

The i5k Workspace@NAL—enabling genomic data access, visualization and curation of arthropod genomes

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ABSTRACT

The 5000 arthropod genomes initiative (i5k) has tasked itself with coordinating the sequencing of 5000 insect or related arthropod genomes. The resulting influx of data, mostly from small research groups or communities with little bioinformatics experience, will require visualization, dissemination and curation, preferably from a centralized platform. The National Agricultural Library (NAL) has implemented the i5k Workspace@NAL (<http://i5k.nal.usda.gov/>) to help meet the i5k initiative's genome hosting needs. Any i5k member is encouraged to contact the i5k Workspace with their genome project details. Once submitted, new content will be accessible via organism pages, genome browsers and BLAST search engines, which are implemented via the open-source Tripal framework, a web interface for the underlying Chado database schema. We also implement the Web Apollo software for groups that choose to curate gene models. New content will add to the existing body of 35 arthropod species, which include species relevant for many aspects of arthropod genomic research, including agriculture, invasion biology, systematics, ecology and evolution, and developmental research.

INTRODUCTION

Insects are an incredibly diverse class, with over 1 million described species. They provide essential pollination services for agriculture (1,2), yet cause substantial damage to crops (3), and are vectors of devastating diseases

(4). Further, they are important ecological, evolutionary, developmental and medical models. The genomes of insects sequenced to date have already provided important insights into genome architecture and evolution (5–7), immune response pathways (8), eusociality (9,10) and speciation (11,12). Sequencing further genomes brings great promise to answer pending questions for basic and applied biology.

Decreasing whole-genome sequencing costs, coupled with moderate genome sizes for insects and their relatives, favor new genome projects for this group. One downside for arthropod comparative genomic research is the great divergence times between arthropod groups, often in the hundreds of millions of years. Comparative analyses would therefore benefit tremendously from sequence information across the breadth of Arthropoda. The 5000 arthropod genomes initiative (i5k) has therefore set the goal to coordinate the sequencing of 5000 insect or related arthropod species (13,14). As such, the i5k initiative should galvanize the generation of large amounts of data of exceptional comparative value. While the i5k initiative will provide guidance on the sequencing of genomes, the onus is still on individual labs with a specific interest in these genomes to organize the sequencing, analysis and curation of their genome projects. To ensure wide re-use of genomic data resulting from the i5k project, it is important that this data is hosted in a centralized location for data sharing, dissemination, visualization and curation. However, developing, deploying and maintaining databases and web servers for genome access and curation ('genome portals') is often beyond the financial and technical reach of smaller genome projects.

Here, we introduce the i5k Workspace@NAL (<https://i5k.nal.usda.gov/>), which the National Agricultural Library (NAL) designed to meet the genome hosting needs of the

