

CrisprBuildr 1.0 | User manual

CrisprBuildr is a web-based tool to aid users in the generation of cloning maps for Crispr-based genome engineering experiments in *Drosophila melanogaster*. The application allows users to delete or tag genes of interest.

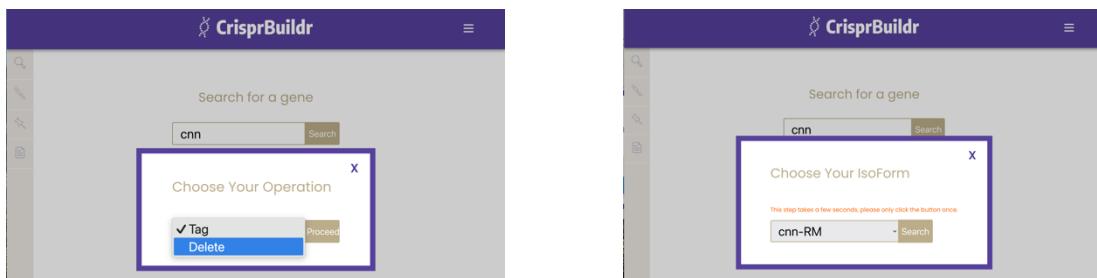
1. Gene deletions

1.1 Search for your gene of interest.

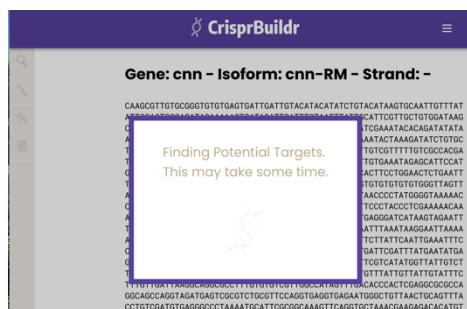
Type the name of your gene of interest into the search field. The application accepts Flybase gene symbols but not full names (e.g cnn instead of centrosomin). Instead of gene symbols, genes can also be searched by Flybase IDs (FBgn's).



1.2 From the pull-down menu, select the 'Delete' function and select the isoform of interest.



1.3 Pick cutting sites. After selecting the desired gene isoform, the application will search for Cas9 cutting sites.



- 1.4** Select efficient cutting sites close to the N-terminus. The menu on the left lists potential cutting sites. The selected cut site is highlighted in yellow in the sequence window. The start codon is highlighted in green. Clicking the target site in the menu on the left selects and locks-in the target site.

Click here to select and lock-in the chosen cut site on the N-terminus.

The screenshot shows the CrisprBuildr interface. On the left, a sidebar titled "N Terminal Targets" lists several potential cutting sites with their efficiency scores. One site, "TACCTCACTAAACTACTGT", has a yellow background, indicating it is selected. The main sequence window displays the "cnn-RM" construct. In the sequence, the start codon "ATG" is highlighted in green, and the selected cutting site "TACCTCACTAAACTACTGT" is highlighted in yellow. Arrows point from the text instructions to the corresponding elements in the interface.

The selected site is highlighted in the sequence.

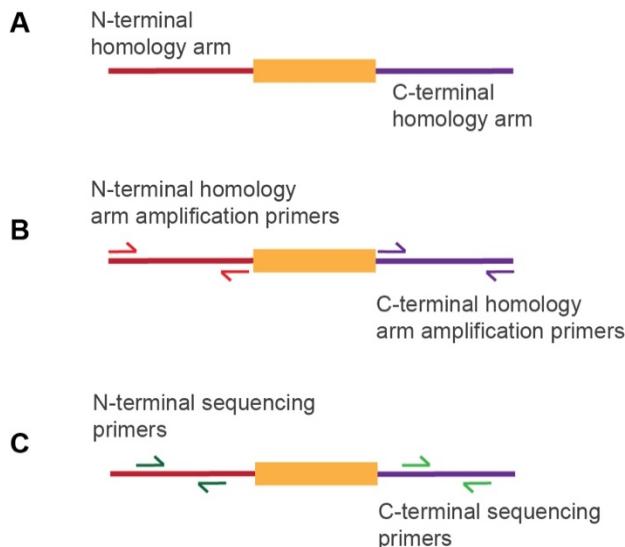
- 1.5** Select efficient cutting sites close to the C-terminus. To view the highlighted sequences, scroll down in the sequence window.

