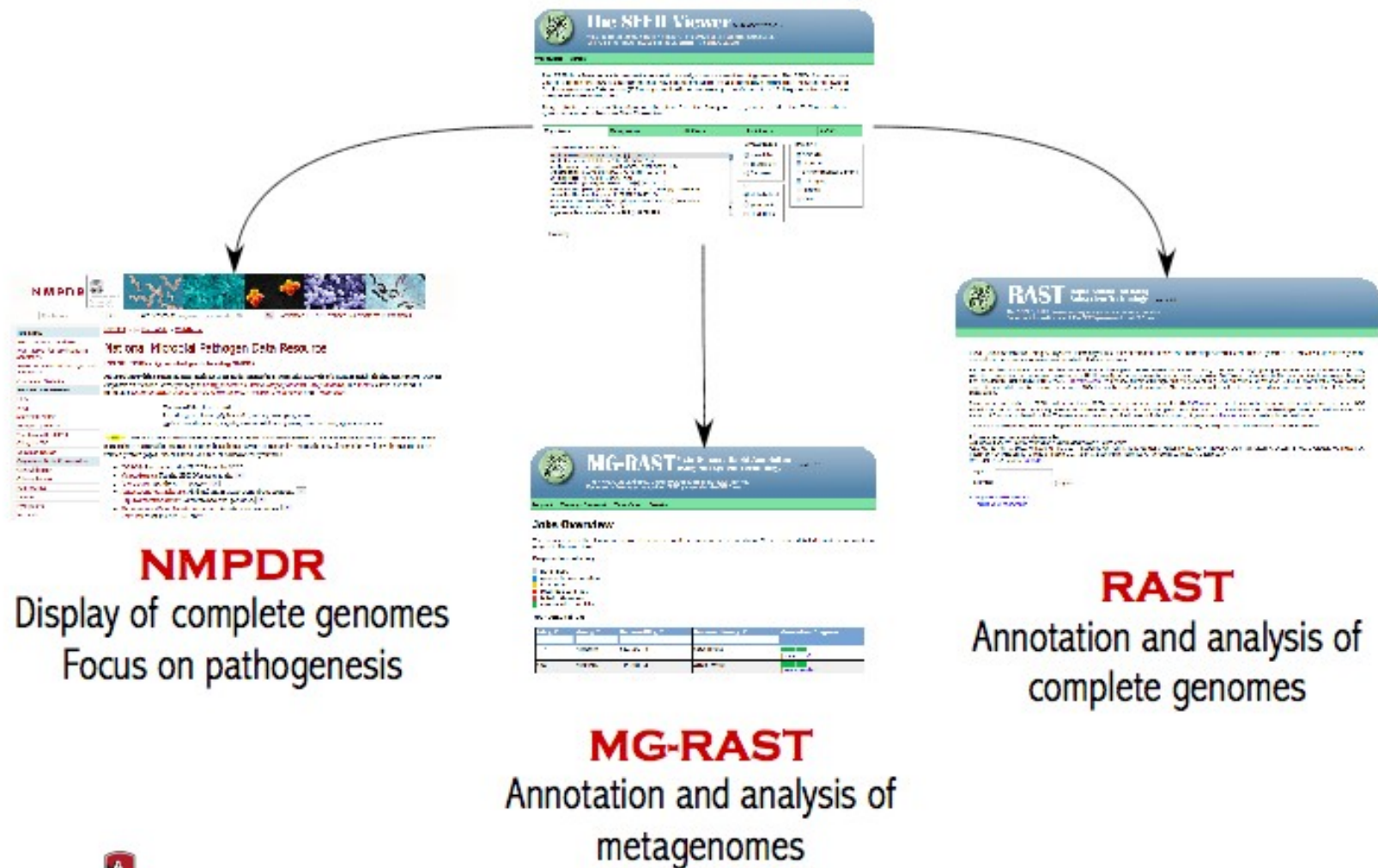
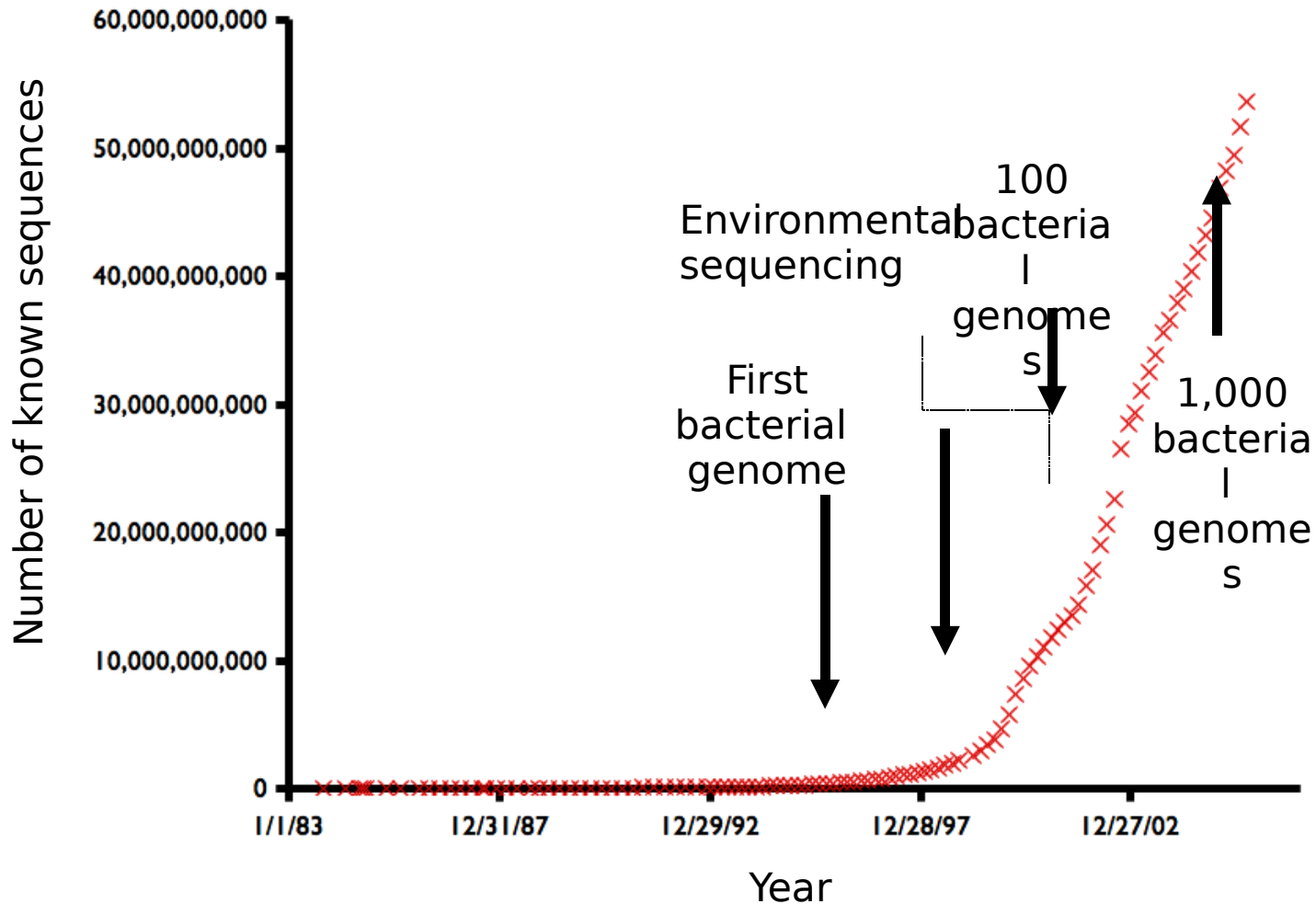


