NEMC Conference: Current Topics in Microbiology • Wednesday, August 7, 2019

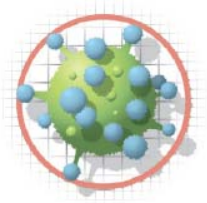
Next Generation Sequencing to Detect Environmental Stress

Krista Ternus, PhD
Signature Science, LLC
kternus@signaturescience.com

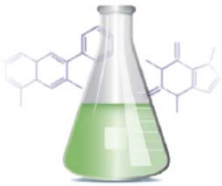
signature science LLC

www.signaturescience.com

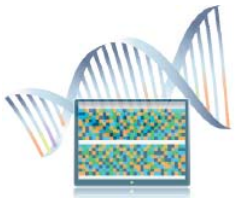
Signature Science, LLC



Life & Physical Sciences
- Environmental genomics
- Proteogenomics
- Applied Materials Science



Laboratory Support Services
- Quality Assurance
- Proficiency Testing
- Third Party Test and Evaluation



DNA Forensics
- Human DNA Casework
- Next Generation Sequencing
- Advanced Analytics

Data Science
- Applied Statistics
- Bioinformatics and Chemometrics
- Natural Language Processing

Intel & Operations
- Training, Exercise, and Mission Rehearsal

Systems Integration/Engineering
- Chemical Sensor Technology Design, Integration, Engineering, and Support
- Environmental Collection and Sampling

More About Us:

2001
- Founded in March 2001
- A Subsidiary of Southwest Research Institute

215
- 215 Employees
- Four Locations Across the U.S.

40
- ~ $40 M Annual Revenue in FY18
- Federal Government Clientele

100
- 100+ Business and Academic Partner Network

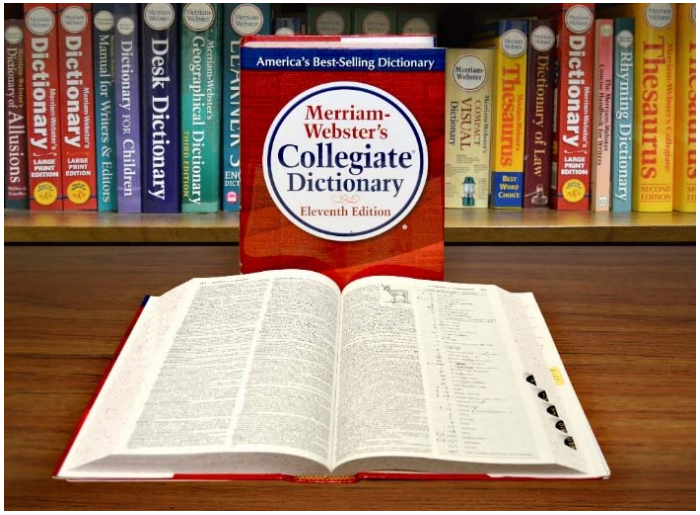
Austin, TX (HQ)

Atlantic City, NJ
Arlington, VA
Charlottesville, VA

signature science LLC

Environmental Metagenomics

https://www.nps.gov/subjects/geology/sri.htm



Great for unbiased, exploratory analyses, as well as when standard assays provide ambiguous or no results

signature science LLC

Metagenomics: Powerful Investigative Tool and Regulatory Challenge

The New York Times

In a First, Test of DNA Finds Root of Illness

Joshua Osborn outside his home in Cottage Grove, Wis., with his father, Clark Osborn. Joshua suffered from swelling in the brain, but tests, a spinal tap and a biopsy were inconclusive. John Maniaci.

www.nytimes.com/2014/06/05/health/in-first-quick-dna-test-diagnoses-a-boys-illness.html

Contains Nonbinding Recommendations

Draft - Not for Implementation

Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

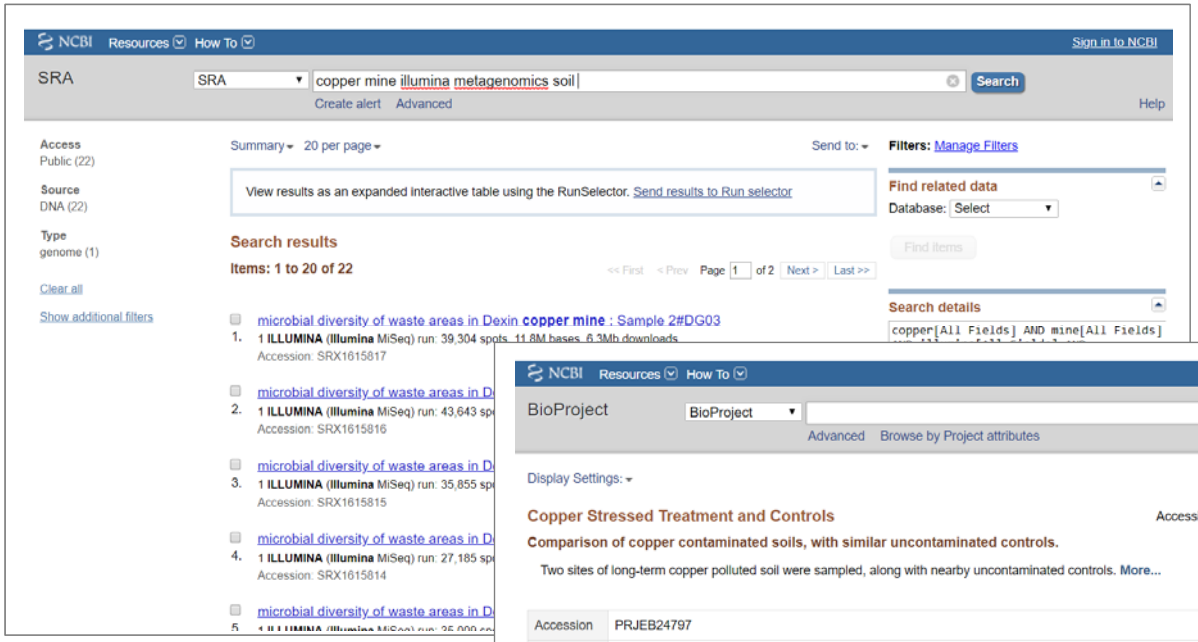
This draft guidance document is being distributed for comment purposes only.

Document issued on: May 13, 2016

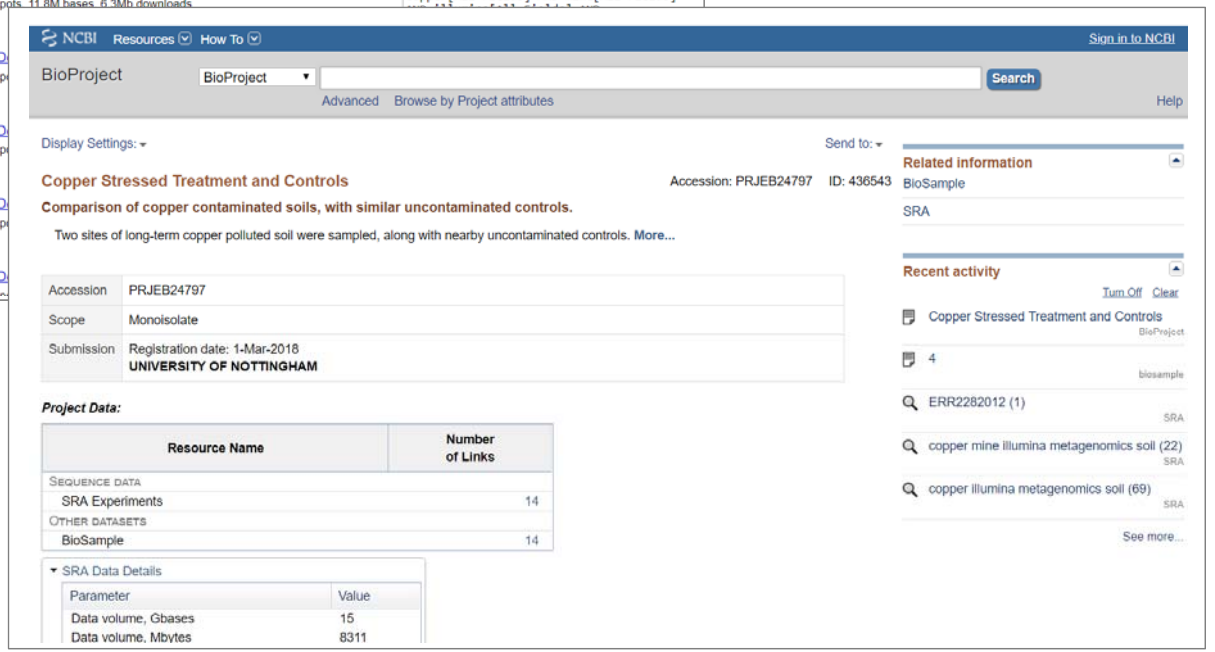
You should submit comments and suggestions regarding this draft document within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. Identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

