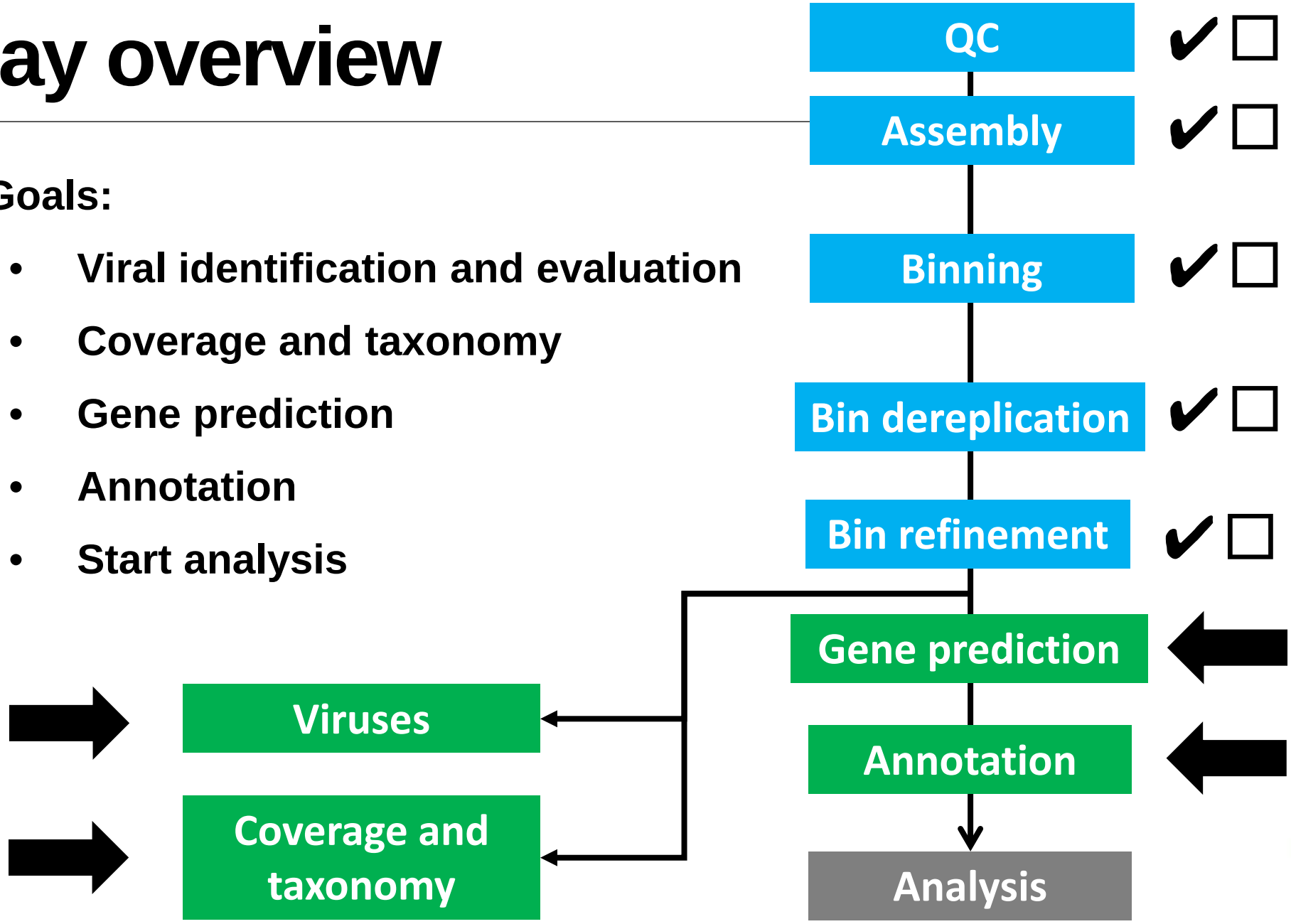
Day 3

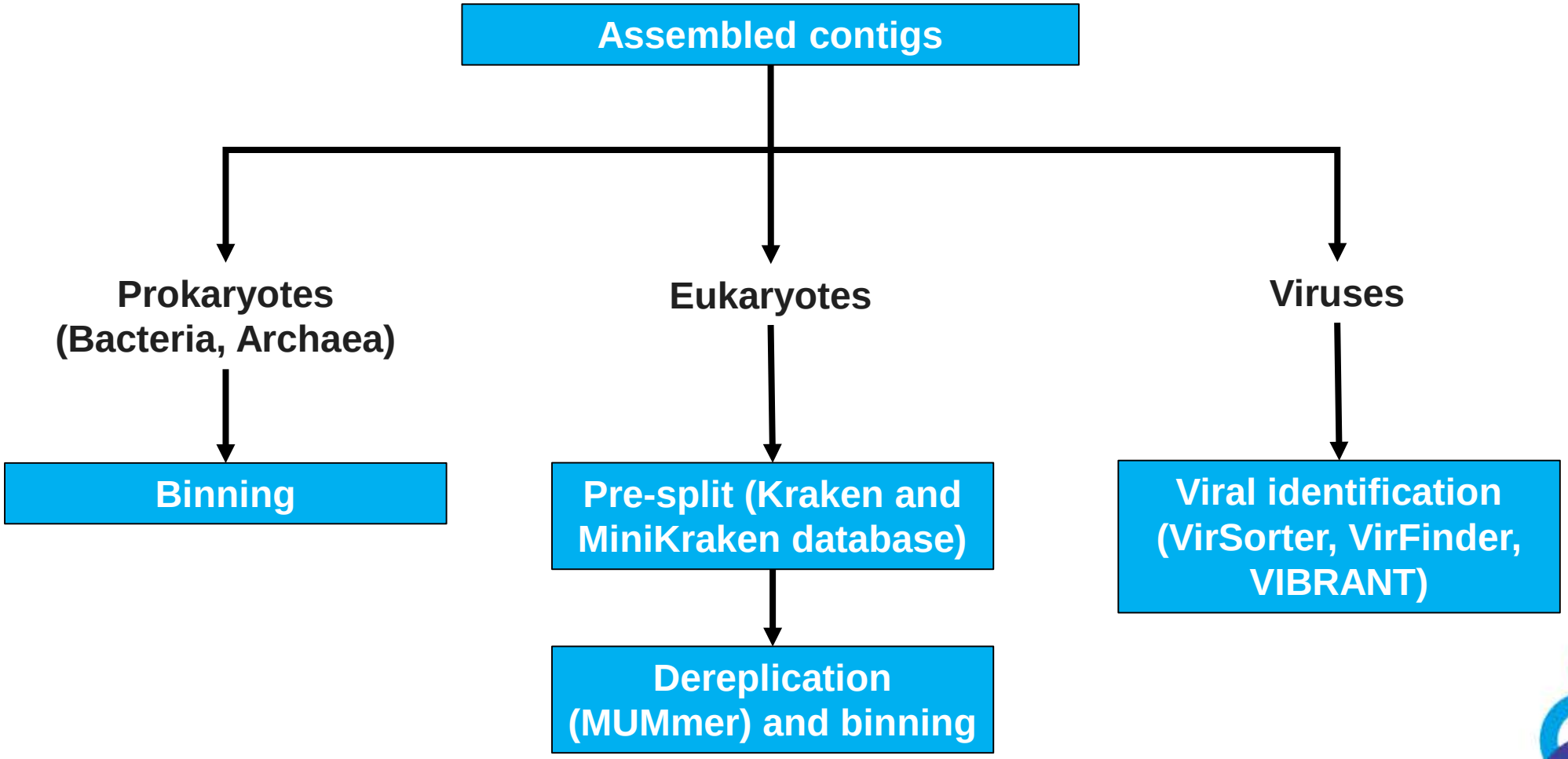
Viruses
Coverage and Taxonomy
Gene prediction
Gene annotation

Day overview

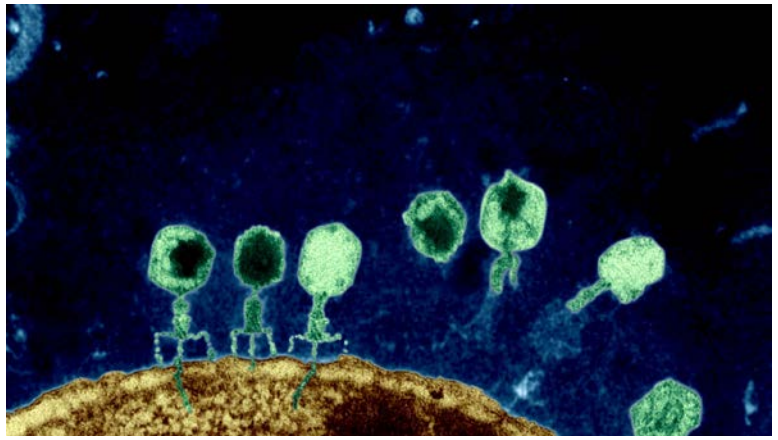
- **Goals:**
  - **Viral identification and evaluation**
  - **Coverage and taxonomy**
  - **Gene prediction**
  - **Annotation**
  - **Start analysis**



Recovering genomes



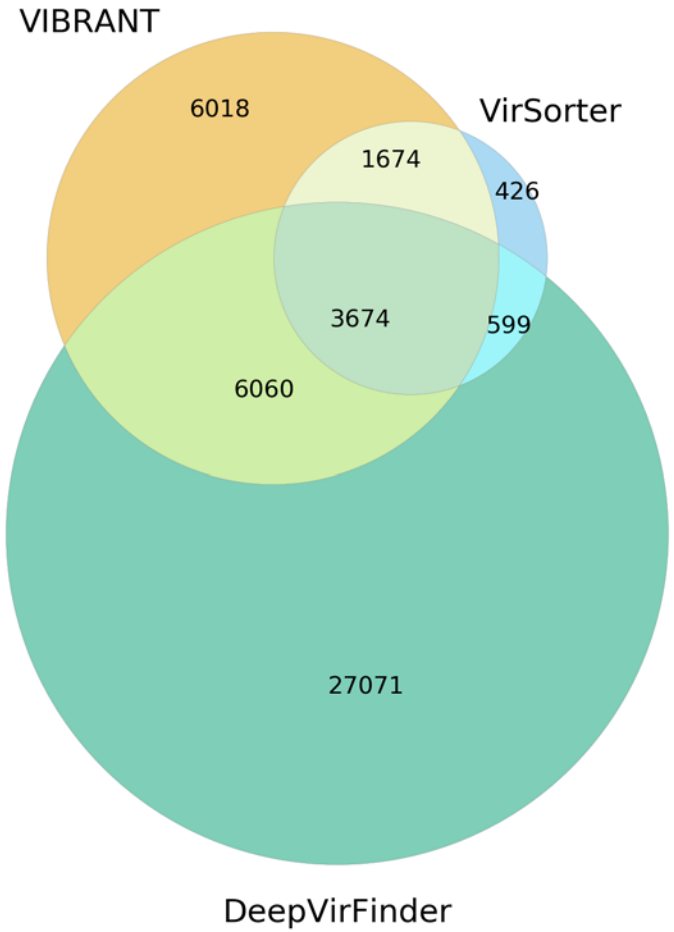
Viruses: Identification

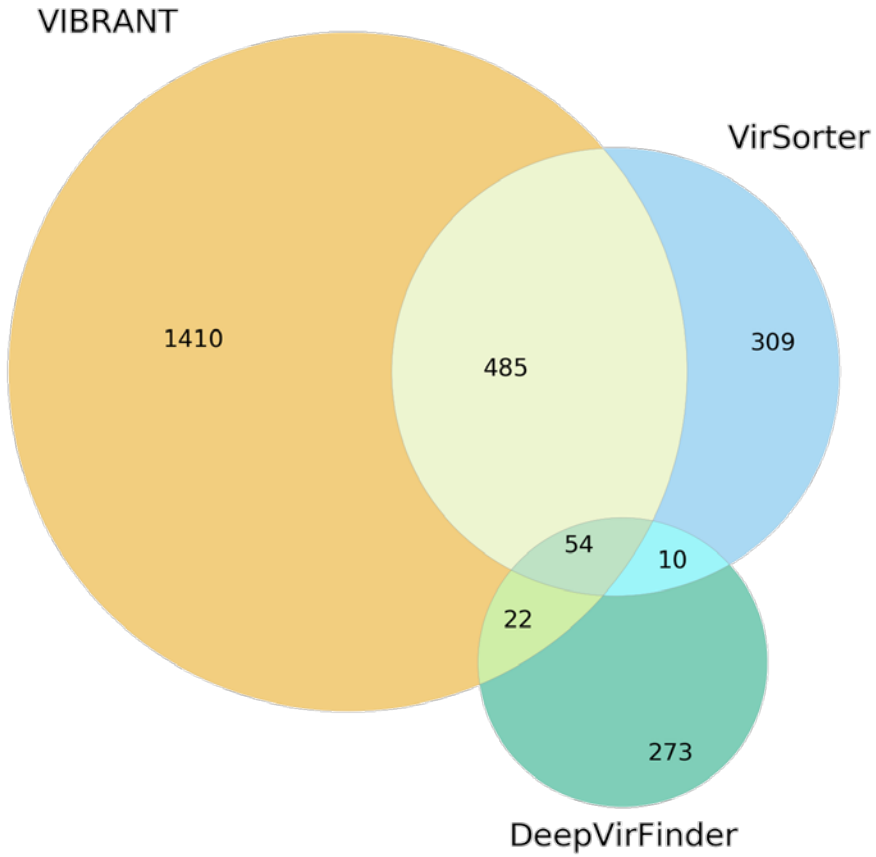


- Assembled contigs
  - Filter to min length > 2000 bp

- Viral identification tools:
  - **VirSorter**
    - Reference database-based + viral genomic features
  - **VirFinder**
    - Kmer frequency-based (machine learning approach)
  - **VIBRANT**
    - Protein similarity-based (machine learning approach)

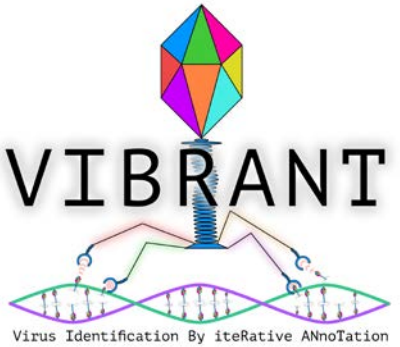
Viruses: Identification





Marine water and sediment

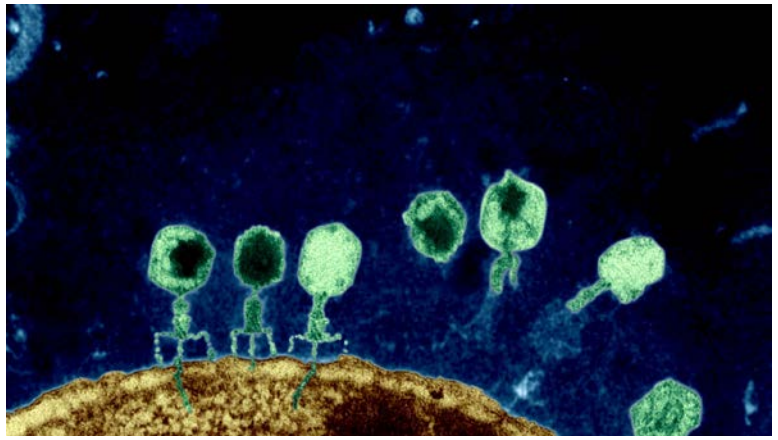
Viruses: Identification



VIBRANT

- Virus Identification By iteRative ANnoTation
- Neural network machine learning of protein annotation signatures
- https://github.com/AnantharamanLab/VIBRANT

Viruses: Evaluation



- **VIBRANT**
  - Includes **v-score**
    - proxy for quality and completeness

- **CheckV**
  - Proviruses: Trims retained host sequence
  - Estimates genome completeness
  - Predicts closed genomes
  - Outputs completeness score consistent with **MIUViG**
    - Minimum Information about an Uncultivated Virus Genome standard
    - Roux et al. (2019) *Nature Biotechnology* 37(1):29-37

Viruses: Evaluation

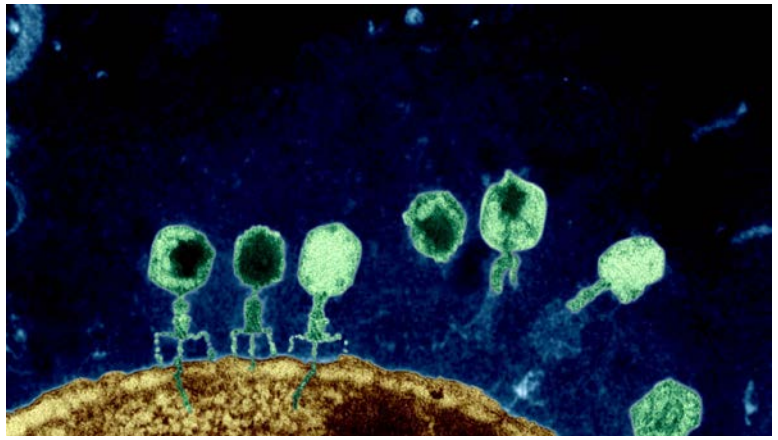
Viruses: CheckV

contig_id	contig_length	genome_copies	gene_count	viral_genes	host_genes	checkv_quality	miuvig_quality
S9.Filter_NODE_9_length_	402524	1	385	0	289	Not-determined	Genome-fragment
S3.Filter_NODE_50_length	223486	1	229	73	17	Medium-quality	Genome-fragment
S8.Filter_NODE_57_length	205905	1	212	72	12	Medium-quality	Genome-fragment
S4.Filter_NODE_43_length	193723	1	199	60	9	Low-quality	Genome-fragment
S5.Filter_NODE_27_length	193264	1	224	56	10	Medium-quality	Genome-fragment
S9.Filter_NODE_52_length	185275	1	179	57	8	Low-quality	Genome-fragment
S8.Filter_NODE_75_length	179522	1	208	86	4	Complete	High-quality

