

Methods in Microbiome Science: Part I

Ivan VC

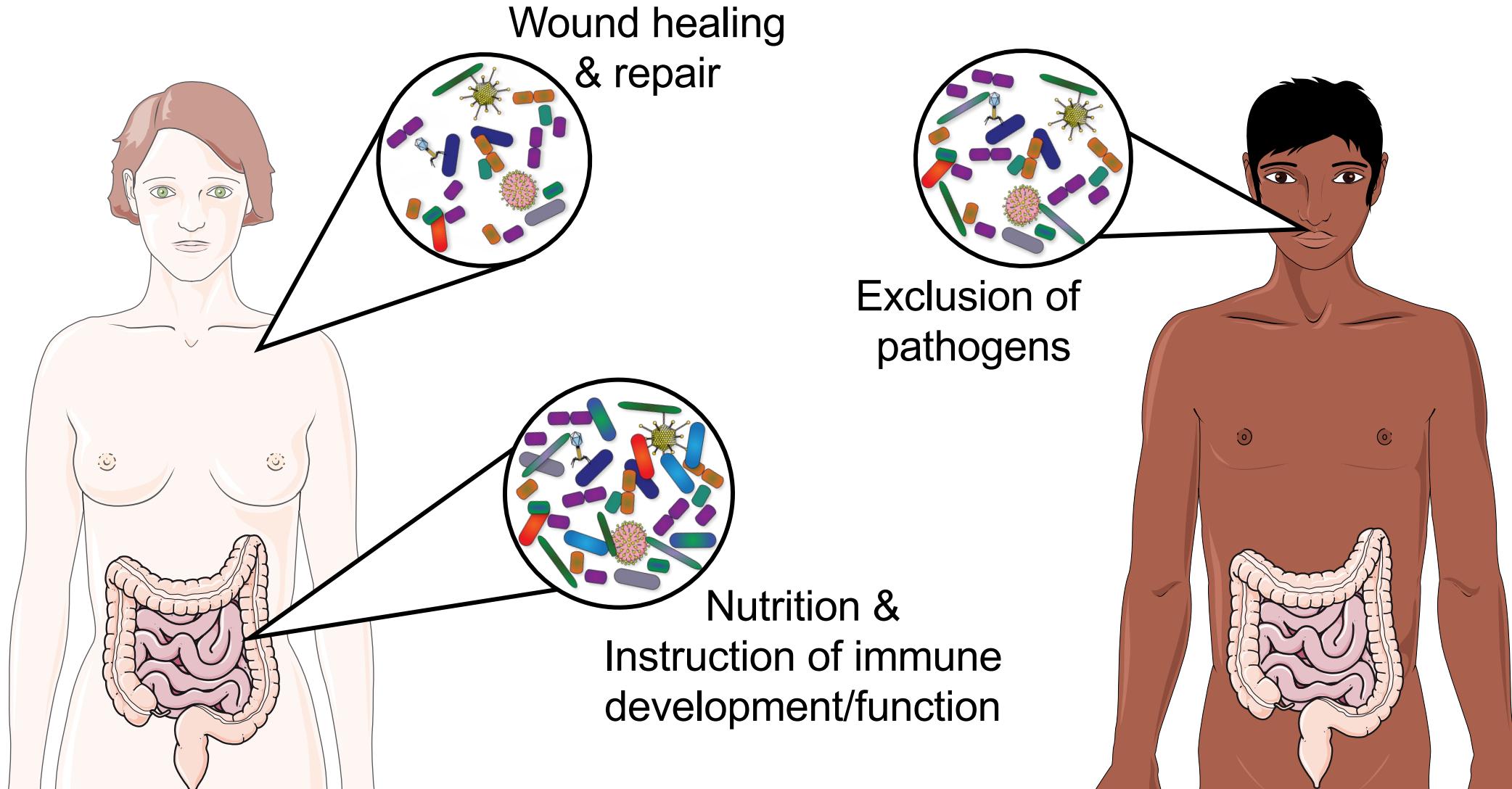
Assistant Professor

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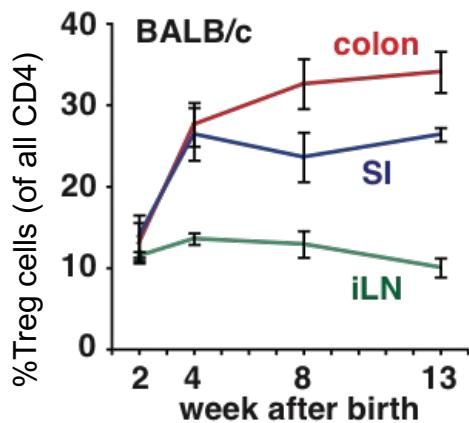
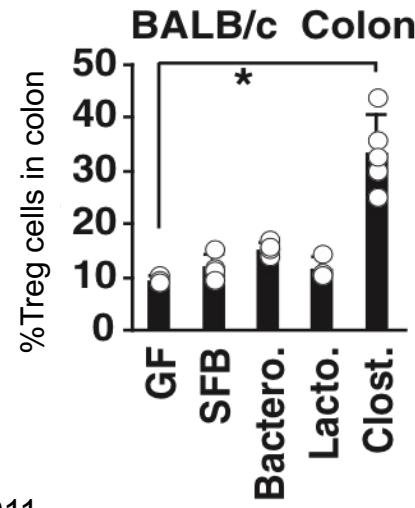
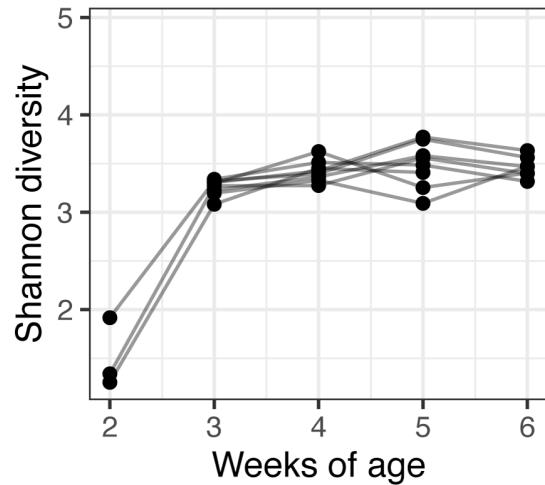
Department of Biomedical Sciences

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The host-associated microbiota is a critical organ with numerous physiological functions

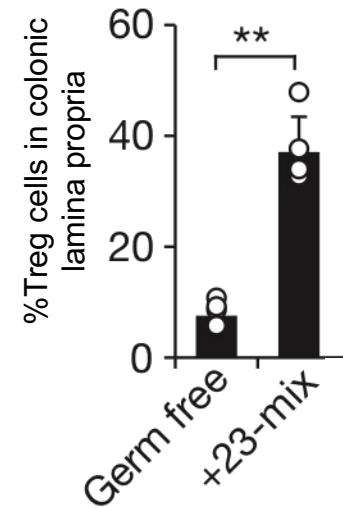


Murine microbiota development induces differentiation of key immune cells



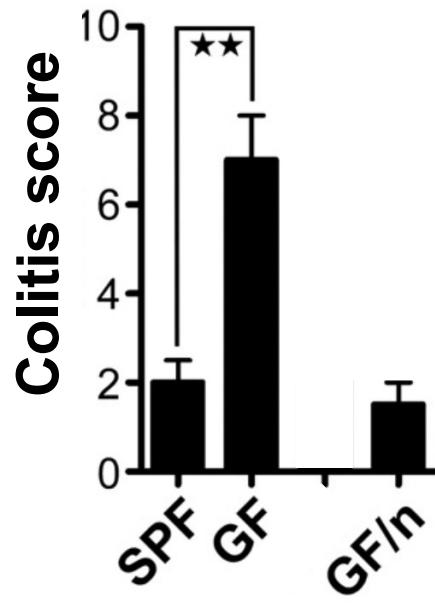
Atarashi et al. *Science* 2011

- T_{reg} :
 - immune cells that dampen immune activity
 - implicated in numerous autoimmune diseases
- Murine *Clostridia* mixture induces T regulatory cells (T_{reg})
- Human-derived “23-mix” of *Clostridia* induces T_{reg}



