Introduction to evolutionary concepts and VMD/MultiSeq - Part I

Characterizing molecular systems

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NIH Center Macromolecular Modeling and Bioinformatics

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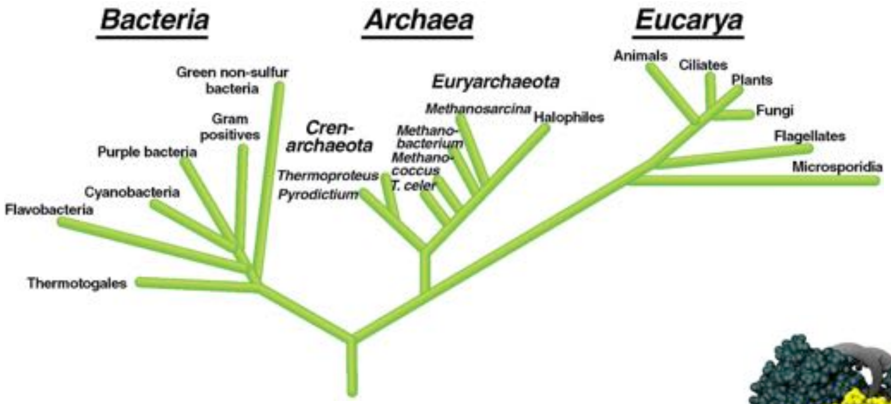
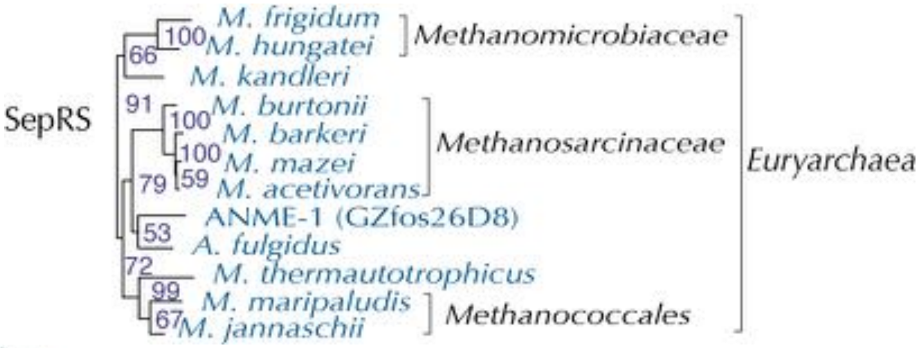
VMD/MultiSeq - “A Tool to Think”

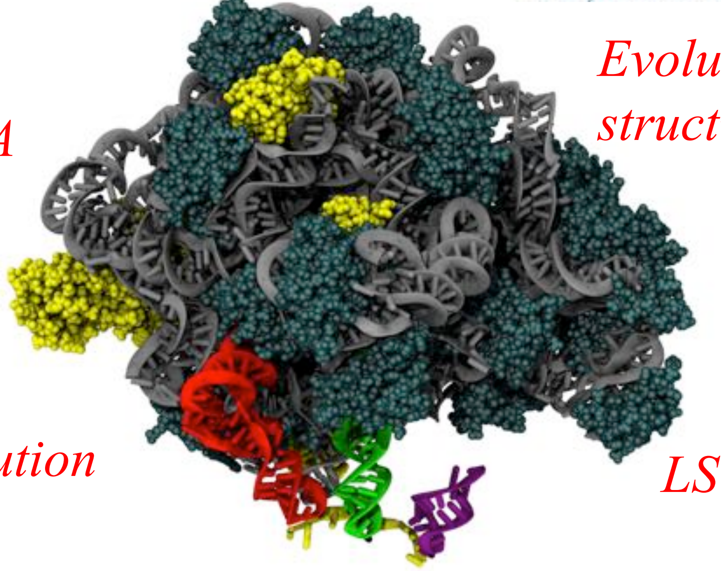
Carl Woese - *“VMD is far from a simple visualization tool for a biologist, it is a true thinking tool. Without it a whole class of biological hypotheses would simply not exist.”*





Evolutionary profiles for protein structure & function prediction

UPT - Woese 16S rRNA

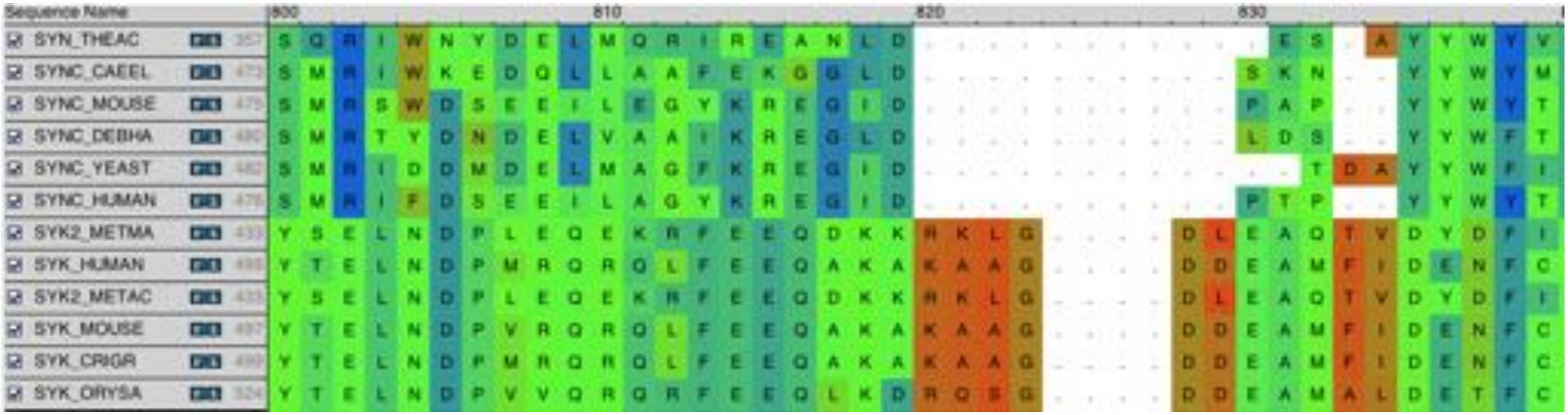


Signatures ribosomal evolution

LSU (23S rRNA + rproteins)

Why Look at More Than One Sequence?

1. Multiple Sequence Alignment shows patterns of conservation



```
Sequence Name        800                 810                 820                 830
SYN_THEAC     357    S Q R I W N Y D E L M Q R I R E A N L D . . . . . . . . . . . E S . A Y Y W Y V
SYNC_CAEEL    473    S M R I W K E D Q L L A A F E K G G L D . . . . . . . . . . S K N . . Y Y W Y M
SYNC_MOUSE    475    S M R S W D S E E I L E G Y K R E G I D . . . . . . . . . . P A P . . Y Y W Y T
SYNC_DEBHA    480    S M R T Y D N D E L V A A I K R E G L D . . . . . . . . . . L D S . . Y Y W F T
SYNC_YEAST    482    S M R I D D M D E L M A G F K R E G I D . . . . . . . . . . . . T D A Y Y W F I
SYNC_HUMAN    476    S M R I F D S E E I L A G Y K R E G I D . . . . . . . . . . P T P . . Y Y W Y T
SYK2_METMA    433    Y S E L N D P L E Q E K R F E E Q D K K R K L G . . . . D L E A Q T V D Y D F I
SYK_HUMAN     495    Y T E L N D P M R Q R Q L F E E Q A K A K A A G . . . . D D E A M F I D E N F C
SYK2_METAC    433    Y S E L N D P L E Q E K R F E E Q D K K R K L G . . . . D L E A Q T V D Y D F I
SYK_MOUSE     497    Y T E L N D P V R Q R Q L F E E Q A K A K A A G . . . . D D E A M F I D E N F C
SYK_CRIGR     499    Y T E L N D P M R Q R Q L F E E Q A K A K A A G . . . . D D E A M F I D E N F C
SYK_ORYSA     324    Y T E L N D P V V Q R Q R F E E Q L K D R Q S G . . . . D D E A M A L D E T F C
```

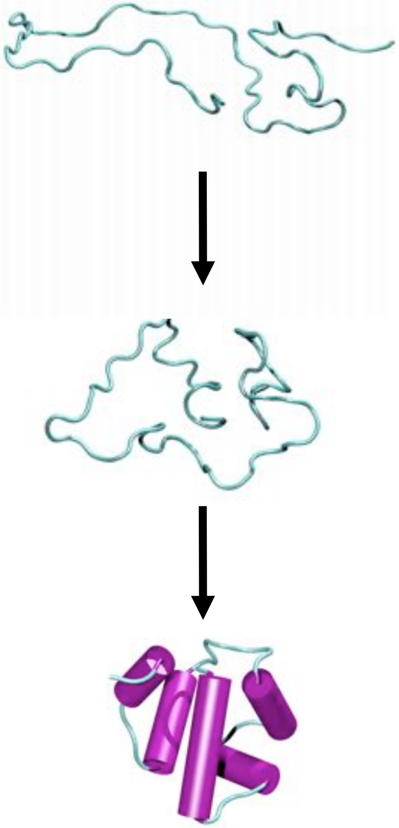
2. Are these positions functionally important? Active sites, folding,..

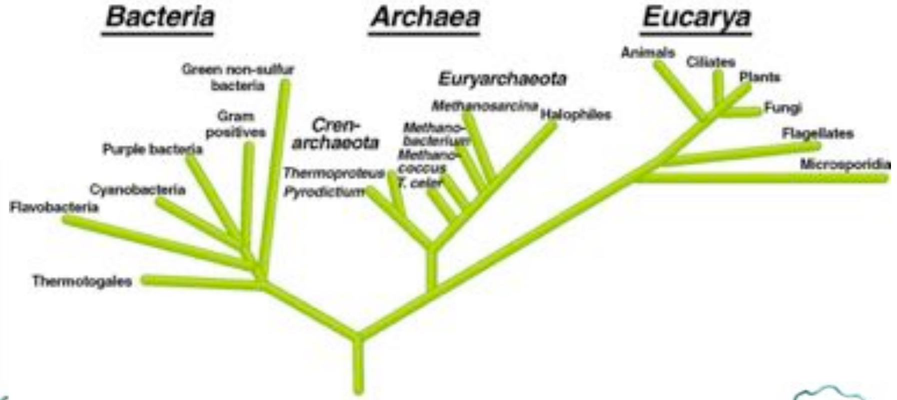
3. What and how many sequences should be included?

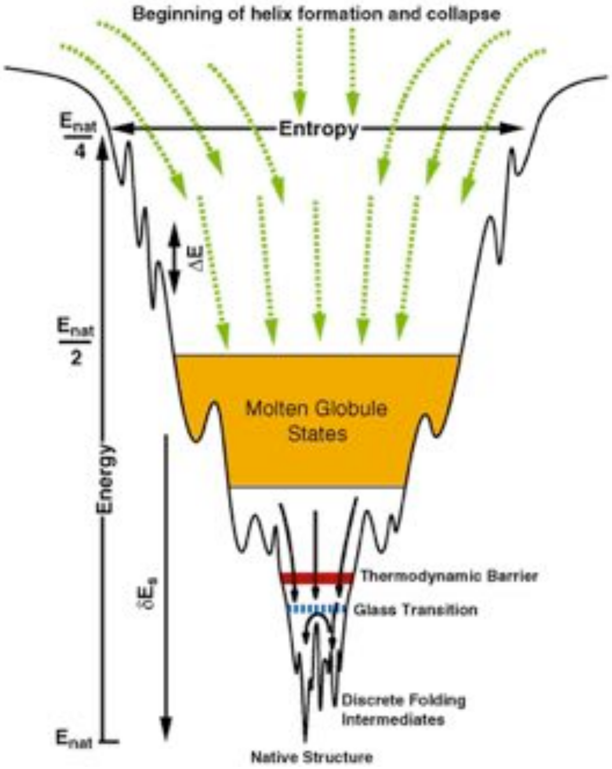
4. Where do I find the sequences and structures for MS alignment?

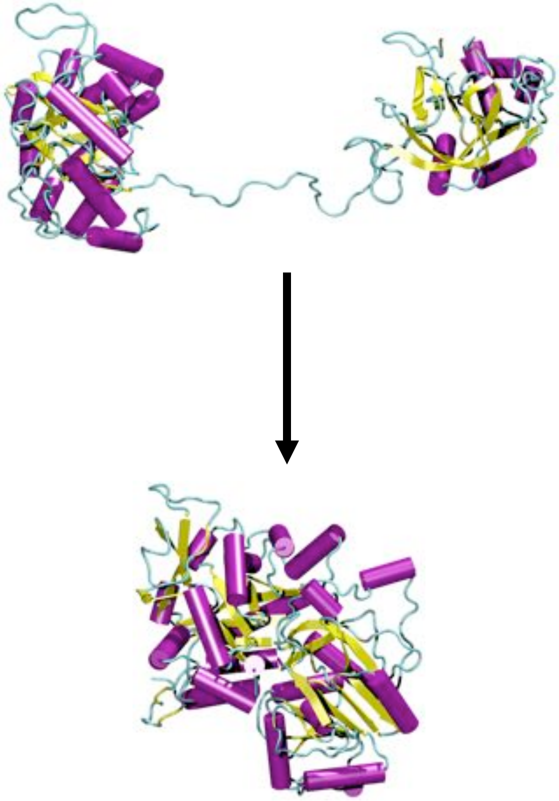
5. How to generate pairwise and multiple sequence alignments?

Protein (RNA) Folding, Structure, & Function









New Tools in VMD/MultiSeq

Protein / RNA Sequence Data

SwissProt DB (400K), Greengenes RNA (100K) Signatures, Zoom

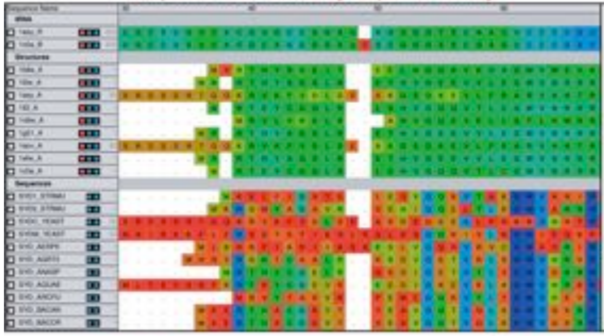
Metadata Information, Clustal, MAFFT & Phylogenetic Trees

RAXml Trees, Genomic Content, Temperature DB

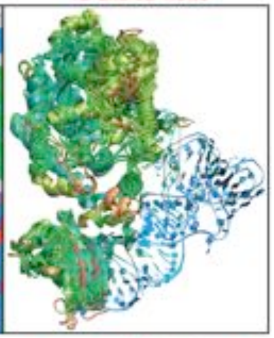
Blast & PsiBlast

Sequence Editor

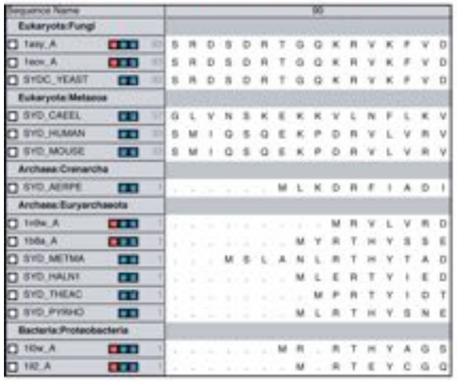
View structural data colored by structural conservation and sequence data colored by sequence identity



