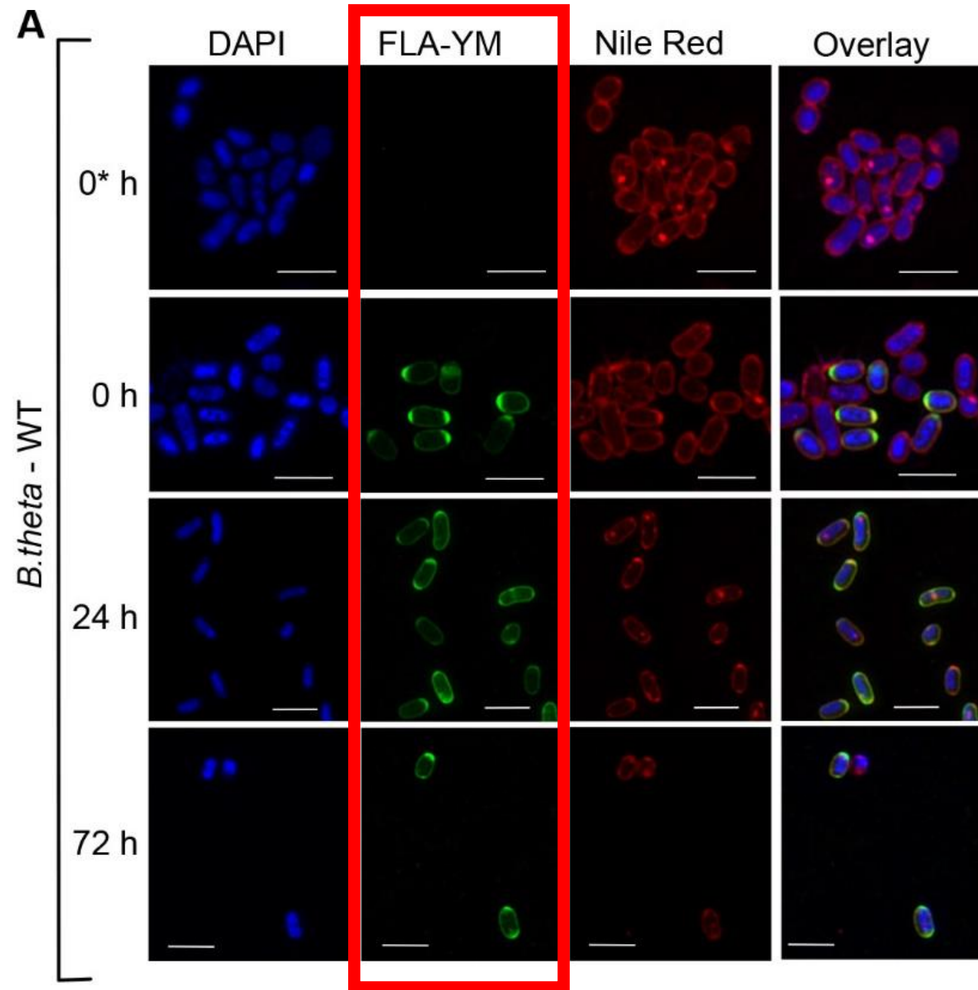
2. Inducible systems allow mechanistic study of essential or toxic gene products.

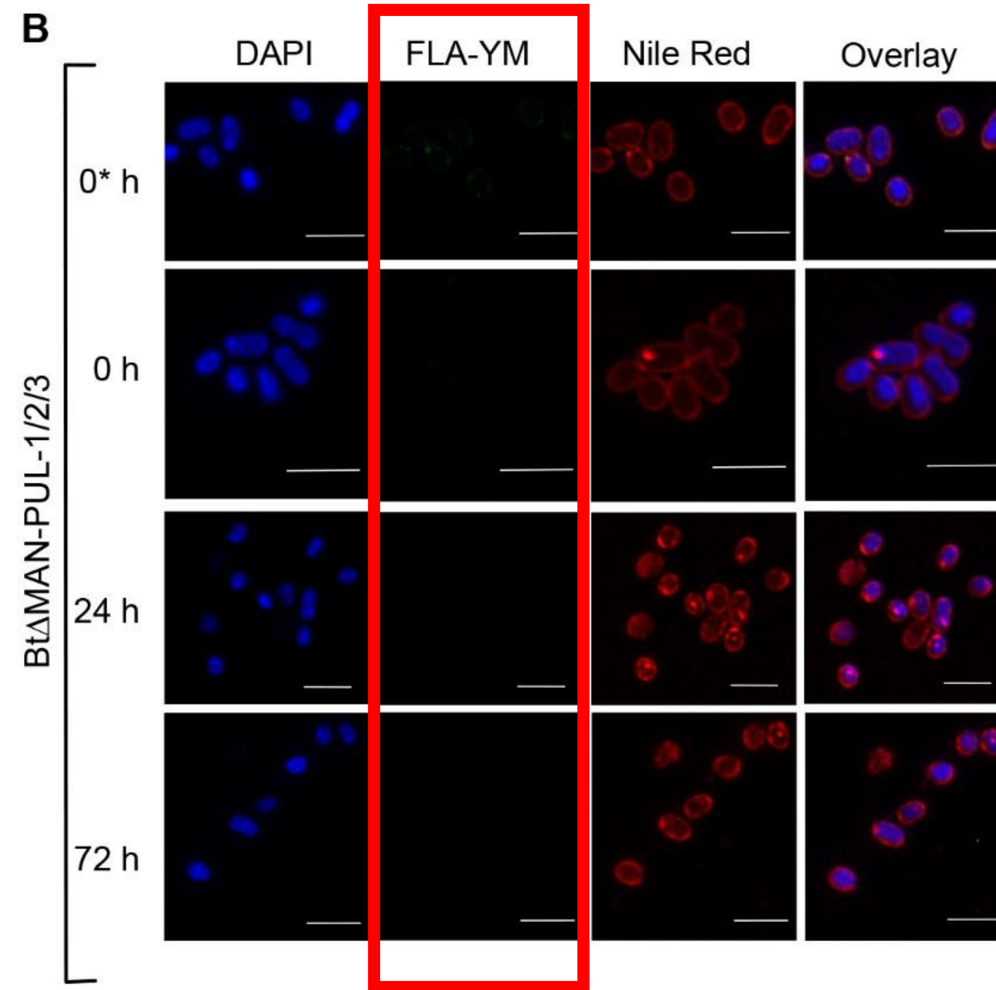
3. Single cell fluorescence imaging of glycan uptake by intestinal bacteria



Hehemann *et al.*, ISME J, 2019

3.1. Single cell fluorescence imaging of glycan uptake by *B. theta* WT and mutant strains





Yeast α-mannan (YM) was labeled with 6-aminofluorescein (FLA) to produce FLA-YM

MAN-PUL-1/2/3 utilization of YM by *B. theta*

DAPI (blue, cellular DNA), FLA-YM (green), and Nile Red (red, membrane lipid bilayer), 0* (true zero), 0 (directly after glycan addition), Visualized by super-resolution structured illumination microscopy (SR-SIM)

