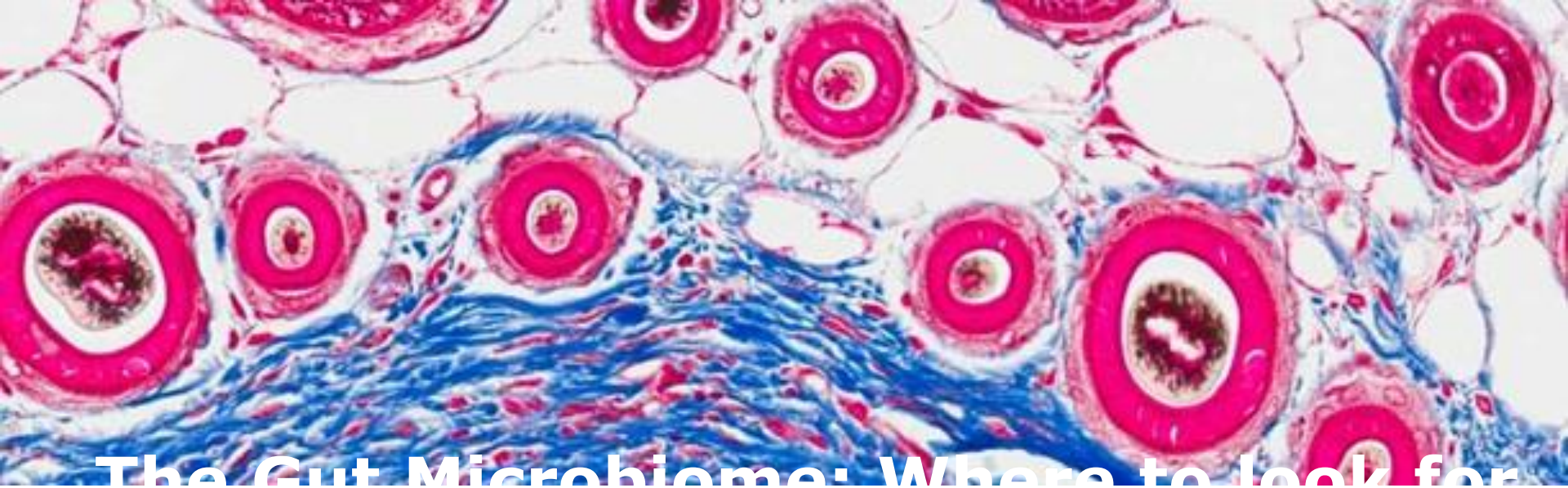


The Gut Microbiome: Where to look for

Microscopic Image showing Skin cells at 20x magnification

Carrie Brodmerkel, PhD
Head, Immunology Biomarkers
June 25, 2018

janssen Immunology

PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Agenda

- Where you can measure and what you can measure
- Considerations in selecting where and what to measure
- Beyond the standard…
- “Watchouts” when interpreting microbiome data
  - Population vs individual

