COLLEGE of AMERICAN PATHOLOGISTS

Microbiome Applications in Pathology

CAP PHC Webinar

Lynn Bry, MD, PhD

April 4, 2019

Webinar Host

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- **This presentation will be recorded. The recording and PDF will go out to all registrants in one week**
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- **Please send in your questions as you think of them via the "Question Box" in your control panel**

Lynn Bry, MD, PhD

- **Medical director in the Clinical Microbiology laboratory and Molecular Pathology service at Brigham & Women's Hospital**
- **Directs the Massachusetts Host – Microbiome Center**
- **Served on the CAP PHC and NGS Lab Accreditation Committees**

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Disclaimer, continued

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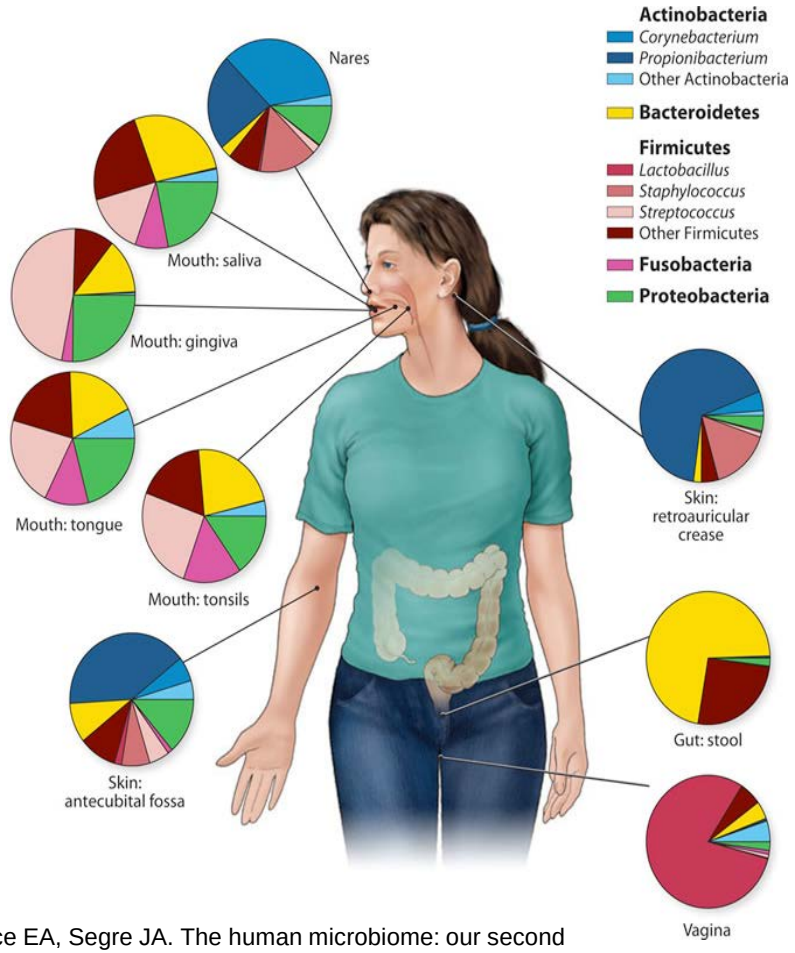
Disclosures

- **I am the founder and SAB Chair of Consortia Therapeutics which is developing live bacteriotherapeutics for the treatment and prevention of human food allergies**
- **SAB member of Inspirata Inc.**

No funding or data from either entity is shown in this presentation.

The Microbiome



Grice EA, Segre JA. The human microbiome: our second genome. *Annu Rev Genomics Hum Genet.* 2012;13:151-70. doi: 10.1146/annurev-genom-090711-163814.

- **Communities of microbes that colonize all body surfaces.**
- **10X more microbial cells in the human body than those of the host.**
- **Important in health and disease.**
- **Exponential growth in publications and commercial opportunities.**

Impact of Host-Microbiome Interactions

100% of the US population has been or will be affected by microbiome-promoted diseases and conditions.

Condition	US residents/yr	Role of the Microbiome
Periodontitis, dental caries	>85,000,000	Confirmed
Take medications where microbial biotransformation causes side effects	>50,000,000	Confirmed
Inflammatory Bowel Diseases	1,300,000	Confirmed
Have had *C. difficile* colitis	500,000	Confirmed
Pre-term birth	400,000	Strong evidence
GI and Oral Cancers	2,500,000	Strong evidence
Food allergies	6,000,000	Strong evidence
Type II Diabetes and pre-diabetic conditions	>70,000,000	Hypothesized
Cardiovascular disease	>84,000,000	Hypothesized

Data from the US Census, MA DPH, CDC, CCFA, AHF, NIH and Partners Healthcare's Research Patient Data Registry

Growing Interest From Industry

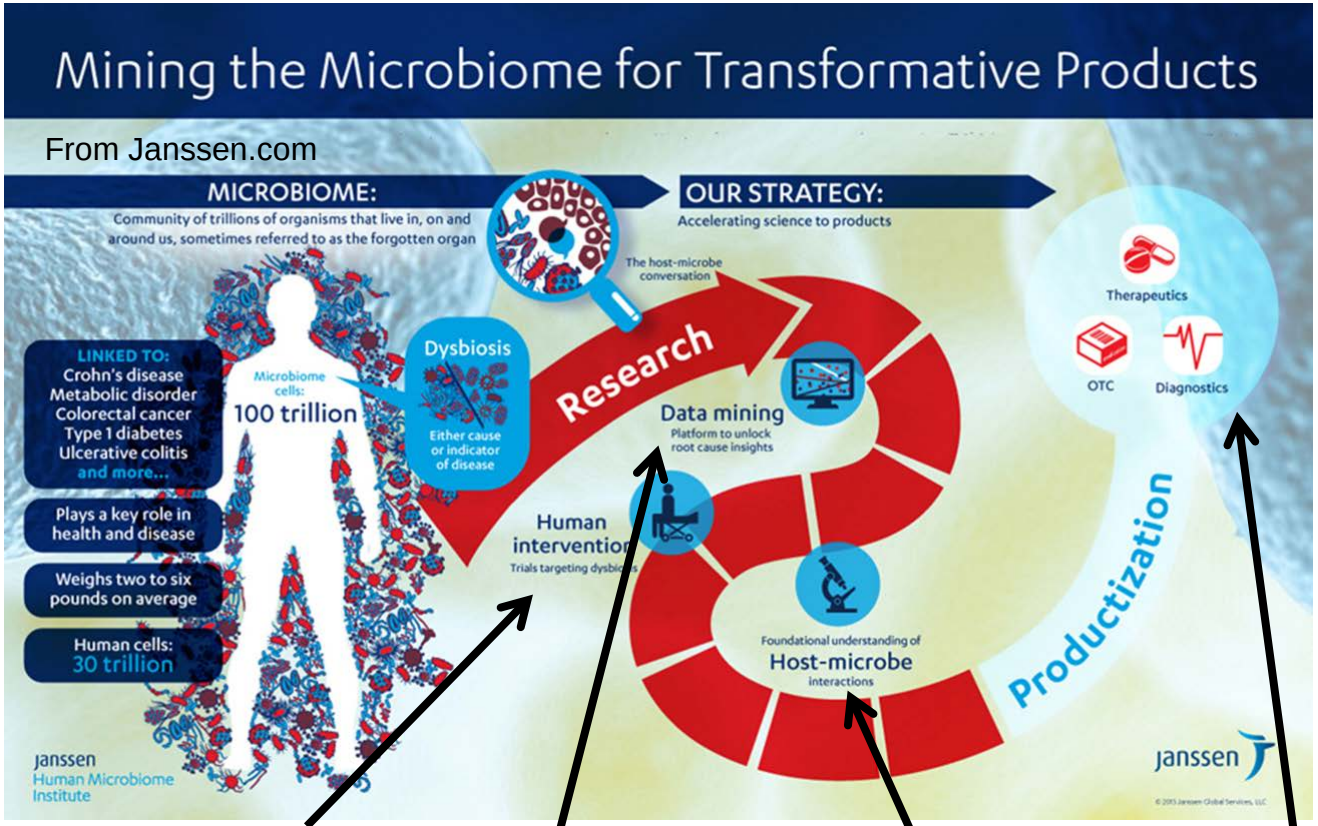


Image provided by Janssen Research & Development, LLC

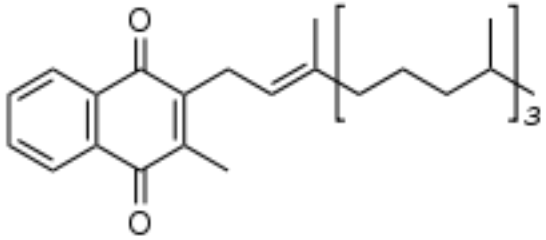
Patients
- CLIA microbiome
- Data/Sample Registries

Novel Computational Models
- Longitudinal Dynamics
- Outcomes prediction

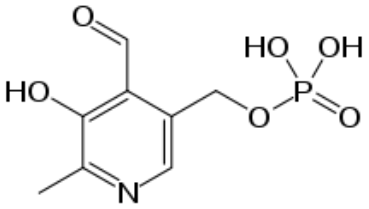
Functional Biology
- Gnotobiotics
- Microbiology/Genetics

Clinical Trials CLIA Lab

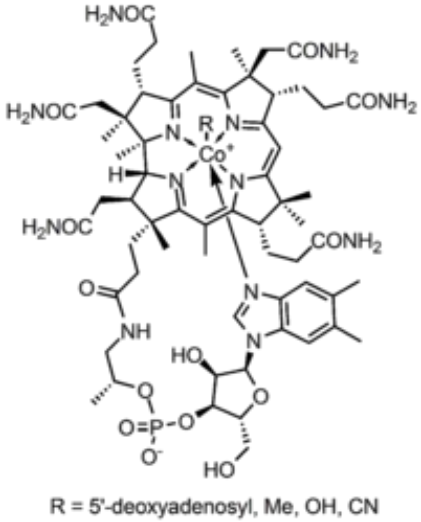
Micronutrients from our Microbiota



Vitamin K (menadione)
-Bacterial electron transporter
-We absorb to also transport electrons, and make blood clotting factors
-Made by many commensals (*E. coli, Bacteroides*)

