

GAL

Genome Annotator *Light*

Version 1.1

User Guide

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Introduction

GAL is a software package for analyzing and visualizing a genome or a group of genomes. GAL is implemented inside Docker. Docker technology is becoming popular throughout the bioinformatics community due to its features, ease with dependencies and more efficient usage of the underlying system and resources. Docker allows deploying an application in a sandbox (called container) to run on the host operating system locally. Docker needs to be installed on the host system (Linux in this case) to proceed with GAL.

Getting Started

GAL can be installed and initiated through Docker. Docker is available in two editions: Community Edition (CE) and Enterprise Edition (EE). Docker CE and EE are available on multiple platforms, on cloud and on-premises.

- Docker website: <https://www.docker.com/>
- Docker Documentation for beginners: <https://docker-curriculum.com/>
- Docker CE and EE are available at: <https://docs.docker.com/engine/installation/#supported-platforms>

System Requirements

GAL can be installed on the following operating systems:

- CentOS 7.1/7.2 & RHEL 7.0/7.1/7.2/7.3 (YUM-based systems)
- Ubuntu 16.04 LTS or higher

Quick Start

1. GAL can be downloaded and installed using following docker command:

```
docker pull rjit17/gal
```

This will fetch the latest version with 'latest' tag. For specific version, use the version number for example: **docker pull rjit17/gal:1.1**

2. To run GAL use the following command:

```
docker run -it -p 8080:80 rjit17/gal
```

This will initiate GAL at port 8080 of local server or *localhost*. User may use another port to initiate another instance

[To manipulate Docker utilities refer to [Docker Documentation](#)]

3. While the GAL instance is running inside Docker container, GAL User Interface (UI) can be accessed through a web browser at following URL:

<http://localhost:port/>

In this case, it is

<http://localhost:8080/>

It can also be:

<http://<IP address of the host computer>:8080>

4. GAL can now be used to upload your data through the browser.

Additional useful Commands

List docker images

To find the pulled docker images in the system user can use the following commands:

```
docker images
```

This will list images as follows,

REPOSITORY	TAG	IMAGE ID	CREATED	VIRTUAL SIZE
rjit17/gal	0.3	8dbdefed7c21	2 days ago	5.722 GB
rjit17/gal	0.2	862e3935ccd8	2 days ago	5.722 GB
rjit17/gal	0.1	2e94bfbe45b9	9 weeks ago	5.665 GB

Set instance name

Docker by default allocates a random name and id for the running instance. User can change the instance name by adding '--name' option in the command line. It will help the user to track an instance later.

Example:

```
docker run --name=test -it -p 8080:80 rjit17/gal
```

Here 'test' is the name of the running instance.

Find docker instances

To find all the available docker instances use the following commands

```
docker ps -a
```

This is the output example of the above command.

CONTAINER ID	IMAGE	COMMAND	CREATED
969ab10373bc	rjit17/gal:0.2	"/bin/sh -c 'service "	26 hours ago
476d22340d5f	rjit17/gal:0.3	"/bin/sh -c 'service "	47 hours ago
a5e4e47e6bdb	rjit17/gal:0.2	"/bin/sh -c 'service "	2 days ago
STATUS	PORTS	NAMES	
Up 26 hours	0.0.0.0:8080->80/tcp	hopeful_visvesvaraya	
Up 47 hours	0.0.0.0:7070->80/tcp	mad_pare	
Exited 2 days ago		trusting_curie	

Exit docker instance

To exit from a running docker instance use 'exit' command.

To exit from docker command line, use **CTRL+p** followed by **CTRL+q**

Re-enter running instance

To re-enter into a running instance, use the following command

```
docker exec -it<Container_id/Name> bash
```

Example:

```
docker exec -ittest bash
```

Here 'test' is the name of the running instance.

Restart Docker instance

To start the stooped instances, use the following command:

```
docker start -i <Container_id/Name>
```

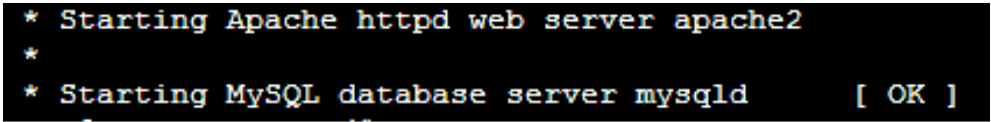
Example:

```
docker start -i test
```

Here **'test'** is the name of the running instance.

Successful GAL Start

On successfuldocker GAL instance start, the following message will appear.