Atarashi et al. *Nature* 2013

Improper microbiota development contributes to immune dysfunction

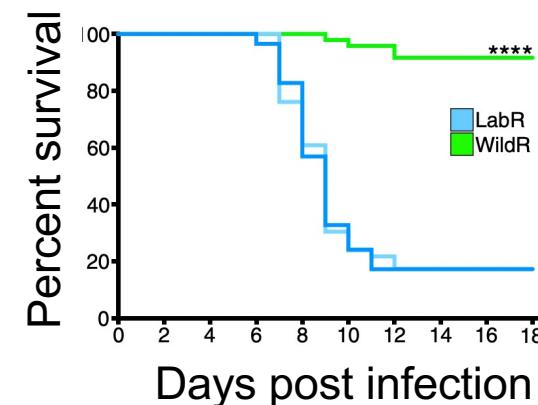
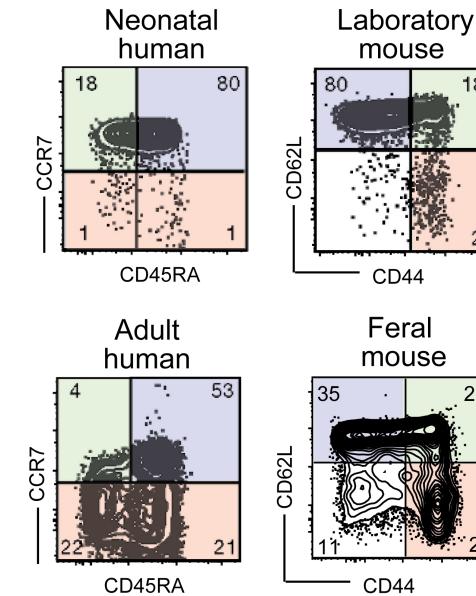


GF/n =
Microbiota
introduced at
1st day of life

GF/a =
Microbiota
introduced at
5th week of life

Beura et al.
Nature 2016

Rosshart et al.
Cell 2017



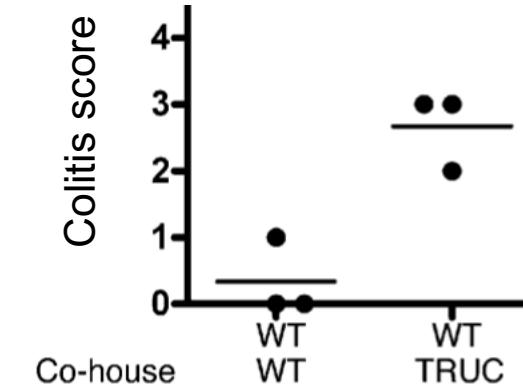
- Lab mice, with limited microbiota diversity, have stunted immune development...

...which impairs survival to immunological challenges (e.g. flu)

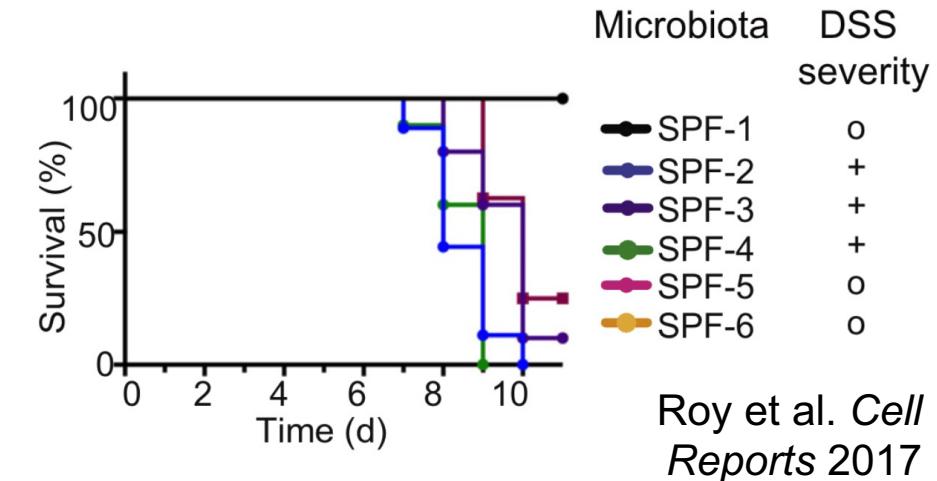
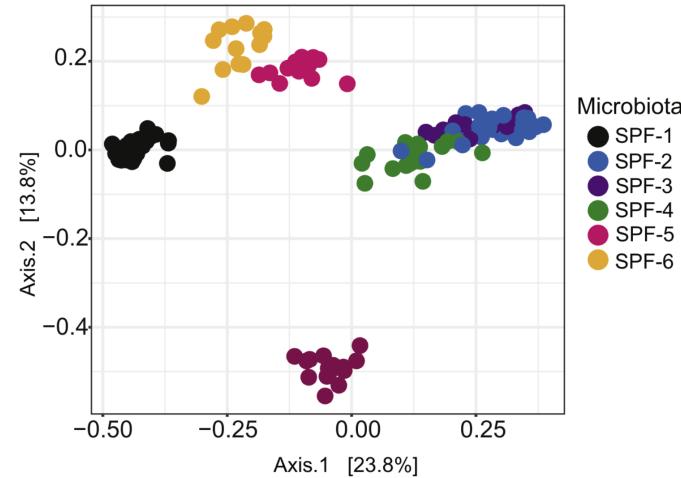
Aberrant gut microbial communities can stimulate inflammatory disease

- Genetic deficiency in immune genes can give rise to aberrant microbiota
 - which is sufficient to cause colitis in wildtype mice

Garrett et al.
Cell 2007



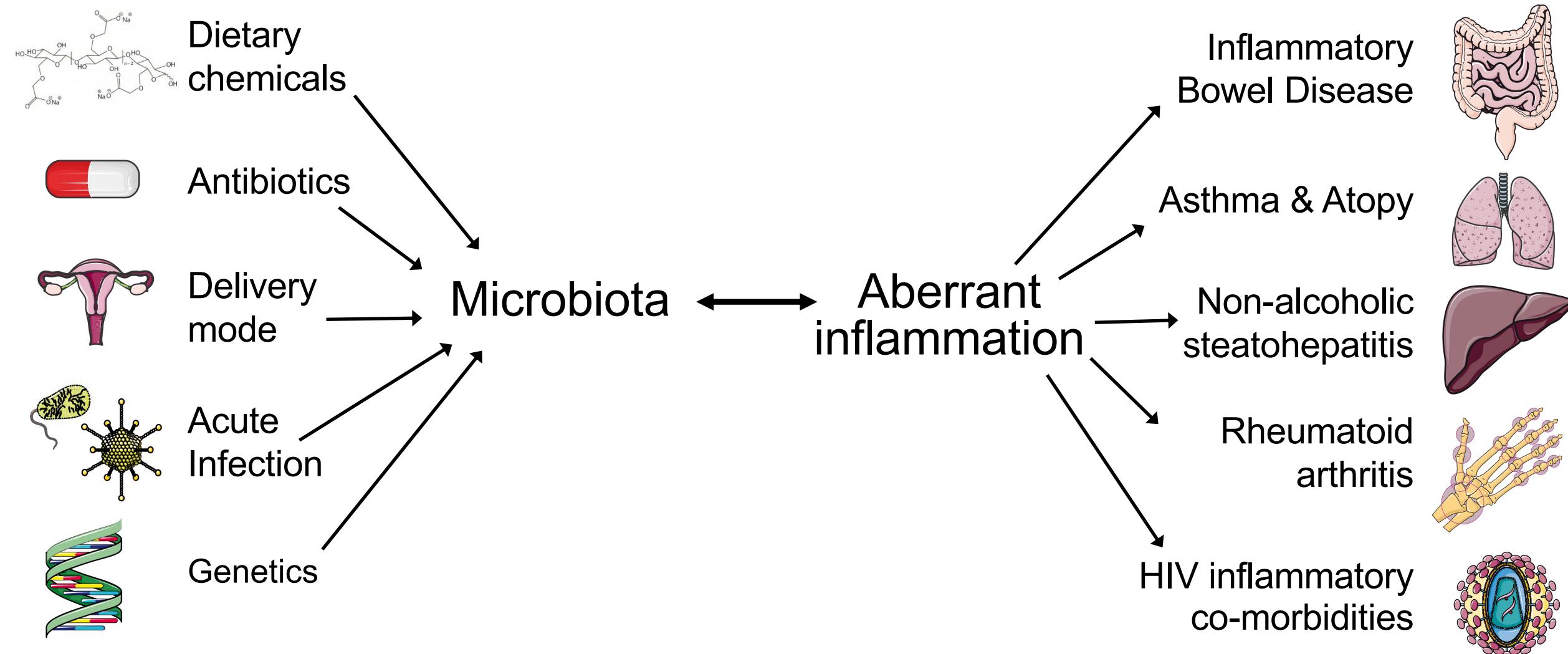
- C57BL/6 mice from different vendors have different microbiota compositions
 - which drastically alter colitis severity



Roy et al. *Cell Reports* 2017

- Similar observations in mouse models of cancer, liver disease, autoimmune disease, you name it: different commensal communities impact phenotypes (in mice)

