COLLEGE of AMERICAN PATHOLOGISTS

Microbiome Applications in Pathology

CAP Webinar

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PARTNERS HEALTHCARE | FOUNDED BY BRIGHAM AND WOMEN'S HOSPITAL AND MASSACHUSETTS GENERAL HOSPITAL

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Lynn Bry, MD, PhD, FCAP

- **Director at the Massachusetts Host-Microbiome Center & Crimson Core**
- **Associate Pathologist at the Brigham and Women's Hospital**
- **Associate Professor of Pathology at the Harvard Medical School**

Disclosures

- **Dr. Bry has no disclosures.**

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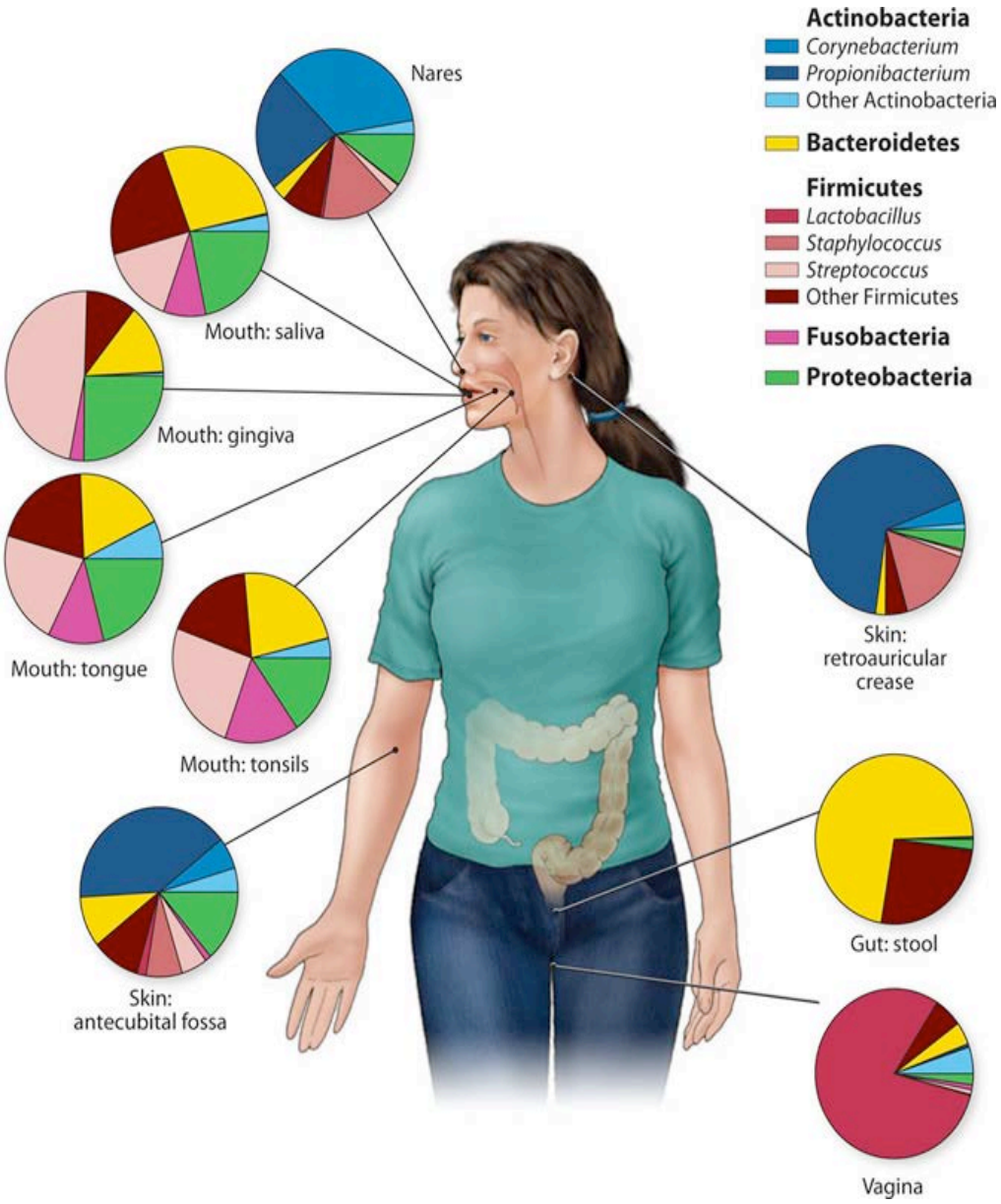
The Microbiome



- **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.**

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.

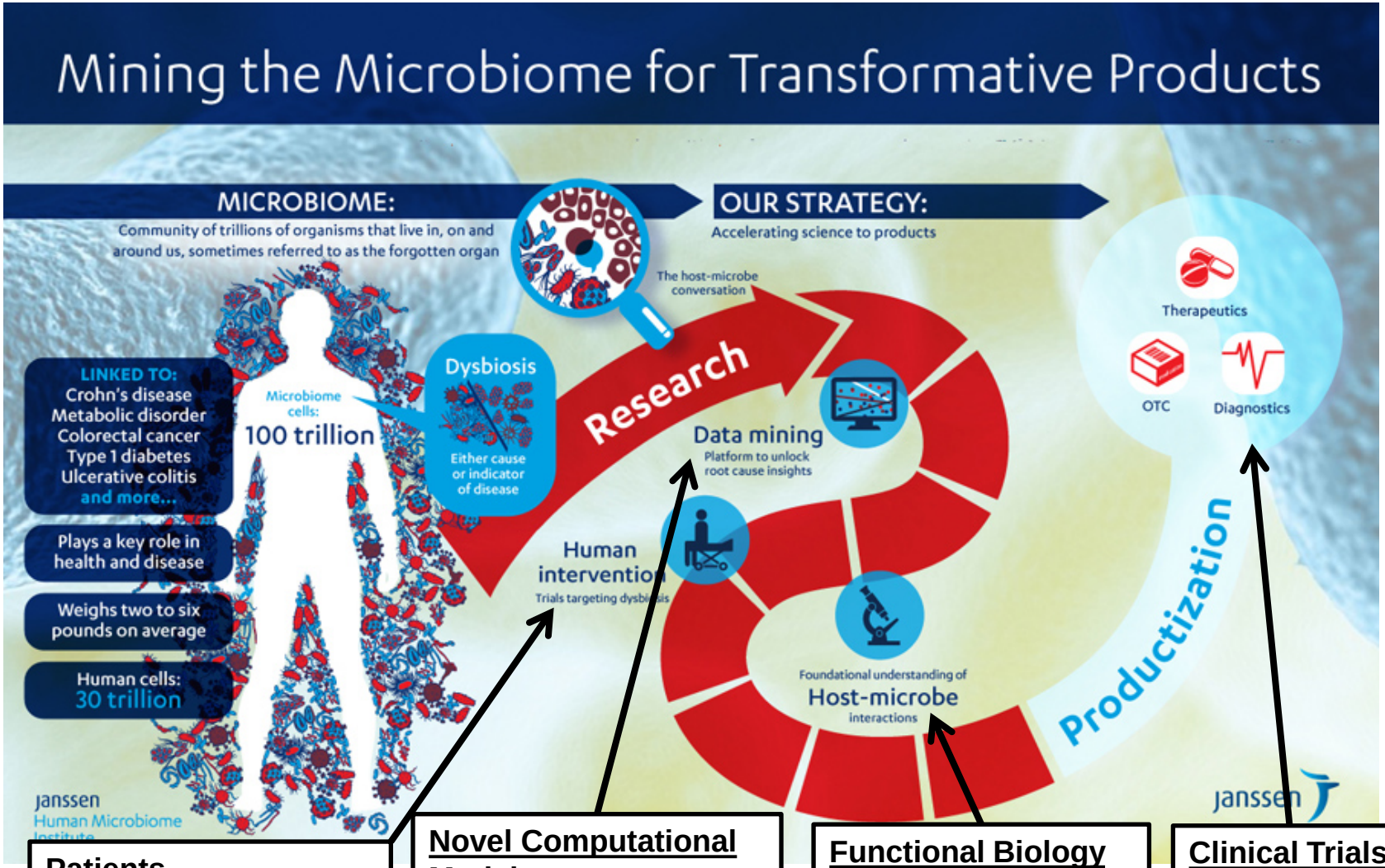
Impact of host-microbiome interactions

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



Patients
- CLIA microbiome
- Data/Sample Registries

Novel Computational Models
- Longitudinal Dynamics
- Outcomes prediction

Functional Biology
- Gnotobiotics
- Microbiology/Genetics

Clinical Trials CLIA Lab

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Janssen Global Services. Mining the Microbiome for Transormative Products. Available at http://www.janssen.com/human-microbiome-institute. Accessed October 6, 2016.