Viruses: CheckV

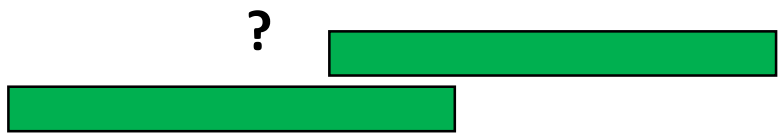
completeness	completeness_method	contamination	provirus	termini	warnings
NA	NA	0	No		'no viral genes detected'
58.01	AAI-based	0	No		
54.2	AAI-based	0	No		
48.57	AAI-based	0	No		
50.48	AAI-based	0	No		
46.04	AAI-based	0	No		
95.59	AAI-based	0	No	121-bp-DTR	
95.14	AAI-based	0	No	121-bp-DTR	

Viruses: Dereplication



- **BBMap's dedupe.sh**
  - Based on min identity fully duplicate or *contained* contigs

- **Problem: overlapping contigs**



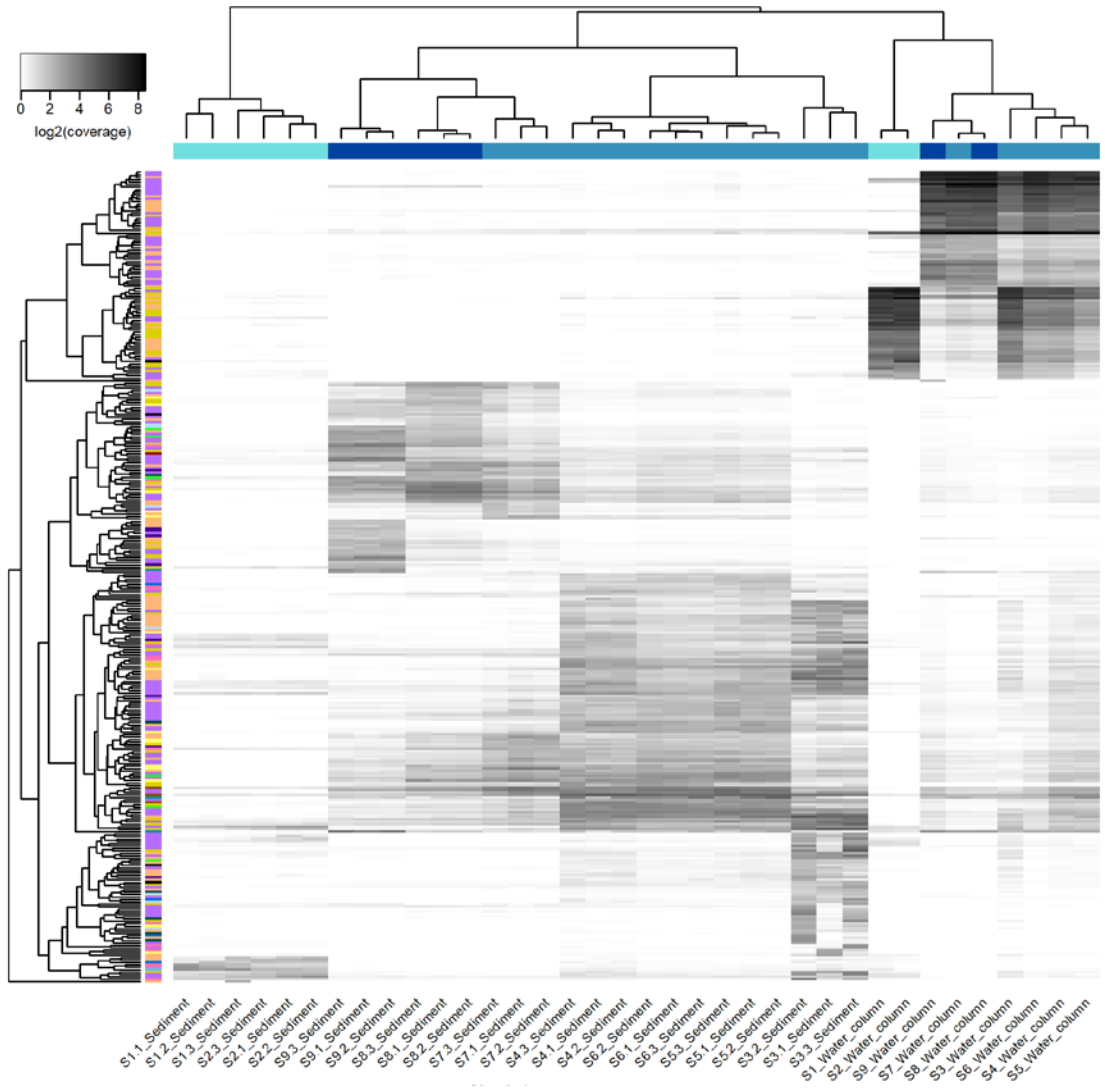
  - **dedupe.sh** can cluster overlapping contigs
  - Custom script required to merge into single representative sequence

Task: Viral identification and evaluation

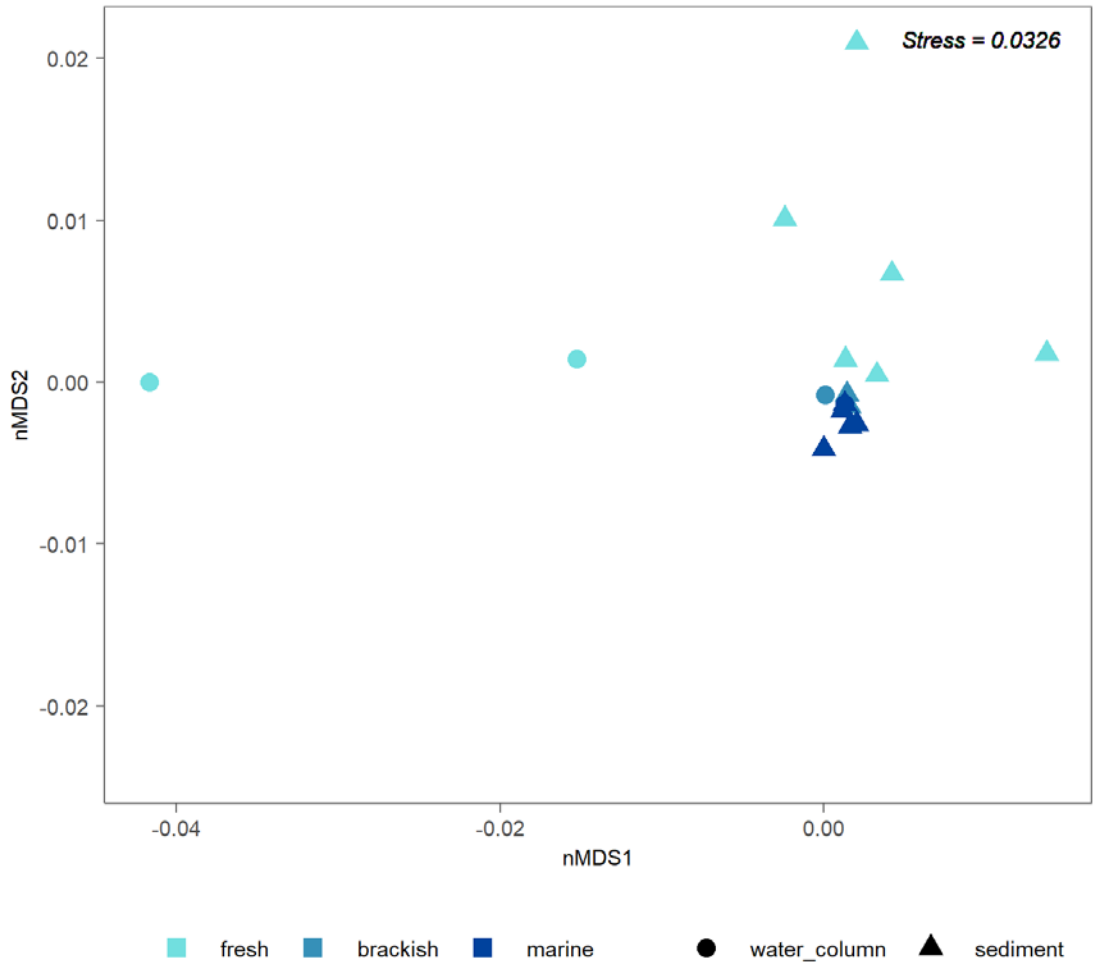
1. Viral identification using VIBRANT
2. Viral contig evaluation using CheckV

Per-sample coverage

Per-sample coverage

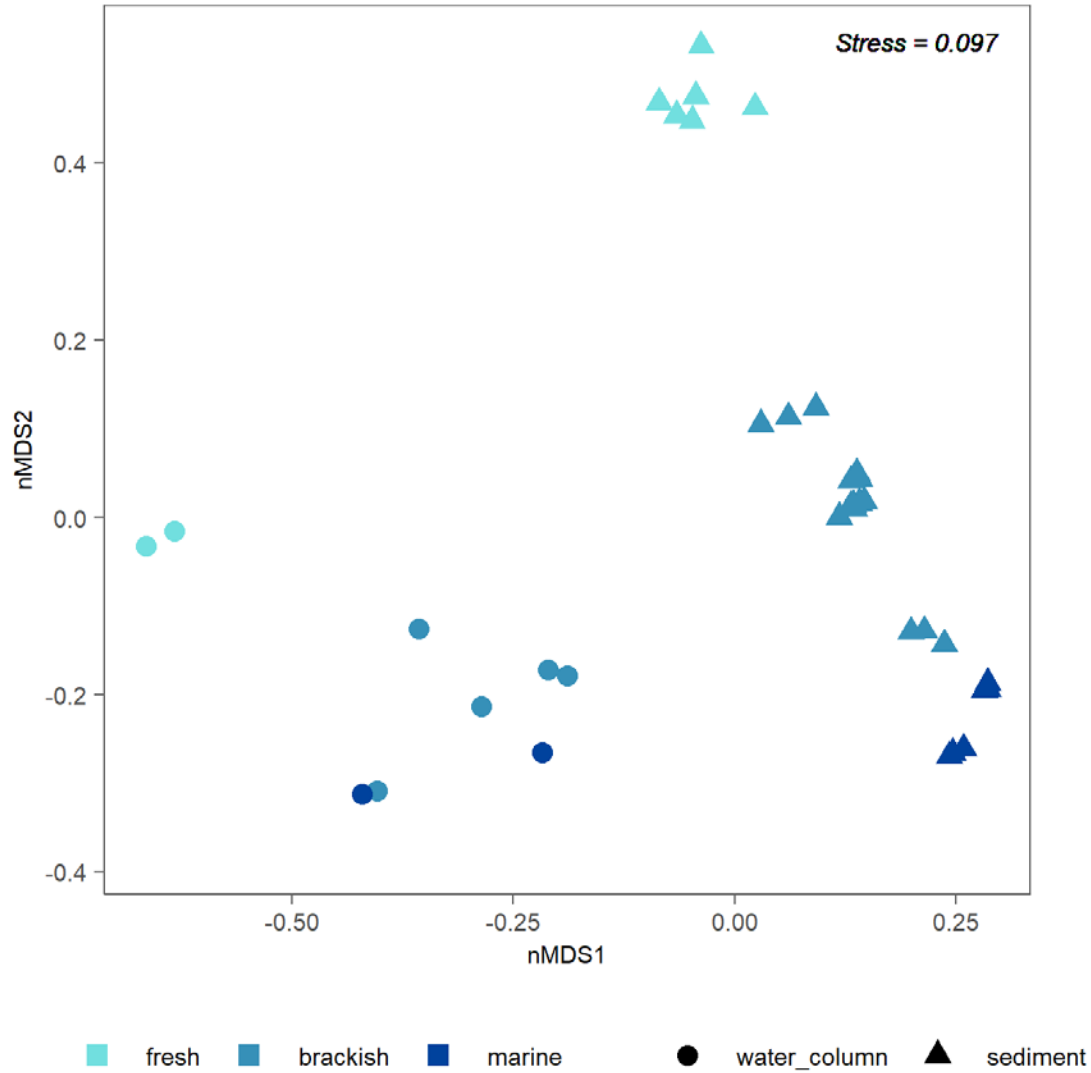


Per-sample coverage



Presence/absence of bins only

Per-sample coverage



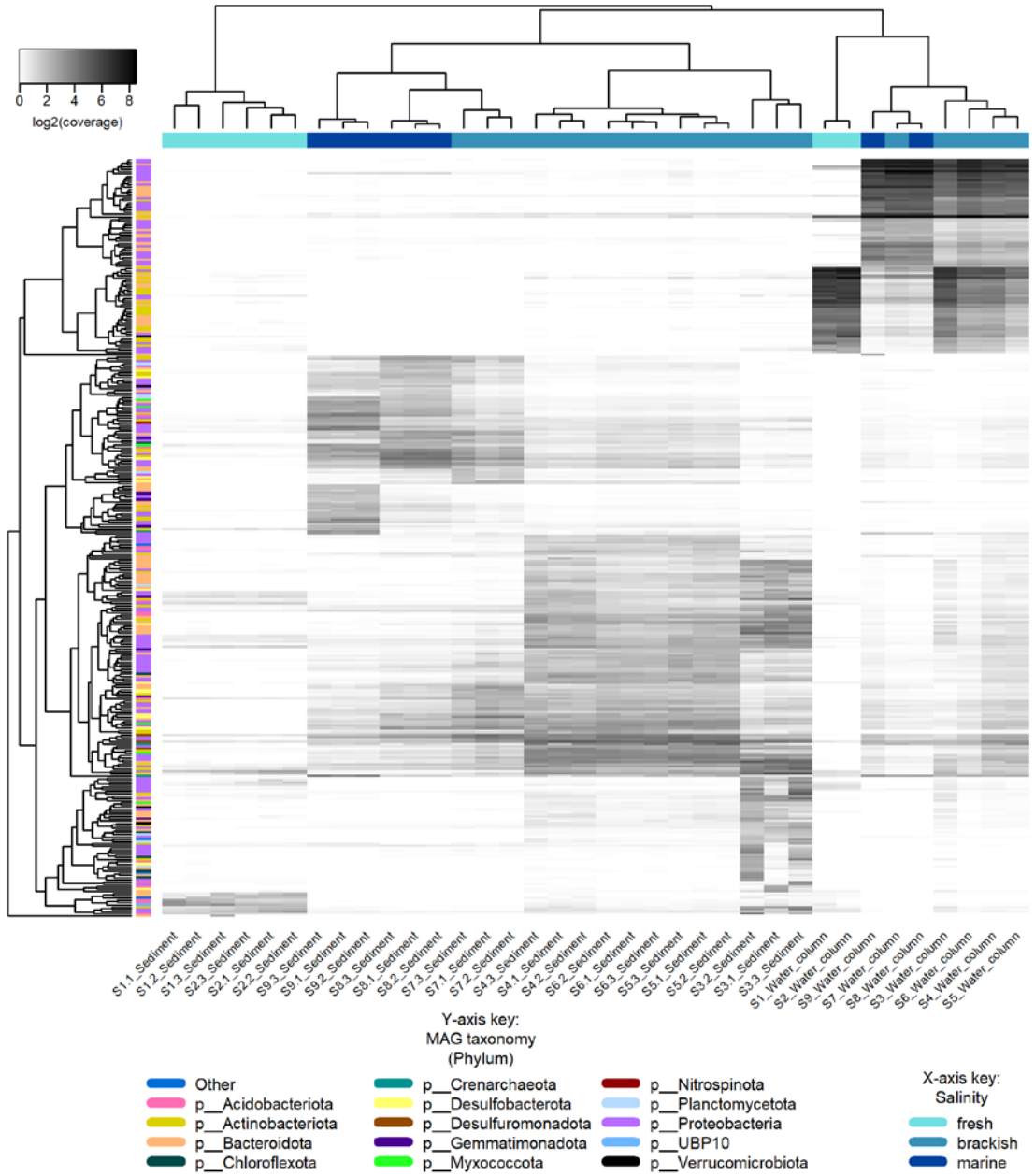
Including coverage profiles

Task: Per-sample coverage

1. Per-sample coverage stats for bin data
2. Per-sample coverage stats for viral contig data

Taxonomy

Taxonomy


0 2 4 6 8
log2(coverage)
S1.1_Sediment
S1.2_Sediment
S1.3_Sediment
S23_Sediment
S2.1_Sediment
S2.2_Sediment
S9.3_Sediment
S9.1_Sediment
S9.2_Sediment
S8.3_Sediment
S8.1_Sediment
S8.2_Sediment
S7.3_Sediment
S7.1_Sediment
S7.2_Sediment
S4.3_Sediment
S4.1_Sediment
S4.2_Sediment
S6.2_Sediment
S6.1_Sediment
S6.3_Sediment
S5.3_Sediment
S5.1_Sediment
S5.2_Sediment
S3.2_Sediment
S3.1_Sediment
S3.3_Sediment
S1_Water_column
S2_Water_column
S9_Water_column
S7_Water_column
S8_Water_column
S3_Water_column
S6_Water_column
S4_Water_column
S5_Water_column
Y-axis key:
MAG taxonomy
(Phylum)
Other
p__Acidobacteriota
p__Actinobacteriota
p__Bacteroidota
p__Chloroflexota
p__Crenarchaeota
p__Desulfobacterota
p__Desulfuromonadota
p__Gemmatimonadota
p__Myxococcota
p__Nitrospinota
p__Planctomycetota
p__Proteobacteria
p__UBP10
p__Verrucomicrobiota
X-axis key:
Salinity
fresh
brackish
marine

