

# Clone Manager

for Windows

Basic Edition

Version 9.1

## Getting Started

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# Getting Started

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Version 9 of the Clone Manager Basic program acts as an expert system to help you with cloning simulation, enzyme operations and graphic map drawing. Clone Manager will find restriction enzyme sites, cut molecules, modify ends, and ligate DNA. Through all of these operations, Clone Manager will track your features and enzyme sites and automatically renumber positions in the new molecules. A very fast and powerful restriction enzyme analysis function will help you to find enzymes to do needed operations. View the resulting data in five different formats (list of sites, map of sites, fragment sizes, gel view of fragments, list of no-cutters) and filter the data to limit the display to only those enzymes of interest at the present time.

Graphic maps are drawn quickly and accurately and high-resolution maps can be sent to your printer or to the Windows clipboard for use in another application. A map format option allows you to add text information (description and notes; map features and enzyme sites) to the printed map page -- a great format for your lab notebook. Use Enhanced Views of the graphic map to customize special molecule maps for use in a presentation or for a figure for a publication. You can adjust each individual feature's style, color, pattern or position and you can drag feature names with the mouse, to position them just where you want them. Extra fonts, colors, styles, line width controls will help you to make some very special maps.

Analysis functions let you identify direct or inverted repeats and dyad symmetries to identify regions that might be associated with biologically active sites. An Open Reading Frame analysis option helps to identify potential protein coding regions. You can also analyze protein (or translated DNA) for hydrophilicity and predict secondary structure (helix, sheet, turn) using the method of Garnier-Osguthorpe-Robson. The hydrophilicity analysis and beta turn prediction can assist you in identifying antigenic regions. A mutagenesis profile feature helps you find sites for silent mutations, places where single base changes can add an enzyme recognition sequence, or locations and base changes needed to exchange one amino acid for another.

You can also use Clone Manager as a quick and easy way to view or edit sequence files, find open reading frames, translate genes, or scan molecule files, searching for genes or text. The molecule viewer window puts all molecule information in one convenient location. Click tabs at the bottom of the window to see the molecule map, restriction map data, sequence, features table, or molecule information. You can set the display style for the sequence data and select additional information to appear along with the sequence (like enzyme sites, sequence labels, or translation). Use the export data feature to handle export (to file, clipboard, or screen capture) of any data from an active window.

## Install Clone Manager

To install an individual or workgroup copy of Clone Manager Basic, follow the instructions below. (If you need to install a network copy of Clone Manager, please refer to the separate instructions accompanying the program disk). Windows should be running on your computer and you should close other open programs before you begin this installation.

- Insert the Clone Manager Basic program CD in your CD-ROM drive
- Allow the disk to autorun or select Start, Settings, Control Panel, and then click Add/Remove Programs.
- Follow the instructions on your screen.

The installation program will ask you to specify the directory where the program files should be copied. Press OK to accept the default directory or type in the directory name you prefer. Once the directory has been specified, the program files will be copied. When you use the program for the first time, you will be asked to designate a home directory. This home directory will be used to hold your personal program configuration

settings and user enzyme lists. Subdirectories will be created below this directory to hold your Work Boxes shortcuts and your DataBook (database utility) files.

## User Preferences

Click File, Preferences to select options to set user configuration options. Click the Basic tab to change your home directory, enter default author information, or select the default file format for saving molecule files. You can save molecule files in the standard Clone Manager molecule format (\*.cm5) or in an xml format (\*.cx5). The \*.cm5 format is preferred and has a file association set (you can double-click on a \*.cm5 file in Explorer and open Clone Manager with this file loaded).

If you are running the program under a multi-user license (Small WorkGroup or Concurrent User license types), you can share enzyme lists or DataBook database files, if these resources have been made available for you. You can also store copies of important molecule files in a central location (repository), perhaps in a standard file format. Click the Share Data tab to identify these locations and the repository data type. Click the Colors tab to change the program default settings for special screen display colors, if needed. These colors are used to bring your attention to important information, to distinguish one type of text from another, or to mark displays with ruler lines or highlighting.

Click the Import Features tab identify the feature keys that should be imported when you open a file in standard GenBank or EMBL format. You can select designated feature keys, elect to use the CDS codon start qualifier, and set the option for handling all other, non-designated features.

**Designated Features** -- Click the Add button to include a new feature key on the import list. When the dialog box appears, select the Feature Key from the list of all keys currently supported in GenBank or EMBL file formats. Select the Draw As feature type to tell the program how to display this feature in Clone Manager. When you next open a GenBank or EMBL file, the program will try to read in features with this feature key.

**All Other Features** -- This option is used to tell the program how to handle all feature keys that are not listed as Designed Features (as described above). You can discard all non-designated features (to keep your features table and file size as small as possible) or import as Information-only features -- (do not appear on the map or annotated sequence but can be viewed on the features table if you press the Info Features toolbar button.)

## Getting Started



Double-click the Clone Manager icon on your desktop to start Clone Manager Basic. All of the functions of the program are accessed by menus, buttons or shortcut keystrokes. The Toolbar will be an important shortcut to the most common operations. To see a Toolbar button name, rest the mouse cursor on the button for a second or two. For those who prefer the keyboard to the mouse, use the Windows Application key to call up a menu of the toolbar-accessible options for a display window.

The Clone Manager Help system provides comprehensive help topics to assist you in using the software or understanding a results display. When working in a dialog box, you can often click a Help button to access a help topic directly related to the task at hand. On results screens or view display screens, use the special Help with View button (far right) to access a help topic related to this view. Or click Help Topics on the Help menu to view application help to browse for general information or review a feature you might plan to use.

# Molecule File Handling

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The molecule file handling functions of the Clone Manager program include opening and loading molecules for use, saving molecules to disk, and creating new molecules. These functions also maintain the list of loaded molecules (Molecule List) and handle selection of molecules for use in subsequent operations.

## Opening Molecule Files

You can use the File, Open command to open existing molecule files and load them into the program for use. You can have multiple molecule files open at one time and you can create new molecule files, as needed. You can use Work Boxes to store location information for groups of files you want to use often. You can read molecule files in Clone Manager file formats, standard GenBank, EMBL or FASTA formats, or plain DOS text sequence files. You can control which feature keys are imported when you open standard GenBank or EMBL files. You can also read molecule data directly from the clipboard. To open files containing molecule information:

- Click the File Open button
- Find the file(s) you want in the Windows file open dialog box
- Click Open.



To select a few, non-contiguous files, click on the first file. Hold down the Control key and click on the remaining files. Click Open to load all of the selected molecules. To select a list of contiguous files, click on the first file in the list. Hold down the Shift key and click on the last file in the list. Click Open to load all of these molecule files.

If 5 or more files are loaded at the same time, you can elect to keep these files together in a group. If you select a file to load that contains multiple molecules, these molecules will automatically be placed in a group for easy handling.

## Molecule File Formats

Molecules in the Clone Manager molecule file formats (\*.cm5, \*.cx5) and the older Clone 4 file format contain sequence data, molecule name and size, and may contain any of the following additional information: features, description, notes, enzyme sites, primer sites. This format should not be edited with a word processor.

Sequences in other standard file formats are imported as double-stranded and blunt-ended. (If the sequence has been identified as a linear molecule and you want it to be circular, you can use the Circularize option during file loading.) Only one strand of a sequence is needed and IUPAC codes for ambiguous bases can be used. A number of sequence databanks and analysis programs will prepare files in a format that is similar (but not always identical) to GenBank or EMBL formats.

If the program cannot automatically load a sequence file, it will identify the closest of the recognized formats (GenBank, EMBL, FASTA or ASCII text). It will try to provide you with information about the problem encountered and provide help with file loading. In the File Help module, the file format and type of molecule are shown at the top left of this information windows. The error is described in the top right. The scrolling data window shows the contents of the file. Usually, you will have one of the common file loading errors. You will want to try and correct the error or split the sequence from the rest of the file and then attempt to reload the file.

## Importing Molecules from the Clipboard

You can read molecule information directly from the Windows clipboard if this information is in one of the standard formats that the program could read from a disk file (like a standard GenBank format). This could be very helpful if you get files using your e-mail system. To read in molecule information:

- In your e-mail document or other Windows workspace, select (highlight) the molecule data
- Click Edit, Copy to place the molecule information on the Clipboard
- In this program, click File, Import from Clipboard

If your e-mail document contains more than one molecule file, the program will read in the information for each molecule in turn and place this collection of molecules on the Molecule List as a group. Select Ungroup to see the individual molecules.

## Retrieving Files from Entrez

You can retrieve sequence files from the Entrez system at the NCBI using a built-in web browser interface. You need an internet connection to use this function. Once you locate the file you need, you can download this file to your hard disk to save the complete GenBank file, including references and annotations, or you can import the molecule for immediate use. To find the file you need:

- Click File, Retrieve from Entrez
- In the search bar, leave the control set to Nucleotide if you are looking for a DNA molecule
- In the Search For box, enter the locus name, accession number or descriptive information.
- Click the Go button.
- When the results are listed, find the file you want and click on the accession number link to view the contents of this file.

If the file contains sequence and molecule information in a standard GenBank format, you can directly use this data. To download the file, click the checkbox to the left of the accession number identifier and then click on the Send To box on the web page and select File to send all the information to file. When the download file message box appears, select the option to save the file to disk. Enter a filename and change the location on your hard disk, if appropriate. You can open this file for use in Clone Manager at any time by clicking File, Open and selecting the file. To view the GenBank annotations information, open the molecule in the molecule viewer window, click the Info tab and then click the GB Annotations button.



To import the molecule for immediate use: click the Get Molecule button on the browser window toolbar (not on the web page). If the active page contained molecule data in the GenBank format, the molecule will be imported into the program for use now. The sequence data, genes read from the features table, and descriptive information will all be a part of the new molecule. You can add enzyme sites or just use the sequence in an alignment or primer design operation.

## Using Work Boxes

The implementation of Work Boxes is similar to the concept of "Favorites" introduced with Windows. The program creates a folder (directory) called "WorkBoxes" and uses this location to store shortcuts you create for folders that you want to use often. To add a new folder shortcut to Work Boxes:

- Click File, Open or use the Open toolbar button.
- Navigate through the directory structure until you see the folder you want in the upper box called "Look in:"
- Click the button Add Work Box

To use the shortcuts in Work Boxes:

- Click the button Work Boxes
- Double-click the folder of interest to see the files it contains.

## Saving Molecule Files

You can save the active molecule to its current name and folder by using the File, Save command or the Save toolbar button. You can also change the name or location of an existing file before you save it, by using the File, Save As... command. If you are saving a file for the first time, you will automatically move to the Save As dialog box so you can name the file and specify its location. You can save molecule files in the Clone Manager binary molecule file format (\*.cm5), in an xml-based molecule file format (\*.cx5), or in the older Clone Manager 4 format. If you always want to save files in the same format, you can set this as a default using File, Preferences.

## Sharing Molecule Data Files

In many cases, you will want to share files with other users in your organization. You can use File, Preferences to set options to prompt you to add author information or send a copy of a molecule file to a specified central location (repository) when it is saved for the first time.

**Author Stamp** -- You can add an author name, date, and notebook reference to the beginning of the Notes field for your Clone Manager molecule files. Adding author information can help you to identify the source of a file or the location of needed documentation. Click Operations, Author Stamp to add author information to the current molecule file now or use the Author Stamp toolbar button when editing molecule information on the Info tab of the molecule viewer window. Use File, Preferences to store the author name.

**Submit to Repository** -- You can instruct the program to send molecule data to a folder (location) you specify. This location may be a central repository for molecule files shared by a group, or a back-up location where you have decided to keep an extra copy of critical files. You can send a copy of the molecule file or just export the sequence, features, and descriptive information in a file format similar to GenBank. If you are saving a file for the first time and are prompted for author information, you can use the checkbox to send the file to the repository location in one easy step during the file save operation. Click File, Submit to Repository to send a copy of the active file to this location now. Use File, Preferences to store the repository location and default file format.

## Exporting Molecule Data

You can export molecule data to a disk file or place this data on the Windows clipboard for later use in another program. You can export the sequence of the molecule or the sequence plus features (in a format like GenBank) using the Export Molecule Sequence option on the Operations menu. If you have a batch of standard Clone Manager files (\*.cm5) that you want to convert to another format, perhaps for file sharing or archival storage in a standard format, you can use a special Multiple File Conversion Utility, found on the File menu. You can select from the following export formats:


**Sequence Only** -- Select this option to export only the sequence of the active molecule. The sequence will be exported in ASCII (DOS text) format in upper case letters, 50 bases per line, with no basepair numbers, and no header or map information.

**FASTA** -- Select this option to export molecule data containing the sequence plus one line of descriptive information (molecule name, description) in FASTA format.

**Compact GenBank** -- Select this option to export essential molecule data in a format that is consistent with the standard GenBank file format. The data will include the molecule name, size, description, features (genes, regions, markers, labels), and sequence data, all with standard GenBank identifiers.

**Commented GenBank** -- Select this option to export a larger subset of molecule data in a format that is consistent with the standard GenBank file format. The data will include the molecule name, size, description, all features (including information-only features), sequence data, information entered in the Notes field, and the 'draw as' type you assigned for each feature.

## Using the Molecule List

 Found on the File menu or use toolbar to access. The molecule list contains molecules loaded into the program or created during the current work session. Newly-created or modified molecules that have not been saved are marked with a diamond symbol. Use this list to identify the molecule to use for the next operation, remove molecules no longer needed, or view additional information about the molecule. You can also look for additional files to add to the molecule list (see below).

You can sort the information in the Molecule List (click on column header) and you can resize the columns as needed to view the data most important to you (drag the join between column headings). If molecules have been loaded in groups, you can temporarily ungroup these files and then regroup them as needed.

**Selecting a Molecule** -- You can identify the molecule to use for the next operation or you can open the molecule viewer window for any molecule loaded in the program. Click on the name of the molecule of interest and then click Select. You can select groups of molecules for alignment operations in the Align Plus program.

**Loading another Molecule** -- Click Browse to access the File Open box to find and load another molecule file, if needed. The file will be opened, entered to the Molecule List and selected for use. Click DataBook to access the built-in database to find and open a specific file. You can search DataBook entries, as needed, to find just the file you're looking for.

