




Microbes and
Microbiome

Julie Segre, PhD

Senior Investigator,
National Human
Genome Research
Institute, NIH



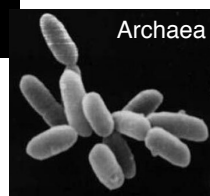
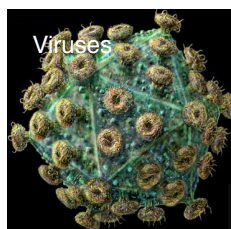
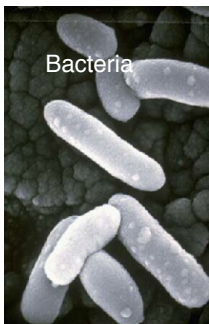
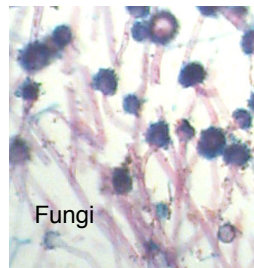
Current Topics in Genome Analysis 2012

Julia Segre

***No Relevant Financial Relationships with
Commercial Interests***

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Why the Human Microbiome?



Each human cell has the same protein-encoding potential. Microbes are more diverse and dynamic than human genome.

3

Human Microbiome Project (HMP) Goals: Baseline to empower future clinical studies

Assess microbial diversity of 250 healthy individuals at 5 sites (gut, nasal, oral, vaginal and skin)



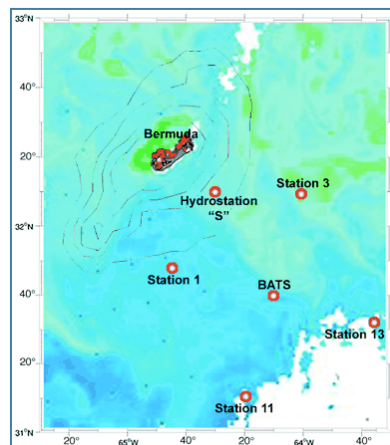
4

HMP Research Goals

- Sequence bacterial reference genomes
- Metagenomics, the analysis of the combined coding potential of a mixed population.
- Correlation of changes in microbial communities with disease states.
- Explore ethical, legal and social implications of this new field of research.

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Microbial Diversity Studied in the Environment



Originally published in *Science Express* on 4 March 2004
Science 2 April 2004:
Vol. 304, no. 5667, pp. 66 – 74
DOI: 10.1126/science.1093857

RESEARCH ARTICLES

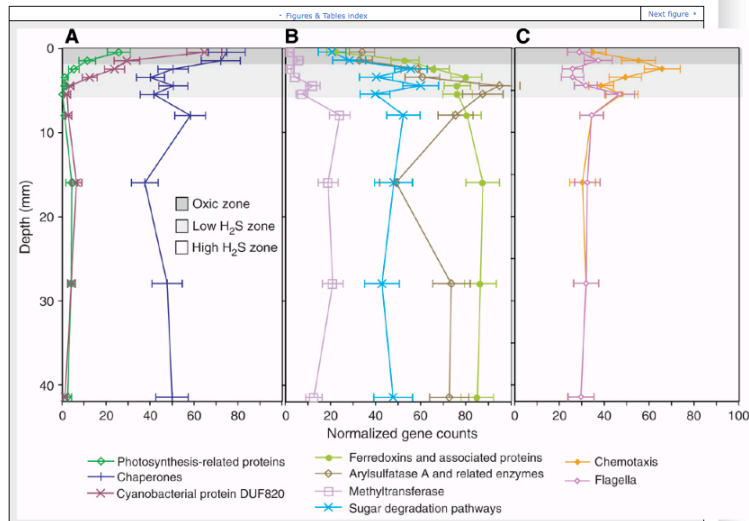
Environmental Genome Shotgun Sequencing of the Sargasso Sea

J. Craig Venter,^{1,2} Karin Remington,¹ John F. Heidelberg,³ Aaron L. Halpern,² Doug Rusch,² Jonathan A. Eisen,³ Dongying Wu,³ Ian Paulsen,³ Karen E. Nelson,³ William Nelson,³ Derrick E. Fouts,³ Samuel Levy,² Anthony H. Knap,⁶ Michael W. Lomas,⁶ Ken Nealson,⁵ Owen White,³ Jeremy Peterson,³ Jeff Hoffman,¹ Rachel Parsons,⁶ Holly Baden-Tillson,¹ Cynthia Pfannkoch,¹ Yu-Hui Rogers,⁴ Hamilton O. Smith¹

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HyperSaline mat diversity Guerro Negro, MX

FROM: **Millimeter-scale genetic gradients and community-level molecular convergence in a hypersaline microbial mat**
 Victor Kunin, Jeroen Raes, J Kirk Harris, John R Spear, Jeffrey J Walker, Natalia Ivanova, Christian von Marung, Brad M Bebout, Norman R Pace, Peer Bork & Phillo Hugenholz
 doi:10.1038/nmsb.2008.35



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And human-environment diversity: shower heads across USA

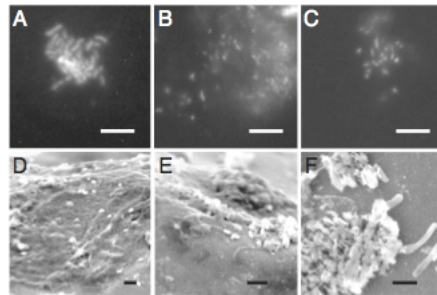


Fig. 1. Fluorescence and SEM images of showerhead biofilm. (A-C) Epifluorescence microscopy of biofilm samples stained with DAPI; scale bars, 10 μ m. (D-F) SEM micrographs of increasing magnification of in situ showerhead

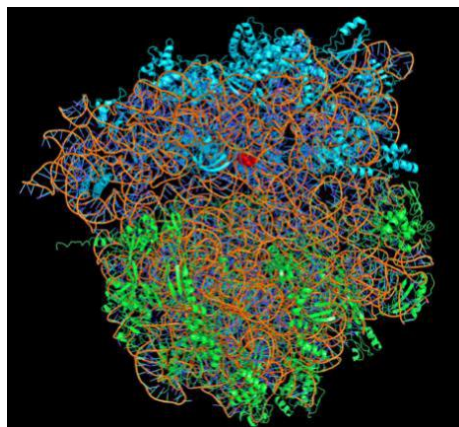
Opportunistic pathogens enriched in showerhead biofilms

Leah M. Feazel^a, Laura K. Baumgartner^a, Kristen L. Peterson^a, Daniel N. Frank^a, J. Kirk Harris^b, and Norman R. Pace^{a,1}

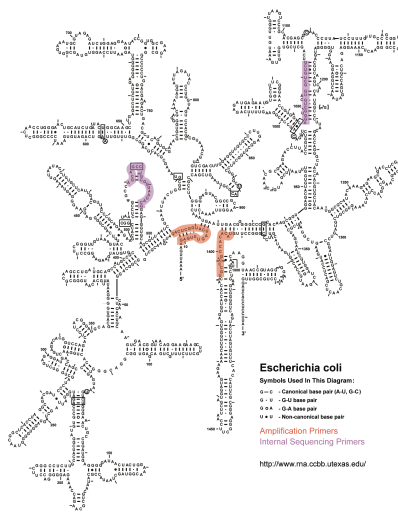
Genus	% of Total	Region	Sample ID	Heatmap % of Library
<i>Acetivibrio</i> sp.	0.1	New York City m-1304	NSHQ0	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ1	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ2	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ3	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ4	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ5	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ6	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ7	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ8	0.1
<i>Acetivibrio</i> sp.	0.1		NSHQ9	0.1
<i>Acetivibrio</i> sp.	0.1	Denver Metro #2A m-1305	DSWQ0	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ1	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ2	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ3	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ4	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ5	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ6	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ7	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ8	0.1
<i>Acetivibrio</i> sp.	0.1		DSWQ9	0.1
<i>Acetivibrio</i> sp.	0.1	Illinois m-112	LSHQ0	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ1	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ2	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ3	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ4	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ5	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ6	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ7	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ8	0.1
<i>Acetivibrio</i> sp.	0.1		LSHQ9	0.1

