ASAP: a resource for annotating, curating, comparing, and disseminating genomic data

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ABSTRACT
ASAP is a comprehensive web-based system for community genome annotation and analysis. ASAP is being used for a large-scale effort to augment and curate annotations for genomes of enterobacterial pathogens and for additional genome sequences. New tools, such as the genome alignment program Mauve, have been incorporated into ASAP in order to improve display and analysis of related genomes. Recent improvements to the database and challenges for future development of the system are discussed. ASAP is available on the web at https://asap.ahabs.wisc.edu/asap/logon.php.

INTRODUCTION
ASAP is a database that supports annotation and curation of genomic data by a distributed community of users through a web interface. The system permits users to upload genome sequence data, annotations and experimental data and it provides an environment for initial annotation of a genome or for updating, viewing and downloading existing annotations and experiments. The database currently provides data for both eukaryotes and bacteria, although the taxonomic focus is on members of the bacterial family Enterobacteriaceae. ASAP was originally created to facilitate the annotation and analysis of the Erwinia chrysanthemi genome by an international group of researchers (1). Since its debut, ASAP was used to complete the initial annotation of this genome and is now used for a number of ongoing genome projects and for maintenance of a larger number of complete genomes. A key feature of the system is a hierarchical curation procedure that provides rapid access to preliminary data that is subject to review by an expert curator. Extensive tracking of annotation data is provided. The system requires that annotators provide the evidence used to support their claims, which enables downstream users to assess the quality of each piece of information.

EXPANDED DATABASE CONTENT
More taxa
The number of genome sequences contained in ASAP has expanded rapidly in recent years to include all published genomes from enterobacteria (currently 15), two mosquito expressed sequence tag (EST) projects (2), two Mycobacterium genome sequences and sequences from a metagenomics study (3) (Table 1). Additional datasets of high-throughput functional genomics data have also been added to the system. The current database contents demonstrate the ability of the system to accommodate diverse data, ranging from unfinished or partial genome sequences to completely annotated genomes with associated experimental data. The choice of genomes to be included in the database is driven by the user community. For several genome projects, particularly the phytopathogenic enterobacteria, ASAP is used as the primary system for genome annotation. For human pathogenic enterobacteria, ASAP is used as part of an NIH-funded project aimed at identifying targets for vaccines, diagnostics and therapeutics by integrating genomic information about these organisms. The EST sequencing project, metagenomics study and Mycobacterium genomes were entered in ASAP by request from user groups who desired to use the functionality available in ASAP for their specific annotation and analysis needs.

More options for access
ASAP was designed to provide access to genome annotation and analysis tools to distributed communities of participants in collaborative genome-scale sequencing and functional genomics experiments. Although the primary focus of the system is to make data available to a wide public audience, there are important practical reasons to restrict access to

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Table 1. Genome data housed in the ASAP database

