



Guide to Insyght user interface (Version 1.1, February 2016)

Insyght is developed by the Maïage lab at INRA.

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1. Introduction

Insyght is a browser that helps users navigate among abundant homologies, syntenies and genes annotations. It is constituted of 3 interconnected views that are detailed in the sections below.

Insyght can be used for the following type of analysis:

- Identification of evolutionary events
- Inference of gene functions (functional annotations)
- Detection of niche-specific genes, analysis of the core genome
- Phylogenetic profiling

Regarding the inference of gene functions, it is worth noting that the error rate of functional annotations is estimated to lie between 5~40% depending on the annotated genome (Jones et al, BMC Bioinformatics, 2007 ; Poptsova et al, Microbiology, 2010 ; Devos et al, Trends Genet, 2001). Errors are mostly due to the transferring of ontologies between “homologs” with a low percentage of similarity (ex 30%) or missing a domain etc.

Examples of challenges that Insyght aims to address:




- Navigating among a large amount of comparative data
- Clear detection of complex rearrangements (scattered, different scales, multiple genomes, etc.)
- Emphasizing both conserved and idiosyncratic genomic regions

Insyght makes use of the Asynchronous JavaScript and XML (AJAX) technology to minimize data transfer between server and clients, send simultaneous server requests and transfer most of the processing load on the client side. The graphical rendering uses the HTML5 canvas

2. The first step: select your reference genome and gene set

Click on the “Select your reference” tab.

2.1. First, select your reference genome

[\[private access\]](#)

HOMESEARCHORTHOLOGS TABLEANNOTATIONS COMPARATORGENOMIC ORGANIZATION

Previous1. Selected reference organism : Enterococcus faecalis V583, taxon_id = 226185 [AE016830]Next

D

Data source: 2688 public organisms are available

A • Type in a few letters of the name or taxon id of your organism or taxonomic group of interest :

Enterococcus faecalis V583, taxon_id = 226185 [AE016830]

B • Or browse the tree of organisms :

Phylum	Class	Order	Family	Genus	Species	
▶ Actinobacteria (290)	▶ Bacilli (495)	Bacillales (270)	▶ Aerococcaceae (1)	▶ Enterococcus (13)	▶ Enterococcus casseliflavus (1)	Enterococcus faecalis 62, taxon_id = 936153 [CP002491]
Aquificae (13)	▶ Clostridia (149)	▶ Lactobacillales (225)	▶ Carnobacteriaceae (3)	▶ Melissococcus (2)	▶ Enterococcus faecalis (6)	▶ Enterococcus faecalis D32, taxon_id = 1206105 [CP003726]
• Armatimonadetes (2)	• Erysipelotrichia (2)		• Enterococcaceae (16)	▶ Tetragenococcus (1)	▶ Enterococcus faecium (4)	▶ Enterococcus faecalis DENG1, taxon_id = 1287066 [CP004081]
Bacteroidetes/Chlorobi group (111)	▶ Negativicutes (6)		Lactobacillaceae (64)		▶ Enterococcus hirae (1)	▶ Enterococcus faecalis OG1RF, taxon_id = 474186
Caldiseptica (1)			Leuconostocaceae (11)		Enterococcus sp. 7L76, taxon_id = 657310 [FP929058]	
Chlamydiae/Verrucomicrobia			Streptococcaceae (130)			

C • Or click on a pre-made example for a quick access:

→ Visualize the genomic organisation for Bacillus subtilis subsp. subtilis str. 168, taxon_id = 224308 [AL009126]




A: A text box with auto completion. Type in a few letters of the organisms you are looking for, a list of matching organisms from the database will show up. If nothing shows up, the organism you are looking for is not in the database; hit enter to find its closest relative. You can also type in the name of taxonomic nodes at any levels (family, genus, etc.)

B: A taxonomic browser synchronized with the text box **A**. Upon selection from the text box **A**, the taxonomic browser will update to show the selected organism. You can select your reference genome by browsing the taxonomic browser directly. In that case the text box **A** will update accordingly.

C: For the purpose of demoing the tool upon a first visit, a list of example is available to click.

D: Once you are done selecting your genome of reference, click “Next” to proceed with the next step.

2.2. Second, choose your visualization and gene set of reference

[private access]

[HOME](#) [SEARCH](#) [ORTHOLOGS TABLE](#) [ANNOTATIONS COMPARATOR](#) [GENOMIC ORGANIZATION](#)

[Previous](#) **2. View genomic organisation or browse gene set with Orthologs table or Annotations comparator** [View orthologs table](#) [Annotations comparator](#)

Visualize the Genomic organisation for reference organism : ☐ Enterococcus faecalis V583, taxon_id = 226185 [AE016830]

Or browse a reference gene set with Orthologs table or Annotations comparator

Browse all CDSs of reference organism : ☐ Enterococcus faecalis V583, taxon_id = 226185 [AE016830]

Or build your reference gene set among 1 available molecules : ☒ complete_genome AE016830 (3,218,031 bp)

Filter genes by:

Genomic locations

start (bp relative to ORI) :

0

stop (bp relative to ORI) :

MAX_STOP (3218031)

Add a new filter

Run query >>

Check query validity

Clear all filters

Filtered genes list :

