

BIONUMERICS Tutorial: Exporting trees to iTOL

1 Introduction

The *Interactive Tree Of Life* or iTOL [1] is a popular online tool for tree visualization and annotation. A publicly accessible instance of this software is available on https://itol.embl.de/ (see Figure 1).



Figure 1: The iTOL home page on https://itol.embl.de/.

The *iTOL integration plugin* in BIONUMERICS allows you to upload dendrograms and meta data from your BIONUMERICS database to the iTOL server.

2 Preparing the database

The **DemoBase Connected** will be used in this tutorial and can be downloaded directly from the *BIONUMERICS Startup* window or restored from the back-up file available on our website:

1. To download the database directly from the *BIONUMERICS Startup* window, click the button, located in the toolbar in the *BIONUMERICS Startup* window. Select **DemoBase**

Connected from the list and select *Database* > *Download* (\square). Confirm the download action.

2. To restore the database from the back-up file, first download the file DemoBase_Connected.bnbk from https://www.bionumerics.com/download/sample-data, under 'DemoBase Connected'.

In the *BIONUMERICS Startup* window, press the b button, select *Restore database*, browse for the downloaded file and select *Create copy*. Specify a name and click < OK >.

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In contrast to other browsers, some versions of Internet Explorer rename the DemoBase_Connected.bnbk database backup file into DemoBase_Connected.zip. If this happens, you should manually remove the .zip file extension and replace with .bnbk. A warning will appear ("If you change a file name extension, the file might become unusable."), but you can safely confirm this action. Keep in mind that Windows might not display the .zip file extension if the option "Hide extensions for known file types" is checked in your Windows folder options.

3 Prerequisites

In order to benefit from the features in the *iTOL integration plugin*, you need an iTOL account with an active subscription and an API key generated.

1. First log in to your iTOL account or create an account on https://itol.embl.de/itol_account.cgi following the instructions outlined on this page.

iTOL requires an active subscription to upload trees, i.e. free access will not work. For more information on iTOL accounts, see https://itol.embl.de/infoReg.cgi.

2. Check if you have an active subscription on the iTOL account information page https://itol. embl.de/userInfo.cgi. If not, purchase your subscription keys and activate your subscription according to the instructions that came with the keys.

Finally, you need to generate an API key which the *iTOL integration plugin* (or other software) uses to identify itself against the iTOL API.

3. On the iTOL account information page, press the "Create API key" button.

Your API key is shown on the same page (see Figure 2).

This API key should be copied and pasted in the *iTOL settings* dialog box during installation of the *iTOL integration plugin* (see 4).

Optionally, a dedicated project can be generated to which trees generated in your BIONUMERICS database are added. A default project called "Sample project" is always generated.

4 Installing the iTOL integration plugin

The *Plugins and Scripts* dialog box can be called from the *Main* window by selecting *File* > *Install* / *remove plugins...* (, c=) (see Figure 3).

When a particular plugin is selected from the list of plugins, a short description appears in the right panel.

A selected plugin can be installed with the *<Install*> button. The software will ask for confirmation before installation. Some plugins are only supported in specific BIONUMERICS configurations. If

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| | | Email: | BE-DAU-INFO@b | piomerieux.com | | Change email | | | | | |
| | s | ubscriptions | | | Abo | ut iTOL subscr | iptions | | | | |
| | | Standard subscription: | 2022-04-28 (357 | days remaining) | a ≙ Ex | tend subscripti | on | | | | |
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| | A | account statistics | | | | | | | | | |

Figure 2: The iTOL account information page with the API key indicated.



Figure 3: The Plugins and Scripts dialog box.

the plugin is not supported by your BIONUMERICS configuration, it cannot be installed and an error message will be generated.

Once a plugin is installed, it is marked with a green V-sign. It can be removed again with the <*Uninstall*> button.

If the selected plugin is documented, pressing < Show Manual > will open its manual in the Help window.

Proceed as follows to install the *iTOL integration plugin*, starting from the *Plugins and Scripts* dialog box:

- 1. Select the *iTOL integration plugin* in the list and press the *<Install*> button.
- 2. Confirm the installation of the plugin and press < OK >.

The *iTOL settings* dialog box pops up (see Figure 4).

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|----------------------------|----------------------|----|-------|
| User API key Project | name | | |
| Server | | | |
| Туре | Public | | |
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| URL | https://itol.embl.de | | |
| | ОК | Са | incel |

Figure 4: The *iTOL settings* dialog box.

In the *API key* text box, an API key as generated on the iTOL account information page should be entered (see ??).

Project name corresponds to the name of the iTOL project under which the exported trees will be listed.

By default, the plugin integrates with the *Public* iTOL server from EMBL (https://itol.embl. de/), which will be applicable for most users. If desired, a *Private* iTOL server can be used after entering the corresponding *URL*.

