Use the Plan Cloning Wizard

Click, Clone, Plan Cloning or use toolbar button. Define what you want to clone, define the vector you want to use, select an enzyme list that the wizard can use, and specify your cloning preferences (or use the default settings). Use toolbar buttons to examine results, construct the recombinant of choice, or redefine the procedure.

Graphic Map

Print or Preview map:

With a working map on screen, click Clone, Send Map to activate print or preview commands. Click preview to see how the map would appear when printed. Click Print or use toolbar Print button to send the high-resolution graphic map to your printer. Use the Print Set Up option (File menu) to change page orientation.

Clipboard or Export map:

Click Clone, Send Map, Clipboard to copy the map to the clipboard. Use Edit, Paste Special to paste it into a word processing document. Click Export to prepare a Windows Metafile (emf or wmf) for use in other Windows applications. Click Save for the Web to prepare a raster graphic file in png format for web page or on-screen presentations.

Set styles, colors for printing or export

Click Clone, Map Print Options or use map window toolbar button to access tabbed dialog box to set options for styles and colors.

Map Basics tab — set font face, text colors and indicate which map text to print; set mapline thickness (normal/heavy) and color.

Features tab — select style and color for feature types, and indicate which to print. Genes are displayed inside mapline, regions on mapline, markers occupy one base position on mapline. Use checkbox to suppress small features on very large molecules.

To set a different color for an important gene, right-click on feature on map and select Customize. Set style and color for this one feature.

To shift feature name, right-click on feature on map and select Shift Name. Enter the number of increments (in units of 1/10 inch) to move the text horizontally or vertically on the printed page.

Sites tab — set font size and color for enzyme or primer names and tick lines. Set options to add basepair number or italicize first 3 characters of enzyme name.

Content tab — change map size or position for Molecule Map Only style. Select Molecule Map and Text to add description, notes or features and map site lists to printed map page.

Quick Reference

Clone Manager Suite 7 for Windows

Getting Started

To install an individual or workgroup copy, insert the program CD in drive and allow disk to autorun or select Start, Settings, Control Panel, and then click Add/Remove Programs. Follow the instructions on your screen.

Click the Clone Manager Suite icon to start the program, which runs in the Sci Ed Central program hub. Select Open to load a molecule file to begin working.

Click Help, Help Topics to view complete application help. Use special Help with View toolbar buttons on complex display screens for related help.
Tip of the Day offers program hints each time the program starts (click Help, Tip of the Day to turn this feature on or off).

Working with Molecules

Open Files—Load Molecules:

Click File, Open or use Open toolbar button. Use Shift or Control keys in conjunction with mouse to open more than one file at the same time.

Information on the clipboard can be read as if it were a disk file using the Paste button found in the file open dialog box.

Click File, Retrieve from Entrez to find sequence files at the NCBI. With file open on screen, click Get Molecule button on browser window toolbar to import molecule for immediate use.

In the file open dialog box, use the WorkBoxes button to open the folder containing the shortcuts to your favorite locations. Use the Add to WorkBoxes button to add a shortcut to the “Look in” location.

Creating new molecules:

Click File, New or use New toolbar button and then follow the wizard instructions. Save new molecule to disk when complete.

Selecting molecules:

Active molecule is in active window (dark title bar). Click on a different window to change the active molecule or select from Molecule List.

Click File, Molecule List or use Molecule List toolbar button to access list of loaded molecules. Click on column heading to sort. Resize columns by dragging column heading join point. Diamond symbol marks unsaved new or modified molecules.

Molecule Viewer Window

Click tabs at the bottom of the window to see molecule Map, Restriction Map data, Sequence, Features or molecule Information. Click Zoom button in tab region (left) to temporarily view just a part of the molecule.

Map tab — Working Map display

Enzyme sites and features (genes, regions, markers) appear on the working map. Click AutoScan button to scan for enzyme sites, click ORF Search button to locate open reading frames and enter as genes. 

Click on column headers in list of map sites to sort by enzyme name or position. Select enzyme and then use toolbar buttons to view site properties or compatible ends, cut at site, view enzyme suppliers or isoschizomers.

Point to a feature or enzyme name on the map and depress left mouse button to see basepair position information and suppressed enzyme sites, if present (++ notation indicates suppressed sites in crowded areas).

RMap Tab — Restriction Map data

Click column headers to sort by enzyme name or number of sites or to select display style. Click No Cutters button to view list of enzymes that do not cut this molecule. Click again to return to prior display. 

Click Filter to set filter options to limit data displayed to just what you need. Click again to remove filter. Click on an enzyme and then use toolbar buttons to view enzyme properties, suppliers, isoschizomers or compatible ends information, add enzyme sites to the map, or cut with this enzyme.

Sequence Tab — Sequence data

View or format sequence of the molecule or use Find options. Click the Format button to set sequence display styles and colors. Click the My Style button to format sequence using your memorized settings. 