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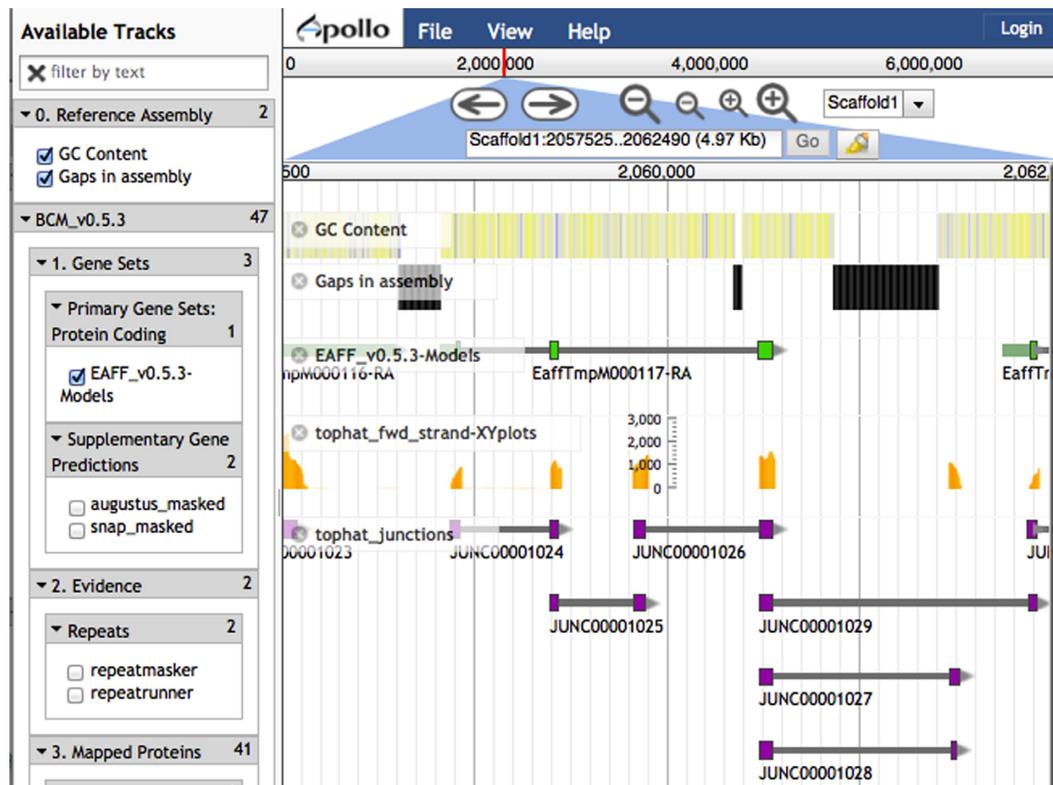


Figure 1. View of the JBrowse genome browser for the copepod *Eurytemora affinis*. G + C content and gap tracks, MAKER-predicted protein-coding gene set, forward-strand RNA-Seq coverage and junction read alignments are shown.

i5k community. The i5k Workspace@NAL has two main goals. First, it aims to help the i5k ‘data producers’, in particular ‘orphaned’ groups without the technical or financial means for genome hosting, at the interface of sequence retrieval and analysis—i.e. how do you access, visualize, curate and disseminate your data once you have received it from the sequencing center? Second, we aim to provide a unified framework for ‘data consumers’ to retrieve relevant genomic information from our data providers. We outline the i5k Workspace, and explain the steps that we take to help i5k data producers disseminate and curate their genome assemblies, and how we present this content to the i5k data consumers. Finally, we explore the future directions the i5k Workspace@NAL will take to continue improving its services for the i5k community, and the arthropod genomics community at large.

USING THE i5k WORKSPACE@NAL

Data producers

Setting up your genome portal. The i5k@NAL hosts genome assemblies for any arthropod genome project that requires our services. There is no hosting preference for a particular taxonomic group or application, but we ask that no agreements with other genome portals be in place, to avoid redundant hosting and curation efforts. Our only requirement is a genome assembly—preferably approved by NCBI (the U.S. National Institutes of Health National Center for Biotechnology Information) or other INSDC

(International Nucleotide Sequence Database Collaboration) members—in FASTA format (scaffold, contig and .agp mapping files). Any sequence features that have been mapped to this assembly can also be submitted, including but not limited to official gene sets (OGS), other consensus gene sets or gene predictions, homology alignments, RNA-Seq mappings and transcriptomes (Figure 1). We provide a tutorial on how to map RNA-Seq reads to a genome in iPlant (<https://i5k.nal.usda.gov/content/performing-rna-seq-alignments-iplant-baylor-i5k-pilot>), and have developed an extension of the exonerate alignment program (15) that generates gff3-formatted output (<https://github.com/hotdogee/exonerate-gff3>), allowing users to map moderately-sized transcriptomes against a genome assembly. We will also transform BAM files to BigWig format in-house if requested. We can advise on what files would be useful to visualize, given the genome community’s specific needs. Finally, we ask for genome communities to provide us with information to populate the organism’s ‘landing’ page, as well as metadata about each file given to us to communicate to other users of the data. Data can be transferred to us via ftp or iPlant (<https://i5k.nal.usda.gov/content/sharing-files-us>). We recommend that each genome community designate a ‘community contact’ to serve as the main contact for data files, as well as mediate the manual curation process if this is to be a part of the genome project.

