Client User's Manual

Ver.1.0.7

Octover 15, 2009

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1. Outline of RECOG

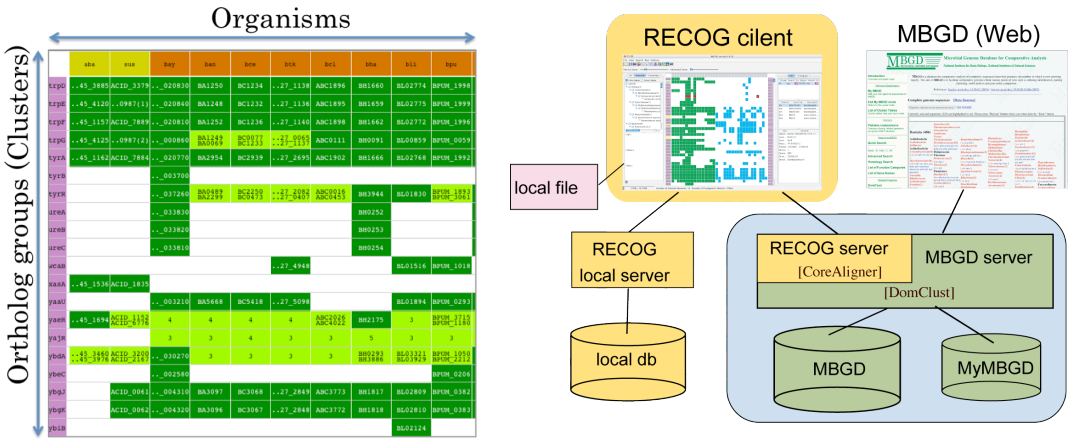
1.1. What is RECOG?

RECOG (Research Environment for Comparative Genomics) is a workbench software program which is used to conduct comparative genome analyses on a massive scale. The main feature of RECOG is the function of ortholog analysis between genomes of numerous species based on the display of an ortholog table (rows: ortholog groups; columns: species). RECOG can also conduct various comparative analyses for detailed examination, based on this table.

RECOG was developed based on the MBGD (Microbial Genome Database for Comparative Analysis). RECOG is a dedicated client software program that is available immediately after connecting to the MBGD server. Meanwhile, the analysis of newly determined microbial genomes, eukaryotic genomes, *etc*. that are not included in the MBGD can also be conducted in a local environment, by installing the RECOG server locally.

The latest version of RECOG is available from http://mbgd.genome.ad.jp/RECOG/.

This manual explains how to use the RECOG Client software program.



2. Operating Environment of RECOG

2.1. Operating system

- Mac OS 10.3 and upward
- Windows XP, Vista
- Linux

2.2. Compatible Java version

- **Java 1.4 and upward**

Note: Make sure that Java JRE 1.4 and upward is installed before installing the RECOG Client software program. If it is not installed, install Java JRE 1.4 and upward before installing the RECOG Client software program.

3. Installation/Uninstallation

3.1. Installation of RECOG for Windows

1. Set 'JAVA_HOME,' an environmental variable, as follows:
   Variable: JAVA_HOME
   Value: the directory in which the JAVA JRE is installed.

2. Double-click **recog-client-<version>.exe** to start the installation.
   Install RECOG by following the instructions on the screen. Upon completion of the installation process, the RECOG menu is added to the start menu.

3.2. Installation of RECOG for Mac

1. Double-click **recog-client.pkg.tgz** to create **recog-client.pkg**.

2. Double-click **recog-client.pkg** to start the installation.
   Install RECOG by following the instructions on the screen.
   During the installation, the administrator's username and password are requested.

3.3. Installation of RECOG for Linux

1. Set 'JAVA_HOME,' an environmental variable, with the following command:

   ```
   bash:   export JAVA_HOME=<JAVA JRE home directory>
   csh:    setenv JAVA_HOME <JAVA JRE home directory>
   ```

2. Decompress **recog-client-<version>.tgz** to create a **recog** directory.
   Create the **recog** directory in an appropriate place.

3.4. Uninstallation of RECOG for Windows

1. Click **Uninstall RECOG** in the start menu to remove the installation directory.
2. If the installation directory is not removed, remove it manually.
3. The data directory **C:¥Documents and Settings¥<user account>¥RECOG** is not removed by the above uninstallation procedure. If you do not need it, remove it manually.

3.5. Uninstallation of RECOG for Mac

1. Remove the following directory manually:

```
/Application/recog.app
/Library/Receipts/recog-client.pkg
```

2. The data directory **/Users/<user account>/RECOG** is not removed by the above uninstallation procedure. If you do not need it, remove it manually.

3.6. Uninstallation of RECOG for Linux

1. Remove the **recog** directory manually.
2. The data directory **/home/<user account>/RECOG** is not removed by the above uninstallation procedure. If you do not need it, remove it manually.

4. Starting and Terminating RECOG

4.1. Starting RECOG

- Windows

  Click **Start** – **All programs** – **RECOG** – **RECOG menu**.

- Mac

  Open **/Applications** in the finder window and double-click the **RECOG icon**.

- Linux

  From the terminal, move to the **recog** directory, and execute the following command:

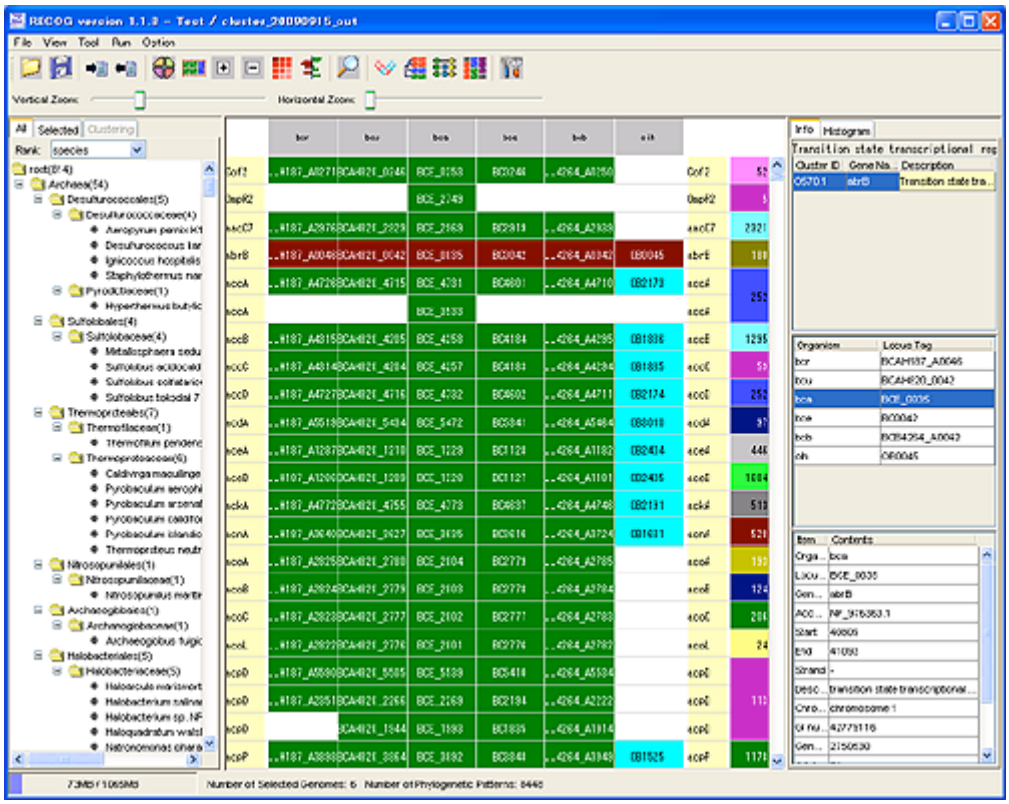
```
./recog.sh
```

4.2. Terminating RECOG

On the menu screen, click **File** – **Exit**.

5. Display and Operation of the RECOG Main Window

5.1. Screen structure

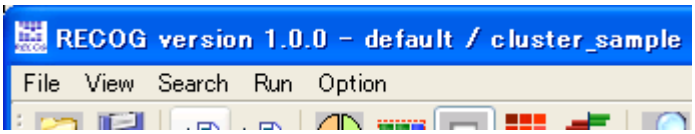


The main window consists of the following components:

- 5.2 Window header
- 5.3 Menu bar
- 5.4 Toolbox
- 5.5 Zooming scale bars
- 5.6 Taxonomy Tree
- 5.7 Phylogenetic Pattern Map（PPM）
- 5.8 Info tab
- 5.9 Histogram tab
- 5.10 Status bar

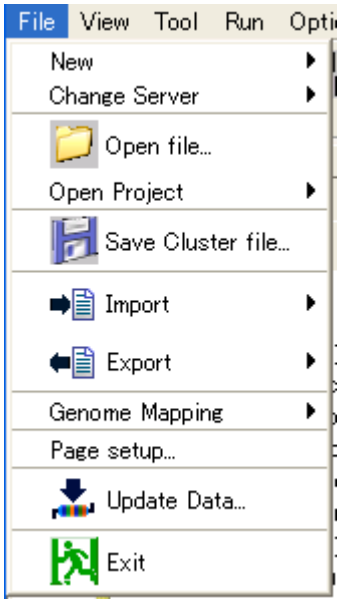
5.2. Window header

The RECOG Client version, name of the current project and name of the DomClust result file are displayed.



5.3. Menu bar

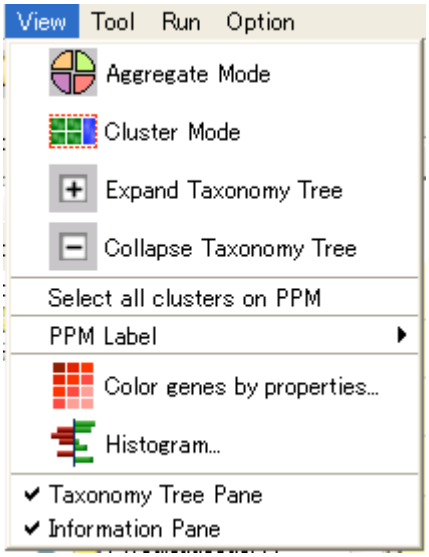
5.3.1. **File** menu



New	New Analysis The currently displayed analysis is cleared. New Project A new project is created.
Change Server…	RECOG server The RECOG server to be used for analysis is specified. Server List… The list of registered RECOG servers is displayed.
Open file	Analysis result files, including DomClust result files, are opened.
Open Project	Project An existing project is opened.

	Project List… The list of existing projects is displayed.
Save Cluster file…	The currently displayed analysis results are saved.
Import	DomClust file… DomClust result files are imported. Gene property file… Gene property files are imported.
Export	DomClust results are output in tab-delimited format, and PPM images are output in PDF format.
Genome Mapping	Regarding imperfect genomes, the contig alignment sequence, direction, *etc*. on the chromosome are set.
Page setup…	The size of the PPM image to be saved is specified.
Update Data	The local genetic data are updated.
Exit	The RECOG Client is terminated.

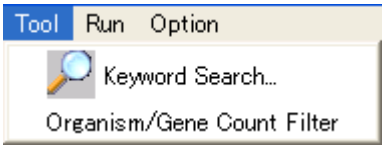
5.3.2. **View** menu



Aggregate Mode / Disaggregate Mode	The PPM display mode is switched between the aggregate mode and the disaggregate mode.
Cluster Mode/Sub-cluster Mode	Regarding the analysis of sort, *etc*., whether to conduct an analysis based on clusters or sub-clusters is specified.
Expand Taxonomy Tree	The tree in the taxonomy browser is expanded one level.
Collapse Taxonomy Tree	The tree in the taxonomy browser is collapsed one level.
Select all clusters on PPM	All the clusters on the phylogenetic pattern map (PPM) are selected.

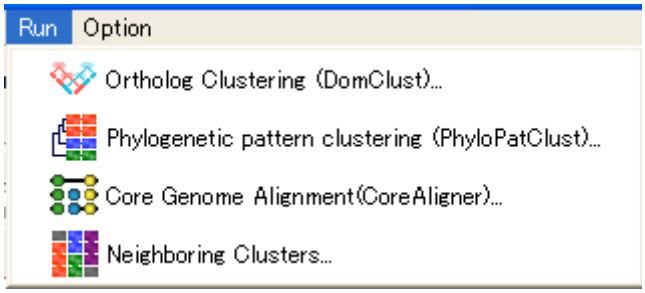
PPM Label	Whether to display the gene name or the cluster ID in the label display fields on both sides of the PPM is specified.
Color genes by properties	Each gene on the PPM is classified by color in proportion to the gene property value.
Histogram	The **Histogram tab** is used to create graphical representations based on the gene property numerical data.
Taxonomy Tree Pane	Whether or not to display the taxonomy tree pane is specified.
Function Category Pane	Whether or not to display the function category pane is specified.

5.3.3. **Tool** menu



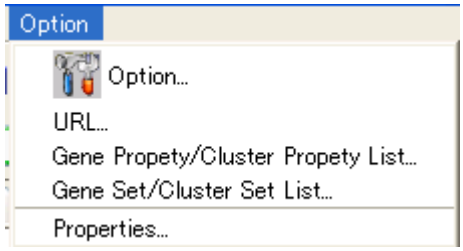
Keyword Search…	A keyword search is conducted regarding the gene/cluster properties.
Organism/Gene Count Filter	The filtering conditions are specified based on the gene count/species in a cluster.

5.3.4. **Run** menu



Ortholog Clustering (DomClust)...	DomClust analysis is conducted.
Phylogenetic pattern clustering (PhyloPatClust)...	Phylogenetic pattern clustering is carried out.
Core Genome Alignment (CoreAligner)...	CoreAligner analysis is conducted.
Neighboring Clusters...	Neighboring gene clustering is carried out.

5.3.5. **Option** menu

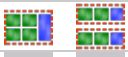


Option...	The option screen is displayed.
URL...	The URL for displaying genetic information from an external resource is set.
Gene Property / Cluster Property List…	The list of registered gene properties/cluster properties is displayed.
Gene Set / Cluster Set List…	The list of registered gene sets/cluster sets is displayed.
Properties...	The properties of the displayed analysis results are displayed.

5.4. Toolbox

The **Toolbox** can be moved by dragging and dropping the left side of the **Toolbox** with the mouse.

To return the **Toolbox** to its original position, click the **Close** button on the upper right of the **Toolbox**.

	Open file
	Save Cluster File
	Import DomClust file
	Export
	Aggregate Mode / Disaggregate Mode
	Cluster Mode / Sub Cluster Mode
	Expand/Collapse Taxonomy Tree
	Color genes by properties
	Histogram
	Keyword Search
	Ortholog Clustering (DomClust)
	Phylogenetic pattern clustering (PhyloPatClust)
	Core Genome Alignment (CoreAligner)

	Neighboring Clusters
	Option

5.5. Zooming scale bars

Each zooming scale bar expands or contracts the horizontal/vertical size of the **PPM**.

- **Vertical Zoom** scale bar

  The longitudinal size of the PPM cell is expanded or contracted by sliding the scale bar laterally.

- **Horizontal Zoom** scale bar

  The transverse size of the PPM cell is expanded or contracted by sliding the scale bar laterally.

5.6. Taxonomy Tree

The **Taxonomy Tree** displays the taxonomic tree of the given species.

1. **All** tab for selecting the species to be analyzed

   The taxonomic tree of all the species registered in the RECOG server is displayed.

   On the tree screen, it is possible to choose the species (ingroup, outgroup) for DomClust analysis and to color the species.

2. **Selected** tab for PPM manipulation

   This tab consists of upper and lower views.

   The upper view displays the taxonomic tree for the organisms (shown on the PPM) that are currently targeted for analysis.

   On the tree screen, phylogenetic pattern filtering conditions, species groups, *etc*. can be set.

   The lower view changes by selecting one of the following upper buttons:

   - **Control**

     A view for controlling the display of ortholog table, including the color settings, filter settings, sort settings, and the switching of the order of display or the display/nondisplay of the PPM species, is displayed.

- **Sp Group**

  Species groups are displayed.

- **Set**

  Currently registered gene sets, cluster sets, compound conditions and species sets are displayed.

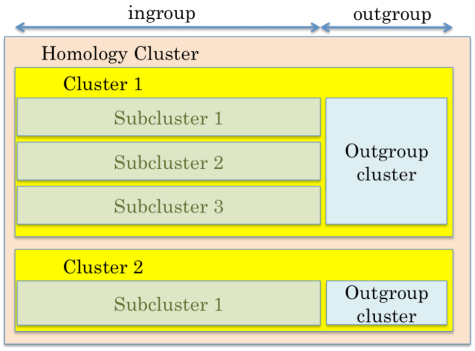
3. **Clustering** tab

   The hierarchical clustering tree obtained from phylogenetic pattern clustering is displayed.

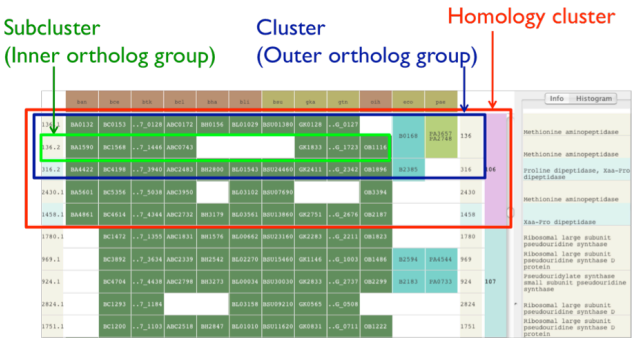
5.7. Phylogenetic Pattern Map（PPM）

The **Phylogenetic Pattern Map** (**PPM**) is the main feature of the RECOG system. In the **PPM**, genes that belong to each ortholog group are listed in a table in which ortholog groups and species are arranged in the rows and columns, respectively. When the display area is reduced, a pattern representing the presence or absence of genes (phylogenetic pattern) is displayed as a heat map. Basically, species are shown in the order of appearance on the taxonomy tree, but if outgroup species are specified, ingroup species are displayed on the left side and outgroup species are displayed on the right side. However, the order of display can be changed via the operation panel. In each cell, the **/locus_tag** of the gene, which belongs to the cell, or the number of genes is displayed. When a cell is clicked, the relevant cluster and gene data are displayed on the **Info** tab at the right end.

If DomClust analysis is carried out upon specifying outgroup species, the cluster table is displayed as a nested table. That is, genes in the outgroup species form an *outgroup cluster* which corresponds to multiple *sub-clusters* that consists of genes in the ingroup species. All of these are included in the (upper-hierarchical) *cluster* (see the figure below). Furthermore, as its upper-hierarchical cluster, a *homologous cluster* is defined as an accumulation of homologous ortholog groups. In normal *disaggregate mode*, the function categories and gene names (or cluster ID's) of the sub-clusters are displayed in the columns on the left side, the function categories and gene names of the clusters are displayed in the columns on the right side, and homologous clusters are displayed on the right side thereof. These columns are called *cluster headers*. On the other hand, in the *aggregate mode*, clusters of the same phylogenetic pattern are displayed in the same row in an aggregated manner, and no cluster headers are displayed.



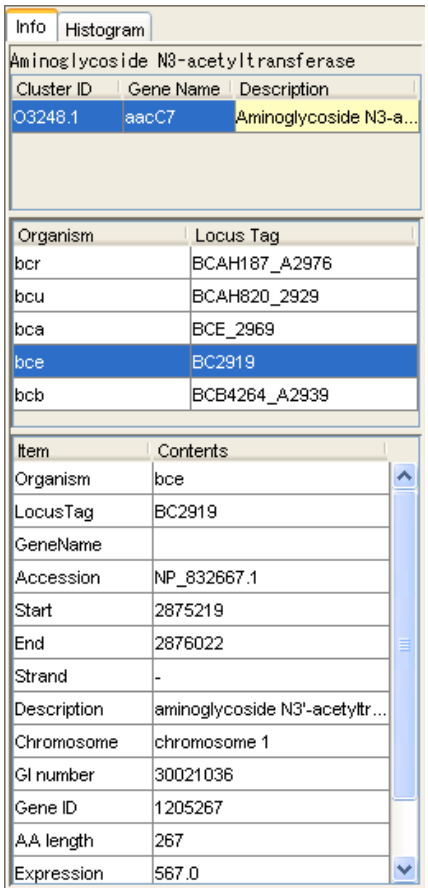
Nested table structure



RECOG screen display

5.8. Info tab

The **Info** tab displays the cluster selected on the PPM and the gene information in that cluster. In the aggregate mode, multiple clusters with the same phylogenetic pattern are displayed.



1. Cluster description field (at the top)

   The cluster selected on the **PPM** is described.

2. Cluster information table (upper table)

The cluster information selected on the **PPM** is displayed. When the table is double-clicked, a browser is activated and the details of the cluster information are shown. Also, by right-clicking the selected cluster on the table, various functions, including **Multiple Alignment** and the display of a **Regional Genome Map**, for the selected gene group can be executed.

Cluster ID	The cluster ID is displayed.
Gene Name	The representative gene name of the cluster (sub-cluster) is displayed.
Description	The representative description of the cluster (sub-cluster) is displayed. As the background color, the color corresponding to the typical function category of the cluster (sub-cluster) is used.

3. Gene information table (center table)

The information on the genes belonging to the cluster selected on the PPM or the cluster information table is displayed. Upon double-clicking, a web browser is activated and the details of the gene information are displayed. If multiple genes on the table are selected and right-clicked, various functions, including **Multiple Alignment** and the display of a **Regional Genome Map**, for the selected gene group can be executed.

Organism	The species code is displayed.
Locus Tag	The locus tag of a gene (domain) is displayed. For the domain, the domain number is shown at the word's end.

4. Detailed gene information table (lower table)

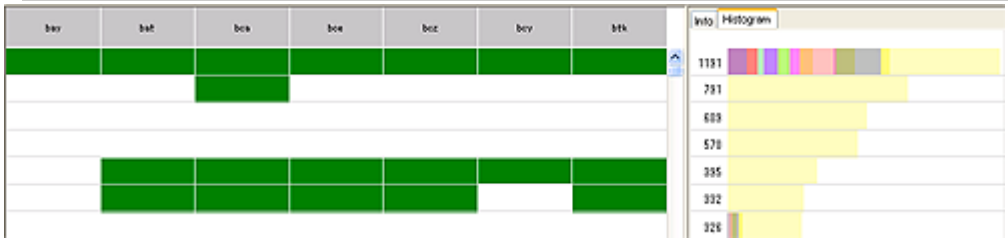
Detailed information on the gene selected on the PPM or the cluster information table is displayed. The registered gene properties are also displayed.

Organism	The species code is displayed.
Locus Tag	Locus Tag
Gene Name	Gene name
Accession (P)	Accession number
Position	Gene region
Direction	Direction of gene
Feature Key	Feature key
GI number	GI number
Gene ID	Gene ID
Description	Description

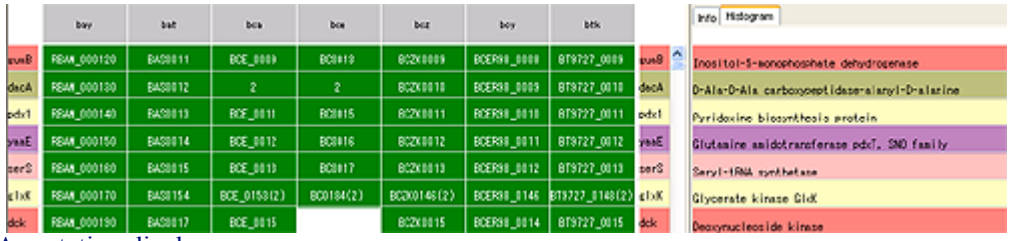
5.9. **Histogram** tab

On the **Histogram** tab, cluster properties are displayed in graphs of various types. In normal disaggregate mode, annotation information is displayed.

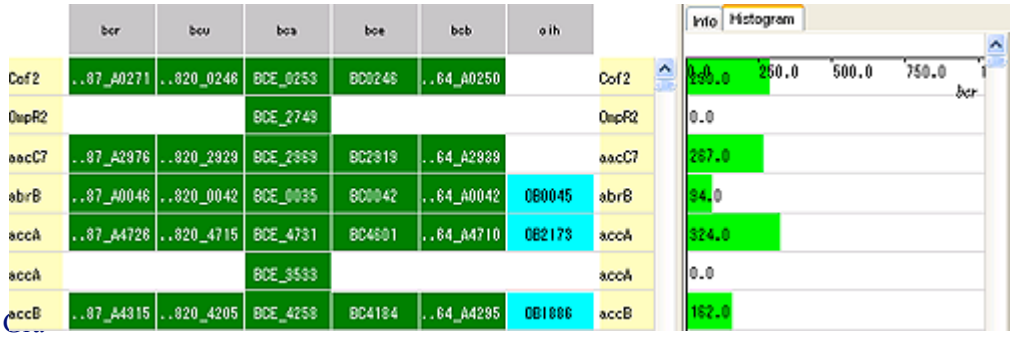
Aggregate mode	Bar graph for phylogenetic pattern frequency
Disaggregate mode	Description / Function Category The description is displayed. For the background color, the color of the sub-cluster's typical function category is displayed. Value 1. The species' numerical data are displayed in a bar graph/line graph. Difference 2. The differences in the species' numerical data are displayed in a bar graph/line graph.



Histogram display in the aggregate mode



Annotation display



Graph display of the property value

5.10. **Status** bar

The **Status** bar displays the amount of used memory, PPM information and application update information.

1. Display of the amount of used memory (left side)

   The amount of memory used by the current application is displayed.

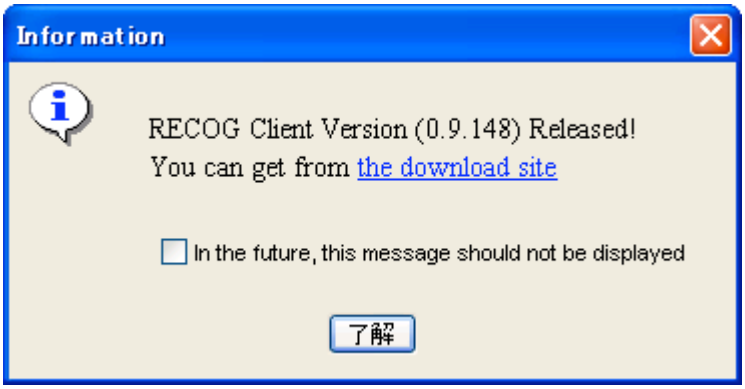
   - Left side: the amount of memory used by the application
   - Right side: the amount of memory allocated to the application

2. Display of PPM size information (center)

   - Left side: the number of species shown on the PPM
   - Right side: the number of clusters (or total number of phylogenetic patterns in the aggregation mode) shown on the PPM

3. Update information (right side)

   The update notice icon is displayed on the right side of the status bar when any application or public data is updated. The update information is accessed by clicking this icon.

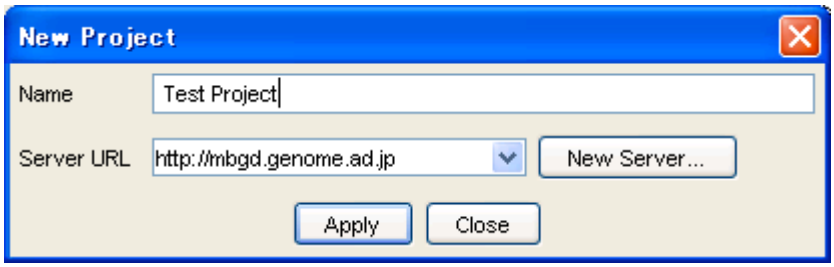


6. Project Creation and Editing

A **Project** is a saved collection of related analysis results. In default mode, the *default project* is selected, and all analysis results are stored therein. It is advisable to create a dedicated project before proceeding with any analysis.

6.1. Creation of a new **Project**

3. Click **File – New – New Project…**.
   The New Project screen appears.



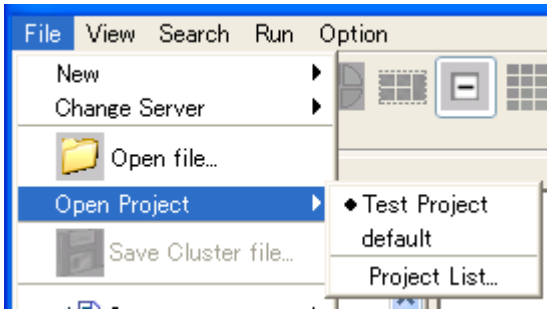
4. On the New Project screen, enter a project name and the URL of the RECOG server that will conduct the analysis. The RECOG server can be selected from the menu from among all the registered servers. If an official server is used, the default settings do not have to be changed. To register the URL for a new server, click the **New Server…** button to register it on the New Server screen.

5. When the **Apply** button on the New Project screen is clicked, the project is registered and opened.

（Note） To register a project, ensure that you can connect to the RECOG server.

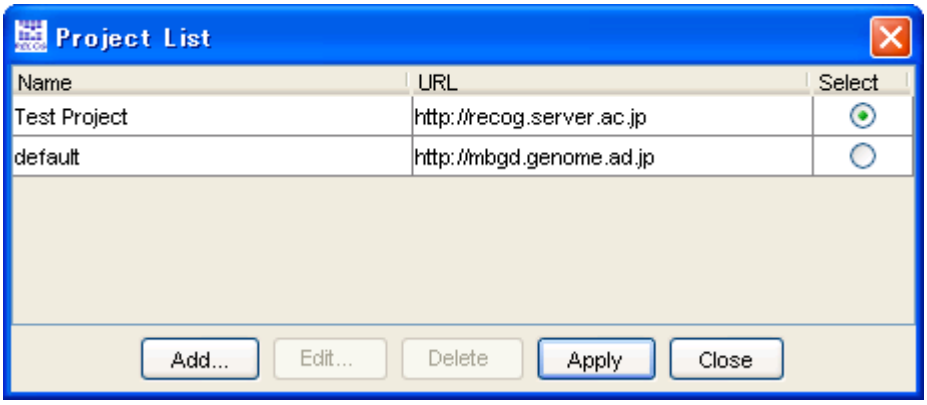
6.2. Open a project

1. Click **File** – **Open Project**, and click the relevant project. The clicked project opens.



6.3. Reference to a list of registered projects

1. Click **File** – **Open Project** – **Project List…** to display the Project List screen.
   The registered project names and the RECOG server URL are displayed on the Project List screen. Also, the **Select** field of the project in use is displayed and checked.



6.4. Project registration

1. Click **File** – **Open Project** – **Project List…** to display the Project List screen.
2. On the Project List screen, click the **Add…** button to display the New Project screen.
3. On the New Project screen, enter the project name and set the URL of the RECOG server that will conduct the analysis.

   To register the URL for a new server, click the **New Server…** button and register it on the New Server screen.
4. On the New Project screen, click the **Apply** button.
5. Click the **Apply** button on the Project List screen.

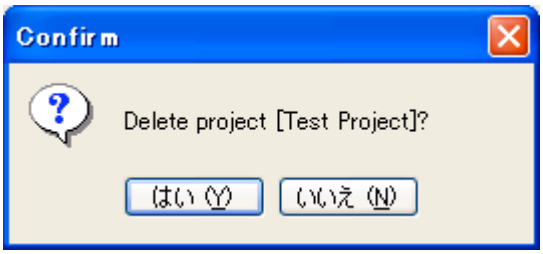
6.5. Project information editing

1. Click **File** – **Open Project** – **Project List…** to display the Project List screen.
2. On the Project List screen, select the project and click the **Edit** button to display the Edit Project screen.
3. On the Edit Project screen, change the project name and server URL.
4. After editing, click the **Apply** button on the Edit Project screen to display the details of the edited information on the Project List screen.
5. Click the **Apply** button on the Project List screen.

6.6. Project removal

1. Click **File** – **Open Project** – **Project List…** to display the Project List screen.

2. On the Project List screen, select the project to be removed and click the **Delete** button. A warning message appears. Click the **OK** button.



3. On the Project List screen, click the **Apply** button.

（**Note**） Upon the removal of the project, all the analysis results, including the DomClust results, of the project are removed.

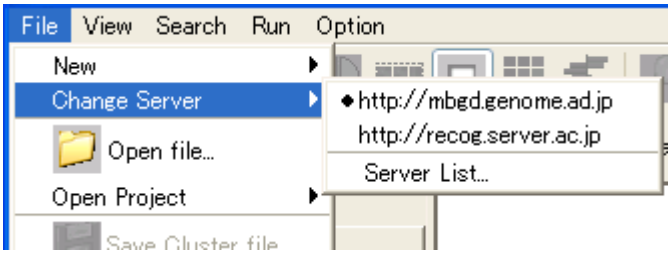
（**Note**） The files created in a project are saved in the following folder in the user's home directory: RECOG/project/*project_name*. So, unnecessary files can be removed by directly accessing this folder.

7. Switching the RECOG Server

The RECOG servers that conduct DomClust analysis and CoreAligner analysis can be switched. When a project is opened, the default RECOG server that is set for the project conducts the analysis.

7.1. Checking the RECOG server used

1. Click **File** – **Change Server** to access the list of URL's of registered RECOG servers. The server currently used for analysis is checked.



