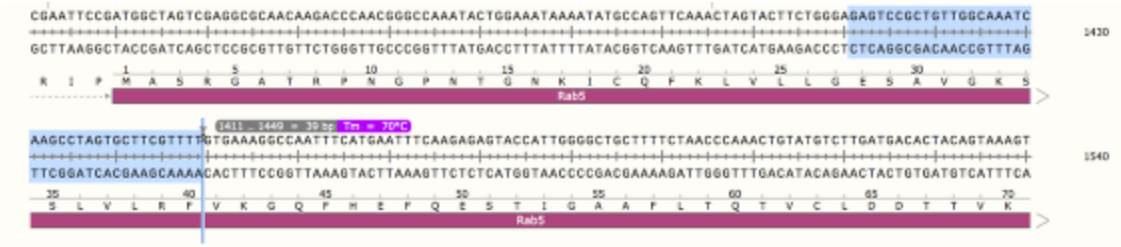
Mutagenesis

How do I simulate site-directed mutagenesis?

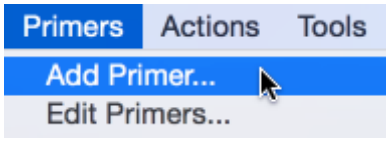
Identify the Mutagenesis Region



First, identify the site you want to change. About 15-18 bases of a mutagenic primer should anneal to the template on each side of the mutagenesis site.

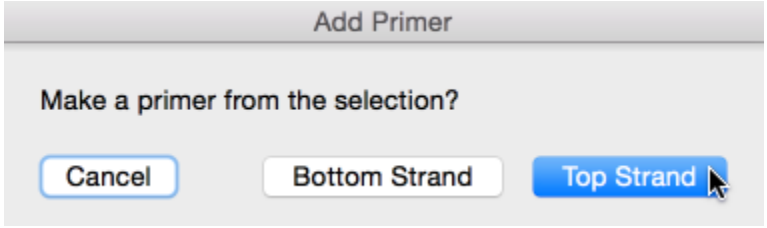
Click to place the cursor 15-18 bp upstream of the mutagenesis site, then drag to create a selection that ends 15-18 bp downstream of the mutagenesis site.

Open the Add Primer Dialog

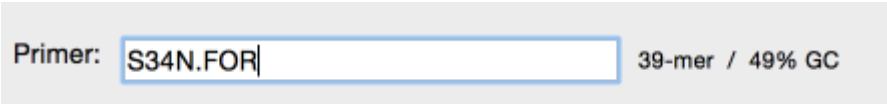


To open the Add Primer dialog, Click **Primers** → **Add Primer...** .

Create the Top-Strand Primer

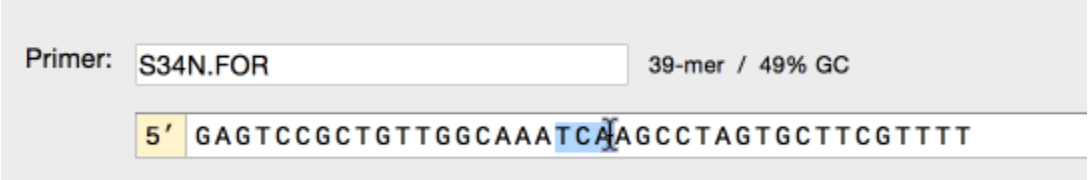


In the Add Primer dialog, click **Top Strand**.