Data processing—what we do with your data. For each new organism and genome assembly, we generate customized organism ‘landing pages’ (e.g. <https://i5k.nal.usda>).

gov/Cimex_lectularius) and ‘analysis pages’ (e.g. <https://i5k.nal.usda.gov/node/85499>) that describe the organism, assembly and annotation method. This is performed within a modified version of the Tripal framework (16), which interfaces the GMOD Chado database schema v1.23 (17) with the Drupal content management system and website creation software (<https://www.drupal.org/>). Data files for each species can be downloaded via the web browser (<https://i5k.nal.usda.gov/content/data-downloads>). For datasets without an OGS, our BLAST+ server (18) is the main point of entry for gene identification. We implemented a BLAST+ back-end in python with RabbitMQ for task queuing. Users may submit multiple query sequences to the BLAST+ server, where every submission is assigned a unique result URL which persists for up to a week. During this time, the user may freely view, share and download BLAST+ results. The results page is an interactive data viewer (Figure 2). Query and subject coverage graphs on the top are drawn dynamically on the HTML5 canvas for every high-scoring segment pairs (HSP). Tabular output from BLAST+ is displayed in a sortable and searchable table on the bottom left, and text output is displayed on the bottom right panel. The four panels on the results page are designed to display the same HSP in different formats, and interacting with any one panel will dynamically redraw the graphs and scroll to the corresponding row in the table and text on the other three panels. Assemblies, transcripts and peptides of OGS and pre-OGS annotations can be searched, and multiple species can be selected simultaneously. For every scaffold searched against, a GFF3 file is generated from tabular BLAST+ output by grouping contiguous HSPs with identical query sequence, subject sequence, strand direction and an overlap length less than six between neighboring HSPs under the same match. This GFF3 file is sent to our next resource, the JBrowse genome browser (19), with a link on the left most column of the results table. JBrowse visualizes annotations and any other mapped features to the genome in a dynamic format. We have generated custom styles to allow for easier identification of feature types across assemblies (e.g. consensus gene sets are green, protein2genome alignments are gold). Metadata for each track is available via the ‘About this track’ field in the track right-click menu. Finally, genomes are provided with the Web Apollo manual curation software (20) if desired (see below). We maintain a mailing list (<http://listserv.nal.usda.gov/cgi-bin/wa?A0=webapollo-users>) for general announcements, and on which information about newly hosted organisms and datasets is disseminated, giving users a potentially broad platform on which their new data is announced.

Manual curation of automated annotations. Computationally predicted gene features often require manual review and editing. To reach a critical mass, manual annotation efforts of groups with little funding often must be distributed across several groups, which may not be in the same location, or even the same time zone (21). The Web Apollo software enables smaller, focused genome groups to coordinate manual annotation efforts across geographically distributed labs (20). We maintain individual Web Apollo instances for each genome assembly that requires curation. Users register with the i5k Workspace to annotate via a custom

registration page built using the Drupal 6 Form API and CAPTCHA modules (<https://i5k.nal.usda.gov/web-apollo-registration>), and are entered into our databases and automatically sent login credentials after community contact approval. Once logged in, any edits the curator makes are immediately written back to the annotation datastores housed in our servers, which are backed up daily (for a full explanation of the Web Apollo software architecture, see (20)). Curators can download all annotations in various formats, such as gff3 or FASTA. Communities are encouraged to annotate across related species. i5k Workspace coordinators can provide guidance on annotation questions, help connect different annotation groups with each other and mediate Web Apollo webinars provided by the Web Apollo development team (<http://genomearchitect.org/about-us>). We provide some annotation guidelines for the i5k pilot project (<https://i5k.nal.usda.gov/content/rules-web-apollo-annotation-i5k-pilot-project>), that may also be applicable to other groups outside of the i5k pilot. We are willing to work with groups to develop an OGS on a case-by-case basis.