Alterations in the human gut microbiota



Hygiene hypothesis: “the sterilization of our environment contributes to inflammatory diseases”

Proc. roy. Soc. Med. Volume 63 January 1970

Dr B M Greenwood and Dr E M Herrick

*(MRC Rheumatism Research Unit,
Canadian Red Cross Hospital,
Taplow, Berkshire)*

and Dr A Voller

*(Nuffield Department of Comparative Medicine,
Zoological Society of London)*

**Can Parasitic Infection Suppress
Autoimmune Disease?**

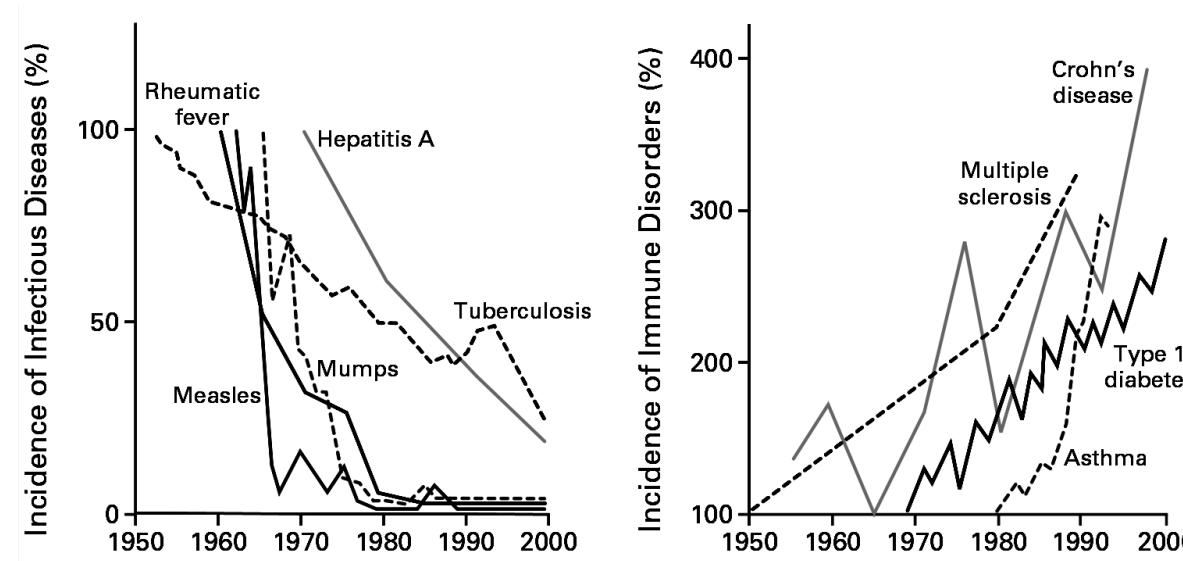
led one of us to suggest that the host response to multiple parasitic infections might in some way interfere with the abnormal immunological processes involved in production of diseases of the autoimmune group (Greenwood 1968). We are

Hygiene hypothesis:

“the sterilization of our environment contributes to inflammatory diseases”

- Elimination of microbial pathogens

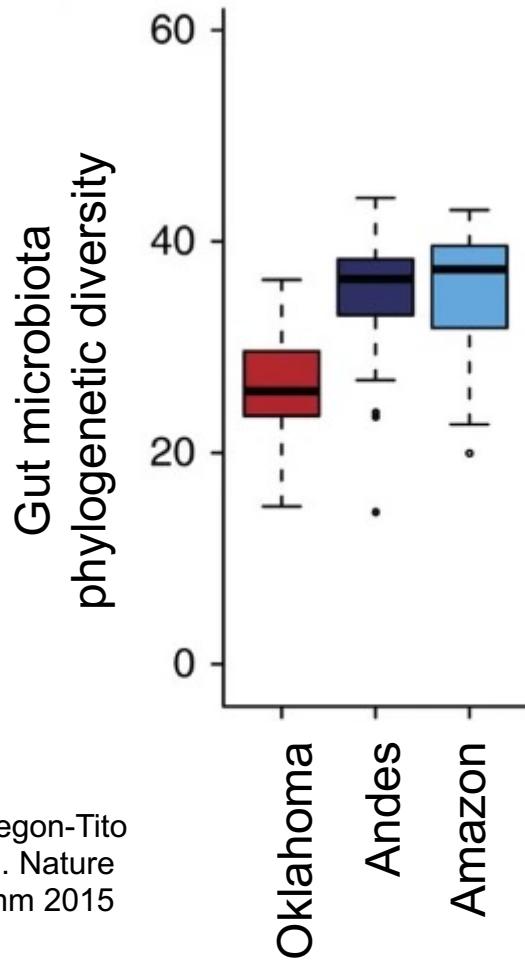
Bach et al.
NEJM 2002



- Average person in industrialized world is exposed to ~600 doses of antibiotics over lifetime

Laxminarayan et al. Science 2016

Industrialized populations are missing microbes



Hygiene hypothesis:

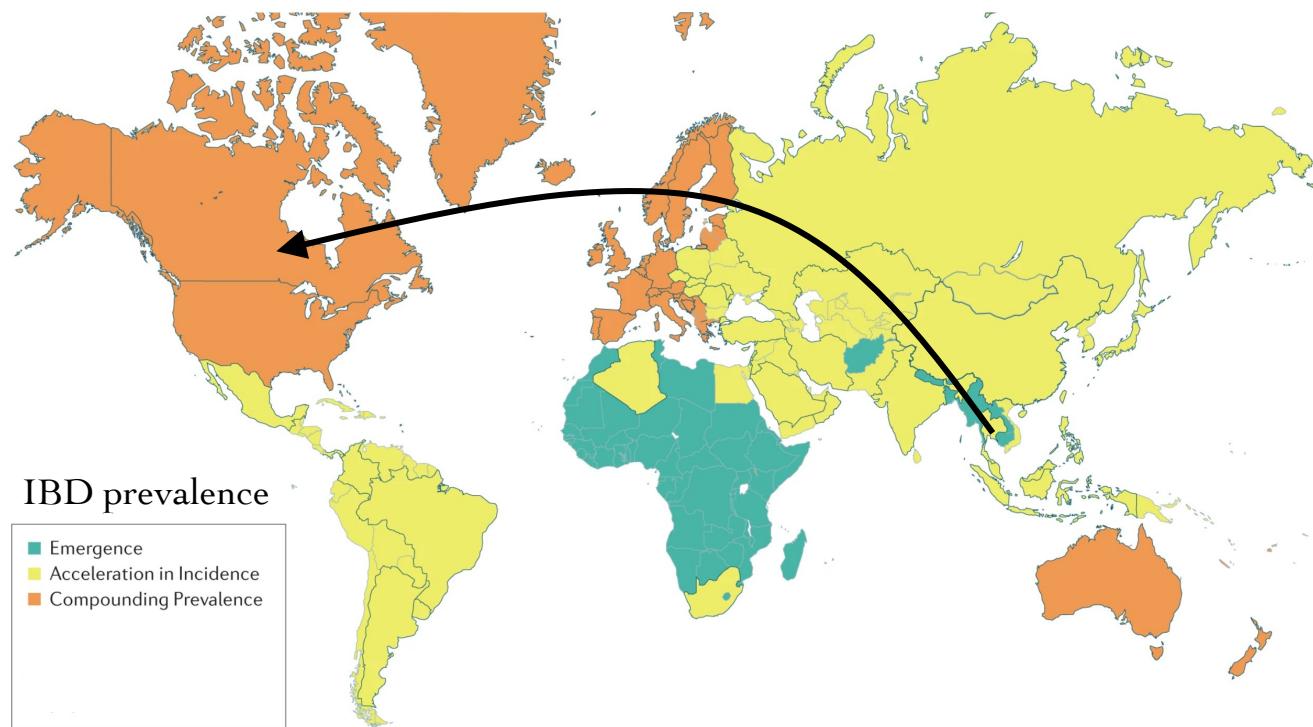
“the sterilization of our environment contributes to inflammatory diseases”

- Correlates of protection from autoinflammatory disorders:
 - Exposure to farm animals (atopy)
 - Day care (atopy)
 - Older siblings (atopy, MS, T1D)
 - Helminth exposure (atopy, IBD)
- Correlate of susceptibility to autoinflammatory disorders:
 - Antibiotic use in early childhood (atopy/asthma, IBD)

