

MacVector 14.5

for Mac OS X

Click Cloning Tutorial

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Overview/Quickstart

The tutorial will show you how to use MacVector to create new cloning construct plasmids by replicating cloning experiments that you might perform in a practical laboratory. The basic steps are to;

- a) Open the source DNA molecule and switch to the **Map** tab.
- b) Hold down the **<shift>** key and click on the two restriction enzyme sites that delineate the ends of your source fragment.
- c) Choose **Edit | Digest**, or click on the **Digest** toolbar button.
- d) Open the target DNA “vector” molecule and switch to the **Map** tab.
- e) Select the destination restriction enzyme site (or hold down **<shift>** and select two sites for a targeted cloning)
- f) Choose **Edit | Ligate**, or click on the **Ligate** toolbar button.
- g) Apply any required sticky end manipulation in the ligation dialog and/or flip the source fragment to get the required orientation.
- h) Click on the **Ligate** button to insert the source fragment into the destination vector.

MacVector always copies and pastes all of the enclosed features from the source molecule into the target molecule, including any custom appearance information you may have added.

Tutorial

Configuring “Auto” Restriction Enzymes

Whenever you open a DNA sequence, MacVector automatically runs a restriction enzyme search on the sequence and displays the results graphically in the **Map** tab. This dynamic or “auto” restriction enzyme search is primarily designed to show you the locations of common enzymes that cut your DNA. There is a more comprehensive restriction enzyme searching function available in the Analyze menu that can be used for in-depth analysis of the restriction enzyme sites in DNA. However, the “Auto” restriction enzyme analysis is often more than adequate for most basic clone construction DNA manipulations.

Open the files *S. coelicolor cosmid SC5A7* and *pUC19*. You will find these in the `/MacVector 12/Tutorial Files/Click Cloning/` folder. Make sure you have the *pUC19*

window frontmost, and then click on the **Map** tab if it is not already active.

While the default settings are usually sufficient for this tutorial, to ensure the Auto restriction enzyme searching parameters are set correctly, click on the **Preferences** icon in the **Map** view toolbar. Ensure **Automatic RE Analysis** is turned on and (for this tutorial) set to **Only use selected enzymes**. If **Common Enzymes** is not shown underneath the **Set Enzyme File...** button, then click on **Set Enzyme File...** to select it. It is located in the /MacVector 12/Restriction Enzymes/ folder.

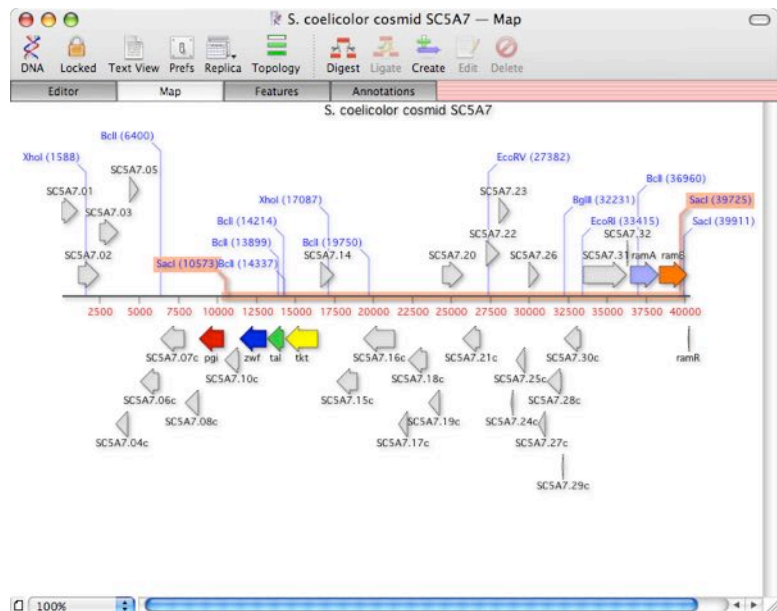
Click on the **Open** button so that the **Common Enzymes** file opens in a Restriction Enzyme Editor window. In this window, you can click on any enzyme name and a check mark appears next to the enzyme indicating it is selected. If you click on the *Bam*HI entry, you will see the checkmark toggle on and off. At the same time, if you look at the pUC19 Map view, you will see the *Bam*HI site turning on and off as you select/deselect the enzyme.

