

Supplemental Help - Clone Manager Professional v 9

The latest update to Clone Manager Professional (ver 9.02) includes a new function to help you to join two sequences to create a larger molecule and an update to the Find Sequence module that lets you find places in the sequence that nearly match a specific pattern of bases (allowing limited mismatches). To help you get started with these new updates, the following help text is provided.

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).

Operation

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.

Resulting 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.


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.

To start a Find sequence operation:

1. Open the molecule viewer window and click the Sequence tab.
2. Click the Find button 
3. Enter the sequence of bases you want the program to search for (click the small button for help with IUPAC ambiguous bases).
4. Use checkboxes to configure the search to permit limited mismatches or mismatches and gaps or insertions.

To jump to matches in the sequence:

5. Set the Search Type to **Go To** Sequence Found.
6. Use checkboxes to limit the search to the upper strand or to begin the search at the start of the sequence.
7. Click Find. If a matching string is found, the string of matching bases will appear as selected text (highlighted) in the sequence view.
8. Click the Find Next button  to locate additional matches.

Or, to view a report of all sites found:

5. Set the Search Type to **Report All Sites** and click Find.
6. View, print, or copy the list of sites found.

Advanced search notes:

When you select the option to allow limited mismatches, the number of mismatches (including gaps or insertions, if applicable) is limited to 1 mismatch for each 10 bases in the search sequence. For short search sequences of less than 10 bases, one mismatch is permitted.

When entering the search string, you can use the check box to indicate that you do not want to expand ambiguous bases. You might want to do this to locate an ambiguous base within your actual sequence data.

Find Sequence Search Results

You can search for matching or nearly matching sites in your sequence and set the search type to report all sites found. When the search has been completed, the results will appear in a scrolling list box with a location map display.

The scrolling list shows each site found, with its start position, sequence of bases, and the number of mismatches (*m*) in the sequence shown. Click on column headers to sort by position, sequence or mismatch number.

For all sites found, the sequence shown is the upper strand sequence. Mismatched bases are shown with upper case (capital) letters, gaps appear as dashes, and inserted bases are shown within parentheses.

The location map display is shown below the list of sites found. Vertical bars above the icon map show the approximate location of the site found and a red arrow marks the site highlighted in the scrolling list.

Add Feature -- click the Add Feature button to enter the highlighted site as a feature for this molecule.

Print List -- print the report of sites found. If you have sorted the list on the screen, the printed list will also be sorted.

Copy List -- copy the report of sites found to the Windows clipboard.