When you load 5 or more molecule files at the same time, you can indicate that these molecules should be kept together as a group for easier handling. In the molecule list display, you can temporarily ungroup these files and then regroup them as needed:

**Ungroup** -- Breaks group structuring and lists all molecules as individual entities. You might want to ungroup to select a single molecule for a specific operation or to open the molecule viewer window to view this molecule's sequence or features table.

**Regroup** -- Restructures groups previously ungrouped (see above). You might want to regroup to select all of the molecules in the group for an alignment procedure using the Align Plus program or to remove all of these molecules from the program in one step.

## Enter to Molecule List

When you create a new molecule by some action you have taken (perhaps by cutting an existing molecule, doing a ligation, or translating a sequence to create a protein), you will need to enter this molecule to the Molecule List. Once on the Molecule List, the new molecule will be available for use in the program and can be saved to a disk file for later use. You can enter a name and description for the new molecule.

## Creating New Molecules

You can create a new molecule file for use in the program and later save this file to disk. The New Molecule Wizard will help you to enter all the information you need for the new molecule. If you just want to use sequence data immediately, you can elect to simply paste a sequence from the clipboard, bypassing the wizard's data entry pages. To create a new molecule:

- Click the New toolbar button
- Select Enter Complete Information and then follow Wizard instructions.
- Or select Paste Sequence and then copy the sequence to the clipboard for import.



When you are finished creating a new molecule, you should save this file to disk if you want to use this molecule again at a later time. (When the new molecule is open, click File, Save As...).

## Entering Molecule Information

You must enter a molecule name and molecule type. You can elect to enter a description or notes about the molecule.

**Basic Molecule Information** (required) -- Enter a name for the molecule and select the molecule type (DNA or Protein). To make changes to this information later, open the molecule viewer window (select the molecule from the Molecule List if necessary) and click the Info tab.

**Molecule Description and Notes** (optional) -- Enter a short description of the molecule and/or a more lengthy notes section. Some users enter keywords, history of construction, or comments in this field. This information is saved as a part of the proprietary program file format and can be edited or printed at a later time.

## Entering Sequence Data

To enter sequence data, type in the complete upper strand sequence in the data entry box provided. You can enter valid or ambiguous bases. All sequence entry is in the Insert mode. Use the Delete key to remove erroneous bases, if necessary. You can also paste the sequence from the clipboard if you already have access to this sequence data in another document. Copy the sequence to the clipboard in your other application and then click the Paste button instead of typing in the sequence. If you do not have sequence for this new molecule, enter the size (in bps) in the space provided.

## Entering Molecule Format, Linear Ends

If your new molecule is DNA, you should indicate if this molecule is circular or linear. If you are creating a linear DNA molecule, you can indicate the restriction enzymes used to create each of the linear ends. Enter restriction enzymes names for one or both ends of the molecule. Enzyme names must be recognized by the program and cannot be asymmetric cutters -- click the small button for help with enzyme names, if required. If you do not enter an enzyme name, the program assumes this to be a blunt end.

## Entering Features

You can enter genes, regions, markers or labels as features for your new molecule. Select the feature type and enter a short feature name. Next, enter start and end basepair positions (markers have only one position). Indicate if on the complementary strand. Enter a feature description, if desired, and select the appropriate feature key. Use the AddNew button to get a new blank data entry area for additional features. Use the Prev or Next buttons to move through the features already entered.

# Molecule Viewer Window

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The molecule viewer window opens when you load a molecule into the program or when you select a molecule from the Molecule List (and are not in the middle of another operation). Tabs along the bottom of the window let you view the map, restriction map, sequence, features table, or information describing this molecule. Use the Help with View button on the window toolbar to access help related to the active view.

The molecule map is the default view and shows a graphic representation of the molecule and its features (genes, regions, or markers). You can view the molecule sequence by clicking on the Sequence tab at the bottom of the viewer window. You can display the sequence in a variety of formats, edit the sequence, and use a search function to find places in the sequence that match a search string. The Features tab gives you access to the features table for this molecule. You can add or remove features or view their properties. The Info tab shows the molecule name, size, description, and notes. You can add or edit this information, as needed. The RMap tab provides detailed restriction map data (described in a later chapter).

## View Map

Click the Map tab at the bottom of the window to display the molecule working map. The map will be either circular or linear, depending on the molecule, and features and enzyme sites will be displayed. You can use the mouse to get more information or initiate actions on this display. You can select a map site to view detailed information about or use an enzyme site, primer site or feature. You can also use other toolbar buttons to find more sites to add to the map or to set options for the working map display or for printing the graphic map.

**Features** -- Features that have been entered to the features table for this molecule will appear as follows: Genes appear as pointers below (inside) the map line. Regions appear as boxes on the map line. Markers appear as short, solid lines crossing the map line. Click the Features tab of the molecule viewer window to add or modify features. Click the ORF search button to locate open reading frames and add them to the map as genes, if desired.

**Enzyme Sites** -- Enzyme recognition sites appear above (outside) the map line. Enzyme sites that are very close together may be displayed on the same line. ++ symbol next to an enzyme name indicates that one or more enzymes very close to this site could not be displayed without overwriting. Point to the enzyme name to see bp position for this enzyme and for any suppressed sites at this location. (Use the Map Sites list on the right to select one of these enzymes.)

In highly congested areas, the enzymes sites will be collected in order and moved to a location above or to the side of the map. Multi-cloning sites will usually be displayed in this way. If your map has no enzyme sites, click the AutoScan button to find enzyme sites using the specified scanning list. Click Clone, Find Enzyme Sites to locate other enzyme sites and add them to the map.

**Primer Sites** -- If you are using Clone Manager Professional, primer sites can be shown above (outside) the map line in much the same way that enzyme sites are displayed. If your map has no primer sites, you can click the Find Primers that Bind button to scan your collection of primer files to find primers that will bind to this molecule. If you are designing primers, you can add these to the molecule map using toolbar buttons in the primer search results list window or in the primer viewer window.



Click the Enzyme Sites/Primer Sites toolbar button to switch from the display of enzyme sites to the display of primer sites. Click the depressed button to return to the display of enzyme sites. Or click on the site list header control (right) to select the list of Enzyme sites, Features, or Primer Sites.

## Working with Map Sites

The Map Sites list is found on the right side of the molecule map display. The scrolling list shows enzyme sites, primers sites, or features entered for the molecule. Click on the site list header control to select what the list displays. Click on a column header to sort the display. Drag the join between column headings to resize a column width. Click to select an enzyme site, primer site, or feature in the list.

**Enzyme Sites** -- list shows the enzyme name, site position, type of ends produced (5' sticky, 3' sticky, blunt) and an indication if this enzyme is a single cutter (sc), cutting this molecule only once. By convention, the enzyme site is marked at the left end of the recognition sequence. You can use toolbar buttons to view the site properties, jump to the formatted sequence at the location of the enzyme site, view enzyme suppliers, enzyme isoschizomers, or compatible ends, or cut at the selected enzyme site. Use the More Actions button to remove all enzyme sites or add an enzyme site when no sequence is present.

**Primer Sites** -- (Clone Manager Professional) list shows the primer name, site position, and an indication if this primer binds to the complement strand. The primer site is marked at the 5' end of the binding site. Complementary strand primers can be shown in a distinctive color (see Format Working Map Display, below). You can use toolbar buttons to view primer properties, jump to the formatted sequence at the location of the primer site, export the primer sequence, create the primer, or start the process to create an amplified product using this primer. Use the More Actions button to change the primer name or remove all primer sites.

**Features** -- list shows the feature name, start and end basepair positions, and an indication if this feature is on the complement strand. A color segment can be shown on the map when a feature is selected (see Format Working Map Display, below). You can use toolbar buttons to view feature properties, jump to the formatted sequence at the start of the selected feature, export the feature sequence, create a protein by translating the feature, or create a fragment containing the feature sequence. Use the More Actions button to customize feature print styles or shift a feature name on the map.

## Format the Working Map Display

You can set several working map (screen) display options. These settings will be remembered and used for all working map displays. Click the Format Map button and then click the Screen Basics tab. Use checkboxes to enable or disable options.

**Selected Features** -- set the checkbox to show a color segment on the map when a feature is selected. This can be helpful in marking the feature extent close to the map line, where enzyme or primer sites are positioned. The color used is a partially transparent form of the Highlighter color set in File, Preferences, Colors.

**Complement Strand Primers** -- Set the checkbox to show complement strand primers in a distinctive color. Normal strand primers appear in the color specified for enzyme or primer sites (Site tab). The color used for complement strand primers is the Auxiliary text color, set in File, Preferences, Colors.

**Mouse Hover** -- When this feature is enabled, you can move the mouse to an enzyme or primer site or a molecule feature and see the basepair position for this element without clicking the mouse. If this feature is disabled, you can point and then left-click with the mouse to get the same base position information.

**Line Smoothing** -- When enabled, anti-aliasing is applied to feature and map lines to improve the appearance of the items in the on-screen map. This does not affect printing and is not applied to maps copied to the Windows clipboard in vector formats. If disabled, the feature and map lines are drawn without smoothing.

## View Sequence

Click the Sequence tab at the bottom of the window to view the molecule sequence. You can view the complete sequence of your molecule or just a part of the sequence and you can select the format for the sequence display. You can copy a portion of the sequence to paste into another application or you can edit the sequence, if needed. You can also find places in the sequence that match a search string you enter or jump to a specified basepair position in the sequence. You can select (highlight) a range of bases and make this sequence into a molecule feature or into a primer (requires Clone Manager Professional).



Click the My Style button to format the sequence in your favorite style. (To set up this feature with your preferences, set the style options on the Format, Style dialog box and then click the Memorize button.)



Click the Go To basepair position button and enter a basepair number. The program will scroll to the part of the sequence containing the basepair number specified.

## Sequence Style Options

You can set the display style for the sequence data and select the additional information that will appear along with the sequence. To set sequence display style:

- With the sequence visible on the screen, click the Format button
- Click the Style tab if not already selected
- In the Sequence options area (center), select single- or double-stranded
- Select block size (10 or 3) and choose upper- or lower-case letters



To select additional information:

- Use check boxes to indicate which items should be displayed with the sequence data
- For selected items, set related options (see below)
- Use the Memorize button to remember the settings for future use.

**Enzyme Sites** -- Enzyme sites are shown above the sequence line. Select the source of the enzyme data: you can use enzyme sites that appear on the working map (Map tab), enzyme sites the program will find using the AutoScan enzyme list, or enzyme sites that are currently shown on the RMap tab.

**Primer Sites** -- Primer sites are shown above the sequence line. You can select the display style and colors to use for primer sites (requires Clone Manager Professional).

**Sequence** -- Single-stranded sequence displays the upper strand for the full sequence and the normal or complementary strand (as indicated) for a partial sequence. Double-stranded sequence displays both strands of the molecule, including sticky ends, where appropriate. Select the block size and case for sequence characters.

**Sequence Labels** -- Shown on the sequence line itself. You can display sequence labels by coloring the bases in the sequence and/or applying color to the background behind the sequence bases, or you can invert the case of the sequence characters.

**Translation** -- You can show 3 frames, single frame 1, 2 or 3 (as selected), or all 6 frames of translation below the sequence line. Show amino acid codes in 1-letter, 1-letter with amino acid numbers, or 3-letter styles. Translated display sample.

**Annotations** -- You can show genes, all map features (genes, regions, markers), or translated genes below the line of sequence. For translated genes, you can select 1-letter, 1-letter with amino acid numbers, or 3-letter amino acid codes.

You can select the default color to be used for each type of sequence text that can be displayed and you can set the base color and background color for sequence labels. To set default sequence text colors, with the sequence visible, click the Format button and then click the Colors tab. Colors selected for features will apply to all text of that type. You can use a custom feature setting to distinguish a special feature, if needed. To set custom feature colors, click the Features tab to move to the features table. Select (highlight) the feature and click the Customize Style button. Select the color for the annotations text for this one feature

## Printed Output Options

You can select the font size to be used for sequence data sent to your printer. The font face will always be Courier New, if this is supported in your printer. Otherwise, the font face will be Courier. (A mono-spaced font is required so that sequence data lines up correctly.) To set printed output options click the Print Options tab and select the font size you want to use for your sequence text.

## Partial Sequences

You can view the complete sequence of your molecule or just a part of the sequence. You can also show a part of the sequence in a distinctive style (perhaps translated or annotated) and the rest of the sequence in a plain format. To view or format part of a sequence, click the Format button, then click the Partial Sequence tab. Set the Partial Sequence option button and enter the start and end basepair positions for the part of the sequence you want. You can use the Features button to see where features start and end. Use the checkbox if you want to show flanking sequence. The sequence you identified as the partial sequence will appear in the style you selected on the Style tab. The flanking sequence will appear as plain sequence.


## Make Selected Sequence Into...

While you are in the sequence display, you can select (highlight) a range of bases and then make this selected sequence into a feature (gene, region, or marker) to add to your molecule's feature table. If you are using Clone Manager Professional, you can also make selected sequence into a primer or complementary strand primer. When you use the mouse to select the sequence bases, you can check the range selected in the upper area, just to the right of the toolbar buttons.

To make selected sequence into a new feature, select (highlight) the sequence bases and click the Make Into... button. Select Enter as Feature from the menu and enter the feature information. This results in the same action as adding a feature by clicking on the Info tab and then adding a new feature to the features table display. If you select to make the sequence into a primer, the primer viewer window will open and you can view the new primer and its evaluation. Modify the primer if needed and save it to disk or add it to a primer collection.


## Using the Find Sequence Tools

You can find places in the sequence that match or nearly match a specific pattern of bases. You can jump to each occurrence found in the sequence display or you can view a report listing all of the sites found on both strands of the entire molecule.

 Click the Find button while the sequence is visible on the screen. Enter the sequence of bases you want the program to search for (click the small button for help with IUPAC ambiguous bases). Use the checkboxes to configure the search to permit limited mismatches or mismatches and gaps or insertions. To jump to matches in the sequence -- set the search type to Go To Sequence Found. If a matching string is found, the string of matching bases will appear as selected text (highlighted). Click the Find Next button to locate additional places with the same sequence of bases. To view a list of sites found -- set the search type to Report All Sites. View or print the list of sites found.

## Editing Sequence Data

You can edit the sequence of a molecule by adding or removing bases. You can be in single-stranded, doubled-stranded, or translated mode, but you can only edit the upper strand of the molecule. You can also use the standard Windows Cut, Copy and Paste operations on your sequence data. To edit sequence data:

 Click the Edit Sequence button on the toolbar in the sequence view window. Make changes to the sequence data as needed and then click the Accept Changes (Update) button to complete the changes you made and modify the sequence of the molecule. Or click the Discard Changes (Cancel) button to abandon your changes and leave the sequence unmodified.

