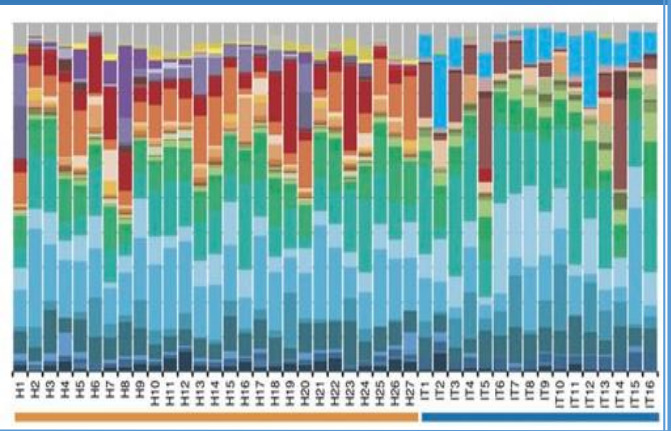
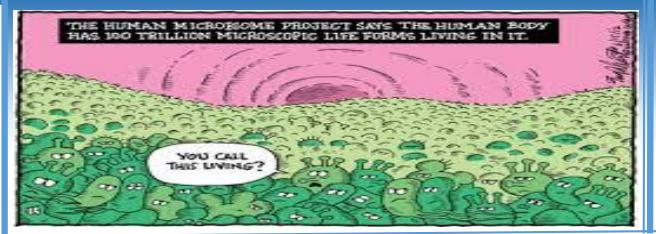

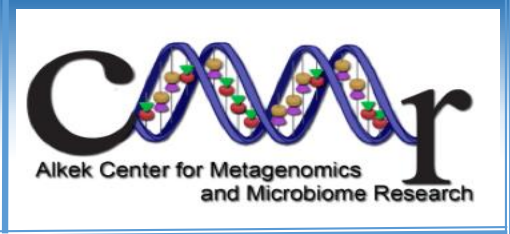



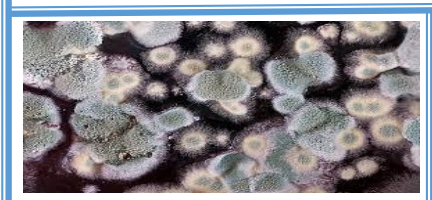

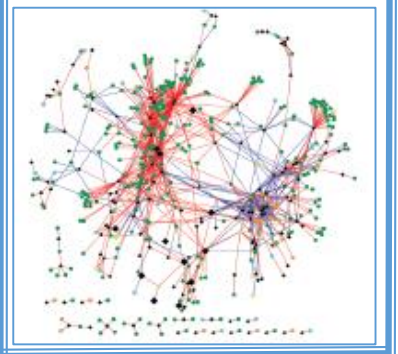
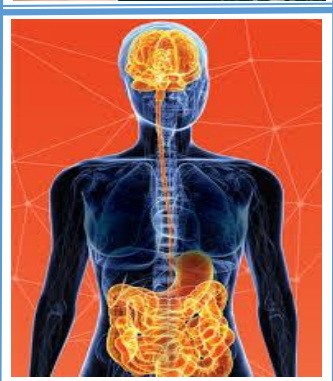
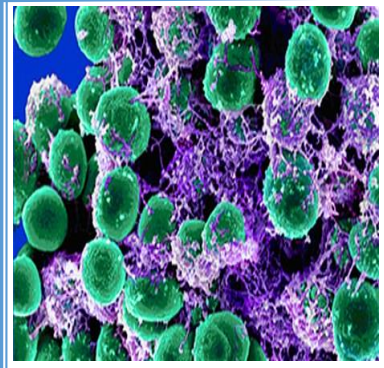


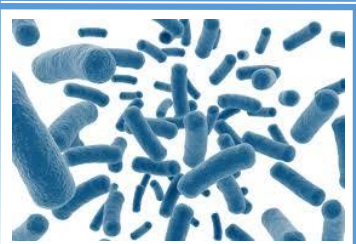


HESI Microbiome Workshop
June 25, 2018

The Tools and Technologies Needed for Microbiome Research

Joseph F. Petrosino
Alkek Center for Metagenomics and Microbiome Research
Department of Molecular Virology and Microbiology
Baylor College of Medicine
Houston, TX

Baylor College of Medicine

Overview

1. Translational microbiome research at Baylor College of Medicine
2. Overview of needs/gaps
3. (Quick) introduction of recent translational projects
    - Immunotherapy--microbiome and PD-1/PD-L1 blockade therapy
    - Maternal high fat diet induced social/synaptic defects in mice
4. Host-microbe models to test causality
5. New enteroid co-culture system for the study of human/host-microbial relationships *in vitro*

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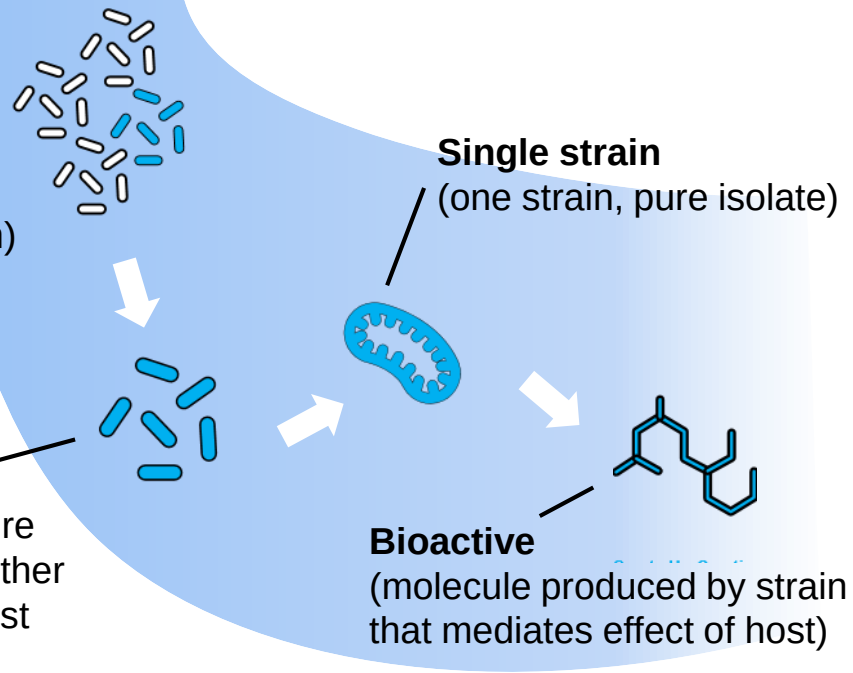
Model for Translational Microbiome Research

Compare microbiota in healthy and ill subjects

ID organisms / genes associated with health / disease

Develop applications / solutions



Community
(100's of strains, undefined composition)

Single strain
(one strain, pure isolate)

Consortium
(defined composition of more than one strain, which together perform a function of interest

Bioactive
(molecule produced by strain that mediates effect of host)

Diagnostic

Probotic / antibiotic

Patient Stratification

- Clinical trials
- Personalized medicine

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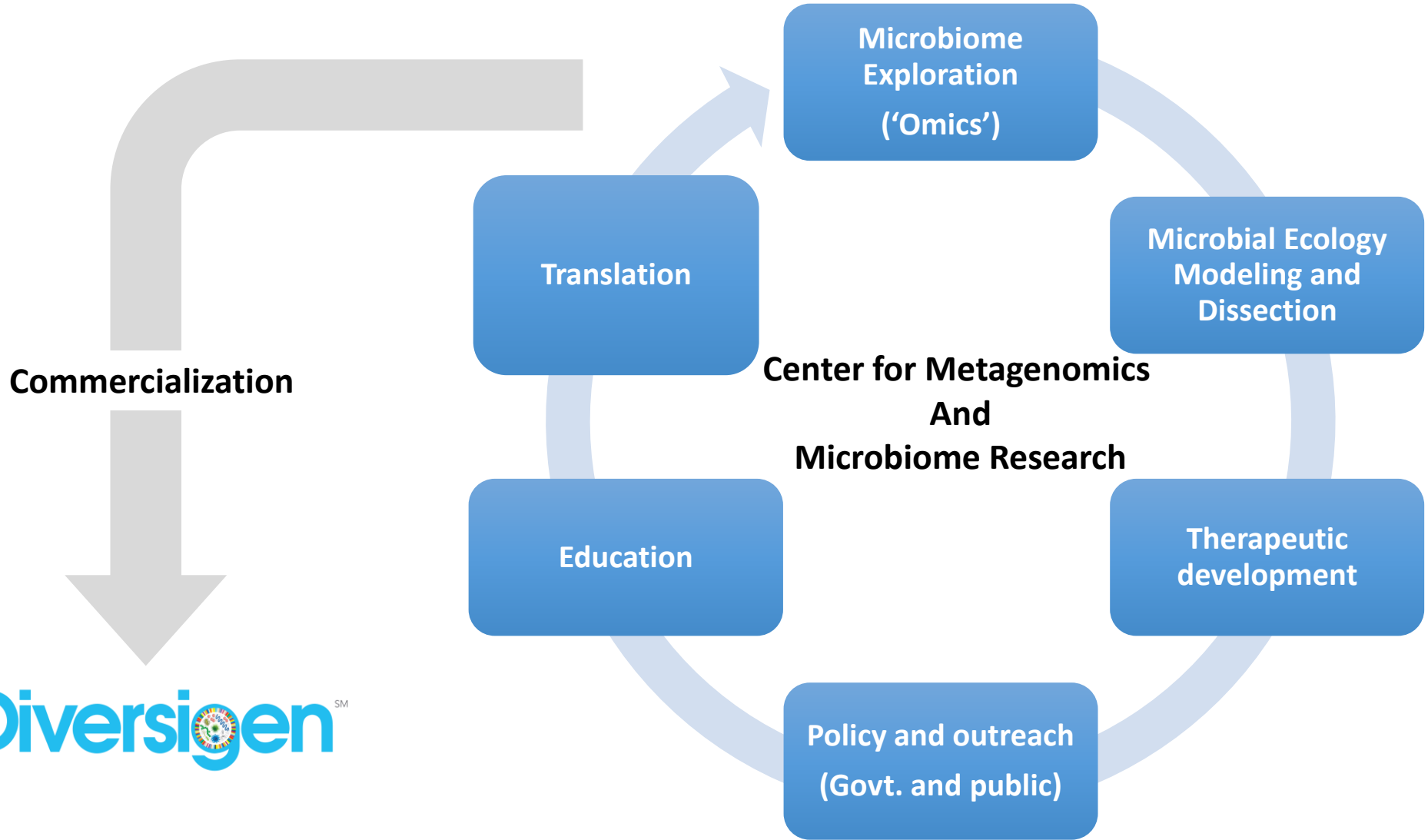
Translational Microbiome Research at Baylor College of Medicine

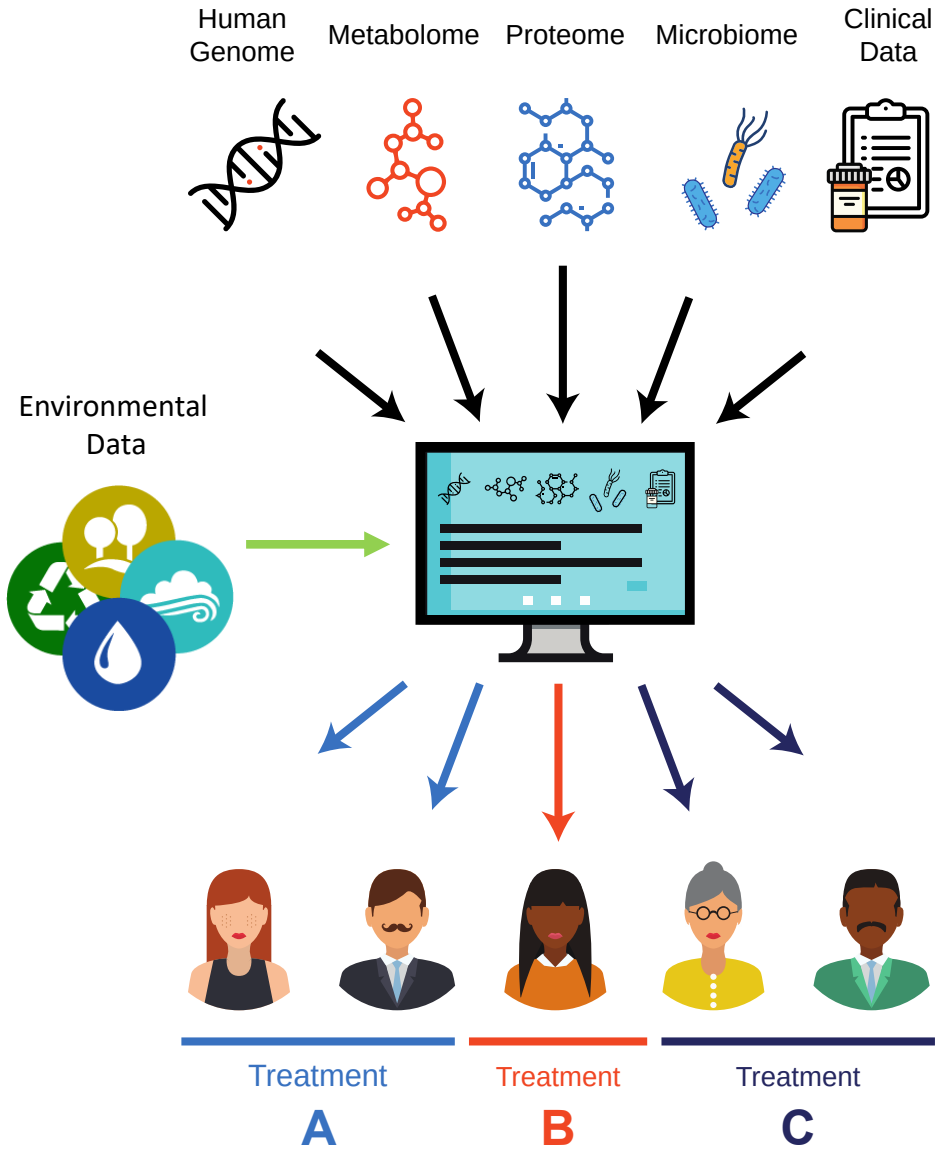
Mission: **To understand how the microbiome impacts health and disease, to translate this understanding for better therapeutics and diagnostics, and to serve as a hub for these activities internationally**



