VIROME

VIRAL INFORMATICS RESOURCE FOR METAGENOME EXPLORATION

A WEB-BASED APPLICATION FOR ANNOTATION AND ANALYSIS OF VIRAL METAGENOMES

by

Jaysheel D. Bhavsar

A thesis submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for the degree of Master of Science in Computer Sciences

Fall 2014

VIROME
VIRAL INFORMATICS RESOURCE FOR METAGENOME EXPLORATION
A WEB-BASED APPLICATION FOR ANNOTATION AND ANALYSIS OF VIRAL METAGENOMES

by

Jaysheel D. Bhavsar

ACKNOWLEDGMENT

I sincerely thank Dr. Li Liao and Dr. K. E. Wommack for their guidance, patience and sharing their keen insight with me throughout this project. I am grateful to you Dr. K. E. Wommack, for teaching me so many aspects of carrying out fruitful research and for your infective perseverance and knowledge. Thank you Dr. S. Polson, D. Nasko, K. Thaper and S. Dhankar and Dr. K. E. Wommack et. al for their patience over my ever-changing crazy ideas of what VIROME should be. I thank the Faculty of the Computer and Information Sciences department, Dr. Cathy H. Wu and Dr. Blake Meyer for making these years of my education truly memorable.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Recent developments in sequencing technology have allowed metagenomic studies to become an increasingly common method for assessment of microbial communities and viral assemblages. Many existing resources for analysis of metagenomic data are designed for the needs of bacterial metagenomic studies, but few address issues specific to viruses. Most notable among these limitations is the lack of solutions for functional and taxonomic annotation of viral metagenomes.

Presented is the Viral Informatics Resource for Metagenome Exploration (VIROME) a web-based application for annotation and analysis of viral metagenomes. VIROME leverages a large database of known and environmental proteins to assess the potential identity of open reading frames (ORFs) within sequences. The analysis pipeline combines a variety of existing and novel resources to automatically form consensus annotations for a given ORF or sequence. The VIROME web interface allows the user to search metagenomes by BLAST, protein family, and functional/taxonomic annotations (among others). Interactive library statistics provide key information summaries at a glance, while enabling the user to directly browse the underlying data by “drilling-down” to increasingly specific levels of resolution. With over 23,028,372 ORFs across 109 libraries from diverse range of environments VIROME acts as a repository for published and contributed metagenome sequence data from viral assemblages

Chapter 1

INTRODUCTION

At the time of inception of the Viral Informatics Resource for Metagenome Exploration (VIROME) in 2005/06 there was a lack of bioinformatics analysis tools/pipelines designed specifically for viral metagenomes, nor were there any visualization tools available that could empower the user to identify and generate categorized data on demand. The analysis tools that did exist were largely adapted from whole genome or bacterial genome pipelines. The pipelines developed for whole genome or bacterial genome analysis largely searched for homologs against sequence databases of known organisms and bacterial reference genomes, this approach does not work well for viral metagenomes because large amount of homologs found in viral metagenomes and viral genomes are to unknown or hypothetical proteins (Wommack, Bhavsar et al. 2008). With the introduction of 454 sequencing and other next generation sequencing technologies it became possible to attain larger sequencing depth and reduce sequencing cost when compared to Sanger sequencing. However increase of short read sequencing data (~ 400 bases) did not translate to an in increase in biological information. Short reads failed to find distant homologs and missed approximately 72% of Clusters of Orthologous Groups (COG) (Tatusov, Galperin et al. 2000) hits when compared to long reads (Wommack, Bhavsar et al. 2008).

Today, there are few metagenome analysis pipelines available, and even fewer analysis pipelines focusing on viral metagenomes. Two of the most popular metagenome analysis pipelines are MG-RAST (Meyer, Paarmann et al. 2008) and MetaVIR (Roux, Faubladier et al. 2011). MG-RAST, which is based on the rapid annotation using subsystem technology (RAST) genome annotation tool (Aziz, Bartels et al. 2008) , has over 117 thousand metagenomes containing 404 billion base pairs of sequence data. Of the 117 thousand metagenomes over 16 thousand metagenomes are publicly available but only small fraction of the publicly accessible metagenomes are viral metagenomes. The core analysis and annotation of MG-RAST depends heavily on the SEED database which is comprises of mainly of bacterial and archaeal genomes (Overbeek, Disz et al. 2004). With little being known about viral communities and the strong sequence divergence and broad gene richness found in viral metagenomes indicate a tremendous genetic diversity (Kristensen, Mushegian et al. 2010) large amount of data is not considered while annotating viral metagenomes. Of all the features available one of the greatest assets of MG-RAST is the metagenome overview summary, which lists several key pieces of information such as:

- Sequence annotation distribution e.g., annotated protein, unknown protein, ribosomal RNA and unknown
- K-mer profile,
- A histogram of hit distribution across subject databases
- A distribution of sequences among top level COGs
- Enzyme groups within the Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa and Goto 2000)

MetaVIR is specifically designed for the analysis of environmental viral communities. MetaVIR compares reads to complete viral genomes from the RefSeq database and performs phylogenetic analysis to explore viral diversity. However MetaVIR has a similar drawback as MG-RAST, by employing a single database of just viral genomes MetaVIR ignores a significant fraction of reads that often exhibit significant overlap to other viral sequences from different locations (Polson, Wilhelm et al. 2010). MetaVIR does have a great metagenome comparative tool, with help of Krona (Ondov, Bergman et al. 2011), makes it possible to compare metagenomes through

- K-mer frequencies
- Taxonomic compositions
- Recruitment plots
- Phylogenetic tree

With just 30% of sequence finding homology to a known functional gene a lot of sequence data is left out of the analysis and annotation of a viral metagenome sequences by both tools. Another feature missing from both these tools is the seamless navigation through various level of data granularity. In each case the primary navigation bar is the only form of navigation from one level of data exploration to next which is an impediment to more intuitive data exploration. Table 1 shows a comparison of features between VIROME, MG-RAST and MetaVIR

Table 1 Compare features between VIROME, MG-RAST and MetaVIR

Features	VIROME	MG-RAST	MetaVIR
Number of subject database	7	1	1
Env. Annotation	Yes	No	No
Comparative tool	Yes	Yes	Yes
MIMARKS standards	Yes	Yes	Unknown
Homology search tool	BLAST	BLAT	BLAST
Interactive interface	✓✓✓✓✓	✓✓✓✓	✓✓✓✓
Download data	✓✓✓✓✓	✓✓✓	✓✓✓
Easy of Navigation	✓✓✓✓✓	✓✓✓	✓✓✓

What distinguishes VIROME from MetaVIR and MG-RAST is the rigorous bioinformatics analysis pipeline; and clean, simple, and intuitive interface, which facilitates natural flow of data exploration. The VIROME bioinformatics analysis pipeline uses six well-known curated databases and an extensive environmental peptide database in contrast to the single database used by MetaVIR for homology search. The VIROME bioinformatics analysis pipeline also uses BLAST for better resolution and accurate alignment as appose to BLAT which is used by MG-RAST. VIROME adheres to data collection and reporting standards as outlined in the minimum information about a marker gene sequence (MIMARKS) and minimum information about (x) sequence (MIxS) specifications (Yilmaz, Kottmann et al. 2011). Inclusion of such metadata empowers VIROME to generate and display well-annotated results, especially in case of environmental annotation. Ensuring extensive annotation with controlled vocabulary also helps in downstream comparative analysis outside VIROME. Based on a rigorous bioinformatics pipeline, VIROME classifies each open reading frame (ORF) into following six classifications:

- Possible functional protein
- Unassigned functional protein
- Top hit viral
- Viral only hit
- Top hit microbial
- Microbial only hit

Additionally VIROME identifies and marks stable RNA genes, in particular transfer RNA (tRNA) and ribosomal RNA (rRNA) genes. Details of each of these categories are available in Chapter 2.

VIROME is a combination of three main components. The VIROME bioinformatics analysis pipeline is the core software engine that conducts bioinformatic analysis and annotation of the sequence data within a viral metagenome library. The VIROME database stores all the data generated by VIROME bioinformatics analysis pipeline and serves data on-demand to the VIROME web application. The VIROME web application is the interface that end-user uses to interact with and explore viral metagenome data. An easy to use interface within intuitive flow of data exploration from one level to next, dynamic data display and scalability were some of the key principles that drove the design of VIROME bioinformatics analysis pipeline, database and web application. Figure 1 shows general overview of data flow through VIROME bioinformatics analysis pipeline, VIROME database and VIROME web application.

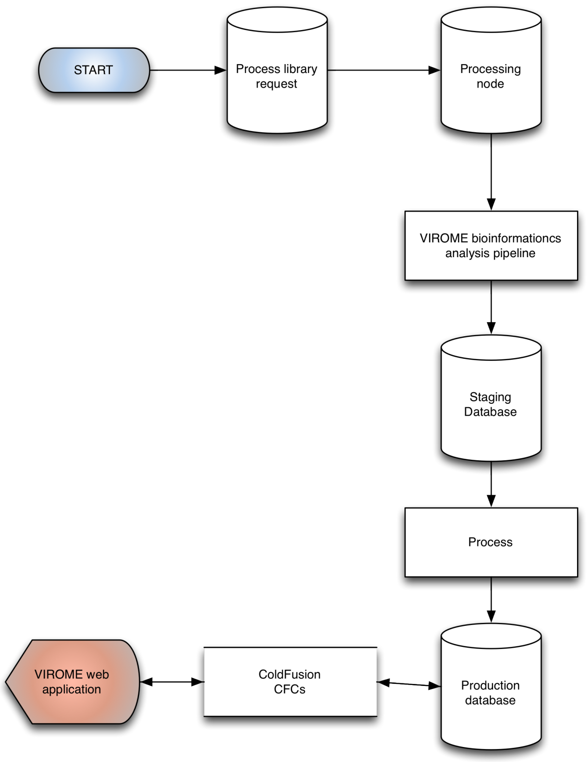


Figure 1 General overview of data flow through VIROME bioinformatics analysis pipeline, VIROME database and VIROME web application.

Chapter 2

VIROME BIOINFORMATICS ANALYSIS PIPELINE

With the advancement in high throughput sequencing, millions of reads are available for analysis in a short amount of time. At the same time pipelines employed to analyze large amount of data have become complex, requiring a large amount of computing power. One approach would be to add more computing power or employ a sophisticated system that can manage and analyze large amounts of data and efficiently and quickly while maintaining accuracy. VIROME bioinformatics analysis pipeline employs one such pipeline management system known as Ergatis (Orvis, Crabtree et al. 2010). Ergatis comprises of two tightly coupled systems. A management system known as Workflow operates behind the scenes and does bulk of the heavy lifting with respect to job management. Workflow is responsible for submitting jobs for execution to a compute cluster or a personal computer, keeping track of job progress, reports on job status, logging and other overhead related to job scheduling. The second part of Ergatis is the visible user interface, which allows a user to setup, execute and maintain a pipeline. The Ergatis front end also shows status of the pipelines as well as its individual components. What differentiates Ergatis from other workflow management systems such as Galaxy (Giardine, Riemer et al. 2005), Taverna (Wolstencroft, Haines et al. 2013) and Kepler (https://kepler-project.org/) is its ability to run multiple components in parallel and serial and its ability to run several scatter/gather processes in parallel of each other. Ergatis is also able to use a single instance of a pipeline to run multiple inputs. For example if five libraries need to be analyzed using the VIROME bioinformatics analysis pipeline five different instances of the pipeline would need to be run in Galaxy, Taverna or Kepler. However, Ergatis could run all five libraries in parallel of each other using a single instance of the VIROME bioinformatics analysis pipeline. This is a useful feature as it provides a single location to monitor, debug and modify multiple libraries. Ergatis's workflow manager is written in JAVA and currently supports Sun Grid Engine or Open Grid Scheduler (http://gridscheduler.sourceforge.net/) along with standalone personal computer. Workflow manager has been previously deployed on HTCondor grid manager (http://research.cs.wisc.edu/htcondor/) but its not actively supported by the Ergatis team. It is possible to add additional support for other grid scheduler such as PBS/Tourque. The Ergatis front end is written in PHP (http://php.net/) and uses common gateway interface requests and responses (CGI) (https://metacpan.org/release/CGI) a Perl module (http://www.perl.org/). Table 2 provides an overview of features between Ergatis, Galaxy, Taverna and Kepler.

Table 2 Feature comparison of various workflow managers.

Features	Ergatis	Galaxy	Taverna	Kepler
Grid support	Yes	Yes	Yes	Yes
Grid type	SGE/OGE	All[1]	Most[2]	Most[2]
Interface	Web	Web	Standalone[3]	Standalone[3]
Open source	Yes	Yes	Yes	Yes
Language	Perl-CGI and JAVA	Perl-CGI and C	JAVA	JAVA
Scatter/Gather	✓✓✓✓✓	✓✓✓	✓✓	✓✓
Multi-input	✓✓✓✓✓	✓✓✓	✓✓	✓✓

[1] Galaxy uses Distributed Resource Management Application API (DRMAA) to submit grid jobs (https://code.google.com/p/drmaa-python/).

[2] Complete list of grid engine support are not listed

[3] Web interface is available for Taverna and Kepler via additional library as web service calls

Figure 2 depicts four main divisions of VIROME bioinformatics analysis pipeline,

- Quality control
- Open reading frame prediction
- Annotation
- Statistics and classification

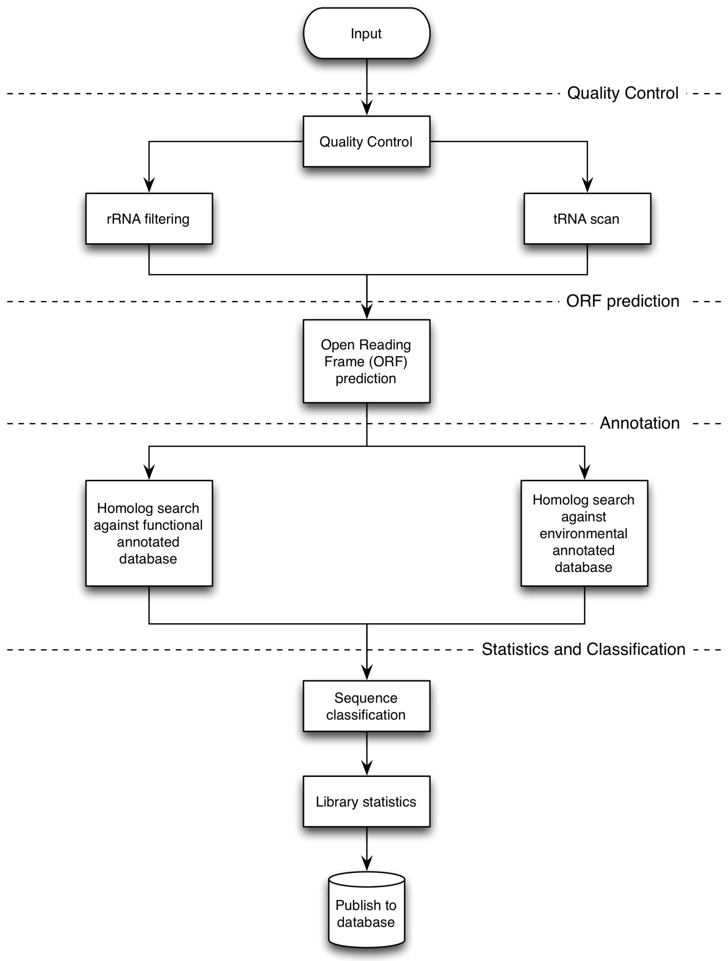
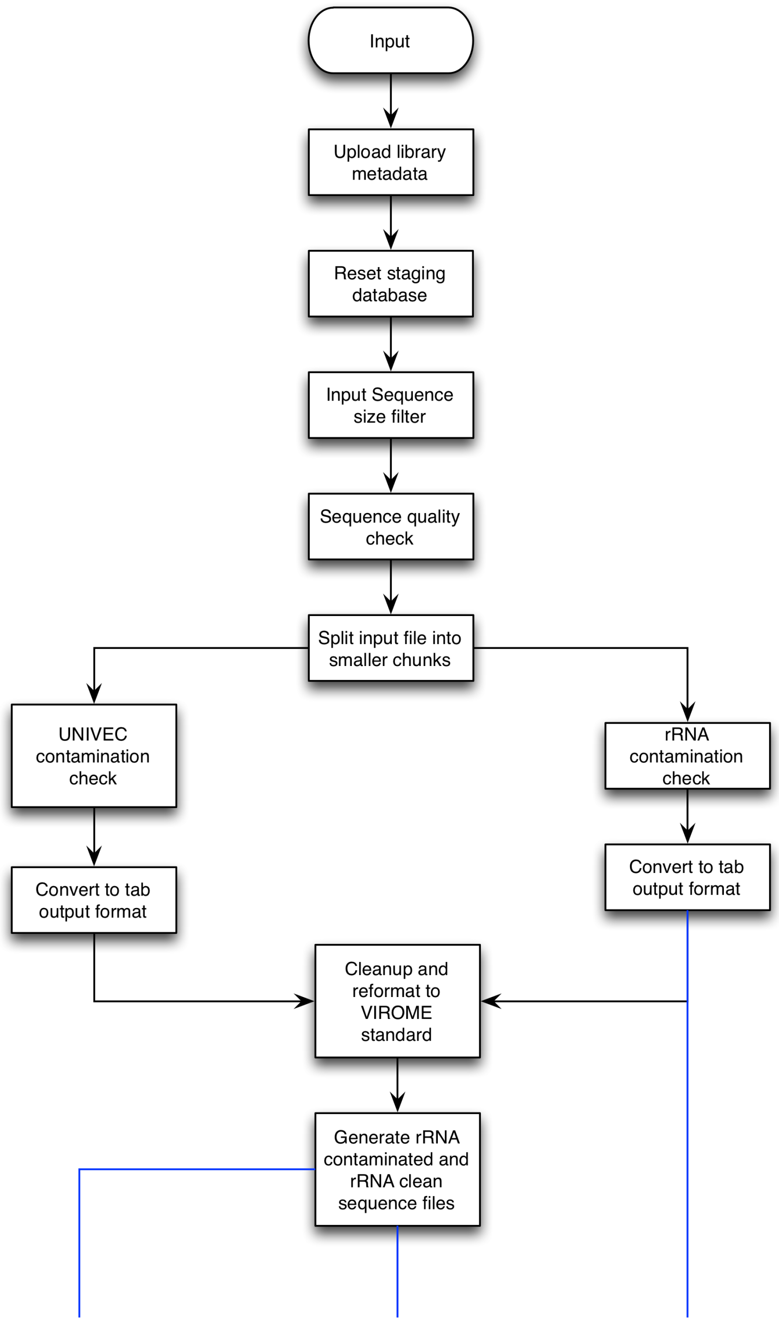
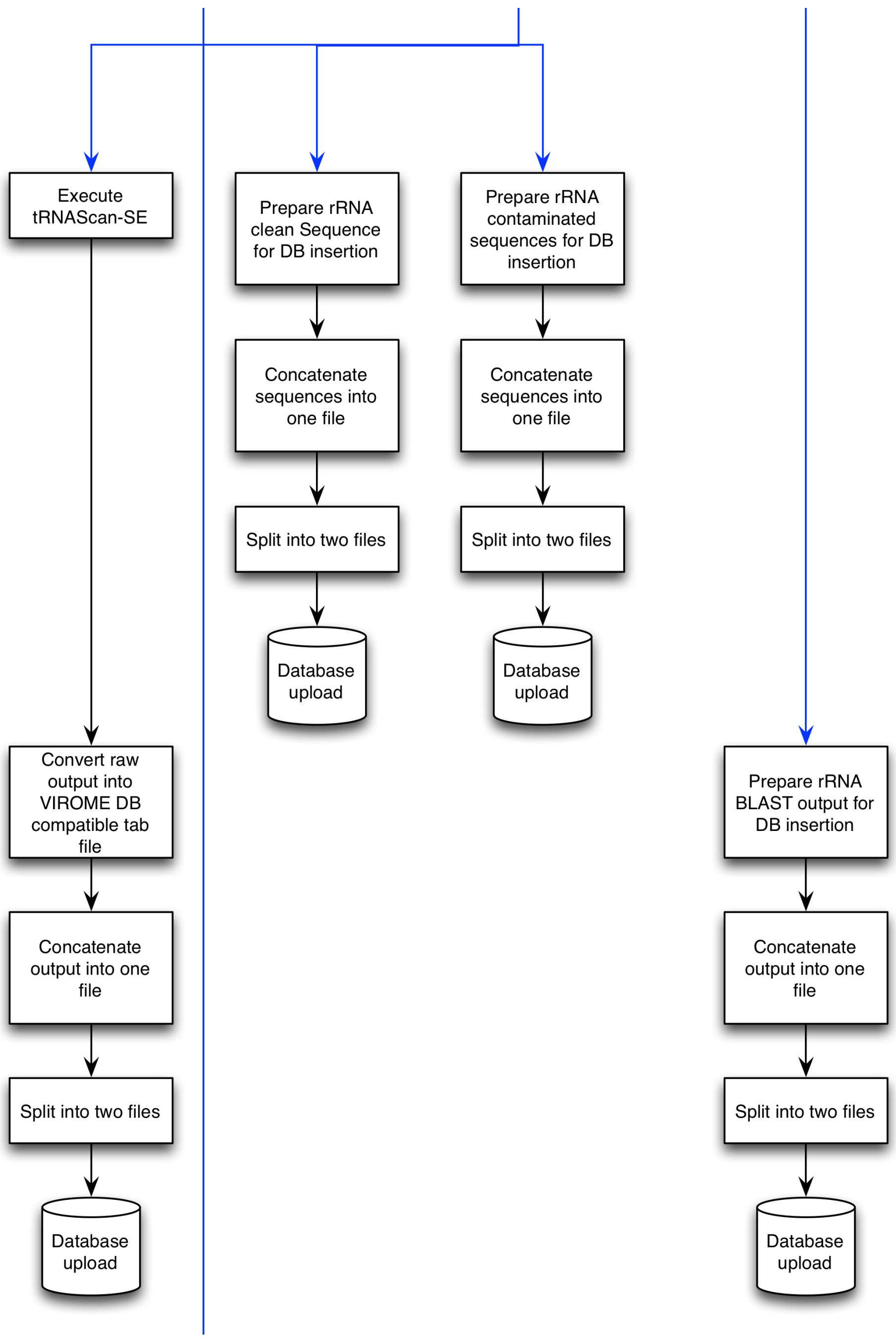
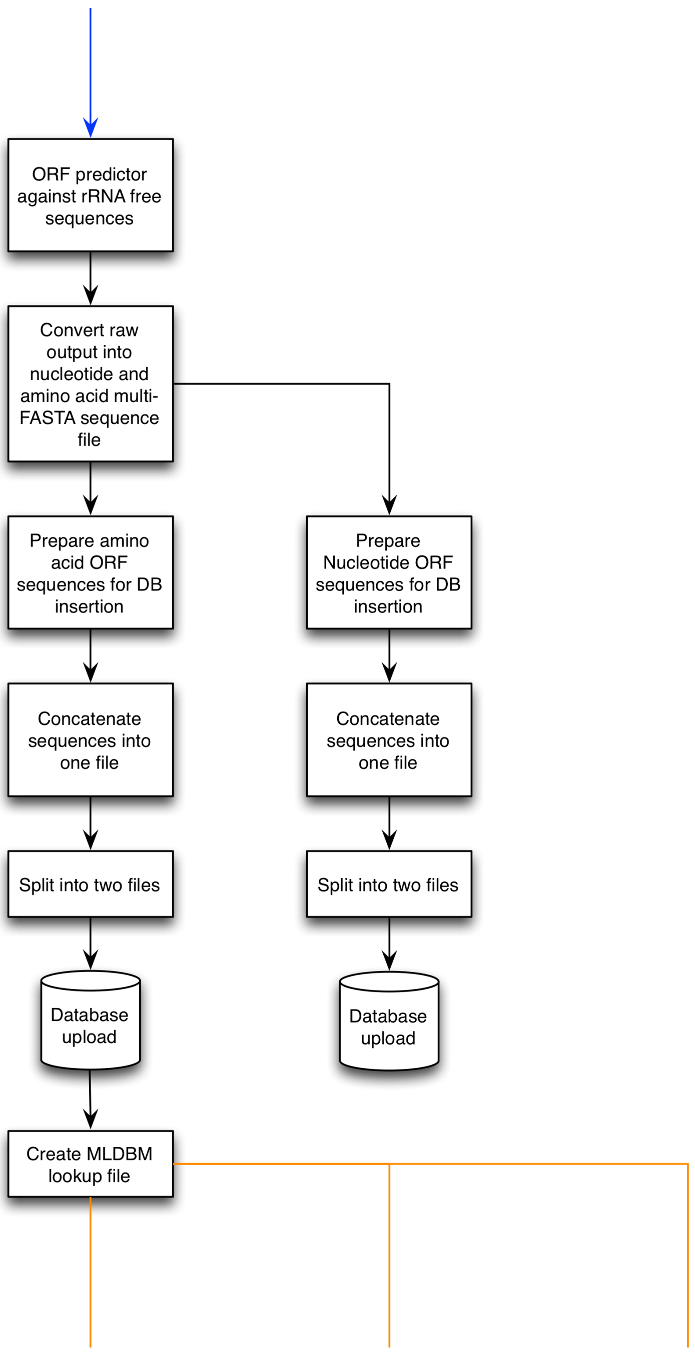

Input
Quality Control
Quality Control
rRNA filtering
tRNA scan
ORF prediction
Open Reading Frame (ORF) prediction
Annotation
Homolog search against functional annotated database
Homolog search against environmental annotated database
Statistics and Classification
Sequence classification
Library statistics
Publish to database

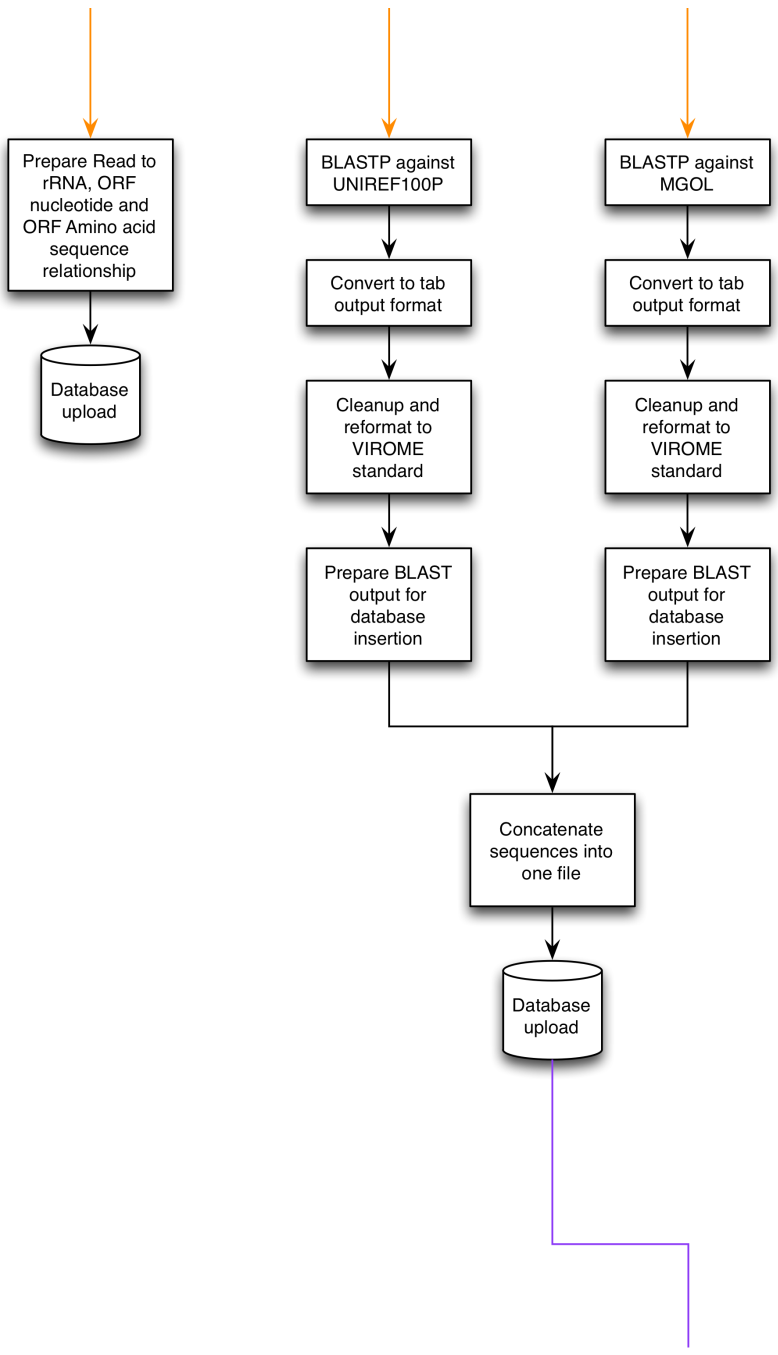
Figure 2 VIROME bioinformatics analysis pipeline overview

Version 1.0 of the VIROME bioinformatics analysis pipeline contains thirty-two unique components of which four components are precompiled algorithms/commands such as BLAST (Altschul, Gish et al. 1990), CD-Hit (Li and Godzik 2006), tRNAScan (Lowe and Eddy 1997), MetaGene (Noguchi, Park et al. 2006) and a UNIX command called “concat”. The other twenty-seven components are Perl scripts and algorithms that were developed by the VIROME team. Due to parallel and serial combinations of these thirty-two components a typical end-to-end Ergatis pipeline consist of sixty-two components. Figure 3 shows all sixty-two components in order or execution.