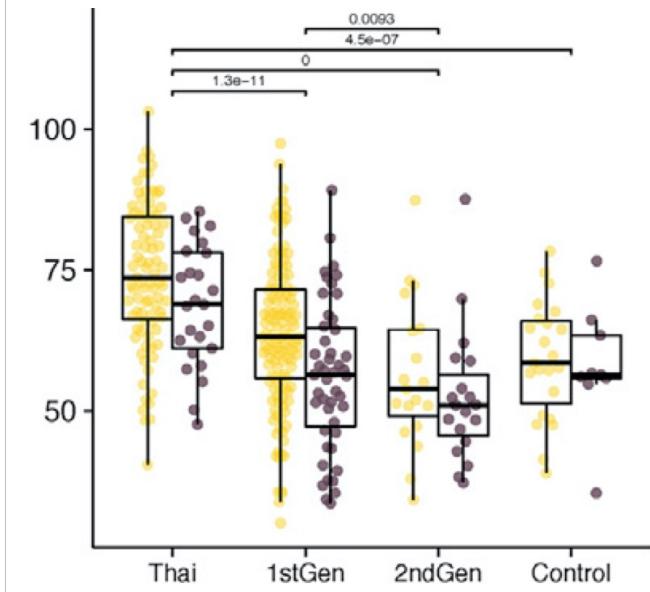
Hygiene hypothesis:

“the sterilization of our environment contributes to inflammatory diseases”

- Migration from low-incidence countries to industrialized countries confers inflammatory bowel disease risk in first and second generation



Faith's Phylogenetic Diversity,
gut microbiota

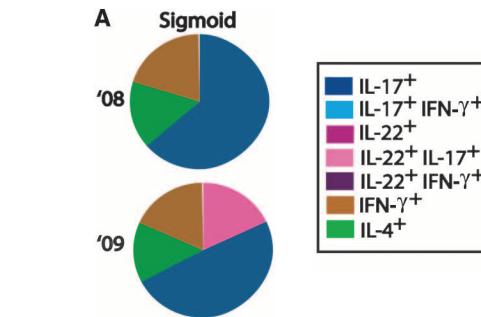
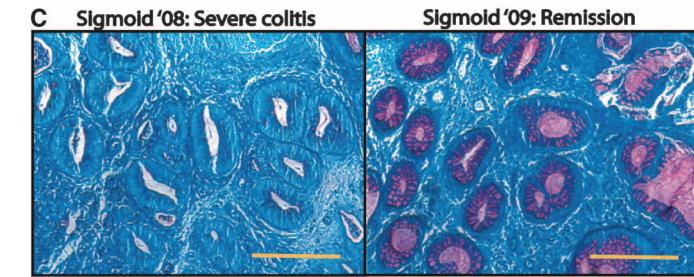
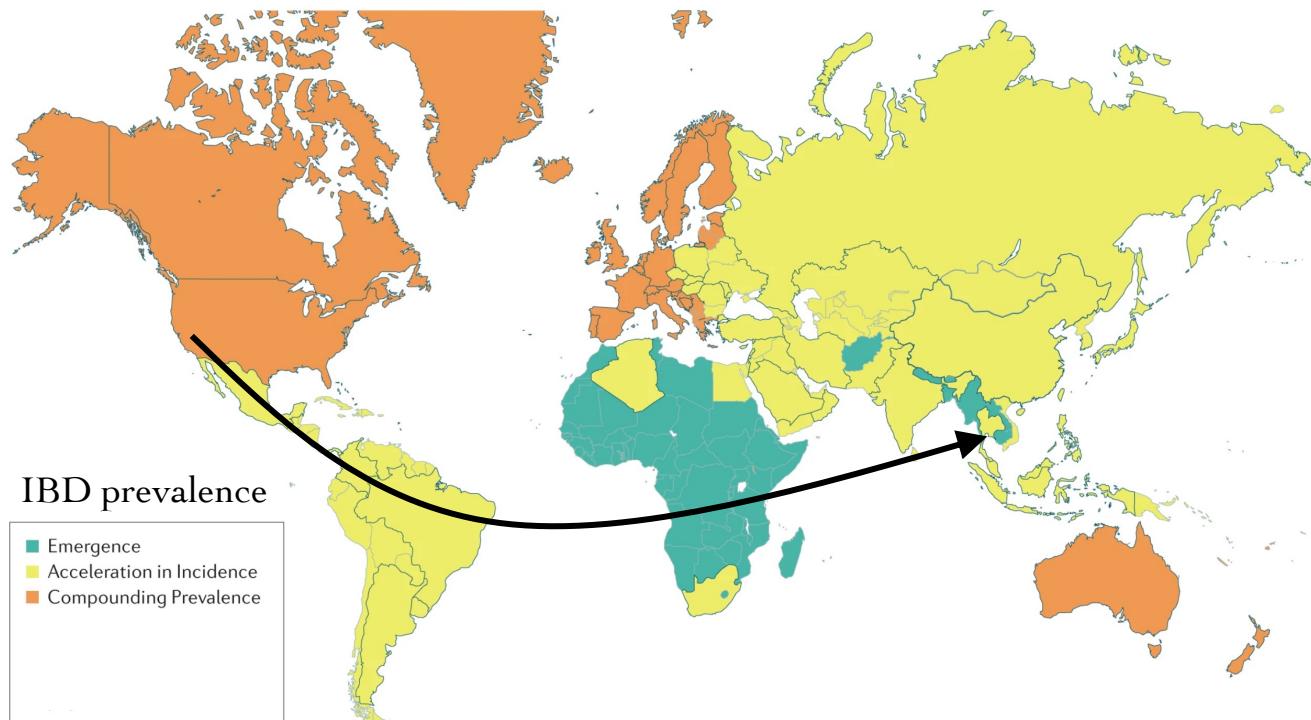


Vangay et al. Cell 2018

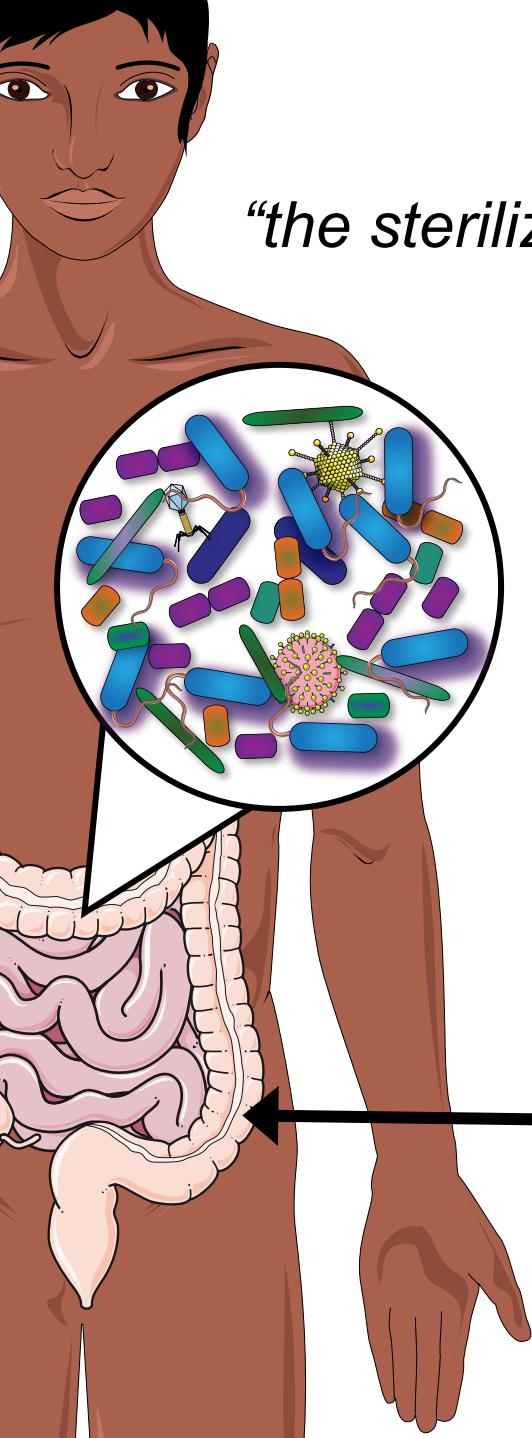
Hygiene hypothesis:

“the sterilization of our environment contributes to inflammatory diseases”

- Severe IBD patient chose to self-inoculate foreign fecal microbiota instead of colectomy, went into remission



