MacVector 13

for Mac OS X

Cloning Clipboard Tutorial

MacVector, Inc.

Software for Scientists

Copyright statement

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Introduction

The **Cloning Clipboard** was introduced in MacVector 12.7. Each time you **Digest** a sequence, the resulting fragment is placed on the **Cloning Clipboard**. You can then join fragments together by clicking and dragging on their ends to build up constructs from individual parts. Finally, you can circularize a fragment to create new plasmids or double-click a fragment to open it in a MacVector sequence window. A ligation sheet is displayed each time you join two ends to give you the opportunity to manipulate the ends (cut back, fill in) if desired. The fragments on the **Cloning Clipboard** are remembered even after you close MacVector and you can delete or rearrange fragments on the list at any time.

Sample Files

All the sample files in this tutorial can be found in;

`/Applications/MacVector/Tutorial Files/Click Cloning/.`

Before You Start!

This tutorial assumes that you are using the `Common Enzymes` file included with MacVector as the file used by the **Automatic RE Analysis** function. If you are not sure, choose the **MacVector | Preferences…** menu item and switch to the **Map View** pane. Click on the **Set Enzyme File** button, navigate to the `/Applications/MacVector /Restriction Enzymes/` folder, choose the `Common Enzymes.renz` file and click on the **Open** button.

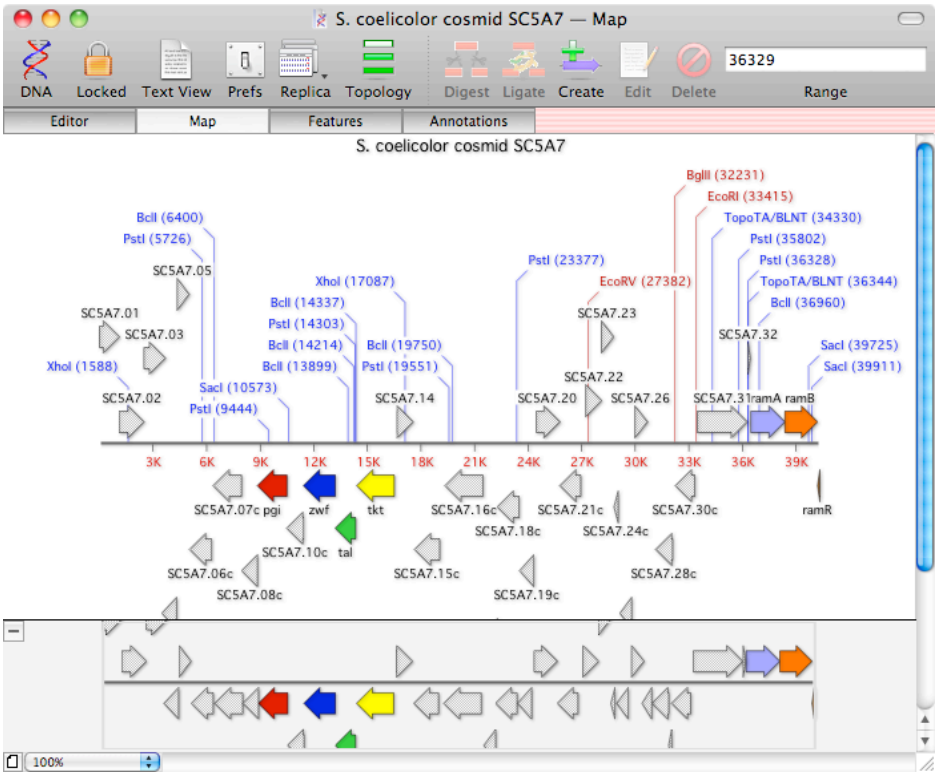
Tutorial

Cloning A Single Fragment Into A Vector

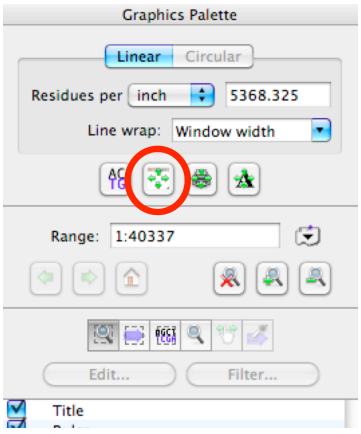
The traditional "click cloning" approach used by MacVector is pretty simple and very effective – open a source DNA sequence, `<shift>`-click on two restriction enzymes to select a fragment, choose **Edit | Copy**, switch to the target vector, select the target restriction enzyme site(s), then choose **Edit | Paste**. Accomplishing the same thing using the **Cloning Clipboard** does take a few more clicks, but it's a good first step to understanding the power that the **Cloning Clipboard** can give you.

