

# 2. Experimental Design

## Golden Gate Assembly

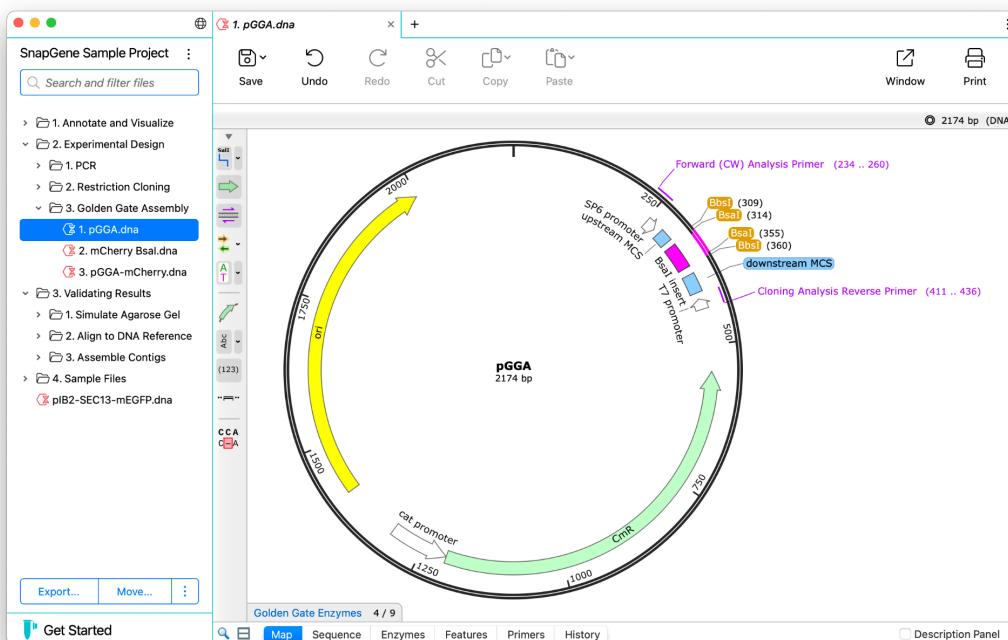
Golden Gate Assembly is a cloning technique that utilizes Type IIS restriction enzymes and T4 DNA ligase to directionally assemble fragments of DNA, and can be simulated in SnapGene using the Golden Gate Assembly action to assemble up to 99 fragments. This tool allows a user to insert sequences that already have Type IIS enzymes present, as well as giving the ability to design primers for the reaction as part of the process, simplifying the design of Golden Gate Assembly experiments.

This document will walk through an example of assembling a mCherry fluorescent protein marker into a pGGA vector, a vector commonly used for Golden Gate Assembly reactions. While it is possible to generate primers for both the vector and insert as part of this cloning dialog, for simplicity these documents already have the Type IIS sites required for assembly. For further information about generating Golden Gate Assemblies in SnapGene, our [User Guide](#) contains more detailed articles. This folder contains the following documents:

1. **pGGA.dna**. This is the vector file to be used in this reaction. This vector contains two Bsal and two BbsI sites to facilitate cloning with these Type IIS sites.
2. **mCherry Bsal.dna**. This is the fragment for this cloning reaction. mCherry is a fluorescent protein marker, and this sequence has already been amplified by PCR to add Bsal sites to the ends of the sequence. The simulation of PCR in SnapGene has already been described in Section 1 of the Experimental Design folder in this Sample Project.
3. **pGGA-mCherry.dna**. This is the final simulated Golden Gate Assembly reaction that has been performed using the above vector and insert.