Pathology applications

Current applications

- *C. difficile* colitis diagnosis and FMT donor screening
- Infectious disease applications using NGS/WGS metagenomic reads
  - Unbiased methods for pathogen detection
- Women's health
- Oral health: periodontitis, dental caries
  - Impact of oral health to other disease conditions: diabetes, pregnancy

Future applications

- Will evolve as we have better mechanistic understanding of microbiota-mediated contributions to disease and targeted therapies
- May leverage existing AP/CP testing (eg, bile acids, immunology, targeted organisms)
- Community effects vs defined biomarkers
- Not limited to effects on colonized surfaces
  - Endocrine, cardiovascular, non-IBD/auto-immune, CNS
  - Carcinogenesis and cancer treatment/prognosis

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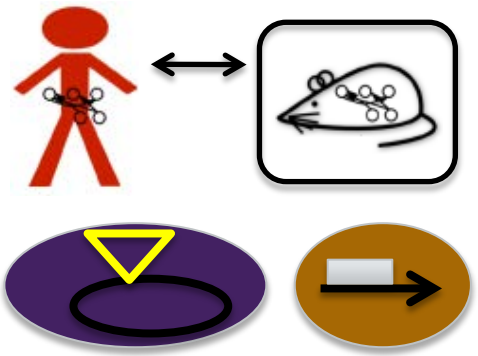
Bedside➡Bench➡Bedside Infrastructure

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

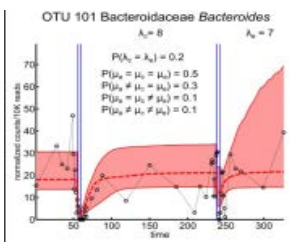


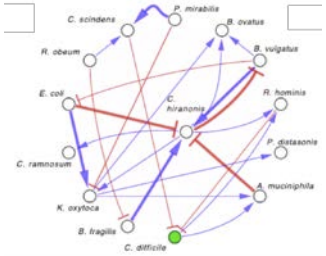
Patient Cohorts
- Biobank/Data Registries
- CLIA Microbiome

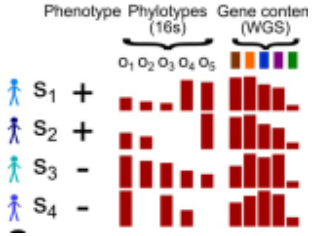
Functional Biology
- Gnotobiotics, in vitro systems
- Microbiology/Genetics, SynthBio

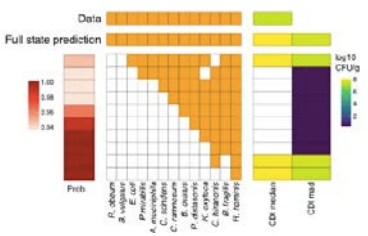
Clinical Trials CLIA Lab

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**

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Methods for studying the microbiome, continued

- **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**

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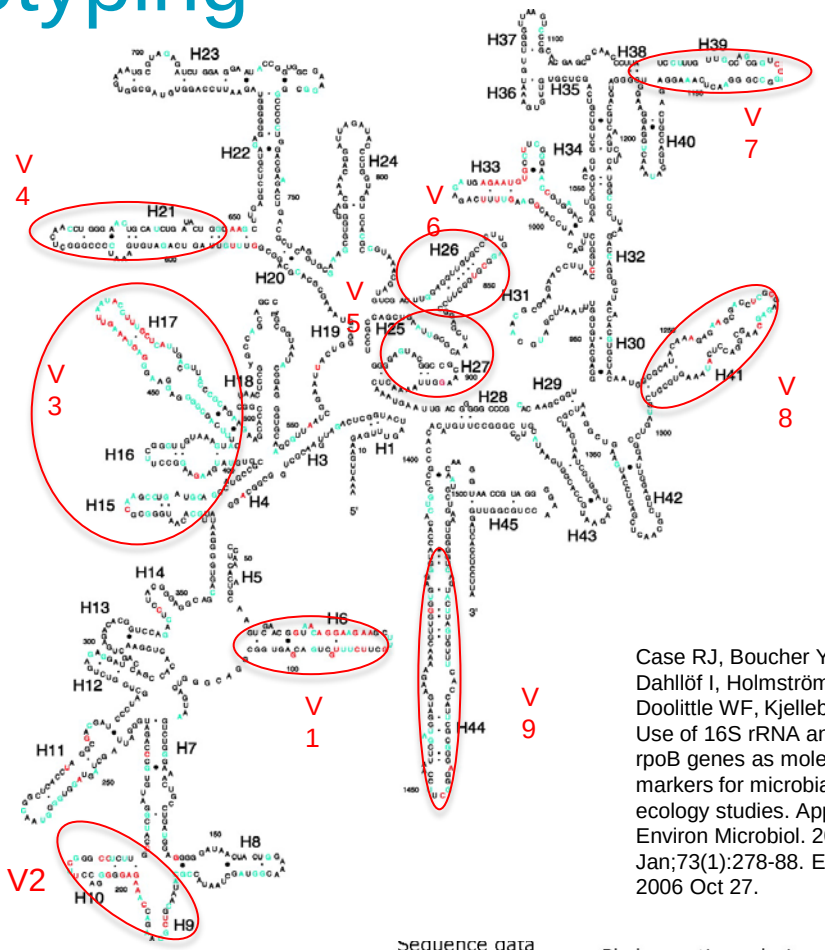
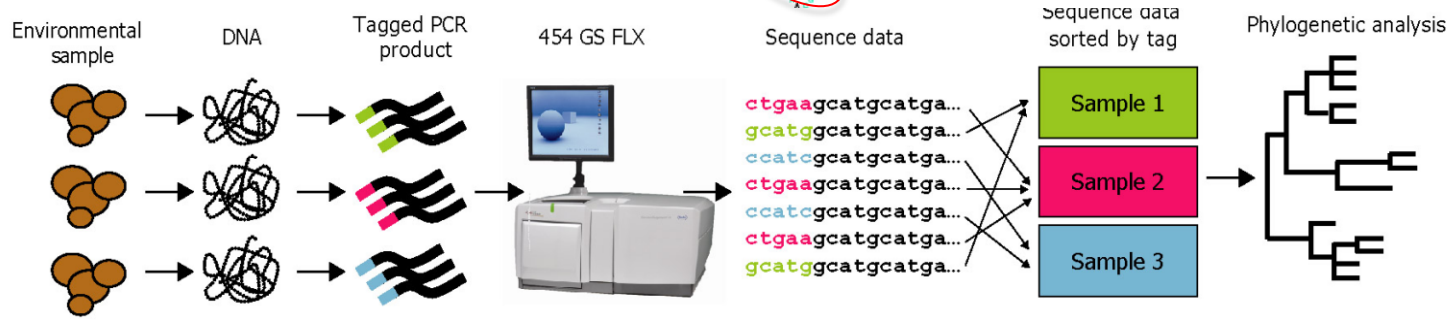
16S rRNA gene phylotyping

- **Amplification over conserved regions of 16S rRNA (ITS, other conserved targets)**
- **NGS of amplified product (MiSeq, IonTorred)**
  - **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.



Credit: Roche Diagnostics

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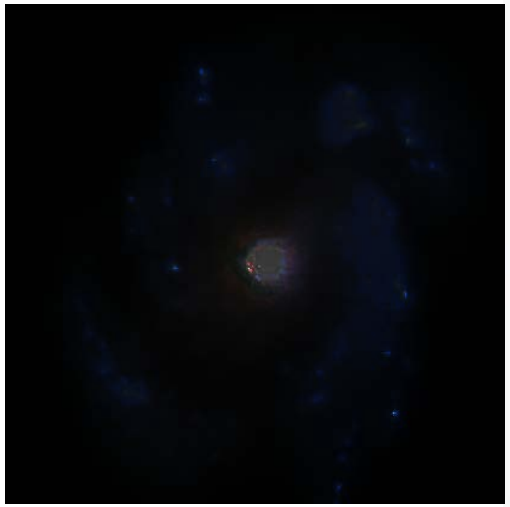
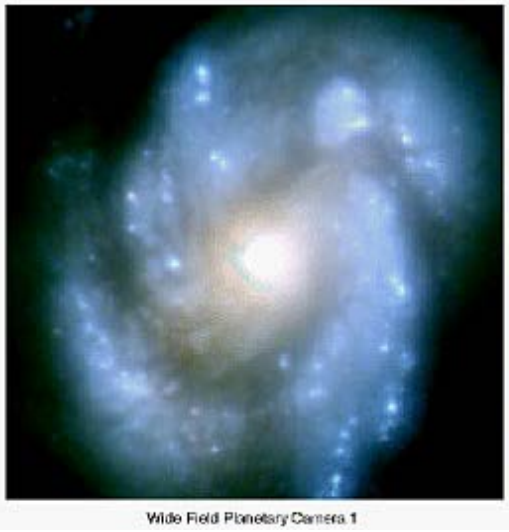
16S rRNA gene phylotyping