- EF_0019 (22,341-22,853)
- EF_0020 (23,088-24,080)
- EF_0021 (24,115-24,918)
- EF_0022 (24,941-25,852)
- EF_0024 (26,014-26,391)
- EF_0025 (26,639-27,166)
- EF_0026 (27,213-27,416)
- EF_0027 (27,614-28,384)
- EF_0028 (28,533-30,101)
- EF_0029 (30,116-31,330)
- EF_0030 (31,430-31,810)
- EF_0031 (32,001-34,592)

0 - 40 : 3113

[Export]

Add Selection >>

Add First 200 >>

[Double-click on a gene for detailed info]

Your selection : 17 out of 200

[Clear Selected / Clear All]

- gyrA (6,674-9,175)
- EF_0024 (26,014-26,391)
- EF_0025 (26,639-27,166)
- EF_0026 (27,213-27,416)
- EF_0027 (27,614-28,384)
- EF_0028 (28,533-30,101)
- EF_0029 (30,116-31,330)
- EF_0030 (31,430-31,810)
- EF_0031 (32,001-34,592)
- EF_0032 (34,629-37,244)
- EF_0033 (37,660-37,809)
- EF_0034 (37,798-38,267)

0 - 17 : 17

[Export]

A: Choose your type of visualization:

- View the Genomic organization of the whole reference genome
- View the Orthologs table or Annotation comparator for all the CDSs of the reference genome
- View the Orthologs table or Annotation comparator for a subset of the CDSs of the reference genome.

A list of elements (complete genome, chromosome, plasmid, etc.) from the reference organism will be available here. Select the element that you wish to list the CDSs from (see section below about the filter box for more details)

B: If you choose to view the genomic organization or to browse all the CDSs of the reference genome with the 2 other views, then you can click directly on the bouton corresponding to the view. If you chose to visualize only a subset of the CDSs of the reference genome, the panel **C-G** will be displayed below for you to choose your reference gene set.

C: The filter box to query genes and populate the gene list **E**. The filter box is detailed in the section below.

D: The buttons to run the query from the filter box, check the query validity (aka translate the query from the filter box into plain English and points out errors in its structure if any), or clear all filters.

E: The gene list that corresponds to the query from the filter box. By default all the CDSs of the selected reference element are shown. Double click on a CDS to show its detailed annotations. Multiple selection of CDSs is allowed. By pressing the control key on the keyboard, multiple genes are selected / deselected by clicking on them. The shift key will select a chunk of contiguous genes. It is possible to combine both the control and shift key.

F: The buttons to add the selected genes from the gene list **E** to your selection cart **G**. You can add to your selection from multiple different elements (chromosomes, plasmids) from the same organism. To do that, choose a different element at the bottom of the panel **A** and add the genes recursively.

G: your selection cart that contains CDSs that will be transferred to the orthologs table view or the annotation comparator. You can select CDSs from this list (multiple selection with the control and shift key is enabled) and remove them (click “clear selected”). You can clear out your selection altogether by clicking “clear all”. Double click on a CDS to show its detailed annotations.

2.3. More on the filter box

Filter genes by:

Presence / absence homology ▾

presence ▾

of homologs in organism(s) :

Bacillus cereus strain
G9842
[taxonId=405531] x

Lactococcus lactis strain
KF147
[taxonId=684738] x

(... AND ...) ▾

absence ▾

of homologs in organism(s) :

Staphylococcus aureus
strain ED98
[taxonId=681288] x

AND/OR...

(...) AND (...) ▾

function, biological process (keyword ▾

containing ▾

ATP binding

(* support POSIX regular
expression)

AND/OR...

(... OR ...) ▾

EC number ▾

starting with ▾

1.2.5

(* support POSIX regular
expression)

AND/OR...

New filter AND/OR...

You can query genes using many different types of filters. The different filters are available from the drop down list at the top of the filter box:

- Genomic localization: query genes whose genomic locations are within a given boundaries. Start and stop must be entered in base pairs relative to the origin of replication ORI.
- Presence/absence homologs: query genes with presence or absence of homologs in a compared species or species under given taxonomic nodes. The core genome is defined as genes having homologs in all the compared organisms under consideration. The disposable gene set is defined as any combination that imply the absence of homologs in at least one compared species.
- Identifier: query genes according to name, synonymous, locus tag, EMBL or Uniprot identifiers. Example: DnaA. BSU00680, P37472. Wherever you are allowed to freely enter a search term, a few modifiers are made accessible to contextualize your search: containing, not containing, starting with, not starting with, ending with, not ending with, strictly, strictly not, is null, is not null, and Posix regex. Posix regular expressions allows to perform more complex query by creating a pattern that can match multiple terms. Please check a resource on the internet for the list of supported posix regular expressions (i.e. https://en.wikipedia.org/wiki/Regular_expression). To give a simple example, searching a gene name that match «^rpo.+» will return all genes whose name start with rpo such as rpoA, rpoB, etc.
- Molecular function: query genes by Gene Ontology keywords or identifiers relative to the gene's molecular function. Example: atp binding, GO:0004422. See the Gene Ontology specification for more information. A few modifiers are made accessible to contextualize your search (containing, not containing, etc.).
- Biological process: query genes by Gene Ontology keywords or identifiers relative to the gene's biological process. Example: DNA replication, GO:0006400. See the Gene Ontology specification for more information. A few modifiers are made accessible to contextualize your search (containing, not containing, etc.).
- EC number: query genes by the Enzyme Commission number (EC number). Example: start with 1.2. See the Enzyme Commission number website for more information. A few modifiers are made accessible to contextualize your search (containing, not containing, etc.).
- Product: query genes by the qualifier "Product". This field may contain various information such as the protein name, putative molecular function, enzymatic reaction, etc. A few modifiers are made accessible to contextualize your search (containing, not containing, etc.).