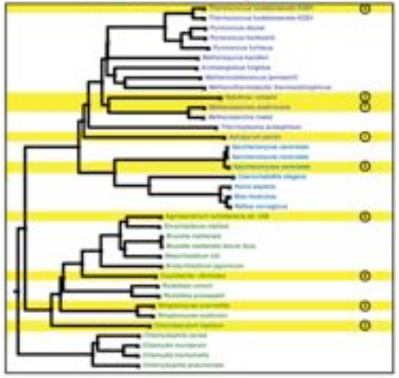
Synchronization between 1D and 3D views



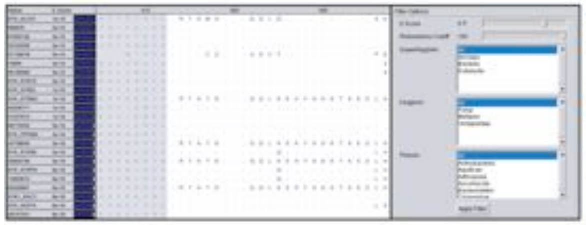
Group data by taxonomic classification



View sequence or structure phylogenies and eliminate redundancy with QR



Import data directly from BLAST databases



Align sequences with Clustal

Sequence Editor: Manually adjust alignments or sequences

Sequence /Structure Alignment

Protein & RNA secondary structure

QR non-redundant seq / str sets

Cluster analysis / Bioinformatics scripting

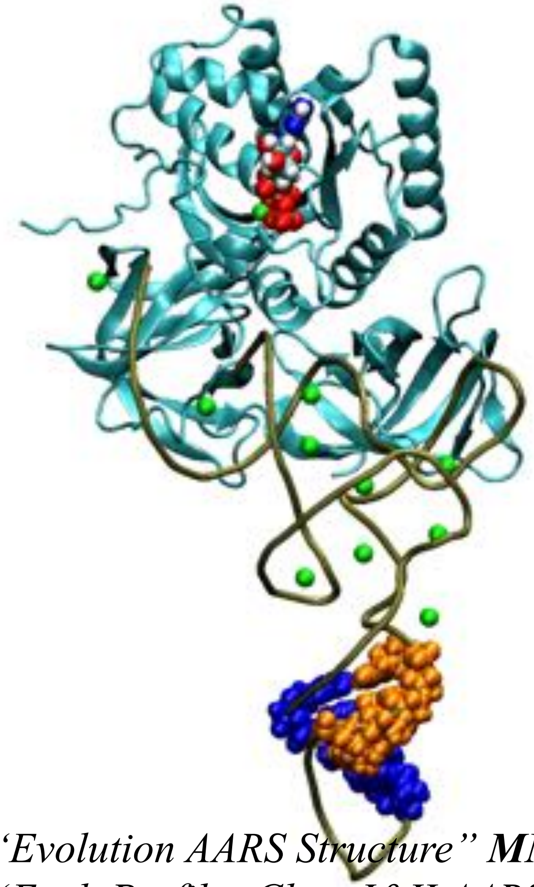
Tutorials MultiSeq/ AARS

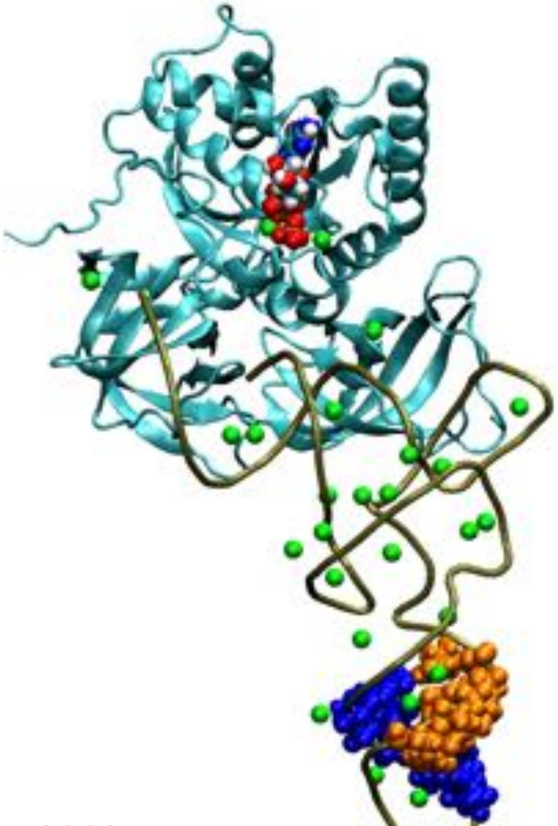
EF-Tu/Ribosome

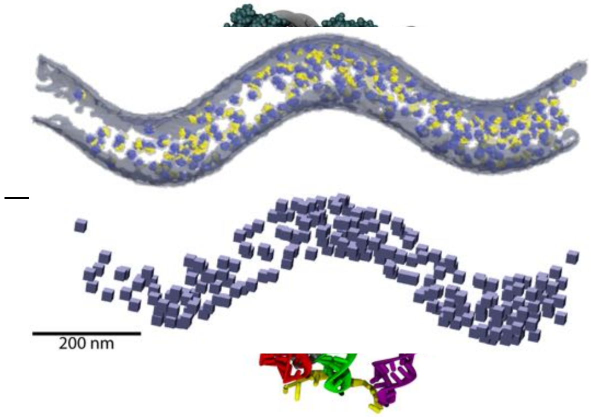
J. Eargle, D. Wright, Z. Luthey-Schulten, *Bioinformatics,* 22:504 (2006)
E. Roberts, J. Eargle, D. Wright, Z. Luthey-Schulten*, BMC Bioinformatics,* 7:382 (2006)

Protein:RNA Complexes in Translation

Evolutionary Analysis & Dynamics







“Evolution AARS Structure” ***MMBR*** 2003
“Evol. Profiles Class I&II AARS” ***JMB*** 2005
“Evolution SepRS/CysRS” ***PNAS*** *2005*
“Dynamic Signaling Network” ***PNAS*** 2009
“Exit Strategy Charged tRNA” ***JMB*** 2010
“Mistransl. in Mycoplasma” ***PNAS*** 2011
*“Capture & Selection of ATP”****JACS*** 2013

“Recognition & tRNA Dynamics”
JMB *2008,****FEBS*** 2010, ***RNA*** 2012
Network Viewer, ***Bioinf., JCTC*** 2012

r-Proteins/r-RNA
Ribosome LSU

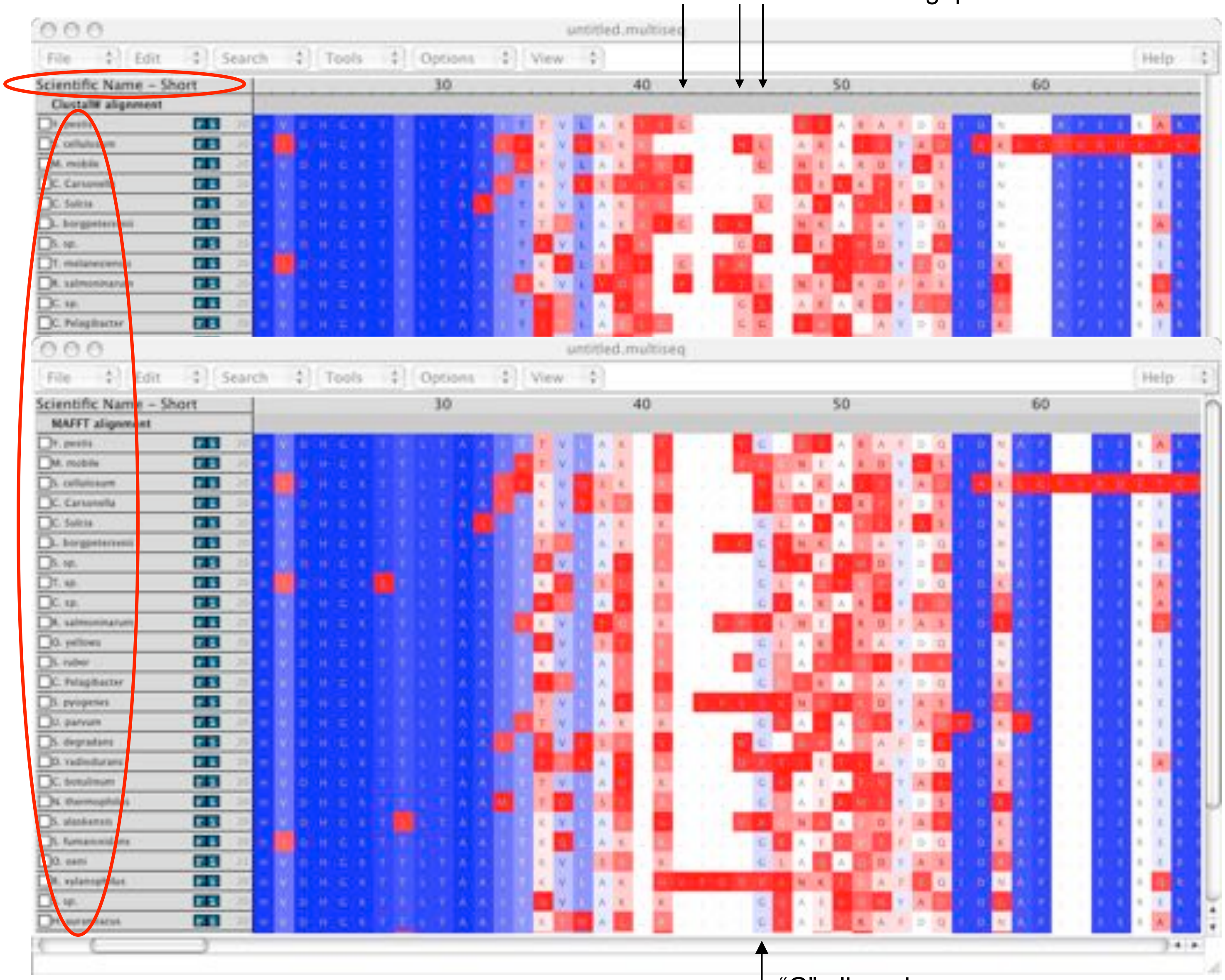
“Signatures ribosomal evolution”
PNAS 2008, ***BMC*** 2009, ***BJ*** 2010
“Motion L1 Stalk:tRNA” ***JMB*** 2010**,**
“Ribosome Biogenesis” ***JPC*** 2012,3
“Whole cell simulations on GPUs“
IEEE 2009,***Plos CB*** 2011,***PRL***2011,
JCC 2013, ***PNAS*** 2013,
PRL 2013, ***CSB*** 2013
Nature *2014,* ***BJ*** *2015*

Basic principles of evolutionary analysis for proteins & RNAs

- Comparative analysis of sequences and structures
- Multiple sequence alignments (gaps and editing)
- Sequence and structure phylogenetic trees*
- Reference to 16S rRNA tree
- Horizontal or lateral gene transfer events
- Genomic context
- Evolutionary profiles representing diversity
- Conservation analysis of evolutionary profiles

*Various models of evolutionary change

Alignment of ~200 EF-Tu sequences in VMD/MultiSeq



“G” scattered around gaps

“G” aligned

“Classic” ClustalW alignment

~ 5 minutes

MAFFT7* alignment

~ 30 seconds

More sequences!

http://www.clustal.org/clustal2/

* MAFFT v7.221, Katoh and Standley, Mol.Biol and Evol. 2015

Sequence Alignment & Dynamic Programming

number of possible alignments:

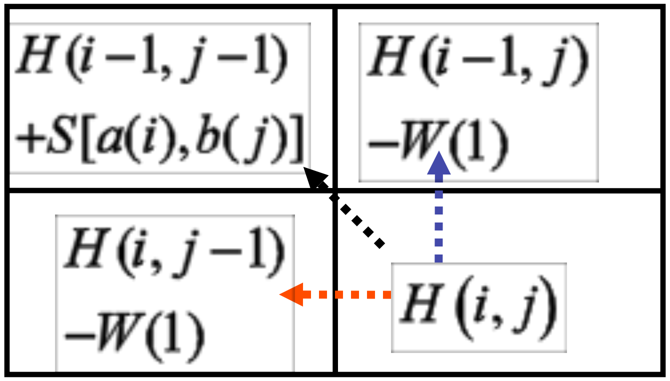
Seq. 1: $a_1\, a_2\, a_3$ - - $a_4\, a_5 \ldots a_n$

Seq. 2: c_1 - $c_2\, c_3\, c_4\, c_5$ - $\ldots c_m$

$$\longrightarrow \; = \binom{2n}{n} = 2^{2n}\left(\sqrt{n\pi}\right)^{-1}$$

Needleman-Wunsch alignment algorithm

$$H(i,j) = MAX \begin{cases} H(i-1,j-1) + S[a(i),b(j)] \\ H(i,j-k) - W(k), \\ H(i-m,j) - W(m) \end{cases}$$



Score Matrix H: Traceback

$\underline{\underline{S}}$: substitution matrix

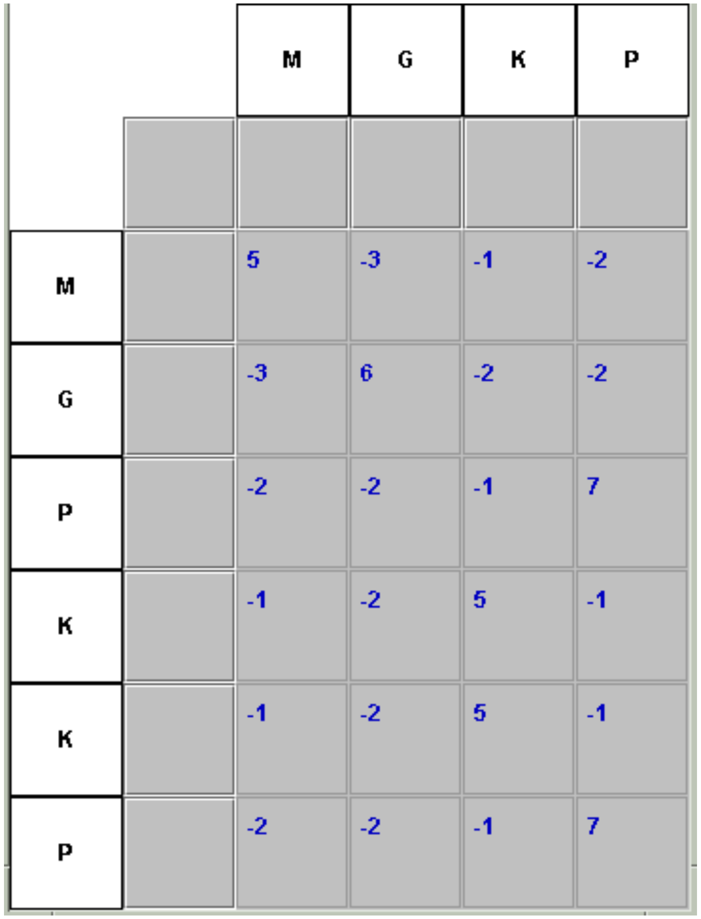
A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	B	Z	X	
5	-2	-1	-1	-2	0	-1	1	-2	-1	-2	-1	-1	-3	-2	1	0	-3	-2	0	-1	-1	0	A
-2	9	0	-1	-3	2	-1	-3	0	-3	-2	3	-1	-2	-3	-1	-2	-2	-1	-2	-1	0	-1	R
-1	0	8	2	-2	1	-1	0	1	-2	-3	0	-2	-3	-2	1	0	-4	-2	-3	4	0	-1	N
-1	-1	2	9	-2	-1	2	-2	0	-4	-3	0	-3	-4	-2	0	-1	-5	-3	-3	6	1	-1	D
-2	-3	-2	-2	16	-4	-2	-3	-4	-4	-2	-3	-3	-2	-5	-1	-1	-6	-4	-2	-2	-3	-2	C
0	2	1	-1	-4	8	2	-2	0	-3	-2	1	-1	-4	-2	1	-1	-1	-1	-3	0	4	-1	Q
-1	-1	-1	2	-2	2	7	-3	0	-4	-2	1	-2	-3	0	0	-1	-2	-2	-3	1	5	-1	E
1	-3	0	-2	-3	-2	-3	8	-2	-4	-4	-2	-2	-3	-1	0	-2	-2	-3	-4	-1	-2	-1	G
-2	0	1	0	-4	0	0	-2	13	-3	-2	-1	1	-2	-2	-1	-2	-5	2	-4	0	0	-1	H
-1	-3	-2	-4	-4	-3	-4	-4	-3	6	2	-3	1	1	-2	-2	-1	-3	0	4	-3	-4	-1	I
-2	-2	-3	-3	-2	-2	-2	-4	-2	2	6	-2	3	2	-4	-3	-1	-1	0	2	-3	-2	-1	L
-1	3	0	0	-3	1	1	-2	-1	-3	-2	6	-1	-3	-1	0	0	-2	-1	-2	0	1	-1	K
-1	-1	-2	-3	-3	-1	-2	-2	1	1	3	-1	7	0	-2	-2	-1	-2	1	1	-3	-2	0	M
-3	-2	-3	-4	-2	-4	-3	-3	-2	1	2	-3	0	9	-4	-2	-1	1	4	0	-3	-4	-1	F
-2	-3	-2	-2	-5	-2	0	-1	-2	-2	-4	-1	-2	-4	11	-1	0	-4	-3	-3	-2	-1	-2	P
1	-1	1	0	-1	1	0	0	-1	-2	-3	0	-2	-2	-1	5	2	-5	-2	-1	0	0	0	S
0	-2	0	-1	-1	-1	-1	-2	-2	-1	-1	0	-1	-1	0	2	6	-4	-1	1	0	-1	0	T
-3	-2	-4	-5	-6	-1	-2	-2	-5	-3	-1	-2	-2	1	-4	-5	-4	19	3	-3	-4	-2	-2	W
-2	-1	-2	-3	-4	-1	-2	-3	2	0	0	-1	1	4	-3	-2	-1	3	9	-1	-3	-2	-1	Y
0	-2	-3	-3	-2	-3	-3	-4	-4	4	2	-2	1	0	-3	-1	1	-3	-1	5	-3	-3	-1	V
-1	-1	4	6	-2	0	1	-1	0	-3	-3	0	-3	-3	-2	0	0	-4	-3	-3	5	2	-1	B
-1	0	0	1	-3	4	5	-2	0	-4	-2	1	-2	-4	-1	0	-1	-2	-2	-3	2	5	-1	Z
0	-1	-1	-1	-2	-1	-1	-1	-1	-1	-1	-1	0	-1	-2	0	0	-2	-1	-1	-1	-1	-1	X

gap penalty W = - 6

Reference: “Biological Sequence Analysis - Probabilistic Models of Proteins and Nucleic Acids” R. Durbin, S. Eddy, A. Krogh, and G. Mitchison, Cambridge U. P.London, 1998; pp. 19-22 (see also other sections)