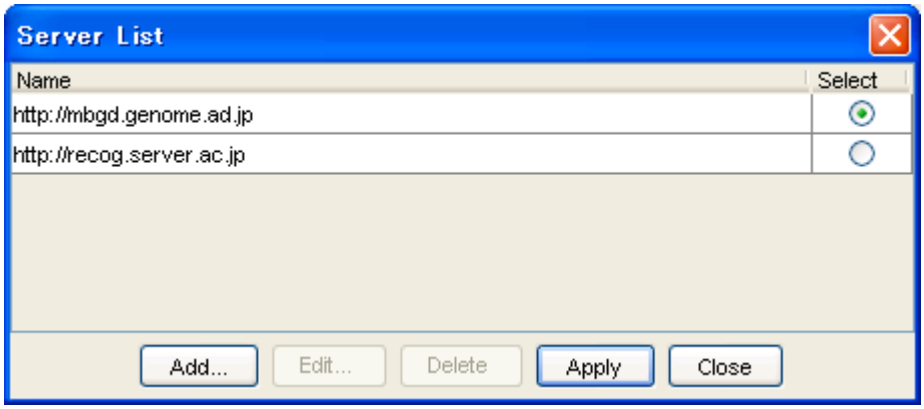
7.2. Switching the RECOG server

1. Click **File** – **Change Server**, and click the RECOG server to be used. The RECOG server in use is switched, and the Taxonomy Tree on the **All** tab is updated using the data from the newly specified RECOG server.

(Note) When the DomClust analysis results are displayed, the RECOG server cannot be switched. Click **File** – **New** – **New Analysis** to clear the display and start a new analysis.

7.3. Reference to the registered RECOG servers

1. Click **File** – **Change Server** – **Server List…** to display the Server List screen. A list of registered RECOG servers is displayed. The RECOG server used for analysis is checked in the **Select** field.



7.4. Registering the RECOG server

1. Click **File** – **Change Server** – **Server List…** to display the Server List screen.
2. On the Server List screen, click the **Add...** button to display the New Server screen.
3. On the New Server screen, enter the URL of the RECOG server and click the **Apply** button.
4. On the Server List screen, click the **Apply** button.

7.5. Editing the RECOG server

1. Click **File** – **Change Server** – **Server List…** to display the Server List screen.
2. On the Server List screen, select the RECOG server to be edited and click the **Edit...** button. The Edit Server screen is displayed.
3. On the Edit Server screen, enter the URL of the RECOG server and click the **Apply** button.
4. On the Server List screen, click the **Apply** button.

7.6. Removal of the RECOG server

1. Click **File** – **Change Server** – **Server List…** to display the Server List screen.

2. On the Server List screen, select the RECOG server to be removed and click the **Delete** button. A warning message appears. Click the **OK** button.

3. On the Server List screen, click the **Apply** button.

8. Display and Manipulation of the Taxonomy Browser

The Taxonomy Browser displays the taxonomy tree of organisms. On the tree, manipulations can be performed, including the selection of the ingroup/outgroup to be subjected to DomClust analysis.

The Taxonomy Browser comprises the **All** tab for specifying the species group to be subjected to analysis from among all the available species, and the **Selected** tab for specifying various manipulations of the species group to be subjected to analysis.

8.1. Expansion/Collapse of the Taxonomy Tree

The classification hierarchy of the Taxonomy Tree can be expanded or collapsed in a stepwise manner by clicking the **Toolbox** buttons.

For expansion, click  (**Expand Taxonomy Tree**) in the **Toolbox**. One expansion increment is made for each click.

For collapsing, click  (**Collapse Taxonomy Tree**) in the **Toolbox**. One collapsing increment is made for each click.

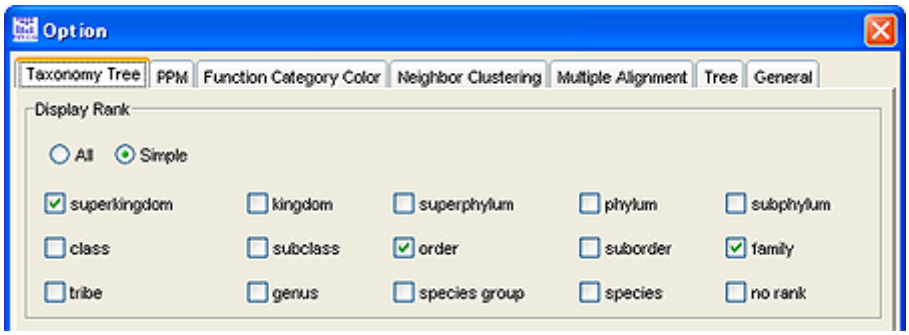
8.2. Specification of a set of classification ranks to be displayed on the Taxonomy Tree

Users can choose a set of taxonomic ranks (species, genus, family, order, etc.) to be displayed on the Taxonomy Tree.

1. Click  (**Option**) in the **Toolbox** to display the Option screen.
2. On the Option screen, click the **Taxonomy Tree** tab.
3. On the **Taxonomy Tree** tab's Display Rank form, specify the classification rank to be displayed.

- All: All the taxonomic ranks are displayed.
- Select: Only the checked taxonomic ranks are displayed.

* Click the **Default** button to return to the default settings.



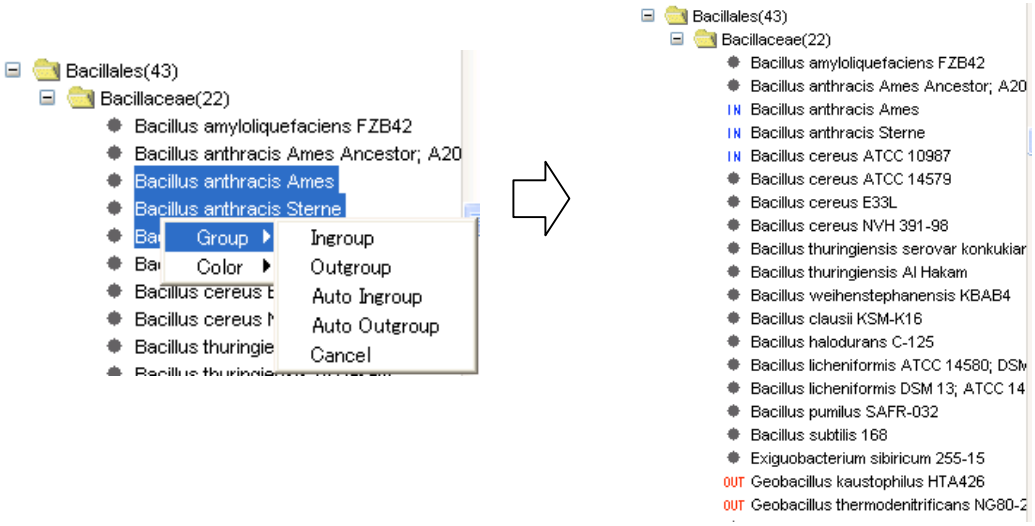
8.3. Specification of the ingroup/outgroup

On the **All** tab, users can choose a set of species to be compared by the DomClust program. Specify a set of species belonging to the interested phylogenetic group as ingroup, and specify, as the need arises, a set of distantly related species for comparison as outgroup (the specification of the ingroup is mandatory, while the specification of outgroup is optional). If the outgroup is specified, groups are severed in creating an ortholog group so that the ingroup species form a single phylogenetic group against the outgroup species.

1. On the Taxonomy Tree, select and click the relevant species or taxonomy nodes.

2. Click the right mouse button, and click **Group** – **Ingroup** or **Outgroup**. The selected species are chosen as the ingroup or the outgroup species, respectively.

   IN is displayed for the ingroup species, and **OUT** is displayed for the outgroup species.



2. To cancel the selection of the ingroup/outgroup species, select and click the relevant species or taxonomic group on the Taxonomy Tree and click **Group** – **Cancel**. To cancel all species or groups, click **Cancel** on the uppermost root node.

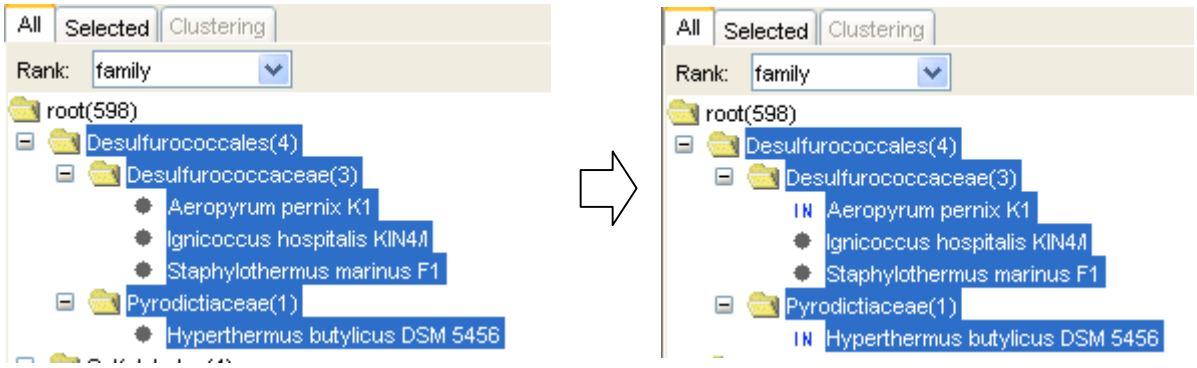
8.4. Automatic ingroup/outgroup specification

The best way to evenly select the species to be analyzed is to select a representative species for each taxonomic rank. RECOG can automatically select a representative species from each taxonomic group for choosing the target species. Here the weight of species is determined by the date of publication of the genome sequence; that is, the earlier the determination of the genome sequence, the higher its significance.

1. From the **Rank** drop-down menu located above the Taxonomy Tree, specify the normative taxonomic rank.

2. On the Taxonomy Tree, select and click the taxonomic group (with a rank higher than the normative rank specified above) to be subjected to analysis.

3. Click the right mouse button, and click **Group** – **Auto Ingroup** or **Auto Outgroup**. From among the selected classification groups, the species with the highest weight is chosen as ingroup/outgroup species for each normative-rank taxonomic group.



(Supplement) Rank item modifications

To modify the **Rank** items in the drop-down menu:

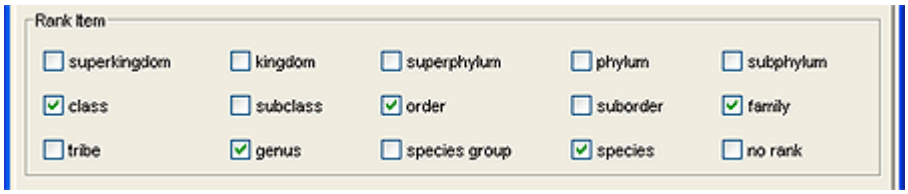
1. Click  (**Option**) in the **Toolbox** to display the Option screen.

2. On the Option screen, click the **Taxonomy Tree** tab.

3. In the Rank Item form on the **Taxonomy Tree** tab, check the taxonomic rank to be

displayed.

4. Click the **Apply** button on the Option screen.



9. Ortholog Clustering (DomClust)

Ortholog clustering is performed upon specifying a species. The result is displayed on the PPM. This is the first analysis conducted in RECOG, and is the basis for all comparative analyses.

9.1. New analysis

If any DomClust analysis result is displayed, clear the result.

1. Click **New** – **New Analysis** to clear the currently displayed DomClust analysis result.

9.2. Execution of DomClust

(Note) DomClust analysis is possible only in an environment where Internet connection is available.

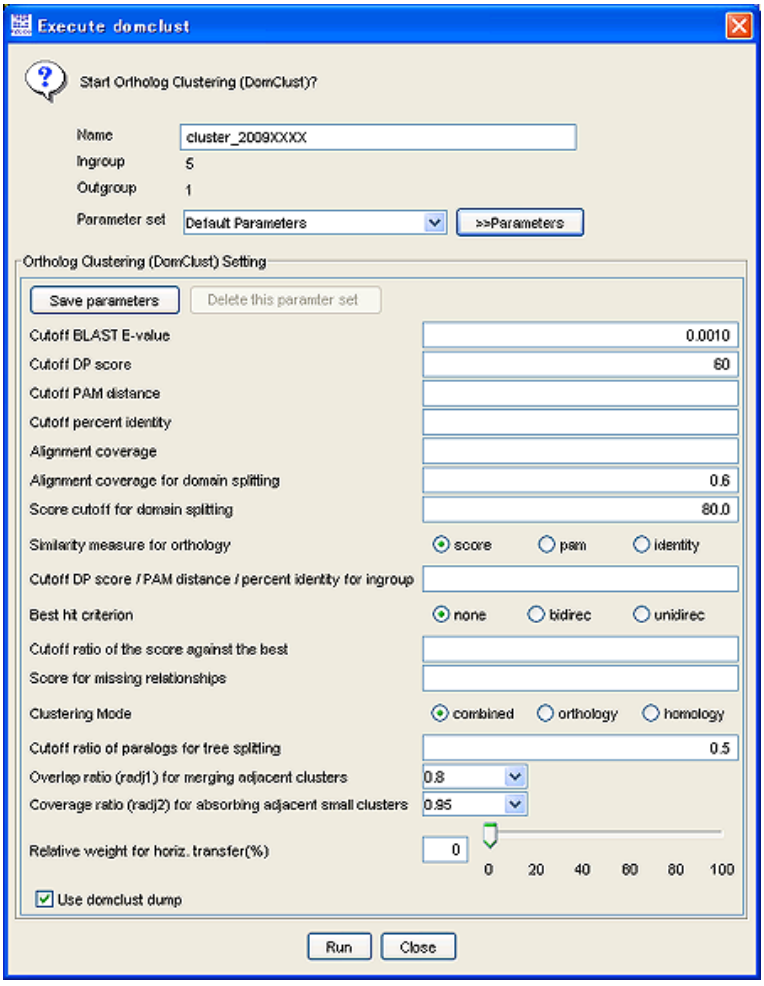
2. Specify the ingroup/outgroup by means of the method shown in 8.3 Specification of the ingroup/outgroup.

3. Click  (**Ortholog Clustering (DomClust)**) in the **Toolbox** to display the Execute domclust screen.

4. On the Execute domclust screen, enter the analysis name in the **Name** field. The result is automatically saved under the analysis name entered here.
   If the default parameter set is used, select 'Default parameters'; if the parameter set executed immediately theretofore is used, select '<<Last Parameters>>'; if DomClust is conducted using a parameter set saved previously, select the parameter set saved in the **Parameter set** field.

5. To specify a new parameter set, click the **Parameters…** button and set it on the displayed parameter-setting screen. For details on the parameters, refer to *37.1 DomClust Parameters*.

6. To save the parameters that were set on the parameter-setting screen, click the **Save parameters** button. To remove the saved setting, specify the parameters to be removed in the **Parameter set** field and click the **Delete this parameter set** button.

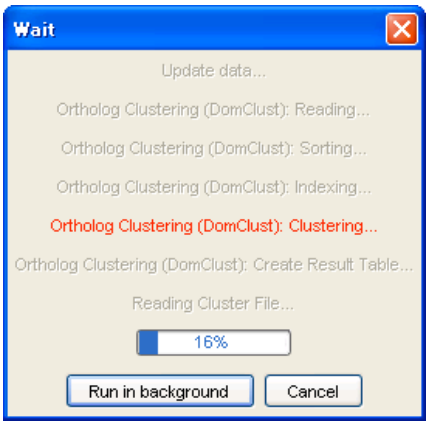


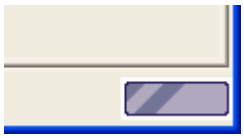
7. After specifying the DomClust analysis result name and parameters, click the **Run** button to display the progress screen and execute the DomClust analysis.

   Click the **Run in background** button on the progress screen to execute the DomClust analysis in the background. In this setting, other operations can be performed concurrently.

   To see the progress screen of the DomClust analysis that is underway in the background,

double-click the progress bar displayed at the bottom right of the screen.





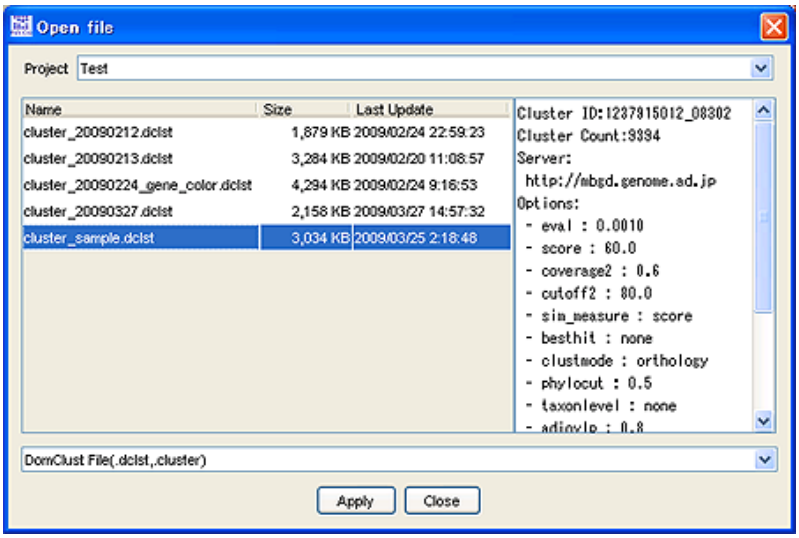
8. Upon the completion of the DomClust analysis, the DomClust analysis result is displayed on the PPM. When the completion message is displayed, click the **OK** button.

   If the analysis is executed in the background, the ”Load DomClust file?” message is displayed. Click the **OK** button.

9.3. Display of the DomClust analysis results

The previous DomClust analysis results are displayed.

1. Click  (**Open files**) in the **Toolbox** to display the Open file screen.



2. On the Open files screen, select the file filter **DomClust File (.dclst, .cluster)**, and then select a project and a DomClust analysis result file.
   Upon selecting the DomClust analysis result file, the relevant information (species set and parameters) on the analysis result is displayed on the right side of the screen.

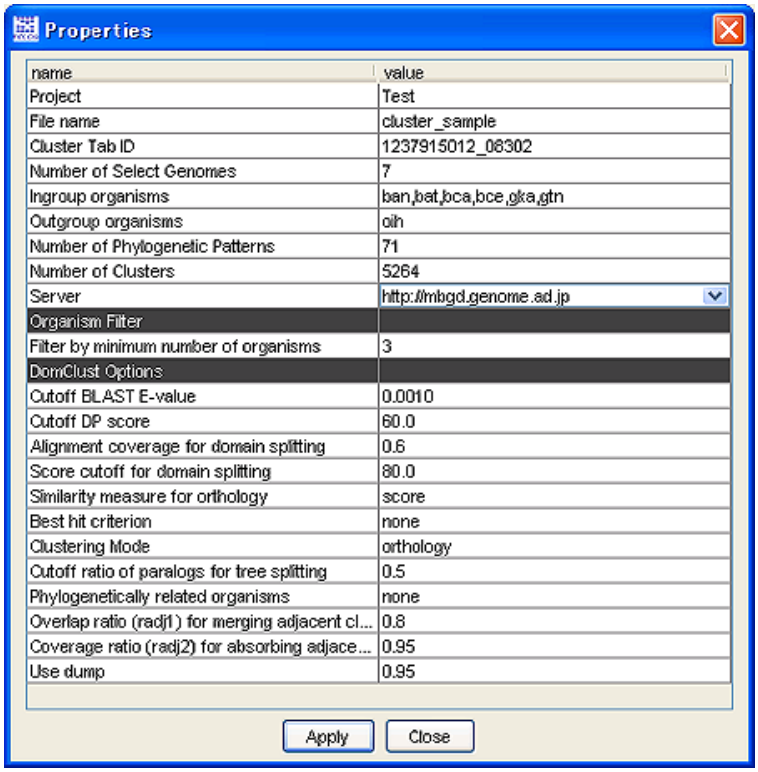
3. Click the **Apply** button on the Open files screen to display the selected DomClust analysis result.

9.4. Display of the DomClust analysis result properties

For the DomClust analysis result, the parameters for the DomClust execution, the applied PPM sorting conditions, *etc*. are displayed.

Also, the RECOG server can be edited for default access by the currently displayed DomClust analysis result.

9. Click **Option** – **Properties…** to display the Properties screen.



10. To edit the RECOG server, select the server from among those listed by double-clicking **value** in the **Server** drop-down menu, and click the **Apply** button.

9.5. Storage of the DomClust analysis results

The DomClust analysis result is automatically saved to the project directory or one of its lower-level directories when the analysis is conducted. To refer to the analysis result using another tool, save the analysis result in DomClust format (.dclst) or tab-delimited format.

Although the tab-delimited format file is useful for loading it into and displaying it on Excel, *etc*., the information on the domain boundary and other types of information are lost. To reload the analysis result into RECOG, save it in DomClust format.

9.5.1. Storing a file in DomClust format

1. Click  (**Save Cluster file**) in the **Toolbox** to display the Save Cluster file screen.

2. On the Save Cluster file screen, specify the file name and the destination folder, and click the OK button.

9.5.2. Storing a file in tab-delimited format

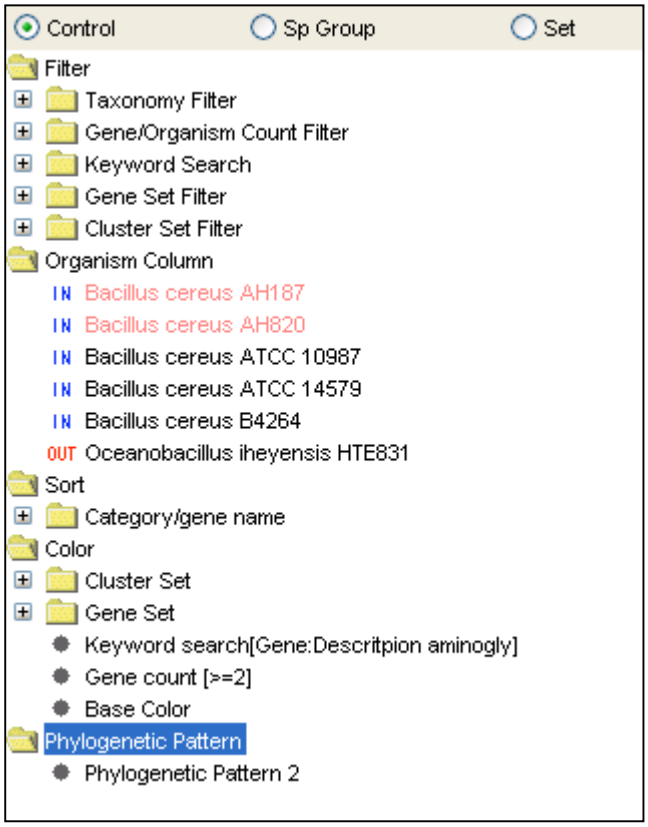
1. Click  (**Export**) in the **Toolbox** to display the Export screen.

2. On the Export screen, specify the file name and the destination folder, and click the OK button.

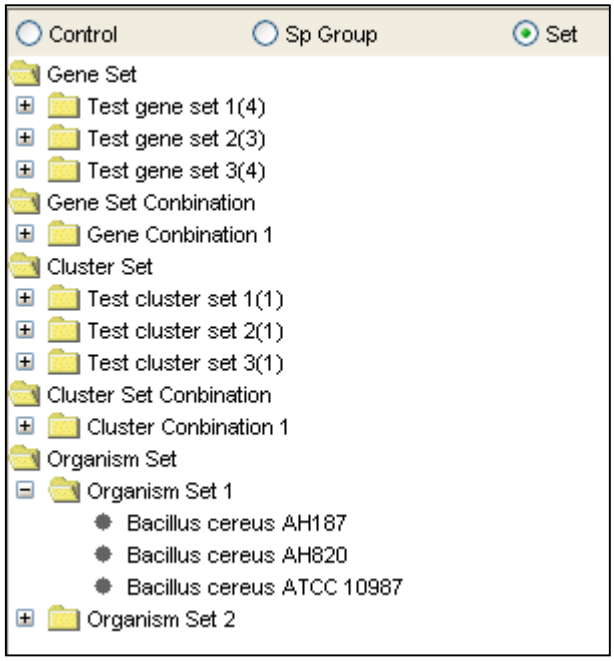
10. Control Panel and Set Management Panel

On the control panel, the filtering settings, alignment sequence settings and display/nondisplay settings for species, sort settings, color settings and phylogenic patterns can be specified.

Also, on the set management panel, the gene sets/cluster sets, combined sets and species sets can be managed.



Control panel



Set management panel

10.1. Display on the control panel

Click the **Selected** tab on the right side of the screen and click the downward **Control** button to display the control panel.

10.2. Control panel operation

10.2.1. Filter settings (**Filter**)

Filter refers to the conditions for selecting the row (cluster) that is displayed on the PPM. In the **Filter** folder on the control panel, the filter conditions that are currently applied to the PPM are displayed. Only those clusters that meet all the filter conditions are displayed on the PPM. Click **Enable/Disable** to enable or disable the filter settings.

The following filter conditions can be specified.

Taxonomy Filter	Phylogenetic pattern filtering. See "15. Taxonomy Filtering."
Gene/Organism Count Filter	Filtering by gene count/species count. See "16. Filtering by Gene Count/Species Count in the Phylogenetic Patterns."
Keyword Search	Filtering by keyword search. See "17. Keyword search."
Gene Set Filter	Filtering by combined gene-set condition. See "31.4 A combined set is set as the filtering condition."
Cluster Set Filter	Filtering by combined cluster-set condition. See "31.4 A combined set is set as the filtering condition."

10.2.2. Sort setting (**Sort**)

The sort condition that is applied to the PPM is displayed in the **Sort** folder of the control panel. For the operational procedure, see "13. PPM sort."

10.2.3. Color setting (**Color**)

In the **Color** folder on the control panel, the gene/cluster color that is displayed on the PPM and the comparative genome map view is set. The coloring is applied in descending order of the alignment sequence on the control panel, and is overwritten (the upper one has priority over the lower one). The order can be changed by dragging and dropping an object. Also, the color settings can be enabled/disabled by clicking **Enable/Disable**.

The following color settings can be specified:

Gene property	Color setting based on gene/cluster property. See "12. Coloring by property."
Neighboring cluster	Color setting based on the result of neighboring gene clustering. See "24. Neighboring gene clustering."
Keyword search	Color setting based on the keyword search result. See "17. Keyword search."
Gene Set	Color setting based on a combined gene-set condition. See "31. Combined set."
Cluster Set	Color setting based on a combined cluster-set condition. See "31. Combined set."
Gene count	Color setting based on the gene count in a cell. See "11.6 Color change according to the gene count in a cell."
Base color	Standard color setting. See "11.5 Changing the color of the PPM cell/boundary."

10.2.4. Phylogenetic pattern registration (**Phylogenetic Pattern**)

The profile used in the similar phylogenetic pattern search function is displayed.

For the operation procedure, see "33. Similar phylogenic pattern search."

10.2.5. Organism column setting (**Organism Column**)

The species to be displayed on the PPM is displayed.

For the operational procedure, see "18. Changing the display order and display/nondisplay of species."

10.3. Display of the set management panel

Click the **Selected** tab on the right of the screen and click the **Set** button below to display the set management panel.

10.4. Operation of the set management panel

On the set management panel, the following sets are displayed and can be operated:

Gene Set	Gene sets are displayed. See "30. Registration and management of gene sets/cluster sets."
Gene Set Combination	Combined gene sets are displayed. See "31. Combined sets."
Cluster Set	Cluster sets are displayed. See "30. Registration and management of gene sets/cluster sets."
Cluster Set Combination	Combined cluster sets are displayed. See "31. Combined sets."
Organism Set	Species sets are displayed. See "32. Species sets."

11. Display and Operation of the Phylogenetic Pattern Map

On the Phylogenetic Pattern Map (**PPM**), the appearance patterns of species belonging to a cluster are displayed.

11.1. Display of the locus tags of genes belonging to a cell

The locus tags of genes belonging to a cell are displayed on the cell. If the cell region is narrow, the gene count is displayed. The display can be switched on/off as follows:

1. Click  (**Option**) in the **Toolbox** to display the Option screen.

   Click the **PPM** tab on the Option screen.

2. Check 'Display gene names or the number of genes' on the PPM tab.

3. Click the **Apply** button.

11.2. Display of the cluster property on the cluster header

The value of the property corresponding to the cluster is displayed in the display area (cluster header) on both sides of the PPM. To change the displayed property, do the following:

1. On the cluster header, click the right mouse button, and on the menu **PPM Label** click the property to be displayed; this displays the property value on the cluster header.

Cluster ID	The homology cluster ID, cluster ID and sub-cluster ID are displayed.
Gene name	The typical gene name of the cluster and the typical gene name of the sub-cluster are displayed (default value).
Cluster score	The cluster score and sub-cluster score are displayed.

Cluster dist	The cluster distance and sub-cluster distance are displayed.
Phylogenetic Pattern Coefficient	In a similar phylogenetic pattern search, the coefficient of correlation with a specified pattern is displayed.

11.3. Display/Nondisplay of the homology cluster header

1. Click the right mouse button on the cluster header, click the menu, and click **Show/Hide homology cluster label**.

11.4. Species color setting

The species color is set. The color set here is reflected in the background color of the PPM species header and the locus tag label of the phylogenetic tree in the multiple alignment analysis.

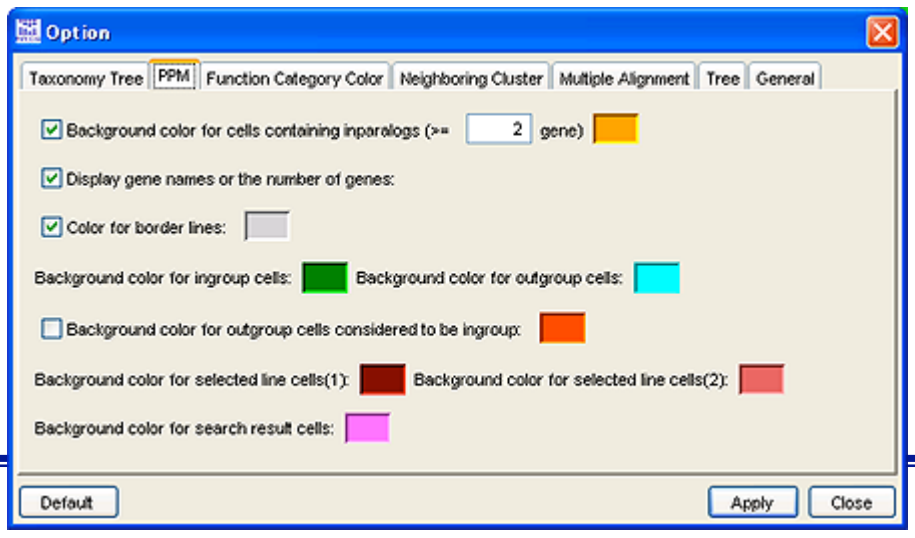
1. Select the species on the Taxonomy Tree at the upper part of the **Selected** tab, click the right mouse button, and click **Color organism – Choose…** in the pop-up menu.

   The Color palette screen is displayed.

2. Set the color on the Color palette screen and click the **OK** button to set the species color.

11.5. Changing the PPM cell/boundary color

1. Select **Color – Base Color** on the control panel, click the right mouse button and click **Edit** to display the **PPM** tab on the Option screen.



2. On the **PPM** tab, the background color of the cells, the display/nondisplay and the color of the boundary are set.

Color for border line	The color of the boundary between cells is set. Untick, and the boundary is not drawn.
Background color for ingroup cells	The background color of the ingroup cells is set.
Background color for outgroup cells	The background color of the outgroup cells is set.
Background color for outgroup cells considered to be ingroup	If the horizontal transfer option is specified, the background color of the outgroup cells that are considered to derive from the ingroup is specified.
Background color for selected line cell (1)	The background color of the cells of the selected cluster is specified.
Background color for selected line cell (2)	The background color of the cells of the selected species is specified.
Background color for search result cells	The background color of the cells of the searched cluster is specified.

3. Click the **Apply** button.

4. To enable/disable the color settings of the PPM cells, double-click **Color** – **Base Color** on the control panel.

11.6. Color change according to the gene count within a cell

The background color of a cell can be changed according to the gene count within the cell by setting a threshold value.

1. Select **Color** – **Gene count** on the control panel, click the right mouse button and click **Edit** to display the **PPM** tab on the Option screen.

2. On 'Background color cells containing inparalogs (>= # genes)' on the **PPM** tab, specify

the threshold gene count and the background color of the cell.

3. Click the **Apply** button to display the color-setting condition on **Color** – **Gene count** on the control panel.

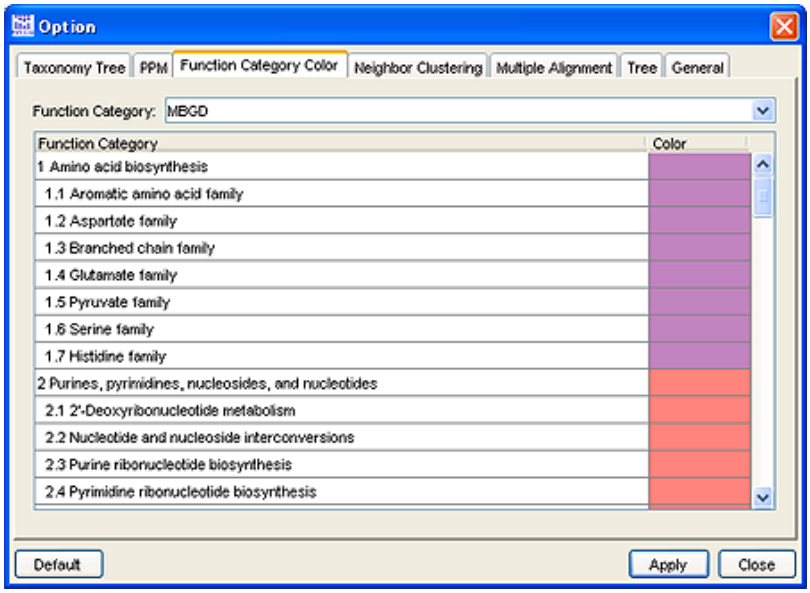
4. To enable/disable the color setting, double-click **Color** – **Gene count** on the control panel.

