Part II

BACTERIAL DIVERSITY, TAXONOMY, PHYLOGENY & TOOLS

Prokaryotic Nomenclature

- Bacterial and Archaeal Names are covered by an official set of rules:
 - "The International Code of Nomenclature of Prokaryotes"
 - Covers ranks from Class to Subspecies.
 - Ranks of Kingdom and Phylum (Domain) are not covered by the code.

Synonymy

9612 Species Names But Only 8062 Species?

New Combination (taxonomic change)

Rhizobium meliloti -> Ensifer meliloti

Homotypic Synonym (same type strain) *Aeromonas caviae = Aeromonas punctata*

Heterotypic Synonym (different type strain)

Wautersia eutropha => Cupriavidis necator



LPSN

List of Prokaryotic names with Standing in Nomenclature

Formerly List of Bacterial names with Standing in Nomenclature (LBSN)

J.P. EUZÉBY SBSV

Author's e-mail

Last full update: August 06, 2009

Minor changes since the last full update: August 08, 2009

URL: www.bacterio.net



Search

Taxonomic categories and changes covered by the Rules of the Code

- Genera and suprageneric taxa: List A-C List D-L List M-R List S-Z
- Suprageneric taxa
- Genera
- Genera of the domain (or empire) of Archaea (or Archaeobacteria)
- Genera of the domain (or empire) of Bacteria (or Eubacteria)
- Approved Lists of Bacterial Names
- Names validly published by announcement in Validation Lists
- Basonyms, new combinations (comb. nov.), nomina nova (nom. nov.)

Taxonomic categories and changes not covered by the Rules of the Code

- Candidatus
- Taxa above the rank of class
- Some prokaryotic names without standing in nomenclature
- Lists of Changes in Taxonomic Opinion

Dalaman January (ID Darkland D. T. Jall)

Search



Search an alphabetical list of bacterial names A - Z

A complete list of the names can be downloaded from our FTP server: bactname.pdf (PDF file), bactname.exe (self-extracting file for MS-DOS systems) or names.txt (uncompressed ASCII text file). To import the list of names into a database or a spreadsheet programme, you can download bactname.zip (compressed Excel file).

Other useful information on bacterial or general nomenclature can be found at:

- J. P. Euzéby, Ecole Nationale Vétérinaire de Toulouse, France
- TOBA Taxonomic Outline of the Bacteria and Archaea
- CCUG, Sweden

>> Catalogue

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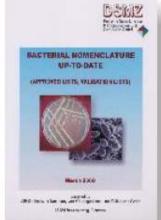
>> Bacterial Nomenclature

>> Schulgeeignete Kulturen

>> Prices

>> Deposit

Species 2000 CheckList



"Bacterial Nomenclature up-to-date" is based on the work of Norbert Weiss, who maintained the database until his retirement in February 2003. The database is based on those names which are validly published according to the Bacteriological Code. The present database is maintained under the supervision of Dorothea Gleim and Manfred Kracht, who may be consulted on technical aspects (database resp. online access). Queries relating to nomenclature or taxonomic interpretation may be addressed to Brian J. Tindall.

New: For more than 1100 species of the Actinobacteria very valuable additional information is available: in the manual Compendium of Actinomycetales by Joachim Wink most of the known



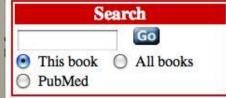
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International Code of Nomenclature of Bacteria

(1990 Revision)

Short Contents | Full Contents

Other books @ NCBI



International Code of Nomenclature of Bacteria (1990 Revision) - 1. General Considerations

General Consideration 6

This Code is divided into Principles, Rules, and Recommendations.

- 1. The *Principles* (Chapter 2) form the basis of the Code, and the Rules and Recommendations are derived from them.
- 2. The Rules (Chapter 3) are designed to make effective the Principles, to put the nomenclature of the past in order, and to provide for the nomenclature of the future.
- 3. The Recommendations (Chapter 3) deal with subsidiary points and are appended to the Rules which they supplement. Recommendations do not have the force of Rules; they are intended to be guides to desirable practice in the future. Names contrary to a Recommendation cannot be rejected for this reason.
- 4. Provisions for emendations of Rules, for special exceptions to Rules, and for interpretation of the Rules in doubtful cases have been made by the establishment of the International Committee on Systematic Bacteriology (ICSB) and its Judicial Commission, which acts on behalf of the ICSB (see Rule 1b and Statutes of the International Committee on Systematic Bacteriology, pp. 137–158 of this volume). Opinions issued by the Judicial Commission become effective after receipt of ten or more favorable votes from Commissioners, but may be rescinded by the ICSB as provided in ICSB Statute 8c (2). The official journal of the ICSB is the International Journal of Systematic Bacteriology (IJSB), formerly the International Bulletin of Bacteriological Nomenclature and Taxonomy (IBBNT). (Some other journal could be specified by the ICSB if required. Such possible future specification is implicit in the use of

Navigation

About this book

1. General Considerations

General Consideration 1

General Consideration 2

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General Consideration 4

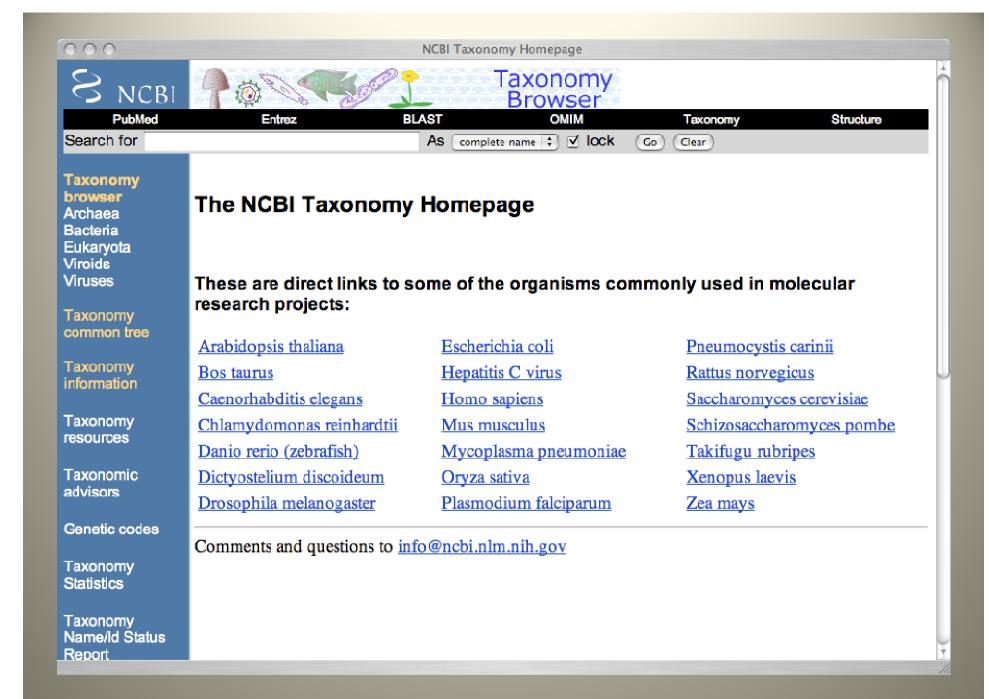
General Consideration 5

→ General Consideration 6 General Consideration 7

Prokaryotic Taxonomy

- There is no formal Prokaryotic taxonomy Taxonomy is a matter of opinion!
- Changes in opinion may require changes in nomenclature
 Rhizobium meliloti -> Ensifer meliloti
- Every taxon must be circumscribed

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The Taxonomic Outline of Bacteria and Archaea

OPEN JOURNAL HOME ABOUT LOG IN REGISTER SEARCH CURRENT **ARCHIVES** SYSTEMS ANNOUNCEMENTS SITE MAP Journal Help Home > TOBA Release 7.7 USER The Taxonomic Outline of Bacteria and Username Archaea Password Remember me Log In TOBA Release 7.7 JOURNAL CONTENT This release of the outline was prepared as part of a disusssion on sequencing the genomes Search. of the type strains of Bacteria and Archaea. Table of Contents Search The Outline Browse Introduction to the Taxonomic Oultine of Bacteria and Archaea PDF By Issue (TOBA) Release 7.7 Bv Author George M Garrity, Timothy G. Lilburn, James R. Cole, Scott By Title 1-5 H. Harrison, Jean Euzeby, Brian J Tindall Part 1 - The Archaea, Phyla Crenarchaeota and Euryarchaeota PDF FONT SIZE George M. Garrity, Timothy G. Lilburn, James R. Cole, 6-31 A A A Scott H. Harrison, Jean Euzeby, Brian J Tindall Part 2 - The Bacteria: Phyla Aquficae, Thermotogae, PDF INFORMATION Thermodesulfobacteria, Deinococcus-Thermus, Chrysiogenetes, Chloroflexi, Thermomicrobia, Nitrospira, Deferribacteres, For Readers Cyanobacteria, and Chlorobi

George M. Garrity, Timothy G. Lilburn, James R. Cole,

For Librarians

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Bergey's Manual Trust

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Bergey's Taxonomic Outlines

The taxonomic outlines for Volumes 3 and 4 of *Bergey's Manual* are available here. Please download the PDF files below.