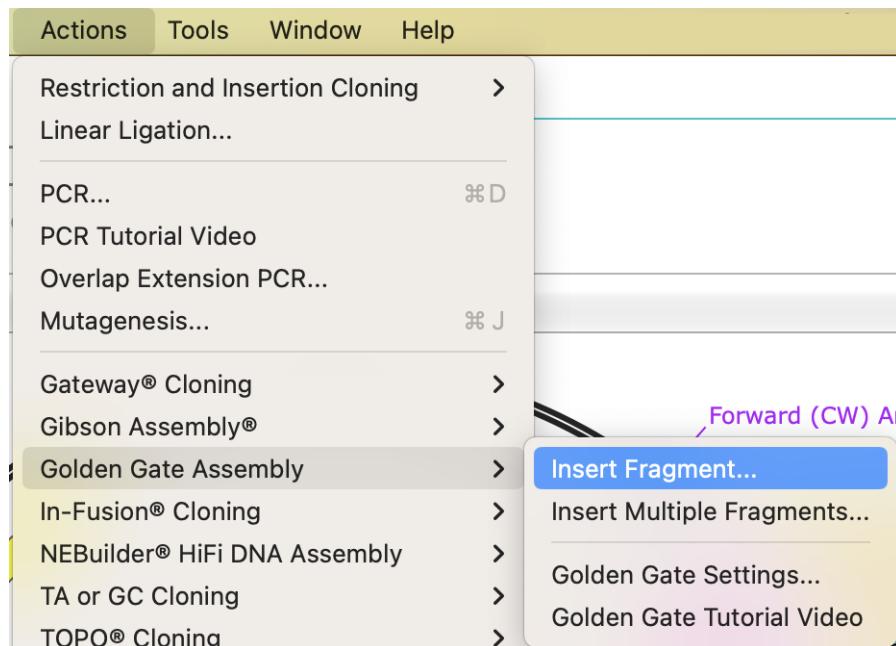
To simulate a Golden Gate Assembly reaction in SnapGene, do the following:

1. Double-click on the pGGA.dna file in the file explorer to open the file in SnapGene. This will bring up the file shown in the screenshot below.

