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Genetic elements of microbes: a comprehensive and integrated genomic database application

Ashlee Benjamin

Genetic Elements of Microbes:
A Comprehensive and Integrated Genomic Database Application

Approved: _____

Director of Bioinformatics

_____

Head, Department of Biological Sciences

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Ashlee Benjamin
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Abstract

The increasing abundance of genomic data has led to the creation of several databases containing the sequence data, metadata about the sequences and information about the organisms. These data are useful in many areas of biological research. Of the many available databases, few contain a significant amount of genome-associated data. In an effort to create a comprehensive microbial genomes database, the Genetic Elements of Microbes (GEM) database application was created. A K-mer analysis tool was also created and added the GEM application to provide an analysis of sequence composition and potential Lateral Gene Transfer (LGT) identification. The GEM application was designed to be convenient to maintain and extend. The K-mer analysis tool's ability to identify islands and to identify LGT events was tested with comparisons to published works. The GEM database application provides another source of genomic sequence and genome-associated data for the scientific community. The K-mer analysis addition provides an easy-to-customize tool to identify regions of dissimilarity and identify potential LGT events. The GEM application interface is publicly accessible at http://bucatini.bioinformatics.rit.edu/~amb4541/cgi-bin/GEMSearch.cgi. The standalone K-mer analysis interface is available at http://bucatini.bioinformatics.rit.edu/~amb4541/cgi-bin/KmerAnalysis.cgi.

Thesis Committee

1. Committee Co-Advisor
   Justin Johnson
   Bioinformatics Manager
   J. Craig Venter Institute

2. Committee Co-Advisor
   Dr. Gary Skuse
   Director of Bioinformatics
   Professor of Biological Sciences
   Rochester Institute of Technology

3. Committee Member
   Dr. David Lawlor
   Associate Professor of Biological Sciences
   Rochester Institute of Technology

4. Committee Member
   Dr. Shibu Yooseph
   Senior Computational Scientist
   J. Craig Venter Institute

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Introduction

Over the past decade, the sequencing of organisms' entire DNA repertoire has exploded with thousands of genomes now available. Sequence data aside, each genome has a significant amount of associated sequence metadata, experimental conditions, organism characteristics, and environmental information. Genomic sequences and this associated data have countless applications in biological research, each application with its own individual data needs. The needs of researchers even vary in terms of sequence data representation; some may need the genome scaffolds while others need the sequences organized by genetic element e.g. chromosomes and plasmids. With so much information and several ways of organizing the sequence data, naturally there are several genomic databases, each with its own advantages and disadvantages.

As one of the most well-known resources in the biological research community, it is no surprise that the NCBI (National Center for Biotechnology Information) has a genomes database [1]. The NCBI Genomes database stores records as genome projects. Each project page provide links to sequence information organized by genetic elements. Sequence length, GC content, number of proteins, and number of RNA's are also given for each genome. NCBI Genome pages have a link to the GOLD (Genomes Online Database) entry [2]. Each page also allows users to browse other genomes at each level of the taxonomy. Most projects have some text describing the organism, associated diseases, environmental information, etc. This information is important and useful, but displaying it in paragraph form may be somewhat disadvantageous.

The Genomes Online Database is a source for both completed and in-progress genome projects. GOLD has a significant amount of additional information for each genome, as well as several links to other genomic databases. Like NCBI, GOLD allows users to browse other genomes at each level of the taxonomy. GOLD does not provide sequence data directly, but links to NCBI and other genomic databases. However, some links are to genomic scaffold pages, others are to genome pages organized by genetic element. This inconsistency may prevent GOLD from being ideal for several research needs.

The Integrated Microbial Genomes (IMG) database is an extremely comprehensive genomic data source [3]. IMG allows users to browse genomic data by genes, full genomes, and biological function. Each genome page has a vast amount of metadata, links to external sources, chromosome maps and other information. Like several other genomic databases, users may browse other genomes at all taxonomy levels. IMG allows users to obtain sequence data as scaffolds, genes, and intergenic sequences. Metadata information is presented in a systematic and concise way, making IMG a very attractive option for computational data retrieval.

Genome Reviews is an European Bioinformatics Institute (EBI) genomic database that provides access to sequences organized by genetic element [4]. Genome Reviews also provides gene and protein information. No metadata or links to external sources are provided, making Genome Reviews useful only for those who need annotated genomic sequence data and no additional information.

The EBI Genomes Server is another EBI genomic database, but is more comprehensive than Genome Reviews [5]. The genomic sequences are organized by genetic

element and the length of each sequence is displayed. Each genome has a link to the Integr8 proteomics database. No additional metadata or links to external data sources are provided.

Integr8 is yet another EBI database and contains the most information by far of the three EBI data sources [6]. Integr8 is a genomics and proteomics database that contains completed genome projects and their associated proteomes. Each genome page has a description of the organism with metadata in paragraph form. Sequence composition and protein statistics are provided for each genetic element and the genome as a whole. Genomic sequence information is organized by genetic element. Integr8 provides DNA and amino acid sequences for genes, while some genome pages also contain orthology, paralogy, and synteny information.

Even all of these well-known genomic databases leave something to be desired in terms of integration of metadata. One feature that seems to be lacking from all of the aforementioned databases is the ability to browse genomes that share metadata. An epidemiologist looking for all microbes involved with a certain disease, or an environmentalist interested in extremophiles must rely on literature searching. GOLD and IMG come close by listing this information in a concise way. However, GOLD's inconsistency in the organization of the sequence data provided by their links can be disadvantageous in many research needs. In an attempt to provide the ability to search by metadata and sequence data organized by genetic element, a comprehensive microbial genomes database was created. The Genetic Elements of Microbes (GEM) database provides metadata information, FASTA sequence files, and Genbank files. GEM also provides a direct link to a K-mer analysis tool, thereby allowing researchers to easily obtain more information.

A K-mer analysis is an analysis of sequence composition and has several applications in genomics research. A K-mer is a K-length segment of DNA. Every DNA sequence has a K-mer frequency signature consisting of a set of frequency values of all possible K-mers in that DNA sequence. K-mer sequence analysis has a wide variety of applications in biological research [7]. K-mer frequency signatures have been shown to be significantly different across species. This can be useful in identifying the source of an unknown DNA sequence, perhaps from metagenomic data. While it is well-known that GC content is helpful in gene identification, K-mer frequency analyses have also proven to be informative. K-mer analyses have been used to identify lateral gene transfer [8]. Because K-mer frequency signatures are generally different across species, the frequency signatures of segments of a sequence can be compared to the organism's overall signature. If these are significantly different, there may have been a lateral gene transfer event.

Lateral gene transfer occurs when some or all of a genetic element (chromosome or plasmid) is transferred from one organism to another by means other than sexual reproduction. This is most significant when it occurs between distinct species or lineages. LGT occurs by three primary mechanisms: transformation, conjugation and transduction. Transformation involves the uptake of naked, exogenous DNA by a cell. This DNA may then be incorporated into the genome. Conjugation is the transfer of DNA from one live cell to another via direct contact and usually involves the transfer of plasmids. Transduction is the transfer of DNA from one cell to another by a virus. LGT is fairly common in prokaryotes but has also been found between prokaryotes and eukaryotes [9]. As with any genetic information, DNA acquired by LGT is subject to natural selection and genetic drift.

Advantageous transfers such as conference of antibiotic resistance or a novel metabolic process could have propagated in the population and accelerated speciation. LGT is a source of genetic diversity and therefore has played a role in evolution. Identification of LGT events can give us a better understanding of their impact on evolution, which in turn will improve our understanding of evolutionary processes in general

With the vast amount of genomic data now available, the scientific community has a better opportunity to identify LGT events via genomic analysis. Several computational methods have already been employed to identify gene transfer events, all having very unique approaches.

Phylogenetic analysis is one of the most commonly used and most reliable methods for identifying LGT events [10, 11, 12]. Phylogenetic methods rely on our current knowledge of evolutionary relationships between species. One approach has been to systematically simulate insertions and deletions, rearranging phylogenetic trees. Trees are typically built using maximum likelihood or maximum parsimony and aim to identify LGT by finding insertions and deletions to explain abnormal gene trees [13]. Some take a similar, but much more drastic approach to tree reconstruction. Horizstory is a phylogenetic approach that builds trees from scratch using a specified gene set [14]. This approach treats almost all phylogenetic detections as signal and very few as noise. Horizstory creates a vertical transfer "backbone" and builds lateral transfer events over this backbone. Another common phylogenetic approach for identifying LGT is comparative genome mapping. Comparative genome mapping is the identification of homologous sequences between genomes, classification homologs into orthologs and paralogs, and identification of sequence stretches

that do not appear to have any homology. Sequence stretches may lack homology because of insertions, deletions, duplications, or LGT. One particular comparative genomics method, MAGIC, makes use of both levels of comparative mapping [15]. MAGIC first identifies all highly homologous sequences and then investigates the remaining sequence for potential LGT events. IslandPick, another comparative genomics method automatically selects the genomes to be compared based on evolutionary distance [16]. The highly homologous regions are then identified and the remaining sequence investigated for genomic islands, or GIs. In general, phylogenetic approaches are based on a gene's abnormal similarity with otherwise dissimilar species. These analyses are very effective, but also tend to be extremely computationally intensive.

As previously stated, sequence composition analysis is commonly used to identify potential LGT events. Sequence composition analyses include GC content, codon bias, nucleotide substitutions, and K-mer frequencies. These approaches focus on regions of unusual sequence composition compared to the full genome. One particular study used nucleotide sequence composition while noting periodicity to provide knowledge of codon boundaries [17]. Like all sequence composition analyses, this study assumed that each genome has a relatively constant and unique sequence composition. Genes that were of an atypical composition were targeted as potential transfers. Another sequence composition method incorporates nucleotide substitution rates [18]. This method takes on the assumption that because different species have different nucleotide compositions, they also have different substitution rates. A gene that has been transferred would undergo a change in substitution rate after it was transferred from the genome of one species to another. This approach uses

the aforementioned hypothesis and calculates the substitution rate matrix for genes within a set of genomes, flagging genes whose rate matrices differ significantly from the other genes in that genome. Sequence composition methods are efficient and moderately effective for LGT identification.

Machine learning approaches have also been employed to identify gene transfers. One such method, SIGI-HMM, utilizes codon usage and Hidden Markov Models (HMMs) to detect genetic islands (GIs) as well as to infer a potential donor for each transfer [19]. The codon usage of each gene in a particular genome is compared with codon usage tables for a set of donors, as well as a set of highly expressed genes using HMMs. Wn-SVM is another machine learning approach that uses nucleotide composition in conjunction with a Support Vector Machine (SVM) to identify potential LGT events [20]. SVM's are machine learning algorithms that create a calculation to achieve a desired result based on input parameters. The SVM is trained using a set of known transfers and run on other genomes to detect transfers. Another common machine learning technique is the use of Bayesian classifiers. One study used a Bayesian classifier in conjunction with oligomer frequencies (i.e. K-mer frequencies) to detect transfers [21]. The classes were individual genomes and the Bayesian classifier was trained with a set of genomes to distinguish among them. A probability of finding a particular sequence belonging to a particular genome was calculated for each candidate transfer sequence. Machine learning approaches have been a recent addition to techniques for identifying LGT, but they have shown success.

Some researchers have also used combinations of these approaches to identify gene transfers. One such approach, DLIGHT, combines evolutionary distances with

multivariate normal theory [22]. DLIGHT calculates pairwise evolutionary distances between genes in different genomes and performs hypothesis testing of LGT vs. no LGT with a multivariate normal distribution. Another study combined a gene clustering method and genome position information to search for LGT events [23]. This method first utilized a gene clustering algorithm to identify foreign genes and sets of foreign genes that are similar to one another. Physical genomic position was then used to reevaluate the classes of genes and reassign genes if necessary. Yet another combinatorial approach utilized codon frequencies and log-odds scores to seek out significantly different areas that could result from transfer events [24]. The codon frequency of each gene in a particular genome was compared to the mean codon frequency of its own genome and other genomes to test relatedness. Combinatorial approaches can be very effective, but are often very stringent. Such approaches can be especially useful to confirm a suspected LGT event.