Hehemann *et al.*, ISME J, 2019

Applications of the 3rd platform

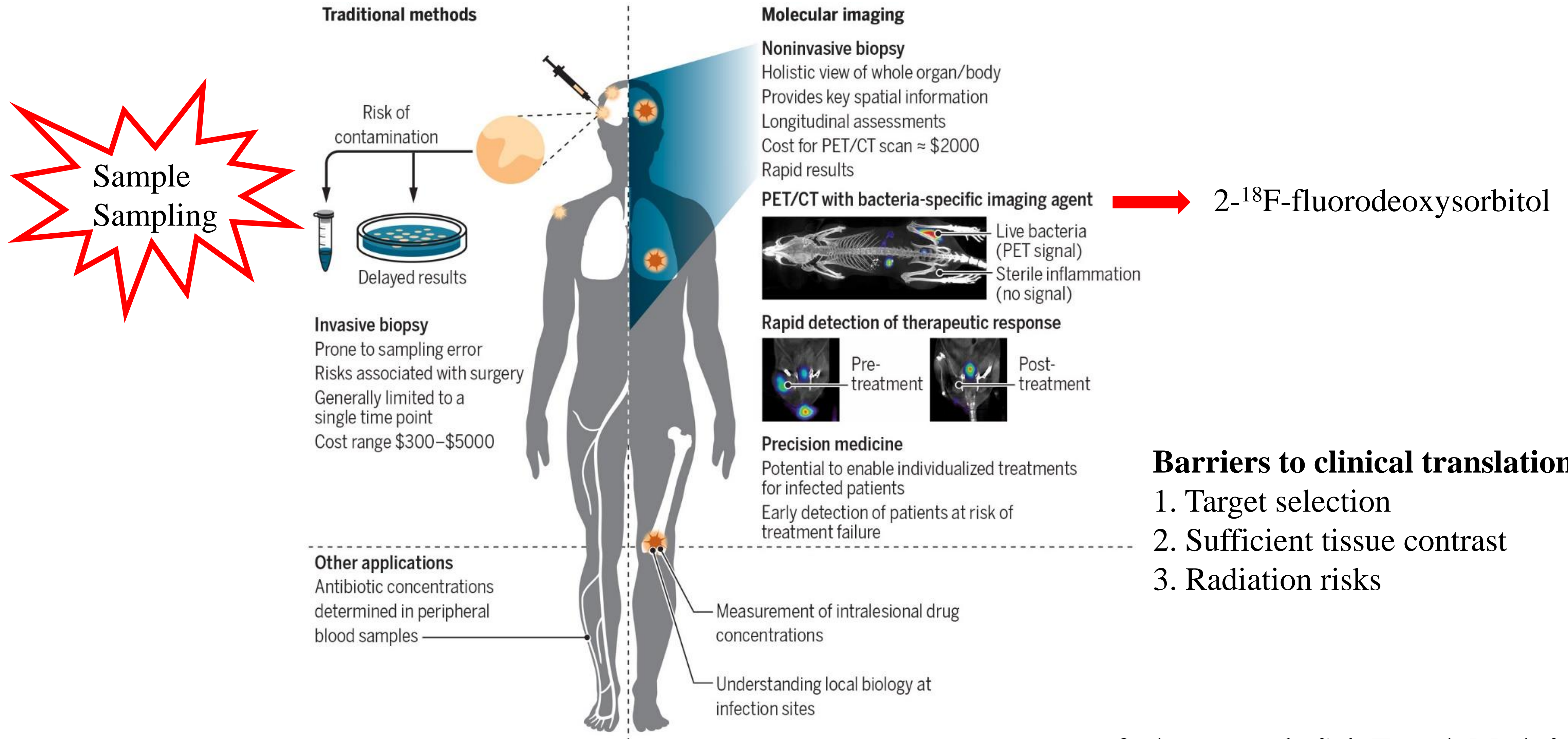
1. This platform provides a direct method to assess specific glycan metabolism in intestinal bacteria at the single cell level.

2. This platform enables rapidly assign metabolic phenotypes to genotypes on the single cell level within a microbial community.

3. This platform is powerful especially when combined with genome editing platforms.

Hehemann *et al.,* ISME J, 2019

4. Molecular imaging of bacterial infections: Overcoming the barriers to clinical translation



2-^{18}F-fluorodeoxysorbitol

Barriers to clinical translation:

1. Target selection
2. Sufficient tissue contrast
3. Radiation risks

Ordonez *et al.*, Sci. Transl. Med.,2019

Summary

These platforms provide good insights for the following studies:

1. Single-cell interactions and connect spatial organization to function

2. Protein function in its native environment

3. Metabolic pathways

4. Host-microbiome interactions

5. Early diagnosis and monitoring of bacterial infections

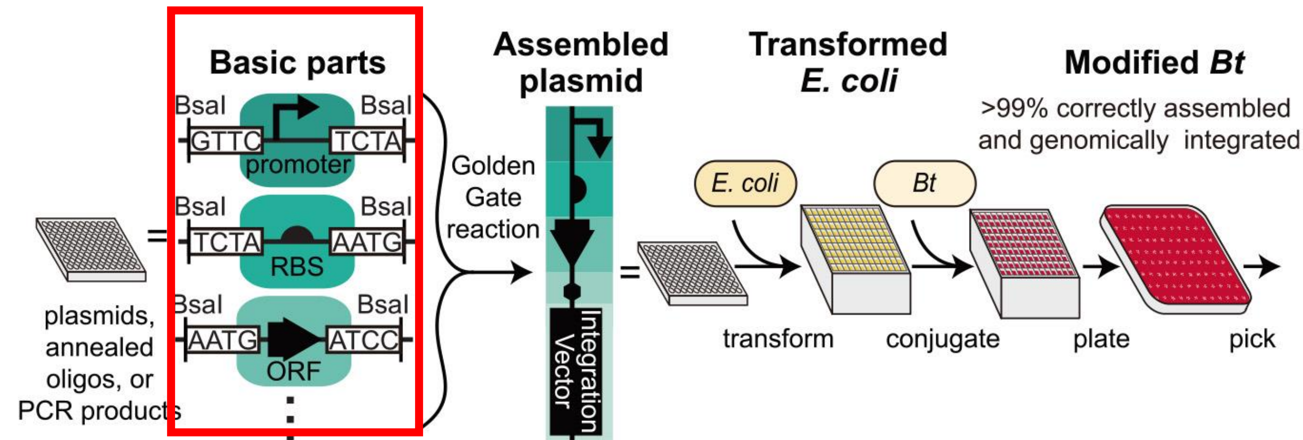
6. Other pathogens especially anaerobes such as Clostridium, Fusobacterium.

References

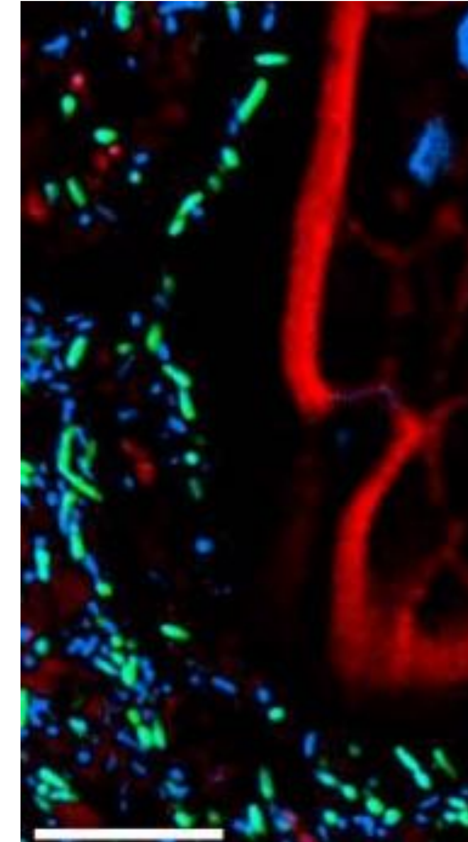
1. Baümler, A. J. &Sperandio, V. Interactions between the microbiota and pathogenic bacteria in the gut. *Nature* vol. 535 85–93 (2016).
2. Boers, S. A., Jansen, R. &Hays, J. P. Understanding and overcoming the pitfalls and biases of next-generation sequencing (NGS) methods for use in the routine clinical microbiological diagnostic laboratory. *European Journal of Clinical Microbiology and Infectious Diseases* vol. 38 1059–1070 (2019).
3. Whitaker, W. R., Shepherd, E. S. &Sonnenburg, J. L. Tunable Expression Tools Enable Single-Cell Strain Distinction in the Gut Microbiome. *Cell* **169**, 538-546.e12 (2017).
4. Tropini, C., Earle, K. A., Huang, K. C. &Sonnenburg, J. L. The Gut Microbiome: Connecting Spatial Organization to Function. *Cell Host Microbe* **21**, 433–442 (2017).
5. Lim, B., Zimmermann, M., Barry, N. A. &Goodman, A. L. Engineered Regulatory Systems Modulate Gene Expression of Human Commensals in the Gut. *Cell* **169**, 547-558.e15 (2017).
6. Joglekar, P. &Segre, J. A. Building a Translational Microbiome Toolbox. *Cell* **169**, 378–380 (2017).
7. Hehemann, J. H. *et al.* Single cell fluorescence imaging of glycan uptake by intestinal bacteria. *ISME J.* **13**, 1883–1889 (2019).
8. Ordonez, A. A. *et al.* Molecular imaging of bacterial infections: Overcoming the barriers to clinical translation. *Sci. Transl. Med.* **11**, eaax8251 (2019).

