Introduction to metagenomic analysis

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Harvard University CFAR Workshop on
Metagenomics and Transcriptomics

16 September 2014

Huttenhower Research Group
Harvard School of Public Health
Department of Biostatistics

Content

- http://huttenhower.sph.harvard.edu/content/cfar2014

Plan

- Informal survey
- Metagenomics concepts & examples
- Tools for taxonomic profiling
  - MetaPhlAn
- Tools for functional profiling
  - HUMAnN
  - ShortBRED
  - PICRUSt
- Tools for testing associations
  - LEfSe
  - MaAsLin
  - CCREPE
- Resources
- Research vignette (time permitting)

What's metagenomics?

Total collection of **microorganisms** within a **community**

Also **microbial community** or **microbiota**

THE MICROFLORA AND THE PRODUCTIVITY OF LEACHED AND NON-LEACHED ALKALI SOIL

J. E. GREAVES[1]

Utah Agricultural Experiment Station

Received for publication July 2, 1926

Chemistry & Biology October 1998, 5:R245–249

Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products

Jo Handelsman[1], Michelle R Rondon[1], Sean F Brady[2], Jon Clardy[2] and Robert M Goodman[1]

ness that they are thought to contain. The methodology has been made possible by advances in molecular biology and eukaryotic genomics, which have laid the groundwork for cloning and functional analysis of the collective genomes of soil microflora, which we term the metagenome of the soil.

Total **genomic potential** of a microbial community

www.sciencemag.org SCIENCE VOL 292 11 MAY 2001

Commensal Host-Bacterial Relationships in the Gut

Lora V. Hooper and Jeffrey I. Gordon*

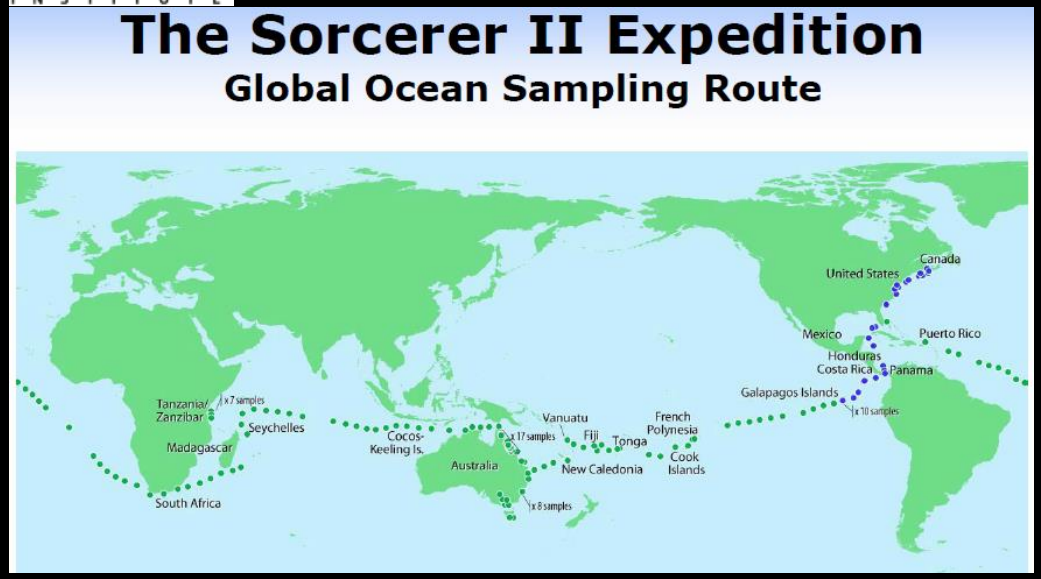
ber our somatic and germ cells (*3*). The Nobel laureate Joshua Lederberg has suggested using the term "microbiome" to describe the collective genome of our indigenous microbes (microflora), the idea being that a comprehensive genetic view of *Homo sapiens* as a life-form should include the genes in our microbiome (*4*).

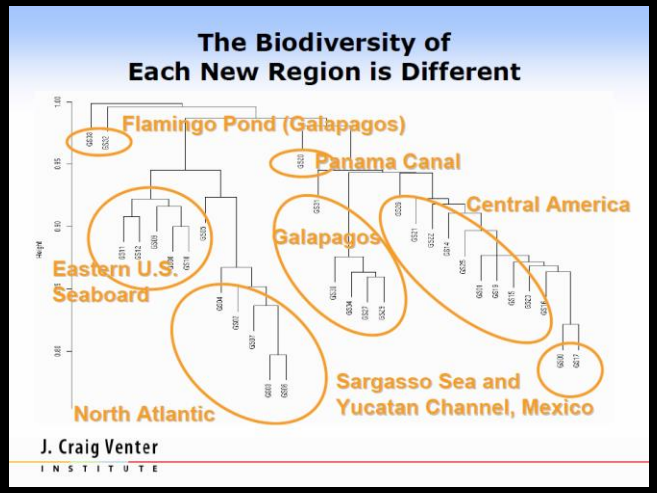
Total **biomolecular repertoire** of a microbial community

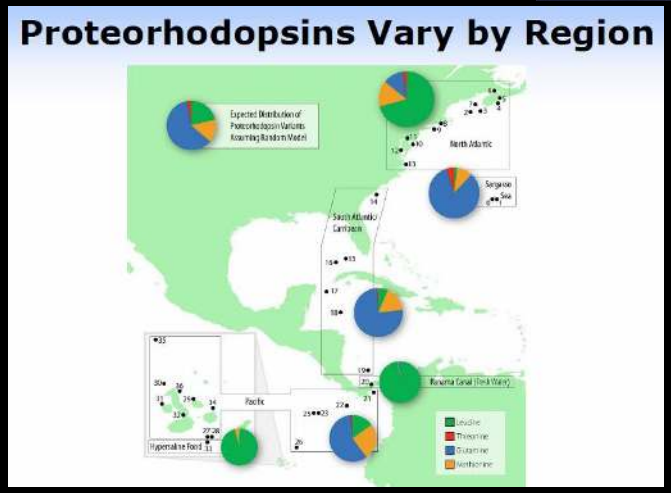
Study of **uncultured microorganisms** from the environment, which can include humans or other living hosts

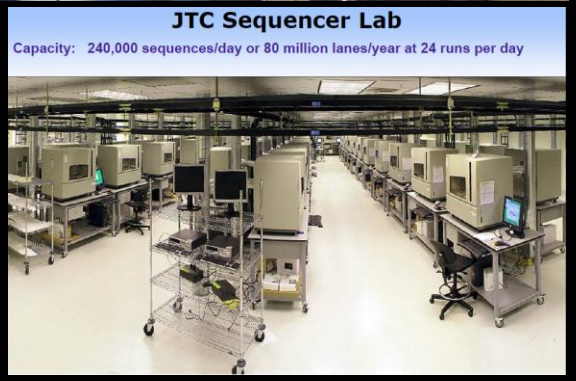
Examples of metagenomic studies: Global Ocean Sampling

J. Craig Venter INSTITUTE 2003/2004 - ongoing









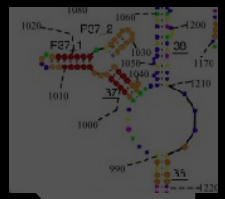
The NIH **H**uman **M**icrobiome **P**roject (**HMP**): A comprehensive microbial survey

- ***What is a "normal" human microbiome?***
- 300 healthy human subjects
- Multiple body sites
  - 15 male, 18 female
- Multiple visits
- Clinical metadata

www.hmpdacc.org

Sequencing as a tool for microbial community analysis

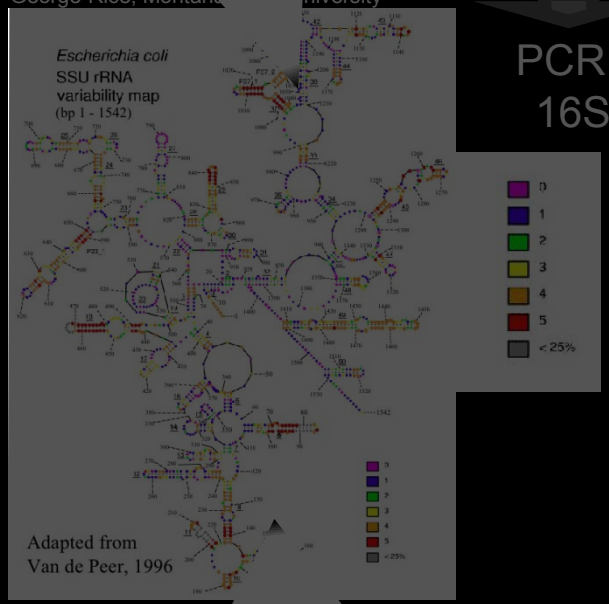
Lyse cells
Extract DNA (and/or RNA)



16S amplicons

Meta'omic

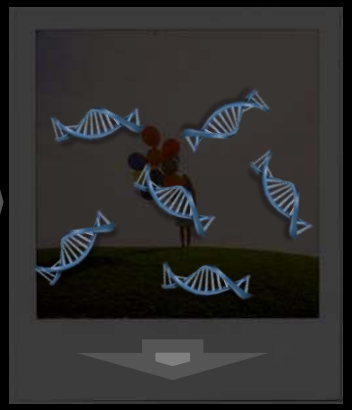
George Rice, Montana [illegible] niversity



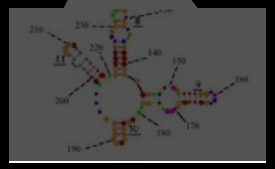
PCR to amplify the single 16S rRNA marker gene

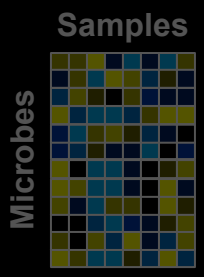
Hello
my name is

Classify sequence
➔ microbe

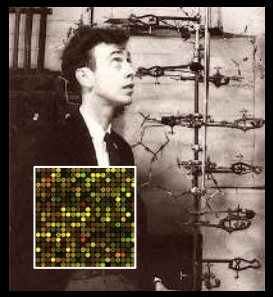


Genes,
Genomes,
Metabolic profiling,
Relative abundances,
Genetic variants...





What to do with your metagenome?



Reservoir of gene and protein functional information

Comprehensive snapshot of microbial ecology and evolution

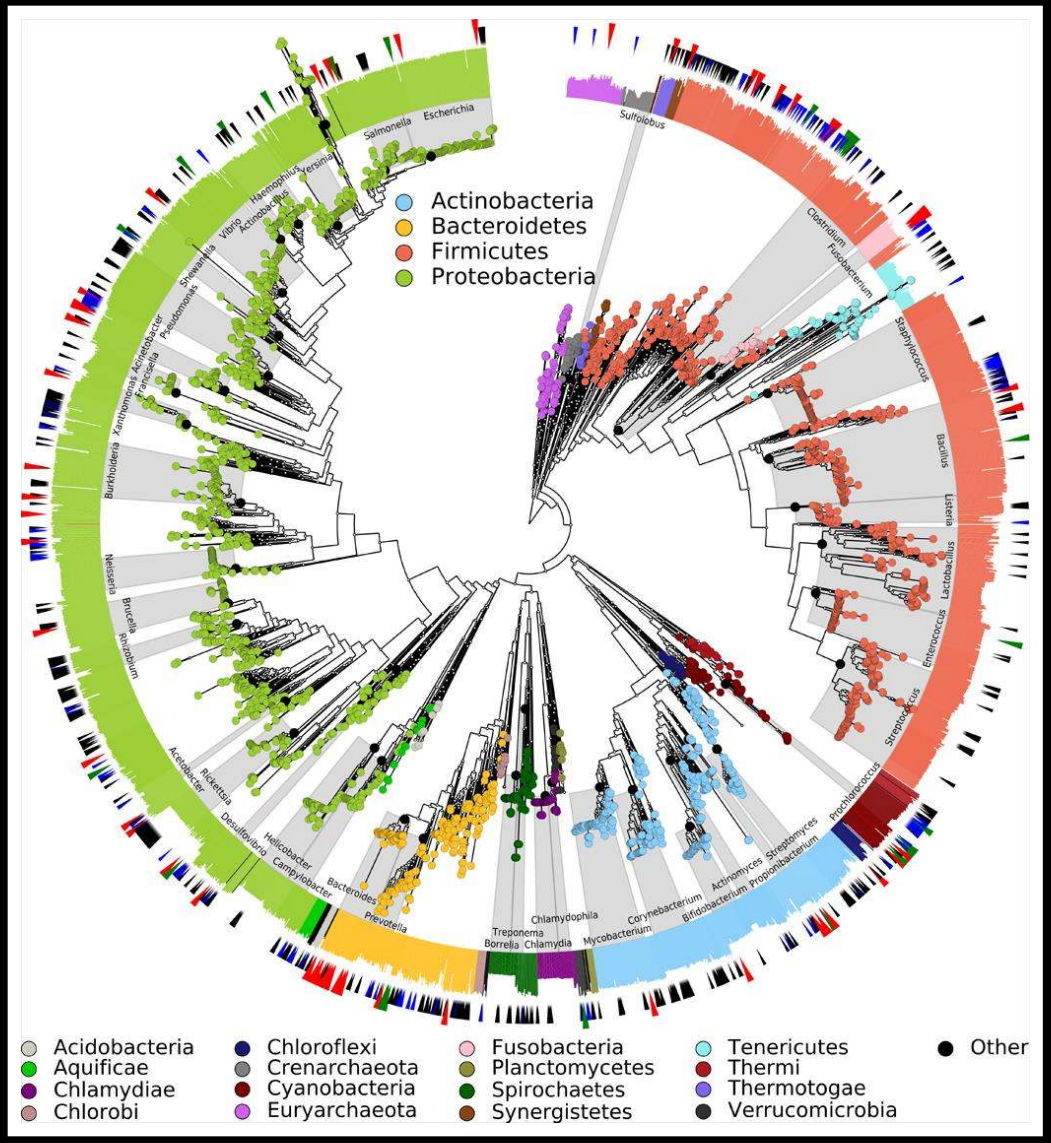
Basic science

Translational

Public health tool monitoring population health and epidemiology

Diagnostic or prognostic biomarker for host disease

Composition-based analyses



Microbiome composition analyses: **phylotypes** and **binning**

RIBOSOMAL DATABASE PROJECT

rdp.cme.msu.edu

green genes

16S rRNA gene database and workbench compatible with ARB

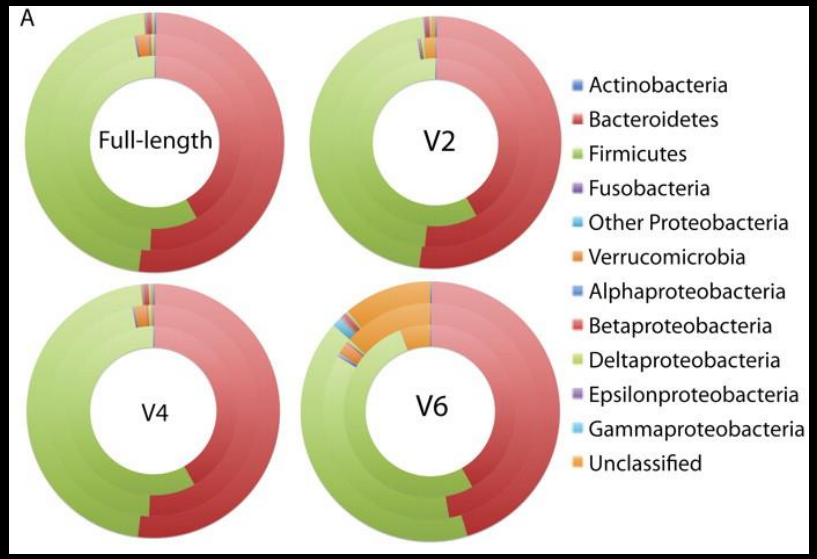
greengenes.lbl.gov

greengenes.lbl.gov

silva

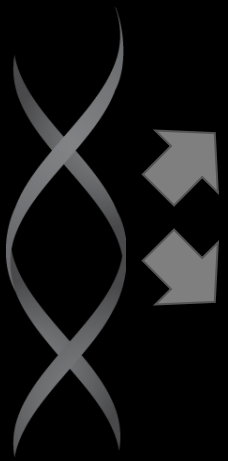
comprehensive ribosomal RNA databases

www.arb-silva.de



Binning: nontrivial assignment of reads to phylotypes

Phylotype or **operational taxonomic unit (OTU)**: organisms clonal to within some tolerance (e.g. 95%); "species"



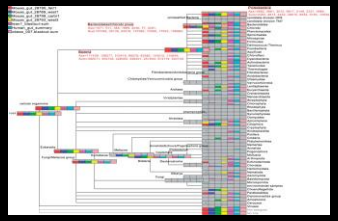
Indirect binning: BLAST etc. Relies on high similarity, reference seq.

Direct binning: analyzes seq. characteristics (GC, codons, etc.) Relies on long reads

Hamady, 2009 PhyloPythia: McHardy, 2007 TETRA: Chan, 2008 Phymm: Brady, 2009 MetaPhlAn: Segata, 2012

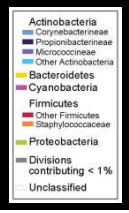
Microbiome composition analyses: **diversity**

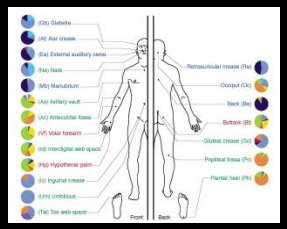
Mitra, 2009



Diversity: broadly, a community's number and distribution of organisms

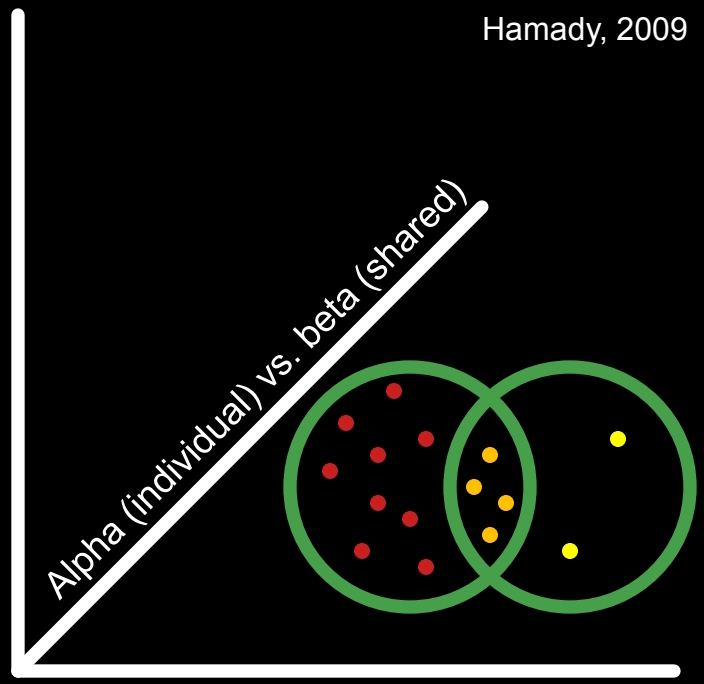
Also **community composition** or **structure**





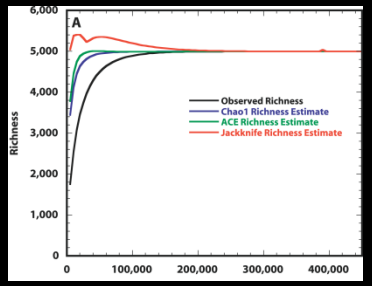
Susan Holmes, Stanford

Hamady, 2009



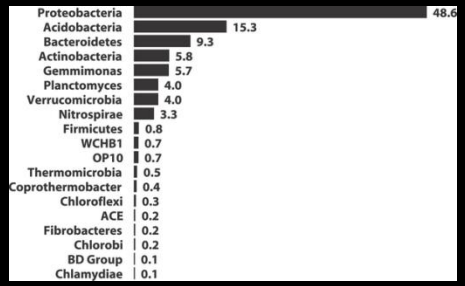
[1 1 0 0]

[10 6 1 4]



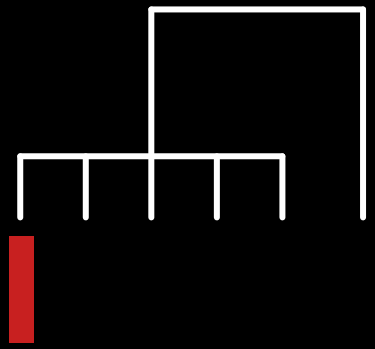
# of sequences

Schloss, 2006

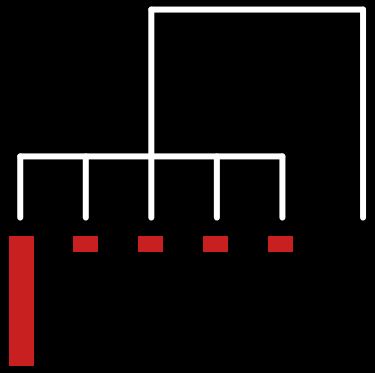


% of sequences

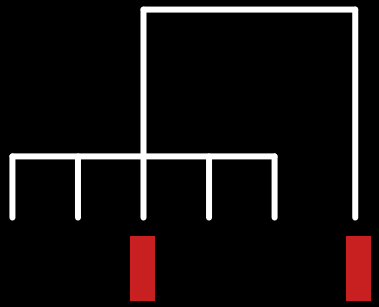
Microbiome composition analyses: **alpha diversity (1-sample) scenarios**



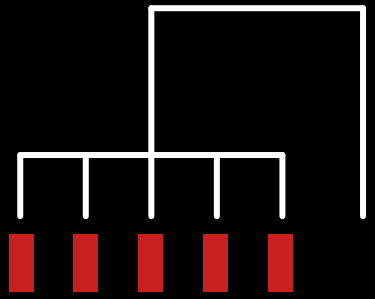
Not diverse



Qualitatively diverse
Taxonomically diverse

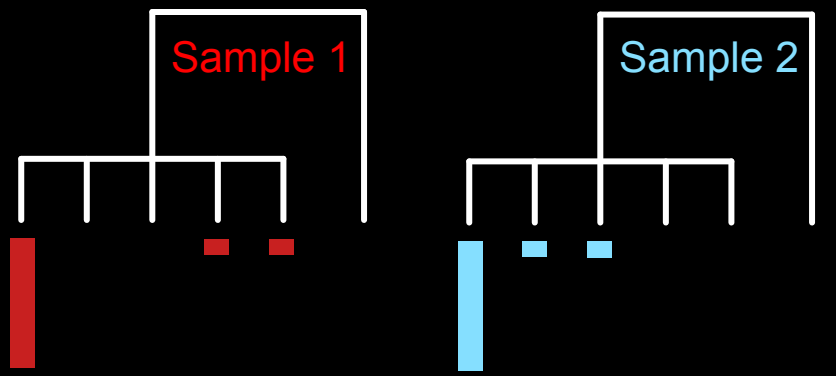


Phylogenetically diverse

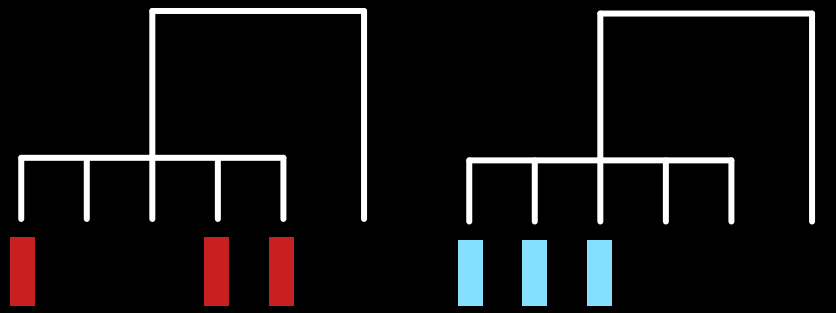


Quantitatively diverse
Taxonomically diverse

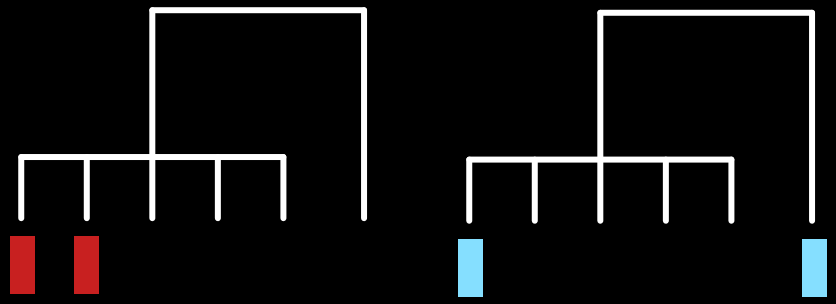
Microbiome composition analyses: **beta diversity (2-sample) scenarios**