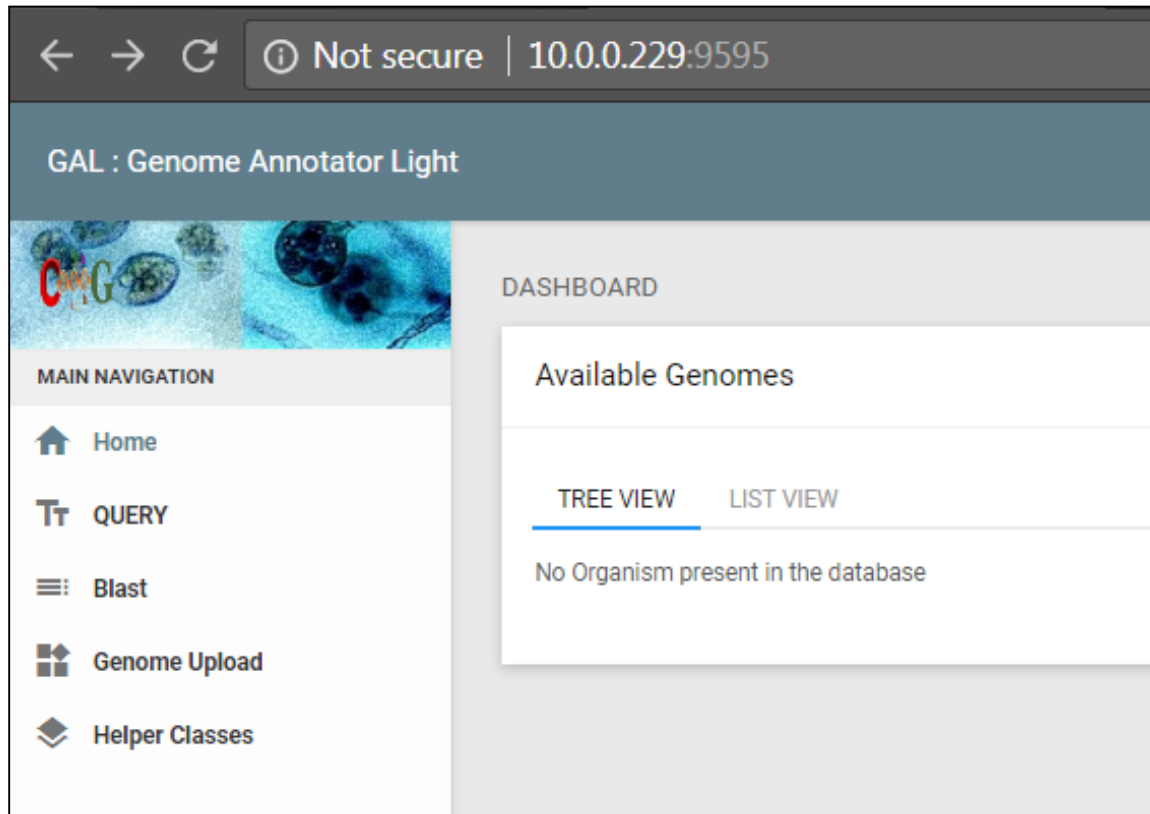
```
* Starting Apache httpd web server apache2
*
* Starting MySQL database server mysqld      [ OK ]
```

[OK] indicates successful initiation.

GAL User Interface (GUI)

GAL GUI is must for data visualization, and it includes several web pages like,

GAL Homepage



- GUI for GAL can be loaded inside a web browser for Genome Upload, Genome Browsing; downstream analyses like Blast Searches, Annotation Query and Sequence Retrieval along with analyses of all the annotated proteins using various EMBOSS tools.
- The Homepage will list the genomes only after they are processed. Until then there will be no data available in the list view or tree view.

It approximately took 28 minutes to process ~5 Mb *E.coli* genome for Genbank Annotation as input on standard Ubuntu Desktop having 4 CPUs and 4 Gb of RAM. The same genome at various annotation levels took proportionate time. e.g. Product Annotation (31 minutes), Minimal Annotation (30 minutes), and No Annotation (175 minutes using GeneMark annotator + NCBI BLAST).

GAL: An Integrated Virtual Machine for Genome Analysis and Visualization

- The Navigation panel to the left will help the user to access various features like:
 - **Genome Upload:** Upload options at any stage of the annotation process.
 - **QUERY:** Gene search using gene name, primary annotation, genomic locus or HMMPFAM/ Signalp/ tmhmm annotations.
 - **BLAST:** Sequence search using NCBI BLAST for protein or gene sequence within the uploaded dataset.
 - **Help:** Help and documentation.

