Supplemental Help - Clone Manager Professional v 9.2

The latest update to Clone Manager Professional (ver 9.2) includes a new module to retrieve sequence files from the Entrez system at the NCBI and a function to help you simulate cloning using Topo® Cloning techniques. To help you get started with these new updates, the following help text is provided.

Retrieving Files from Entrez (E-utils)

You can retrieve sequence files from the Entrez system at the NCBI using a new module that can directly access the NCBI file system. You need an internet connection to use this function. Once you locate the file you need, you can import the molecule for immediate use in Clone Manager or you can download this file to your hard disk to save the complete GenBank file, including references and annotations.

To find the file you need:

1. Click File, Retrieve from Entrez (E-utils)
2. In the Search In control, select Nucleotide for DNA molecules or select protein
3. In the Query box, enter the locus name, accession number or descriptive information.
4. If you need a special sort order for results, set using the Sort control.
5. Click the Search button.
6. When the results are listed, find the file you want and click to select.

Search results are listed in summary format, 20 items per page. Use the Next or Prev buttons to move from one page of items to another. Drag the lower right corner of the window to increase the window size. Drag the join between data columns to change the column width.

To display more information about each file:

Use the Display control to switch from the Essentials (concise) display of file contents to the Expanded (all columns) display. The Expanded display includes additional columns of information such as CreateDate, UpdateDate, or TaxId.

To view the selected molecule file:

1. Select (click on) the file you want
2. Click the Show GenBank File button.

To import the molecule for immediate use:

1. Select (click on) the file you want
2. Click the Get Molecule button
3. Set the checkbox if you want to also save the complete GenBank file to your hard disk

The molecule will be imported into the Clone Manager 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 AutoScan to add enzyme sites or just use the sequence in an alignment or primer design operation. If you set the checkbox to also save the complete GenBank file, you will be able to access the references and annotations in this file at a later time.

File formats information

Clone Manager files contain essential molecule information (sequence, selected features, description), as well as restriction enzyme sites and notes you may add to the file. (Use File, Preferences, Import Features to select which features are imported). GenBank files contain essential molecule information and may also contain references, comments, and additional features that are not imported into Clone Manager.

If all of this information is important to you, you will probably want to have both a Clone Manager and a GenBank file for the molecule. When you create a Clone Manager file from a GenBank file (either by using the import for immediate use operation or by opening a GenBank file previously saved to disk), Clone

Manager will automatically store a link between the two files so that you can view the extra information while working in Clone Manager.

Topoisomerase Cloning Wizard

This wizard will help you to simulate cloning using Topo® Cloning techniques (Invitrogen Corp). It will help you select the appropriate components, show you the proposed result, and create the resulting recombinant molecule. Please refer to the technical publications received with the Invitrogen products or available at www.invitrogen.com for more information about the recombination reactions or possible use restrictions.

Selected vector files are provided. These files are copied to your home directory and can be found in the subfolder Topo_Vectors.

Getting Started

Select the Topoisomerase Cloning Wizard from the Clone menu. Next, identify the procedure you want to do. You can do TA, directional, or blunt Topo cloning or you can prepare a PCR product for use in a subsequent cloning procedure.

Select Insert and Vector Molecules (Topo cloning)

Identify the molecule that contains the region you want to clone. Click the Change button to access the Molecule List or Browse. For TA cloning, the program will automatically add a 3' terminal A base to each strand if not already present. For directional cloning, the program will automatically add 5' terminal CACC to the appropriate strand if not already present.

Next, identify the vector molecule you want to use. In many cases, a vector will be suggested. Click the Change button to select another vector. The vector you select must have Topo sites that the program can recognize. For TA cloning, the program will automatically add a 3' terminal T base to each strand if not already present. For directional cloning, the program will automatically add a complementary GTGG overhang if not already present.

Prepare PCR product

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.

Next select the cloning ends required for use in the subsequent cloning procedure. You can select blunt, TA cloning, or directional cloning ends. For TA cloning, the program will add 3'A overhangs to each end of the amplified product. For directional cloning, 5' CACC will be added to the Lead In primer (see below).

When amplifying a region on the normal strand, Primer A will lead into the expressed sequence. When amplifying a region on the complement strand, Primer B will lead into the expressed sequence. Use the boxes below the primer designations to type in other bases that you want to add to the 5' extension 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 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.

View Topoisomerase Cloning Results

After using the wizard to simulate the construction of a clone using Topo® 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 Create Molecule button to have the program automatically do the required steps to simulate the cloning experiment, produce the recombinant molecule, and enter to the Molecule List. You can use this new molecule now or save to disk for later use.

If you are using Clone Manager Professional, you can design and evaluate primers to generate PCR products for use in subsequent topoisomerase cloning procedures. 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. The area below the map includes information about the amplified product and about the primers used to generate this product. When you select the option to design Better Primers, if either primer (or the primer pair) does not meet the criteria set, the notation 'Caution -- check primer pair report' appears in the primer description area.

 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.

 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.

Note: In the case of directional cloning, if two or more bases at the 3' end of the insert are homologous to the CACC overhang, you will be alerted that this may result in bidirectional cloning. You can continue or abandon the procedure and perhaps modify the insert molecule or add a cap to one of the primers.

Verify Results

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.

Redefine Cloning Experiment

 Click the Redefine toolbar button to make changes to the cloning experiment just completed. You may want to do this if you find you inserted a gene in the wrong orientation or selected the wrong vector.

Segmented Features

Genes and regions may consist of joins. In the features table, the symbols for these segmented features appear similar to the standard symbols, but with the cyan arrow (gene) or green box (region) shown in two segments. To see the basepair positions for the join segments or to view feature qualifiers, follow the instructions below.

To view GB location/qualifiers:

1. Select (highlight) the name of the feature.
2. Click the GB Location/Qualifiers button  or use the mouse cursor (see below).

The information displayed will include feature location data (basepair positions) and qualifiers available for this feature. GenBank format files imported in version 9.2 or later of Clone Manager will read in all feature qualifiers and retain this information when saved as a Clone Manager (*.cm5) file.

 When you move the mouse over the small symbol to the left of the feature name on a line in the features table, the cursor changes to a rectangle shape. Use this special cursor to click on the feature to see the GB location/qualifiers for this feature. Click anywhere else to dismiss this box.

Change your GenBank import filter

If you routinely retrieve GenBank files that contain joins, you will want to change your import filter at this time. (If you do not change the import filter settings, Clone Manager will read in the CDS features with joins as well as the exons for each feature, resulting in maps with apparent duplicate features.)

To change your import filter:

1. Click File, Preferences, Import Features
2. In the section Designated Features, confirm that the feature key CDS appears and has the Draw As style Gene 1.
3. Select the feature key exon, click the Edit button, and change the Draw As style from Gene to Info only.

Following this change, exons will not appear on molecule maps or the annotated sequence. They will be shown on the features table if you click the toolbar button Info Features. If you want certain exons to appear on a molecule map, just select this feature in the features table for that molecule, and edit the feature to have a Draw As type of gene or region.