The SEED Family

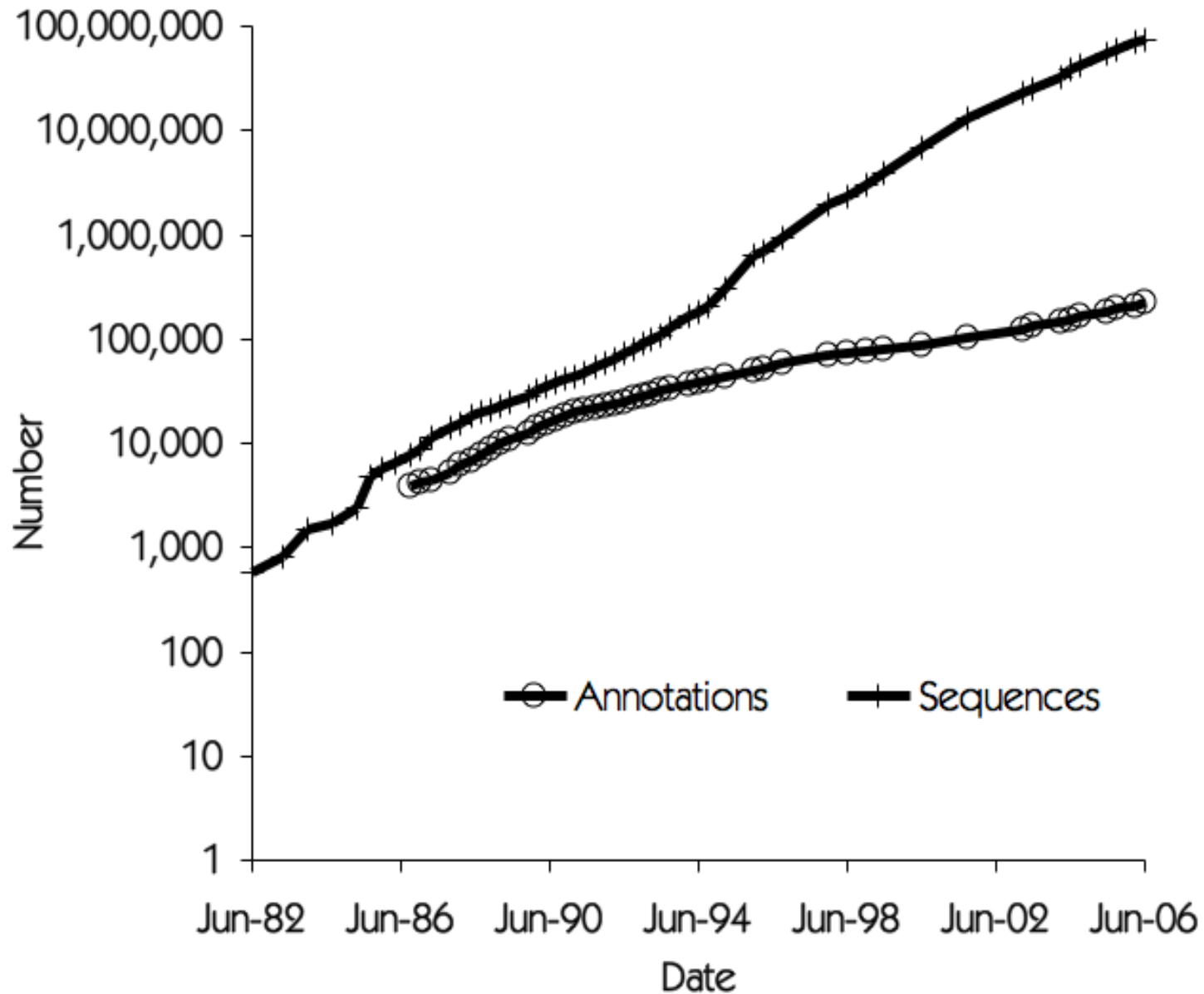
THE SEED Environmental, Viral, Bacterial, Archaeal, and Eukaryal Genome Interpretation



How much has been sequenced?



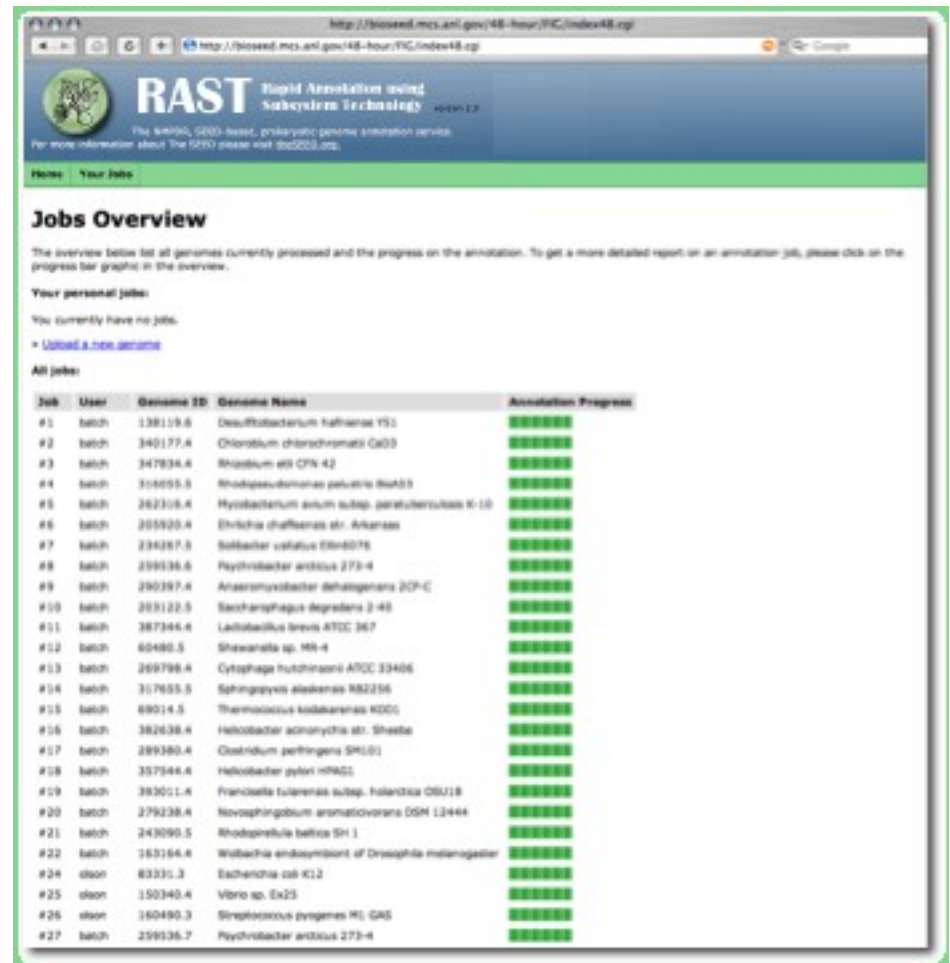
Annotations vs. sequences



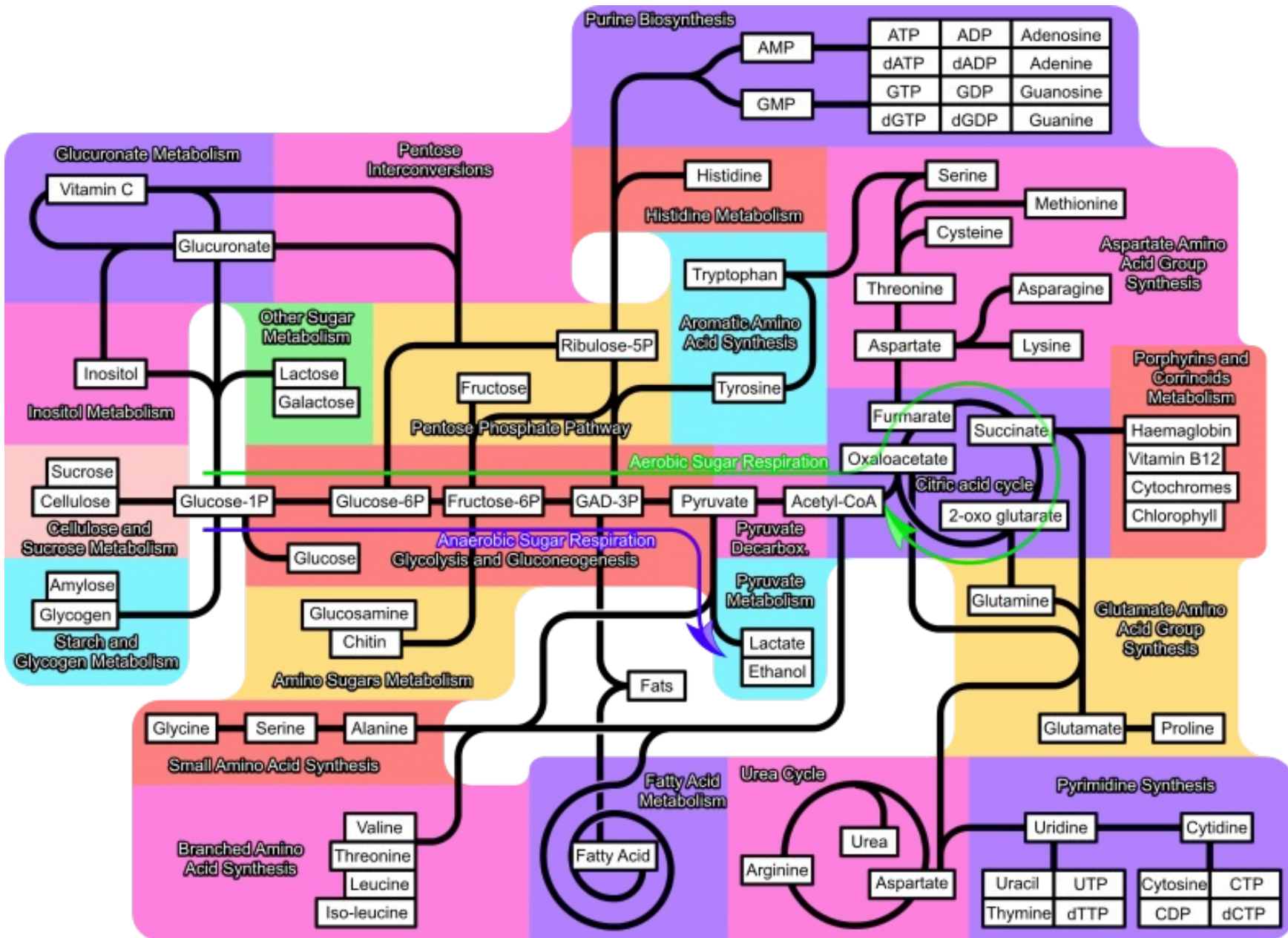
Annotation of Complete Genomes

<http://rast.nmpdr.org/>

- Automated user originated processing
- Takes 1-7 hours depending on size and complexity of the genome
- ~2,000 external submissions, including hundreds of genomes not yet publicly released.
- Reannotation of >500 genomes complete
- 1,000 users, 200 organizations, 25 countries.