GAL Data Upload Options

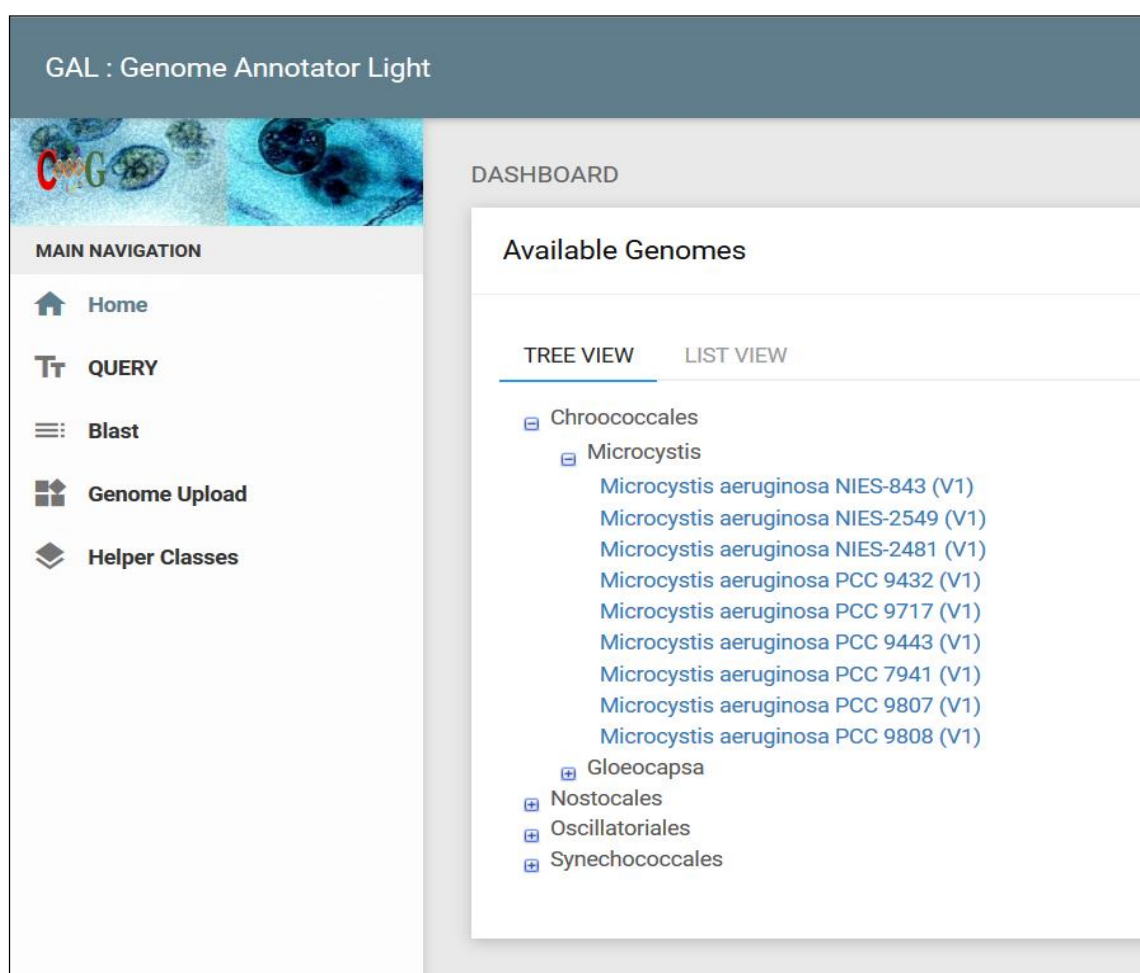
The screenshot displays the GAL: Genome Annotator Light web interface. On the left is a 'MAIN NAVIGATION' sidebar with icons and links for Home, QUERY, Blast, Genome Upload (which is highlighted), and Helper Classes. The main content area is titled 'Genome Upload' and features four tabs: 'Genbank' (selected), 'Product Annotation', 'Minimal Annotation', and 'No Annotation'. Under the 'Genbank' tab, there are input fields for 'Organism Name' (with placeholder text 'Enter your Organism Name') and 'Organism Version' (with placeholder text 'Version'). Below these is a 'Genbank File' section containing a 'Choose File' button, the text 'No file chosen', and a prompt 'Please provide genbank file of your genome'. At the bottom of the form are three blue buttons: 'Submit', 'Load Demo Data', and 'Download Demo Data'.

