

User Guide to



SeqBuilder Pro

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Welcome to SeqBuilder Pro

SeqBuilder Pro is Lasergene's application for sequence editing and map creation, as well as plasmid auto-annotation, automated virtual cloning and primer design. Easily navigate between synchronized views with a click of a button, and rearrange views and toolbars by simply dragging and dropping them where you want them to be.

The following video is a brief walk-through of the SeqBuilder Pro application.

These support pages contain written help and tutorials, as well as video tutorials.

- For help **INSTALLING** Lasergene, see our separate [Installation Guide](#).
- For written **TUTORIALS** with free data, see [SeqBuilder Pro Tutorials](#).
- [Click here](#) for a list of all topics that include **VIDEOS**.
- To **PRINT** the current page of the User Guide, click the printer icon in the top right corner ().
- To download a **PDF** of the entire User Guide, scroll to the bottom of the table of contents on the left, and press **Download as PDF**.

SeqBuilder Pro Tutorials

SeqBuilder Pro tutorials are typically located in the table of contents just under the help topic they are designed to illustrate. For convenience, all tutorials are also listed below.

Primer tutorials:

- [Create and modify primers for TA cloning](#)

Cloning tutorials:

- [Gateway cloning](#)
- [MultiSite Gateway Pro cloning](#)
- [Gibson cloning with a custom vector](#)
- [Use a PCR insert in TA cloning](#)
- [PCR-directed restriction cloning](#)

Restriction enzyme tutorials:

- [Apply a restriction enzyme to the sequence](#)
- [Find restriction sites on either side of a feature](#)

Miscellaneous tutorials:

- [Open an Entrez sequence by accession number](#)
- [Create and modify a feature](#)
- [Create a style set for CDS features](#) (for plasmid auto-annotation)

Open an Existing Sequence

SeqBuilder Pro is so powerful that you can import the entire human genome or as many as 5,000 bacterial genomes into a single SeqBuilder project file (.sbp).

The following topics describe three ways to open an existing sequence:

- [Open a file stored on your computer](#)
- [Open a sequence from SeqBuilder Pro's Project window](#)
- [Open a sequence from an online database](#) (e.g., NCBI's BLAST or Entrez databases or Vector NTI)

[Click here](#) for a list of supported file formats.

Open a local sequence

To open a local sequence file:

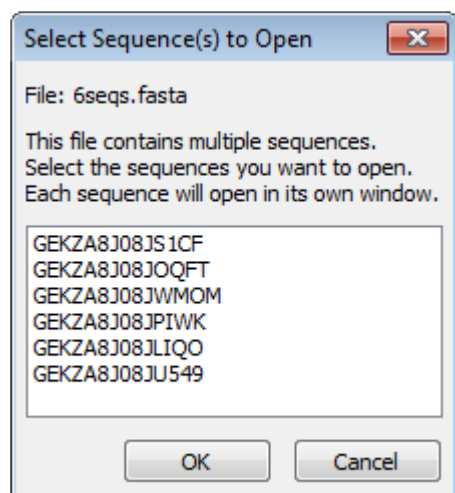
Choose **File > Open** or use the keyboard shortcut **Ctrl/Cmd+O** to open a nucleotide or protein sequence. If the sequence shape (circular or linear) is not defined in the file, SeqBuilder Pro will assume the sequence is linear.

When using **File > Open**, note that *.txt* format files are hidden in the Open File dialog by default. If you are attempting to open a Uniprot (EMBL) file in *.txt* format, change the file format drop-down menu from **All Known Formats** to **All Files** before browsing for the location of the *.txt* file(s).

Note that SeqBuilder Pro can also open *.structure* files from DNASTAR's Protean 3D protein structure application, but the residue numbering may be different in the two applications. SeqBuilder Pro always numbers sequence residues starting at '1.' By contrast, Protean 3D uses residue numbering provided by the author of the experimental structure. In addition, if there are gaps or extra residues in the *.structure* sequence, then features mapped to the version of the file in SeqBuilder Pro will not be synchronized with features in Protean 3D. Because of these differences, any edits made in SeqBuilder Pro can be saved for later viewing in SeqBuilder Pro. However, these edits will not appear if the *.structure* document is reopened in Protean 3D.

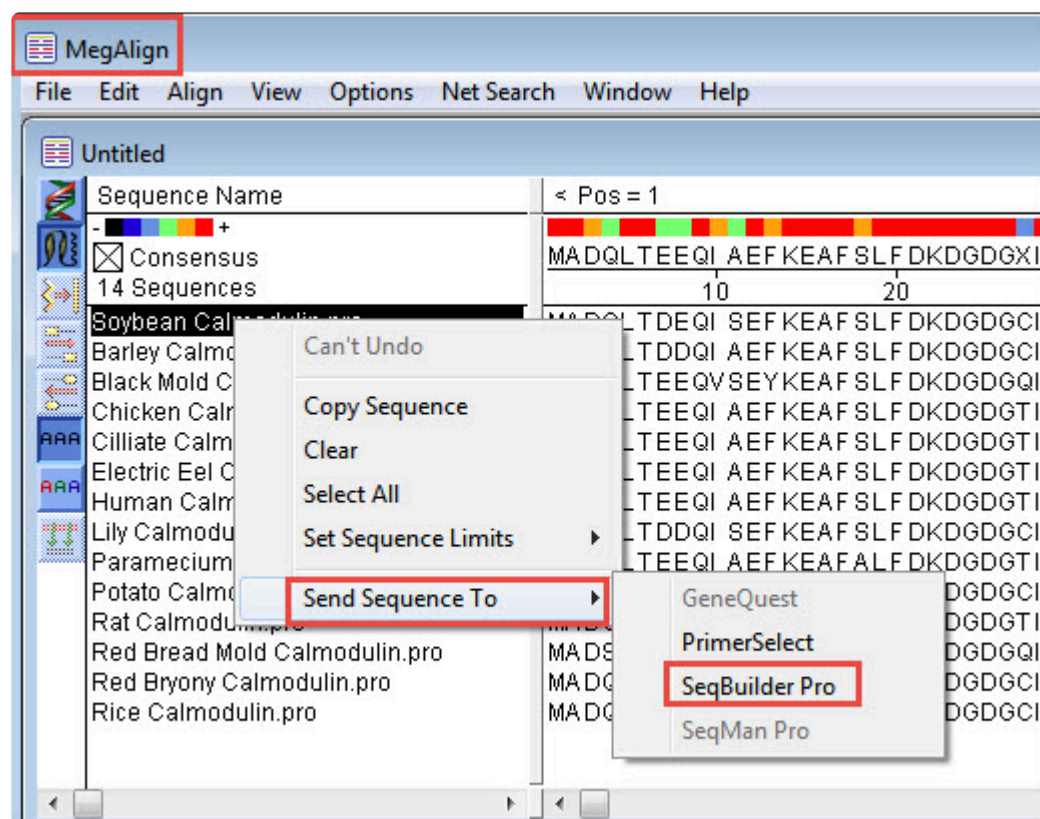
To open a local sequence file containing multiple sequences:

Choose **File > Open**. In the ensuing dialog, select one or more files using click, **Shift** + click or **Ctrl/Cmd** + click, and then press **OK**. Each selected sequence will open as a separate SeqBuilder Pro document.



To send an open file from another Lasergene application to SeqBuilder Pro:

Select the sequence and use **File > Send Sequence To > SeqBuilder Pro**. SeqBuilder Pro is one of several Lasergene applications that support “synchronous updating.” This means that changes made to a sequence in one application (e.g. SeqBuilder Pro) are also applied to the sequence in the other application.



To batch import pre-2008 Vector NTI files:

Select **File > New Project** and then drag and drop the Vector NTI files (DNA *.gb*; protein *.gp*; or database archives *.ma4* and *.pa4*) onto the Document window. A separate SeqBuilder Project will be created for each file. SeqBuilder Pro only supports pre-2008 Vector NTI files, and does not support newer files, including those from Vector NTI Express.

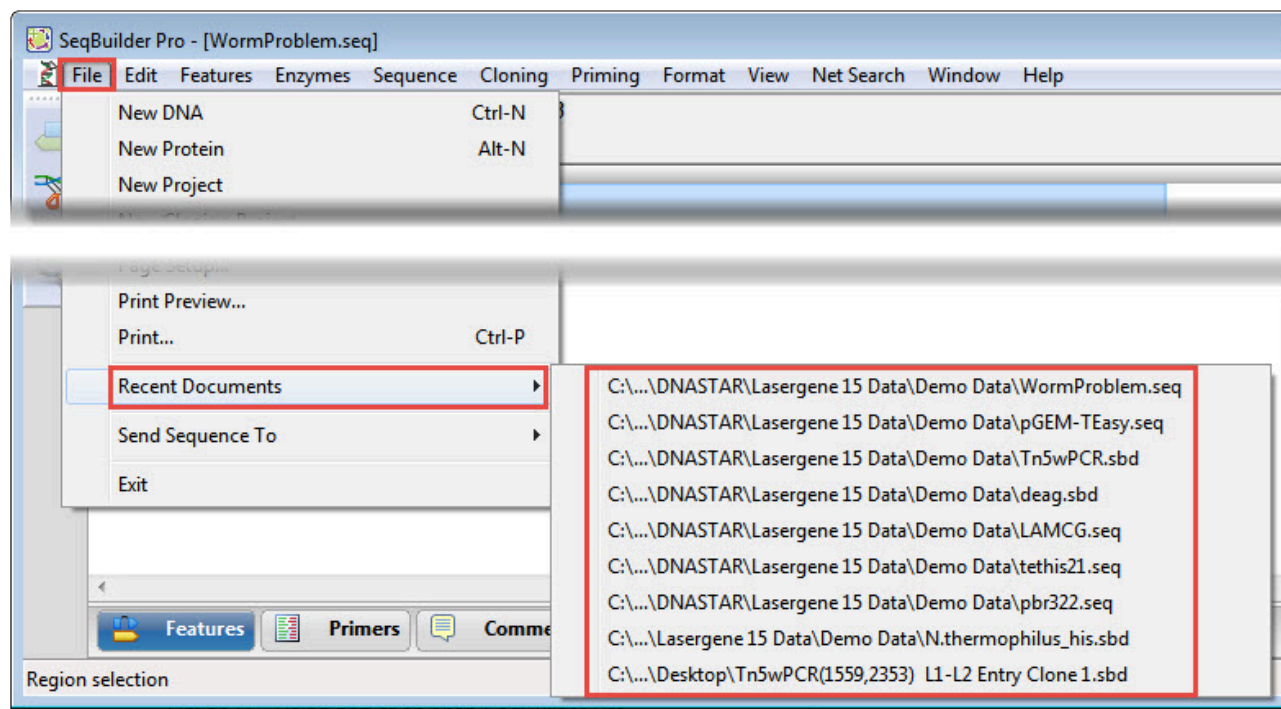
✿ **Note for Macintosh users:** Vector NTI only runs on Macintosh macOS 10.3 or lower, while Lasergene runs only on macOS 10.10 and higher. If you are using Vector NTI on Macintosh 10.3 or earlier, you must first archive each sequence as an *.ma4* or *.pa4* file. Then, you can upgrade your operating system to a current version, and import.

To import one or more sequences into an existing project:

Select **File > Import Sequence(s) to Project**. Importing creates a full copy of each imported file that is not related to the original file.

To re-open a recently-opened document:

Choose it from the **File > Recent Documents** list.



✿ Non-sequence files, such as enzymes or selectors, do not appear in this list.

Change the default application for opening Lasergene files

On Windows:

1. Open the **Control Panel**.
2. Go to **Programs > Default Programs > Associate a file type or protocol with a specific program**.
3. Select extension you wish to change in the **Extensions** list and then click the **Change program** button.
4. Click the **Browse** button in the Open With dialog.
5. Navigate to and select the application you want the files to open in. (Lasergene applications are located by default in **C:\Program Files (x86)\DNASTAR\Lasergene 'x'**, where 'x' represents the version number.
6. Click **Open**.
7. Click **OK** to close the Open With dialog, and then click **Close**.

On Macintosh:

1. Select a file of the type you wish to change in Finder.
2. Go to **File > Get Info**.
3. Click the drop-down arrow in the **Open With** field, and navigate to the application you want the files to open in. (Lasergene applications are located by default in **Applications:DNASTAR:Lasergene 'x'**, where 'x' represents the version number).
4. Click the **Change All** button to make the application you selected the default for all files of this type.

To change the default application for opening Lasergene files:

[Click here](#) for detailed information.

Open a sequence from the Project window

To open one or more sequences from the [Project window](#) :

1. Open a populated Project window by selecting it from the **Window** menu.
2. Select the desired sequences using click, **Shift+click** or **Ctrl/Cmd+click**.
3. Choose **File > Open Selections**.
4. If you have selected multiple sequences, you will prompted whether you wish to **Open 'n' sequences**. Click **OK**.

Each sequence opens as a separate document within the project.

Open a sequence from an online database

SeqBuilder Pro's Net Search menu allows you to search online databases (e.g., the National Center for Biotechnology Information's BLAST and Entrez servers), for sequences that match all or part of the current sequence. Once a match has been located, you may add it to the current project or save it for use in other Lasergene applications.

When you perform BLAST or Entrez searches using a Lasergene application, you can enter your choice of sequences from the match list directly to the Lasergene application; the application will also automatically save copies of each file in Lasergene format. You may also use an accession number to directly download sequence data from NCBI and automatically open it in the Lasergene application.

See the following topics for detailed instructions:

- [Open an Entrez database file by locus name or accession number](#)
- [Open an Entrez database file using a text search](#)
- [Open a BLAST database file using a similarity search](#)
- [Import a Vector NTI database](#)

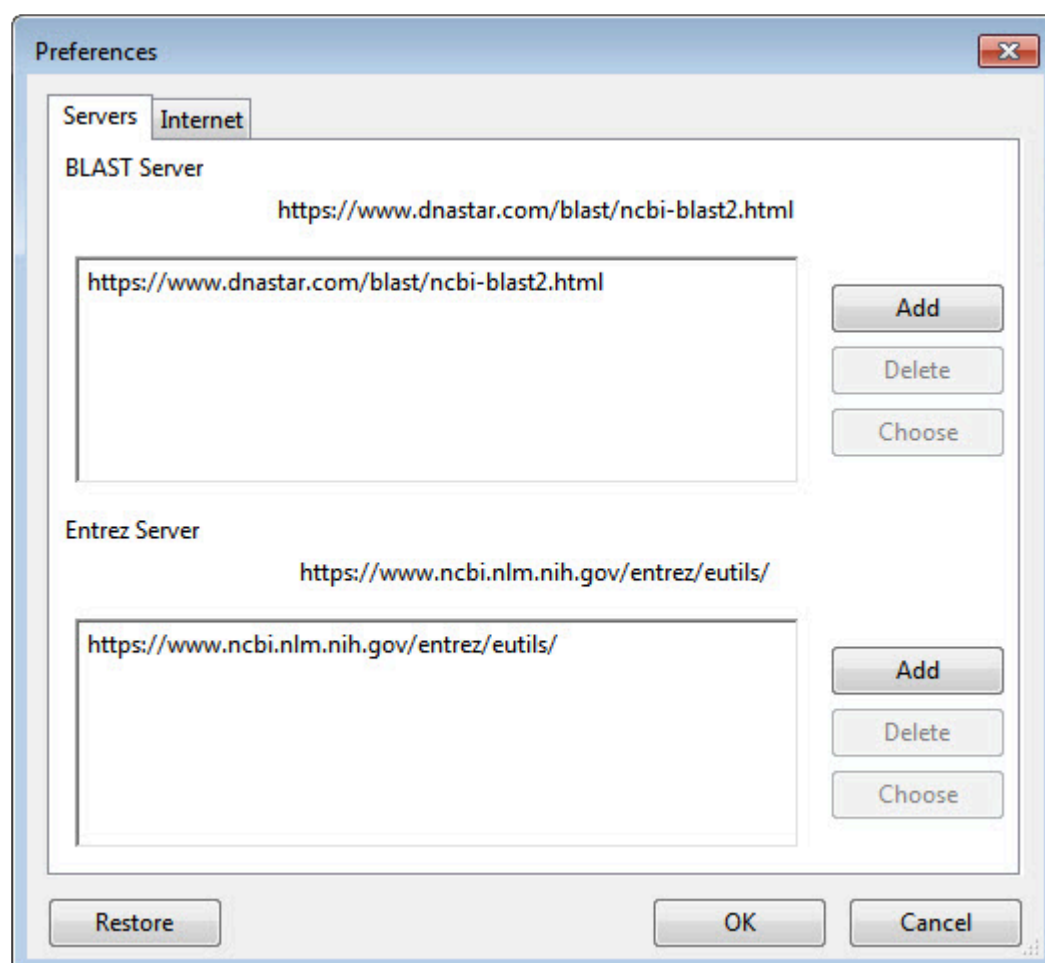


At the time of each Lasergene release, the SeqBuilder Pro Preferences dialog contains current URLs for searching NCBI's BLAST and Entrez databases. If NCBI changes URLs at a later date, you will need to update the Preferences dialog as instructed in [Specify URLs for BLAST and Entrez searches](#).

Specify URLs for BLAST and Entrez Searches

To specify URLs to use for [online database searching](#) :

1. Choose **Edit > Preferences** (Windows) or **Seqbuilder Pro > Preferences** (Macintosh) and select the **Servers** tab. The upper and lower portions of this dialog let you specify BLAST and Entrez server URLs, respectively. Unless “overwritten” by substituting custom addresses, the **Servers** tab uses the NCBI BLAST and Entrez server URLs that were current at the time of the Lasergene release. At present, these addresses are <https://www.dnastar.com/blast/ncbi-blast2.html> and <https://www.ncbi.nlm.nih.gov/entrez/eutils/>.

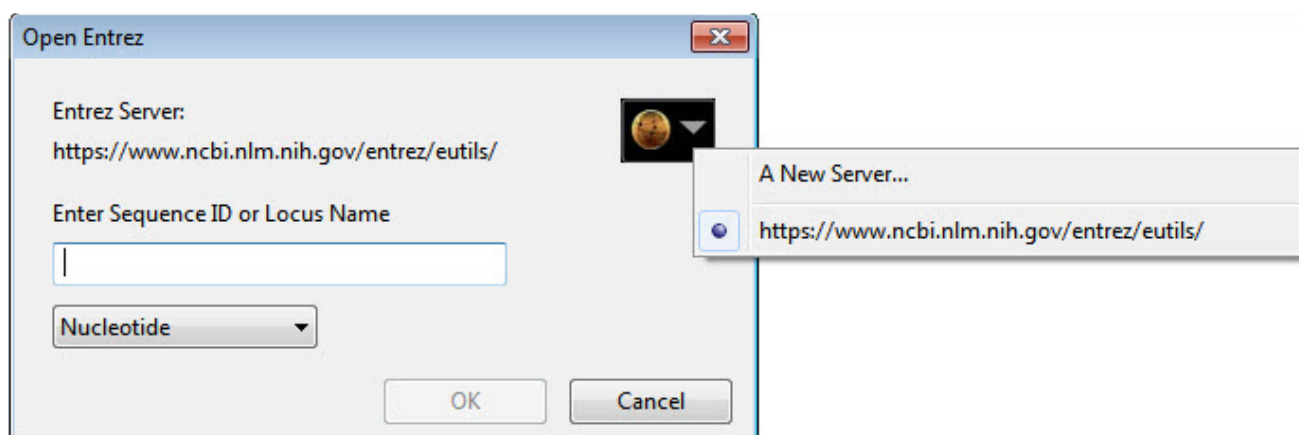


- To add a new address to either box, click the associated **Add** button and type in the URL.
 - To delete an address, select the URL from the list and click the corresponding **Delete** button.
 - To set the default server for performing a search, select the URL from the list and then click the corresponding **Choose** button.
2. To save changes and edit from the Preferences dialog, click **OK**. To return to the default NCBI server URLs, click **Restore**.

Open an Entrez database file by locus name or accession number

If you know the locus name or accession number for a sequence, SeqBuilder Pro can retrieve it from the Entrez database at the National Center for Biotechnology Information (NCBI):

1. Use **File > Open Entrez Sequence** or the keyboard shortcut **Ctrl/Cmd+R**.
2. The **Server** field lists the currently selected Entrez server. You may change to a different server by selecting it from the list on the right under the globe icon; or choose **A New Server** and then type a new URL.



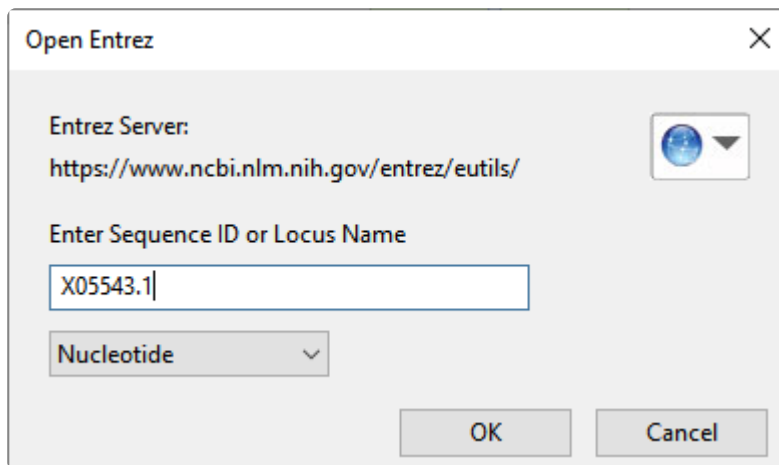
3. In the **Enter Sequence ID or Locus Name** text box, type a locus name or accession number.
4. Specify the database name using the drop-down menu.
5. Click **OK**.

If the search was successful, the Save As dialog will appear. The default file name is the accession number or locus name followed by the sequence extension (*.seq* or *.pro*). After saving, the sequence will be opened as a new SeqBuilder Pro document.

Try it! – Open an Entrez sequence by accession number

In this part of the tutorial, you will open a histone DNA sequence from NCBI's Entrez database. In order to complete this tutorial, your computer must be connected to the Internet.

1. Select **File > Open Entrez Sequence**. The Open Entrez dialog appears.
2. Under **Enter Sequence ID or Locus Name**, enter **X05543.1**, which is the sequence's "accession.version" number.
3. Keep the drop-down menu default selection of **Nucleotide** and click **OK**.



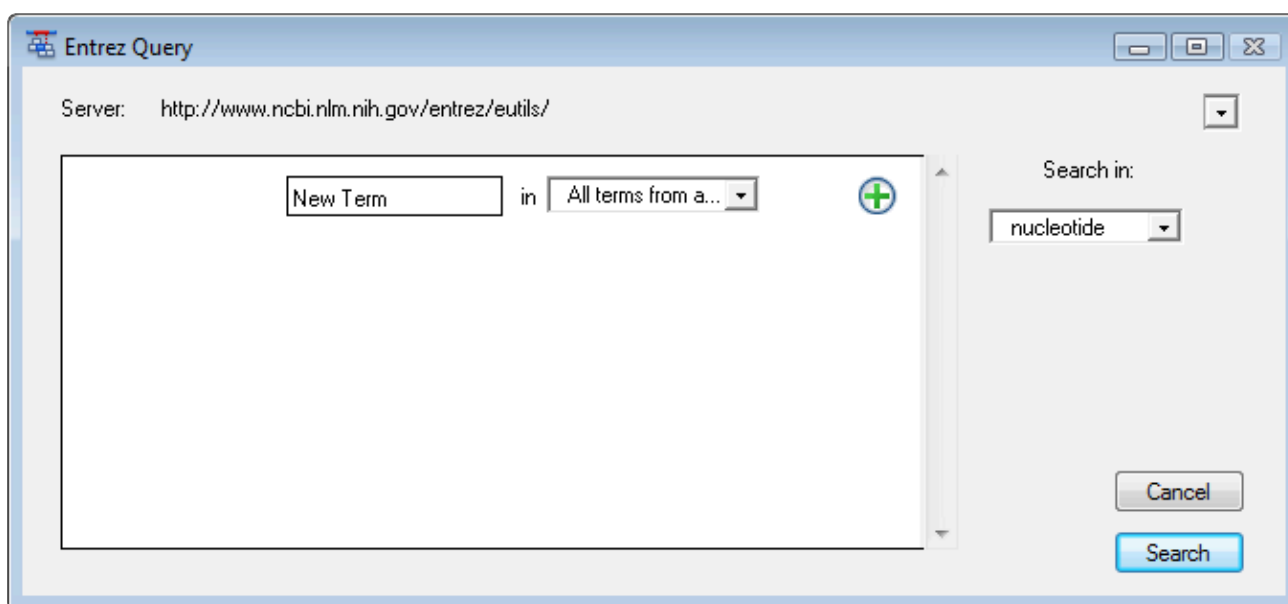
4. Select a location to store the sequence and click **Save** to open the sequence as a SeqBuilder Pro document.
5. (optional) Use **File > Save** to save the sequence. The sequence is used in the tutorial [Try it! – Find restriction sites on either side of a feature](#).

Open an Entrez database file using a text search

SeqBuilder Pro provides a graphical interface for constructing Boolean queries of text databases like NCBI's Entrez database. Search results are returned as a list from which you may select any or all sequences, then save them or open them as documents in the current application.

To perform a text search of the NCBI Entrez database:

1. Select **Net Search > New Text Search**. The Entrez Query dialog appears.





- The **Server** field lists the currently selected Entrez server. You may change to a different server by choosing one from the drop-down menu, or choose **A New Server** and then type a new URL.
 - Use the drop-down menu in the lower right of the window to choose the database to search. This menu will reflect the available databases on the selected server.
2. Create the search criteria using the fields provided. To access a context menu of editing commands (**Cut**, **Copy**, **Paste**, etc.), right-click anywhere in the Entrez Query dialog.

The screenshot shows the 'Entrez Query' window. At the top, the server URL is 'http://www.ncbi.nlm.nih.gov/entrez/eutils/'. Below this is a table for building a query:

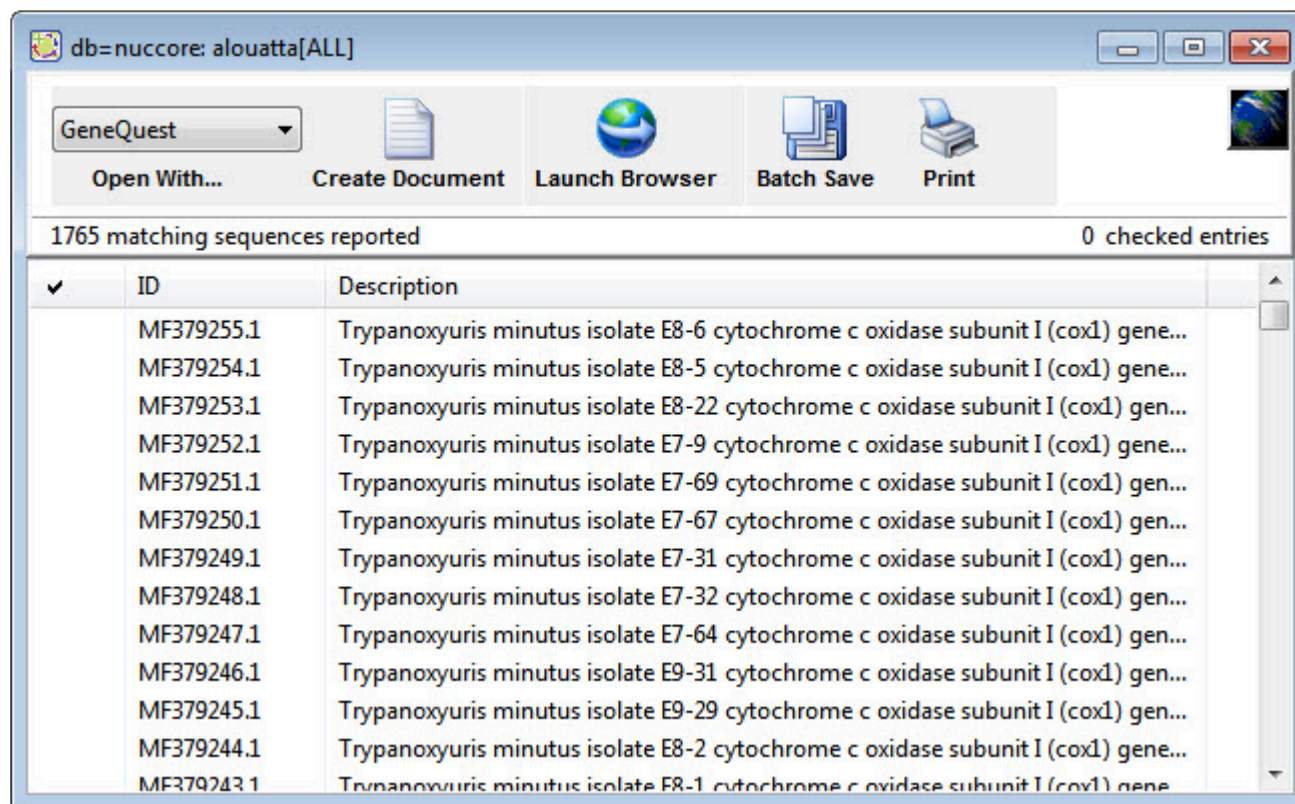
Operator	Term	Field	Buttons
	Smith	Author(s) of pu...	+
And	human	Scientific and ...	- +
Or	mythic	Name of protei...	- +
And Not	21	Volume numbe...	- +

To the right of the table is a 'Search in:' dropdown menu currently set to 'nucleotide'. At the bottom right are 'Cancel' and 'Search' buttons.

- - Enter the first (or only) criterion into the text box on the left.
 - You may change the search field heading from the drop-down list to the right of that text box. The default is **All terms from all searches**, which will search every word of every listing for the query term in the database. The field headings vary depending upon the online database that you are using.
 - To add a criterion to the query, click the  button and choose **And**, **Or**, or **And Not** from the drop-down menu on the left of the new row. **And** requires both the first and the second criterion to be present. **Or** will look for matches containing either criterion. **And Not** will search for matches containing the first criterion but not the second.
 - To remove a criterion, click the  button in the row that you want to remove.
- 3. Click **Search** to send the query to the database. Once the search is completed, any matches are displayed in the [Entrez Search Results window](#).

Entrez Search Results window

When an [Entrez database search](#) is complete, the Entrez Search Results window appears, showing a list of sequences that match your query.



The buttons at the top of the window are used to open, save, copy or print results.

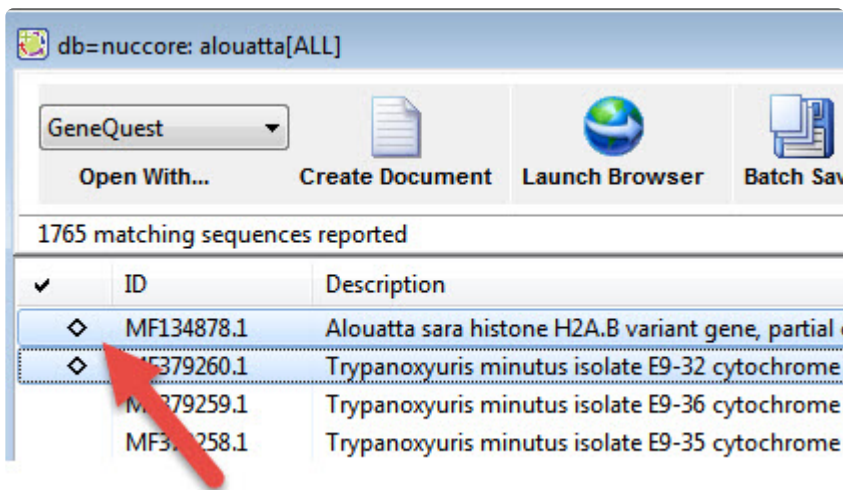
Task	Button to use...
To open one or more results in the selected Lasergene application	Open With
To open one or more results in the current application	Create Document.
To open one or more results in your default web browser	Launch Browser
To save a group of results as sequence files	Batch Save
To print one or more results	Print

The following table describes tasks that can be performed in the lower portion of the window:

Task	How to...
To sort results	Click on a column header to sort the rows by that column.

To narrow the results of a text query	Make sure the text search results window is active by clicking on it. If you have several text results windows open, select Net Search > Current Results to locate the desired window. Select Net Search > Search These Results and enter the desired search criteria. Click Search .
To change the search query	To modify a text query to obtain different results without having to completely recreate a complex query, select Net Search > Change This Query . The Entrez Query dialog appears containing the same search criteria that you last used, allowing you to edit as necessary before performing a new search.

After using [Batch Save](#) or [Create Document](#), selections that have been downloaded or opened are denoted with a black diamond or arrow.



View search results in a web browser

To view one or more results from the [Entrez](#) or [BLAST](#) Search Results windows in a web browser:

Do either of the following:

- Double-click on any result from the window.
- Select one or more results from the window using click, **Ctrl/Cmd+click**, or **Shift+click**, or by adding a checkbox to the left of the row(s) of interest. Then click the **Launch Browser** button or use the **Net Search > Launch Browser** menu command.

Each selected match will be displayed in a separate browser window.



Popup blocking software may prevent Lasergene from opening multiple web browser windows simultaneously. Check the documentation for your popup blocking software for additional information.

Open results in a Lasergene application

To open one or more results from the [Entrez](#) or [BLAST](#) Search Results windows:

1. Select one or more results from the Search Results window using click, **Ctrl/Cmd+click**, or **Shift+click**, or by adding a checkbox to the left of the row(s) of interest.
2. Do any of the following:
 - - To open the results in a Lasergene application other than SeqBuilder, select its name from the drop-down menu above the words **Open With**.
 - To open the results in SeqBuilder Pro, click **Create Document**. In the resulting dialog, select one of the following options from the **Save** field:
 - **Top** opens the specified number of matches, starting with the first sequence on the list. Specify a number in the text box (default is 10).
 - **Next** opens the specified number of sequences, starting with the first one currently highlighted. Specify a number in the text box (default is 10).
 - **Checked** opens sequences with a checkmark beside them.
 - **Selected** opens only highlighted sequences.
 - **All** opens all sequences.
 - To save the file(s) in a location other than the default directory, click the **Set Location** button.
3. Click **OK**.

Print search results

To print results from the [Entrez Search Results](#) window:

1. Select one or more results from the Search Results window using click, **Ctrl/Cmd+click**, or **Shift+click**, or by adding a checkbox to the left of the row(s) of interest.
2. Click the **Print** button to proceed with printing using the standard Print dialog for your computer's operating system.

To print results from an [BLAST Search Results](#) window:

1. Select one or more results from the Search Results window using click, **Ctrl/Cmd+click**, or **Shift+click**, or by adding a checkbox to the left of the row(s) of interest.
2. Click the **Print** button.
3. In the resulting dialog, select one of the following options from the **Print** field:
 - **Top** prints the specified number of matches, starting with the first sequence on the list. Specify a number in the text box (default is 10).
 - **Next** prints the specified number of sequences, starting with the first one currently highlighted. Specify a number in the text box (default is 10).
 - **Checked** prints sequences with a checkmark beside them.
 - **Selected** prints only highlighted sequences.
 - **All** prints all sequences.
4. Click **Print** to proceed with printing using the standard Print dialog for your computer's operating system.

Copy search results

Selected results may be copied to the clipboard from the [Entrez](#) or [BLAST](#) Search Results windows.

To copy detailed information:

1. Select one or more results from the Search Results window using click, **Ctrl/Cmd+click**, or **Shift+click**, or by adding a checkbox to the left of the row(s) of interest.
2. Choose **Edit > Copy** to move results to the clipboard. At a minimum, copied results will include an identifier and description for each selected result.
3. Paste into a spreadsheet or text document.

To copy exact text sequence only:

1. In the bottom pane of the BLAST search results window, select the desired text by dragging your mouse.
2. Choose **Edit > Copy** to move the selected text to the clipboard.
3. Paste into a spreadsheet or text document.



When pasting data into a word processing application, choose a mono-spaced font such as Courier so that the alignments display correctly.

Save search results

To save selected results from the [Entrez](#) or [BLAST](#) Search Results windows as a text file:

1. (optional) [Specify a default download folder for online search results.](#)
2. Make the [Entrez](#) or [BLAST](#) Search Results window the active window.
3. Select **File > Save As**.

By default, the file will be saved with a text file extension (.txt).

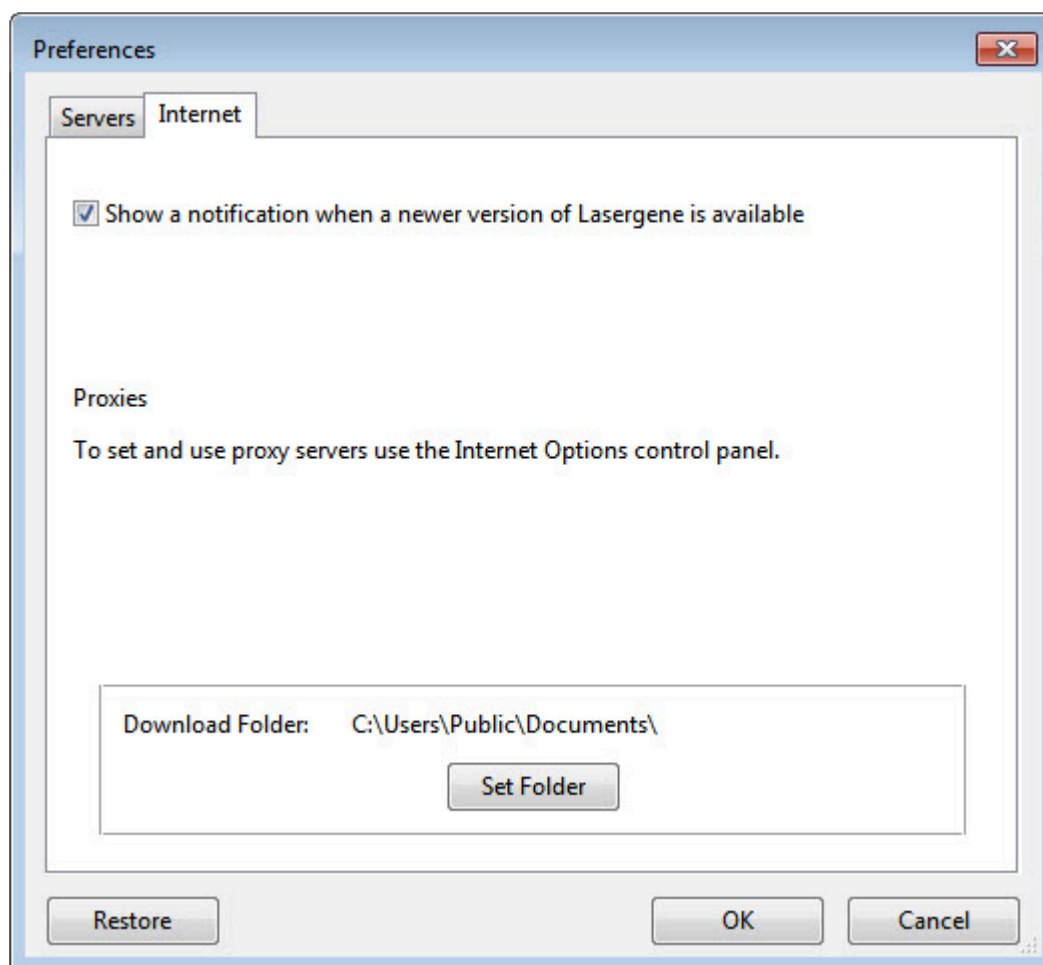
To save multiple search results as sequence files:

1. (optional) [Specify a default download folder for online search results.](#)
1. Select one or more results from the Search Results window using click, **Ctrl/Cmd+click**, or **Shift+click**, or by adding a checkbox to the left of the row(s) of interest.
2. Click the **Batch Save** button.
3. In the resulting dialog, select one of the following options from the **Save** field:
 - **Top** opens the specified number of matches, starting with the first sequence on the list. Specify a number in the text box (default is 10).
 - **Next** opens the specified number of sequences, starting with the first one currently highlighted. Specify a number in the text box (default is 10).
 - **Checked** opens sequences with a checkmark beside them.
 - **Selected** opens only highlighted sequences.
 - **All** opens all sequences.
4. (optional) To save the file(s) in a location other than the default directory, click the **Set Location** button.
5. Click **OK** again to save the sequence files to the specified directory.

Specify a download folder for online search results

To specify a download folder for results from an [Entrez](#) or [BLAST](#) Search Result window:

1. Choose **Edit > Preferences** (Windows) or **SeqBuilder Pro > Preferences** (Macintosh) and select the **Internet** tab.

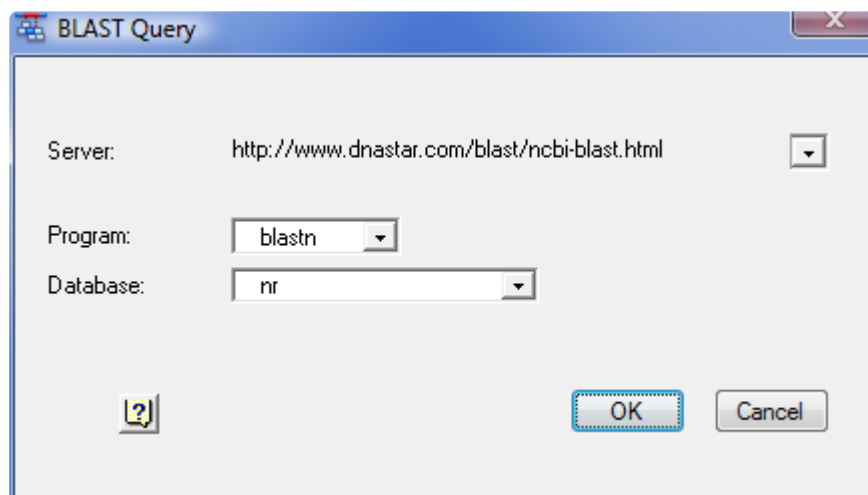


2. (optional) To automatically check for Lasergene software updates, check the box next to **Show a notification when a newer version of Lasergene is available**.
3. To specify the default directory for downloading results from online database searches, press the **Set Folder** button.
4. Navigate to the desired location and press **Select Folder**.
5. To save changes and exit from the Preferences dialog, click **OK**.

Open a BLAST database file using a similarity search

To perform a BLAST search:

1. Select the sequence or portion of sequence that you want to search for.
2. Choose **Net Search > BLAST Selection**.



- The **Server** field lists the currently selected BLAST server. You may change to a different server by choosing one from the drop-down list provided, or choose A **New Server** and then type a new URL.
- The **Programs** field lists the currently selected BLAST program. The following programs are available through this list, though some servers may not offer all of these programs:
 - **blastn** searches a nucleotide sequence database with a nucleotide query sequence
 - **blastp** searches a protein sequence database with an amino acid query sequence
 - **blastx** searches a protein sequence database with the six-frame translation of a nucleotide query sequence
 - **tblastn** searches the six-frame translation of a nucleotide sequence database with a protein query sequence
 - **tblastx** searches the six-frame translation of a nucleotide sequence database with the six-frame translation of a nucleotide query sequence
- The **Database** field lists the database that will be searched. Choose from the databases

available on the server by selecting one from the list provided.

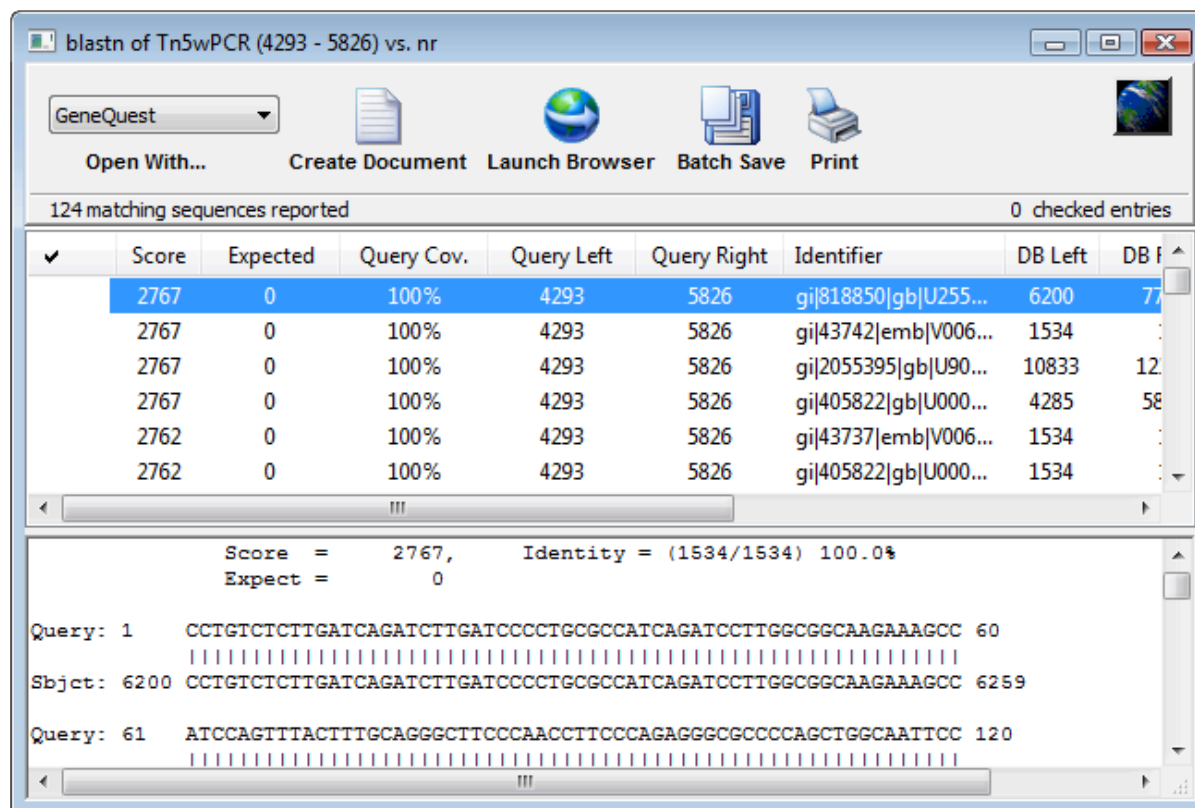
* Using less common databases may cause a noticeably slower search. According to NCBI, this is because the less frequently used databases may not already be loaded in memory on an available server. In contrast, frequently used databases such as **nr** or **nt** are already loaded in memory on a number of servers.

* Available databases may include the **refseq_representative_genomes** database. This database contains representative bacterial/archaeal genomes, as opposed to all the genomes (some of which are multiples) now in Genbank or RefSeq. The **refseq_representative_genomes** database can be helpful when matches to *Escherichia coli/Shigella*, for example, occupy all 100 of the top BLAST results. For example, say you want to find the location of a particular sequence in the curated "model organism version" of the E. coli K-12 MG1655 genome (U00096.3/NC_000913.3). Here is the location of the true sequence match when using different databases: **refseq_genomic** - absent from top 100 hits. **nr** - far down the list of the top 100 hits. **refseq_representative_genomes** – the top hit.

3. After confirming the values in the Query dialog, click **OK** to initiate the search. Once the search is completed, any matches are displayed in the [BLAST Search Results window](#). You may continue to work with other Lasergene application activities while waiting for BLAST search results, but you cannot launch another BLAST search until you close the current BLAST window.

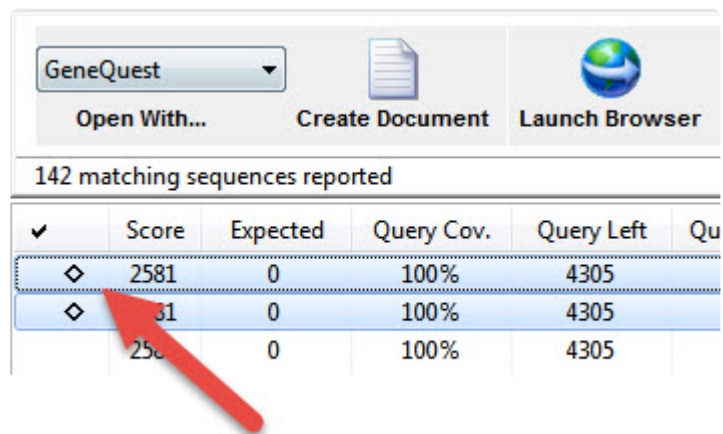
BLAST Search Results window

When a [BLAST database search](#) is complete, the BLAST Search Results window appears, showing a list of sequences that match your query.



Task	Button to use...
To open one or more results in the selected Lasergene application	Open With
To open one or more results in the current application	Create Document
To open one or more results in your default web browser	Launch Browser
To save a group of results as sequence files	Batch Save
To print one or more results	Print

The lower pane of the BLAST Search Results window contains the alignment of the query (upper sequence) to the highlighted database entry (lower sequence). The upper pane of the window contains the name of possible matches in order of probability. After using [Create Document](#) or [Batch Save](#), selections that have been downloaded or opened are denoted with a black diamond or arrow.



The following columns are available in the upper part of the window. Click on a column header to sort the rows by that column.

Column Name	Description
Score	The similarity score for the result.
Expected	<p>The expectation value. This corresponds to the Expect value displayed in the lower pane of the window.</p> <p>Note: Detailed information about how Score and Expected are derived is available at www.ncbi.nlm.nih.gov/BLAST. In general, a higher score and lower expectation connote a better match.</p>
Query Cov.	<p>The percentage of the queried sequence that aligns to a match in the database. For example, if you BLAST a range of 596 bases, and 565 of those bases align to a sequence in the database, then the Query Cov. value will be 565/596, or 95%.</p> <p>Note: Query Cov. differs from the Identity value shown in the lower portion of the BLAST window. Identity is the percentage of bases within the alignment that match. For example, if 565 bases align to a sequence in the database, and there are 3 mismatches within the alignment, then the Identity value would be 562/565, or 99.5%.</p>
Query Left	The left end coordinate of the queried sequence that corresponds to the left end coordinate of the matching result (DB Left).
Query Right	The right end coordinate of the queried sequence that corresponds to the right end coordinate of the matching result (DB Right).
Identifier	The sequence identifier for the result.
DB Left	The left end coordinate of the result sequence that corresponds to the left end coordinate of queried sequence (Query Left).
DB Right	The right end coordinate of the result sequence that corresponds to the right end coordinate of

	queried sequence (Query Right).
Description	The description, from NCBI, of the result sequence.

Import a Vector NTI database

Invitrogen's Vector NTI software will no longer be sold after December 31, 2019. In preparation for this event, Vector NTI users are encouraged to move their databases to SeqBuilder Pro using the following automated method:

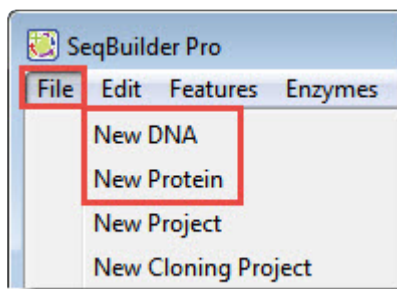
1. Download the Vector NTI Data Export Tool for your operating system from [this Invitrogen website page](#).
2. Run this export tool on the same computer where the Vector NTI database is installed. This creates a folder called *VNTI Advanced Data* that contains subfolders named in the step below.
3. Open SeqBuilder Pro version 16.0 or later and choose **File > Import VNTI Data into Project**. Here is what happens to the Vector NTI subfolder files upon use of the command:
 - *DNA and RNA sequence files* – Imported.
 - *Protein molecules* – Imported.
 - *Oligos* – Imported. You will be prompted to save the file *Oligos.csv* into an primer catalog file that is usable by SeqBuilder Pro named *Invitrogen Primers.pri*.
 - *Enzyme files* – Not imported. Rebase enzyme files are already present in SeqBuilder Pro.
 - *Gel markers* – Not imported. SeqBuilder Pro's [agarose gel simulation](#) feature provides most commercially available gel markers and lets you easily add additional gel markers, if needed.

Create a New Sequence

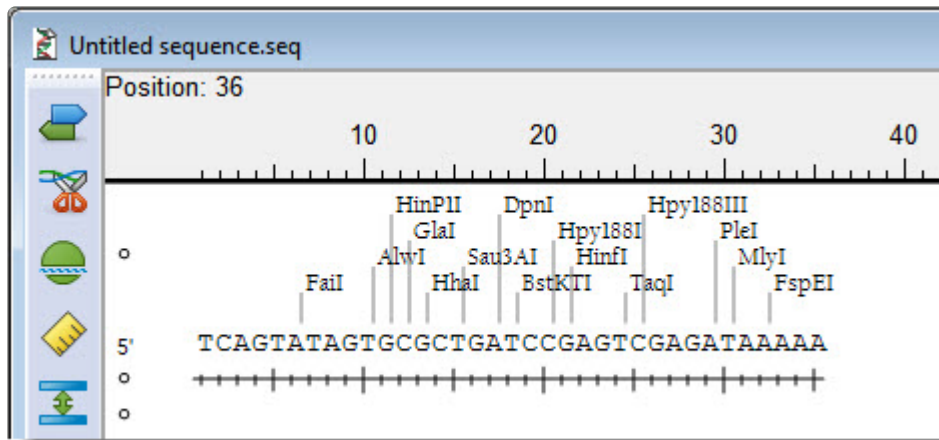
The following video is a brief overview of sequence creation and editing in SeqBuilder Pro.

To create a new DNA or protein sequence from scratch:

1. Choose the appropriate menu command or keyboard shortcut.
 - DNA: **File > New DNA** or **Ctrl/Cmd+N**.
 - Protein: **File > New Protein**, **Alt+N** (Win), or **Option+N** (Mac).

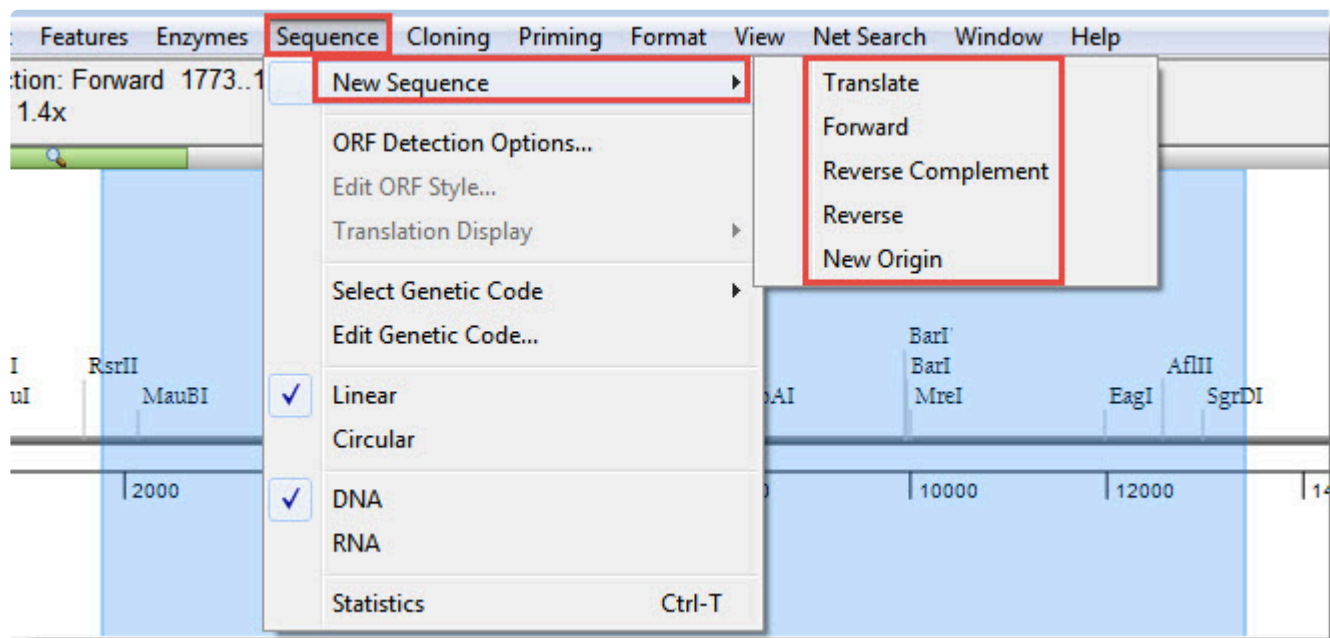


2. Using the keyboard, type in the new sequence using the appropriate [IUPAC codes](#).



To create a new sequence related to a currently-open sequence:

1. (optional) Choose a portion of sequence (e.g., by clicking on a feature). Otherwise, the entire sequence will be used.
2. Use the options in the **Sequence > New Sequence** sub-menu to create a new sequence project that uses the selection (or the entire sequence) as a starting point.



* Note: To activate all of the options in the sub-menu, you must first select all or part of the open sequence.

Menu option	Description
-------------	-------------

Translate	To open the translation of the currently selected nucleotide sequence as a new, unnamed SeqBuilder protein project. The currently selected genetic code is used in the translation. If the range of sequence to be translated was selected by clicking on a feature, then the /codon_start qualifier for that feature will be used to determine the starting frame of the translation, and the feature name will be used to determine the name of the translated sequence. For information on using translations with features, see Edit information about a feature .
Back Translate	To open the back-translation of the currently selected protein sequence as a new, unnamed SeqBuilder Pro nucleotide project. The currently selected genetic code is used in the back translation.
Forward	To open a copy of the currently selected nucleotide sequence as a new, untitled SeqBuilder Project.
Reverse	To open the reverse of the currently selected nucleotide sequence in a new, untitled SeqBuilder Pro window.
Reverse Complement	To open the reverse complement of the currently selected nucleotide sequence in a new, untitled SeqBuilder Pro window.
New Origin	To open a copy of a circular nucleotide sequence in a new, untitled SeqBuilder Project, but with the current position or selection as the origin. Warning: If the sequence is linear, this command will append upstream nucleotides to the downstream end, thereby yielding an incorrect result.

Additional topics of interest:

- [IUPAC codes](#)
- [Use editing commands](#)
- [Select an object or range](#)
- [Go to a location on a sequence](#)

IUPAC codes

IUPAC codes for amino acids:

1-Letter Code	3-Letter Code	Amino Acid
A	Ala	Alanine
B	Asx	Aspartic Acid or Asparagine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
J	Xle	Leucine or Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
O	Pyl	Pyrrolysine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
U	Sec	Selenocysteine
V	Val	Valine
W	Trp	Tryptophan
X	Xaa	Unspecified or unknown
Y	Tyr	Tyrosine

Z	Glx	Glutamic Acid or Glutamine
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IUPAC codes for nucleotides:

Symbol	Description	Bases Represented			
A	adenosine/adenine	A			
C	cytidine/cytosine		C		
G	guanosine/guanine			G	
T	thymidine/thymine				T
U	uridine/uracil				U
W	weak	A			T
S	strong		C	G	
M	amino	A	C		
K	keto			G	T
R	purine	A		G	
Y	pyrimidine		C		T
B	not A		C	G	T
D	not C	A		G	T
H	not G	A	C		T
V	not T	A	C	G	
N or -	any base (not a gap)	A	C	G	T



The non-standard nucleotide codes “I” (Inosine) or “X” (xanthine) are not supported.

Use editing commands

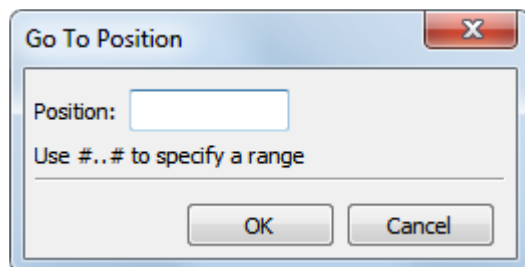
The following editing commands can be used in multiple views, panels and dialogs.

Task	How to...
To remove the current selection from the project and place it on the clipboard	<p>Choose Edit > Cut or use keyboard shortcut Ctrl/Cmd+X. Among other things, you can cut range and text selections, comments in the Comment view, features in the Features view and translation rulers.</p> <p>You can also use Edit > Select All within the Sequence, Circular, Linear, or Minimap and then use Edit > Cut to remove the contents.</p> <p>Note: Cut data does not remain strand-specific. If you copy data from a forward strand, you may copy it to the reverse strand and vice versa.</p>
To paste the contents of the clipboard into an insertion point	Choose Edit > Paste or use the keyboard shortcut Ctrl/Cmd+V .
To delete the selection from the project without placing the contents in the clipboard	Choose Edit > Delete or press the Delete key
To select the entire contents of the active window	Choose Edit > Select All or use the keyboard shortcut Ctrl/Cmd+A . Neither option is available within the Features view.
To reverse the effect of the previous operation(s)	Choose Edit > Undo or the keyboard shortcut Ctrl/Cmd+Z . You may use these options multiple times to undo multiple actions.
To reverse the effect of the last Edit > Undo command	Choose Edit > Redo or the keyboard shortcut Ctrl/Cmd+Shift+Z . You may use these options multiple times to redo multiple actions.

Go to a location on a sequence

To go to a numerical position or select a range on the sequence:

1. Choose **Edit > Go To Position** or use the keyboard shortcut **Ctrl/Cmd+J**.



2. Type the desired position or range into the box in the dialog. To specify a range, type in the left coordinate, then two periods (..), then the right coordinate. For example, to include the range from 35-141, type **35..141** into the text box. The “hint” uses number symbols (#) to show the format for entering a range. However, when you enter the range, you must substitute an integer for each number symbol.

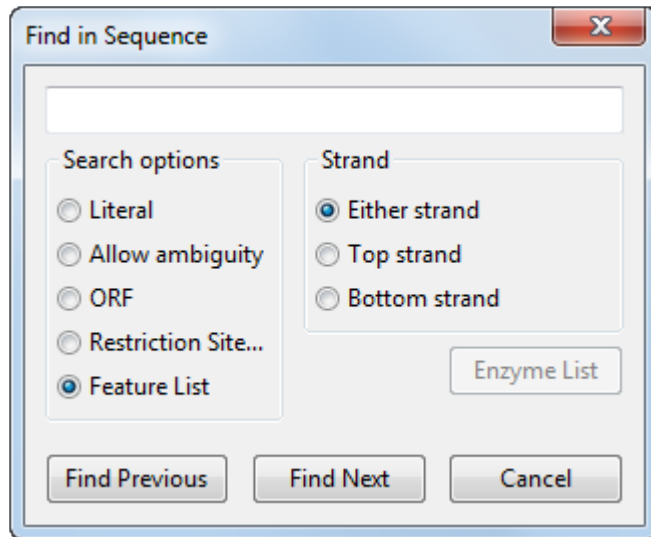
Inclusive ranges for the forward strand are typed with the symbol > or < or .. (two dots) between the two positions (e.g., 2500 > 3000). Regardless of the symbol used, sequence selection is always on the forward strand.

3. Click **OK**.

SeqBuilder Pro positions the cursor at the specified numerical position or selects (highlights) the specified range. If you enter a number that is outside the range of the sequence, you will receive the message “Error: out of bounds.”

To search for a text string in a sequence:

1. Choose **Edit > Find** or the keyboard shortcut **Ctrl/Cmd+F**.



You can use this dialog to locate a sequence string (including sequence from a primer), an ORF, a restriction site, or the text for any qualifier field in a feature.

2. Specify one of the following search options:

- - **Literal** or **Allow Ambiguity** (DNA or protein) searches for a string like GCTTTAG of up to 256 characters in length. Enter the search string into the text box. When using **Literal**, searching for the string ACNC will only find the exact match, ACNC. When using **Allow Ambiguity** searching for the string ACNC will find ACTC, ACCC, ACGC, ACAC, or any other ambiguous base.

✿ If you selected **Allow Ambiguity**, one or more X's added *before* the search term act as wildcards for an equal number of bases. One or more X's added *after* the search term act as wildcards for an equal number of bases minus one. (e.g. XXGAT will return 5-base matches; GATXX will return 4-base matches).

✿ When performing an ambiguous search with an 'N' on the 3' end of the search query, searches will find matches, but the match selection will not include the base corresponding to the 3' N in the search. Example: An ambiguous search for NGCN will find NGCNs located anywhere on the sequence, but only the part of the match corresponding to NGC will be selected.

- - **ORF** (DNA only) searches for open reading frames based on the ORF criteria.
 - **Restriction Site** (DNA only) searches for a restriction site. When selected, the **Select Enzyme** dialog will appear. Choose a site to search for, and then click **Done** to return to the **Find in Sequence** dialog.
 - **Feature List** searches the qualifier fields for all features in the [Features view](#) for the specified search string. This option also applies to primer searches.

3. Choose the strand to search using the Strand options, which are only enabled for nucleotide sequences. Choices are normally **Forward**, **Reverse**, or **Either strand**. When using **ORF**, you may search either the **Forward** or **Reverse** strand.
4. Click **Find Next** to search right or **Find Previous** to search left.

Once the **Find in Sequence** dialog is closed, select **Edit > Find Again** or use the keyboard shortcut **Ctrl/Cmd+G** to continue the search based on the last criteria entered.

Create Sequences Based on Other Sequences (Batch Editing)

To create new sequences related to locally-stored sequences

SeqBuilder Pro 15.1 and later support batch sequence editing. See the following topics to learn how to create new sequences:

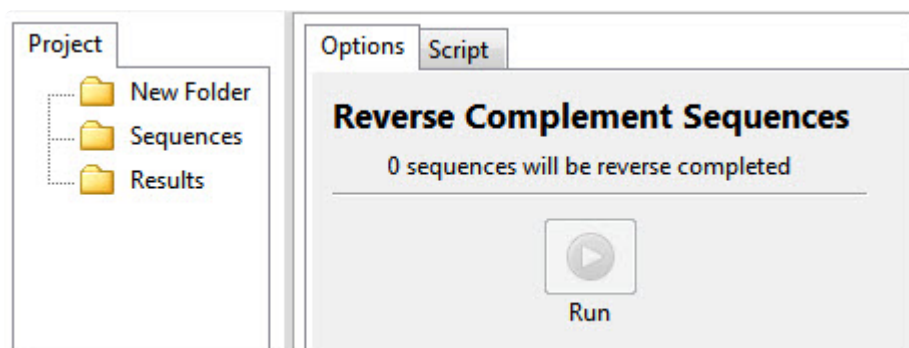
- ...by [reverse complementing one or more sequences](#)
- ...by [translating one or more sequences](#)
- ...by [joining multiple sequences together](#)
- ...from [specified features in another sequence](#)
- ...by [exporting sequences into a single file](#)
- ...by [sampling \(filtering\) a group of sequences](#) to choose ones of special interest

An efficient way to group your sequences in preparation for batch editing is to first open the sequences of interest, then use the command **File > Collect Open Sequences into New Project**. This puts all open sequences into one Project File. This makes it very easy to select the files when using the batch editing commands.

Create reverse complements of multiple sequences

To reverse complement one or more sequences:

1. Choose **Tools > Batch Reverse Complement**. The [Project window](#) opens with the Project and Options tabs active.

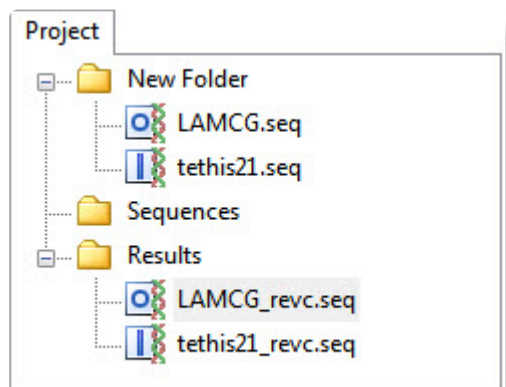


2. In the Project tab, the sequences you wish to reverse complement should be placed in the **Sequences** folder. To add sequences to a folder, select the folder, then right-click it and choose **Import**. Select the desired sequence files, and press **Open**. Next, select a single sequence, or use **Shift+click** or **Ctrl/Cmd+click** to select multiple sequences.

In the Options tab, the number of sequences selected on the left appears in the message: “‘n’ sequences will be reverse complemented.’

3. In the Options tab, press the **Run** button.

The new sequence(s) appear in the Project tab’s **Results** folder.



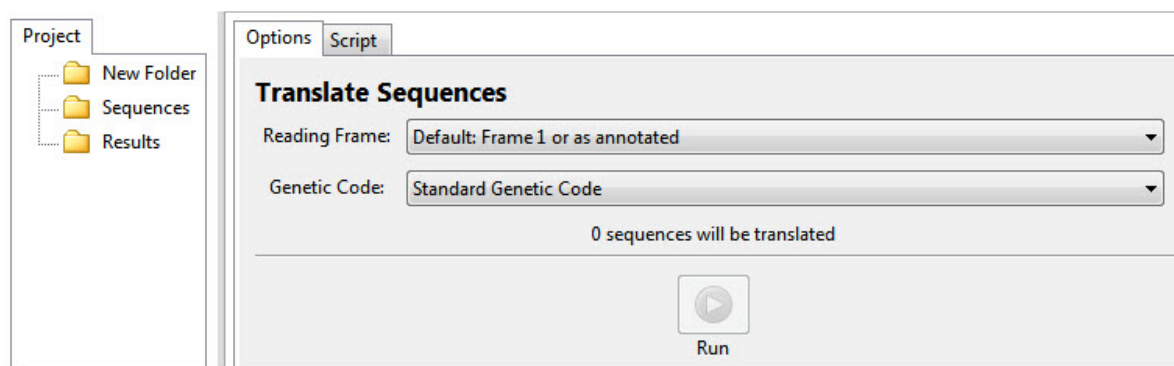
4. (optional) If you wish to see the script that was used to run this process, press the Script tab.

5. (optional) If you want to see the history of the run, including the location of the output file(s), press the History tab.

Create translations of multiple sequences

To translate one or more nucleic acid sequences into protein sequences using a specified translation table:

1. Choose **Tools > Batch Translate**. The [Project window](#) opens with the Project and Options tabs active.



2. In the Project tab, the sequences you wish to translate should be placed in the **Sequences** folder. To add sequences to a folder, select the folder, then right-click it and choose **Import**. Select the desired sequence files, and press **Open**. Next, select a single sequence, or use **Shift+click** or **Ctrl/Cmd+click** to select multiple sequences.