Subsystems Make Up Metabolism



Subsystem spreadsheet (conceptually)

	Chaperone	Subunit	Usher	Adhesin
<i>S. enterica</i> Enteritidis	2389	2388	2387	2386
<i>E. coli</i> HS	3068	3067	3066	3065
<i>B. cenocepacia</i> J2315	2604	2603	2602	2601
<i>S. maltophilia</i>	1085	1088	1087	1086

Over 1,000 Subsystems

Three level “hierarchy”

- Amino Acids and Derivatives
 - Alanine, serine, and glycine
 - Serine Biosynthesis
- Amino Acids and Derivatives
 - Lysine, threonine, methionine, and cysteine
 - Methionine Biosynthesis

Make your own subsystems!

Class	# SS	Class	# SS
Amino Acids and Derivatives	56	Nucleosides and Nucleotides	14
Carbohydrates	97	Phosphorus Metabolism	6
Cell Division / Cycle	10	Photosynthesis	9
Cell Wall and Capsule	50	Potassium metabolism	3
Clustering-based ss	193	Protein Metabolism	52
Cofactors, Vitamins, Pigments	43	RNA Metabolism	39
DNA Metabolism	30	Regulation/signaling	23
Fatty Acids, Lipids, and Isoprenoids	22	Respiration	44
Membrane Transport	41	Secondary Metabolism	24
Metabolism of Aromatic Compounds	30	Stress Response	37
Motility and Chemotaxis	8	Sulfur Metabolism	12
Nitrogen Metabolism	11	Virulence	116

The annotation process (complete genomes)

- Find the phylogenetic neighborhood of your genome
- Look for proteins that related organisms have
 - Core proteins
 - Subset of all subsystems
- Use those calls as a training set for critica/glimmer
 - Intrinsic training set!



The SEED Viewer

SEED Viewer version 2.0

Welcome to the SEED Viewer - a read-only browser of the curated SEED data.
For more information about The SEED please visit theSEED.org.

»Navigate »Organism »Comparative Tools »Help

find



Rob Edwards

Organism Overview for *Haloferax denitrificans* (35745.3)

Genome	<i>Haloferax denitrificans</i> (Taxonomy ID: 35745)
Domain	Archaea
Size	3,830,000 bp
Number of Contigs	19
Number of Subsystems	219
Number of Coding Sequences	3769
Number of RNAs	56

Browse Compare Download Annotate

Browse through the features of [Haloferax denitrificans](#) both graphically and through a table. Both allow quick navigation and filtering for features of your interest. Each feature is linked to its own detail page.

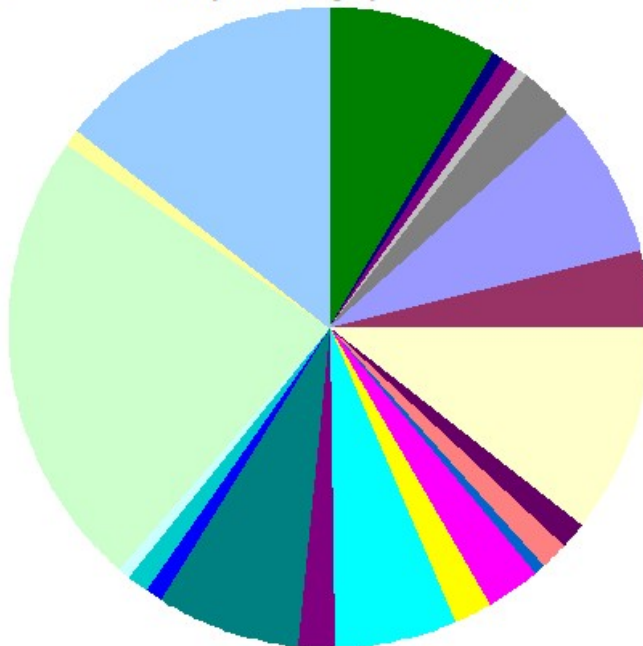
Click [here](#) to get to the Genome Browser

Subsystem Statistics Features in Subsystems

Subsystem Coverage



Subsystem Category Distribution



Subsystem Feature Counts

- ☐ Phages, Prophages, Transposable elements (0)
- ☐ Cofactors, Vitamins, Prosthetic Groups, Pigments (94)
- ☐ Cell Wall and Capsule (5)
- ☐ Photosynthesis (0)
- ☐ Potassium metabolism (8)
- ☐ Miscellaneous (6)
- ☐ Membrane Transport (31)
- ☐ Nucleosides and Nucleotides (81)
- ☐ RNA Metabolism (43)
- ☐ Protein Metabolism (110)
- ☐ Cell Division and Cell Cycle (16)
- ☐ Motility and Chemotaxis (14)
- ☐ Regulation and Cell signaling (6)
- ☐ Secondary Metabolism (0)
- ☐ DNA Metabolism (29)
- ☐ Virulence (22)
- ☐ Fatty Acids, Lipids, and Isoprenoids (64)
- ☐ Nitrogen Metabolism (20)
- ☐ Dormancy and Sporulation (0)
- ☐ Respiration (76)
- ☐ Stress Response (9)
- ☐ Sulfur Metabolism (12)
- ☐ Metabolism of Aromatic Compounds (7)
- ☐ Amino Acids and Derivatives (247)
- ☐ Phosphorus Metabolism (9)
- ☐ Carbohydrates (152)

Automatic metabolic reconstruction

- Subsystem, GO, and KEGG connections
 - KEGG EC numbers
 - KEGG reaction numbers
 - SEED reaction numbers (Chris Henry)
- Metabolic flux models
 - Automatically generate FBA matrices (Aaron Best/Matt DeJongh; Hope College)



The SEED Viewer

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»Organism

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Rob Edwards

Diagram

Functional Roles

Subsystem Spreadsheet

Description

Additional Notes

Group Alias	Abbrev.	Functional Role	Reactions	Scenario Reactions	GO
all					
	SdaC	Serine transporter	-	-	GO:0005279 , GO:0006865 , GO:0009847 , GO:0016020 , GO:0016021
*Sda	SdaA	L-serine dehydratase (EC 4.3.1.17)	R00220	-	GO:0003941
Pyruvate_route, *Sda	SdhA	L-serine dehydratase, alpha subunit (EC 4.3.1.17)	-	-	GO:0003941
Pyruvate_route, *Sda	SdhB	L-serine dehydratase, beta subunit (EC 4.3.1.17)	-	-	GO:0003941
Pyruvate_route	TdcB	Threonine dehydratase, catabolic (EC 4.3.1.19)	R00220	-	GO:0004794
Pyruvate_route	DsdA	D-serine dehydratase (EC 4.3.1.18)	R00221	-	GO:0008721
Pyruvate_route	DsdC	D-serine dehydratase transcriptional activator	-	-	-
	DsdX	D-serine permease DsdX	-	-	-
	GlyA	Serine hydroxymethyltransferase (EC 2.1.2.1)	R00945	-	GO:0004372
	GcvP	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P protein) (EC 1.4.4.2)	R03425	-	GO:0004375
*GcvP, Glycine_cleavage_system	GcvP1	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P1 protein) (EC 1.4.4.2)	R03425	-	GO:0004375
*GcvP, Glycine_cleavage_system	GcvP2	Glycine dehydrogenase [decarboxylating] (glycine cleavage system P2 protein) (EC 1.4.4.2)	R03425	-	GO:0004375
*GcvP, Glycine_cleavage_system	GcvT	Aminomethyltransferase (glycine cleavage system T protein) (EC	-	-	-