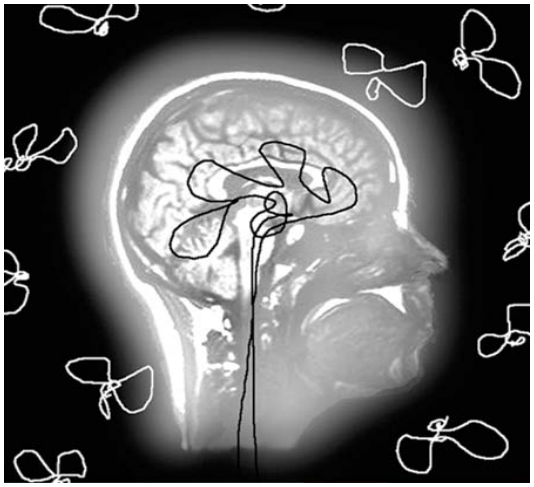


Vitamin B6 (pyridoxine)
-Bacterial transfer factor (amines, -COOH, -SH..)
-Produce blood cells, neurotransmitters
-Made by many commensals - *Clostridia*, lactobacilli, others



Vitamin B12 (cobalamin)
-Only made by microbes.
-Most complex vitamin
-Many microbial forms

-*Proprionibacteria*, some *Lactobacilli*, rumen flora
-Needed to make the building blocks of DNA



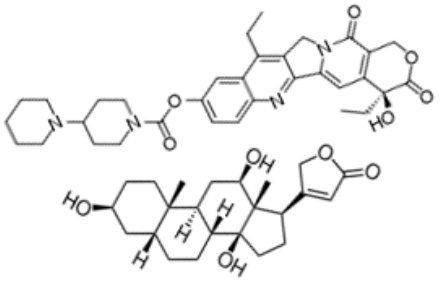
Micronutrients From our Microbiota

Short Chain Fatty Acids

-End products of microbial fermentation
-We can absorb them
-Convert to fats, proteins
-Healthy colon, unhealthy waistline?

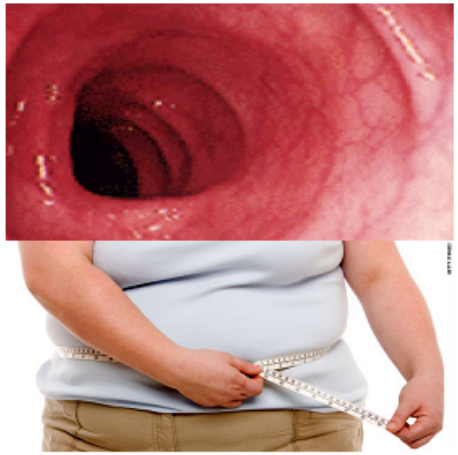
Ethanol

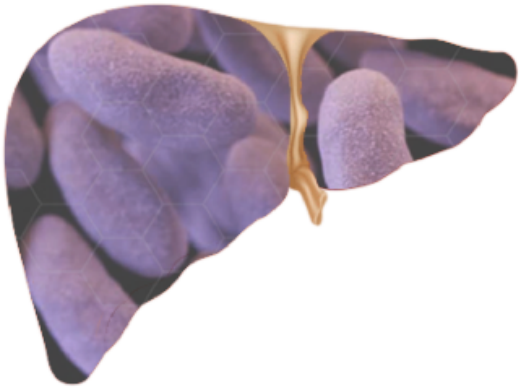
-Common end-product from fermenting sugars.
-Long-term sugary diet -> selects fermenters -> potential cause of NASH?



Drug Metabolism

-Irinotecan (cancer drug)
-Digoxigenin (heart drug)
-Microbes can inactive OR create toxic compounds, causing side effects.
-Microbial metabolic organ





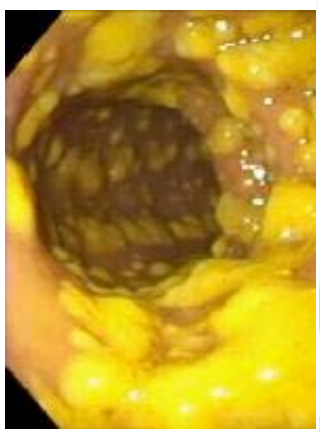
Microbiota: Infection and Immunity

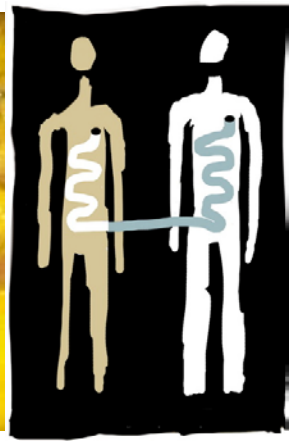
Dysbioses

- Microbial communities that trigger aberrant immune responses
- IBD, auto-immunity
- Atopic diseases
- Microbial manipulation.

Susceptibility to Infections

- Crowd control: good guys in, bad guys out
- Mature immune system
- *Clostridium difficile*
- Dysentery
- Childhood infections





Pathobionts

- Single or community effects -> “ill humors”
- Behavior can depend upon environment (you are what you eat)
- *Bilophila wadsworthia*

Targeted therapies: Prebiotics, probiotics, antibiotics, immunotherapeutics
Microbial modifications: Dietary changes, fecal/community transplantation

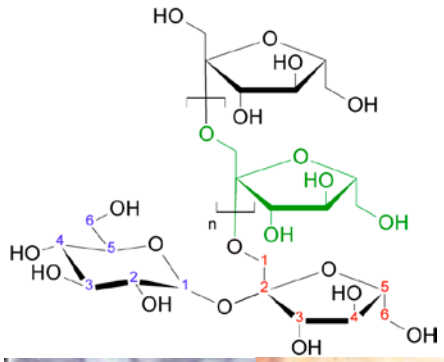
Manipulating our Microbiota

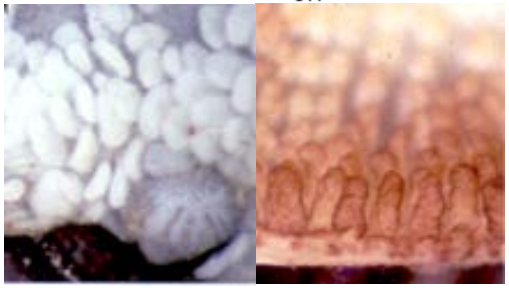
Diet, Environment

- Dietary factors
- Rapid change in microbial communities relative to oral intake
- Skin exposures
- Environmental exposures

Prebiotics

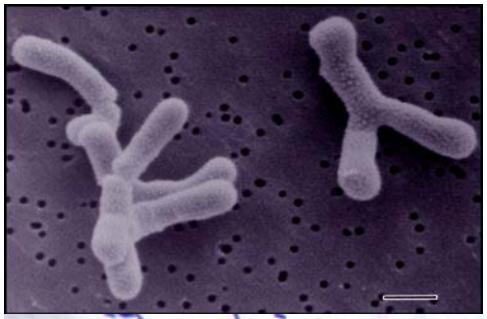
- Small molecules that promote specific microbes and/or communities
- Complex sugars, micronutrients, others





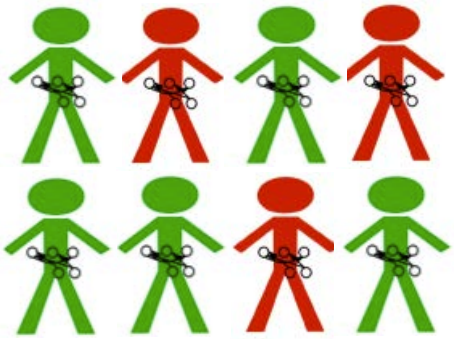
Probiotics

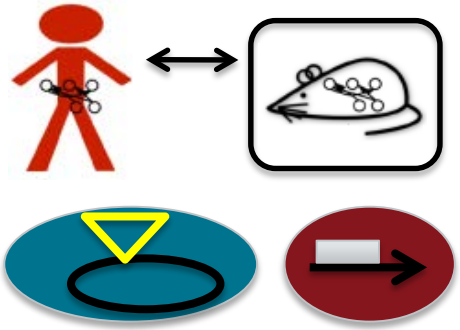
- Administered communities
- Foodstuffs
- “Bugs as Drugs”
- Nutraceuticals



Identifying Causal Effects of the Microbiota

Move the field from descriptive associations to causal effects of the microbiota *in vivo*



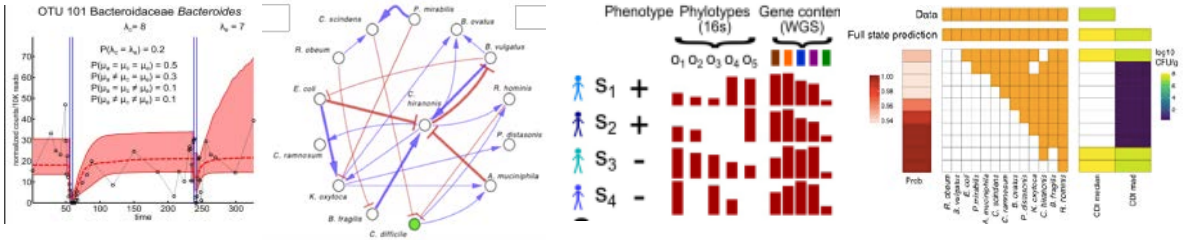


Patient Cohorts
- Biobank/Data Registries
- CLIA Microbiome

Functional Biology
- Gnotobiotics, in vitro systems
- Microbiology/Genetics, SynthBio