signature science LLC

Finding Real World Data



www.ncbi.nlm.nih.gov/sra



www.ncbi.nlm.nih.gov/bioproject/

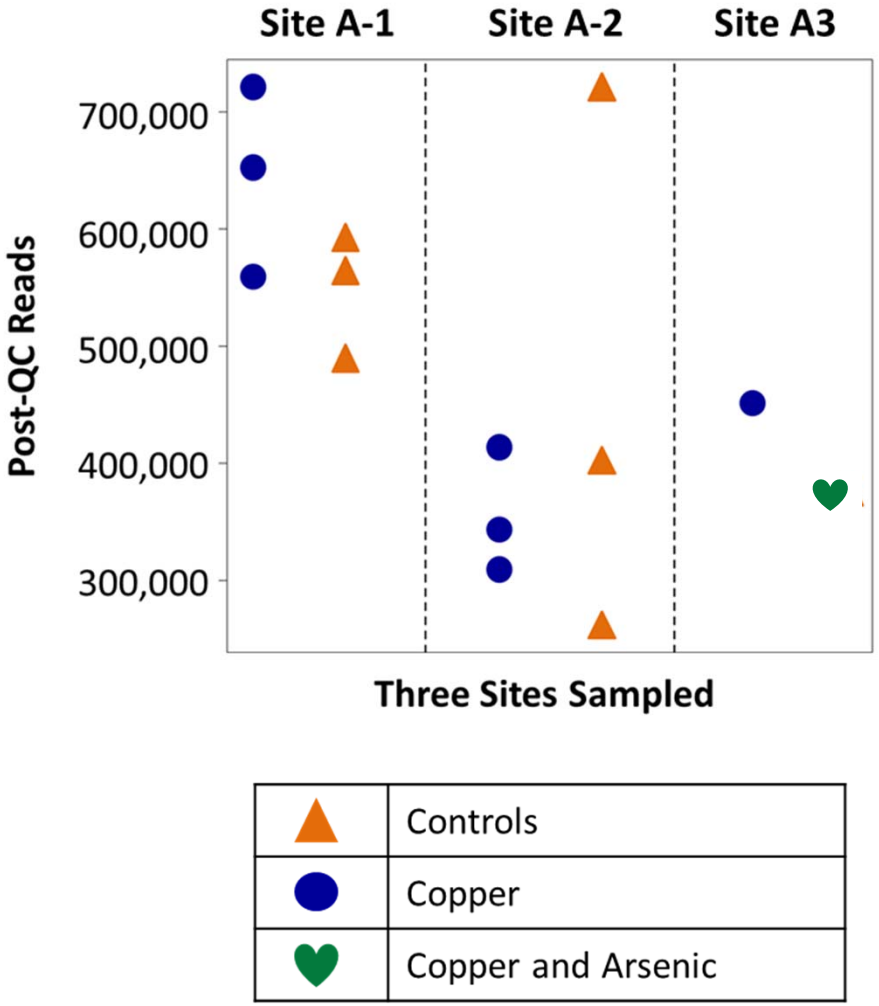
signature science LLC

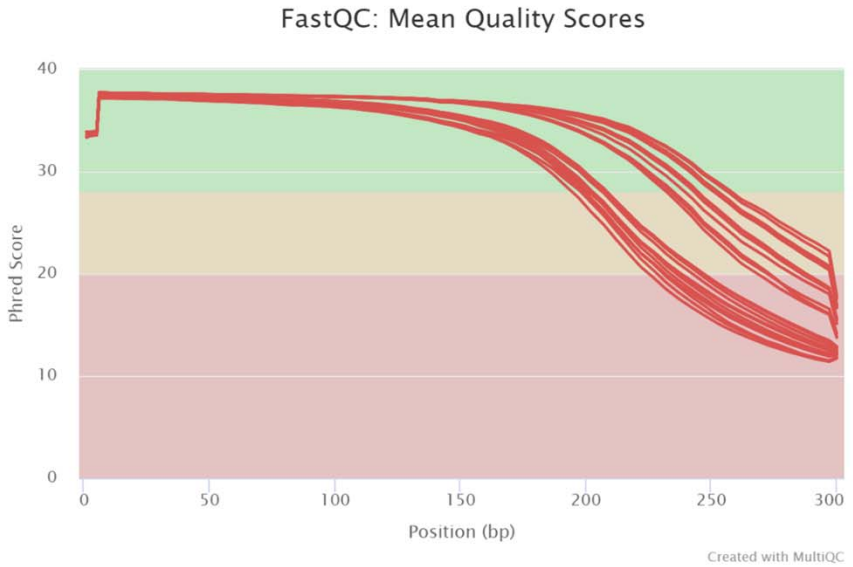
Acid Mine Drainage

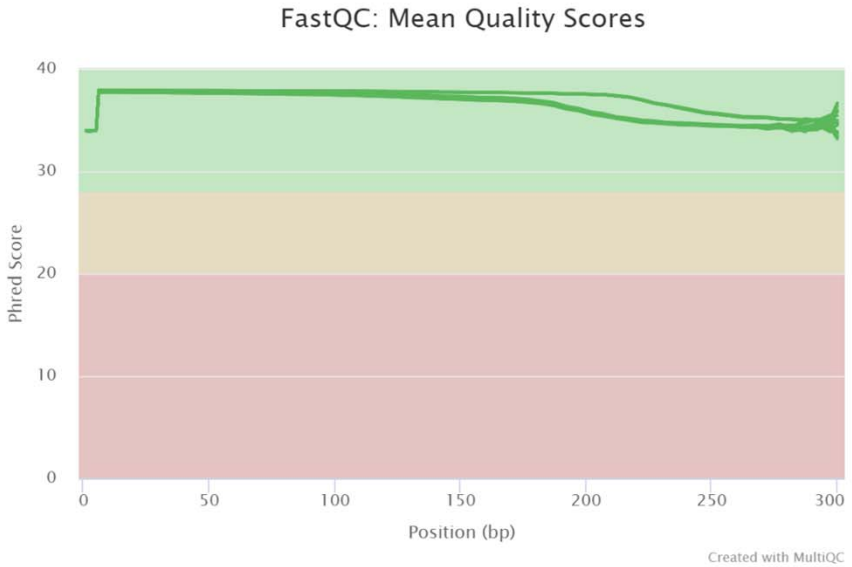
https://www.fws.gov/mountain-prairie/contaminants/acidMineDrainage.php

