



JELLYFISH
Version 3.3
USER GUIDE
Printable Version

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Introduction

Jellyfish is a user-friendly software package for research biologists. Its graphical user interface allows you to perform gene analysis quickly and efficiently. Translate DNA, align sequences, perform restriction digests, and submit sequences to BLAST and other analysis websites just by pointing and clicking.

The continued evolution of Jellyfish depends on user feedback. We want to know what you think about the software. Let us know your suggestions, ideas, complaints, questions, and overall impression. Be a part of the biological community that's helping to make Jellyfish one of the most valuable and efficient research tools available. Contact us at jellyfish@fieldscientific.com.

Installation

To begin, download the Installer program from the Jellyfish web site. Once the icon for the installer is on your desktop, double-click it to begin the installation process. Follow the on-screen instructions. When installation is complete, click on the Jellyfish desktop icon to start the program. A dialogue box appears asking for your registered email address and password. (If you are not a registered Jellyfish user, please go to <http://www.jellyfishsoftware.com> to register before using this program.) You only need to enter your email address once. The next time you open Jellyfish, your email address will be listed automatically as a user; just click on your name and click the login button.

Your initial login, as well as most of the functions of Jellyfish, requires an Internet connection.

Proxy/Firewall Settings:

For some corporate systems, it may be necessary to establish proxy or firewall settings. If this is the case, a dialog box will pop up with the New User Dialog box, asking you for proxy settings. See your system administrator for the exact information to enter. Once you have opened Jellyfish, proxy settings may be changed by clicking on Internet Options under Tools in the main menu above the Project Window.

Getting Started

When you log in to Jellyfish, the Jellyfish screen opens on your desktop. You will see a Project Window on the left-hand side of the screen and a panel labeled "Manipulate" on the right-hand side of the screen. The functions of Jellyfish revolve around selecting a file (usually a sequence) in the Project Window and then manipulating the file using one of the panels on the right.

There are two ways to add a sequence to the Project Window:

1) To import a sequence from Genbank, choose "Import from Genbank" from the File menu at the top left of the screen. Enter the search term and select a database from the pulldown menu. Click "Search." Select one or more sequences from the results that appear and click "Fetch." Your selection is automatically entered into the Project Window. If you select only one sequence, it is displayed as its own file in the Project Window. If you select more than one sequence, they are grouped together in the Project Window in their own folder under the search term.

2) If you already have a sequence saved in a file on your computer, choose "Open" from the File menu. In the dialog box that appears, select the file you want to open. The sequence automatically appears in your Project Window.

When you close Jellyfish, all of the files you have created are automatically saved in the Project Window. They will appear in the Project Window the next time you open Jellyfish.

Project Window

The Project Window organizes your sequences. When you select a sequence in the Project Window, that sequence becomes active in the Panels.

Sequence Files

Each sequence is its own file. All oligos and Web tool search results involving a sequence are stored in the sub-folder of that sequence in the Project Window.

To create a file:

For a new DNA sequence, select "New -> DNA sequence" from the File pulldown menu. For other new files, select the appropriate selection from the "New" menu in the File pulldown menu. You can also use the File menu to import a sequence from Genbank or open a file on your computer.


To rename a file:

Click on a file to highlight it, right-click on the file name (for PC users) or hold down the control key and click on the file name (for Mac users) and select "**Rename**" from the pop-up menu. Type in the new name and press **Enter**.

To duplicate a file:


Right-click on the file name (for PC users) or hold down the control key and click on the file name (for Mac users), and select "**Save As...**" from the pop-up menu. Select the location in the dialog box that appears.

To export a file:

Right-click on the file name (for PC users) or hold down the control key and click on the file name (for Mac users), and select "**Export**" from the pop-up menu. Select the location in the dialog box that appears. Additionally, you can use the  button.

To delete a file:

Right-click on the file name (for PC users) or hold down the control key and click on the file name (for Mac users), and select "**Delete**" from the pop-up menu.

Additionally you can use the  button.

Supported Formats:

Jellyfish uses the "readseq" package and supports the following formats:

- * IG/Stanford, used by Intelligenetics and others
- * GenBank/GB, genbank flatfile format
- * NBRF format
- * EMBL, EMBL flatfile format
- * DNASTrider, for common Mac program
- * Fitch format, limited use
- * Pearson/Fasta, a common format used by Fasta programs and others
- * Zuker format, limited use.
- * Olsen, format printed by Olsen VMS sequence editor.
- * Phylip3.2, sequential format for Phylip programs
- * Phylip, interleaved format for Phylip programs (v3.3, v3.4)
- * MSF multi sequence format used by GCG software
- * PAUP's multiple sequence (NEXUS) format
- * PIR/CODATA format used by PIR
- * ASN.1 format used by NCBI
- * GCG, single sequence format of GCG software (use MSF format instead)
- * Plain/Raw, sequence data only (no name, document, numbering)

Panel by Panel

Panels Introduction

Each of the panels performs different analysis tasks on the sequences selected in your Project Window. Some panels are independent and others work in conjunction with each other. Tasks performed in one panel are maintained while you work in other panels.