Clinical Trials CLIA Lab

Novel Computational Models
- Longitudinal Dynamics
- Outcomes prediction



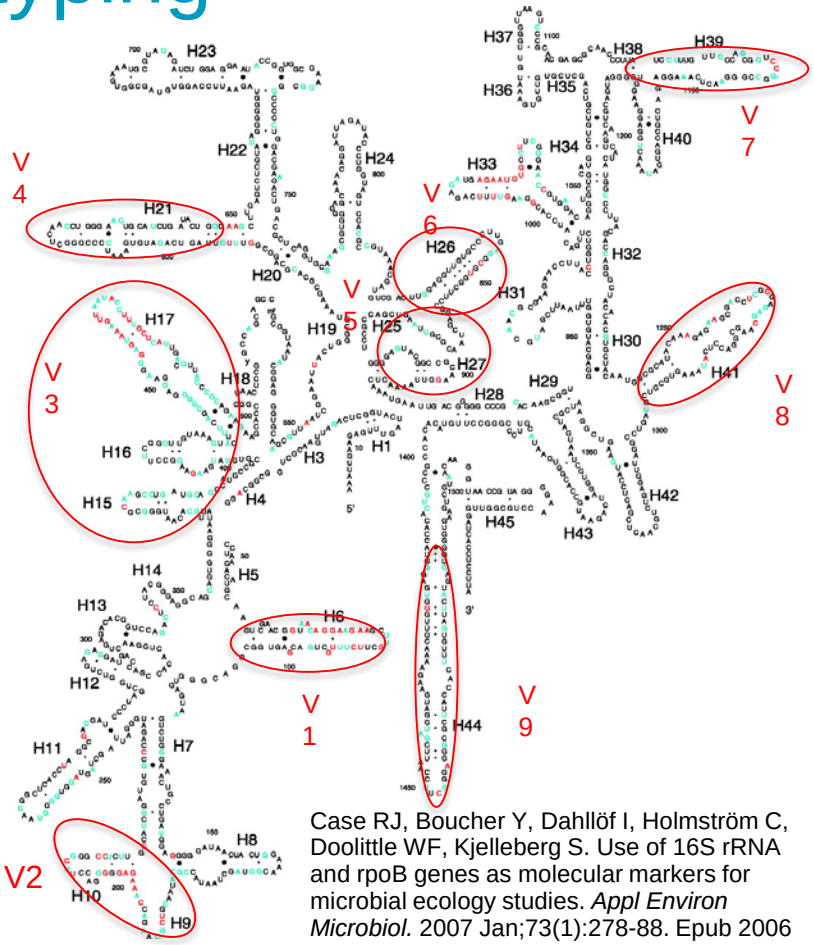
Bucci V, Tzen B, Li N, Simmons M, Tanoue T, Bogart E, Deng L, Yeliseyev V, Delaney ML, Liu Q, Olle B, Stein RR, Honda K, Bry L, Gerber GK. MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. Genome Biol. 2016 Jun 3;17(1):121. doi: 10.1186/s13059-016-0980-6.

Methods for studying the microbiome

- **Next generation sequencing**
  - 16S rRNA gene phylotyping
  - Metagenomics
  - Virome
  - Eukaryotic colonizers (fungi, protozoa, parasites)
- **Other molecular methods**
  - Targeted probes
  - Hybrid methods: immune or metabolite capture
- **Metabolomics**
  - Microbial metabolites in directed vs undirected fashion
  - SCFA GC/LC
  - Mass-spec profiles
- **Microbiologic**
  - Culture-based methods
  - Antigen detection
  - Microbial genetics and synthetic biologic techniques
- **Computational**
  - Bioinformatic tools: OTU clustering, metagenomics
  - Longitudinal dynamics, outcomes prediction, principled models
  - Essential to make sense of complex datasets and distinguish signal from noise

16S rRNA Gene Phylotyping

- Amplification over conserved regions of 16S rRNA (ITS, other conserved targets)
- NGS of amplified product (MiSeq, IonTorrent)
  - Short read platforms limit to V4, V1-3, V3-4 or 3-5
- Bioinformatic/computational methods to get to operational taxonomic units (OTU)

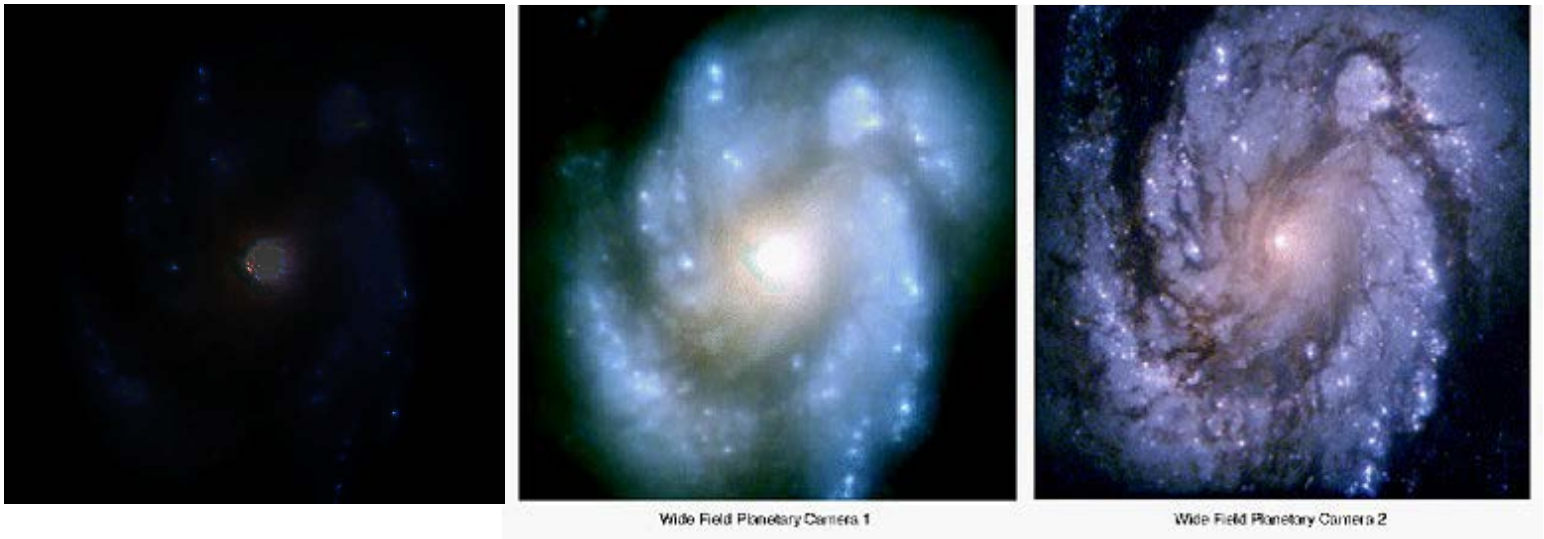


Case RJ, Boucher Y, Dahllöf I, Holmström C, Doolittle WF, Kjelleberg S. Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. *Appl Environ Microbiol.* 2007 Jan;73(1):278-88. Epub 2006 Oct 27.

454 GS FLX Figure Credit: Roche Diagnostics

16S rRNA Gene Phylotyping

- Has revolutionized evaluation of complex microbial ecosystems
- Limits with resolution and detection of ecosystem members
- Research use only assay



Before phylotyping methods

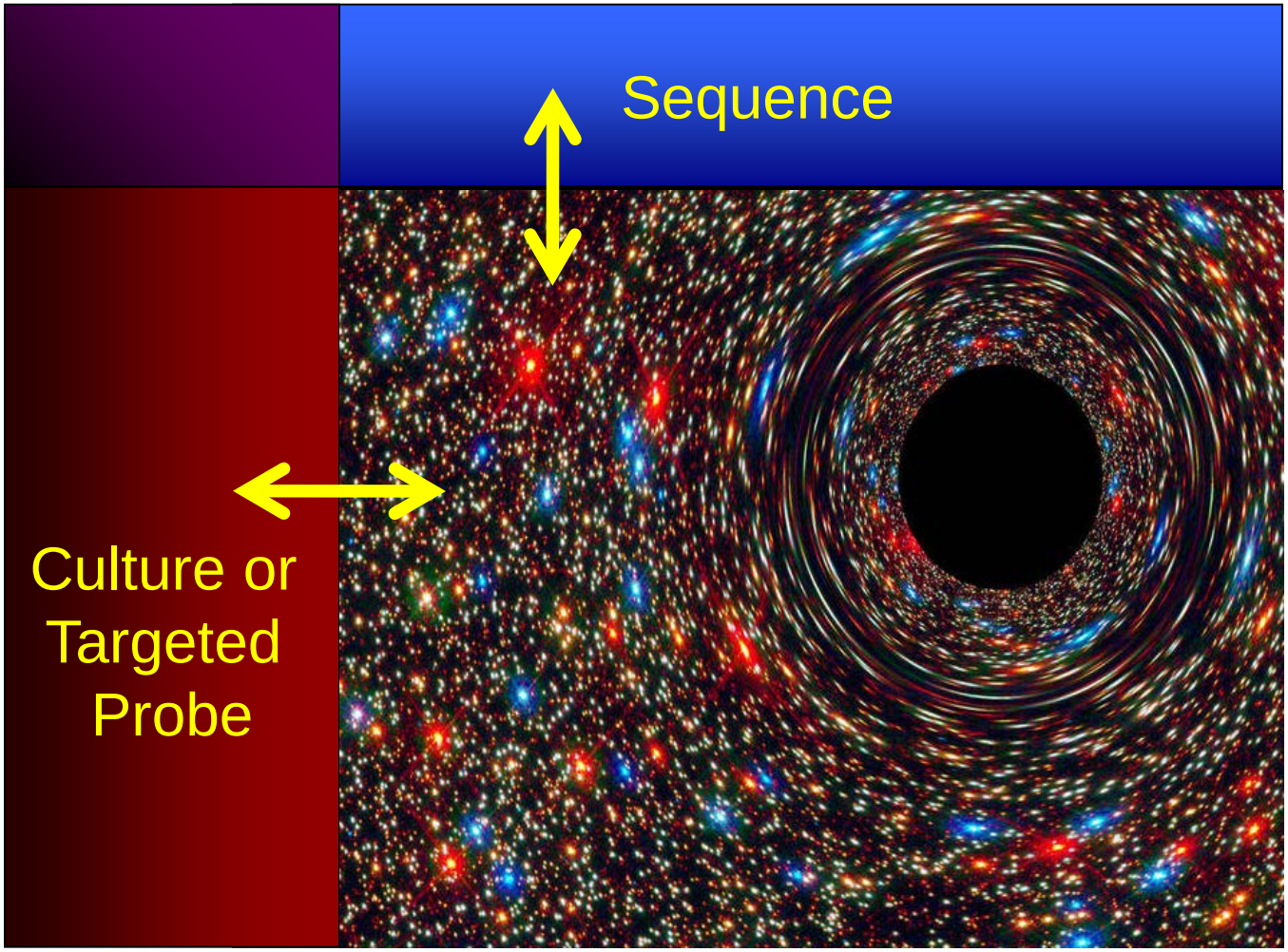
16S phylotyping on short-read platforms..