In the Options tab, the number of sequences selected on the left appears in the message: “n’ sequences will be translated.”

3. In the Options tab, use the **Reading Frame** drop-down menu to choose the reading frame for the translation.
 - **Default: 1 or as annotated** – Honor any existing /codon_start annotations in the input file(s); or otherwise, to begin at the first base.
 - **Ignore ‘/codon_start’ annotations** – Begin translating at the first base and to ignore any /codon_start sequence annotations. Note that this selection is equivalent to the **Default: 1 or as annotated** selection (above) for any input sequence other than a .gbk or .seq file with /codon_start qualifiers.
 - **1, 2 and 3** – Begin the translation at the first, second or third base, respectively, ignoring any /codon_start annotations. For an input sequence containing /codon_start qualifiers, selecting the start codon implicitly is similar to choosing **Ignore ‘/codon_start’ annotations**, but allows a specific frame to be set.
4. In the **Genetic Code** drop-down menu, choose a code for the translation. All NCBI and Lasergene tables are available, and are denoted by number and/or description. By default, the translation uses

the genetic code identified in the source data (e.g., the GenBank /transl_table qualifier), if available; otherwise, it uses the standard genetic code (NCBI:1).

5. Press the **Run** button.

If an input sequence was annotated, each CDS feature is translated separately, and any translation table and/or codon_start annotations are honored. If the input file was not annotated, or did not contain CDS features, the entire sequence is translated. Note that translation halts when there are fewer than 3 bases left. An “extra” base or two at the end will not be reflected in the output.

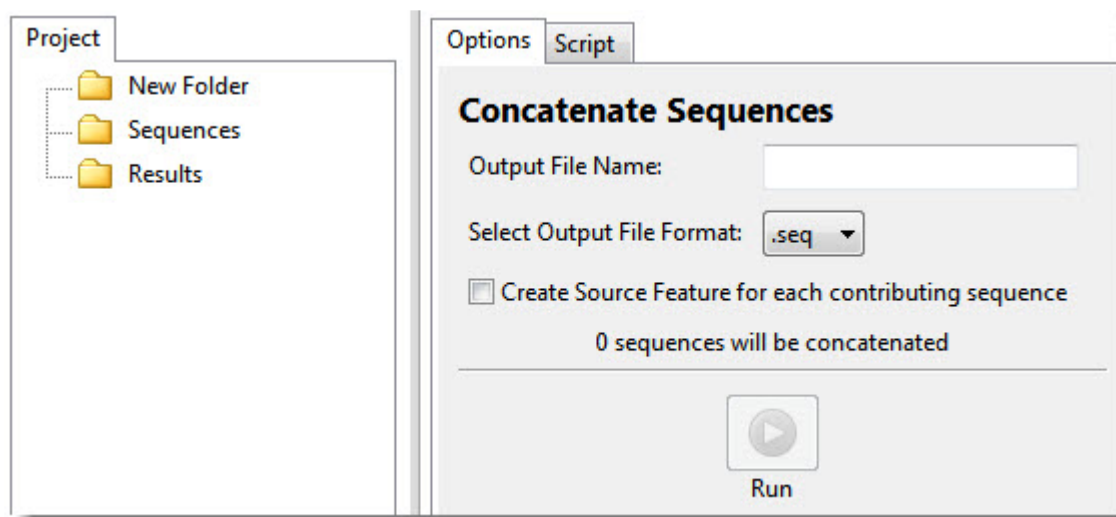
The new sequence(s) appear in the Project tab's **Results** folder.

6. (optional) If you wish to see the script that was used to run this process, press the Script tab.
7. (optional) If you want to see the history of the run, including the location of the output file(s), press the History tab.

Create one sequence from multiple sequences

To join two or more sequences end-to-end:

1. Choose **Tools > Concatenate**. The [Project window](#) opens with the Project and Options tabs active.



2. In the Project tab place the sequences you wish to join into the **Sequences** folder. To add sequences to a folder, select the folder, then right-click it and choose **Import**. Select the desired sequence files, and press **Open**. Use **Shift+click** or **Ctrl/Cmd+click** to select two or more sequences.

In the Options tab on the right, the number of sequences selected on the left appears in the message: “n’ sequences will be concatenated.’

3. In the Options tab, type a name (and extension, if desired) in the **Output File Name** text box.
4. Select an output file format from the drop-down menu. Choices include *.gbk* (preserves annotations) or *.fas* (no annotations).
5. If desired, check the **Create Source Feature for each contributing sequence** box. Checking this box creates a “source” feature for each sequence that contributes to the concatenated sequence. This can assist you in keeping track of where each sequence originated.
6. Press the **Run** button.

The new sequence appears in the Project tab’s **Results** folder under the name specified. No file extension is appended to the name unless one was specified in the **Output File Name** box.

7. (optional) If you wish to see the script that was used to run this process, press the Script tab.

8. (optional) If you want to see the history of the run, including the location of the output file(s), press the History tab.

Example:

Input sequence 1: GGAGTCTCCC

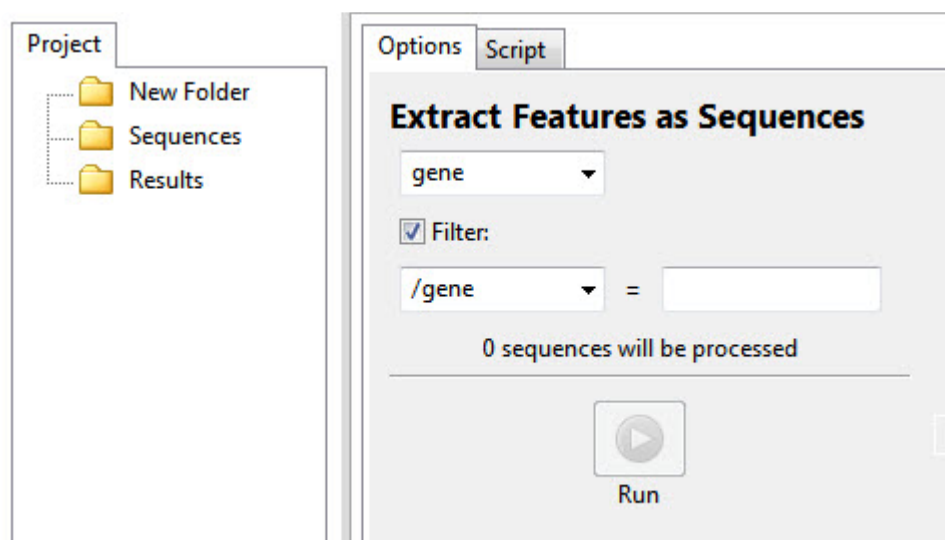
Input sequence 2: ATTGATTACA

Output sequence: GGAGTCTCCCATTGATTACA

Create sequences from features in another sequence

To create new sequences from existing sequence features:

1. Choose **Tools > Extract Features as Sequences**. The [Project window](#) opens with the Project and Options tabs active.



2. In the Project tab, place the sequences whose features you wish to extract into the **Sequences** folder. To add sequences to a folder, select the folder, then right-click it and choose **Import**. Select the desired sequence files, and press **Open**. Use **Shift+click** or **Ctrl/Cmd+click** to select two or more sequences.

In the Options tab on the right, the number of sequences selected on the left appears in the message: “‘n’ sequences will be processed.’

3. In the Options tab, use the upper drop-down menu to choose the type of feature to extract (e.g., **CDS**, **mRNA**, etc.)
4. If you wish to further limit the search to features that contain particular qualifiers, check the **Filter** box and use the lower drop-down menu to choose a qualifier. To the right, enter the text that the qualifier must match (e.g., */gene = thrL*). You may use wildcards in this box if you wish (e.g., */gene = thr**)
5. Press the **Run** button.

The extracted features appear as individual sequences in the Project tab’s **Results** folder. Sequences are named automatically according to a contained qualifier (e.g., */protein_id*, */locus*, or */accession*).

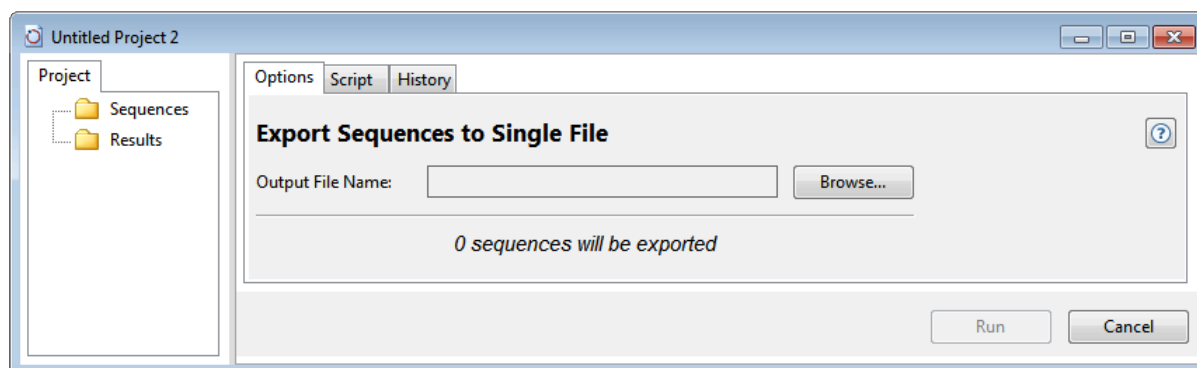
6. (optional) If you wish to see the script that was used to run this process, press the Script tab.

7. (optional) If you want to see the history of the run, including the location of the output file(s), press the History tab.

Export sequences into a single file

To create a single multi-sequence file composed of exported sequences:

1. Choose **Tools > Export Sequences into Single File**. The [Project window](#) opens with the Project and Options tabs active.



2. In the Project tab, place the sequences you wish to export into the **Sequences** folder. To add sequences to a folder, select the folder, then right-click it and choose **Import**. Select the desired sequence files, and press **Open**. Use **Shift+click** or **Ctrl/Cmd+click** to select two or more sequences.

In the Options tab on the right, the number of sequences selected on the left appears in the message: “‘n’ sequences will be exported.’

3. In the Options tab, click the **Browse** button. In the ensuing dialog, navigate to the location for the output file. Type in a name and select an extension (.fas or .gbk), then click **Save**. The **Output File Name** text box now contains the name and file path where the output file will be saved.
4. Press the **Run** button.

The new multi-sequence file appears in the Project tab’s **Results** folder with the name chosen above. The new file is represented by a folder icon and its contained sequences are named automatically according to a qualifier (e.g., /protein_id, /locus, or /accession).

5. (optional) If you wish to see the script that was used to run this process, press the Script tab.
6. (optional) If you want to see the history of the run, including the location of the output file(s), press the History tab.

Create a file by sampling sequences

The **Tools > Sample By** command is used to create an output file that contains a filtered set of sequences from a source file. Source file sequences can be filtered according to one or more specified conditions, such as length, contents, and start/end sequence characters.

To create a sequence by sampling other sequences:

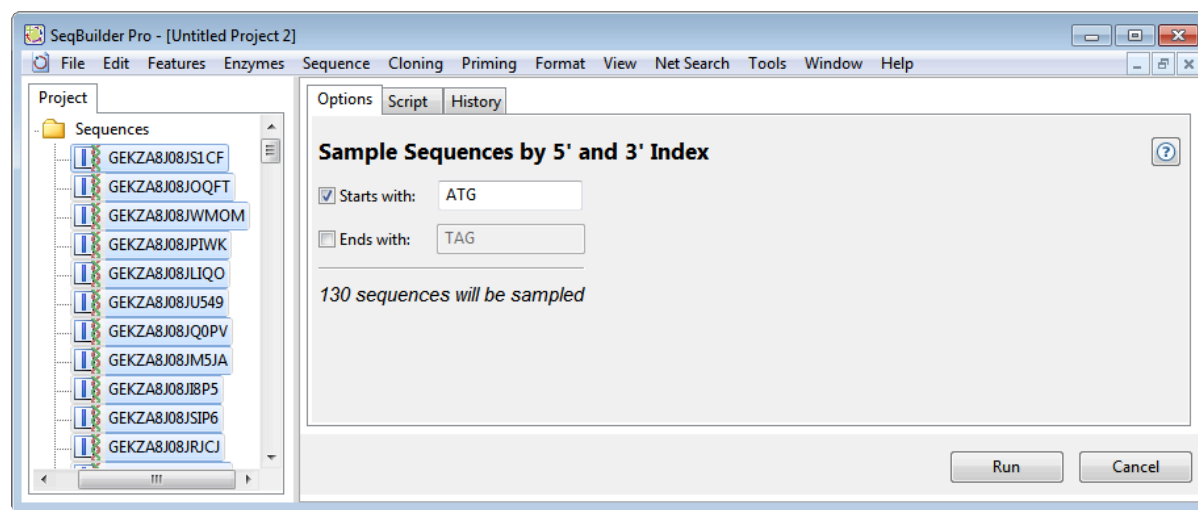
1. Choose **Tools > Sample By > (Subcommand)**. Available subcommands are: **5' and 3' End Tags**, **Contains Sequence**, **Probability or Skipping**, **Length**, and **Name**.

The [Project window](#) opens with the Project and Options tabs active.

2. In the Project tab, the sequences you wish to sample should be placed in the **Sequences** folder. To add sequences to a folder, select the folder, then right-click it and choose **Import**. Select the desired sequence files, and press **Open**. Next, select a single sequence, or use **Shift+click** or **Ctrl/Cmd+click** to select multiple sequences.
3. The Options tab varies in appearance, depending on which subcommand was chosen. The number of sequences selected in the Project tab appears in the message: “n’ sequences will be sampled.”
4. Change settings in the Options tab as applicable for the chosen subcommand.

5' and 3' End Tags:

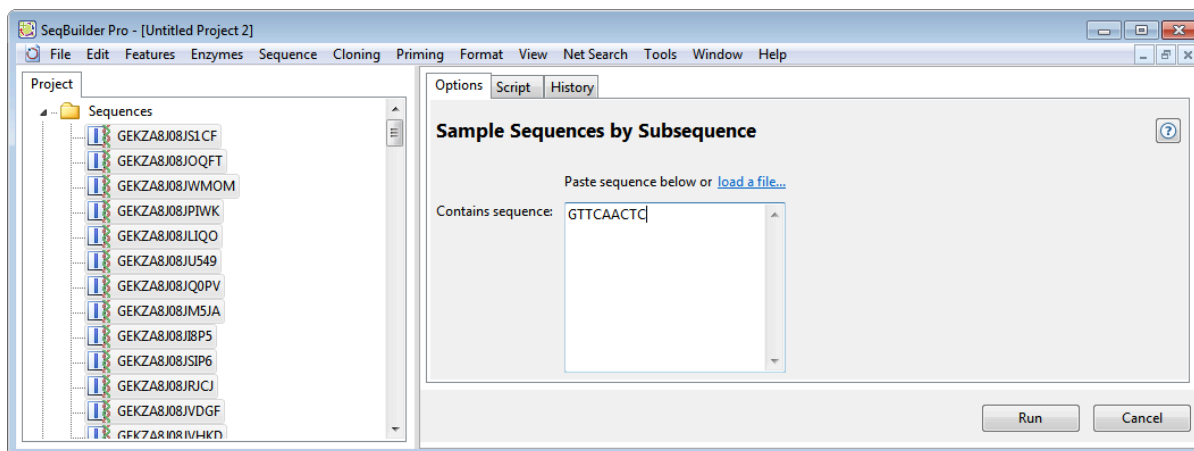
Tools > Sample By > 5' and 3' End Tags samples only those sequences beginning and/or ending with a specified sequence fragment or “index tag” on the 3' or 5' end. For sequences of DNA or unknown type, matches can occur at the ends of either strand.



Check the **Starts with** and/or **Ends with** boxes, then enter the search strand(s) to the right of the checked box(es).

Contains Sequence:

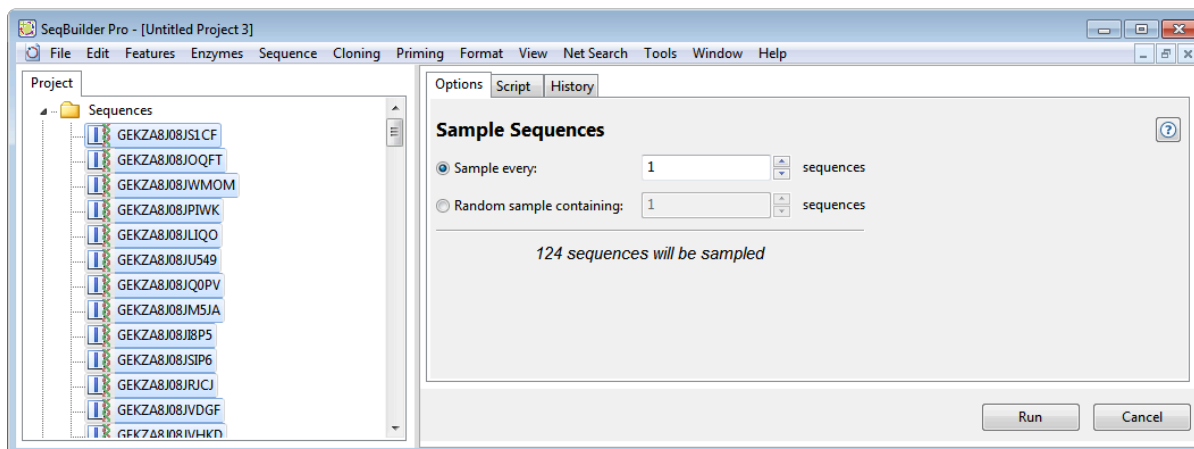
Tools > Sample By > Contains Sequence samples only those sequences containing a specified sequence fragment. This provides a way to sample a set of sequences that contain the same subsequence. For sequences of DNA or unknown type, matches can occur on either strand.



Either type the subsequence or paste copied sequence into the text field, or use the “load a file” link to navigate to a sequence or text file.

Probability or Skipping:

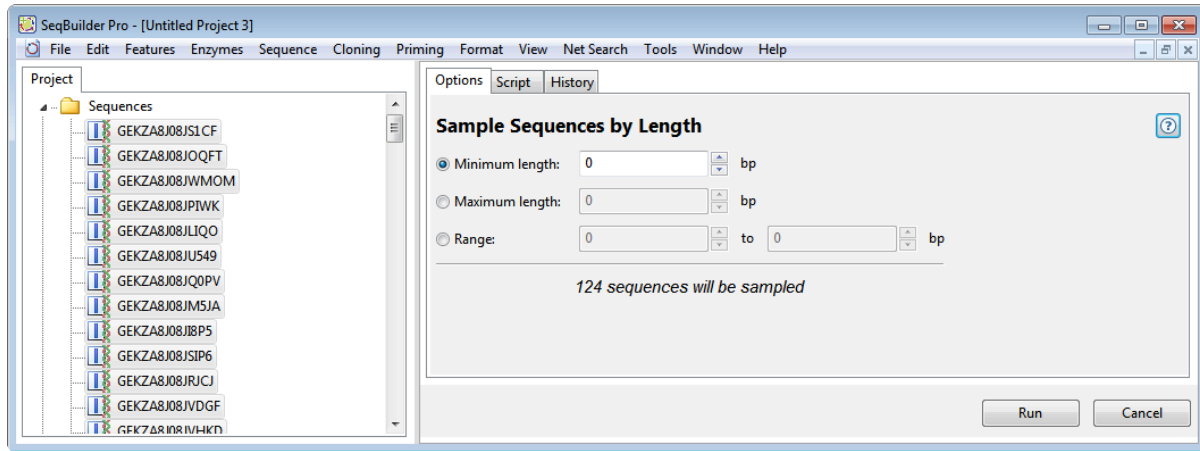
Tools > Sample By > Probability or Skipping samples a random subset of sequences.



To choose every n^{th} sequence from the file, select **Sample every** and type the ‘n’ value (an integer) at the right. To sample a certain number of random sequences instead, select **Random sample containing** and type the number of sequences at the right.

Length:

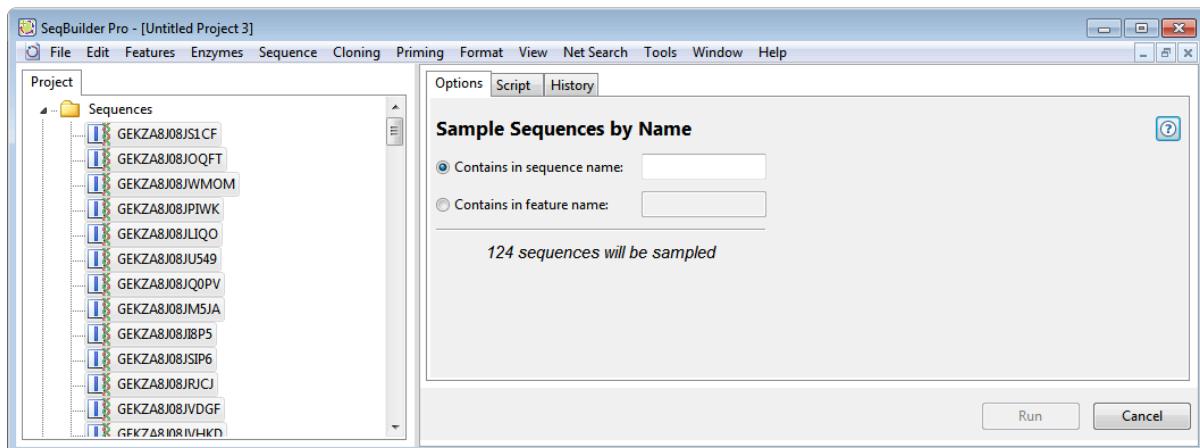
Tools > Sample By > Length samples only those sequences with a length greater and/or less than a specified length.



To specify a length threshold for sampled sequences, choose between **Minimum length**, **Maximum length** and **Range**. Then type the cutoff value(s) into the box at right.

Name:

Tools > Sample By > Name samples only sequences with the specified name.



Choose whether sampled sequences need to have the specified text in the **sequence name** or a **feature name**. Type in the text to the right of the selected option.

5. In the Options tab, press the **Run** button.

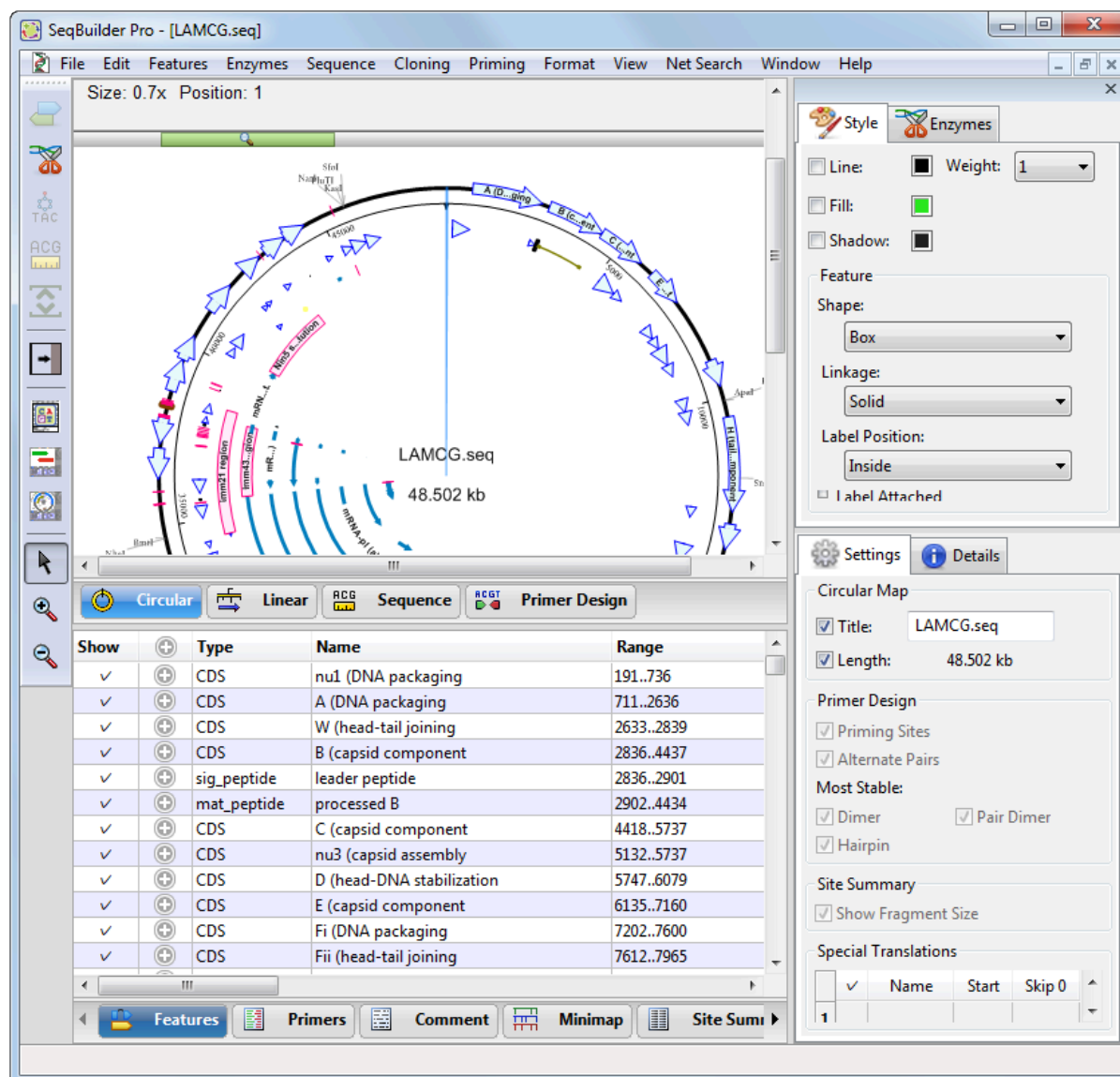
The resulting sequence(s) appear in the Project tab's **Results** folder. The results file has the same name as first sequence file that was sampled, followed by "_sampled."

6. (optional) If you wish to see the script that was used to run this process, press the Script tab.
7. (optional) If you want to see the history of the run, including the location of the output file(s), press the History tab.

Windows, Views, Panels and Tools

The Document window opens when you open a nucleotide or protein sequence or a SeqBuilder (.sbd) document. This is the window where you can [edit the sequence](#) or add/edit [features](#), [restriction enzymes](#), [ORFs](#), [translations](#) and/or [rulers](#) to the sequence.

- On the left of the window is a group of [shared button tools](#).
- The main/center portion of the window contains two view panes, each showing a different [view](#). Depending on the active sequence, views may include: [Circular](#), [Linear](#), [Sequence](#), and [Primer Design](#), [Features](#), [Primers](#), [Comment](#), [Minimap](#), [Site Summary](#) or [History](#).
- The right side of the window consists of four [panels](#): [Style](#), [Enzymes](#), [Settings](#) and [Details](#).



The following video is a general overview of the SeqBuilder Pro windows and panels:

Document window views

The following video contains a short tour of each of the Document window views:

To change from one view to another:

Click the tab with the name of the desired view.



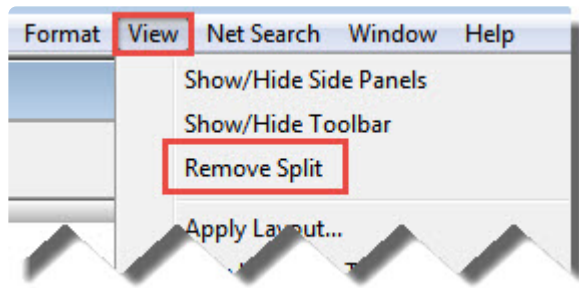
By default, the upper portion of the window lets you choose any of 1-4 views, depending whether the sequence is nucleotide or protein. Views may include: [Circular](#), [Linear](#), [Sequence](#), and [Primer Design](#). The default view is Circular or Linear, depending on the sequence type. The lower portion of the window lets you choose any of 2-6 views, also depending on the sequence type. Views may include: [Features](#), [Primers](#), [Comment](#), [Minimap](#), [Site Summary](#) and [History](#).

To display a “lower pane” view on the upper pane, or vice versa:


Use the mouse to drag and drop the view tab at the new location.

To toggle between displaying one or two views:

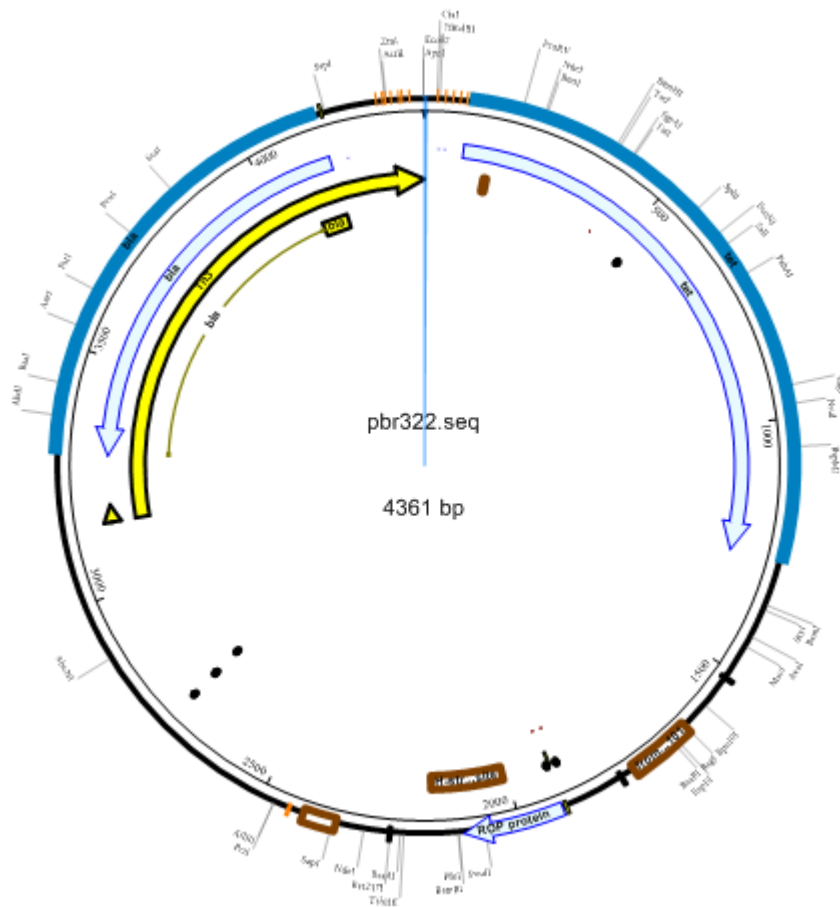
Use **View > Split** or **View > Remove Split**.




Circular view

The Circular view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Circular** tab ( **Circular**).

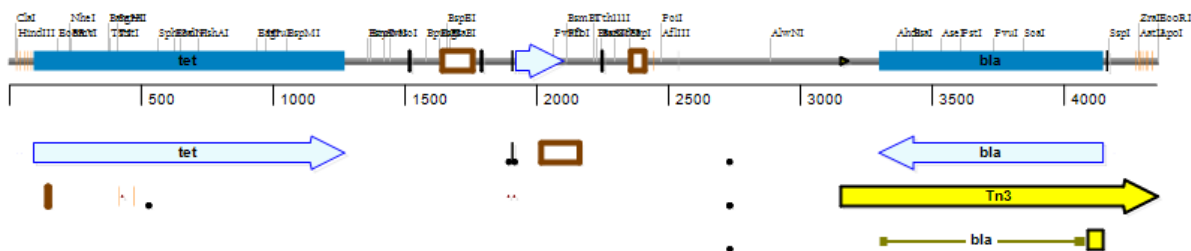
The Circular view is only available for nucleotide sequences, and displays a circular map of the current sequence. The default view shows any restriction enzymes on the outside of the circular map and features on the inside, with title and sequence length in the center.




Linear view

The Linear view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Linear** tab ( **Linear**).

The Linear view is only available for nucleotide sequences, and displays a linear map of the current sequence. The default view shows restriction sites above and feature graphics below the linear map. If you attempt to view a circular sequence in the Linear view, note that ORFs and restriction sites that cross the origin will be impacted.

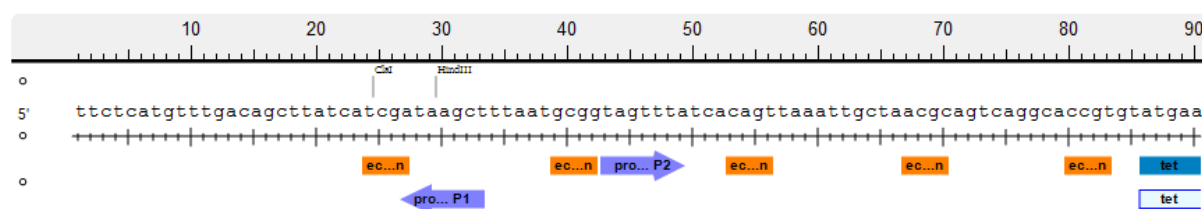


Sequence view

The Sequence view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Sequence** tab ( **Sequence**).


The Sequence view displays the detailed, residue-level sequence display. By default, the display contains:

- For protein sequences – The protein sequence and a ruler.
- For nucleotide sequences – The top strand DNA sequence, a ruler, and any annotations except “Source.”

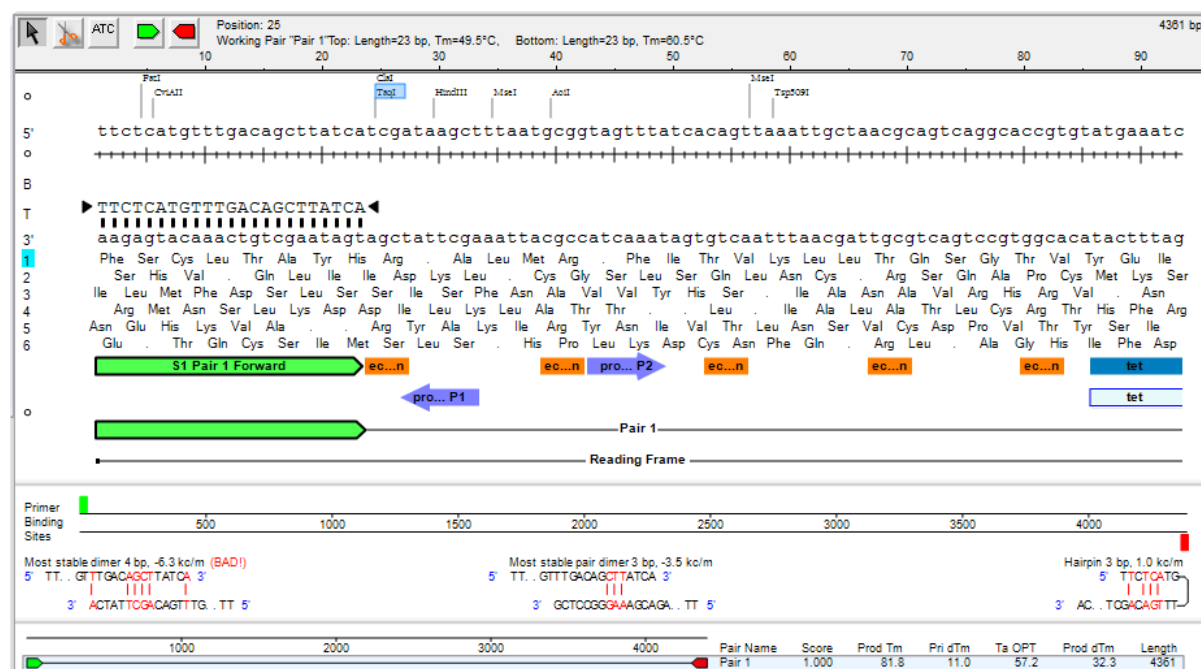


Primer Design view

The Primer Design view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. It also has its [own set of tools](#).

To make it the active view (or one of two active views), click on the **Primer Design** tab ( **Primer Design**).

The Primer Design view lets you design and analyze primers for a nucleotide sequence. Data in the Primer Design view appears in a header (featuring [tools](#) and details about the primers) and three distinct panes below that header. See [Work with Primers](#) for detailed information about performing tasks within this view.



Sequence details in the header:

Details about the primers, if any, are displayed in the header of the Primer Design view, just to the right of the tools.

Selection/ Position	The current position of the cursor. If a range is selected, the length of the range and strand (forward or reverse) is specified, along with the sequence coordinates.
Working Pair	The name of the currently selected primer pair.
Forward	The length and melting temperature of the forward primer. This value is updated automatically if settings are changed.

Reverse	The length and melting temperature of the reverse primer. This value is updated automatically if settings are changed.
Sequence Length	The total length of the sequence. This value is shown in the top right corner of the header.

Residue pane:

Displays the base level nucleotide sequence, restriction sites, translations, and features, as well as the currently selected primer sequence. This pane can be used to modify your primers.

Once you have done this, a green arrow represents the forward strand primer; red indicates the reverse strand primer. Primer sequences are displayed in 5' to 3' orientation, and include modifications that you have made. Edited nucleotides appear in lowercase.

Mispriming pane:

Shows primer binding sites, and the most stable conformation of dimers, pair dimers, and hairpins, if they exist. As the current primer pair is changed, the information displayed in the Mispriming Pane is updated automatically.

At the top of this pane, **Primer Binding Sites** displays where the primer binds to the template as well as any additional sites the primer may bind to on either strand. Any sites on the template matching at least the 12-mer at the 3' end of the primer are displayed. Primers on the top strand are indicated in green; primers on the reverse strand are shown in red.

Beneath **Primer Binding Sites**, the most stable conformation of dimers, pair dimers, and hairpins are shown, if they exist. To calculate this, SeqBuilder Pro locates secondary structure interactions using the nearest-neighbor model for all three types of duplexing. For self and pair dimer duplexing, oligonucleotide sequences are compared at every potential overlapping position by aligning sequences and advancing one residue at a time.

- **Most stable dimer** - Displays the primer binding to itself in the most stable conformation, if there is one.
- **Most stable pair dimer** - Displays the primer binding to the opposite strand primer in the most stable conformation, if there is one.
- **Most stable hairpin** - Displays the primer binding to itself by folding in the most stable conformation, if there is one.

✱ **Note:** If the word **BAD!** appears in red, it indicates that the final pentamer value of the dimer exceeds the **3' pentamer stability** threshold defined in the Primer Characteristics section of the [Create Primer Pairs](#) dialog. The **Ignore Duplexing n bp from 3' parameter** also affects duplexing. Avoid using primers with dimers or hairpins labeled “BAD!” unless you have experimental evidence that they will function as desired, or if you have no other option.






Alternate pairs pane:

Displays the list of primer pairs discovered by your search, along with additional information for each pair, including product length, melting temperatures, and a quality score. The following information is displayed in columns for each pair:

Name/Abbrev.	Description
Pair Name	The name of the primer pair.
Score	The quality score assigned to the primer pair.
Prod Tm	The melting temperature of the product.
Pri DTm	The difference in melting temperature between the primers in the pair.
Ta OPT	The optimal annealing temperature for the primer pair.
Prod dTm	The difference in melting temperature between the product and the lower primer Tm*. *
Length	The length of the product.

Primer Design tools

The following tools are located in the upper right of the [Primer Design view](#):

Tool	Description
Selection Mode 	To enable selection of a region of sequence using the mouse. After selecting this tool, click on a translated amino acid to select its codon. Click and hold on a restriction enzyme to show the cut sites, represented by thin blue lines. Click and hold on a restriction enzyme while holding down the Alt (Win) or Option (Mac) key to show the cut represented by bold black arrows, as well as the recognition sequence.
Introduce Restriction Site 	To introduce a restriction site into the sequence. After selecting this tool, click between the residues where the restriction site should be added, and choose an enzyme from the list provided. The restriction enzymes identified to cleave the sequence at your specified location will be organized into 3 groups: Silent substitutions , Non-silent substitutions , and Introduce a stop .
Introduce Codon Change/Mutation 	To introduce a mutation into your sequence. After selecting this tool, the cursor will highlight each codon as you hover over the sequence. Click the codon of interest to display a list of alternate codons available for a silent mutation, as well as a list of alternate amino acids available for non-silent mutations. Selecting an alternate amino acid will replace the sequence via the least disruptive alternate codon.
Show Top Primer Strand 	To change the focus of the Residue Pane to the top primer. The Mispriming Pane is updated automatically to reflect the associated primer.
Show Bottom Primer Strand 	To change the focus of the Residue Pane to the bottom primer. The Mispriming Pane is updated automatically to reflect the associated primer.

Quality score calculation


SeqBuilder Pro assigns a quality score for each primer pair it identifies. These scores are displayed in the Alternate Pairs pane of the [Primer Design view](#).

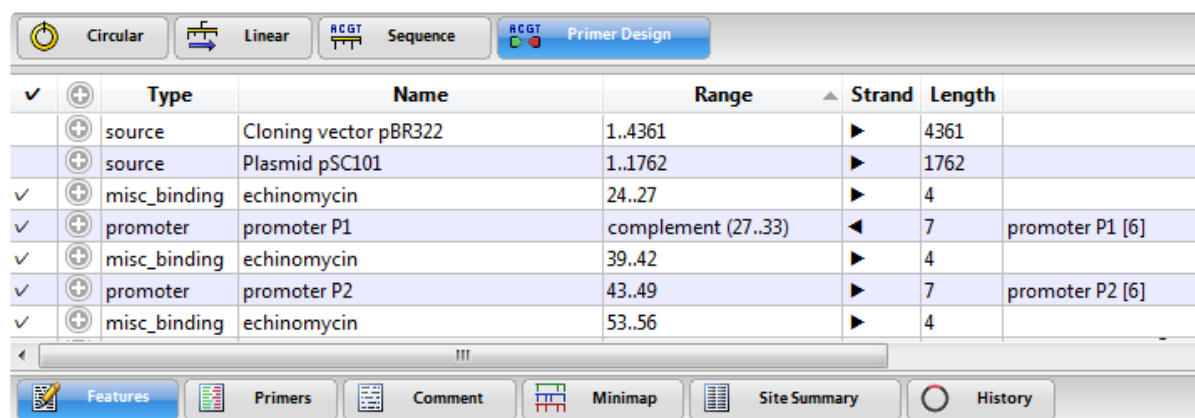
The following considerations are made when determining the primer pair quality score:

- The distance of each primer from its respective end.
- Each primer's melting temperature in relation to the Target T_m.
- The number of false priming sites for each primer.
- Whether or not a primer is located in a repeated region.
- Uniqueness at the 3' end of the primer.
- Internal repetitiveness of the primer.
- The stability profile of the primer.
- The length of most stable dimer.
- The length of most stable hairpin.

Each of the above characteristics is assigned a score. The individual scores are then combined and normalized to a range from 0 to 1, where 1 is the best possible score. The primer pairs are sorted from highest to lowest by score and displayed in the Alternate Pairs Pane.

Features view

The Features view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Features** tab ( **Features**).



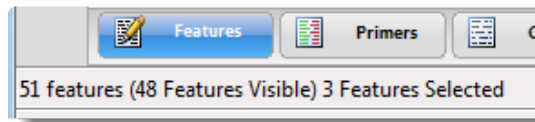
✓	+	Type	Name	Range	Strand	Length
	+	source	Cloning vector pBR322	1..4361	▶	4361
	+	source	Plasmid pSC101	1..1762	▶	1762
✓	+	misc_binding	echinomycin	24..27	▶	4
✓	+	promoter	promoter P1	complement (27..33)	◀	7
✓	+	misc_binding	echinomycin	39..42	▶	4
✓	+	promoter	promoter P2	43..49	▶	7
✓	+	misc_binding	echinomycin	53..56	▶	4

The Features view displays a table of features (annotations) that have previously been identified in the DNA or protein sequence. Features may be user-defined, or they may be part of the input file from GenBank or from other Lasergene applications. For information on creating, editing or deleting features, see [Features](#).



The following table describes how to perform various tasks within this view:

Task	How to...
To select one or more features in all graphical views	Single click on one feature row; or select multiple features using Ctrl/Cmd+click , Shift+click , or by dragging the mouse across the desired rows. Selected features are simultaneously selected in the Circular and Linear views.
To select the feature and its corresponding sequence, and to scroll to its location	Double-click on a compacted feature row or select the feature row and choose Feature > Select Sequence .
To expand/contract a feature row	Click a plus icon to expand the row and show details about the feature; click a minus icon to hide feature details. As a shortcut, you can use Ctrl/Cmd+Alt to toggle between expanding/contracting all rows.
To edit feature information	Expand a row, as described above. Insert your cursor in a field and type the desired information.
To sort data alphabetically within a column	Click on the column header.
To see the number of the features in total, the number	Look at the footer at the bottom left of the view.


visible, and the number
selected



Columns in the Features view are described below:

Column name	Description
	Rows with a checkmark in this column denote a feature that is currently visible in the graphical views (Circular, Linear, Sequence). To hide a feature, click its checkmark.
	See “To expand/contract a feature row” in the table above.
Type	The feature type.
Name	The feature display name.
Range	The range of the sequence included in the feature.
Strand	The strand where the feature is located (forward or reverse).
Length	The length of the feature in base pairs.
Description	Often the contents of the first /note qualifier.

Primers view

The Primers view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Primers** tab ( **Primers**).

✓	▲	Set Name	Name	F/R	Primer Sequence	Length	Tm	Ta OPT	dG	Score	Current
✓	▼	Set 1	Pair 1		<TTCTCATGTTTGACAGCTTATCA>..<CGAG 4361		Tm=81.8, dTm= TaOPT=57.2			1.000	
		Set 1	S1 Pair 1 Forv F		TTCTCATGTTTGACAGCTTATCA	23	49.5		-38.1		
		Set 1	S1 Pair 1 Rev R		TTCTTGAAGACGAAAGGGCCTCG	23	60.5		-46.6		
	▶	Set 2	Pair 1		<CGAGGCCCTTTCGTCTTCAA>..<CGAGG(23		Tm=51.9, dTm= TaOPT=38.5			1.000	✓
		Set 2	S2 Pair 1 Forv F		CGAGGCCCTTTCGTCTTCAA	20	56.6		-41.5		✓
		Set 2	S2 Pair 1 Rev R		TTCTTGAAGACGAAAGGGCCTCG	23	60.5		-46.6		✓

The Primers view is available for nucleotide sequence only, and displays tabular results from primer searches, including the primer sequence and its length, product (for primer pairs), melting temperature, and free energy (ΔG). For information on creating, editing or deleting primers and primer pairs, see [Work with Primers](#).

A checkmark next to a primer pair indicates that it is the currently selected pair. Select a row to also select that primer pair in the graphical views (Circular, Linear, Sequence). The table supports selection of groups of primer pairs using **Ctrl/Cmd+click**, **Shift+click**, or by dragging the mouse across the desired rows.


You can rename a primer by expanding its entry in this view (i.e. by clicking on the plus sign) and then clicking on a **Name** cell. Type in any desired name and press **Enter**.

The table below describes each of the columns in the Primers view.

Column	Description
Show	A checkmark in this field denotes a feature that is currently visible in the Primer Design view, as well as the other graphical views (Circular, Linear, Sequence). To hide a feature, click its checkmark.
Active	A bullet point in this field indicates that the pair is the currently selected pair in the current working set. The current pair is always displayed in the Primer Design view.
Set Name	The name of the set. One set is created for each primer search. By default, SeqBuilder Pro assigns a generic name, like Set 1, Set 2, etc. However, this field is editable so that you can assign a more descriptive name for the set of primers, if desired.
Pair Name	The name of the primer pair. By default, SeqBuilder Pro assigns a generic name, like Pair 1, Pair 2, etc. However, this field is editable so that you can assign a more descriptive name for your primer pair, if desired.
F/R	An F in this column indicates that the primer is found on the forward (top) strand of the template sequence; an R indicates that it is on the reverse (bottom) strand.

Primer Sequence	For primer pairs, the sequences are shown for both members of the pair, forward strand followed by reverse strand. For primers, the sequence of the primer is shown in the 5' to 3' orientation, and is editable. Changes made to the primer sequence are automatically reflected in the Primer Design view.
Length	For primer pairs, this value indicates the length (in bp) of the PCR product. For primers, it indicates the length (in bp) of the primer.
Tm	<p>For primers, the melting temperature of the primer is displayed. For primer pairs, 3 different values are listed:</p> <ul style="list-style-type: none"> * L: The length of the product. * Tm: The melting temperature of the product. * dTm: The melting temperature difference between the product and the lower primer Tm. * TaOPT: The optimal annealing temperature for the primer pair. * dG: The delta-G value, free energy, that is computed over of the whole primer. Note that the dG value is only calculated for individual primers, not primer pairs.


Comment view

The Comment view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Comment** tab ( **Comment**).

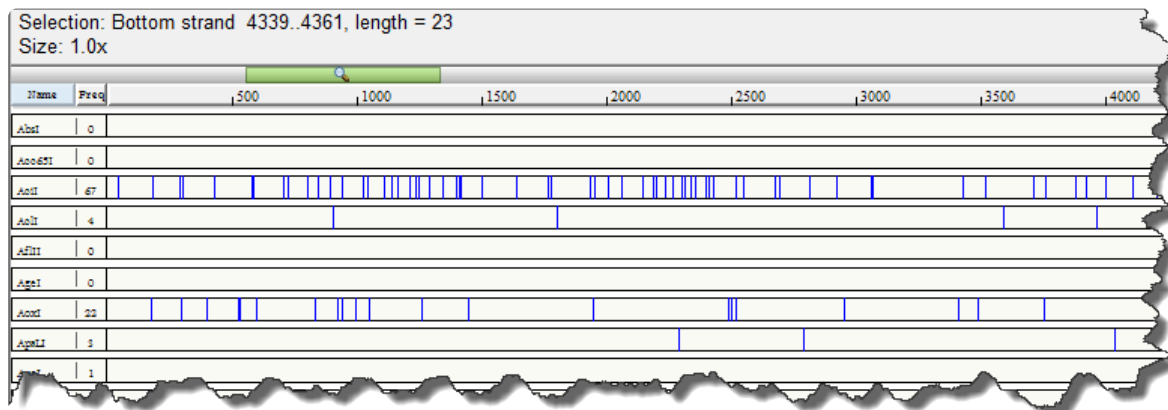
The Comment view displays any descriptive text associated with a sequence; for example, the header text of a GenBank entry. Comments are optional, and not all sequences have them. To edit a comment, insert the cursor anywhere and edit as desired. You may type in new text, delete existing text, or paste in text from the clipboard.

```
LOCUS      SYNpBR322          4361 bp      DNA      circular SYN 19-JUN-2002
DEFINITION Cloning vector pBR322, complete genome.
ACCESSION  J01749 K00005 L08654 M10282 M10283 M10286 M10356 M10784 M10785
           M10786 M33694 V01119
VERSION    J01749.1  GI:208958
KEYWORDS   ampicillin resistance; beta-lactamase; cloning vector; drug
           resistance protein; origin of replication; plasmid; tetracycline
           resistance.
SOURCE     Cloning vector pBR322
  ORGANISM Cloning vector pBR322
           artificial sequences; vectors.
REFERENCE  1  (bases 1 to 3; 3259 to 4361)
AUTHORS    Sutcliffe,J.G.
TITLE      Nucleotide sequence of the ampicillin resistance gene of
           Escherichia coli plasmid pBR322
JOURNAL    Proc. Natl. Acad. Sci. U.S.A. 75 (8), 3737-3741 (1978)
MEDLINE    79012484
```

Minimap view


The Minimap view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Minimap** tab ( **Minimap**).

The Minimap view is available only for nucleotide sequence and displays a smaller version of the current sequence. The Minimap is scaled to show the whole sequence in a single line. Each row in this view shows where a particular restriction site falls along the sequence.



Because the entire sequence is shown in a single line, the font size in this view tends to be quite small. To enlarge the font size, use the command **Format > Size > Larger** one or more times.






Site Summary view

The Site Summary view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **Site Summary** tab ( **Site Summary**).

The Site Summary view is only available for nucleotide sequences and displays a table of restriction sites and their locations on the sequence. This view shows any restriction sites that were chosen for display in this or another pane. The current position of the cursor appears in the upper left corner of the view.

Position: 190 4361 bp

Name	Freq	Position
Absent Sites	0	AarI, AbsI, Acc65I, AflII, AgeI, AjuI, AjuI', AleI, Aloi, Aloi', ApaI, ArsI, ArsI', AscI, AsiSI, AvrII, BglII, BlnI, BmgBI, BplI, BplI', BsaXI, BsaXI', BseRI, BsiWI, BsrGI, BssHII, BstBI, BstEII, BstXI, EcoICRI, Fall, Fall', FseI, HpaI, KpnI, MauBI, MfeI, MluI, MreI, MteI, NcoI, NotI, NsiI, PacI, PspXI, PstI, PstI', RsrII, SacI, SacII, SanDI, SbfI, SexAI, SfiI, SgrDI, SmaI, SnaBI, SpeI, SrfI, St
AatII	1	4289 (4361)
AclI	4	902 (899), 1801 (1792), 3593 (373), 3966 (1297)
AcuI	2	3022 (1012), 4034 (3349)
AfeI	4	235 (262), 497 (281), 778 (952), 1730 (2866)
AhdI	1	3367 (4361)
AlfI	6	1016 (34), 1050 (397), 1447 (34), 1481 (187), 1668 (34), 1702 (3675)
AlfI'	6	1016 (34), 1050 (397), 1447 (34), 1481 (187), 1668 (34), 1702 (3675)
AlwNI	1	2890 (4361)
ApaLI	3	2290 (498), 2788 (1246), 4034 (2617)
AseI	1	3539 (4361)
BamHI	1	376 (4361)
BbsI	3	731 (863), 1594 (2751), 4345 (747)
BcgI	3	732 (1355), 2087 (1785), 3872 (1221)
BcgI'	3	698 (1355), 2053 (1853), 3906 (1153)
BciVI	2	2677 (1527), 4204 (2834)
BdaI	8	656 (34), 690 (372), 1062 (34), 1096 (922), 2018 (34), 2052 (493), 2545 (34), 2579 (2438)


 Primers
  Comment
  Minimap
  Site Summary
  History

The table contains a row for each restriction enzyme currently applied to the sequence. Enzymes that have no cut sites all appear in a single row.

Column Name	Description
Name	Enzyme name
Frequency	Number of cutting sites
Position	Specific cutting locations for the enzyme. Use the Settings panel to specify whether to display or hide the fragment length in parentheses to the right of the enzyme position.

The default font size in this view tends to be quite small. To enlarge the font size, use the command **Format > Size > Larger** one or more times.

History view

The History view is one of the ten views available in the [Document window](#), and shares a sub-set of [button tools](#) with the other views. To make it the active view (or one of two active views), click on the **History** tab ().

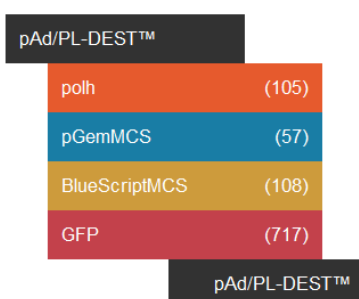
The History view is empty until one of the following happens:

- You [accept suggested annotation matches](#) in the [Annotation Results window](#). In this case, the view displays a list of the accepted annotations.
- You perform a cloning simulation and open any of the resulting files (i.e., entry clones, inserts, expression clones) in the SeqBuilder Pro Document window. In this case, the History view displays the Cloning Summary.

DNASTAR®
Software for life scientists

Cloning Summary

Fragments



Steps

- 1) Amplify polh using primers:
Top: 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGATATCATGGAGA-3'
Bottom: 5'-GGGGACAACCTTTGTATACAAAAGTTGTAATATTTATAGGTTTTTTTA-3'
- 2) Recombine step 1 with pDONR221-P1P5r
- 3) Amplify pGemMCS using primers:
Top: 5'-GGGGACAACCTTTGTATACAAAAGTTGGGTTAGGTAAGTTCGAGCTCGGTACCC-3'
Bottom: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTTGAGCTTCGATGCCTGCAGGTC-3'
- 4) Recombine step 3 with pDONR221-P5P4
- 5) Amplify BlueScriptMCS using primers:
Top: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTTAGGTACCGGGCCCCCTC-3'
Bottom: 5'-GGGGACAACCTTTGTATAATAAAGTTGGGTTGAGCTCCACCGCGGTGGC-3'
- 6) Recombine step 5 with pDONR221-P4rP3r
- 7) Amplify GFP using primers:
Top: 5'-GGGGACAACCTTTGTATAATAAAGTTGGGTTAATGAGTAAAGGAGAAGAACTT-3'
Bottom: 5'-GGGGACCACCTTTGTACAGAAAAGCTGGGTTCTATTTGTATAGTTTCATCCA-3'
- 8) Recombine step 7 with pDONR221-P3P2
- 9) Recombine steps 2, 4, 6, and 8 with pAd/PL-DEST™

Fragment Junctions

pAd/PL-DEST™-polh

```
S Q V C T K K Q A S V A X
H K F V Q K S R L Q L L
T S L Y K K A G F S C .
TCACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGA
V L K Y L F A P K L Q Q
C T Q V F F C A E T A S
. L N T C F L L S . N S X
```

polh-pGemMCS

```
. I L Q L C I Q K L L E F E
K Y Y N F V Y K S C . N S X
I N I T T L Y T K V V R I R X
ATAAATATACAACTTTGTATACAAAAGTTGTAgaattcgag
F I V V K Y V F T T L I R X
Y I N C S Q I C F N N S N S X
L Y . L K T Y L L Q . F E L
```

pGemMCS-BlueScriptMCS

```
C K L . Q L F S I Q S C R Y R X
A S F N N F F L Y K V V G T G
Q A L T T F F Y T K L S V P X
tgcaagcttTAACAACTTTTCTATACAAAAGTTGTCggtacc
C A K V V K K . V F N D T G P
L S . C S K E I C L Q R Y R X
A L K L L K K R Y L T T P V P X
```

BlueScriptMCS-GFP

```
G A Q P N C I I K L G . . V X
V E L N P T V . . S W V N E . X
W S S T Q L Y N K V G L M S K
gtggagctcAACCCAACTGTATATAAAGTTGGGTTAATGAGT
H L E V W S Y L L T P N I L L X
P A . G L Q I I F N P . H T F
S S L G V T Y Y L Q T L S Y X
```

GFP-pAd/PL-DEST™

```
T N R T Q L S C T K W Y H K
Q I E P S F L V Q S G I T S
Y K . N P A F L Y K V V S Q V
TACAAATAGAACCAGCTTTCTTGTACAAAAGTGGTATCACAAG
L Y F G A K K Y L T T D C T
V F L V W S E Q V F H Y . L N
C I S G L K R T C L P I V L
```

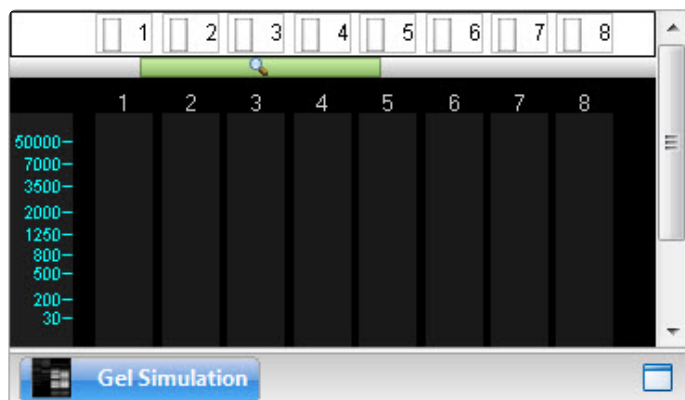
In the event that both of the above apply, the History view shows both cloning and annotation results. Newer

results are appended underneath earlier results.

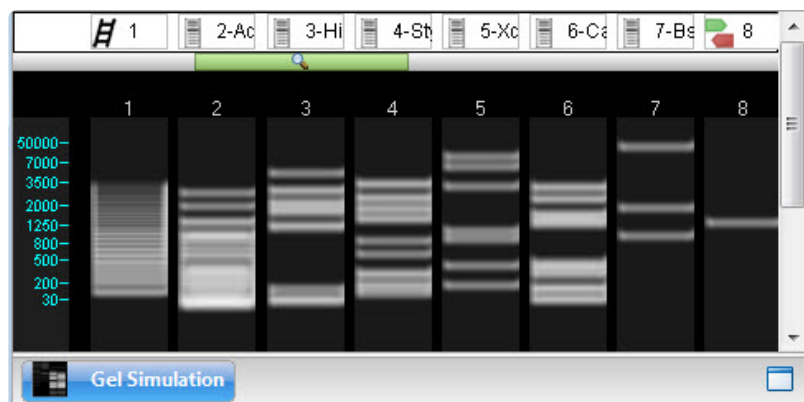
If you would like to remove all information from this view, choose **View > Clear History**. You will be prompted to confirm your decision.

Gel Simulation view

The Gel Simulation view is available in the [Document window](#) after using the **File > New Agarose Gel** command. By default, the view contains eight empty lanes and has a 10 cm width and 7 cm height., but these parameters can be changed as desired. The following image shows how the view appears before setting up a gel simulation. The view contains a ruler on the left, and lane numbers at the top of each empty lane and in the status bar (i.e., the header).



After setting up the gel simulation, the header shows the marker(s), enzyme(s) and/or primer(s) that have been applied to each lane. A ladder icon is used to represent a marker, a “gel” icon to represent an enzyme, and green/red primer arrows to represent a primer pair.



The following table shows tasks pertaining to the Gel Simulation view.

Task	How to...
Specify the number of lanes, size of the gel, percent agarose and run time	Use the Settings panel .
Apply markers, enzymes, and primer	See Agarose Gel Simulation .



pairs	
Select a lane	Click on the lane number header, or click anywhere in the lane.
View information about what has been applied to the lane	Hover the mouse over the status bar above that lane to reveal a tooltip.
View information about a band or fragment size notation	Hover the mouse over it to reveal a tooltip.
Select a fragment and its range on the sequence	Click on a band. The fragment is shown in red, and the footer is populated with information about the selection. Any fragment from the same sequence as the selected fragment, and which overlaps the selected fragment in any way, will appear in blue.

Fragment Sizes view

The Fragment Sizes view is available in the [Document window](#) after using the **File > New Agarose Gel** command. The view initially appears empty, or may contain one or more faintly outlined boxes.

After [setting up an agarose gel simulation](#), the view is populated with columns, each representing a lane in the [Gel Simulation view](#). Fragment lengths are shown in base pair (bp) units. The column headers show the marker(s), enzyme(s) and/or primer(s) that have been applied to each lane.

1 100 bp PCR Molecular Ruler	2 N.thermophilus_his AclI	3 N.thermophilus_his HinP1I	4 N.thermophilus_his StyI	5 N.thermophilus_his XcmI	6 N.thermophilus_his Cac8I	7 N.thermophilus_his BstXI	8 N.thermophilus_his S1 Pair 1 Forward S1 Pair 1 Reverse
3000 2900 2800 2700 2600 2500 2400 2300 2200 2100 2000 1900 1800 1700 1600 1500 1400 1300 1200 1100 1000 900 800 700 600 500 400 300 200 100	2588 1873 1345 1289 998 974 962 945 906 899 890 738 614 522 414 384 364 362 347 345 304 289 248 205 194 169 127 99 91 86 79 76 69 46 41 32 21 20 15 14 7 3 (2) 2 (2)	4458 2790 2736 2103 1987 1763 1615 1225 1134 119 51 11 9	3411 3174 2445 2212 1803 1732 1444 1373 813 591 322 280 178 143 80	8760 5628 3112 1076 860 402 163	3127 2921 2264 2218 1615 1512 1466 1279 1220 459 413 409 342 282 151 144 104 32 31 12	17260 1792 949	1284


Fragment Sizes


The following table shows tasks pertaining to the Fragment Sizes view.


Task	How to...
Select a lane	Click anywhere in the lane.
View information about what has been applied to the lane	Hover the mouse over the lane to reveal a tooltip.
Select a fragment	Click on a fragment size notation. The fragment is shown in red, and the footer is

and its range on the sequence	populated with information about the selection. Any fragment from the same sequence as the selected fragment, and which overlaps the selected fragment in any way, will appear in blue.
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Document window panels

The right side of the [Document window](#) consists of four panels: [Style](#), [Enzymes](#), [Settings](#) and [Details](#).

To display/hide the panels:

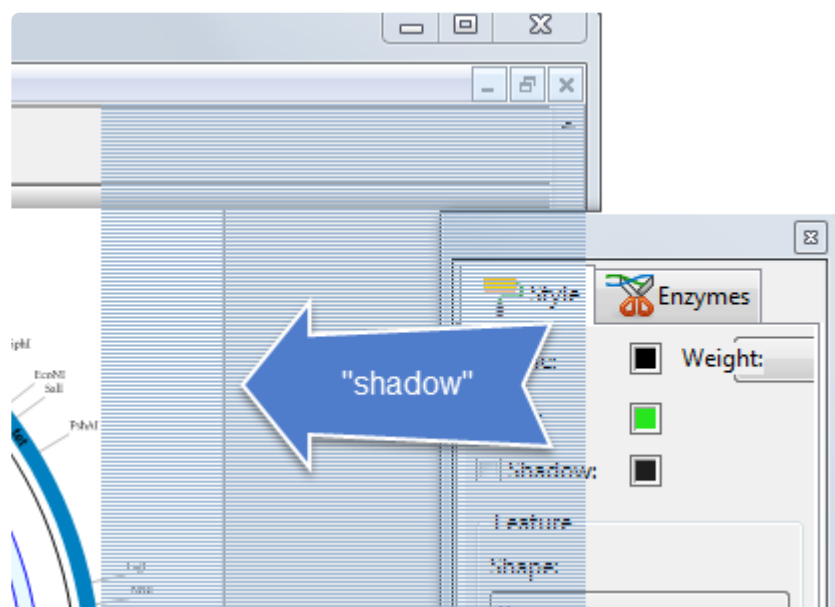
Choose **View > Show/Hide Side Panels** or use the **Show/Hide Settings** tool () on the left of the Document window.

To move a panel from the upper part to the lower part of the window, or vice versa:

Each panel has an tab labeled with its name. Drag the tab up or down using the mouse. When you see a “shadow,” drop the tab and the panel will snap into the new position.

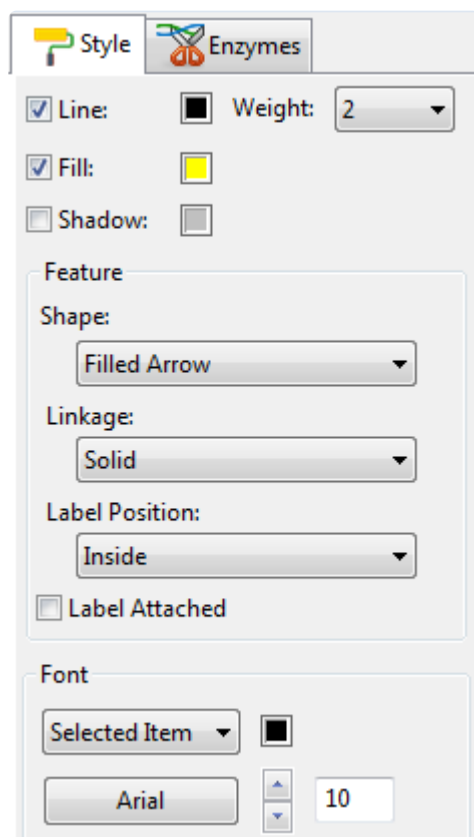
To undock and redock the panels:

To undock, use the mouse to hover at the top of the associated frame. When the cursor changes to a 4-pointed arrow, drag the frame to the desired position. To redock, follow the same procedure, moving the frame near its original position. When a “shadow” appears, as shown in the image below, drop the frame and it will snap into position




Style panel

The Style panel, represented by a paint roller icon (), provides the ability to change the appearance of a currently selected item in the [Document window](#).



Open the Style panel using any of these methods:

- Click on the **Style** tab to bring that panel to the front. If the tab is hidden, reveal it by clicking the **Show/Hide Settings Panel** tool () on the left of the Document window.
- Right-click on a feature and choose **Edit Feature Style** from the context menu.
- Select a feature and choose **Features > Edit Feature Style**.

To learn about functions that can be performed in this panel, see [Change the rendering style or font](#).

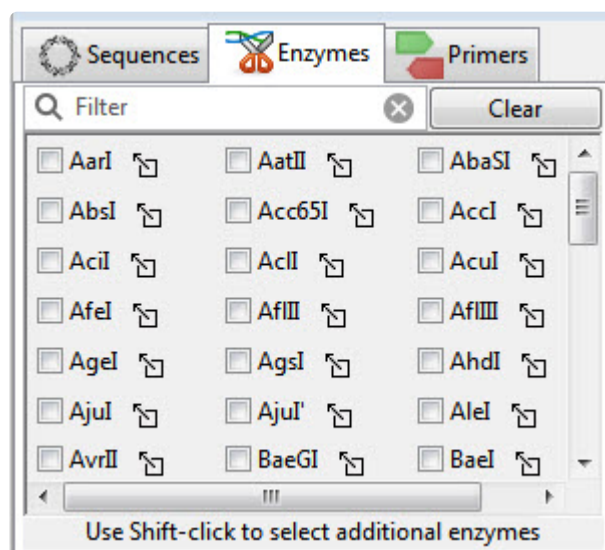
Enzymes panel

The Enzymes panel varies depending on which views are active. For detailed information, see:

- [Enzymes panel for agarose gel simulations](#)
- [Enzymes panel for other situations](#)

Enzymes panel for gels

A specialized version of the Enzymes panel becomes available after you use **File > New Agarose Gel** to initiate an [agarose gel simulation](#). It can be opened by clicking on the **Enzymes** tab, represented by a scissors icon (✂) and located to the right of the [Gel Simulation view](#).