11.7. Color display corresponding to each functional category

The color corresponding to the typical functional category of a cluster is displayed in the gene name display field.

1. Click  (**Option**) in the **Toolbox** to display the Option screen. Click the **Function Category Color** tab on the Option screen.

2. On the **Function Category Color** tab, specify the functional category for drawing.



3. To change the functional-category color on the lower list, click the **Color** column, specify the color on the displayed Color palette screen, and click the **OK** button.

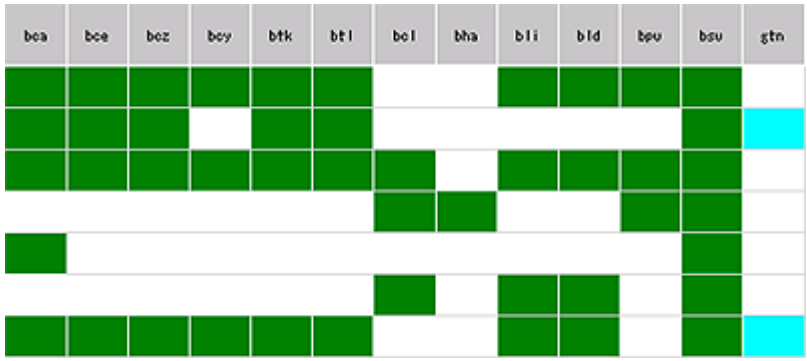
4. Click the **Apply** button.

11.8. Aggregated display of the PPM

In aggregate PPM mode, clusters of the same phylogenetic pattern are aggregated into a single line.

1. Click  (**Aggregate Mode**) in the **Toolbox** to display the PPM in aggregate mode.

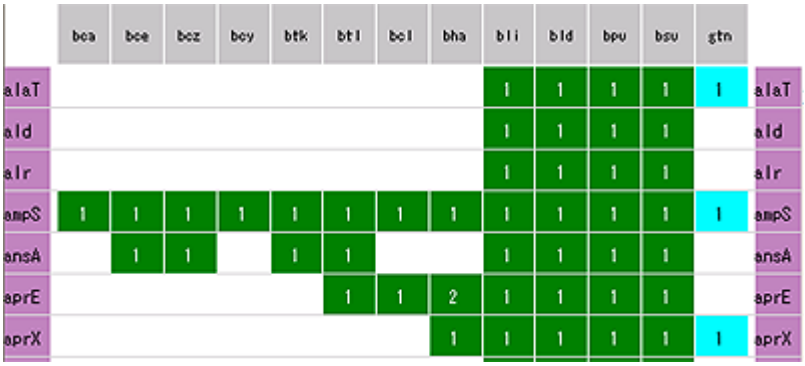
2. The PPM can also be displayed in aggregate mode by clicking **View** – **Aggregate Mode**, or by clicking **Aggregate Mode** after right-clicking the PPM.



To exit from the aggregate mode, do the following:

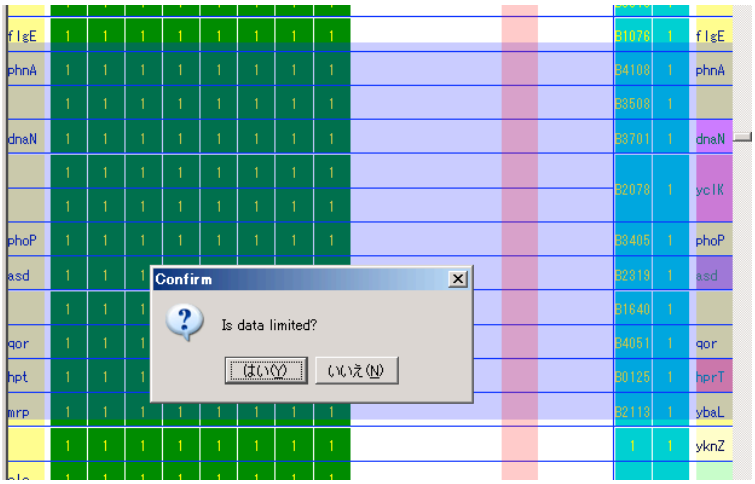
1. Click  (**Disaggregate Mode**) in the **Toolbox** to exit from the aggregate mode.

3. One can also exit from the aggregate mode by clicking **View** – **Disaggregate Mode**, or by clicking **Disaggregate Mode** after right-clicking the PPM.

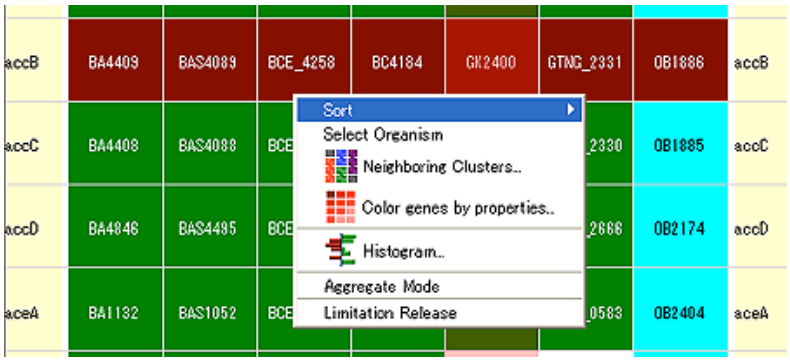


11.9. Limited display of a selected region on the PPM

The limited display of a selected region is possible by dragging the mouse on the PPM to specify the region.



To exit from the limited display mode, click the right mouse button and click **Limitation Release** on the menu.

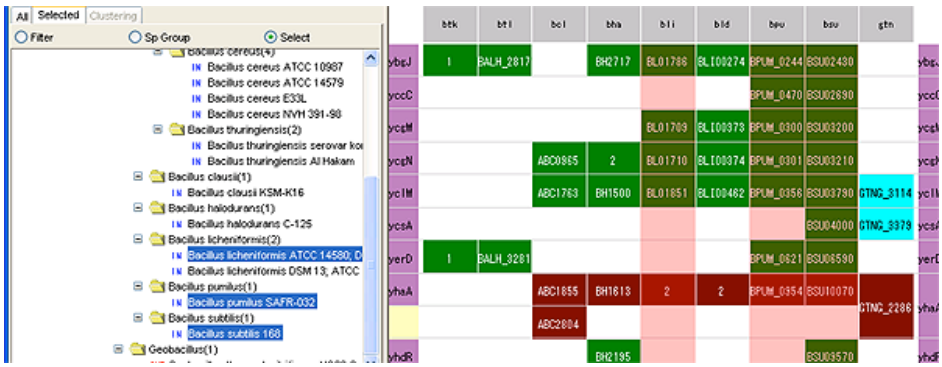


11.10. Highlighting a species by selecting it on the Taxonomy Tree

Select and click a species on the Taxonomy Tree above the **Selected** tab to highlight the relevant species on the PPM.

Also, to exit from the species selection mode on the Taxonomy Tree, do the following:

- Windows/Linux: Ctrl + left click on the selected species
- Mac: Apple-key + left click on the selected species



11.11. Selection of a species (phylogenetic pattern) in a cluster

The species in a cluster specified on the PPM is selected and displayed on the Taxonomy Tree on the **All** tab/**Selected** tab. This function is useful when searching a phylogenetic pattern similar to that of a specified ortholog group, *etc*.

1. Click to select a cluster on the PPM.
2. Click the right mouse button on the PPM and click **Select Organism** on the pop-up menu to select the relevant species on the Taxonomy Tree on the **All** tab/**Selected** tab.

12. Color Display by Properties

Each cell on the PPM can be colored and displayed using the gene properties registered as described in "29.1 Registration of gene properties," the correlation coefficient determined based on the similar phylogenetic pattern search function, *etc*.

12.1. Color display setting by properties

1. Click  (**Color genes by properties**) in the **Toolbox** to display the Color genes by properties screen.

2. Specify the coloring conditions on the Color genes by properties screen.

   - Organism: Specify a species.
   - Property: Specify properties.
     Value-type or enumeration-type properties can be colored and displayed.
   - Color: Set the color on the PPM.

     If the properties are of the value type:

     ✧ Threshold setting
     The color as per a specified property value is set (labels 1 and 2 in the above figure). Click the **Add color** button to set up to four values and colors. The color(s) between them is determined by the linear interpolation method. Click the **Remove color** button to remove the intermediate values.

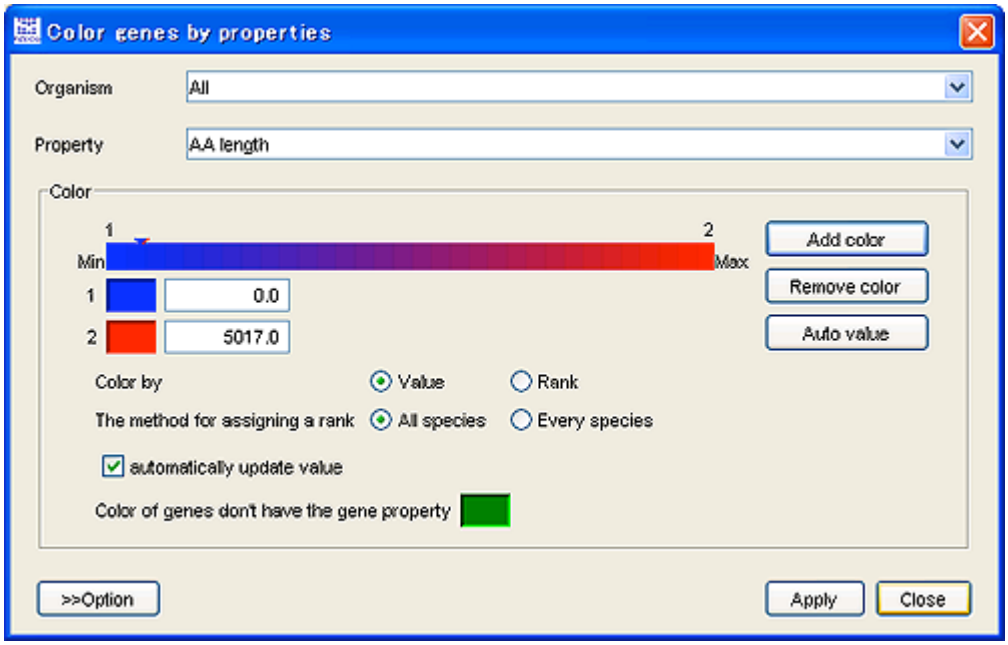
     ✧ "**Color by"**
     Specify whether to set the color based on value (**Value**) or ranking (**Rank**).

     ✧ "**The method for assigning rank**"
     If the color is set as per ranking, specify whether to rank all the species specified (**All species**) or each species (**Every species**).

     ✧ "**Automatic update value**"
     If this is checked, the threshold value is automatically divided equally in consideration of the possible range of the properties immediately after the change thereof.

✧ **"Color of genes don't have the gene property"**

This is used to set the color of the gene with no specified property value.

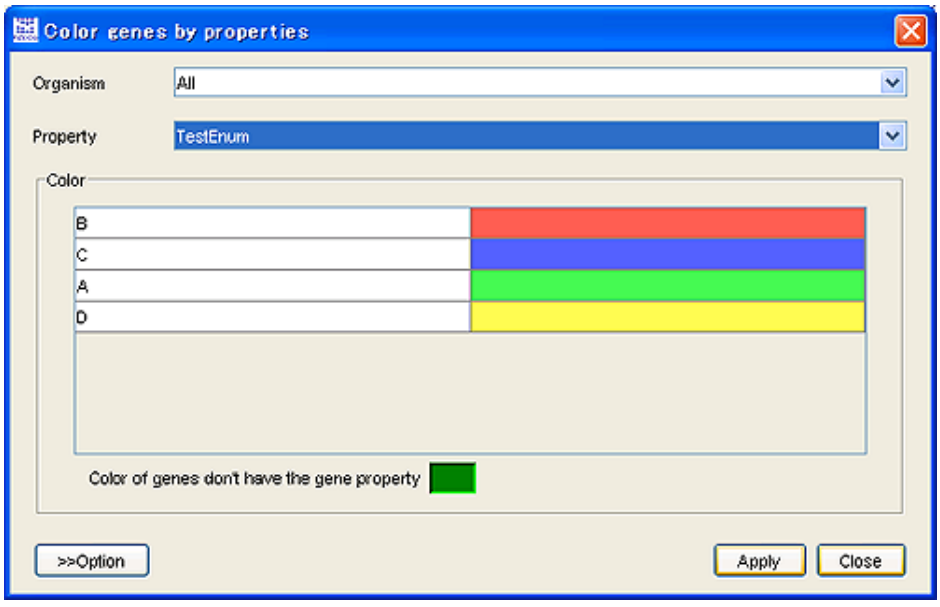


If the properties are of the enumeration type:

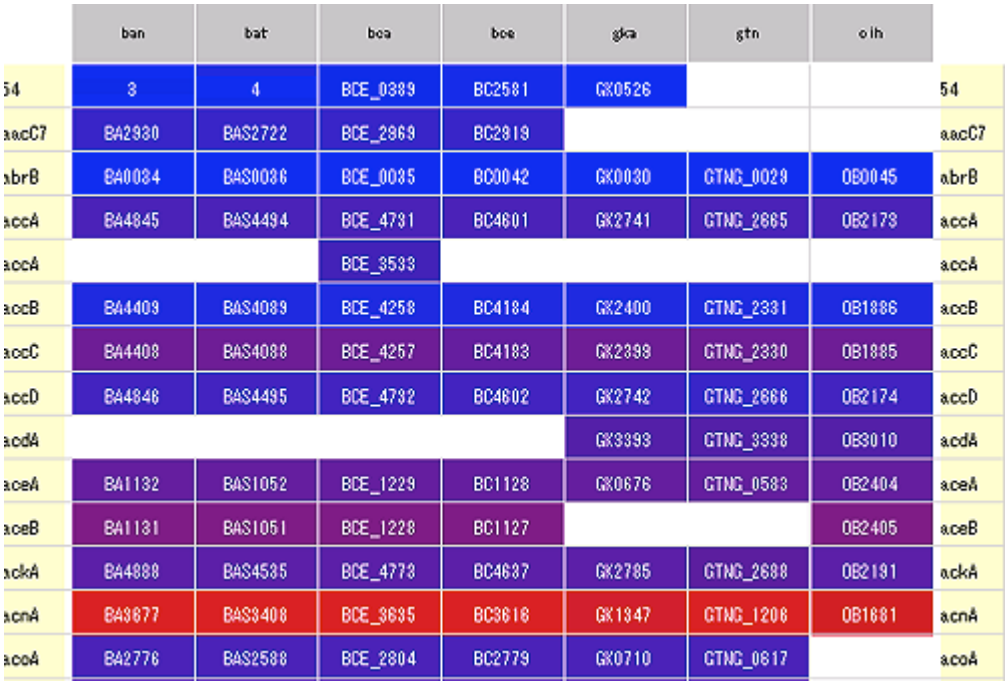
✧ Set the color for each possible value.

✧ **"Color of genes don't have the gene property"**

This is used to set the color of the gene with no specified property value.

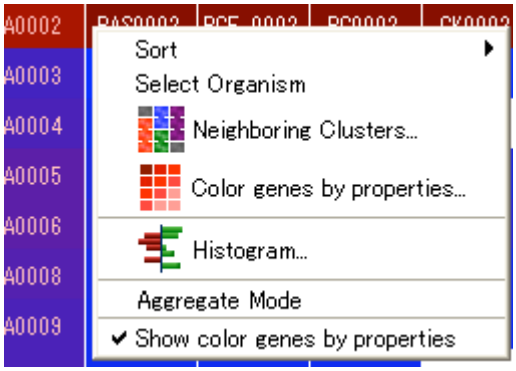


3. Click the **Apply** button on the Color genes by properties screen.
   Each cell is displayed in color as per the conditions specified on the PPM.



	ban	bat	bca	bce	gka	gtn	oih	
54	3	4	BCE_0389	BC2581	GK0526			54
aacC7	BA2930	BAS2722	BCE_2869	BC2819				aacC7
abrB	BA0034	BAS0036	BCE_0035	BC0042	GK0030	GTNG_0029	OB0045	abrB
accA	BA4845	BAS4494	BCE_4731	BC4601	GK2741	GTNG_2665	OB2173	accA
accA			BCE_3533					accA
accB	BA4409	BAS4089	BCE_4258	BC4184	GK2400	GTNG_2331	OB1886	accB
accC	BA4408	BAS4088	BCE_4257	BC4183	GK2399	GTNG_2330	OB1885	accC
accD	BA4846	BAS4495	BCE_4732	BC4602	GK2742	GTNG_2666	OB2174	accD
acdA					GK3393	GTNG_3338	OB3010	acdA
aceA	BA1132	BAS1052	BCE_1229	BC1128	GK0676	GTNG_0583	OB2404	aceA
aceB	BA1131	BAS1051	BCE_1228	BC1127			OB2405	aceB
ackA	BA4888	BAS4535	BCE_4773	BC4637	GK2785	GTNG_2688	OB2191	ackA
acnA	BA3677	BAS3408	BCE_3635	BC3616	GK1347	GTNG_1206	OB1681	acnA
acoA	BA2776	BAS2588	BCE_2804	BC2779	GK0710	GTNG_0617		acoA

The display/nondisplay of the color presentation can be switched on/off by ticking/unticking **Show color genes by properties** on the pop-up menu on the PPM.



12.2. Enable/Disable property color setting

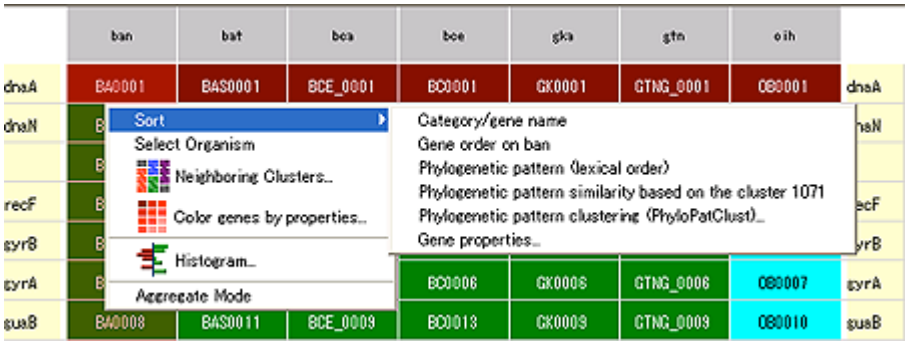
1. To enable or disable the property color setting, double-click **Color** – **Gene property** on the control panel.

13. PPM Sort

The PPM can be sorted and displayed according to various conditions based on the phylogenetic pattern.

13.1. PPM sort in disaggregate mode

In the disaggregate mode, the rows are sorted for each cluster or sub-cluster.



1. If the DomClust result contains an outgroup, click the **Cluster Mode/Sub-Cluster Mode** button in the **Toolbox** to specify whether to sort for each cluster  (**Cluster Mode**) or for each sub-cluster  (**Sub-Cluster Mode**). If no outgroup is specified, the designation is not effective.

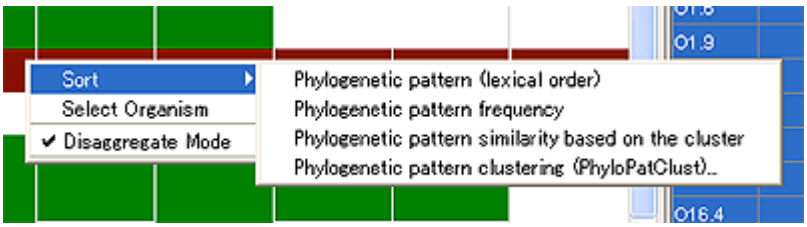
Cluster Mode	Sorting is carried out for each cluster. If a sub-cluster belonging to a cluster contains at least one species, sorting is carried out, given that the cluster contains at least one species.
Sub-Cluster Mode	Sorting is carried out for each sub-cluster.

2. Click the right mouse button on the PPM, select **Sort** on the pop-up menu and click the sort method on the sub-menu. Sort and display the phylogenetic pattern on the PPM as per the specified sort method. However, for **Gene order** and **Phylogenetic pattern similarity**, the species or cluster to be sorted depends on the location on the table of the clicked point.

Sort method	Details
Category/gene name	The data are sorted by function category/gene name.
Gene order on <genome name>	The data are sorted in ascending order of the position of the specified species on the genome.
Phylogenetic pattern (lexical order)	The data are sorted in lexical order of the phylogenetic patterns.
Phylogenetic pattern similarity based on the cluster #	The data are sorted in order of similarity to the phylogenetic pattern of a specified cluster. As the similarity indicator, specify one of the following: 1. Normalized hamming distance 2. Correlation coefficient 3. Correlation coefficient, absolute 4. Mutual information
Phylogenetic pattern cluster (PhyloPatClust)	Phylogenetic pattern clustering is carried out, and the data are sorted according to the hierarchical tree.
Homology Cluster ID	The data are sorted by the homology cluster ID/cluster ID/sub-cluster ID.
Gene properties…	The data are sorted based on the specified gene properties (see 12.3).

13.2. PPM sort in the aggregate mode

In the aggregate mode, the data are sorted based on the phylogenetic pattern of the aggregated clusters.



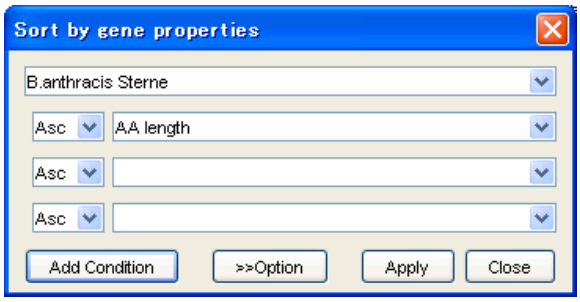
1. Click the right mouse button on the PPM, click **Sort** on the menu, and click the sort method on the sub-menu to sort and display the phylogenetic patterns on the PPM.

Sort method	Details
Phylogenetic pattern (lexical order)	The data are sorted in lexical order of the phylogenetic patterns.
Phylogenetic pattern frequency	The data are sorted in descending order of the occurrence frequency of the sub-clusters with phylogenetic patterns.
Phylogenetic pattern similarity based on the cluster	The data are sorted in order of similarity to the phylogenetic pattern of a specified cluster. As the similarity indicator, specify one of the following: 1. Normalized hamming distance 2. Correlation coefficient 3. Correlation coefficient, absolute 4. Mutual information
Phylogenetic pattern cluster (PhyloPatClust)	Phylogenetic pattern clustering is carried out, and the data are sorted according to the hierarchical tree.

13.3. Sort based on properties

The phylogenetic patterns are sorted based on the gene properties provided by the RECOG server or the gene properties registered as described in "29.1 Registration of gene properties."

1. Click the right mouse button on the PPM and click **Sort – Gene properties…** to display the Sort by gene properties screen.



2. On the Sort by gene properties screen, specify the gene properties for the sorting and the ascending order (Asc)/descending order (Desc).

   To add the sorting conditions, click the **Add Condition** button.

Click the **Option** button, and the following setting column is displayed.

- "**Representative value of multiple values set for a gene property**"

  If multiple values are set for a gene property of a gene, specify the method of determining the representative value for the sorting.

  - ✧ Min: The minimum value among multiple values is used.
  - ✧ Max: The maximum value among multiple values is used.
  - ✧ Median: The median among multiple values is used.
  - ✧ Average: The mean value among multiple values is used.*

- "**Representative value of multiple genes in a cell**"

  If a cell contains multiple genes, specify the method of determining the representative value for the sorting.

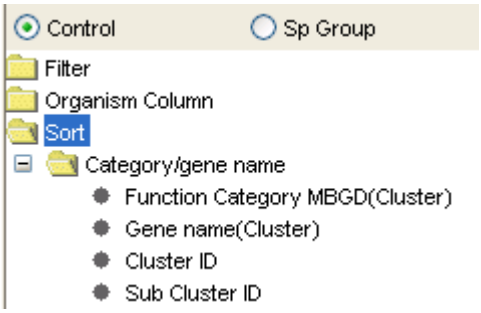
  - ✧ Min: The minimum value among the multiple genes is used.
  - ✧ Max: The maximum value among the multiple genes is used.
  - ✧ Median: The median among the multiple genes is used.
  - ✧ Average: The mean value among the multiple genes is used.*

* Only numerical-type gene properties apply.

3. After specifying the sort conditions, click the **Apply** button to sort and display the phylogenetic patterns on the PPM according to the sort conditions.

13.4. Display of the sort conditions

The currently valid sort conditions are displayed in the **Sort** folder on the control panel.



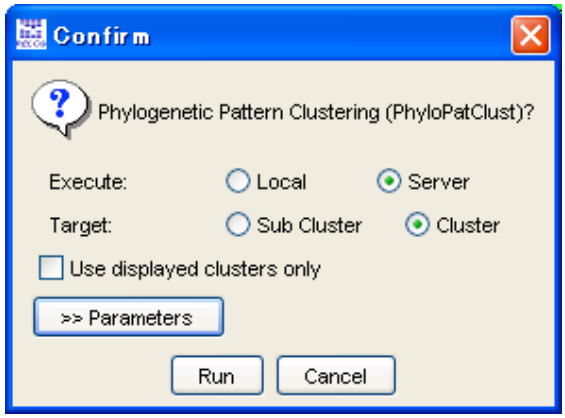
14. Phylogenetic Pattern Clustering (PhyloPatClust)

Upon the analysis of the phylogenetic pattern clustering (PhyloPatClust), each ortholog group is clustered based on the similarity of the phylogenetic patterns and sorted based on the results. Also, the clustering tree is displayed on the **Clustering** tab.

14.1. Execution of PhyloPatClust

1. Click  (**Phylogenetic pattern clustering (PhyloPatClust)**) in the **Toolbox** to display the Confirm screen.

2. Specify the conditions on the Confirm screen.



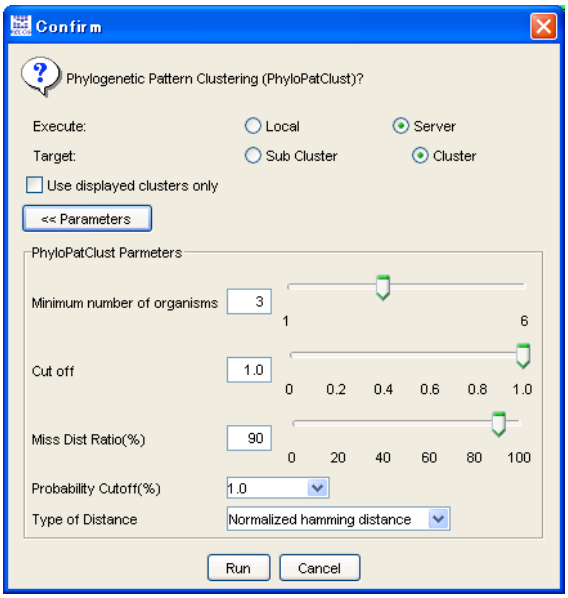
- **Execute**: If the clustering is carried out locally, specify **Local**. If the clustering is carried out on the RECOG server, specify **Server**.
  (Note) Server is available only in an environment with an Internet connection.

- **Target**: If the clustering is carried out based on the phylogenetic pattern of a cluster, specify **Cluster**. If the clustering is carried out based on the phylogenetic pattern of a sub-cluster, specify **Sub Cluster**.

- **Use displayed clusters only**: If this is checked, phylogenetic pattern clustering is carried out only for the clusters currently displayed on the PPM. This reduces the processing time when there are many clusters.

3. Click the **Parameters** button to set the parameters on the parameter-setting screen.

4. Click the **Apply** button on the Confirm screen to execute PhloPatClust. Upon completion of the process, a dendrogram (clustering tree) is displayed on the **Clustering** tab based on the clustering result, and the PPM is sorted based on its arrangement.



14.2. Operation of the clustering tree

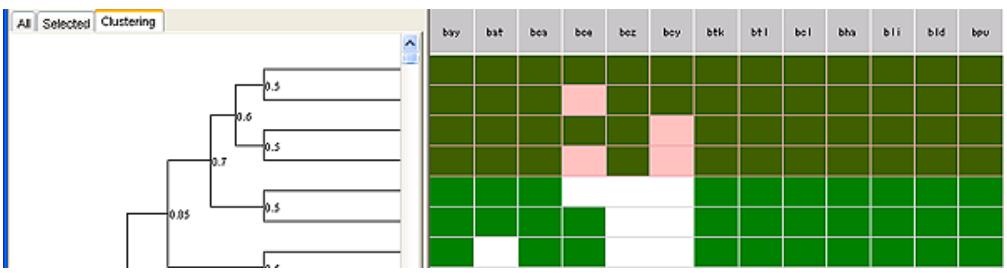
On the clustering tree, the display of distance can be switched on/off, and by clicking a branch point, clusters belonging to points not higher than the branch point can be selected.

To switch on the display of distance, do the following:

1. Click the right mouse button on the **Clustering** tab and click and check **Show Distance** to display the distance on the clustering tree.

To select a cluster not higher than a given branch point:

1. On the **Clustering** tab, click a point near the desired branch point of the clustering tree to select the clusters not higher than the clicked branch point.



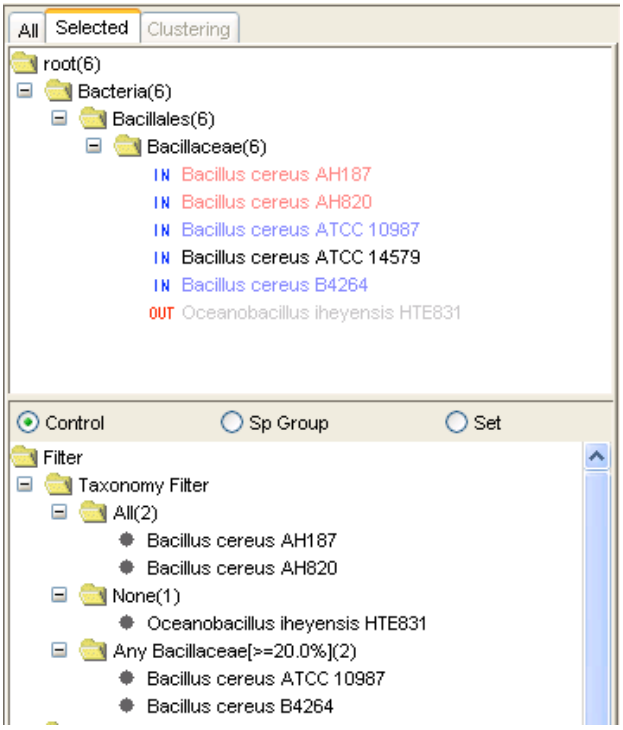
15. Taxonomy Filtering

Specify the filtering conditions on the Taxonomy Tree on the **Selected** tab to display on the PPM only the clusters with phylogenetic patterns that meet the conditions. In setting the filtering conditions, specify a set of species and conditions therefor at the same time. One of three sets of species listed in the table below can be specified. Of the three sets, 'All' and 'None' are special sets with fixed conditions, whereas for 'Any,' the conditions can be set freely. If simply specifying a condition of existence/nonexistence in a genome, use 'All'/'None,' respectively. Using 'Any,' more complicate conditions can be specified, such as 'Existence in more than half of each of bacteria and archaea.'

Species set	Display on the PPM	Species color
All	The clusters that are present in all the species in the set are displayed.	Light red
Any	The clusters that are present in more/less than a certain number/percentage of species in the set are displayed.	Light blue
None	The clusters that are absent in all species in the set are displayed.	Grey

15.1. Displaying the taxonomy filtering conditions

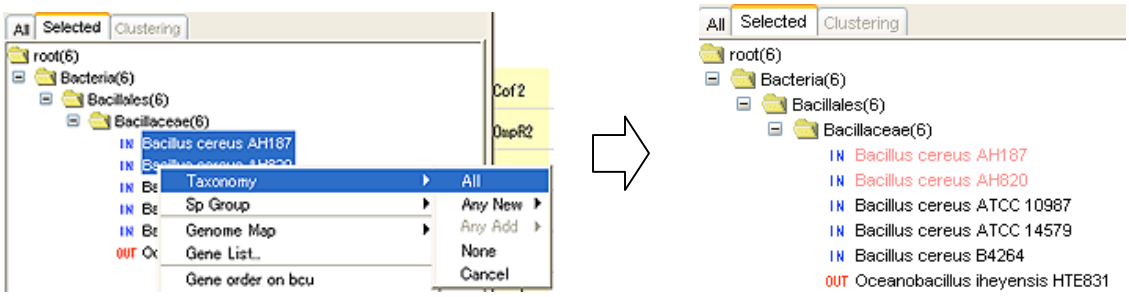
The taxonomy filtering conditions are displayed in **Filter – Taxonomy Filter** on the control panel.