3. Enter **API key** and **Project name** and press <**OK**> to close the *iTOL settings* dialog box.

A message appears to indicate that the *iTOL integration plugin* is installed, with the recommendation to restart the database.

4. Press < OK > to close the message.

Once the plugin is successfully installed, it is marked with a green V-sign in the *Plugins and Scripts* dialog box.

- 5. Press < *Close* > to close the *Plugins and Scripts* dialog box.
- 6. Close and reopen the database to activate the features of the *iTOL integration plugin*.

The *iTOL integration plugin* adds items to the menu of the *Comparison* window.

5 Exporting trees to iTOL

Any tree in the *Comparison* window, regardless the dataset it was calculated on, can be exported to iTOL with the *iTOL integration plugin*. It works according to the WYSIWYG (What You See Is What You Get) principle, meaning that – within the limits of what can be visualized in iTOL – whatever is shown in the *Comparison* window, will be included in the export. In summary, following data is exported:

- The dendrogram currently displayed in the *Dendrogram* panel.
- Any experiment data currently visualized in the *Experiment data* panel. All experiment types will be interpreted as characters and can be visualized in iTOL as heat maps. Note that sequences longer than 4,000 bases will not be exported, as they cannot be displayed in iTOL anyways.
- The entry information displayed in the Information fields panel.
- When defined, comparison groups, with their group labels and group colors.
- The similarity matrix as shown in *Similarities* panel.

As an example we will export a RFLP based tree together with experiment data, the similarity matrix and meta data to iTOL for tree annotation.

1. Select all entries in the database with the **Ctrl+A** and unselect the three entries which have the information "STANDARD" in the *Genus* information field (see Figure 5).

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| | 4 PhenoTest | Character type | | | 2020-06-12-09-22-00 | Vercingetorix | nalustria | 42815 | | | | | | | |
| n. | 5 FAME | Character type | | G@Gel07@005 | 2020-06-12 09:22:00 | Ambiorix | sylvestris | 52416 | | | | | | | |
| | 6 165 rDNA | Sequence type | | G@Gel07@006 | 2020-06-12 09:22:00 | Ambiorix | 50 | 52415 | | | | | | | |
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Figure 5: The main window with the entry selection.

- 2. Highlight the *Comparisons* panel in the *Main* window and select *Edit* > *Create new object...* (+) to create a new comparison for the selected entries (see Figure 6).
- 3. Make sure the *RFLP1* experiment is selected in the *Experiments* panel (see Figure 7) and select *Clustering* > *Calculate* > *Cluster analysis (similarity matrix)...*.



Figure 6: Create a new comparison.

| Experiments | | | | | | | | |
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| | b. | DNA-Hybrid | <default></default> | | | | | |
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Figure 7: The *RFLP1* experiment highlighted.

- 4. Click <*Next*> and <*Finish*> to generate a tree based on the default selected similarity coefficient and clustering method.
- 5. In the *Information fields* panel, right-click the *Genus* information field header, select *Groups* > *Create groups from database field* (see Figure 8) and click <*OK*> to create groups of entries with the same genus name.

| Info | rmation fields | | | Similarities | | | | |
|------|---------------------|----------------------------|--------------------------------------|---|--|--|--|--|
| R | ¦ ∐\ ३ î | ↓ ^{100%} ↑ | Ļ | i ↓†∔ 122 | | | | |
| | | | | RFLP1 0 _ 20 _ 40 _ 60 _ 80 _ 100 | | | | |
| | fied date | Genus | | | | | | |
| ~ | 06-12 09:22:00 | Ambiorix | ↓ Arrange entries by field | | | | | |
| ~ | 06-12 09:22:00 | Ambiorix | Arrange entries by field (inverted) | | | | | |
| ~ | 06-12 09:22:00 | Ambiorix | Arrange entries by field (numerical) | | | | | |
| ~ | 06-12 09:22:00 | Ambiorix | Arrange | antries by field (inverted+ numerical) | | | | |
| ~ | 06-12 09:22:00 | Ambiorix | Anange | entries by field (inverted+fidffefical) | | | | |
| ~ | 06-12 09:22:00 | Ambiorix | Create groups from database field | | | | | |
| 1 | 06-12 09:22:00 | Ambiorix | Create gr | oups norm autobase meta | | | | |

Figure 8: Creating groups.

- 6. Click on the eye icon next to the *PhenoTest* experiment in the *Experiments* panel (see Figure 9) to make the experiment data visible in the *Experiment data* panel.
- 7. Press *Clustering* > *Similarity matrix* > *Show values* (Im) to make the similarity values visible in the *Similarities* panel.