This version of the panel differs from the [standard Enzymes panel](#) in three ways:

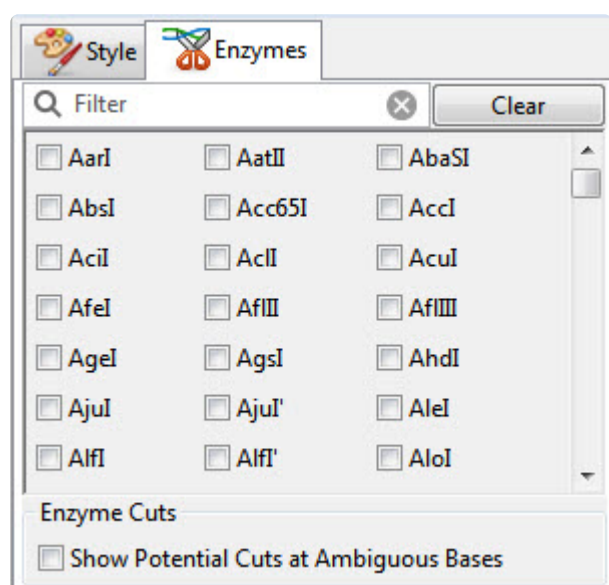
- A **Clear** button appears at the top right of the panel.
- A “drag & drop” icon is added to the right of each enzyme.
- There is no **Enzyme cuts** checkbox under the enzyme list.

For detailed information about using the specialized Enzymes panel to apply and remove enzymes from lanes in the Gel Simulation view, see [Apply or remove enzymes in the Gel Simulation view](#).

Enzymes panel for other situations

The Enzymes panel, represented by a scissors icon (), lets you choose which enzymes to display/hide in the following [Document window views](#) : [Circular](#), [Linear](#), [Sequence](#), [Primer Design](#), [Minimap](#) and [Site Summary](#). This panel is one of two ways to display/hide restriction enzymes. See [Enzymes](#) for in-depth information.

The Enzymes panel opens automatically when you click on the **Enzymes** tool () and choose **Pick Specific Enzymes**. It can also be opened manually by clicking on the **Enzymes** tab, located by default in the top right portion of the Document window. If the tab is hidden, reveal it by clicking the **Show/Hide Settings** tool () on the left of the Document window.




For detailed information about using the Enzymes panel to apply and remove enzymes from the views, see [Show or hide enzymes in other views](#).


Settings panel

The Settings panel varies depending on which views are active. For detailed information, see:

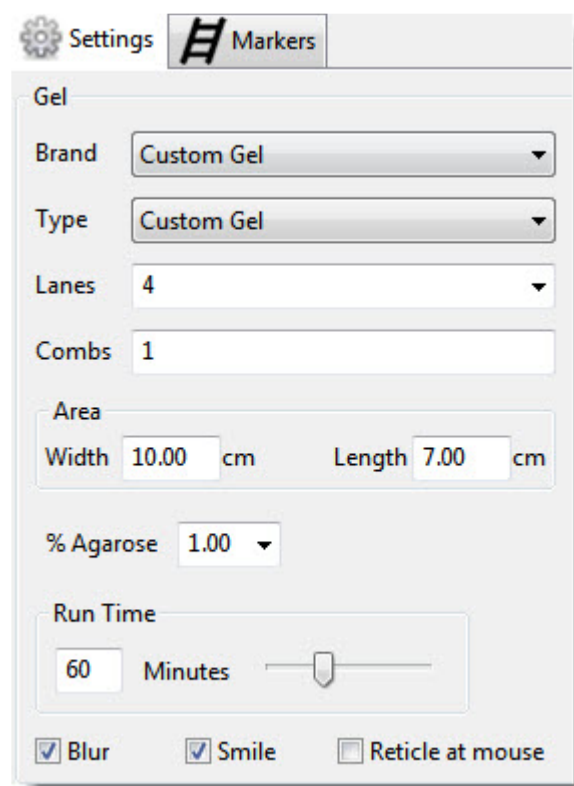
- [Settings panel for agarose gel simulations](#)
- [Settings panel for other situations](#)

Settings panel for gels

 **Note:** When not performing an agarose gel simulation, the Settings panel has different options, which are described in [Settings panel for other situations](#).

When performing an agarose gel simulation, the Settings panel, represented by a gear icon (), is located on the lower right of the [Document window](#). This panel lets you change properties of the simulated agarose gel displayed in the [Gel Simulation view](#).

The Settings panel is only available after using the **File > New Agarose Gel** command, and can then be accessed by clicking on the **Settings** tab.





- **Brand** – Use this drop-down menu to choose the gel manufacturer.
- **Type** – Use this drop-down menu to select the “model” of gel from the **Brand** manufacturer. The choice made here affects the lane layout, which includes the next three settings below.
- **Lanes** – By default, the number of lanes is determined by the selections made in **Brand** and **Type**. However, you can type in a different value, if desired.
- **Combs** – When using agarose gels, combs are plastic inserts with teeth, each the width of one lane. The spaces between the teeth are equal to the spaces between the lanes. By default, the number of

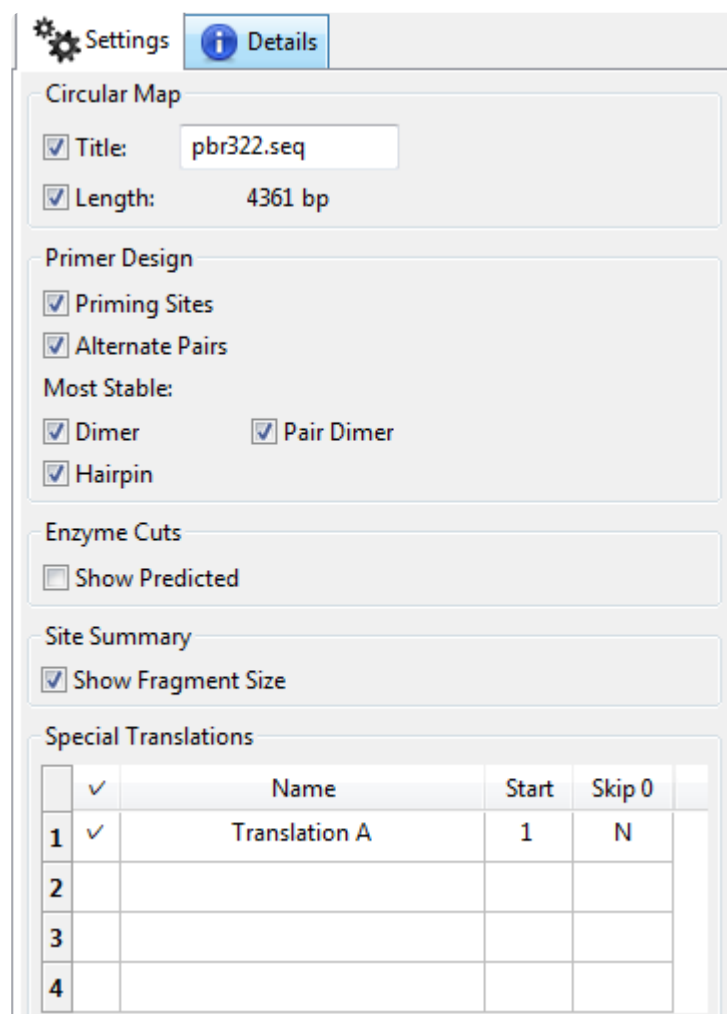
combs in the Settings panel is determined by the selections made in **Brand** and **Type**. If desired, you may type in a different value. Numbers greater than 1 cause duplicate sets of lanes to appear in the Gel Simulation view.

- **Area** – By default, the area of the gel is determined by the selections made in **Brand** and **Type**. However, you can type in any desired **Width** and **Length**, in cm.
- **% Agarose** – The concentration of agarose controls the distance that fragments propagate in a given time. A higher concentration causes fragments to slow down. Therefore, to resolve larger DNA fragments, use a lower **% Agarose**, and vice versa. By default, **% Agarose** is set to “1.00.” If desired, make another selection from the drop-down menu.
- **Run Time** – Use the slider to select a run time between 1-180 minutes. Or type in a number to define a run time of any length.
- **Blur** – Check this box to slightly blur the bars in the Gel Simulation view, more closely approximating the look of a lab-produced gel. This option is checked by default.
- **Smile** – Check this box to make the bars in the Gel Simulation view take on a slight “smile” shape, more closely approximating the look of a lab-produced gel. This option is checked by default.
- **Reticle at mouse** – Check this box to add a blue line that travels vertically with the mouse as it hovers over lanes in the Gel Simulation view. This option is unchecked by default.

Settings panel for other situations

 **Note:** When performing an agarose gel simulation, the Settings panel has different options, which are described in [Settings panel for gels](#).

The Settings panel, represented by a gear icon (), is located on the right of the [Document window](#) and lets you display or hide various components of some of the views on the left.



Circular Map

☒ Title:

☒ Length:

Primer Design

☒ Priming Sites

☒ Alternate Pairs

Most Stable:

☒ Dimer ☒ Pair Dimer

☒ Hairpin

Enzyme Cuts


☐ Show Predicted

Site Summary

☒ Show Fragment Size

Special Translations

	✓	Name	Start	Skip 0
1	✓	Translation A	1	N
2				
3				
4				

The Settings panel can be opened by clicking on the **Settings** tab. If the tab is hidden, reveal it by clicking the **Show/Hide Settings** tool () on the left of the Document window.

Circular Map - Use the checkboxes to choose whether to show/hide the **Title** and **Length** of the sequence in the center of the Circular view. To change the title text, type new text into the box provided. An alternate way

Primer Design - Use the checkboxes to specify whether to show/hide **Priming Sites**, **Alternate Pairs**, and



the most stable **Dimer**, **Hairpin** and/or **Pair Dimer** in the Primer Design view.

Site Summary - Use the checkbox to show/hide the **Fragment Size** in the Site Summary view table. When shown, the size appears in parentheses to the right of the enzyme position.

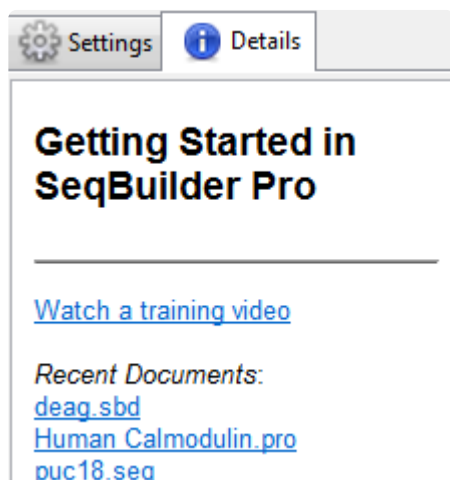
Translations – The table lists any translations created in the [Sequence view](#) using the [Create Translation command](#).

Task	How to...
To show/hide a partial translation	Add/remove the checkmark from its row
To change the name of the partial translation	Type the desired text in the Name column
To specify the starting number for the partial translation ruler	Type a number into the Start column.
To toggle between including/excluding the zero coordinate	Click the cell in the Skip 0 column. When numbering a partial translation from the negatives into the positive values, the Skip 0 functionality lets you specify whether to include or exclude the zero coordinate. Protein chemists often number the mature peptide starting with one. In cases where a signal peptide precedes the mature peptide and the signal peptide gets cleaved in the final product, a common convention is to number those residues negatively, counting back from the mature peptide. Since this is an arbitrary convention, and the coordinates do not actually go from left to right, the zero coordinate is usually skipped. In combination, Start and Skip 0 give you the ability to create such a numbering.

Details panel

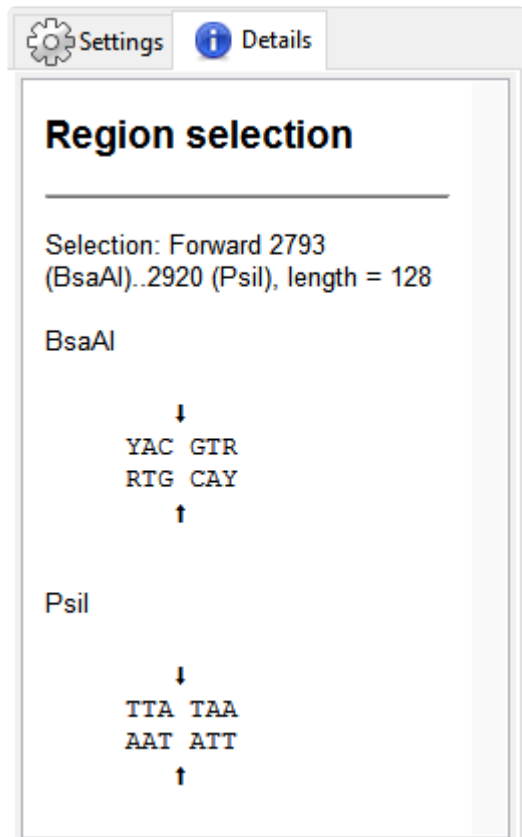
The Details panel, represented by a blue “information” icon () displays information about an active view or selection in the [Document window](#). The Details panel can be opened by clicking on the **Details** tab. If the tab is hidden, reveal it by clicking the **Show/Hide Settings** tool () on the left of the Document window.

- If nothing is selected, the panel displays links to help you get started in SeqBuilder Pro. Click on the links to watch a training video or open a recent document.



- When a sequence range is selected, including the entire sequence, the panel shows the following information:
 - **Selection** – Whether the selection is on the forward or reverse strand and the endpoints of the selection.
 - **Length** – The length of the selected region in base pairs.

If you specifically [select the range between two enzymes](#), the [Details panel](#) will show the information above plus the enzyme names and their cut sites.



- If a single feature is selected and represents a gene or CDS, the panel displays an analysis of the protein. In other situations, it displays the base composition. Among other dinucleotides, the analysis table may include the %CG dinucleotide. This dinucleotide is relevant to mammalian biology, as it is methylated in cells and clusters of CG are associated with certain types of promoters.

Feature selection

Feature Information:

Feature Type: CDS
 Location: 1559..2353
 Orientation: Forward
 citation: [2]
 codon_start: 1
 db_xref: GI:405969
 dnas_title: aminoglycoside-3'-O-phosphotransferase
 experiment: experimental evidence, no additional details recorded
 function: neomycin/kanamycin resistance
 product: aminoglycoside-3'-O-phosphotransferase
 protein_id: AAA73390.1
 transl_table: 11
 translation: MIEQDGLHAGSPAAWV

Statistics:

% A	18.11 [144]
% A+T	40.38 [321]
% Ambiguous	0.00 [0]
% C	28.30 [225]
% G	31.32 [249]
% G+C	59.62 [474]
% T	22.26 [177]
% diCG	9.32 [74]
Total number of bases	795
Amino Acids	264
Charge at PH 7.0	-18.026153
Hydrophobic Amino Acids (A,I,L,F,W,V)	109
Isoelectric Point	4.502942
Polar Amino Acids (N,C,Q,S,T,Y)	43
Strongly Acidic(-) Amino Acids (D,E)	43
Strongly Basic(+) Amino Acids (K,R)	24
% AA	3.27 [26]
% AC	4.28 [34]

- The **Feature Information** section is compiled from annotations included in the sequence when it was opened (e.g., a Genbank file); or added later using SeqBuilder Pro's Features view).

- The **Statistics** table contains mathematical calculations performed by SeqBuilder Pro.
- If multiple features are selected, the panel contains information about how many features are selected and how many are currently visible in the view.

Feature selection

Selection:

Number of features	192
Selected	3
Visible	191
CDS	1
mat_peptide	All(1)
sig_peptide	All(1)

The Selection table contains information calculated by SeqBuilder Pro:

- **Number of features** – The total number in the project.
- **Selected** – The number of features currently selected.
- **Visible** – The number of features in the selection that have a checkmark in the Show column
- **CDS** – The number of CDS features in the current selection.
- **mat_peptide** – The coding sequence for the mature protein product following post-translational modification. Does not include a stop codon. “All (#)” means that all Features of that type are selected. This information is useful when making global changes by Feature type.
- **sig_peptide** – The signal peptide coding sequence. “All (#)” means that all Features of that type are selected. This information is useful when making global changes by Feature type.
- When one or more enzymes are individually selected, the panel displays the name of each selected enzyme, along with a portion of sequence showing its cut sites on the top and bottom strands.

Enzyme Cut

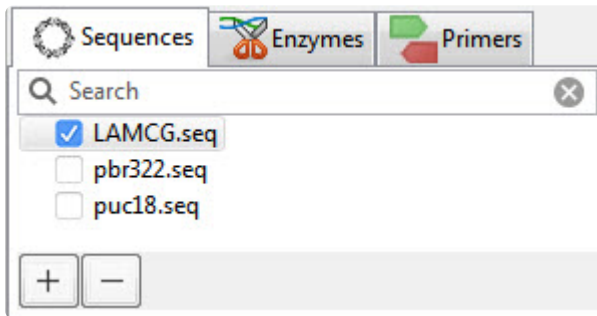
SgrDI

↓
CG TCGA CG
GC AGCT GC
↑

Sequences panel

The Sequences panel, represented by a circular sequence icon, lets you choose which sequence to use when performing an [agarose gel simulation](#).

This panel is enabled only after you use the **File > New Agarose Gel** command. Sequences that were open in the [Document window](#) or selected in a [Project window](#) prior to using this command will appear in the panel.



If no sequences were open, the panel will appear empty.

To add sequences to the panel:

Use any of the following methods. Note that multiple sequences can be added to the panel, though only one can be used for the simulation.

- Click the plus sign in the bottom left of the panel. Select the file of interest and press **Open**.
- Select a sequence and choose **File > Import (Sequences to Project)**. Select the file of interest and press **Open**.
- Right-click anywhere in the panel and choose **Import (Sequences to Project)**. Select the file of interest and press **Open**.
- Drag and drop a file from your file explorer onto the panel.

To remove sequences from the panel:

Use any of the following methods:

- Select a sequence by clicking on its name, then press the minus sign in the lower left of the panel. When prompted to confirm, click **OK**.
- Select a sequence by clicking on its name, then choose **Edit > Cut**.

- Right-click on the sequence and choose **Cut** or **Delete**.



Note: If you attempt to remove a sequence that is being used in the agarose gel simulation, you will receive a warning message.

To add a sequence to a lane in the Gel Simulation view:

Do any of the following:

- Drag a sequence from the Sequences panel and drop it in a lane in the Gel Simulation view.
- Select a lane in the Gel Simulation view, then put a checkmark next to a sequence in the Sequences panel.
- Select an empty lane in the Gel Simulation view, then add a sequence to the Sequences panel, as above. The first sequence added will be applied to the selected lane.

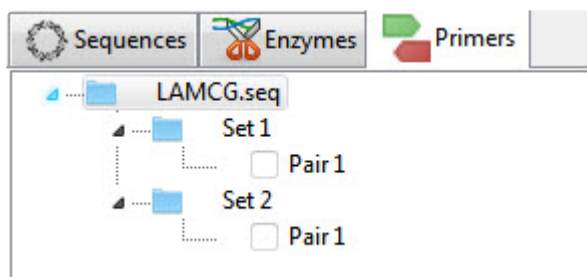
In each case, the sequence is applied in linear fashion to the gel in the Gel Simulation view, running the full width of the gel. If you select a different sequence, the previous sequence will become deselected.

Primers panel

The Primers panel, represented by a green/red arrow icon, lets you choose which primer pairs to apply to a lane in the [Gel Simulation view](#).

The Primer panel becomes available only after you initiate an [agarose gel simulation](#) using the **File > New Agarose Gel** command.

If you performed at least one [primer pair search](#) prior to beginning the agarose gel simulation, the primer pair(s) are listed here in an expandable tree.



If you did not perform a primer pair search this panel is empty, and cannot be populated. Note that unpaired primers are not supported in agarose gel simulations.

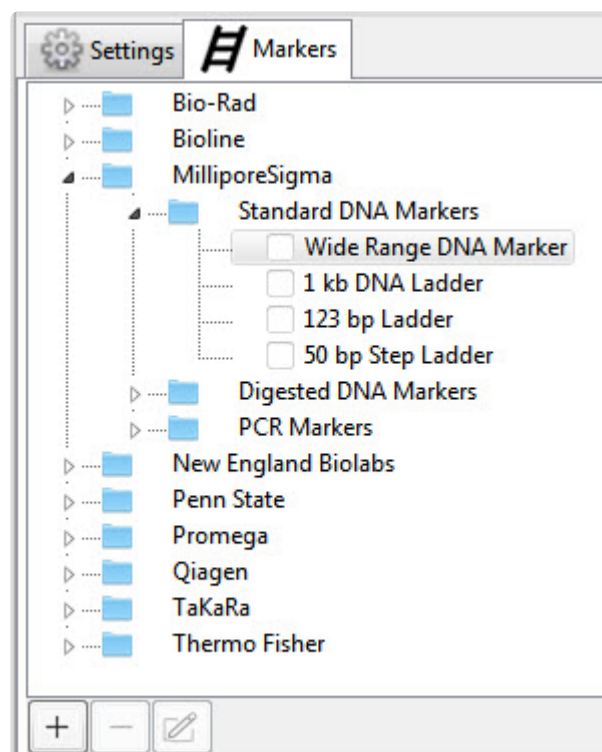
To apply a primer to a lane in the Gel Simulation view, either:

- Select a lane in the Gel Simulation view, then put a checkmark next to one or more primer pairs in the Primers panel to add them to the selected lane. To check multiple primer pairs, hold down the **Shift** key while making selections. If the **Shift** key is not held down, any new primer pair selection will replace the previous selection. If you add a primer in error, use **Edit > Undo primer choice** to revert the lane to its previous state.
- Use the mouse to grab a primer pair in the Primers panel, then drag it and drop it in the desired lane in the Gel Simulation view.

Markers panel

The Markers panel, represented by a ladder icon, lets you choose which molecular weight marker to apply to a lane in the [Gel Simulation view](#).

The Markers panel becomes available only after you initiate an [agarose gel simulation](#) using the **File > New Agarose Gel** command.



Expand the file tree as needed to locate the marker of interest from an extensive list of commercially-available markers or custom markers. Any custom markers created using the procedure further down this topic are located in a folder called *User*.

To apply a marker to a lane in the Gel Simulation view:

The marker is typically applied to the first lane but may be applied to any desired lane.

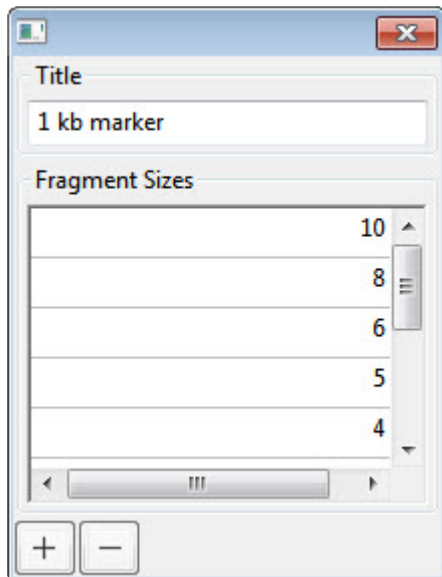
- Use the mouse to drag the marker from the Markers panel and drop it in a lane in the Gel Simulation view.
- Select a lane in the Gel Simulation view and then go to the Markers panel and check the box next to the marker you wish to add.

If you added a marker in error, use **Edit > Undo marker choice** to revert the lane to its previous state.

To create a custom marker:

Common custom markers include digests of Lambda, phiX174, pBR322, and pUC19, but you can add any custom marker using this procedure.

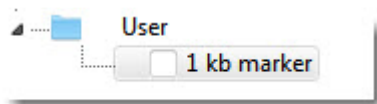
1. Click the plus sign in the bottom left of the panel.
2. Type in a **Title** for the marker.
3. Type each **Fragment Size** on a separate line.



To add a line, click the plus sign icon. To delete a line, select it and click the minus sign icon.

4. When all information has been entered, save and close the marker by clicking on the red 'x' in the corner.

The marker now appears at the bottom of the Markers panel in a folder called *User*.

To edit a custom marker at a later time:

1. Select the marker from the *User* folder.
2. Press the pen & paper icon in the bottom left of the Markers panel.
3. Follow the directions above to change the title or fragment sizes.








To remove a custom marker from the panel:







Select a custom marker in the *Users* folder by clicking on its name. Then press the minus sign in the lower left of the panel. When prompted to confirm, click **OK**.

Shared button tools

The [Document window](#) features a set of shared button tools. Tools apply to the currently-active view, and different subsets of the tools are available for different views.

By default, the shared button tools are located on the left side of the window. However, the set of tools can be repositioned as desired.

Tool	Descriptions
Strand 	To choose whether to show the sequence for the Top Strand , or for Both top & bottom strands. (Sequence and Primer Design views only)
Enzymes 	To choose which group of restriction enzyme cut sites to display on the sequence, if any. Use None if you don't wish to display any cut sites.
Translations and ORFs 	To choose whether to display translation Reading Frame(s) (Sequence and Primer Design views only), ORFs (open reading frames; Linear and Sequence views only), or Primer Product Coding Frames (after choosing primers in the Primer Design view). Each of these options has a sub-menu for displaying the translation for All or No Frames , only Top or Bottom frames, or one specific frame. To create a translation for only the selected portion of the sequence, choose the Create Translation command (called a “partial translation” in SeqBuilder 14.1 and earlier).
Rulers 	To choose whether to have No Rulers , a ruler for the Sequence , Translation , or Both . Choosing Translation Ruler or Both causes a translation of the sequence to appear, along with the sequence. (Sequence and Primer Design view only)
Vertical Spacing 	To choose the amount of spacing between view components. Use Vertical Spacing spreads to add some empty space between the components of the view. Choose No Vertical Spacing to make the view more compact.
Show/Hide Settings Panel 	To toggle between displaying/hiding the panels on the right of the Document window.
Compact EditSeq Layout 	To display the Sequence view in the upper half of the Document window, without spacers or rulers, and the Features view in the lower half.
Full Sequence Layout	To display the Sequence view in the upper half of the Document window, with both strands, all features, translations for all frames, and both rulers displayed. The Features view appears in the lower half of the window.

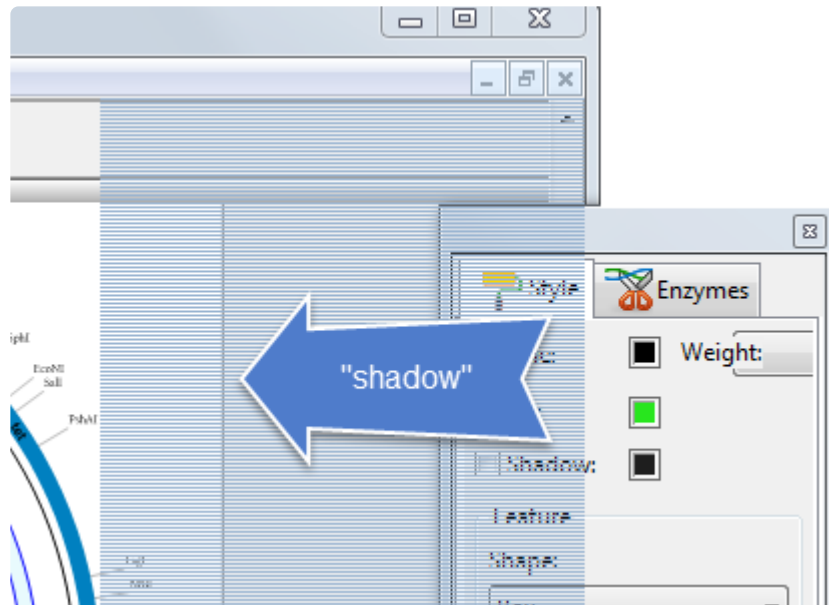
	
Primer Design Layout 	To display the Primer Design view in the upper half of the Document window, and the Primers view in the lower half.
Default Layout 	To display the Circular, Linear, or Sequence view (depending on the sequence type and length) in the upper half of the Document window, and the Features view on the lower half. SeqBuilder respects the detected sequence type (linear vs. circular) unless the sequence is over 100 kbp, in which case the default is to display the Sequence view.
Range 	To activate the Range utility. Once the tool is active, use your mouse to select a sequence range in the view.
Zoom In 	To activate the Zoom In utility. Once the tool is active, click one or more times on the view to zoom in.
Zoom Out 	To activate the Zoom Out utility. Once the tool is active, click one or more times on the view to zoom out.

To undock/redock the tools from the Document window:

To undock the tools, hover the mouse near the top of the tool column. When the cursor changes to a 4-pointed arrow, drag the tools to the desired position

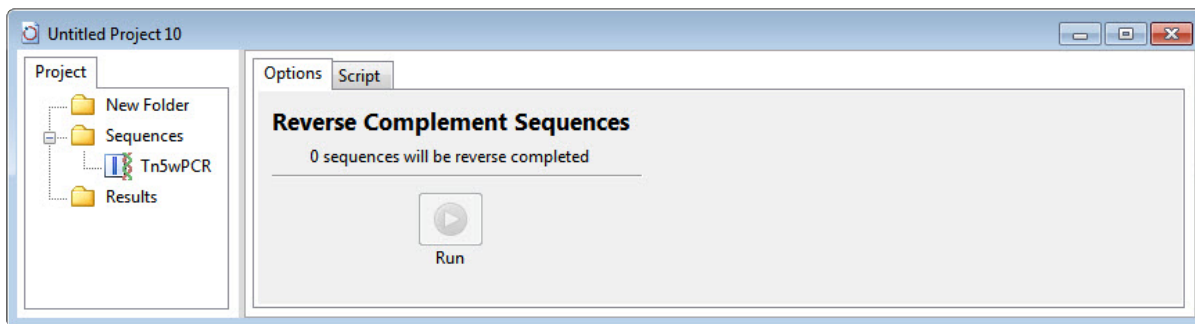


To re-dock the tools, follow the same procedure, moving the tools near their original position. When a “shadow” appears, as shown in the image below, drop the tools and they will snap into position



Project window

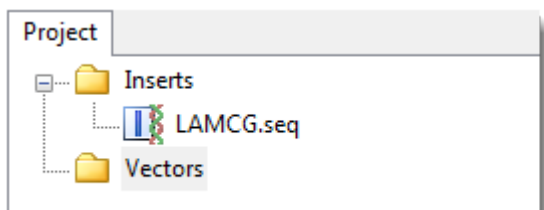
The Project window is a single- or multi-tabbed window that launches in the following situations:



- Choosing **File > New Project**
The window opens with an unpopulated folder named *New Folder* in the Project tab.
- Choosing **File > New Cloning Project**
The window opens with two unpopulated folders named *Inserts* and *Vectors* in the Project tab.
- Choosing one of many **Cloning** menu commands
The window opens with two unpopulated folders named *Inserts* and *Vectors* in the Project tab. The Clone and Summary tabs are visible on the right.
- Making any selection from the **Tools** menu (available in version 15.1 and later)
The window opens with three folders named *New Folder*, *Sequences* and *Results* in the Project tab. The Options and Script tabs are visible on the right.
- Saving and reopening a project that utilized the Project window.

Each Project window tab is described below. Note that only 1-3 tabs will be visible in a given workflow.

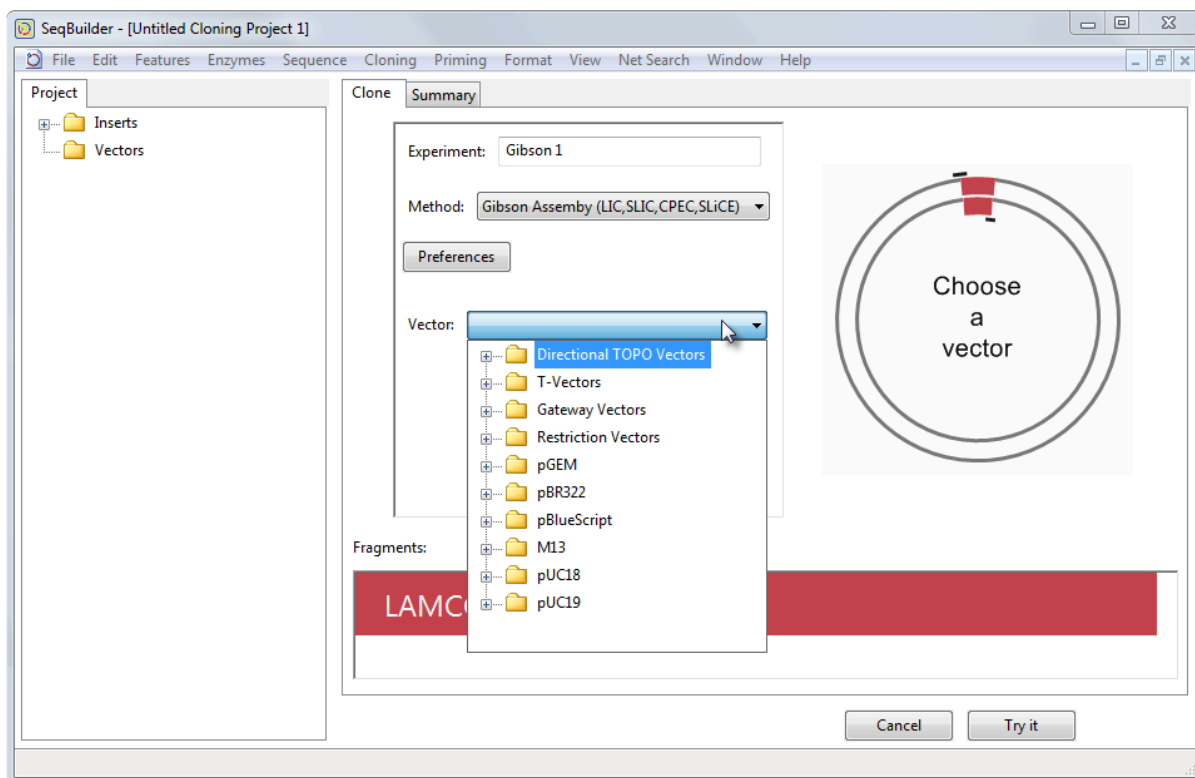
Project tab:



Task	How to...
------	-----------

To display/hide the contents of each folder	Click the plus/minus signs on the left.
To add a file to a folder	Select the folder, then right-click on it and choose Import
To rename a sequence or folder	Select the sequence or folder, then right-click on it and choose Rename .
To export a sequence	Select the sequence, then right-click on it and choose Export
To open a file in the Document window	Double-click on any sequence. Linear sequences open in the Linear view , while circular sequences open in the Circular view .

Clone tab:



When [designing a virtual clone](#), this tab prompts you to select the cloning method, choose a vector; digest or linearize the vector (if needed); and begin the cloning simulation.

Summary tab:

When [designing a virtual clone](#), this tab displays the Cloning Summary, which can be reviewed prior to completing the simulation. The Cloning Summary provides details about the components of the cloning simulation, including fragment inserts, primers, and the steps needed to prepare the vector and amplify inserts. It also displays the reading frame around all fragment junctions graphically, allowing you to verify

whether promoters and genes are in synchrony.

Options tab:

When [batch editing sequences](#), this tab lets you select options to use. For instance, when translating a group of sequences, you can choose which genetic code to use.

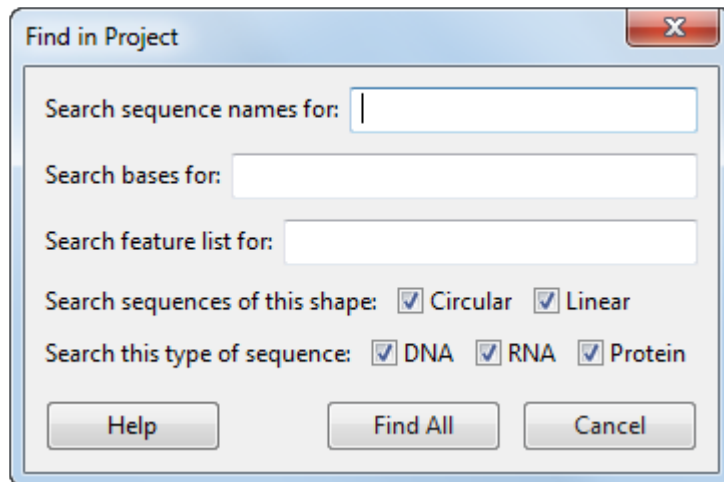
Script tab:

When [batch editing sequences](#), this tab lets you see the script (“program”) being used in the procedure.

Find a sequence in the Project window

To find a sequence in the [Project window](#) :

1. Select **Edit > Find**.



2. Specify the search criteria as follows:

- **Search sequence names for:** To search for sequences whose names contain the text input in this text box.
- **Search bases for:** To search for sequences containing the sequence string input in this text box.
- **Search feature list for:** To search for sequences containing a feature whose name or title tag matches the text in this text box.
- **Search sequences of shape:** Check **Circular**, **Linear** or both.
- **Search sequence of type:** Check **DNA**, **RNA**, and/or **Protein**, and only sequences that match one of the checks will be found.

3. When you are done selecting criteria, click **Find All**.

Any matching sequences will be displayed in the Search Results tab of the [Project window](#). You may work with the sequences in this tab as you would with any cloning project. However, while in this view, you may not rename the sequences or use the **File > Import** or **Edit > Paste** commands.

To return to the full view, click on that tab at the top of the project window. Alternatively, right-click on a sequence and choose **Show in Project** to switch back to that sequence.

Customize the Sequence Display

To specify sequence characteristics and how a sequence is displayed:

Task	How to...
To add, delete, or change the letters in a sequence	Open the Sequence view and insert the cursor where desired. Delete letters, type in new letters, or overwrite existing letters.
To change the font case	Select a portion of the sequence and choose Format > Case .
To change the font color, size or style	Use the Style panel .
To change the way residues are grouped in the Sequence view	Choose Format > Residue Grouping . Select from the following options. <ul style="list-style-type: none"> • None: (default) The base pairs appear in an unbroken line. • Group by 3: To visually break up the sequence after every trio of base pairs. • Group by 10: To visually break up the sequence after every ten base pairs.
To specify whether a sequence is linear or circular	Choose Sequence > Linear or Sequence > Circular . Regardless of the selection made here, SeqBuilder Pro can display any sequence in both the Linear and Circular views. Specifying Circular impacts ORFs and restriction sites that cross the origin.
To specify whether the sequence contains DNA or RNA	Choose Sequence > DNA or Sequence > RNA . If RNA is chosen, the sequence is displayed with U's instead of T's.

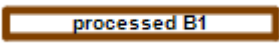
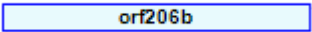
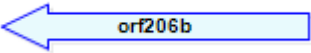
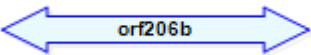
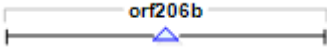
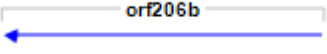
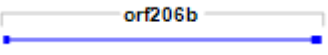
Additional topics related to sequence display:

- [Change the rendering style or font](#)
- [Move an object or row](#)
- [Switch to a preset layout](#)
- [Handle overlapping graphics](#)
- [Zoom in or out](#)

Change the rendering style or font

To change the rendering style or font of a selected feature, enzyme or range:

Open the [Style panel](#) and perform one of the tasks in the table below.

Task	How to...
To show/hide an outline around a selected item	To show/hide an outline, check/uncheck the Line box. Choose a line Weight using the drop-down menu. Change the line color, if desired, by clicking on the colored box and making a selection from the color chooser.
To show/hide a solid fill or drop shadow for a selected item	To show/hide a solid fill or drop shadow, check/uncheck the Fill or Shadow box. Change the fill or shadow color, if desired, by clicking on the colored box and making a selection from the color chooser.
To change the shape of a selected feature	<p>By default, a new misc_feature is an brown-bordered box with the feature's name inside.</p>  <p>Use the Shape drop-down menu to select the shape of the feature. The following shapes are available:</p> <p>Box </p> <p>Filled Arrow </p> <p>Filled Double Arrow </p> <p>Triangle Glyph  (may be disabled for some very long features)</p> <p>Stroked Arrow </p> <p>Stroked Goalpost </p>

To choose how to link multi-segment features	Make a selection from the Linkage drop-down menu. Choices are Solid , Dashed and None .
To change the position of a feature label	A feature's label is the topmost note as seen in the Features view. If you are working with a multi-segment feature, Inside will display segment names inside each segment box, while Above and Below will instead display only the corresponding feature name above or below the segment box.
To include a label inside or adjacent to the feature in the Circular view	Check Label Attached and use the Label Position drop-down menu to specify the location. For instance, you may wish to move a long feature name above or below the feature shape. If the Label Attached box is unchecked, the label appears outside the circular map with a line connecting it to the feature.
To change the font style, color or size	<p>Choose the items to which changes should apply using the Font drop-down menu. Choices include the Selected Item, All Features, All Enzymes, the Sequence, a Circular (Map) Title, or the Ruler. To select a different font color, click on the colored box to open the color chooser. The current font name is shown as a button. Click on the button to open a dialog where you can change the font, font style or size. Alternatively, the font size can be changed within the Style panel by typing a new font size into the text box or using the up/down arrows.</p> <p>Note: To increase/decrease the font for a single selected feature, choose Format > Size > Larger or Smaller.</p>

Move an object or row

To move an object in the [Circular](#), [Linear](#), or [Sequence](#) views:

Double-click on an object and then drag it in the desired direction.

To move a row:

Change the order of rows in a tabular view by dragging the identifying character at the left of the row up or down with the mouse.

Use preset layouts or create new ones

The following table discusses use of menu commands that affect the layout of a sequence in the [Document window](#).

Task	How to...
To choose a template and apply it to the current file	Choose View > Apply Layout .
To save the current layout as a template to be applied to other projects	Choose View > Save Layout as Template .
To save the current layout as the default layout	To save the current layout as the default layout for all SeqBuilder Pro files that do not already have layout information included (e.g., <i>.seq</i> , <i>.pro</i> , or <i>.gbk</i> files), choose View > Save Layout as Default Template .
To reformat the Linear or Circular views to the default layout	Choose View > Restore DNASTAR Default Layout . This minimizes white space and places objects in the following order (from top to bottom): enzyme cut sites, features, ORFs. Note that the position of the ruler does not change after using this command.
To recalculate the display	To rerun the dynamic/force-directed graph for enzyme display, and to refresh the feature display, choose View > Recalculate Display .
To resize the current view so that the printed portion of the view appears as large as possible	To resize the current view so that the printed portion of the view appears as large as possible while showing the entire printed portion of the page, choose View > Fit to View . This option is not available in all views.
To resize the current view so that the whole printed page appears in the selected pane	Choose View > Fit to Page . This view is smaller than when using View > Fit to View as it includes the margins of the page as well as the printed portion of the page.




Installed templates can also be applied using any of the three [Layout button tools](#) on the left of the Document window.

Handle overlapping graphics

If there are overlapping items in a view (e.g., restriction enzyme labels), right-click on one of the items and choose **Move to Back**.

Zoom in or out

In the [Circular](#), [Linear](#) and [Minimap](#) views, you can use the zoom slider () at the top of a view to increase or decrease the size of the view.

Select an Object or Range

When you select an object or range in one SeqBuilder Pro view, the selection is made in all other applicable views simultaneously.

To select an object in any SeqBuilder Pro view:

Click the object (e.g., a feature, translation, enzyme, ruler, etc.).

To select a range in the [Circular](#), [Linear](#), or [Sequence](#) views:



Click and drag the mouse across the portion of the sequence you want to select. To instead select the inverse range, hold down the **Ctrl** (Win) or **Cmd** (Mac) key while dragging the mouse over the portion you do not wish to select.

To select the range between two enzymes in the [Circular](#), [Linear](#), or [Sequence](#) views:

Do either of the following:

- Click the first enzyme label, then **Shift+click** on the second label.
- Click the first enzyme label to select it, then drag your mouse until the second enzyme of interest is selected.

When you select the range between two enzymes, the [Details panel](#) will show the **Region selection** information, including the enzyme names and their cut sites.

 Settings  Details

Region selection

Selection: Forward 2793
(BsaAI)..2920 (Psil), length = 128

BsaAI

↓

YAC GTR
RTG CAY

↑

Psil

↓

TTA TAA
AAT ATT

↑

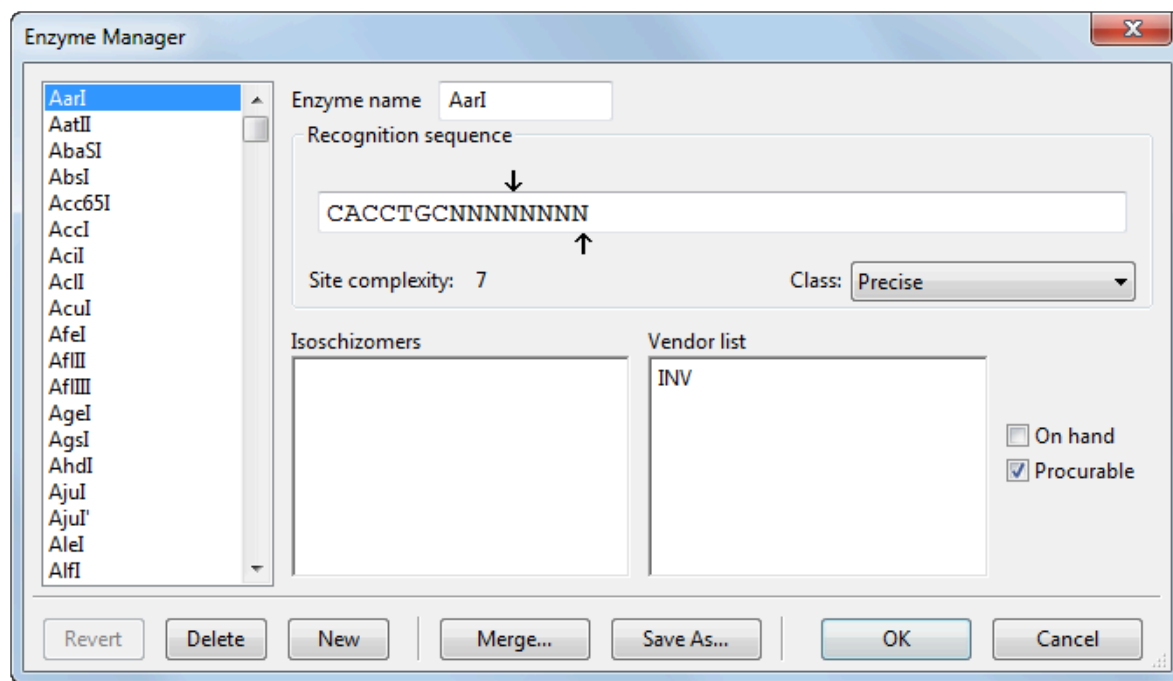
Enzymes

For information on working with enzymes, see the following topics:

- Create and manage [individual enzymes](#) or [sets of enzymes](#)
- [Apply or remove enzymes in the Gel Simulation view](#)
- [Show or hide enzymes in other views](#)
- [Change the way enzymes are displayed](#)
- [View cut sites and recognition sequence](#)

Create and manage individual enzymes

The Enzyme Manager allows you to create, modify or delete enzymes for use in SeqBuilder Pro or other Lasergene applications. If you want to create a new enzyme, open the manager using **Enzymes > New Enzyme**. To edit an existing enzyme, select the enzyme in a view and choose **Enzymes > Enzyme Manager**.



The list on the left contains all of the defined restriction enzymes in use by Lasergene.

In the main portion of the dialog:

- **Enzyme name** displays the name of the enzyme currently selected in a view, or chosen from the list in the Enzyme Manager dialog.
- **Recognition sequence** is the pair string that identifies where the restriction enzyme will cut the strand. (Note that *Bgh I* and *Taq II* have [unusual cleavage properties](#).)
- **Class**: There are three classes of enzymes: Precise, Random, or Unknown. If the class is Precise, you can set the forward and reverse strand cut sites. Drag the arrows above and below the recognition sequence to the appropriate positions. The upper arrow indicates the top strand cut site and the lower arrow indicates the reverse strand cut site.
- **Isoschizomers** lists any commercially available isoschizomers.
- **Vendor list** contains one or more three letter vendor codes.

- **On Hand**/***Procurable** defines the availability of the enzyme.

The buttons at the bottom of the window are used as follows:

Revert	To return a modified enzyme to its original (default) configuration. Note that Revert will not bring back a recently deleted enzyme. Instead, click Cancel before closing the Enzyme Manager to prevent the enzyme's deletion.
Delete	To remove an enzyme from Lasergene. Use caution, as Enzyme deletion cannot be undone by selecting Edit > Undo . If you delete an enzyme from the Enzyme Manager dialog, you will have to recreate it manually if you need it later.
New	To create a new restriction enzyme. In this case, you will enter data into the Enzyme Manager dialog's fields.
OK	to accept any changes (modifications to enzymes, deleted enzymes, or new enzymes) made since opening the Enzyme Manager and return to SeqBuilder Pro.
Cancel	To reject changes made since opening the Enzyme Manager and return to SeqBuilder Pro.
Merge	To import enzymes from another SeqBuilder Pro enzyme file (<i>Enzymes.ezd</i>). This function does not overwrite any existing enzymes, but rather only imports enzymes from the selected file that do not exist in the current file. If you want to update an existing enzyme using the Merge feature, you should first delete that enzyme and then merge the file containing the updated enzyme.
Save As	To save a copy of your current enzyme file. This can be useful if you wish to share your enzyme file with others.

Enzymes with unusual cleavage properties (Bgh I and Taq II)

Most restriction enzymes follow a simple cutting pattern, with one forward and one reverse cut site. Several enzymes have two forward and two reverse cut sites. For these enzymes, two restriction sites need to be entered into the database rather than one. For example, Bgh I:



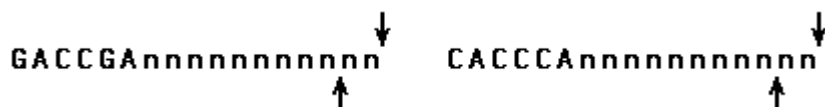
would be listed as BghI:



and BghI':




Taq II has two distinct recognition sequences which may be represented as Taq II and Taq II', respectively:



Create and manage sets of enzymes

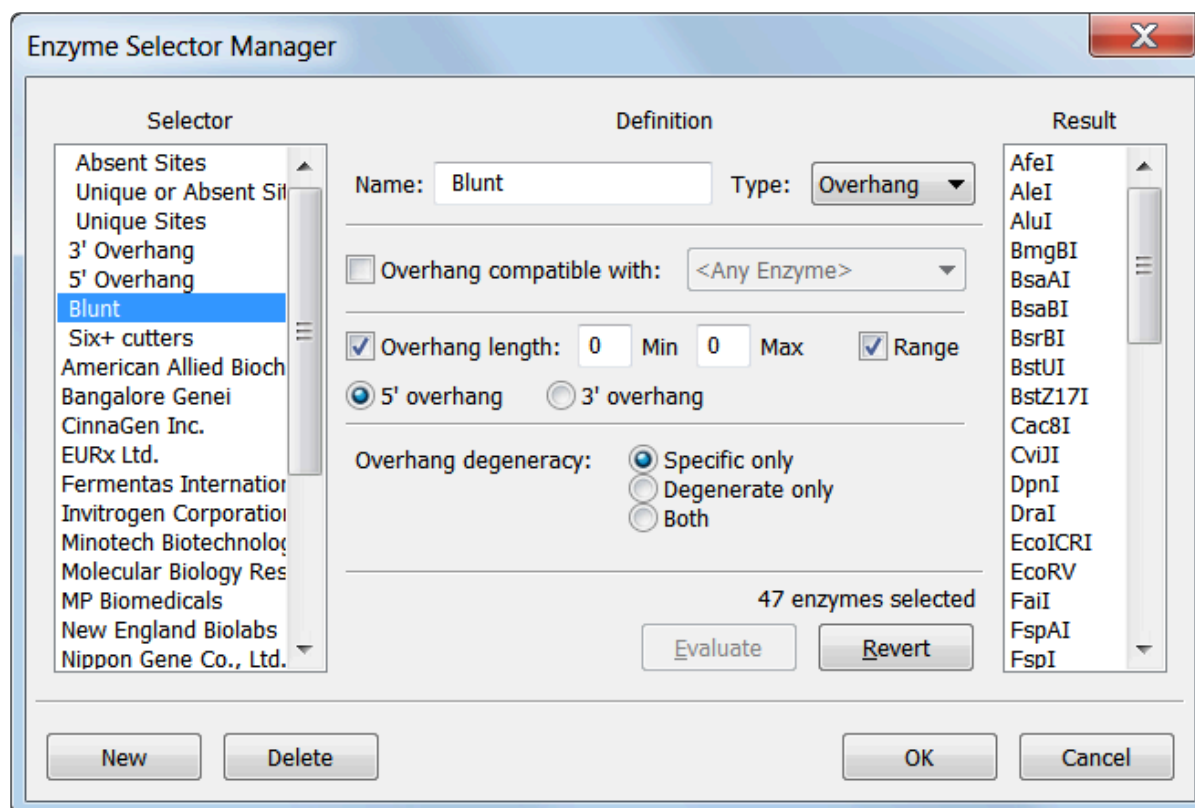
Enzyme selectors provide a way to define a group of restriction enzymes based on specific criteria. Once created, the selector will appear in the list of preset enzyme categories that appears when you click the

Enzymes tool (). Once defined, enzyme selectors are available for use in SeqBuilder Pro or GeneQuest.


- To create a new selector, open the manager using **Enzymes > New Selector**.
- To create a new selector for enzymes that do/don't cut a selected region, select a region of sequence and choose **Enzymes > Show Enzymes That Cut the Selection** or **Enzymes > Show Enzymes That Don't Cut the Selection**. Note that if you apply multiple selectors to the sequence, only the enzymes that meet the criteria for all applied selectors will be displayed. For example, if the **Unique Sites** selector is already applied to your sequence, and you then select a region and choose **Enzymes > Show Enzymes That (Don't) Cut the Selection**, the resulting enzymes will be those that are both unique and cut (or don't cut) within the selected range.
- To create a new selector for enzymes compatible with a selected enzyme, double-click on an enzyme to select it. Then choose **Enzymes > Show Enzymes Compatible With**. The new selector is named after the enzyme you chose.
- To edit an existing selector, choose **Enzymes > Enzyme Selector Manager**.

Enzyme Selector Manager

The Enzyme Selector Manager is opened by choosing **Enzymes > New Selector** or **Enzymes > Selector Manager**.



This topic describes the layout of the Manager. Additional information about using the Manager is in the topic [Create and manage sets of enzymes](#).

- The **Selector** list on the left contains a list of all the selectors available for use. Choose a selector to modify by clicking it. The selector's criteria will then appear in the Definition section of the dialog, and the enzymes that are included in this selector group appear in the Results section of the dialog (if this selector chooses any enzymes).
- The **Definition** portion of the dialog contains the criteria that the selector uses for displaying specific enzymes using the **Enzymes** button tool (). This portion of the dialog contains the following items:
 - **Name** contains the name of the current selector. You can enter a name for a new selector here when you wish to create a new selector.
 - **Type** sets the type of selector and changes the options offered in this dialog. See the table below for details:

Type	Description
Class	<p>To create a selector that uses class, complexity, availability (or some combination of these) as its criteria.</p> <ul style="list-style-type: none"> • Only precise – Check to select class II sites. • Only random – Check to select class I sites. <p>Check both boxes to select enzymes from either class.</p> <ul style="list-style-type: none"> • Site complexity – Check to limit the enzymes in this selector by the minimum length of the recognition sequence. Enter the minimum length in the Min box. Check the Range box if you wish to set a maximum length for the enzyme as well as a minimum length. • Commercial – Check to limit the enzymes in this selector to those that are commercially available. • On hand – Check to limit the enzymes in this selector to those that are currently available in the laboratory. To use this, each enzyme that is on hand should be so marked.
Overhang	<p>To create a selector based on a set of overhang criteria.</p> <ul style="list-style-type: none"> • Overhang compatible with – Check to enable a drop-down menu that lets you specify whether the overhang be compatible with any enzyme or with one specific enzyme. • Overhang length – To set a minimum length in base pairs for the overhang for either 3' or 5' overhangs. Set a range of base pairs by checking Range: <p>The previous two options (Overhang compatible with and Overhang length) are mutually exclusive.</p> <ul style="list-style-type: none"> • Overhang degeneracy – Choose whether this selector should use only specific, only degenerate, or both types of enzymes.
Frequency	<p>To create a selector based on criteria that specifies the frequency in which cutting sites appear within a specified range in the sequence. Use this also to find unique cloning sites or fragment exclusion sites.</p> <ul style="list-style-type: none"> • Cut frequency: To specify the frequency of cutting sites within a range. Check Range if you wish to specify a Min imum and Max imum number of cuts that fall within the

	<p>range.</p> <ul style="list-style-type: none"> • Target sequence: To select a sequence other than the current sequence; either another open sequence, or another sequence stored on your computer. Click Browse to select another sequence from the hard drive Or use the drop-down menu to select a currently open sequence from the list. Generally, frequency filters should not have a specific target. To apply the filter to any sequence, do not browse to a specific sequence, but instead select <Set when applied> from the drop-down menu. The program will not evaluate the Result and display it on the right side of the window. Instead, the results will be evaluated when the selector is applied to the sequence. • Sequence range: Check to specify the Begin ning and End of a sequence segment where this enzyme must cut the strand the number of times specified in Cut frequency.
Pick	To create a selector that only displays specific enzymes. To add an enzyme to the selector, click its name in Remaining Enzymes and click the left arrow. To remove an enzyme from the selector, click its name in Selected Enzymes and then click the right arrow.
Combine	<p>To create a selector that is a combination of other selectors. Choose a selector in the center column list and then select a logical connector in the first column. You can choose not for the first selector to choose enzymes that are not in that selector. You can choose not, or, and, or not, or and not for the second and subsequent selectors.</p> <p>Click + to add another selector and another row to the selector list. Click – to remove a selector and a row from the selector list along with any associated logical connector.</p>

- The **Results** list on the right displays the enzymes that this selector will display on the sequence when chosen. Results cannot always be evaluated, so this section could potentially remain blank. In a case where the results cannot be evaluated, red text appears on the dialog explaining why the results can't be evaluated. The buttons in this dialog are used as follows:

Type	Description
Evaluate	To apply new criteria to the selector. Enzymes that will be selected by this selector appear in the Results list on the right of the dialog. If the selector displays the desired set of enzymes in the Result box, click OK to save the Selector. There are some cases when the selector cannot be evaluated. You can create a Frequency filter where the Target Sequence is set to <Set When Applied> . In this case, the filter is not evaluated until the filter is actually applied to a specific sequence. This allows you to create a generic “unique” filter that will show only enzymes that cut a specific sequence one time. The set of enzymes will vary from sequence to sequence. Such selectors are not evaluated until applied to a sequence.
Revert	To remove all changes made to the current selector and revert to the original criteria. This option does not apply to changes made to the selector name or type.
New	To create a new selector.

Delete	To remove the selector.
OK	To accept any changes made since opening the Enzyme Selector Manager and return to SeqBuilder Pro. The new or modified selector will appear in the Restriction Enzymes > Selectors folder.
Cancel	To reject changes made since opening the Enzyme Selector Manager and return to SeqBuilder Pro. If you click Cancel when creating a new selector, the new selector will not be added to the Restriction Enzymes > Selectors folder.

Try it! – Find restriction sites on either side of a feature

In this tutorial, you will look for a single enzyme capable of excising a histone coding feature from a sequence named X05543.1.

1. To open the sequence, follow the tutorial [Try it! – Open an Entrez sequence by accession number](#).


You'll need an enzyme that cuts in just two places. So you'll start by creating and applying a frequency selector that displays enzymes that cuts the sequence in a maximum of two places:

2. Select **Enzymes > New Selector**. The Enzyme Selector Manager appears. New Selector is highlighted in the Selector list and the Name is **Untitled 1**.
3. In the Name box, type **Two cuts max**.
4. From the Type drop-down menu, select **Frequency**.
5. Overwrite the number **1** in the Max box with the number **2**. Leave all other parameters at their default settings.

Enzyme Selector Manager

Selector	Definition	Result
<ul style="list-style-type: none"> Unique or Absent Sites Two cuts max Unique Sites 3' Overhang 5' Overhang Blunt Six+ cutters Unique Six+ cutters Agilent Technologies EURx Ltd. Life Technologies Minotech Biotechnology Molecular Biology Resou New England Biolabs Nippon Gene Co., Ltd. Promega Corporation Roche Applied Science SibEnzyme Ltd. Sigma Chemical Corpor 	<p>Name: <input type="text" value="Two cuts max"/> Type: <input type="text" value="Frequency"/></p> <hr/> <p>Cut frequency <input type="text" value="1"/> Min <input type="text" value="2"/> Max <input checked="" type="checkbox"/> Range</p> <p>Target sequence: <input type="text" value="<Set when appli..."/> <input type="button" value="Browse..."/></p> <p><input type="checkbox"/> Sequence range:</p> <p>Note: This selector is used by all sequences; resulting enzymes cannot be determined at this time.</p> <hr/> <p>Selector not evaluated yet.</p> <p><input type="button" value="Evaluate"/> <input type="button" value="Revert"/></p>	

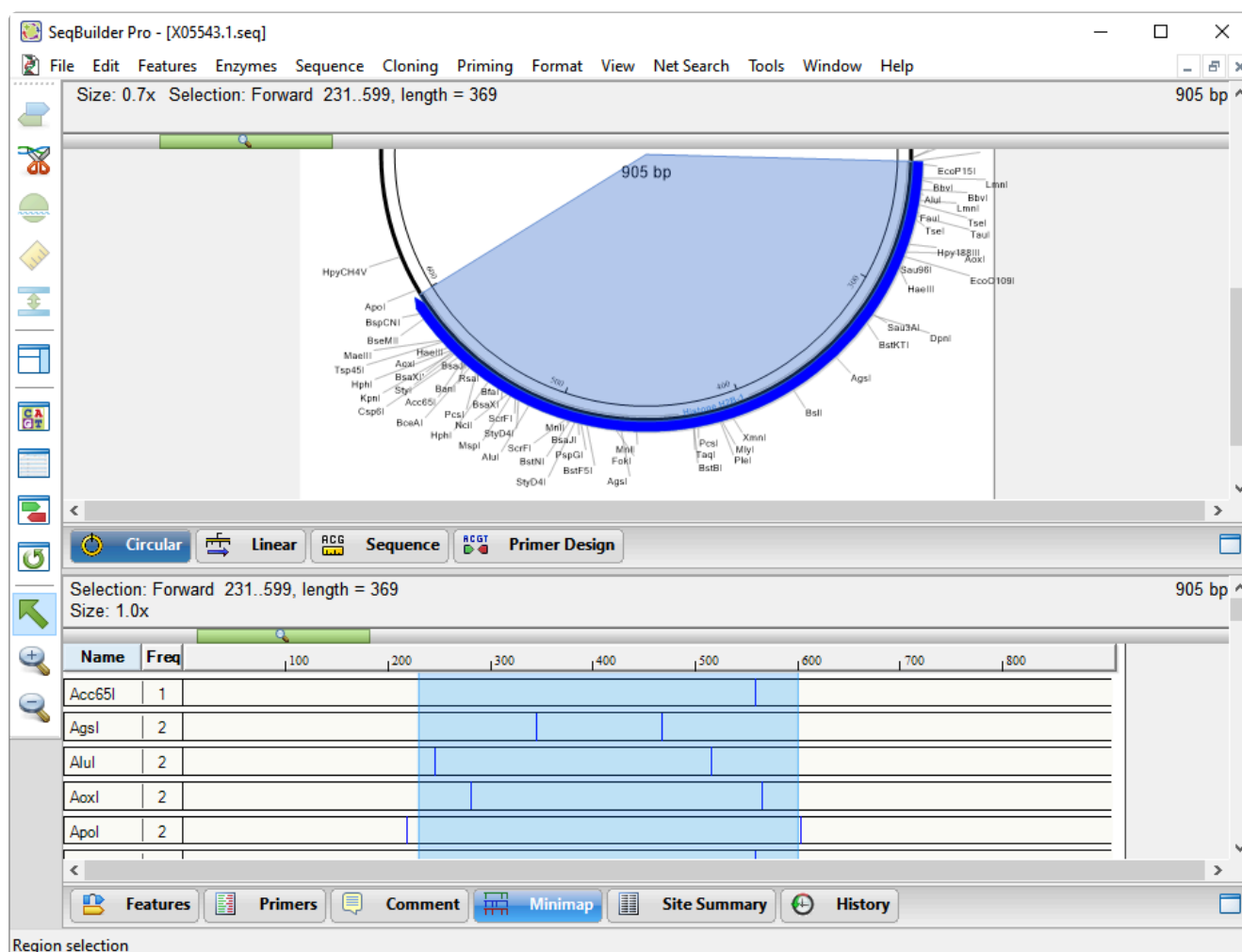
6. Click **OK** to create the new selector and return to the main view.

7. Click on the **Enzymes** tool () on the upper left of the window, then choose the new selector **Two cuts max** to display only the restriction enzymes that will cut this sequence one or two times.

✿ Even if you were to exit now without saving the project, the Enzyme Selector “Two cuts max” would be saved automatically and available through the **Enzymes** tool.

Many restriction sites remain, so you can now use the Minimap view to find the best restriction enzyme for the job:

8. In the Circular view (top) or Features view (bottom), single-click on the feature **Histone H2B-1** to select the feature in all views. Histone H2B-1 is a CDS feature that was included in the original Genbank-formatted sequence.
9. At the bottom of the window, click the Minimap tab to move to that view. The Minimap is scaled to show the whole sequence in a single line. In this view, restrictions enzyme names appear as individual rows in the left-hand column. Their corresponding cut sites are shown in the same row on the right.




10. At the top of the Minimap view, click on the ruler to sort the enzymes by which cut closest to the selection



The restriction sites are sorted so that those cutting closest to and outside the feature appear near the top, those cutting further away from the outside appear further down, and those cutting inside the feature's range appear at the bottom. Note that the **ApoI** restriction site cuts just to the left and right of the histone gene, and thus may be used for excising the gene.

Name	Freq	200	400	600	800
SwaI	1				
DraI	2				
ApoI	2				
HpyCH4V	2				
BsrI	1				
PsiI	1				
HpyCH4III	1				
SfiI	1				

11. To remove all of the currently-applied enzymes from the sequence, click the **Enzymes** tool () and choose **None**.
12. To apply only the **ApoI** enzyme, click the tool again and choose **Pick Specific Enzymes**. This opens the Enzymes panel on the right. Check the box next to **ApoI**.

This is the end of this tutorial topic.

Apply or remove enzymes in the Gel Simulation view

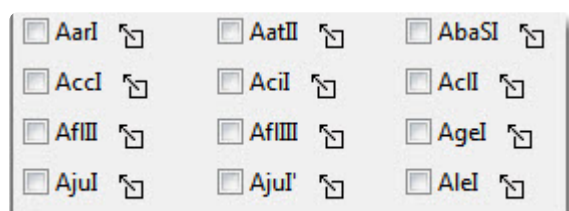
After using **File > New Agarose Gel** to initiate an [agarose gel simulation](#), a specialized version of the [Enzymes panel](#) can be used to apply or remove enzymes from a lane in the [Gel Simulation view](#).

To apply enzymes to an agarose gel lane:

First select a non-empty gel lane. If the gel lane of interest is empty, first add a sequence to it by clicking on the **Sequences** tab and checking the box next to a sequence. If no sequences appear in the [Sequences panel](#), right-click in the white area and choose **Import**.

Next, either:

- Use the mouse to grab the icon to the right of an enzyme in the Enzymes panel, then drag it and drop it in the desired lane.



- Select a lane in the Gel Simulation view, then put a checkmark next to one or more enzymes in the Enzymes panel to add them to the selected lane. To check multiple enzymes, hold down the **Shift** key while making selections. If the **Shift** key is not held down, any new enzyme selection will replace the previous selection.

To clear enzymes from a lane:


Select the lane, then press the **Clear** button at the top right of the Enzymes panel.

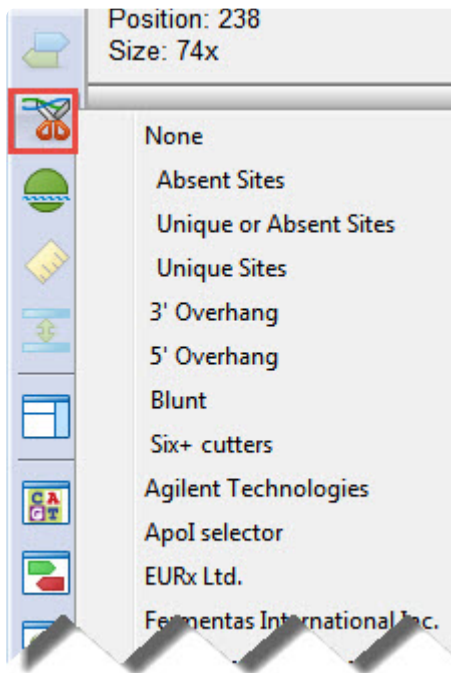
To remove an enzyme added in error:

Use **Edit > Undo enzyme choice** to revert the lane to its previous state.

Show or hide enzymes in other views

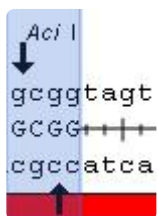
To show/hide sets of enzymes using the **Enzymes** tool:

To apply groups of enzymes to the sequence based on the frequency of cuts, type or class, site complexity, or overhang compatibility, click the **Enzymes** tool () and make a selection from the list. Choosing **None** hides all enzymes from the display. The image below shows some of the categories included with the software installation. If you have [created sets of enzymes](#) using the [Enzyme Selector Manager](#), these will appear at the bottom of the list.



If desired, additional sets of enzymes can be applied to intersect with those in the first selector. When selecting subsequent sets of enzymes, hold down the **Ctrl**, **Alt**, or **Shift** keys (Windows) or **Cmd**, **Ctrl**, **Option**, or **Shift** (Macintosh).

Once restriction enzyme(s) have been applied to the [Sequence view](#), click and hold on a restriction enzyme label in this view to show the cut sites, represented by thin blue lines. Or do the same while pressing the **Alt** (Win) or **Option** (Mac) key to show the cut represented by bold black arrows, and to display the recognition sequence.