- Volume 3
- Volume 4
- Volume 5 The Actinobacteria

Earlier releases of the complete taxonomic outline are available here:

- Release 1.0 (April 2001)
- Release 2.0 (January 2002)
- Release 3.0 (July 2002)
- Release 4.0 (October 2003)
- Release 5.0 (May 2004)

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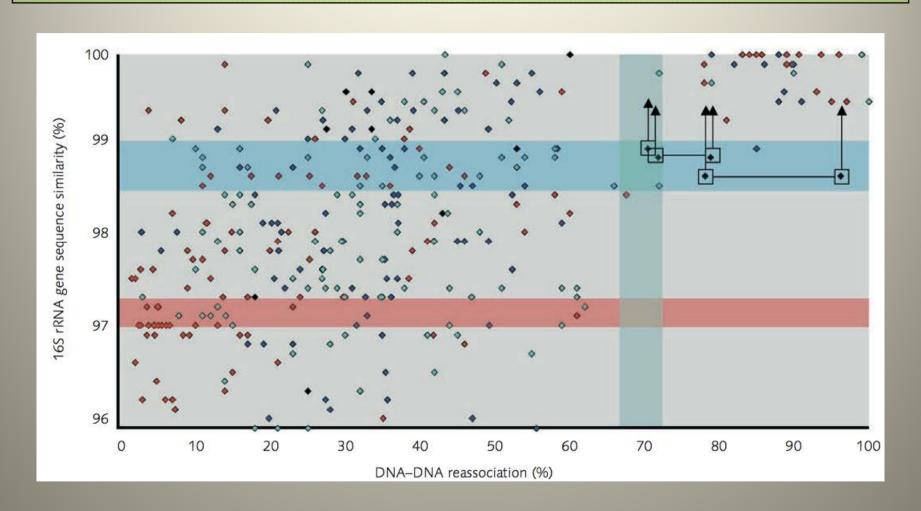
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This site last updated 11 August 2009

Bacterial Species

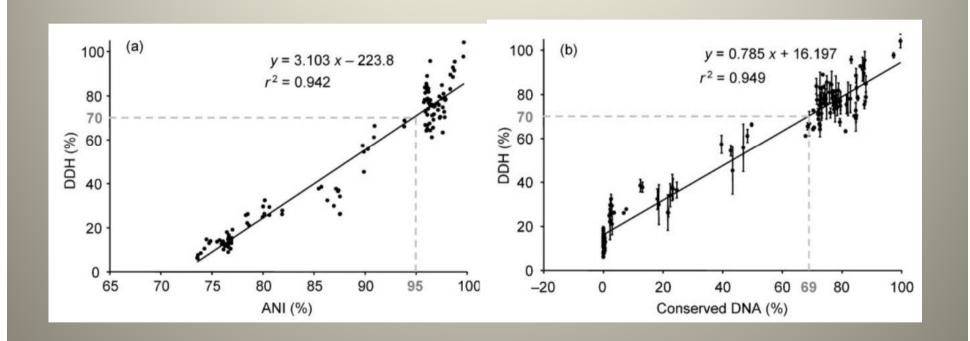
- One strain of a species is designated as the "type strain". Other closely related strains are of the same species.
- There is no completely objective method for species delimitation.
- The current accepted standard is 70% DNA-DNA hybridization (DDH)

DDH vs 16S Similarity



Stackebrandt, E. and J. Ebers. (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiology Today:152-155.

DDH vs Genome Similarity



Goris, J., Konstantinidis, K.T., Klappenbach, J.A., Coenye, T., Vandamme, P., Tiedje, J.M. (2007) DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. **IJSEM** 57:81–91. doi:10.1099/ijs.0.64483-0

Bacterial Species Definition

- The species concept in bacteria is not well defined.
- As an ad-hoc measure, 70% DHH has several limitations.
- Less than 98.5% 16S similarity indicates different species, but greater than 98.5% does not indicate the same species.
- ANI may be a good candidate to replace DHH as an ad-hoc species definition.

Nomenclatural References

- The International Code of Nomenclature of Prokaryotes: http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid =icnb
- List of Prokaryotic Names with Standing in Nomenclature http://www.bacterio.cict.fr/index.html
- Bacterial Nomenclature Up-to-Date <u>http://www.dsmz.de/microorganisms/bacterial</u> <u>nomenclature.php</u>

Taxonomy References

- NCBI Taxonomy http://www.ncbi.nlm.nih.gov/Taxonomy/
- TOBA http://www.taxonomicoutline.org/
- Bergey's Taxonomy <u>http://www.bergeys.org/outlines.html</u>

Phylogenetic Inference

Use simplest method that works.

Why rRNA?

- Universal
- No ortholog paralog problem
- No horizontal gene transfer
- Easy to amplify and sequence
- Large database

Jukes & Cantor

$$a = u dt$$

$$p = \frac{3}{4} (1 - e^{-\frac{4}{3}ut})$$

$$ut = -\frac{3}{4} \log_e (1 - \frac{4}{3}p)$$

(ut = evolutionary distance)

Substitution Models

- Jukes & Cantor (0 parameters)
 - All rates equal
- Kimura (2 parameters)
 - Transition & transversion different
- HKY (6 parameters)
 - Plus different frequency of four nucleotides
- Generalized time reversible
 - 8 parameters

Alignment Mask

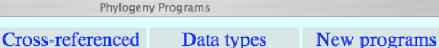
- Use to remove unstable regions from phylogenetic analysis
 - Inserts and deletes make homolog assigment difficult
- Rapidly changing regions add noise
- Should be reported in publication (but often are not)
- Alignment masks should be provided with alignment
- Can be created by using ad-hoc rules
 - Eg 50% residues in column

Phylogenetic Methods

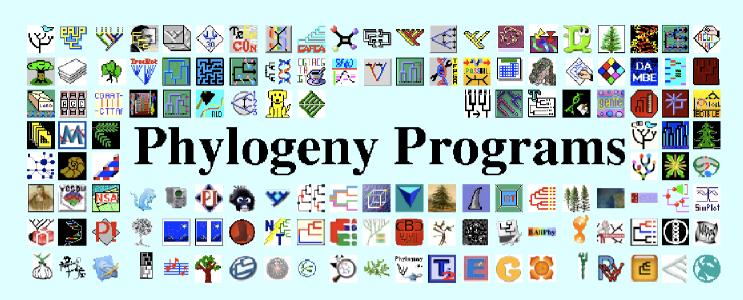
- UPGMA
 - Assumes clock
- Neighbor Joining
 - No clock, distance based
- Parsimony
 - Slow, used in other fields
- Maximum Likelihood
 - Slow, but fast converging, allows different rates
- Bayesian
 - Can be faster then maximum likelihood

Confidence Estimation

- Bootstrapping alignment columns
 - Estimates confidence for individual branch order
 - Sensitive to "unstable" sequenes
- Kishino-Hasegawa-Templeton test
 - Compares whole trees
 - Asks one is significantly better
 - Uses Maximum Likelihood



Submitting



Methods

By computer

Changes Waiting list Other lists Old programs Not listed ???

Here are 385 phylogeny packages and 52 <u>free servers</u>, all that I know about. It is an attempt to be completely comprehensive. I have not made any attempt to exclude programs that do not meet some standard of quality or importance. Updates to these pages are made roughly weekly. <u>Here</u> is a "waiting list" of new programs waiting to have their full entries constructed. Many of the programs in these pages are available on the web, and some of the older ones are also available from <u>ftp server machines</u>.