Diversigen℠

Alkek Center for Metagenomics and Microbiome Research

Precision Medicine Paradigm

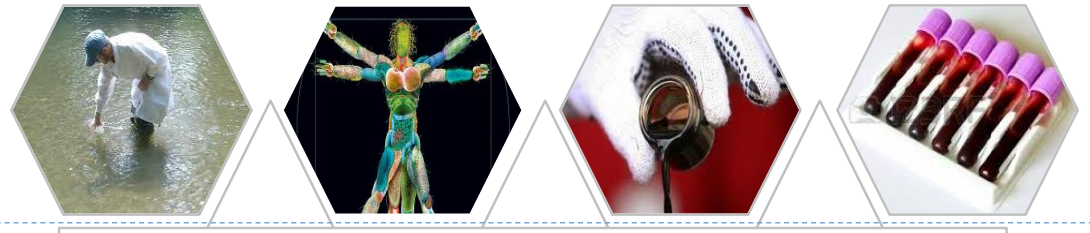


Keys to microbiome input on precision medicine

- How widely applicable is knowledge from one study or cohort
- How translatable is a pre-clinical model observation

Metagenomic/genomic applications in CMMR service center

Experimental Design



Sample:
Collection
Storage
Shipping
Processing

Microbial Biomass Enrichment

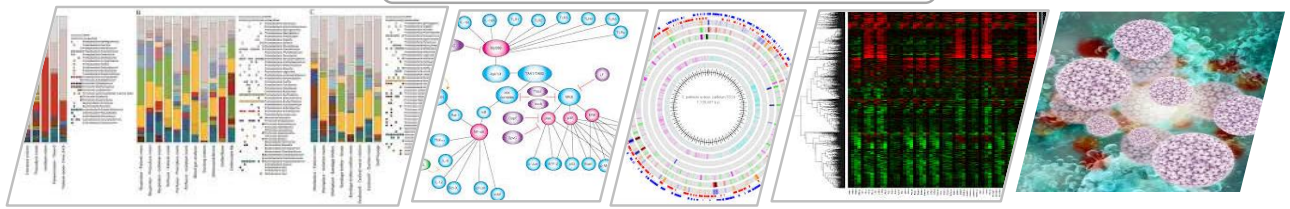
DNA/RNA Extraction and Purification

Library preparation/QC
Sequencing

Sequencing Platform Flexibility

Bioinformatics

OUTPUTS



Community Structure | Bacterial Pangenome Assemblies | Genome | Microbial Viral Detection RNASeq

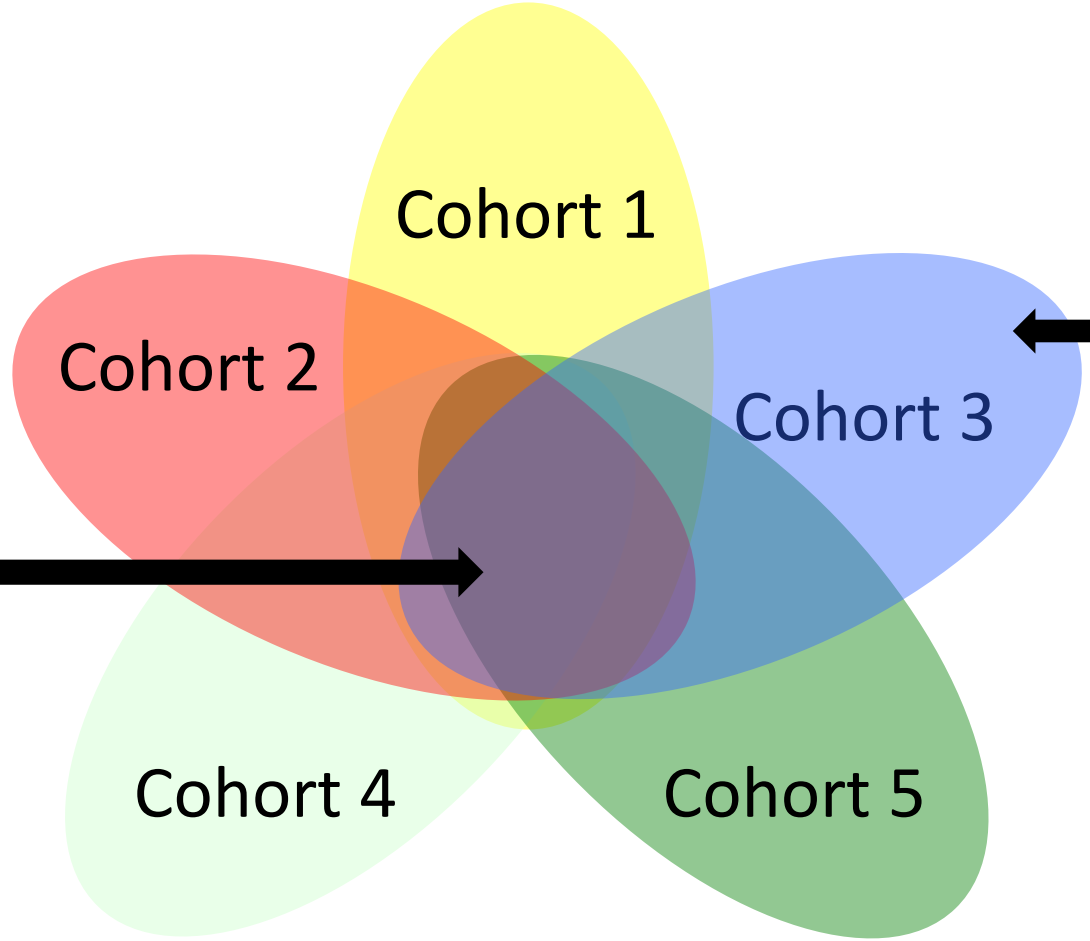
Baylor College of Medicine

Alkek Center for Metagenomics and Microbiome Research

Baylor College of Medicine

Advantages of metagenomics at scale

Studying multiple disease cohorts can facilitate dissection of disease mechanisms and lead to better diagnostics and therapeutics



Universal factors:
- Common exposures
- Common genetic risks

*Universal target discovery

Unique regional factors:
- Diet
- Race/ethnicity
- Access to health care
- Environment

*Personalized target discovery

Uniformity in sample processing and data generation

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Disease and Disease Model Projects
> 300 projects over the last 6 years, and growing…

- Chronic Kidney Disease (Raj, George Washington University)
- NASA extreme environments (JPL, Pasadena)
- Autism (Mazmanian, Cal Tech)
- Malnutrition (Preidis, Texas Children's)
- Type 1 Diabetes (Krischer; Burkhardt, USF; Atkinson, UF)
- Microbiome of death (Bucheli and Lynne, SHSU)
- Microbial surveillance (Klotman (BCM-MC); Maresso (elementary school))
- Synthetic probiotic organisms (Tabor, Rice)
- Necrotizing enterocolitis (Burrin; Premkumar, BCM)
- Fecal transplants (Graham, BCM; Wilkerson, MD Anderson; DuPont, UTHSC)
- HIV (Vigil, UT; Bryson, UTH; San Juan, U. Norte; Klotman, BCM)
- Colon cancer (El Serag, BCM; Daniel-MacDougall, MD Anderson)
- Type 2 Diabetes (Fisher-Hoch, UTSPH)
- COPD cancer (Liu, BCM)
- *Clostridium difficile* (Graham and Koo, BCM; DuPont, UTSPH)
- IBS/IBD (DuPont, BCM (adults); Versalovic, TCH/BCM (children); Dann UTMB; Round, Utah)
- Leukemia (Adachi, MD Anderson)
- Pancreatic cancer and cachexia (Fogelman, MD, MDACC)
- Diet and cognition during development (Costa-Mattioli, BCM)
- Anorexia and bulimia (Pinho, Albert Einstein Medicina Diagnóstica, Brazil)
- Suicide and suicidal ideation (Salas, BCM)

CMMr
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Gaps/needs in clinical microbiome research

Experimental Design

Microbial Biomass Enrichment

Sample:
Collection
Storage
Shipping
Processing

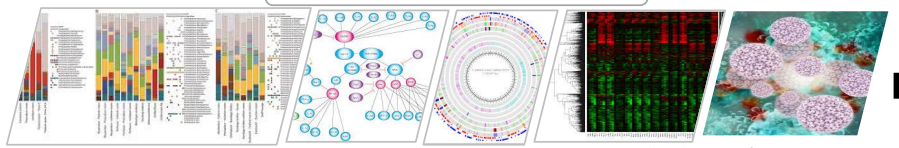
DNA / RNA Extraction and Purification

Sequencing Platform Flexibility

Library preparation/QC
Sequencing

OUTPUTS

Bioinformatics



Community Structure | Bacterial Pangenome | Genome Assemblies | Microbial RNASeq | Viral Detection

Universal sampling kits—one kit fits all omics
- Take home
- Easy
- Stable at room temp

Removal/inactivation of host DNA/RNA
- Nuclease txt before extraction
- PMA (propidium monoazide) txt

Lower cost automated library prep
- < ng levels of input DNA/RNA

Statistical models built for microbiome data
- Accounting for sparse data
- Longitudinal sampling
- Integrating multi-omics

Gaps/needs in clinical microbiome research

Experimental Design

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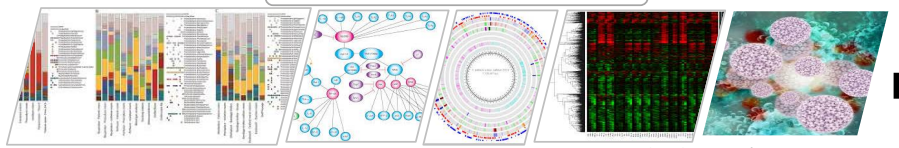

Community Structure | Bacterial Pangenome | Genome Assemblies | Microbial RNASeq | Viral Detection

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Statistical models built for microbiome data
- Accounting for sparse data
- Longitudinal sampling
- Integrating multi-omics

When you have all of this, then what???

