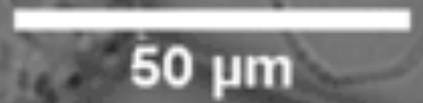
PERSONALIZING PREBIOTIC REGIMENS *for* ASTRONAUTS

LAWRENCE DAVID

Assistant Professor, Molecular Genetics & Microbiology

Associate Director, Duke Microbiome Center

Duke University



MICROBIOME

Charis Tsevis

- **All animals** are colonized by microbial communities

Charis Tsevis

MICROBIOME

- **All animals** are colonized by microbial communities
- In humans:
  - **~100 trillion** bacteria colonize epithelial surfaces
  - **100 billion bacteria *per gram*** of fecal matter in the colon

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Gut Bacteria From Thin Humans Can Slim Mice Down

By GINA KOLATA
Published: September 5, 2013

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Meddling gut bacteria get in the way of heart therapy

Only some patients who get a cardiac drug have bacteria that inactivate it.

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The Bacteria That May One Day Cure Food Allergies

Mandy Oaklander
@mandyoaklander
Aug. 25, 2014

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Gut Bacteria May Play a Role in Autism

Evidence is mounting that intestinal microbes exacerbate or perhaps even cause some of autism's symptoms

Aug 14, 2014 | By Melinda Wenner Moyer

The Telegraph

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Drinking milk could be behind rise in bowel disease

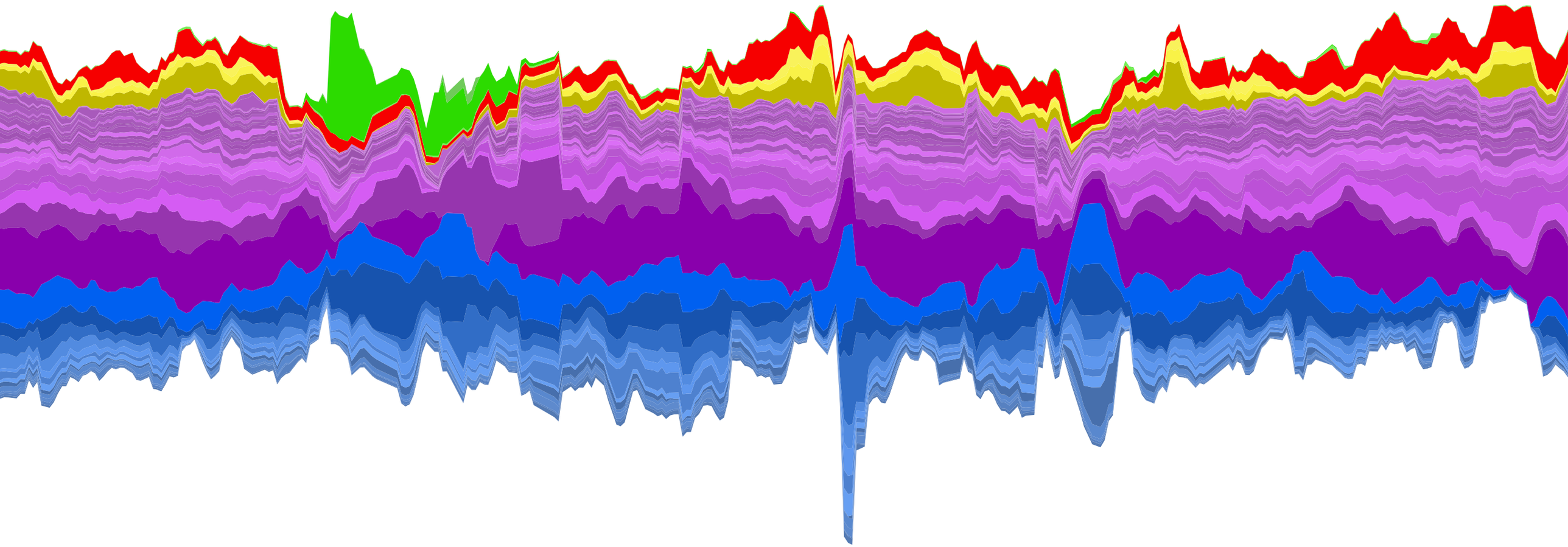
Diets rich in milk could be behind the growth in cases of inflammatory bowel disease because they fill our guts with "bad bacteria", a study on mice suggests.

The Atlantic SUBSCRIBE SEARCH MENU

HEALTH

When Feces Is the Best Medicine

GUT MICROBIOME IS
DYNAMIC



David et al., *Genome Biol* 2014

DIET CAN BE A WAY TO MANIPULATE

Peter Turnbaugh

Morgan Walker/NPR

DIET CAN BE A WAY TO
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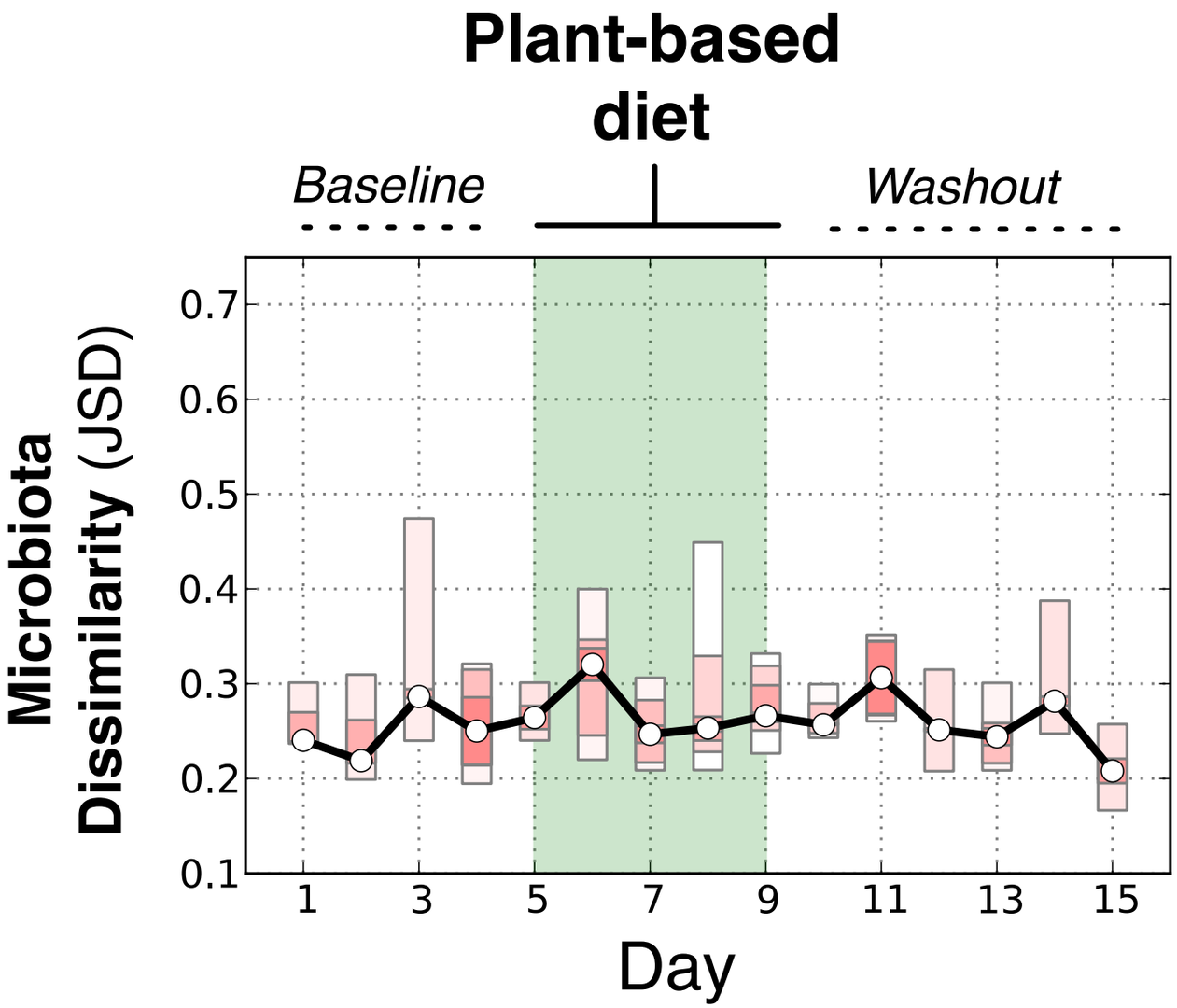

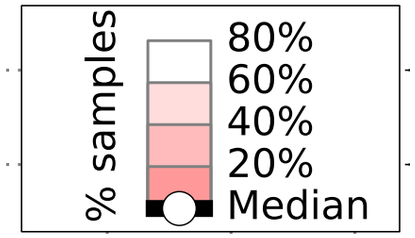

David et al., *Nature* 2014

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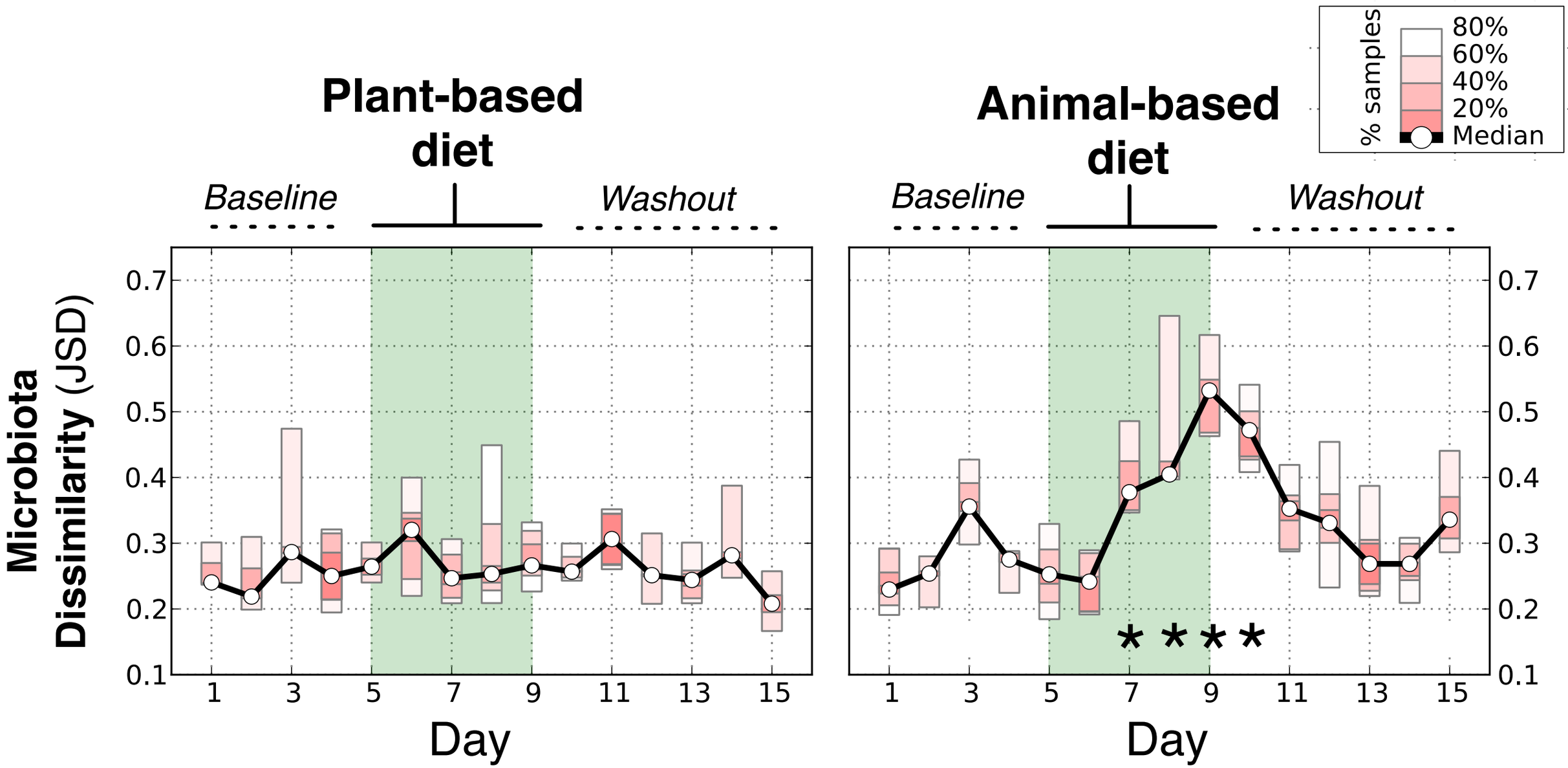

David et al., *Nature* 2014

PREBIOTICS

- Prebiotics are **indigestible dietary carbohydrates** known to stimulate gut microbiota

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Body Site	Lag (days)	Host factor	Representative OTUs (#)	ρ
Subject A Gut	0	Stool:Hardness	Eggerthella/Clostridium(11)	-0.30
	0	Stool:TimeOfDay	Eggerthella/Clostridium(11)	0.27
	1	Nutrition:Fiber	Clostridium(6)	-0.38
	1	Nutrition:Fiber	Ruminococcaceae/F.prausnitzii(4)	-0.44
	1	Nutrition:Fiber	Eggerthella/Clostridium(11)	-0.39
	1	Nutrition:Fiber	Ruminococcus/R.gnavus/Clostridium(4)	-0.51
	1	Nutrition:Fiber	Ruminococcus/R.gnavus/Clostridium(5)	-0.51
	1	Nutrition:Fiber	Blautia(3)	-0.38
	1	Nutrition:Fiber	Bifidobacteriales(13)	0.36
	1	Nutrition:Fiber	Coprococcus(8)	0.44
	1	Nutrition:Fiber	Clostridium(1)	-0.42
	1	Nutrition:Fiber	Ruminococcus/R.gnavus/Clostridium(6)	-0.44
	1	Nutrition:Fiber	Roseburia/E.rectale(30)	0.37
	1	Food:OrangeJuice	Clostridium(1)	0.28
	1	Food:BreakfastBar	Ruminococcus/R.gnavus/Clostridium(4)	-0.27
	1	Food:BreakfastBar	Ruminococcus/R.gnavus/Clostridium(5)	-0.40
	1	Food:BreakfastBar	Bifidobacteriales(13)	0.27
	1	Food:BreakfastBar	Clostridium(1)	-0.43
	1	Food:Yogurt	Bifidobacteriales(2)	0.45
	1	Food:Fruits:Fresh	Clostridiales(4)	-0.27
	1	Food:Fruits:Citrus	Ruminococcaceae/F.prausnitzii(4)	0.36
	1	Food:Soup	Clostridiales(1)	-0.25
	1	Food:Soup	Blautia(21)	-0.26
	1	Food:Soup:Other	Clostridiales(1)	-0.27
	1	Food:Soup:Other	Blautia(21)	-0.28

David et al., *Genome Biology* 2014

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	1	[illegible]	[illegible]	[illegible]
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	1	Food:Fruits:Fresh	Clostridiales(4)	-0.27
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PREBIOTICS

- Prebiotics are **indigestible dietary carbohydrates** known to stimulate gut microbiota
- Of interest because gut microbial fermentation leads to short-chain fatty acids like **butyrate**, a primary energy source for colonic epithelial cells
- Also of relevance to **spaceflight** because **shelf-stable** and tend to originate in **plants**

HOW CAN PREBIOTIC REGIMENS BE RATIONALLY DESIGNED?

- **Dozens** of dietary fiber species exist

Prebiotics: Oligo- and Polysaccharides			
Amylopectin (corn)	Galactomannan	Lichenin	Pullulan
Amylopectin (potato)	Glucomannan	Lignin	Rhamnogalacturonan I
Arabinan	Glycogen	Oat spelt xylan	Starch (rice)
Arabinogalactan	Gum (guar)	Pectic galactan (lupin)	Starch (corn)
Arabinoxylan (wheat)	Gum (rosin)	Pectic galactan (potato)	Starch (wheat)
Cellobiose	Heparin	Pectin (apple)	Starch (potato)
Chitin	Hyaluronan	Pectin (citrus fruit)	Xyloglucan
Chondroitin sulfate	Inulin	Pectin (citrus peel)	α-cellulose
Dextran	Laminarin	Polygalacturonate	α-mannan

HOW CAN PREBIOTIC REGIMENS BE RATIONALLY DESIGNED?