signature science LLC

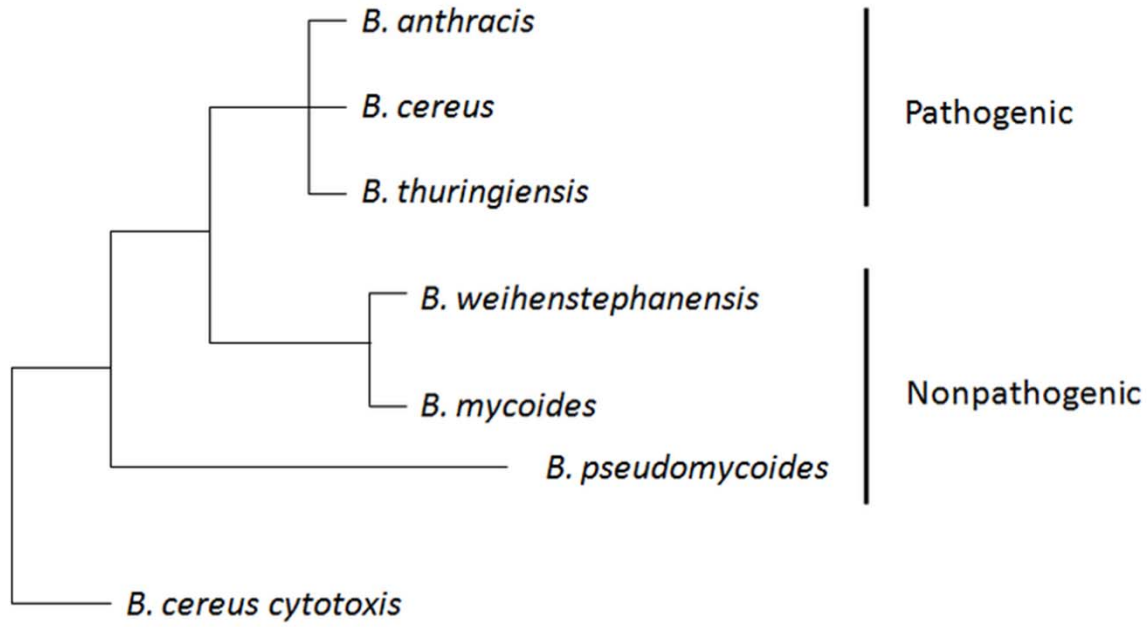
Quality Control







Methods for Taxonomic Classification




```
RLA0_PYRAE -MMLAIGKRRYVRTRQYPARKVKIVSEATELLQKYPYVFLFDLHGLSSRILHEYRYRLRRY-GVIKIIKPTLFKIAFTKVYGG---IPAE   85
RLA0_METAC ------MAEERHHTEHIPQWKKDEIENIKELIQSHKVFGMVGIEGILATKMQKIRRDLKDV-AVLKVSRNTLTERALNQLG-----ETIP   78
RLA0_METMA ------MAEERHHTEHIPQWKKDEIENIKELIQSHKVFGMVRIEGILATKIQKIRRDLKDV-AVLKVSRNTLIERALNQLG-----ESIP   78
RLA0_ARCFU ------MAAVRGS---PPEYKVRAVEEIKRMISSKPVVAIVSFRNVPAGQMQKIRREFRGK-AEIKVVKNTLLERALDALG-----GDYL   75
RLA0_METKA MAVKAKGQPPSGYEPKVAEWKRREVKELKELMDEYENVGLVDLEGIPAPQLQEIRAKLRERDTIIRMSRNTLMRIALEEKLDER--PELE   88
RLA0_METTH ------------MAHVAEWKKKEVQELHDLIKGYEVVGIANLADIPARQLQKMRQTLRDS-ALIRMSKKTLISLALEKAGREL--ENVD   74
RLA0_METTL -------MITAESEHKIAPWKIEEVNKLKELLKNGQIVALVDMMEVPARQLQEIRDKIR-GTMTLKMSRNTLIERAIKEVAEETGNPEFA   82
RLA0_METVA -------MIDAKSEHKIAPWKIEEVNALKELLKSANVIALIDMMEVPAVQLQEIRDKIR-DQMTLKMSRNTLIKRAVEEVAEETGNPEFA   82
RLA0_METJA --------METKVKAHVAPWKIEEVKTLKGLIKSKPVVAIVDMMDVPAPQLQEIRDKIR-DKVKLRMSRNTLIIRALKEAAEELNNPKLA   81
RLA0_PYRAB ------------MAHVAEWKKKEVEELANLIKSYPVIALVDVSSMPAYPLSQMRRLIRENGGLLRVSRNTLIELAIKKAAQELGKPELE   77
RLA0_PYRHO ------------MAHVAEWKKKEVEELAKLIKSYPVIALVDVSSMPAYPLSQMRRLIRENGGLLRVSRNTLIELAIKKAAKELGKPELE   77
RLA0_PYRFU ------------MAHVAEWKKKEVEELANLIKSYPVVALVDVSSMPAYPLSQMRRLIRENNGLLRVSRNTLIELAIKKVAQELGKPELE   77
RLA0_PYRKO ------------MAHVAEWKKKEVEELANIIKSYPVIALVDVAGVPAYPLSKMRDKLR-GKALLRVSRNTLIELAIKRAAQELGQPELE   76
RLA0_HALMA -----MSAESERKTETIPEWKQEEVDAIVEMIESYESVGVVNIAGIPSRQLQDMRRDLHGT-AELRVSRNTLLERALDDVD-----DGLE   79
RLA0_HALVO -----MSESEVRQTEVIPQWKREEVDELVDFIESYESVGVVGVAGIPSRQLQSMRRELHGS-AAVRMSRNTLVNRALDEVN-----DGFE   79
RLA0_HALSA -----MSAEEQRTTEEVPEWKRQEVAELVDLLETYDSVGVVNVTGIPSKQLQDMRRGLHGQ-AALRMSRNTLLVRALEEAG-----DGLD   79
RLA0_THEAC ------------MKEVSQQKKELVNEITQRIKASRSVAIVDTAGIRTRQIQDIRGKNRGK-INLKVIKKTLLFKALENLGD----EKLS   72
RLA0_THEVO ------------MRKINPKKKEIVSELAQDITKSKAVAIVDIKGVRTRQMQDIRAKNRDK-VKIKVVKKTLLFKALDSIND----EKLT   72
RLA0_PICTO ------------MTEPAQWKIDFVKNLENEINSRKVAAIVSIKGLRNNEFQKIRNSIRDK-ARIKVSRARLLRLAIENTGK----NNIV   72
     ruler 1.......10........20........30........40........50........60........70........80........90
```


signature science LLC

Scrabble Analogy: One Possibility

signature science LLC

Scrabble Analogy: Thousands of Possibilities!

spandril
spandrils
spang
spangle
spangled
spangles
spanglier
spangliest

spado
spadones
spae
spaed
spaeing
spaeings
spaes
spaetzle

spanks
spanless
spanned
spanner
spanners
spanning
spanokopita
spanokopitas

spanceling
spancelled
spancelling
spancels
spandex
spandexes
spandrel
spandrels

spaniel
spaniels
spank
spanked
spanker
spankers
spanking
spankings

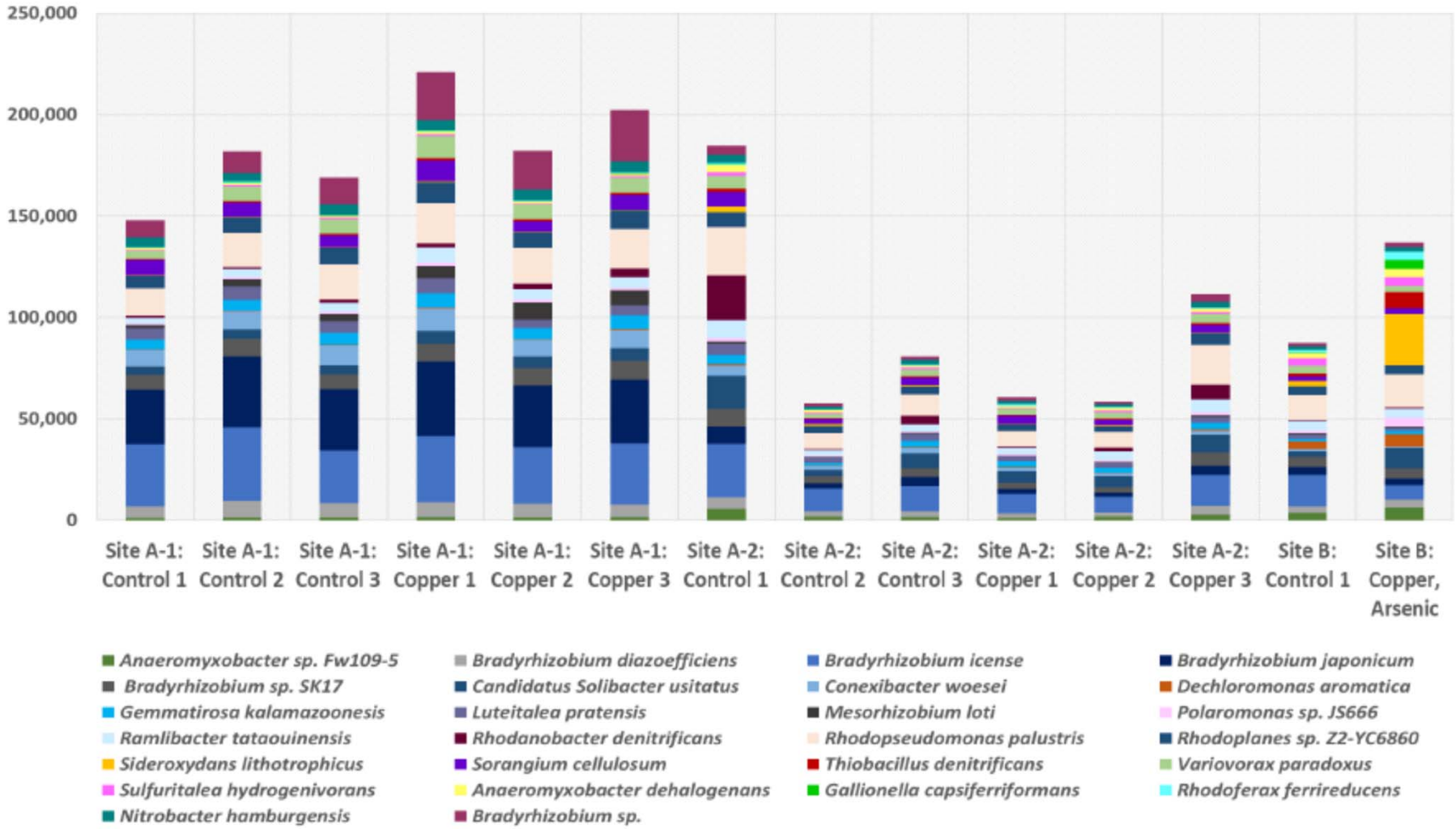
spaes
spaetzle
spaetzles
spaghetti
spaghettilike
spaghettini
spaghettinis
spaghettis

