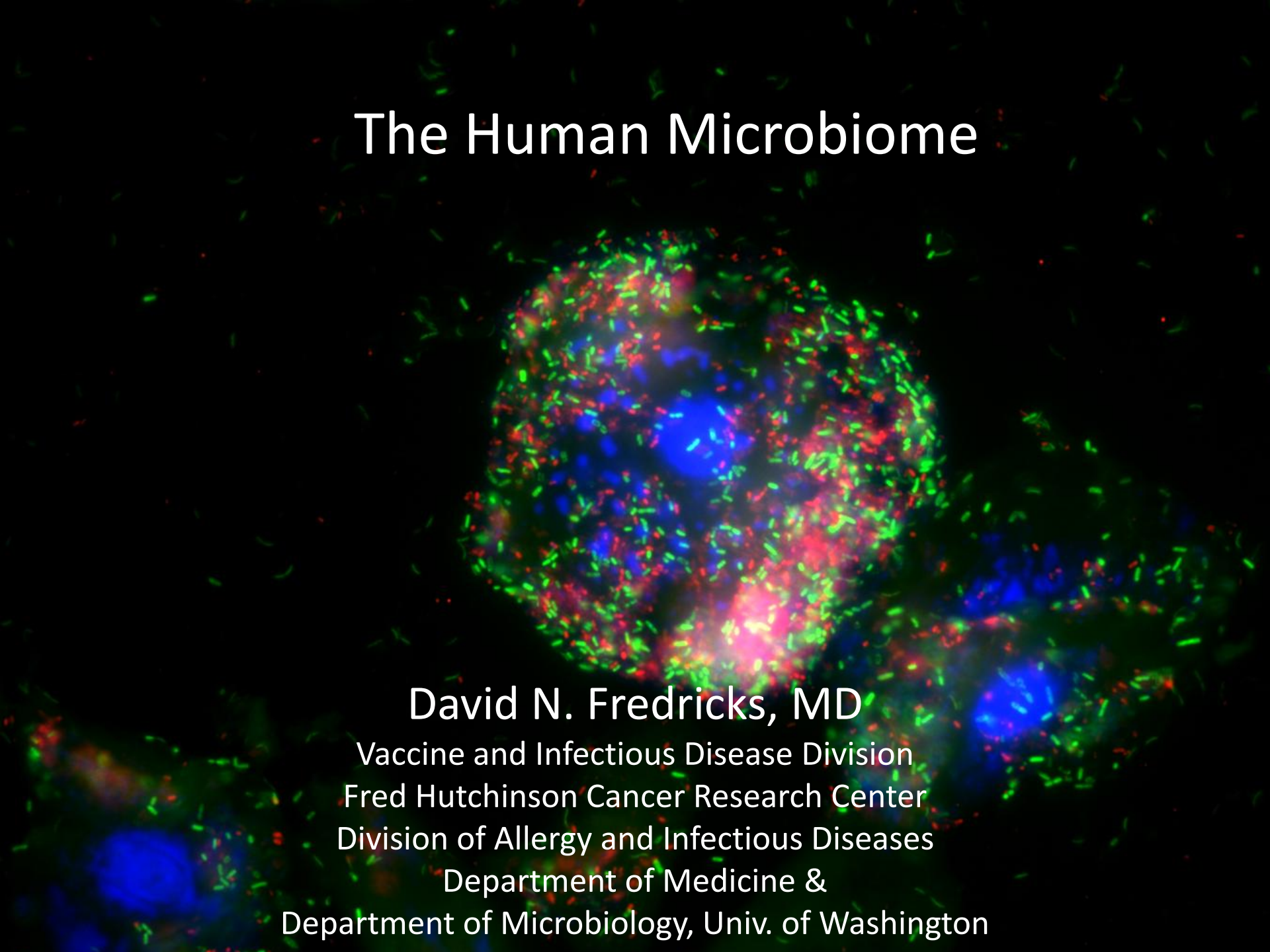
The Human Microbiome



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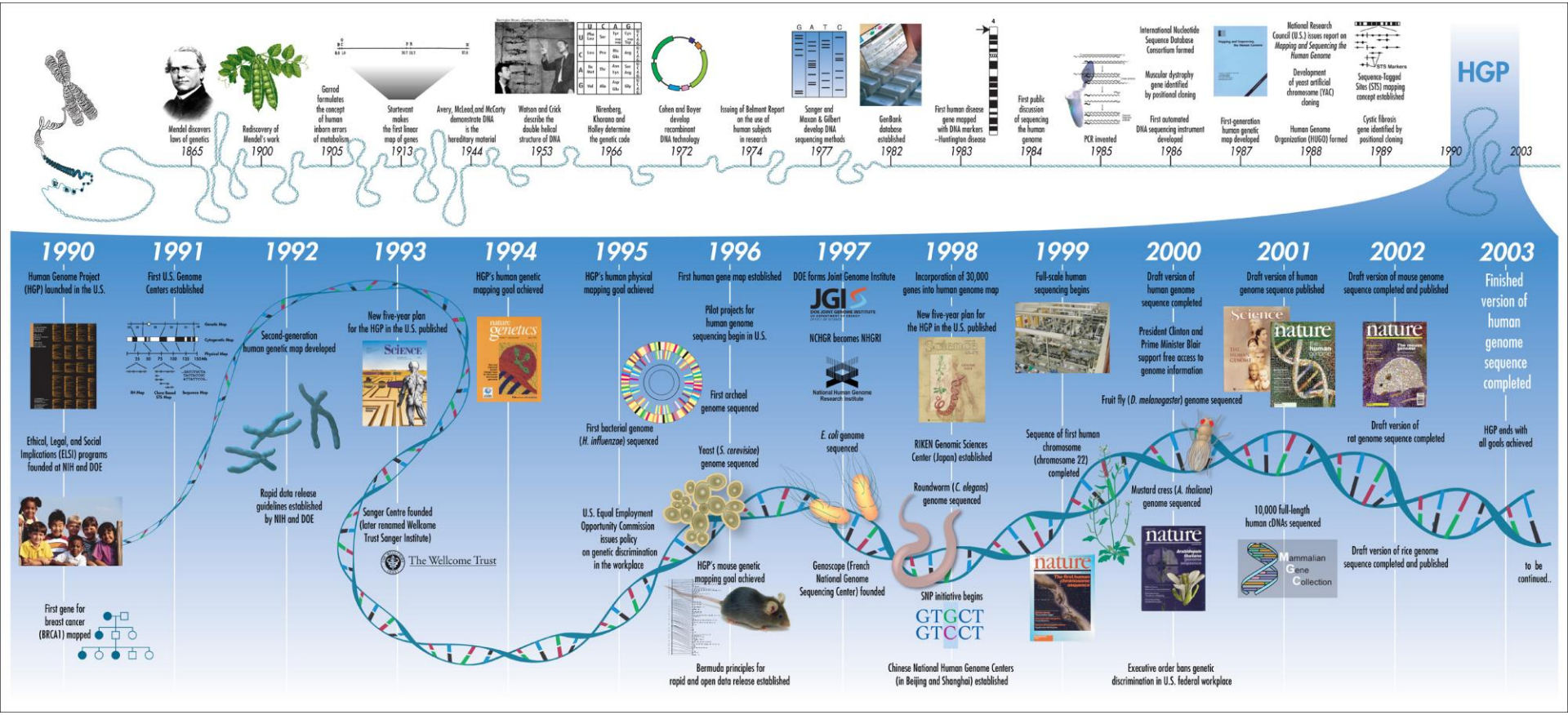
Outline

- Role of the indigenous microbiota in human health and disease
  - The human microbiota and microbiome: definitions
- Introduction to molecular methods for characterizing the microbial inhabitants of humans
- The human vaginal microbiota
  - Diversity: What is the bacterial census of the human vagina?
    - Species richness, composition, and concentration
  - Dynamism: how stable are vaginal bacterial communities and what factors influence the composition and concentrations of bacteria?
  - Dysbiosis: What changes ensue with the onset of bacterial vaginosis (BV) and what is the impact of antibiotic treatment for BV?
- Use of “omics” approaches to characterize the genetic and functional capabilities of microbial communities
    - Single cell genomics and metagenomics (genes)
    - Metatranscriptomics (mRNA and rRNA)
    - Proteomics and metabolomics (proteins and metabolites)

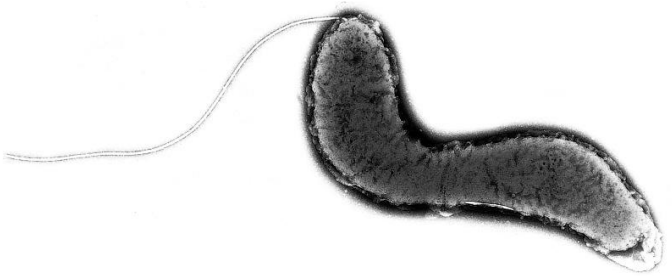
Human Genome Project (HGP)



- 3.16 billion base pairs of DNA in genome; cost of HGP: $2.7 billion
- Anticipated number of human genes at initiation of project: >100,000
  - Fruit fly ~ 14,000 genes, Chicken ~23,000 genes, Corn ~59,000 genes
- Humans ~25,000 genes!