Thank you for your attention !

1.1.1. Constructing plasmids for Expression of fluorescent proteins



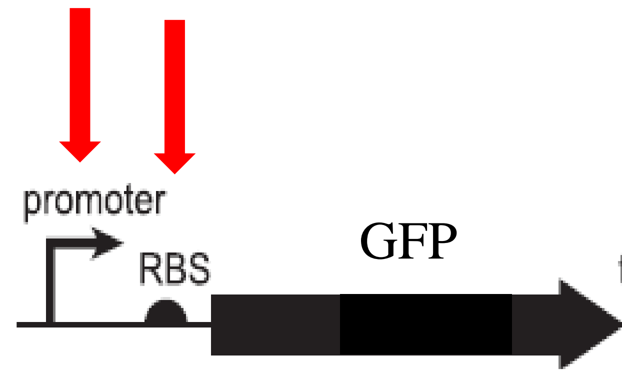
Vector: NBU2 integration plasmid



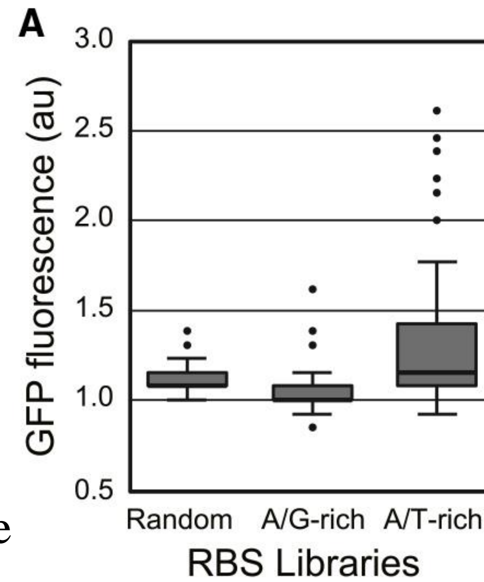
Host tissue (red, actin), GFP expressing Bacteroides (green) and non-expressing Bacteroides (blue, DAPI)

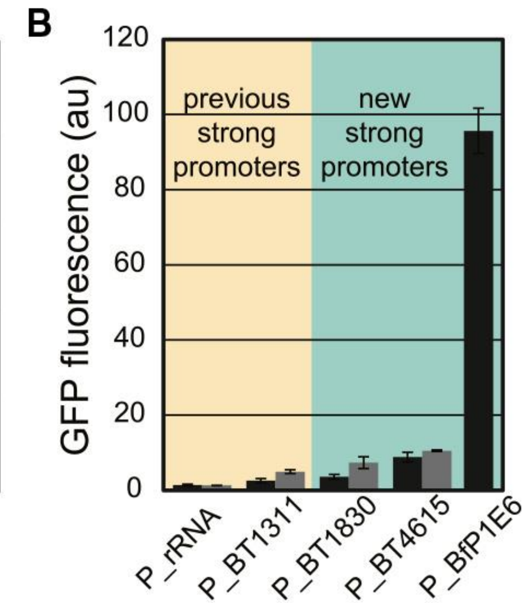
Whitaker *et al.*, Cell, 2017

1.1.2. Developing High-Expression Tools



RBS libraries
- Random degenerate sequence
- A/G-rich degenerate sequence
- A/T-rich degenerate sequence

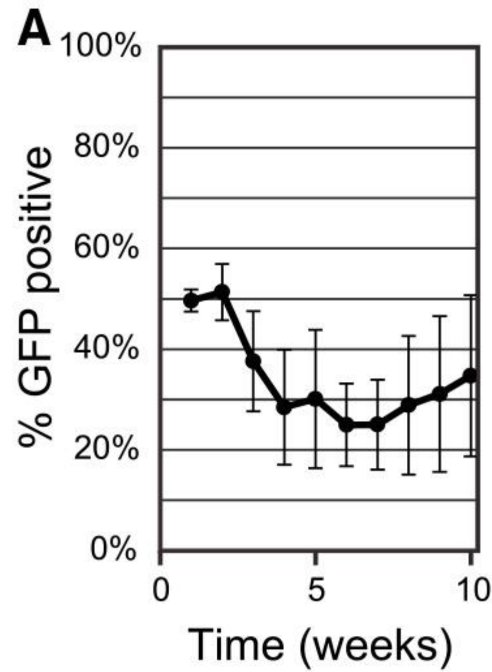


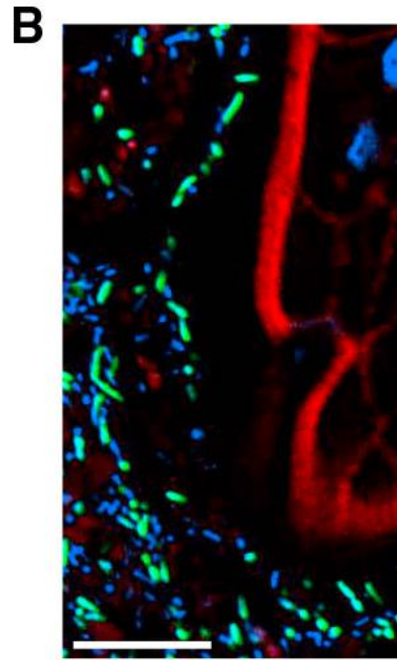


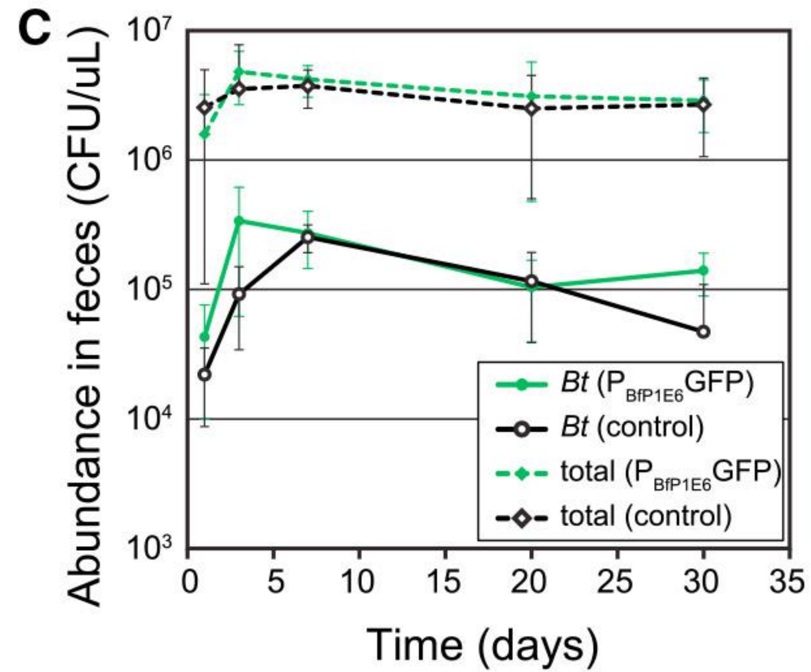
phage promoter

Whitaker *et al.*, Cell, 2017

1.1.3. High protein expression will not result in a loss of Bt fitness







A 50:50 mix of the two strains was inoculated into germ-free mice to assess the fitness burden