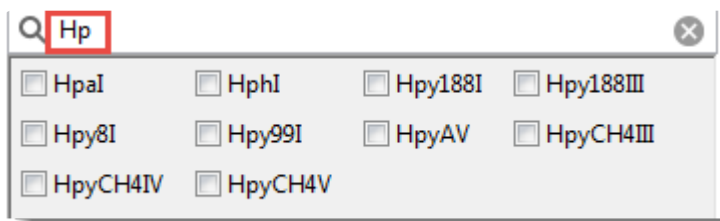
✿ **Note:** Use of the **Enzymes** tool to display a preselected set of enzymes overrides and selections made manually in the Enzymes panel (described below).

To hide an individual enzyme:

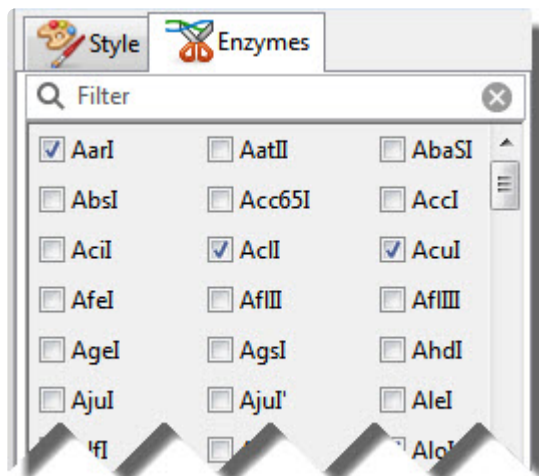
Right-click on the enzyme and choose whichever command is available: **Hide Enzyme** or **Hide Selected Enzyme**. Alternatively, use the procedure below.

To show/hide individual enzymes using the Enzymes panel:

1. Open the [Enzymes panel](#) by clicking on its tab or by clicking the **Enzymes** tool () and choosing **Pick Specific Enzymes**.
2. In the Enzymes panel, search for enzymes of interest using the responsive search bar at the top.
 - To filter the selection to enzymes beginning with particular letters, start typing the enzyme name.



- To remove a filter, click the 'x' at the right side of the search bar.
3. Check the boxes next to enzymes you wish to display and remove check marks from enzymes you wish to hide.



The [Details panel](#) will list any restriction enzymes that are suppressed due to very high cut frequency.

4. (optional) To predict enzyme cuts for a sequence with ambiguous bases, check the **Show Potential Cuts at Ambiguous Bases** box under the enzyme list. This box is unchecked by default.

Potential cuts occur when the enzyme recognition sequence and the template sequence are both ambiguous, as can happen when examining a back-translated protein. If the ambiguous residues have a non-empty intersection, then a cut is possible. However, if the intersection does not cover the whole ambiguous set, the cut is not certain. With a fully characterized sequence, all cuts are certain, so there will be no potential cuts.

When **Show Potential Cuts at Ambiguous Bases** is checked, any restriction enzymes so detected will appear in gray, rather than in blue (Minimap) or black (other views). If you edit the sequence to remove some of the unmatched ambiguity, some of these gray sites will turn black to reflect that the recognition sequence pattern now matches the sequence pattern. [Click here](#) to see an example of how this option is used.

✿ **Note:** Back-translations of serine and arginine are overly degenerate and will cause the prediction of sites that cannot possibly exist. Caution should be used for sites matching those codons.

1. If you have not already done so, follow the tutorial [Try it! – Create and modify primers for TA cloning](#). If you closed the project since following that tutorial, use **File > Open** to open the file saved at the end of that tutorial.

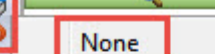
The screenshot displays the SeqBuilder Pro software interface. The main window shows a linear map of the COX2 gene, with a scale from 0 to 600 bp. The gene is represented by a blue bar, and the restriction sites are indicated by vertical lines and labels. The enzymes listed include BstNI, Sau3AI, DpnI, XbaI, EcoRI, PstI, Hpy8I, CviAII, Hpy188I, BsaXI, DdeI, HpyAV, PstI, HpyCH4IV, NlaIII, BslI, MfeI, TstI, BsaXI, CviJI, MmeI, TspGWI, BclII, and EcoP15I. The position is 1 and the size is 0.8x. The page is 1 of 1.

On the right side, there is a panel for Style and Enzymes. The Style panel includes options for Line, Fill, and Shadow, with a Weight of 1. The Enzymes panel includes a Feature section with Shape and Linkage options. The Settings and Details panels are also visible.

At the bottom, there is a table showing the features of the COX2 gene:

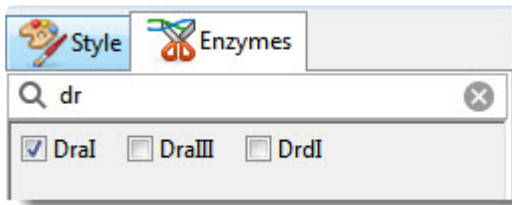
Show	Type	Name	Range	Strand	Length	Description
<input checked="" type="checkbox"/>	gene	COX2	1..696	►	696	
<input checked="" type="checkbox"/>	CDS	COX2	1..696	►	696	
<input checked="" type="checkbox"/>	source	COX2	1..696	►	696	

Below the table, there is a status bar indicating "35 enzymes displayed, 35 total cuts, 0 do not cut".

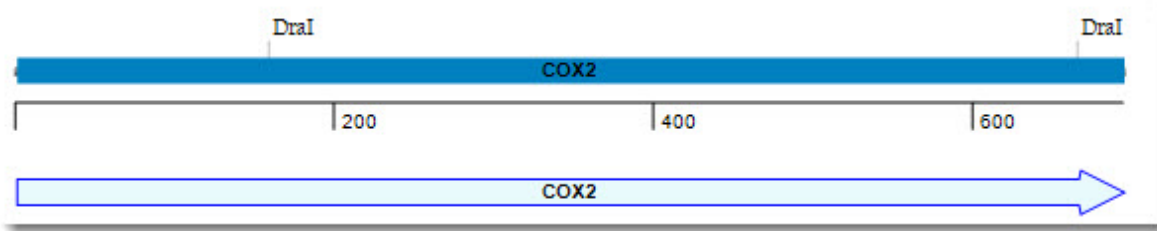


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Filter search area, type '**dr**,' the first two letters of *DraI*. Add a checkmark next to **DraI**.



5. Observe that two *DraI* cut sites now appear in the view.



This completes the “Try it!” for displaying restriction enzyme sites.

Example of showing potential cuts at ambiguous bases

The following example shows the use of the **Show Potential Cuts at Ambiguous Bases** option, located in the Enzymes panel:

1. Create a new DNA project and paste in the following sequence, which contains many ambiguous bases:

ATGWSNGCNAARGCNGARAGGATCCARAARCCNGCNWSNAARGCNCCNGCNGARAARAARCCNGCN

Several enzymes are applied to the sequence automatically and are visible in the Sequence view.

2. From the Enzymes panel, check the box next to *AluI*, which has the recognition sequence AGCT.

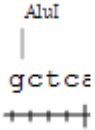
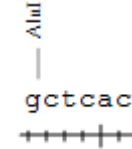
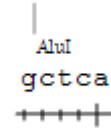
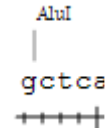
There are two possible outcomes, depending on whether **Show Potential Cuts at Ambiguous Bases** is checked or unchecked in that panel:

- Unchecked (default): Since the literal sequence AGCT is not present in the DNA sequence, no *AluI* enzymes appear on the sequence.
- Checked: Even though AGCT is not present, RGCN and NGCN are both recognized as potential sites, and the enzyme *AluI* appears in six locations on the sequence.

ATGWSAA(RGCN)GARAGGATCCARAARCCWSNAACC(NGCN)GARAARAARCC

Change the way enzymes are displayed

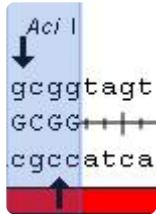
To change the way that enzymes appear on a particular view:

Description	Command
<p>To position the label parallel to the sequence (default).</p> 	<p>Format > Horizontal Labels</p>
<p>To position the label perpendicular to the sequence.</p> 	<p>Format > Vertical/Radial Labels</p>
<p>To set the lead lines above the enzyme label when the enzyme display has been moved to be under or inside of the sequence.</p>  <p>Note: This command is only enabled in the Sequence and Primer Design views.</p>	<p>Enzymes > Lead Lines Above Label</p>
<p>To set the lead lines for the enzymes beneath the label.</p>  <p>Note: This command is only enabled in the Sequence and Primer Design views.</p>	<p>Enzymes > Lead Lines Below Label</p>

View cut sites and recognition sequence

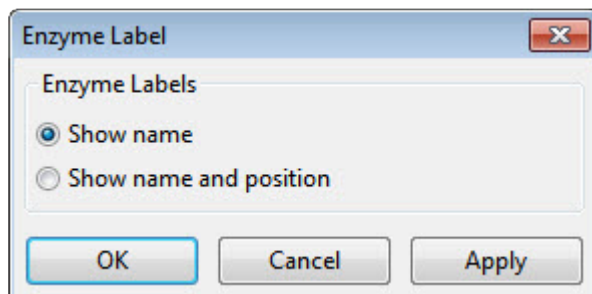
In the [Sequence](#) and [Primer Design](#) views:

- To show cut sites, click and hold on a restriction enzyme label. Cut sites are represented by thin blue lines.
- To display the recognition sequence, press the **Alt** (Win) or **Option** (Mac) key while clicking and holding on an enzyme label. Cut sites are represented by thick black arrows.

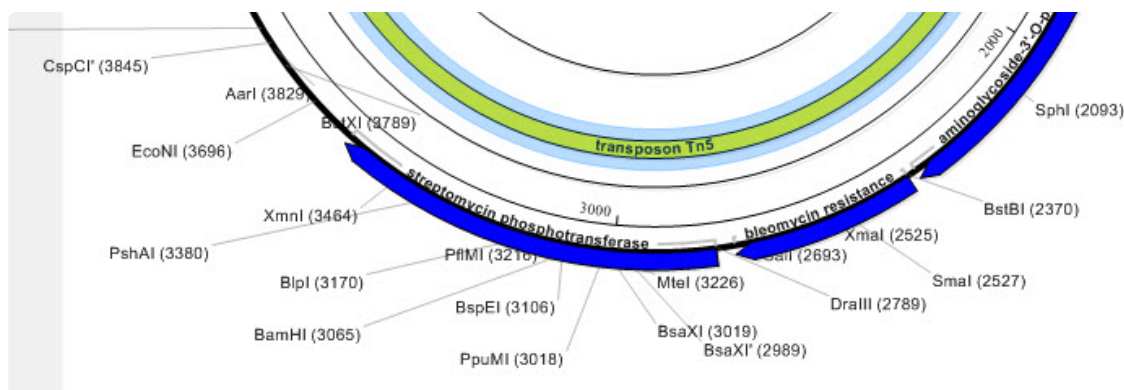


In the [Sequence](#), [Linear](#), or [Circular](#) views:

To choose whether to display enzyme names or both names and positions, use **Enzymes > Show Name/Position**. Make the desired selection and press **Apply**.



The following image shows the result of choosing **Show name and position**.



ORFs

To display open reading frames (ORFs) in the [Linear](#) or [Sequence](#) views:

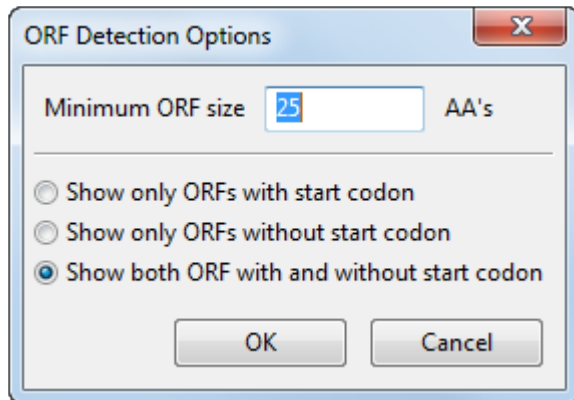
Click on the [Translations and ORFs tool](#) () and make a selection from the ORFs sub-menu.

Displayed ORFs appear as color-coded boxes and lines. Multiple ORFs can be displayed on the same line, and stops and starts appear within the color-coded boxes for each ORF. ORFs will change as you edit the sequence. When ORFs wrap across a line break or, in the case of a circular sequence, across the “end” of the sequence, there is no end marker at the end of the frame. Symbols within ORFs have the following meanings:

▶	Start codon in top frames
◀	Start codon in bottom frames
▷	Terminator as start in top frames
◁	Terminator as start in bottom frames
	Terminator (stop codon, end of ORF)
	Sequence start (linear sequences only)
	Sequence end (linear sequences only)
×	Invalid base (non IUB code) in ORF

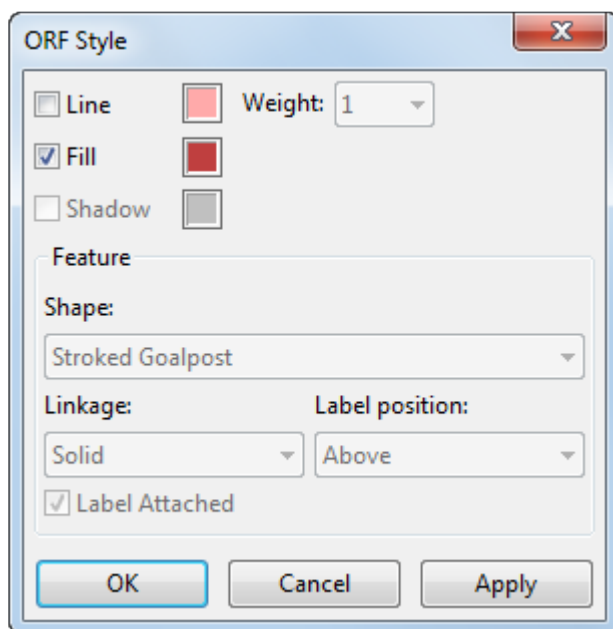
To hide a single ORF strand, right-click on the strand and choose **Hide ORF Strand**.

To specify search criteria for ORFs and designate which ORFs should appear in the views, choose **Sequence > ORF Detection Options**. The selection criteria specified in this dialog are used by the **Edit > Find** and **Find Again** when searching for an ORF. Use **Minimum ORF size** to set the smallest number of amino acids that make up the ORF. Choose whether to show ORFs with or without a start codon, or both. Click **OK**.




To change the display style for an ORF:

Right-click on it and choose **Edit ORF Style**. Or double-click on the ORF to select it and choose **Sequence > Edit ORF Style**.



In the ensuing dialog:

- Check **Line** to turn on the outline for the ORF. Change the line color by clicking on the corresponding color box.
- Use the **Weight** drop-down menu to set the thickness of the line.
- Check **Fill** to have the ORF use a fill color, if the shape permits one. Set the fill color by clicking the color box to the right of the **Fill** option.
- Check **Shadow** to have the ORF use a shadow. Set the shadow color by clicking the color to the right of the Shadow option.

 **Note:** The other options on this dialog are not available for ORFs.

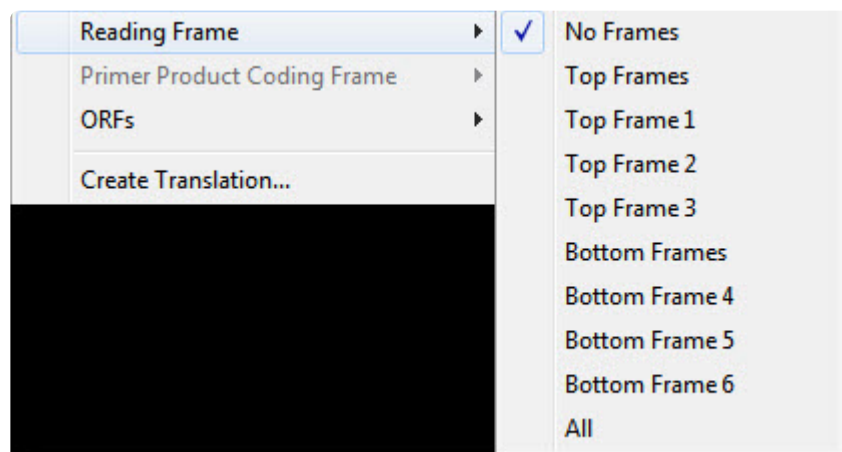
Click **Apply** to apply the changes before closing the dialog.

Translations


SeqBuilder Pro lets you view the translation for part or all of an open nucleotide sequence or create a new protein sequence based on the translation of a nucleotide sequence.

To display translations for an open nucleotide sequence:

In the [Sequence](#) or [Primer Design](#) view, click on the [Translations and ORFs tool](#) () and make a selection from the **Reading Frame** sub-menu.



After displaying a translation, you can modify it as follows:

- To hide a translation strand, right-click on it and choose **Hide Translation Strand**.
- To create a translation for only the selected portion of the sequence, in the Sequence view, click on the [Translations and ORFs tool](#) () and choose **Create Translation**. Type a name and press **OK**.
- To control the display and numbering of the translation, use the Translations table located in the [Settings panel](#).
- To toggle between displaying amino acids as 1- or 3-letter codes, choose **Sequence > Translation Display > Three Letter Code** or **Sequence > Translation Display > One Letter Code**.

To save the translation of a DNA/RNA sequence as a new protein sequence:

- To create a new protein sequence from one nucleotide sequence, see [Create a New Sequence](#).
- To create new protein sequences from multiple nucleotide sequences simultaneously, see [Create translations of multiple sequences](#)

To change or modify the genetic code used for translations:

See the topics [Choose the genetic code](#) and [Modify the genetic code](#).

The following video provides a brief walkthrough of translation in SeqBuilder Pro.

Choose the genetic code

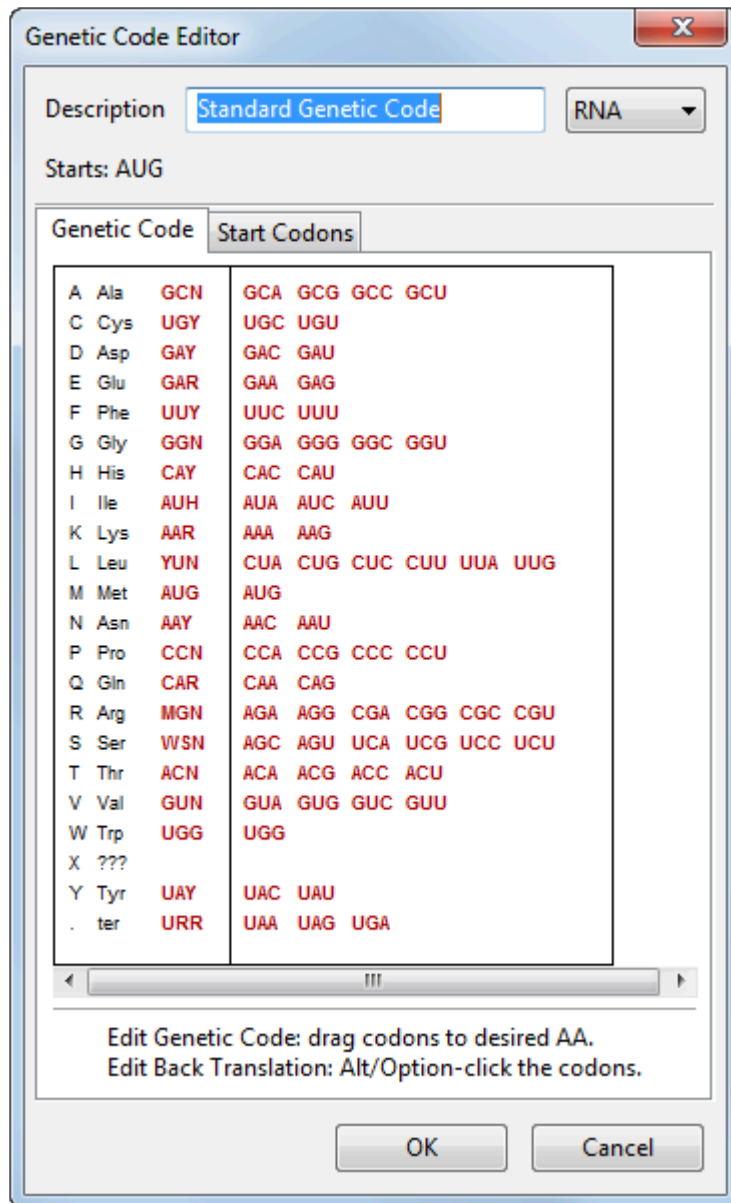
To select a genetic code to use in translations:

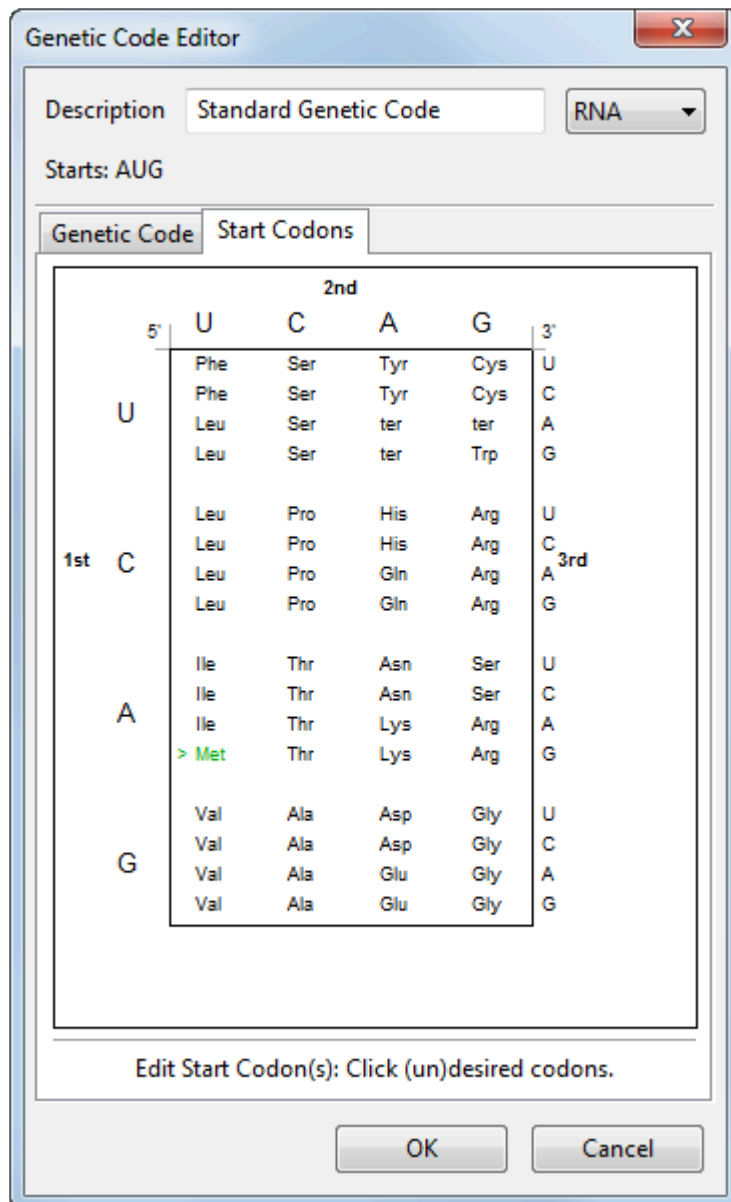
1. Select **Sequence > Select Genetic Codes**. The active genetic code is preceded by a check mark.
2. Click on a code to select it. The default genetic code used is the Standard Genetic Code.
3. If prompted, navigate to the location of the genetic code file. By default, this location is:
 - **Windows 7 & Windows 8:** *C:\Users\Public\Public Documents\DNASTAR\Lasergene x Data\GeneticCodes*
 - **Macintosh:** *Hard Drive:Applications:DNASTAR:Lasergene x:GeneticCodes*

Modify the genetic code

To modify and rename a genetic code:

1. Open a sequence or SeqBuilder Pro project.
2. Select **Sequence > Edit Genetic Code**. The two-tabbed Genetic Code Editor appears.

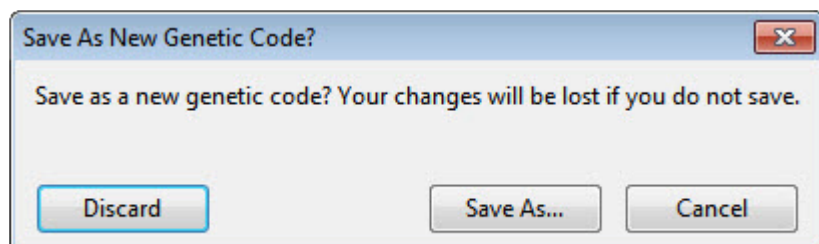




Task	How to...
To assign a name for the code	Type a name into the Description field. Warning: If you do not make changes to this field, the original genetic code will be overwritten with any changes made below.
To specify whether the code is for RNA or DNA	Select RNA or DNA from the drop-down menu in the upper right of the dialog.
To modify codon assignments,	In the Genetic Code tab, use the mouse to drag a codon to the appropriate amino acid row. The color of the codon will change from red to black to show it has been moved. SeqBuilder Pro only considers red codons when it calculates the aggregate codon for the set.

including stop codons	
To include the newly moved codon when calculating the aggregate codon	In the Genetic Code tab, Alt+click (Win) or Option+click (Mac) on the codon to change it back to red. The sum of all codons in the row is recalculated to give the revised reverse translation codon. The same recalculation occurs instantly when a codon is removed from a row. Reverse translation codons, shown in the third column from the left, are the codons used in reverse translating a protein sequence.
To change the codons that will be used for back translation without changing the forward translation	In the Genetic Code tab, Alt+click (Win) or Option+click (Mac) on codons to exclude. For example, in the Standard Genetic Code, there are two codons for Cysteine (UGU and UGC) and the default reverse translation codon is UGY (Y means pyrimidine, i.e. C or U). To reverse translate Cysteine residues as UGC, Alt+click (Win) or Option+click (Mac) on the UGU codon to change its color to black so it is not considered for reverse translation. The reverse translation codon for Cysteine will now read UGC instead of UGY.
To edit start codons	In the Start Codons tab, select the desired start codons visually by finding the row containing the first base (on the left), following that over to the column belonging to the second base (in the center) and selecting the row in the table that aligns with the third base (on the right). Click an amino acid to turn it green, and cause > to appear beside it. The codon representing the amino acid now appears at the top of the screen to the right of the word Starts .
To remove a start codon from the list at the top of the page	In the Start Codons tab, click on the corresponding amino acid in the body of the table.

- Click **OK**. If you have changed the description, SeqBuilder Pro requests confirmation.



Task	How to...
To refuse the changes and return to using the original configuration of the genetic code	Select Discard .
To save the genetic code under a new name	Select Save As . In the Save dialog, type a name for the


	modified code. Do <u>not</u> change the save location. Press Save .
To return to the Genetic Code Editor and continue modifying the genetic code	Select Cancel .



Caution: Changes made to a genetic code in SeqBuilder Pro will affect the same genetic code in all other Lasergene applications.

Rulers

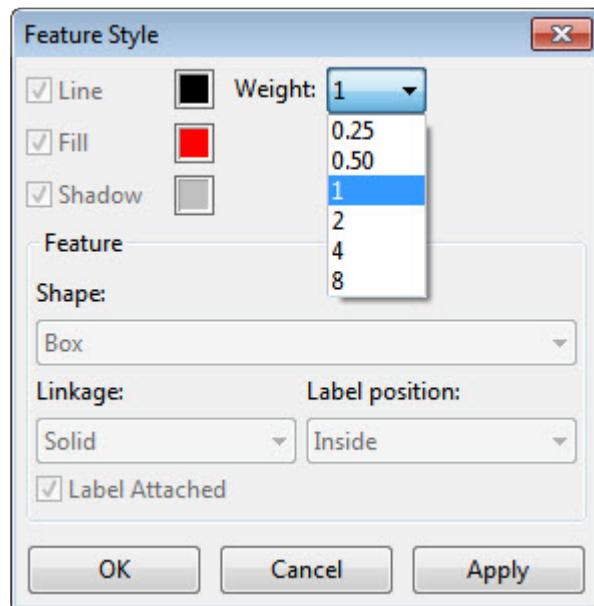
To display or hide rulers:

Use the [Rulers tool](#) (). You can also hide a specific ruler by right-clicking on it and choosing whichever command is available: **Hide Ruler** or **Hide Translation Ruler**.

Rulers can only be edited in the circumstances described below:

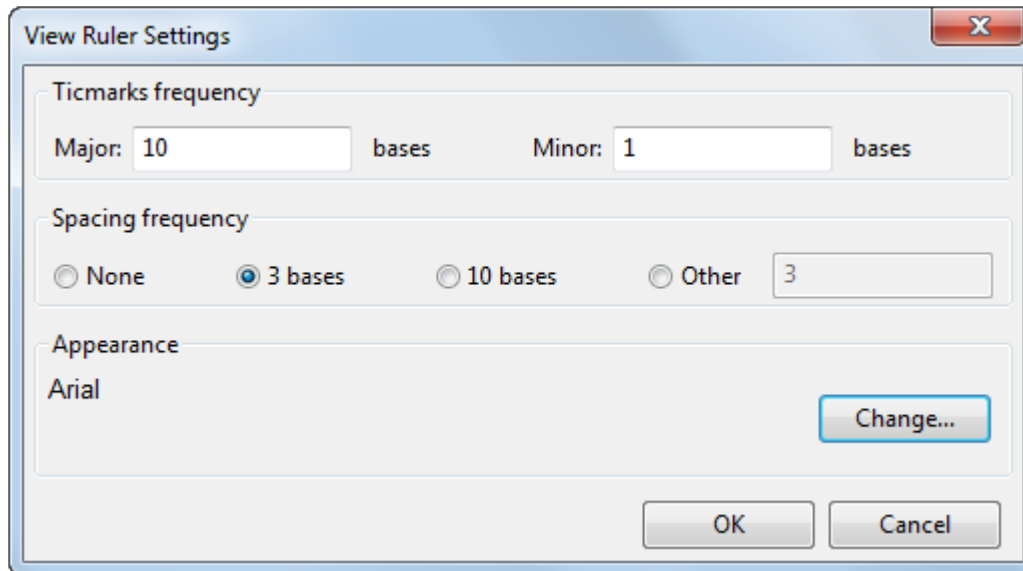
To change the line weight of the ruler in the [Linear view](#) :

Right-click on the ruler and choose **Edit Ruler Style** to open the Edit Feature Style dialog. Select the desired weight from the Weight drop-down menu. All other options are disabled.



To edit the header ruler in the [Sequence view](#) :

Right-click on the ruler and choose **Ruler Settings**.



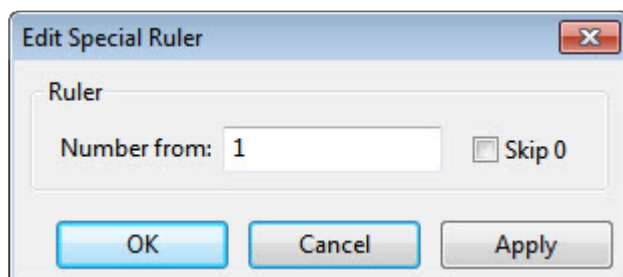
- Enter values for **Major** and **Minor** tick marks. **Major** must be a larger number than **Minor** or else a warning will be displayed.
- To display the sequence without grouping, keep the default selection of **None**. To display it in groups of **3 bases** or **10 bases**, select the corresponding radio button. To display the sequence in any other grouping, choose **Other** and type a number into the text box.
- The font style is shown in the Appearance section. To change the font, click **Change** and choose from available font, style and size options.

After making any desired changes, click **OK**.

To edit the translation ruler in the [Sequence view](#) :

After following the steps in Create a translation for only the selected portion of the sequence, a partial translation ruler is displayed. There are two ways to edit this ruler. One way is described in the topic [Settings panel](#). Another way is described below:

Choose **Format > Translation Ruler** or right-click on a selected ruler, and choose **Edit Ruler**.



- To specify that the ruler start with a number other than one (including negative numbers), enter it in

Number from.

- To skip the number zero when a translation ruler numbers amino acids from a negative number to a positive number, check **Skip 0**.

Example:

If **Skip 0** was unchecked, the sequence would be -3, -2, -1, 0, 1, 2, 3

If **Skip 0** was checked, the sequence would be -3, -2, -1, 1, 2, 3, 4

Features

Features are annotations made to a specified range on a sequence. They can be imported as part of a sequence file (e.g., Genbank, EMBL, etc.), or they can be created within SeqBuilder Pro. See the following topics to learn how to:

- [Create or delete features](#)
- [Edit information about a feature](#)
- [Show or hide features](#)
- [Join feature segments](#)

✿ TUTORIAL: [Try it! – Create and modify a feature.](#)

The screenshot displays the SeqBuilder Pro interface with a circular plasmid map of Tn5wPCR (5834 bp). A feature is selected on the map, and its details are shown in the 'Feature selection' panel. The 'Feature selection' panel includes a table of features and a detailed view of the selected 'repeat_region' feature.

Show	Type	Name	Range	Strand	Length	Description
<input type="checkbox"/>	conflict	A	1692..1692	1	1	A missing 'C' in the sequence published
<input checked="" type="checkbox"/>	CDS	bleomycin resistance	2374..2754	1	381	
<input checked="" type="checkbox"/>	CDS	streptomycin phosphotransferase	2793..3593	1	801	This protein confers streptomycin resistance
<input checked="" type="checkbox"/>	repeat_region		4293..5826			
	/standard_name = IS50R /experiment = experimental evidence, no additional details recorded /note = IS50R is functional in transposition /citation = [1] /function = insertion sequence IS50R /rpt_type = inverted /rpt_unit_range = 1..1534 /rpt_unit_range = 4285..5818					
<input checked="" type="checkbox"/>	feature	putative IHF binding site	4294..4317	1	24	putative IHF binding site
<input checked="" type="checkbox"/>	feature	Apal primer with clamp	5828..5834	1	7	Apal primer with clamp
<input checked="" type="checkbox"/>	feature	putative DnaA box	5811..5819	1	9	putative DnaA box
<input checked="" type="checkbox"/>	feature	complement (4305..5735)	4305..5735	1	1431	
<input checked="" type="checkbox"/>	feature	complement (4305..5570)	4305..5570	1	1266	
<input checked="" type="checkbox"/>	feature	complement (4308..5826)	4308..5826	1	1519	

Feature selection

Feature Information:

Feature Type: repeat_region
 Location: 4293..5826
 Orientation: Forward
 citation: [1]
 experiment: experimental evidence, no additional details recorded
 function: insertion sequence IS50R
 note: IS50R is functional in transposition
 rpt_type: inverted
 rpt_unit_range: 4285..5818

Create or delete features

To create a new feature:

1. Within any [Document window view](#) that permits range selection, select a range of the sequence. To select a range that wraps the origin, position the cursor at one end of the feature, and then use **Ctrl/Cmd+Shift+Click** to select the other end of the feature.
2. Create the new feature as follows:
 - To create a new misc_feature using the currently highlighted range of sequence as the first segment, select **Features > New Feature** or use the keyboard shortcut **Ctrl/Cmd+K**.
 - To create a new CDS feature using the currently highlighted range of sequence as the first segment, select **Features > New Feature with Translation**. The new feature will also have a **/translation** qualifier attached to it. If no sequence is selected, this command is inactive.

The new feature is selected in all views, and appears, by default, in graphical views as a brown box.



If you are in the Minimap or Site Summary views, you will not be able to see the new feature until you view the sequence in Circular, Linear, or Sequence views.

3. (optional) [Change the appearance of a feature](#) (e.g., color, rendering style, text placement, etc.) using options in the [Style panel](#).
4. (optional) For a multi-segment feature, [add the next feature segment](#).

To delete a feature:

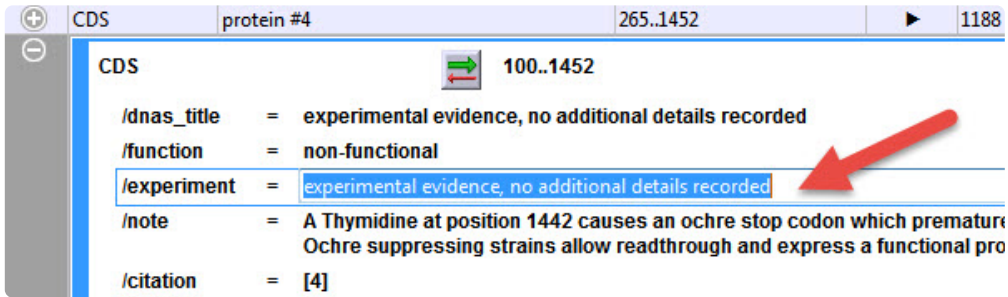


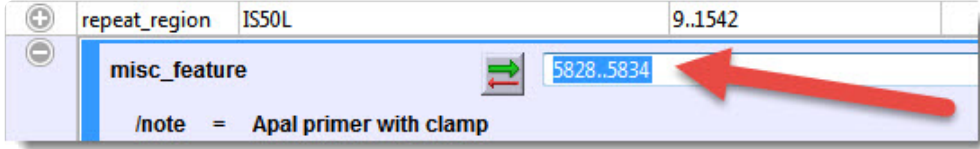
right-click or **Ctrl+click** on the feature and select **Delete**. A confirmation dialog appears. Click **Yes** to delete the feature.


Deletion is permanent, and thus differs from [hiding a feature](#). However, if you change your mind immediately, you may undo the deletion by selecting **Edit > Undo Feature Delete**.

Edit information about a feature

To edit information about a feature:

1. Open the [Features view](#).
2. Expand the row(s) for the feature(s) of interest:
 - Expand a single row by clicking on the plus sign on the left of the row.
 - Expand all rows by right-clicking anywhere in the view and choosing **Expand All** from the context menu.
3. Do any of the tasks described in the table below.

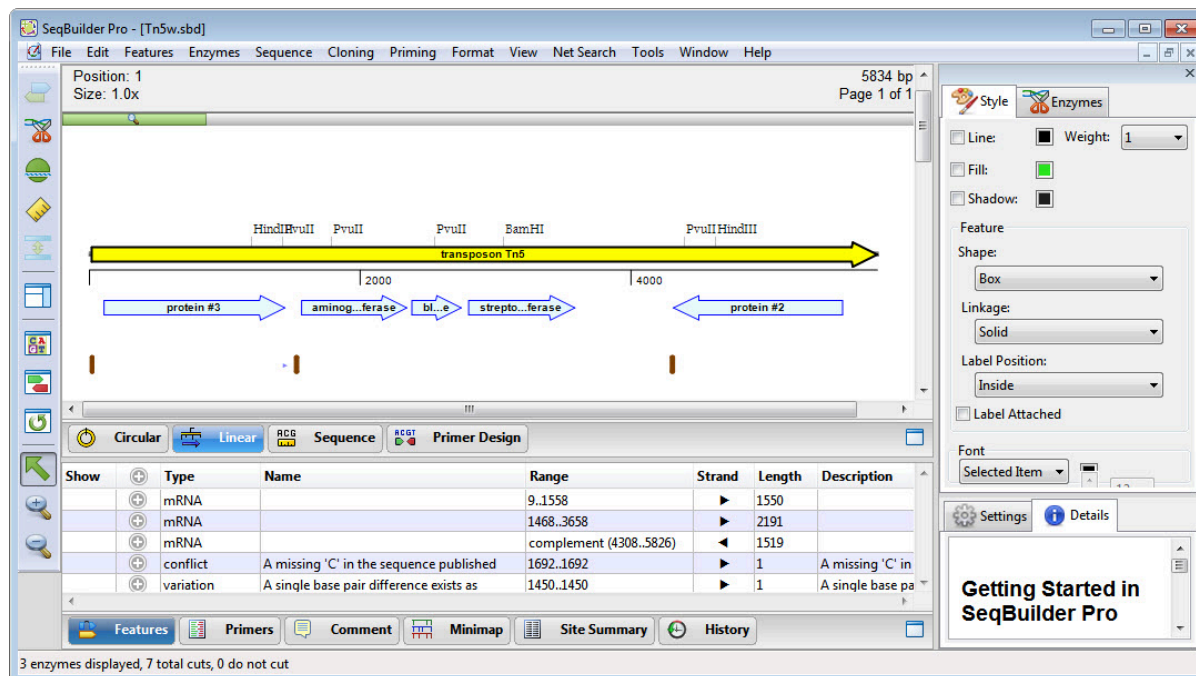
Task	How to...
To edit existing text	<p>Place your cursor in any text field and edit or overwrite the existing entry for that field.</p> 
Reverse the direction of the feature	<p>To reverse the direction of the feature so that it references the opposite strand (top or bottom), click the direction tool () or select the feature and choose Edit > Reverse Strand. If the feature has previously been translated, it will initially appear in red text. If this happens, you will need to choose Features > Update All Translations to update the translation for the feature.</p>
To extend or shrink the length or to change the segment	<p>Click the numbered range next to the direction tool (). Overwrite one or both numbers to redefine the area included in the feature. Leave two periods between the numbers. To extend the feature by adding a segment to it, type in a comma and then the new segment coordinates. Alternatively, follow the instructions in Joining Features.</p> 
To select	<p>The text following the /dnas_title field is used as the feature label throughout SeqBuilder Pro's</p>

text to use as the feature label	views. To select text from another qualifier for this purpose, right-click on that qualifier and choose Use as Feature Label or select it and choose Features > Use As Feature Label .
To add a new /note or other qualifier	Right-click on the feature and choose New Note . A /note qualifier field appears and you may type or paste text into it. To change the /note to another type of qualifier, click on the text /note . A menu appears containing several qualifiers. Select a new qualifier type, or click All to see a complete list of available qualifiers.
To delete a selected qualifier field	Right-click on the feature and choose Delete Note ; or select the feature and choose Features > Delete Qualifier Field . Translations that have not been updated after a modification has been made appear in red.
To rename a feature	Right-click on the feature and choose Rename Feature or Edit Feature Description ; or select the feature and choose Features > Rename Feature or Features > Edit Feature Description . The following conventions apply to feature names: a) Using the punctuation symbols left parenthesis, left square bracket, semi-colon or slash in the name will cause the feature name to be truncated just before the symbol, even if more text follows. b) If the feature description is ≤ 40 characters in length, the entire description is accepted, including spaces. c) If the feature description is ≥ 41 characters and does not contain spaces, the entire description is accepted. d) If the feature description is ≥ 41 characters and contains spaces, the entire description will be truncated just to the left of the first space.
To permanently remove a feature	Right-click on the feature and choose Delete Feature , or select the feature and choose Features > Delete Feature . If you only want to hide the feature from view, turn display of that feature 'off' instead.
To add a /translation qualifier to the feature	Right-click on the feature and choose New Translation or select the feature and choose Features > New Translation . SeqBuilder Pro automatically calculates the translated protein sequence for the selected feature. A /translation qualifier appears in the feature in the Features view. If you later use the direction tool () to change the selected strand, you must select Features > Update All Translations to update the /translation qualifier.
To automatically update the translation	Right-click on the feature and choose Update Feature's Translation . Translations should be updated if you have used Features> Join to Feature , or if you have modified a segment within a feature. To update all translations, use Features > Update All Translations .

Try it! – Create and modify a feature

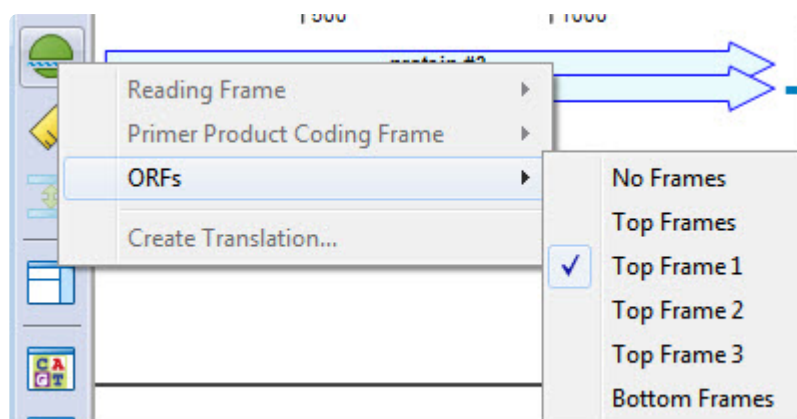
In the following tutorial, you'll locate open reading frames (ORFs), create a feature at the location of a pair of ORFs, and then change the way the feature is displayed.

1. If you have not yet download and extracted the tutorial data, [click here to download it](#). Then decompress (unzip) the file archive using the method of your choice.
2. Use **File > Open** to open the demo file *Tn5w.sbd*.

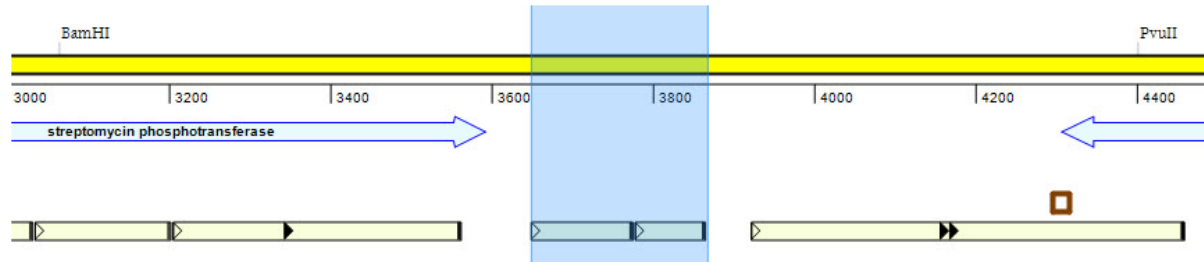


The upper pane of the window displays the [Linear view](#), while the lower pane shows the [Features view](#).

3. To locate ORFs, use the **Translations and ORFs** tool on the left and select **ORFs > Top Frame 1**.

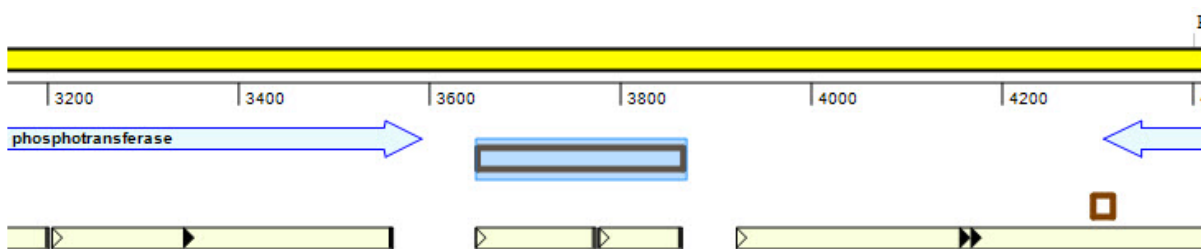


4. Hold down the **Shift** key and select each of the adjacent ORFs around position 3700.



In the header, check that the current Selection is “Forward 3649..3867, length = 219.”


5. Select **Features > New Feature**. A feature appears in the Linear view as an orange-brown box.



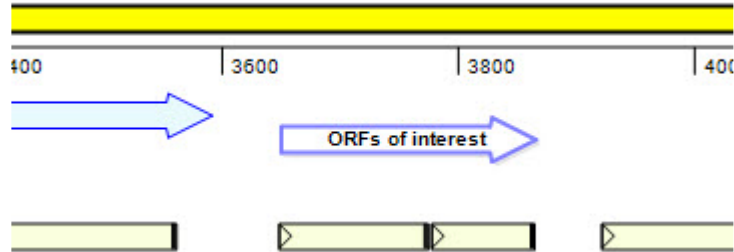
6. In the bottom pane of the screen, note the flashing cursor to the right of the word **/note**. Type **ORFs of interest** and press **Enter/Return**; this notation now appears as the label for the feature in the Linear view.

Show		Type	Name	Range
	+	mRNA		complete
✓	-	misc_feature	3649..3867	
			/note = ORFs of interest	
	+	conflict	A missing 'C' in the sequence published	1692..1
	+	variation	A single base pair difference exists as	1450..1

7. To make this the permanent label for the feature, right-click on **/note** and choose **Use As Feature Label**.
8. Near the top-right of the SeqBuilder Pro window, click on the **Style** tab to make the [Style panel](#) active. In the Style panel:
- Click the orange-brown box to the right of **Line** and pick any shade of purple from the color chooser. Press **OK**.
 - Change the **Line Weight** to **2**.
 - Add a shadow by checking the box next to **Shadow**.

- d. Use the **Shape** drop-down menu to select **Filled Arrow**.
9. (optional) If desired, move the green zoom slider () to the right to zoom in on the feature.

The feature now appears as below.



Show or hide features

To choose which features to show/hide:

In the **Show** column of Features view, rows with check marks represent the features currently displayed in the graphical views. Rows without check marks are not displayed in the views. Click the **Show** column for a particular row to add/remove a check mark.



To facilitate (un)checking multiple features at once (e.g., CDS features, features starting with a particular word, etc.) you may wish to click on the heading for the relevant column to sort it before adding/removing check marks.

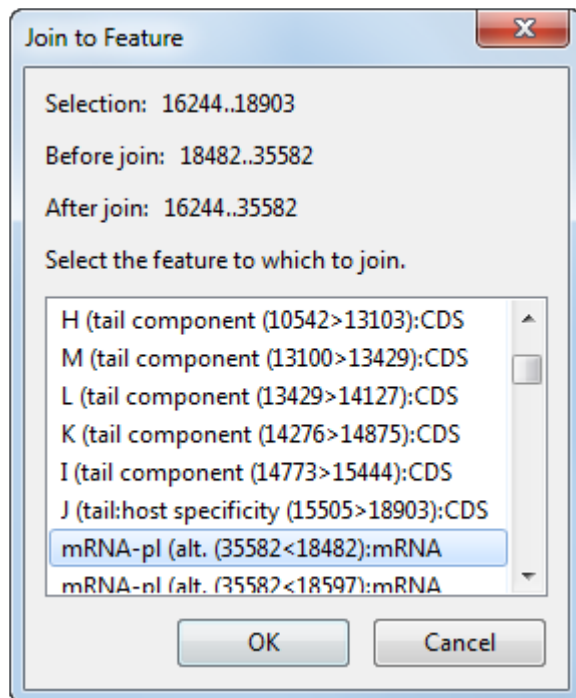
To hide a feature:

An alternate method for hiding a feature is to right-click on the feature in a graphical view and choose **Hide Feature**.

Join feature segments

To join a new segment to an existing feature:

1. (optional) If an initial feature segment doesn't exist, [create](#) one.
2. Select a region of a sequence to join to the existing feature.
3. Select **Features > Join to Feature**. The Join to Feature dialog appears. This dialog contains a list of all the features in the current sequence.



4. Select a feature from the list to which you will join the currently selected region. The dialog displays that feature's segment(s) both before and after joining the highlighted region.
5. Click **OK** to join the highlighted region to the selected feature.
6. In the Features view, the feature name for the multisegment feature will appear in the **/dnas_title** qualifier field in the following format:

feature name;segment 1+segment 2+segment 3+segment 4...

For example:

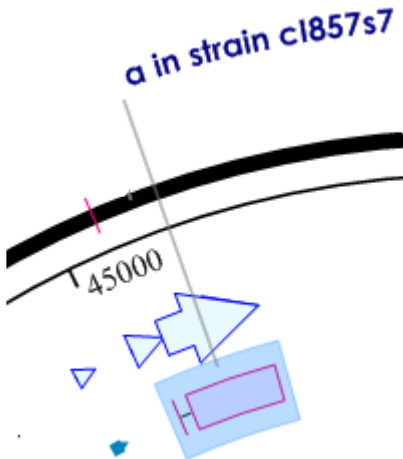
variation		45352..45352, 45493..46427
/note	=	a in strain cl857s7 ([25]); g in wild-type
/replace	=	a
/dnas_title	=	a in strain cl857s7;b in strain cl857s7+unknown segment

To change the name of the entire feature and/or each individual segment name, click in the /dnas_title qualifier field to open the Multisegment Name Editor.

	Name
Feature	a in strain cl857s7
Segment 45352, 45352	b in strain cl857s7
Segment 45493, 46427	unknown segment

OK Cancel

- In the Circular view, you may move the feature name entirely outside the circular map, where it may be easier to read. To do this, right-click on the feature and select **Detach Feature Label**.



The outcome of this procedure is different from simply [modifying the length of a feature](#). The updated feature will include two orange boxes (or other shapes, as set up in the [Style panel](#)) when displayed in the [Sequence](#), [Linear](#), or [Circular](#) views. One is for the original feature and the other is for the newly joined segment. If the two segments are not adjacent, the default style in the Sequence view is to show them connected by a line.

Primers

SeqBuilder Pro lets you design primers for regions of interest on your sequence. The following video is a brief overview of SeqBuilder Pro's primer design workflow.

To learn more about working with primers, see:

- [Create primer pairs automatically](#)
- [Create a primer based on a selected region of sequence](#)
- [Import primers](#)
- [Pair primers manually](#)
- [Edit primers](#)
- [Choose, show or hide primers](#)
- [Export primers](#)
- [Delete primers](#)
- [Methods used in primer design](#)

Create primer pairs automatically

To design primer pairs:

Open the [Sequence](#), [Linear](#), [Circular](#) or [Minimap](#) view and use the mouse to select the region you wish to amplify. If none of the sequence is selected, the entire sequence will be amplified. Choose **Priming > Create Primer Pairs**.

Create Primer Pairs

Locations

☒ Primers end exactly at selection
☐ Choose optimal primer location

Stay within bp of selection

Display at most primer pairs

Amplify 5' - to - 3'

▶ Conditions
 ▶ Primer Characteristics

In the Create Primer Pairs dialog, define the required conditions for identifying primer candidates.

Locations settings:

The Locations section of the Create Primer Pairs dialog is expanded by default and allows you to specify parameters that define where SeqBuilder Pro will search for primers on your sequence.

Locations

☐ Primers end exactly at selection
☒ Choose optimal primer location

Stay within bp of selection

Display at most primer pairs

Amplify 5' - to - 3'

Specify allowable primer locations by selecting one of the two radio buttons:

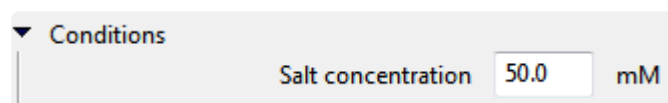
- **Primers end exactly at selection** searches for primers that lie exactly within the selected region.

- **Choose optimal primer location** searches for a specified maximum number of primers that begin and end within the distance specified outside of the selected region. When this option is selected, two additional options are enabled. The defaults are to **Stay within 0 bp of the selection** and **Display at most 1 primer pair**. If desired, you may type different numbers in these boxes.

The region selected for amplification can be changed by editing the coordinates in the **Amplify 5' to 3' fields**. If you wish to create a product that wraps the origin, enter a 3' coordinate that is less than the 5' coordinate. Alternately, you can select a feature that wraps the origin prior to opening the dialog. To create product on the reverse strand, select the region you wish to amplify as a feature on the reverse strand before opening the dialog.

Conditions settings:

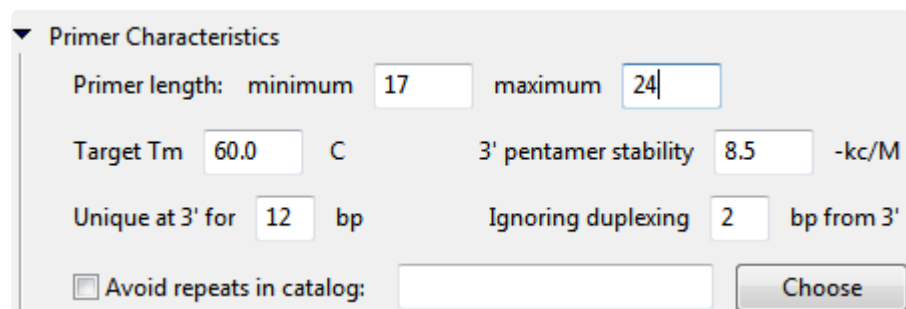
The Conditions section of the Create Primer Pairs dialog allows you to specify the initial salt concentration, which affects the calculation of predicted melting temperature. This section is minimized by default and can be expanded by clicking on the small arrow to the left of **Conditions**.



By default, the **Salt concentration** is 50.0 mM. This value may be adjusted to correspond to actual experimental conditions.

Primer Characteristics settings:

The Primer Characteristics section of the Create Primer Pairs dialog enables you to limit your search for primers based on primer length, target melting temperature, and primer interactions at the 3' end. You also have the option to avoid known repetitive sequences that are likely to cause mispriming. This section is minimized by default and can be expanded by clicking on the small arrow to the left of **Primer Characteristics**.



- **Primer length minimum** defines the minimum acceptable primer length. Primers must be at least this length (in bp) in order to be considered for selection.

- **Primer length maximum** defines the maximum acceptable primer length. Primers must not exceed this length (in bp) in order to be considered for selection.
- **Target Tm** defines the target primer melting temperature. SeqBuilder Pro will attempt to locate primers as close to this value as possible.
- **3' pentamer stability** defines the threshold for pentamer stability at the 3' end of the primer. Pentamer dG values at the 3' end exceeding this value will not be considered for selection. See [The Pentamer Profile](#) for additional information.
- **Unique at 3' for n bp** defines the number of base pairs at the 3' end of the primer that must be unique in order to be considered for selection. Uniqueness of at least 12 or more bases reduces the likelihood of mispriming at another site.
- **Ignore duplexing n bp from 3'** defines the number of base pairs from the 3' end of the primer where duplexing can be ignored.
- Check the box next to **Avoid repeats in catalog** if you have known repetitive sequences that you wish for SeqBuilder Pro to ignore when selecting primer locations. Click **Choose** to locate and select your file containing the repetitive sequences. The file must be a **tab-delimited .txt** file created manually through a text editor. If a .txt file is used, each line should include, from left to right, the sequence fragment, then a tab, then the name or notation.

After making changes, you can return to the original settings, if desired, by clicking the **Defaults** button. Once the settings are as you wish, click **OK** to begin the search.

After attempting to create primer pairs automatically, there are two possible outcomes:

- If primer pairs were not located, a dialog appears saying that “No primer pairs were found.” In most cases, this issue can be resolved by changing the settings in the Primer Characteristics dialog. This time, allow SeqBuilder Pro to choose the primer locations by using or relaxing the **Choose optimal primer location** parameter. Alternatively, you can create primers using **Priming > Create Primer from Selection** and then manually pair them together.
- If primer pairs were located, SeqBuilder Pro creates and displays a **PCR_product** feature, two **PCR_primer** features, and a **PCR_frame** feature for each located primer pair. Just like any other feature, these are visible in the Primer Design view and the graphical views.

Create a primer based on a selected region of sequence

To create a primer based on a selected region of sequence:

1. Open the [Primer Design view](#).
2. Select some or all of the sequence.
3. Select **Priming > Create Forward Primer** to create the primer on the forward strand, or **Priming > Create Reverse Primer** to create the primer on the reverse strand.

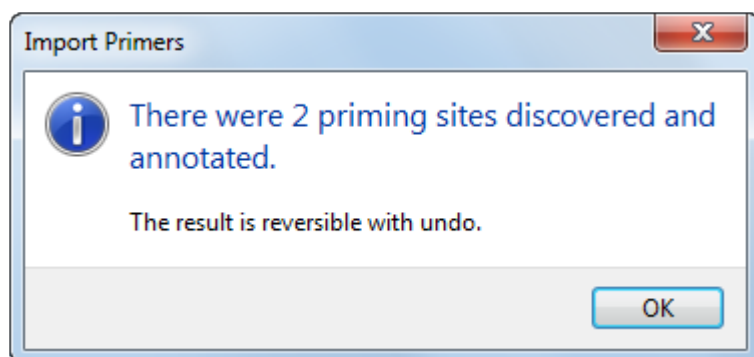
A new primer feature is added automatically and is visible in all graphical views. The forward/reverse primer is also added as a row in the [Primers view](#).

Import primers

To import primers from an external primer catalog:

1. Select **File > Import Primers from a Catalog**.
2. Choose a primer catalog (.pri).

SeqBuilder Pro attempts to locate each primer in the catalog on the current sequence by searching for sites on the template that match at least the 12-mer at the 3' end of the primer. Those that are found are annotated as **PCR_primer** features and added to the [Primers view](#). If primers were located, a message confirming the new primers will appear:



Each **PCR_primer** feature added as a result of importing primers will have three qualifiers:

- **/primer** - the primer sequence
- **/note** - any notes for the primer that were contained in the catalog
- **/set** - the set number SeqBuilder Pro assigns to the group of imported primers. The Primers view uses set numbers to organize primers into groups.

Note: After designing primers, a feature of the amplified segment is created. When you import the primers from a primer catalog, however, the amplification features are lost. This is by design, as primers can be imported onto an unrelated sequence. However, if you import primers from a catalog, the **PCR_primer** features appear in both the [Features](#) and [Primers](#) views. If you then select a pair of primers from the Primers view and choose **Priming > Pair Selected Primers**, a new **PCR_product** feature will be added to the Features view

Pair primers manually

To manually create a new primer pair from two existing primers (as an alternative to [creating primer pairs automatically](#)):

1. Select the primers you wish to pair using either of these methods:
 - - From within the [Primers view](#), click on the row of the first primer, and then **Ctrl/Cmd+click** on the row for the second primer.
 - From within the [Primer Design view](#), click on the first primer feature, and then **Shift+click** on the second primer feature.
2. Select **Priming > Pair Selected Primers**.

A new primer pair is created from the two selected primers, and becomes the active pair.

Edit primers

There are several ways to edit a primer in the [Primers view](#) or the [Primer Design view](#):

- [Modify a base in the primer](#)
- [Modify the primer length](#)
- [Introduce a codon change or mutation](#)
- [Introduce restriction sites](#)


Change the name of a primer

Once you have created primers, you can change the name of the primer as follows.

1. Open the [Primer Design](#) view.
2. Expand the primer row by clicking on the plus sign.
3. In the **Name** column, click on the cell for the name you wish to change.
4. Type a new name into the field and press **Enter**.

Modify a base in the primer


To modify a base in the primer:

1. [Create or import primers or primer pairs.](#)
2. Modify bases using either of these methods:
 - For single primers only (i.e., the primer cannot be part of a primer pair) – From within the [Primers view](#), click inside the **Primer Sequence** column and edit the existing nucleotide sequence.
 - For single or paired primers – From within the [Primer Design view](#), click on the **Selection Mode** tool () at the top left of the window. Overwrite the nucleotide character with the desired character. The edited nucleotide and the resulting changes in translation are shown in magenta. Restriction enzymes whose recognition sites are eliminated by the edit are shown in blue; sites that are introduced by the edit are shown in magenta.

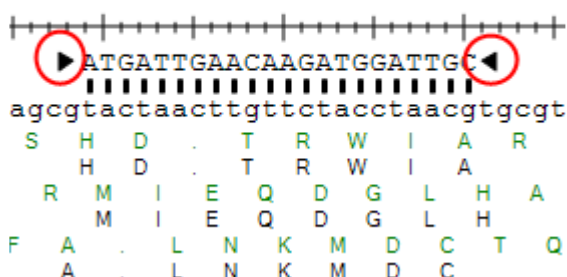


Modify the primer length

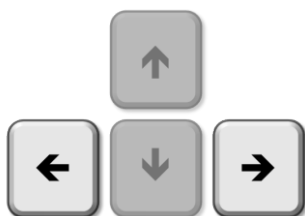
To modify the primer length:

1. [Create or import primers or primer pairs.](#)
2. From the [Primer Design view](#), click on the **Selection Mode** tool () at the top left of the window.
3. Do any of the following:
 - Use the mouse to drag one or both handles at the ends of each primer in either direction.

tgcgatgattgaacaagatggattgcacgca




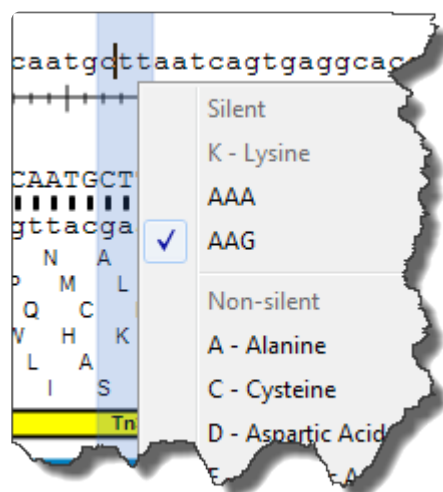
- Select a base at either end of the primer and press the **Delete** key.
- Select a base at either end of the primer and press the **Ctrl/Cmd** key while pressing the left or right arrow keys on the keyboard.



Introduce a codon change or mutation

To introduce a codon change or mutation:

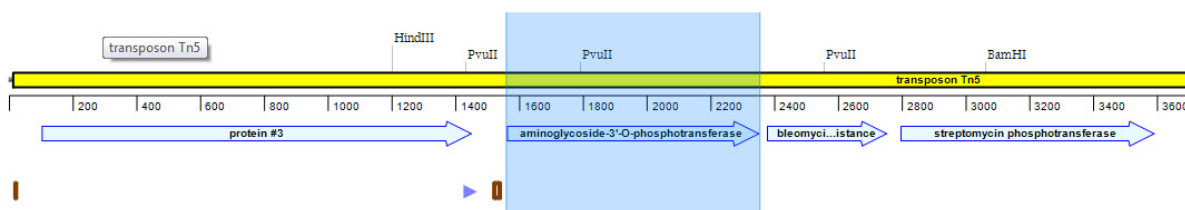
1. [Create or import primers or primer pairs.](#)
2. Open the [Primer Design view.](#)
3. Select the **Introduce Codon Change/Mutation** tool () at the top left of the window; or choose **Priming > Introduce Codon Change/Mutation.**
4. Click on the codon of interest to open a codon menu showing the current codon checked. The menu lists synonymous codons and other amino acids for introducing non-synonymous changes.
5. Select an alternate codon (for silent mutations), or an alternate amino acid (for non-silent mutations). Selecting an alternate amino acid will replace the sequence via the least disruptive alternate codon. Codons which have been changed cause an edit to the primer, with changes appearing in magenta.



Try it! – Create and modify primers for TA cloning

To create a primer pair for a region of interest on the sequence to use in TA cloning:

1. If you have not yet download and extracted the tutorial data, [click here to download it](#). Then decompress (unzip) the file archive using the method of your choice.
2. Use **File > Open** to open the demo file *Tn5w.sbd*. The file opens in the [Linear view](#).
3. Click on the neomycin/kanamycin resistance feature labeled **aminoglycoside-3'-O-phosphotransferase** to select that range of sequence.



4. Select **Priming > Create Primer Pairs**. The Create Primer Pairs dialog appears.
5. Click the triangles next to **Conditions** and **Primer Characteristics** to expand those sections.

Create Primer Pairs

Locations

☒ Primers end exactly at selection
☐ Choose optimal primer location

Stay within bp of selection

Display at most primer pairs

Amplify 5' - to - 3'

Conditions

Salt concentration mM

Primer Characteristics

Primer length: minimum maximum

Target Tm C 3' pentamer stability -kc/M

Unique at 3' for bp Ignoring duplexing bp from 3'

☐ Avoid repeats in catalog:

In most cases, you will not need to change the settings in this dialog. For the purposes of this tutorial, however, you are going to deliberately make a change such that the default **Primer length** values will not yield optimal results.

- Adjust the **Target Tm** to **55.0 C**. Leave the rest of the values as they are, and then click **OK**.

SeqBuilder Pro will choose the best primer pair with the characteristics specified that lies within the selected region. The upper view of the SeqBuilder document is now the Primer Design view, focused on the forward strand of the primer. The lower view is the Primers view.


- If necessary, drag the dividing bar between the Primer Design and Primers views so that you can see all three sections of the Primer Design View: Residue, Mispriming and Alternate Pairs.

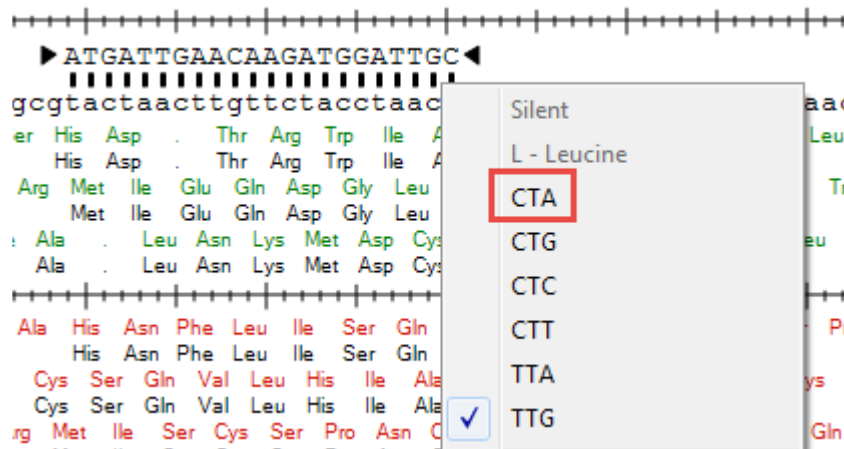


In the Primer Design view (upper pane), notice that, in the Mispriming section, the **Most stable dimer** and **Most stable pair dimer** are both labeled “BAD!” This indicates that the final pentamer value of the dimer exceeds the **3’ pentamer stability** threshold defined in the Primer Characteristics section of the Create Primer Pairs dialog. In general, you should avoid using primers with dimers or hairpins labeled “BAD!” unless you have experimental evidence that they will function as desired, or unless you have no other option.

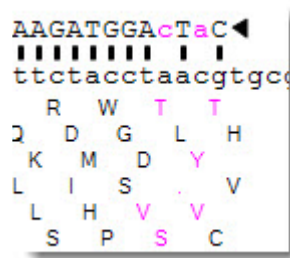
- In the Primers view (lower pane) the single primer pair located by your search is displayed. Click the plus sign next to **Set 1** to expand the primer pair and view each individual primer within the pair. Notice that the melting temperature for the Forward primer is lower than the Target Tm of 55°C.

