

Supplemental Help - Clone Manager Professional v 9

A recent update to Clone Manager Professional (ver 9.01) includes a new function to help you design a set of sequencing primers. To help you get started with this new wizard, the following help text is provided.

Sequencing Primer Wizard

This wizard will help you to design a set of primers for sequencing a molecule region. The wizard will select three non-overlapping primers per sequencing block, where possible. You can re-order the primers in a block to pick another primer as your top choice, where needed. You can export primer sequences, save to a primer collection or view a map of sequencing coverage.

Getting Started

Select the Sequencing Wizard from the Primer menu. Next, follow the wizard instructions to specify the molecule region and strand you want to sequence, enter primer design preferences and specify which design criteria are most important to you so that primers will be ranked and selected accordingly.

Define What you Want to Sequence

Identify the molecule that contains the region you want to sequence. 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 sequence and use the option buttons to indicate if the region to sequence is on the upper/normal strand or on the lower/complement strand.

You can use the Features... button to look at the features table for this molecule and select a gene, if appropriate. The program will enter the base positions for you.

Set the Primer Design Options

For automated DNA or cycle sequencing, set the primer type to PCR Primer to set the appropriate primer evaluation criteria. You can leave the criteria set to default conditions, or you can edit the criteria settings for this primer search. If the annealing temperature (shown below the Criteria button) is not correct for this protocol, click the Criteria button and change this value.

Enter the optimal primer length you would like and indicate the acceptable primer size range. Enter the number of bases preferred for each sequencing block and enter the values that reflect the trace constraints, or accept the default values.

Specify Weight Settings

You can change the weight settings that are used to rank and select the best primers. You can indicate if distance from the optimal target position is more or less important to you, if the penalty for false priming should be increased or ignored, or if avoiding primer dimers is more or less critical to you.

The standard (default) settings will usually give very good results.

Specify Search Preferences

If the search for primers is not successful for any sequencing block, the program will attempt to adjust the block positioning and/or adjust the acceptable GC range and repeat the primer search procedure. If primers still cannot be found for a given sequencing block, the program will adjust (relax) the primer evaluation criteria for primers in this block to try and find primers you can use.

You can accept the default settings or indicate which adjustments you will allow for each of these categories.

View Sequencing Wizard Results

The primers designed for the sequencing project are shown in a text display, grouped by sequencing block. For each primer listed, the data display shows the primer rank in this block (1, 2 or 3), the binding position

for the 5' end of the primer to the template molecule, the primer sequence (5' to 3'), the primer length, and the %GC and Tm °C. If the primer was selected using relaxed primer criteria, the symbol !! follows the Tm value.

You can see one primer per sequencing block or three alternate primers per block. Use the drop-down control in the display toolbar to select First Choice or Three Alternates to control the number of primers to display. If difficult areas are encountered and primers can not be found or if primers are not positioned to provide sequence for the entire block, the notation ** coverage alert ** is added to the block header line.



Use the Map Sequencing Coverage button to view an alternate display, showing the approximate extent of reliable sequence resulting from each primer-associated sequencing reaction. A map line for the region to be sequenced is shown at the top of the display. Blocks with possible incomplete coverage are marked with red.

Each of the expected sequences is shown by a colored bar on a separate line. The position of the bar shows the approximate location of the sequence. A blue bar indicates sequence is being aligned 5' to 3', while a light red bar indicates the sequence is being aligned in the reverse direction (3' to 5').

You can use the drop-down control in the toolbar to view one primer-associated sequence per block or three alternates per block. Select (highlight) a sequence bar and return to the text display to identify the primer or its binding position. Click the depressed Map Sequencing Coverage button to return to the standard list display.



Use the Rotate Alternate Primers button to rotate (re-order) the primers in the block containing the selected (highlighted) primer. This will move an alternate primer to the First Choice position at the top of the list for this sequencing block. The rank numbers (1, 2, 3) reflect the wizard's rank assignment and do not change with the rotation. (You can use these numbers to return the primers to the original order if needed.)



Use the Make New Primer Collection button to create a new primer collection containing all of the primers displayed -- either 1 primer (first choice) per block or 3 alternate primers per block, depending on the display style selected. You will be asked to enter a short identifier that can be used as part of the name for your collection and its primers.



Use the Export All Primer Sequences button to export primer sequences to a disk file or copy to the Windows clipboard for later use in another program. The wizard will export the primer data displayed -- either 1 primer (first choice) per block or 3 alternate primers per block, depending on the display selected. You can export primer sequences only, primer names and primer sequences, or a tab-delimited data set which also adds the primer description to the data.

Single-primer actions -- With the highlight bar on one of the primers, you can use the first three toolbar buttons to view the primer report for the selected primer, enter this primer to the Primer List for later use, or export the sequence for this primer.

Redefine Sequencing Primer Search



Click the Redefine toolbar button to change (fine-tune) the sequencing primer search. You may want to change the preferred size of the sequencing blocks or modify the primer selection criteria or annealing temperature.

Export Primer Sequences

When you use the Export All Primer Sequences option in the sequencing primer wizard results, you can export primer sequences only, primer names and primer sequences, or a tab-delimited data set which also adds the primer description to the data.

Sequences only

Select this option to export only the sequences of the primers, one primer per line, 5' to 3'. The order of the primers exported will be the same as the order in the results display.

Primer name and primer sequence

This option will construct a name for each primer, using the 5-character identifier you entered, and the basepair position of the binding site in the molecule sequence. The default identifier is the first 5 characters of the molecule name.

Tab-delimited data set

Select this option to export the following information, separated by tab characters. This format can be imported into other applications, like an Excel spreadsheet.

Name -- primer name consisting of the 5-character identifier you entered plus the basepair position of the binding site in the molecule sequence.

Sequence -- the primer sequence 5' to 3'

Characteristics -- the primer length (in parentheses), followed by the primer %GC value and the T_m value in degrees C.

Description -- includes the number of the sequencing block this primer was designed for, the rank number assigned for this primer in the group of 3 alternates, and the name of the molecule that contains the region to be sequenced.