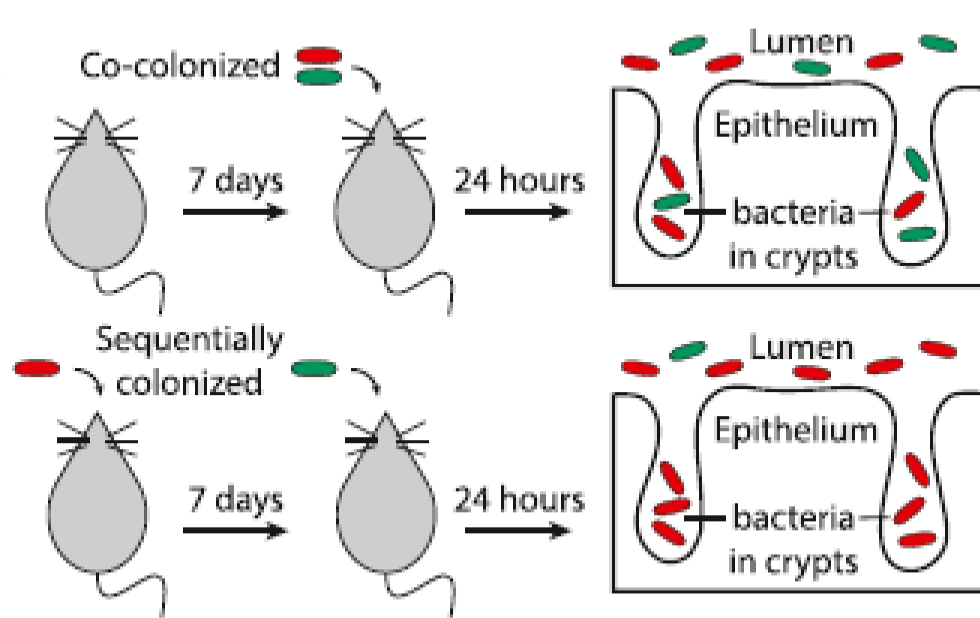
Only a minor fitness defect and stable colonization over a 10-week period in gnotobiotic mice

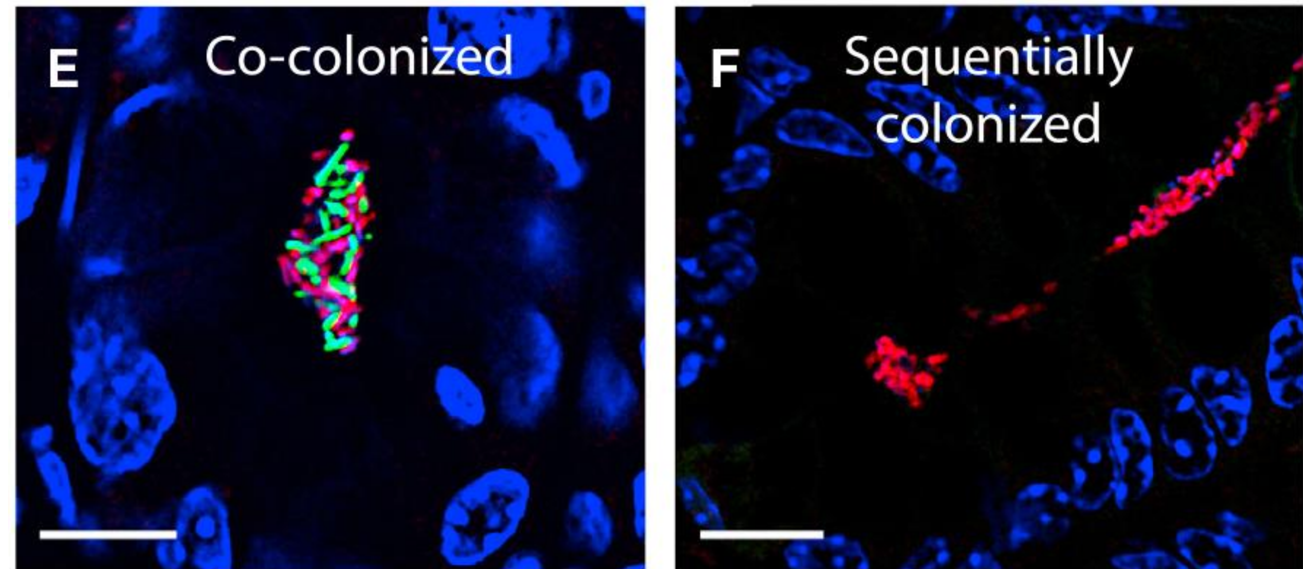
Sufficient for single-cell detection *in vivo*