For this tutorial, make sure the enzymes *Bam*HI, *Eco*RI, *Eco*RV, *Sma*I and *Sac*I are selected (you can leave others selected as well). You can permanently save your favorite selections in the file if you wish by selecting **Save** in the **File** menu.

A simple cloning

In the *S. coelicolor* cosmid SC5A7 window, click on the **Map** tab. You will see a graphical map of the cosmid with the locations of the cuts sites displayed. There should also be a floating palette window with the title **S. coelicolor cosmid** available. If you don't see this, look in the **Windows** menu and select **Show Graphics Palette**.

Click on the **Fit to Window** button in the floating palette window.



The display should resize so that you can see the entire cosmid on a single line. At this stage you may notice that the two *Sac*I sites at the extreme right end of the cosmid have truncated labels – to display the entire label, click in the **Line Wrap** edit box of the floating palette and increase the wrap to 9.

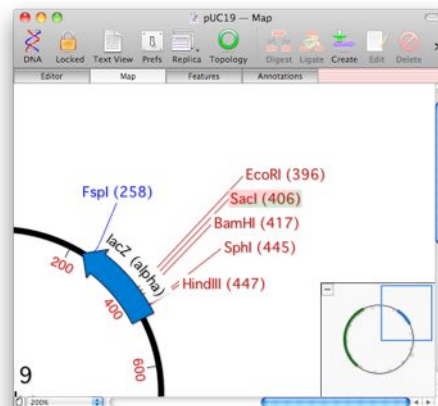
You should be able to see a *Sac*I site at 10573. Click on the site label and it should select.

Hold down the <shift> key and select the *Sac*I site at 39725 (near the end of the cosmid). Now both *Sac*I sites should be selected.

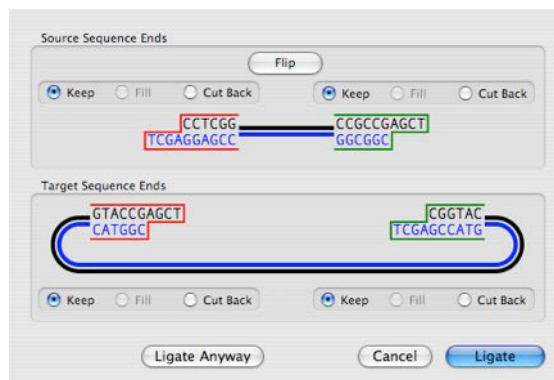
Choose **Digest** from the **Edit** menu (or click on the **Digest** toolbar icon). This copies the fragment, along with sticky end information and all overlapping annotations and feature information, onto the clipboard. A dialog will appear confirming the fragment has been copied.



Switch to the pUC19 sequence window, make sure the **Map** tab is selected and click on the single *Sac*I site at 406. This site will be highlighted in red/green to show that it is compatible with both ends of the digested fragment.



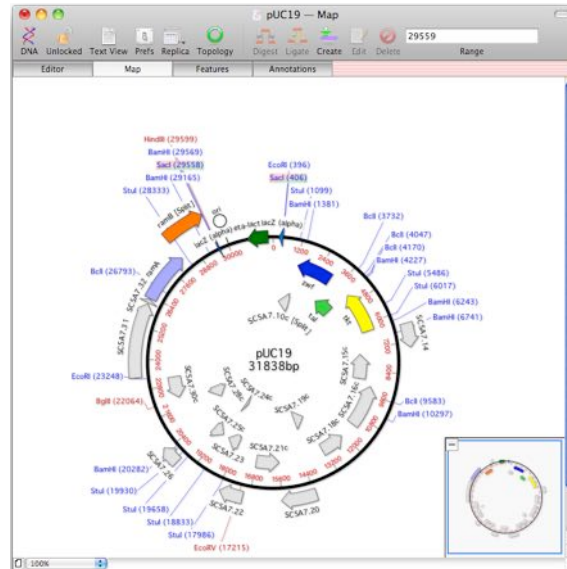
Select **Ligate** from the **Edit** menu (or click on the **Ligate** toolbar button). You should be prompted to unlock the pUC19 sequence. This is to prevent you from accidentally modifying an important sequence file. A ligation dialog will appear displaying the structure of the sticky ends.