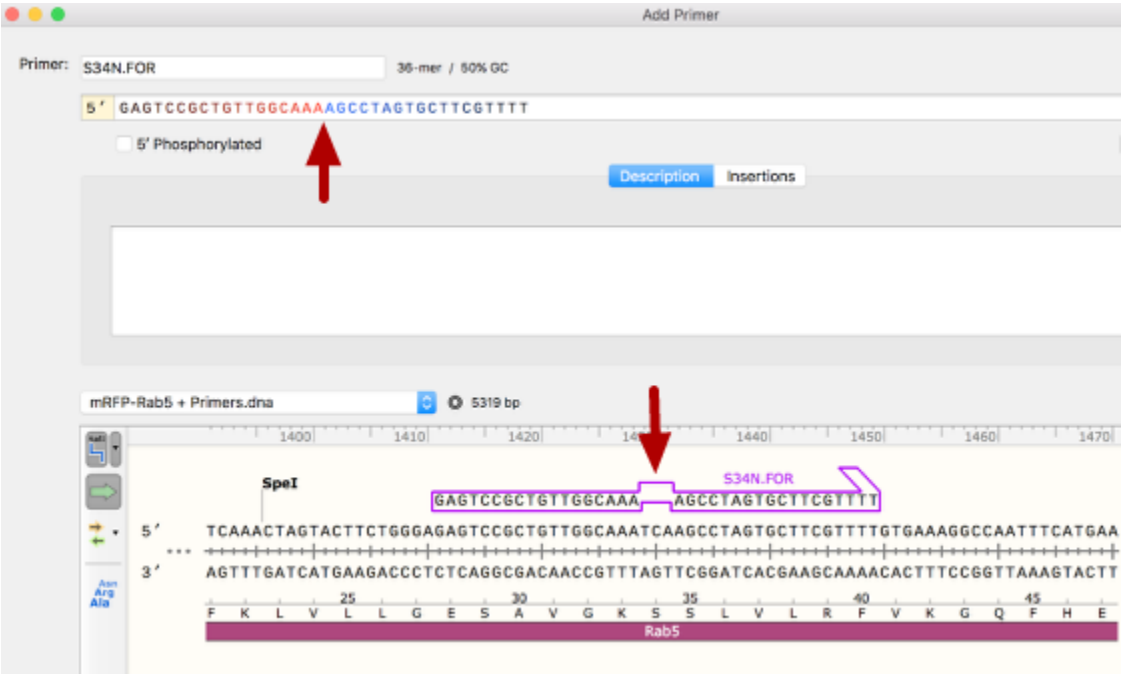
If desired, edit the primer name.

Choose the Mutagenesis Site



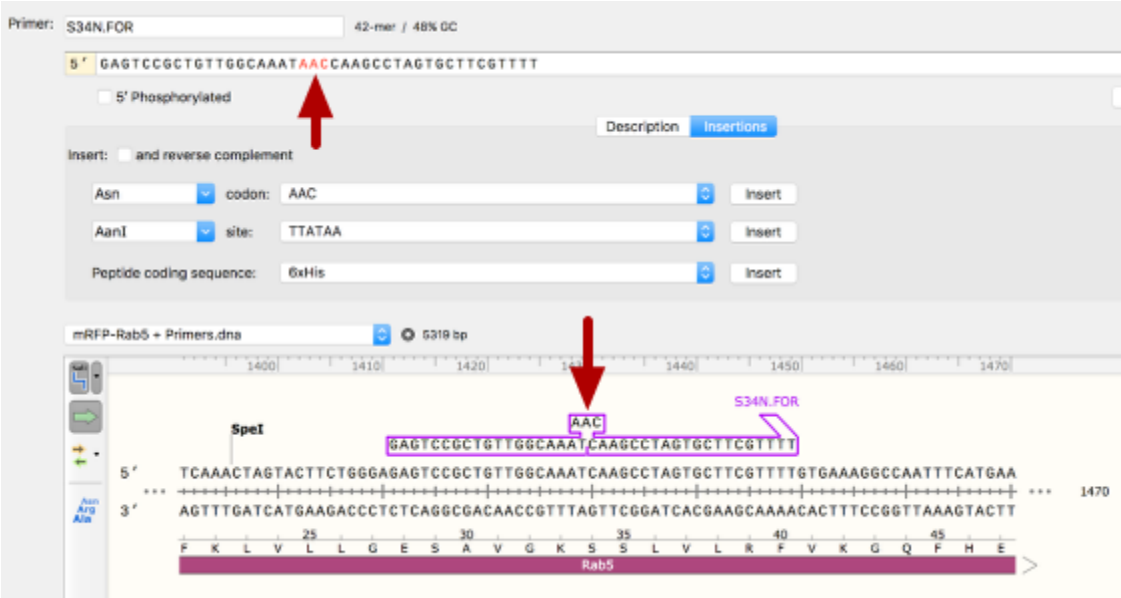
Choose the mutagenesis site in the text box. For a deletion or replacement, select the relevant bases. For an insertion, click to place a cursor between the flanking bases.