The Populated Subsystem



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Organism	Domain	Variant	active	SdaC	*Sda	TdcB	DsdA	DsdC	DsdX	GlyA	GcvP	*GcvP	GcvH	CysB	CysA	SerB	SerC	SerA	GlxK	Kbl	TynA	SerS	
	all	[?] = all	yes	All	All	All	All	All	All	All	All	All	All	All	All	All	All	All	All	All	All	All	All
Haloferax denitrificans	Archaea	1.2	yes	?		?	?	?	?	3392 , 812	?	2593 , 2594 , 2596	2595	1197	1945 , 896	1963 , 357	?	1005 , 1966 , 3266 , 3285 , 3545	?	?	?	3085	
	Bacteria	1.1234	yes	1557 , 3344	2030 , 2589	?	?	?	?	2233 , 2990	352 , 5119	2029 , 4404	4403 , 5118	2313	2312	2 , 3633 , 3849 , 4510	3294	1560 , 2287 , 3436 , 351	2841 , 2842	?	?	4964	
Halomicrobium mukohataei DSM 12286	Archaea	*1.13	yes							1636 , 955		1954 12, 1955 11, 1959 13	1958			3000 , 765		1293 , 3023				3077	
Pyrobaculum arsenaticum DSM 13514	Archaea	1.2	yes							48						1912 , 2009		1670				1601	
Pyrobaculum islandicum DSM 4184	Archaea	1.2	yes							1078						1629 , 1794		560				522	
Pyrococcus furiosus DSM 3638	Archaea	1.1	yes							1828		1387 13, 2053 11, 2054 12	1539					1441		270		1245	
Pyrobaculum aerophilum str. IM2	Archaea	1.2	yes							542						1393		2343				2223	
Pyrococcus horikoshii OT3	Archaea	1.1	yes							1616		1118 13, 1951 12, 1952 11	1299			1841		1367		274		693	

Automatically compare metabolic reconstructions



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Compare Metabolic Reconstruction of [Haloferax mediterranei](#) (A) and [Haloferax denitrificans](#) (B)

display items per page

[«first](#) [«prev](#)

displaying 25 - 39 of 39

Presence ▲▼ <input type="text" value="B"/>	Category <input type="text" value="all"/>	Subcategory <input type="text" value="all"/>	Subsystem ▲▼ <input type="text"/>	Role ▲▼ <input type="text"/>	Organism A ▲ <input type="text" value="find"/>	SS active A <input type="text" value="no"/>	Organism B ▲▼ <input type="text" value="fig 35745.3.peq.1330"/>	SS active B <input type="text" value="yes"/>
B	Membrane Transport	ABC transporters	ABC transporter branched-chain amino acid (TC 3.A.1.4.1)	Branched-chain amino acid transport ATP-binding protein livF (TC 3.A.1.4.1)	<input type="text" value="find"/>	no	fig 35745.3.peq.1330	yes
B	Membrane Transport	ABC transporters	ABC transporter branched-chain amino acid (TC 3.A.1.4.1)	Branched-chain amino acid transport system permease protein livM (TC 3.A.1.4.1)	<input type="text" value="find"/>	no	fig 35745.3.peq.1417	yes
B	Metabolism of Aromatic Compounds	Peripheral pathways for catabolism of aromatic compounds	Benzoate catabolism	Muconate cycloisomerase (EC 5.5.1.1)	<input type="text" value="find"/>	no	fig 35745.3.peq.767	yes
B	Nitrogen Metabolism	no subcategory	Denitrification	Nitrous oxide reductase maturation protein, outer-membrane lipoprotein NosL	<input type="text" value="find"/>	no	fig 35745.3.peq.1359	yes

Find and suggest candidate functions

- Rapidly correct missing annotations
- Add more members to subsystems
- Improves future genome annotations!
(especially with new subsystems)

The Live ASM Test

Philadelphia, 2009

- 10 genomes submitted on Thursday at 6 pm
- First annotation complete before 8 am Friday
- Remaining annotations completed Friday before noon
- (there were others in the pipeline too!)
- Presentation ASM 2009 Tuesday, 8pm

Subsystems coverage of sequenced Archaea

Genome	Percent of Proteins in Subsystems
Haloferax denitrificans	20%
Haloferax mediterranei	19%
Haloferax sulfurifontis	19%
Haloferax volcanii DS2	19%
Haloarcula sp 33800	19%
Haloarcula sp 33799	18%

Prophages

PHANTOME
NSF
Mya Breitbart, Matt Sullivan, Jeff Elhai, Rob Edwards



Comparing complete genomes to metagenomes

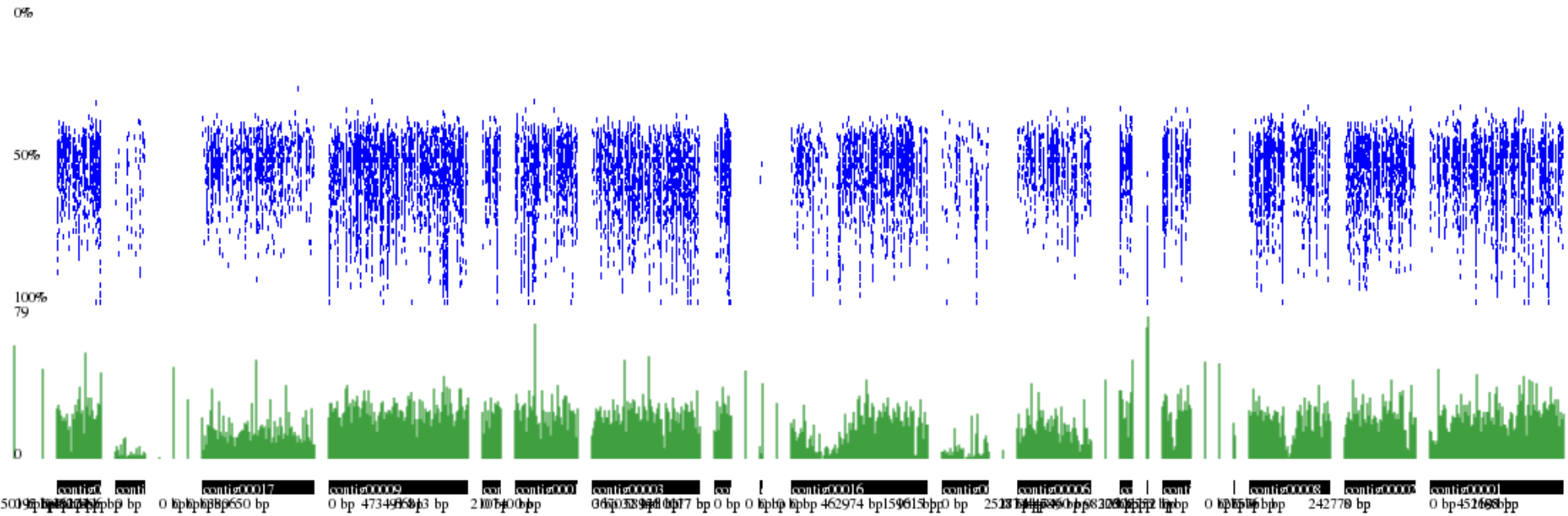
Metagenomics RAST has 300 public
metagenomes

Compared using tblastx

Human Poop



High Salinity Salterns San Diego, July 2004



Thanks Nick Celms, Beltran Rodriguez-Mueller, Mya Breitbart, & Forest Rohwer

The metagenomics RAST server




MG-RAST Meta Genome Rapid Annotation
using Subsystem Technology

Metagenomics SEED Viewer version 2.0

Welcome to the Metagenomics SEED Viewer.

For more information about The SEED please visit theSEED.org.

»Navigate »Help

 Rob Edwards

MG-RAST is a fully-automated service for annotating metagenome samples.

It provides:

- **annotation** of sequence fragments,
- their **phylogenetic classification**,
- **metabolic reconstructions** and
- **comparison tools**

The service is built as a modified version of the RAST server which was originally designed to support high-quality annotation of complete or draft microbial genomes.



more

If you use our service, please cite:

The Metagenomics RAST server - A public resource for the automatic phylogenetic and functional analysis of metagenomes F. Meyer, D. Paarmann, M. D'Souza, R. Olson, E. M. Glass, M. Kubal, T. Paczian, R. Stevens, A. Wilke, J. Wilkening and R. A. Edwards
submitted



» [Manage your uploaded data](#) » [Register a new account](#) » [Upload new metagenome to MG-RAST](#)

You have access to the following metagenomes:

Number of publicly available metagenome: 158

start typing to narrow selection
Public: 5-Way (CG) Acid Mine Drainage Biofilm (4441137.3) from project
Public: 640F6 (4440355.3) from project Cow Rumen
Public: 710F6 (4440387.3) from project Cow Rumen
Public: 80F6 (4440356.3) from project Cow Rumen
Public: ALVINELLA (4441102.3) from project Alvinella Pompejana Epibio
Public: ArcticVir2002 (4440306.3) from project Ocean Viruses
Public: Australian Phosphorus Removing (EBPR) Sludge (4441092.3) from
Public: BBCVir96to04 (4440305.3) from project Ocean viruses

[View Metagenome](#)

Automated Processing

User uploads
DNA sequences

Pre-processing:

✓ Genome Upload has been successfully completed.

Metagenome ID - Name:

Job:

User:

Date:

Number of uploaded sequences:

Total uploaded sequence length:

Jobs Details #570

» [Browse annotated metagenome in SEED Viewer](#)

» Available downloads for this job:

» [Share this metagenome with selected users](#)

» [Make this metagenome publicly accessible](#)

» [Back to the Jobs Overview](#)

✓ Preprocessing has been successfully completed.