Where methods need to go

Credit: NASA

Sequence vs Culture or Probe-Based Methods

Complex ecosystems >10^{10} CFU/g

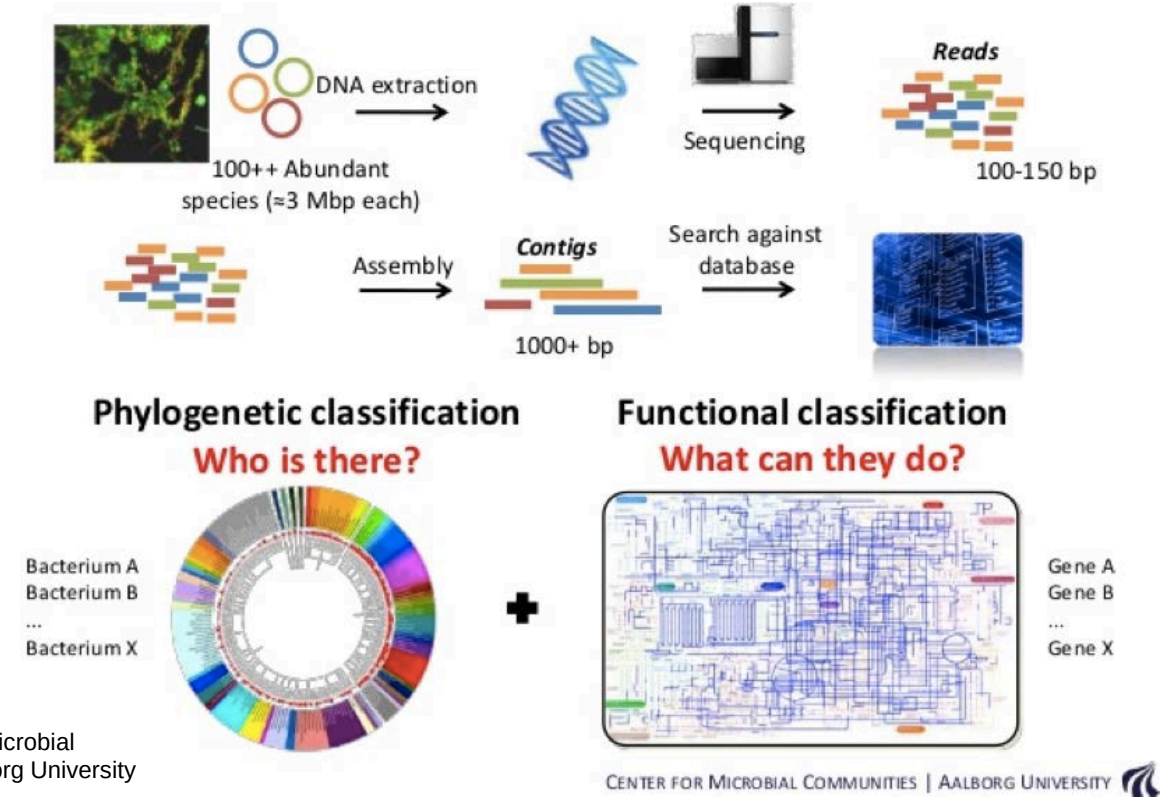


Log^{10} CFU/g

Organisms Present

Credit: NASA

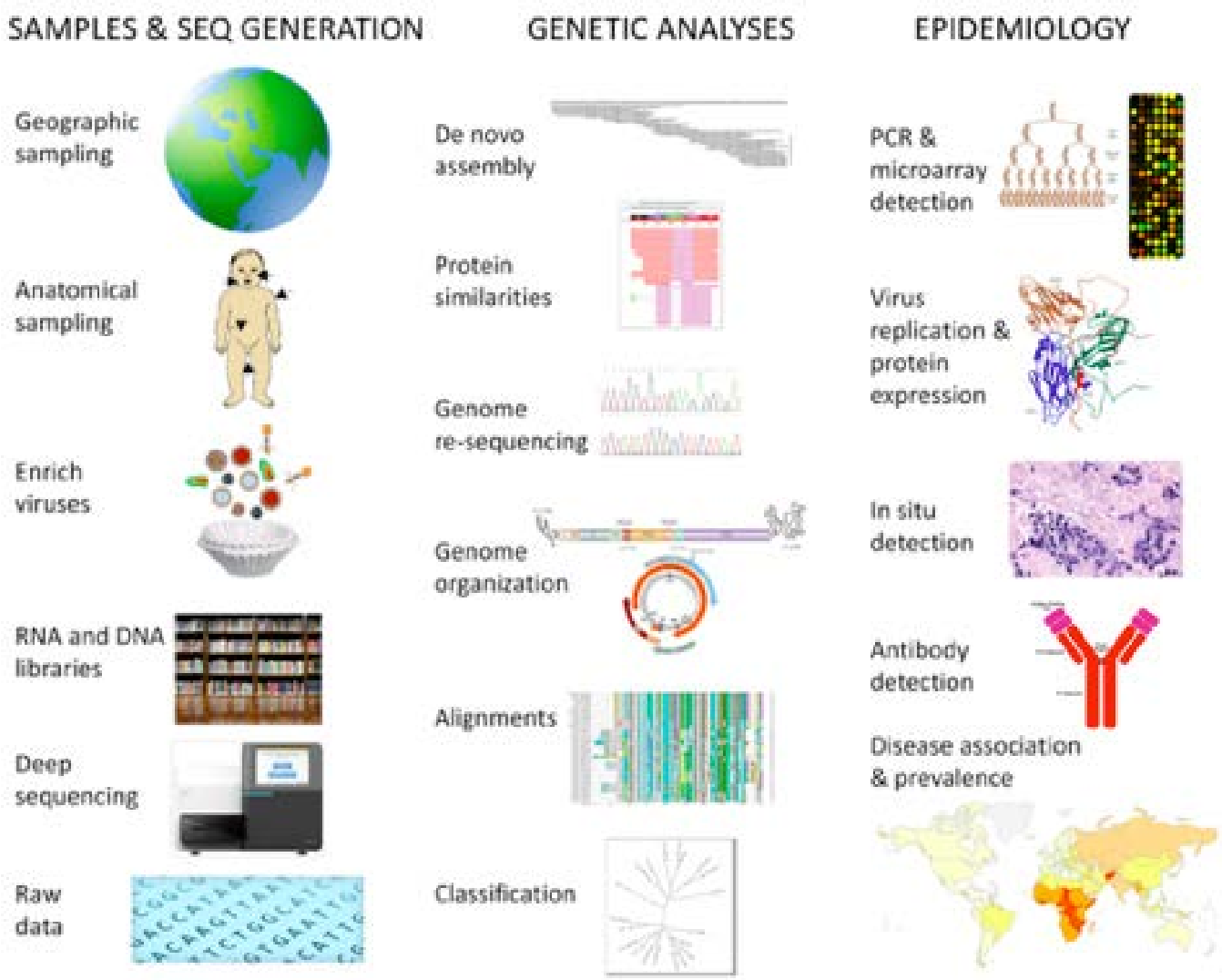
Metagenomic WGS Reads



Credit: Center for Microbial Communities, Aalborg University

- Component members and gene content
- More expensive and computationally intensive; needs for curated reference data
- **Clinical applications for *unbiased* pathogen detection**
  - CNS and other sterile body sites
  - Immunocompromised patients (urine in kidney transplant patients, e.g.)

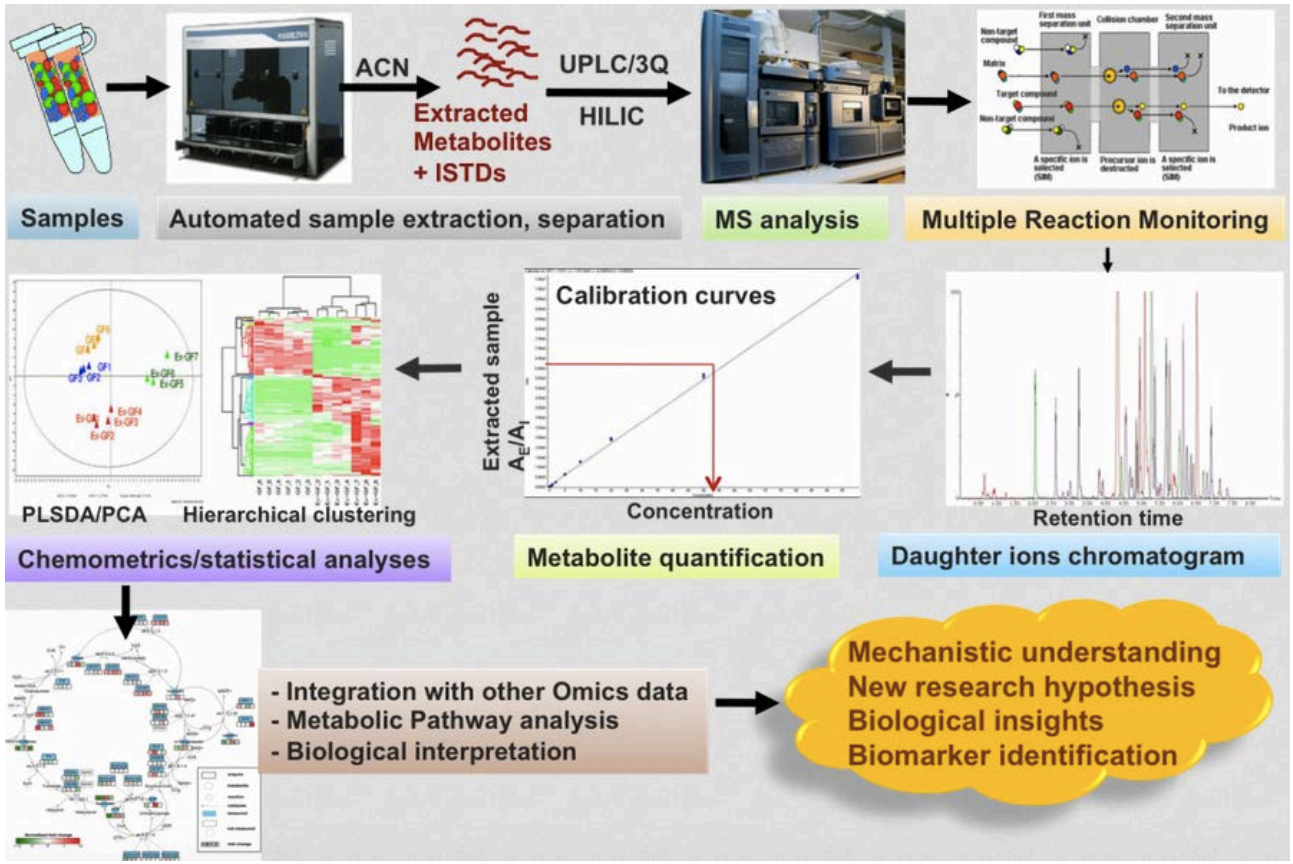
Virome Analyses (Host and Phage)



Delwart E. A roadmap to the human virome. *PLoS Pathog.* 2013 Feb;9(2):e1003146. doi: 10.1371/journal.ppat.1003146.