- Cellular component: query genes by Gene Ontology keywords or identifiers relative to the gene's cellular location. Example: cytoplasm, GO:0016021. See the Gene Ontology specification for more information. A few modifiers are made accessible to contextualize your search (containing, not containing, etc.).
- Dbxref: List of supported external identifiers are: InterPro, HOGENOM, HSSP, GO, SubtiList, PDB, Rfam, UniParc, and TIGR.
- Type of evidence: List of supported types of evidence are: "Evidence at protein level", "Evidence at transcript level", "Inferred from homology", and "Predicted". Sometimes this field is simply not empty with a reference to a protein identifier for example.

You can combine multiple filters by clicking the bouton “Add a new filter” at the bottom of the filter box. The operators AND (intersection) and OR (union) are supported. You can combine an unlimited number of filters. The goal of this functionality is to allow you to build a biologically relevant query according to multiple criteria: for example list the genes that are niche specific / core genome and related to a given molecular function or biological process.

There are 4 different choices for the operators:

- (...AND...
- , ...)AND(...
- (...OR...)
- ...)OR(...

In each case, the dots represent the arguments to be linked. When the parenthesis encloses the dots, such as in (...AND...) and (...OR...), the link will be interpreted as an inner level. When the dots are outside the parenthesis, such as in ...)AND(...) and ...)OR(..., the link will be interpreted as an outer level. Therefore, you can specify which operator or suite of operators have a higher precedence by enclosing them within a parenthesis. For example, the query "(1 AND 2) OR 3" will yield a different result than the query "1 AND (2 OR 3)". It is forbidden to mix union and intersection operators within the same parenthesis, but different types of operators are authorized if they are separated by an operator outside a parenthesis. Similarly all operators outside of the parenthesis must be of similar type. Use the button “Check query validity” to translate the query from the filter box in to plain English.

2.4. Select a reference taxonomic node: direct access to the core / dispensable genome

If you select a reference taxonomic node instead of a reference organism during the first step (see section above), then an additional option is given to you during the second step: browse the core / dispensable gene set for reference node (A).

The screenshot shows the InSight web interface. At the top, there is a navigation bar with links: HOME, SEARCH, ORTHOLOGS TABLE, ANNOTATIONS COMPARATOR, and GENOMIC ORGANIZATION. The ANNOTATIONS COMPARATOR link is highlighted. To the right of the navigation bar are logos for INRA (SCIENCE & IMPACT) and MaIAGE. Below the navigation bar, there is a section titled "2. View genomic organisation or browse gene set with Orthologs table or Annotations comparator". This section contains a "Previous" button and two options: "View orthologs table" and "Annotations comparator". The "Annotations comparator" option is selected. Below this, there is a section labeled "A" with the text "Visualize the Genomic organisation for reference organism : Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]". This is followed by three radio button options: "Or browse the core / dispensable gene set for reference node : Enterococcus (13 organisms)", "Or browse all CDSs of reference organism : Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]", and "Or build your reference gene set among 1 available molecules : complete_genome CP004856 (3,427,276 bp)". The first option is selected. Below this, there is a section labeled "C" with three radio button options: "Assert for presence of homolog(s) in 10 organism(s)", "Assert for absence of homolog(s) in 1 organism(s)", and "Either presence or absence of homolog(s) in 1 organism(s)". The first option is selected. To the right of these options, there is a text "⇒ Resulting reference gene set : 98 CDS" followed by a red "D". Below this, there is a section labeled "B" which is a taxonomic browser. It shows a tree structure with "Enterococcus" as the root. Under "Enterococcus", there are several sub-nodes: "Enterococcus casseliflavus (1)", "Enterococcus faecalis (6)", "Enterococcus faecium (4)", "Enterococcus hirae (1)", and "Enterococcus sp. 7L76, tax_id = 657310 [FP929058]". The "Enterococcus casseliflavus (1)" node is selected and highlighted in blue. To the right of the tree, there is a box containing the text "Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]".

Visualize the Genomic organisation for reference organism : ☐ Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]

A Or browse the core / dispensable gene set for reference node : ☒ Enterococcus (13 organisms)

Or browse all CDSs of reference organism : ☐ Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]

Or build your reference gene set among 1 available molecules : ☐ complete_genome CP004856 (3,427,276 bp)

C Assert for presence of homolog(s) in 10 organism(s)

Assert for absence of homolog(s) in 1 organism(s)

Either presence or absence of homolog(s) in 1 organism(s)

⇒ Resulting reference gene set : 98 CDS **D**

↓ To change the reference genome (currently choice by default : Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]) or which genomes to assert for the presence or absence of homologs, double-click on an organism or node in the taxonomic browser below :

Reference taxonomic node : Enterococcus (13)

Species

- Enterococcus casseliflavus (1) ▶
- Enterococcus faecalis (6) ▶
- Enterococcus faecium (4) ▶
- Enterococcus hirae (1) ▶
- Enterococcus sp. 7L76, tax_id = 657310 [FP929058]

B

Enterococcus casseliflavus EC20, tax_id = 565655 [CP004856]

B: a taxonomic browser will appear with the root being the reference taxonomic node. A reference genome is chosen by default and can be changed as well as the genomes to assert for the presence or absence of

homologs. A menu pops up when double-clicking on a node or an organism that allow to change its category (presence / absence / either).