- **Dozens** of dietary fiber species exist
- Each may **vary in effect**

	Butyrate			
Group[b]	Before	During	Change (%)	*P* value
Accessible starch ($n = 39$)	13 ± 6.1	15 ± 8.3	+13	0.18
Hi-Maize ($n = 43$)	9.3 ± 4.1	9.7 ± 5.6	+5	0.81
Potato ($n = 43$)	13 ± 6.0	16 ± 7.5	+29	**<0.001**
Inulin ($n = 49$)	11 ± 6.0	13 ± 7.0	+17	0.14

Baxter et al., *mBio* 2019

HOW CAN PREBIOTIC REGIMENS BE RATIONALLY DESIGNED?

- **Dozens** of dietary fiber species exist
- Each may **vary in effect**
- **Individuals also vary** in response

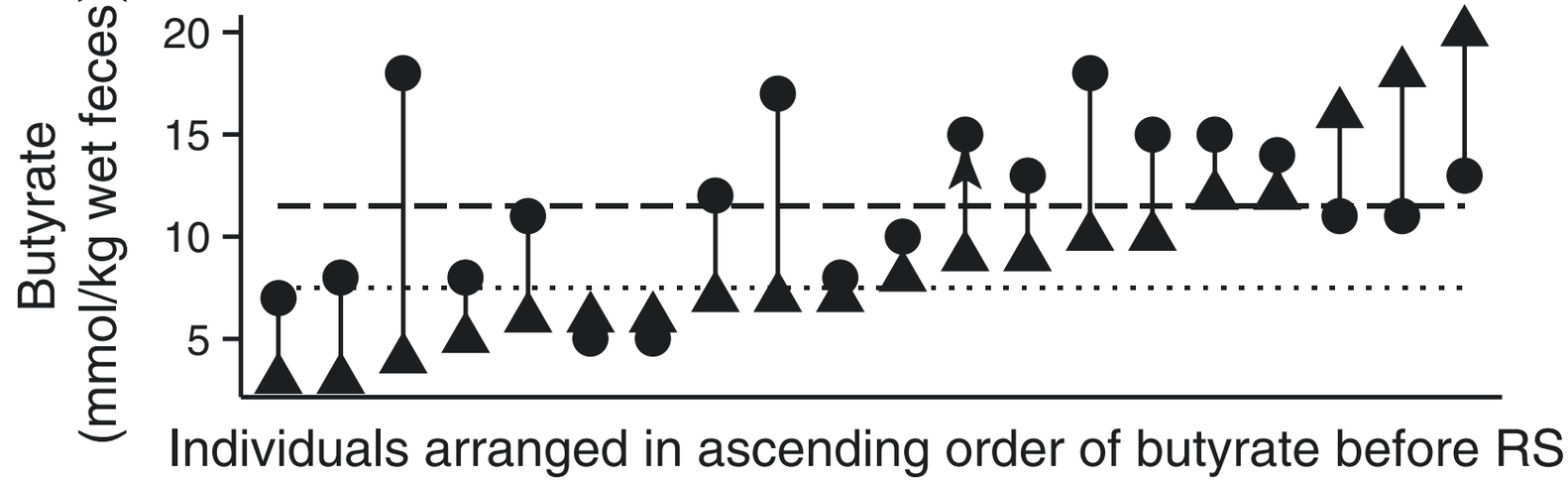

Fig. 2 Median butyrate concentrations for each individual before (*triangles*) and during consumption of RS (*circles*). *Dotted* and *dashed lines* denote the median values for butyrate before and during RS, respectively, for the entire study population

Venkataraman et al., *Microbiome* 2016

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- **Dozens** of dietary fiber species exist
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- **Individuals also vary** in response

How do we avoid provisioning astronauts with prebiotics that don't work?

GOALS OF OUR WORK

Answer three questions for astronauts:

- **Which prebiotics** should they take?
- **Who** should take them?
- And, **when**?

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CHALLENGES
TO SCREENING PREBIOTICS

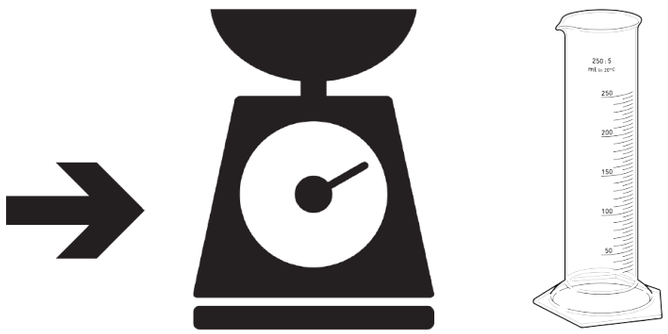
- Potentially a large number of prebiotics to screen
- Human studies can be logistically demanding
- *In vivo* physiological variation (e.g. SCFA absorption) may mask microbial response

A SIMPLE
HOST-FREE PREBIOTIC ASSAY

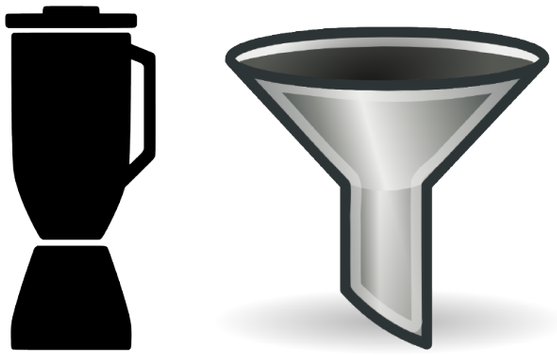
Zack Holmes

PUMP: Prebiotic Utilization and Metabolite Production

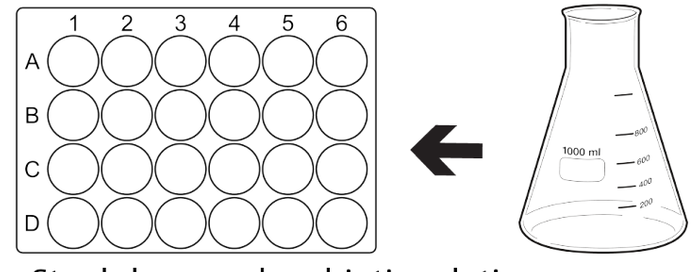
1 Stool is self-collected and frozen.
Transported on ice to laboratory



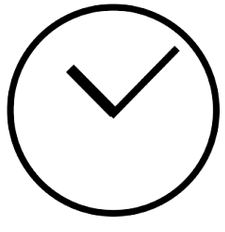
2 Thawed stool is weighed, and
appropriate volume of PBS is added



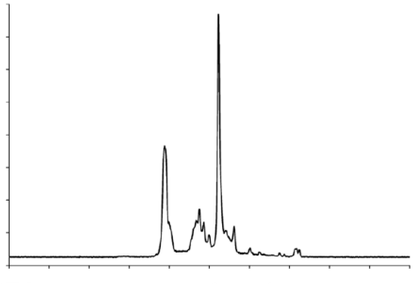
3 Stool and PBS mixed, slurry flitered
through mesh to remove particulates



4 Stool slurry and prebiotic solution
are added to 24-well plates



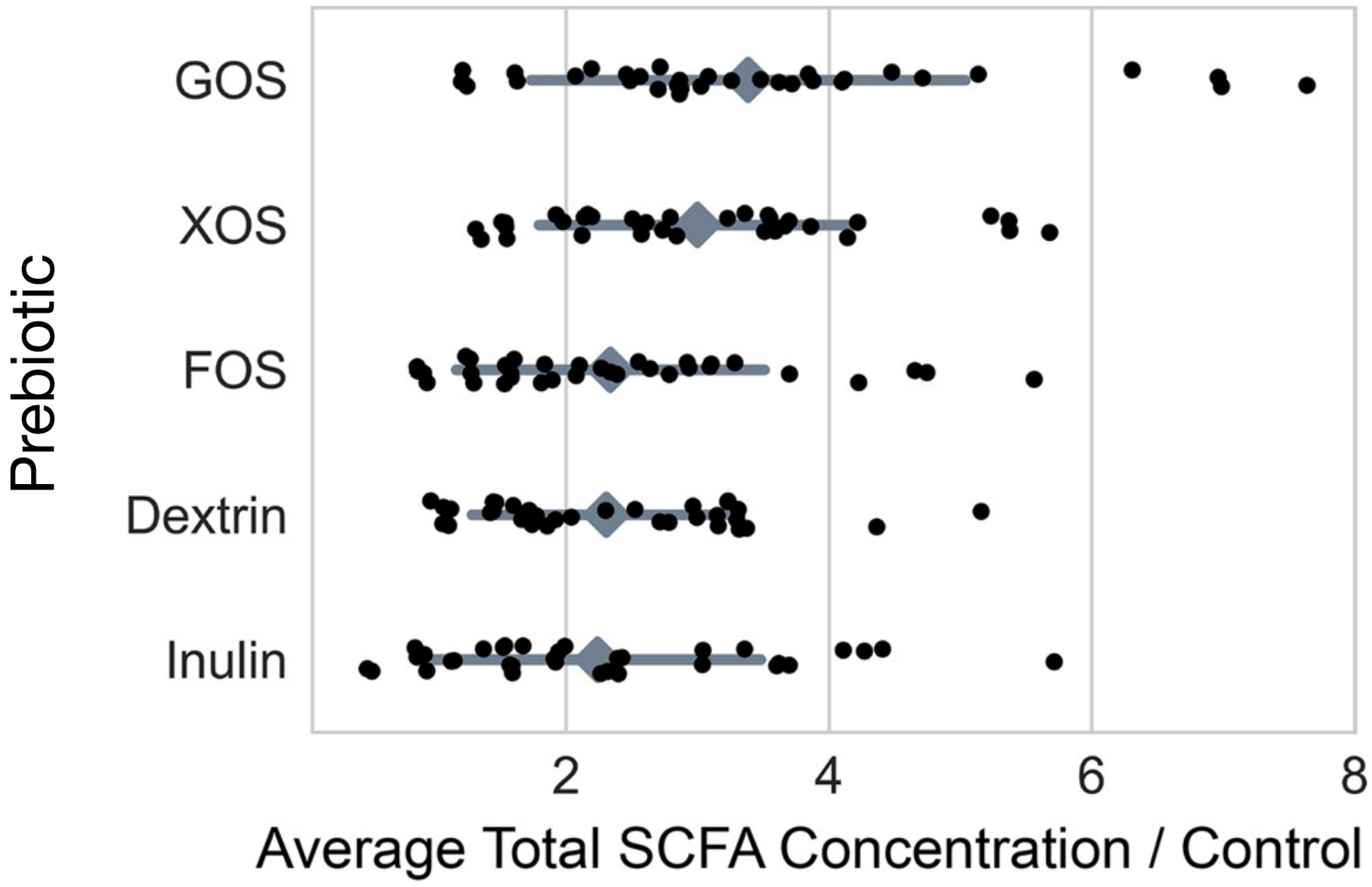
5 Plates are incubated at 37 degrees
in an anaerobic chamber for 24 hours



6 SCFA concentration is measured
using a gas chromatograph FID

Holmes et al., *mBio* 2020

OBSERVED SIGNIFICANT VARIATION
BETWEEN PREBIOTICS

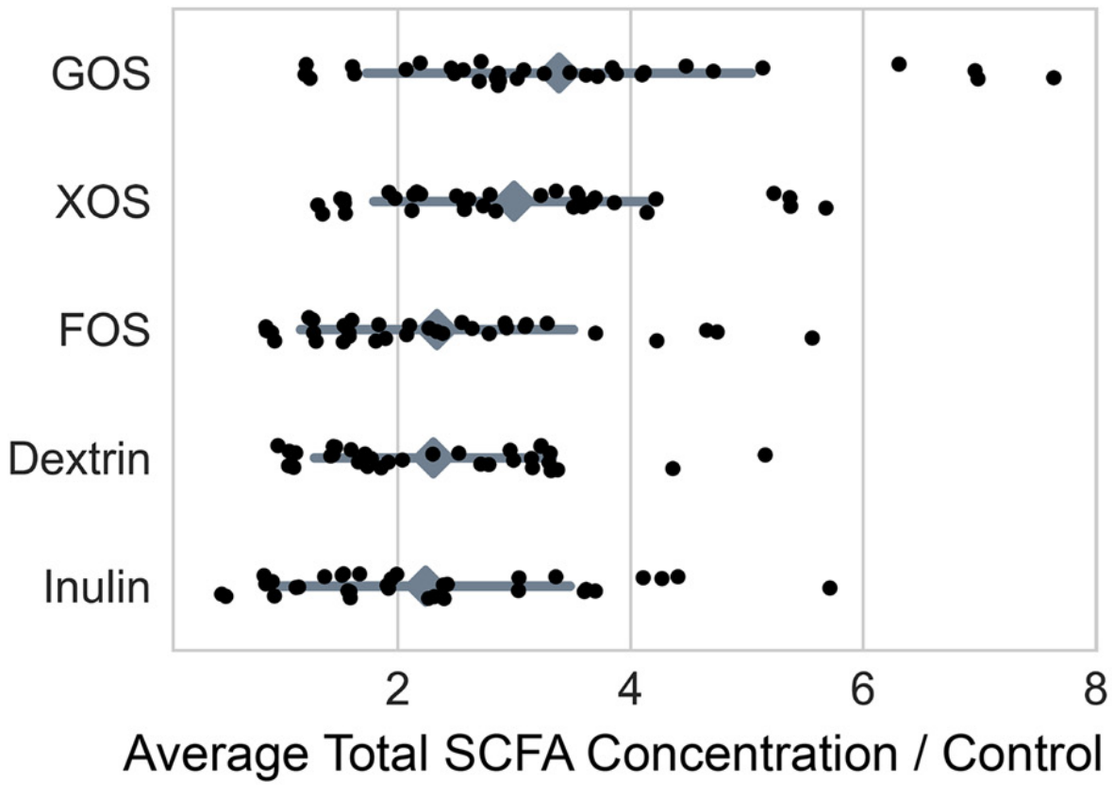


n=17 stool donors
$p < 0.0001$, two-way ANOVA

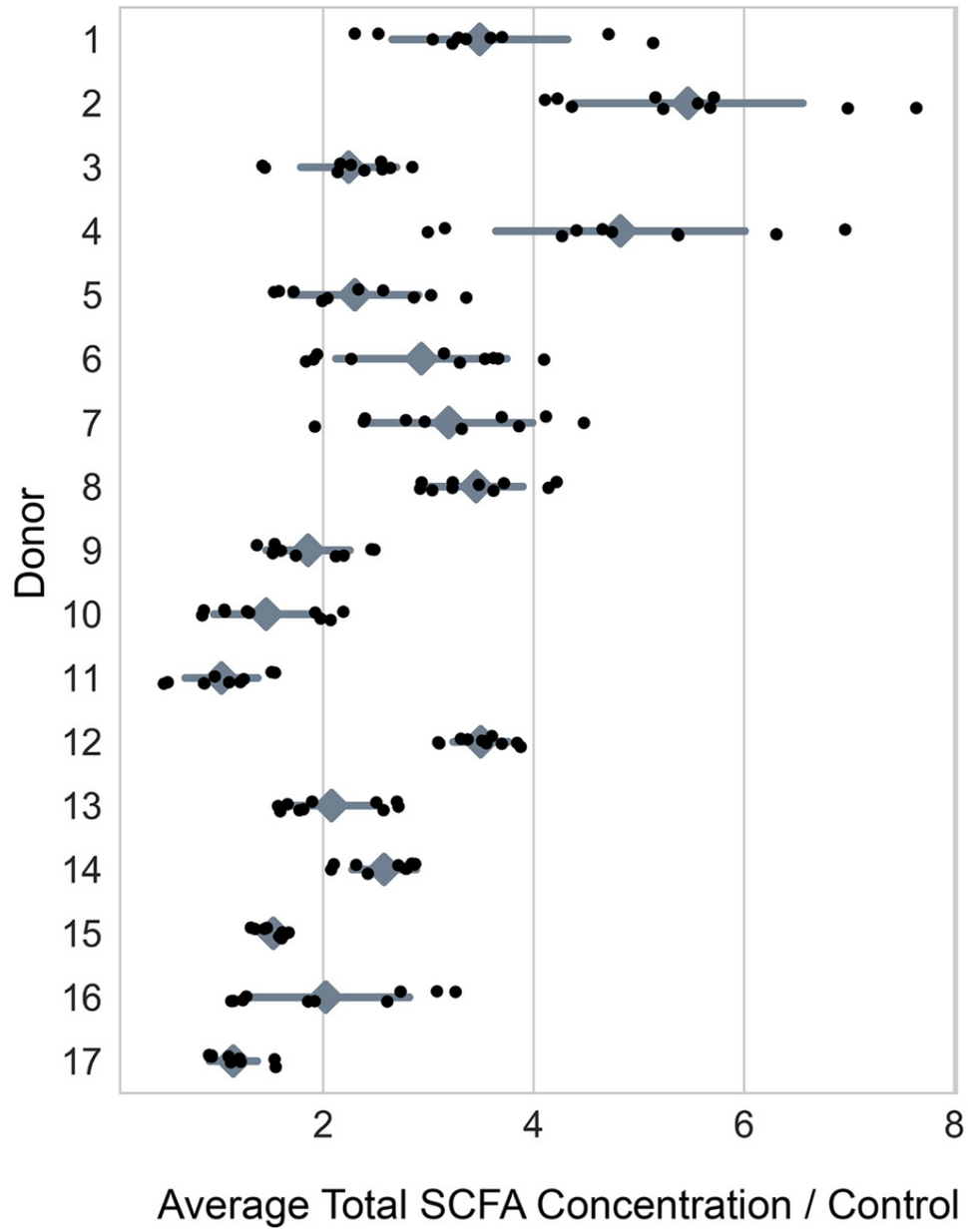
Holmes et al., *mBio* 2020

YET, VARIATION
BETWEEN DONORS LARGER

Grouped by
PREBIOTICS



Grouped by
DONOR



Holmes et al., *mBio* 2020

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HOW CAN WE STUDY INDIVIDUAL VARIATION?