Input
Upload library metadata
Reset staging database
Input Sequence size filter
Sequence quality check
Split input file into smaller chunks
UNIVEC contamination check
rRNA contamination check
Convert to tab output format
Convert to tab output format
Cleanup and reformat to VIROME standard
Generate rRNA contaminated and rRNA clean sequence files


Execute tRNAScan-SE
Prepare rRNA clean Sequence for DB insertion
Prepare rRNA contaminated sequences for DB insertion
Concatenate sequences into one file
Concatenate sequences into one file
Split into two files
Split into two files
Database upload
Database upload
Convert raw output into VIROME DB compatible tab file
Prepare rRNA BLAST output for DB insertion
Concatenate output into one file
Concatenate output into one file
Split into two files
Split into two files
Database upload
Database upload


ORF predictor against rRNA free sequences
Convert raw output into nucleotide and amino acid multi-FASTA sequence file
Prepare amino acid ORF sequences for DB insertion
Prepare Nucleotide ORF sequences for DB insertion
Concatenate sequences into one file
Concatenate sequences into one file
Split into two files
Split into two files
Database upload
Database upload
Create MLDBM lookup file


Prepare Read to rRNA, ORF nucleotide and ORF Amino acid sequence relationship
Database upload
BLASTP against UNIREF100P
Convert to tab output format
Cleanup and reformat to VIROME standard
Prepare BLAST output for database insertion
BLASTP against MGOL
Convert to tab output format
Cleanup and reformat to VIROME standard
Prepare BLAST output for database insertion
Concatenate sequences into one file
Database upload

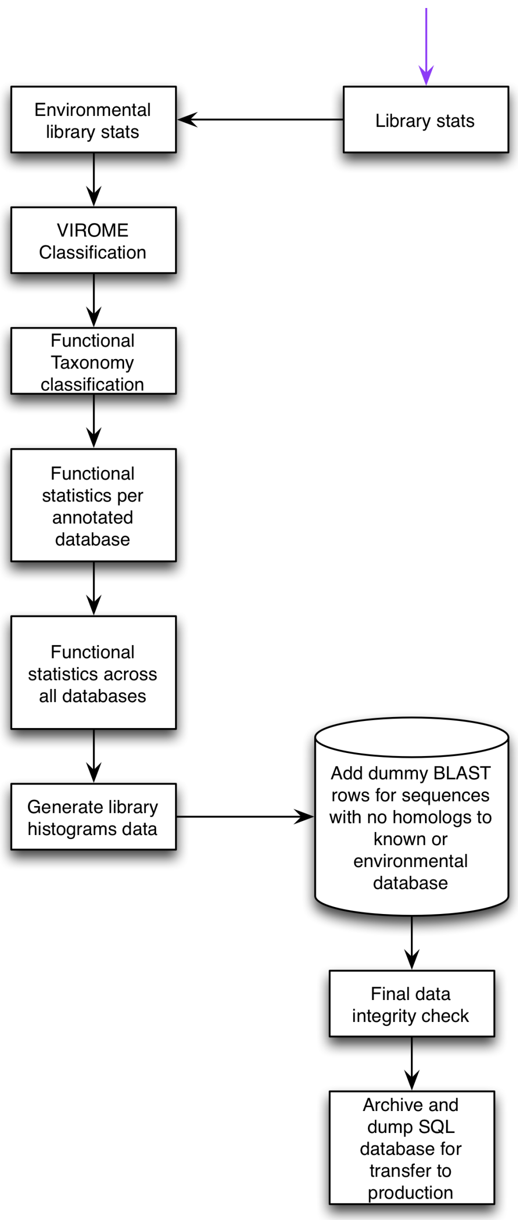

Library stats
Environmental library stats
VIROME Classification
Functional Taxonomy classification
Functional statistics per annotated database
Functional statistics across all databases
Generate library histograms data
Add dummy BLAST rows for sequences with no homologs to known or environmental database
Final data integrity check
Archive and dump SQL database for transfer to production

Figure 3 Detail VIROME bioinformatics analysis pipeline

The VIROME bioinformatics analysis pipeline v1.0 accepts FASTA, FASTQ or SFF inputs. All input sequences are filtered for minimum length of 300 bases for unassembled reads or 600 bases for assembled contigs. In the case of FASTQ and SFF input average phred base quality score (Ewing, Hillier et al. 1998) must be greater than 20. In the case of 454 pyrosequencing data (typically in SFF files) artificial duplicates are removed using CD-HIT-454 (Li and Godzik 2006) at 98% identity. Ambiguous base such as N, R, and Y must be under 8% per sequence. The UniVec (http://www.ncbi.nlm.nih.gov/tools/vecscreen/univec) database is used to screen reads for the presence of contaminating vector sequences within metagenome sequence reads/contigs. A taxonomically diverse collection of ~30,000 ribosomal RNA (rRNA) genes (5S, 16S, 18S, and 23S) is used to detect the presence of ribosomal RNA homologs within sequence libraries rRNA contaminant using NCBI BLASTN v2.2.28+ (Altschul, Gish et al. 1990) with minimum expected value of $1e^{-75}$. The VIROME bioinformatics analysis pipeline v1.0 scans for transfer RNA (tRNA) using tRNAScan-SE v1.3.1 (Lowe and Eddy 1997).

The VIROME bioinformatics analysis pipeline v1.0 is an open reading frame (ORF) based pipeline; it uses predicted ORFs for functional and environmental annotation. Long ORFs are especially helpful in identifying candidate protein coding regions in a DNA sequence. The VIROME bioinformatics analysis pipeline v1.0 uses MetaGene (Noguchi, Park et al. 2006) to predict open reading frames. Because of the short read length of Illumina and Ion Torrent sequences users must assemble these data and submit contigs of ≥ 600 bases, rather than single pass reads. Assembly drastically reduces the CPU time necessary for analysis and contigs greater than 600 bases are known to provide substantially more biological information than short reads (Wommack, Bhavsar et al. 2008)

The VIROME bioinformatics analysis pipeline v1.0 performs homology searches against a functional and an environmental sequence database using NCBI BLAST. Functional homologs are found using BLASTP search against a well-curated database called UniRef100-Plus (UniRef100P). UniRef100P is made up of seven de-replicated databases:

1. ACLAME: A classification of mobile genetic elements, which contains the collection and classification of mobile genetic elements (MGEs) from various sources, comprising all known phage genomes, plasmids and transposons (Leplae, Hebrant et al. 2004)

2. COG: Cluster of orthologous groups of proteins from the sequenced genomes of prokaryotes and unicellular eukaryotes and the construction of clusters of predicted orthologs (Tatusov, Galperin et al. 2000)

3. GO: Gene ontology provides a set of structured, controlled vocabularies for community use in annotating genes, gene products and sequences (Ashburner, Ball et al. 2000)

4. KEGG: Kyoto encyclopedia of genes and genomes is a knowledge base for systematic analysis of gene functions, linking genomic information with higher order functional information. The genomic information is stored in the GENES database, which is a collection of gene catalogs for all the completely sequenced genomes and some partial genomes with up-to-date annotation of gene functions (Kanehisa and Goto 2000)

5. PhageSEED: A tool kit for high-quality annotation of available phage sequences (http://www.phantome.org)

6. SEED: A toolkit to view and manipulate genomic data, automatically append annotations to it, and exchange information (Overbeek, Disz et al. 2004)

7. UniRef100: The UniRef (UniProt Reference Clusters) provide clustered sets of sequences from the UniProt Knowledgebase (UniProtKB) and selected UniProt Archive records to obtain complete coverage of sequence space at several resolutions while hiding redundant sequences (Suzek, Huang et al. 2007). UniRef100 Contains peptides (>11 aa in length) gathered from the major sequence repositories (e.g., GenBank NR).

Environmental homologs are found using a BLASTP search against a well-curated database known as Metagenomes Online (MgOl) (S. Polson, K. E. Wommack at el, unpublished). Currently the MgOl database contains 258 libraries, of those, 159 libraries are viral, 88 prokaryotic and remaining 10 are eukaryotic or mixed organism libraries. MgOl contains 56,254,299 proteins for a total of 6,480,011,292 bases of amino acid sequences. MgOl is a manually annotated resource of predicted proteins identified in viral and microbial shotgun metagenomes. MgOl libraries are annotated with an abundance of sample metadata including sample provenance, geographical description, environmental parameters, sampling and preparation methodologies, and Environmental Ontology (ENVO) (http://www.ebi.ac.uk/ontology-lookup/browse.do?ontName=ENVO) terms. Samples are further categorized using MgOl sample descriptors, easy to understand terms allowing samples to be easily grouped for comparison (http://metagenomesonline.org).

The VIROME bioinformatics analysis pipeline v1.0 classifies each sequence into one of seven categories:

1. Possible functional protein: These are sequences that have an homolog against UniRef100P database with expected value (e-value) of at least $1e^{-3}$ and the top homolog sequence is well curated i.e. BLAST hit description not unclassified, hypothetical protein or unknown.

2. Unassigned protein: These are sequences that have an homolog against UniRef100P database with e-value of at least $1e^{-3}$ but the top homolog sequence is not well curated i.e. BLAST hit description is unclassified, hypothetical protein or unknown.

3. Top viral: These are sequences that have a homolog against MgOl database with e-value of at least $1e^{-3}$ and the top homolog sequence is in one of the MgOl viral libraries.

4. Top microbial: These are sequences that have a homolog against MgOl database with e-value of at least $1e^{-3}$ and the top homolog sequence is in one of the MgOl prokaryotic or eukaryotic libraries.

5. Viral only: These are sequences that have a homolog against MgOl database with e-value of at least $1e^{-3}$ and all homologs are to MgOl viral libraries.

6. Microbial only: These are sequences that have a homolog against MgOl database with e-value of at least $1e^{-3}$ and all homologs are to MgOl prokaryotic or eukaryotic libraries.

7. ORFans: These are sequences that do not show homology to any UniRef100P or MgOl peptide with an e-value of $1e^{-3}$ or does not have any homologs at all to either UniRef100P or MgOl database.

Aside from the above seven categories, each sequence is scanned for tRNA sequence using tRNAScan-SE v1.3.1 and for rRNA contamination. Transfer RNA results are stored in a

MySQL (https://www.mysql.com) database (See chapter 3: VIROME database) and can be visualized using the VIROME web-application, sequence detail view. Sequences containing ribosomal RNA are removed from the sequence library in quality control stage of the VIROME bioinformatics analysis pipeline v1.0 and are stored in MySQL database. These sequences are displayed by selecting the rRNA pipe slice in the browse view of the VIROME web-application, see Chapter 4 for details.

The VIROME bioinformatics analysis pipeline v1.0 generates several statistical summaries for each library. These analyses cover a broad range of levels of biological organization from groups of thousands of sequences to a single ORF. The statistical summaries generated by VIROME bioinformatics analysis pipeline v1.0 are:

1. Analysis overview: Analysis overview shows the distribution of ORFs, in raw counts across UniRef100P, MgOl, both UniRef100P and MgOl and ORFans.

2. VIROME Classification: VIROME Classification shows percent distribution of ORFs across all seven categories described above (possible functional protein, unassigned protein, ACLAME, COG, GO, KEGG, SEED, PhageSEED) along with percent distribution of transfer RNA and ribosomal RNA.

3. Detailed hierarchical distributions for each functional databases ACLAME, COG, GO, KEGG, SEED and PhageSEED are also generated. Due to arbitrary depth of ACLAME and GO hierarchy a limit of six levels is imposed while viewing these statistics using the VIROME application.

4. Taxonomy: Hierarchical distribution of functional taxonomic lineage of all ORFs with top BLAST hit to UniRef100P subject database.

Using the environmental feature annotations of MgOl sequences and the VIROME informatics pipeline, it is possible to group viral metagenome peptides according to significant BLAST hits against MgOl peptides. The MgOl environmental feature annotations used for grouping viral metagenome peptides are: MgOl library id; library type; genesis; sphere; ecosystem; or extreme environment; and its physiochemical characteristics. The summaries of MgOl BLAST hit data for viral metagenome peptides are provided according to each of these environmental features using a weighted scheme (Wommack, Bhavsar et al. 2012).

Along with the above statistics, the VIROME bioinformatics analysis pipeline v1.0 also generates histograms of read/contig lengths, ORF lengths and GC content per library. Raw data produced by the VIROME bioinformatics analysis pipeline v1.0 can be downloaded via the VIROME application. There are several how-to videos related to VIROME application are available online at http://www.virome.dbi.udel.edu

All the components of the VIROME bioinformatics analysis pipeline are written in Perl, using an object oriented programing paradigm. All Perl modules and scripts related to VIROME bioinformatics analysis pipeline and all scripts and modules required to setup an Ergatis instance are available in a private Github (https://github.com) repository URL: https://github.com/bjaysheel/virome_pipeline. Efforts are under way to create purely Perl based workflow, which can then be distributed as a virtual machine or as open-source package, for individuals to maintain their private VIROME bioinformatics analysis pipeline.

Chapter 3

VIROME DATABASE

All data generated by VIROME bioinformatics analysis pipeline is stored in a MySQL (https://www.mysql.com/) database. The VIROME database is organized into two major databases. The first database, known as APP_INFO, holds all the metadata related to each library and all VIROME registered user information. The second database is known as VIR_DATA where all data generated by the VIROME bioinformatics analysis pipeline is stored. Figure 4 shows organization of VIROME database.

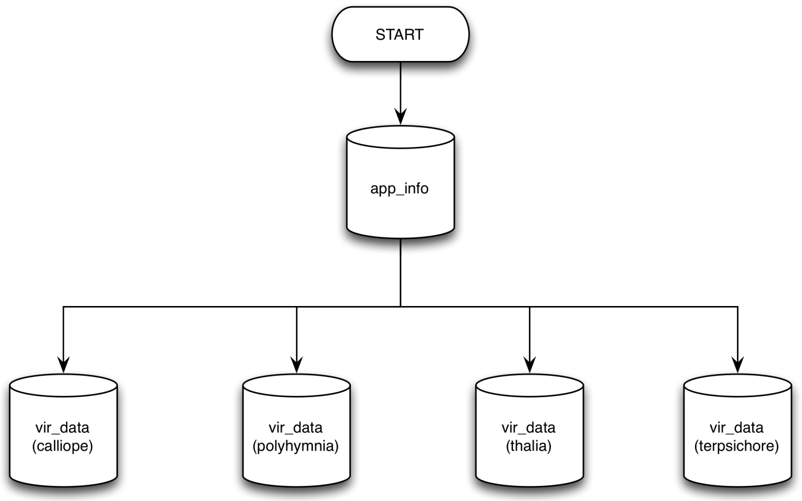


Figure 4 VIROME database organization

All metadata for each library, registered user information and bookmarks of saved searches are stored in APP_INFO database. Figure 5 shows detail schema of APP_INFO.

The user table contains basic information for each user such as name, email address, institute affiliation, username and password. Passwords are encrypted using external ColdFusion (http://www.adobe.com/products/coldfusion-family.html) encryption as opposed to inherent MySQL password encryption this allows the flexibility to retrieve user password if required.

Groups table creates a grouping of users. A group can contain multiple users, and a user can belong to more than one group. By default when a new user is generated a new group is generated by default with the new user as its member. All libraries uploaded by the user will automatically be assigned this user's respective group ID. A user can request access to a private library not assigned to them by sending an email to virome@virome.dbi.udel.edu and once verified a confirmation email will be sent.

The library table contains basic library information such as library name, project name, library description, environment, sequencing method, and whether or not respective library should be made public to the world. Each library is assigned a group ID, which establishes ownership and user access, however a library can only be assigned to one group ID.

Lib_summary table holds metadata such as library type (viral/microbial), NCBI accession (if any), data source (e.g.: CAMERA, NCBI), sequencing type, amplification, number of sites, site ID, genesis, sphere, ecosystem, physical chemical modifiers, physical substrate, Envo terms, geographic region, geographic place and geographic coordinates. A complete list of metadata types in the Lib_Summary table can be found in the VIROME database documentation (in digital format).

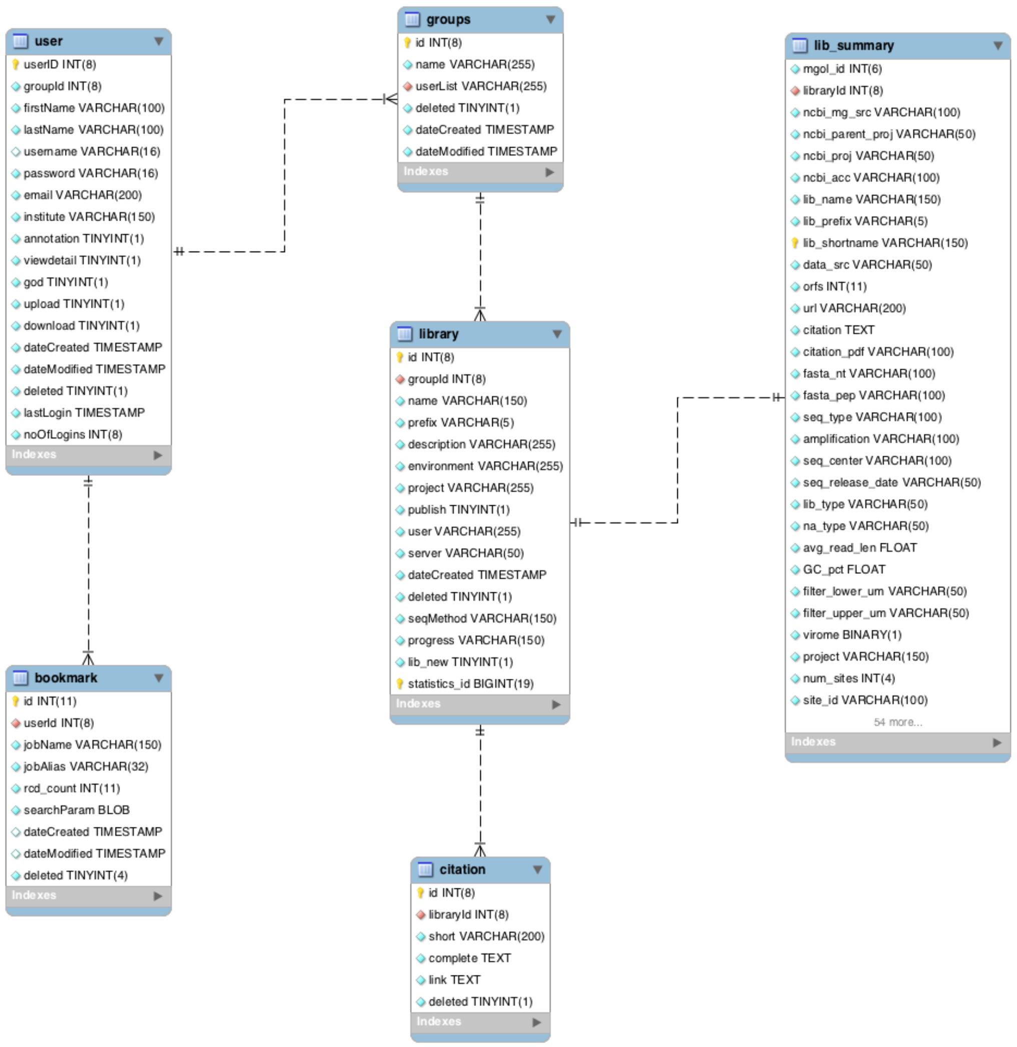


Figure 5 APP_INFO database schema

The library table links data in APP_INFO with data in VIR_DATA. VIR_DATA stores all sequence data, transfer RNA, ribosomal RNA, results of open reading frame prediction, BLAST results and summary output from the VIROME bioinformatics analysis pipeline. Due to budgetary constraints the VIROME database is not hosted on a dedicated database machine, which could be capable, of responding to large number of queries and complex queries quickly and efficiently. For this reason a choice was made to distribute the data stored in the VIR_DATA database into four different databases across four different physical machines. This architecture served to reduce load on single machine, and enable quick queries over a smaller database as opposed to one large table in a single database. This architecture is depicted in Figure 4; each instance of VIR_DATA has an identical structure over all four servers (Calliope, Polyhymnia, Thalia and Terpsichore). The environment metadata type in the library table is used as a category for dividing the data over each of the four servers; Table 3 shows each server and environments they represent.

Table 3 Server and Environment organization of VIROME analysis data.

Server	Environment
Calliope	Organismal Substrate Sediment
Polyhymnia	Soil
Thalia	Extreme Solid Substrate
Terpsichore	Water

Figure 6 shows details of the VIR_DATA database and relationships between each table. The sequence table is an important table in VIR_DATA as it ties all tables together within VIR_DATA and serves to connect VIR_DATA with APP_INFO. The sequence table contains all sequences reads, contigs and ORFs. Type ID column in sequence table identifies if a sequence is an ORF, read/contig or ribosomal RNA, while the sequence_relationship table establishes the relationship between read/contig and ORFs. The blastp and blastn tables both hold all homolog results from VIROME bioinformatics analysis pipeline.

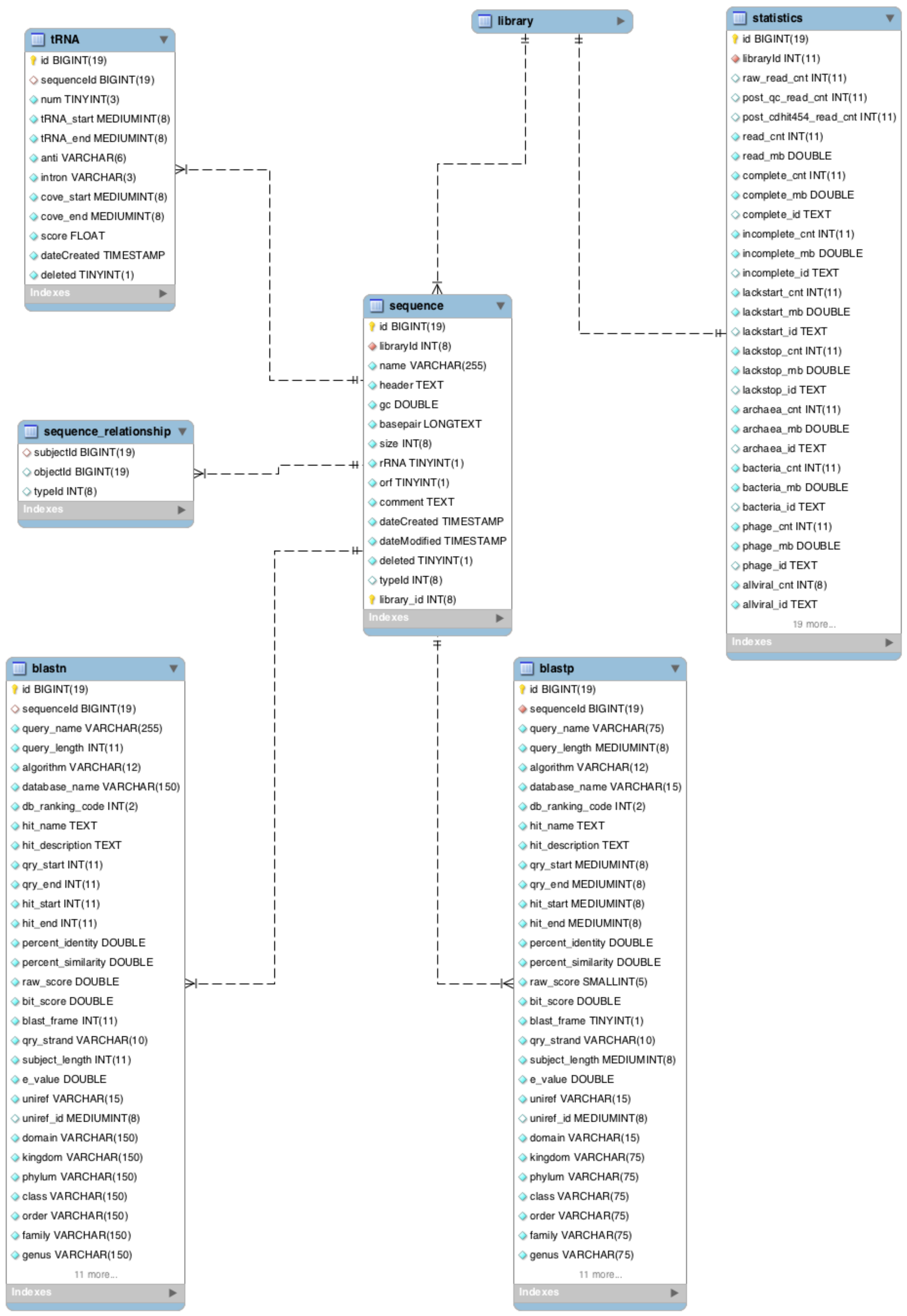


Figure 6 VIR_DATA database schema. Library table is not part of VIR_DATA its shown here for visual purpose linking VIR_DATA to APP_INFO database.