| Sequence project | Project type | Taxonomic group | Status | Availability |
|------------------|--------------|-----------------|--------|--------------|
| Aedes aegypti, bacteria-inoculated hemocyte | EST | Eukaryote | Ongoing data collection | Public/Private |
| Armigeres subalbatus, bacteria-inoculated hemocyte | EST | Eukaryote | Ongoing data collection | Public/Private |
| Buchnera aphidicola (Batzongia pistaicae) | Genomic | Enterobacteraeae | Complete | Public |
| B.aphidicola strain Sg (Schicaphis graminum) | Genomic | Enterobacteraeae | Complete | Public |
| Buchnera sp. APS | Genomic | Enterobacteraeae | Complete | Public |
| Candidatus Blochmannia floridanus | Genomic | Enterobacteraeae | Complete | Public |
| Environmental BAC clone | Metagenomic | Uncharacterized | Unfinished | Private |
| Erwinia amylovora strain Ea273 | Genomic | Enterobacteraeae | Complete | Private |
| Erwinia carotovora subsp. atroseptica strain SCR1043 | Genomic | Enterobacteraeae | Complete | Public |
| Erwinia chrysanthemi strain 3937 | Genomic | Enterobacteraeae | Complete | Public |
| Escherichia coli K-12 strain MG1655 | Genomic | Enterobacteraeae | Complete, under curation | Public |
| E.coli O157:H7 strain EDL933 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| E.coli O157:H7 strain RIMD 0509952 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| E.coli strain CT073 | Genomic | Enterobacteraeae | Complete | Public |
| E.coli strain RS218 | Genomic | Enterobacteraeae | Complete, undergoing annotation | Private |
| Mycobacterium avium | Genomic | Actinobacteria | Complete | Private |
| M.avium subsp. paratuberculosis K-10 | Genomic | Actinobacteria | Complete | Private |
| Pantoea stewartii DC283 | Genomic | Enterobacteraeae | Unfinished | Private |
| Photobadus luminescens subsp. laumondii TTO1 | Genomic | Enterobacteraeae | Complete | Public |
| Salmonella Choleraesuis | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Salmonella typhimurium LT2 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Salmonella Paratyphi A str. ATCC 9150 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Salmonella enterica serovar Typhi plasmid R27 | Plasmid | Enterobacteraeae | Complete, ERIC curation | Public |
| S.enterica serovar Typhi strain CT18 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| S.enterica subspecies enterica serovar Typhi Ty2 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Salmonella typhimurium SR1 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| S.flexneri 2a strain 2457T | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| S.flexneri 2a strain 301 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| S.flexneri virulence plasmid pWR100 | Plasmid | Enterobacteraeae | Complete, ERIC curation | Public |
| S.flexneri virulence plasmid pWR501 | Plasmid | Enterobacteraeae | Complete, ERIC curation | Public |
| Wigglesworthia brevipalpis | Genomic | Enterobacteraeae | Complete | Public |
| Yersinia pestis KIM | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Y.pestis biovar Medievalis strain 91001 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Y.pestis strain CO92 | Genomic | Enterobacteraeae | Complete, ERIC curation | Public |
| Yersinia pseudotuberculosis IP 32953 | Genomic | Enterobacteraeae | Complete | Public |

In the status column, projects referred to as ‘complete’ are finished, annotated genome projects open for community annotation, ‘unfinished’ projects are incomplete genome sequences undergoing completion and annotation, ‘complete, ERIC curation’ refers to complete genome projects that are being curated by members of the Enteropathogen Resource Integration Center (ERIC, http://www.ericrc.org/).

Certain information in the database is made available to a subset of users. As indicated in Table 1 the majority of projects in ASAP allow any guest user to access all data and allow anyone to sign up as a community annotator. Annotators, as opposed to guests, need a user name and password to log on to the system. This is simply so that we can record the identity of the annotator alongside their contributions, which provides a means of assigning credit and creating a mechanism for users or curators to request additional information about particular annotations. There are important reasons for restricting access to some projects. For example we have used ASAP in classrooms to teach genomic analysis to students. Students were provided with a copy of a genome project and they used ASAP to analyze and annotate gene functions. By restricting access to students in the class we can store the project in the same database as the public data without affecting the information available to a typical user. In other situations, such as with the Mycobacterium sequences, the data are used only by select users for specific purposes and are not made available to the general public (that has access to these sequences through other sources).

The inclusion of all available genomes from enterobacteria in ASAP reflects our desire to create a resource for updating and comparing genomic data from this phylogenetically cohesive bacterial family. The enterobacteria are phenotypically diverse organisms that include a number of medically and agriculturally important pathogens. As described below, ASAP has a number of enhancements that facilitate comparative genomic analyses and standardization of annotations across genomes.