Click here to select and lock-in the chosen cut site on the C-terminus.

The screenshot shows the CrisprBuildr interface. On the left, a sidebar titled "C Terminal Targets" lists several potential cutting sites with their efficiency scores. One site, "TCGGTTAACTCGTCAATG", has a yellow background, indicating it is selected. The main sequence window displays the "cnn-RM" construct. In the sequence, the selected cutting site "TCGGTTAACTCGTCAATG" is highlighted in yellow. A large portion of the sequence is visible at the bottom of the window. Arrows point from the text instructions to the corresponding elements in the interface.

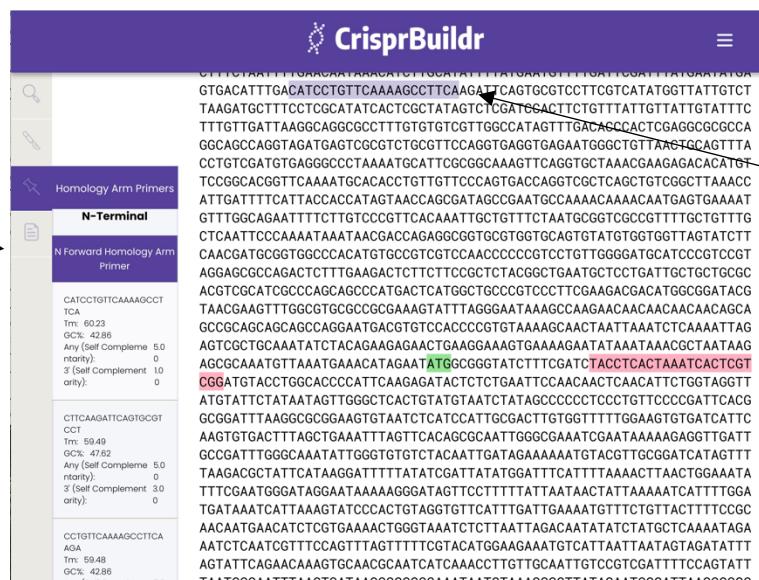
The selected site is highlighted in the sequence.

- 1.6** Design amplification and sequencing primers for the homology arms. **(A)** In this step, users will pick primers to amplify a N-terminal and a C-terminal homology arm. These primers are used to amplify and then sequence the homology arms to ensure that the target locus of the host strain corresponds to the published sequence. In each instance, 4 primers are selected: **(B)** Forward and reverse amplification primers for the N-terminus and C-terminus. **(C)** Forward and reverse sequencing primer for the N-terminus and C-terminus. It is important to note that users might want to design their own final cloning primers based on their respective needs.



The images below only show the **primer selection for the N-terminus**. The process is the same for the C-Terminus

Click here to select and lock-in forward amplification primers for the N-terminal homology arm.