spadework
spadeworks
spadices
spadille
spadilles
spading
spadix
spadixes

spagyric
spagyrics
spahee
spahees
spahi
spahis
spail
spails

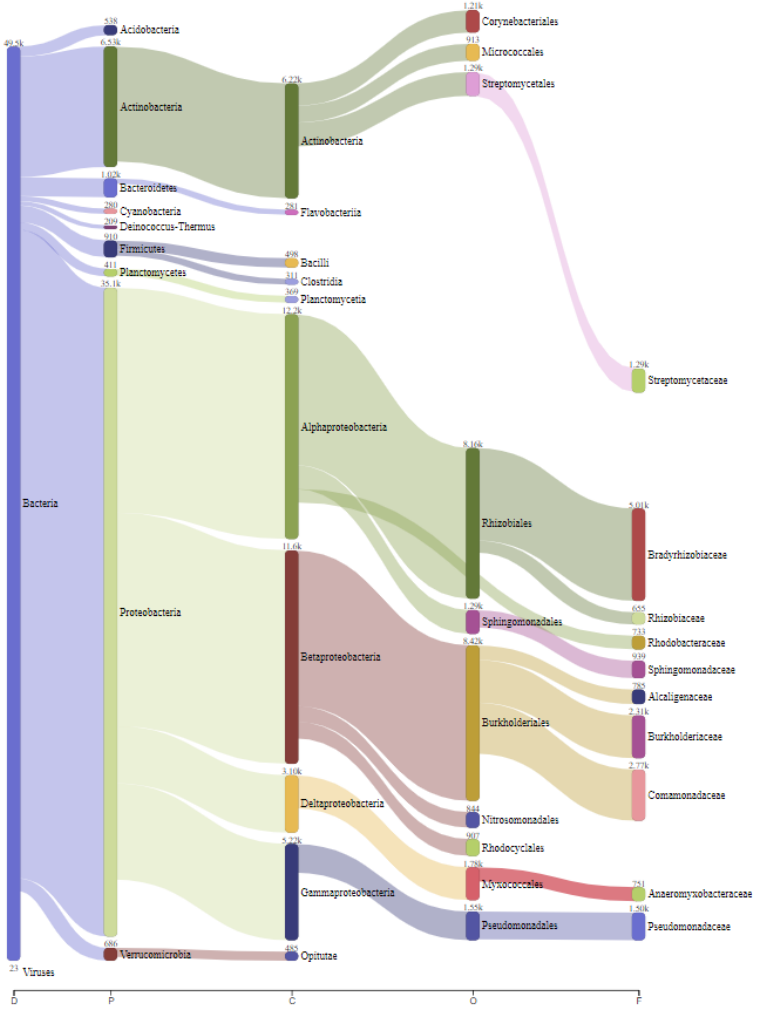
signature science LLC

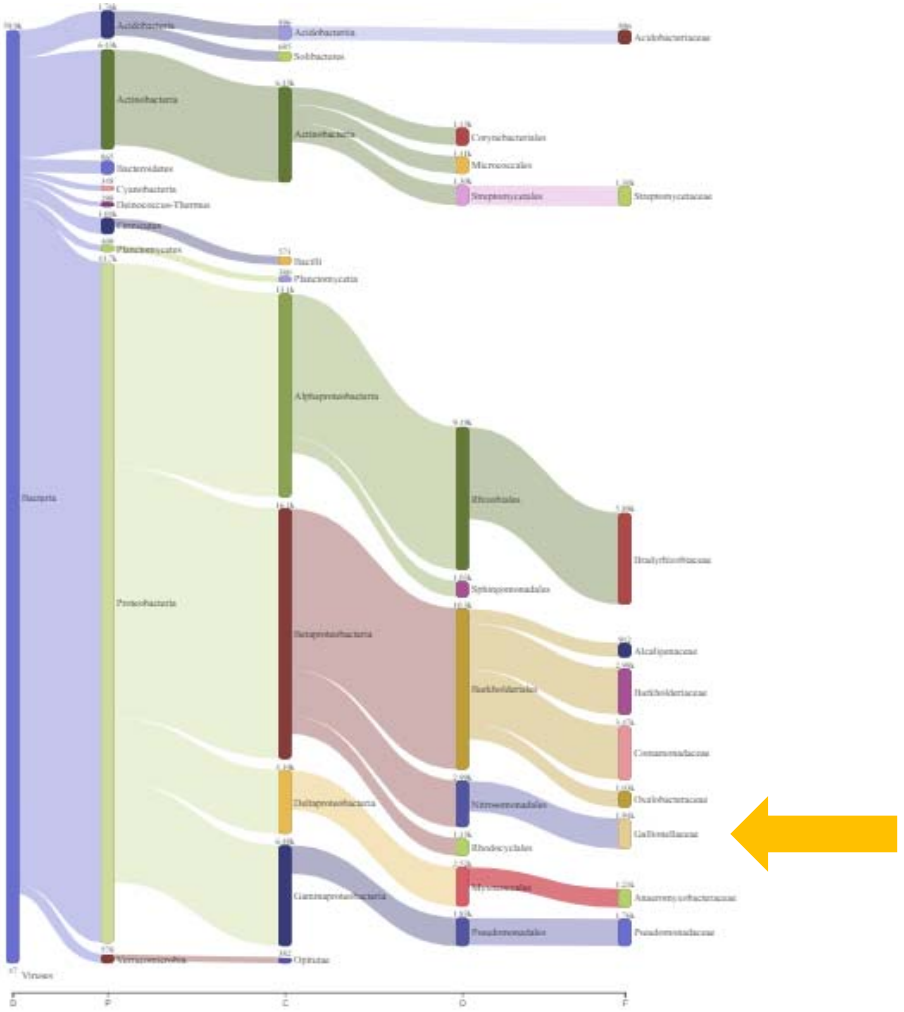
Taxonomic Classification



signature science LLC

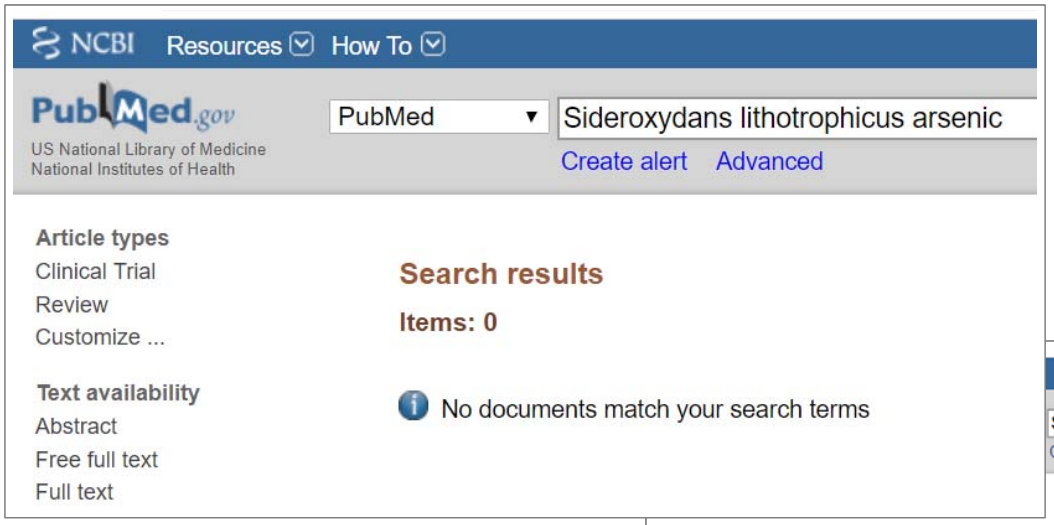
Visualizing Taxonomies



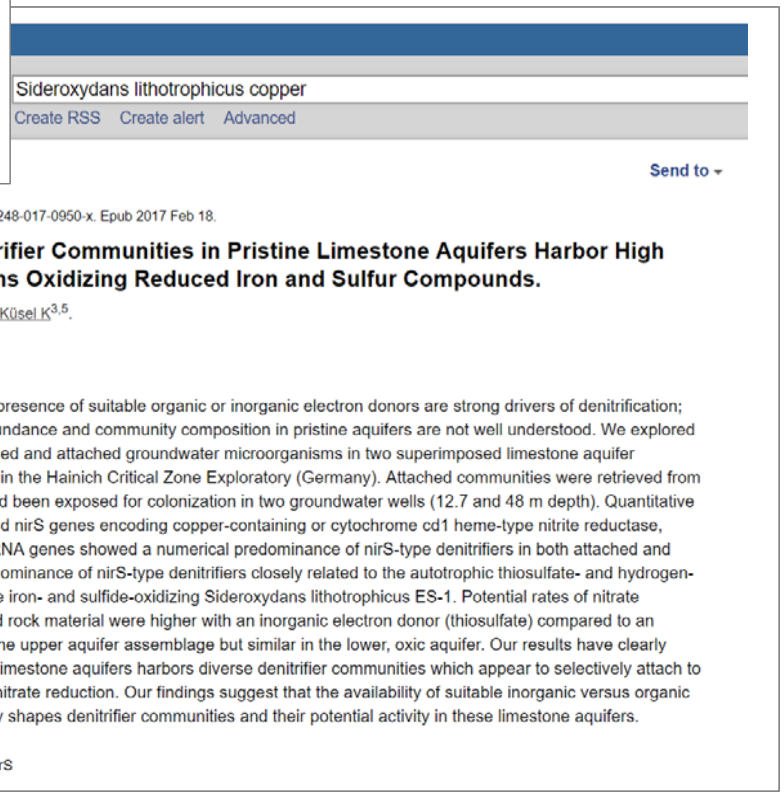


signature science LLC

Sideroxydans lithotrophicus



Limited publications are available for this species, especially with the words "arsenic" or "copper"



While this may not have been an obvious species ahead of time, it was successfully identified with an unbiased sequencing approach

Sideroxydans lithotrophicus

Organism Overview ID: 1862

Sideroxydans lithotrophicus

Sideroxydans lithotrophicus overview

Lineage: Bacteria[24883]; Proteobacteria[7898]; Betaproteobacteria[1124]; Nitrosomonadales[141]; Gallionellaceae[26]; Sideroxydans[2]; Sideroxydans lithotrophicus[1]

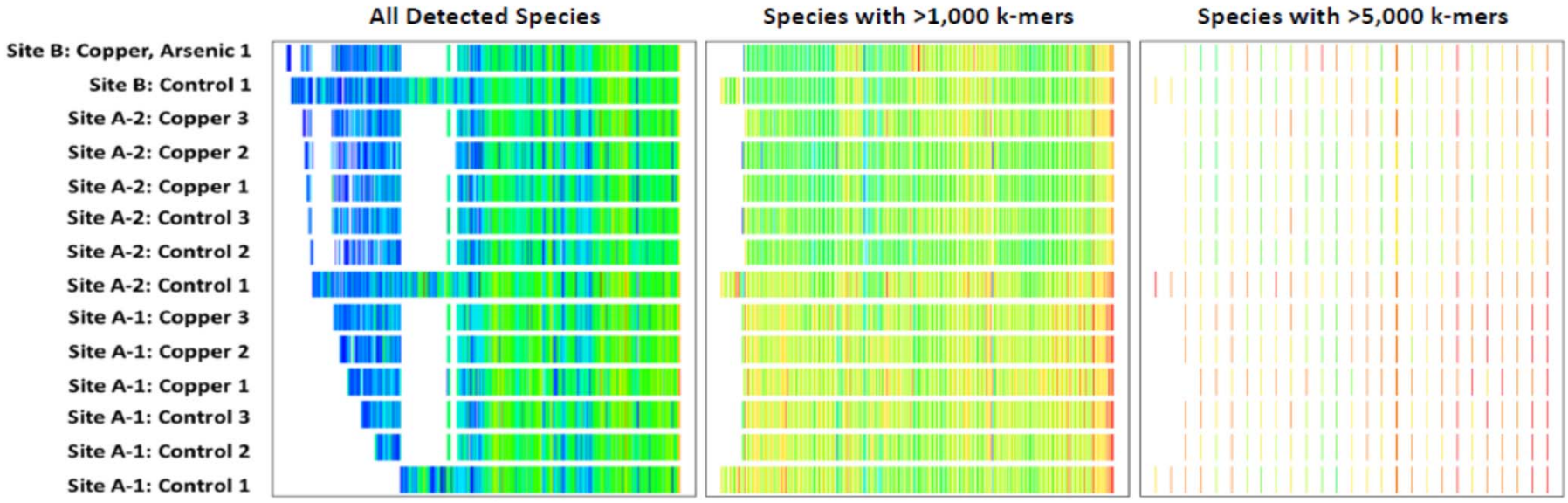
Sideroxydans lithotrophicus. *Sideroxydans lithotrophicus* is an iron-oxidizing freshwater bacterium that has been isolated from iron contaminated groundwater.