Humans as “Super-organisms”

Human: 10^{13} human cells



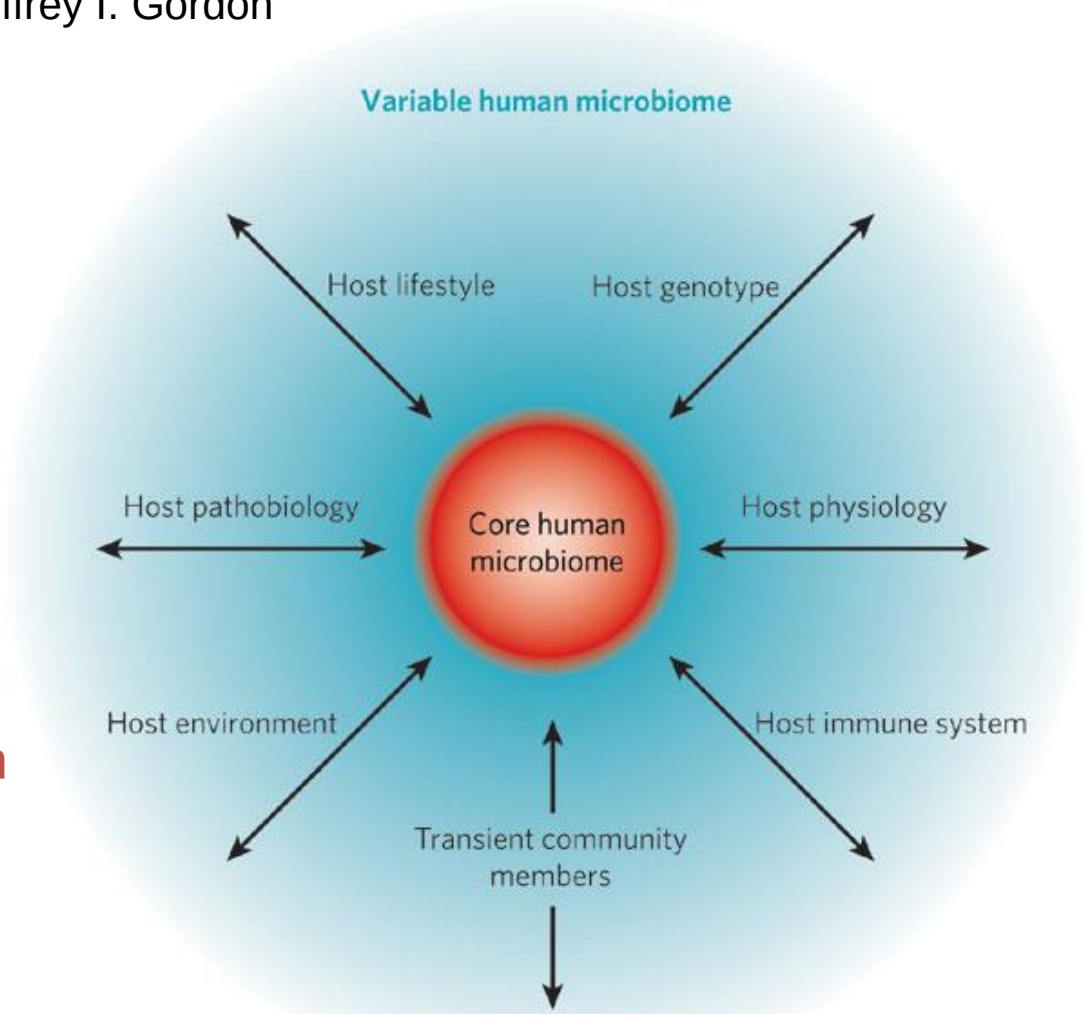
Human: 10^{14} bacterial cells

- The microorganisms that live on and inside humans (the microbiota) are estimated to outnumber human somatic and germ cells by a factor of ten
- Together, the genomes of these microbial symbionts provide traits that humans did not need to evolve on their own
  - Microbiome #1: collection of microbial genes associated with humans
  - Microbiome #2: collection of microbes within the human biome
- We are a genetic and metabolic composite of microbial and human cells, leading to the concept of the human super-organism
  - More than 3,000,000 genes provided by our gut microbiome!

The Human Microbiome Project Peter J. Turnbaugh, Ruth E. Ley, Micah Hamady, Claire M. Fraser-Liggett, Rob Knight & Jeffrey I. Gordon
***Nature* 449**, 804-810(18 October 2007

HMP Goals:

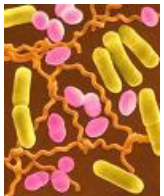
1. Determining whether individuals share a core human microbiome
2. Understanding whether changes in the human microbiome can be correlated with changes in human health



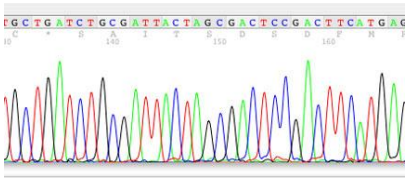
The core human microbiome (red) is the set of genes present in a given habitat in all or the vast majority of humans. Habitat can be defined over a range of scales, from the entire body to a specific surface area, such as the gut or a region within the gut. The variable human microbiome (blue) is the set of genes present in a given habitat in a smaller subset of humans.

Cultivation vs. Molecular Analyses of the Human Microbiome

- Cultivation of microbes
  - Description of species (phenotypic or genotypic)
  - Sequence genomes from isolates
- Cultivation-independent analysis of microbial populations and their genes (molecular)
  - PCR of 16S rRNA genes from bacteria to detect and identify species; no information on other elements of the microbiome
  - Metagenomic analysis: extract nucleic acid directly from a sample and perform high throughput sequencing to catalog the microbes and genes represented



DNA



SCIENCE VOL 312 2 JUNE 2006 Science. 2006 Jun 2;312(5778):1355-9.

The Human Gut Microbiome

Metagenomic Analysis of the Human Distal Gut Microbiome

Steven R. Gill,[1*‡] Mihai Pop,[1†] Robert T. DeBoy,[1] Paul B. Eckburg,[2,3,4] Peter J. Turnbaugh,[5] Buck S. Samuel,[5] Jeffrey I. Gordon,[5] David A. Relman,[2,3,4] Claire M. Fraser-Liggett,[1,6] Karen E. Nelson[1]

The human intestinal microbiota is composed of 10^{13} to 10^{14} microorganisms whose collective genome ("microbiome") contains at least 100 times as many genes as our own genome. We analyzed ~78 million base pairs of unique DNA sequence and 2062 polymerase chain reaction–amplified 16*S* ribosomal DNA sequences obtained from the fecal DNAs of two healthy adults. Using metabolic function analyses of identified genes, we compared our human genome with the average content of previously sequenced microbial genomes. Our microbiome has significantly enriched metabolism of glycans, amino acids, and xenobiotics; methanogenesis; and 2-methyl-D-erythritol 4-phosphate pathway–mediated biosynthesis of vitamins and isoprenoids. Thus, humans are superorganisms whose metabolism represents an amalgamation of microbial and human attributes.