Data consumers

Content at the i5k Workspace. The i5k initiative strives to coordinate the sequencing of 5000 insect or related arthropod genomes. The i5k Workspace is committed to hosting content for projects under the i5k umbrella that require our services. Therefore, our content is dynamic and grows with the i5k community itself. We currently host data from 35 arthropod species and counting (Table 1), including two non-insect classes: four Arachnid and two Maxillopodan species. Within class Insecta, we host data on 2 Palaeopteran, 4 Hymenopteran, 4 Coleopteran, 1 Trichopteran, 10 Dipteran, 6 Hemipteran, 1 Thysanopteran and 1 Lepidopteran species. The main source for this data is the Baylor College of Medicine’s i5k pilot project (<https://www.hgsc.bcm.edu/arthropods/i5k-pilot-project-summary>), which jump-started the i5k initiative with the coordinated sequencing and annotation of 29 arthropod genomes. Among the species are several of interest to invasion biology (the Asian long-horned beetle (*Anoplophora glabripennis*), the Colorado potato beetle (*Leptinotarsa decemlineata*), the copepod (*Eurytemora affinis*) and the infamous bed bug (*Cimex lectularius*). The Baylor College of Medicine’s modENCODE project sequenced eight *Drosophila* species, which are available (<https://www.hgsc.bcm.edu/arthropods/drosophila-modencode-project>). We have also started hosting organisms that were not part of the i5k pilot species: the Asian citrus psyllid (*Diaphorina citri*) (<http://psyllid.org/>), the copepod (*Tigriopus californicus*), the tobacco hornworm (*Manduca sexta*) and the Hessian fly (*Mayetiola destructor*). In addition to genome assemblies, all species that we host have at least one set of consensus gene models (.gff3, .faa, .fna file formats), and component ‘evidence’ data files of the consensus gene models. Each assembly and annotation set has a separate analysis page that describes how the analysis was performed. Two recently added species, *M. sexta* and *M. destructor*, have an OGS, and we are in the process of developing more rigorous search options via the

