

Studying the Human Microbiome in Epidemiologic Studies

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Metabolic Epidemiology Branch

Division of Cancer Epidemiology and Genetics

The Human Microbiome

- Vast array of microorganisms in and on the body
- Perform critical physiological functions
- Communities, not single taxa
- NGS allows new forms of characterization that couldn't be approached using culture-based methods



Epidemiologic studies

Biosample collection methods must be:

- Reproducible, stable, and accurate
- Suitable for multiple assay platforms
- Cost efficient when collecting tens of thousands
- Patient acceptable in healthy subjects

Quality control standards:

- To evaluate reproducibility across and between studies
- To facilitate data pooling

Statistical methods:

- Multivariate models
- Compositional data

Optimizing biosample collection

Fecal collection methods



No Solution



RNA later

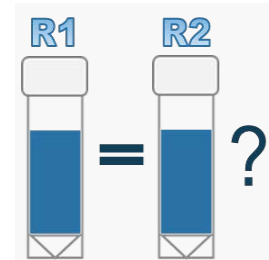


95% Ethanol

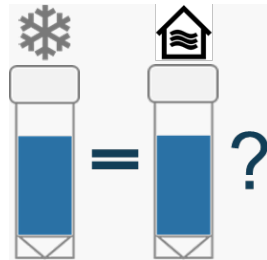


Optimizing biosample collection

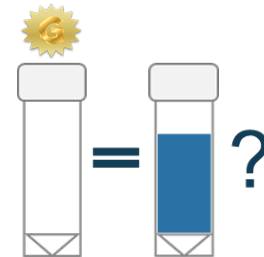
1. Technical reproducibility



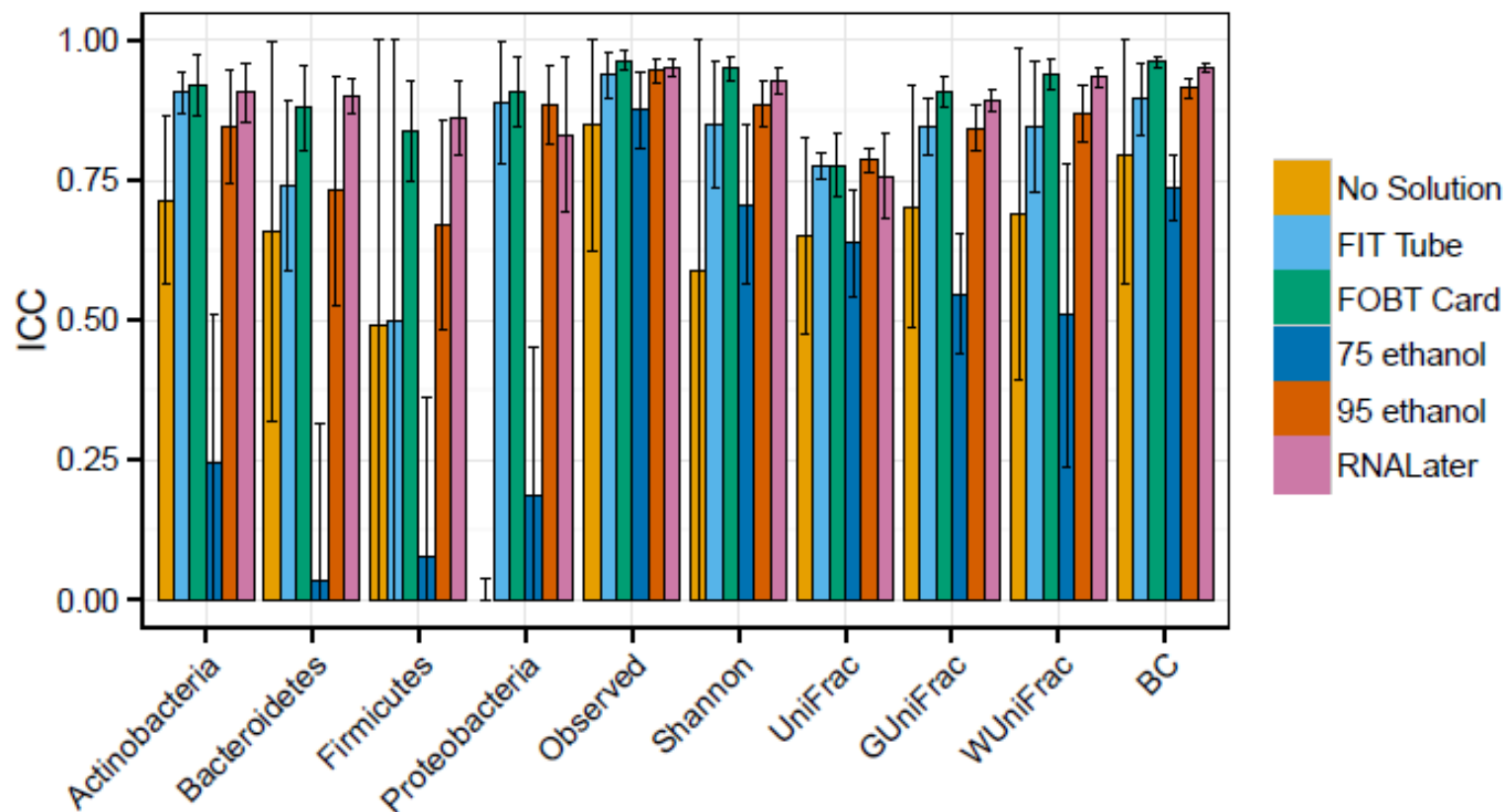
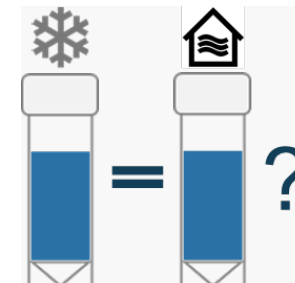
2. Stability