Qualitatively diverse
Taxonomically diverse

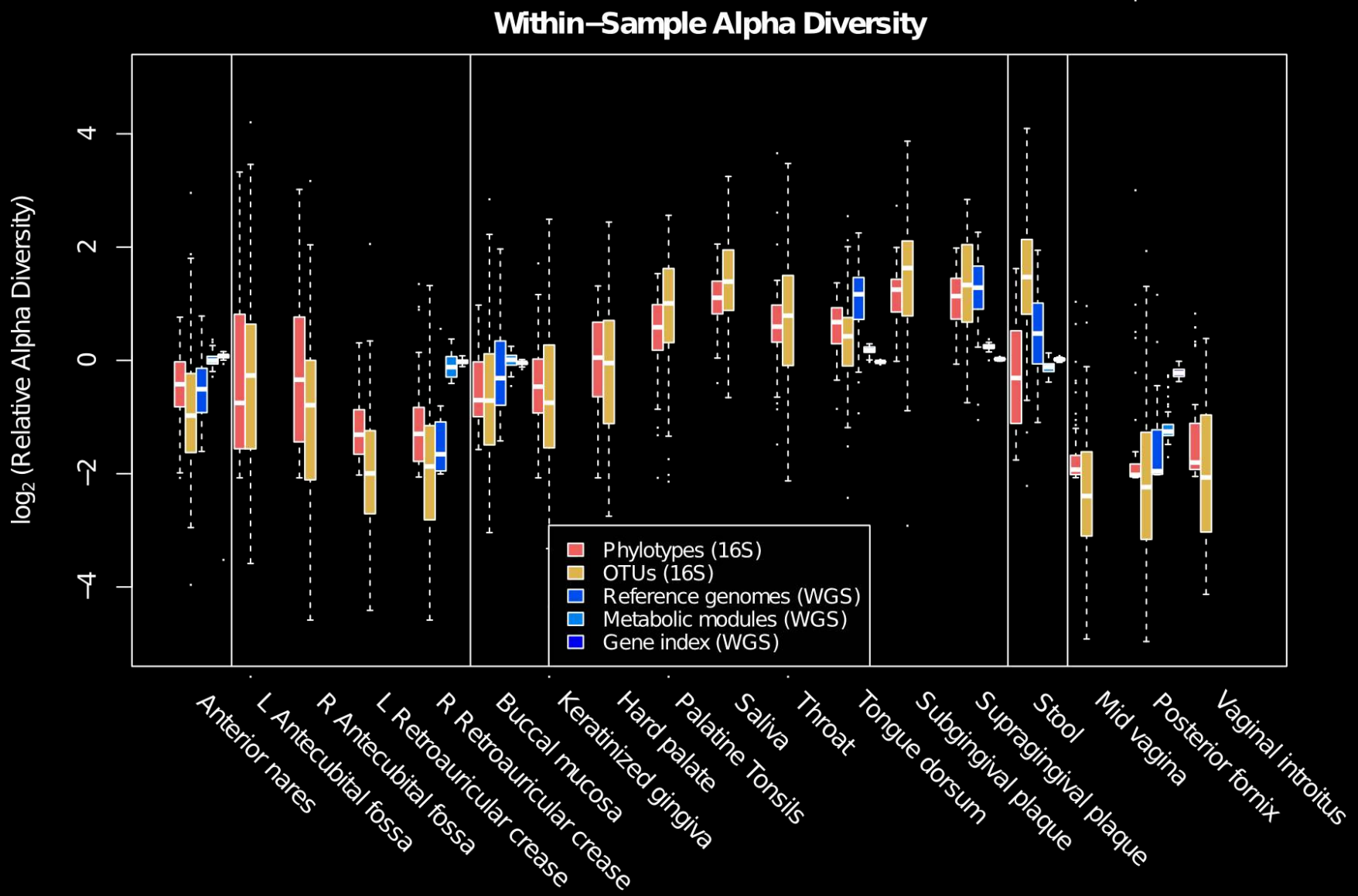


Quantitatively diverse
Taxonomically diverse

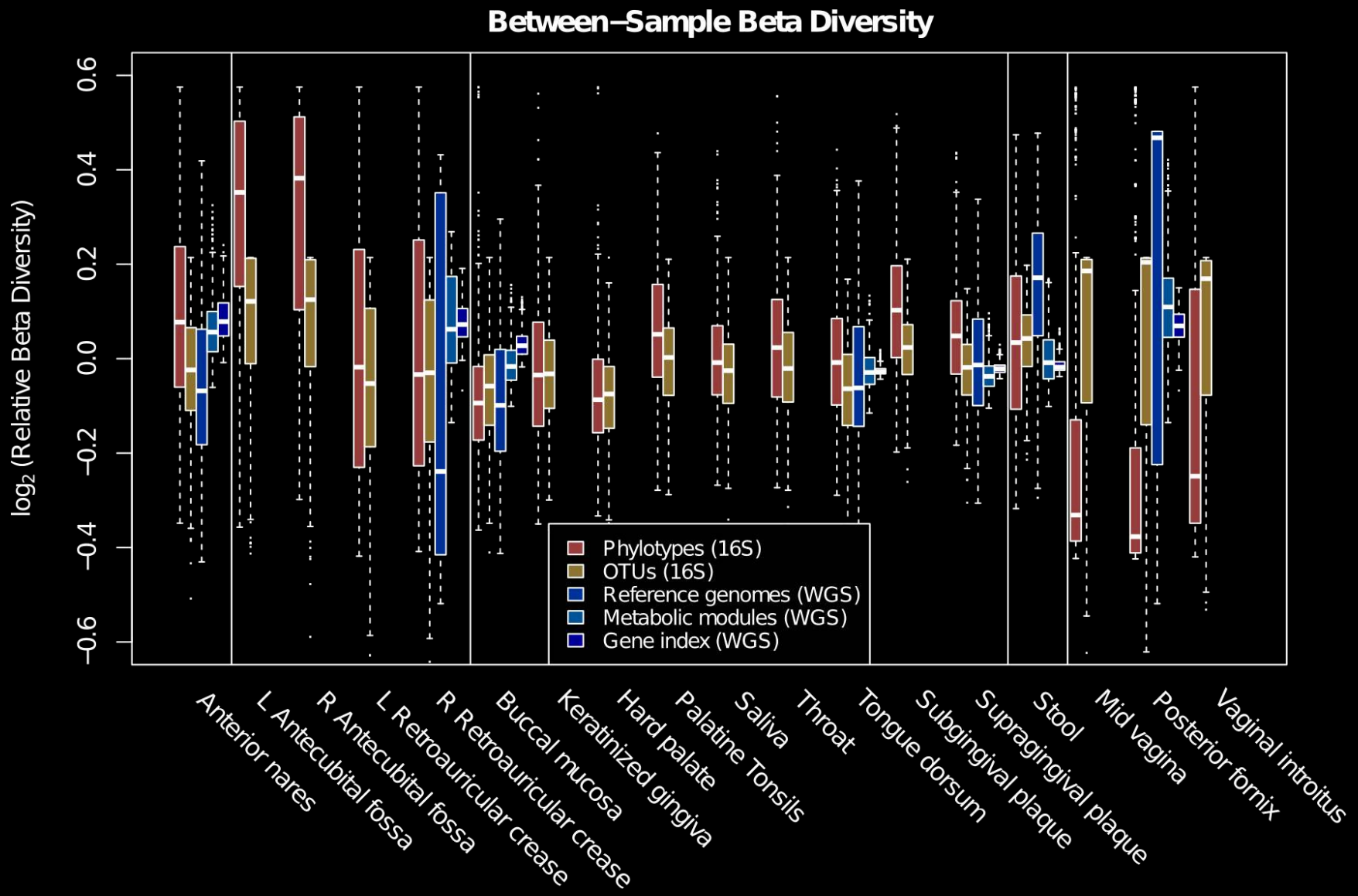


Quantitatively diverse
Phylogenetically diverse

Which human body sites harbor the greatest microbial diversity per individual?

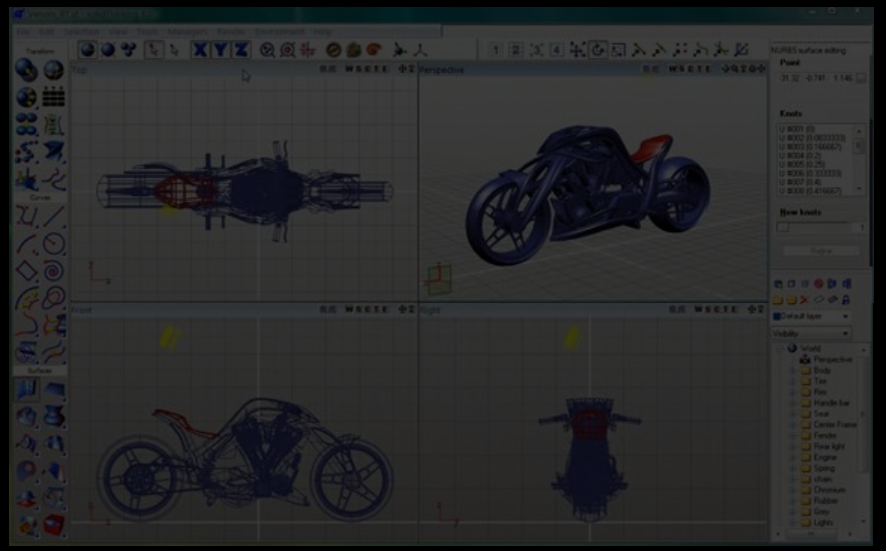


Which human body sites share the greatest microbial diversity among individuals?

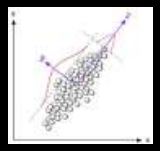


Microbiome composition analyses: **ordination**

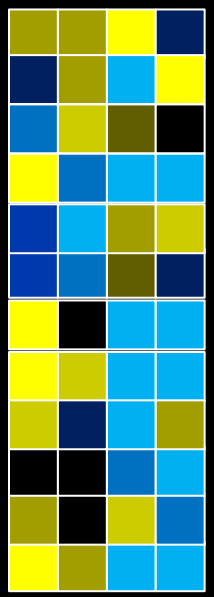
Ordination is the constrained projection of high-dimensional data into fewer dimensions.



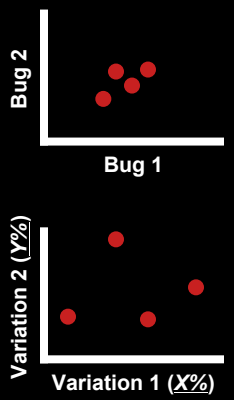
PCA or **Principal Component Analysis** guarantees the new dimensions maximize normal variation.

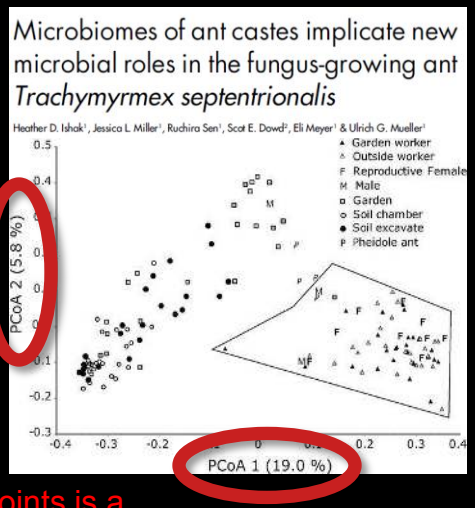


NMDS or **Nonmetric Multidimensional Scaling**, also called **PCoA** or **Principal Coordinates Analysis**, guarantees the new dimensions maximize an arbitrary similarity score (such as UniFrac beta-diversity).

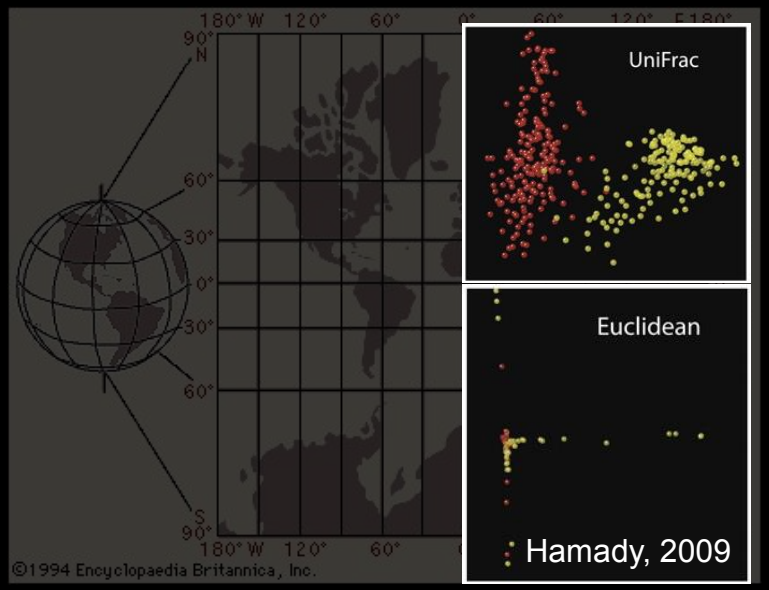


Distance between points is Euclidean

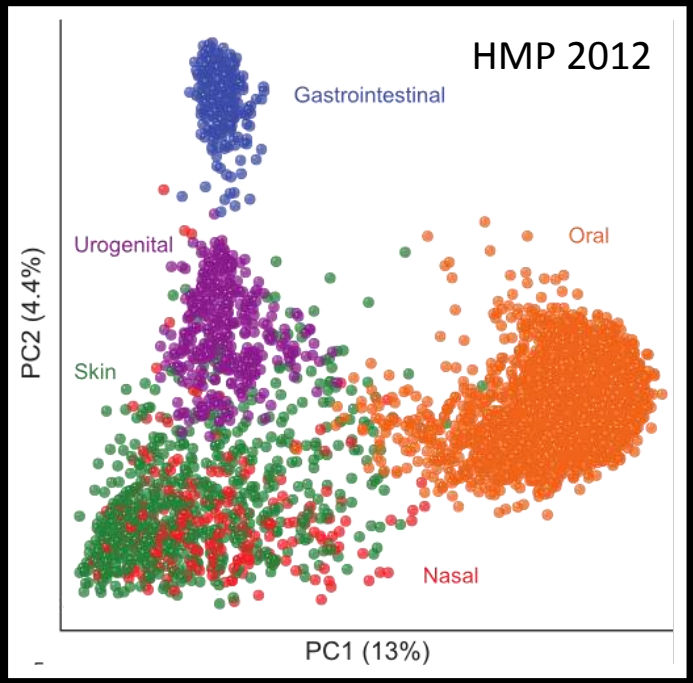


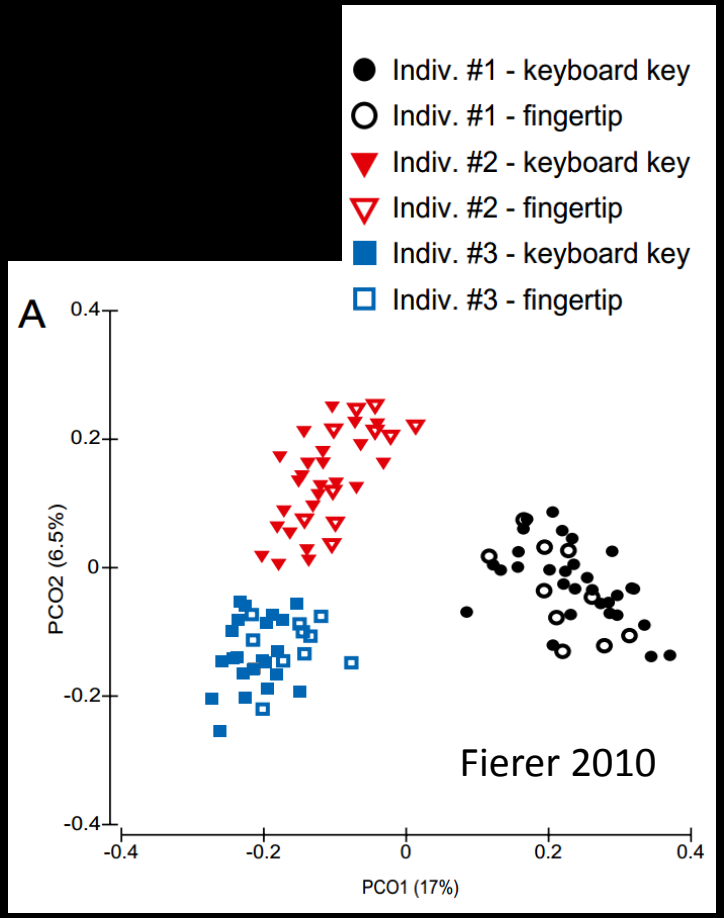


Distance between points is a ***proportional function*** of their ***similarity***



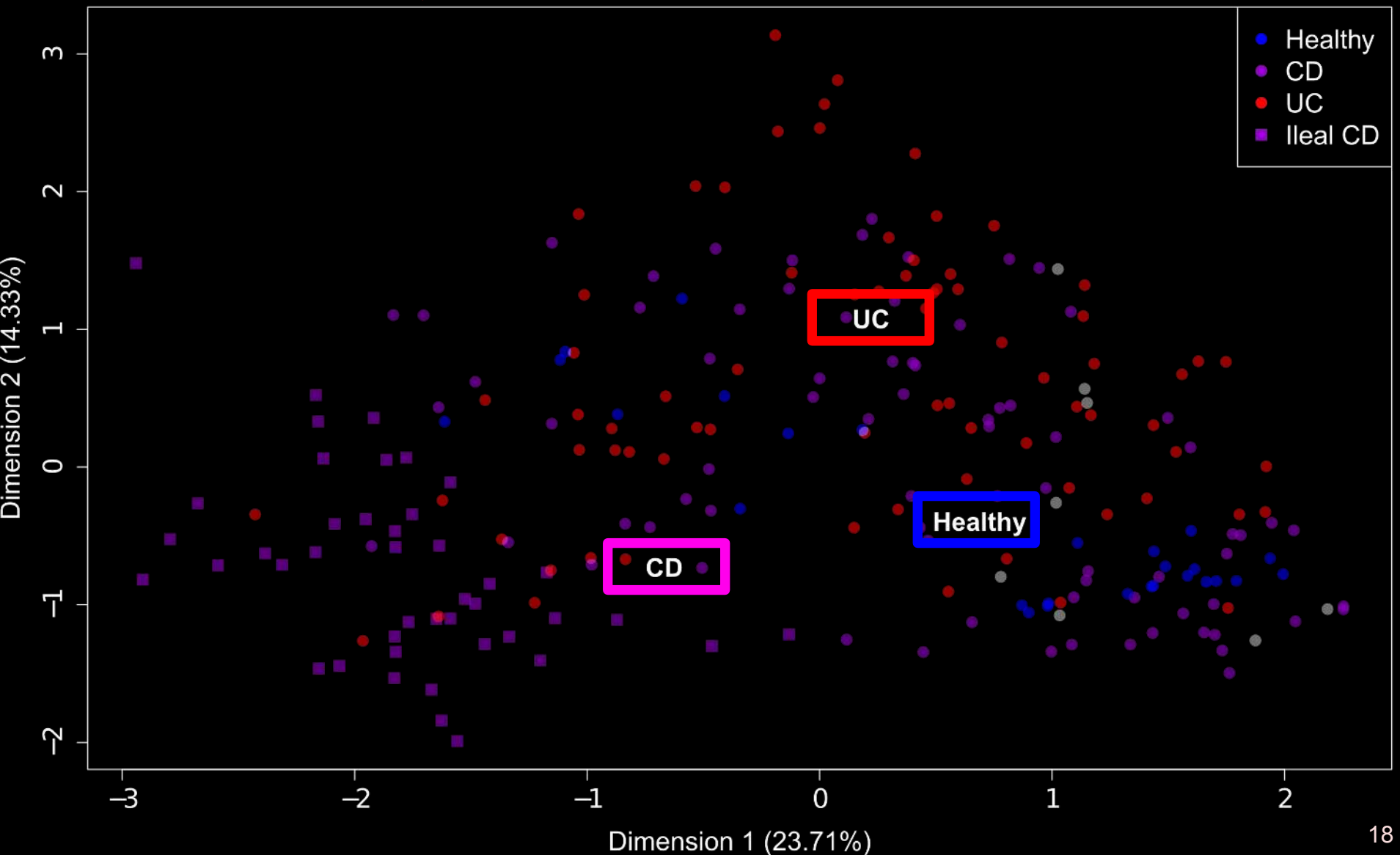
Microbiome composition analyses: **ordination examples**





How is the gut microbiome disrupted during IBD and its treatment?

With Ramnik Xavier, Bruce Sands



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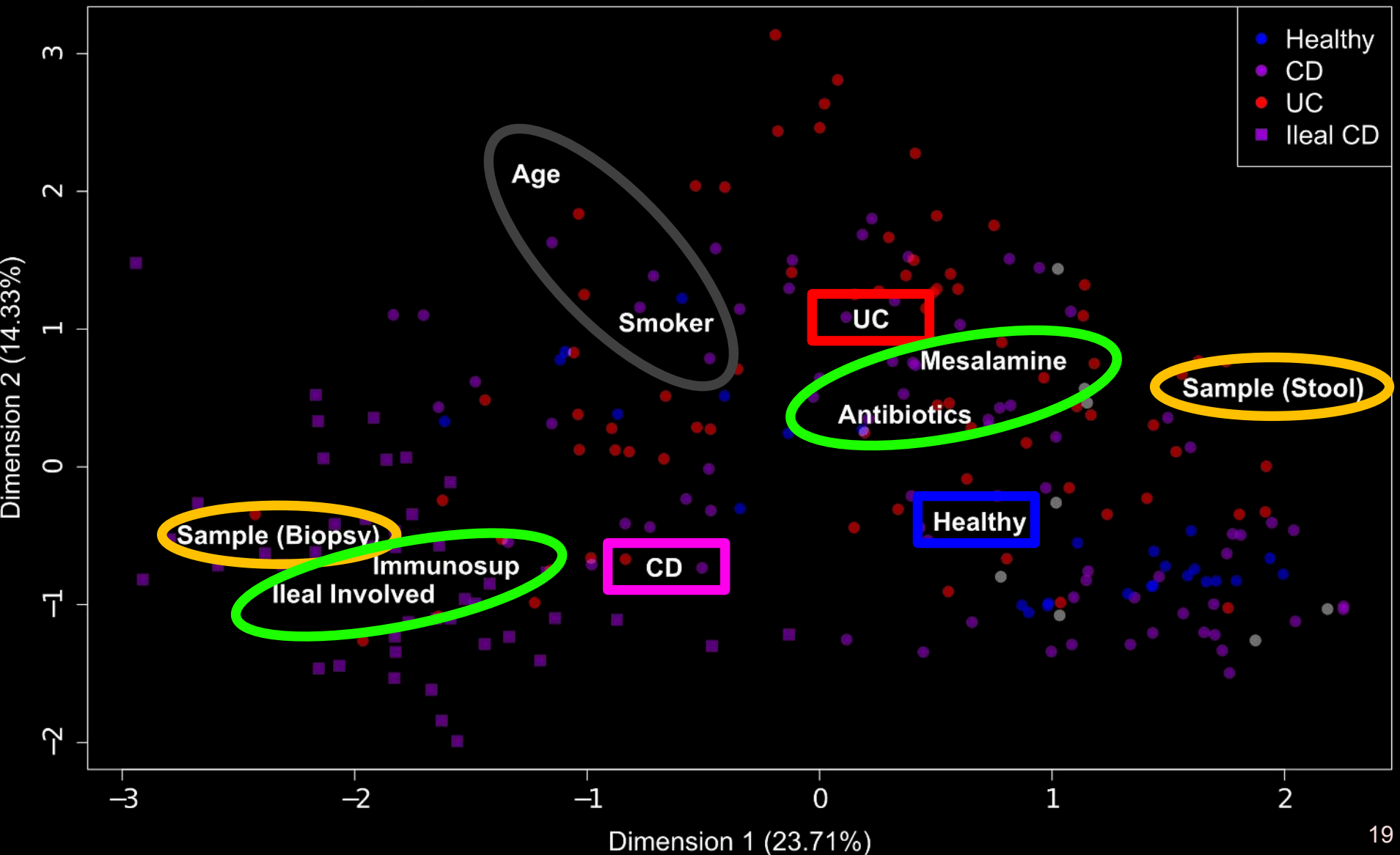
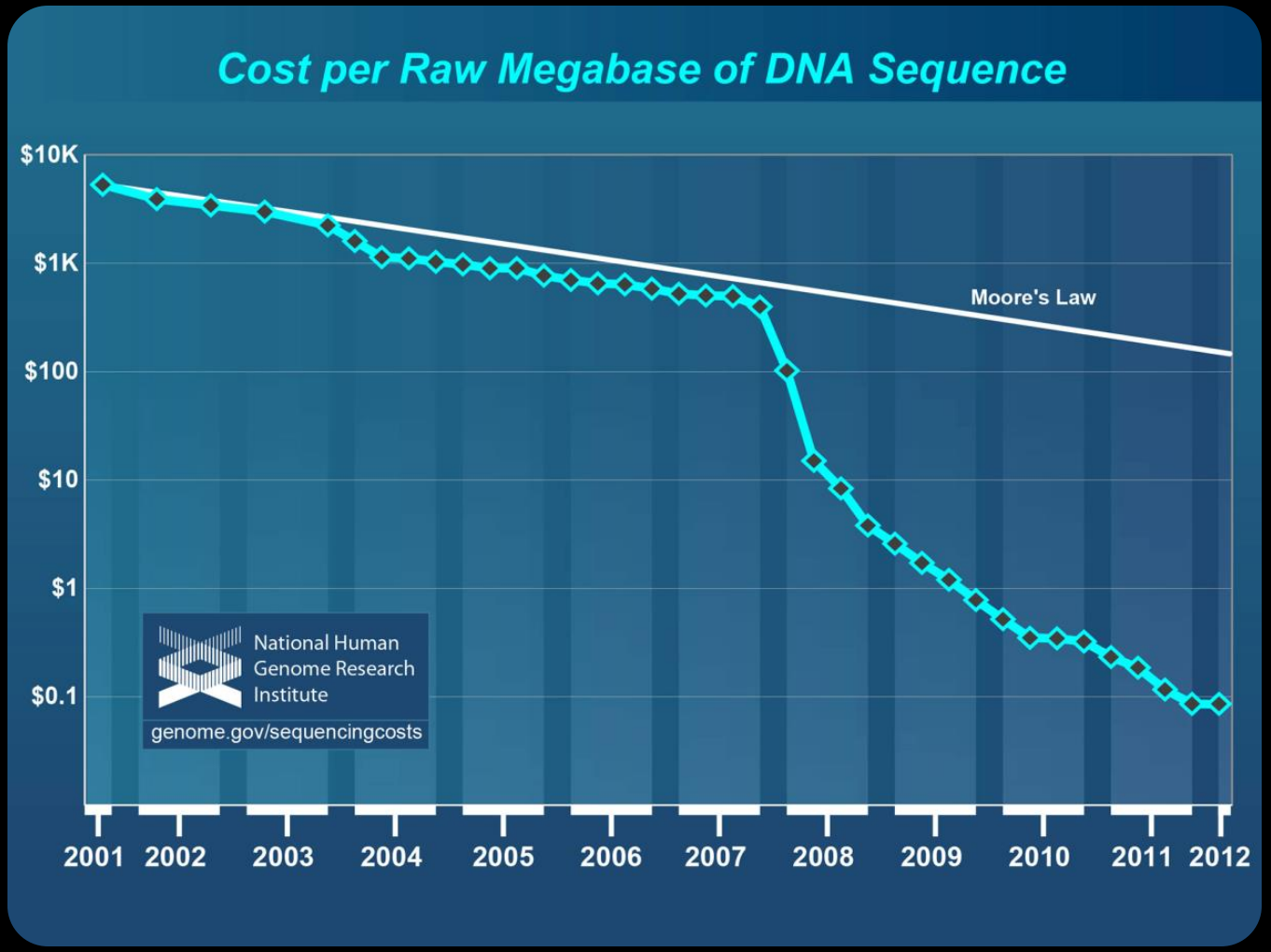
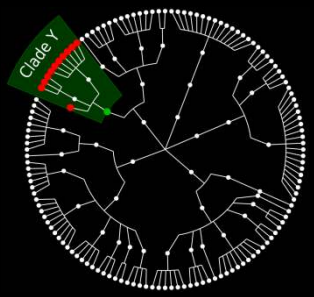