Selected forward amplification primer


CrisprBuildr

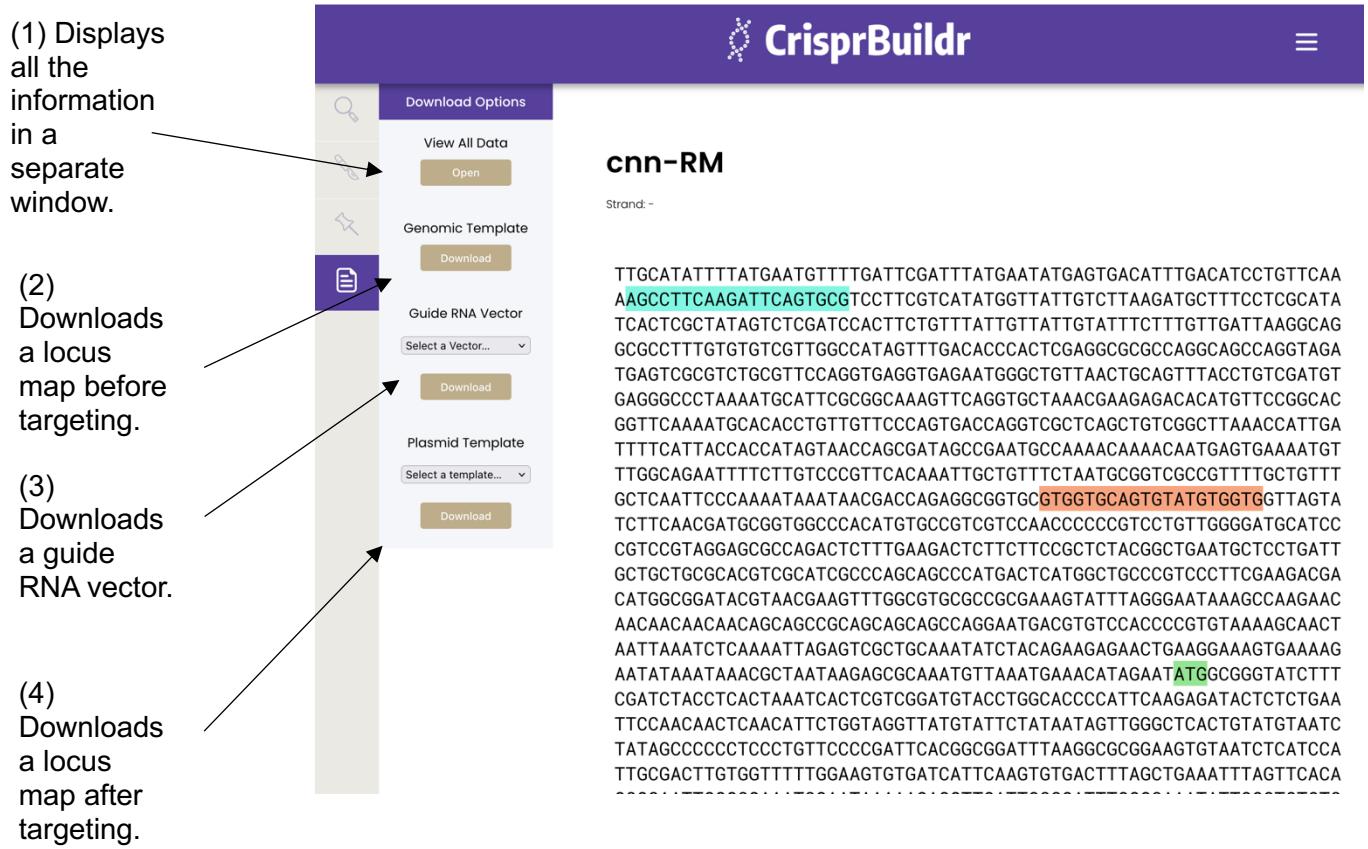
Selected forward amplification primer

Selected reverse amplification primer

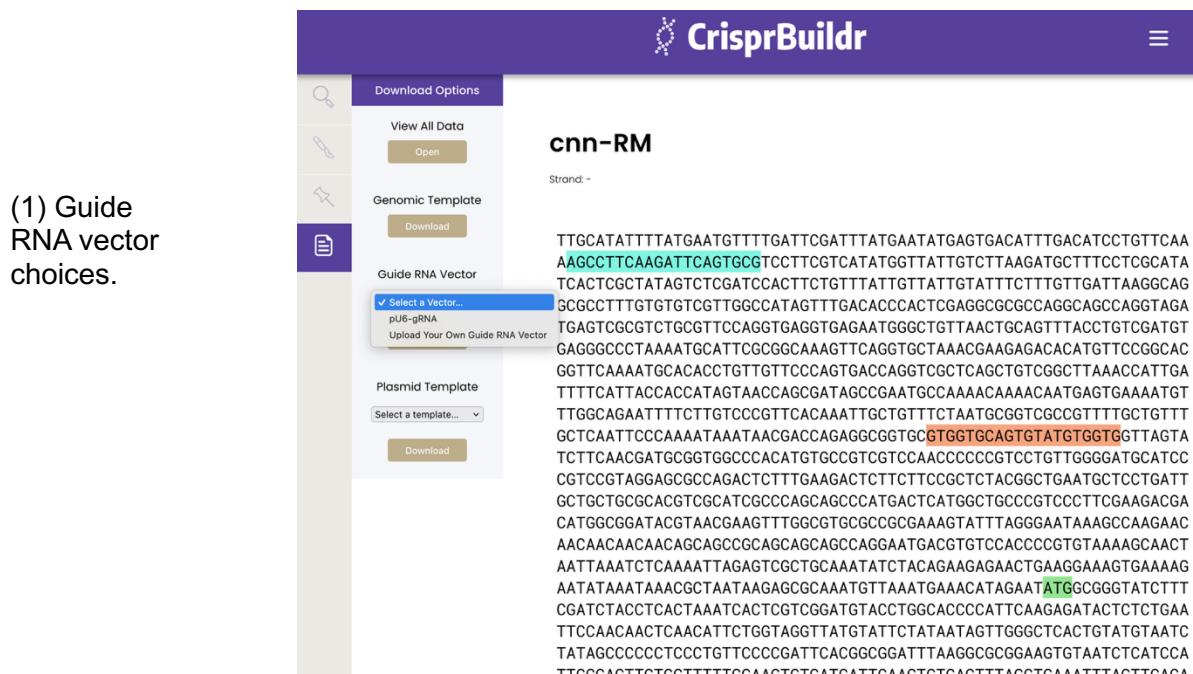
Selected forward amplification primer

Selected forward sequencing primer

- 1.7** Choose your cloning maps. In the last step, users can (1) view and print all the data, (2) download the genomic template without any alterations, or (3) pick a deletion vector and download a locus map that shows the chosen deletion vector after a hypothetical successful targeting event.



For the guide RNA and plasmid template vectors, users can either chose from a small selection of vectors or upload their own guide RNA or plasmid template vector. **IMPORTANT:** if users chose their own guide RNA or plasmid template vectors, the corresponding vectors need to be modified to be compatible with CrisprBuildr. **See section 2.6** on how to modify the vectors accordingly. These modifications only need to be done once. See also 1.8 for steps to upload your own vector.



cnn-RM

Strand: -

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TTGCATATTTATGAAATGTTGATTGATTGATTGAATATGAGTGACATTGACATCTGTTCA
AAGCCTTCAAGATTCACTGGCTCCTCGTCATATGGTTATTCCTTAAGATGCTTCCCTGCCATA