The programs listed below include both free and non-free ones; in some cases I do not know whether a program is free. I have listed as free those that I knew were free; for the others you have to ask their distributor. Usually when I say that a program is downloadable from a web site, this means that it is available free.



PHYLIP

PHYLIP version 3.68 was released on 13 August 2008. This is a "stable" bug-fix release. An "alpha" pre-release of PHYLIP 3.7, called 3.7a, will be released in a few months.

PHYLIP is a *free* package of programs for inferring phylogenies. It is distributed as source code, documentation files, and a number of different types of executables. These Web pages, by <u>Joe Felsenstein</u> of the <u>Department of Genome Sciences</u> and the <u>Department of Biology</u> at the <u>University of Washington</u>, contain information on **PHYLIP** and ways to transfer the executables, source code and documentation to your computer.

- A general description of PHYLIP.
- Programs in the PHYLIP package
- About the Executables
- About the Source code ... compiling it yourself
- The documentation web pages for PHYLIP can be read here
- Get me PHYLIP
- How to install PHYLIP
- · Frequently asked questions
- An excellent guide to using PHYLIP with molecular data is available here.
- PHYLIP on the web (HTML documentation, server services)
- Current and future versions of PHYLIP (including new features)
- Older versions of DHVI ID including version 2.5





Download MEGA 🖈 (

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(PDF Manual)

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Links

FAQ

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User Discussion Forum

Suggestions Box

Acknowledgements

Contact Us

Kumar S, Dudley J, Nei M & Tamura K (2008) MEGA: A biologist-centric software for evolutionary analysis of DNA and protein sequences.

Briefings in Bioinformatics 9: 299-306.





New Features

Excel and CSV Output
Update Notification
Interface Improvements

MEGA 4: Molecular Evolutionary Genetics Analysis

MEGA is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, and testing evolutionary hypotheses.

MEGA 4 has been tested on the following Microsoft Windows® operating systems:

Windows 95/98, NT, 2000, XP, and Vista.

New Features:

Real-Time Caption Expert Engine
 A unique facility to generate detailed captions for different types of analyses and results. These captions are intended to provide detailed, natural language descriptions of the methods and models used in

Clearcut

The reference implementation for Relaxed Neighbor Joining (RNJ)

Evans, J., Sheneman, L., Foster, J.A., (2006) Relaxed Neighbor-Joining: A Fast Distance-Based Phylogenetic Tree Construction Method, *Journal of Molecular Evolution*, **62**:785-792.

Extremely efficient phylogenetic tree reconstruction



Neighbor joining (NJ) is a popular distance-based phylogenetic tree reconstruction method. It has nice theoretical properties, but suffers from an $O(N^3)$ time complexity. Popular implementations of traditional NJ cannot process datasets with more than a few thousand taxa.

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The weighbor Homepage

Weighbor: Weighted Neighbor Joining

Created by William J. Bruno, Nicholas D. Socci, and Aaron L. Halpern.

New:

Weighbor 1.2 is here! Better and faster than weighbor 1.0. Upgrade now! Read more.

Please cite: William J. Bruno, Nicholas D. Socci, and Aaron L. Halpern Weighted Neighbor Joining: A Likelihood-Based Approach to Distance-Based Phylogeny Reconstruction, Mol. Biol. Evol. 17 (1): 189-197 (2000).

Weighbor is a weighted version of Neighbor Joining that gives significantly less weight to the longer distances in the distance matrix. The weights are based on variances and covariances expected in a simple Jukes-Cantor model. The criterion for which pair is joined is based on a likelihood function on the distances. The resulting trees are less perturbed by adding distant taxa compared to Neighbor Joining, and negative branch lengths are avoided. The method does not suffer from long branch attraction as maximum parsimony and other methods do. The method is much faster than maximum likelihood, usually faster than maximum parsimony, and a lot slower than Neighbor Joining.

HOW TO USE WEIGHBOR

Weighbor (or weighted neighbor joining) is PHYLIP compatible. You must create a distance matrix, such as by using the PHYLIP program DNADIST, or, the least-squares method of Goldstein and Pollock.

Distances should always be given in units of substitutions per site; scaling distances by a constant can radically change the tree weighbor makes! If your distance matrix represents percent change, the values must be multiplied by .01 before passing them to weighbor. If your data includes a distance of infinity, it should be



MrBayes: Bayesian Inference of Phylogeny

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MrBayes is a program for the Bayesian estimation of phylogeny. Bayesian inference of phylogeny is based upon a quantity called the posterior probability distribution of trees, which is the probability of a tree conditioned on the observations. The conditioning is accomplished using Bayes's theorem. The posterior probability distribution of trees is impossible to calculate analytically; instead, MrBayes uses a simulation technique called Markov chain Monte Carlo (or MCMC) to approximate the posterior probabilities of trees.

The program takes as input a character matrix in a NEXUS file format. The output is several files with the parameters that were sampled by the MCMC algorithm. MrBayes can summarize the information in

Phylogeny Programs

- Phylogeny Program List: <u>http://evolution.genetics.washington.edu/phylip/software.html</u>
- Phylip <u>http://evolution.genetics.washington.edu/phylip</u>
- MEGA http://www.megasoftware.net/
- Clearcut <u>http://bioinformatics.hungry.com/clearcut/</u>
- Weighbor
 http://www.t6.lanl.gov/billb/weighbor/index.html
- Mr. Bayes
- http://mrbayes.csit.fsu.edu/

