

Clone Manager 10

TUTORIALS

This file contains ten tutorials for those who are new to Clone Manager.

This document is in Portable Document Format (PDF) and can be read with a PDF file reader, such as Adobe Acrobat Reader DC or a web browser, such as Google Chrome or Microsoft Edge.

When viewing the file, you can set the option to display Bookmarks or Contents so that you can see an interactive list of the topics covered. Just click on a bookmark to move to that place in the PDF file

The complete contents of this file can be printed on letter-sized paper (approx. 40 pages). Alternatively, individual tutorials of interest can be printed (each 4 pages).

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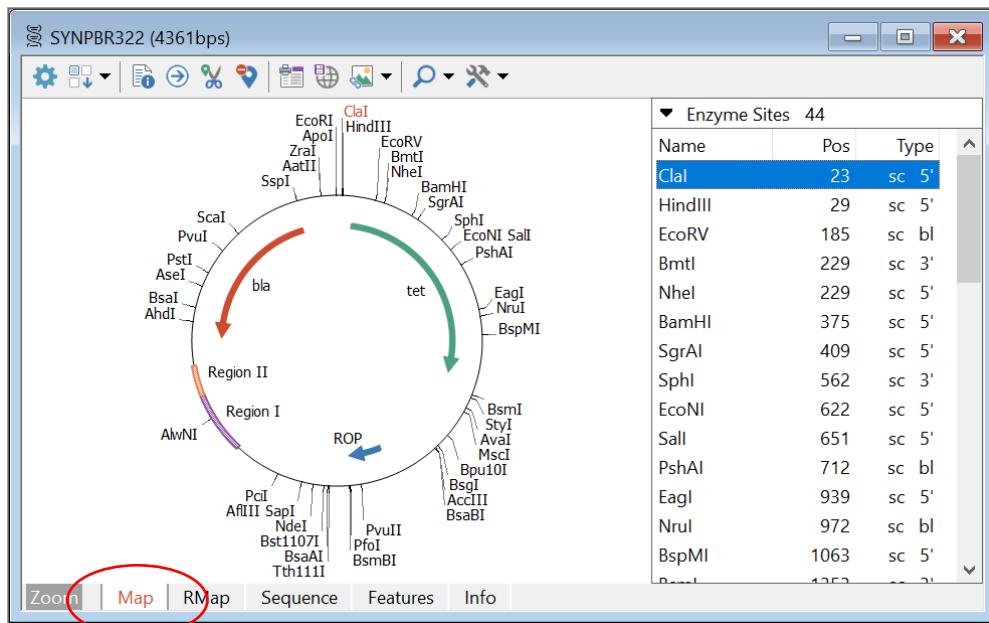
Molecule Maps

Clone Manager draws molecule maps on screen (working maps) that you can use to locate molecule features and enzyme sites or initiate actions. Clone Manager also sends high-resolution maps (graphic maps) to your printer, to a disk file, or the Windows clipboard for use in other applications.

Working Maps

To view a molecule working map:

- Open a molecule file or select a molecule from the Molecule List to open the molecule viewer window.
- If the map is not displayed, click the **Map** tab at the bottom of the window.



The basic working map will be either circular or linear, depending on the molecule. The map will show features (genes, regions, markers) and enzyme sites, primer sites or label sites, as selected.

Genes -- appear on the map inside (below) the map line. The default style is solid arrow.

Regions (user-defined sections) -- appear on the map line. The default style is shaded box.

Markers -- appear as short, solid lines, crossing the map line. (not shown on sample above)

Enzyme sites (or primer or label sites) -- appear outside (above) the map line.



Click the **Find** toolbar button in the map window to find more information that can be added to this molecule file. You can find enzyme sites (autoscans), find open reading frames (ORFs), find primers that bind to this molecule or find sequence phrases present here.

To set the working map display options:



- Click the **Settings** toolbar button.
- Click the **Screen Basics** tab to set the working map display options (mark feature segment with color, distinguish primers on complement strand, enable mouse hover, enable line smoothing)
- Click the **Features** tab to set default map styles and colors for each feature type.

These settings will be remembered and used for all working map displays until you change the settings.

Mouse Actions

- Point to a feature or enzyme name to see basepair position information.
- Left-click on a site on map to select an enzyme or primer site (turns red when selected) or a feature (segment shown) and then use toolbar buttons to initiate action or get information.
- Right-click on a site to see a menu of common options, including **GoTo Sequence** that will jump you to the formatted sequence at the position of this site.

Working with the Map Sites List

The Map Sites list is found on the right side of the molecule map display. This scrolling list shows enzyme sites, primer sites, label sites, or features entered for this molecule. Click on a column header to sort the display. Drag the join between column headings to resize column width.



Click the **Change Sites** button in the window toolbar to select to view enzyme sites, features, label sites, or primer sites in the map sites list box. Features will always be displayed on the working map, but they can also be listed in the map sites list. Enzyme sites are replaced with label sites or primer sites both on the map and in the list, when selected.

Enzyme sites – the map sites list shows enzyme name, site position (marked at left end of recognition sequence), type of ends produced (5' sticky, 3' sticky, or blunt) and an indication if this enzyme is a single cutter (sc), cutting this molecule only once.

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 (use Settings to enable or disable color).

Label sites – list shows name, start and end positions and an indication is this on the complement strand.

Primer sites – list shows primer name, site position (marked at 5'end of binding site), and an indication if this primer binds to the complement strand.

Click on a site or feature in the map sites list to select an item, or click on the item on the map. A selected feature will show a color segment on the map, an enzyme name will turn red.



When you use the **Tools** button in the map window toolbar, you have access to functions that are specific for the selected item. If a feature is selected, tools include create protein, create fragment or customize feature style. If an enzyme site is selected, tools include suppliers, isoschizomers or compatible ends for the enzyme.

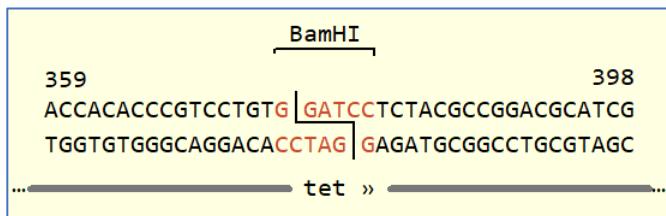


Use the **GoTo Sequence** button to move to the sequence view, formatted to show the selected item. Click the Map tab to return to the map view.



Use the **Properties** button to view information about the selected item. For a feature, the properties information includes feature details, translation, composition and codon usage.

For an enzyme site, the properties display shows the enzyme recognition sequence (in color) displayed in the context of the flanking sequence. The cut position is marked by a staggered vertical line. Genes in this area are shown as a line below the sequence.



Graphic Maps

The graphic map is a high-resolution map that can be sent to your printer, copied to the clipboard, or exported to a disk file for use in other programs. You can use the Settings button to change the appearance or content of the high-resolution graphic map before printing.

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

To set map print options:

- Open a molecule file and click the **Map** tab to view working map
 - Click the **Settings** toolbar button to set map default styles for printing.
 - Click the **Print Basics** tab to set font face, indicate which items to print, and assign text colors.
 - Click the **Features** tab to set default map styles and colors for each feature type.
 - Click the **Sites** tab to set font size, color for site names and ticklines and indicate if you want to use basepair numbers or italics for enzyme sites.
 - Click the **Content** tab to add molecule description and notes and/or a list of features and map sites to the printed map page or to change the molecule map size.

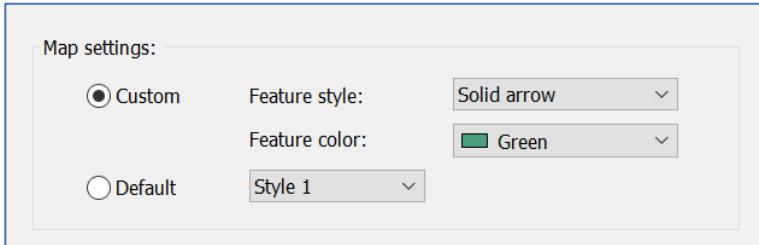


When you select a graphic map style for printing that adds additional text to the map page, the map will automatically be reduced to 90% of full size and it will be moved upward from the center to make room for the extra text.

To change the style or color of an individual feature:

- Click on the feature to select.
 - Click the **Tools** toolbar button and select **Customize Feature Style**.

- Or right-click on the feature and select **Customize**
- Set the style and color for this one feature or reset to a default style.



Print, Copy, Export Graphic Map

Graphic maps can be sent to your printer, copied to the clipboard or exported to a disk file for use in other programs. To get started open a molecule file and click the **Map** tab to view the working map on the screen.



Click the **Print** button on the main toolbar to send the map to your printer. Click **File, Other Tools > Print Preview** if you want to check the appearance of your map before printing.



Click the **Send View to Clipboard** button on the main toolbar to send the map to the Windows clipboard for use in other programs.



Click the **Send View to File** button on the main toolbar to create a disk file in Enhanced Windows Metafile (*.emf) or Windows Metafile (*.wmf) format.



For web page use, click the **Save for Web** toolbar button in the map window to prepare a special raster graphic map in a web-friendly (*.png) format.

Enhanced Views of the Graphic Map

You can use an Enhanced View to create a special graphic map, perhaps for a presentation or publication. When you start a new enhanced view, you begin with the standard molecule map, formatted for printing. You can then 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. You can control the thickness and color of lines and even switch between circular or linear map formats.

To start an enhanced view map of the active molecule:

- Open the molecule viewer window and click the **Map** tab.
- Click the **Enhanced View** button in the window toolbar and select **New View**.



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. Please refer to the on-line help or the user manual for more detailed information about using this function.

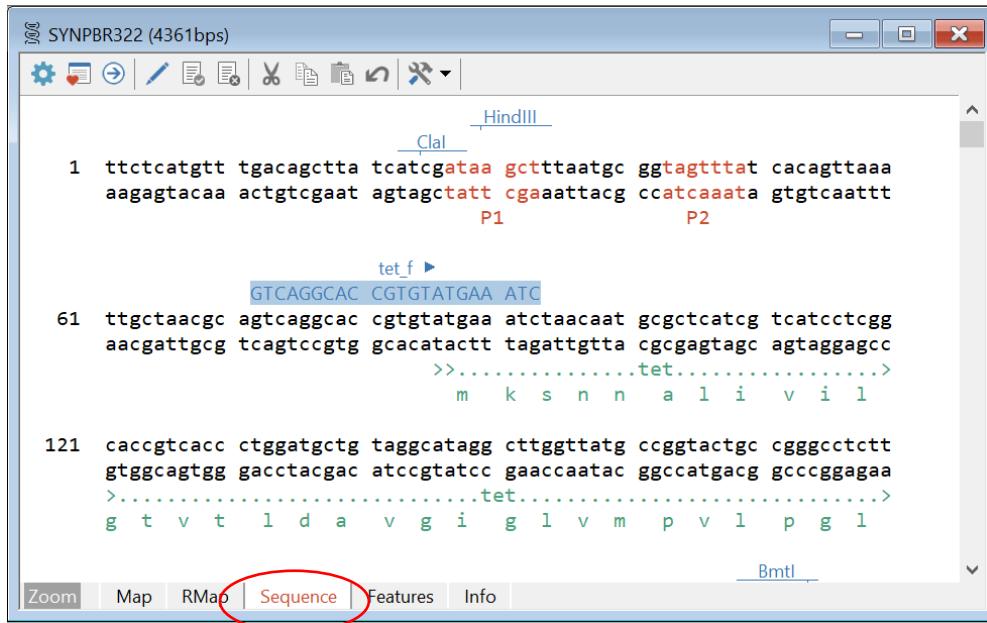
Sequence Display

Clone Manager has a wide variety of formatting options for sequence and associated data elements. You can set the display style for the sequence and you can select the additional information that will appear along with the sequence – display any combination of sequence, enzyme sites, sequence labels, primer sites, translation, annotation. Once the sequence display is just the way you want it, you can remember the style for later use. You can copy the formatted sequence to the Windows clipboard or send it to your printer or to a disk file for use in another application.

