Insyght's
Standalone Virtual Machine
-
User guide
(Version 2.1.1, Feb 2019)

Citation Insyght:
Lacroix T., Loux V., Gendrault A., Hoebeke M., and Gibrat J-F. (2014) Insyght: navigating amongst abundant homologues, syntenies and gene functional annotations in bacteria, it's that symbol! Nucleic Acids Res. 42(21):e162. PMID: 25249626

Content:

1 Getting started with visualizing your data

~~ Remark: the screen will lock itself automatically after a period of inactivity or if you lock it intentionally. The default password to unlock it is: insyght

~~ Remark: Insyght expects a transcript per CDS and is therefore only suitable for Bacteria and Archea.

~~ Remark: the requirements for Insyght's standalone virtual machine can be found at https://sourcesup.renater.fr/wiki/insyght/requirements_for_insyght_s_standalone_virtual_machine

1.1 Launch Insyght

To launch your local instance of the Insyght web application, double click on the "launch_insyght_web_app" icon on the desktop. It will open Firefox to its home address: **http://localhost:8080/Insyght/**.

If you already inserted some data previously, you should be able to browse them. If you haven't, a message will appear saying that the database is empty. Follow this guide to populate the database with your data. Links are also available to showcase the tool's features or to access different manuals and guides (https://sourcesup.renater.fr/frs/?group_id=3679).

1.2 [Optional] Remove all current data in your instance of the database

Optionally, you can remove all the current data in your instance of the database before inserting new data. Insyght relies on this database to fetch any information it displays. Skip this section if you want to keep the current data and add yours along with them. The process of adding data in Insyght is incremental and can be done multiple times without deleting the current data.

If you wish to remove the current data in the database, type in the following command in a terminal:
perl -I $SCRIPTS_DIR $SCRIPTS_DIR/truncate_all_db_tables.pl

~~ Warning: this action cannot be undone

*~~ Remark: throughout the document, the text that is **highlighted in red** is to be copied/paste or typed in a terminal.*

~~ Remark: The database should now be empty. If you open the Insyght web application, you should see that no organism is available.

1.3 Add your own data

This section explains how to add and visualize your own data with Insyght.

~~ Remark: we suggest that you try loading the demo data first to check that the pipeline is working correctly. See the steps below to load the demo data.

~~ Remark: Do not shut down the VM while the pipeline runs else it will terminate the process. If an unexpected error occurs (i.e. computer crash, power shutdown, etc.) before the pipeline is finished, you can re-launch it. On the other hand, you can close the terminal that runs the pipeline anytime as the command thereafter uses screen. You can open another terminal anytime and re-attach the process of the pipeline as needed (see steps below). Closing the terminal will not end the screen command but using crtl-C in the terminal or shutting down the VM while the command runs will end it.

~~ Benchmark: When information on benchmarking is provided, it was carried out on the following machine: Intel(R) Core(TM) i7-4800MQ CPU @ 2.70GHz, 32 GO RAM, 64 bits. Virtualbox was used as the virtualization host environment. The VM was launched with the following parameters: 9654 Mo RAM, 1 CPU, 69 Mo video RAM. Those parameters (including the number of available internal processors CPU) can be configured before launching the VM.

1.3.1 Open a Terminal

1.3.2 Delete the temporary and log files generated by the pipeline from the previous run

```
rm -f -r ${DATA_DIR}/*
rm -f -r ${LOG_DIR}/*
rm -f -r ${BANK_DIR}/*
```

1.3.3 [Optional] Download genome files from ncbi or annotate raw fasta file

See the section "*Different ways to download a raw fasta or annotated genome file from the ncbi or ebi repositories*" for more details. if you want to annotate raw fasta files, you can quickly annotate them with prokka which will automatically generate a genbank file. Prokka (http://www.vicbioinformatics.com/software.prokka.shtml) is a software tool for the rapid annotation of prokaryotic genomes developed by the Victorian Bioinformatics Consortium. For more details, see the section "Run prokka to

quickly annotate raw fasta file".

