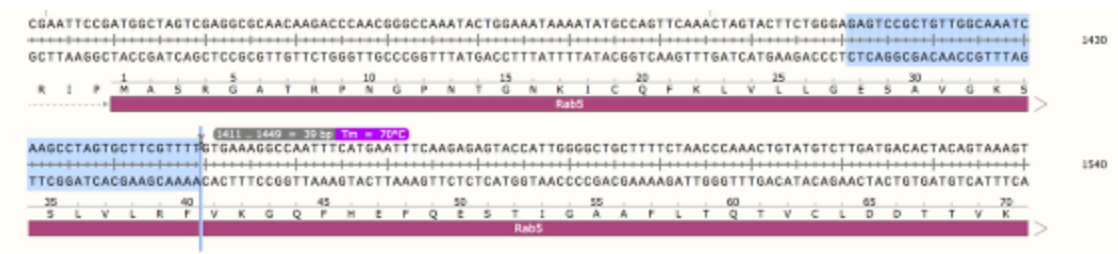




# Mutagenesis

How do I simulate site-directed mutagenesis?

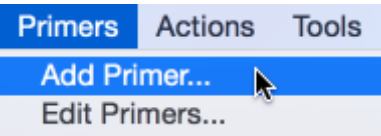
## Identify the Mutagenesis Region



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.

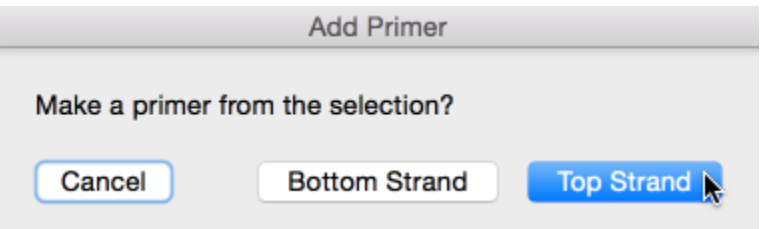
Click to place the cursor 15-18 bp upstream of the mutagenesis site, then drag to create a selection that ends 15-18 bp downstream of the mutagenesis site.

## 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.

Primer:  39-mer / 49% GC

If desired, edit the primer name.

## Choose the Mutagenesis Site

Primer:  39-mer / 49% GC

5'

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

Primer:  36-mer / 50% GC

5'

☐ 5' Phosphorylated

Description Insertions

mRFP-Rab5 + Primers.dna 5319 bp

SpeI

5' TCAAACTAGTACTTCTGGGAGAGTCCGCTGTTGGCAAATCAAGCCTAGTGCTTCGTTTTGTGAAAGGCCAATTCATGAA

3' AGTTTGATCATGAAGACCTCTCAGGCGACAACCGTTTATGTTTCGATCACGAAGCAAAACACTTCCGGTTAAAGTACTT

F K L V L L G E S A V G K S S L V L R F V K G Q F H E

Rab5

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

The screenshot shows a software interface for primer modification. At the top, the primer is labeled 'S34N.FOR' and is 42-mer with 48% GC. The sequence is 5' GAGTCCGCTGTTGGCAAAT**AAC**CAAGCCTAGTGCTTCGTTTT. A red arrow points to the 'AAC' insertion, which is highlighted in red. Below the sequence, there are options for '5' Phosphorylated' (unchecked) and tabs for 'Description' and 'Insertions'. Under the 'Insertions' tab, there are three sections: 'Insert: and reverse complement' (unchecked), 'Asn' with codon 'AAC' and an 'Insert' button, 'AanI' with site 'TTATAA' and an 'Insert' button, and 'Peptide coding sequence: 6xHis' with an 'Insert' button. At the bottom, a DNA map shows the primer binding to a sequence with a 'SpeI' site. The 'AAC' insertion is shown as a purple box. The map also shows the 'S34N.FOR' primer binding site and the 'Rab5' gene structure.

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

The screenshot shows a software interface for primer modification. At the top, the primer is labeled 'S34N.FOR' and is 39-mer with 49% GC. The sequence is 5' GAGTCCGCTGTTGGCAAAT**CA**AGCCTAGTGCTTCGTTTT. The 'CA' bases are highlighted in blue. Below the sequence, there are options for '5' Phosphorylated' (unchecked) and tabs for 'Description' and 'Insertions'. The 'Insertions' tab is selected and highlighted with a red circle. Under the 'Insertions' tab, there are three sections: 'Ala' with codon 'GCA' and an 'Insert' button, 'AanI' with site 'TTATAA' and an 'Insert' button, and 'Peptide coding sequence: 6xHis' with an 'Insert' button.

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.