15.2. Setting the 'All' conditions

1. Select the species on the Taxonomy Tree in the upper part of the **Selected** tab.

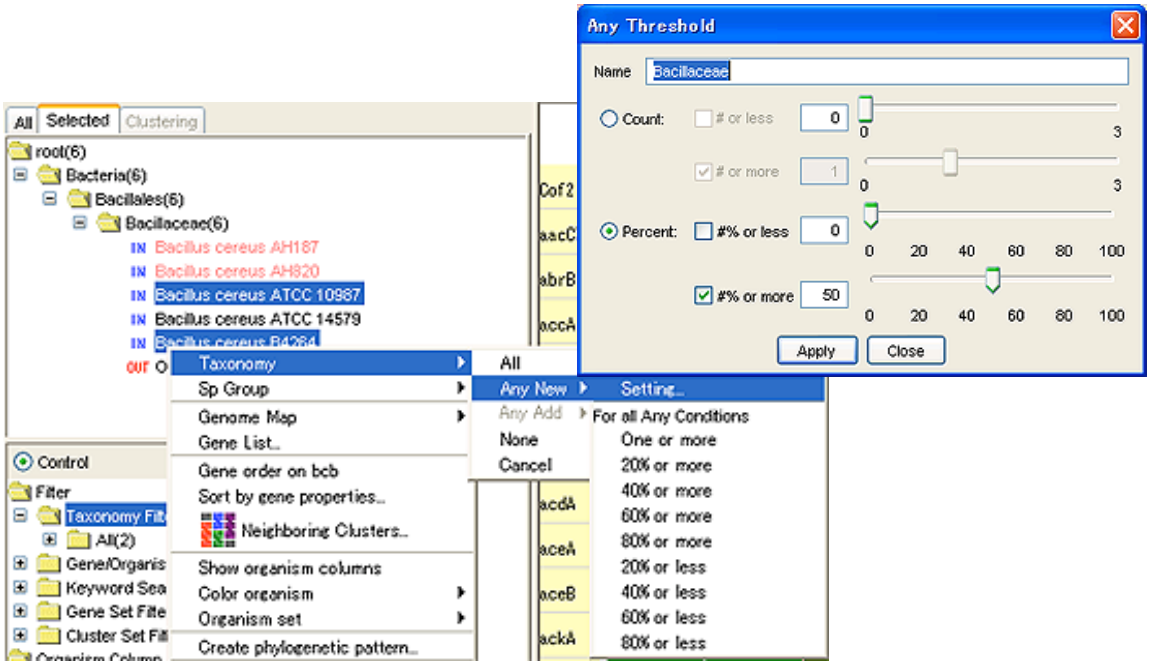
2. Click the right mouse button and click **Taxonomy** – **All** to display the registered species in **Filter** – **Taxonomy Filter** – **All** on the control panel and to display the relevant species names on the tree in light red.



15.3. Setting the 'Any' conditions

1. Select two or more species names on the Taxonomy Tree in the upper part of the **Selected** tab.

2. Click the right mouse button and click **Taxonomy** – **Any New** – **Setting…** to display the Any Threshold screen.



3. Specify the 'Any' conditions on the Any Threshold screen.

- **Count**: Specify the conditions for the number of species in a cluster.
  - ✧ # or less: The number of species is lower than or equal to #.
  - ✧ # or more: The number of species is higher than or equal to #.

  Example 1: The conditions [# or more: 3, # or less: 5] are met if the number of species in a cluster is 3 or higher *and* 5 or lower.

  Example 2: The conditions [# or more: 5, # or less: 3] are met if the number of species in a cluster is 5 or higher *or* 3 or lower.

  (Note the use of *and* and *or* in the two examples.)

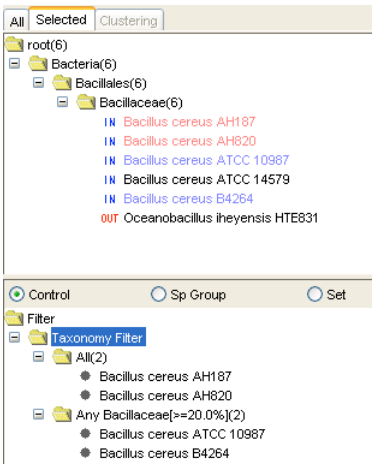
- **Percent**: Specify the conditions for the percentage of species in a cluster.
  - ✧ # or less: The maximum number of species accounts for #% of all.
  - ✧ # or more: The minimum number of species accounts for #% of all.

  Example 1: The conditions [# or more: 30, # or less: 50] are met if a species in a cluster accounts for between 30-50%.

  Example 2: The conditions [# or more: 50, # or less: 30] are met if a species in a cluster accounts for 50% or more or 30% or less.

4. After specifying the 'Any' conditions, click the **Apply** button on the Any Threshold screen to display the 'Any' conditions in **Filter – Taxonomy Filter – Any** on the control panel. Also, the species names meeting the 'Any' conditions are displayed on the tree in light blue.

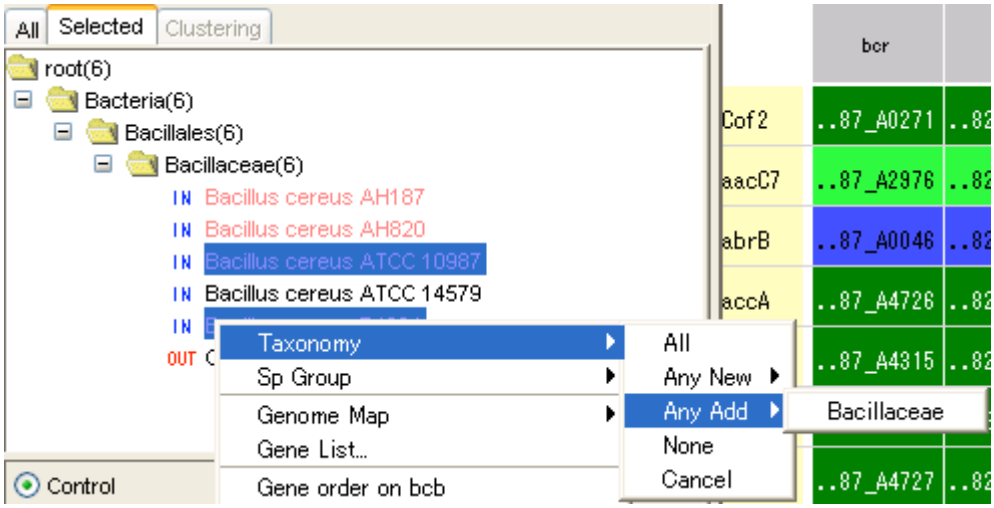
   A name can be attached to each of the 'Any' conditions in order to distinguish between the conditions.



5. To specify the 'Any' conditions more easily, select more than one species name, click the right mouse button and click **Taxonomy – Any New - For all any conditions: 'Any'**

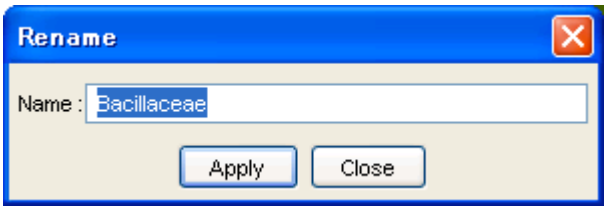
condition shown below to display in the lower view the species names registered for the 'Any' conditions.

6. To add species to the 'Any' conditions, select the species name or names, click the right mouse button and click **Taxonomy – Add – Any Add – (Additional 'Any' condition)**. The relevant species are added to the 'Any' conditions in the lower view and the relevant species names are displayed on the tree in light blue.



15.4. Changing the names of the 'Any' conditions

1. On the control panel, select **Filter – Taxonomy Filter – (name of 'Any' condition),** click the right mouse button and click **Rename** to display the Rename screen.



2. On the Rename screen, edit the name, and click the **Apply** button.

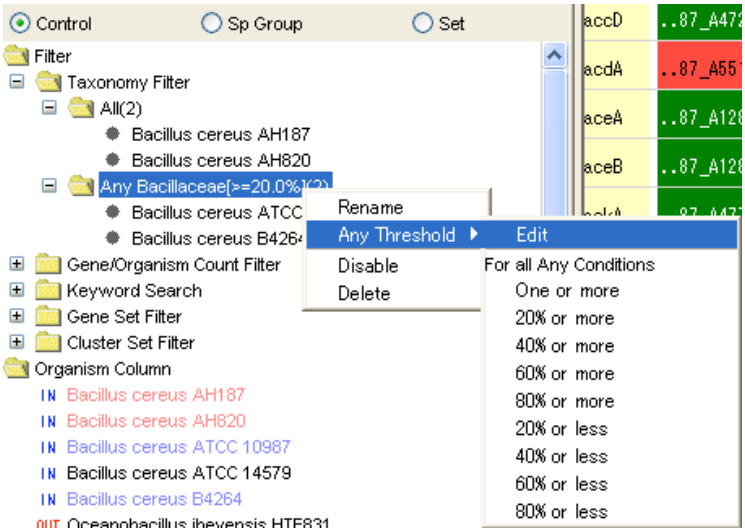
15.5. Changing the threshold value of the 'Any' conditions

1. On the control panel, select **Filter** – **Taxonomy Filter** – **(Name of 'Any' condition)** to display the conditions for phylogenetic pattern filtering.

2. From among the conditions for phylogenetic pattern filtering, select the 'Any' condition, click the right mouse button and click **Any Threshold** – **Edit** to display the Any Threshold screen.

3. On the Any Threshold screen, specify the 'Any' conditions.
   For details, refer to Item 3 of "15.2 Setting the 'Any' conditions."

4. To specify the 'Any' conditions more easily, select and click 'Any', click the right mouse button and click **Taxonomy** – **Any New** – **For all any conditions: 'Any' condition shown below**. The 'Any' conditions are changed to the clicked 'Any' conditions.

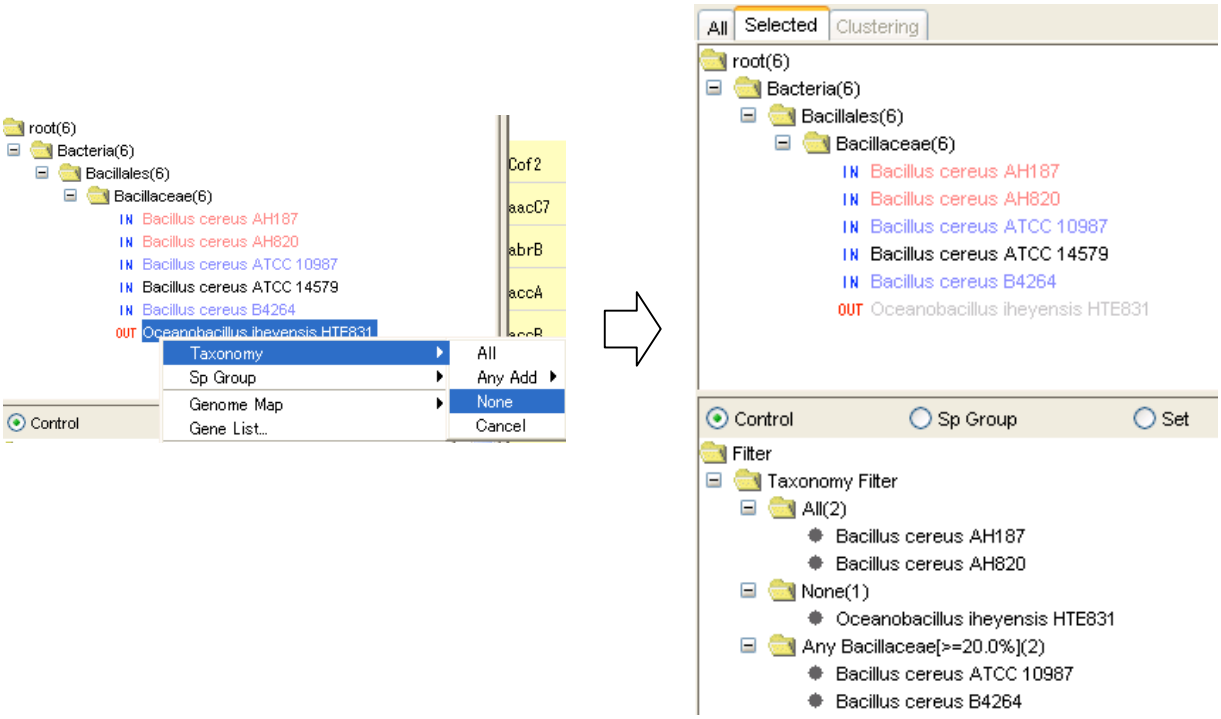


15.6. Setting the 'None' conditions

1. Select the species on the Taxonomy Tree in the upper part of the **Selected** tab.

2. Click the right mouse button and click **Taxonomy** – **None** to display the registered species names in **Filter** – **Taxonomy Filter** – **None** on the control panel. The relevant species

names are displayed on the tree in grey.



15.7. Enable/Disable conditions

1. In **Filter** – **Taxonomy Filter** on the control panel, select the conditions, click the right mouse button and click **Enable/Disable** to enable/disable the selected conditions.

15.8. Removal of conditions

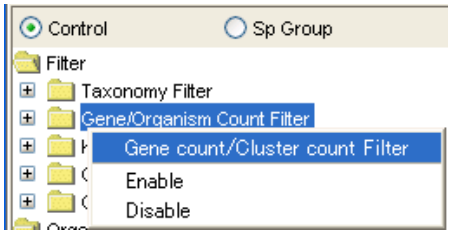
1. Select the conditions in **Filter** – **Taxonomy Filter** on the control panel, click the right mouse button and click **Delete** to remove the selected conditions.

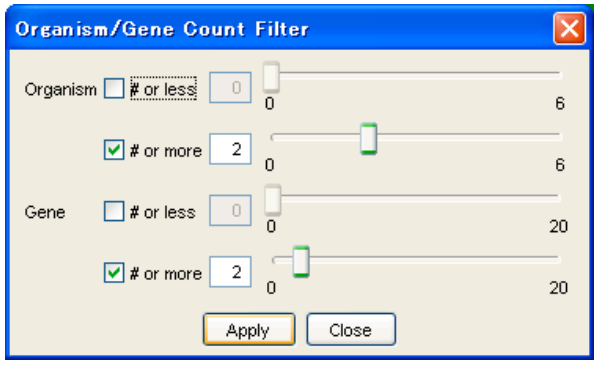
16. Filtering by Gene Count/Species Count in the Phylogenetic Patterns

Set a threshold value for the gene count/species count in the phylogenetic patterns and filter the data based thereon. The results are displayed on the PPM.

16.1. Setting the conditions

1. Select **Filter** – **Gene/Organism Count Filter** on the control panel, click the right mouse button and click **Gene/Organism Count Filter** to display the Gene/Organism Count Filter screen.





2. Specify the conditions on the **Gene/Organism Count Filter** screen, and click the **Apply** button to display the conditions in **Filter** – **Gene/Organism Count Filter** on the control panel.

16.2. Enable/Disable conditions

1. Select **Filter** – **Gene/Organism Count Filter** on the control panel, click the right mouse button and click **Enable/Disable**.

17. Keyword Search

Regarding the cluster results, both clusters and genes can be searched. The gene search is conducted in concert with the RECOG server.

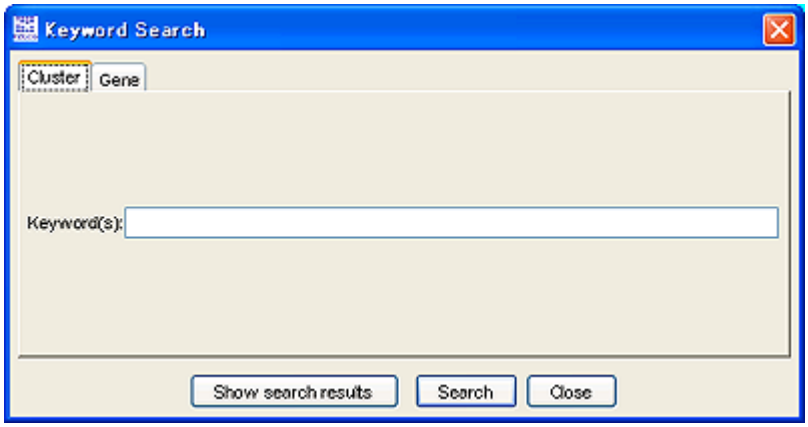
	Search of clusters	**Search of genes**
Search target	・Representative gene name ・Representative description of each cluster	・Gene properties provided by the RECOG server (description, gene name, *etc*.) ・Gene/cluster properties registered through the use of the import function, *etc*.

17.1. Search of clusters

1. Click  (**Keyword Search**) in the **Toolbox** to display the Keyword Search screen.

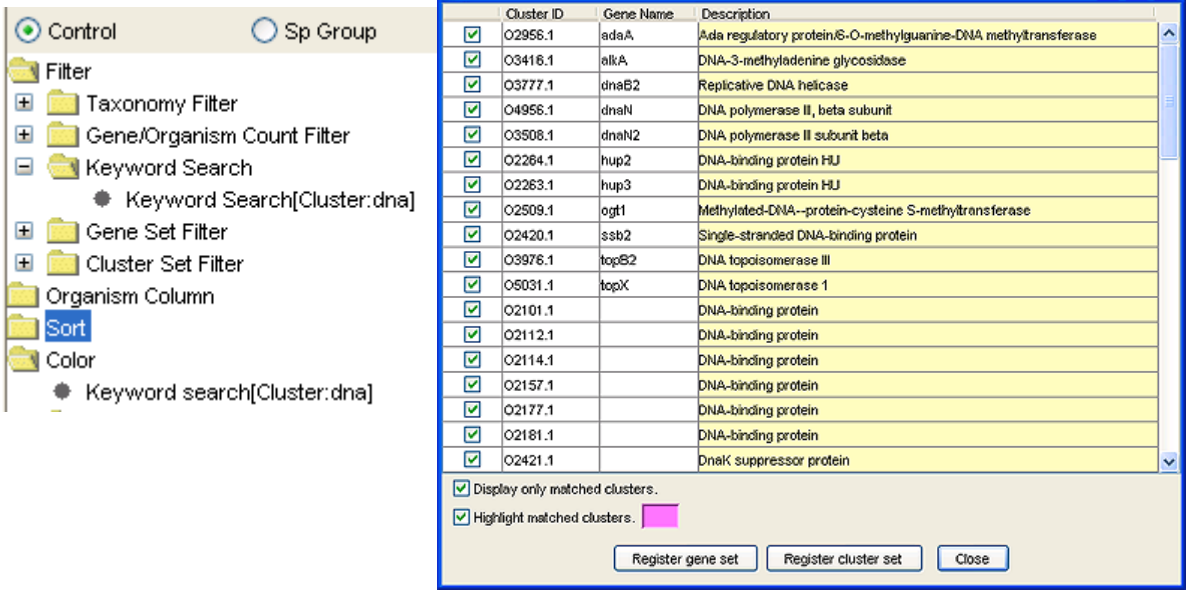
2. On the Keyword Search screen, click the **Cluster** tab.

3. Enter the keyword(s) in the **Keyword(s)** column and click the **Search** button to begin the search.



4. Upon the completion of the search process, the Keyword Search Result screen is displayed. The conditions corresponding to the search results are displayed in **Filter** - **Color** on the

control panel. On the PPM, only the searched clusters are highlighted.



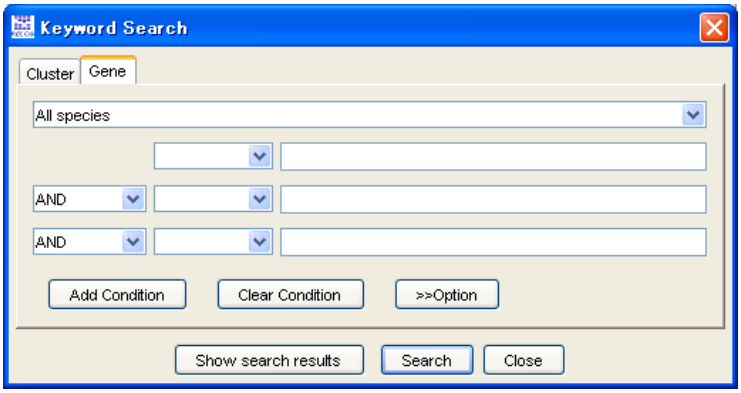
5. Check 'Display only matched clusters' on the Keyword Search Result screen to display on the PPM only the searched clusters. Upon unchecking, all the clusters are displayed.

   Check 'Highlight matched clusters' to highlight the searched clusters. Upon unchecking, and the highlight is cancelled. Also, by clicking the color-setting column, the color of the highlight can be set.

17.2. Search of genes

1. Click  (**Keyword Search**) in the **Toolbox** to display the Keyword Search screen. On the Keyword Search screen, click the **Gene** tab.

2. Specify an item and a keyword, and click the **Search** button.
   To specify multiple conditions, click the **Add Condition** button to add conditions.
   To clear a condition, click the **Clear Condition** button.
   Upon specifying the condition(s), click the **Search** button.



For the search, the following marks can be entered in the keyword entry column:

Type of search	Example	
Match search	Word	Genes that include a phrase matching 'word' are searched**.
Partial match search	* word *	Genes that include the phrase '～word～' are searched.
Prefix search	word *	Genes that include the phrase 'word～' are searched.
Suffix search	* word	Genes that include the phrase '～word' are searched.
Or more	>=10	Ten or more genes are searched. #
Or less	<=10	Ten or less genes are searched. #
More than	>10	More than ten genes are searched. #
Less than	<10	Less than ten genes are searched. #
With keyword inside	+ABC	Genes with 'word' inside are searched.
Without keyword inside	-word	Genes without 'word' inside are searched.
Multiple word search	word1 word2	Genes that include word1 or word2 are searched.
Phrase search	"word1 word2"	Genes that include word1 and word2 as a phrase are searched.

** In the 'Description' field, a partial match search is conducted.

The inequality sign is valid only for numerical-type gene properties.

3. Click the **Option** button to specify the following conditions:

   - **'Search on the server'**

     If this is checked, the gene properties retrievable on the RECOG server are searched on that server. If unchecked, all the gene properties are searched locally.

   - **'Representative value of multiple values set for a gene property'**

     If **Value** and **Difference** are specified, and if multiple values are set for a gene property of a gene, the method of applying the search conditions is specified.

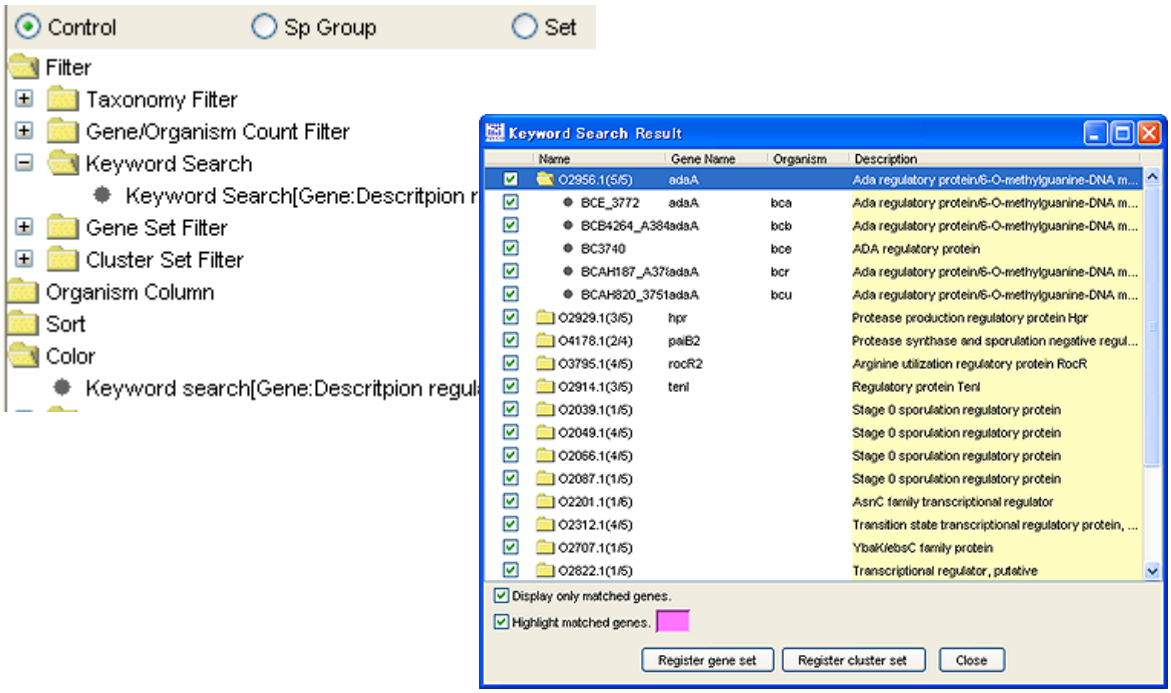
     ✧ **At Least One**: If at least one of the multiple values meets the condition, it is considered that the search condition is met.

     ✧ **All**: If all the multiple values meet the condition, it is considered that the search condition is met.

     ✧ **Average**: If the mean value of the multiple values meets the condition, it is considered that the search condition is met.*

     * This applies only to numerical-type gene properties.

4. Upon the completion of the search process, the Keyword Search Result screen is displayed. In **Filter - Color** on the control panel, the conditions corresponding to the search result are displayed. On the PPM, only the clusters containing the searched genes are highlighted.



3. Check 'Display only matched clusters' on the Keyword Search Result screen to display and only the searched clusters on the PPM. Upon unchecking, all the clusters are displayed.

   Check 'Highlight matched clusters' to highlight the searched clusters. Upon unchecking, the highlighting is cancelled. Click the color-setting column to set the highlight color.

17.3. Redisplaying the search results

1. Click  (**Keyword Search**) in the **Toolbox** to display the Keyword Search screen.

2. On the Keyword Search screen, click the **Show search results** button to display the last search results.

17.4. Enable/Disable filter settings by the search results

1. Select **Filter** – **Keyword Search** on the control panel, click the right mouse button, and click **Enable/Disable**.

   The same operation can be carried out by checking/unchecking 'Display only matched clusters' on the Keyword Search Result screen.

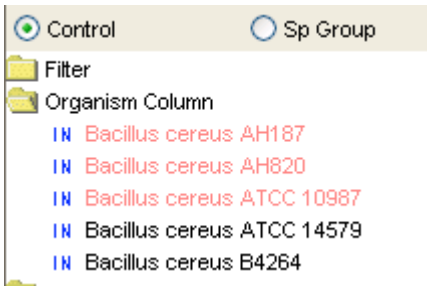
17.5. Enable/Disable color settings by the search results

1. Select **Color** – **Keyword Search** on the control panel, click the right mouse button, and click **Enable/Disable**.

   The same operation can be carried out by checking/unchecking 'Highlight matched clusters' on the Keyword Search Result screen.

18.Changing the Display Order of Species or Display/Nondisplay Status of Species

In the **Organism Column** on the control panel, the order of species displayed on the PPM can be changed or the display/nondisplay of species can be set.



18.1. Changing the order of display of species

1. To change the order of display of species, drag the species in the **Organism Column** on the control panel to the destination.
   On the PPM, only the species displayed on the Organism Column are displayed.

18.2. Setting the display/nondisplay of species

1. Double-click the species in the **Organism Column** on the control panel.

   You can also click the right mouse button on the species and choose **Show/Hide** to set the display/nondisplay modes.

18.3. Adding species to be displayed

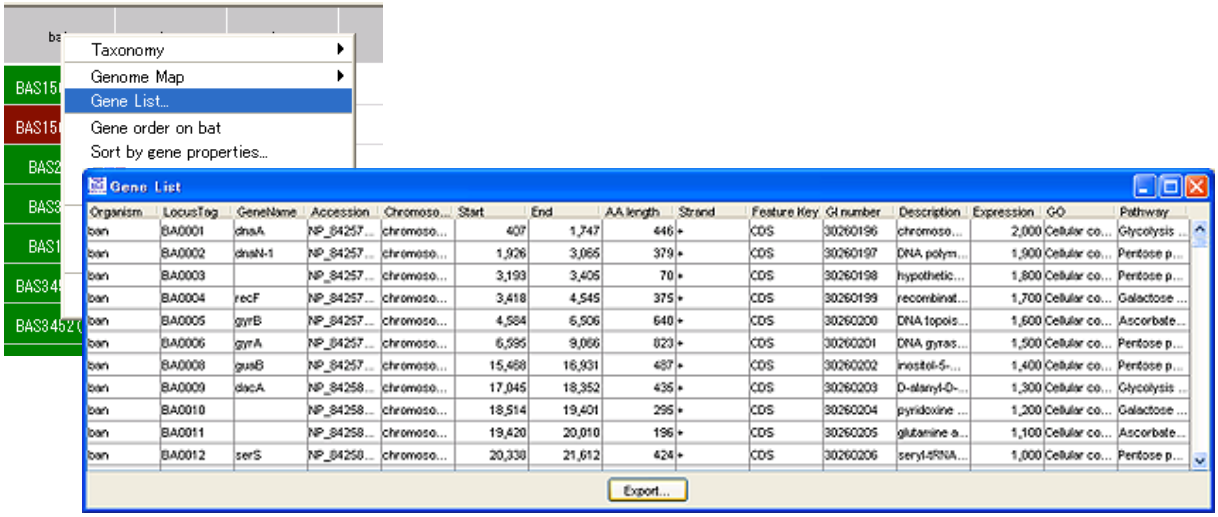
1. To display the selected species on the PPM, select the species on the Taxonomy Tree on the **Selected** tab, click the right mouse button and click **Show organism columns**.

18.4. Removing displayed species

1. Select species in the **Organism Column** on the control panel, click the right mouse button and click **Delete organism columns**. When the warning message is dislayed, click the **OK** button.

19. List of Genes

A list of genes of the selected species is displayed.



19.1. Displaying the list of genes

1. To display the Gene List screen, select the species on the Taxonomy Tree on the **Selected** tab, click the right mouse button and click **Gene List…** on the pop-up menu. You can also click the right mouse button on an abbreviated species name in the header row of the PPM table to open the Gene List screen.

19.2. Sorting the list of genes

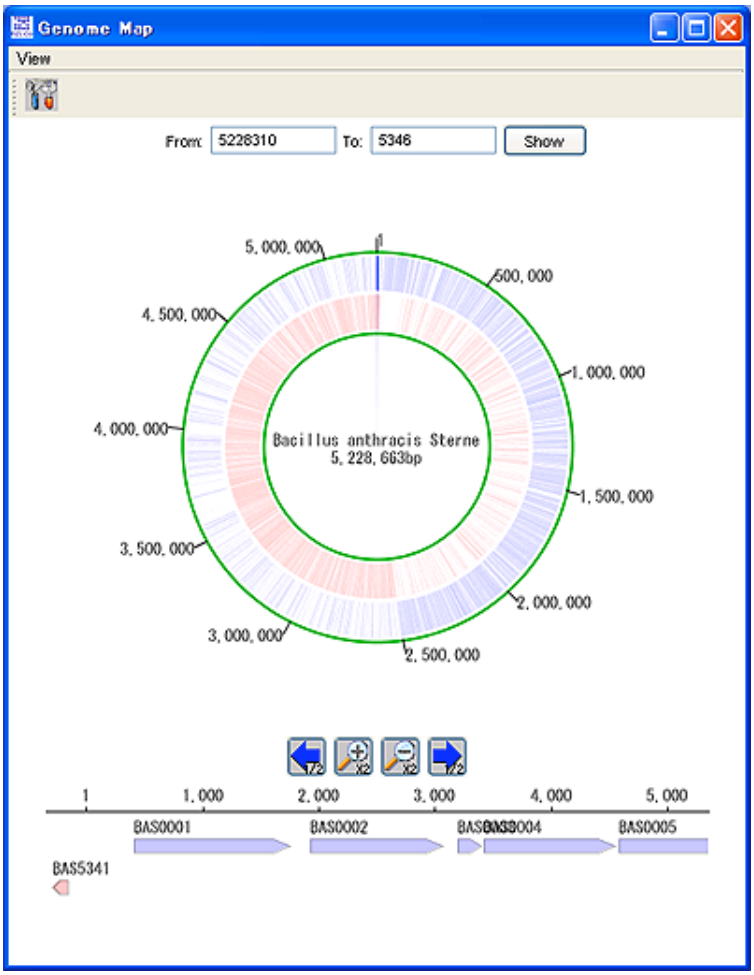
1. To sort the gene properties in the ascending/desceding order and display the gene property values, click the gene property name on the Gene List screen.

19.3. Saving the list of genes

1. To output the list of gene property values in tab-delimiting format, click the **Export…** button on the Gene List screen to display the Save gene list screen, enter the output file name and click the **OK** button.