Whitaker *et al.*, Cell, 2017

1.2. Colonization by Bt Prevents Crypt Localization of an Isogenic Strain

Isogenic strains of *B. thetaiotaomicron*

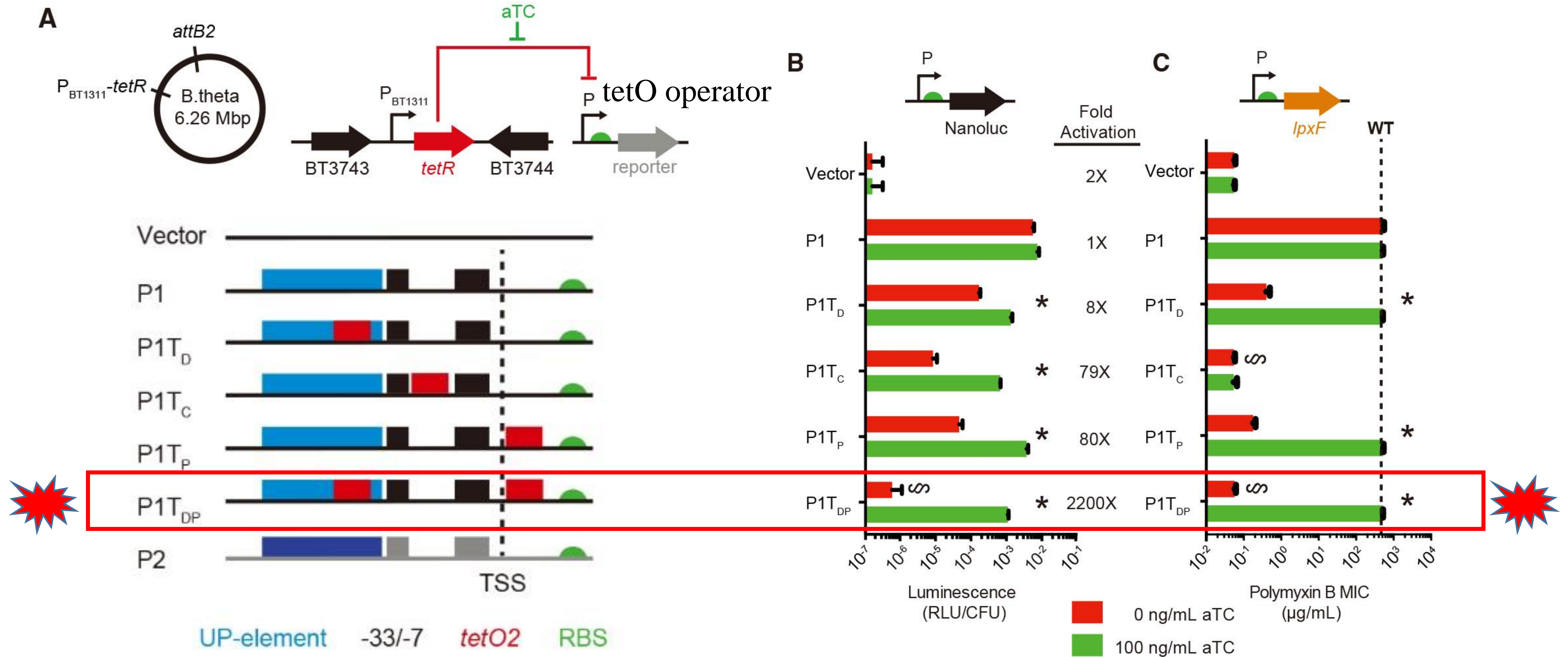




Green: 52% VS 0.73%

Whitaker *et al*., Cell, 2017

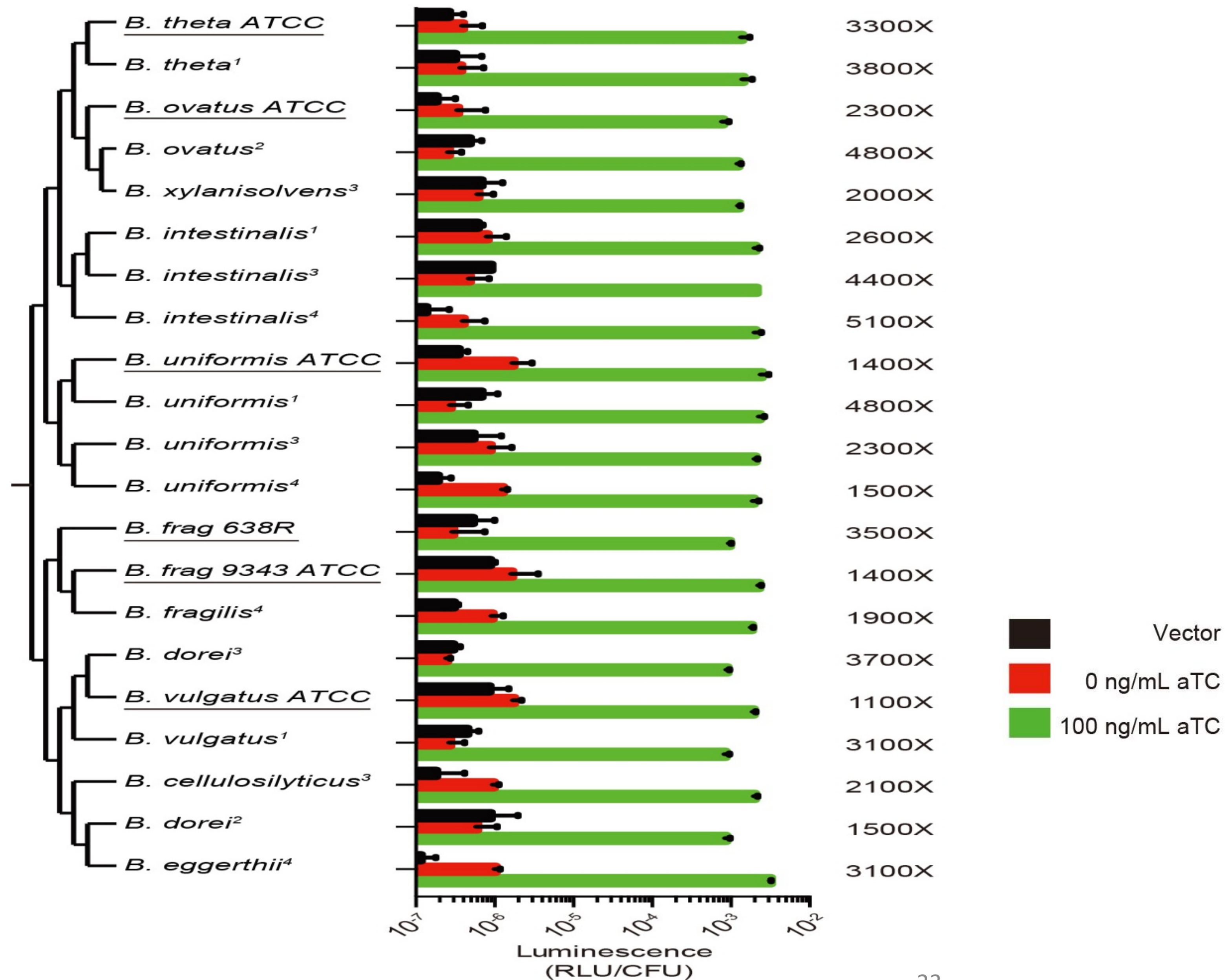
2.1.1. Engineered tetO2-Containing Promoters Maintain High Levels of Gene Expression



TetR has a high affinity (~10^{-11} M) for the tetracycline analog anhydrotetracycline (aTC), and aTC biding to TetR decreases its affinity for tetO by six to ten orders of magnitude

Lim *et al.*, Cell, 2017

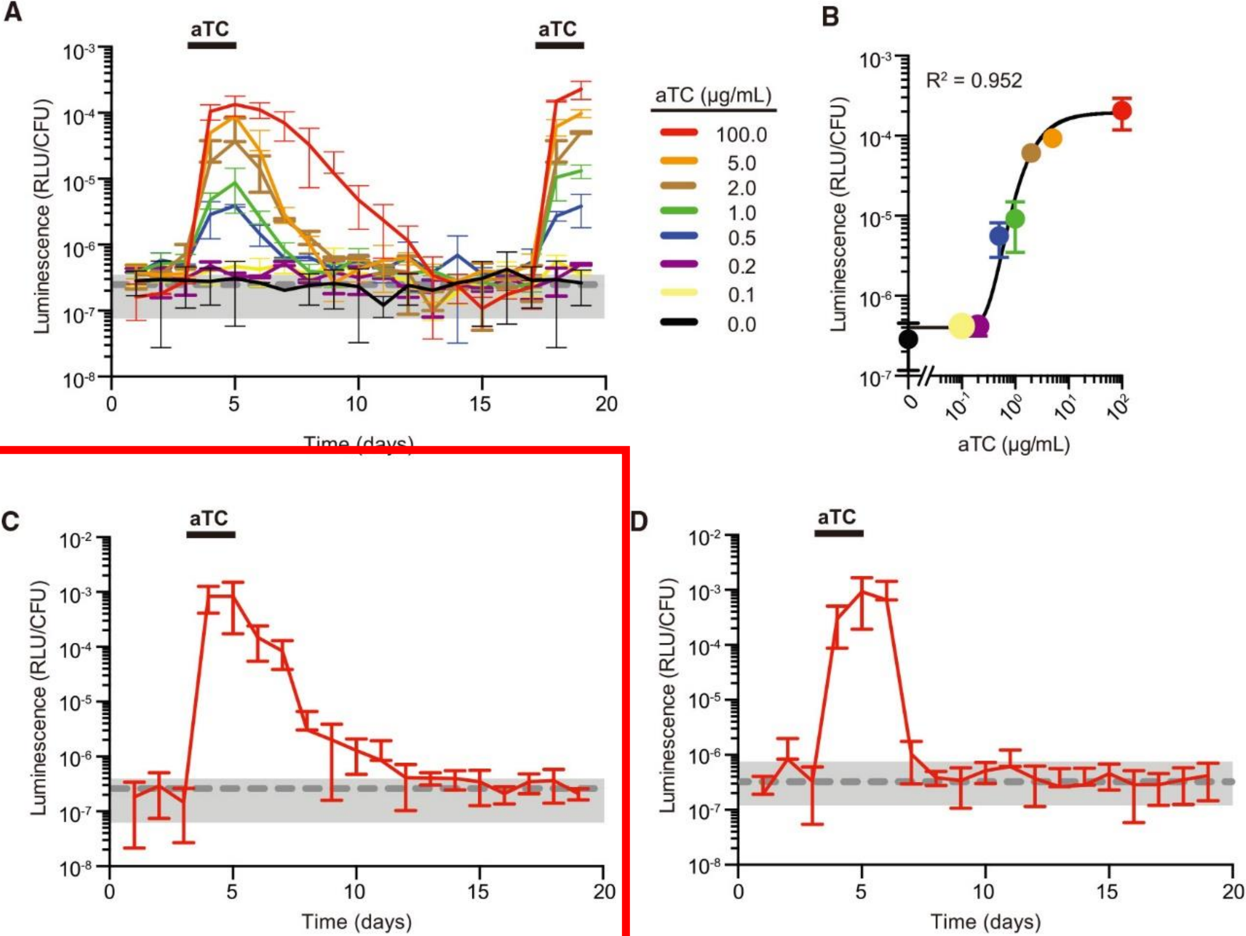
2.1.2. Evaluation and Performance of the P1TDP Platform in Different Bacteroides Species