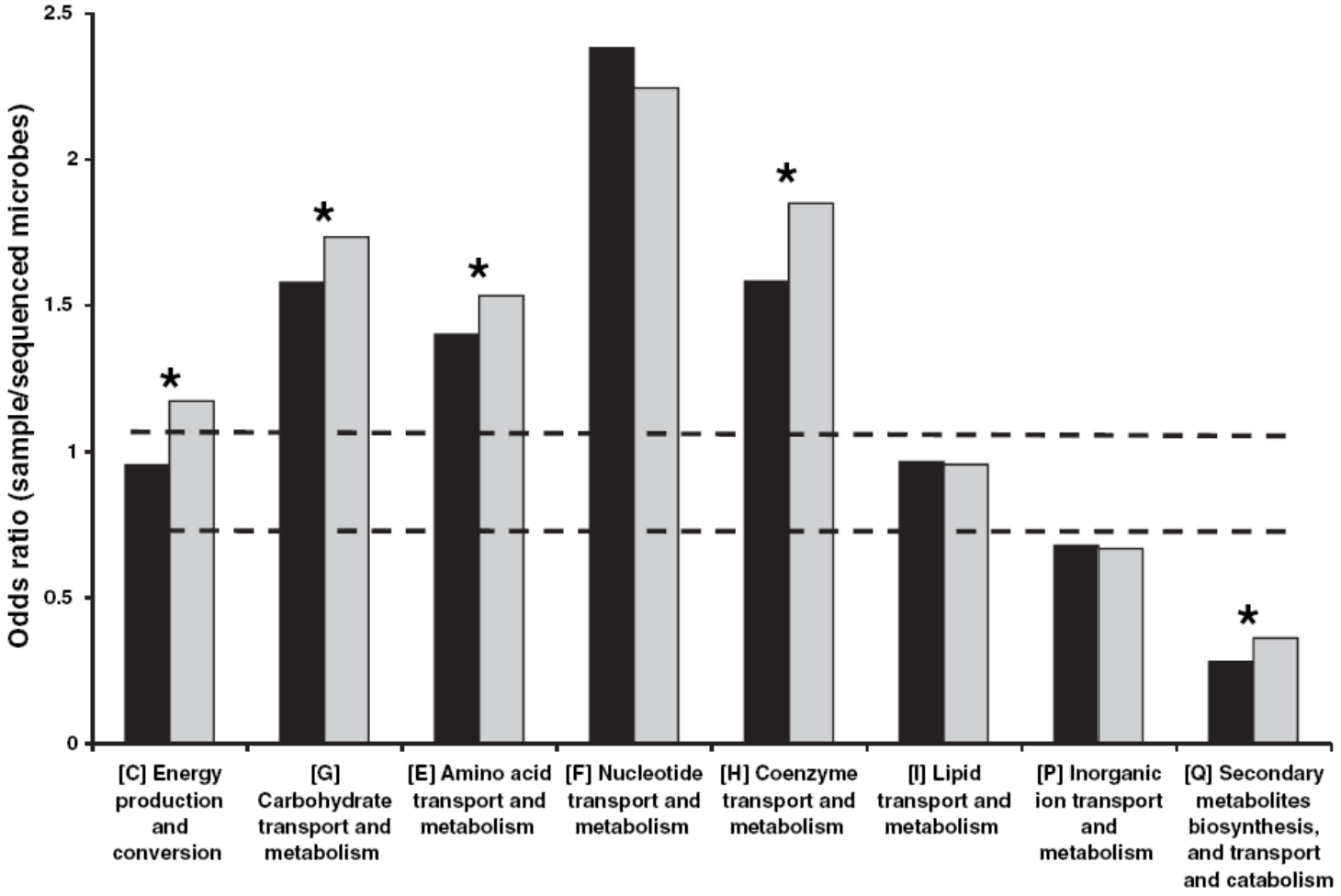


Fig. 2. COG analysis reveals metabolic functions that are enriched or underrepresented in the human distal gut microbiome (relative to all sequenced microbes). Color code: black, subject 7; gray, subject 8. Bars above both dashed lines indicate enrichment, and bars below both lines indicate underrepresentation ($P < 0.05$). Asterisks indicate categories that are significantly different between the two subjects ($P < 0.05$). Secondary metabolites biosynthesis includes antibiotics, pigments, and nonribosomal peptides. Inorganic ion transport and metabolism includes phosphate, sulfate, and various cation transporters.

Vol 464 | 4 March 2010 | doi:10.1038/nature08821

A human gut microbial gene catalogue established by metagenomic sequencing

Junjie Qin[1]*, Ruiqiang Li[1]*, Jeroen Raes[2,3], Manimozhiyan Arumugam[2], Kristoffer Solvsten Burgdorf[4], Chaysavanh Manichanh[5], Trine Nielsen[4], Nicolas Pons[6], Florence Levenez[6], Takuji Yamada[2], Daniel R. Mende[2], Junhua Li[1,7], Junming Xu[1], Shaochuan Li[1], Dongfang Li[1,8], Jianjun Cao[1], Bo Wang[1], Huiqing Liang[1], Huisong Zheng[1], Yinlong Xie[1,7], Julien Tap[6], Patricia Lepage[6], Marcelo Bertalan[9], Jean-Michel Batto[6], Torben Hansen[4], Denis Le Paslier[10], Allan Linneberg[11], H. Bjørn Nielsen[9], Eric Pelletier[10], Pierre Renault[6], Thomas Sicheritz-Ponten[9], Keith Turner[12], Hongmei Zhu[1], Chang Yu[1], Shengting Li[1], Min Jian[1], Yan Zhou[1], Yingrui Li[1], Xiuqing Zhang[1], Songgang Li[1], Nan Qin[1], Huanming Yang[1], Jian Wang[1], Søren Brunak[9], Joel Doré[6], Francisco Guarner[5], Karsten Kristiansen[13], Oluf Pedersen[4,14], Julian Parkhill[12], Jean Weissenbach[10], MetaHIT Consortium†, Peer Bork[2], S. Dusko Ehrlich[6] & Jun Wang[1,13]

To understand the impact of gut microbes on human health and well-being it is crucial to assess their genetic potential. Here we describe the Illumina-based metagenomic sequencing, assembly and characterization of 3.3 million non-redundant microbial genes, derived from 576.7 gigabases of sequence, from faecal samples of 124 European individuals. The gene set, ~150 times larger than the human gene complement, contains an overwhelming majority of the prevalent (more frequent) microbial genes of the cohort and probably includes a large proportion of the prevalent human intestinal microbial genes. The genes are largely shared among individuals of the cohort. Over 99% of the genes are bacterial, indicating that the entire cohort harbours between 1,000 and 1,150 prevalent bacterial species and each individual at least 160 such species, which are also largely shared. We define and describe the minimal gut metagenome and the minimal gut bacterial genome in terms of functions present in all individuals and most bacteria, respectively.

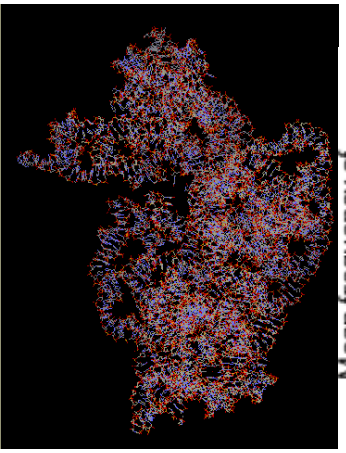
- 3.3 million non-redundant microbial genes
- >1000 gut bacterial species in cohort of 124
  - But only ~160 bacterial species/individual
- About 500,000 microbial genes/individual
  - 40% of genes present in at least half of cohort