You can use the standard Windows Cut, Copy and Paste operations on your sequence data. Sequence is stored in the program single-stranded and all operations work on sequence in this way. To copy sequence from the molecule viewer window select (highlight) the sequence to be copied using the mouse or use Control+A to select the entire sequence. (Look in the toolbar area to see the range of bases selected.) Click the Copy button to copy the selected sequence to the clipboard.






To paste sequence into the molecule viewer window, copy single-stranded sequence to the clipboard. Then open the molecule viewer window and click the Sequence tab. Click to position the edit cursor where you want to paste the sequence currently on the clipboard and click the Paste button. The program will check for valid bases and paste the sequence into the location specified. The sequence will be displayed in the format selected.


## View Features

Click the Features tab in the molecule viewer window to view the features table. Molecule features appear in the listing with a symbol for the feature type, the feature name, the start and end basepair positions, the feature description, and the feature key, if assigned. Click the column headers to sort the features table by name, start or end position, descriptive text or key. Click toolbar buttons in this window to add, edit or delete features, view feature properties, create a protein or fragment, export the feature sequence, set custom print style settings for an individual feature, filter the data displayed, or show or hide the display of information-only features.


## Feature Types

Feature types are used to tell the program how to draw the feature on the molecule map or show the feature on the annotated sequence. Molecule feature types include Genes, Regions, Markers, Labels and Information-only features. You can set the style and color to be used for each feature type when printing your molecule maps or annotated sequence.


-  **Genes** -- usually represent translated sequences, have start and end basepair positions, and can be on the complementary or normal strand. Genes can appear on the molecule map and annotated sequence.
-  **Regions** -- zones of special significance for the user, have start and end basepair positions, and can be on the complementary or normal strand. Regions can appear on the molecule map and annotated sequence.
-  **Markers** -- markers have a direction, occupy one base position, and can be on the complementary or normal strand. Markers can appear on the molecule map and annotated sequence.
-  **Labels** -- areas within the sequence that are of special interest to the user, and have start and end basepair position. Labels can be shown on the annotated sequence, but they do not appear on the molecule map.
-  **Info only** -- not shown on the molecule map or on the annotated sequence. Use for features where you want to preserve the information about the feature but do not want it to clutter your map or features table.

 Click the Add Feature button to add a new feature, then enter the feature information. To edit feature information, select (highlight) the name of the feature and click the Edit Feature button. Modify the feature information, as required. To delete a feature, move the highlight to the name of the feature you want to remove and click the Delete Feature button

## Viewing Feature Properties

 To view feature properties, select (highlight) the name of the feature and click the Feature Properties button. The information displayed will depend on the feature type. For Genes, properties include basic information about this gene, its translation, and the amino acid composition, codon usage, molecular weight and pI for the translation product. For Regions, properties include basic information about the region defined and its base composition. For Markers, Labels and Information-only features, properties are limited to basic information (name, position) for this feature.

## Using the Features Table Filter


 If your molecule has a very large number of features, you can click the Filter button to set filter options to transiently limit the data displayed in the molecule viewer window features table by location or feature type. When a filter has been applied, the Filter toolbar button will be depressed. Click the depressed Filter button to remove the filter. Use the toolbar Info Features button to show or hide information-only features.

**Filter by Feature Type** -- Use the check boxes to indicate the feature types that you want to display. You can select more than one option. Leave all of the checkboxes blank if you want to list all feature types.

**Filter by Location** -- Check the box for this option and then enter the two basepair position numbers that define the region where you want the features listed. All features that are found within this region will be shown, even if the feature is not fully contained within the region.

## Customize Feature Styles

In the lower area of the features table display, a boxed area is used to show the styles set for the selected (highlighted) feature. If you have not set any custom styles for this feature, the settings will reflect the default values for printed map features or for annotated sequence display. To assign custom styles:

- Select (highlight) the name of the feature.
- Click the Customize Style button 
- Set the style and color to be used for this feature for the printed graphic map.
- Or set the text color to be used for this feature in the annotated sequence display.

To turn off the custom style settings, click the Customize Style button and then click the Clear button to set the styles back to your default values.

## View Info

Click the Info tab in the molecule viewer window to view molecule information. You can view or edit the molecule name, size, description, or notes. Click toolbar buttons in this window to add author information to the notes field, change the starting basepair number for this molecule, or access GB Annotations (header information) if the file is a GenBank or EMBL format file. You can also view molecule properties information in the lower right section. For DNA molecules, you can see base composition. For protein molecules, you can see molecular weight, pI, and amino acid composition. Molecule descriptive information includes:

**Molecule name** -- appears in the Molecule List, on program screens and in maps and other printed output.

**Size in bases** -- if the molecule does not contain actual sequence data, you can change the size by typing in the number of bases. If sequence is present, you will have to edit the sequence (add or remove bases) to change the size of the molecule.

**Description** -- appears on many types of printed output and can optionally be printed on molecule map pages.

**Notes** -- more detailed information about molecule. Can be keywords, history of construction, comments. This can also be printed on molecule map pages.

## Adding Author Information



You can add an author name, date, and notebook reference to the beginning of the Notes field for your Clone Manager molecule (\*.cm5) files. This can help you to identify the source of a file or the location of needed documentation. As part of the Notes field, this information can be printed on the page with your molecule map or written to a DataBook entry. To add author information, click the Author Stamp button. Enter your name and a notebook reference, if applicable.

## Viewing GenBank Annotations



Annotations refers to the header information found in a GenBank or EMBL format file. To view GenBank Annotations click the GB Annotations button. After modification to the molecule file (such as adding enzyme sites or performing cloning operations), you will want to save the file. Files are saved in a proprietary format which includes all of the information you entered about this molecule: features, enzyme sites, description, notes. The annotation information is not saved as a part of this file format, but the program will automatically save a link to the original GenBank file so that you can later find this information.

## Base Renumbering



You can designate a number other than 1 to use as the first basepair number for the sequence of the active molecule. You might want to renumber your sequence if you are using part of a larger molecule and want to maintain consistent base numbers for features or enzyme sites. To assign a starting basepair number, click the Base Numbering button and enter the starting basepair number for this molecule.

## Zoom Molecule

You can select to display only a part of a large molecule. This is often helpful if there are a large number of features, with a very cluttered map, or if the features are very small relative to the size of the molecule and cannot be displayed clearly. To zoom a molecule just click the Zoom button (left end of tab bar at bottom of window) and enter the start and end basepair positions for the region to display. The data display is regenerated and the window title identifies the zoomed region.

The Map view shows features and enzyme or primer sites for the region specified, always as a linear map. A mapline bar and colored box at the top of the map window show the approximate location of the zoomed region. If features are not fully contained within the zoomed region, a set of dots before or after the feature name indicate that the feature extends further in that direction.

The Restriction map view shows restriction enzyme sites found within the zoomed region. Gel View of Fragments and Fragment Size displays are not available for a zoomed molecule. The Sequence view includes only the sequence of bases in the zoomed region. The Features table view lists features that are found within the zoomed region, even if they are not fully contained within this region.

# Graphic Maps

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When a molecule has been loaded into the Clone Manager program or when a molecule is selected from the Molecule List, the molecule viewer window will open, showing a working map of this molecule. The program can also draw a high-resolution graphic map that can be sent to your printer, copied to the Windows Clipboard, or exported as a file for later use by another program. You can also create and save as many as six Enhanced Views of the graphic map for each molecule file. These special graphic maps might be used to customize a molecule map specifically for use in a presentation or for a figure for a publication.

## Standard Graphic Maps

By design, all of the standard graphic maps will look consistent in style. You store your map preferences (like typeface, gene style, region color, etc.) in the map print options settings. Each time you print a map or copy to the clipboard, the program uses the map print option settings to format your map. If the settings are unchanged, each of your maps will look similar. If you do change the settings, all maps that you print or copy thereafter will use the new settings.



Click the Format Map button to change the appearance or content of the high-resolution graphic map.

You can turn on or off the printing of types of basic text information, select feature styles and colors, select enzyme or primer site styles, or change the size or position of the graphic map or add descriptive text to the map page. Changing map print options will not affect the appearance of the working map in the molecule viewer window (with the exception of feature colors), but will affect printed, copied or exported maps.

## Format Map | Print Basics

You can select the font face and colors used to print text information on your graphic maps and you can control the thickness and color of the mapline. To set basic options for the graphic map, click the Format Map button and then click the Print Basics tab. Set one typeface (font) for all text on your map. Arial is a simple, modern, sans serif typeface, while Times Roman is a more classic, slender, serif typeface. Use checkboxes to indicate which text items to print, select a color for each item type and select a color and weight for the mapline.

## Format Map | Features

You can control the style and color for feature types (genes, regions, and markers) to be printed on your graphic map. These style and color settings apply to all features of that type. You can use a custom feature setting to distinguish a special feature, if needed, and you can move a feature name to avoid overwriting if necessary. If you are using very large molecules, you can elect to suppress the drawing of very small features.

Feature positions are preset -- genes will be displayed inside the map line, regions will be displayed on the map line, and markers occupy one base position and will be drawn around this base position on the map line. To set feature print styles click the Format Map button and then click the Features tab.

Use checkboxes to indicate which feature types to print, then select a style and color for each feature type. To set custom feature settings, with the map visible on the screen, right-click on a feature and select Customize. Select the style and color for this one feature or Clear to remove this custom setting. To shift a feature name, right-click on the feature and select Shift Name. Enter the number of increments (in units of 1/10 inch) to move the text horizontally or vertically on the printed page. If you are using large molecules (greater than 100,000 bp), you can elect to suppress the drawing of features that are very small relative to the size of the molecule. Use the checkbox on the Features tab to enable this option.

## Format Map | Sites

You can select one of two preset font sizes for enzyme and primer names and you can control the color to be used for these names and tick lines. You can also indicate if the program should print basepair numbers for each enzyme or primer site or if it should italicize the first three letters of the enzyme name. To set enzyme print styles, click the Format Map button and then click the Sites tab. Set the font size for enzyme or primer names to Standard or Small and select a color for names and tick lines. Use the checkboxes to print basepair numbers or italicize enzyme names.

## Format Map | Content

You can print stand-alone graphic maps, or you can select a pre-defined map format that adds additional text information to the printed map page. If you do not add additional text, you can move or resize the graphic map before printing or copying to the clipboard. In almost all cases, the default map size and position will print a complete, centered, high-resolution graphic map in either landscape or portrait orientation. (To change the page orientation, select Print Setup from the File menu).

**Maps with Additional Text** -- You can select a graphic map style for printing that adds additional text to the map page. You can select to include the molecule description and notes and/or a list of features and map sites. To print a map with text annotations, click the Format Map button and then click the Content tab. Select Molecule Map and Text and use checkboxes to indicate which text you would like to include. The graphic map will automatically be reduced to 90% of full size and it will be moved upward from the center to make room for the extra text. (This format is intended for printing in portrait orientation.) The text information will be printed on the lower section of the map page and may go on to a second page, if required.

**Changing Map Size or Position** -- You can change the size of a graphic map before printing. You can also resize a map before copying to the clipboard or exporting. You can change the position of a graphic map on the printed page, if necessary. You can shift the map up or down on the page, or you can shift the map slightly to the left or right. To change the size of the graphic map, click the Format Map button and then click the Content tab. Select Molecule Map Only and select 80% or 90% to decrease the map size, or select 110% or 120% to increase the map size. To change the position of the map on the page, click the Format Map button and then click the Content tab. Select Molecule Map Only. Move the map left or right by using the horizontal position slider control. Move the map up or down by using the vertical position slider control. The default position is centered horizontally and vertically on the printed page. Click the Defaults button to return the map to its original position (centered) and size (100%).

## Enhanced Views of Graphic Map

Found on the Map menu when a molecule map is visible in the active window. You can use an Enhanced View to create a special graphic map, perhaps for a presentation or a publication. You might want to enhance the molecule map to differentiate or emphasize an important set of features, show only the most significant enzyme or primer sites, or control line thickness and color to give the best visual image.

When you start a new Enhanced View, you begin with the molecule map, formatted for printing using your Clone Manager preferences. Then you can adjust each individual feature's style, color, pattern, or position. You can drag feature names with the mouse, to position them just where you want them. You can directly control the font face, font style, point size and color for enzyme, primer, or feature names, or the molecule name or size text. If needed, you can remove features or enzyme sites from this map, without removing them from your molecule file. You can control the thickness and color of the enzyme and basepair interval tick lines, as well as the map line. You can even switch between circular and linear map formats.

When complete, you can save the Enhanced View to the molecule file for later use. As many as 6 enhanced views can be saved with any molecule file. You can print the enhanced map, copy it to the clipboard, or export it to a disk file for use in other programs.

To create a special, enhanced map of the active molecule:

- Open the molecule viewer window and click the Map tab.
- On the main menu, click Map, Enhanced View, New View.

The new enhanced view window will open with your molecule map, drawn using the Format Map print options settings stored in Clone Manager. Use your mouse to drag on the lower right corner of the window to increase the window size, as needed. Toolbar buttons and controls let you modify the appearance of text, lines, and features drawn on the molecule map. You can click the Undo button to reverse the last action taken, if needed.

**Text** -- Use the font controls in the toolbar to change the text characteristics for a selected text item. Similar items (enzyme/primer names, bp interval numbers, feature names) will all be formatted to the same font face, style, point size. Click the Color button to change text color.

**Features** -- Click on a feature (gene, region, marker) drawn on the map to activate the toolbar buttons that enhance feature drawing. You can change the feature style, position, fill color, fill pattern, and feature name text color. To remove a feature not needed for this map, click to select and then click the Delete toolbar button.

**Enzyme sites** -- Click the Enz BP Num button to display or hide the basepair numbers for all sites. Click the Enz Italics button to italicize the first three letters of enzyme names. To remove enzyme sites that are not needed, click to select and then click the Delete toolbar button. To select more than one site, select the first site and then hold down the Control key while selecting additional sites.

**Adding features or sites** -- The enhanced view of the molecule map is built from the data in the Clone Manager molecule file. You can add features and enzyme sites or primer sites that are a part of the molecule but not currently displayed on the enhanced view map. Click the Add button and select Add Feature, Add Enzyme Sites or Add Primer Sites, as needed.