Manipulate Panel

This panel allows you to explore and manipulate your sequence graphically. Select a sequence from the Project Window. In the Manipulate panel, the sequence is represented graphically at the top in the Graphic Overview window, and textually at the bottom in the Sequence Window.

The **red bar** represents the entire sequence.

The **grey slider** can be dragged with your mouse to navigate the sequence. The portion of the sequence within the **grey slider** is the same portion in the Sequence window.


1. Annotations

The **blue-colored bars** beneath the red bar represent annotations for the selected sequence. Click an annotation and the corresponding sequence is highlighted in the Sequence Window below.

Extend or shrink the bottom margin of the Annotations window by placing your cursor at the bottom edge of the frame; when you get the up-and-down arrow in place of the cursor, drag the frame boundary to the desired place. This allows you to hide the annotations or to display some or all of them.

2. Add Annotations

There are four ways to add new annotations to the sequence you are working with:

- 1) highlight the code in the Sequence window, then click the  Add Annotation button above the Annotation window;
- 2) click the Add Annotation button and type the code location numbers into the boxes;
- 3) highlight the code in the Sequence window, then right-click on the highlighted section (for PC users) or press the control key and click on the highlighted section (for Mac users). Select "Add Annotation" from the pop-up menu;
- 4) highlight the code in the Sequence window, then choose "Add Annotation" in the Actions menu.

On all four of these, you will be prompted to enter a name and description of the annotation, along with the feature type (e.g. gene, allele, D-loop, etc.).

3. BLAST, Save as, Edit and Delete Annotations

If you right-click an annotation (for PC users) or control-click an annotation (for Mac users), you'll see a pop-up window with options to BLAST, Save as, delete or edit that annotation.

a) BLAST: select "BLAST" from the pop-up menu. Unless you have disabled it, a security notice pops up indicating that sequence data is about to be sent off-site. You are taken to the Internet panel, or to an external browser, where your BLAST is queued.

Click the "Format results" button. The Internet panel tells you how many seconds it will take to get your results back, and then displays them as soon as they're available. The annotation is saved as a child of the selected sequence and the BLAST result is saved as a sub file of the annotation.

The following BLAST types are used in Jellyfish:



- if the sequence is DNA, blastn is used
- if the sequence is a protein, blastp is used

Both of these are used against the non-redundant GenBank database.

b) Save as: select "Save as" from the pop-up menu. A dialog box appears asking you to rename the annotation. Click "OK." The new file appears in your Project Window as a sub file of the original sequence.

c) Edit: select "Edit" from the pop-up menu to rename and reposition the annotation. The Edit Annotation button allows you to rename and reposition the annotation. Your changes show up graphically in the Annotations window.

d) Delete: select "Delete" from the pop-up menu. You are asked to confirm the removal of the annotation. Your changes show up graphically in the Annotations window.

N.B.: You can also delete and edit annotations by clicking an annotation and then clicking the Delete Annotation button  or Edit Annotation button  (both located above the Annotations window).

4. Zoom In / Zoom Out buttons

To view annotations more closely, position the purple slider over the annotation and click the Zoom In button. Click again as necessary for a closer view. To return to the normal view, click the Zoom Out button as often as necessary.

5. Reverse Complement button

Clicking this button performs a reverse complement of your entire sequence. The reverse complement is shown both in the Sequence window and in the Annotations window. Additionally, the Reverse Complement icon is displayed beside your File name (at the top of the Manipulate panel) so you know its orientation. Click the Reverse Complement button again to return to the original sequence. You can also perform "Reverse" or "Complement" functions by selecting these options from the Actions pulldown menu.

6. Change Case button

Use this button to change the case of a selected portion of sequence. For example, if you would like a selected portion to show up in all capital letters, highlight the portion and then click the Change Case button. Click this button twice and the highlighted portion will go to all lowercase letters.

7. BLAST Sequence button

Submit a selected sequence to the NCBI BLAST server by clicking the BLAST button. (If you have made any changes to the sequence up to this point, you will be asked whether you want to save the changes before the sequence is BLASTed.) The Internet panel opens with your BLAST request. Click the "Format results" button. The Internet panel tells you how many seconds it will take to get your results back, and

then displays them as soon as they're available. The BLAST request is automatically added as a sub file to the original sequence in the Project Window.