*Need **lots** of cultures:*

- Each individual estimated to harbor **hundreds of unique** gut bacterial strains

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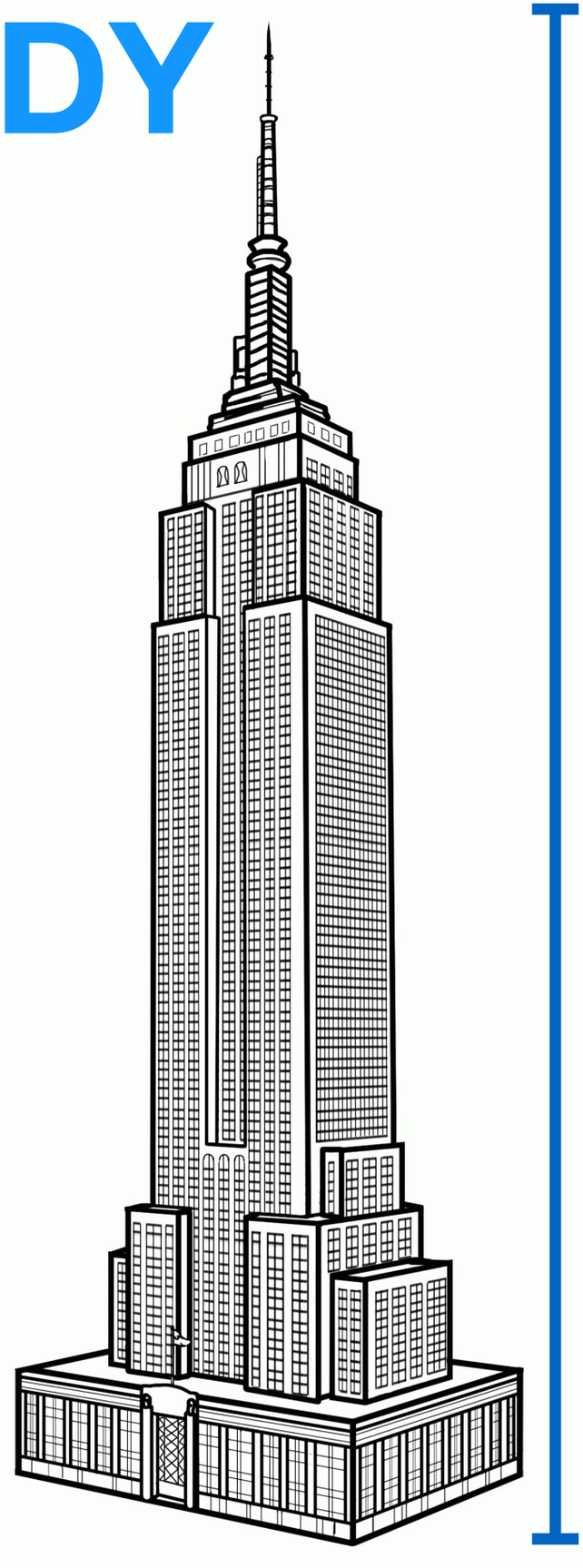

Petri dishes to capture **90% of gut bacterial species**

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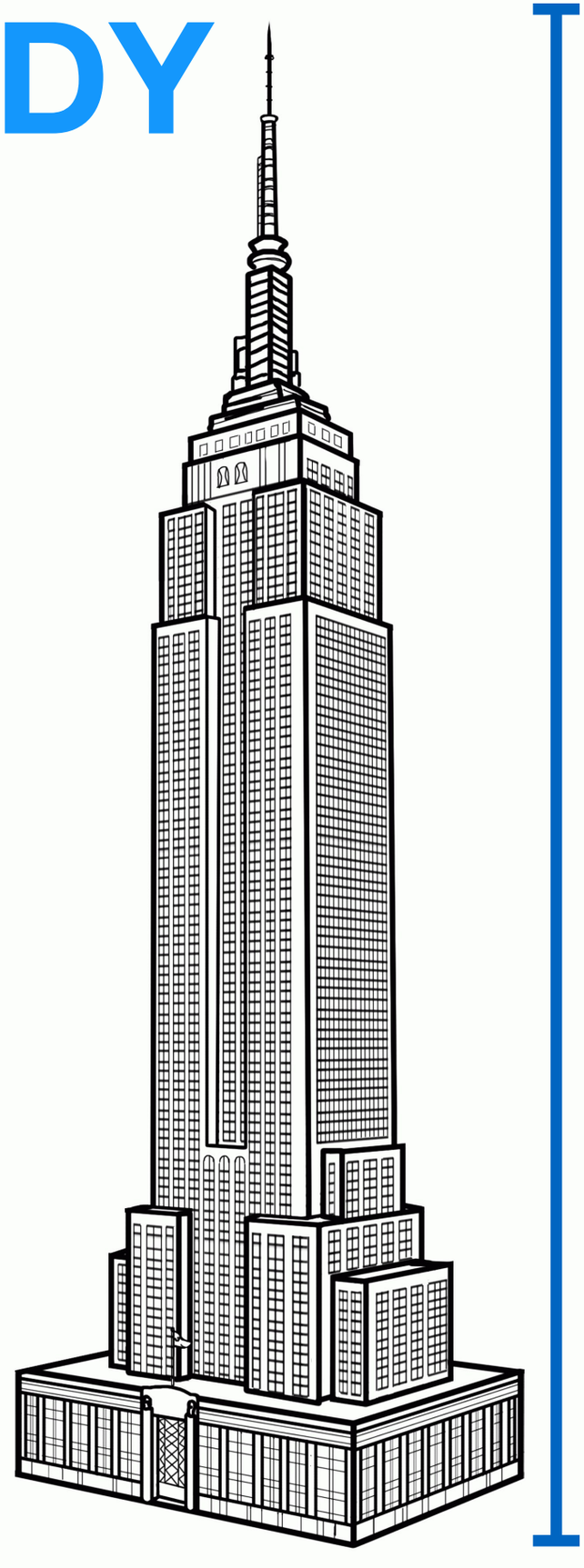
*Culture can be **hard to scale**:*

- Manual isolation can be **tedious** and **time-consuming**



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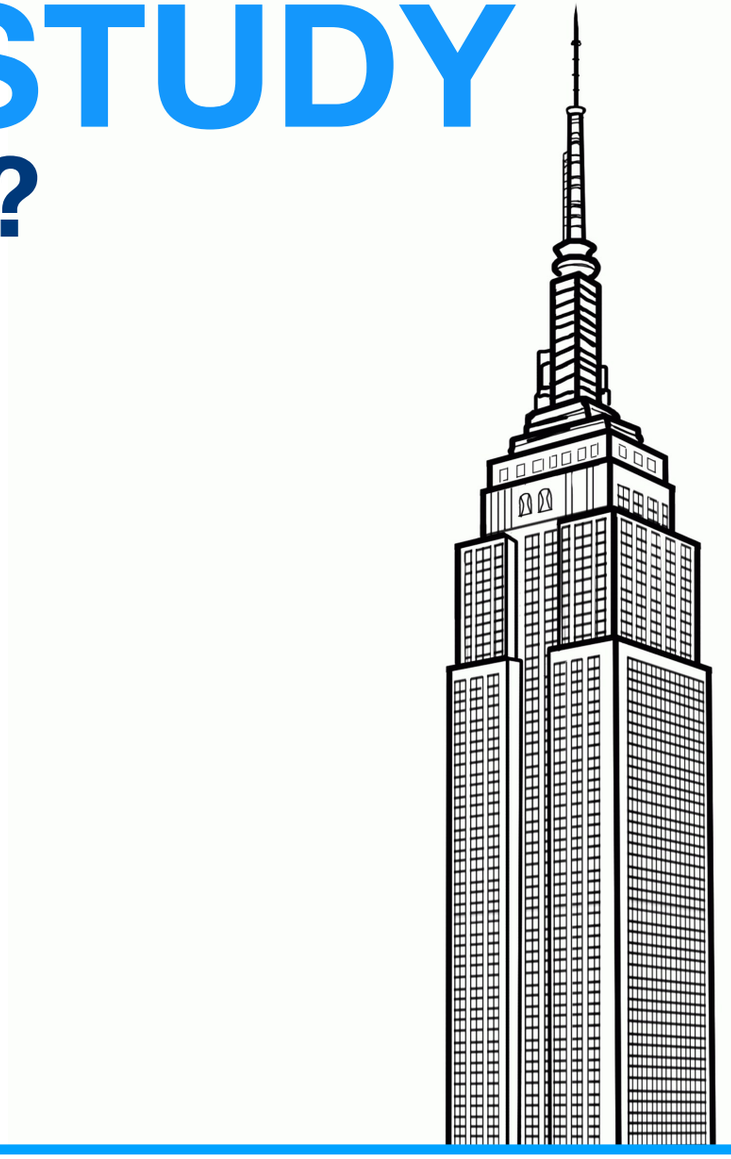
- **Millions of colonies** needed to match sensitivity of DNA sequencing

*Culture can be **hard to scale**:*

- Manual isolation can be **tedious** and **time-consuming**
- Automation may require **expensive** and/or **bulky** robotics that need to be enclosed anaerobically