Sequence Style

To view sequence data:

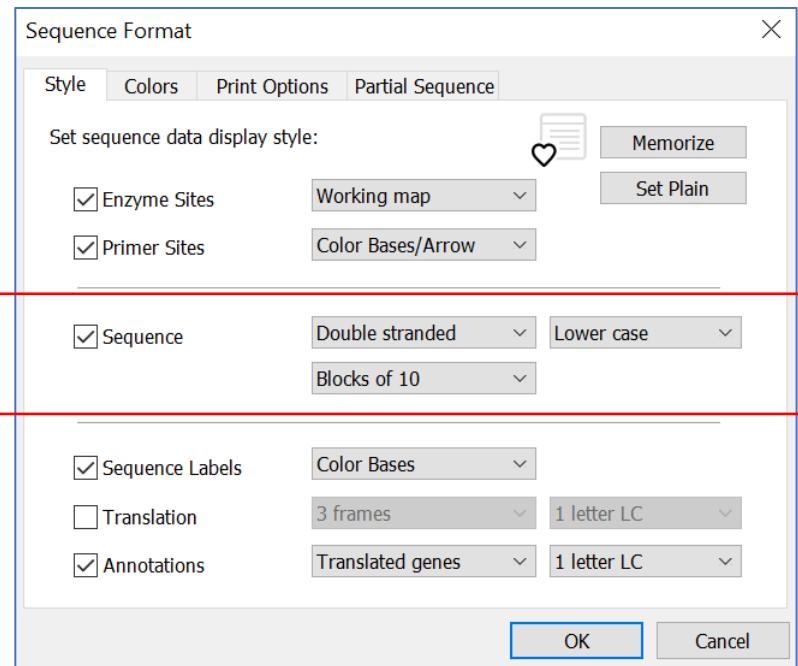
- Open a molecule file or select a molecule from the Molecule List to open the molecule viewer window.
- Click the **Sequence** tab at the bottom of the window.



If you want to view only part of a sequence, click the **Zoom** button (left end of tab bar at bottom of window). Select the option **Zoom to Show Region Specified** and enter the start and end basepair positions for the region to display.

To set the sequence format options:

- Click the **Settings** toolbar 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



Show Additional Information

You can use the Settings dialog box to select the additional information that will appear along with the sequence and you can set the default colors for sequence text items.

Use the check boxes to indicate which items should be displayed with the sequence data and set related options (see below).

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

Marking the enzyme recognition position above the line of sequence shows the precise location of important enzyme cuts. When combined with other annotation information, enzymes can be quickly selected based on the cloning or analysis task needed.

Primer Sites – shown above the sequence line. You can select to show primers with colored bases and an arrow indicating direction or you can use two colors, using colored bases in a rectangle of color. (Primer sites display requires Clone Manager Professional.)

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.

Labels are most useful for short sequences that have special significance, like a binding site for a regulatory cofactor or a processing site.

Translation – shown below the sequence line. Show 3 frames, single frame 1, 2 or 3 (as selected), or all 6 frames of translation. Show amino acid codes in 1-letter, 1-letter with amino acid numbers, or 3-letter styles.

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

Set Default Colors

You can select the default color to be used for each type of sequence text that can be displayed. Colors can help to distinguish one type of text from another. Colors selected for feature annotations will apply to all features of that type, but you can use a custom feature setting to distinguish a special feature (see below).

To set default sequence text colors:

- Click the **Settings** toolbar button 
- Click the **Colors** tab
- Select the preferred text color for each item type. Different sequence annotation colors can be set for genes, regions, and markers.
- Select the preferred background color (second option box) for primer sites or sequence labels. This background color will be used if two color style selected.

To remember your settings:

- Click the **Style** tab
- Click the **Memorize** button



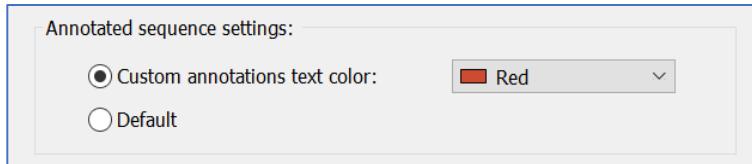
When you start a new work session and want your sequence display to use your remembered settings, click the **My Style** toolbar button in the sequence display window.

Customize Sequence Annotations Colors for Features

You can set a different color for one individual feature, to make this feature stand out in the sequence display. This may be especially useful for sequence labels, where you want to distinguish one special part of the sequence from the rest of the sequence. Or you may want to color the sequence annotation for individual genes in a distinctive color, perhaps the color used for this feature on the graphic map.

To set custom feature colors:

- Click the **Features** tab to move to the features table.
- Select (highlight) the feature you want to change.
- Click the **Customize Feature** button on the window toolbar. 
- Select the color for the sequence annotations text in the lower area.



In the features table view, a boxed area below the list is used to show the styles set for the selected feature. If this box is not visible, click the **Settings** toolbar button and select Show selected feature styles in lower area.

Click the **Sequence** tab to return to the sequence view.

Sequence Data Tools

While viewing sequence data, 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. When sequence is highlighted, either with a found match or because you selected it with your mouse, you can use options on the Tools menu to enter this highlighted sequence as a feature or create a primer.

To find a sequence match:

- With the sequence visible on the screen, click the **Tools** button 
- Select **Find** and enter the sequence of bases to search for
- Use the checkboxes to configure the search to permit limited mismatches or mismatches and gaps or insertions.
- Select **Go To** to jump to and highlight the sequence match found
- Use checkboxes to search only the upper strand and to start at the beginning of the sequence
- Click **Tools** again and then **Find Next** or use Ctrl + I to locate additional matches

To enter selected sequence as a feature:

- Select (highlight) sequence bases with the mouse (hold down left mouse button and drag).
- Sequence basepair numbers for the start and end of the selected region are shown in the window toolbar at the right, along with the length (number of bases)
- Click the **Tools** button and select **Enter as Feature**
- Give the feature a name and description and select the Draw as Type (gene, region, marker, label) and set the default style.

Edit Sequence

You can edit the sequence of a molecule by adding or removing bases. You can be in single-stranded, double-stranded, or translated mode, but you can only edit the upper strand of the molecule.

Click the **Edit** toolbar button and make changes 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.

Clicking either button will leave the edit mode and allow you to move to another task.

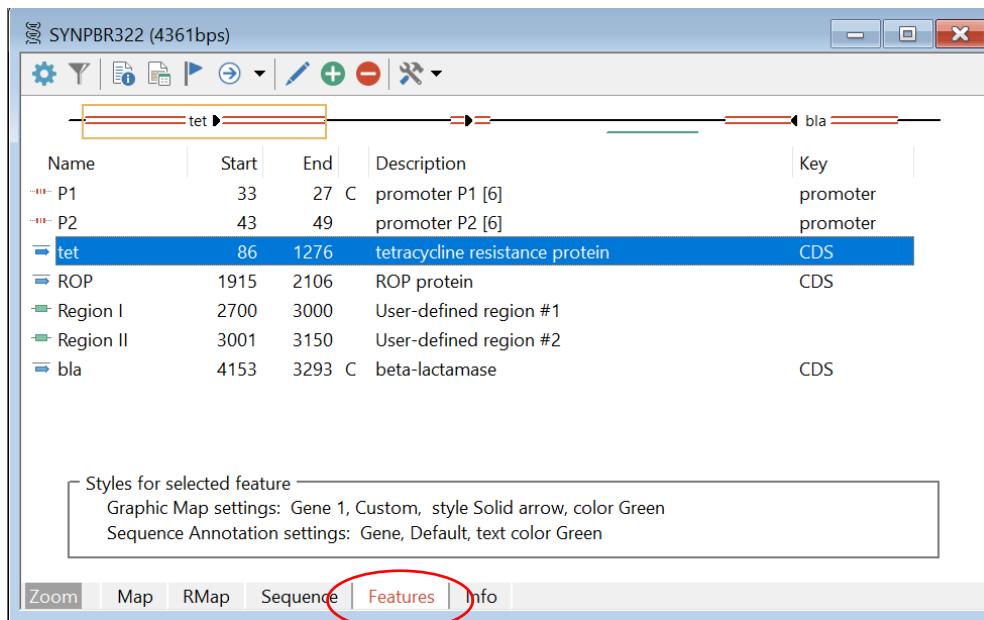
Molecule Features

Clone Manager has a features table display in the molecule viewer window. Molecule features appear in the listing with a symbol for the feature type, the feature name, basepair positions, description, and the feature key, if assigned. You can show a simple features map above the list and/or a box showing the feature styles for the selected feature below the list. 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 styles or filter the data displayed.

Features Table Display

To view the features table:

- Open a molecule file or select a molecule from the Molecule List to open the molecule viewer window.
- Click the **Features** tab at the bottom of the window.



Feature Types

Feature Draw as 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 for molecule maps or annotated sequence.

- **Genes** – usually represent translated sequences, have start and end basepair positions, can be on the complementary or normal strand. Shown on the molecule map and annotated sequence.

- **Regions** – zones of special significance for the user, have start and end basepair positions, can be on the complementary or normal strand. Shown on the molecule map and annotated sequence.
- **Markers** – markers have a direction, occupy one base position, can be on the complementary or normal strand. Markers can appear on the molecule map and annotated sequence.
- **Labels** – sequence areas of special interest to the user, have start and end basepair positions. Labels can be shown on the annotated sequence and appear on the molecule map as label sites.
- i Info-only** – not shown on the molecule map or on the annotated sequence. Use for features where you want to preserve the information but do not want it to clutter your map.

To set the features table display options:



- Click the **Settings** toolbar button
- Select to show feature bp numbers start-to-end (standard) or left-to-right
- Use checkboxes to select to show information-only features, the simple features map above the list and the selected feature styles in a boxed-area below the list.
- Click the **Memorize** button to remember these settings

Use 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 by location or feature type. Click the depressed **Filter** button to remove the filter.

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

View Features Information

To get more information or use feature tools, open a molecule file and click the **Features** tab and then select (highlight) a feature.



Click the **Feature Properties** toolbar button to get more information about the selected feature. 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.



Click the **GB Location/Qualifiers** button to view GenBank location or qualifiers. The information displayed includes feature location data (basepair positions) and qualifiers available for this feature. GenBank format files imported into Clone Manager will read in all feature qualifiers and retain this information when saved as a Clone Manager file. Click outside the pop-up box to dismiss.

Use Feature Tools



Click the **GoTo Sequence** button and select Go To Sequence **Start** or Go To Sequence **End** to move to the sequence view, formatted to show the selected feature. Click the Features tab to return to the features table display.



Click the **Tools** button and select options to **Make Protein**, **Make Fragment**, or **Export Feature Sequence** for the selected feature.

When you select to make a fragment from a feature, you create a new molecule containing the feature sequence, with the option of including extra bases at each end and you can set the fragment orientation for the new molecule. When you select to export the feature sequence, you can select to export to a disk file or to copy to the Windows clipboard for use in another application.



Click the **Customize Feature** button to set a custom style and color to be used for this feature for the graphic map or set a custom text color to be used in the annotated sequence display.

You can set an individual style or color for this feature or select a default setting.

Map settings:

Custom Feature style: Solid arrow
 Default Feature color: Green
 Custom Style 1

Annotated sequence settings:

Custom annotations text color: Red
 Default

Add or Edit a Feature

Click the **Add Feature** toolbar button to add a new feature or the **Edit Feature** button to make changes to the information for an existing feature. Then enter or modify the feature information.

Feature Name:	T24H18_30	Feature Key:	CDS
Description:	2-oxoglutarate/malate translocator precursor-li	Codon Start:	1
Draw as Type:	Gene	Style:	1
Location:	Start: 11237	End: 9245	<input checked="" type="checkbox"/> Complement

For a standard feature, enter the feature name, feature key, and feature description in the upper area of the dialog box. Select the Draw as Type (gene, region, marker, label or info-only) and assign a default Style. (Default styles are set using Settings, Style on the Map tab.) You may choose to Edit a feature to change the Draw as Type for some Info-only features so that they appear on the map or annotated

sequence, or you may want to change a region to a gene if you want to translate this feature and create a protein.

When entering new features, you can select the appropriate feature key from the drop-down list of keys supported. Your designated feature keys appear first, but you can click More... to see all of the possible feature keys. (You can tell Clone Manager which features should be imported, based on feature key, when you open a file in standard GenBank or EMBL format. These are your designated feature keys – click File, Preferences, then click the Import Features tab to set this.)