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TOPIC 1. Bacterial Diversity: 16S rRNA gene



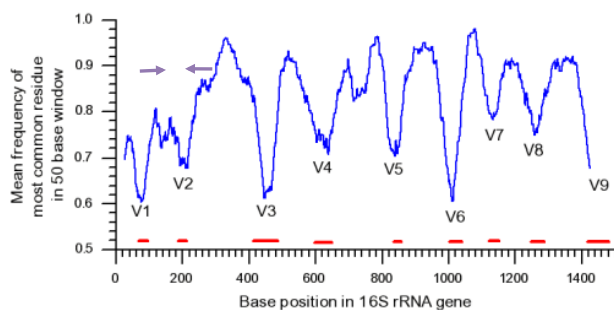
Orange= rRNA ;
Blue = small subunit proteins
Green = large subunit proteins



Secondary Structure: 16S small subunit ribosomal RNA

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Bacterial Load: qPCR wth primers in conserved regions



16S gene was amplified using forward primer 63F (5-GCAGGCCTAACACATGCAAGTC-3) and reverse primer 355R (5-CTGCTGCCTCCCGTAGGAGT-3) to yield a 292-bp PCR product. (Castillo M...Gasa J...2006)

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Calculating Bacterial Load

Human DNA	300 pg		Bacterial DNA 30 pg		3 pg	
	Ct	copy #	Ct	copy #	Ct	copy #
0 g	17.85	54924.50	20.92	6951.93	24.24	743.61
0.3 ng	17.78	57575.00	20.93	6905.28	24.42	658.74

C_t of qPCR of bacterial DNA to calculate relative bacterial counts of each sampling method. The function used to calculate copy number is as follows: $C_t = -3.42x + 34.06$; $R^2 = 0.99$; where C_t = threshold cycle and $x = \log$ copy number.

- Swab yields 10,000 bacteria/cm²
- Scrape yields 50,000 bacteria/cm²
- Biopsy yields 1,000,000 bacteria/cm²

Grice et al, Genome Research 2008

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How to study microbial diversity

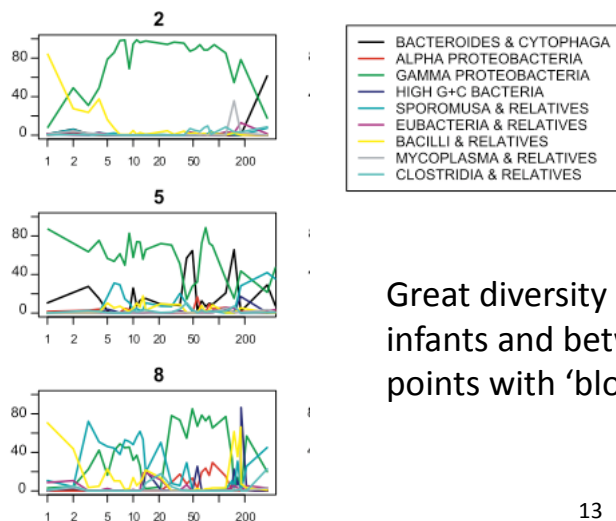
- Fingerprinting: cheapest, but very limited (Anderson and Cairney, Envir Microbiol 2004)
- PhyloChip or GeoChip: like microarray,
 - will be powerful to assess changes in diversity (when predominate species enumerated) but like all Chips will never find UNIQUE species (Wilson Appl Environ Microbiol 2002 and He ISME J 2007)
- Sequencing: taxonomic classification and function, dynamic range and compare multiple complex samples.

For a SMALL study, SEQUENCE is limiting;
 For a LARGE study, BIOINFORMATICS is limiting.

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PhyloChip to examine intestinal microbiota in first year of life

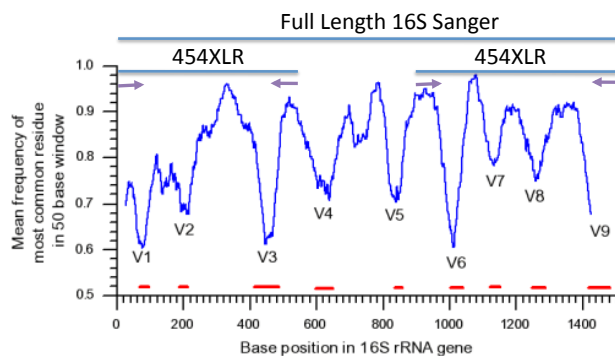
Palmer, Relman, Brown 2007 PLOS Bio



Great diversity between infants and between time points with 'blooms'

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16S Bacterial rRNA gene conserved, variable and hypervariable regions. Primers put into conserved regions, phylogeny determined by variable regions, 'species' by hypervariable regions.



PRIMERS SIGNIFICANTLY DETERMINE MICROBIAL DIVERSITY RECOVERED. CAN NOT A PRIORI COMPARE YOUR DATASET TO SOMEONE ELSE'S IF DIFFERENT PRIMER OR AMPLIFICATION CONDITIONS WERE USED

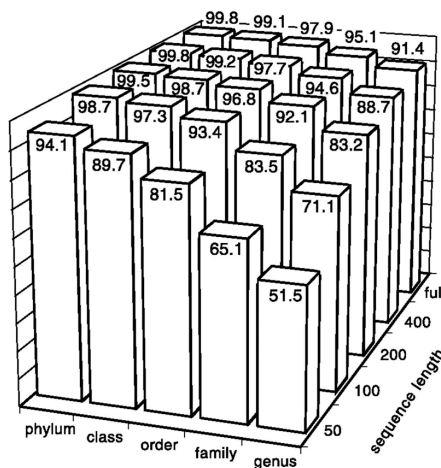
14

How many reads do you need? Depends on site diversity (slide 34,35) and taxonomic aim of study

- Sanger: Full-length 1.6 kb gives you a match to a cultured isolate, 384 sequences/sample
- 454/Roche: 400 bp V1-V3 or V6-V9 region, allows you to assign to genera, 3,000 reads/sample
- Illumina: 100 bp tags (2x150 bp on MiSeq) identify bacterial genera, not species (and great for whole genome bacterial sequencing)

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FIG. 1. Overall classification accuracy by query size (exhaustive leave-one-out testing using the Bergey corpus). Numbers are percentages of tests correctly classified.



Applied and Environmental Microbiology, August 2007, p. 5261-5267, Vol. 73, No. 16
Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial
Taxonomy · Qiong Wang,¹ George M. Garrity,^{1,2} James M. Tiedje,^{1,2} and James R. Cole¹
Also see: Liu, DeSantis, Andersen and Knight, NAR 2008

16

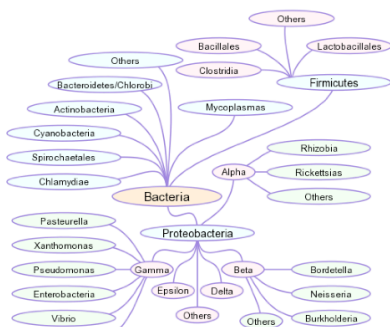
How to identify a bacterial sequence and align sequences?



BLAST with bacterial genomes (text table)

Enter your query sequence as Accession/GI or FASTA:

Select type of query and database or BLAST program
 Query: DNA Database: Genomic Both Blast-program: blastn MegaBlast
 You may change BLAST options
 Expect: 10 Filter: default Descriptions: 100 Alignments: 100



Matches MANY sequences.
 Maybe your sequence is
 previously UNCULTURED?