Petri dishes to capture **90% of gut bacterial s**

Colony Picking Robot

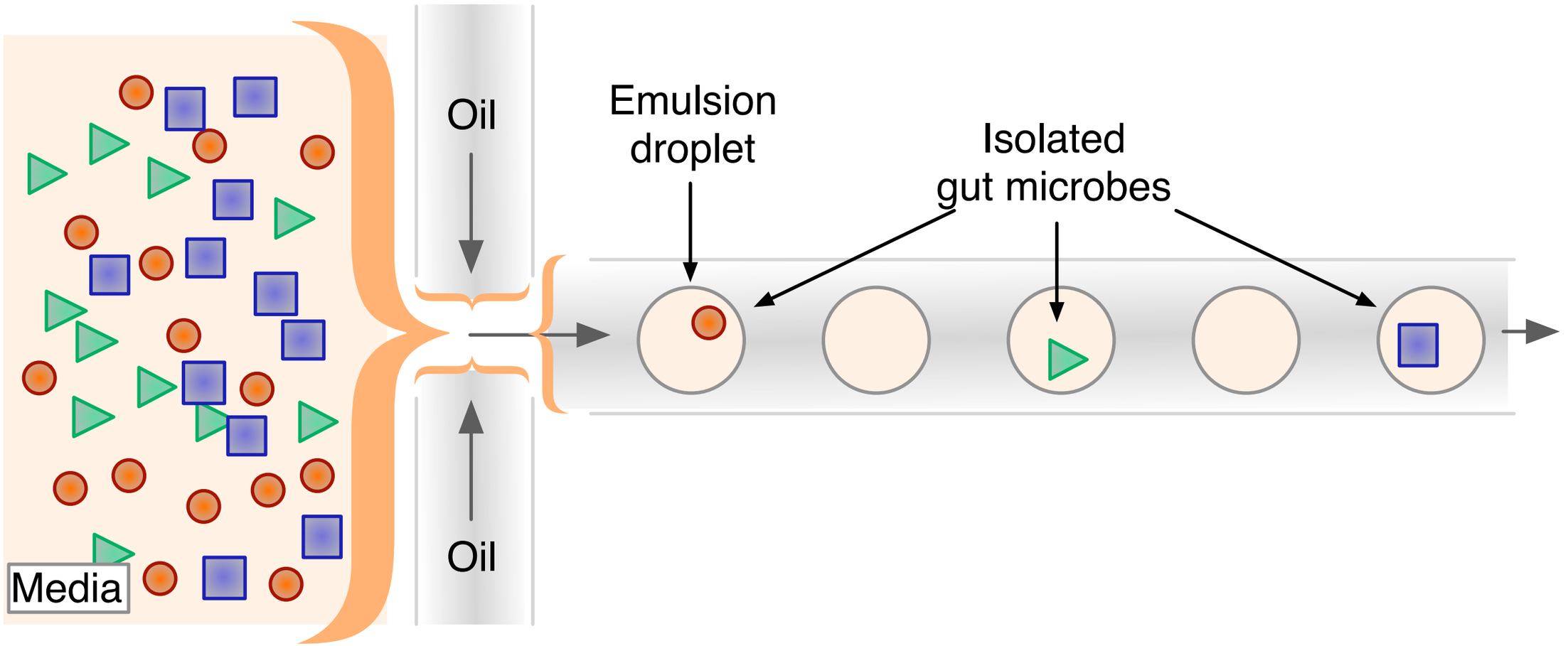
Anaerobic Chamber

MICROFLUIDIC
BACTERIAL CULTURE

Rachael Bloom

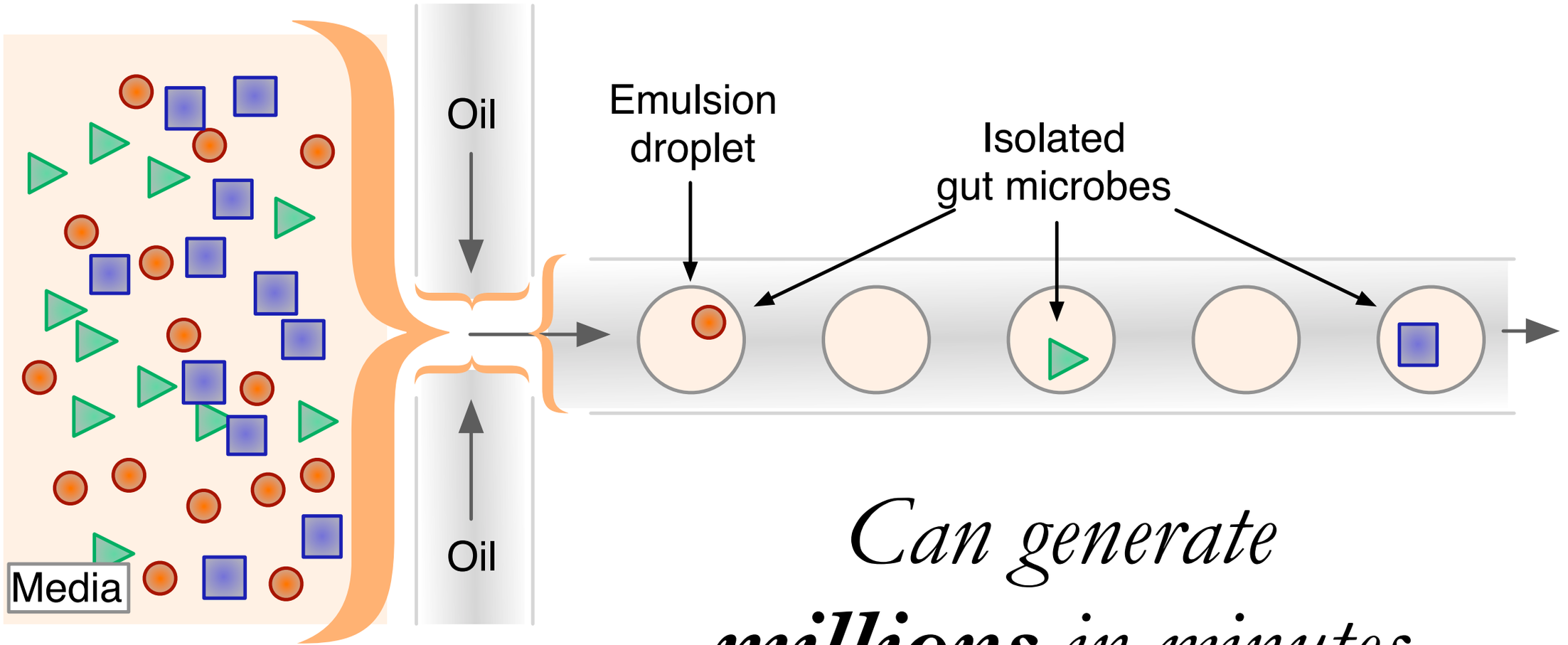
MICROFLUIDIC BACTERIAL CULTURE

Rachael Bloom



MICROFLUIDIC
BACTERIAL CULTURE

Rachael Bloom



Can generate
millions *in minutes*

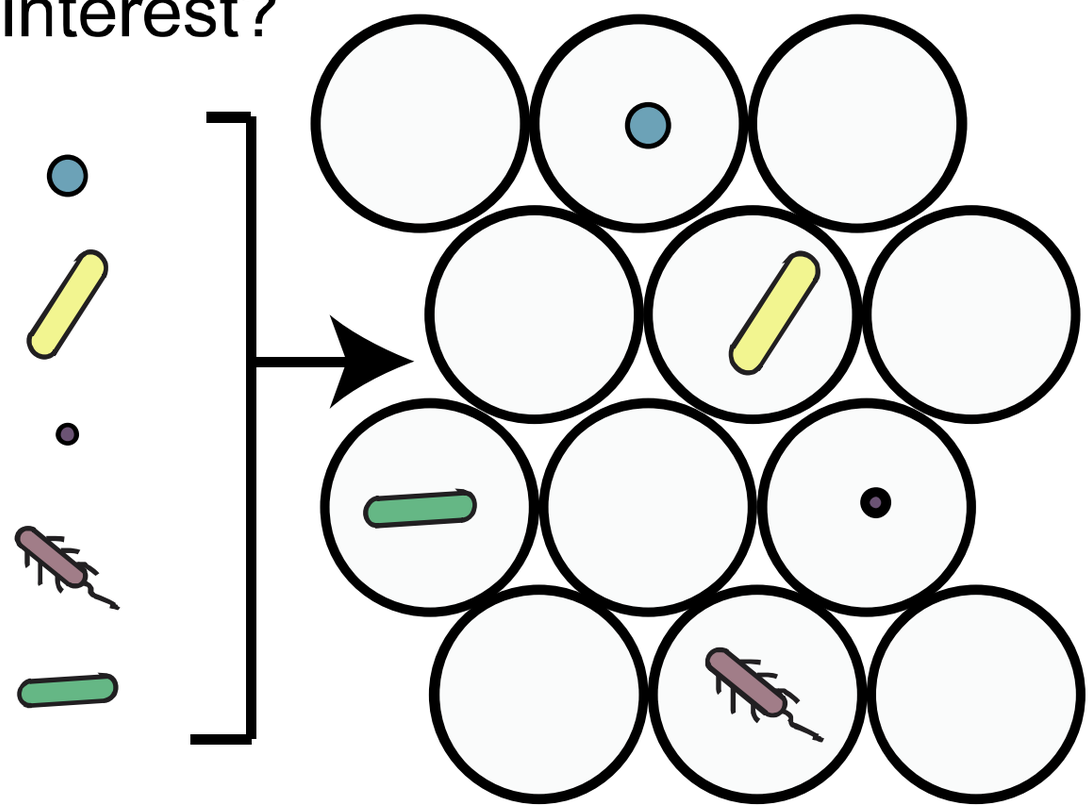
APPLY TOWARDS

PREBIOTIC UTILIZATION

Max Villa

Metabolize carbon source of interest?

Isolate with sole carbon source



APPLY TOWARDS

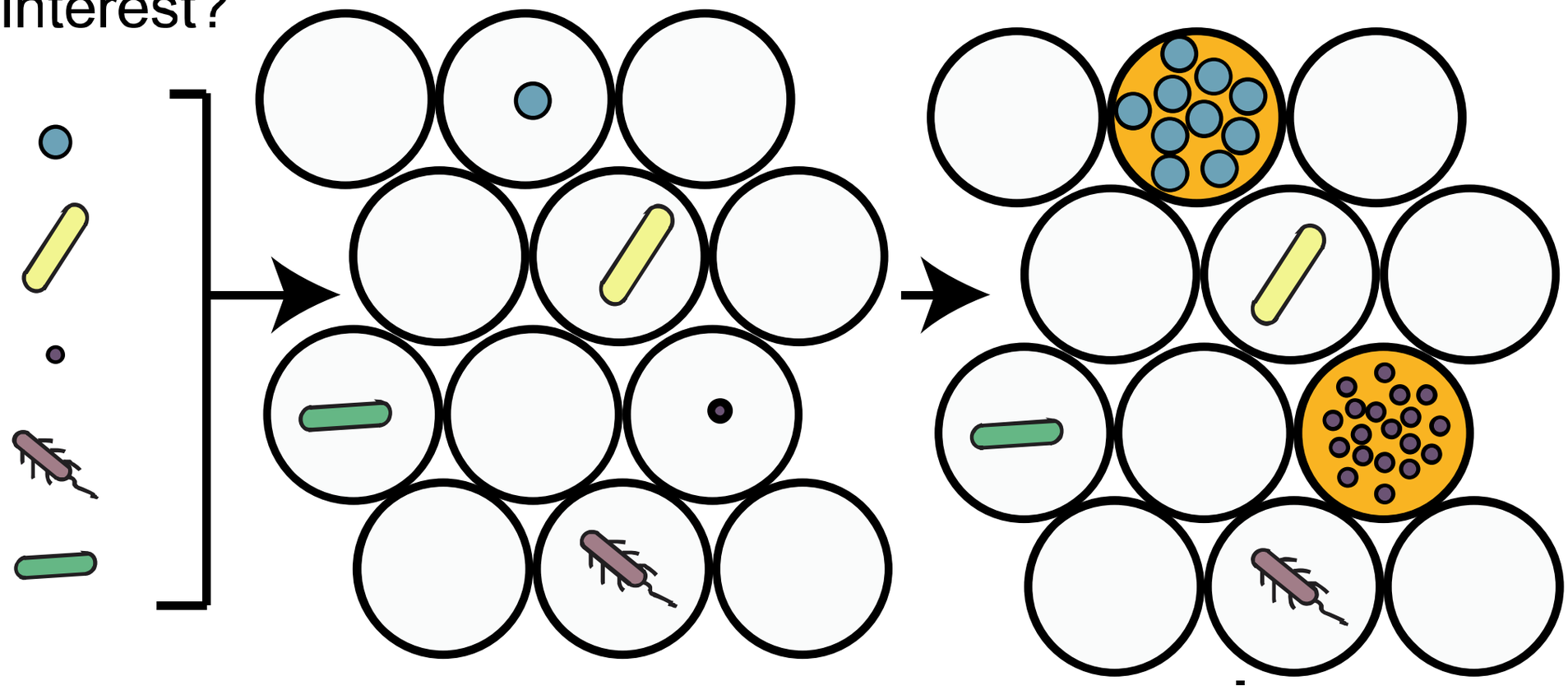
PREBIOTIC UTILIZATION

Max Villa

Metabolize carbon source of interest?

Isolate with sole carbon source

Growth restricted to microbes that utilize carbon source



APPLY TOWARDS

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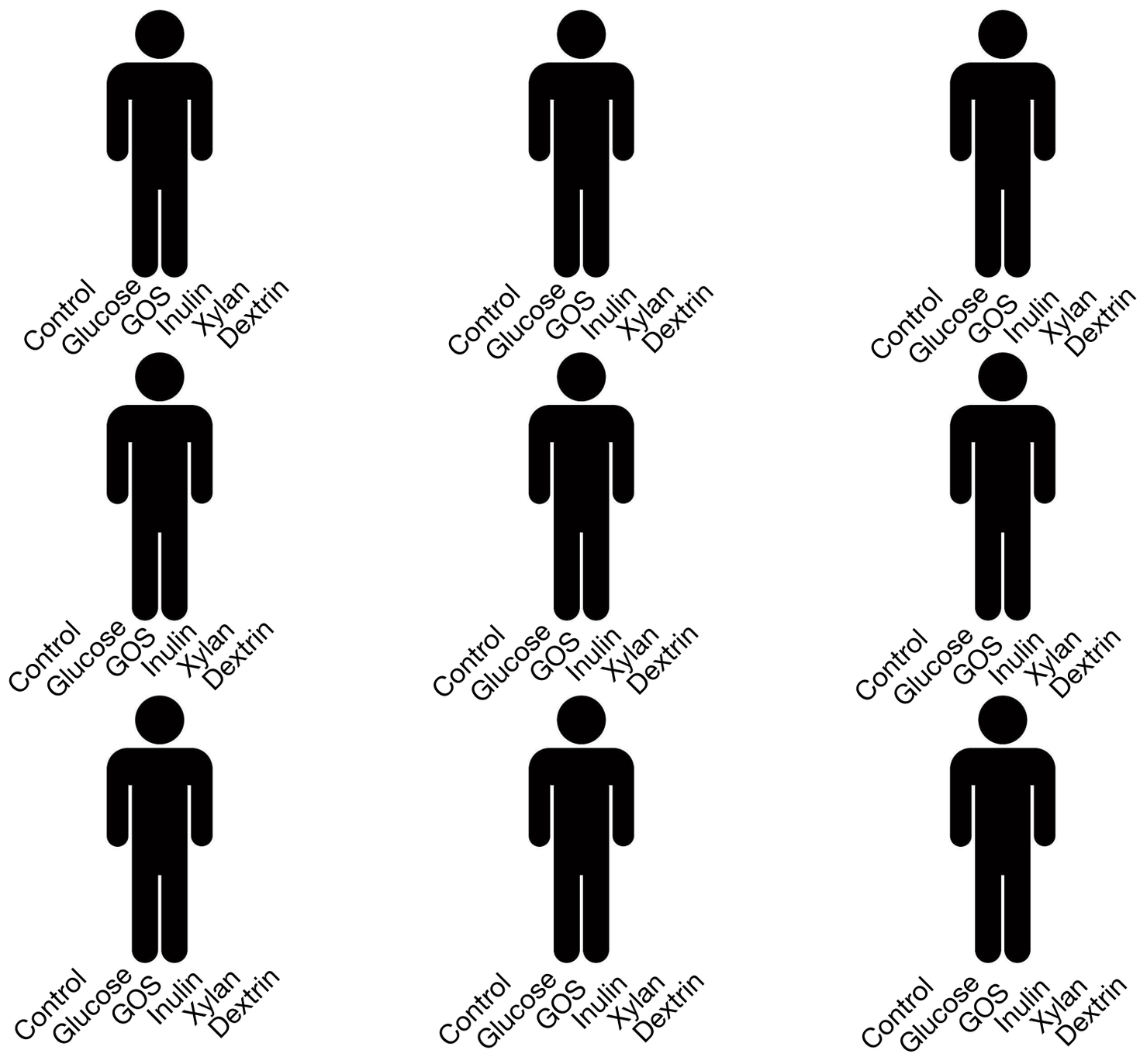

APPLY TOWARDS
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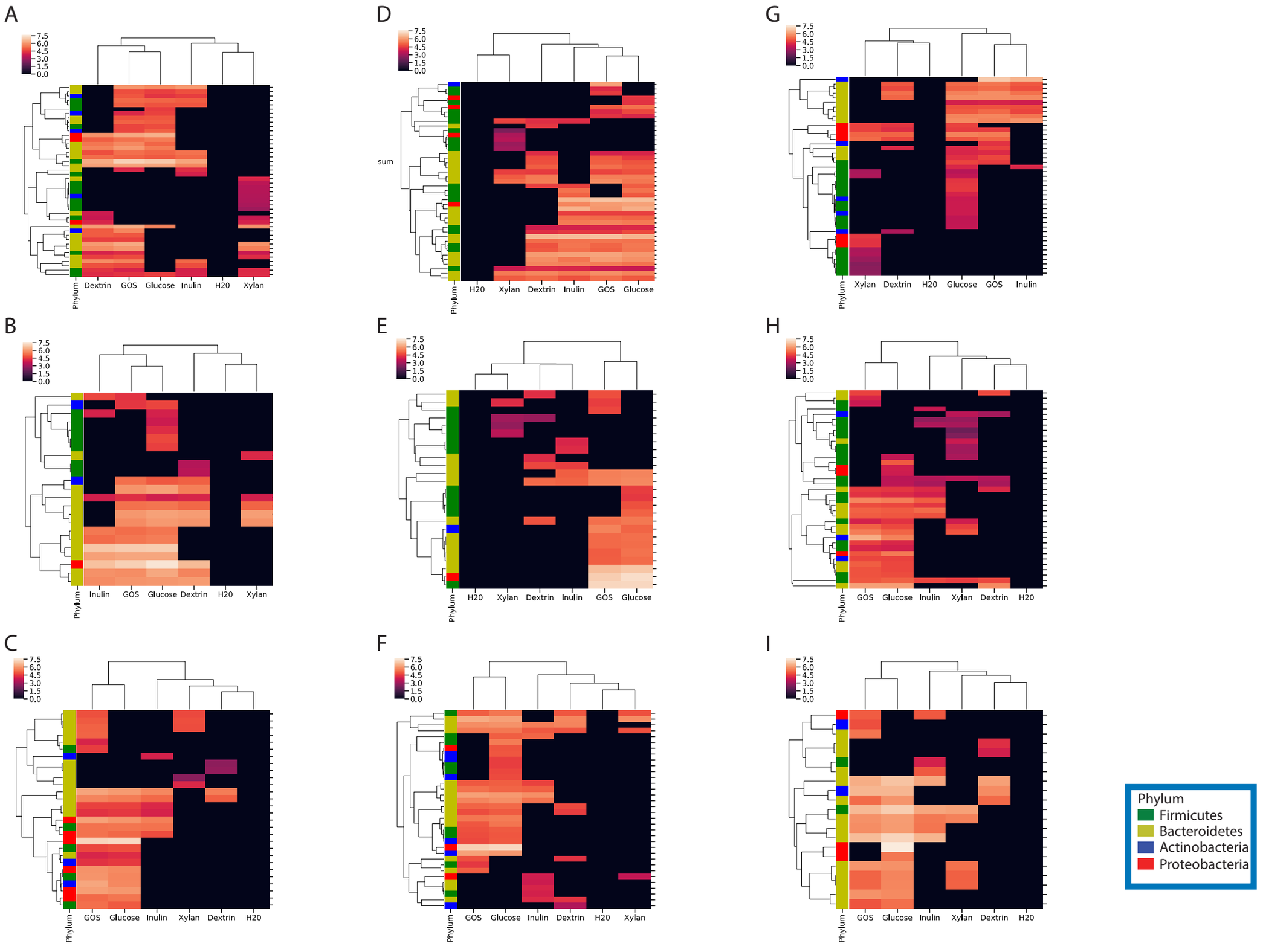
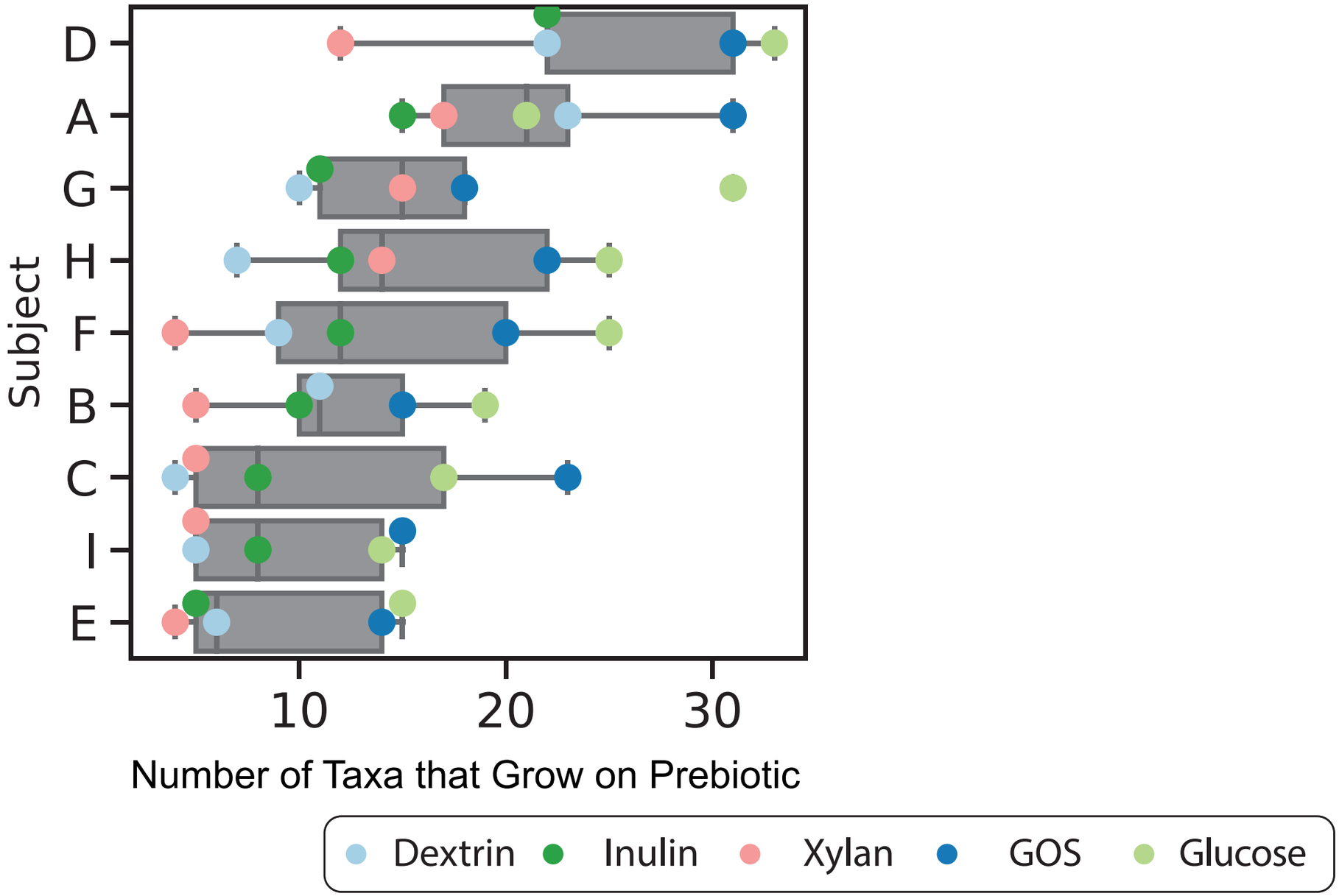