3. Concordance or accuracy



Optimizing biosample collection



Relative Abundance

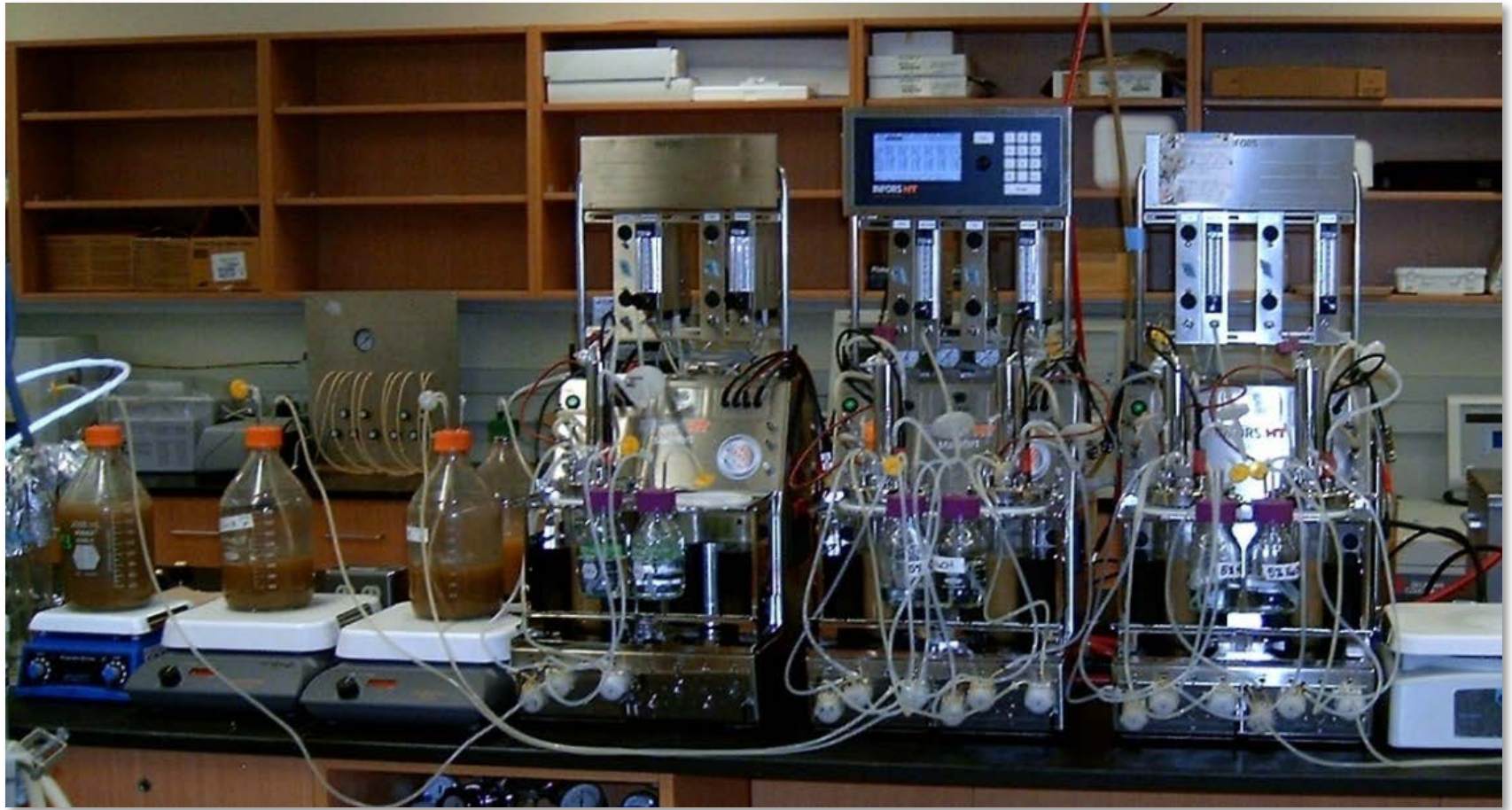
Alpha-

Beta-Diversity

Optimizing fecal collection

Collection method	Analytic Method			
	<u>16S rRNA gene</u>	<u>Shotgun sequencing</u>	<u>Transcriptomics</u>	<u>Metabolomics</u>
No additive	Samples are not stable after storage at ambient temperature			
75% ethanol	Poor	Not tested	Not tested	Not tested
95% ethanol	Good	Fair/Good	Being analyzed	Good
FOBT	Good	Good	Being analyzed	Fair/Good
FIT	Good	Good	Being analyzed	Poor
<i>RNAlater</i>	Good	Good	Being analyzed	Failed GC/MS