Primer: S34N.FOR 39-mer / 49% GC

5' GAGTCCGCTGTTGGCAAATCAAGCCTAGTGCTTCGTTTT

☐ 5' Phosphorylated

Description Insertions

Insert: ☐ and reverse complement

Ala codon: GCA Insert

Ala site: TTATAA Insert

Arg site: TTATAA Insert

Asn site: TTATAA Insert

Asp site: TTATAA Insert

Cys site: TTATAA Insert

Gln site: TTATAA Insert

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.

Primer: S34N.FOR 39-mer / 49% GC Color: [dropdown]

5' GAGTCCGCTGTTGGCAAATCAAGCCTAGTGCTTCGTTTT

☐ 5' Phosphorylated

Description Insertions

Insert: ☐ and reverse complement

Asn codon: AAG Insert

AanI site: TTATAA Insert

Peptide coding sequence: 6xHis Insert

mRFP-Rab5 + Primers.dna 5319 bp

SpeI

5' TCAAAC TAGTACTTCTGGGAGAGTCCGCTGTTGGCAAATCAAGCCTAGTGCTTCGTTTTGTGAAAGGCCAATTTTCATGAA

3' AGTTTGATCATGAAGACCTCTCAGGCGACAACCGTTTAGTTCGGATCACGAAGCAAAACACTTTCGGTTAAAGTACTT

Rab5

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

☐ Close after adding primer

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



36 annealed bases /  $T_m = 63^\circ\text{C}$

Close Add Primer to Template

Click Add Primer to Template.

## Create the Bottom-Strand Primer

Color: Black

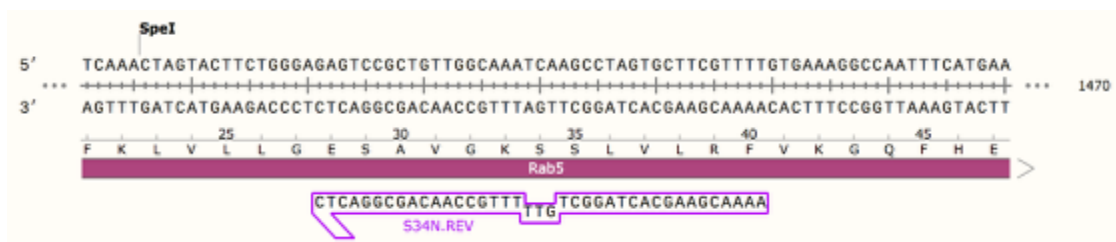
3'

Reverse Complement

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

Primer: S34N.REV 39-mer / 49% GC

If desired, edit the primer name.



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

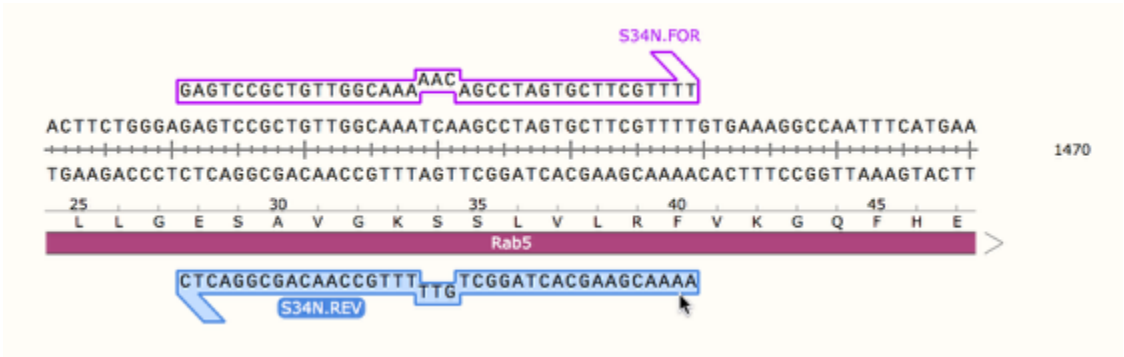
36 annealed bases /  $T_m = 63^\circ\text{C}$

2 Close 1 Add Primer to Template

Click Add Primer to Template. Then click Close.