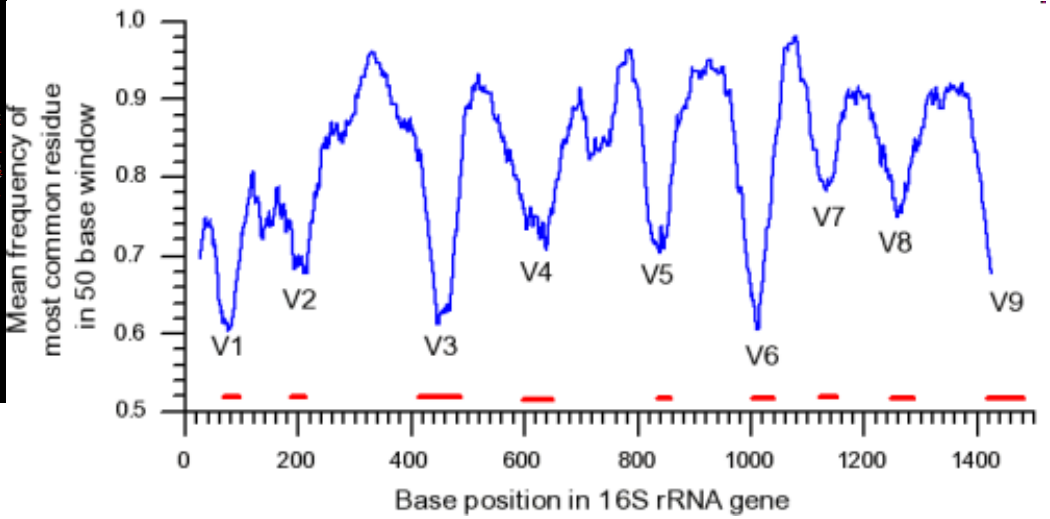
The gut microbiome affects…

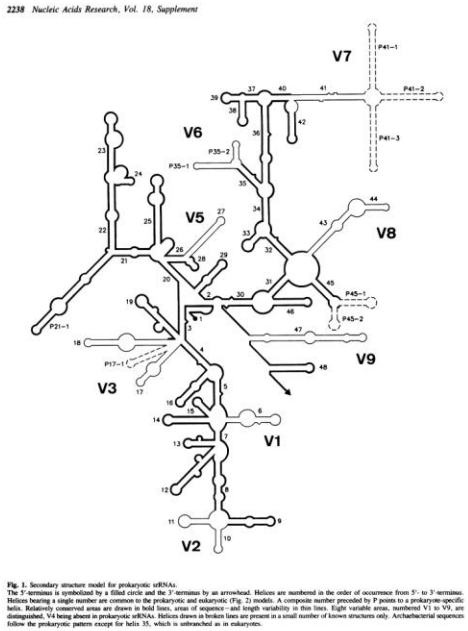
- Vitamin production (vitamin K)
- Development of innate and adaptive immunity
- Turnover of gut epithelial cells (malignancy?)
- Metabolism of xenobiotics (drugs)
- Harvest of nutrients/energy metabolism (physiology)
  - Propensity to develop obesity
- Organ size: Heart, intestine
  - Anatomy and development
- Locomotor activity (behavior)

***Nature* 449**, 804-810(18 October 2007)

The 16S rRNA gene







- Present in all bacteria (essential: codes for small subunit of ribosomal RNA complex, necessary for protein synthesis)
- Has properties of a molecular clock
  - rDNA sequence similarities between species correlate with evolutionary relatedness (time to common ancestor)
  - Little evidence of horizontal gene transfer or recombination
- Conserved regions: useful for broad range PCR
- Variable regions: useful for species identification

The bacterial 16S rRNA gene

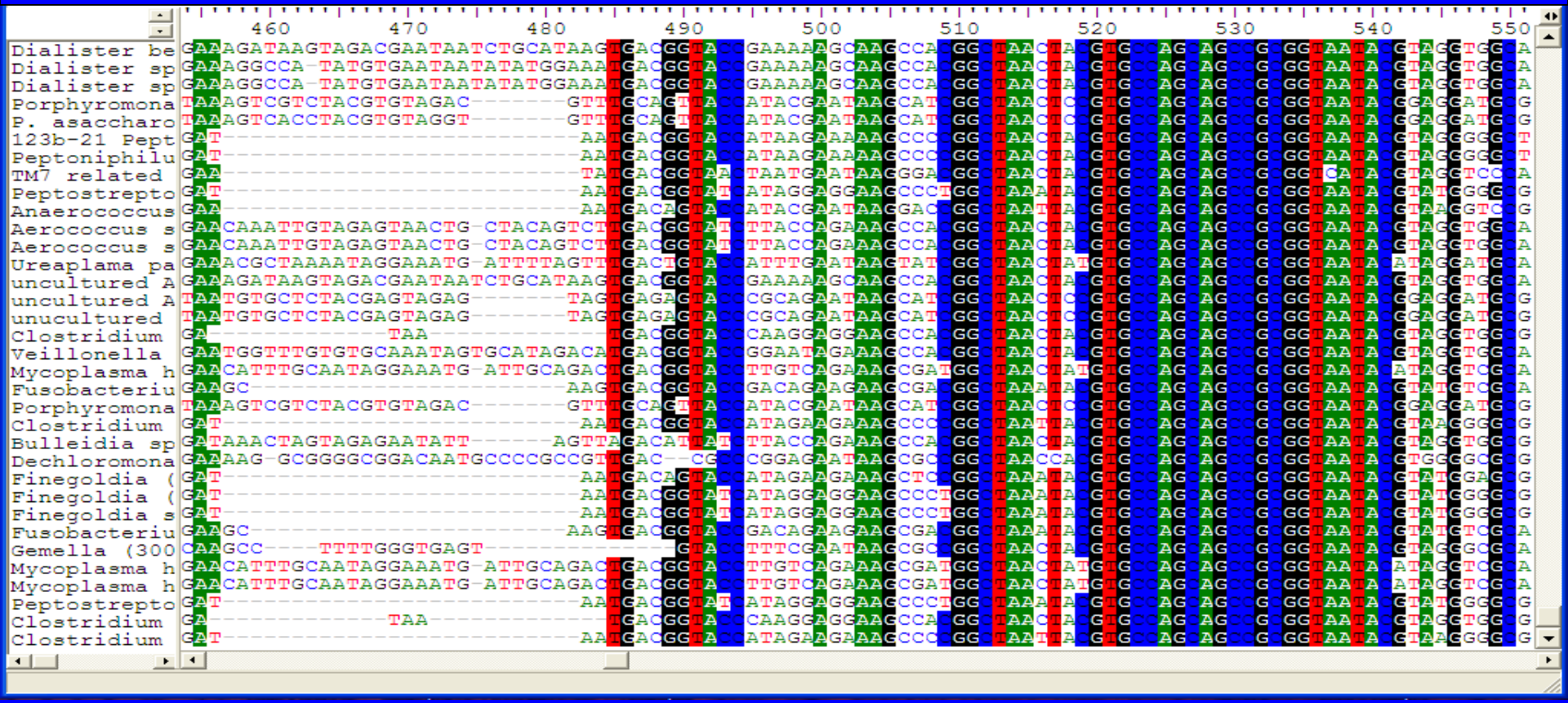
Present in all bacteria

Little evidence of horizontal gene transfer

Accurate phylogenies

Variable

Conserved



Why Study the Vaginal Microbiota?

- The vaginal microbiota affects the health of women and impacts the success of pregnancy
  - *E. coli* colonization of the vagina may precede UTI
  - Group B streptococcus and neonatal sepsis
- The vagina hosts unique consortia of microbes suggesting selection for these key organisms
- Bacterial vaginosis (BV) is a condition linked to numerous health problems, including:
  - ❖ Preterm birth
  - ❖ Pelvic inflammatory disease (infection of upper tract)
  - ❖ HIV acquisition and shedding
  - ❖ Increased risk of other sexually transmitted diseases (GC, CT, Trich, HSV, HPV)
  - ❖ Post hysterectomy vaginal cuff cellulitis and other surgical infections

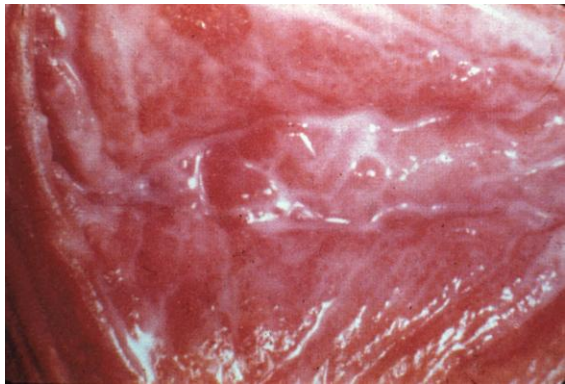
Epidemiology. 2007;18:702-8.