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RDP Database <http://rdp.cme.msu.edu/>

- RDP 10.18 consists of 920,643 aligned and annotated 16S rRNA sequences. Naïve Bayesian classifier based on Bergey's taxonomy. (Note: other taxonomies such as Euzebey and NCBI exist).
- Tools: RDP classifier, Seqmatch, Probematch

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 2007, p. 5261-5267
 0099-2240/07/508.00+0 doi:10.1128/AEM.00062-07
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Vol. 73, No. 16

Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy[†]

Qiong Wang,¹ George M. Garrity,^{1,2} James M. Tiedje,^{1,2} and James R. Cole^{1*}

RDHP HOME | ABOUT | ANNOUNCEMENTS | CITATION | CONTACTS | RESOURCES | RELATED SITES | TUTORIALS

RIBOSOMAL DATABASE PROJECT

BROWSERS | CLASSIFIER | LIBCOMPARE | SEQMATCH | PROBE MATCH | TREE BUILDER | PYRO | TAXOMATIC | SEQCART | ASSIGNGEN

RDP Release 10, Update 18 :: Jan 25, 2010 :: 1,358,426 16S rRNAs

The Ribosomal Database Project (RDP) provides ribosome related data and services to the scientific community, including online data analysis and aligned and annotated Bacterial and Archaeal small-subunit 16S rRNA sequences.

[Cite RDP's NAR article](#)

login

News

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RDP Pyrosequencing Pipeline

About the RDP's Pyrosequencing Pipeline

The Ribosomal Database Project's Pyrosequencing Pipeline aims to simplify the processing of large 16S rRNA sequence libraries obtained through pyrosequencing. This site processes and converts the data to formats suitable for common ecological and statistical packages such as SPADE, EstimateS, and R.

Data Processing Steps:

- **Pipeline Initial Process** - sort and trim the raw reads, filter low quality sequences.
- **Aligner** - align sequences using the fast, secondary-structure aware Infernal aligner.
- **Complete Linkage Clustering** - cluster sequences by the complete-linkage clustering method.

Formats for Common Programs:

- **SPADE Formatter** - make a SPADE compatible input format.
- **R Formatter** - make a R compatible input format.
- **EstimateS Formatter** - make an EstimateS compatible input format. Can also be used with PAST.
- **Mothur: Column Distance Matrix** - create a column distance matrix compatible with Mothur.
- **Mothur: Phylip Distance Matrix** - create a matrix and sample group file compatible with Mothur's LIBSHUFF function.

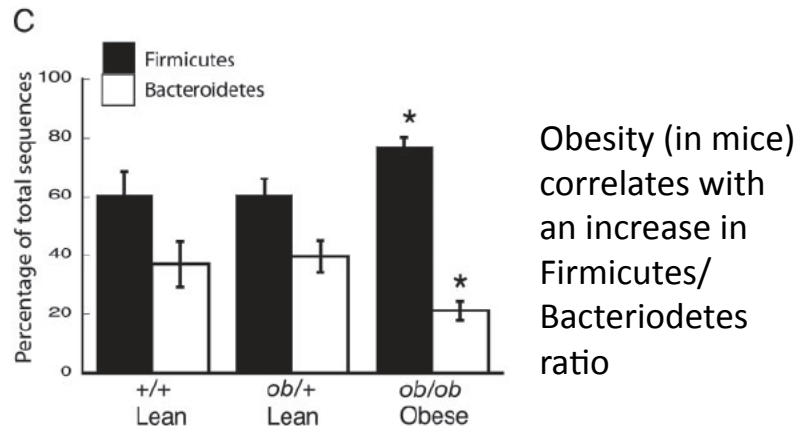
Analysis Tools:

- **Shannon & Chao1 Index** - calculate Shannon Index & Chao1 estimator from a single sample file.
- **Rarefaction** - calculate Rarefaction from a single sample file.
- **RDP Classifier** - assign 16S rRNA sequences to our taxonomical hierarchy.
- **RDP LibCompare** - compare two sequence libraries using the RDP Classifier [19](#)

Host Sequence Contamination

- Important when dealing with human-derived samples
- Ethically, projects should attempt to filter human subject sequences before submission to public databases
- This is actually harder than it sounds

Gordon: lean versus obese mice



Ley, ... Gordon PNAS 2005

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Also true in humans

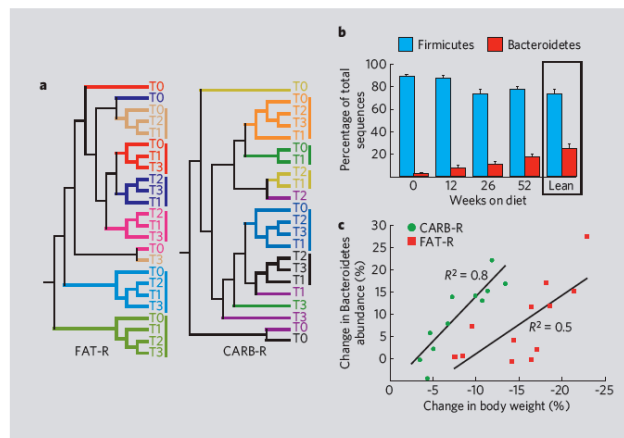


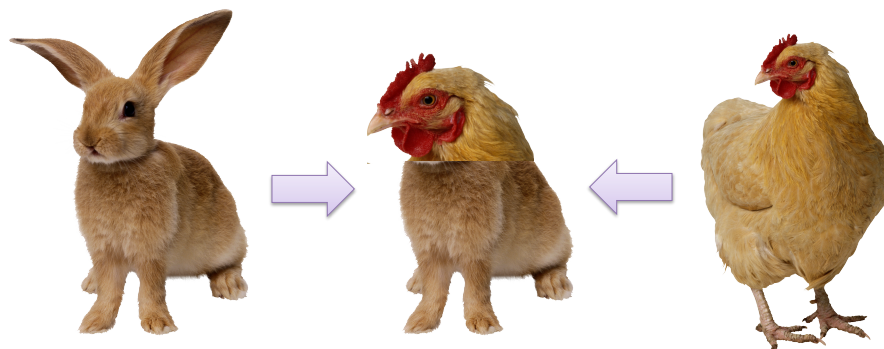
Figure 1 | Correlation between body-weight loss and gut microbial ecology. **a**, Clustering of 16S ribosomal RNA gene sequence libraries of faecal microbiota for each person (in different colours) and time point in diet therapy (T0, baseline; T1, 12 weeks; T2, 26 weeks; T3, 52 weeks) in the two diet-treatment groups (fat restricted, FAT-R; carbohydrate restricted, CARB-R), based on UniFrac analysis of the 18,348-sequence phylogenetic tree. **b**, Relative abundance of Bacteroidetes and Firmicutes. For each time point, values from all available samples were averaged (n was 11 or 12 per time point). Lean-

NATURE | Vol 444 | 21/28 December 2006

Ruth E. Ley, Peter J. Turnbaugh, Samuel Klein,
 Jeffrey I. Gordon

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Chimeras: PCR generated (template switching)

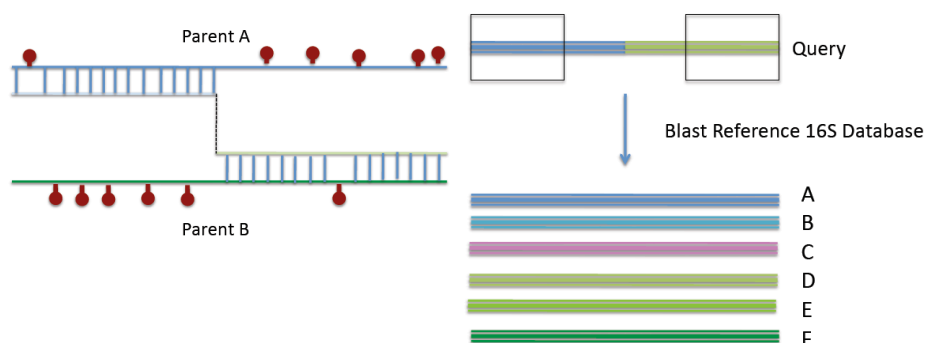


Evaluate Accuracy:

- True Positives (TP): artificial chimeras flagged
- False Positives (FP): reference (non-chimera) flagged

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How Do Chimeras Occur? Incomplete extension of PCR, Template Switching at Conserved Regions



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ChimeraSlayer Detection Program

<http://microbiomeutil.sourceforge.net>

Compatible with near-full length Sanger sequences and shorter 454-FLX sequences (~500 bp).

