

3. Validating Results

Simulate Agarose Gel

The **Simulate Agarose Gel** tool in SnapGene makes it possible to simulate and visualize the migration of DNA fragments through an agarose gel. It is also possible to simulate cutting a sequence with restriction enzymes, or amplifying a sequence by PCR from directly within this tool. This tool can be found by selecting the **Tools>Simulate Agarose Gel** menu. This will bring up the following dialog which will allow you to select the sequence(s) you wish to simulate. The sequences can be imported in one of three ways:

1. Select the Choose DNA Sequences dropdown menu at the top of the dialog to choose files from your computer.
2. Drag files directly from the project browser into SnapGene directly into this window.
3. Select the files directly in the project browser prior to opening this dialog. You will need to select two or more DNA files, then SnapGene will ask whether you wish to simulate an agarose gel with the selected files. Select **Simulate agarose gel** to open the gel dialog with the selected sequences prepopulated.



After importing your sequences into this dialog, select OK to simulate the gel.

This folder contains a single gel file that has been generated using the Simulate Agarose Gel tool in SnapGene as described above. Select Plasmid Gel.gel to open this file in SnapGene. This will show the following:

The screenshot displays the SnapGene software interface. On the left is a sidebar with a search bar and a tree view of project folders. The main window is titled 'Plasmid Gel.gel' and contains a simulated agarose gel and a plasmid map.

Agarose Gel Simulation:

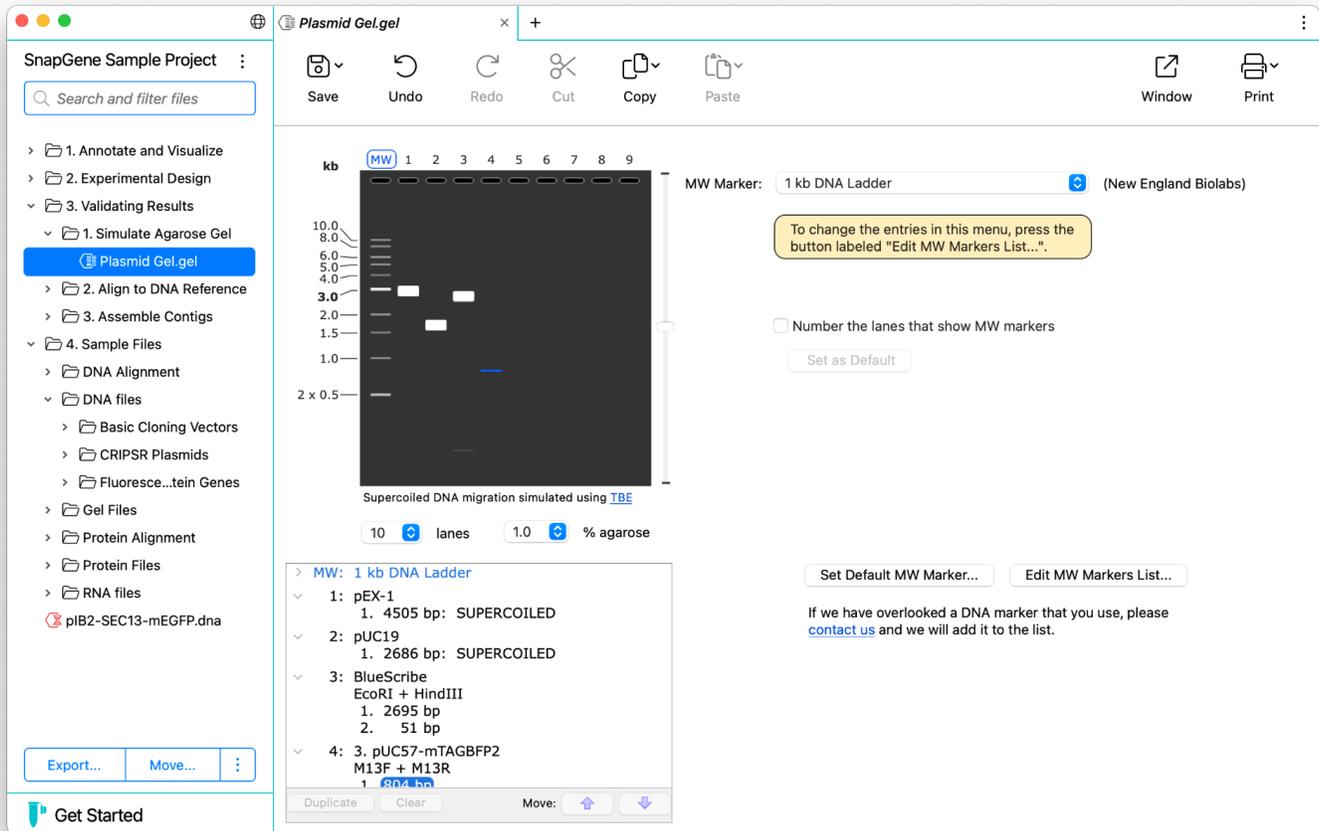
- Lane 1 (MW):** 1 kb DNA Ladder with markers at 10.0, 8.0, 6.0, 5.0, 4.0, 3.0, 2.0, 1.5, and 1.0 kb.
- Lanes 2-9:** Simulated lanes for pEX-1 and its digests. Lane 2 shows a single band at 4.505 kb (SUPERCOILED). Lane 3 shows two bands at 2.695 kb and 51 bp (EcoRI + HindIII). Lane 4 shows two bands at 1.300 kb and 1.250 kb (ori).
- Settings:** 10 lanes, 1.0% agarose.