Meta'omic analyses



Typical shotgun metagenome and metatranscriptome analyses

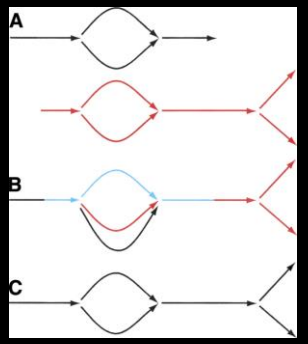
Taxonomic Profiling

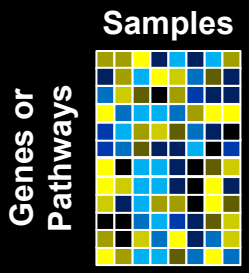


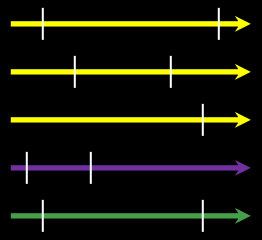
Functional Profiling

eggNOG4.0

Assembly







Profiling microbial communities and ecology at species-level resolution

ARTICLE

Arumugam Nature 2012

doi:10.1038/nature09944

Enterotypes of the human gut microbiome

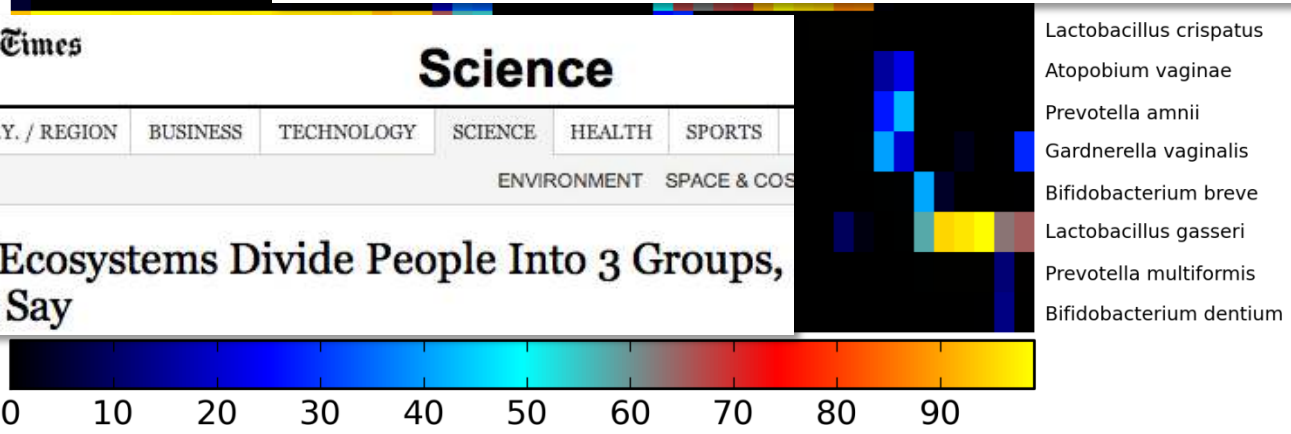
The New York Times

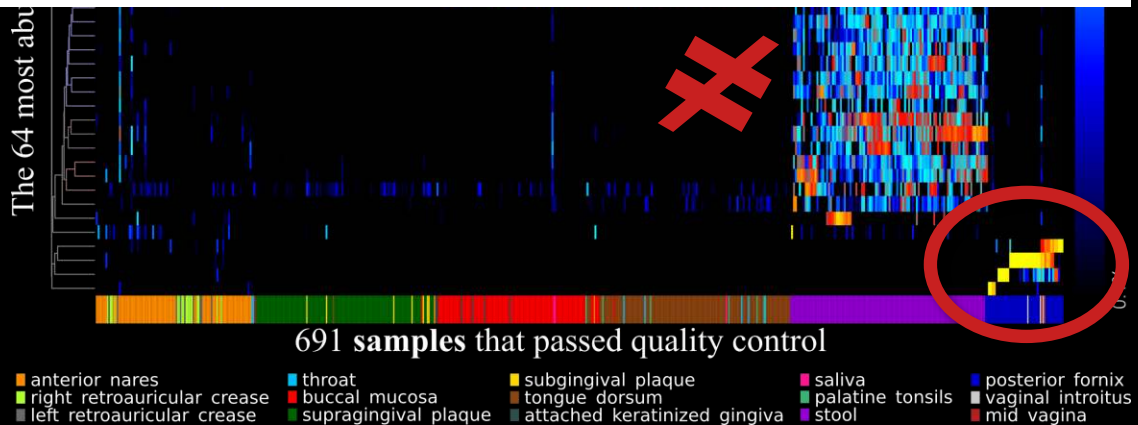
Science

WORLD | U.S. | N.Y. / REGION | BUSINESS | TECHNOLOGY | SCIENCE | HEALTH | SPORTS

ENVIRONMENT | SPACE & COS

Bacterial Ecosystems Divide People Into 3 Groups, Scientists Say

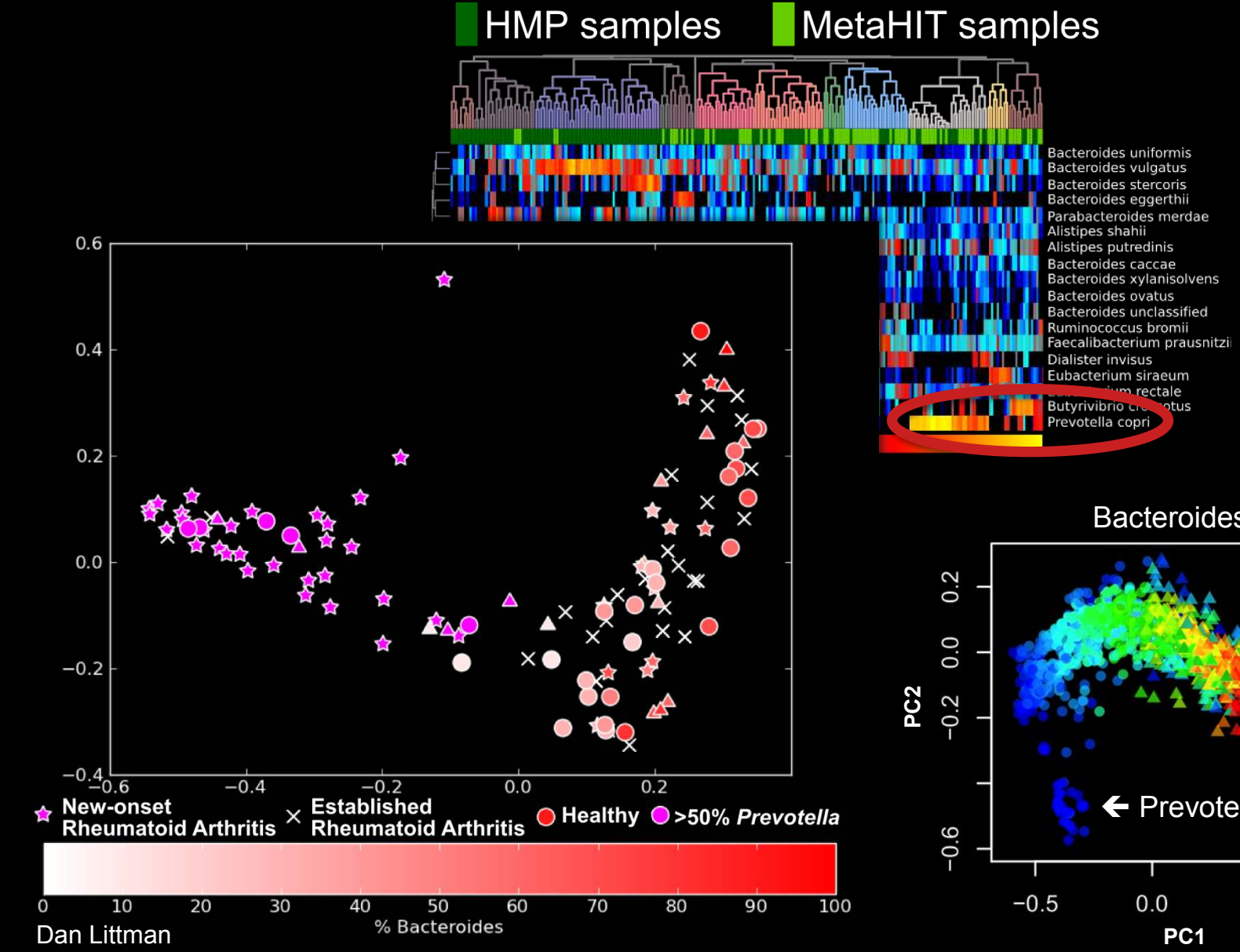




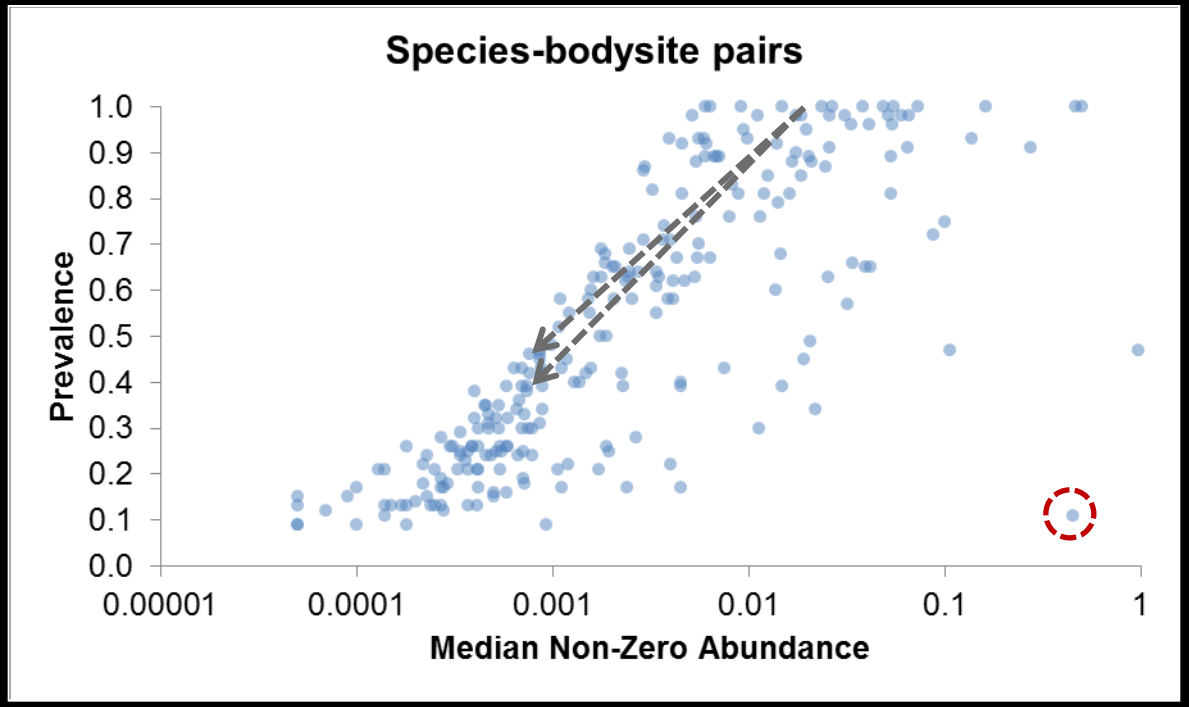
Gut

Vaginal

Are there discrete “types” of typical human microbiomes?



Species prevalence vs. species abundance

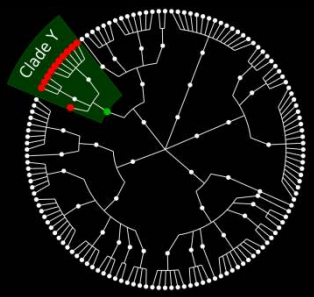


site	bug	log10 med non0 abund	prevalence	tp 1-2 stability
Stool	s__Prevotella_copri	-0.3	0.11	1.00

site	bug	log10 med non0 abund	prevalence	tp 1-2 stability
Tongue_dorsum	s__Actinomyces_odontolyticus	-1.8	1.00	1.00
Buccal_mucosa	s__Actinomyces_odontolyticus	-3.1	0.47	0.78
Supragingival_plaque	s__Actinomyces_odontolyticus	-3.1	0.38	0.50

Typical shotgun metagenome and metatranscriptome analyses

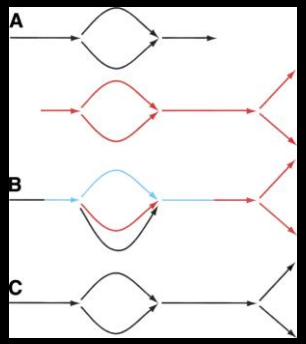
Taxonomic Profiling

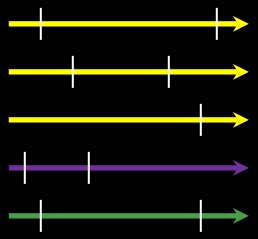


Functional Profiling

eggNOG4.0

Assembly



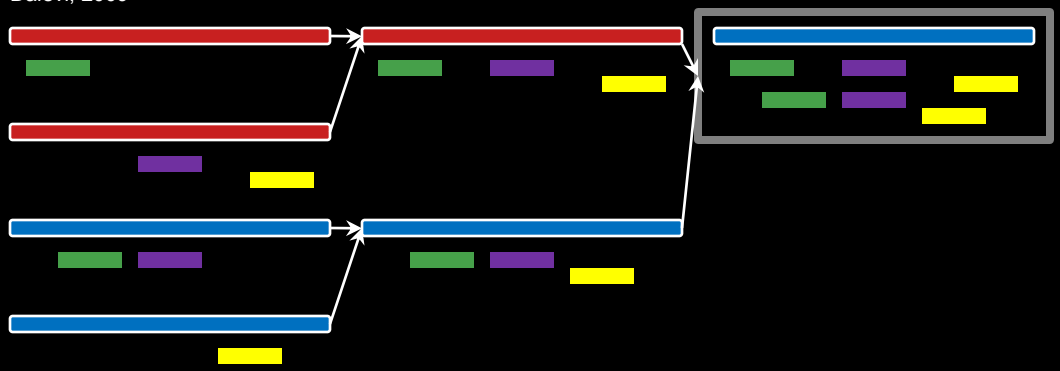


Metagenomic analyses: gene calling and proxygenes

Extrinsic gene calling: BLAST etc. (proxygenes)

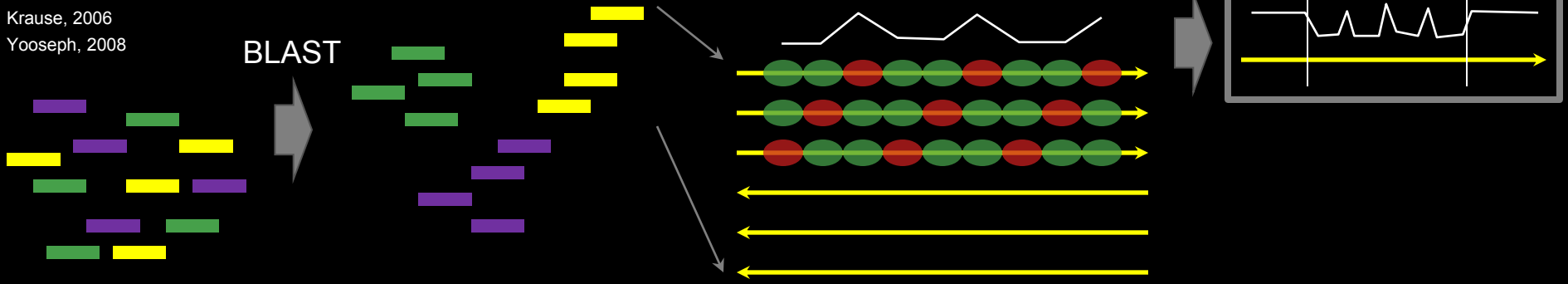
Intrinsic gene calling: ORF detection from seq.

Dalevi, 2009



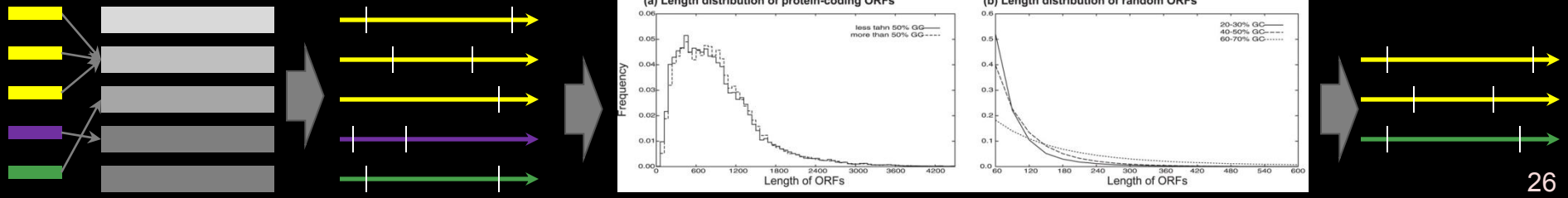
Krause, 2006
Yooseph, 2008

BLAST



Orphelia: Hoff, 2009
MetaGene: Noguchi, 2006

HMM models

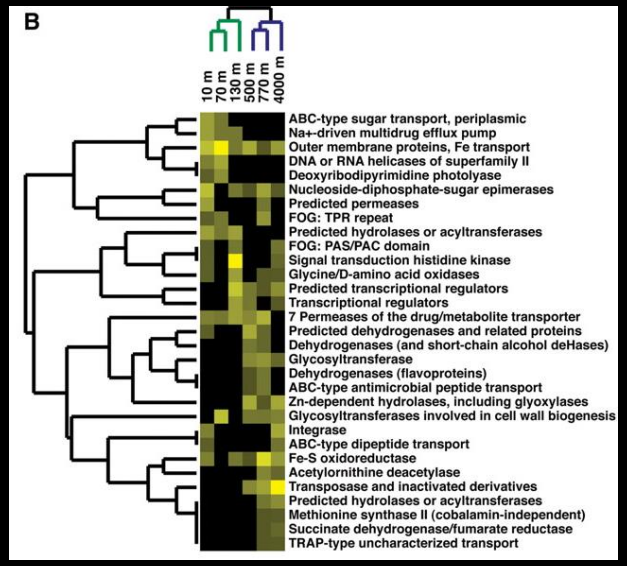


Metagenomic analyses: molecular functions and biological roles

Orthology:
Grouping genes by conserved sequence features
COG, KO, FIGfam…

Structure:
Grouping genes by similar protein domains
Pfam, TIGRfam, SMART, EC…

Biological roles:
Grouping genes by pathway and process involvement
GO, KEGG, MetaCyc, SEED…

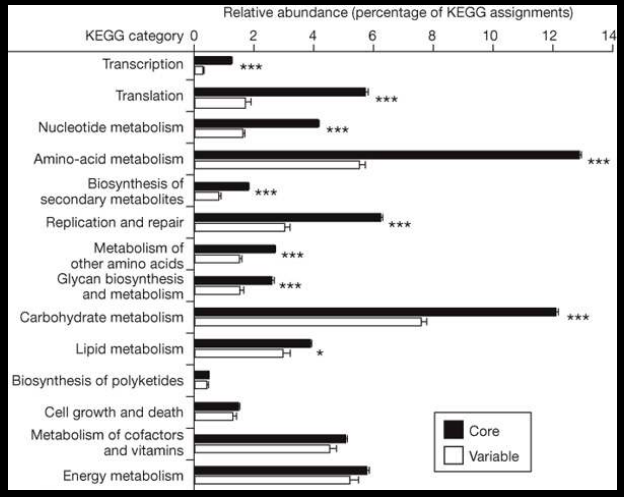


DeLong, 2006

Table 1 | Glycoside hydrolases and carbohydrate-binding modules

CAZy family*	Pfam HMM name†	Known activities‡	Termite gut community§
Glycoside hydrolase catalytic domains**††			
GH1	Glyco_hydro_1	β-Glucosidase, β-galactosidase, β-mannosidase, others	22
GH2	Glyco_hydro_2_C	β-Galactosidase, β-mannosidase, others	23
GH3	Glyco_hydro_3	β-1,4-Glucosidase, β-1,4-xylosidase, β-1,3-glucosidase, α-L-arabinofuranosidase, others	69
GH4	Glyco_hydro_4	α-Glucosidase, α-galactosidase, α-glucuronidase, others	14
GH5	Cellulase	Cellulase, β-1,4-endoglucanase, β-1,3-glucosidase, β-1,4-endoxylanase, β-1,4-endomannanase, others	56
GH8	Glyco_hydro_8	Cellulase, β-1,3-glucosidase, β-1,4-endoxylanase, β-1,4-endomannanase, others	5
GH9	Glyco_hydro_9	Endoglucanase, cellobiohydrolase, β-glucosidase	9
GH10	Glyco_hydro_10	Xylanase, β-1,3-endoxylanase	46
GH11	Glyco_hydro_11	Xylanase	14
GH13	Alpha-amylase	α-Amylase, catalytic domain, and related enzymes	48
GH16	Glyco_hydro_16	β-1,3(4)-Endoglucanase, others	1
GH18	Glyco_hydro_18	Chitinase, endo-β-*N*-acetylglucosaminidase, non-catalytic proteins	17
GH20	Glyco_hydro_20	β-Hexosaminidase, lacto-*N*-biosidase	15