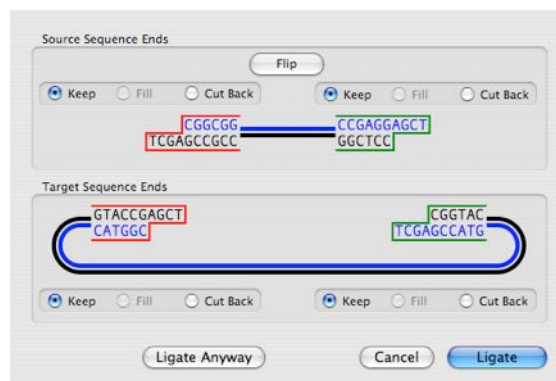
The dialog shows red and green outlines around the source and target sticky ends. This indicates that both sets of ends are compatible and can be ligated together.

Click on the **Ligate** button.

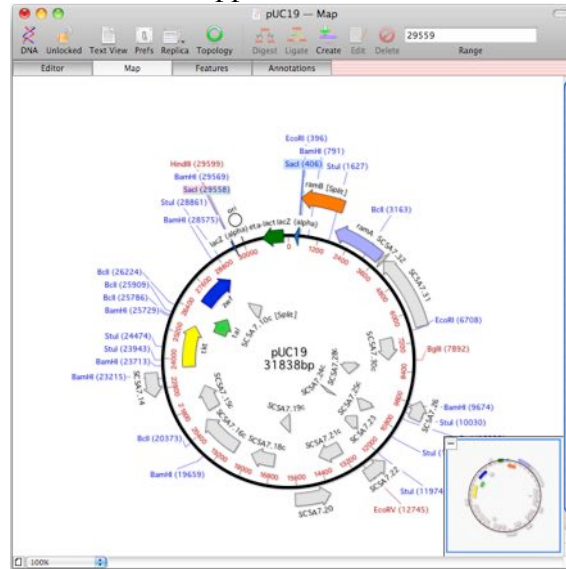
The fragment from the clipboard becomes inserted into the *Sac*I site of pUC19. Note that the pasted fragment retains all of the features and feature appearance of the source DNA molecule (compare the two screenshots).



Note the direction that the fragment was inserted. Now choose **Edit | Undo Ligate** – pUC19 is restored back to its original appearance. Once again, click on the *Sac*I site and choose **Edit | Ligate**. This time, click on the **Flip** button in the ligation dialog. You should now see that the strands in the source fragment have changed colors – the upper strand is now blue and the lower strand is black. This provides a visual indication that the fragment is “upside down” i.e. it has been reversed and complemented.

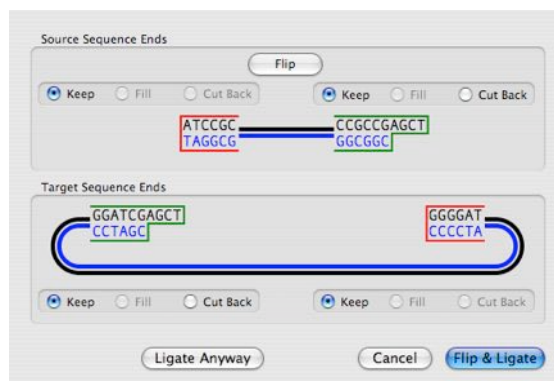


Now when you click on the **Ligate** button, the source fragment is inserted in the opposite orientation;



Two Enzyme Directed Cloning

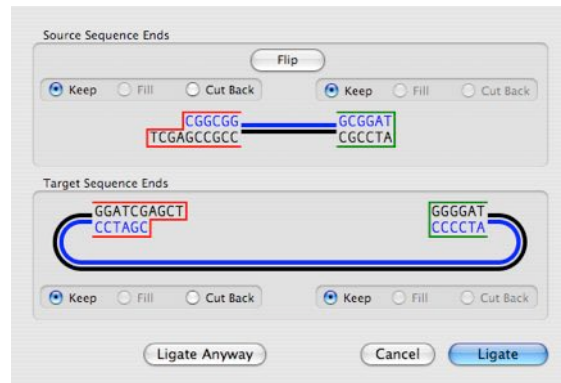
The ligation dialog is sufficiently aware of the compatibility of sticky ends to know when a fragment needs to be flipped to be able to be ligated. To illustrate this, first undo the last ligation into pUC19 (or close without saving and re-open pUC19 from disk). Next, switch to the *S. coelicolor* Cosmid window and select the *EcoRV* (27382) – *SacI* (39725) fragment (remember to hold the **<shift>** key down) and choose **Edit | Digest** (or click on the **Digest** button). Switch to pUC19, select the *SacI* (406) and *SmaI* (414) sites and choose **Edit | Ligate** (or click on the **Ligate** button) (please note that for circular sequences you must select the sites in a 5' to 3' order as the selection extends from the first site to the second site in a 5' to 3' order.0).