Sobel JD. Infect Dis Clin N Am 2005;19:387-406

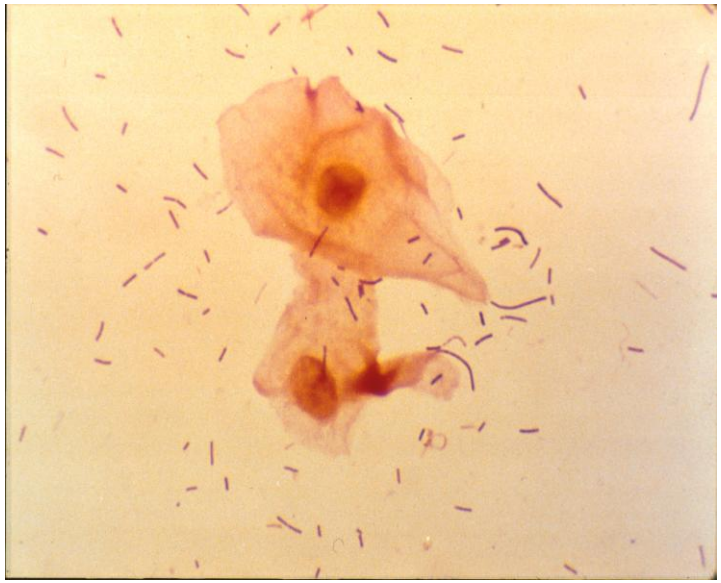
Bacterial Vaginosis

The most prevalent cause of vaginal symptoms among women of childbearing age

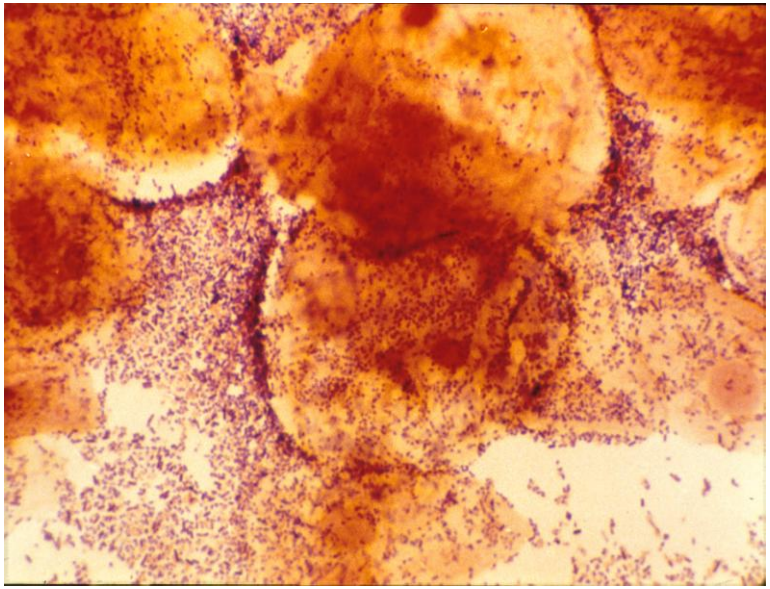
- ~ 4 million doctor visits/year in U.S.
  - ≥10% of women experience BV
  - NHANES survey in US: overall prevalence 29%
  - Prevalence >50% in settings with high HIV burden (SS Africa)
- Abnormal vaginal discharge in ~50% of women
  - Increased amount -glycosidase activity of GNR on vaginal mucous
  - Odor from volatilization of amines produced by anaerobic metabolism → trimethylamine
- High rate of relapse: causes unknown



Bacterial Vaginosis (BV)



Gram stain of normal vaginal fluid with many GPR (lactobacilli), normal epithelial cells



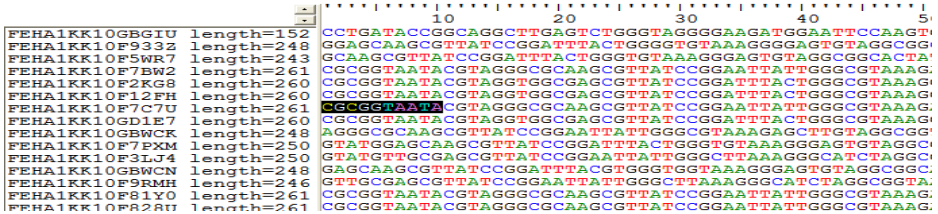
Gram stain of BV with few GPR, greater diversity of morphotypes, and clue cells

Schematic for Pyrosequencing Approach

16S rRNA Gene

Fusion primer A: broad range Fw primer

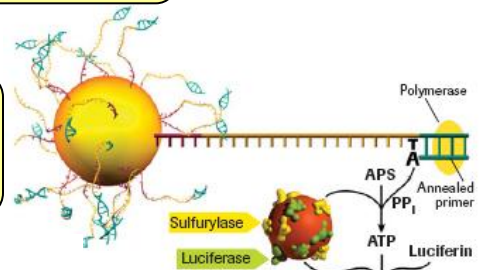
Fusion primer B: Bar code: Broad range Rev