- **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

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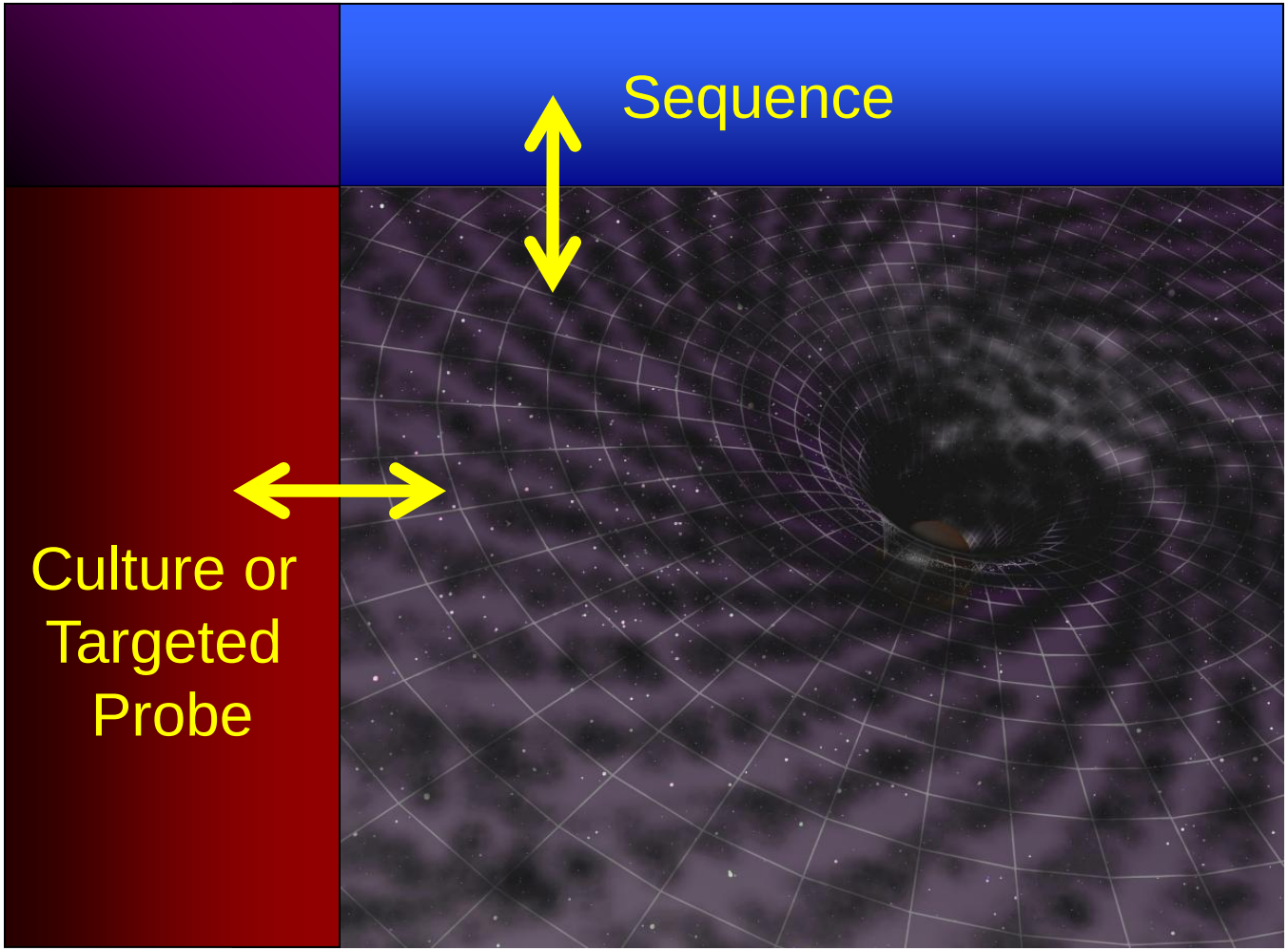
Photo credit: NASA

Sequence vs culture or probe-based methods

Complex ecosystems >10^{10} CFU/g

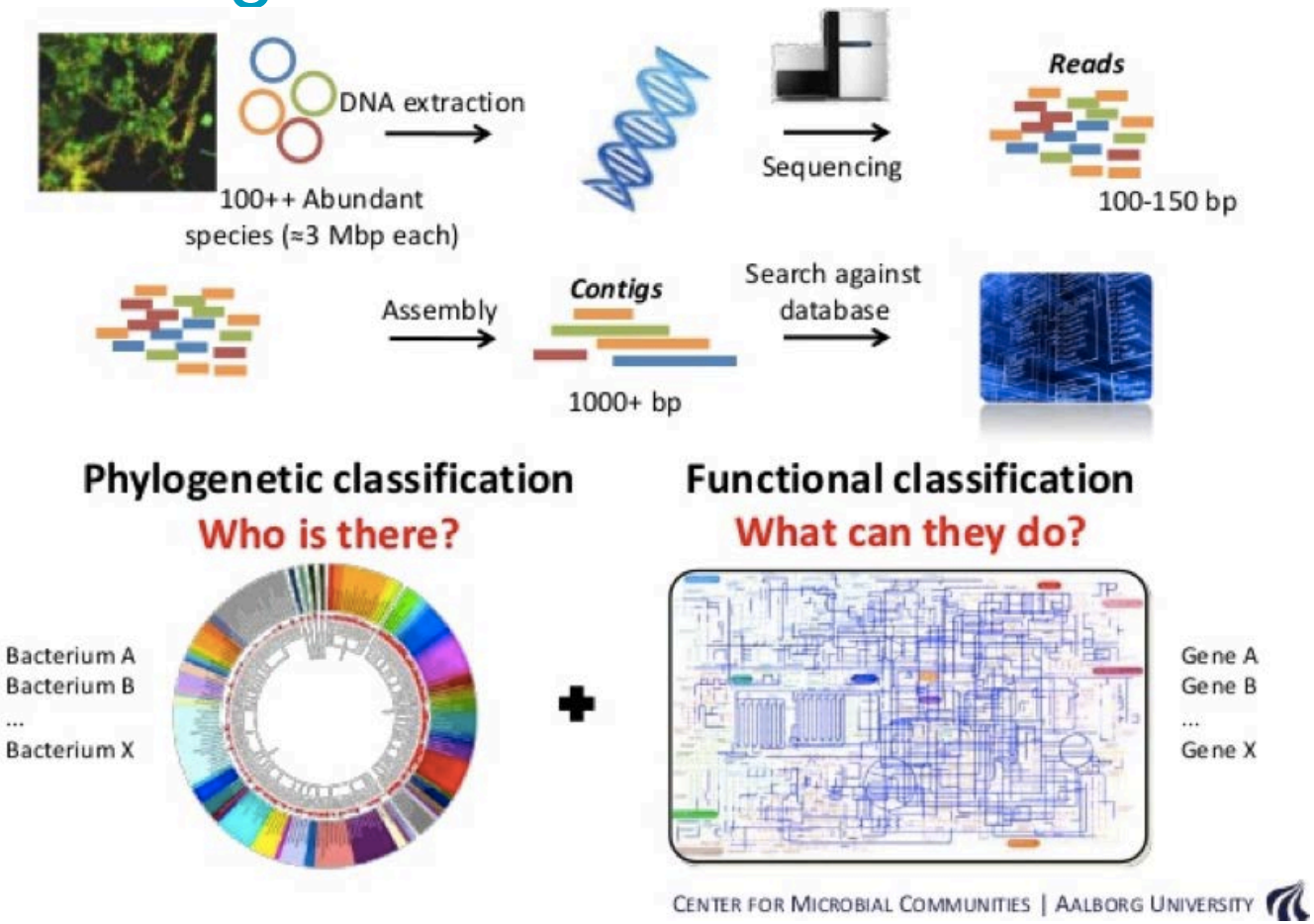


Log^{10} CFU/g

Organisms Present

Credit: Felipe Esquivel Reed

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 other sterile body sites**
  - **Immunocompromised patients (urine in kidney transplant patients)**

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Virome analyses (host and phage)