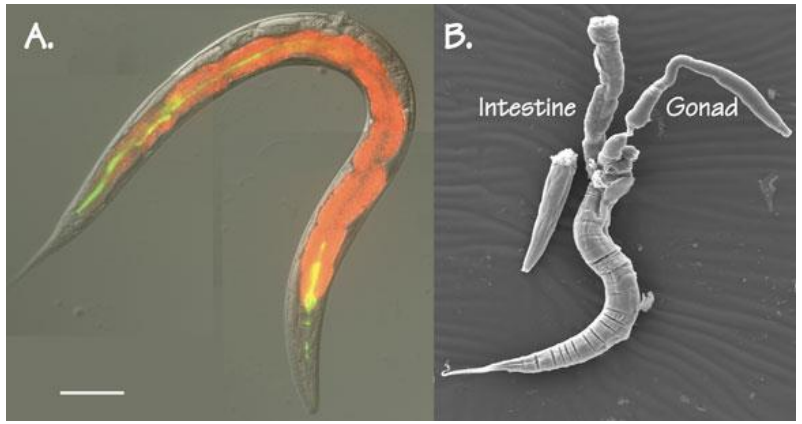
Model systems for host-microbe interactions: Microbiome phenotyping pipeline

Baylor College of Medicine

In vitro culturing in mini bioreactors

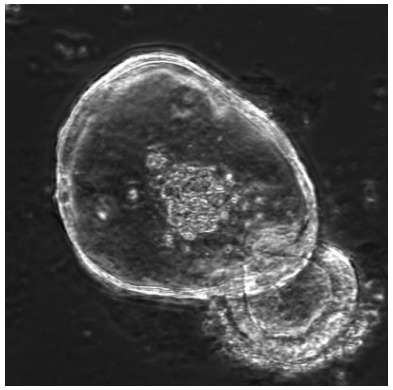
Robert Britton

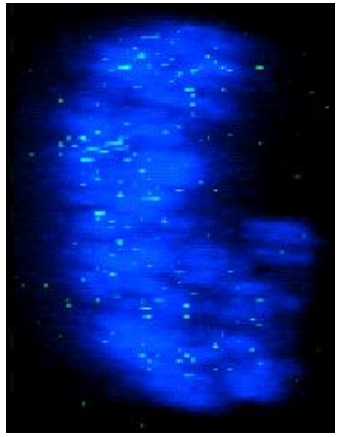
In vivo studies in *C. elegans*



Buck Samuel

Enteroids from various GI locations





Mary Estes

In vivo studies in germ free mice

Alton Swennes

Alkek Center for Metagenomics and Microbiome Research

Baylor College of Medicine

ARTICLE

doi:10.1038/nature25178

Dietary trehalose enhances virulence of epidemic *Clostridium difficile*

J. Collins[1], C. Robinson[2], H. Danhof[1], C. W. Knetsch[3], H. C. van Leeuwen[3], T. D. Lawley[4], J. M. Auchtung[1] & R. A. Britton[1]

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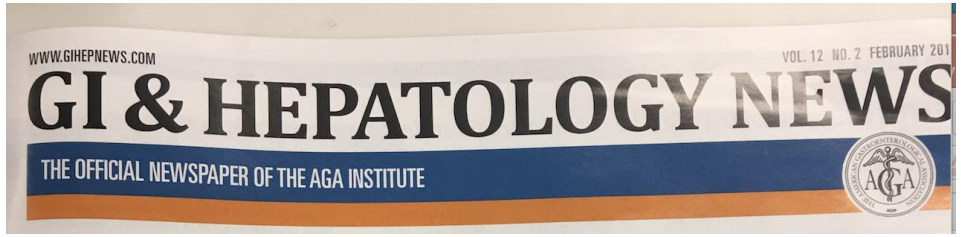


James Collins

Rob Britton

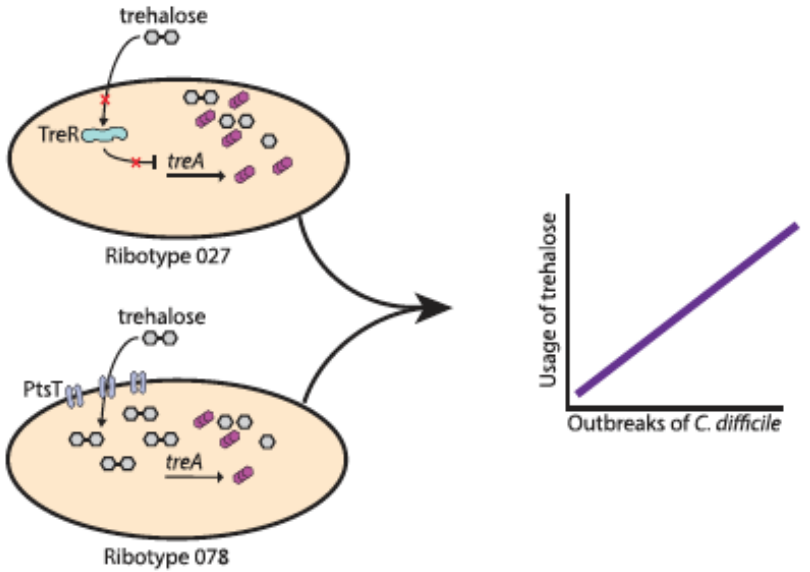


Figure 1. Two Independent Trehalose Utilization Mechanisms in Distinct Epidemic Strains of *C. difficile*

Collins et al. (2018) report two unique mechanisms of trehalose utilization in the ribotype 027 and 078 lineage of *C. difficile*. Ribotype 027 strains have a mutation in the trehalose transcriptional repressor (TreR), and ribotype 087 strains encode for an additional trehalose transporter (PstT). The emergence of these ribotypes as epidemic strains of *C. difficile* has coincided with increased usage of trehalose as a food additive.

Alkek Center for Metagenomics and Microbiome Research

Baylor College of Medicine

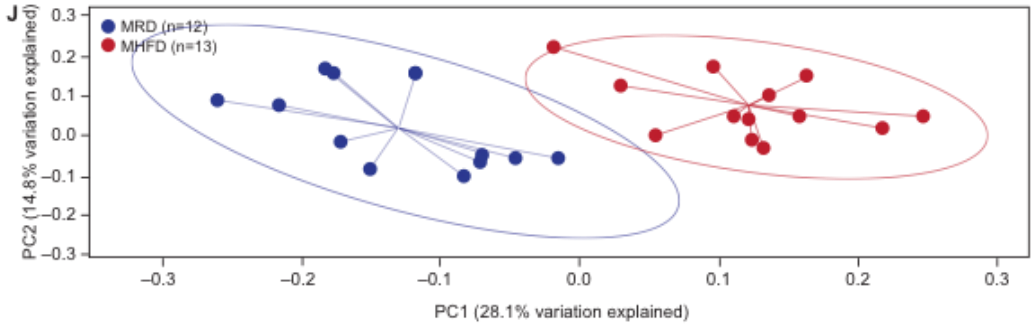
Cell 2016, Cell 165, 1762–1775

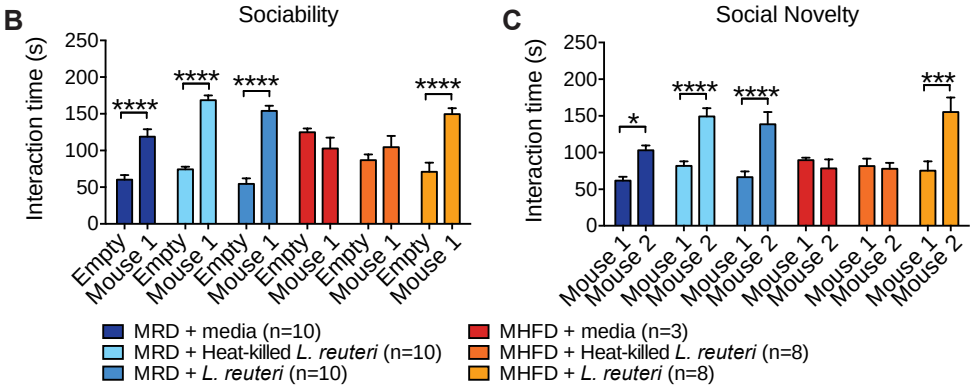
Article

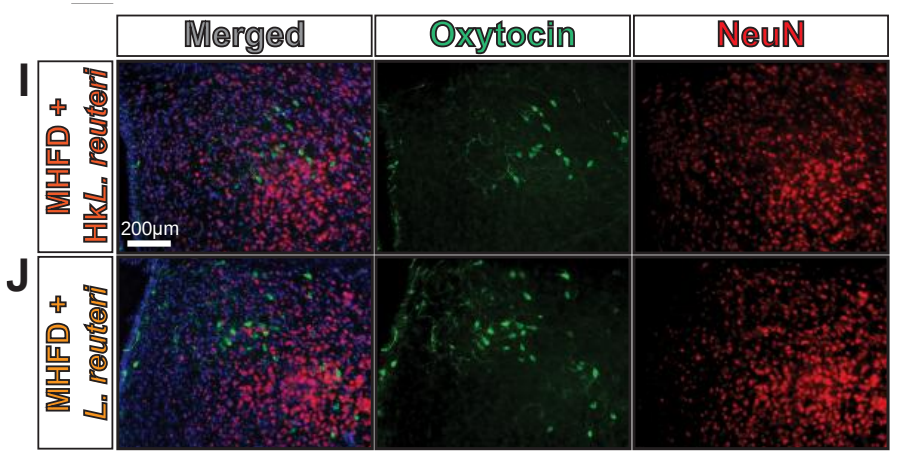
Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring

Shelly A. Buffington,[1,2] Gonzalo Viana Di Prisco,[1,2] Thomas A. Auchtung,[3,4] Nadim J. Ajami,[3,4] Joseph F. Petrosino,[3,4] and Mauro Costa-Mattioli[1,2,*]

[1]Department of Neuroscience
[2]Memory and Brain Research Center
[3]Alkek Center for Metagenomics and Microbiome Research
[4]Department of Molecular Virology and Microbiology
Baylor College of Medicine, Houston, TX 77030, USA
*Correspondence: costamat@bcm.edu
http://dx.doi.org/10.1016/j.cell.2016.06.001








Shelly Buffington


Mauro Costa-Mattioli

Alkek Center for Metagenomics and Microbiome Research

Baylor College of Medicine

Recent Immunotherapy Advances

COVER Illustration of gut microbial species assembling into the shape of a cancer awareness ribbon. Cancer patients with an abundance of different gut bacteria have better survival outcomes after checkpoint inhibitor immunotherapy, whereas patients with depleted gut flora respond poorly to this treatment. Thus, modulating the microbiome may provide hope for improved cancer patient responses. See pages 32, 91, 97, and 104. Illustration: V. Altounian/*Science*

RESEARCH

CANCER IMMUNOTHERAPY

Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients

V. Gopalakrishnan,[1,2*] C. N. Spencer,[2,3*] L. Nezi,[3*] A. Reuben,[1] M. C. Andrews,[1] T. V. Karpinets,[3] P. A. Prieto,[1†] D. Vicente,[1] K. Hoffman,[4] S. C. Wei,[5] A. P. Cogdill,[1,5] L. Zhao,[3] C. W. Hudgens,[6] D. S. Hutchinson,[7] T. Manzo,[3] M. Petaccia de Macedo,[6‡] T. Cotechini,[8] T. Kumar,[3] W. S. Chen,[9] S. M. Reddy,[10] R. Szczepaniak Sloane,[1] J. Galloway-Pena,[11] H. Jiang,[1] P. L. Chen,[9§] E. J. Shpall,[12] K. Rezvani,[12] A. M. Alousi,[12] R. F. Chemaly,[11] S. Shelburne,[3,11] L. M. Vence,[5] P. C. Okhuysen,[11] V. B. Jensen,[13] A. G. Swennes,[7] F. McAllister,[14] E. Marcelo Riquelme Sanchez,[14] Y. Zhang,[14] E. Le Chatelier,[15] L. Zitvogel,[16] N. Pons,[15] J. L. Austin-Breneman,[1||] L. E. Haydu,[1] E. M. Burton,[1] J. M. Gardner,[1] E. Sirmans,[17] J. Hu,[18] A. J. Lazar,[6,9] T. Tsujikawa,[8] A. Diab,[17] H. Tawbi,[17] I. C. Glitza,[17] W. J. Hwu,[17] S. P. Patel,[17] S. E. Woodman,[17] R. N. Amaria,[17] M. A. Davies,[17] J. E. Gershenwald,[1] P. Hwu,[17] J. E. Lee,[1] J. Zhang,[3] L. M. Coussens,[8] Z. A. Cooper,[1,3¶] P. A. Futreal,[3] C. R. Daniel,[4,2] N. J. Ajami,[7] J. F. Petrosino,[7] M. T. Tetzlaff,[6,9] P. Sharma,[5,19] J. P. Allison,[5] R. R. Jenq,[3#] J. A. Wargo[1,3#**]

RESEARCH

CANCER IMMUNOTHERAPY

The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients

Vyara Matson,[1*] Jessica Fessler,[1*] Riyue Bao,[2,3*] Tara Chongsuwat,[4] Yuanyuan Zha,[4] Maria-Luisa Alegre,[4] Jason J. Luke,[4] Thomas F. Gajewski[1,4†]

Anti–PD-1–based immunotherapy has had a major impact on cancer treatment but has only benefited a subset of patients. Among the variables that could contribute to interpatient heterogeneity is differential composition of the patients' microbiome, which has been shown to affect antitumor immunity and immunotherapy efficacy in preclinical mouse models. We analyzed baseline stool samples from metastatic melanoma patients before immunotherapy treatment, through an integration of 16S ribosomal RNA gene sequencing, metagenomic shotgun sequencing, and quantitative polymerase chain reaction for selected bacteria. A significant association was observed between commensal microbial composition and clinical response. Bacterial species more abundant in responders included *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*. Reconstitution of germ-free mice with fecal material from responding patients could lead to improved tumor control, augmented T cell responses, and greater efficacy of anti–PD-L1 therapy. Our results suggest that the commensal microbiome may have a mechanistic impact on antitumor immunity in human cancer patients.