Some criticize all of the above approaches because the results may be explained by more than just LGT events [8]. Duplications, deletions, and genetic drift among other things can account for potential LGT events. Granted, no computational approach to finding LGT is completely definitive, but *in silico* analyses are faster than wet lab approaches, and they allow us to utilize the massive amounts of sequence data.

K-mer frequency analysis is one of the most common and efficient approaches used by bioinformaticists. As previously indicated, this approach does assume that K-mer frequencies are distinct features of each prokaryotic genome, like all sequence composition analyses. As ancient LGT events will have likely undergone mutation to better match the host genome, K-mer frequency analysis cannot detect all transfer events. However, this same

disadvantage brings light to an advantage of K-mer analysis in comparison to phylogenetic approaches. Requiring sequence alignments may overlook a significant portion of LGT events due to mutations that interrupt alignments. K-mer analysis may identify more ancient transfers by lifting the strict sequence conservation restraints.

There are several research applications for K-mer frequency analysis, which is why a K-mer frequency analysis tool was created and incorporated into the GEM database application. The analysis tool is linked directly with all genomes in the database and provides great flexibility with regard to algorithm parameters and analysis output. This flexibility provides researchers with an opportunity to run an analysis relevant to their research.

The GEM database and K-mer analysis tool are a beneficial contribution to the scientific research community; allowing users to browse thousands of microbial and viral genomes by genome metadata and carry out customized sequence composition analyses.

Materials and Methods

GEM Program Design

The GEM database application was developed with a Model-View-Controller (MVC) software engineering architectural pattern. MVC design separates program functionality and viewing, data manipulation, and data representation into multiple components. This design allows modification of one component without disturbing the functionality of another. The GEM database application has five main functional units: the Database, a Database Abstraction, a Data Cleanser, a Data Retriever, and the web interface. A diagram of the GEM application components and their interactions is shown in Figure 1 below.

Figure 1 - GEM Application Design

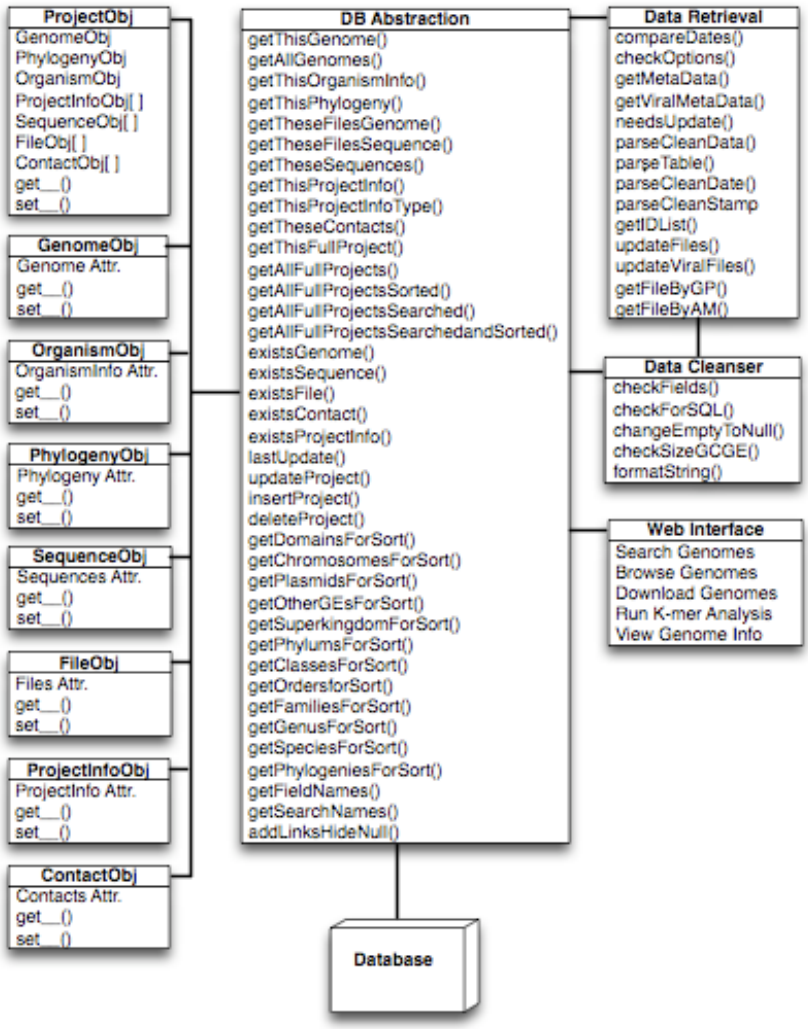


Figure 1 shows the multiple components of the GEM application. The value objects (shown on the left) are used to represent the data in the database. The Data Retrieval is used to obtain genomic data from external sources. The Data Cleanser checks and fixes data before it goes into the database. The Web Interface is where the user makes views and downloads information, and runs K-mer analyses. The Data Abstraction links the database with all other components.

The Database Abstraction component provides connections to the database, presents data from the database in a meaningful way (as value objects), and takes care of all database queries and edits. The Data Cleanser maintains Data Integrity and helps ensure the accuracy of the data going into the database. The Data Retriever is the main procedural program on the

server side, and obtains data from outside resources to update the database. Code was checked into Subversion (SVN) for all major revisions in an effort to adhere to standard Software Engineering practices. All components were written in Perl and are described in further detail below.

Database Design

The data for GEM is stored in a mySQL database. The database schema is shown in Figure 2.

Figure 2 - GEM Database Schema

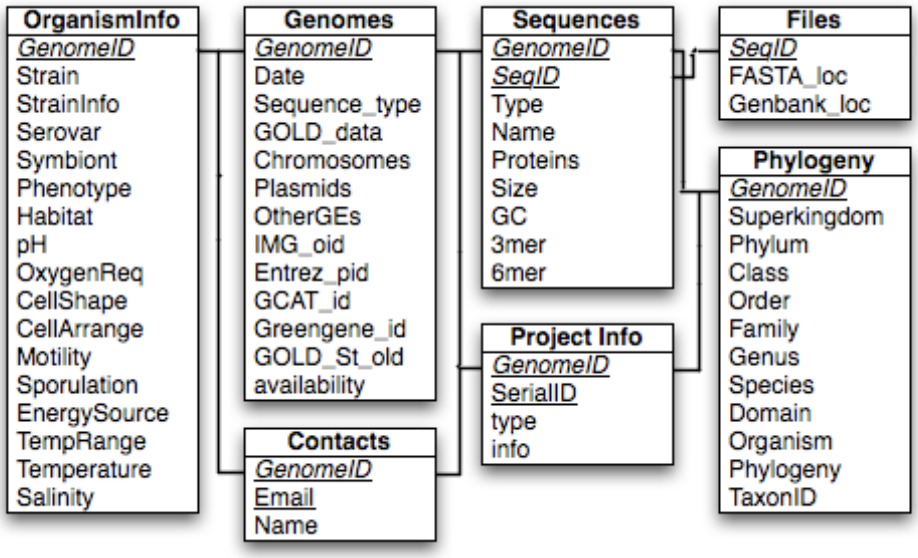


Figure 2 shows the database schema for GEM. There are seven tables containing information about the organism, information about the overall genome, sequences and their associated metadata, files for each sequence, phylogeny for the organism, contacts for the project, and miscellaneous project information.

The data is normalized into seven tables. These tables store a wide variety of information as well as file system locations for the sequence and Genbank files. The main primary key in most tables is the Genome ID. This key is either the GOLD Stamp ID from GOLD, or the

NCBI Genome ID from the Viral Genomes Database. The type of ID is specified in the Genomes table, Sequence_type attribute. The Genomes table also includes general information about the project and the number of genetic elements. The date in the Genomes table is the date this entry was last updated in GEM. The IMG_oid, Entrez_pid, GCAT_id, Greengene_id, and GOLD_St_old are all identifiers for external genomic databases. Availability is simply whether the genome project is public or private. The GOLD_data in the Genomes table is a string containing the original IDs from GOLD (not necessarily IDs split by chromosome or plasmid). The OrganismInfo table contains a significant amount of metadata for that particular organism. The Phylogeny table contains taxonomy information at all levels for that particular organism, as well as NCBI's taxon ID. The Contacts table simply contains a name and an e-mail address or website for the contacts of the genome project. In the Sequences table, the NCBI sequence ID is used in conjunction with the Genome ID to form a unique composite key. Type refers to the type of genetic element and is either chromosome, plasmid, or other. The sequence name, number of proteins, size, GC content and the 3-mer and 6-mer signatures are also stored. The Files table is connected to the Sequences table via the sequence ID and only contains the file system paths to the FASTA and Genbank files for that sequence. The ProjectInfo table is slightly different. Because of the variety of information and the abundance of many-many relationships in GOLD, the sequencing method, sequencing depth, isolation, country, databases, institute, funding, and publications information are condensed into a single table. Each entry in the project info table will have a Genome ID, a serial ID, an information type, and information. Using databases as an example, there can be many databases for a single genome. The type in the

ProjectInfo entry would be database (or institute, publication, etc). The serial ID is an auto-increment integer to identify a single database entry for that genome. If we had 2 database entries for genome Gc123456, the genome ID, type, and serial IDs (1 and 2) would form a composite primary key for the two entries, where info would contain the name of each database. Each Genome has a single entry each in the Genomes, Phylogeny, and OrganismInfo tables, and may have many entries in the Sequences, Files, ProjectInfo, and Contacts tables.

Value Objects

Value Object classes were created to represent the data in a meaningful way for the rest of the components. As shown in Figure 1, there are eight value objects: one for each table in the database, and one that combines all other value objects into one single genome project. This Project object has a Genome Object, Organism Info Object, Phylogeny Object, and lists of Contact Objects, Sequence Objects, File Objects, and Project Info Objects. These Value Objects are used by the Data Retriever, Data Cleanser, and Web Interface to interact with the Data Abstraction, and are used by the Data Abstraction to form SQL statements to interact with the database.

Data Abstraction

The Database Abstraction component handles database queries, inserts, updates, and deletes. All SQL statements are contained within this layer. The Data Abstraction functions include genome insertion, genome updates, genome deletes, checks to see if a genome or other object exists in the database, checks to see when a genome entry was last updated, value object retrievals by Genome ID, queries to build the options for the Web

Interface, and queries to carry out user searches. The Data Retriever obtains genomic information and calls the insert and update methods of the Data Abstraction to update the database. Any data coming in to the Database Abstraction Layer will be passed through the Data Cleanser component to ensure its integrity.

Data Retrieval and Sources

The Data Retrieval component obtains genomic metadata and sequence data in two main steps: gathering a list of genomes and their associated metadata, and then sequence retrieval. For the first step, a different procedure and data source are used for Viruses than for Archaea and Bacteria. When retrieving the genome list and metadata for Archaea and Bacteria, GOLD is used as the resource [2]. Because GOLD has so much metadata for each genome, it is a fantastic reference. Unfortunately, GOLD does not store Viral Genomes. For this reason, the source of Viral Genome information is the Entrez Genomes Database [1]. This database contains limited metadata, but is a reliable resource for a recently completed Viral genomes. The list and metadata retrieval for both types of genomes is carried out using the curl command in unix. This command obtains the html source of a web address. The html is then parsed for the desired information. The second step is the same for both genome types; the sequence data for each genetic element of each genome is retrieved from NCBI via BioPerl. The Data Retriever is run via a cron job weekly to update the database. If any new genomes from GOLD or NCBI are encountered, they are inserted into the database. If any of the genomes have been updated since the date in the database, that genome entry is updated. Some of the metadata fields retrieved from GOLD contain multiple entries separated by commas. This text is split into multiple Project Information Objects within the single Project

Object. These metadata types include disease, industrial relevance, database, institution, and country.