Chapter 4

VIROME WEB-APPLICATION

Modern day metagenomics and metagenome analysis pipelines generate data at such an enormous scale that text files, shell scripts and spreadsheets are not a viable option for extracting meaningful biological information from analysis pipeline results. A level of expertise is required to write and execute scripts, not to mention a higher probability of introducing an error while manually curating results and running scripts. The central challenge in designing the VIROME web-application was to create a tool capable of providing key statistical information and data summary with intuitive ease of use. A tool, which would empower its user to search, bin and categorize data in multiple ways with few simple clicks and give complete access to input raw data. The VIROME web-application also needed to adapt and respond to large data requests efficiently and quickly. Instead of providing static tables, static charts and lots of text, I hoped to develop a tool that was adaptive, dynamic, interactive and responsive, a tool that would make data exploration exciting and engaging. It took several iterations and evaluation of various technologies to get the right combination of server side and client side technology to present VIROME web-application in its current state. Chapter 6 Sequence Detail then and now a case study, highlights how advancement in sequencing technology generated an explosion of data which prompted a major change in the VIROME user interface. The new interface not only allows the VIROME web-application to adapt to large influx of data in the future, it also gives the VIROME web-application an ability to display various data types such as assembled long contigs, raw reads that makeup a contig and ORFs predicted from assembled long contigs. The new user interface can also adapt and display N-number of subject databases, only requirement is that VIROME bioinformatics analysis pipeline add respective BLAST results in appropriate tables.

At the time of initial VIROME web-application development in late 2005 early 2006 the technology choices for generating a high quality and interactive web-application were limited. Some of the popular options for server side and client side technology were:

- JAVA servlet (http://docs.oracle.com/javaee/6/tutorial/doc/bnafd.html),
- JAVA server faces (http://docs.oracle.com/javaee/6/tutorial/doc/bnaph.html)
- PHP (http://php.net/)
- Perl-CGI (https://metacpan.org/release/CGI)
- JavaScript (https://en.wikipedia.org/wiki/JavaScript)
- HTML/XHTML (https://en.wikipedia.org/wiki/XHTML)

Combination of these technologies helped produce usable tools but VIROME needed more than long page view, multiple scroll bars, tables and graphs broken in to several sub sections. In particular, representation of hierarchical relationships was difficult with these technologies as multiple graphs needed to be pre computed and stored on the server. This made the process inefficient, requiring large amount of disk space and a cluttered user-interface. HTML5 (https://en.wikipedia.org/wiki/HTML5) was years away; JQuery (http://jquery.com/) wasn't introduced until August of 2006 and in its early versions wasn't as powerful as it is today. The tug-of-war for browser supremacy among various vendors hampered the ability to create a sophisticated and interactive web-application will work flawlessly across all browser platforms. Even today with most browsers converging towards a single standard when it comes to displaying data using HTML5 and cascading style sheets (CSS) version 3, some code redundancy is required to ensure that data is presented in similar fashion between Microsoft Internet Explorer (http://windows.microsoft.com/en-us/internet-explorer), Apple

Safari (https://www.apple.com/safari) and Mozilla Firefox (https://www.mozilla.org/en-US/firefox). With frequent updates and new version of browsers being released developers are frequently required to test and modify existing applications just to make sure that their applications are functioning to their potential or restrict end user to a specific browser version to ensure the application and its features are working as intended. This takes time away from future enhancement and feature addition that would improve user experience.

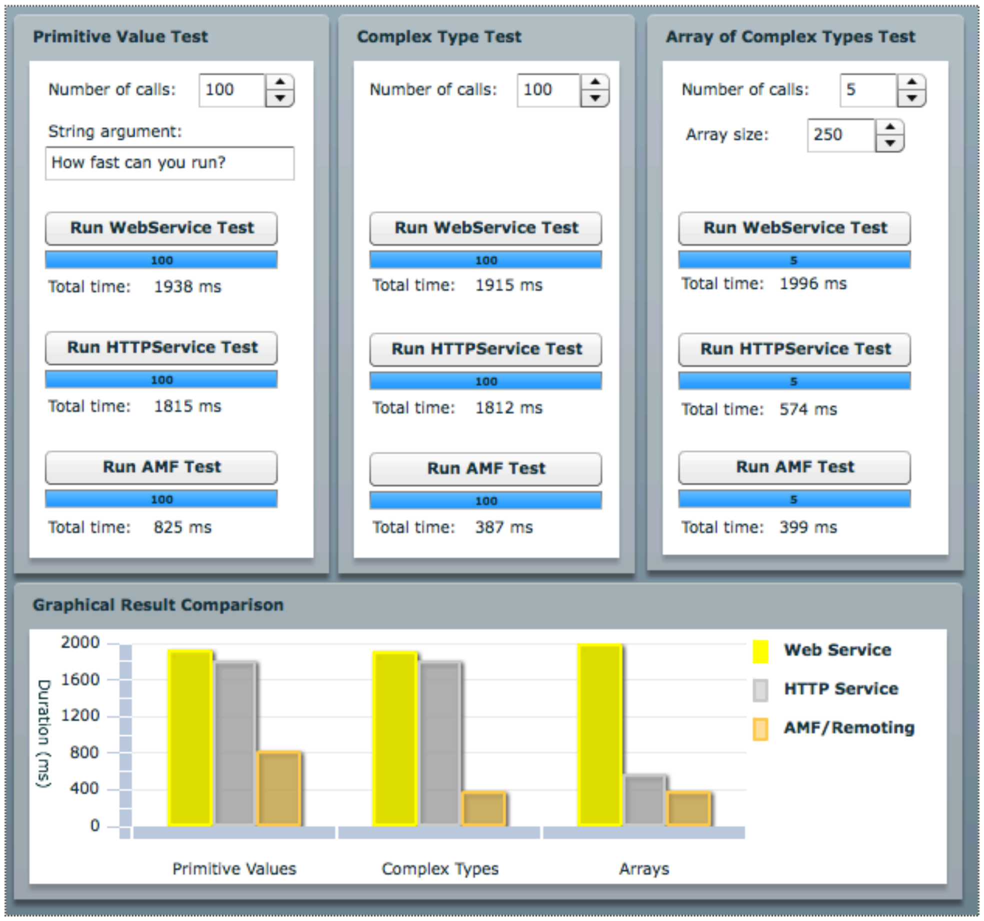
In its current version, the VIROME web-application performs exactly the same, on any browser[4] making it truly platform independent. To achieve this end, the VIROME web-application was built using combination of open source and proprietary software. The VIROME web-application was developed using Apache Flex (http://flex.apache.org), previously know as Adobe Flex (https://www.adobe.com/products/flex.html), an open source application framework for all end-user facing content display and interaction, along with an Adobe ColdFusion (http://www.adobe.com/products/coldfusion-family.html) application server as the middleware, and a MySQL database server in the backend to store all data generated by VIROME bioinformatics analysis pipeline (see Chapter 2 VIROME bioinformatics analysis pipeline, and Chapter 3 VIROME database for details).

The advantage of using Flex was that the compiled application is turned into an interactive Adobe Flash (http://www.adobe.com/products/flashruntimes.html) movie which can be deployed on all major browsers, desktops and even mobile device, and in each instance the look, feel, and behavior of the web application remains consistent. The only requirement to launch the VIROME web-application is Adobe Flash player, which according to Adobe's statistics about 99% of all desktop browsers have Adobe Flash player installed (https://www.adobe.com/products/flashruntimes/statistics.html). This provided a significant

[4] Adobe Flash player plugin must be installed (https://www.adobe.com/products/flashplayer.html)

advantage over other available technologies at the time. Flex allowed VIROME web-application development to focus on features and enhancements impacting the end-user experience directly versus ensuring web-application behavior was consistent across various browsers.

The VIROME web-application employs ColdFusion as its middleware to communicate between client (end-user) and data stored on the server. ColdFusion was chosen over other application servers such as JAVA or PHP for its efficiency in communicating with Flex applications using Action Message Format (AMF) when compared to traditional approach to communication such as web services or HTTP post. AMF is a binary format used to serialize object graphs such as ActionScript objects (an object-oriented programming language originally developed by Macromedia Inc.) and XML (eXtended Markup Language), or send messages between an Adobe Flash client and a remote service. AMF is optimized to keep the size of the AMF messages small compared to the Simple Object Access Protocol (SOAP)/XML used by web services. Figure 7 shows benchmark test using three different communication methods from a Flex application across various data types.



Primitive Value Test
Number of calls: 100
String argument: How fast can you run?
WebService: 100 — Total time: 1938 ms
HTTPService: 100 — Total time: 1815 ms
AMF: 100 — Total time: 825 ms

Complex Type Test
Number of calls: 100
WebService: 100 — Total time: 1915 ms
HTTPService: 100 — Total time: 1812 ms
AMF: 100 — Total time: 387 ms

Array of Complex Types Test
Number of calls: 5
Array size: 250
WebService: 5 — Total time: 1996 ms
HTTPService: 5 — Total time: 574 ms
AMF: 5 — Total time: 399 ms

Figure 7 Benchmark test of data communication time over three different protocol and various data types (http://www.themidnightcoders.com/products/weborb-for-net/developer-den/technical-articles/amf-vs-webservices.html)

MySQL database server is the de facto choice for backend database. MySQL is widely used, well-supported and open source.

The VIROME web-application followed a modular development practice, which loosely followed model-view-controller design fundamentals. The VIROME web-application is a combination of three components. Each component is modular and performs very specific roles. The visual element is the first component, a web-interface developed in Flex. The middle-ware which process all data and requests from user interface developed in

ColdFusion is the second; and the backend MySQL database which stores all the data is the third. All code for the user interface and middleware data processing is modular and follows object oriented programing practices. Figure 8, shows division of VIROME web-application and communication interface between each component.

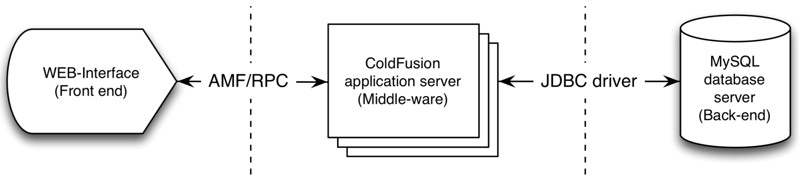


Figure 8 The components of VIROME web-application, and their method of communication

The web-interface is an event based, asynchronous system. The application listens and anticipates events; upon hearing or receiving such event appropriate actions are taken. The asynchronous nature of the web-interface allows the user to explore other aspects of the web-interface while appropriate actions are performed in the background. The visual components for user interaction, such a buttons, accordions, tabs, bar charts, pie charts, etc. are written in XML-based user interface markup language introduced by Macromedia known as MXML. Organization of data, communication between Flex and the ColdFusion application server using AMF or remote procedure call (RPC), and logic to determine a course of action based on an event are all written in ActionScript.

Visual components and ActionScript classes are modular, based on their specific roles; for example, all visual components related to the statistics view of the VIROME web interface and organized together under “stats” package/module. Similarly, all components related to the search view are organized under “search” package/module. All ActionScripts, which support or create custom visual components, are organized as "com.component" modules. In addition to these internal modules there are two external modules, user and bookmarks for the VIROME web-interface. The user module is responsible for all end-user actions such as login, registration, password retrieval and tracking user log-ins. The bookmark module is responsible for keeping track of all saved searches, recalling past bookmarks or creating new ones. Communication between the main VIROME web-interface and the User and Bookmark modules is handled via an Interface known as VIROMELib. The role of this interface is quite simple; VIROMELib interface passes messages and sets internal parameters of respective modules.

Middle-ware does the heavy lifting in the VIROME web-application. The middle-ware is responsible for interpreting requests from the user, gathering all the data from the database, organizing all the data as expected by the web-interface and returning the requested data. There are six major classes that support various aspects of the web-interface, one utility class and an application level class, which sets global parameters related to VIROME web-application. The six major classes are:

- Library
- Search
- Statistics
- ORF
- READ
- Export

Individual functions of each class, and detailed commands are available in an independent interactive web form application called VIROME ColdFusion Component

Documentation. Organization of the MySQL database can be found in Chapter 3: VIROME Database.

The VIROME web-interface was designed for seamless and intuitive exploration of data generated by the VIROME bioinformatics analysis pipeline. Data generated by the VIROME bioinformatics analysis pipeline can be explored at five different levels with the assistance of various views provided by the web-interface. Figure 9, shows the pyramid of data exploration from the highest level, Level 1: Home view to the most granular level individual ORF's BLAST results Level 5: Sequence detail view.

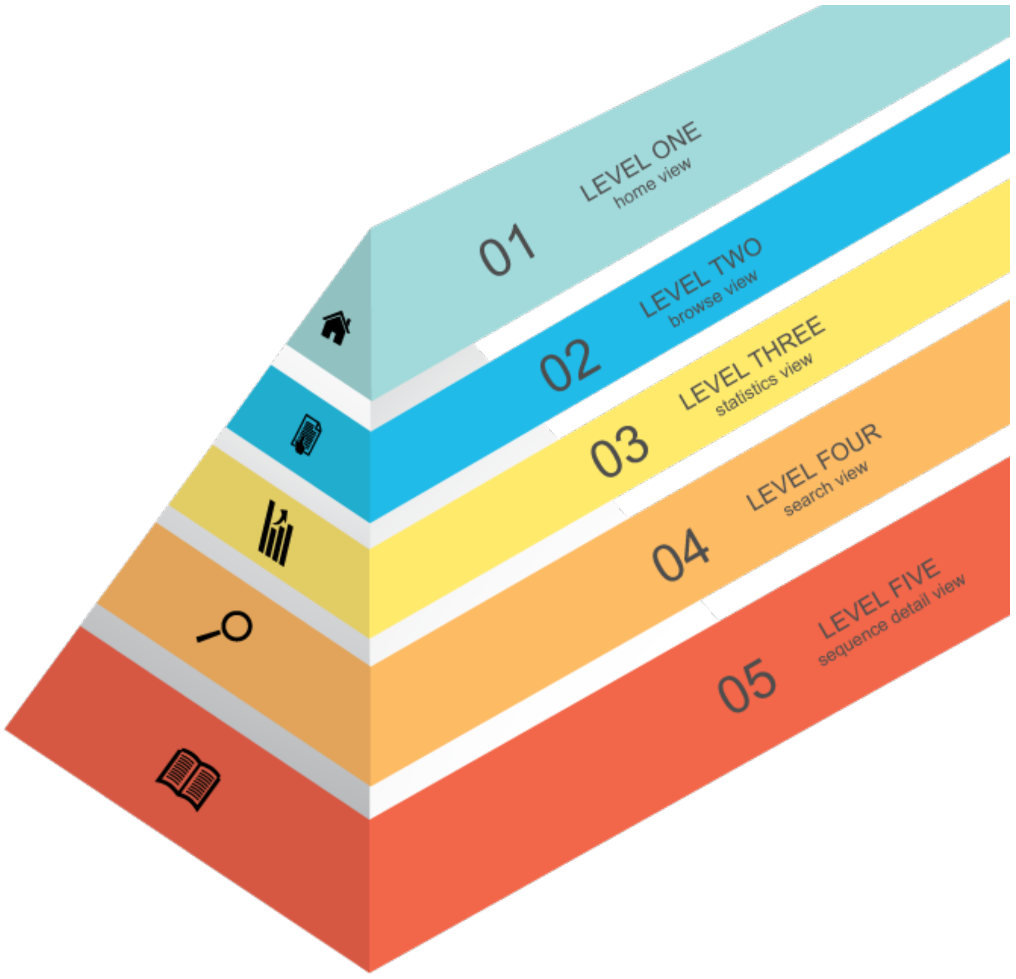


Figure 9 VIROME web-application data exploration pyramid. Graphic by freepik.com

Level 1:
Home View

The home view gives a bird's eye view of all the data currently stored in the VIROME database and available for exploration via the web-application. Data is presented in two ways, a summary table and an interactive bar chart. The summary table organizes data based on environmental classification it shows total number of libraries, total number of reads/contigs, megabases of reads/contigs, mean read/contig length, total number of ORFs, megabases of ORFs and mean ORF length. The interactive bar chart also shows the distribution of ORFs across each environment, but instead of displaying raw ORF counts, ORF counts are $\log_{10}$ normalized for ease of comparison across environments. Clicking on any one of the bars shows a deeper resolution of ORF counts across all VIROME ORF categories: tRNA, rRNA, possible functional protein, unassigned functional protein, top viral hit, top microbial hit, viral only hits, microbial only hits and ORFans. See Chapter 2: VIROME bioinformatics analysis pipeline for details on VIROME ORF categories.

Level 2:
Browse View

The browse view lists all libraries and three pie charts per library that shows the composition of a library based on biological and genetic data obtained through homology searches for each ORF. The three pie charts shown in the browse view are:

1. VIROME ORF categories (See Chapter 2: VIROME Bioinformatics analysis pipeline)
2. ORF status such as missing 3', missing 5', complete ORFs or missing both 3' and 5' ends
3. Functional taxonomy distribution at domain level.

The browse view is organized using two visual component tabs. The tabs divides libraries based on public or private domain and accordions within each tab organizes each library by environment. The following use case illustrates the importance of the browse view and how the browse view can be used as a visual comparison feature between two libraries of similar characteristics.

Use case one: Consider two libraries as shown in Figure 10. No background information is assumed except that the two libraries or part of same project. The first column of Figure 10 shows basic library statistics such as number of raw reads/contigs, megabases of reads/contigs, mega bases of ORFs, and library coding percentage

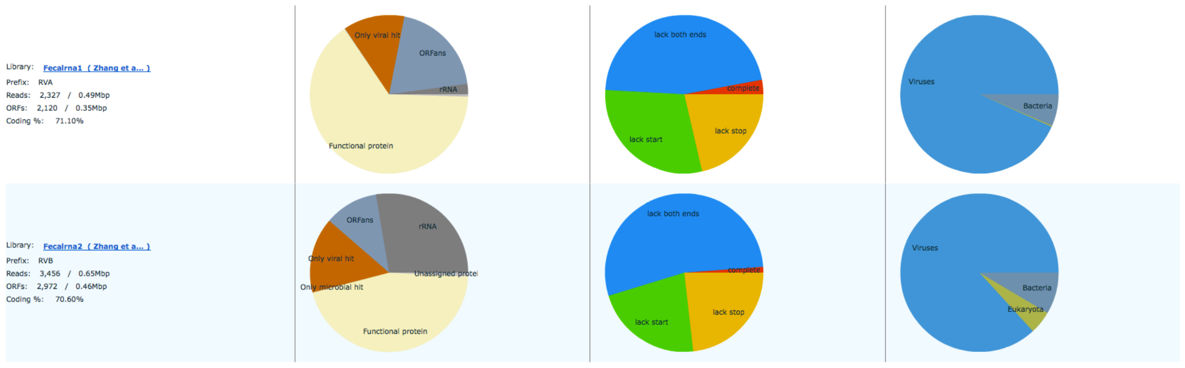
$$Library\ Coding\ Percentage = \frac{ORF\ megabases}{read/contig\ megabases} * 100$$

Low coding percentage could potentially indicate short read lengths, or a problem during sequencing/library preparation as the ORF predictor identified very few potential proteins in the library.

The second column shows the distribution of ORFs based on their biological classification from homology search. This pie chart can provide a wealth of information. For example a high percentage of ribosomal RNAs could point to potential contamination of cellular DNA within the viral metagenome library, while a high percentage of unassigned functional proteins warrants further investigation as an abundance of ORFs found homologs to only unclassified or hypothetical proteins.

The third column shows ORF distribution of a library based on completeness. Given the short read length of next-generation sequencing technologies many libraries show a low concentration of complete ORFs across all environments and library types. Fourth column shows the distribution of ORFs based on functional taxonomy at the domain level based on homology search.

Presentation of library data at Level 2 also facilitates a quick comparison between two libraries. The two libraries shown in Figure 10 RVA (top library) and RVB both have similar coding percentage and distribution of ORF completeness. However comparing VIROME ORF category distribution points to a potential contamination of the RVB library by cellular DNA as the ribosomal RNA pie slice is quite large.


Library: Fecalrna1 (Zhang et a...)
Prefix: RVA
Reads: 2,327 / 0.49Mbp
ORFs: 2,120 / 0.35Mbp
Coding %: 71.10%

Library: Fecalrna2 (Zhang et a...)
Prefix: RVB
Reads: 3,456 / 0.65Mbp
ORFs: 2,972 / 0.46Mbp
Coding %: 70.60%

Figure 10 Level 2: Browse view shows organization of ORF by biological and genetic data. Pie charts from left to right VIROME ORF categories, ORF type and Functional taxonomy at domain level

Level 3:
Statistics View

Similar to Level 2: Browse view, the statistics view also shows compositional features of a library using biological and genetic data obtained through homology search of each ORF. Unlike the browse view, the statistics view provides an in depth look at the distribution of ORFs within a library with the help of interactive bar charts and pie charts. The interactive charts provide a good way to explore hierarchical data such as functional taxonomy levels from domain level to species level or hierarchical structure of Kyoto Encyclopedia of Genes and Genomes pathway (KEGG). The data used to generate all charts in Statistics view and any other charts presented in VIROME web-application can be downloaded for further independent analysis outside of VIROME.

Use case two: Continuing with the same two libraries as presented in Use case one. From figure 10 we know that 1,407 ORFs, approximately 65% of ORFs for RVA library, have a homolog to the functional database UNIREF100P. Figure 11 of the statistics view shows a more detailed distribution of these 1,407 ORFs across various functional databases, and Figure 12 further explores the distribution of 1,390 ORF that have homologs to the Gene Ontology database. One could use this feature of the statistics view to bin ORFs, in this case get all ORFs that are classified as facilitators of biological process according to the Gene ontology database and download these ORFs for further independent analysis such as phylogenetic tree construction as demonstrated by Schmidt and colleagues (Schmidt, Sakowski et al. 2014). One could also use the functional taxonomy chart as show in Figure 13 to download all ORFs that had a homology to Tymoviridae family of functional taxonomy for fragment recruitment analysis as demonstrated by Douglas B. Rusch (Rusch, Halpern et al. 2007).