SAMPLES & SEQ GENERATION

Geographic sampling

Anatomical sampling

Enrich viruses

RNA and DNA libraries

Deep sequencing

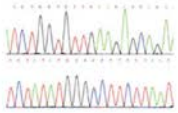
Raw data

GENETIC ANALYSES

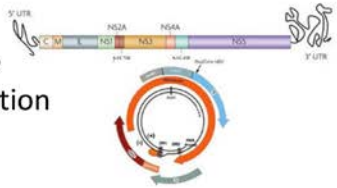
De novo assembly

Protein similarities

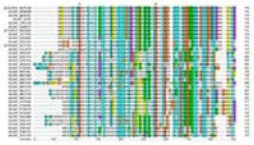
Genome re-sequencing



Genome organization



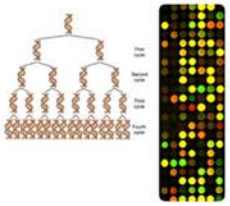
Alignments



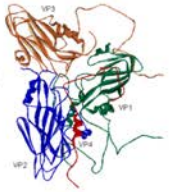
Classification

EPIDEMIOLOGY

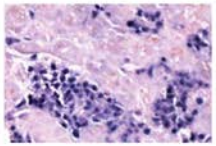
PCR & microarray detection



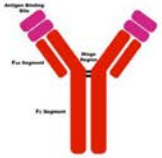
Virus replication & protein expression



In situ detection



Antibody detection



Disease association & prevalence

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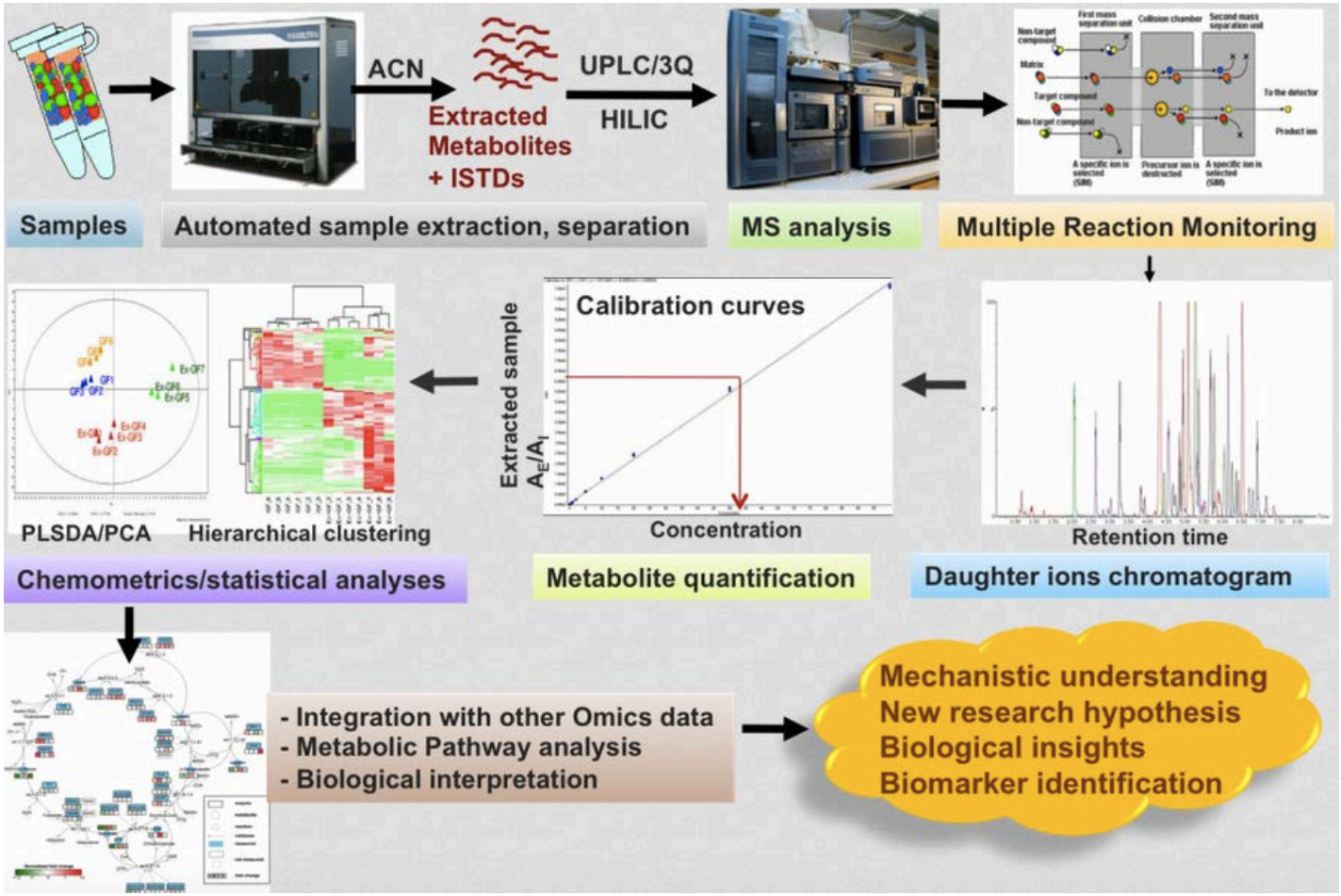
Delwart E. A roadmap to the human virome. PLoS Pathog. 2013 Feb;9(2):e1003146. doi: 10.1371/journal.ppat.1003146.

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**



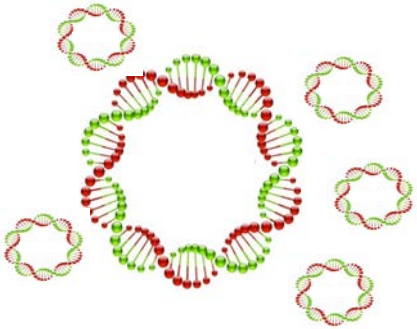
Institute for Molecular Medicine Finland (FIMM). FIMM Metabolomics Work Flow. Available at https://www.fimm.fi/en/services/technology-centre/metabolomics. Accessed October 6, 2016.

Pathogen genome sequencing

- **NGS-platforms, commonly MiSeq**
  - **HIV/HCV genotyping: used in other countries. FDA-approved tests not yet available in the US**
  - **Hospital and national surveillance activities, difficult to culture species**

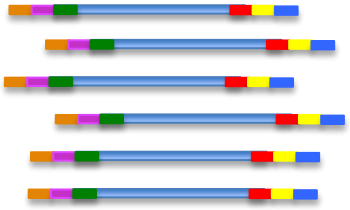
Credit: Ronald Kloberdanz

Clinical isolates



Prep total DNA
Qiagen high-throughput extractors

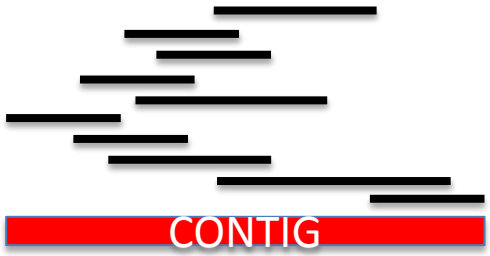
[Lynn Bry] © 123RF.com



Nextera or Truseq library -> SciClone

MiSeq
(250PE reads, 50-100x)

Credit: Illumina, Inc.



