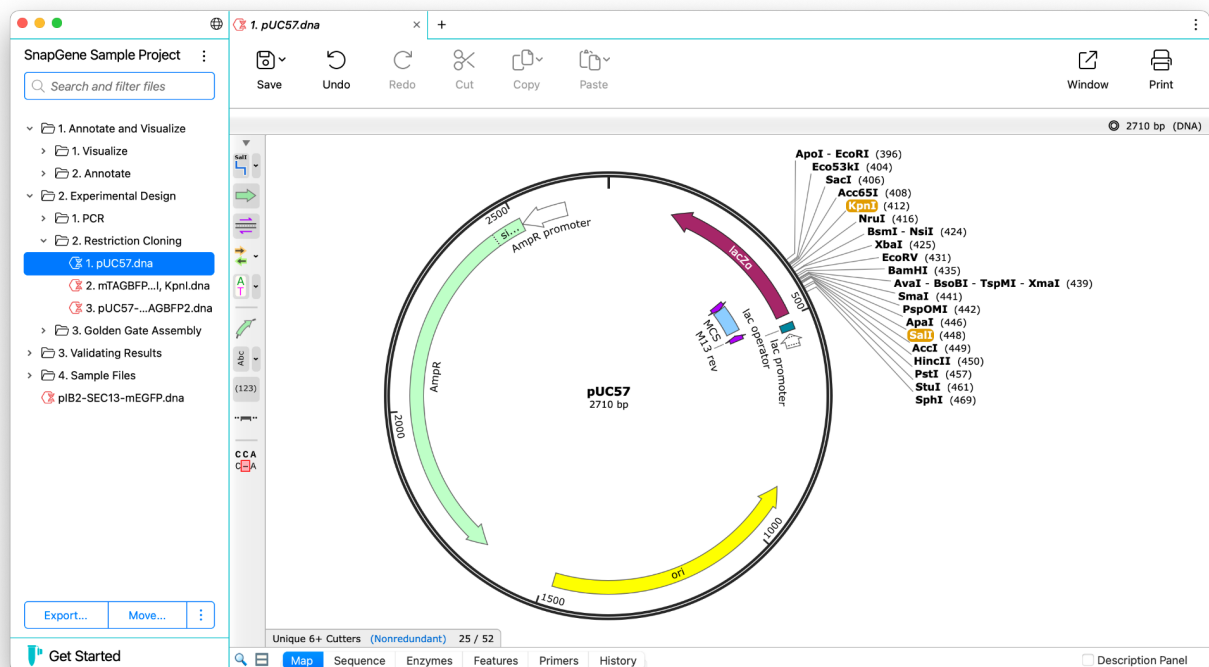# 4. Experimental Design

Restriction Cloning

The **Restriction Cloning** action makes it possible to easily simulate restriction cloning reactions of up to 99 fragments in SnapGene. This is one of many cloning techniques able to be simulated in SnapGene. The step-by-step video guides on our website provide a summary of each of the cloning techniques available in SnapGene.

This document will walk through an example of cloning a **mTAGBFP2** fluorescent marker into a **pUC57** vector. This folder contains 3 documents:

1. **pUC57.dna**. pUC57 is a common cloning vector, and contains a multiple cloning site (MCS) with a large number of enzyme sites that can be used for cloning reactions.
2. **mTAGBFP2 SalI, KpnI.dna**. This file contains the mTAGBFP2 fluorescent protein sequence that has been amplified by PCR with primers that introduce *Sal*I and *Kpn*I restriction enzyme sites to facilitate this restriction cloning reaction. For instructions on how to do this, please see the **PCR** section in the **2. Experimental Design** folder of the **SnapGene Sample Project**.
3. pUC57-mTAGBFP2.dna. Is the final file produced by simulating the restriction cloning reaction in SnapGene.
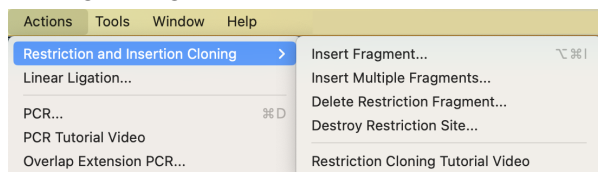
To simulate a restriction cloning reaction in SnapGene, do the following:

1. Open **pUC57.dna** in SnapGene by double-clicking on the file in the file explorer. This will open the file shown below.
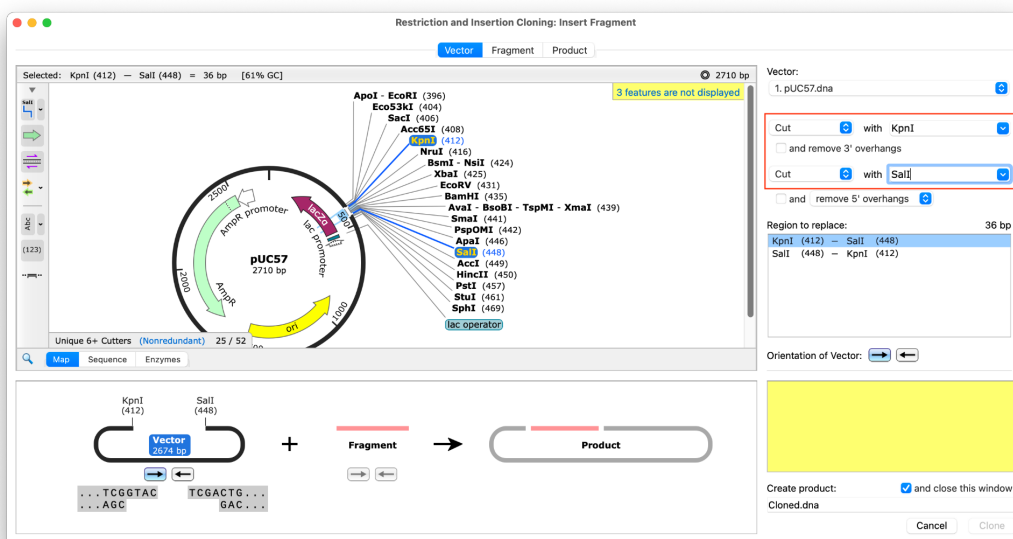