Show	Active	Set Name	Name	F/R	Primer Sequence	Score	Length	Tm	dTm	Ta OPT	dG
✓	•	Set 1	Pair 1		<ATGATTGAACAAGATGGATTGC>...<GCCTTC 1.000	795	83.5	32.7	58.8		
		Set 1	S1 Pair 1 Forward	F	ATGATTGAACAAGATGGATTGC	22	50.8	3.6			-38.6
		Set 1	S1 Pair 1 Reverse	R	TCAGAAGAAGCTCGTCAAGAAGGC	23	54.4	3.6			-41.4

- In the Primer Design view (upper pane), select the codon change mode by clicking on the **Introduce Codon Change/Mutation** button () near the top left of the view. Notice that the cursor changes to an arrow, and that each codon is highlighted as you hover over the primer sequence.
- Introduce a codon change by clicking on the **TTG** triplet in the primer, located at 1577-1579 of your sequence. From the list of codons given, select **CTA**.




In the sequence area, the two changes to the primer sequence, and the corresponding changes to the translation, are shown in magenta in lower case.



(In the Primers view, these edits appear in lower case, but without any color change).


In the mispriming section, note the good news that the dimers/hairpins have either disappeared or are no longer marked “BAD!.” The bad news is that the header shows the T_m has now dropped further from the target temperature of 55.0°C, moving from 50.8°C to 44.1°C.

11. Click the **Selection mode** tool () to exit from the current “codon change” mode.
12. Referring to both the T_m in the header, and the Mispriming section at the bottom of the view, drag the triangle on the 3' (right) end of the primer sequence to the right. Increase the length of the currently **22 bp** primer, stopping when these two conditions are met: 1) the T_m is close to 55.0°C, 2) Mispriming contains no items labeled “BAD!.” This occurs when the primer is **31 bp** in length and the T_m is 55.3°C.
13. In the Primers view, name the modified pair by clicking on **Pair 1** in the **Name** column and typing **55 degree pair**.
14. Save the project with any name and in any desired location using **File > Save As**.

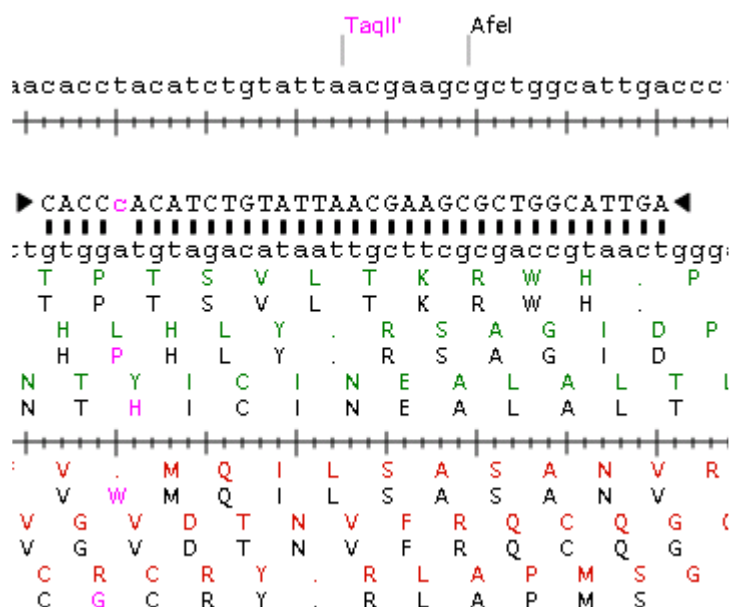
The saved project is used in the tutorial [Try it! – Use a PCR insert in TA cloning](#).

Introduce restriction sites

To introduce restriction sites for primers:

1. [Create or import primers or primer pairs.](#)
2. From the [Primer Design view](#), either click on the **Introduce Restriction Site** tool () at the top left of the window, or choose **Priming > Introduce Restriction Site**.
3. Click between the residues where the restriction site should be added. This opens a top level menu with the restriction enzymes identified to cleave the sequence at a specific location:
 - **<Specific enzymes by name>**
 - **Non-silent substitutions**
 - **Any enzyme** – Select this option to choose an enzyme from a submenu. If you are looking for a particular enzyme, click on the top-level menu, then type the first 1-2 letters of that enzyme. This will automatically navigate to the part of the submenu list where the enzyme is located.
 - **Enzymes that don't cut product** – See note for **Any enzyme**, above.
 - **Introduce a stop**

New restriction sites are displayed in magenta, as well as nucleotides in the primer sequence and their translations that differ from the template. Restriction enzymes whose recognition sites are eliminated by the edit are shown in blue.



Choose, show or hide primers

To choose a selected primer pair (i.e. make it the active pair):

Within the [Primers view](#), select a primer pair and choose **Priming > Set as Active Primer**. The selected primer pair will be opened in the [Primer Design view](#), where it can be edited or analyzed.

To display the forward/reverse (i.e., top/bottom) strand primer of the current primer pair in the center of the Primer Design view, choose **Priming > Show Forward Primer** or **Priming > Show Reverse Primer**.

To show or hide primer pairs:

Open the [Primers view](#). In the **Show** column, checked rows represent primers currently displayed in the graphical views. If you wish to sort the rows before selecting the primer pairs to show/hide, click on the column header of interest. In the **Show** column, add check marks in rows you wish to display and remove them from rows you wish to hide.

To show or hide alternate pairs:

In the Alternate Pairs section (lowermost pane) of the [Primer Design view](#), right-click on a primer pair and choose an item from the context menu:

Context menu option	Function
Hide Pair	To hide a selected pair.
Delete Pair	To delete a selected pair.
Show Only This Pair	To hide all but the selected pair.
Show All Pairs	To show all found pairs.

Export primers

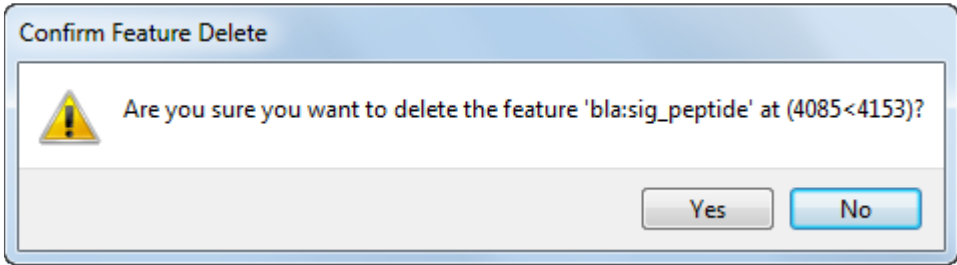
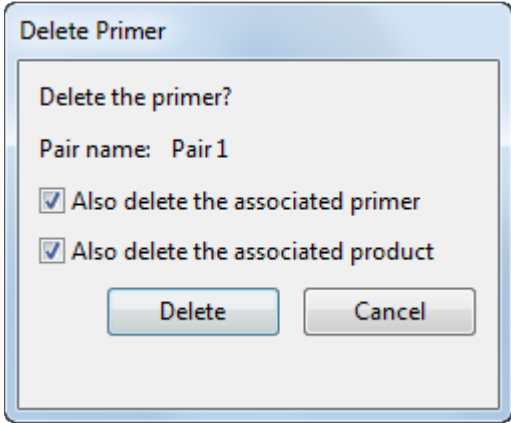
To export primers:

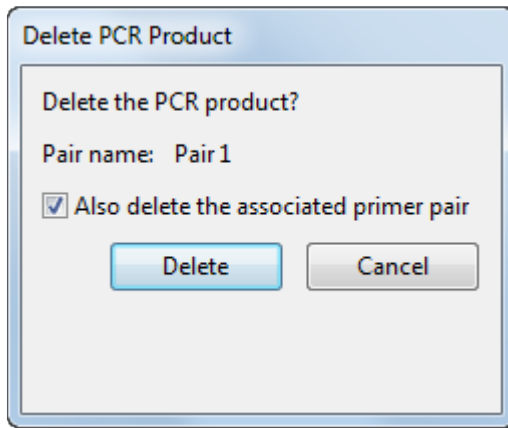
1. [Create or import primers](#).
2. Open the [Primers view](#).
3. Choose **File > Save Primer Catalog**.
4. Select one of the following three formats:
 - - **Tab-delimited text** (.txt) – Values from the Set Name, Pair Name, T/B, and Primer Sequence columns are exported from the Primers, as well as the sequence range of the primer, and saved as a tab-delimited text file.
 - **FASTA** (.fas) * – * The primer sequences are exported as a single FASTA file. A name for each sequence is derived from the sequence range, the pair and set names, and whether the primer is on the top (T) or bottom (B) strand. For example: **T_1712..1731_Pair_1,Set_1**
 - **Primer catalog** (.pri) * – * The primers in the Primers are exported as a primer catalog for future use in SeqBuilder Pro.

Delete primers

To delete a primer or PCR product:

1. Open the [Primers](#) or [Primer Design](#) view.
2. Select the corresponding feature and select **Features > Delete** or **Edit > Delete Feature**. Or right-click on the feature and choose **Delete** or **Delete Feature** from the context menu.
3. Confirm the deletion as directed in the table below.

Feature type	Confirmation dialog
General feature	<p>“Are you sure you want to delete the feature...?”</p>  <p>Confirm the deletion by pressing Yes.</p>
Primer that is part of a primer pair	<p>“Delete the primer?”</p>  <p>To delete both primers from a pair, not only the selected primer, check Also delete the associated primer. To also delete the PCR product associated with the selected primer, check Also delete the associated product. After checking or unchecking the boxes, press Delete.</p>
PCR product	<p>“Delete the PCR product?”</p>



To delete both of the primers associated with the selected PCR product, check **Also delete the associated primer pair**. After checking or unchecking the box, press **Delete**.

Methods used in primer design

To learn about considerations for calculating primers and primer pairs, see the following topics:

- [Thermodynamic models](#)
- [Pentamer profile](#)
- [Annealing temperature](#)
- [Melting temperature](#)

Thermodynamic models

The stability of a particular double stranded sequence is related to the number of hydrogen bonds formed. GC rich sequences pair with greater stability than AT rich sequences, but sequence ordering is also a component of stability. For example, the duplex AA/TT forms a more stable pair than the duplex AC/GT due to the more favorable interaction of neighboring bases in the duplex. SeqBuilder Pro uses the nearest neighbor model to measure the stability of a given duplex. It is based upon the free energy of adjacent dinucleotides and calculated from dinucleotide entropy and enthalpy data presented in [Bresslauer et al.](#) for DNA, and [Freier et al.](#) for RNA.

The calculation of free energy (ΔG) is $\Delta G = \Delta H - T\Delta S$, where ΔH is enthalpy, ΔS is entropy and T is temperature. The value of T is 25°C.

The calculation of ΔH and ΔS is where the dinucleotide data come in. For a given sequence, $S(abcde)$: ΔH is the sum of dinucleotide ΔH values [$\Delta H(abcde) = \Delta H(ab) + \Delta H(bc) + \Delta H(cd) + \Delta H(de) + \Delta H_{ini}$], where ΔH_{ini} is a helix formation constant.

ΔS is the sum of dinucleotide ΔS values [$\Delta S(abcde) = \Delta S(ab) + \Delta S(bc) + \Delta S(cd) + \Delta S(de) + \Delta S_{ini}$], where ΔS_{ini} is a helix formation constant.

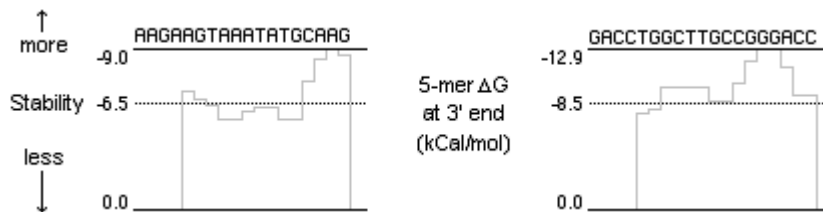
SeqBuilder Pro uses ΔG values computed over the entire template sequence and ΔG values computed in pentamer windows to evaluate the stability of a given primer. Selected primers will have ΔG values near the mean ΔG computed over the entire template, pentamer ΔG values lower than the mean at the 3' end, and for pair scoring, a stability profile of pentamer ΔG values above the mean near 5' and descending toward 3'.

Wherever possible SeqBuilder Pro uses the nearest neighbor method for computing the ΔG and the T_m of primers. These models are valid for sequences of less than 50 bases. For long primers, probes and products a less accurate method of computing free energy is used, which is based upon the GC content of the sequence.

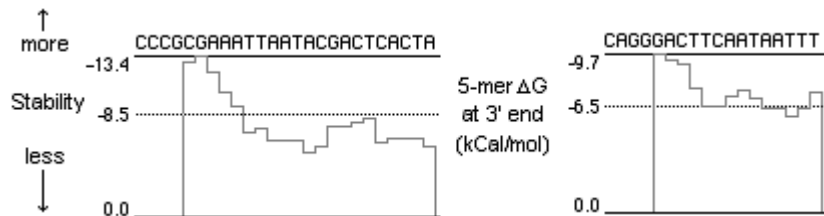
Pentamer profile

Pentamer free energy is one of the most important considerations in designing good primers. To perform well a primer needs to have low stability at the 3' end (usually higher than -8.5 kCal/mol) and higher stability toward the 5' end. This will help to eliminate false priming, as stability is low at the 3' end (where thermostable DNA polymerase binds), and higher upstream. This means that if a primer hybridizes at all (3' first), it will bind even more strongly with a good 5' upstream match. This is why a descending 5' -3' free energy profile is important.

For example, the primers below performed poorly due to their low pentamer ΔG values at the 3' end. Their high 3' stability makes it easy to bind in many template locations.



By contrast, the following primers performed well, due to their higher pentamer ΔG values at the 3' end. Pentamers with higher ΔG value (less negative!) are less stable. They would not hybridize in as many template locations due to the low 3' stability, but would bind more strongly upstream.



Annealing temperature

To amplify products with high fidelity it is important to choose an annealing temperature that is optimal for the specific combination of primers and template sequence. A carefully chosen annealing temperature (T_a) will significantly affect product yield and reduce the amplification of partially polymerized products. T_a calculations used by SeqBuilder Pro are as presented in [Rychlik et al.](#) :

$$T_a = 0.3 T_m \text{ Primer} + 0.7 T_m \text{ Product} - 14.9$$

Where T_m Primer is the minimum T_m of the two primers using the appropriate method and T_m Product is the product T_m using the appropriate GC content formula.

Melting temperature

Melting temperature is an important factor in primer design. After the template sequence has been evaluated for free energy, SeqBuilder Pro can then determine melting temperatures for the template and subsequently located primers. PCR primer pairs which perform well have closely matched melting temperatures to promote amplification of both strands in equal amounts. The T_m of primers is calculated using entropy and enthalpy data from the nearest neighbor models:

$$T_m = 16.6 \log[\text{salt}] - \frac{273.15 + \Delta H}{(\Delta S + R \ln(\frac{c}{4}))}$$

...where salt is the concentration of Na^+ in moles, R is the molar gas constant 1.987 cal/mol°C, and c is the initial concentration of the primer.

For long primers and probes (greater than 50 bases) and for products, a formula based upon GC content ([Baldino et al. 1989](#)) is used to calculate T_m .

For DNA:

$$T_m = 81.5 + 16.6 \log[\text{salt}] + \frac{41(\text{GC}\%)}{100} - \frac{65(\% \text{formamide})}{100} - \frac{675}{\text{length}}$$

For RNA:

$$T_m = 79.8 + 18.5 \log[\text{salt}] + \frac{58.4(\% \text{GC})}{100} + \frac{11.8(\% \text{GC})^2}{100} - \frac{35(\% \text{formamide})}{100} - \frac{820}{\text{length}}$$

For DNA-RNA:

Replace 0.35 (%formamide) with 0.50 (%formamide), as presented in [Wahl et al.](#).

Plasmid Auto-Annotation

SeqBuilder Pro's built-in plasmid Feature Library contains over 150 curated features, and allows you to add your own features, as well.

If your vector sequences lack some annotations, or contain incomplete or obsolete versions of annotations, you can effortlessly annotate them from the curated versions. Conversely, if you prefer your vector sequence annotations to the curated versions, you can automatically update the Feature Library with the new information.

Watch this video to see how easy it is to update vector file annotations:

While the actual [auto-annotation](#) takes only a few seconds, there are several optional procedures you may wish to do before auto-annotating:

- [Create a custom feature database for auto-annotation searches](#)
- [Specify the features to use in auto-annotation](#)
- [Specify the appearance of features in the Annotation Results window](#)

Create a custom feature database for auto-annotation searches

When you [auto-annotate vector sequences](#), SeqBuilder Pro searches for potential matches in the installed Feature Library, and in a custom database of features named “User” features. Creating a custom “User” database is optional.

To create a “User” database and add features to it:

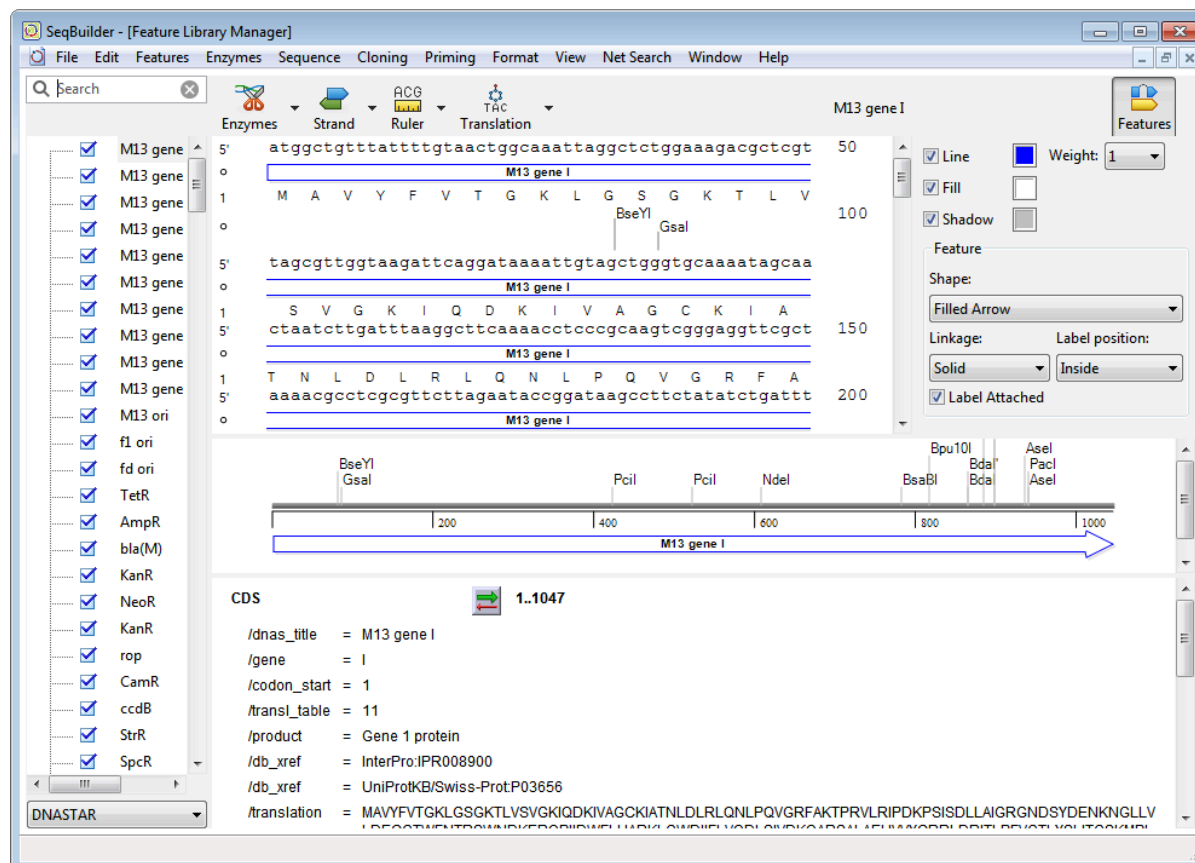
1. Move sequences to the Feature Library by doing either of the following:
 - - From the [Circular](#), [Linear](#), or [Sequence](#) views, click on a feature or select a range containing the desired feature(s). The selection must be at least 15 bp in length. Then choose **Features > Send to Feature Library**; or select the feature and use **Features > Send to Feature Library**.
 - Use **Features > Feature Library Manager** to open the [Feature Library Manager](#). Then drag a sequence at least 15 bp in length from your computer’s file explorer and drop it on the library window.

If you send a sequence range or an entire sequence to the database, any features are stripped out of the sequence and saved as separate entries in the database.

2. Repeat the above step until you have added all the desired features. Note that if you add the same feature multiple times, all of them will be saved in the database. However, during auto-annotation, only one will be selected as the “best” match to the query sequence.
3. Select **File > Save Project**. In most cases, you should save the file with the original name and location. An exception is if you will be creating different User databases for different organisms. In that case, save each database using a unique descriptive name and the default *.library* extension.

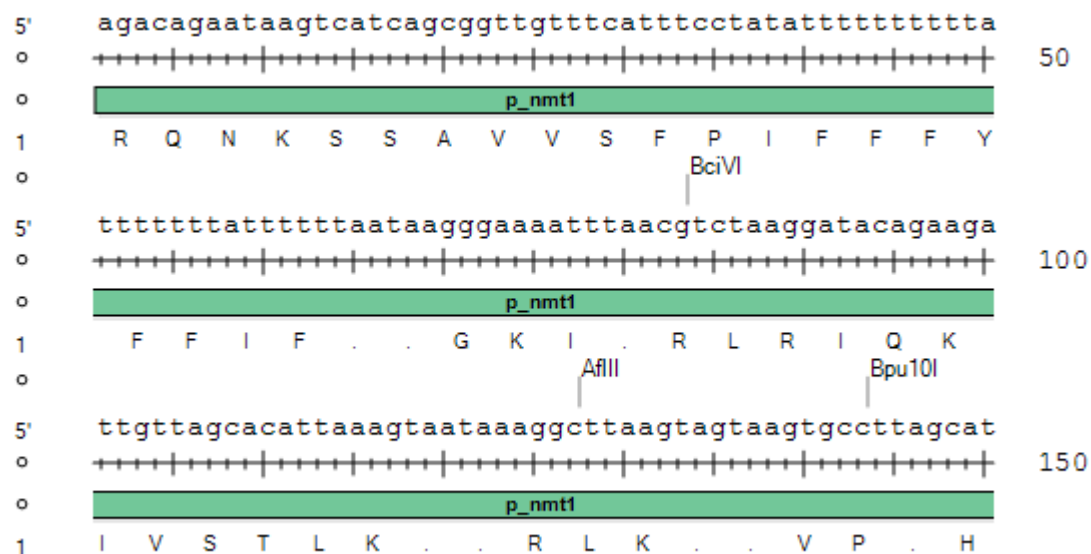
Feature Library Manager

The Feature Library Manager is a browser for the over 150 entries in SeqBuilder Pro's built-in plasmid Feature Library, and allows you to add your own features to the database. This window also lets you [specify the appearance of features](#) in the [Annotation Results window](#).

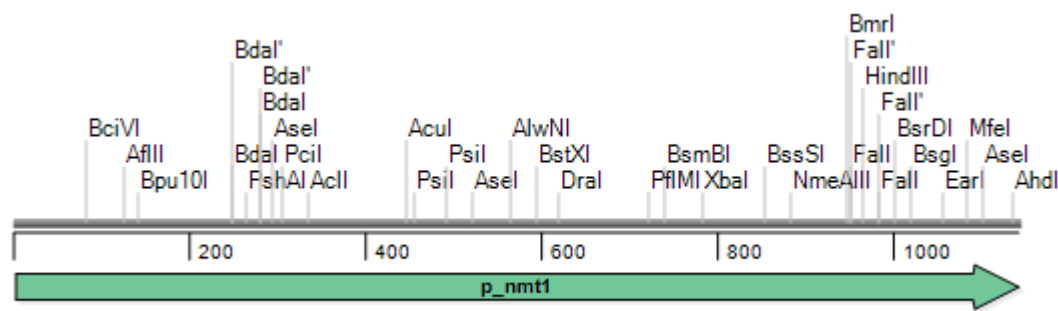


The tools at the top of the window control aspects of how the Sequence view, and sometimes the Linear view, are displayed or formatted in this window. The selected formatting will also be used for any auto-annotations that originate from the Annotation Results window. Use of these tools is described in [Specify the appearance of features in the Feature Library Manager and the Annotation Results window](#).

The top pane in the main portion of the window shows the [Sequence view](#) for the selected feature. The display of cut sites, sequence(s), translations, rulers and features in this section depends on choices made using the tools at the top of the window. When multiple features are selected from the Feature list (see below), sequences and translations in this view are displayed as a series of n's, and no cut sites can be displayed.



The middle pane of the Feature Library Manager displays the Linear view for the selected plasmid feature. The display of cut sites and features in this section depends on choices made using the tools at the top of the window. When multiple features are selected from the Feature list (see below), no cut sites can be displayed in this view.



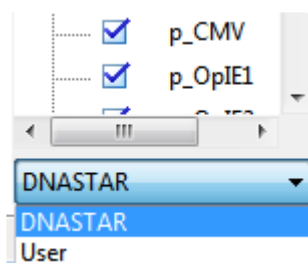
The bottom pane of the Feature Library Manager shows the Feature list for the selected plasmid feature. Items in this section can be edited by clicking on them and typing, just as in SeqBuilder Pro's main [Features view](#). If multiple items are selected in the Feature list, this pane is blank.

Specify the features to use in auto-annotation

To specify the features to use in auto-annotation:

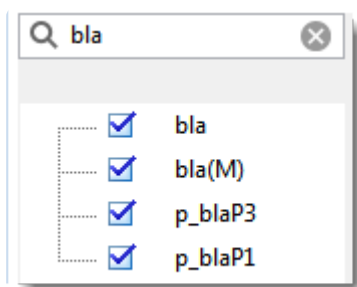
Specifying which features to use in [auto-annotation](#) is optional.

1. Choose **Features > Feature Library Manager**. This automatically loads the installed database file named *feature.library*. The Feature list on the left of the [Feature Library Manager](#) is populated with the name of each feature in the installed database, but the rest of the window is unpopulated.
2. (optional) To choose a different feature database, use the drop-down menu under the list. **DNASTAR** represents the installed library features, while **User** represents a [custom database](#).

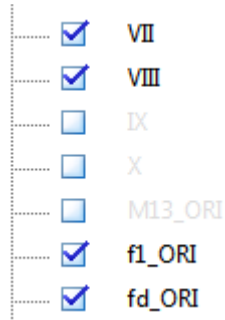


3. To populate the remainder of the window, select a feature from the Feature list by clicking on its name. You can select multiple features using **Ctrl/Cmd+click** or **Shift+click**, but sequence bases, translations and cut sites will not be displayed in this case. Multiple selection is used primarily to apply style changes to a number of library items simultaneously (see below).

To find a feature of interest, type its name (or a portion of its name) in the search bar above the list and press **Enter/Return** on the keyboard. To clear search results and return to the full list, press the 'x.'



4. To specify which features to include/exclude from an [auto-annotation search](#), check or uncheck the boxes next to their names. By default, all features on the list are checked. Unchecked features are grayed out.






5. To save changes made in the Feature Library Manager, choose **File > Save Project**. These are the two types of changes that are preserved even when you update from one Lasergene version to another:
 - - The presence or absence of check marks in the Feature list on the left. Unchecked items will not be considered when searching for matches in the Annotation Results window.
 - Changes made in the Features pane (for changing the format style) are used in auto-annotation of features in the [Annotation Results window](#).

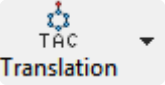
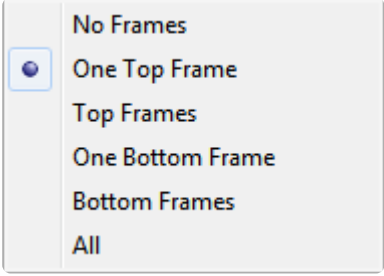

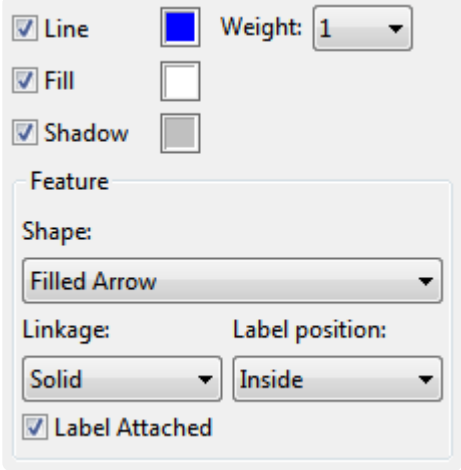
Specify the appearance of features in the Feature Library Manager and Annotation Results window

You can use the tools at the top of the [Feature Library Manager](#) to control how features are displayed in the Manager, itself, and in the [Annotation Results window](#) after [performing the auto-annotation procedure](#). This step is entirely optional.

For example, you could specify that all *lacZ* features be shown using light blue arrows outlined in purple. Later, when you looked for query matches in the Annotation Results window, all *lacZ* features would be displayed in the specified style. To try a similar workflow, see [Try it! – Create a style set for CDS features](#).

The following table describes how to use the tools to change the appearance of features.

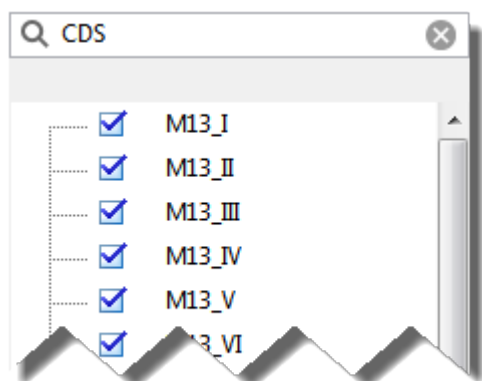
Tool	Menu options	Lets you specify...
 <p>Enzymes</p>	<div> None Unique Sites <input checked="" type="radio"/> Six+ cutters Blunt 5' Overhang 3' Overhang Two cuts max AbaSI Compatible Fnu4HI Compatible BstKTI Compatible AbaSI Compatible </div>	<p>Whether to display a particular class of enzyme cut sites, or no cut sites, on both the Sequence and Linear views. The available selectors are a subset of those available using the Filter by Selectors check boxes in the upper pane of the main SeqBuilder Pro Document window.</p> <p>Note: When multiple features are selected from the Features view, no cut sites are displayed, regardless of the selection made here.</p>
 <p>Strand</p>	<div> <input checked="" type="radio"/> Top Strand Both </div>	<p>Whether to show the sequence for the top strand, or for both top & bottom strands, on the Sequence view.</p>
 <p>Ruler</p>	<div> <input checked="" type="radio"/> No Rulers Sequence Ruler Translation Ruler Both </div>	<p>In the Sequence view, whether to place a ruler under the sequence, under the translation, neither, or both. Choosing Translation Ruler or Both causes a translation of the sequence to appear, along with the sequence.</p>

<p>Translation</p>  <p>Translation</p>		<p>Whether to display translations in the Sequence view for 1 or 3 reading frames and for top and/or bottom strands. The options are similar to the Full Translation checkboxes in the upper pane of the main SeqBuilder Pro Document window.</p>
<p>Features</p>  <p>Features</p>		<p>Style options for annotations in the Sequence and Linear views.</p> <ul style="list-style-type: none"> • To display an outline around the feature, check the Line box and choose a line Weight using the drop-down menu. • To select a different line color from the current color, click on the colored box to open the color chooser. • To hide the outline, uncheck the Line box. • To provide a solid fill color or drop shadow for the feature, check the Fill or Shadow box. If a different color is desired, click on the corresponding colored box to open the color chooser. • To remove the fill color or drop shadow, uncheck the box on the left. • To choose a different shape for the feature, select an option from the Shape drop-down menu. • To choose what type of line to use in linking multi-segment features, make a selection from the Linkage drop-down menu. The “DNASTAR” (installed) library does not contain multi-segment features, but such features can be added as “User” features. • To include a label for the feature, check Label Attached. Use the Label Position drop-down menu to specify a location for the label.

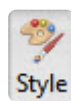
Try it! – Create a style set for CDS features

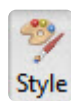
The [Feature Library Manager](#) can be used to specify how to display particular features or groups of features when auto-annotating plasmids/vectors in the [Annotation Results window](#). This tutorial shows how to specify a particular style for displaying all CDS features, but can be adapted to design style sets for any individual feature or group of features in the Feature Library Manager.

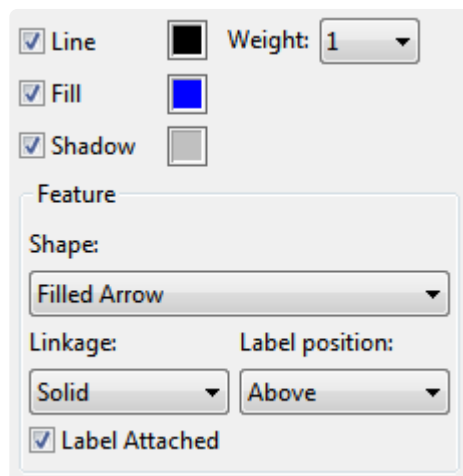
1. Choose **Features > Feature Library Manager**.
2. In the Search bar on the top left, type in *CDS* and press **Return/Enter** on your keyboard. All non-CDS features will be removed from the Feature list below the search bar.



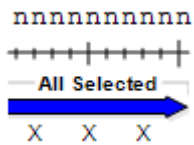
3. Select all items remaining in the Feature list (i.e., the CDS features) by clicking on the first feature on the list, and then **Shift+clicking** on the last feature.



4. Click on the **Style** tool at the top right of the window () to open the Style pane.

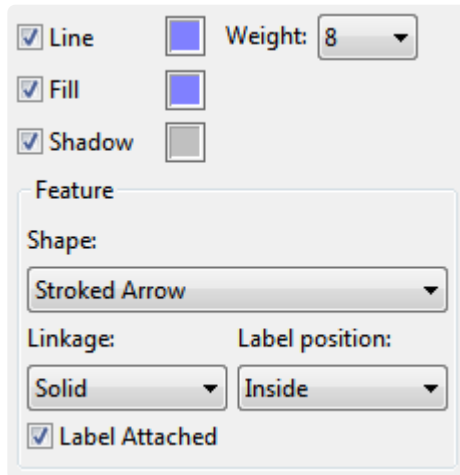


The feature is currently displayed as:

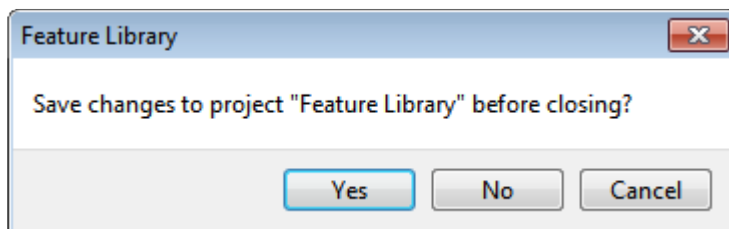


5. Change the **Line** and **Fill** colors to lilac by clicking on the corresponding color boxes. Change the **Weight** to **8**, and change the **Shape** to **Stroked Arrow**. Specify a **Label position** of **Inside**.

The feature is now displayed as:



6. Press the 'x' in the top corner of the window to close it. When prompted to save, choose **Yes**.



7. Follow the steps in [Perform the auto-annotation procedure](#). CDS features will preserve the style formatting set up in the steps above.

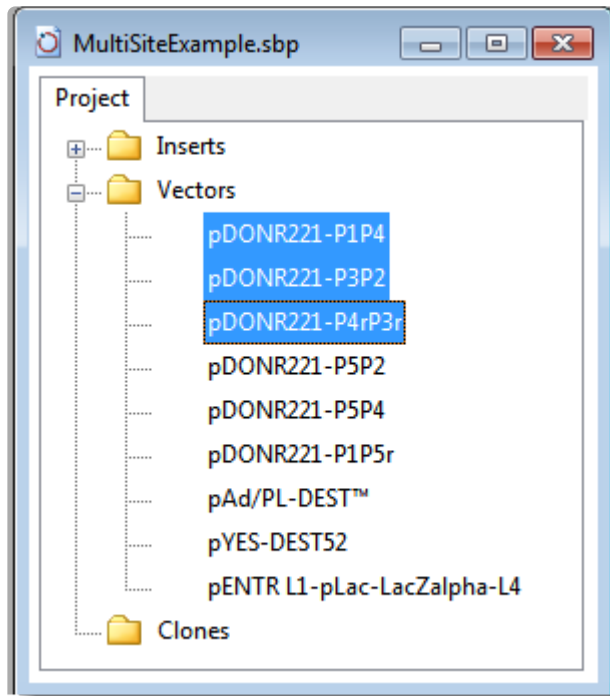
Perform the auto-annotation procedure

If your vector sequences lack some annotations, or contain incomplete or obsolete versions of annotations, the following procedure lets you effortlessly copy features from a curated [Feature Library](#), or from your own [custom features](#), to the query sequences. Conversely, if you prefer the query annotations to the library versions, you can automatically update the Feature Library version to match the annotations in the query sequences.

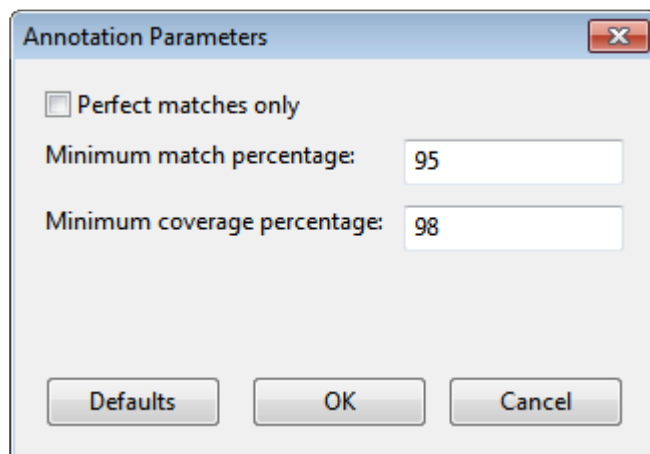
Before following the steps below, you may wish to [create a custom feature database](#), [specify the features to use in auto-annotation](#), or [specify the appearance of features in the Feature Library Manager and Annotation Results window](#). These preliminaries are entirely optional.

To auto-annotate a vector sequence from the installed Feature Library and/or your own custom feature database:

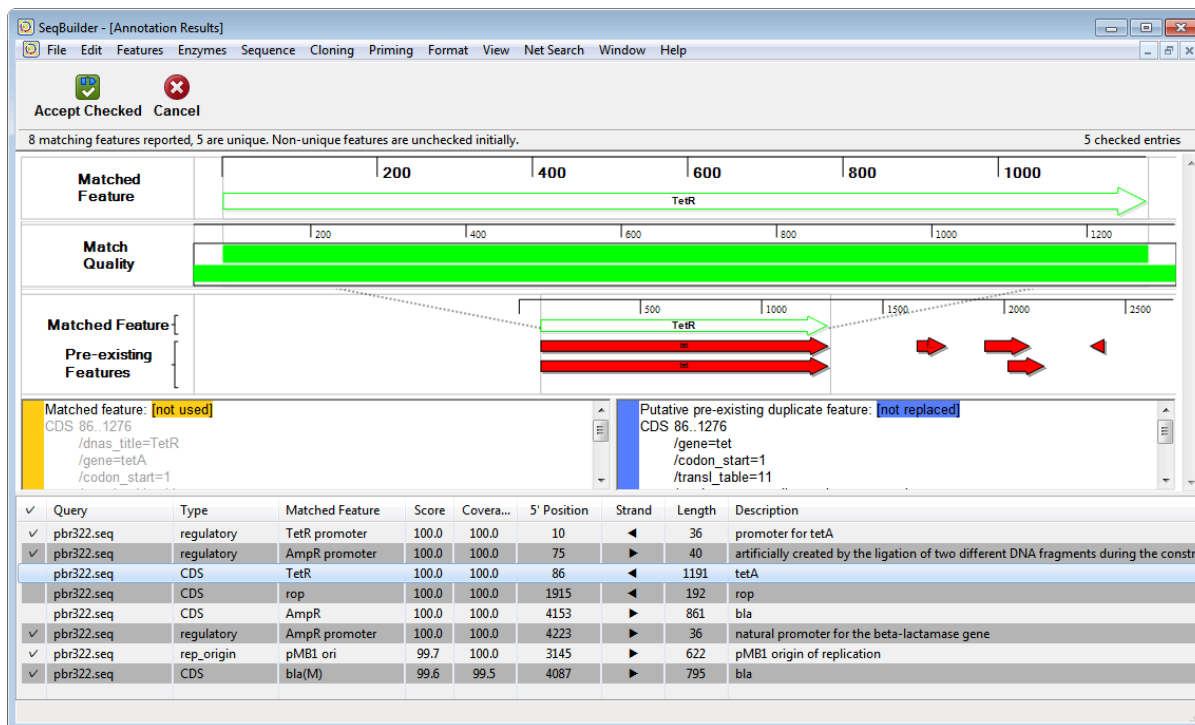
1. (optional) If you have [created multiple](#) “User” databases (e.g. for different organisms), you must change one of the databases to the default library name before proceeding. To do this, navigate to *C:\Users\UserName\AppData\Local\DNASTAR\UserFeatures.library* (Win) or *~/Library/Preferences* (Mac) and temporarily rename the file *UserFeatures.library*.
2. Use either of the following methods to specify which vector sequence(s) to auto-annotate:
 - - Open the desired sequence in the [Document window](#).
 - Open a cloning project in the [Project window](#). Expand the **Vectors** folder and select one or more files or folders.



3. Choose **Features > Annotate Sequence**. In the ensuing Annotation Parameters dialog, specify the thresholds for calling a match, or check **Perfect matches only** to effectively set each value to 100%. By default, SeqBuilder Pro calls a match when a feature sequence matches at least 95% of bases in the query sequence (**Minimum match percentage**) and overlaps the query sequence by 98% (**Minimum coverage percentage**). After making the desired selections, press **OK**.



The [Annotation Results window](#) opens. If the query sequence was already up-to-date, a message will appear: “No new features were found.” In this case, click **OK** to exit from the annotation procedure. Otherwise, the Annotation Results window will populate.



The sortable table at the bottom of the window will likely contain both checked and unchecked rows. A row with a checkmark in its leftmost column represents an annotation that is absent in the query sequence. A row without a checkmark represents an annotation that already exists in the query sequence. The version from SeqBuilder Pro's plasmid Feature Library may be more accurate or up-to-date than the annotation currently in the query sequence, so you may also wish to examine these entries. See The Annotation Results Window for detailed information about the contents of each pane of the window.

✿ Features shorter than 15 bases in length are omitted from searches, even if they exist in the Feature Library as "User" features.

- Click on a feature row to make it the active feature and to populate the rest of the window with information relevant to that feature.
- Use evidence in this window (e.g., match quality, shared endpoints, etc.) to determine whether or not to copy a feature to the query sequence. For instance, you may wish to copy the feature if the plasmid database version is more complete, or if it represents a new feature that does not yet exist in the query sequence.

Example:

An unchecked row was selected in the annotation match table at the bottom of the window. The Matched Feature / Putative Duplicate Feature panes (below) reveal that both features cover an identical range, good evidence that they are duplicates. The feature in the query sequence is named *Km(R)*, while the plasmid Feature Library calls it *KanR* but refers to "Kanamycin kinase" and "Geneticin®" in the /note and /function fields, hinting further at a possible match.

Matched feature: [not used] CDS 3111..3920 /dnas_title=KanR /gene=nptI /gene_synonym=aph(3')-Ia /gene_synonym=neo /gene_synonym=aphA1 /gene_synonym=aph(3')-Ia /codon_start=1 /transl_table=11 /product=Aminoglycoside 3'-phosphotransferase /EC_number=2.7.1.95 /note=APH(3')-I; Kanamycin kinase, type I; Neomycin-kanamycin phosphotransferase /function=confers resistance to neomycin, kanamycin, and G418 (Geneticin) /translation=MSHIQRETSRPLNSNMDADLYGYKWARDNVGQSGATYRL	Putative pre-existing duplicate feature: [not replaced] CDS 3111..3920 /dnas_title=Km(R) /invitrogen=1200000 /ntifkey=4 /label=Km(R)
---	--

As it happens, Invitrogen/Life Technologies' Geneticin® is the antibiotic also called G418. It is an aminoglycoside related to Gentamicin. The enzyme encoded by this gene confers resistance to Geneticin as well as the related antibiotics Neomycin and Kanamycin. So *Km(R)* and *KanR* are indeed the same gene. However, the plasmid Feature Library version on the left contains considerably more detailed information, and you will probably want to use this information to overwrite the feature currently in the query sequence.

6. To copy checked features from the Feature Library to your query sequence(s) (or vice versa, for rows

in which **Replace** has been specified), click on the **Accept Checked** tool (). If you

instead wish to exit without copying any features, click the **Cancel** tool ().

7. If you copied any features to the query, we recommend immediately using **File > Save Project** to save them. Otherwise, if you later close the project without saving, any changes will be lost.

Annotation Results Window

The Annotation Results window displays putative matches between SeqBuilder Pro's plasmid Feature Library and one or more query sequences, usually vector sequences from a cloning project. If the query sequence lacks some/all of the located features from the plasmid Feature Library, or contains incomplete or obsolete versions of certain features, this window allows you to copy features from the database to the query sequence. To open the window, select one or more sequences from the Project window and then choose **Features > Annotate Sequence**.

This topic describes the layout of the window. To learn how to perform a search, evaluate putative feature matches and add them to the query sequence, refer to [Perform the auto-annotation procedure](#).

SeqBuilder - [Annotation Results]

File Edit Features Enzymes Sequence Cloning Priming Format View Net Search Window Help

Accept Checked Cancel

8 matching features reported, 5 are unique. Non-unique features are unchecked initially. 5 checked entries

Matched Feature: 200 400 600 800 1000

Match Quality: 200 400 600 800 1000 1200

Matched Feature: 500 1000 1500 2000 2500

Pre-existing Features: 500 1000 1500 2000 2500

Matched feature: [not used]
CDS 86..1276
/dnas_title=TetR
/gene=tetA
/codon_start=1

Putative pre-existing duplicate feature: [not replaced]
CDS 86..1276
/gene=tet
/codon_start=1
/transl_table=11


Query	Type	Matched Feature	Score	Covera...	5' Position	Strand	Length	Description
✓ pbr322.seq	regulatory	TetR promoter	100.0	100.0	10	◀	36	promoter for tetA
✓ pbr322.seq	regulatory	AmpR promoter	100.0	100.0	75	▶	40	artificially created by the ligation of two different DNA fragments during the construction
✓ pbr322.seq	CDS	TetR	100.0	100.0	86	◀	1191	tetA
✓ pbr322.seq	CDS	rop	100.0	100.0	1915	◀	192	rop
✓ pbr322.seq	CDS	AmpR	100.0	100.0	4153	▶	861	bla
✓ pbr322.seq	regulatory	AmpR promoter	100.0	100.0	4223	▶	36	natural promoter for the beta-lactamase gene
✓ pbr322.seq	rep_origin	pMB1 ori	99.7	100.0	3145	▶	622	pMB1 origin of replication
✓ pbr322.seq	CDS	bla(M)	99.6	99.5	4087	▶	795	bla

Components of the Annotation Results window are described below from top to bottom. However, note that the selection made in the lowermost pane determines the contents of all other panes.

After you have examined putative feature matches in the table, use the **Accept Checked** tool (



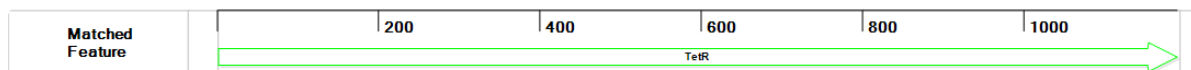
) at the top of the window to copy all features checked in the table below to the query

sequence. Alternatively, click **Cancel** () to close the Annotation Results window without copying any features.

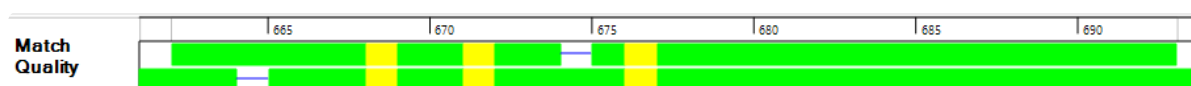
The header area shows how many matching features were found, and how many were unique.

8 matching features reported, 5 are unique. Non-unique features are unchecked initially.

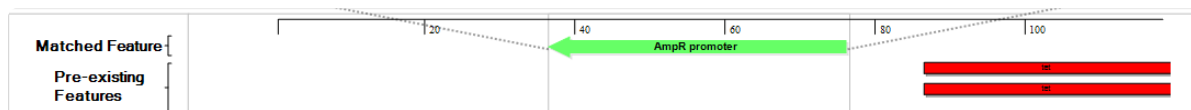
The Matched Feature pane near the top of the window shows the full width of the selected feature in the style specified in the Feature Library Manager. If no style has been specified, it is instead shown in the default style.



The Match Quality pane displays an alignment histogram that uses the query vector's coordinate system to show where a plasmid Feature Library feature matches the query sequence. The top bar represents the feature from the plasmid database, while the bottom bar shows the location of that match on the query. Gaps appear as empty space, mismatches are displayed in yellow, and matches appear in green.



The Matched Feature/Context Features pane uses the query vector's coordinate system to show features that overlap or are near to the feature selected in the table. Gray vertical lines at the endpoints of the feature can be used to find features with identical or near-identical endpoints. Imperfect matches can still represent duplicates. For corroborating evidence, look for significant overlap in features, as well as text field matches.



The Matched feature and Putative feature panes just above the “annotation match table” lets you compare text information from the feature in the query sequence (on the right) and the putative plasmid Feature Library match (on the left). If the query sequence does not already contain the putative feature (i.e., the row in the table below is checked by default), the right pane will say: “No putative duplicate found.”



The Annotation match table in the lower half of the window consists of a list of annotations that match the query sequence at the specified thresholds for match and coverage percentage. To sort entries alphanumerically based on a particular column, click on the corresponding column header.

✓	Query	Type	Matched Feature	Score	Coverage	5' Position	Strand	Length	Description
✓	pbr322.seq	regulatory	TetR promoter	100.0	100.0	10	◀	36	promoter for tetA
✓	pbr322.seq	regulatory	AmpR promoter	100.0	100.0	75	▶	40	artificially created by the ligation of two different DNA fragments during ...
	pbr322.seq	CDS	TetR	100.0	100.0	86	◀	1191	tetA
	pbr322.seq	CDS	rop	100.0	100.0	1915	◀	192	rop
	pbr322.seq	CDS	AmpR	100.0	100.0	4153	▶	861	bla
✓	pbr322.seq	regulatory	AmpR promoter	100.0	100.0	4223	▶	36	natural promoter for the beta-lactamase gene
✓	pbr322.seq	rep_origin	pMB1 ori	99.7	100.0	3145	▶	622	pMB1 origin of replication
✓	pbr322.seq	CDS	bla(M)	99.6	99.5	4087	▶	795	bla

The table columns are described below:

Column name	Description
✓	<p>A check mark at the left of a row signifies a unique annotation: it exists in the plasmid Feature Library, but not in the query sequence. If multiple versions of an annotation match the query, only the best match is checked, by default. An unchecked row signifies either a non-unique annotation or a duplicate annotation where one of the other duplicates was a better match. The leftmost table cell for each row is used to add or remove these check marks manually. When you click on the cell, a menu opens with the following choices:</p> <div> <div>✓ Accept</div> <div>↺ Replace</div> <div>Ignore</div> </div> <ul style="list-style-type: none"> • Accept – To transfer the Feature Library version of this annotation to the query sequence. • Replace – To overwrite the pre-existing query sequence version of this feature with the Feature Library version. • Ignore – To remove an Accept or Replace designation from the row.
Query	The name of the query sequence that the plasmid Feature Library match pertains to.
Type	Feature type (e.g., CDS, regulatory, rep_origin, etc.)
Matched Feature	Name of the feature for which a match was found.
Score	Likelihood that the plasmid database feature is a match for the query sequence, based on calculations made within SeqMan NGen.
Coverage	(Length of the plasmid database feature / length of the putative match in the query sequence) x 100%
5' Position	5' Position of the plasmid database feature on the query sequence's coordinate system.
Strand	Contains an arrow showing the strand direction, left (◀) or right (▶).
Length	Length of the plasmid database feature.
Description	Description of the plasmid database feature.

Automated Virtual Cloning

SeqBuilder Pro supports automated virtual cloning using a wide variety of cloning techniques. Simply select your insert and vector sequences, and SeqBuilder Pro will automatically construct the virtual clone. Choose vectors from the vector catalog provided, or add your own custom vectors with ease. Once your clone is made, the Cloning Summary view shows details about the fragment inserts, primers, fragment junctions, as well as the steps needed to prepare the vector and amplify the insert. The following video is a brief introduction to virtual cloning in SeqBuilder Pro:

Virtual cloning consists of optional preliminary steps and the main cloning workflow.

Preliminary steps:

These optional steps take about 2-20 minutes each, but can provide benefits for many future cloning workflows.

- Add custom vectors to the cloning vector catalog
 - [Create a custom vector for Gateway cloning](#)
 - [Create a custom vector for TA cloning](#)
 - [Create a custom vector for directional TOPO cloning](#)
- [Auto-annotate vector sequences](#)

Main cloning workflow::

The main cloning workflow takes only a few minutes from start to finish. However, for purposes of explanation, it has been divided into seven steps.

1. [Start a cloning project and specify the insert](#)
2. [Specify the method and options](#)
3. [Specify the vector and entry clone](#)
4. [Digest/linearize a circular vector](#)
5. [Run the cloning simulation](#)
6. (optional) [Verify the clone](#)
7. [View and print cloning results](#)

Preliminary Step (optional): Add custom vectors to the cloning vector catalog

A comprehensive cloning vector catalog named *CloningVectors.sbp* is installed with Lasergene. Before you begin a cloning project, you can create and add custom vectors to this catalog to use with current and future projects. As you update Lasergene in the future, DNASTAR will update the vector catalog, but will not overwrite any vectors that you have imported or customized.

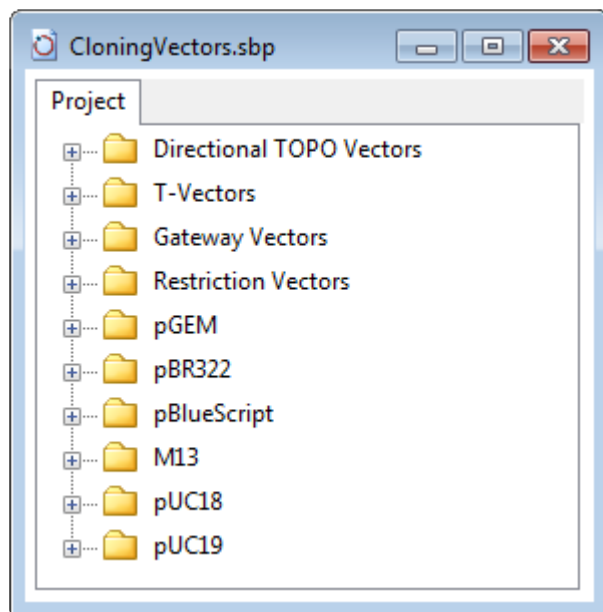
To add a vector to the installed catalog:

1. Use **File > Open** to open the following file:

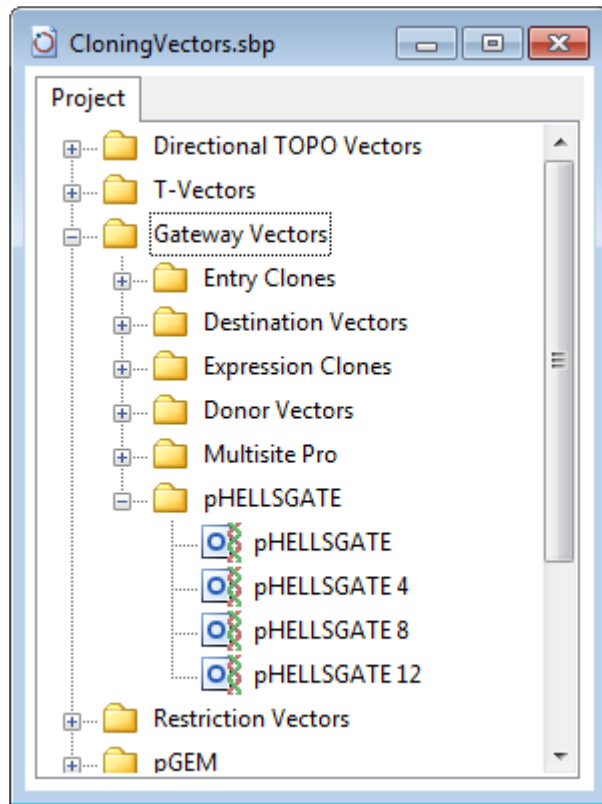
- **Windows:** *C:\ProgramData\DNASTAR Data\Lasergene 'x' Data\CloningVectors.sbp*
- **Macintosh:** *Hard Drive:Applications:DNASTAR:Lasergene 'x' Data:CloningVectors.sbp*

... where 'x' represents the version number.

The Project window opens to display folders containing different categories of vectors.



2. Click on the plus sign to the left of a folder to expand it and view its contents. Some folders contain vectors; others contain sub-folders that must also be expanded in order to view the vectors.



3. Decide which folder is best for your custom vector. Then right-click on the folder and select Import.
4. Select your custom vector sequence and press **Open** to add a copy of it to SeqBuilder Pro's vector catalog.
5. Choose **File > Save Project**.
6. Exit from SeqBuilder Pro.

When you next open SeqBuilder Pro and want to add a vector to a cloning project, you should see your vector in the folder to which you added it.

To learn how to create specialized vectors for different cloning methods, see:

- [Create a custom vector for Gateway cloning](#)
- [Create a custom vector for TA cloning](#)
- [Create a custom vector for directional TOPO cloning](#)



Important: Be sure to use the formatting described in these subtopics. This includes saving

the vector in linear or circular format, as required by the cloning method. Only vectors that have the specified formatting will appear in the **Vector** list when you do a cloning project.

Create a custom vector for Gateway cloning

If desired, a Gateway cloning vector may be constructed manually and then [added to the Cloning Vector Catalog](#) prior to beginning the cloning workflow. Prepare the vector as follows and refer to the example at the bottom of this topic.

- Set the type to circular by selecting **Sequence > Circular**.
- Add **misc_recomb** features to your sequence on the appropriate sub-sequences where recombination occurs. Note that while it is preferred for the entire recombination site to exist within your sequence, SeqBuilder Pro at minimum requires the appropriate att core sequence for each of the five types of recombination sites. SeqBuilder Pro uses these core sequences along with the feature labels to correctly identify each Gateway plasmid:
 - Type 1: **GTACAAA** (forward strand)
 - Type 2: **CTTGTAC** (reverse strand)
 - Type 3: **ATTATAC** (reverse strand)
 - Type 4: **GTATAGA** (forward strand)
 - Type 5: **GTATACA** (forward strand)
- Label the features with the following **/dnas_title** tags:
 - For Expression Clones: **attB1, attB2, attB3, attB4, and attB5**
 - For Donor Vectors: **attP1, attP2, attP3, attP4, and attP5**
 - For Entry Clones: **attL1, attL2, attL3, attL4, and attL5**
 - For Destination Vectors: **attR1, attR2, attR3, attR4, and attR5**



The classic Gateway cloning protocol uses only types **1** and **2** of recombination sites. MultiSite Gateway Pro cloning utilizes all 5 types of recombination sites, which allows you to recombine multiple regions at one time.

Example:

The pAd-PL-DEST sequence provided in SeqBuilder Pro's Vector Catalog is shown below as an example of how to use these tags:

misc_recomb 512..636

/dnas_title="attR1"

/note="attR1"

/label=attR1

/note="recombination site for the Gateway® LR reaction"

misc_recomb complement(2092..2216)

/dnas_title="attR2"

/note="attR2"

/label=attR2

/note="recombination site for the Gateway® LR reaction"

After creating and saving the vector, [add it to the Cloning Vector Catalog](#) so it can be used with future projects.

Create a custom vector for TA cloning

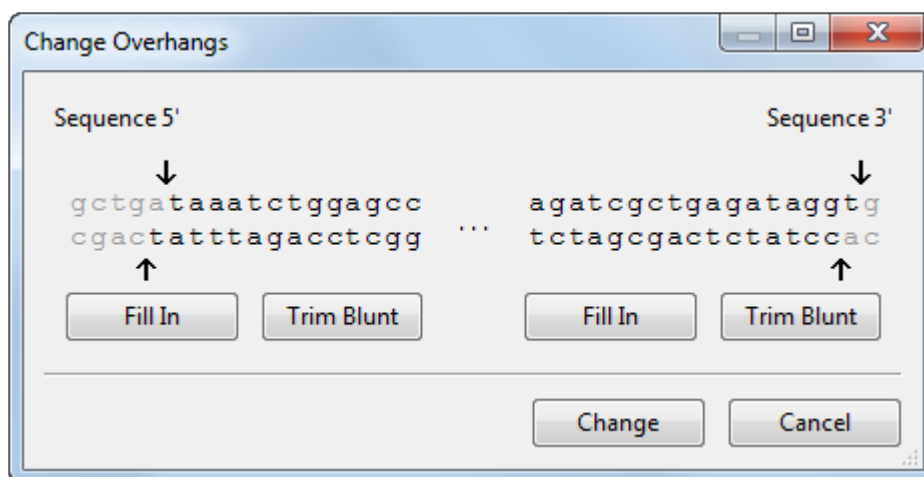
If desired, a T-vector for TA cloning may be constructed manually and then [added to the Cloning Vector Catalog](#) prior to beginning the cloning workflow.

To make the vector recognizable for TA cloning, a specific overhang must be created on both strands at the 3' ends.

1. Use **File > Open** to open the file or SeqBuilder project containing the vector sequence.
2. Select **Cloning > Change Overhangs**.

In this dialog, you can use the **Trim Blunt** or **Fill In** buttons to simulate modification of overhangs for incompatible ends, or manually manipulate the overhangs by dragging the arrows located above and below the two strands. The **Revert** button lets you return to the default overhangs.

- a. Use the arrow sliders to leave one overhanging thymine (T) residue on the bottom left and another on the top right.



- b. Click **Change**.
3. Choose **File > Save**.
4. [Add the new vector to the Cloning Vector Catalog](#) so it can be used with future projects.

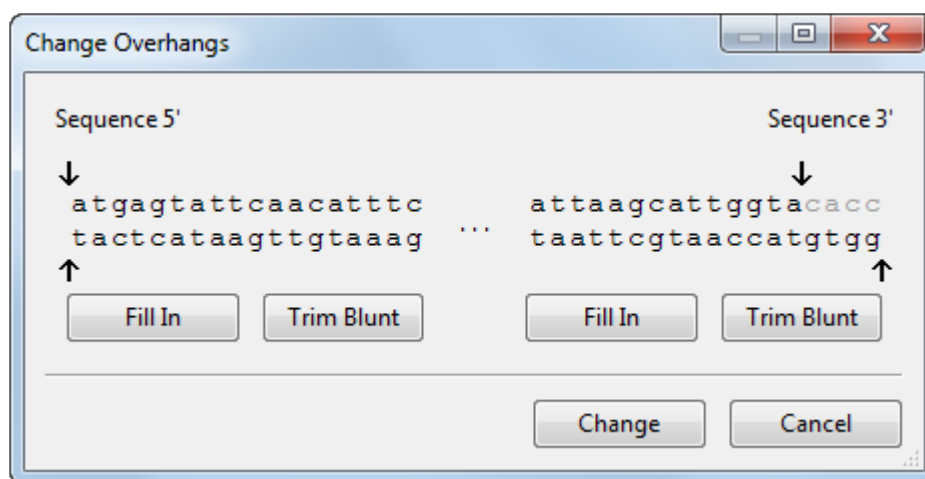
Create a custom vector for directional TOPO cloning

If desired, a directional TOPO cloning vector may be constructed manually and then [added to the Cloning Vector Catalog](#) prior to beginning the cloning workflow. To make the vector recognizable for directional TOPO cloning, a specific overhang must be created at the 3' end of the forward strand.

1. Use **File > Open** to open the file or SeqBuilder Project containing the vector sequence.
2. To the 3' (right) end of the vector sequence, add the bases **cacc**.
3. Choose **Cloning > Change Overhangs**.

In this dialog, you can use the **Trim Blunt** or **Fill In** buttons to simulate modification of overhangs for incompatible ends, or manually manipulate the overhangs by dragging the arrows located above and below the two strands. The **Revert** button lets you return to the default overhangs.

- a. On the left, make sure the pair is blunt, i.e. does not contain any overhangs. On the right, drag the top arrow to the left to “mask” the bases you just added (**cacc**). This results in a **gtgg** overhang on the bottom right.



- b. Click **Change**.
4. Choose **File > Save**.
5. [Add the new vector to the Cloning Vector Catalog](#) so it can be used with future projects.

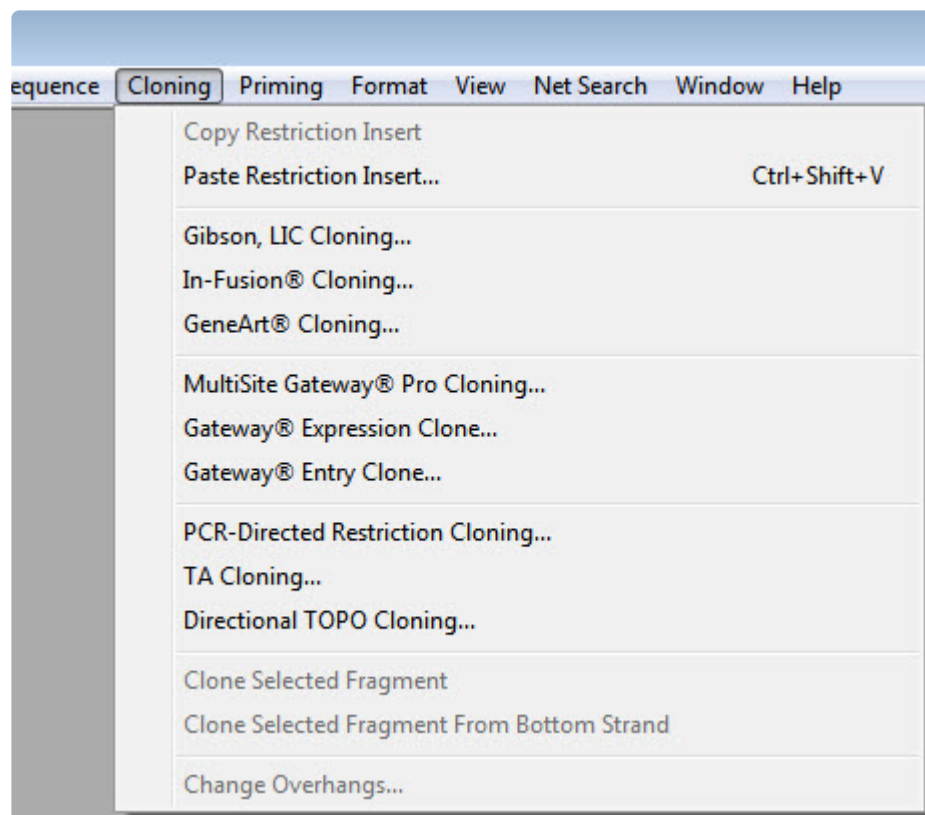
Step 1: Start a cloning project and specify the insert

The first step in performing a cloning simulation is to specify the insert. The following topics describe six different methods of specifying the insert to use with your cloning project:

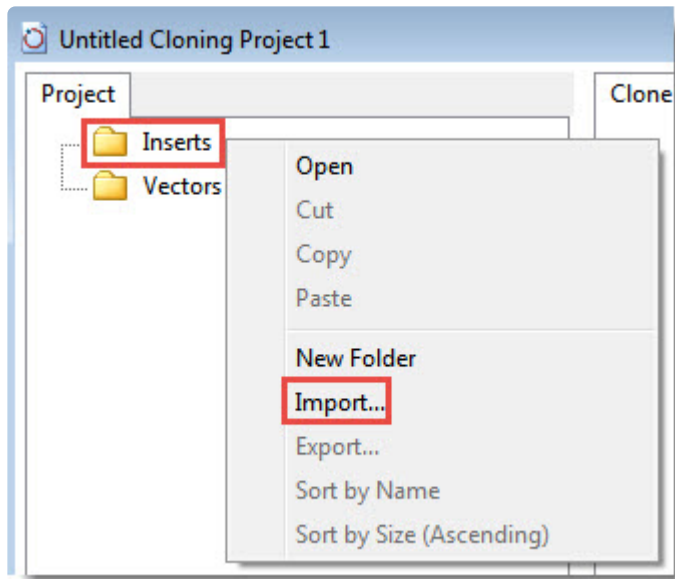
- [Option A – Use a sequence file on your computer as the insert](#)
- [Option B – Copy inserts from another SeqBuilder Pro cloning project](#)
- [Option C – Use an entire open SeqBuilder Pro sequence as the insert](#)
- [Option D – Use part of an open SeqBuilder Pro sequence file as the insert](#)
- [Option E – Use the PCR product of the current primer pair as the insert](#)
- [Option F – Use restriction enzyme cut sites to create the insert](#)

Option A – Use a sequence file on your computer as the insert

1. From the **Cloning** menu, select one of the following commands: **Gibson, LIC Cloning**; **In-Fusion Cloning**, **GeneArt Cloning**; **MultiSite Gateway Pro Cloning**; **Gateway Expression Clone**; **Gateway Entry Clone**; **Restriction Enzyme Cloning**; **TA Cloning**; **Directional TOPO Cloning**.



2. In the **Project** tab on the left of the [Project window](#), right-click on **Inserts** and choose **Import**.



3. Make a selection in the file chooser and click **Open**.
4. Expand the **Inserts** folder, if necessary, by clicking on the plus sign.
5. Drag the inserts from the **Inserts** folder and drop them in the **Fragments** section near the bottom right of the Project window.