Select **File | Open** and navigate to the `/Applications/MacVector /Tutorial Files/Click Cloning/` folder. Select `S. coelicolor cosmid SC5A7` and click **Open** to open the sequence then repeat and open `pUC19`.

The `S. coelicolor cosmid SC5A7` sequence should look something like this;

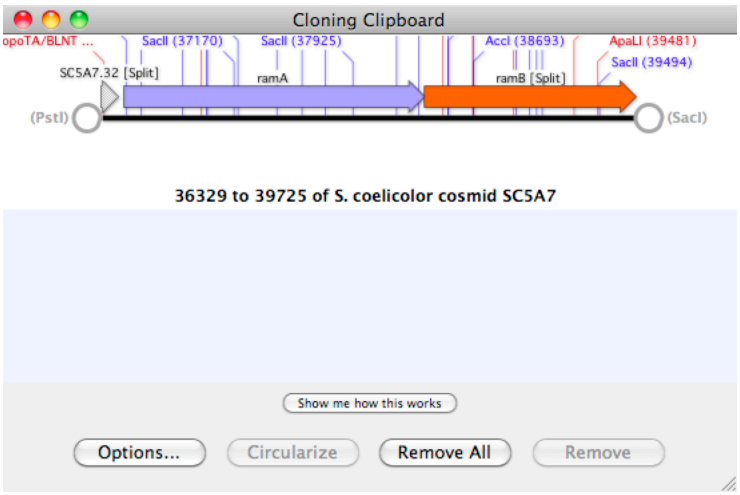


If the entire sequence does not fit on a a single line in the window as shown above, then click on the **Fit To Window** button in the floating **Graphics Palette**;



Click on the *Pst*I site at 36,328, hold down the `<shift>` key and click on the *Sac*I site at 39,725. Then click on the **Digest** button on the toolbar

The **Cloning Clipboard** window will automatically open and you will see the fragment you have just "digested".



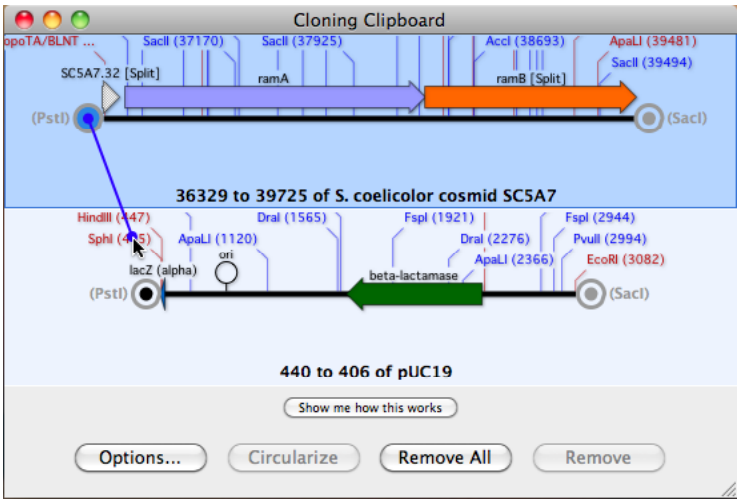
Switch to the `pUC19` window. Click on the *Pst*I site at 439, hold down the `<shift>` key then click on the *Sac*I site at 406. Finally click on the **Digest** toolbar button.

Note that the order that you click on the sites is important. For this circular vector, we want to copy the main body of the vector to the **Cloning Clipboard**. MacVector uses "clockwise" selection, so we have to click on the *Pst*I site first to ensure the main part of the vector becomes selected.

You should now have two fragments on the **Cloning Clipboard**. The next step is to join the end of one fragment to the other.

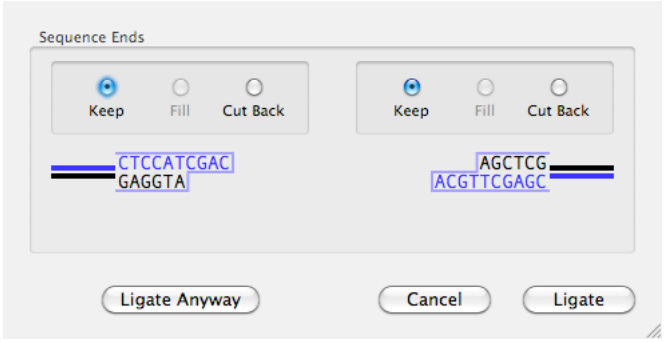
In the **Cloning Clipboard** window, click on the *Pst*I site at the left end of the `S. coelicolor fragment`, hold down the mouse button and drag it towards the *Pst*I site at the left end of the `pUC19` fragment.