The Ribosomal Database Project

Sequences and tools

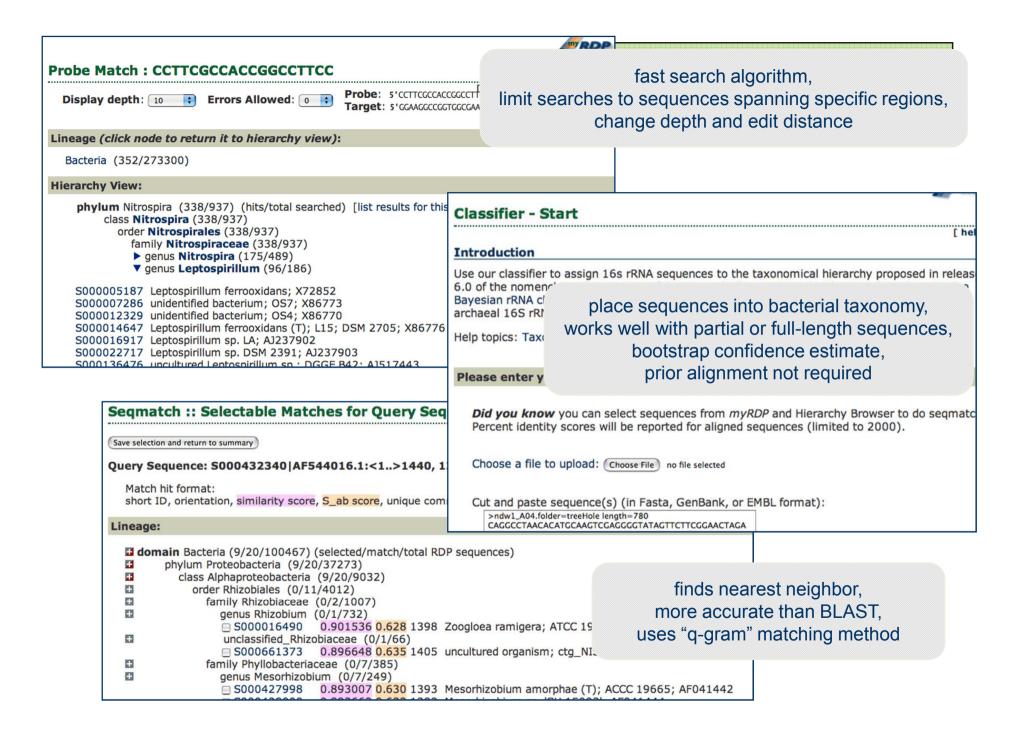
Aligned and annotated sequences

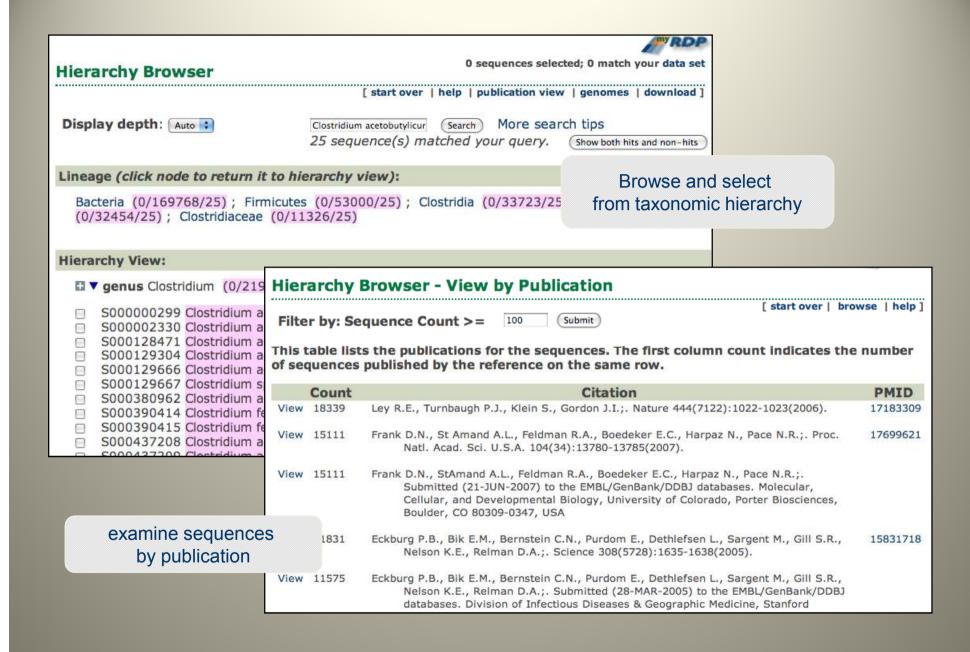
Ribosomal Database Project II



ANNOUNCEMENTS RESOURCES RELATED SITES ABOUT CITATION CONTACTS Release 9.57 :: Jan 7, 2008 :: 471,792 16S rRNAs upload and align (More on Release 9 and monthly is available in the release notes tutorials and help your own 16S RDP video tutorials sequences in RDP Analysis Tools your private News myRDP - Align and Classify your 16S rRNA sequences. Use the RDP Pipeline to process mvRDP space and sequence libraries from raw sequencer output to analysis. 01/07/2008 use the new RDP 9, Update 57 Relea Tree Builder Create a phylogenetic tree. includes a new Genome interactive online tools viewing sequences fron analysis *Pipeline* genome project. [more, Hierarchy Browser - Browse a phylogenetic h sequences for download or use. 08/08/2007 RDP 9, Update 53 Released using TOBA Release 7.7 (The Taxonomic Classifier - Assign 16S rRNA sequences to our taxonomical hierarchy. Outline of Bacteria and Archaea). RDP HOME | BROWSER | CLASSIFIER | LIBCOMPARE | SEQMATCH | PROBE MATCH | TREE | myRDP | seqCART Library Compare - Compare two sequence library Sequence Match - Upload your sequence and 7 sequences selected; 7 match your data set **Hierarchy Browser** [start over | help | publication view | genomes | download] Probe Match - See what your probe targets in c view by publication or genome Other Resources - Alignment files, ASM poster Display depth: Auto : e coli 146 seauence powerful search and Release 8.1 - The old dataset. selection features 'click node to return it to hierarchy view): The Ribosomal Database Project (RDP) provides rib Bacteria (7/164872/146): Proteobacteria (7/56215/122): Gammaproteobacteria (7/26787/91) scientific community, including online data analysis small-subunit 16S rRNA sequences. **Hierarchy View:** order Enterobacteriales (7/5454/73) (selected/total/search matches) [options] Sponsors: Ŧ family Enterobacteriaceae (7/5454/73) Ŧ ▶ genus Buchnera (0/56/1) 3 Biological and ▶ genus Citrobacter (0/428/3) -▼ genus Photorhabdus (7/93/7)

S000528568 Photorhabdus luminescens subsp. laumondii TTO1; BX571859 S000528570 Photorhabdus luminescens subsp. laumondii TTO1; BX571860 S000528572 Photorhabdus luminescens subsp. laumondii TTO1; BX571861





Genome: Clostridium acetobutylicum ATCC 824

This organism is a known Type Strain.

RDP Taxonomy Lineage: domain Bacteria; phylum Firmicutes; class Clostridia; order Clostridiales; family Clostridiaceae; genus Clostridium

NCBI Taxonomy Lineage: no rank root; no rank cellular organisms: superkingdom Bacteria: phylum Firmicutes; class Clostridia; order Clostridiales; family Clostridiace

Sequencing Status from NCBI: COMPLETE

rRNA sequence from genome projects

Estimated Genome size: 4.13 Mbp

References (1)

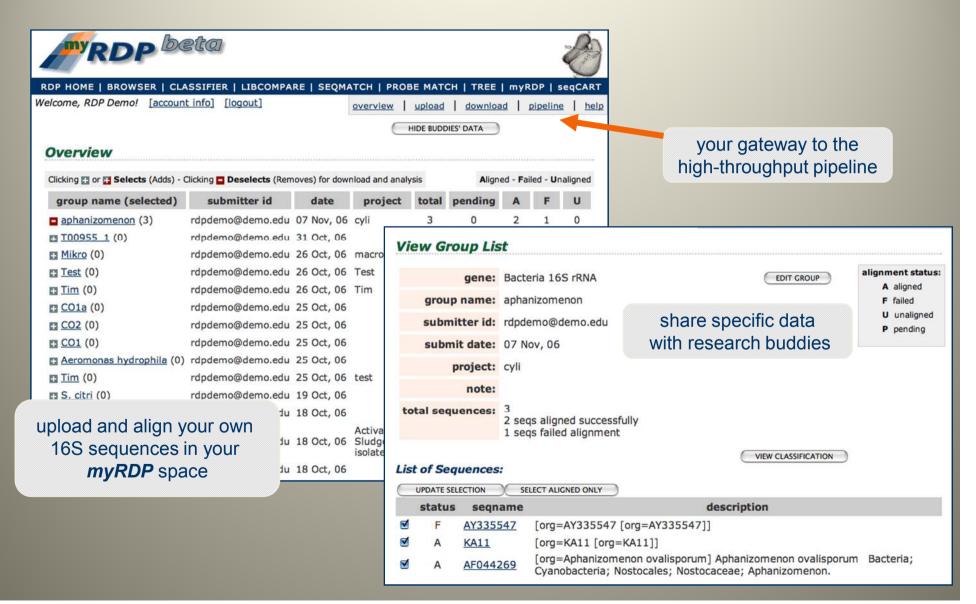
Outside Links (5)

16S rRNA Sequences (11)

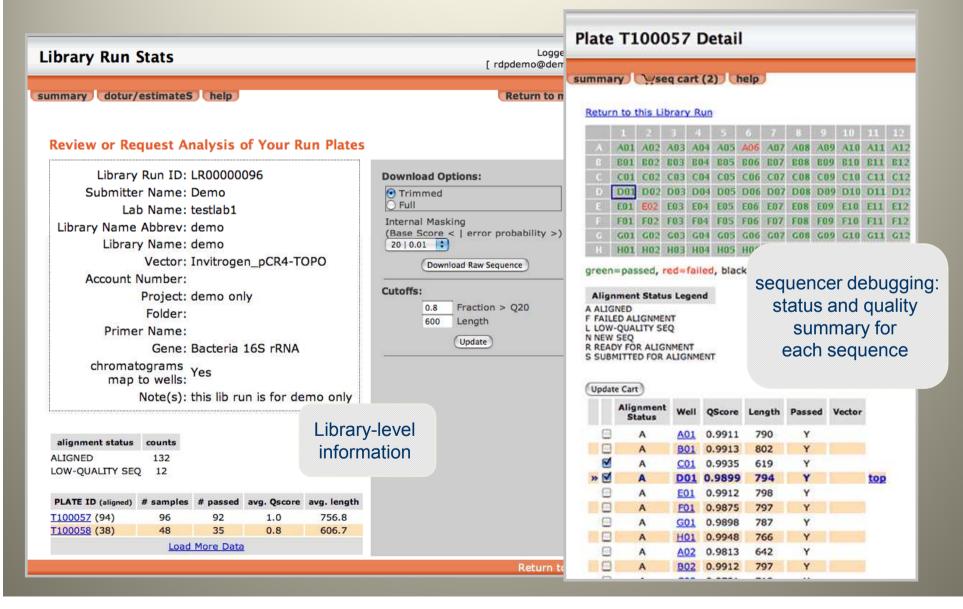
Links provided by the Genomic Rosetta Stone