Using GenBank Feature Qualifiers

For more complex features with joins or qualifiers, you can enter or edit this information in the lower areas of the Add or Edit Feature dialog box. Clone Manager supports all of the GenBank/EMBL feature keys and has support for mandatory qualifiers.

The screenshot shows two sections of a dialog box:

- Join Segments:** A table with columns #, Start, and End. It contains three rows:

#	Start	End
1	11237	10149
2	9919	9698
3	9607	9245

Buttons: Add, Del, Edit. Instructions: Click Add button to enter new join segment. Select segment and then click Edit or Del button.
- Qualifiers:** A table with columns Qualifier and Value. It contains five rows:

Qualifier	Value
gene	T24H18_30
note	strong similarity to 2-oxoglutarate/malate translocato..
codon_start	1
product	2-oxoglutarate/malate translocator precursor-like pro...
protein_id	CAB88250.1

Buttons: Add, Del, Edit. Instructions: Select segment and then click Edit or Del button.

When you entered a new feature, you selected the appropriate feature key from the drop-down list of keys supported. Standard qualifiers will be generated based on the Feature Key you selected and mandatory qualifiers will be identified (if any) and shown at the top of the list.

Click the **Add** button to enter a new qualifier. Select the qualifier name from the list provided and enter the qualifier value. When you select a mandatory qualifier and then click **Edit** you will see the list of possible qualifier values or you can type in text, if appropriate. Do not enter quotation marks.

Click the **Edit** or **Del** button to modify or remove the selected qualifier. Right-click to move a qualifier up or down in the qualifier list.

New Sequence

Clone Manager can read molecule files in standard GenBank, EMBL, or FASTA formats. It can also import plain sequence data, perhaps from a sequencer-generated data file or sequence data sent in an email from a colleague. Sequence can be in upper- or lower-case letters and the program knows how to handle basepair numbers and spaces between blocks of bases. Non-sequence lines (comments), if present, should start with a semi-colon.

On occasion, you may have to get a sequence for a vector you plan to use. The manufacturer's website usually has vector sequence data for your use and you may be able to download a file prepared in GenBank format. If a prepared file is not available, you can copy and paste the sequence data to use in Clone Manager.

Once you have imported your new sequence data, you can explore the sequence and add additional information to complete a more complex molecule file. You can add features (like genes), enzyme recognition sites, a molecule name and description, and notes or comments about the sequence.

To read in sequence data from the clipboard:

- In your e-mail document or other Windows workspace, select (highlight) the sequence data.
- Click Edit, Copy (or Ctrl + C) to place the sequence data on the Windows clipboard
- In Clone Manager, click **File, Other Tools, Import from Clipboard**

If the sequence data can be read, Clone Manager will automatically get ready to do an AutoScan operation to find enzyme sites.

Problems with Reading Sequence Data

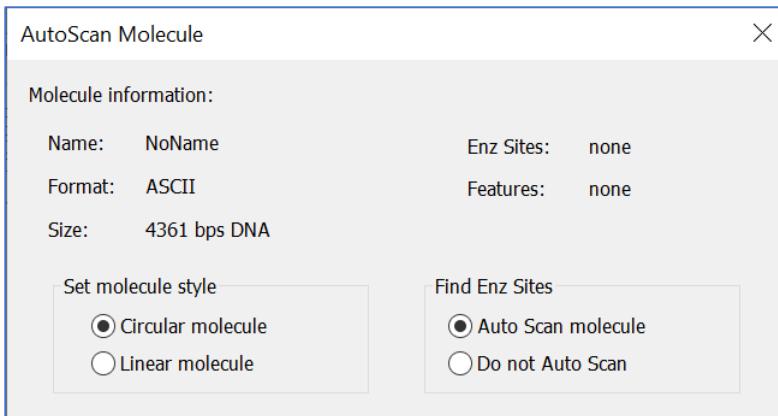
If the program cannot automatically load the sequence, it will identify the closest of the recognized formats and try to provide you with information about the problem encountered and provide help with file loading. In the File Help module, you will find the error described in the top right. The scrolling data window shows the contents of the file. Usually, you will have one of the common file loading errors (like an unrecognized character in file). You can click the Go To Error button to move to the problem point and correct it. Then click Retry Load. (Please refer to the Help Topic "Problems with File Loading" in Clone Manager for more detailed information.)

Find Enzyme Sites

Once your sequence data has been read into Clone Manager, a new molecule file is in the process of being created. The AutoScan operation is the next step. This operation will look for single cutters and automatically enter the enzyme sites to the molecule map in one easy operation. The enzymes used for the default AutoScan operation are major, commercially-available enzymes with recognition sequences of 6 or more bases.

In the AutoScan dialog box, you can check that the number of bases imported is correct, you can set the molecule style to be circular or linear and you can initiate the AutoScan operation. The molecule does

not yet have a name, but you can add one, as well as descriptive information or comments (see later section). Click **OK** to move on and perform the autoscan.



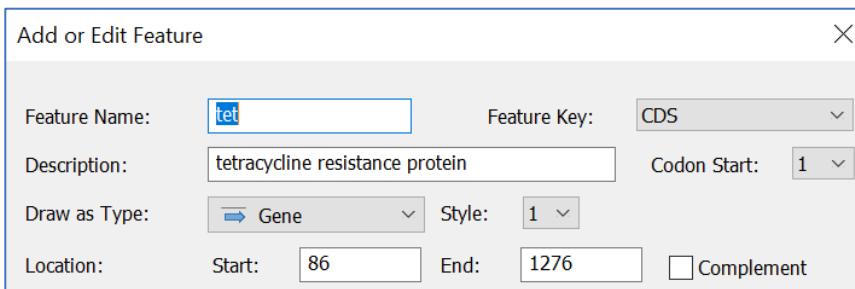
Alternatively, you might want to add all of the enzyme sites for just the enzymes you have in your freezer. This is where user enzyme lists come in handy. To add these additional enzyme sites to your molecule:

- Click **Clone, Find Enzyme Sites**
- Set the Enzyme List to your user list (on right)
- Set the option for List of Enzymes (on left)
- Select find All Sites User List

Enter Features Information

If your vector data from the manufacturer's website had information about genes, regions of interest, or special parts of the sequence (like promoters), you will want to add this information to the Features Table in Clone Manager. (If you were able to download a file in GenBank format, this information was automatically entered for you, based on your GenBank import filter settings.)

- Click the **Features** tab and then click the **Add Feature** toolbar button 
- Enter the Feature **Name** (a short name looks good on the map) and select a Feature **Key** (optional)
- Enter the Feature **Description** and select the **Draw as** Type and Style
- Enter the start and end basepair positions and indicate if on the complement strand



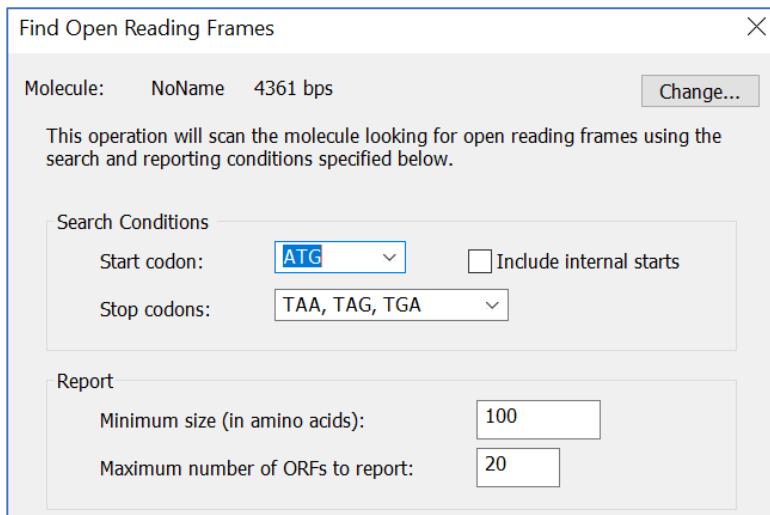
You may also want to do a search for open read frames to see if there are other features you might want to add to your new molecule file or to verify the positions of the features you may have entered from published data.

Find Open Reading Frames

When you do an ORF search, you can identify the start and stop codons you require and set the minimum acceptable ORF size. When the search is completed, you can select ORFs to add to your molecule as possible genes.

To start an ORF search:

- Select **Find Open Reading frames** on the **Discover** menu
- Identify the **Start codon** and the **Stop codons** you require
- Set the minimum size (in amino acids)



- When the results appear, click on a column header to sort by position, frame or size
- Select (highlight) an ORF (shown on map in lower area as red arrow)

The screenshot shows a table titled 'ORFs: 7' with columns 'Start', 'Frame', and 'AAs'. The first row is highlighted with a blue background. The table lists the following data:

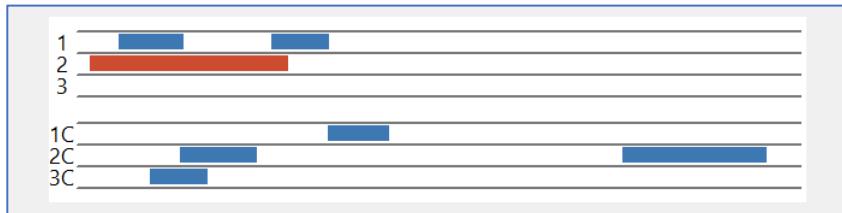
Start	Frame	AAs
86	2	396
4153	2	C 286
1081	2	C 152
259	1	127
1883	1	C 122
780	2	C 116

Next to the table is a text input field: 'Enter the selected Open Reading Frame as a Gene or Enter All.' with buttons 'Enter Gene' (highlighted in blue) and 'Enter All'. Below the table are buttons 'Print List' and 'Show Overview'. In the bottom left corner of the window, there is a red arrow pointing to the right, indicating the direction of the molecular map.

- Click **Enter Gene** to add this ORF as a gene in the features table
- Add the feature name, description, draw as style in the Add Feature dialog box.

If your molecule already has genes in the features table, they will be shown in the molecule map display in the lower area.

To see all of the listed open reading frames positioned in their approximate locations in the six translation frames, click on the **Show Overview** option. The overview display will replace the iconic map display. The highlighted ORF in the scrolling list is shown in red.



Enter Molecule Information

To begin with, your molecule has a place holder name. You can enter or change the molecule name, and add a description and notes for the new molecule file.

- Click the **Info** tab and then click the **Edit** toolbar button 
- Enter the Molecule Name and Description
- Enter Notes information. (The first line of Notes text can be seen in the Molecule List window, so put what's most important at the top.)
- If you got vector sequence data from the manufacturer's website, you can add this information in the notes field.

Once you have saved this file to disk (see below), Clone Manager will add the file name and file location to the molecule information display.

Save Molecule File



Remember to save your file to disk when you've finished adding all your new information. Just click the Save button on the main toolbar.

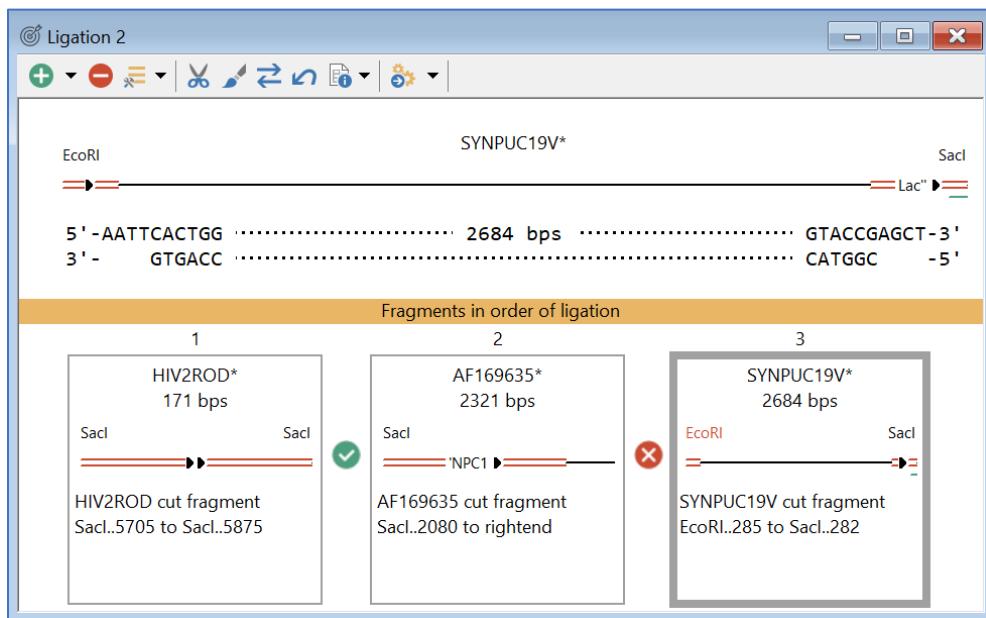
Simulate Cloning

Clone Manager 10 has an updated Ligate module to help you simulate cloning. You can use the Ligate option to join together two or more pieces of DNA or to join the two ends of the same molecule (self-ligate). The ligate display window lets you see the molecules as they are being prepared for ligation.