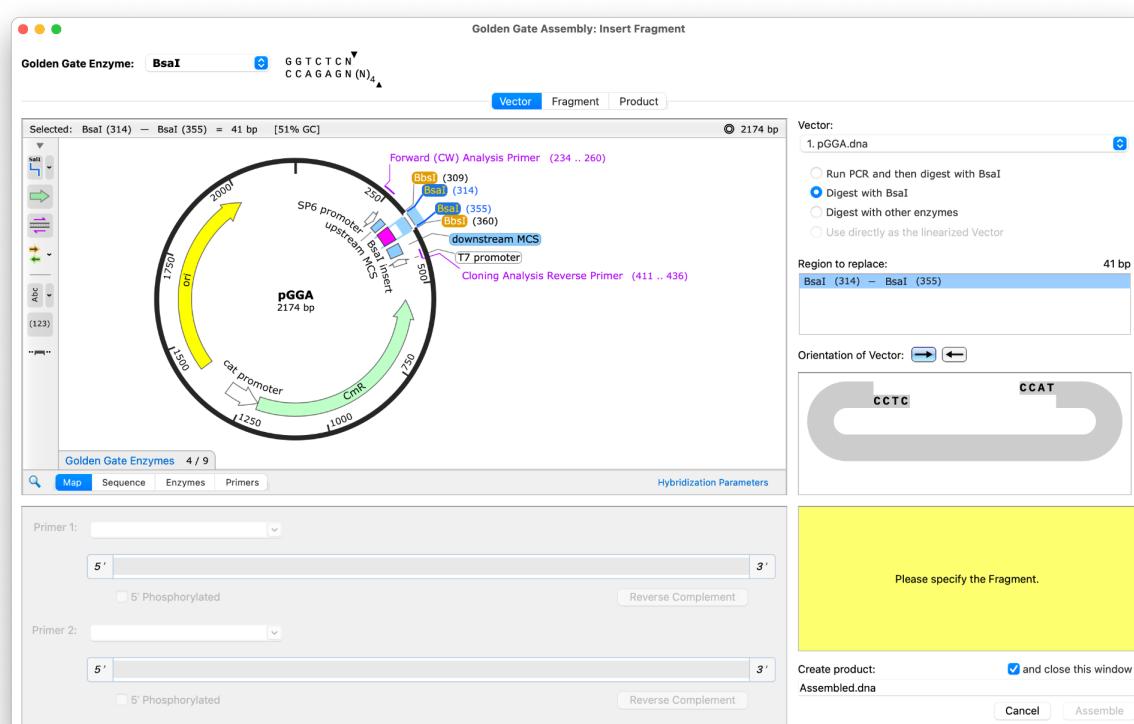


The *Bsal* and *BbsI* Type IIS enzyme sites are highlighted in yellow on this sequence. The *Bsal* sites will be used for this reaction.

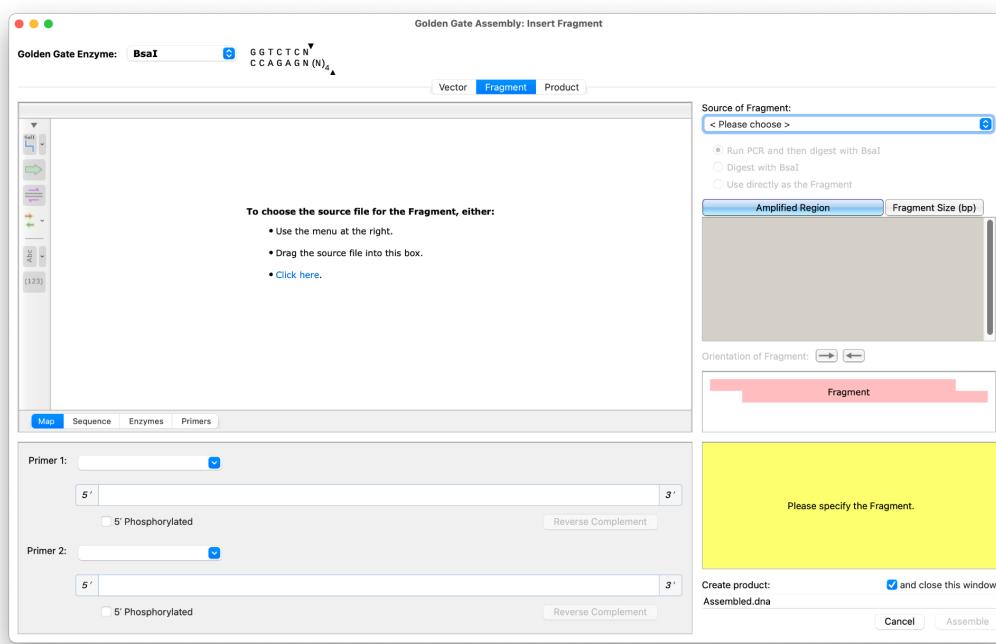
2. Open the Golden Gate Assembly dialog by selecting **Actions>Golden Gate Assembly>Insert Fragment...**