The following BLAST types are used in Jellyfish:

- if the sequence is DNA, blastn is used
- if the sequence is a protein, blastp is used

Both of these are used against the non-redundant GenBank database.

8. Translate button

In the Project Window, select the sequence you wish to translate. On the Manipulate Panel, click the Translate button. A pop-up menu allows you to select a reading frame relative to the origin of the sequence. Once you click on a reading frame, your sequence is automatically translated in the Sequence window.

To add additional reading frames, click the Translate button again. Select a new reading frame. This translation shows up below the first translation in the Sequence window.

An asterisk (*) in the translation represents a stop codon. A blank space is added for translations with degenerate bases.

You may edit the translation and the program will translate along with your edits.

To save the translation, click "Save Translation" under the Actions Menu. A dialog box appears asking you to designate the reading frames you wish to save. When you have made your selections, click "OK." The translated sequences appear in the Project Window as sub files of the original sequence.

To clear the screen of translations and return to the original sequence, click the Translate button and select "Remove all" from the pop-up menu.

9. Find ORFs (Open Reading Frames) button

Use this button to search DNA sequences for open reading frames larger than 100 residues. Select a sequence from the Project Window, then click on the Find ORFs button. Annotations are added for all predicted ORFs greater than 100 residues.

10. Create Oligo button

To create an oligo, highlight the DNA sequence you want (must be 200 bases or less) in the Sequence window. Click the Create Oligo button. The Oligos panel opens with information about your oligo (please see Oligos Panel below for a detailed explanation). Your oligo is automatically added as a sub file to the original sequence in the Project Window. Jellyfish gives the oligo a name based on the parent sequence and the number of bases. You can rename the oligo by clicking on its name in the Project Window, typing the new name, and then hitting the Enter key.

11. Convert Circular / Linear button

Use this button to make your DNA construct circular or linear. The current sequence will be analyzed as circular instead of as linear. Additionally, the "Convert Circular / Linear" icon is displayed beside your File name (at the top of the Manipulate panel) when circular so that you know its orientation. On the Manipulate panel, clicking the Find ORF's button while "Make Circular" is selected finds ORF's across the circular sequence. On the Restriction Enzymes panel, the digest statistics show the correct digest sizes for circular analysis when "Make Circular" is selected. To convert the analysis back to linear, click the "Convert Circular / Linear" button again.

12. Search Sequence button

Use this button to search for specific sub-sequences within your sequence. Type the text you are searching for in the text box to the left of the Search button, and then click the Search button. The first instance of the text is highlighted in the Sequence window. Click Search again and the next instance of the text is highlighted, and so on.

Restriction Enzymes Panel

This panel creates a map of a restriction enzyme digest of your sequence. The sequence you are working with is displayed graphically in the Annotations window. Enzyme cut sites are displayed in the window below the Annotations window.

1. Restriction Enzyme Sets


- a. Under "Cut with," select either a set of enzymes or "Edit Sets." The defaults are:
 - My Favorite Enzymes--contains enzymes selected by you
 - All Enzymes--includes all enzymes
 - Include Isoschizomers--includes all enzymes and all isoschizomers
 - Edit Sets--allows you to edit, add, and delete your own sets of enzymes. If you select "Edit Sets," a dialog box appears with "My Favorite Enzymes" as the default active set.
 - To add enzymes to this set, select an enzyme from the list to the left, then click the + button.
 - To delete enzymes, select them in the "My Favorite Enzymes" set and then click the - button.
 - You can rename "My Favorite Enzymes" just by typing over the highlighted name.
 - To add a separate customizable set, click on the new file button in the dialog box. Add enzymes to this new set from the list to the left.
 - To delete a set, select it in the pull-down list and then click on the trash button.
- b. Select the number of cut sites you want. If you specify a certain number of cut sites, press **Enter** after typing in the number.

2. Graphical Enzyme Display

- a. The cut sites show up as black lines on the **red bar** in the Annotations frame.

- b. Scroll through the sequence in the lower frame until you reach the name of a restriction enzyme (in red). You may also navigate using the grey slider on the red bar.
- c. View the recognition sequence and the exact cut site (highlighted in yellow) by placing the mouse arrow on the enzyme name.
- d. Click on the enzyme name to see information about digest buffers and conditions for that enzyme in the upper right hand box. Right-clicking on the name (for a PC) or holding down the control key as you click the mouse (for a Mac) displays the same information.


3. Project Statistics

- a. Click on the Digest Statistics button  for a separate window listing the number of times each enzyme in the current set cuts, and the location of each cut.