The user can provide data in four ways, viz. type1: Genbank Annotation, type2: Only Genome Fasta files, type3: Genome fasta and gff files; type 4: Genome Fasta, gff files and product files

- **Genbank Annotation:** This allows data input through NCBI annotated Genbank file (GBFF).

- **Product Annotation:** This allows genome FASTA, GFF (genome feature file) and product information file.
- **Minimal Annotation:** This allows the basic annotation information provided by the user where user provides genome FASTA (FNA) file and GFF file.
- **No Annotation:** This allows data through only genome FASTA (FNA) file with annotation options using AUGUSTUS or Genmark for eukaryotic and prokaryotic genomes using related reference genomes, respectively.

GAL Sample Data



Clicking the genome name will direct the browser to Genome Summary Page for respective organism where organism details and links to the Scaffold wise Genome browser links are provided.

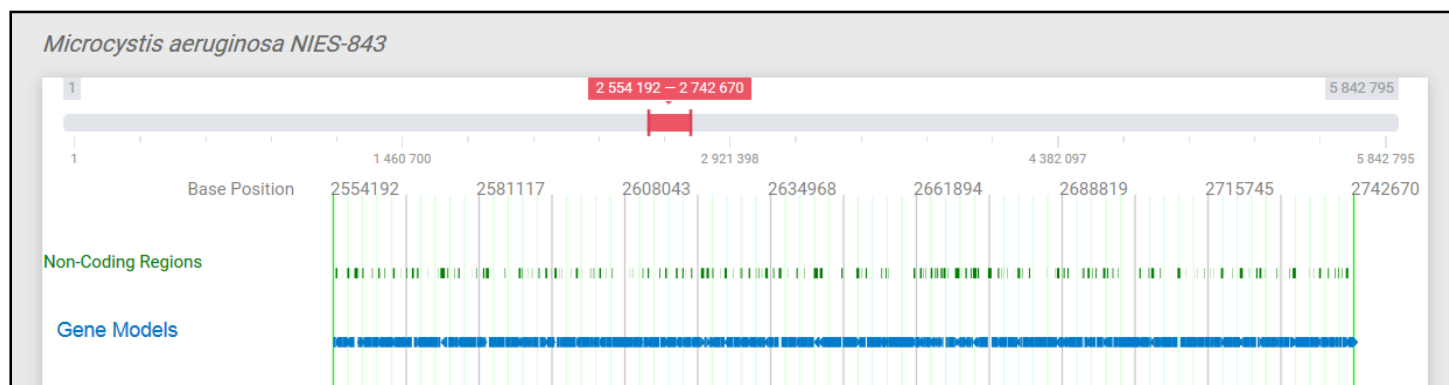
GAL: An Integrated Virtual Machine for Genome Analysis and Visualization

From genome browser, each coding and non-coding regions can be visualized in details with exon-intron boundaries along with sequence download links and analysis options.