## Basic Molecule Drawing



Click the Basic Properties button to modify basic settings for the molecule map, features, or enzyme/primer sites. Basic settings include map format, line widths, line colors, basepair interval markings, special text display settings and font characteristics. Click the Tools button to quickly change the map size by 10% or edit a selected feature

**Map Basics** -- Select to draw your molecule as a circular or linear map. If the molecule is defined in its data file as circular and you select to draw it in the linear format, the program will open out the molecule at basepair position 1. If the molecule is defined as linear and you select to draw it in the circular format, the program will draw the molecule map as a circle, where the two molecule ends meet at the top. When a molecule is defined as linear, features cannot go over the top.

You can draw the map smaller or larger than the normal (100%) size. Select 80, 90, 100, 110, or 120% of normal. Adjusting the size of the map can give more room for features or enzyme sites or change the appearance and balance of the map elements.

Click the Font buttons to set font characteristics for the molecule name and molecule size text. (This text can be moved on the map, if needed. Just select the text (click on it) and then drag the text using your mouse.) Clear the check boxes to turn off the display of either the molecule name or the molecule size text.

Select the line width and color to be used for the Map Line.

Basepair intervals can be numbered in bps or kbps and you can set the font for the interval numbers. Clear the checkbox if you do not want to display the basepair interval numbers. Basepair interval ticks and numbers can be displayed inside the circular map, at the map line, or in an intermediate position. For linear maps, these intervals can be displayed at the map line, on a ruler line below the map (select Standard), or in an intermediate position. Clear the checkbox if you do not want to display interval tick lines or numbers in any position. Select the width and color to be used for the basepair interval tick lines.

**Feature Basics** -- Select the Font Face, Font Style, and Point Size to be used for all feature names. The most common settings are Arial, Regular, 10 points. If you have a large number of features, select a smaller point size. You can also select a color to be used for all feature names or you can select individual colors for each feature name. (To change color for one feature name, select the feature name on the map and then use the Color toolbar button to select a color for this one feature name.)

To move a feature name, select (click on) the feature name and drag to a new position using the mouse. If you expect to change the size of the molecule map or adjust the position of the features, move the feature names after you have made the other changes.

To change a feature name, right-click on the feature name and select Edit Feature. Click in the Name field and make any changes to the feature name -- shorter names are often helpful for a crowded map.


**Enzyme/Primer Site Basics** -- Select the Font Face, Font Style, and Point Size to be used for all enzyme and primer names. The most common settings are Tahoma, Regular, 10 points. If you have a large number of enzyme or primer sites, select a smaller point size. You can select one color for all enzyme names and one color for all primer names.


You can use the checkboxes to set the display of basepair numbers for all enzyme and primer sites or to italicize the first three letters of enzyme names.


Select the width and color to be used for enzyme/primer site tick lines. (These lines extend from the map line to the enzyme or primer name). When you have many enzyme sites and are using a small point size for enzyme name text, select a thin tick line. If your enzyme names are large or you are using a thick map line, select a thicker tick line for balance.


## Enhance Feature Drawing

You can set the drawing style, color, fill pattern, and position for each individual feature in the enhanced view map. Select (click on) the feature and then use the toolbar buttons described below.

**Feature Position** -- Nine pre-set positions can be used. Four positions are inside/below the map line, one position is on the map line, and four positions are outside/above the map line. Click the  Move Feature Out/Up button to move the feature to a position further from the center of a circular map or to a higher position on a linear map. Click the Move Feature In/Down button to move the feature to a position closer to the center of a circular map or to a lower position on a linear map.

**Feature Style** -- Eight drawing styles are available for genes or regions. Narrow styles are most useful for maps that have a large number of features, while wider styles provide greater impact.  Markers have a subset of drawing styles, drawn around the single Marker basepair position. Click the Feature Style button and select the drawing style for this feature.

**Feature Pattern** -- Eight fill patterns can be used for genes or regions. Select Open (no fill), Solid, Shaded, or patterned with vertical, horizontal, or diagonal lines. Markers are always drawn with the Open style. Fill patterns can be printer- and resolution-dependent. Click the Feature Pattern button and select the fill pattern for this feature. 

**Feature Color** -- You can select the color for the feature drawn on the map and you can select the color for the individual feature name. Twenty standard colors can be used or you can click More  Colors to select a non-standard (custom) color. Click the Color button and select the color for this feature or feature name text.

**Move feature name** -- On the molecule map, click on the feature name to select. Then drag the name with the mouse to move to a new location, as needed.

## Working with Color

Color can help to make your enhanced view maps more interesting and easier to understand. Bold, bright colors catch the viewer's eye and indicate the importance of an object. Too much color, however, can lessen this impact. You can change the color for individual features or for text elements.

- Select (click on) the item.
- Click the Color button
- Click on the color you want or click More Colors to select a color not shown.

When you click the Color button, you will see a color selector that shows the 20 standard colors. In some cases you just need one or two different colors to complete your figure or match a color in use elsewhere. To do this, you can select custom colors, using controls to mix the color components or you can type in numerical values.

Easy steps to a custom color:


- Click the Color button, then click More Colors.
- Click on a basic color or on an area in the color matrix that's close to what you want.
- Check the Color|Solid sample box (lower right)
- Use the slider at the far right to make the color lighter or darker
- When the color is right, click OK to use this color.

The next time you click the Color button, your custom color will be displayed in the bottom row (above More Colors), so that you can easily use this color again.

You can change the color for the molecule map line, basepair interval tick lines, and enzyme or primer site tick lines only in the basic properties dialog boxes. Click the Basic Properties button and select Map Basics (map line, bp interval lines) or Site Basics (enzyme/primer lines) and select colors and line widths.

## Saving Enhanced Views

When you have completed work on your enhanced view map, you can save this special map data to the molecule file. You can later re-open this enhanced view for further work or print or export the graphic map. You can have a maximum of 6 enhanced views saved in each molecule file. You can organize the enhanced views, renaming the views or deleting ones that are no longer needed.

 Click the Save View toolbar button in the enhanced view display window. The enhanced view data will be saved as a part of the molecule file. The view name assigned consists of the date and time the view data was saved, but you can change this to a more meaningful name (see below). Remember to save the molecule file to disk before exiting the program.

To organize the enhanced views saved to a molecule file, open the molecule in the molecule viewer window and click the Map tab. Click the Map menu and select Enhanced View, Organize Views. In the Organize Enhanced Views dialog box, select (click on) the view that you want to rename or delete. To change the name

of the selected view, click the Rename button and then type in the new name. To permanently remove the selected view, click the Delete button.

## Graphic Map Output

You can use options on the Map menu to preview or print the high-resolution graphic map or to copy or export the graphic map for use in another program. The highest resolution graphic maps are exported as vector graphic files (\*.wmf or \*.emf), which can easily be scaled. You can create an enhanced view map and export as a raster graphic file, if needed.

**Print** -- Click Print or use the toolbar Print button to send the map to your printer. To change the orientation of the printed page, click File, Print SetUp. Click Print Preview to check the appearance of your map before printing, copying to the clipboard, or exporting to a disk file.

**Clipboard** -- Click Copy to Clipboard or use the toolbar Send View to Clipboard button to copy the high-resolution graphic map to the Windows clipboard for use in other Windows applications. The styles and colors set for printing will be used for clipboard output. To paste your map in a Word document, open your word processing program and move to the place where you want your map and then click Edit, Paste Special and select Picture (Enhanced Metafile).

**Export** -- Use this option to export the high-resolution graphic map as a Windows Metafile (\*.wmf) or an Enhanced Windows Metafile (\*.emf) for use in other Windows applications capable of importing these file formats. The styles and colors set for printing will be used for the exported map. To use your map metafile in a Word document, open your word processing program and move to the place where you want to insert your map and then click Insert, Picture, From File.

**Save for Web** -- Use this option to prepare a raster graphic map in the Portable Network Graphics (\*.png) format, particularly useful for computer screen display (such as on a web page or in an on-screen presentation). For standard graphic maps, the map size is automatically set to 80%, bp interval numbers are turned off, and the use of italics or small fonts for enzyme names is disabled. Enhanced views of the graphic map are not automatically modified before export.

### Additional options for Enhanced View Maps



For enhanced views of the graphic map, click the Copy/Export View button in the enhanced view display window to send your enhanced view map to the Windows clipboard or to a disk file in vector or raster (bitmap) file formats.

Vector file formats (emf, wmf) provide high-resolution images that can be resized accurately. Selected raster file formats (tif, gif, png) provide high quality map images combined with small file size. For raster copy and export options, you can select a low-resolution bitmap (looks similar to the map on the screen) or a high-resolution bitmap (looks similar to the printed map).

# Enzyme Operations

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The Clone Manager program can find restriction enzyme recognition sites -- for one enzyme at a time, or for all enzymes on an enzyme list meeting specified criteria (like single or double-cutters). Once an enzyme site has been entered, you can view this site in detail (recognition sequence, cut position, cohesive ends produced). The program uses enzyme lists to store the information needed to work with restriction enzymes. You can view, print or update enzyme lists and you can use a helpful wizard to build user-designated enzyme lists. You can keep your master enzyme list up-to-date by doing an update procedure using the latest REBASE enzyme data file (available monthly over the Internet).

Clone Manager has a powerful restriction enzyme analysis module that displays information on restriction enzyme sites as a page in the molecule viewer window (click the RMap tab to get to this display). You can view the restriction enzyme analysis in one of five display styles, including a list of no-cutters. You can also set filter options so that you see only the results you need.

## Finding Enzyme Sites



Found on the Clone menu or use toolbar button to access. You can find recognition sites for specific restriction enzymes or you can find sites for lists of enzymes (all single or double cutters for any enzyme list, or all sites for all enzymes on a user-defined enzyme list). The Rebuild map sites list option prepares a list of the enzymes used in the current molecule map and then automatically rescans for each enzyme, rebuilding the list of map sites. This is useful after a ligation of two molecules or after a change to the sequence of a molecule.

### Finding Sites for Enzymes

You can search a molecule looking for recognition sites for one or more restriction enzymes. Once found, you can see the number and location of these sites, enter all of these sites to the molecule map, if desired, or cut the DNA with one enzyme or at one specific site. To find recognition sites for enzymes:

- Click Clone, Find Sites and then click Enzyme(s).
- Type in the names of the enzymes, separated by a comma, or find the enzymes in the lookup list to the right (see below).
- Click OK to find sites for these enzymes.

**Enzyme List Lookup** -- The enzymes on the selected enzyme list are shown along with their recognition sequence. Press a letter key to move to enzymes in the list that begin with that letter. Double-click to paste an enzyme name from the list to the text box. To view a different collection of enzymes, change the enzyme list, using the combo box above the list of enzymes. You can select one of your user enzyme lists, create an On-the-Fly list of enzymes with specific characteristics, or use one of the program-defined enzyme lists.

### Finding Sites for a List of Enzymes

You can find recognition sites for all enzymes on an enzyme list in one easy operation. Once found, you can see the number and location of these sites, enter all of these sites to the molecule map, if desired, or cut the DNA with an enzyme. You can find all Single Cutters (enzymes that cut the molecule only once) or all Double Cutters (enzymes that cut the molecule in two places) using any enzyme list. You can find all sites for all enzymes on the list for user-designated enzyme lists. To find recognition sites for lists of enzymes:

- Click Clone, Find Sites and then click List of Enzymes.


- Click Single Cutters, Double Cutters or All Sites User List.
- Select the enzyme list using the Enzyme List combo box on the right.
- Click OK to find sites for these enzymes.

To change the enzyme list, use the combo box above the list of enzymes on the right. You can select one of your user enzyme lists, create an On-the-Fly list of enzymes with specific characteristics, or use one of the program-defined enzyme lists.

## Find Sites Results

When a search for enzyme sites has been completed, the results will be presented in a scrolling list box and location map display. Enzyme recognition sites are listed, showing the enzyme name and the site position. Click on the column headers to sort by enzyme name or position. Vertical bars above the icon map show the approximate location of the enzyme sites and a red arrow marks the highlighted position. You can add all of the enzyme recognition sites found to the molecule map, cut the molecule at all the sites found for the enzyme highlighted, or cut the molecule at the one enzyme site highlighted.

## Auto Scan Molecule

 Found on the Operations menu or use toolbar button in map window to access. Also available during import of files that contain no enzyme site information (such as GenBank or EMBL files). You can use this operation to scan a molecule using the AutoScan-designated enzyme list, looking for enzyme sites. Sites found will automatically be entered for this molecule in one batch operation. If the molecule already has enzyme sites, you can decide if you want to replace the existing site list or simply add the new sites (append).

AutoScan will look for single cutters using the Scanner enzyme list unless you designate an alternate enzyme list. The Scanner list contains the major, commercially-available enzymes with recognition sequences of 6 or more bases. To designate a different enzyme list, click Set (List) in the AutoScan dialog box. For program-defined enzyme lists (Commercial(Main), All Enzymes, Scanner List), you can find all single cutters during the AutoScan operation. If you designate a user-defined enzyme list instead, you can find all sites for enzymes on your list or all single cutters.

If you load molecules in groups, the AutoScan operation will be bypassed. When you open the molecule viewer for a newly-loaded molecule with no enzyme site information, you can click the AutoScan button in the map window toolbar to start this operation.

## Enzyme Lists

The Clone Manager program uses enzyme lists to hold the information about each enzyme that is necessary for its use. You can view, search or print enzyme lists and you can build or modify user enzyme lists to meet your individual needs. You can also update your master enzyme file monthly using REBASE data files so that you are always up-to-date with new enzyme information. Types of enzyme lists include program lists, user lists, shared lists, special enzymes, and the on-the-fly list.

When you install Clone Manager, the master enzyme file is copied to the installation directory. When you do a REBASE update, the new enzyme master file will be written to your home directory. On start-up, the program will check your home directory for a new master enzyme file and use the latest file found. Your user-specific enzyme information (user lists, special enzymes) will always be stored in files in your home directory.

## Viewing Enzyme Lists

Click Clone, Enzyme Lists to open the enzyme list resource. Click on Program Lists or My User Lists to see enzyme lists in these categories. Use the Tab key or Shift + Tab to move between the left and right panels or click with the mouse. Click on the Folder Options toolbar button to add a new empty enzyme list, or to rename, delete, import or export a user enzyme list.