TCACTCGCTATAGTCGATCCACTCTGTGTTATGGTTATTCCTGTTGATTAAGGCAG
GCCCTTGTGTCGTTGCCCATAGTTGACACCCACTCGAGGCCGCCAGGCCAGCAGTAGA
TGAGTCGCTGTCGTTCCAGGTGAGGTGAGAAATGGGCTTAACGTGAGTTACCTGTCGATGT
GAGGGCCTAAATGATTGGCAGCAAGTTCAAGGTGAAACGAAGACACATGTTCCGGCAC
GGTCAAATAGCACCTGTTCCCGTGAACAGGTCGCTCAGCTGTCGGCTTAACCATTG
TTTCAATTACACCATAGTAACACAGCATGGCAATGCCAAACAAACAAATGAGTGAATG
GCAAGAATTCTGTCCGGTACAATTGCTGTTCAATGCCGTGCCGTTTGTGTT
CAATTCCAAAATAAAATAACGACAGAGGGGTGCGTGGTCAAGTGTGTTGAGGATGATCC
TCAACGATGCCGTCGCCCCACATGTCGCTGTCACCCCGTCTGTTGGGATGATCC
CGCTGCTGCGCACGTCGATGCCAGGCCAGCTGACTCATGCGTCCCCTGAGACGA
CATGGCGGATACGTAACGAAGATTGGCGTGCAGCGAAGATTTAGGAATAAGCAAGAAC
AACACAAACACAGCAGCCCCAGCAGCAGGAATGACGTGTCACCCCGTGTAAAGCAACT
AATTAATCTCAAATTAGCTGCTGCAAAATCTACAGAAAGAGAAACTGAAGGAAGTGAAGA
AATATAATAAACGCTAATAAGGCCAAATGTTAAATGAAACATAGAATATGCGGTATCTT
CGATCTACCTACTAAATCACTGTCGGATGTCCTGGCACCCATTCAAGAGATACTCTGAA
TTCCAACAACCACTTCTGGTAGGTTATGTTCTATAATAGTTGGCTACTGTATGTAATC
TATAGCCCCCTCCCTGTTCCCGATTCAAGGCCGATTAAAGGCCGAAAGTGAATCTCATCC
TTGCAGTTGTTGGGTTGGAGAGTGTGATCATTCAGTGTGAACTTGTGACTTTAGTT

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1.8 Choose your own vectors:

Here only shown for the Guide RNA vector upload (after modifications – see 2.6 - have been done). The same steps apply for the Plasmid Template vector.