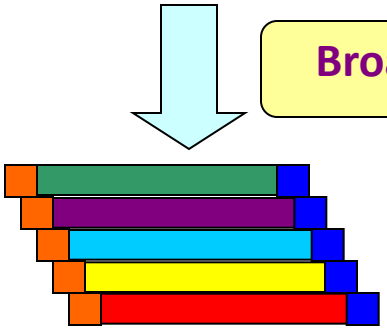


Alignment /Data Analysis

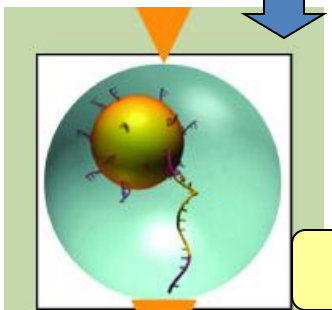
Broad-range PCR

Sequencing by synthesis



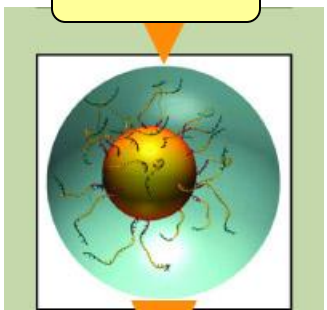


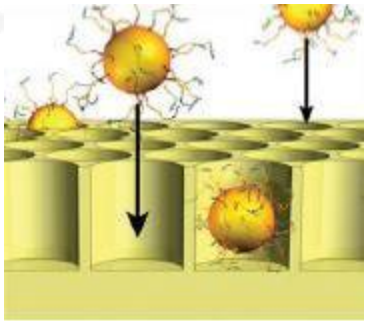
PCR Products w/ Fusion Primers



Attachment to Bead

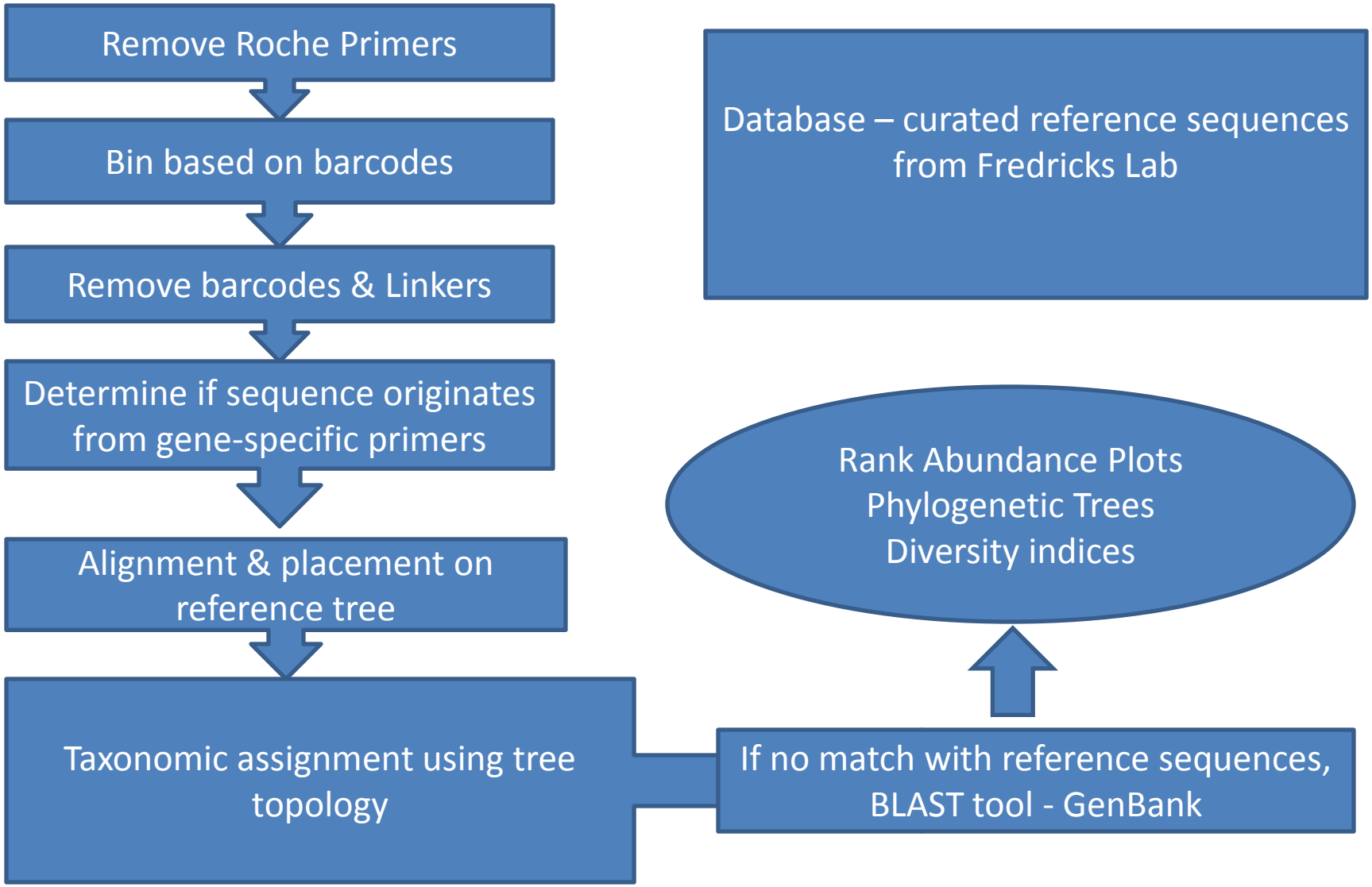
emPCR





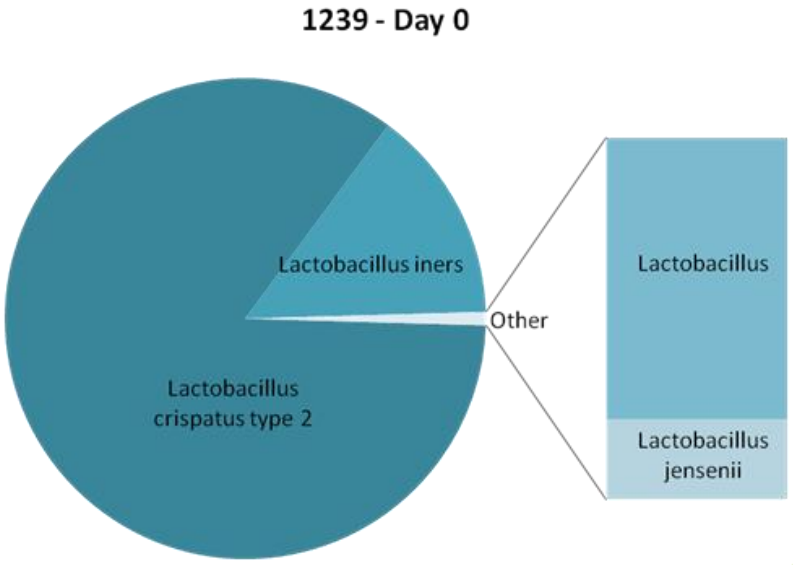
Beads to Picotiter Plate

Pyrosequencing Pipeline

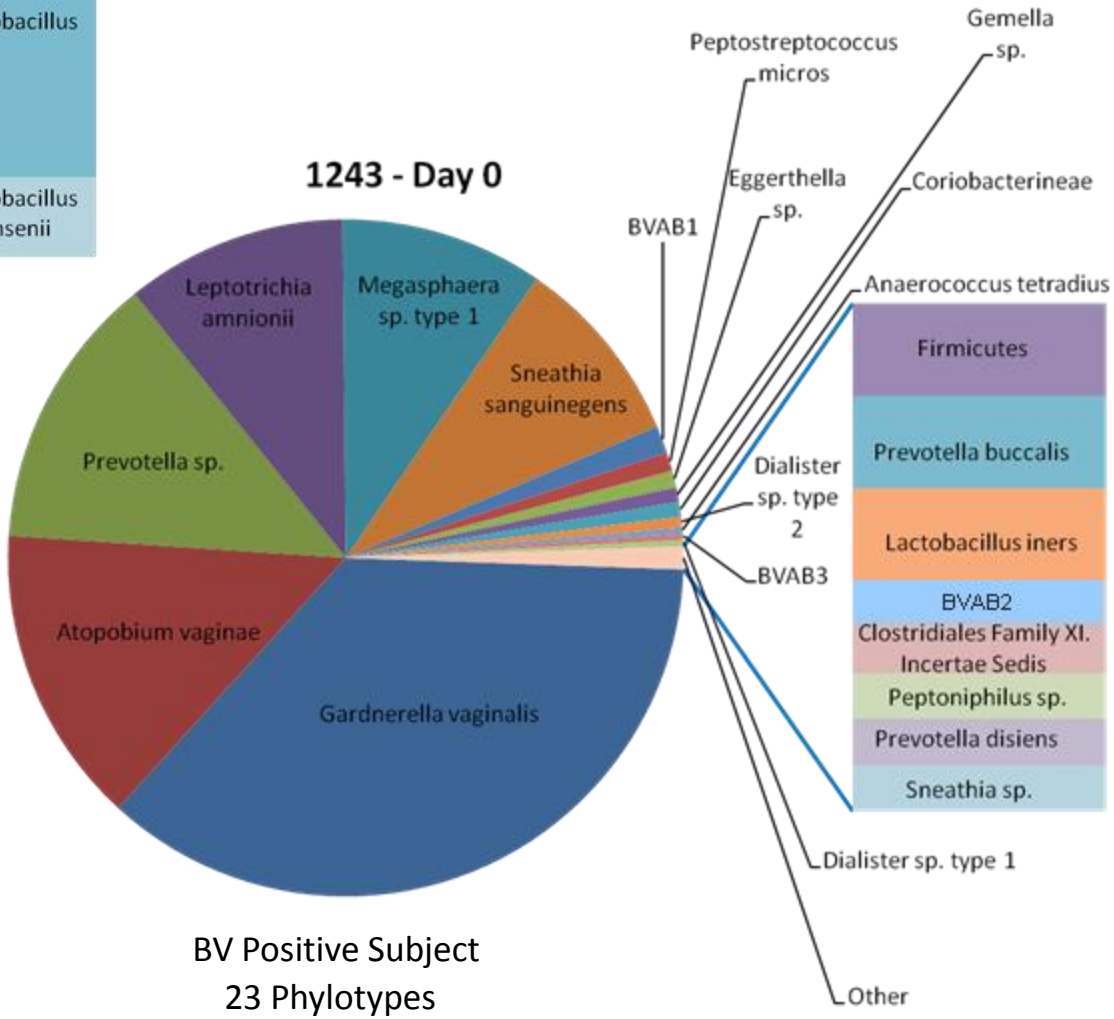


Computational Biologists : Erick Matsen, Noah Hoffman, Martin Morgan

BACTERIAL DIVERSITY – PYROSEQUENCING



BV Negative Subject
4 Phylotypes



1000 sequences analyzed

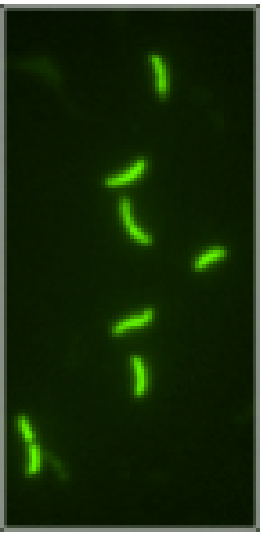
Species richness increased in BV
Species diversity increased in BV