Edit sequence — click the Edit Sequence button to change the sequence by adding or removing bases. You can also use cut, copy and paste operations on sequence data. Sequence is stored in the program single-stranded and all operations work on the upper strand of sequence.

Features Tab — Features table

Click Features tab to view features table and add or edit information about genes, regions, or markers. Click Filter to limit data by feature type or location. Click Info Features to show or hide information-only features.

Click Customize button to set custom feature styles for map or sequence. Click Feature properties button to see codon usage, amino acid composition, molecular weight and pI for gene translation products. 

Info Tab — Molecule information

Click Info tab to view or edit molecule name, description, notes, change starting bp number or view molecule composition.

Enzyme Operations

Find Enzyme sites:

Click Clone, Find Sites or use toolbar button. Find sites for specific enzymes or all enzymes on a list that meet a specific requirement (like single cutters) or all enzymes on a user list. 

View or build enzyme lists:

To view, click Clone, Enzyme Lists and then click the View List tab. To edit a user-designated list, clear the Read-Only checkbox and then click Add Enz or Del Enz. To build a new user list, click Clone, Enzyme Lists and then click the Build New button. Follow wizard instructions.

REBASE updates:

Update your master enzyme file using the latest REBASE data file (updated monthly). Each time you update, the All Enzymes, Commercial (Main), and Scanner enzyme lists will be regenerated. User-designated enzyme lists and any special enzymes you entered will not be affected.

To update: Click Clone, Enzyme Lists and then click the Update tab. Click on the option for the REBASE file source and complete the required information. Click Update Now. You can get REBASE files from the REBASE web site: http://rebase.neb.com.

Instant enzyme information:

Click toolbar buttons on the Map or RMap displays to view Properties, Suppliers, Isoschizomers or Compatible Ends for the selected enzyme. Enzyme information is updated when you do a REBASE update.

Cloning Operations

Cut molecules:

Click Clone, Cut or use toolbar button. Cut a molecule with specific enzymes, cut at a single map site, or cut at specified bp positions. 

Ligate molecules:

Click Clone, Ligate or use toolbar button. Join together two pieces of DNA or join the ends of the same molecule (self-ligate). Use the small buttons to the right of each molecule in the ligate display to change the molecule, cut the DNA, or modify the molecule ends. 

After ligation, a circular recombinant will be rotate to restore the original reference frame of the vector (in upper molecule window). Use double arrowhead button to flip molecules between windows, if needed.

Modify molecule ends:

Click Clone, Modify Ends or use small button on ligate display. Modify the ends of a linear molecule using Klenow Fill-In, 5'->3' exonuclease, 3'->5' exonuclease, Partial Fill-In, or custom end modification.

Comparing Two Sequences

Click Align, Compare Two Sequences or use toolbar button. Select type of comparison, set sequence type (DNA or amino acid), and set method or scoring matrix. Click Next to identify sequences to compare.

Local homology search methods:

FastScan uses rapid search lookup table method. MaxScore favors finding high scoring regions. MaxQual favors finding shorter regions of higher homology. Needleman-Wunsch uses exhaustive base-by-base comparisons to find long regions of homology, even if they have numerous mismatches.

Identify sequences to compare:

For Sequence box 1, click molecule list button at right and select sequence from molecule list. Repeat for Sequence box 2.

Viewing local homology search results:

Sort list of results found by score, percent matches, length, or the start position of the region in either sequence 1 or 2.

Scanning for Similarities

Click Align, Scan for Similarity or use toolbar button. Click New Search and identify the query sequence (search molecule name and region). Set translation option and click Next to identify files or folders to scan.

Identify search locations:

Click Add Folder button to select a folder location to add to the search list. (Subfolders are included.) Click Add File button to select a library file to add to the search list. Click Save List button if you want to use the same search locations for later scans.

Set scan options:

Set Speed to make the search more or less sensitive. Set lower Cutoff to accept results of lower significance.

Scan Results Wizard:

Use the wizard to select molecules to load in the program for use now, extract molecules from a database file for use later, or set up a multiple sequence alignment, using the scan results as a starting point.

Remote Searches

Click Align, BLAST Internet Search. Select the BLAST program to use and the BLAST database to search. You need an internet connection to send search requests to the NCBI BLAST server. A built-in web browser shows search results. Click Get Molecule button to import molecule in GenBank format for use now.

Designing Primers

Click Primer, Design or use toolbar button. Set primer type, enter primer length, click Next. Change molecule, if necessary and set Search for Primers or Create from Molecule and then follow instructions below.

Search for Primers:

Enter target region or position and select design objective. Primers found are listed. Sort by rank (quality) or position. Click Primer Report button to open primer viewer for information or analyses.

Click Search Results/Search Stats button to review search summary. To find more primers: Click Auto Adjust Length to repeat search, varying primer length ±1 or ±2 bases. Click Auto Adjust Criteria to repeat search, adjusting (relaxing) primer criteria as instructed.