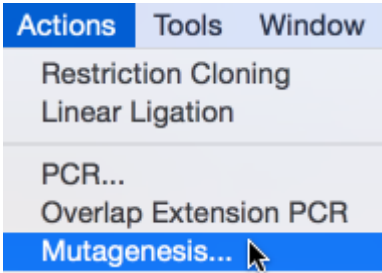


# Select a Mutagenic Primer



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

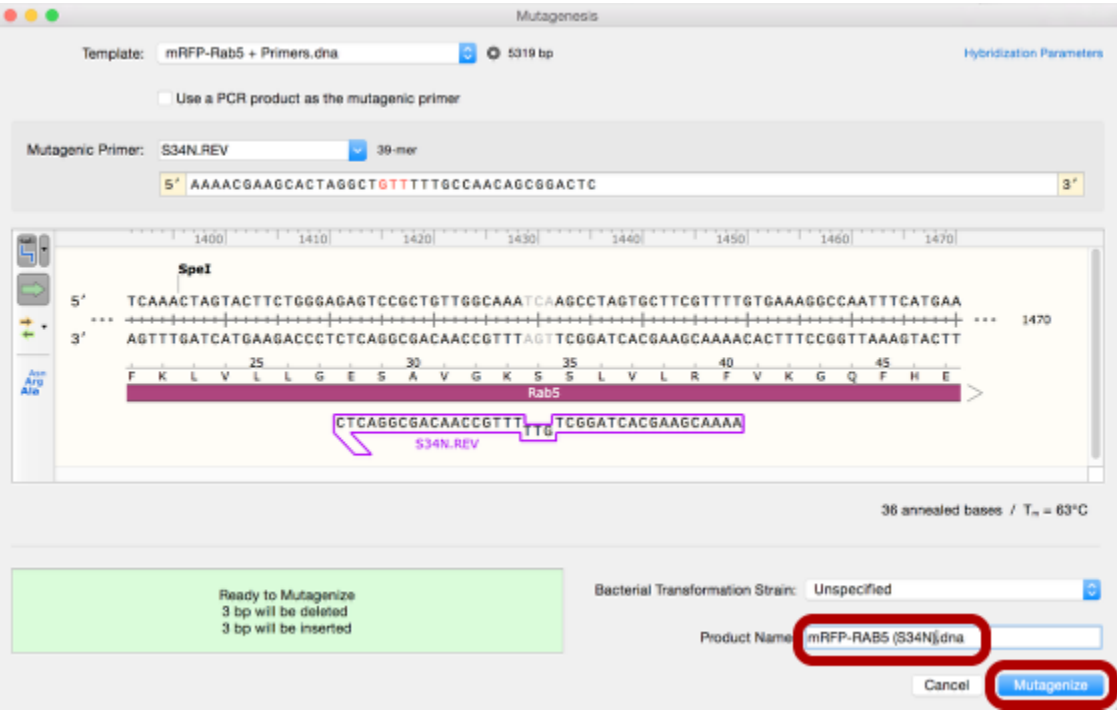
# Open the Mutagenesis Dialog



Click Actions → Mutagenesis... .

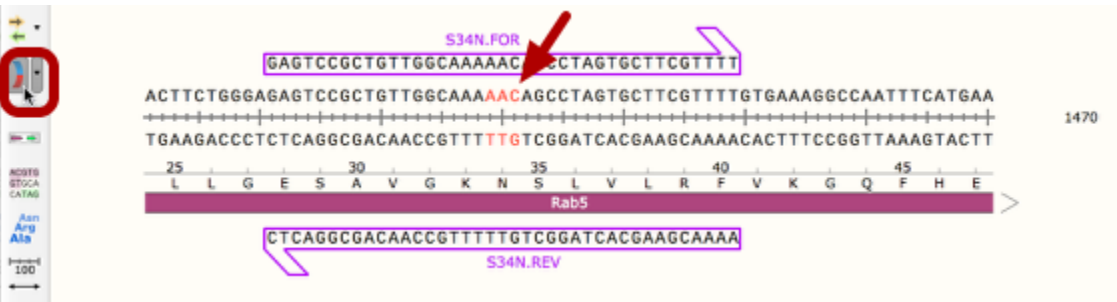


# Edit the Plasmid Name



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

| 4 Primers                           |                                                                                                |        |  |               |  |      |
|-------------------------------------|------------------------------------------------------------------------------------------------|--------|--|---------------|--|------|
|                                     | Primer                                                                                         | Length |  | Binding Sites |  | Tm   |
| <input checked="" type="checkbox"/> | <b>Rab5.FOR</b><br>/sequence = acagcgTCCGGAATGGCTAGTCGAGGCGCA<br>63% GC / 9282.1 Da            | 30-mer |  | 1330 .. 1347  |  | 61°C |
| <input checked="" type="checkbox"/> | <b>S34N.FOR</b><br>/sequence = GAGTCCGCTGTTGGCAAAACAGCCTAGTGCTTCGTTTT<br>49% GC / 11,988.8 Da  | 39-mer |  | 1411 .. 1449  |  | 70°C |
| <input checked="" type="checkbox"/> | <b>S34N.REV</b><br>/sequence = AAAACGAAGCACTAGGCTGTTTTTGCCAACAGCGGACTC<br>49% GC / 11,984.9 Da | 39-mer |  | 1411 .. 1449  |  | 70°C |
| <input checked="" type="checkbox"/> | <b>Rab5.REV</b><br>/sequence = GATCAGTTATCTAGATCCGGTGGATCC<br>48% GC / 8290.5 Da               | 27-mer |  | 1978 .. 2004  |  | 60°C |

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.