The ligation dialog outlines compatible ends in colors to indicate what will happen when you click on the **Ligate** button. The left end of the source sequence is always outlined in red, with the right end

outlined in green. In this case the source *EcoRV* and destination *SmaI* are both blunt cutters and outlined in red, with the compatible *SacI* sites outlined in green. Because the colors are diagonally opposed, this indicates that the fragment needs to be flipped before it can be ligated. The blue **Ligate** button text has changed to **Flip & Ligate** to indicate this.

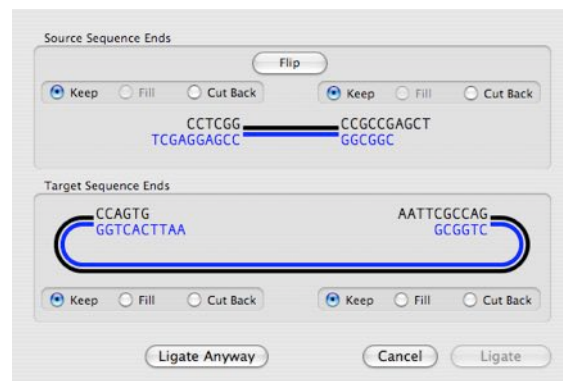
If you click on the **Flip** button, the dialog changes so that the source sequence is reversed and complemented – the blue and black strands are swapped and the ends switch so that the sticky *SacI* site is now at the left end of the source. In addition, the text of the **Ligate** button has changed from **Flip & Ligate** to simply **Ligate** to indicate that no additional flipping is required.



Manipulating Sticky Ends

It is often necessary to fill in or cut back the ends produced by restriction enzymes so that fragments can be blunt end ligated into a vector. The ligation dialog lets you fill in 5' overhangs and/or cut back 5' or 3' overhangs to blunt the ends of either the source or target sequence ends.

We will illustrate this using the *SacI* fragment from earlier, but this time we will try to insert it into the *EcoRI* site of pUC19. Undo your last ligation into



pUC19 (or close without saving and re-open pUC19) then switch to the *S. coelicolor* Cosmid window. Select the large *SacI* fragment (10573-39725), **Digest** it, switch to pUC19, select the *EcoRI* (396) site and choose **Ligate**. This time there are no outlines around the ends and the **Ligate** button is disabled because the ends are not compatible. Note that the source fragment has 3' overhangs that cannot be filled in, they can only be cut back, and so only the **Cut Back** radio buttons are enabled. Conversely, the target fragment

has 5' overhangs, so both the **Fill** and **Cut Back** radio buttons are active.



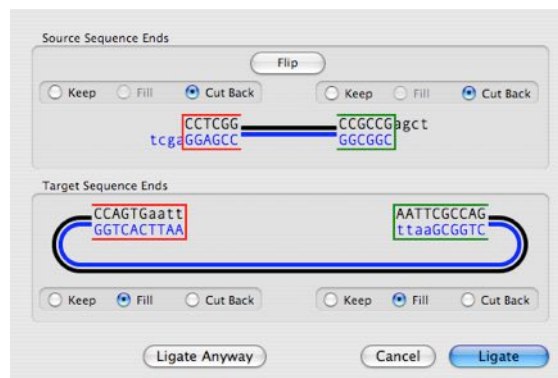
Click on the **Cut Back** radio button for the source fragment right end. Note how the AGCT 3' overhang is now displayed in lower case – this indicates that you want to remove those residues. Select the **Cut Back** radio button

for the left end as well.