De novo assembly of raw reads into contigs (SPAdes) -> computational cluster

QC:
N50
Genome coverage

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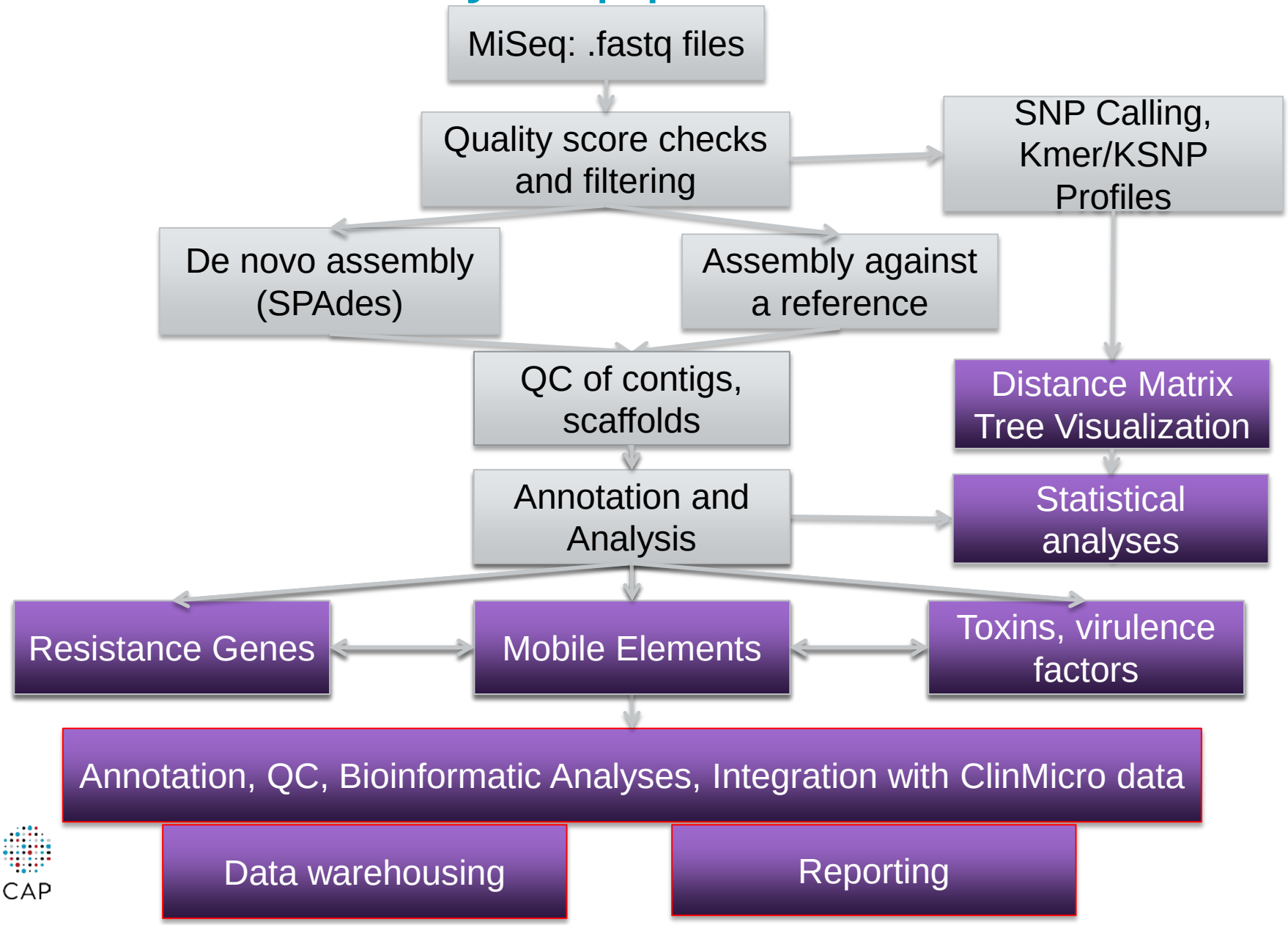
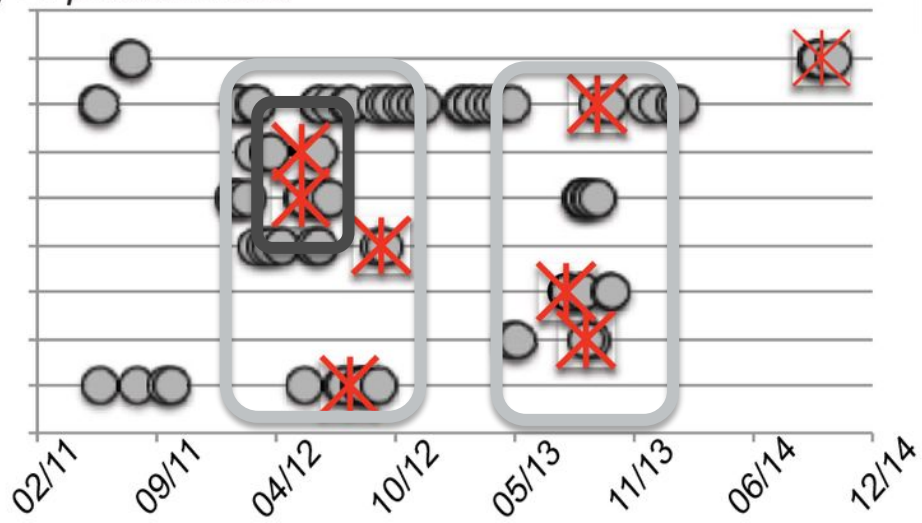
Genomic analysis pipeline



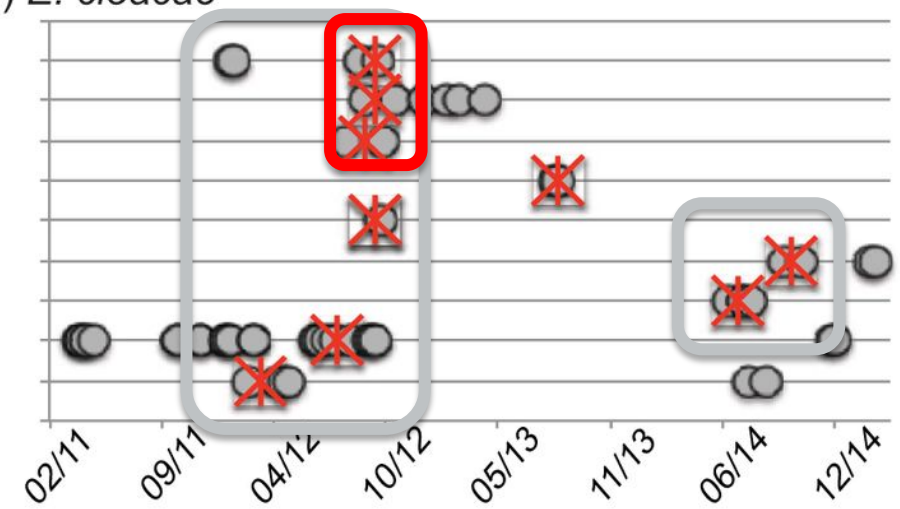
CRE: Epidemiologic Analyses

A) *K. pneumoniae*



Plasmid	MLST	Strain
P34399-43.500kb	ST-258	BWH-C10
P34399-43.500kb	ST-258	BWH-C8
pKPC-484	ST-258	BWH-C1
pKPC-484	ST-258	BWH-C2
chromosomal	ST-258	BWH-C5
chromosomal, pBK15692	ST-258	BWH-C6
pBWH-C7-KPC	ST-258	BWH-NC12
No KPC	ST-258	BWH-C7

B) *E. cloacae*



Plasmid	MLST	Strain
pBWH-C13-KPC	ST-171	BWH-C14
pBWH-C13-KPC	ST-171	BWH-C15
pBWH-C13-KPC	ST-171	BWH-C13
No KPC	ST-78	BWH-NC28
pBWH-C16-KPC	ST-78	BWH-C16
pBWH-C16-KPC	ST-78	BWH-C17
ME-BWH-C11-KPC	ST-78	BWH-C11
ME-BWH-C11-KPC	ST-78	BWH-C12
No KPC	ST-78	BWH-NC16

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Nicole D. Pecora et al. *mBio* 2015; doi:10.1128/mBio.01030-15 mBio
Journals.ASM.org

Case Studies: Host-Microbiome Systems

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

(2) **Food allergies: Integrated systems identifying therapeutic human microbiota**

(1) **Dysbiosis in disease: Therapeutic microbiota and small molecule targets in IBD**

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

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

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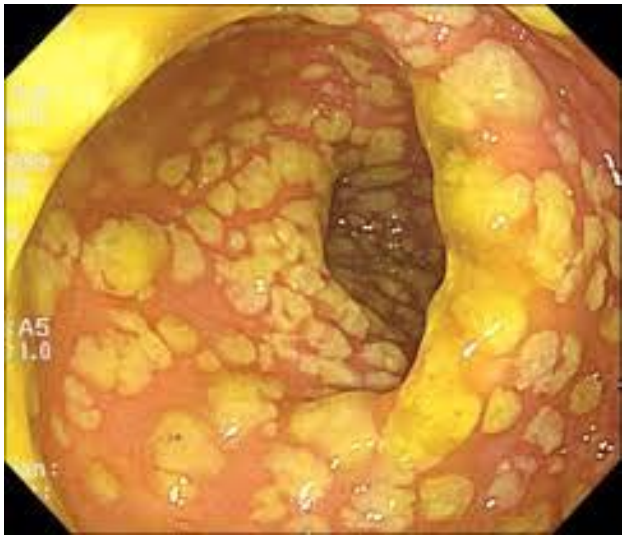
Clostridium difficile colitis

- **Pseudomembranous colitis**
  - **5-10% population colonized with *C. difficile***
    - **Not all strains are toxigenic**
  - **Substantive morbidity and mortality, particularly in immunocompromised patients**
  - **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**



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. https://doi.org/10.4166/kjg.2010.56.6.387

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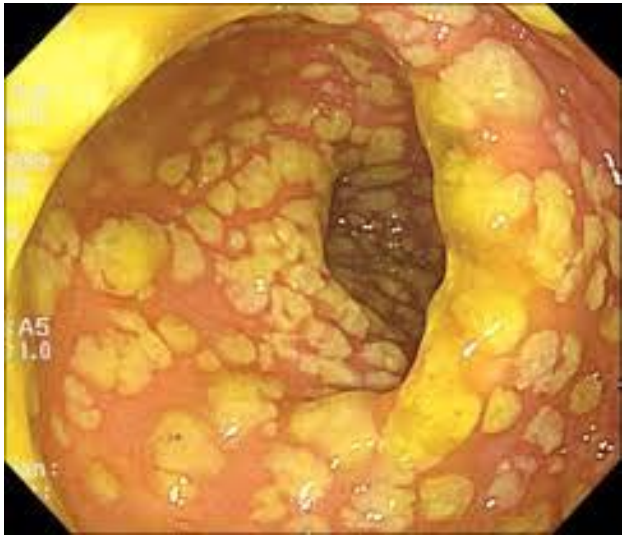
Clostridium difficile colitis, continued

- **Therapy for recurrent infection: Fecal Microbiota Transplant/FMT**
  - **Colonoscope vs oral capsule**
  - **OpenBiome**
  - **Seres Therapeutics, others**
- **Target therapy per missing microbial activities**