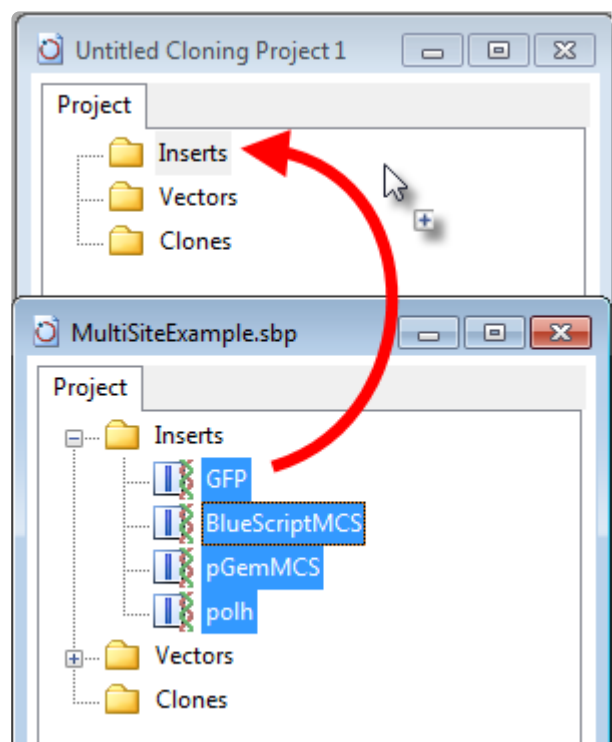
or...

1. Open your computer's file explorer and locate the insert sequence(s) of interest.
2. In SeqBuilder Pro, choose **File > New Cloning Project**.
3. Drag each insert from the file explorer to the **Inserts** folder of the Project window.
4. Expand the **Inserts** folder, if necessary, by clicking on the plus sign. Drag the inserts from the **Inserts** folder and drop them in the **Fragments** section near the bottom right of the window.

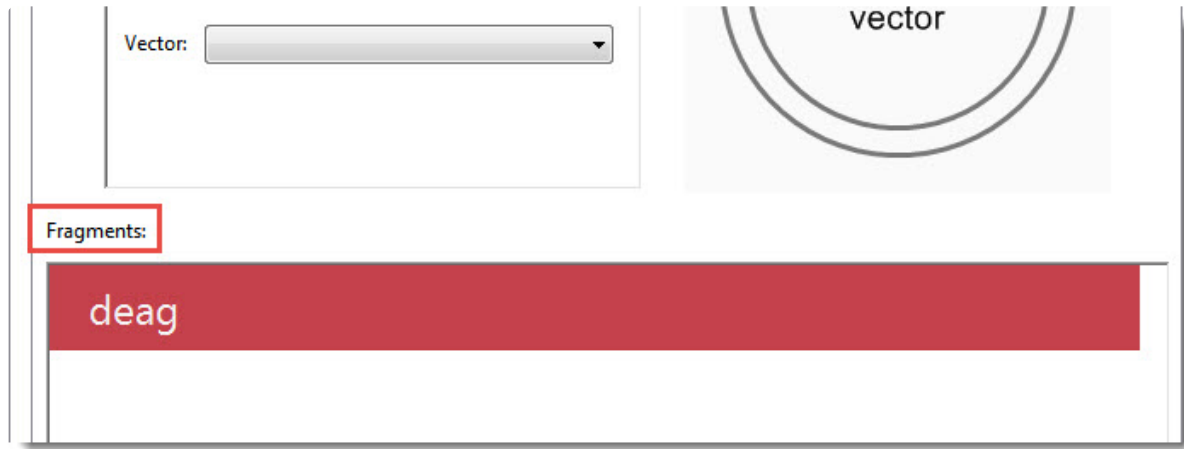
Proceed to [Step 2: Specify the method and options.](#)

Option B – Copy inserts from another SeqBuilder Pro cloning project

1. Open an existing SeqBuilder Pro cloning project that has the desired inserts in its **Inserts** folder.
2. Press **File > New Cloning Project**.
3. Drag the inserts from the original project's **Inserts** folder into the new project's **Inserts** folder.



4. Close the [Project window](#) for the older project.
5. Expand the new **Inserts** folder, if necessary, by clicking on its plus sign.
6. Drag the inserts from the **Inserts** folder and drop them in the **Fragments** section near the bottom right of the Clone tab.

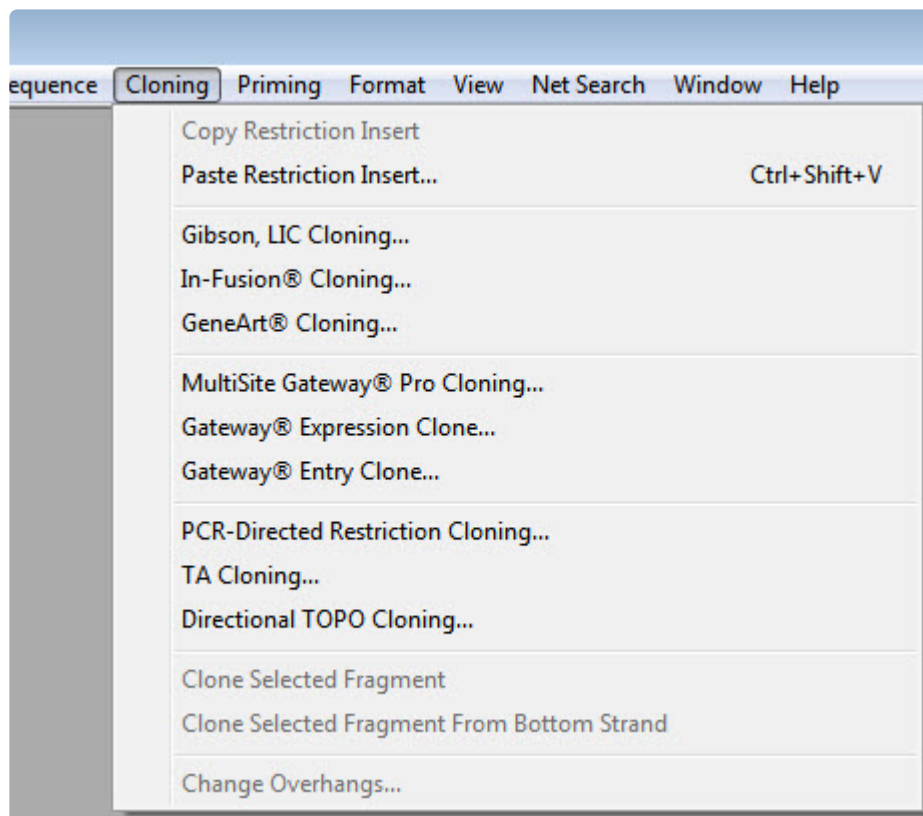


The screenshot displays the SeqBuilder Pro interface. At the top left, there is a 'Vector:' label followed by a dropdown menu. To the right of this is a diagram of a circular plasmid vector, labeled 'vector'. Below the 'Vector:' label, the word 'Fragments:' is highlighted with a red rectangular box. Underneath this box, a large red horizontal bar is visible, containing the text 'deag' in white. The rest of the interface is currently blank.

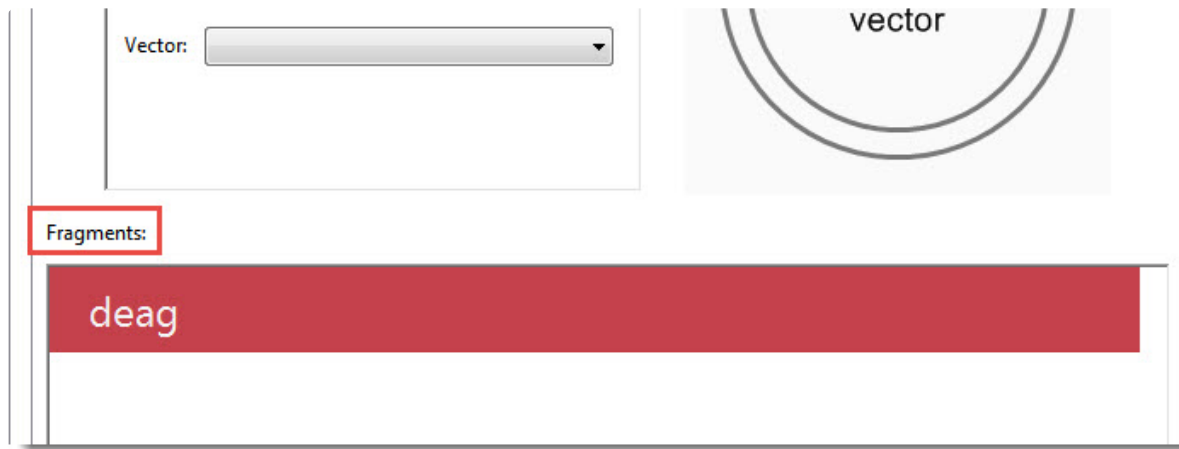
Proceed to [Step 2: Specify the method and options.](#)

Option C – Use an entire open SeqBuilder Pro sequence as the insert

1. Use **File > Open** to open the file or SeqBuilder Project (.sbd) containing the insert.
2. From the **Cloning** menu, select one of the following commands: **Gibson, LIC Cloning**; **In-Fusion Cloning**, **GeneArt Cloning**; **MultiSite Gateway Pro Cloning**; **Gateway Expression Clone**; **Gateway Entry Clone**; **Restriction Enzyme Cloning**; **TA Cloning**; **Directional TOPO Cloning**.



3. Choose **File > Add [sequence name]** to Untitled Cloning Project 'n,' where 'n' is a number. The [Document window](#) will become active again.
4. Return to the [Project window](#) by clicking on it. In the **Project** tab, drag and drop the sequence into the **Inserts** folder.
5. Expand the new **Inserts** folder, if necessary, by clicking on its plus sign.
6. Drag the inserts from the **Inserts** folder and drop them in the **Fragments** section near the bottom right of the Clone tab.



Vector:

vector

Fragments:

deag

Proceed to [Step 2: Specify the method and options.](#)

Option D – Use part of an open SeqBuilder Pro sequence file as the insert

1. Use **File > Open** to open the file or SeqBuilder Project (.sbd) containing the sequence fragment you wish to clone.
2. Use any of the following methods to select the sequence fragment to use as the insert:
 - - Manually select a portion of sequence by dragging the mouse across it.
 - Click on an existing feature to select the corresponding sequence.
 - Use the method described in [Using a PCR product of the current primer pair as the insert](#)
 - Use the method described in [Using restriction enzyme cut sites to create the insert](#). Note that you do not actually need use a restriction enzyme cut site as the sequence fragment. The sequence fragment can be anything you want: an open reading frame (ORF), multiple cloning site (MCS; polylinker), feature, or a random selection.
3. Choose **Cloning > Clone Selected Fragment**, or **Cloning > Clone Selected Fragment From Opposite Strand**, as needed. The latter command selects the opposite orientation of the sequence.

The [Project window](#) opens with the Clone tab active. The selection from Steps 1-2 is placed in the **Inserts** folder in the Project tab, as well as in the **Fragments** section near the bottom right of the Clone tab.

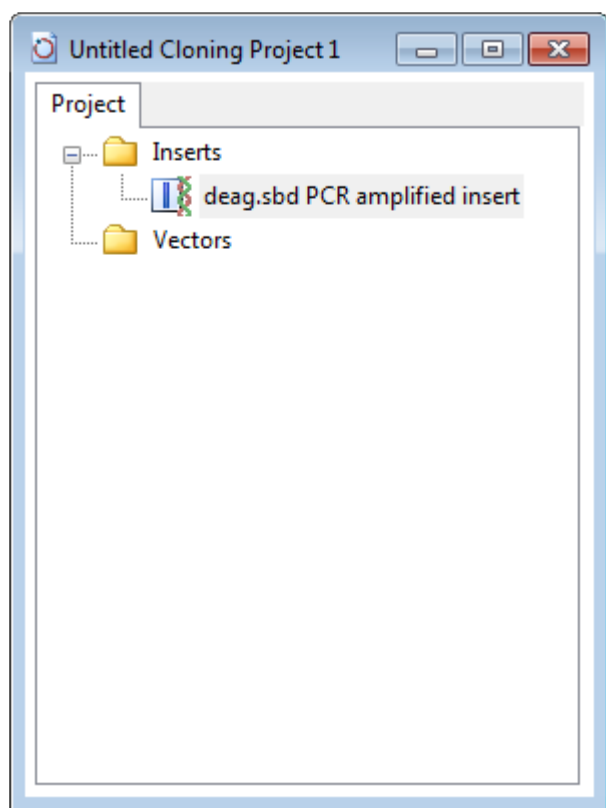
Proceed to [Step 2: Specify the method and options](#).

Option E – Use the PCR product of the current primer pair as the insert

If you will be using the TA cloning method, you can use the PCR product of the current primer pair as an insert.

1. Use **File > Open** to open the file or SeqBuilder Pro project (.sbd) containing the sequence of interest.
2. [Create primer pairs](#).
3. With the [Primer Design](#) or [Primers](#) view active, choose **Priming > Create Insert by PCR**.


SeqBuilder Pro extracts the sequence of the PCR product, including any modifications you have made, and simulates its amplification with Taq polymerase including the addition of 3' A overhangs to each end. Primer and product features are carried forward with the insert. The [Project window](#) opens with the new Taq-amplified insert in the **Inserts** folder.



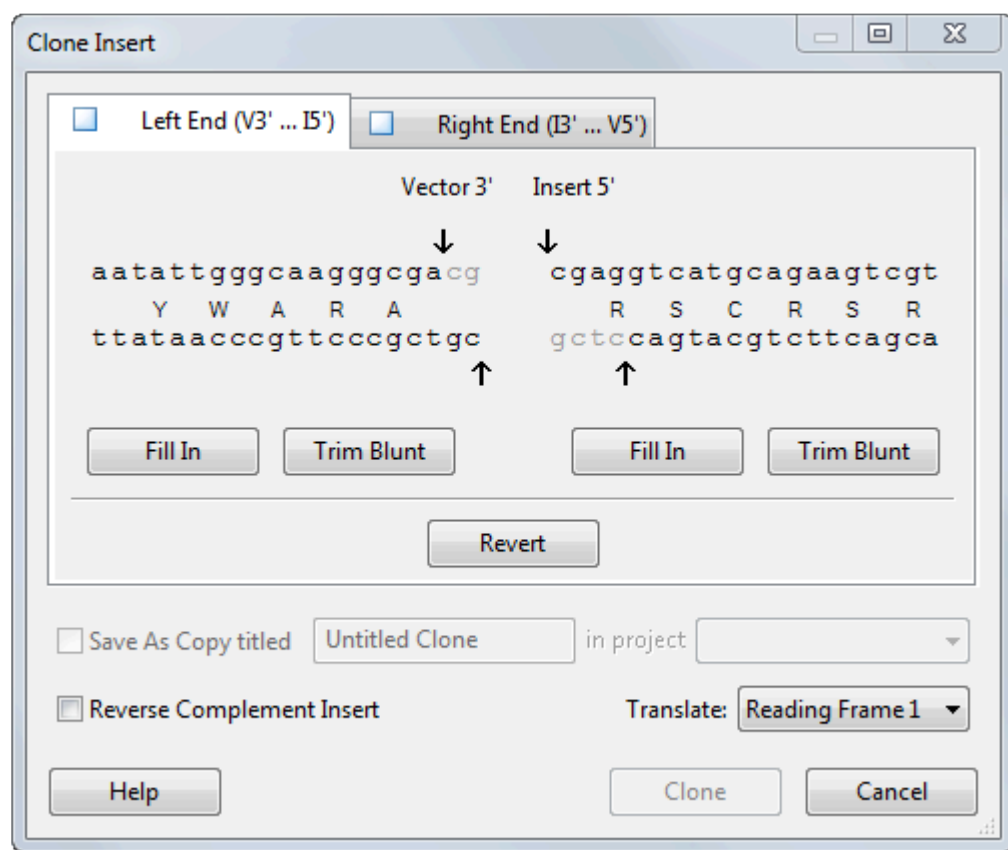
4. Drag the insert from the **Inserts** folder and drop it in the **Fragments** section at the bottom of the Cloning tab.

Proceed to [Step 2: Specify the method and options](#).

Option F – Use restriction enzyme cut sites to create the insert

1. Use **File > Open** to open the file or SeqBuilder Pro project (.sbd) containing the vector sequence.
2. Use the [Enzymes tool](#) () to apply the set of **Unique Sites**.
3. When you find suitable left and right cut sites, select the region between them by clicking on the label for the restriction site on one end and then **Shift+clicking** on the label on the other end.
4. Choose **Cloning > Copy Restriction Insert** or use the keyboard shortcut **Ctrl/Cmd+Shift+V**.
5. Choose **Cloning > Paste Restriction Insert**.

The two-tabbed Clone Insert dialog opens, with separate tabs for the **Left (5') End** and **Right (3') End**. The corresponding ends of the vector sequence are displayed adjacent to insert ends on each panel.



There are several operations you can perform while in this dialog:

- To simulate modification of overhangs for incompatible ends, use the **Trim Blunt** or **Fill In**

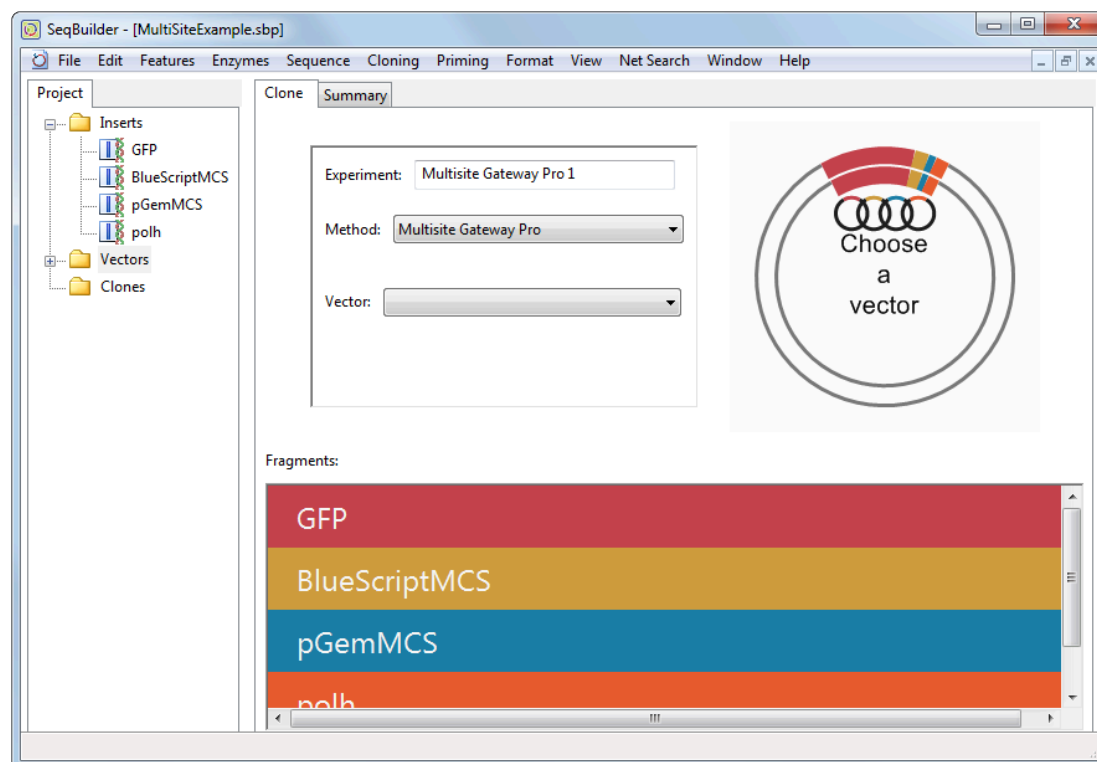
buttons.

- To manipulate the overhangs manually, move the arrows above and below the two strands. This can be used to simulate partial fill or end protection.
 - To return to the default overhangs, click **Revert**.
 - To flip the insert, with respect to the vector, check the **Reverse Complement Insert** box.
 - The translation for the reading frame specified in the **Translate** field is shown between the top and bottom strands for both the vector and insert sequences. As the ends of the vector and insert are adjusted, the translation is updated automatically. To view the translation for a different reading frame, select the desired reading frame from the **Translate** drop-down menu. Reading frames in this list correspond to the reading frames displayed on the [Primer Design](#), [Sequence](#), and [Linear](#) views.
6. Once the vector and insert have been constructed with compatible overhangs, click the **Clone** button.
 7. Choose **Cloning >Restriction Enzyme Cloning**.
 8. The Project window opens with the Clone tab active. The selection from Steps 1-2 is placed in the **Inserts** folder in the Project tab, as well as in the **Fragments** section at the bottom of the Clone tab.

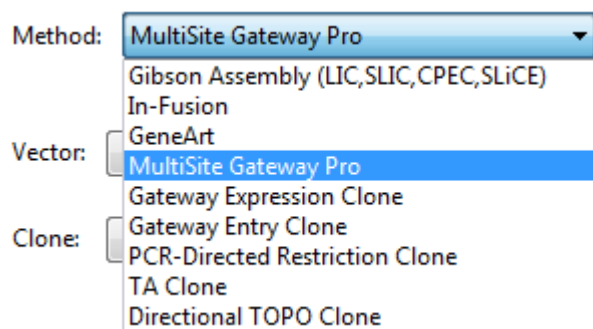
Proceed to [Step 2: Specify the method and options](#).

Step 2: Specify the method and options

After [beginning a cloning project and specifying the insert](#), the cloning project should appear similar to the one below. This example shows four inserts, but your project may contain any allowable number for the cloning method you will be using. The circular cartoon in the upper right corner displays inserts in the same order in which they appear in the Fragments section. The cartoon text prompts you to “Choose a vector,” which you will do in the step following this current one.

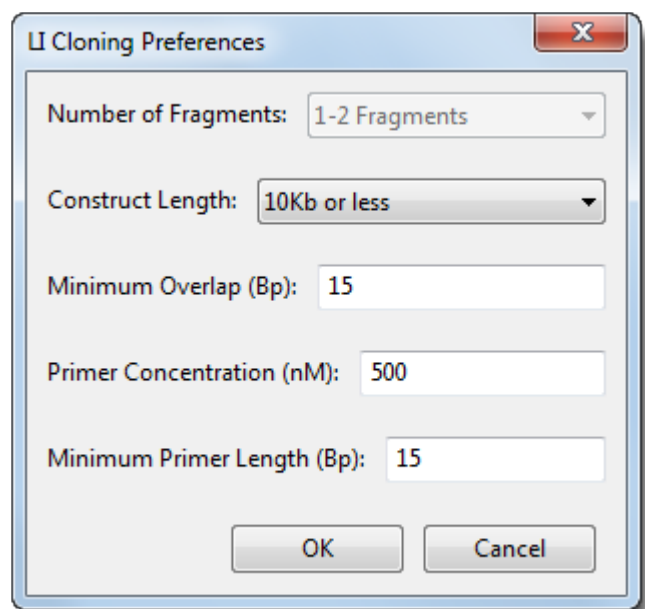


1. In the **Experiment** text box, keep the default name or overwrite it with another name.
2. If the desired [cloning method](#) is not already selected in the **Method** drop-down menu, choose it from the list.



3. (optional) SeqBuilder Pro optimizes method settings, but if you wish to change method options for the

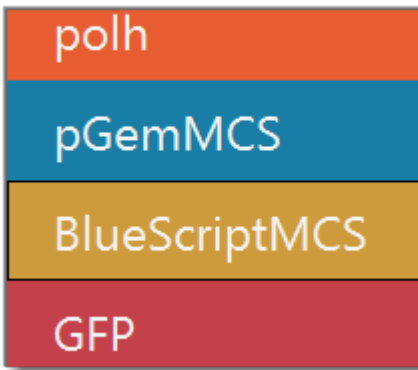
Gibson Assembly, In-Fusion, or GeneArt methods, you can use the **Preferences** button.



Option	Description
Number of Fragments	Most cloning methods allow just one insert, but Gibson, In-Fusion, and MultiSite Gateway Pro cloning each allow different numbers of inserts. The default for this menu is optimized for the selected cloning method, but you can override this by making another selection. Choices are 1-2 Fragments, 3-4 Fragments, or Adjust Automatically.
Construct Length	The anticipated length of the finished expression clone. Choices are 10Kb or less , More than 10Kb , or Anything .
Minimum Overlap (Bp)	Relevant to ligation-independent cloning methods and constrains the length of overlap to the specified value or higher. The maximum value allowed is 60 Bp; higher values are ignored.
Primer Concentration (nM)	(This option is currently disabled, so changing the value has no impact.)
Minimum Primer Length (Bp)	Desired minimum threshold for the primer length. The maximum value allowed is 60 Bp; higher values are ignored.

- In the **Fragments** section, drag and drop the inserts until they are in the desired order. For instance: a promoter, one or two cloning sites, and Green Fluorescent Protein (GFP). If there is only one insert, skip this step.

Fragments:



Proceed to [Step 3: Specify the vector and entry clone.](#)

Available cloning methods

SeqBuilder Pro supports the following cloning methods:

MultiSite Gateway Pro Cloning

MultiSite Gateway Pro technology enhances the classic Gateway cloning protocol by allowing you to clone up to four regions at one time. In MultiSite Gateway Pro cloning, three additional types of recombination sites have been engineered to recombine uniquely between the two ends of a source sequence and a host vector. MultiSite Gateway Pro cloning can be simulated in SeqBuilder Pro using the five-step cloning workflow.

The recombination features used in MultiSite Gateway cloning are as follows:

- For Expression Clones: attB1, attB2, attB3, attB4, and attB5
- For Donor Vectors: attP1, attP2, attP3, attP4, and attP5
- For Entry Clones: attL1, attL2, attL3, attL4, and attL5
- For Destination Vectors: attR1, attR2, attR3, attR4, and attR5



Note: When an r is added to any of these feature names, that indicates the reverse of the specified site. For example, L5r indicates an L5 site in reverse.

As in classic Gateway cloning, attL and attR sites recombine, and attB and attP sites recombine. The recombined sites transform by swapping opposite halves into the opposite set, so attL and attR become attB and attP and vice versa.

Click [here](#) for information on the specific 2, 3, and 4 fragment MultiSite Gateway protocol from Invitrogen.

For detailed information on creating a suitable vector, see the section “Preparation of Gateway cloning vectors” in [Add custom vectors to the Cloning Vector Catalog](#).



Note: During the cloning simulation, primers are altered to match the donor vector in the first clone. When creating inserts with attB1-attB2 primers, SeqBuilder Pro automatically adds four guanines (GGGG) to the 5' end of the primer, as per the requirement specified in in Thermofisher's online [Gateway Technology Manual](#).

Gateway Cloning

Gateway cloning is based upon the site specific recombination of lambda bacteriophage. Two types of recombination sites have been engineered to recombine uniquely between the two ends of a source sequence and a host vector. The sites have also been engineered to allow the recombination reactions to be completely reversible using two sets of catalytic proteins. These two reactions are called LR and BP, so named because of the recombination sites involved in each; LR recombines attL and attR sites, BP recombines attB and attP sites. The recombined sites transform by swapping opposite halves into the opposite set, so attL and attR become attB and attP and vice versa. Vectors engineered to contain these sites have been given the names: Entry Clone (attB), Expression Clone (attL), Donor Vector (attP), and Destination Vector (attR).

When you perform a Gateway cloning simulation in SeqBuilder Pro, you first create an Entry Clone for a specified fragment insert. This Entry Clone (rather than a fragment insert) is then used as input for making an Expression Clone. Both type of clones are created using the cloning workflow. You will follow the workflow once to create the Entry Clone, then again to create the Expression Clone.

A special type of Gateway cloning is RNA interference (RNAi) cloning. This procedure uses Gateway recombination technology and a special vector (pHELLSGATE and its derivatives) containing two pairs of recombination sites opposing each other on opposite strands. The cloning system is intended to create an RNA transcript under expression that interferes with the translation of the cloned gene, thereby suppressing it.

- To construct inverted repeat Entry Clones, use the vectors pHELLSGATE and pHELLSGATE 4.
- To construct inverted repeat Expression Clones from the single site Entry Clones, use the vectors pHELLSGATE 8 and pHELLSGATE 12.

For detailed information on creating a suitable vector, see the section “Preparation of Gateway cloning

vectors” in [Add custom vectors to the Cloning Vector Catalog](#).

Gibson (LIC, SLIC, CPEC, SLiCE) Cloning

Gibson assembly and Ligation Independent Cloning (LIC) are techniques offered by New England Biolabs. Both cloning methods can be simulated in SeqBuilder Pro using the five-step cloning workflow. Note that Gibson cloning allows up to 6 fragment inserts. When using this cloning method, restriction insert size must be relatively large in order to prevent primer overlaps that could keep the fragment from being incorporated into the expression clone.

In-Fusion Cloning

In-Fusion cloning is a technique offered by Clontech/Takara, and allows a maximum of 2 fragment inserts. This cloning method can be simulated in SeqBuilder Pro using the five-step cloning workflow.

GeneArt Cloning

GeneArt cloning is offered by ThermoFisher Scientific and allows up to 4 inserts. This cloning method can be simulated in SeqBuilder Pro using the five-step cloning workflow.

PCR-Directed Restriction Cloning

PCR-directed restriction cloning utilizes the unique activity of site-specific restriction enzymes. First use restriction enzyme cut sites to create the insert. If no convenient restriction sites are available, you may instead select the region you want to clone and SeqBuilder Pro will engineer primers to create sites compatible with the selected sites of the chosen vector. In either case, proceed to follow Steps 2-5 of the cloning workflow.

Using a cloning vector with compatible restriction sites, the insert and vector can be digested with restriction enzymes to generate complementary ends, thereby enabling the vector and insert to be efficiently ligated. SeqBuilder Pro uses the basic idea very similar to the copy/paste of text editing. The primary difference in SeqBuilder Pro is the way the sequence endpoints are selected and what happens after the paste command. The cloning workflow simplifies this further by making the selection of restriction sites easier and by eliminating the need to find matching sites in the cloned region.

TA Cloning

TA Cloning is a cloning method that takes advantage of the terminal transferase activity of some DNA Polymerases that add a 3'-A overhang to each end of a PCR product. A linearized vector with prepared 3'-T overhangs facilitates ligation of the PCR product. TA cloning can be simulated in SeqBuilder Pro using the

five-step cloning workflow. In Step 1, follow the instructions for Option E – Using the PCR product of the current primer pair as the insert.

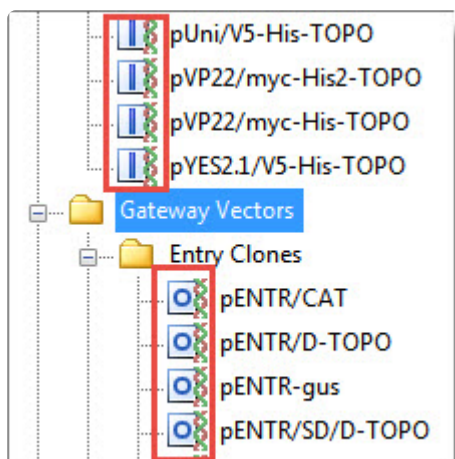
Directional TOPO Cloning

Directional TOPO cloning utilizes the binding activity of Topoisomerase I, and can be simulated in SeqBuilder Pro using the five-step cloning workflow. A sequence of interest is PCR amplified using a forward strand primer with four additional bases (CACC). Commercial vector sequences have been engineered with topoisomerase bound at 3' and a complementary 5' GTGG overhang. The overhang invades the 5' end of the PCR product and anneals to the added bases. The 5' hydroxyl group of the PCR product attacks the topoisomerase bound to the vector, releasing it and causing the ligation of the phosphate backbone. Orientation of the insert is determined by the 5' overhang, allowing the other end to be blunt.

Step 3: Specify the vector and entry clone

After [specifying the cloning method and options](#), the next step is to choose the vector. In addition, if you are performing Gateway Expression cloning, you will need to specify which previously-saved Gateway Entry clone to use.

1. From the Clone tab of the [Project window](#), select a vector from the **Vector** drop-down menu. (Learn how to [add your own vectors to this list](#)). The menu has been optimized to contain suitable vectors for the chosen cloning method. The icon next to each vector shows whether it is linear (required for TA & TOPO cloning) or circular.



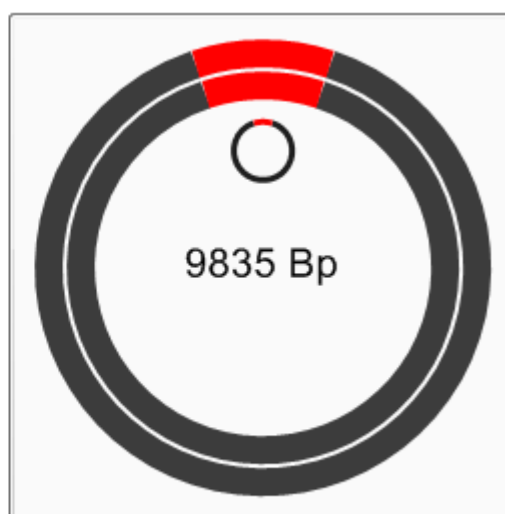
2. If you are doing Gateway Expression cloning, the **Clone** drop-down menu will also be present. From this menu, select the entry clone that was created in an earlier Gateway Entry cloning project. Alternatively, you may select the entry clone by dragging it from its Project tab folder and dropping it onto the **Clone** drop-down menu.

Experiment:

Method:

Vector:

Clone:

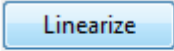



The cartoon on the upper right of the tab will update its appearance and text based on your selections.

Proceed to [Step 4: Digest/linearize a circular vector](#).


Step 4: Digest/linearize a circular vector

After you [specify the vector or entry clone](#), a **Digest** or **Linearize** button may appear. This button lets you specify where in the vector to insert the fragment. The appearance (or not) and name of this button depends on which method you chose, and whether your vector is linear or circular.

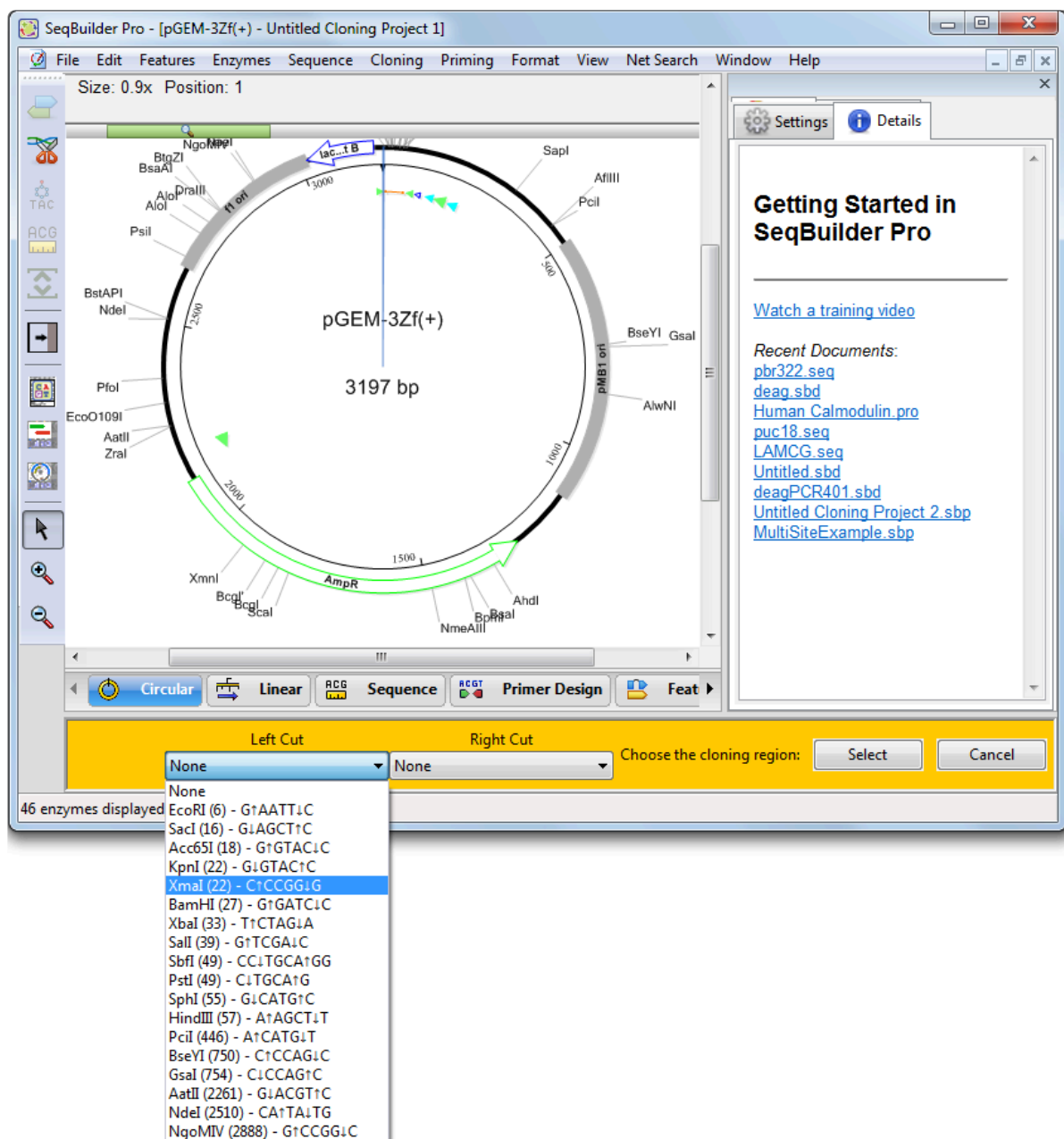
Method	Button name
Gibson Assembly	
In-Fusion, GeneArt	Need to linearize the vector
PCR-Directed Restriction Cloning	 Need to digest the vector
TA & TOPO	No button (linear vectors required)
Gateway methods	No button (uses recombination)

To digest/linearize the vector:

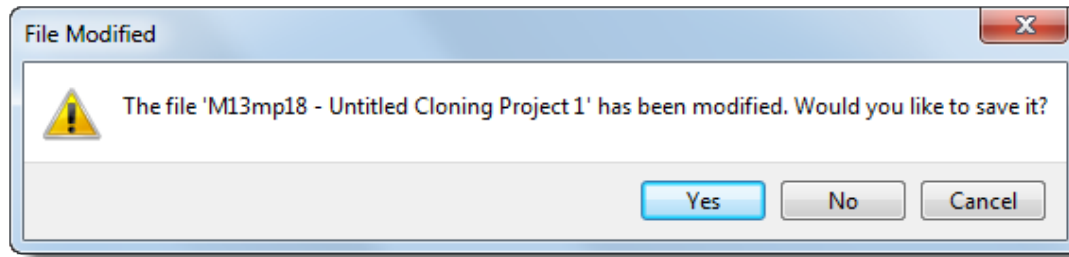
1. Press the **Digest** or **Linearize** button opens the [Document window](#) and displays the vector and its cut sites. Providing the vector and insert have already been specified, only enzymes that are unique in the vector and that do not cut the insert are displayed.

 **Note:** The same window also opens if you use the **Cloning > Clone Restriction Insert** from the Document window without first specifying a selection. The procedure described below also applies to that situation.

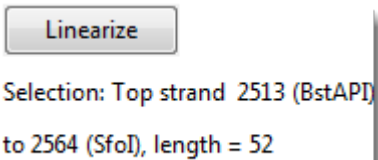
2. Select one or two cut sites using any of the following methods:
 - Click on one restriction enzyme in the [Circular view](#), and then **Shift+click** on the second enzyme (if any).
 - Select one or two enzymes using the **Left Cut** and/or **Right Cut** drop-down menus in the gold area at the bottom of the window. SeqBuilder Pro automatically populates the menus with enzymes that are unique in the vector being displayed and are blunt or have a non-degenerate overhang. In addition, if you have ever manually changed the selector to display other enzymes and chosen a site that does not meet the criteria above, that site will be displayed in the menus from that point on.



- Select one custom cut location by clicking on that base in the Circular view. Or select two cut locations by dragging the mouse across the area that should be removed.
 - When you have made your selection(s), press the **Select** button on the bottom right.
3. To return to the cloning window without cutting the vector choose **Cancel**. Otherwise, press **Select** to confirm the cloning region. When prompted to confirm file modification, select **Yes**.



If you selected cut sites, information about them now appears below the **Linearize** or **Digest** button.



Proceed to [Step 5: Run the cloning simulation](#).

Step 5: Run the cloning simulation

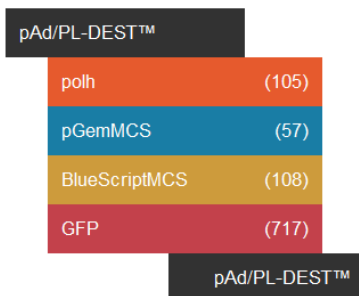
When you have specified the [insert](#) and [vector](#), and set up additional options as desired, you are ready to begin the cloning simulation:

1. Press the **Try It** or **Clone** button in the bottom right corner of the [Project window](#). The view switches from the Clone tab to the Summary tab.

DNASTAR®
Software for life scientists

Cloning Summary

Fragments



Steps

- 1) Amplify polh using primers:
Top: 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGATATCATGGAGA-3'
Bottom: 5'-GGGGACAACCTTTGTATACAAAAGTTGTAATATTTATAGTCTTTTITA-3'
- 2) Recombine step 1 with pDONR221-P1P5r
- 3) Amplify pGemMCS using primers:
Top: 5'-GGGGACAACCTTTGTATACAAAAGTTGGGTTAGAAATTCGAGCTCGGTACCC-3'
Bottom: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTTGAGCTTCGATGCCTGCAGGTC-3'
- 4) Recombine step 3 with pDONR221-P5P4
- 5) Amplify BlueScriptMCS using primers:
Top: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTTAGGTACCGGGCCCCCTC-3'
Bottom: 5'-GGGGACAACCTTTGTATATAAAGTTGGGTTGAGCTCCACCGCGGTGC-3'
- 6) Recombine step 5 with pDONR221-P4rP3r
- 7) Amplify GFP using primers:
Top: 5'-GGGGACAACCTTTGTATATAAAGTTGGGTTAATGAGTAAAGGAGAAGAACTT-3'
Bottom: 5'-GGGGACAACCTTTGTACAGAAAAGCTGGGTTCTATTGTATAGTTTCATCCA-3'
- 8) Recombine step 7 with pDONR221-P3P2
- 9) Recombine steps 2, 4, 6, and 8 with pAd/PL-DEST™

Fragment Junctions

pAd/PL-DEST™-polh

```
S Q V C T K K Q A S V A X
H K F V Q K S R L Q L L
T S L Y K K A G F S C .
TCACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGA
V L K Y L F A P K L Q Q
C T Q V F F C A E T A S
. L N T C F L L S . N S X
```

polh-pGemMCS

```
. I L Q L C I Q K L L E F E
K Y Y N F V Y K S C . N S X
I N I T T L Y T K V V R I R X
ATAAATATACAACTTTGTATACAAAAGTTGTTGaatcgag
F I V V K Y V F T T L I R X
Y I N C S Q I C F N N S N S X
L Y . L K T Y L L Q . F E L
```

pGemMCS-BlueScriptMCS

```
C K L . Q L F S I Q S C R Y R X
A S F N N F F L Y K V V G T G
Q A L T T F F Y T K L S V P X
tgcaagcttTAACAACTTTTCTATACAAAGTTGTCggtacc
C A K V V K K . V F N D I G P
L S . C S K E I C L Q R Y R X
A L K L L K K R Y L T T P V P X
```

BlueScriptMCS-GFP

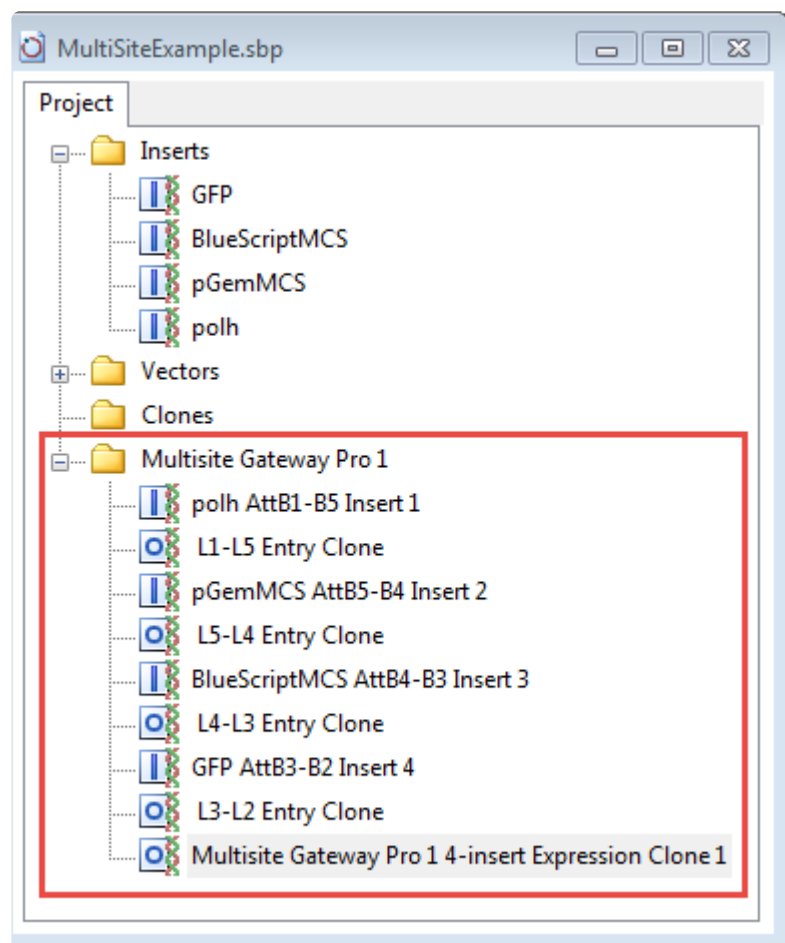
```
G A Q P N C I I K L G . . V X
V E L N P T V . . S W V N E . X
W S S T Q L Y N K V G L M S K
gtggagctcAACCAACTGTATAAAGTTGGGTTAATGAGT
H L E V W S Y L L T P N I L L X
P A . G L Q I I F N P . H T F
S S L G V T Y Y L Q T L S Y X
```

GFP-pAd/PL-DEST™

```
T N R T Q L S C T K W Y H K
Q I E P S F L V Q S G I T S
Y K . N P A F L Y K V V S Q V
TACAAATAGAACCAGCTTCTTGTACAAAAGTTGATCACAAG
L Y F G A K K Y L T T D C T
V F L V W S E Q V F H Y . L N
C I S G L K R T C L P I V L
```

2. Review the three Summary sections: Fragments, Steps and Fragment Junctions. If the summary procedure does not meet your requirements, click **Change** to switch back to the Clone tab, where you can make changes. Otherwise, click **Make It** to proceed with the cloning simulation.

If simulation is successful, a new folder is added to Project tab of the Project window. Depending on the method used, this folder may contain a copy of each insert and entry clone, as well as the expected product that will be created after recombination occurs (i.e., the finished expression clone). Any item can be opened in the SeqBuilder Pro Document window by double-clicking on its name.



If simulation is unsuccessful, you may receive the error message “The vector is not digested by restriction enzyme(s).” This message may be seen when performing a cloning simulation where restriction enzyme cut sites were used to create the insert. Click **Change** to return to the Clone tab, then try again using a different insert.

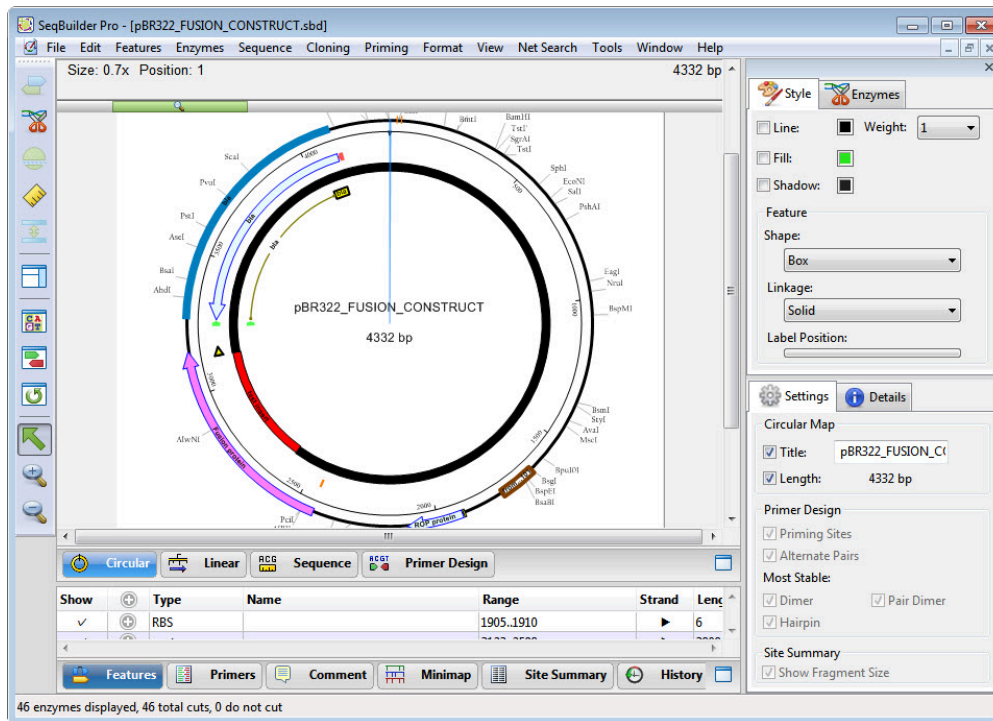
3. The Setup tab asks whether you wish to verify the clone. You can perform cloning verification at any time. If you have already prepared the clone in the laboratory and sequenced it, proceed to [Step 6 \(optional\): Verify the clone](#). Otherwise, continue to the next step.
4. Save the cloning project using **File > Save Project**. Then proceed to [Step 7: View and print cloning results](#).

Step 6 (optional): Verify the clone

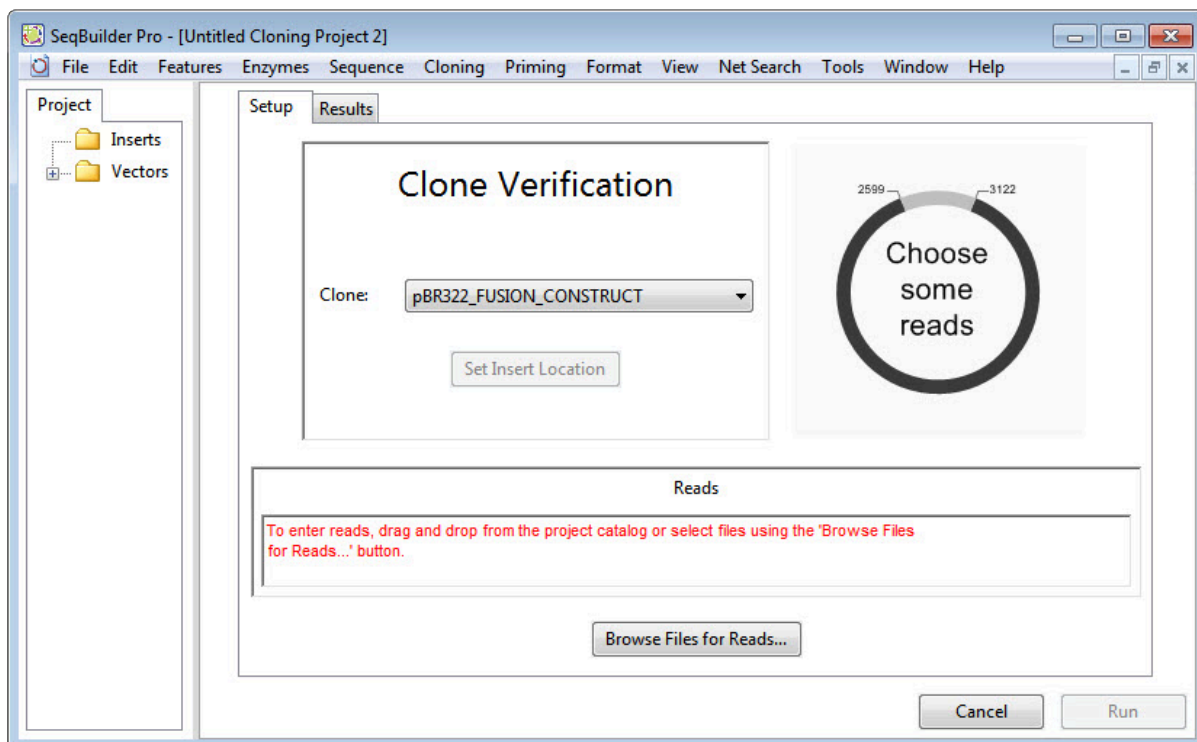
SeqBuilder Pro 15.1 and later offers the ability to verify whether or not a clone produced in the laboratory matches the version designed in SeqBuilder Pro. To learn how, watch this brief video or follow the written instructions below:

To verify a clone:

1. Use SeqBuilder Pro to [design a virtual clone](#). After completing [Step 5: Run the cloning simulation](#), you will be asked if you wish to verify the clone, discussed in this topic. If you have already created and sequenced the clone in the laboratory, skip to step 5 of the instructions below. Most users, however, will continue to step 2 of the instructions below.
2. Save the cloning project.
3. Create the clone “in real life” in the laboratory.
4. Sequence the lab clone via Sanger sequencing and put the resulting *.abi* files on the same computer used to design the virtual clone.
5. Launch SeqBuilder Pro and use **File > Open** to open the original cloning project.



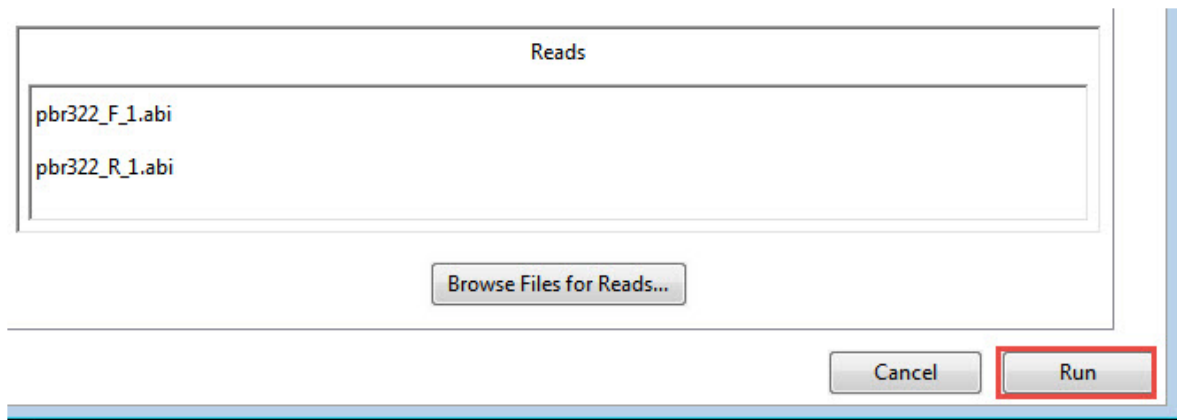
6. Choose **Cloning > Verify Clone**. The [Project window](#) opens with the Setup tab active.



7. Use the **Clone** drop-down menu to specify the clone of interest, if it is not already specified.

8. If SeqBuilder Pro cannot locate the cloning insert, the **Set Insert Location** button will be enabled. Press the button to navigate to the insert.

9. Press the **Browse files for Reads** button at the bottom of the Setup tab. Navigate to the Sanger *.abi* files obtained from sequencing the laboratory clone.
10. Press the **Run** button.



Behind the scenes, DNASTAR's SeqMan Pro's alignment algorithm runs an alignment to verify whether or not the "real life" clone matches the virtual clone designed in SeqBuilder Pro.

Proceed to the topic [Interpret results from clone verification](#).

Interpret results from clone verification

After following the steps in [Step 6 \(optional\): Verify the clone](#), SeqBuilder Pro displays the results in the Results tab of the [Project window](#). The results consist of up to three sections, and may feature graphics, messages and links.

Interpreting messages in the Results tab:

Situation	Results tab shows...																
No issues	<div>The actual clone appears to agree with the computer simulation.</div> <div>No significant variations reported.</div>																
Minor differences	<div>The actual clone appears to agree with the computer simulation.</div> <div>There may be some mismatches worth examining listed below</div> <div>Example of a clone with a frameshift:</div> <div>Notable variations:</div> <table><thead><tr><th>Clone Position</th><th>Location</th><th>Dist</th><th>Type</th><th>Base</th><th>Call</th><th>Codon</th><th>Alignment Position</th></tr></thead><tbody><tr><td>2597</td><td>vector</td><td>-1</td><td>Indel</td><td>A</td><td>-</td><td>EGAA > X:GAX</td><td>1904</td></tr></tbody></table>	Clone Position	Location	Dist	Type	Base	Call	Codon	Alignment Position	2597	vector	-1	Indel	A	-	EGAA > X:GAX	1904
Clone Position	Location	Dist	Type	Base	Call	Codon	Alignment Position										
2597	vector	-1	Indel	A	-	EGAA > X:GAX	1904										
Major differences	Example of a clone with a large mismatch:																



1 - pbr322_R_1.abi(1>540)

2 - pbr322_F_1.abi(1>398)

Highlighted regions contain conflicts of a density that suggest that the actual clone differs from the computer simulation.

CAUTION: Highlighted regions suggest that the clone is not in agreement with the computer simulation.

[Show the reads assembled with the clone.](#)

Notable conflicting regions:

Clone Position	Conflicts	Location	Alignment Position
1870 1976	15.3%	vector	1870 1976

Notable variations:

Clone Position	Location	Dist	Type	Base	Call	Codon	Alignment Position
2549	vector	-40 ▶	Indel	G	-	G:GGG > G:GGx	1861
2550	vector	-39 ▶	Indel	G	-	G:GGG > G:GGx	1862
2551	vector	-38 ▶	Indel	G	-	V:GTC > S:TCx	1863
2552	vector	-37 ▶	Indel	T	-	V:GTC > A:GCx	1864
2665	insert	◀ 76	Indel	G	-	V:GTG > X:TGx	1977
2700	insert	◀ 111	SNP	T	C	P:CCT > P:CCc	2012
2774	insert	◀ 185	SNP	G	C	.:TGA > S:TcA	2086
2858	insert	◀ 269	SNP	G	C	S:AGT > T:AcT	2170
2860	insert	◀ 271	SNP	T	A	F:TTC > t:aTC	2172
2934	insert	217 ▶	SNP	C	G	S:AGC > R:AGg	2246
3042	insert	109 ▶	SNP	T	A	N:AAT > K:AAa	2354
3098	insert	53 ▶	Indel	-	T	C:TGT > L:TtG	2410
3131	insert	20 ▶	Indel	A	-	K:AAG > X:AGx	2444
3150	insert	1 ▶	SNP	A	T	L:CTA > L:CTt	2463

Using links in the Results tab:

Link	Result
------	--------

“Show the reads assembled with the clone”	Launches SeqMan Pro’s Alignment view .
“Clone Position” link	Opens SeqBuilder Pro’s Features view ; also opens the Sequence view with the position or range selected.
“Alignment Position” link (Notable Conflicting Regions section)	Launches SeqMan Pro’s Strategy view with the position or range selected.
“Alignment Position” link (Notable Variations section)	Launches SeqMan Pro’s Alignment view with the position or range selected and the trace data expanded.

If desired, proceed to [Step 7: View and print cloning results](#).

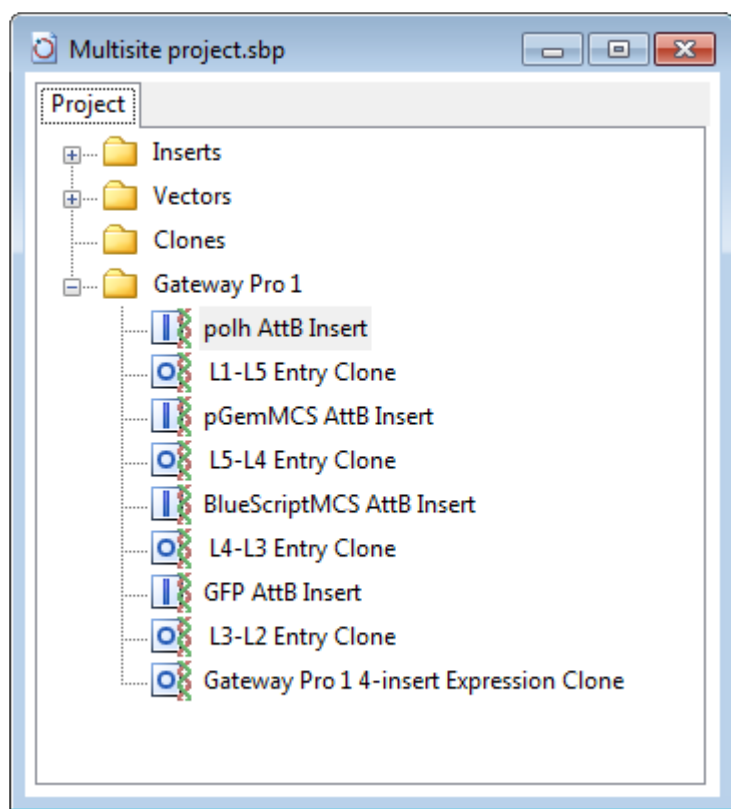
Step 7: View and print cloning results

The Cloning Summary provides details about the components of the cloning simulation, including fragment inserts, primers, and the steps needed to prepare the vector and amplify inserts. It also displays the reading frame around all fragment junctions graphically, allowing you to verify whether promoters and genes are in synchrony.

The Summary is available once you complete a cloning project and open any of the resulting files (i.e., entry clones, inserts, expression clones) in the [Document window](#).

To access the Cloning Summary:

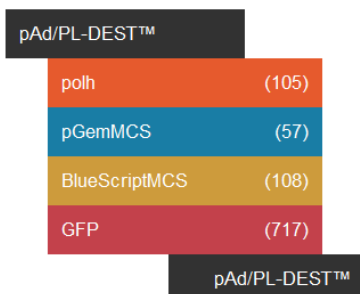
1. [Design a virtual clone](#).
2. From the [Project window](#), expand the folder containing the cloning project of interest.



3. Double-click on the name of any result file to open it in the [Document window](#).
4. Open the [History view](#) to see the Cloning Summary.

Cloning Summary

Fragments



Steps

- 1) Amplify polh using primers:
 Top: 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGATATCATGGAGA-3'
 Bottom: 5'-GGGGACAACCTTTGTATACAAAGTTGTAATATTATAGTGTITTTTA-3'
- 2) Recombine step 1 with pDONR221-P1P5r
- 3) Amplify pGemMCS using primers:
 Top: 5'-GGGGACAACCTTTGTATACAAAGTTGGGTTAGGATCGAGCTCGGTACCC-3'
 Bottom: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTGAAGCTTGCATGCCGTCAGGTC-3'
- 4) Recombine step 3 with pDONR221-P5P4
- 5) Amplify BlueScriptMCS using primers:
 Top: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTTAGGTACCGGGCCCCCCTC-3'
 Bottom: 5'-GGGGACAACCTTTGTATAATAAAGTTGGGTTGAGCTCCACCGGTGGC-3'
- 6) Recombine step 5 with pDONR221-P4rP3r
- 7) Amplify GFP using primers:
 Top: 5'-GGGGACAACCTTTGTATAATAAAGTTGGGTTAATGAGTAAAGGAGAAGAAGTT-3'
 Bottom: 5'-GGGGACCACTTTGTACAGAAAAGCTGGGTTCTATTTGTATAGTTTCATCCA-3'
- 8) Recombine step 7 with pDONR221-P3P2
- 9) Recombine steps 2, 4, 6, and 8 with pAd/PL-DEST™

Fragment Junctions

pAd/PL-DEST™-polh

```

S Q V C T K K Q A S V A X
H K F V Q K S R L Q L L
T S L Y K K A G F S C .
TCACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGA
V L K Y L F A P K L Q Q
C T Q V F F C A E T A S
. L N T C F L L S . N S X
  
```

polh-pGemMCS

```

. I L Q L C I Q K L L E F E
K Y Y N F V Y K S C . N S X
I N I T T L Y T K V V R I R X
ATAAATATACAACTTTGTATACAAAGTTGTTAgaattcgag
F I V V K Y V F T T L I R X
Y I N C S Q I C F N N S N S X
L Y . L K T Y L L Q . F E L
  
```

pGemMCS-BlueScriptMCS

```

C K L . Q L F S I Q S C R Y R X
A S F N N F F L Y K V V G T G
Q A L T T F F Y T K L S V P X
tgcaagcttTAACAACCTTTTCTATACAAAGTTGTCggtacc
C A K V V K K . V F N D T G P
L S . C S K E I C L Q R Y R X
A L K L L K K R Y L T T P V P X
  
```

BlueScriptMCS-GFP

```

G A Q P N C I I K L G . . V X
V E L N P T V . . S W V N E . X
W S S T Q L Y N K V G L M S K
gtggagctcAACCAACTGTATAATAAAGTTGGGTTAATGAGT
H L E V W S Y L L T P N I L L X
P A . G L Q I I F N P . H T F
S S L G V T Y Y L Q T L S Y X
  
```

GFP-pAd/PL-DEST™

```

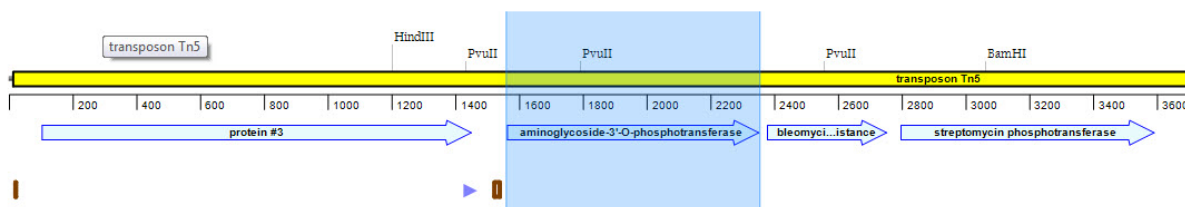
T N R T Q L S C T K W Y H K
Q I E P S F L V Q S G I T S
Y K . N P A F L Y K V V S Q V
TACAAATAGAACCCAGCTTCTTGTACAAAGTGGTATCACAAG
L Y F G A K K Y L T T D C T
V F L V W S E Q V F H Y . L N
C I S G L K R T C L P I V L
  
```

- - To print the Cloning Summary, choose **File > Print**.
 - To copy the entire Cloning Summary to the clipboard, choose **Edit > Select All**, then select **Edit > Copy**. The summary can be pasted from the clipboard into many commonly-used text editors.

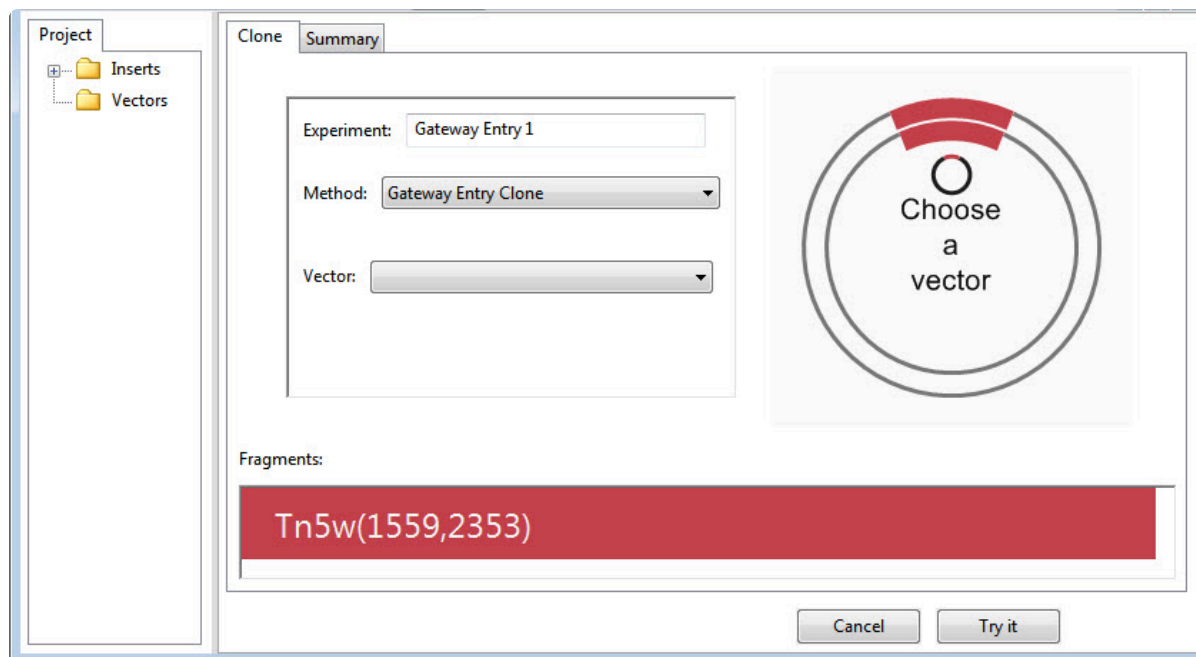
Try it! – Gateway cloning

[Gateway cloning](#) is based upon the site-specific recombination of lambda bacteriophage, and is a two-part process. In the first part of the tutorial, you will create a Gateway entry clone. In the second part, you will use the entry clone as input for constructing the final Gateway expression clone.

1. If you have not yet download and extracted the tutorial data, [click here to download it](#). Then decompress (unzip) the file archive using the method of your choice.
2. Use **File > Open** to open the demo file *Tn5w.sbd*. The sequence opens in the [Linear view](#).
3. Click on the neomycin/kanamycin resistance feature labeled **aminoglycoside-3'-O-phosphotransferase** to select that range of sequence.