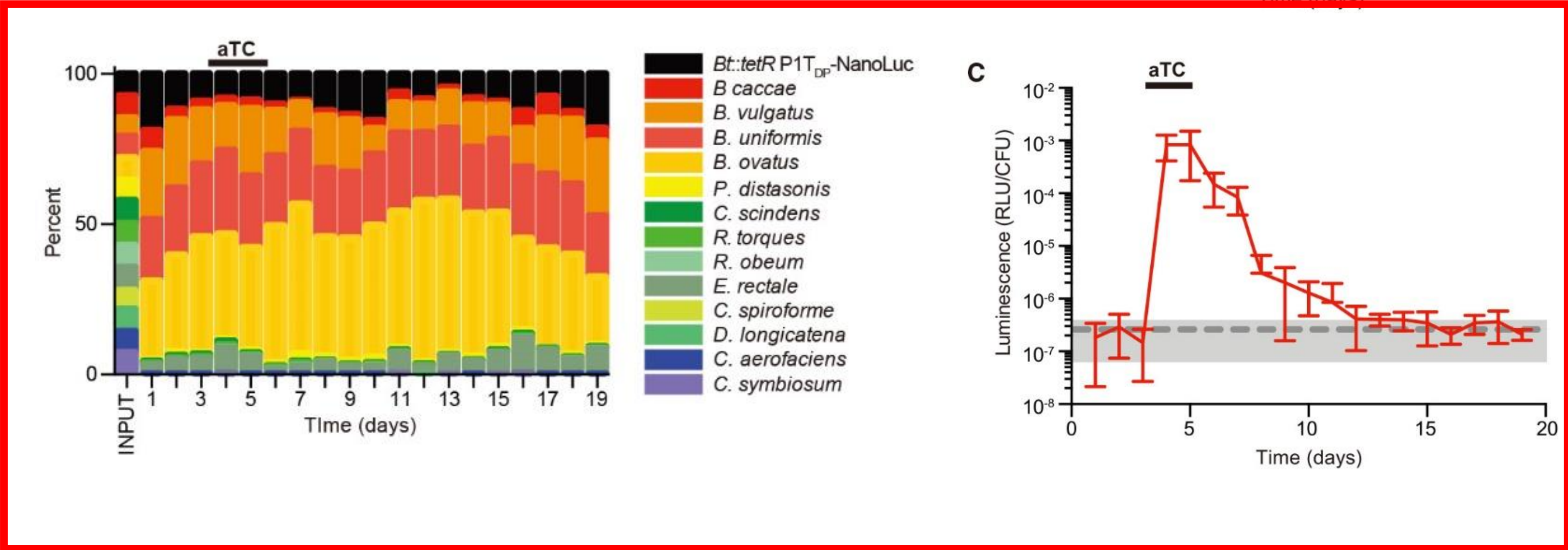


Lim *et al*., Cell, 2017

2.1.3. Exogenous Control of Bacteroides Gene Expression in the Mouse Gut via a Synthetic Inducer

Working concentration: 100 μg/mL
The inducer (aTC) is effective within 24 hr and undetectable 6 days after removed .