CANCER IMMUNOTHERAPY

Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors

Bertrand Routy,[1,2,3] Emmanuelle Le Chatelier,[4] Lisa Derosa,[1,2,3] Connie P. M. Duong,[1,2,5] Maryam Tidjani Alou,[1,2,3] Romain Daillère,[1,2,3] Aurélie Fluckiger,[1,2,5] Meriem Messaoudene,[1,2] Conrad Rauber,[1,2,3] Maria P. Roberti,[1,2,5] Marine Fidelle,[1,3,5] Caroline Flament,[1,2,5] Vichnou Poirier-Colame,[1,2,5] Paule Opolon,[6] Christophe Klein,[7] Kristina Iribarren,[8,9,10,11,12] Laura Mondragón,[8,9,10,11,12] Nicolas Jacquelot,[1,2,3] Bo Qu,[1,2,3] Gladys Ferrere,[1,2,3] Céline Clémenson,[1,13] Laura Mezquita,[1,14] Jordi Remon Masip,[1,14] Charles Naltet,[15] Solenn Brosseau,[15] Coureche Kaderbhai,[16] Corentin Richard,[16] Hira Rizvi,[17] Florence Levenez,[4] Nathalie Galleron,[4] Benoit Quinquis,[4] Nicolas Pons,[4] Bernhard Ryffel,[18] Véronique Minard-Colin,[1,19] Patrick Gonin,[1,20] Jean-Charles Soria,[1,14] Eric Deutsch,[1,13] Yohann Loriot,[1,3,14] François Ghiringhelli,[16] Gérard Zalcman,[15] François Goldwasser,[9,21,22] Bernard Escudier,[1,14,23] Matthew D. Hellmann,[24,25] Alexander Eggermont,[1,2,14] Didier Raoult,[26] Laurence Albiges,[1,3,14] Guido Kroemer,[8,9,10,11,12,27,28*] Laurence Zitvogel[1,2,3,5*]

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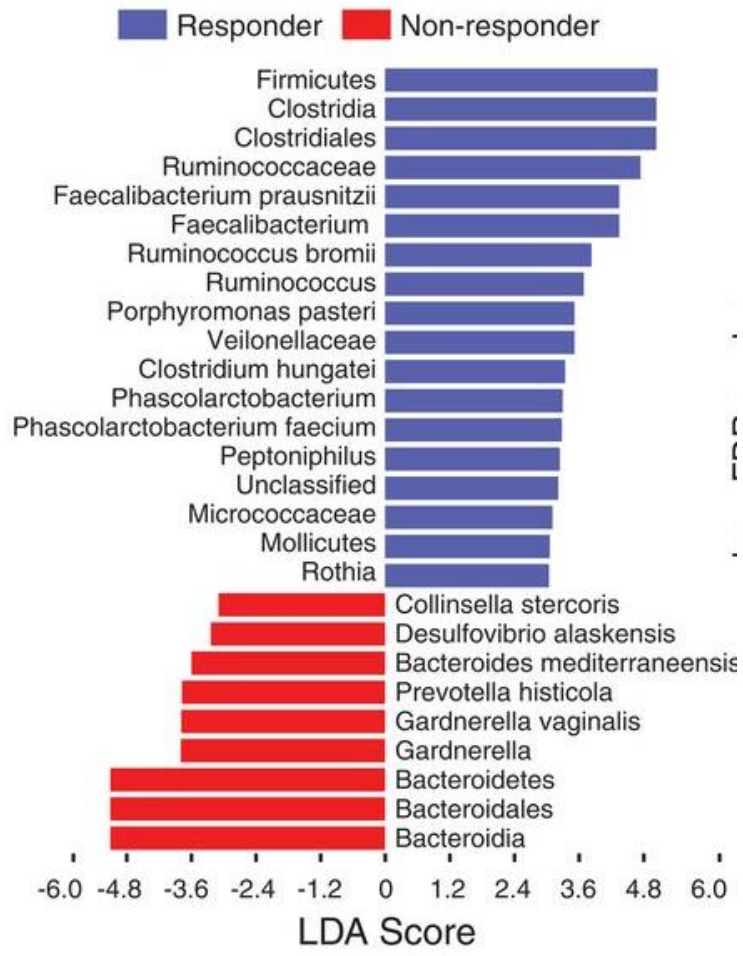
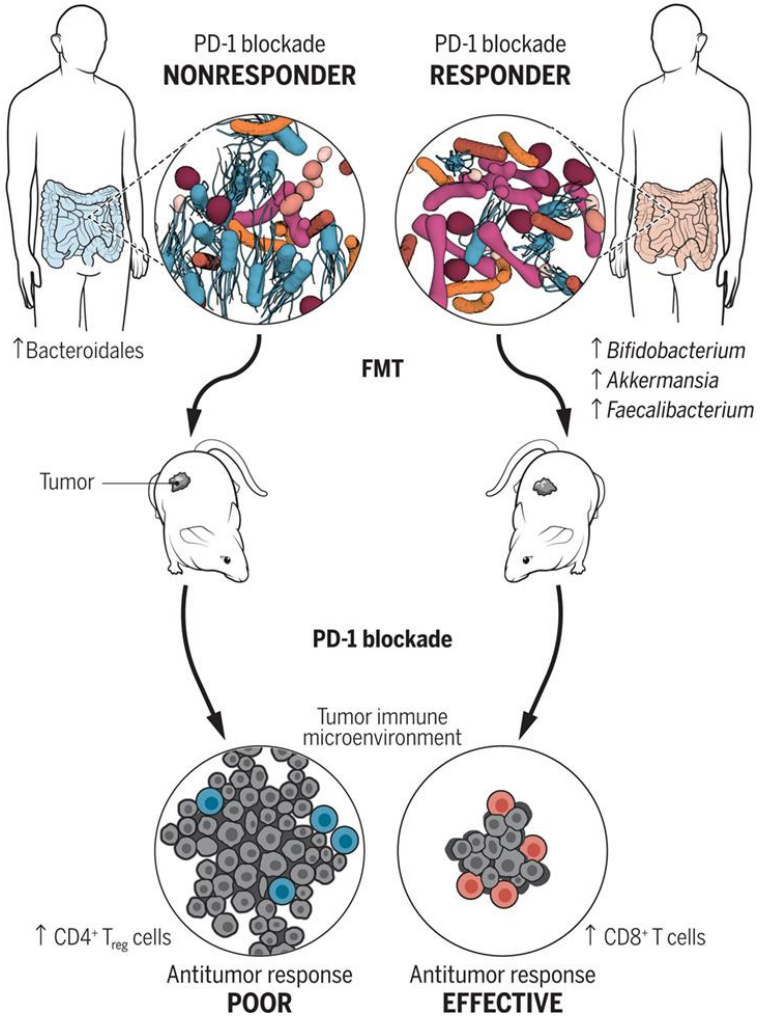
Microbiome alone can improve an immunotherapy response





Science 05 Jan 2018: Vol. 359, Issue 6371, pp. 32-34

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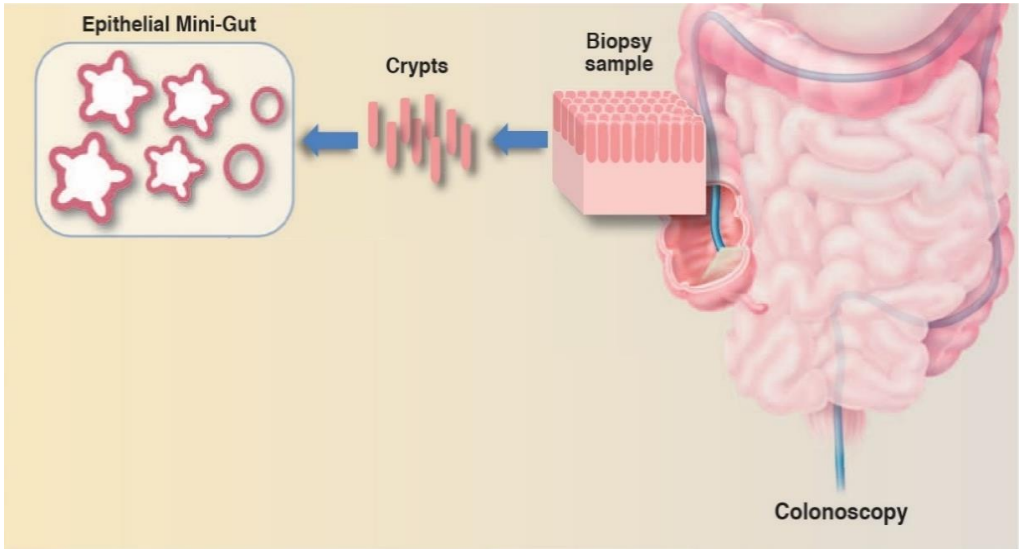
Enteroids: a unique opportunity for studying human disease at the epithelial level



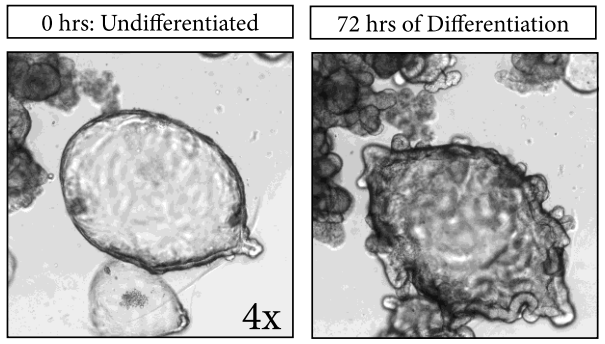
Mechanistic investigations of host-microbe interactions in the gut are limited because of two principle challenges:

1. Epithelium is oxygen dependent while many gut bacteria are facultative or obligate anaerobes

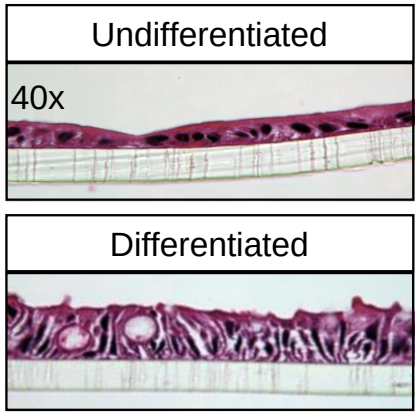
2. Intestinal epithelium is in a state of chronic low-grade hypoxia which is exacerbated in inflammatory conditions such as Inflammatory Bowel Disease (IBD).

OXYGEN CONTROL IS IMPORTANT!