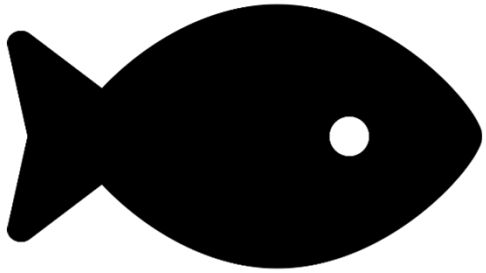
```
gene            978988..980019
                /locus_tag="Slit_0997"
CDS             978988..980019
                /locus_tag="Slit_0997"
                /inference="protein motif:TFAM:TIGR00832"
                /note="KEGG: lch:Lcho_3118 arsenical-resistance protein;
                TIGRFAM: arsenical-resistance protein;
                PFAM: Bile acid:sodium symporter"
                /codon_start=1
                /transl_table=11
                /product="arsenical-resistance protein"
                /protein_id="ADE11235.1"
                /db_xref="InterPro:IPR002657"
                /db_xref="InterPro:IPR004706"
                /translation="MSIFERYLTLWVFLCIIIGVVLGQTIPAPFHWLGGLEIAKVNLP
                VGLLIWVMIIPMLLRIDFGALHEVRKHWRGIGVTLFINWAVKPFSMALLGWLFIRHLF
                AAYLPAEQLDSYIAGLVLLAAAPCTAMVFVWSRLVNGEPLFTLSQVALNDTIMVFAFA
                PLVALLLGISSIVVPWNTLLVSVVLYIVLPVLLSQVWRRLLLARGEDALQRTLHALGP
                ISIAALLLTLVLLFAFQGKAIVEQPLIILMLAVPITIQVYFTSGLAYWLNRRFGEAHC
                VAGPSALIGASNFFELAVAAAISLFGFESGAALATVVGVLIEVPVMLSVVHIVKRSQG
                WYQTGGAAN"
```