Alignments Panel

This panel lets you align sequences with a click of the mouse. Alignments are performed with the Clustal W algorithm internally (Align) or at the NPS server (WebAlign) using the default setting.

1. There are two ways to select sequences for alignment:

- a. Select sequences in the Project Window and drag them with the mouse into the Alignment box, or
- b. Select a sequence in the Project Window and click the Add  button on the Alignment Panel. Repeat this process for the second sequence (and any additional sequences).

2. Click the Align –or- WebAlign button.

3. In the dialog box that appears, enter a name for the new alignment. Click "OK." Unless you have disabled it, a security notice pops up indicating that sequence data is about to be sent off-site.

4. The alignment results are displayed in the Alignment panel. It may take up to a few minutes for the results to appear, depending on the server load and the length and number of the sequences being aligned.

To rename the sequences in an alignment:


- a. Select "Rename Sequences" from the Actions menu, or click the Rename Sequences button.
- b. Type the new sequence name(s) in the dialog box that appears.

5. When complete, the alignment is added to the Project Window.

N.B. To change the colors of the alignments, select "Set Colors" under the Actions menu. Click on a color box for a choice of colors, click on a new color, and then click "OK."

Oligos Panel

This panel displays an analysis of oligos created in the Manipulate panel.

The oligo sequence you are working with is displayed at the top of the Sequence window. Change the nucleotides from one base to another (or specify degeneracy) by highlighting the base you wish to change, then clicking the specify degeneracy button (). Select the degeneracy you prefer and click the OK button.

Oligo Features:

Color boxes display qualitative ratings of hairpins, dimers, runs, and repeats in the selected oligo:

Above Average	No hairpins, dimers, runs, or repeats found in this oligo
Average	Hairpins, dimers, runs, and/or repeats <u>found</u> in this oligo
Below Average	Many hairpins, dimers, runs, and/or repeats <u>found</u> in this oligo. Undesirable.

Click a color box for specific information on each element.

Oligo Statistics:

This box gives you all the vital data concerning your oligo, including length, percent GC, molecular weight, Tm, dG, dH, dS, End Stability, and GC Clamp.

PCR Panel

Use this panel to design, select, and modify primers for PCR. Activate one of the top three windows ("Forward Primer," "Reverse Primer," or "Product") by clicking anywhere in that window.

Forward Primer Window:

This window allows you to set the parameters for your forward primer. For primer location, select either "Exact" or "Range." Then highlight the forward primer in the Sequence Window below. If you selected "Exact," the exact beginning and end are indicated automatically in the Forward Primer Window. If you selected "Range," the exact beginning and end are indicated automatically in the Forward Primer window,

but you can now modify the length by typing in the desired base pairs (18 and 27 are the default base pairs).

In both the Forward Primer Window and the Reverse Primer Window, if you have a predefined sequence, you can search for it in the Sequence Window by pasting or typing the sequence in "Search for Primer." Jellyfish will only find exact matches or complements.

Reverse Primer Window:


This window allows you to set the parameters for your reverse primer. Directions for this window are the same as for the Forward Primer Window.

Product Window:

Use the Product Window to set the size range for your product. Type in the numbers under "Length."

Modify Selection Parameters button


Jellyfish comes with preset default parameters for primer selection. To change these parameters, click the Modify Selection Parameters button. Type in your changes in the window that pops up. If you enter an invalid value, you'll get an error message.

Restore a default setting by clicking on the Restore Default button (). Hover your mouse over a parameter name for an explanation of that parameter.

Generate Primers button

Once you've set your options for primer selection, click the "Generate Primers" button. The PCR Panel then displays your primers and products under the heading "Results." (If you wish to return to "Primer Design," click on "Primer Design" at the top of the panel.) Each primer's name consists of the first 3 characters of the sequence file, the start and end location of the primer, an underscore, and a capital F or capital R depending on whether the primer is a forward or reverse primer. Each product's name consists of the first 3 characters of the sequence file and the start and end locations of the product.

To modify a primer, drag your primer (and its pair) from the "Recommended Pairs" table to the "Modify Primers and Combinations" table. Double-click each name to rename the primer or to make sequence or chemical modifications.

A "Modify Primer" window pops up. Select an enzyme or a modification from the lists below. Click the Add button (). The restriction site or modification is automatically entered into the sequence at the location of your cursor.

The color boxes next to the name of each oligo indicate the quality of that oligo:

Above Average	Above average overall – for all categories: dimers, hairpins, etc.
Average	Average overall - for all categories: dimers, hairpins, etc.
Below Average	Below average overall – for all categories: dimers, hairpins, etc.