Click on an enzyme list in the left panel to display the contents of this list in the right panel. For each enzyme in this list, the name, recognition sequence, cut position, ends produced and overhang bases are shown. Click on column headers to sort by the values in that column. Select an enzyme by clicking on the enzyme in the list in the right panel. Type in the initial letters of an enzyme name to quickly move to that enzyme in the list. Use the toolbar buttons to view enzyme suppliers, isoschizomers, compatible ends, or enzyme properties. Use the Ambiguous Bases toolbar button to see a list of IUPAC ambiguous codes.

## Program Enzyme Lists

The Clone Manager program constructs and maintains three general-purpose enzyme lists. Each time you update your enzyme lists using the latest REBASE enzyme file, these lists will be regenerated. You cannot edit these program-defined enzyme lists.


**Commercial (Main)** -- This list contains the major, commercially-available enzymes recognized by the Clone Manager Program (approximately 200 enzymes). It is a subset of the master enzyme list. Duplicate enzymes with the same recognition sequence and cut position are eliminated. This list is the program default for most enzyme operations.

**All Enzymes** -- This is the master list containing all of the enzymes recognized by the program. There are more than 600 enzymes in this list, including many isoschizomers for the common commercially-available enzymes found in the Commercial (Main) List.


**Scanner List** -- This list contains the major, commercially-available enzymes with recognition sequences of 6 or more bases. This list is the program default for the AutoScan operation.

## User Enzyme Lists

You can create user-defined enzyme lists to hold the enzymes you routinely use or enzymes you plan to use for a specific task. Clone Manager provides a wizard to help you through the process of building new user enzyme lists. To create a new enzyme list:

- Click Clone, Enzyme Lists
- Click the Build User List toolbar button. 
- Follow the wizard instructions.

Once your enzyme list has been created, you can later add or remove enzymes, as necessary. You can add enzymes that the program recognizes or Special Enzymes (perhaps not commercially available) that you define. To add an enzyme to an existing list:

- Click Clone, Enzyme Lists
- In the left panel, select the user-defined enzyme list that you want to add enzymes to
- Click the Unlock toolbar button to allow changes to this list. 
- Click the add Enzyme toolbar button and type in the enzyme name in the data entry box provided. An enzyme lookup list box will help you get the name exactly right.
- Click OK to add this enzyme to the selected user enzyme list.

To remove an enzyme on the unlocked user list, select (highlight) the enzyme and then click the Delete Enzyme toolbar button.

You can export or import user enzyme lists, as needed, to provide a list to a colleague or to move your list from one computer to another. You can also rename or delete a user enzyme list, if required. Just right-click on the list in the left panel and select the appropriate option.



While viewing filtered restriction map data, you can build a new user enzyme list using the enzymes shown on the display (including the list of No Cutters). Just click the toolbar button Build User List in the restriction map window to get started.

## On the Fly Enzyme List

You can create a transient enzyme list just when you need it and discard the list at the end of the current operation. Or you can create a transient list in the Enzyme Lists resource, where you can sort the list and review pertinent information about the enzymes. To create and use a transient enzyme list:

- Start the operation you want to perform or click Clone, Enzyme Lists.
- Select the enzyme list On-The-Fly.
- Specify enzyme characteristics (see below).


**Recognition Element Size** -- Select recognition element sizes of 4, 5, 6, or more than 6 bases. You can select more than one size. Leave all boxes clear to permit any size element.

**Ends Produced** -- Select the type of ends required: 5' overhang, 3' overhang, or blunt ends. You can select more than one end type. Leave all boxes clear to permit any type of ends.

**Selection Criteria** -- You can specify if only commercially available enzymes should be included in your transient list. If you do not check this box, a small number of enzymes with unique recognition sequences will be included although they are not commercially available. You can also indicate the supplier you would prefer to use. During enzyme selection, if more than one enzyme with the same recognition sequence and cut position is available, the enzyme from your preferred supplier will be used.

## Updating Enzyme Lists

You can update the Clone Manager master enzyme file monthly using REBASE data files so that you are always up-to-date with new enzyme information. (Latest REBASE review, Nucleic Acids Research 33:D269-D270, 2007). Each time you do an update, the program-defined enzyme lists (Commercial (Main), All Enzymes and Scanner) will be regenerated. The update procedure will not discard your user-defined enzyme lists or any special enzymes that you may have entered. To do a REBASE update:

- Click Clone, Enzyme Lists
- Click the REBASE Update toolbar button 
- If you already have the latest REBASE file (via email or from the web), set the option **Have the File** and provide the complete filename and path.
- Set the option **Get the File Now** to have the program get the file for you using anonymous FTP.
- Click **Update**

The program will locate and process the REBASE data file and report the changes found. Once the enzyme list update is complete, you must exit the program and then re-start it in order to use the new enzyme lists. To get the latest REBASE data file, connect to the REBASE web site <http://rebase.neb.com>, click on the REBASE Files icon, click on file format #35, Clone Manager, and then click Get File. Save the data file to your computer. When you do an update procedure, you will need to identify this file.

## Viewing Restriction Map Data

Restriction map data for the active molecule (or the zoomed region of the molecule) is displayed in the molecule viewer window. Click the RMap tab at the bottom of the window to move to this display. You can print the data in this view window, copy this view to the clipboard, or export the view data to a disk file.

Restriction enzyme recognition site information is listed in table format or displayed in a graphic format. Click the data column header to change the display style. Click the column headers to sort the data by enzyme name or number of sites. Use the combo box in the toolbar to select a different enzyme list if you want to regenerate the restriction map data using another enzyme list (perhaps a list of enzymes you constructed that are routinely used in your lab).



Click the No Cutters button to see a list of enzymes on the specified enzyme list that do not cut within the active molecule (or that do not cut within the zoomed region of the molecule). Click the depressed No Cutters button to return to the prior data display.

**Special Notations** -- Enzymes with a large number of sites (more than 50), have the data display suppressed. Click the small plus sign next to the enzyme name to display or hide this data. Enzyme names shown in color denote possible methylation sensitivity (click Format button to enable this setting.)

**Enzyme Information** -- If you find an enzyme on the Restriction Map display that looks like it will cut in just the right place, but you need more information about this enzyme, you can use toolbar buttons to get enzyme properties, suppliers, isoschizomers, or compatible ends. Select (highlight) the enzyme and then click one of the toolbar buttons.

**Enzyme Actions** -- While you are looking at the Restriction Map display, you can select an enzyme and cut with this enzyme or enter the sites to your molecule map. If you are looking at restriction map data that has been filtered to find just the enzymes you need at this time, you can enter enzyme sites to the molecule map for all or some of the enzymes shown, or you can use these enzymes to build a new user enzyme list.

### Restriction Map Format



In the restriction map window, click the Format button to change the enzyme list, data display style, or sort order (see below). You can also make these selections using the toolbar in the restriction map window, but you must use this dialog box to mark enzymes with possible methylation sensitivity or to remember your settings.

**Memorize Settings** -- Once you have selected the enzyme list, display style and sort order, click the Memorize button to instruct the program to remember these settings and use them whenever you initially open a restriction map window. You can use the toolbar buttons to change the display as needed when viewing data.

**Enzyme List Selection** -- By default, the Commercial (Main) enzyme list is used to generate the restriction map data, but you can select any other enzyme list and regenerate the data. To select a different enzyme list, use the Enzyme List combo box in the restriction map window toolbar or in the format options dialog box.

**Methylation Sensitivity Alert** -- Some enzymes may be sensitive to methylation. If you want to be able to identify these enzymes, click the Format button and then check the Mark Methylation Sensitivity box. The names of the enzymes with possible methylation sensitivity will be shown in color.

You can sort the restriction map data by enzyme name or by the number of sites (or fragments) for each enzyme. Click on the column header in the display window or select the sort option in the combo box in the format options dialog box.

**Sort by Enzyme Name** -- Restriction map data is sorted alphabetically by enzyme name.

**Sort by Number of Sites (or Fragments)** -- Restriction map data is sorted by the number of recognition sites found for each enzyme (or the number of fragments that will result when cut by each enzyme). Enzymes with one recognition site or fragment are listed first. Within each number grouping, enzymes are listed in alphabetical order.

You can display restriction map data in the styles described below. To select a style, click on the data column header in the restriction map window and select a style from the menu offered. If you always want to use a specific display style, click the Format button and then select the display style in the Format Restriction Map dialog box. Click the Memorize button to remember this selection.

**List of Recognition Sites** -- Shows restriction map data in a table format. The enzyme name is shown at the left and then the number of sites for this enzyme. The basepair position for each recognition site for this enzyme appears in ascending basepair order.

**Map of Recognition Sites** -- Shows a graphic representation of the restriction map. Each enzyme on the list selected that has sites in this molecule is listed. The enzyme sites are marked by diamonds at their approximate location relative to the molecule map, shown in the top of this window.

**Fragment Sizes** -- Shows restriction map data in a table format. The enzyme name and the number of fragments is shown at the left, and then each fragment (in bps) produced by cutting with this enzyme. Fragments for each enzyme are listed in descending size order. (Not available for zoomed molecules.)

**Gel View of Fragments** -- Shows a graphic representation of a gel produced by cutting with each enzyme. The enzyme name and number of fragments is shown at the left and horizontal lanes are marked. A logarithmic scale (bottom of the simulated gel view) relates to fragment size in bps. A vertical bar shows the approximate position at which a band would appear on a gel for a fragment of the size predicted by cutting with the enzyme. (Not available for zoomed molecules.)

**No Cutters** -- List of all the enzymes on the specified enzyme list which do NOT cut the molecule. The list is in alphabetical order, reading from left to right. Filter settings have no affect on the list of non-cutting enzymes.

## Using the Restriction Map Filter

In the restriction map window, click the Filter button to set filter options to limit the data displayed to just what you need at this time. You can filter based on how enzymes will cut your molecule or based on enzyme characteristics. When a filter has been applied, the Filter toolbar button will be depressed. Click the depressed Filter button to remove the filter.

**Filter by Cut Information** -- You can set filter options to limit the data display to enzymes that cut a specified number of times, or to show only enzymes that cut inside a specified region and/or enzymes that cut outside a specified region. You can use both cut region filter settings to identify two cut zones.

Use the Cut Outside Region option to designate a region you would like to protect -- enzymes that cut in this region will not be included in the display. Use the Cut Inside Region option to specify the minimum and maximum basepair positions within which you need an enzyme to cut. For enzymes which cut at multiple sites, if any cuts fall within the region specified, this enzyme and all of its recognition sites will be included in the display.

**Filter by Enzyme Characteristics** -- You can set filter options to show only enzymes that produce specific end types (like 5' overhang) or that have a specific recognition element size. Use the check boxes to select the characteristics required. Leave all of the check boxes blank to accept any type of ends or to accept any size recognition elements.

# Cloning Operations

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Clone Manager can cut DNA at enzyme cut sites or at specified basepair positions. Molecule features and enzyme sites are automatically recalculated after a cut operation. The program can ligate DNA, checking for compatible ends. You can modify molecule ends, if needed for ligation, and you can select the cloning orientation. For fast simulated cloning, you can cut, ligate, and modify ends from the same display window showing sticky ends and molecule icon maps.

The Plan Cloning Wizard lets you describe your objectives, review the list of possible solutions, and instruct the Wizard to construct the recombinant of your choice. The new PCR Cloning Wizard will help you to design PCR primers to amplify the region of a molecule you want to clone, use enzymes to prepare the vector or the region for insertion, and then generate recombinant molecules (Clone Manager Professional required).

## Cutting Molecules



Found on the Clone menu or use toolbar button to access. You can cut a molecule by selecting an enzyme from an enzyme list and cutting at all sites for this enzyme. Or you can cut at one specific enzyme site already marked on your molecule map or at specified basepair positions.

### Cutting with Enzymes

You can cut a molecule using restriction enzymes. The molecule will be cut at all of the sites for an enzyme. (To cut at only one site, use the cut at Map Site option). You can select 1, 2 or 3 enzymes and cut with these enzymes in one operation. To cut a molecule with enzymes:

- Click Clone, Cut and then click Enzyme(s).
- Type in the names of the enzymes, separated by a comma, or find the enzymes in the lookup list to the right (see below).
- Click OK to cut at all sites for these enzymes.

**Enzyme List Lookup** -- The enzymes on the selected enzyme list are shown along with their recognition sequence. Press a letter key to move to enzymes in the list that begin with that letter. Double-click to paste an enzyme name from the list to the text box. To view a different collection of enzymes, change the enzyme list, using the combo box above the list of enzymes. You can select one of your user enzyme lists, create an On-the-Fly list of enzymes with specific characteristics, or use one of the program-defined enzyme lists.

### Cutting at Map Sites

You can cut a molecule by selecting one enzyme site already marked on the molecule map. By convention, the enzyme site is marked at the left end of the recognition sequence for that enzyme. The program will cut at the correct cut position for this enzyme site. To cut a molecule at one specific map site:

- Click Clone, Cut
- Click Map Site and select one enzyme site from the list box.
- Click OK to cut.

Alternatively, you can use the working map display screen to cut at a single map site. Select (click on) an enzyme site on the working map and click the Cut at Site toolbar button. If you want to check a cut position first, select the enzyme and then click the Properties toolbar button to see the exact cut position and ends produced.

## Cutting at Base Positions

You can cut a molecule by specifying one or two basepair positions. You might want to do this to cut out a section of the molecule. The positions you specify do not have to be enzyme sites. To cut at base positions:

- Click Clone, Cut and then click Base Position(s).
- Type in 1 or 2 basepair numbers, separated by a comma
- Click OK to cut after these basepair positions.

The molecule will be cut blunt-ended into one or more fragments and you can select the fragment that you wish to keep.

## Cut Results


Following a cut operation, a copy of the original molecule will be cut into one or more fragments. Resulting fragments can be entered to the molecule list, making them available for use within the program. (If needed for further use, you should save these new molecules to disk.) If a cut action results in only one molecule fragment, you can immediately enter this fragment to the molecule list.

When multiple fragments result from the cut action, you can view the resulting fragments and identify which to enter to the molecule list for immediate or later use. Multiple fragments are listed, showing the start and end basepair positions and the fragment size. Click on the column header to sort on the values in that column. The red horizontal bar above the icon map shows the approximate location of the highlighted fragment. If cutting with enzymes, the enzyme producing the left end of the fragment is shown to the left and above the red bar; the enzyme producing the right end is shown to the right and below the bar.