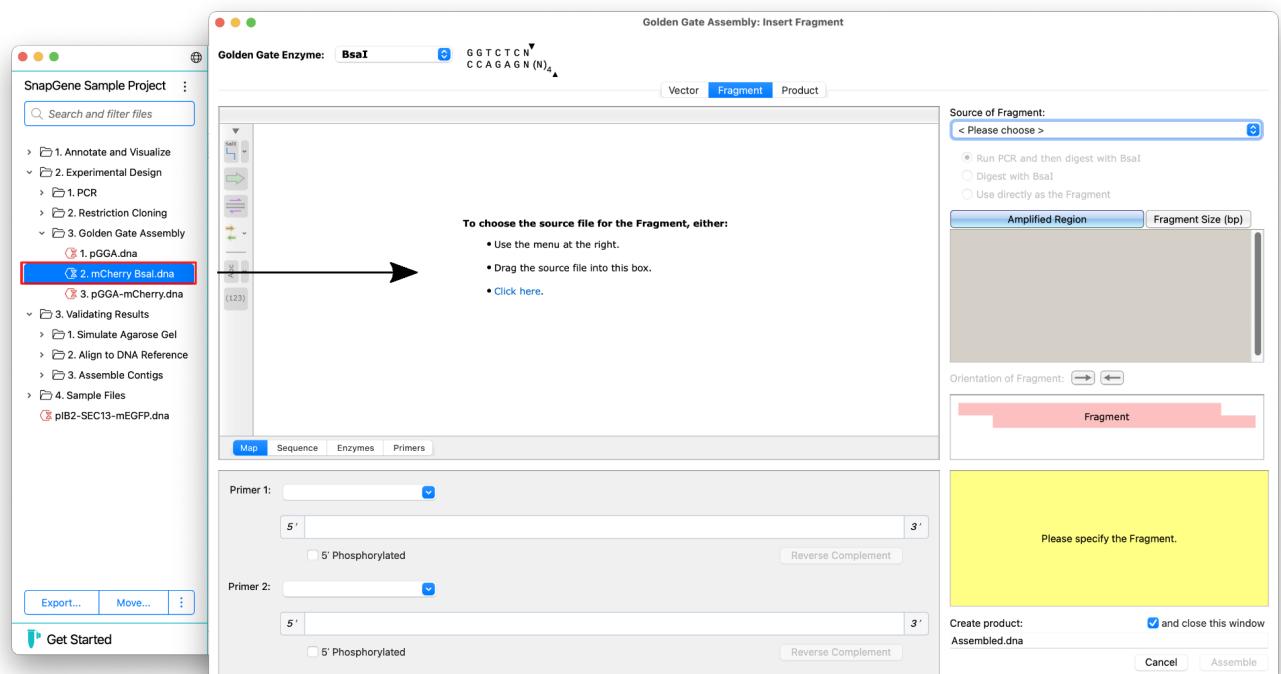
3. This will open the pGGA.dna file as the vector in the Golden Gate Assembly dialog. In this case SnapGene has automatically detected the *Bsal* sites and highlighted **digest with *Bsal*** as the method of cloning. For other sequences you may need to use the other settings provided (see our [User Guide](#) for other examples). **Run PCR and then digest with *Bsal*** will design PCR primers, allowing any sequence, even those without the appropriate Type IIS sites, to be used for Golden Gate Assembly. **Digest with other enzymes** will allow you to use enzymes other than Type IIS enzyme sites. For the purposes of this assembly, use the default settings that SnapGene has specified.