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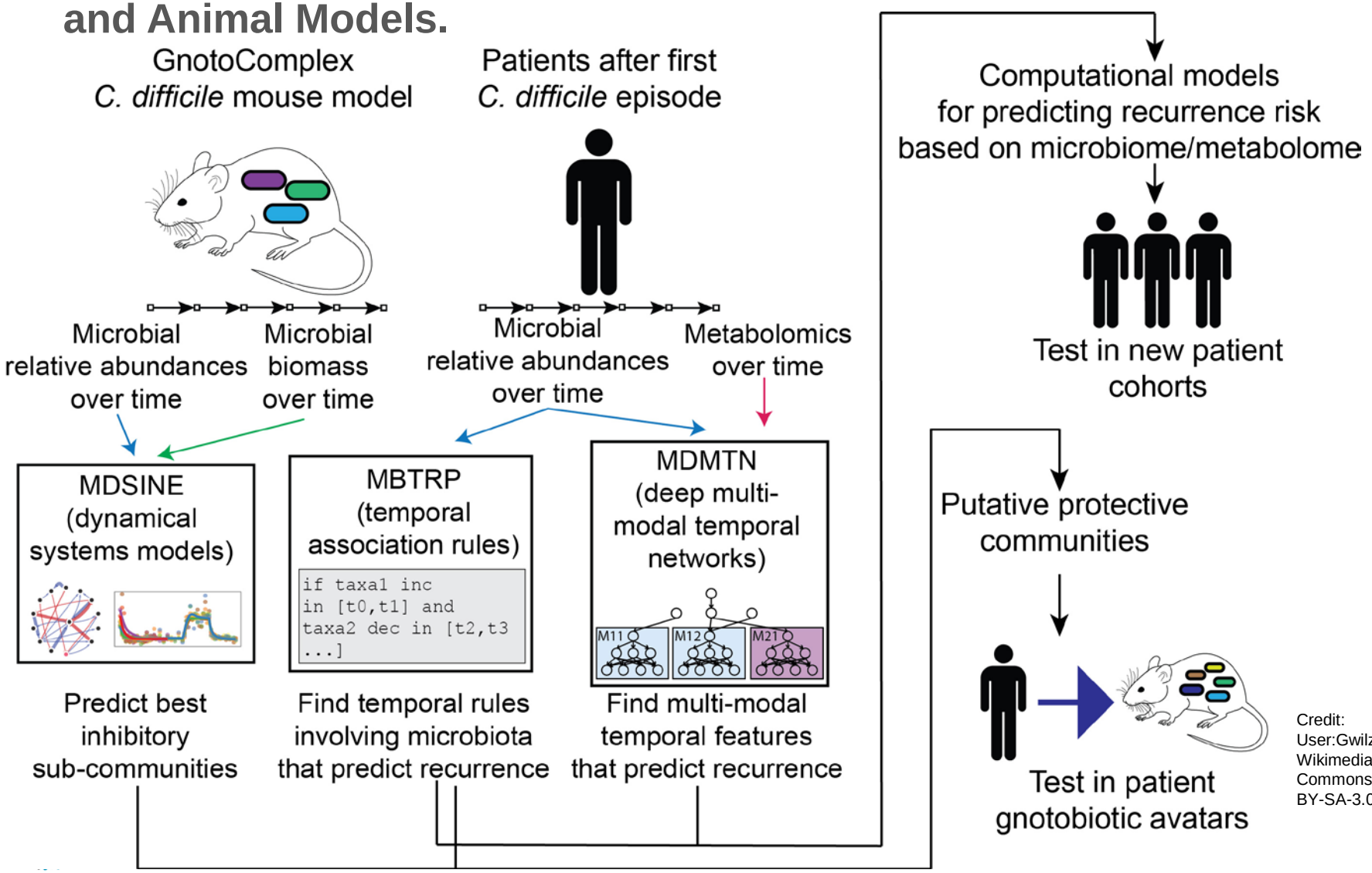
Infectious Disease Models: *C. difficile* colitis

Integrated Infrastructure: Clinical, Microbiologic, Computational, and Animal Models.



Credit: User:Gwilz / Wikimedia Commons / CC-BY-SA-3.0

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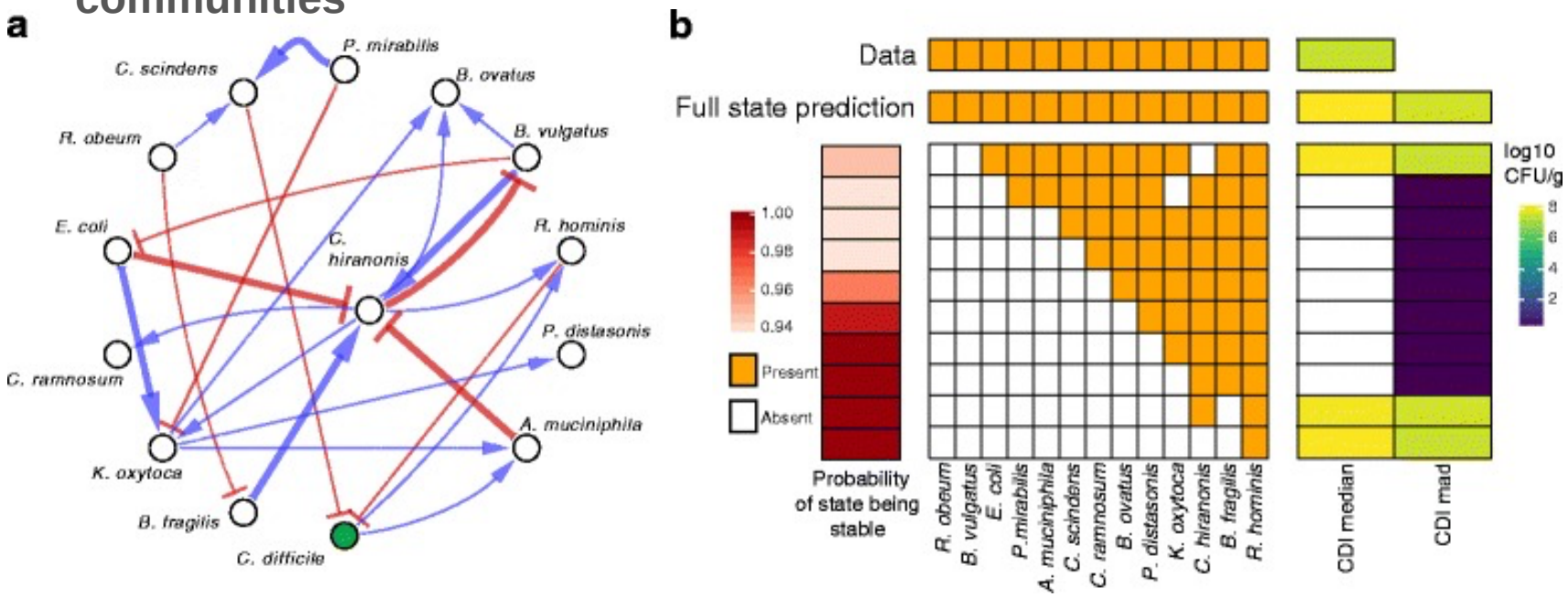
Bucci V, Tzen B, Li N, Simmons M, Tanoue T, Bogart E, Deng L, Yeliseyev V, Delaney ML, Liu Q, Olle B, Stein RR, Honda K, Bry L, Gerber GK. MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. Genome Biol. 2016 Jun 3;17(1):121. doi: 10.1186/s13059-016-0980-6.

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**

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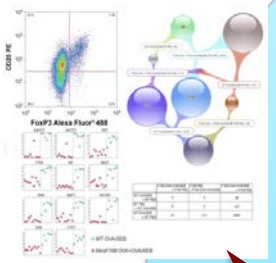
Bucci V, Tzen B, Li N, Simmons M, Tanoue T, Bogart E, Deng L, Yeliseyev V, Delaney ML, Liu Q, Olle B, Stein RR, Honda K, Bry L, Gerber GK. MDSINE: Microbial Dynamical Systems INference Engine for microbiome time-series analyses. Genome Biol. 2016 Jun 3;17(1):121. doi: 10.1186/s13059-016-0980-6.