Data Cleanser

The Data Cleanser component is a layer that checks (and fixes if necessary) any data going into the Data Abstraction layer for subsequent insertion into the database. Data going in must be checked to ensure that all necessary information is present, does not contain SQL insertions, and is as accurate as possible. The Data Cleanser examines a Project Object to make sure all required fields (such as Genome ID) are present. If a Project passes this check, all allowed undefined values are changed to NULL to allow the Data Abstraction to insert or update this Project. The Data Cleanser also removes all leading and trailing whitespace on the text fields within the Project Object. This prevents problems with inserting the information in the database as well as displaying the information in the Web Interface. The Data Cleanser also checks each sequence in the genome project to make sure the GC content and size values are accurate. The Genome Object is also examined to ensure the accuracy of the genetic element counts based on the sequence type attribute of each Sequence Object. The final check before allowing access to the Data Abstraction component is a screen for SQL injections. Although unlikely, it is possible that a Project may contain characters or phrases that could compromise the integrity of the database by incorrectly modifying or even deleting data. Only when a Project passes this screening is it allowed to access the database via the Data Abstraction.

Web Interface

The web interface for the GEM database application was written using Perl CGI, incorporating some additional html and CSS. Like the other components, the web interface uses Value Objects and Data Abstraction methods. Refer to the Results and Discussion sections for functionality and features of the interface.

K-mer Analysis

The K-mer analysis is an add-on to the GEM database application. A suite of programs all written in Perl carry out a sequence composition analysis, identify K-mer islands, plot differences in sequence composition, and compare island signatures to other genetic elements in the GEM database.

K-mer Analysis Algorithm

Running the K-mer analysis has four main steps: calculating the overall K-mer frequency vector for the sequence, determining thresholds for island identification, scanning the sequence for islands, and refining the island boundaries. The first step when calculating the overall K-mer frequency vector is to identify a list of all possible K-mers. This list is then trimmed of one of each of the reverse complement pairs. Only one of the pairs is counted to avoid strand bias. Then, the K-mers are counted for the entire sequence, overlapping included. For example, if we were to count the 2-mers in the sequence "AAA", there would be 2 "AA" 2-mers. Once we have the counts, we divide each by the total number of possible K-mers, giving us a frequency vector whose sum is one. A detailed example is shown in Figure 3 below.

Figure 3 - K-mer Signature Example

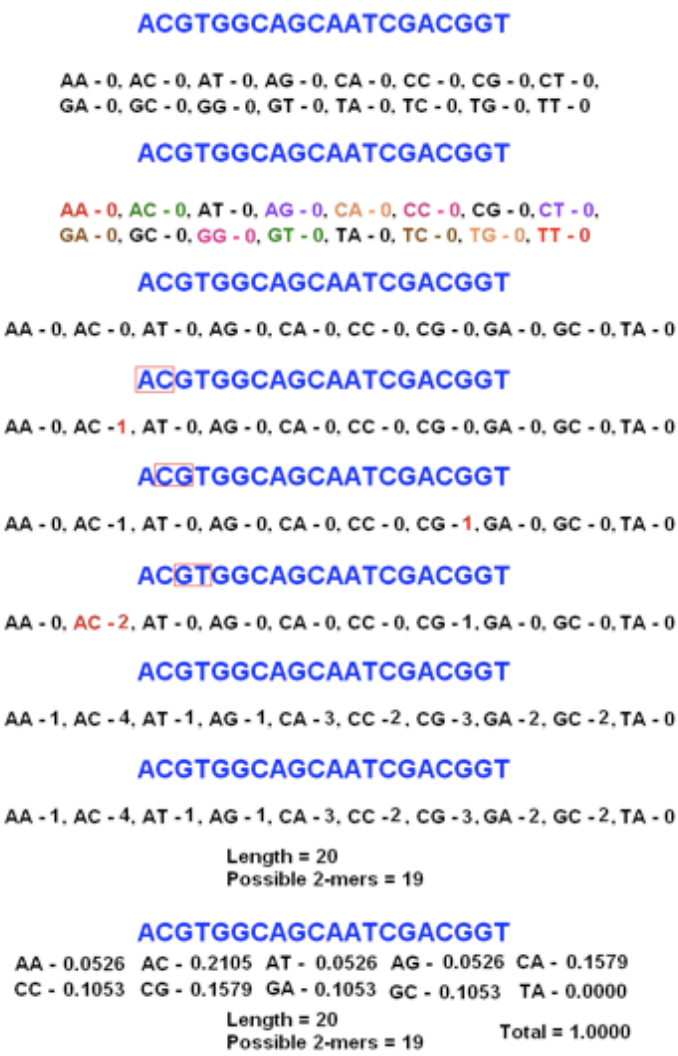


Figure 3 shows a step-by-step example of a K-mer signature calculation. First, all possible 2-mers are identified. Then, all reverse compliment pairs are removed. The 2-mers are then counted, and the each value is divided by all possible 2-mers to produce a frequency vector.

The example in Figure 3 shows the calculation of the 2-mer frequencies of a short sequence. In the first step, all possible 2-mers are identified, and there are sixteen possibilities. In the second step, all reverse compliments are identified and highlighted in matching colors. These possibilities are then removed from the vector, resulting in ten possibilities in the third step.

Next, first 2-mer in the sequence is obtained (AC) and the matching vector position is incremented. This is highlighted by the red box on the sequence and the AC count changed from zero to one. The next step in the figure is similar, identifying the next 2-mer and incrementing the CG count to reflect its presence. In the following step a 2-mer that was deprecated earlier in the process is encountered. In this case, the count of the reverse complement which in this instance is AC gets incremented. This counting process continues until the end of the sequence is reached. Finally, it is determined that for a sequence of length 20, there are 19 possible 2-mers. Dividing each count by 19 results in the K-mer frequency vector, or K-mer signature. After determining the K-mer signature for the sequence the thresholds for island identification are determined. The algorithm uses a Monte Carlo style approach to determining thresholds. Several random sequences of the same length and sequence composition are generated and analyzed. To analyze a sequence, the frequency vector of a segment of the given window size is calculated starting at the beginning of the sequence. The Euclidean distance between this vector and the frequency vector for the overall sequence is calculated. This process continues for segments of the same window size, sliding the specified number of base pairs for the remainder of the sequence. This process is illustrated in Figure 4 below.

Figure 4 - K-mer Signature Distance Analysis Example

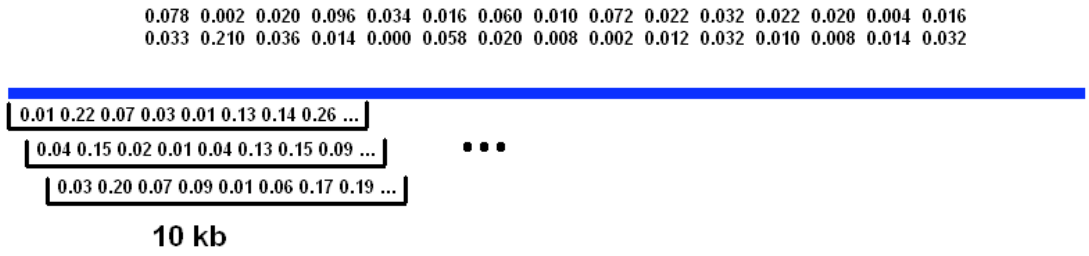


Figure 4 shows an example of a K-mer signature distance analysis. K-mer frequency vectors are calculated along the sequence for segments of a specified window size. The distance between the K-mer frequency vector of each segment and the frequency vector of the overall sequence is calculated.

If the window size for an analysis is 10kb, the K-mer signature is calculated for the first 10,000 base pairs using the method shown in Figure 3 above. The Euclidean distance between the K-mer signature for the full sequence is calculated. This 10kb window then "slides" down the sequence by the specified slide value. If the slide value was 1,000, the first window would be positions 1-10,000, the second would be positions 1,001-11,000, and so on. When determining thresholds, all of the calculated distance values are stored. The average and standard deviation of those distances is calculated. Using the specified percentile threshold, the threshold for island identification is set to be the average plus or minus a certain number of standard deviations. The number of standard deviations away from the mean is based on the placement of the threshold percentage on the normal curve. This threshold for islands is calculated for the initial scan as well as the rescan using the appropriate window size and threshold percentile. Once the thresholds are determined, the sequence is scanned for islands. To identify islands, each sequence is analyzed in the same

manner as the random sequences in the threshold determination step - calculating vectors for segments of the specified window size and calculating the distance from the overall sequence vector. If the distance is above the initial scan threshold, this segment is stored for subsequent refinement. Once the initial scan is complete, the island boundaries are refined. The segments that were above the initial scan threshold are then analyzed a second time with a 10X smaller window size. If the distance of a segment is above the rescan threshold, that segment is (or is part of) an island. Segments above the threshold that are directly adjacent to one another (or overlapping) are automatically joined. However, if an island is within a specified number of base pairs of another island those two islands will be merged. All parameters which include the K value, window size, slide value, threshold percentiles, and the merge threshold are specified by the user.

Sequence Composition Distance Plots

Plots of the distances between the window K-mer signatures and the overall sequence signature are generated using GNUplot. The frequency output file is re-formatted to serve as an input file for GNUplot and the initial scan distances, rescan distances, initial scan threshold, and rescan threshold are plotted. A sample plot is shown in Figure 5 below.

Figure 5 - Sample K-mer Signature Distance Plot

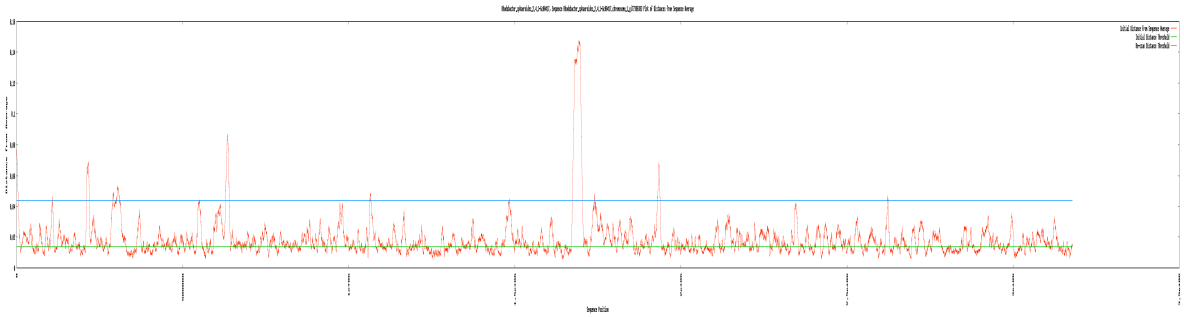


Figure 5 shows a sample plot of distances between segment K-mer signatures and the full sequence signature. The sequence position is shown on the x-axis and the Euclidean distance is shown on the y-axis.

Island Signature Comparisons

The K-mer signatures of identified islands can be compared with the full sequence K-mer signatures of all genetic elements in the GEM database. The distance between the K-mer signature of the island and the K-mer signature of each genetic element is calculated. All comparisons may be stored and written to the output file. However, the top three matches are output by default. This is currently only available for 3-mers and 6-mers, as those are the signatures stored in the database.

Web Interface

Like the web interface for the GEM database application, the K-mer analysis interface was written using Perl CGI, html and CSS. Refer to the Results and Discussion sections for functionality and features of the interface.

