

Supplemental Help - Clone Manager Professional v 9.1

The latest update to Clone Manager Professional (ver 9.1) includes a new function to help you simulate cloning using Gateway® Recombination Cloning techniques. To help you get started with this new wizard, the following help text is provided.

Gateway® Cloning Wizard

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

Selected donor and destination vector files are provided. These files are copied to your home directory and can be found in the subfolder GWC_Vectors. You can use your own donor and destination vector files. The last section of this document provides some helpful suggestions.

Getting Started

Select the Gateway Cloning Wizard from the Clone menu. Next, identify the procedure you want to do. You can make an entry clone (BP reaction), an expression clone (LR reaction), prepare a PCR product with attB sites, or simulate a complex multisite procedure.

Select Insert and Vector Molecules (BP and LR reactions)

To make an Entry Clone, identify the molecule that contains the region you want to clone, flanked by attB sites. Click the Change button to access the Molecule List or Browse. Next, identify the donor vector you want to use. In many cases, a donor vector will be suggested. Click the Change button to select another vector. The donor vector you select must have attP sites that match the attB sites in your insert.

To make an Expression Clone, identify the insert (entry clone) with attL sites that you want to use. Click the Change button to access the Molecule List or Browse. Next, select the destination vector. Click the Change button to select another vector. The destination vector must have matching attR sites. If you want to use more than one entry clone in this reaction, set the checkbox and click Next to move to a page where you can add multiple entry clones.

Define What you Want to Amplify (PCR product generation)

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.

Select the attB extensions you want to add to each primer. For a simple one fragment recombination, attB1 and attB2 are standard. When amplifying a region on the normal strand, the attB extension for Primer A will lead into the expressed sequence. When amplifying a region on the complement strand, the attB extension for Primer B will lead into the expressed sequence. Use the box below the attB extension to type in other bases that you want to add after the attB extension and 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 attB 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.

Multisite Pro kits

Select the 2, 3 or 4 fragment procedure and indicate the method to use. If inserts have already been prepared, identify the molecule or PCR product that contains the region you want to insert for each fragment, flanked by the needed attB sites. The first line (Make Fragment...) shows the attB sites required for this fragment. Click the Change button to access the Molecule List or Browse. The donor vector with matching attP sites is suggested. Continue to create the additional fragments needed.

If you need to prepare the PCR products, begin by identifying the molecule, region and strand to amplify for this fragment. The required attB extensions are preset for primers A and B for each fragment. You can add extra bases between the attB extension and the template sequence, if needed. This PCR product will be generated and used for the subsequent BP reaction. The donor vector with matching attP sites is suggested. Continue this two-step process to create the additional fragments needed.

When all fragments have been prepared, they will be collected and automatically added as entry clones for the subsequent LR reaction. Select a destination vector to prepare the expression clone. The destination vector you select must contain attR1 and attR2 sites.

View Gateway® Cloning Results

After using the wizard to simulate the construction of an entry or expression clone using Gateway® Recombination 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 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.



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. Following a multisite procedure, you can also collect the intermediate molecules generated during the procedure (entry clones, PCR products).

If you are using Clone Manager Professional, you can design and evaluate primers to generate PCR products with attB extensions for use in Gateway cloning steps. 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, flanked by the attB sites. The area below the map includes information about the amplified product and about the primers used to generate this product. If either primer (or the primer pair) does not meet the criteria set, the notation 'Caution -- check primer pair report' appears above the primer descriptions.



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. Following a multisite procedure, the Create Molecule option allows you to add all the primers to a new primer collection or export all primer sequences in one easy step.

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.

Create Molecules - MultiSite Procedure

After using the wizard to simulate a complex multisite procedure, you can have the program automatically do the required steps to simulate the cloning experiment and produce the recombinant molecule and all intermediate molecules in one batch operation. If you are using Clone Manager Professional, you can also create and save the primers used to prepare PCR products and export the primer sequences.



Click the Create Molecule button to start the process.

Molecules

Use the checkboxes to indicate which molecules you would like the program to create and enter to the Molecule List for you. You can select to create the recombinant shown on the results screen (Expression Clone) and the entry clones prepared in each of the BP reactions. (These entry clones were then used in the final LR reaction to produce the expression clone.) If you prepared PCR products in order to make the entry clones, you can also elect to create and enter these molecules.

Molecule names follow the suggested naming conventions: Expression clones and entry clones begin with pEXP or pENTR, as appropriate, followed by a short vector name and then the subcloned gene name. PCR products begin with pcr followed by the two flanking attB sites and the amplified gene name. Once molecules are on the Molecule List, you can open these molecules to review their contents or save them to disk for later use. When saving to disk, it may be helpful to create a new folder and store all of the molecules from one recombinant experiment in the same location.

Process Molecules

Enzyme sites for the new molecules can 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.

You can clear the AutoScan checkbox, instructing the program to rebuild the map sites list by rescanning with enzymes currently listed for the molecules being recombined. This is the procedure used following a standard Clone, Ligate operation, but it may result in a number of enzyme sites that are not single-cutters for the newly-created molecule.

Primers

If you are using Clone Manager Professional you can use the checkboxes to enter or use primer data generated in this experiment. You can elect to create each of the primers used to prepare PCR products and save these primers to a new primer collection. The new collection will be named GateWiz_ followed by the subcloned gene name (as found in the expression clone). Primer names will indicate the attB site appended and the gene to be amplified. 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.

Using your own Donor and Destination Vector Files

You can use your own donor and destination vector files in the Gateway Cloning Wizard. When you need to select a donor or destination vector, you can navigate to your own files and select them for use. If you place these files in the subfolder Dest vectors or Donor vectors, located in the GWC_Vectors folder in your Home

directory, they will appear in the selection list for the default location, avoiding the need to navigate to another location.

In your own files, recombination sites should be entered using a standard nomenclature (shown below). Clone Manager knows how to handle features labeled in this way and will not carry over partial features in the recombinant molecule. Recombination sites can be entered on either strand. Clone Manager scans the sequence of the recombination site and uses the appropriate strand.

Example:

Feature name = attB1 (or attP4, etc)

Feature key = misc_recomb