Given a candidate chimera query sequence, candidate parental sequences of a chimera are identified by a homology search. The ends of the query sequence are searched separately to identify candidate parental sequences. ... Those candidate parents identified by this alignment fitting procedure are tested in all pairwise combinations as potential parents of the putative chimeric query sequence using a modified Bellerophon-like algorithm.

Microbiome Utilities Portal of the Broad Institute



[Genome Res.](#) 2011 Mar;21(3):494-504.

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How to align sequences?

```

Query  225  ATTAGCTAGTTGGTAAGGTAACGGCT---TACCAAGGC-A-ACG-ATGCATAGCC-GACC  277
      |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct  212  ATTAGCTAGTAGGTGGGGTAACGGCTCCATCCCTAGGCGAGCCGAATCCTTAGCCTGGTC  271


Query  278  TGAGAGG-GTGATCGGCCACACTGGAAGTGAAG-ACACGGTCCAGACTCCTACGGGAGGCA  335
      |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||  |||
Sbjct  272  TGAGAGGAATGACCAGCCACACTGGGACTGAGAACACGGTCCAGACTCCTACGGGAGGCA  331
    
```

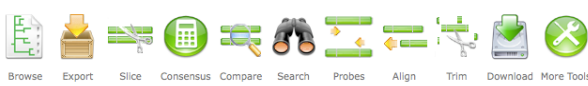
**WANT TO USE A PROGRAM THAT TAKES 16S
 STRUCTURE INTO CONSIDERATION. GAPS
 ARE MORE LIKELY IN LOOPS THAN STEMS**

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NAST and NASTier

fixed-width character alignment format


16S rRNA gene database and workbench compatible with ARB
greengenes.lbl.gov



W394-W399 Nucleic Acids Research, 2006, Vol. 34, Web Server issue
doi:10.1093/nar/gkl244

NAST: a multiple sequence alignment server for comparative analysis of 16S rRNA genes

T. Z. DeSantis^{1,4,*}, P. Hugenholtz², K. Keller^{5,4}, E. L. Brodie¹, N. Larsen³, Y. M. Piceno¹, R. Phan^{1,4} and G. L. Andersen^{1,4,*}


NAST-iEr


The NAST-iEr alignment utility ([download](#)) aligns a single raw nucleotide sequence against one or more NAST formatted sequences.

The alignment algorithm involves global dynamic programming alignment to a fixed template sequences without any end-gap penalty similar in principle to Pearson's align0 program with a fixed template sequence containing arbitrary gap positions.

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Silva Database (ARB): <http://www.arb-silva.de/> Build a Phylogenetic Tree and Calculate Branch Length


comprehensive ribosomal RNA databases



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SILVA

Welcome to the SILVA rRNA database project

A comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data, free for academic use.

SILVA provides comprehensive, quality checked and regularly updated databases of aligned small (16S/18S, 5S) and large subunit (23S/28S, LSU) ribosomal RNA (rRNA) sequences for all three domains of life (*Bacteria*, *Archaea* and *Eukarya*).

SILVA are the official databases of the software package ARB.

For more background information - [Click here](#)

News

Latest News

12.06.09
EMBL/SILVA release 99 skipped
Our next release will be EMBL/SILVA 100, expected for July/August. [\[more\]](#)

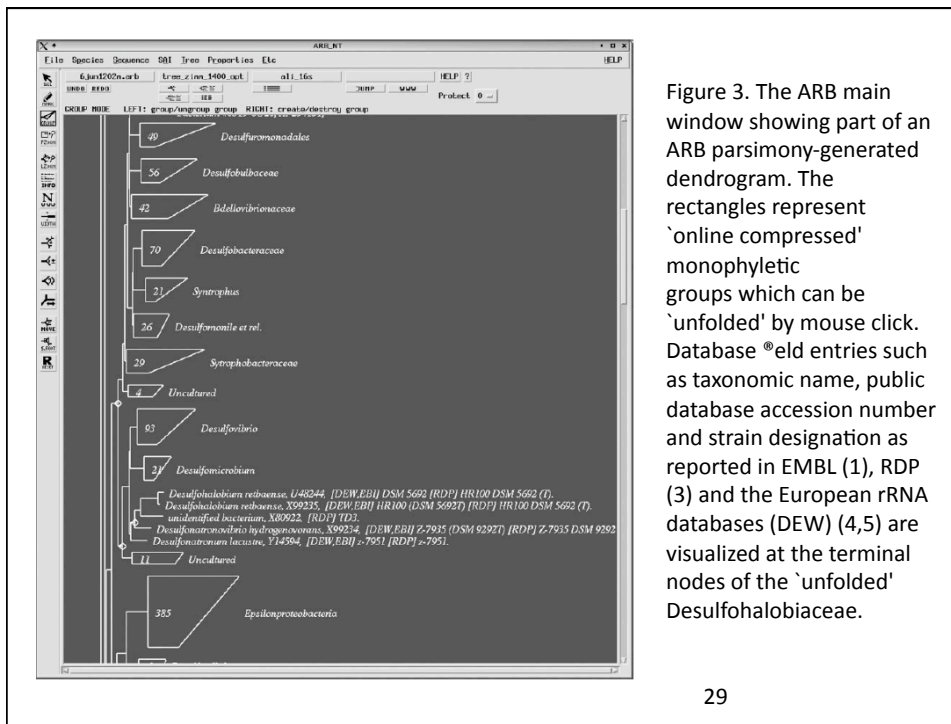
09.06.09
Next regular ARB/SILVA workshop already in August 2009!
Announcement of the the next regular ARB/SILVA workshop on short notice! It will be held August 18 - 21, 2009 in Bremen, Germany. [\[more\]](#)

Pruesse, E., C. Quast, K. Knittel, B. Fuchs, W. Ludwig, J. Peplies, and F. O. Glöckner.
SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB.
[Nuc. Acids Res. 2007; Vol. 35, No. 21, p. 7188-7196](#) Nucleic Acids Research, 2004, Vol. 32, No. 4 1363-1371
DOI: 10.1093/nar/gkh293

ARB: a software environment for sequence data

Wolfgang Ludwig¹, Oliver Strunk, Ralf Westram, Lothar Richter, Harald Meier¹, Yadhukumar, Arno Buchner, Tina Lai, Susanne Steppi, Gangolf Jobb¹, Wolfram Förster¹, Igor Brettske, Stefan Gerber, Anton W. Ginhart¹, Oliver Gross, Silke Grumann¹, Stefan Hermann¹, Ralf Jost¹, Andreas König¹, Thomas Lies¹, Ralph Lüßmann¹, Michael May¹, Björn Nonhoff¹, Boris Reichel¹, Robert Strehlow¹, Alexandros Stamatakis¹, Norbert Stuckmann¹, Alexander Vilbig¹, Michael Lenke¹, Thomas Ludwig², Arndt Bode¹ and Karl-Heinz Schleifer

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Defining Taxonomic Groups by sequence similarity: DOTUR, SONS and MOTHUR <http://www.mothur.org>

mothur

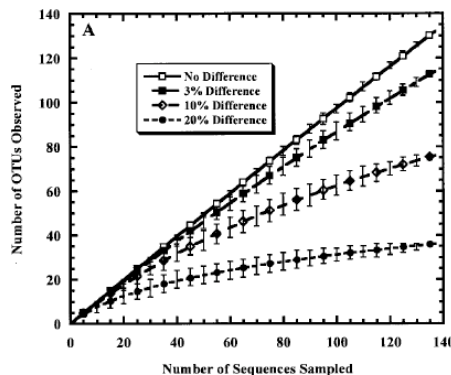
Download **Wiki** **Forum** **facebook**

Welcome to the website for the mothur project, initiated by [Dr. Patrick Schloss](#) and his software development team in the [Department of Microbiology & Immunology](#) at [The University of Michigan](#). This project seeks to develop a single piece of open-source, expandable software to fill the bioinformatics needs of the microbial ecology community. In February 2009 we released the first version of mothur, which had accelerated versions of the popular DOTUR and SONS programs. Since then we have added the functionality of a number of other popular tools including s-libshuff, TreeClimber (i.e. the parsimony test), UniFrac, distance calculation, visualization tools, a NAST-based aligner, and many other features. If you would

OTU: Operational Taxonomic Unit

Cluster Sequences Based on Furthest Joining Method; i.e. Every sequence is at most X% different from every other sequence in the group

% identity within group determines the number of OTUs produced. This should be done on the TOTAL dataset. Most experiments classify at the 97% or 99% identity.








APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 2005, p. 1501-1506
 0099-2240/05/\$08.00+0 doi:10.1128/AEM.71.3.1501-1506.2005
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Introducing DOTUR, a Computer Program for Defining Operational Taxonomic Units and Estimating Species Richness

Patrick D. Schloss and Jo Handelsman*

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Comparing Bacterial Diversity: Community Membership & Structure

	Grp A	Grp B
	60	50
	34	50
	2	0
	2	0
	2	0

Community Membership

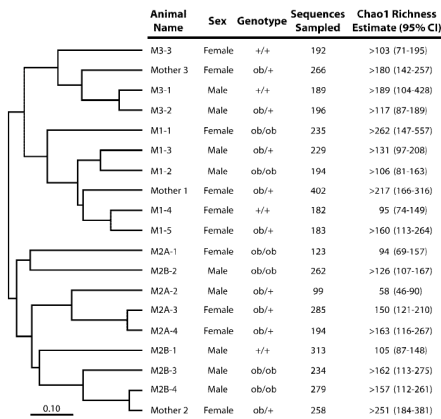
(Categories of fruit in common)
 $= 2/5 = 0.4$

Community Structure

(Pieces of fruit in common)
 $= \sim 0.9$

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Community Membership: Pups are most like their mothers



APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2006, p. 6773-6779
 0099-2240/06/\$08.00+0 doi:10.1128/AEM.00474-06
 Copyright © 2006, American Society for Microbiology. All Rights Reserved.

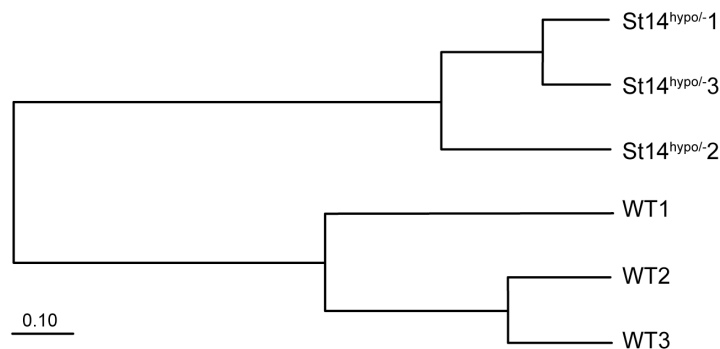
Vol. 72, No. 10

Introducing SONS, a Tool for Operational Taxonomic Unit-Based
 Comparisons of Microbial Community Memberships and Structures

Patrick D. Schloss† and Jo Handelsman*

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Community Structure: Pups cluster according to genotype



Scharschmidt et al. JID 2009

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UniFrac: Unique Fraction Metric

- Measures fraction of branch length in a tree that is unique to a community
- Weighted or unweighted for abundance
- Can be used with multivariate statistical methods (UPGMA and PCA) for visualization
- Calculate parsimonious changes to obtain p value

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 2005, p. 8228–8235
0099-2240/05/\$08.00+0 doi:10.1128/AEM.71.12.8228–8235.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Vol. 71, No. 12

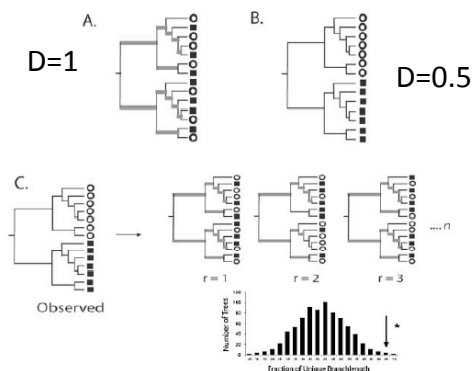
UniFrac: a New Phylogenetic Method for Comparing Microbial Communities

Catherine Lozupone¹ and Rob Knight^{2*}

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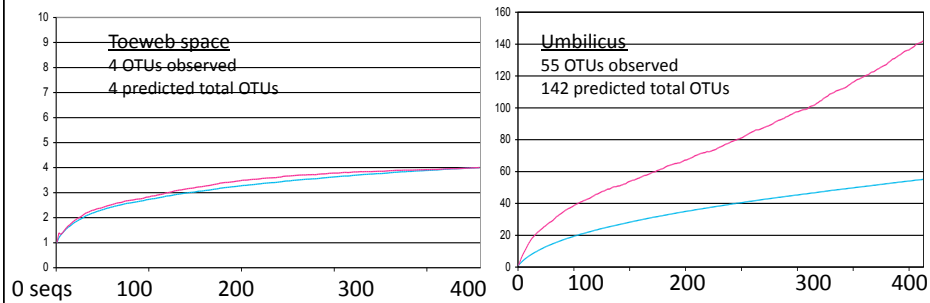
UniFrac allows you to:

1. Determine if the environments in the input phylogenetic tree have significantly different microbial communities.
2. Determine if community differences are concentrated within particular lineages of the phylogenetic tree.



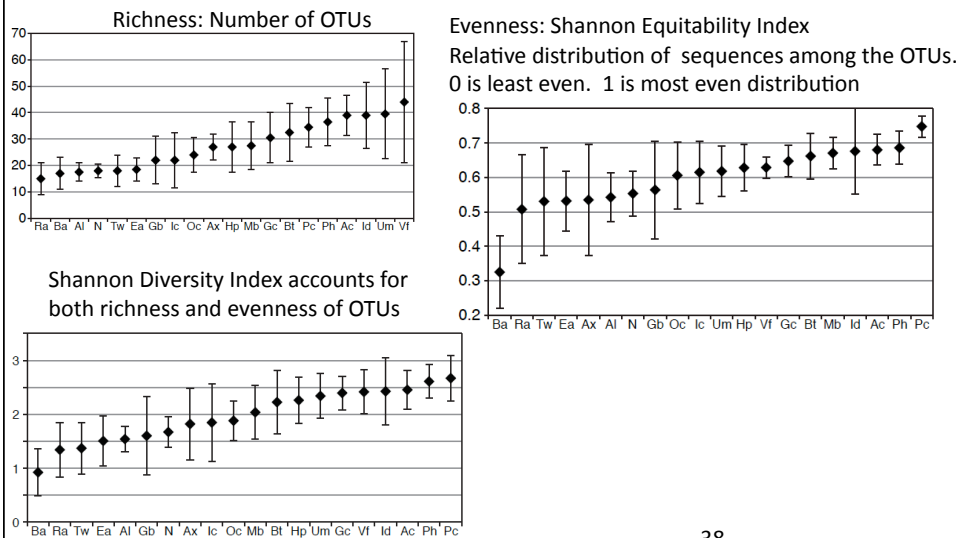
36

How much diversity is there in the population? Have you sequenced enough to capture the diversity? Chao1 rarefaction curves



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Richness, evenness, diversity: Shannon and Simpson diversity



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If you are using 454 sequences,
 consider VAMPS to form OTUs
<http://vamps.mbl.edu/>

Revised: October 9th, 2009.