Use the Ligate Function

To get started:

- Open a molecule file or select a molecule from the Molecule List to open the molecule viewer window.
- Click **Clone, Ligate** to open the Ligate window.
- Click the **Add Molecule** toolbar button to add the other molecules or fragments needed for the cloning simulation.



In the upper half of the Ligate window, an icon map of the active 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. The molecule in this upper window can be modified (see below).

In the lower half of the window, all of the molecules or fragments are shown in boxes. Click the **Add Molecule** button to select additional molecules to add to this window, if needed. You should arrange these molecules or fragments in the order in which they should be joined. Click on a molecule and drag to move to the right or left, as needed, or use the **Move or Select Fragment** toolbar button.

Small icons appear between each fragment box to indicate if ligation is possible. If changes are needed to a fragment to enable ligation, click to select the fragment, causing it to appear in the upper box, where modifications can be made.



Use the **Fragment Information** button to view the features table for the active molecule or to find enzymes that could prepare ends that would be compatible to the fragment to the left or right of this fragment in the lower window.

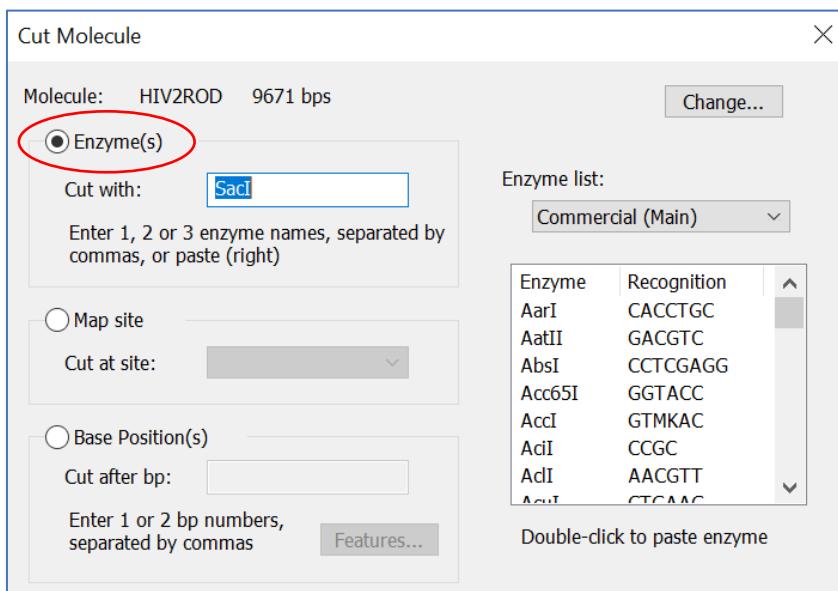
Compatible Ends information is available for the fragment ends at the far right and far left of the fragment display so that you can check for compatibility, assuming you will want the ligation procedure to circularize the end molecule.

Preparing Molecules/Fragments for Ligation

You can use the Cut operation to prepare DNA for ligation. 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. Clone Manager can cut DNA at enzyme cut sites or at user-specified basepair positions. You can use enzyme sites already entered for this molecule, or Clone Manager can find sites for 1, 2, or 3 enzymes you select and then cut with these enzymes at all sites found.

To use the cut operation:

- Select (click on) the molecule to cut.
 - Click the **Cut** toolbar button.
-
- In the Cut Molecule dialog box, click **Enzymes** to cut with an enzyme
 - Type in the enzyme name or select it from the list at right.



If the cut operation results in multiple molecule fragments, you can select one fragment for use in the current operation or identify one or more fragment to create for later use. Each fragment is listed with

the start and end basepair position and the length of the fragment. The highlighted fragment is displayed on the iconic map below the list.

The screenshot shows a 'Fragments' dialog box. On the left, a table lists four fragments with columns for Start, End, and Size. The fourth fragment, '5705 5875 171', is highlighted with a blue background. On the right, instructions say 'Select one fragment to use now or create fragment(s) for later use.' Below this are three buttons: 'Use Now' (circled in red), 'Create', and 'Print List'. Below the buttons is an iconographic map of a DNA double helix with restriction sites: EcoRI at the top left, SmaI at the top center, SacI at the top right, and KpnI at the bottom right. Arrows indicate the direction of the DNA strands.

Start	End	Size
1	5288	5288
5289	5704	416
5705	5875	171
5876	9671	3796

Select (highlight) the fragment you want and click the **Use Now** button. This fragment will replace the original molecule in the ligation window.

Modify Molecule Ends

You can use the Modify Ends operation to change the ends of a linear molecule to prepare it for ligation. You can use Klenow Fill-In, 5' -> 3' exonuclease, 3' -> 5' exonuclease, or Partial Fill-In modifications to change the molecule ends.

To modify the fragment ends:

- Select (click on) the molecule to modify.
- Click the **Modify Ends** toolbar button.
- Select the end modification you want to use.

The screenshot shows the 'Modify Ends' dialog box. At the top, it says 'Modify Ends'. The main area displays a DNA sequence with restriction sites EcoRI, SmaI, SacI, and KpnI. Below the sequence, the 5' and 3' ends are shown with their nucleotide sequences: 5' - AATTCCTGG 2684 bps GTACCGAGCT-3' and 3' - GTGACC CATGGC - 5'. A dropdown menu under 'Specify modification' is set to 'Type: 5'-3' exo'. There is also a 'Cancel' button in the top right corner.

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.

5' -> 3' Exonuclease – treatment with 5' -> 3' exonuclease will remove bases from the 5-prime single-strand ends of the molecule.

3' -> 5' Exonuclease – treatment with 3' -> 5' exonuclease will remove bases from the 3-prime single-strand ends of the molecule.

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 proposed end modification is shown on the display in red base letters, where bases are added, or green crossed-out letters, where bases are removed.

Select **Modify** to change the molecule ends as shown and alter the sequence for this DNA.



Click the **Invert Fragment** button if you need to reverse (invert) the sequence of the selected fragment.



If a change you made to a fragment is not what you expected, click the **Undo Last Operation** to undo the last modification to the selected fragment.

Complete Ligation



When all of the fragments are ready, click the **Ligate** button to join all compatible ends of the fragments in the order shown.

After ligation, a circular recombinant will be rotated to restore the original reference frame of the vector. If you do not want the ligation operation to circularize the molecule, click on the drop-down arrow to the right on the Ligate button and select **Do Not Circularize**.

Once ligation is complete, you can enter the molecule to the Molecule List for further use or save it to disk.

Notes:

The newly-ligated product has been scanned using all enzymes present in all starting fragments and the map sites list updated. If you would prefer to limit the enzyme sites for the new molecule to only single cutters, you can click the **Find** button in the molecule map window toolbar and select **Find Enzyme Sites (Autoscan)** and select to Replace Existing List with New Sites Found.

If you would prefer to have a different base position at the top (origin) of the map, you can click **Tools** on the main menu and select **Process Molecule**, and then select **Rotate Molecule**.

Cloning Wizards

Clone Manager 10 has a number of wizards that will help you to simulate cloning using a variety of techniques. The wizards will help you select the appropriate components, show you the proposed result, and create the resulting recombinant molecule. There are often a number of settings you can adjust, if desired, but the default settings will usually be just what you need.

Gateway Cloning -- The Gateway cloning wizard will help you to simulate cloning using Gateway® Recombination Cloning techniques (Invitrogen Corp). It will help you make an entry clone (BP reaction), make an expression clone (LR reaction), prepare a PCR product with attB sites, or perform complex MultiSite procedures. 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.

Topoisomerase Cloning -- The Topoisomerase cloning wizard supports cloning using Topo® Cloning techniques (Invitrogen Corp). You can do TOPO TA Cloning, Directional TOPO cloning, Blunt TOPO cloning, or prepare PCR products with added terminal bases as needed. You can indicate your primer design goal and your preferences for GC content adjustment or primer criteria stringency.

PCR Cloning -- The PCR Cloning wizard can 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. It will help you with Topoisomerase cloning, Classic TA cloning, restriction enzyme cloning and blunt end cloning, all using PCR amplification. You can view several solutions and save selected primers, amplified products and recombinants for later use.

Assembly Cloning -- You can choose from several assembly cloning wizards to help you simulate cloning using Gibson Assembly™ (Synthetic Genomics, Inc), In-Fusion® Cloning (Clontech Laboratories Inc), Ligation-Independent Cloning, or related cloning techniques. Use the Ligation-Independent Cloning wizard to join fragments previously prepared with correct overlaps. Use the Gibson cloning or In-fusion cloning wizards to use PCR to create amplified fragments with overlaps, and then assemble the fragments, using the assembly procedure selected.

After using one of the wizards, you can view the proposed result and create the recombinant molecule. If you are using Clone Manager Professional and have designed primers to amplify the fragments and create the overlaps, you can view more detailed information about the primers, save the primers to a collection file, or export the primer sequences.

Example: Simulate Classic TA Cloning

To get started:

- Click **Clone, PCR Cloning Wizard**. Click Next.
- Select **Classic TA Cloning** and click Next.
- Click the **Change** button to select or change the molecule that contains the region you want to amplify for cloning
- Enter the upper strand coordinates of the region you want to clone (you can set the orientation of the insert later)

Define what you want to amplify:

Identify the molecule that contains the region you want to amplify for cloning and enter the basepair positions for this region.

Molecule:	HIV2ROD 9671 bps
Change molecule > Change...	
Region to clone:	Start bp: <input type="text" value="2309"/> End bp: <input type="text" value="4939"/>
Enter upper strand coordinates	
View Features Table > Features...	

You can look in the Features Table for the basepair position numbers of the gene you want to clone

To set the primer design options (next screen):

- Set the primer type to **PCR Primer**
- Leave all of the options set to default, unless you specifically want to override
- You can modify the primer type, optimal primer length, and search extent, if desired.

To specify preferences (next screens):

- Leave all of the adjustment settings at default values, unless you need to override
- You can adjust the GC content acceptable range, the criteria adjustments allowed, and search region adjustments, if needed when a search for PCR primers is not successful.
- On the next screen, for bases at the 5' end of your PCR primers, leave the setting marked to **Avoid 5'T if possible**.

To define the vector you want to use (next screens):

- Click the **Change** button and select a vector molecule. The wizard uses circular molecules.
- Identify the region you want to clone into and click Next.
- Select the enzyme you want to use to cut the vector and set the orientation of the insert. The enzymes shown cut only inside the region specified and do not cut the insert molecule.

Select the enzyme you want to use to cut the vector from the list below.
Change the enzyme list, if needed, to consider other enzymes.