Metabolomics/Metabolite Phenotyping

- MassSpec, GC/LC: Microbial ID via MALDI-TOF, SCFA profiles
- Important for defining “dysbiosis” and microbial factors that can be used diagnostics or to predict patient outcomes
  - Incorporate with host-makers



Courtesy: A/Prof. Vidya Velagapudi

Case Studies: Host-Microbiome Systems

(1) **Infectious Disease:** C. difficile colitis: development of therapeutic microbiota

(2) **Dysbiosis in disease**: Therapeutic microbiota and small molecule targets in IBD and other diseases

(3) **Microbiota-mediated effects in cancer** development and treatment

(4) **Drug and xenobiotic metabolism**: IBD and cancer therapeutics; other classes

(5) **Microbiota-mediated effects in cardiovascular disease**

Clostridium difficile colitis

- **Pseudomembranous colitis**
  - 5-10% population colonized with *C. difficile*
    - Not all strains are toxigenic
  - Substantive morbidity and mortality, particularly in immunocompromised patiens
  - 10-20% of patients fail antibiotic treatment to develop recurrent C. diff.

- Microbiota-mediated protection
  - Positive and negative effects from primary and secondary bile acids on C. diff germination
  - Competition for nutrients, colonization niches

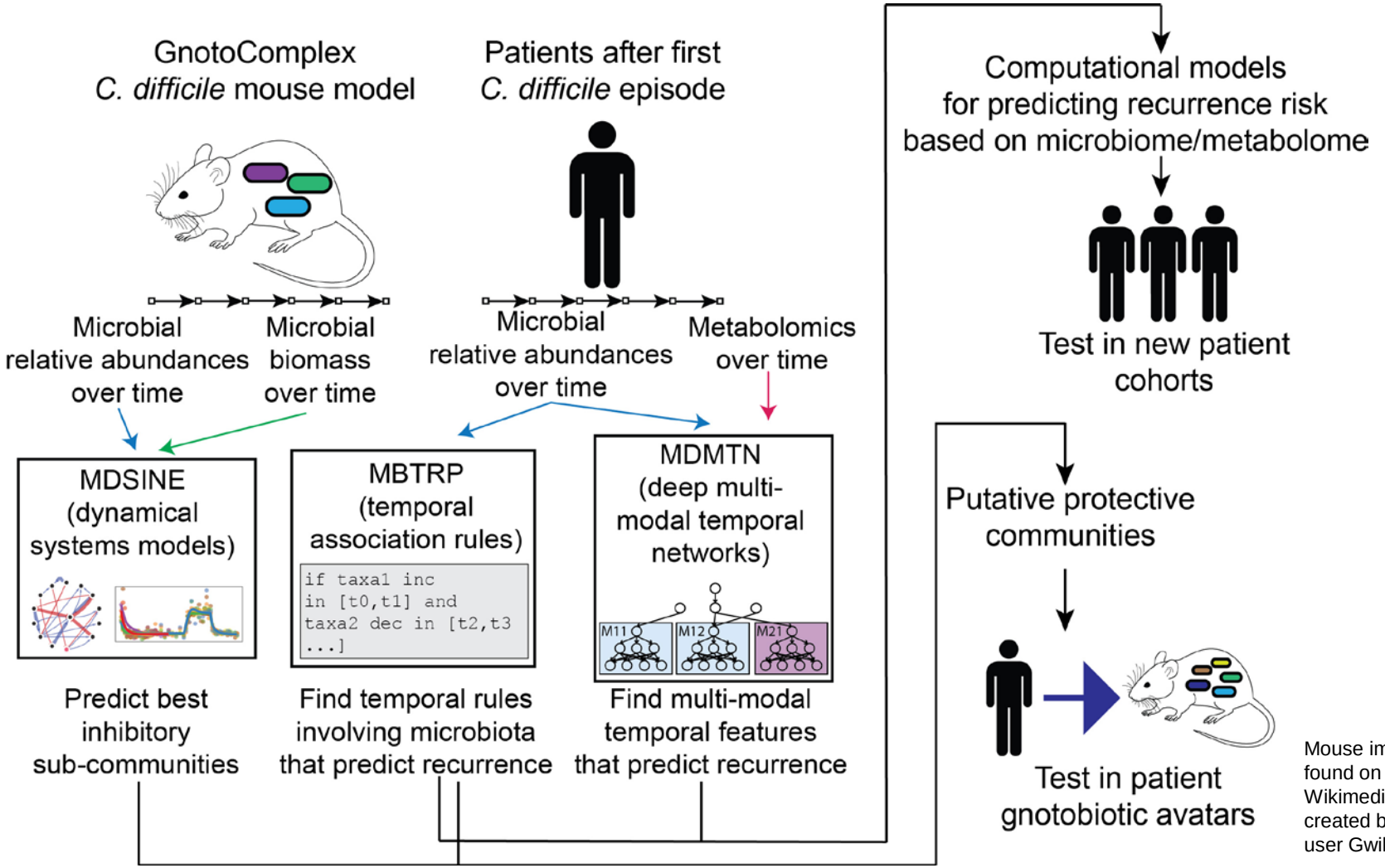
- Therapy for recurrent infection: Fecal Microbiota Transplant/FMT
  - Colonoscope vs oral capsule
  - OpenBiome
  - Seres Therapeutics, Rebiotix, Finch Therapeutics others

- Target therapy per missing microbial activities

Yu J, Kim NY, Lee HM, Lee HN, Ahn HJ, Kim SW, Choi KY. A Case of Pseudomembranous Colitis in a Juvenile Rheumatoid Arthritis Patient Taking Methotrexate. *Korean J Gastroenterol*. 2010 Dec;56(6):387-390.

Infectious Disease Models: *C. difficile* colitis

Integrated Infrastructure: Clinical, Microbiologic, Computational and Animal



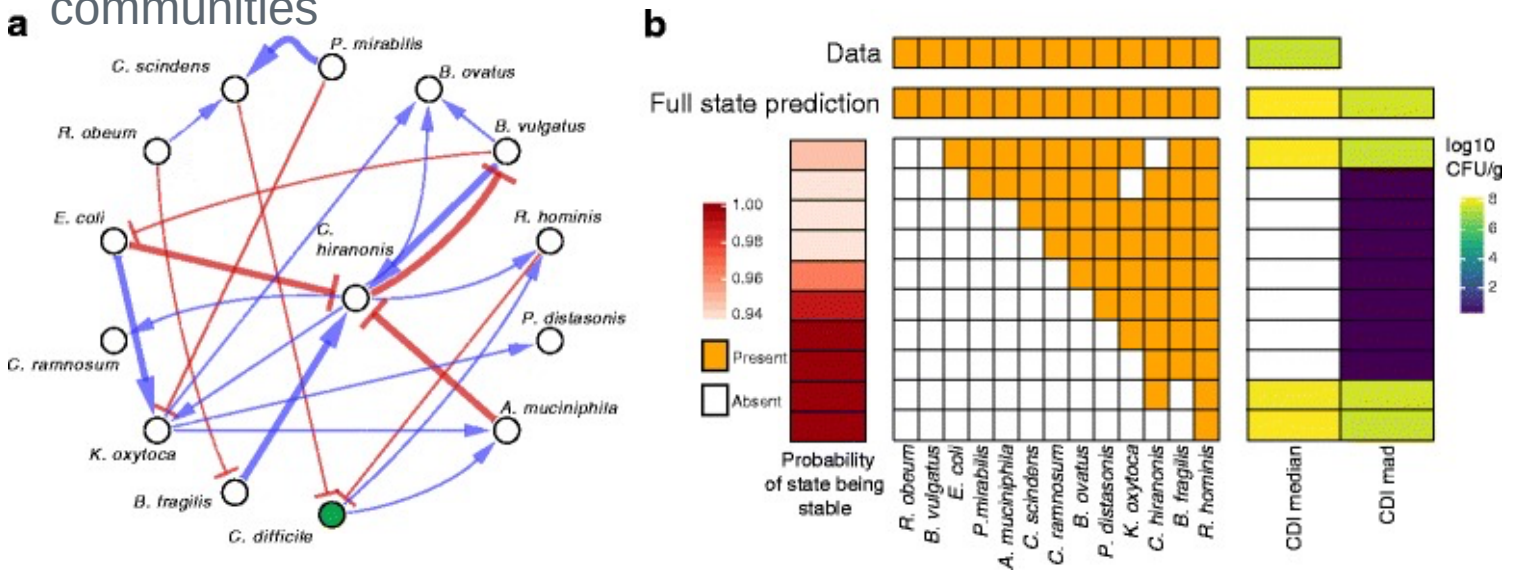
Mouse image found on Wikimedia, created by user Gwilz

Bucci V, Tzen B, Li N, Simmons M, Tanoue T, Bogart E, Deng L, Yeliseyev V, Delaney ML, Liu Q, Olle B, Stein RR, Honda K, Bry L, Gerber GK. MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. *Genome Biol.* 2016 Jun 3;17(1):121. doi: 10.1186/s13059-016-0980-6.

Infectious Disease Models: *C. difficile* colitis

Microbe Interaction Network communities

Prediction of therapeutic defined



- Defined formulations will be available for treatment of recurrent *C. diff*

Pathology applications

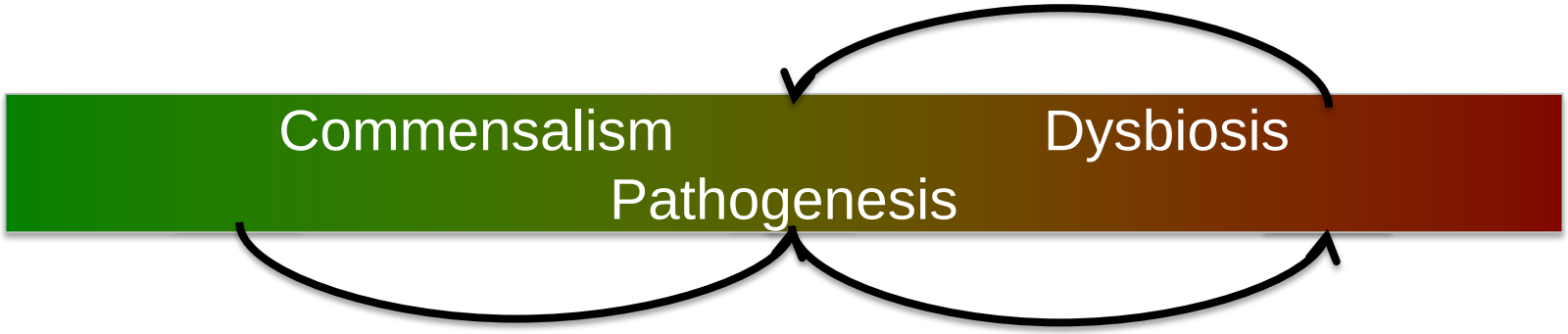
- Biomarkers to assess patients at risk of recurrence, predict successful therapy
- Stool-based analytes vs microbial community signatures
- As blood banks manage blood transfusion will micro labs manage therapeutic microbial communities, whether from vendor or in-house sources.