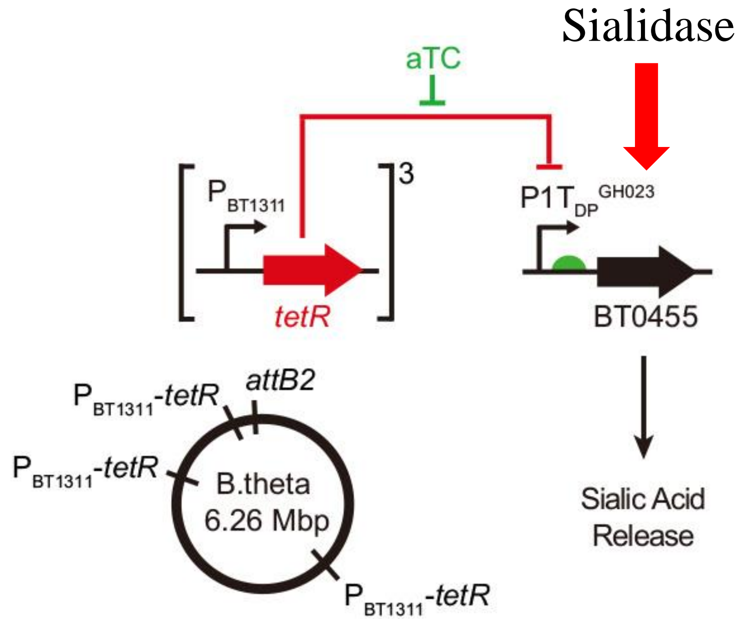




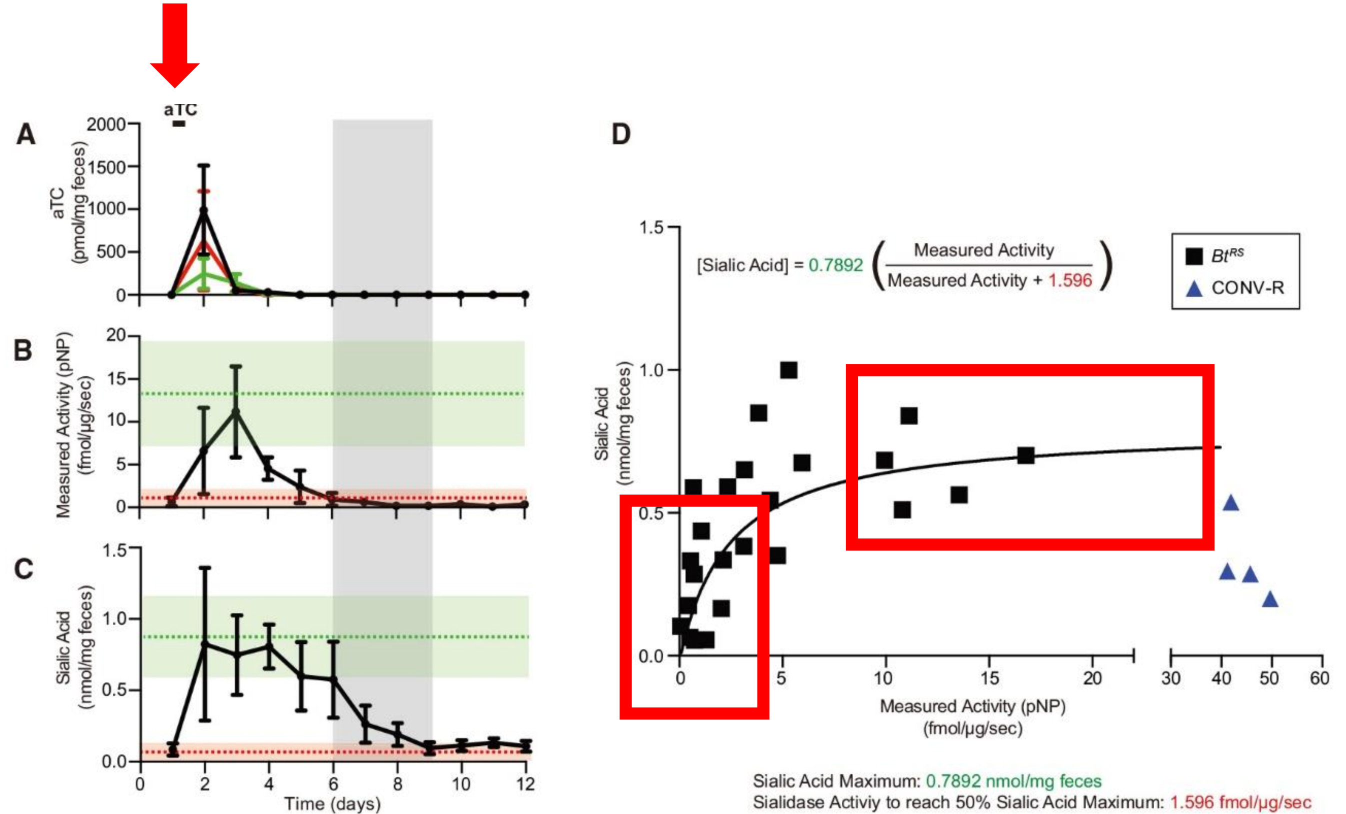
Lim *et al.*, Cell, 2017

2.1.4 Inducible Expression Platforms Reveal New Dynamics of Host-Microbiome Interactions

Sialidase



B. thetaiotaomicron



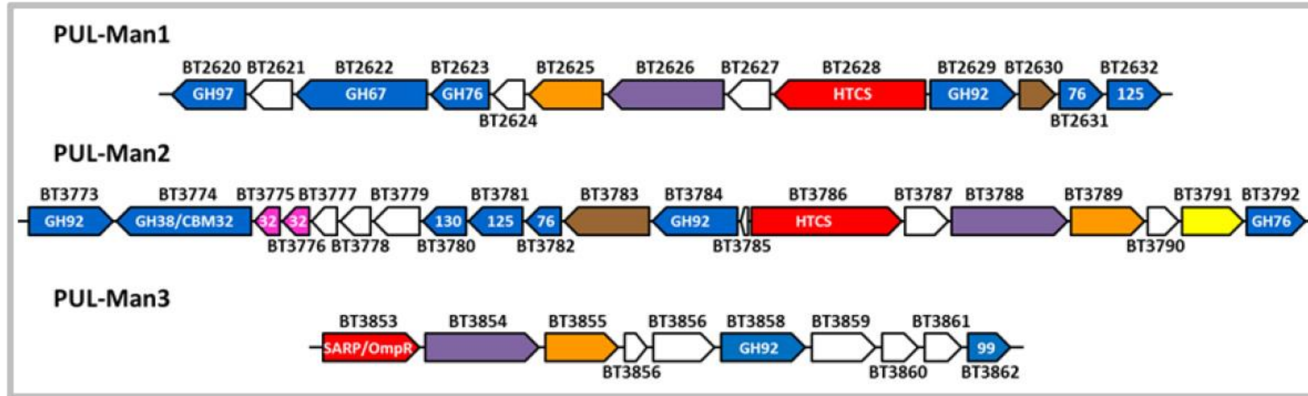
Substrate-limited

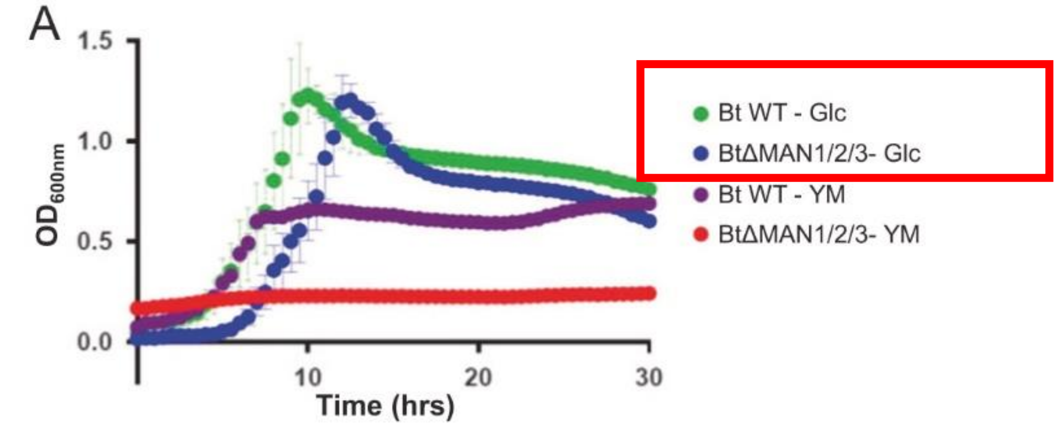
Bt::3xtetR ΔDBT0455
$P1T_{DP}^{GH023}$-BT0455

Lim *et al*., Cell, 2017

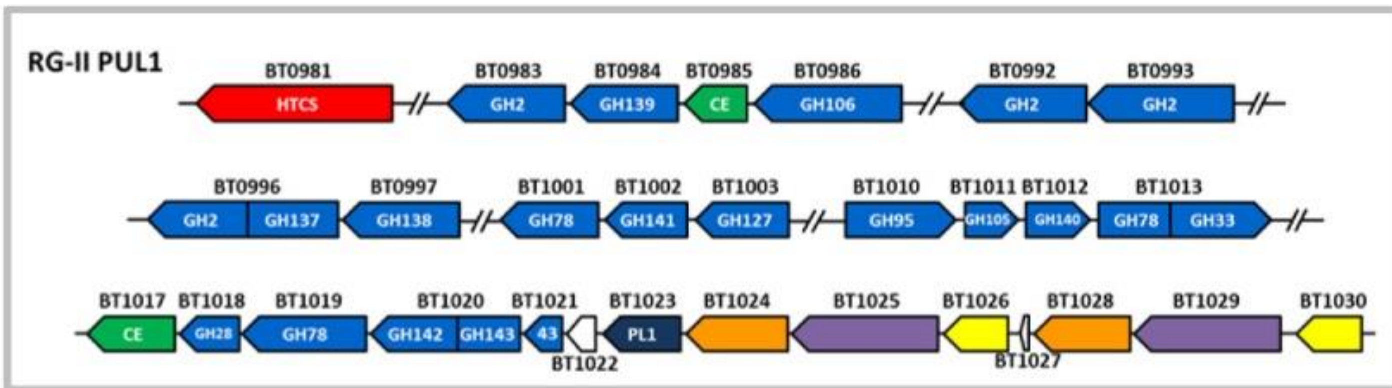
3.1.1. Choose substrates for metabolic pathway study

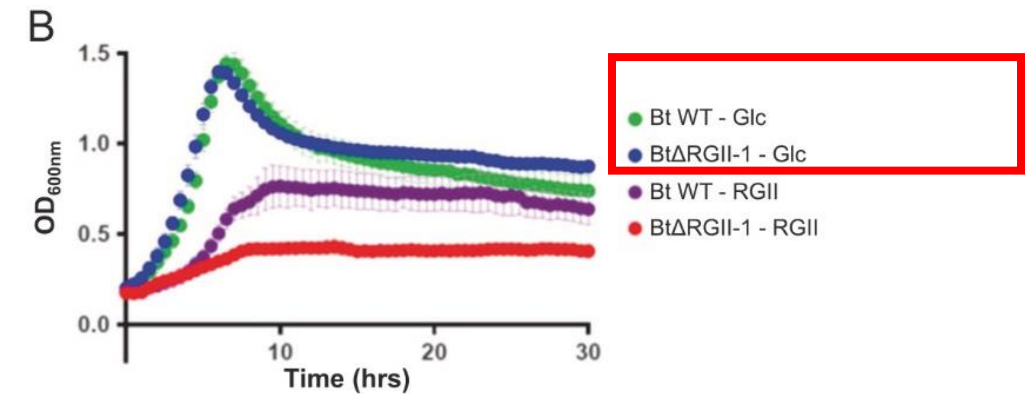
Yeast α-mannan (YM) and rhamnogalacturonan-II (RGII), two structurally distinct glycans were fluorescently labeled and fed to *Bacteroides thetaiotaomicron* VPI-5482.