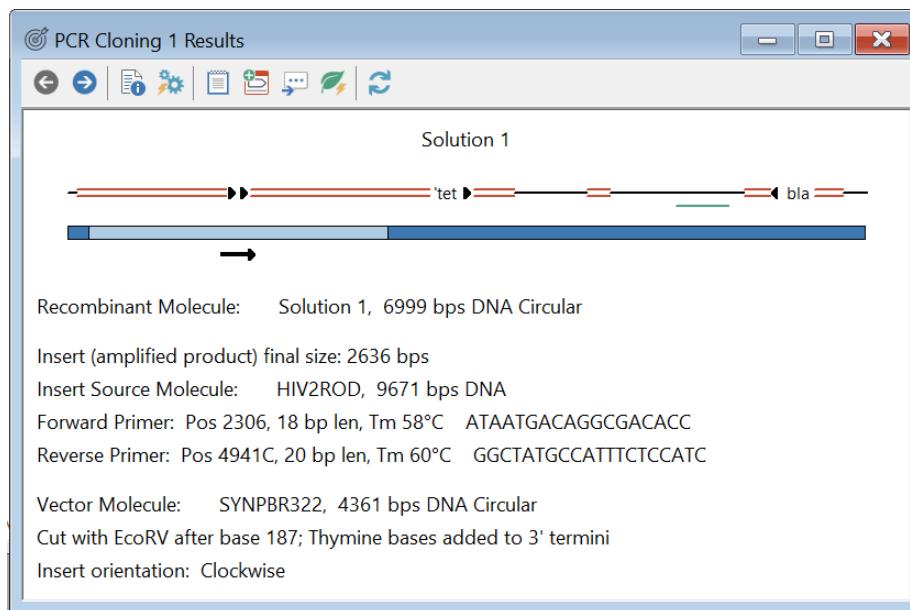
Enzyme	Recognition	Cuts after
EcoRV	GATATC	187
NruI	TCGCGA	974
PshAI	GACNNNNNGTC	716

Select the orientation of the insert: TA Cloning: Add 3' terminal T base

- Click **Next** and review the instructions, selecting the number of solutions to display.
- Click **Finish** to view the cloning simulation results.

Classic TA Cloning Results

If possible solutions can be found for the cloning experiment you described, the selected number of best solutions can be viewed sequentially. For each solution, 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 molecules, primers, enzymes used.



Use the **Next Solution** button to view the next solution in the set. Use the Previous Solution button to return to a prior solution in the set.

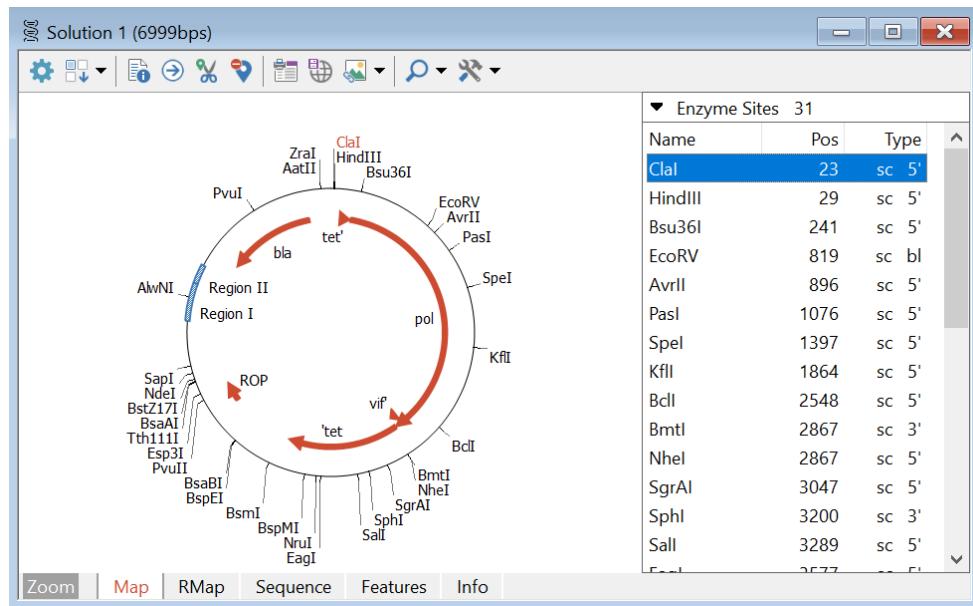
The **Solution Details** button lets you view, print or copy a detailed summary of the cloning experiment, including primer sequences, positions, lengths and Tm values.

Click the **Create Molecule** button to have the program automatically do the required steps to simulate the cloning experiment, produce the recombinant molecule, and enter it to the Molecule List. Enter a molecule name for your new recombinant and modify the description, if needed.

For Gateway cloning results, you can also 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.

For Topoisomerase cloning results, if you have just prepared a PCR product, you can click the Use Now button to take the amplified product shown and use it in a TA, directional, or blunt topoisomerase cloning procedure.

After clicking the Create Molecule button, the new molecule will open in a new molecule viewer window. You can examine your results and return to the PCR cloning results window to select another recombinant or view primer information. Here's our new recombinant molecule:



Viewing More Primer Information

If you are using Clone Manager Professional and have designed primers to amplify the fragments and create the overlaps, you can return to the cloning solution results screen and view more information about the primers. For some results displays, you can use the drop down list box to move from the Summary view to the view of primer pairs for each fragment.

In our results view, you can view a primer report, enter the primers to the primer list, export the primer sequences or create the amplified product.

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

 Click the **Enter to Primer List** button to add these primers to the list of loaded/active primers and also add these to a collection file.

 Click the **Export Primer Sequences** button to export or copy the two primer sequences sequentially to a disk file or to the Windows clipboard.

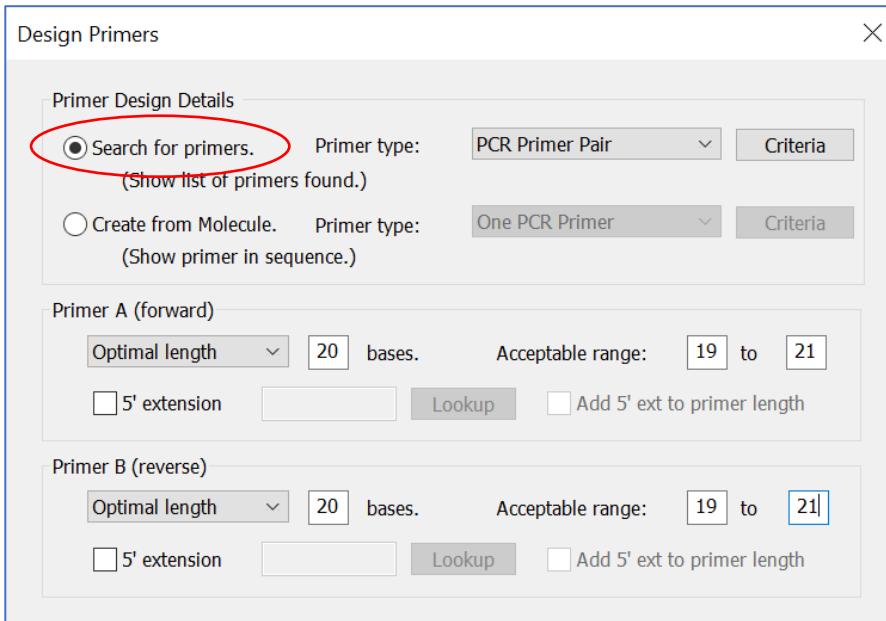
 Click the **Create Amplified Product** button to use the two primers and source molecule shown to prepare the PCR product. You can enter this to the molecule list and open for use now, save to a disk file, or export the product sequence to an ASCII text file.

Search for Primer Pairs

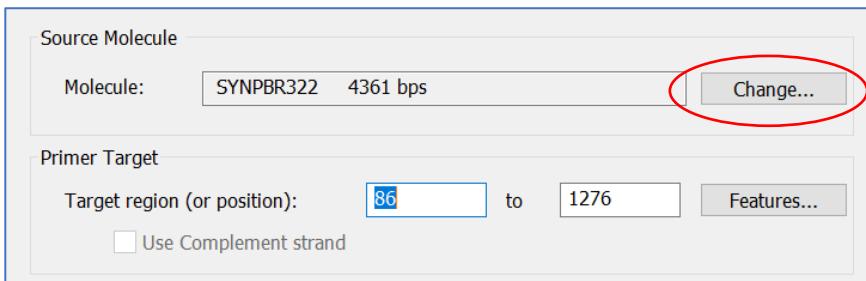
Clone Manager can search the molecule and region you specify, looking for primers that meet the evaluation criteria you set. For PCR primer pairs, you can indicate if you want to amplify a complete target region or just a fragment within a specified size range. Results are listed with basic information about each primer pair. You can sort the list and analyze or use any of the primers found. An alternate view lets you see which evaluation criteria eliminated possible primers during the search.

To get started:

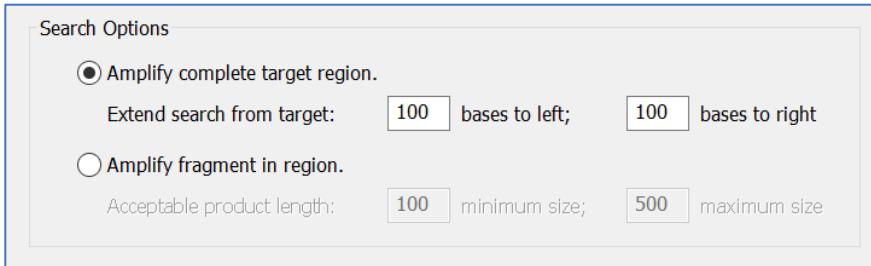
- Click **Primer, Design**.
- Set the option **Search for Primers** and select the Primer Type.
- In the lower area, set the option to **Optimal Length or Required Length**, and enter the number of bases for Primers A and B.
- If using Optimal Length, set the acceptable range of bases for each primer.



- Click **Next** to identify the source molecule and target region.



- Confirm that the source molecule shown is the one you want to use or click **Change** to select a different molecule.
- Next, enter a target region. You can use the Features button to access the Features Table for this molecule and paste in the start and end basepair positions for a feature.



- Indicate if you want to amplify the complete target region or just a fragment in this region and enter the search extent or acceptable product length.
- Click **Finish** to complete the instructions for the primer search.

Primer Search Results

When a search has been completed, the results will be displayed in the Search Results or Search Statistics windows. If the search was successful, primers found will be listed in a table format showing basic information about each primer or primer pair. You can use the combo box in the window toolbar to sort the list by quality (rank sort), position, or product length.

Search 6										
Rank sort										
Molecule: SYNPBR322 4361 bps Search for: PCR Primer Pair Possible Pairs: 9 Target Region: 86 to 1276; Amplify target region										
Rank	Position	GC %	Tm°C	Max Dimers	FPrim	Product				
	A	B	A	B	A	B	3' Any	A	B	Length
1	74	1317	50	50	60	61	2	4	...	1244
2	74	1316	50	52	60	59	2	4	...	1243
3	74	1362	50	50	60	63	2	6	...	1289
4	74	1361	50	52	60	62	2	6	...	1288
5	70	1316	57	52	62	59	2	4	3	1247
6	74	1362	50	52	60	65	2	6	...	1289
7	74	1361	50	55	60	65	2	6	4	1288
8	74	1368	50	50	60	62	2	6	14	1295
9	74	1369	50	52	60	65	2	6	14	1296

If you want to adjust the search to find additional primers, you can try the Auto Adjust Length or Auto Adjust Criteria buttons to make quick adjustments and repeat the search to try to find other primers (particularly useful if your search yielded only a short list of primers or pairs).



Click the **Enter to Primer List** button to add the primer or pair to the Primer List, entering a primer name and description. When entering to the Primer List, you can also add these primers to one of your collection files.



Click the **Enter Primer Site to Map** button to add the primer binding site for the selected primer or pair to your molecule file as primer map sites that can appear on your molecule map or annotated sequence.



Click the **Primer Report** button to view a report for the selected primer or pair. The Primer Viewer Window will open with primer report data visible and access to more detailed analyses.

With the Primer Viewer Window open, you can click the **Info** tab to view information about the primer sequences and the amplified product.

The screenshot shows the 'Primer Pair' window with the title '[Search 6] Primer Pair'. The window contains the following information:

- Selected Pair:**
 - Rank: 1 PCR Primer Pair Search
 - Primer A: Molecule: SYNPBR322 Pos: 74
 - Primer B: Molecule: SYNPBR322 Pos: 1317 Compl
- Primer Sequences:**
 - A ✓ Length: 20 CAGGCACCGTGTATGAAATC
 - B ✓ Length: 20 TGGAGTGGTGAATCCGTTAG
- Amplified Product:** Size: 1244 bps Product GC 61%
Calc temperatures: Melting T 84°C, Annealing T 57°C
- Linked molecule:** SYNPBR322 Pos: 74, 1317 C
- Molecular Diagram:** A horizontal line representing a DNA molecule with several segments. Red segments indicate regions where primers A and B are located. Green segments indicate other regions of the molecule. Arrows point to specific genes: 'tet' on the left and 'bla' on the right.