Gut Microbes as the Source of Biomarkers

Mucosal Biopsy

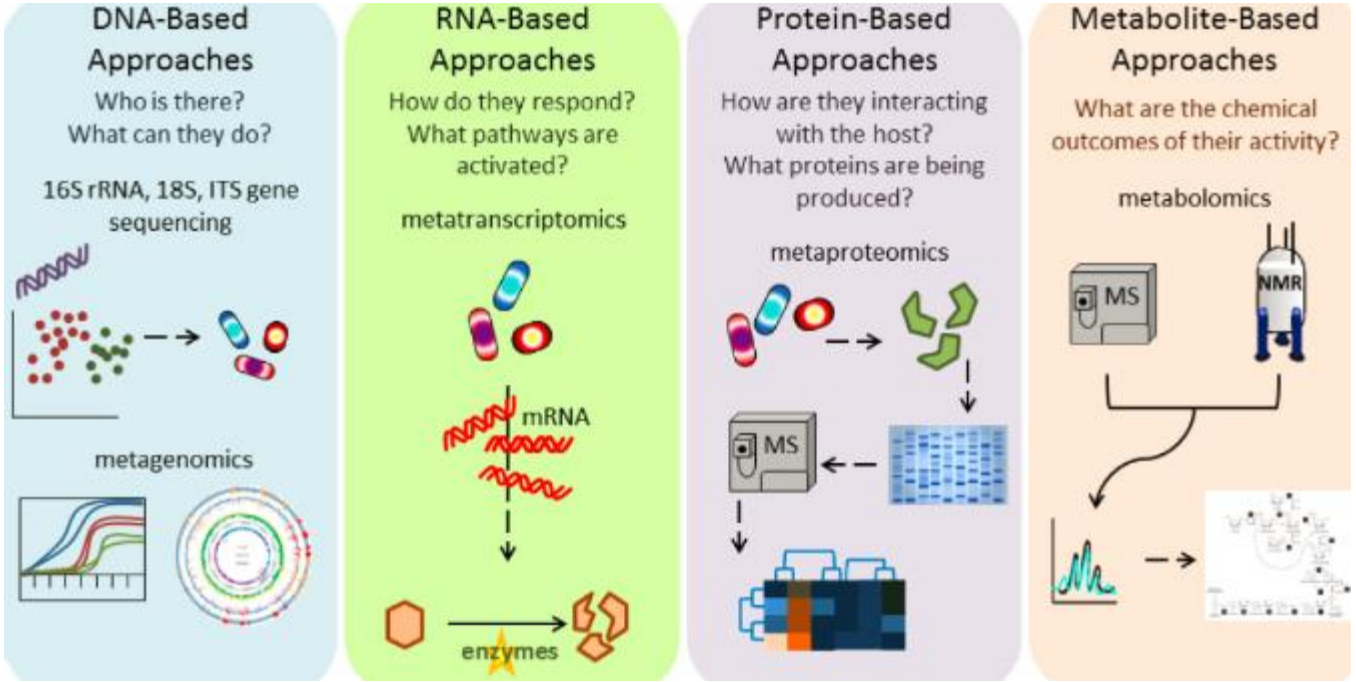
Colonic Brushing

Rectal Swab

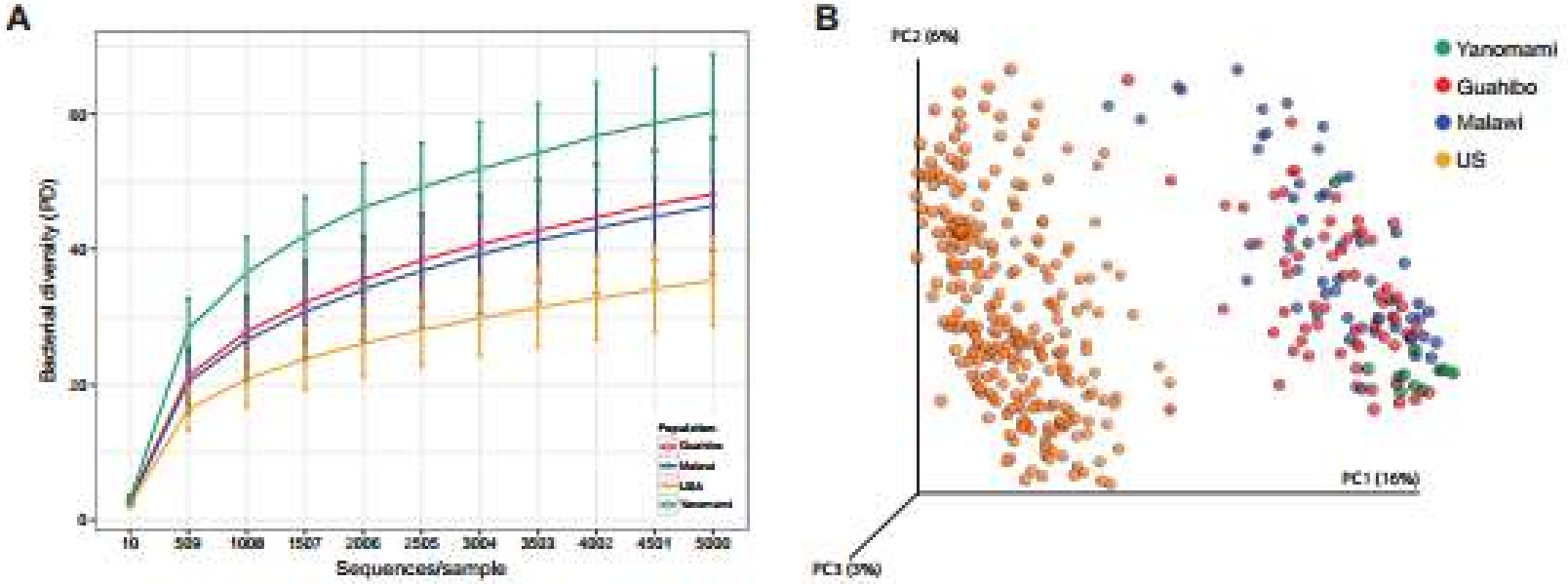
Urine

Stool

Standard Molecular Methods for Microbiome



Diversity is commonly used to assess “health”

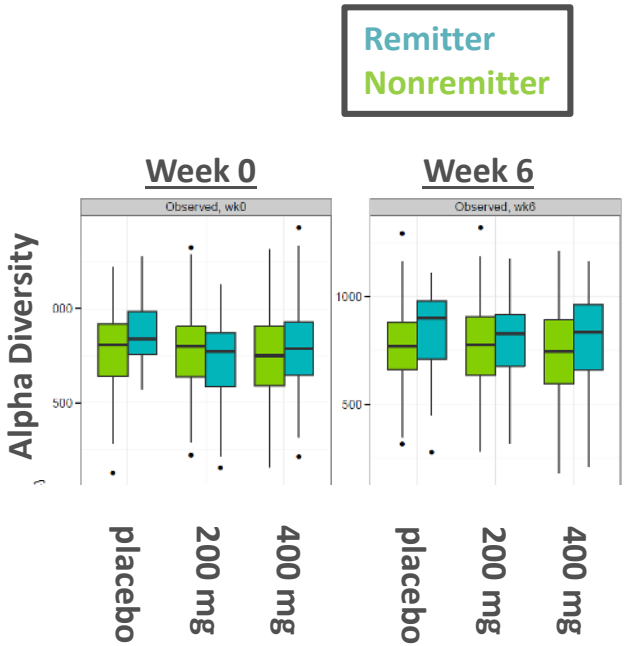


The microbiome of uncontacted Amerindians

Clemente et al. Sci. Adv. 2015;1:e1500183 17 April 2015

Response to Anti-TNF therapy in Ulcerative Colitis: Remitters More Diverse Than Nonremitters

- Diversity associated with remission
  - Remitters more diverse compared to nonremitters post-treatment
  - Does not appear to be specific to golimumab
  - UC has less evidence of dysbiosis then CD



World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/e
Help Desk: http://www.wjgnet.com/esps/helpd
DOI: 10.4251/wjgo.v8.i5.459

**Urinary metabolites as
gastrointestinal disease**

Irene Sarosiek, Rudolf Schicho, Pedro Blan

SCIENTIFIC REPORTS

OPEN

Hippurate as a metabolomic marker of gut microbiome diversity: Modulation by diet and relationship

Sci Transl Med. 2016 June 15; 8(343): 343ra81. doi:10.1126/scitranslmed.aad0917.