Genomes OnLine Database: Gc00060	GOLD: Genomes Online Database, is a World Wide Web resource for comprehensive access to information regarding complete and ongoing genome projects around the world.		
Genome Catalogue: 000550_GCAT	Database holding genome reports that conform to the MIGS/MIMS specification, as defined by the Genomic Standards Consortium		
NCBI Taxonomic Database: 272562	The NCBI taxonomy database contains the names of all organisms that are represented in the genetic databases with at least one nucleotide or protein sequence.		
NCBI Genome Project: 77	The NCBI Entrez Genor collection of complete a assembly, annotation, a	Provides links to other genome-related information	chable Jencing,
Integrated Microbial Genomes (IMG): 637000076	The Integrated Microbial Genomes (IMG) system (Nucleic Acids Research, 2006, Vol. 34, Database issue D344-D348) provides a framework for comparative analysis of the genomes sequenced by the Joint Genome Institute. Its goal is to facilitate the visualization and exploration of genomes from a functional and evolutionary perspective.		

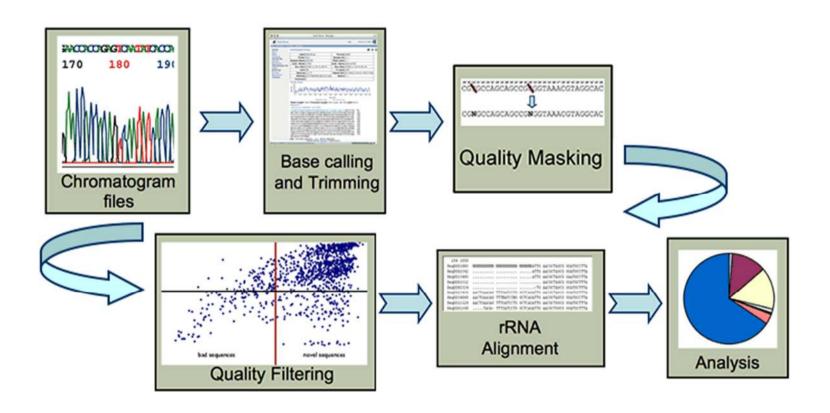
Ribosomal Database Project-II *myRDP* Personal/Collaborative Workspace



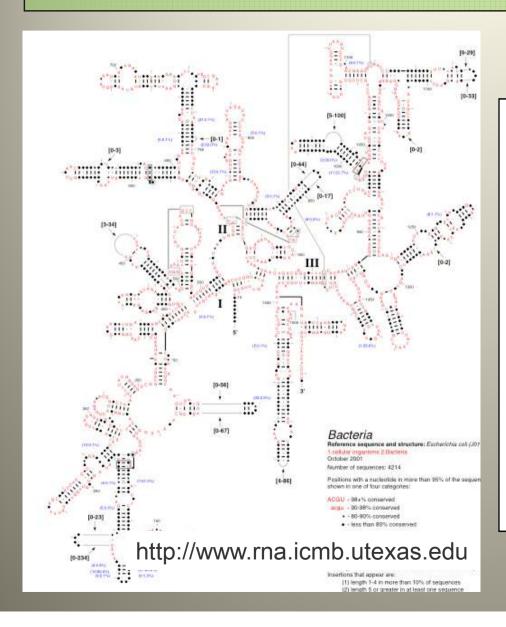
Ribosomal Database Project-II *myRDP* High-Throughput Pipeline



Ribosomal Database Project-II *myRDP* Single-Read Pipeline

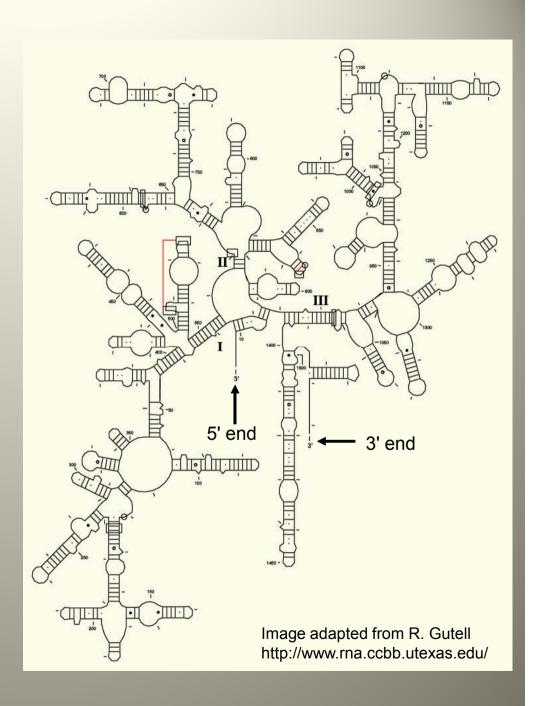


RDP 10 Alignment



- INFERNAL Stochastic Context-Free Grammar Aligner (Eddy)
- INFERNAL (Eddy)
 - Fast
 - Replaces RNAcad (Brown)
- Incorporates secondary structure information
- Probabilistic model
- New training set

Secondary structure of small subunit ribosomal RNA



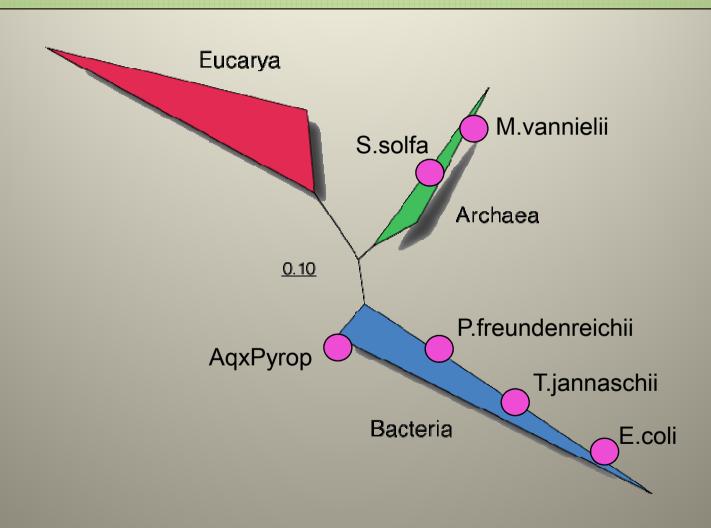
Unaligned rRNA sequences in a multiple alignment editor

Taxa																															
67	Arb.globi	U	G	С	U	U	G	С	Α	С	С	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С	G	G	G
68	Arb.globi	Α	Α	G	U	С	G	Α	Α	С	G	Α	U	G	Α	U	С	С	G	G	U	G	С	U	U	G	С	Α	С	С	G
69	Arb.oxyda	G	С	U	U	G	С	U	С	С	U	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	C	G	G	G	U	G
70	Arb.pascn	С	С	С	С	Α	G	C	U	U	G	С	U	G	G	G	G	G	G	Α	U	U	Α	G	U	G	G	C	G	Α	Α
71	Arb.plych	G	G	G	Α	G	С	U	U	G	С	U	С	С	U	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С	G
72	Arb.ramos	С	С	С	Α	G	С	U	U	G	С	U	G	G	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С
73	Ren.salmo	G	G	U	Α	С	U	U	G	С	Α	G	Α	Α	G	Α	Α	G	С	Α	С	С	G	G	С	U	Α	Α	С	U	Α
74	Ren.salmo	Α	G	U	С	G	Α	Α	С	G	Α	U	G	Α	Α	G	С	G	G	U	С	G	U	U	G	С	G	С	С	G	U
75	Arb.agili	С	U	С	Α	С	U	U	G	U	G	G	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С	G	G
76	Arb.aures	U	G	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С	G	G	G	U	G	Α	G	U	Α	Α	С
77	Arb.citru	U	U	U	G	U	G	C	U	U	G	С	Α	С	Α	Α	Α	Α	U	G	Α	U	U	Α	G	U	G	G	С	G	Α
78	Arb.hstdn	G	С	U	U	N	С	U	G	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С	G	G	G	U	G
79	Arb.ilici	С	С	Α	G	С	U	U	G	С	U	G	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	C	G	G
80	Arb.nic∨o	С	С	Α	G	С	U	U	G	С	U	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С	G	G	G
81	Arb.ureaf	С	G	G	U	G	С	U	U	G	С	G	С	С	G	G	G	G	Α	U	U	Α	G	U	G	G	С	G	Α	Α	С