Its reference genome contains genes involved in arsenic resistance

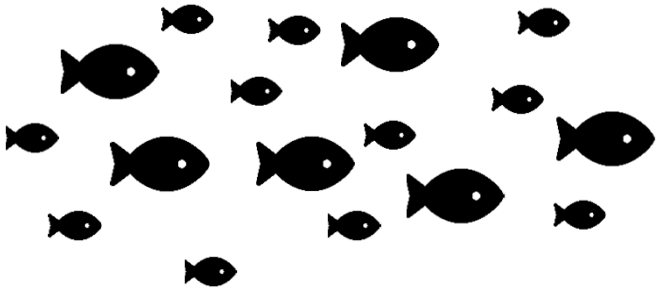
signature science LLC

Complex Environmental Samples

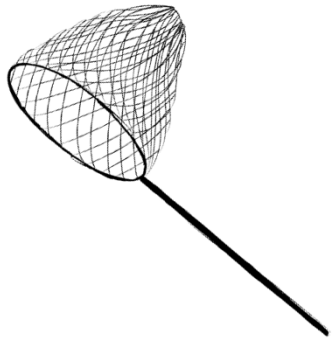




Genome



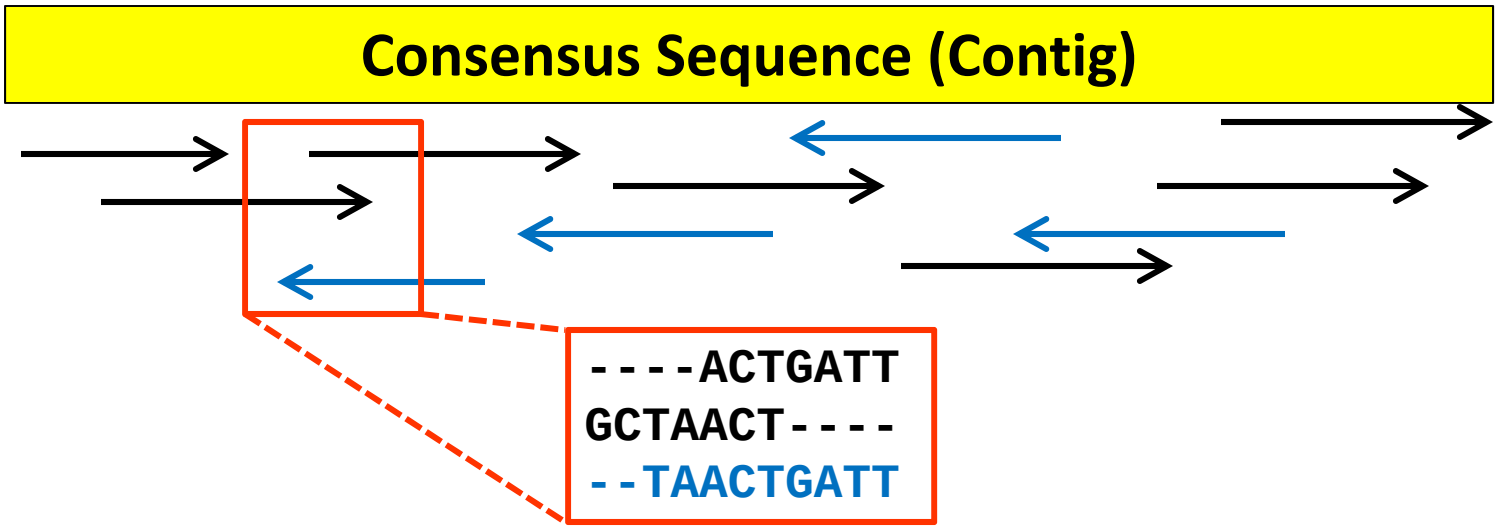
Genes



signature science LLC

Metagenome Assembly

```
   Sequence Name   @HWI-EAS367_0010:3:1:2380:6567#0/1
        Sequence   TAATTTCCATTCATCATGACAGCCCTCCAGAGGTTAGACAAC
   Sequence Info   +HWI-EAS367_0010:3:1:2380:6567#0/1
Quality in ASCII   GGG?GEG@GB?<EBE>C8E8ECCEEGGDD>CC89AGD8BBA8
   Sequence Name   @HWI-EAS367_0010:3:1:2585:6567#0/1
        Sequence   ACAGTATTCTGGGGAGGATTAAATTAGATAAACATGCAAGAA
   Sequence Info   +HWI-EAS367_0010:3:1:2585:6567#0/1
Quality in ASCII   EGGGGGGGFECG+CDADDGD4BAB9D<G+G?/?;7E,8CAGG
```



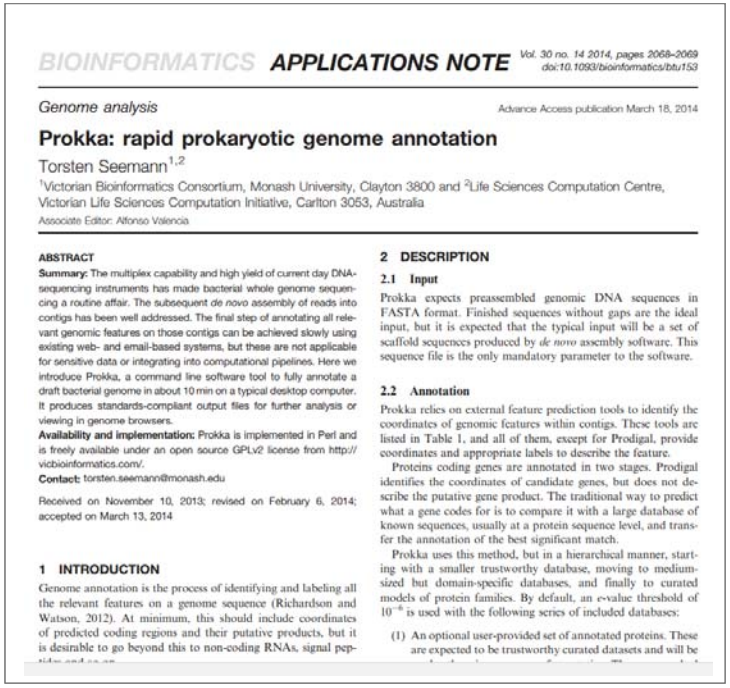
signature science LLC

Functional Inference

GENEONTOLOGY
Unifying Biology

COGs
Phylogenetic classification of proteins encoded in complete genomes

NCBI


BIOINFORMATICS APPLICATIONS NOTE

Vol. 30 no. 14 2014, pages 2068–2069
doi:10.1093/bioinformatics/btu153

Genome analysis

Advance Access publication March 18, 2014

Prokka: rapid prokaryotic genome annotation

Torsten Seemann[1,2]

[1]Victorian Bioinformatics Consortium, Monash University, Clayton 3800 and [2]Life Sciences Computation Centre, Victorian Life Sciences Computation Initiative, Carlton 3053, Australia

Associate Editor: Alfonso Valencia

ABSTRACT

Summary: The multiplex capability and high yield of current day DNA-sequencing instruments has made bacterial whole genome sequencing a routine affair. The subsequent *de novo* assembly of reads into contigs has been well addressed. The final step of annotating all relevant genomic features on those contigs can be achieved slowly using existing web- and email-based systems, but these are not applicable for sensitive data or integrating into computational pipelines. Here we introduce Prokka, a command line software tool to fully annotate a draft bacterial genome in about 10 min on a typical desktop computer. It produces standards-compliant output files for further analysis or viewing in genome browsers.

Availability and implementation: Prokka is implemented in Perl and is freely available under an open source GPLv2 license from http://vicbioinformatics.com/.