cnn-RM

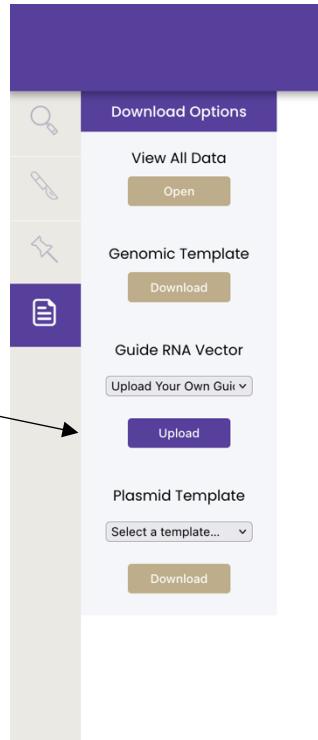
Strand: -

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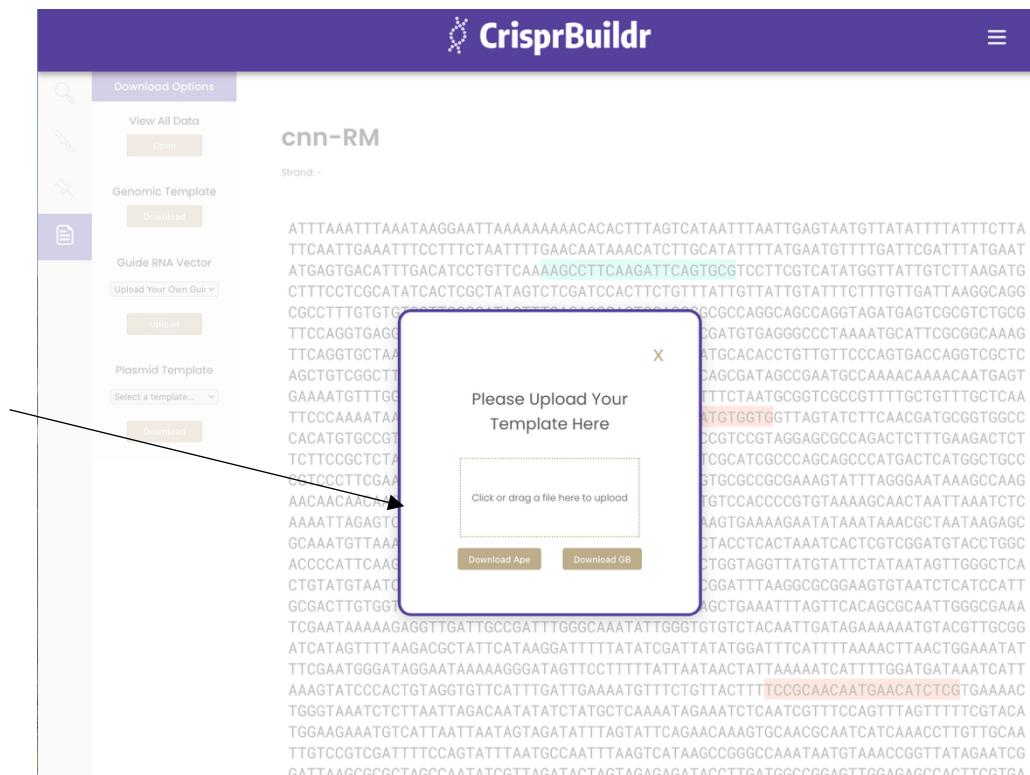
ATTTAAATTTAAATAAGGAATTAAGGACACACTTGTATAATTAAATTGAGTAATGTTATTTCTTA
TTCAATTGAAATTCTCTTAATTTGAAATAAACTCTGCAATTGTTATGGTTATGTTGATTGATTTGATTG
ATGAGTGCATTTGACATCTGTCATAGTCGATCCACTCTGTGTTATGGTTATGTTGATTAAGGCAGG
TTTCTCGCATATCACTCGCTATAGTCGATCCACTCTGTGTTATGGTTATGTTGATTAAGGCAGG
GCTTGTGTCGTTGGCCTAAGTGTGACGCCCCACTCGAGGCCGCCAGGCCAGGTAGATGAGTCGCCCTGCG
.TCCAGGTGAGGTGAGAAATGGCTTTAACTGCACTTACCTGTCGATGTCGAGGGCCTTAAATGCAATTCCGGCAAAAG
TTCAGGTGCTAACAGAAGAGACATGTCGCGCACGGTCAAATGCAACCTGTTCCAGTGACCGGGTGCCT
AGCTGCGCTAACACATTGTTTCAATTACCCACATGCAACCGGATAGCCGAATGCCAAACAAACAAATGAGT
GAAATGTTGGCAGAACATTCTGTCGGCTTCAAAATTGCTGTTCAATGCGGTGCGGTGTTGCTGTTGCTCAA
TTCCCAAAATAAAATAACGACAGAGGGGGTGGCTGTCGAGTGTGTTGAGGAAATGCTTCAACGATGCCGTTGCC
CACATGCGCGTCAACCCCGTCTGTTGGGATGTCATGCCGTCAGGAGGGCCAGACTCTTGAAGACTCT
TCTCCCGTCACTCGCTGAATGCTCTGTCGCCAGTCGCCATGCCCAAGGCCATGACTCATGCC
CGTCCCTGCAAGACGACATGCCGATAGCTAACGAAGTTGGCTGCCGGAAAGTTAGGAATAAGGCCAG
AAACAAACAAACACAGCAGCCCCAGCAGCAGCAGGAATGACGTGTCACCCGGTAAAGCAACTAATTAACGCTAATAAGAGC
AAATTAATGAGTCGCTGCAAAATCTACAGAATATGCGGTATCTTCACTCTACTAAATCACTGTCGGATGACCTGCC