Double-click on a color box to see that primer's or product's complete characteristics. In the window that pops up, color boxes with the same ranking scheme as above indicate a primer's or product's rank for hairpins, dimers, runs, and repeats. Click on those color boxes for more details on each category.

To create custom forward and reverse pair combinations, drag and drop the desired primers into their respective Custom Combination windows below. A new product and rank are automatically generated. Click on the names and color boxes for further information.

Notes Panel

This panel allows you to store notes concerning each file in your Project Window. Select a sequence, then select the Notes panel. You will see two boxes:

1. The upper box is the Notes area for the project, which contains the selected sequence. A table to the right displays information about the project sequence ("Attributes").
2. The lower box is the Notes area for the selected sequence. It also has a table to the right, which displays information about that sequence ("Text").

You may edit notes at any time. They are saved automatically.

Genbank Panel

This panel displays the Genbank record for a selected sequence if that sequence was downloaded from Genbank or imported from Genbank files. This panel will be blank if the selected sequence did not come from Genbank.

Note: Annotations you make in the Manipulate panel will not be reflected in the Genbank record.

Statistics Panel

Use this panel to get instant base composition statistics, plus molecular weight and length, for a selected sequence.

Web Tools Panel

This panel is made up of two sub panels: Find Sequences and Query Sequences panels. Click on either of the sub panels to access it.

1. Find Sequences: The Find sequences panel is the Import from GenBank interface. You can access this panel by selecting "Import from GenBank" from the File pulldown menu –or- by click the Web Tools / Find Sequences panel.

Then, enter the search term and select a database from the pulldown menu. Click "Search." Select one or more sequences from the results that appear and click "Fetch." Your selection is automatically entered into the Project Window. If you select only one sequence, it is displayed as its own file in the Project Window. If you select more than one sequence, they are grouped together in the Project Window in their own folder under the search term.

2. Query Sequences: This panel allows you to select and submit your sequence to a number of Web-based analyses. The sequence that is selected in the Project window is subject to analysis and only Web tools that are relevant to that sequence are displayed in the Query Sequences window. Select the tool set you want to use for analysis by selecting "My Favorite Tools" or "All Tools" from the "Work with:" pulldown menu. You may also edit the tool set.

To perform a BLAST or other Web-based sequence analysis:

- a. Select the checkbox for each Web tool you wish to activate. You can activate as many tools as you like to perform multiple searches simultaneously. Click the "Run" button when you have made your selections.
- b. Your submitted analyses will appear in the results window. While the analysis is in progress, it will be labeled as "Connecting" in the Status column of the Results window. When the analysis is done, it will be labeled "Done" in the Status column of the Results window.
- c. When an analysis is complete, its results will automatically be stored in the Project window as a sub file of your sequence.
- d. Double click the tool name –or- the results file stored in the Project window to view your results. This takes you to an external browser, where the results are displayed.

Menus

File

This menu provides another way of performing the functions discussed under Project Window.

In addition, the "Export as JPEG" option allows you to export from the current panel any file that can be converted into an image.

"Print" and "Print Preview" operate for any currently selected file.

Edit

Use these commands to manipulate your sequence.

Tools

Access each of the panels by selecting them from this menu. (You can also do this by clicking on the tabs at the top of each panel.)

Use "Configure Tools" to select which panels you want to appear in the main window (for example, if you don't need the Oligos panel, you can hide this from view in the main window).

Use "Internet Options" to establish proxy settings.

Actions

This menu contains options pertaining to the currently selected panel.

Help

The **New User Intro** is a series of informative text boxes that you see the first time you start Jellyfish. It gives an overview of the functions of Jellyfish.

Contact Us takes you to the contact page at Field Scientific.com. We try to answer all requests within 24 hours.

Go to JellyfishSoftware.com opens a separate browser and takes you to <http://www.jellyfishsoftware.com>, where you can download Jellyfish and manage your Jellyfish account.

About Jellyfish gives you the version number of the Jellyfish software you are using.

Security

All of the files you generate through Jellyfish are stored only on your computer. Sequence information is never sent to our servers. Certain functions of Jellyfish, however, submit your sequence information over the Web, as if you were working directly through your own Internet browser. The security issues involved in submitting sequence information over the Web with Jellyfish are the same as with your own Internet browser, and you are notified of this with a pop-up dialog box (unless you elect to hide it) each time you submit information over the Web via Jellyfish.

The only information sent to Field Scientific is your email address and password at initial login, and then your email address and session time at each subsequent login.

Additional Resources

Please feel free to email jellyfish@fieldscientific.com if you have any further questions.

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