The end of the `pUC19` fragment should show a dark black target spot, indicating that the end is compatible with the end you are dragging. The other ends will have a lighter gray spot, indicating that they are not directly compatible.



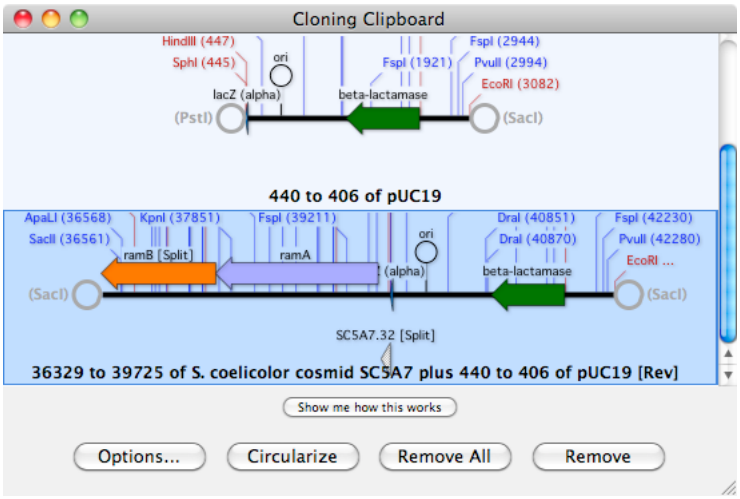
Continue dragging until the pointer is over the target *Pst*I site – it will become highlighted – then release the mouse button.

A **Ligation Sheet** will appear, allowing you to optionally modify the ends prior to ligation.



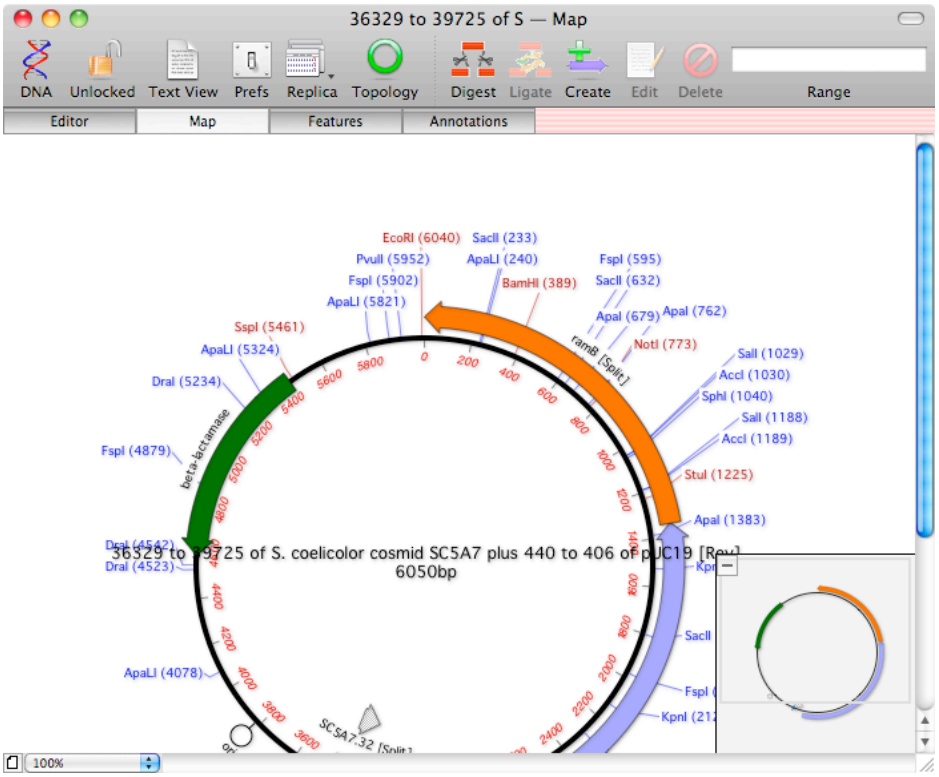
In this case, we don't need to modify the ends so click on the **Ligate** button.