Received: 18 May 2017
Accepted: 25 September 2017
Published online: 20 October 2017

Natural history of the infant gut microbiome and impact of antibiotic treatments on strain-level diversity and stability

Moran Yassour[1,2], Tommi Vatanen[1,3], Heli Siljander[4,5,6], Anu-Maaria Hämäläinen[7], Taina Härkönen[4,5], Samppa J Ryhänen[4,5], Eric A Franzosa[8], Hera Vlamakis[1], Curtis Huttenhower[1,8], Dirk Gevers[1,‡], Eric S Lander[1,9,10,†], Mikael Knip[4,5,6,11,†], on behalf of the DIABIMMUNE Study Group[§], and Ramnik J Xavier[1,2,12,13,†,*]

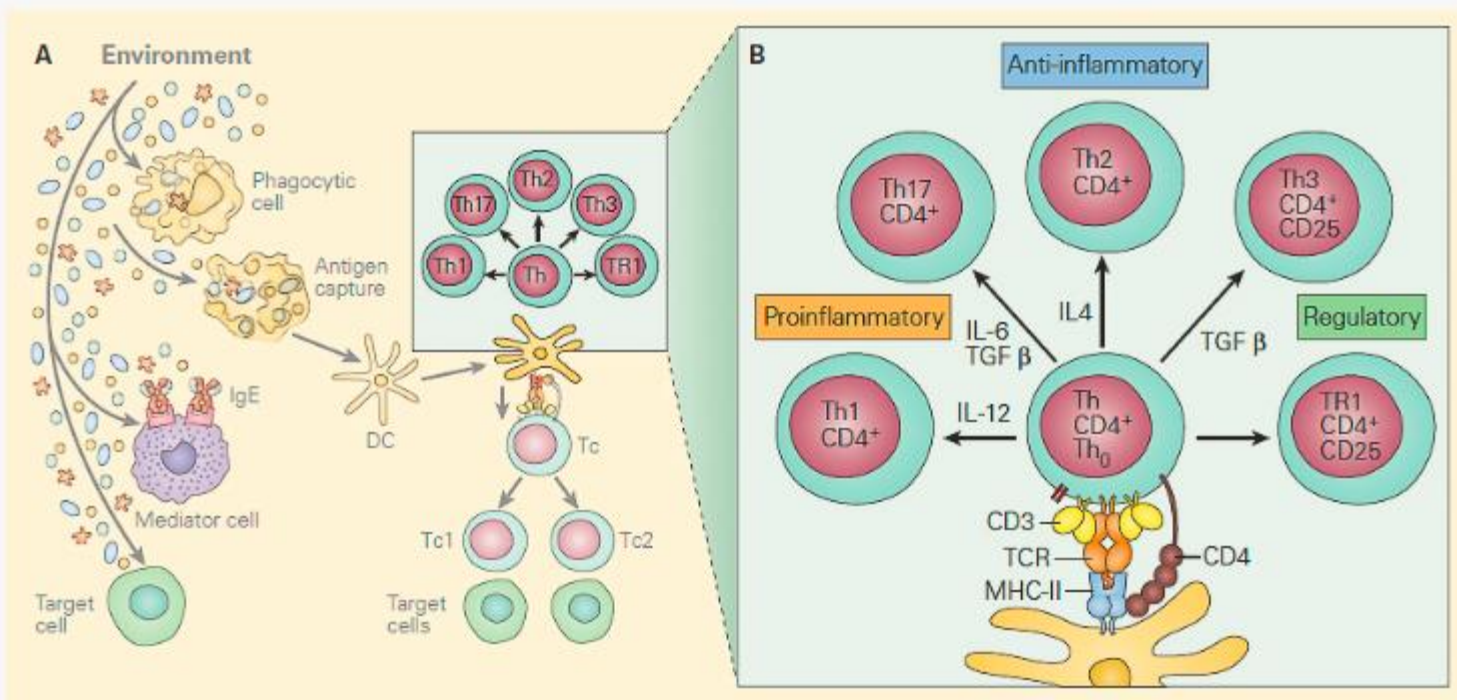
Host as the Source of Biomarkers

Mucosal Biopsy

Stool

Serum/Plasma

Peripheral Blood



The gut microbiome: host vs microbe influence on the immune system, disease and response to treatment

DISEASE MECHANISMS

Human genetic variation and the gut microbiome

Andrew Brantley Hall[1,2], Andr

Abstract | Taxonomic and functi
been implicated in multiple hum
studies reveal that variants in ma
associated with an altered comp
the microbial organisms residing
that certain host genetic variant
condition, in which the normal m
disorders of metabolism and imr

T induction by a rationally selected mixture of Cl

Koj
Nis
V. F
Sak

Affi

Nat.