Note the *Kpn*I and *Sal*I restriction enzyme sites highlighted in yellow. These are the sites that will be used for this cloning reaction. It is also important to note that the lac promoter in this sequence (shown in white) is in the reverse orientation. This will also become important later on.
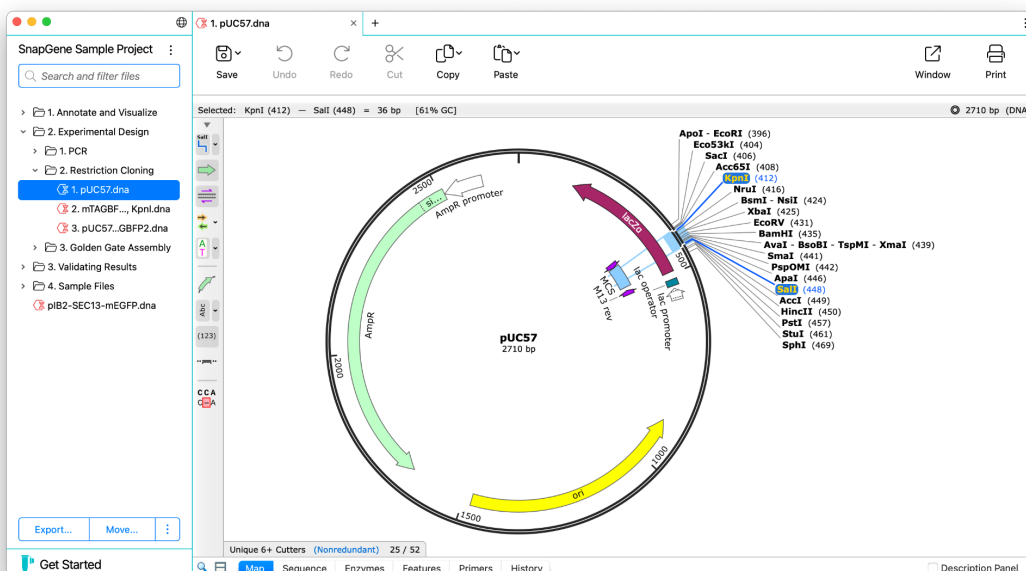
2. Select **Actions>Restriction and Insertion Cloning>Insert Fragment…** This will open the restriction cloning dialog with the **pUC57.dna** sequence you have opened as the vector.
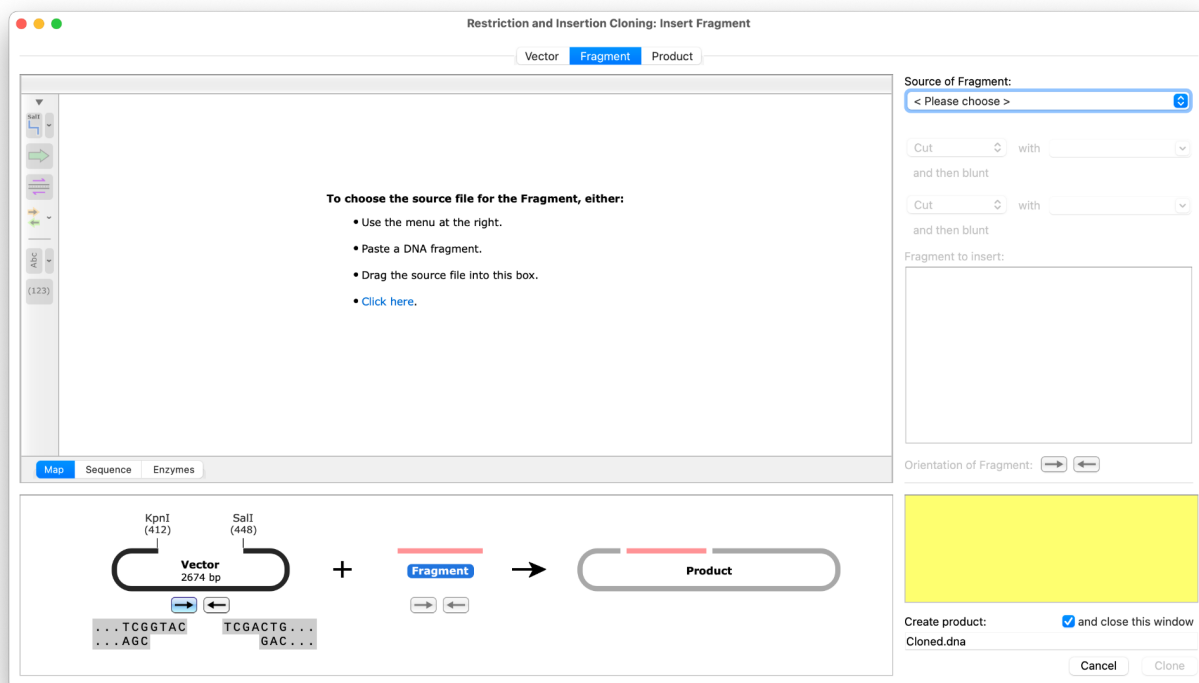

3. *Kpn*I and *Sal*I will be used for this restriction cloning reaction. This information can be inputted in a number of different ways:

1. Type the enzyme names into the textbox on the right-hand side of the restriction cloning dialog.

2. Select the region between the *Kpn*I and *Sal*I sites in the sequence view by selecting the highlighted *Kpn*I site, holding shift, then selecting the highlighted *Sal*I site.
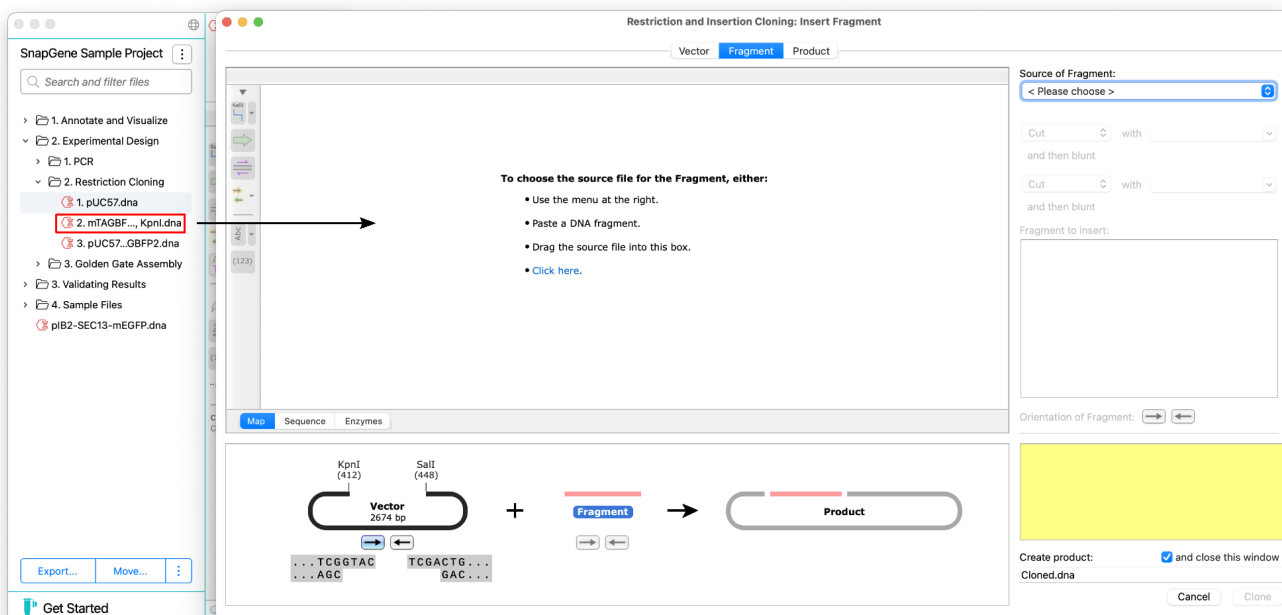

3. It is also possible to select the region between the *Kpn*I and *Sal*I sites on the sequence prior to opening the restriction cloning dialog. This will result in the dialog being prepopulated with the enzyme sites that you have selected.
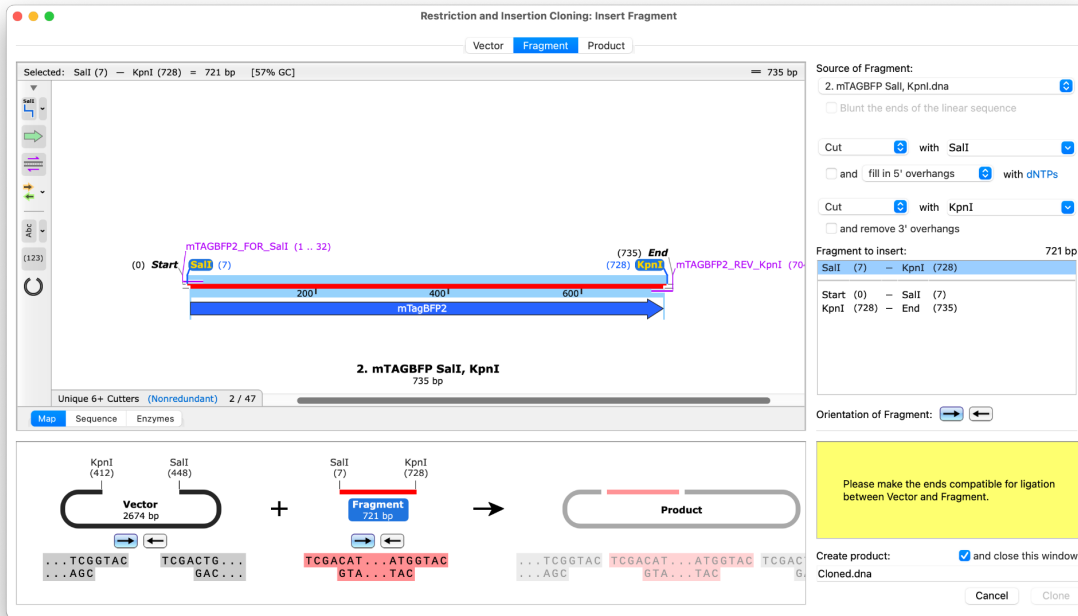
4. Switch to the **Fragment** tab to select your insert. This will bring up the dialog that allows you to select the **mTAGBFP2 KpnI, SalI.dna** fragment file. You can bring your fragment into this tab in a number of ways:

1. Use the **Source of Fragment** dropdown menu on the right-hand side of the dialog window. If your file has been recently opened you will see it listed here, otherwise you will be able to open the file browser on your computer to access the file.