The **Cloning Clipboard** updates to display the new constructed fragment;

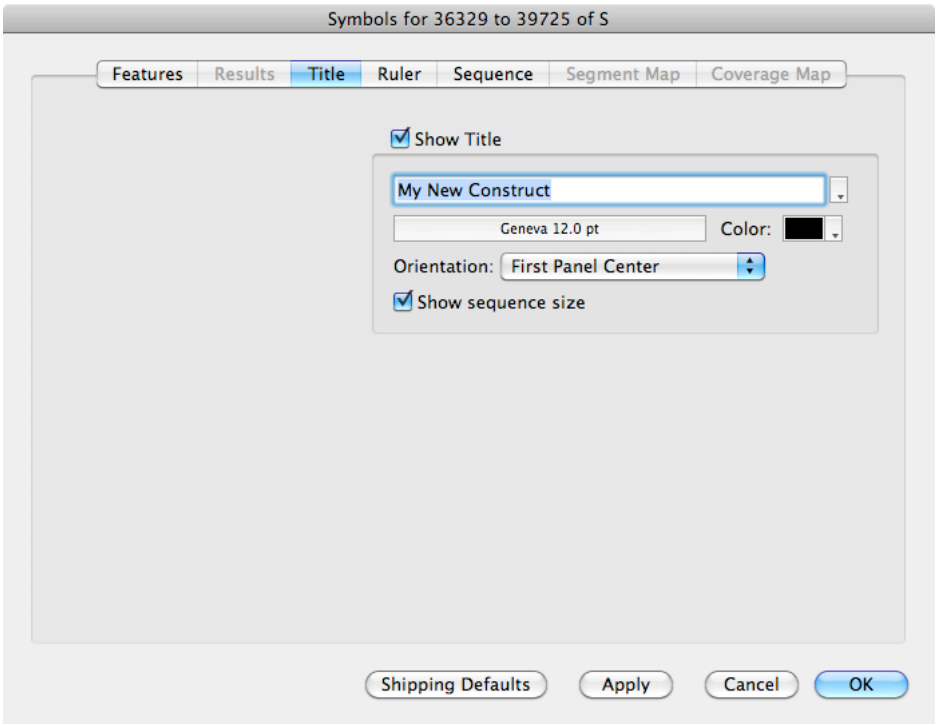


Make sure the new fragment is selected (as shown above) then click on the **Circularize** button.

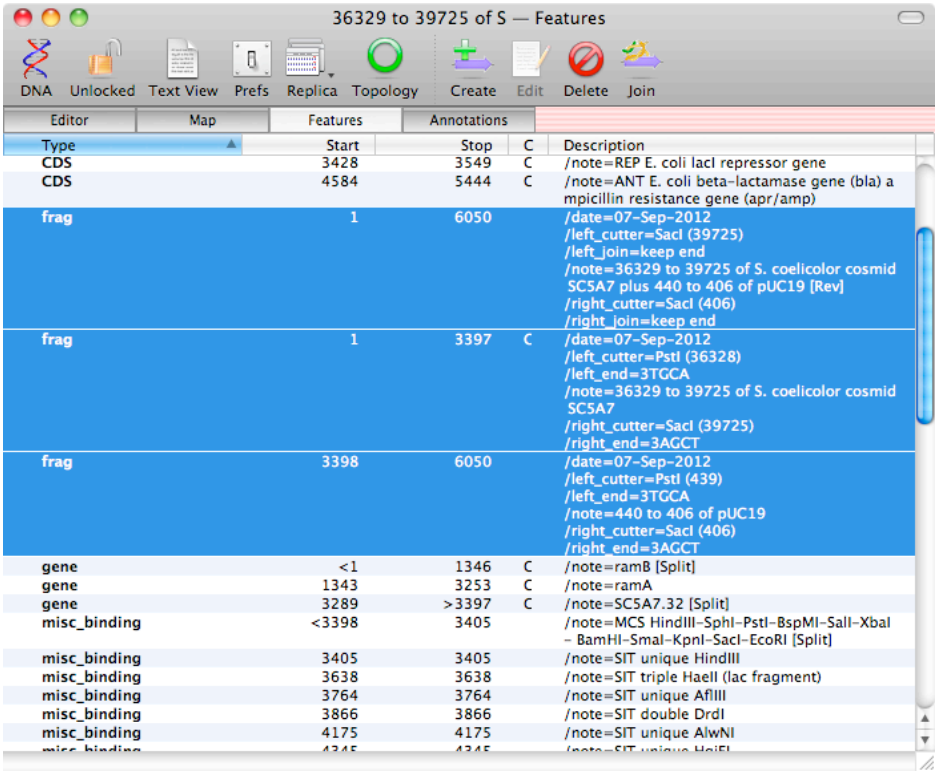
If you like to drag things, you can achieve the same effect by dragging the *Sac*I site from one end of the fragment and dropping it on the *Sac*I site at the other end. Either way, after you **OK** the **Ligation Sheet**, a MacVector sequence window will open with the new construct.