At the bottom of the window, there are tabs for **Info** (which is selected) and **Analyze**.



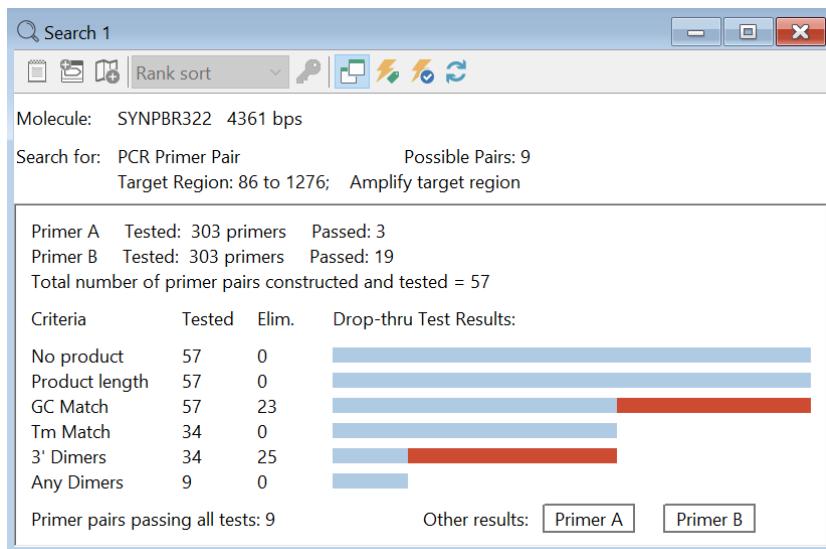
Click the **Create Product** button in this window to create a new DNA molecule that is the PCR-amplified product of the two primers and associated molecule shown.

Alternate Search Results View

The Primer Search Statistics Display is an alternate to the search results display and shows which criteria eliminated primers or pairs during this search. The program initially identifies all possible primers in your search region. It then begins to eliminate primers that fail to pass the tests shown. The test steps are sequential, and only primers that pass a given test are considered in the following test(s). The number of primers tested and eliminated in each step is reported numerically and displayed as a bar chart on the right (eliminated primers are shown in red).



Click the **Search Results/Search Stats** toolbar button to see where criteria eliminated primers. (If no primers were found initially, the Search Statistics display is the only display available.)



Finding more primers

When a search has been unable to find sufficient primers that meet all of the evaluation criteria, you can modify the search conditions (vary primer length or relax evaluation criteria) or you can redefine the search region to increase the size of the search range. (Use the **Redefine** button in the results window to return to the search setup dialog box to change the search range.)

Changing the primer length by one or two bases may have an effect on the number of primers found within the area specified and meeting the criteria selected. Or you may be willing to accept a slightly longer run of bases or an increase in non-3' dimers, without changing primer length.



Auto Adjust Primer Length – use this function to start an automated sequence that will repeat the primer search, adjusting the primer length as specified, and then display the new results.



Auto Adjust Primer Criteria – use this function to start an automated sequence that will adjust (relax) primer evaluation criteria, repeat the search, and then display the new results.

In the Auto Adjust Primer Criteria dialog box, select **Conservative** or **More Aggressive** to adjust (relax) the settings marked at the right with gray checks. Or select **Custom** and then check the individual criteria settings that you want to adjust (relax), using the Search Stats display to see which criteria eliminated primers.

Finding More Primer Pairs

The Search Stats display for a primer pair search has three results. If pairs have been successfully found, the display shows the pairs results. Buttons in the lower right can be used to access statistics for Primer A or Primer B separately. When a search has found Primer A candidates and Primer B candidates, but is unable to find A:B pairs meeting your criteria, you can modify the matching criteria to accept larger differences between the two primers. On the Auto Adjust Primer Criteria dialog box, click **Custom** and check the boxes for **Pairs GC Match** and/or **Pairs Tm Match** to relax these matching criteria.

Create Primer from Molecule

Clone Manager can create a PCR primer, sequencing primer, or probe using the sequence of a source molecule. The program will position a primer of the length you specify at the initial position you specify. You can then move the primer along the sequence or adjust its length until the primer evaluation meets the criteria set. This design option is especially useful if you must have a primer very close to a specific location in your molecule.

To get started:

- Click **Primer, Design**.
- Set the option **Create from Molecule** and select the Primer Type.
- Enter the primer length (required length) and the 5' extension (if any).

Design Primers

Primer Design Details

Search for primers. Primer type: **PCR Primer Pair**

Create from Molecule. Primer type: **One PCR Primer**

Primer

Required length **24** bases. Acceptable range: to
 5' extension **GAATTC** Add 5' ext to primer length

Required length bases. Acceptable range: to
 5' extension Add 5' ext to primer length

- Click **Next** to identify the source molecule and target position.

Source Molecule

Molecule: **SYNPBR322 4361 bps**

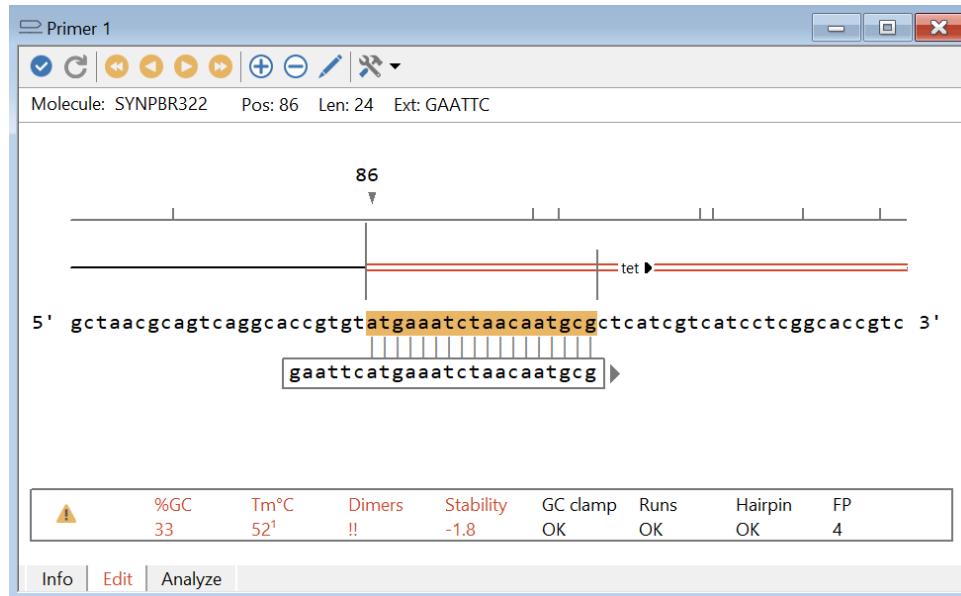
Primer Target

Target region (or position): **86** to
 Use Complement strand

- Confirm that the source molecule shown is the one you want to use or click **Change** to select a different molecule.

- Enter a target position for initial placement of the primer and use the checkbox if you want to design a primer using the complement strand.
- Click **Finish** to create the primer and enter a primer name and description when prompted.

The Primer Viewer Window will open, displaying your new primer and its evaluation. The screen display for the new primer provides information about the primer and its evaluation, and about the molecule in the region around the primer.



The molecule sequence is shown across the center of the screen, with the 5' and 3' ends marked. For DNA molecules, a simple map showing genes or regions appears above the sequence. A horizontal line above the map may show tick marks at some locations. These are neighboring primer sites that will meet the criteria set.

The primer sequence is shown in a boxed area below the molecule sequence. If a 5' extension is present, these bases are shown as non-homologous to the molecule sequence (no vertical match lines).

Primer Evaluation Bar

The primer evaluation bar appears in a boxed display area at the bottom of the primer viewer window on the Edit tab view. The primer is evaluated for GC content, melting temperature (Tm), presence of dimers, stability, GC clamp, presence of runs or hairpins and possible false priming. The icon at the left edge of the evaluation bar indicates whether or not the current primer satisfies all of the criteria set.

- The primer meets all of the criteria set. Click the **Analyze** tab to find out more about this primer or print a primer design report. Click the **Info** tab for molecule-associated information and to save the primer to disk for later use, if desired.

 The primer does not meet one or more of the criteria set. Values that do not meet criteria will appear in color. You can click on the value in the Evaluation Bar to jump to the appropriate view on the Analyze tab for more details. (Click the **Edit** tab to return.)

If the primer does not meet criteria, you can adjust the primer position or primer length until you find a primer that does meet criteria. If necessary, you can also change the primer 5' extension.

Adjust Primer Position

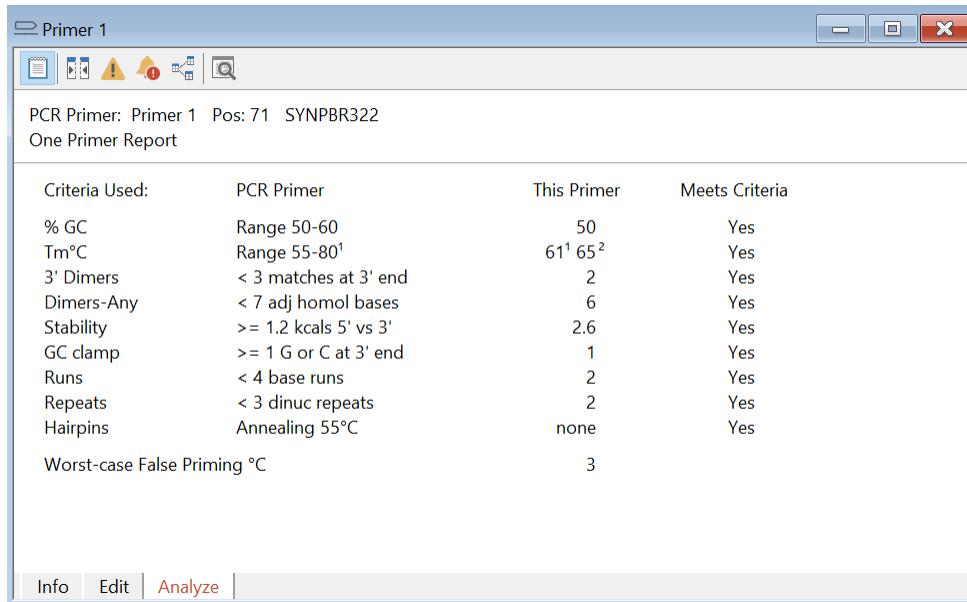
Four toolbar buttons can be used to move the primer to the left or right within the sequence, thereby changing the bases in the primer sequence. You can move the primer one basepair position at a time or you can jump to the next position that meets the criteria specified. The nearby positions that will meet the criteria set appear as tick lines on the horizontal line above the map.

 Use the **Move Left** or **Move Right** (single arrowhead) buttons or the keyboard left or right arrow keys to move the primer one bp position in the direction of the arrow. Use the **Scan Left** or **Scan Right** (double arrowhead) buttons or the Ctrl + arrow keys on the keyboard to move the primer in that direction to the next position that meets the criteria set. Once moved, the primer will be re-evaluated in its new position.

Adjust Primer Length or Change Primer 5' Extension

 You can increase or decrease the length of the primer, as needed. Click the **Increase Primer Length** button to add one base to the 3' end of the primer. Click the **Decrease Primer Length** button to remove one base from the 3' end of the primer. You can modify the primer 5' extension, if necessary. Click the **Edit 5' Extension** button and then enter or edit the 5' extension for this primer.

Our sample primer was moved to the left using the Scan Left button to the nearest position that would meet the criteria set. Click the **Analyze** tab to see detailed analysis information about this primer.



The screenshot shows the 'Primer' software window with the 'Analyze' tab selected. The window title is 'Primer 1'. The main area displays a report for 'PCR Primer: Primer 1 Pos: 71 SYNPBR322 One Primer Report'. The report table lists various criteria and their results:

Criteria Used:	PCR Primer	This Primer	Meets Criteria
% GC	Range 50-60	50	Yes
Tm°C	Range 55-80 ¹	61 ¹ 65 ²	Yes
3' Dimers	< 3 matches at 3' end	2	Yes
Dimers-Any	< 7 adj homol bases	6	Yes
Stability	>= 1.2 kcals 5' vs 3'	2.6	Yes
GC clamp	>= 1 G or C at 3' end	1	Yes
Runs	< 4 base runs	2	Yes
Repeats	< 3 dinuc repeats	2	Yes
Hairpins	Annealing 55°C	none	Yes
Worst-case False Priming °C		3	

At the bottom of the window, there are tabs for 'Info', 'Edit', and 'Analyze'.