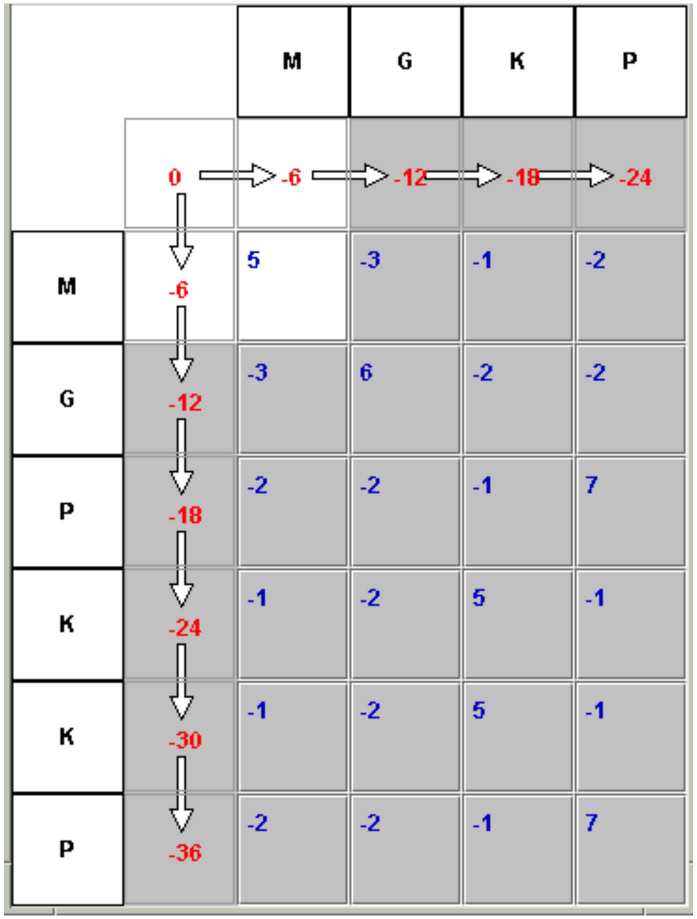
Needleman-Wunsch Global Alignment

Similarity Values



		M	G	K	P
M		5	-3	-1	-2
G		-3	6	-2	-2
P		-2	-2	-1	7
K		-1	-2	5	-1
K		-1	-2	5	-1
P		-2	-2	-1	7

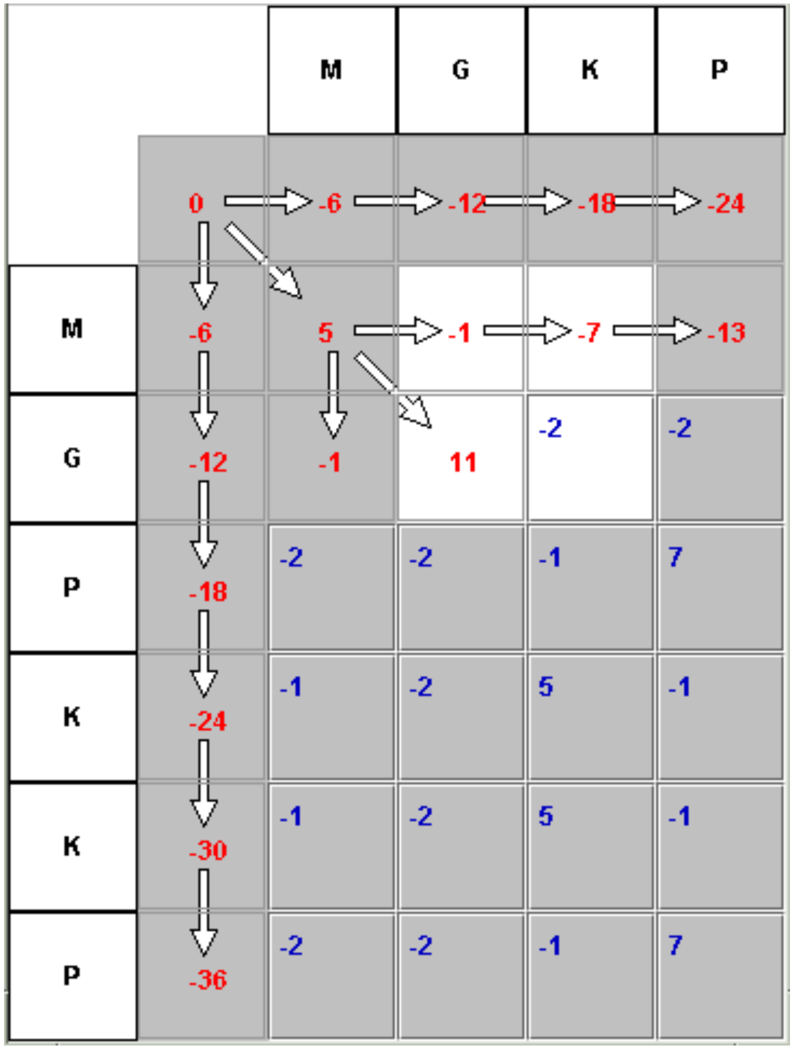
Initialization of Gap Penalties



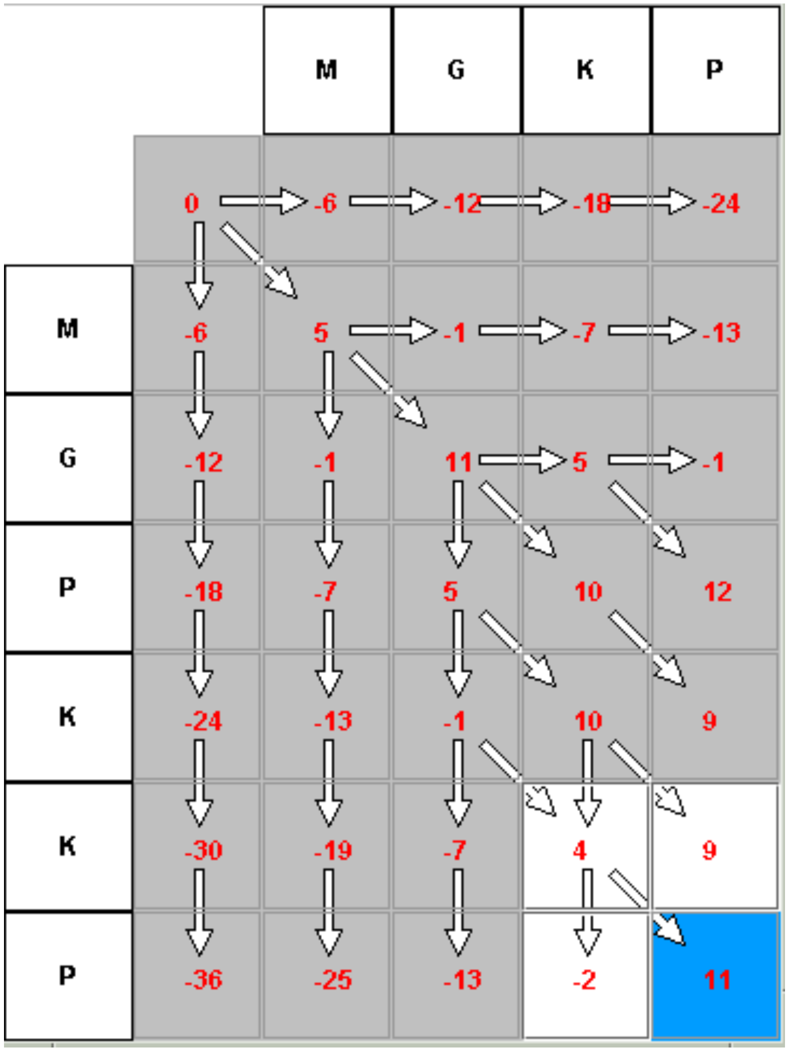
		M	G	K	P
	0	-6	-12	-18	-24
M	-6	5	-3	-1	-2
G	-12	-3	6	-2	-2
P	-18	-2	-2	-1	7
K	-24	-1	-2	5	-1
K	-30	-1	-2	5	-1
P	-36	-2	-2	-1	7

http://genome.dkfz-heidelberg.de/husar/fileadmin/handouts/02pairwise_method.pdf

Filling out the Score Matrix H

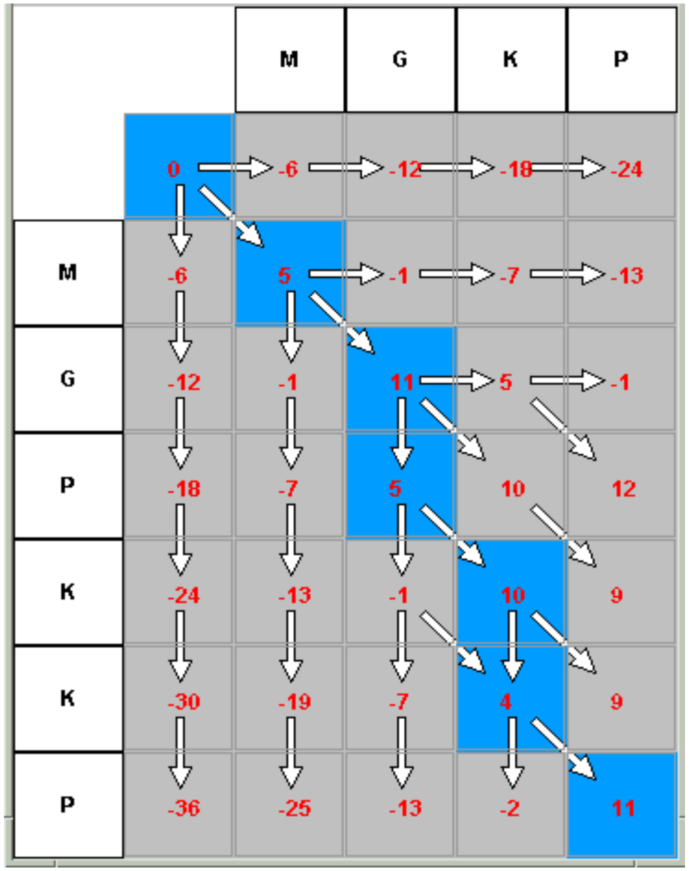


		M	G	K	P
	0	-6	-12	-18	-24
M	-6	5	-1	-7	-13
G	-12	-1	11	-2	-2
P	-18	-2	-2	-1	7
K	-24	-1	-2	5	-1
K	-30	-1	-2	5	-1
P	-36	-2	-2	-1	7



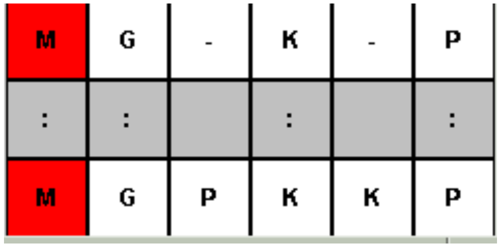
		M	G	K	P
	0	-6	-12	-18	-24
M	-6	5	-1	-7	-13
G	-12	-1	11	5	-1
P	-18	-7	5	10	12
K	-24	-13	-1	10	9
K	-30	-19	-7	4	9
P	-36	-25	-13	-2	11

Traceback and Alignment



		M	G	K	P
	0	-6	-12	-18	-24
M	-6	5	-1	-7	-13
G	-12	-1	11	5	-1
P	-18	-7	5	10	12
K	-24	-13	-1	10	9
K	-30	-19	-7	4	9
P	-36	-25	-13	-2	11

The Alignment



M	G	-	K	-	P
:	:		:		:
M	G	P	K	K	P

Traceback (blue) from optimal score

STAMP - Multiple Structural Alignments

1. Initial Alignment Inputs

- Multiple Sequence alignment
- Ridged Body "Scan"
- Pairwise Alignments and Hierarchical Clustering

2. Refine Initial Alignment & Produce Multiple Structural Alignment

$$P_{ij} = \left\{ e^{-d_{ij}^2/2E_1} \right\} \left\{ e^{-s_{ij}^2/2E_2} \right\}$$

probability that residue i on structure A is equivalent to residue j on structure B.

d_{ij} – distance between i & j

s_{ij} – conformational similarity; function of rms bewteen i-1, i, i+1 and j-1, j, j+1.

- •Dynamic Programming (Smith-Waterman) through P matrix gives optimal set of equivalent residues.
- •This set is used to re-superpose the two chains. Then iterate until alignment score is unchanged.
- •This procedure is performed for all pairs with no gap penalty

R. Russell, G. Barton (1992) *Proteins* **14:** 309 R.B. Russel, T. Walsh, G. Barton, STAMP version 4.4: User Guide, 2010.

Multiple Structural Alignments

STAMP – cont’d

2. Refine Initial Alignment & Produce Multiple Structural Alignment

Alignment score:

$$S_C = \frac{S_p}{L_P} \frac{L_p - i_A}{L_A} \frac{L_p - i_B}{L_B}$$

$$S_p = \sum_{\text{aln.path}} P_{ij}$$

L_p, L_A, L_B – length of alignment, sequence A, sequence B

i_A, i_B – length of gaps in A and B.

Multiple Alignment:

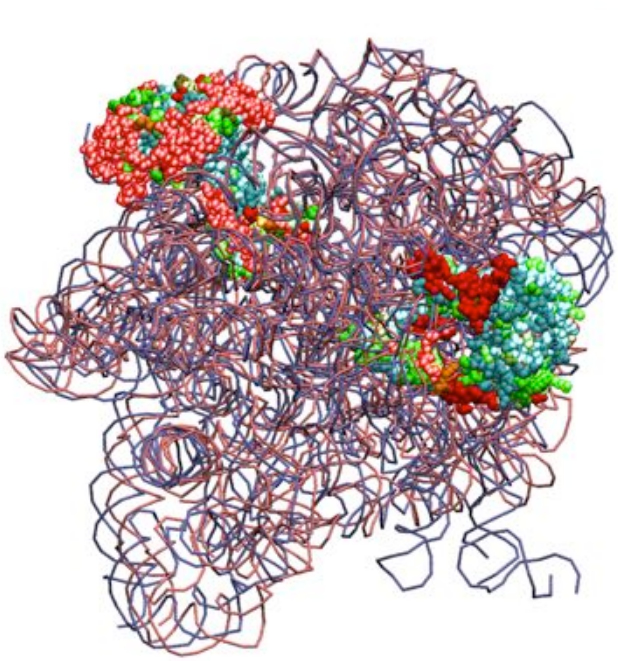
•Create a dendrogram using the alignment score.

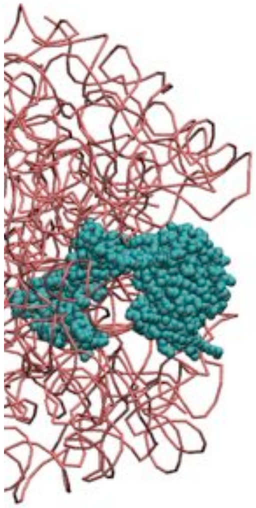
•Successively align groups of proteins (from branch tips to root).

•When 2 or more sequences are in a group,
then average coordinates are used.

Structural Overlaps - STAMP

Ribosome large subunit showing ribosomal proteins L2 and L3
180,000 atoms in 4 rRNAs and 58 proteins





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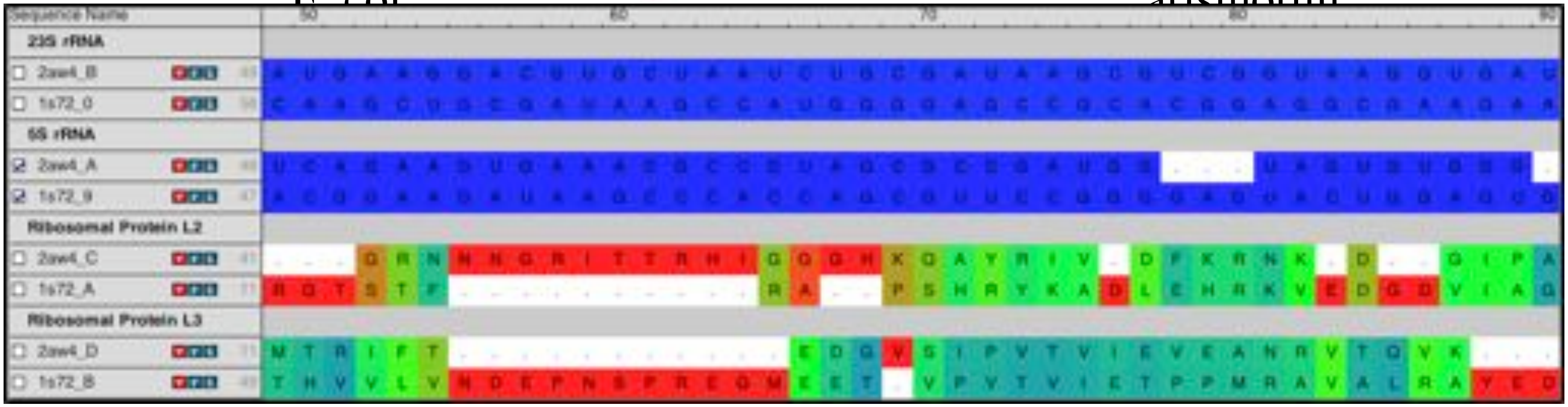
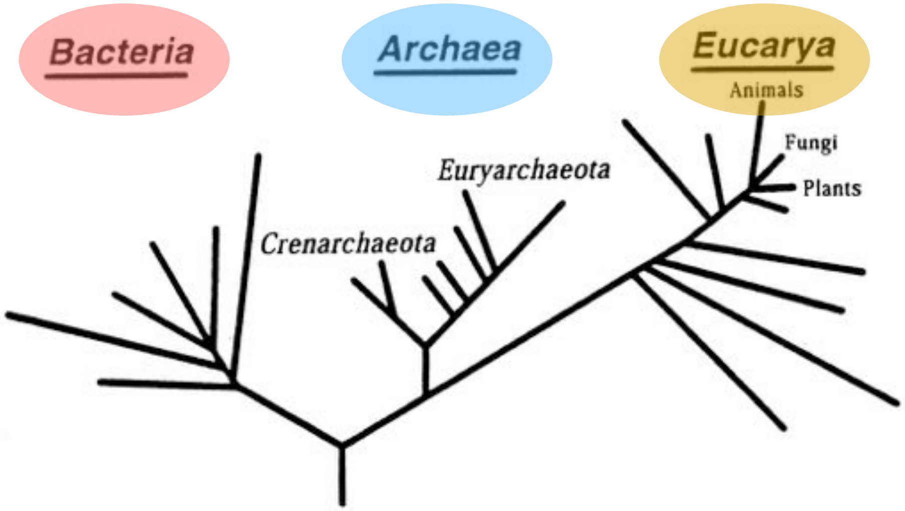
arismortui



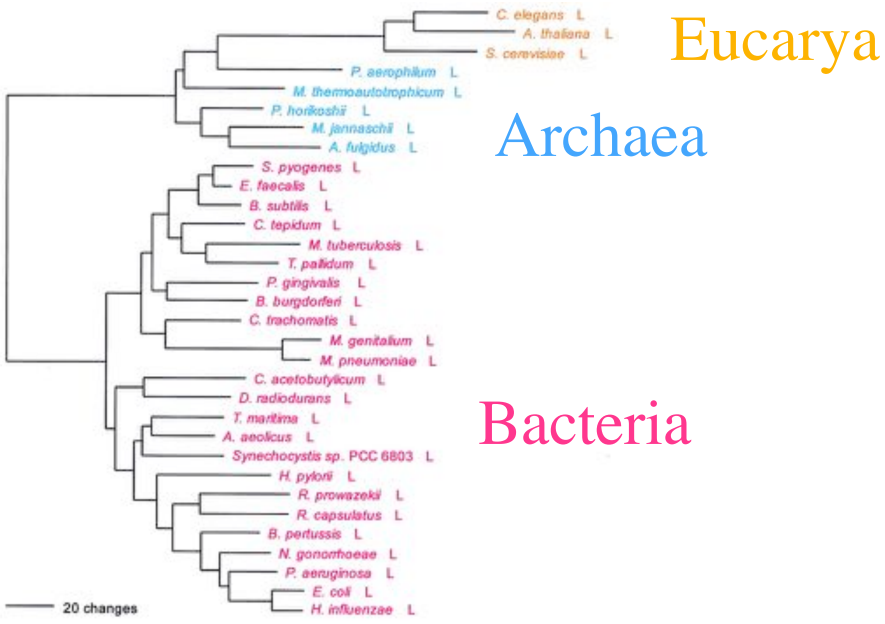
Sequence Name		50 · 60 · 70 · 80 · 90
23S rRNA		
□ 2aw4_B	49	A U G A A G G A C U U G C U A A U C U G C G A U A A G C U U C G G U A A G G U G A C
□ 1s72_0	50	C A A G C G G C G A U A A G C C A U G G G G A G C C G C A C G G A G G C G A A G A A
5S rRNA		
☑ 2aw4_A	48	U C A G A A G U G A A A C G C C G U A G C G C C G A U G G . . . U A G U G U G G G .
☑ 1s72_9	47	A C G G A A G A U A A G C C C C A C C A G C G U U C C G G U G A G U A C U U G A G C G
Ribosomal Protein L2		
□ 2aw4_C	41	. . . G R N N N G R I T T R H I G G G H K G A Y R I V . D F K R N K . D . . G I P A
□ 1s72_A	11	R G T S T F . . . . R A . . P S H R Y K A D L C H R K V E D G D V I A G
Ribosomal Protein L3		
□ 2aw4_D	31	M T R I F T . . . . E D G V S I P V T V I E V E A N R V T Q V K . . .
□ 1s72_B	49	T H V V L V N D E F N S P R E G M E E T . V P V T V I E T P P M R A V A L R A Y E D