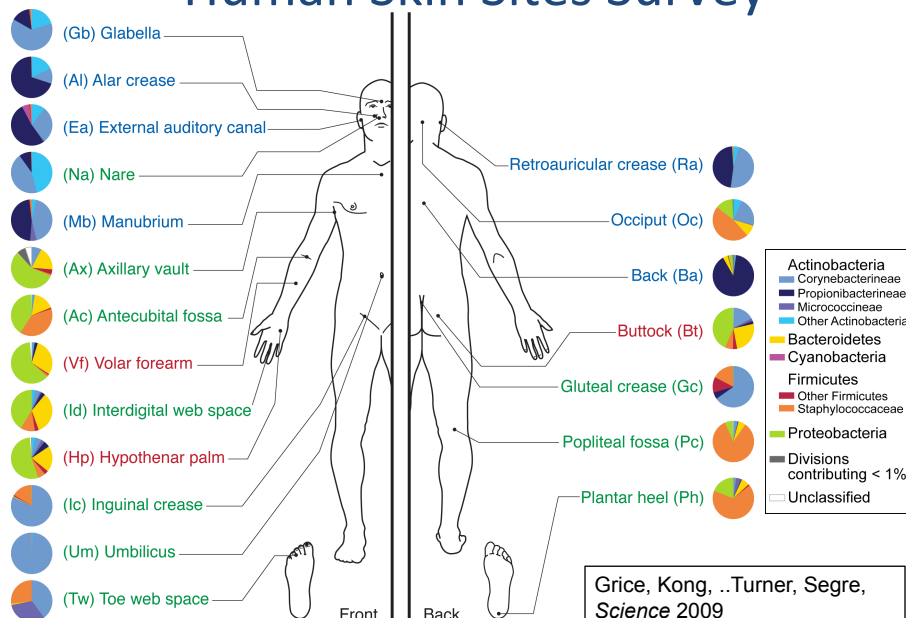
VAMPS The Visualization and Analysis of Microbial Population Structures

VAMPS is an integrated collection of tools for researchers to visualize and analyze data for microbial population structures and distributions. For more information on the VAMPS project, visit our [VAMPS Overview](#) page.

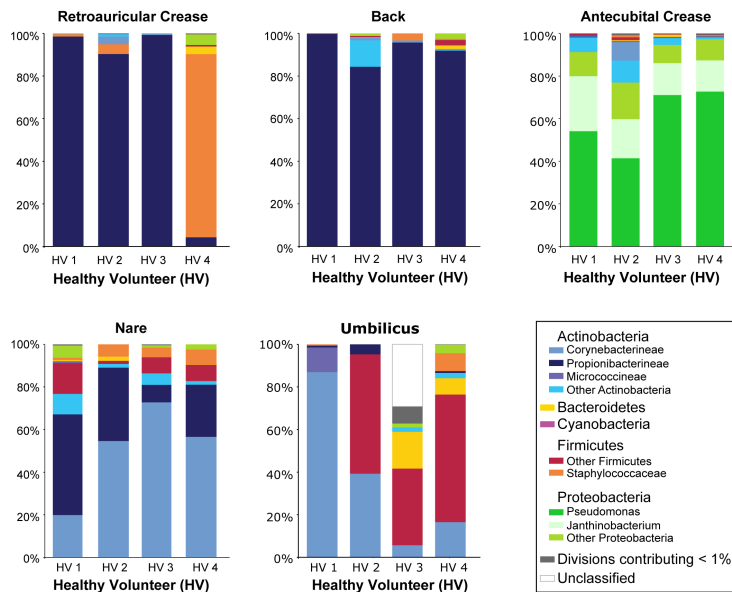
There are two essential elements to VAMPS:

- **Visualization and Analysis - *Community Visualization*** including heat maps and comparative pie charts, as well as diversity estimates, rarefaction curves and spreadsheet-style output provide researchers with analytical tools for assessing individual microbial populations, based on either taxonomic assignments or independently-derived operational taxonomic units (OTUs).
- **Data Ramp** - Researchers who want to use the VAMPS tools with their own data can enter their sequence or taxonomy data to the VAMPS website and merge it with the existing shared datasets for individual or comparative analyses. Researchers will be given a user name and password, and their data will be visible only to registered users of their choice.

Human Skin Sites Survey



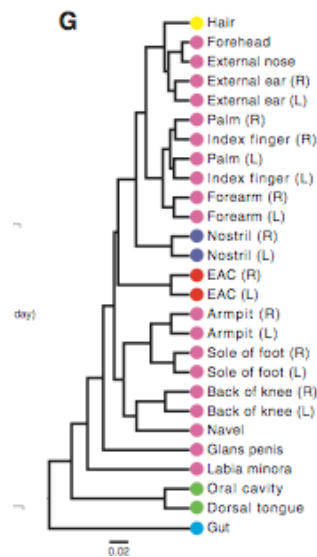
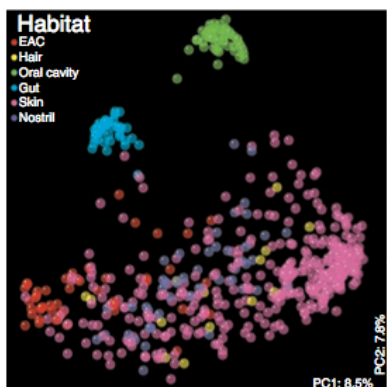
Sub-site inter-personal variation



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Bacterial Community Variation in Human Body Habitats Across Space and Time

Elizabeth K. Costello,¹ Christian L. Lauber,² Micah Hamady,³ Noah Fierer,^{2,4} Jeffrey I. Gordon,⁷ Rob Knight^{1,4*}



16S rRNA sequences cluster according to body site rather than individual

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**Microbial community profiling for human microbiome projects:
Tools, techniques, and challenges**

Micah Hamady and Rob Knight


Genome Res. 2009 19: 1141-1152 originally published online April 21, 2009
Access the most recent version at doi:[10.1101/gr.085464.108](https://doi.org/10.1101/gr.085464.108)

INSIGHT FEATURE NATURE Vol 449 | 18 October 2007 | doi:10.1038/nature06244

The Human Microbiome Project

Peter J. Turnbaugh, Ruth E. Ley, Micah Hamady, Claire M. Fraser-Liggett, Rob Knight & Jeffrey I. Gordon

A strategy to understand the microbial components of the human genetic and metabolic landscape and how they contribute to normal physiology and predisposition to disease.



The NIH Human Microbiome Project

The NIH HMP Working Group, Jane Peterson, Susan Garges, et al.

Genome Res. 2009 19: 2317-2323 originally published online October 9, 2009
Access the most recent version at doi:[10.1101/gr.096651.109](https://doi.org/10.1101/gr.096651.109)

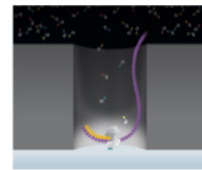
43

Fungal Diversity

- Similar strategy can be used to classify the 18S rRNA or the intervening sequence (ITS) of fungi

Topic 2: Sequencing Bacterial Genomes

- Roche/454 generates 1, 250,000 reads of ~400+ bp (5 Gbp).
- Illumina generates shorter reads (100+ bp) but generate more sequence data per run for cheaper price/base pair.