**Use Now** -- enter the highlighted fragment to the molecule list and then continue to use this as the active molecule. All other fragments will be discarded.

**Create** -- enter the highlighted fragment to the molecule list and then return to this dialog box to create additional fragments.

## Ligating Molecules

 Found on the Clone menu or use button on the main toolbar to access. Use this option to join together two pieces of DNA or to join the two ends of the same molecule (self-ligate). The ligate display window lets you see the two molecules as they are being prepared for ligation. An icon map of the molecule is shown, with features (if any). If enzymes were used to create the molecule's ends, these enzyme names appear above the respective end. The actual sequence of each end is shown below the map. Use the Features or CompEnds buttons (lower left) to view the features tables for the molecules or to find enzymes that could prepare ends that would be compatible to the molecule end you select.

**Changing molecules** -- Use the molecule list button to the right of the molecule window to select a molecule for this window or to change the molecule currently shown. You will want to put the vector in the upper window -- after ligation, a circular recombinant will be rotated to restore the original reference frame of the vector (in the upper molecule window). If needed, you can flip the contents of the two windows. Use the double arrowhead button to the right, between the two molecule windows, to put the lower molecule in the upper window and the upper molecule in the lower window.

**Cutting the DNA** -- Use the cut button on the right to access the Cut Molecule operations for this molecule. You can cut a circular molecule to make it linear, cut out a region to be cloned, or cut with an enzyme to make a compatible end.

**Modifying the Molecule Ends** -- Use the modify ends button on the right to access the Modify Ends operations for this molecule. You can use standard or custom end modifications to prepare molecule ends for ligation.

**Perform ligation** -- Click the Ligate button to join all compatible ends or the Partial button to ligate only the two molecule ends you designate.

## Ligation Results

When you click the Ligate button in the Ligation working window, a ligation will be performed if the ends of the two molecules of DNA are compatible. All possible ends will be ligated. If only one ligation orientation is possible, you will be ready to enter the new molecule to the Molecule List.

If you added a piece of DNA that could be inserted in more than one orientation, you can select the orientation you prefer. The insert is marked in color below the molecule icon map, with an arrow indicating direction. Use the Enter1 or Enter2 buttons (lower left) to enter the product with the desired orientation to the molecule list. If you want to save both products, enter each to the molecule list sequentially.

When you click the Partial button in the Ligation working window, a partial ligation will join only the two molecule ends you select. The resulting product will be linear. Set the option that joins the ends in the orientation you want, using the drawings to visualize the ligation event. After you add the new molecule to the molecule list, you can return to the Ligate display to select additional ligation products.

## Modifying Molecule Ends

Found on the Clone menu or access using the Modify Ends buttons from the Ligate display window. Use this option to modify the ends of a linear molecule. An icon map of the molecule is shown, with features (if any). If enzymes were used to create the molecule's ends, these enzyme names appear above the respective end. The actual sequence of each end is shown below the map.

Select the type of end modification using the combo box in the lower left to do Klenow Fill-In, 5' ->3' exonuclease, 3' -> 5' exonuclease, or Partial Fill-In operations (see below). The proposed modification will appear in the sequence of the ends, if this type of modification has an effect on the molecule.

Select Modify to change the molecule ends, as shown, and alter the sequence for this DNA. You can also directly edit the single stranded extensions by selecting Custom (see below).

## Standard End Modifications

**Klenow Fill-In** -- Treatment with DNA polymerase I Klenow fragment will fill in 5-prime overhang extensions of the molecule to create a blunt end. The bases to be added appear as lower-case color letters in the display.

**5' -> 3' Exonuclease** -- Treatment with 5' -> 3' exonuclease will remove bases from the 5-prime single-strand ends of the molecule (i.e. a 5-prime sticky end will be trimmed down to a blunt end). The extent of exonucleolytic action is limited to the single-strand extensions. The bases to be excised appear as lower case letters with a cross-out line in color.


**3' -> 5' Exonuclease** -- Treatment with 3' -> 5' exonuclease will remove bases from the 3-prime single-strand ends of the molecule (i.e. a 3-prime sticky end will be trimmed down to a blunt end). The bases to be excised appear as lower case gray letters with a cross-out line in color.

**Partial Fill-In** -- Select this option and then use the check boxes to select the bases A, C, G or T to partly fill in 5-prime overhang extensions. The bases to be added appear as lower case color letters.

## Custom End Modification

Click the Custom button to directly edit the single-strand extensions. Type in bases or remove bases, as needed. The single-strand extension is limited to a maximum of 9 bases. To replace a 5' sticky end with a 3' sticky end, remove the 5' end first and then add bases for the new 3' overhang.

## Use the Plan Cloning Wizard

 You can describe a cloning experiment and have the Plan Cloning Wizard suggest some possible cloning solutions for your review. You can select one of the suggested solutions and view more details about this procedure, or create the resultant recombinant with the click of a button. To use the Plan Cloning Wizard, click the Plan Cloning toolbar button and follow the wizard instructions. The wizard will help you to identify the insert and vector sequences and indicate your preferences for the cloning experiment.

**Define What You Want to Clone** -- Identify the molecule that contains the region you want to clone. Use the Change button to select another molecule if needed. Use the Features button to look at the features table for this molecule and select a gene, if appropriate. The program will enter the base positions for you.

**Define the Vector You Want to Use** -- Click the Change button to select a molecule that you want to use as the vector. The Wizard can only use circular vector molecules. You can use the Features button to look at the features table for this molecule. You might want to select a gene (such as a gene for antibiotic resistance) in order to do recombinant selection by insertional inactivation. If the vector molecule contains a multi-cloning site, the basepair positions for this region will automatically be entered for you.

**Select an Enzyme List** -- In finding possible solutions for your cloning experiment, the program will consider only those enzymes contained in the list you select. The default list is the one you designated as your AutoScan list. You might want to try more than one enzyme list, depending on how many or how few possible solutions you get. When you are viewing the results, you can easily Redefine your search without entering all of the information again.

**Specify Your Cloning Preferences** -- You can accept the default settings for all preferences or you can select your own individual preferences. You can indicate whether to use 1 or 2 enzymes to cut the insert, if you will allow the use of blunt-cutting enzymes or Klenow fill-in, if you have a preference for orientation of the insert, or if you want to limit extra insert DNA.

When you select an option that says "Prefer ..." the program will increase the weight for results of this type and move them closer to the top of the list of possible solutions. When you select an option that says "Require ..." the program will eliminate results that do not satisfy this requirement. The preferred enzyme supplier selection is used to collect specific information about each enzyme shown in the details of construction. This information includes buffers, incubation temperature, heat inactivation status.

## View Plan Results

If possible solutions can be found for the cloning experiment you described, these results will be shown in a table display, sorted with the best results (based on your preferences) at the top. Select (highlight) the result of interest and use toolbar buttons to view the details of a suggested solution, construct a recombinant, or redefine the experiment, if needed.

## Use the PCR Cloning Wizard

If you are using Clone Manager Professional, this wizard will help you to design PCR primers to amplify the region of a molecule you want to clone, use enzymes to prepare the vector or the region for insertion, and then generate recombinant molecules. You can view several solutions and save selected primers, amplified products and recombinants for later use. Select the PCR Cloning Wizard from the Primer or Clone menus. Next, identify the type of PCR cloning you want to do and then follow the wizard instructions.

**Define What you Want to Amplify for Cloning** -- Identify the molecule that contains the region you want to clone. Use the Change... button to select another molecule if needed. Enter the upper strand coordinates of the region you want to clone. Use the Features... button to look at the features table for this molecule and select a gene, if appropriate. The program will enter the base positions for you.

**Set the Primer Design Options** -- Set the primer type to PCR Primer to set the appropriate primer evaluation criteria. You can leave the criteria set to default conditions, or you can edit the criteria settings for this primer search. Enter the optimal primer length you would like and indicate the acceptable primer size range. Enter the number of bases the search can extend to the left for the forward primer, and to the right for the reverse primer.

**Specify Preferences** -- If the search for PCR primers is not successful, the program will attempt to extend the acceptable GC range, adjust (relax) the primer evaluation criteria, or increase the search regions to try and find primers you can use. You can accept the default settings or indicate your preferences for each of these categories. For Topoisomerase or Classic TA Cloning, you can indicate your preferences with regard to initial residues at the 5' end of your PCR primers that can affect cloning efficiency. These preferences will affect the quality score for each primer and help to sort the best primers to the top of the list. In addition, the program will automatically eliminate primers that contain the recognition sequence for Vaccinia Topoisomerase I.

**Define the Vector you Want to Use** -- Click the Change... button to select a molecule that you want to use as the vector. The Wizard uses circular vector molecules. If the vector molecule contains a multi-cloning site, the basepair positions for this region will be entered for you. For Topoisomerase cloning, the program will find the Topo sites and enter the most likely cut positions for you. You can change these positions, if necessary, and you can select the orientation of the insert.

**Select Enzymes** -- For Blunt End cloning and Classic TA cloning, select the enzyme you want to use to cut the vector molecule. The program will show you enzymes from the specified enzyme list that cut only inside the region specified and do not cut the insert molecule. If you want to consider other enzymes, you can select a different enzyme list. For Restriction Enzyme cloning, you can select different enzymes to cut the left and right sites of the vector molecule and you can designate a cap for each enzyme (recommended). The program will show you enzymes that cut only once in the region specified, produce sticky ends, do not have ambiguous bases, and do not cut the insert molecule. The enzyme recognition sequence and cap will be added to the 5' end of the appropriate primer, increasing the length of the primer sequence you specified. Select the orientation of insert -- clockwise (cw), or counter-clockwise (ccw).

## View PCR Cloning Results

If possible solutions can be found for the PCR cloning experiment you described, the selected number of best solutions can be viewed sequentially. Use the Next Solution button to move to the next result.

For a solution, you can use toolbar buttons to view a complete text summary of the solution, do the required steps to simulate the cloning experiment and produce the recombinant molecule, or enter PCR primers to the primer list, saving the primers to disk or adding them to your primer collection. Click the Redefine toolbar button to change the cloning experiment, if needed.

## Use the Gateway® Cloning Wizard

This wizard will help you to simulate cloning using Gateway® Recombination Cloning techniques (Invitrogen Corp). It will help you select the appropriate components, show you the proposed result, and create the resulting recombinant molecule. If you are using a Multisite Pro kit, the wizard will walk you through constructing multiple fragments with the right attB sites and donor vectors. Please refer to the technical publications received with the Gateway® products or available at [www.invitrogen.com](http://www.invitrogen.com) for more information about the recombination reactions or possible use restrictions. Selected donor and destination vector files are provided. These files are copied to your home directory and can be found in the subfolder GWC\_Vectors.

Select the Gateway Cloning Wizard from the Clone menu. Next, identify the procedure you want to do. You can make an entry clone (BP reaction), an expression clone (LR reaction), prepare a PCR product with attB sites, or simulate a complex multisite procedure.

**Select Insert and Vector Molecules (BP and LR reactions)** -- To make an Entry Clone, identify the molecule that contains the region you want to clone, flanked by attB sites. Click the Change button to access the Molecule List or Browse. Next, identify the donor vector you want to use. In many cases, a donor vector will be suggested. Click the Change button to select another vector. The donor vector you select must have attP sites that match the attB sites in your insert. To make an Expression Clone, identify the insert (entry clone) with attL sites that you want to use. Click the Change button to access the Molecule List or Browse. Next, select the destination vector. Click the Change button to select another vector. The destination vector must have matching attR sites. If you want to use more than one entry clone in this reaction, set the checkbox and click Next to move to a page where you can add multiple entry clones.

**Define What you Want to Amplify (PCR product generation)** -- Identify the molecule that contains the region you want to amplify. Use the Change button to select another molecule if the one shown is not the one you want to work with. Enter the upper strand coordinates of the region you want to amplify and set the strand (normal or complement) to amplify. Use the Features button to look at the features table for this molecule and select a gene, if appropriate. The program will enter the base positions for you.

Select the attB extensions you want to add to each primer. For a simple one fragment recombination, attB1 and attB2 are standard. When amplifying a region on the normal strand, the attB extension for Primer A will lead into the expressed sequence. When amplifying a region on the complement strand, the attB extension for Primer B will lead into the expressed sequence. Use the box below the attB extension to type in other bases that you want to add after the attB extension and before the template sequence. These additional nucleotides may assist in protein expression or maintain proper reading frame. For both Primer A and Primer B, enter the additional bases 5' to 3'. If you are using Clone Manager Basic, the region to be amplified will be extracted and the attB extensions added to simulate PCR product generation.

If you are using Clone Manager Professional, primers can be designed and evaluated. The primer type, criteria, and primer length within the sequence template have been set and should not need to be changed. Set the primer design goal to Restrict to Amplified Region if you do not want any extra sequence before or after the region you selected to amplify. The program will construct the possible primers of different lengths at the two fixed positions and select the best primer pair. If you need higher quality primers and can accept some extra sequence, set the design goal to Better Primers. Specify the GC content range and criteria adjustment that you will allow, if needed, to find better primers. The program will search the region 20-40 bases before and after the bounds of the region to amplify, attempting to find primers that meet all criteria settings and then selecting the best primers.

**Multisite Pro kits** -- Select the 2, 3 or 4 fragment procedure and indicate the method to use. If inserts have already been prepared, identify the molecule or PCR product that contains the region you want to insert for each fragment, flanked by the needed attB sites. The first line (Make Fragment...) shows the attB sites required

for this fragment. Click the Change button to access the Molecule List or Browse. The donor vector with matching attP sites is suggested. Continue to create the additional fragments needed.

If you need to prepare the PCR products, begin by identifying the molecule, region and strand to amplify for this fragment. The required attB extensions are preset for primers A and B for each fragment. You can add extra bases between the attB extension and the template sequence, if needed. This PCR product will be generated and used for the subsequent BP reaction. The donor vector with matching attP sites is suggested. Continue this two-step process to create the additional fragments needed.

When all fragments have been prepared, they will be collected and automatically added as entry clones for the subsequent LR reaction. Select a destination vector to prepare the expression clone. The destination vector you select must contain attR1 and attR2 sites.