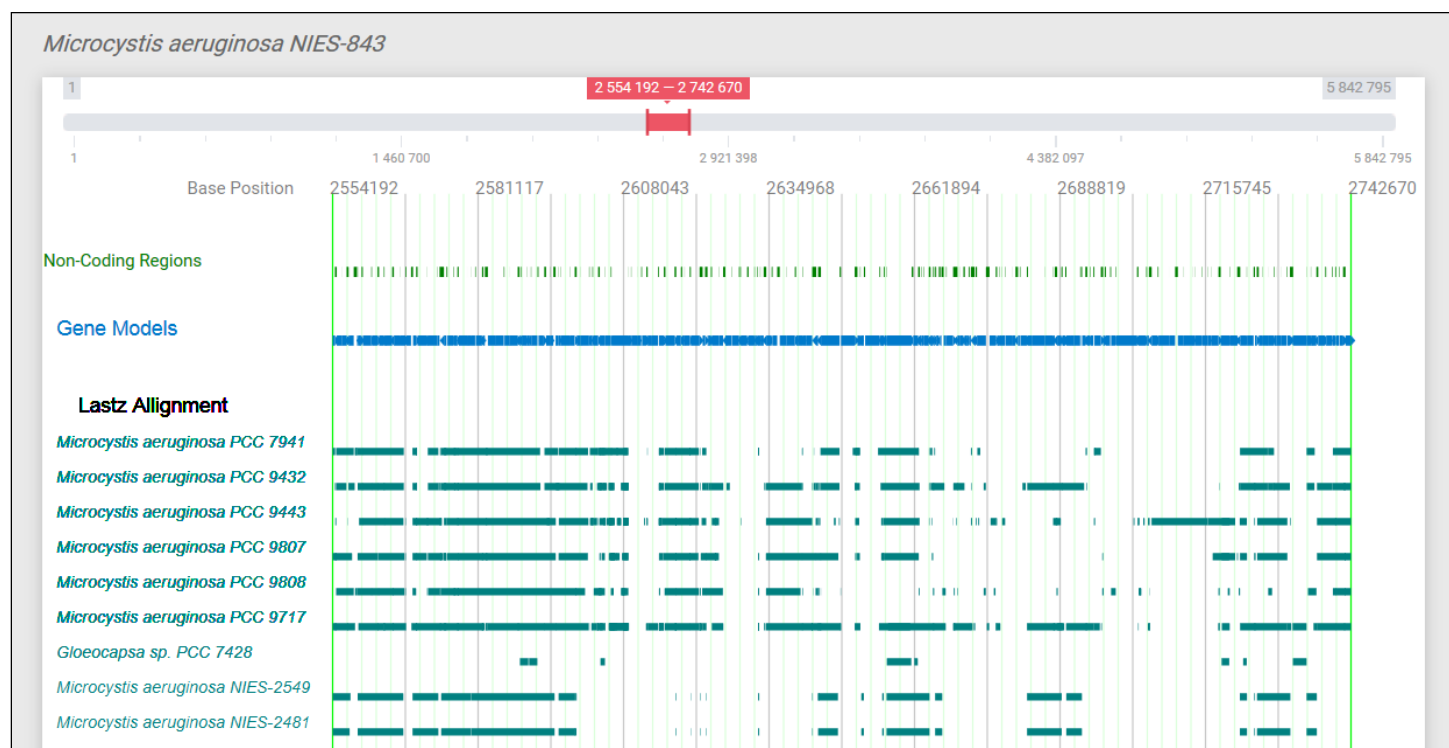
GAL Genome Browser

GAL Genome browser can visualize **coding** and **non-coding** regions in selected locus range of selected genome, as shown in the following image.

SINGLE GENOME BROWSER MODE



MULTI GENOME BROWSER MODE



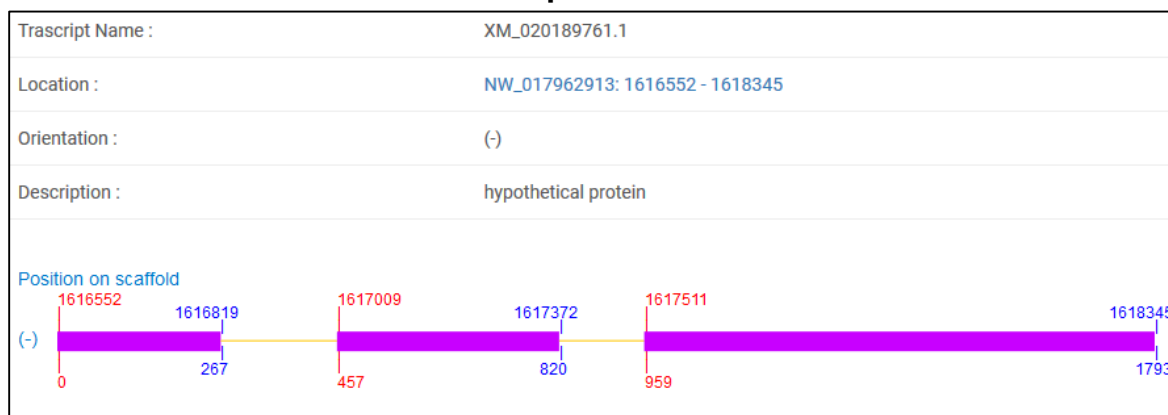
Additionally, GAL can automatically visualize respective regions from multiple taxonomically related species (if present in given dataset) based on LastZ Alignments.

Each highlighted region links to the individual gene details page with annotation details, gene analysis options and sequence download options.