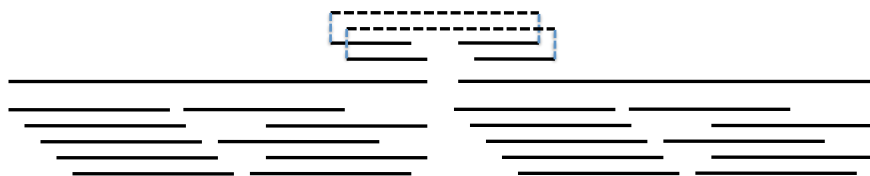
Roche/454-XLR <i>Pyrosequencing</i>	Illumina Gaii, HiSeq, MiSeq <i>Sequencing by synthesis</i>
<ul style="list-style-type: none"> •Emulsion PCR •400-bp read (avg) 	<ul style="list-style-type: none"> •Bridge PCR •100+-bp read, paired end



45

* Manufacturer specifications from Holt and Jones, Genome Research 18:839-46 (2008)

Paired end reads (8 kb inserts) scaffold contigs



Unidirectional reads form contigs

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Assemblers (*de novo*)

- Phrap
- Newbler (454)
- Velvet
- ALL-PATHS, SSAKE, VCAKE, SHARCGS, Edena, AMOS
- CAP3/PCAP



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Newbler (gsAssembler)

- Works in base-space and flow-space
- Overlap-Layout-Consensus method
- Homopolymer correction
- 1. Identify pairwise read overlaps
- 2. Build graph
 - 1. Nodes are contiguous alignments
 - 2. Edges connect nodes with branch points representing repeat boundaries
- 3. Detangle
- 4. Build consensus alignment

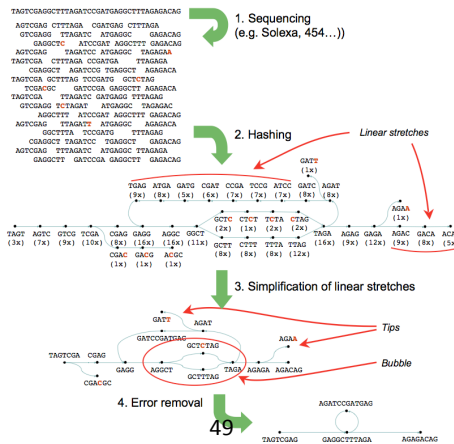
48

Velvet (Zerbino and Birney, 2008)

- Works in base-space and color-space
- Good for small genomes
- Agnostic of read length

1. Construct k-mer hash
2. Build De Bruijn graph
3. Simplify graph
4. Resolve

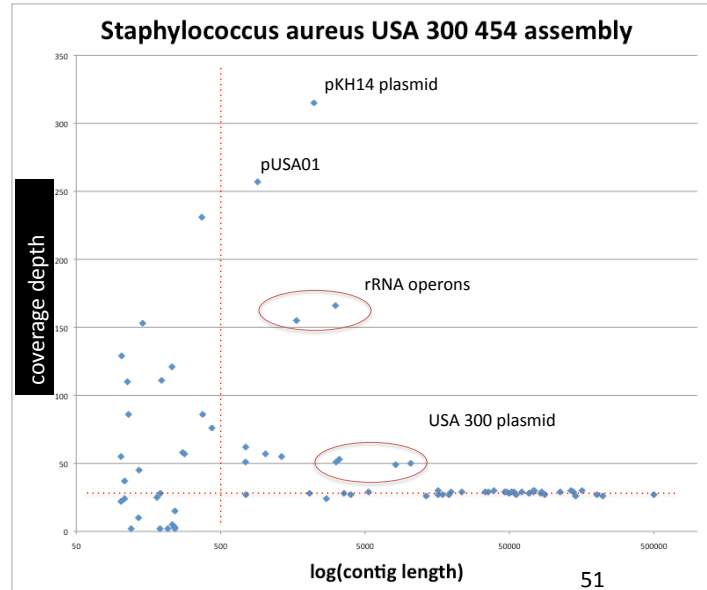
1. Tips
2. Bubbles



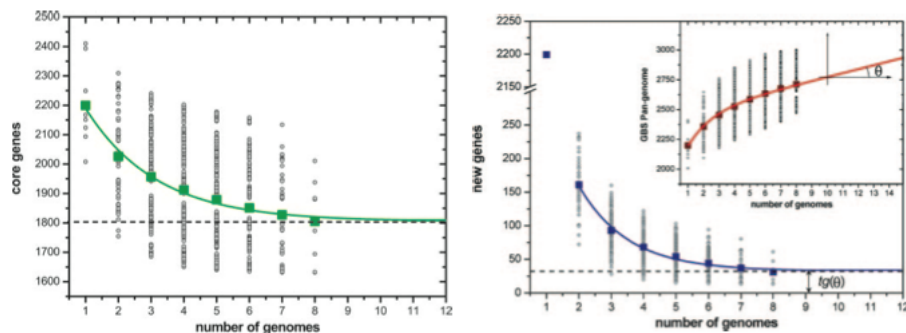
Evaluating Assemblies

- Coverage is a measure of how deeply a region has been sequenced
- The Lander-Waterman model predicts 8-10 fold coverage is needed to minimize the number of contigs for a 1 Mbp genome
- The N50 size is the point at which 50% of bases are in contigs this size or greater

Evaluating High Coverage Contigs



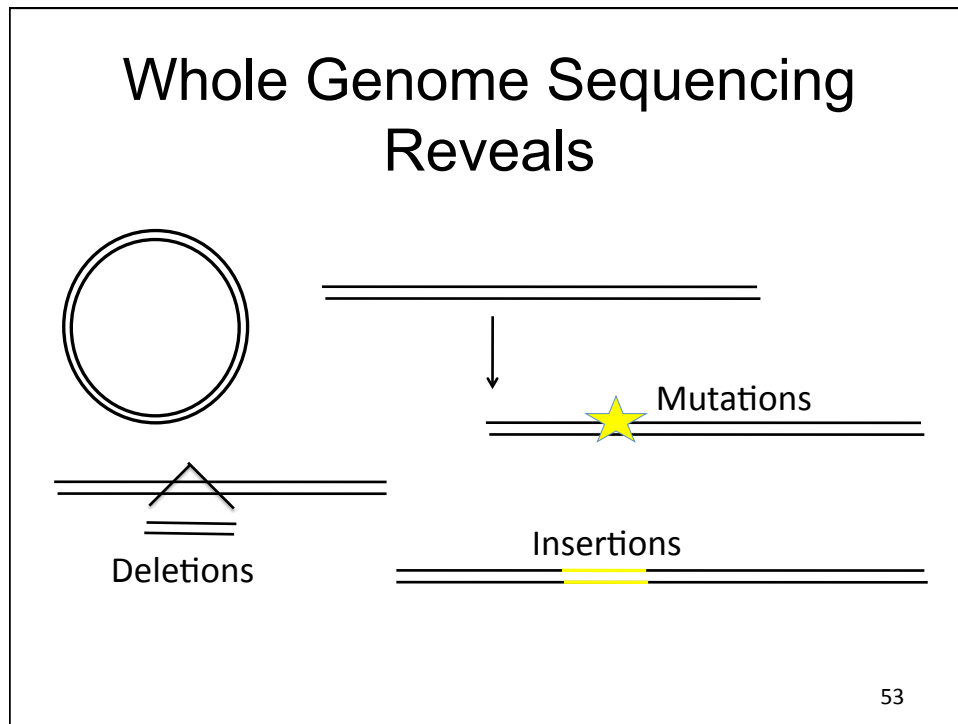
Is there a reference genome? Is it a fixed genome? Bacteria exchange information with horizontal gene transfer



Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae*: Implications for the microbial "pan-genome"

Hervé Tettelin^{1,2}, Vega Masignani^{1,2}, Michael J. Cieslewicz^{1,2,3,4}, Claudio Donati¹, Duccio Medini¹, Naomi L. Ward^{1,2}, Samuel V. Anguolli¹, Jonathan Crabtree¹, Amanda L. Jones¹, A. Scott Durkin¹, Robert T. DeBoy¹, Tanja M. Davidsen¹, Marimosa Mora¹, Maria Scarselli¹, Immaculada Margarit y Ros¹, Jeremy D. Peterson¹, Christopher R. Hauser¹, Jaideep P. Sundaram¹, William C. Nelson¹, Ramana Madupati¹, Lauren M. Brinkac¹, Robert J. Dodson¹, Mary J. Rosovitz¹, Steven A. Sullivan¹, Sean C. Daugherty¹, Daniel H. Haft¹, Jeremy Selengut¹, Michelle L. Gwinn¹, Liwei Zhou¹, Nikhat Zafar¹, Hoda Khouri¹, Diana Radune¹, George Dimitrov¹, Kisha Watkins¹, Kevin J. B. O'Connor¹, Shannon Smith¹, Teresa R. Utterback¹, Owen White¹, Craig E. Rubens¹, Guido Grandi¹, Lawrence C. Madoff¹, Dennis L. Kasper¹, John L. Telford¹, Michael R. Wessels^{1,2}, Rino Rappuoli^{1,2}, and Claire M. Fraser^{1,2,3,4,5,6}

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TOPIC 3. Identifying Novel Virus: Transplant Associated Arenavirus (also SARS, Merkel cell carcinoma)

Resequencing the human genome to identify viral associated disease is getting EASIER and CHEAPER. Once you find them once, finding them again is PCR-based. Very cheap and easy!