C: A recap of the organisms in each categories appear at the top of the taxonomic browser. Clicking on a category will open a pop up that list the organisms in that category.

D: The number of reference genes that corresponds to the query is indicated on the right. By clicking on it, a pop up opens that recap the gene list. Click on a gene to obtain information about its annotation. At the bottom of this pop up, 2 buttons allow you to transfer this gene set to the orthologs table and the annotation comparator respectively.

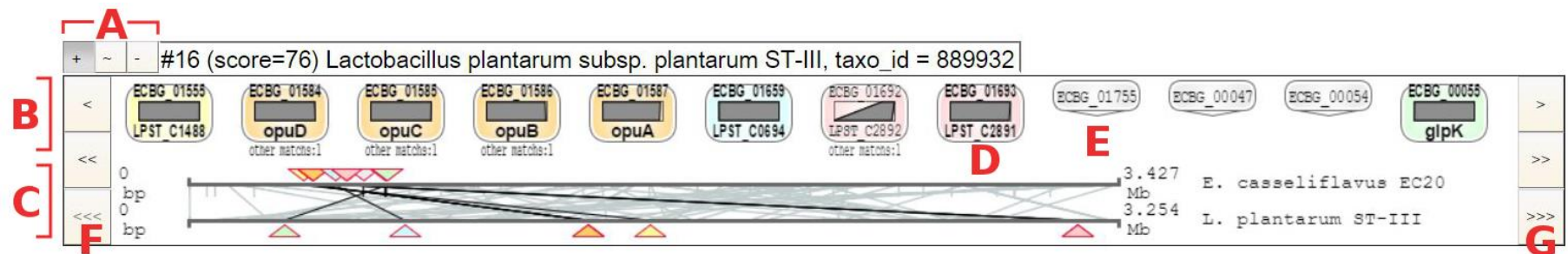
The "orthologs" table view provides a familiar layout with genes as columns and organisms as rows. It allows to easily check for the presence, absence or multiple copies of homologs. It also allow to spot genes that are in synteny. The genes that are presented in columns correspond either to all the CDSs of the organism or only a subset (selection cart) depending on the options chosen in the previous step (see section above).

Info and options Contextual menu: browse multiple homologies per gene

This view is organized into two different parts:

3.1. The comparison results (right side / central part)

On the right side / central part, the comparison results are stacked-up on top of each other, each within its own window. Below is a picture detailing a result window:



There are 3 sizes available to adapt the height of each result window (A):

- Maximum height: it shows both the symbolic and the trapezoid representation (default for the genomic organization view). The symbols provide legibility while the proportional display simultaneously allows grasping genomic locations. In other words, you can visualize the genomic location of the genes while simultaneously interacting with the symbols.
- Average height: it shows only the symbolic representation (default for the orthologs table view).
- Hide: it only shows the name of the organism.

When the result window is set to the maximum height like in the example picture above, there are two main parts per window: the upper part (B) depicts symbols representing the different genomic annotations, while the lower part (C) consists of the trapezoid view.

A triangular marker on the trapezoid view indicates the genomic location of each symbol. In both views, the representation for the reference genome is displayed at the top, the representation for the compared

genome at the bottom, and homologous region span over both top and bottom. Each symbol can be selected by clicking on it. Upon selection, both the symbolic and the trapezoid views are highlighted in red and additional information is displayed on the left panel (see section below for details). A contextual menu pops-up when the user double-clicks on a symbol and provides options that allow for example to transfer gene(s) from one type of view to the others (see section below for details).

There are 2 different types of symbols in this view:

- Gene homology (**D**): it is comprised of the reference gene name at the top, a proportional display of the sequence alignment in the middle, and the name of the subject gene at the bottom. Multiple homologs can be stacked in 1 cell ("off shoots", see below). Homologs are background colored according to synteny. Genes that are located side by side and have a similar background color belong to the same synteny. In the example picture above, 4 genes whose background color is orange are in synteny and 2 other whose background color is pink are part of another synteny. There are more syntenies per organism than color that can be easily distinguished by the human eye however. As a result, the same set of colors will be used in rotation for the background of different syntenies. The same color will never be used for 2 adjacent syntenies however, therefore 2 adjacent different colors indicates 2 different syntenies. The best way to make sure that 2 genes belong to the same synteny is to look at the synteny id on the left panel under the stack "detailed info -> synteny info". Similar background color between 2 different result windows is meaningless.
- Gene without homolog (**E**): it contain only the reference gene name at the top. Its background is grey.

Users can navigate among the annotation symbols either downstream (**F**) or upstream (**G**). In this view, navigation among the genes is synchronized for all the results windows so that a column always corresponds to one reference gene.

As mentioned above with regards to synteny, the game is to find homologs that are located side by side and have a similar background color; it means they belong to the same synteny.

The result window at the very top refers to the reference organism against itself; it is convenient to visualize paralogs. By default, this windows is set on hide.

From the perspective of the reference genome, a gene may sometimes have multiple homologous copies at different locations in the compared genome (due to paralogs or duplication of the synteny region). Multiple overlapping homologies are stacked in one cell and displayed as “offshoots” (text underneath the symbol). By clicking on this text, the user can browse among all those offshoots. It is also possible to access the offshoots through the contextual menu: Selected symbol -> List off shoots.