Aligned rRNA sequences in editor

Taxa																																	
67	Arb.globi	G	U	G	-	G	U	U	-	U	U	G	G	Α	U	G	-	-	G	Α	С	U	С	G	С	G	G	С	С	U	Α	U	С
68	Arb.globi	G	U	G	-	G	U	U	-	U	U	G	G	Α	U	G	-	-	G	Α	С	U	С	G	С	G	G	С	С	U	A	U	С
69	Arb.oxyda	U	G	U	G	G	U	U	-	U	U	G	G	Α	U	G	-	-	G	Α	С	U	С	G	С	G	G	С	С	U	Α	U	С
70	Arb.pascn	G	U	G	-	G	U	U	-	U	U	G	G	Α	U	G	-	-	G	Α	С	U	С	G	С	G	G	С	С	U	Α	U	С
71	Arb.plych	U	G	U	-	G	U	U	-	U	U	G	G	Α	U	G	-	-	G	Α	С	U	С	G	С	G	G	С	С	U	Α	U	С
72	Arb.ramos	G	U	G	-	G	U	U	-	U	U	G	G	Α	U	G	-	-	G	Α	С	U	С	G	С	G	G	С	С	U	Α	U	С
73	Ren.salmo	G	C	G		G	U	U	% = 1	U	U	G	G	A	U	G		2 4 7	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
74	Arb.agili	G	U	G		G	U	U		U	U	G	G	A	U	G		8 <u>14</u>	G	A	C	U	C	G	C	G	G	C	C	U	A	U	С
75	Arb.aures	G	U	G	3(0)	G	U	U	35 - 1	U	U	G	G	A	U	G	3 .	88	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
76	Arb.citru	G	C	G	ep u e	G	U	U	si x c	U	U	G	G	A	U	G	ii - c	:: - c	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
77	Arb.hstdn	G	U	G	(4	G	U	U	7 4	U	U	G	G	A	U	G		9 .5 1	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
78	Arb.ilici	G	U	G	3	G	U	U		U	U	G	G	A	U	G		535 286 286	G	A	C	U	C	G	C	G	G	C	C	U	A	U	С
79	Arb.nicvo	G	U	G	85 - 8	G	U	U	88=8	U	U	G	G	A	U	G	85=8	88	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
80	Arb.ureaf	G	U	G	() + C	G	U	U	ejo u c	U	U	G	G	A	U	G	() - C	sp ic	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
81	Arb.nicna	U	C	G	24	G	U	G	984	G	G	G	G	A	U	G	(14)	28	G	A	C	U	C	G	C	G	G	C	C	U	A	U	C
82	Arb.protp	U	C	G	-	G	U	U	-	G	G	G	G	A	U	G	-	32	G	A	C	U	C	G	C	G	G	C	C	U	A	U	С

The Biosphere



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Acknowledgement of rRNA secondary structure image:

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Sequence Alignment

Accuracy, Time, Memory

Multiple Sequence Alignment

- Pairwise dynamic programming
 - Smith-Waserman, Needleman Wunsch
 - Can be transformed into probabilistic framework
- Multidimensional dynamic programming
 - Not practical
- Progressive alignment
 - Muscle, ClustalW
 - Both are progressive iterative

BLAST

- Heuristic search strategy
- Locate high-scoring short matches
 - 3aa or 5 to 11 bases
- Extend short matches
- Determine significance using extreme value distribution statistics

BLAST (cont.)

- E value
 - Database dependent
- Bits
 - Database independent
- % Similarity (identity)
 - For aligned segment s
 - NOT overall % identity

Model Based Alignment

- Profile Hidden Markov Models
 - Protein and nucleic acid
 - Models primary sequence
- Stochastic Context-Free Grammars
 - Incorporates RNA secondary structure

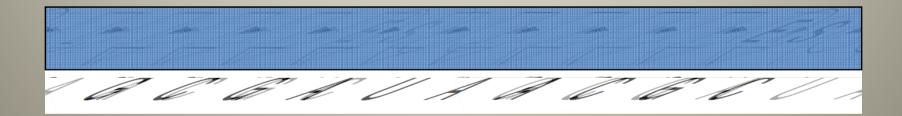
Hidden Markov Model



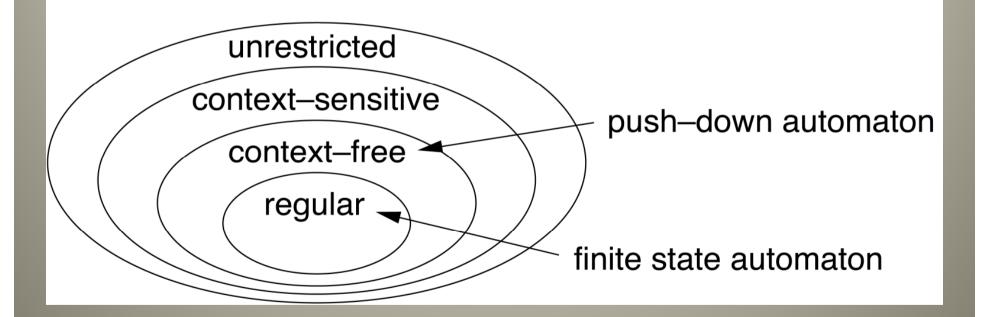
Hidden Markov Model



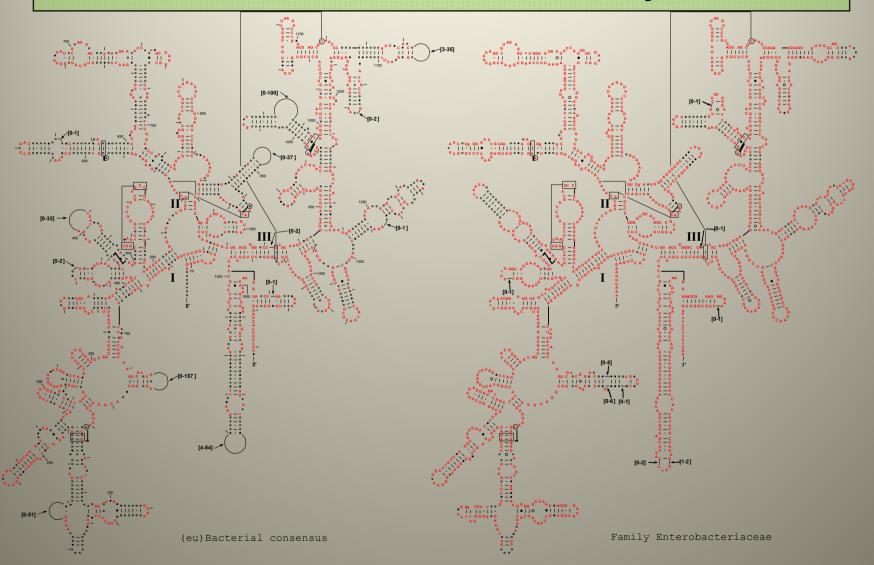
Hidden Markov Model



Chomsky Transformational Grammars



2D Structure Conserved from Domain to Family



Diagrams from the Gutell Lab Comparative RNA Web Site (http://www.rna.icmb.utexas.edu)

Aligner References

- MUSCLE <u>http://www.drive5.com/muscle/</u>
- BLAST <u>http://blast.ncbi.nlm.nih.gov/</u>
- HMMER http://hmmer.janelia.org/
- INFERNAL http://infernal.janelia.org/

Distance Calculation

 Phylogenetic methods only score base substitution, not insertion or deletion.