1.3.4 Gather all the .gbk, .dat and .gbff genomes files you want to add in ${BANK_DIR}

Copy or move the genomes files (.gbk, .dat, and .gbff) in the directory ${BANK_DIR}.
cp ***PATH_TO_YOUR_FILE* ${BANK_DIR}**
Repeat the command for each file to copy. For example, if you did download the complete genomes files from Refseq for Enterococcus faecalis, Acidobacteriia, and Escherichia albertii following the command line given as an example in "*Download genome files from ncbi using script*", do:
cp ~/Downloads/* ${BANK_DIR}

List the files that are going to be inserted:
ll $BANK_DIR/

~~ Remark: A set of demo genome files can be found under the directory ${SCRIPTS_DIR}/../../ORIGAMI/DEMO_FILES/ANNOTATED_GENOMES/BATCH1/ If you want to load the demo data to check that the pipeline is working correctly, use the command:
cp ${SCRIPTS_DIR}/../DEMO_FILES
/ANNOTATED_GENOMES/BATCH1/* ${BANK_DIR}/

~~ Remark: To download genome files from ncbi or ebi, see the section "Different ways to download a raw fasta or annotated genome file from the ncbi or ebi repositories". If you use the script download_genome_files_from_ncbi.pl, the files will be downloaded directly into the ${BANK_DIR} directory so there is no need to copy them.

~~ Remark: If you did annotate some fasta file with prokka, do not forget to copy them as well, typically with the command:
cp /root/mydisk/prokka_output/*.gbk ${BANK_DIR}/

~~ Remark: Compressed files with the .gz extension are supported by the pipeline, no need to extract them.

1.3.5 Run the Insyght pipeline to add the data

This script will perform preliminary preparations of the genome files, retrieve primary data (organisms, genes, features, etc.), compute the cross comparison of all the CDSs, infer the syntenies, and insert the data into the database. For an overview of the pipeline, see the section "Overview of the pipeline". To run the script:
**screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl **

-PROGRAM_TO_USE PLAST

This script may take some time hence the use of screen to detach the process from the terminal as needed and to avoid closing the process if the terminal closes. To detach the process, you can close the terminal that runs this script or type “ctrl-a d”. At a later time, open another terminal and re-attach the process of the pipeline as needed by typing:
screen -D -R
Closing the terminal will not end the screen command but using crtl-C in the terminal while the command runs or shutting down the VM will end it.
If you have detached the process and want to check whether the script is still running or not, use the command:
screen -ls
If the script is done, you should see an output such as:
No Sockets found …
If the script is still running, you should see an output such as:
There are screens on…
Alternatively, you can use **top** to see the list of all the programs that are running or check the log file for this step (see below).

~~ Remark: Information will be printed on the screen while the script run. If the script is successful, screen will exit. You should see:
run_Insyght_pipeline.pl successfully completed at DATE
printed on the last line of the log file:
tail ${LOG_DIR}/run_Insyght_pipeline/run_Insyght_pipeline.log
To access the whole log file, use
less ${LOG_DIR}/run_Insyght_pipeline/run_Insyght_pipeline.log

~~ Benchmark: The time and resources needed to process the data grow with the number of genomes to compare. When using Plast (see below):

Number of genomes	*Processing time*	*Final size of database*	*Max size needed for OS (~10 GB) + tmp files*	*Min local disk space when choosing master VM*
17 (demo dataset)	*~1h*	*~325 MB*	*~14 GB*	*20 GB*
50	*~8h45min*	*~1.7 GB*	*~18 GB*	*20 GB*
100	*~34h*	*~4.8 GB*	*~22 GB*	*40 GB*
150	*~108h*	*~10 GB*	*~33 GB*	*40 GB*
200	*~152h*	*~17 GB*	*~45 GB*	*80 GB*
250	*~259h*	*~25 GB*	*~65 GB*	*80 GB*

When using multiple parallel processes (multicore VM or cluster, see below), it approximately divides the time it takes to process the genomes by the number of jobs in parallel (option -MAX_JOBS, see below) plus an extra hour. For example, with -MAX_JOBS 20 (20 processes in parallel, either on local CPU or cluster) it takes ~7 hours to process 150 genomes instead of 108 hours with a single thread. The pipeline

can be used incrementally without deleting previous entries. In this case, each new organism will to be compared to new and previous entries.