Structure of PUL-MAN1, 2 and 3, pathways responsible for the utilization of YM by *B. theta*.

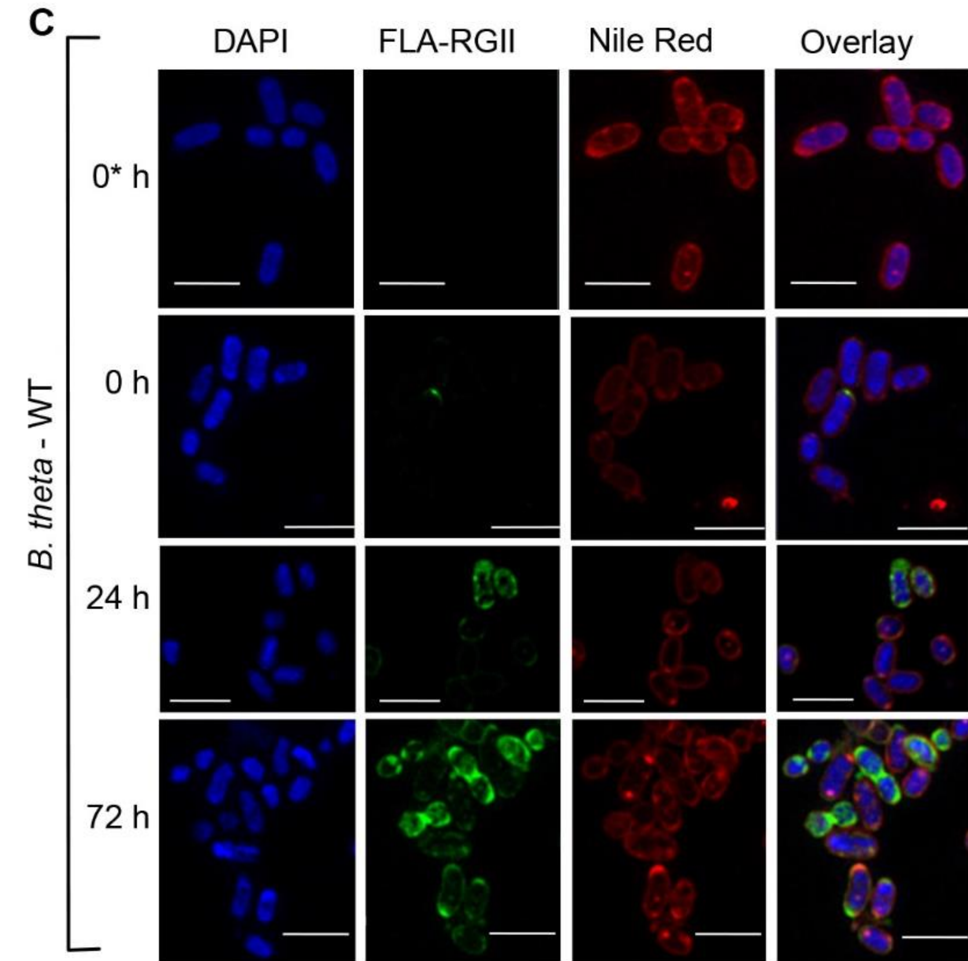


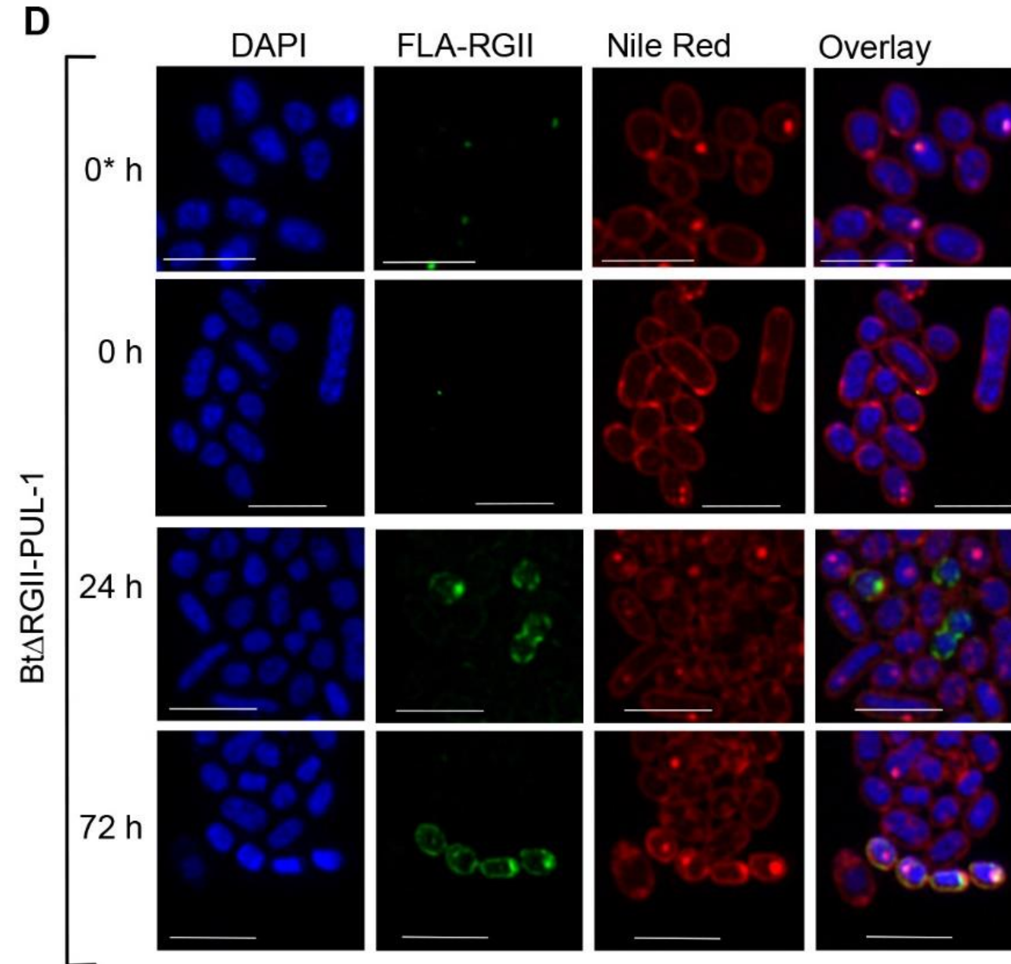


Structure of PUL-RGII1 which responsible for the utilization of RGII by *B. theta.*

Hehemann *et al.*, ISME J, 2019

3.2. Full panel display of fluorescently labeled *B. theta* mutant strains





RGII was conjugated at free diols with 6-aminofluorescein (FLA) using a cyanogen-bromide activation chemistry: FLA-RGII

DAPI (blue, cellular DNA), FLA-YM (green), and Nile Red (red, membrane lipid bilayer), 0* (true zero), 0 (directly after glycan addition), Visualized by super-resolution structured illumination microscopy (SR-SIM)

Hehemann *et al.*, ISME J, 2019

4. Molecular imaging of bacterial infections: Overcoming the barriers to clinical translation

Background:

Clinical diagnostic tools requiring direct sample testing cannot be applied to infections deep within the body, and clinically available imaging tools lack specificity. New approaches are needed for early diagnosis and monitoring of bacterial infections and rapid detection of drug-resistant organisms.

Highlight:

Molecular imaging allows for longitudinal, noninvasive assessments and can provide key information about infectious processes deep within the body.

Ordonez *et al*., Sci. Transl. Med.,2019