Bucci V, Tzen B, Li N, Simmons M, Tanoue T, Bogart E, Deng L, Yeliseyev V, Delaney ML, Liu Q, Olle B, Stein RR, Honda K, Bry L, Gerber GK. MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. *Genome Biol.* 2016 Jun 3;17(1):121. doi: 10.1186/s13059-016-0980-6.

Dysbiosis in Disease

Many factors promote dysbiosis => IBD, IBS, NASH, others..

2. Infections alter the microbiota



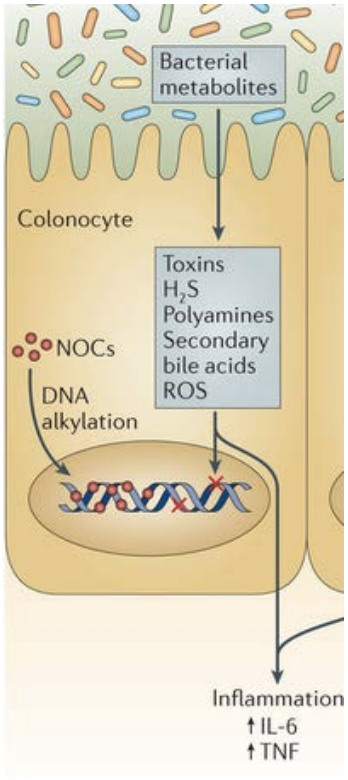
1. Diet, drugs, patient's immune status, changes in gut barrier or lumenal environment.

3. Dysbiosis alters host susceptibility to infections

- **<u>Pathology Applications</u>**:
  - Specific biomarkers that are predictive of dysbiotic conditions
    - Microbial metabolites or products
      - NASH, subsets of diabetic/pre-diabetic patients
    - Host markers
      - Metabolic, immune, hormonal
      - IBS, auto-immune and allergic diseases
  - Computational modeling of patient-microbial dynamics

Microbiome in Cancer

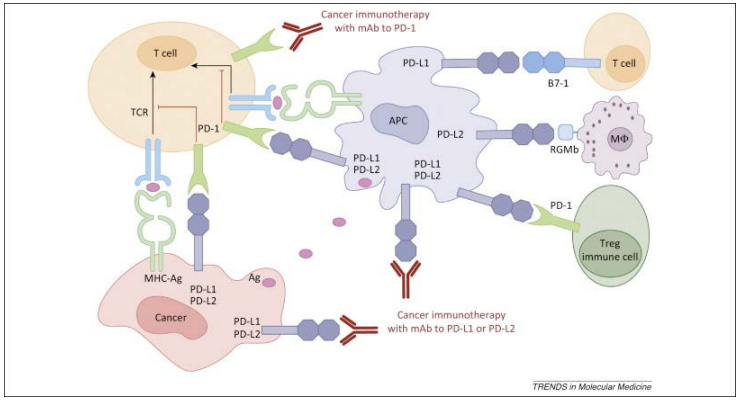
- **Direct and indirect effects on carcinogenesis**
  - Microbial biotransformations: carcinogens, ingested cyanogenic glycosides, environmental toxicants.
  - Chronic inflammation: *H pylori,* HCV
  - Epithelial cell turnover -> commensal interactions with APC/MIN and other cell division pathways
- **Impact on drug efficacy and novel drug targets**
  - Immunomodulatory functions => efficacy of checkpoint inhibitors
  - Direct biotransformation of oral and IV-administered anti-neoplastics: Irinotecan => microbiota-mediated toxicities
  - Gut commensal communities and host susceptibility to GVHD post-SCT.



Louis, P., et al. (2014). "The gut microbiota, bacterial metabolites and colorectal cancer." Nat Rev Micro 12(10): 661-672.

Pathology Applications:

- Assessment of microbial communities impacting drug efficacy or toxic profiles
- Identify pathobionts -> pathogens important in diagnosis and management
- Small molecule targets on host or microbial side important for dx and therapeutic implications



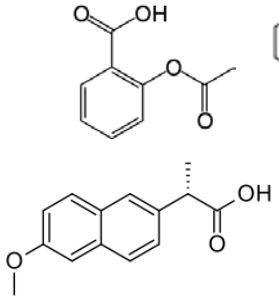
Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1 and PD-L1 pathway. Trends Mol Med. 2015 Jan;21(1):24-33. doi: 10.1016/j.molmed.2014.10.009.

Drug Metabolism

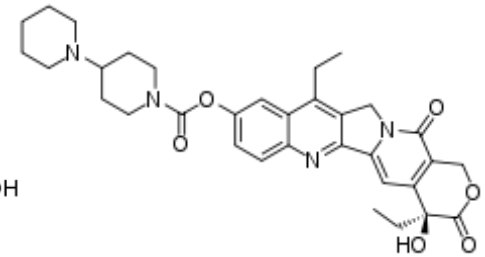
- Microbiota-mediated biotransformation of drugs

Microbiota-mediated Toxicities

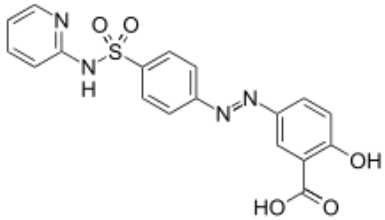
Altered or Reduced Efficacy



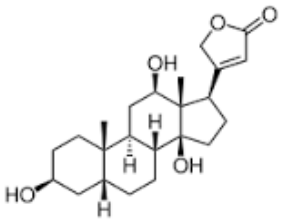
NSAIDS



Irinotecan



Sulfasalazine

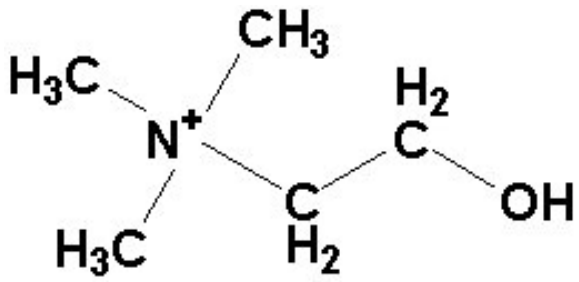


Digoxigenin

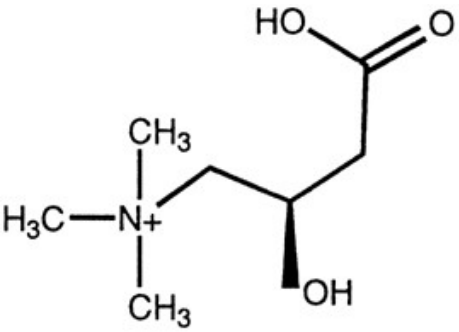
- **Pathology Applications:**
  - Assay for microbial drug-transforming activities
    - Microbial beta-glucuronidases, sterol metabolizing enzymes
  - Contributions to dysbiosis
    - Immunomodulators, anti-microbials, altered host factors (bile acids, antibodies)
  - Immunologic competence for therapeutics to act
    - Checkpoint inhibitors – necessary microbial activities or post-intervention to boost activities (microbial and immunologic markers)
  - Incorporate with therapeutic drug monitoring (TDM)