Modify the Top-Strand Primer by Deletion



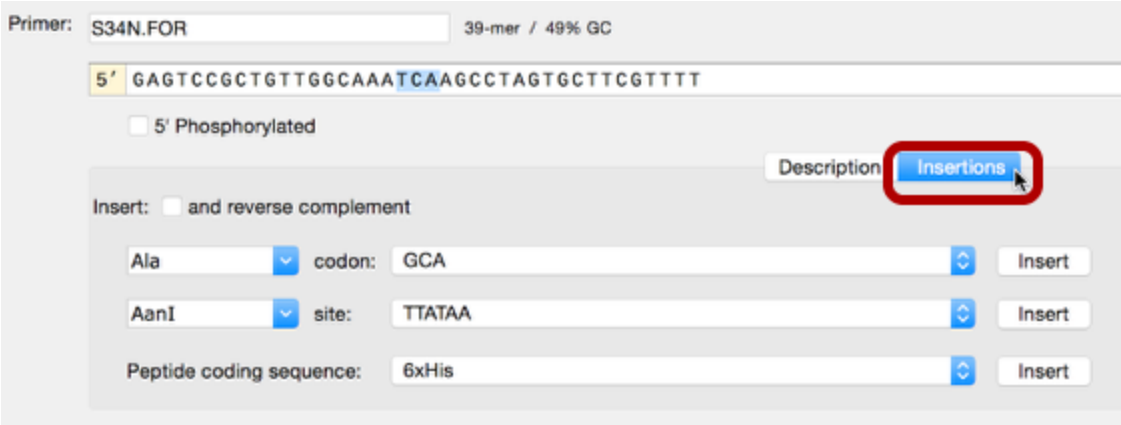
If the mutagenesis involves a deletion, the site of the deletion will be marked by red and blue color gradients in the text box and by an elevated placement in the primer display.

Modify the Top-Strand Primer by Insertion

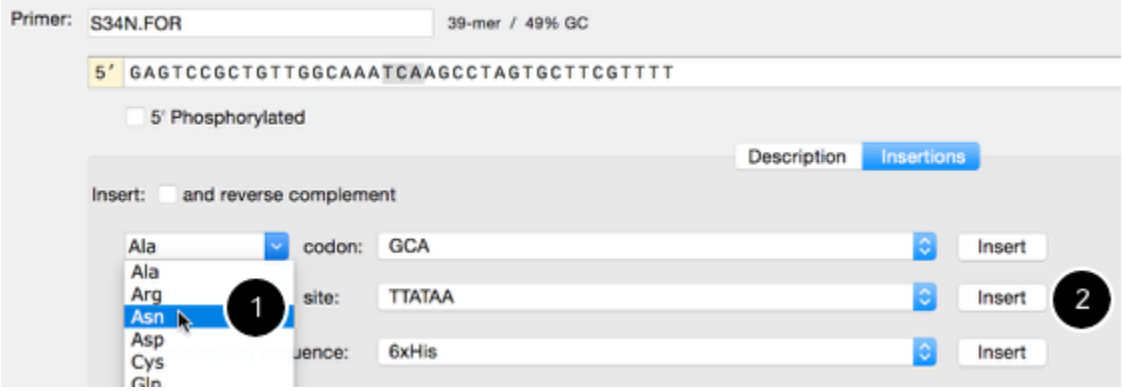


If the mutagenesis involves an insertion, the inserted bases will be marked by red color in the text box and by an elevated placement in the primer display.