Universal Phylogenetic Tree

3 domains of life



Reference 16S rRNA tree

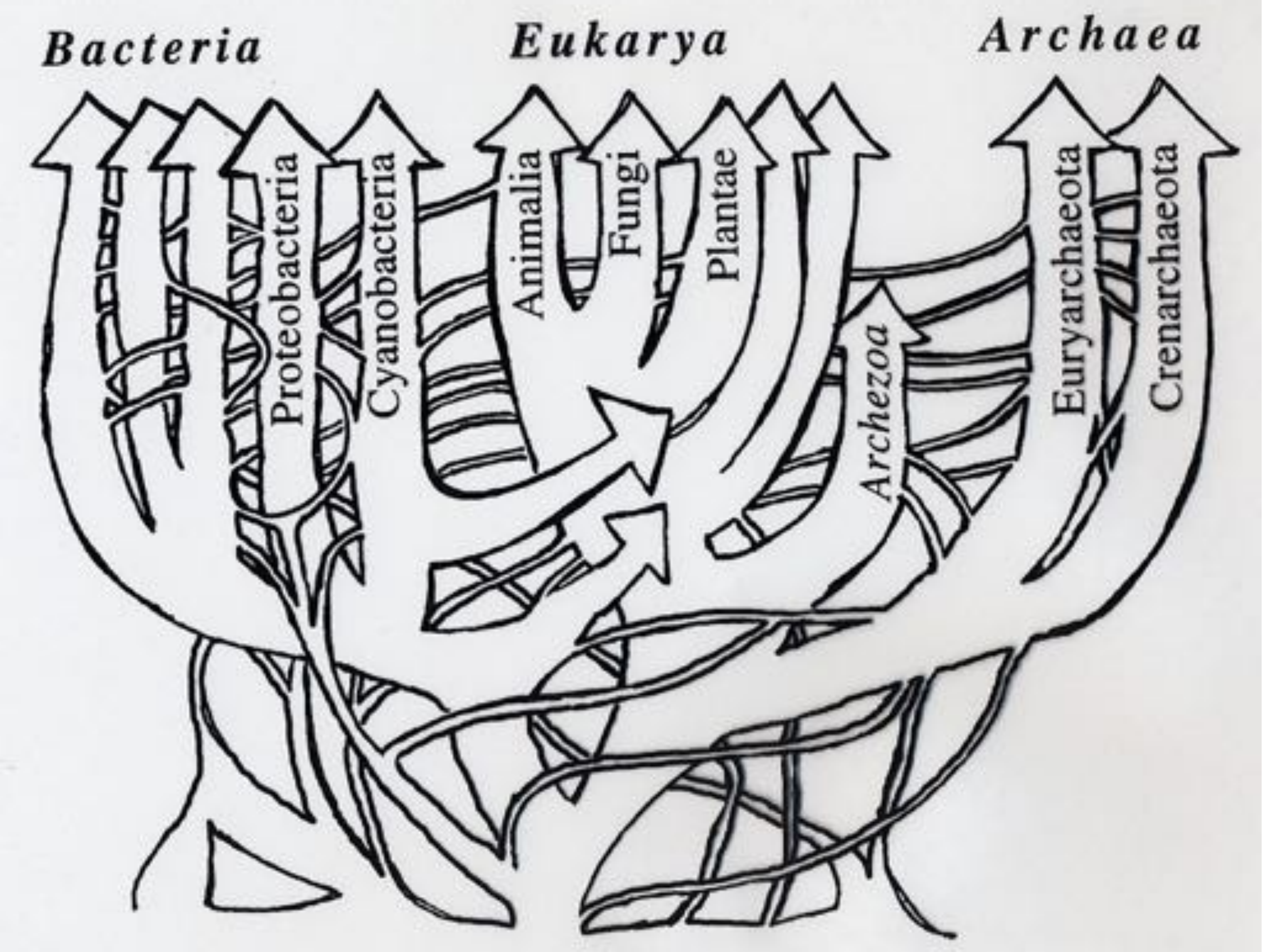


Leucyl-tRNA synthetase displays the full canonical phylogenetic distribution.

For review see Woese *PNAS* 2000

Woese, Olsen, Ibba, Soll *MMBR* 2000

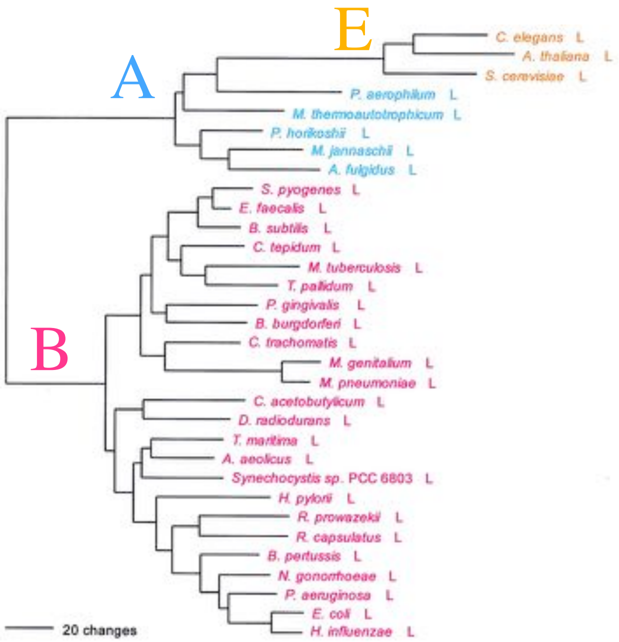
Look for horizontal gene transfer events



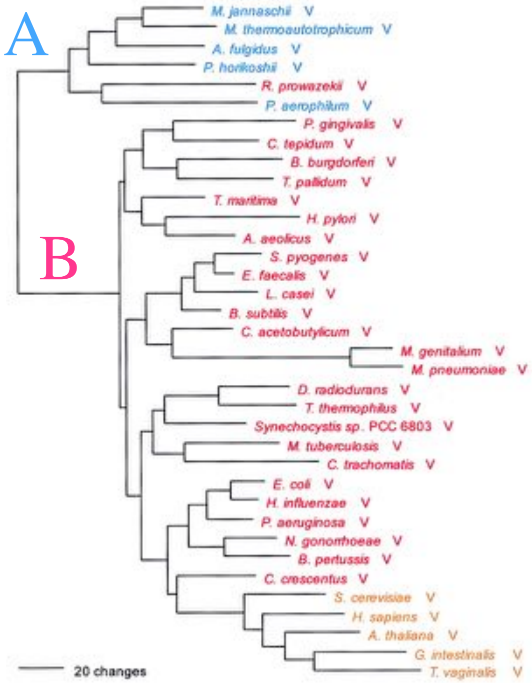
After W. Doolittle, modified by G. Olsen

Phylogenetic Distributions

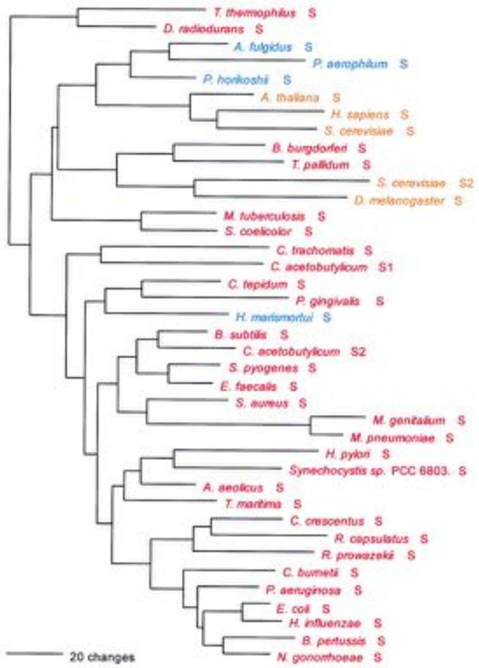
Full Canonical



Basal Canonical



Non-canonical



increasing inter-domain of life Horizontal Gene Transfer

"HGT erodes the historical trace, but does not completely erase it…." G. Olsen

Woese, Olsen, Ibba, Soll *MMBR* 2000

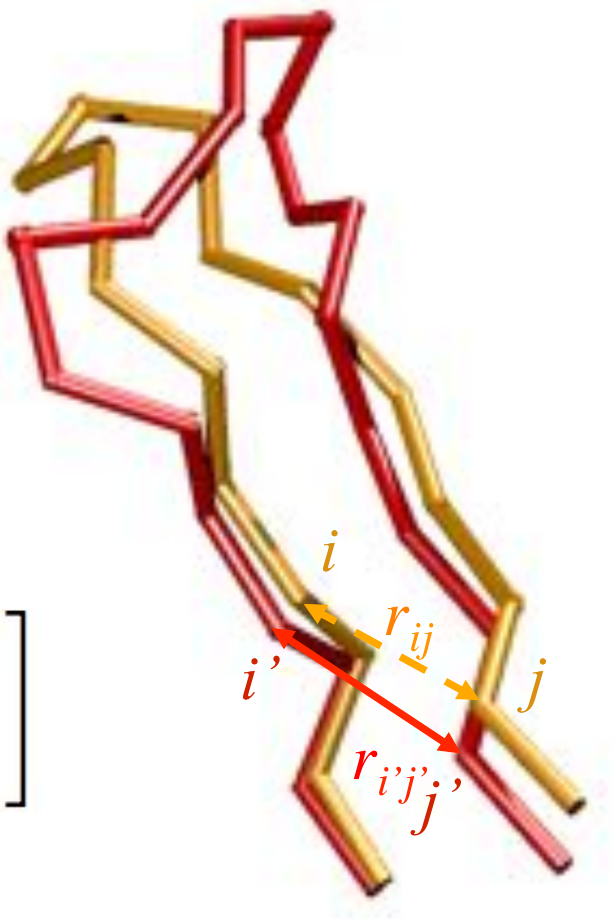
Protein Structure Similarity Measure

Q_H Structural Homology

fraction of native contacts for aligned residues + presence and perturbation of gaps

$$Q_H = \aleph \left[q_{aln} + q_{gap} \right]$$

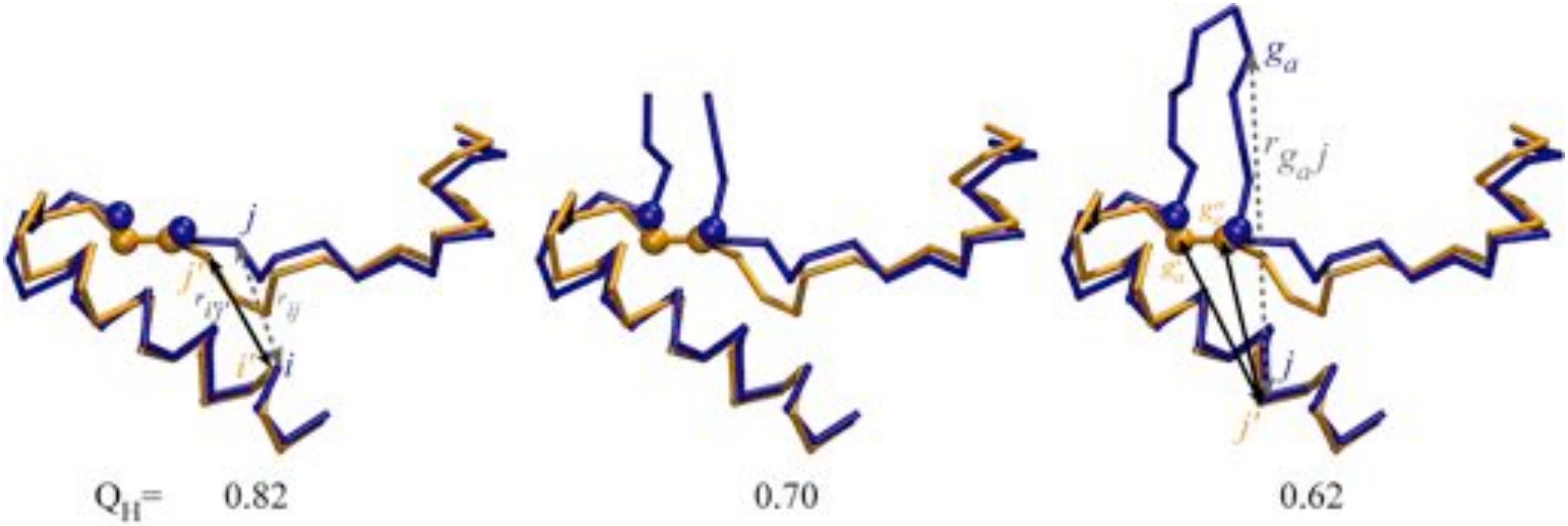
$$q_{aln} = \sum_{i<j-2} \exp\left[-\frac{(r_{ij} - r_{i'j'})^2}{2\sigma_{ij}^2} \right]$$

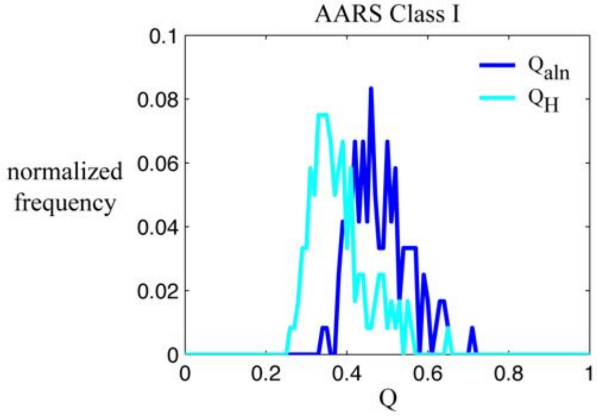


O'Donoghue & Luthey-Schulten *MMBR* 2003.

Structural Similarity Measure: The effect of insertions

“Gaps should count as a character but not dominate” C. Woese



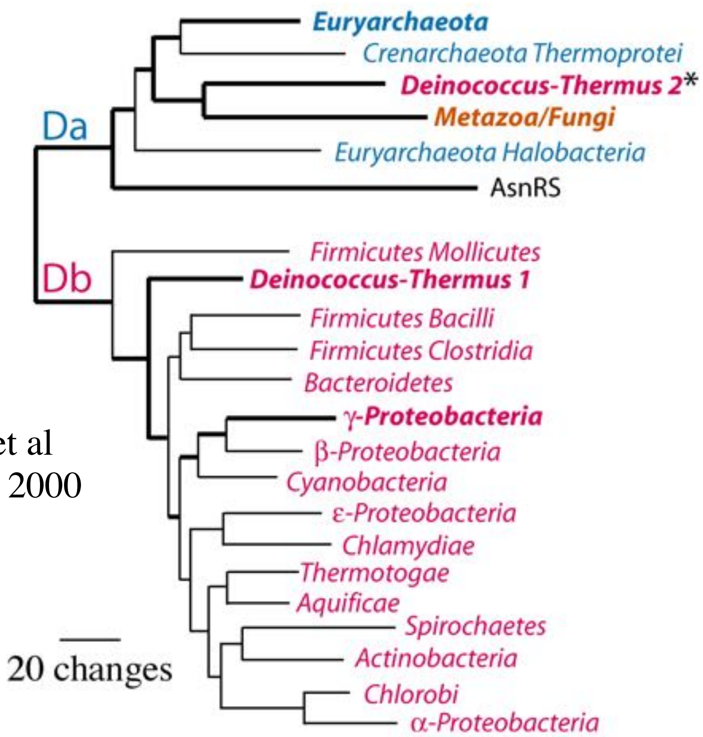


$$q_{gap} = \sum_{g_a} \sum_{j}^{N_{aln}} \max \left\{ \exp\left[-\frac{\left(r_{g_a j} - r_{g'_a j'}\right)^2}{2\sigma^2_{g_a j}} \right], \exp\left[-\frac{\left(r_{g_a j} - r_{g''_a j'}\right)^2}{2\sigma^2_{g_a j}} \right] \right\}$$

$$+ \sum_{g_b} \sum_{j}^{N_{aln}} \max \left\{ \exp\left[-\frac{\left(r_{g_b j} - r_{g'_b j'}\right)^2}{2\sigma^2_{g_b j}} \right], \exp\left[-\frac{\left(r_{g_b j} - r_{g''_b j'}\right)^2}{2\sigma^2_{g_b j}} \right] \right\}$$

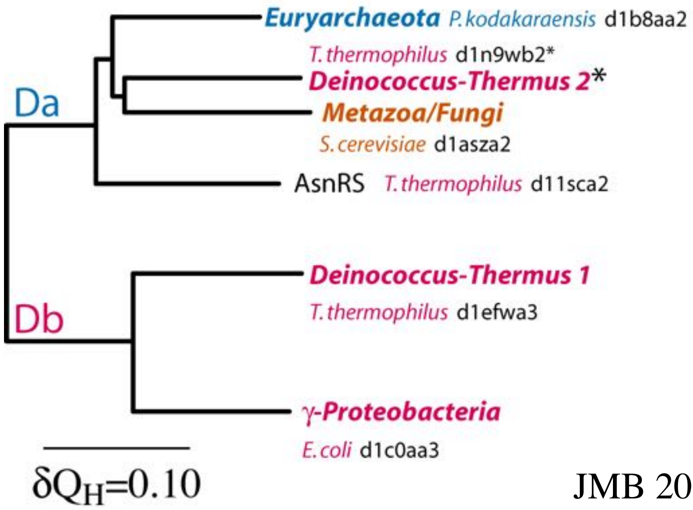
Structure encodes evolutionary information!