Science

Gut microbio immunothera

Bertrand Routy,[1,2,3] Emm
Maryam Tidjani Alou,[1,2,
Maria P. Roberti,[1,2,5] Ma
Christophe Klein,[7] Kristi
Gladys Ferrere,[1,2,3] Célin
Solenn Brosseau,[15] Cour
Nathalie Galleron,[4] Ben
Patrick Gonin,[1,20] Jean-C
Gérard Zalcman,[15] Franç
Alexander Eggermont,[1,2
Laurence Zitvogel[1,2,3,5*]

Short Article

REPORTS

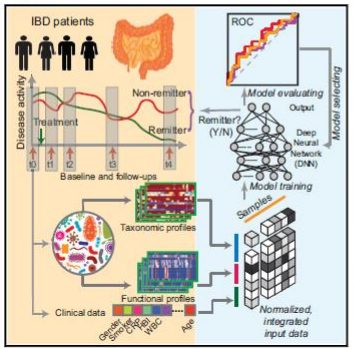
B. Routy *et al.*, *Science*
cience.aan3706 (2017).

Conrad Rauber,[1,2,3]
le Opolon,[6]
Bo Qu,[1,2,3]
altet,[15]
lin,[1,19]
helli,[16]
25

Cell Host & Microbe

Gut Microbiome Function Predicts Response to Anti-integrin Biologic Therapy in Inflammatory Bowel Diseases

Graphical Abstract



Authors

Ashwin N. Ananthakrishnan, Chengwei Luo, Vijay Yajnik, ..., Betsy W. Stevens, Thomas Cleland, Ramnik J. Xavier

Correspondence

xavier@molbio.mgh.harvard.edu

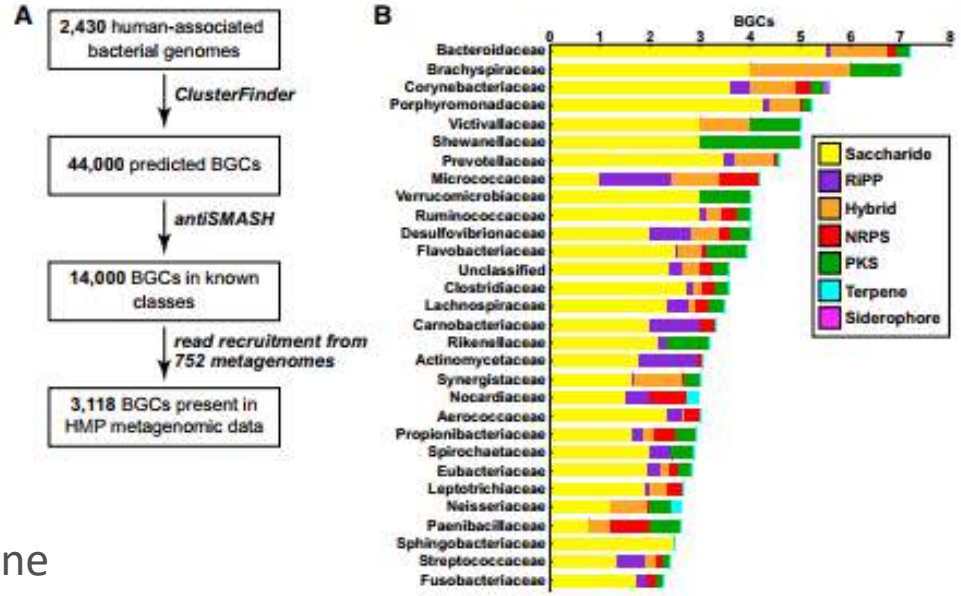
In Brief

Gut microbiome may predict responses to clinical therapy. Ananthakrishnan et al. conducted a prospective study with IBD patients initiating anti-integrin therapy. Higher abundance of butyrate producers and enrichment of 13 microbial pathways at baseline in therapy-responsive CD patients was observed. Early microbial changes persist up to 1 year in responders.

Beyond the standard: Better to Assess Sequence and Function?

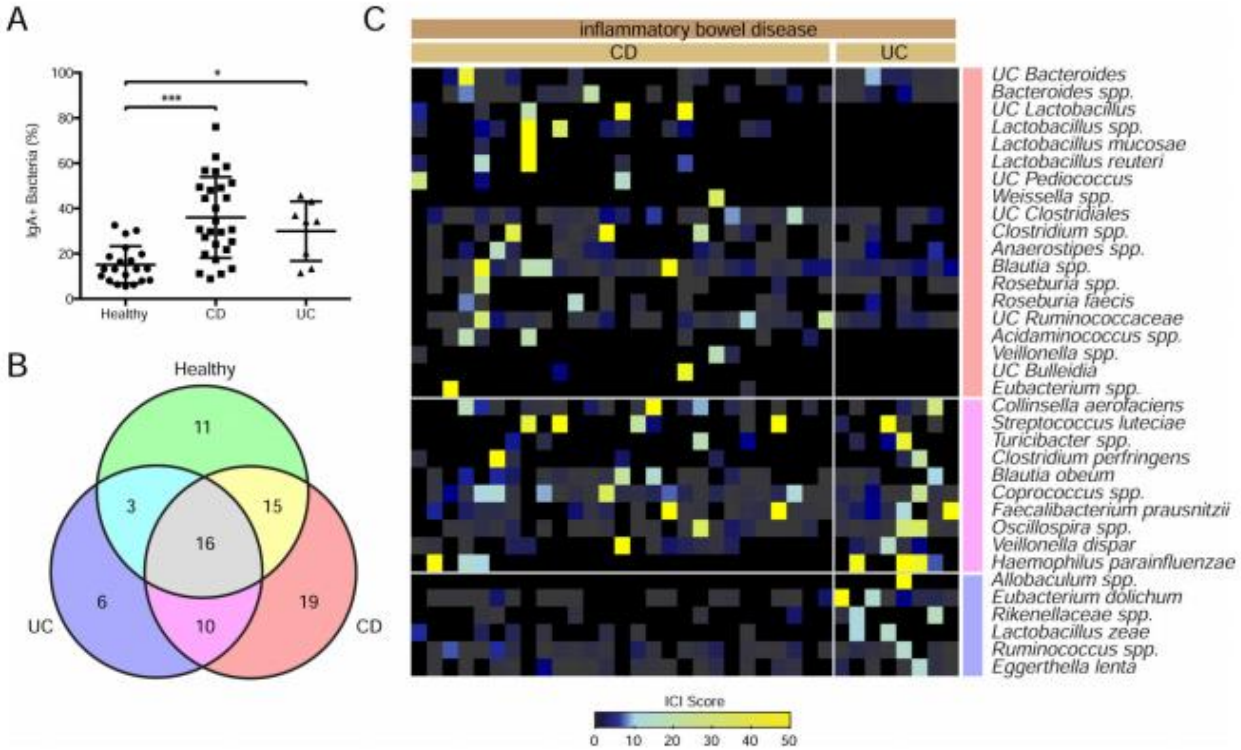
- Knowing what bugs make can lead to better understanding of microbe-host and microbe- microbe interactions