QC standards



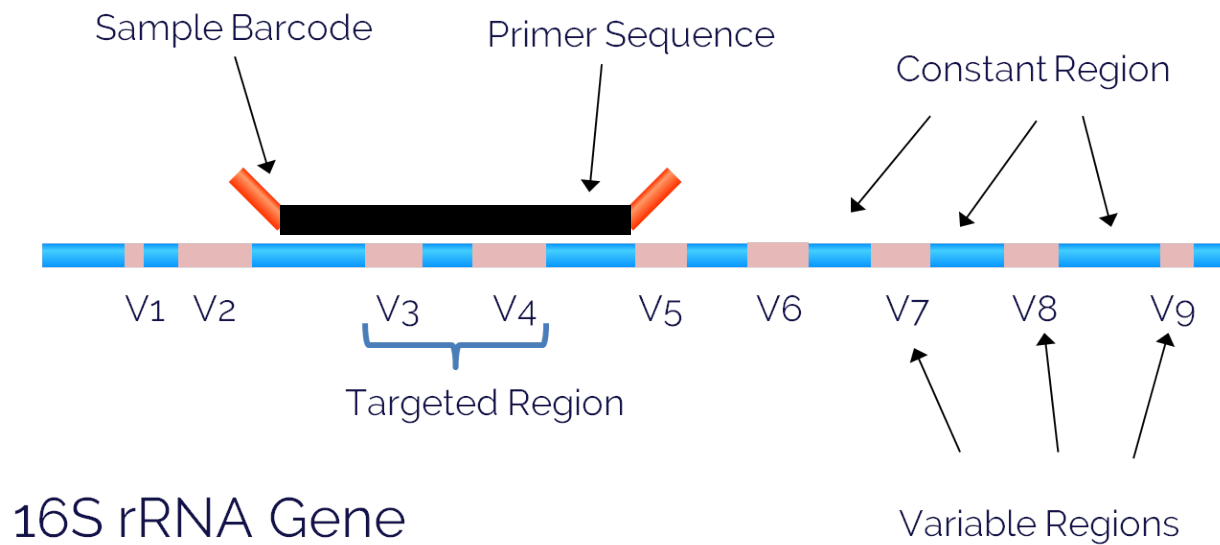
QC standards

Not currently available from NIST

We constructed three

- Robogut/Chemostat provides liters of identical complex 'natural' sample
- Aliquots from generous donors
- Artificial communities with dozens to hundreds of known bacteria

DNA extraction, PCR, and sequencing



Microbiome Quality Control Project: MBQC



OPEN LETTER

Open Access

The microbiome quality control project: baseline study design and future directions



Rashmi Sinha^{1*} , Christian C. Abnet¹, Owen White², Rob Knight³ and Curtis Huttenhower⁴

Microbiome Quality Control Project: MBQC



OPEN LETTER

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The microbiome quality control project: baseline study design and future directions








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MBQC

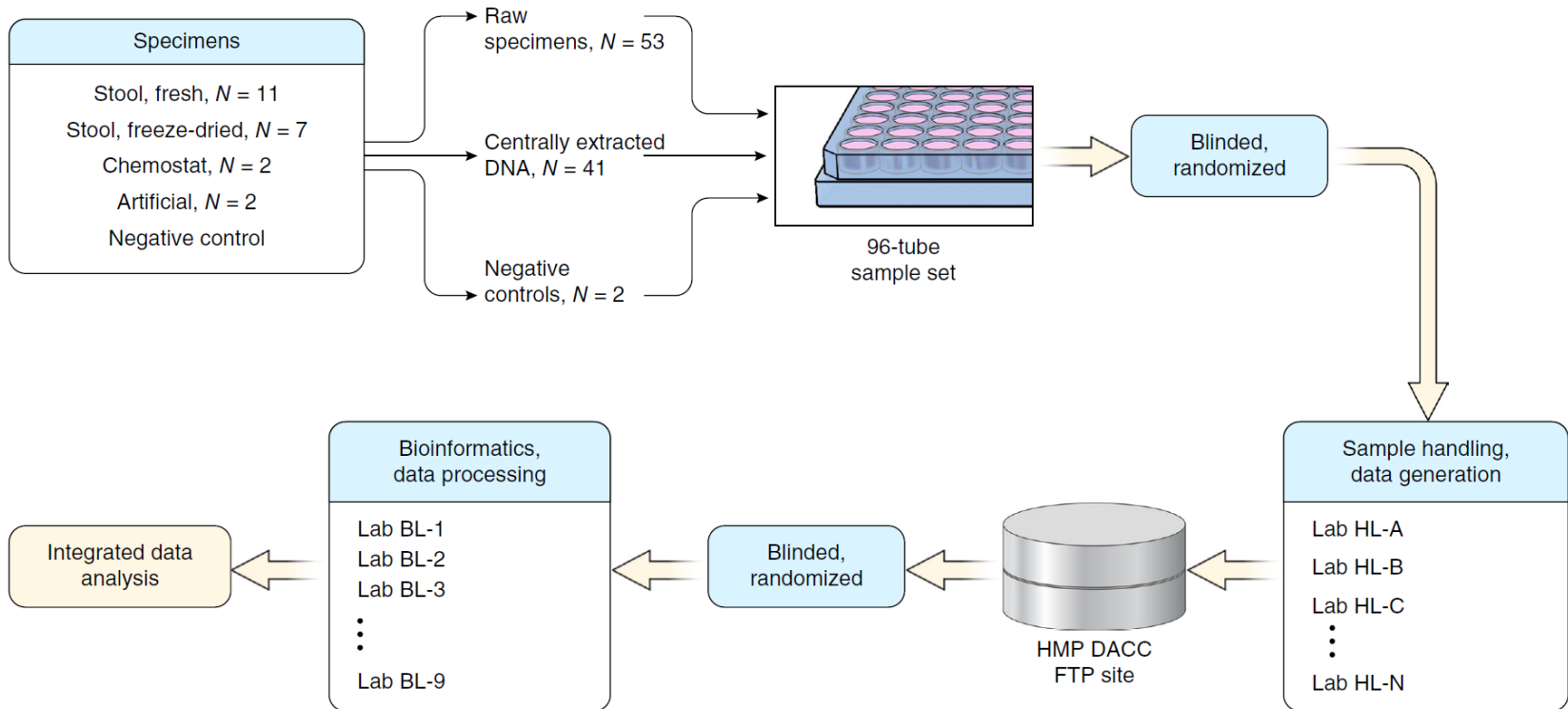
**nature
biotechnology**

Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium

Rashmi Sinha¹, Galeb Abu-Ali^{2,3}, Emily Vogtmann¹ , Anthony A Fodor⁴, Boyu Ren², Amnon Amir⁵, Emma Schwager^{2,3} , Jonathan Crabtree⁶, Siyuan Ma^{2,3}, The Microbiome Quality Control Project Consortium⁷, Christian C Abnet¹ , Rob Knight^{5,8} , Owen White⁶ & Curtis Huttenhower^{2,3} 