Modify the Top-Strand Primer by Replacement



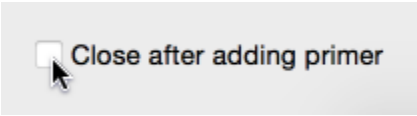
To modify the primer, select the bases to be replaced. Type the new bases. Alternatively, click **Insertions** to switch the tab, and then proceed as described below.



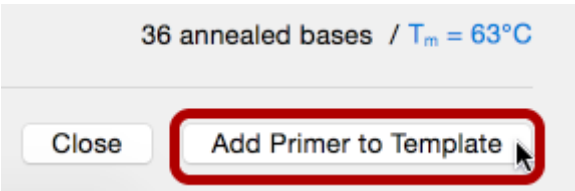
Replace the selected bases as follows. Choose a codon, site, or peptide coding sequence using the menu controls, and then click the appropriate **Insert** button.

The inserted bases will be marked by red color in the text box and by an elevated placement in the primer display.

Add the Primer to the Template

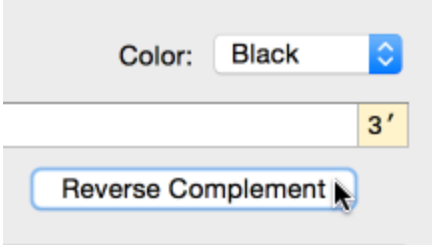


If a bottom-strand primer will be created as well, uncheck the box labeled **Close after adding primer**.

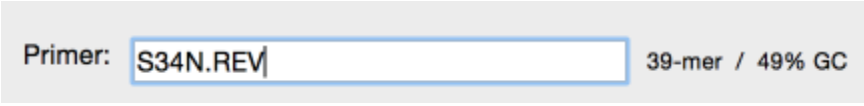


Click **Add Primer to Template**.

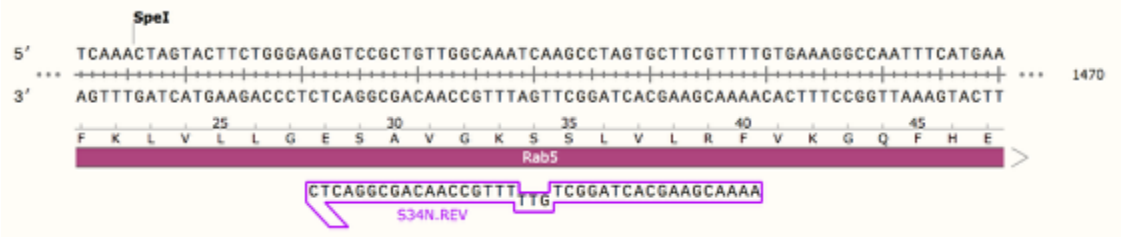
Create the Bottom-Strand Primer



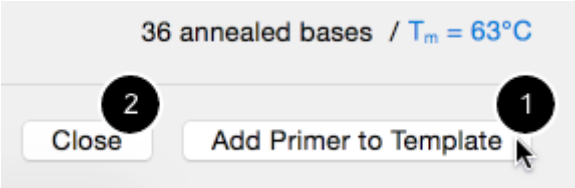
If a bottom-strand primer is desired, click **Reverse Complement**.



If desired, edit the primer name.