Broadhurst et al. Sci Trans Med 2010



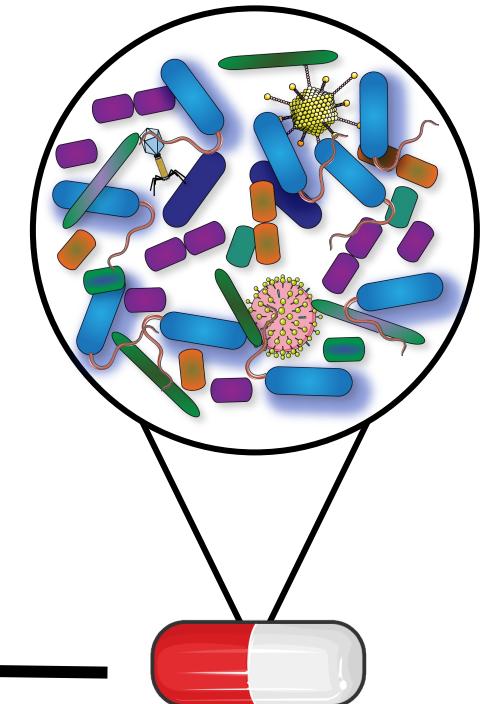
Hygiene hypothesis:

“the sterilization of our environment contributes to inflammatory diseases”

- Fecal microbiota transplant

- Cancer immunotherapy (Davar et al. Science 2021)
- Inflammatory bowel disease (Moayyedi et al. 2015, Rossen et al. 2015, Paramsothy et al. 2017, Costello et al. 2019)
- Autism spectrum disorder (Kang et al. Microbiome 2017)
- Recurrent Clostridioides difficile infection (van Nood NEJM 2013)
- Atopic dermatitis (Mashiah et al. Immun Inflamm Dis. 2022)

Donor fecal
microbiome



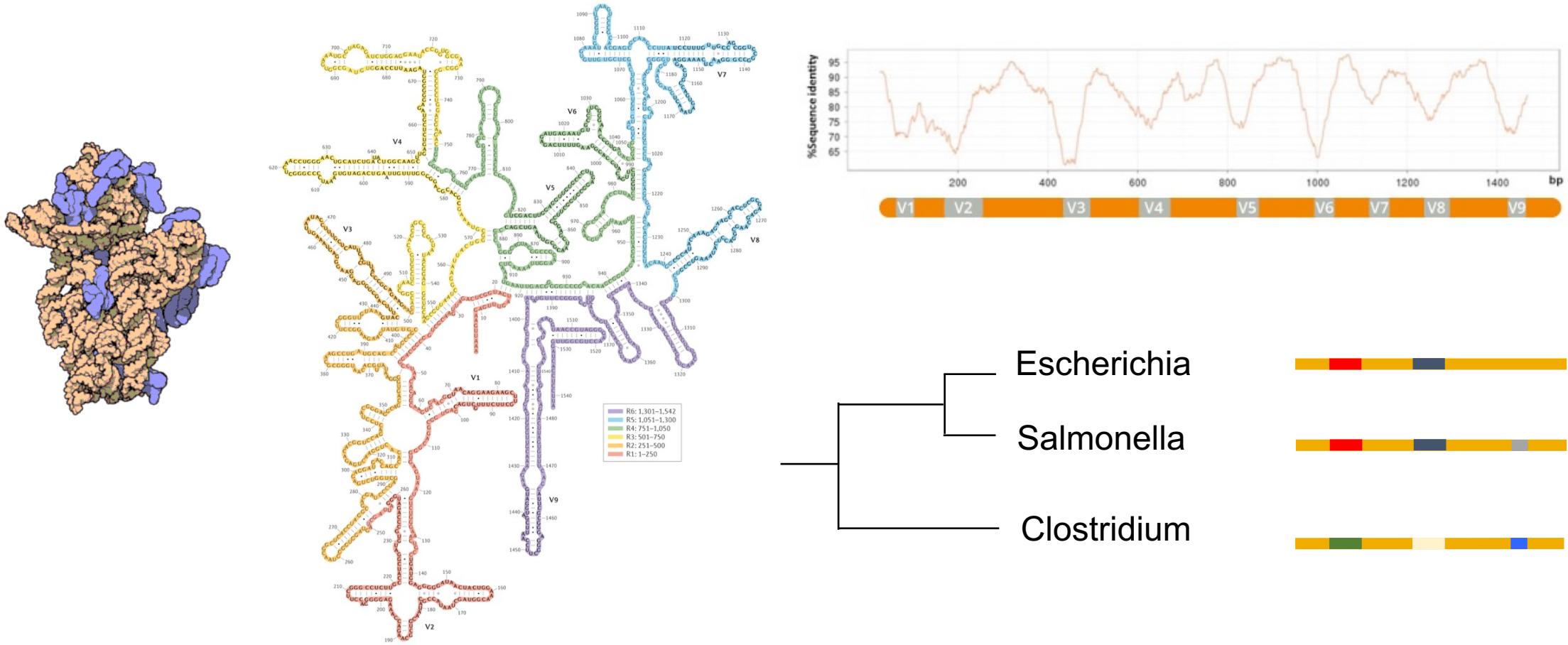
Tools of the trade

- “Dark Ages”: culture-dependent microbiota profiling
 - Estimates for cultivable species:
 - human gut microbiota: 50-60%
 - mouse gut microbiota: 20%
 - Low throughput



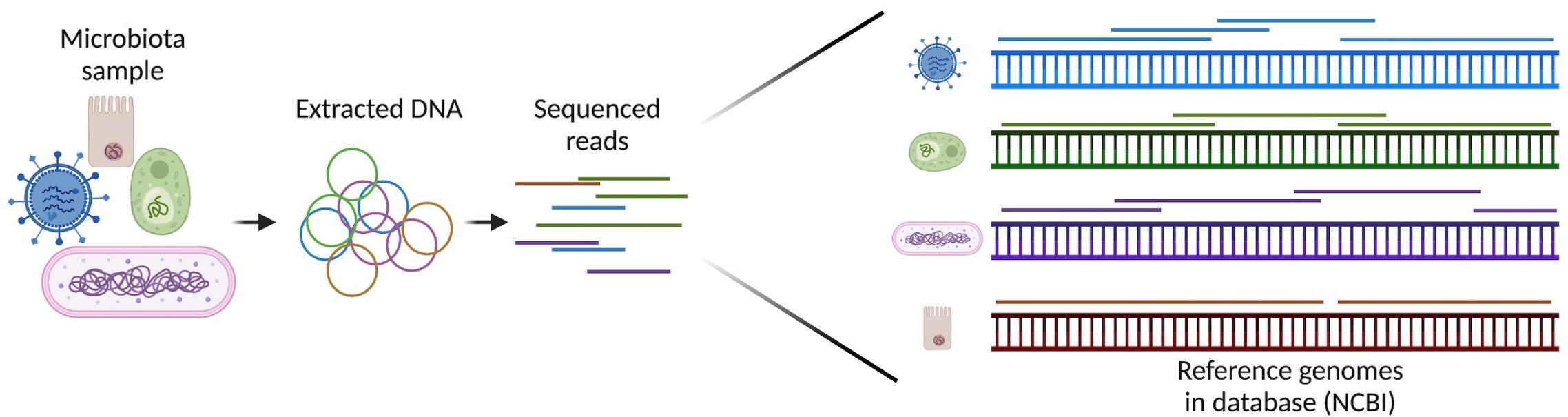
Tools of the trade

- “Age of Enlightenment”: Genetic profiling of the microbiota
 - Bacterial 16S ribosomal RNA sequencing