Plasmid Map (pEX-1, 4505 bp):

- Features include: Amp^r promoter, f1 ori, MCS, T7 terminator, lacI promoter, and lacI.
- Restriction sites for 6+ cutters are shown: BsaBI, BseRI, BspDI, AscI, BglII, HindIII, NheI, BmtI, RsrII, MluI, NotI, AvaI, BsoBI, PmeI, FseI, BlnI, StyI, EcoO109I, BtgI, SacII, PflMI, BstAPI, BclI, BstEII, PspOMI, ApaI, HincII, HpaI, and BsmBI-Eco3I.

On the left side of the file you will see the gel. Each lane is numbered, with a molecular weight marker in the first lane (MW). This marker shows the size of each of the fragments. The settings below this gel will allow you to alter the number of lanes in the gel, as well as change the percentage of agarose used to simulate the gel.

To change the molecular weight marker, select the MW lane on the top of the gel. SnapGene contains a large database of common molecular weight markers from different supplies. To change the marker, use the dropdown menu at the top of the window. Only a small selection of common markers are in this list, but the list of those shown can be altered by selecting the **Edit MW Markers List...** option at the bottom of the window. Once you have selected the marker you wish to use, you can also opt to set this as the default marker when generating gels by selecting the **Set Default MW Marker...** button.

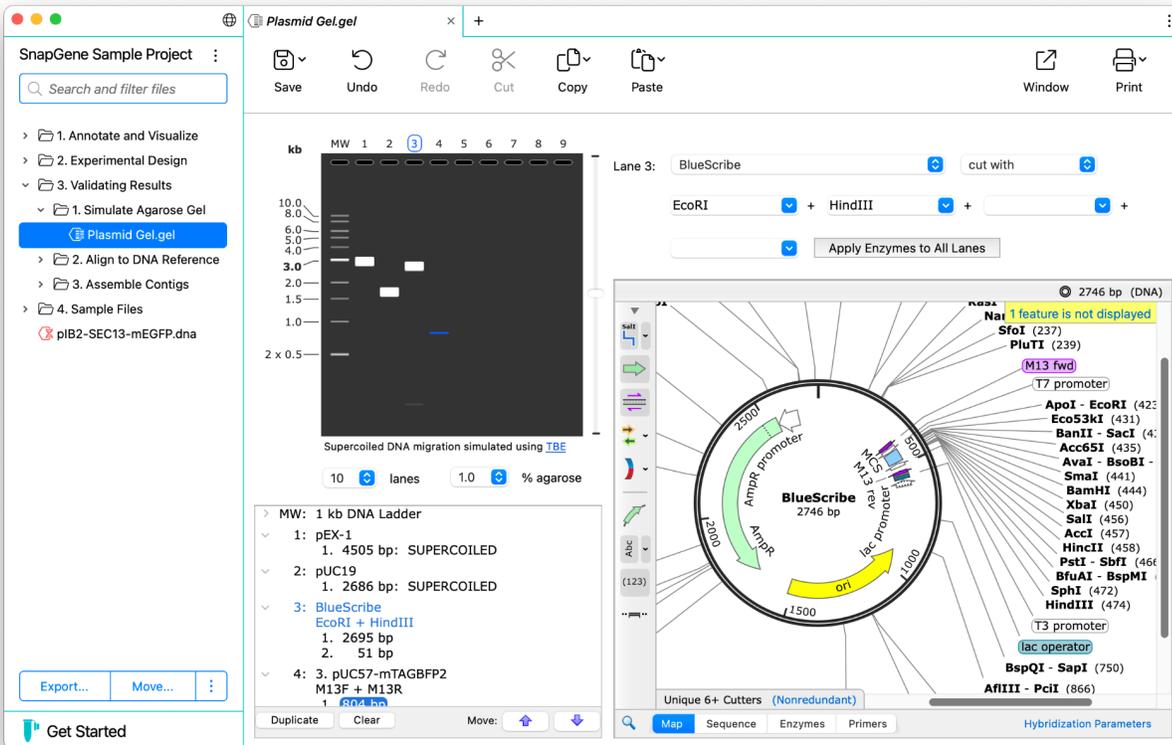


Switching back to any of the numbered lanes by selecting the number will take you back to the sequence view for the selected sequence.