Warnecke, 2007

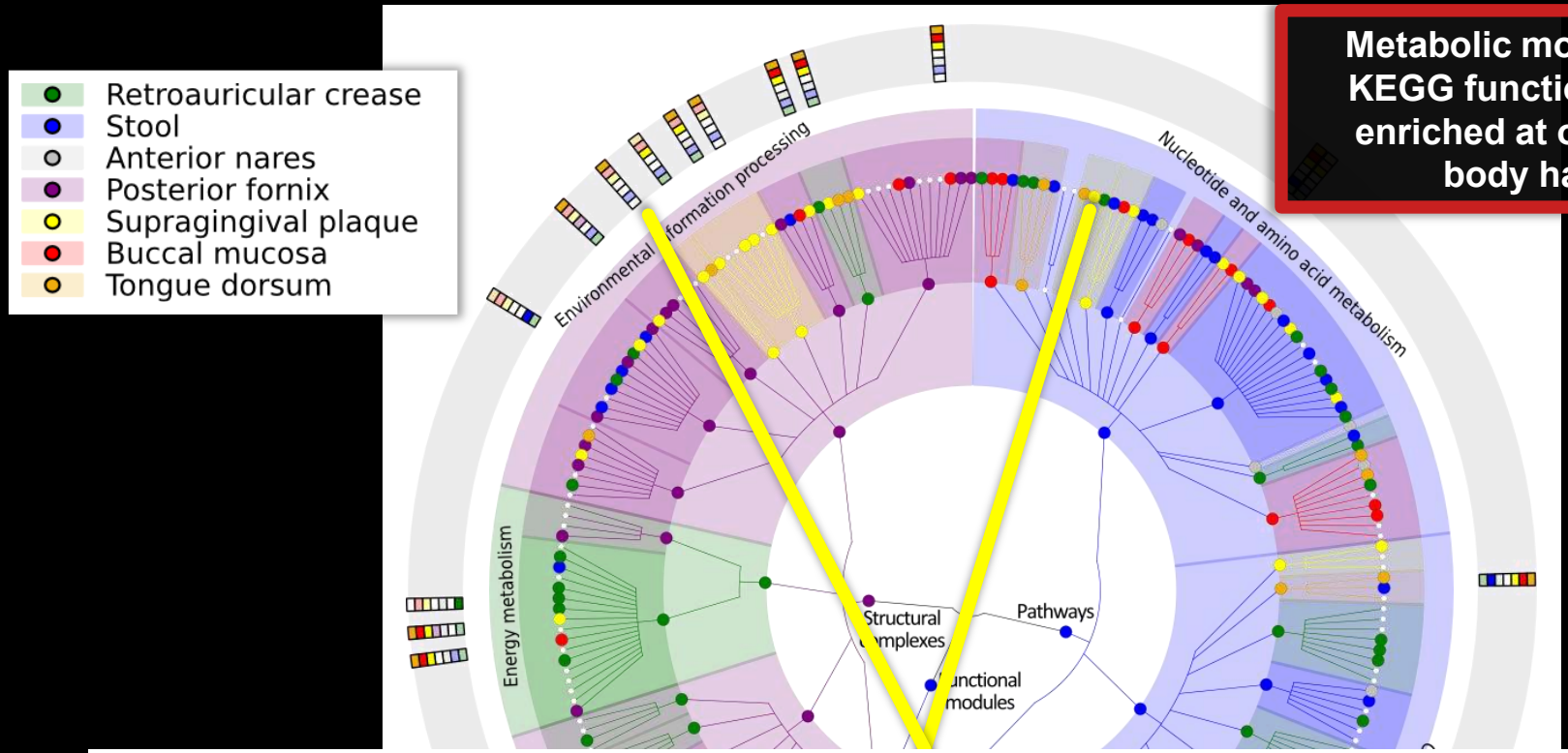


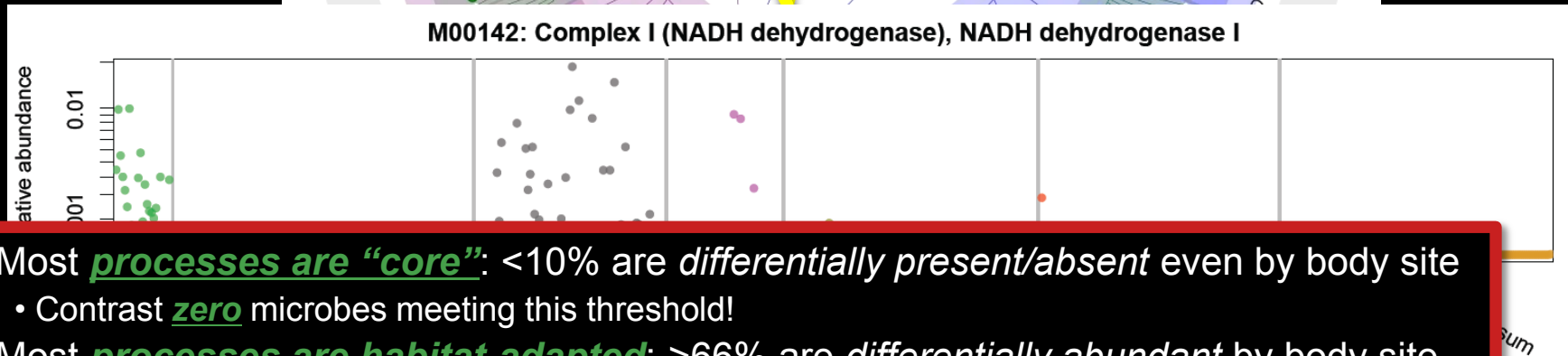
Turnbaugh, 2009

Niche specialization in human microbiome function

LEfSe:
LDA Effect Size
Nonparametric test for microbial and metagenomic biomarkers
http://huttenhower.sph.harvard.edu/lefse

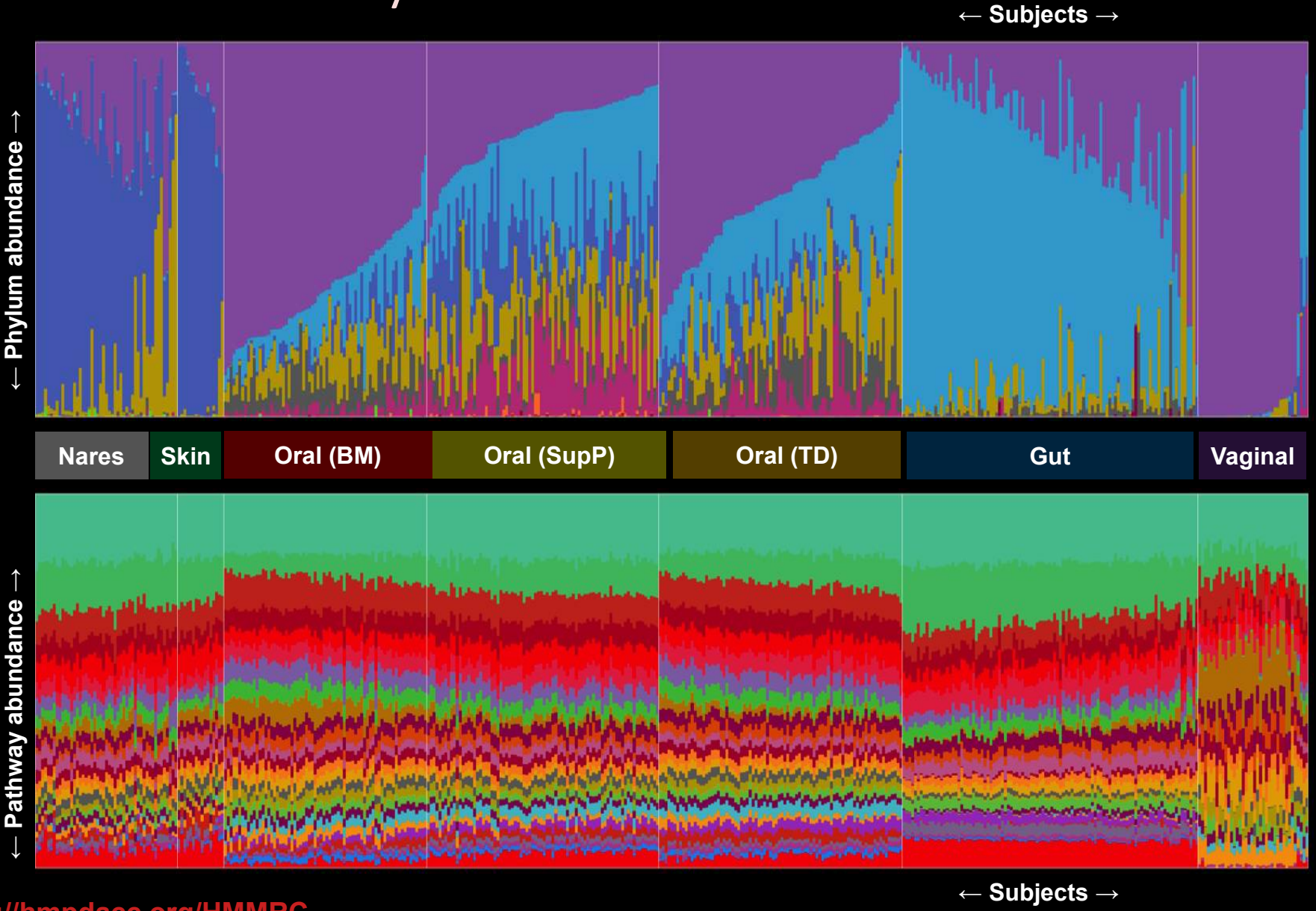
Metabolic modules in the KEGG functional catalog enriched at one or more body habitats





- Most ***processes are "core"***: <10% are *differentially present/absent* even by body site
  - Contrast ***zero*** microbes meeting this threshold!
- Most ***processes are habitat-adapted***: >66% are *differentially abundant* by body site

"Who's there," versus, "What they're doing," in the healthy human microbiome



Which *functions* of the gut microbiome are disrupted by IBD?

- Over *six times* as many microbial metabolic processes disrupted in IBD as microbes.
  - If there's a transit strike, everyone working for the MBTA is disrupted, not everyone named Smith or Jones.
  - Phylogenetic distribution of function is *consistent* but *diffuse*
- During IBD, microbes...

Stop

- Creating most amino acids
- Degrading complex carbs.
- Producing short-chain fatty acids

Start

- Taking up more host products
- Dodging the immune system
- Adhering to and invading host cells

Plan

- Informal survey
- Metagenomics concepts & examples
- Tools for taxonomic profiling
  - MetaPhlAn
- Tools for functional profiling
  - HUMAnN
  - ShortBRED
  - PICRUSt
- Tools for testing associations
  - LEfSe
  - MaAsLin
  - CCREPE
- Resources
- Research vignette (time permitting)

The two big questions…

Who is there?
(taxonomic profiling)

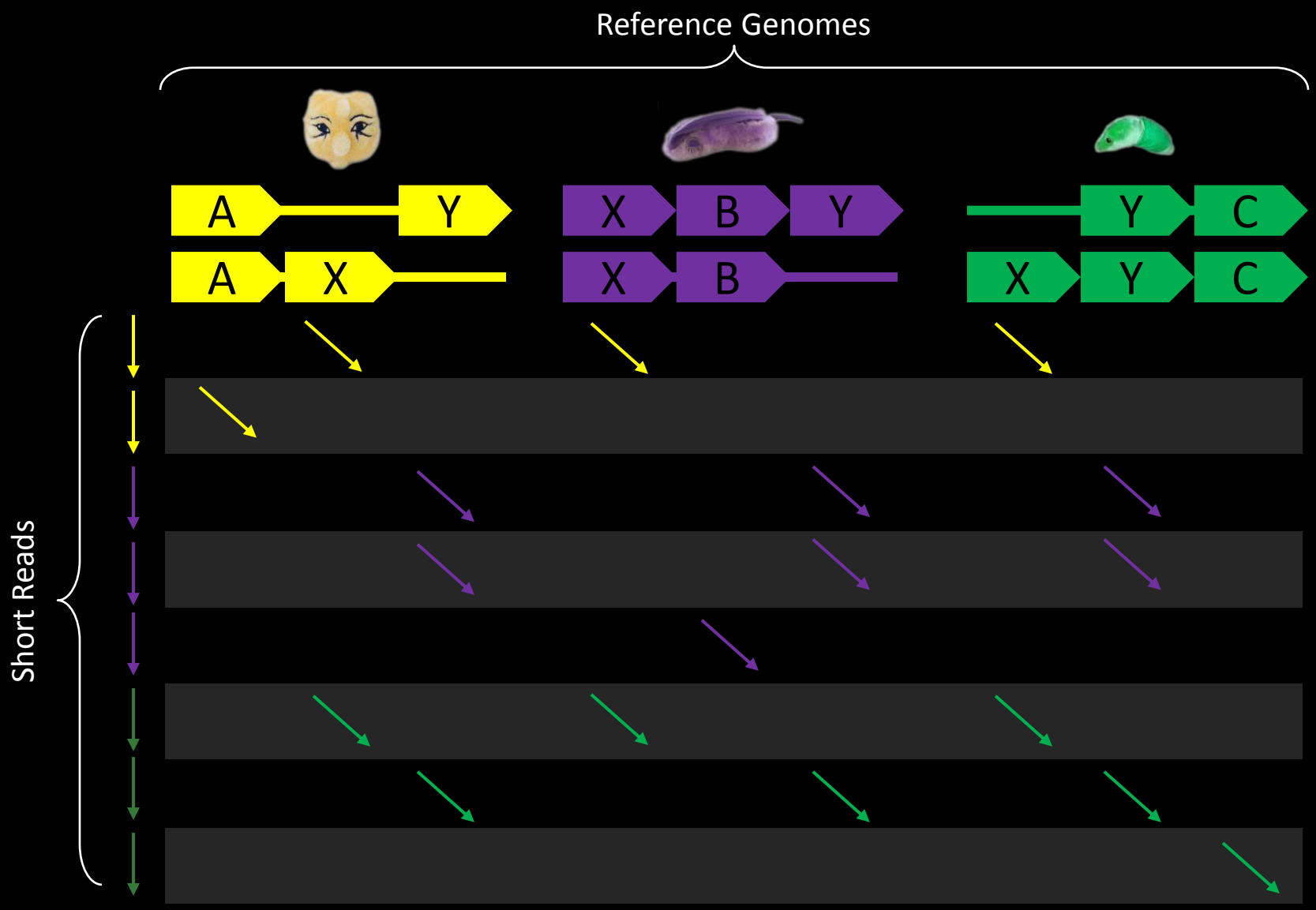
What are they doing?
(functional profiling)

Reference genomes

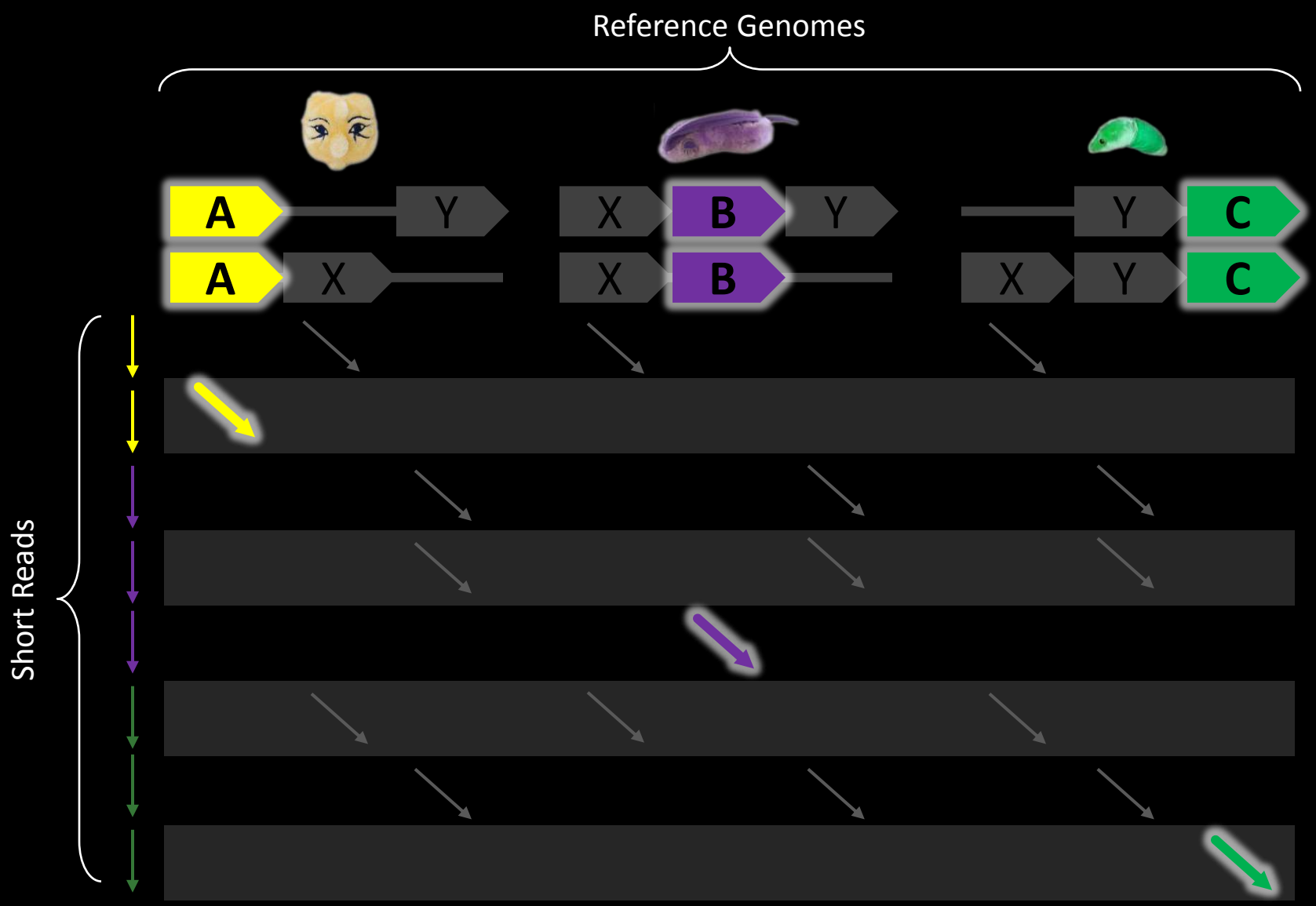
- IMG alone now contains ~3,100 bacterial genomes
  - Plus ~100 archaeal, ~100 eukaryotic, and a few thousand viruses
  - About half final and half draft
- These comprise 1,222 bacterial species
  - 652 genera, 278 families, 130 orders, 66 classes, 33 phyla
  - 2,383 total clades
- **And roughly 10M genes**

- These genes and genomes are a tremendous resource to:
  - Identify conserved markers that can be used to infer phylogeny
  - Identify unique markers that can be used to infer taxonomy
  - Relate the microbial members of a community to their metagenomic functional potential

MetaPhlAn
Metagenomic Phylogenic Analysis



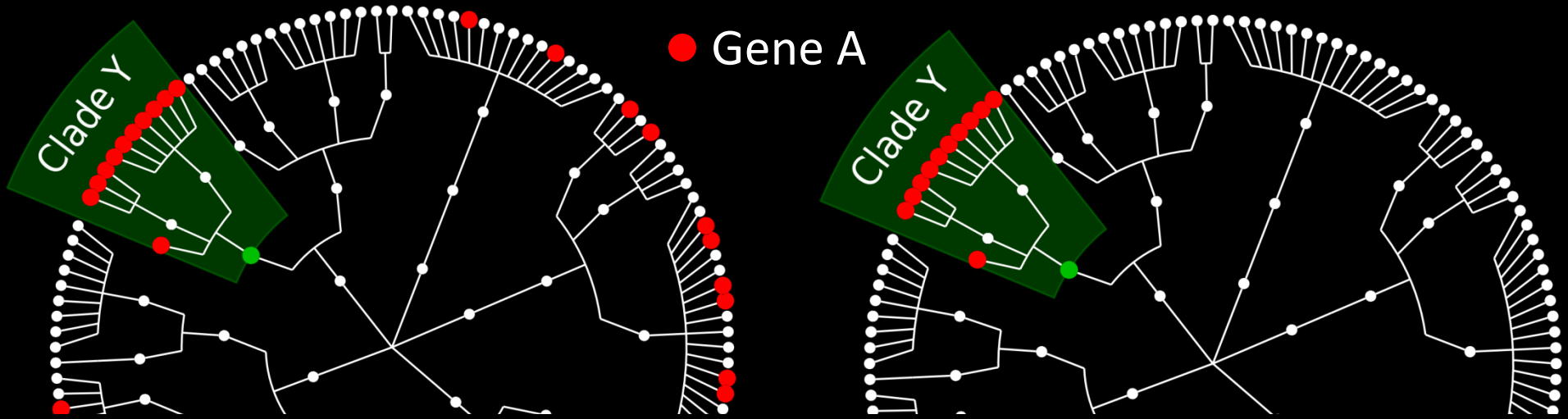
MetaPhlAn
Metagenomic Phylogenic Analysis



MetaPhlAn overview

A is a core gene for clade Y

A is a unique marker gene for clade Y



ChocoPhlAn (offline pipeline)

- Identify all **core genes** for all clades
- Screen core genes for unique **marker genes**
- Select most representative marker genes

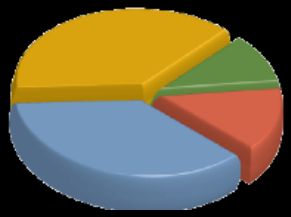
Unique marker genes DB

Available reference genomes

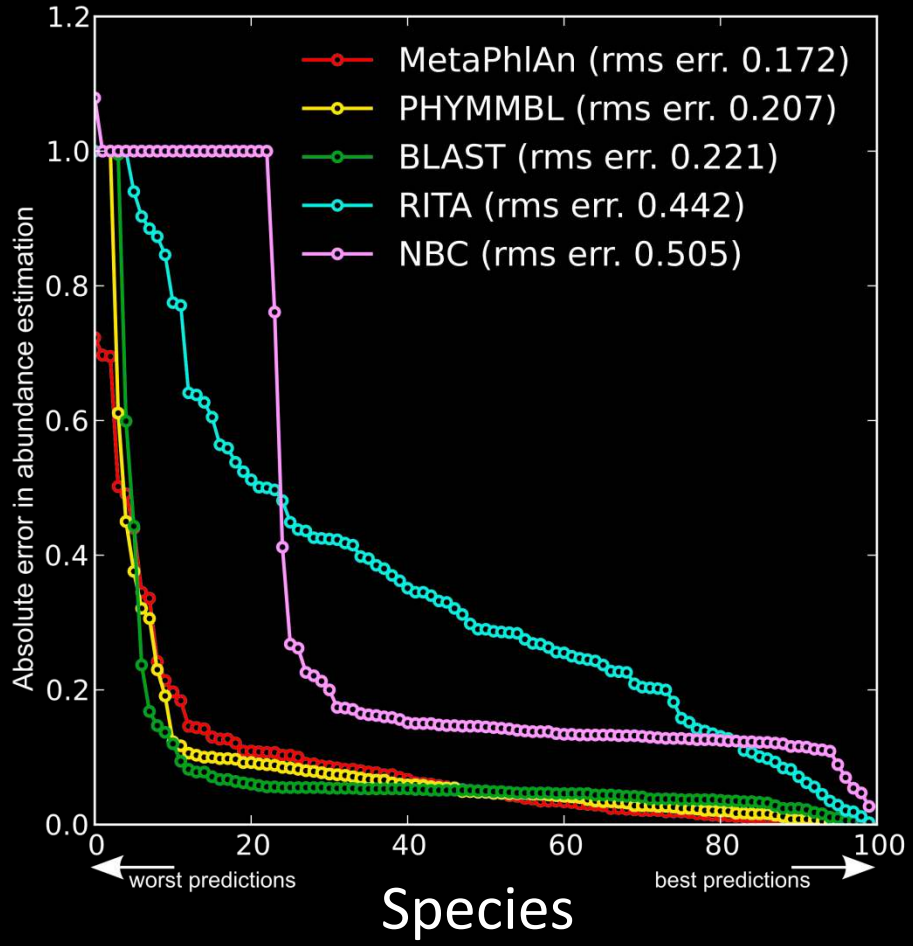
MetaPhlAn

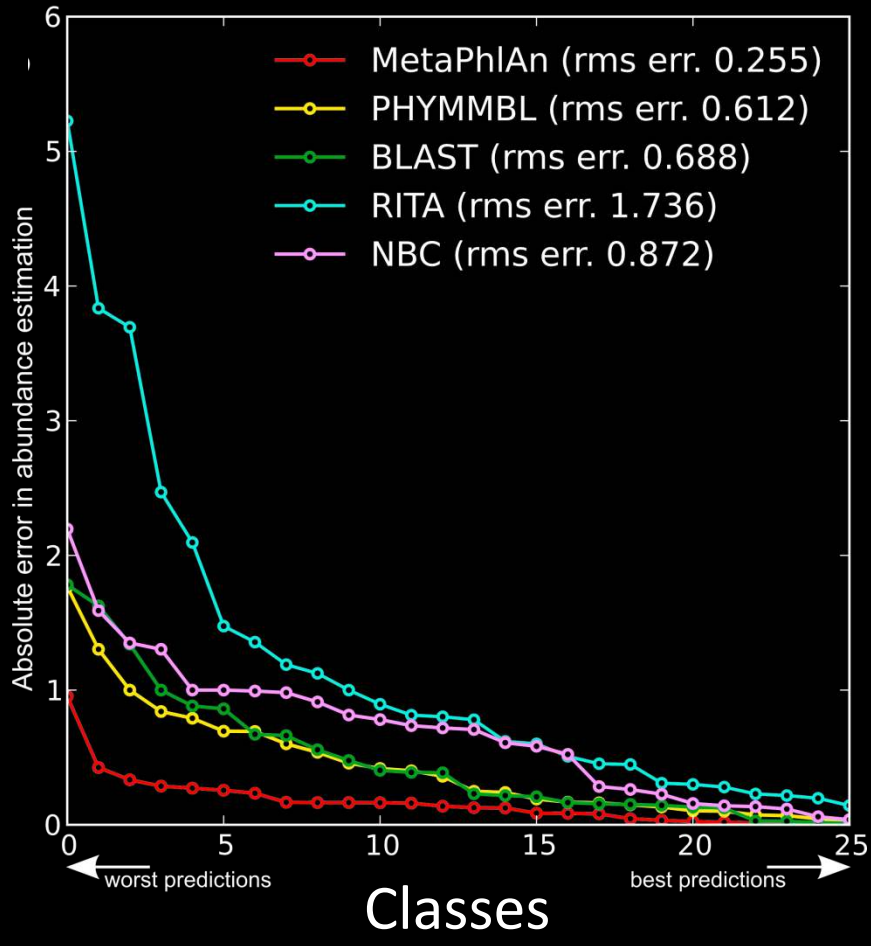
- Blast reads agains the marker genes
- Assign, count, normalize reads

Metagenome



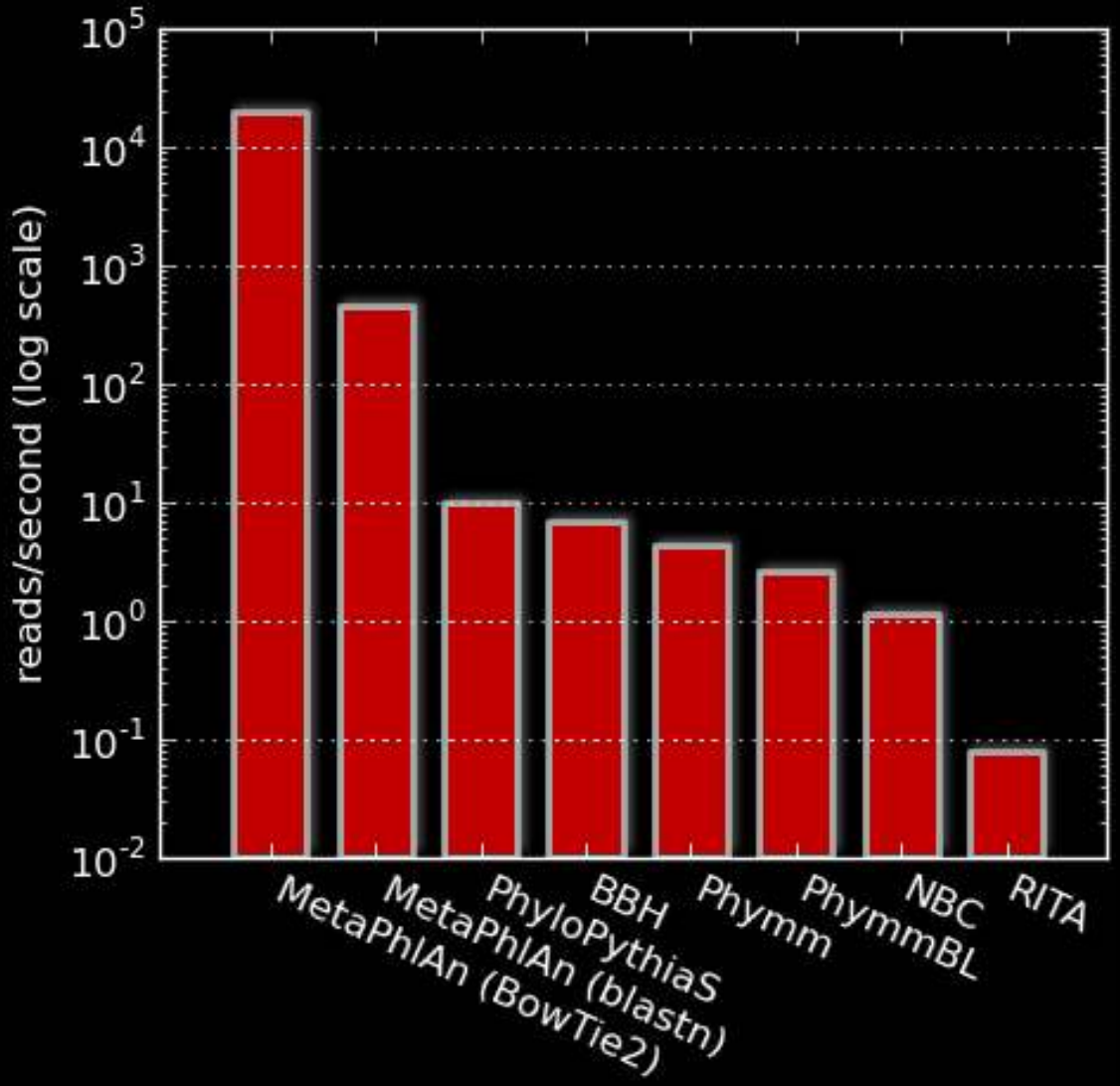
Evaluation of MetaPhlAn accuracy





(Validation on high-complexity uniformly distributed synthetic metagenomes.)