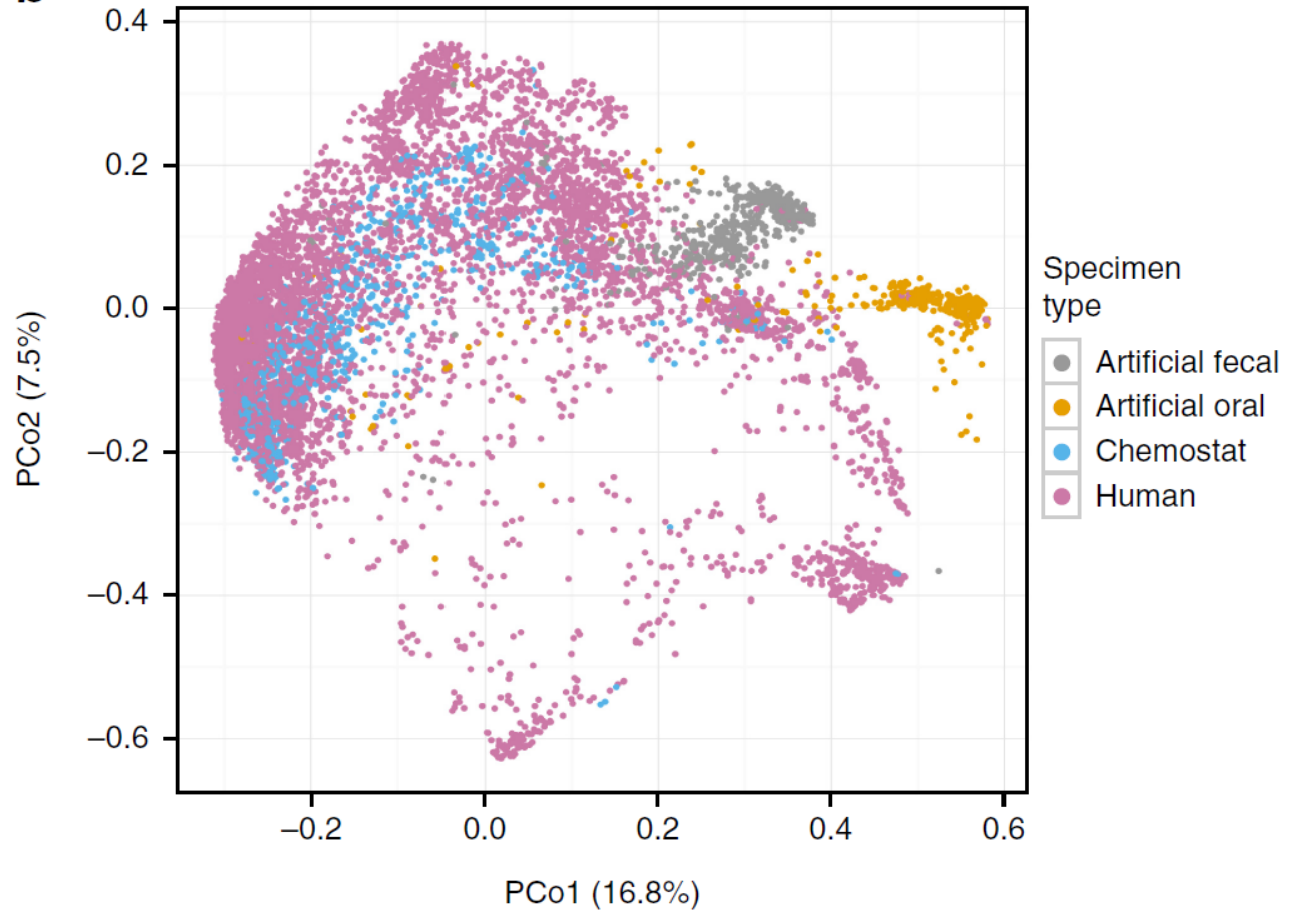
NATURE BIOTECHNOLOGY VOLUME 35 NUMBER 11 NOVEMBER 2017

MBQC

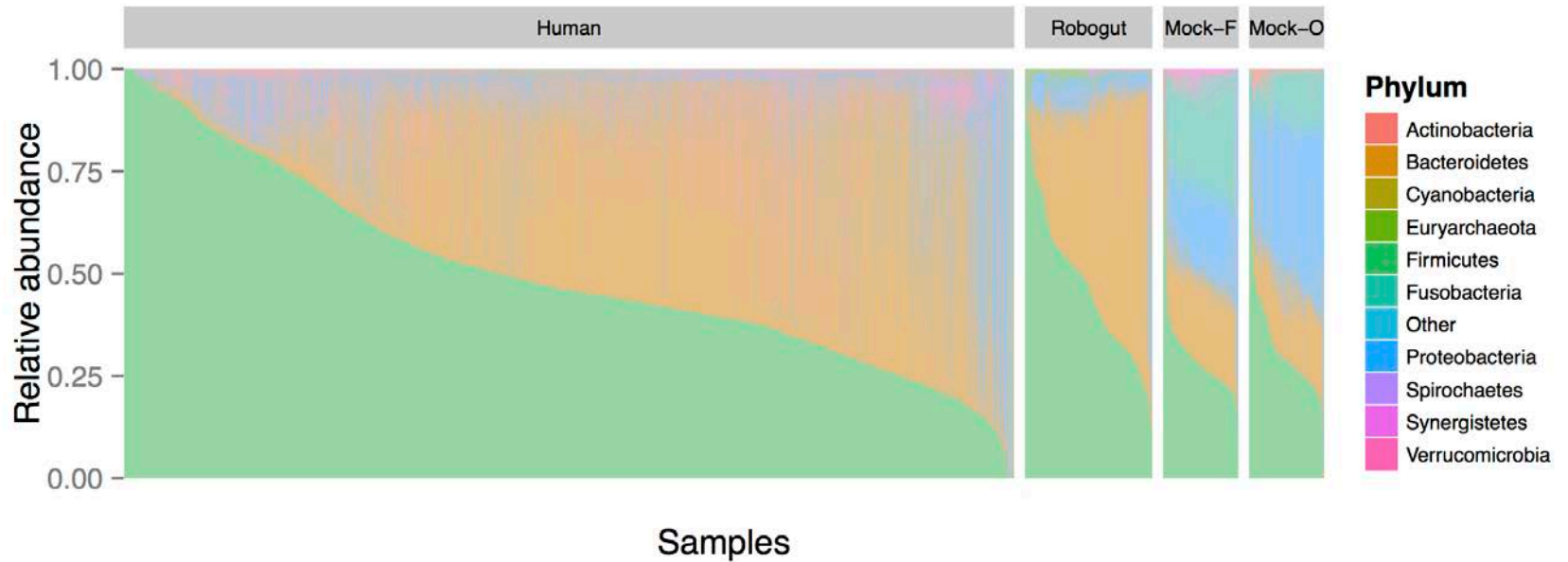


MBQC

b



MBQC



Supplementary Figure 1: Taxonomic profiles of the MBQC-base samples. Proportions of 10 bacterial phyla that were detected with a minimum relative abundance of 0.01% in at least 10% of the 16,554 samples that were subjected to integrated analysis.