- Closely related bugs by sequence may produce different small molecules leading to different interpretations of the microbial diversity of a subject than use of taxonomic classification alone



Cell 158, 1402–1414

Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease: Use of IgA-SEQ



Microbial Antibodies

- Microbial antibodies detected in various immune diseases
- Only limited number of antibodies measured (ASCA, OmpC, etc.)
- Opportunity exists to expand

Front Med (Lausanne). 2018 May 23;5:153

TABLE 1 | Microbial proteins reactive to plasmas from Crohn's and Sjogren's patients.

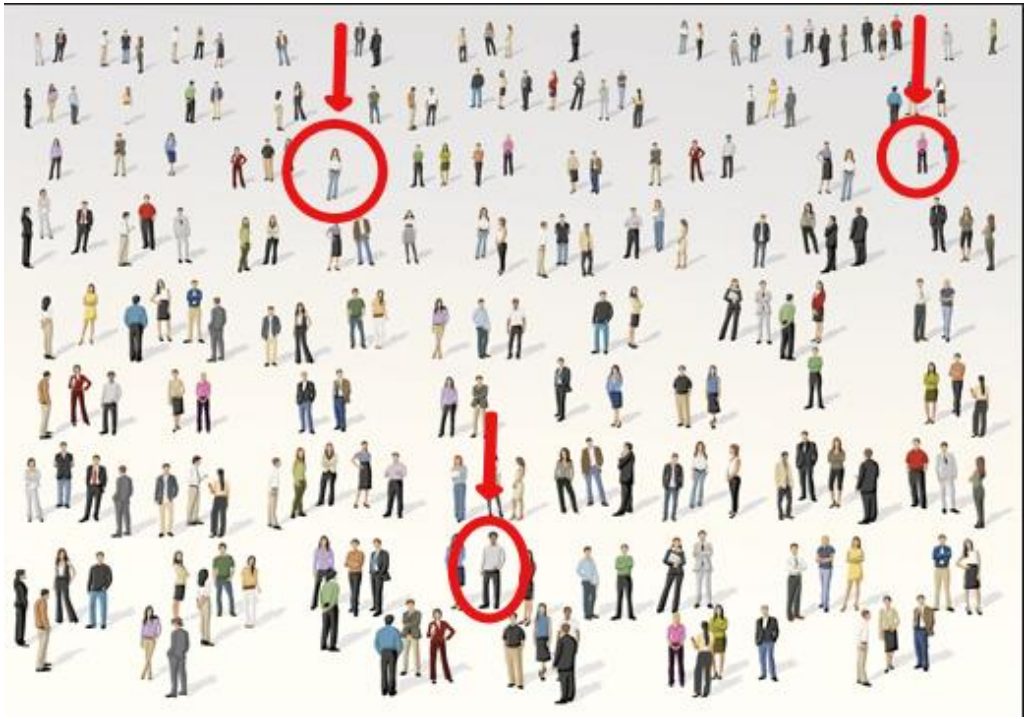
Name	MW(Kd)	Microbial species
DNA-directed RNA polymerase subunit B (RPOB)	135	*S. aureus*
Elongation factor G (EF-G)	75	*S. aureus/ pseudintemedius*
ATP synthase subunit alpha (ATP5a)	55	*S. aureus/ pseudintemedius*
Uncharacterized lipoprotein SACOL0083 (unknown)	27	*S. pseudintemedius*
Elongation factor Tu (EF-Tu)	42	*Escherichia coli*
Outer membrane porin protein C (ompC)	39	*Escherichia Coli*
Heat shock protein 65 (hsp65)	60	*M. paratuberculosis*

J Clin Immunol. 2009 March ; 29(2): 190

The Frequency of Positive Seroreactivity to Tissue Transglutaminase (tTG) Anti-*Saccharomyces cerevisiae* (ASCA), I2, and OmpW among Patients (55) with Celiac Disease at the Time of Diagnosis and After Gluten-free Diet

	CD at the time of dg	CD GFD	*p* value
tTG	50/55 (90.9%)	7/54 (13.0%)	<0.001
ASCA IgA and/or IgG	27/55 (49.1%)	12/55 (21.8%)	<0.001
I2	47/55 (85.5%)	41/55 (74.5%)	0.070
OmpW	35/55 (60%)	27/55 (49.1%)	0.070