Evaluation of MetaPhlAn performance



>50 times faster than earlier methods

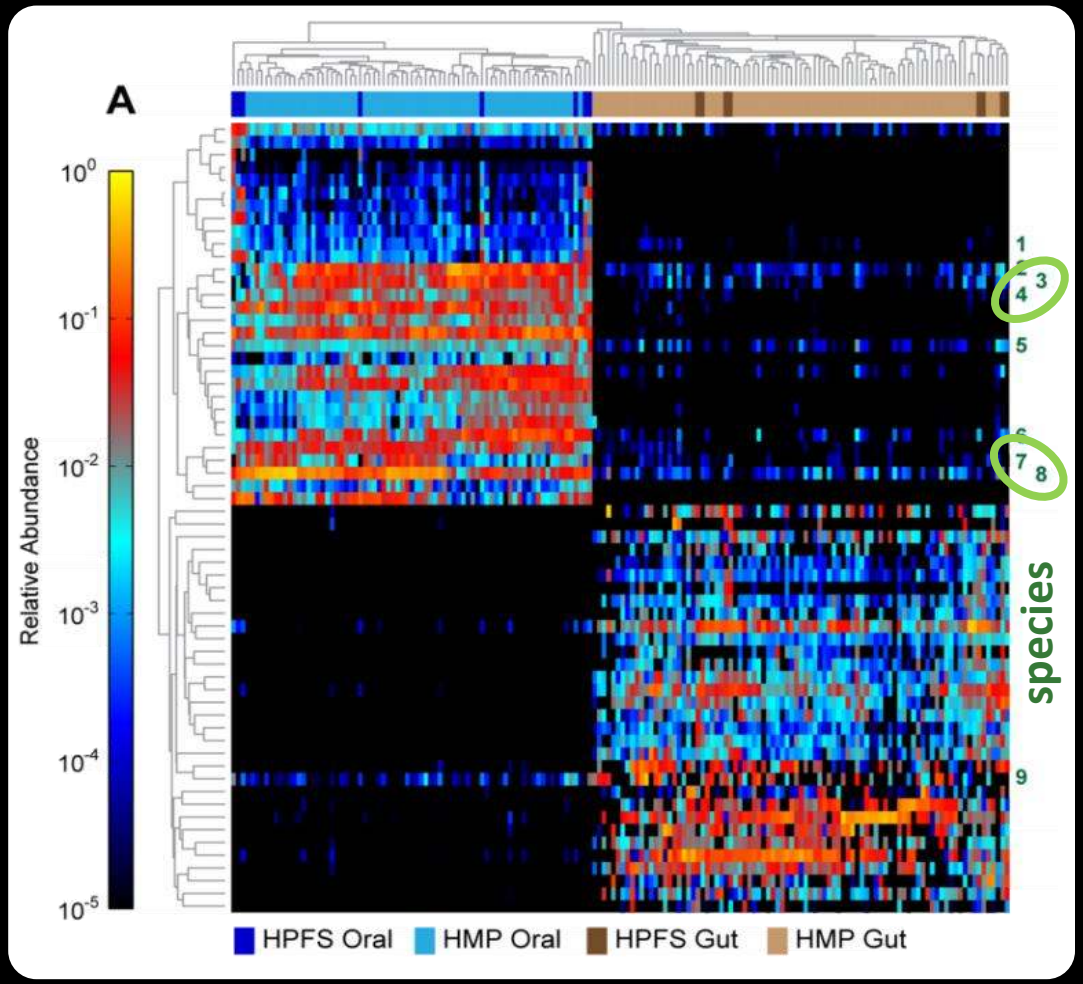
450 reads/sec (BLAST)

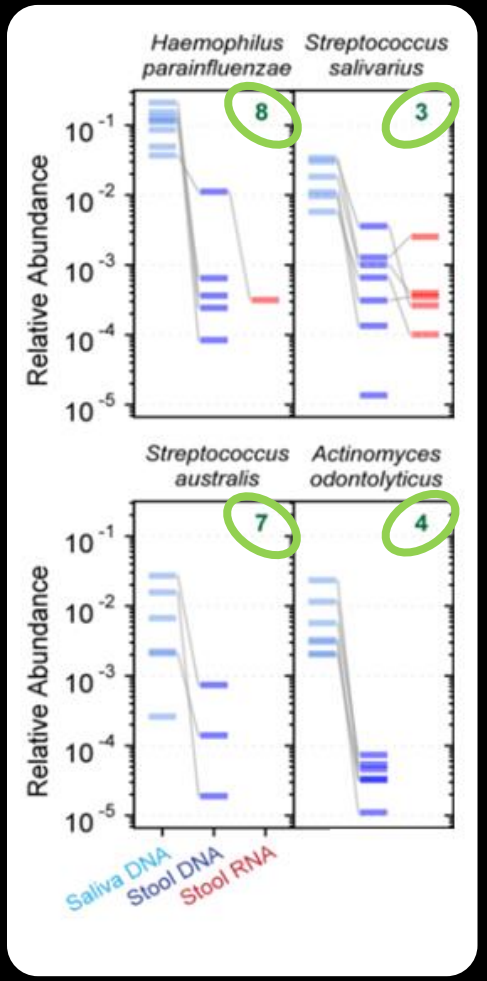
Up to 25,000 reads/sec (bowtie2)

Multi-threaded

Easily parallelizable

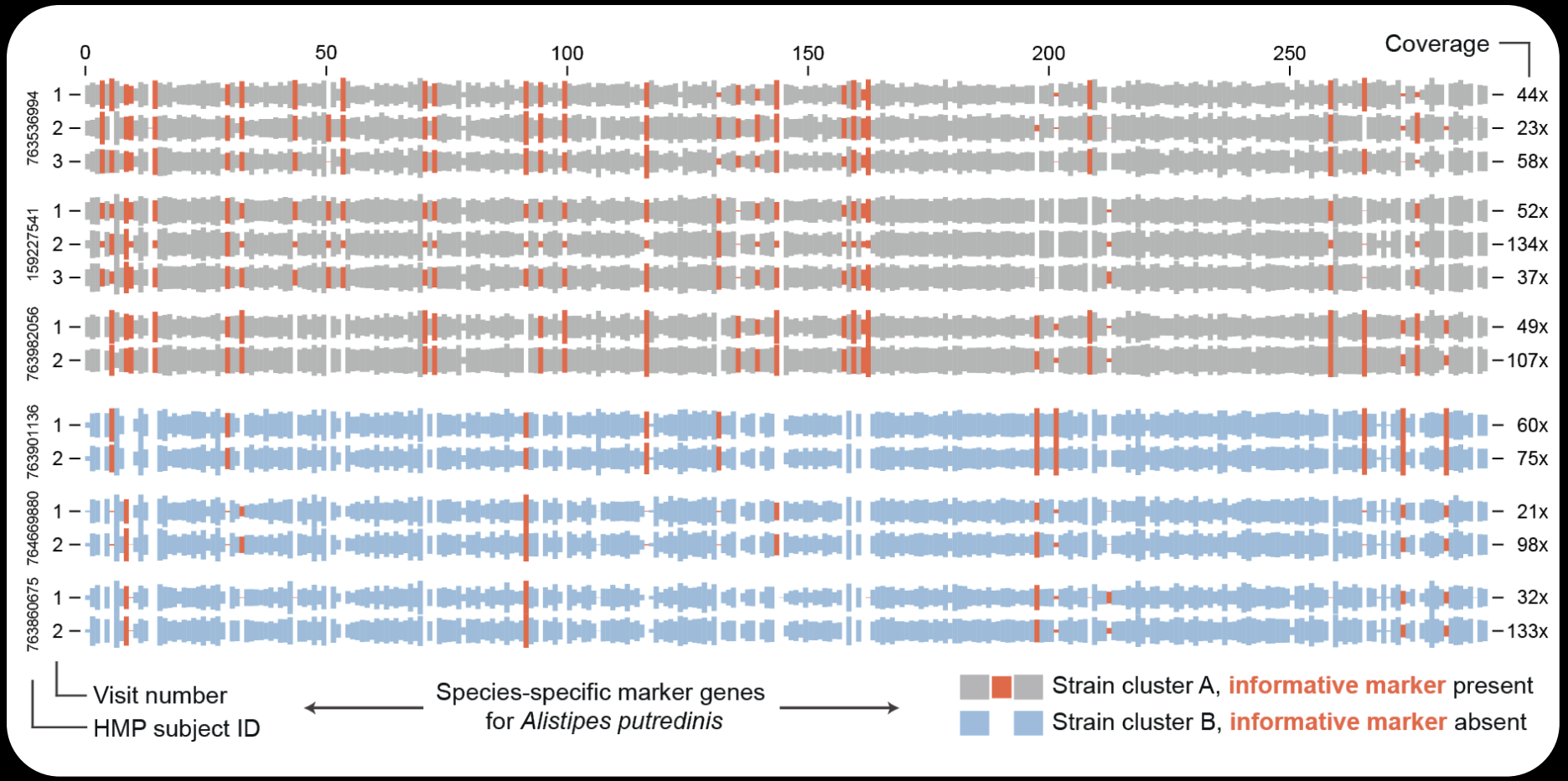
MetaPhlAn in action





Franzosa et al. *PNAS* 11:E2329-38 (2014)

MetaPhlAn in action: *strain profiling*



- In practice, not all markers are present
- Individual-specific marker “barcodes”
- Often very stable over time

Plan

- Informal survey
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  - MetaPhlAn
- Tools for functional profiling
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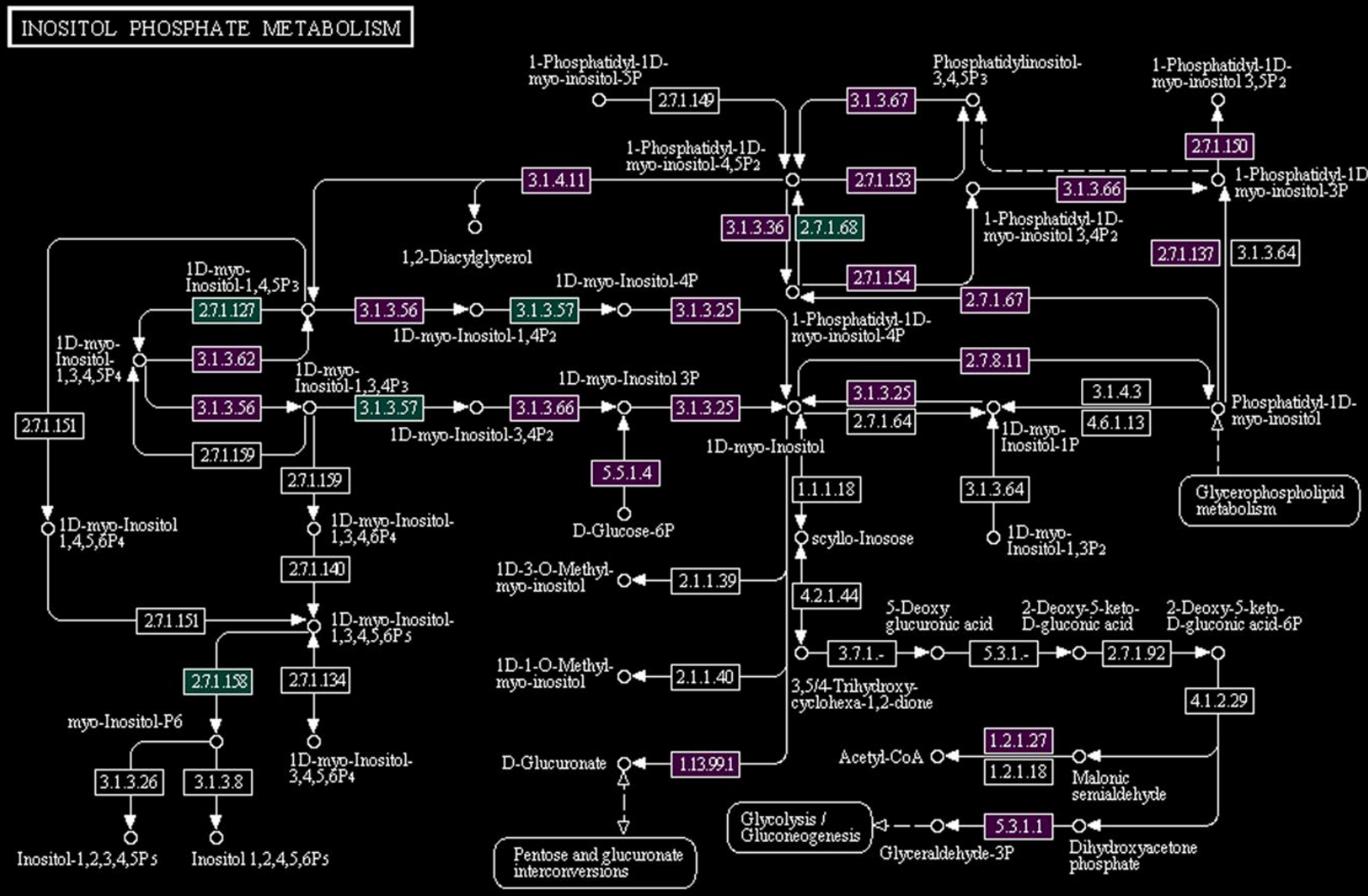
  - ShortBRED
  - PICRUSt
- Tools for testing associations
  - LEfSe
  - MaAsLin
  - CCREPE
- Resources
- Research vignette (time permitting)

The two big questions…

Who is there?
(taxonomic profiling)

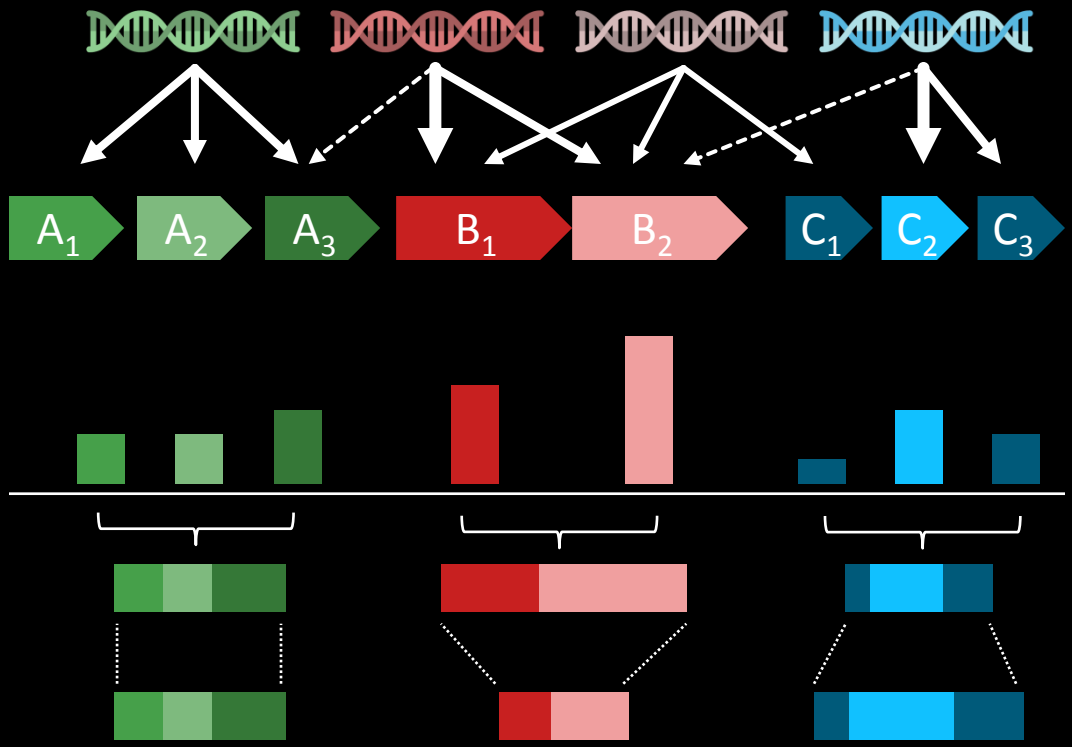
What are they doing?
(functional profiling)

(What we mean by “function”)



HUMAnN

HMP Unified Metabolic Analysis Network



Short reads + protein families

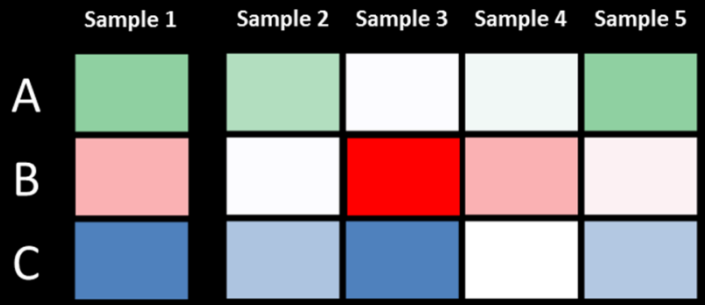
Translated BLAST search

$$c(g) = \frac{1}{|g|} \sum_{r} \frac{\sum_{a(r)} (1 - p_a) \Delta(a = g)}{\sum_{a(r)} 1 - p_a}$$

Weight hits by significance

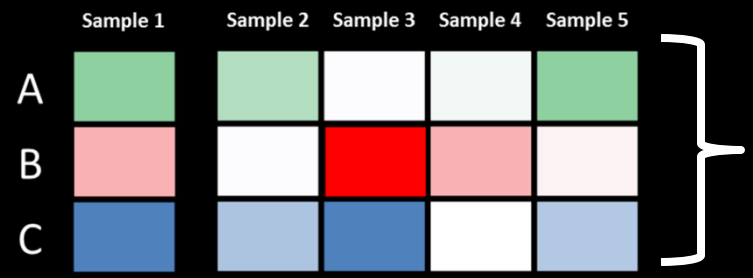
Sum over families

Adjust for sequence length

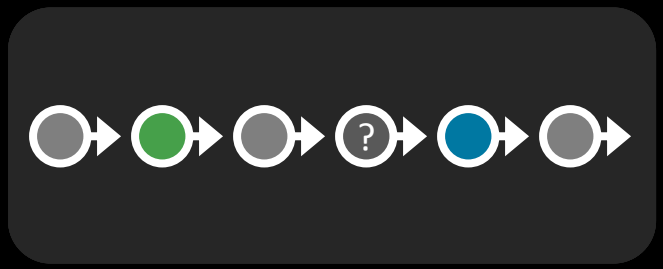


Repeat for each metagenomic or metatranscriptomic sample

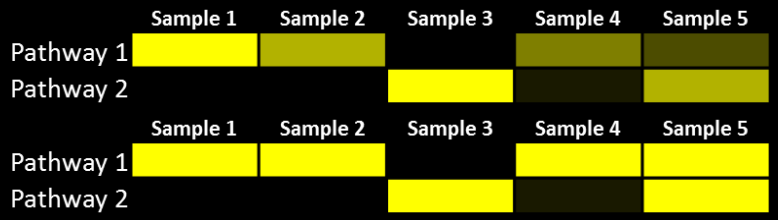
HUMAnN
HMP Unified Metabolic Analysis Network



Millions of hits are collapsed into thousands of gene families (KOs) (*still a large number*)

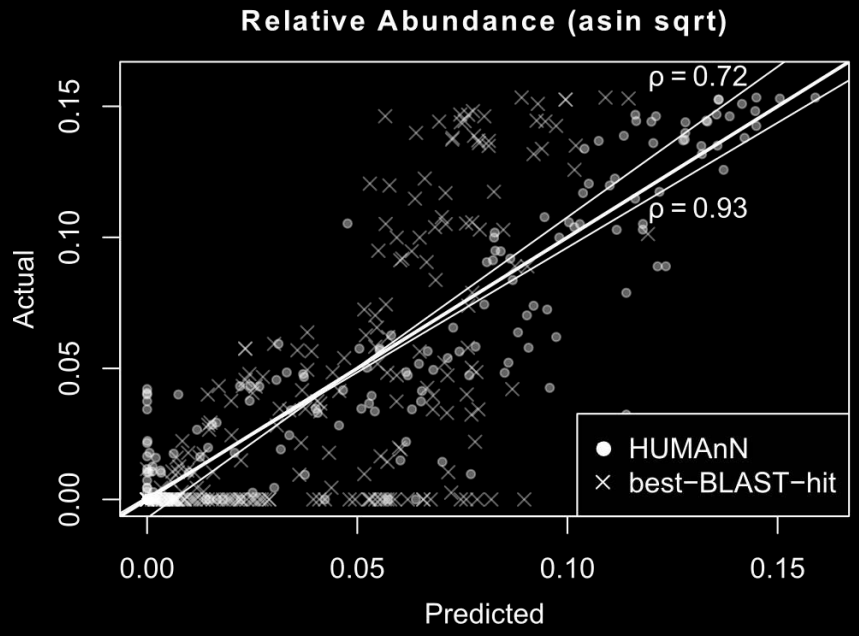


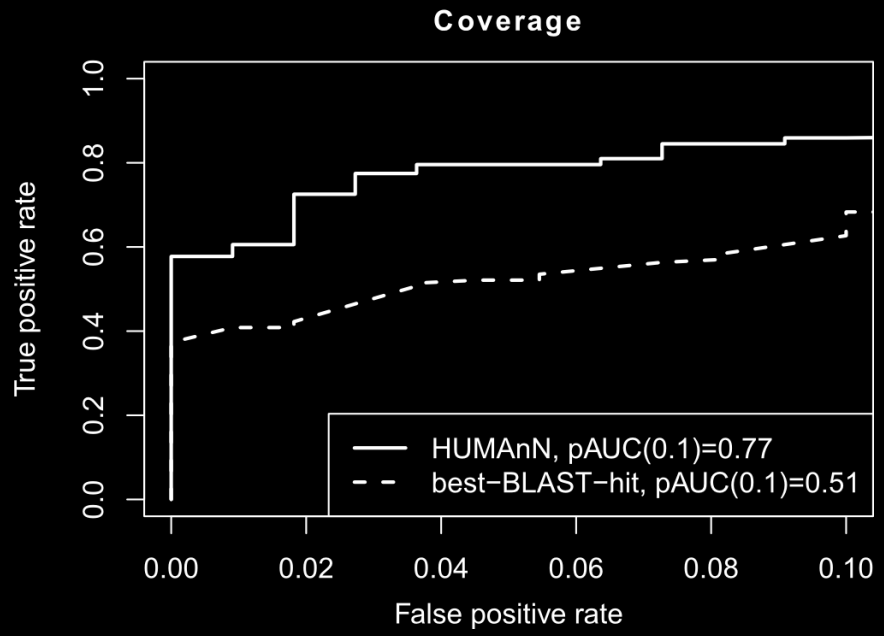
- Map genes to KEGG pathways
- Use MinPath (Ye 2009) to find simplest pathway explanation for observed genes
- Remove pathways unlikely to be present due to low organismal abundance
- Smooth/fill gaps



Collapsing KO abundance into KEGG pathway abundance (or presence/absence) yields a smaller, more tractable feature set