Immunology: Microbiota-based Therapy of Food Allergy

1. Patient Cohorts: FAMILI

2. High-throughput Immunophenotyping



7. Prospective Clinical Trials

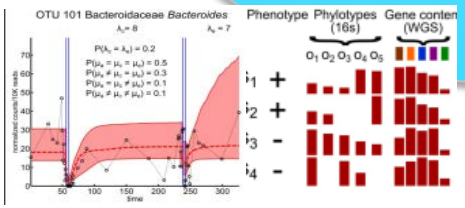
Food Allergy and Microbiota-Immune Longitudinal Interactions

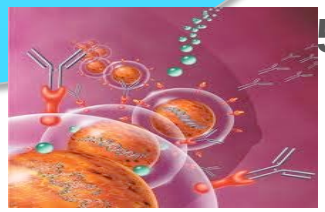
Integrated Systems to study Immune-Microbiota Effects Across Patient Cohorts

3. Longitudinal Microbiome Studies

6. Validation in Animal Models

4. Computational Analyses





5. Diagnostic and Therapeutic Targets

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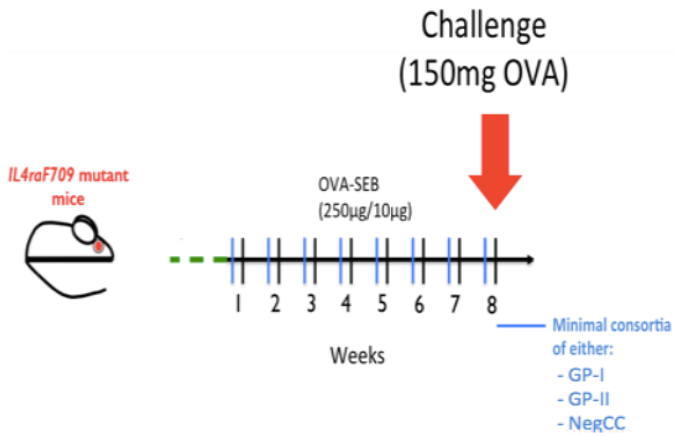
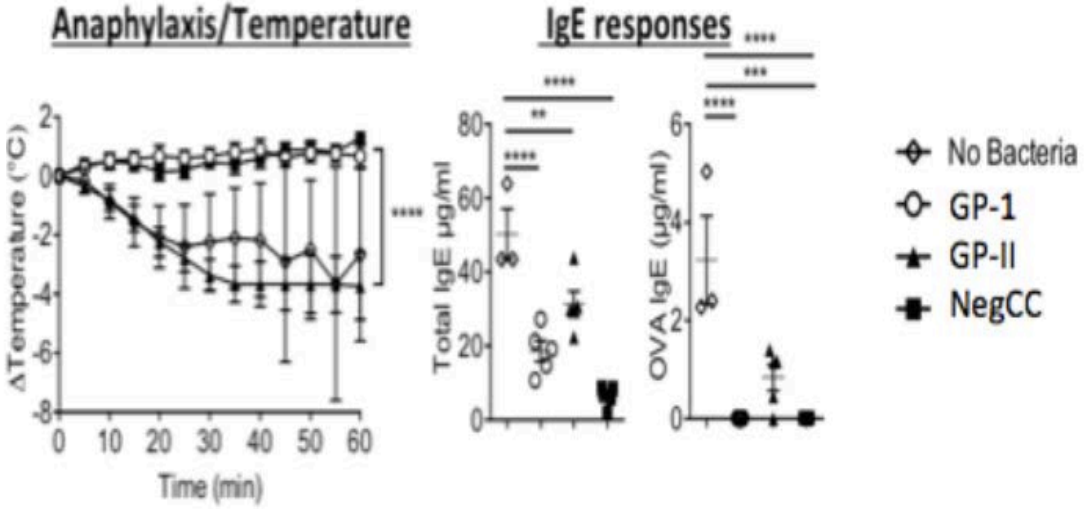
Therapeutic microbiota

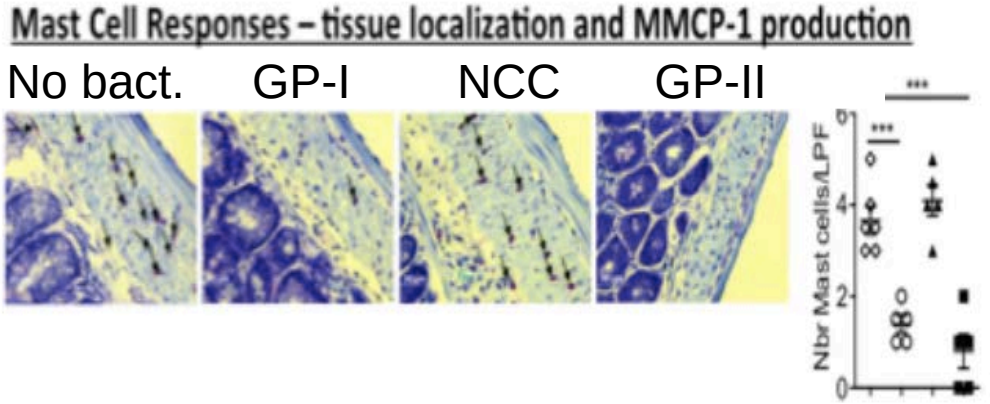


Therapeutic microbiota for treat-to-prevent and treat-to-cure regimens



Pathology applications:

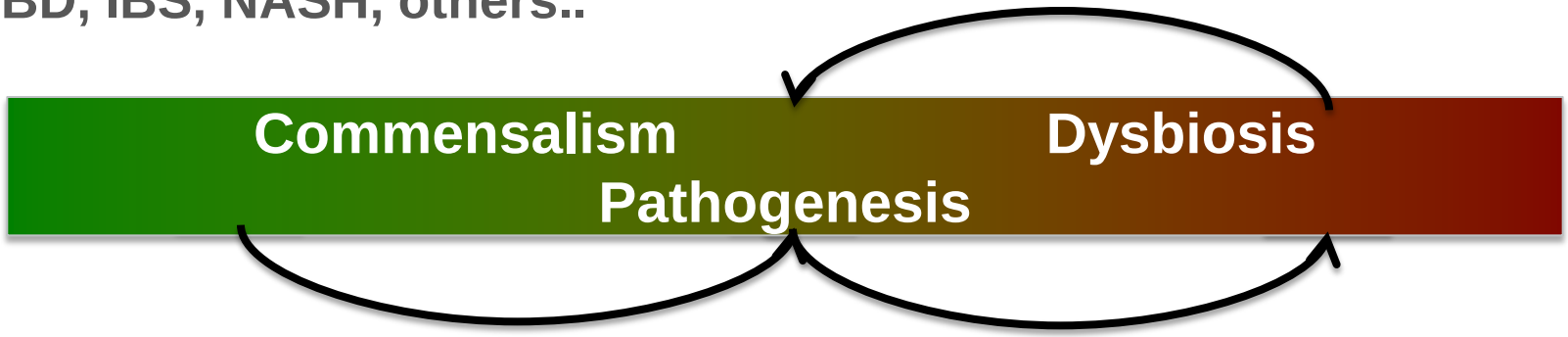
- Identify dysbiotic communities vs. absence of critical members
- Causative microbial pathways and/or products and metabolites
- Follow response to treatment (microbiome and routine immunologic analyses)



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

Pathology Applications:

- **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**

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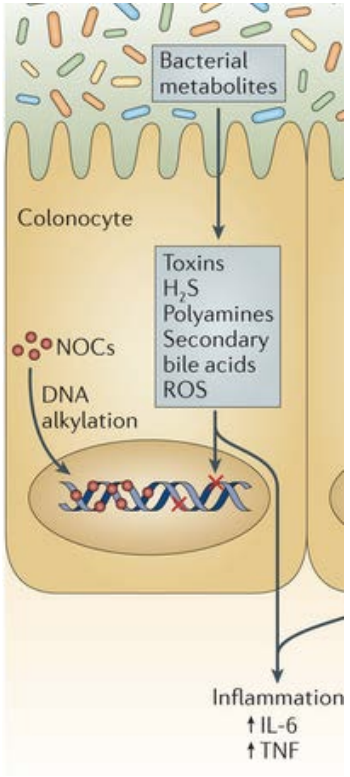
Microbiome in Cancer

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

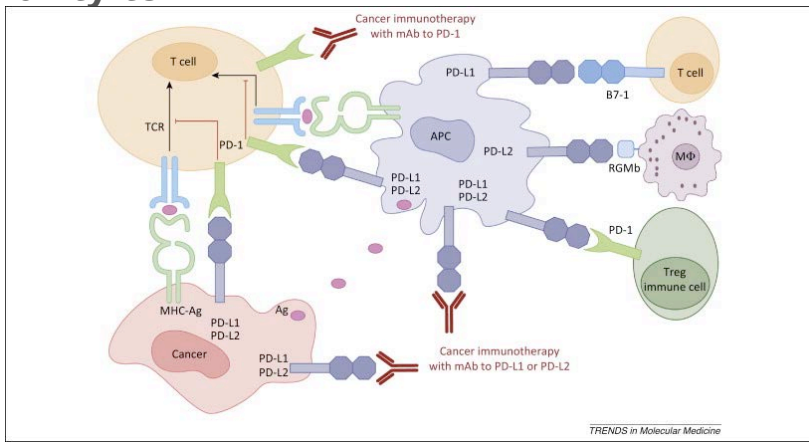
- **Immunomodulatory functions => efficacy of checkpoint inhibitors**
- **Direct biotransformation of oral and IV-administered anti-neoplastics: Irinotecan => microbiota-mediated toxicities**
- **Gut commensal communities and host susceptibility to GVHD post-SCT**



Ohaegbulam KC, Assal A, Lazar-Molnar E, Yao Y, Zang X. Human cancer immunotherapy with antibodies to the PD-1