ACCCCATTAAGAGATACTCTGTAATTCCAACAACTCAACATTCTGTAAGGTATGTTCTATAATAGTTGGCTCA
CTGTTGTTAATCTATAAGCCCCCTCTGTCGCGGATTCAAGGGGATTTAGGCGGAGTGTAACTCATCCATT
GGGACTGTGTTGGGTTGGAGGTGATCTGCAATTAGCTGAAAGTGTGAAATTAGTCAGCAGGCCATGGGGCAA
TCGAATAAAAGGGTTGATTGGCATTGGCAAAATTTGGGTTGTCACATTGATAGAAAAAATGTAACGTTGCGG
ATCATAGTTAAAGCCCTATTCTACAGGATTTATGATGTTTCAATTGATAGAAAAAATGTAACGTTGCGG
TTCAATGGGATAAGGAATAAAAGGGATAGTTCTTTTAAATAATTAACATTGTTGGGATGATAATCATT
AAAGATCCCAGTAGGTGTCATTGTTGAAATGTTCTGTTACTTTCCGCAACATGCAACATCTCGTGGAAAC
TGGTAATCTTAAATTAGACAATATCTATGCTCAAATAGAAATCTCAATGCTTCCAGTTAGTTTCTGACA
TGGAAGAAATGCTCAATTAAATAGTAGATATTAGTATTGAGAACAGCAATCTCAACCTTGTGCAA
TTGTCGCTGATTTCAGTTGAGGTTAGTGCAGTAACTGAGGAGTACCTGATGGCCGGAGTTGAGAGCCACTCTGTA
GATTAAGCCGGTAGCCAAATCTGTAAGTACTAGAGAGATACCTGATGGCCGGAGTTGAGAGCCACTCTGTA
CTGGAGCCCCCTGGGAATGGTCGATCAAGTTGGCTGAAAGTGTGAGGAGTACTCCGGTTGCTGTTCTGTTCCAGGG
ATTCCCTGCTGGGATTGGTCGATCAAGTTGGCTGCAAGTGGCCACTCTACATACGTTGATGTCGGAAATCGGAAT
TACCCCTGCTGGGATTGGTCGATCAAGTTGGCTGCAAGTGGCCACTCTACATACGTTGATGTCGGAAATCGGAAT
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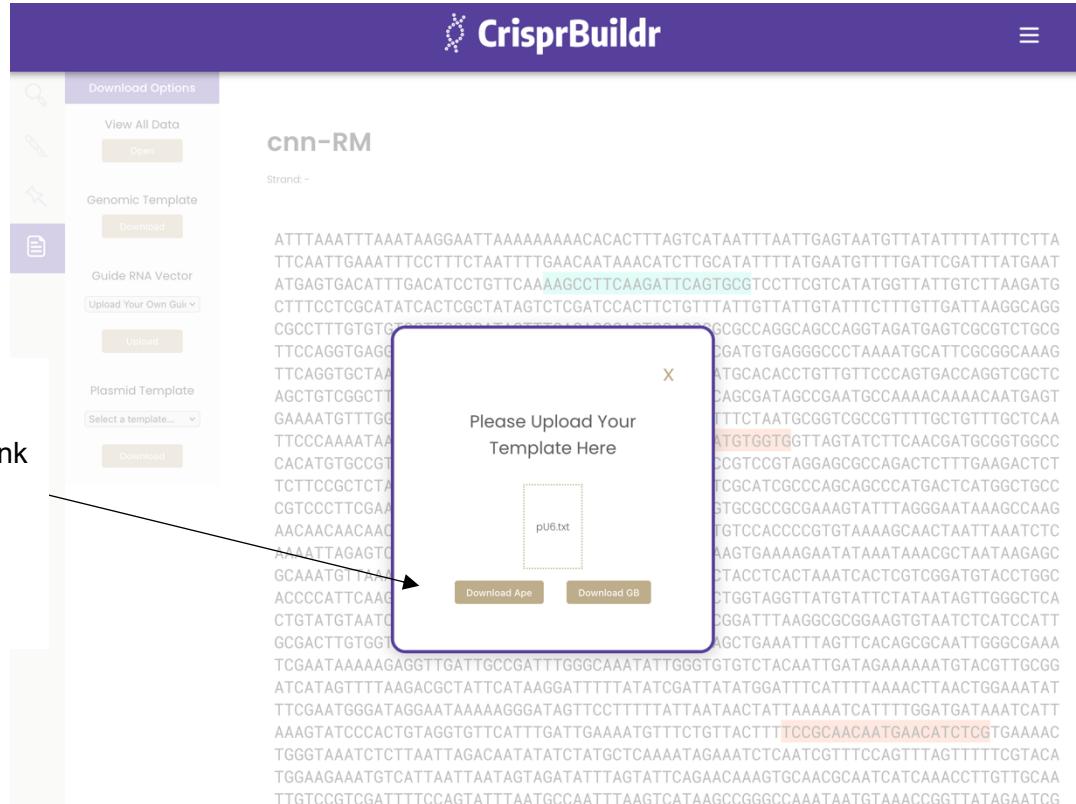
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(2) Hit Upload. This will open a new window.