Next Steps in Studying the Human Microbiome and Health in Prospective Studies

Bethesda, MD

May 16 – 17, 2017

Sponsored by

Metabolic Epidemiology Branch, DCEG

Epidemiology and Genomics Research Program, DCCPS

25 speakers

More than 200 registrants

Academia, government, industry

Next steps

Collect fecal and oral samples in cohorts now

Include one common biosample collection method per cohort if feasible to aid pooling projects

Include QC samples and blanks routinely

Use shared QC samples to aid interpolation

Continue work on standardizing DNA processing, bioinformatics, metabolomics

Deeper engagement with biostatisticians

Cancer Epidemiology, Biomarkers & Prevention
Comparison of Collection Methods for Fecal Samples for Discovery Metabolomics in Epidemiologic Studies
 Enrika Lottfeld, Emily Vogtmann, Joshua N. Sampson, Steven C. Moore, Heidi Nelson, Rob Knight, Nicholas Chia, and Rashmi Sinha

Cancer Epidemiology, Biomarkers & Prevention
Research Strategies for Nutritional and Physical Activity Epidemiology and Cancer Prevention
 Sondat Mahabir, Walter C. Willett, Christine M. Friedenreich, Gabriel Y. Lai, Carol J. Bouchez, Charles E. Matthews, Rashmi Sinha, Graham A. Cozzit, Joseph A. Rothwell, Jill Reedy, Alpa Y. Patel, Michael F. Leitzmann, Gary E. FRASER, Sharon Ross, Stephen D. Hunting, Christian C. Abnet, Lawrence H. Kushi, Philip R. Taylor, and Ross Prentice

JOURNAL of the NATIONAL CANCER INSTITUTE
Investigation of the Association Between the Fecal Microbiota and Breast Cancer in Postmenopausal Women: a Population-Based Case-Control Pilot Study
 James J. Goedert, Gielra Jones, Xing Hua, Xia Xu, Guoqin Yu, Roberto Flores, Roni T. Falk, Mitchell H. Gail, Jianxin Shi, Jacques Ravel ... Show more

frontiers
 in Cellular and Infection Microbiology
Molecular Characterization of the Human Stomach Microbiota in Gastric Cancer Patients
 Guoqin Yu, Javier Torres, [...], and Alica M. Goldstein

PLOS ONE
TENTH ANNIVERSARY
Colorectal Cancer and the Human Gut Microbiome: Reproducibility with Whole-Genome Shotgun Sequencing
 Emily Vogtmann, Xing Hua, Georg Zeller, Shirochi Sunagawa, Anita Y. Voigt, Rajna Herczeg, James J. Goedert, Jianxin Shi, Peer Dork, Rashmi Sinha

Cancer Causes & Control
Association between tobacco use and the upper gastrointestinal microbiome among Chinese men
 Emily Vogtmann, Roberto Flores, Guoqin Yu, Neal D. Freedman, Jianxin Shi, Mitchell H. Gail, Bruce A. Dye, Guo-Qing Wang, Vanja Klepac-Ceraj, Bruce J. Paster, Wen-Qiang Wei, Hui-Qin Guo, Sanford M. Dawsey, You-Lin Qiao, Christian C. Abnet

Gut
Human oral microbiome and prospective risk for pancreatic cancer: a population-based nested case-control study FREE
 Xiaozhou Fan¹, Alexander V Alekseyenko², Jing Wu³, Brandilyn A Peters¹, Eric J Jacobs², Susan M Gapstur², Mark P Purdue⁴, Christian C Abnet⁵, Rachael Stolzenberg-Solomon⁶, George Miller^{3,4,7}, Jacques Ravel⁸, Richard B Hayes^{1,7}, Jyoung Ahn^{1,7}

PLOS ONE
TENTH ANNIVERSARY
Fecal Microbiota, Fecal Metabolome, and Colorectal Cancer Interrelations
 Rashmi Sinha, Jyoung Ahn, Joshua N. Sampson, Jianxin Shi, Guoqin Yu, Xiaoqin Xiong, Richard B. Hayes, James J. Goedert

Obesity
Beta-diversity metrics of the upper digestive tract microbiome are associated with body mass index
 Shih-Wen Lin, Neal D. Freedman, Jianxin Shi, Mitchell H. Gail, Emily Vogtmann, Guoqin Yu, Vanja Klepac-Ceraj, Bruce J. Paster, Bruce A. Dye, Guo-Qing Wang, Wen-Qiang Wei, Jin-Hu Fan, You-Lin Qiao, Sanford M. Dawsey, Christian C. Abnet

BMC Public Health
The association between the upper digestive tract microbiota by HOMIM and oral health in a population-based study in Linxian, China
 Guoqin Yu, Bruce A Dye, Mitchell H Gail, Jianxin Shi, Vanja Klepac-Ceraj, Bruce J Paster, Guo-Qing Wang, Wen-Qiang Wei, Jin-Hu Fan, You-Lin Qiao, Sanford M Dawsey, Neal D Freedman and Christian C Abnet

SCIENTIFIC REPORTS
Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia
 Dariush Nasrollahzadeh, Reza Malekzadeh, Alexander Pioner, Ramin Shakeri, Masoud Sotoudeh, Saman Fahimi, Siavosh Nasreri-Moghaddam, Farin Kamangar, Christian C. Abnet, Björn Wöckler, Farhad Islami, Paolo Doffetta, Paul Brennan, Sanford M. Dawsey & Weimin Ye