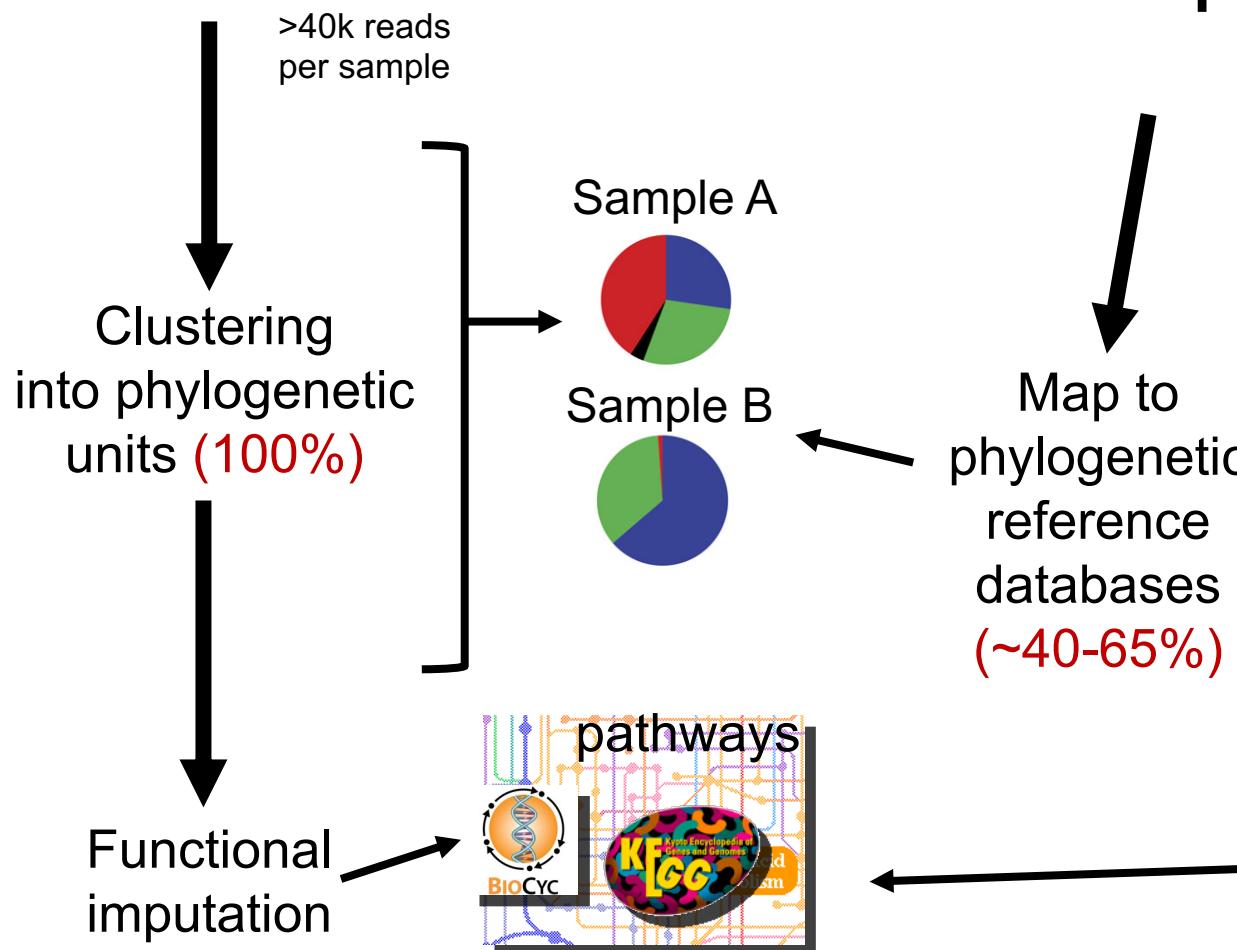
Tools of the trade

- “Age of Enlightenment”: Genetic profiling of the microbiota
 - Shotgun metagenomic sequencing



Tools of the trade: Workflow

16S rRNA sequencing:



Shotgun metagenomic sequencing:

~10M reads per sample

Strengths and Weaknesses

16S rRNA sequencing:

Reveals *phylogenetic identities* of bacteria within a community

- Advantages:
 - fewer reads necessary → cost
 - effective on low biomass samples
 - can quantify bacteria not represented in databases
- Disadvantages:
 - taxonomic resolution for most taxa only up to genus level

E. coli V4

A sequence alignment of two 16S rRNA gene fragments. The top sequence is labeled 'E. coli V4' and the bottom sequence is labeled 'Shigella dysenteriae V4'. The sequences are color-coded by nucleotide: Adenine (red), Thymine (green), Cytosine (blue), and Guanine (orange). The alignment shows high conservation between the two sequences, with minor differences highlighted by the color coding.

ACCGGCTTA_CTCCGTGCCAGCA_GCCGGGTTA_TACGGAGGGTGCAAGCGTTA_TCGGAATTACTGGCGTAAAGCGCACGCCAGGCG
ACCGGCTTA_CTCCGTGCCAGCA_GCCGGGTTA_TACGGAGGGTGCAAGCGTTA_TCGGAATTACTGGCGTAAAGCGCACGCCAGGCG

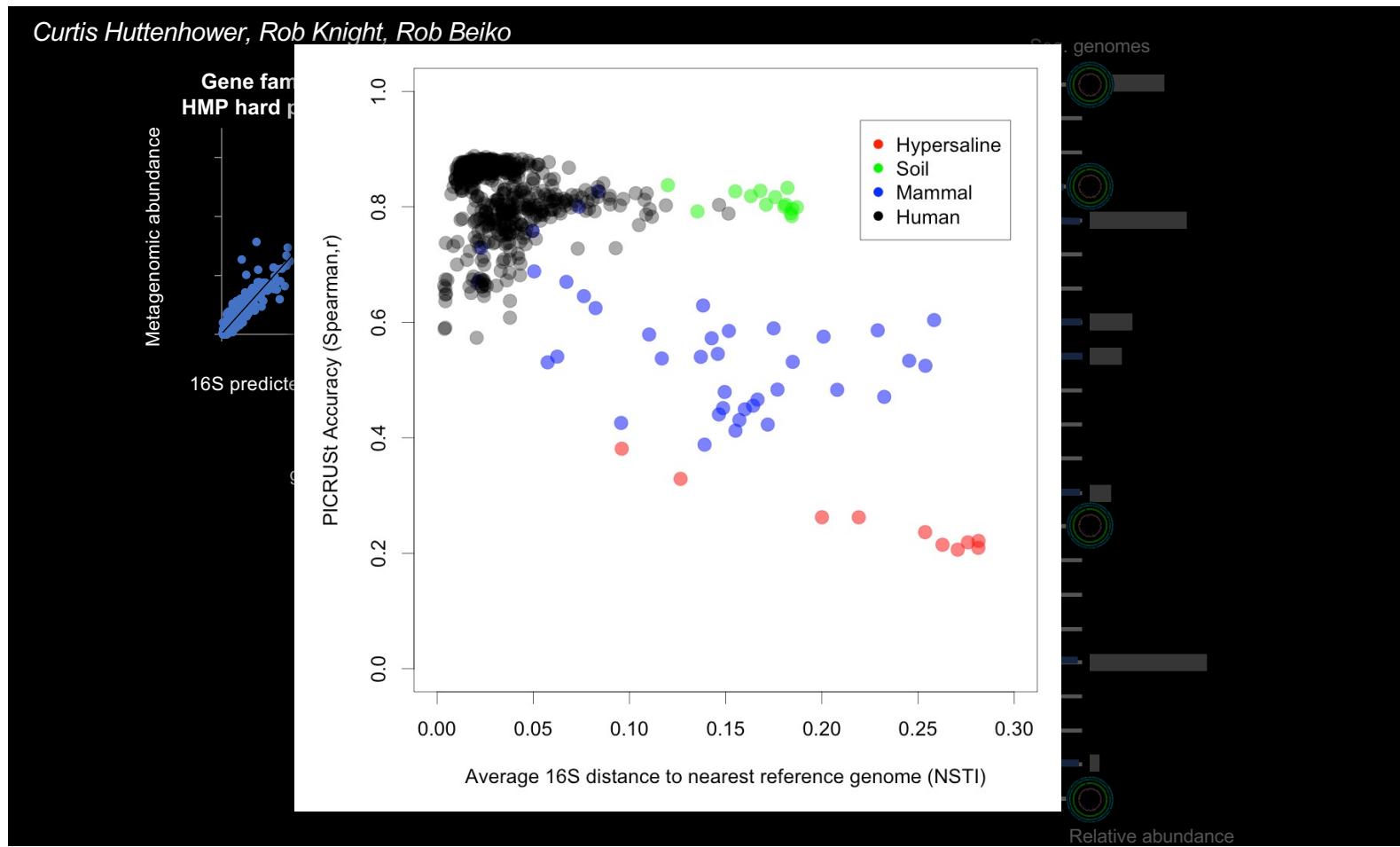
Shigella dysenteriae V4

Shotgun metagenomic sequencing:

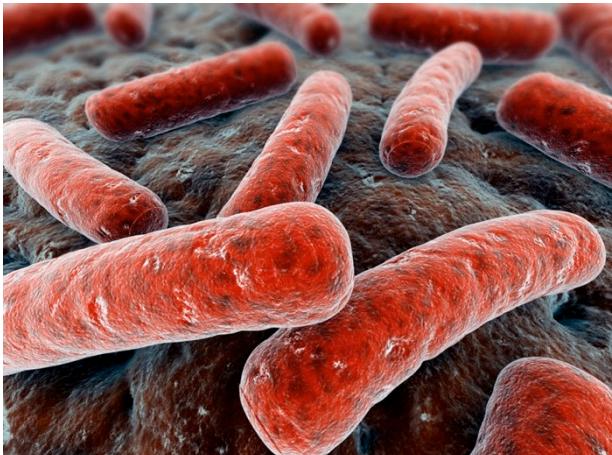
Identifies *gene content* of bacteria within a community

- Advantages:
 - directly characterizes functional potential (enzymatic, virulence factors)
 - can provide higher-resolution taxonomic, genomic information
- Disadvantages:
 - quantification of previously uncultured bacteria is poor (database dependent)
 - not feasible for low biomass samples (biopsies, skin, airway, internal organs)
 - ~50% of genes have no putative function