4. Select **Cloning > Gateway Entry Clone** to launch the [Project window](#).



The selected sequence fragment has been placed in the Clone tab's **Fragments** area and in the Project tab's **Inserts** folder. To expand the **Inserts** folder and reveal its contents, click the plus icon (Win) or arrow (Mac).

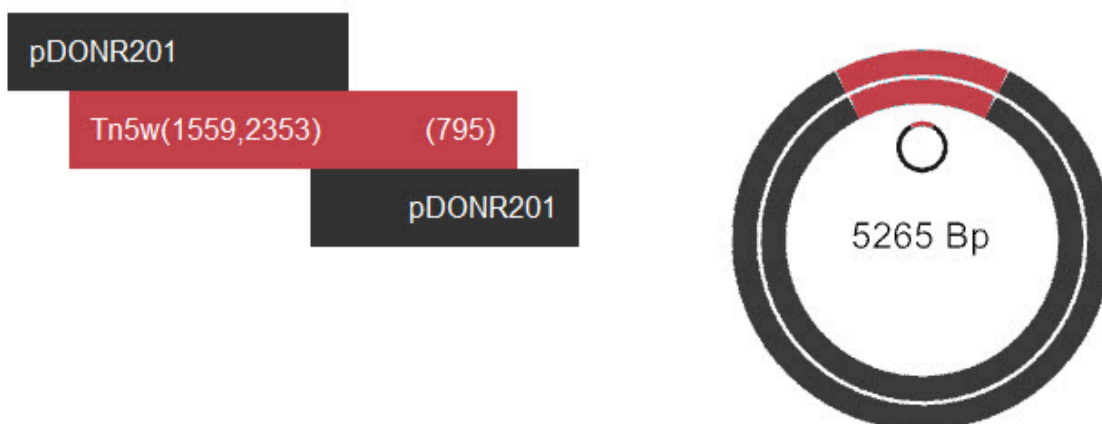
The circular cartoon in the upper right corner displays the insert (in red) and prompts you to "Choose

a vector.” The cartoon updates its appearance and text based on your selections.

5. Use the **Vector** drop-down menu to select the donor vector **pDONR201**. Note that the menu has been optimized to contain suitable vectors for the chosen cloning method. In the current project, only circular vectors () are offered.
6. Press the **Try It** button in the bottom right corner of the window. The Project view switches from the Clone tab to the Summary tab.

Cloning Summary

Fragments



Steps

- 1) Amplify Tn5w(1559,2353) using primers:
 Top: 5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGATTGAACAAGATGGATTGC-3'
 Bottom: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTATCAGAAGAACTCGTCAAGAAGG-3'
- 2) Recombine amplified Tn5w(1559,2353) with pDONR201.

Fragment Junctions

pDONR201-Tn5w(1559,2353)

```

F I M P S L Y K K A G F M I E
L . C Q V C T K K Q A S . L
Y N A K F V Q K S R L H D .
TTTATAATGCCAAGTTTGTACAAAAAAGCAGGCTTCatgattg
. L A L N T C F L L S . S Q
I I G L K Y L F A P K M I S
K Y H W T Q V F F C A E H N F
  
```

Tn5w(1559,2353)-pDONR201

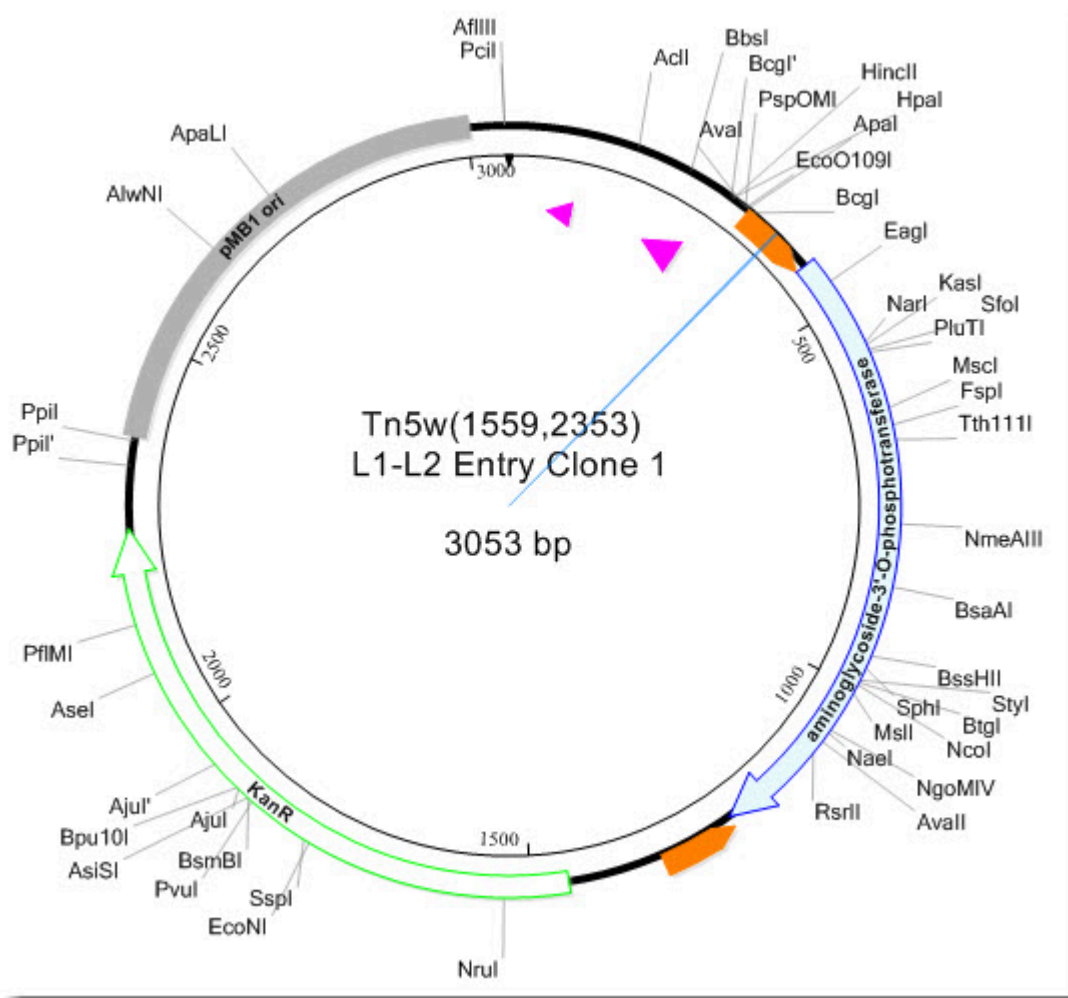
```

F F . Y P A F L Y K V G I I R X
S S D T Q L S C T K W A L . E
L L I P S F L V Q S G H Y K X
  
```

7. Briefly look at the contents of the three sections: Fragments, Steps and Fragment Junctions. Then click **Make It** to proceed with the cloning simulation.

* If the contents had not met your requirements, you could have clicked **Change** to switch back to the Clone tab in order to make changes there.

The *L1-L2 Entry Clone 1* opens automatically in a new [Document window](#). By default, the top pane shows the simulated clone in the [Circular view](#).



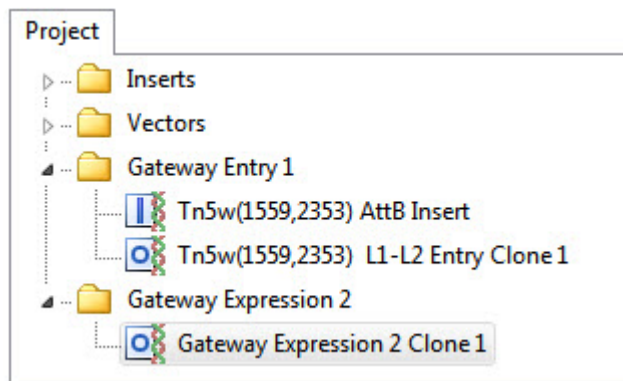
8. Click on the **History** tab at the bottom of the window to open the [History view](#). This view shows the Cloning Summary last seen in Step 6. Since this summary contains the steps to make the entry clone, you may wish to print a copy.
9. To see a print preview, click anywhere on the Cloning Summary to select it, and then choose **File > Print**. You could change the scale and/or print the summary from this window, if desired. However, there is no need to do so for this tutorial.
10. On Windows, click the 'x' in the top corner to close the Print Preview window. On Macintosh, click the **Cancel** button.

Next, you will create the expression clone and (optionally) print a document that includes the steps to

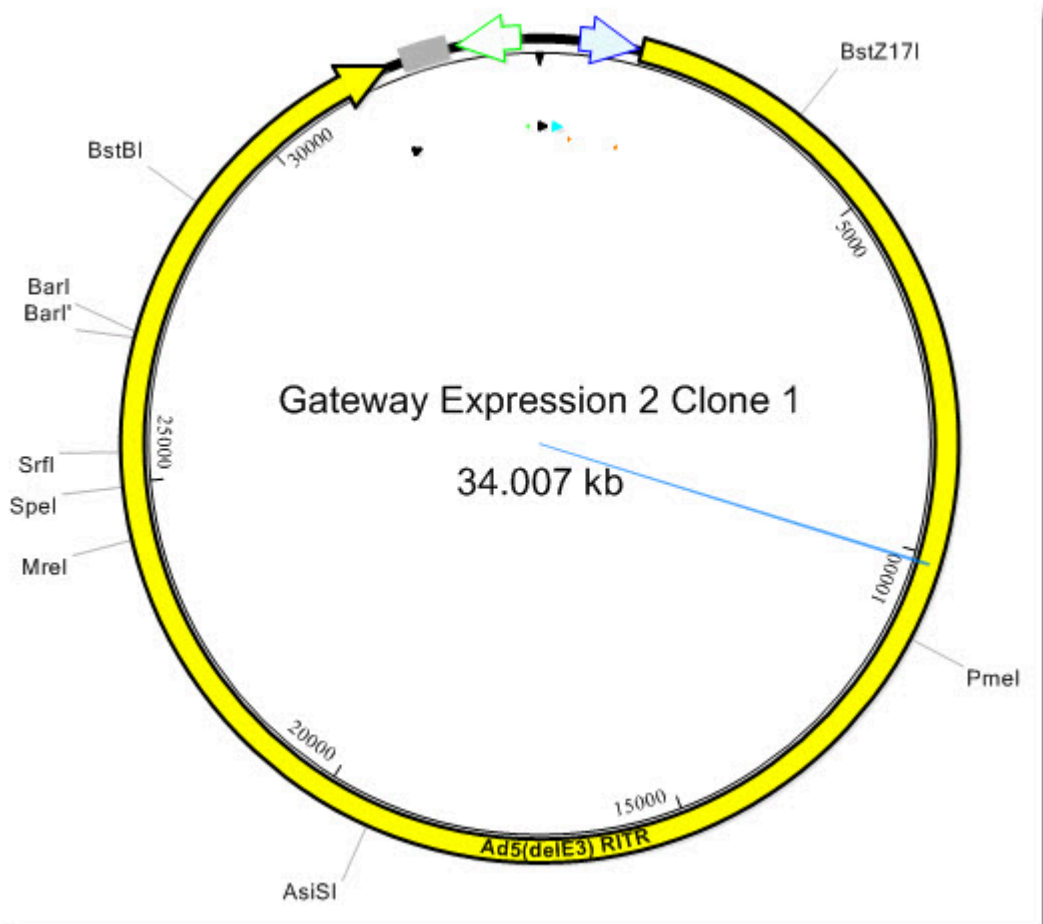
create the entry clone:

11. Choose **Cloning > Gateway Expression Clone** to again open the Project window.
12. Use the **Vector** drop-down menu to select the destination vector **pAd/BLOCK-iT-DEST**.
13. Use the **Clone** drop-down menu to select **Tn5w(1559,2353) L1-L2 Entry Clone 1** from the bottom of the list.
14. Press **Try It** and then **Make It**.
15. The Setup tab asks whether you wish to verify the clone. Press the **Cancel** button.

A new folder, *Gateway Expression 2*, is added to Project tab.



The *Gateway Expression 2 Clone 1* opens automatically in a new [Document window](#). As before, the top pane shows the simulated expression clone in the [Circular view](#).



As before, the Cloning Summary appears in the [History view](#).

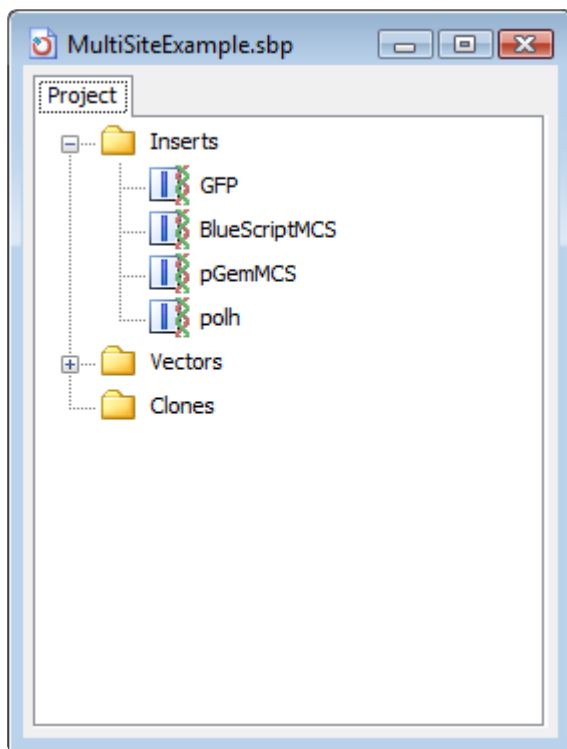
16. Click once on the Cloning Summary pane to select it.
17. (optional) Use **Edit > Select All**, then **File > Print** to print the summary.

Try it! – MultiSite Gateway Pro cloning

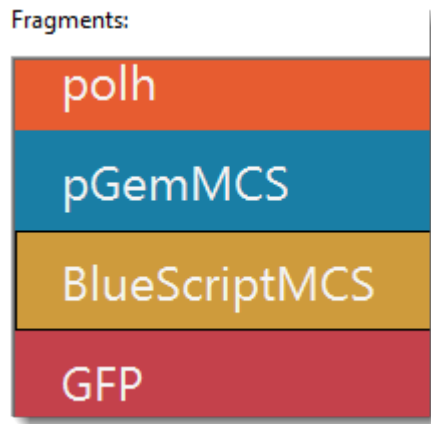
[MultiSite Gateway Pro](#) technology enhances the classic Gateway cloning protocol by allowing you to clone up to four regions at one time. In MultiSite Gateway Pro cloning, three additional types of recombination sites have been engineered to recombine uniquely between the two ends of a source sequence and a host vector.

In this tutorial, you will create a clone that has a *P_{ol}* promoter, two multiple cloning sites, and a reporter gene.

1. If you have not yet download and extracted the tutorial data, [click here to download it](#). Then decompress (unzip) the file archive using the method of your choice.
2. Use **File > Open** to open the file *MultiSiteExample.sbp* in the [Project window](#).
3. Click on the plus sign next to **Inserts** to expand the folder.



4. Choose **Cloning > Multisite Gateway Pro Cloning**.
5. Use **Shift+click** to select all four inserts, then drag and drop them in the **Fragments** area to the bottom right of the Clone tab
6. Reorder the fragments to match the image below. To do this, click on a fragment to select it, then drag and drop it in the new position.

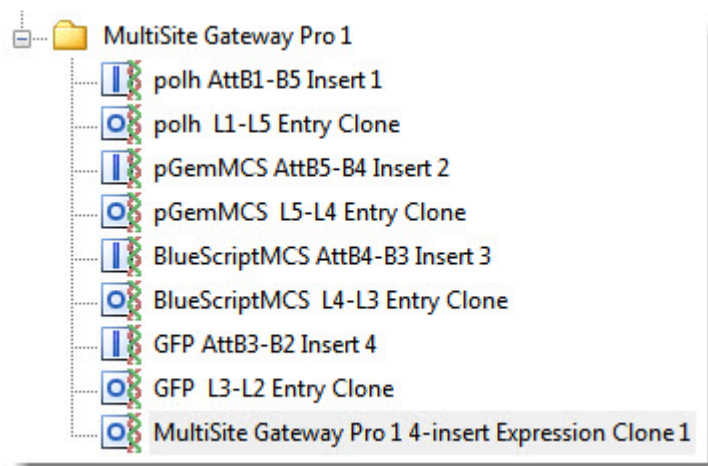


7. In the Clone tab, click on the **Vector** drop-down menu and select **pDEST8**.

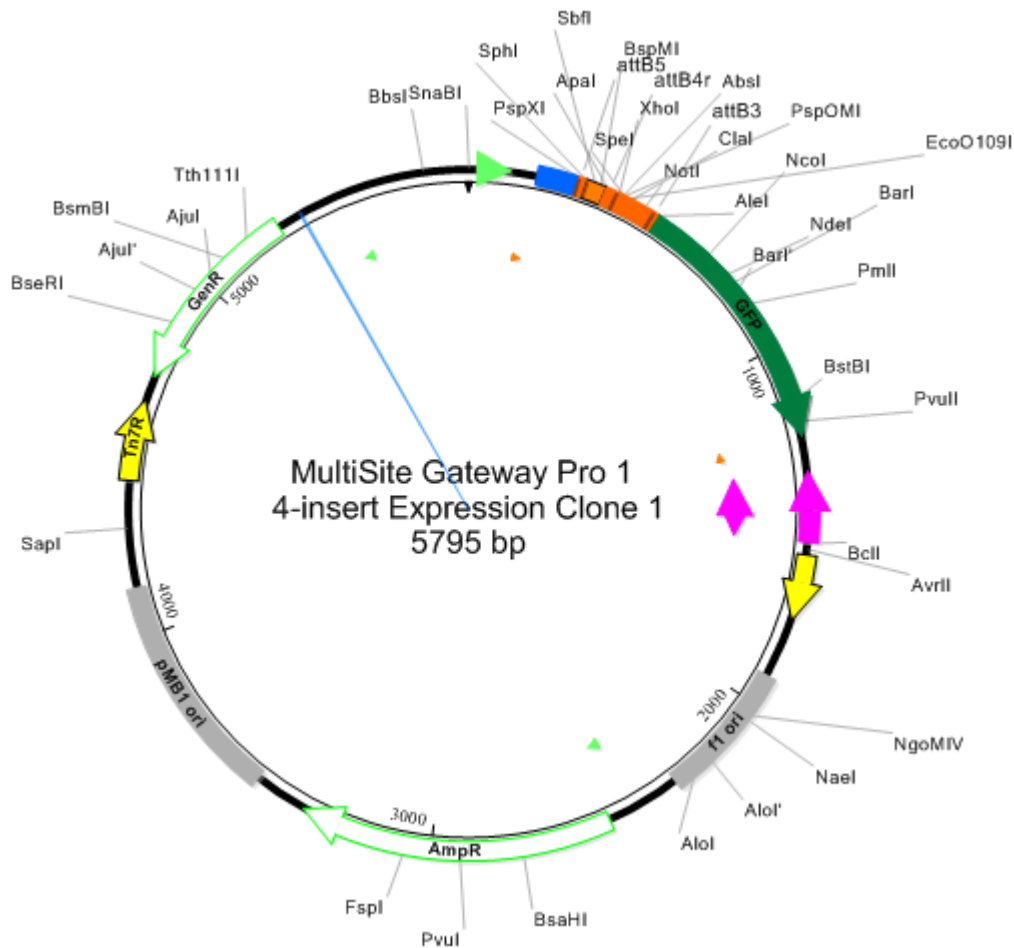
✿ Windows users can navigate using a vertical scroll bar, while Macintosh users should use a mouse roller or similar tool to scroll.

8. Click **Try It** and then **Make It**.
9. The Setup tab asks whether you wish to verify the clone. Press the **Cancel** button.

In the Project tab, a new folder is added containing four amplified inserts (all linear), as well as four entry clones and the final expression clone (all circular).



The Multisite Gateway Pro 1 4-insert Expression Clone 1_ opens automatically in a new [Document window](#). By default, the top pane shows the simulated expression clone in the [Circular view](#).



- Click on the **History** tab at the bottom of the window to open the [History view](#), which contains the Cloning Summary. The summary includes a nine-step cloning procedure and lists the primers needed for each step.

Steps

- 1) Amplify polh using primers:
 Top: 5'-GGGGACAAGTTTGTACAAAAAGCAGGCTTCAGTTGCTGATATCATGGAGA-3'
 Bottom: 5'-GGGGACAACCTTTGTATACAAAGTTGTAATATTTATAGGTTTTTTTA-3'
- 2) Recombine step 1 with pDONR221-P1P5r
- 3) Amplify pGemMCS using primers:
 Top: 5'-GGGGACAACCTTTGTATACAAAGTTGTAGAATTCGAGCTCGGTACCC-3'
 Bottom: 5'-GGGGACAACCTTTGTATAGAAAAGTTGGGTGAAGCTTGCAATGCCTGCAGGTC-3'
- 4) Recombine step 3 with pDONR221-P5P4
- 5) Amplify BlueScriptMCS using primers:
 Top: 5'-GGGGACAACCTTTCTATACAAAGTTGTAGGTACCGGGCCCCCCTC-3'
 Bottom: 5'-GGGGACAACCTTTATTATACAAAGTTGTGAGCTCCACCGCGGTGGC-3'
- 6) Recombine step 5 with pDONR221-P4rP3r
- 7) Amplify GFP using primers:
 Top: 5'-GGGGACAACCTTTGTATAATAAGTTGTAATGAGTAAAGGAGAAGAACTT-3'
 Bottom: 5'-GGGGACCACTTTGTACAAGAAAGCTGGGTACTATTTGTATAGTTCATCCA-3'
- 8) Recombine step 7 with pDONR221-P3P2
- 9) Recombine steps 2, 4, 6, and 8 with pDESTTM8

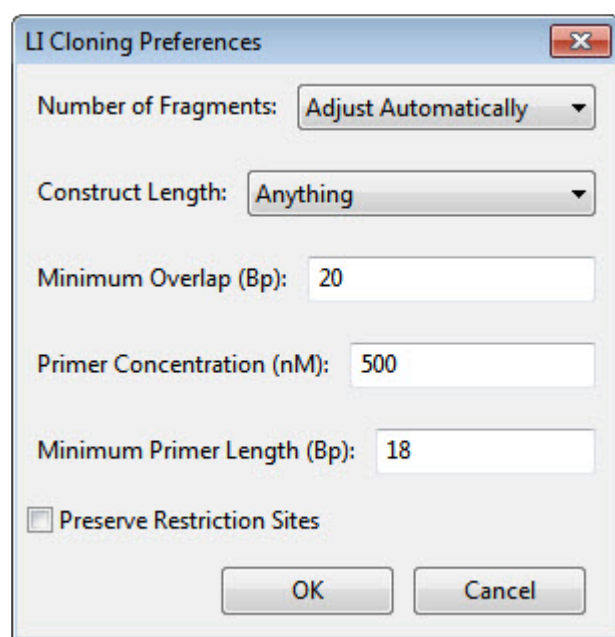
To print the Cloning Summary or copy it to the clipboard, see [View and print cloning results](#).

Try it! – Gibson cloning with a custom vector

The **Vectors** drop-down menu in the [Project window](#) is a shortcut to all of the vectors contained in the *CloningVectors.sbp* project, also known as the “Vector Catalog.” It is possible to add a custom vector to an open project, or to add it to the Vector Catalog for use with future projects. The first part of this tutorial deals with the former case, and takes you through the steps needed to add a custom vector to a particular Gibson cloning project.

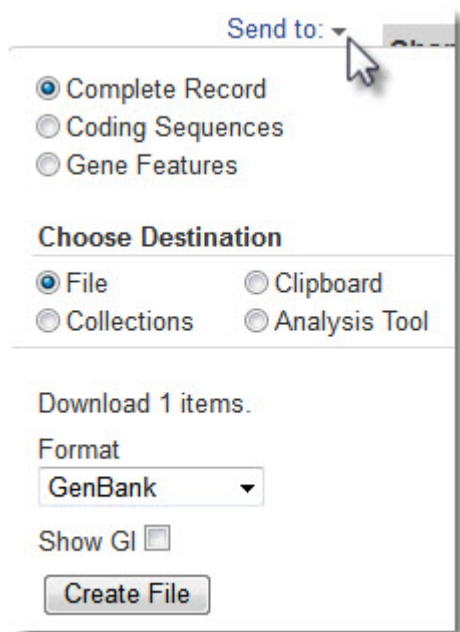
You will then make the Gibson clone as verification that you added the custom vector correctly. [Gibson](#) assembly, offered by New England Biolabs, is a technique which allows up to 6 fragment inserts. When using Gibson assembly, restriction insert size must be relatively large in order to prevent primer overlaps that could keep the insert from being incorporated into the expression clone.

1. If you have not yet download and extracted the tutorial data, [click here to download it](#). Then decompress (unzip) the file archive using the method of your choice.
2. Use **File > Open** to open the demo file *Tn5w.sbd*. The sequence opens in the [Linear view](#).
3. Click on the neomycin/kanamycin resistance feature labeled **aminoglycoside-3'-O-phosphotransferase** to select that range of sequence.
4. Choose **Cloning > Gibson, LIC Cloning** to open the [Project window](#).
5. Some of the cloning methods in SeqBuilder Pro have editable options. Click on the **Preferences** button to see the options available for Gibson cloning.



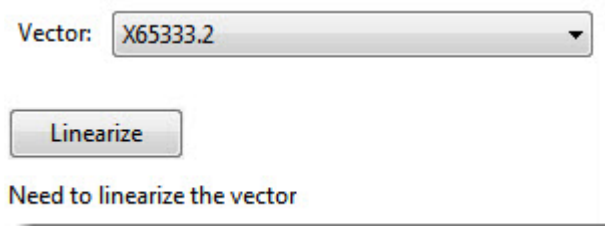
Leave all settings at the defaults and click **Cancel** to exit.

6. You'll be using a custom vector that is not in the Vector Catalog, and therefore not in the **Vector** drop-down menu on the right of the Project window. To specify the custom vector:
 - a. Go to the NCBI page for [Promega cloning vector pSP73](#).
 - b. Click on the **Send to** link near the top of the page, and make the following selections:

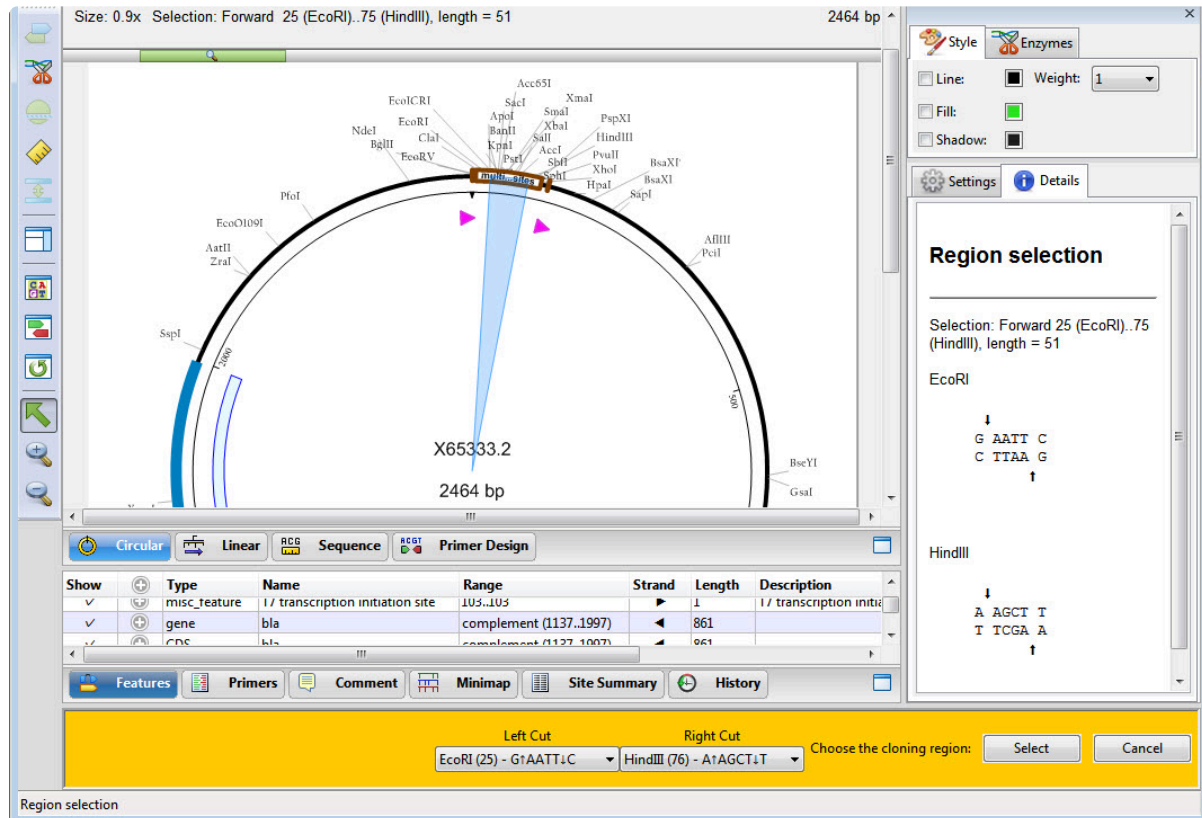


- c. Press **Create File** and then save the file when prompted.
 - d. Use your file explorer to find the saved file, then drag and drop it on the **Vector** drop-down menu in SeqBuilder Pro's Clone tab.

The vector is selected automatically, and a new **Linearize** button also appears.

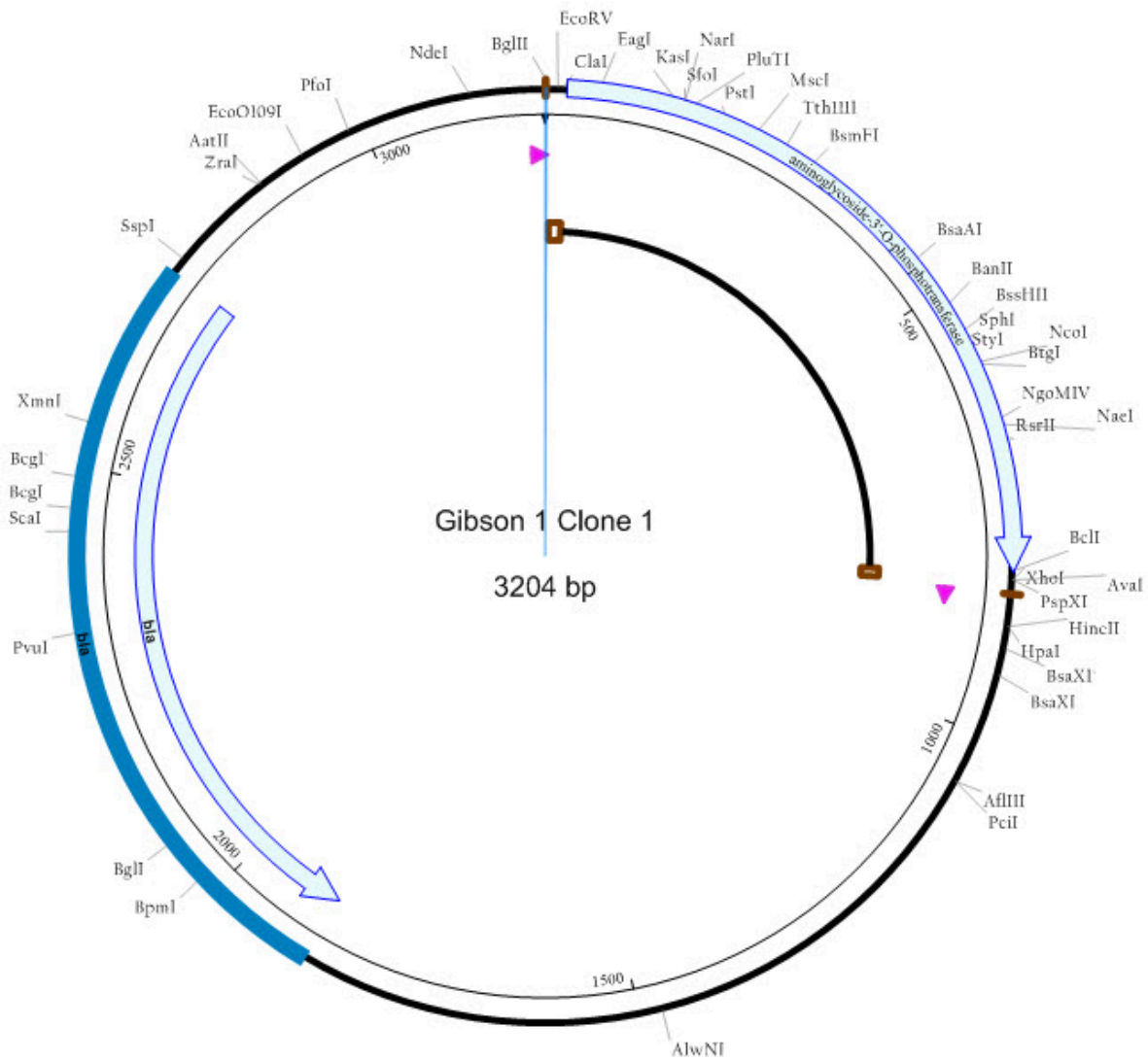


7. Press **Linearize** to open the pSP73 vector in the [Circular view](#). “Unique sites,” enzymes that cut the sequence only once, are displayed by default.
8. Use the **Left Cut** and **Right Cut** drop-down menus in the yellow bar at the bottom of the window to specify **EcoRI** and **HindIII**, respectively.



9. In the yellow bar, press **Select**.
10. Back in the Project window, press **Try It** and then **Make It**.
11. The Setup tab asks whether you wish to verify the clone. Press the **Cancel** button.

The *Gibson 1 Clone 1* opens automatically in a new [Document window](#). By default, the top pane shows the simulated clone in the [Circular view](#).

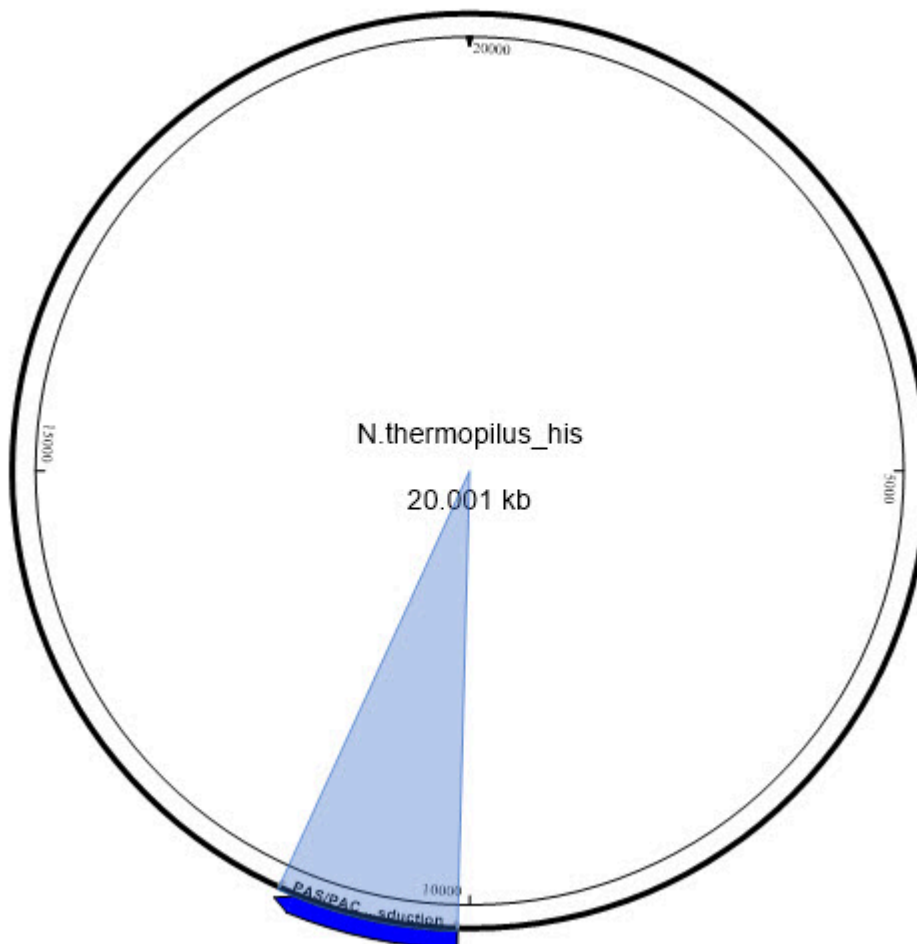


This marks the end of the tutorial.

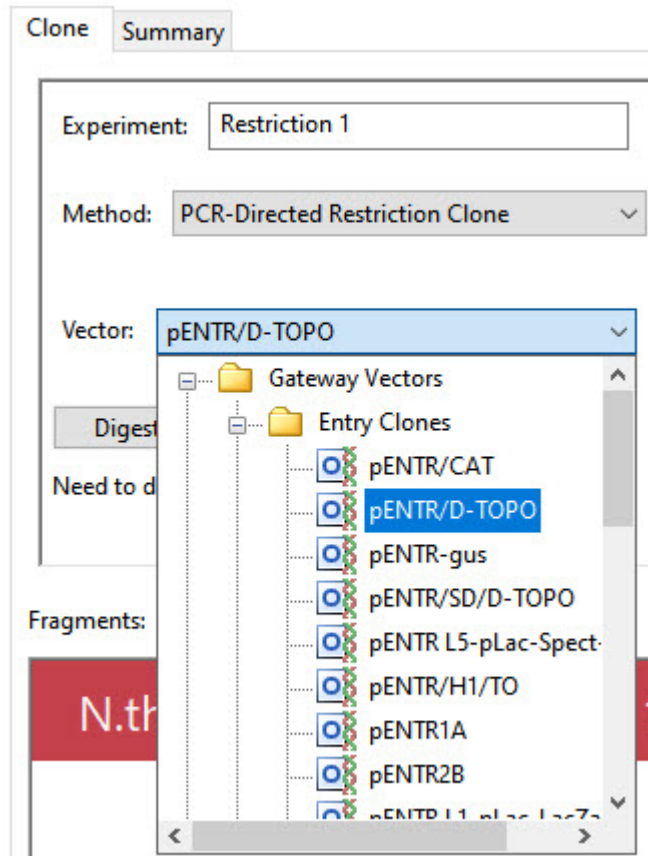
Try it! – PCR-directed restriction cloning

This tutorial demonstrates how to cut and clone a signal transduction gene into the pENTR/D-TOPO vector.

1. If you have not yet download and extracted the tutorial data, [click here to download it](#). Then decompress (unzip) the file archive using the method of your choice.
2. Use **File > Open** to open *N.thermophilus_his.sbd*. The sequence opens in the Circular view.
3. Click on the **PAS/PAC sensor signal transduction** feature to select it.



4. Choose **Cloning > Clone Selected Fragment**.
5. In the **Clone** tab on the right, choose the **Method** named **PCR-Directed Restriction Clone**.
6. In the **Vector** drop-down menu, click on the plus sign next to **Gateway Vectors**, then the plus sign next to **Entry Clones** and select **pENTR/D-TOPO**.



7. Press the **Digest** button. A specialized version of the Circular view opens.
8. Select the range between the cut sites **SacII** and **Ascl**, both located just below the “3 o’clock” position in the Circular view. Use any of these methods to select the range:
 - Click on the enzyme label **SacII** in the Circular view and then **Shift+click** on the enzyme **Ascl**.
 - Click on the enzyme label **SacII** and then drag the mouse until the enzyme **Ascl** is also selected.
 - In the yellow bar at the bottom of the view, choose the **Left Cut** site **SacII** and the **Right Cut** site **Ascl**.

Region selection

Selection: Forward 673 (SacII)..697 (AscI), length = 25

SacII

```

      ↓
    CC GC GG
    GG CG CC
      ↑
  
```

AscI

```

      ↓
    GG CGCG CC
    CC GC CG GG
      ↑
  
```

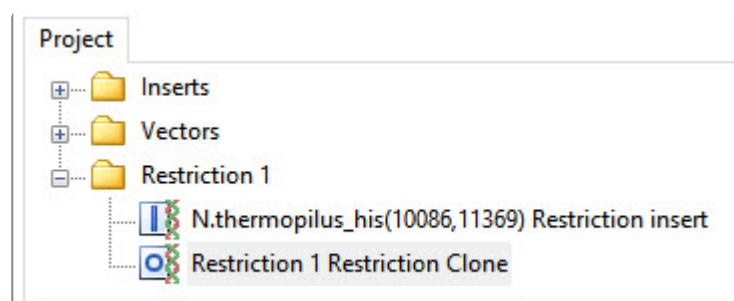
Type	Name	Range	Strand	Length	Description
CDS	KanR	974..1783	►	810	Aminoglycoside 3'-phospho
regulatory	T7 promoter	complement (823..840)	◄	18	T7 promoter
misc_feature	TOPO	680..693	►	14	vaccinia virus DNA topoisom

Left Cut: SacII (673) - CC↓GC↑GG
Right Cut: AscI (697) - GG↑CGCG↓CC
Choose the cloning region: [Help] [Select] [Cancel]

9. Press the **Select** button. The **Clone** tab comes back into focus.

10. Press **Try it**. Then press **Make it**.

In the **Project** tab on the left, note the appearance of two new files: the *Restriction insert* and *Restriction Clone*.



The *Restriction Clone* opens automatically in a new [Document window](#). By default, the top pane shows the simulated clone in the [Circular view](#).

11. Click on the **History** tab at the bottom of the SeqBuilder Pro window to see the Cloning Summary, including the steps needed to create this clone in your lab.

Cloning Summary

Fragments



Steps

- 1) Amplify *N.thermophilus_his*(10086,11369) using primers:
 Top: 5'-CCGCGGATGCGCTCTGTTTTTTTA-3'
 Bottom: 5'-GGCGCGCCTTAATCTGGTTTGCATAATC-3'
- 2) Digest pENTR/D-TOPO and *N.thermophilus_his*(10086,11369) using restriction enzymes:
 SacII and AscI

Fragment Junctions

pENTR/D-TOPO-*N.thermophilus_his*(10086,11369)

```

      R L R G C A X
    A G S A D A L X
      Q A P R M R S
    GCAGGCTCCGCGGatgcgctct
      C A G R I R E X
      L S R P H A R
      P E A S A S X
  
```

N.thermophilus_his(10086,11369)-pENTR/D-TOPO

```

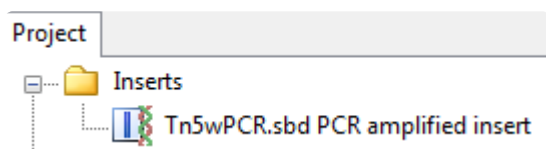
      N Q I K A R X
    Q T R L R R A X
      K P D . G A P
    caaaccagattaaGGCGGCGCCG
      L G S . P A G X
      F W I L A R R
      V L N L R A X
  
```



Try it! – Use a PCR insert in TA cloning

The tutorial [Try it! – Create and modify primers for TA cloning](#) demonstrated how to select and modify a primer pair. This tutorial shows how to extract the PCR insert and create a virtual TA clone.

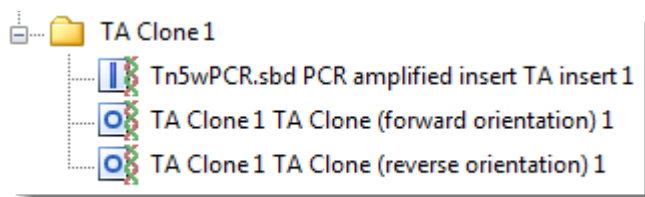
1. If you have not already done so, follow the tutorial [Try it! – Create and modify primers for TA cloning](#). If the project is still open from following the tutorial, double-click on the white space in the Primers view to remove the current selection. If you closed the project since following that tutorial, use **File > Open** to open the file saved at the end of that tutorial.
2. Choose **Priming > Create Insert by PCR** to extract the sequence of the PCR product and simulate its amplification with Taq polymerase including the addition of 3' A overhangs to each end.

A [Project window](#) opens with a new Taq amplified insert inside the **Inserts** folder.



3. Select **Cloning > TA Cloning**.
4. Drag the amplified insert from the **Inserts** folder of the Project tab and drop it on the **Fragment** area of the Clone tab.
5. Look in the **Vector** drop-down menu and note that the set has been filtered to include only linear vectors, as required for TA (and TOPO) cloning. Linear vectors have the icon . Select **pBAD-TOPO** from the **Vector** drop-down menu.
6. Click **Try It** and then **Make It**.

In the **Project** tab on the left, note that new forward and reverse orientation clones now appear in the Project tab in the *TA Clone 1* folder.



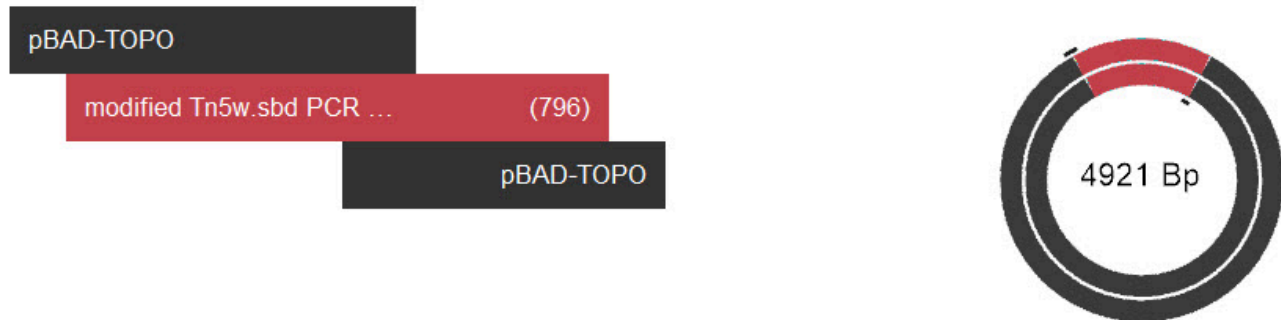
The forward and reverse orientation clone files open automatically in new [Document windows](#). By default, each top pane shows the simulated clone in the [Circular view](#). Notice that the primers and PCR product appear as features. These features can also be seen in the [Features view](#), as well as all graphical views.



- Click on the **History** tab at the bottom of the SeqBuilder Pro window to see the Cloning Summary, including the steps needed to create this clone in your lab.

Cloning Summary

Fragments



Steps

- 1) Amplify modified Tn5w.sbd PCR amplified insert using primers:
 Top: 5'-ATGATTGAACAAGATGGACTACA-3'
 Bottom: 5'-TTCAGAAGAACTCGTCAAGAAG-3'

Fragment Junctions

pBAD-TOPO-modified Tn5w.sbd PCR amplified insert

```

L A L M I E
S P L . L
R P Y D .
CTCGCCCTTATGATTGAA
R G . S Q
A R I I S
E G K H N F

```

modified Tn5w.sbd PCR amplified insert-pBAD-TOPO

```

F . R A S
S S E G R A
L L K G E
TCTTCTGAaGGGCGAGCT
R R F P S S
K Q L A L
E S P R A

```

Automated Batch Cloning

Automated batch cloning lets you automatically clone large numbers of fragments into the same vector. Most of the steps involved are similar or identical to those in regular [automated virtual cloning](#), but the cloning window has been optimized for batch cloning.

Preliminary steps:

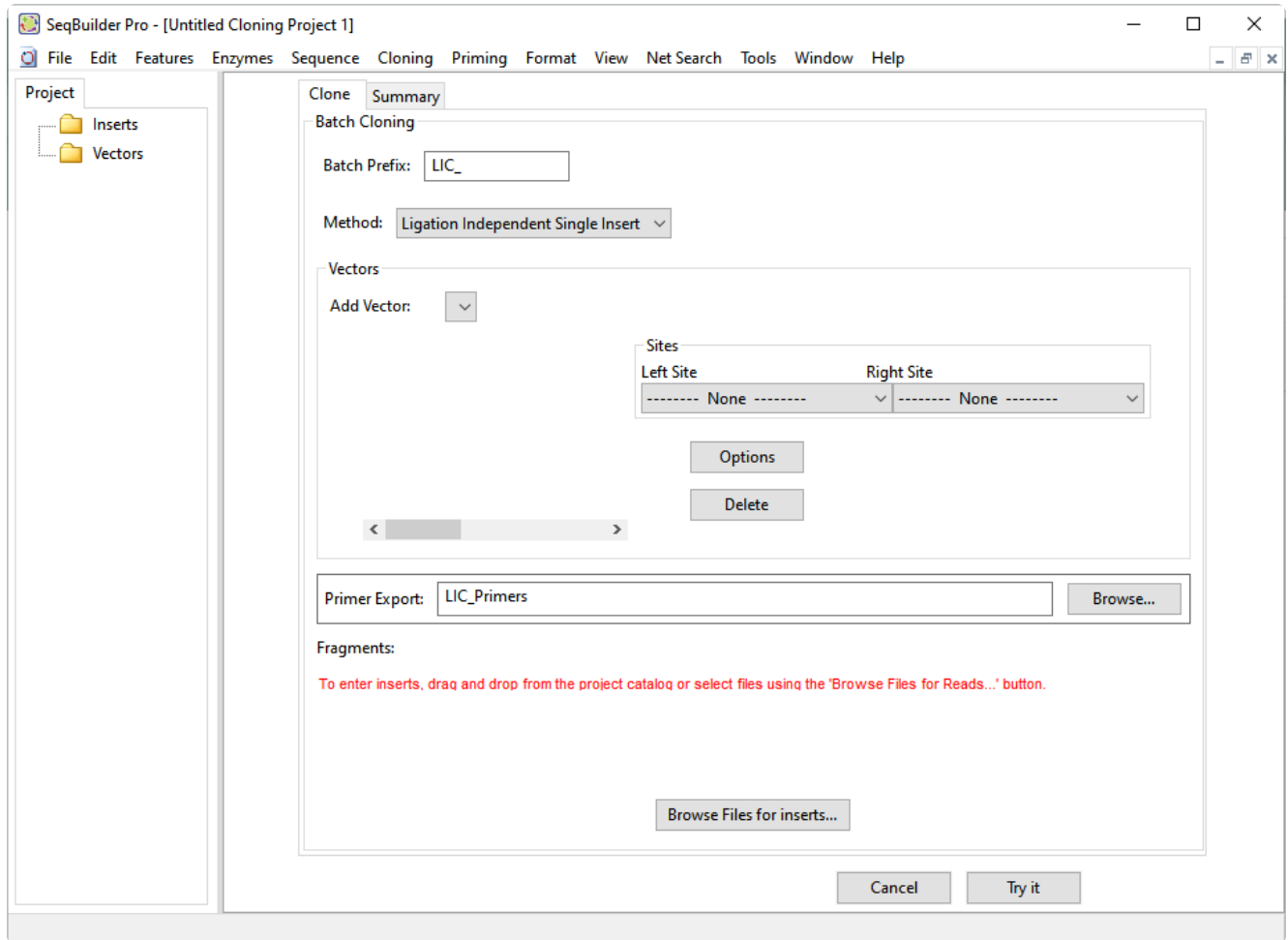
These optional steps take about 2-20 minutes each, but can provide benefits for many future cloning workflows.

- [Add custom vectors to the Cloning Vector Catalog](#)
- [Auto-annotate vector sequences](#)

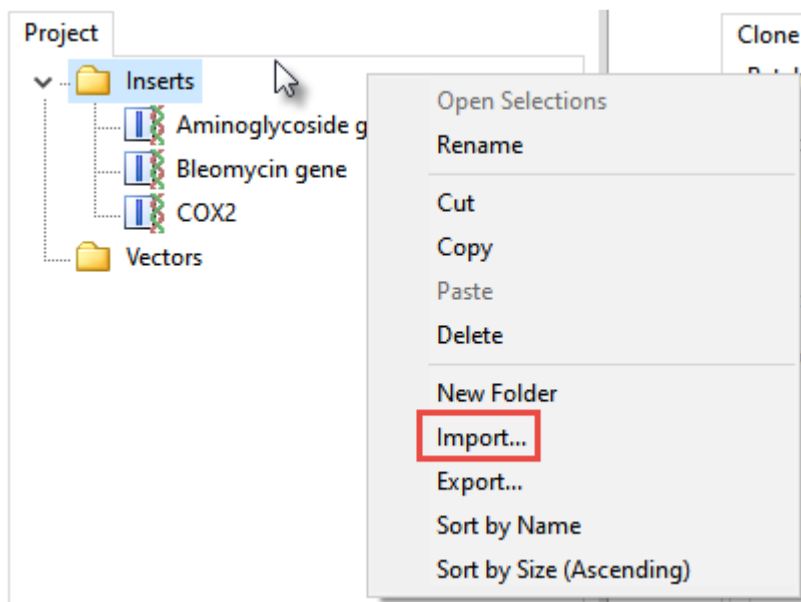
Main cloning workflow::

The main cloning workflow takes only about two minutes from start to finish. However, for purposes of explanation, it has been divided into nine steps.

1. Choose **Cloning > Batch Clone** or **Tools > Batch Clone** to open a specialized version of the Cloning window.



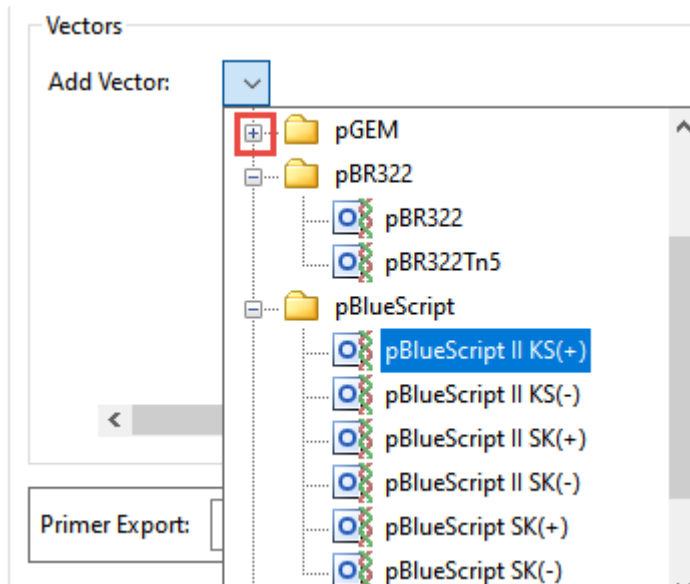
2. Load the inserts into the **Inserts** folder by clicking and then right-clicking on the folder and choosing **Import**. Select the file and click **Open**.



✿ **Note:** Other methods for choosing inserts are described in [this topic](#).

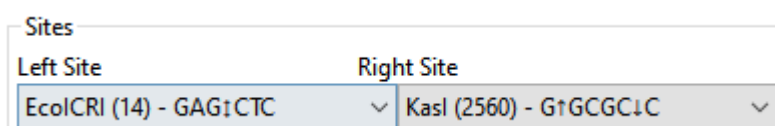
3. Use the **Method** drop-down to choose between **Ligation Independent Single Insert**, **Gateway Single Insert** or **PCR Directed Restriction Insert**.
4. The **Batch Prefix** box is populated with a prefix that will be used to begin each clone's name. This prefix relates to the current cloning method. If you would like to override this, type a substitute prefix into the box.
5. If you are doing Ligation (LIC) or PCR cloning, choose one or more vectors from the **Add Vector** drop-down. If you are doing Gateway cloning, you will be prompted to choose both a **Donor Vector** and a **Destination Vector**.

Within the Add Vector drop-down menu, expand or hide folder contents by pressing the +/- buttons.

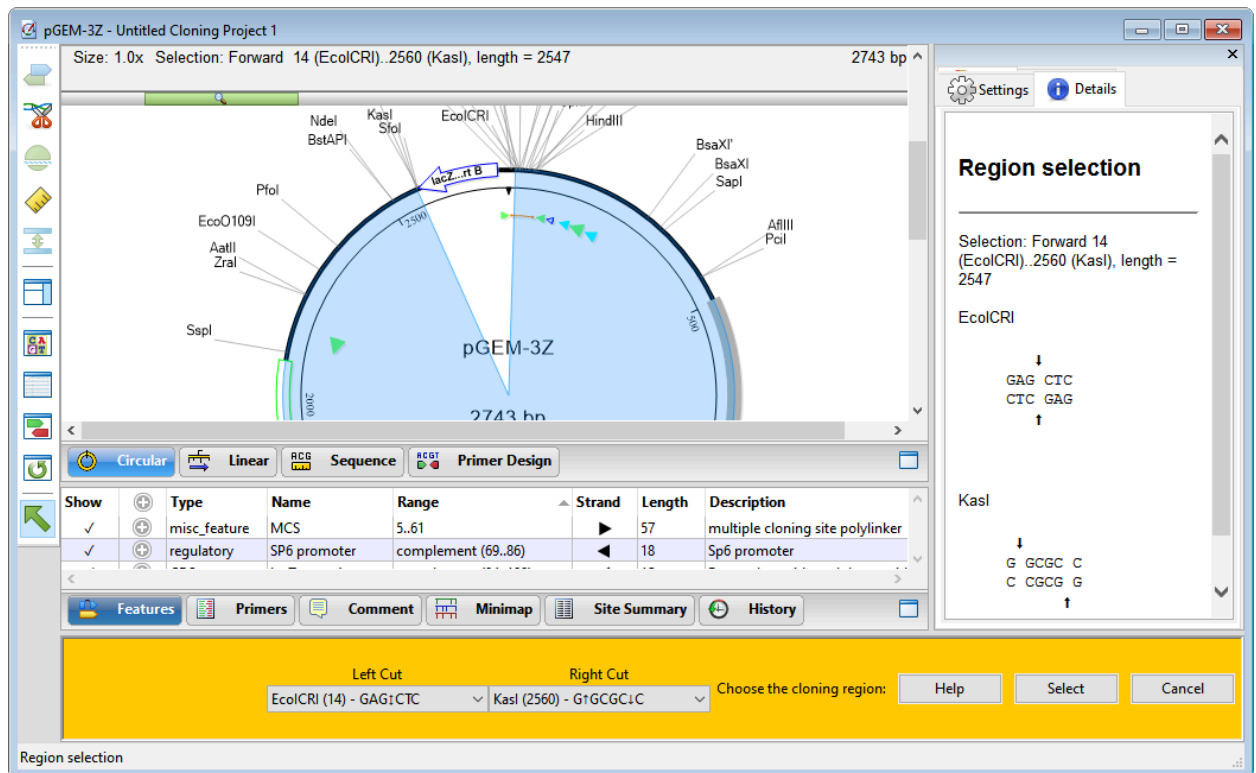


To add multiple vectors, use the same drop-down menu to choose each additional vector. To delete a vector once it appears in the list below the drop-down menu, right-click on its name and choose **Delete**.

6. If you are doing LIC or PCR cloning, specify the cloning region using either of the following methods:
 - Choose enzymes from the **Left Site** and **Right Site** drop-down menus. If you change your mind, simply make a different selection from either/both menus.

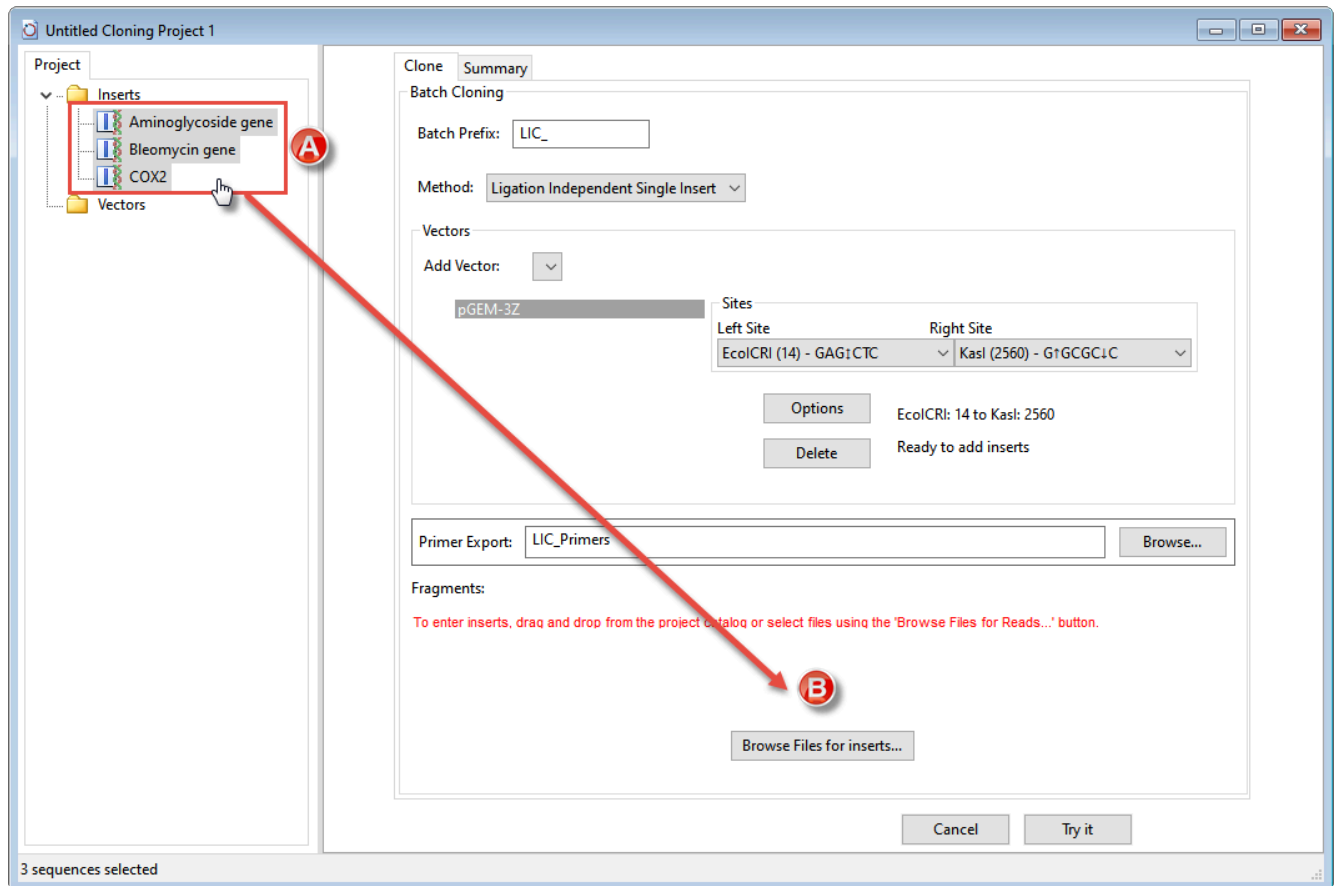


- Press the **Options** button to open the Circular view and choose restriction sites from the two drop-down menus there. After specifying the cloning region, press **Select**.



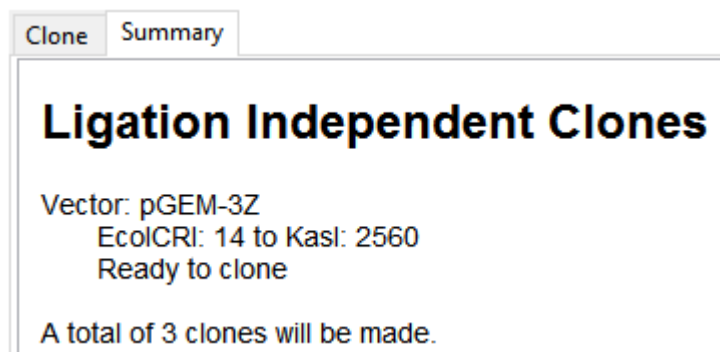
To remove selections and return the cloning region menus to their default states, press **Delete**.

- Specify inserts by pressing the **Browse files for inserts** button or by dragging the insert(s) from the **Inserts** folder (A) on the left of the window and dropping them into the area near the button (B).



To remove an insert from the list, right-click on it and choose **Delete**.

8. Press **Try It**. A summary appears.



If you wish to change something before running the cloning simulation, press **Change**. Otherwise, proceed to the next step.

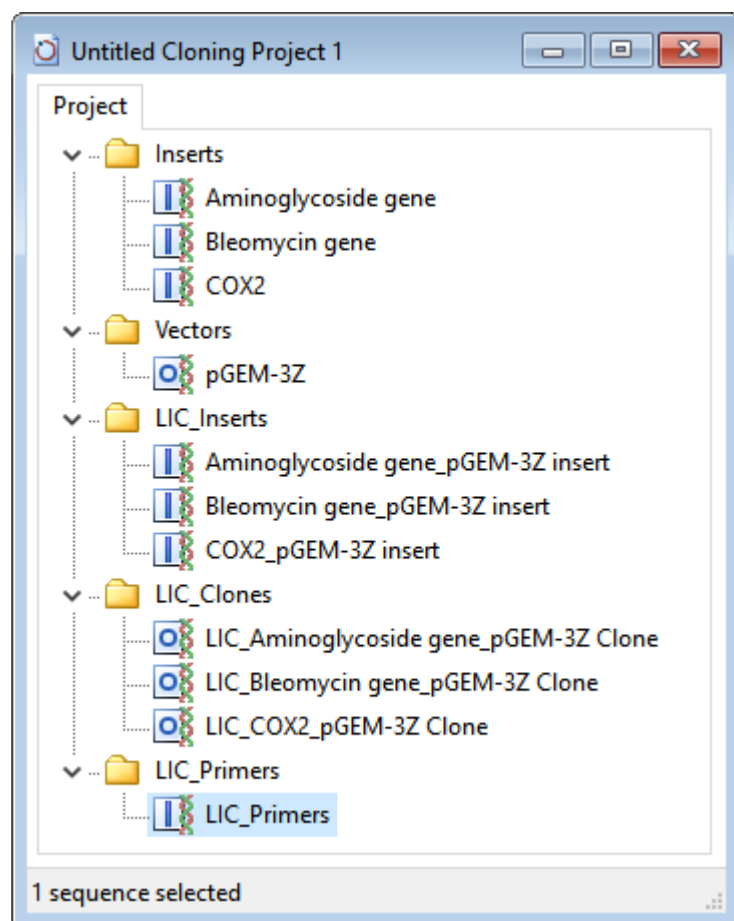
9. Press **Make it**. A window pops up prompting you to save the primers file. Choose between FASTA (.fas), primer catalog (.pri) and text (.txt) formats, then click **Save**. This primer file can be used for ordering primers. An example of a .fas file opened in a text editor appears below.

```

GTAATACGACTCACTATAGGGCGAATTCGAGCTCATGATTGAACAAGAT   LIC_Aminoglycoside gene_pGEM-3Z Forward insert primer
GCGAATGGCGCCTTCAGAAGAACTCGTCAAGAAGG LIC_Aminoglycoside gene_pGEM-3Z Reverse insert primer
TCACTATAGGGCGAATTCGAGCTCATGACCGACCA LIC_Bleomycin gene_pGEM-3Z Forward insert primer
CGAATGGCGCCTTCATGAGATGCCTGCAAGCAATT LIC_Bleomycin gene_pGEM-3Z Reverse insert primer
GACTCACTATAGGGCGAATTCGAGCTCATTAATAATTT LIC_COX2_pGEM-3Z Forward insert primer
CGAATGGCGCCTTTATTCTATAGTACCAAAACATCAT LIC_COX2_pGEM-3Z Reverse insert primer

```

Results are saved in the Project window. Folder contents are described below.



- **Inserts** – Contains any files added to the folder prior to cloning.
- **Vectors** – Contains any vectors added to the folder prior to cloning, as well as the vector(s) actually used in the cloning simulation.
- **Inserts** – The inserts resulting from the cloning simulation. These are named with the suffix *-insert* to differentiate them from the original files.
- **Clones** – The clones. Each name begins with the selected **Batch Prefix** and has the suffix *Clone*.

- **Primers** – Each clone and insert combination has its own set of primers. This file, which begins with the selected **Batch Prefix**, has all primers concatenated together. Double-click on the file to open it in SeqBuilder Pro's main window and view primer details in the **Primers** tab.

Show	Active		Set Name	Name	F/R	Primer Sequence
✓			Set 1	LIC_Aminoglycoside gene_pGEM-3Z		<GTAATACGACTCACTATAGGGCGAATTCGAGCTCATGATTGAACAAGAT>.. <CCTTCTTGACGAGTTCTTCTGAAGGCGCCATTGCG>
			Set 1	LIC_Aminoglycoside gene_pGEM-3Z	F	GTAATACGACTCACTATAGGGCGAATTCGAGCTCATGATTGAACAAGAT
			Set 1	LIC_Aminoglycoside gene_pGEM-3Z	R	GCGAATGGCGCCTTCAGAAGAACTCGTCAAGAAGG
✓			Set 2	LIC_Bleomycin gene_pGEM-3Z		<TCACTATAGGGCGAATTCGAGCTCATGACCGACCA>.. <AATTGCTTGCAAGGCATCTCATGAAGGCGCCATTG>
✓			Set 3	LIC_COX2_pGEM-3Z		<GACTCACTATAGGGCGAATTCGAGCTCATTAATAATT>.. <ATGATGTTTTGGTACTATAGAATAAAGGCGCCATTG>

Features
 Primers
 Comment
 Minimap
 Site Summary
 History

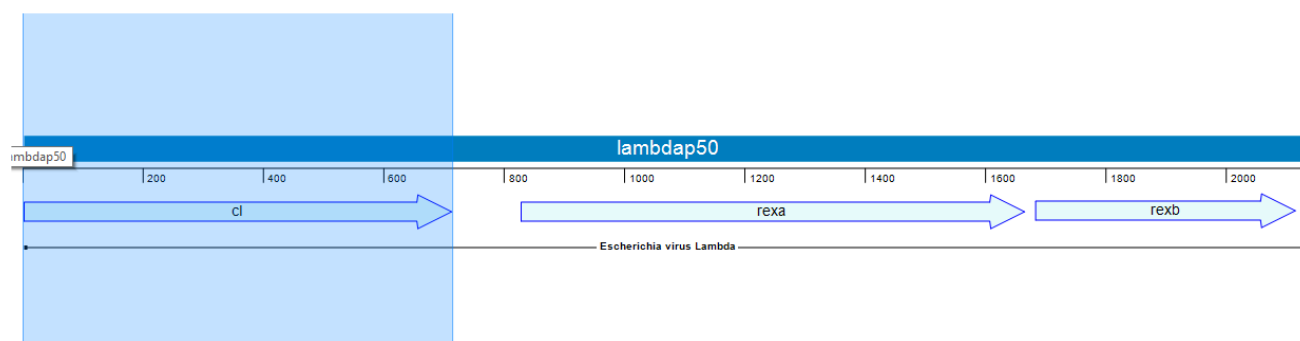
Site-Directed Mutagenesis

Site-directed mutagenesis is a molecular biology method that is used to make specific changes to the DNA sequence of a gene. As of Lasergene 16.0, you can perform site-directed mutagenesis using Protean 3D's Protein Design service together with SeqBuilder Pro. The following case study is based on [a poster that DNASTAR presented](#) at the 2019 PEGS Summit in Boston.