sequence-based phylogeny

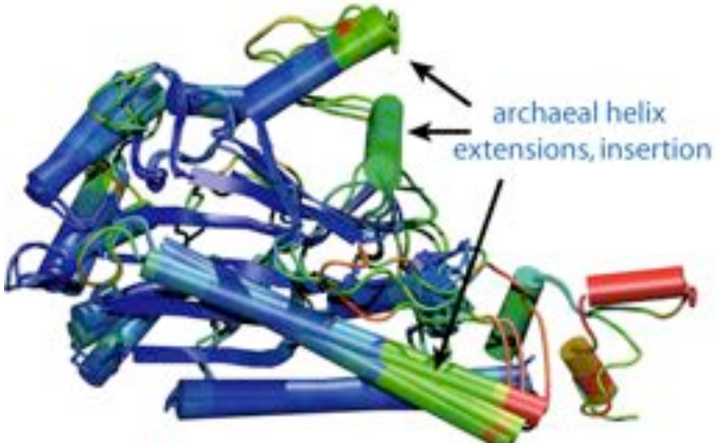


Woese et al
MMBR 2000

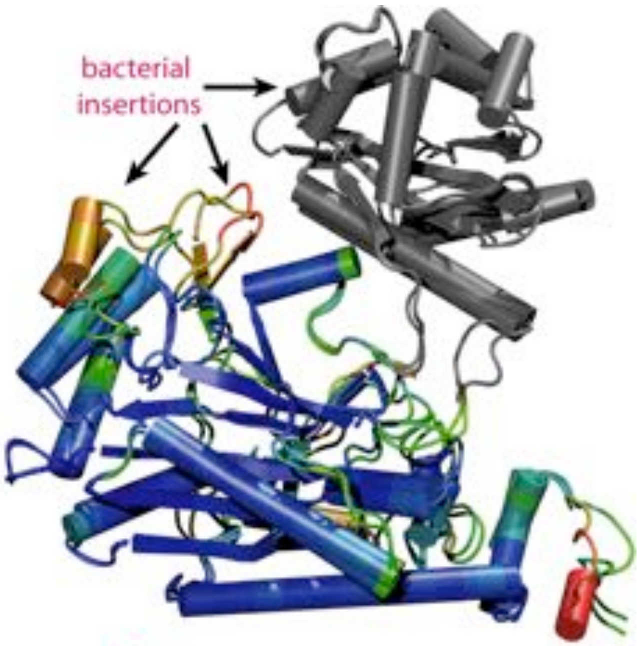
structure-based phylogeny



JMB 2005
MMBR 2003



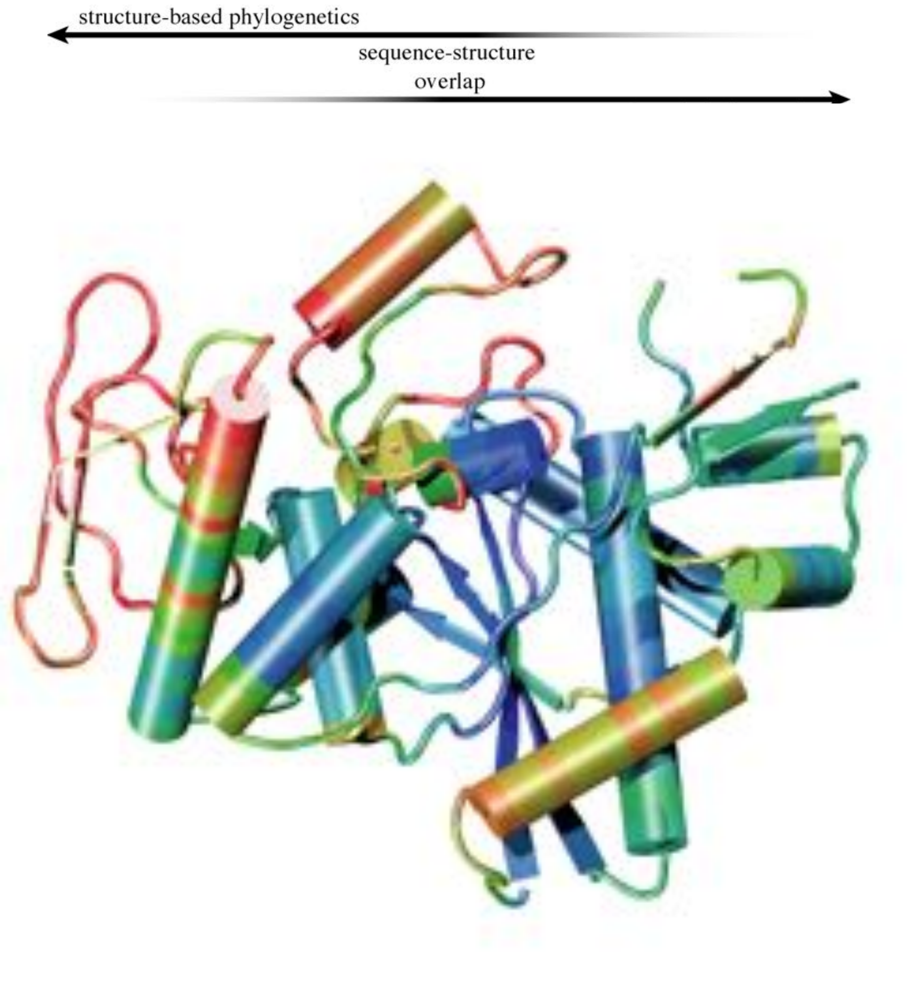
Da - AspRS archaeal genre



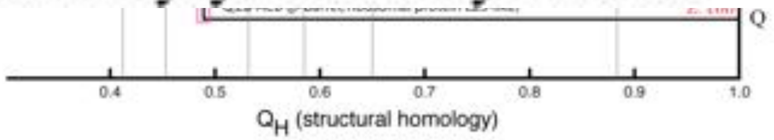
Db - AspRS bacterial genre

Structure reveals distant evolutionary events

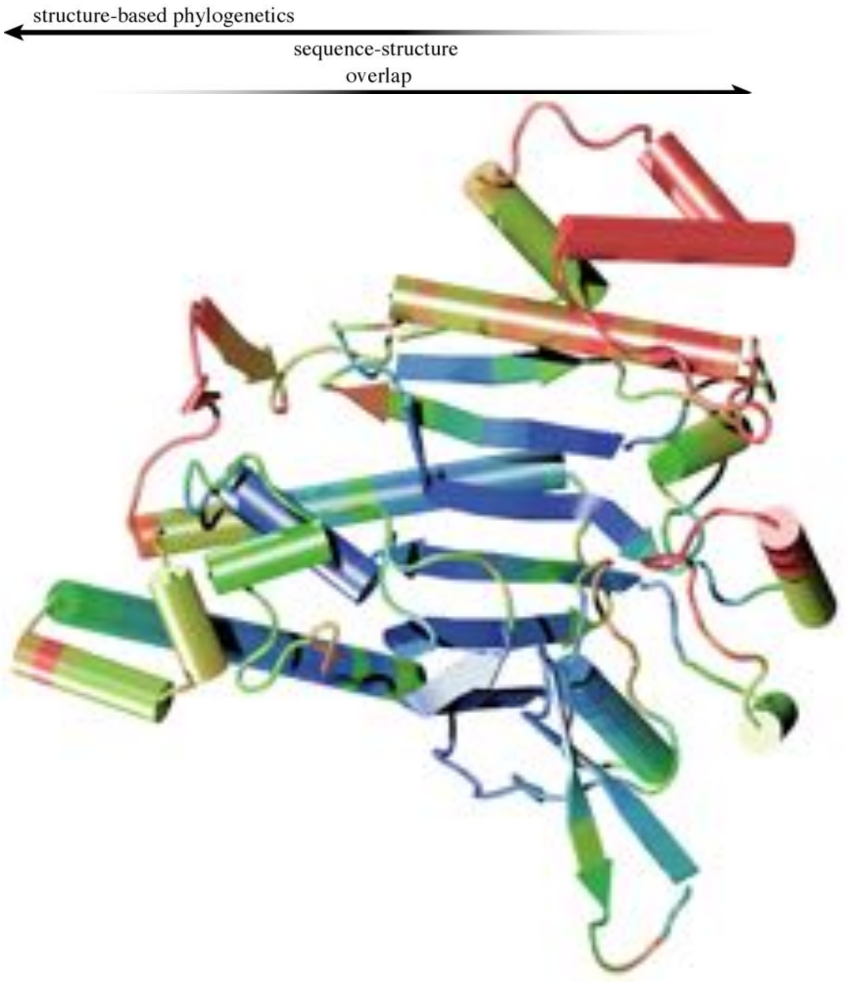
Class I AARSs



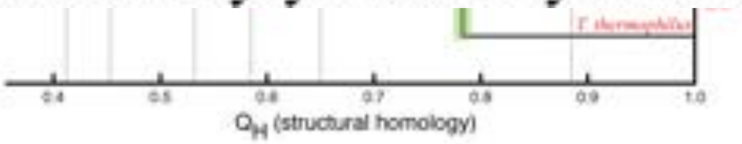
Class I Lysyl-tRNA Synthetase



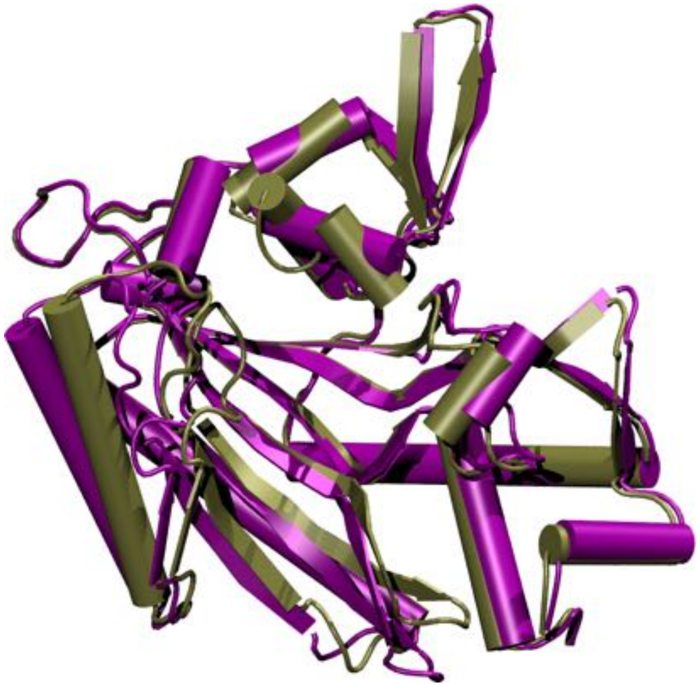
Class II AARSs



Class II Lysyl-tRNA Synthetase



Sequences define more recent evolutionary events



Conformational changes in the same protein.

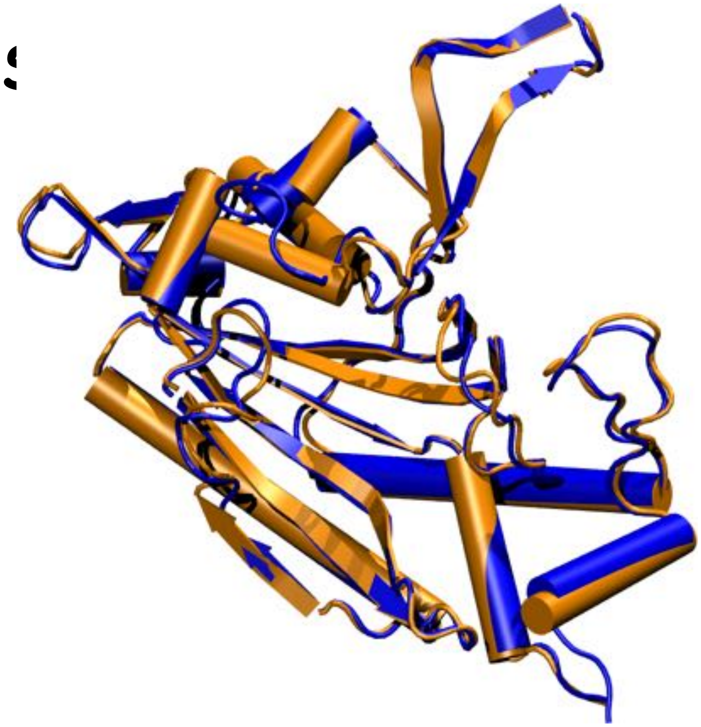
ThrRS

T-AMP analog, 1.55 A.

T, 2.00 A.

$Q_H = 0.80$

Sequence identity = 1.00



Structures for two different species.

ProRS

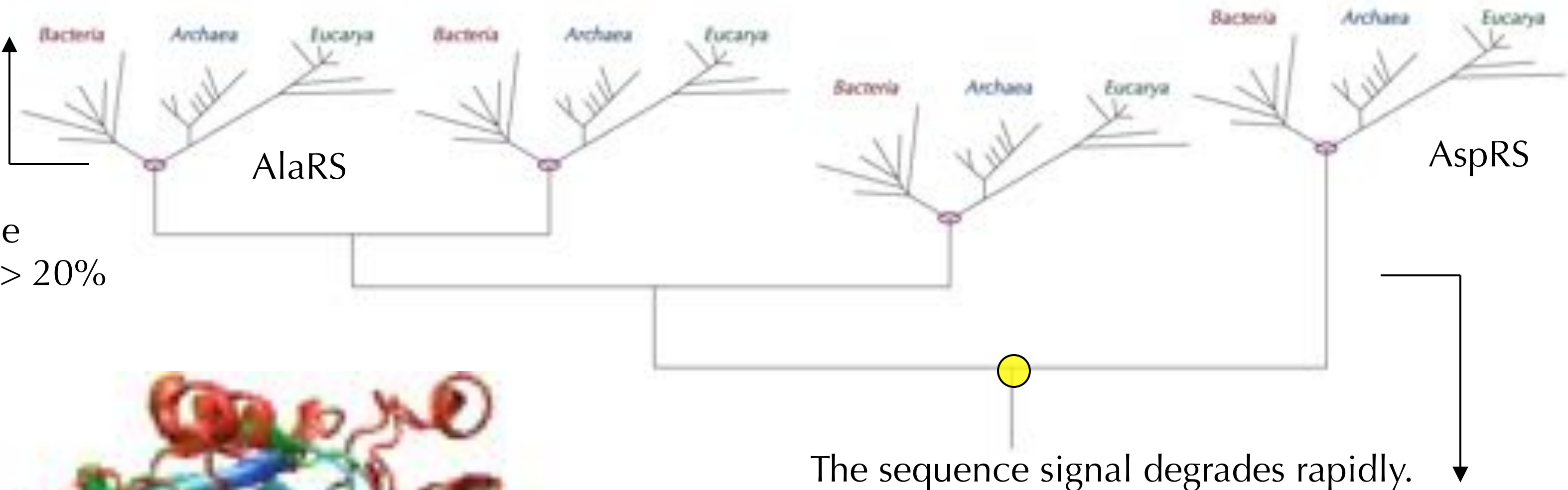
M. jannaschii, 2.55 A.

M. thermoautotrophicus, 3.20 A.

$Q_H = 0.89$

Sequence identity = 0.69

Relationship Between Sequence & Structure

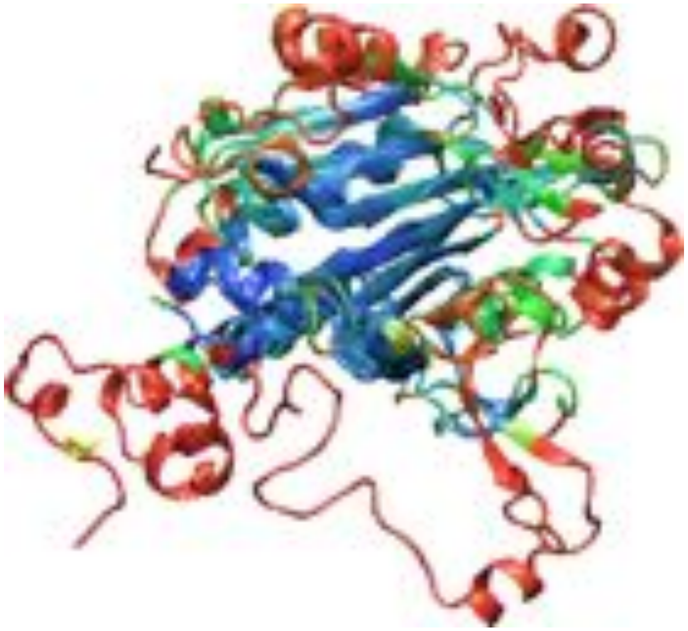


AlaRS

AspRS

sequence
identity > 20%

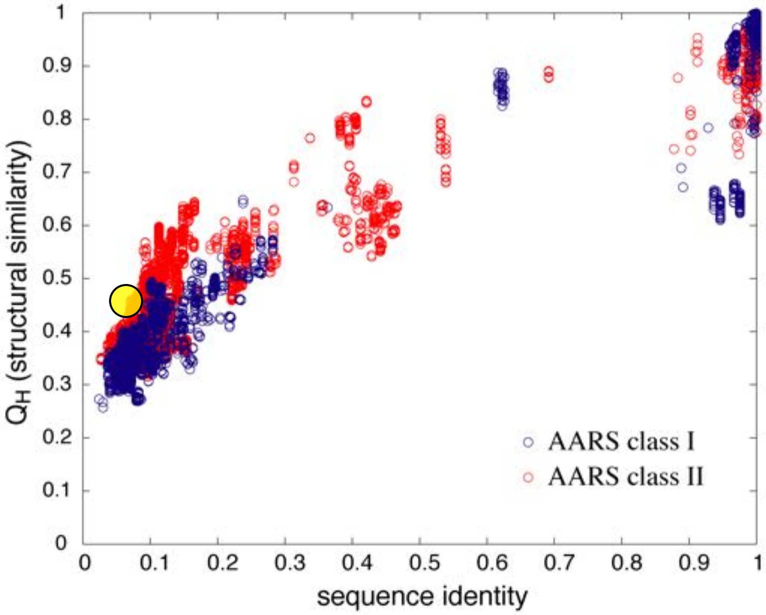
The sequence signal degrades rapidly.
sequence identity < 10%



Structural superposition of AlaRS & AspRS.

