



## Click Cloning Tutorial

### Files you will need

(All are distributed with MacVector, including the trial version)

- pUC19 – in the MacVector 10.5/Tutorial Files/Click Cloning/ folder
- S.coelicolor Cosmid SC5A7 – in the MacVector 10.5/Tutorial Files/Click Cloning/ folder
- Common Enzymes – in the MacVector 10.5/Restriction Enzymes/ folder

### Overview

The tutorial will show you how to use MacVector to replicate a subcloning operation that you might perform in a practical laboratory. We will subclone an EcoRI-SacI fragment from a cosmid into pUC19. The steps will be (a) select the enzymes to use in the cloning experiment, (b) copy the target fragment onto the clipboard and (c) paste the fragment between the EcoRI and SacI sites of pUC19.

### Procedure

#### 1.1 Selecting Restriction Enzymes

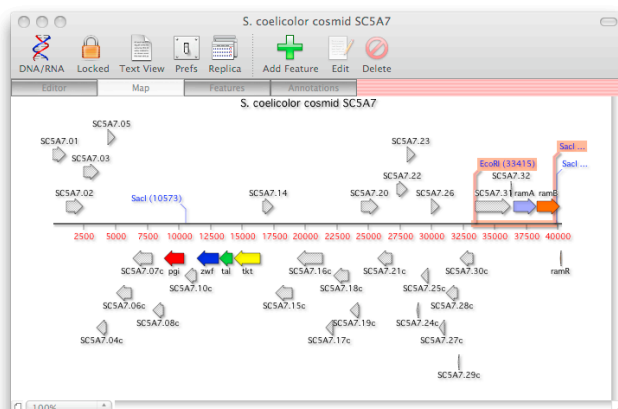
Click on the Preferences icon in the Map view toolbar. Ensure **Automatic RE Analysis** is turned on and set to **Only use selected enzymes**. If Common Enzymes is not shown in the RE file window then click on **Set Enzyme File...** to select it. It is located in the /MacVector 10.5/Restriction Enzymes/ folder. Then click on **Open** to be able to select enzymes in this file. Click **OK** to close this dialog. Now click on the Common Enzymes window. You can click on an enzyme name and a check mark appears next to the enzyme. Click on the “deselect” toolbar button – this is the one that has an image of a checkmark with a cross through it. Select the enzymes BamHI, EcoRI and SacI. You can permanently save the selections in the file if you wish by selecting **Save** in the File menu.

#### 1.2 Copying the Cosmid Fragment

Open the file S. coelicolor cosmid SC5A7. You will find this in the /MacVector 10.5/Tutorial Files/Click Cloning/ folder.

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.



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You should be able to see an EcoRI site at 33415. Click on the site label and it should select.

Hold down the <shift> key and select the SacI site at 39725 (near the end of the cosmid). Now both it and the EcoRI site should be selected.

Choose **Copy** from the **Edit** menu. This copies the fragment, along with all overlapping annotations and feature information, onto the clipboard.

### 1.3 Pasting into the Vector



Open the vector file pUC19 – you should find it in the Tutorial Files/Click Cloning/ folder.

Again switch to the **Map** tab, and in the same way as previously, select the EcoRI and SacI sites (remember to hold down the <shift> key).

Select **Paste** from the **Edit** menu. You should be prompted to unlock the pUC19 sequence. This is to prevent you from accidentally modifying an important sequence file. The fragment from the clipboard will then replace the small EcoRI-SacI fragment in pUC19. Note that the pasted fragment retains all of the features and feature appearance of the source DNA molecule (compare the two screenshots).

### 1.4 Additional Information

The example here uses a double digest, but you can use the same workflow for single digested DNA as well – in that case you will only need to select the one site in the target vector.

Please note that this tutorial uses the Automatic RE analysis function. In addition to this there is a more complex and powerful function available in **Analyze | Restriction Analysis**. This function uses a separate Map window, but has more functionality, such as being able to exclude regions from the map (e.g. only show me sites that do not cut in the gene I want to excise), being able to show enzymes that only cut in a specific region, but not anywhere else in the entire sequence, and other functions. You may prefer to use this to design more complex digests.

Note that when the restriction map is regenerated, it honors the original restriction enzyme settings i.e. MacVector will use the settings for the destination sequence. If those settings are different from the source molecule, you may not see the restriction enzyme pattern you expect. For example, if you paste a BamHI fragment into a unique BamHI site, but you set the destination restriction analysis to **number of cuts <= 1**, then you will not see the BamHI sites you used in the regenerated map because the new molecule will contain two BamHI sites.

The copy/paste is sticky end aware, so you will get a warning dialog if the ends of the source and destination molecules are not compatible. MacVector will automatically flip the pasted fragment if required to match the ends of the target molecule.

If you need to “flip” a fragment to get the orientation correct, hold down the <option> key while pasting and MacVector will flip the fragment before inserting it into the destination.