The long title in the middle of the sequence can be annoying, so double-click on it to give it a more suitable name;



Finally, you can see a history of the construct in the **frag** features in the **Features** tab;

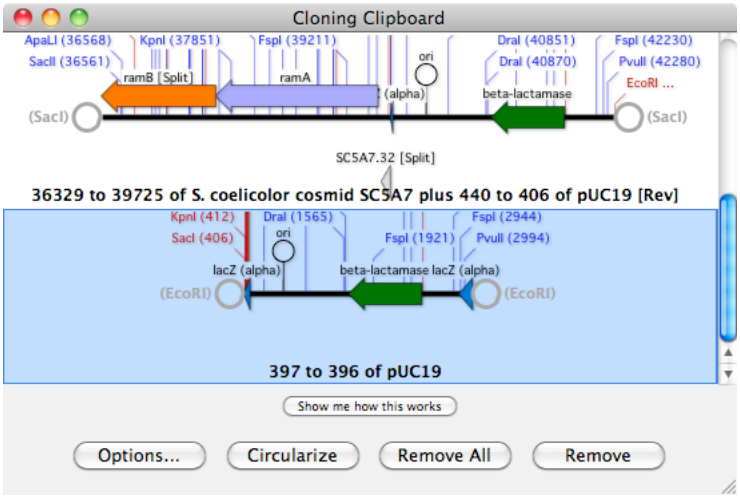


Removing A Restriction Site From A Vector

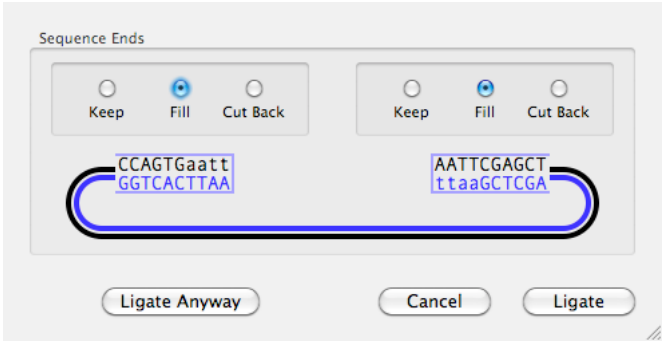
One common cloning operation that was difficult to replicate with earlier versions of MacVector is when you want to open up a plasmid by cutting with a restriction enzyme, fill or cut back the ends, then re-ligate to remove the site. This is now extremely easy with MacVector.

Bring the `pUC19` window to the front. Click on the *Eco*RI site at 396 and click on the **Digest** button

The Cloning Clipboard updates to show the linearized vector;

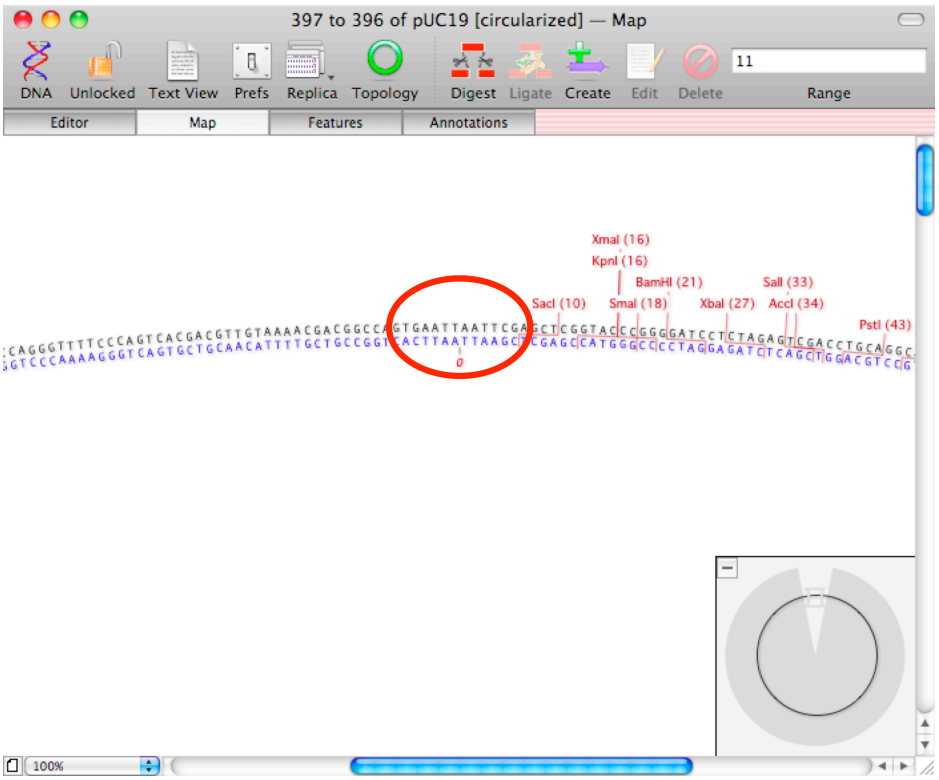


Click on the **Circularize** button then select the two **Fill** radio buttons to fill in the two ends of the fragment.