3D Enteroid

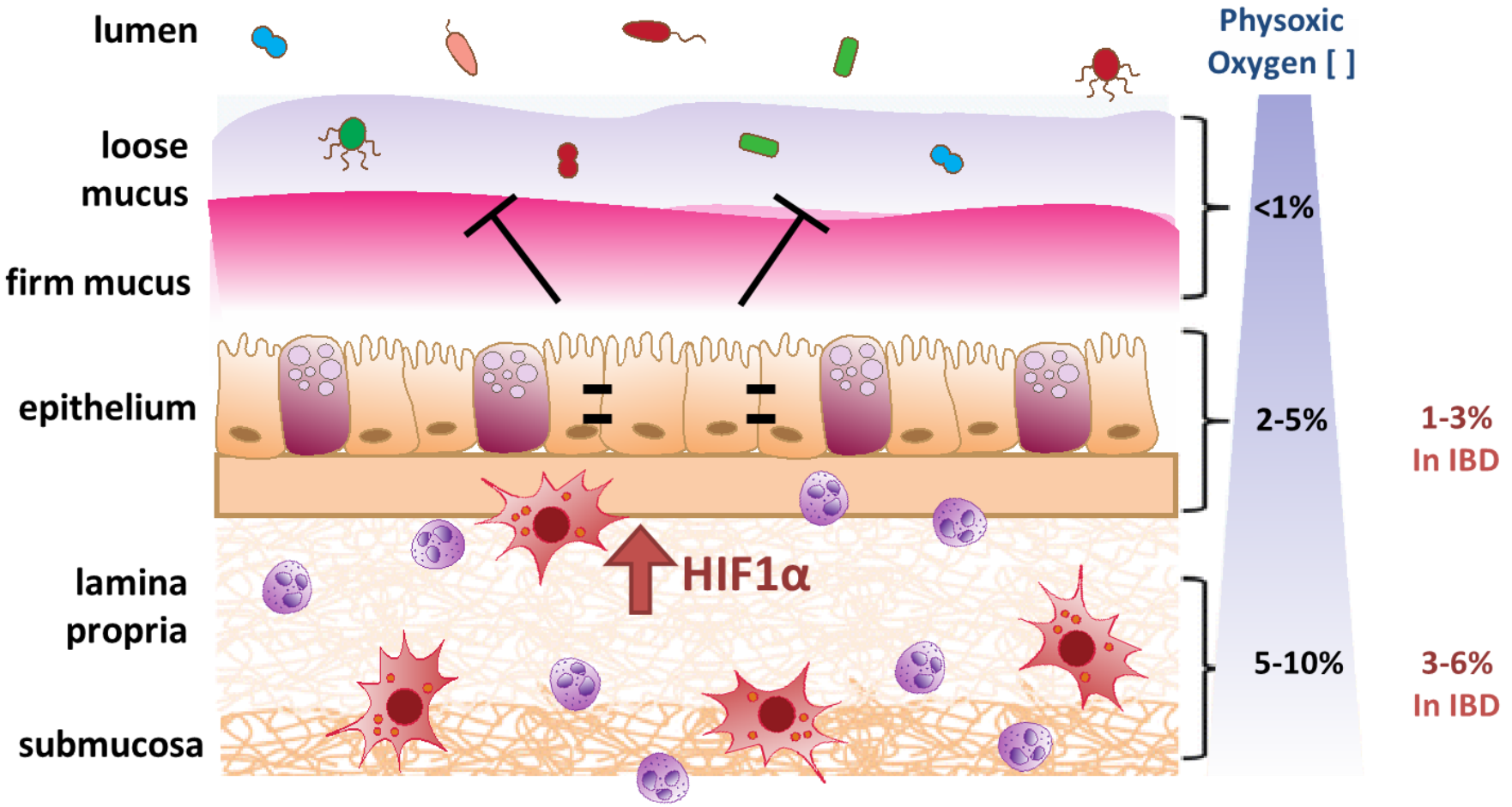


Enteroid Monolayer



Stelzner, M. et al, J. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2012**, *302* (12), G1359–G1363.
Moon, C. et al, *Mucosal Immunol.* **2014**, *7* (4), 818–828.

Physiologic and inflammatory hypoxia in the gut



Physiologic Epithelium = 2-5% O_2 *in vivo*

Hypoxic Epithelium = 1-3% O_2 *in vivo*

Monolayers in Standard Incubator = 7-18% O_2

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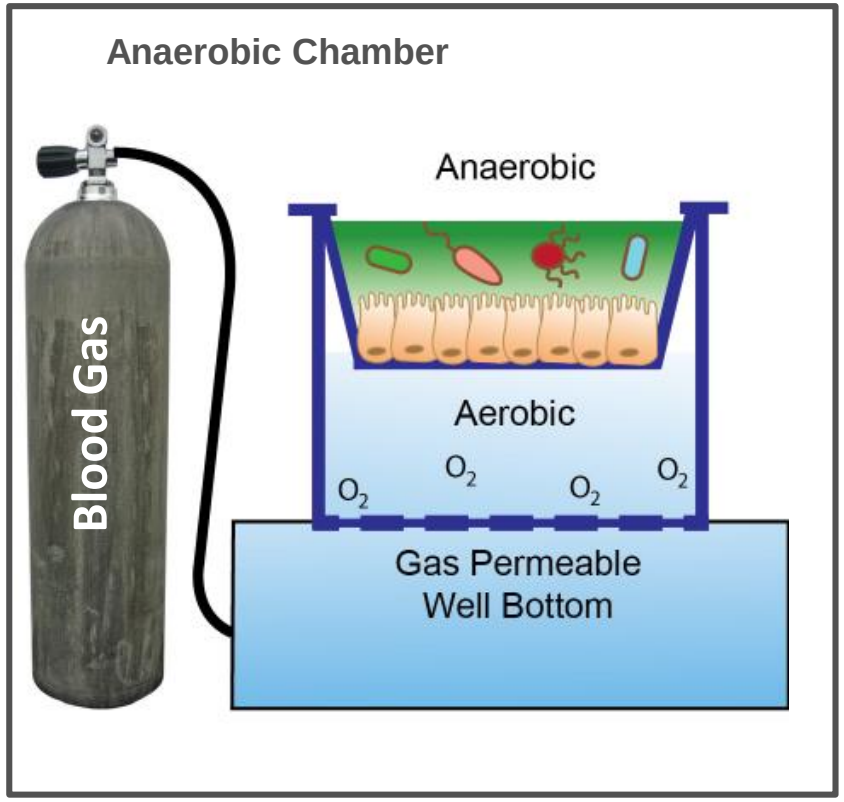
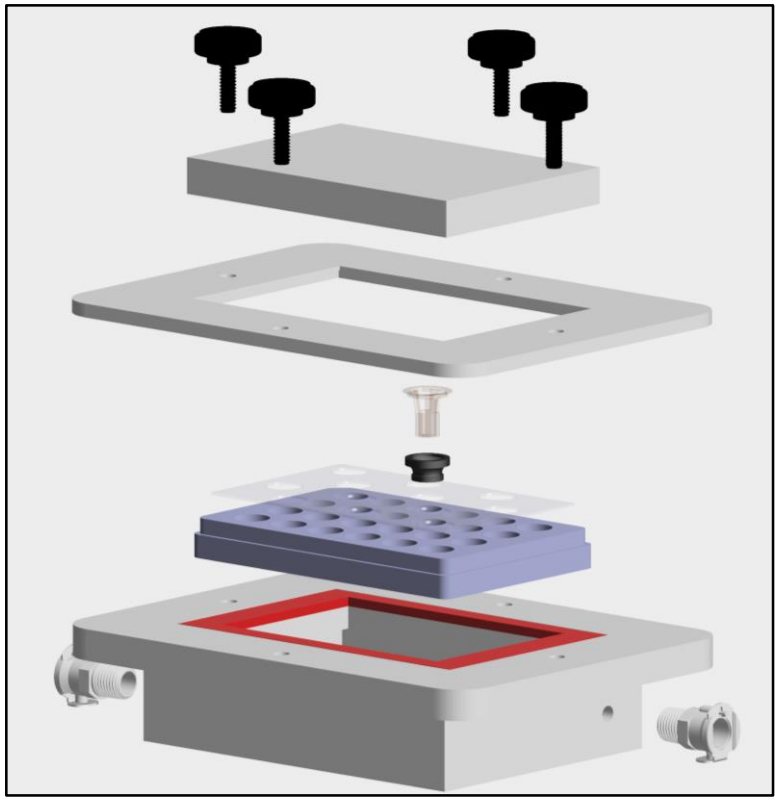
Assembly and validation of a novel enteroid-anaerobe co-culture system






Tatiana Fofanova


Chris Stewart


Jenny Auchtung

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Experimental Design

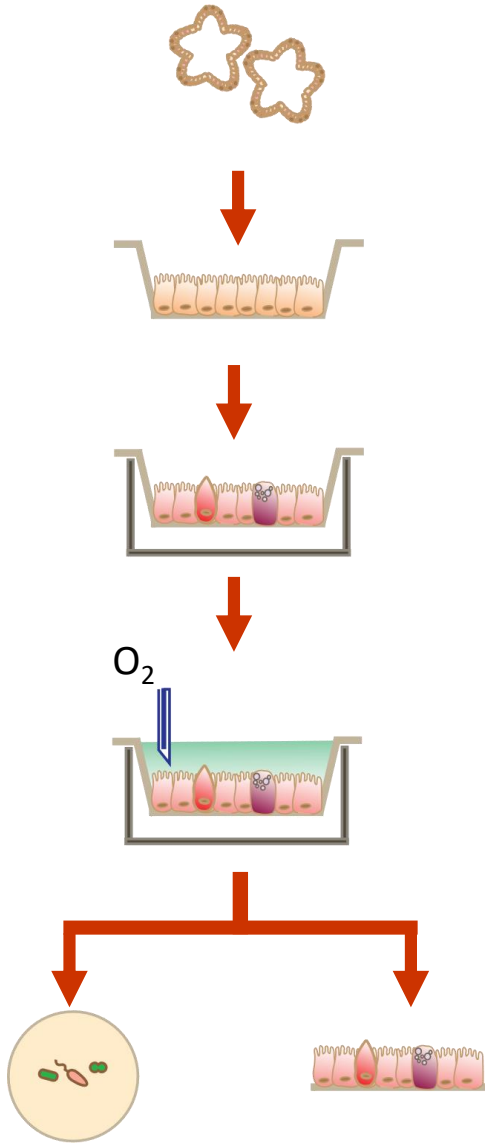
3D Jejunal enteroids, p10-p15

Seeded $5x10^5$ / transwell

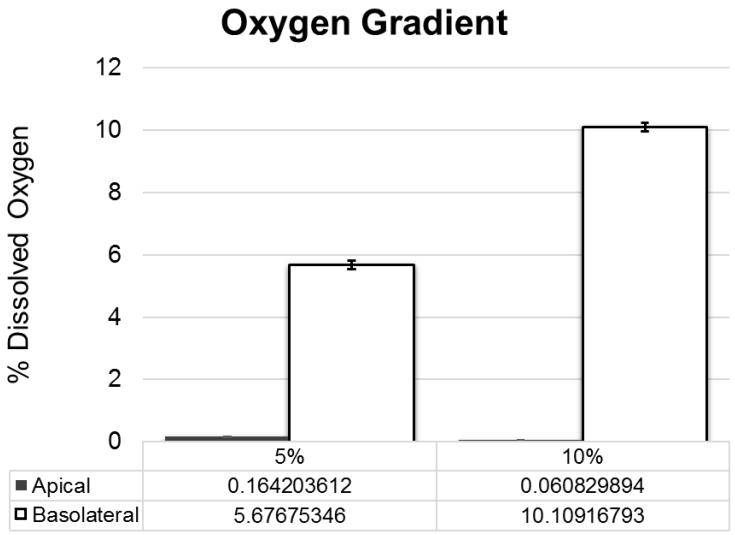
4 days differentiation

Fitted into Co-Culture System
1-2hr equilibration at 5% Oxygen
O_2 measurements

O_2 measurements
Contamination checks at 0h, 24hr
Transepithelial electronic resistance (TEER) at 0hr, 24hr
Endpoint: histology, RNA, survival



System recapitulates *in vivo* oxygen gradients



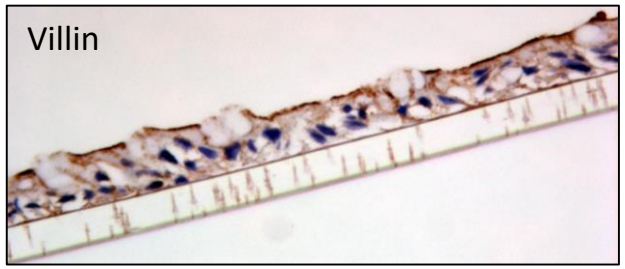
Steep Oxygen Gradient:

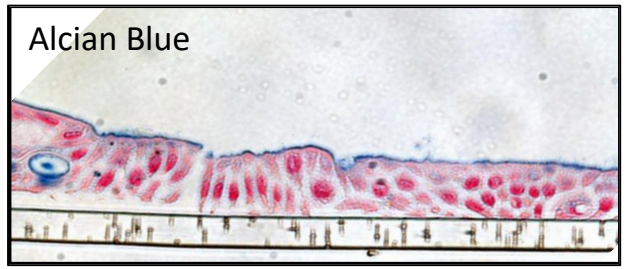
-delivery of 5.6% and 10.2% O_2 blood gas,

-basolateral side is oxygenated while the apical side is effectively anaerobic.