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**



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

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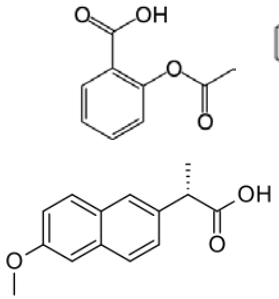
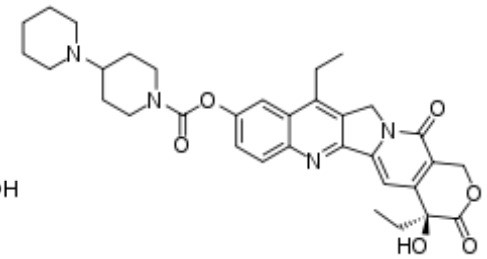
Drug metabolism

- **Microbiota-mediated biotransformation of drugs**

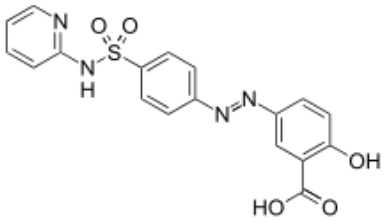
Microbiota-mediated Toxicities **Altered or Reduced Efficacy**



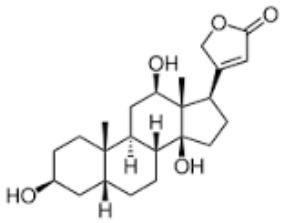
NSAIDS



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)**

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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**

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- NLM ORISE program
- BWH Clinical Laboratories

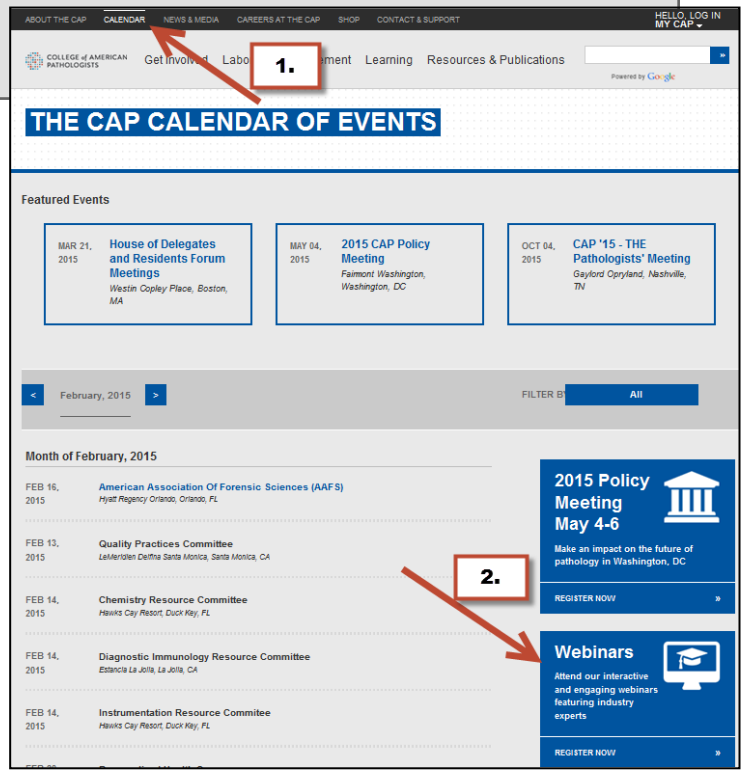
Credit: Anthony Howe

Exploring Korea. Modo Sculpture Park. Available at http://www.exploringkorea.com/modo-sculpture-park-full-house-set/. Accessed October 6, 2016.

Save the Date for Upcoming Complimentary CAP PHC Webinars

DATE	TOPIC	SPEAKER
Nov 3 **11 AM CT**	**PD-L1: Immune Check Point Blockade in Cancer**	**Kenneth J. Bloom, MD, FCAP**

Register for upcoming webinars:
www.CAP.org > Calendar > Webinars



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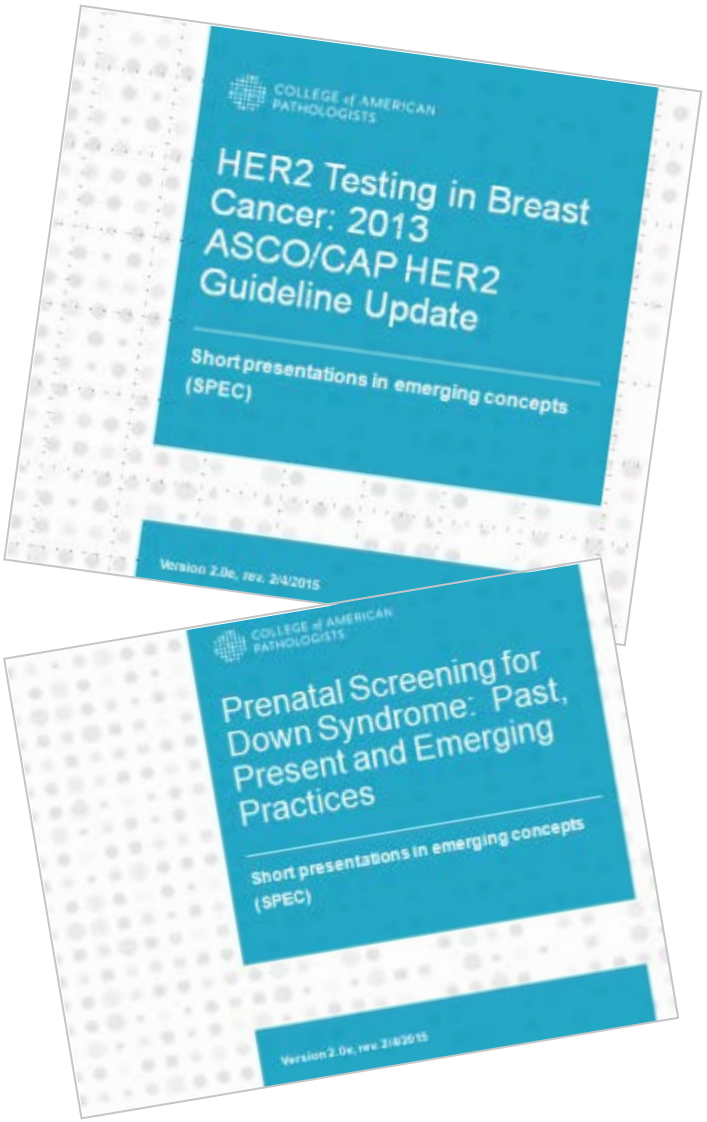
Short Presentations on Emerging Concepts (SPECS)

- **Pathology SPECs are:**
  - **short PowerPoints, created for pathologists**
  - **Focused on diseases where molecular tests play a key role in patient management**
- **New topics are Renal Tumors, cell free DNA (cfDNA), and PD-L1 as well as other emerging topics**
- **Access them www.cap.org > Resources and Publications**



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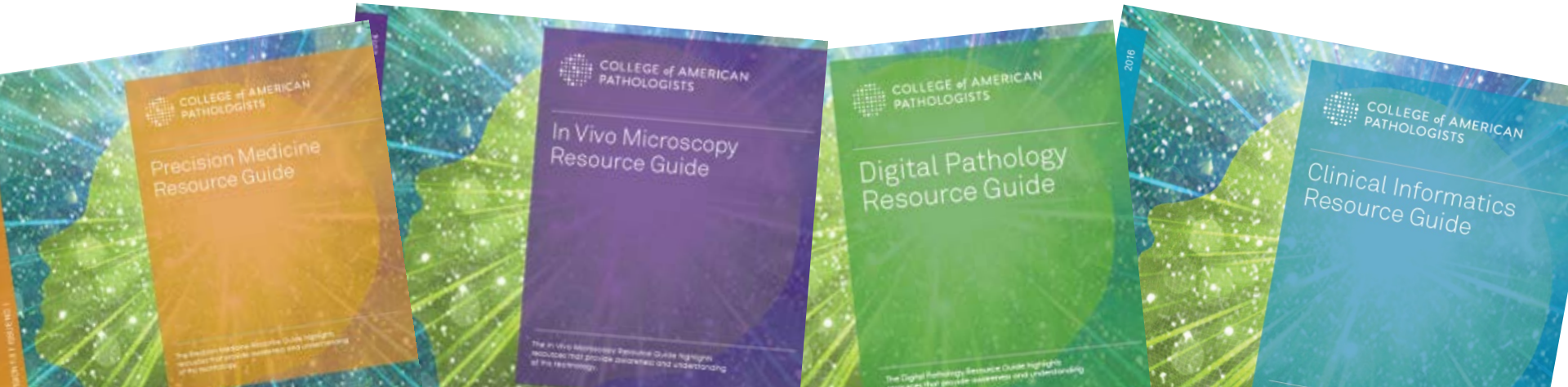
CAP's Pathology Resource Guide: Precision Medicine

- **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, **“Microbiome Applications in Pathology” by Lynn Bry, MD, PhD, FCAP.**

- 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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