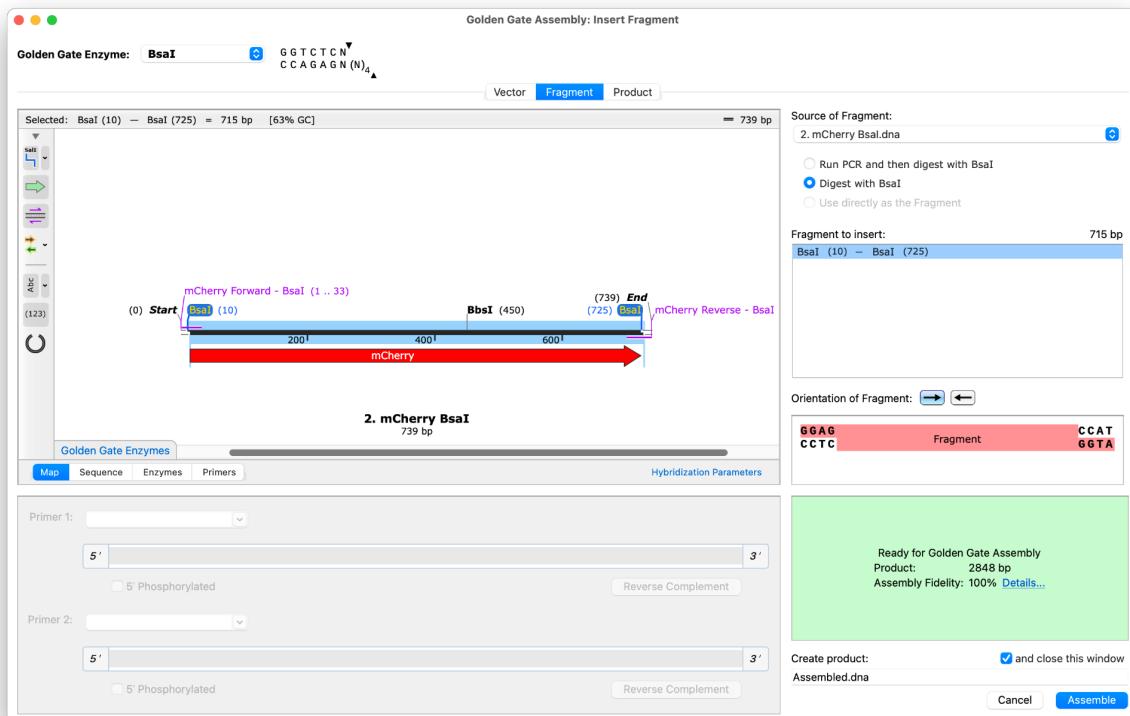
4. Switch to the **Fragment** tab by selecting the **Fragment** button at the top of the window. This will bring up the dialog that allows you to select the **mCherry BsAl.dna** fragment file. You can bring your fragment into this tab in a number of ways:
1. Use the **Source of Fragment** dropdown menu 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.



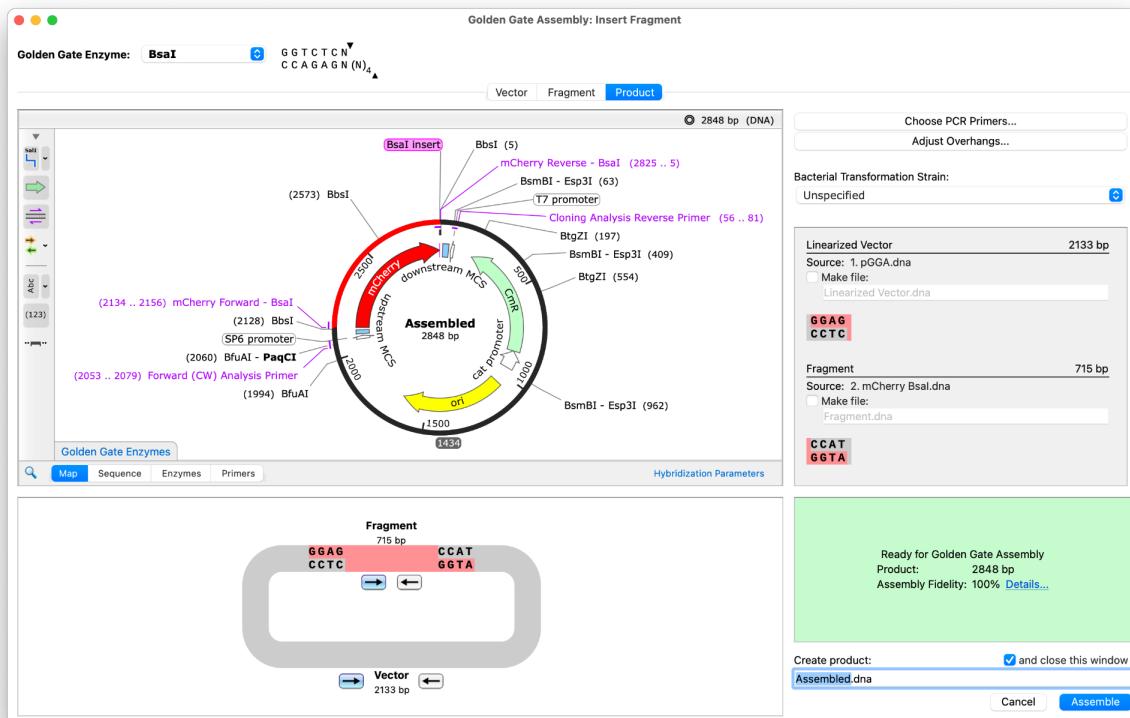
3. Drag the **mCherry BsAl.dna** fragment file directly from the project browser window into the cloning dialog window.