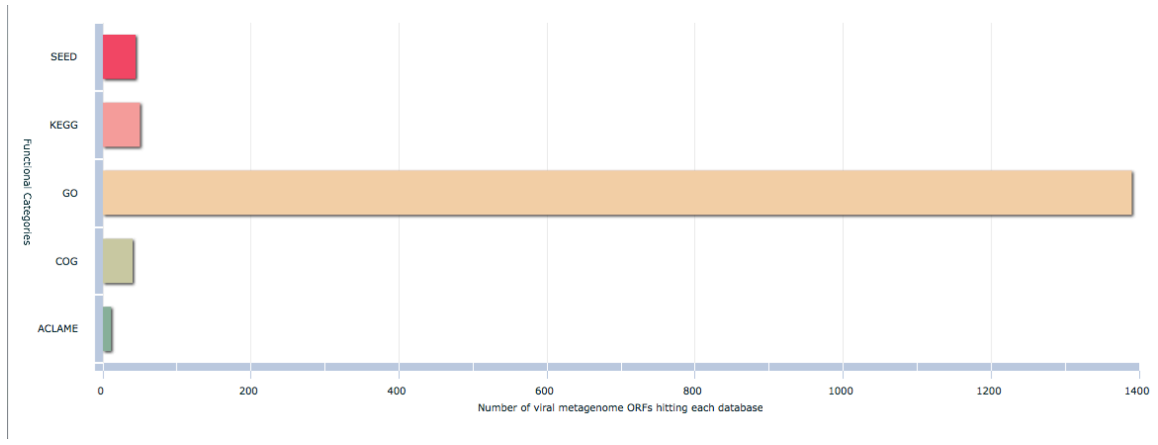


Figure 11 Distribution 1407 ORF classified as potential functional protein ORFs for RVA library

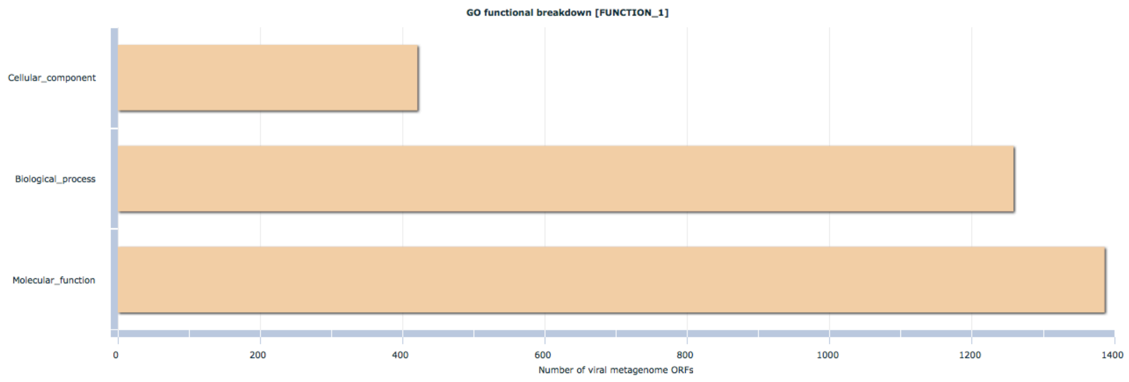


Figure 12 Function 1 distribution of 1390 ORF that were homologous to GO database for RVA library

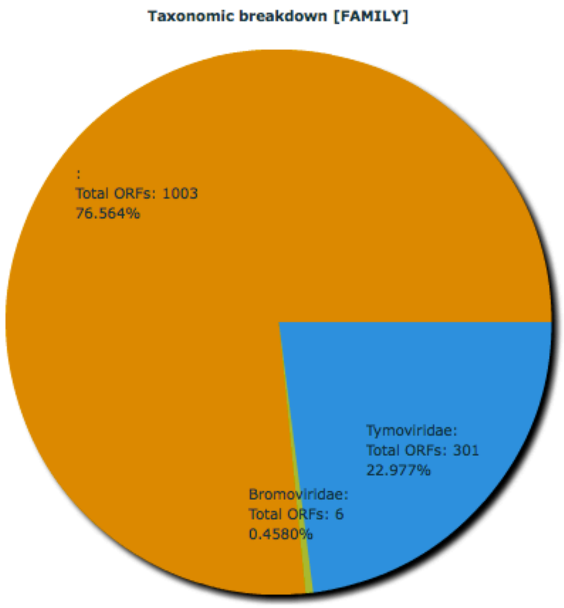
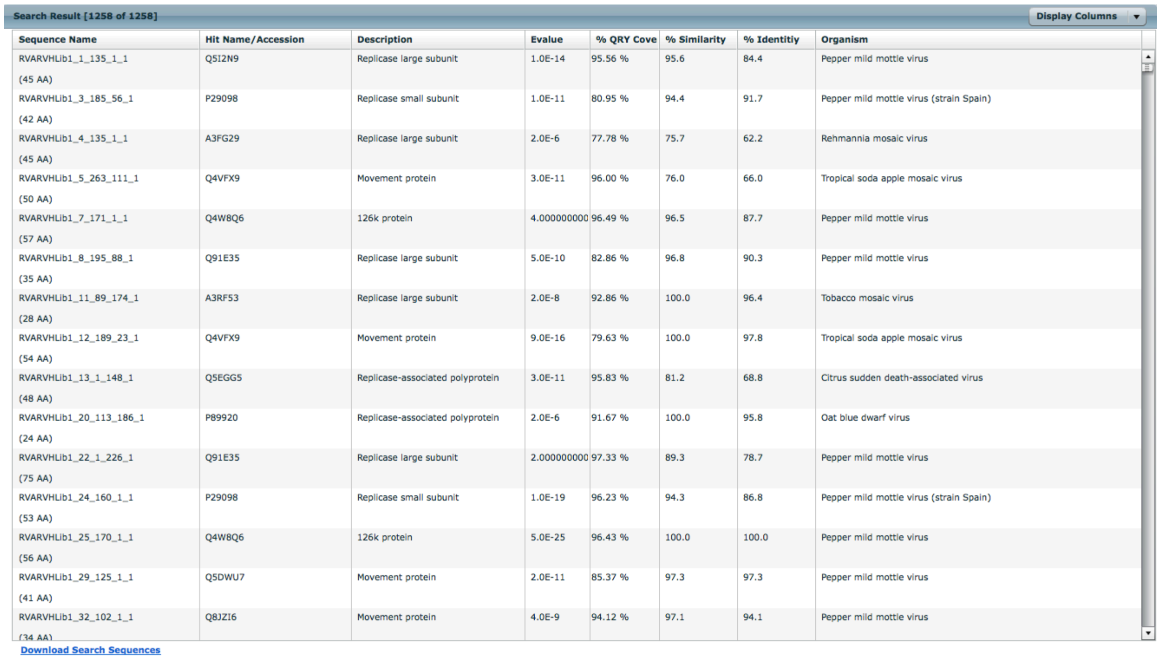


Figure 13 Family level view of RVA functional taxonomy statistics

Level 4:
Search View

The search view provides an ability to search the VIROME database across various biological and environmental criteria and list individual ORFs. It is the search view that facilitates downloads of ORFs that are binned according to various biological and environmental criteria for subsequent analysis outside of VIROME see Figure 14.

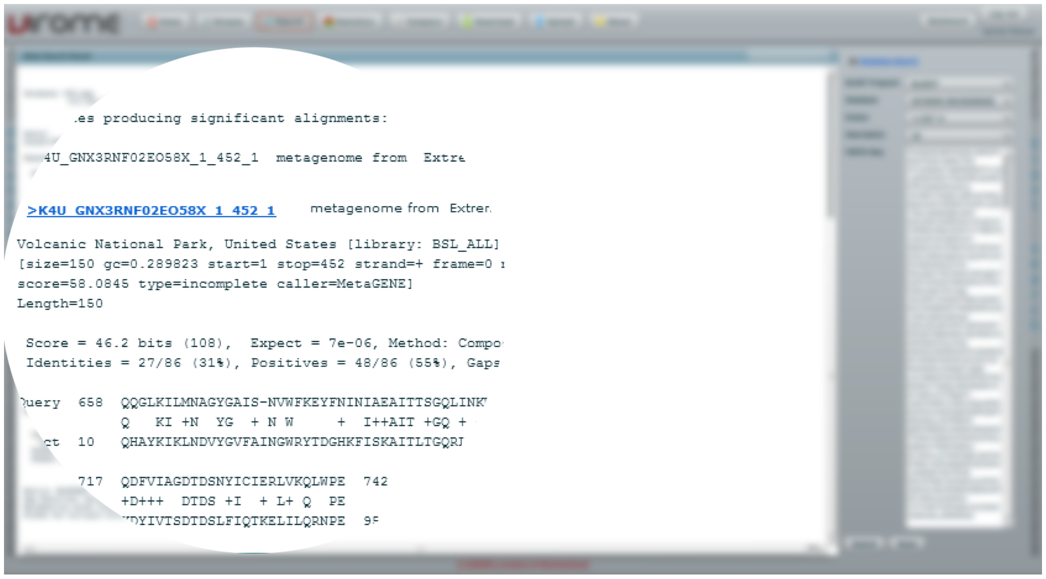
The search view not only allows a user to search the VIROME database, but also BLAST their target sequence against any combination of VIROME libraries, from within VIROME web-application. Should the query sequence find any homologs to sequences in the VIROME database the search view provides the ability to explore and get details about the subject sequence all from within the VIROME web-application. Figure 15 shows the BLAST function of the VIROME web-application. Due to budgetary constraints and resources currently only one query sequence can be submitted to BLAST against VIROME database. To BLAST multiple sequences users are encouraged to submit a request to virome@virome.dbi.udel.edu.



Search Result [1258 of 1258]

Sequence Name	Hit Name/Accession	Description	Evalue	% QRY Cove	% Similarity	% Identitiy	Organism
RVARVHLib1_1_135_1_1 (45 AA)	Q5I2N9	Replicase large subunit	1.0E-14	95.56 %	95.6	84.4	Pepper mild mottle virus
RVARVHLib1_3_185_56_1 (42 AA)	P29098	Replicase small subunit	1.0E-11	80.95 %	94.4	91.7	Pepper mild mottle virus (strain Spain)
RVARVHLib1_4_135_1_1 (45 AA)	A3FG29	Replicase large subunit	2.0E-6	77.78 %	75.7	62.2	Rehmannia mosaic virus
RVARVHLib1_5_263_111_1 (50 AA)	Q4VFX9	Movement protein	3.0E-11	96.00 %	76.0	66.0	Tropical soda apple mosaic virus
RVARVHLib1_7_171_1_1 (57 AA)	Q4W8Q6	126k protein	4.000000000	96.49 %	96.5	87.7	Pepper mild mottle virus
RVARVHLib1_8_195_88_1 (35 AA)	Q91E35	Replicase large subunit	5.0E-10	82.86 %	96.8	90.3	Pepper mild mottle virus
RVARVHLib1_11_89_174_1 (28 AA)	A3RF53	Replicase large subunit	2.0E-8	92.86 %	100.0	96.4	Tobacco mosaic virus
RVARVHLib1_12_189_23_1 (54 AA)	Q4VFX9	Movement protein	9.0E-16	79.63 %	100.0	97.8	Tropical soda apple mosaic virus
RVARVHLib1_13_1_148_1 (48 AA)	Q5EGG5	Replicase-associated polyprotein	3.0E-11	95.83 %	81.2	68.8	Citrus sudden death-associated virus
RVARVHLib1_20_113_186_1 (24 AA)	P89920	Replicase-associated polyprotein	2.0E-6	91.67 %	100.0	95.8	Oat blue dwarf virus
RVARVHLib1_22_1_226_1 (75 AA)	Q91E35	Replicase large subunit	2.000000000	97.33 %	89.3	78.7	Pepper mild mottle virus
RVARVHLib1_24_160_1_1 (53 AA)	P29098	Replicase small subunit	1.0E-19	96.23 %	94.3	86.8	Pepper mild mottle virus (strain Spain)
RVARVHLib1_25_170_1_1 (56 AA)	Q4W8Q6	126k protein	5.0E-25	96.43 %	100.0	100.0	Pepper mild mottle virus
RVARVHLib1_29_125_1_1 (41 AA)	Q5DWU7	Movement protein	2.0E-11	85.37 %	97.3	97.3	Pepper mild mottle virus
RVARVHLib1_32_102_1_1 (34 AA)	Q8JZI6	Movement protein	4.0E-9	94.12 %	97.1	94.1	Pepper mild mottle virus

Figure 14 List of all ORF that are part of Biological Process as annotated by Gene ontology database



```
.es producing significant alignments:

4U_GNX3RNF02EO58X_1_452_1  metagenome from  Extre

>K4U_GNX3RNF02EO58X_1_452_1    metagenome from  Extrer

Volcanic National Park, United States [library: BSL_ALL]
[size=150 gc=0.289823 start=1 stop=452 strand=+ frame=0
score=58.0845 type=incomplete caller=MetaGENE]
Length=150

 Score = 46.2 bits (108),  Expect = 7e-06, Method: Compo
 Identities = 27/86 (31%), Positives = 48/86 (55%), Gaps

uery  658  QQGLKILMNAGYGAIS-NVWFKEYFNINIAEAITTSGQLINK
           Q   KI +N  YG  + N W     +  I++AIT +GQ +
 ct   10   QHAYKIKLNDVYGVFAINGWRYTDGHKFISKAITLTGQRJ

      717  QDFVIAGDTDSNYICIERLVKQLWPE  742
           +D+++  DTDS +I  + L+ Q  PE
           DYIVTSDTDSLFIQTKELILQRNPE  9
```

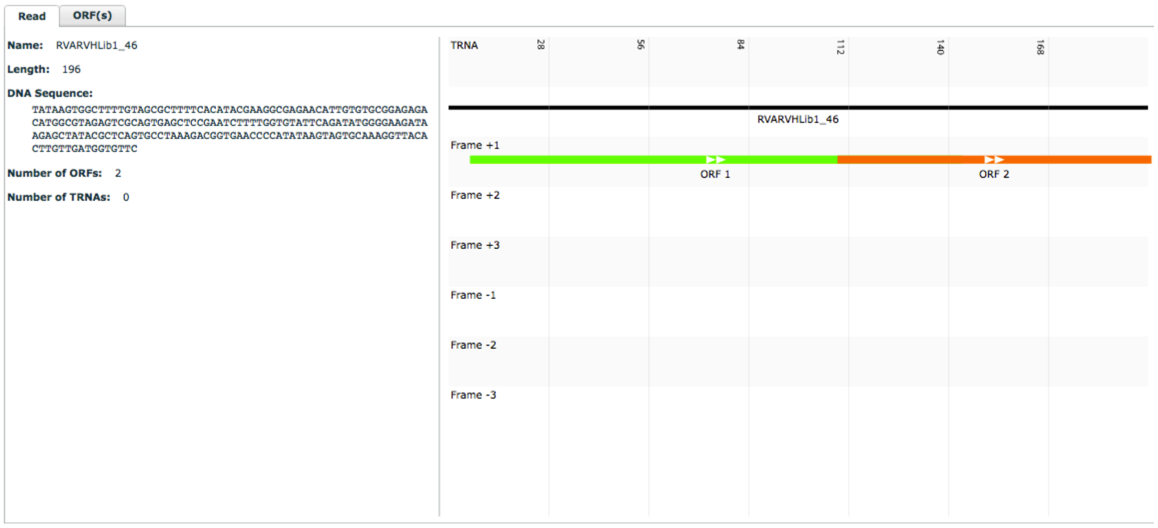
Figure 15 BLAST function and results with VIROME web-application

Level 5:
Sequence Detail View

The sequence detail view shows the finer details of each read/contig and ORF produced by the VIROME bioinformatics analysis pipeline. The sequence detail view is organized into two tabs, the read/contig tab and the ORF tab. The read tab contains raw read or contig information such as the number of ORFs, the number of transfer RNA(s), read length and the number of ORF(s) predicted. The read tab also includes an image that overlays all ORF(s) predicted along with location of transfer RNA as reported by tRNAScan-SE. The image shown in Figure 16 illustrates the distribution and coverage of ORFs across a read/contig and if the read contains any transfer RNA sequences. The ORFs in the image are color coded to indicate the completeness of the ORF:

- Green: An ORF with start and stop codon
- Orange: An ORF missing stop codon
- Blue: An ORF missing start codon
- Pink: An ORF missing both start and stop codons

The arrow on each ORF indicates direction of the ORF. Along with the image, the read tab also presents table of top BLAST hits against functional database UNIREF100P, the functional taxonomy lineage of ORFs that had homologs to the functional database, top BLAST hit against environmental database MgOl and an environmental summary of each ORF that had homolog to the environmental database. The information displayed in these tables can help infer functional taxonomy lineage, and environmental summary of a read/contig.


Read ORF(s)

Name: RVARVHLib1_46

Length: 196

DNA Sequence:

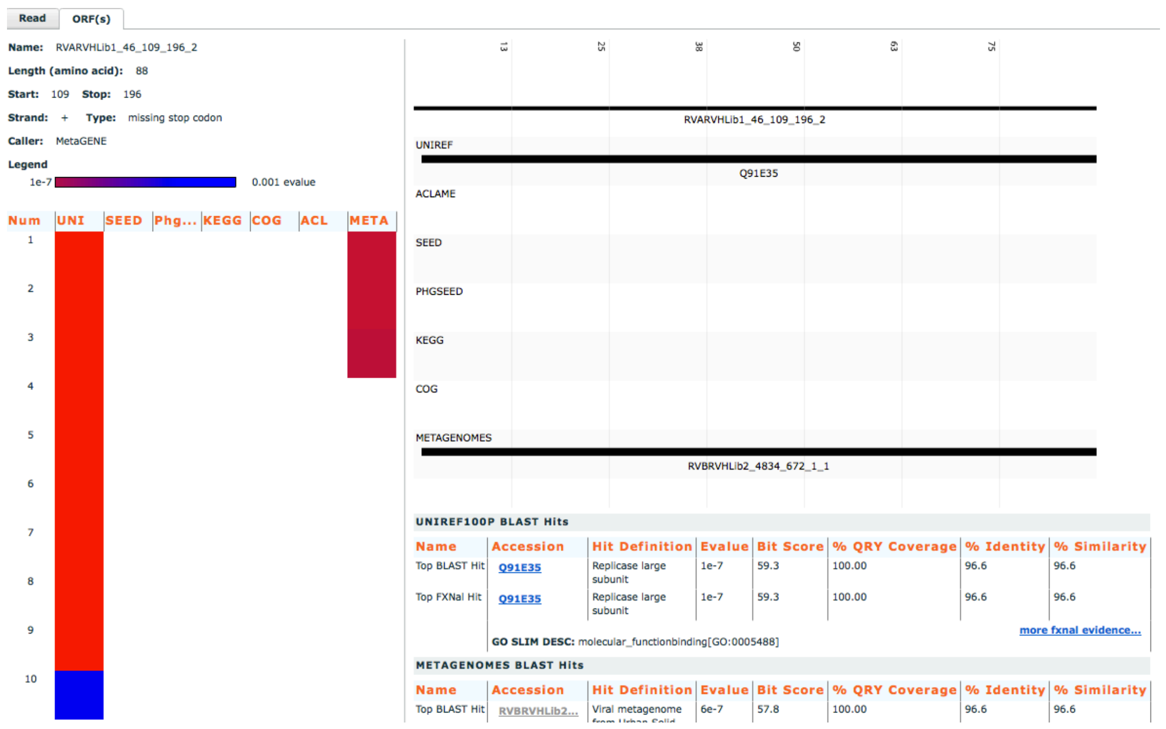
```
TATAAGTGGCTTTTGTAGCGCTTTTCACATACGAAGGCGAGAACATTGTGTGCGGAGAGA
CATGGCGTAGAGTCGCAGTGAGCTCCGAATCTTTTGGTGTATTCAGATATGGGGAAGATA
AGAGCTATACGCTCAGTGCCTAAAGACGGTGAACCCCATATAAGTAGTGCAAAGGTTACA
CTTGTTGATGGTGTTC
```

Number of ORFs: 2

Number of TRNAs: 0

Figure 16 Read tab of sequence detail view. Raw read information on the left, and distribution of ORFs across a read/contig

Similar to the read tab, the ORF tab shows an image of top BLAST hits across all functional databases and environmental database. The BLAST imager is a great way to quickly visualize the coverage and quality of the BLAST homologs. The ORF tab also shows a heat map of e-value of the top ten BLAST hits across all functional databases and the environmental database. Subtle change in the hit map across databases indicates strong homology against subject database as opposed to an abrupt change as shown in Figure 17. Selecting any square of a heat map will show a detailed tab delimited BLAST report of the top 50 hits as reported by the VIROME bioinformatics analysis pipeline.



Name: RVARVHLib1_46_109_196_2
Length (amino acid): 88
Start: 109 **Stop:** 196
Strand: + **Type:** missing stop codon
Caller: MetaGENE

UNIREF100P BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	Q91E35	Replicase large subunit	1e-7	59.3	100.00	96.6	96.6
Top FXNal Hit	Q91E35	Replicase large subunit	1e-7	59.3	100.00	96.6	96.6

more fxnal evidence...

GO SLIM DESC: molecular_functionbinding[GO:0005488]

METAGENOMES BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	RVBRVHLib2...	Viral metagenome	6e-7	57.8	100.00	96.6	96.6

Figure 17 ORF tab of sequence detail view

Comparinator

With advances in sequencing technology, and varying nature of the sample to sequence pipeline for each library is it difficult to predict all type of analysis and summarization needs users may desire. For this reason VIROME emphasizes the ability to put data in the hands of the user in a format that can easily be transferred to other analysis platforms. Aside from the data exploration levels, the VIROME web-application also facilitates comparative analysis across multiple libraries using Quantitative Insights Into Microbial Ecology (QIIME) package (Caporaso, Kuczynski et al. 2010). QIIME is an open source software package for comparison and analysis of microbial communities, primarily based on high-throughput amplicon sequencing data generated on a variety of platforms, but also supports analysis of other types of data such as shotgun metagenomic data. Data required for comparative analysis using QIIME package can be downloaded from the VIROME web-interface using a compare feature known as the Comparinator. The Comparinator allows an end-user to download biological observation matrix format files (McDonald, Clemente et al. 2012) for two or more libraries which can be directly plugged into various QIIME scripts. The Comparinator currently supports the following metrics for library comparison:

1. Functional taxonomy
2. A classification of mobile genetic elements (ACLAME) hierarchy
3. Clusters of Orthologous Groups (COG) hierarchy
4. Kyoto Encyclopedia of Genes and Genomes (KEGG) hierarchy
5. SEED hierarchy

Flex and ColdFusion code related to VIROME web-application are available in a private Github repository URL: https://github.com/bjaysheel/virome_app. Now that Apache Flex support for PHP application server is stable and has matured to support large data driven application such as VIROME web-application, efforts are under way to convert all ColdFusion classes in to PHP classes and modules. Moving to PHP as a middleware application server will remove dependency on proprietary software making all components of VIROME web-application open source. This will permit us to distribute VIROME web-application as a virtual machine or as open-source package that can be used as private mirrored application.

Chapter 5

VIROME SUBMISSION PORTAL

The VIROME submission portal allows users to submit their libraries for analysis via the VIROME bioinformatics analysis pipeline and subsequently explore analyzed data using the VIROME web-application. To ensure that data submitted is consistent across all libraries, and that the VIROME data is in accordance with minimum information about any sequence (MIxS) standard (Yilmaz, Kottmann et al. 2011) the VIROME data submission portal requires that all fields be completed for a successful library submission. By enforcing strict policy for collecting both primitive and derived metadata, the VIROME application provides a more complete disclosure of metadata that can be pertinent to comparative analyses between environmental metagenomes. This is especially important when considering that over 70% viral metagenome ORFs find homologs to only peptides within the environmental database.

The VIROME submission portal was developed using HTML5 and PHP. The submission portal interacts with ColdFusion server and leverages methods written for the VIROME web-application to allow end users to add, update and delete their libraries, see Figure 18. Each library goes through three stages when submitted to the VIROME submission portal:

1. Stand by: Library has been submitted once the data has been manually curated it will be queued for processing via the VIROME bioinformatics analysis pipeline
2. Processing: Library is being analyzed via VIROME bioinformatics analysis pipeline

3. Complete: Library has been analyzed via VIROME bioinformatics analysis pipeline, and is ready for data exploration via VIROME web-application.

Manual intervention is required as a library progresses through each stage, to ensure data integrity and the quality of data. The VIROME submission portal URL: http://virome.dbi.udel.edu/submission/index.php, shares a single login between VIROME web application and VIROME submission portal.

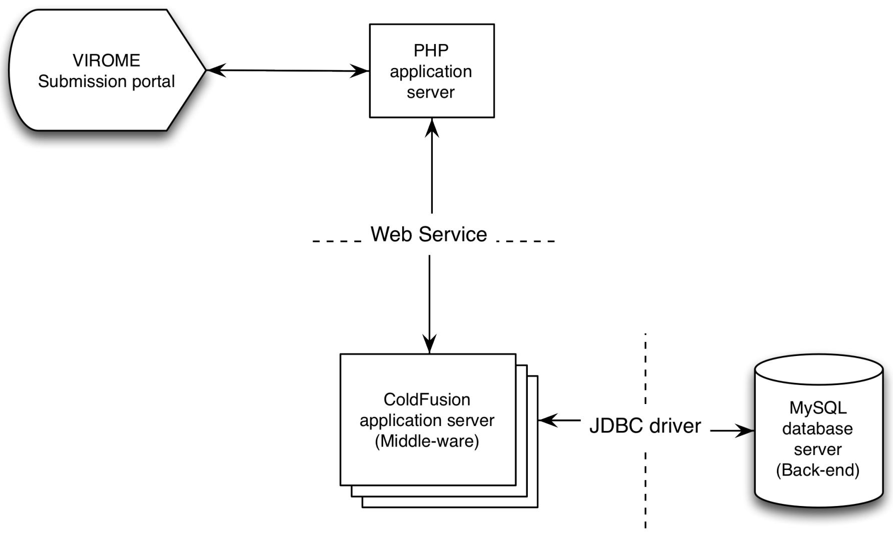

VIROME Submission portal
PHP application server
Web Service
ColdFusion application server (Middle-ware)
JDBC driver
MySQL database server (Back-end)

Figure 18 VIROME submission portal and its interaction with existing ColdFusion classes, and database

Chapter 6

SEQUENCE DETAIL THEN AND NOW: A CASE STUDY

The Viral Informatics Resource for Metagenome Exploration (VIROME) is a web-based application specializing in analysis of viral metagenomes. Among the various modules that make up the VIROME web application, the sequence detail is an important module as it allows the user to dig deep in to granular details of each ORF. At any given point a user is only one, at the most two clicks away from viewing: the distribution of ORF across an entire read/contig or whole genome; a heat map of e-value distribution across all databases; a detailed blast table highlighting the top functional hits (if any) and the top blast hits based on best e-value; and a quick view of top blast hits across all databases along with its functional lineage (if any). These views also allow a user to download data in various formats at the level of individual ORF, read/contig or whole genome level.

At the inception of VIROME, next-generation sequencing technology was in its infancy. Next-generation sequencing technologies such as 454-pyrosequencing dramatically reduced the cost of sequencing but the quality of information gathered, compared to Sanger sequencing, was less appropriate for phylogenetic and functional characterization of metagenomes (Wommack, Bhavsar et al. 2008). Therefore a conscious decision was made to design the sequence detail modules based on ORFs called from Sanger reads of approximate length of 750 bases.

The average number of ORF (> 50 bases) called from Sanger environmental metagenome libraries were ~2 and maximum of 5. Based on this finding a tabbed user interface was chosen see Figure 19 and 20. This interface quickly allowed a user to see a number of surrounding ORFs, navigate to any one of the neighboring ORFs with a single click, and give access to read level information with a single click.

At the read level, a static image (designed similar to the BLAST imager view for NCBI-BLAST) provided a view of the distribution of ORFs across an entire read, as well as the position of tRNA(s) if present. Top BLAST hits based on e-value across all ORFs per database was displayed in a static table, which gave at quick glance a look at type and quality of BLAST hits. A functional taxonomy table across all ORFs provided a naive consensus for read, at the same time showing taxonomic similarities or differences in each ORFs. An entire read in nucleotide bases was displayed for convenience to copy and paste for further analysis.

At the ORF level, a static blast imager showed top BLAST hits across all databases. Top BLAST hits based on e-value across all databases along with functional lineage provides some indication of the possible taxonomic origin of the sequence. A heat map of the top ten blast hits across all databases shows at a glance similarities between BLAST hits, and how quickly quality of hits changes. Upon clicking on a specific region of the heat map a detailed BLAST report is presented where the top functional and top blast hits are highlighted along with functional taxonomy or environmental metadata. Reads in nucleotide format with the ORF sequence highlighted is displayed to give a visual representation of how an ORF spans over a given read, see Figure 19 and 20.

With the advancement in sequencing and improved assembly algorithms it is now possible to get better quality information from next-generation sequences. When displaying information from long read contigs from assembled metagenome or viral whole genomes the tabbed view became cumbersome to the point of unusable, see Figure 21 and 22. Labels on each of the tabs were unreadable, navigation to a desired ORFs was difficult involving, multiple scroll bars which made viewing the relevant information very difficult and confusing. It became apparent that the user interface for the sequence detail view needed to be overhauled to account for long read data.

Thus the vision for version 2.0 was to present the same information in a more efficient manner, with interactive images and tables to allow a user to download information right from the sequence detail view.

Instead of multi tab view, there is now only one tab per feature (currently only the read and ORF tab). The static fixed length BLAST image was replaced by a dynamic, interactive, native BLAST imager, which scales based on read length that allowed for an even spaced ORF overlay. Dynamic tables that can be sorted by any given column replaced static simple tables. The entire content of the tables can now be downloaded in a tab-delimited file, for further analysis. Instead of displaying all bases of read sequences only the first few are displayed, and a mechanism is in place to display and download the entire read sequence see Figure 23 and 24.