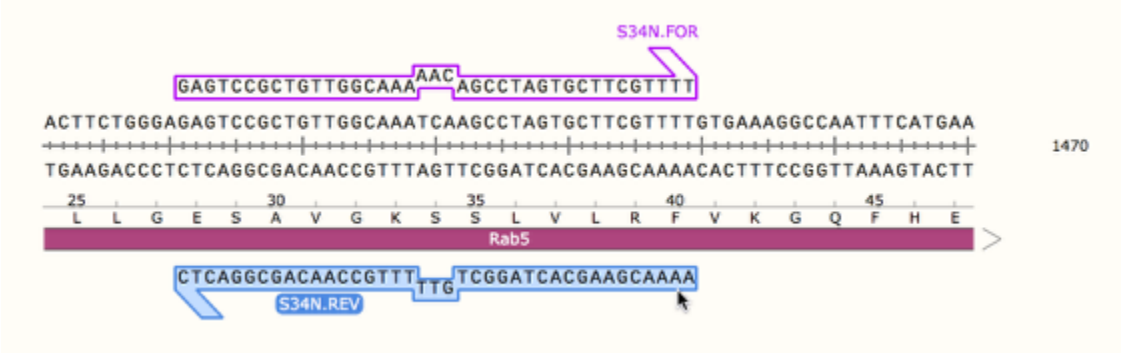


The bottom-strand primer will be displayed below the double-stranded template sequence.



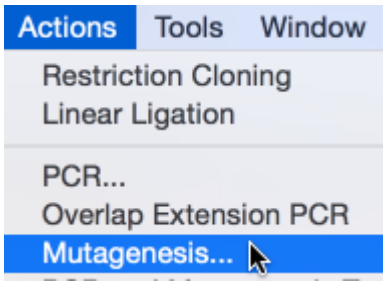
Click **Add Primer to Template**. Then click **Close**.

Select a Mutagenic Primer



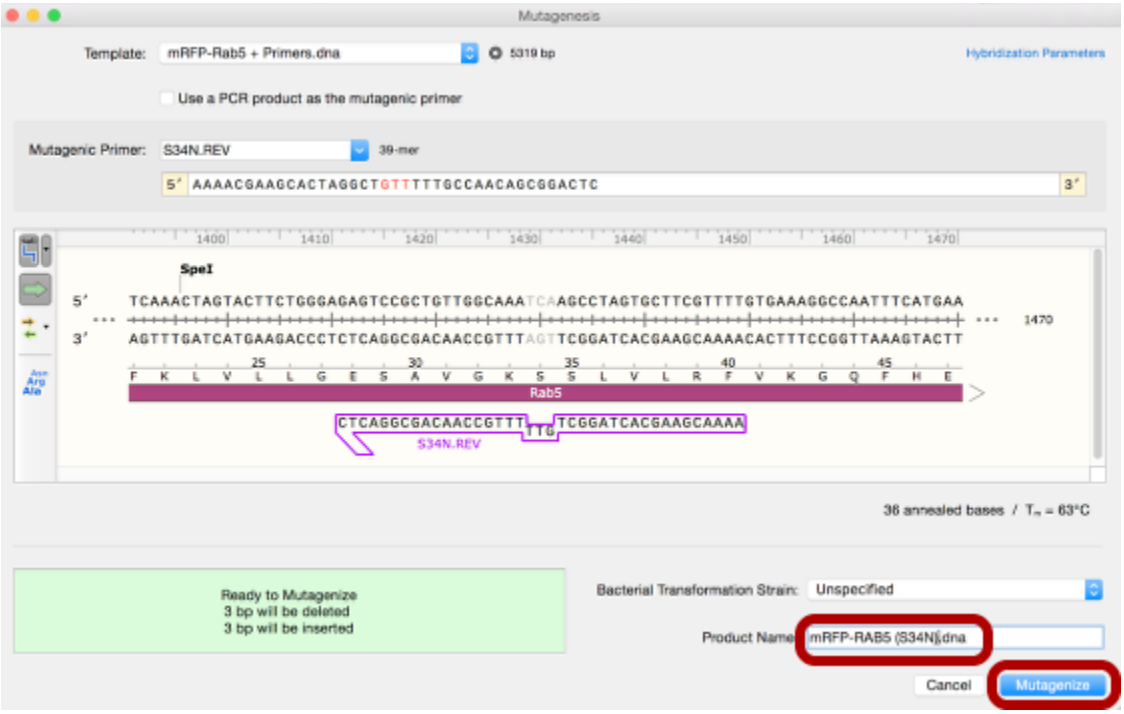
Select either a top-strand or a bottom-strand mutagenic primer.