2. Select the **Click here** button in the empty sequence viewer to immediately be taken to your file browser so that you can select the file.

3. Drag the **mTAGBFP2 KpnI, SalI.dna** fragment file directly from the project browser window into the cloning dialog window.

5. Once the mTAGBFP2 file is open in the fragment window, you will need to select the enzymes you wish to cut the fragment with. To do this, either select the *Kpn*I and *Sal*I sites in the sequence view, or type them in the **Cut with** textboxes on the right-hand side of the window. Once you do this, you will notice an issue highlighted in the yellow textbox.
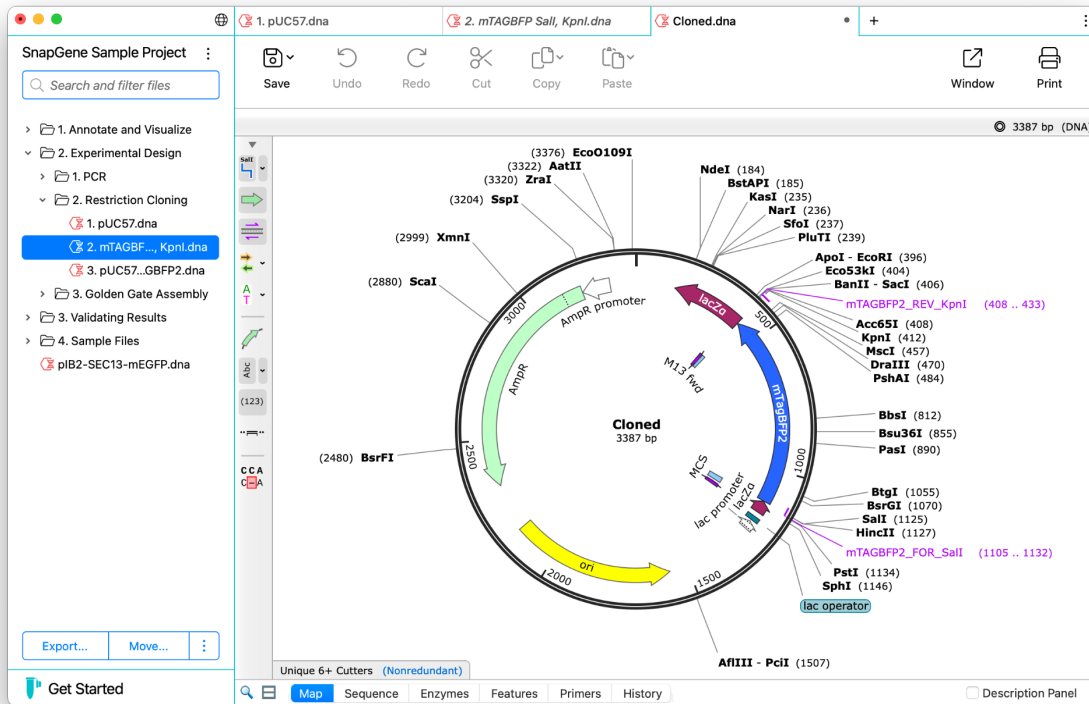

Looking at the product overview at the bottom of the window, it will become apparent that the overhangs for pUC57 and the mTAGBFP2 fragment do not match. At the beginning of this document it was stated that the lac promoter in the pUC57 vector is in the reverse orientation. In order for mTAGBFP2 to be expressed in this vector, the fragment will also need to be in the reverse orientation.

6. To reverse the fragment, select the reverse arrow next to **Orientation of Fragment** on the right-hand side, above the yellow warning message. SnapGene will immediately pick up the correct overhangs and allow you to proceed with the cloning reaction.

7. Select the **Clone** button on the bottom-right corner of the cloning dialog to simulate your cloning reaction. This will generate a file called Cloned.dna, a pUC57 vector containing mTAGBFP2 in the *Kpn*I and *Sal*I sites.

8. Switch to the history tab by selecting **History** at the bottom of the window. This will give further information of the cloning reaction that you have just performed. Select the name of any of the ancestor files to open them in SnapGene.