More diverse projects

A key reason that ASAP is able to support such diverse projects is the capacity of the database to handle multiple sequences associated with a project, such as short sequences, assembled contigs, ESTs or complete genomes that may have multiple chromosomes and plasmids. We have expanded the query tools available to users to search for annotations across genomes and to restrict queries to specific subsets of annotations by nearly any characteristic stored in the database. ASAP queries produce lists of database objects (features) matching the search criteria. Each feature corresponds to a span of nucleotide coordinates in a given sequence within the genome.

More ontologies

Uniformity in the type, quality and display of genome information makes comparisons across genes and organisms more useful. We have incorporated several ontologies that make standardization of annotations easier. For descriptions of gene functions and products we currently employ the Gene Ontology (GO) (http://www.geneontology.org/).
On 26 July 2018 by guest

Enhancements for comparative genomics

ASAP provides extensive support for analyses of related genome sequences. We maintain curated lists of relationships between the annotated proteins (orthology, paralogy, etc.). There is a semi-automatic implementation of the reciprocal best hits BLAST algorithm for identifying potential orthologs. PHP-scripts are used to download sequences, run the searches, parse the results, import the results, detect best hits and add ortholog candidates. Adding orthologs from the list of reciprocal best hits is done using an interface that provides statistics and predictions of orthologous genes and a number of alignments related to a particular genome are presented to a user viewing that genome. For example, a user viewing one of the *Escherichia coli* O157:H7 genomes is presented with options for viewing an alignment of four related *E. coli* genomes or an alignment of *E. coli* with other enterobacteria. When browsing annotations for a particular gene a user can choose to view the multiple alignment by launching a java applet that displays a visualization of the genome alignment centered on the selected gene (Figure 1). Users can zoom in and out, scroll through the alignment and browse genome annotations through the applet. Selecting an annotated feature will bounce the user to the ASAP annotation page with more detailed information about the entity. The tool is particularly useful for identifying conserved blocks of sequences between genomes and regions corresponding to genomic islands. Curators often use the tool when reviewing homology relationships between genes. Since the alignments generated by Mauve are based on nucleotide sequences they are also useful for analyzing regions other than just protein-coding gene sequences, such as conserved DNA binding sites and functional RNAs.

Handling experimental data

ASAP serves as a repository for experimental data associated with genomes in the database. There are examples of microarray hybridization data, high-throughput phenotypic data and results from IVET experiments (7) in ASAP currently, and the system is flexible enough to accommodate most other forms of experimental information associated with genome-scale functional characterization. Nearly any genomic data that can be represented in a tabular format can be imported into the system. To comply with standards such as MIAME for microarray experiments, users can attach nearly any sort of metadata associated with an experiment when uploading results. This can include information such as detailed protocols, array design files, analytical methodology, and links to additional resources.

There are two main ways in which ASAP users can interact with the experimental data. The most direct route is to select a genome and view a list of available experimental data sets. These data are organized into sets by the depositors, where a set is a collection of experiments related by a common theme, such as data from a single publication. After selecting a set, a user is presented with a table describing each of the experiments in the set along with the associated metadata. When experiments are selected, the user can choose from several options that specify the format of the data requested and can query for results for specific genes. The second route to the data is through the gene annotations. When there is experimental data in ASAP corresponding to a particular gene, users are shown a list of relevant experimental datasets on the gene annotation page. Selecting a set will return all of the data for that gene from the experiments in the selected set.

Comparison of ASAP to other software tools

*coliBASE* (8) is a web-based database for comparative genome analysis of *E. coli* and related species. It includes many of the same types of data as ASAP including genome alignments and predictions of orthologous genes and a number of

Whole-genome multiple alignments

Annotation and visualization of genomes have been greatly enhanced by integrating Mauve, a whole-genome alignment and viewing system (6). The Mauve aligner is used to construct multiple alignments of relevant genomic sequences that are uploaded into the ASAP system. All pre-built multiple
additional automated predictions. Key features that distinguish the ASAP system include a focus on providing users with tools for contributing to the genome annotations, detailed descriptions of evidence used to infer gene functions and support for curators to evaluate and correct automated predictions and contributed information. coliBase is not packaged for redistribution although it is also used for databases of other microbes.