~~ *Recommended options for this script (you can combine them):*

-PROGRAM_TO_USE {PLAST,BLAST}
As an alternative to Blast, you have the option to use Plast (https://plast.inria.fr/). With the default parameters and for most cases, Plast is about 5 times faster than Blast but less sensitive (about 2 times less alignments found). However, regarding BiDirectional Best Hits (BDBH), this decrease in sensitivity is not significant. As Insyght relies primarily on BDBH, Plast therefore gives results comparable to Blast for our purpose. Blast is the default sequences alignment tool if you omit the -PROGRAM_TO_USE option or if you use:
**screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl **
-PROGRAM_TO_USE BLAST
Using Blast, this step took ~6 hours for the 17 organisms provided as demo.
If you wish to use Plast instead, use the following option:
**screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl **
-PROGRAM_TO_USE PLAST
Using Plast, this step took ~1 hours for 17 organisms provided as demo.

-MAX_JOBS {POSITIVE_DIGIT}
To parallelize the processes and make this step run faster, you can use the option -MAX_JOBS. The total time for this step will decrease proportionally with the number of jobs launched in parallel. With -MAX_JOBS 17, this step took ~1 hour for the 17 organisms provided as demo:
**screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl **
-MAX_JOBS 17
The -MAX_JOBS value must be at most the number of available processors. To know the number of internal processors available, type in the command:
grep -c processor /proc/cpuinfo
You can configure your VM to have as much internal processors as the host machine. If the number of available processors is limited on your host machine, a solution to greatly increase the number of available processors is to use a cluster (external processors, see option -CMD_CLUSTER below).

-CMD_CLUSTER {**COMMAND_SUBMIT_CLUSTER*}
See the section "Using a cluster (external processors)" for more details. If the CMD_CLUSTER argument is omitted (default), then the command is executed by the bash locally on the internal processors.

~~ *Other options for this script:*

- To change the default threshold parameters for homologs and syntenies, see the section "Changing the threshold parameters for the programs blast (used to find gene

similarity) and align (used to compute the synteny)".

-FORCE_SPLIT_ACCNUM_INTO_SEPARATE_ENTRIES {ON, OFF} # default OFF
If you want each element (chromosomes, plasmids, etc.) of an organism to be treated as its own entry instead of being grouped by organism/assembly, turn this option ON:

```
screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl \
 -FORCE_SPLIT_ACCNUM_INTO_SEPARATE_ENTRIES ON
```

1.3.6 See the results

That is it, launch Insyght (see section "Launch Insyght") to see the results!

2 How to update the virtual machine

2.1 How to update the entire virtual image

As the project develops, you can benefit from the latest version and update your local virtual image. You can either update the entire virtual image (this section) or only the individual components that have changed (see the section "How to update the web application (war file) only").

- To know what your current version is, see the file Insyght_virtual_image_version_XXX_and_licencing.txt on the desktop of your virtual image.
- The latest version of the virtual image can be found at **https://migale.jouy.inra.fr/redmine/projects/insyght/news** under the section "**Latest version of the Insyght virtual machine**".
- Import the .ova file into your favorite virtualization software package (ie VirtualBox).
- If you wish to keep in the new virtual image the data that you have computed in the database of the old virtual image, then you will need to migrate them as follow:
- Dump the data from the database of the old virtual image into a file:

su - postgres
mdp: postgres
pg_dump -Fc origami_prod > /home/insyght/Documents/dump_origami/
PATH_WHERE_DUMP_FILE_WILL_BE_SAVED/my_dump_file.dump