System Information

This application currently runs on an x86_64 Linux server at the Rochester Institute of Technology. Versions 5.8.6, 1.6, and 4.1.20 are used for Perl, BioPerl, and mySQL, respectively.

LGT Identification with K-mer Analysis

To test the validity of using this K-mer analysis to identify LGT events, six different analyses were carried out on genomes and gene sets with previously established LGT or genomic islands. The first comparison was completed with 13 bacterial genomes for four genes known to be products of LGT. The genomes were *Buchnera aphidicola* APS, *Escherichia coli* K12, *Haemophilus influenzae* rd, *Pseudomonas aeruginosa* PAO1, *Pasteurella multocida* Pm70, *Salmonella typhimurium* LT, *Vibrio cholerae*, *Wigglesworthia brevipalpis*, *Xanthomonas axonopodis*, *Xanthomonas campestris*, *Xylella fastidiosa*, *Yersinia pestis*, and *Yersinia pestis* KIM. The genes were ileS, bioB, mviN, and tadA. The genomes were run through the K-mer analysis with a K value of 3, window size of 10,000, slide value of 100, and threshold percentiles of 99.99. A BLASTN search for each gene in each species was performed on the set of island sequences to determine if the genes, or segments of the genes had been identified. The second analysis was run on *Neisseria meningitidis* to search for sodC, bioC, a conserved hypothetical protein, Type III Restriction enzyme, Type III methyltransferase, and Virulence Associated Protein, all LGT events from *Haemophilus influenzae*. The analysis was carried out in the same manner as the first. The third analysis was very similar to the previous analyses, except it was carried out with *Wolinella succinogenes* searching for a genomic island at the tRNAMet gene, the nif genes, and for

synteny with the pVir plasmid. Very similar to the first three, the fourth analysis searched for genomic islands at Tn4371, glyV, and the clc element in *Pseudomonas putida* KT2440. The remaining two analyses were a comparison of genomic islands identified by the K-mer analysis method (same parameters as all other analyses) and those published by other researchers. These analyses were for *Escherichia coli* CFT073 and *Thermotoga maritima* MSB8. All sequence data was obtained from NCBI [1].

Results

Genomic Data

The GEM Database currently contains 3,016 genomes. Fifty three of those genomes are Archaea, 764 are Bacteria, and 2,199 are Viruses.

GEM Interface

The GEM Interface provides the user with three main functions: browsing genomes (searching or browsing all), downloading genomes, and running a K-mer analysis on a genome. Figure 6 shows the home page for the GEM database application.

Figure 6 - GEM Database Application Home Page

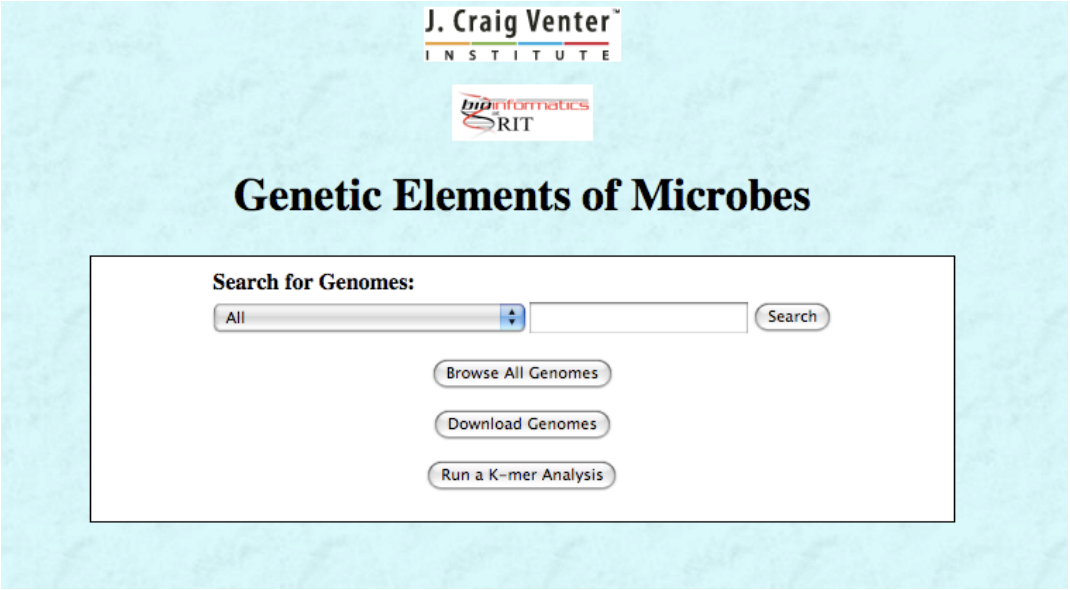


Figure 6 shows a screenshot of the GEM Database Application Home Page.

Both the "Search" button and the "Browse All Genomes" button will bring the user to a page similar to the one shown in Figure 7.

Figure 7 - GEM Database Application Browsing Page

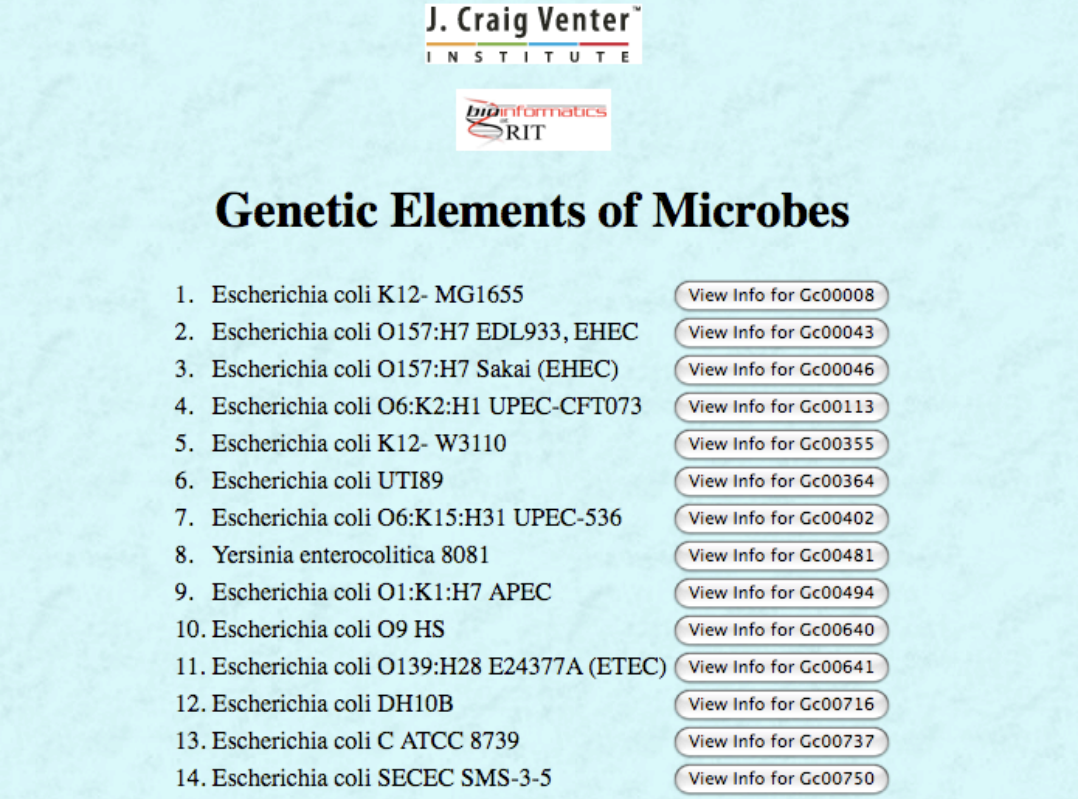


Figure 7 shows a screenshot of the GEM Database Application Browsing Page.

This is the standard browsing page. The organism name and strain are listed with a button to bring up that genomes information page. If this page is reached via the “Browse All Genomes “ button, all genomes in the database will be displayed. If the user arrives at this page from a search, only genomes matching their search criteria will be present. Clicking on the “View Info” button for a genome, brings up the information page. An example is shown in Figure 8.

Figure 8 - GEM Database Application Genome Information Page

J. Craig Venter INSTITUTE

bioinformatics RIT

Genetic Elements of Microbes

Download Genome Gc00737 | Analyze K-mers for Gc00737

General Information

Organism	Escherichia coli C	N/A
Domain	BACTERIAL	Browse Other BACTERIAL Genomes
Superkingdom	Bacteria	Browse Other Bacteria Genomes
Phylum	Proteobacteria	Browse Other Proteobacteria Genomes
Class	Gammaproteobacteria	Browse Other Gammaproteobacteria Genomes
Order	Enterobacteriales	Browse Other Enterobacteriales Genomes
Family	Enterobacteriaceae	Browse Other Enterobacteriaceae Genomes
Genus	Escherichia	Browse Other Escherichia Genomes
Species	Escherichia coli	Browse Other Escherichia coli Genomes
Phylogeny	PROTEOBACTERIA-GAMMA	Browse Other PROTEOBACTERIA-GAMMA Genomes
Chromosomes	1	N/A
Plasmids	0	N/A
Other Genetic Elements	0	N/A
Strain	ATCC 8739	N/A
Habitat	Host	Browse Other Host Genomes
Oxygen Requirements	Facultative	Browse Other Facultative Genomes
Cell Shape	Rod-shaped	Browse Other Rod-shaped Genomes
Cell Arrangement	Pairs, Singles	Browse Other Pairs, Singles Genomes
Motility	Motile	Browse Other Motile Genomes
Sporulation	Nonsporulating	Browse Other Nonsporulating Genomes
Temperature Range	Mesophile	Browse Other Mesophile Genomes
Availability	Public	N/A
GOLD Data	NC_010468	N/A
IMG OID	641522623	N/A
Taxon ID	481805	N/A
Entrez PID	18083	N/A
GCAT ID	003790_GCAT	N/A

Project Information

Info Type	Info	Browse Other Genomes
Institute	DOE Joint Genome Institute	Browse Other DOE Joint Genome Institute Genomes
Project Status	Complete and Published	Browse Other Complete and Published Genomes
Sequencing Status	Complete	Browse Other Complete Genomes
Database	NCBI	Browse Other NCBI Genomes
Database	DOE Joint Genome Institute	Browse Other DOE Joint Genome Institute Genomes
Publication	Unpublished	Browse Other Unpublished Genomes
Funding	DOE	Browse Other DOE Genomes

Sequences

Sequence ID	Sequence Type	Sequence Name	Proteins	Size	GC Content
CP000946	Chromosome	Chromosome	0	4746218	0.508665215124969

Contacts

Name	Email Address
Ingram L	ingram@ufl.edu

Figure 8 shows a screenshot of a sample GEM Database Application Genome Information Page.

The information page displays all data from the database for that genome. Some of the data have “Browse Other Genomes” buttons. These buttons carry out a search for genomes sharing that data point and displays them on the browsing page shown in Figure 7. Clicking

the “Download Genomes” button on the home page in Figure 6 brings up the genome selection page shown in Figure 9.