3.2. The stack panels (left side)

On the left side, different stack panels are available that provide the user with additional information and functionalities:

3.2.1. Detailed info

When clicking on a symbol in a comparison result such as an orthology, this stack panel display information on the gene(s) annotations by category: genome, element, synteny (if applicable), gene(s), and alignment (if applicable). Click on a header of a category to open/close the information. Some of those information are linked to relevant external database (Ensembl, ncbi, uniprot, EC number, etc.)

3.2.2. Result quick navigation

The list of compared genome sorted according to a given score (see section below for details). The organisms in this list that are highlighted in bold with a grey background are currently displayed in the comparison results display on the right side. Clicking on an organism update the comparison results to display this particular organism. You can use Control-F to search for an organism of interest in the list.

3.2.3. Sort result list by

Three scopes can be combined with 4 sorting types in descending or ascending order. You can sort the result list according to 3 different scopes:

- Selected reference gene: sort by a single reference gene. This is equivalent to sorting by a single column. To use this scope, select a reference gene previous to changing the scope.

- Selected reference gene set: The scope is the gene set that you selected under the search tab. This is equivalent to sorting by all column simultaneously.
- Whole organism (default): The scope is the whole reference organism, regardless of the gene set or displayed region

Those scopes can be associated with 4 different "sort by" types:

- Abundance of homologs (default): the more homologs with the reference, the higher the organism will score.
- Synteny score: organisms that have the longer stroke of co-localized homologies will score higher.
- Alignment score: it is a sum of all the blast alignment score of the homologies for the selected scope, so the more pair base similarity with the reference, the higher the subject organism will score.
- Abundance of homologs' annotations: the result with the more genomic annotation such as molecular function, product, etc. will score higher.

There are 2 different ways to order the results:

- Descending (default) : the bigger scores first
- Ascending: the lower scores first

Not all combinations are possible at this time. For example abundance of homolog's annotation is not available for the scope "selected reference gene set" and "whole organism" but only for "selected reference gene".

3.2.4. Display options

You can increase / decrease the font size or number of rows and columns by clicking on the appropriate buttons. In the orthologs table view, the synchronize options are not relevant; they are relevant only for the genomic context view.

4. The annotations comparator view

This view compares the functional annotations of the reference gene and its homologs and classifies the annotations into three categories:

- Shared: annotations present in the reference gene and at least in one homolog
- Missing: annotations present in at least one homolog but missing in the reference gene
- Unique: annotations present in the reference gene but missing in homologs.

By selecting one of these categories, the user has the possibility to navigate in the following cascading subcategories: the functional annotation classes (molecular function, biological process, cellular component, EC number), the functional annotations assigned to the gene(s), the list of compared organisms, the homologous genes, a summary of the sequence alignment, and detailed information about the compared gene:

HOME SEARCH HOMOLOGS TABLE ANNOTATIONS COMPARATOR GENOMIC ORGANIZATION

Detailed Info

Reference gene: dnaA [BSU00010]

Type of feature : CDS
Location : 410..1750
Product : Chromosomal replication initiator protein dnaA
Molecular Function : ATP binding
Molecular Function : DNA replication origin binding
Molecular Function : nucleoside-triphosphatase activity
Molecular Function : protein binding
Biological Process : DNA replication initiation
Biological Process : regulation of DNA replication

Reference organism : Bacillus subtilis strain 168, taxo_id = 224308

Reference genes	Comparison categories	Annotation classes	Annotations	Compared organisms	Compared Genes	Detail of the compared gene
dnaA [BSU00010]	[Shared] Annotations present in the reference gene and at least in one homolog (6)	Molecular Function (3)	DNA replication origin binding GO:0003688 (391 = 96.1%)	Bacillus anthracis strain Ames ancestor, taxo_id = 261594 (1)	dnaA [GBAA0001]	Type of feature : CDS Location : 407..1747 Product : Chromosomal replication initiator protein dnaA Molecular Function : ATP binding Molecular Function : DNA replication origin binding Molecular Function : nucleoside-triphosphatase activity Biological Process : DNA replication initiation
dnaN [BSU00020]		Biological Process (2)	ATP binding GO:0005524 (391 = 96.1%)	Bacillus anthracis strain Ames, taxo_id = 198094 (1)		
yaaA [BSU00030]	[Missing] Annotations present in at least one homolog but missing in the reference gene (5)	Cellular Component (1)	nucleoside-triphosphatase activity GO:0017111 (392 = 96.3%)	Bacillus anthracis strain Steme, taxo_id = 260799 (1)		
recF [BSU00040]		EC Number (0)		Arthrobacter aureus strain TC1, taxo_id = 290340 (1)		
yaaB [BSU00050]	[Unique] Annotations present in the reference gene but missing in homologs (1)			Lactococcus lactis strain MG1363, taxo_id = 416870 (1)		
gyrB [BSU00060]	Show More			Lactococcus lactis strain SK11, taxo_id = 272622 (1)		
gyrA [BSU00070]				Lactococcus lactis strain		
yaaC [BSU00080]						
guaB [BSU00090]						
dacA [BSU00100]						
pdxS [BSU00110]						
pdxT [BSU00120]						
serS [BSU00130]						

Number of species with homolog(s) sharing this annotation.

When this last subcategory is shown, the similarities and discrepancies between the reference gene functional annotations and its homolog are highlighted in green and red respectively. The number of items that belong to a given subcategory is shown within parentheses. For example, in parentheses next to a given reference gene functional annotation is the number of species with homolog(s) sharing this annotation. This number is an indication of the degree of commonality of a given annotation among homologs.