Three organ-transplant recipients died with a month of the transplant

Table 1. Characteristics of the Organ-Transplant Recipients.

Recipient No.	Age yr	Diagnosis	Organ Transplanted	Clinical Course	Interval between Transplantation and Death days
1	63	End-stage renal failure due to polycystic kidney disease	Kidney	Fever, sepsis, encephalopathy, acute tubular necrosis, graft rejection, radiographic evidence of chest infiltrates	36
2	64	Decompensated cirrhosis and hepatocellular cancer due to hepatitis C infection	Liver	Fever, confusion, encephalopathy with myoclonus, chest infiltrates	30
3	44	End-stage renal failure due to polycystic kidney disease	Kidney	Fever, graft rejection, intraabdominal hematomas and effusion, transplant nephrectomy, encephalopathic illness	29

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The needle(s) in the haystack...

103,632 reads from 454 FLX lane
 (length= 45-337 nt, mean=162.)
 94,043 reads after filtering

BLASTN largely uninformative

BLASTX analysis identified 14 fragments that were consistent with Old World arenaviruses (12 S-segment and 2 L-segment).

PCR using primers based on the pyrosequencing reads and consensus information from sequenced Arenaviruses

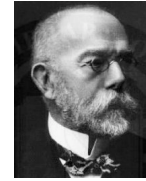
Table 2. Nucleotide and Amino Acid Homologies of the New Arenavirus to Other Arenaviruses.^a

Gene	Accession No.	LCMV Strain	Homology	
			Amino Acid	Nucleotide
GPC	AB261990	M2	94	86
NP	AB261990	M2	97	87
L	DQ286932	Marseille 12	82	79
Z	DQ286932	Marseille 12	79	72

^a LCMV denotes lymphocytic choriomeningitis virus.

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Sequencing is just the start... Koch's postulates



- The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy animals.
- The microorganism must be isolated from a diseased organism and grown in pure culture.
- The cultured microorganism should cause disease when introduced into a healthy organism.
- The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

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TOPIC 4. METAGENOMICS: DNA sequence from multiple organisms

Fungal, Bacterial, Viral, Archaeal DNA all together
(with human DNA).

Very Complex mixture and very complex computationally.

Vol 455|25 September 2008

nature

MICROBIOLOGY

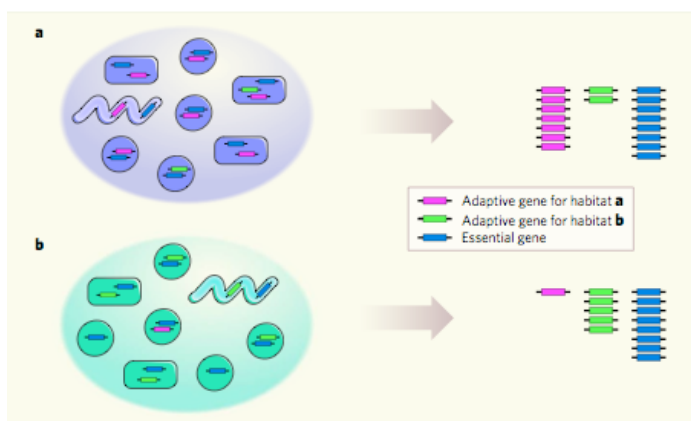
Metagenomics

Philip Hugenholtz and Gene W. Tyson

Ten years after the term metagenomics was coined, the approach continues to gather momentum. This culture-independent, molecular way of analysing environmental samples of cohabiting microbial populations has opened up fresh perspectives on microbiology.

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Metagenomics: types of bacteria similar between 2 populations, but pink genes enriched in top population



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A core gut microbiome in obese and lean twins

Peter J. Turnbaugh¹, Micah Hamady³, Tanya Yatsunenok¹, Brandi L. Cantarel⁵, Alexis Duncan², Ruth E. Ley¹, Mitchell L. Sogin⁶, William J. Jones⁷, Bruce A. Roe⁸, Jason P. Affourtit⁹, Michael Egholm⁹, Bernard Henrissat⁵, Andrew C. Heath², Rob Knight⁴ & Jeffrey I. Gordon¹

A human gut microbial gene catalogue established by metagenomic sequencing

Junjie Qin^{1*}, Ruiqiang Li^{1*}, Jeroen Raes^{2,3}, Manimozhayan Arumugam⁷, Kristoffer Solvsten Burgdorf⁴, Chaysavanh Manichanh⁵, Trine Nielsen⁶, Nicolas Pons⁶, Florence Levenez⁶, Takuji Yamada⁷, Daniel R. Mende⁷, Junhua Li^{1,7}, Junming Xu¹, Shaochuan Li¹, Dongfang Li^{1,8}, Jianjun Cao¹, Bo Wang¹, Huiqing Liang¹, Huisong Zheng¹, Yinlong Xie^{1,7}, Julien Tap⁹, Patricia Lepage⁹, Marcelo Bertalan⁹, Jean-Michel Batto⁹, Torben Hansen⁹, Denis Le Paslier¹⁰, Allan Linneberg¹¹, H. Bjørn Nielsen⁹, Eric Pelletier¹⁰, Pierre Renault⁹, Thomas Sicheritz-Ponten⁹, Keith Turner¹², Hongmei Zhu¹, Chang Yu¹, Shengting Li¹, Min Jian¹, Yan Zhou¹, Yingrui Li¹, Xiuqing Zhang¹, Songgang Li¹, Nan Qin¹, Huanming Yang¹, Jian Wang¹, Søren Brunak⁹, Joel Doré⁹, Francisco Guarner⁹, Karsten Kristiansen¹³, Oluf Pedersen^{4,14}, Julian Parkhill¹², Jean Weissenbach¹⁰, MetaHIT Consortium¹, Peer Bork², S. Dusko Ehrlich⁶ & Jun Wang^{1,15}

The human body contains about ten times as many microbes as human cells, and most of them live in the gut. The new study, published today in *Nature*¹, shows that, between them, those microbes contain 3.3 million genes, dwarfing the human genome's 23,000. The authors also find that the bacterial species in one person's gut are not as different from those of others as had been expected.

Tools do not yet exist to catalogue and comprehend metagenomic complexity

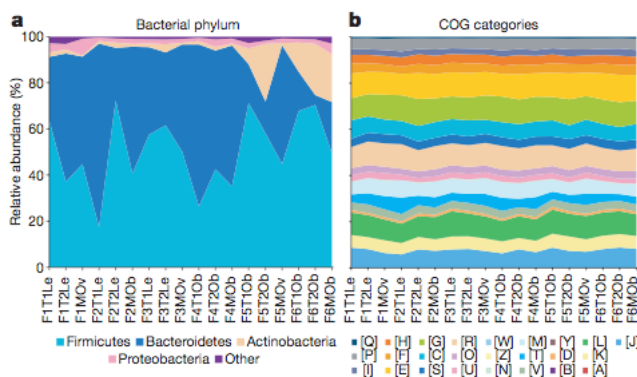


Figure 3 | Comparison of taxonomic and functional variations in the human gut microbiome. a, Relative abundance of major phyla across 18 faecal microbiomes from monozygotic twins and their mothers, based on BLASTX