The following statistics are based on:

Number of sequences: 23

Total sequence length: 53295251

Average read length: 227.606717772416

Longest sequence id: >58459919

Longest sequence length: 374

Shortest sequence id: >58696741

Shortest sequence length: 36

✓ Similarity Computation has been successfully completed.

✓ Similarity postprocessing has been successfully completed.

✓ Final Assignment has been successfully completed.

Summary View



MG-RAST Meta Genome Rapid Annotation
using Subsystem Technology

Metagenomics SEED Viewer version 2.0

Welcome to the Metagenomics SEED Viewer.
For more information about The SEED please visit theSEED.org.

»Navigate »Metagenome »Compare Metagenomes »Management »Help

Rob Edwards

Metagenome Overview for MG_Soybean_Field_Sample (4440698.3)

Project:	Fermi_metagenomics_samples
Metagenome	MG_Soybean_Field_Sample
Metagenome ID:	4440698.3
Description:	No description available.
Uploaded on:	Sun Mar 23 20:49:40 2008
Total no. of sequences	234,155
Total sequence size	53,295,251
Shortest sequence length	36
Longest sequence length	374
Average sequence length	227.61
Average GC content	not computed

Overview **Metabolic Analysis** Phylogenetic Analysis Compare

The metagenome overview page provides basic information and a summary regarding the selected metagenome. Information includes project name, project description, metagenome name and unique id as well as sequence length and percent GC statistics. Histograms of sequence length and GC content is also provided. In order to provide a brief overview of the taxonomic distribution, a table is provided with domain distribution for RNA and protein based analysis.

The Overview is accessible through the menu via
» Metagenome » [Overview](#)

Summary and Statistics

The MG_Soybean_Field_Sample data set contains 234,155 contigs totaling 53,295,251 basepairs with an average fragment length of 227.61 (you can [download](#) the entire data set). A total of 61,041 sequences (26.07%) could be matched to proteins in [SEED subsystems](#) (using an e-value cut-off of 1e-5), you can explore metabolic reconstructions based on different parameters on the [Metabolic Reconstruction Page](#). Based on 94,012 hits against the SEED protein non-redundant database (40.15 % of the fragments) and on the 127 hits against the ribosomal RNA database [Greengenes](#) (0.05%) we computed the following table (using an e-value cut-off of 1e-5 and a minimum alignment length of 50bp).

The [Phylogenetic Reconstruction](#) page will allow you to view taxonomic distributions in greater detail, change parameters and incorporate additional databases into your analysis.

The [MG-RAST manual](#) has more pointers for working with the system.

	Protein based	16s based
Archaea	2.07% (1946)	0.00% (0)
Bacteria	84.76% (79682)	90.55% (115)
Eukaryota	1.04% (980)	0.00% (0)
Virus	0.00% (0)	0.00% (0)
Other	12.13% (11404)	9.45% (12)



www.nmpdr.org

www.theseed.org



Metagenomics Tools

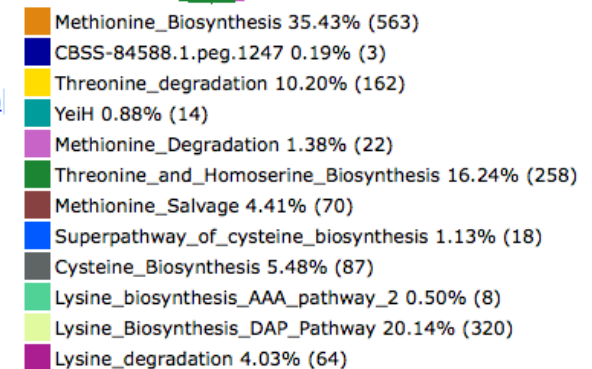
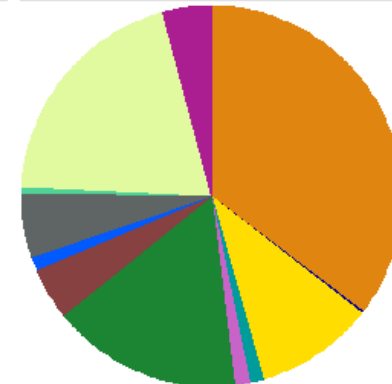
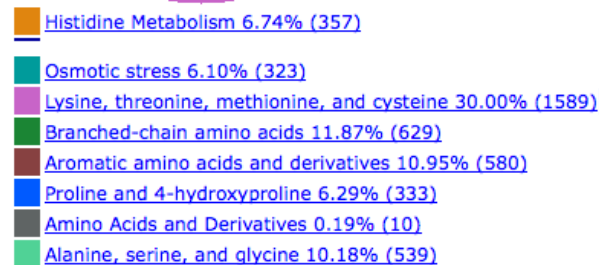
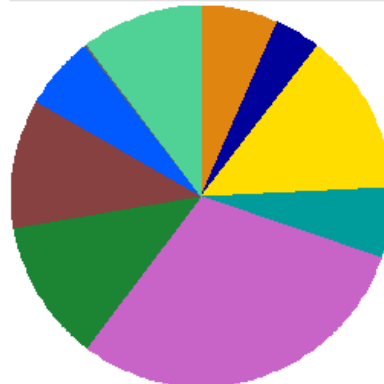
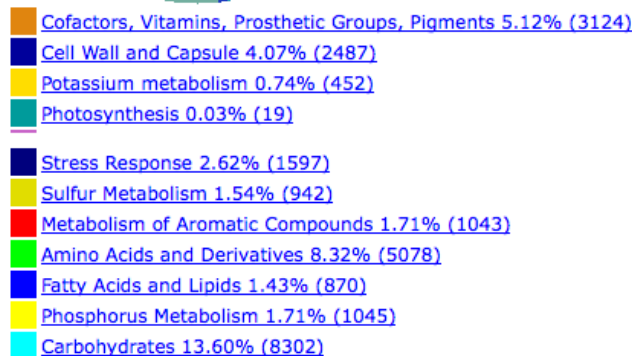
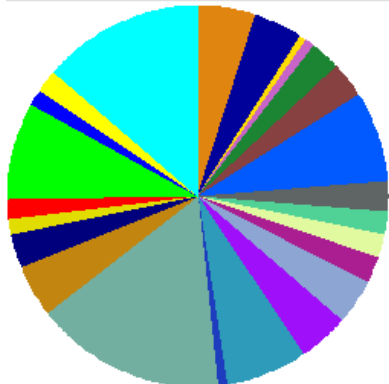
Annotation & Subsystems