Cancer Epidemiology, Biomarkers & Prevention
Collecting Fecal Samples for Microbiome Analyses in Epidemiology Studies
 Rashmi Sinha, Jun Chen, Amnon Amir, Emily Vogtmann, Jianxin Shi, Kristin S. Inman, Roberto Flores, Joshua Sampson, Rob Knight, and Nicholas Chia

American Journal of Epidemiology
Comparison of Collection Methods for Fecal Samples in Microbiome Studies
 Emily Vogtmann, Jun Chen, Amnon Amir, Jianxin Shi, Christian C. Abnet, Heidi Nelson, Rob Knight, Nicholas Chia, Rashmi Sinha Author Notes

PLOS ONE
TENTH ANNIVERSARY
Sex, Body Mass Index, and Dietary Fiber Intake Influence the Human Gut Microbiome
 Christina Dominanni, Rashmi Sinha, James J. Goedert, Zhihang Pei, Liying Yang, Richard B. Hayes, Jyoung Ahn

IJC International Journal of Cancer
Gastric microbiota features associated with cancer risk factors and clinical outcomes: A pilot study in gastric cardia cancer patients from Shanxi, China
 Guoqin Yu, Nan Hu, Leming Wang, Chaoyu Wang, Xiao-You Han, Mike Humphry, Jacques Ravel, Christian C. Abnet, Philip R. Taylor, Alica M. Goldstein

BJC British Journal of Cancer
Postmenopausal breast cancer and oestrogen associations with the IgA-coated and IgA-noncoated faecal microbiota
 James J. Goedert, Xing Hua, Agata Bielicka, Isaac Okoyos, Ginger L. Mitts, Giera S. Jones, Hebaoui Fujisawa, Rashmi Sinha, Yunhu Wu, Xia Xu, Jacques Ravel, Hainan Shi, Noah W. Palm & Heather Spencer-Felgason

Carcinogenesis
Fecal metabolomics: assay performance and association with colorectal cancer
 James J. Goedert, Joshua N. Sampson, Steven C. Moore, Qian Xiao, Xiaoqin Xiong, Richard B. Hayes, Jyoung Ahn, Jianxin Shi, Rashmi Sinha

nature biotechnology
Assessment of variation in microbial community amplicon sequencing by the Microbiome Quality Control (MBQC) project consortium
 Rashmi Sinha, Galib Abu Ali, Emily Vogtmann, Anthony A Fedor, Boyu Ren, Amnon Amir, Emma Schwager, Jonathan Crabtree, Siyan Ma, The Microbiome Quality Control Project Consortium, Christian C. Abnet, Rob Knight, Owen White & Curtis Huttenhower

Applied and Environmental Microbiology
Comparison of Fecal Collection Methods for Microbiota Studies in Bangladesh
 Emily Vogtmann, Jun Chen, Muhammad G. Kibriya, Yu Chen, Tariqul Islam, Mahbul Eunes, Alauddin Ahmed, Jabun Naher, Anisur Rahman, Amnon Amir, Jianxin Shi, Christian C. Abnet, Heidi Nelson, Rob Knight, Nicholas Chia, Habibur Ahsan and Rashmi Sinha

JOURNAL of the NATIONAL CANCER INSTITUTE
Human Gut Microbiome and Risk for Colorectal Cancer
 Jyoung Ahn, Rashmi Sinha, Zhiheng Pei, Christine Dominanni, Jing Wu, Jianxin Shi, James J. Goedert, Richard B. Hayes, Liying Yang

Genome Biology
The microbiome quality control project: baseline study design and future directions
 Rashmi Sinha, Christian C. Abnet, Owen White, Rob Knight and Curtis Huttenhower

DCEG microbiome portfolio

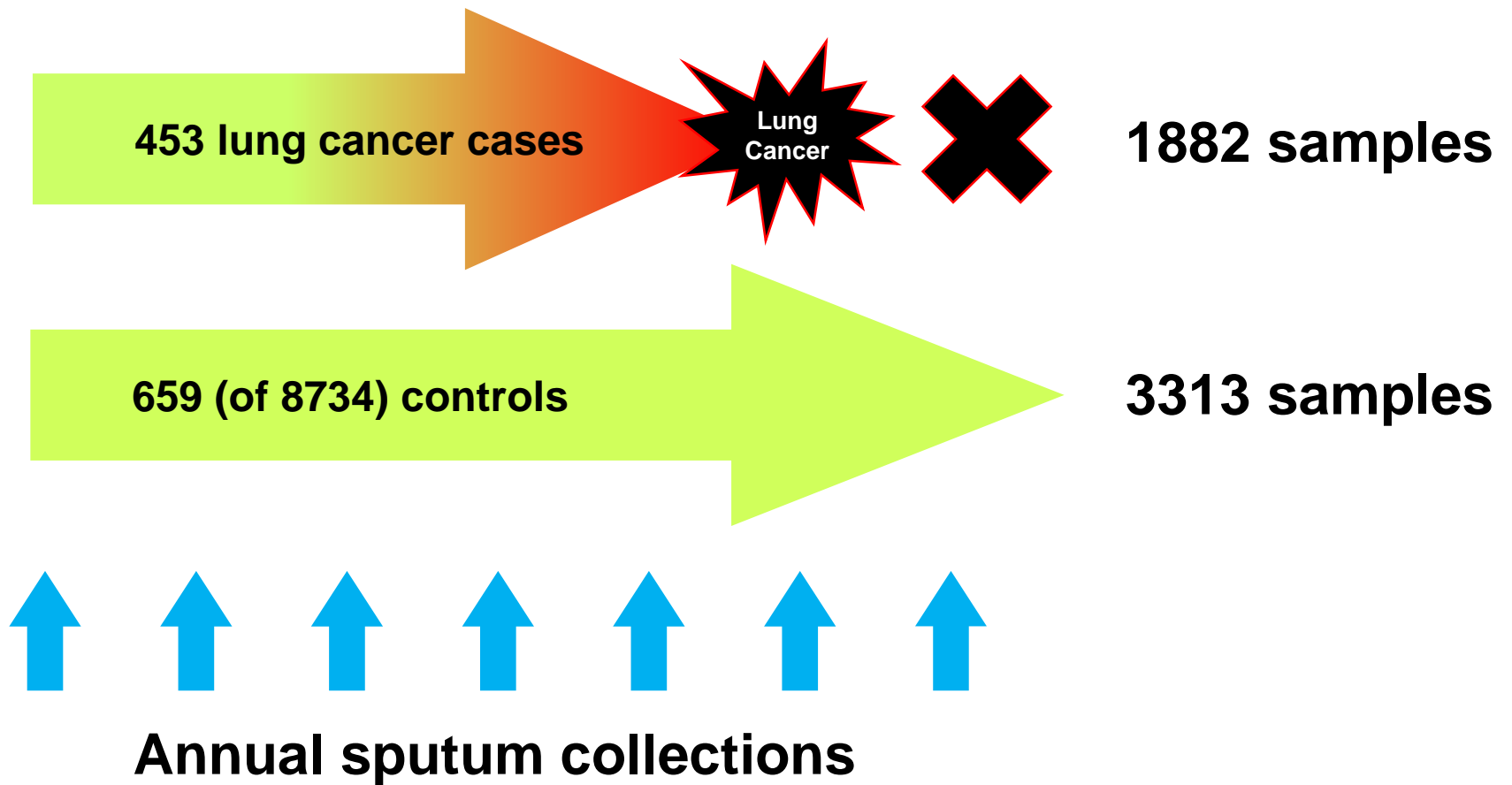
- **Methods studies (Collection, MBQC etc.)**
- **Using existing oral/sputum collections**
- **New cohorts**