The annotations comparator relies mostly on functional annotations based on a controlled vocabulary such as gene ontology (i.e. molecular function, biological process); it is less relevant for fields that are typically more heterogeneous like product.

On the left panel, two options are accessible to refine your search:

- Under the panel “Compared organisms”, the set of compared organisms can be restricted to a subset of the taxonomy. The default taxonomic node is root which is all the organisms in the database. To only compare the references genes with homolog of a taxonomic nodes or organism of your choice, delete the root node by clicking on it and hitting the “del” stroke on your keyboard or clicking on the X next to it. Then type in a few letters of the organism or taxonomic node you wish to add to the comparison. Multiple organisms or taxonomic nodes can be added. Click on Submit to take your new criterion into account.
- Under the panel “Filter homologs by...”, homologs can be filtered according to e-value, minimum percentage identity, minimum percentage query alignment length, and whether or not they correspond to orthologs BDBH. Click on Submit to take your new criterion into account.

To have access to the contextual menu (see section below for details), double click on a reference gene. For example you can then export this table in a .csv format.

5. The genomic context view

Detailed Info

[Double click on a symbol to access the contextual menu.]

- Genome Info
- Element Info
- Genomic region Info

Synteny type : not reversed
 Synteny id : 57,263,667
 pairs : 3
 Query start (pb) : 153,744
 Query stop (pb) : 157,378
 Subject start (pb) : 1,745,878
 Subject stop (pb) : 1,749,315

Score : 12
 Orthologs : 3
 Homologs : 0
 Mismatches : 0
 Gaps : 0

Results Quick

Sort result list by

Display options

Reference organism : **Bacillus cereus strain ZK = E33L**, **taxo_id = 288681**

Previous Next

Genomic organisation view : displaying results 25 - 28 of 406

#25 (score=2843) **Lysinibacillus sphaericus strain C3-41**, **taxo_id = 444177**

#26 (score=2765) **Bacillus halodurans strain C-125 = ATCC BAA-125 = JCM 9153 = FERM 7344 = DSM**

#27 (score=2751) **Bacillus clausii strain KSM-K16**, **taxo_id = 66692**

#28 (score=2740) **Bacillus pseudofirmus strain OF4**, **taxo_id = 398511**

Info and options

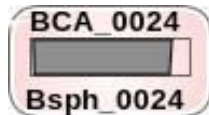
Selection. Double click for contextual menu

Comparison windows

Navigate results

The overall layout for this view is similar to the orthologs table view but its philosophy is different; it proposes a new way to explore the landscape of conserved and idiosyncratic genomic regions across multiple pairwise comparisons. The variety of symbols in this view represents the diversity of events regarding genomic loci conservation and idiosyncrasy:

- Gene homology (same as the orthology table view):



The gene homology symbol is comprised of the reference gene name at the top, a proportional display of the sequence alignment in the middle, and the name of the subject gene at the bottom. Homologs are background colored according to synteny.

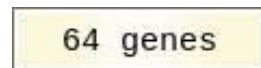
- Gene without homolog [reference or compared genome]:



The gene without homolog symbol contain either only the reference gene name at the top [reference] or only the subject gene name at the bottom [compared genome].

- Conserved synteny:

Orientation 5'-3' conserved:



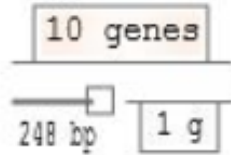
Off shoots: 2

Or reversed:



Represented by a rectangle or a bowtie if the synteny is reversed. It is centered in the middle to reflect that it belongs to both the reference and the compared genomes. Its text represents the number of CDSs comprised within that conserved synteny.

- Genomic region without homolog [reference or compared genome]:



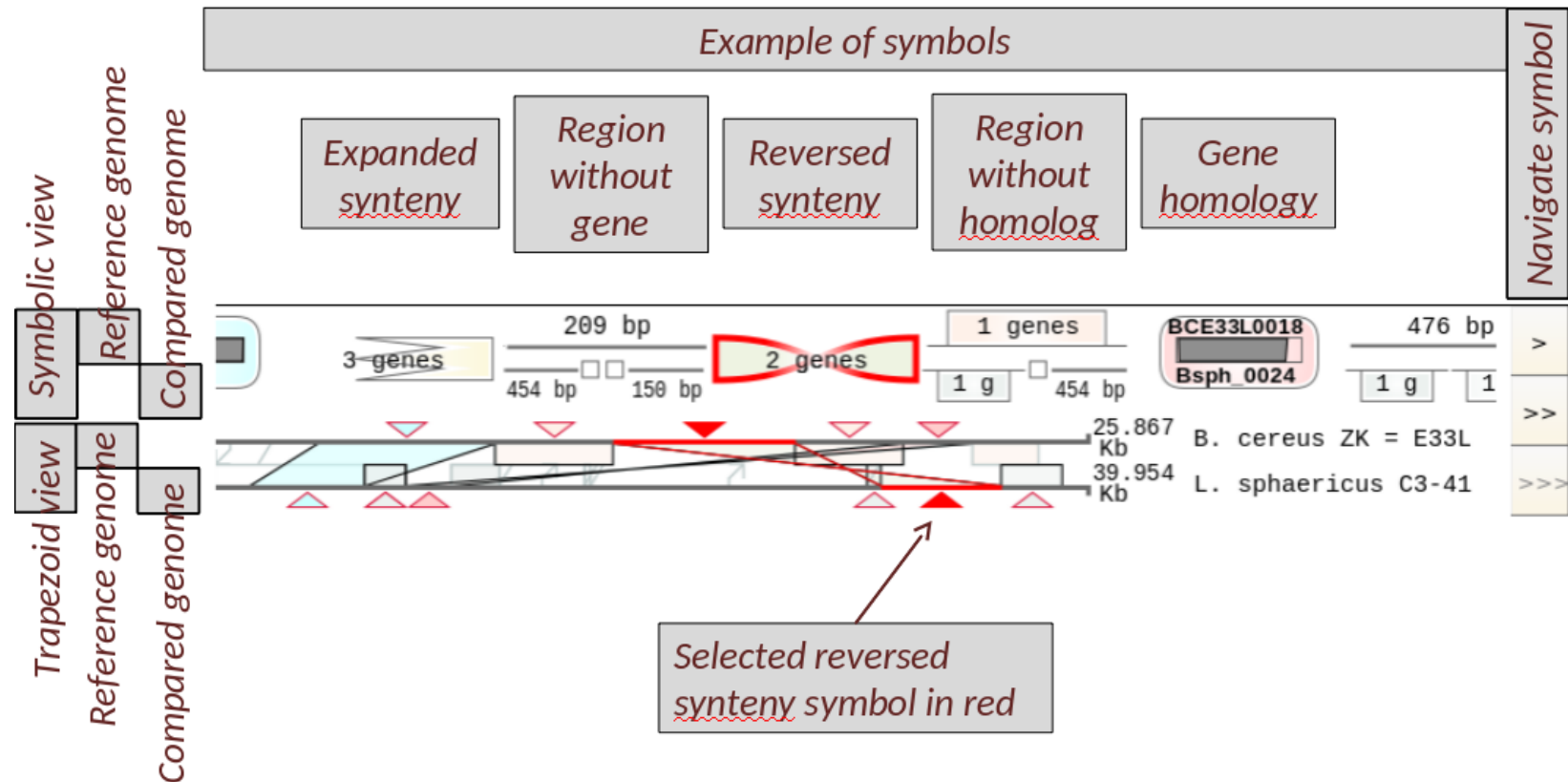
When there is no gene within that region, it looks like a line symbol. When there are genes within that region, it looks like a rectangle either located in the upper half [reference] or in the lower half [compared genome]. The representation of the genomic region on the compared genome is trickier as their location can appear scattered. We choose to represent them at half scale surrounding the bottom part of a syntenic region or homolog. If the symbol for the genomic region without homolog appears at the bottom left of the symbol of a given synteny, the region is located upstream of the syntenic region. If the symbol for the genomic region insertion appears at the bottom right of the symbol of a given synteny, the region is located downstream of the syntenic region.

- Genes overlap:



This symbol is displayed when the genomic location of 2 genes are overlapping.

Symbols are ordered according to their start position on the reference genome and the user has the possibility to browse among them. The resulting display for the reference genome appears as a succession of homologous symbols followed by non-homologous symbols. This cyclic partitioning contributes to a better legibility of the genomic rearrangements and idiosyncrasies. The proportional / trapezoid view at the bottom allows to simultaneously grasp genomic locations and complex rearrangements:



When browsing a result window, the other results windows are not synchronized by default. The user has the option to synchronize the navigation in the “display option” panel on the left. All the other options accessible through the left panel work similarly as the orthology table view.

It is also possible to synchronize all results window on a specific gene through the contextual menu: double click on a gene -> quick navigation -> Synchronize all displays on this reference gene. When double clicking on a synteny symbol, the user has the possibility to transfer all those genes to the orthologs table view for example to have an overview of their conservation in other genomes. It is also possible to expand a synteny to show the genes directly in the genomic organization view.

From the perspective of the reference genome, a gene or subset of genes within a shared synteny may sometimes have multiple homologous copies at different locations in the compared genome (due to paralogs or duplication of the synteny region). A menu below the symbolic representation is shown whenever such offshoot events occur, allowing the user to browse among them by clicking on this text. It is also possible to access the offshoots through the contextual menu: Selected symbol -> List off shoots.

The different chromosomes or plasmids of an organism are displayed back to back and delimited by "T" markers. You can highlight the different elements by going in the contextual menu and choosing "zoom in [elements]" (see the "Search and navigate" section).

6. The contextual menu

You can access it by double clicking on a gene or a symbol. Here is the hierarchy of functionalities accessible from this menu (**in bold**) with a few comments (*in italic*):

Transfert / find in other view

Reference gene in ...

Orthologs browsing table

Annotations comparator

Genomic organization view

Reference gene set in ... *From the genomic organisation view, you can transfer genes from a synteny or a gene region without homolog. From the orthologs table or annotation comparator view, you can transfer all the genes displayed.*

Orthologs browsing table

Annotations comparator

Reference displayed region in ... *Only available in the genomic organization view. It allow to transfer all the genes of the genome. If you define a region by zooming, only genes from this region are transferred.*

Orthologs browsing table

Annotations comparator

Selected symbol

Expand / collapse interlocked symbols *See or hide the genes within a conserved synteny or gene region without homolog.*

List off shoots... *Only available if there are multiple homologs that overlap with regard to their genomic location on the reference. After clicking this option, a pop up containing the list of all the overlapping homologs will appear, select the one to be displayed in place of the current symbol. This functionality allows to navigate among the z axis / third dimension. By clicking directly on the text "other matches" or "off shoots" underneath a given symbol, it automatically loops through all the copies one by one.*