Gene Details Page

All the annotated genes, transcript or proteins can be analyzed separately into Gene details page,

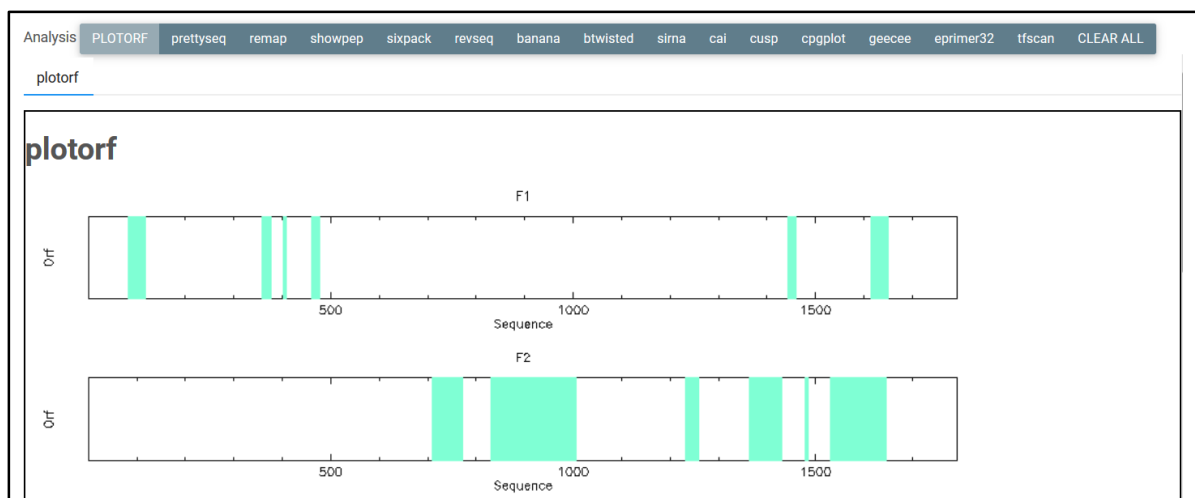
Exon Intron Boundaries for transcripts:



Annotation summary tables for various methods are also displayed on the same page for more details.

EMBOSS TOOLKIT

The protein analysis supported by various EMBOSS tools is available at each gene details page. The outputs can be visualized on the same page by just clicking the name of the package. All the outputs can be downloaded as image or text format wherever suitable.



The above screenshot shows various EMBOSS tools incorporated into the GAL analysis. The example output for the given transcript by plotorf tool is shown here. All the adjacent tabs with the name of these tools can generate the standard outputs. These tools include **banana**, **cpgplot**, **eprimer32**, **sixpack**, **showpep**, **tfscan** etc.

Gene Sequence Page

The gene sequence page provides the option for retrieving nucleotide sequences of the genomic region as well as protein sequence of the translated gene. The green highlighted sequence indicates the exons for easy understanding and reporting.

Predicted Gene Model(green marked regions are exons, white marked are introns)

Length: 1793 bases

```
ccctgctttgttggcgaccactttaccggagggtctacttggattagtagttgttcaggggtcaggtcgatccagttgtcgatgattctgatccggagaaggttgttagga
gttagttgagttaacacgagctgcatcagataaggctgctgttaagtaggtagttgtcttgttgtttaatcataaggataaccgctggttgatctggtcaataaactag
gtaattgacttatgaggggacttagttgactattttaaatgttggcggttgttgggtgagaggaaaatggttttaagttctgaatagagttataagttactaagtttag
attataggagttaaacggctccttgatgattgacaggttgttgaagtggtagttaaacaggttgaagatgtcttgaacagtaagggtattccttagtttcagaaacctg
taccgtagctatttaatggttatagttttgatttaagttaaaaggagggttgtagctgcttcattgttcttagttgacatagaaggatctgcgggatttttagccgagttg
gaataccgaggaacacttttagtatttccggacacgtggagttaggggataaaaaatatttgaatgtactctgtgcttcgacgttgttctttaaattactggttgatagga
ggaaagatctgttgaaggtagtggtttaaggacttttctaactatggaggataaaagtgttttctttaaactttgttagtaggtgccaagaaattgaaactgatttgatt
ttgattaagatttatctgattagttgctttttatagggtaaaattgttgaaatggtcaagtttgtgtgtcgttgaggttgtgtgttagagttgttgagttgttag
agttgttggagttgttggagttgttagagttgttggagttgttggagttattgtagaattatctttatgttttagtttagtttcagttgttggatttttgagagattact
acttttttaattatagttttatgatgttgagtagttagggtttgttatctgagtaagccattgtctatttgatttggaaatactttcgtctcagaagtgagggtgacctttt
agaattgacttgatcgtttaccaaccgggttagctggaagttatgccaaaccatttgccttcctcagtaggtaaaagctaaccgttttgaattacagacatctattatact
ttctactttaatggtagtagaaatcgctagtgtaagataagggtttttaatatctgcttttgatgatgttggtttagttctttttgtttcttttagttcttttgattcta
gttccagttctttaaattgttaggtgagtcctatgattatttggtagtgtctctactattactattattactattacttttgggtcttttagttaaagtaaaactag
atgagttactacttctaatactactgttataggtatggggtagttatagtcctatagaattacttaagggttaggttactttcaggttaatgtttatagggttcgatttcc
aaagaccaaccggctataaagtttacaaaaggggagttggatattgtctaccacagaaaaatgtcaagaacaatgacctttaccttagtttaacctagggttagttgggtc
tcgattgggtgtgtactcttttatatttgattcgctagggtttatttaatttactaccgatatttttagtatcttaattagttattttcgattttaagatttgttttcgagag
ggaaagtagaaaagttt
```