Create from Molecule (display in sequence):

Enter target position for initial primer placement. Primer viewer window opens and shows primer and molecule sequence. Map with genes (if any) appears above sequence. Ticks on top line mark locations of neighboring primers that will meet criteria set.

Evaluation bar below primer indicates if primer satisfies criteria set. Click on evaluation value to see more details on appropriate page in Analyze tab. Click Edit tab to return. Move primer or adjust length using toolbar buttons until primer meets criteria set.

Direct Entry of primer:

Click Primer, Direct Entry to start wizard. Enter primer name, type, primer sequence. When complete, view primer report in primer viewer window. Click File, Save to save new primer to disk. Click Primer, Find Molecule Sites to scan your molecule collection to see if this new primer will bind effectively to any of the scanned molecules.

Primer Viewer Window

Select primer from Primer List to open the primer viewer window. Click tabs at bottom of window to see primer information or to edit or analyze primers. Click File, Save to save the active primer to disk.

Info — Shows detailed primer information and map (if applicable), amplified product information (for primer pairs). Enter or modify primer name or description, view criteria, link to molecule, or create amplified product.

Edit — Shows primer in context of molecule sequence. Change primer position or length, if needed. Edit primer sequence to correct direct entry error or introduce mutation in primer linked to molecule. Add site to map.

Analyze — View primer reports or detailed analysis results or check for false priming or any homologies against a specified molecule.

Analyzing Primers

To analyze one primer, open primer viewer window and click Analyze tab, or click Primer Report button from search list, or click Primer, Analyze and select one or two primers to analyze. Click toolbar buttons on Analyze tab for:

Primer Reports — general analysis information summary
Dimers — homologies between primers (primer dimers)
Cautions — summary of Stability, GC Clamp, Runs of bases, Repeats, Hairpins analyses
False Priming or Any Homologies — 3'-end homologies or any significant homologies between primer and template

Analyze Mix Wizard:
Found on Primer menu. Use wizard to identify list of primers and molecules and set parameters for analysis. Analyze possible products formed, binding sites found, and analysis cautions noted (primer dimers that might affect results or molecules or primers not used.)

Primer Values Profile:
Click Operations, Primer Values profile. Calculates Tm, GC content and stability for primers of specified length and plots values.

Mutagenesis Profile:
Click Operations, Mutagenesis profile. View sequence, enzyme recognition sites, translation. Find 'almost' enzyme sites, construct mutants.

Primer Operations

Link to Molecule:
Found on Info tab. Select primer from primer list to open primer viewer window. Click Link to Molecule button and set Link option to link a copy of this primer to the specified molecule.

Create Product:
Found on Info tab. Perform pair analysis or click Primer Report button for primer pair in search list and then click Info tab. If amplified product shown, click Create Product button.

Find Primer Sites:
Found on Primer menu or use toolbar. Find sites where primers in your collection could bind effectively to the active molecule. Add primer sites to molecule for display on map or sequence.

Display Primer Sites (after adding to molecule):
To display on molecule map, click Enzyme Sites/Primer Sites toolbar button to switch to primer sites. To display in sequence, click sequence Format button, check Primer Sites on Style tab and select display style. Select primer site colors on Color tab.

Aligning Multiple Sequences

Click Align, Align Multiple Sequences or use toolbar button. Select type of alignment, set sequence type (DNA or amino acid) and select scoring matrix. Click Next to identify sequences to align.

Identify sequences to align:
Click molecule list button at right and select sequences or group from molecule list. Use small up or down arrowhead buttons to move sequences within list. Click Edit button to identify region to align, if less than full molecule. Click Save (lower left) to save this alignment setup file to disk for later use. Click Finish to start the alignment.

Subclone Locator:
Click Align, Subclone Locator to prepare an alignment to locate clones on the primary sequence. Identify the primary sequence molecule and the location of the subclone files that you want to scan. Prepares an assembled multiple sequence alignment with a special Picture tab view.

Viewing Alignment Results

Results are displayed in a comprehensive, tabbed viewing window and can be printed, copied to the Windows clipboard (click View, Send View to Clipboard) or exported to a disk file (click View, Send View to File).

Info tab — contains alignment summary information table (multiple sequence alignments) or list of homologies found (local homology searches or scans for similarity). Move mouse over molecule name and click for more information about molecule.

Picture tab — shows phylogeny dendrogram (multi-way alignment), subclone coverage (assembled alignment using subclone locator), or list of homologies found (local homology searches or scans for similarity).

Sequence tab — shows actual aligned sequences for entire alignment of multiple sequences. Click Switch Format button to quickly toggle between formats emphasizing the similarities or differences between the sequences. Click Format button to access full set of sequence formats and page style options. For local homology searches or scans for similarity, sequence tab shows aligned sequences for the selected homology block. Click Next Result or Previous Result button to move through results list.

Map tab — shows similarity maps for multiple sequence alignments. Click Compress View to fit more data in the results window. Point with mouse and click to see basepair position number for area of interest on map. For local homology searches or scans for similarity, map tab shows a detailed location map for the selected homology block.