Charts **Tabular View**

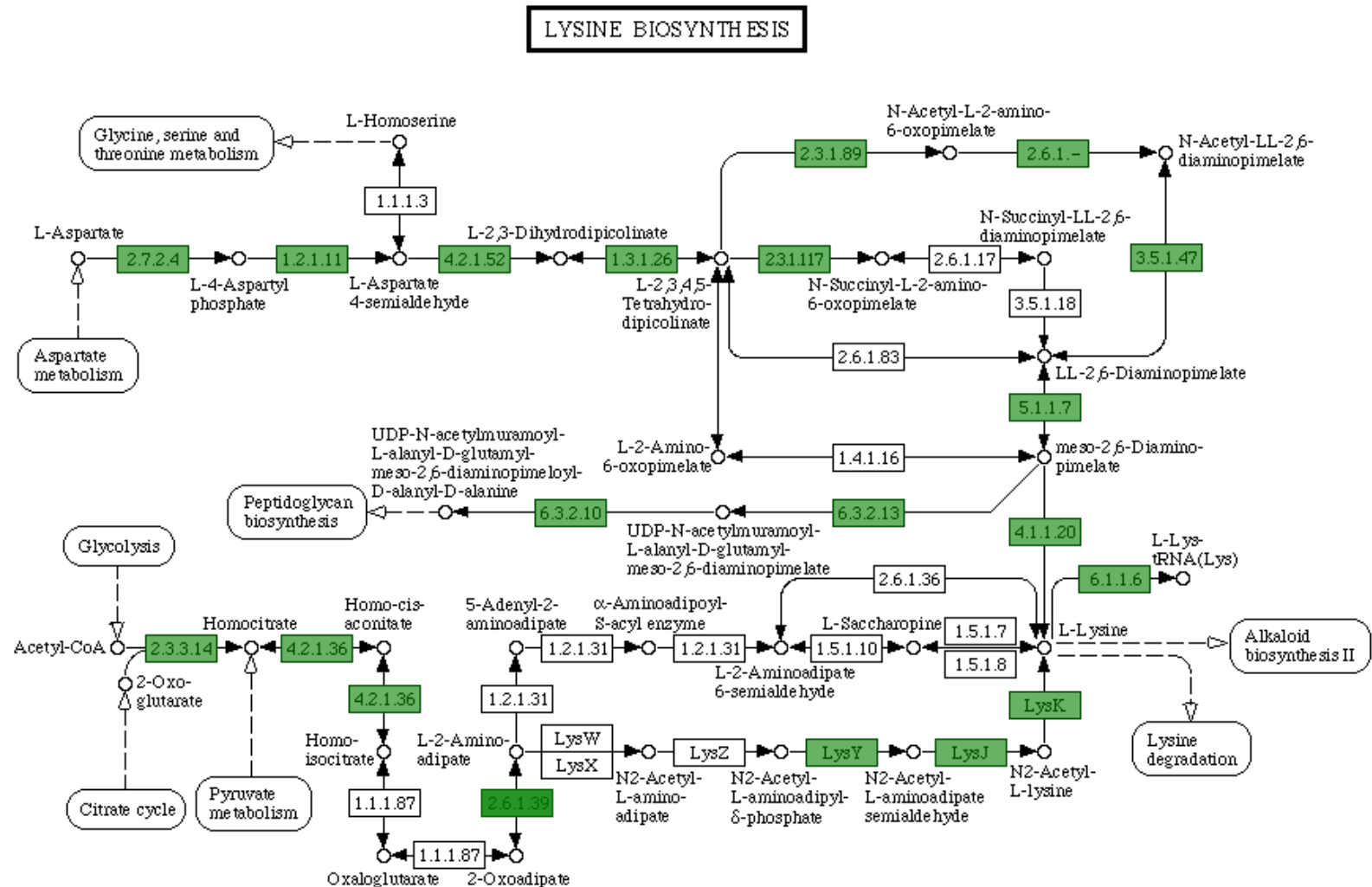
Group

Amino Acids and Derivatives

Lysine, threonine, methionine, and cysteine



Metagenomics Tools Annotation & KEGG maps



Metagenomics Tools

Recruitment Plots

Recruitment Plot from MG_Soybean_Field_Sample (4440698.3)

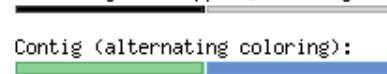
Select filter options

Maximum e-value	<input type="text" value="0.001"/>
Minimum p-value	<input type="text" value="leave blank for all"/>
Minimum percent identity	<input type="text" value="leave blank for all"/>
Minimum alignment length	<input type="text" value="leave blank for all"/>

Fragment e-value coloring:

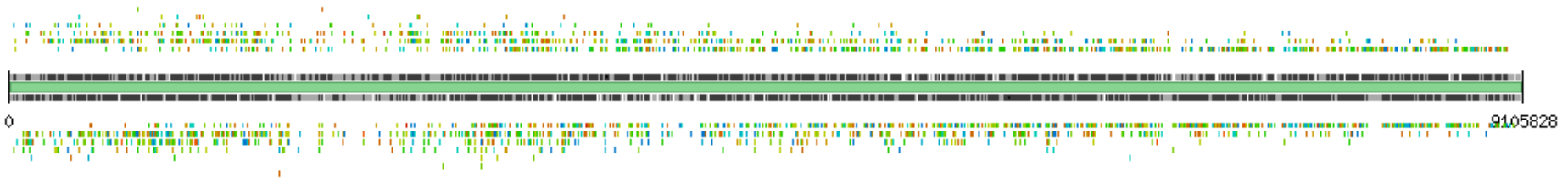
1e-50 1e-40 1e-30 1e-25 1e-20 1e-15 1e-10 1e-07 1e-05 0.001

PEG (fragment mapped / no fragments mapped):

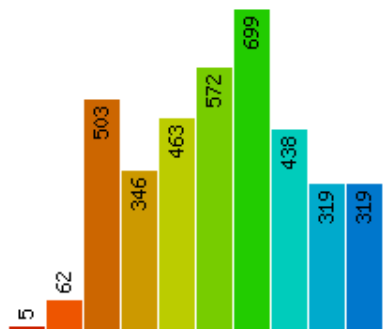


Re-compute results [« click here to reset »](#)

Plot



Evalue histogram



Summary

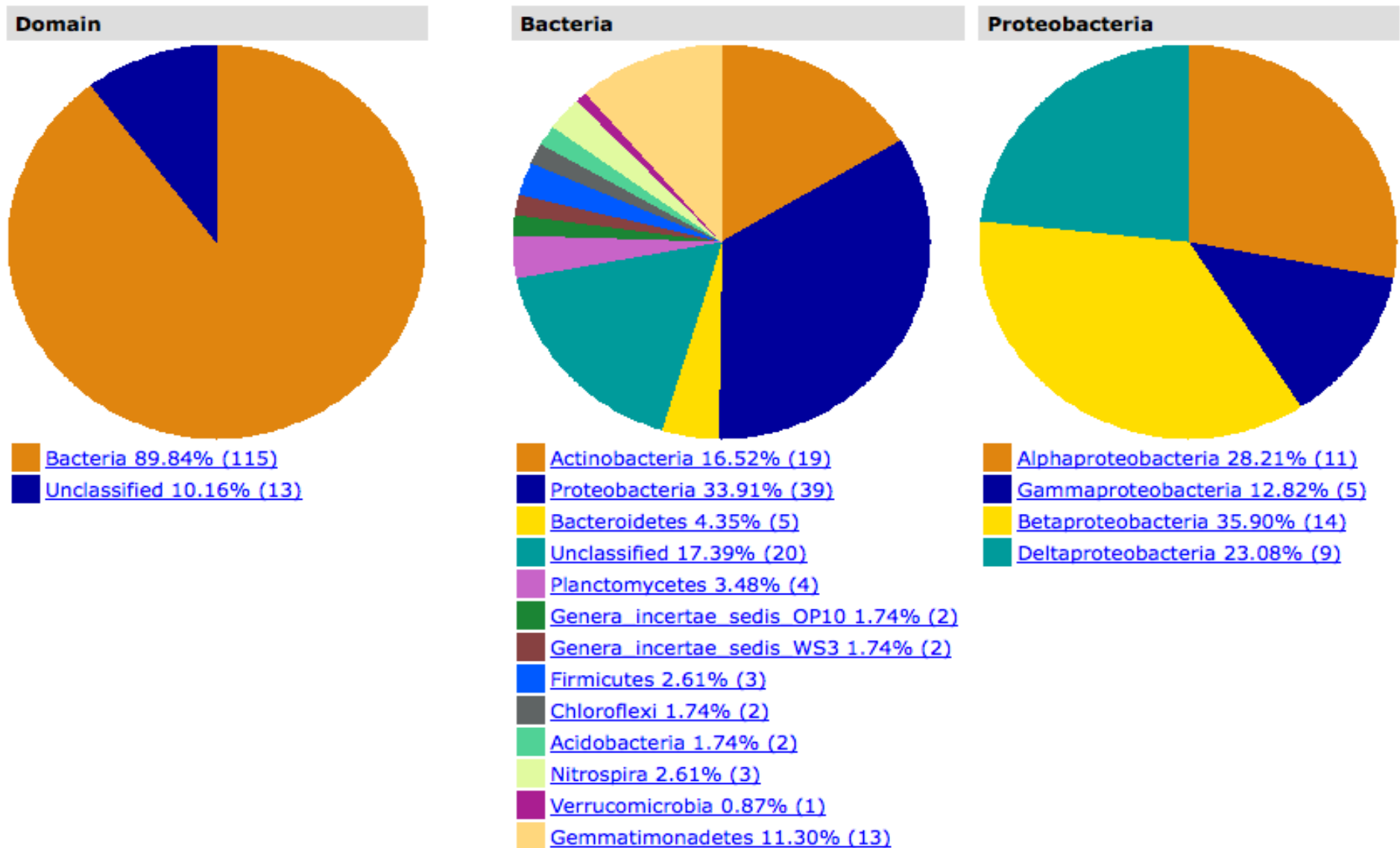
The reference genome *Bradyrhizobium japonicum* USDA 110 ([224911.1](#)) contains 1 contig(s) and 9.1 Mbp.

3,726 fragments hit 2,099 of 8,770 features from the *Bradyrhizobium japonicum* USDA 110 genome totaling 248,873 bp or 0.027X coverage.