Predicted protein sequence

Length: 489 amino acids

```
WDETTAGEMASQMWLIINKSPVQLGQQLRLGLFQQSSINSIVLDVVYSDDNSSIKQNNKLVLFGDQLDQLFDPLTEYSPESDVKIYKPPNKLSEFYQNSRLISIFNDSN
LISSICQELLTVQTNFTINLVNLFQNFVIPLRIKVLHGIKLPISKLSIFPPTIDEVTRINCIFLDALKSAQPYGSFEIHKACGTSIPYFYKAYMRHEAATRNFDQLS
SFLDNFHHQIPERIDTSYFTKRRIETIIHGSNLTKLKLILNRLINEKISHLNTFTINNHNKSLMMKKLISKYNNSSIQTIDSFGNDKLKPYESRVFTPTGKILTELANGW
PIDLQYGWNNRRVISIFDCENLMSVDNMKDEITIIIFSDHILFLKIIDENYNNQIKKKQRKSRLKRSSPITNIPKLKVSQWADISNVFPSTYNDGVFLQFFVTGNGIKLDPN
QPELTQHMRKYKLSDPNKLNLDGYKIIELINKAKILNKSSPFHLFK
```

BLAST Page

As the genomes are available in the database after processing the genomes uploaded by the user, any nucleotide or protein sequences can be BLASTed against the available genomes. The selection of any of the genomes or all the is possible from the checkboxes near organism names. The genomes are shown as tree view for the blast options.

Local Blast

Copy and Paste your sequence

```
>sequence
ttttgagagattactacttttttaattatagttttatgatgttgagtagttaggt
ttgttatctgagtaagccattgctatttgattttggaatactttcgtctcagaa
gtgaggttgacctttttagaattgacttgatcgttaccaaccggttagctg
gaagttagccaaccatttgccttcagtataggtaaaagctaacgcttt
tqaattacaqacatctattatactttctactttaatqgtaqtaaaatcqcta
```

Select Blast Program NCBI-BLASTN

Select Database

- Enterobacterales
 - Escherichia
- Saccharomycetales
 - Ascoidea
 - ☒ Ascoidea rubescens DSM 1968 (V1)
 - ☐ Ascoidea rubescens DSM 1968 (V2)
 - ☐ Ascoidea rubescens DSM 1968 (V3)

Evalue(E):

Cutoff Value(S):

Substitution matrix BLOSUM62

Maximum Alignments(B): 10

Set up Filter Option YES

The screenshot of the BLAST page showing various options for sequence input and parameter as well as genome selection.

Command Line Options

How to run GAL in command line mode?

GAL can easily be run from a web browser. Optionally, for users familiar with Docker command line and Ubuntu Terminal can run GAL through command line.

Accessing host directory

The host directory can be accessed through the following command:

```
docker run -it -v [host_directory_path]:[GAL_file  
system_path] -p 8080:80 rjit17/gal:[GAL version]
```

Example:

```
docker run -it -v /home/arijit/test:/usr/GAL_data -p  
8080:80 rjit17/gal
```

After running the above command, the host operating directory will be available to the GAL file system. In that way user can process data from the host directory. Now you will enter to GAL container.

```
root@container_id:/#
```

Running the programs

GAL is based on Python. Python 3.4 or above is required to use GAL. The main program for GAL is **main.py** present at: **/usr/GAL** path.

To run the GAL control script use following command:

```
python3 /usr/GAL/main.py --orgconfig=[config_file_path]
```

Setting up the configuration file

User needs to provide configuration file in INI format.