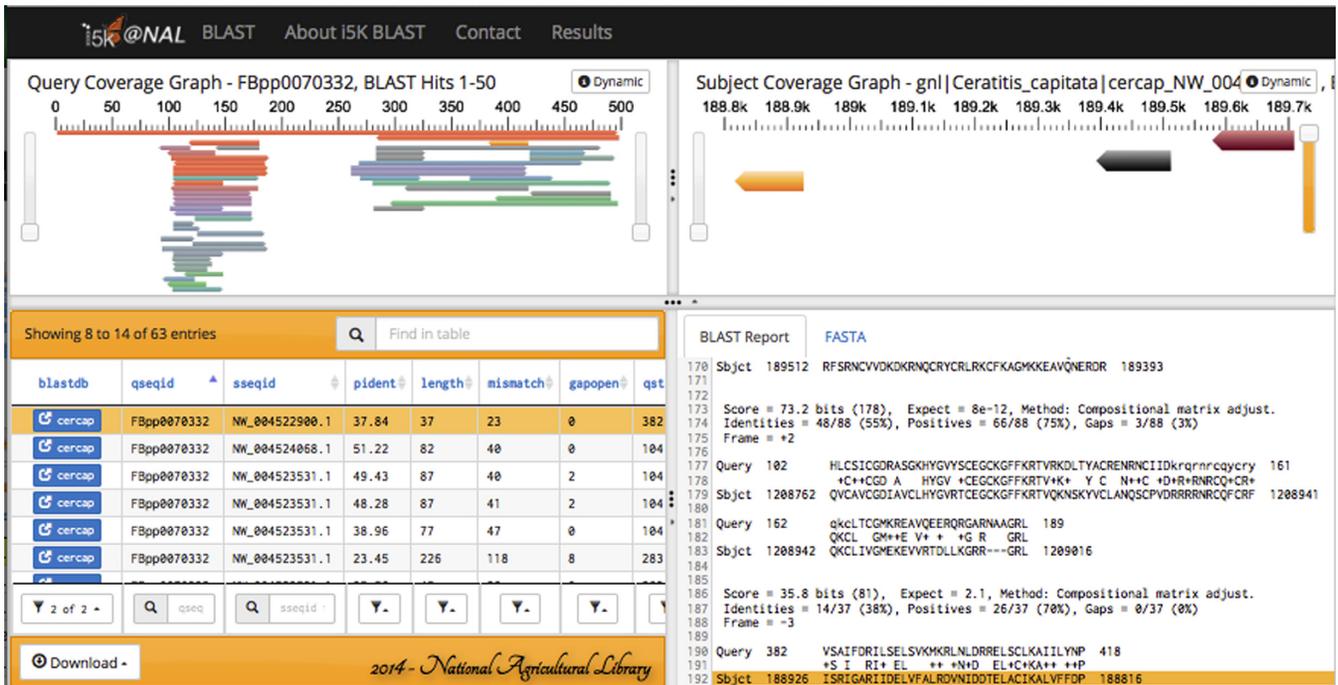


Figure 2. The interactive BLAST+ result viewer displaying a TBLASTN search of one query sequence on two genomes with 63 HSPs. The focused HSP is highlighted in orange.

Tripal framework for species with an OGS. For the genome browsers, our data processing pipeline generates two tracks based on the reference assembly per project: G + C content as a heatmap and the location of gaps in the assembly. Many groups have also provided additional user contributed data files, such as mapped RNA-Seq reads and transcriptomes to aid manual curation (Figure 1). We can make any of the data hosted in the genome browsers available on our ‘downloads’ page upon request. With the exception of the manually curated genes, which require registration to access, all data is publicly available. After the curation process is completed for each genome, the manually curated genes will be made freely available.

Accessing and visualizing i5k Workspace content. Users can navigate through the available species’ organism or genome browser pages via drop-down menus on the i5k Workspace home page. Data downloads are available via a central downloads page (<https://i5k.nal.usda.gov/content/data-downloads>). All organisms’ genomes, predicted peptide and transcript sequences can be accessed via our BLAST page (<https://i5k.nal.usda.gov/blast>), and multiple species can be searched against simultaneously if the same residue type is selected (e.g. either nucleotide or protein). This allows for straightforward retrieval of individual sequences of interest. Performing BLAST searches against a genome generates a link to the JBrowse genome browser, in which the resulting HSPs are visualized as a track. The user can then log in to their Web Apollo account straight from the browser window to begin annotating. For example, an annotator can use BLAST to align a sequence from a well-annotated reference species, such as *Drosophila melanogaster*, to multiple genome assemblies at once, open

the results in multiple genome browsers and begin annotating if they notice that the computationally predicted gene feature needs editing. We also provide a tutorial for using BLAST in the i5k Workspace (<https://i5k.nal.usda.gov/content/blast-tutorial>). We use Google Analytics to identify user metrics such as duration of stay, aggregated user behavior and entry/exit points. Identification of resources that are in high demand, such as BLAST, is critical for determining what may need refactoring to increase capacity. Alternatively, low traffic resources need to be assessed to determine why they are under utilized and whether steps may be taken to improve usage.