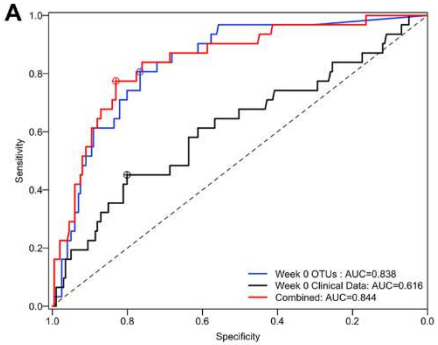
Biomarkers: Population vs Individual



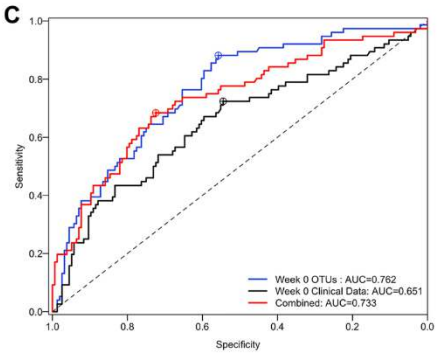
Going from Population to Individuals

Baseline microbiome predicting week 6 clinical readout post Ustekinumab (anti-p40 mAb) in mod/severe CD

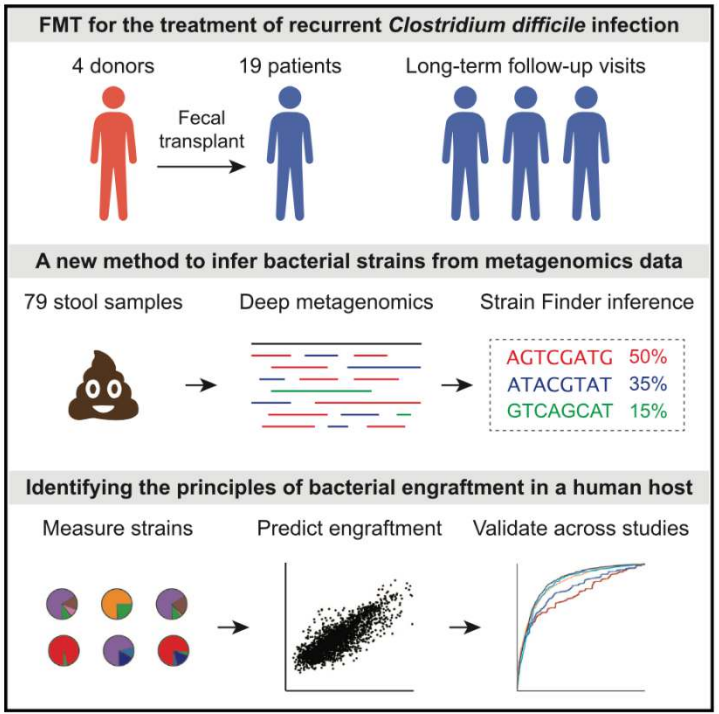
Wk6 clinical response



Wk6 clinical remission



Baseline microbiome predicting engraftment post FMT in rCDI subjects



Are we ready?

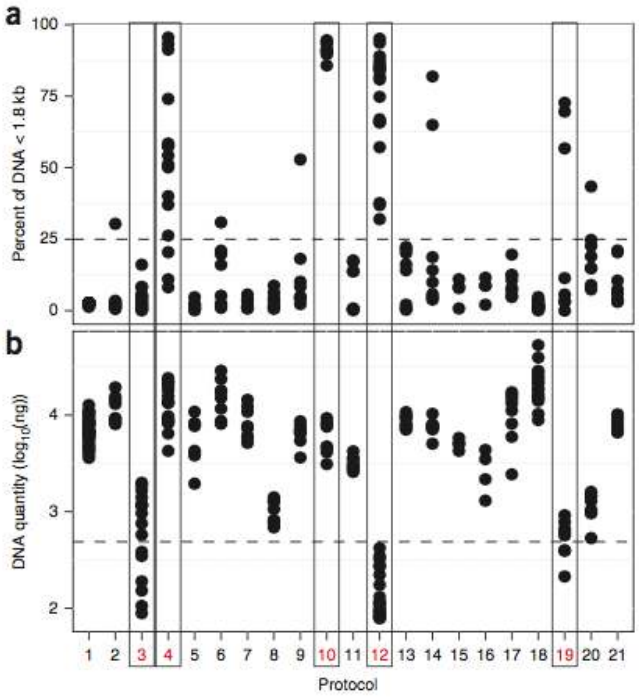
Methods are continually evolving and current understanding may shift based on improvements…

- Extraction methods
- Absolute vs relative abundance of microbes
- Sequencing depth and tools to characterize microbes

Why DNA extraction protocol matters

DNA quality (line=1.8kb)