II. Microbiota effects on platelet activation -> cardiovascular disease

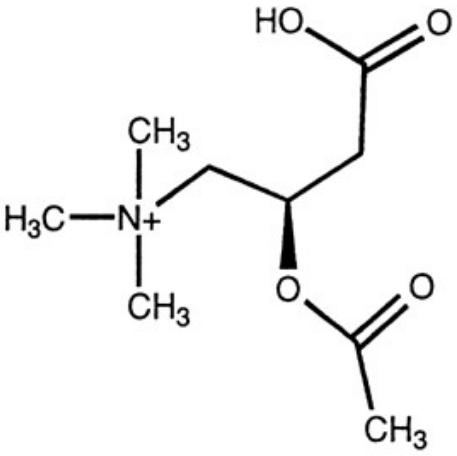
Dietary, host sources



Choline

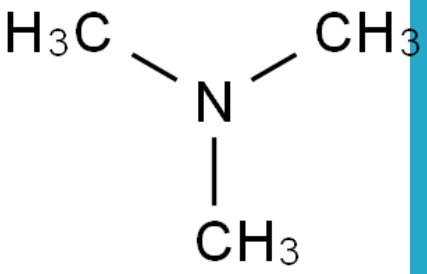


L-Carnitine



Acetyl-L-Carnitine

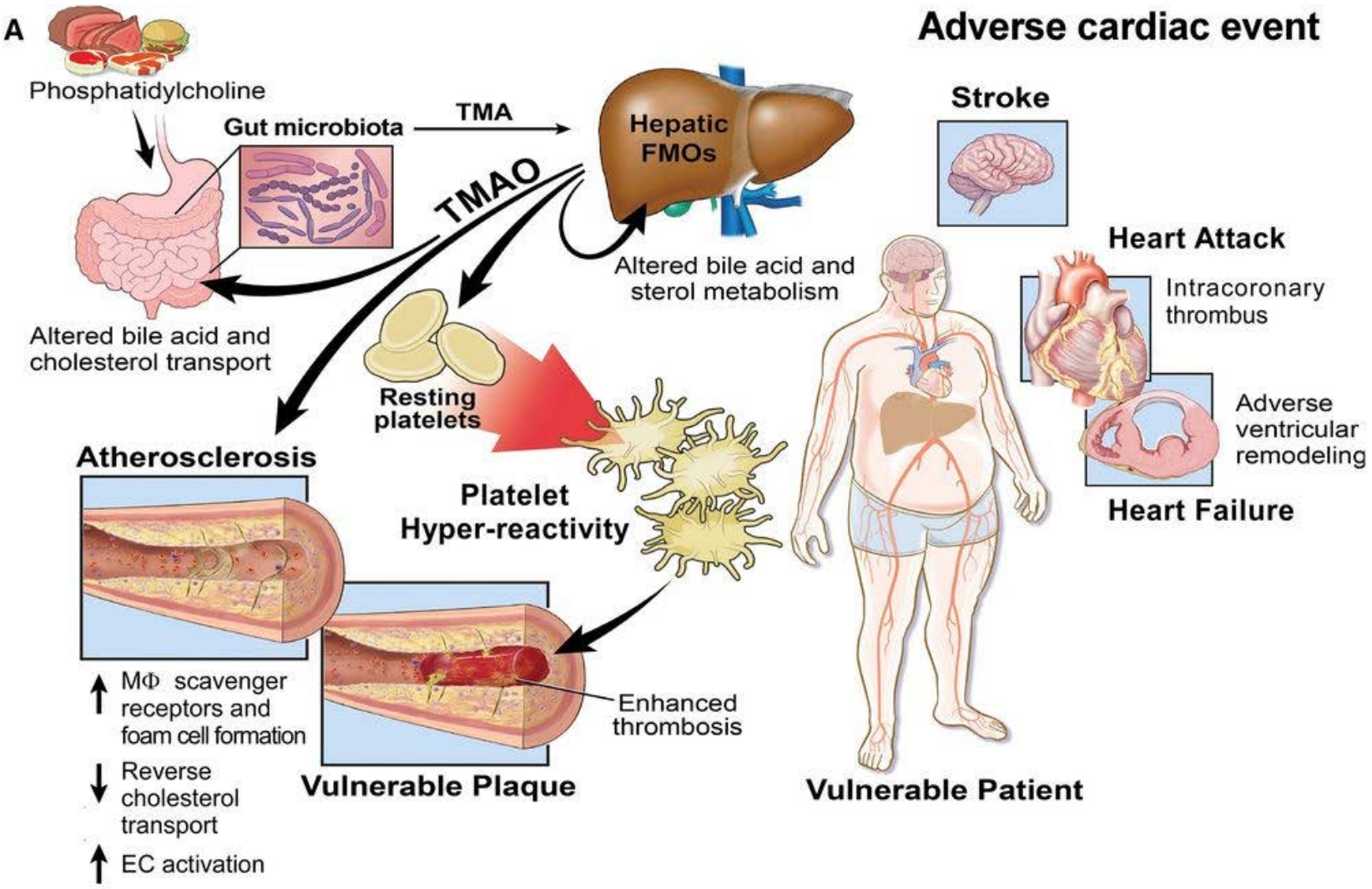
Microbial Ammonia lyases



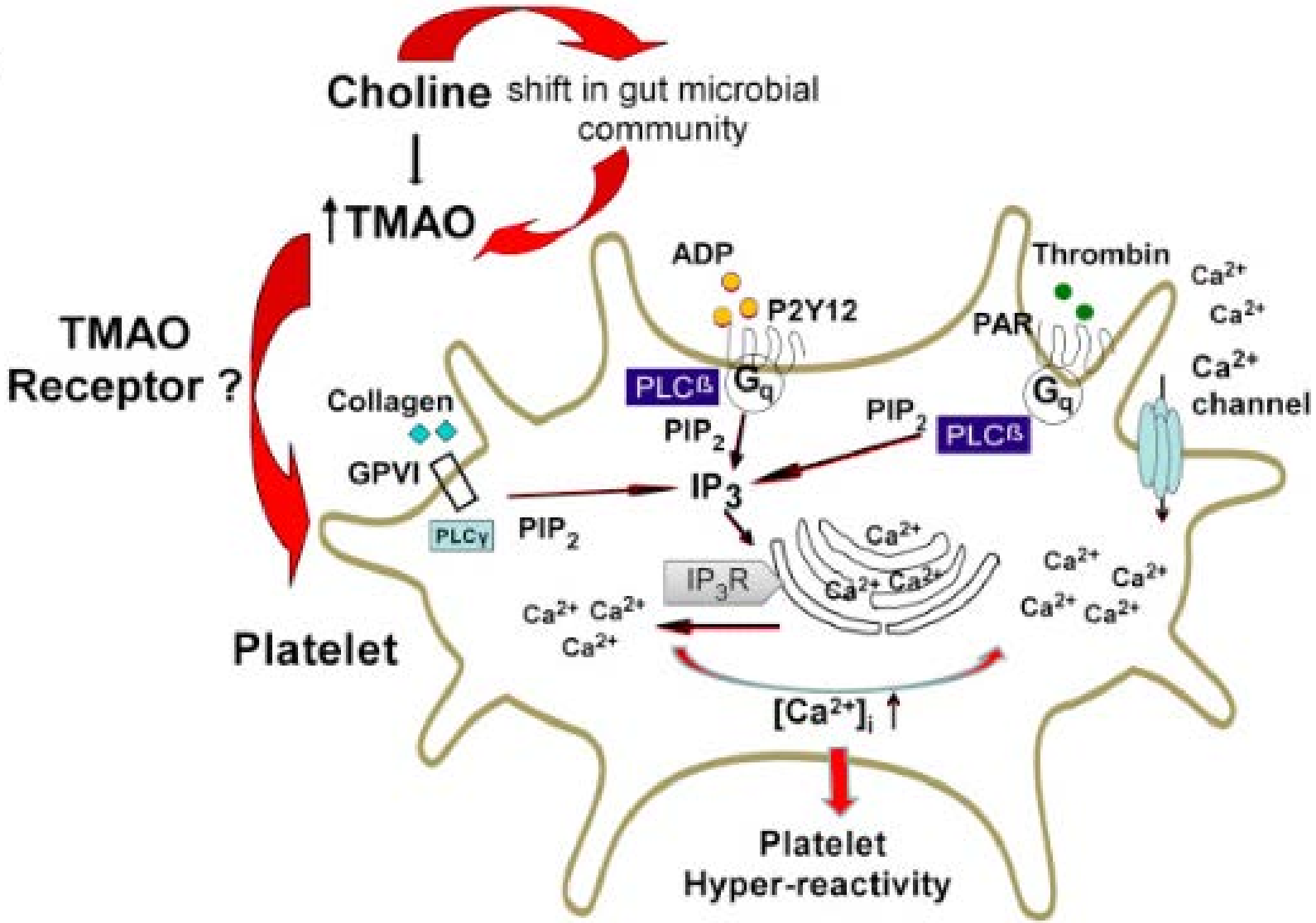
Trimethylamine (TMA)

Microbial cutC/cutD genes

- Homologs present in many species of commensal bacteria
- Best described in *E. coli* and related species of Enterobacteriaceae (Proteobacteria).
- Present in Bacteroidetes, Firmicutes and species from other microbial phyla
- ***cutC: ammonia lyase***
- ***cutD: activating protein***
- cut and eut operon systems (ethanolamine utilization) -> carboxysome or carboxysome-like cellular structure
  - Sequester radical enzymatic and/or highly O2 sensitive reactions
  - Assay by PCR signatures, enzymatic activity, or metabolic products in stool vs plasma.



Zhu W, Gregory JC, Org E, et al. Gut Microbial Metabolite TMAO Enhances Platelet Hyperreactivity and Thrombosis Risk. *Cell*. 2016;165(1):111-124. doi:10.1016/j.cell.2016.02.011



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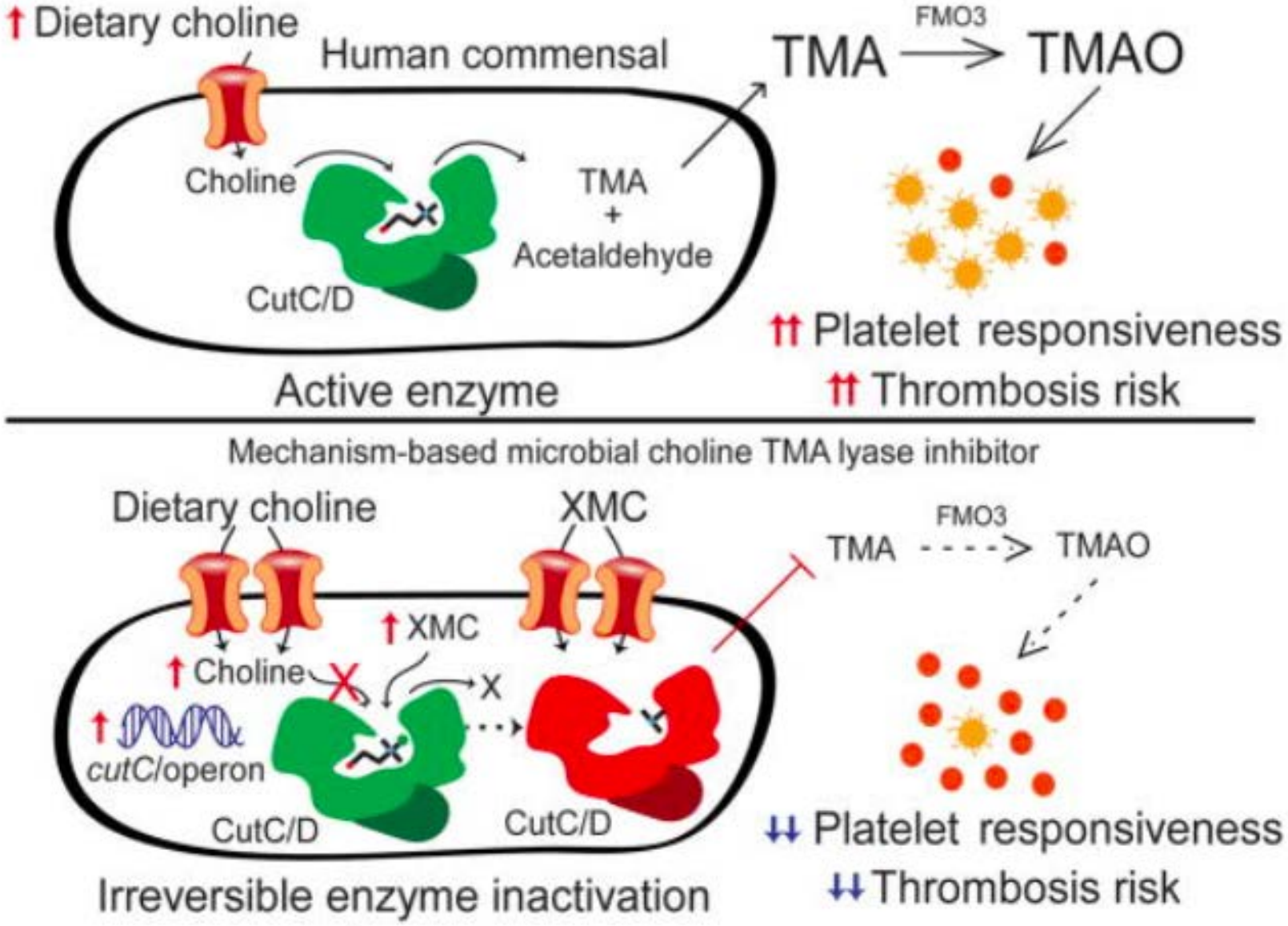