- Copy the .dump file onto an USB key or the hard drive of the host machine. This file needs to be copied or made accessible to the new virtual image.
- Delete the data in the database of the new virtual image (see the section "[Optional] Remove all current data in your instance of the database")
- Restore the data into the database of the new image from the dump file

su - postgres
pg_restore -Fc -d origami_prod /home/insyght/Documents/dump_origami/
PATH_WHERE_DUMP_FILE_HAS_BEEN_COPIED/my_dump_file.dump

- reclaim disk space and update index of the new image:

vacuumdb --full --analyze -h localhost -p 5432 -U origami_admin origami_prod
~~ Remark: safely ignore the warnings on the terminal

- Check that everything is working as expected within the new virtual image by launching Insyght. Delete the old virtual image.

Often it is faster and more efficient to update only the individual components that have changed instead of the entire virtual image. Another benefit of this approach is that your database needs not to be migrated. See the sections below to carry out an update of the web application (war file) only.

2.2 How to update the web application (war file) only

- Launch insyght locally (step 1.1/) and go under “Home -> Last news” to know what is the version of Insyght you are currently running.
- Check the latest version of the war file available at **https://migale.jouy.inra.fr/redmine/projects/insyght/news** under the section "**Latest version of the .war file**".
- If you want to update the web application on your virtual image, download the new Insyght.war file and deploy it in tomcat as follow:
- Launch firefox
- Type in **http://localhost:8080/manager/html** in the address bar

user: tomcat
password: tomcat

- Locate the application path /Insyght among the list of web application and undeploy it (button on the right side of the table).
- Go to the section «WAR file to deploy» at the bottom of the page.
- Browse to the new war file you just downloaded using the file selector and press «deploy». If everything went fine you should see the message “Ok” at the top of the page.
- Type in **http://localhost:8080/Insyght/** in the address bar in firefox or hit the “home” button, you should have the latest version!

3 Other information

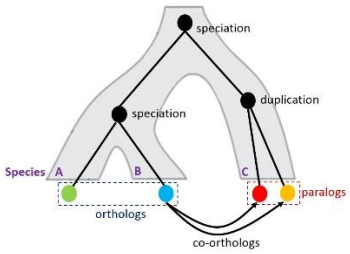
3.1 Contact and report error

• Email us: insyght[AT]inra.fr
• Report a bug: Go to https://migale.jouy.inra.fr/redmine/projects/insyght and then click on "Issues" to see the list of already reported bugs and features. You can browse the list anonymously but you will need to register / login to submit a new one. Once you register / login (click on "sign in / register" on the top right hand corner), a tab "New issue" appears. If you want to report a bug, please describe the organism / element / gene / action that led to it and the web browser you are using. You can also attach documents such as screenshots.

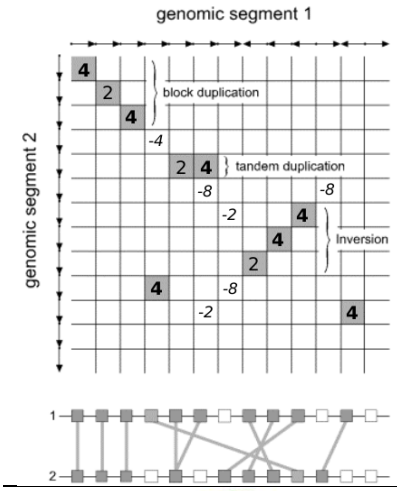
3.2 Overview of the pipeline

I/ The automated pipeline carries out the following tasks:

1. Process the genome files (.embl, .gbk, ou .gbff format) to extract the primary data (genomic annotations).



2. Find homologs (BLASTp).



3. Find syntenies.

4. Insert the data into the relational database.

II/ The Insyght web application is used to visualise the data and carry out the data mining.

3.3 How to manually connect to the PostgreSQL database

Click on the Terminal item in the menu bar on the left. Type in or paste the following command:
psql -h localhost -p 5432 -U origami_admin -d origami_prod

You are now connected to your instance of the origami database. If you wish, you can use plain sql commands to query the data as you like.