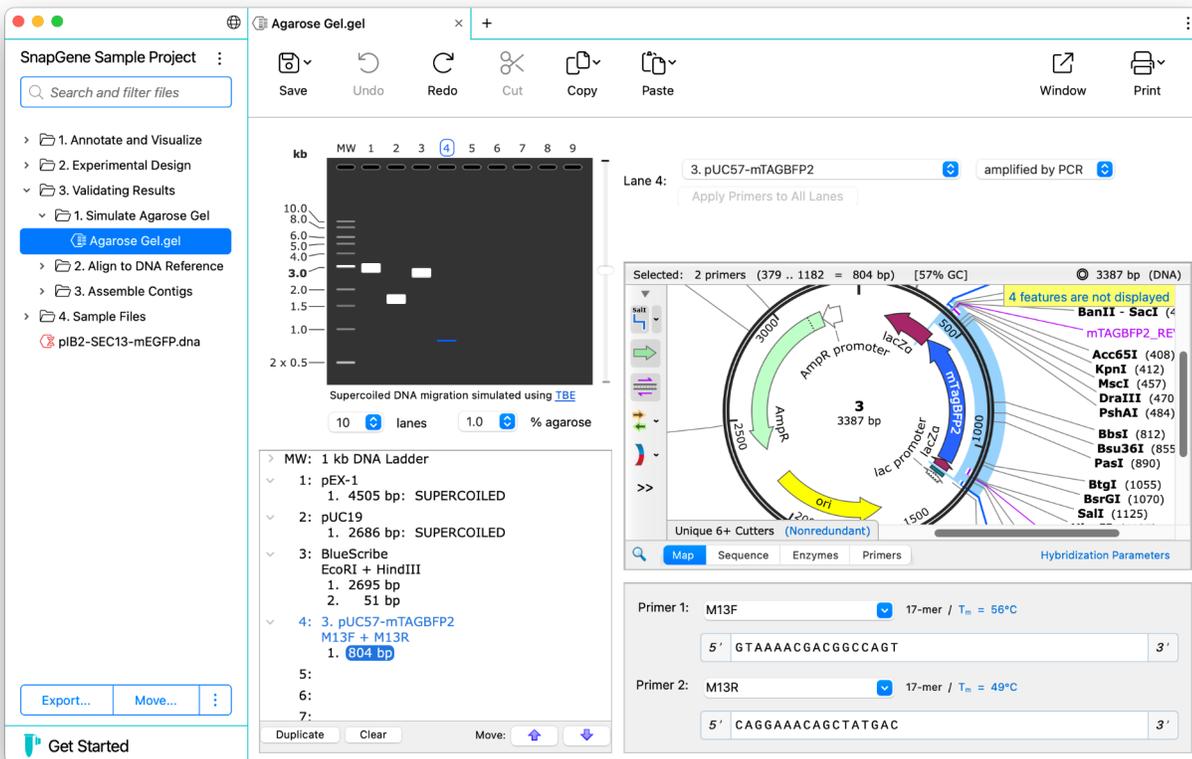
Below the gel on the left-hand side is a list of all the files in the gel. This gel contains four sequences that have been treated in different ways.

1. pEX-1
2. pUC19
3. BlueScribe
4. pUC57-mTAGBFP2

Both pEX-1 and pUC19 remain untouched and are supercoiled, circular sequences. BlueScribe has instead been digested by two enzymes: EcoRI and HindIII. Select lane 3 on either the gel or in the list below to show information about the BlueScribe sequence. Here you will see the EcoRI and HindIII enzymes are listed in the dropdown boxes on the right-hand side of the file. This is where digestion with restriction enzymes can be simulated for a sequence. Use the dropdown boxes to specify up to four enzymes to cut your sequence with. Selecting Apply Enzymes to All Lanes will apply your changes to all sequences in the gel, if they have not already been modified to instead be amplified by PCR.



Selecting lane 4 will bring up the pUC57-mTAGBFP2 sequence which has instead been amplified using the M13F and M13R primers.



The **amplified by PCR** option is not selected by default, instead the cut with setting used for restriction enzymes is the default option for agarose gels. If you wish to simulate PCR you will need to do this by selecting the **Cut with** dropdown menu and changing this to **amplified by PCR**. This will then bring up the dialog that will allow you to enter the primer information. If the primers are already annotated on your sequence, you can select these using the **Primer 1** and **Primer 2** dropdown boxes, otherwise the primer sequences can be entered manually using the text box below. If the primers bind, you will then see the fragment shown on your gel.