Click on the **Ligate** button.

A new window appears containing the recircularized plasmid, but now missing the *Eco*RI site., here shown zoomed in to the residue level in the **Map** tab;



Multi-Fragment Gateway Cloning

The real power of the **Cloning Clipboard** becomes most apparent when you are trying to replicate 3-fragment cloning experiments. One common example of this type of approach is the MultiSite Gateway® Technology from Invitrogen. This uses *in vitro* recombination driven by a series of related phage *att* sites to quickly generate constructs made from multiple fragments with high efficiency. For more details, check out the information on the Invitrogen web site;

http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cloning/Gateway-Cloning/MultiSite-Gateway-Technology.html

For this example, we will see how simple it is to generate a construct consisting of three separate fragments joined end to end and inserted into a suitable target vector. Each fragment has been inserted into an "Entry" vector – the constructs can all be found in the `/Applications/MacVector/Tutorial Files/Click Cloning/` folder.

`vectorA.nucl` – contains **fragA** flanked by *att*L4 and *att*R1 sites

`vectorB.nucl` – contains **fragB** flanked by *att*L1 and *att*L2 sites

`vectorC.nucl` – contains **fragC** flanked by *att*R2 and *att*L3 sites

`pDESTR4R3.nucl` – this is a "Destination" vector with *att*R4 and *att*R3 sites.

The strategy here is that we will join the fragments together to form a construct;

…*att*L4-**fragA**-*att*R1/*att*L1-**fragB**-*att*L2/*att*R2-**fragC**-*att*L3…

and then insert that into `pDESTR4R3`. For the purposes of manipulation of the *att* sites in MacVector, we can treat them just like restriction enzymes with long asymmetrical overhangs. While the experimental *in vitro* operation is a recombination rather than a ligation, the end results will be identical.

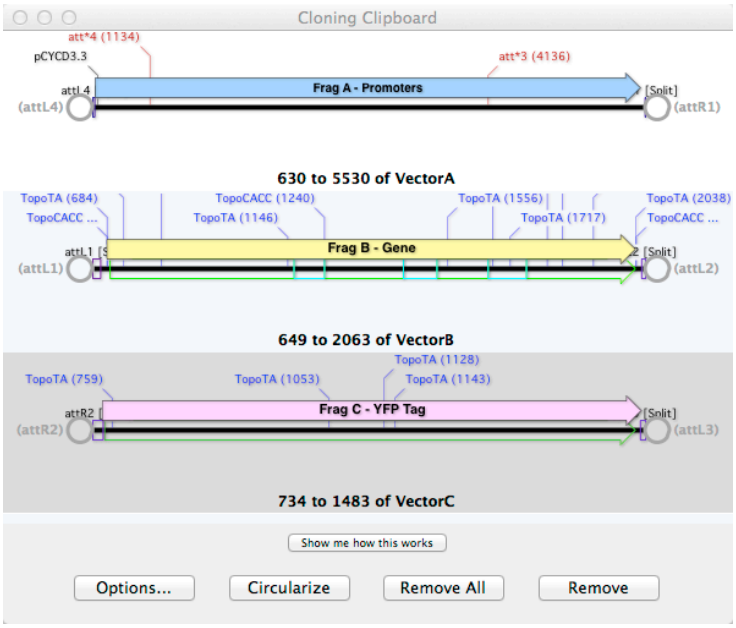
Choose **File I Open** and open each of the sequences `vectorA.nucl`, `vectorB.nucl`, `vectorC.nucl` and `pDESTR4R3.nucl`.

In the **Map** tab of `vectorA.nucl`, click on the *att*L4 site at 629, hold down the `<shift>` key and click on the *att*R1 site at 5530, then click on the **Digest** toolbar button.

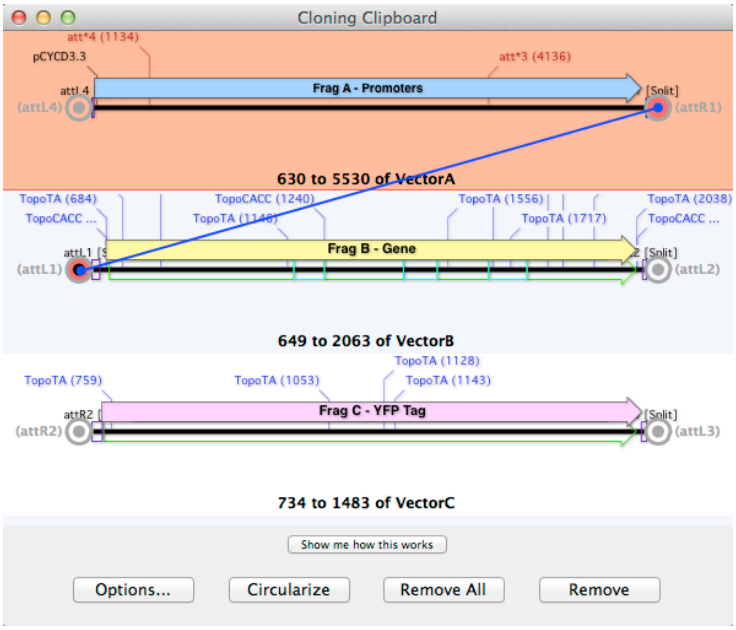
Switch to `vectorB.nucl`, select from the *att*L1 site at 648 to the *att*L2 site at 2063 and **Digest**.