Figure 9 - GEM Database Application Genome Selection Page

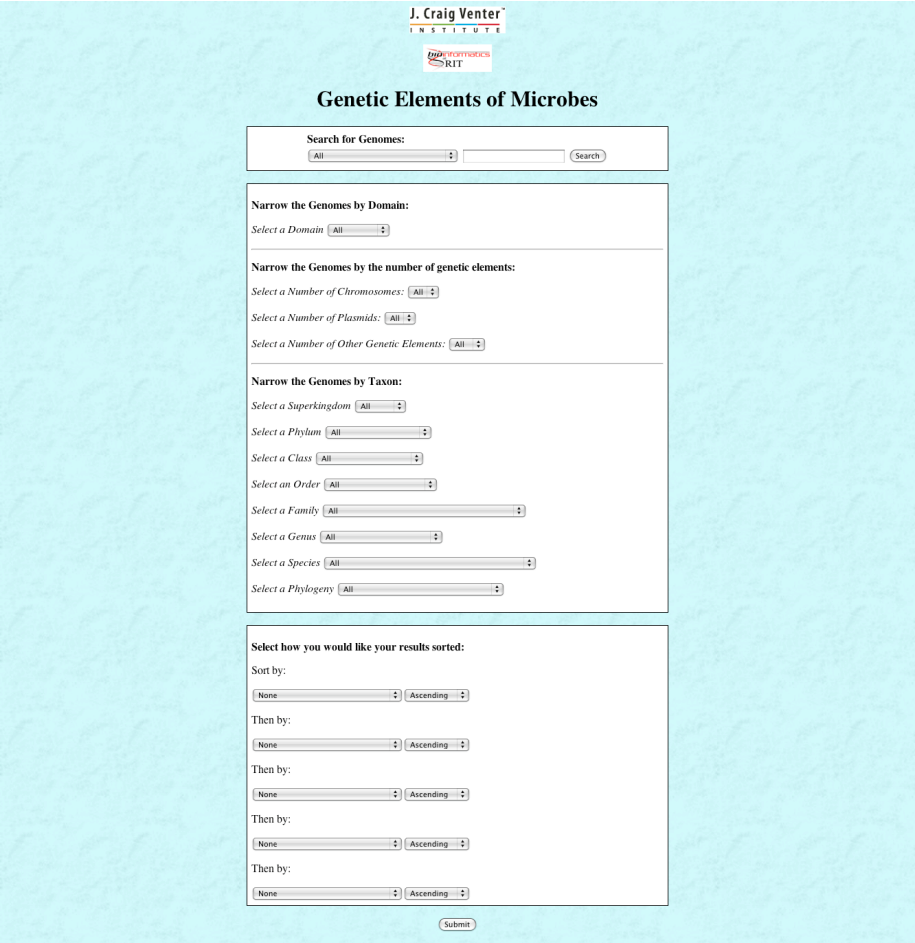
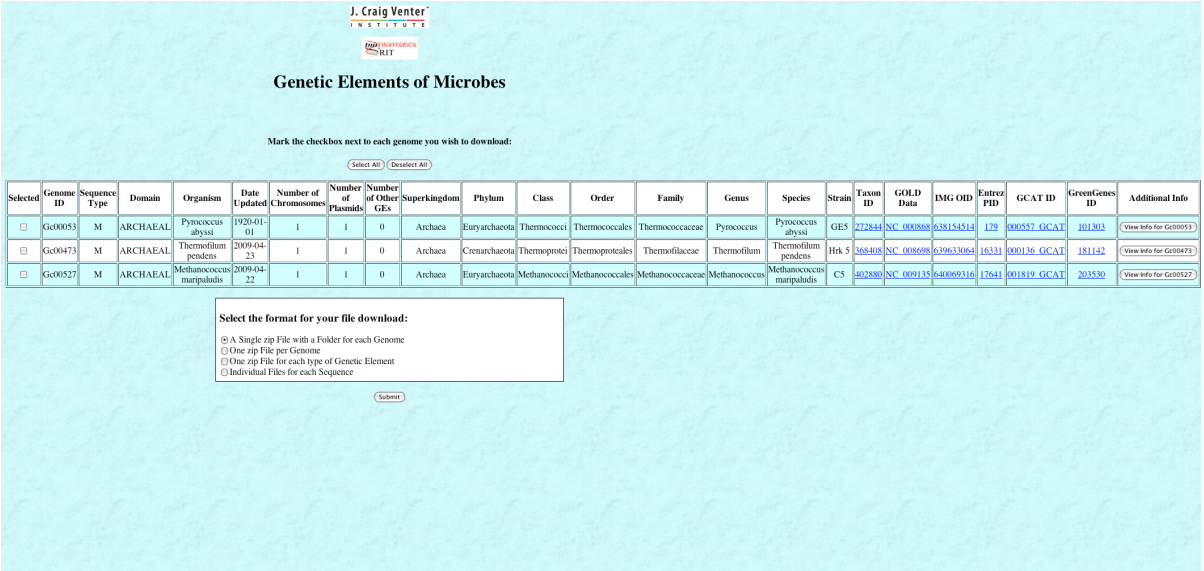


Figure 9 shows a screenshot of the GEM Database Application Genome Selection Page.

The genome selection page has genome properties from the database for narrowing and sorting the genomes that appear on the download selection page. Searching for genomes to appear on the download selection page is also allowed. The download selection page is shown in Figure 10.

Figure 10 - GEM Database Application Download Selection Page



Selected	Genome ID	Sequence Type	Domain	Organism	Date Updated	Number of Chromosomes	Number of Plasmids	Number of Other GEs	Superkingdom	Phylum	Class	Order	Family	Genus	Species	Strain	Taxon ID	GOLD Data	IMG OID	Entrez PID	GCAT ID	GreenGenes ID	Additional Info
☐	Gc00053	M	ARCHAEAL	Pyrococcus abyssi	1920-01-01	1	1	0	Archaea	Euryarchaeota	Thermococci	Thermococcales	Thermococcaceae	Pyrococcus	Pyrococcus abyssi	GE5	272844	NC_000868	638154514	179	000557_GCAT	101303	View Info for Gc00053
☐	Gc00473	M	ARCHAEAL	Thermofilum pendens	2009-04-23	1	1	0	Archaea	Crenarchaeota	Thermoprotei	Thermoproteales	Thermofilaceae	Thermofilum	Thermofilum pendens	Hrk 5	368408	NC_008698	639633064	16331	000136_GCAT	181142	View Info for Gc00473
☐	Gc00527	M	ARCHAEAL	Methanococcus maripaludis	2009-04-22	1	1	0	Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanococcaceae	Methanococcus	Methanococcus maripaludis	C5	402880	NC_009135	640069316	17641	001819_GCAT	203530	View Info for Gc00527

Figure 10 shows a screenshot of the GEM Database Application Download Selection Page.

The download selection page displays genomes matching the criteria specified on the genome selection page. Select information from the database is displayed, along with a button leading to the information page (see Figure 8) for each genome. Once the user selects one or more genomes, chooses a download format, and clicks submit, a new page with a link to download the results appears. The download page is shown in Figure 11.

Figure 11 - GEM Database Application Download Results Page

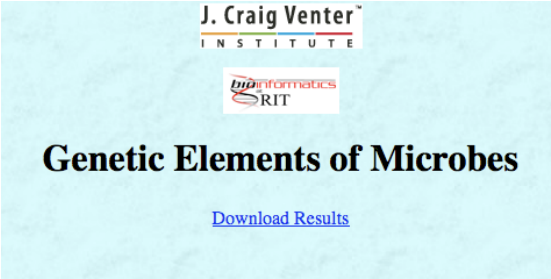
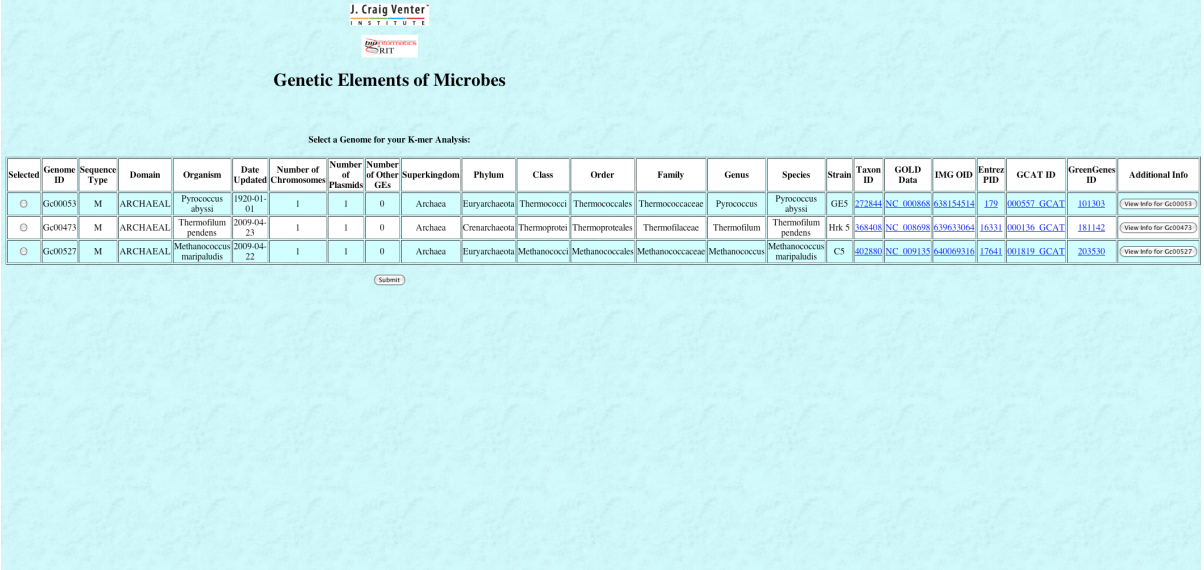


Figure 11 shows a screenshot of the GEM Database Application Download Results Page.

Running a K-mer analysis on with the GEM Interface is very similar to downloading a set of genomes. The "Run a K-mer Analysis" button on the home page (shown in Figure 6) leads to the same genome selection page shown in Figure 9. When the criteria is specified, the matching genomes are displayed in the K-mer analysis selection page. This page is shown in Figure 12 below.

Figure 12 - GEM Database Application K-mer Analysis Selection Page



Selected	Genome ID	Sequence Type	Domain	Organism	Date Updated	Number of Chromosomes	Number of Plasmids	Number of Other GEs	Superkingdom	Phylum	Class	Order	Family	Genus	Species	Strain	Taxon ID	GOLD Data	IMG OID	Entrez PID	GCAT ID	GreenGenes ID	Additional Info
○	Gc00053	M	ARCHAEAL	Pyrococcus abyssi	1920-01-01	1	1	0	Archaea	Euryarchaeota	Thermococci	Thermococcales	Thermococcaceae	Pyrococcus	Pyrococcus abyssi	GE5	272844	NC_000868	638154514	179	000557_GCAT	101303	View Info for Gc00053
○	Gc00473	M	ARCHAEAL	Thermofilum pendens	2009-04-23	1	1	0	Archaea	Crenarchaeota	Thermoprotei	Thermoproteales	Thermofilaceae	Thermofilum	Thermofilum pendens	Hrk 5	368408	NC_008698	639633064	16331	000136_GCAT	181142	View Info for Gc00473
○	Gc00527	M	ARCHAEAL	Methanococcus maripaludis	2009-04-22	1	1	0	Archaea	Euryarchaeota	Methanococci	Methanococcales	Methanococcaceae	Methanococcus	Methanococcus maripaludis	C5	402880	NC_009135	640069316	17641	001819_GCAT	203530	View Info for Gc00527

Figure 12 shows a screenshot of the GEM Database Application K-mer Analysis Selection Page.

The K-mer analysis selection page is very similar to the download selection page. The same information from the database is displayed, along with a button leading to the information page. Once the user selects a genome for the analysis and clicks submit, the K-mer Analysis page is displayed. This page is shown in Figure 13 below.