Contact: torsten.seemann@monash.edu

Received on November 10, 2013; revised on February 6, 2014; accepted on March 13, 2014

1 INTRODUCTION

Genome annotation is the process of identifying and labeling all the relevant features on a genome sequence (Richardson and Watson, 2012). At minimum, this should include coordinates of predicted coding regions and their putative products, but it is desirable to go beyond this to non-coding RNAs, signal pep-

2 DESCRIPTION

2.1 Input

Prokka expects preassembled genomic DNA sequences in FASTA format. Finished sequences without gaps are the ideal input, but it is expected that the typical input will be a set of scaffold sequences produced by *de novo* assembly software. This sequence file is the only mandatory parameter to the software.

2.2 Annotation

Prokka relies on external feature prediction tools to identify the coordinates of genomic features within contigs. These tools are listed in Table 1, and all of them, except for Prodigal, provide coordinates and appropriate labels to describe the feature.

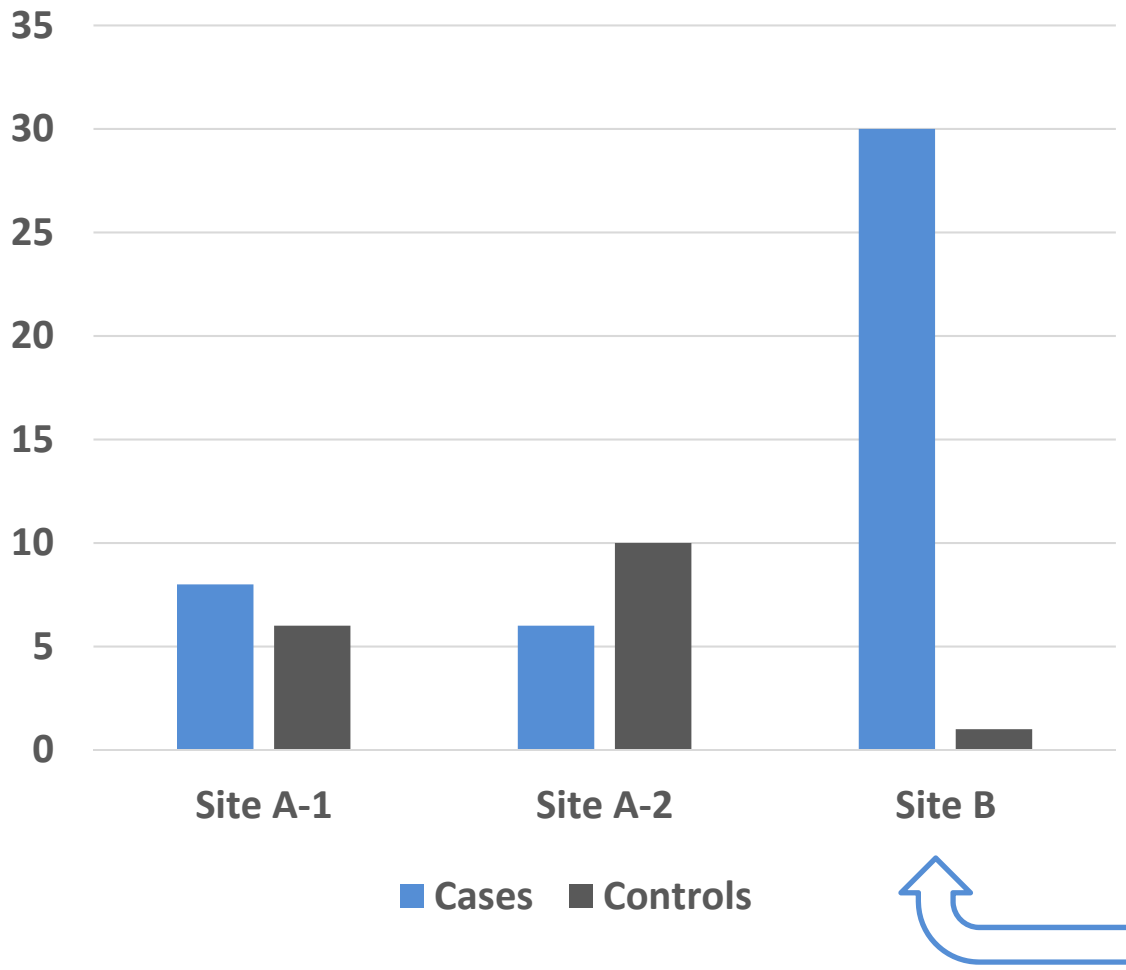
Proteins coding genes are annotated in two stages. Prodigal identifies the coordinates of candidate genes, but does not describe the putative gene product. The traditional way to predict what a gene codes for is to compare it with a large database of known sequences, usually at a protein sequence level, and transfer the annotation of the best significant match.

Prokka uses this method, but in a hierarchical manner, starting with a smaller trustworthy database, moving to medium-sized but domain-specific databases, and finally to curated models of protein families. By default, an e-value threshold of 10^{-6} is used with the following series of included databases:

(1) An optional user-provided set of annotated proteins. These are expected to be trustworthy curated datasets and will be

signature science LLC

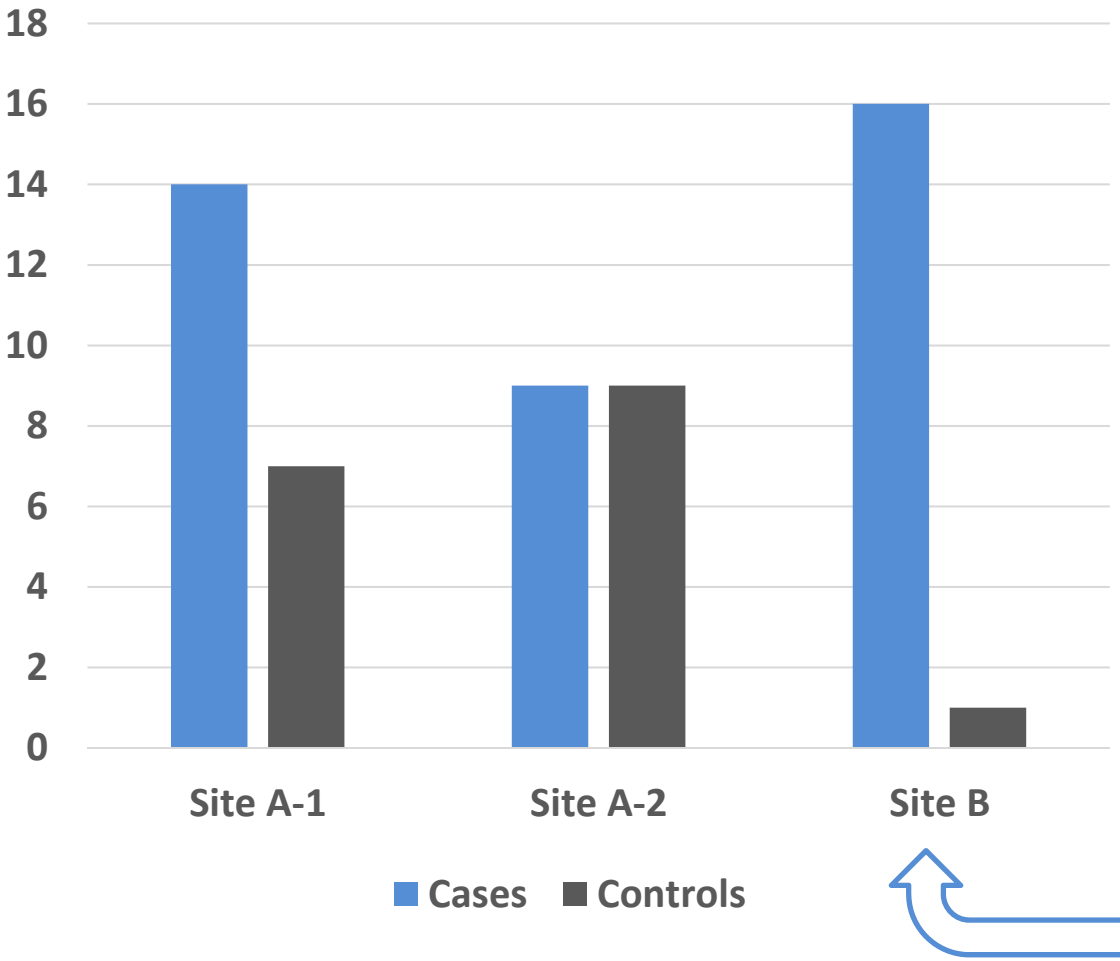
“Arsenic” in Predicted Gene Products



Site B

Arsenate reductase	7	0
Arsenical-resistance protein Acr3	6	0
Protein ArsC	4	0
Arsenical pump-driving ATPase	3	0
Arsenical resistance operon trans-acting repressor ArsD	3	0
Arsenite methyltransferase	3	0
Arsenic resistance transcriptional regulator ArsR1	2	0
Arsenite oxidase subunit AioA	1	0
Arsenite oxidase subunit AioB	1	0
Arsenical pump membrane protein	0	0
Glutaredoxin arsenate reductase	0	0
NADPH-dependent FMN reductase ArsH	0	1