Task: Taxonomy

1. Assign taxonomy to the refined bins using GTDB-TK
2. Examine example outputs from viral taxonomy prediction via vConTACT2

Gene prediction

Gene prediction and annotation

- Genome prediction annotation is the process of attaching biological information to sequences
- It consists of three main steps:
  - Gene prediction
  - Prediction of protein sequences
  - Functional annotation: Attaching biological information to these elements

Gene prediction

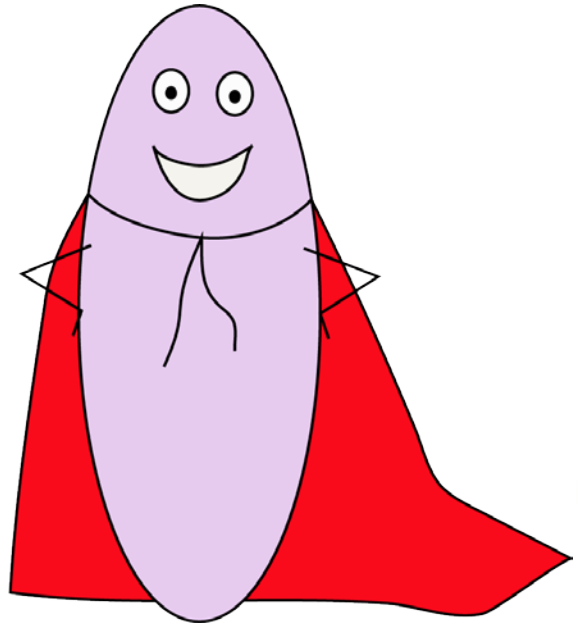
Aim:

- To identify regions of genomic DNA that encode putative genes present in high quality genomes

About 1/1000th of a human genome in size, but with only 1/10th less coding DNA sequence → 100 x more power packed!!!

Prokaryote genomes:

- High gene density
- Genes = continuous stretches of coding DNA
- Absence of introns in the protein coding regions



Gene prediction

Gene finding algorithms for prokaryotes

- Homology:
  - Search by sequence similarity to homologous sequences
  - Based on the assumption that functional regions are more conserved evolutionarily than non-functional regions

- *Ab initio*:
  - Search by content: find genes by statistical properties that distinguish protein-coding DNA from non-coding DNA
  - Search by signals/sites, e.g. promoters, start and stop codons

Gene prediction

Homology: Sequence similarity searches

- Finding similarity in gene sequences between expressed sequence tags (ESTs), proteins, or other genomes to the input genome
- Local alignment:
  - BLAST family tools: https://blast.ncbi.nlm.nih.gov/Blast.cgi
  - Global alignment
  - GeneWise: https://www.ebi.ac.uk/Tools/psa/genewise/

Gene prediction

***Ab initio* search by content algorithms:**

- Markov Models
- Dynamic Programming
- Linear discriminant analysis
- Linguist methods
- Neural Network

Gene prediction

***Ab initio* search by content:** Markov Model Based Algorithms

- Most widespread algorithms for gene finding in prokaryotes are based on Markov Models
- Aim is to capture compositional differences among coding regions, “shadow” coding regions (coding on the opposite DNA strand) and non-coding DNA

Gene prediction

Markov Model Based Algorithms: Glimmer

- https://ccb.jhu.edu/software/glimmer/
- Interpolated Markov model (IMM) DNA discriminator
- Log-likelihood that a given interval on a DNA sequence was generated by a model of coding versus non-coding DNA

Gene prediction

Markov Model Based Algorithms: GeneMark/GeneMarkHMM/MetaGeneMark

- http://exon.gatech.edu/GeneMark/
- GeneMark is a family of gene prediction tools
- Genomic sequences can be analysed either by the self-training program GeneMarkS (sequences >50 kb) or using Heuristic Models by GeneMarkHMM
- Pre-trained model parameters are available for many species
- Metagenomics sequences can be analysed with MetaGeneMark

Gene prediction

Prodigal (PROkaryotic Dynamic Programming Genefinding ALgorithm)

- http://compbio.ornl.gov/prodigal/
- Based on Dynamic Programming, not Markov Models
- Gene-finding algorithm for prokaryote genomes developed to predict translation initiation sites more accurately.
- High accuracy in high GC content genomes
- Tends to predict longer genes rather than more genes (minimising number of false positives)

Gene prediction

Prodigal for metagenomics:

- Use anon (meta) mode with metagenomic data (or short sequence data)
  - Copes with diverse genomes
  - Unlike normal mode, it does not attempt to study the input sequence, and predict based on these assumptions
  - Uses pre-calculated training files, and predicts genes based on the best results
- Alternatively, use normal mode on each individual genome bin

Gene prediction

Prodigal for metagenomics:

- Caveat: unusual genetic codes
  - First uses genetic code 11 (stop codons TAA, TGA, TAG)
  - If genes are too short, uses alternative code 4 (TGA not a stop codon)
  - Will not try code 25, but will issue warning if genes are short
  - Must manually select code 25

Gene prediction

Prodigal for metagenomics:

- Important note:
  - Prodigal predicts coding DNA sequence ONLY
  - Provides nucleic acid (.fna) and amino acid (.faa) files
  - DOES NOT identify other features (e.g. rRNA, tRNA)
  - Combine with other prediction tools

Gene prediction

Predicting RNA features and non-coding regions:

- MeTaxa2: predicts ribosomal RNA sequences in a genome
- Aragorn: predicts tRNA and tmRNA sequences

Gene prediction

Predicting protein coding sequences in unassembled (short) reads

- FragGeneScan:
    - Tuning parameters for short sequences (and hence incomplete genes)
    - Model sequence error

Task: Gene prediction

Preparing data for gene prediction

1. Identify and prepare input files for each gene prediction tool (Prodigal, MeTaxa2 and Aragorn)
2. Configure parameters for gene prediction

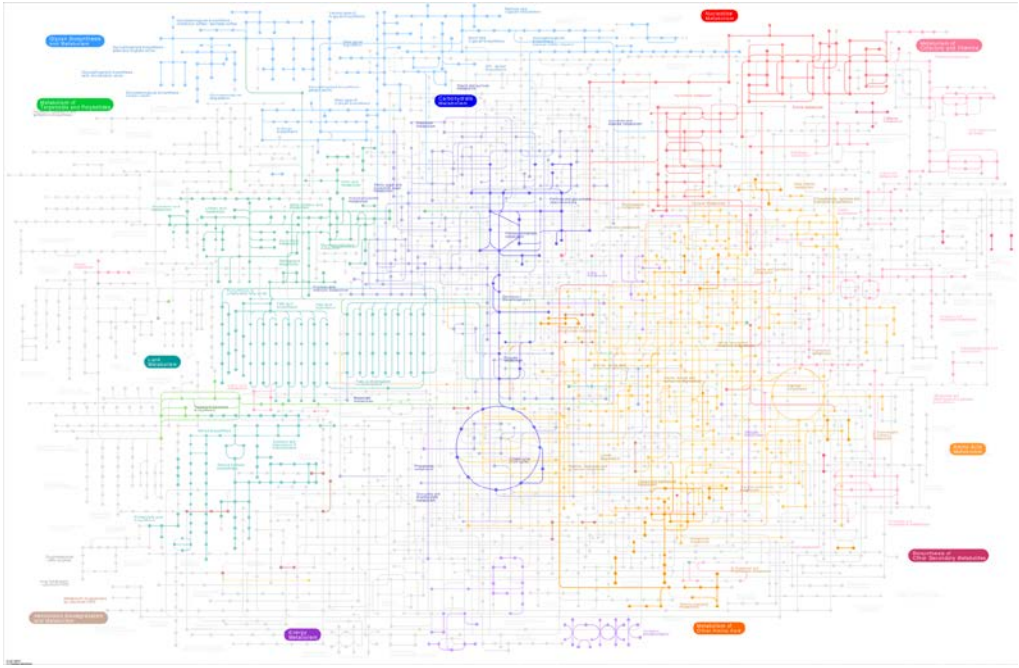
Perform gene prediction

1. Run each job directly from the node (no slurm script required)

Gene annotation

Gene annotation

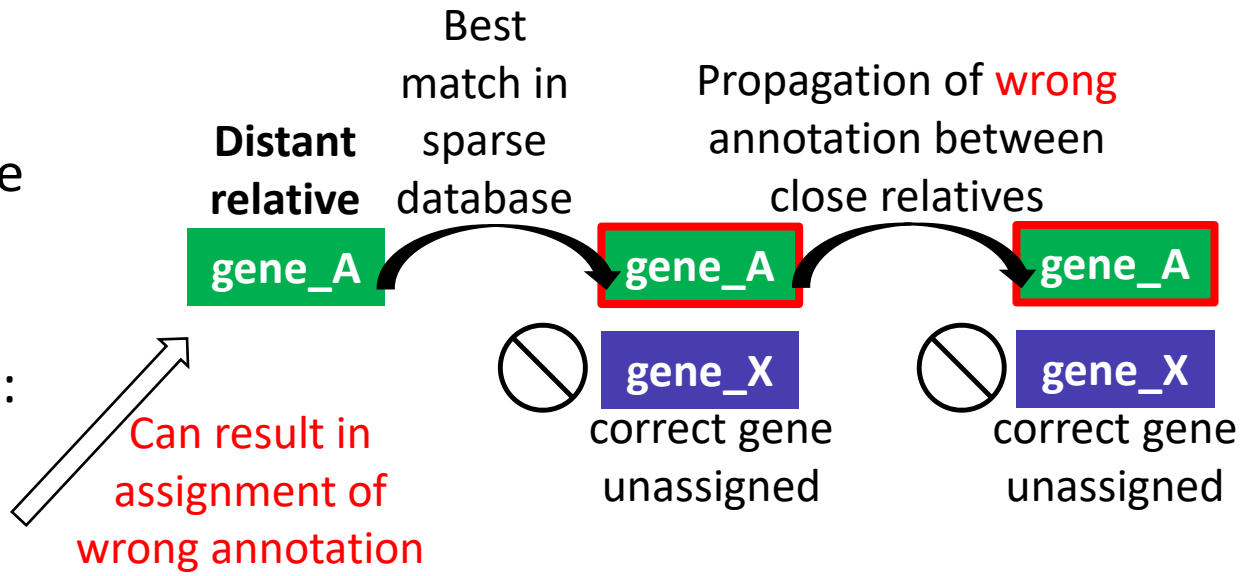
- Genome annotation attempts to predict gene function
- Predicted genes or protein sequences are compared against a curated set of reference sequences for which function is known, or is strongly suspected



Gene annotation

Caveat:

- Annotations are dependent on the reference database
- Environmental genomes can have:
  - Genes with distant homology matches to unrelated taxa
  - Large numbers of “hypothetical” gene annotations (= genes of unknown function)



Gene annotation

Caveat:

- Annotations are “advice”
- Automated annotations often need to be manually curated
- Interrogate if: expected functional gene is missing from annotations
- Gene synteny is a useful for missing gene discovery, e.g.:
  - check genes co-located in operons for putative functions
  - check for operon truncation (due to contig break)

(Handley et al., 2014, Environmental Microbiology)

Gene annotation

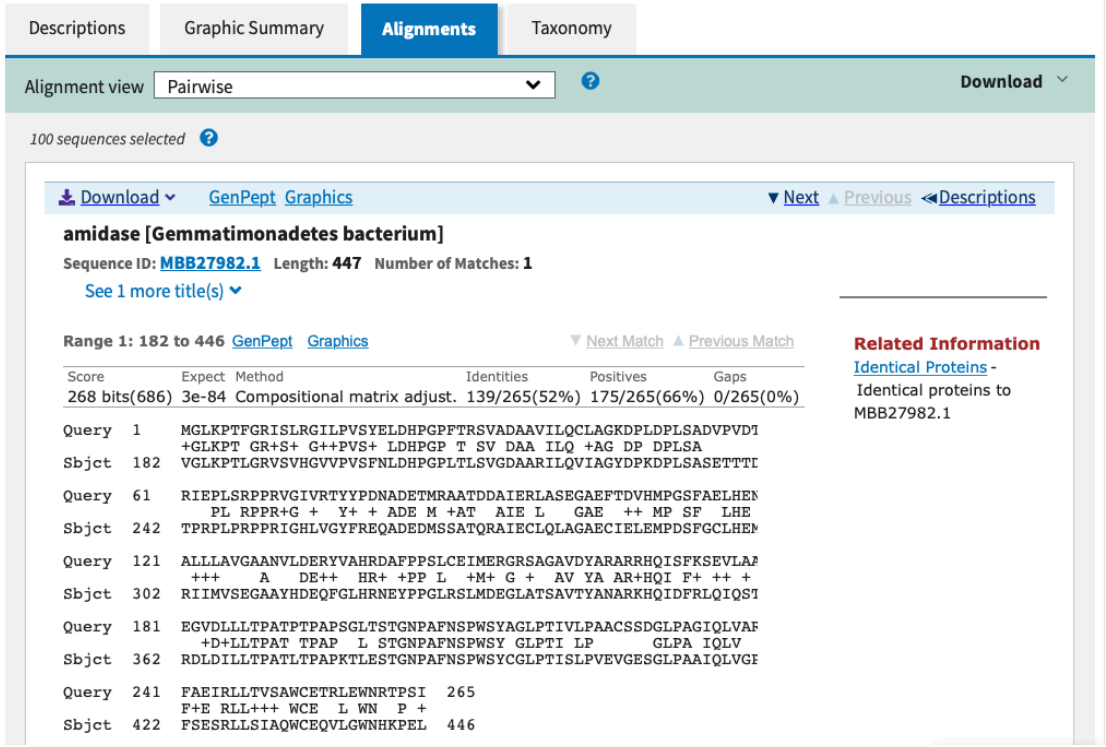
There are two main ways to perform gene annotation with protein sequences:

- BLAST-like gene annotation
- Domain annotation

Gene annotation

BLAST-like gene annotation

- Pairwise local alignment between the gene of interest (query sequence) and the sequences in the database (target sequence)
- Tools:
  - BLAST: web-based and stand alone (usually too slow for metagenomics)
  - USEARCH (64-bit): fast (subscription needed)
  - Diamond: fast



Gene annotation

HMM-profiling of domains:

- Considers the query sequences as a collection of independently functioning protein folding domains
- Uses database of Hidden Markov models built from a collection of proteins that share a common domain
- Profiles build from statistical map of the
  - amino acid transitions (from position to position),
  - variations (differences at a position),
  - insertions/deletions between positions
- Tools: HMMer software (http://hmmer.org/)

Gene annotation

Common functional databases

- KEGG (Kyoto Encyclopedia of Genes and Genomes) (https://www.kegg.jp)

  Very popular, each entry is well annotated, and often linked into “Modules” or “Pathways” (Full access now requires a license fee)

- COGs (Clusters of Orthologous Groups of proteins) (https://www.ncbi.nlm.nih.gov/COG/)

  Classify proteins from completely sequenced genomes on the basis of the orthology concept

- PFAM (https://pfam.xfam.org)

  Focused more on protein domains based on hidden Markov models

- TIGRfam (https://www.jcvi.org/tigrfams)

  Database of protein family definitions based on hidden Markov models

Gene annotation

Common functional databases (continued)

- The PANTHER (**P**rotein **AN**alysis **TH**rough **E**volutionary **R**elationships) Classification System (http://pantherdb.org)

  Proteins are classified according to Family and subfamily, molecular function, biological process and pathway

- UniRef (UniProt Reference Clusters) (https://www.uniprot.org/)

  Protein clustering at different levels (e.g. UniRef100, UniRef90, UniRef50)

- BioCyc Database Collection (https://biocyc.org)

  14735 Pathway/Genome Databases (PGDBs), plus software tools

  Subscriptions are required to access most of BioCyc

- MetaCyc Metabolic Pathway Database (https://metacyc.org)