~~ *Remark: type in* **\q** *to exit the psql mode.*

3.4 Reclaim disk space of deleted rows and re-calculate the indexes of the database

To reclaim disk space following important changes to the database or if over time the web application feels sluggish, it is best to clean up the database underlining storage and indexing mechanisms. Type in the following command in a terminal:
vacuumdb --full --analyze -h localhost -p 5432 -U origami_admin origami_prod

~~ Remark: safely ignore the warnings on the terminal

3.5 Other useful command line tips

- Check free disk space: **df -h .**

- Check database size: connect to the database and then type: **\l+**

- Count inserted data into the database:
perl -I $SCRIPTS_DIR $SCRIPTS_DIR/count_inserted_data.pl

- For processes that are running for a long time, detach the process from the terminal by using nohup (https://en.wikipedia.org/wiki/Nohup) or screen (https://www.gnu.org/software/screen/manual/screen.html).

- Check what programs are running: **top**

- To access the log files of tomcat: **cd /var/lib/tomcat8/logs**

3.6 If you want to change the default passwords

– Linux users:
root (default pssd= insyght): **sudo passwd root**
insyght (default pssd= insyght): **sudo passwd insyght**
postgres (default pssd= postgres): **sudo passwd postgres**

~~ Remark: if you install or remove softwares that need you to authenticate, use the root password (default pssd= insyght)

– postgresql database users :
postgres (default pssd= postgres): **ALTER USER postgres WITH PASSWORD 'new_pssd';**
origami_admin (default pssd= origami_admin): **ALTER USER origami_admin WITH PASSWORD 'new_pssd';**

~~ Remark: It is not advisable to change the password for origami_read as this parameter is encoded in the web application to access the data. origami_read has no administrative rights on the database anyway so there is no security risk.

3.7 Changing the threshold parameters for the programs blast (used to find gene similarity) and align (used to compute the synteny)

Prior to running the pipeline, you can configure the blast threshold parameter (e-value). Open the file BlastConfig.pm and change the threshold value to your liking (default is -thresh => '5e-2').
gedit ${SCRIPTS_DIR}/BlastConfig.pm

Synteny regions are computed with a dynamic programming algorithm called “align” to determine the highest scoring paths among the chain of collinear homologs. By default, small gaps are allowed within the conserved synteny. You can customize those parameters using the following arguments:

-MAIN_ALIGN_OPTION_os*: defines the ortholog (BDBH) score; default value = 4; must be integer > 0.*

-MAIN_ALIGN_OPTION_hs*: defines the homolog (non-BDBH but significative blast result) score; default value = 2; must be integer > 0.*

-MAIN_ALIGN_OPTION_mp*: defines the mismatch penalty score within a synteny; default value = -4; must be integer < 0.*

-MAIN_ALIGN_OPTION_gc*: defines the gap creation score within a synteny; default value = -3; must be integer < 0. Must be different than the mismatch penalty score due to a current bug.*

-MAIN_ALIGN_OPTION_ge*: defines the gap extension score within a synteny; default value = -3; must be integer < 0. Must be identical to the gap creation score due to a current bug.*

-MAIN_ALIGN_OPTION_m*: defines the minimum size for a synteny (number of CDS); default value = 1; must be integer > 0.*

-MAIN_ALIGN_OPTION_c*: defines the cutoff score for a synteny; default value = 8; must be integer > 0.*

-MAIN_ALIGN_OPTION_o*: defines if the program must include all orthologs regardless of the cutoff score; default value = 1; must be integer 1 (Yes) or 0 (No).*

-MAIN_ALIGN_OPTION_pf*: defines the minimum protein fraction for Ortholog; default value = 0.5; must be double between 0 and 1.*

For example, here is a command that uses two of the optional arguments above:

```
screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl \
 -MAIN_ALIGN_OPTION_os 8 \
 -MAIN_ALIGN_OPTION_hs 4
```

~~ Remark: As you can add data incrementally, it is possible (although not advised) to insert data computed with 2 different sets of blast or align parameters. Comparing the results between data generated with different sets of parameters is strongly discouraged however. A warning that the database is deemed not stable will be raised.