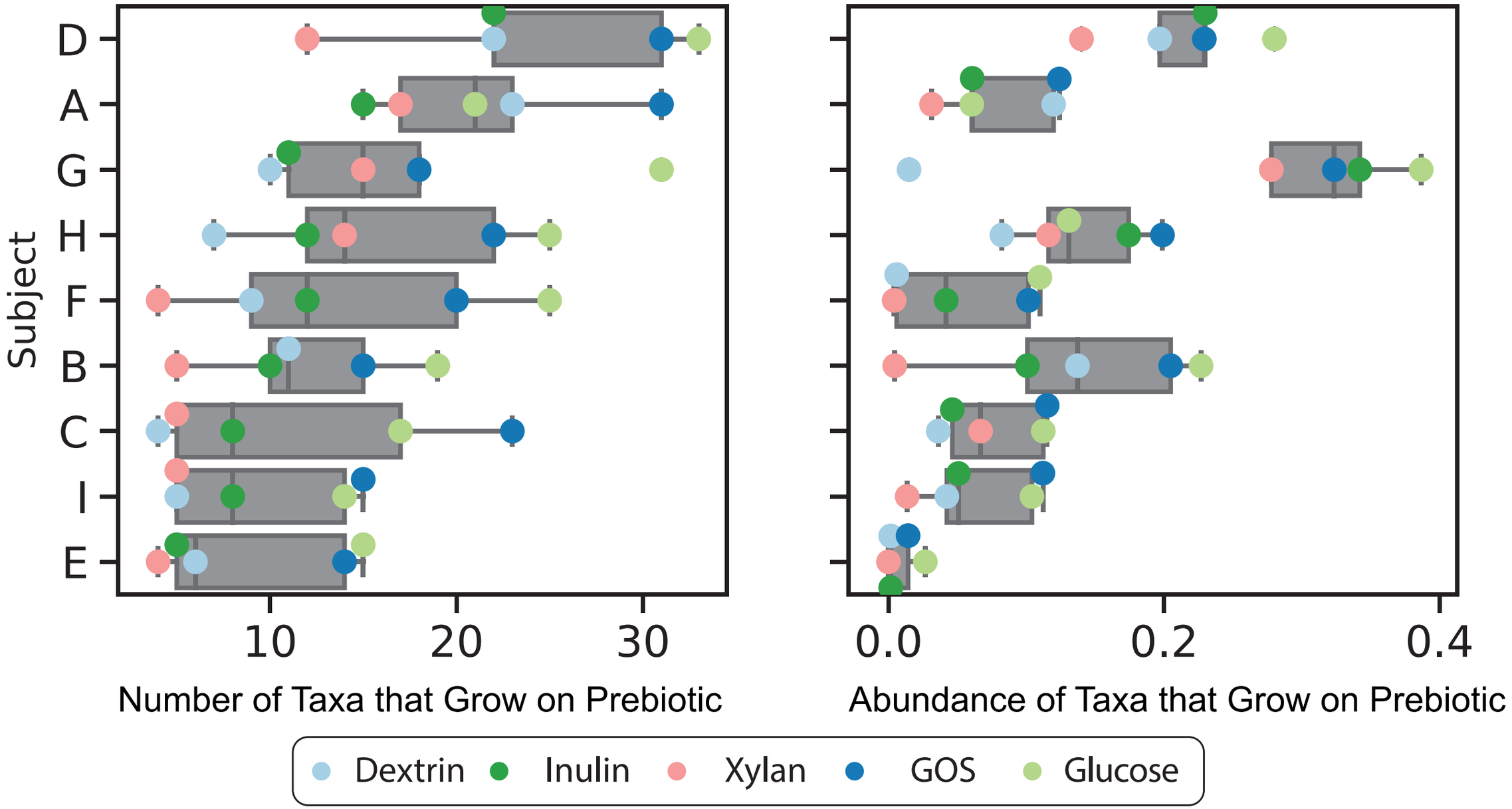
EVERYONE
HOSTS PREBIOTIC UTILIZERS



Villa*, Bloom*, et al., *mSystems* 2020

EVERYONE
HOSTS PREBIOTIC UTILIZERS

But, people vary in abundance of utilizers across prebiotics



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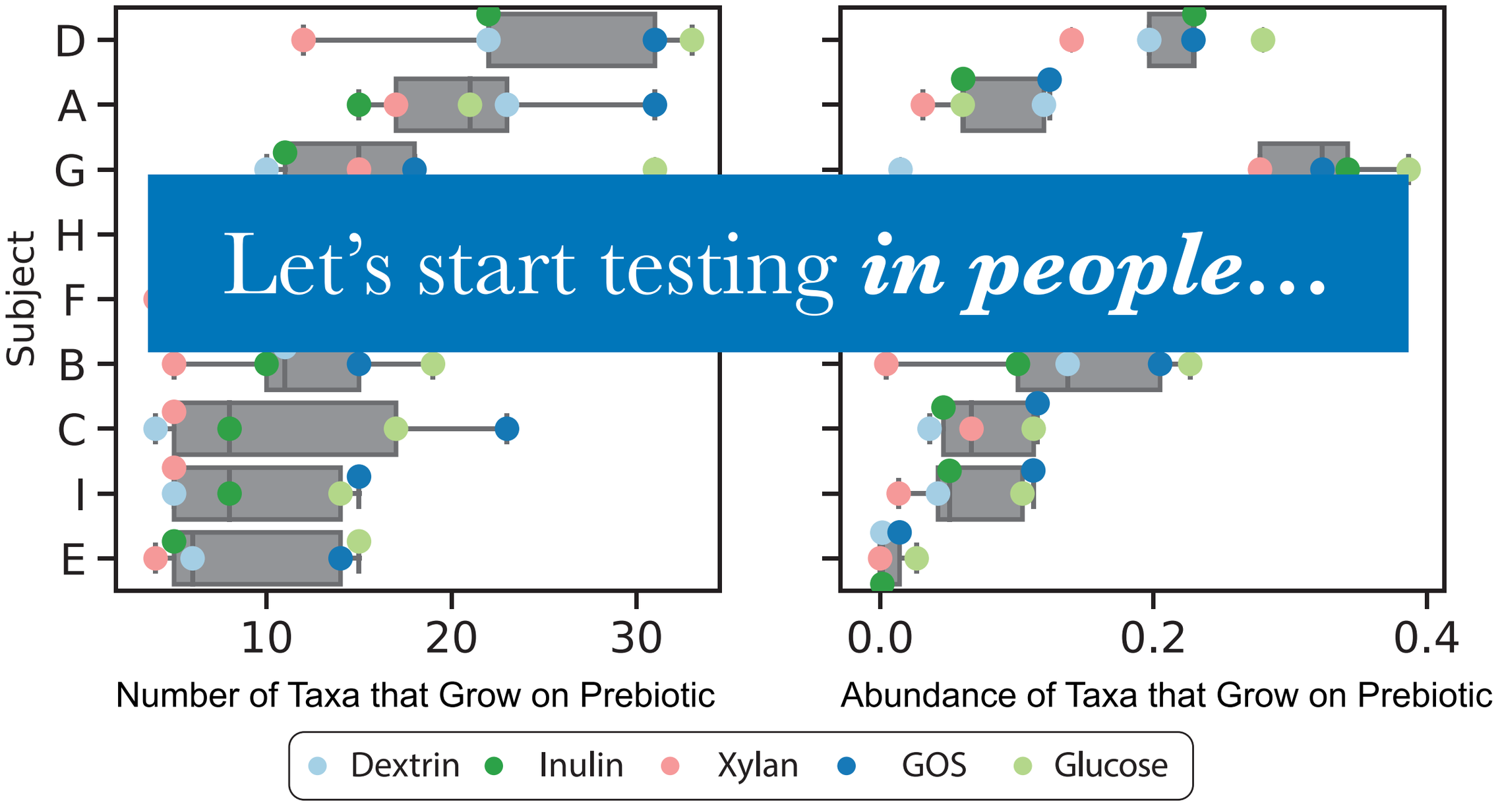

Villa*, Bloom*, et al., *mSystems* 2020

STUDY GOAL

- Test the hypothesis that individuals will vary in prebiotic metabolic potential, ***regardless of prebiotic***

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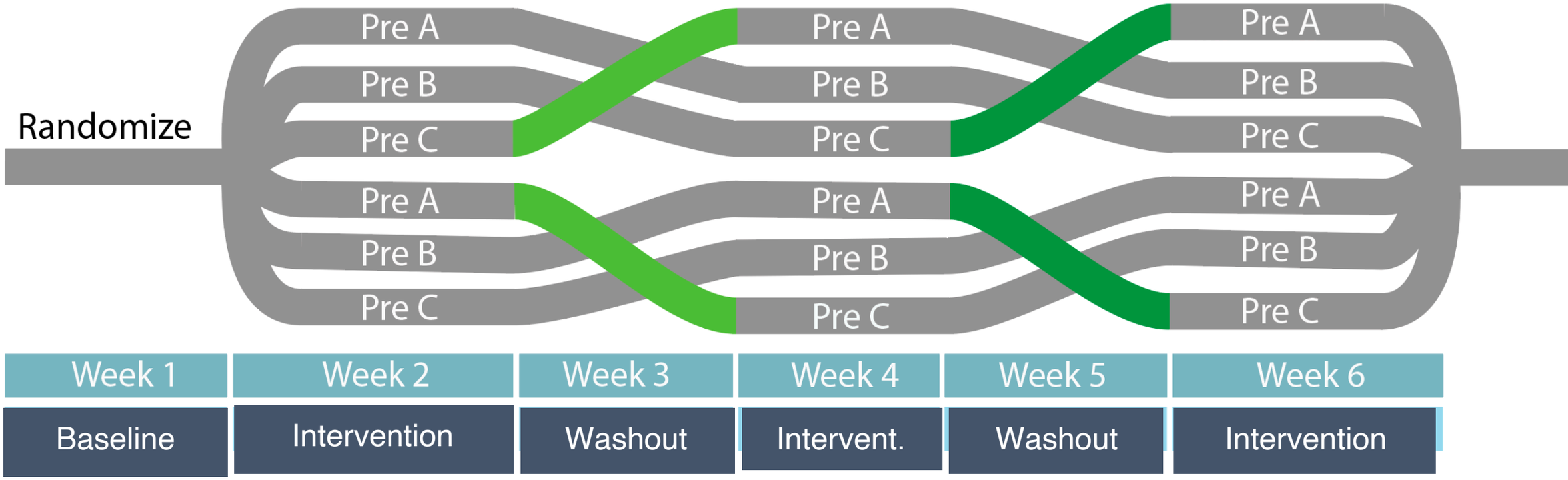
- Test the hypothesis that individuals will vary in prebiotic metabolic potential, ***regardless of prebiotic***
- Requires a ***unique study design*** where all individuals consume the same battery of prebiotics

CHoosing the OptiMal Prebiotic (CHOMP)