- Score comparable positions
 - Mask out unaligned regions, insertions
 - Ignore positions with deletion

Other Common Distances

- Hamming distance
 - No gap insert
 - Original Blast
- Edit distance
 - Penalize for gaps
 - RDP Probe Match
- Matching word percentage (q-gram)
 - Does not require alignment
 - RDP Sequence Match

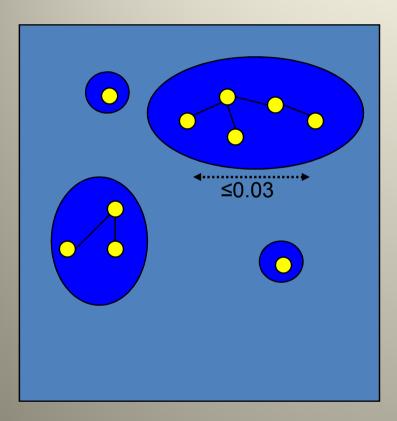
Clustering

Accuracy, Time, Memory

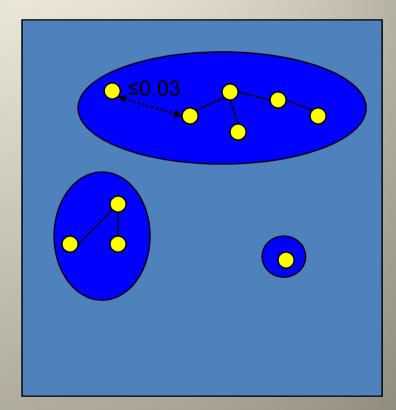
Unsupervised Classification (Clustering)

- Hierarchical Agglomerative
 - Single Linkage (Nearest neighbor)
 - Average Linkage (UPGMA)
 - Compete Linkage (Furthest Neighbor)
- Partitional Clustering
 - K-Means
 - Not often used in this field
- Self Organizing Maps
 - Using word frequency

Hierarchical Clustering



Complete Linkage



Single Linkage





Contents

- Download!
- News & Update
- CD-HIT Home
- · Author's Home
- Manual
- Project Page
- Project CVS
- Project FTP
- Project BUGS
- References
- Related Resources
- Support
- Thanks!





Welcome to the CD-HIT Project Main Page

CD-HIT stands for Cluster Database at High Identity with Tolerance. The program (cd-hit) takes a <u>fasta format</u> sequence database as input and produces a set of 'non-redundant' (nr) <u>representative sequences</u> as output. In addition cd-hit outputs a <u>cluster</u> file, documenting the sequence 'groupies' for each nr sequence representative. The idea is to reduce the overall size of the database without removing any sequence information by only removing 'redundant' (or highly similar) sequences. This is why the resulting database is called <u>non-redundant</u> (nr). Essentially, cd-hit produces a set of closely related <u>protein families</u> from a given fasta sequence database.

CD-HIT uses a 'longest sequence first' <u>list removal algorithm</u> to remove sequences above a certain <u>identity</u> threshold. Additionally the algorithm implements a very fast heuristic to find high identity segments between sequences, and so can avoid many costly full alignments.

With recent developments, cd-hit package offers new programs for DNA sequence clustering and comparing two databases. It also has lots of new options for clustering control.

CD-HIT was originally written by Weizhong Li and is now an open source project!

Bugs

There are a number of <u>outstanding bugs</u> in the current implementation. We are always looking for hard working and enthusiastic volunteers (people like <u>Luc Ducazu</u>) to shoot these problems down.

Sub Projects

The CD-HIT project provides a number of opportunities for interesting research activities. If one of these sub-projects takes your interest why not join up and take part? We are especially keen to work closely with bioinformatics MSc students working on their MSc projects.

- MyCD-HIT. A CD-HIT implementation embedded in a MySQL UDF!
- Clustering Benchmarks. Develop and implement benchmarks to test clustering behavior.
- CD-HIT CGI. On-line access to the algorithm.





FastGroupII

Tools Home

FastGroupII

Database

Calculation

User's Guide

FastGroupII

FastGroupII analysis ClustalW analysis

Download

FastGroup1.0
Converter

Welcome to FastGroupII

Very little is known about prokaryotic diversity on coral reefs, the most biodiverse of all marine ecosystems. To address this issue we have been sequencing 16S rDNAs from *Archaea* and *Bacteria* associated with corals (1, 2). To help with the analyses, the FastGroup project was started in 2001. FastGroup will take large 16S rDNA datasets, trim them, and determine community structure (e.g., dereplication).

The first version of FastGroup ⁽³⁾ was a Java program and can be downloaded here. FastGroupII is a web-based tool. It dereplicates large 16S rDNA libraries using several different algorithms, including the original FastGroup Percentage Sequence Identity (PSI), PSI with Gaps, Tree-parsing (ClustalW global alignment) ⁽⁴⁾, and Seq-Match (Sequence Match in Ribosomal Database Project) ⁽⁵⁾. FastGroupII also automatically calculates standard diversity and richness indexes, including the Shannon-Wiener index ⁽⁶⁾, Chao1 ⁽⁷⁾, and rarefaction ⁽⁸⁾.

Full online article: FastGroupII: A web-based bioinformatics platform for analyses of large 16S rDNA libraries, Yanan Yu, Mya Breitbart, Pat McNairnie and Forest Rohwer, BMC Bioinformatics 2006,7:57. Click here.

To use FastGroupII, click here.

Supervised Classification

- K-Nearest Neighbors
 - SeqMatch, Megan, easyTaxon
 - Last Common Ancestor
- Bayesian
 - RDP Classifier
- Kernel methods
 - Support Vector Machines

Thirty-One Years of rRNA Sequencing

Proc. Natl. Acad. Sci. USA Vol. 75, No. 10, pp 4801–4805, October 1978 Biochemistry

Complete nucleotide sequence of a 16S ribosomal RNA gene from Escherichia coli

(recombinant plasmids/DNA sequence analysis/rrnB cistron)

JÜRGEN BROSIUS, MARGARET L. PALMER, POINDEXTER J. KENNEDY, AND HARRY F. NOLLER

Thirmann Laboratories, University of California, Santa Cruz, California 95064

Communicated by Robert L. Sinsheimer, July 26, 1978

The complete nucleotide sequence of the 16S ABSTRACT RNA gene from the rrnB cistron of Escherichia coli has been determined by using three rapid DNA sequencing methods. Nearly all of the structure has been confirmed by two to six independent sequence determinations on both DNA strands. The length of the 16S rRNA chain inferred from the DNA sequence is 1541 nucleotides, in close agreement with previous estimates. We note discrepancies between this sequence and the most recent version of it reported from direct RNA sequencing [Ehresmann, C., Stiegler, P., Carbon, P. & Ebel, J. P. (1977) FEBS Lett. 84, 337-341]. A few of these may be explained by heterogeneity among 16S rRNA sequences from different cistrons. No nucleotide sequences were found in the 16S rRNA gene that cannot be reconciled with RNase digestion products of mature 16S rRNA.

sequence have been confirmed, and additional errors have been found involving oligonucleotide sequences, ordering of oligonucleotides, and, in one instance, the location of a larger section of the primary structure. No nucleotide sequences were found that cannot be accounted for from the RNase digestion products of mature 16S rRNA.