The Comparison window should now look like Figure 10.

| Experiments | | | | | | | | |
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| | | RFLP2 | <all band="" classes=""></all> | | | | | |
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| | b. | DNA-Hybrid | <default></default> | | | | | |
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Figure 9: Showing the *Phenotest* experiment data.

| Comparison | | | | - 🗆 X |
|---|--|---|--|--|
| File Edit Layout Groups Clustering | Statistics Fingerprints Characters Sequence TrendData Read | Sets Spectra Composite iTOL Window Help | | |
| 周局 局 ≭ 創作 | PhenoTest 🔘 🗗 🗽 | Ba h | | |
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| Experiments | | | | |
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| C III RFLP2 | | | | RFLP1 |
| C E AFLP | RFLP1 | 8 8 8 8 8 8 8 8 | | 0 20 40 60 80 100 |
| C PhenoTest | 0 40 50 60 70 80 90 100 | | hed date Genus Species V | |
| C FAME | | 2.528 0.412 2.674 0.836 2.649 0.594 2.068 2.566 | C 06-12 09:22:00 Ambiorix sylvestris | 995 100 932 907 909 919 929 847 895 883 8 |
| 16S rDNA | [| 2.500 0.300 2.707 0.529 2.753 0.408 2.472 2.457 2.394 0.516 2.819 0.782 2.364 0.311 2.866 2.449 | CONTRACTOR AND | 92.8 93.2 100 68.3 69.8 68.5 91.4 84.2 68.9 68.2 7 |
| C L DNA-Bybrid | | 1.477 0.882 2.218 1.143 2.503 1.828 2.529 2.612 | C 06-12 09:22:00 Ambiorix sp. | 90.9 90.7 88.3 100 95.7 92.7 92.9 89.8 89.6 89.1 8 |
| < > | | 2.423 0.354 2.797 0.747 2.375 0.684 2.839 2.036 | 06-12 09:22:00 Ambiorix sylvestris | 91.3 90.9 89.8 95.7 100 92.6 94.1 91.8 89.5 87.6 9 |
| Analyses Local composite datasets | -16 | 2.353 0.425 2.429 0.524 2.730 0.383 2.815 2.698 | C6-12 09:22:00 Ambiorix sylvestris | 92.5 91.9 88.5 92.7 92.6 100 98.5 92.4 91.0 80.2 9 |
| 51 0 | | 2.127 0.429 2.775 0.539 2.781 0.314 2.483 1.212 | O6-12 09:22:00 Ambiorix aberrans | 93.3 92.9 91.4 92.9 94.1 98.5 100 91.9 89.7 83.3 9 |
| | | 2.815 0.353 2.581 0.531 2.689 0.213 2.812 2.007 | O6-12 09:22:00 Ambiorix sylvestris | 85.2 84.7 84.2 89.8 91.8 92.4 91.9 100 95.2 83.5 9 |
| Name 🗸 🗸 | | 2.239 0.383 2.731 0.610 2.850 0.694 1.965 2.398 | 06-12 09:22:00 Ambiorix sylvestris | 874 883 882 891 876 802 833 835 895 100 7 |
| E RFLP1 | - II | 2493 0380 2447 0534 2898 0592 2245 2783 | C 06-12 09-22:00 Ambiorix sylvestris | 81.0 80.1 79.6 88.5 91.0 92.8 91.9 92.6 80.3 75.6 1 |
| | | 2,756 0,410 2,428 0,524 2,360 0,286 2,609 2,644 | C 06-12 09:22:00 Ambiorix svivestris | 80.5 79.9 79.2 83.7 88.5 91.9 90.8 93.1 89.4 72.5 9 |
| | | 2.820 0.513 2.425 0.839 2.466 0.495 2.492 2.642 | 06-12 09:22:00 Ambiorix sylvestris | 82.3 81.9 76.8 83.2 83.7 92.9 90.8 88.2 81.7 70.0 9 |
| | | 2.124 1.208 1.632 1.373 2.528 1.549 2.686 2.843 | C6-12 09:22:00 Ambiorix sp. | 74.5 74.8 78.0 89.8 87.0 76.3 78.7 80.4 75.5 80.9 8 |
| | | 2.624 0.414 2.871 0.963 2.362 0.395 2.498 2.732 | 06-12 09:22:00 Perdrix pseudoarchaeus | 77.7 78.0 81.3 87.1 89.8 77.2 80.3 84.0 80.8 82.1 8 |
| | | 1.496 0.546 1.934 0.545 2.542 0.493 2.768 2.850 | O6-12 09:22:00 Ambiorix sylvestris | 89.1 88.8 86.2 92.6 92.2 82.4 83.8 83.6 86.2 78.3 8 |
| | | 2.043 1.112 2.187 1.235 2.477 1.277 2.484 1.895 2.307 0.356 2.720 0.569 2.420 0.655 2.580 2.505 | 06-12 09:22:00 Ambiorix sp. 06 12 09:22:00 Ambiorix subjective | 81.0 81.4 83.1 80.5 84.7 80.9 82.0 77.9 77.6 77.6 7 |