Analyze Primer

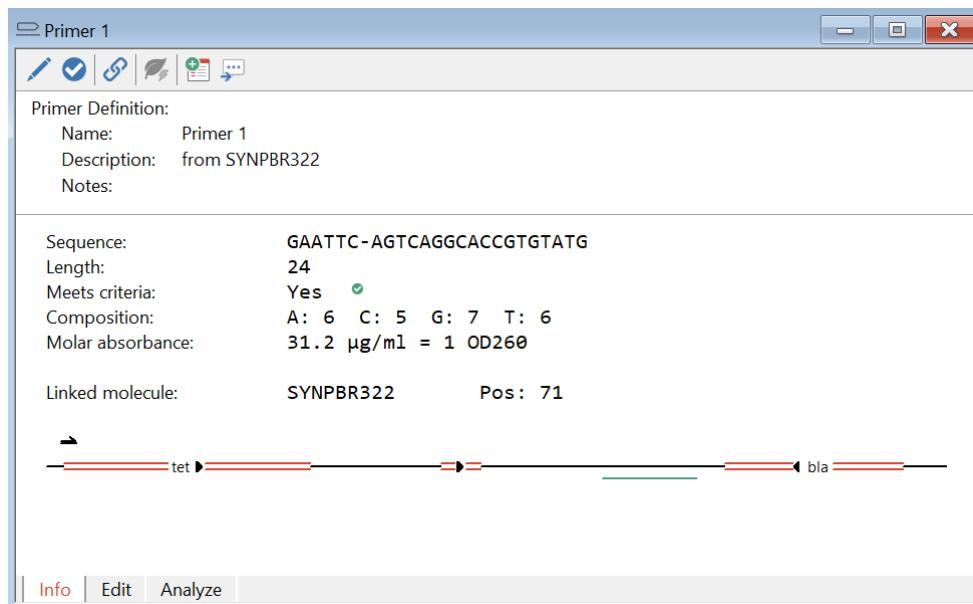
The **Primer Report** (shown above) gives the criteria settings used to evaluate this primer, the actual values for each item, and an indication of whether the criteria were met.

Click toolbar buttons in this window to get more detailed information about primer **Dimers**, **Cautions** (stability, runs, repeats, hairpins), **False Priming**, or **Any Homologies** found.

View Primer Information

Click the **Info** tab to review or edit information about this primer, add the primer to one of your primer collections or export the primer sequence.

The Info display shows the primer sequence, including the 5' extension, primer length, evaluation results, composition, molar absorbance. A molecule map shows the approximate primer location and the basepair position in the linked molecule. Click the **Print** button on the main toolbar to print a comprehensive report for your records.



Click the **Add to Collection** button to add this primer to one of your primer collection files. Select the collection file from the list shown.



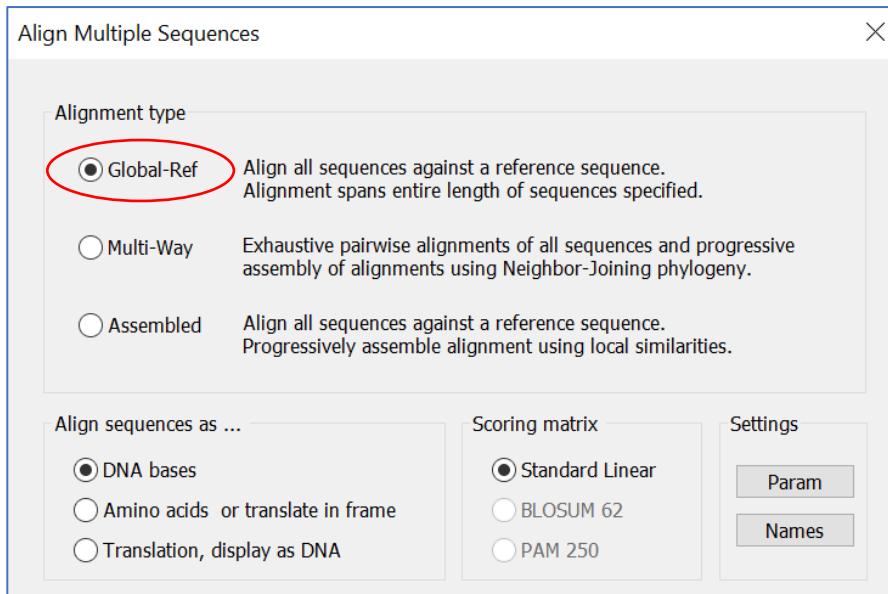
Click the **Export Primer Sequence** button to export the primer sequence to a plain ASCII (DOS) text file or to copy to the Windows Clipboard for use in another application.

Align Multiple Sequences

Clone Manager can align multiple sequences against a reference sequence using a global or assembled procedure or do an exhaustive pairwise global alignment of all sequences with a progressive assembly of the alignments (multi-way alignment). Alignment results are displayed in a tabbed window – view a table of information summarizing the alignment results, the actual aligned sequences in a variety of formats, a set of simple maps showing areas of significant similarity for each sequence, or a dendrogram for multi-way phylogeny. You can save the alignment setup or results to a disk file.

To get started:

- Click **Align, Align Multiple Sequences**.
- Set the Alignment Type to **Global-Ref**, Multi-Way or Assembled (see below).
- In the lower area, set the option to align sequences as **DNA bases**, Amino acids (translate in frame), or Translation (display as DNA).
- Select the Scoring Matrix or use the default setting.
- Click **Next** to identify the sequences to align.



Global-Ref Alignment – will align each of the sequences against a reference sequence (always in position 1). The objective is to maximize the number of matching bases over the full length of each molecule. This type of global alignment works best when sequences are of similar size and reasonably related to the reference molecule.

Multi-Way Alignment – will do exhaustive pairwise global alignments of all sequences and progressive assembly using Neighbor-Joining phylogeny. When the alignment is complete, you will have the option to re-order the sequences based on the phylogenetic data produced.

Assembled Alignment – will compare each sequence against the reference sequence (always in position 1), looking for significant regions of local homology to lock in (using the FastScan – MaxQuality method). The alignment is then completed using a global alignment procedure to align the rest of each sequence against the reference sequence. This type of alignment can be useful when one or more areas of strong similarity are found in otherwise poorly-related sequences.

To select sequences to align:

Pos	Molecule	Start	End	Length	C
*	Ref ARBLKSM	1	2958	2958	
2	ARBL2KSM	1	2961	2961	
3	ARBLKSP	1	2958	2958	
4	ARBL2KSP	1	2961	2961	

- Click the **Add Molecule** button (at the right)
- Select a sequence or group from the molecule list or click Browse to open another file.
- To remove a sequence, select (highlight) it and then use the **Remove Molecule** button.
- Use the Move Up or Move Down buttons to move the highlighted sequence within the list, if needed. The reference molecule should always be in position 1.
- Click **Finish** to complete the setup and start the alignment.

To align only a part of a molecule sequence or to change the molecule name to display:

- Select (highlight) the name of the molecule.
- Click the **Identify Region** button (at the right)
- Click Partial Sequence to align less than the full molecule and enter the start and end basepair positions. The start base should always be the first nucleotide to be aligned.
- Use the Complement checkbox if the region to be aligned is on the complement strand.
- Type in a name to display if you need to shorten or change a molecule name.
- Click **OK** to complete this item.

Alignment Results

When an alignment operation has been performed, the alignment results will be displayed in a comprehensive, tabbed viewing window. Click the tabs to access alternate views of your results.

Toolbar buttons let you sort data, navigate through results lists, format the display style for aligned sequences, or view additional data, where applicable. You can print the data in a view window, copy this view to the clipboard, or export the view data to a disk file.

Info Tab

The Info tab provides a table of information that gives a concise overview of the multiple sequence alignment results, including the sequences and regions aligned, the number of matches found, the number of non-matches found, and the calculated percent matching bases.

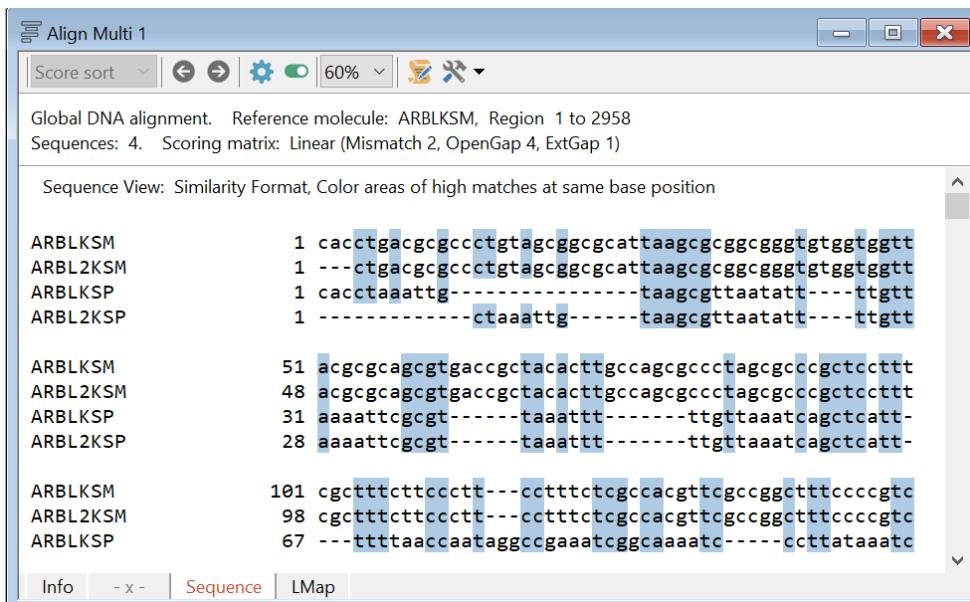
Global DNA alignment. Reference molecule: ARBLKSM, Region 1 to 2958 Sequences: 4. Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)					
Sequence	Start	End	#Match	NonMatch	%Match
ARBLKSM	1	2958			
ARBL2KSM	1	2961	2950	14	99
ARBLKSP	1	2958	2711	321	89
ARBL2KSP	1	2961	2703	335	88

- When you move the mouse over a molecule name in the table, the cursor changes to a rectangle. Use this special cursor to click on a molecule name to see a pop-up box that will give you more information about this molecule (name, size, file name, description).

You can use **Tools, Redefine** to return to the alignment setup dialog box to change the alignment setup, if needed, and then repeat the alignment.

Sequence Tab

You can view the actual aligned sequences in a variety of formats, emphasizing the differences or similarities between the sequences. Click the **Sequence** tab to access this view.





Click the **Show Similarity/Difference** button to toggle between the default similarity and default difference formats to get a rapid impression of which view will give you the best display.

The default similarity format shows all bases and uses color to distinguish areas with high matches. The default difference format shows only bases that do not match the reference sequence and uses background color to bring these to your attention. In the difference formats, dots indicate matches.

Some formats apply color if the similarity exceeds a specified value (Color areas of high matches or Similarity summary bars format). Use the Similarity Significance Value combo box in the toolbar to set the level of significance (range 50-100%) you want to apply for these formats.

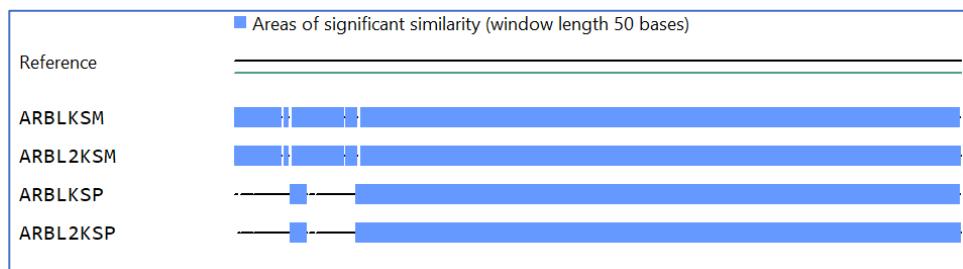


Click the **Settings** button to access the full set of sequence format options or to set page style options. When the format is just the way you want it, you can print, copy or export the data.