  2722 pathways from 3009 different organisms

Gene annotation

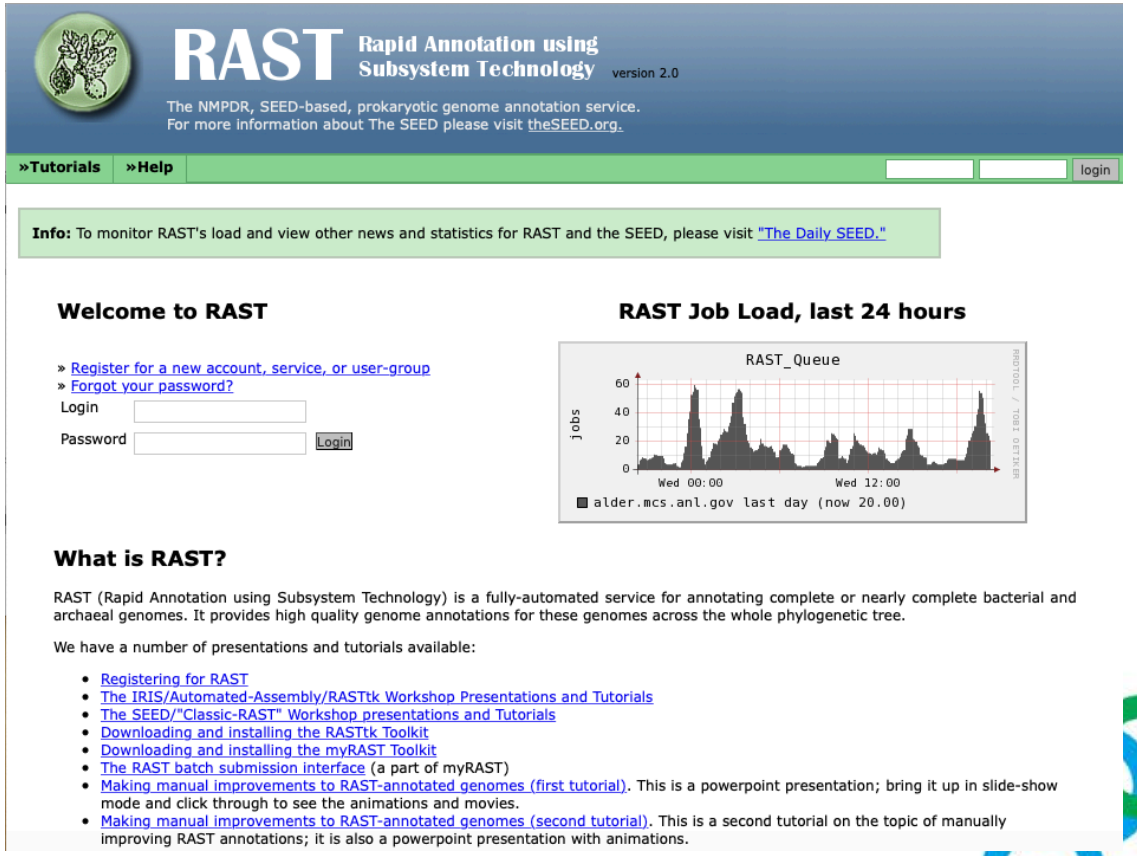
Some web-based annotation tools:

- Web BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi)
- RAST/MG-RAST (Rapid Annotation using Subsystem Technology) Annotation Server
- KEGG Automatic annotation and KEGG mapping service
  - BLAST-Koala: BLAST search (https://www.kegg.jp/blastkoala/)
  - GHOST-Koala: GHOSTX search (https://www.kegg.jp/ghostkoala/)
  - KofamKOALA: HMM profile search (https://www.genome.jp/tools/kofamkoala/)
- IMG/M (The Integrated Microbial Genomes and Microbiomes) (https://img.jgi.doe.gov)

Gene annotation

RAST Annotation Server (Rapid **Annotation** using Subsystem Technology):

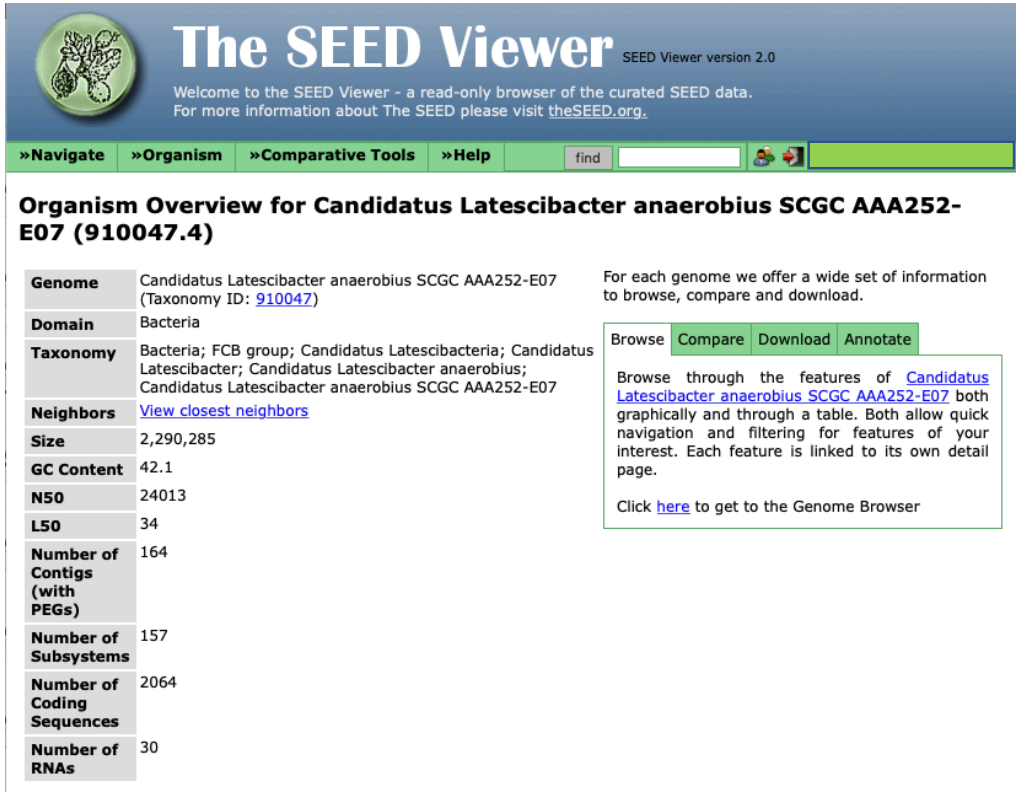
- Fast annotation (~1 genome/day)
- Can use for individual genome bins
- It works well for genomes similar to large groups of reference genomes
- As usual: requires manual curation after initial annotation

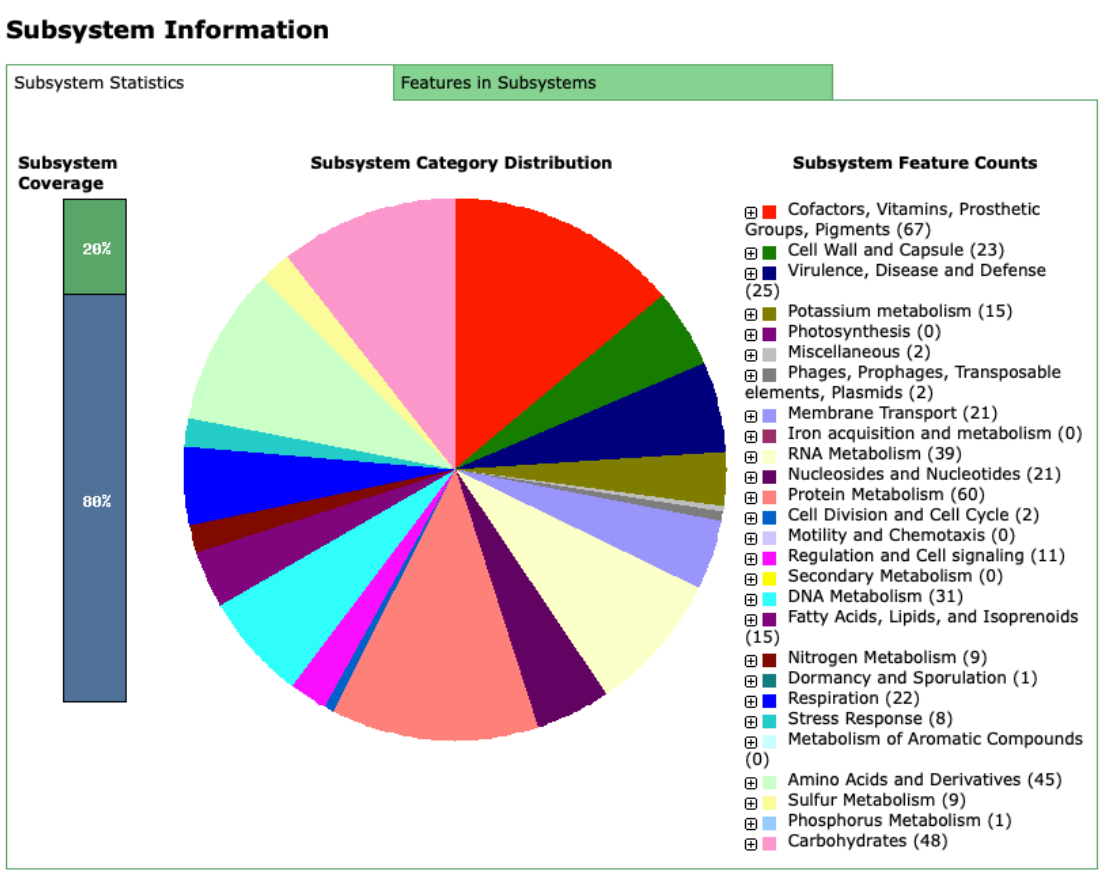


http://rast.theseed.org/FIG/rast.cgi

Gene annotation

RAST Annotation Server (Rapid **Annotation** using Subsystem Technology)





Gene annotation

Distilling and Refining Annotations of Metabolism (DRAM; Shaffer et al. 2020. Nucleic Acids Research 48(16)**)**

- Tool for gene prediction and gene annotation of MAGs (DRAM-v for viruses)
  - Functional annotation:
    - BLAST-style searches:
      - KEGG (if provided),
      - UniRef 90 (if desired)
      - MEROPS
    - HMM searches
      - Kofam, Pfam, dbCAN2 (CAZy)
      - VOGDB
    - tRNAs and rRNAs also detected

Gene annotation

Distilling and Refining Annotations of Metabolism (DRAM; Shaffer et al. 2020. Nucleic Acids Research 48(16)**)**

- Genome annotations to metabolic functions in three levels:

1. RAW
Each gene nucleotide and amino acid sequence with annotations

	fasta	scaffold	gene_positio	start_positio	end_position	strandedness	rank	kegg_id	kegg_hit	uniref_id	uniref_hit	uniref_taxon	uniref_RBH
bin_0_1f935	bin_0	1f9359e86e6	1	205	1371	1	B	K02338	DNA polyme	Q7V9E7_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	2	1375	2151	1	B			Q7V9E6_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	3	2191	4593	1	B	K23269	phosphoribos	PURL_PROM	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	4	4653	6110	1	B	K00764	amidophospl	Q7TV87_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	5	6146	8635	-1	B			A0A163AH7(	UniRef90_A(	Cyanobacteri	TRUE
bin_0_1f935	bin_0	1f9359e86e6	6	8713	9606	-1	B			Q7V9E3_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	7	9616	10590	-1	B	K18979	epoxyqueuos	Q7V9E2_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	8	10677	11291	1	B			Q7V9E1_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	9	11363	12112	1	B			Q7V9E0_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	10	12142	12777	1	B	K03625	transcription	A0A162EFM(	UniRef90_A(	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	11	12777	14231	1	B	K03110	fused signal	Q7V9D8_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	12	14355	15698	1	B	K07315	phosphoserir	A0A163R2M	UniRef90_A(	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	13	15728	17140	1	B	K01755	argininosucc	ARLY_PROM	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	14	17264	17872	1	C			Q7V9D6_PR(	UniRef90_Q	Cyanobacteri	FALSE
bin_0_1f935	bin_0	1f9359e86e6	15	17882	18886	-1	B	K05539	tRNA-dihydr	A0A163N6K8	UniRef90_A(	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	16	18956	19462	1	C	K07305	peptide-met	A0A163N6J4	UniRef90_A(	Prochlorococ	FALSE
bin_0_1f935	bin_0	1f9359e86e6	17	19434	20711	1	B			Q7V9D3_PR(	UniRef90_Q	Prochlorococ	TRUE
bin_0_1f935	bin_0	1f9359e86e6	18	20686	21966	-1	B	K02653	type IV pilus	Q7V9D2_PR(	UniRef90_Q	Cyanobacteri	TRUE
bin_0_1f935	bin_0	1f9359e86e6	19	21983	23059	-1	B	K02669	twitching mo	A0A163N6E5	UniRef90_A(	Prochlorococ	TRUE
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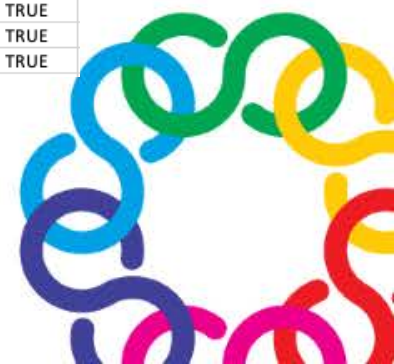


Gene annotation

Distilling and Refining Annotations of Metabolism (DRAM; Shaffer et al. 2020. Nucleic Acids Research 48(16)**)**

- Genome annotations to metabolic functions in three levels:

2. DISTILLATE
Taxonomy (GTDB-tk), quality statistics (checkM), and key metabolisms summarized by genome

9	K02303	uroporphy	Siroheme b	Antibiotic Resistance		1	1	3	1	1	1	0	1	1	1
0	K02304	precorrin-2	Siroheme b	Antibiotic Resistance		0	1	1	1	1	0	0	1	1	1
1	K02492	glutamyl-tf	Siroheme b	Antibiotic Resistance		1	1	1	1	1	0	1	1	1	1
2	K02496	uroporphy	Siroheme b	Antibiotic Resistance		0	0	1	1	1	0	0	0	0	0
3	K03794	sirohydroc	Siroheme b	Antibiotic Resistance		0	0	0	0	0	0	0	0	0	0
4	K13542	uroporphy	Siroheme b	Antibiotic Resistance		0	0	0	0	0	0	0	0	1	1
5	K13543	uroporphy	Siroheme b	Antibiotic Resistance		0	0	0	0	0	0	0	0	0	0
6	K14163	glutamyl-tf	Siroheme b	Antibiotic Resistance		0	0	0	0	0	0	0	0	0	0
7	K07464	cas4; CRISP	Subtype I-A	CRISPR	Type I CRISI	0	0	0	0	0	1	0	0	1	2
8	K07725	csa3; CRISP	Subtype I-A	CRISPR	Type I CRISI	0	0	0	0	0	0	0	0	0	0
9	K19074	csa2; CRISP	Subtype I-A	CRISPR	Type I CRISI	0	0	0	0	0	0	0	0	0	0
0	K19075	cst2, cas7;	Subtype I-A	CRISPR	Type I CRISI	0	0	0	0	0	0	0	1	0	1

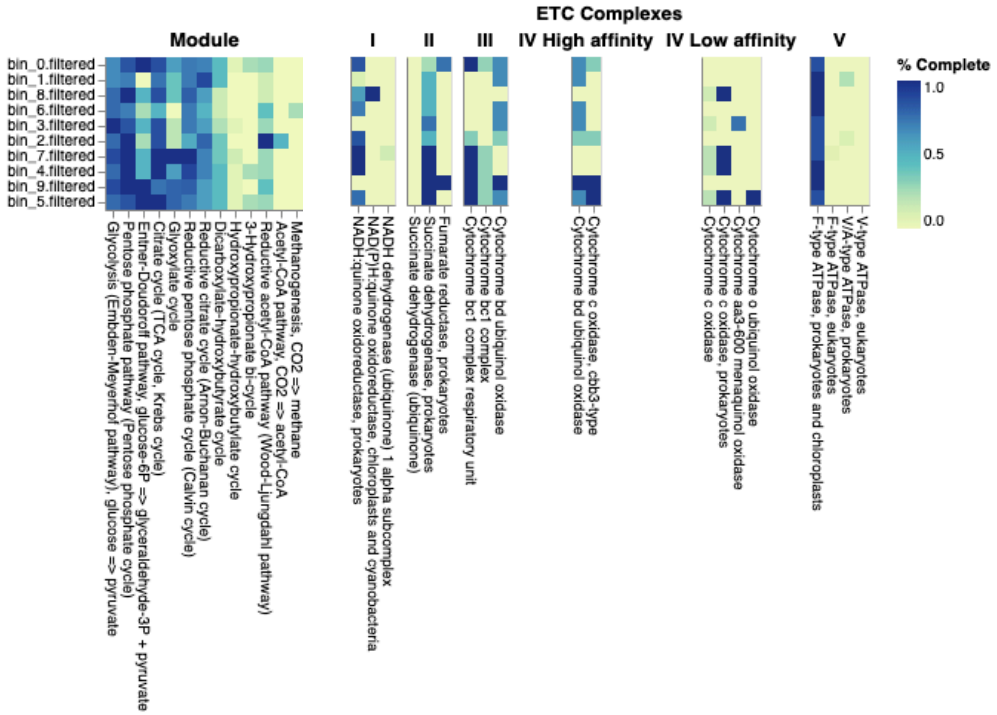
MISC | carbon utilization | Transporters | Energy | Organic Nitrogen | rRNA | tRNA | +

Gene annotation

Distilling and Refining Annotations of Metabolism (DRAM; Shaffer et al. 2020. Nucleic Acids Research 48(16)**)**

- Genome annotations to metabolic functions in three levels:

3. PRODUCT
Interactive heatmap of key metabolic functions by genome



Gene annotation

Distilling and Refining Annotations of Metabolism (DRAM; Shaffer et al. 2020. Nucleic Acids Research 48(16)**)**