HUMAnN accuracy

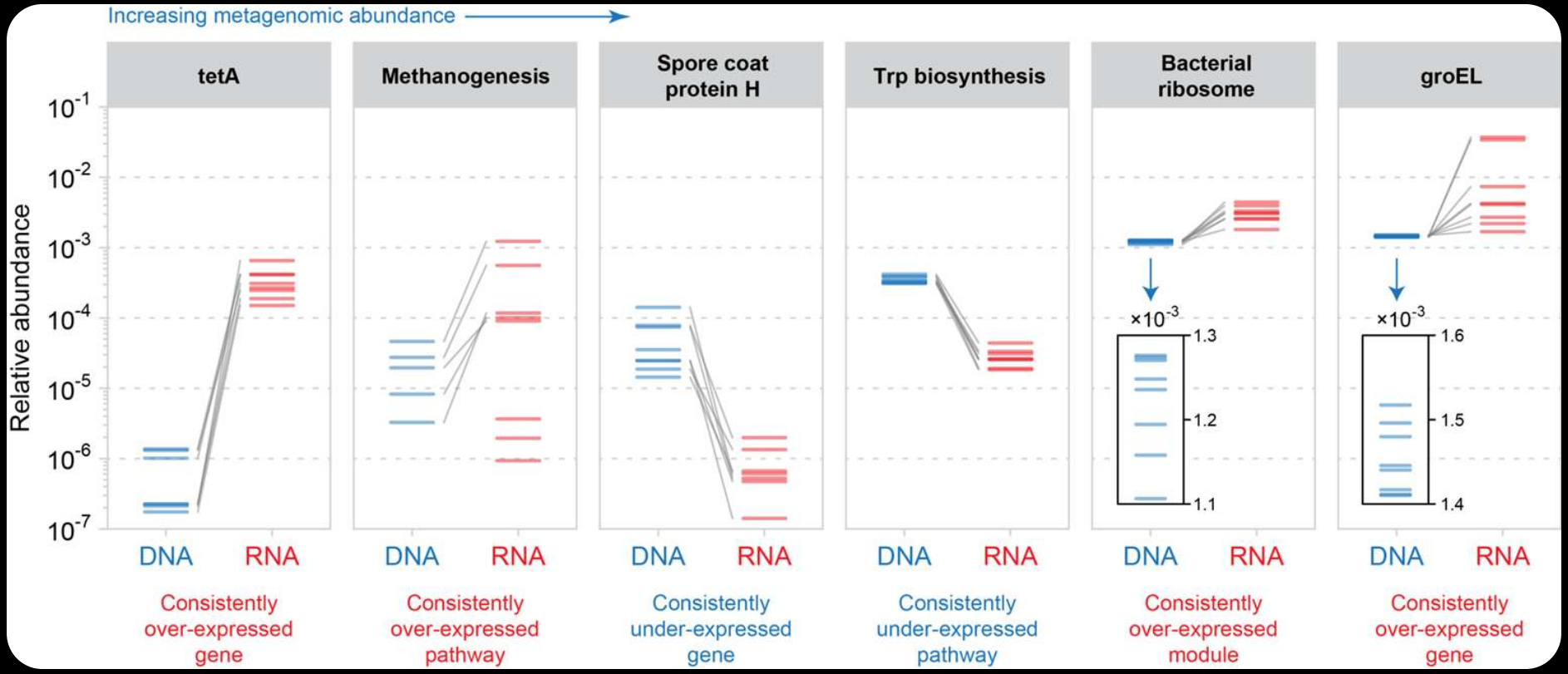




Validated against synthetic metagenome samples
(similar to MetaPhlAn validation)

Gene family abundance and pathway presence/absence
calls beat naïve best-BLAST-hit strategy

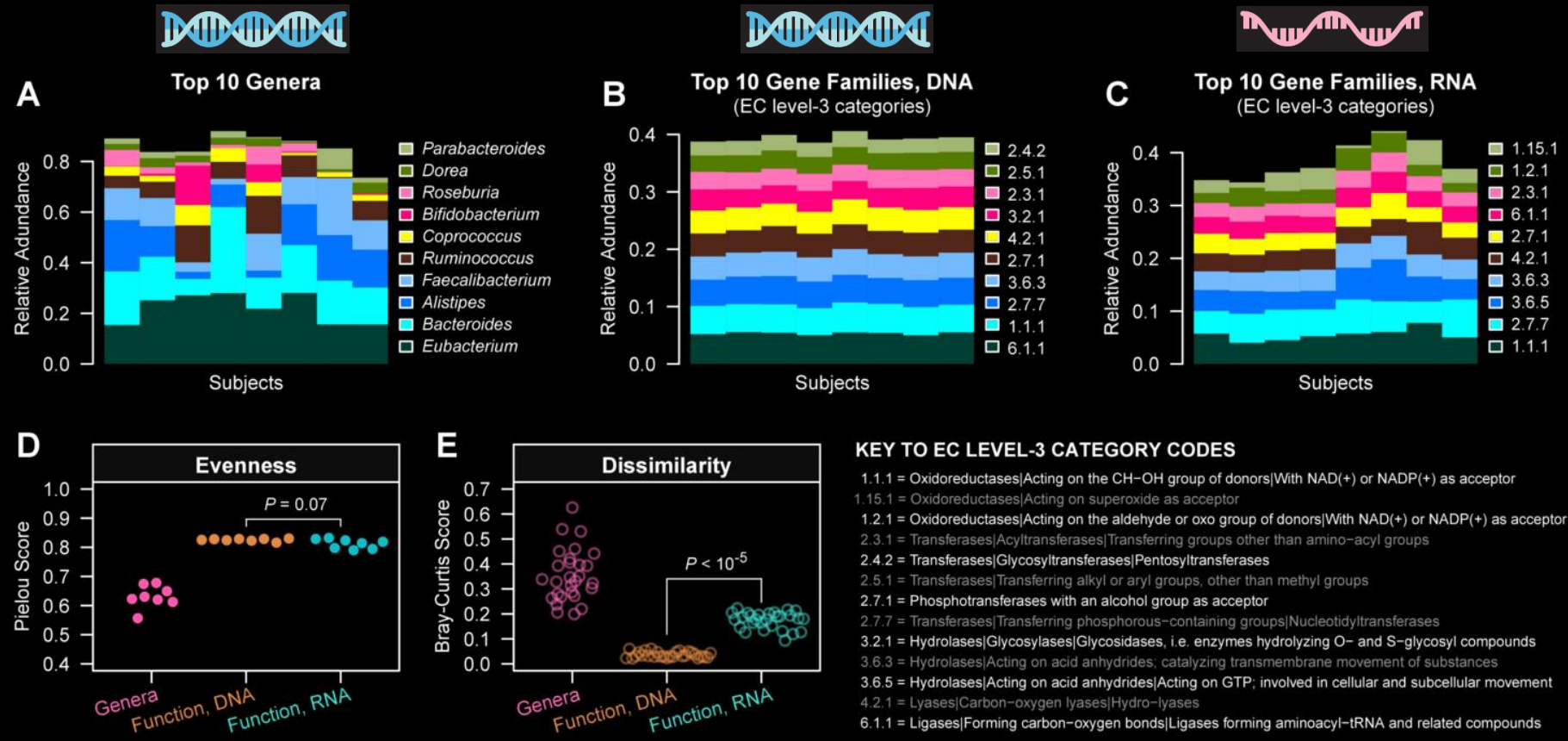
HUMAnN in action
Functional potential (DNA) vs activity (RNA)



Functional metagenomics & metatranscriptomics of 8 heathy human stool samples

Franzosa et al. *PNAS* 11:E2329-38 (2014)

HUMAnN in action
Conserved potential & variable activity



KEY TO EC LEVEL-3 CATEGORY CODES

1.1.1 = Oxidoreductases|Acting on the CH−OH group of donors|With NAD(+) or NADP(+) as acceptor
1.15.1 = Oxidoreductases|Acting on superoxide as acceptor
1.2.1 = Oxidoreductases|Acting on the aldehyde or oxo group of donors|With NAD(+) or NADP(+) as acceptor
2.3.1 = Transferases|Acyltransferases|Transferring groups other than amino−acyl groups
2.4.2 = Transferases|Glycosyltransferases|Pentosyltransferases
2.5.1 = Transferases|Transferring alkyl or aryl groups, other than methyl groups
2.7.1 = Phosphotransferases with an alcohol group as acceptor
2.7.7 = Transferases|Transferring phosphorous−containing groups|Nucleotidyltransferases
3.2.1 = Hydrolases|Glycosylases|Glycosidases, i.e. enzymes hydrolyzing O− and S−glycosyl compounds
3.6.3 = Hydrolases|Acting on acid anhydrides; catalyzing transmembrane movement of substances
3.6.5 = Hydrolases|Acting on acid anhydrides|Acting on GTP; involved in cellular and subcellular movement
4.2.1 = Lyases|Carbon−oxygen lyases|Hydro−lyases
6.1.1 = Ligases|Forming carbon−oxygen bonds|Ligases forming aminoacyl−tRNA and related compounds

Franzosa et al. *PNAS* 11:E2329-38 (2014)

What's there: ShortBRED

Jim Kaminski

- **ShortBRED** is a tool for quantifying protein families in metagenomes
  - Short Better REad Dataset

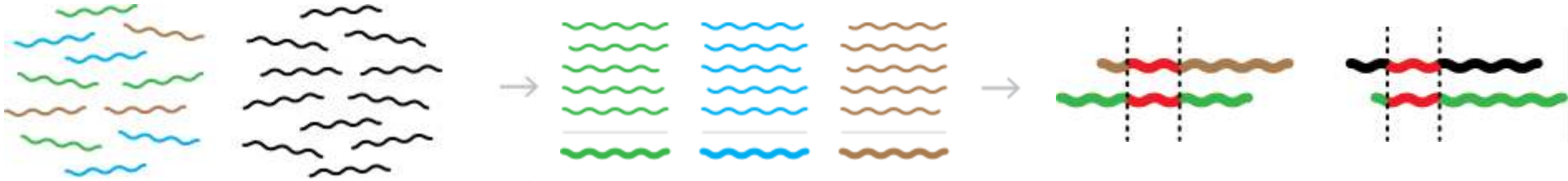
- Inputs:
  - FASTA file of proteins of interest
  - Large reference database of protein sequences (FASTA or blastdb)
  - Metagenomes (FASTA/FASTQ nucleotide files)
- Outputs:
  - Short, unique markers for protein families of interest (FASTA)
  - Relative abundances of protein families of interest in each metagenome (text file, RPKM)

- Compared to BLAST (or HUMAnN), this is:
  - Faster
  - More specific

What's there: ShortBRED algorithm

- Cluster proteins of interest into families
  - Record consensus sequences
- Identify unique and common areas among proteins
  - Compared against each other
  - Compared against reference database
  - Remove all of these
- Remaining subseqs. uniquely ID a family
  - Record these as markers for that family

What's there: ShortBRED marker identification

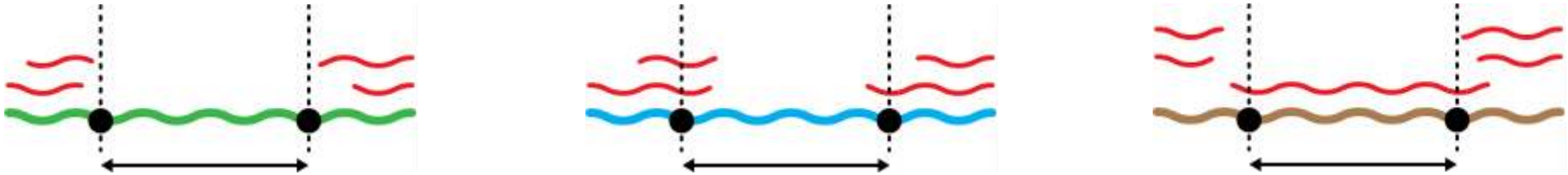


Prots of interest

Reference database

Cluster into families

Identify short, common regions

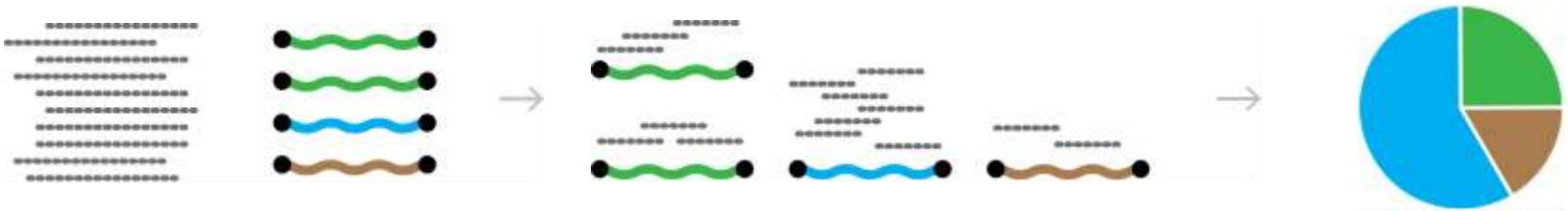


True Marker

Junction Marker

Quasi Marker

What's there: ShortBRED family quantification



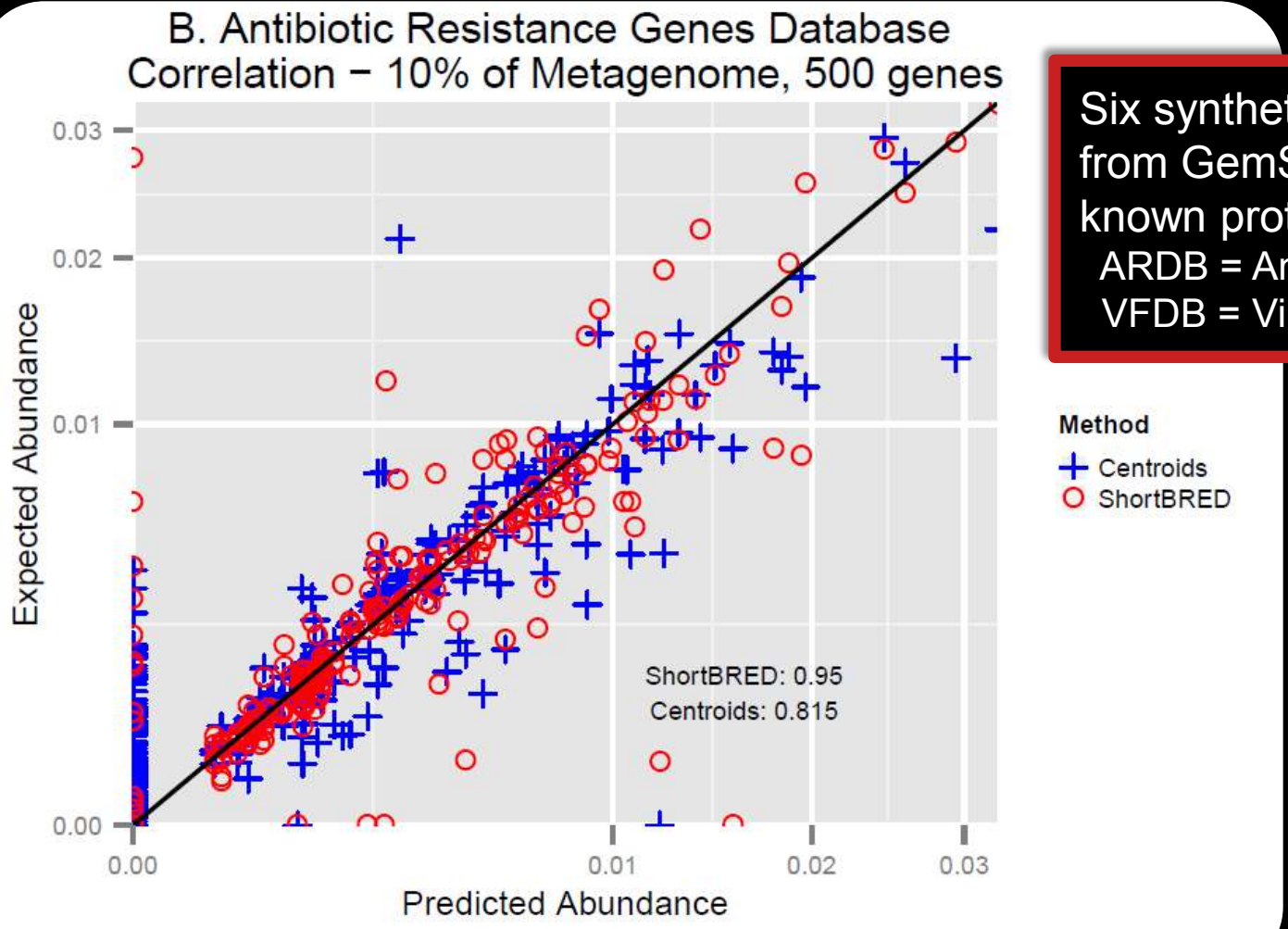
Metagenome reads

ShortBRED markers

Translated search for high ID hits

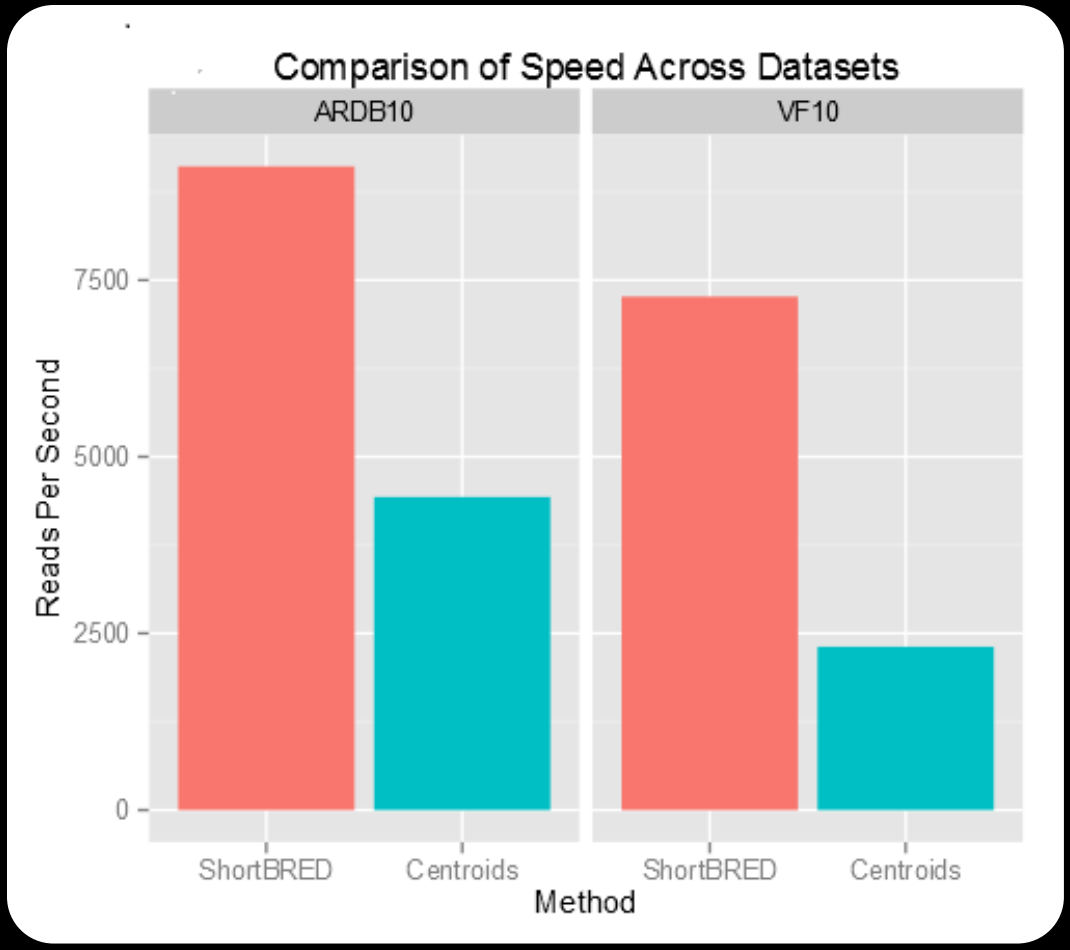
Normalize relative abundances

What's there: ShortBRED is accurate



Six synthetic metagenomes from GemSim, spiked with known proteins of interest:
ARDB = Antibiotic Resistance
VFDB = Virulence Factors

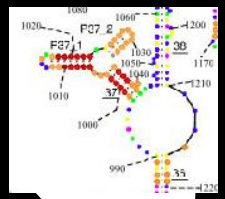
What's there: ShortBRED is fast



Six synthetic metagenomes from GemSim, spiked with known proteins of interest:
ARDB = Antibiotic Resistance
VFDB = Virulence Factors

Can we infer anything about function from 16S data?

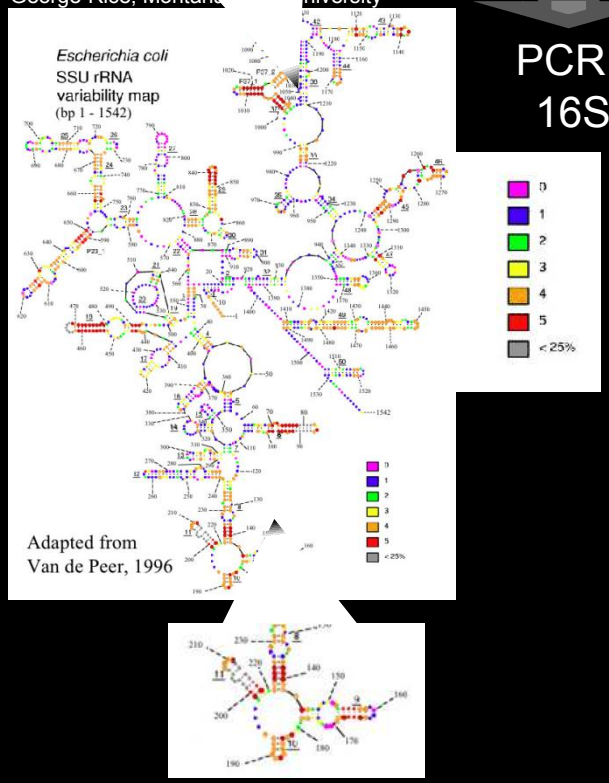
Lyse cells
Extract DNA (and/or RNA)



16S amplicons

Meta'omic

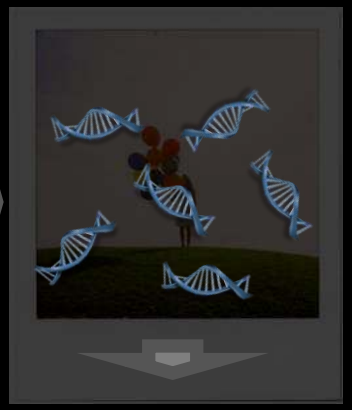
George Rice, Montana [illegible] niversity



PCR to amplify the single 16S rRNA marker gene

Hello
my name is

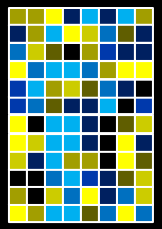
Classify sequence
➔ microbe



Genes,
Genomes,
Metabolic profiling,
Relative abundances,
Genetic variants...

Samples

Microbes



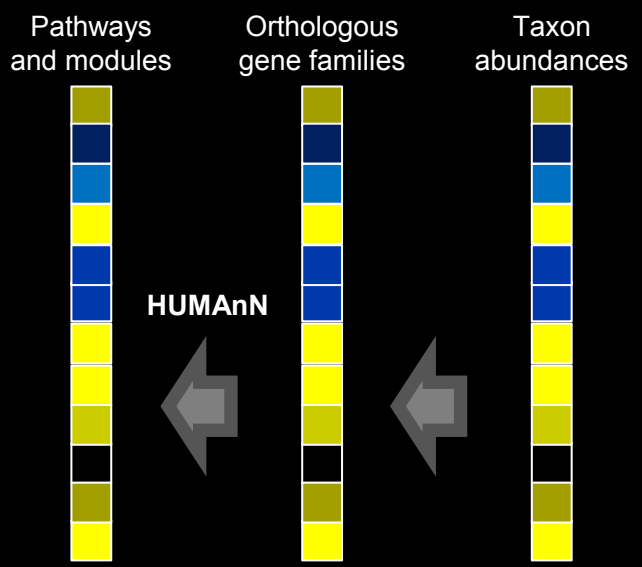
Relative abundances

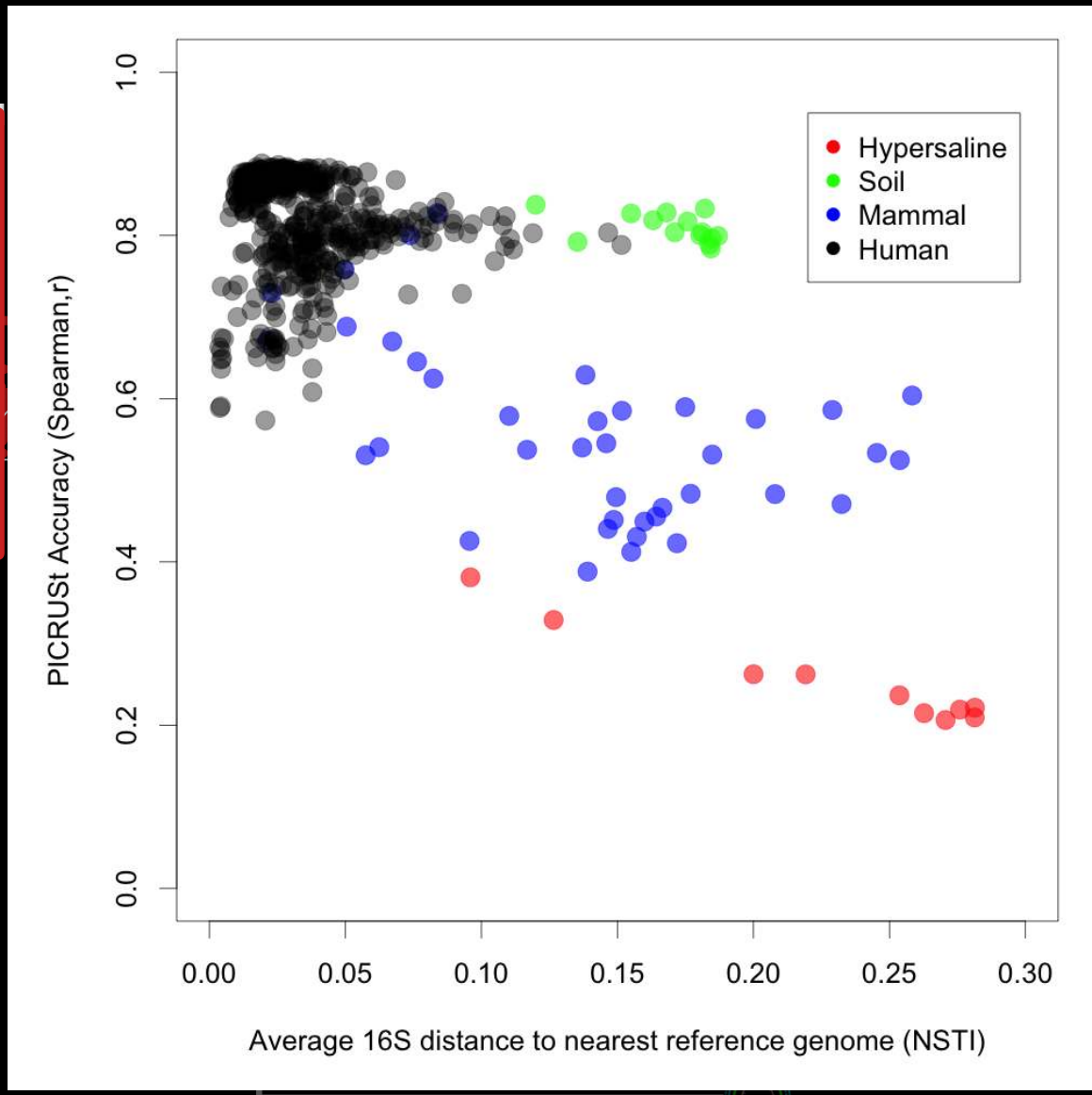
PICRUSt: Inferring community metagenomic potential from marker gene sequencing

With Rob Knight, Rob Beiko

One can recover ***general*** community function with reasonable accuracy from 16S profiles.

http://picrust.github.com





Relative abundance

Plan

- Informal survey
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- Tools for functional profiling
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  - PICRUSt
- Tools for testing associations
  - LEfSe
  - MaAsLin
  - CCREPE
- Resources
- Research vignette (time permitting)

The two big questions…

Who is there?