BV Positive Subject
23 Phylotypes

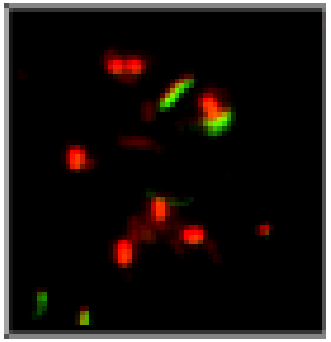
Molecular Identification of Bacteria Associated with Bacterial Vaginosis

Fredricks DN et al. NEJM 2005;353:1899-911

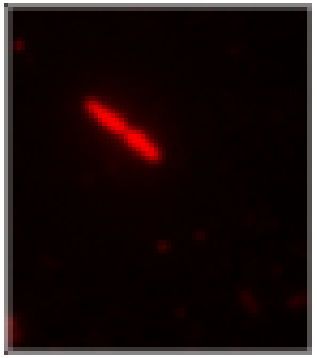
BVAB1

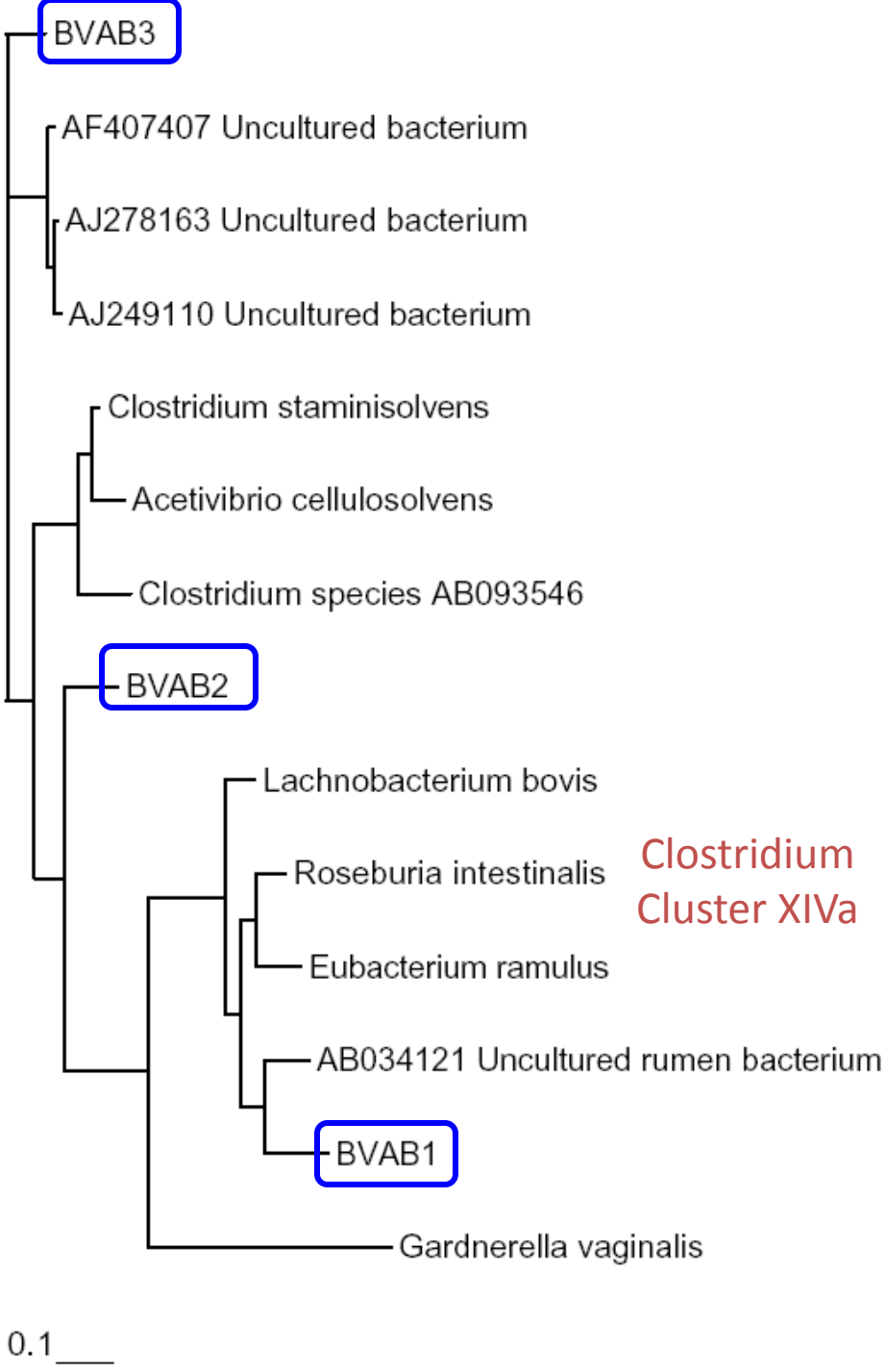


BVAB2



BVAB3





Relationship of Specific Vaginal Bacteria and Bacterial Vaginosis Treatment Failure in Women Who Have Sex with Women

Jeanne M. Marrazzo, MD, MPH; Katherine K. Thomas, MS; Tina L. Fiedler, BS; Kathleen Ringwood, MSW; and David N. Fredricks, MD

Ann Intern Med. 2008;149:20-28.

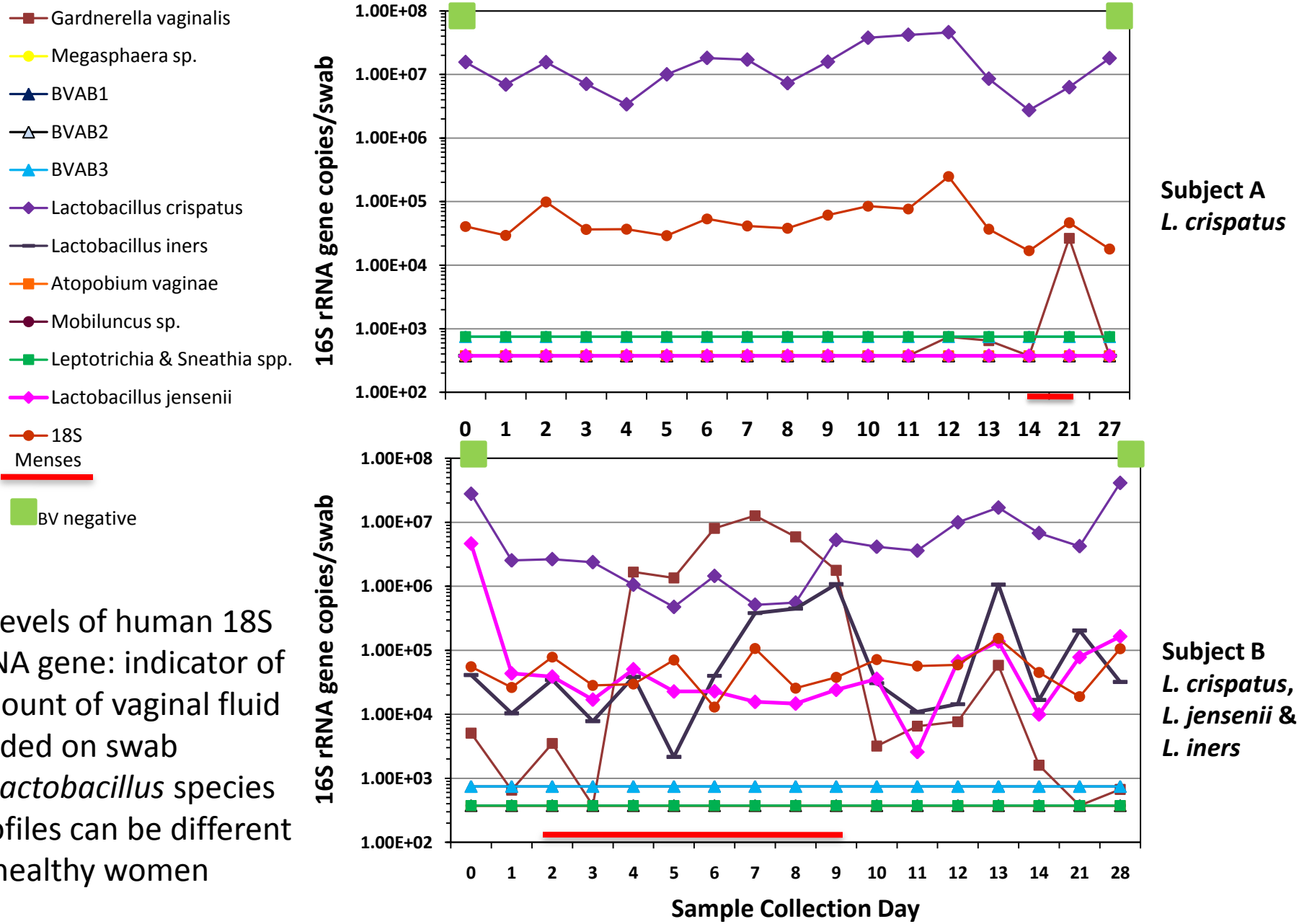
Table 3. **Multivariable Analysis of Factors Associated with Persistence of Bacterial Vaginosis (*BV*) in 113 Women, Adjusted for Nonadherence to Treatment**