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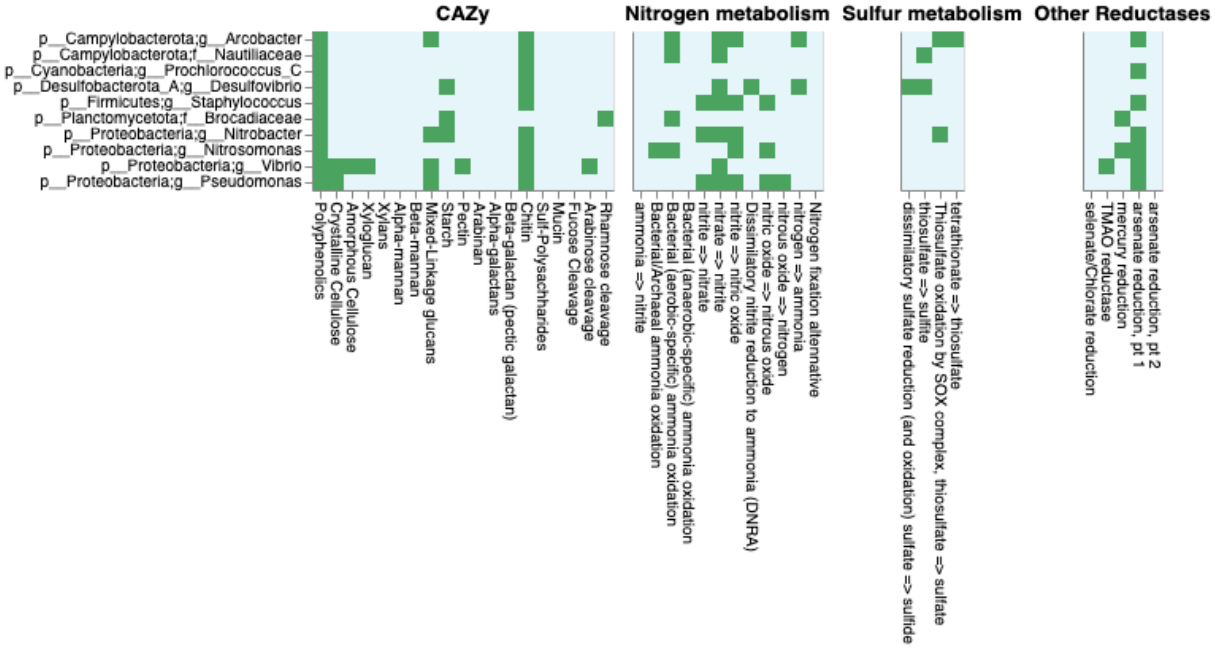
Interactive heatmap of key metabolic functions by genome



Task: Gene annotation

Preparing data for gene annotation

1. Identify and prepare input files for gene annotation with Diamond and hmmer
2. Configure parameters for gene annotation

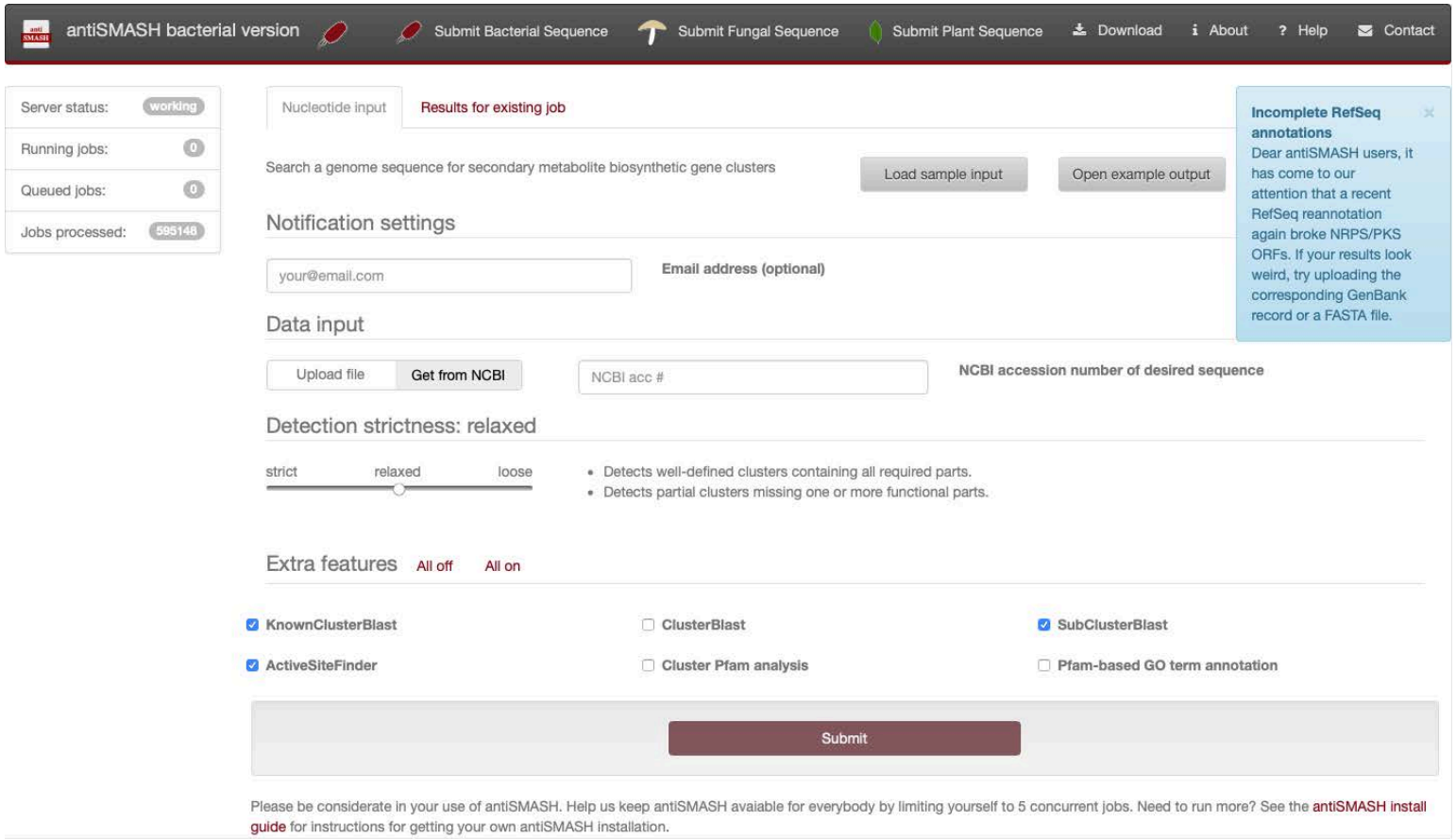
Perform gene annotation

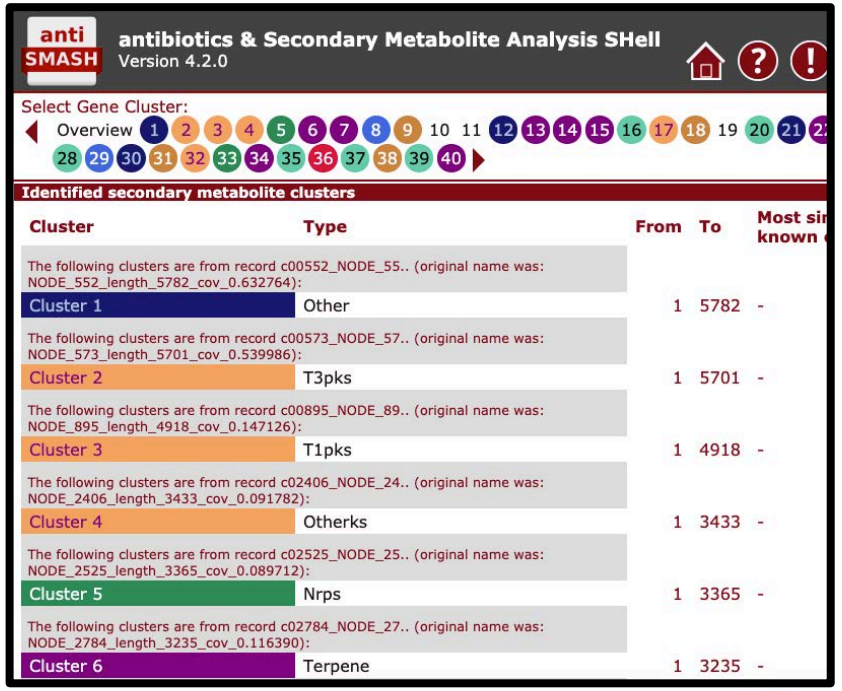
1. Prepare annotation jobs (Diamond and hmmer) to run under slurm

Online resources and data analysis

Gene annotation

Identification of Biosynthetic Gene Clusters with **antiSMASH**

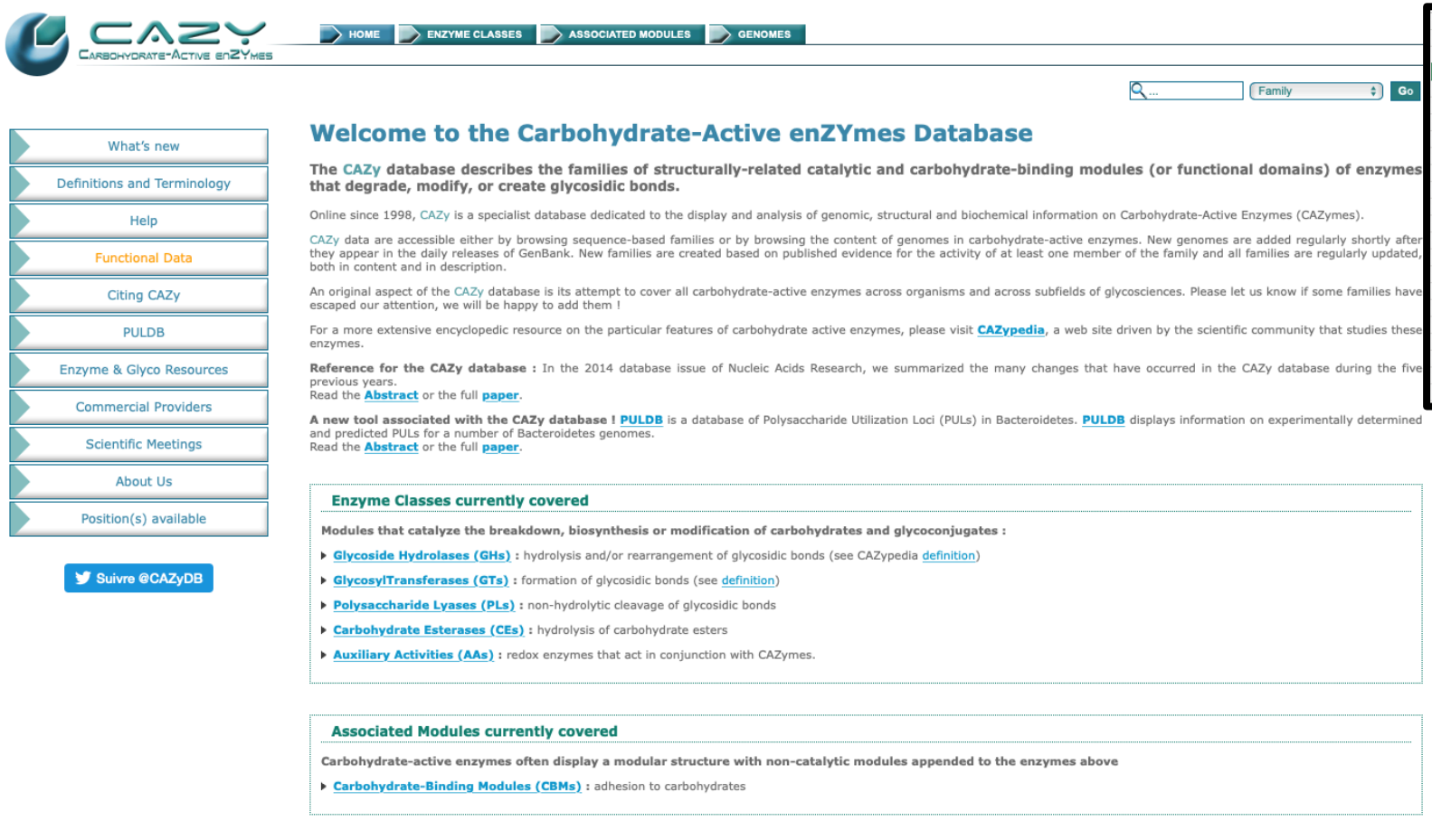




https://antismash.secondarymetabolites.org/

Gene annotation

Identification of Carbohydrate-Active enZYmes - **CAZY Database**



Query	Subject	E-value	Subject start	Subject end	Query start	Query end	Covered fraction
fig|6666666.197029.peg.1003	GH109.hmm	6.90E-12	1	117	2	112	0.920634921
fig|6666666.197029.peg.1015	GT4.hmm	3.00E-35	8	157	193	340	0.93125
fig|6666666.197029.peg.1034	PL17.hmm	0.00017	2	84	577	648	0.589928058
fig|6666666.197029.peg.1083	GH38.hmm	3.20E-28	25	182	33	183	0.583643123
fig|6666666.197029.peg.1124	GH117.hmm	2.60E-06	64	161	39	125	0.45971564
fig|6666666.197029.peg.1151	CE14.hmm	1.10E-16	1	124	9	127	0.991935484
fig|6666666.197029.peg.1183	GH109.hmm	2.70E-09	63	121	23	84	0.46031746
fig|6666666.197029.peg.1208	GH109.hmm	2.30E-12	3	112	6	106	0.865079365
fig|6666666.197029.peg.1232	GH109.hmm	1.70E-07	2	121	25	145	0.944444444
fig|6666666.197029.peg.1233	GH74.hmm	2.70E-11	30	117	164	254	0.373390558
fig|6666666.197029.peg.1233	GH74.hmm	4.70E-08	43	118	228	301	0.321888412
fig|6666666.197029.peg.1247	PL12.hmm	1.70E-27	1	138	388	516	0.992753623
fig|6666666.197029.peg.127	GH109.hmm	1.60E-12	2	122	5	119	0.952380952
fig|6666666.197029.peg.1289	GH109.hmm	1.60E-07	1	122	10	133	0.96031746
fig|6666666.197029.peg.1297	GH109.hmm	2.20E-08	4	121	5	111	0.928571429
fig|6666666.197029.peg.130	GH32.hmm	1.80E-08	43	171	93	232	0.436860068
fig|6666666.197029.peg.1325	GH109.hmm	1.20E-12	4	105	9	105	0.801587302
fig|6666666.197029.peg.1326	GH109.hmm	1.10E-09	4	118	10	115	0.904761905
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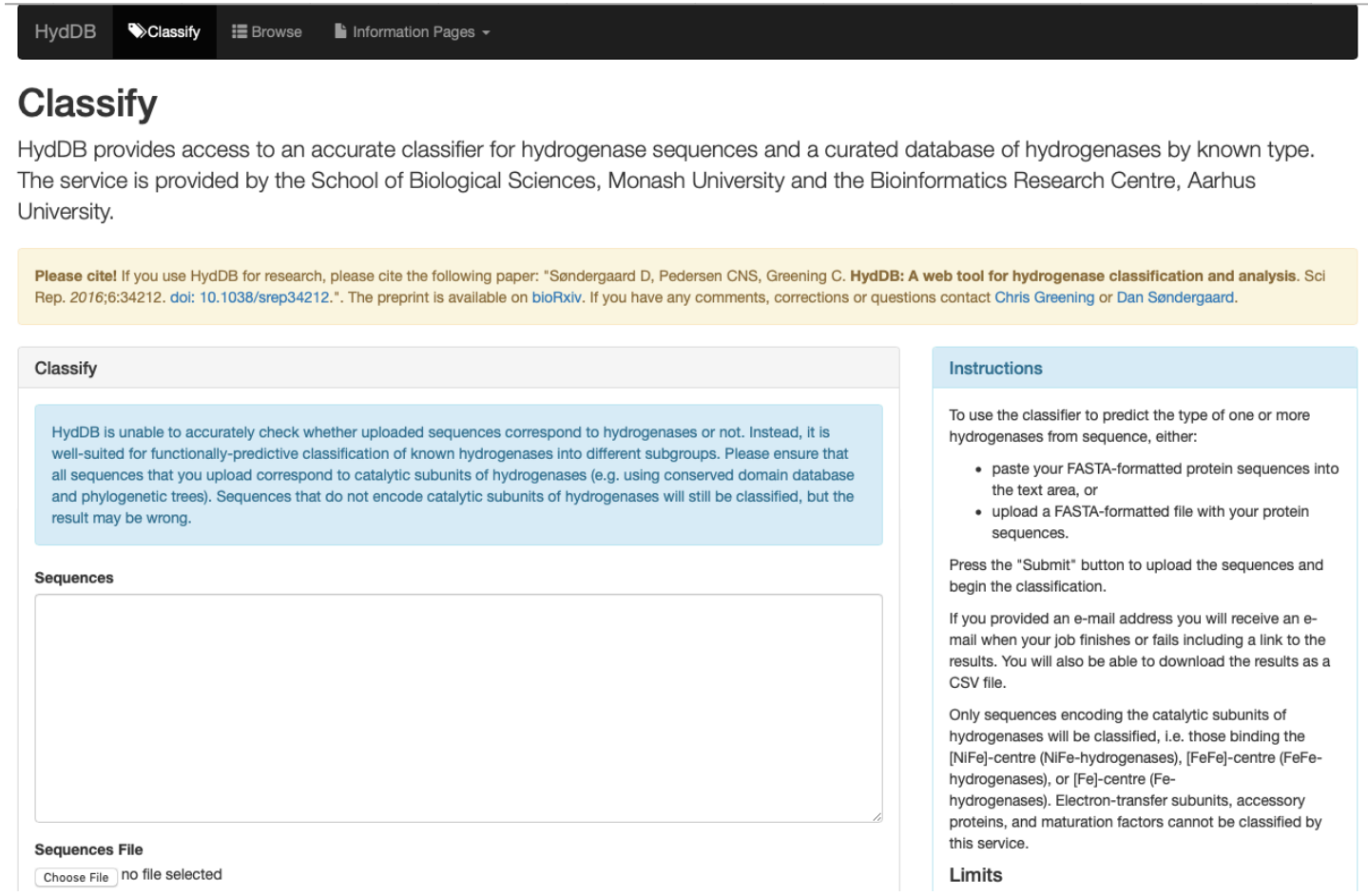
fig|6666666.197029.peg.1334	CE10.hmm	9.80E-16	99	316	3	221	0.636363636
fig|6666666.197029.peg.1340	GH28.hmm	7.60E-07	59	217	148	307	0.486153846
fig|6666666.197029.peg.1359	CBM9.hmm	1.60E-32	1	181	39	240	0.989010989