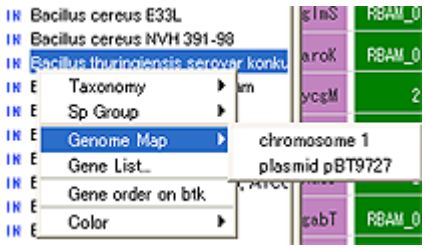
20. Display and Operation of the Circular Genome Map (CGM)

The Circular Genome Map (CGM) draws a circular/linear genome map of the selected species.



20.1. Displaying the CGM

1. To display the CGM, select the species on the Taxonomy Tree on the **Selected** tab, click the right mouse button and click **Genome Map - Chromosome name**.



20.2. Changing the selected region

1. Enter the region to be selected in **From** and **To** in the upper part of the CGM screen, and click the **Show** button. The entetred region is highlighted and the displayed region of the genome map in the lower part of the CGM screen changes.
   The selected region can also be changed by dragging the mouse on the circular genome.

2. To move the selected region in the clockwise/counterclockwise direction, click the  (**Previous**)/  (**Next**) button in the lower part of the CGM screen, respectively. It is also possible to change the moving distance on the pop-up menu upon right-clicking the button.

3. To zoom in/zoom out on the selected region, click the  (**Zoom in**)  (**Zoom out**) button in the lower part of the CGM screen, respectively. By right-clicking on the button, the zoom ratio can be changed on the pop-up menu.

20.3. Linkage between the PPM and CGM

1. Click a gene on the genome map in the lower part of the CGM screen to select on the PPM the cluster to which the clicked gene belongs.

2. Click a cell on the PPM to highlight the location of the clicked gene on the circular genome on the CGM. Also, the displayed region of the lower genome map changes and the clicked gene is displayed.

3. Click the header in the upper part of the PPM to change the genome displayed on the CGM. You can compre the locations on the chromosome of the genes in a given cluster between genomes by selecting a cluster and switching the displayed genome one after another.

20.4. Changing the color of genes

1. Click  (**Option**) in the **Toolbox** on the CGM screen to display the Genome Map Options screen.

2. Specify the gene color.

- **'Function Category'**

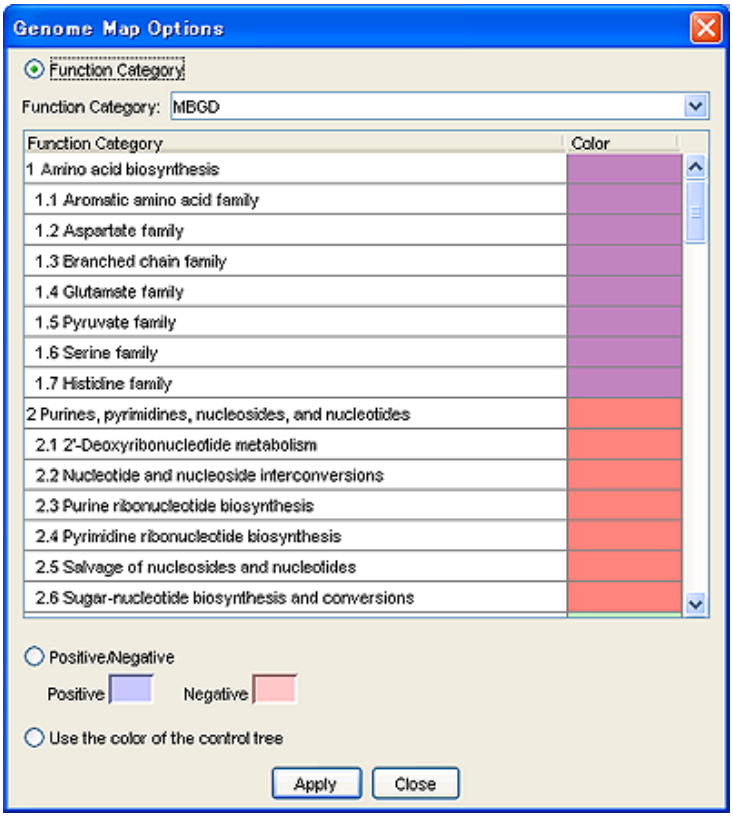
  The gene is displayed in the color corresponding to the representative function category of the cluster to which the gene belongs.

- **'Positive/Negative'**

  The gene is displayed in the color corresponding to the direction of the gene.

- **'Use the color of the control tree'**

  The gene is displayed in the color specified by the user in **Color** on the control panel.



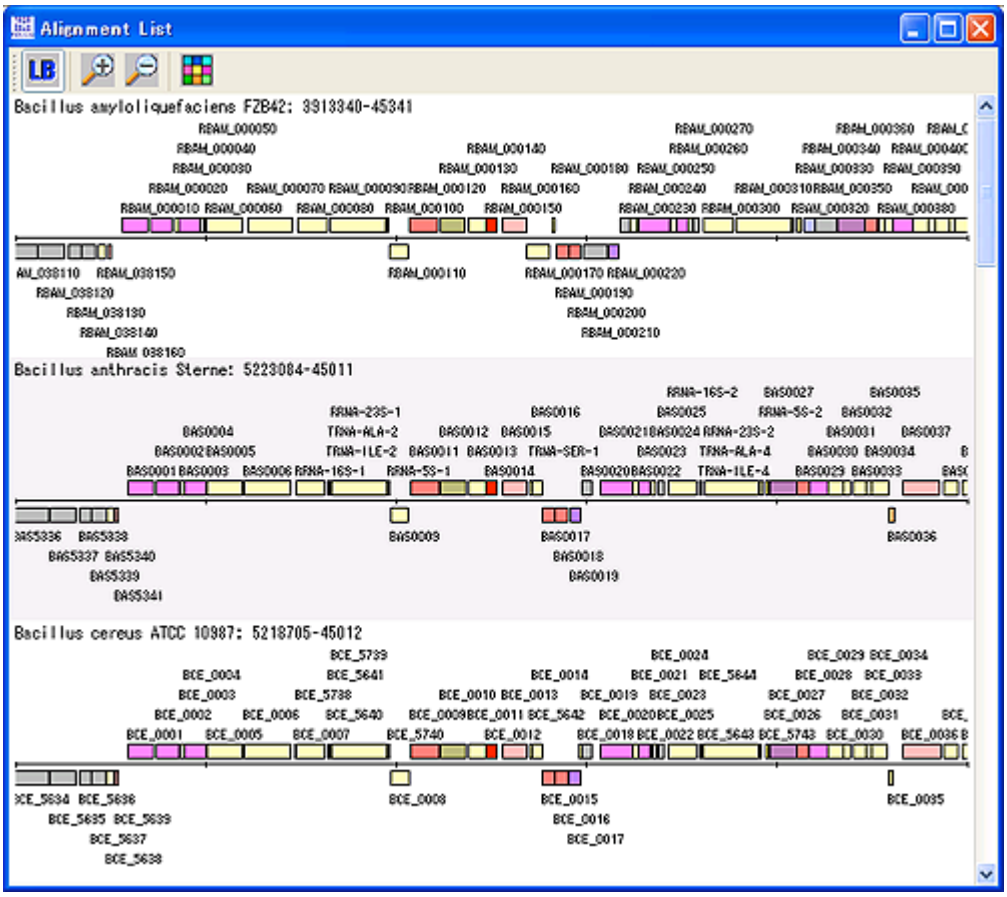
20.5. Displaying gene information in a browser

1. Double-click a gene on the genome map in the lower part of the CGM screen to display the information on the default external resource specified in '35. **External Resource URL Management**' in the browser.

   Click the external resource URL displayed upon right-clicking, and the information on the external resource is displayed in the browser.

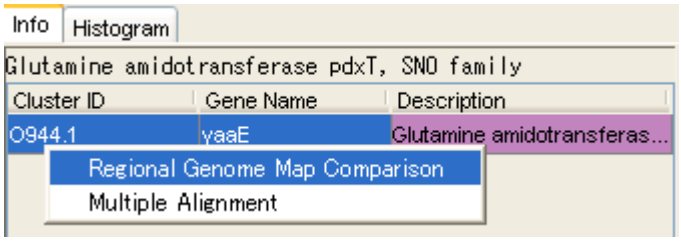
21. Display and Operation of the Regional Genome Map（RGM）

To allow users to compare the chromosome map around the genes belonging to a specified ortholog cluster, the Regional Genome Map (RGM) displays the genome map, where these genes are arranged at the center.



21.1. Displaying the RGM

1. To display the Regional Genome Map (RGM) screen, select a cluster on the **Info** tab, click the right mouse button and click **Regional Genome Map** on the pop-up menu.



21.2. Zooming in/out on the RGM

1. To zoom in/out on the RGM, click  (**Zoom in**)/  (**Zoom out**) in the **Toolbox**, respectively, on the Regional Genome Map screen.

21.3. Display/Nondisplay of the Locus Tag

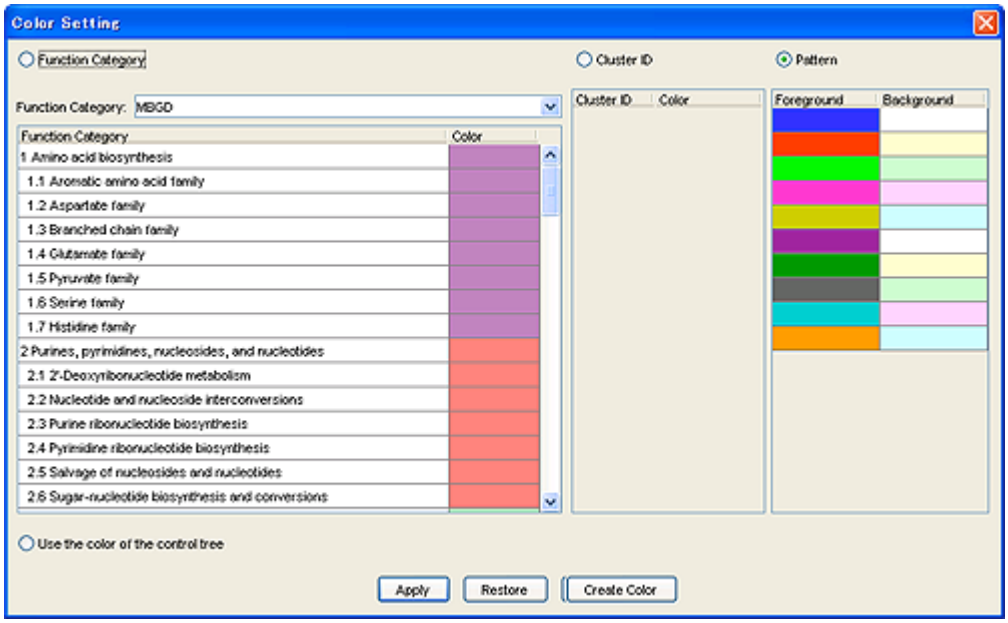
1. Click  (**Label ON/OFF**) in the **Toolbox** on the Regional Genome Map screen. When the button is displayed in color, the Locus Tag is displayed, and when the button is displayed in grey, the Locus Tag is hidden.

21.4. Setting the gene color

1. To display the Color Setting screen, click  (**Color Setting**) in the **Toolbox** on the Regional Genome Map screen.
2. Set the gene color on the Color Setting screen.

Sort	Display on the PPM
Function Category	The color allocated to the current Function Category is used.
Cluster ID	A unique color is allocated to each cluster. The color is allocated according to the gradation based on the cluster ID. Although it is difficult to distinguish between the colors, the allocation remains the same even if the dsplayed region changes.
Pattern	A unique color and pattern are allocated to each cluster. The color and pattern are allocated to each currently displayed cluster in the order of cluster size. Although the color and pattern are easily distinguishable, the allocation changes with the displayed region.
Use the color currently assigned on the PPM	The color currently in use on the PPM (the color set in **Color** on the control panel) is used.

3. Click the **Apply** button.



21.5. Displaying gene information in a web browser

1. By double-clicking a gene, you can display in a web browser the informationof the default external resource that is set according to "35. External Resource URL Management." You can also click the right mouse button on a gene and choose an external resource URL to display.

22. Multiple Alignment and Phylogenetic Tree

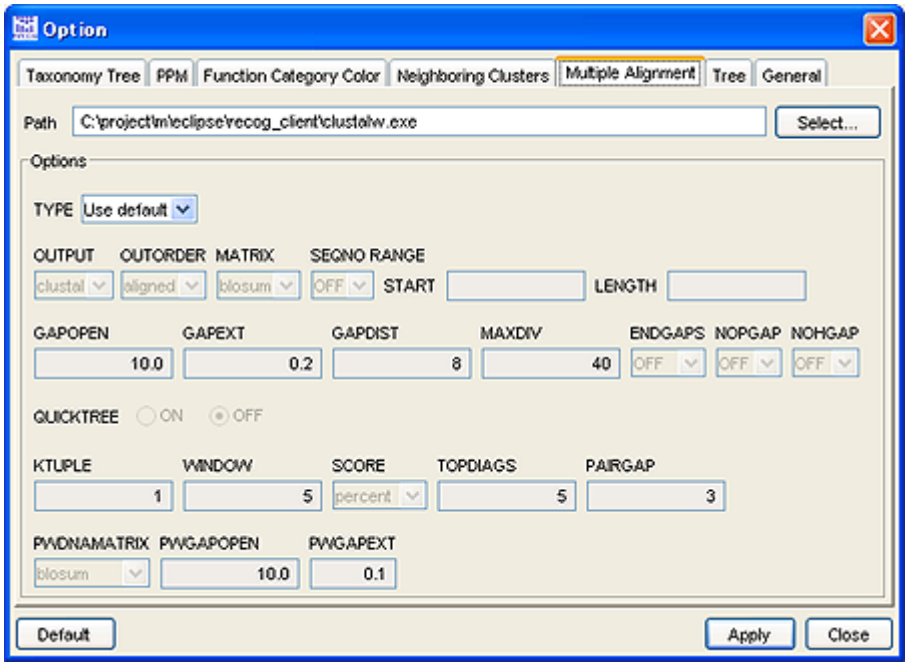
Multiple alignment among genes belonging to a cluster can be created using the ClustalW program. Also, a phylogenetic tree can be created and displayed based on the results of the multiple alignment.

22.1. Execution of multiple alignment

(Note) Multiple alignment can be executed only in an environment where Internet connection is available.

1. Specify the execution parameters of ClustalW.

   Clicking  (**Option**) in the **Toolbox** to display the Option screen, and specify the ClustalW conditions on the **Multiple Alignment** tab on the Option screen.



- Path

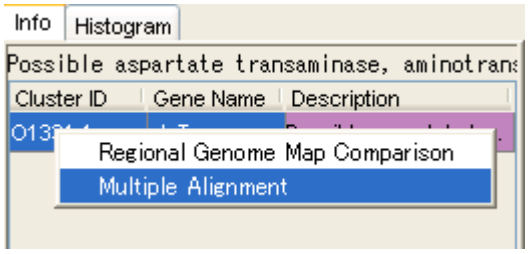
  Specify the path of ClustalW. The default setting is the ClustalW attached to the installer.

- TYPE
  - ・Use the default:

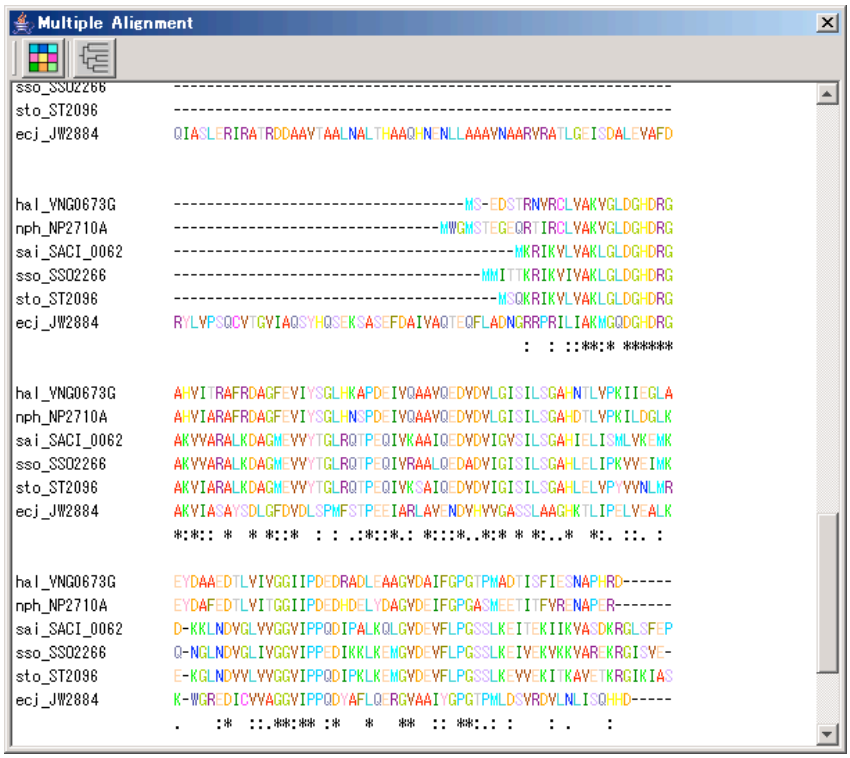
    Multiple alignment is executed with the default parameter of ClustalW.
  - ・Protein:

    Multiple alignment is executed as per the setting specified on the screen.

2. In the **Disaggregate Mode**, select the cluster in the cluster information display table on the **Info** tab, click the right mouse button, and click **Multiple Alignment** on the pop-up menu to display the progress screen and execute the multiple alignment. When the Confirm screen is displayed, click the **OK** button.

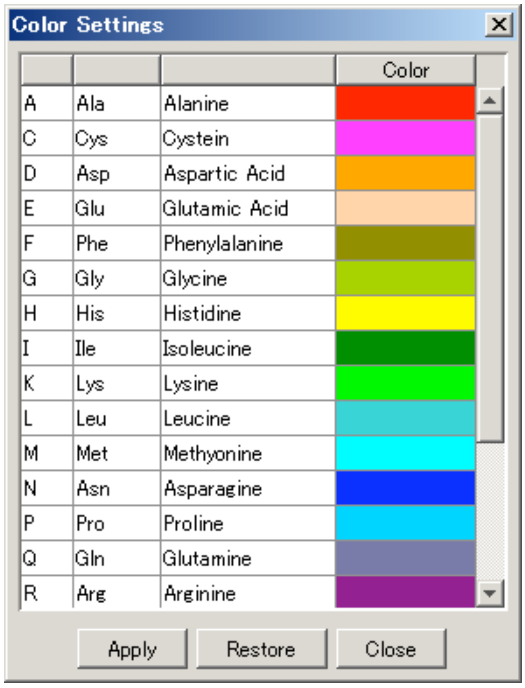


3. After the completion of the multiple alignment process, the Multiple Alignment screen is displayed.



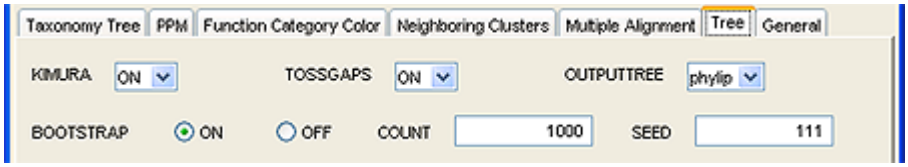
22.2. Changing the colors of the amino-acid letter strings

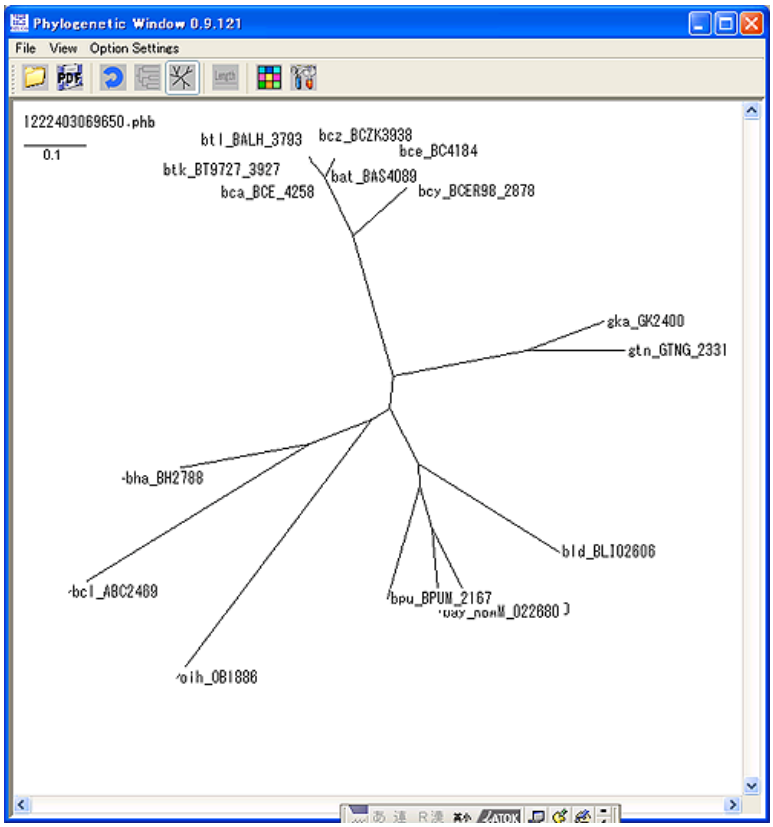
1. Click the  (**Color Setting**) button on the Multiple Alignment screen to display the Color Setting screen.
2. On the Color Setting screen, you can specify the colors of the amino-acid letter strings.



22.3. Displaying the phylogenetic tree

1. To display the Phylogenetic Tree screen, click  (**Clustal Tree**) on the Multiple Alignment screen.
2. To change the parameters used in the creation of the phylogenetic tree, click  (**Option**) in the **Toolbox**. Specify the parameters on the **Tree** tab on the Option screen.





3. On the Phylogenetic Tree screen, the following operations are possible:

- Reading the dnd files:

  Click  (**Open DND file**), select the dnd file and click the **OK** button.

- Outputting the phylogenetic tree to a PDF file:

  Click  (**PDF**), specify destination and file name, and click the **OK** button.

- Switching between the rooted phylogenetic tree and the unrooted phylogenetic tree:

  To display the rooted phylogenetic tree, click  (**Rooted Horizontal**), and to display the unrooted phylogenetic tree, click  (**Unrooted**).

- Displaying the distance:

  Click  (**Show Length ON/OFF**).

- Various settings:

  Click  (**Option Settings**).

  ✧ **Directory** tab

  Set the default directory for loading DND files and the defaut directory for outputting PDF files.

  ✧ **Style**

  Specify the node style.

  ✧ **Color**

  Specify the **Locus Tag** colors.

  ・Species

  Each species is displayed by the species color (the color of the species header in the PPM table) specified in the **Color Organisms** menu on the **Texonomy Tree** in the upper part of the **Selected** tab.

  ・OUT / IN Group

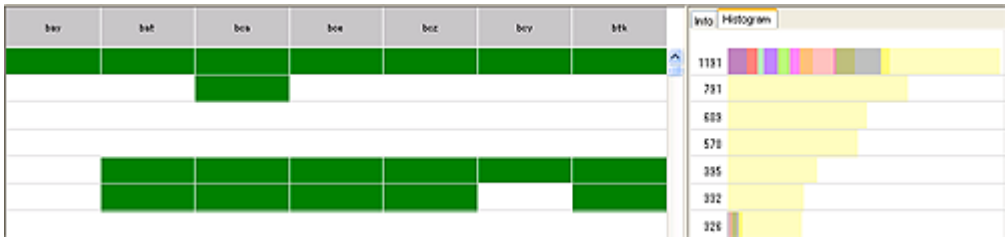
  Each species is displayed by the ingroup/outgroup color specified in **Font Color** on the **Taxonomy Tree** tab on the Option screen, according to the current ingroup/outgroup specification.

23. Function Category Frequency Graph/ Numerical Data Graph

On the **Histogram** tab, the frequency of the function category in the same phylogenetic pattern can be displayed by graphs, or the numerical data of the specified gene properties can be displayed by bar graphs.

23.1. Function category frequency graphs

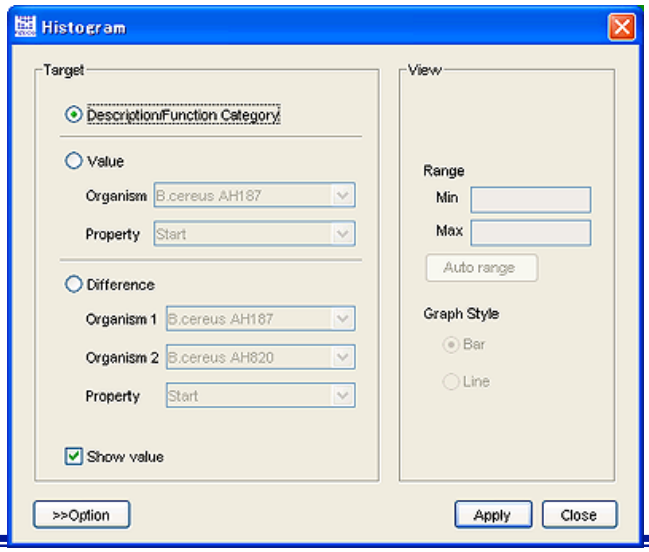
In the **Disaggregate Mode**, the frequency of each function category in the same phylogenetic pattern is displayed in a graph on the **Histogram** tab.



23.2. Displaying a numerical data graph, a description or the function category

In the **Disaggregate Mode**, a graph of numerical gene property values or the description and the function category of each cluster is displayed on the **Histogram** tab.

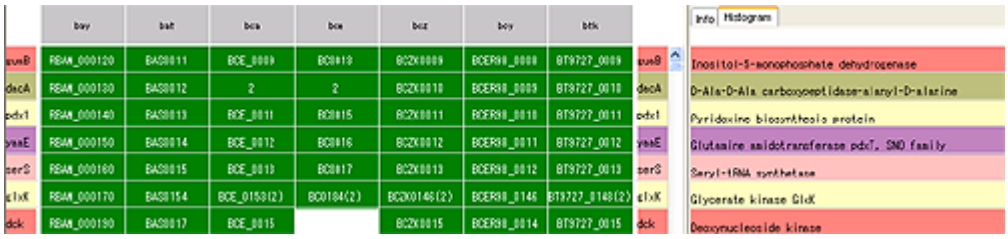
1. To display the Histogram screen, click  (**Histogram**) in the **Toolbox**.



2. Specify the graph to be displayed on the **Histogram** tab.

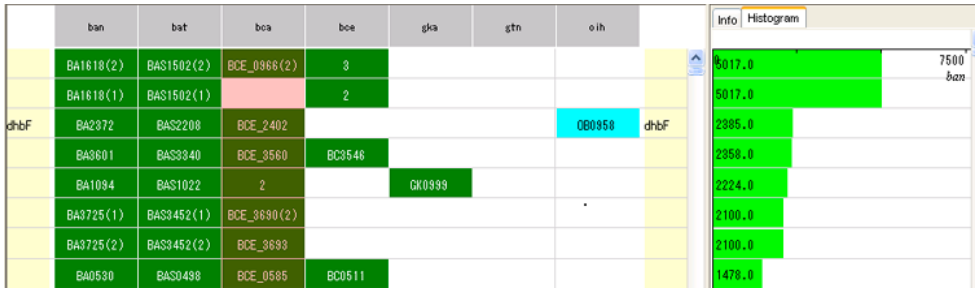
- **Description/Function Category**

  The description of the cluster is displayed with the color of the function category of the cluster in the background.



- **Value**

  The specified numerical property data are displayed by a bar graph. Cluster property data or gene property data of a specified species can be displayed.



- **Difference**

  The difference in the numerical gene property data between two species is displayed by a bar graph. Two species and their gene properties for comparison should be specified.

If **Value** and **Difference** are specified, the numerical data are displayed on the graph upon checking 'Show value.'

3. If **Value** and **Difference** are specified, specify also the display range and graph style in the 'View' column.

   - **Range**

     Specify the display range. To automatically specify the display range as between the minimum and the maximum values of the target property, click the **Auto range** button.

   - **Graph Style**

     Select one of the two graph styles, 'Bar' (bar graph) or 'Line' (line graph).

4. To set the following conditions, click the **Option** button.

   - Representative value of multiple property values for a gene

     If **Value** and **Difference** are specified, and if multiple values are set for a gene property of a gene, specify the method of determining the representative value for graph display.

     ✧ Min: The minimum value among multiple values is used.
     ✧ Max: The maximum value among multiple values is used.
     ✧ Median: The median among multiple values is used.
     ✧ Average: The mean value among multiple values is used.

   - Representative value of multiple genes in a cell

     If **Value** and **Difference** are specified, and if a cell contains multiple genes, specify the method of determining the representative value for graph display.

     ✧ Min: The minimum value among multiple genes is used.
     ✧ Max: The maximum value among multiple genes is used.
     ✧ Median: The median among multiple genes is used.
     ✧ Average: The mean value of the properties of multiple genes is used.*

5. To display the graph on the **Histogram** tab, click the **Apply** button on the Histogram screen.

* The numerical values displayed on the **Value** and **Difference** graph can be switched between display/nondisplay on the popup menu **Show value**, displayed upon right-clicking on the graph.

23.3. Switching between the display/nondisplay of the

Histogram tab

1. To display the **Histogram** tab on the right of the screen, choose and check from the menu **View - Information Pane**.

2. To hide the **Histogram** tab, uncheck **View - Information Pane**.

24. Clustering Neighborhood Genes

This function groups genes that are located in the vicinity of each other on the phylogenetic pattern map (PPM) table and in terms of the genome sequence, and assigns a color for each group.

24.1. Execution of the clustering of neighborhood genes

1. To display the Neighboring Clusters screen, click  (**Neighboring Clusters**) in the **Toolbox**.

2. Specify the conditions for neighborhood gene clustering on the Neighboring Clusters screen.

   - **Search range of clusters**

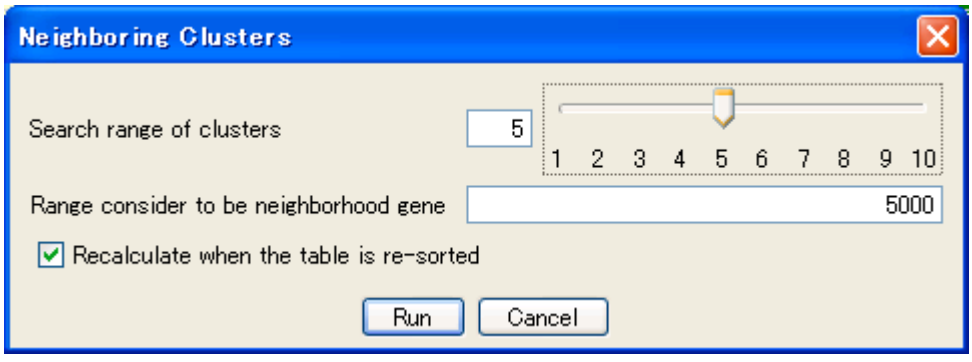
     Specify the range of clusters considered to be neighborhood on the PPM table. If the range specified is *N*, *N* clusters above and below each cluster are considered to be neighborhoods of that cluster.

   - **Range within which genes are considered to be in each other's neighborhood**

     Specify the distance between two genes on the chromosome considered to be in each other's neighborhood.

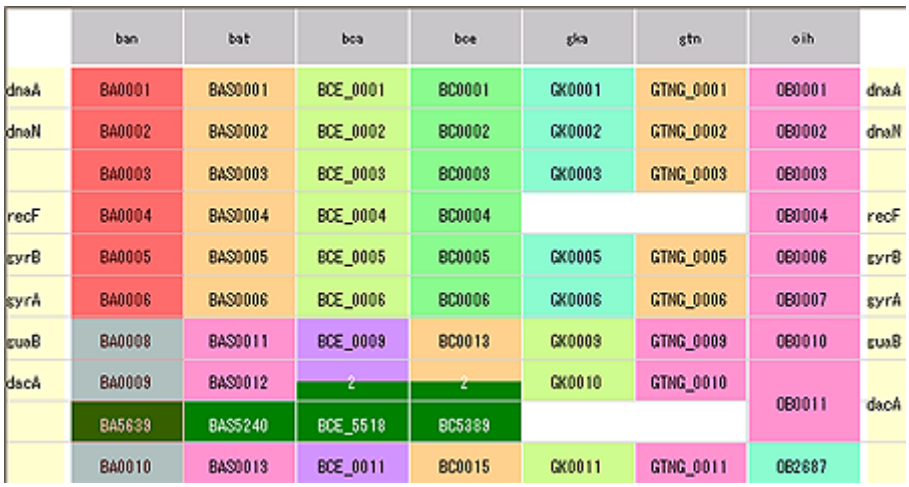
   - **Recalculate when the table is re-sorted**

     If the order of clusters is changed due to the sort, *etc*. of clusters on the PPM, and if this column is checked, neighborhood gene clustering is automatically executed under the conditions specified immediately before. If unchecked, the results of the neighborhood gene clustering are cleared after the order of clusters is changed.