## View Gateway® Cloning Results

After using the wizard to simulate the construction of an entry or expression clone using Gateway® Recombination Cloning techniques (Invitrogen Corp), you can view the proposed result and create the recombinant molecule. An iconic map of the recombinant molecule is shown, with the insert marked in a lighter color, inserted in the direction indicated by the arrow. If features were present in the vector or insert molecules, these features will be shown above the map line. The area below the map shows detailed information about the recombinant molecule, the insert molecule, and the vector used.



Click the Use Now button to take the molecule shown and use it in the next logical step in Gateway cloning. If you have just done a BP reaction, for example, the Use Now action will start an LR reaction using the entry clone you just created.



Click the Create Molecule button to have the program automatically do the required steps to simulate the cloning experiment and produce the recombinant molecule. You can enter this new molecule to the molecule list to view now or save to disk for later use. Following a multisite procedure, you can also collect the intermediate molecules generated during the procedure (entry clones, PCR products).

If you are using Clone Manager Professional, you can design and evaluate primers to generate PCR products with attB extensions for use in Gateway cloning steps. If you requested more than one solution, you can view the solutions sequentially using the Next and Previous toolbar buttons. For each solution, the iconic map shows the amplified product, flanked by the attB sites. The area below the map includes information about the amplified product and about the primers used to generate this product. If either primer (or the primer pair) does not meet the criteria set, the notation 'Caution -- check primer pair report' appears above the primer descriptions.



Click the Primer Pair Report button to view the evaluation report and related primer analysis screens for the primers associated with this solution.

You can use other toolbar buttons to export primer sequences or add these primers to the primer list, saving the primers to disk or adding them to your primer collections. Following a multisite procedure, the Create Molecule option allows you to add all the primers to a new primer collection or export all primer sequences in one easy step.

When the simulated recombinant molecule has been generated, you will want to review this new molecule to verify that the results are as expected. You might want to view the Features Table to verify your inserts are present in the correct position and orientation. Select an inserted gene and use the toolbar button Go To Start of Sequence to open the sequence view with the translated gene highlighted in the context of the full molecule sequence. Other helpful tools you might want to use include ORF Search and Analyze Molecule, Open Reading Frames, both found on the Operations Menu.

# Analysis Functions

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Clone Manager can search for Open Reading Frames and easily enter found ORFs as genes in the features table for the molecule. It can provide information about cohesive ends to help you select enzymes for use. You can use Clone Manager to analyze DNA molecules to find direct or inverted repeats and dyad symmetries and you can analyze protein (or translated DNA) molecules for hydrophilicity and look at protein structure (helix, sheet, and turn configurations). A more detailed Open Reading Frames analysis is a part of the Analyze Molecule option.

## Open Reading Frame Search



Found on the Operations menu or use toolbar button in map window to access. Use this option to locate Open Reading Frames for the active molecule. You can indicate if you require that each ORF start at a specific codon. Select ATG, GTG, or type in the base letters for the start codon required. Similarly, you can indicate specific stop codons or accept the default settings. Select the checkbox if you want the program to include open reading frames that start within longer ORFs. You can also enter the minimum size cutoff (in amino acids) and the maximum number of open reading frames to be reported.

When a search for Open Reading Frames has been completed, the results will appear in a scrolling list box and location map display. The scrolling list shows each ORF found, with its start position (in bps), frame, and the length (in amino acids). Click on the column header to sort by the value in that column. The red arrow above the molecule map shows the approximate location of the highlighted ORF. The arrow points right for normal strand, left for complementary strand ORFs.

**Enter Gene** -- enter the highlighted ORF as a gene in the features table for this molecule, with the option to enter a gene name.

**Enter All** -- enter every ORF as a gene in the features table, automatically entering generic names (ORF-n) with numbers incrementing in the order shown. Sort the display to change the order, if needed.

You can also set the checkbox to Show Overview to see all of the listed open reading frames positioned in their approximate locations in the six translation frames. The highlighted ORF is shown in red.

## Compatible Ends



Clone Manager can show you the cohesive end that would be produced by cutting with a specified restriction enzyme and a list of enzymes that could be used to produce compatible cohesive ends. This function can be accessed from the molecule map or restriction map displays, or while viewing enzyme lists or working in the ligate window.

The list of enzymes that could produce compatible ends is limited to prototype enzymes and neoschizomers. That is, if more than one enzyme could produce exactly the same compatible end, only one enzyme is shown (the prototype for this group). If another enzyme has the same recognition sequence but cuts in a different position (a neoschizomer), this enzyme will also be listed if it will produce a compatible end.

**Molecule Map** -- Click on an enzyme site and then click the Compatible Ends button. The display will show the cohesive end produced by cutting with this enzyme at this site, as well as enzymes that could produce compatible ends. Even if the enzyme has a degenerate recognition sequence (it can recognize more than one sequence), the sequence of bases at this specific enzyme site is known.

**Restriction Map** -- Click on an enzyme and then click the Compatible Ends button. If the enzyme has a degenerate recognition sequence, ambiguous bases in the overhang will be expanded and each of the possible overhangs shown. For each overhang shown, enzymes that could produce compatible ends are listed (if any).

**Enzyme Lists** -- When you are viewing enzyme lists (Clone, Enzyme Lists), you can click on an enzyme and then click the Compatible Ends button to view enzyme-specific compatible ends information, as described for Restriction Map data (above).


**Ligate Window** -- When you are in the process of performing a ligation, you can cut or modify molecule ends, as needed. If you need help with finding an enzyme that can produce cohesive ends compatible to one of the ends in the ligation window, you can click the Comp Ends button and then select the end you need to match. Site-specific compatible ends information will be displayed, as described for the Molecule Map (above).

## Analyze Molecule

You can do some basic analyses on your DNA molecules. You can analyze a molecule to find open reading frames, viewing an ORF map of start and stop codons, which help to identify potential protein coding regions. A numerical value (Fickett's TESTCODE) can help you to determine if a region is likely to encode a protein. You can analyze direct or inverted repeats and dyad symmetries to identify regions that might be associated with biologically active sites such as control regions, transcription terminator regions, or RNA processing sites.

You can also do some basic analyses on protein (or translated DNA) molecules. You can analyze protein structure for alpha-helix, beta-sheet (extended), beta-turn and coil (random) states using the method of Garnier-Osguthorpe-Robson. You can use hydrophilicity analysis with beta turn prediction to assist in identifying antigenic regions. To analyze your molecule:

- Click Operations, Analyze Molecule
- Select the type of analysis
- Identify which part of the sequence to analyze

 When viewing analysis results, use the Help with Results toolbar button to view help related to the analysis displayed.

## Open Reading Frame Analysis

You can analyze open reading frames for a full molecule sequence, or for a portion of the sequence. You can set the minimum size of the open reading frame (in amino acids) that the program should report. As a part of this analysis, the program uses Fickett's TESTCODE algorithm to generate a score that can help you determine whether or not an open reading frame is likely to be a protein coding region. This method is based on the observed asymmetry of base utilization within coding regions.

The analysis results are shown in a table format of numerical values and as a picture overview showing all 6 possible reading frames with stop and start codons. Click the toolbar buttons to display analysis results with Any, ATG or GTG start codons. Use the toolbar buttons to select Text or Graphic displays. Use the Redefine button to return to the Analyze Molecule setup dialog box to select another analysis type or another region of the molecule to analyze.

**Text Display** -- for each open reading frame, the program reports start and end basepair position, length in amino acids, frame, and Fickett's TESTCODE score values. The first amino acids of the open reading frame sequence are displayed. You can sort the list of open reading frames found by frame, length (in descending order), start position, or score (in descending order). You can also use the Add Feature button to enter the selected ORF as a gene in your molecule's features table.

**Graphics Display** -- an ORF map shows all 6 possible reading frames. Open reading frames that exceed the minimum size specified and that exceed 5% of the analysis region length are shaded in pale blue. Full height black bars mark terminators. Half height colored bars can be used to mark start codons.

## DNA Repeats Analysis

You can analyze direct or inverted repeats (and dyad symmetries using a separate option) to identify regions that might be associated with biologically active sites such as control regions, transcription terminator regions, or RNA processing sites. The repeats found are shown in a table display. Use the toolbar buttons in this window to move between the three repeats displays (direct, inverted or palindromes) or return to the analyze set-up dialog box (Redefine). You can sort the list of repeats by length (in descending order), or by position of the first or second repeat. If the number of repeats found exceeds 100, the program will eliminate from its list all repeats containing the smallest number of bases (for example, all 4-base repeats may be dropped).

**Direct Repeats** -- Click the Direct Repeats toolbar button in the Analyze Repeats display window to view. The program identifies all repeated sequences of four or more bases that are oriented in the same direction (head-to-tail) in the region to be analyzed. The repeated sequences can be abutted (tandem repeats) or separated by unrelated sequence.

**Inverted Repeats** -- Click the Inverted Repeats toolbar button in the Analyze Repeats display window to view. The program identifies all inverted repeats of four or more bases in the region to be analyzed. (If you want to limit the search to those inverted repeats that would be able to form stable stem-loops or hairpin structures, use the Dyad Symmetries analysis.)

**Palindromes** -- Click the Palindromes toolbar button in the Analyze Repeats display window to view. The program identifies inverted repeats of four or more bases that are not separated by any unrelated sequence. Such DNA sequences read the same on both strands.

## Dyad Symmetries

You can analyze dyad symmetries (and direct or inverted repeats using a separate option) to identify regions that might be associated with biologically active sites such as control regions, transcription terminator regions, or RNA processing sites. This option identifies sequences which could form stable stem-loop or hairpin structures. The program looks for sequences that are repeated in the opposite direction on the same strand and then uses RNA energy parameters to determine if each stem loop would be stable.

The program reports the largest and most stable duplexes which could be formed in descending order (up to a maximum of 100 duplexes with a negative delta-G value). Please note that G:U basepairs can be stable under these circumstances and may be a part of duplexes found. You can use the Redefine toolbar button to return to the Analyze Molecule set-up dialog box to select another analysis type or another region of the molecule to analyze.

## Hydrophilicity (Protein) Analysis

You can analyze protein (or translated DNA) molecules for hydrophilicity. Predictions are calculated using methods derived from Kyte & Doolittle and Hopp & Woods, and for surface exposure. The analysis results are shown in a table format of numerical values and as a set of line graphs. Use the toolbar buttons to select Text or Graphic displays. Use the Redefine button to return to the Analyze Molecule setup dialog box.

**Text Display** -- for each amino acid residue, numerical values are given for Kyte-Doolittle, Hopp-Woods, and Surface-Exposure analyses. All three methods provide an indication of the hydrophilic character of the environment, and the range for each analysis is shown at the top of the column.

**Graphics Display** -- three line graphs plot the values calculated for each of the three analyses. For all three graphs, values above the axis line are hydrophilic or predicted to be exposed at the surface of the protein.

**Kyte & Doolittle Analysis** -- Kyte & Doolittle values fall within a range of +4 to -4, with hydrophilic residues having a negative score. The most hydrophilic residue has a value of -4.5 (arginine). On the graphic display, values above the axis line are hydrophilic; values below the axis line are hydrophobic. Kyte & Doolittle represents a composite hydrophobicity scale derived from interpretation of free energy changes on a water-vapor phase transition and an analysis of buried side chains. Each value is the average of the values of 5 adjacent residues and is plotted at the middle residue. The range of values is approximately  $\pm 4$  relative units.

**Hopp & Woods Analysis** -- Hopp & Woods values fall within a range of -3 to +3, with hydrophilic residues having a positive score. The most hydrophilic residues have a value of +3.0. On the graphic display, values above the axis line are hydrophilic; values below the axis line are hydrophobic. Hopp & Woods derived hydrophilicity values from a study of antigenicity and adjusted the values to maximize the accuracy of predicting antigenic determinants. Each value is the average of the values of 6 adjacent residues and is plotted at the middle point. The range of values is approximately  $\pm 3$  relative units.

**Surface Exposure Analysis** -- The surface exposure value is presented as a proportion of the residue which is exposed on the surface of the protein. These values fall within a range of 0 to 1.000. The most exposed amino acid has a value of 0.97 (lysine). On the graphic display, peak values which fall above the axis line are predicted to be exposed on the surface of the protein. Surface exposure analysis uses the data of Janin, et al, which provides values representing the fraction of residues of a given amino acid that have a surface area of greater than 20 angstroms squared. High values therefore represent amino acids that are likely to be exposed on the surface of the protein. Plotted values are the average of 6 residues and are plotted at the middle point.

## Protein Structure Analysis

You can analyze protein structure for alpha-helix, beta-sheet (extended), beta-turn and coil (random) states. Predictions of secondary structure are calculated using the method of Garnier-Osguthorpe-Robson. You can use hydrophilicity analysis with beta turn prediction to assist in identifying antigenic regions. The analysis results are shown in a table format of numerical values and as a set of line graphs. Use the toolbar buttons to select Text or Graphic displays. Use the Redefine toolbar button to return to the Analyze Molecule set-up dialog box to select another analysis type or another region of the molecule to analyze.

**Text Display** -- for each amino acid residue, numerical values show the likelihood for that residue to be in helix, sheet, turn or coil states. The columns on the far right use a plus symbol (+) to indicate which state is most likely.

**Graphics Display** -- three line graphs plot the predicted values for alpha-helix, beta-sheet and beta-turn configurations. For all three graphs, values above the axis line represent regions which are likely to form helix, sheet or turn structures.

# Operations and Utilities

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Clone Manager includes operations and utilities to rotate, invert, circularize or translate a molecule, export results data, find text in molecule files and show a mutagenesis profile that provides a complex view of a sequence reacting to base changes. A simple database utility, DataBook, can be used to organize and store information about your molecule or data files.

## Process Molecule

Found on the Operations menu. You can invert the active DNA molecule, or rotate a circular DNA molecule. If a DNA molecule was brought into the program as a linear molecule (perhaps because the "circular" descriptor was missing), you can circularize the molecule. You can also translate all or a part of a DNA molecule. When the process has been completed, you will want to enter the new molecule to the Molecule List, changing the name or description, as needed. The inverted, rotated, circularized, or translated molecule exists only within the current program and should be saved to disk if needed for further use.

**Invert Molecule** -- Use this option to invert a copy of the active molecule, changing the positions of all sites and features.

**Rotate Molecule** -- Use this option to rotate a circular molecule to bring a different point to the top of the map. The program will rotate a copy of the molecule, changing the positions of all sites and features. To rotate a molecule, enter the basepair position to appear at the top of the map (the new origin of the molecule).