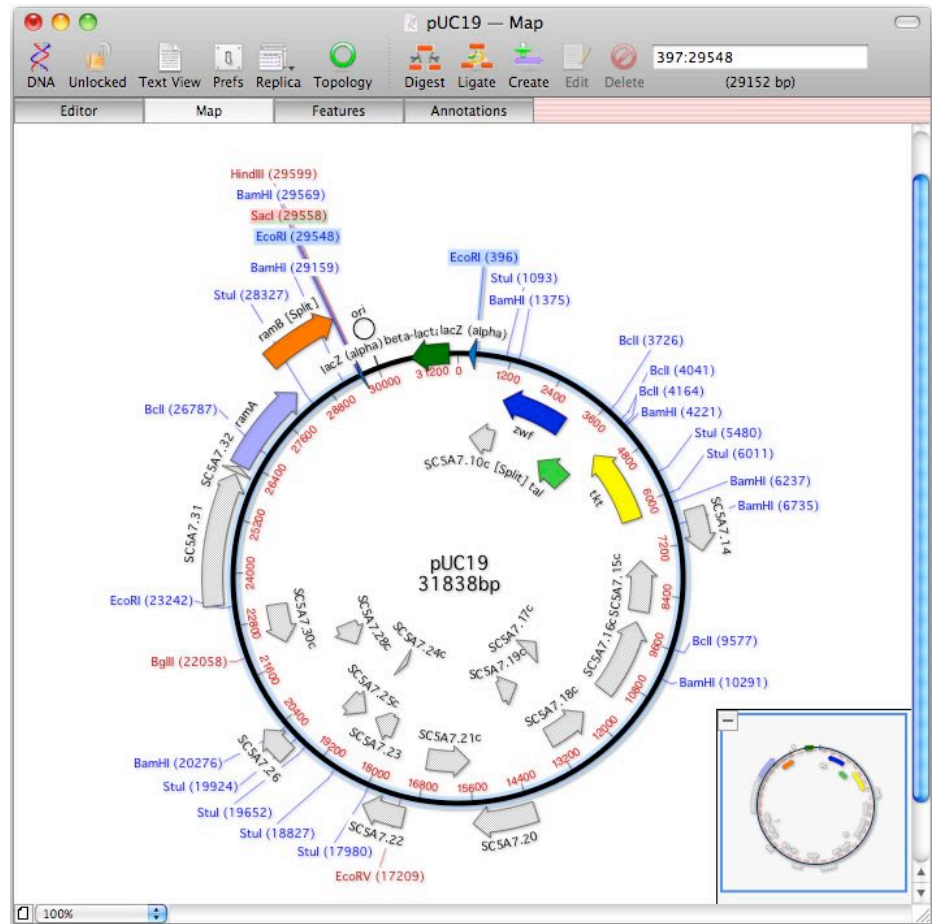
Now click on the **Fill** radio button for the left target end. The end gets filled with lower case residues and the end becomes outlined to indicate it is now compatible with the source left end. You can also see that by filling in the end the target sequence will have the sequence

...GAATT at the end. The cut back source will start with CCT... so the ligated molecule should have the

sequence GAATTC at the junction, which will regenerate an *Eco*RI site. When you click on the **Fill** button at the target right end, that will be compatible with the second source end and the **Ligate** button becomes enabled.



Click on **Ligate** – the resulting molecule does indeed have *Eco*RI sites regenerated at the junctions of the two molecules (locations 396 and 29548)(shown highlighted in the screenshot for clarity). Also note that the *Sac*I site (29558) is highlighted in red and green to show that it is still compatible with the *Sac*I – *Sac*I digested fragment on the clipboard.



Additional Information

Although the examples shown here used the **Digest** and **Ligate** buttons, you can accomplish the same things using the **Edit | Copy** and **Edit | Paste** functions. The differences are that (a) **Copy** places a copy of the graphical image on the clipboard in PDF format as well as copying the DNA sequence and (b) **Paste** only displays the **Ligation** dialog if the source and target ends are incompatible.

If you click on the **Ligate Anyway** button, MacVector will ignore the end information and treat all of the cut sites as if they were blunt ended.

Whenever you paste a fragment into a target molecule in this way, a special “frag” feature is created in the target with a note describing the source of the segment of DNA. You can use this to keep track of the history of your constructs.