

3. Experimental Design

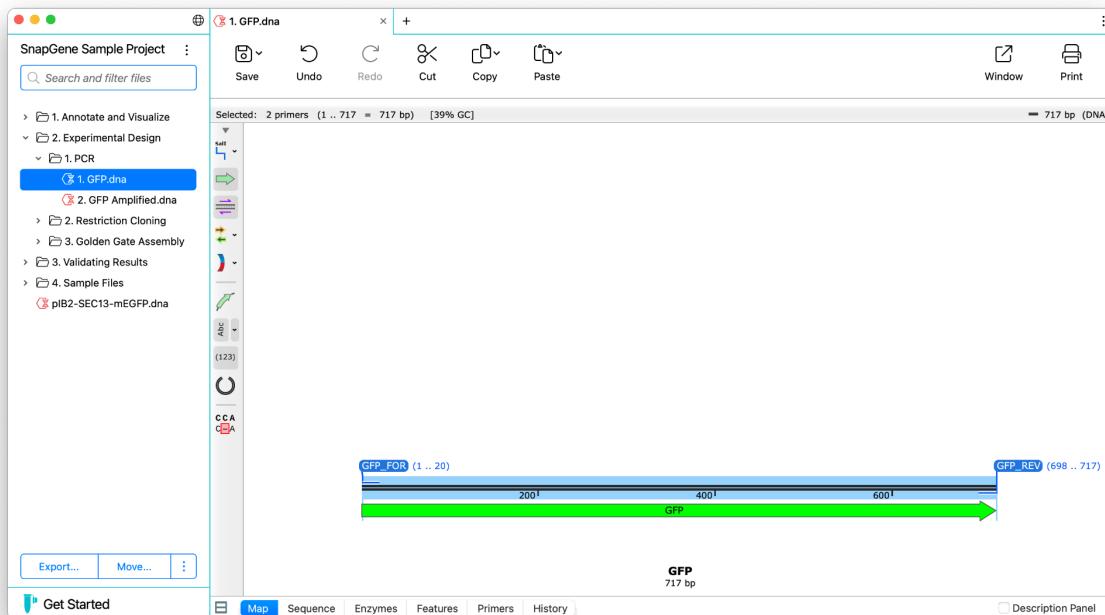
PCR

The **PCR** action allows you to simulate polymerase chain reaction (PCR) with primer sequences in SnapGene. This folder contains two documents that provides instructions for a PCR simulation:

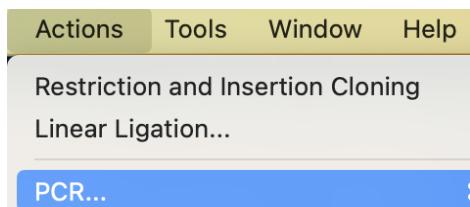
1. **GFP.dna**. This file is a sequence for the Green Fluorescent Protein (GFP) annotated with forward and reverse primers that introduce restriction enzyme sites. This is the type of PCR a user might perform for restriction cloning reactions if your insert does not already possess the restriction enzyme sites needed for cloning.
2. **GFP Amplified.dna**. This file is the final amplified GFP.dna product with the two annotated primers listed above.

A PCR reaction can be simulated in SnapGene with this document by doing the following:

1. Open the **GFP.dna** document in SnapGene by double-clicking the **GFP.dna** file in the file explorer panel.
2. Select the **GFP_FOR** primer annotated on the sequence, hold the shift key on your keyboard, then select the **GFP_REV** primer on your sequence. If you already have primers annotated on your sequence, this is the easiest way to pre-populate the PCR dialog with your desired primers.