List reference genes *A pop up that allow you to navigate among the reference genes of the selected symbol.*

Quick navigation

Find gene... *Quickly find your gene of interest. Center the view on your gene of interest either for only one selected result or for all the results. "Synchronize all displays on this reference gene" works similarly to finding the selected reference gene for all the results.*

Synchronize all displays on this reference gene *Only available in the genomic organization view.*

Center this display at click position *This option allows to quickly jump to a specific location on the genome. To activate this option, double click on the genomic scaffold at the place you want to focus the symbolic view and choose this option in the contextual menu.*

Navigate symbols

Find next

Gene homology

Synteny region

Synteny region > 3 genes

Synteny region > 20 genes

Reference gene / genomic region without homolog

Find previous

Gene homology

Synteny region

Synteny region > 3 genes

Synteny region > 20 genes

Reference gene / genomic region without homolog

Zoom (Alpha) *See the section "How to zoom in Insyght" below.*

Reference genome

zoom in [elements...]

zoom out...

2X

5X

MAX

Compared genome

zoom in [elements...]

zoom out...

2X

5X

MAX

Export

Gene(s) sequence(s)...

Table...

Graphics... *Insyght uses the canvas technology, right click on an image to save it as a high-quality png image that can be resized.*

7. The zoom in Insyght (experimental feature)

The zoom feature is Alpha, meaning it is in an experimental stage of development and its accessibility needs improvement.

Zooming is useful to focus the display of the symbolic and proportional representation onto a region of interest. When the results are first displayed, the scale is the whole organism. It means that we get to look at minimum magnification and we see all the chromosomes and plasmids back to back like in a bird view. The numbers on the left of the genome scaffolds indicate the start base pair for the display (0 is the origin). The numbers on the right of the genome scaffolds indicate the stop base pair for the display in scientific format. There are few ways to zoom in / out:

- Click and drag: Define a zoom region by clicking on the genomic scaffold and drag the mouse lengthwise along it. The selected region appears in red and when the mouse button is released, it triggers the zoom. The start and stop of the genomic scaffolds change to reflect the newly displayed region. Zooming in the reference and the compared genomes is dissociated, only one of them is magnified at a time. Zooming is recursive, it can be done multiple times. To cancel a zoom before releasing the mouse button, move the mouse away from the genomic scaffolds and the zooming will break. After zooming, the navigation among the symbols stays unchanged, the only difference is that the set of symbols is reduced and altered (see explanation below). The symbolic representation (at the top) reflects the proportional region that is displayed (at the bottom). By default after a zoom, the upper part centers itself to show the first symbol that is mapped on the reference.
- Zoom in [elements]: Another way to zoom is by using the contextual menu "zoom in [elements]". A list of the accession numbers (chromosomes, plasmids or complete genome) of the organism appears. While scrolling over the list, the red zoom region appears over the location of the highlighted element. Click on it to automatically zoom on that element. This is useful to quickly compare two elements.
- Zoom out: To zoom out, use the contextual menu "zoom out -> 2X, 5X or MAX". You can also drag the mouse to encompass the whole genomic scaffold on the proportional view.

Synchronized zooming in the genomic organization view (accessible through the “display options” stack)

means that zooming occurs for all the compared genomes simultaneously. Enable synchronized zooming in the "Display options" on the left. The selected region is highlighted in red in all results windows and zooms occur simultaneously. The same reference region is displayed for all the results. Synchronized zooming is not available for the compared genome, only for the reference. Synchronized zooming is automatic in the homology browsing table view to keep the table layout aspect.

The symbols are altered by the zooming. Two main events can happen:

- Partial [left or right, on the reference or the compared genome]:



After a zoom, a symbol could be cut between its start and stop position. It then becomes of type "partial". If the missing base pair are on the left of the display, it is a partial left and is represented by a rectangular symbol open at its left with an arrow inside pointing left. If the missing base pair are on the right of the display, the symbol is mirror left/right to the one described in the previous sentence. When clicking on a "partial" symbol, the detailed info panel gives information about what was the previous type of symbol before it was cut and the gene names if appropriate.

- Missing target [left or right, on the reference or the compared genome]:



Because the zooming between the reference and the compared genomes is dissociated, another event that can occur is "missing target". For example it happens when a zoom occurs on the compared genome and, as a result, a given reference gene that is part of a homologous pair is still in full display on the reference genome but its compared genome homolog is out of range. The symbol for missing target resemble an arc-parallelogram open on top [compared genome] or bottom [reference genome].

8. Supported url parameters

You can directly access data in Insyght by passing url parameters. Supporting url parameters are:

- referenceSpecies
- referenceStrain
- referenceSubstrain
- referenceAccnum
- referenceGeneSetNames
- referenceGeneSetLocusTag

Examples url with supported parameters:

- <http://genome.jouy.inra.fr/Insyght/#&referenceSpecies=Bacillus+subtilis&referenceStrain=168&referenceGeneSetNames=dnaA>
- <http://genome.jouy.inra.fr/Insyght/#&referenceSpecies=Bacillus+subtilis&referenceStrain=168&referenceGeneSetLocusTag=BSU00020>
- <http://genome.jouy.inra.fr/Insyght/#&referenceSpecies=Bacillus+subtilis&referenceStrain=168&referenceGeneSetNames=yaaA,gyrB>
- http://genome.jouy.inra.fr/Insyght/#&referenceAccnum=NC_000964&referenceGeneSetLocusTag=BSU00050,BSU00080