NHANES

- CDC/National Center for Health Statistics
- Representative sample of the US population
- Extensive phenotyping
- 10,000 oral wash specimens



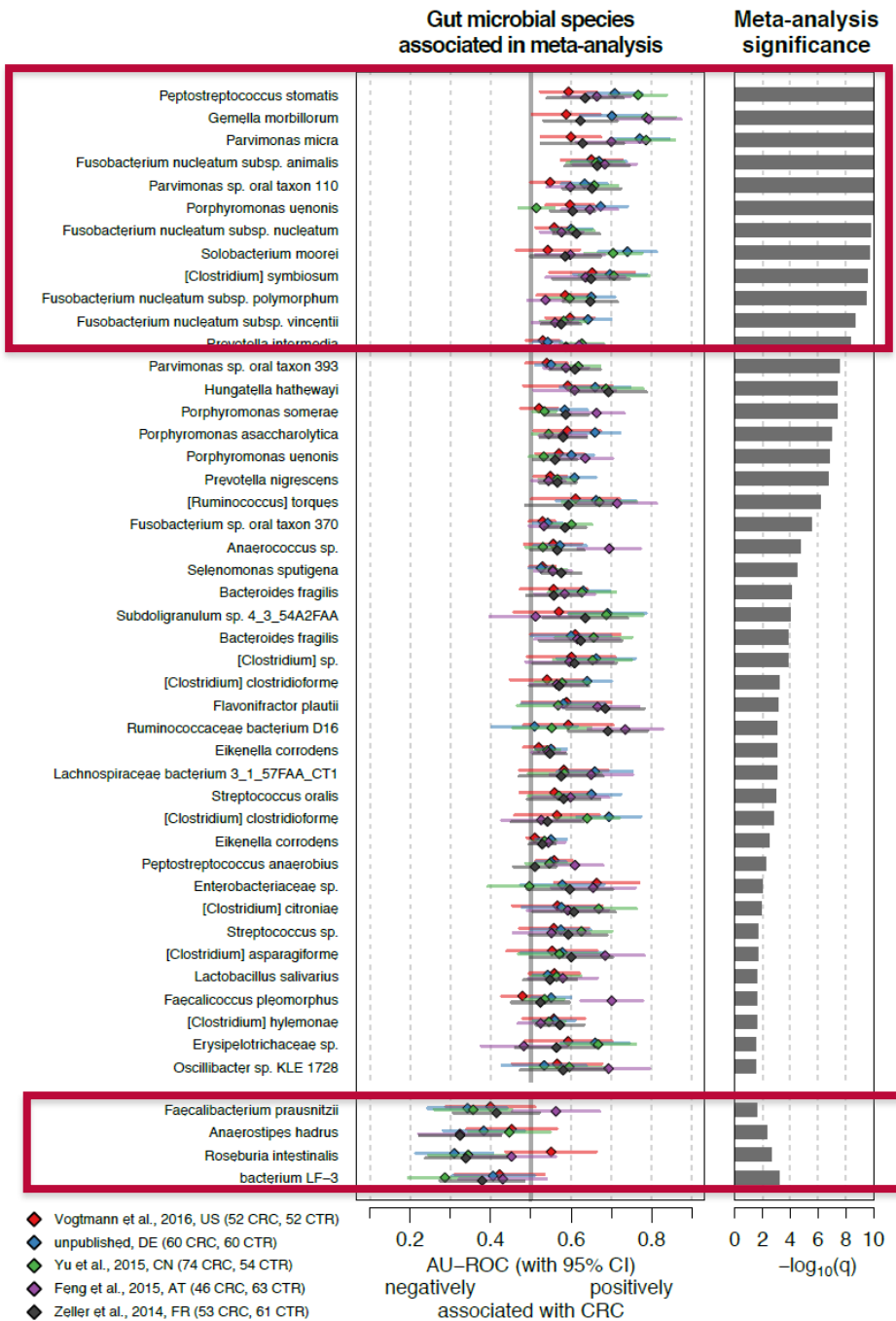
Yunnan Tin Miners



Oral microbiome case-cohort study

- Oral samples from 3 US Cohorts
- Follow-up for more than 10 years

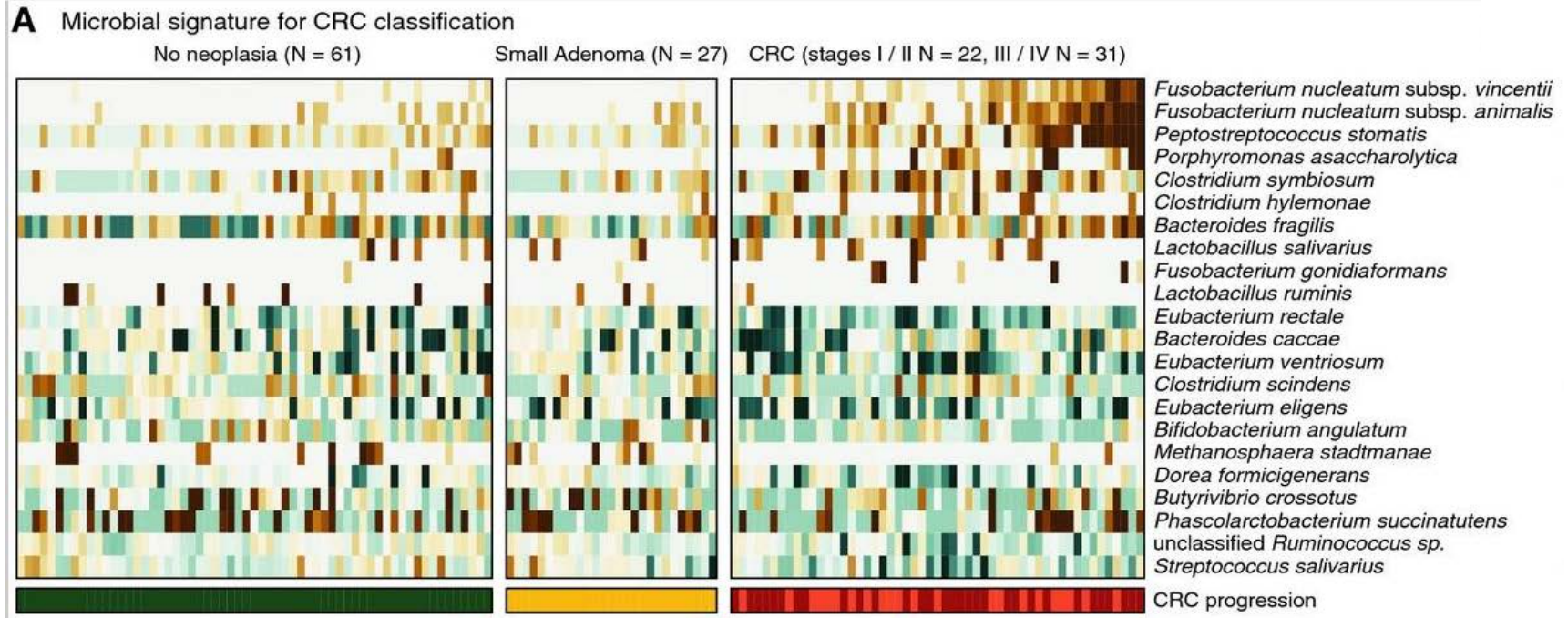
Cancer site	AHS	PLCO	NIH-AARP	Total
Bronchus/lung	204	751	542	1497
Colorectum	294	525	331	1150
Esophagus	26	-	43	69
Head/neck	57	-	60	117
Hepatobiliary tract	19	91	47	157
Pancreas	43	-	129	172
Small intestine	14	31	29	74
Stomach	44	60	58	162
Sum of cases	701	1458	1239	3398
Referent group	1000	1289	1300	3589
Grand Total				6987



Meta-analyses of shotgun sequence data for CRC from five case-control studies

Relative abundances of 22 gut microbial species

(fold change over the median relative abundance observed in controls)



CANCER

Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer

Susan Bullman,^{1,2} Chandra S. Pedomallu,^{1,2} Ewa Sicinska,¹ Thomas E. Clancy,³ Xiaoyang Zhang,^{1,2} Diana Cai,^{1,2} Donna Neuberg,¹ Katherine Huang,² Fatima Guevara,¹ Timothy Nelson,¹ Otari Chipashvili,¹ Timothy Hagan,¹ Mark Walker,² Aruna Ramachandran,^{1,2} Begoña Diosdado,^{1,2} Garazi Serna,⁴ Nuria Mulet,⁴ Stefania Landolfi,⁴ Santiago Ramon y Cajal,⁴ Roberta Fasani,⁴ Andrew J. Aguirre,^{1,2,3} Kimmie Ng,¹ Elena Élez,⁴ Shuji Ogino,^{1,3,5} Josep Tabernero,⁴ Charles S. Fuchs,⁶ William C. Hahn,^{1,2,3} Paolo Nuciforo,⁴ Matthew Meyerson^{1,2,3*}

Prospective study of oral microbiome and colorectal cancer risk

- Oral wash from 1150 future colorectal cancer cases and 3500 controls
- Presence of the *Fusobacterium* genus was not associated with CRC risk
- Ongoing analyses
 - 16S resolution may not be sufficient
 - Predictors of positivity
 - Polyp Prevention Trial tissue collection

DCEG New Cohorts: Planning stage

Next Gen cohort

U.S. Department of Veterans Affairs

Million Veteran Program

Fecal immunochemical test cohorts

Kaiser Northern California

Piedmont colorectal screening program

Chinese CRC screening trial cohort

Acknowledgements

DCEG

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Anil Chaturvedi
Neal Freedman
Laure Beane Freeman
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Xing Hua
Jianxin Shi
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Bin Zhu

DCCPS

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Peer Bork
Georg Zeller

Harvard

Curtis Huttenhower

University of California San Diego

Rob Knight

University of Maryland

Owen White

Mayo Clinic

Nick Chia
Jun Chen

MD Anderson

Maura Gillison

NIDCR

Bruce Dye

New York University

Marty Blaser

Northern Arizona University

Greg Caporaso



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