Zack Holmes

Heather Durand



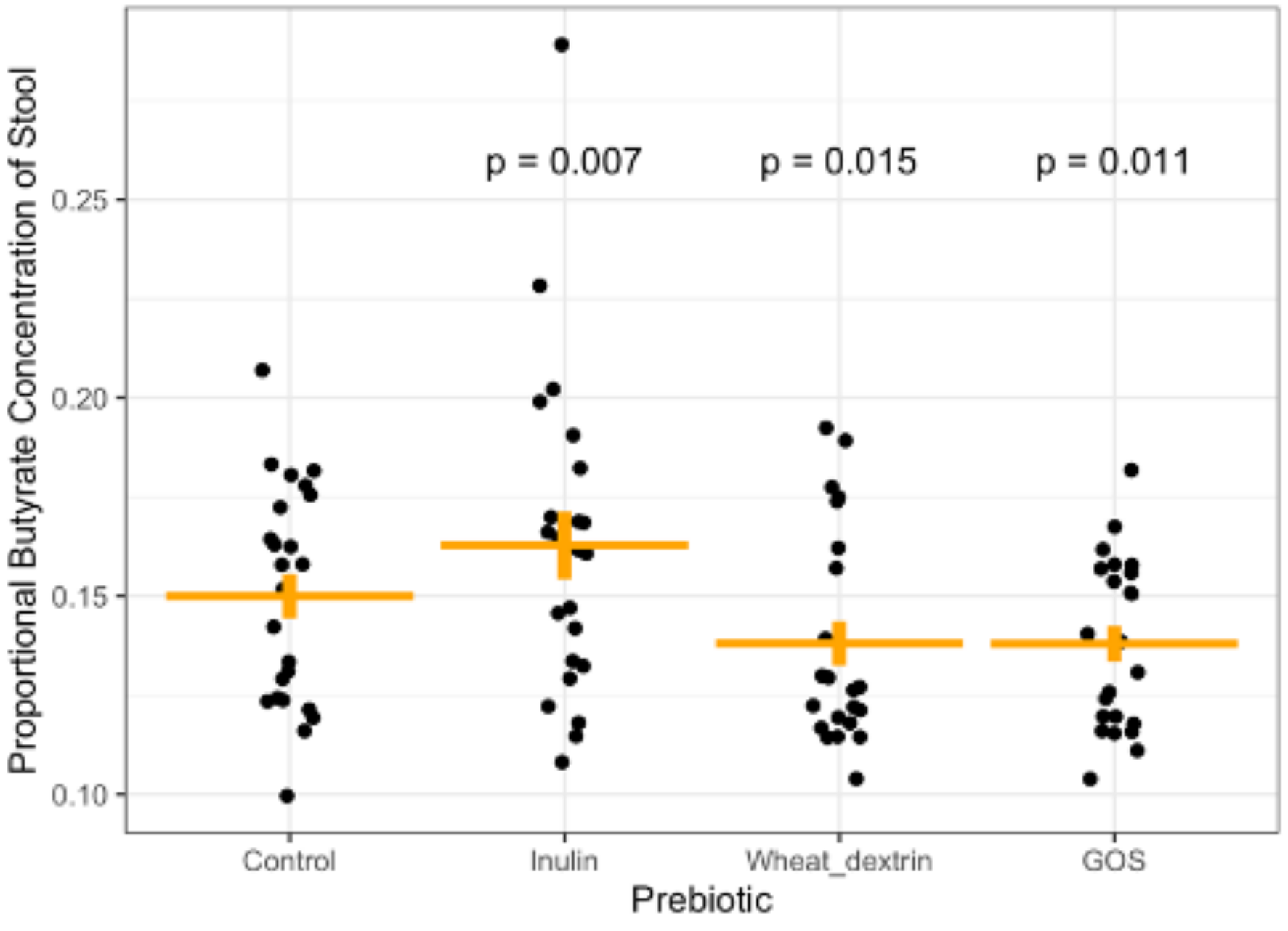
Pre A: Inulin
Pre B: Dextrin
Pre C: Galactooligosaccharides

***n* = 28** healthy volunteers completed

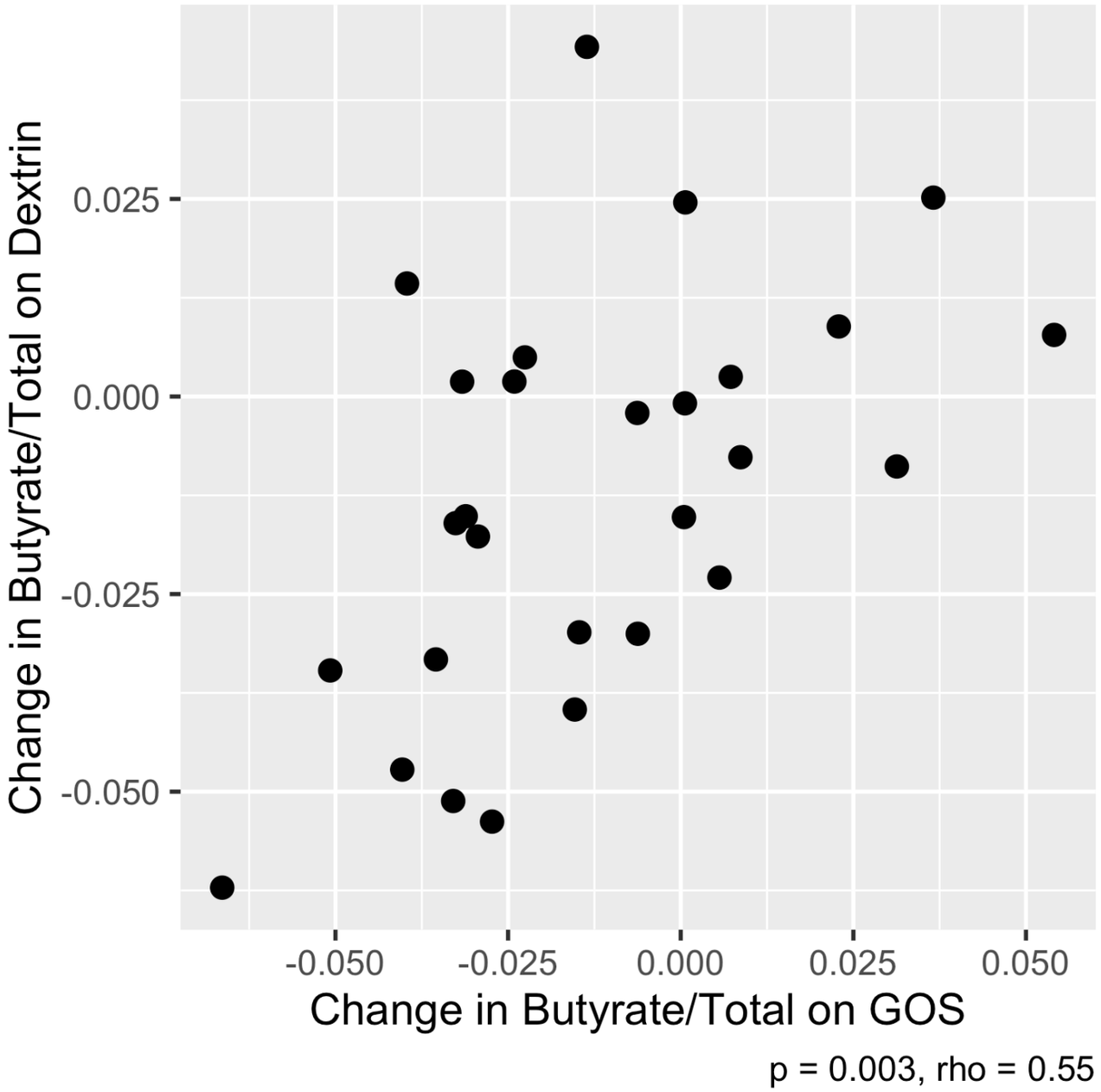
ClinicalTrials.gov ID: NCT03595306

Holmes, et al., *In prep*

AGAIN, SIGNIFICANT VARIATION
BETWEEN PREBIOTICS



IMPORTANTLY, INDIVIDUAL VARIATION CORRELATED ACROSS PREBIOTICS



WHAT DRIVES
INDIVIDUAL VARIATION?

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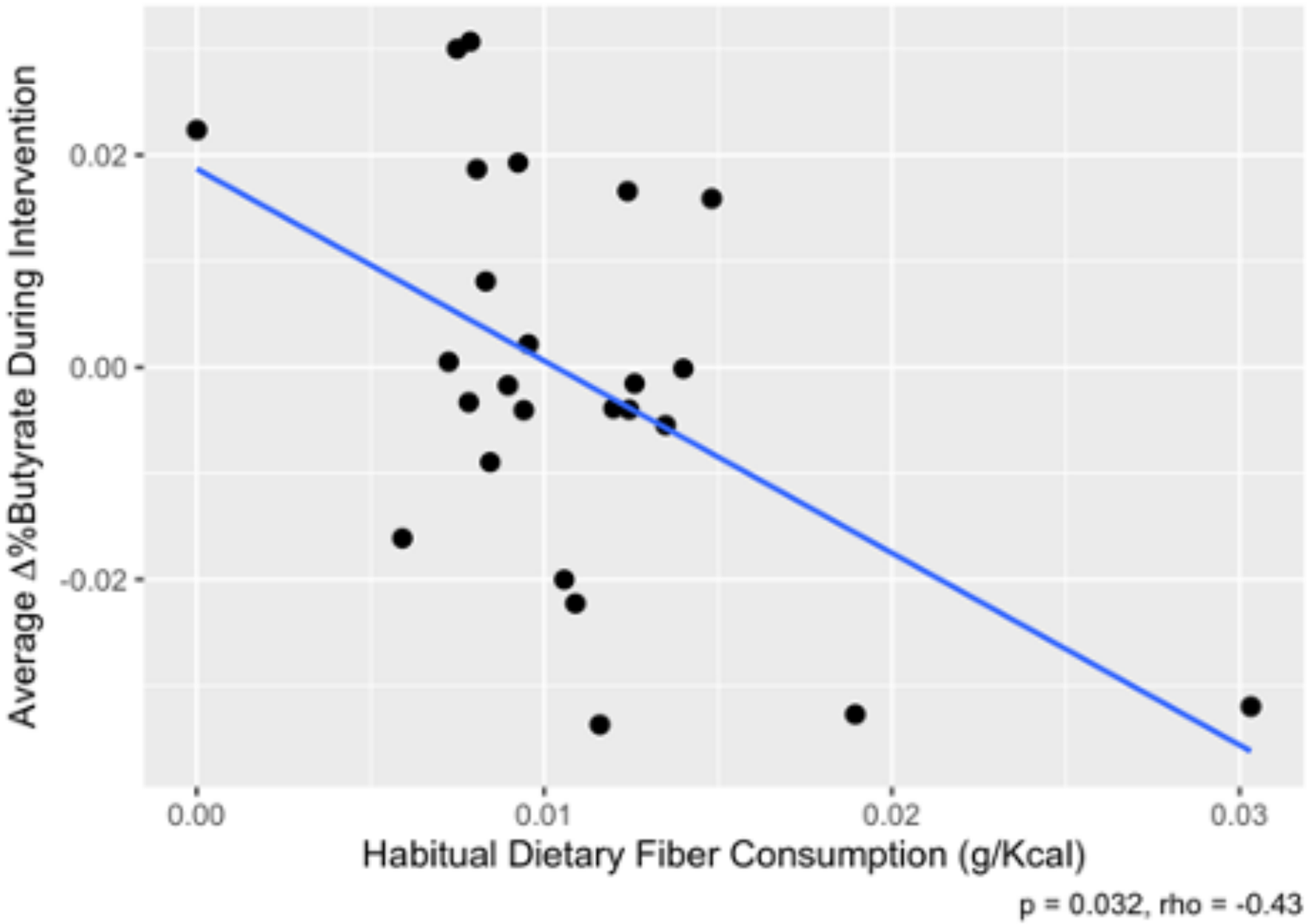
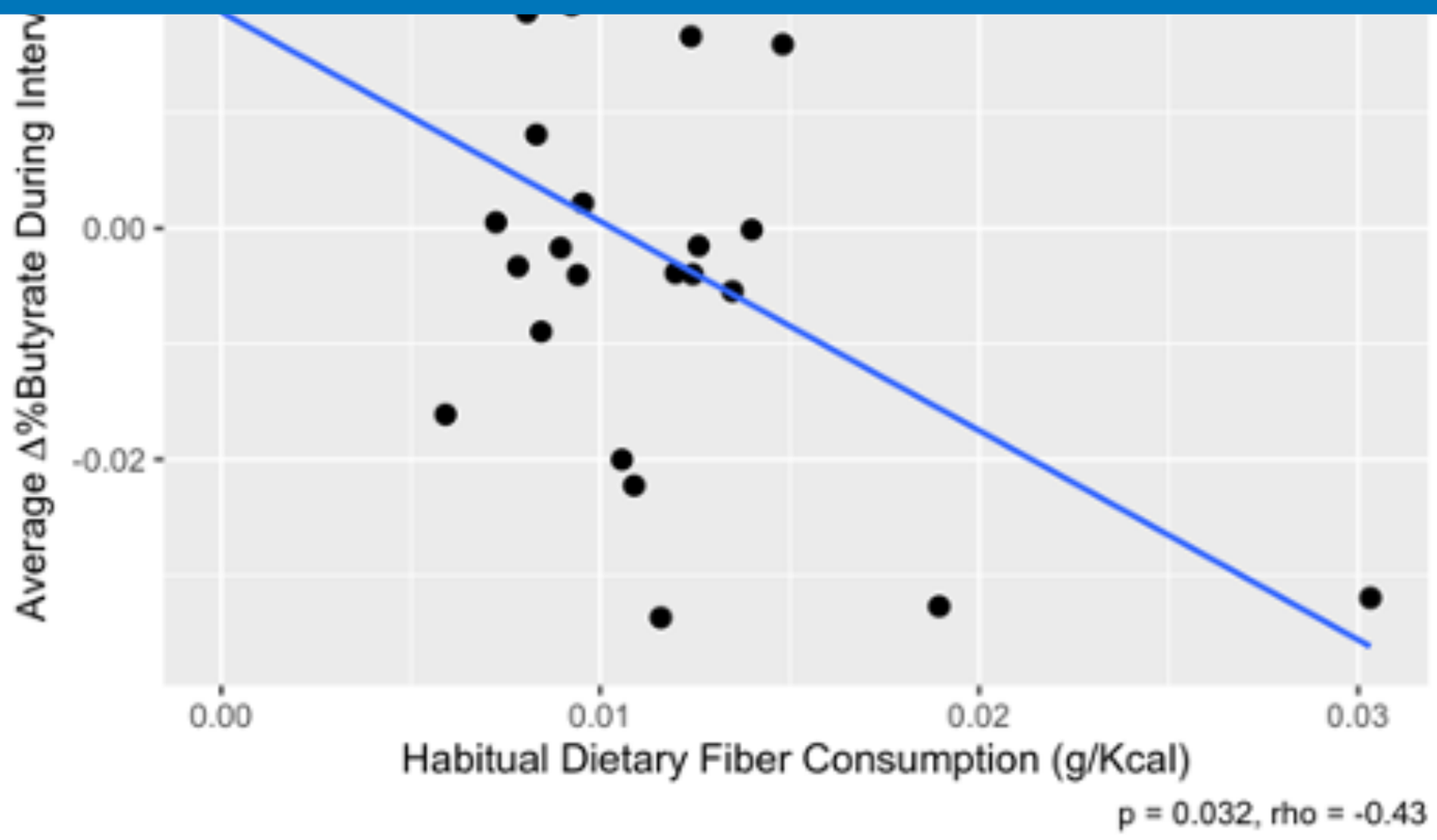


WHAT DRIVES
INDIVIDUAL VARIATION?

Can recent fiber intake affect prebiotic response?



GOALS OF OUR WORK

Answer three questions for astronauts:

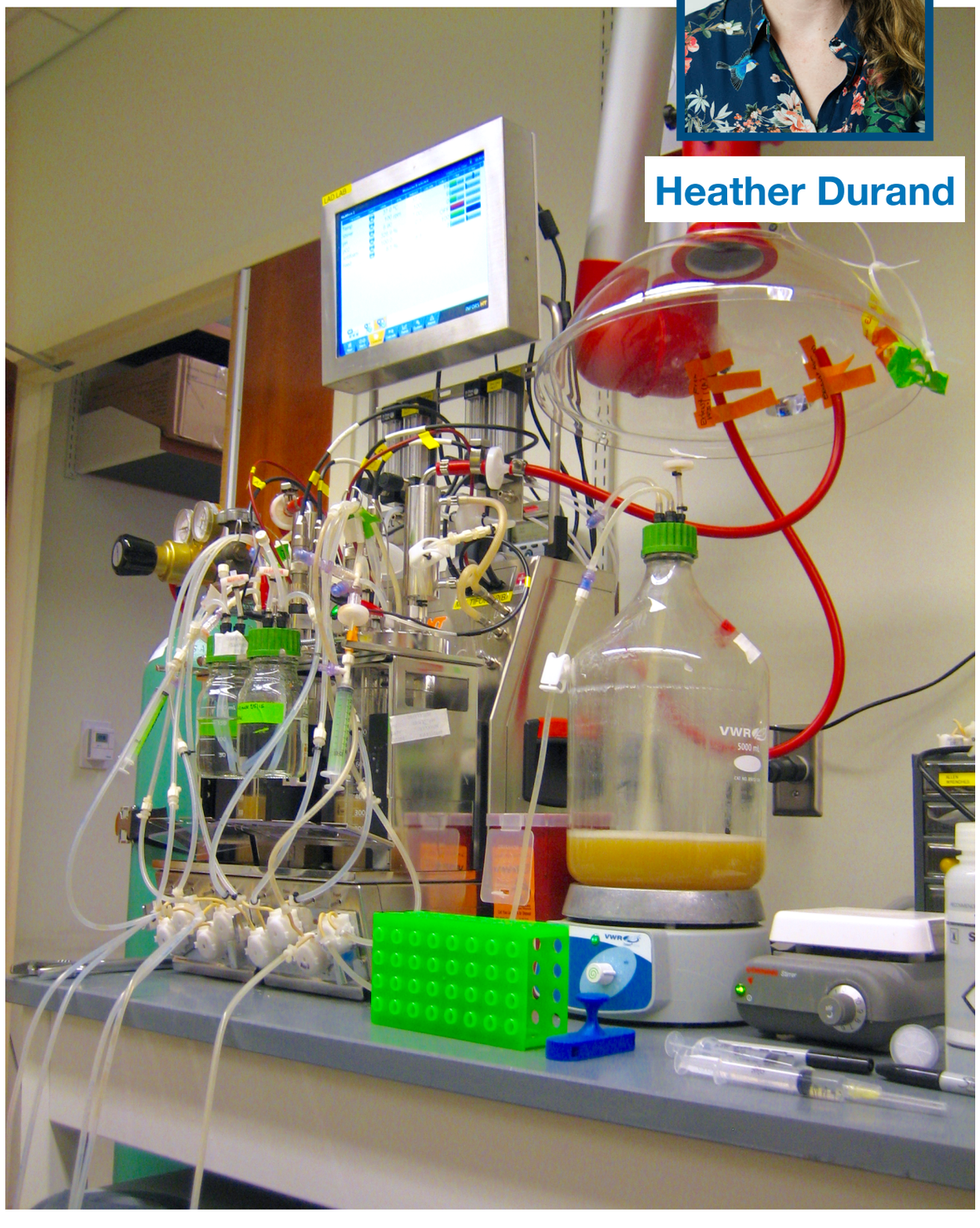
- **Which prebiotics** should they take?
- **Who** should take them?
- And, **when**?