| | | 2.296 0.574 2.750 1.324 2.825 0.814 1.489 2.783 | 66-12 09:22:00 Ambiorix sylvestris | 88.3 89.1 89.4 81.8 83.9 75.4 79.7 72.6 80.9 89.2 4 |
| | | 1.539 0.385 2.435 0.995 2.260 0.474 2.505 2.358 | 06-12 09:22:00 Ambiorix sylvestris | 84.8 85.7 85.0 79.1 81.1 78.0 81.8 09.3 74.7 81.8 |
| Groups | | 1.646 0.458 2.583 0.572 2.462 0.392 2.130 2.658 | 06-12 09:22:00 Perdrix pseudoarchaeus | 67.8 67.7 67.8 67.7 71.0 65.8 66.8 72.1 62.9 66.9 6 |
| ▶◎ 誓 誓 點 | | 2.405 0.200 2.721 0.553 2.659 0.019 1.836 1.885 | 06-12 09:22:00 Perdrix pseudoarchaeus | 67.5 57.8 01.2 00.9 69.6 60.7 03.2 70.9 03.6 02.9 0 |
| Size Name | | 1.994 0.248 2.554 0.551 2.745 0.279 2.524 2.485 | 06-12 09:22:00 Perdrix pseudoarchaeus | 64.5 64.8 67.5 68.2 70.4 69.6 84.1 60.7 65.6 66.3 5 |
| 22 Amb | | 1.842 0.418 2.188 0.888 2.225 0.150 1.909 2.474 | C 06-12 09:22:00 Perdrix pseudoarchaeus | 63.7 63.8 63.0 67.0 63.2 63.0 63.3 60.6 64.8 63.3 6 67.2 65.6 71.6 69.1 72.2 59.9 64.0 63.0 57.0 64.6 6 |
| 16 Perdrix | | 1.002 0.202 2.150 0.041 2.241 0.047 1.072 1.924 | OS-12 09-22 00 Perdrix pseudoarchaeus OS-12 09-22 00 Perdrix pseudoarchaeus | 02.9 03.1 07.2 09.3 72.6 02.6 05.9 05.5 05.6 05.6 0 |
| 8 Ver | | 1.975 0.236 2.900 0.557 2.857 0.524 2.827 2.569 | O6-12 09-22:00 Perdrix pseudoarchaeus | 67.1 67.3 73.7 74.0 77.4 68.2 69.3 73.5 68.1 70.6 7 |
| | | 1.815 0.204 1.493 1.536 2.509 0.100 2.233 2.760 | 06-12 09:22:00 Perdrix pseudoarchaeus | 07.2 05.7 75.6 74.1 77.2 07.5 70.4 72.2 05.6 05.8 0 |
| | L | 1.650 0.244 2.897 1.702 2.420 0.714 1.853 2.628 | 06-12 09:22:00 Perdrix pseudoarchaeus | 84.4 84.0 71.5 70.9 72.0 66.0 67.7 69.5 66.7 65.2 6 |
| | | 2.709 0.277 2.875 0.543 2.220 0.116 2.341 2.734 | 06-12 09:22:00 Perdrix pseudoarchaeus | 67.2 67.8 06.1 07.2 68.4 58.7 81.1 08.0 02.6 04.8 6 |
| | | 2.021 0.279 2.597 0.960 2.605 0.527 2.737 2.630 | Contraction of the second seco | 07.1 07.8 05.2 04.9 07.3 00.7 80.5 03.8 58.5 04.7 0 |
| | | 1.038 1.497 1.668 1.144 1.583 0.546 1.841 2.056 | C 06-12 09:22:00 Perdrix pseudoarchaeus | 75.4 75.5 62.1 74.6 60.3 71.5 75.3 73.6 67.8 69.2 7 |
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Figure 10: The Comparison window.

Select *iTOL* > *Upload to iTOL...* to upload the tree, experiment data, metadata and similarity values to iTOL (see Figure 11).

The tree is uploaded under its **Dendrogram name**, as provided in the *Page 2* wizard page of the *Similarity coefficient* wizard and can be further annotated and customized in iTOL and exported in different formats. The **PhenoTest** experiment data, the similarity matrix, the defined groups and information fields can be made visible next to the tree by toggling on the respective dataset (see Figure 11). Each dataset is also associated with a dataset configuration button to access dataset specific options. For a description of the iTOL annotation functionality, we refer to the iTOL help pages.



The uploaded tree can only be saved in your project when you are logged in with your iTOL account.



Figure 11: The iTOL interface with the uploaded tree and the group labels and similarities datasets toggled on.

Bibliography

[1] Ivica Letunic and Peer Bork. Interactive tree of life (itol) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research*, 04 2021. gkab301.