Supplemental Help - Clone Manager Professional v 9.3

The latest update to Clone Manager Professional (ver 9.3) includes a new function to help you simulate cloning using Gibson Assembly™ or related cloning techniques. To help you get started with these new updates, the following help text is provided.

Assembly Cloning Wizard

This wizard will help you to simulate cloning using Gibson Assembly™ (Synthetic Genomics, Inc.) or related cloning techniques. 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 any Gibson Assembly or other products for more information about the recombination reactions or possible use restrictions.

Getting Started

Select Assembly Cloning from the Clone menu to start the wizard. Next, identify the procedure you want to do. You can assemble a set of overlapping fragments, previously prepared, or use PCR to create amplified fragments with overlaps, then assemble the fragments. Procedure options such as minimum number of bases of overlap, minimum fragment size, or assembly reaction temperature, have been set for Gibson Assembly, but can be changed for other methods. If primer design will be used, additional options can be configured. Click Next to identify your fragments.

Select Fragments with Overlaps to Assemble

Click the Add Fragment button to access the Molecule List or Browse to identify the fragment with overlaps to add to the list of fragments to assemble. Click the Add Vector button to identify a linearized vector fragment with overlaps to add to the list. A vector with overlaps at both ends can result in a circular recombinant molecule.

Starting with the first fragment in the list, overlaps will be used to determine the order of assembly. The fragment in the top position is used to seed the assembly and sets the start and orientation. A vector fragment will automatically be placed in the first position of the list. If you are not using a vector, you can move an important fragment to the first position.

PCR Amplify Fragments to Assemble

Click the Add Fragment button to add fragments to the list of fragments to assemble. For each fragment, 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. Set the check box if you need to add extra bases before (lead in) or after (lead out) this fragment. Enter the extra bases as they would appear in the upper strand of the final fragment assembly.

Click the Add Vector button to identify the vector molecule you want to clone into. For circular vectors, you can enter the basepair positions (upper strand) that immediately flank the site where the assembled insert will be placed. When you select to enter basepair numbers, you have the option to PCR amplify the vector. The PCR primers generated for the vector will not have insert overlaps. Alternatively, you can select one or two enzymes to cut the vector, creating the insert site. The drop-down controls list the single-cutting enzymes you can use for this molecule. If a single cut is to be used, enter the same enzyme name in both boxes. (Note that 5' overhangs of a restriction site will be removed during the Gibson assembly procedure.) Set the orientation for the assembled insert.

In the list of fragments to assemble, the fragments should be listed in the correct order for assembly. Use the Move Up or Move Down buttons to re-order the fragment list as needed. If a vector is used, the vector fragment will automatically be placed in the first position of the list of fragments to assemble.

If you are using Clone Manager Basic, the region to be amplified will be extracted and the overlap extensions added to simulate PCR product generation. If you are using Clone Manager Professional, primers can be designed and evaluated. The primer type and criteria have been set and should not need to be changed. The program will construct the possible primers of different lengths at the two fixed positions, adding overlaps and extra bases (if needed), and select the best primer pair. If the option has been

selected, the overlap segments will be split between the two adjacent fragments, when possible. This permits greater flexibility in primer design and may result in primers of shorter length. When the junction is between a fragment and a vector, the entire overlap segment will be a part of the insert primer.

Default values

Default values set for Gibson Assembly procedures are as follows: Minimum number of bases of overlap = 16; minimum fragment size (bp) = 150; Assembly reaction temperature (°C) = 50; Minimum overlap Tm (°C) = 60; Split overlap between fragments where possible = Checked; Overlap end base must be G or C = Unchecked; Primer type = Gibson primer pair.

View Assembly Cloning Results

After using the wizard to simulate the assembly of a molecule using assembly cloning techniques, you can view the proposed result and create the recombinant molecule. An iconic map of the recombinant molecule is shown, with the inserted fragments drawn in alternating lighter colors. Fragment numbers appear below the map segments. If features were present in the vector or insert molecules, these features will be shown above the map line. If primers were created, these appear above or below the map line at the fragment junctions. The area below the map shows detailed information about the recombinant molecule, the insert fragments, and the vector used.

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. Use the drop down list box to move from the Summary view to the view of primer pairs for each fragment. These detailed views give the primer description, primer sequence, possible primer cautions and a three-frame translation of the primer sequence (5' to 3'). Bases in upper case letters are homologous to the template; bases in lower case letters represent the overlap and extra bases added (if any).

 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. When entering to the Molecule List, you can change the molecule name or description. You can also indicate if enzyme sites for the new molecule should be auto-scanned using 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 will be entered, replacing the existing enzyme sites.

If you are using Clone Manager Professional, you can also create and save the primers used to prepare PCR amplified fragments. Use the check boxes to add all the primers to a new primer collection, export all primer sequences, or add primer sites to the recombinant molecule in one easy step. The new primer collection will be named AsmWiz_ followed by the name you entered (or the default Assembly 1). If you select to export all primer sequences, the list of primers (name and sequence) will be copied to the Windows clipboard. When you see the message indicating that this data is on the clipboard, you can paste this information into another document. You might want to do this to order oligos or document your work.

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.

If you are using Clone Manager Professional and have selected to add the primer sites to your recombinant molecule file, you can view these primer sites on the molecule map. Click the Enzyme Sites/Primer Sites toolbar button to toggle between enzyme sites and primer sites. Right click on a primer site and select Go To Sequence to view the molecule sequence at the position of the primer, with primers shown above the sequence bases.

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.