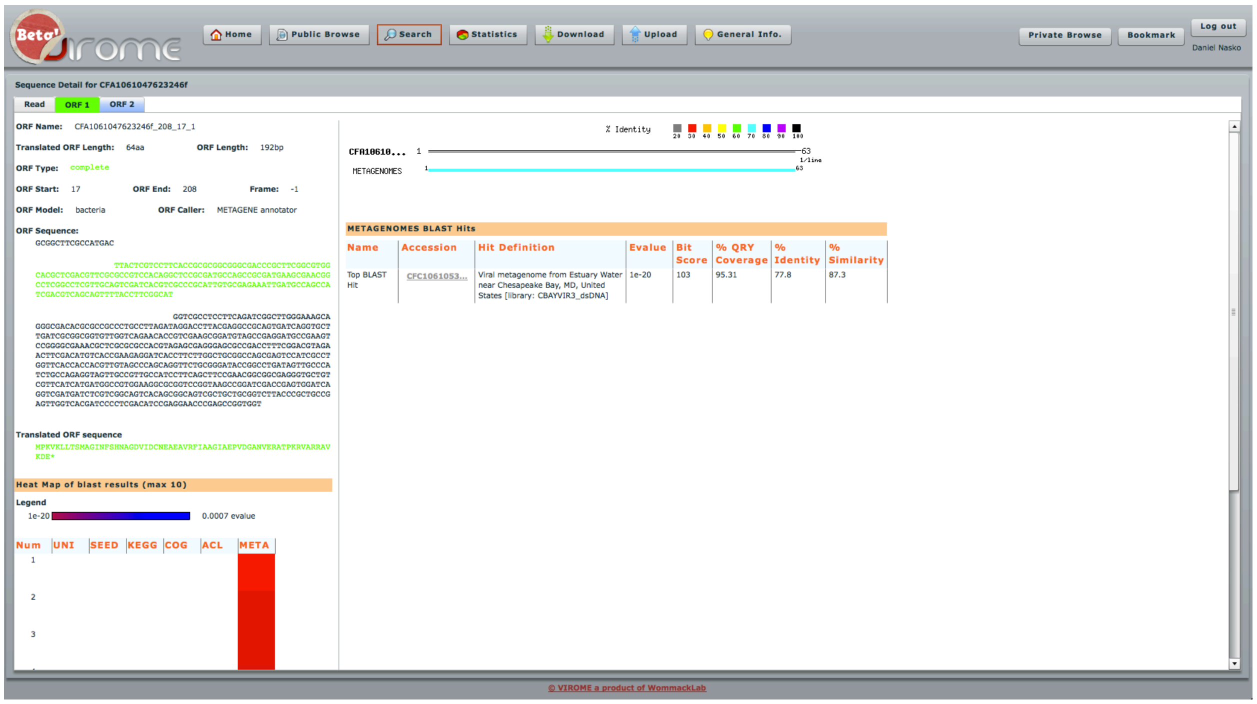
There is now a single tab for ORF. Each ORF is displayed in a sliding window format. Navigation to an ORF is possible via "Next" and "Previous" buttons or by simply entering desired ORF number in the field provided. This eliminates the clutter of tabs as occurred in the previous version, while at the same time keeping navigation to neighboring ORFs simple and quick. A dynamic, high quality, native BLAST imager replaced the static BLAST imager from Figure 19 and 20. With the inclusion of a dynamic, high quality BLAST imager in the read tab, which provides a clear view of the ORFs distribution across the read, the long string of nucleotide read with an ORF overlay has been omitted from this version. The heat map of the top 10 BLAST hits across all databases, and view of top BLAST hits per database along with functional or environmental metadata lineage remains unchanged from the previous version, see Figure 25.

In conclusion with the overhaul of sequence detail view; information presented is more clear, concentrated, and easily accessible. The new sequence detail view provides multiple ways to navigate to a desired ORF, sort data in any way desired, make use of entire viewable screen, reduce the amount of scrolling, all this while still providing same high quality information. This version also provides a mechanism to download and bin data at an individual read level across all BLAST databases, taxonomy and environmental metadata for further analysis. Below is a table comparing two versions of the sequence detail view.

Table 4 Feature comparison between sequence detail view version 1 and version 2.

Features / Version	v1.0	v2.0
Interactive high quality image	No	Yes
Interactive tables	No	Yes
Download data	No	Yes
Quickly identify ORF type	Yes (via tab color)	No (available via text only)
Easy of navigation	Yes (limited number of ORFs)	Yes
Scalability	No	Yes

There are few features that are missing from new version, most notable being the colored ORF tab based on ORF type (complete, missing stop codon, missing start codon or missing start and stop codons) and ORF amino acid sequence string from ORF view. Both of which were determined to be a low priority feature, and will be added in subsequent version release of VIROME web application.



Sequence Detail for CFA1061047623246f

ORF Name: CFA1061047623246f_208_17_1

Translated ORF Length: 64aa   ORF Length: 192bp

ORF Type: complete

ORF Start: 17   ORF End: 208   Frame: -1

ORF Model: bacteria   ORF Caller: METAGENE annotator

ORF Sequence:

```
GCGGCTTCGCCATGAC

                    TTACTCGTCCTTCACCGCGCGGCGGGCGACCCGCTTCGGCGTGG
CACGCTCGACGTTCGCGCCGTCCACAGGCTCCGCGATGCCAGCCGCGATGAAGCGAACGG
CCTCGGCCTCGTTGCAGTCGATCACGTCGCCCGCATTGTGCGAGAAATTGATGCCAGCCA
TCGACGTCAGCAGTTTTACCTTCGGCAT

                                   GGTCGCCTCCTTCAGATCGGCTTGGGAAAGCA
GGGCGACACGCGCCGCCCTGCCTTAGATAGGACCTTACGAGGCCGCAGTGATCAGGTGCT
TGATCGCGGCGGTGTTGGTCAGAACACCGTCGAAGCGGATGTAGCCGAGGATGCCGAAGT
CCGGGGCGAAACGCTCGCGCGCCACGTAGAGCGAGGGAGCGCCGACCTTTCGGACGTAGA
ACTTCGACATGTCACCGAAGAGGATCACCTTCTTGGCTGCGGCCAGCGAGTCCATCGCCT
GGTTCACCACCACGTTGTAGCCCAGCAGGTTCTGCGGGATACCGGCCTGATAGTTGCCCA
TCTGCCAGAGGTAGTTGCCGTTGCCATCCTTCAGCTTCCGAACGGCGGCGAGGGTGCTGT
CGTTCATCATGATGGCCGTGGAAGGCGCGGTCCGGTAAGCCGGATCGACCGAGTGGATCA
GGTCGATGATCTCGTCGGCAGTCACAGCGGCAGTCGCTGCTGCGGTCTTACCCGCTGCCG
AGTTGGTCACGATCCCCTCGACATCCGAGGAACCCGAGCCGGTGGT
```

Translated ORF sequence

```
MPKVKLLTSMAGINFSHNAGDVIDCNEAEAVRFIAAGIAEPVDGANVERATPKRVARRAV
KDE*
```

Heat Map of blast results (max 10)

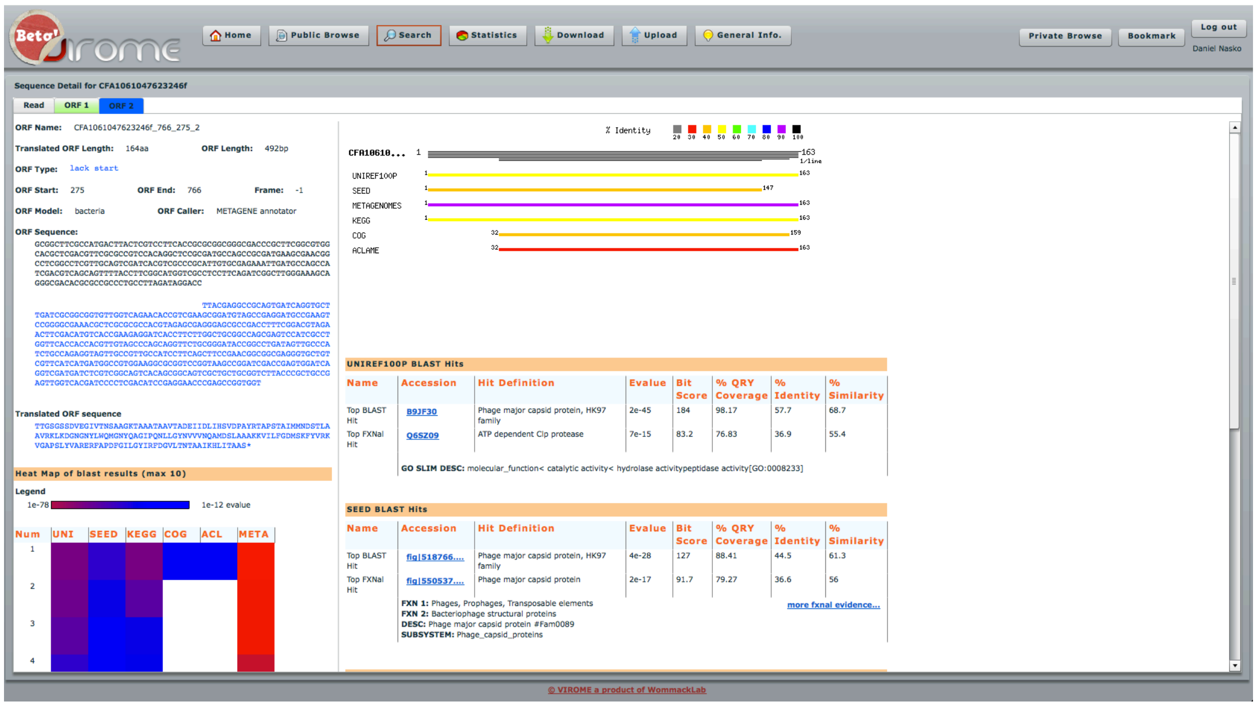
Legend

1e-20 0.0007 evalue

METAGENOMES BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	CFC1061053...	Viral metagenome from Estuary Water near Chesapeake Bay, MD, United States [library: CBAYVIR3_dsDNA]	1e-20	103	95.31	77.8	87.3

Figure 19 Sequence detail view v1.0 ORF view. A tab for each ORF, plus a read tab, ORF details on top left, a heat map across all database on bottom left, static blast imager on top right. Detail top blast hits per database bottom right.



Sequence Detail for CFA1061047623246f

ORF Name: CFA1061047623246f_766_275_2

Translated ORF Length: 164aa **ORF Length:** 492bp

ORF Type: lack start

ORF Start: 275 **ORF End:** 766 **Frame:** -1

ORF Model: bacteria **ORF Caller:** METAGENE annotator

ORF Sequence:

```
GCGGCTTCGCCATGACTTACTCGTCCTTCACCGCGCGGCGGGCGACCCGCTTCGGCGTGG
CACGCTCGACGTTCGCGCCGTCCACAGGCTCCGCGATGCCAGCCGCGATGAAGCGAACGG
CCTCGGCCTCGTTGCAGTCGATCACGTCGCCCGCATTGTGCGAGAAATTGATGCCAGCCA
TCGACGTCAGCAGTTTTACCTTCGGCATGGTCGCCTCCTTCAGATCGGCTTGGGAAAGCA
GGGCGACACGCGCCGCCCTGCCTTAGATAGGACC

                                    TTACGAGGCCGCAGTGATCAGGTGCT
TGATCGCGGCGGTGTTGGTCAGAACACCGTCGAAGCGGATGTAGCCGAGGATGCCGAAGT
CCGGGGCGAAACGCTCGCGCCACGTAGAGCGAGGGAGCGCCGACCTTTCGGACGTAGA
ACTTCGACATGTCACCGAAGAGGATCACCTTCTTGGCTGCGGCCAGCGAGTCCATCGCCT
GGTTCACCACCACGTTGTAGCCCAGCAGGTTCTGCGGGATACCGGCCTGATAGTTGCCCA
TCTGCCAGAGGTAGTTGCCGTTGCCATCCTTCAGCTTCCGAACGGCGGCGAGGGTGCTGT
CGTTCATCATGATGGCCGTGGAAGGCGCGGTCCGGTAAGCCGGATCGACCGAGTGGATCA
GGTCGATGATCTCGTCGGCAGTCACAGCGGCAGTCGCTGCTGCGGTCTTACCCGCTGCCG
AGTTGGTCACGATCCCCTCGACATCCGAGGAACCCGAGCCGGTGGT
```

Translated ORF sequence

```
TTGSGSSDVEGIVTNSAAGKTAAATAAVTADEIIDLIHSVDPAYRTAPSTAIMMNDSTLA
AVRKLKDGNGNYLWQMGNYQAGIPQNLLGYNVVVNQAMDSLAAAKKVILFGDMSKFYVRK
VGAPSLYVARERFAPDFGILGYIRFDGVLTNTAAIKHLITAAS*
```

Heat Map of blast results (max 10)

Num	UNI	SEED	KEGG	COG	ACL	META
1						
2						
3						
4						

UNIREF100P BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	B9JF30	Phage major capsid protein, HK97 family	2e-45	184	98.17	57.7	68.7
Top FXNal Hit	Q6SZ09	ATP dependent Clp protease	7e-15	83.2	76.83	36.9	55.4

GO SLIM DESC: molecular_function< catalytic activity< hydrolase activitypeptidase activity[GO:0008233]

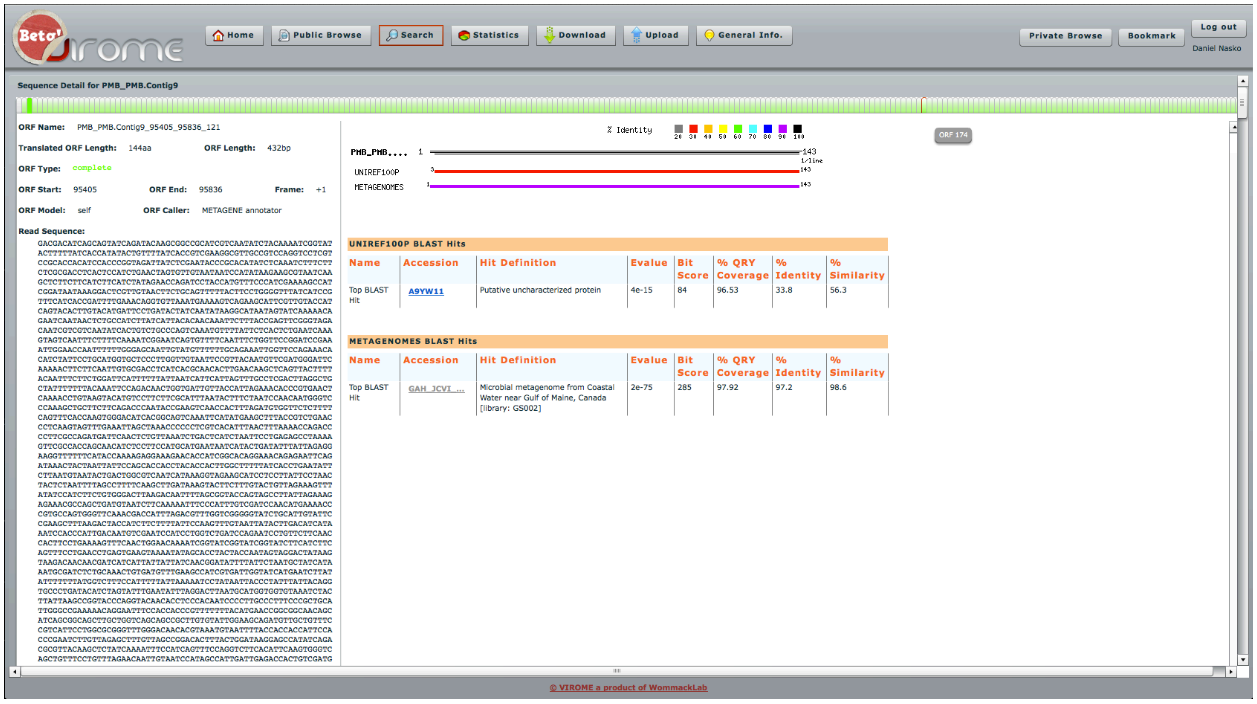
SEED BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	fig|518766....	Phage major capsid protein, HK97 family	4e-28	127	88.41	44.5	61.3
Top FXNal Hit	fig|550537....	Phage major capsid protein	2e-17	91.7	79.27	36.6	56

FXN 1: Phages, Prophages, Transposable elements
FXN 2: Bacteriophage structural proteins
DESC: Phage major capsid protein #Fam0089
SUBSYSTEM: Phage_capsid_proteins

more fxnal evidence...

Figure 20 Sequence detail view v1.0



Sequence Detail for PMB_PMB.Contig9

ORF Name:	PMB_PMB.Contig9_95405_95836_121				
Translated ORF Length:	144aa	ORF Length:	432bp		
ORF Type:	complete				
ORF Start:	95405	ORF End:	95836	Frame:	+1
ORF Model:	self	ORF Caller:	METAGENE annotator		

Read Sequence:

```
GACGACATCAGCAGTATCAGATACAAGCGGCCGCATCGTCAATATCTACAAAATCGGTAT
ACTTTTTATCACCATATACTGTTTTATCACCGTCGAAGGCGTTGCCGTCCAGGTCCTCGT
CCGCACCACATCCACCCGGTAGATTATCTCGAATACCCGCACATATCTCAAATCTTTCTT
CTCGCGACCTCACTCCATCTGAACTAGTGTTGTAATAATCCATATAAGAAGCGTAATCAA
GCTCTTCTTCATCTTCATCTATAGAACCAGATCCTACCATGTTTCCCATCGAAAAGCCAT
CGGATAATAAAGGACTCGTTGTAACTTCTGCAGTTTTTACTTCCTGGGGTTTATCATCCG
TTTCATCACCGATTTTGAAACAGGTGTTAAATGAAAAGTCAGAAGCATTCGTTGTACCAT
CAGTACACTTGTACATGATTCCTGATACTATCAATATAAGGCATAATAGTATCAAAAACA
GAATCAATAACTCTGCCATCTTATCATTACACAACAAATTCTTTACCGAGTTCGGGTAGA
CAATCGTCGTCAATATCACTGTCTGCCCAGTCAAATGTTTTATTCTCACTCTGAATCAAA
GTAGTCAATTTCTTTTCAAAATCGGAATCAGTGTTTTCAATTTCTGGTTCCGGATCCGAA
ATTGGAACCAATTTTTTGGGAGCAATTGTATGTTTTTTGCAGAAATTGGTTCCAGAAACA
CATCTATTCCTGCATGGTGCTCCCTTGGTTGTAATTCCGTTACAATGTTCGATGGGATTC
AAAAACTTCTTCAATTGTGCGACCTCATCACGCAACACTTGAACAAGCTCAGTTACTTTT
ACAATTTCTTCTGGATTCATTTTTTATTAATCATTCATTAGTTTGCCTCGACTTAGGCTG
CTATTTTTTTACAAATTCCAGACAACTGGTGATTGTTACCATTAGAAACACCCGTGAACT
CAAAACCTGTAAGTACATGTCCTTCTTCGCATTTAATACTTTCTAATCCAACAATGGGTC
CCAAAGCTGCTTCTTCAGACCCAATACCGAAGTCAACCACTTTAGATGTGGTTCTCTTTT
CAGTTTCACCAAGTGGGACATCACGGCAGTCAAATTCATATGAAGCTTTACCGTCTGAAC
CCTCAAGTAGTTTGAAATTAGCTAAACCCCCCTCGTCACATTTAACTTTAAAACCAGACC
CCTTCGCCAGATGATTCAACTCTGTTAAATCTGACTCATCTAATTCCTGAGAGCCTAAAA
GTTCGCCACCAGCAACATCTCCTTCCATGCATGAATAATCATACTGATATTTATTAGAGG
AAGGTTTTTCATACCAAAAGAGGAAAGAACACCATCGGCACAGGAAACAGAGAATTCAG
ATAAACTACTAATTATTCCAGCACCACCTACACCACTTGGCTTTTTATCACCTGAATATT
CTTAATGTAATACTGACTGGCGTCAATCATAAAGGTAGAAGCATCCTCCTTATTCCTAAC
TACTCTAATTTTAGCCTTTTCAAGCTTGATAAAGTACTTCTTTGTACTGTTAGAAAGTTT
ATATCCATCTTCTGTGGGACTTAAGACAATTTTAGCGGTACCAGTAGCCTTATTAGAAAG
AGAAACGCCAGCTGATGTAATCTTCAAAAATTTCCCATTTGTCGATCCAACATGAAAACC
CGTGCCAGTGGGTTCAAACGACCATTTAGACGTTTGGTCGGGGGTATCTGCATTGTATTC
CGAAGCTTTAAGACTACCATCTTCTTTTATTCCAAGTTTGTAATTATACTTGACATCATA
AATCCACCCATTGACAATGTCGAATCCATCCTGGTCTGATCCAGAATCCTGTTCTTCAAC
CACTTCCTGAAAAGTTTCAACTGGAACAAAATCGGTATCGGTATCGGTATCTTCATCTTC
AGTTTCCTGAACCTGAGTGAAGTAAAATATAGCACCTACTACCAATAGTAGGACTATAAG
TAAGACAACAACGATCATCATTATTATTATCAACGGATATTTTATTCTAATGCTATCATA
AATGCGATCTCTGCAAACTGTGATGTTTGAAGCCATCGTGATTGGTATCATGAATCTTAT
ATTTTTTTATGGTCTTTCCATTTTTATTAAAAATCCTATAATTACCCTATTTATTACAGG
TGCCCTGATACATCTAGTATTTGAATATTTAGGACTTAATGCATGGTGGTGTAAATCTAC
TTATTAAGCCGGTACCCAGGTACAACACCTCCCACAATCCCCTTGCCCTTTCCCGCTGCA
TTGGGCCGAAAAACAGGAATTTCCACCACCCGTTTTTTACATGAACCGGCGGCAACAGC
ATCAGCGGCAGCTTGCTGGTCAGCAGCCGCTTGTGTATTGGAAGCAGATGTTGCTGTTTC
CGTCATTCCTGGCGCGGGTTTGGGACAACACGTAAATGTAATTTTACCACCACCATTCCA
CCCGAATCTTGTTAGAGCTTTGTTAGCCGGACACTTTACTGGATAAGGAGCCATATCAGA
CGCGTTACAAGCTCTATCAAAATTTCCATCAGTTTCCAGGTCTTCACATTCAAGTGGGTC
AGCTGTTTCCTGTTTAGAACAATTGTAATCCATAGCCATTGATTGAGACCACTGTCGATG
```

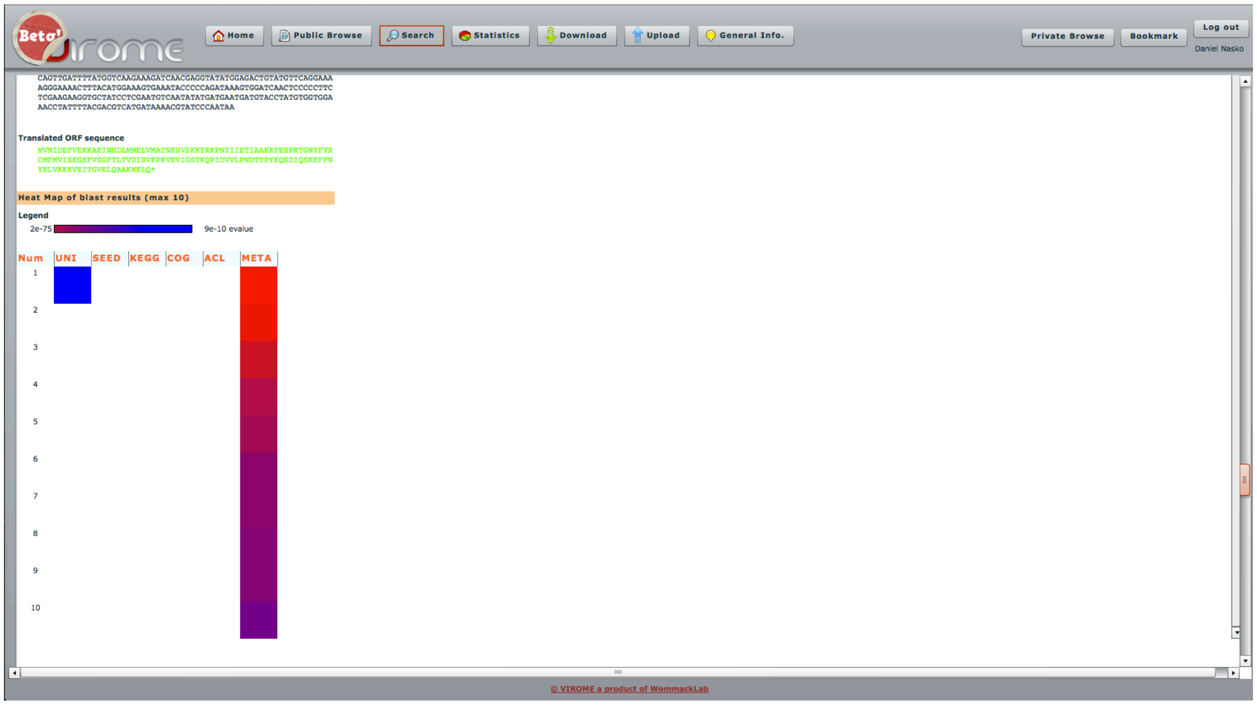
UNIREF100P BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	A9YW11	Putative uncharacterized protein	4e-15	84	96.53	33.8	56.3

METAGENOMES BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	GAH_JCVI_...	Microbial metagenome from Coastal Water near Gulf of Maine, Canada [library: GS002]	2e-75	285	97.92	97.2	98.6

Figure 21 Long reads in tab view with over 100 ORFs. Based on screen resolution, with over 5 ORFs per read tab labels are not visible and navigation difficult. Read tab is also not visible. Notice multiple scroll bars to the right.



```
CAGTTGATTTTATGGTCAAGAAAGATCAACGAGGTATATGGAGACTGTATGTTCAGGAAA
AGGGAAAACTTTACATGGAAAGTGAAATACCCCCAGATAAAGTGGATCAACTCCCCCTTC
TCGAAGAAGGTGCTATCCTCGAATGTCAATATATGATGAATGATGTACCTATGTGGTGGA
AACCTATTTTACGACGTCATGATAAAACGTATCCCAATAA
```