3. To execute neighborhood gene clustering, click the **Run** button on the Neighboring Clusters screen.

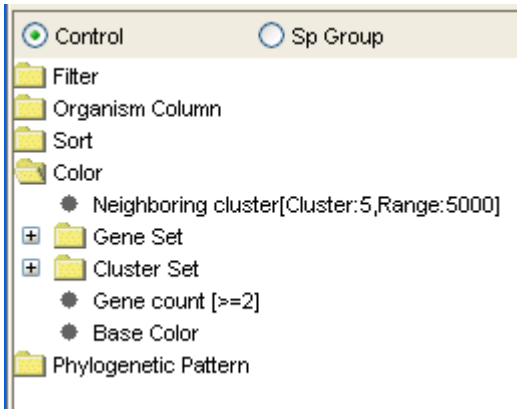
   Upon the completion of the process of neighborhood gene clustering, the group of genes considered to be in each other's neighborhood on the PPM is clustered and displayed in the same color. Also, **Neighboring cluster** is displayed in **Color** on the control panel.



In this figure, only the cells having the same color in the same neighborhood in the table on the same genome belong to a *neighborhood gene cluster*. Note that the same color may be used for different clusters; cells in different genomes or cells far away from each other are not related even if they have the same color in the table.

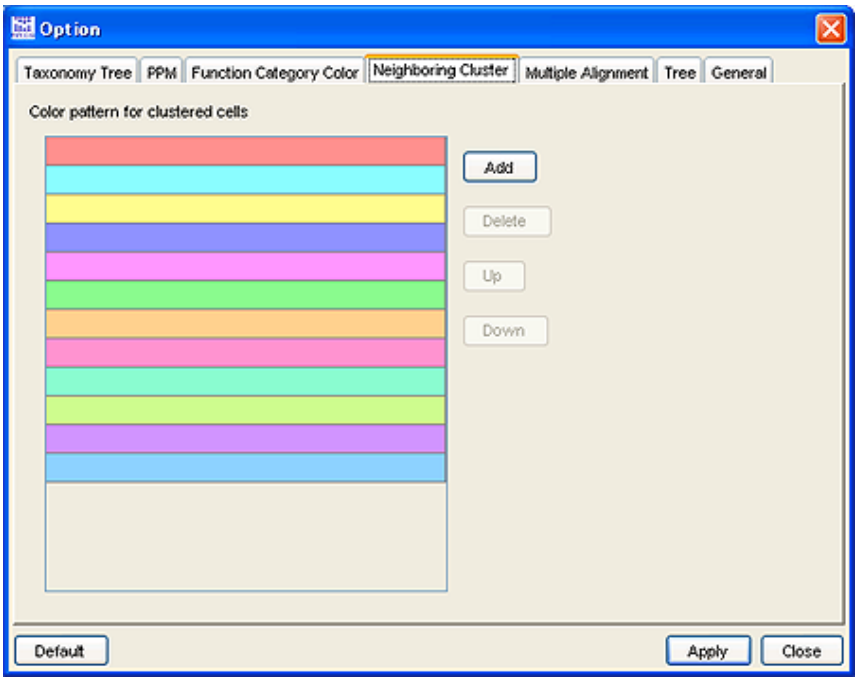
Display/Nondisplay of the clustering results

1. To switch between display/nondisplay, double-click **Color - Neighboring cluster** on the control panel.



24.2. Changing the color of a group of neighborhood genes

1. To display the Option screen, click  (**Option**) in the **Toolbox**. On the Option screen, click **Neighboring Cluster**.



2. Set the color pattern.

- **Color pattern for clustered cells**

  Set the color pattern for coloring genes clustered by neighborhood gene clustering. The color is assignmed to the neighborhood clusters in accordance with the order specified on the list. The assignment is repeated from the top of the list after reaching the end of the list.

  - **Add** button

    Add a color pattern.

  - **Delete** button

    Delete the color pattern selected from the list.

  - **Up** button/**Down** button

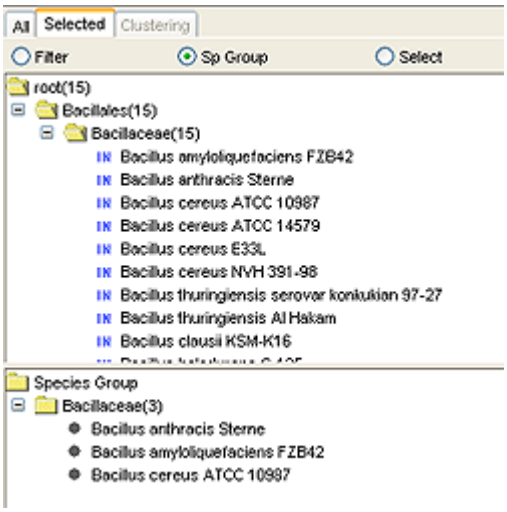
    Shift the selected color pattern upward/downward.

25. Species Groups

Multiple species closely related to each other can be registered as a species group. The species groups registered here can be used in the analysis such as the CoreAligner program.

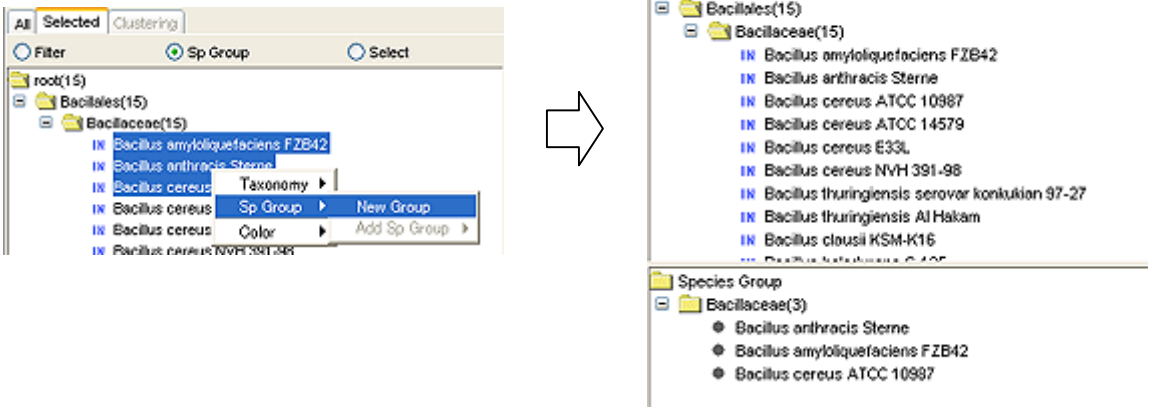
25.1. Displaying species groups

1. To display the species groups set in the lower view of the Taxonomy Tree, click the **Sp Group** button in the upper view of the Taxonomy Tree on the **Selected** tab.



25.2. Registration of species groups

1. To create a new species group with a specified set of species, select the species on the Taxonomy Tree in the upper view of the **Selected** tab, click the right mouse button, and choose **Sp Group - New Group**. The species group is displayed in the lower view,



25.3. Editing species group names

1. Select **Sp Group** on the **Selected** tab.
2. Select the species group to be renamed in the lower view, click the right mouse button, and click **Rename**. Rename screen is displayed.
3. To rename the species group, edit the name on the Rename screen and click the **Apply** button.

25.4. Removing species groups and removing species from a species group

1. Select **Sp Group** on the **Selected** tab.
2. Select the species groups or species to be removed in the lower view, click the right mouse button, and click **Delete**.

26. Genome Core Structure Alignment (CoreAligner)

Genome core structure analysis consists of the extraction of genomic structures that are well conserved among related genomes. That is, a pair of orthologous groups are extracted whose genomic neighborhood relationship is conserved above a certain level, and the orthologous groups are realigned based on this neighborhood relationsships. The CoreAligner program for such analysis is run on the RECOG server, and the extracted core structures are displayed.

26.1. Running the CoreAligner program

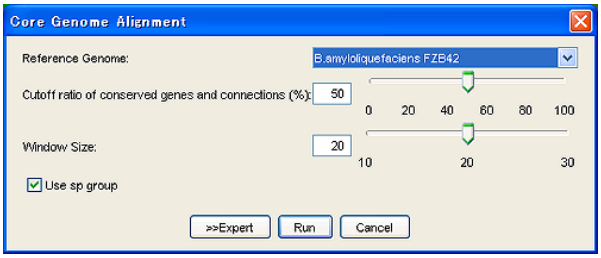
1. The ortholog analysis is performed and the results are displayed as described in "9.2 Execution of DomClust" and "9.3 Display of the DomClust analysis results."

2. Click  (**Core genome alignment (CoreAligner)**) in the **Toolbox**. The Core Genome Alignment screen is displayed.

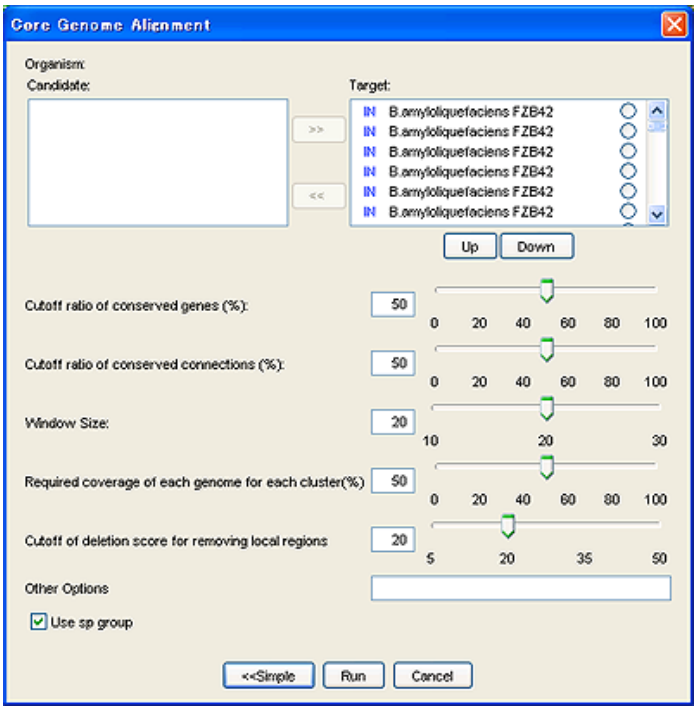
3. Specify the conditions for the analysis carried out by the CoreAligner program on the Core Genome Alignment screen. There are two ways of specifying the analysis conditions: Simple Mode and Expert Mode.

	Simple Mode	Expert Mode
Assignable item	- Reference genome - Cutoff ratio of conserved orthologs and neighborhood relations - Window size - Use/unuse species group	- Reference genome - Cutoff ratio of conserved orthologs - Cutoff ratio of conserved neighborhood relations - Window size - Use/unuse species group - Display/Nondisplay of species - Display order of species

- **Simple Mode**

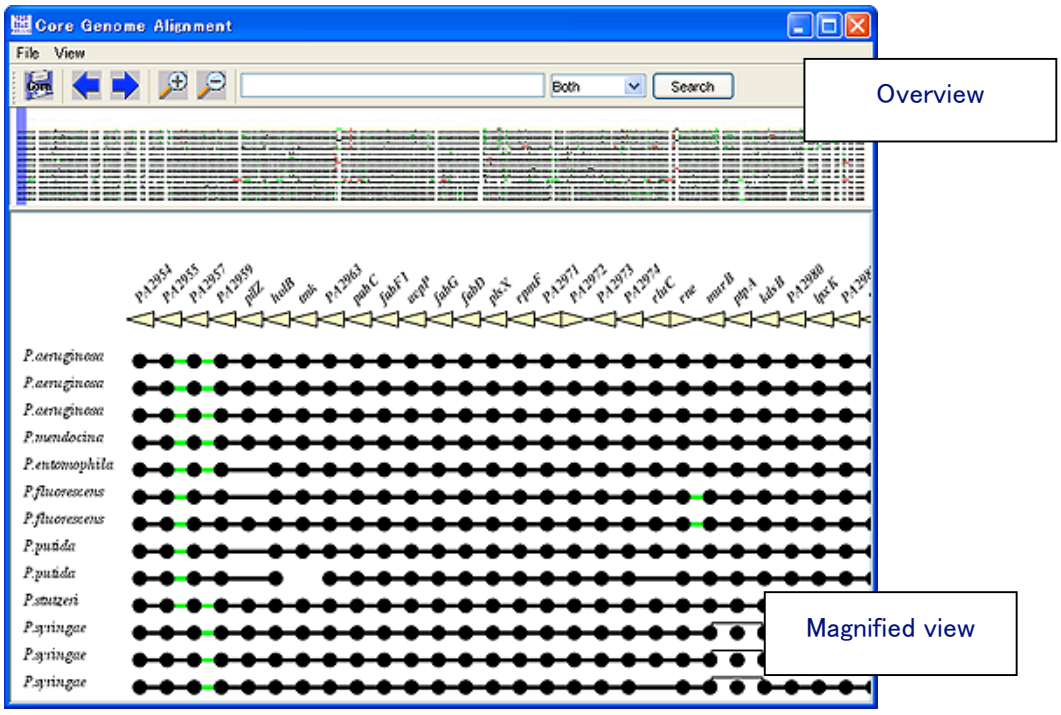


- **Expert Mode**



4. After specifying the conditions, click the **Run** button. The progress screen is displayed and the analysis by the CoreAligner program starts.

5. Upon the completion of the analysis, the Core Genome Alignment screen and the **Genome Comparison** Viewer screen are displayed.



26.2. Displaying the CoreAligner analysis results

The previously executed CoreAligner analysis results are displayed.

1. Click  (**Open files**) in the **Toolbox**. The Open file screen is displayed.

2. On the Open file screen, select the file filter 'Core Genome File (.coaln, .coregenome)' and then select the project and CoreAligner analysis result file.
   Upon selecting the CoreAligner analysis result file, the information on the analysis results is displayed on the right of the screen.

3. Click the **Apply** button on the Open files screen to display the selected CoreAligner analysis results and DomClust results.

26.3. Components of the core structure display

- **Ortholog group**
  A longitudinal gene group corresponds to an ortholog group.

- **Node**
  Each node is represented by a circle or square.

Shape	Details
●	Contains only one gene.
■	Contains two or more genes (inparalogs).

- **Line**
  Each line is color-coded in accordance with the conditions.

Color	Details
Black	There is no insertion between genes.
Green	There is an insertion between genes.
Red	The relative direction of the genes is reversed (inversion).

- **Gene Direction**
  The triangular arrow in the upper part of ortholog group indicates the direction.
  The background is displayed in the color corresponding to the typical function category of the ortholog group.

26.4. Changing the display position

1. To scroll the screen in the direction of the clicked button, click  (**Move Left**)/  (**Move Right**) in the **Toolbox** on the Core Genome Alignment screen.
2. The display position can be changed by dragging the mouse on the **Overview** window.

26.5. Selecting an ortholog group

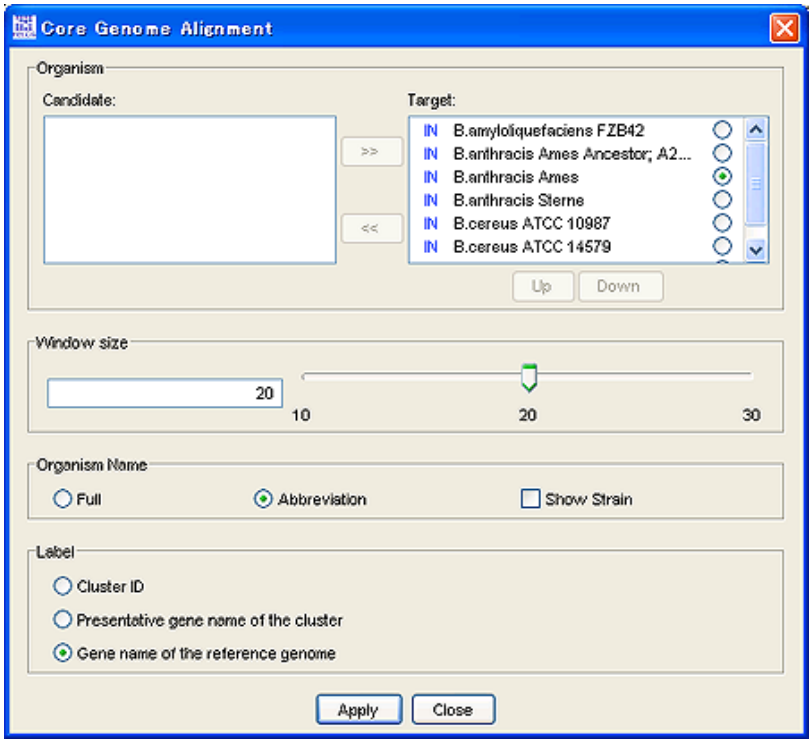
1. To highlight an ortholog group, click the ortholog group in the magnified view on the Core Genome Alignment screen. Also, in the Genome Comparison Viewer, the corresponding ortholog group is highlighted.

26.6. Locating an ortholog group at center

1. Double-click the relevant ortholog group on the Core Genome Alignment screen to display it at center. Also, on the Genome Comparison Viewer screen, the corresponding ortholog group is mainly displayed.

26.7. Setting a reference genome

1. To display the Core Genome Alignment display modification screen, click **View – View Change….** on the Core Genome Alignment screen.
2. In the 'Target' column, check the column on the right of the species to be set as the reference genome.
3. Click the **Apply** button.



26.8. Display/Nondisplay of species

1. To display the screen for changing the Core Genome Alignment view, click **View - View Change...** on the Core Genome Alignment screen.
2. To display species, select the species in the **Candidate** column and click the >> button.
3. To hide species, select the species in the **Target** column and click the << button.
4. Click the **Apply** button.

26.9. Changing the display order of species

1. To display the screen for changing the Core Genome Alignment view, click **View - View Change...** on the Core Genome Alignment screen.
2. To change the display order of species, select the species in the **Target** column on the screen for changing the Core Genome Alignment view, and click the **Up** or **Down** button.
3. Click the **Apply** button.

26.10. Resetting the window size

1. To display the screen for changing the Core Genome Alignment view, click **View - View Change...** on the Core Genome Alignment screen.
2. To reset the window size of the neighborhood relation, set the value in the **Window Size** section.
3. Click the **Apply** button.

26.11. Changing the display style of species names

1. To display the screen for changing the Core Genome Alignment view, click **View - View Change...** on the Core Genome Alignment screen.
2. Select the display style in the **Organism** column on the screen for changing the Core Genome Alignment view.
   - Normal: Species are displayed according to their official names.
   - Abbreviation: Species are displayed according to their abbreviated names.
   - Show Strain: If this is checked, the strains are displayed.

26.12. Changing the ortholog group labels

1. Click **View - View Change...** on the Core Genome Alignment screen. The Core Genome Alignment view is displayed
2. Specify the items to be displayed as labels for the ortholog group in the **Label** section on the screen for changing the Core Genome Alignment view.
   - Cluster ID*

     The cluster ID corresponding to the ortholog group is displayed.
   - Representative gene name of the cluster*

     The representative gene name of the cluster corresponding to the ortholog group is displayed.
   - Gene name of the reference genome

     The gene name of the reference genome is displayed. If gene name is undefined in that

genome, the Locus Tag is displayed.

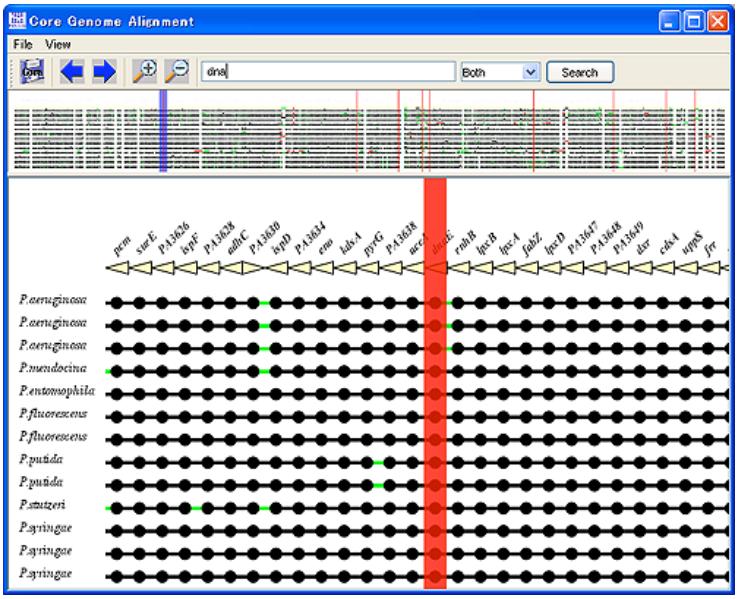
* If these items are specified, the gene name display column on the PPM is switched synchronously.

26.13. Zoom

1. To zoom in/zoom out on the core structure image, click  (**Zoom in**)/  (**Zoom out**) in the **Toolbox** on the Core Genome Alignment screen.

26.14. Searching by gene name/Locus Tag

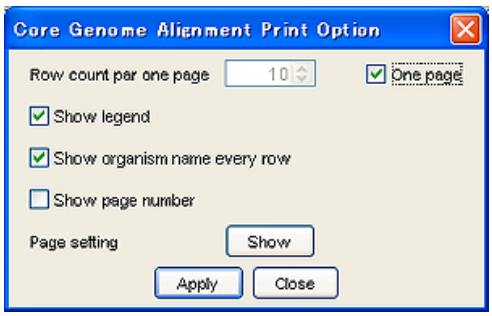
1. In the column for item selection in the **Toolbox** on the Core Genome Alignment screen, select **Gene Name**, **Locus Tag** or **Both**. If **Both** is selected, a search according to both the gene name and the locus tag is conducted.
2. Enter a keyword in the keyword entry column.
3. Click the **Search** button, and the ortholog group to which the searched gene belongs is highlighted on the **Overview,** and the viewing area of the magnified view is scrolled so that one of the ortholog groups hit by the search is displayed in the center of the screen.
4. If the **Search** button is clicked under the same conditions, the viewing area of the magnified view is scrolled so that the next ortholog group containing the searched gene is displayed in the center of the screen.



26.15. Printing the core structure image

1. Click **File** – **Preview** on the Core Genome Alignment screen. The Core Genome Alignment Preview screen is displayed.

2. Click the **Option** button on the Core Genome Alignment Preview screen to display the Core Genome Alignment Print Option screen, and specify the option.



Option	Details
Row count per page	Specify the number of rows displayed on a page.
One page	Print so that the **Core Genome Alignment** image fits into a page.
Show legend	If this is checked, the legend is displayed.
Show organism name on every row	If this is checked, the species names are displayed in all the rows. If unchecked, the species names are displayed only in the first row of each page.
Show page number	If this is checked, the page number is displayed.
Page setting	Specify the paper size and orientation.

3. Click the **Print** button on the Core Genome Alignment Preview screen, and the printer selection screen is displayed. Specify the printing conditions and click the **OK** button.

26.16. Saving the CoreAligner results

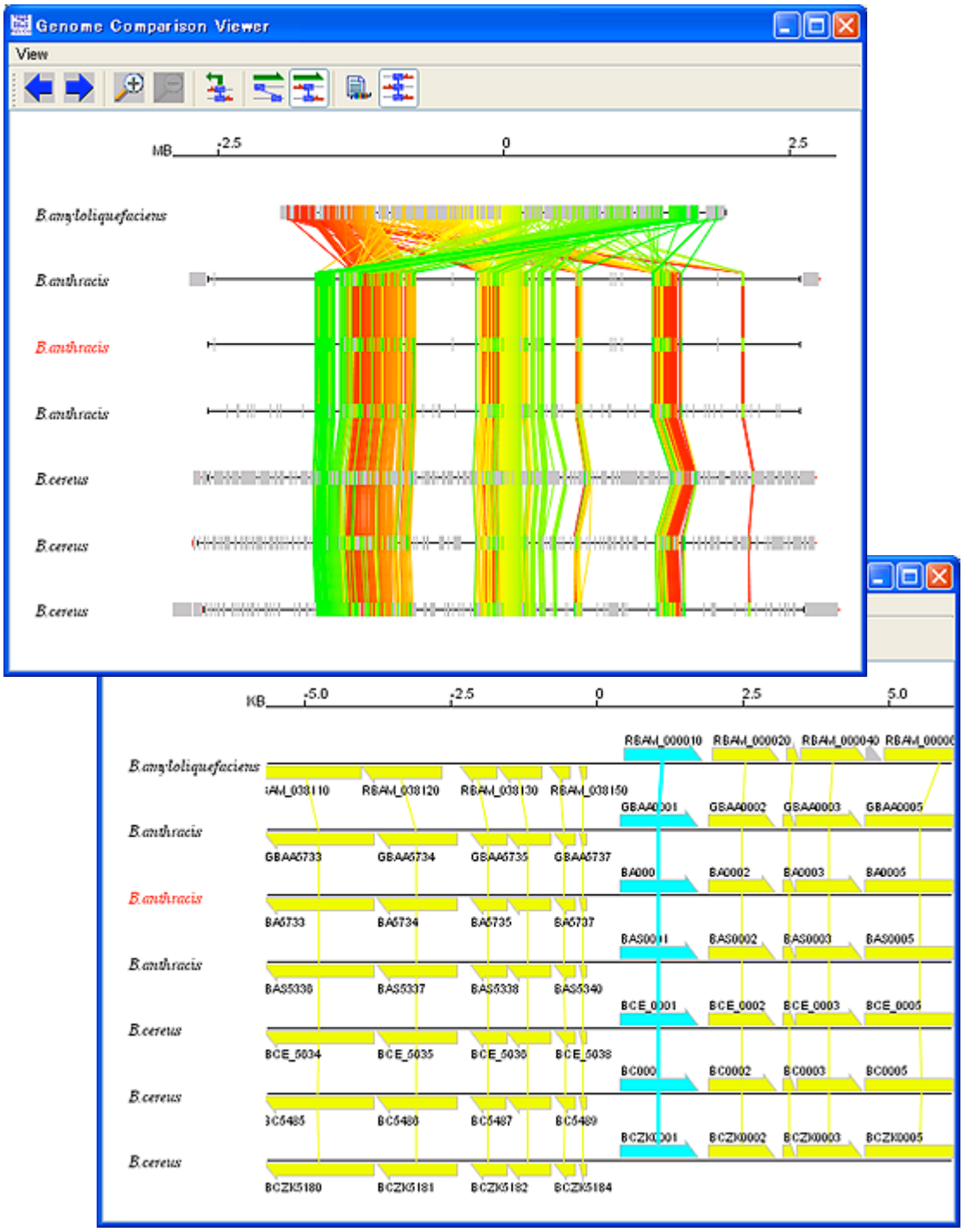
The CoreAligner analysis results are saved automatically in the Project directory or its sub-directory when an analysis is conducted. Save the analysis results in the CoreAligner format (.coaln).

1. Click 

(**Save Core Genome File**) in the **Toolbox**, specify the destination for saving and the file name, and click the **OK** button.

27. Genome Comparison Viewer

The Genome Comparison Viewer assigns colors to each gene based on the core structure extracted by the CoreAligner analysis, and displays a genome map that connects the corresponding ortholog groups by straight lines. Upon zooming in, the Viewer automatically switches to the detailed gene view (see the figure below).



27.1. Displaying the Genome Comparison Viewer

The Genome Comparison Viewer is displayed after the CoreAligner procedure is finished. Therefore, the Viewer can be displayed in the same manner as that described in "26.1 Running the CoreAligner program" and "26.2 Displaying the CoreAligner analysis results."

Also, the viewer can be displayed by clicking **View - Genome Comparison Viewer** on the Core Genome Alignment screen.

27.2. Changing the display area

1. To scroll the view area in the direction of the clicked button, click  (**Move Left**)/  (**Move Right**) in the **Toolbox** on the Genome Comparison Viewer screen.

   For changing the display area, there are two modes, as shown below. The two modes can be switched by clicking the relevant button in the **Toolbox**.

   -  (**Simple Mode**)

     In this mode, the display area is moved within a certain interval without adjusting the gene display position.

   -  (**Adjust Mode**)

     In this mode, after changing the view area, the display is made upon relocating so that the genes belonging to the ortholog group that contains the gene near the center of the view area on the reference genome align or stand in a straight line. Also, regarding the orientation of the genes, the display is made upon making the gene orientation uniform according to the settings described in "27.14 Automatic correction of gene orientation."

27.3. Zooming

1. To zoom in/zoom out on the display area, click  (**Zoom in**)/  (**Zoom out**) in the **Toolbox** on the Genome Comparison Viewer screen, respectively.

2. If the zooming in exceeds a certain scale, the screen automatically switches to the detailed

gene view.

27.4. Moving a specified ortholog group to the center of the screen

1. In normal mode (the mode without the operation set out in 26.5 below), upon double-clicking on the genes belonging to the ortholog group on the Genome Comparison Viewer screen, the double-clicked ortholog group moves to the center of the screen. Also, on the Core Genome Alignment screen as well, the clicked ortholog group is relocated to the center of the screen.

27.5. Displaying gene information in a browser

1. Click  (**Show the gene information at clicking gene**) in the **Toolbox** on the Genome Comparison Viewer screen. Upon double-clicking in this state, the information on the default external resource set in "**35**. External Resource URL Management" is displayed in a browser.
2. To display the information on the external resource in a browser, click the right mouse button, and click the displayed external resource URL.

27.6. Saving the origin

1. To save the current origin (center point), click **View** -  **Save Origin** on the Genome Comparison Viewer screen.

27.7. Recovering the origin

1. To relocate the genome map with the origin saved immediately before as the center point, click  (**Recover Origin**) in the **Toolbox** on the Genome Comparison Viewer screen.

27.8. Display/Nondisplay of species

Specify the species set to be displayed on the Genome Comparison Viewer screen.

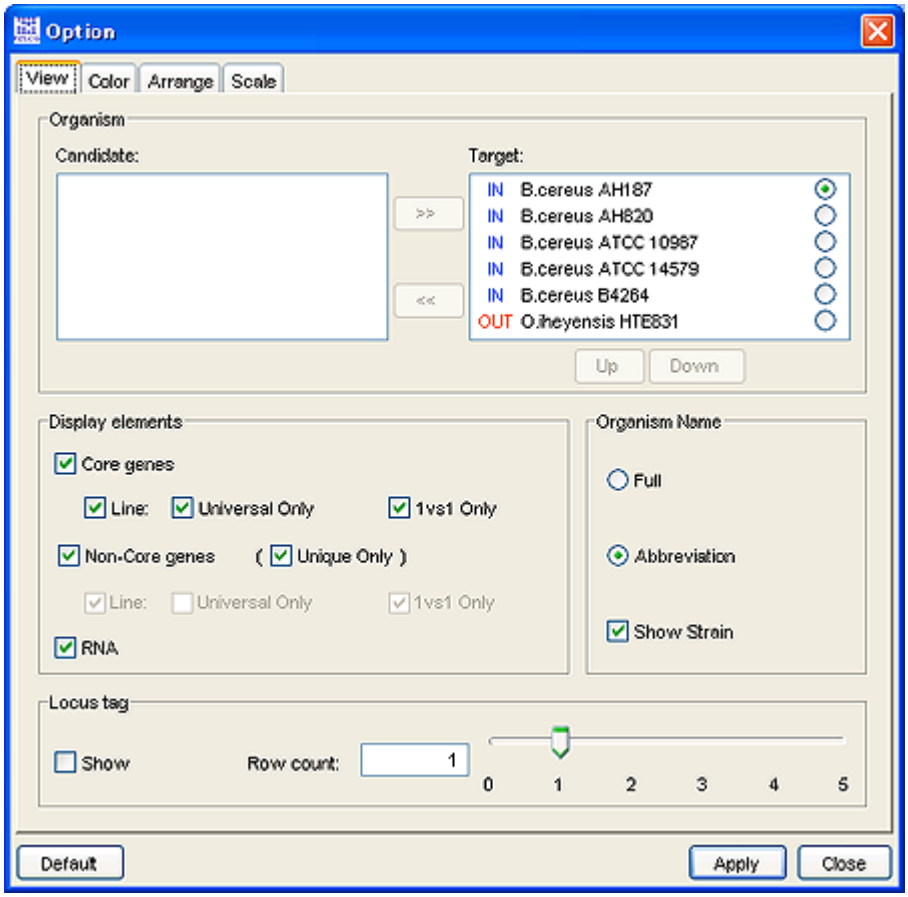
1. Click **View - View Change...** on the Genome Comparison Viewer screen. The screen for changing the display of the Genome Comparison Viewer screen is displayed.

   Click the **View** tab on the screen for changing the display of the Genome Comparison Viewer screen.

2. To display species, select the species in the **Candidate** column and click the >> button.

3. To hide species, select the species in the **Target** column and click the << button.

4. Click the **Apply** button.



27.9. Changing the display order of species

1. Click **View - View Change...** on the Genome Comparison Viewer screen. The screen for changing the display of the Genome Comparison Viewer screen is displayed.

   Click the **View** tab on the screen for changing the display of the Genome Comparison Viewer screen.

2. To permute the species, select the species in the **Target** column on the **View** tab and click the **Up** or **Down** button.

3. Click the **Apply** button.

27.10. Display/Nondisplay of genes or ortholog lines

1. Click **View - View Change...** on the Genome Comparison Viewer screen.

   Click the **View** tab on the screen for changing the display of the Genome Comparison Viewer screen.

2. In the **Display elements** column on the **View** tab, specify the display/nondisplay of genes and the display/nondisplay of the lines that represent ortholog relationships.

Genes and RNA display switching options

Option	Details
Core genes	The genes extracted by the CoreAligner analysis are called Core genes. If this column is checked, the Core genes are displayed.
Non-Core genes	Genes that were not extracted by the CoreAligner analysis are called Non-Core genes. If this column is checked, the Non-Core genes are displayed.
RNA	If this column is checked, the RNA genes are displayed.