DNA quantity



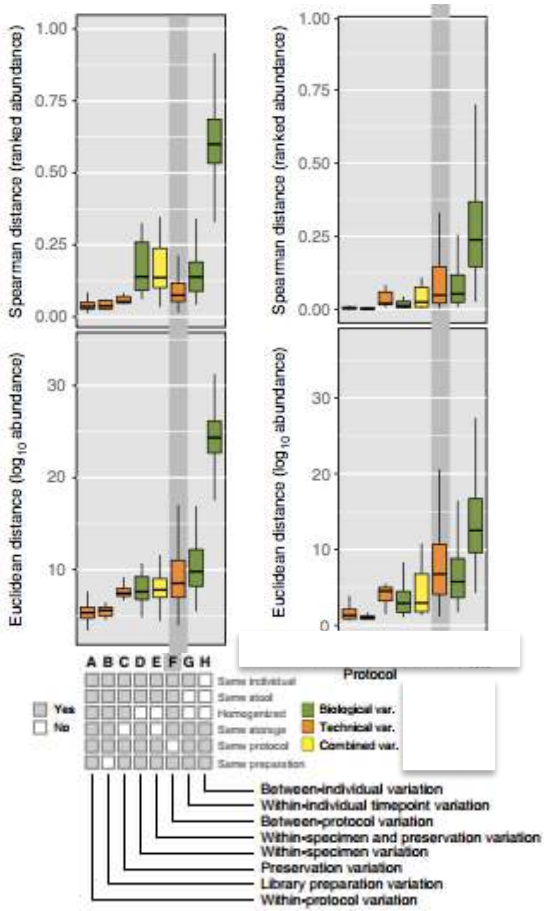
- 21 DNA extraction protocols evaluated on same fecal samples (11 countries/3 continents)
- Cutoff drawn at >500ng of DNA with <25% fragmentation
- Compared with library prep, sample storage, biologic variation within sample and within subject over time

DNA extraction had largest effect on outcome of metagenomic analysis

Costea et al Nature Biotechnology Oct 2017

Why DNA extraction protocol matters

Species Function ⟸ Abundance



- Relative effect size of variation tested:
  - Using Spearman distance to rank abundances found comparable species rankings between protocols
  - Using Euclidean distance to assess abundance there was high variability between protocols
- Inter-individual variation as expected is largest source of variation

Costea et al Nature Biotechnology 2017

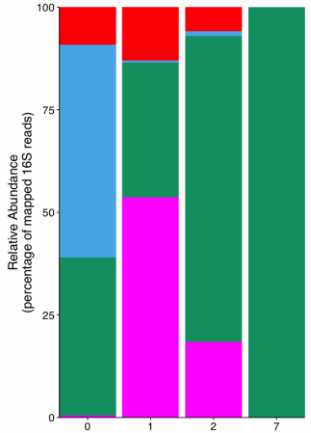
Relative Abundance vs Absolute Abundance

- Relative composition doesn’t allow for understanding of absolute abundance considering the variability across specimens

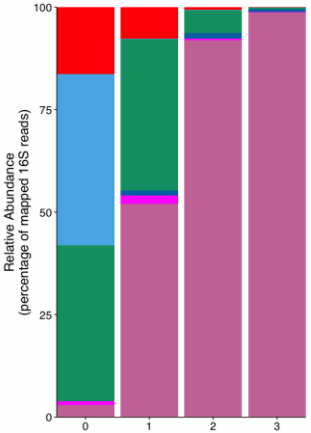
- Vastly different interpretations about diversity can be made depending on use of relative vs absolute abundance

Relative vs Absolute Abundances Yield Different Conclusions

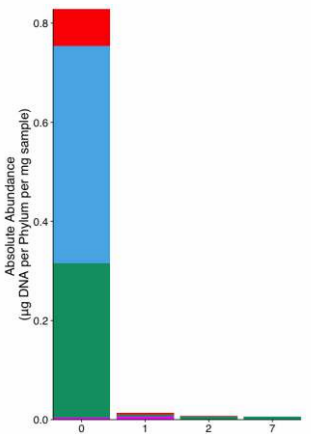
Same stool samples from mice treated with vancomycin