3.8 Making your local instance of Insyght publicly available to the web

The virtual machine with your own data can be turned into a web server and shared across the web if you wish to do so. Ask your administrator to authorize external access to the virtual machine through a proxy, he will provide you with an url accessible worldwide.

3.9 Different ways to download a raw fasta or annotated genome file from the ncbi or ebi repositories

3.9.1 Download genome files from ncbi using script

The files downloaded by this script will be stored directly into the ${BANK_DIR} directory. To see the files already in ${BANK_DIR}:

```
ll ${BANK_DIR}
```

Run the script download_genome_files_from_ncbi.pl. For example, to download complete genomes files from Refseq for Enterococcus faecalis (taxon id 1351), Acidobacteriia (taxon id 204432), and Escherichia albertii (taxon id 208962), use the following command:

```
perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/download_genome_files_from_ncbi.pl \
 -NCBI_ASSEMBLY_SUMMARY_FILE DEFAULT_refseq_bacteria \
 -RESTRICT_TO_LIST_TAXON_IDS_AND_SUBNODES 1351 \
 -RESTRICT_TO_LIST_TAXON_IDS_AND_SUBNODES 204432 \
 -RESTRICT_TO_LIST_TAXON_IDS_AND_SUBNODES 208962 \
 -RESTRICT_TO_COMPLETE_GENOME ON \
 -RESTRICT_TO_LATEST_ASSEMBLY ON
```

The option -NCBI_ASSEMBLY_SUMMARY_FILE can be a path to the assembly file of your own or one of the 4 default values:
DEFAULT_genbank_archaea, DEFAULT_refseq_archaea,
DEFAULT_genbank_bacteria, DEFAULT_refseq_bacteria.
You can mix archaea and bacteria assembly summary files if you want by repeating this option multiple times, but do not mix genbank and refseq assembly files that are redundant over genomes else their elements will be duplicated in the database as well.

At least one -NCBI_ASSEMBLY_SUMMARY_FILE option or -UNIPROT_GENOME_TABLE_FILE option (see below) is mandatory in order to provide the script with assembly accession. If neither is provided, the script will not download anything. If you wish to use Uniprot, you can download a Uniprot genome table file from http://www.uniprot.org/proteomes/ by creating you list of genomes of interest, clicking on “Columns” to add all columns, and then clicking on “Download”.
If the option -RESTRICT_TO_LIST_TAXON_IDS_AND_SUBNODES is omitted, all the assemblies in the assembly file will be downloaded. The option -RESTRICT_TO_LIST_TAXON_IDS_AND_SUBNODES expects NCBI taxon ids (browse the NCBI Taxonomy database to get the taxon id for your taxonomic node of interest: https://www.ncbi.nlm.nih.gov/taxonomy).

The complete list of options for the script download_genome_files_from_ncbi.pl is as follow:
-NCBI_ASSEMBLY_SUMMARY_FILE {**PATH_TO_FILE**,

DEFAULT_genbank_archaea, DEFAULT_refseq_archaea,
DEFAULT_genbank_bacteria, DEFAULT_refseq_bacteria}
 -UNIPROT_GENOME_TABLE_FILE {**PATH_TO_FILE**}
 -RESTRICT_TO_TAXA_DOMAIN {archaea, bacteria}
 -RESTRICT_TO_DATABANK {genbank, refseq}
 -RESTRICT_TO_LATEST_ASSEMBLY {ON, OFF} #default ON
 -RESTRICT_TO_COMPLETE_GENOME {ON, OFF} #default ON
 -RESTRICT_TO_LIST_TAXON_IDS_AND_SUBNODES {NCBI taxon id}
 -LIMIT_TO_X_RANDOM_SAMPLES {POSITIVE DIGIT}
 -LIMIT_TO_X_FIRST_ORDERED_SAMPLES {POSITIVE DIGIT}
 -LIST_AND_EXIT {ON, OFF} #default OFF
 -SOFTLINK_LOCAL_PUBLIC_REPOSITORY_IF_AVAILABLE {**PATH_TO_DIR**}