(3) Either click the dashed square to select your file or drag it into the box.

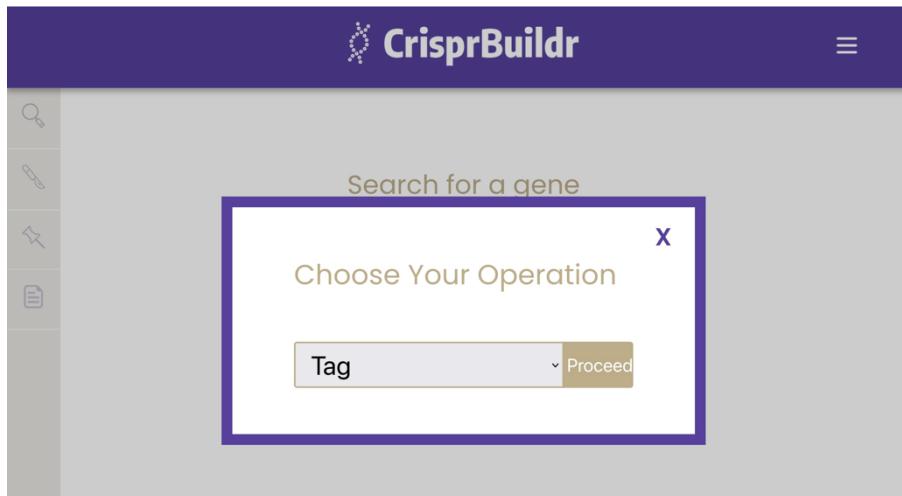




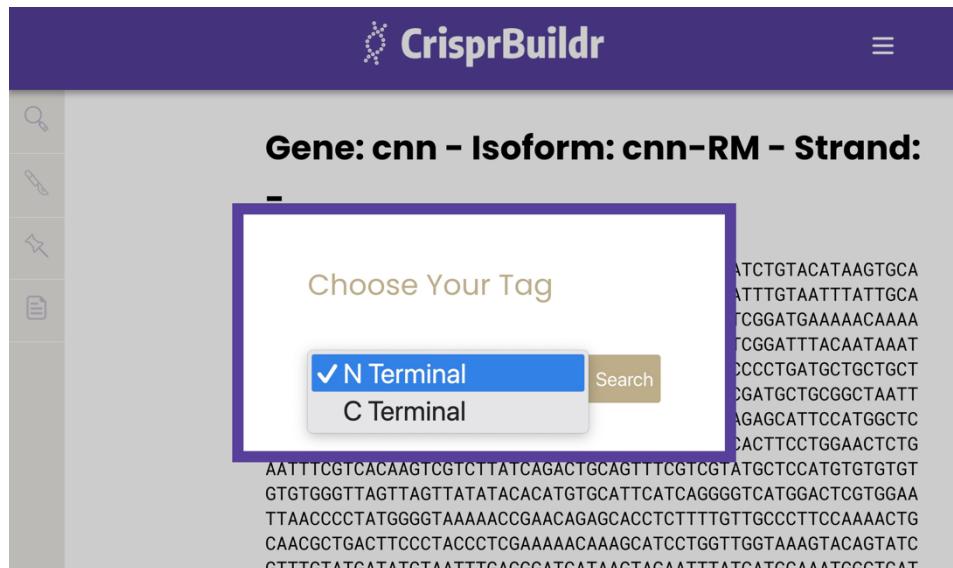
(4) Hit ‘Download BG or ‘Download Ape’. The Genbank (GB) file can be opened in most cloning applications.

2. Gene tagging

- 2.1 Pick the tagging operation (below) and isoform (not shown).



- 2.2 Chose the tag location from the drop-down menu. Only N-terminal selection is shown hereafter.



- 2.3 Select the desired cut site. For N-terminal tagging, only 1 cut site close to the start codon is needed. For C-terminal tagging, the selections are close to the stop codon (not shown).

The menu on the left lists several cut sites.

CrisprBuildr

▼ Select Cut Site

- TACCTCAACTAAATCACTC GTCGG
Efficiency: 3.66153
Strand: -
Off Targets: 0
- AAATGAAACATAGAATAT GCGCG
Efficiency: 4.22992
Strand: -
Off Targets: 0
- GTTAAATGAAACATAGAAT ATGG
Efficiency: 5.43676
Strand: -
Off Targets: 0
- ATCCGACGAGTGATTAG TGAGG

GCCCTAAAAATGCATTGCGGAAAGTTAGGTGCTAACAGAAGAGACACATGTTCCGGC