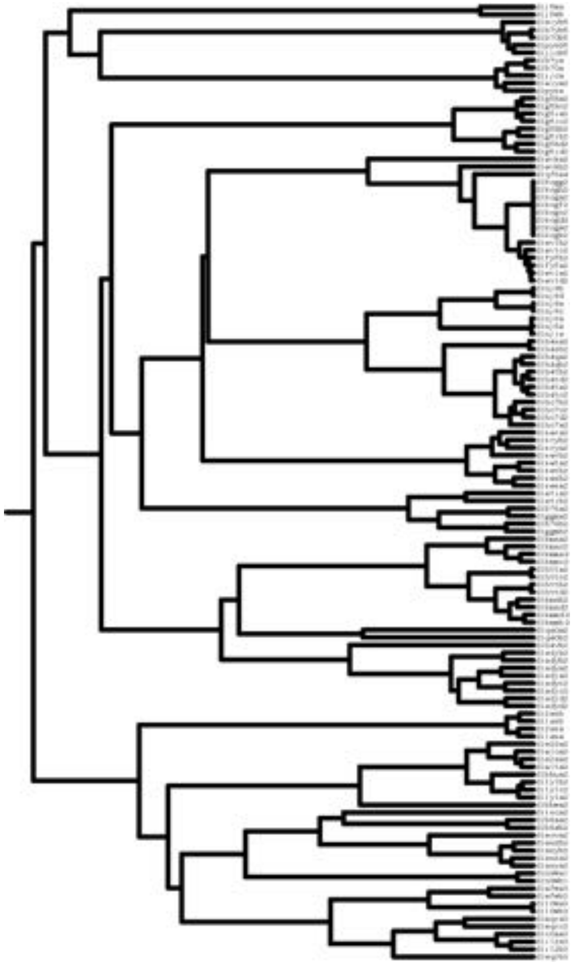
● Sequence id = 0.055, Q_H= 0.48

O'Donoghue & Luthey-Schulten (2003) *MMBR* 67: 550–73.
Structural alignment & visualization software MultiSeq/VMD



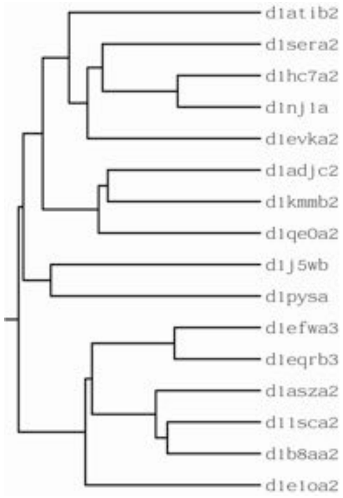
Non-redundant Representative Profiles

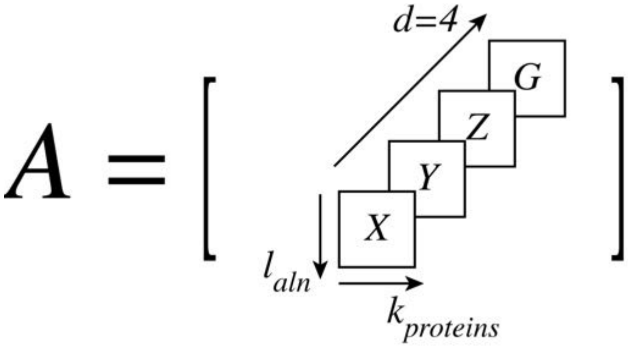
Too much information
129 Structures

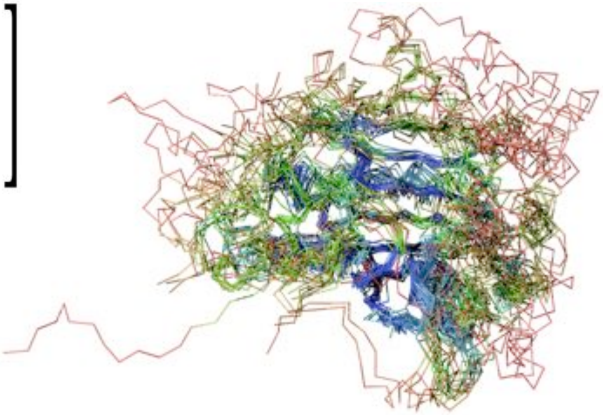


Multidimensional QR factorization of alignment matrix, *A*.

Economy of information
16 representatives







QR computes a set of maximal linearly independent structures.

P. O’Donoghue and Z. Luthey-Schulten (2003) *MMBR* **67**:550-571.

P. O’Donoghue and Z. Luthey-Schulten (2005) *J. Mol. Biol.*, **346**, 875-894.

Numerical Encoding of Proteins in a Multiple Alignment

Encoding Structure

Rotated Cartesian + Gap = 4-space

Aligned position $(x_{C_\alpha}, y_{C_\alpha}, z_{C_\alpha}, 0)$

Gapped position $(0, 0, 0, g)$

Gap Scaling $g = \gamma \dfrac{\|X\|_{F_4} + \|Y\|_{F_4} + \|Z\|_{F_4}}{\|G\|_{F_4}}$

adjustable parameter

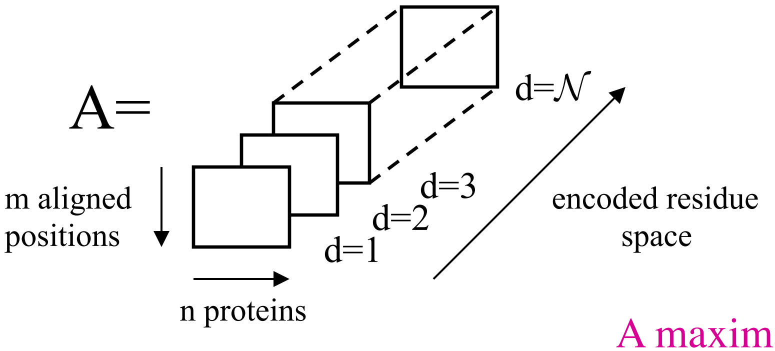
Sequence Space

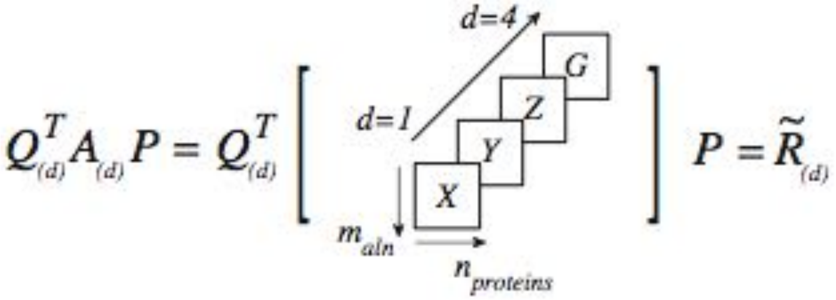
Orthogonal Encoding = 24-space

23 amino acids (20 + B, X, Z) + gap

A = (1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
B = (0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
C = (0,0,1,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0)
…
GAP = (0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,1)

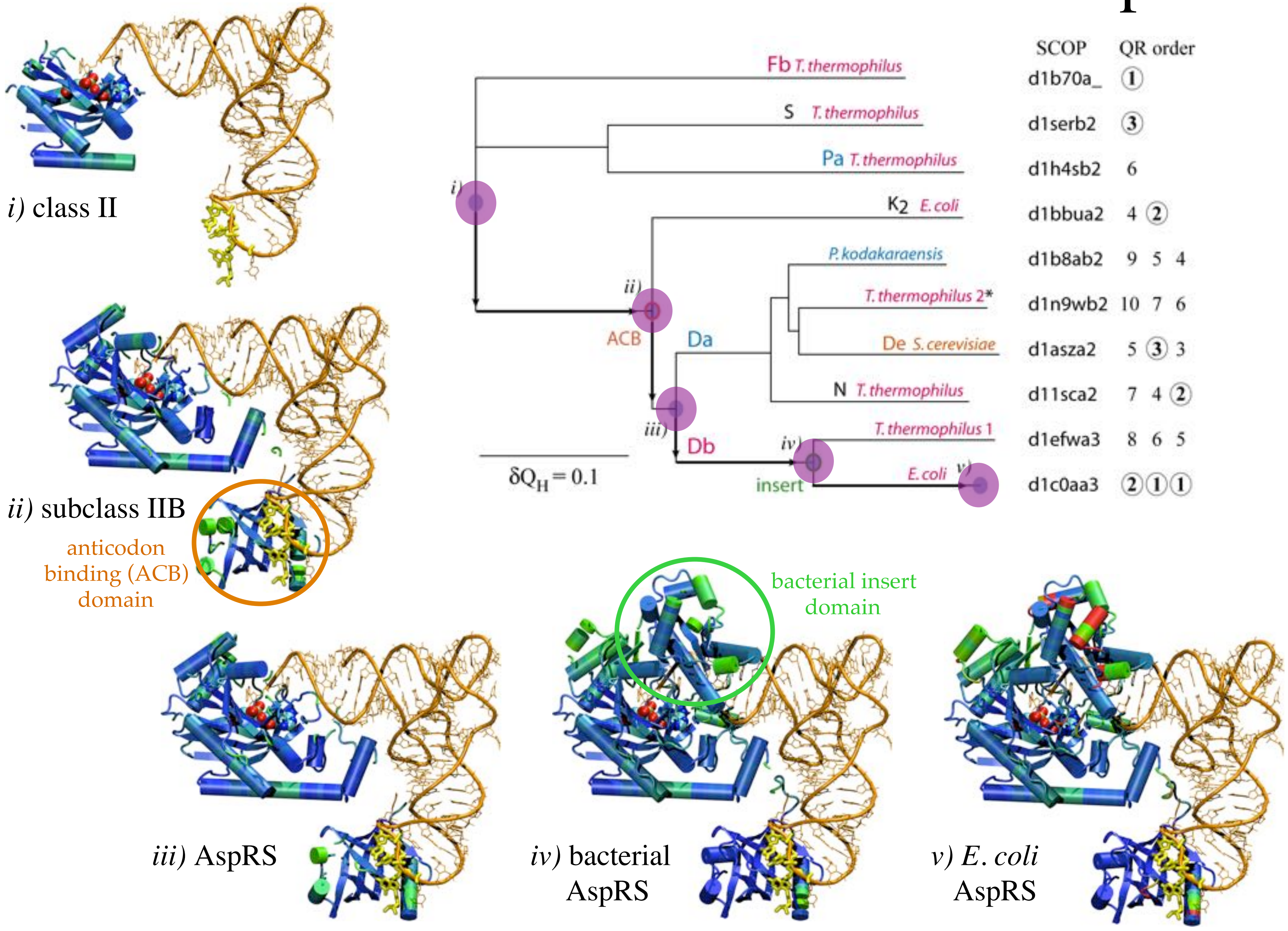
Alignment is a Matrix with Linearly Dependent Columns





A maximal linearly independent subset can be determined with respect to a threshold, e.g., similarity measure threshold.

Evolution of Structure and Function in AspRS



SCOP	QR order		
d1b70a_	①		
d1serb2	③		
d1h4sb2	6		
d1bbua2	4	②	
d1b8ab2	9	5	4
d1n9wb2	10	7	6
d1asza2	5	③	3
d11sca2	7	4	②
d1efwa3	8	6	5
d1c0aa3	②	①	①

Summary Structural Evolutionary Profiles

1. Structures often more conserved than sequences!! Similar structures at the Family and Superfamily levels.

Add more structural information to identify core and variable regions

2. Which structures and sequences to include? Use evolution and eliminate redundancy with QR factorization

New Tools in VMD/MultiSeq

Protein / RNA
Sequence Data

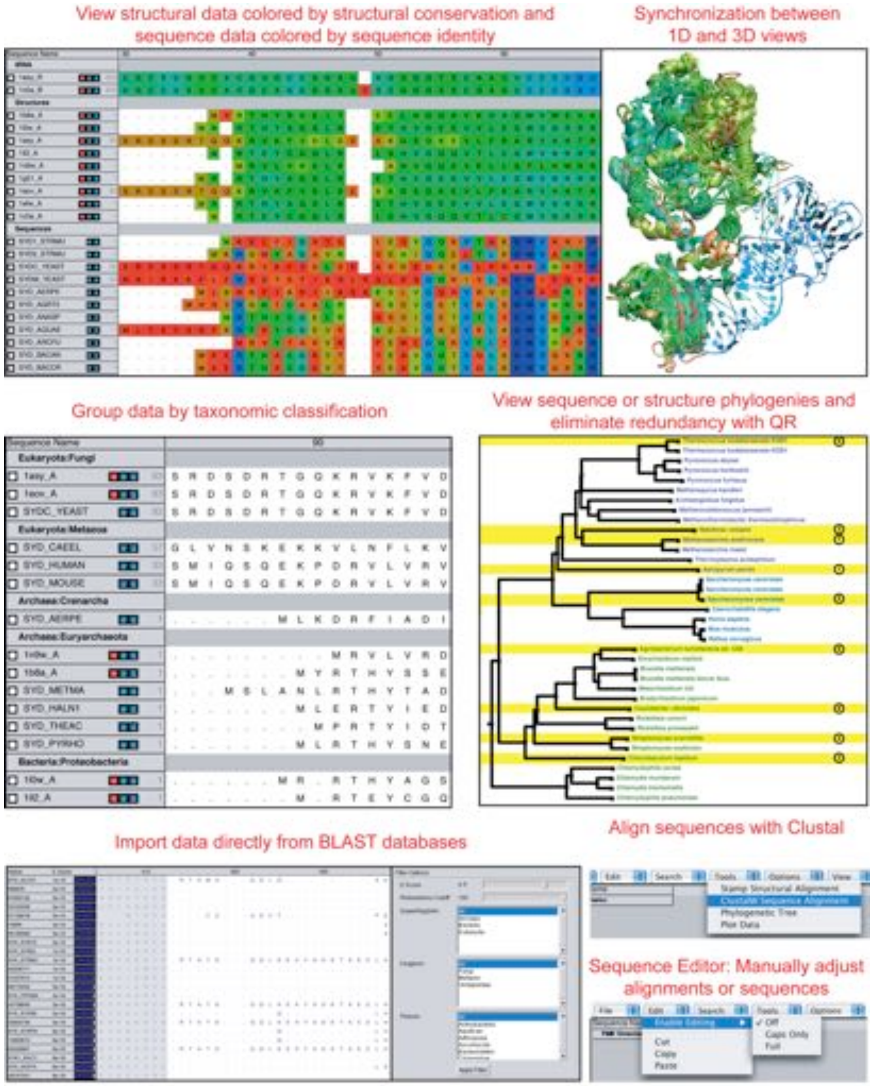
SwissProt DB (400K),
Greengenes RNA (100K)
Signatures, Zoom

Metadata Information,
Clustal &
Phylogenetic Trees

RAXml Trees,
Genomic Content,
Temperature DB

Blast & PsiBlast

Sequence Editor


View structural data colored by structural conservation and sequence data colored by sequence identity

Synchronization between 1D and 3D views

Group data by taxonomic classification

View sequence or structure phylogenies and eliminate redundancy with QR

Import data directly from BLAST databases

Align sequences with Clustal

Sequence Editor: Manually adjust alignments or sequences

Sequence /Structure
Alignment

Protein & RNA
secondary structure

QR non-redundant
seq / str sets

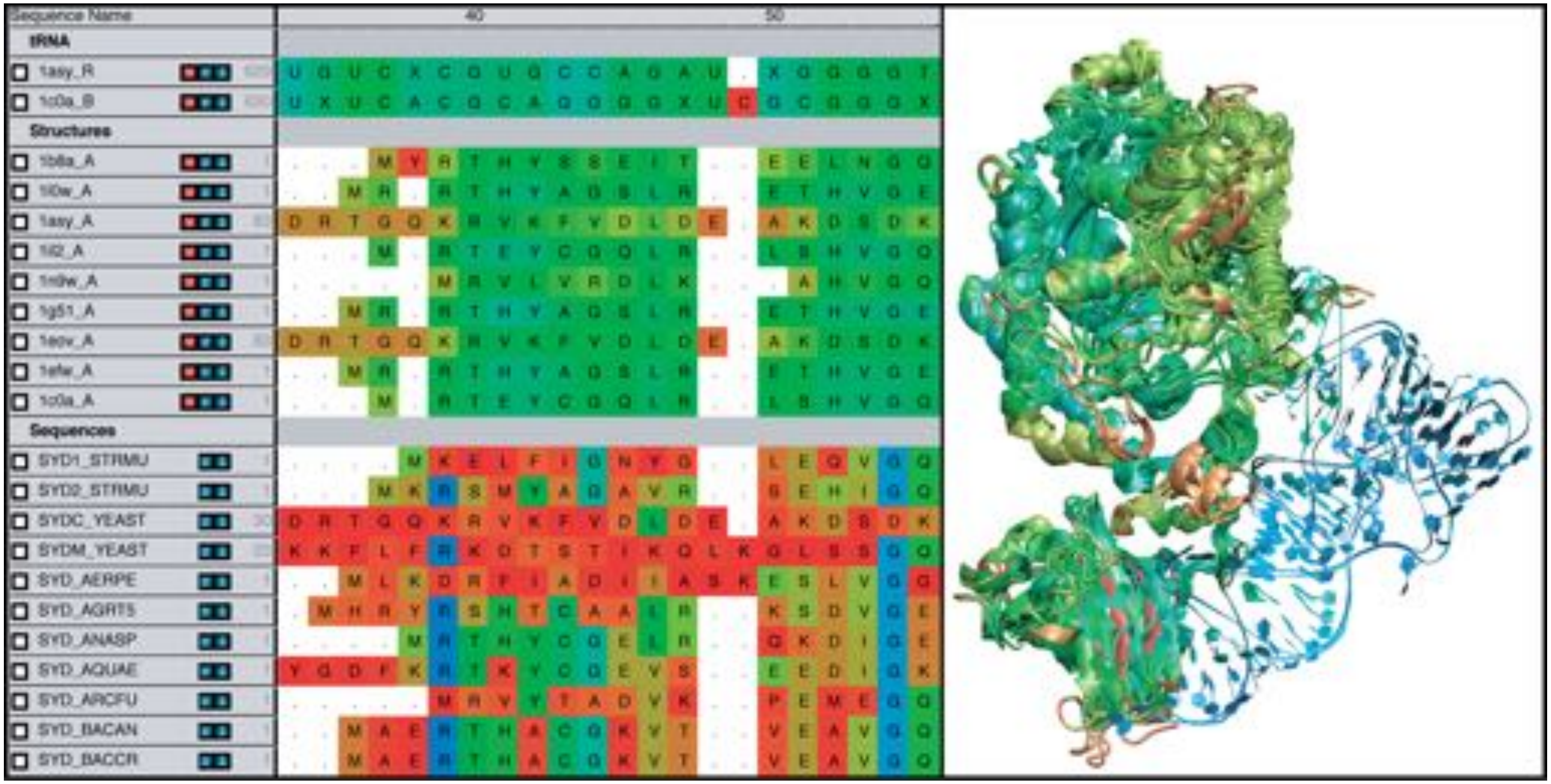
Cluster
analysis /
Bioinformatics
scripting