Figure 13 - GEM Database Application K-mer Analysis Page

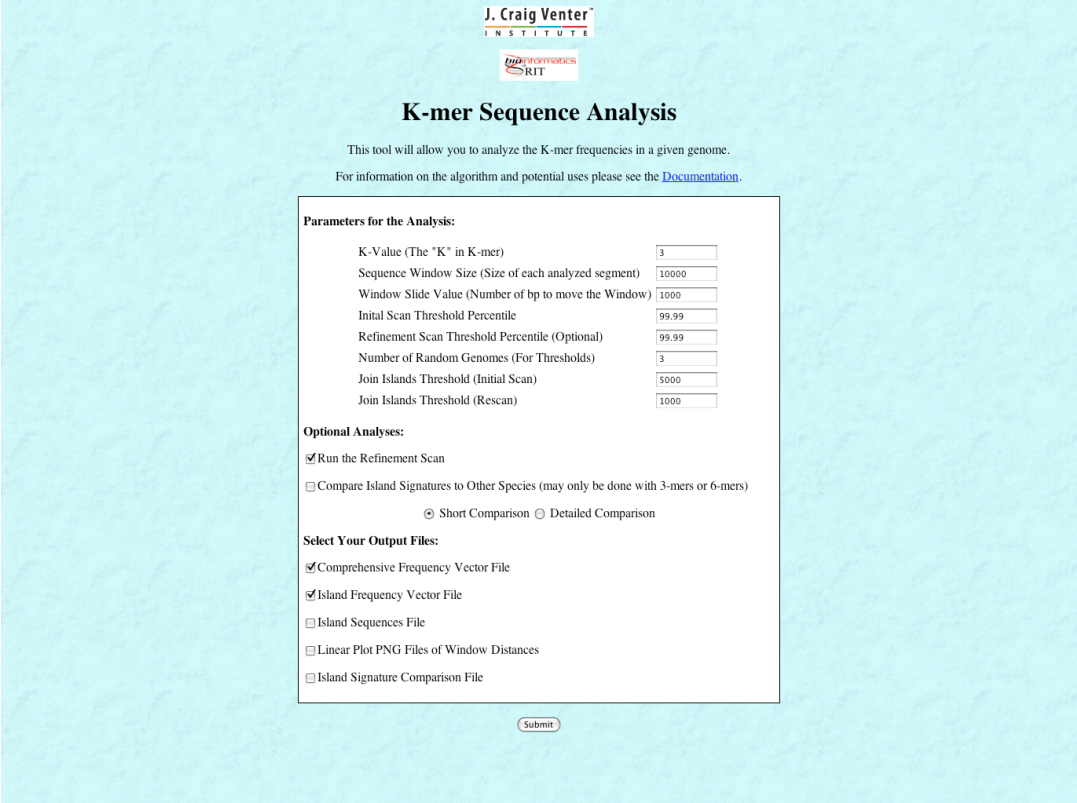


Figure 13 shows a screenshot of the GEM Database Application K-mer Analysis Page.

The K-mer analysis page is where the user specifies all parameters and requested output information for their K-mer analysis. See the Discussion section for a more detailed description of parameters that may be specified by the user. Once the parameters are set and the user clicks submit, the K-mer analysis is run and the results are displayed and posted for downloading as shown in Figure 14.

Figure 14 - GEM Database Application K-mer Analysis Results Page

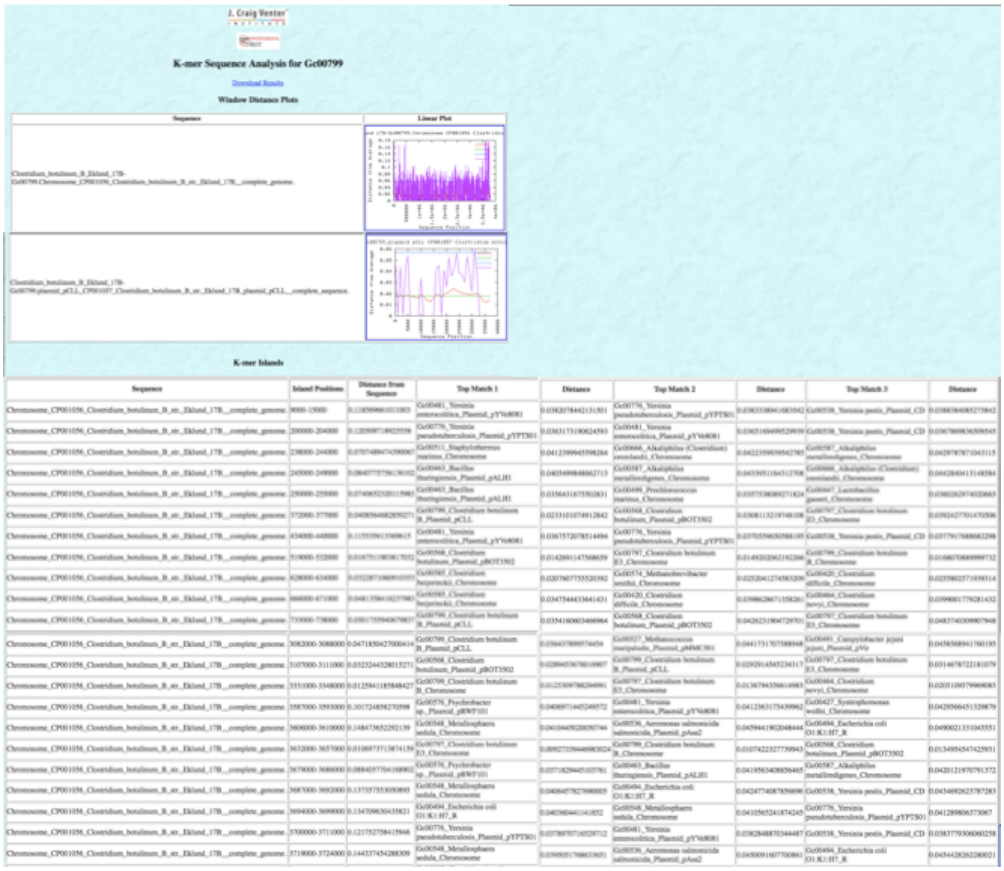


Figure 14 shows a screenshot of the GEM Database Application K-mer Analysis Results Page.

To summarize the pages, a flow chart with all the page types and three functions is shown in Figure 15.

Figure 15 - GEM Database Application Page Navigation Paths

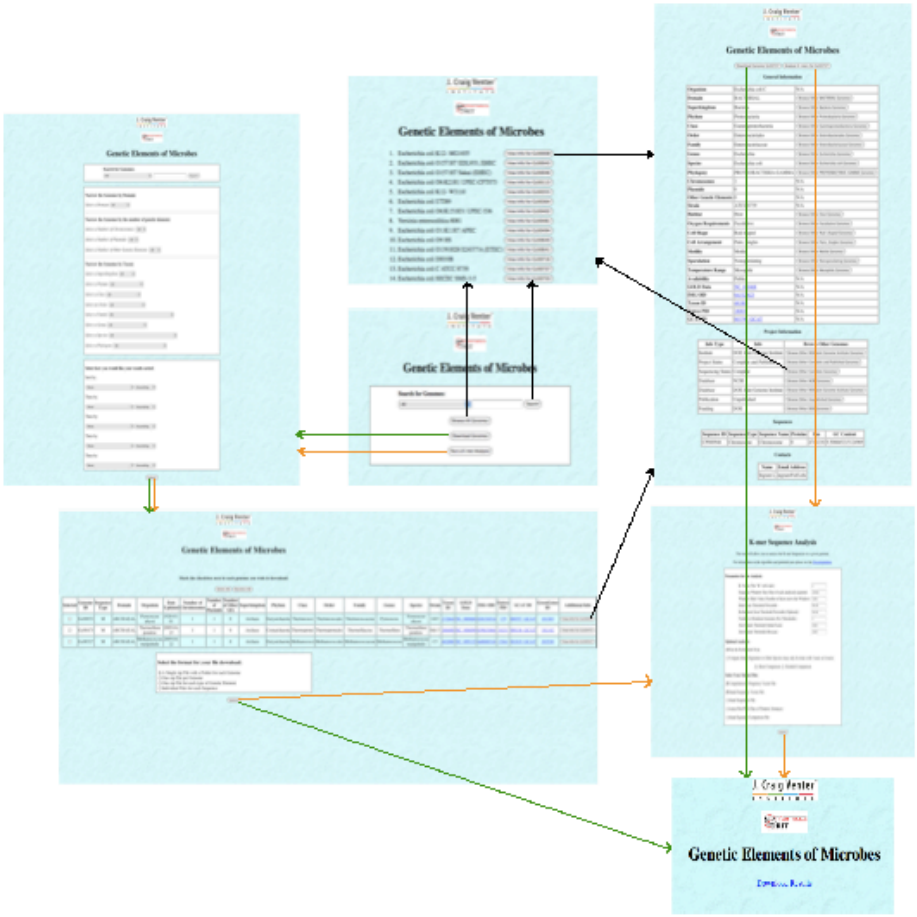


Figure 15 shows an overview of the GEM Database Application page navigation paths. The browsing path is shown with black arrows, download path with green, and K-mer analysis with orange.

The main page is shown in the center of Figure 15. Black arrows indicate the browsing navigation through the various pages. Likewise, green indicates the genome downloads and orange indicates the K-mer analysis. Starting with browsing navigation from the home page, the “Search” and “Browse all Genomes” buttons lead to the main browsing page (top and center) with the appropriate genomes displayed. From the browsing page, each “View Info” button leads to the information page (top right) for that particular genome. From the information page, all “Browse Additional Genomes” buttons lead back to the main browsing

page with the appropriate genomes displayed. The information pages can also be accessed with the "View Info" buttons on the download and K-mer selection pages (bottom left). If the user is using the genome download navigation, the "Download Genomes" button on the home page leads to the genome selection page (top left). The "Search" or "Submit" button on this page leads to the download selection page (bottom left). Once genomes are selected, the submit button leads to the download page (bottom right). The "Download Genome" button on any information page also leads to the download page for that single genome. The K-mer analysis navigation is very similar to the download genomes navigation. The "Run A K-mer Analysis" button on the home page leads to the same genome selection page. The "Search" or "Submit" button on this page leads to the K-mer selection page (bottom left). Figure 14 does not show the K-mer selection page, but because it is so similar to the download selection page this is used in its place. Once a genome is selected, the "Submit" button on the K-mer selection page directs the user to the K-mer analysis page. Once the parameters are specified and the "Submit" button is pressed, the analysis is run and the user is brought to the results page. To save space, the actual K-mer analysis results page is not shown in Figure 15. Refer to Figure 14 for a sample K-mer analysis results page. A K-mer analysis can also be run from a genome's information page by clicking the "Run a K-mer Analysis" button. This will bring the user to the K-mer analysis page to specify their parameters.

Standalone K-mer Analysis Interface

The K-mer Analysis can also be run on sequences that are not in the GEM database by using the standalone K-mer Analysis Interface. This K-mer analysis is exactly

the same as the GEM-linked K-mer analysis except the user uploads a sequence for analysis. The standalone K-mer Analysis Interface is shown in Figure 16.