Healthy phenotype:

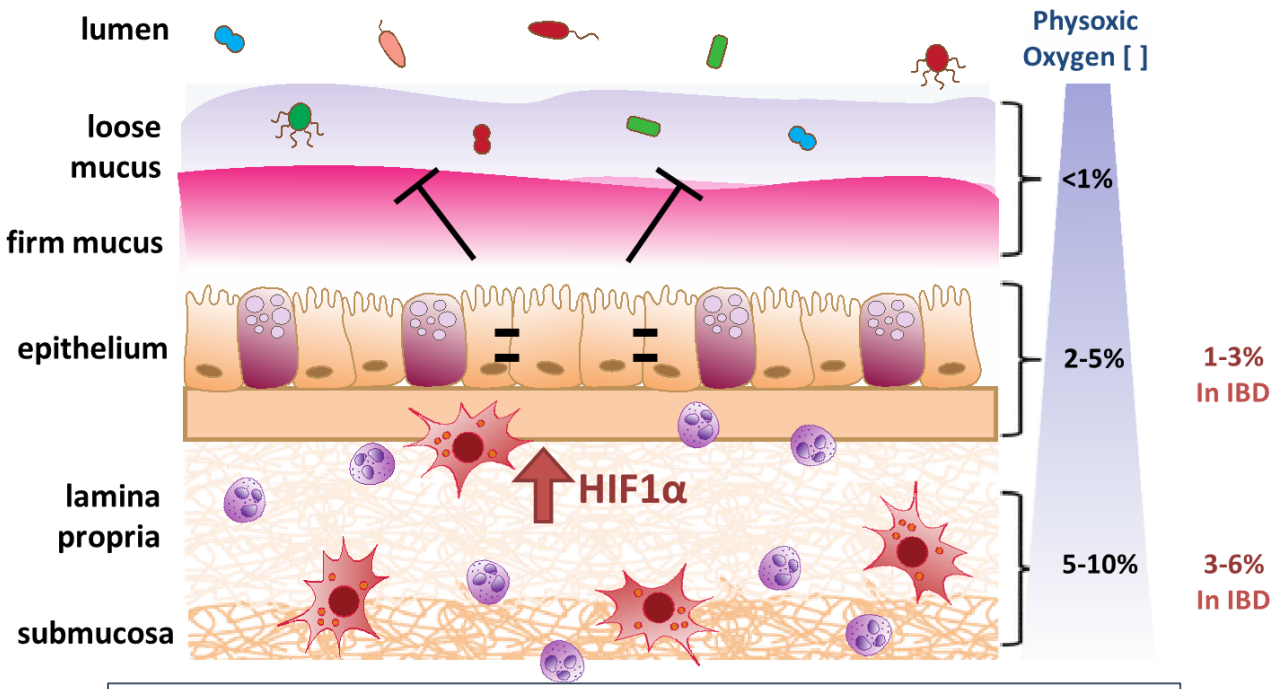
Enteroids monolayers imaged after 24hrs at 5% basolateral oxygen are polarized (Villin stain in brown), show an intact mucus layer (Alcian blue stain) and normal morphology





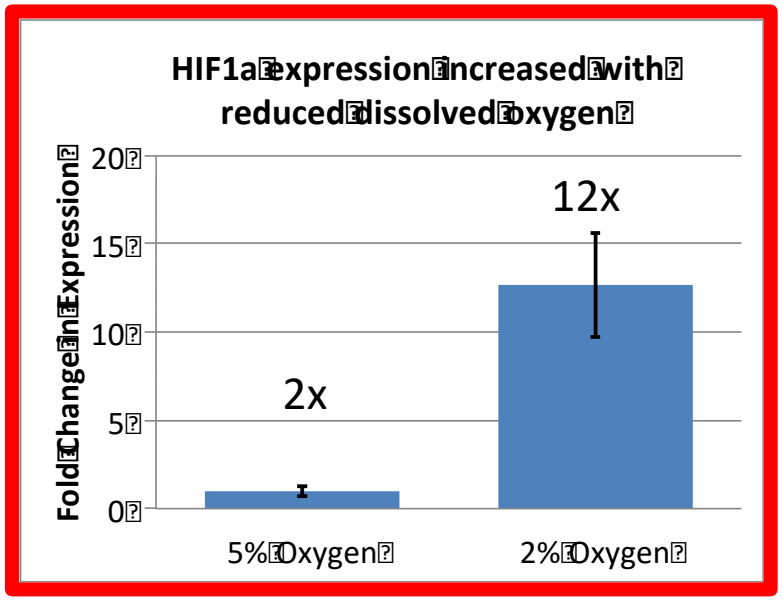
Impact of physiological levels of oxygen

Hallmark of Physiological Hypoxia: HIF1a



Physiologic Hypoxia = 2-5% O_2 *in vivo*
Inflammatory Hypoxia = 1-3% O_2 *in vivo*
Monolayers in Standard Incubator = 8.5-10% O_2

- HIF1a expression
  - Increased barrier integrity
  - Activation of NFkB and other innate immunity genes
  - Increased mucin production



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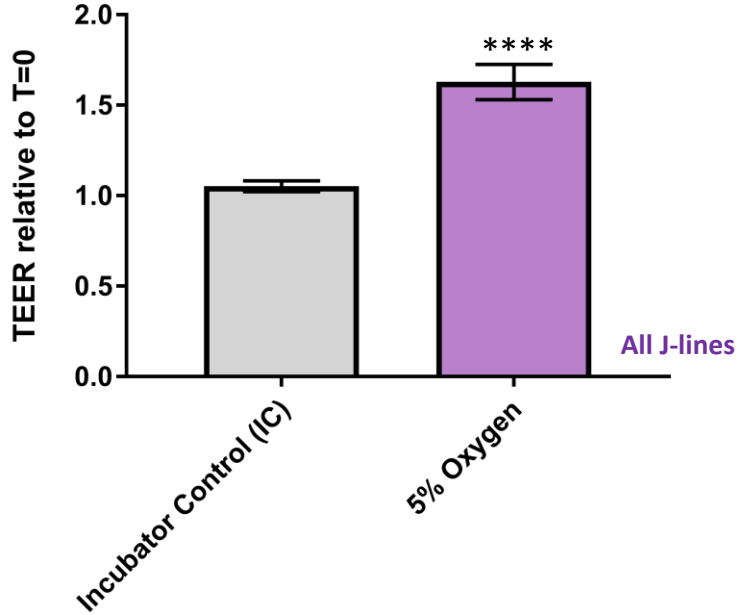
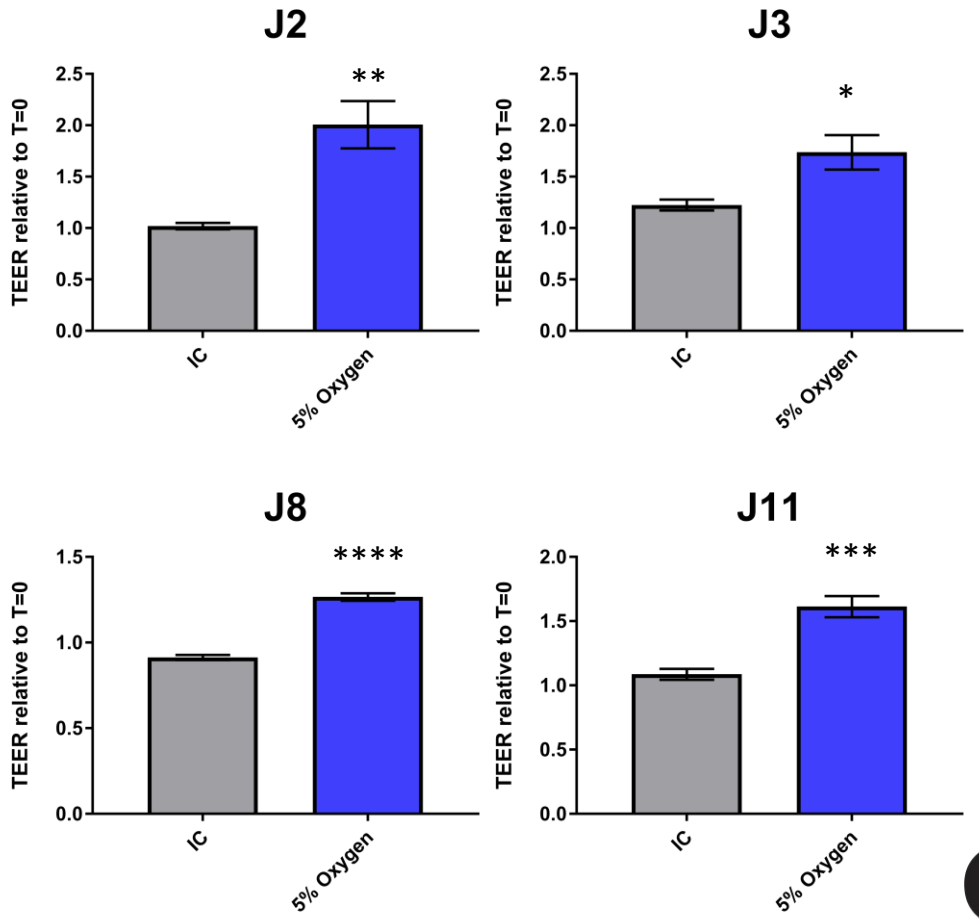
Trans-epithelial resistance increases in response to hypoxia, independent of patient line

Hallmark of Physiological Hypoxia: Increased Barrier Integrity

24 hr incubation in 5% basolateral oxygen

Reduced Oxygen Conditions (5% oxygen) Increase Epithelial Barrier Integrity





Gene expression under physiologic hypoxia

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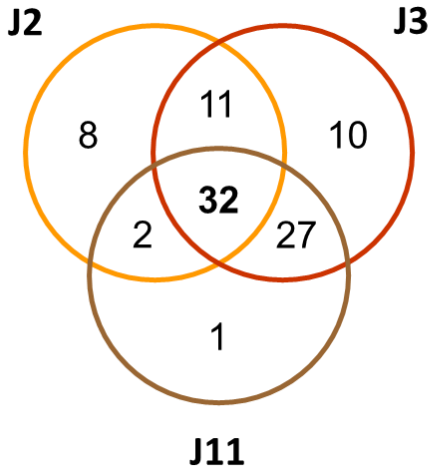
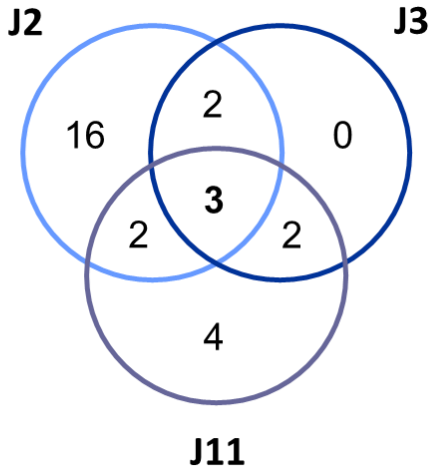
Hallmark of Physiological Hypoxia: Antimicrobial Response and Barrier Integrity

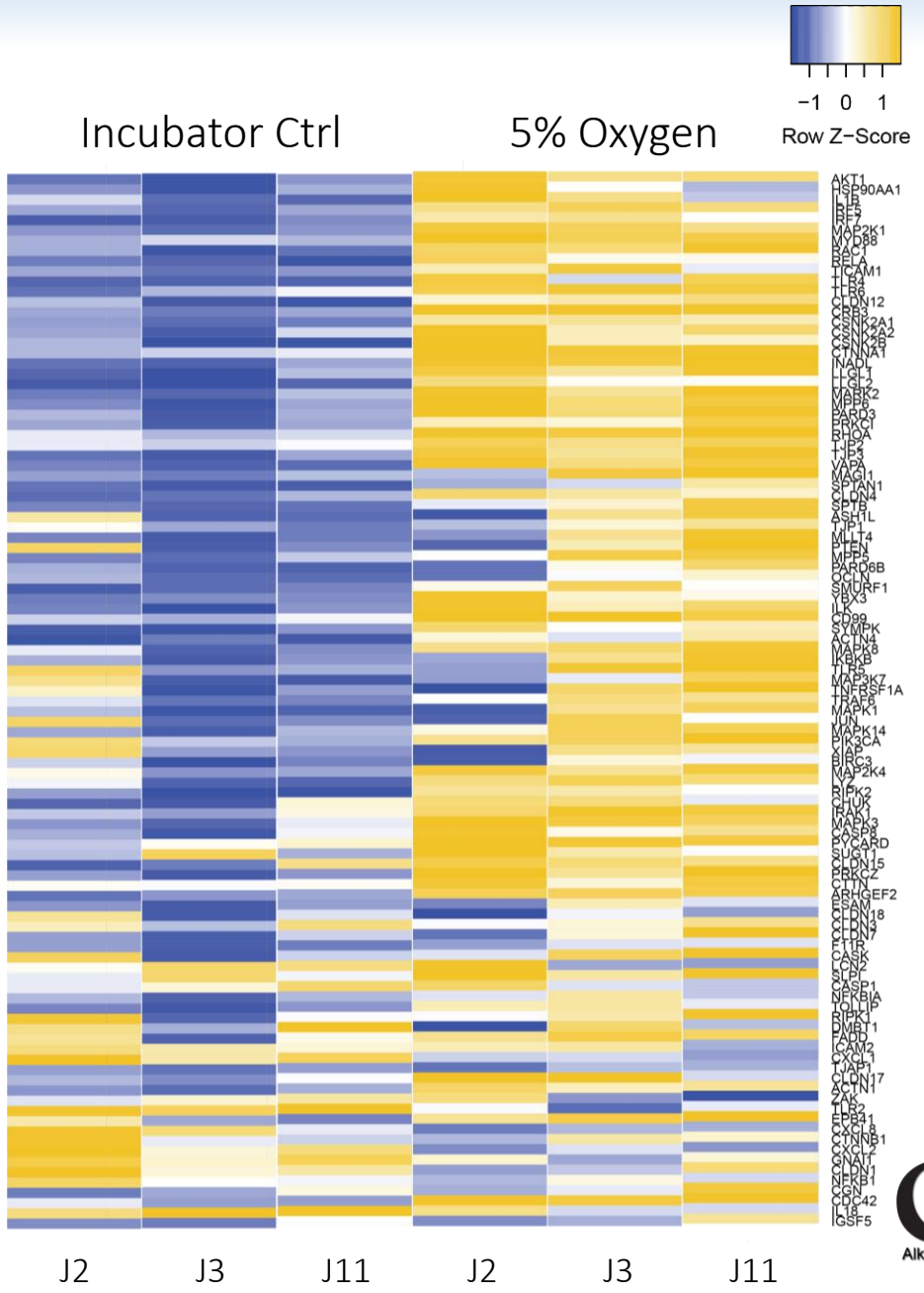
- RT2 Profiler Arrays: Tight Junction Array, Anti-Microbial Response Array
- 168 genes (84 per pathway)
- 106 retained in analysis after QC