Switching options for ortholog line display

Options	Details
Universal Only	Ortholog groups that contain the genes of all species are called universal ortholog groups. If this column is selected, only the universal ortholog groups are displayed.
1 vs. 1 Only	Ortholog groups that contain only a gene for each species are called 1 vs. 1 groups. If this column is checked, only the 1 vs 1 ortholog groups are displayed.
Unique Only * Non-Core genes only	If this column is checked, only unique genes that form no ortholog groups are displayed.

3. Click the **Apply** button.

27.11. Changing the display style of species names

1. Click **View - View Change...** on the Genome Comparison Viewer screen.

   Click the **View** tab on the screen for changing the display style of the Genome Comparison Viewer screen.

2. Select the display stype of species names in the **Organism** column on the **View** tab.

   - Normal: The official species names are displayed.
   - Abbreviation: The abbreviated species names are displayed.
   - Show Strain: If this is checked, the strains are displayed.

27.12. Display/Nondisplay of the Locus Tag

1. Click **View - View Change...** on the Genome Comparison Viewer screen.

   Click the **View** tab on the screen for changing the display style of the Genome Comparison Viewer screen.

2. To display the Locus Tag on the genome map, check **Show** in the **Locus Tag** column on the screen for changing the display style of the Genome Comparison Viewer screen. In the **Row count**, specify the number of rows for displaying the Locus Tag. If multiple rows are specified, their space is used so that names do not overlap each other.

   To hide the Locus Tag on the genome map, uncheck **Show** in the **Locus Tag** column.

   If **0** is specified in the **Row count**, the distance between genomes takes its minimum value and the Locus Tag is not displayed regardless of whether **Show** is checked or unchecked.

3. Click the **Apply** button.

27.13. Color setting

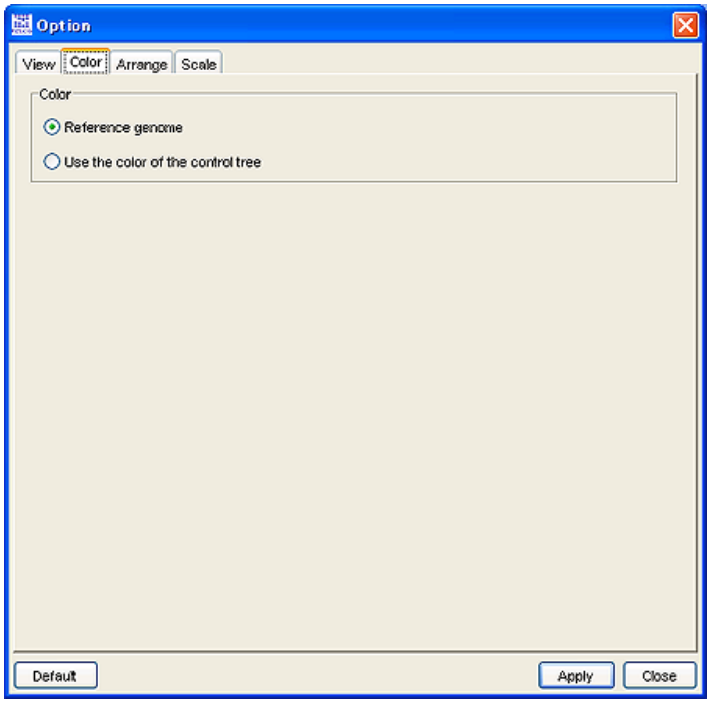
The color can be set based on the gene position on the reference genome or it can be set on the **Color** tab on the control panel.

1. Click **View - View Change...** on the Genome Comparison Viewer screen. Click the **Color** tab on the screen for changing the display of the Genome Comparison Viewer screen.

2. On the **Color** tab, set the colors for genes and ortholog lines.

   - **Reference genome**
     Core genes are colored with color gradations from green to red based on the gene positions on the reference genome. Non-Core genes and RNA are colored in grey and deep blue, respectively.

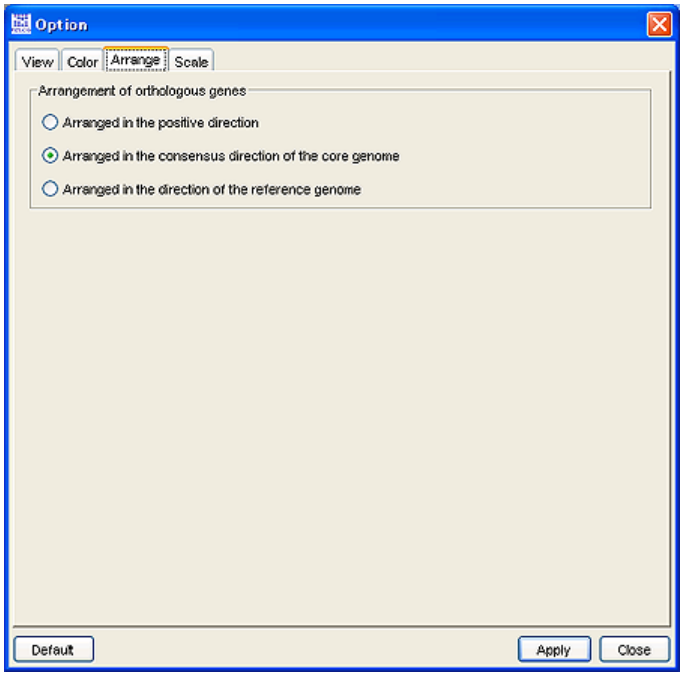
   - **Use the color of the control tree**
     For coloring, the color settings on the **Color** tab on the control panel (i.e. the same color settings in the PPM table) are used.



27.14. Automatic correction of the gene orientation

In accordance with the setting for the automatic correction of the gene orientation, the gene orientation is made uniform in the ortholog group displayed in the center.

The setting for the automatic correction of the gene orientation is valid only in Adjust Mode.



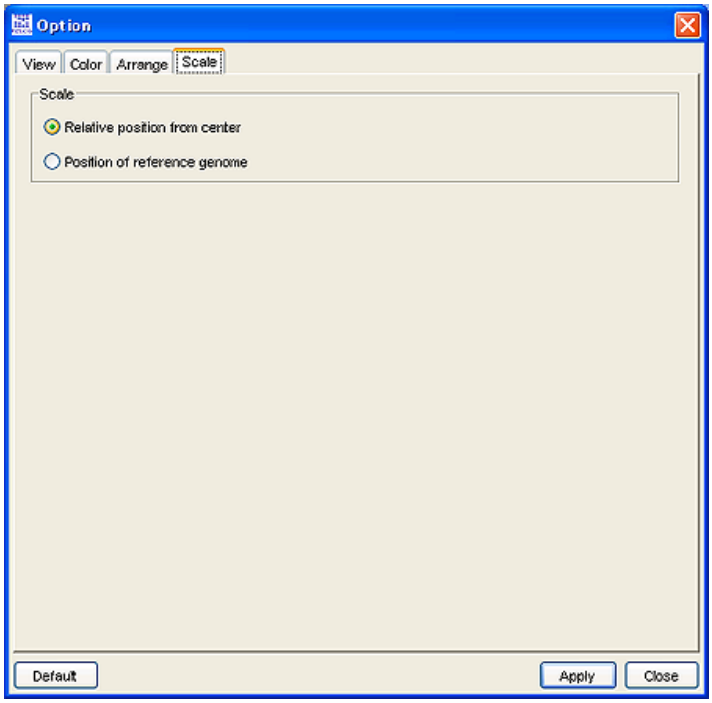
1. Click **View - View Change...** on the Genome Comparison Viewer screen.

Click the **Arrange** tab on the screen for changing the display of the Genome Comparison Viewer screen.

2. Specify the method of automatic correction of the gene orientation on the **Arrange** tab.

- **Arrange in the positive direction**
  Place all genes in the positive direction.
- **Arrange in the consensus direction of the core genome**
  Place all genes in the consensus direction of the ortholog group obtained by the CoreAligner analysis.
- **Arrange in the direction of the reference genome**
  Place all genes in the direction of the reference genome genes.

27.15. Changing the display style of the scale marks



1. Click **View - View Change…** on the Genome Comparison Viewer screen.

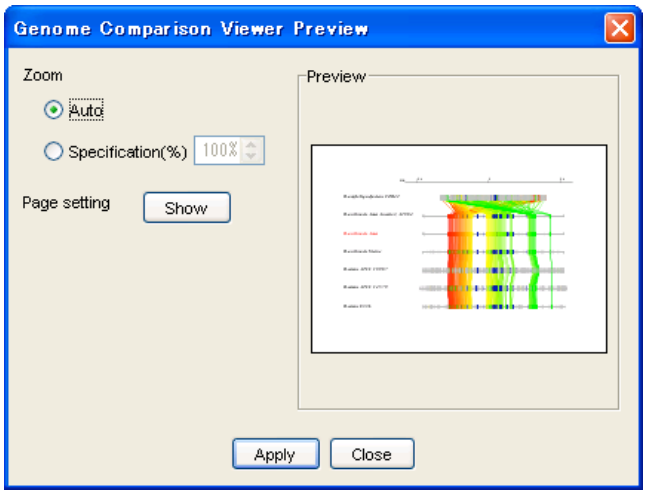
2. On the **Scale** tab, set the display style of the scale marks.

- **Relative position from center**
  The relative position from the center is displayed on the scale marks.

- **Position of reference genome**

  The position based on the coordinates of the reference genome is displayed on the scale marks.

27.16. Printing



1. Click **File** – **Preview…** on the Genome Comparison Viewer screen. The Genome Comparison Viewer Preview screen is displayed.

2. On the Genome Comparison Viewer Preview screen, the following settings are possible:

   - **Zoom**

     Specify the image magnification.

     If **Auto** is specified, the image magnification is adjusted so that the image fits onto a single sheet of paper.

   - **Page settings**

     Specify the paper size, *etc*.

3. Click the **Apply** button.

   The print setting screen is displayed. Printing is carried out in accordance with the screen display.

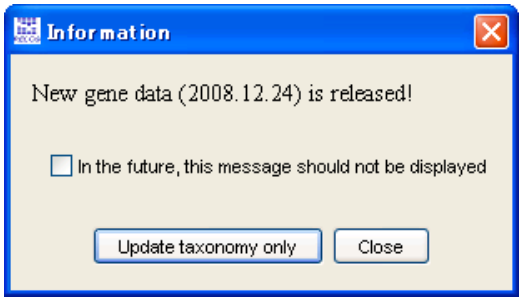
   To directly print without displaying the Genome Comparison Preview screen, click **File** – **Print…** on the Genome Comparison Viewer screen.

28. Updating the Gene Information

Using the gene information update function of the RECOG Client, the gene information, chromosome information, Taxonomy Tree information and Function Category information can be updated.

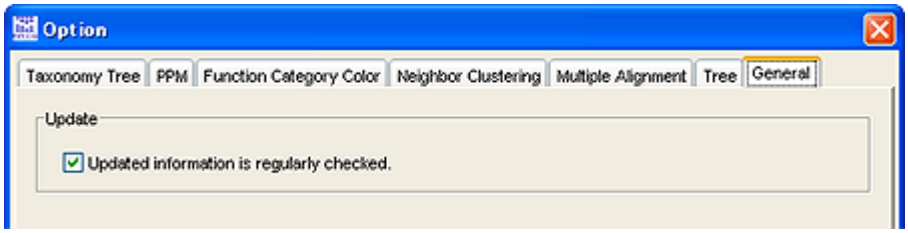
28.1. Updating the Taxonomy Tree based on the update notice

If any gene information provided by the RECOG server is updated, the  icon is displayed on the lower right of the screen. To display the update notice, click the icon. To update the Taxonomy Tree data on the **All** tab, click the **Update taxonomy only** button in the notice message.



* If "In the future, this message should not be displayed" is checked and the **Close** button is clicked, no update notices will be displayed thereafter.

To display update notices again, click  (**Option**) in the **Toolbox**, check "Update information is regularly checked" on the **General** tab on the Option screen, and click the **Apply** button.

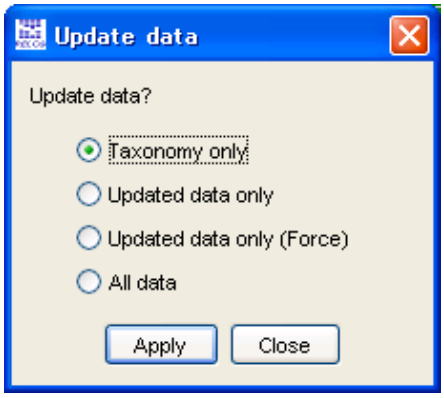


28.2. Updating gene information through Update Data

The RECOG Client can download data from the RECOG server. This function is used to update the RECOG Client data when the RECOG server data have been updated.

1. To display the Update data screen, click **File - Update Data…**.

2. Select one of the following on the Update data screen, and click the **Apply** button.



- **Taxonomy data only**

  Only the Taxonomy Tree data are updated. Other data are retrieved as the need arises. Usually, this mode is the most efficient.

- **Updated data only**

  Regarding the Taxonomy Tree data and the gene information currently held by the RECOG Client, only the data updated by the server are updated. This function is used to update necessary information collectively.

- **Updated data only（Force）**

  The Taxonomy Tree data and all the gene information held by the RECOG Client are updated. This function is used to force an update in cases where the RECOG Client's information is defective.

- **All data**

  All the gene data are downloaded.

(Note) If **All data** is specified, it may take about several tens of minutes to download the data, depending also on the network transfer rate.

29. Registration and Management of Gene/Cluster Properties

It is possible to incorporate any type of gene information by importing gene property files. Cluster properties are properties defined for each ortholog cluster, which typically calculated using gene properties. Gene properties and cluster properties can be utilized for various analyses such as sorting and PPM coloring.

29.1. Registration of gene properties

1. Create a gene property file.
   The format of a gene property file is as shown below:

- The first row describes the header.
  - ✧ In the first column, "sp" (a three-letter code for a species) is given, and in the second column, "locustag" is given.
  - ✧ In the third column, the arbitrary gene property name and type are given.

    (Example) If the gene property name is Expression and the type is numerical, the description should be Expression (Num).

  The following four types of gene properties can be specified:

Type	Code	Example
String type	Char	BC2639
Numerical type	Num	-10.3
Enumeration type	Enum（element1, element2,…） * element#: possible value, that is, only the element specified here is assignable as a value.	Yes, No * Enum(Yes,No), that is, Yes and No are the specified elements.
Hierarchical type	Hierarchy	1.2.1

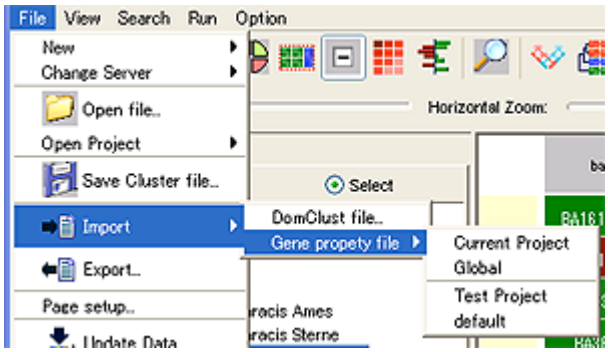
- In the second row, gene property values are described.
  - ✧ In the first column, a three-letter code for a species is given, and in the second column, Locus Tag is given.
  - ✧ In the third column, arbitrary gene property values are given.

    If a gene has two values, the type should be specified as "Multi," and the two values should be delimited by ";" (semicolon).

(Example)

sp	locustag	GO(Char)	Expression(Num)	Pathway(Char,Multi)
ban	BA0001	Cellular component	2000	Glycolysis / Gluconeogenesis;Citrate cycle
…				

2. Click **File** – **Import** - **Gene properties file**, and select the destination for registering the gene property.



Select the destination from among the following:

- **Current Project**, project name
  Register the gene property so that it becomes available only in the current project or in the specified projects.

- **Global**
  Register the gene property so that it becomes available in all projects.

3. The Import gene property file screen opens. To register the gene property, specify the gene property file created, and click the **Open** button.

29.2. Referencing a list of gene/cluster properties

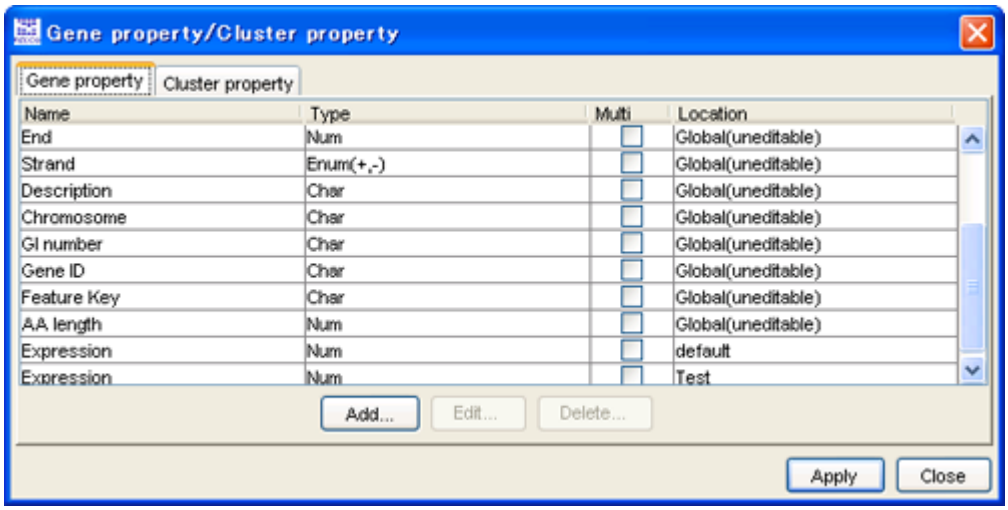
On the Gene property / Cluster property screen, the registered properties can be checked.

1. To display the Gene property/Cluster property screen, click **Option** - **Gene Property/Cluster Property List…**.

2. To display a gene property, click the **Gene** tab, and to display a cluster property, click the **Cluster** tab. The details of each screen that can be displayed are as follows.

   - **Name**: property name

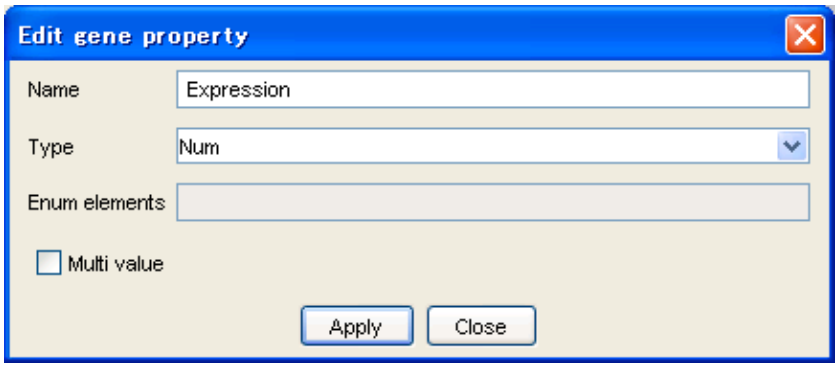
- **Type**: type
- **Multi**: multiple attribute value flag. If this is checked, the gene has multiple attribute values.
- **Location**: location for storage. * Gene properties only
  - ✧ **Global (uneditable)**
    System defined gene properties provided by the RECOG server.
    They cannot be edited or removed.
  - ✧ **Global**
    Gene properties that can be referred to in all projects.
  - ✧ **Project name**
    Gene properties that can be referred to only within a project.
- **Category**: category. * Cluster properties only.
  - ✧ **DomClust**: Property corresponding to the DomClust results.
  - ✧ **Homology Cluster**: Property corresponding to a homology cluster.
  - ✧ **Cluster**: Property corresponding to a cluster
  - ✧ **Sub Cluster**: Property corresponding to a sub-cluster



29.3. Editing properties

The property name and type can be modified.

1. To display the Gene Property/Cluster Property screen, click **Option - Gene Property/Cluster Property List…**.
2. To display the Edit gene property screen, select the property to be edited on the Gene Property / Cluster Property screen, and click the **Edit** button.



3. On the Edit gene property screen, specify the gene property name (**Name**), type (**Type**) and the presence or absence of multiple attribute values (**Multi** value).
   If the enumeration type is specified, specify also the possible values (**Enum** elements) in comma-delimited form.
4. On the Edit gene property screen, click the **Apply** button.
5. On the Gene Property / Cluster Property screen, click the **Apply** button.

29.4. Removing a property

1. To display the Gene Property / Cluster Property screen, click **Option - Gene Property / Cluster Property List…**.
2. On the Gene Property / Cluster Property screen, select the property to be removed, and click the **Delete** button. When the Confirm screen is displayed, click the **OK** button.
3. To remove the property, click the **Apply** button on the Gene Property / Cluster Property screen.

30. Registration and Management of Gene/Cluster Sets

Multiple genes/clusters can be registered as a set. The registered gene/cluster sets can be utilized for sorting, color setting and filter setting.

30.1. Registration of a gene/cluster set

A gene/cluster set can be registered by the following three methods:

- Registration from a file
- Registration from a cluster selected on the PPM
- Registration from the keyword search results

30.1.1. Registration from a file

1. Create a gene/cluster set file.

   The format for the gene/cluster set file should be one of the following three:

   a. **dclust** format

   The **dclust** format should comprise the following:

   <Species code>:<LocusTag>[|,|¥t]<Species code>:<LocusTag>...

   <Species code>:<LocusTag>...

   (Example)

```
ban:BA0001, ban:BA0002
bca:BCE_0009,bce:BC0013,oihOB0010
```

   b. **clusttab** format

   The file format should be one of the following:

   - ✧ The **clusttab** format output by clicking **File - Export**
   - ✧ The file format output by clicking **Export gene/cluster set**

   c. Gene property format

   This is the file format described in "29.1 Registration of gene properties."

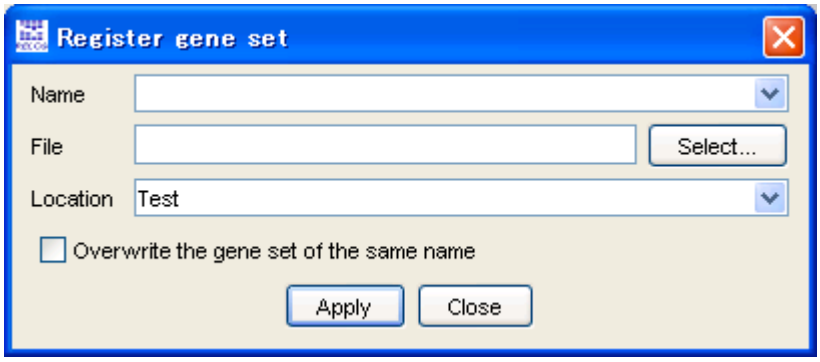
2. Click **File** – **Import - Gene set file**, and select the destination for registering the gene set. Select from among the following:

   - **Current Project**, project name

     Register the gene set so that it becomes available only in the current project or in the specified projects.

   - **Global**

     Register the gene set so that it becomes available in all projects.



3. The Register gene set screen opens. To register the gene/cluster set, specify the set name, gene/cluster set file and the destination for saving, and click the **Apply** button.

   If 'Overwrite the gene/cluster set of the same name' is checked, the registration is made upon overwriting the gene/cluster set of the same name if such a set exists.

   The registered gene/cluster set is displayed in **Gene Set/Cluster Set** on the set control panel.

30.1.2. Registration from the cluster selected on the PPM

1. Select the cluster on the PPM.

2. To open the Register gene/cluster set screen, click the right mouse button and click **Create gene/cluster set**.

3. To register the gene/cluster set, specify the set name and the destination for registration on the Register gene/cluster set screen, and click the **Apply** button.

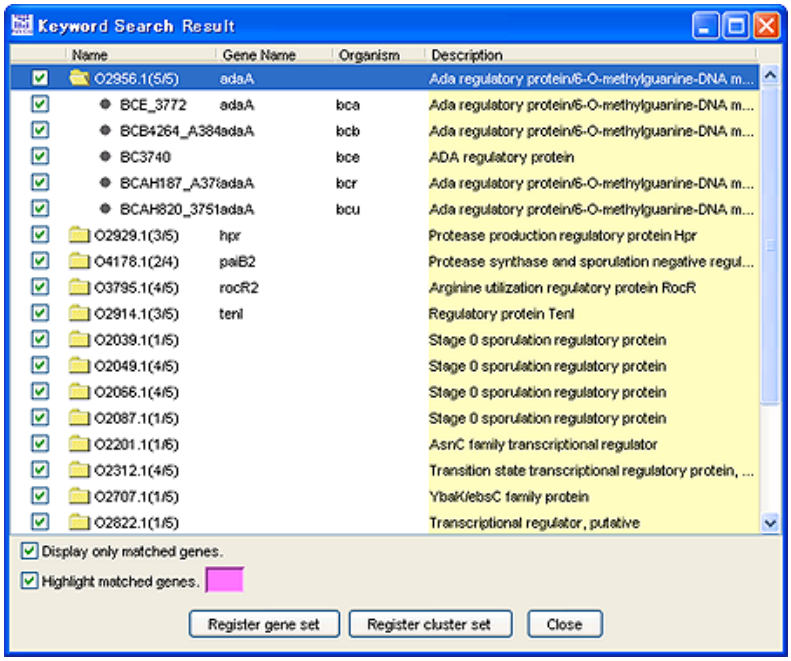
   If 'Overwrite the gene/cluster set of the same name' is checked, the registration is made upon overwriting the gene/cluster set of the same name if such a set exists.

   Registered gene/cluster sets are displayed in **Gene Set/Cluster Set** on the set control

panel.

30.1.3. Registration from the keyword search results

1. Display the Keyword Search Result screen.



2. To display the Register gene/cluster set screen, check the column to the right of the gene/cluster to be registered, and click the **Register gene set/Register cluster set** button.

3. To register the gene/cluster set, specify the set name and the destination for registration on the Register gene/cluster set screen, and click the **Apply** button.

   If 'Overwrite the gene/cluster set of the same name' is checked, registration is made upon overwriting the gene/cluster set of the same name if such a set exists.

   Registered gene/cluster sets are displayed in **Gene Set/Cluster Set** on the set control panel.

30.2. Outputting a gene/cluster set to a file

1. To display the Export gene/cluster set screen, select gene/cluster set in **Gene Set/Cluster Set** on the set control panel, click the right mouse button, and click **Export gene/cluster set**.

2. On the Export gene/cluster set screen, select the file name to be saved, and click the **OK** button.

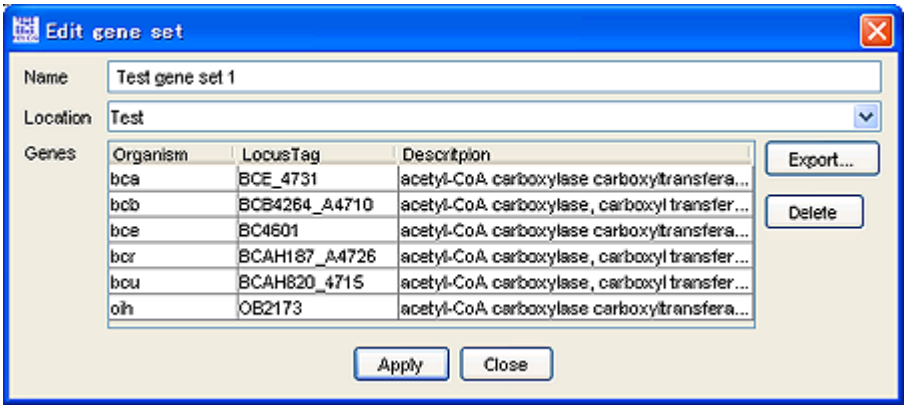
30.3. Editing a gene/cluster set (removing genes)

1. To display the Edit gene/cluster set screen, select a gene/cluster set in **Gene Set/Cluster Set** on the set control panel, click the right mouse button, and click **Edit gene/cluster set**.

2. On the Edit gene/cluster set screen, change the name/destination for registration (only for gene sets), or remove a gene/cluster.

   To output the list of genes/clusters registered as a gene/cluster set to a file, click the **Export** button.

3. On the Edit gene/cluster set screen, click the **Apply** button.



30.4. Registering additional genes/clusters to a gene/cluster set

The cluster and the genes contained in a cluster selected on the PPM can be added to a registered gene/cluster set.

1. Select a cluster on the PPM.
2. To add the genes/cluster to a registered gene/cluster set, select a gene/cluster set in **Gene Set/Cluster Set** on the set control panel, click the right mouse button, and click **Add selected genes/clusters to**.

30.5. Removing a gene/cluster set

1. In **Gene Set/Cluster Set** on the set control panel, select a gene/cluster set, click the right mouse button, and click **Delete gene/cluster set**.
2. To remove the gene/cluster set, click the **OK** button when the warning message is displayed.

30.6. Referencing the list of gene/cluster sets

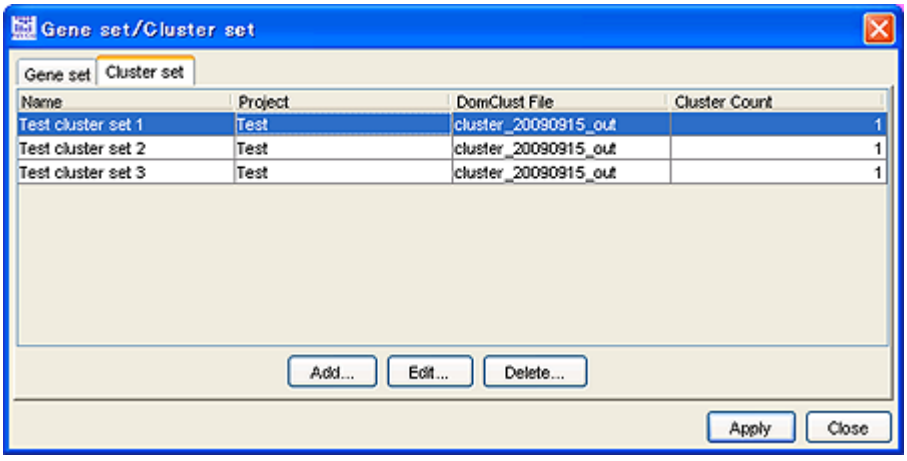
On the Gene set/Cluster set screen, the list of registered gene/cluster sets can be checked.

1. To display the Gene set/Cluster set screen, click **Option - Gene Set/ Cluster Set List…**.
2. To display the gene property, click the **Gene** tab, and to display the cluster property, click the **Cluster** tab. On each screen, the following items are displayed:

- **Name**: property name.
- **Location**: location for saving. *Gene properties only.
  - ✧ **Global**

    A gene property that can be referred to in all projects.
  - ✧ **Project name**

    A gene property that can be referred to only in the named project.
- **Gene Count**: the gene count of a gene set. *Gene sets only.
- **Project**: registration destination project. *Cluster sets only.
- **DomClust File**: registration destination DomClust result file name. *Cluster sets only.
- **Cluster Count**: registration destination DomClust result file name. *Cluster sets only.



3. To register, edit or remove a gene/cluster set, click the **Add…**, **Edit…** or **Delete…** button, respectively.

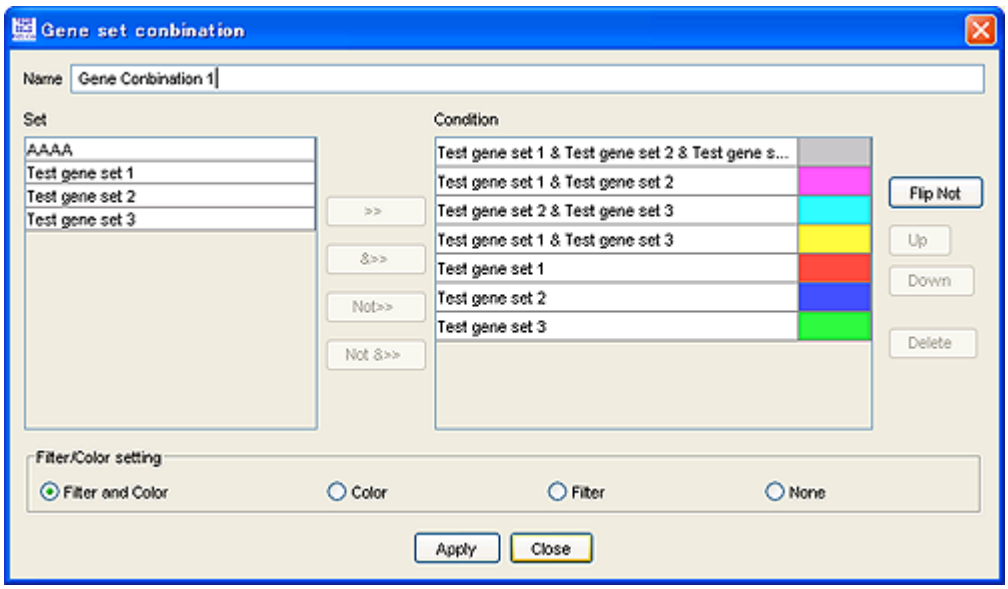
31. Combined Set

A combined set consisting of multiple gene/cluster sets can be created. The combined set can be utilized for coloring, filtering, *etc*. based on the results of set operations using multiple sets.