http://www.cazy.org

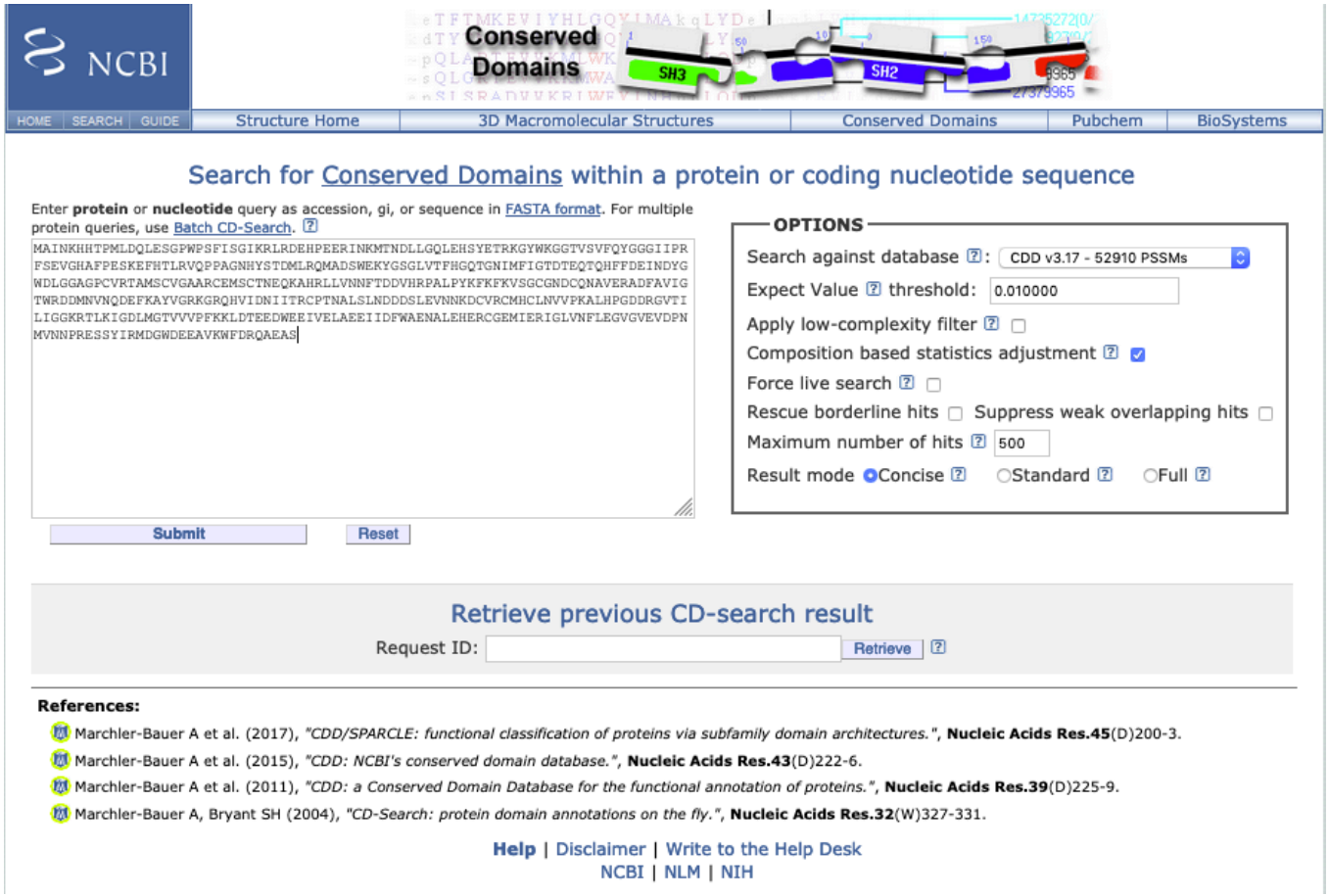
Gene annotation

Accurate classifier for hydrogenase sequences - **HydDB**



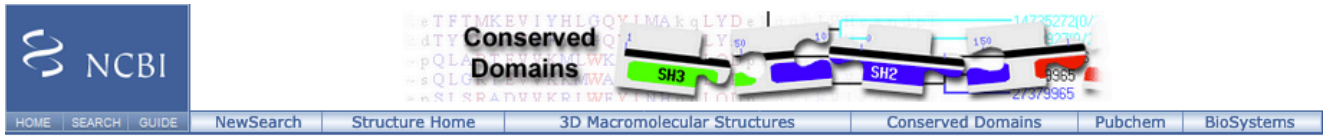
https://services.birc.au.dk/hyddb/

NCBI Conserved Domain Search



Search nucleotide/protein sequence(s) for conserved domains

Individual search: https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
Batch: https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi

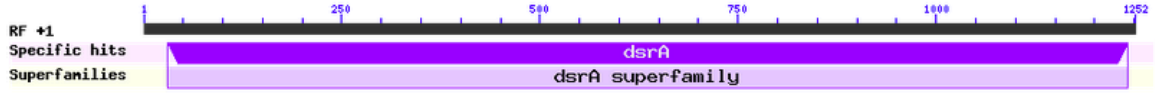


Conserved domains on [lcl|1]

View: Concise Results

NZ_JRAA01000001.1:c722683-721433 Solemya velum gill symbiont strain WH SV_sym_Scaffold_1, whole genome shotgun sequence

Graphical summary Zoom to residue level show extra options »



Search for similar domain architectures | Refine search

List of domain hits

Name	Accession	Description	Interval	E-value
dsrA	TIGR02064	sulfite reductase, dissimilatory-type alpha subunit; Dissimilatory sulfite reductase catalyzes ...	31-1242	0e+00

sulfite reductase, dissimilatory-type alpha subunit; Dissimilatory sulfite reductase catalyzes the six-electron reduction of sulfite to sulfide, as the terminal reaction in dissimilatory sulfate reduction. It remains unclear however, whether trithionate and thiosulfate serve as intermediate compounds to sulfide, or as end products of sulfite reduction. Sulfite reductase is a multisubunit enzyme composed of dimers of either alpha/beta or alpha/beta/gamma subunits, each containing a siroheme and iron sulfur cluster prosthetic center. Found in sulfate-reducing bacteria, these genes are commonly located in an unidirectional gene cluster. This model describes the alpha subunit of sulfite reductase. [Central intermediary metabolism, Sulfur metabolism]

:

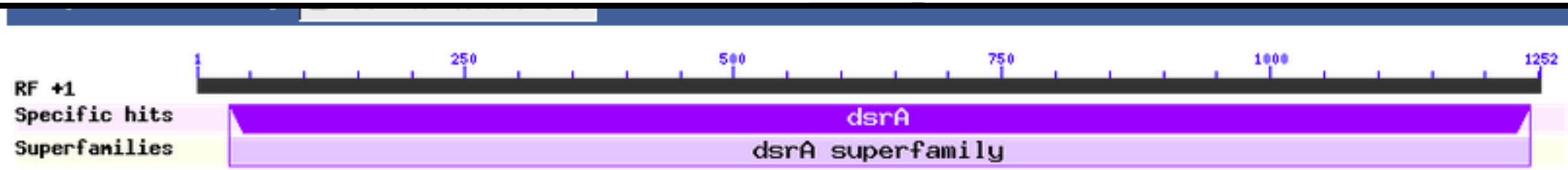
Pssm-ID: 273948 [Multi-domain] **Cd Length:** 402 **Bit Score:** 667.31 **E-value:** 0e+00

```
                    10        20        30        40        50        60        70        80
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1              11 LDQLESGPWPSFISGIKRLRDEHPEERINKMTNDLLGQLEHSYETRKGYWKGGTVSVFQYGGGIIPRFSEVGHAFPESKE 90
Cdd:TIGR02064   1 LDQLEKGPWPSFVSEIKKTAAYRADYQVPVDPEDLLGVLELSYDERKTHWKGGIVSVFGYGGGVIGRYSDQGEKFPGVAE 80

                   90        100       110       120       130       140       150       160
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1              91 FHTLRVQPPAGNHYSTDMLRQMADSWEKYGSGLVTFHGQTGNIMFIGTDTEQTQHFFDEINDYGWDLGGAGPCVRTAMSC 170
Cdd:TIGR02064  81 FHTVRVAQPSGKFYSTDYLRQLCDVWEKYGSGLTNFHGQTGDIVFLGTQTPQLQEIFEELTNLGTDLGGSGSNLRTPESC 160

                  170       180       190       200       210       220       230       240
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1             171 VGAARCEMSCTNEQKAHRLLVNNFTDDVHRPALPYKFKFKVSGCGNDCQNAVERADFAVIGTWRDDMNVNQDEFKAYVGR 250
Cdd:TIGR02064 161 VGPARCEFACYDTLKACYELTMEYQDELHRPAFPYKFKFKFSGCPNDCVAAIARSDFAVIGTWKDDIKVDQEAVKAYIAG 240

                  250       260       270       280       290       300       310       320
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1             251 KGRQHVIDNIITRCPTNALSLNDDDSLEVNNKDCVRCMHCLNVVPKALHPGDDRGVTILIGGKRTLKIGDLMGTVVVPFK 330
Cdd:TIGR02064 241 WGKFDIENEVVNRCPTKAISWDGSKELSIDNRECVRCMHCINKMPKALHPGDERGVTILIGGKAPILDGAQMGWVVVPFV 320

                  330       340       350       360       370       380       390       400
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1             331 klDTEEDWEEIVELAEEIIDFWAENALEHERCGEMIERIGLVNFLEGVGVEVDPNMVNNPRESSYIRMDGWDEEAVKWFD 410
Cdd:TIGR02064 321 --EAEEPYDEIKELVEKIIDWWDEEGKNRERIGETIKRLGLQKFLEVIGIEPDPQMVKEPRTNPYIFFKVEDEVPGGWDA 398

                    ....
1             411 RQAE 414
Cdd:TIGR02064 399 DIAE 402
```

References:

Marchler-Bauer A et al. (2017), *"CDD/SPARCLE: functional classification of proteins via subfamily domain architectures."*, **Nucleic Acids Res.45**(D)200-3.

Conserved Domain Search results:
dsrA gene of *Solemya velum* gill symbiont strain WH



Search for similar domain architectures | Refine search

List of domain hits

Name	Accession	Description	Interval	E-value
dsrA	TIGR02064	sulfite reductase, dissimilatory-type alpha subunit; Dissimilatory sulfite reductase catalyzes ...	31-1242	0e+00

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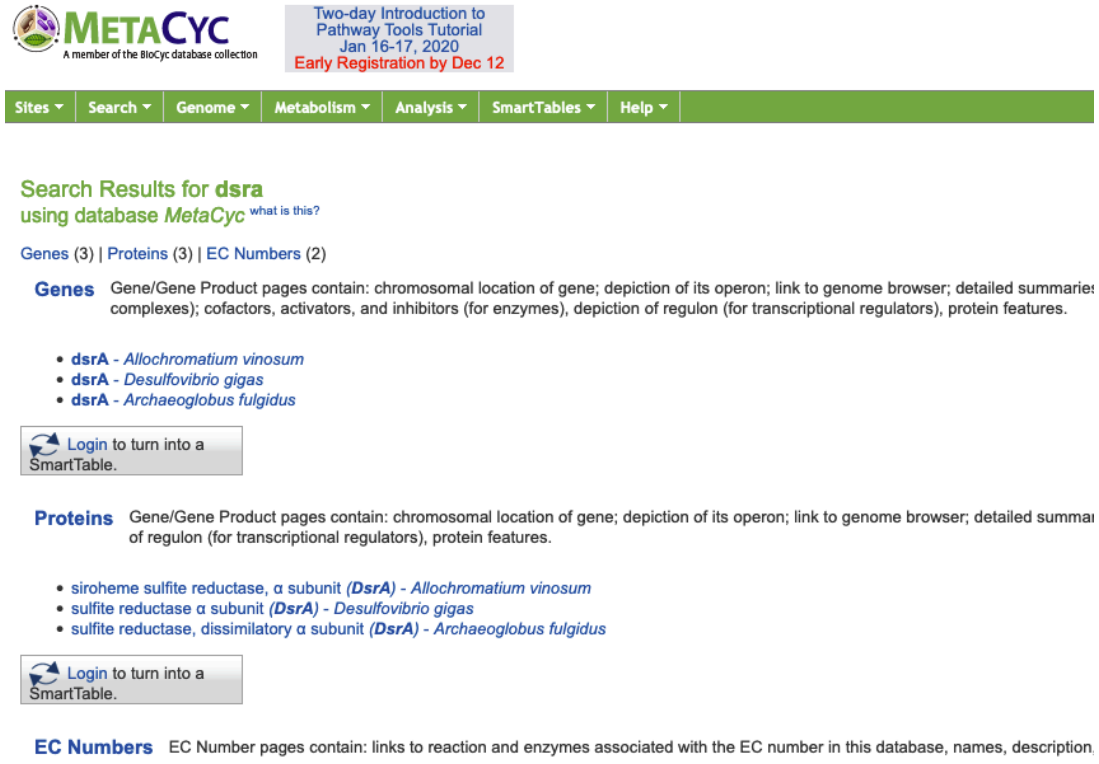
```
                    170       180       190       200       210       220       230       240
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1           171 VGAARCEMSCTNEQKAHRLLVNNFTDDVHRPALPYKFKFKVSGCGNDCQNAVERADFAVIGTWRDDMNVNQDEFKAYVGR 250
Cdd:TIGR02064 161 VGPARCEFACYDTLKACYELTMEYQDELHRPAFPYKFKFKFSGCPNDCVAAIARSDFAVIGTWKDDIKVDQEAVKAYIAG 240

                    250       260       270       280       290       300       310       320
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1           251 KGRQHVIDNIITRCPTNALSLNDDDSLEVNNKDCVRCMHCLNVVPKALHPGDDRGVTILIGGKRTLKIGDLMGTVVVPFK 330
Cdd:TIGR02064 241 WGKFDIENEVVNRCPTKAISWDGSKELSIDNRECVRCMHCINKMPKALHPGDERGVTILIGGKAPILDGAQMGWVVVPFV 320

                    330       340       350       360       370       380       390       400
                    ....*....|....*....|....*....|....*....|....*....|....*....|....*....|....*....|
1           331 klDTEEDWEEIVELAEEIIDFWAENALEHERCGEMIERIGLVNFLEGVGVEVDPNMVNNPRESSYIRMDGWDEEAVKWFD 410
Cdd:TIGR02064 321 --EAEEPYDEIKELVEKIIDWWDEEGKNRERIGETIKRLGLQKFLEVIGIEPDPQMVKEPRTNPYIFFKVEDEVPGGWDA 398

                    ....
1           411 RQAE 414
Cdd:TIGR02064 399 DIAE 402
```

References:

Marchler-Bauer A et al. (2017), *"CDD/SPARCLE: functional classification of proteins via subfamily domain architectures."*, **Nucleic Acids Res.45**(D)200-3.