METHODS

Cloning and Mapping of DNA. The 16S rRNA gene from the rrnB cistron of E. coli was cloned from two EcoRI restriction fragments of $\lambda rif^{\rm d}18$ (17, 18) in the Co1E1 plasmid vector. Determination of the location of the 16S rRNA sequences and restriction enzyme cleavage sites will be described elsewhere.

rRNA is becoming increasingly important in our current per-

Twenty-Eight Years Later

Proc. Natl. Acad. Sci., USA Vol. 103, No. 32, pp 12115-12120, August 2006 www.pnas.org/cgi/doi/10.1073/pnas.0605127103

Microbial diversity in the deep sea and the underexplored "rare biosphere"

Mitchell L. Sogin*[†], Hilary G. Morrison*, Julie A. Huber*, David Mark Welch*, Susan M. Huse*, Phillip R. Neal*, Jesus M. Arrieta^{‡§}, and Gerhard J. Herndl[‡]

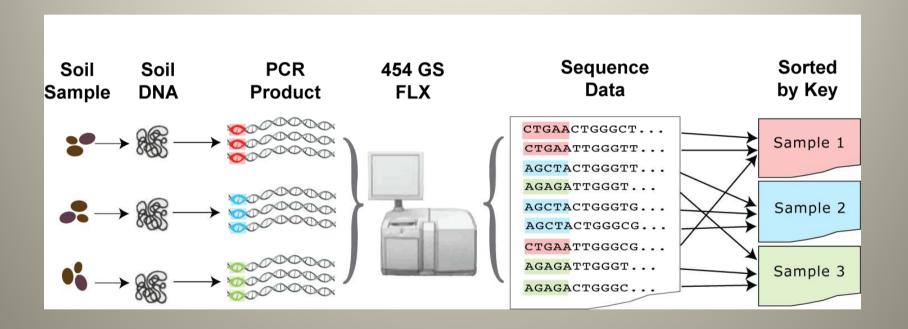
*Josephine Bay Paul Center, Marine Biological Laboratory at Woods Hole, 7 MBL Street, Woods Hole, MA 02543; and [‡]Royal Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB, Den Burg, Texel, The Netherlands

Communicated by M. S. Meselson, Harvard University, Cambridge, MA, June 20, 2006 (received for review May 5, 2006)

The evolution of marine microbes over billions of years predicts that the composition of microbial communities should be much greater than the published estimates of a few thousand distinct kinds of microbes per liter of seawater. By adopting a massively parallel tag sequencing strategy, we show that bacterial communities of deep water masses of the North Atlantic and diffuse flow hydrothermal vents are one to two orders of magnitude more complex than previously reported for any microbial environment. A relatively small number of different populations dominate all samples, but thousands of low-abundance populations account for most of the observed phylogenetic diversity. This "rare biosphere" is very ancient and may represent a nearly inexhaustible source of genomic innovation. Members of the rare biosphere are highly divergent from each other and, at different times in earth's history, may have had a profound impact on shaping planetary processes.

Gene sequences, most commonly those encoding rRNAs, provide a basis for estimating microbial phylogenetic diversity (5, 7. 14-18) and generating taxonomic inventories of marine microbial populations (5, 7, 14-18). Evolutionary distances between orthologous sequences (19) or similarities to database entries identified through BLAST (20), FASTA (21), or Bayesian classifiers (22) identify operational taxonomic units (OTUs) that correspond to species or kinds of organisms. A variety of parametric and nonparametric methods extrapolate information from observed frequencies of OTUs or species abundance curves to predict the number of different microbial taxa in a local sample (23-26). Richness estimates of marine microbial communities through comparisons of rRNAs range from a few hundred phylotypes per ml in the water column (19) to as many as 3,000 from marine sediments (27, 28). One of the largest water column surveys (1,000 PCR amplicons) described the presence of only 516 unique sequences and estimated occurrence of

Multiplexed Amplicon Pyrosequencing





RDP'S 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.

NCBI/EBI Submission Tools:

- myRDP SRA Prepkit Beta helps prepare and manage the complicated xml documents required for submitting your Pyrosequencing data to NCBI and EBI's Short Read Archive.
- Fastq A java web start program that creates a Fastq file from a fasta and quality file. Requires Java 5 or above.

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.

Miscellaneous Utilities:

- Alignment Merger merge multiple alignment files into one alignment.
- Dereplicate use this tool to make representative sequences.
- FASTA Sequence Selection make a sub-selection of FASTA sequences from the original sequence file.

RDP Pyrosequencing Pipeline



Initial Processing Steps

- Sort by barcode (key)
- Quality filter
 - Forward & (optional) reverse primers
 - Ambiguities
 - –Length
- Trim key & primer sequences

Two Analysis Tracks

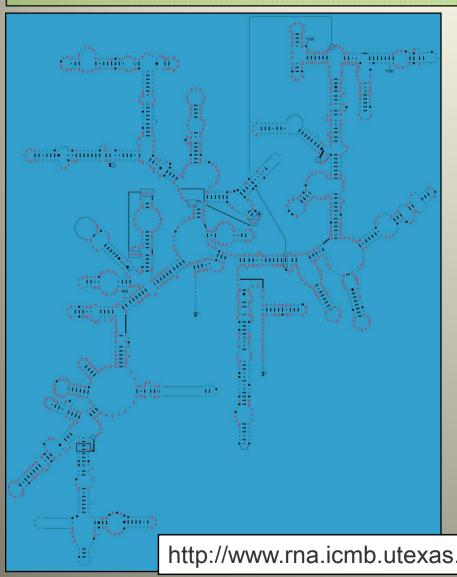
Taxonomy Independent

- Global Alignment
- Cluster Based OTU Assignment
- Standard Ecological Metrics
- Many 3rd Party Data Formats

Taxonomy Dependent

- RDP Classifier
- Sequence Match
- Many 3rd Party Data Formats

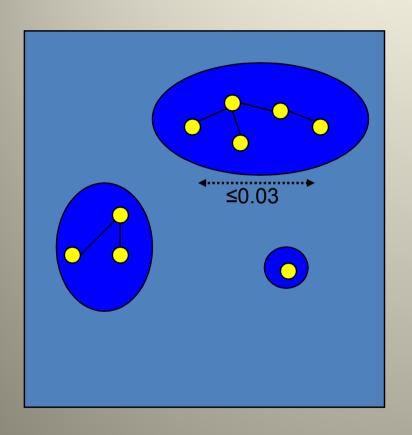
Model Based Alignment



- **Infernal Aligner**
 - (Nawrocki and Eddy. 2007, **PLoS Comput Biol)**
- Fast 500/min
- **Probabilistic Model**
 - Model describes shared features
- Incorporates 2d Structure
 - Cannone et al. 2002, **BioMed Central Bioinformatics**

http://www.rna.icmb.utexas.edu

Complete Linkage Clustering (Operational Taxonomic Units)

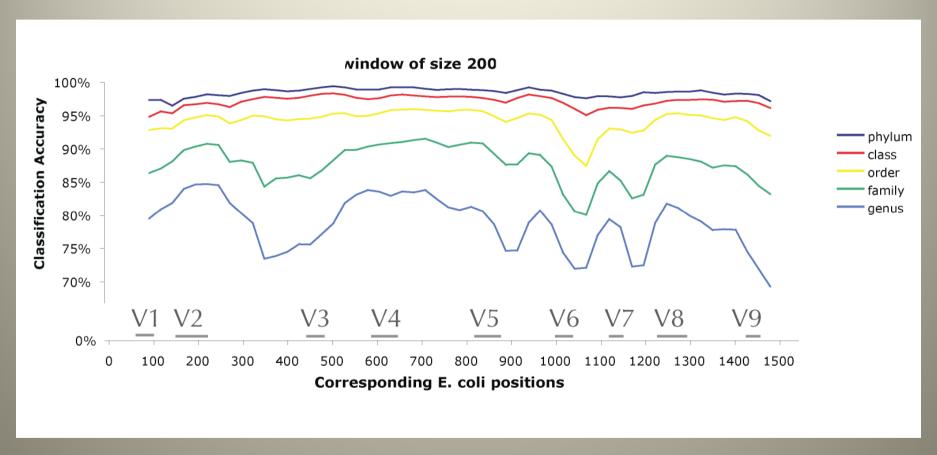


- Distance based method
- Guaranteed intra-cluster distance
- N² algorithm
- Current online limit
 150,000 unique reads
- Memory-efficient version in testing

RDP Naive Bayesian Classifier

- Fast 3000/min
- Places sequences into bacterial taxonomy
- Works well on partial or full-length sequences
- Does not require alignment
- Easily re-trained to match new taxonomies
- Bootstrap confidence estimates
- Online GUI Soap service Open source

Classifier Accuracy on 200 bp Regions



From Wang et. al., AEM, 2007

RDP Classifier Bootstrap Performance (Genus Level - Short Reads)

		V3				V6			V4				
Bootstrap cutoff	0%	50%	80%		0%	50%	80%	0%	6 50%	80%			
Human Gut													
% classified	100	92.4	82.3		100	73.5	40.4	10	97.0	87.9			
% matching	92.0	95.0	98.1		79.0	96.5	98.7	92.	94.5	95.7			
				S	oil								
% classified	100	71.3	48.3		100	32.7	16.7	10	74.4	56.3			
% matching	70.0	85.5	94.6		48.0	80.0	84.3	84.	1 93.3	96.8			