What are they doing?

Sample #	1	2	3	4	5	6
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

The ~~two~~ three big questions…

Who is there?

What are they doing?

What does it all mean?

Sample #	1	2	3	4	5	6
Profession	Student	Postdoc	Postdoc	Professor	Student	Student
Gender	Male	Female	Female	Male	Male	Female
Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
Clade1|Bug2	0.00	0.30	0.36	0.37	0.04	0.05
Clade2	0.60	0.13	0.57	0.32	0.53	0.68
Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

Properties of microbiome data

- Compositional nature (Σ = 1)
  - Abundance is relative, not absolute
- High dynamic range
- Often sparse (sample dominated by a few species)
- Noisy
- Hierarchical organization

Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
Clade1|Bug1	0.40	0.56	0.07	0.31	0.42	0.27
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Clade2|Bug3	0.11	0.00	0.10	0.32	0.15	0.23
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

Properties of microbiome data

- General problem: correlate microbiome features with metadata (potentially controlling for other features)
- Intuitively summarize the results

Sample #	1	2	3	4	5	6
Profession	Student	Postdoc	Postdoc	Professor	Student	Student
Gender	Male	Female	Female	Male	Male	Female
Site	Oral	Gut	Oral	Gut	Oral	Gut
Clade1	0.40	0.87	0.43	0.68	0.47	0.32
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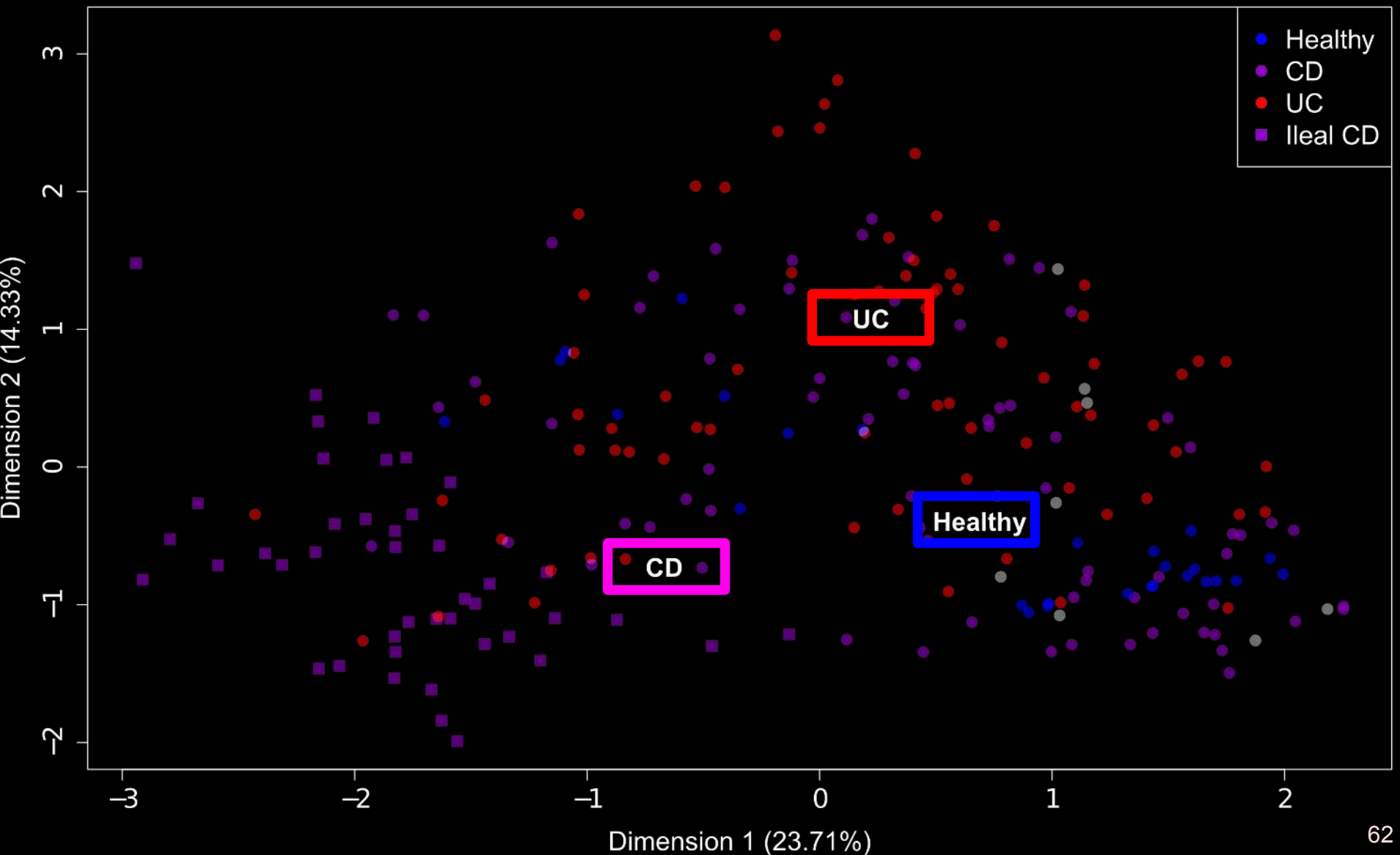
Clade2|Bug4	0.49	0.13	0.47	0.00	0.39	0.45

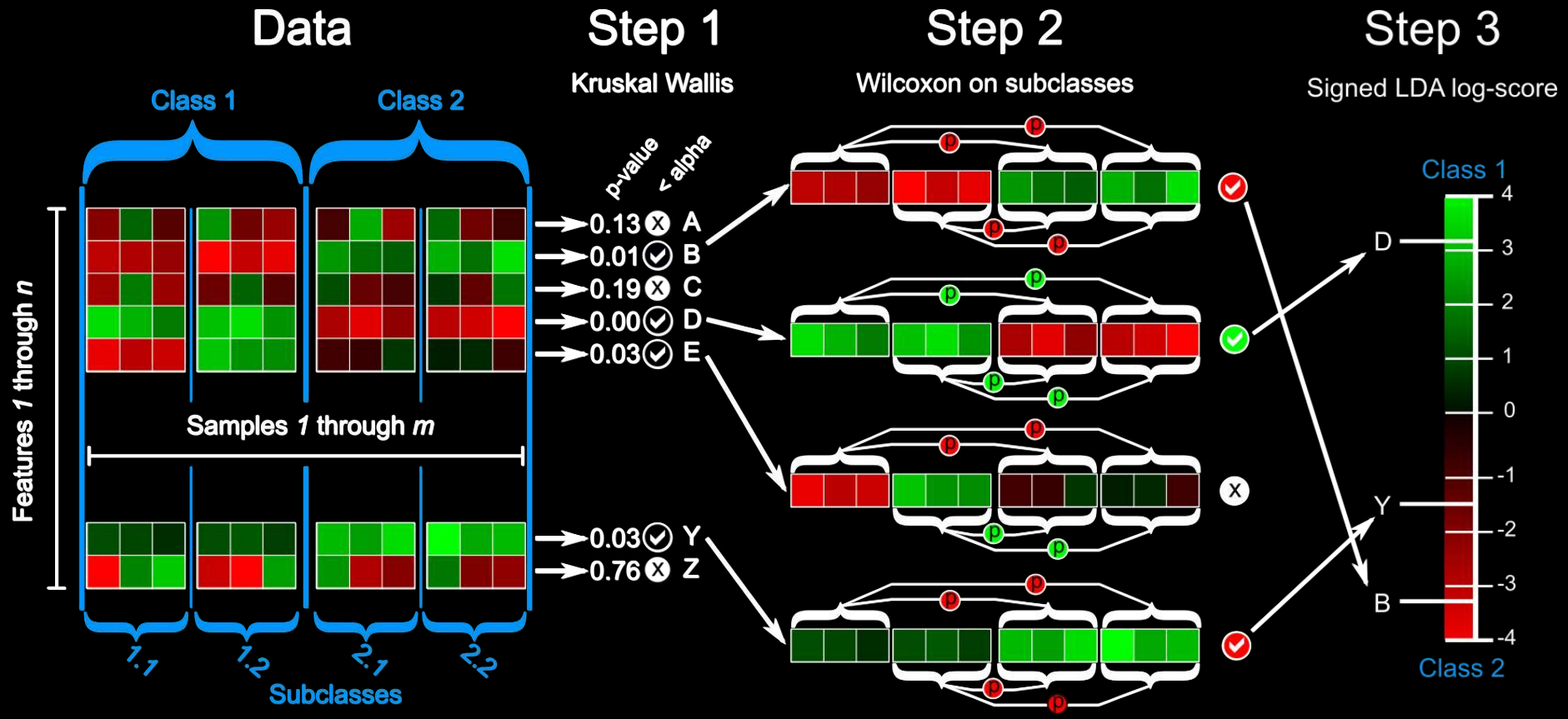
Recall that ordination is exploratory (no *p*-values for a trend, for example)



LEfSe: LDA Effect Size

Finding metagenomic biomarkers

Nicola Segata



A

Statistical consistency

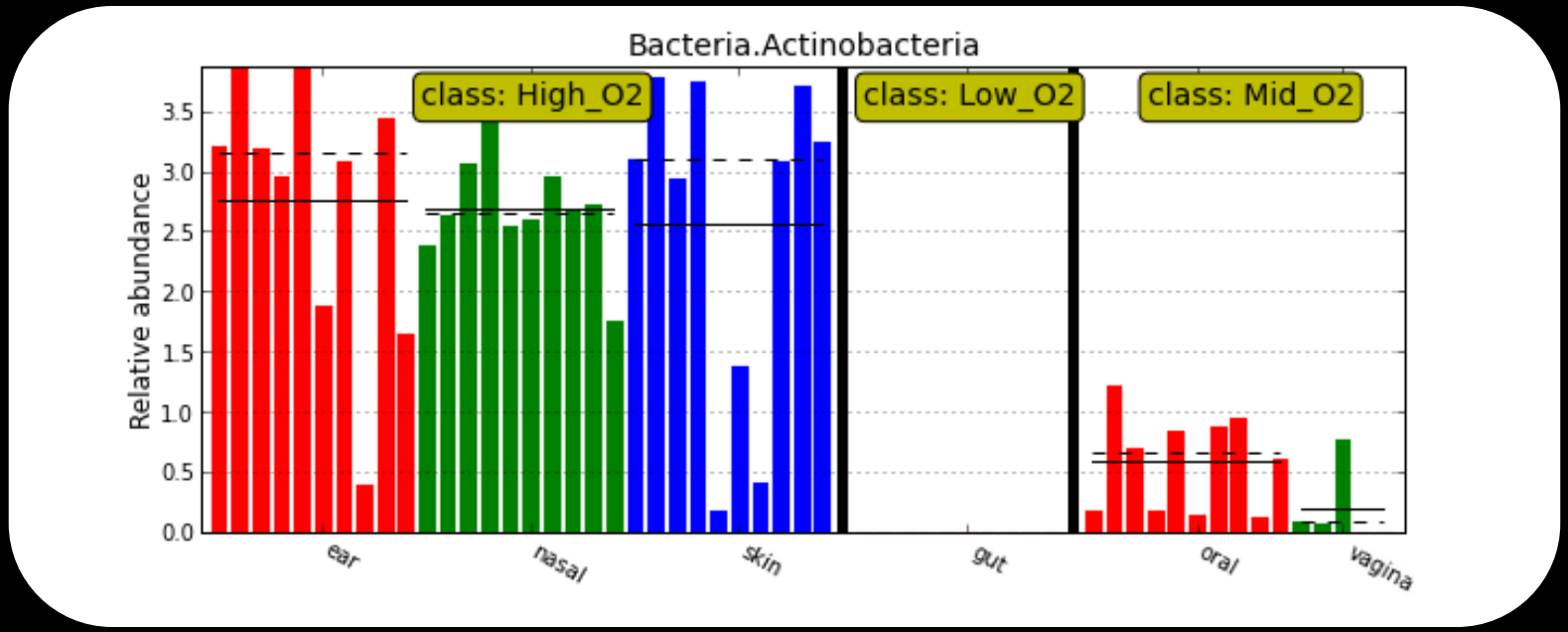
B

Biological consistency

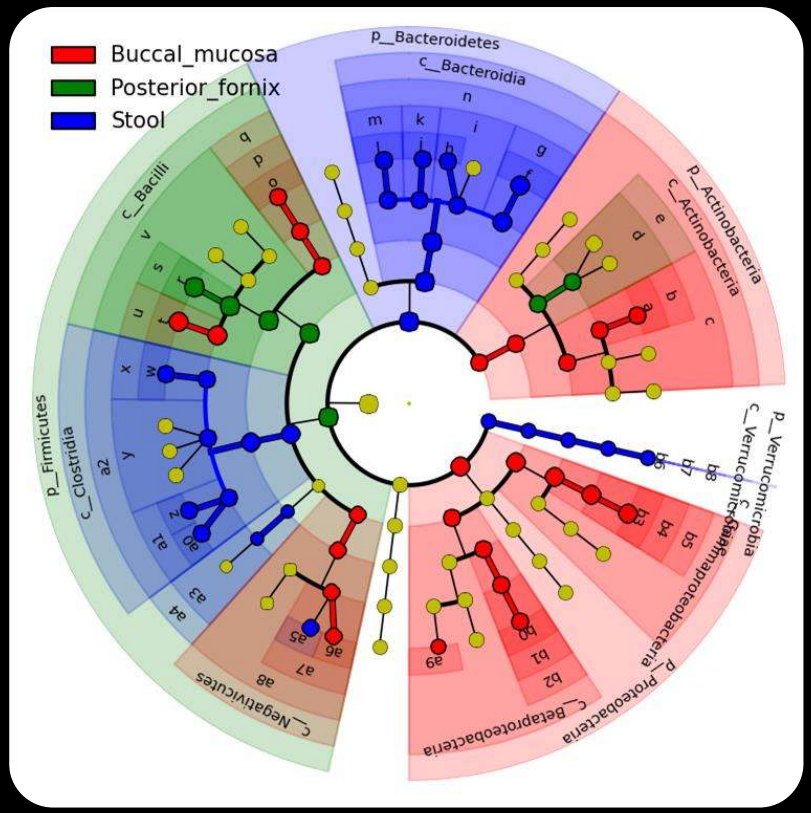
C

Biological Effect size

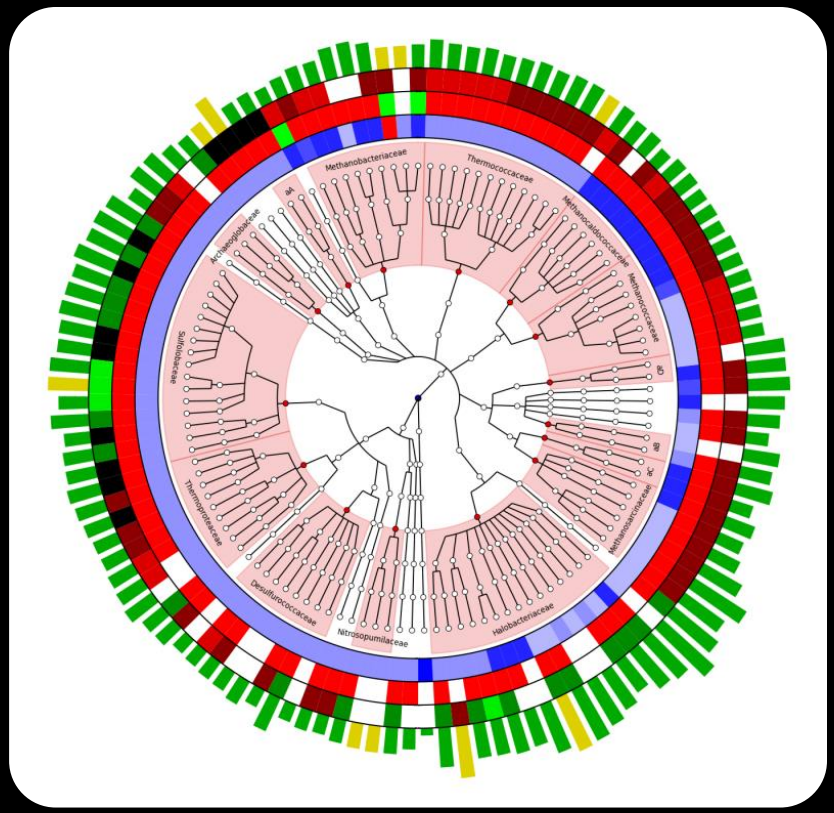
Example LEfSe application:
Find O_2-loving bugs (controlling for body site)



Superimpose enrichments on the tree of life using GraPhlAn

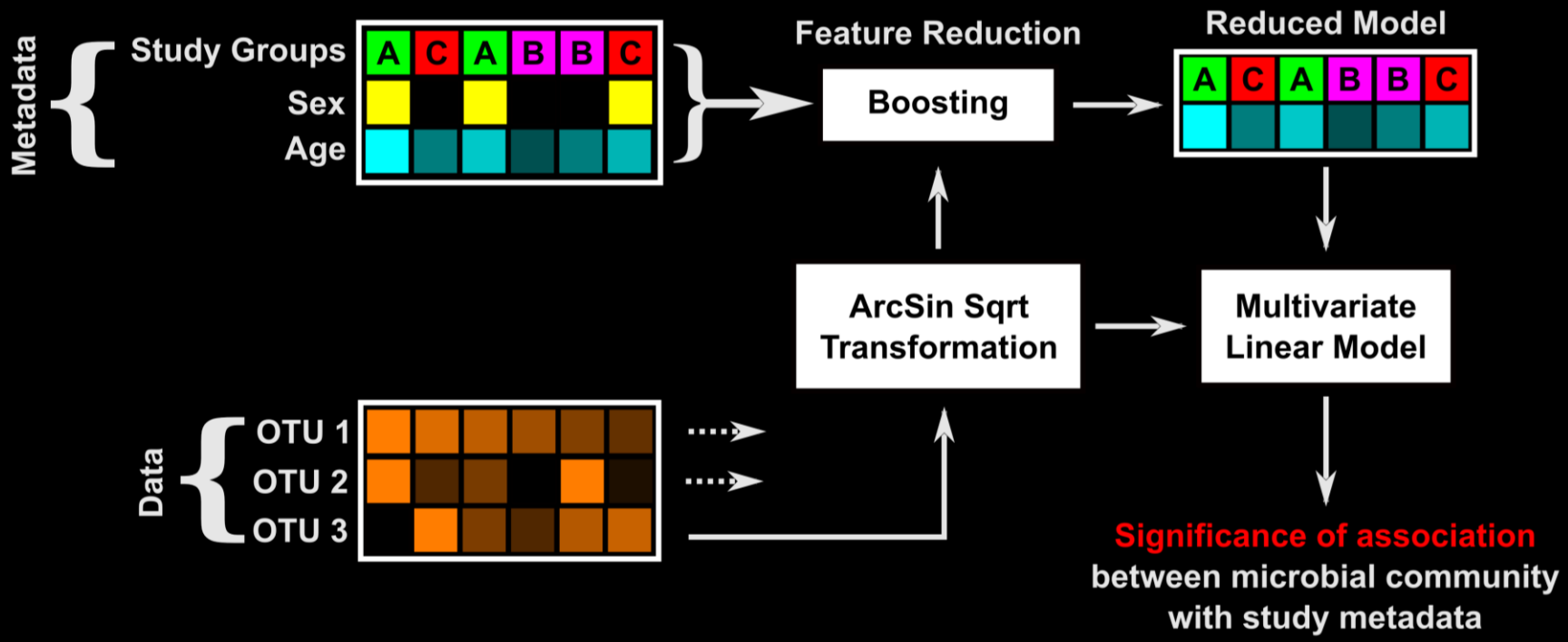


LEfSe Associations



Metadata Rings

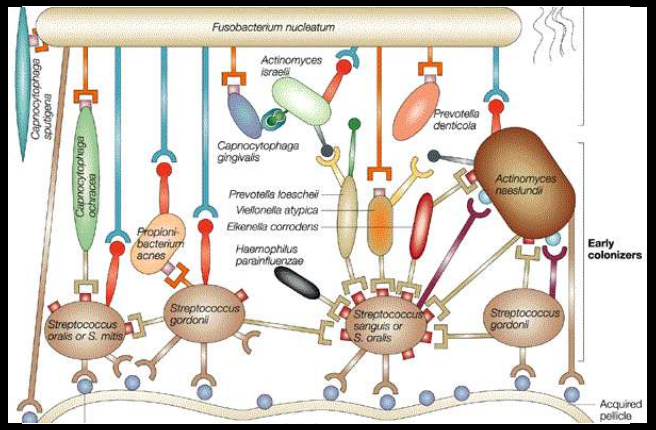
MaAsLin
Multivariate Association with Linear Models



- A more general solution for finding significant metagenomic associations in metadata-rich studies

Tim Tickle

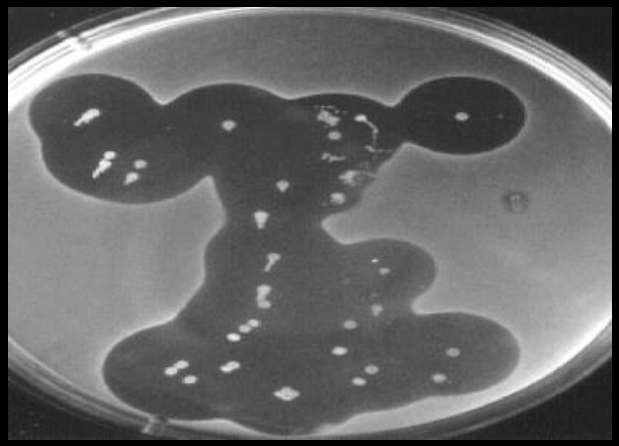
Microbiome downstream analyses: interaction network reconstruction



It's a jungle in there – microbial interactions follow patterns from classical macro-ecology.