Translated ORF sequence

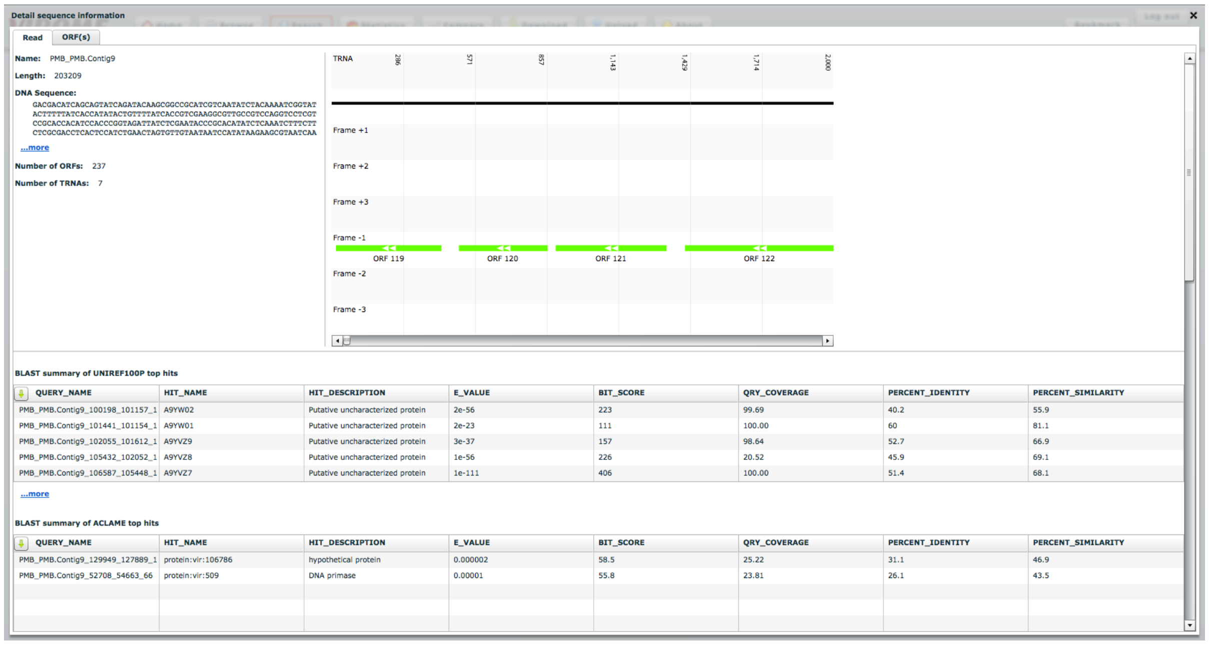
```
MVNIDEFVEKKAEINHDLMNELVMATNKHVSKKYKKPNYIIETIAAKKFEHPKTGNYFYR
CMFMVISKGSFVSGFTLTVDIRVKPKVEVIGSTKQPIDVVLPNDTTPYEQSDIQSKEFFN
YELVKKKVEITGVELQAAKNKLQ*
```

Heat Map of blast results (max 10)

Legend

2e-75 9e-10 evalue

Figure 22 Need to use both scroll bars to view heat map of ORF in Figure 21. Notice individual tabs are not visible anymore. The ORF overlay on read nucleotide is rendered unusable in this view, as you can't see entire read in one screen


Detail sequence information

Read | ORF(s)

Name: PMB_PMB.Contig9

Length: 203209

DNA Sequence:

```
GACGACATCAGCAGTATCAGATACAAGCGGCCGCATCGTCAATATCTACAAAATCGGTAT
ACTTTTTATCACCATATACTGTTTTATCACCGTCGAAGGCGTTGCCGTCCAGGTCCTCGT
CCGCACCACATCCACCCGGTAGATTATCTCGAATACCCGCACATATCTCAAATCTTTCTT
CTCGCGACCTCACTCCATCTGAACTAGTGTTGTAATAATCCATATAAGAAGCGTAATCAA
```

...more

Number of ORFs: 237

Number of TRNAs: 7

BLAST summary of UNIREF100P top hits

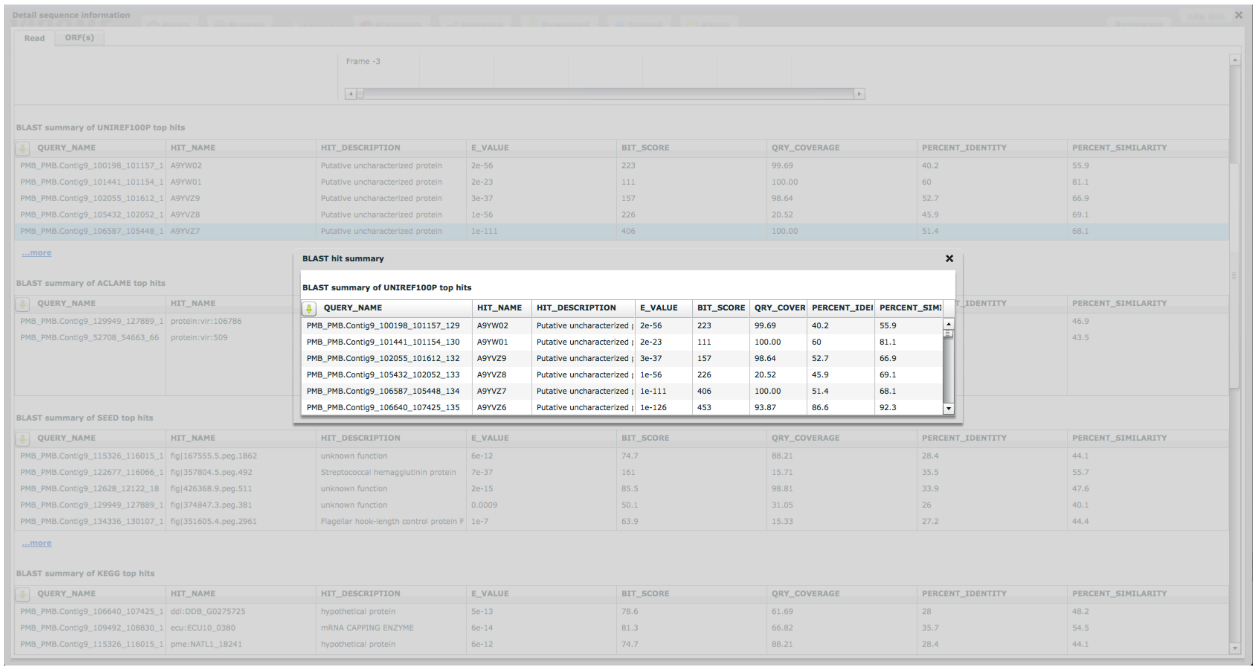
QUERY_NAME	HIT_NAME	HIT_DESCRIPTION	E_VALUE	BIT_SCORE	QRY_COVERAGE	PERCENT_IDENTITY	PERCENT_SIMILARITY
PMB_PMB.Contig9_100198_101157_1	A9YW02	Putative uncharacterized protein	2e-56	223	99.69	40.2	55.9
PMB_PMB.Contig9_101441_101154_1	A9YW01	Putative uncharacterized protein	2e-23	111	100.00	60	81.1
PMB_PMB.Contig9_102055_101612_1	A9YVZ9	Putative uncharacterized protein	3e-37	157	98.64	52.7	66.9
PMB_PMB.Contig9_105432_102052_1	A9YVZ8	Putative uncharacterized protein	1e-56	226	20.52	45.9	69.1
PMB_PMB.Contig9_106587_105448_1	A9YVZ7	Putative uncharacterized protein	1e-111	406	100.00	51.4	68.1

...more

BLAST summary of ACLAME top hits

QUERY_NAME	HIT_NAME	HIT_DESCRIPTION	E_VALUE	BIT_SCORE	QRY_COVERAGE	PERCENT_IDENTITY	PERCENT_SIMILARITY
PMB_PMB.Contig9_129949_127889_1	protein:vir:106786	hypothetical protein	0.000002	58.5	25.22	31.1	46.9
PMB_PMB.Contig9_52708_54663_66	protein:vir:509	DNA primase	0.00001	55.8	23.81	26.1	43.5

Figure 23 Read Detail on top right. Interactive and dynamic blast imager top right. Blast hits per database, functional taxonomy and environmental metadata available in interactive and downloadable tables in the bottom half of the view


Detail sequence information

Read | ORF(s)

Frame -3

BLAST summary of UNIREF100P top hits

QUERY_NAME	HIT_NAME	HIT_DESCRIPTION	E_VALUE	BIT_SCORE	QRY_COVERAGE	PERCENT_IDENTITY	PERCENT_SIMILARITY
PMB_PMB.Contig9_100198_101157_1	A9YW02	Putative uncharacterized protein	2e-56	223	99.69	40.2	55.9
PMB_PMB.Contig9_101441_101154_1	A9YW01	Putative uncharacterized protein	2e-23	111	100.00	60	81.1
PMB_PMB.Contig9_102055_101612_1	A9YVZ9	Putative uncharacterized protein	3e-37	157	98.64	52.7	66.9
PMB_PMB.Contig9_105432_102052_1	A9YVZ8	Putative uncharacterized protein	1e-56	226	20.52	45.9	69.1
PMB_PMB.Contig9_106587_105448_1	A9YVZ7	Putative uncharacterized protein	1e-111	406	100.00	51.4	68.1

...more

BLAST summary of ACLAME top hits

QUERY_NAME	HIT_NAME					PERCENT_IDENTITY	PERCENT_SIMILARITY
PMB_PMB.Contig9_129949_127889_1	protein:vir:106786						46.9
PMB_PMB.Contig9_52708_54663_66	protein:vir:509						43.5

BLAST hit summary

BLAST summary of UNIREF100P top hits

QUERY_NAME	HIT_NAME	HIT_DESCRIPTION	E_VALUE	BIT_SCORE	QRY_COVER	PERCENT_IDEI	PERCENT_SIMI
PMB_PMB.Contig9_100198_101157_129	A9YW02	Putative uncharacterized ¡	2e-56	223	99.69	40.2	55.9
PMB_PMB.Contig9_101441_101154_130	A9YW01	Putative uncharacterized ¡	2e-23	111	100.00	60	81.1
PMB_PMB.Contig9_102055_101612_132	A9YVZ9	Putative uncharacterized ¡	3e-37	157	98.64	52.7	66.9
PMB_PMB.Contig9_105432_102052_133	A9YVZ8	Putative uncharacterized ¡	1e-56	226	20.52	45.9	69.1
PMB_PMB.Contig9_106587_105448_134	A9YVZ7	Putative uncharacterized ¡	1e-111	406	100.00	51.4	68.1
PMB_PMB.Contig9_106640_107425_135	A9YVZ6	Putative uncharacterized ¡	1e-126	453	93.87	86.6	92.3

BLAST summary of SEED top hits

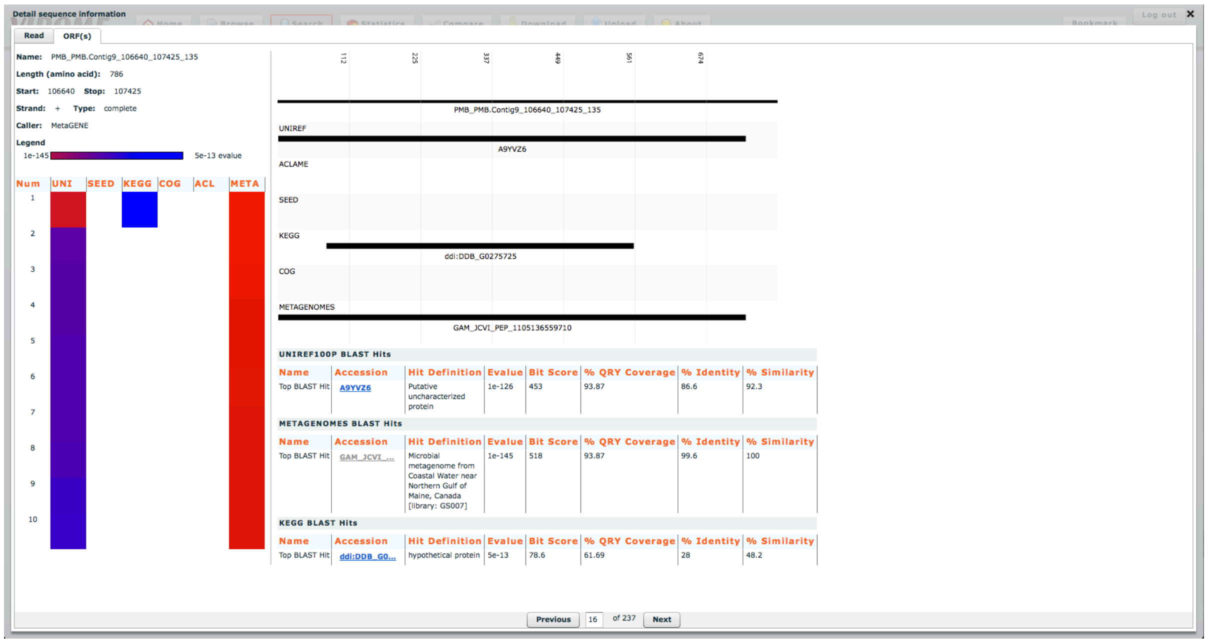
QUERY_NAME	HIT_NAME	HIT_DESCRIPTION	E_VALUE	BIT_SCORE	QRY_COVERAGE	PERCENT_IDENTITY	PERCENT_SIMILARITY
PMB_PMB.Contig9_115326_116015_1	fig|167555.5.peg.1862	unknown function	6e-12	74.7	88.21	28.4	44.1
PMB_PMB.Contig9_122677_116066_1	fig|357804.5.peg.492	Streptococcal hemagglutinin protein	7e-37	161	15.71	35.5	55.7
PMB_PMB.Contig9_12628_12122_18	fig|426368.9.peg.511	unknown function	2e-15	85.5	98.81	33.9	47.6
PMB_PMB.Contig9_129949_127889_1	fig|374847.3.peg.381	unknown function	0.0009	50.1	31.05	26	40.1
PMB_PMB.Contig9_134336_130107_1	fig|351605.4.peg.2961	Flagellar hook-length control protein F	1e-7	63.9	15.33	27.2	44.4

...more

BLAST summary of KEGG top hits

QUERY_NAME	HIT_NAME	HIT_DESCRIPTION	E_VALUE	BIT_SCORE	QRY_COVERAGE	PERCENT_IDENTITY	PERCENT_SIMILARITY
PMB_PMB.Contig9_106640_107425_1	ddi:DDB_G0275725	hypothetical protein	5e-13	78.6	61.69	28	48.2
PMB_PMB.Contig9_109492_108830_1	ecu:ECU10_0380	mRNA CAPPING ENZYME	6e-14	81.3	66.82	35.7	54.5
PMB_PMB.Contig9_115326_116015_1	pme:NATL1_18241	hypothetical protein	6e-12	74.7	88.21	28.4	44.1

Figure 24 Figure 1: Detail view of UNIREF100 table.



Name: PMB_PMB.Contig9_106640_107425_135

Length (amino acid): 786

Start: 106640 **Stop:** 107425

Strand: + **Type:** complete

Caller: MetaGENE

UNIREF100P BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	A9YVZ6	Putative uncharacterized protein	1e-126	453	93.87	86.6	92.3

METAGENOMES BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	GAM_JCVI_...	Microbial metagenome from Coastal Water near Northern Gulf of Maine, Canada [library: GS007]	1e-145	518	93.87	99.6	100

KEGG BLAST Hits

Name	Accession	Hit Definition	Evalue	Bit Score	% QRY Coverage	% Identity	% Similarity
Top BLAST Hit	ddi:DDB_G0...	hypothetical protein	5e-13	78.6	61.69	28	48.2

Figure 25 Version 2.0 ORF view. Notice ORF navigation bar at the bottom. ORF navigation bar, and tabs on top are always in view in the same place no matter how large or small ORF content gets.

Chapter 7

CONCLUSION

We have successfully created a framework, consisting of an end-to-end bioinformatics analysis pipeline coupled with a rich and dynamic web user-interface that is platform independent and a portal for data submission, all of which is backed by a single storage structure that shares data across all three applications. This framework addresses a need in the research community, namely a complete bioinformatics analysis pipeline for environmental viral metagenomes. This analysis pipeline leverages all the information available not just homology to known database(s) along with an adaptive, interactive and intuitive user interface that engages user in data exploration and puts the control of data in users hand.

The process begins with the VIROME submission portal, where a strict policy of collecting metadata insures that data is consistent across all libraries and that a controlled vocabulary is used, which can be leveraged downstream for exhaustive comparative analysis. The VIROME bioinformatics analysis pipeline runs all incoming data through extensive quality checks and filters. All sequences that pass various quality measures are analyzed using a suite of open source tools and custom scripts. The VIROME bioinformatics analysis pipeline leverages seven well-known databases and a comprehensive environmental metagenomes database to annotate ORFs based on their biological and genetic data obtained from homology search. This approach of using both environmental and known databases allows VIROME users to annotate a library using majority of the sequence data not just the 30% of sequences that find homology with known databases. Finally, the VIROME web-application puts the user in the driver's seat with dynamic, interactive, intuitive and engaging user-interface where an intuitive flow of data allows users to easily explore and download data for further analysis outside of VIROME using a powerful quantitative comparison and analysis tool known as QIIME.

Ongoing efforts directed towards making the VIROME framework independent of third party tools such as ColdFusion and Ergatis, would minimize dependencies on other tools and modules. This would enable VIROME framework to be installed easily and run locally with or without a computing grid and empower users to analyze and explore data in a seamless fashion at a push of button.

By providing a complete framework to annotate and explore environmental viral metagenomes freely to the community we have created an important resource. VIROME currently has over 250 unique active users across 10 countries. VIROME framework has analyzed over 300 libraries and seven thousand megabases of sequence data. Fifteen different peer review articles have cited VIROME.

REFERENCES

Altschul, S., W. Gish, W. Miller, E. Myers and D. Lipman (1990). "Basic local alignment search tool." Journal of molecular biology 215(3): 403-410.

Ashburner, M., C. Ball, J. Blake, D. Botstein, H. Butler, J. Cherry, A. Davis, K. Dolinski, S. Dwight, J. Eppig, M. Harris, D. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J. Matese, J. Richardson, M. Ringwald, G. Rubin and G. Sherlock (2000). "Gene ontology: tool for the unification of biology. The Gene Ontology Consortium." Nature genetics 25(1): 25-29.

Aziz, R., D. Bartels, A. Best, M. DeJongh, T. Disz, R. Edwards, K. Formsma, S. Gerdes, E. Glass, M. Kubal, F. Meyer, G. Olsen, R. Olson, A. Osterman, R. Overbeek, L. McNeil, D. Paarmann, T. Paczian, B. Parrello, G. Pusch, C. Reich, R. Stevens, O. Vassieva, V. Vonstein, A. Wilke and O. Zagnitko (2008). "The RAST Server: rapid annotations using subsystems technology." BMC genomics 9: 75.

Caporaso, J., J. Kuczynski, J. Stombaugh, K. Bittinger, F. Bushman, E. Costello, N. Fierer, A. Peña, J. Goodrich, J. Gordon, G. Huttley, S. Kelley, D. Knights, J. Koenig, R. Ley, C. Lozupone, D. McDonald, B. Muegge, M. Pirrung, J. Reeder, J. Sevinsky, P. Turnbaugh, W. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld and R. Knight (2010). "QIIME allows analysis of high-throughput community sequencing data." Nature methods 7(5): 335-336.

Ewing, B., L. Hillier, M. C. Wendl and P. Green (1998). Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Research. 8: 175-185.

Giardine, B., C. Riemer, R. C. Hardison, R. Burhans, L. Elnitski, P. Shah, Y. Zhang, D. Blankenberg, I. Albert, J. Taylor, W. Miller, W. J. Kent and A. Nekrutenko (2005). "Galaxy: a platform for interactive large-scale genome analysis." Genome Res 15(10): 1451-1455.

Kanehisa, M. and S. Goto (2000). "KEGG: kyoto encyclopedia of genes and genomes." Nucleic acids research 28(1): 27-30.

Kristensen, D. M., A. R. Mushegian, V. V. Dolja and E. V. Koonin (2010). New dimensions of the virus world discovered through metagenomics. Trends Microbiol., Elsevier. 18: 11-19.

Leplae, R., A. Hebrant, S. Wodak and A. Toussaint (2004). "ACLAME: a CLAssification of Mobile genetic Elements." Nucleic acids research 32(Database issue): 9.

Li, W. and A. Godzik (2006). "Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences." Bioinformatics (Oxford, England) 22(13): 1658-1659.

Lowe, T. and S. Eddy (1997). "tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence." Nucleic acids research 25(5): 955-964.

McDonald, D., J. Clemente, J. Kuczynski, J. Rideout, J. Stombaugh, D. Wendel, A. Wilke, S. Huse, J. Hufnagle, F. Meyer, R. Knight and J. Caporaso (2012). "The Biological Observation Matrix (BIOM) format or: how I learned to stop worrying and love the ome-ome." GigaScience 1(1): 7.

Meyer, F., D. Paarmann, M. D'Souza, R. Olson, E. Glass, M. Kubal, T. Paczian, A. Rodriguez, R. Stevens, A. Wilke, J. Wilkening and R. Edwards (2008). "The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes." BMC bioinformatics 9: 386.

Noguchi, H., J. Park and T. Takagi (2006). "MetaGene: prokaryotic gene finding from environmental genome shotgun sequences." Nucleic acids research 34(19): 5623-5630.

Ondov, B., N. Bergman and A. Phillippy (2011). "Interactive metagenomic visualization in a Web browser." BMC bioinformatics 12: 385.

Orvis, J., J. Crabtree, K. Galens, A. Gussman, J. M. Inman, E. Lee, S. Nampally, D. Riley, J. P. Sundaram, V. Felix, B. Whitty, A. Mahurkar, J. Wortman, O. White and S. V. Angiuoli (2010). "Ergatis: a web interface and scalable software system for bioinformatics workflows." Bioinformatics 26(12): 1488-1492.

Overbeek, R., T. Disz and R. Stevens (2004). "The SEED: a peer-to-peer environment for genome annotation." Communications of the ACM 47(11): 46-51.

Polson, S. W., S. W. Wilhelm and K. E. Wommack (2010). Unraveling the viral tapestry (from inside the capsid out). ISME J. 5: 165-168.

Roux, S., M. Faubladier, A. Mahul, N. Paulhe, A. Bernard, D. Debroas and F. Enault (2011). "Metavir: a web server dedicated to virome analysis." Bioinformatics (Oxford, England) 27(21): 3074-3075.

Rusch, D. B., A. L. Halpern, G. Sutton, K. B. Heidelberg, S. Williamson, S. Yooseph, D. Wu, J. A. Eisen, J. M. Hoffman, K. Remington, K. Beeson, B. Tran, H. Smith, H. Baden-Tillson, C. Stewart, J. Thorpe, J. Freeman, C. Andrews-Pfannkoch, J. E. Venter, K. Li, S. Kravitz, J. F. Heidelberg, T. Utterback, Y.-H. Rogers, L. I. Falcón, V. Souza, G. Bonilla-Rosso, L. E. Eguiarte, D. M. Karl, S. Sathyendranath, T. Platt, E. Bermingham, V. Gallardo, G. Tamayo-Castillo, M. R. Ferrari, R. L. Strausberg, K. Nealson, R. Friedman, M. Frazier and J. C. Venter (2007). The Sorcerer II Global Ocean Sampling Expedition: Northwest Atlantic through Eastern Tropical Pacific. Plos Biol. 5: e77.

Schmidt, H. F., E. G. Sakowski, S. J. Williamson, S. W. Polson and K. E. Wommack (2014). "Shotgun metagenomics indicates novel family A DNA polymerases predominate within marine virioplankton." ISME J 8(1): 103-114.

Suzek, B., H. Huang, P. McGarvey, R. Mazumder and C. Wu (2007). "UniRef: comprehensive and non-redundant UniProt reference clusters." Bioinformatics (Oxford, England) 23(10): 1282-1288.

Tatusov, R. L., M. Y. Galperin, D. A. Natale and E. V. Koonin (2000). The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Research. 28: 33-36.

Wolstencroft, K., R. Haines, D. Fellows, A. Williams, D. Withers, S. Owen, S. Soiland-Reyes, I. Dunlop, A. Nenadic, P. Fisher, J. Bhagat, K. Belhajjame, F. Bacall, A. Hardisty, A. Nieva de la Hidalga, M. P. Balcazar Vargas, S. Sufi and C. Goble (2013). "The Taverna workflow suite: designing and executing workflows of Web Services on the desktop, web or in the cloud." Nucleic Acids Res 41(Web Server issue): W557-561.

Wommack, K., J. Bhavsar, S. Polson, J. Chen, M. Dumas, S. Srinivasiah, M. Furman, S. Jamindar and D. Nasko (2012). "VIROME: a standard operating procedure for analysis of viral metagenome sequences." Standards in genomic sciences 6(3): 427-439.

Wommack, K., J. Bhavsar and J. Ravel (2008). "Metagenomics: read length matters." Applied and environmental microbiology 74(5): 1453-1463.

Yilmaz, P., R. Kottmann, D. Field, R. Knight, J. Cole, L. Amaral-Zettler, J. Gilbert, I. Karsch-Mizrachi, A. Johnston, G. Cochrane, R. Vaughan, C. Hunter, J. Park, N. Morrison, P. Rocca-Serra, P. Sterk, M. Arumugam, M. Bailey, L. Baumgartner, B. Birren, M. Blaser, V. Bonazzi, T. Booth, P. Bork, F. Bushman, P. Buttigieg, P. Chain, E. Charlson, E. Costello, H. Huot-Creasy, P. Dawyndt, T. DeSantis, N. Fierer, J. Fuhrman, R. Gallery, D. Gevers, R. Gibbs, I. San Gil, A. Gonzalez, J. Gordon, R. Guralnick, W. Hankeln, S. Highlander, P. Hugenholtz, J. Jansson, A. Kau, S. Kelley, J. Kennedy, D. Knights, O. Koren, J. Kuczynski, N. Kyrpides, R. Larsen, C. Lauber, T. Legg, R. Ley, C. Lozupone, W. Ludwig, D. Lyons, E. Maguire, B. Methé, F. Meyer, B. Muegge, S. Nakielny, K. Nelson, D. Nemergut, J. Neufeld, L. Newbold, A. Oliver, N. Pace, G. Palanisamy, J. Peplies, J. Petrosino, L. Proctor, E. Pruesse, C. Quast, J. Raes, S. Ratnasingham, J. Ravel, D. Relman, S. Assunta-Sansone, P. Schloss, L. Schriml, R. Sinha, M. Smith, E. Sodergren, A. Spo, J. Stombaugh, J. Tiedje, D. Ward, G. Weinstock, D. Wendel, O. White, A. Whiteley, A. Wilke, J. Wortman, T. Yatsunenko and F. Glöckner (2011). "Minimum information about a marker gene sequence (MIMARKS) and minimum information about any (x) sequence (MIxS) specifications." Nature biotechnology 29(5): 415-420.