Open the Mutagenesis Dialog



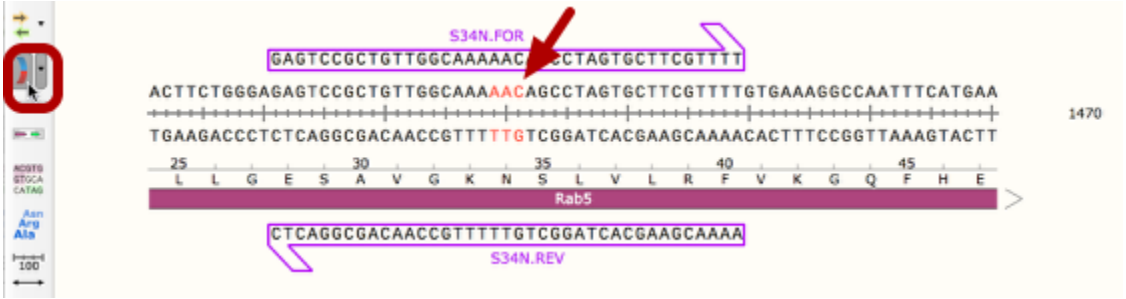
Click Actions → Mutagenesis... .

Edit the Plasmid Name



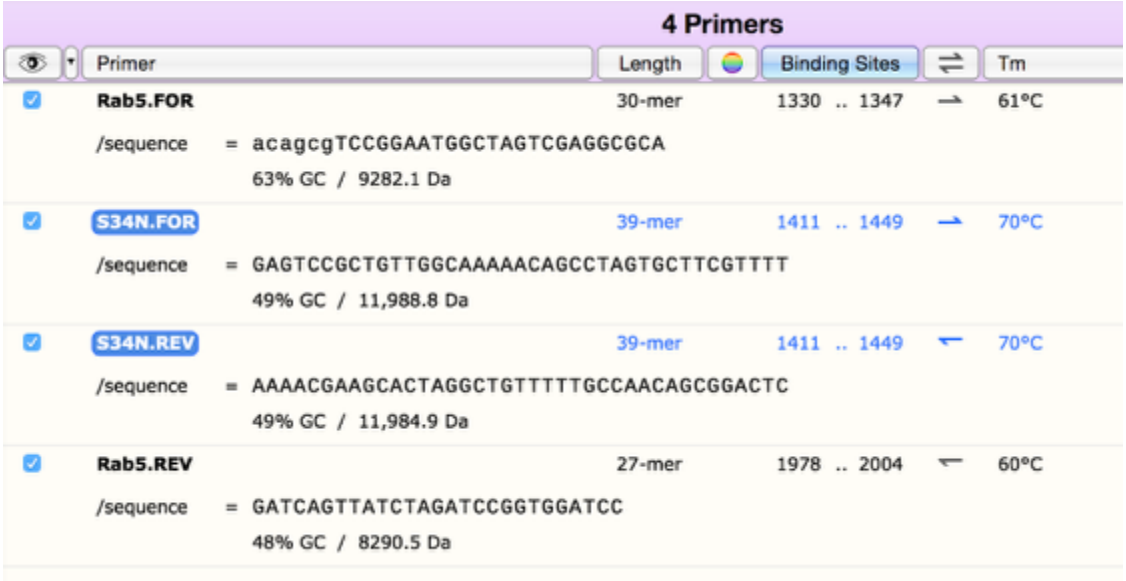
If desired, edit the name of the mutagenized plasmid, then click **Mutagenize**.

Show History Colors



To highlight the mutated region in the new sequence window, click the "Show history colors" button in the side toolbar. Inserted or replaced bases will be marked in red, or a deletion will be marked by red and blue color gradients.

Export the Primer Data



To export the mutagenic primers, switch to Primers view. Select the primers of interest, then click **Primers → Export Selected Primer Data**. See the "Export Primers" lesson for more details.