**Circularize Molecule** -- Use this option to blunt-end ligate a linear molecule to make it circular. (If the molecule has cohesive ends, the upper strand of sequence will be treated as if it were blunt-ended.)

**Translate Molecule** -- Use this option to translate the active DNA molecule to create a protein. Translation will proceed from the starting point specified, on the strand specified, and continue until a stop codon or the end of the molecule is encountered. To translate a molecule, enter the starting basepair position for translation. Use the check box to indicate if you want to translate the complement strand.

## Join Sequences

Found on the Clone or Operations menu. You can use this function to join two sequences to create a larger molecule. Molecule features (genes, regions, etc) will be retained during this operation and enzyme sites can be auto-scanned anew or rebuilt from existing site lists. After joining, you can enter the new molecule to the Molecule List, entering a molecule name and description.

**Sequences** -- Identify the molecules to join. Use the Change... button to access the molecule list to select a molecule to use for Sequence A and then for Sequence B. The sequence selected for Sequence A should be the first part of a new linear molecule or the sequence into which the other sequence (Sequence B) will be inserted.

Use the Invert checkbox to invert (reverse complement) a molecule sequence, if needed. The positions of all sites and features will be adjusted. The join operations work on upper strand sequence. If a molecule has cohesive ends, the upper strand of sequence will be treated as if it were blunt-ended. If a molecule is circular, it will be cut after the last base to open the molecule at the origin. For an Insert operation, Sequence A will retain its original format (it can be circular or linear with sticky ends).

You can select the method to use to join the two sequences:

**Append** -- simply add Sequence B to the end of Sequence A. A blunt-end ligation will attach the 3' end of sequence A to the 5' end of sequence B. Molecule features will be retained and basepair positions recalculated.

**Splice** -- remove overlap when appending B to A. Prior to ligating the sequences, the program will check the bases at the 3' end of sequence A and at the 5' end of sequence B, looking for exact homology. If at least 10 bases of exact matches are found at the ends of the molecules, the matching bases of Sequence B will be trimmed. The blunt-end ligation will then be performed, as in the Append option, above.

**Insert** -- insert Sequence B after bp number n in Sequence A. After selecting this option, use the edit box provided to enter the basepair number in Sequence A after which the insert should be made. Use the Features... button to look at the features table for the Sequence A molecule.

Following an Append or Splice operation, the new molecule can be either linear or circular. If you select a circular molecule format, the program will blunt-end ligate the linear molecule to make it circular. For an Insert operation, the resulting molecule retains the original format of Sequence A.

Enzyme sites for the new molecule can be auto-scanned at the completion of the join operation. The AutoScan procedure uses your designated AutoScan enzyme list, looking for enzyme sites (single cutters or all on user list, as specified in the AutoScan settings). The enzyme sites found will be entered, replacing the existing enzyme sites. You can clear the AutoScan checkbox, instructing the program to rebuild the map sites list by rescanning with enzymes currently listed for either molecule. This is the procedure used following a standard Clone, Ligation operation, but it may result in a number of enzyme sites that are not single-cutters for the newly-joined molecule.

## Export Data

Found on the File menu. Use this option to export data that is in the active window at the time you select the Export option. You can export the complete set of data in the view or capture a pixel image of just the data shown in the window. You can also export basic molecule, primer, or alignment data, depending on the contents of the active window. You can export the data to a disk file or copy it to the Windows clipboard for later use in another program.

**Data in View** -- Select this option to export all of the results information in the view. The complete set of information is automatically selected and then sent to a disk file or copied to the clipboard. If the active window shows a molecule map, the high-resolution (printed format) graphic map will be exported, using the styles and colors set for printing.

**Window Snapshot** -- Select this option to do a screen capture of just the data that is currently shown in the active window, exactly as it appears. You can send this pixel image to a disk file or copy it to the Windows clipboard. Files are saved in the Portable Network Graphics (\*.png) format, particularly useful for computer screen display such as on a web page or in an on-screen presentation.

**Primary Data** (where applicable) -- If the active window is a part of the Molecule Viewer Window or the Alignment Viewer Window, you can export primary molecule data or primary alignment data. If the active window is a part of the Primer Viewer Window, you can export the sequence of the primer in plain text format, or the primer name and sequence in FASTA format.

**Converted Map Data** (where applicable) -- If the active window shows an enhanced view of the graphic map, you can convert the map data into raster images for use in other Windows programs that require a raster file format. You can export in Tif, Png, Jpg, Gif or Bmp file formats (requires GDI Plus, which is a part of the Windows XP or Vista operating systems).

## Find in Files

Found on the Operations menu. You can use this function to scan molecule files to look for certain genes or specific text in the molecule name or description. For Clone Manager files (\*.cm5), you can also look for specific text in the Notes field. To find text in a molecule file:

- Click Operations, Find in Files
- Enter the feature name or the text you want the program to search for
- Identify the file or folders of files that should be searched
- Use the checkboxes to identify the locations in each file where the program should look

When the search has been completed, you can view the list of molecules found to contain the search text. Click on column headers to sort the list by molecule name, file name, date, or description. Drag on the join between columns to resize. Double-click on a join to make the column size fit the data and then scroll to view longer molecule descriptions, if needed. You can print or copy the results list or load a molecule file for use.

## Multiple File Conversion

You can convert molecule files in standard Clone Manager file formats (\*.cm5, \*.cx5) to another file format for sharing with others, archival storage, web applications, or use by other programs. You can also construct primer collections using your individual primer files in standard primer file formats (\*.pd4, \*.pd2) or construct DataBook tables from your molecule or data files in one easy batch operation. The file conversion utility will attempt to open and convert the individual files you specify and all appropriate files found in the folders you specify, including those contained in subfolders. To start the file converter:

- Click File, Multiple File Conversion.
- Select the file conversion process (molecule files, primer collections or DataBook tables).
- Identify the files or folders of files to convert. Click the Add Folder button to select a folder location to add to the list. Subfolders will be included. Click the Add File button to select one or more molecule files to add to the list.
- Click Next to identify the output format and destination.
- For molecule file conversions, select the file format you want to convert to, using the guidelines provided in the application help, and select the location (folder) where you want the program to save the converted molecule files.
- For primer collection or databook table construction, enter the name for the new file or folder. The program will place this in the Collections or DataBook folder in your home directory.
- For databook table construction, use the checkboxes if you want to include primer or alignment files in the new databook table under construction.

Click Finish to complete the setup and begin the file conversion process.

## The DataBook Database



Found on the Operations menu or use toolbar button to access. DataBook is a simple, built-in database you can use to organize and store information about your molecule or data files, including primers or alignment results. You can think of it as a list of the files that you want to keep track of and just enough information about each file to let you find and open the file when you need it.

You can construct a databook from existing molecule or data files using a batch procedure in the Multiple File Conversion utility. Or you can add molecules or data files directly to an existing databook with the click of the

Add to DataBook toolbar button. In either case, the program will create new records and pre-enter most of the important information for you. You can create new databook files, as needed, and create folders to organize your databook files. All your databook files are stored within the folder DataBook, located in your home directory. These files have the extension \*.dx5.

Once you have records in your databook files, you can view, edit, or organize the records within the databooks. You can also search the databooks to find a specific molecule or data file and open the file for use. With the DataBook module open, click on the My DataBooks folder to open folders or files in this location. Use the Tab key or Shift + Tab to move between the left and right panels or click with the mouse. Click the Folder Options toolbar button to add new folders or new empty databook files, rename or delete any of the files or folders you have created, or export or import databook records.

Click on a databook file in the left panel to display the contents of this file in the right panel. For each databook record, the information in the Data Info, Description and File Name fields will be shown. Click on column headers to sort by the values in that column. Move the mouse to the join between two column headings, depress the mouse button and drag to resize the column. Use the mouse to drag out the dialog box borders to resize the DataBook window.

**View or Edit DataBook Record --** Select a record by clicking on the entry in the list in the right panel. Use the toolbar buttons to edit the information in this record or view additional information, delete this entry, copy the entry to paste into another databook file, or view the properties of the actual molecule or data file. When changes have been made to a databook record, these changes are automatically saved for you.

**Load a File for Use --** Click on a record and then click the Load File button at the lower right to use this molecule in an on-going operation or to open this file for use now. If more than one file can be selected for an operation, use the Shift or Control keys in conjunction with the mouse to select additional files.

## DataBook Searches


You can search in your databook files to locate a specific molecule, entries with certain genes, or records that have a common text string. You can search using a molecule name or any text that could be found in the data info, features/details, description, or comments/history fields. To start a search:

- Open the DataBook module
- Click on the databook file or folder in the left panel that you want to search
- In the Find edit box (lower area), type in the text to search for
- Click the Go button

If records are found that meet the search criteria, these records will be listed in a new folder called Search Results, found at the bottom of the folder list in the left panel. (Click on the Search Results folder to open this folder, if it is not already open.) You can select a file to open for use from this list or you can print the list of entries or copy it to the clipboard. When you close the DataBook module, the Search Results folder will be discarded. If you do not want to search all four of the default fields, click the small question mark button to the right of the Go button. Click to uncheck fields that you do not want to search. You can also search within the Search Results folder, to further refine your search, as needed.

## Add to DataBook

You can add a molecule, primer, or alignment results file to the built-in DataBook database in a very simple, efficient procedure. The program will pre-enter most of the important information for you. You can leave the entry as is, or you can change or add additional information that will appear in the databook only (your molecule, primer, or data file will not be changed). To add a new molecule or data file:

- Open the molecule, primer or alignment results file.
- Click the Add to DataBook button (main toolbar) 
- Identify the databook where you want to place this new record
- Use the checkbox (lower right) if you want to view the record as soon as it is created.
- Add or modify information in the data entry fields, as needed, and click OK to save.

You can also enter a new record manually. Just open the DataBook database, click the Add New Entry toolbar button, and fill in the fields with the information about your file. You can also copy a record already in a databook -- when viewing records in your databook files, select a record you want to copy and then click the Copy Entry button. Select the databook file you want to add this record copy to and click OK.


## Mutagenesis Profile

Found on the Operations menu. You can view 50 bases of sequence in your molecule around the target position specified. Restriction enzyme sites are shown above the sequence and one frame of translation below. You can change one or more bases in the sequence and see if the change affects enzyme sites or the translation. You can also find 'almost' enzyme sites (one-base mismatches), view base changes required to change from one amino acid to another, and view possible silent changes. You can instruct the program to create a mutant primer or molecule incorporating your changes.


The sequence will be scanned using restriction enzymes recognized by the program (commercially-available, 6 base cutters with no ambiguous bases). Enzyme sites are shown by a horizontal line, indicating the length of the recognition sequence. The cut position is marked by a small pointer and the enzyme name appears over the line. One frame of translation is shown. You can use the toolbar control to change the frame, if necessary. If you identified the target position using the features table, the gene selected is shown in the lower left and the frame is automatically set. Termination codons are shown as ---.

**Changing Bases in the Sequence** -- You can change bases (mutate) the sequence shown in this display. Click on the base that you want to change or move the vertical ruler using the toolbar or keyboard left or right arrow keys. The basepair position is reported at the top of the ruler line. With the vertical ruler line on the base that you want to change, click on the toolbar A, C, G or T buttons or press these letter keys on the keyboard to change the base to the letter key pressed. Base changes appear in red (or the assigned alert color) below the sequence lines. When a base change adds an enzyme site, the enzyme name appears in color. When a base change removes an enzyme site, the enzyme name is crossed out with a colored line. When a base change results in a change to the protein translation, the amino acid specified by the mutation appears in color below the original amino acid.


## Finding 'Almost' Enzyme Sites

 You can use this option to find 'almost' enzyme sites (one-base mismatches to the recognition sequence of a restriction enzyme). The sequence will be scanned using restriction enzymes recognized by the program (commercially-available, 6 base cutters with no ambiguous bases) and a list of 'almost' enzyme sites constructed. The list shows each single base change that could introduce an enzyme recognition sequence, the position for the change, the name of the enzyme site that would be introduced and the type of end produced by cutting with this enzyme. You can sort this list by position or enzyme name by clicking on the column header. Once you identify the change you want to make, click the Change Base button. The sequence on the Mutagenesis Profile display will be changed at the position specified. Newly-introduced enzyme sites appear in red (or the assigned alert color) above the sequence.


## Mutate - Change Amino Acid

 You can use this option to find out which bases you would need to change in the sequence so that an existing amino acid is replaced with another amino acid. Click on the amino acid you want to change or move the vertical ruler line to the amino acid. Click the Change Amino Acid button and then use the Change To combo box to select the amino acid you would like to introduce. The list shows the codon positions and the base changes that would need to be made in each position to specify the amino acid shown. You can move the highlight bar in this list to select the row containing the base changes you would like to make and then click the Make Changes button. The sequence on the Mutagenesis Profile display will be changed, as instructed, and the change in the translation will appear in red (or the assigned alert color). If the base changes remove an enzyme site, the enzyme name is crossed out with a colored line. If an enzyme site is added, the new enzyme site appears in color.

## Mutate - Show Silent Changes

 You can use this option to see which bases in the sequence you could change without changing the translation shown. When you click the Show Silent Changes button, possible silent base changes are shown directly below the upper strand of sequence. If only one base change can be made at a position without changing the translation, this base is shown as a letter (a, c, g or t). If more than one base change is possible at this position, an asterisk character (\*) is used to indicate the multiple possibilities. Click on the base that you want to change or move the vertical ruler line to the base. Use the toolbar A, C, G or T buttons or press the letter keys on the keyboard to make the change to the sequence base. If the base change removes an enzyme site, the enzyme name is crossed out with a colored line. If an enzyme site is added, the new enzyme site appears in color. Click the depressed Show Silent Changes button to clear this display.

## Creating a Mutant Primer or Molecule

 After you have made changes to the bases shown in this display (introduced a mutation), you can use the Create Mutant toolbar button to have the program create a primer or molecule that contains the modified bases. (Primer creation requires Clone Manager Professional.) If you select the option to create a mutated molecule, the program will enter the molecule to the molecule list and then open the molecule viewer window for this new molecule. If you select the option to create a primer with the mutation, you can specify the criteria type you want to use to evaluate the primer (usually PCR). The program will select a primer length that includes your modified bases and you can indicate if you want the complement strand to be used. The program will analyze all possible primers that would include the mutated bases and select the best choice, opening the primer viewer window for this primer. You can analyze this primer, give it a more meaningful name and description, and save the primer to disk.