APPENDIX
VIROME BIOINFORMATICS ANALYSIS PIPELINE DOCUMENTATION

ALLFXNALDBBREAK.PL

NAME

```
      AllFxnalDBBreak.pl
```

SYNOPSIS

```
      USAGE: AllFxnalDBBreak.pl
             --input=/input/filename
             --outdir=/path/to/output/dir
             --server=server-name
             --env=dbi
             --library=libraryId
             [
                   --log=/path/to/logfile
                   --debug=N
             ]
```

OPTIONS

```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server, -s OPTIONAL if input and/or server is defined.
      Server/database name where library data is stored

      --library, -l OPTIONAL if input and/or server is defined. Specific
      library ID

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
Create XML document that conations information to draw all functional
count chart. i.e.: number of hits in KEGG, SEED, UNIREF100P, COG, and
ACLAME
```

INPUT

```
The input is defined with --server,  --library. Or by an input file
which is a tab delimited text file with libraryId, prefix and library
name

    e.g. of input file (tab delimited):
      #LibraryID  NAME   Prefix  SERVER
      1  ABC  ABC_NAME
```

OUTPUT

```
A well defend XML file with number of ORFs with significant hit to
functional databases such as
      SEED
      KEGG
      COG
      ACLAME
      GO and
      PhageSEED

A secondary xml document with IDDOC prefix is created which store
individual IDs for each ORF.  IDDOC and XMLDOC files are linked via
TAG_##

egg: XMLDOC
<?xml version="1.0" encoding="UTF-8"?>
   <root>
         <CATEGORY LABEL="ACLAME" VALUE="57" TAG="TAG_1"
      IDFNAME="FXNAL_OVERVIEW_IDDOC_27.xml" />
         <CATEGORY LABEL="COG" VALUE="16" TAG="TAG_2"
      IDFNAME="FXNAL_OVERVIEW_IDDOC_27.xml" />
         <CATEGORY LABEL="GO" VALUE="117" TAG="TAG_3"
      IDFNAME="FXNAL_OVERVIEW_IDDOC_27.xml" />
         <CATEGORY LABEL="KEGG" VALUE="16" TAG="TAG_4"
      IDFNAME="FXNAL_OVERVIEW_IDDOC_27.xml" />
         <CATEGORY LABEL="SEED" VALUE="2" TAG="TAG_5"
      IDFNAME="FXNAL_OVERVIEW_IDDOC_27.xml" />
   </root>

e.g.: IDDOC
<?xml version="1.0" encoding="UTF-8"?>
   <root>
      <TAG_1
      IDLIST="23125,23133,23142,23145,23169,23187,23212,23218,23225,
      ...," />
      <TAG_2
      IDLIST="23149,23188,23226,23229,23255,23381,23383,23533,23541,235
      59,23583,23630,23654,23666,23672,23743" />
```

```
        <TAG_3
        IDLIST="23132,23142,23145,23149,23154,23165,23166,23188,23190,231
        96, ..., " />
        <TAG_4
        IDLIST="23145,23188,23255,23288,23319,23336,23337,23381,23389,234
        16,23533,23541,23630,23666,23672,23743" />
        <TAG_5 IDLIST="23288,23381" />
     </root>
```

EXAMPLE

```
       AllFxnalDBBreak.pl --server=calliope --env=dbi --library=31
```

BLAST-RESULT-PREP.PL

```
NAME
        blast-result-prep.pl - prepare blast btab file for MySQL upload

SYNOPSIS
      USAGE: blast-result-prep.pl
            --input=/path/to/blast/btab/output
            --liblist=/library/list/file/from/db-load-library
            --lookupDir=/dir/where/mldbm/lookup/files/are
            [
               --log=/path/to/logfile
               --debug=N
            ]

OPTIONS
      --input, -i REQUIRED The full path to blast btab output

      --liblist, -ll REQUIRED Library list file, and output of db-load-
library.

      --lookupDir, -ld REQUIRED Dir where all lookup files are stored.

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message

DESCRIPTION
        Given raw tab delimited BLAST results, this script will rearrange,
        edit and add information to each input row so that each row is in
        sync with the columns of blastp table of VIROME database.

        Based on subject database a ranking integer is added to identify
        database hierarchy

INPUT
        The input to this is defined using the --input.  This should point to
        the BLAST btab output

       Fields that are load are

         1   query_name
         2   query_length
         3   algorithm
         4   database_name
         5   hit_name
         6   qry_start
         7   qry_end
         8   hit_start
         9  hit_end
```

```
          10  percent_identity
          11  percent_similarity
          12  raw_score
          13  bit_score
          14  hit_description
          15  blast_frame
          16  qry_strand (Plus | Minus)
          17  hit_length
          18  e_value

          if UNIREF100P blast result then following are also added

          19  domain
          20  kingdom
          21  phylum
          22  class
          23  order
          24  family
          25  genus
          26  species
          27  organism
          28  fxn_topHit
```

OUTPUT

A tab delimited file of BLAST records, which are in sync with each columns of VIROME blast table.

Output of this script can be uploaded directly to VIROME blast table without any further modification or column specification.

EXAMPLE

```
blast-result-prep.pl --input=/path/to/file.name --
outdir=/path/to/output/dir --liblist=/path/to/file.name --
lookupDir=/path/to/file.name
```

BTABTRIMMGOL.PL

NAME

btabTrimMGOL.pl - remove limits the number of hits per query in a btab file

SYNOPSIS

```
USAGE: btabTrim.pl
        --input_file_base=input_btab_file_base-name
        --input_file_path=input_btab-file-path
        --input_file_extension=input_btab-file-extension
        --output=/path/to/trim_file.btab
        [
              --log=/path/to/logfile
              --debug=N
        ]
```

OPTIONS

--btab_file_base, -n REQUIRED The base name of input btab file.

--output,-o REQUIRED btab blast file trimmed to allow a maximum of N hits per query

--log, -l OPTIONAL Log file location

--debug, -d OPTIONAL Debug level

--help, -h This help message

DESCRIPTION

This script is used to limit the number of hits per query in a btab blast result file

INPUT

The input to this is defined using the --btabIN_file_base, --btabIN_file_extension, --fasta_file_path and -btab option. This should point to the btab ncbi-blast output and original FASTA file information.

OUTPUT

The output is defined using the --output. This file is a btab format file.

CONTACT

Shawn Polson
polson@dbi.udel.edu

DB-LOAD-LIBRARY.PL

NAME

db-load-library.pl - load Library output to DB

SYNOPSIS

```
USAGE: db-load-library.pl
        --id=/id the id of the library
        --user=/user/who/is/uploading/component
        --outdir=/path/to/output/dir
        --env=/env where to execute script
        [
                --log=/path/to/logfile
                --debug=N
        ]
```

OPTIONS

--id, -i REQUIRED The id number of the library you are running. You can find this in the library table on Jabba.

--user, -u REQUIRED User who is uploading library information.

--outdir, o REQUIRED Path to output dir where a tab file of library id and library prefix is stored.

--env, -e REQUIRED Specific environment where this script is executed. Based on these values DB connection and file locations are set.

Currently supports

```
igs
dbi
ageek or
test
```

--log, -l OPTIONAL Log file location

--debug, -d OPTIONAL Debug level

--help, -h This help message

DESCRIPTION

Add Library Information to VIROME MySQL database

INPUT

The input to this is defined using the --id. This should be the id number of the library you wish to run. If the library you wish to run does not have an id than you will need to add the information tot he table.

OUTPUT

Library information is added to VIROME library table.

CONTACT

Daniel J. Nasko
dan.nasko@gmail.com

DB-LOAD-NOHIT.PL

```
NAME
      db-load-nohit.pl

SYNOPSIS
      USAGE: db-load-nohit.pl
             --server=server-name
             --env=dbi
             --library=libraryId
             --input=/path/to/input/file
             --outdir=/path/to/output/dir
             [
                   --log=/path/to/logfile
                  --debug=N
             ]

OPTIONS
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server,-s REQUIRED Server/database name

      --library,-l REQUIRED Specific library ID

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

      Currently supports
             igs
             dbi
             ageek or
             test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message

DESCRIPTION
      For all ORFs that  are defined as ORFans by VIROME pipeline, add a
      blank/dummy row in VIROME blast table, So they can be displayed and
      treaded similar to Functional or Metagenome BLAST hits.

INPUT
      The input is defined with --server, --library.

OUTPUT
      Add no-hit row to BLAST tables.
```

EXAMPLE

```
      db-load-nohit.pl --server=calliope --env=dbi --library=31
```

DB-LOAD-UNIREF-LOOKUP.PL

NAME

db-load-uniref-lookup.pl - load uniref100+ cluster output

SYNOPSIS

```
USAGE: db-load-blast.pl
      --input=/path/to/cluster/output
      [
            --log=/path/to/logfile
            --debug=N
      ]
```

OPTIONS

```
--input, -i REQUIRED The full path to cd-hit cluster output

--log, -l OPTIONAL Log file location

--debug, -d OPTIONAL Debug level

--help, -h This help message
```

DESCRIPTION

DEPRICATED: This script is used to load uniref100+ cd-hit cluster output in to MySQL database.

INPUT

The input to this is defined using the --input. This should point to the cd-hit cluster output

EXAMPLE

```
db-load-uniref-lookup.pl --input=/path/to/file.name
```

DB-TO-LOOKUP.PL

NAME

```
      db-to-lookup.pl: Create a MLDBM lookup file
```

SYNOPSIS

```
      USAGE: db-to-lookup.pl
            --input=/library/info/file
            --table=/tablename
            --env=/env/where/executing
            --outdir=/output/dir
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input, -i REQUIRED Tab delimited library info file.

      --table, -t REQUIRED MySQL DB table name

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

      Currently supports
            igs
            dbi
            ageek or
            test

      --outdir, -o REQUIRED Output dir where lookup file will be stored

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

Convert MySQL table into MLDBM file base database. This is used by VIROME pipeline as a lookup file per library, so as to reduce MySQL look ups and subsequently reduce load on MySQL server.

INPUT

A library and VIROME table name to convert into MLDBM database

Currently only supports VIROME sequence table

OUTPUT

A MLDBM database.

EXAMPLE

```
      db-to-lookup.pl --input=/path/to/file.name --outdir=/path/to/output/dir
      --table=table.name --env=dbi
```

DB-UPLOAD.PL

NAME

```
      db-upload.pl: Upload file into MySQL DB
```

SYNOPSIS

```
      USAGE: db-upload.pl
            --input=/file/name
            --table=/tablename
            --env=/env/where/executing
            --outdir=/output/dir/loc
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input, -i REQUIRED The full path to tab delimited

      --table, -t REQUIRED MySQL DB table name

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

      Currently supports
            igs
            dbi
            ageek or
            test

      --outdir, -o REQUIRED Output dir where lookup file will be stored

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      This script is used to upload data already formatted using various prep
      scripts into VIROME MySQL tables
```

INPUT

```
      A tab delimited file. Each column is in sync with respective VIROME SQL
      table

      Currently following tables are supported
            sequence_relationship
            sequence
            blastx
            blastp
            blastn
            tRNA and
            ORF
```

OUTPUT

All data is uploaded into respective SQL tables

EXAMPLE

```
      db-upload.pl --input=/path/to/file.name --outdir=/path/to/output/dir --
      table=table.name --env=dbi
```

DEREP-BLAST-OUTPUT.PL

NAME

derep-blast-output.pl - format library header after cd-hit

SYNOPSIS

```
      USAGE: derep-blast-output.pl
             --file_part=/blast/btab/file/name
             --file_path=/blast/btab/file/path
             --clstr=/path/to/cdhit/clstr/list
             --output=/full/path/to/output/file
             [
                    --log=/path/to/logfile
                    --debug=N
             ]
```

OPTIONS

--file_path, -f REQUIRED The full path to blast btab file output

--file_part, -p REQUIRED The file part of blast btab file

--clstr, -c REQUIRED The full path to cdhit clstr output file list

--output, -o REQUIRED The full path to output file

--debug,-d Debug level. Use a large number to turn on verbose debugging.

--log, -l OPTIONAL Log file location

--debug, -d OPTIONAL Debug level

--help, -h This help message

DESCRIPTION

This script is used to de-replication cluster file so blast btab includes all sequence part of a cluster.

INPUT

The input to this is defined using the --file_part. This should point to the blast btab file part, --file_path is the blast btab file path --clstr should point to cdhit clstr file list, --output points to full path to new btab output file.

OUTPUT

A tab delimited output file where each UNIREF100P has been expanded to its respective SEED, KEGG, COG, ACLAME and PhageSEED records with respective accession number, description and taxonomy. Other BLAST information such as e-value, bit score, length, hsp information remains the same

EXAMPLE

```
derep-blast-output.pl --file_part=/blast/btab/file/name --
file_path=/blast/btab/file/path --clstr=/path/to/cdhit/clstr/list --
output=/full/path/to/output/file
```

ENV_LIB_STATS.PL

NAME
```
       env_lib_stats.pl
```

SYNOPSIS
```
      USAGE: env_lib_stats.pl
            --server=server-name
            --env=dbi
            --library=libraryId
            --lookupDir=/location/of/mldbm/dir
            --input=/path/to/input/file
            --outdir=/path/to/output/dir
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS
```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server, -s OPTIONAL if input and/or server is defined.
      Server/database name where library data is stored

      --library, -l OPTIONAL if input and/or server is defined. Specific
      library ID

      --lookupDir, -ld REQUIRED Directory of MLDBM lookup files

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION
```
      This script will process all libraries on a given server.  Get a break
      down of environmental statistics as per
            genesis,
            sphere,
        eco-system,
        extreme,
        physio_chem,
        library type and
        various env. library type.

    Counts for each category is stored in _cnt field, and all sequence IDs
    each categories are stored in an external file.
```

INPUT

```
    The input is defined with --server, which is a domain name only.
        e.g.: calliope (if server name is calliope.dbi.udel.edu)
```

OUTPUT

```
    All counts for each category are stored in "env_statistics" table on
    the "server" given as input.  All sequence IDs for each category are
    stored in an external file, and its location is stored in db.
```

EXAMPLE

```
    env_lib_stats.pl --server=calliope --env=dbi --library=31
```

FRAGGENESCAN2VIROMEORF.PL

NAME

```
      fragGeneScan2viromeORF.pl
```

SYNOPSIS

```
      USAGE: fragGeneScan2viromeORF.pl
            -i=input dir
            -p=inputFilePrefix
            -r=originalReadFile.fsa
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input_dir,-i REQUIRED Input directory where all fragGeneScan output
      files are expecting PREFIX.frag_gene_scan.raw file PREFIX.faa
      PREFIX.ffn and PREFIX

      --inputFilePrefix, -p REQUIRED Input file name prefix

      --read_file, -r REQUIRED Original read file input to FragGeneScan

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

Convert FragGeneScan output (A JCVI in house ORF predictor) into VIROME ORF predictor format so all ORF can be treated and displayed via VIROME app the same.

INPUT

Raw fragGeneScan output along with the original FASTA file used to predict ORF by fragGeneScan

OUTPUT

ORF predictor as expected by VIROME.

Currently support fragGeneScan to Metagene conversion.

EXAMPLE

```
      fragGeneScan2viromeORF i=input dir -p=inputFilePrefix -
      r=originalReadFile.fsa
```

FXN-DB-TO-LOOKUP.PL

NAME
```
            fxn-db-to-lookup.pl: Create a MLDBM lookup file
```

SYNOPSIS
```
      USAGE: fxn-db-to-lookup.pl
            --input=/file/name
            --table=/tablename
            --env=/env/where/executing
            --outdir=/output/dir/loc
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS
```
      --input, -i REQUIRED The full path to tab delimited

      --table, -t REQUIRED MySQL DB table name

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

      Currently supports
            igs
            dbi
            ageek or
            test

      --outdir, -o REQUIRED Output dir where lookup file will be stored

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION
```
      Convert functional tables from MySQL table to MLDBM database. For
      faster lookup and reduce MySQL load by VIROME pipeline
```

INPUT
```
      A functional database name to be converted into MLDBM database.

      Currently supports
            SEED
            PhageSEED
            KEGG
            COG
            ACLAME and
            UNIREF
```

OUTPUT

A MLDBM lookup database

EXAMPLE

```
fxn-db-to-lookup.pl --outdir=/location/of/outdir --env=dbi --table=seed
```

FXNALDBBREAK.PL

NAME

```
      FxnalDBBreak.pl
```

SYNOPSIS

```
      USAGE: FxnalDBBreak.pl
            --input=/input/filename
            --outdir=/path/to/output/dir
            --server=server-name
            --env=dbi
            --library=libraryId
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server, -s OPTIONAL if input and/or server is defined.
      Server/database name where library data is stored

      --library, -l OPTIONAL if input and/or server is defined. Specific
      library ID

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Create a XML document for each functional database with hierarchical
      structure as represented by each functional database annotation.

      Since the hierarchical tree structure of GO and ACLAME database can be
      arbitrarily long,  a hard limit of 6 levels is set for GO and ACLAME
      tree structure
```

INPUT

    The input is defined with --server, --library.

OUTPUT

    A well-defined XML file with number of ORFs within each database
    hierarchy.
    A secondary xml document with IDDOC prefix is created which store
    individual IDs for each ORF. IDDOC and XMLDOC files are linked via
    TAG_##

    egg: XMLDOC

```
<?xml version="1.0" encoding="UTF-8"?>
<root>
<FUNCTION_1 NAME="Metabolism" LABEL="Metabolism" VALUE="15"
TAG="TAG_1" IDFNAME="KEGG_IDDOC_27.xml">
   <FUNCTION_2 NAME="Amino Acid Metabolism" LABEL="Amino Acid
   Metabolism" VALUE="8" TAG="TAG_2" IDFNAME="KEGG_IDDOC_27.xml">
      <FUNCTION_3 NAME="Cysteine and Methionine Metabolism"
      LABEL="Cysteine and Methionine Metabolism" VALUE="7"
      TAG="TAG_3" IDFNAME="KEGG_IDDOC_27.xml"/>
      <FUNCTION_3 NAME="Glycine, Serine and Threonine Metabolism"
      LABEL="Glycine, Serine and Threonine Metabolism" VALUE="1"
      TAG="TAG_4" IDFNAME="KEGG_IDDOC_27.xml"/>
   </FUNCTION_2>
   <FUNCTION_2 NAME="Carbohydrate Metabolism" LABEL="Carbohydrate
   Metabolism" VALUE="1" TAG="TAG_5" IDFNAME="KEGG_IDDOC_27.xml">
      <FUNCTION_3 NAME="Fructose and Mannose Metabolism"
      LABEL="Fructose and Mannose Metabolism" VALUE="1"
      TAG="TAG_6" IDFNAME="KEGG_IDDOC_27.xml"/>
      <FUNCTION_3 NAME="Butanoate Metabolism" LABEL="Butanoate
      Metabolism" VALUE="1" TAG="TAG_7"
      IDFNAME="KEGG_IDDOC_27.xml"/>
   </FUNCTION_2>
   <FUNCTION_2 NAME="Lipid Metabolism" LABEL="Lipid Metabolism"
   VALUE="1" TAG="TAG_8" IDFNAME="KEGG_IDDOC_27.xml">
      <FUNCTION_3 NAME="Linoleic Acid Metabolism" LABEL="Linoleic
      Acid Metabolism" VALUE="1" TAG="TAG_9"
      IDFNAME="KEGG_IDDOC_27.xml"/>
   </FUNCTION_2>
   <FUNCTION_2 NAME="Xenobiotics Biodegradation and Metabolism"
   LABEL="Xenobiotics Biodegradation and Metabolism" VALUE="1"
   TAG="TAG_10" IDFNAME="KEGG_IDDOC_27.xml">
      <FUNCTION_3 NAME="Tetrachloroethene Degradation"
      LABEL="Tetrachloroethene Degradation" VALUE="1"
      TAG="TAG_11" IDFNAME="KEGG_IDDOC_27.xml"/>
      <FUNCTION_3 NAME="Bisphenol a Degradation" LABEL="Bisphenol
      a Degradation" VALUE="1" TAG="TAG_12"
      IDFNAME="KEGG_IDDOC_27.xml"/>
   </FUNCTION_2>
   <FUNCTION_2 NAME="Nucleotide Metabolism" LABEL="Nucleotide
   Metabolism" VALUE="4" TAG="TAG_13"
   IDFNAME="KEGG_IDDOC_27.xml">
```

```
            <FUNCTION_3 NAME="Purine Metabolism" LABEL="Purine
            Metabolism" VALUE="3" TAG="TAG_14"
            IDFNAME="KEGG_IDDOC_27.xml"/>
            <FUNCTION_3 NAME="Pyrimidine Metabolism" LABEL="Pyrimidine
            Metabolism" VALUE="4" TAG="TAG_15"
            IDFNAME="KEGG_IDDOC_27.xml"/>
         </FUNCTION_2>
         <FUNCTION_2 NAME="Energy Metabolism" LABEL="Energy Metabolism"
         VALUE="3" TAG="TAG_16" IDFNAME="KEGG_IDDOC_27.xml">
         …
         …
         …
      </root>

      egg: IDDOC
      <?xml version="1.0" encoding="UTF-8"?>
      <root>
         <TAG_1
         IDLIST="23188,23190,23255,23288,23381,23389,23416,23456,23533,
         23541,23630,23654,23666,23739,23743"/>
         <TAG_2
         IDLIST="23188,23255,23381,23389,23416,23630,23666,23743"/>
         <TAG_3 IDLIST="23188,23255,23389,23416,23630,23666,23743"/>
         <TAG_4 IDLIST="23381"/>
         <TAG_5 IDLIST="23190"/>
         <TAG_6 IDLIST="23190"/>
         <TAG_7 IDLIST="23190"/>
         <TAG_8 IDLIST="23190"/>
         <TAG_9 IDLIST="23190"/>
         <TAG_10 IDLIST="23190"/>
         <TAG_11 IDLIST="23190"/>
         <TAG_12 IDLIST="23190"/>
         <TAG_13 IDLIST="23288,23533,23541,23654"/>
         <TAG_14 IDLIST="23288,23533,23654"/>
         <TAG_15 IDLIST="23288,23533,23541,23654"/>
         <TAG_16 IDLIST="23381,23456,23739"/>
       …
      </root>
```

EXAMPLE

```
      FxnalDBBreak.pl --server=calliope --env=dbi --library=31
```

GEN_LIB_STATS.PL

NAME
```
      gen_lib_stats.pl
```

SYNOPSIS
```
      USAGE: gen_lib_stats.pl
            --server=server-name
            --env=dbi
            --library=libraryId
            --lookupDir=/location/of/mldbm/dir
            --input=/path/to/input/file
            --outdir=/path/to/output/dir
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS
```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server, -s OPTIONAL if input and/or server is defined.
      Server/database name where library data is stored

      --library, -l OPTIONAL if input and/or server is defined. Specific
      library ID

      --lookupDir, -ld REQUIRED Directory of MLDBM lookup files

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION
```
      This script will process all libraries on a given server.  Get a break
      down of
            ORF types (missing 3', missing 5', incomplete, complete)
            ORF model (bacteria, archaea, phage)
            LIB type (viral only, microbial only, top viral, top microbial)
            FUNCTIONAL and Unclassified
      Counts for each category are stored in _cnt field, and all sequence IDs
      for each category are stored in an external file.
```

INPUT

```
      The input is defined with --server which is a domain name only.
            e.g.: calliope (if server name is calliope.dbi.udel.edu)
```

OUTPUT

```
      All counts for each category are stored in "statistics" table on the
      "server" given as input.  All sequence IDs for each category are stored
      in an external file, and its location is stored in db.
```

EXAMPLE

```
      gen_lib_stats.pl --server=calliope --env=dbi --library=31
```

METAGENE-PREP.PL

NAME

```
      metagene-prep.pl - prepare metagene raw output for MySQL upload
```

SYNOPSIS

```
      USAGE: metagene-prep.pl
            --input=/path/to/metagene/raw/output
            --liblist=/library/list/file/from/DB-load-library
            lookupDir=/dir/where/mldbm/lookup/files/are
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input, -i REQUIRED The full path to metagene raw output

      --liblist, -ll REQUIRED Library list file, and output of DB-load-
      library.

      --lookupDir, -ld REQUIRED Dir where all lookup files are stored.

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      DEPRICATE. Convert raw Metagene output into well-defined data that is
      in sync with VIROME ORF table

      egg: raw Metagene output

      # EB8_MMETSP1129-20121227-18562
      # gc = 0.642596, rbs = -1
      # self: -
      gene_1  1   891 +   0   01  94.2685 a   -   -   -
      # EB8_MMETSP1129-20121227-18563
      # gc = 0.516753, rbs = -1
      # self: -
      gene_1  39  1502    -   0   11  42.0969 p   -   -   -
      # EB8_MMETSP1129-20121227-18564
      # gc = 0.60627, rbs = -1
      # self: p
      gene_1  1   7494    +   0   01  410.446 s   -   -   -
      # EB8_MMETSP1129-20121227-18565
      # gc = 0.579521, rbs = -1
      # self: -
      gene_1  1   201 +   0   01  8.40845 p   -   -   -
      gene_2  351 918 -   1   01  67.0183 p   -   -   -
```

INPUT

The input to this is defined using the --input. This should point to the metagene raw output file.

OUTPUT

A tab delimited file of ORF details in sync with VIROME ORF table

EXAMPLE

```
metagene-prep.pl --input=/path/to/file.name --
outdir=/path/to/output/dir --liblist=/path/to/file.name --
lookupDir=/path/to/file.name
```

MGA2SEQ_PEP.PL

NAME

mga2seq_pep.pl - convert metagene raw output to FASTA nucleotide and peptide file

SYNOPSIS

```
USAGE: mga2seq_pep.pl
       --input=/path/to/FASTA
       --mga=/path/to/mga/output
       --prefix
       --outdir
       [
              --log=/path/to/logfile
              --debug=N
       ]
```

OPTIONS

```
--input, -i REQUIRED The full path to FASTA sequence file.

--mga, mga REQUIRED Metagene output file

--log, -l OPTIONAL Log file location

--debug, -d OPTIONAL Debug level

--help, -h This help message
```

DESCRIPTION

This script is used to convert metagene output to nucleotide and peptide sequence file.