5. SnapGene will automatically detect the *BsaI* sites on the mCherry fragment and assemble the fragment as appropriate.

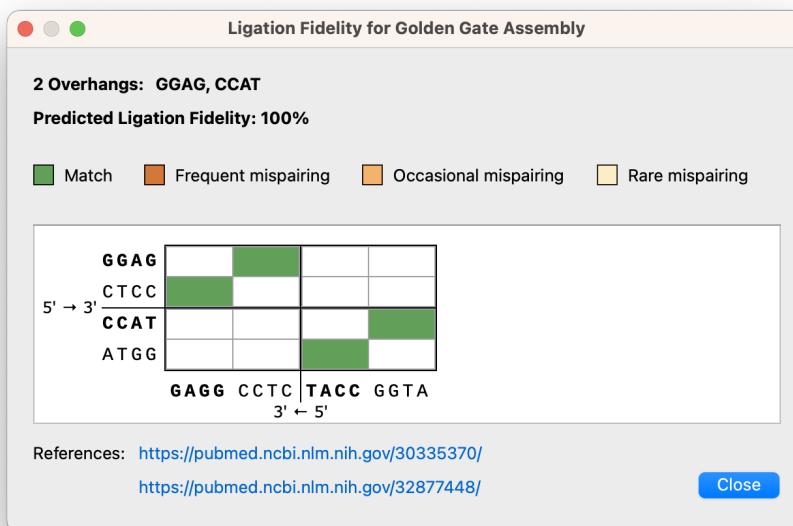


6. To check that everything is correct before assembly, select the product tab and look at the assembled product. You will see that mCherry has been cloned into pGGA between the two *BsaI* sites. The overhangs are shown on the product overview below.

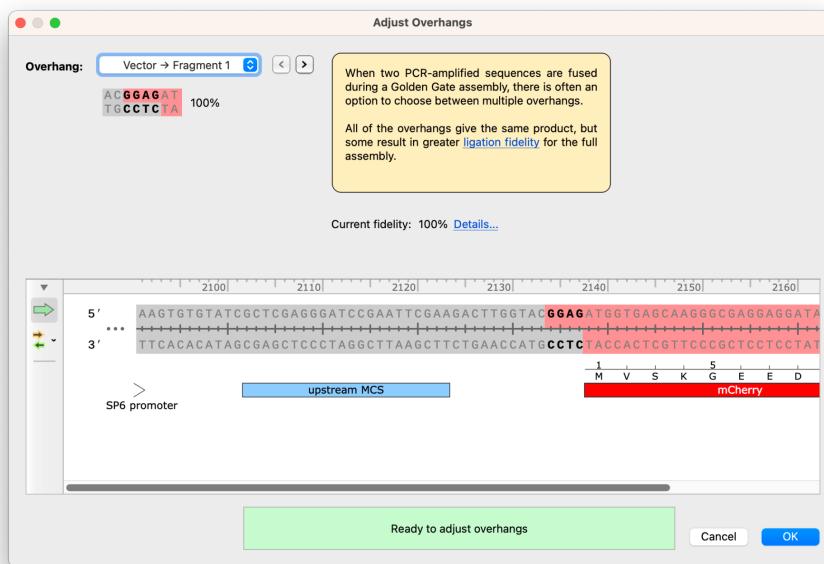


7. The assembly fidelity of the reaction is also reported. SnapGene uses empirical data from Potapov et al. (2018) to choose compatible combinations of unique nucleotide overhangs for Golden Gate Assembly.