Finally, switch to `vectorC.nucl` and digest the fragment from the *att*R2 site at 733 to the *att*L3 site at 1483.

The **Cloning Clipboard** should now look like this;

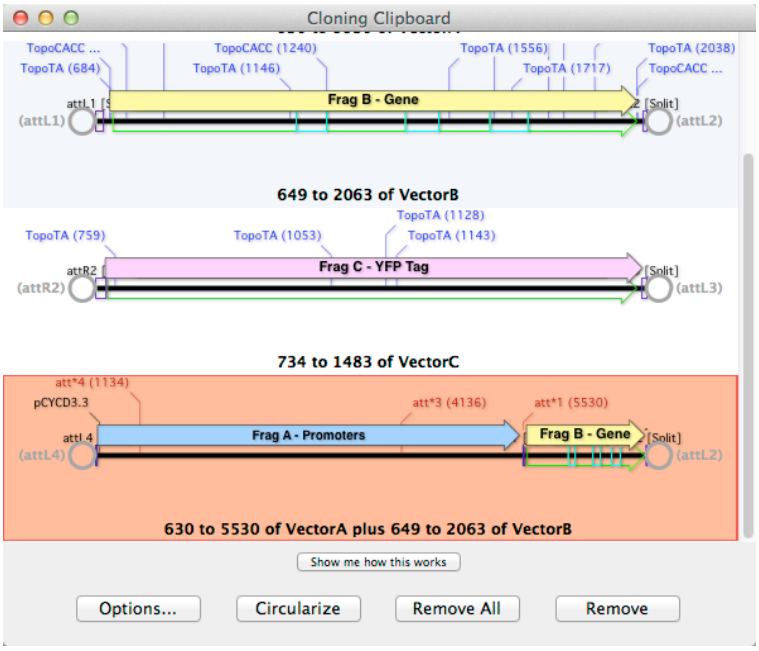


Click on the circle at the right end of **fragA** (marked as *att*R1), hold down the mouse and drag the line onto the *att*L1 circle at the left end of **fragB**, then release the mouse.



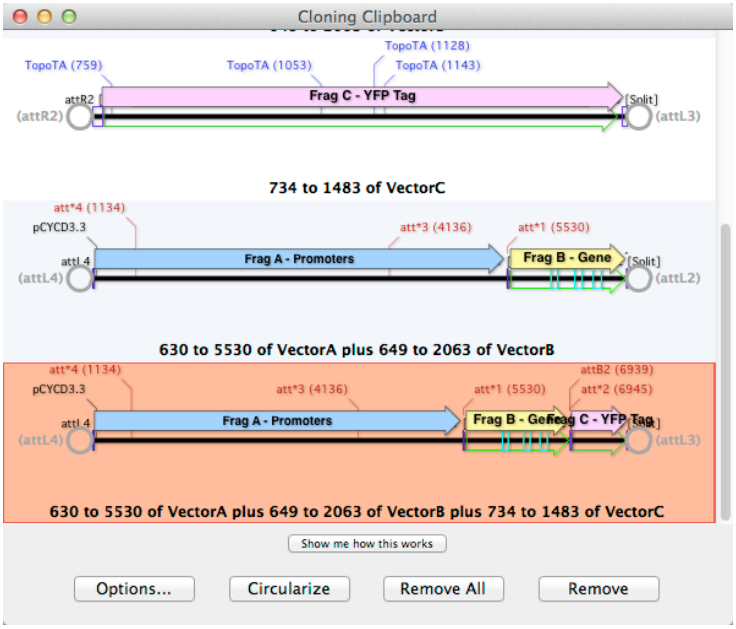
In the ligation sheet that drops down, click on the **Ligate** button.