HYPOTHESIS

Exposure to **prior doses** of a prebiotic will affect microbiota response **over time**

TEST USING AN ARTIFICIAL GUT

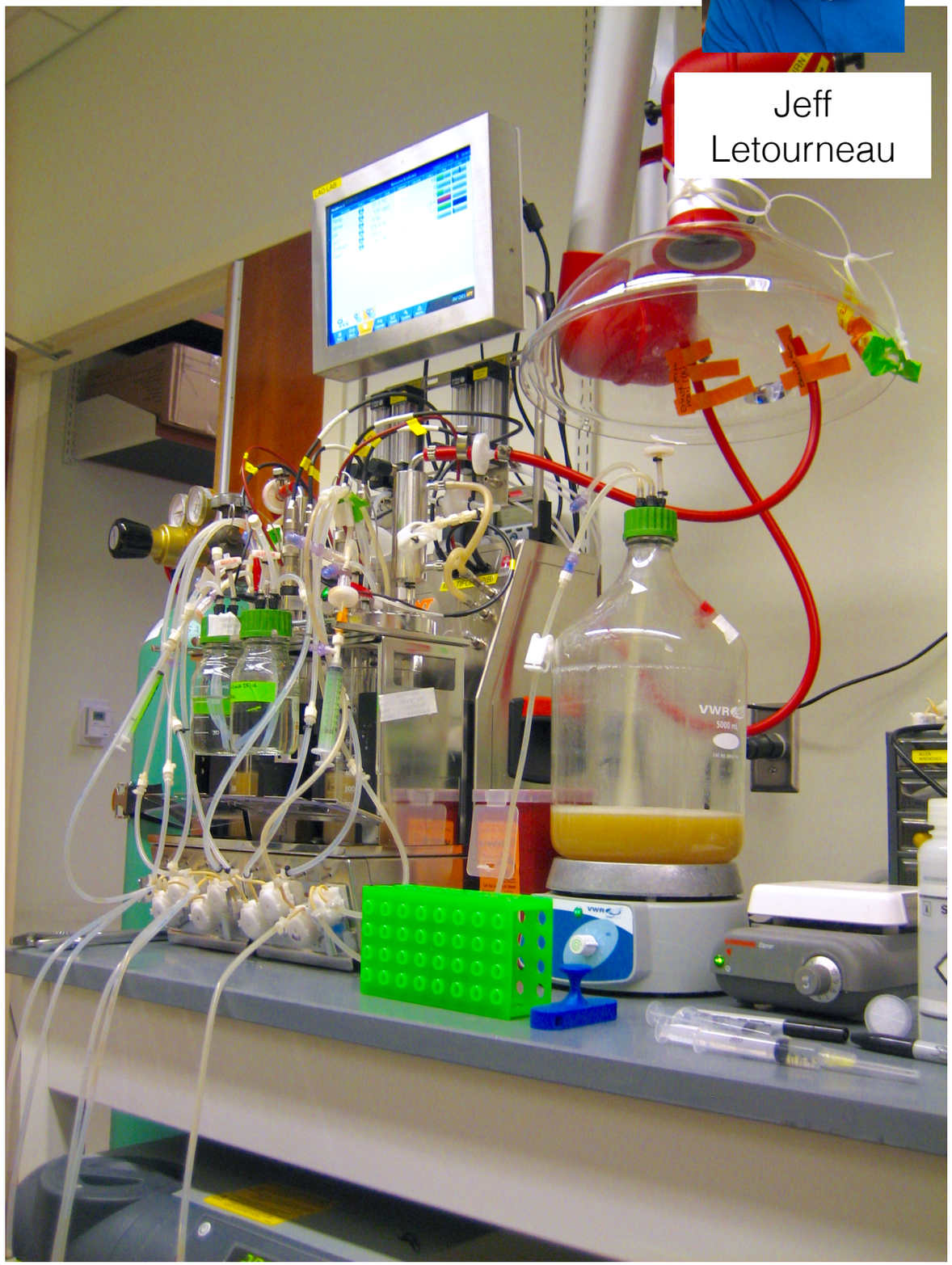
Heather Durand

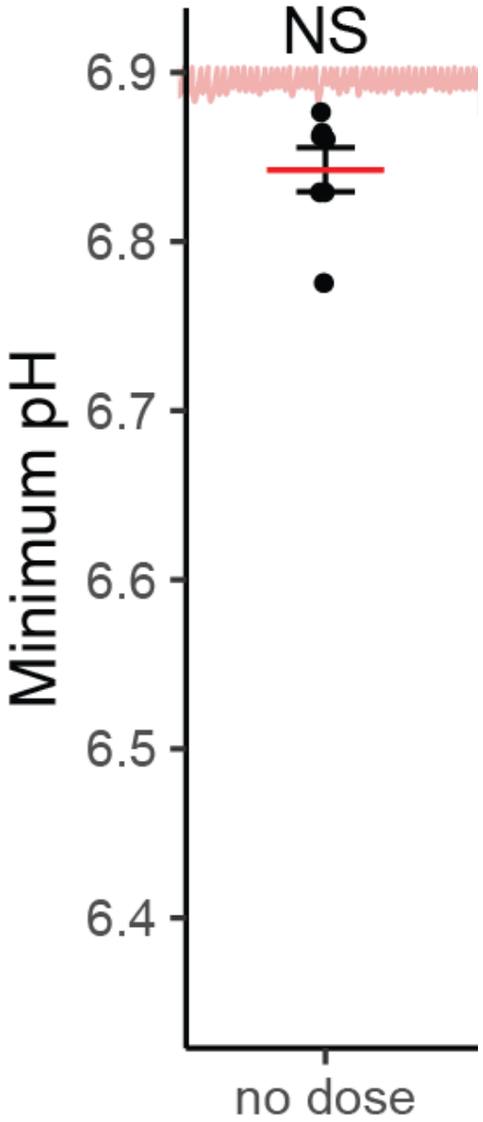


Silverman, Durand, et al, *Microbiome* 2018

TEST USING AN ARTIFICIAL GUT

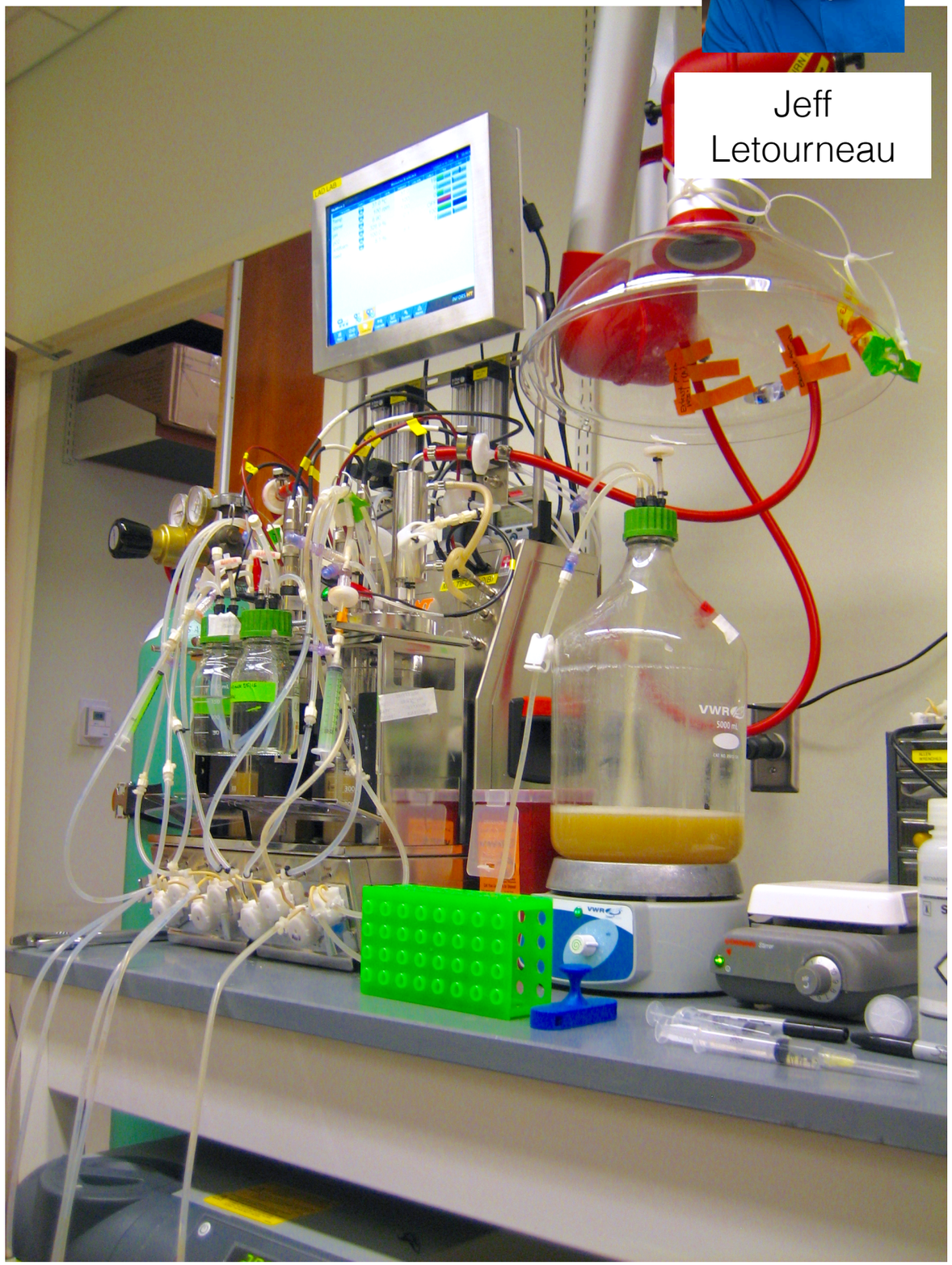
Jeff Letourneau

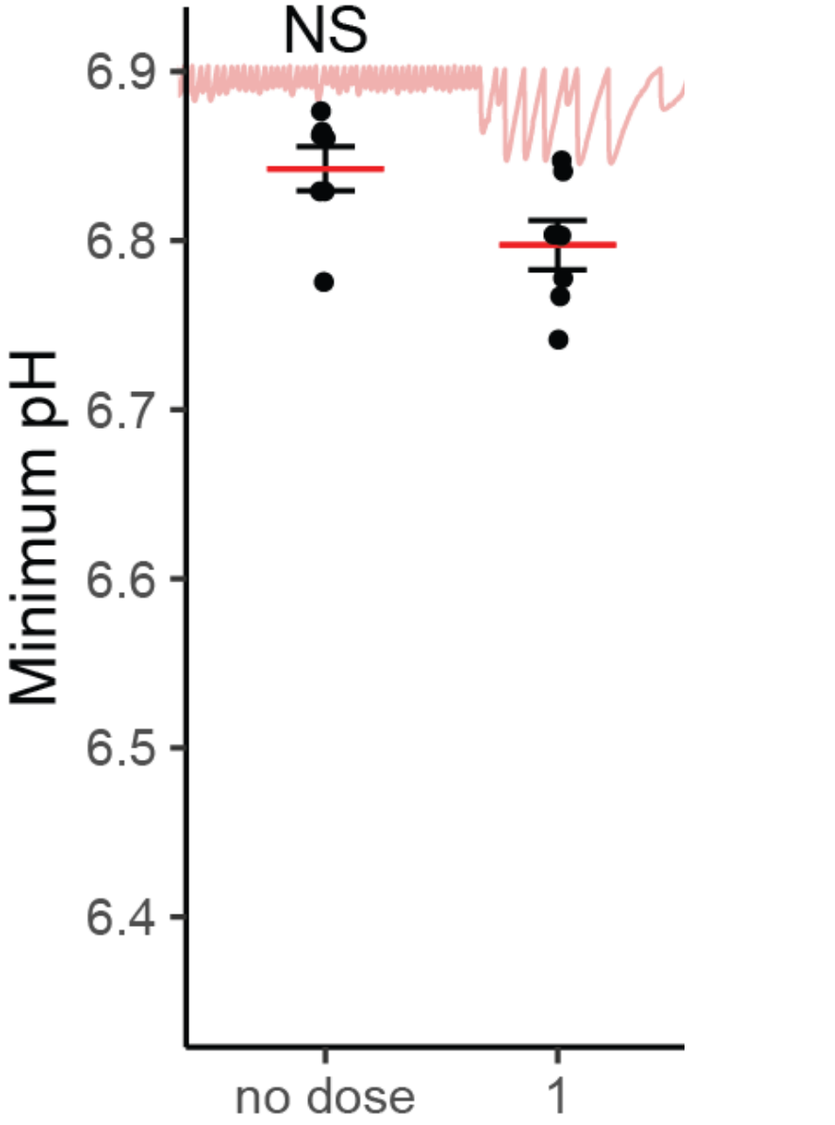




TEST USING AN ARTIFICIAL GUT

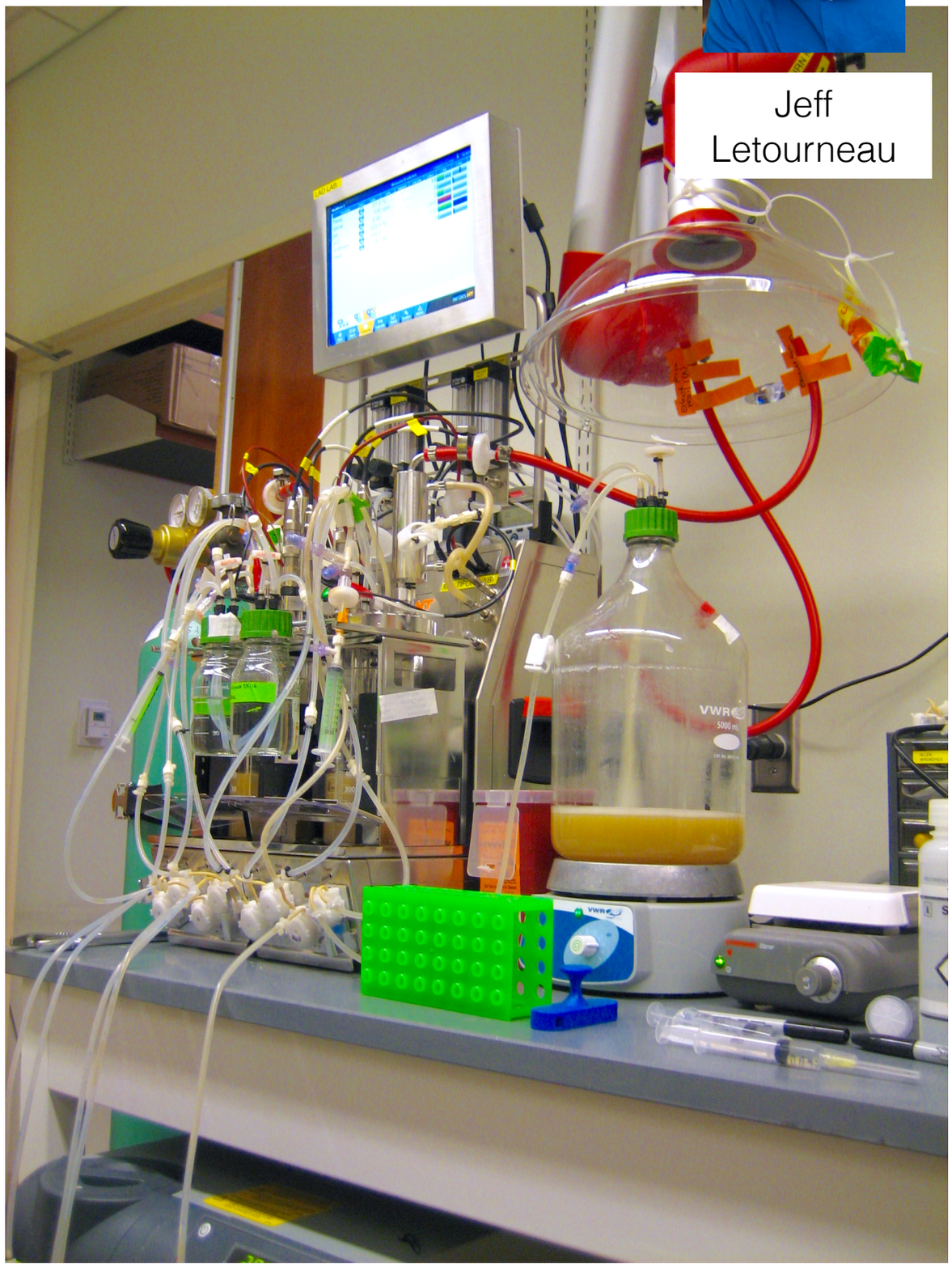
Jeff Letourneau

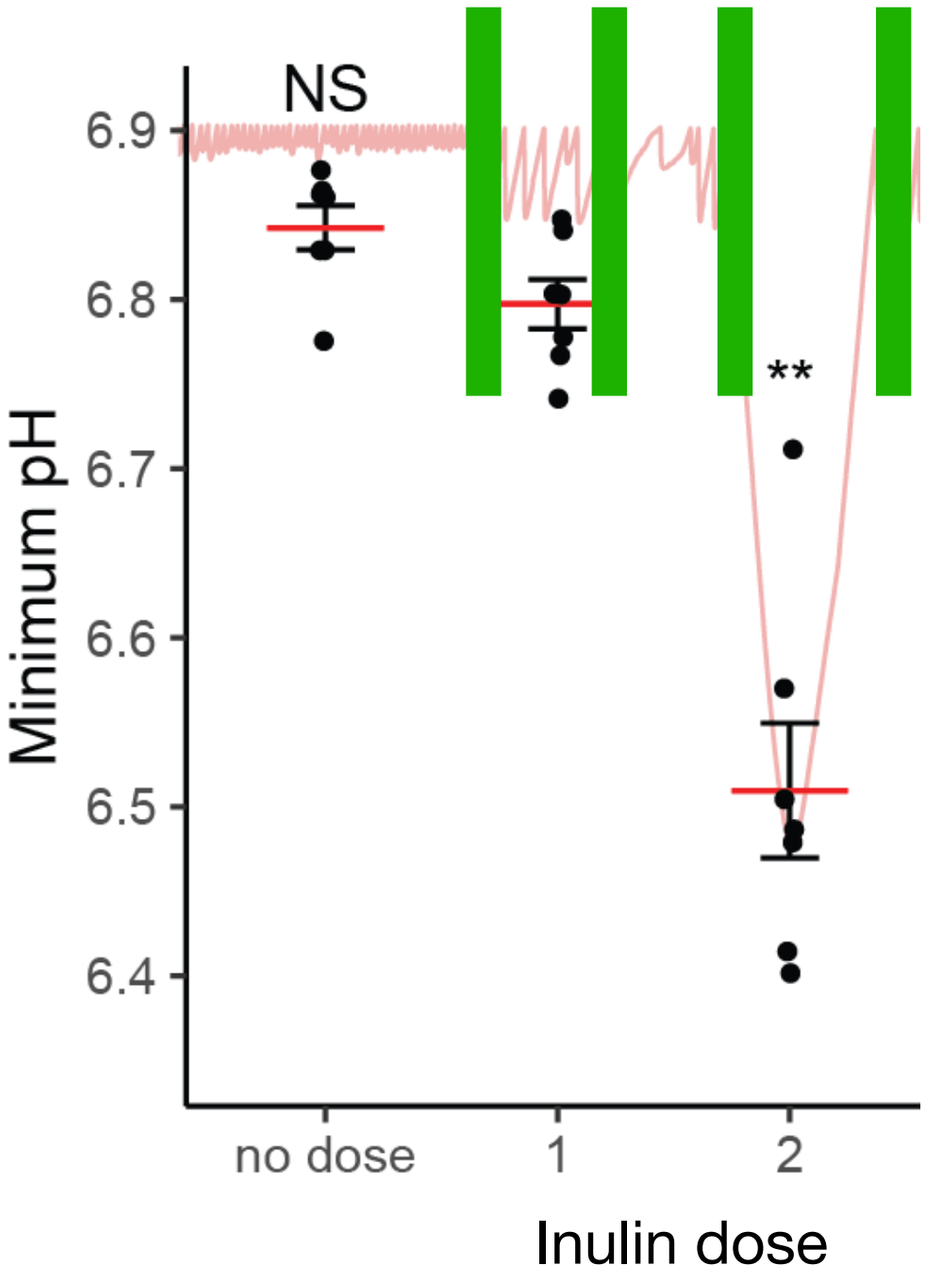




TEST USING AN ARTIFICIAL GUT

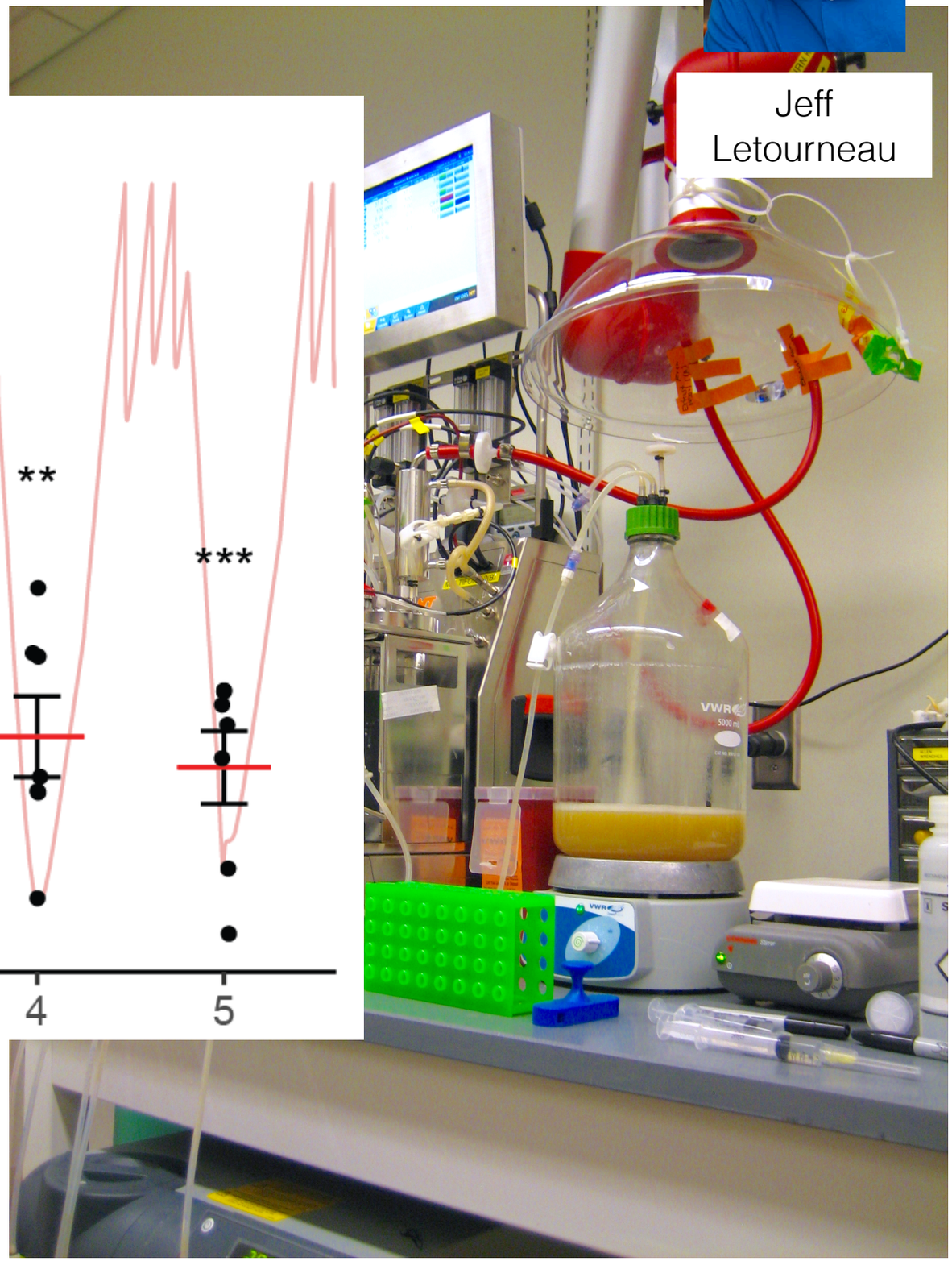
Jeff Letourneau



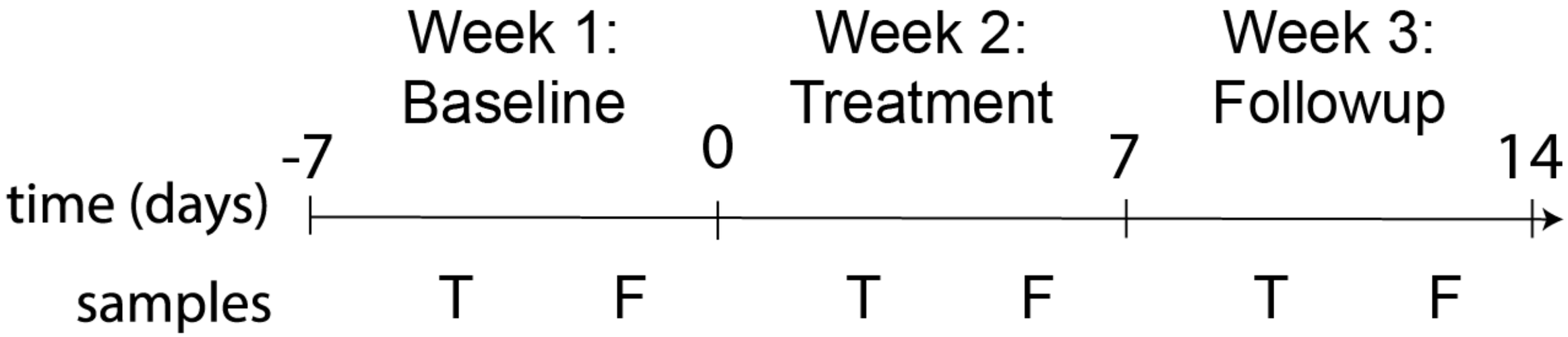


TEST USING AN ARTIFICIAL GUT

Jeff Letourneau



IS A SINGLE DOSE SUFFICIENT IN PEOPLE?



Placebo group (n=8)

▼ ▼ ▼ ▼ ▼

9g maltodextrin
2x/day

Prebiotic group (n=8)

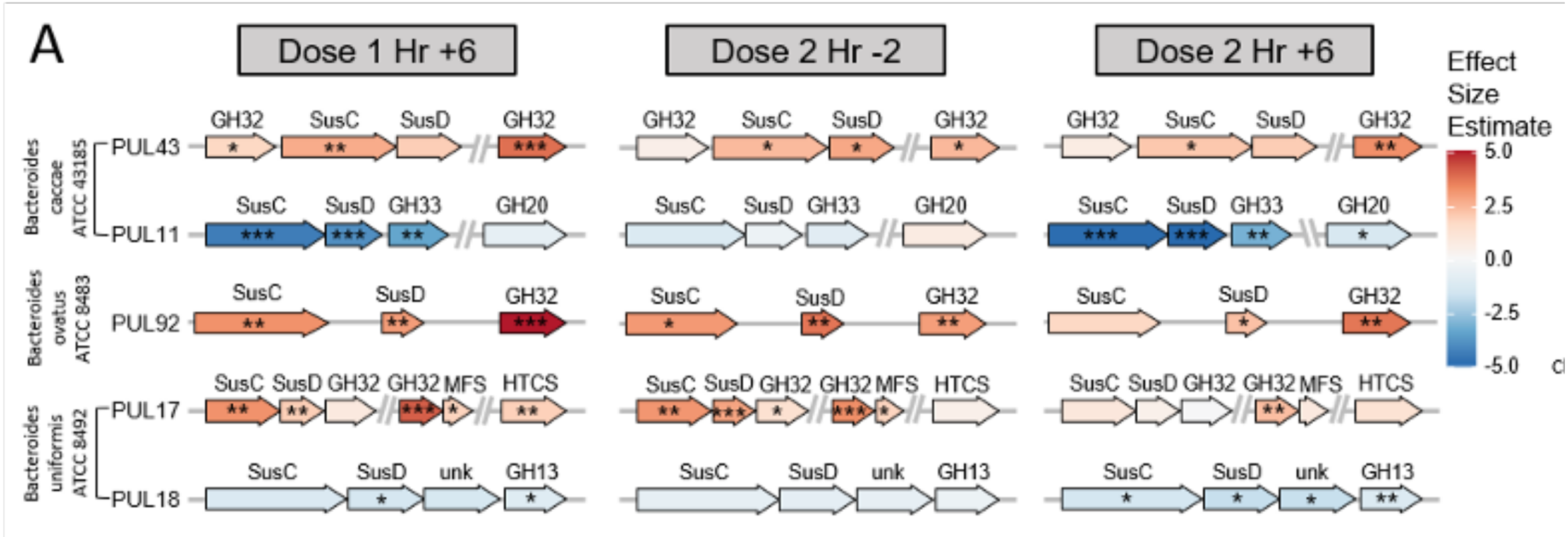
▼ ▼ ▼ ▼ ▼

9g inulin
2x/day

Jeff Letourneau

***n* = 40** healthy volunteers

HOW DOES THIS WORK?



Within 6 hrs of prebiotic exposure, human gut bacteria **activate polysaccharide utilization loci**

SUMMARY

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- **Prebiotics vary** in effect on gut microbiota

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- But, **stronger individual variation** in response

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SUMMARY

- **Prebiotics vary** in effect on gut microbiota
- But, **stronger individual variation** in response
- Variation can be **linked to gut microbial function**, which in turn is **linked to prior diet**
- Even **initial exposure** to prebiotics will **alter response to subsequent doses**

VISION FOR ASTRONAUTS

Three questions:

VISION FOR ASTRONAUTS

Three questions:

1. **Which prebiotics** should they take?

VISION FOR ASTRONAUTS

Three questions:

1. **Which prebiotics** should they take?
    - Might **not matter** too much.

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VISION FOR ASTRONAUTS

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VISION FOR ASTRONAUTS

Three questions:

1. **Which prebiotics** should they take?
    - Might **not matter** too much.
2. **Who** should take them?
    - **Greatest benefit** among those who **normally eat the least fiber**
3. And, **when**?
    - Poor responders may start **shortly before** spaceflight

ONGOING WORK

What are the effects of prebiotics on **cognition** and **mental performance**?

Ken Racicot

Mathias Basner

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