Gene annotation

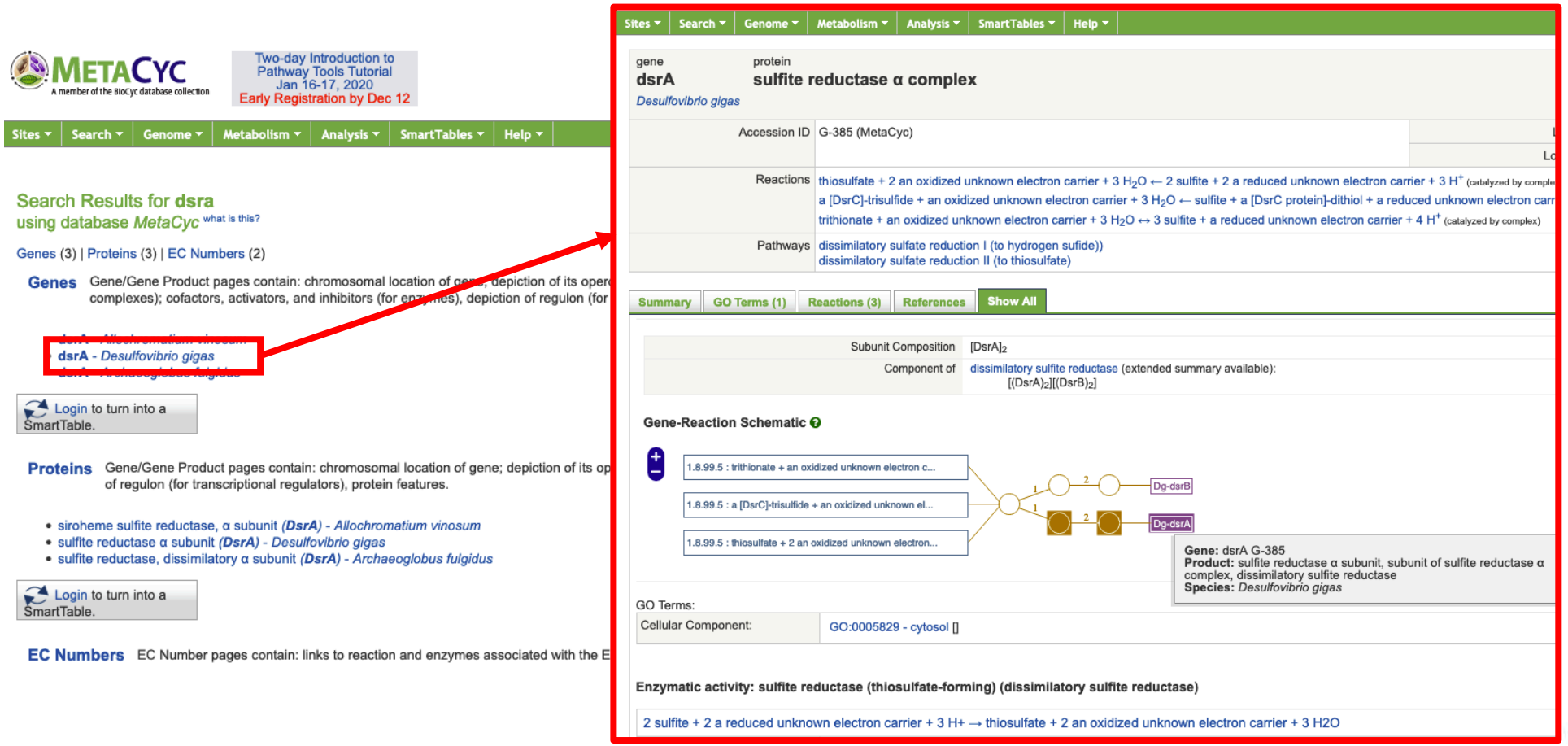
Metacyc: experimentally curated metabolic pathways



https://metacyc.org/

Gene annotation

Metacyc: experimentally curated metabolic pathways



https://metacyc.org/

Gene annotation

- The **PSORT** family - prediction of protein localization sites in cells.
- Useful for making cell schematics!

psortb

Updates | Documentation | Resources | Contact

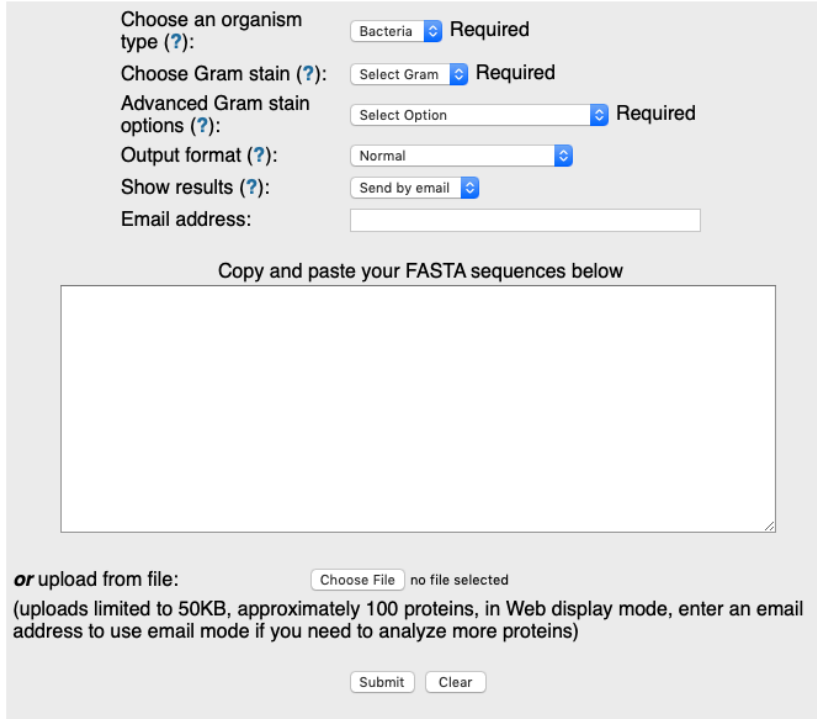
Submit a Sequence to PSORTb version 3.0.2

Based on a study last performed in 2010, PSORTb v3.0.2 is the most precise bacterial localization prediction tool available. PSORTb v3.0.2 has a number of **improvements** over PSORTb v2.0.4. Version 2 of PSORTb is maintained **here**.

You can currently submit one or more Gram-positive or Gram-negative bacterial sequences or archaeal sequences in FASTA format **(?)**. Copy and paste your FASTA-formatted sequences into the textbox below or select a file containing your sequences to upload from your computer. Web display mode is limited to the analysis of approximately 100 proteins. For larger analyses, either enter your email address in the form below (results of up to 5000 per submission returned by email) or for even larger analyses we can help you or you can download the standalone version.

See also:

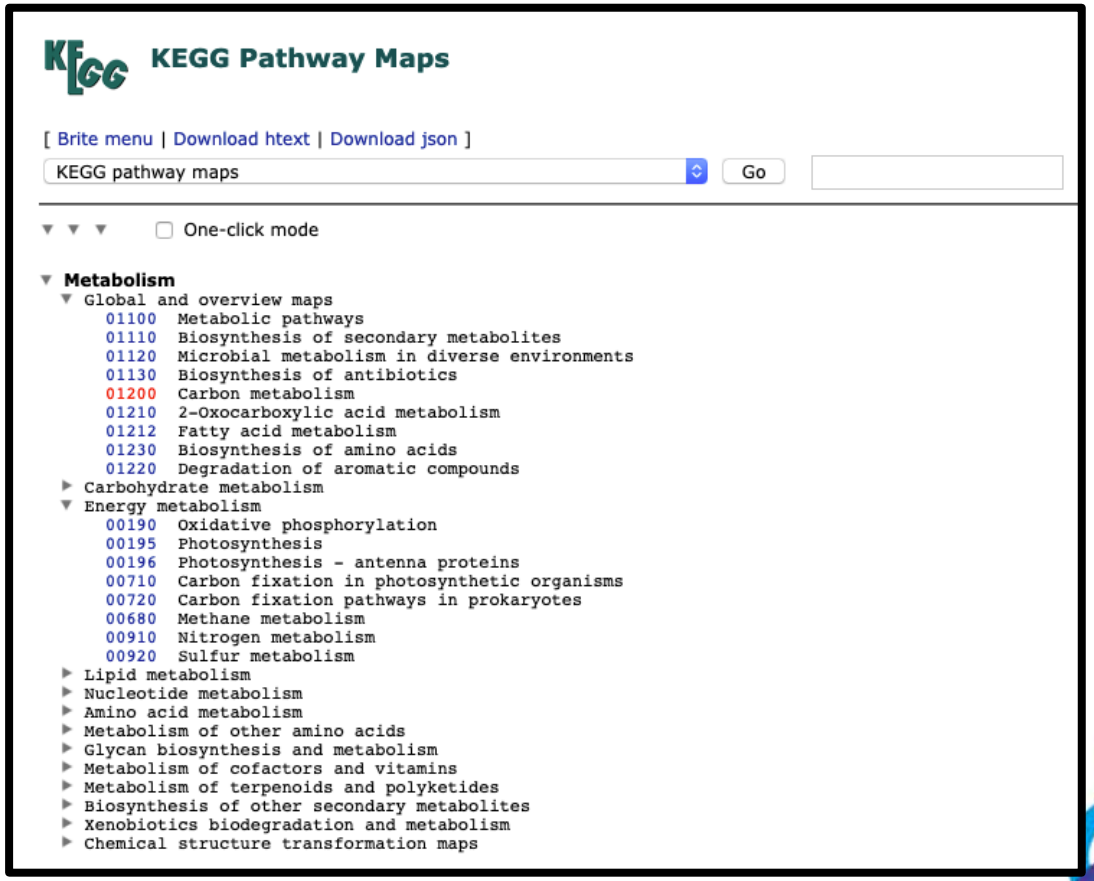
- **Updates**
- **Precomputed genome results**
- **Limitations of PSORTb v.3.0**
- **PSORTb User's Guide**
- **Docker PSORTb web service** (what is **docker**?)
- **Download standalone PSORTb**
- **Docker standalone PSORTb** (what is **docker**?)



http://psort.org/

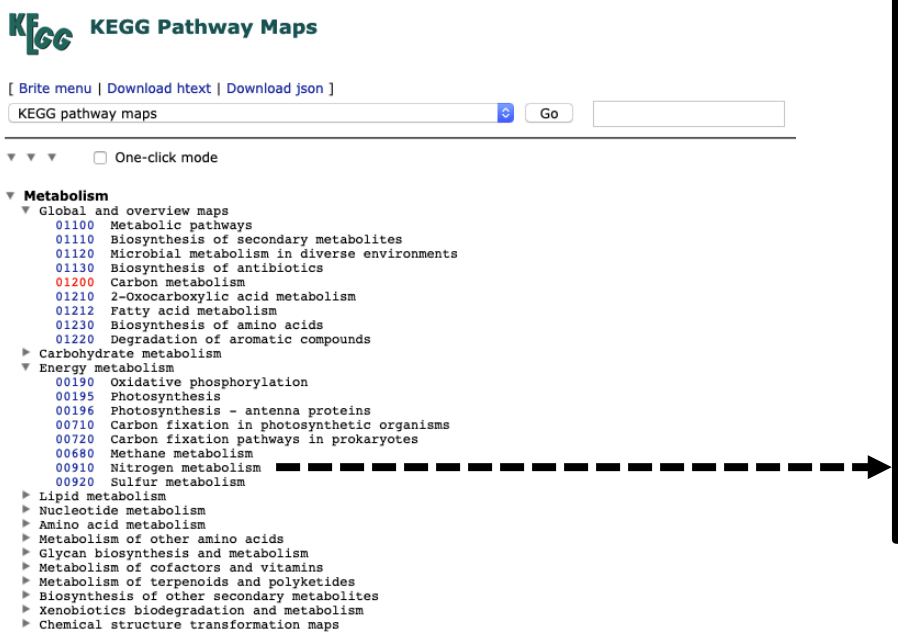
Gene annotation

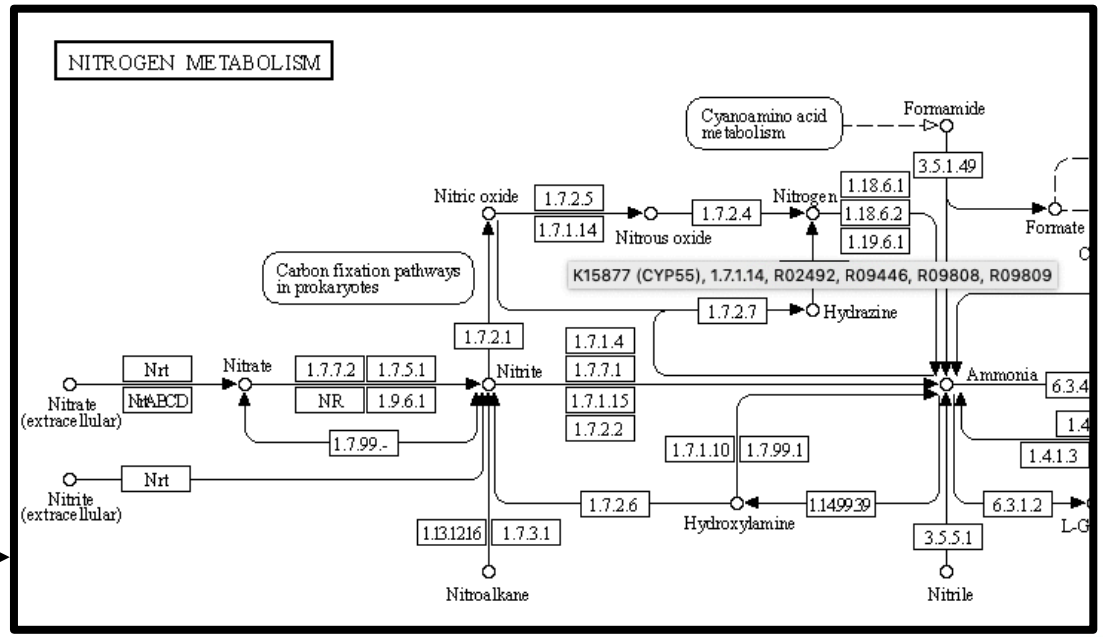
KEGG: https://www.genome.jp/kegg/pathway.html



Gene annotation

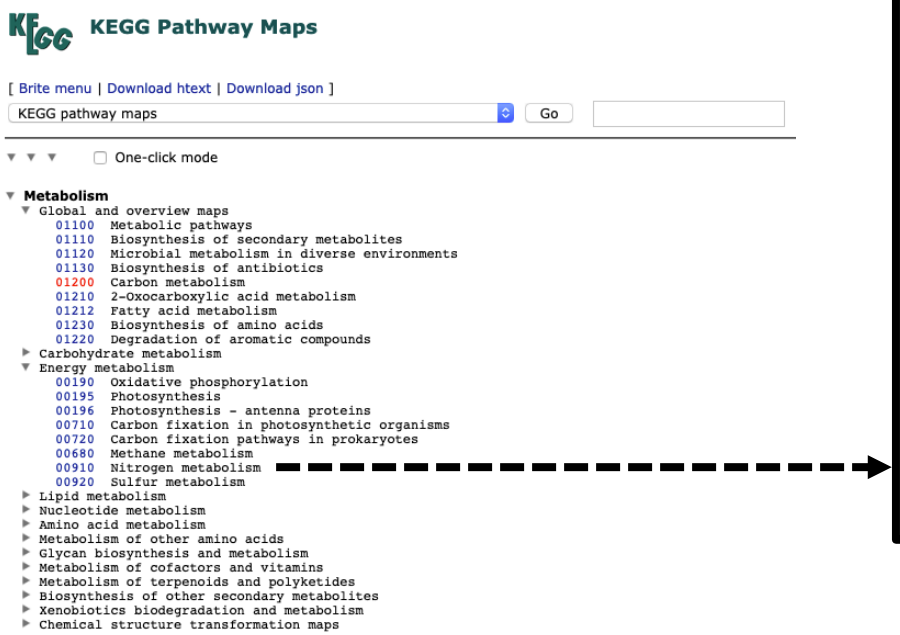
KEGG: https://www.genome.jp/kegg/pathway.html