This data is used to calculate predicted ligation fidelity for individual overhangs, then predicts an overall assembly fidelity by multiplying the predicted fidelities of each unique overhang used in the assembly. Selecting the Details... link next to the Assembly fidelity will bring up the fidelity matrix.

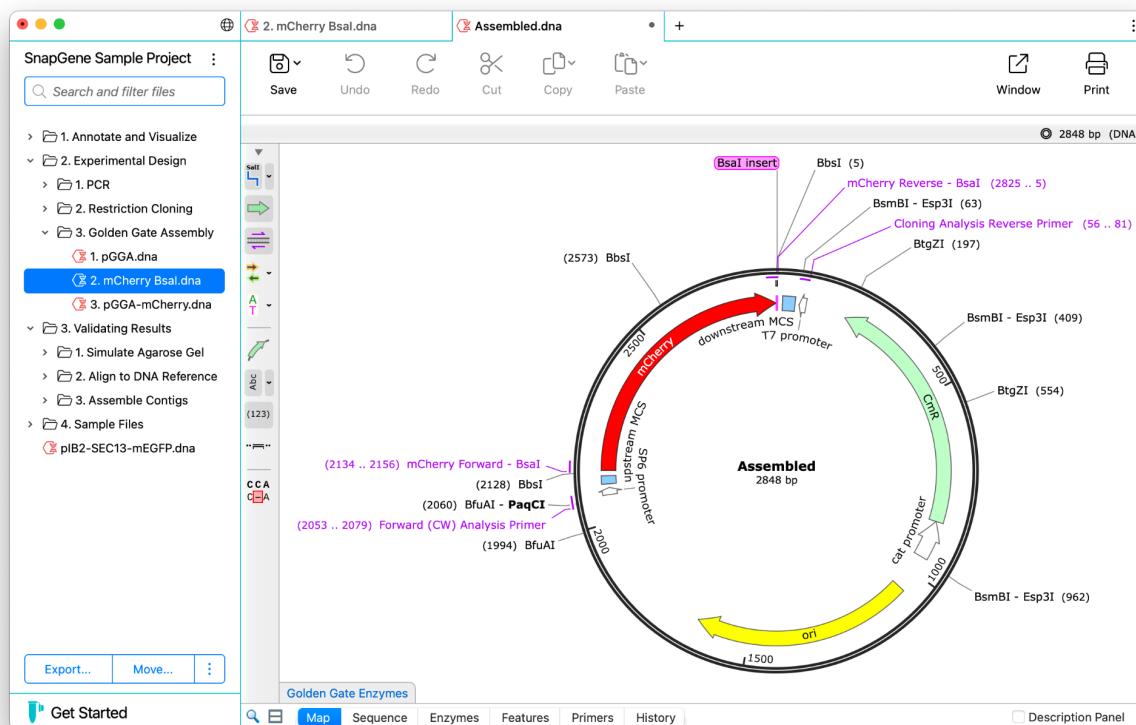


SnapGene will automatically choose the combination of overhangs that produces the highest fidelity for the assembly. However, the overhangs can be changed if desired when using SnapGene to design the PCR primers for the reaction by selecting the **Adjust overhangs...** button. This will bring up the dialog shown below, with the different options for the overhangs displayed. As this reaction is using predesigned fragments, no alternative options are provided.



More information about the assembly ligation fidelity values and adjusting overhangs in SnapGene can be found at our [User Guide](#).

8. Cancel out of the Adjust Overhangs dialog and select **Assemble** to complete the simulation with the existing settings. This will generate a file called **Assembled.dna**, with mCherry assembled into pGGA via the *BsaI* sites.



9. Selecting the **History** tab at the bottom of the window will show you the history of the file, and display the cloning simulation that you have just performed. Selecting on the names of any of the ancestral files will open these files in SnapGene.