Figure 16 - Standalone K-mer Analysis Interface

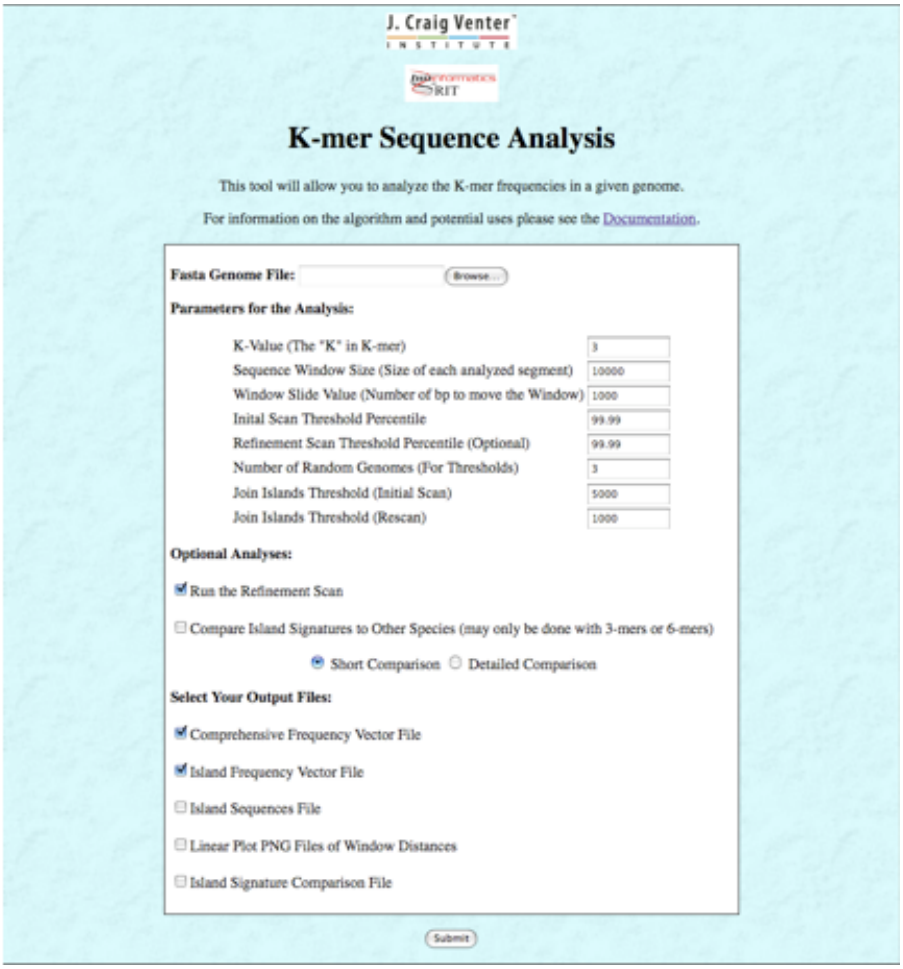


Figure 16 shows a screenshot of the Standalone K-mer Analysis Interface.

LGT Identification with K-mer Analysis

Six different analyses were carried out on genomes and gene sets with previously established LGT or genomic islands in an attempt to validate the use of the K-mer Analysis for LGT identification.

LGT in Thirteen Gamma-Proteobacterial Genomes

The *Buchnera aphidicola* APS, *Escherichia coli* K12, *Haemophilus influenzae* rd, *Pseudomonas aeruginosa* PAO1, *Pasteurella multocida* Pm70, *Salmonella typhimurium* LT, *Vibrio cholerae*, *Wigglesworthia brevipalpis*, *Xanthomonas axonopodis*, *Xanthomonas campestris*, *Xylella fastidiosa*, *Yersinia pestis*, and *Yersinia pestis* KIM genomes were analyzed for LGT of the genes ileS, bioB, mviN, and tadA in comparison to a study by X. Wei et al [25]. The results of the analysis are shown in Table I.

Table I - Results from Gamma-Proteobacterial Genomes

Genome	Gene	Identified as an Island
Buchnera aphidicola APS	ileS	Yes
Buchnera aphidicola APS	bioB	No
Buchnera aphidicola APS	mviN	No
Buchnera aphidicola APS	tadA	Not present in genome
Escherichia coli K12	ileS	Yes
Escherichia coli K12	bioB	Yes
Escherichia coli K12	mviN	No
Escherichia coli K12	tadA	No
Haemophilus influenzae rd	ileS	Yes
Haemophilus influenzae rd	bioB	No
Haemophilus influenzae rd	mviN	No
Haemophilus influenzae rd	tadA	Not present in genome
Pseudomonas aeruginosa PAO1	ileS	No
Pseudomonas aeruginosa PAO1	bioB	Yes
Pseudomonas aeruginosa PAO1	mviN	No

Genome	Gene	Identified as an Island
Pseudomonas aeruginosa PAO1	tadA	No
Pasteurella multocida Pm70	ileS	No
Pasteurella multocida Pm70	bioB	No
Pasteurella multocida Pm70	mviN	No
Pasteurella multocida Pm70	tadA	No
Salmonella typhimurium LT	ileS	No
Salmonella typhimurium LT	bioB	No
Salmonella typhimurium LT	mviN	No
Salmonella typhimurium LT	tadA	Not present in genome
Vibrio cholerae	ileS	Yes
Vibrio cholerae	bioB	Yes
Vibrio cholerae	mviN	No
Vibrio cholerae	tadA	Not present in genome
Wigglesworthia brevipalpis	ileS	No
Wigglesworthia brevipalpis	bioB	No
Wigglesworthia brevipalpis	mviN	No
Wigglesworthia brevipalpis	tadA	Not present in genome
Xanthomonas axonopodis	ileS	No
Xanthomonas axonopodis	bioB	Yes
Xanthomonas axonopodis	mviN	No
Xanthomonas axonopodis	tadA	Not present in genome
Xanthomonas campestris	ileS	Yes
Xanthomonas campestris	bioB	Yes
Xanthomonas campestris	mviN	No
Xanthomonas campestris	tadA	Not present in genome
Xylella fastidiosa	ileS	Yes
Xylella fastidiosa	bioB	Yes
Xylella fastidiosa	mviN	No

Genome	Gene	Identified as an Island
Xylella fastidiosa	tadA	Not present in genome
Yersinia pestis CO92	ileS	No
Yersinia pestis CO92	bioB	Yes
Yersinia pestis CO92	mviN	Yes
Yersinia pestis CO92	tadA	Yes
Yersinia pestis KIM	ileS	No
Yersinia pestis KIM	bioB	Yes
Yersinia pestis KIM	mviN	No
Yersinia pestis KIM	tadA	Yes

Table I shows the results from the analysis of 4 genes in 13 Gamma-Proteobacterial genomes.

LGT In Neisseria meningitidis

The *Neisseria meningitidis* genome was analyzed to search for LGT of sodC, bioC, a conserved hypothetical protein, Type III Restriction enzyme, Type III methyltransferase, and Virulence Associated Protein. The results of this analysis are shown in Table II.

Table II - Results from *Neisseria meningitidis*

Gene	Identified as an Island
sodC	Yes
bioC	Yes
CHP	Yes
Type III RE	Yes
Type III MT	Yes
VAP	No

Table II shows the results from the analysis of 6 genes in *Neisseria meningitidis.*

LGT in Wolinella succinogenes

Wolinella succinogenes was analyzed for an LGT event at the tRNAMet gene, the nif genes, and an island having synteny with the pVir plasmid. Results are shown in Table III.

Table III - Results from *Wolinella succinogenes*

Gene/Island	Identified as an Island
tRNAMet	Yes
nif	No
pVir island	No

Table III shows the results from the analysis of 3 genes in *Wolinella succinogenes.*

LGT in Pseudomonas putida KT2440

The *Pseudomonas putida* KT2440 genome was analyzed for LGT of Tn4371, glyV, and the clc element. The results are shown in Table IV below.

Table IV - Results from *Pseudomonas putida* KT2440

Gene	Identified as an Island
Tn4371	No
glyV	Yes
clc element	Yes

Table IV shows the results from the analysis of 3 genes in *Pseudomonas putida* KT2440.

Genomic Islands in Escherichia coli CFT073

The *Escherichia coli* CFT073 genome was analyzed for genomic islands to compare to another study. The islands from the previous study, their approximate positions, and whether or not the K-mer Analysis identified the same island are shown in Table V.

Table V - Results from *Escherichia coli* CFT073

Published Island	Approximate Positions	Identified as an Island
aspV Island	270,000 - 390,000	Yes
thrW and betA Island	400,000 - 460,000	Yes
cryptic prophage 1	900,000 - 950,000	Yes
serX island	1,125,000 - 1,240,000	Yes
cryptic prophage 2 and 3	1,325,000 - 1,390,000	Yes
cryptic prophage 4	1,400,000 - 1,460,000	Yes

Published Island	Approximate Positions	Identified as an Island
serU, asnW, asnT, asnU, cobU, and galF Island	2,200,000 - 2,400,000	Yes
argW Island	2,725,000 - 2,775,000	Yes
metV Island	3,225,000 - 3,260,000	Yes
pheV Island	3,475,000 - 3,525,000	Yes
selC Island	4,250,000 - 4,350,000	Yes
pheU Island	4,950,000 - 5,000,000	Yes
leuX Island	5,100,000 - 5,150,000	Yes

Table V shows the results from the genetic island analysis in *Escherichia coli* CFT073.

Genomic Islands in Thermotoga maritima MSB8

Thermotoga maritima MSB8 was also analyzed for genomic islands and compared to another study. The results are shown in Table VI.

Table VI - Results from *Thermotoga maritima MSB8*

Approximate Positions	Identified as an Island
1,000 - 3,000	Yes
16,000 - 20,000	No
22,000 - 33,000	No
68,000 - 78,000	No
95,000 - 97,000	Yes
167,000 - 170,000	No
190,000 - 198,000	Yes
312,000 - 325,000	Yes
362,000 - 364,000	No
386,000 - 390,000	No

Approximate Positions	Identified as an Island
408,000 - 415,000	Yes
426,000 - 435,000	No
450,000 - 458,000	Yes
582,000 - 584,000	No
632,000 - 638,000	Yes
660,000 - 680,000	Yes
690,000 - 692,000	Yes
774,000 - 786,000	Yes
965,000 - 967,000	Yes
970,000 - 978,000	Yes
1,000,000 - 1,020,000	Yes
1,066,000 - 1,080,000	Yes
1.130,000 - 1,132,000	No
1,160,000 - 1,165,000	No
1,196,000 - 1,198,000	Yes
1,200,000 - 1,208,000	Yes
1,210,000 - 1,212,000	No
1,216,000 - 1,238,000	Yes
1,250,000 - 1,256,000	No
1,260,000 - 1,268,000	No
1,296,000 - 1,298,000	Yes
1,310,000 - 1,314,000	No
1,322,000 - 1,330,000	Yes
1,332,000 - 1,334,000	Yes
1,354,000 - 1,358,000	Yes
1,366,000 - 1,376,000	No
1,414,000 - 1,416,000	No
1,420,000 - 1.422.000	Yes

Approximate Positions	Identified as an Island
1,510,000 - 1,512,000	No
1,576,000 - 1,578,000	Yes
1,624,000 - 1,630,000	Yes
1,720,000 - 1,734,000	Yes
1,766,000 - 1,768,000	Yes
1,772,000 - 1,776,000	Yes
1,786,000 - 1,790,000	No

Table VI shows the results from the genetic island analysis in *Thermotoga maritima* MSB8.

Discussion

Features

The GEM database application has several features that would be useful for researchers. As previously discussed, GEM has three main navigation paths: browsing, downloading, and running K-mer analyses. Figure 15 illustrates the workflow between pages. The browsing navigation path has several features for researchers searching for specific genomes and identifying other genomes of interest. The user has the ability to browse all of the genomes or search for genomes they are interested in. When using the search feature, the user may choose to search by 42 different data types, as well as search in all data types. The 42 types include all levels of taxonomy, genome identifiers, environmental information, project information, number of genetic elements, organism morphology, and even project contacts. When a user chooses a genome and views the information page, many more browsing features are available. Each genome information page has links to external data sources including NCBI Nucleotide, IMG, Greengenes, GCAT, and Entrez. There are also buttons on each information page that allow users to browse genomes sharing certain types of data. These browse buttons are present for all levels of taxonomy, disease, relevance, organism habitat, oxygen requirements, cell morphology, temperature range, motility, sporulation, and all project information. This allows the user to view all genomes associated with a certain disease, all from a particular database, etc. and is perhaps the most valuable feature of the GEM database application. Each genome information page also has a button to download the sequence and genbank files for that genome, and a button to run a K-mer analysis for that genome.