Artemis (9) and the Artemis Comparison Tool (ACT) (10) are stand-alone applications that are widely used for genome annotation, viewing and comparison. Artemis is particularly useful for initial annotation of a complete microbial genome sequence and, in fact, several of the genomes in the ASAP database were initially annotated using this tool. Artemis does not include a database and relies on flat files for input. ACT uses components of Artemis and provides an interactive visualization of comparisons of complete genomes based on BLAST (11) or MUMmer (12) searches to identify conserved segments. As a web-based application, ASAP provides an environment for multiple users to collaborate on genome annotation simultaneously, a feature lacking in the Artemis system. Additionally ASAP supports genome projects in progress, which may consist of many individual sequence files, and provides means to track features across multiple versions of a project, tasks that are not easy to accomplish using Artemis.

GenColors (13) is a new web-based system for annotation of prokaryotic genomes that considers data from related genomes and genome comparisons. It offers integration of data from ongoing sequencing projects and annotated genomic sequences obtained from GenBank. The database does not require annotators to record the evidence for new input and does not offer any support for high-throughput experimental data associated with genome projects. Since ASAP will export genome annotations as Genbank flat files, it is simple to create files that are suitable for input into either Artemis or Gencolors.

**Interfacing with the community of users**

Since its initial release the ASAP user community has grown and their needs have diversified. ASAP is used for curatorial review of complete annotated bacterial genomes, primary annotation of incomplete sequences and annotation of several eukaryotic EST projects. The latest implementation of ASAP provides tools that allow users to customize the look and feel of the interface. A user logged onto the system is provided with a set of custom links to database contents on their front page, and new links to any database content can be added while browsing. The general look of the ASAP display (colors, logos, etc.) can be modified for individual genomes, since some user communities prefer that the style of the ASAP interface reflect their unique content or personal tastes.

Uploading data into ASAP is facilitated by a number of scripts. New genomes can be entered by importing GenBank flat files or FASTA files. Information about the genome, such as coordinates of predicted genome features, annotations, external links, coordinate updates, orthologs and experimental data can be uploaded using tab-delimited text files. The specific formats required for uploading data are documented on the individual upload pages.
The results of all queries to ASAP can be downloaded as tab-delimited text files. When querying genome annotations, users can opt to download a text file containing the protein or DNA sequences or annotations for the set of features returned by the query. Downloads are available for complete genome sequences and annotation in a number of formats including complete Genbank formatted files or spreadsheets formatted for Genbank submission.

We encourage the installation of ASAP at other sites and have made the source code available under a GNU public license.

Future challenges

An ongoing challenge in genomic sciences is the standardization of data formats across different database systems. To facilitate exchange of sequences and annotations between different systems we are planning to implement the use of GFF3 format (http://song.sourceforge.net/gff3.shtml) as an option for download. We plan to support additional formats for download of data from ASAP as they develop.

To allow users another avenue for querying of the database we plan to implement a BLAST server integrated into the ASAP system. Users could enter query sequences, do searches against the ASAP contents and receive formatted results that would link back to entries in ASAP. Additional resources that we would like to add to ASAP include pre-built alignments and phylogenetic trees for related sequences. Results from other software to predict protein function such as Interpro scans (14) for each protein and PSORTB subcellular predictions (15) will be added in the near future.

As the number of complete genome sequences grows, the prospect of annotating and curating the sequences by careful manual inspection by a dedicated expert scientist, the current state of the art, becomes daunting. This problem is currently mitigated by solutions that attempt to automate much of the annotation procedure. However, there are many complexities in genome analyses that cannot be readily automated. Where possible we will develop and incorporate computational solutions to these knottier problems. At the same time we will augment and improve the tools and interfaces used by scientists to manually interact with the data.

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