INI format:

```
[section]  
name=value
```

Structure of the organism configuration file:

```
[OrganismDetails]  
Organism:  
version:  
source_url:  
  
[SequenceType]  
SequenceType:  
  
[AnnotationInfo]  
Blastp:  
signalp:  
pfam:  
tmhmm:  
  
[filePath]  
GenBank:  
FASTA:  
GFF:  
Product:  
LastZ:  
SignalP:  
pfam:  
TMHMM:  
Interproscan:  
  
[other]  
Program:  
ReferenceGenome:
```

Sample configuration file is present at: **/usr/GAL/config/organism_config_format.ini**

Data Format

We have defined input data type in four ways,

Data Type Name	Input files
Genbank Annotation	Genbank Sequence File
No Annotation	Genome Fasta File
Minimal Annotation	Genome Fasta File, GFF file
Product Annotation	Genome Fasta File, GFF File, Product file

Sample organism data upload using command line mode:

Data type	Commands to upload Sample genomes
Genbank Annotation	python3 main.py -org=/usr/GAL/SampleFiles/genbank_annotation/org_config.Ini
No Annotation	python3 main.py -org=/usr/GAL/SampleFiles/no_annotation/org_config.Ini
Minimal Annotation	python3 main.py -org=/usr/GAL/SampleFiles/minimal_annotation/org_config.Ini
Product Annotation	python3 main.py -org=/usr/GAL/SampleFiles/product_annotation/org_config.Ini

List of Reference Genomes

AUGUSTUS Reference Genomes	
Organism Name	Organism code for configuration file
Animals	
Aedes aegypti	aedes
Amphimedon queenslandica	amphimedon
Acyrtosiphon pisum	pea_aphid
Brugia malayi	brugia
Caenorhabditis elegans	caenorhabditis
Drosophila melanogaster	fly
Homo sapiens	human
Nasonia vitripennis	nasonia
Tribolium castaneum	tribolium
Trichinella spiralis	trichinella
Alveolata	
Tetrahymena thermophila	tetrahymena

Toxoplasma gondii	toxoplasma
Plants and Algae	
Arabidopsis thaliana	arabidopsis
Galdieria sulphuraria	galdieria
Solanum lycopersicum	tomato
Zea mays	maize
Fungi	
Aspergillus fumigatus	aspergillus_fumigatus
Aspergillus nidulans	aspergillus_nidulans
Aspergillus oryzae	aspergillus_oryzae
Aspergillus terreus	aspergillus_terreus
Botrytis cinerea	botrytis_cinerea
Candida albicans	candida_albicans
Candida guilliermondii	candida_guilliermondii
Candida tropicalis	candida_tropicalis
Chaetomium globosum	chaetomium_globosum
Organism Name	Organism code for configuration file
Coccidioides immitis	coccidioides_immitis
Coprinus cinereus	coprinus
Cryptococcus neoformans	cryptococcus_neoformans_neoformans_B
Debaryomyces hansenii	debaryomyces_hansenii
Encephalitozoon cuniculi	encephalitozoon_cuniculi_GB
Eremothecium gossypii	eremothecium_gossypii
Fusarium graminearum	fusarium_graminearum
Histoplasma capsulatum	histoplasma_capsulatum
Kluyveromyces lactis	kluyveromyces_lactis
Laccaria bicolor	laccaria_bicolor
Lodderomyces elongisporus	lodderomyces_elongisporus
Magnaporthe grisea	magnaporthe_grisea
Neurospora crassa	neurospora_crassa
Phanerochaete chrysosporium	phanerochaete_chrysosporium
Pichia stipitis	pichia_stipitis
Rhizopus oryzae	rhizopus_oryzae
Saccharomyces cerevisiae	saccharomyces_cerevisiae_S288C
Schizosaccharomyces pombe	schizosaccharomyces_pombe
Ustilago maydis	ustilago_maydis
Yarrowia lipolytica	yarrowia_lipolytica

GeneMark Reference Genomes	
Organism Name	Organism code for configuration file
Vibrio fischeri ES114	Aliivibrio_fischeri_hmm.mod
Azotobacter vinelandii DJ	Azotobacter_vinelandii_hmm.mod
Bacillus subtilis subsp. subtilis str. 168	Bacillus_subtilis_hmm.mod
Escherichia coli str. K-12 substr. MG1655	Escherichia_coli_hmm.mod
Mycoplasma genitalium G37	Mycoplasma_genitalium_hmm.mod
Pseudomonas fluorescens SBW25	Pseudomonas_fluorescens_hmm.mod
Synechocystis sp. PCC 6803	Synechocystis_sp._PCC_6803_hmm.mod

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