Tutorials MultiSeq/
AARS
EF-Tu/Ribosome

J. Eargle, D. Wright, Z. Luthey-Schulten, *Bioinformatics,* 22:504 (2006)
E. Roberts, J. Eargle, D. Wright, Z. Luthey-Schulten, *BMC Bioinformatics,* 7:382 (2006)

MultiSeq Combines Sequence and Structure

- Align sequences or structures; manually edit alignments
- View data colored by numerous metrics including structural conservation and sequence similarity
- Synchronized coloring between 1D and 3D views



```
Sequence Name                    40                            50
tRNA
□ 1asy_R        U G U C X C G U G C C A G A U . X G G G G T
□ 1c0a_B        U X U C A C G C A G G G G X U C G C G G G X
Structures
□ 1b8a_A        . . . M Y R T H Y S S E I T . . E E L N G Q
□ 1l0w_A        . . M R . R T H Y A G S L R . . E T H V G E
□ 1asy_A        D R T G Q K R V K F V D L D E . A K D S D K
□ 1il2_A        . . . M . R T E Y C G Q L R . . L S H V G Q
□ 1n9w_A        . . . . . M R V L V R D L K . . . A H V G Q
□ 1g51_A        . . M R . R T H Y A G S L R . . E T H V G E
□ 1eov_A        D R T G Q K R V K F V D L D E . A K D S D K
□ 1efw_A        . . M R . R T H Y A G S L R . . E T H V G E
□ 1c0a_A        . . . M . R T E Y C G Q L R . . L S H V G Q
Sequences
□ SYD1_STRMU    . . . . M K E L F I G N Y G . . L E Q V G Q
□ SYD2_STRMU    . . . M K R S M Y A G A V R . . S E H I G Q
□ SYDC_YEAST    D R T G Q K R V K F V D L D E . A K D S D K
□ SYDM_YEAST    K K F L F R K D T S T I K Q L K G L S S G Q
□ SYD_AERPE     . . M L K D R F I A D I I A S K E S L V G G
□ SYD_AGRT5     . M H R Y R S H T C A A L R . . K S D V G E
□ SYD_ANASP     . . . . M R T H Y C G E L R . . G K D I G E
□ SYD_AQUAE     Y G D F K R T K Y C G E V S . . E E D I G K
□ SYD_ARCFU     . . . . . M R V Y T A D V K . . P E M E G Q
□ SYD_BACAN     . . M A E R T H A C G K V T . . V E A V G Q
□ SYD_BACCR     . . M A E R T H A C G K V T . . V E A V G Q
```

Variation in structures

Variation in sequences

Load large sequence sets*

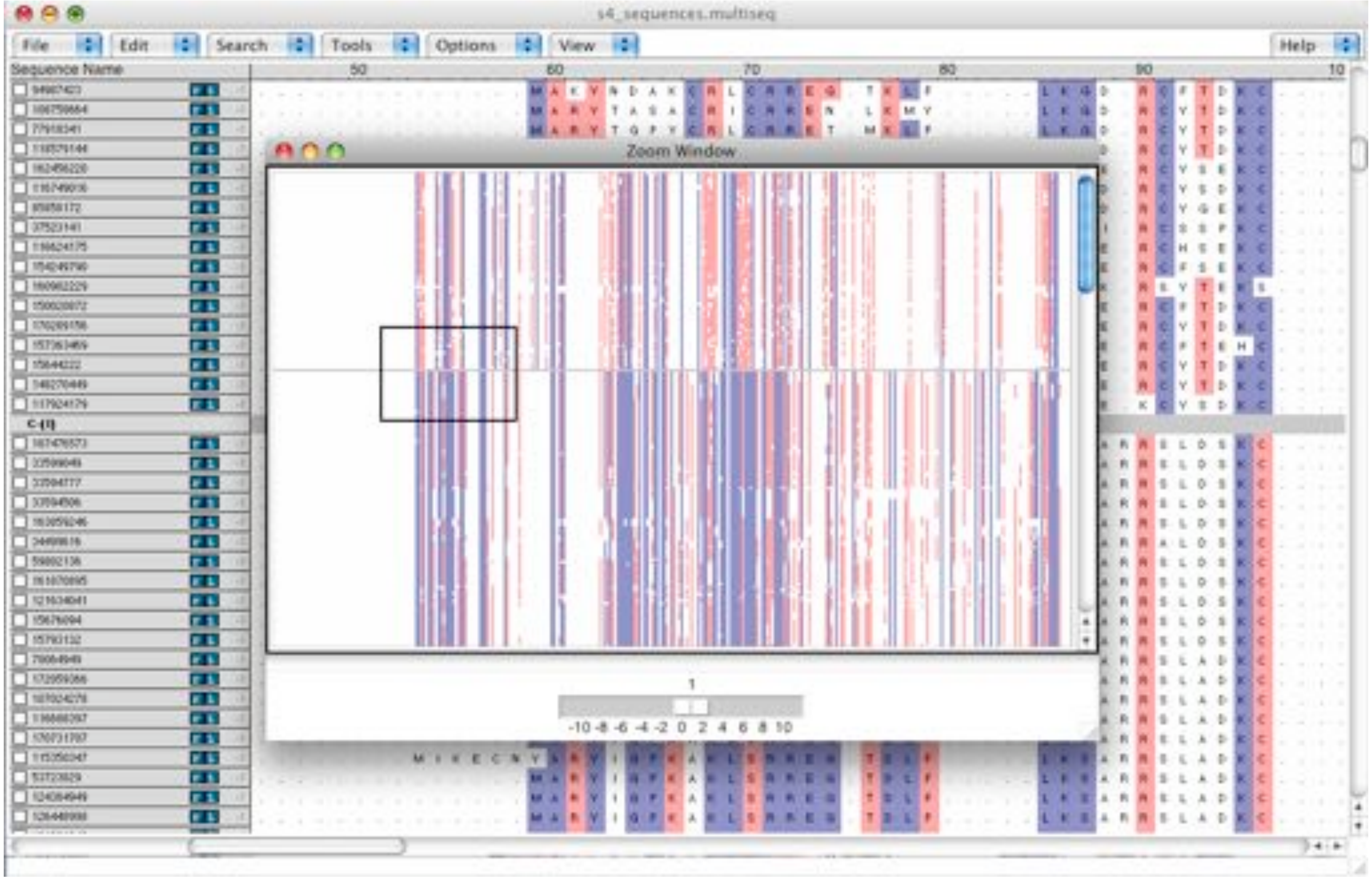
Swiss-Prot (Proteins)	Greengenes (RNA)*
Curated sequences	Environmental 16S rRNA
392,667 sequences	90,654 entries
Unaligned	Aligned (7682 positions)
177 MB on disk	670 MB on disk
2 minutes to load	2.5 minutes to load *
2.4 GB memory used	4.0 GB memory used*

*"Signatures of ribosomal evolution" with Carl Woese, PNAS (2008)

*Release May 2013 contains 1.2 million sequences – Memory??

Sequence editor

- New sequence API allows editing of large alignments. Align closely related sequences by group, combine groups, and then manually correct.
- Zoom window gives an overview of the alignment, quickly move the editing window to any part of the alignment.

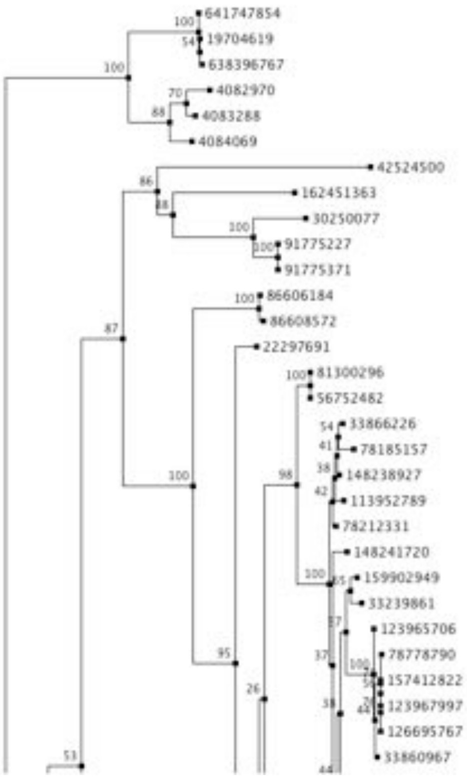


660 sequences of ribosomal protein S4 from all complete bacterial genomes*.

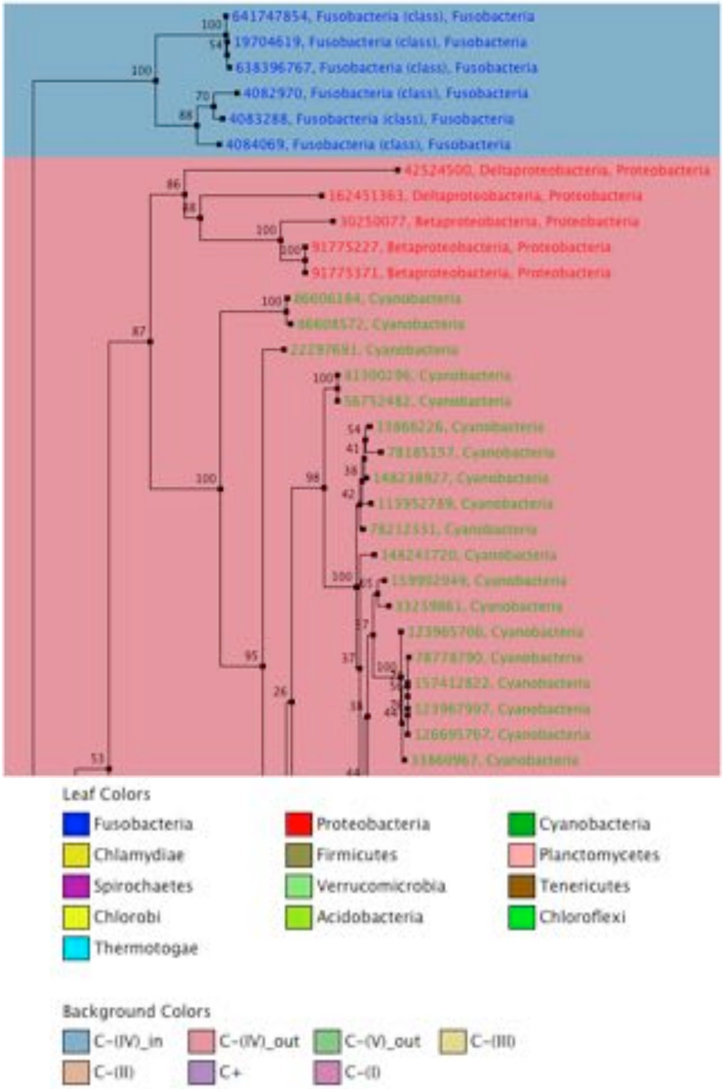
* K. Chen, E. Roberts, Z Luthey-Schulten (2009) BMC Bioinformatics

Phylogenetic tree editor

- Automatically add annotations and colors to phylogenetic trees based on taxonomy, enzyme, temperature class, and/or MultiSeq groupings.



Maximum likelihood tree of 660 S4 sequences reconstructed using RAxML.



A cluster of five proteobacterial sequences branch near the cyanobacterial sequences. These are cases of horizontal gene transfer.

Elijah Roberts 2009

Scripting MultiSeq

- All MultiSeq functions can be scripted.
- Scripting an analysis provides benefits:
  - It can be checked for correctness.
  - It can be quickly repeated by anyone.
  - It can be modified later with new functionality.
  - It can be run on a cluster in VMD text mode. (if it can be easily broken into independent chunks)
- Many functions are too user specific and/or too complex to be turned into a GUI.
- Some examples of MultiSeq scripts…

Genome content

- When using sequence from fully sequenced genomes, additional information is available in the genome content.
- Conservation of gene ordering, neighbors, or intergenic regions can provide additional evolutionary information not contained in the sequence.
- Gene names and ordering can be obtained from the genome PTT files, want to organize the information in an evolutionarily meaningful manner.

```
Location          Strand Length PID         Gene  Synonym     Code  COG          Product
3437638..3438021   -     127    16131173    rplQ  b3294 -     COG0203J     50S ribosomal subunit protein L17
3438062..3439051   -     329    16131174    rpoA  b3295 -     COG0202K     RNA polymerase, alpha subunit
3439077..3439697   -     206    16131175    rpsD  b3296 -     COG0522J     30S ribosomal subunit protein S4
3439731..3440120   -     129    16131176    rpsK  b3297 -     COG0100J     30S ribosomal subunit protein S11
3440137..3440493   -     118    16131177    rpsM  b3298 -     COG0099J     30S ribosomal subunit protein S13
3440640..3440756   -     38     16131178    rpmJ  b3299 -     COG0257J     50S ribosomal subunit protein L36
3440788..3442119   -     443    16131179    secY  b3300 -     COG0201U     preprotein translocase membrane subunit
3442127..3442561   -     144    16131180    rplO  b3301 -     COG0200J     50S ribosomal subunit protein L15
3442565..3442744   -     59     16131181    rpmD  b3302 -     COG1841J     50S ribosomal subunit protein L30
3442748..3443251   -     167    16131182    rpsE  b3303 -     COG0098J     30S ribosomal subunit protein S5
```

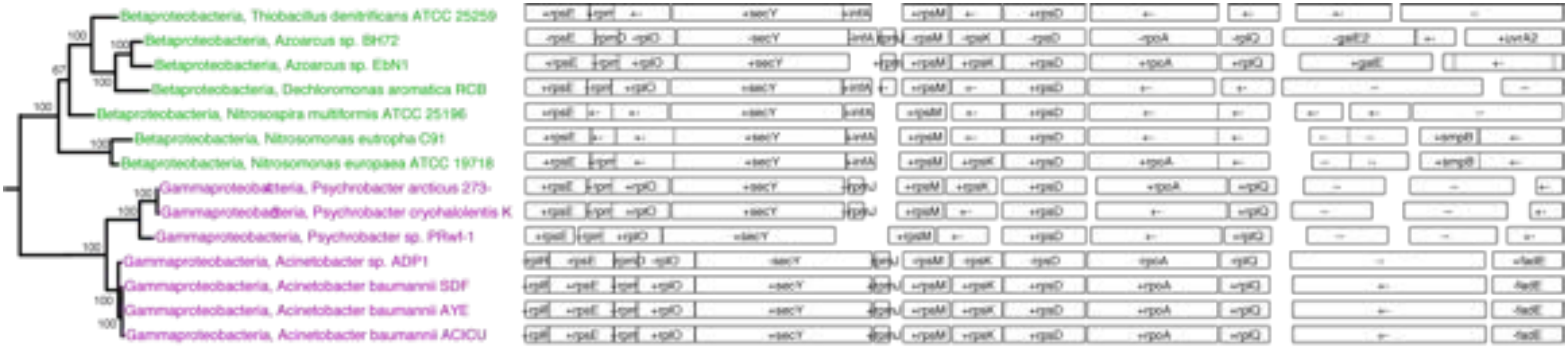
Combined genomic context/phylogenetic tree

- Use a script to walk through a phylogenetic tree, find the genome content near the source gene, create a graphical representation of the combined data.

```
proc draw_genome_context_of_phylogeny {args} {

    # Load the sequences.
    set alignment [::SeqData::Fasta::loadSequences $alignmentFilename]

    # Load the tree
    set tree [::PhyloTree::Newick::loadTreeFile $treeFilename]

    # Reorder the alignment by the tree.
    set treeAlignment {}
    set leafNodes [::PhyloTree::Data::getLeafNodes $tree]
    foreach node $leafNodes {
        set foundNode 0
        set nodeName [::PhyloTree::Data::getNodeName $tree $node]
        foreach sequence $alignment {
            if {$nodeName == [::SeqData::getName $sequence]} {
                lappend treeAlignment $sequence
                set foundNode 1
                break
            }
        }
    }

    # Draw the genomic context.
    drawGenomicContextOfAlignment $outputFilename $treeAlignment $contextDistance $scaling $genomeDirectory
}
```

Combined genomic context/phylogenetic tree

```
proc drawGenomicContextOfAlignment {outputFilename alignment contextDistance scaling genomeDirectory} {

    foreach sequence $alignment {

        # Make sure we have the GI number for this sequence.
        set giNumber [::SeqData::getSourceData $sequence "gi"]

        # Make sure we can tell which genome this sequence is from.
        set taxonomy [join [::SeqData::getLineage $sequence 1 0 1] ","]
        if {![info exists genomeTaxonomyMap($taxonomy)]} {
            error "ERROR) Unknown genome for sequence [::SeqData::getName $sequence]: $taxonomy"
        }

        # Go through each of the genome context files for the genome.
        set foundGene 0
        foreach genomeName $genomeTaxonomyMap($taxonomy) {
            ...
        }
    }

    # Draw the genomic context.
    drawMultipleGenomicContext $outputFilename $alignment $geneFiles $genePositions $geneStrands $contextDistance
}
```