A new ligated fragment should appear on the **Cloning Clipboard**;



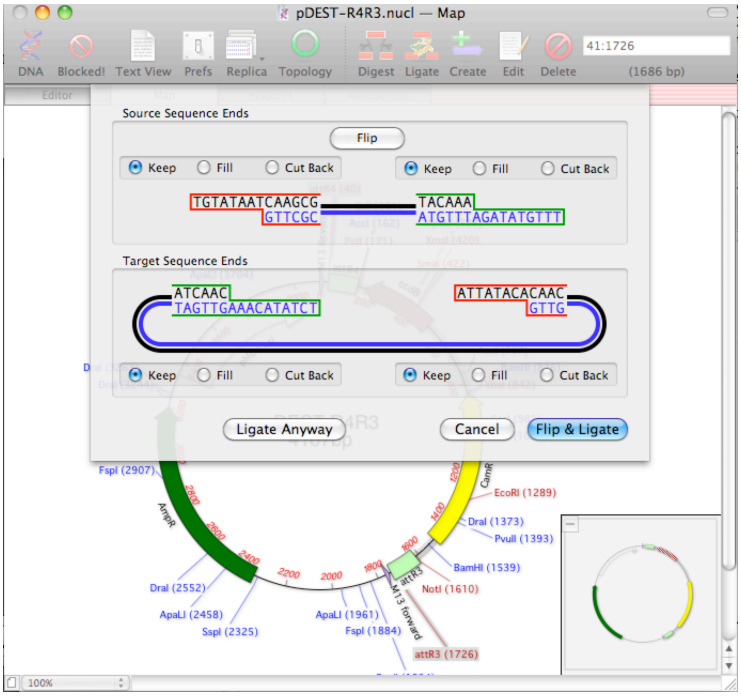
Now drag the *att*R2 end of the **fragC** fragment to the *att*L2 end of the new **fragA+fragB** fragment and **Ligate**.

Again, a new **fragA+fragB+fragC** fragment should appear in the **Cloning Clipboard** with an *att*L3 site at one end and an *att*L4 site at the other.

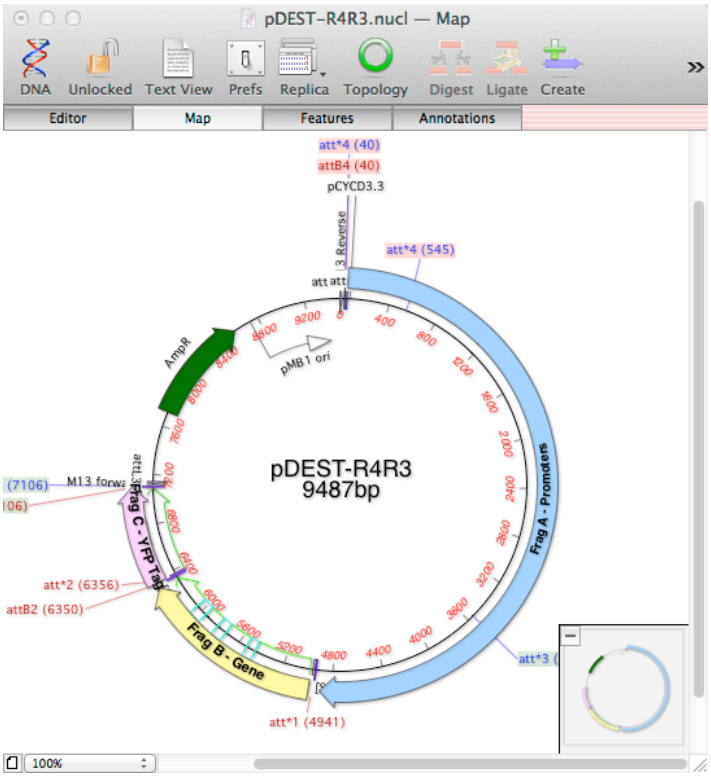


Leave the new fragment selected in the **Cloning Clipboard** and switch to the `pDESTR4R3.nucl` window. Select the *att*R4 site at 40 followed by the *att*R3 site at 1726, then click on the **Ligate** button on the toolbar.

The **Ligate** toolbar button always tries to paste in the currently selected fragment on the **Cloning Clipboard**.



Click on the **Ligate** button.



The final construct has the three fragments inserted in the correct orientation and exactly duplicates results of an *in vitro* recombination experiment of a mix of all four starting plasmids.

These general approaches can be used to simulate any multi-fragment cloning, whether using regular restriction enzymes or Gateway *att* sites. The fragments on the **Cloning Clipboard** can be selected and deleted, or the entire clipboard cleared using the buttons at the bottom of the window. The fragments can also be re-ordered by dragging and dropping into a new location. Finally, you can **Edit | Copy** any selected fragment on the **Cloning Clipboard** to copy it to the regular shared system clipboard (also known as the “pasteboard”) if you want to paste the sequence into another application.