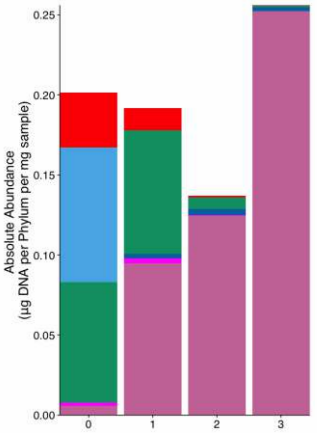
High-Dose Vancomycin



Low-Dose Vancomycin

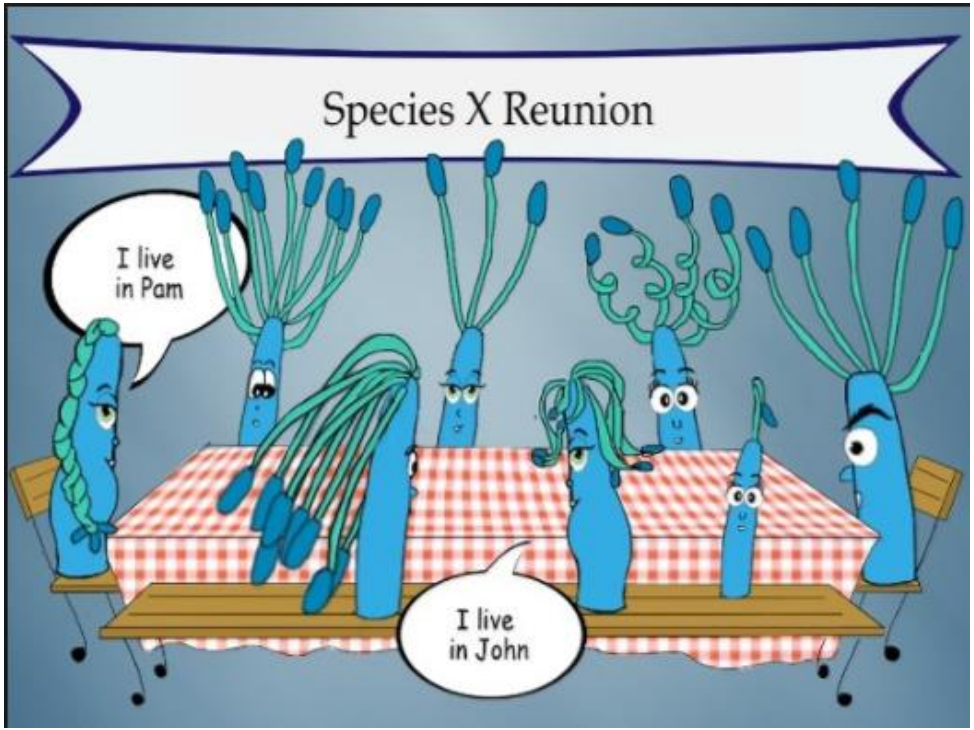


High-Dose Vancomycin



Low-Dose Vancomycin

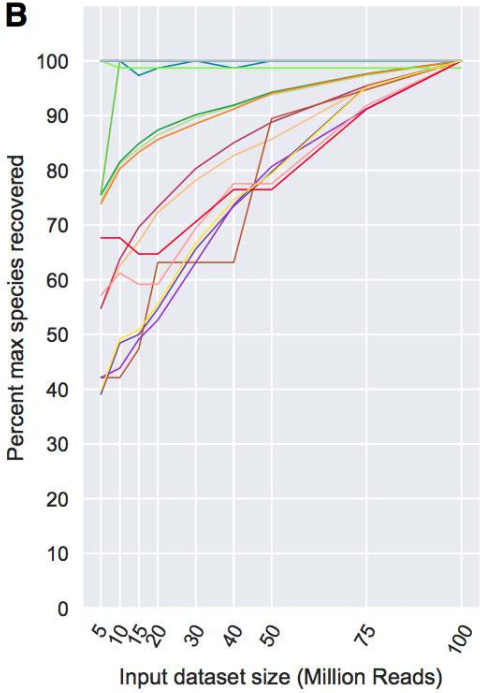
How do you define a species or strain in a complex environment?



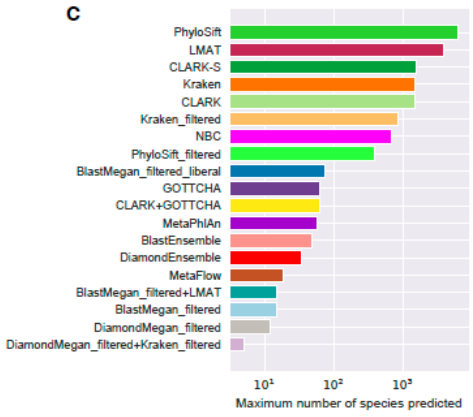
- In human stool a single species may be <0.1% of reads

Dana C, Thomas

Sequencing depth and tool matter



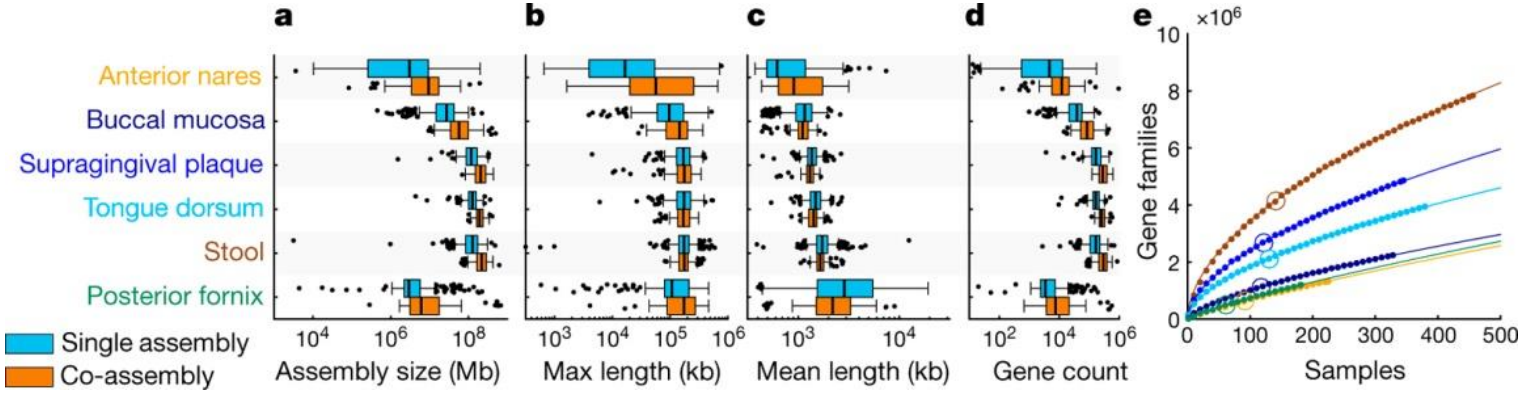
Downsampling of deep seq environmental sample



- Depth of sequencing can significantly change the results of a metagenomic study, depending on the tool used
- Marker-based tools identified far more species as depth of sequencing increased
- Essential to standardize sequencing depth and analytical tool to compare between similar studies

McIntyre et al bioRxiv 2017

Recent data from HMP



Still more to learn…# of gene families has not yet leveled off

J Lloyd-Price *et al. Nature* 1–6 (2017) doi:10.1038/nature23889

Key considerations

- What question are you trying to answer
  - What sample
    - Microbial and/or Host
    - Site of action
    - Static vs Functional
  - What methodology
    - Is there consensus on how to analyze and interpret the data?
    - What biases might be inherent in the data
  - How can the data be interpreted
    - Population vs Individual

THANK YOU

Janssen

- Ling-Yang Hao
- Lani San Mateo

Ichan School of Medicine

- Eduardo Contijoch
- Jeremiah Faith