The download genomes path also has many features worth noting. Selecting genome download from the home page directs the user to the genome selection page. The genome selection page allows a user to narrow the list of genomes for download selection. This is achieved with a search or a “narrow and sort”. The search is just like that of the initial browsing search. The “narrow and sort” option allows the user to narrow the results by Domain, number of genetic elements, and each level of taxonomy. This set can may then be sorted at up to five different levels. Results may be sorted by each of the 42 data types with the search function, and can be in ascending or descending order. Once the user clicks the search or submit button, the genomes matching the criteria will be displayed on the download selection page. The user may select or deselect all using the buttons at the top of the page. A limited amount of information for each genome is displayed in the selection table, as well as links to external data sources and a button leading to the information page for that genome. The user may select one or more genomes to download, and then choose between one of four download types. The available download types include a singe zip file containing a folder for each genome, one zip file per genome, one zip file for each type of genetic element, and individual files for each sequence.

The features of the K-mer analysis navigation path are very similar to those in the download sequences path. Selecting K-mer analysis from the home page leads to the same genome selection page, allowing the user to search or “narrow and sort” the list of genomes they see on the following K-mer selection page. The K-mer selection page displays the same information for each genome, including the external links and button to access the information page. The user may select one genome to analyze and continue to the K-mer

analysis page. On the K-mer analysis page, the user may completely customize their analysis by specifying the K value, window size, slide value, initial scan threshold percentile, rescan threshold percentile, number of random genomes to generate for threshold determination, island joining threshold for the initial scan, island joining threshold for the rescan, and minimum island size to consider. The user may also specify which analyses he or she would like to run, and which output files to include. The rescan may be included or omitted, and the resulting islands may be compared to the K-mer signatures of all other genetic elements in GEM. If the user chooses to run a comparison, they have the choice between a short comparison and a detailed comparison. The short comparison only gives the top three hits in GEM, while the detailed comparison gives distance measures for every genetic element. There are five output files available to the user. The Comprehensive Frequency Vector File contains frequency vectors for every window of every sequence analyzed, the overall K-mer frequency vector for each sequence, and the thresholds for islands. The Island Frequency Vector File contains the frequency vectors and positions for every island identifies. The Island Sequences File is a FASTA file containing a the sequence of every identified island. The Island Signature Comparison File contains the results of the genetic element signature comparisons for every island. Finally, the Linear Plot files are PNG files for each sequence, containing a plot of the initial scan window distances, rescan window distances, initial scan threshold, and rescan threshold. This provides the user with a graphical representation of the islands in each sequence. All requested results files are posted for downloading, and all applicable plots, islands, and top three comparison matches for each island are displayed on

the results page. If plots are displayed on the results page, smaller plots are displayed in the table and when clicked on, open a new window or tab with the full size plot.

To prevent multiple users (or single users running many analyses) from overloading the host server, computationally intensive steps run a check for available resources and wait to continue if the server is very busy. This check is implemented before obtaining genome files for the user, and before running the K-mer analysis. If the cpu or memory usage is above sixty percent on every processor, the application waits for ten seconds and checks again. The application proceeds when resources become available.

Potential Uses

The GEM database application will allow researchers to easily obtain FASTA and Genbank files for their analyses. Sets of genomes can be easily found and retrieved using the download navigation of the application. Scientifically relevant metadata may also be viewed for each genome, providing a fast and easy way to learn more about a particular organism.

There are several potential uses for K-mer analyses. Researchers may use the analysis simply for identification of genomic islands in the sequence(s) of a genome. Genomic island identification has been shown to be useful in gene identification. As discussed previously, this analysis may also be used for potential LGT identification. There are several other uses for K-mer analysis not mentioned here and this tool will allow researchers to customize their analyses to fit their research needs.

Advantages of Design

As previously indicated, a MVC design approach was used to create the GEM database application. There are several advantages to this design approach. The most

beneficial advantage is the convenience of changing components. For example, changing the database would only require changing the value objects and the Data Abstraction layer, at most. Entries in the database are represented by the Value Objects, and the Data Abstraction is the only portion that directly communicates with the database. If the database language were changed to oracle, for example, only the SQL calls in the Data Abstraction would need to be modified. If the database structure were changed completely, the Value Objects and the Data Abstraction would need to be updated, but the Data Cleanser, Data Retriever and the Web Interface could all remain the same. Because the only component communicating with the database is the Data Abstraction and not the Interface, the design makes the application more secure. The separation of the user input and the database helps prevent malicious use. The Data Cleanser also contributes to the security. All calls to modify the database are checked for SQL injections to protect the data integrity. The MVC design also facilitates code re-use, increasing the efficiency for developers. This will make it very easy for future developers aiming to extend or modify the application. Similarly, this design is very extensible. Using the same server-side components, it would be very simple to create a second web interface with a different function. This is also a significant advantage for future developers who may add to this application.

Disadvantages of Design

There are disadvantages to the design approach and language choices of the GEM database application. The major caveat of the MVC design approach is the complexity. It would be challenging for a developer to take on the project and learn the roles and limitation of all the components. This would be especially challenging for someone with little to no

knowledge about Object Oriented Programming or MVC. Following from the complexity caveat, the large number of files for this project may make it difficult to move or copy to another server. The language choice for this application is also somewhat of a disadvantage. Perl was used for its easy string manipulation, CGI module, and BioPerl modules. However, Perl is not a memory-efficient language. This could slow down the application as well as the host server as the number of available genomes, and thus the size of the database increases.

Limitations

The GEM database currently contains 3,016 genomes. On the current host server, loading all genomes for browsing takes 10 to 15 seconds. At this time, this is merely an inconvenience. However, if the database is extended to include eukaryotic genomes or if the number of microbial genomes available increases (as it most definitely is) this time will increase and could become a more serious concern. When a user downloads many genomes at one time, the archive creation can take several minutes, not including the download time for the user. Again, this is an inconvenience that is worth the time at the moment, but if the user were to attempt to download every single genome or if the database were extended to eukaryotic genomes this would be a serious concern.

Running the K-mer analysis is a computationally intensive and thus time consuming step. The *Escherichia coli* K12 genome took 9 minutes to analyze using 3-mers, a window size of 10kb, a slide value of 1kb, generating 2 random genomes, and threshold percentiles of 99.99. Analyzing a larger genome, increasing the K value, decreasing the window size, decreasing the slide value, and increasing the number of random genomes, and

decreasing the threshold percentiles would all increase the analysis time. The K-mer analysis interface creates an auto-refresh waiting page to prevent a browser time-out.

The resource check also creates a limitation. At any given time, there can only be as many K-mer analyses or genome downloads running as there are processors.

LGT Identification with K-mer Analysis

The K-mer Analysis of thirteen Gamma-Proteobacterial genomes was compared to that in a study by Wei et al. [25]. The results of the K-mer analysis are shown in Table I. Wei et al. implemented a distance-based phylogeny method to rank genes based on their predicted occurrence of LGT. This distance-based method calculated evolutionary distances between orthologs and compared the distances to the overall evolutionary distances between species. One particular validation of this method analyzed the same 13 species previously discussed in the results section. Out of the 13 species for the 4 known LGT genes, they found 4 occurrences of LGT for ileS, 2 for bioB, 2 for mviN, and 1 for tadA. The K-mer analysis identified 6 occurrences for ileS, 8 for bioB, 1 for mviN, and 2 for tadA. Phylogenetic analyses tend to be more stringent than sequence composition techniques. When investigating closely related species, sequence similarity tends to be more highly conserved than sequence composition. This explains why the K-mer analysis identified more LGT events than the published technique.

A study by Sandberg et al. found LGT events from *H. influenzae* to *Neisseria meningitidis* for genes sodC, bioC, a conserved hypothetical protein, Type III restriction enzyme, Type III methyltransferase, and virulence associated protein [21]. The *Neisseria meningitidis* genome was subjected to K-mer analyses, and resulting islands were searched

for the aforementioned genes using BLASTN (results shown in Table II). All six genes were present in the identified islands, further indicating that the K-mer analysis is able to identify LGT events.

A review by Dobrindt et al. presented identified LGT events in *Wolinella succinogenes* at the tRNAMet gene, nif genes, and a genomic island having high synteny with the pVir virulence plasmid of *C. jejuni* [26]. The genome was subjected to K-mer analysis and the islands searched for the above genes, as in the comparison for *Neisseria meningitidis*. A part of an island matched the tRNAMet gene, but the nif genes and the pVir sequence were not found. The same review presented LGT events in *Pseudomonas putida* KT2440 including the Tn4371 transposable element, the clc transposable element, and the glyV gene. The genome was analyzed in the previously noted method. The Tn4371 element was not identified, but sections of the clc element and glyV gene were present in the identified islands. This lack of identification of some of the LGT events indicates that the K-mer analysis approach does have its limitations. As ancient LGT events, and events under less selective pressure will have likely undergone mutation to better match the K-mer signature of the host genome, K-mer frequency analysis cannot detect all transfer events.

The K-mer analysis was also compared to two studies of genomic island identification, a study of *Escherichia coli* CFT073 [27], and a study of *Thermotoga maritima* MSB8 [28]. In the *E. coli* study, 24.98 % of the genomic sequence was identified as islands. The K-mer analysis identified 20.66 % of the genome. A comparison of all islands over 4kb is shown in Table V. The K-mer analysis identified all 13 of the islands identified in the study. When analyzing the *T. maritima* genome, the K-mer analysis identified 10.31% as

islands. A comparison of all islands over 2kb was completed, results shown in Table VI. Of the 45 islands over 2kb from the study, the K-mer analysis identified 27, or 60%. Running the analysis with parameters optimized for smaller islands may increase the identified islands.

Overall, the K-mer analysis is certainly a useful tool for identifying regions of dissimilarity in a given genome. This is achieved with results comparable to other island identification techniques. This analysis may also be used for identifying potential LGT events, with limited certainty. Validation with published LGT events showed that most transfers were detected by the K-mer analysis, but were not the only islands identified. This is the result of a high false positive rate. However, it is likely that we as a scientific community do not know every LGT event that has occurred, artificially inflating the appearance of false positives in the analysis. Also, not all genomic islands are necessarily LGT events. Genomic islands can arise from evolutionary phenomena such as genetic drift and highly conserved DNA sequence due to strong positive selection. The nature of a sequence composition analysis is optimal for the identification of genomic islands which include LGT, but are not solely the results of transfers. Due to the relatively high occurrence of false positives in this analysis, it is recommended that the results be used with another LGT identification method. This could be easily achieved by using the sequence file of identified islands.

Potential Improvements to GEM

Some improvements could be made to the GEM database application, mostly with the web interface. Incorporating javascript to provide a more user-friendly and aesthetically

pleasing interface would be one of the first priorities for future work. Allowing users to dynamically sort their results on the browsing and selection pages would improve their experience with GEM. In addition, the dynamically generated information pages for each genome could be implemented as "pop-outs" instead of in the same page as the CGI. The Data Retrieval component could also be extended to obtain more information from NCBI and other external sources. For example, storing NCBI's description for each genome would provide users with even more information about the organism. Finally, a more sophisticated estimation of required resources for each download or K-mer analysis would be beneficial.

Potential Improvements to the K-mer Analysis

The K-mer Analysis could be improved in many ways. First, the analysis program could be examined for further optimization to reduce the analysis time. Providing users with the option to turn off reverse compliment masking would increase their ability to customize their analysis. Also, adding knowledge of reading frame to the analysis would provide greater flexibility for researchers in their application of the results. Finally, allowing the user to input an email address to be notified when their analysis finishes would make the K-mer analysis more convenient.

Conclusion

The GEM database application allows researches to quickly and easily obtain sequence information for one or more genomes organized by genetic element. Genomes may be searched and viewed in organized, scientifically relevant ways. Genomes may also be accessed by their metadata information, which is a beneficial and time-saving feature. This application was implemented with a design that facilitates maintenance and further

development. The K-mer analysis addition to the GEM database application provides a flexible tool to identify regions of dissimilarity and contribute to an LGT identification study.

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