BVAB Detected at Baseline	Risk Ratio (95% CI)*	Expected Risk for BV Persistence among Adherent Participants (95% CI)†
BVAB3	2.6 (1.4–5.45)	0.20 (0.04–0.44)
Peptoniphilus lacrimalis	2.8 (1.2–13.3)	0.22 (0.10–0.36)
Neither BVAB nor *P. lacrimalis*	Referent	0.08 (0.02–0.15)

BVAB= bacterial vaginosis–associated bacteria.

* Risk ratios and 95% CIs were obtained by using Poisson regression with bootstrap CIs.

Fluctuation of bacteria in women without BV



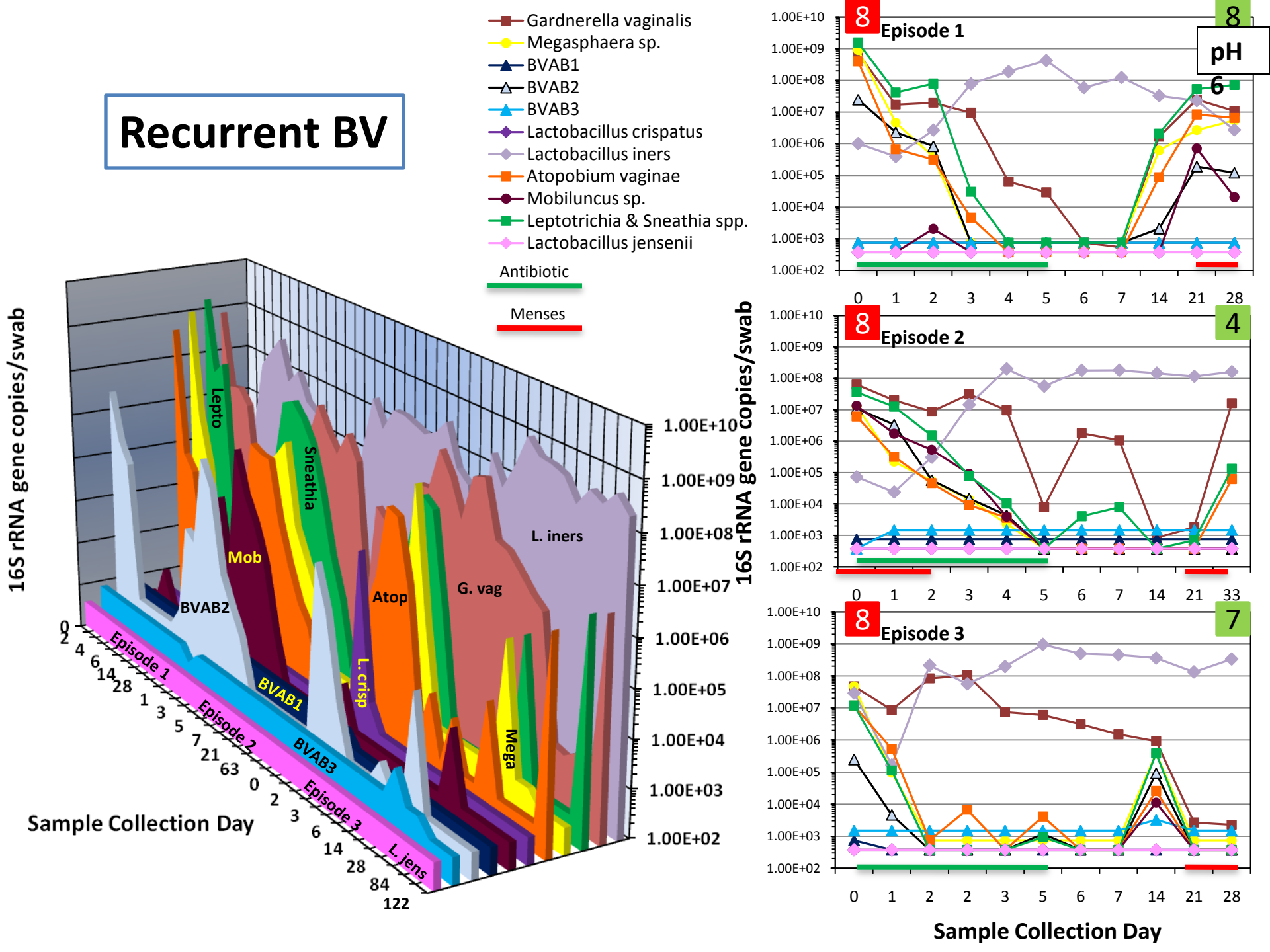
- Levels of human 18S rRNA gene: indicator of amount of vaginal fluid loaded on swab
- *Lactobacillus* species profiles can be different in healthy women

Differences in levels of bacteria by qPCR during menstruation

Bacterium	mean log_{10} adjusted difference (95% CI), p-value* during menstruation
Lactobacillus crispatus	-0.60 (-0.94, -0.25), p=0.001
Lactobacillus jensenii	-0.39 (-0.79, 0.01), p=0.06
Lactobacillus iners	0.10 (-0.23, 0.43), p=0.56
Gardnerella vaginalis	1.38 (0.83, 1.93), p<0.001

Srinivasan S et al. PLoS One 2010.

Recurrent BV



Summary: Vaginal Microbiota

- The human vagina harbors communities of bacteria that are very different from other human body sites
- Bacterial diversity in subjects without BV is limited, whereas subjects with BV have a high degree of species richness that includes many novel and fastidious bacteria
- Treatment of BV with antibiotics results in a rapid decline of anaerobic bacteria, though relapse is common
- The nature of the interactions among BV-associated bacteria is poorly understood
  - Functional redundancy to explain heterogeneity?
  - Are there syntrophic metabolic interactions?

The Human Microbiome and Omics

- Single cell genomics: NIH sequencing initiative
  - What are the functional capabilities of individual microbes?
- Metagenomics: assessing community gene content by high throughput sequencing
  - What are the functional capabilities of microbial communities as assessed by gene representation?
- Metatranscriptomics: mRNA and rRNA
  - Which genes are expressed in certain communities under defined conditions?
- Proteomics: Which proteins are present and how do they change with host factors or community composition?
- Metabolomics: Which small molecule metabolites are present in a given habitat and how do fluxes illuminate the biochemistry of the community?

Acknowledgements

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Roger Bumgarner, PhD

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