Up-regulated



Down-regulated





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Pathways differentially regulated during physiologic hypoxia

GO-ID	Description	Corrected P-Value	Cluster Frequency	Genes
45429	Positive regulation of nitric oxide biosynthesis process	1.9214 e-8	6/33 (18.1%)	HSP90AA1 IL1B AKT1 TICAM1 TLR4 TLR2
43123	Positive regulation of I-kB kinase/NF-kB cascade	5.1691 e-8	8/33 (24.2%)	VAPA IL1B TLR6 TICAM1 TLR4 RELA RHOA MYD88
2221	Pattern recognition receptor signaling pathway	6.9208 e-8	5/33 (15.1%)	TLR6, TICAM, TLR4, RELA, TLR2
326755	Regulation of IL-6 production	6.9208 e-8	6/33 (18.1%)	IL1B, TLR6, TICAM, TLR4, MYD88, TLR2
10647	Positive regulation of cell communication	1.1126 E-7	11/33 (33.3%)	VAPA IL1B RAC1 TLR6 TICAM1 HIF1A TLR4 RHOA RELA MYD88 TLR2
6954	Inflammatory Response	1.1785 e-7	10/33 (30.3%)	CXCL8 IL1B AKT1 CXCL1 RAC1 TLR6 TICAM1 HIF1A TLR4 RELA
7163	Establishment or maintenance of cell polarity	3.195 e-6	5/33 (15.1%)	CDC42 PRKCI PARD3 LLGL1 MARK2
Generated via Cytoscape using the BiNGO Plugin (*BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks*)				

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Summary: System Design and Induction of Hypoxia

- Precision control of delivered basolateral oxygen
- System reaches steady-state within 2 hours
- Operating range is approximately between 1-8% Oxygen
- Induction of physiological hypoxia in enteroid monolayers
  - HIF1a Expression
  - Barrier Integrity
  - Upregulation of tight junction and anti-microbial response genes
- Gene expression profile reflects activation of
  - NO biosynthesis pathway as a regulator of hypoxia
  - NFkB cascade

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Can we add bacteria to the mix

Bacteroides thetaiotaomicron

- Bacteroidetes
- Gram-negative nanoanaerobe
- Common, abundant commensal
- Acetate production

Clinical implications:
Associated with remission in UC/Crohns

Blautia sp.

- Firmicutes
- Gram-positive obligate anaerobe
- Common, abundant commensal
- Lactate and acetate production

Clinical implications:
Reduced incidence of GvH disease

Griffiths, S. *FST Journal*, 2015

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Experimental Design

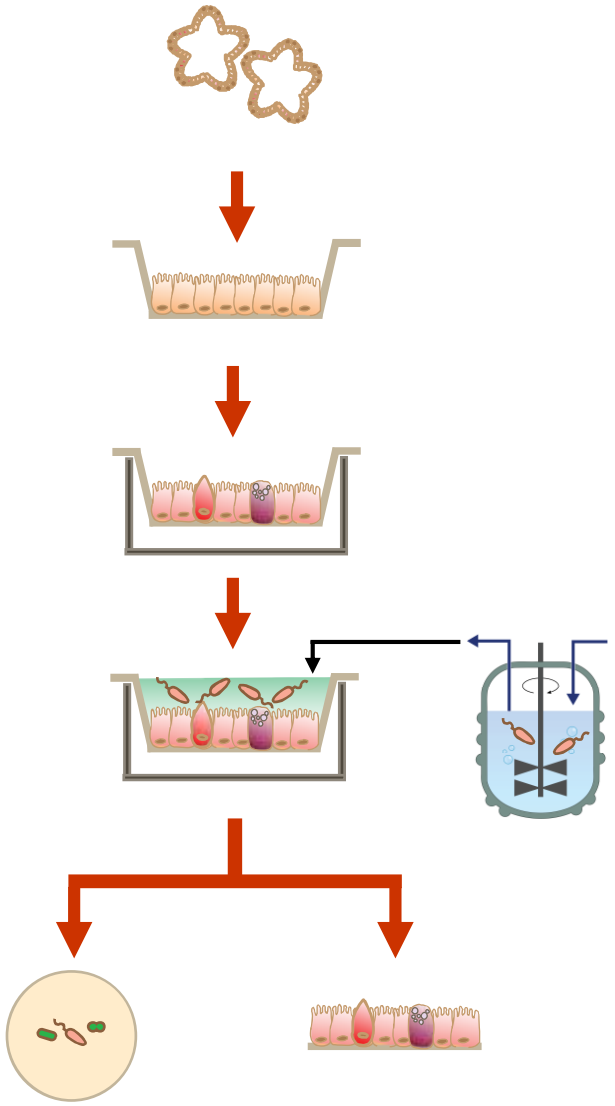
3D Jejunal enteroids, p10-p15

Seeded $5x10^5$ / transwell

4 days Differentiation

Fitted into Co-Culture System
1-2hr equilibration at 5% Oxygen
+ $3x10^4$ bacteria in 300uL for 24hr

CFU counts at 0h, 8hr, 24hr
TEER at 0hr, 8hr, 24hr
Endpoint: histology, RNA, survival



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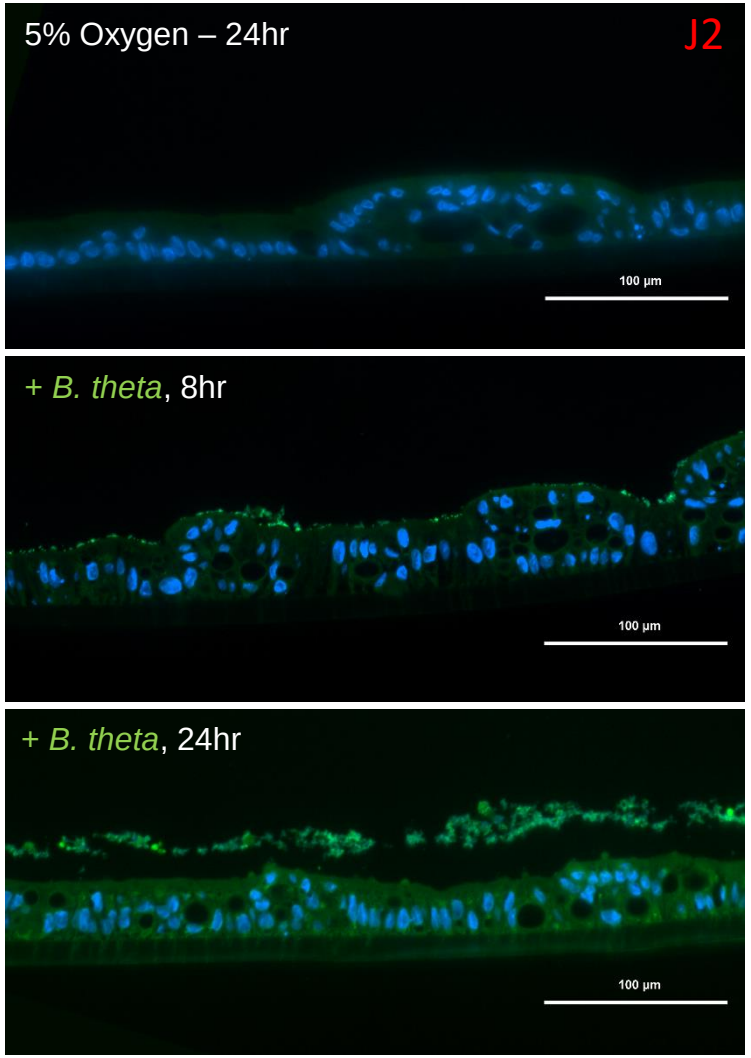
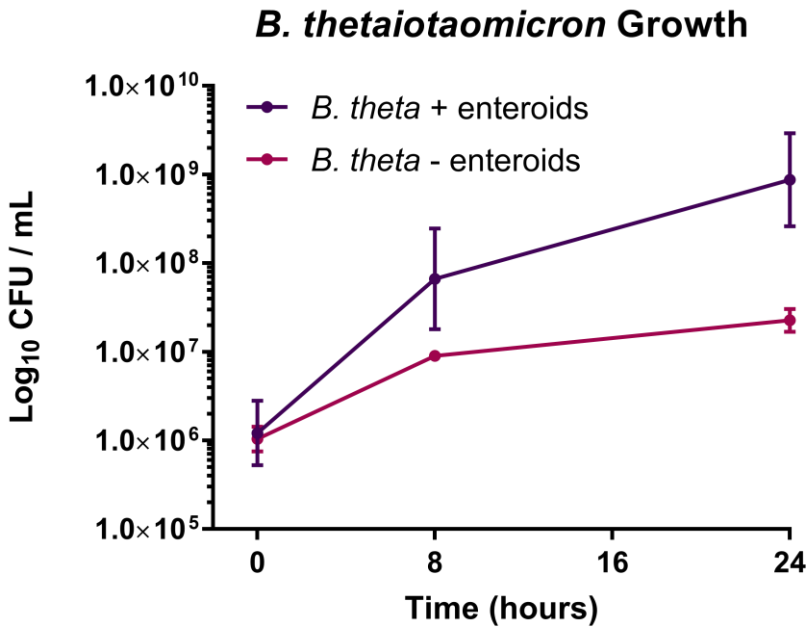
16S FISH Stain