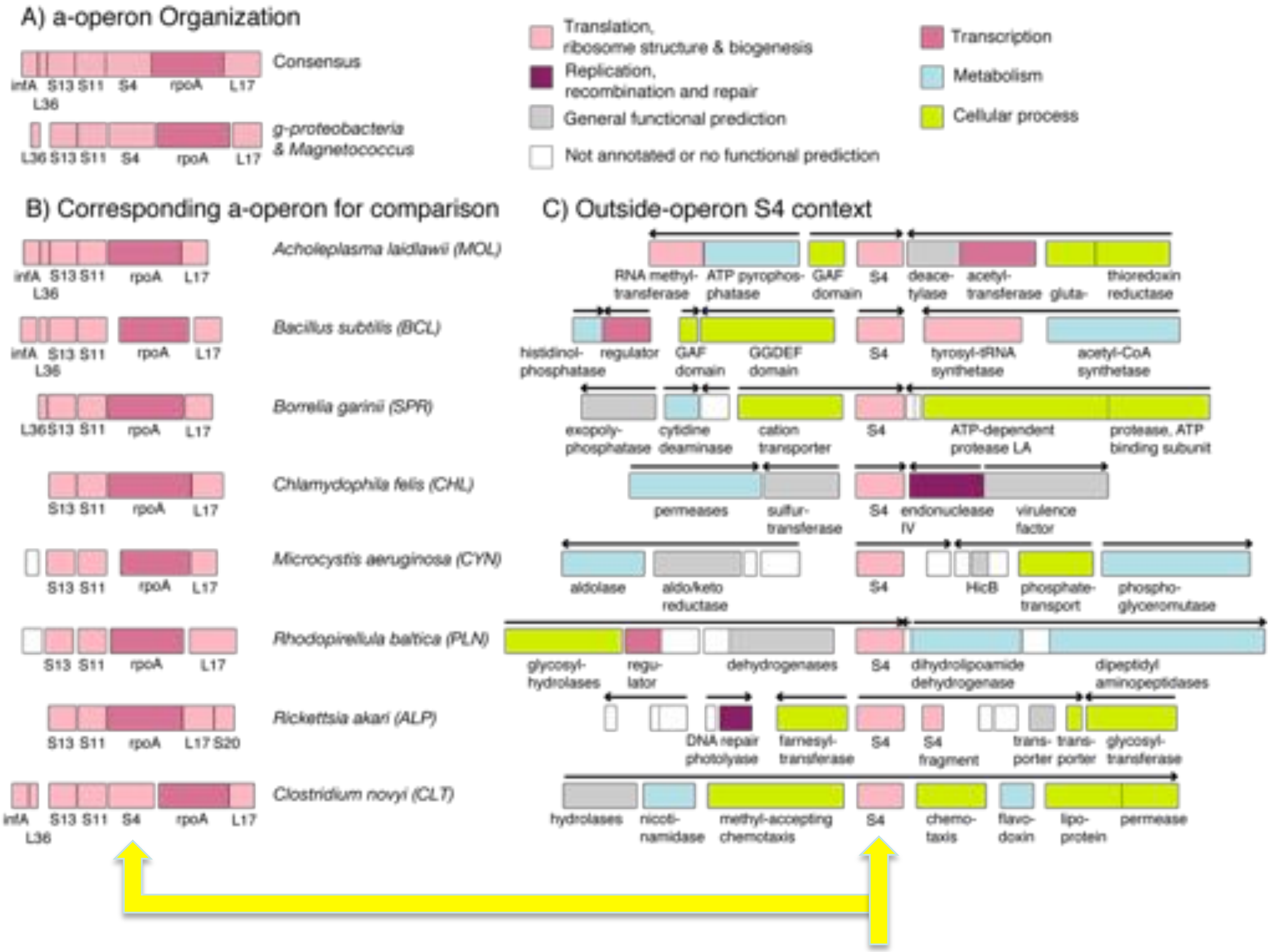

Betaproteobacteria, Thiobacillus denitrificans ATCC 25259
Betaproteobacteria, Azoarcus sp. BH72
Betaproteobacteria, Azoarcus sp. EbN1
Betaproteobacteria, Dechloromonas aromatica RCB
Betaproteobacteria, Nitrosospira multiformis ATCC 25196
Betaproteobacteria, Nitrosomonas eutropha C91
Betaproteobacteria, Nitrosomonas europaea ATCC 19718
Gammaproteobacteria, Psychrobacter arcticus 273-
Gammaproteobacteria, Psychrobacter cryohalolentis K
Gammaproteobacteria, Psychrobacter sp. PRwf-1
Gammaproteobacteria, Acinetobacter sp. ADP1
Gammaproteobacteria, Acinetobacter baumannii SDF
Gammaproteobacteria, Acinetobacter baumannii AYE
Gammaproteobacteria, Acinetobacter baumannii ACICU
100
100
67
100
100
100
100
100
100
100
100

Genome content future directions

- Genome content still a work in progress.
- Good candidate for a GUI: combined phylogenetic tree/ genome content viewer.
- Can also use COG codes to color by gene function.
- Still need API for manipulating PTT files.

Roberts, Chen, ZLS, **BMC Evol. Bio**. 2009

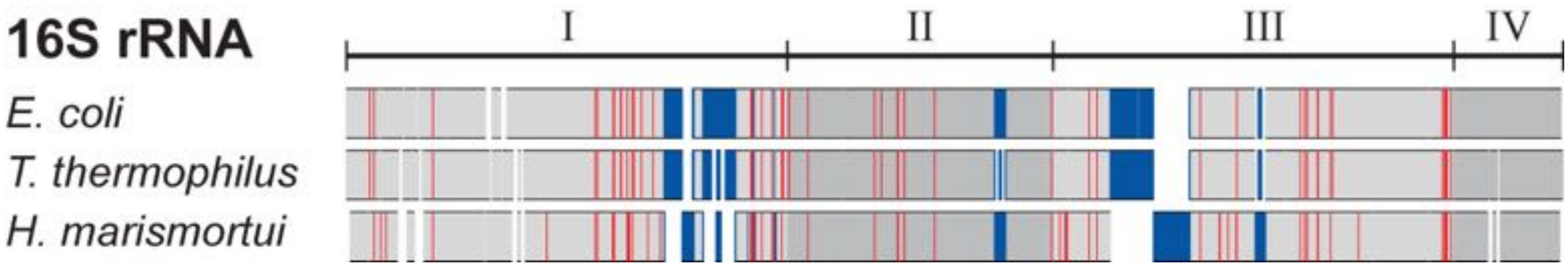
See also ITEP for microbial genomes, Benedict et al. **BMC Genomics 2014**



Genome content of ribosomal protein S4 by occurrence of the gene in the alpha operon.

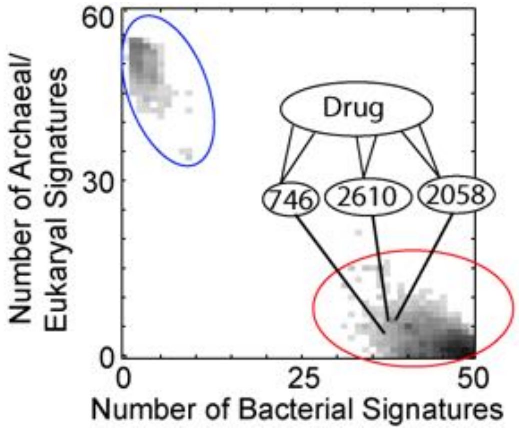
Fifteen Clostridia genomes contain two copies of S4: one zinc-binding and one zinc-free.

Molecular Signatures of Translation- Drug Targets

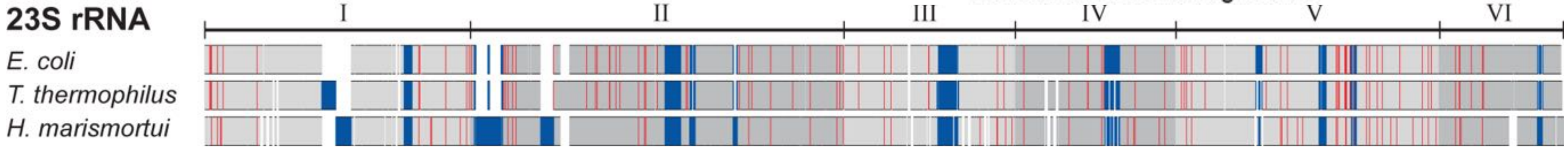


Ribosomal Signatures: Idiosyncrasies in rRNA and/or r-proteins characteristic of the domains of life

69 (119) & **6 (14)** in 16S (23S)



MultiSeq Zoom

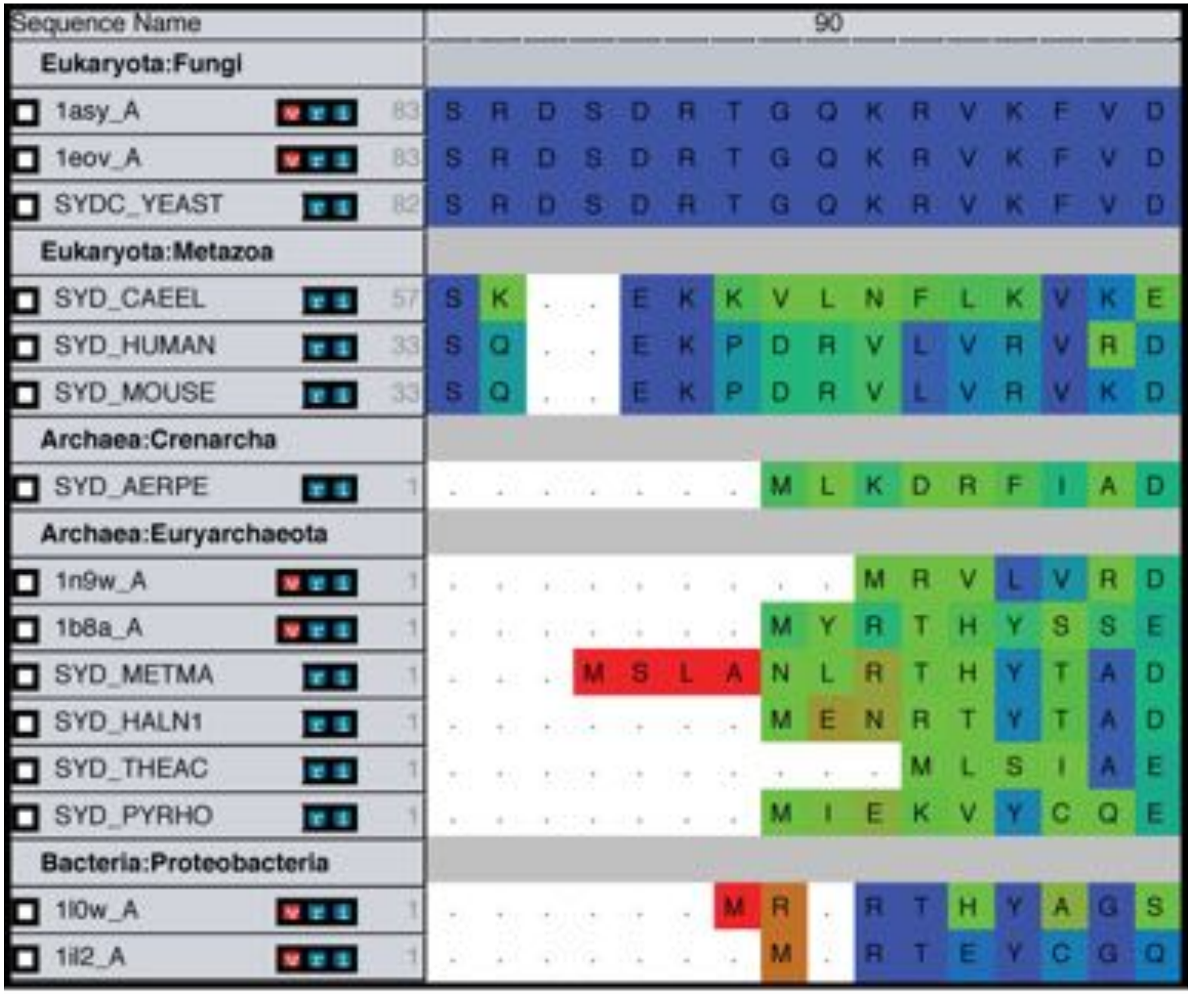


E. Roberts, A. Sethi, J. Montoya, C. R. Woese & Z. Luthey-Schulten. ***PNAS*** *"Molecular Signatures of Ribosomal Evolution" (2008)*

Kim,… Luthey-Schulten, Ha, and Woodson, ***Nature*** *"Protein-guided RNA dynamics during early ribosome assembly (2014)*

Flexible Grouping of Data

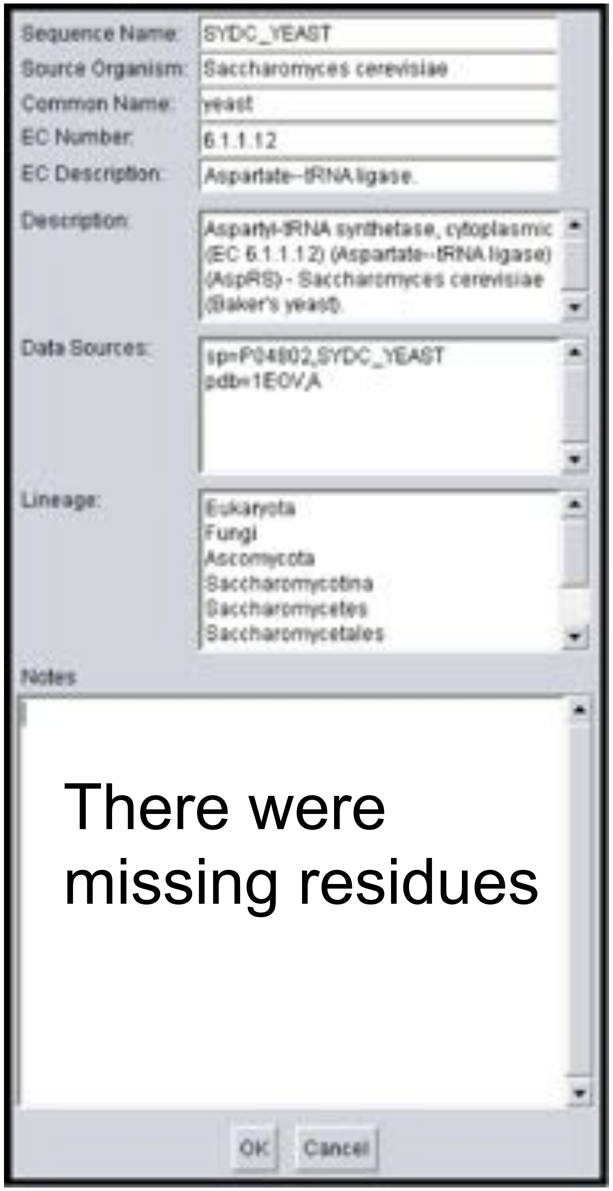
- Automatically group data by taxonomic classification to assist in evolutionary analysis (HGT) or create custom groups
- Apply metrics to groups independently, e.g bacterial signal



Sequence Name										90							
Eukaryota:Fungi																	
□ 1asy_A	83	S	R	D	S	D	R	T	G	Q	K	R	V	K	F	V	D
□ 1eov_A	83	S	R	D	S	D	R	T	G	Q	K	R	V	K	F	V	D
□ SYDC_YEAST	82	S	R	D	S	D	R	T	G	Q	K	R	V	K	F	V	D
Eukaryota:Metazoa																	
□ SYD_CAEEL	57	S	K	.	.	E	K	K	V	L	N	F	L	K	V	K	E
□ SYD_HUMAN	33	S	Q	.	.	E	K	P	D	R	V	L	V	R	V	R	D
□ SYD_MOUSE	33	S	Q	.	.	E	K	P	D	R	V	L	V	R	V	K	D
Archaea:Crenarcha																	
□ SYD_AERPE	1	.	.	.	.	.	.	.	M	L	K	D	R	F	I	A	D
Archaea:Euryarchaeota																	
□ 1n9w_A	1	.	.	.	.	.	.	.	.	.	M	R	V	L	V	R	D
□ 1b8a_A	1	.	.	.	.	.	.	.	M	Y	R	T	H	Y	S	S	E
□ SYD_METMA	1	.	.	.	M	S	L	A	N	L	R	T	H	Y	T	A	D
□ SYD_HALN1	1	.	.	.	.	.	.	.	M	E	N	R	T	Y	T	A	D
□ SYD_THEAC	1	.	.	.	.	.	.	.	.	.	.	M	L	S	I	A	E
□ SYD_PYRHO	1	.	.	.	.	.	.	.	M	I	E	K	V	Y	C	Q	E
Bacteria:Proteobacteria																	
□ 1l0w_A	1	.	.	.	.	.	.	M	R	.	R	T	H	Y	A	G	S
□ 1il2_A	1	.	.	.	.	.	.	.	M	.	R	T	E	Y	C	G	Q

MultiSeq: Display and Edit Metadata

- External databases are cross-referenced to display metadata such as taxonomy (lineage), data source (sp, Uniprot #), EC, enzymatic function
- Changes to metadata should periodically be updated!!!
- Electronic Notebook: Notes and annotations about a specific sequence or structure can be added – and saved



Sequence Name: SYDC_YEAST

Source Organism: Saccharomyces cerevisiae

Common Name: yeast

EC Number: 6.1.1.12

EC Description: Aspartate--tRNA ligase.

Description: Aspartyl-tRNA synthetase, cytoplasmic (EC 6.1.1.12) (Aspartate--tRNA ligase) (AspRS) - Saccharomyces cerevisiae (Baker's yeast).

Data Sources:
sp=P04802,SYDC_YEAST
pdb=1EOV,A

Lineage:
Eukaryota
Fungi
Ascomycota
Saccharomycotina
Saccharomycetes
Saccharomycetales

Notes

There were missing residues