Mutualism

Predation

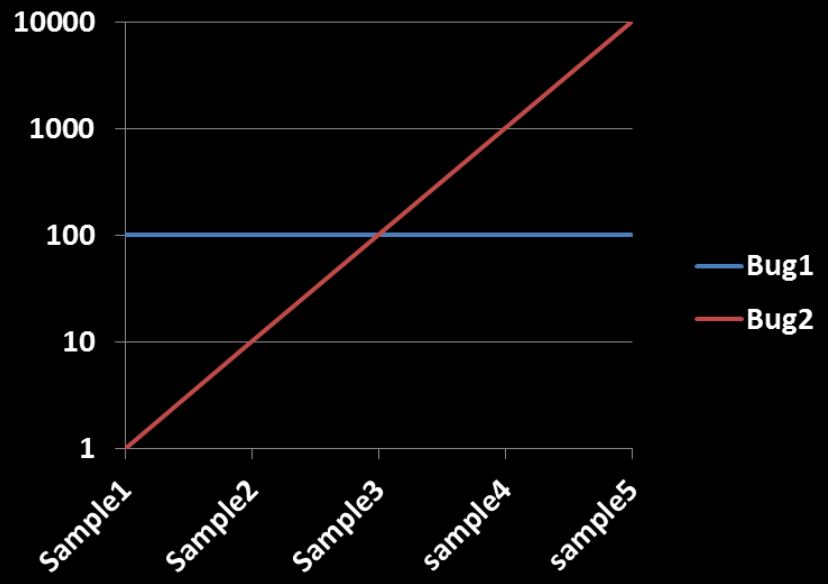


Competition

Given microbial relative abundance measurements over many samples, can we detect *co-occurrence and co-exclusion relationships?*

Relative abundance data poses a problem for correlating metagenomic features

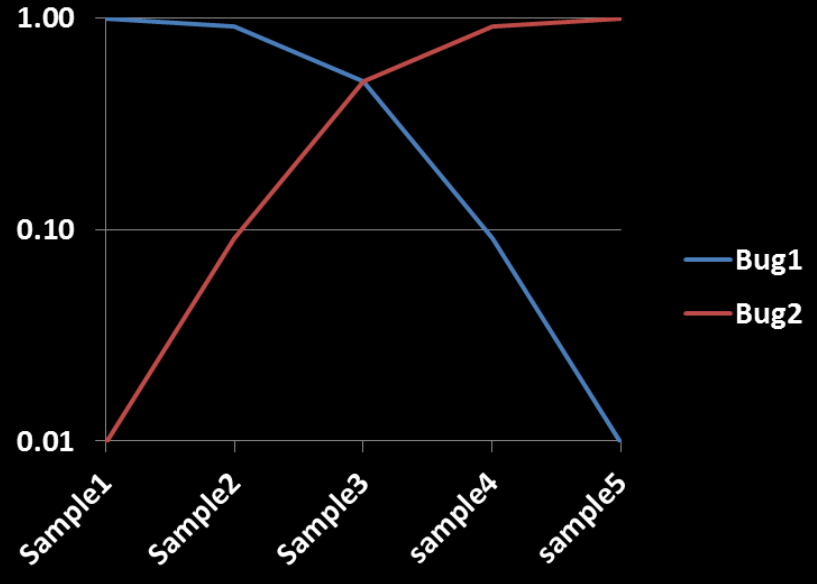
	Sample1	Sample2	Sample3	sample4	sample5
Bug1	100	100	100	100	100
Bug2	1	10	100	1000	10000



Absolute (cell) counts

No bug1-bug2 correlation

	Sample1	Sample2	Sample3	sample4	sample5
Bug1	0.99	0.91	0.50	0.09	0.01
Bug2	0.01	0.09	0.50	0.91	0.99

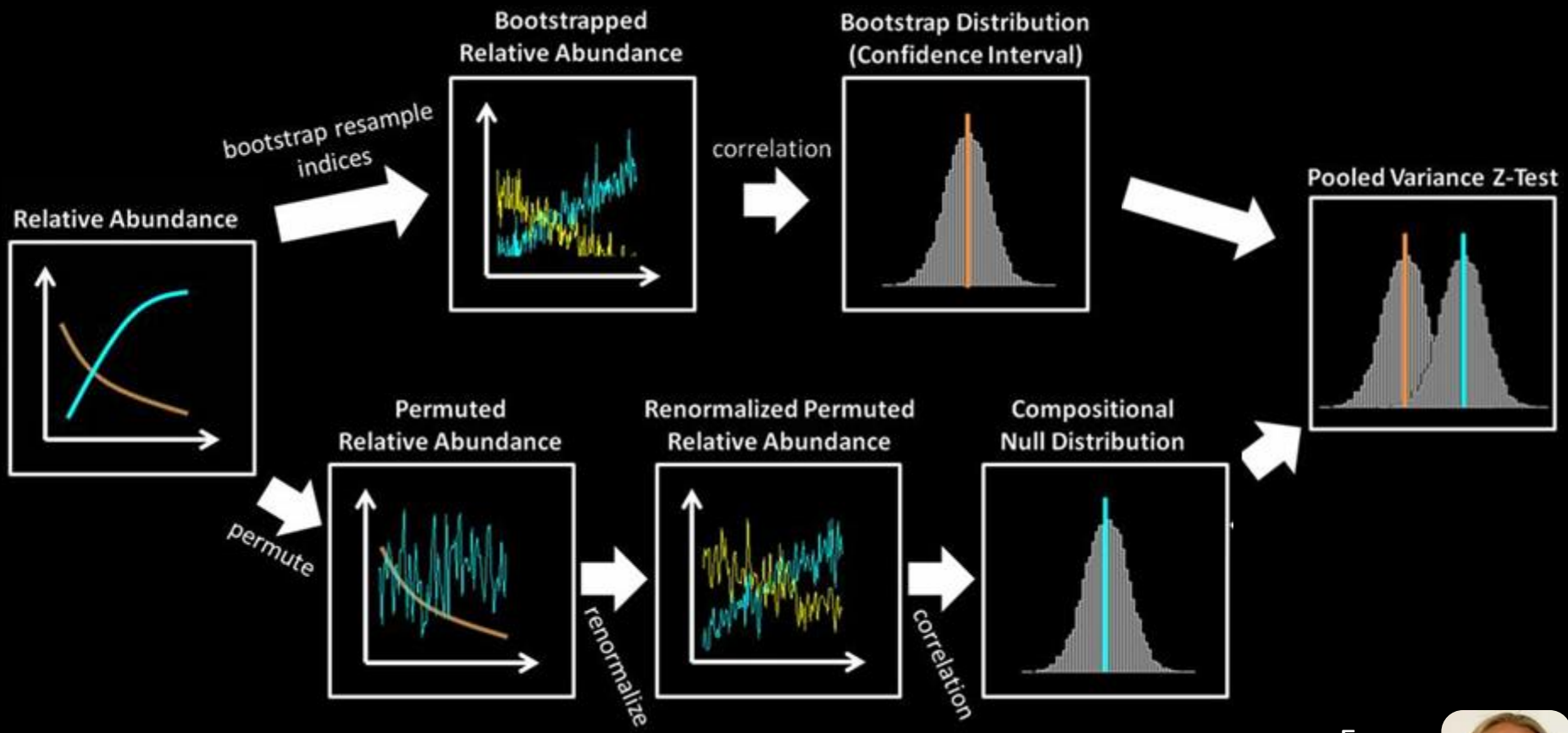


Relative abundance

Spurious bug1-bug2 correlation

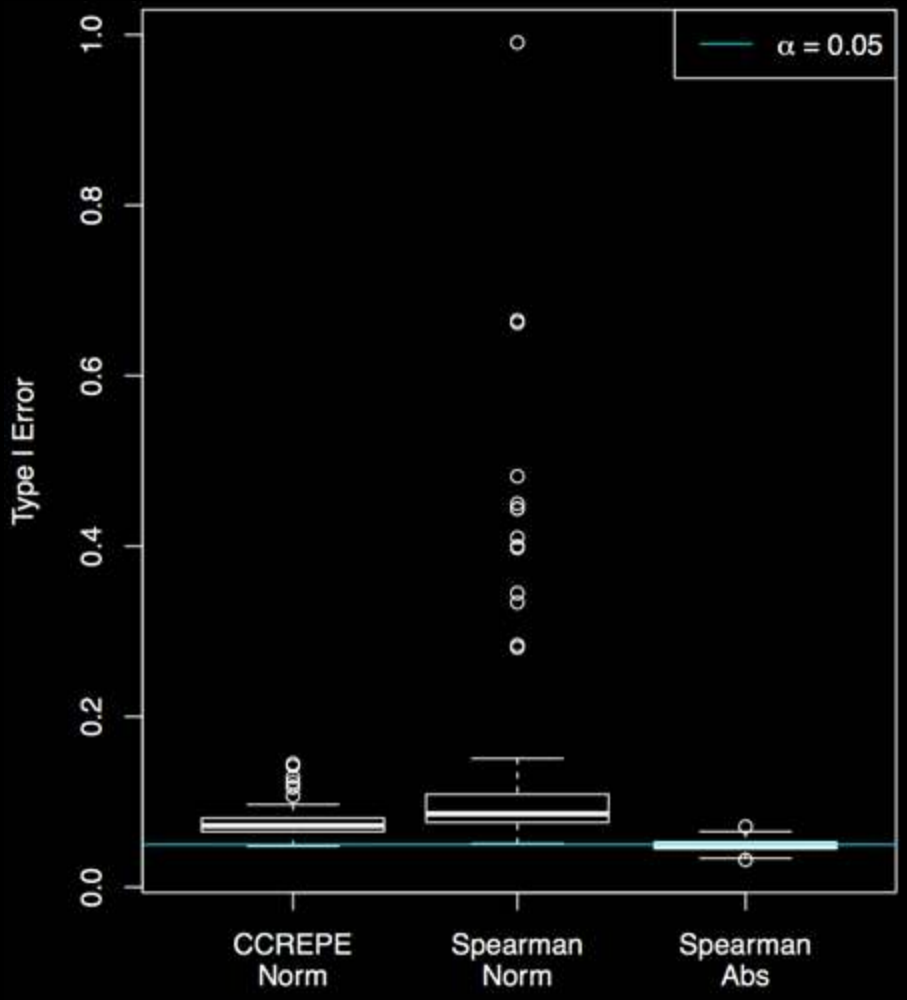
(sequencing yields rel. ab.)

CCREPE: Compositionality Corrected by REnormalization and PErmutation



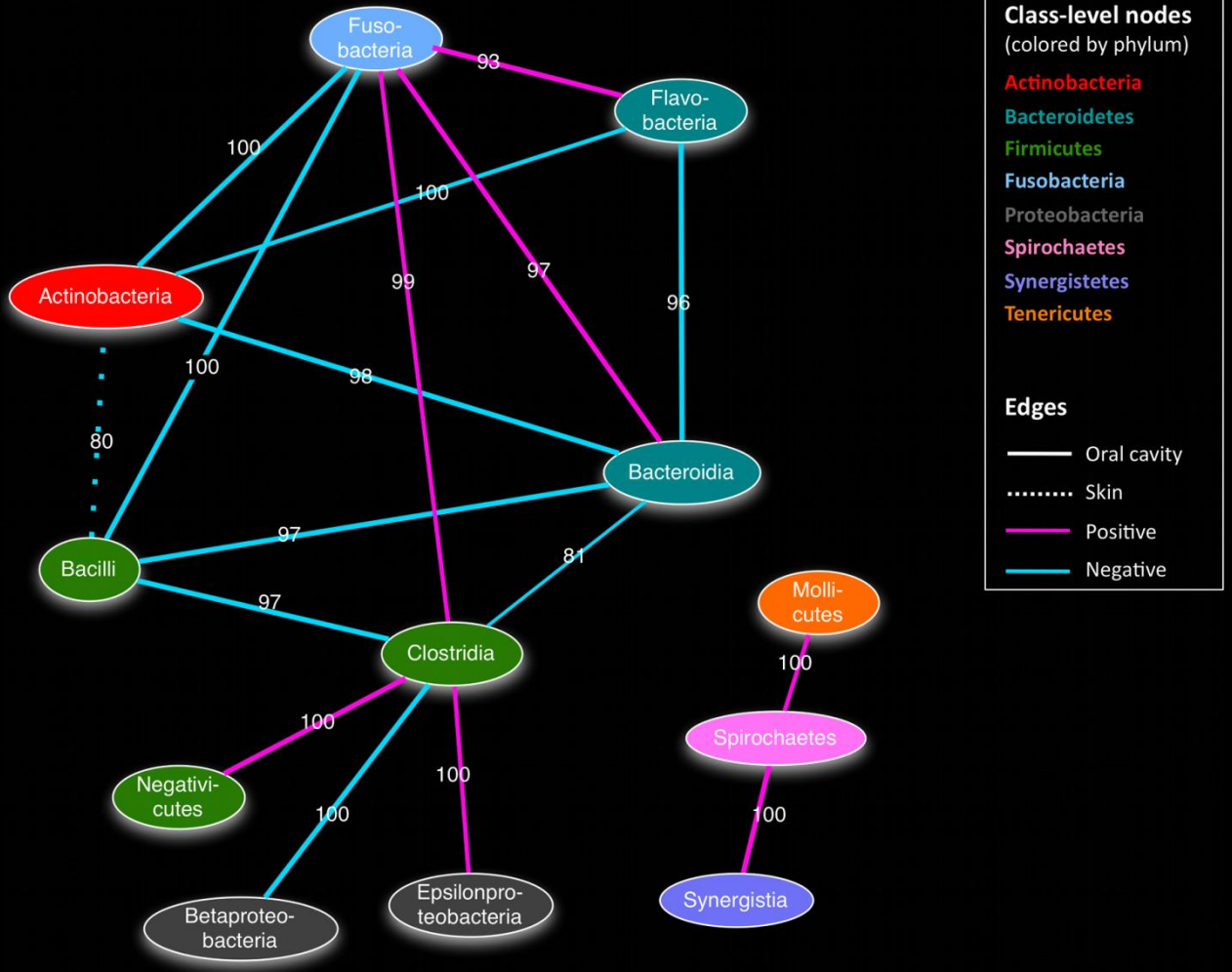
Emma Schwager

CCREPE: Compositionality Corrected by REnormalization and PErmutation



- Synthetic evaluation
- Random sample feature/tables
- No built-in correlation structure

CCREPE: Compositionality Corrected by REnormalization and PErmutation



"Microbial co-occurrence relationships in the human microbiome."
Faust, et al. *PLoS Comp Biol*, 8:e1002606 (2012).

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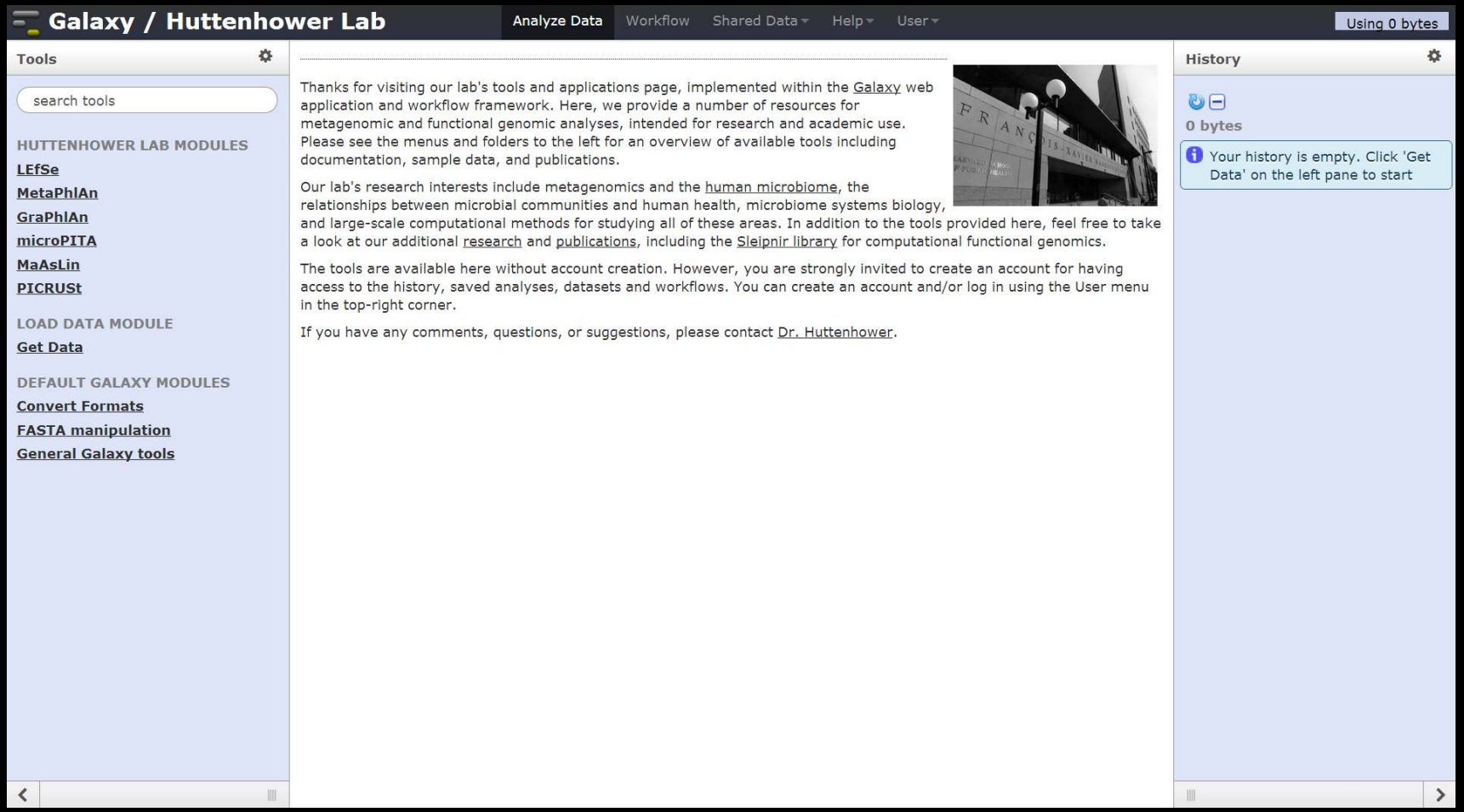
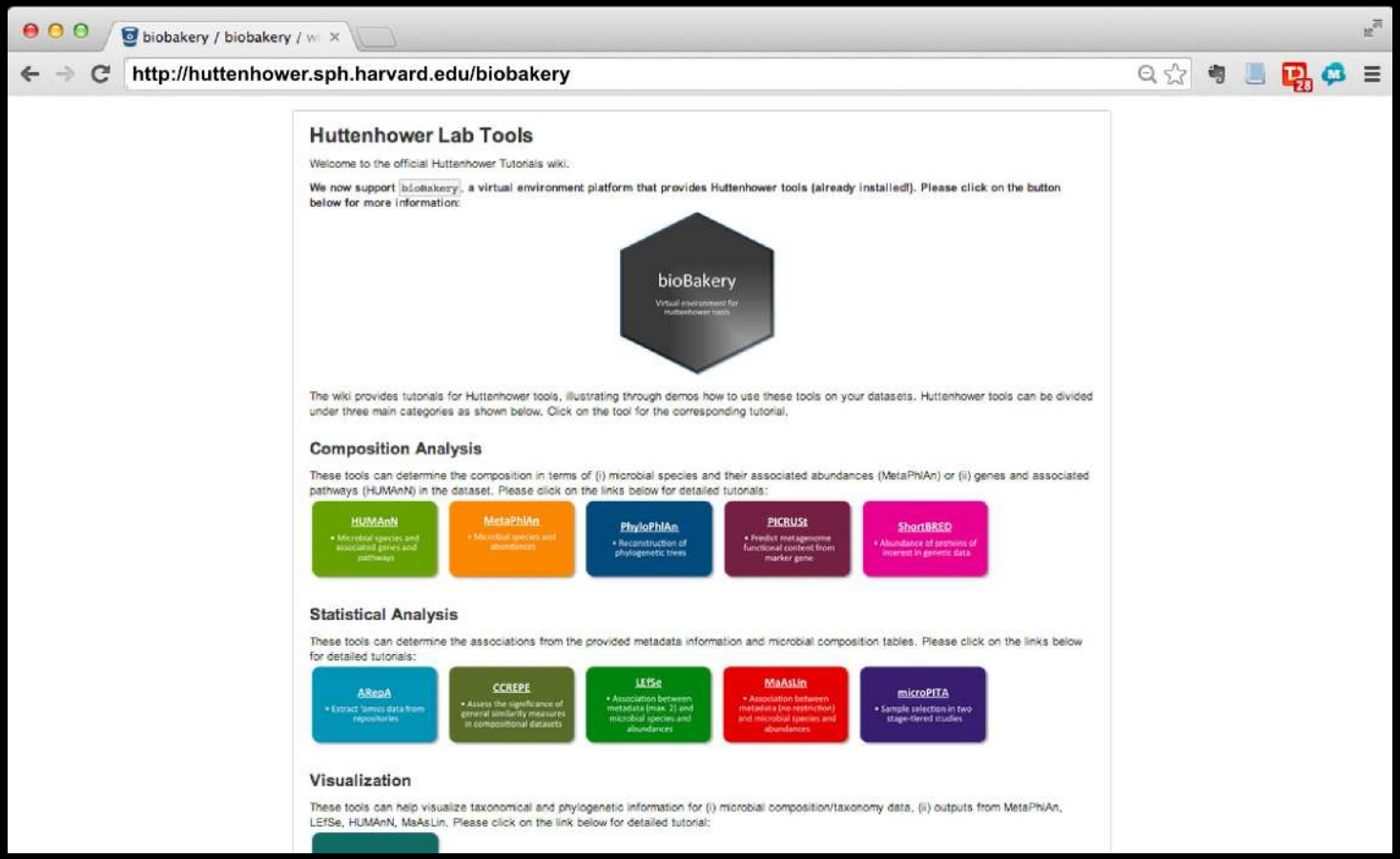
Using tools through Galaxy



http://huttenhower.sph.harvard.edu/galaxy

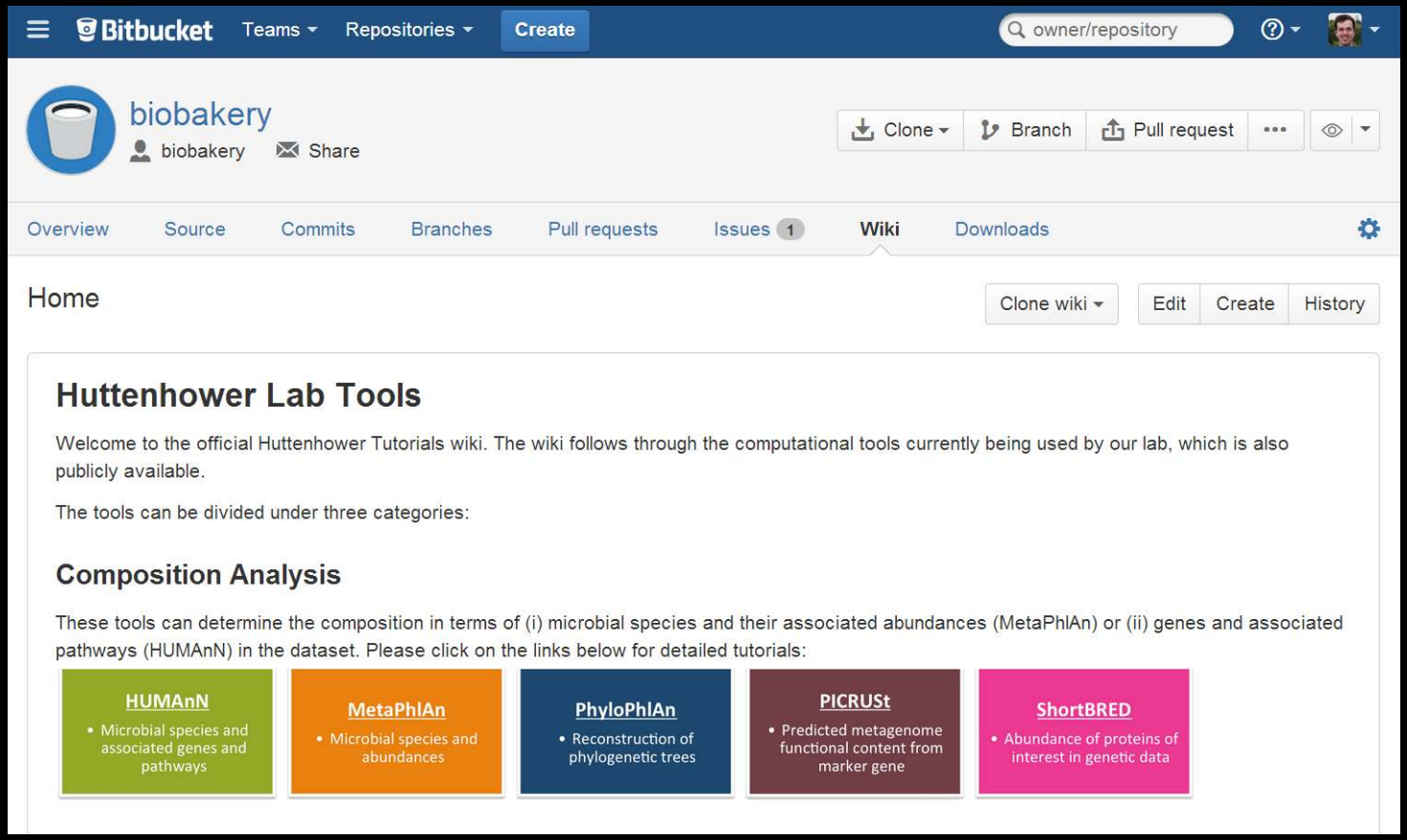
Tutorials available online



http://huttenhower.sph.harvard.edu/biobakery

(click on your tool-of-interest)

All tools are open source



http://bitbucket.org/biobakery/biobakery

The bioBakery Virtual Machine

https://bitbucket.org/biobakery/biobakery/wiki/biobakery_wiki

Ubuntu base image preloaded and configured to run all Huttenhower lab tools; one click up-and-running via Vagrant

Thank you!


Curtis Huttenhower


Xochitl Morgan


Afrah Shafquat


Keith Bayer


George Weingart


Regina Joice


Aleksandar Kostic


Chengwei Luo

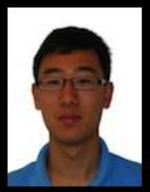

Tiffany Hsu


Emma Schwager


Koji Yasuda


Kevin Oh


Boyu Ren


Andy Shi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Bruce Sands

Human Microbiome Project

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George Weinstock
Karen Nelson
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Erica Sodergren
Anthony Fodor
Marty Blaser
Jacques Ravel
Pat Schloss
Sahar Abubucker
Brandi Cantarel
Alyx Schubert
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Beltran Rodriguez-Mueller
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Yuzhen Ye
Mihai Pop
Larry Forney
Barbara Methe

Bruce Birren Mark Daly
Doyle Ward Ashlee Earl

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