To access the log file for this script:
less $LOG_DIR/download/download_genome_files_from_ncbi.pl

To check the list of files that were downloaded:
ll ${BANK_DIR}

3.9.2 Browse and download http content with a web browser

- ncbi http server : http://www.ncbi.nlm.nih.gov/guide/genomes-maps/
- ebi http server : http://www.ebi.ac.uk/genomes/bacteria.html or http://bacteria.ensembl.org/index.html

~~ Remark: Upon download, the files will be stored in the default "Downloads" directory associated with the web browser (i.e. "/home/insyght/Downloads").

3.9.3 Browse and download ftp content with a web browser

- ncbi ftp server: ftp://ftp.ncbi.nih.gov/

~~ Remark: To access the genbank content go to ftp://ftp.ncbi.nih.gov/genomes/genbank/. To access the refseq content go to ftp://ftp.ncbi.nih.gov/genomes/refseq/. The ncbi refseq repository is less exhaustive than genbank but contains only annotated genomes.

~~ Remark: To search a particular word within the web page, use ctrl-f. You can use this trick to find your genome of interest among the long list of organisms.

~~ Remark: Prefer the latest assembly for a given organism (directory latest_assembly_versions). The .fna files are the raw fasta file, the .gbff files are the annotated genome files (if any).

- ebi ftp server: ftp://ftp.ensemblgenomes.org/pub/bacteria/current

3.10 Run prokka to quickly annotate raw fasta file

Prokka (http://www.vicbioinformatics.com/software.prokka.shtml) is a software tool for the rapid annotation of prokaryotic genomes developed by Victorian Bioinformatics Consortium. It is pre-installed on this virtual machine. The following commands delete any previous output and run prokka on the example file:

```
rm -f -r /root/mydisk/prokka_output/*
prokka --compliant --addgenes --mincontiglen 200 \
--outdir /root/mydisk/prokka_output \
--genus Acetohalobium --species arabaticum --strain DSM_5501 \
--centre JOUY --locustag AarD \
--force \
$SCRIPTS_DIR/../DEMO_FILES/GENOMIC_FASTA/GCF_000270085.1_ASM27008v1_genomic.fna
```

To annotate your own fasta file, just substitute the example filename above with the path to your file and adapt the different arguments of prokka (--genus, --species etc.) accordingly.

~~ Remark: Information will be printed on the screen while the script run. If the script is successful, you should see you should see a list of output files followed by a reference to the prokka paper printed on the last lines. To see the resulting files generated by prokka, type:
ll /root/mydisk/prokka_output
The file that we are interested in is the .gbk file; to display it, type:
less /root/mydisk/prokka_output/*.gbk

~~ Remark on the other prokka arguments: type:
prokka -help

~~ Benchmark: This step took ~10 minutes for the fasta file provided as demo with 1 CPU in use.

3.11 Using a cluster (external processors)

Using a cluster allow you to parallelize the resources-intensive steps. If you have access to a cluster from your network, you should be able to use it from the insyght standalone VM as well. Please contact your local administrator on how to mount access to the cluster's master. Configuration of the cluster's master to allow the ip of the VM may be required as well. On the VM, install the client packages to allow for job submission. For example, if the cluster runs SGE (Sun Grid Engine), install the utilities

for the Grid Engine queue management as follow:

sudo apt-get install gridengine-client

password: **insyght**

You should now be able to submit jobs to the cluster. Here is an example of a command that make use of a hypothetical cluster of 200 processors:

```
screen perl -I $SCRIPTS_DIR/ $SCRIPTS_DIR/run_Insyght_pipeline.pl \
 -MAX_JOBS 200 -CMD_CLUSTER "qsub -m ea"
```