Roberts AB, Gu X, Buffa JA, et al. Development of a gut microbe–targeted nonlethal therapeutic to inhibit thrombosis potential. *Nature Medicine*. 2018;24(9):1407-1417. doi:10.1038/s41591-018-0128-1

Microbiome Modulation of CV Disease

- **Diagnostic opportunities**
  - Stool and plasma-based testing
  - Dietary, microbial and host metabolites
  - Integrate with functional platelet/coagulation testing
  - Integrate with other cardiovascular risk markers
  - Therapeutic drug monitoring of cutC/D inhibitors to assess therapeutic efficacy (stool vs plasma testing)

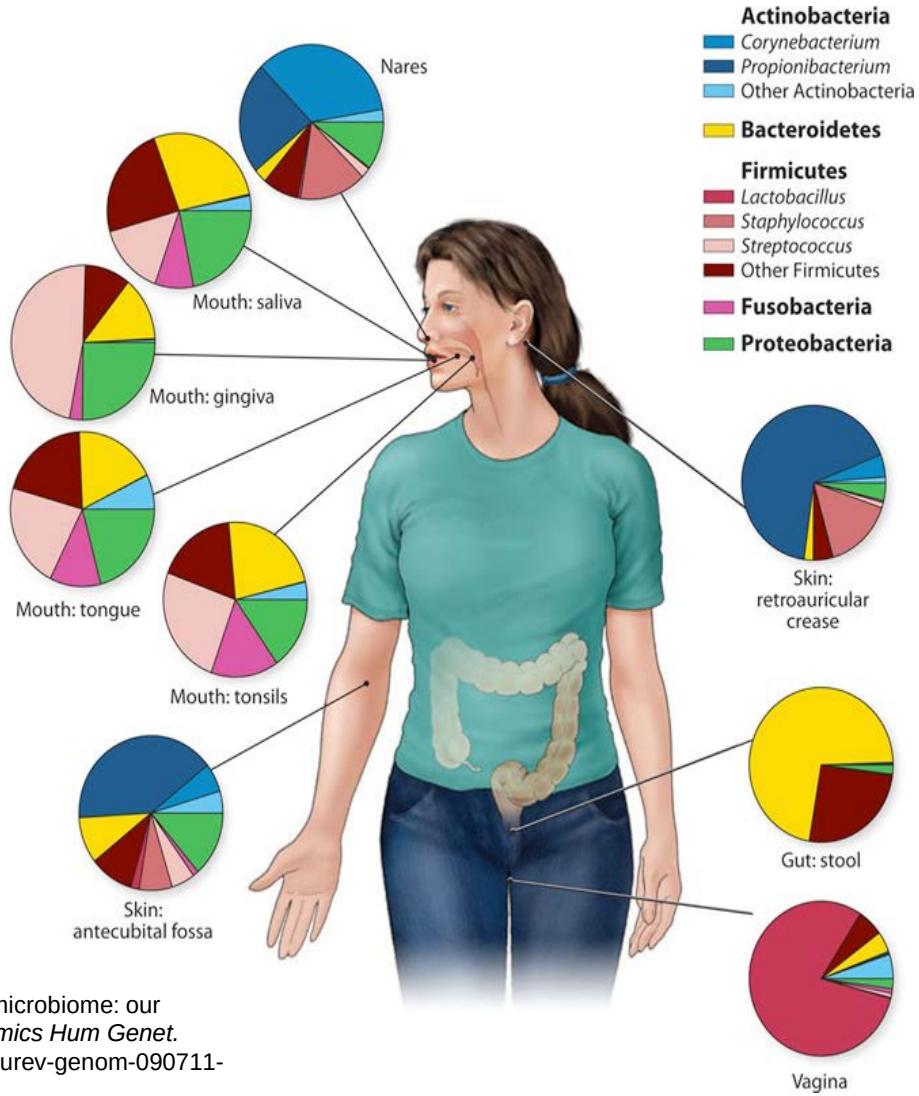
- **Needs**
  - Further study of effects in patient populations to understand credible contributions to disease risk and progression
  - Optimization of existing CLIA platforms vs new modalities
    - MALDI-TOF, GCLC, Immunoassay vs molecular methods

Pathology, Pathologists and the Microbiome

- **Sanity check for moving things to productive clinical use**
  - Assessment of the literature
  - Assuring robust evidence for clinical use
  - Participating in, and providing infrastructure for clinical trials

- **New and existing methods and resources will be used**
  - Microbiology, chemistry, TDM, immunology, AP services, others
  - Need for incorporation of computational models
  - Warehousing of microbiome and pathogen genomic information
  - “Bugs as drugs” – oversight and quality programs
  - CLIA-level accreditation programs that support existing areas of the lab that will be used, and potential new testing modalities

Questions

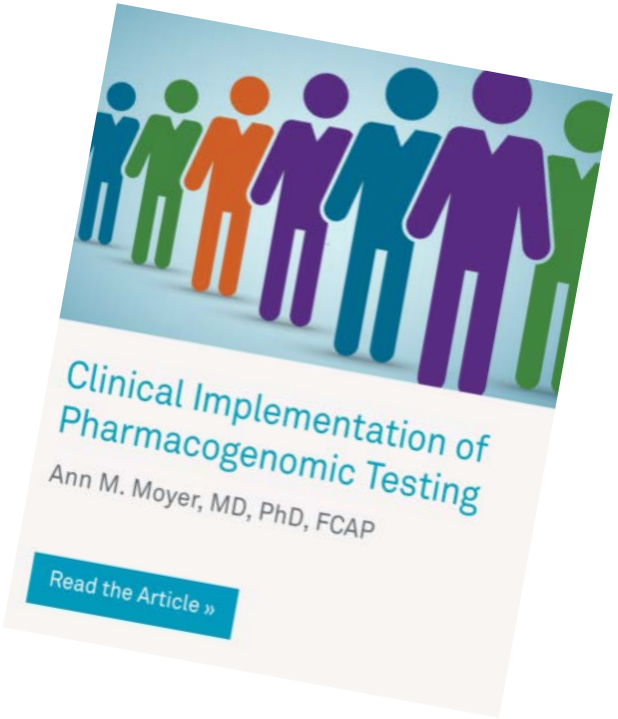


Grice EA, Segre JA. The human microbiome: our second genome. *Annu Rev Genomics Hum Genet.* 2012;13:151-70. doi: 10.1146/annurev-genom-090711-163814.

CAP's Precision Medicine Webpage

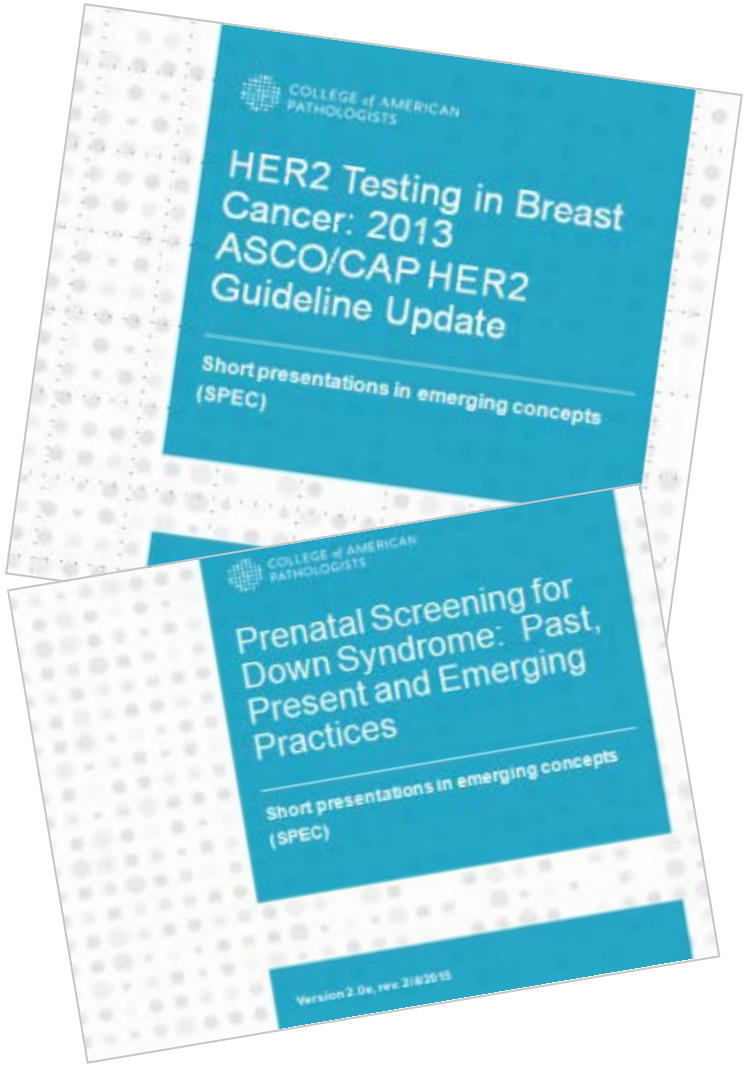
- **The webpage includes brief, relevant articles by CAP members that enable the reader to gain a better understanding of a particular area of precision medicine.**
  - **Examples include pharmacogenetics, immune response genes, and the latest in the molecular drivers of cancer.**
  - **Access them www.cap.org >**

    Member Resources > Precision Medicine



Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **Short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **Recent topics include:**
  - **Microbiome**
  - **Biomarkers in Lung Cancer**
  - **MDS**
  - **Other emerging topics**
- **Access them at www.cap.org > Resources and Publications**



CAP's Pathology Resource Guide: Precision Medicine

- **The CAP has created the Pathology Resource Guides to assist pathologists in understanding key emerging technologies.**
  - **Printed guides are now available for members ($39) and non-members ($69)**
  - **The digital copy of the Resource Guides are a complimentary member benefit**
  - **Access them www.cap.org > Resources and Publications**

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- Women learn the importance of preventive care through annual exams, a Pap test, Mammogram and a healthy lifestyle

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THANK YOU!

Thank you for attending our webinar, "Diagnostic Applications of the Microbiome" by

Lynn Bry, MD, PhD

For comments about this webinar or suggestions for upcoming webinars, please contact phcwebinars@cap.org.

NOTE: There is no CME/CE credit available for today's free webinar. The PDF of the presentation will be sent out in a week.

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