31.1. Registering a combined set

1. Registering a combined set of gene sets: Select gene sets in **Gene Set** on the set control panel to display the Gene set combination screen, click the right mouse button, and click **New gene set combination…**.

   Registering a combined set of cluster sets: select cluster sets in **Cluster Set** on the set control panel to display the Cluster set combination screen, click the right mouse button, and click **New cluster set combination…**.

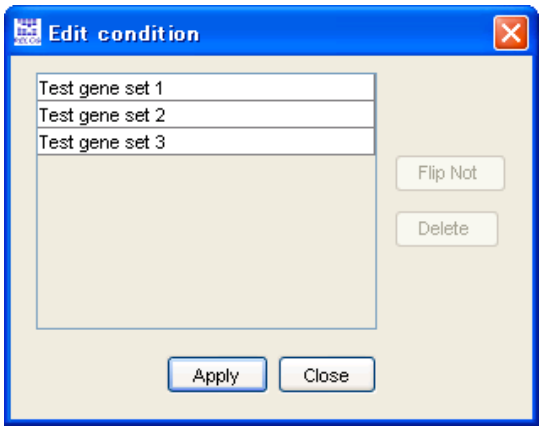


2. On the Gene/Cluster set combination screen, specify the gene/cluster set names and the logical conditions between the sets.

   - To add gene/cluster sets to the combined conditions, select the sets in the **Set** column and click the >> button.
   - To add multiple gene/cluster sets to the combined conditions as a common set, select the sets in the **Set** column and click the **&>>** button.
   - To add gene/cluster sets to the combined conditions as a negative condition, select the

sets in the **Set** column and click the **Not>>** button.

- To add multiple gene/cluster sets to the combined conditions as a common set of negative conditions, select the sets in the **Set** column and click the **Not&>>** button.
- To remove a condition from the combined conditions, select the condition in the **Condition** column and click the **Delete** button.
- To modify the order of priority of the combined conditions, select a condition in the **Condition** column and click the **Up/Down** button.
- To change a condition in the combined conditions into a negative condition, select the condition in the **Condition** column and click the **Flip Not** button.
- To modify the negative condition in each set contained in the combined conditions, double-click the condition name in the **Condition** column and modify it on the Edit condition screen displayed.



- To modify the color applied to the combined conditions, double-click the **Color** column in the **Condition** column and modify it on the Color selection screen displayed.

3. In the **Filter/Color setting** column, specify whether or not to apply a combined set to the color/filter setting.

- **Filter and Color**

  Register a combined set to add it as the condition for the color/filter setting.

- **Color**

  Register a combined set to add it as the condition for the color setting.

- **Filter**

  Register a combined set to add it as the condition for the filter setting.

- **None**

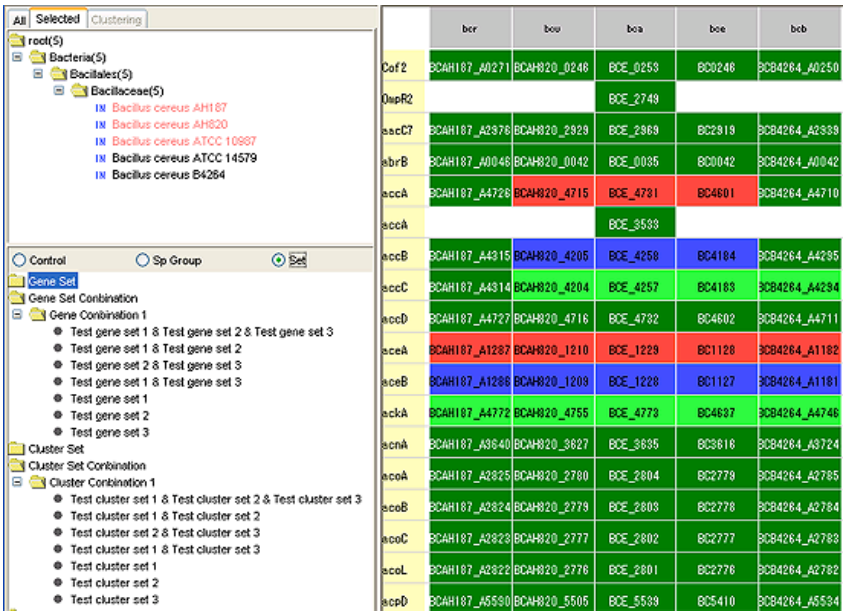
  Register only combined sets.

4. To display the combined conditions in **Gene Set Combination/Cluster Set Combination** on the set control panel, specify the conditions on the Gene/Cluster set combination screen and click the **Apply** button.

   If **Filter and Color** or **Filter** is specified in the **Filter/Color setting** column, the filter conditions are displayed in **Filter - Gene Set Filter/Cluster Set Filter** on the control panel.

   If **Filter and Color** or **Color** is specified in the **Filter/Color setting** column, the filter conditions are displayed in **Color - Gene Set/Cluster Set Filter** on the control panel.

   The specified filter settings and color settings are reflected on the PPM, in the comparative genome map view, *etc*.



31.2. Editing a combined set

1. To display the Gene/Cluster set combination screen, select a combined set in **Gene Set Combination/Cluster Set Combination** on the set control panel, click the right mouse button, and click **Edit gene/cluster set combination**.
2. Modify the conditions on the Gene/Cluster set combination screen. For the method of setting the conditions, refer to "31.1 Registering a combined set."
3. After modifying the conditions, click the **Apply** button.

31.3. Removing a combined set

To remove a combined set, select the combined set in **Gene Set Combination/Cluster Set Combination** on the set control panel, click the right mouse button, and click **Delete set combination**. When the warning message is displayed, click the **OK** button.

31.4. Specifying a combined set as a filter condition

1. To set a combined set as a filter condition in **Filter - Gene Set Filter/Cluster Set Filter** on the control panel, select the combined set in **Gene Set Combination/Cluster Set Combination** on the set control panel, click the right mouse button, and click **Register filter**.

31.5. Specifying a combined set as a color condition

1. To set a combined set as a color condition in **Filter - Gene Set/Cluster Set** on the control panel, select the combined set in **Gene Set Combination/Cluster Set Combination** on the set control panel, click the right mouse button, and click **Register color**.

31.6. Enabling/Disabling a filter setting

1. Select **Filter - Gene Set Filter/Cluster Set Filter** on the control panel, click the right mouse button, and click **Enable/Disable**.
2. To enable/disable each individual condition of a combined set, double-click the condition in **Filter - Gene Set Filter/Cluster Set Filter - Combined Set Name** on the control panel.

31.7. Enabling/Disabling a color setting

1. Select **Color - Gene Set/Cluster Set** on the control panel, click the right mouse button, and click **Enable/Disable**.
2. To enable/disable each individual condition of a combined set, double-click the condition in **Color - Gene Set/Cluster Set – Combined Set Name** on the control panel.

32. Species Set

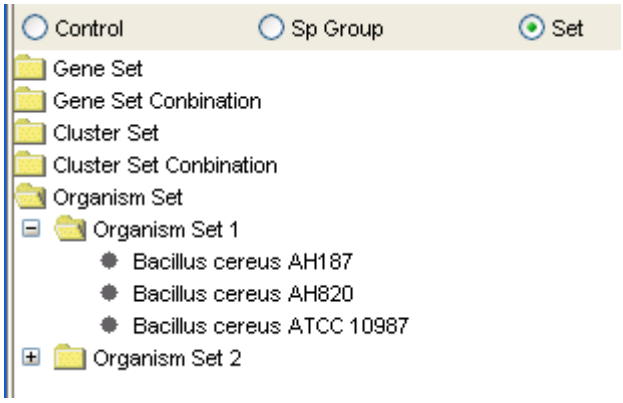
Multiple species can be specified as a set. The species set can be utilized for the phylogenetic pattern filtering setting, the species color setting, and profile editing and operation.

32.1. Registering a species set

1. To display the Organism Set screen, select a species on the Taxonomy Tree in the upper part of the **Selected** tab, click the right mouse button, and click **Organism set - New organism set…**.

2. Enter the name on the Organism Set screen. In the **Target** column, specify one of the following: **New** (new registration), **Overwrite** (overwriting of an existing species set), **Add** (additional registration).

3. To display the species set in **Organism Set** on the set control panel, specify the conditions and click the **Apply** button.



32.2. Editing a species set name

To display the Organism Set screen, select a species set in **Organism Set** on the set control panel, click the right mouse button, and click **Edit**.

1. Modify the species set name and click the **Apply** button.

32.3. Removing a species set

Select a species set in **Organism Set** on the set control panel, click the right mouse button, and click **Delete organism set**. When the warning message is displayed, click the **OK** button.

32.4. Setting colors using a species set

1. In **Organism Set** on the set control panel, select a species set, click the right mouse button, and click the sub-menu of **Color organism**.

   For the color setting method for species, refer to "11.4 Species color setting."

32.5. Taxonomy filtering using a species set

1. In **Organism Set** on the set control panel, select a species set, click the right mouse button, click **Taxonomy**, and click the condition menu for the phylogenetic pattern filtering.

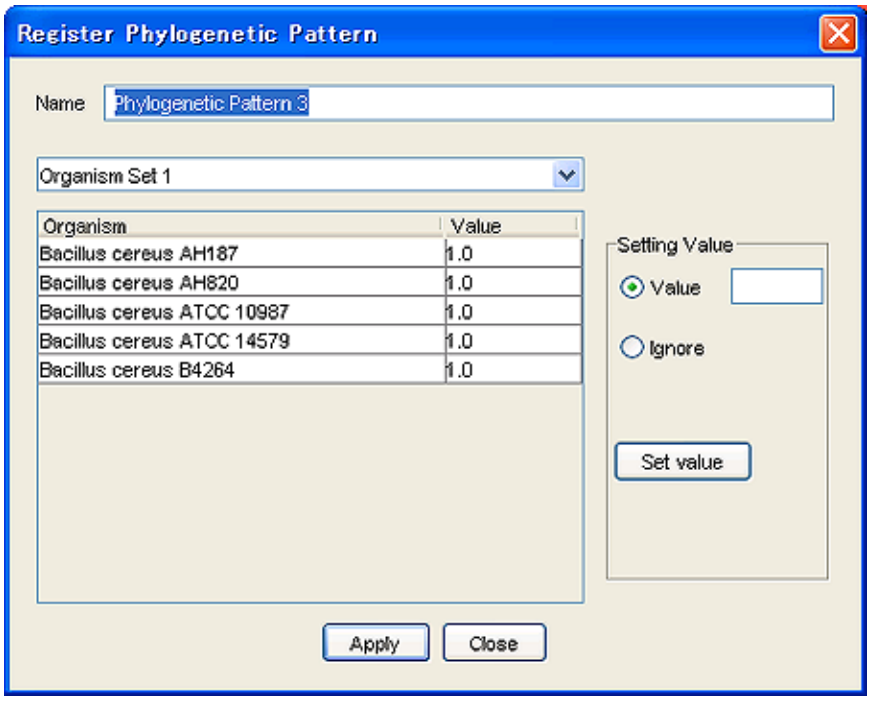
   For the operation procedure of phylogenetic pattern filtering, refer to "15. Taxonomy Filtering."

33. Similar Phylogenetic Pattern Search

Register a reference phylogenetic profile to evaluate the similarity between the profile and the phylogenetic pattern of each cluster. The results can be utilized for color setting, filtering, sorting, *etc*.

33.1. Profile registration from a cluster

1. Select a cluster on the PPM, click the right mouse button, and click **Create phylogenetic pattern**. The Register phylogenetic Pattern screen is displayed, and phylogenetic patterns according to the presence or absence of genes in the selected cluster are displayed thereon.



2. On the Register Phylogenetic Pattern screen, specify the profile name and weight for each species.

- To change the weight, select the species in the list of species on the lower right and specify the weight in the **Setting Value** column.

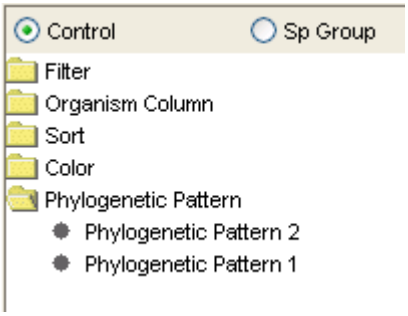
  ✧ **Value**: Specify the weight value.

  ✧ **Ignore**: Specify the species to be ignored in determining the coefficient of correlation.

After selection, click the **Set Value** button.

- You can change the weight using a species set. To do so, select the species set in the **Species Set** column above the list of species. Upon selection, the species contained in the species set are selected in the list of species. Then, set the weight of specified species in the **Setting Value** column.

3. After specifying the conditions, click the **Apply** button. The profile is displayed in **Phylogenetic Pattern** on the control panel.



33.2. Editing a profile

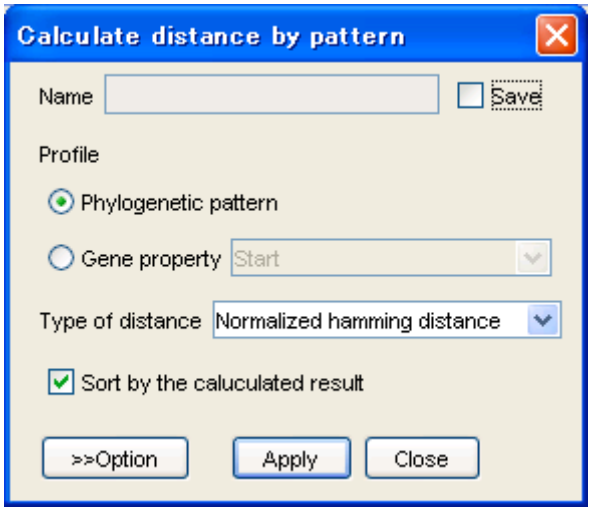
1. To display the Register phylogenetic Pattern screen, select a profile in **Phylogenetic Pattern** on the control panel, click the right mouse button, and click **Edit pattern**.
2. Edit the profile. For the editing method, refer to "33.1 Profile registration from a cluster."
3. After editing the profile, click the **Apply** button.

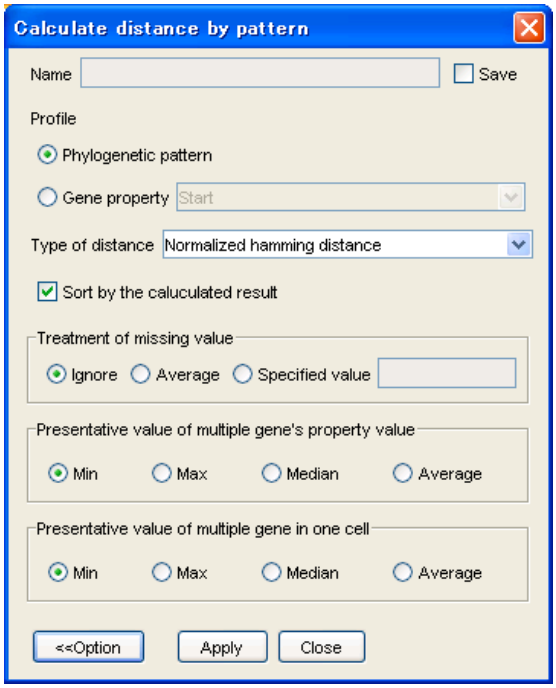
33.3. Removing a profile

1. Select a profile in **Phylogenetic Pattern** on the control panel, click the right mouse button, and click **Delete pattern**. When the warning message is displayed, click the **OK** button.

33.4. Similar phylogenetic pattern search

1. To display the Calculate distance by pattern screen, select a profile in **Phylogenetic Pattern** on the control panel, click the right mouse button, and click **Calculate distance by pattern**.





2. On the Calculate distance by pattern screen, specify the conditions for determining the pattern similarity.

- **Name** column, **Save** column

  To save the calculation results to a file, check the **Save** column and enter the name. If the calculation results are saved to a file, they can be used even if the DomClust result file is reloaded.

- **Profile** column

  Specify the profile type.

  ✧ **Phylogenetic pattern**

  Use as a profile the binary vector representing the occurrence pattern of species (expressing the presence or absence of species as 0 or 1, respectively).

✧ **Gene property**

Use as a profile the numerical vector representing a property value of each gene based on the specified gene property.

- **Type of distance**

Specify the index for calculation to be used as the value of dissimilarity, in which 0 is the nearest and 1 is the farthest.

✧ **Normalized hamming distance**

✧ **Correlation coefficient**

✧ **Correlation coefficient, absolute**

✧ **Mutual information**

* If 'Gene property' is specified in the **Profile** column, only the 'Correlation coefficient' can be specified.

- **Sort by the calculated result**

If this is checked, the PPM table is sorted using the calculated distance values.

3. To set the following conditions, click the **>>Option** button.

- **Treatment of missing values**

In cases where there is no gene corresponding to a species in a cluster, specify the calculation method to cope with any missing values. This setting becomes effective when **Gene property** is specified in **Profile**.

✧ **Ignore**

Calculate in disregard of the species.

✧ **Average**

Calculate by applying the mean of the values of the other genes in the same cluster.

✧ **Specified value**

Calculate by applying a specified value.

- **Representative value of multiple gene property values**

  Specify the method of determining the representative value when multiple values are defined for a gene.

  - ✧ **Min**: The minimum value among multiple values is used.
  - ✧ **Max**: The maximum value among multiple values is used.
  - ✧ **Median**: The median among multiple values is used.
  - ✧ **Average**: The mean value of multiple values is used.*

- **Representative value of multiple genes in one cell**

  Specify the method of determining the representative value when there are multiple genes in a cell.

  - ✧ **Min**: The minimum value among multiple genes is used.
  - ✧ **Max**: The maximum value among multiple genes is used.
  - ✧ **Median**: The median among multiple genes is used.
  - ✧ **Average**: The mean value of the gene properties of multiple genes is used.*

4. To calculate the dissimilarity of the specified pattern with each cluster, click the **Apply** button after specifying the conditions.

   After the phylogenetic pattern similarity search is completed, the calculated dissimilarity value is displayed on the label on the side of the PPM. Also, if **Sort by the calculated result** is checked, the PPM is sorted based on the value of dissimilarity.

   The dissimilarity value is registered as a cluster property under the following name for use in analysis:

   - If the **Save** column is checked: the entered name.
   - If the **Save** column is unchecked: "Phylogenetic Pattern Coefficient."

33.5. Uses of the results of the phylogenetic pattern similarity search

The results of the phylogenetic pattern search can be used in the following functions:

- Display on the cluster header
- PPM sorting (see "13 PPM Sort")

- Filtering by keyword search (see "17 Keyword Search")
- Color setting by properties (see "12 Color Display by Properties")

33.6. Removing the phylogenetic pattern similarity search

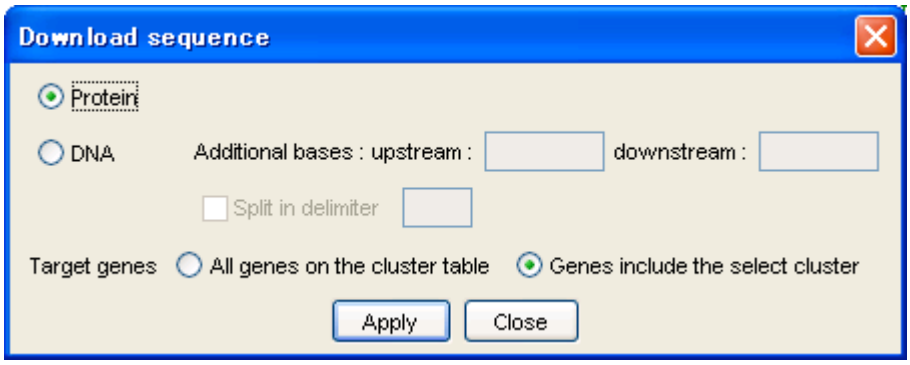
1. The coefficient of correlation saved to a file upon checking the **Save** column can be removed on the **Cluster property** tab on the Gene property/Cluster property screen. For the method of removal, refer to "29.2 Referencing a list of gene/cluster properties".

34. Downloading the sequence information

Download the protein/DNA sequences of the genes contained in the cluster selected on the PPM.

34.1. Downloading the sequence information

1. Select a cluster on the PPM.
2. Click the right mouse button and choose **Download sequence…**. The Download sequence screen is displayed



3. On the Download sequence screen, select amino-acid sequence or DNA sequence.

- **Protein**

  Download the amino-acid sequence.

- **DNA**

  Download the DNA sequence. For the DNA sequence, the extra sequence count obtained upstream and downstream and the delimiting letter between the extra sequence obtained and the gene DNA sequence can be specified.

4. In **Target genes**, specify the target gene.

- All genes on the cluster table

  The genes contained in all the clusters displayed on the PPM are the targets.

- Genes included in the selected cluster

  The genes contained in the cluster currently selected on the PPM are the targets.

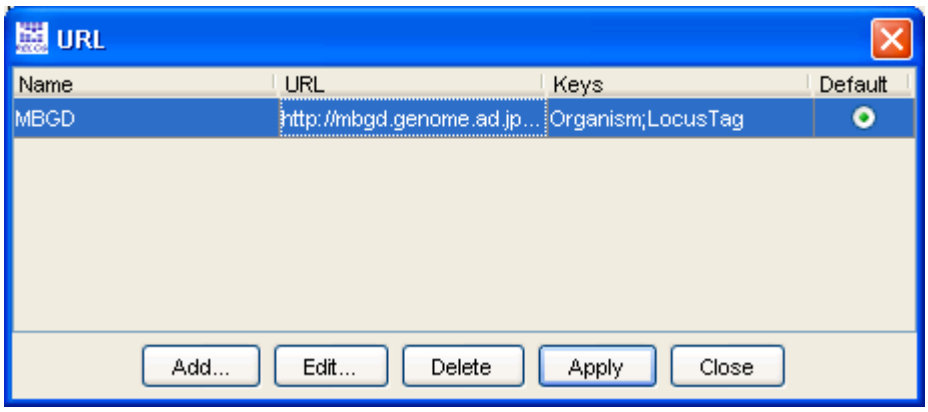
5. After specifying the conditions, click the **Apply** button. When the screen for file saving is displayed, enter the file name and click the **OK** button.

35. Management of External Resource URL's

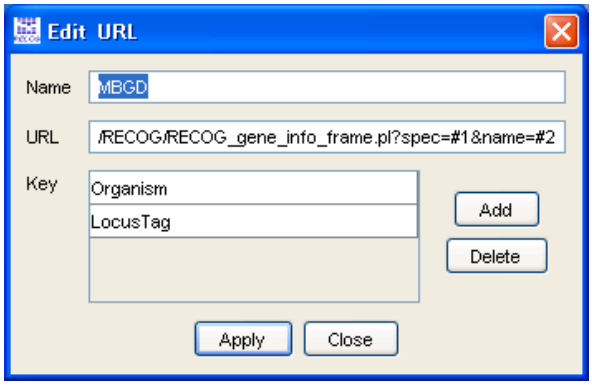
To display the information on external resources in a web browser from the **Info** tab and the Regional Genome Map, register the external resource URL's.

35.1. Registering an external resource URL

1. Click **Option** - **URL…** to display the URL screen.



2. To display the Edit URL screen, click the **Add** button on the URL screen.



3. On the Edit URL screen, specify the name of an external resource (**Name**), the **URL**, and the gene property (**Key**) to be used as the key for the URL.
   To embed a gene property value in the URL, enter "**#<number>**" in the URL, click the **Add** button, and specify the gene property in the **Key** column.

   (Example) MBGD gene data URL

   http://mbgd.genome.ad.jp/htbin/RECOG/RECOG_gene_info_frame.pl
   ?spec=#1&name=#2

#1: Gene property **Organism**

#2: Gene property **Locus Tag**

4. To display the registered external resource URL on the URL screen, click the **Apply** button on the Edit URL screen.

5. Click the **Apply** button on the URL screen.

35.2. Editing an external resource URL

1. Click **Option - URL…** to display the URL screen.

2. To display the Edit URL screen, specify the external resource to be edited on the URL screen and click the **Edit** button.

3. On the Edit URL screen, edit the external resource information.

4. On the Edit URL screen, click the **Apply** button.

5. On the URL screen, click the **Apply** button.

35.3. Removing an external resource URL

1. To display the URL screen, click **Option - URL…**.

2. On the URL screen, specify the external resource to be removed, and click the **Delete** button. When the Confirm screen is displayed, click the **OK** button.

3. On the URL screen, click the **Apply** button.

36. Appendix

36.1. DomClust parameters

Parameter	Details
Cutoff BLAST E-value	This value specifies a cutoff E-value for the BLAST results. The maximum value is 1e-2. Note that, in MBGD, the E-value is adjusted so that the size of the search space (the database size times the query length) is 1e9.
Cutoff DP score	Cutoff score of the optimal local alignment with the JTT-PAM250 scoring matrix (Jones *et al*., 1992). The same cutoff is used for both the selection and the clustering steps when score is used as a similarity measure.
Cutoff PAM distance	PAM is a unit of evolutionary distance defined as the number of accepted point mutations per 100 residues (Dayhoff *et al*., 1978). The PAM distance is estimated based on the PAM substitution matrix which gives the best alignment score. The same cutoff is used for both the selection and the clustering steps when PAM is used as a dissimilarity measure.
Cutoff percent identity	Percent identity is defined as ***{the number of identical residue pairs} / {alignment length} * 100***. The alignment length includes the internal gaps.
Alignment coverage	Alignment coverage is defined as ***{alignment length} / {length of the shorter sequence} * 100***. Raising this parameter removes matches only in short regions *before* the clustering procedure. MBGD does not conduct this check by default.
Alignment coverage for domain splitting	In MBGD, a domain-splitting procedure is incorporated in the hierarchical clustering algorithm. When merging two most similar sequences (or clusters), the algorithm searches for another sequence (S3) that matches one of the merged sequences (S1) in the region outside the alignment between the merged sequences. The algorithm splits the sequence S1 if such a sequence S3 is found and the alignment between S1 and S3 satisfies the coverage condition specified by this parameter and score condition specified by the next parameter. Raise this parameter to avoid excessively short domains generated due to partial matches.
Score cutoff for domain splitting	Cutoff score for the match between S1 and S3 described above to split the sequence. The effect of this parameter is similar but possibly complementary to that of the previous parameter.
Similarity measure for orthology	This option specifies which similarity or dissimilarity measure (score or PAM) for use in orthology identification or clustering. Note that the scores depend on the alignment lengths while PAMs do not.
Best hit criterion	The bi-directional best hit criterion (i.e. gene pairs ***(a,b)*** of genomes A and B, such that ***a*** is the most similar gene to ***b*** in A and vice versa), is a conventional approach for ortholog identification between two genomes. The uni-directional version

	is also routinely used for predicting gene functions. MBGD does not use such a criterion in the selection step by default, since the UPGMA algorithm itself must involve it, but in some situations, it might be useful for the purpose of filtering out some apparent paralogs before clustering. See the next section for details.
Cutoff ratio of the score against the best	This parameter is not effective when the best-hit criterion above is not used. Orthology need not be a one-to-one relationship. As the bi-directional best-hit criterion, two genes ***(a,b)*** are considered to be orthologs when score***(a,b)*** satisfies ***score(a,b) / max(max_y(score(a,y)), max_x(score(x,b))) * 100 >= cutoff_ratio,*** where ***x*** and ***y*** are any genes of genomes A and B, respectively. Using ***cutoff_ratio*** =100 corresponds to the exact bi-directional best-hit criterion. Similarly, as the unidirectional best-hit criterion, two genes ***(a,b)*** are considered to be orthologs when ***score(a,b) / min(max_y(score(a,y)), max_x(score(x,b))) * 100 >= cutoff_ratio.***
Score for missing relationships	Although the usual hierarchical clustering algorithm requires a complete similarity/dissimilarity matrix, here only significant similarities found by the search are used. This option specifies a value to be assigned for the relationships missed by the search. The value must be smaller (larger) than the similarity (dissimilarity) cutoff. Specifying an extremely small (large) value will result in a classification similar to that by complete linkage clustering, whereas specifying a value close to the cutoff gives results similar to that by single linkage clustering. The default value (=blank) is ***{score_cutoff * 0.95} or {pam_cutoff / 0.95}.***
Clustering Mode	This option specifies whether orthologous or homologous groups shall be created. It is simply equivalent to omitting the tree-splitting procedure described below by specifying ***phylocut*** **> 1**.
Cutoff ratio of paralogs for tree splitting	In MBGD, orthologous groups are created by splitting the trees of homologous clusters created by the hierarchical clustering algorithm. A node with two children, A and B, is split when **| Intersect(Ph(A),Ph(B)) | / min(|Ph(A)|, |Ph(B)|) > *phylocut***, where Ph(A) denotes the set of species contained in node A (phylogenetic pattern), |Ph| denotes the cardinality of Ph, and Intersect(A,B) is the intersection of sets A and B. This parameter is not effective when ClusteringMode = 'homology' is specified.
Phylogenetically related organisms	When counting the number of species in the above calculation, one can incorporate taxonomic information by counting related species only once. A taxonomic rank can be specified to determine which set of organisms are considered to be related.
Overlap ratio (r_{adj1}) for merging	After the tree-splitting procedure described above, two clusters

adjacent clusters	of domains are joined when they are almost always adjacent to each other. More precisely, two clusters A and B are joined when $\|adjacent(A,B)\| / max(\|A\|,\|B\|) >= r_{adj1}$ or $\|adjacent(A,B)\| / min(\|A\|,\|B\|) >= r_{adj2}$, where adjacent(A,B) is a set of domains belonging to A and B that are adjacent to each other, and r_{adj1} and r_{adj2} are parameters satisfying $0 <= r_{adj1} <= r_{adj2} <= 1$.
Coverage ratio (r_{adj2}) for absorbing adjacent small clusters	See above. Note that this parameter is not effective if $r_{adj2} <= r_{adj1}$.
Relative weight for horizontal transfer	Relative weight for horizontal transfer ($0 <= x <= 1$)
Use domclust dump	If this is checked, the DomClust analysis is conducted using the cache for previous DomClust analysis results. This shortens the processing time of the analysis.

37. Glossary

A

Aggregation

Integration of clusters of the same phylogenetic pattern into a single row in the phylogenetic pattern map.

C

Cell

A square corresponding to a species belonging to an ortholog group on the PPM

CoreAligner（http://mbgd.genome.ad.jp/CoreAligner/）

A software program for creating a core structure based on the preservation of gene alignment sequences between affinity genomes

Circular Genome Map (CGM)

A gene map on which genes, *etc*. are drawn in a circle

ClustalW

A software program for performing the Multiple Alignment

Cluster

A group of genes grouped together based on DomClust analysis results

Cluster ID

A unique ID given to each cluster

COG（http://www.ncbi.nlm.nih.gov/COG/）

Clusters of Orthologous Groups of proteins (COGs)

D

DomClust（http://mbgd.genome.ad.jp/domclust/）

Hierarchical clustering program for orthologous protein domain classification, which is the standard method to construct ortholog groups in RECOG.

E

Extra Taxonomy Tree

The Taxonomy Tree shown on the **Selected** tab

F

Function category

Functional classification attendant on and characterizing genes and clusters

G

Gene Property

Property values associated with each gene, which can be given by the user.

Genome Comparison Viewer

A genome map in which the gene core structure is colored based on the results of DomClust analysis and CoreAligner analysis

I

Ingroup

The set of target species in phylogenetic analysis. In the DomClust analysis, ingroup species is specified as the set of related species on which the attention of the user is focused

M

MBGD (http://mbgd.genome.ad.jp)

Microbial Genome Database for Comparative Analysis, which is the standard genome database in RECOG.

N

Neighborhood gene clustering

A method for identifying genes that are located near both in the phylogenetic pattern map and in the genomic sequence.

O

Ortholog group (cluster)

A group of homologous genes made by clustering based on the orthologous relationships between genes. In RECOG, ortholog groups are constructed by the DomClust program and an ortholog table created based on the ortholog groups is used as a basis for any comparative anaylsis. In the genomic core structure analysis, a core structure alignment is generated by reordering ortholog groups based on the conserved gene order along each chromosome.

Ortholog table

A table showing orthologous relationships among species, where each row represents ortholog group and each column represents species. In RECOG, an ortholog table is represented

as a phylogenetic pattern map (PPM).

Outgroup

The set of control species in phylogenetic analysis, which are located outside of the ingroup on the phylogenetic tree. In the DomClust analysis, outgroup species is specified as the group of species which do not belong to the ingroup.

P

Phylogenetic pattern

In a strict sense, a binary vector that indicates the presence (1) or absence (0) of a gene for each species defined for each orthologous group. In a more broad sense, a numeric vector that indicates some gene property value of each species.

Phylogenetic Pattern Map (PPM)

A matrix that shows the presence or absence of species based on the DomClust analysis results

R

RECOG（http://mbgd.genome.ad.jp/RECOG/）

Research Environment for Comparative Genomics

A client server-type software program that is a comparative genome workbench for conducting various comparative analyses based on DomClust analysis results

RECOG server

The server that conducts the DomClust analysis and CoreAligner analysis and provides gene information in concert with the RECOG Client

Regional Genome Map (RGM)

A genome map for ortholog comparison

T

Taxonomy Tree

A phylogenetic species classification in tree form