INPUT

The input to this is defined using the --input/-i or mga/-m. This should point to the FASTA file containing sequence(s), and metagene output file

OUTPUT

Two FASTA files one with predicted ORFs in amino acid format, and one with predicted ORFs in nucleotide format

EXAMPLE

```
mga2seq_pep.pl --input=/path/to/FASTA --mga=/path/to/mga/output --
prefix=abc --outdir=/location/to/dir
```

NT_FASTA_CHECK.PL

NAME

```
      nt_fasta_check.pl
```

SYNOPSIS

```
      USAGE: nt_fasta_check.pl
```

OPTIONS

```
      --FASTA, -f REQUIRED Input FASTA file

      --outdir, -o REQUIRED Output directory to store results.

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

Takes as input a FASTA file, and library prefix/library file Prefix each sequence id with PREFIX from library file, and check the quality of read bases.

INPUT

FASTA file in .fsa, .fa, .FASTA or .txt file format OUTPUT dir where updated input file is stored (output dir cannot be same as where input file is)

OUTPUT

An updated FASTA file with each sequence ID prefixed by PREFIX A ref file with original and new sequence ID

EXAMPLE

```
      nt_fasta_check.pl -i=input.fsa -o=/output_dir -ll=library_list_file
      or
      nt_fasta_check.pl -i=input.fsa -o=/output_dir -lf=library_file
```

QC_FILTER.PL

NAME

```
      QC_filter.pl
```

SYNOPSIS

```
      USAGE: QC_filter.pl
             --FASTA FASTA-file
              --qual qual-file-list
             --cutoff cutoff-value
             --minlen min-length
             --outdir output-dir
```

OPTIONS

```
       --FASTA, -f A FASTA file

      --qual, -q A quality file is original input was SFF or FASTQ

      --cutoff, -c Cuff off threshold

      --minlen, -m Minimum length cutoff for each input read

      --outdir, -o

      --help,-h This help message
```

DESCRIPTION

Give input sequence check for minimum length of each read, and mean sequence quality

INPUT

Raw sequences

OUTPUT

FASTA file where low quality sequences and sequences less than minimum length requirement are removed from input file.

EXAMPLE

```
      QC_filter.pl --FASTA=FASTA-file --qual=qual-file --cutoff=cutoff-value
      --minlen=min-length --outdir=output-dir
```

RESET-PROCESSING-DB.PL

NAME

```
      reset-processing-db.pl
```

SYNOPSIS

```
      USAGE: reset-processing-db.pl
             --lib_file=/tab/delimited/lib/info/file
             --env=/execution/loc
             --output_dir=/path/to/output/dir
```

OPTIONS

```
      --lib_file,-lf Absolute file path that contains tab delimited library
      information

             egg: id   name    prefix    server

      --output_dir,-o Output directory

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

               Currently supports
                   igs
                   dbi
                   ageek or
                   test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Create backup of processing DB, empty processing DB, set ids of
      processing DB to respective server info.  This is only for igs
      processing
```

INPUT

```
      The input is defined with --lib_file --env.
```

OUTPUT

```
      MySQL dump of processing db.
```

EXAMPLE

```
      reset-processing-db.pl --lib_file=file_name.tab --env=igs
```

RRNA-SCRUB.PL

NAME

rRNA-scrub.pl - remove rRNA sequences identified by blast from the input FASTA.

SYNOPSIS

```
      USAGE: rRNA-scrub.pl
            --fasta_file_base=FASTA file base name
            --fasta_file_path=/path/to/fasta_file
            --fasta_file_extension=FASTA file extension
            --btab=/path/to/input_file.btab
            --outputA=/path/to/rRNA_minus_original.fasta
            --outputB=/path/to/rRNA_identified_sequences.fasta
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --fasta_file_base, -n REQUIRED The base name of FASTA file.

      --fasta_file_path, -b REQUIRED The full path to FASTA file.

      --fasta_file_extension, -e REQUIRED The file extension

      --btab,-b REQUIRED The input btab blast output from the ncbi-blastn
suite.

      --outputA,-oA REQUIRED The file to which all sequence except for rRNA
      identified sequence will be written.

      --outputB, -oB REQUIRED The file to which all rRNA identified sequences
      will be written

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

This script is used to scrub rRNA identified sequences from ncbi-blast suite, and create a new FASTA file.

INPUT

The input to this is defined using the --fasta_file_base, --fasta_file_extension --fasta_file_path and -btab option. This should point to the btab ncbi-blast output and original FASTA file information.

OUTPUT

The output is defined using the --outputA and -outputB option. These files created are FASTA files of original FASTA minus the rRNA sequences, and FASTA file of rRNA identified sequences.

EXAMPLE

```
rRNA-scrub.pl --fasta_file_base=FASTA-file-base-name --
fasta_file_path=/path/to/fasta_file --fasta_file_extension=FASTA file
extension --btab=/path/to/input_file.btab --
outputA=/path/to/rRNA_minus_original.fasta --
outputB=/path/to/rRNA_identified_sequences.fasta
```

SEQUENCE_RELATIONSHIP.PL

NAME

```
      sequence_relationship.pl - prepare sequence info for upload to DB
```

SYNOPSIS

```
      USAGE: sequence_relationship.pl
             --input=/library/list/file
             --outdir=/output/dir
             --env=execution env name
             [
                   --log=/path/to/logfile
                   --debug=N
             ]
```

OPTIONS

```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Create a sequence relation tree between
            reads/contigs -> ORFs (peptide) / ORF (nuclotide) and
            reads/contigs -> rRNAs
```

INPUT

```
      A tab delimited file with library and its metadata to be processed.

      egg:
            #libaryID    libary name    library prefix    server
           220     M6O1K   POA     terpsichore
```

OUTPUT

```
      A tab delimited file of read/contig, ORF and rRNA id parent/child
      relationship tree.
```

EXAMPLE

```
      sequence_relationship.pl --input=/path/to/input --
output=/path/to/outdir --env=dbi
```

SEQUENCE-PREP.PL

NAME

sequence-prep.pl - prepare sequence info for upload to DB

SYNOPSIS

```
USAGE: sequence-prep.pl
          --input=/path/to/FASTA
          --outdir=/output/dir
          --libListFile=/library/list/file
          --type=sequence type
          --outdir=/output/dir
          [
                --log=/path/to/logfile
                --debug=N
          ]
```

OPTIONS

--input, -i REQUIRED The full path to FASTA sequence file. # start a comment. # File format >ABC125234 .... # where ABC is three letter library prefix.

--outdir, -od REQUIRED Output dir location

--libListFile, -ll REQUIRED Library list file, an output of DB-load-library.

--type, -t REQUIRED Sequence type read=1, rRNA=2, orf (aa)=3, orf (dna)=4

--log, -l OPTIONAL Log file location

--debug, -d OPTIONAL Debug level

--help, -h This help message

DESCRIPTION

This script is used to prepare sequence for MySQL upload.

INPUT

The input to this is defined using the --input. This should point to the FASTA file containing sequence(s). Input must be a muiltfasta file, each file contain sequences for one library.

OUTPUT

A tab delimited file with columns in sync with VIROME sequence table.

EXAMPLE

```
sequence-prep.pl --input=/path/to/input --outdir=/path/to/outdir --
liblist=/path/to/lib/list --typeId=2
```

SPLIT_BTAB.PL

```
NAME
      split_btab.pl -- splits btab results

SYNOPSIS
      split_btab.pl
            --btab /Path/to/infile.btab
            --out /Path/to/outdir/
            --splits=25
            [--help]
            [--manual]

DESCRIPTION
      Split btab files such that they don't break up query results.

OPTIONS
      -b, --btab=FILENAME Input file in BTAB format. (Required)

      -o, --outdir=FILENAME Output directory. (Required)

      -s, --splits=INT Number of splits. (Required)

      -h, --help Displays the usage message. (Optional)

      -m, --manual Displays full manual. (Optional)

DEPENDENCIES
      Requires the following Perl libraries.

AUTHOR
    Written by Daniel Nasko, Center for Bioinformatics and Computational
    Biology, University of Delaware.

REPORTING BUGS
    Report bugs to dnasko@udel.edu

COPYRIGHT
    Copyright 2012 Daniel Nasko. License GPLv3+: GNU GPL version 3 or later
    <http://gnu.org/licenses/gpl.html>. This is free software: you are free
    to change and redistribute it. There is NO WARRANTY, to the extent
    permitted by law.

    Please acknowledge author and affiliation in published work arising from
    this script's usage <http://bioinformatics.udel.edu/Core/Acknowledge>.
```

SPLITMULTIFASTA.PL

NAME

split_multifasta.pl - split a single FASTA file containing multiple sequences into separate files.

SYNOPSIS

```
USAGE: split_multifasta.pl
      --input_file=/path/to/some_file.fsa
      --output_dir=/path/to/somedir
      [
            --output_list=/path/to/somefile.list
            --output_subdir_size=1000
            --output_subdir_prefix=FASTA
            --seqs_per_file=1
            --total_files=1
            --compress_output=1
      ]
```

OPTIONS

--input_file,-i The input multi-FASTA file to split.

--output_dir,-o The directory to which the output files will be written.

--output_list,-s Write a list file containing the paths of each of the regular output files. This may be useful for later scripts that can accept a list as input.

--output_file_prefix,-f If defined, each file created will have this string prepended to its name. This is ignored unless writing multiple sequences to each output file using the --seqs_per_file option with a value greater than 1, else each file created will just be a number.

--output_subdir_size,-u If defined, this script will create numbered subdirectories in the output directory, each containing this many sequences files. Once this limit is reached, another subdirectory is created.

--output_subdir_prefix,-p To be used along with --output_subdir_size, this allows more control of the names of the subdirectories created. Rather than just incrementing numbers (like 10), each subdirectory will be named with this prefix (like prefix10).

--total_files, -t Used if the user wants to specify the total outputs files to be created. The script will determine the amount of sequences per file to meet this parameter. Cannot be used in conjunction with the seqs_per_file parameter.

--compress_output,-c Output FASTA files will be gzipped when written.

```
       --debug,-d Debug level. Use a large number to turn on verbose
       debugging.

       --log,-l Log file

       --help,-h This help message
```

DESCRIPTION

This script is used to split a single FASTA file containing multiple sequences into separate files containing one sequence each.

INPUT

The input is defined with --input_file and should be a single FASTA file. File extensions are ignored. When creating this multi-entry FASTA file, one should take care to make the first *word* after the > symbol a unique value, as it will be used as the file name for that sequence. For example:

```
  >gi53791237 Tragulus javanicus p97bcnt gene for p97Bcnt

ACAGGAGAAGAGACTGAAGAGACACGTTCAGGAGAAGAGCAAGAGAAGCCTAAAGAAATGCAAGAAGTTA

AACTCACCAAATCACTTGTTGAAGAAGTCAGGTAACATGACATTCACAAACTTCAAAACTAGTTCTTTAA

AAAGGAACATCTCTCTTTTAATATGTATGCATTATTAATTTATTTACTCATTGGCGTGGAGGAGGAAATG

  >gi15387669 Corynebacterium callunae pCC1 plasmid

ATGCATGCTAGTGTGGTGAGTATGAGCACACACATTCATGGGCACCGCCGGGGTGCAGGGGGGCTTGCCC

CTTGTCCATGCGGGGTGTGGGGCTTGCCCCGCCGATAGAGACCGGCCACCACCATGGCACCCGGTCGCGG

GGTGATCGGCCACCACCACCGCCCCCGGCCACTCTCCCCCTGTCTAGGCCATATTTCAGGCCGTCCACTG
```

Whitespace is ignored within the input file. See the OUTPUT section for more on creation of output files.

OUTPUT

The name of each output sequence file is pulled from the FASTA header of that sequence. The first *word* after the > symbol will be used as the file name, along with the extension .fsa. The word is defined as all the text after the > symbol up to the first whitespace.

If the above example were your input file, two files would be created:

```
gi53791237.fsa
gi15387669.fsa
```

Any characters other than a-z A-Z 0-9 . _ - in the ID will be changed into an underscore. This only occurs in the file name; the original FASTA header within the file will be unmodified.

You can pass a path to the optional --output_list to create a text file containing the full paths to each of the FASTA files created by this script.

Two other optional arguments, --output_subdir_size and --output_subdir_prefix, can be used on input sets that are too large to write out to one directory. This depends on the limitations of your file system, but you usually don't want 100,000 files written in the same directory.

If you have an FASTA file containing 95000 sequences, and use the following option:

```
      --output_dir=/some/path
      --output_subdir_size=30000
```

The following will be created:

```
  directory              file count
  ---------------------------------
  /some/path/1/          30000
  /some/path/2/          30000
  /some/path/3/          30000
  /some/path/4/           5000
```

If you choose to create a list file (and you probably want to), it will contain these proper paths.

You may not want the subdirectories to simply be numbers, as above, so you can use the --output_subdir_prefix option. For example:

```
  --output_dir=/some/path
  --output_subdir_size=30000
  --output_subdir_prefix=FASTA
```

The following will be created:

```
  directory              file count
  ---------------------------------
  /some/path/fasta1/     30000
  /some/path/fasta2/     30000
  /some/path/fasta3/     30000
  /some/path/fasta4/      5000
```

Finally, you can write multiple sequences to each output file using the --seqs_per_file option, which can be used along with --outupt_subdir_size and --output_subdir_prefix. The main difference to note is that, if you use --seqs_per_file, the FASTA file created will no longer be named using values taken from the header, since it will contain multiple headers. Instead, the file will simply be named using sequential numbers starting at 1 (like 1.fsa). For example:

```
  --output_dir=/some/path
```

```
        --output_subdir_size=3000
        --output_subdir_prefix=FASTA
        --seqs_per_file=10

    The following will be created:

        directory              file count
        ---------------------------------
        /some/path/fasta1/     3000
        /some/path/fasta2/     3000
        /some/path/fasta3/     3000
        /some/path/fasta4/      500
```

CONTACT

```
        Joshua Orvis
        jorvis@tigr.org
```

TRNASCAN-PREP.PL

NAME

```
      tRNAScan-prep.pl - prepare tRNAScan raw output for MySQL upload
```

SYNOPSIS

```
      USAGE: tRNAScan-prep.pl
            --input=/path/to/metagene/raw/output
            --outdir=/output/directory
            --liblist=/library/list/file/from/DB-load-library
            --lookupDir=/dir/where/mldbm/lookup/files/are
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input, -i REQUIRED The full path to input file

      --output, -d REQUIRED Path to output dir

      --liblist, -ll REQUIRED Library list file, an output of DB-load-
      library.

      --lookupDir, -ld REQUIRED Directory of MLDBM lookup files

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Convert tRNAScan raw output into a tab delimited file with columns that
      are in sync with VIROME tRNA table.
```

INPUT

```
      The input to this is defined using the --input.  This should point to
      the tRNAScan-SE raw output file list.  One file per line.

      egg:
            PVP_GO0QTYD02JISH2  1   218 146 Lys TTT 0   0   75.04
            PVP_GO0QTYD02GTM67  1   58  131 Met CAT 0   0   72.35
            PVP_GO0QTYD02GTM67  2   192 274 Leu TAA 0   0   68.29
            PVP_GO0QTYD02GTM67  3   281 352 Cys GCA 0   0   55.94
```

OUTPUT

```
      A tab delimited output of tRNA information

      egg:
      #seqeunceid    tRNA #    start    stop    anti    intron cove_start
      cove_end    score
```

```
123     1       265     336     Pseudoudo       GTT     0       0
28.16
123     2       4186    4260    Met     CAT     0       0       48.36
123     3       3876    3804    Undefet ???     0       0       36.26
456     1       577     652     Undefet ???     0       0       22.12
989     1       149     217     Asn     GTT     0       0       25.99
```

EXAMPLE

```
tRNAScan-prep.pl --input=/path/to/input --outdir=/path/to/outdir --
liblist=/path/to/lib/list --lookupDir=/path/to/mldbm/lookup/dir
```

UPDATE_MGOL_NAMES_IN_FASTA.PL

NAME

```
      update_mgol_names_in_fasta.pl
```

SYNOPSIS

```
      USAGE: update_mgol_name.pl
             --FASTA=/path/to/input/file.fasta
             --outdir=/path/to/output/dir
             [
                   --log=/path/to/logfile
                   --debug=N
             ]
```

OPTIONS

```
      --FASTA, -f REQUIRED Fasta file name from where MGOL sequence(s) names
      needs to be updated

      --outdir, -o REQUIRED Output directory where updated MGOL FASTA file
      will be stored.

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Update MGOL sequence name to have appropriate 3 letter prefix as
      defined in mgol_library table.
```

INPUT

```
      The input is defined with --FASTA and --map.
```

OUTPUT

```
      Updated sequence name in the given FASTA file
```

EXAMPLE

```
      update_mgol_names_in_fasta.pl --FASTA=filename.fasta --
      outdir=/path/to/output-dir
```

UPDATEQUALITYFILE.PL

NAME

```
      updateQualityFile.pl
```

SYNOPSIS

```
      USAGE: updateQualityFile.pl
            --FASTA=/path/to/fasta.fsa
            --quality=/path/to/quality_file.qual
            --outdir=/path/to/output/dir
            --prefix=prefix
```

OPTIONS

```
      --FASTA, -f REQUIRED The full path to FASTA sequence file.

      --quality, -q REQUIRED The full path to the associated quality file.

      --outdir, -o REQUIRED Path to output dir

      --prefix, -p REQUIRED Unique 3 digit alpha/numeric library identifier

      --help,-h This help message
```

DESCRIPTION

Script is designed to look at the sequence file and pull out only those relevant quality scores. This is necessary as the FASTA file is passed thru QC components that will throw out certain sequences.

INPUT

The input to this is defined using the --FASTA/-f AND --quality/-q flags. These should point to the files containing the FASTA and quality files, respectively.

CONTACT

Daniel J. Nasko
dan.nasko@gmail.com

VIROMECLASSIFICATION.PL

NAME

```
      viromeClassification.pl
```

SYNOPSIS

```
      USAGE: viromeClassification.pl
            --input=/input/filename
            --outdir=/path/to/output/dir
            --server=server-name
            --env=dbi
            --library=libraryId
            [
                  --log=/path/to/logfile
                  --debug=N
            ]
```

OPTIONS

```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server, -s OPTIONAL if input and/or server is defined.
      Server/database name where library data is stored

      --library, -l OPTIONAL if input and/or server is defined. Specific
      library ID

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Create XML document with number of ORF binned per VIROME category
            i.e.:
                  tRNA
                  rRNA
                  Possible functional
                  Unassigned functional
                  Top viral
                  Top microbial
                  Viral only
                  Microbial only and
                  ORFan

            ORF counts are log normal.
```

INPUT

```
      The input is defined with --server,  --library.
```

OUTPUT

```
      A XML document with ORF counts per VIROME category

      egg:
      <?xml version="1.0" encoding="UTF-8"?>
      <root>
            <CATEGORY LABEL="tRNA" CAT="tRNA" VALUE="1" />
            <CATEGORY LABEL="rRNA" CAT="rRNA" VALUE="0" />
            <CATEGORY LABEL="Possible functional protein" CAT="fxn"
            VALUE="20" />
            <CATEGORY LABEL="Unassignfxn protein" CAT="unassignfxn"
            VALUE="38" />
            <CATEGORY LABEL="Top-hit viral" CAT="topviral" VALUE="1" />
            <CATEGORY LABEL="Viral only" CAT="allviral" VALUE="22" />
            <CATEGORY LABEL="Top-hit microbial" CAT="topmicrobial" VALUE="0"
            />
            <CATEGORY LABEL="Microbial only" CAT="allmicrobial" VALUE="0" />
            <CATEGORY LABEL="ORFan" CAT="orfan" VALUE="21" />
      </root>
```

EXAMPLE

```
      viromeClassification.pl --server=calliope --env=dbi --library=31 --
      outdir=/path/to/output/dir
```

VIROMEORF-PREP.PL

NAME

    viromeorf-prep.pl - prepare orf calls for MySQL upload

SYNOPSIS

```
USAGE: viromeorf-prep.pl
      --input=/path/orf/output
      --liblist=/library/list/file/from/DB-load-library
      --lookupDir=/dir/where/mldbm/lookup/files/are
      --outdir=/path/to/output/dir
      [
            --log=/path/to/logfile
            --debug=N
      ]
```

OPTIONS

    --input, -i REQUIRED The full path to input file

    --output, -d REQUIRED Path to output dir

    --liblist, -ll REQUIRED Library list file, an output of DB-load-
    library.

    --lookupDir, -ld REQUIRED Directory of MLDBM lookup files

    --log, -l OPTIONAL Log file location

    --debug, -d OPTIONAL Debug level

    --help, -h This help message

DESCRIPTION

    DEPRICATED. Prepare and update ORF data to sync with VIROME ORF table

INPUT

    The input to this is defined using the --input. This should point to
    orf output file.

OUTPUT

    A tab delimited file with ORF detail in sync with VIROME ORF table.

EXAMPLE

```
viromeorf-prep.pl --input=/input/dir --outdir=/path/to/output/dir --
liblist=31 --lookupDir=/path/to/lookup/mldbm/dir
```

VIROMETAXONOMYXML.PL

NAME

```
      viromeTaxonomyXML.pl
```

SYNOPSIS

```
      USAGE: viromeTaxonomyXML.pl
             --input=/input/filename
             --outdir=/path/to/output/dir
             --server=server-name
             --env=dbi
             --library=libraryId
             [
                   --log=/path/to/logfile
                   --debug=N
             ]
```

OPTIONS

```
      --input, -i OPTIONAL if server and library is defined Tab delimited
      file of library and its metadata information

      --outdir, -o REQUIRED Complete path to output dir.

      --server, -s OPTIONAL if input and/or server is defined.
      Server/database name where library data is stored

      --library, -l OPTIONAL if input and/or server is defined. Specific
      library ID

      --env, -e REQUIRED Specific environment where this script is executed.
      Based on these values DB connection and file locations are set.

              Currently supports
                  igs
                  dbi
                  ageek or
                  test

      --log, -l OPTIONAL Log file location

      --debug, -d OPTIONAL Debug level

      --help, -h This help message
```

DESCRIPTION

```
      Create hierarchical XML document of all Uniref functional ORFs
```

INPUT

```
      The input is defined with --server,  --library.
```

OUTPUT

```
      XML output per library of taxonomic information
```

e.g.:

```
<root>
<DOMAIN LABEL="Archaea" NAME="Archaea" VALUE="2"
IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_1">
  <KINGDOM LABEL="Archaea" NAME="Archaea" VALUE="2"
  IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_2">
     <PHYLUM LABEL="Euryarchaeota" NAME="Euryarchaeota" VALUE="2"
     IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_3">
        <CLASS LABEL="Thermoplasmata" NAME="Thermoplasmata" VALUE="2"
        IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_4">
           <ORDER LABEL="Thermoplasmatales" NAME="Thermoplasmatales"
           VALUE="2" IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_5">
              <FAMILY LABEL="Thermoplasmataceae"
              NAME="Thermoplasmataceae" VALUE="2"
              IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_6">
                 <GENUS LABEL="Thermoplasma" NAME="Thermoplasma"
                 VALUE="2" IDFNAME="TAXONOMY_IDDOC_27.xml"
                 TAG="TAG_7">
                    <SPECIES LABEL="volcanium" NAME="volcanium"
                    VALUE="2" IDFNAME="TAXONOMY_IDDOC_27.xml"
                    TAG="TAG_8">
                       <ORGANISM LABEL="Thermoplasma volcanium
                       GSS1" NAME="Thermoplasma volcanium GSS1"
                       VALUE="2" IDFNAME="TAXONOMY_IDDOC_27.xml"
                       TAG="TAG_9" />
                    </SPECIES>
                </GENUS>
              </FAMILY>
           </ORDER>
        </CLASS>
     </PHYLUM>
  </KINGDOM>
</DOMAIN>
<DOMAIN LABEL="Bacteria" NAME="Bacteria" VALUE="369"
IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_10">
  <KINGDOM LABEL="Bacteria" NAME="Bacteria" VALUE="369"
  IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_11">
     <PHYLUM LABEL="Bacteroidetes" NAME="Bacteroidetes" VALUE="342"
     IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_12">
        <CLASS LABEL="Bacteroidia" NAME="Bacteroidia" VALUE="340"
        IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_13">
           <ORDER LABEL="Bacteroidales" NAME="Bacteroidales"
           VALUE="340" IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_14">
              <FAMILY LABEL="Bacteroidaceae" NAME="Bacteroidaceae"
              VALUE="173" IDFNAME="TAXONOMY_IDDOC_27.xml"
              TAG="TAG_15">
                <GENUS LABEL="Bacteroides" NAME="Bacteroides"
                VALUE="167" IDFNAME="TAXONOMY_IDDOC_27.xml"
                TAG="TAG_16">
                    <SPECIES LABEL="UNKNOWN SPECIES" NAME="UNKNOWN
                    SPECIES" VALUE="53"
                    IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_17">
```

```
                                      <ORGANISM LABEL="Bacteroides fragilis"
                                      NAME="Bacteroides fragilis" VALUE="1"
                                      IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_18"
                                      />
                                      <ORGANISM LABEL="Bacteroides sp. 2_2_4"
                                      NAME="Bacteroides sp. 2_2_4" VALUE="13"
                                      IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_19"
                                      />
                                      <ORGANISM LABEL="Bacteroides sp. 9_1_42FAA"
                                      NAME="Bacteroides sp. 9_1_42FAA" VALUE="15"
                                      IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_20"
                                      />
                                      <ORGANISM LABEL="Bacteroides sp. D1"
                                      NAME="Bacteroides sp. D1" VALUE="23"
                                      IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_21"
                                      />
                                      <ORGANISM LABEL="Bacteroides sp. D4"
                                      NAME="Bacteroides sp. D4" VALUE="1"
                                      IDFNAME="TAXONOMY_IDDOC_27.xml" TAG="TAG_22"
                                      />
                                    </SPECIES>
                                    <SPECIES LABEL="caccae" NAME="caccae"
                                    VALUE="34" IDFNAME="TAXONOMY_IDDOC_27.xml"
                                    TAG="TAG_23">
                                       <ORGANISM LABEL="Bacteroides caccae ATCC
                                       43185" NAME="Bacteroides caccae ATCC 43185"
                                       VALUE="34" IDFNAME="TAXONOMY_IDDOC_27.xml"
                                       TAG="TAG_24" />
                                    </SPECIES>
                                    <SPECIES LABEL="capillosus" NAME="capillosus"
                                    VALUE="1" IDFNAME="TAXONOMY_IDDOC_27.xml"
                                    TAG="TAG_25">
                                       <ORGANISM LABEL="Bacteroides capillosus
                                       ATCC 29799" NAME="Bacteroides capillosus
                                       ATCC 29799" VALUE="1"
                                       IDFNAME="TAXONOMY_IDDOC_27.xml"
                                       TAG="TAG_26" />
                                       ...
                                       ...
                                       ...
                       ...
                       ...
                    </root>
```

EXAMPLE

```
    viromeTaxonomyXML.pl --server=calliope --env=dbi --library=31
```