Location Map Tab

You can click the **LMap** tab to view a summary of the extent and location of similarity of the sequences and additional information about regions of DNA or protein molecules. Each of the sequences is shown on a separate line. The entire search region is shown for each molecule, scaled to fit in the data box.

For global alignments, each sequence is compared to the reference sequence. For multi-way or two-way alignments, each sequence is compared with a virtual composite sequence. You can set the significance value for this display using the Similarity Significance Value combo box in the toolbar.



Regions where similarity is greater than or equal to the significance value are marked with colored bars. For assembled alignments, a blue bar indicates that the molecule is being aligned 5' to 3', while a light red bar indicates the molecule is being aligned in the reverse direction (3' to 5'). You can point with the mouse at a map area and depress the left mouse button to see the basepair position at the cursor location. You can also view composition data as an overlay on top of the similarity data. Click **Tools** to compress the view to show more lines of data or to overlay composition data.

Picture Tab

If a multi-way alignment has been performed, you can click the **Picture** tab to view a dendrogram which suggests the pattern of relatedness of all of the sequences aligned. The dendrogram is constructed with a distance-based tree-building method using the neighbor-joining algorithm. The amount of dissimilarity (the distance) between two aligned sequences is used to derive the tree. The method is very fast and results in a single tree, similar to the phylogenetic guide trees produced using the CLUSTAL W approach.

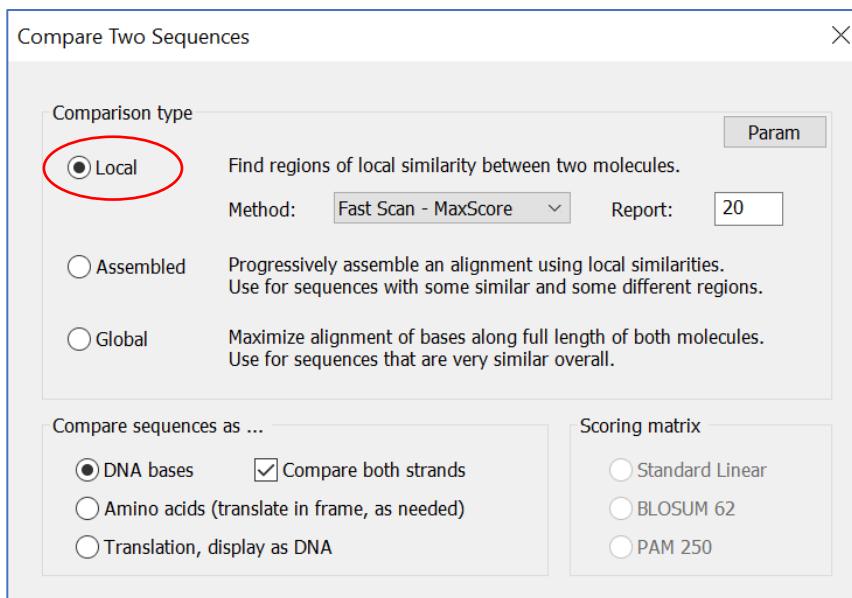
Compare Two Sequences

Clone Manager can compare two sequences by searching for regions of local homology or by aligning the two sequences using a global or an assembled alignment procedure. Local homology searches can be done using the FastScan method or the Needleman-Wunsch method. Alignment results are displayed in a tabbed window.

For local homology searches, you can view a list of the local homologies found or a set of simple maps locating these local homologies. For each homology block listed, you can view the actual aligned sequences or a more detailed map showing the local homology in each molecule.

To get started:

- Click **Align, Compare Two Sequences**.
- Set the Comparison Type to **Local**, Assembled, or Global (see below).
- In the lower area, set the option to compare sequences as **DNA bases**, Amino acids (translate in frame), or Translation (display as DNA).
- Set the checkbox if both DNA strands should be compared.
- Click **Next** to identify the sequences to compare.



Compare with Local Homology Searches – compare two sequences by searching for regions of local homology. Select to use the FastScan or Needleman-Wunsch method. Once the local homology search is complete, you can view a list of best regions found, the aligned sequences or view a location map.

Compare with Assembled Alignment – compare two sequences by finding significant regions of local homology (using the FastScan – MaxQuality method) and then completing the alignment using a global alignment procedure. This type of comparison can be useful when one or more areas of strong similarity are found in otherwise poorly-related sequences.

Compare with Global Alignment – compare two sequences using a global alignment procedure. The objective is to maximize the number of matching bases over the full length of each molecule. This type of global alignment works best when sequences are of similar size and reasonably related to each other.

To select sequences to compare:

Identify the two molecule sequences to compare.

Sequence 1

Molecule: SYNPBR322 4361 bps

Full sequence Compl

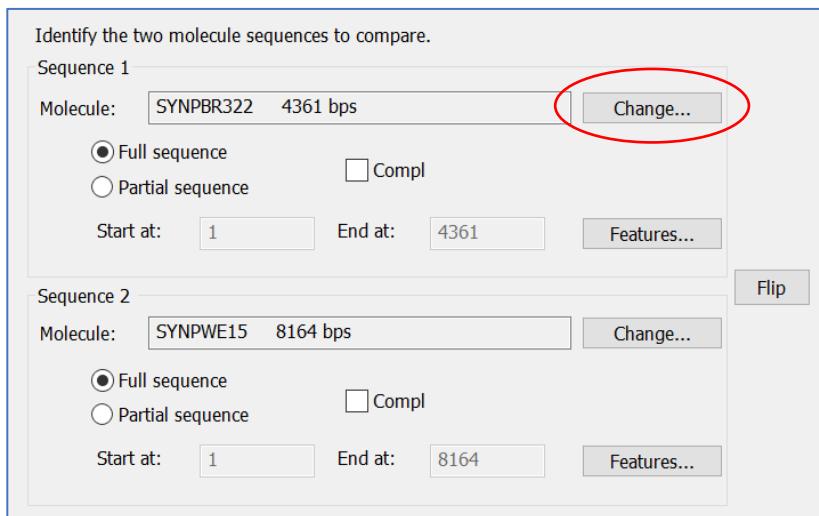
Start at: End at: Features...

Sequence 2

Molecule: SYNPWE15 8164 bps

Full sequence Compl

Start at: End at: Features...



- Click the **Change** button to the right of the molecule name box for Sequence 1
- Select a sequence from the molecule list or click Browse to open another file.
- Repeat this procedure for Sequence 2
- Click the Flip button to exchange the sequences between the boxes, if needed.

To compare only a part of a molecule sequence:

- Below the molecule name, click **Partial Sequence**
- Enter the start and end basepair positions of the region. The start base should always be the first nucleotide to be compared.
- Use the Complement checkbox to indicate that the region is on the complement strand.
- Click **Finish** to complete the setup instructions and begin the sequence comparison.

[View Results](#)

Results of local alignments include a sortable list of homologies found, shown in table or graphic format. For each homology block listed, you can also view the aligned sequences or a detailed map of the local homology region.

[Info Tab](#)

The **Info** tab shows a table that lists the regions of local homology found in two-sequence comparisons using the Local Homology option. You can use the combo box in the toolbar to sort the results by score, percent matches, length, or the start position of the region of homology in either molecule. Score is a composite value that reflects the number of aligned matching bases with penalties for mismatched bases or gaps in the alignment.

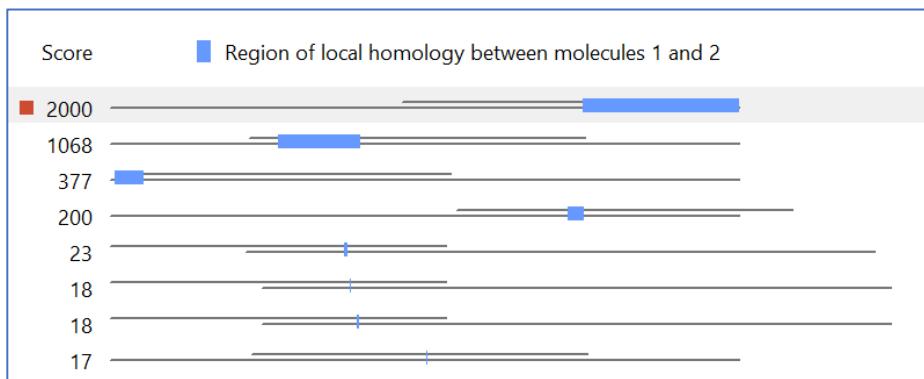
Local DNA homologies. Mol 1 SYNPBR322 (1 to 4361) Mol 2 SYNPWE15 (1 to 8164)
 Both strands. Method: FastScan - Max Score

Percent Matches	Score	Length	Mol 1		Mol 2	
			Start	End	Start	End
99	2000	2011	2348	4358	6143	8164
99	1068	1070	376	1445	2182	3251
99	377	379	1	379	68	446
100	200	200	1449	1648	5947	6146
71	23	41	3046	3086	6892	6852 C
80	18	27	3110	3136	7038	7009 C
80	18	30	3203	3232	6942	6916 C
86	17	20	2262	2281	4059	4037 C

Our sample results are sorted by score. You can move the highlight bar to any region of homology found. When you click the Sequence or LMap tabs, you will see the aligned sequence data or a detailed map for this selected homology block.

Picture Tab

Click the **Picture** tab to get a graphic view of the list of regions of homology found. This view consists of a set of simple maps locating these local homologies. Each entry shows the region of local homology as a colored bar, between lines representing the two molecules. Like the Info tab view, you can sort these results and move the highlight bar to select a region in order to view sequence or detailed map data.



Sequence Tab

You can view the actual aligned sequences in a variety of formats, emphasizing the differences or similarities between the sequences. Click the **Sequence** tab to access this view. The default view shows only non-matching bases (difference format), with a dot used to indicate a match. The background area behind non-matching bases is colored to assist with finding these non-matches.

Some formats apply color if the similarity exceeds a specified value (Color areas of high matches or Similarity summary bars format). Use the Similarity Significance Value combo box in the toolbar to set the level of significance (range 50-100%) you want to apply for these formats.



Click the **Show Similarity/Difference** button to toggle between the default similarity and default difference formats to get a rapid impression of which view will give you the best display.



Click the **Settings** button to access the full set of sequence format options or to set page style options. When the format is just the way you want it, you can print, copy or export the data.

Score sort ▾

60% ▾

Homology Block: Percent Matches 99 Score 2000 Length 2011

Sequence View: Difference Format, Color behind non-matches

SYNPBR322	2348	gcgtctccgttcccgctactgactcgctggcgttcggc
SYNPWE15	6143
SYNPBR322	2398	tgcggcgagcggtatcagctcactaaaggcgtaatacggttatccaca
SYNPWE15	6193
SYNPBR322	2448	gaatcaggggataacgcaggaaagaacatgtgagcaaaaggccagaaaa
SYNPWE15	6243
SYNPBR322	2498	g-----gccaggaaccgtaaaaaggcccgttgctggcgtttt
SYNPWE15	6293	.gcagcaaaag.....
SYNPBR322	2537	ccataggctccgccccctgacgagcatcacaaaatcgacgctcaagtc

Info | Picture | Sequence | LMap



Click the **Next Result** or Previous Results toolbar buttons to navigate through the list of local homologies found without leaving the sequence view.

Location Map Tab

For each region of local homology, you can view a map showing the approximate position of the region of homology within each of the molecules. Click the **LMap** tab to access this view. Molecule names are shown, along with homology block basepair coordinates. The regions of local homology appear as colored bars. Homologous regions on the normal strand appear in blue, while regions on the complement strand appear in light red. Click the Next Result or Previous Result toolbar buttons to navigate through the list of local homologies found without leaving this view window.

■ Homology Block: Percent Matches 99 Score 2000 Length 2011

SYNPB322 (1 to 4361)

Homology Block: 2348 to 4358

SYNPWE15 (1 to 8164)

Homology Block: 6143 to 8164

tet ROP bla

5' —————— 3'

ORF-4 ORF-1 ORF-3 ORF-2