[View fragments](#)

Metagenomics Tools

Phylogenetic Reconstruction



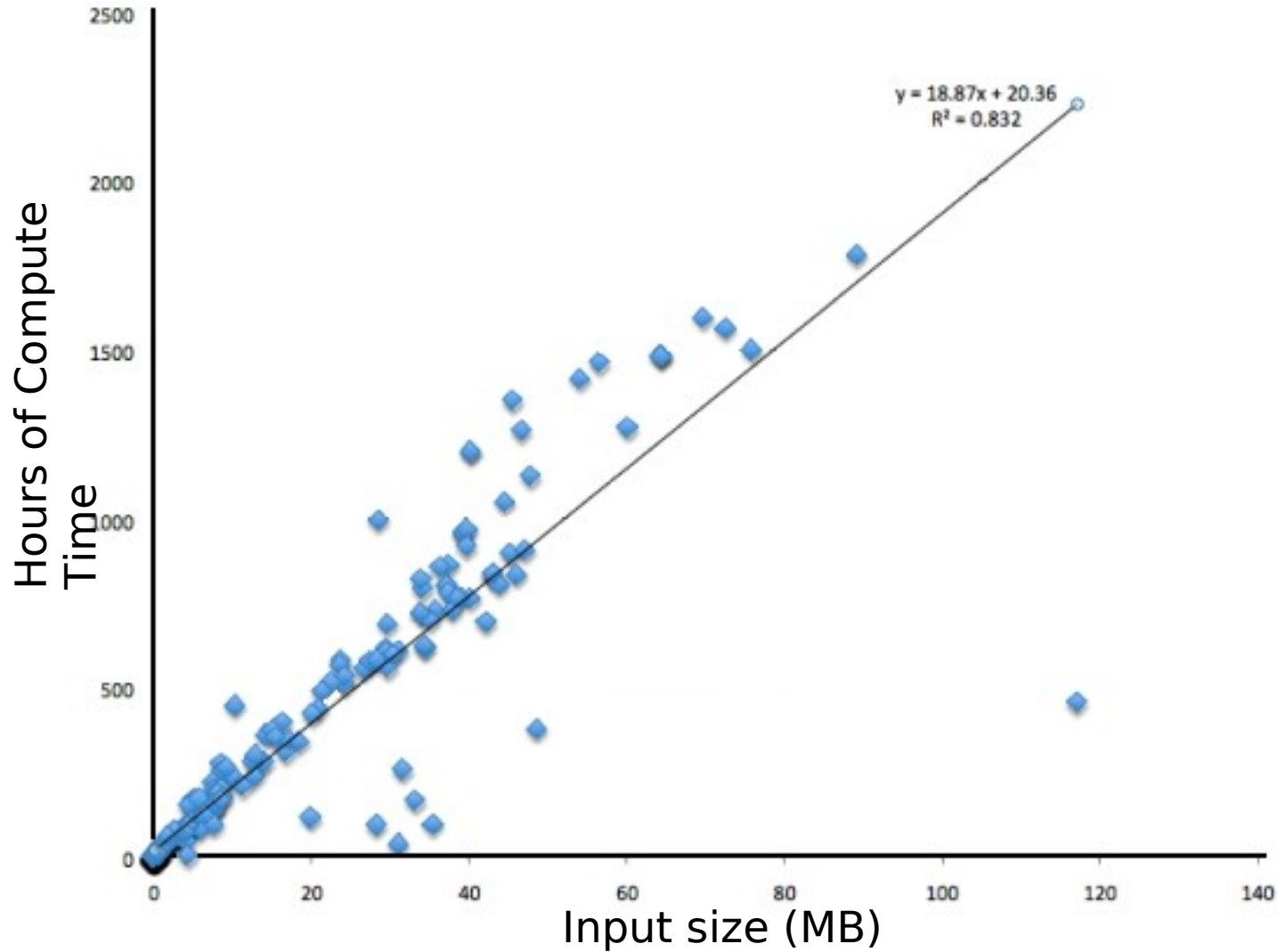
Metagenomics Tools

Comparative Tools

Subsystem Hierarchy 1 ▲▼ all	4440690.3 ▲▼	4440740.3 ▲▼	4440739.3 ▲▼	4440698.3 ▲▼
Carbohydrates	0.0059	0.0074	0.0084	0.0106
Clustering-based subsystems	0.0035	0.0045	0.0051	0.0066
Amino Acids and Derivatives	0.0030	0.0038	0.0042	0.0053
Virulence	0.0030	0.0036	0.0039	0.0048
Cofactors, Vitamins, Prosthetic Groups, Pigments	0.0024	0.0029	0.0032	0.0042
Respiration	0.0021	0.0025	0.0027	0.0035
Protein Metabolism	0.0020	0.0024	0.0028	0.0035
Cell Wall and Capsule	0.0018	0.0022	0.0026	0.0032
Unclassified	0.0018	0.0022	0.0024	0.0030
Metabolism of Aromatic Compounds	0.0015	0.0018	0.0020	0.0025
RNA Metabolism	0.0015	0.0018	0.0020	0.0024
Stress Response	0.0013	0.0017	0.0018	0.0022
Membrane Transport	0.0010	0.0012	0.0014	0.0017
DNA Metabolism	0.0009	0.0012	0.0013	0.0017
Regulation and Cell signaling	0.0009	0.0012	0.0011	0.0016
Nucleosides and Nucleotides	0.0008	0.0010	0.0012	0.0014

Computational Requirements

~19 hours of compute per input megabyte



How much so far

Total:

2,854 metagenomes
265,126,281 sequences
68,490,338,939 bp (68 Gbp)

Public:

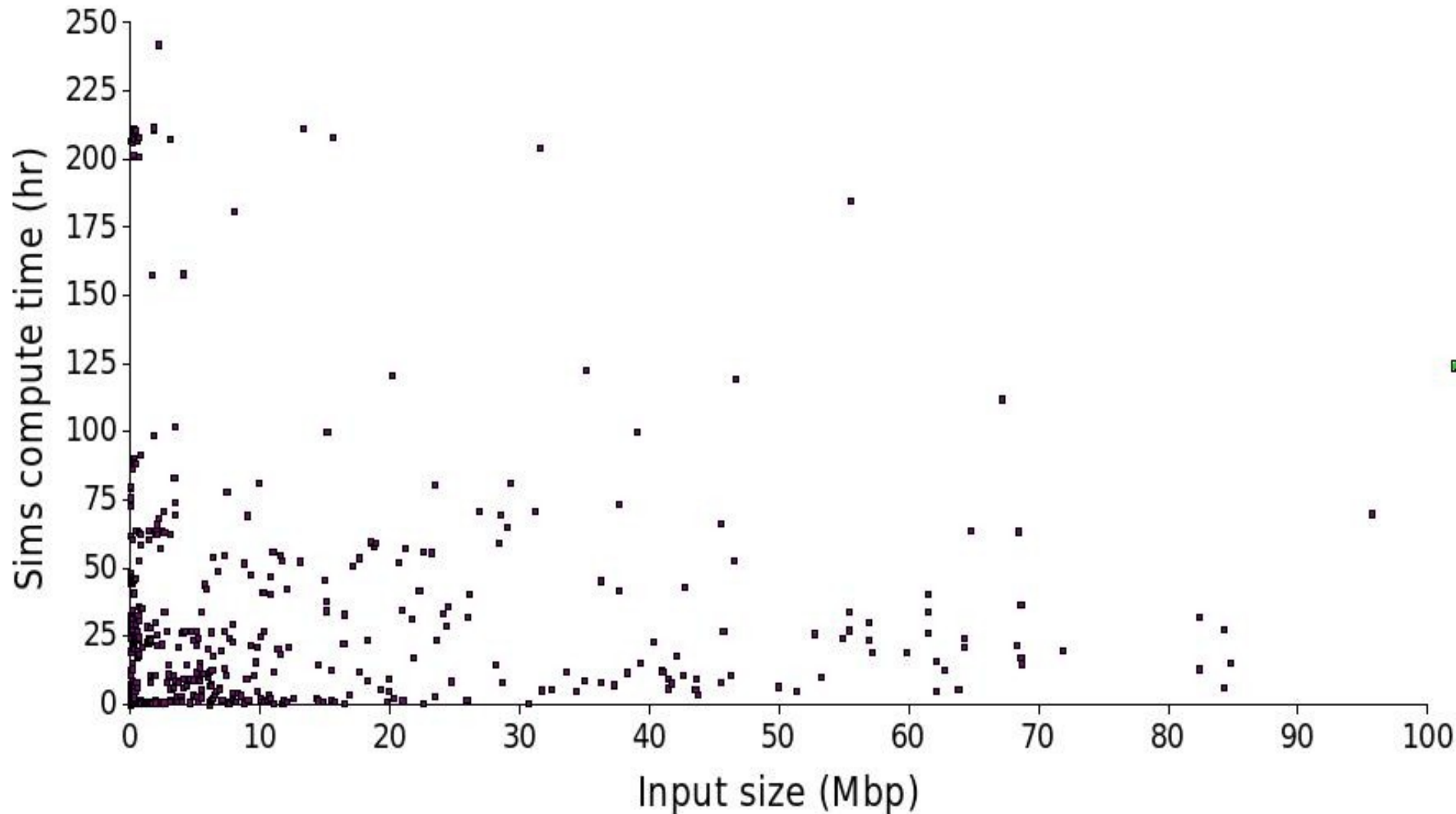
299 Metagenomes
45,445,163 sequences
19,341,509,132 bp (19 Gbp)

Compute time (on a single CPU):