Functional imputation from 16S data using PICRUSt



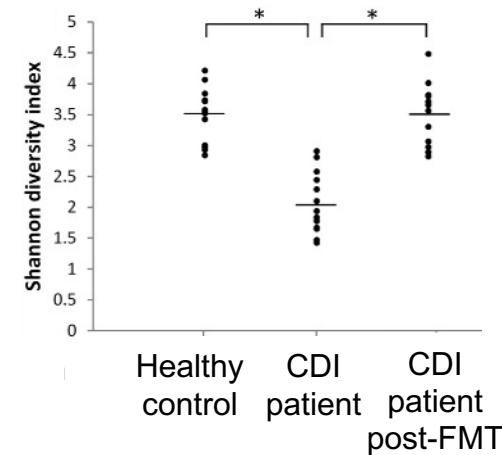
Prototypical microbiome-mediated disease: Recurrent *Clostridioides difficile* infection



- Causes recurrent diarrhea that can escalate to life-threatening inflammation of the colon
- Up to 450,000 new cases and **20,000** deaths each year in the USA with \$5 billion annual cost
- Most common predisposing factors include immunosuppression and **antibiotics** use

Prototypical microbiome-mediated disease: Recurrent *Clostridioides difficile* infection

- *C. difficile* infection is associated with a loss of diversity of the endogenous microbiota
- Is generally treated with antibiotics targeting *C. difficile* (e.g. vancomycin)
- Antibiotic treatment commonly results in recurrence (~35%)
- Restoration of the endogenous microbiota via fecal microbial transplantation (FMT)
 - cures up to 94% of patients (van Nood, NEJM, 2013)



Weingarden et al. Am J Phys 2015

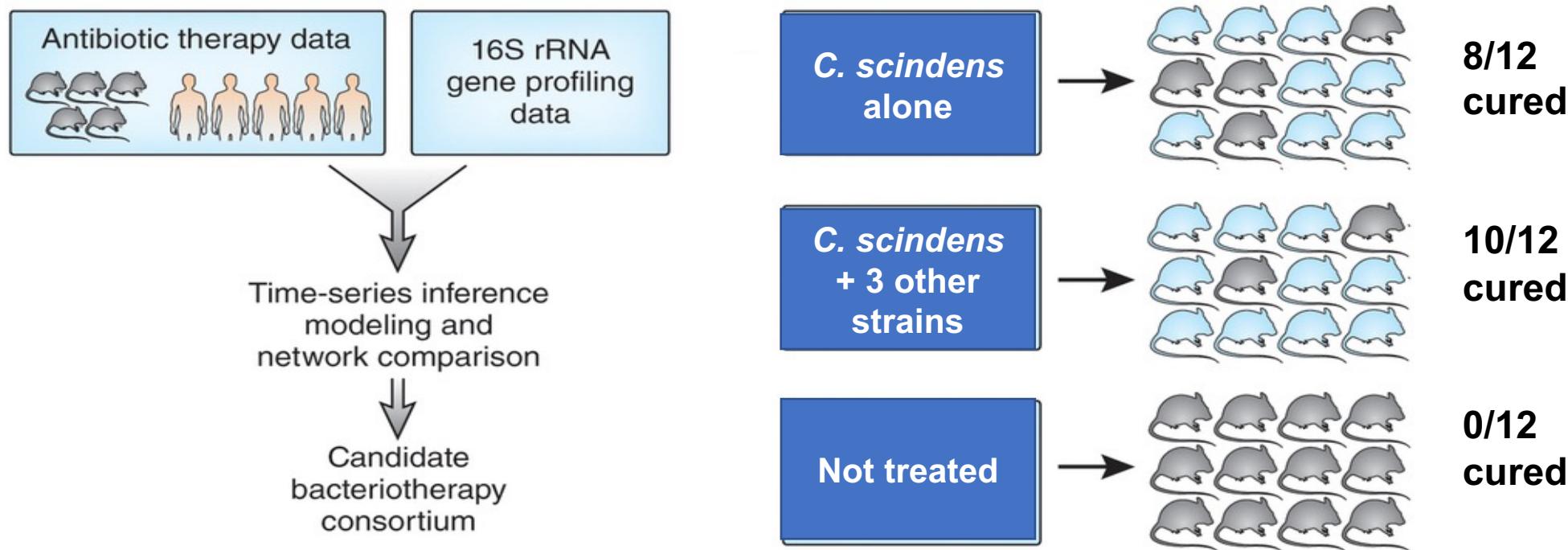
Question: What are the key gut-resident microbes that cure *C. difficile* infection?

Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*

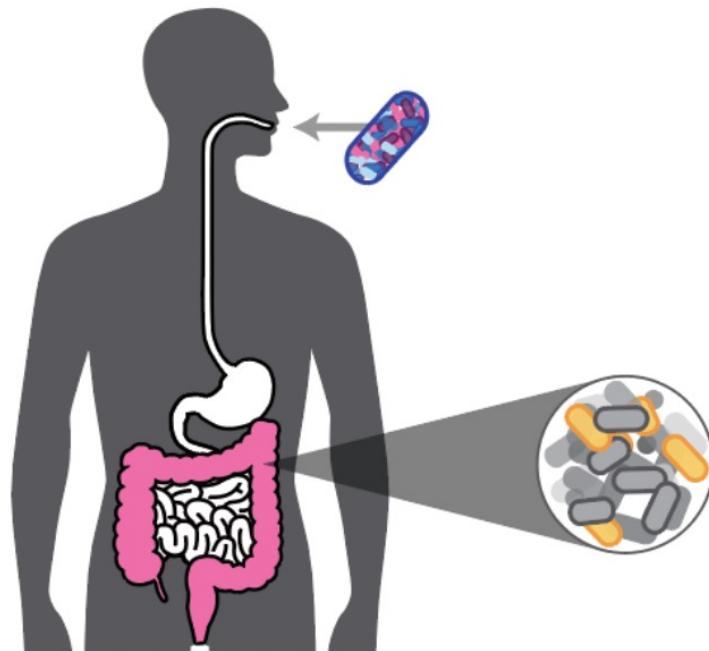
Charlie G. Buffie^{1,2}, Vanni Bucci^{3,4}, Richard R. Stein³, Peter T. McKenney^{1,2}, Lilan Ling², Asia Gobourne², Daniel No², Hui Liu⁵, Melissa Kinnebrew^{1,2}, Agnes Viale⁶, Eric Littmann², Marcel R. M. van den Brink^{7,8}, Robert R. Jenq⁷, Ying Taur^{1,2}, Chris Sander³, Justin R. Cross⁵, Nora C. Toussaint^{2,3}, Joao B. Xavier^{2,3} & Eric G. Pamer^{1,2,8}

206 | NATURE | VOL 517 | 8 JANUARY 2015

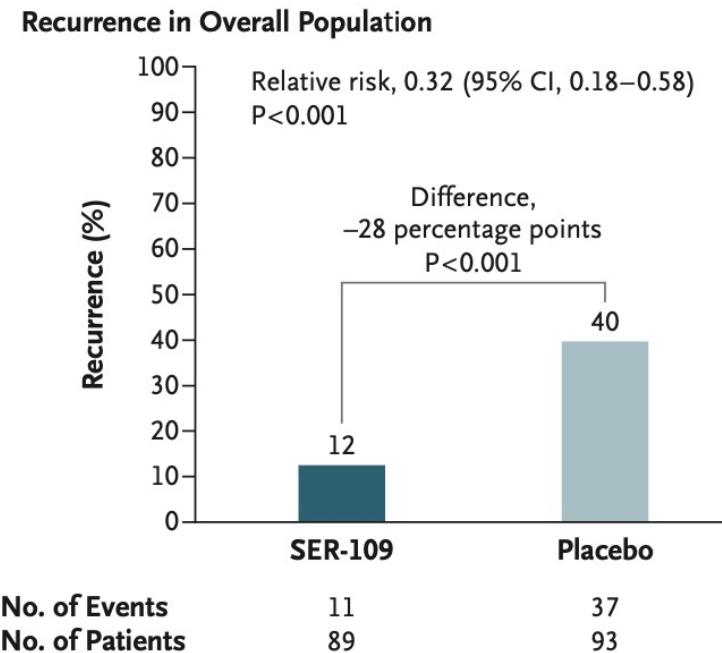
Systematic discovery of bacterial therapeutics



Precision microbiota restoration prevents recurrent CDI



3 Seres Therapeutics, Inc. © 2021
Henn et al Gastroenterology, 2021



Feuerstadt et al. NEJM. 2022

- Seres Therapeutics identified gut taxa that could help prevent CDI, bottled them up, and made a therapeutic