“Copper” in Predicted Gene Products



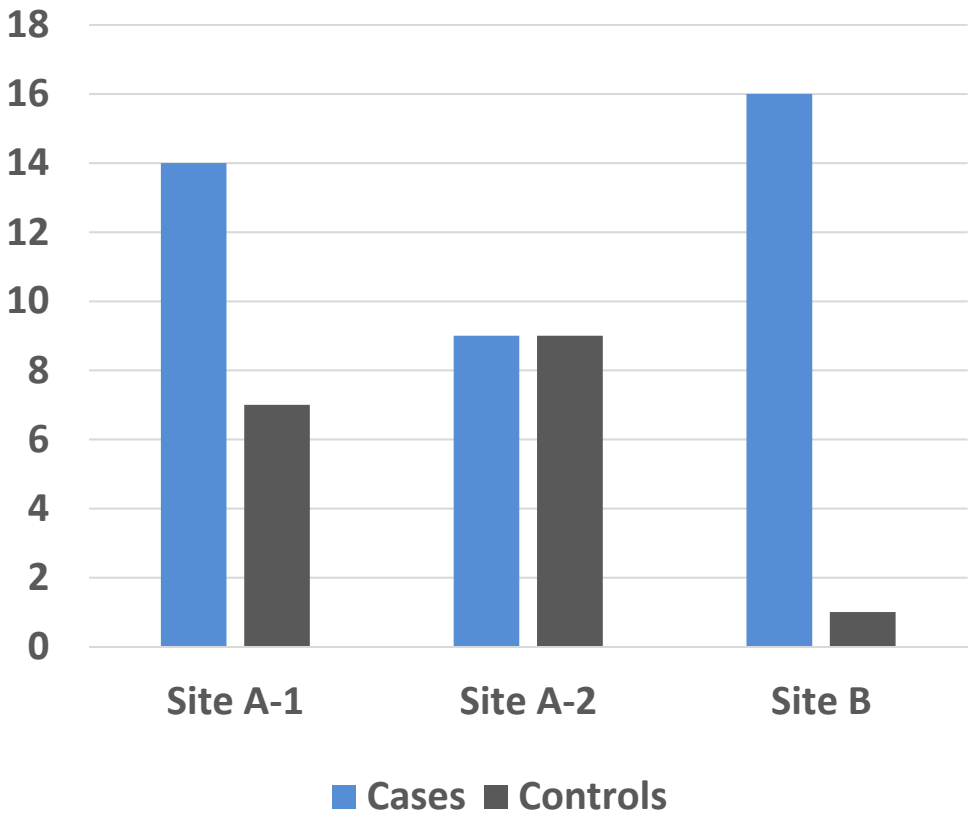
Site B

Copper-exporting P-type ATPase	4	1
Copper resistance protein A	3	0
Copper-exporting P-type ATPase B	3	0
Copper resistance protein B	2	0
Copper-transporting P-type ATPase	2	0
Copper chaperone CopZ	1	0
Multicopper oxidase MmcO	1	0
Multicopper oxidase mco	0	0
Copper-sensing transcriptional repressor CsoR	0	0
Copper-containing nitrite reductase	0	0
Copper homeostasis protein CutC	0	0
Copper resistance protein C	0	0

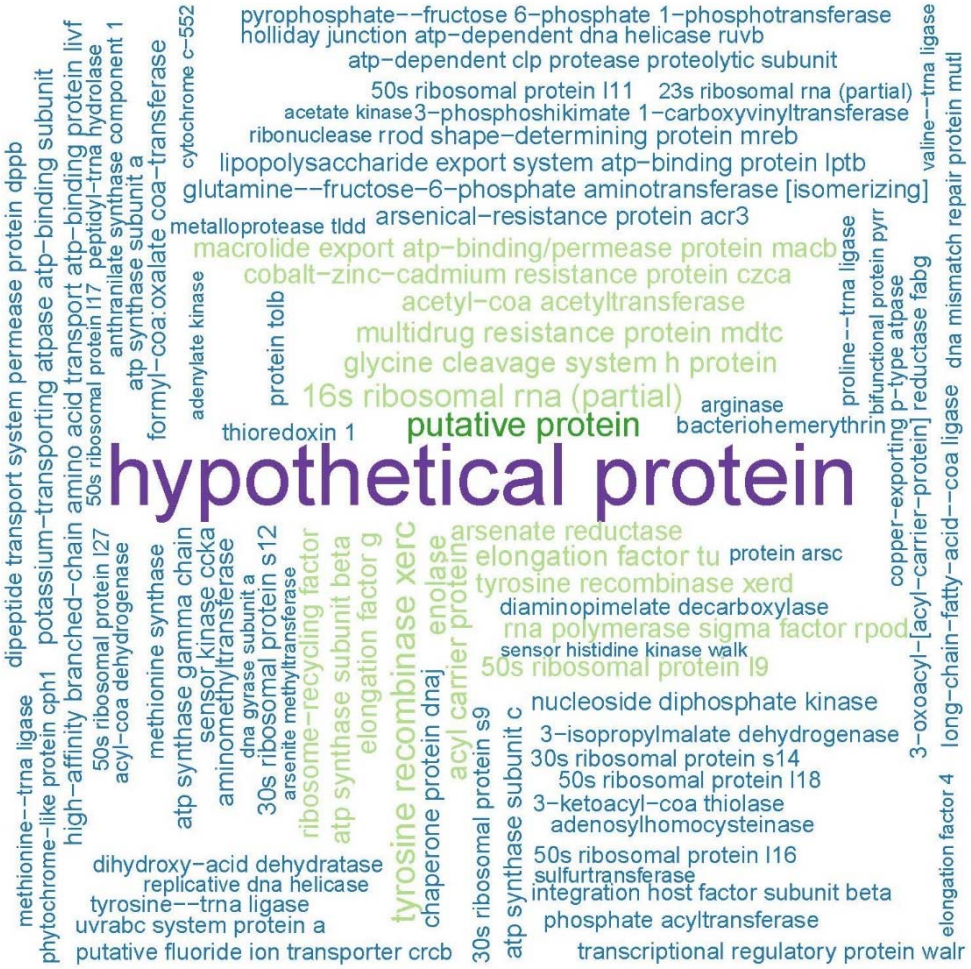
signature science LLC

“Copper” in Predicted Gene Products

	Site A-1		Site A-2	
Copper homeostasis protein CutC	3	3	1	2
Multicopper oxidase MmcO	2	0	0	1
Copper chaperone CopZ	2	0	0	0
Copper-transporting P-type ATPase	2	0	1	2
Multicopper oxidase mco	2	1	0	1
Copper-sensing transcriptional repressor CsoR	1	0	3	0
Copper resistance protein C	1	0	1	0
Copper-exporting P-type ATPase B	1	0	3	3
Copper resistance protein B	0	1	0	0
Copper-containing nitrite reductase	0	1	0	0
Copper resistance protein A	0	0	0	0
Copper-exporting P-type ATPase	0	1	0	0



Hypothetical Proteins



Predicting the functions of unknown proteins is an active area of research for my group.

Please contact me if we can help you!

Krista Ternus, PhD

kternus@signaturescience.com

@KristaTernus

signature science LLC

Questions?

signature science LLC