System supports enteroid-anaerobe co-culture for at least 24 hours: *B. theta*



N=12/group

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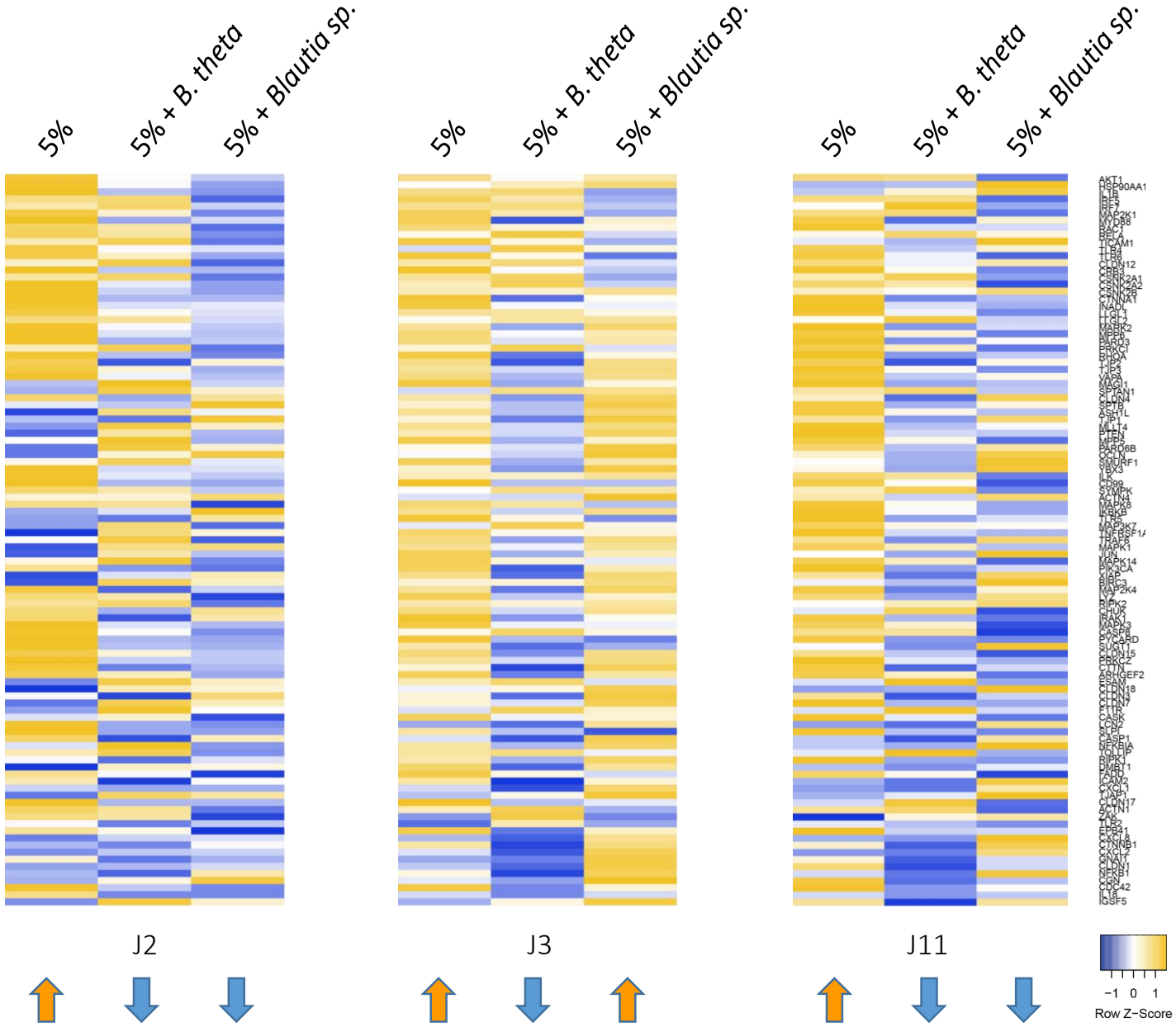
Unique hypoxia/tight-junction related gene expression changes with bacterial co-culture



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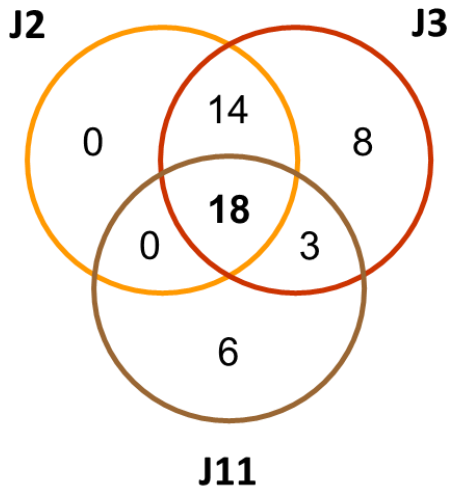
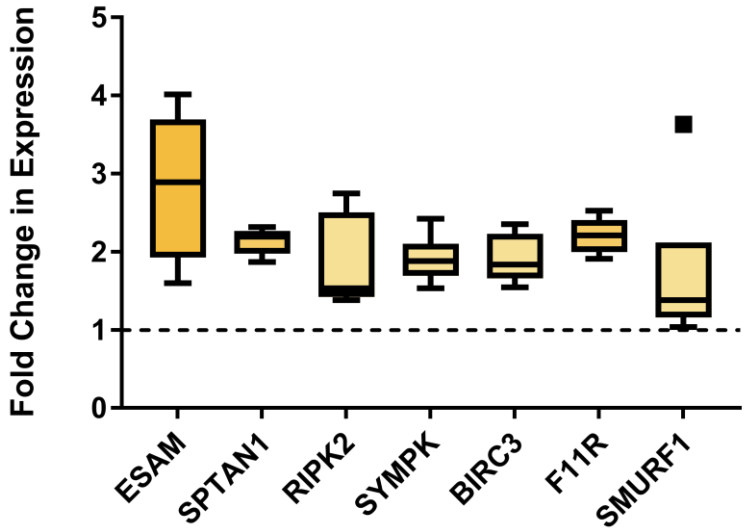
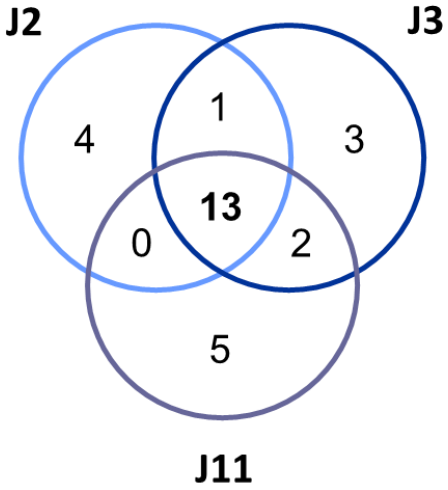
Shared genetic expression changes following *B. theta* co-culture



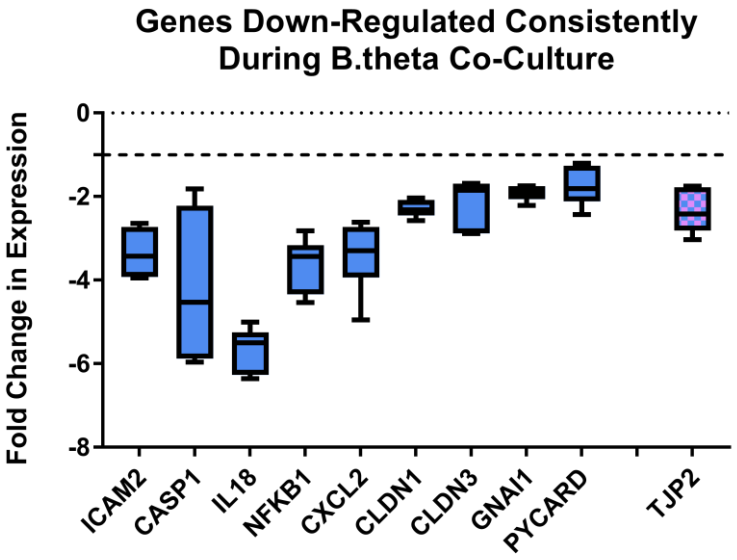
11/18 are also upregulated in Hypoxia



Down-Regulated Up-Regulated



3/13 are also down-regulated in Hypoxia (CXCL1, CXCL8, TLR2)



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Pathways differentially regulated during physiologic *B. theta* co-culture

GO-ID	Description	Corrected P-Value	Cluster Frequency	Genes
42127	Regulation of Cell Proliferation	9.79E-03	7/31 22.5%	MAP2K1 CXCL8 RIPK2 IL18 CXCL1 TLR4 RELA
6954	Inflammatory Response	3.26E-05	8/31 25.8%	CXCL8 RIPK2 CXCL1 F11R CXCL2 TLR4 RELA NFKB1
44419	Interspecies Interaction Between Organisms	7.92E-04	6/31 19.3%	IRF7 F11R CLDN1 TLR4 RELA TLR2
2758	Innate Immune Response Activating Signal Transduction	1.86E-05	4/31 12.9%	RIPK2 TLR4 RELA TLR2
1819	Positive Regulation of Cytokine Production	1.86E-05	6/31 19.3%	PYCARD RIPK2 CASP1 IL18 TLR4 TLR2
51092	Positive Regulation of NFkb TF Activity	1.86E-05	5/31 16.1%	PYCARD RIPK2 TLR4 RELA TLR2
2237	Response To Molecule of Bacterial Origin	2.41E-04	5/31 16.1%	RIPK2 CASP1 TLR4 RELA TLR2

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Summary: Enteroid-anaerobe co-culture

- Short term co-culture of commensal nano-anaerobe (*B. theta*) and obligate anaerobe (*Blautia sp.*) with enteroid monolayers permits/enables survival of both
- Co-culture decreases TEER in a patient-line specific manner
- *B. theta* growth is independent of enteroid line
- *Blautia sp.* growth is potentially affected by enteroid line (DNS)
- Gene expression profile, in response to *B. theta* co-culture, similar between patient lines
  - Reduced expression of some genes previously up-regulated in physiological-hypoxia
  - Expression profile corroborates interaction with molecules of bacterial origin
- Gene expression profile, in response to *Blautia* co-culture, varies between lines

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Opportunities with enteroid anaerobe co-culture

<u>The enteroid system may be used to:</u>

- reveal mechanism in host microbe associations
- predict translatability of animal model studies
- Characterize the individual responses to potential microbiome therapeutic and diagnostic candidates
- Amenable to: RNAseq, metabolomics, proteomics, single-cell sequencing

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Summary: Gaps/tools needed in translational microbiome research

- Large cohort friendly (and lab friendly) collection protocols/reagents
- Enrichment of target molecules for analyses of non-stool samples
- Statistical models appropriate for complex aspects of microbiome studies/data
- Evolution of pre-clinical models to more accurately dissect host-microbial relationships
    - Include precision medicine/individualized nuances of responses

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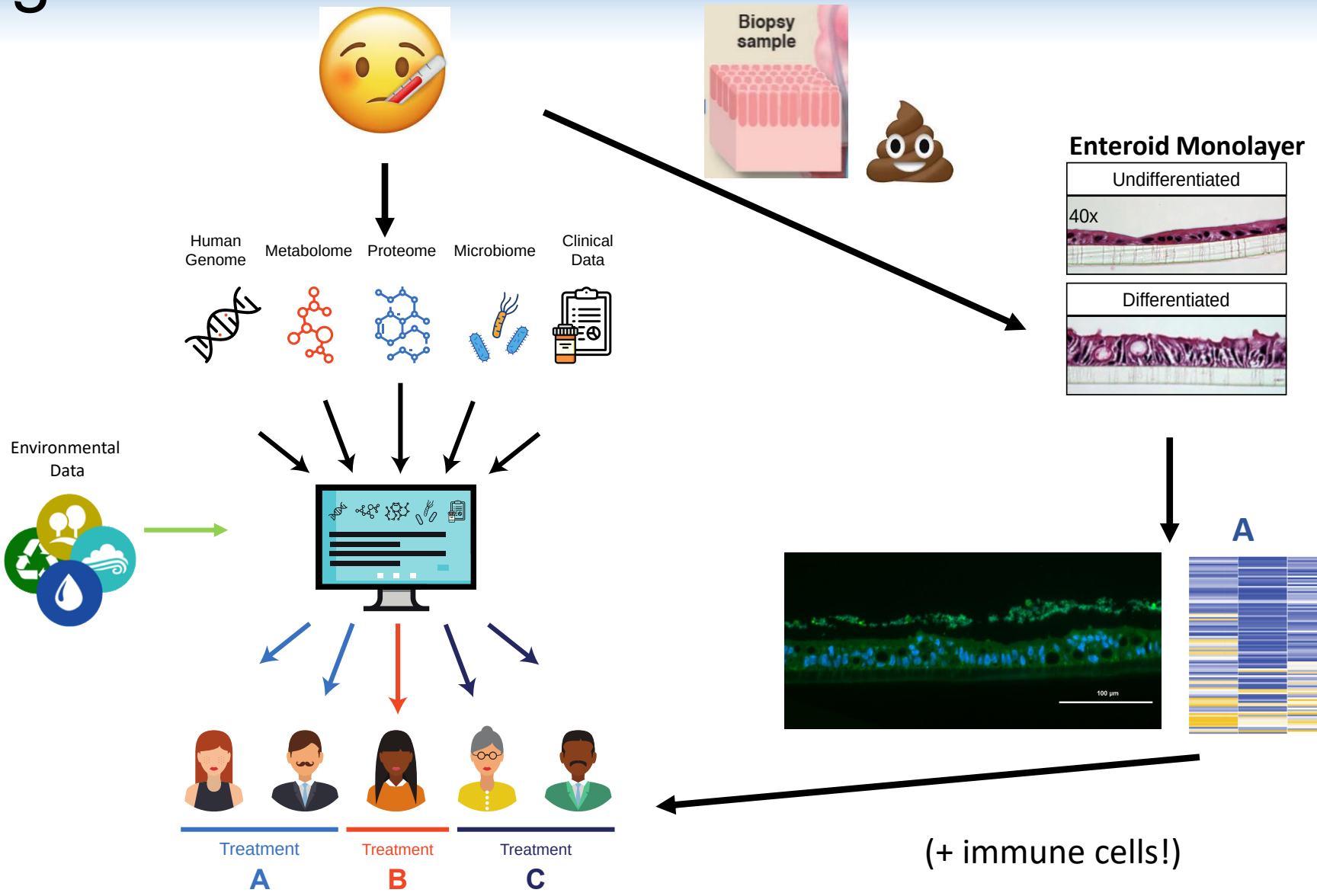
Moving toward…



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