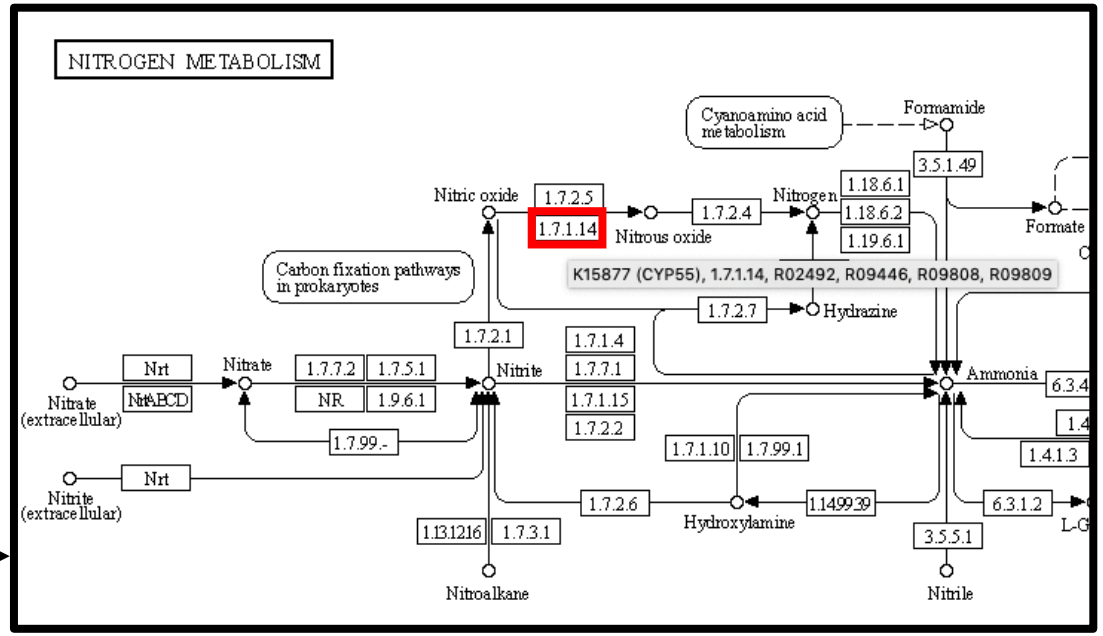




Gene annotation

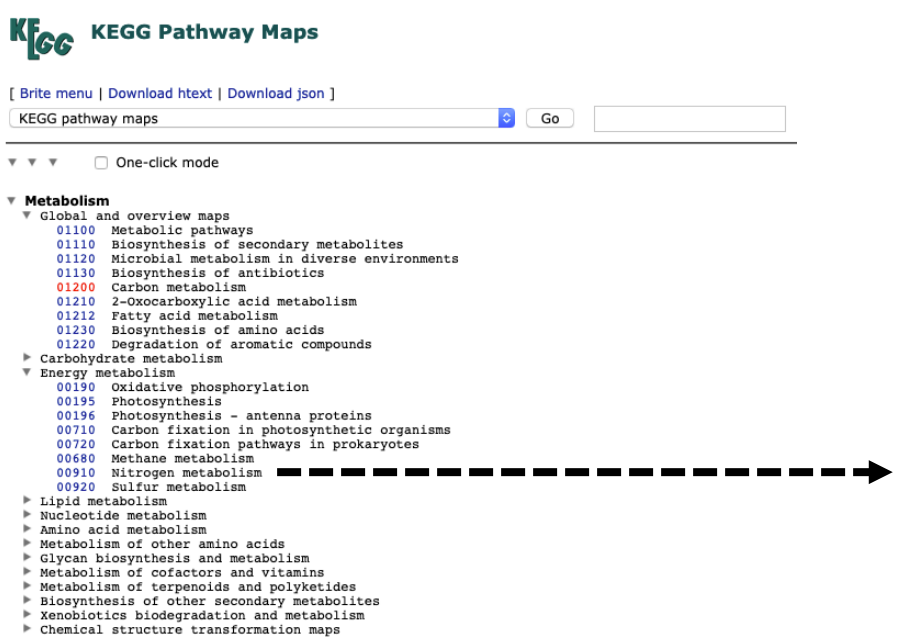
KEGG: https://www.genome.jp/kegg/pathway.html

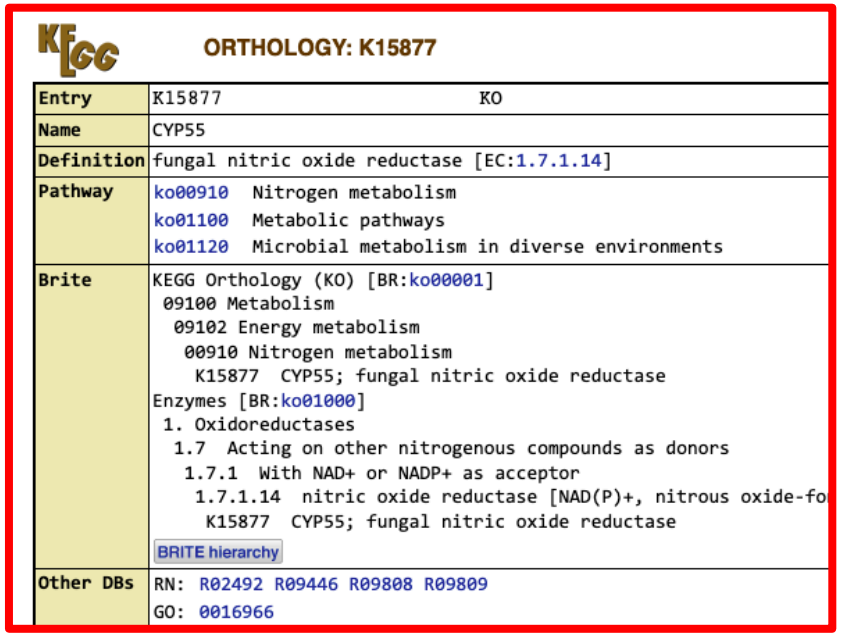




Gene annotation

KEGG: https://www.genome.jp/kegg/pathway.html





Summary of online resources

Resources to help interpret your data:

- **KEGG:** https://www.genome.jp/kegg/pathway.html
- **BioCyc:** https://biocyc.org/
- **MetaCyc:** https://metacyc.org/
- **HydDB:** https://services.birc.au.dk/hyddb/
- **PSORT:** https://psort.hgc.jp/

Task: Gene annotation

1. View KEGG annotation in website

Bin taxonomic classification

Bin taxonomic classification

- **16S rRNA commonly not recovered by *de novo* assembly**

- **Can recover 16S and 18S using EMIRGE**
- **Caveat: can be difficult to assign to genomes in complex communities with many similar taxa**

Bin taxonomic classification

Solution:

- **Use one or more single copy core genes**
- **Concatenate protein sequences of multiple single copy core genes**

Concatenated protein sequence tree:
Phylogenetic placement of cyanobacterial genome bins (Wai-iti River, Nelson)

Bin taxonomic classification

- **Genome Taxonomy Database Toolkit (GTDB-Tk)**
- **Use to classify bins against reference genome trees (GTDB)**

- **Uses set of 120 concatenated protein sequences (of single copy core genes)**

https://github.com/Ecogenomics/GtdbTk
https://gtdb.ecogenomic.org/

(Chaumeil et al., 2019, Bioinformatics)

Rank assignment based on:

- Tree placement
- Relative Evolutionary Divergence (value between 0=root and 1=tip)
- Species assignment:
  - Average Nucleotide Identity

Discriminate species

Proxy for DNA-DNA hybridization

Pairwise genome comparisons:

- **Average Nucleotide Identities (ANI)**
  - **gene comparisons**
- **Average Amino Acid Identities (AAI)**
  - **predicted protein comparisons**
- **Alignable Fraction (AF)**
  - **proportion of genes that align**

Determine via: Pairwise BLAST-like search

Discriminate species

- Pairwise AAI comparisons between genomic bins from the Gulf of Mexico seafloor
- All unique species (i.e. <95-96% AAI)
- Figures shows clusters of similar genomes
- Red = more similar
- Blue = dissimilar

(Handley et al., 2017, ISME J)

Discriminate species

Alternative to gANI (AAI):

- https://ggdc.dsmz.de/

Viral taxonomic classification

vConTACT v.2.0 (https://bitbucket.org/MAVERICLab/vcontact2/src/master/)

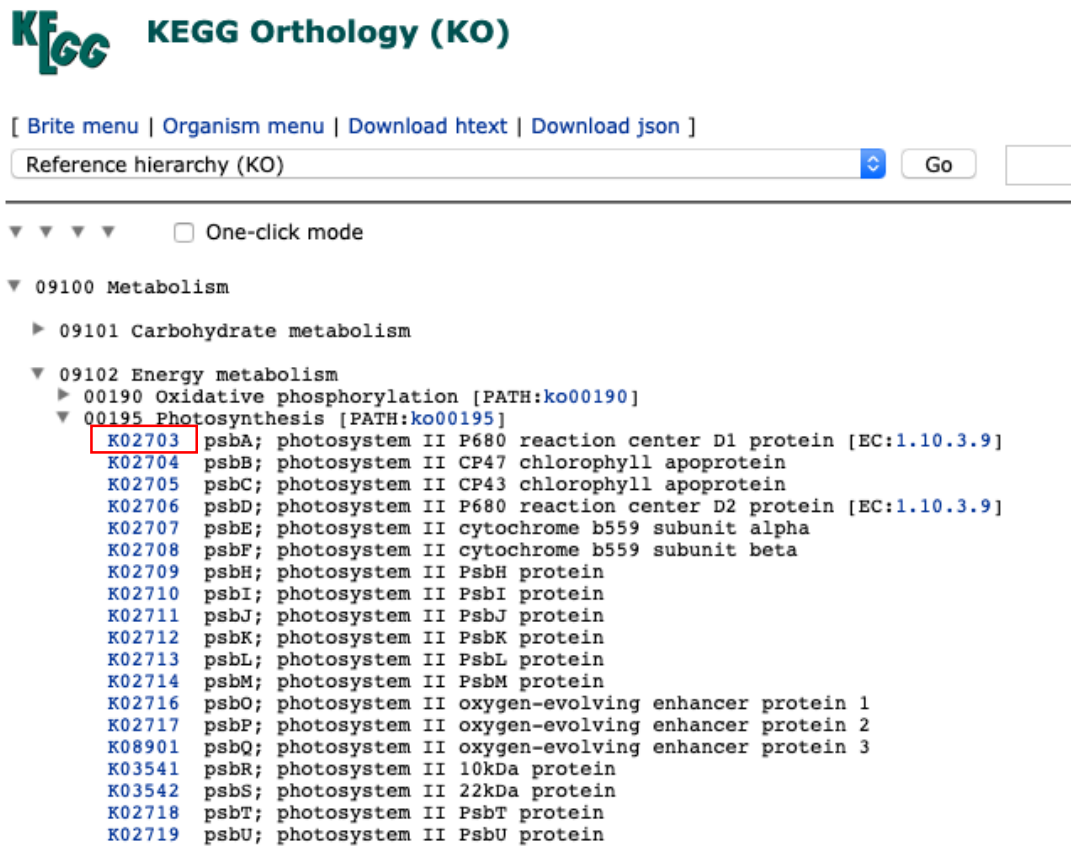
- Clustering-based: guilt-by-contig-association taxonomic prediction
- Reference database (Viral RefSeq) + identified viral contigs

Phylogenetic trait distributions

- Interactive phylogenetic and trait based tree
- Annotree (http://annotree.uwaterloo.ca/app/)
- Trait searches by:
  - Taxonomic hierarchy
  - KEGG (KO number)
  - Pfam
  - TIGRFAM

Phylogenetic trait distributions

Get KEGG KO number from the KEGG website or your annotations

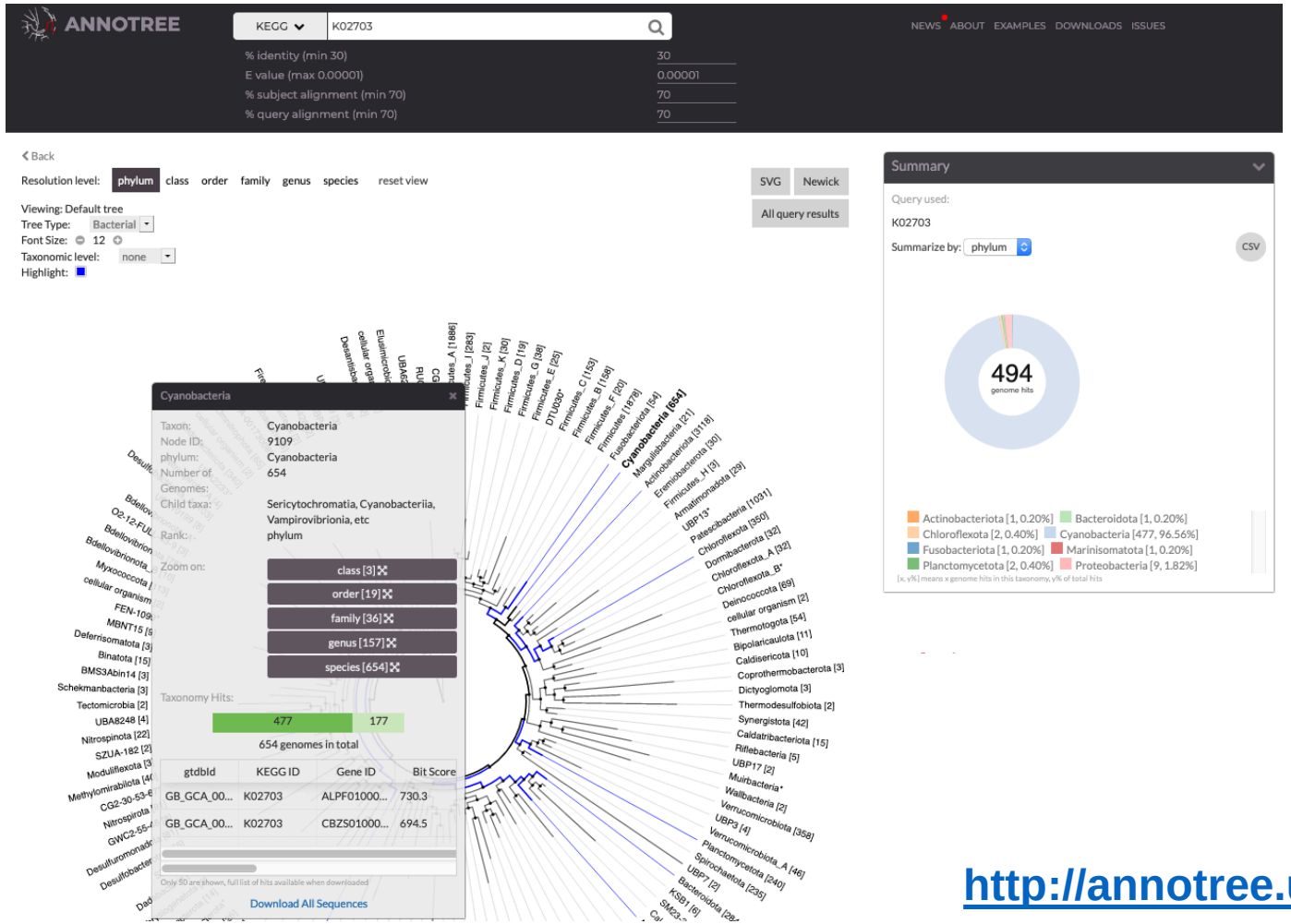


K02703

https://www.genome.jp/kegg-bin/get_htext#C17

Phylogenetic trait distributions

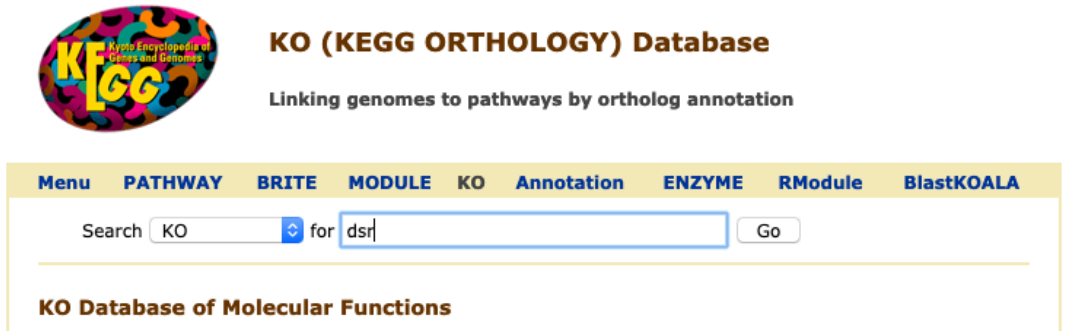
Add to ANNOTREE search box and select hierarchy

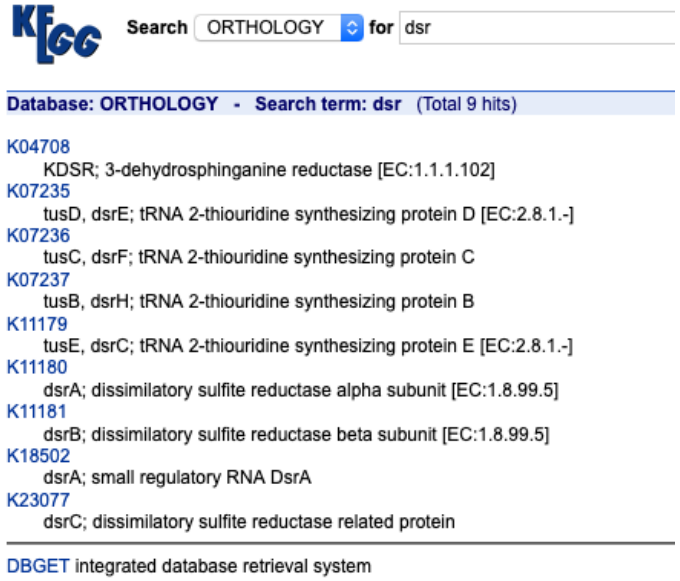


http://annotree.uwaterloo.ca/app/

Task 1: Use ANNOTREE

- Use ANNOTREE to explore the phylogenetic distribution of functions
- Try using attribute annotations for your group task
- You can use your KEGG Orthology (KO) numbers
- **Note:** You can also get KO numbers from the KEGG website (https://www.genome.jp/kegg/ko.html) by searching for gene names





Task 2: Gene annotation (cont)

Perform gene annotation with DRAM

1. Prepare DRAM annotation job to run under slurm

Task 3: Pick group challenge!

Determine which genome(s) have the following attributes, and the genetic mechanisms used for these attributes:

1. **Denitrification** (Nitrate or nitrite to nitrogen)
2. **Ammonia oxidation** (Ammonia to nitrite or nitrate)
3. **Anammox** (Ammonia and nitrite to nitrogen)
4. **Sulfur oxidation** (SOX pathway, thiosulfate to sulfate)
5. **Sulfur reduction** (DSR pathway, sulfate to sulfide)
6. **Photosynthetic carbon fixation**
7. **Non-photosynthetic carbon fixation** (Reverse TCA or Wood-Ljundahl)
8. **Non-polar flagella expression due to a chromosomal deletion**
9. **Plasmid-encoded antibiotic resistance**
10. **Aerobic (versus anaerobic) metabolism**