Introduction:

Nature commonly evolves proteins to provide sufficient fold stability to optimize its activity—not its energetic stability. Lasergene's site-directed mutagenesis workflow is designed to help improve the developability of a protein by predicting which mutations may lead to increased protein stability and developability.

In the following example, we'll use Lasergene's site-directed mutagenesis workflow to replicate the findings of Lim, et al., 1994 (*Proc. Natl. Acad. Sci. USA*, 91:423-7). Lim made three specific mutations to the repressor protein *cl*. This protein is part of the *lambda P50* operon (PDB ID: 1LMB), which also includes the genes *rexa* and *rexb*.



Lim's study found that mutating the repressor protein *cl* at three specific points conferred 0.5 kcal/mol more stability compared to the wild type.

As we describe below, Lasergene's site-directed mutagenesis workflow not only replicates Lim's findings, but early on—in Step #2—reveals other mutations that could be even more stabilizing. Our results were presented by us in a poster at the 2019 PEGS conference.

Note: The coordinates in this blog post match the current PDB structure, not the coordinates reported from the 1994 paper or the poster cited above. UniProt indicates position 1 is a removed initiator methionine and the chain starts at position 2 (serine). By contrast, RCSB numbers the start of the chain after post-translational modification as position 1. This blog post uses a sequence that follows the UniProt convention rather than the RCSB convention.

Part I: Scan for hotspots and create variants on protein structure

Since we have a protein structure as our starting point, we will begin with the following steps. If a protein

structure was not available, we could instead start by creating variant primers as shown in Part II.

1. Launch Protean 3D and use **File > Open from PDB** to open the protein sequence **1LMB**.

We will begin by performing “hot spot scanning” using alanine. This hot spot workflow is used to predict folding hot spots; residues that contribute to fold stability more significantly than others. The more destabilizing the alanine variant, the greater the probability the position and residue identity is important to the fold.

2. Choose **Modeling > Protein Design > Scan for Hot Spots With > Document**. Keep all defaults and choose **Run**.

In approximately a minute or less, the results of the alanine scan appear in the Report view. The Prediction column indicates that the positions referenced by Lim—36, 40 and 47—are all predicted to be “destabilizing.”

Report 2: Hot Spot Scan for 1LMB (179 positions)

Hot Spot Scan for 1LMB (179 positions)

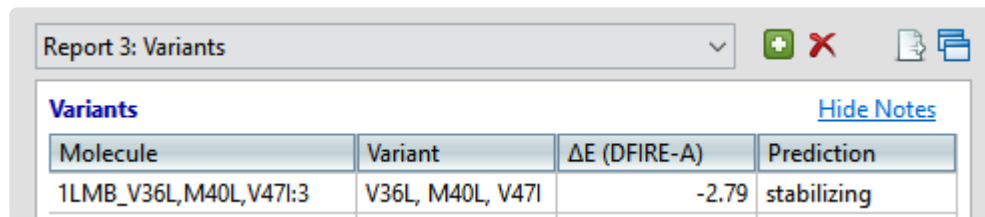
Molecule	Variant	ΔE (DFIRE-A)	Prediction
1LMB:3	E34A	-0.20	
1LMB:3	S35A	-0.44	
1LMB:3	V36A	1.34	destabilizing
1LMB:3	A37A	-0.00	
1LMB:3	D38A	-0.41	
1LMB:3	K39A	-0.40	
1LMB:3	M40A	0.94	destabilizing
1LMB:3	G41A	-0.25	
1LMB:3	M42A	0.47	
1LMB:3	G43A	-0.37	
1LMB:3	Q44A	-0.17	
1LMB:3	S45A	-0.34	
1LMB:3	G46A	-0.73	stabilizing
1LMB:3	V47A	1.00	destabilizing
1LMB:3	G48A	-0.86	stabilizing
1LMB:3	A49A	-0.01	

The Report contains several residues that are even more destabilizing (i.e., have higher DFIRE-A potentials) than the ones in Lim’s 1994 paper. Ideally, these would be our first choice for increasing the developability of the protein. However, for purposes of replicating Lim’s findings, we will proceed with the three positions referenced above.

3. To manually insert variants into these positions of interest, choose **Modeling > Protein Design > Create Variant With > Document**. Then use the **Substitute** drop-down menus to change position 36 from **V** to **L**; position 40 from **M** to **L**; and position 47 from **V** to **I**.

If we weren't replicating Lim's study, which amino acids would we substitute for the originals? Unfortunately, there is no "magical" way to determine which variants will work best. We can simply run different combinations and see which ones lead to increased stability.

Press **Run**. The new Report shows that this combination of variants is predicted to be "stabilizing."



Molecule	Variant	ΔE (DFIRE-A)	Prediction
1LMB_V36L,M40L,V47I:3	V36L, M40L, V47I	-2.79	stabilizing

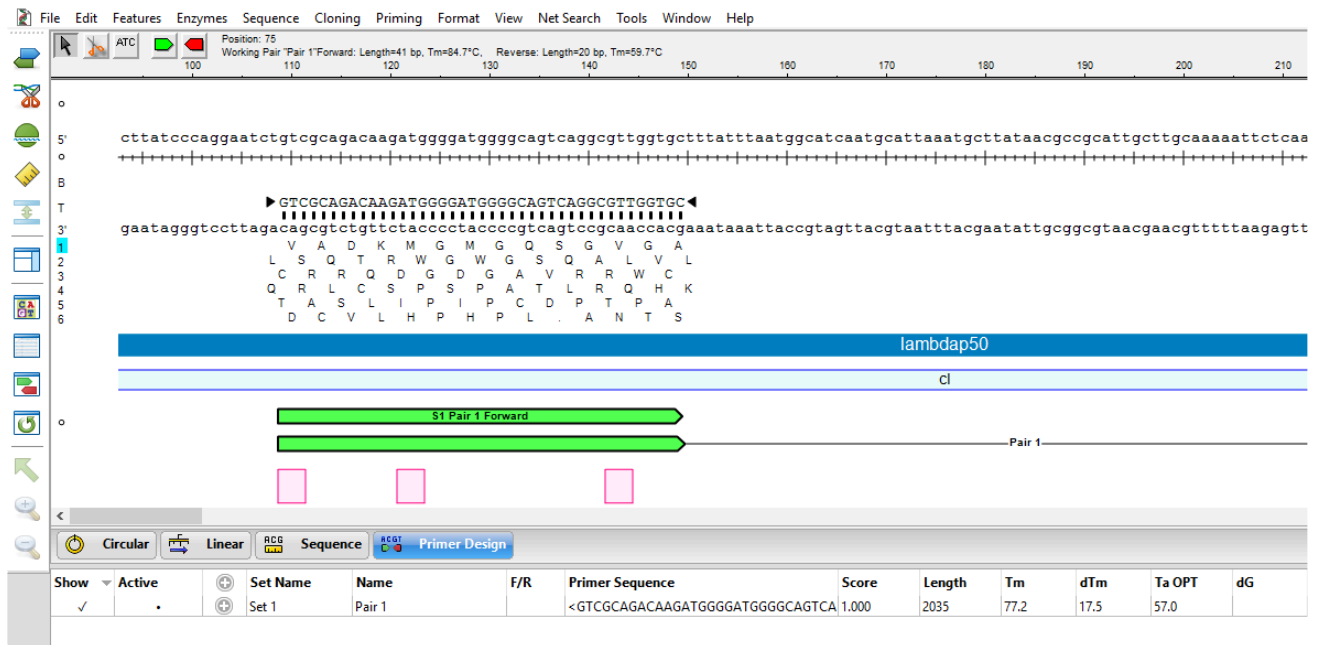
Part II: Introduce variants in sequence and create mutated primer

1. Launch SeqBuilder Pro and use **File > Open** to open the original (unmutated) 1LMB protein sequence.
2. [Reverse translate](#) the sequence to DNA. (We also created annotations showing the locations of the three variants—these appear in the image in Step 5). Choose **File > Save**.
3. Select all nucleotides from the beginning of the *cl* gene to the right (downstream) of the sequence and delete them. Then use **File > Save As** to save the remaining fragment as *Upstream fragment.sbd*.
4. Open the sequence saved in Step 2, above. Select all nucleotides to the left (upstream) of the *cl* gene and delete them. Then use **File > Save As** to save the remainder of the sequence as *cl mutated fragment.sbd*.

We now have two sequence files that—laid end to end—would be identical to the original nucleotide sequence.

5. In this step, we will use the [Primer Design view](#) to create PCR primer pairs at the exact desired location with respect to features and translational frames. Choose **Edit > Select All**, then **Priming > Create Primer Pairs**. Keep the default setting Primers end exactly at selection and press **OK**. Once the primers are created, the forward primer is extended to 41 bp in length to fully cover all three mutation areas.

The image below shows that the three codons where the variants will be introduced (pink) all occur on the forward primer (green).



Position: 75
Working Pair "Pair 1" Forward: Length=41 bp, Tm=84.7°C, Reverse: Length=20 bp, Tm=59.7°C

5' cttatcccaggaatctgtcgcagacaagatggggatggggcagtcaggcgttggtgctttatttaatggcatcaatgcattaaatgcttataacgcgcattgcttgcaaaaattctcaa
3' gaatagggtccttagacagcgtctgttctaccctaccgcgtcagtcgcgaaccacgaataaattaccgtagttacgtaatttacgaatattgcggcgtaacgaacgtttttaagagtt

► GTCGCAGACAAGATGGGGATGGGGCAGTCAGGCCTTGGTGC ◀

V A D K M G M G Q S G V G A
L S Q T R W G W G A V R R W C L
Q R L C S P S P A T L R Q H K
T A S L I P I P C D P T P A K
D C V L H P H P L . A N T S

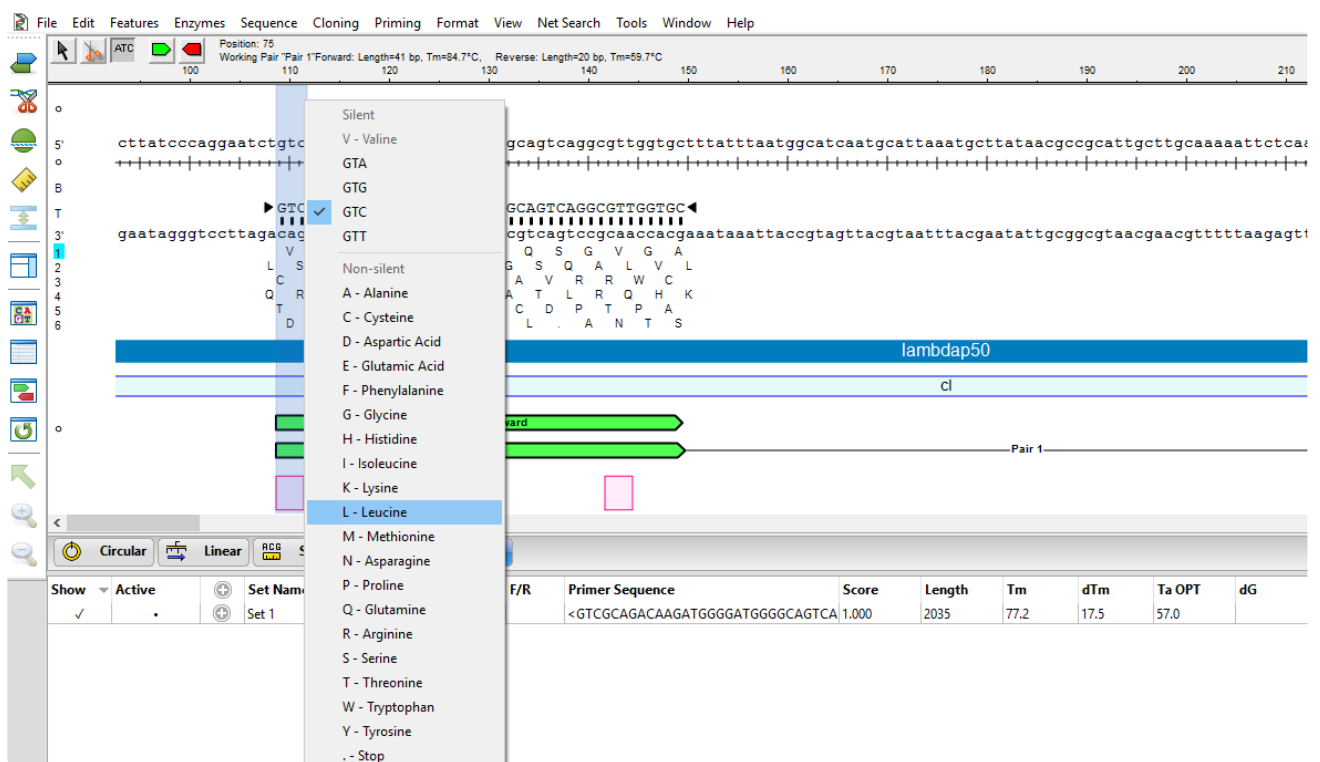
lambdap50
cl

S1 Pair 1 Forward
Pair 1

Circular Linear Sequence Primer Design

Show	Active	Set Name	Name	F/R	Primer Sequence	Score	Length	Tm	dTm	Ta OPT	dG
✓	•	Set 1	Pair 1		<GTCGCAGACAAGATGGGGATGGGGCAGTCA	1,000	2035	77.2	17.5	57.0	

6. Next, we need to recreate the variants of interest using the Introduce **Codon Change/Mutation** tool (ATC). This tool allows us to easily modify triplet codons within primers to introduce the desired mutations. Select the tool, then hover over the first codon (V in Reading Frame 1) and choose the desired substitute: L.



Position: 75
Working Pair "Pair 1" Forward: Length=41 bp, Tm=84.7°C, Reverse: Length=20 bp, Tm=59.7°C

5' cttatcccaggaatctgtcgcagacaagatggggatggggcagtcaggcgttggtgctttatttaatggcatcaatgcattaaatgcttataacgcgcattgcttgcaaaaattctcaa
3' gaatagggtccttagacagcgtctgttctaccctaccgcgtcagtcgcgaaccacgaataaattaccgtagttacgtaatttacgaatattgcggcgtaacgaacgtttttaagagtt

► GTCGCAGACAAGATGGGGATGGGGCAGTCAGGCCTTGGTGC ◀

V A D K M G M G Q S G V G A
L S Q T R W G W G A V R R W C L
Q R L C S P S P A T L R Q H K
T A S L I P I P C D P T P A K
D C V L H P H P L . A N T S

lambdap50
cl


S1 Pair 1 Forward
Pair 1

Circular Linear Sequence Primer Design

Show	Active	Set Name	Name	F/R	Primer Sequence	Score	Length	Tm	dTm	Ta OPT	dG
✓	•	Set 1	Pair 1		<GTCGCAGACAAGATGGGGATGGGGCAGTCA	1,000	2035	77.2	17.5	57.0	

Silent
V - Valine
GTA
GTG
GTC
GTT
Non-silent
A - Alanine
C - Cysteine
D - Aspartic Acid
E - Glutamic Acid
F - Phenylalanine
G - Glycine
H - Histidine
I - Isoleucine
K - Lysine
L - Leucine
M - Methionine
N - Asparagine
P - Proline
Q - Glutamine
R - Arginine
S - Serine
T - Threonine
W - Tryptophan
Y - Tyrosine
- Stop

7. In the same way, introduce the planned variants at the second and third codons. The mutated bases are represented by a pink lower case font, as shown below.



Position: 2124
Working Pair 1 Forward: Length=37 bp, Tm=84.9°C, Reverse: Length=22 bp, Tm=83.5°C

Sequence: aaaaaaagaaaaatgaacttggttatccaggaatctgtcgcagacaagatggggatggggcagtcaggcggttggtgctttatttaatggcatcaatgcattaaatgcttataacgccgattgc

Protein: L A D K L G M G Q S G I G
L L Q T S W G W G S Q A L G
S C R Q V G D G A V R H W
E Q L C L T P S P A T L C H Q
P A S L N P I P C D P M P
K C V L Q P H P L A N T

lambdap50
cl

S1 Pair 1 Forward
Pair 1

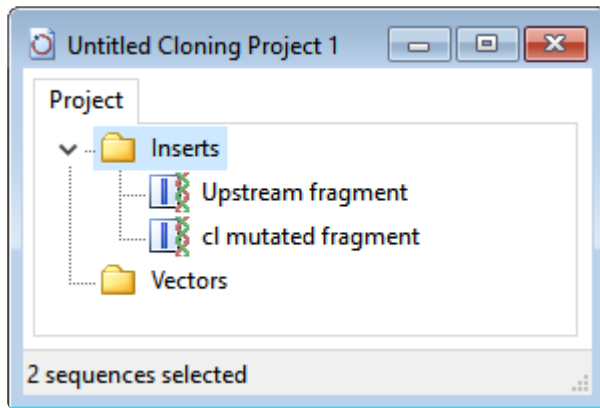
Show	Active	Set Name	Name	F/R	Primer Sequence	Score	Length	Tm	dTm	Ta OPT	dG
✓	*	Set 1	Pair 1		<cTIGCAGACAAGITGGGGATGGGGCAGTCAGGCaTTG>	1.000	2035	77.2	13.7	58.2	
		Set 1	S1 Pair 1 Forward	F	cTIGCAGACAAGITGGGGATGGGGCAGTCAGGCaTTG		37	64.9	1.5		-63.5
		Set 1	S1 Pair 1 Reverse	R	CAAGAAAAACCCGGCGCTGAGG		22	63.5	1.5		-48.4

At this point, either or both primers could be further adjusted to improve T_m differences and primer-dimer formation.

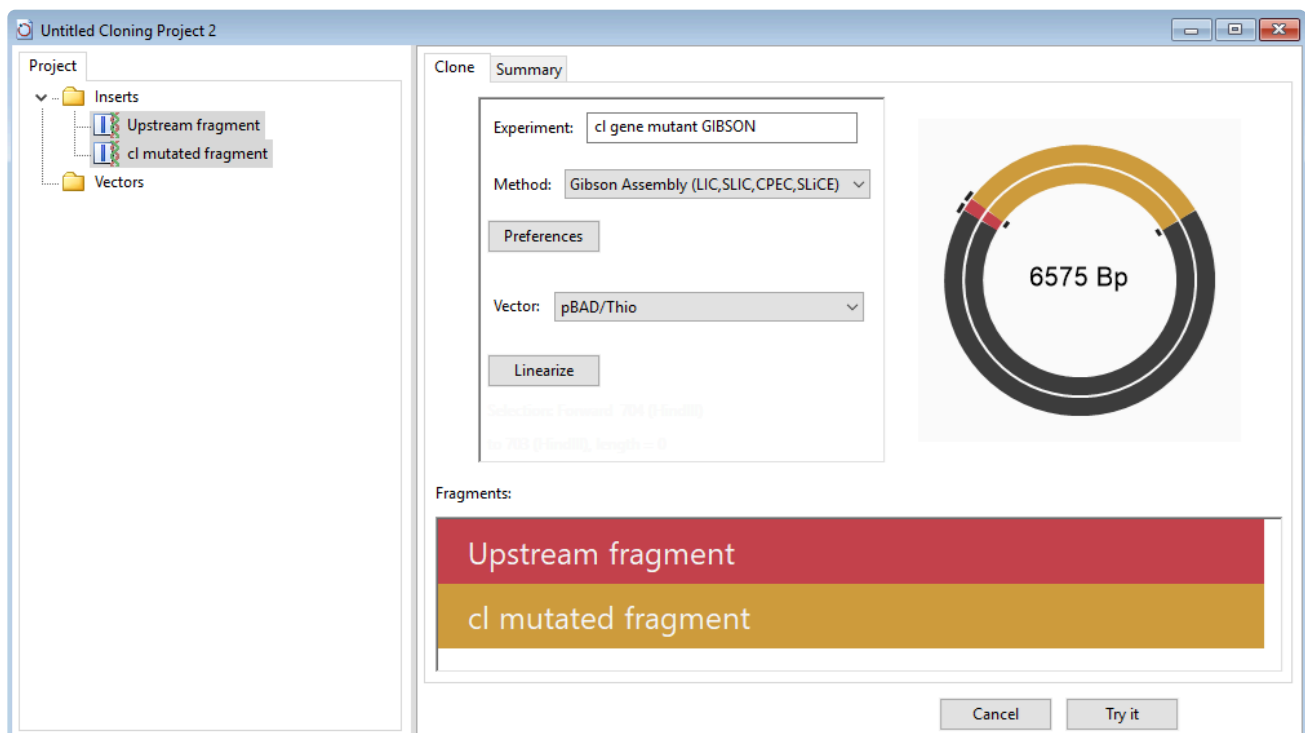
- Save the *cl mutated fragment.sbd* project using **File > Save**.
- Reopen the sequence fragment *Upstream fragment.sbd* and create primer pairs using the commands in Step 4, above. Save the project using **File > Save**.

SeqBuilder Pro provides multi-fragment PCR based cloning options so that the mutated gene of interest can be cloned into an appropriate expression vector. In the next steps, we will clone the mutated *cl* gene (and the rest of the *lambdap50 operon*) and the upstream (non-mutated) fragment of the *cl* gene into an *E.coli* expression vector system (pBAM/Thio) using PCR-based Gibson assembly.

- Open a new, empty cloning project using **File > New Cloning Project**.
- Click on the **Inserts** folder to select it. Then right-click on the **Inserts** folder and choose **Import**. Use **Ctrl+click** (Win) or **Cmd+click** (Mac) to select the files *cl mutated fragment.sbd* and *Upstream fragment.sbd*, then click **Open**.



12. Choose **Cloning > Gibson, LIC Cloning**.
13. Next to **Experiment**, type in **cl gene mutant**.
14. Use the **Vector** drop-down menu and click the plus sign next to **Restriction Vectors** to expand the folder. Then click on the first vector in that folder, **pBAD/THIO**.
15. Drag the two sequence fragments from the **Inserts** folder and drop them in the **Fragments** area on the bottom right. Make sure the upstream fragment is on top. In the cloning dialog, name it **cl gene mutant**.

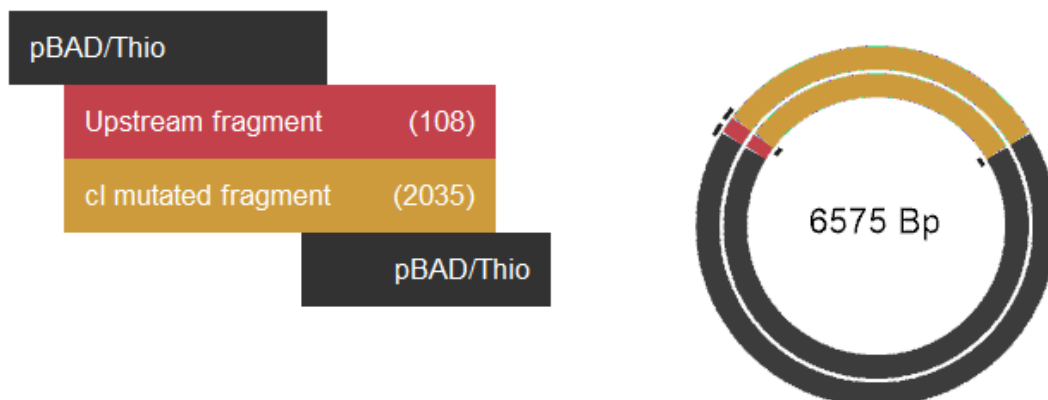


16. Press the **Linearize** button and specify a **Left Cut** at **NaeI (676)** and **Right Cut** at **HindIII (703)**. Press **Select**.
17. Press **Try it**, then **Make it**. The [History view](#) shows a rendering of the fragments and clone, all primer

information, and the list of steps needed to create the clone in the lab. The red text in the Primers area is the junction that puts the two sequence fragments back together.

Cloning Summary

Fragments



Primers

Overlap	Primer	Anneal	T/B	Tm	Ta
pBAD/Thio	ATCCGGTGATGACGATGACA ATGAGCACAAAAAGAAACC	Upstream fragment	T	60.45	52.85
cl mutated fragment	CAACTTGTCTGCAAG AGATTCTGGGATAAGCCAAGTT	Upstream fragment	B	60.48	52.88
Upstream fragment	CTTGGCTTATCCCAGGAATCT CTTGCAGACAAGTTG	cl mutated fragment	T	60.51	52.91
pBAD/Thio	ATAGGCTTACCTTCA CAAAGAAAACCCGGCGCTGA	cl mutated fragment	B	60.53	52.93

Steps

1) Cut pBAD/Thio with HindIII

2) Amplify Upstream fragment using primers:

Top: 5'-ATCCGGTGATGACGATGACAATGAGCACAAAAAGAAACC-3'
 Bottom: 5'-CAACTTGTCTGCAAGAGATTCTGGGATAAGCCAAGTT-3'

3) Amplify cl mutated fragment using primers:

Top: 5'-CTTGGCTTATCCCAGGAATCTCTTGCAGACAAGTTG-3'
 Bottom: 5'-ATAGGCTTACCTTCACAAAGAAAACCCGGCGCTGA-3'

18. Open expression clone *cl gene mutant GIBSON Clone 1* in SeqBuilder Pro by double-clicking on it.

The [Linear view](#) shows all the of the vector and insert feature annotations and lets us apply ORF detection and translation to confirm that the gene of interest remains in-frame with the fusion protein.

19. Apply the ORF for “Top Frame 1,” shown as a red bar in the image below. The small white triangles along the ORF denote the *atg* start sites.

20. Click on the start site corresponding to *hpTrxA*, a mutated version of the *E. coli* protein thioredoxin (*trxA*) used to create a metal-binding domain that allows purification of thioredoxin fusion on metal-chelating resins.

The screenshot displays the SeqBuilder Pro interface. The top panel shows a genomic map with a selection of 346,1416 bp (length = 1071) and a size of 7.7x. The map includes a pBAD thio promoter (black bar), a mutated *hpTrxA* gene (red bar), and a *mutate* region (yellow bar). A blue arrow indicates the *hpTrxA* gene. Below the map, a cloning summary is provided.

Cloning Summary

Fragments

Fragment	Length (bp)
pBAD/Thio	
Upstream fragment	(108)
<i>cl</i> mutated fragment	(2035)
pBAD/Thio	

A circular plasmid map is shown on the right, labeled 6575 Bp.

The selection contains the entire *cl* gene feature; verification that the transcript is in-frame.

Agarose Gel Simulation

Agarose gel simulation lets you find the location of restriction enzymes on a gel and perform 'mock' digests to determine the expected size of digest products on an electrophoresis gel. Gel simulations let you find the ideal percent agarose and set of molecular weight markers to resolve your DNA fragments. This digital simulation replaces manual plotting of DNA fragment mobility on graph paper. Once you run the actual gel in your laboratory, you can place the gel or its image side-by-side with an image of the simulation to confirm gels that have complex banding patterns.

The following video is a brief walkthrough of the agarose gel simulation workflow:

To perform an agarose gel simulation:

1. (optional) [Open the sequence](#) you wish to use in the simulation.
2. (optional) If you plan to apply primer pairs to one or more lanes of the simulated gel, [locate the primer pairs](#).
3. Choose **File > New Agarose Gel**.

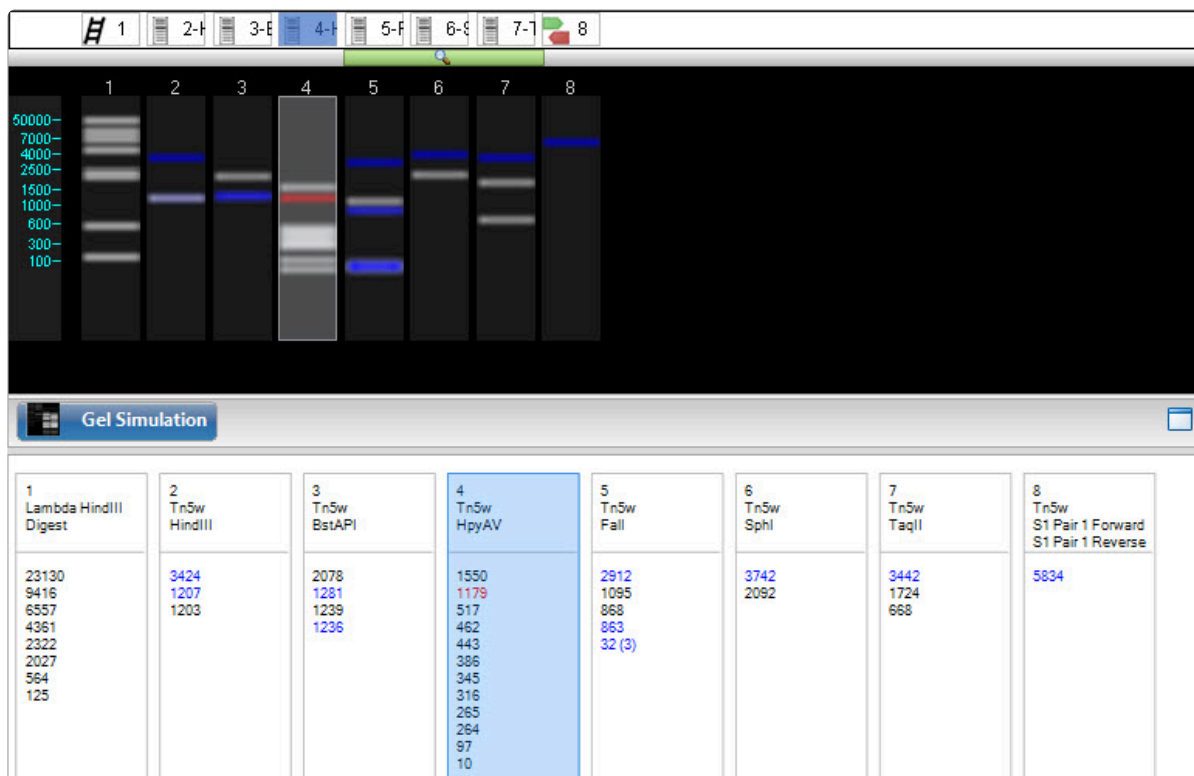
A new project window opens with the [Gel Simulation](#) and [Fragment Sizes](#) views active. On the right, the [Sequences](#), [Enzymes](#) and [Primers](#) panels appear at the top, and the [Settings](#) and [Markers](#) panels appear on the bottom. All of these views and panels are synchronized, meaning that each of them will update simultaneously when a change is made in any of the other views or panels. If a file was already open in SeqBuilder Pro, it will be added to the Sequences panel.

4. Click on the **Settings** tab and [define gel parameters](#) such as dimensions, percent agarose, and run time. Choose from a variety of commercial gels, and/or enter custom parameters.

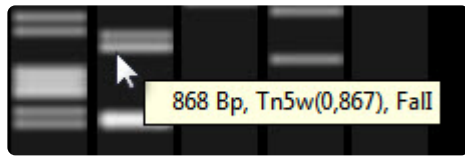
5. Select the lanes one by one and apply a molecular weight marker, sequence, enzyme, or primer pair.
 - To apply a molecular weight marker, select a gel lane. Then click on the **Markers** tab and check the box next to the desired marker. For more information, see [Markers panel](#).
 - To apply a sequence, select a gel lane. Then click on the **Sequences** tab and check the box next to the sequence of interest. To add sequences to the panel, right-click within the panel and choose **Import**. For details, see [Sequences panel](#).
 - To apply an enzyme, select an already-populated gel lane. (Note: If a lane is unpopulated, follow the bullet point above to first apply a sequence to the lane). Then click on the **Enzyme** tab and check the box next to the enzyme of interest. For more information, see [Enzymes panel for gels](#) and [Apply or remove enzymes in the gel simulation view](#).
 - To apply a primer, select a gel lane. Then click on the **Primers** tab and check the box next to the desired primer pair. For details, see [Primers panel](#).

To analyze the results:

- To select a fragment and its range on the sequence, click on a band in the Gel Simulation view, or on a fragment size notation in the Fragment Sizes view. The fragment is shown in red, and the footer is populated with information about the selection. Any fragment from the same sequence as the selected fragment, and which overlaps the selected fragment in any way, will appear in blue.



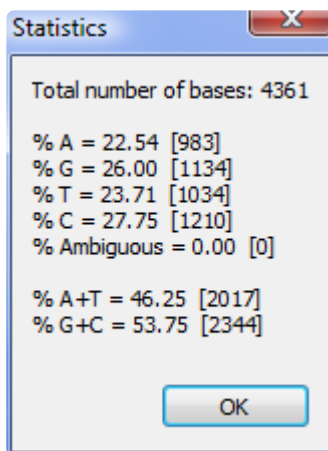
- To view information about a band or fragment size notation, hover the mouse over it to reveal a tooltip.



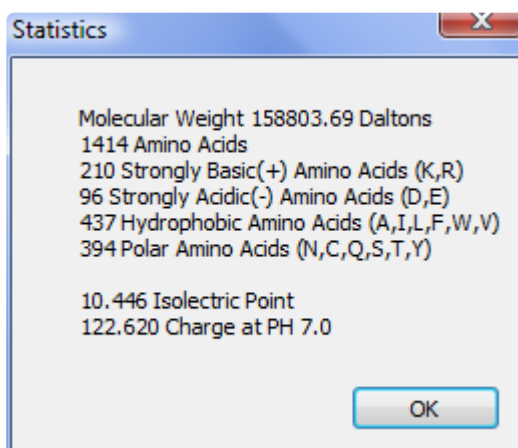
Sequence Statistics

To view a statistical summary of a selected portion of sequence:

1. Select a portion of the sequence in a graphical view.
2. Choose **Sequence > Statistics**.
 - For nucleotide sequences, the display shows the number and composition of bases, the percentage of A, G, T, C, ambiguous bases, and A+T and C+G in the selected region. The actual numbers of each type of base follows the percentage in brackets.



- For protein sequences, the summary includes the number of strongly basic, strongly acidic, hydrophobic, and polar amino acids, as well as the molecular weight, isoelectric point, and charge at pH 7.0 for the selected region.



Motion Library

Lasergene applications are designed to work together seamlessly. One useful workflow is to create or edit a protein sequence in SeqBuilder Pro, fold it in [Novafold](#) and view the finished product in [Protean 3D](#).

Whether or not you are already licensed to use Protean 3D, SeqBuilder Pro lets you browse protein animations from Protean 3D's "Motion Library." The Motion Library consists of over 500 animated and annotated macromolecular motions illustrating structural conformational changes.

To launch Protean 3D with the Motion Library open, choose **Help > Molecules in Motion**. To learn about the Motion Library and how to use it, see the Protean 3D help topic, [The Motion Library](#).

Copy, Print, Save and Export

From within SeqBuilder Pro, you can do the following:

- [Copy a sequence or image](#)
- Print ([Windows](#) / [Macintosh](#))
- [Save](#)
- [Export sequences](#)

Copy a sequence or image

To copy selected text (including sequence) to the clipboard:

Choose **Edit > Copy** or use the keyboard shortcut **Ctrl/Cmd+C**.

To copy selected text that will be pasted in a particular format:

Use **Edit > Copy As**.

- **Unformatted Sequence** – Copies the sequence only.
- **Formatted Sequence** – Copies the sequence with spacing and line breaks, as it appears in the Sequence view.
- **Translated sequence** – Copies the protein translation of the highlighted range.
- **Spliced sequence** – Copies only the sequence corresponding to the segments of the selected feature, rather than the whole region. For example, if a feature consisted of two segments — 100-129 and 200-232, Spliced sequence would copy a string consisting of bases 100-129, immediately followed by bases 200-232. In contrast, Unformatted Sequence would copy *every* base from 100-232.

To copy an image of a view:

To copy an image of the [Circular](#), [Linear](#), [Sequence](#), [Gel Simulation](#) or [Fragment Sizes](#) view, make it active and choose **Edit > Copy As Picture**. When using this command from most views, only the selected information is copied to the clipboard. From within the Circular view, the entire map is copied, regardless of the selection.

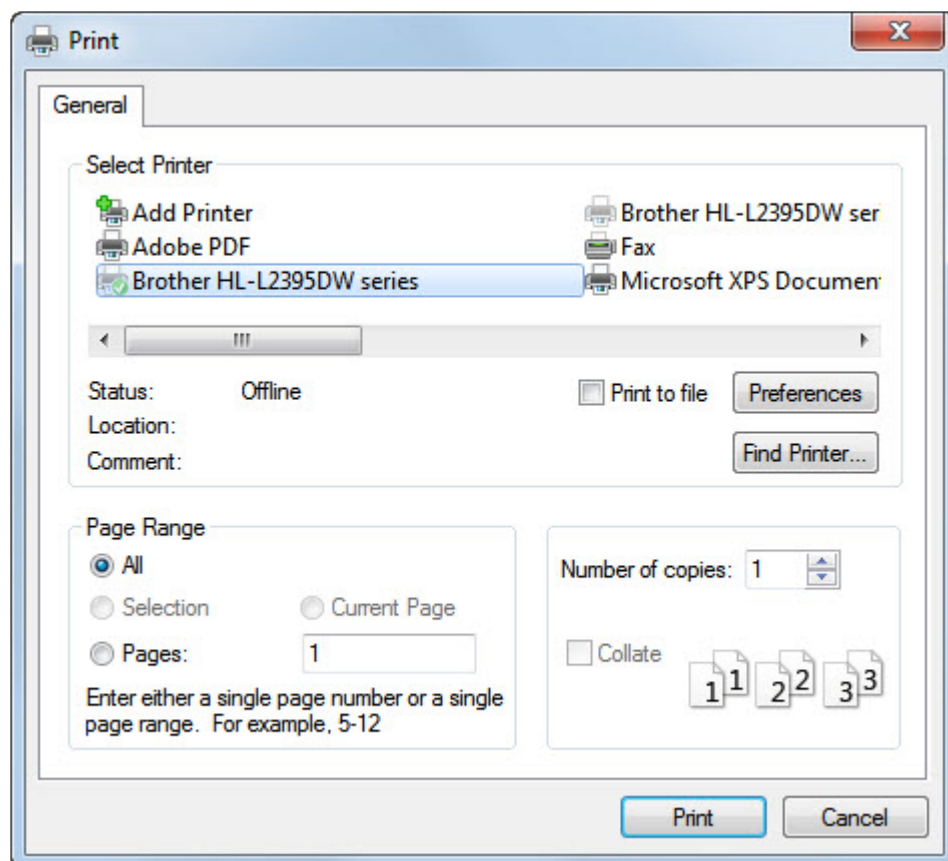


When pasting the copied picture into a Microsoft Office application, we recommend using **Edit > Paste Special** and selecting **Picture (Enhanced Metafile)**.

Print on Windows

To print from within SeqBuilder Pro on Windows:




Select **File > Print** or the keyboard shortcut **Ctrl/Cmd+P** or use **File > Print Preview** (see below) and select the **Print** tool. Select a printer and choose the pages and quantities to print.



To view a preview before printing:

Use **File > Print Preview**.

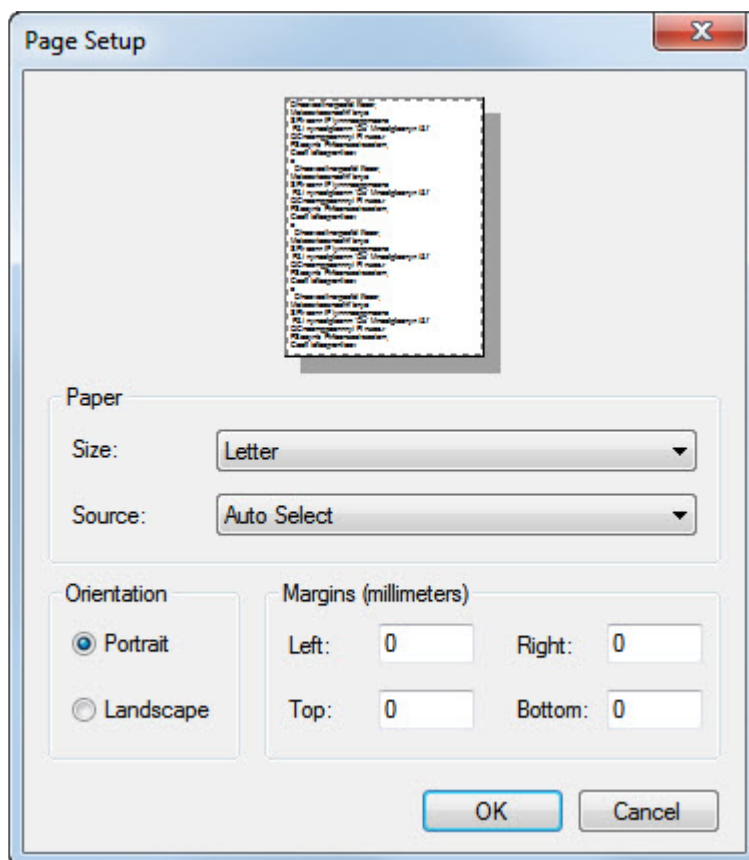
Several navigation and zooming tools are provided in this window.

	Opens the Print dialog; identical to selecting File > Print .
	Returns to the first page in the project.
	Returns to the previous page.

	Shows the total number of pages, and allows you to go to a particular page by typing in the box on the left.
	Advances to the next page.
	Advances to the last page in the project.
	Use the plus/minus buttons or drop-down menu to choose the zoom scale for the preview, from 10-200%. Note: To change the actual printing scale, see File > Page Layout , below.
	To exit from the Print Preview window.

To change the printing size, orientation or margins:

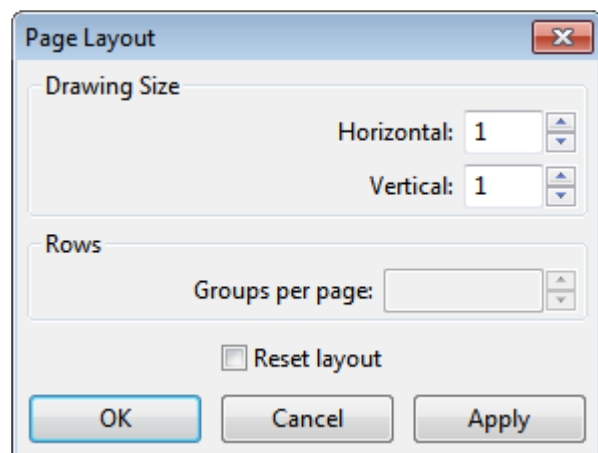
Choose **File > Page Setup**.



The default printing size for all DNASTAR applications is U.S. “Letter” size (8.5 × 11 inches = 21.6 × 28 cm).

To change the page layout:

From within the [Circular](#), [Linear](#) or [Sequence](#) views, select **File > Page Layout**.



- In the Drawing Size area, use the **Horizontal** and/or **Vertical** text boxes to set the number of pages that will be used to display the current view. By increasing the number of pages, you will enhance the resolution in any of these views. One or both of these options may be available depending on the view.
- Enter a number in **Groups per page** sets the number of rows that will print per page in Linear view. To view a linear map in one continuous line on the page, change this value to 1.
- (optional) Check the box next to **Reset layout** to restore the default values.

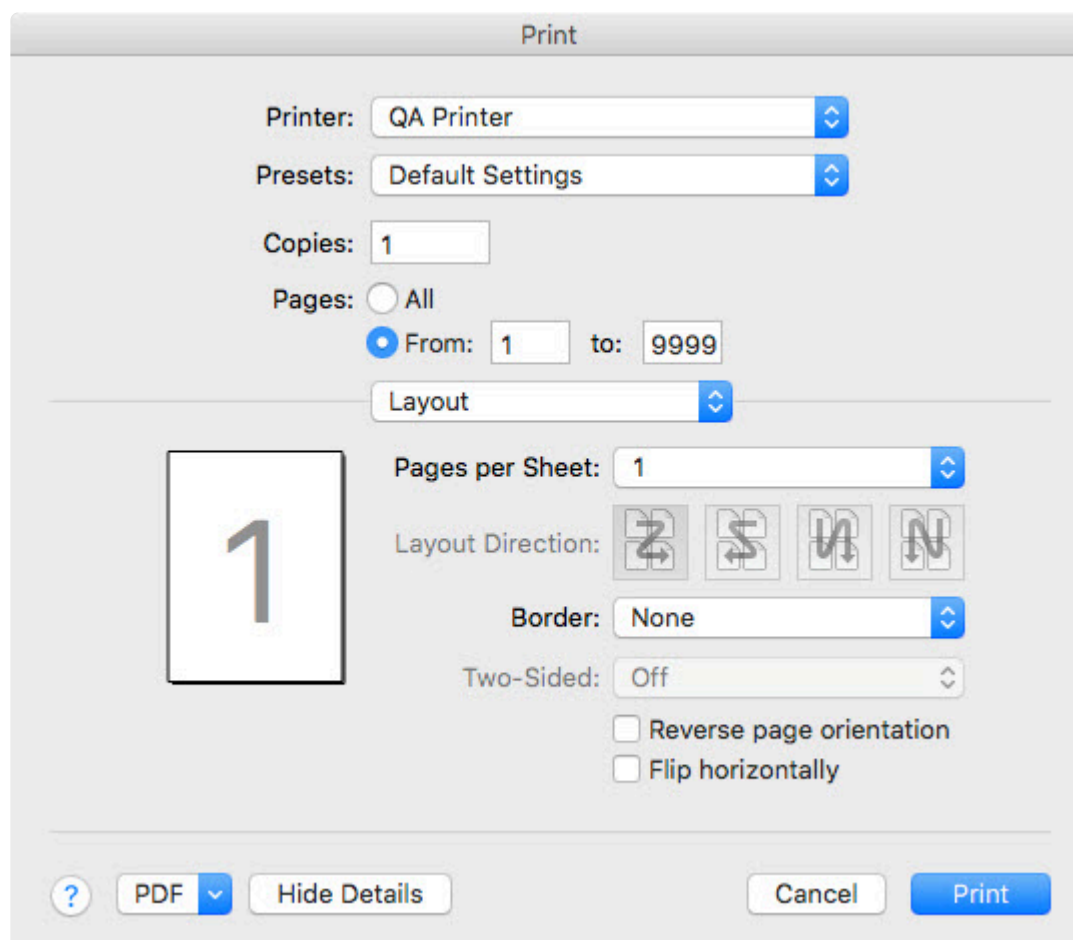
When you have made the desired selections, press **Apply** to save changes and then exit the dialog by clicking **OK**. Or press **Cancel** to exit without saving changes.

Print on Macintosh

To print from within SeqBuilder Pro on a Macintosh:

Select **File > Print** or the keyboard shortcut **Cmd+P**.

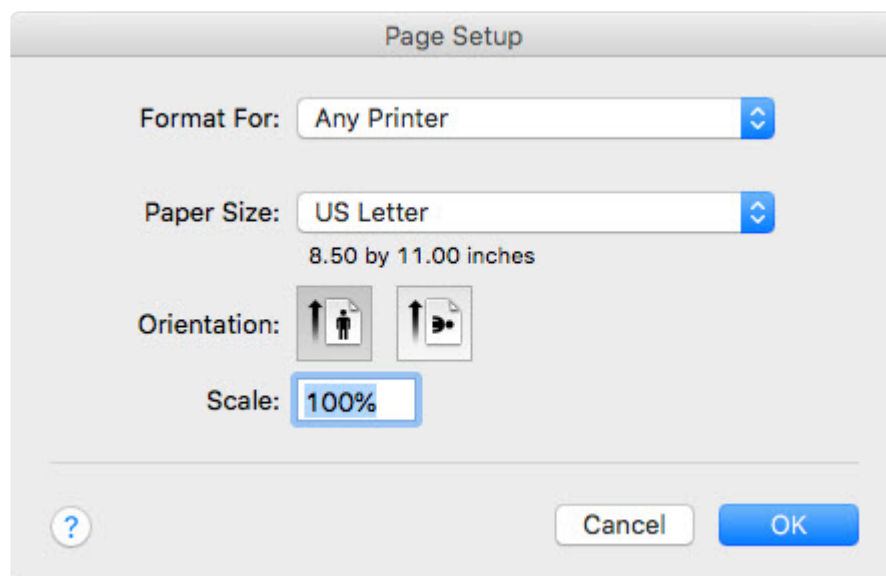
Initially, an abbreviated version of the Print dialog is displayed. To view all print options, press **Show Details**. Once expanded, the selection you make in the middle drop-down menu (with the default of **Layout**) determines which options are available in the lower portion of the dialog. For example, if the default value is retained, the dialog appears as follows:



Other print options may be available, depending on the specific printer.

To change the printing size, orientation or margins:

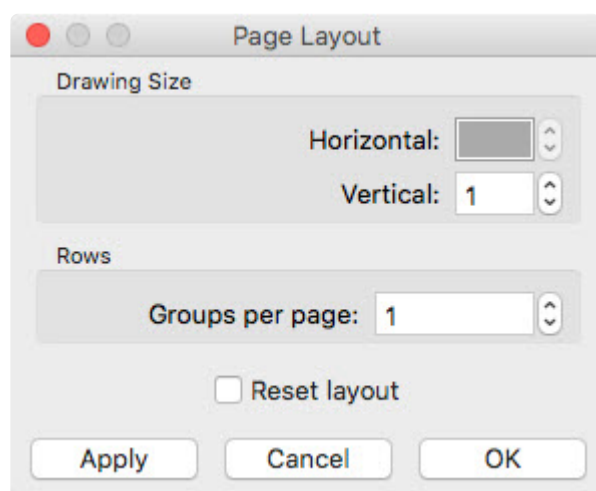
Choose **File > Page Setup**.



The default printing size for all DNASTAR applications is U.S. “Letter” size (8.5 × 11 inches = 21.6 × 28 cm).

To change the page layout:

1. From within the [Circular](#), [Linear](#) or [Sequence](#) views, select **File > Page Layout**.



- In the Drawing Size area, use the **Horizontal** and/or **Vertical** text boxes to set the number of pages that will be used to display the current view. By increasing the number of pages, you will enhance the resolution in any of these views. One or both of these options may be available depending on the view.
- Enter a number in **Groups per page** sets the number of rows that will print per page in Linear view. To view a linear map in one continuous line on the page, change this value to 1.
- (optional) Check the box next to **Reset layout** to restore the default values.

When you have made the desired selections, press **Apply** to save changes and then exit the dialog by clicking **OK**. Or press **Cancel** to exit without saving changes.

Save

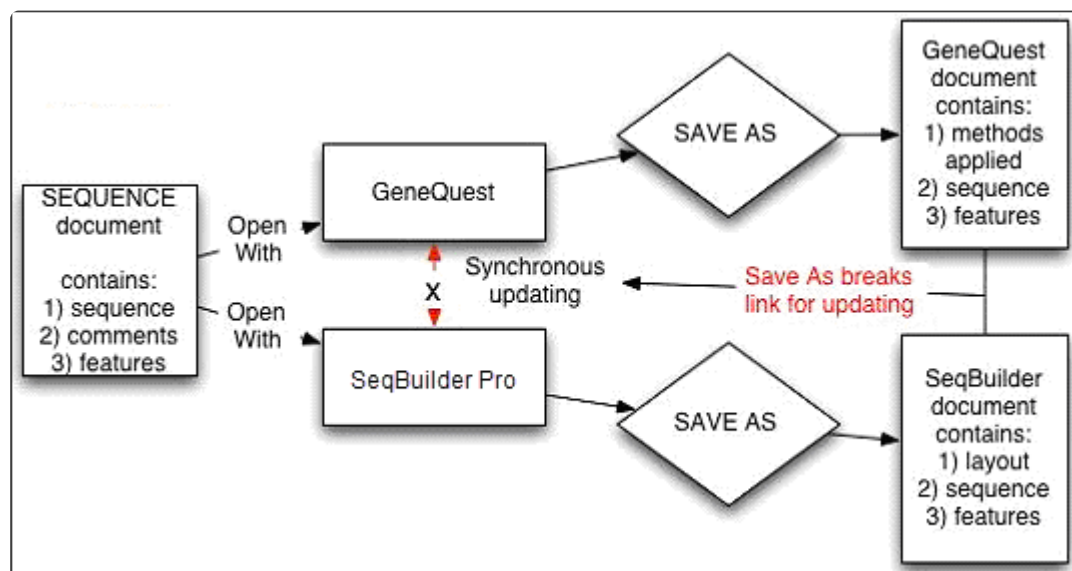
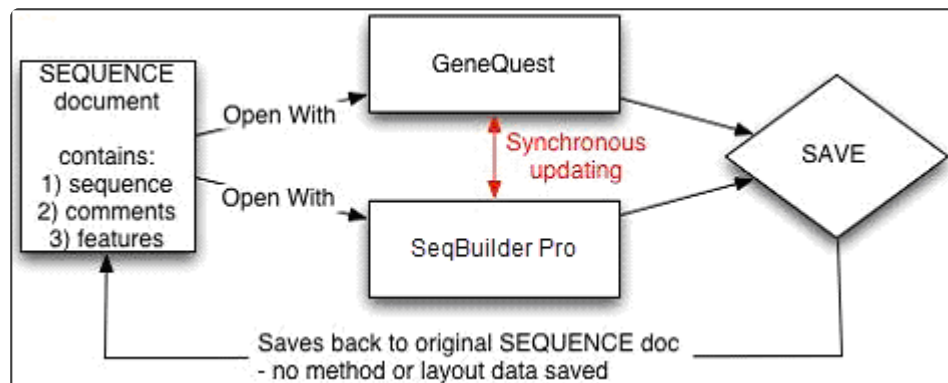
You can save a sequence or project using **File > Save (Project)** or **File > Save As (Project)**. See the table below for restrictions. Most SeqBuilder Pro projects are saved with the extension *.sbd* (single document project) or *.sbp* (multiple document project), but agarose gel simulations are saved with the extension *.sbgel*.

If you have a large number of sequences open, we recommend first collecting them into a Project Folder before saving. To do this, use the command **File > Collect Open Sequences into New Project** before using one of the **Save** commands.

Ability	File > Save or Ctrl/ Cmd+S	File > Save As
Save to new name ¹		x
Save to new location		x
Save a nucleotide sequence file (<i>.seq</i>) in <i>.sbd</i> , <i>.seq</i> , <i>.gbk</i> , <i>.embl</i> , <i>.fas</i> , and <i>.abi</i> formats. Save a protein sequence file (<i>.pro</i>) in <i>.sbd</i> , <i>.pro</i> , <i>.gbk</i> , <i>.embl</i> , or <i>.fap</i> formats.		x
Preserve synchronous updating ² with GeneQuest	x	
Preserve sequence information, features, and comments	x	x
Preserve layout and formatting ³		x

¹ If you change the name of a SeqBuilder project using the file explorer, rather than the **Save As** command, the name of the sequence in the project will retain its original name.

² As shown in the illustrations below, once you use **File > Save As**, you can no longer update the original sequence file [synchronously with other Lasergene applications](#) because the newly-saved project file has copied the information from that sequence file into the new project file along with additional project information.



You can re-establish a synchronous updating link between SeqBuilder Pro and the other application by opening the newly-created project file in the other application. You do not need to close the original project file. However, because **File > Save As** has been used in another open application, that file will no longer be updated when you make changes in the application where you last used **File > Save As**.

³ If you are saving a sequence file (e.g., *.seq*, *.pro*, *.gbk*, *.fas*) that you have changed, **File > Save** does not save any layout information like active views, colors, and elements visible on the views. If you need to save the layout information as well, use **File > Save As** to save the file as a SeqBuilder Pro document.

Export sequences

To export one or more sequences in the Project window to individual sequence files:

To do this, you must have already imported one or more sequences to the [Project window](#).

1. Select one or more sequences from the Project tab.
2. Choose **File > Export Sequence(s) From Project** or right-click on the selection and choose **Export**.
3. Choose a format and location for saving the exported sequence(s).
 - If you selected a single sequence, the sequence name is used as the default file name. If you selected multiple sequences, the project name is used instead.
 - Available file formats may include: *.sbd*, *.seq*, *.gbk*, *.embl*, and *.fas*. For formats that do not support multiple sequences, each sequence is saved as a separate file.

Synchronous updating allows you to work on the same file in multiple Lasergene applications at the same time, and is available in SeqBuilder Pro and GeneQuest, and—on a limited basis—in Protean 3D. Changes made to a sequence in one application are automatically applied in the other shared applications.

To export one or more sequences to a single sequence file:

See [Export sequences into a single file](#).

To export a sequence to another Lasergene application with synchronous updating:

1. Open the sequence of interest.
2. Send the sequence to 1-3 additional applications using **File > Send Sequence To > [Lasergene application]**. For example, you could open the same document in:
 - SeqBuilder Pro – to edit the sequence
 - GeneQuest – to further analyze a region of conservation

For information about how **File > Save** and **File > Save As** affect synchronous updating, see [Save](#).

Note that Protean 3D does not allow the editing of sequences or features, and synchronous updating is not supported for *.pdb* files. As a result, Protean 3D has limited, unidirectional synchronous updating capacity compared to some other Lasergene applications. Changes made in another application may be

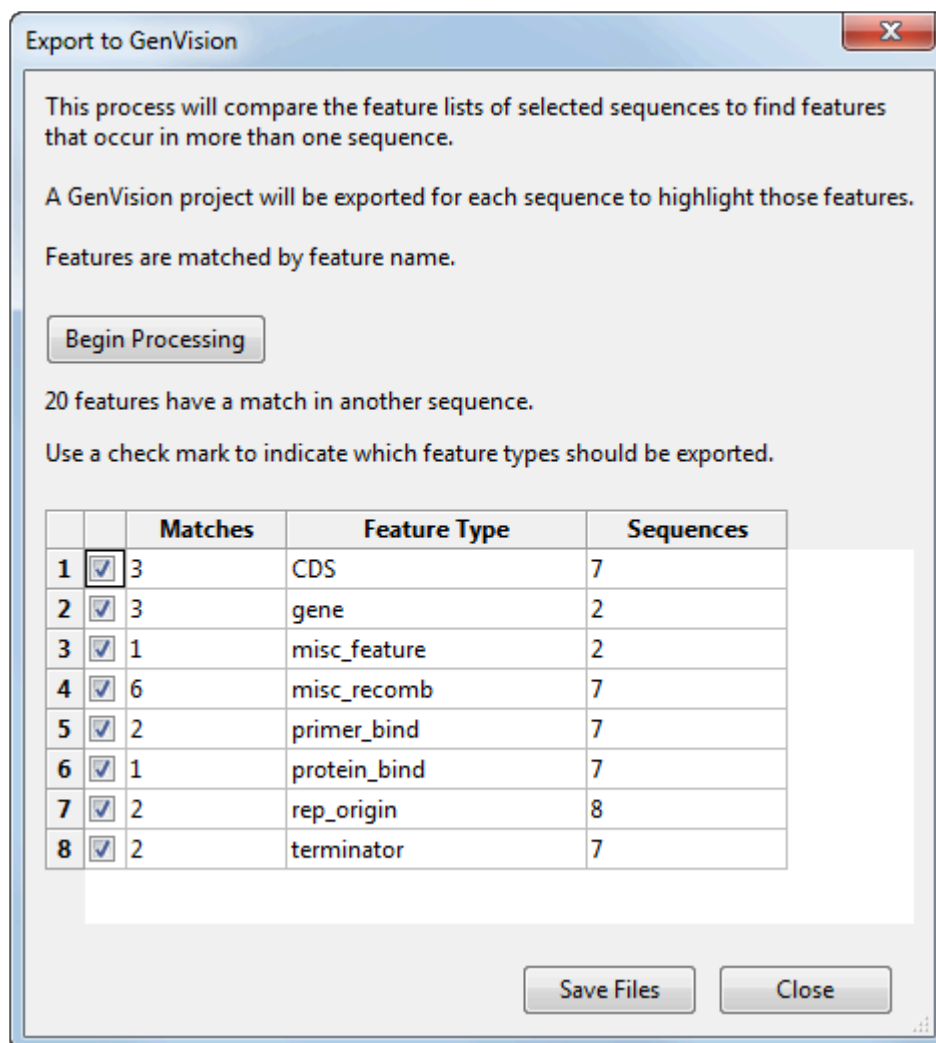
synchronized to Protean 3D, but not the reverse, as in these two scenarios:

- A file is open in another Lasergene application and you have made unsaved changes to it (e.g., trimming, adding a feature). When you initially open the same file in Protean 3D, any changes made to the original sequence or its features will also appear there.
- The same file is already open in both Protean 3D and another application. You make changes to the file in the other application. Only feature edits will synchronize to Protean 3D; sequence edits are ignored.

To export one or more sequences to GenVision:

1. Either make a selection in the [Linear](#), [Circular](#), or [Sequence](#) views, or select a sequence in the [Project window](#).
2. Choose **File > Export as GenVision Project** and click the **Begin Processing** button.

The Export to GenVision dialog compares the features lists for all of the sequences you selected in the Project window in order to find features with the same name that occur in more than one sequence. Once the process is complete, a message will be displayed giving the number of features SeqBuilder Pro identified that occur in more than one of the selected sequences. The table at the bottom of the window lists the number of matches for each feature type, and how many sequences contain a matched feature of the given type.



3. (optional) To exclude certain feature types from the export, remove the check mark from the row beside that feature type. If a feature type has 100 or more matches, it will be un-selected by default.
4. Click the **Save Files** button.

One GenVision project (*.gnv*) will be created for each of the selected sequences, and will display the features from one sequence connecting to the same features in all of the other sequences where it occurs. A *.txt* file will also be created for each exported panel. A new folder containing the exported text files will be created within the same directory as your GenVision project and will be named the same as your GenVision project with a *.data* extension.

Considerations when exporting sequences from the Project window:

- The use of color in the resulting GenVision project differs based on whether one feature type or multiple feature types were exported. For a single type, each matching set of features will have its own color. When multiple types are exported, each feature type will have its own color.
- Two text panels are created for the feature names. One panel displays the feature names as radial

text around the outside of the circular image and the other panel displays the feature names in an arc inside the circular image. By default, only the radially displayed text panel is displayed. However, you may display the other text panel if you wish by adjusting its priority within its data file.

- In the exported image, features are sorted so that feature types that have the most features appear near the center of the circle.

Considerations when exporting sequences from the Linear, Circular, or Sequence views:

- When exporting from the Circular view, the GenVision figure will be exported as circular. When exporting from the Linear or Sequence view, the GenVision figure will be exported as linear. Protein sequences are exported as linear.
- The colors for fonts, ORFs, features, and the Circular map title are chosen in the Style panel, and are preserved during export. For features, the **Fill** color is used for the feature color in GenVision. If a feature does not use the fill color (e.g., stroked shapes), then the line color is used. Unchecking the **Fill** checkbox will also cause the line color to be used. Feature labels are exported in the same color as the **Shape**.
- For linear images, the **Vertical** value in the Page Layout dialog ([Windows](#) / [Macintosh](#)) is used for the “Pages High” GenVision parameter.
- The page size and margin settings specified in the Page Setup dialog will be preserved in the exported GenVision project.
- An End Legend file is created for all of the panels within the export, and is named the same as your GenVision project with “_table” appended. For example, **GenVisionProject_table.txt**.
- All exported text files will be prefixed with **SB** to denote that they were exported from SeqBuilder Pro.

Considerations regarding features:

- Features are grouped into panels by feature type. One panel is created for each feature type.
- For each feature panel created, a corresponding panel is created for the feature labels. Feature labels are exported to GenVision if the **Label Position** is either **Above** or **Below**. Labels are not exported if position is **Inside**. For additional information, see The Style Panel.
- If your features have detached labels, note that the label settings found in **Format > Horizontal Labels** or **Format > Radial Labels** (for the Circular view and **Format > Horizontal Labels** or **Format > Vertical Labels** (for the Linear view) are used when exporting feature labels to GenVision.
- Features in SeqBuilder Pro that have the **Fill** checkbox checked in the [Style panel](#) will be exported to

GenVision as a filled arrow. When the **Fill** checkbox is not checked, the feature will be exported as an open arrow.

- For protein sequences, the export does not include segment names for multi-segmented features.

* The visibility of feature labels within GenVision is determined by the **priority** set in the GenVision text panel dialog or in the **priority** column of the text panel data file. (The values in the data file supersede those in the panel.) If you are unable to see a feature label exported from SeqBuilder Pro, try adjusting the **priority** within GenVision.

Close and Exit

To close a single project:

Select **File > Close** or use the keyboard shortcut **Ctrl/Cmd+W**.

To close all projects and leave SeqBuilder Pro:

Windows: Select **File > Exit**

Macintosh: Select **SeqBuilder Pro > Quit**.

If you are closing a project that has been edited since the last save, you will be prompted to save before closing/quitting.

Appendix

Research references

Baldino et al. (1989). *Methods in Enzymology*, Vol. 168, pp. 761-777.

Bresslauer KJ et al. (1986). *Proceedings of the National Academy of Science, USA*. Vol. 83, pp. 3746-3750.

Frier SM et al. (1986). "Improved free-energy parameters for predictions of RNA duplex stability." *Proc. Natl. Acad. Sci.* 83: 9373-9377.

Rychlik W, Spencer WJ and Roads RE (1990). "Optimization of the annealing temperature for DNA amplification in vitro." *Nucleic Acids Research*, Vol. 18 No. 21, pp. 6409-6412.

Wahl GM et al. (1987). *Methods in Enzymology*, Vol. 152, pp. 399-407.

Installed Lasergene file locations

The following file names use 'x' to represent the version number.

File Category	Application	Path
Application ²	ArrayStar	Windows: C:\Program Files (x86)\DNASTAR\Lasergene x\ ArrayStar
	SeqNinja (command line)	Windows: C:\Program Files (x86)\DNASTAR\Lasergene x\ SeqNinjaCL
	All others	Windows: C:\Program Files (x86)\DNASTAR\Lasergene x Macintosh: /Applications/ DNASTAR/Lasergene x
Data Manager ² (<i>DMx</i> , <i>DMx.exe</i>)	SeqBuilder Pro, Protean 3D, GeneQuest, MegAlign Pro	Windows: C:\Program Files (x86) \DNASTAR\Lasergene x Macintosh: /Applications/ DNASTAR/Lasergene x
Data Manager State File	SeqBuilder Pro, Protean 3D, GeneQuest, MegAlign Pro	Windows: C:\Program Data\ DNASTAR\DataManager Macintosh: ~/Library/ Application Support/ DNASTAR/DataManager, ~/Library/Preferences/ DNASTAR/DataManager
License Manager ²	All	Windows: C:\Program Files (x86)\DNASTAR\License Manager Macintosh: /Applications/ DNASTAR/License Manager
Server License File (<i>/servrc</i>), Server License Manager ² , <i>Server Executables</i> (<i>_Iserv</i> , <i>Iservnt.exe</i>)	All	Windows: C:\Program Files (x86)\DNASTAR- LicenseServer\Server

		Macintosh: ~/Library/ DNASTAR-LicenseServer
Standalone & Trial Licenses (*.license), License Server Client License (*.lshost), Key Server Client License (*.keyhost)	All	Windows: C:\Program Data\ DNASTAR\Licenses Macintosh: ~/Library/ Application Support/ DNASTAR/Licenses
Preferences	Protean 3D, Navigator, SeqNinja (DNA*), GenVision Pro	Windows: C:\Users\<user>\DNASTAR Macintosh: ~/Library/ DNASTAR
	ArrayStar	Windows: C:\Users\<User>\AppData\ Roaming\DNASTAR\ArrayStar
	All others	Windows: C:\Users\<user>\AppData\ Local\DNASTAR\ Macintosh: ~/Library/ Preferences



AppData is a hidden folder in Windows. To unhide the folder, go to **Organize > Folder and Search Options > View > Show Hidden files and folders**.

Installed enzyme file locations

SeqBuilder Pro contains representatives of a wide variety of restriction sites for available enzyme isotypes. By default, Lasergene's enzyme and enzyme selector files are installed in the following directory:

- **Windows:** C:\Program Files (x86)\DNASTAR\Lasergene x
- **Macintosh:** Hard Drive:Applications:DNASTAR:Lasergene x

Once an enzyme file or enzyme selector file has been edited, the revised file is saved to:

- **Windows:** C:\Program Files (x86)\DNASTAR\DataManager\1.0
- **Macintosh** (depends on version):
 - Hard Drive:Library:Preferences:DNASTAR:DataManager:1.0
 - Hard Drive: Users:<user ID>:Library: Preferences: DNASTAR: DataManager: 1.0
 - Hard Drive: Applications:DNASTAR:Lasergene x

At any time, you may see what enzyme or selector file SeqBuilder Pro is using by selecting **Help > About SeqBuilder Pro** (Windows) or **SeqBuilder Pro > About SeqBuilder Pro** (Macintosh).

If the enzyme file is write-protected, a copy of the file will be made in a user-specified location. This copy will track changes made to enzymes, new enzymes, and deleted enzymes. Re-installing Lasergene will not overwrite the changes, but likewise this file will not receive updated enzyme definitions.

For vendor information and a list of abbreviations used by SeqBuilder Pro, see the [REBASE Suppliers](#) web page.

Turn off usage logging

By default, [usage logging](#) is enabled in Lasergene version 11 and later. To opt out of usage logging, launch the DNASTAR Navigator and go to **View > Preferences > Lasergene** (Win) or **DNASTAR Navigator > Preferences > Lasergene** (Mac). Uncheck the box and click **Apply** and then **OK**.