FMT shows efficacy in ulcerative colitis

Study (year)	Patients (n)	Results (primary outcome)
Moayyedi et al. (2015) ⁵	70	24% FMT group versus 5% placebo group ($P=0.03$)
Rosser et al. (2015) ⁶	37	30.4% FMT group versus 20% placebo group ($P=0.51$)
Paramsothy et al. (2017) ⁷	85	27% donor FMT group versus 8% autologous FMT group ($P=0.021$)
Costello et al. (2019) ⁴	73	32% donor FMT group versus 9% autologous FMT group ($P=0.03$)

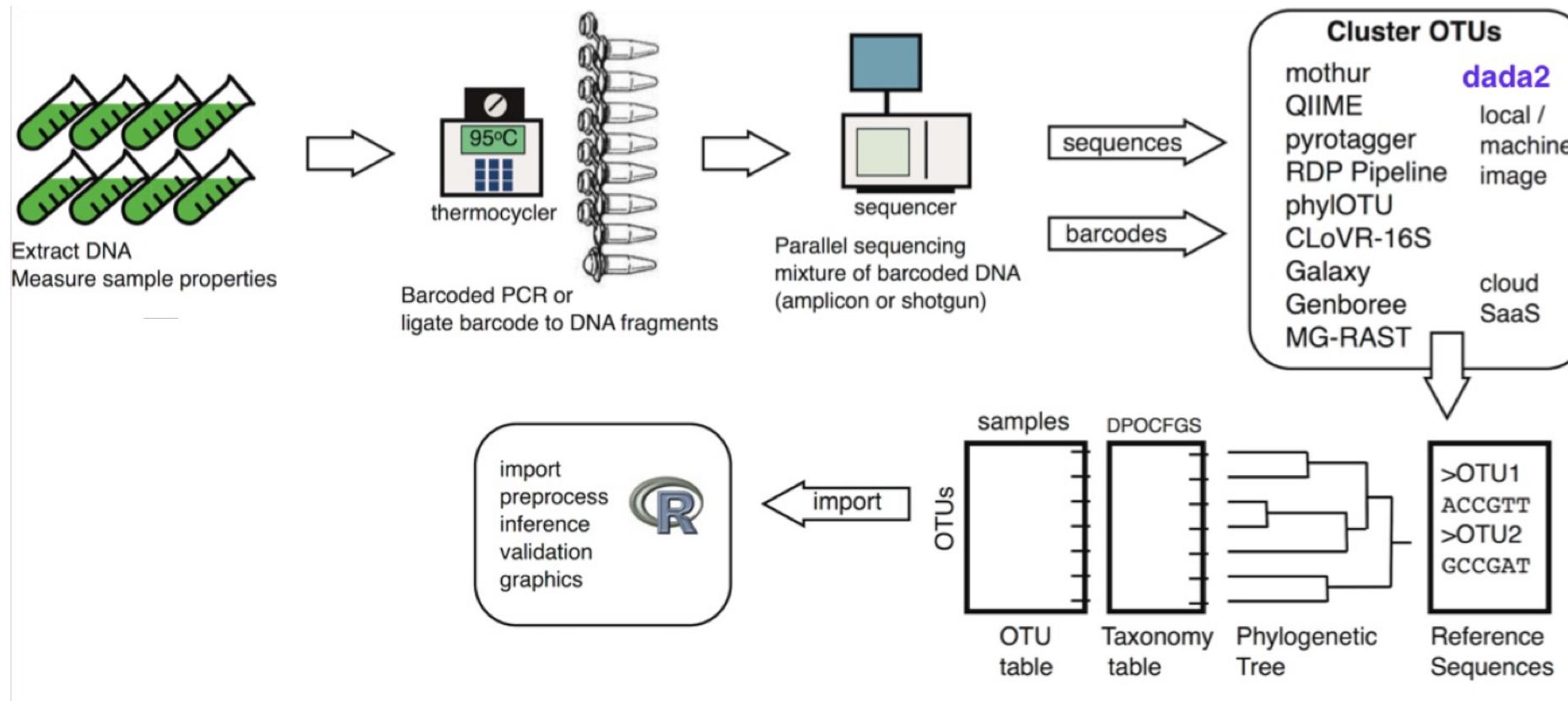
Cammarota & Ianiro,
Nature Rev Gastro 2019

Note: ~30% treatment response is similar to standard of care

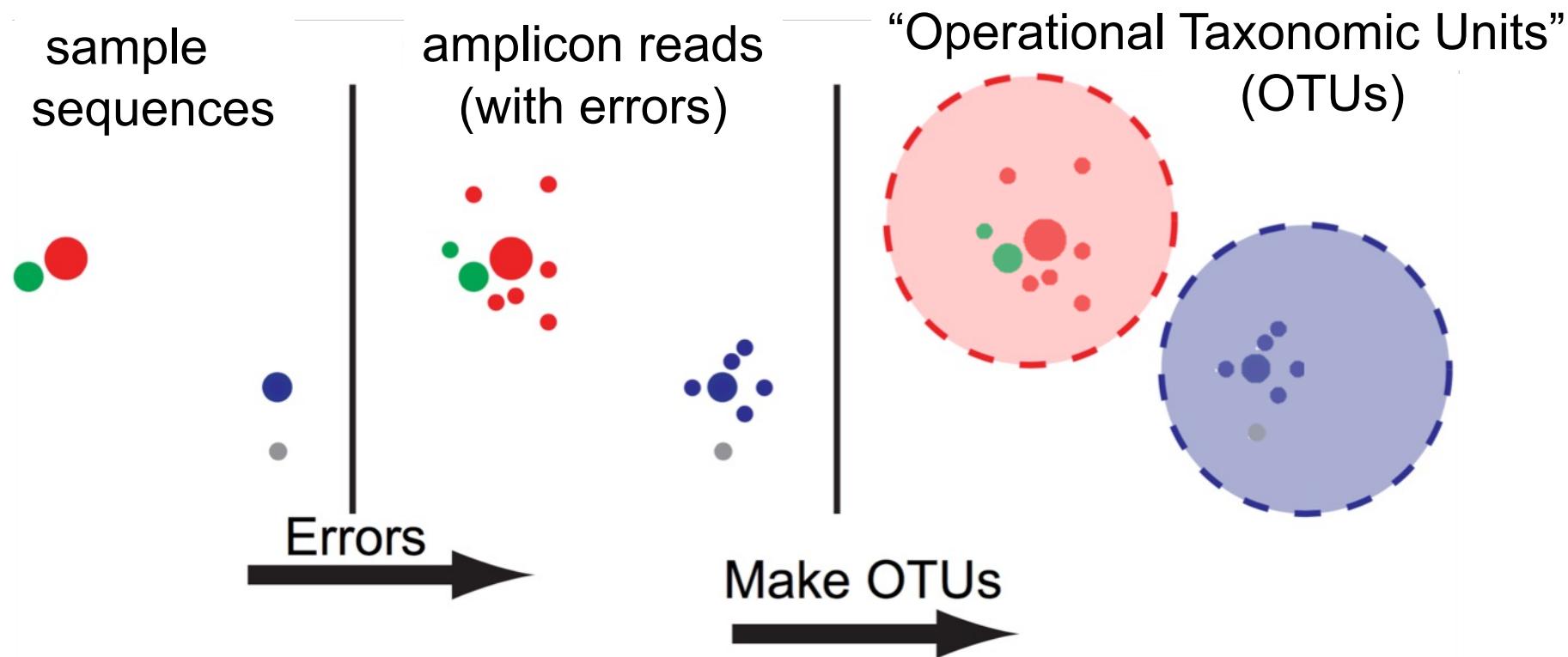
Questions remain:

- Which donor gut microbes are critical for response?
 - Which recipient microbes must be displaced?
- Is there person-specificity for either of above questions?
- Can targeted approaches be designed to obviate FMT?

A closer look at 16S rRNA sequencing



16S rRNA sequencing analysis: From Operational Taxonomic Units (OTU)



16S rRNA sequencing analysis: dada2

Read 1 C T A A G A C C G G A T A G G T A

Read 2 C T A A G A C C C G G A T A G G T A

Read 3 C T A A G A C C C G G G C T A G G T A

Read 4 C T A A G A C C C G G A T A G G T A

Read 5 C T A A G A C C G G A T A G G T A

Illumina ‘quality scores’
low high


Quality Score Q(X)	Error Probability P(~X)
Q40	0.0001 (1 in 10,000)
Q30	0.001 (1 in 1,000)
Q20	0.01 (1 in 100)
Q10	0.1 (1 in 10)

Discussion/Questions