FUTURE DIRECTIONS

The i5k Workspace is available for any arthropod genome project. We therefore anticipate that our content will expand in the future. To prepare for future expansions, we are developing in two directions: automation and delegation. First, we strive to automate as many tasks as possible. We are currently working on an improved and streamlined data submission system that will harvest metadata from submitters in a user-friendly way, and are also developing tools for more consistent processing of gff3 files. Second, not all tasks can be automated; for those that cannot, we will develop tools to delegate most of the responsibility to the community contacts. Finally, we intend to streamline the tools that we offer, and only expand in a few more limited directions. For example, we strive to improve our organism browser to accommodate more species; therefore, we are identifying other options to search our content, for example via tree-based viewers. We will implement tools to visualize pre-computed orthology and paralogy data of official

Table 1. Species hosted at the i5k Workspace@NAL, their common name and source

Species name	Common name	Source
<i>Agrilus planipennis</i>	Emerald ash borer	BCM i5k pilot
<i>Anoplophora glabripennis</i>	Asian long-horned beetle	BCM i5k pilot
<i>Athalia rosae</i>	Turnip sawfly	BCM i5k pilot
<i>Centruroides exilicauda</i>	Bark scorpion	BCM i5k pilot
<i>Ceratitis capitata</i>	Mediterranean fruit fly	BCM i5k pilot
<i>Cimex lectularius</i>	Bed bug	BCM i5k pilot
<i>Copidosoma floridanum</i>	Parasitic wasp	BCM i5k pilot
<i>Diaphorina citri</i>	Asian citrus psyllid	International Psyllid Consortium
<i>Drosophila biarmipes</i>	NA	BCM modENCODE project
<i>Drosophila bipectinata</i>	NA	BCM modENCODE project
<i>Drosophila elegans</i>	NA	BCM modENCODE project
<i>Drosophila eugracilis</i>	NA	BCM modENCODE project
<i>Drosophila ficusphila</i>	NA	BCM modENCODE project
<i>Drosophila kikkawai</i>	NA	BCM modENCODE project
<i>Drosophila rhopaloa</i>	NA	BCM modENCODE project
<i>Drosophila takahashii</i>	NA	BCM modENCODE project
<i>Ephemera danica</i>	Mayfly	BCM i5k pilot
<i>Eurytemora affinis</i>	Common copepod	BCM i5k pilot
<i>Frankliniella occidentalis</i>	Wester flower thrips	BCM i5k pilot
<i>Gerris buenoi</i>	Water strider	BCM i5k pilot
<i>Homalodisca vitripennis</i>	Glassy-winged sharpshooter	BCM i5k pilot
<i>Ladona fulva</i>	Scarce chaser	BCM i5k pilot
<i>Latrodectus hesperus</i>	Western black widow spider	BCM i5k pilot
<i>Leptinotarsa decemlineata</i>	Colorado potato beetle	BCM i5k pilot
<i>Limnephilus lunatus</i>	Caddis fly	BCM i5k pilot
<i>Loxosceles reclusa</i>	Brown recluse spider	BCM i5k pilot
<i>Manduca sexta</i>	Tobacco hornworm	Kansas State University
<i>Mayetiola destructor</i>	Hessian fly	Purdue University
<i>Oncopeltus fasciatus</i>	Milkweed bug	BCM i5k pilot
<i>Onthophagus taurus</i>	Bull-headed dung beetle	BCM i5k pilot
<i>Orussus abietinus</i>	Parasitic wood wasp	BCM i5k pilot
<i>Pachypsylla venusta</i>	Hackberry petiole gall psyllid	BCM i5k pilot
<i>Parasteatoda tepidariorum</i>	Common house spider	BCM i5k pilot
<i>Tigriopus californicus</i>	Harpacticoid copepod	UC San Diego
<i>Trichogramma pretiosum</i>	Parasitic wasp	BCM i5k pilot

The Baylor College of Medicine is abbreviated as BCM.

gene models when this data becomes available. For projects with an OGS, we will enable searches for gene names and IDs via the Tripal interface for the Drupal Views module. Finally, we are developing new ways to display manual curation data from our active curation communities, which will draw annotation statistics directly from the Web Apollo Berkeley data stores and display them on the i5k Workspace front page; statistics will be re-calculated and refreshed hourly. The NAL has committed to sustaining this project for the longer term, provided the site resources are being sufficiently used.

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Conflict of interest statement. None declared.

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