1,292,000 hours = 53,833 days = 147 years

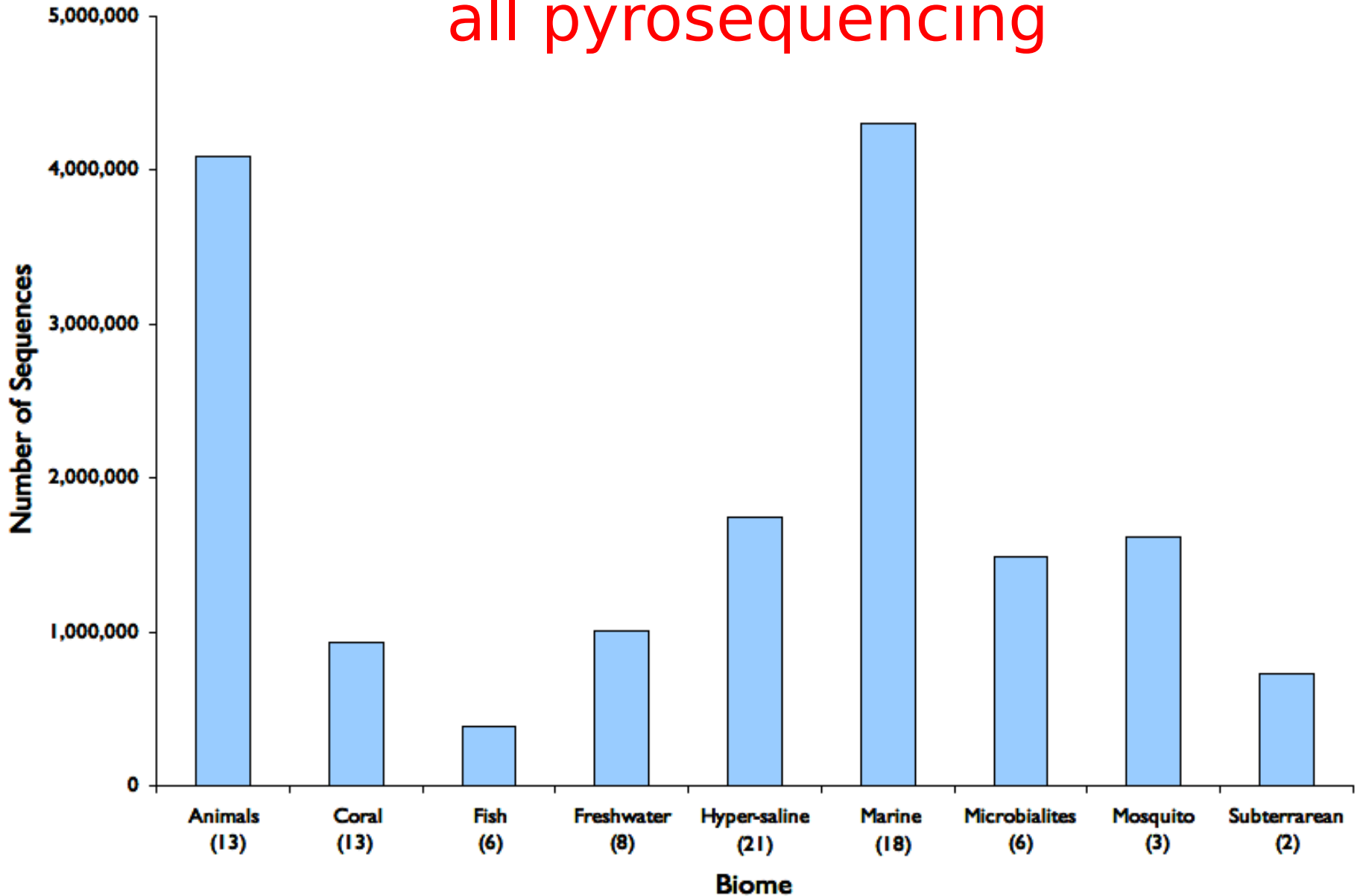
Lots of computers, no pattern

Sims compute time (<100 Mbp)



Does it work?

Lots of sequences
all pyrosequencing

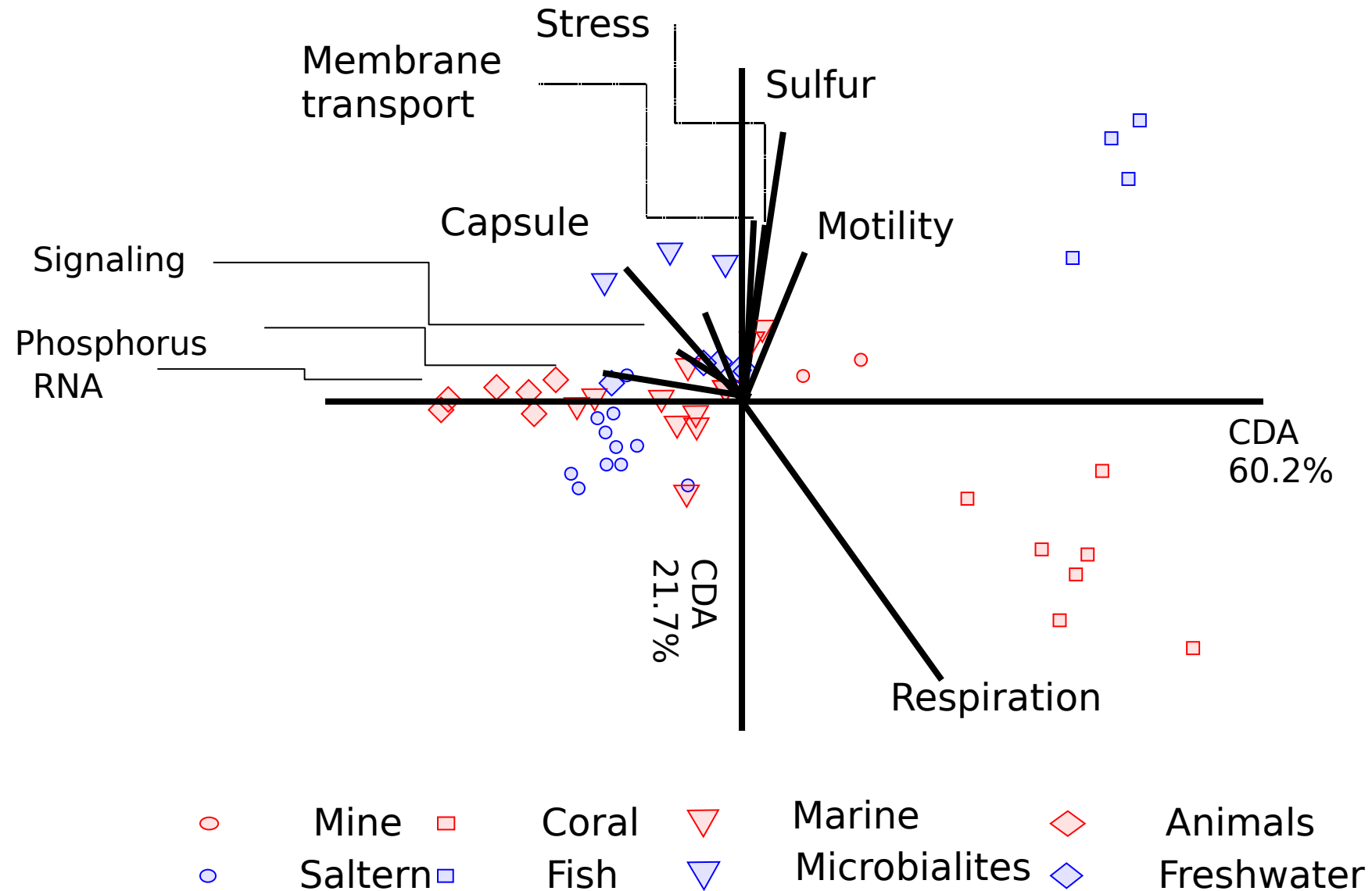


Metagenomics Tools

Functional Heat Maps

Subsystem Hierarchy 1 ▲▼ all ▲▼	4440690.3 ▲▼	4440740.3 ▲▼	4440739.3 ▲▼	4440698.3 ▲▼
Carbohydrates	0.0059	0.0074	0.0084	0.0106
Clustering-based subsystems	0.0035	0.0045	0.0051	0.0066
Amino Acids and Derivatives	0.0030	0.0038	0.0042	0.0053
Virulence	0.0030	0.0036	0.0039	0.0048
Cofactors, Vitamins, Prosthetic Groups, Pigments	0.0024	0.0029	0.0032	0.0042
Respiration	0.0021	0.0025	0.0027	0.0035
Protein Metabolism	0.0020	0.0024	0.0028	0.0035
Cell Wall and Capsule	0.0018	0.0022	0.0026	0.0032
Unclassified	0.0018	0.0022	0.0024	0.0030
Metabolism of Aromatic Compounds	0.0015	0.0018	0.0020	0.0025
RNA Metabolism	0.0015	0.0018	0.0020	0.0024
Stress Response	0.0013	0.0017	0.0018	0.0022
Membrane Transport	0.0010	0.0012	0.0014	0.0017
DNA Metabolism	0.0009	0.0012	0.0013	0.0017
Regulation and Cell signaling	0.0009	0.0012	0.0011	0.0016
Nucleosides and Nucleotides	0.0008	0.0010	0.0012	0.0014

From Sequences To Environments



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