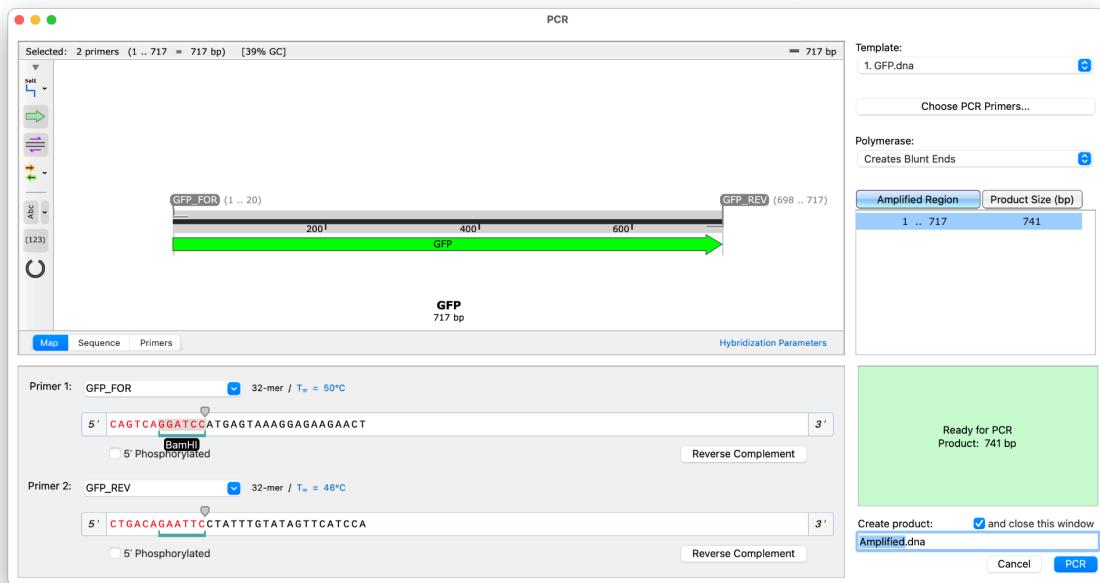


3. Select the **Actions>PCR...** menu item. This will bring up the PCR dialog.

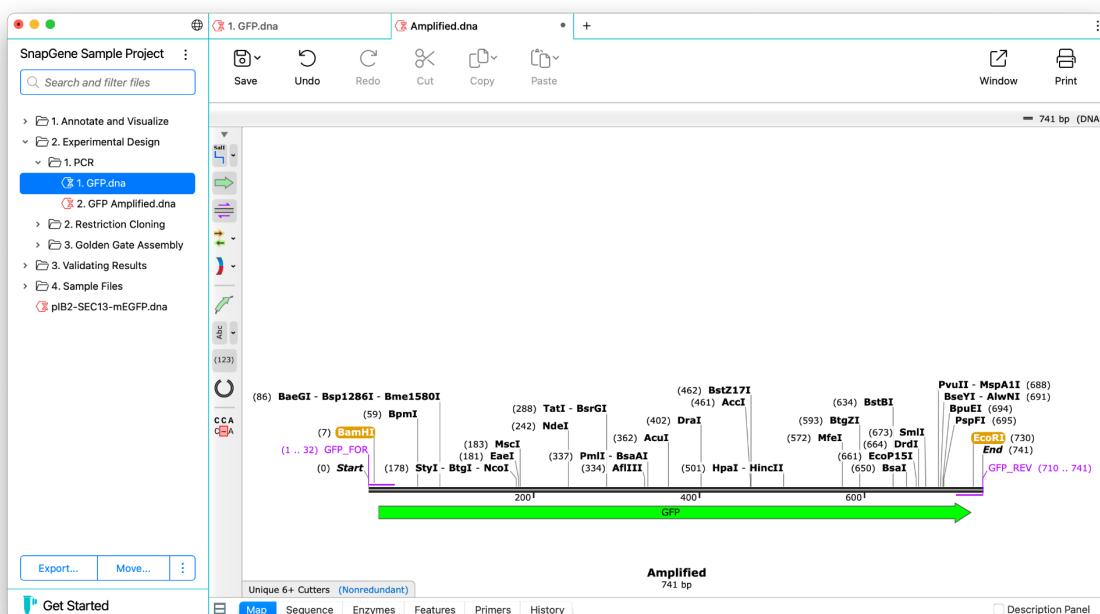


4. As you have selected the primers before opening the PCR dialog, the primer information will be pre-populated in the dialog window and the PCR is ready to be performed. You will see that the *BamHI* and *EcoRI* enzyme sites are annotated on this primer in this window. To see the name of each of these sites, hover over the teal-colored line in the sequence box.

If you have not preselected your primer sequences before opening this dialog, you can also type the sequence into the sequence boxes, or use the dropdown boxes to select primers that are already annotated on your sequence. Then select **PCR** in the bottom-right of the window to simulate the PCR.



5. This will generate a document called **Amplified.dna** that has been amplified by the primers that you specified and SnapGene will open to this document automatically. If you toggle on the **enzymes** view at the top-left of the window, you will see that the *BamHI* and *EcoRI* sites have been introduced by this reaction.



6. Select the **History** tab at the bottom of the window to see more details of your PCR simulation. This gives information of the reaction that has been performed, and what primers were used. Selecting the primer names will give more information about the sequences of those primers, and selecting the GFP.dna file name will open the original ancestral document.

The screenshot shows the SnapGene software interface. On the left, the project tree is visible with '1. GFP.dna' selected. The main workspace displays a DNA sequence with a green arrow labeled 'GFP' spanning from position 2001 to 6001. Above this, a blue arrow labeled 'Amplified' spans from 741 bp to 741 bp. A PCR reaction is shown below, with arrows indicating the primers 'GFP_FOR' and 'GFP_REV' at positions 1 and 717 respectively, amplifying a region from 1 to 717. The resulting product is a green arrow labeled 'GFP' from position 1 to 717 bp. The top menu bar includes options like Save, Undo, Redo, Cut, Copy, Paste, Window, and Print. The bottom navigation bar includes Map, Sequence, Enzymes, Features, Primers, and History, with History being the active tab.