ACGGTCAAATGCACACCTGTTGTCAGTGACCAGGTCGCTCAGCTGTCGGCTTAA
ACCATTGATTTCATTAACCCATAGTAACCAGCGATAGCGAATGCCAAACAAAACA
ATGAGTGAAGAATGTTGGCAGAATTTCCTGTCAGCTCAGCTGTCGGCTTAA
CGGTGCGCTTGTGCTCAATCCAAAATAAACGACAGAGGGCGGTGCG
TGGTGCAGTGTATGTTGGTTAGTATCTCAACGATGCGTGGCCACATGTCGCG
GTCAACCCCCCGTCTGTTGGATGATCCCGTCCGTAGGAGGCCAGACTCTTGA
AGACTCTCTCGCTCACGGCTGAATGCTCTGATTGCTGCGCACGTCGATCG
CCCAGCAGCCATGACTCATGGCTGCCGTCCCTCGAAGACGACATGGCGATACGTA
ACGAAGTTGGCGTGCGCCGCAAAGTAGTTAGGAATAAGCCAAGAACACAAC
AACAGCAGCCGAGCAGCAGCAGGAATGACGTGTCACCCGTAAAAGCAACTAAT
TAAATCTCAAATTAGAGTCGCTCAAATATCTACAGAAGAGAACTGAAGGAAGTGA
AAGAATATAAAACGCTATAAGAGCGCAAATGTTAATGAAACATAGAATATGGCG
GGTATCTTCGATCTACCTCAACTAACACTCGTCGATGTCAGTGCACCCATTCAA
GAGATACTCTGAATTCCAACAACACTCAACATTCTGGTAGTTATGATTCTATAATAG
TTGGCCTACTGTATGTAATCTATAGCCCCCTCCCTGTTCCCGATTCAACGGGATT
TAAGGCGCGGAAGTGTAACTCATCATTGCGACTTGTGGTTTGGAAAGTGTGATCAT
TCAAGTGTGACTTTAGCTGAATTAGTCACAGCGCAAATGGGCAAATCGAATAAAA
AGAGGTTGATTGCCATTGGCAAATATTGGGTGTTGCTACAATTGATAGAAAAAATG
TACGTTGCCGATCATAGTTAAGACGCTATTCTATAAGGATTTTATATCGATTATG
GATTCTATTAAAACCTTAACGGAAATATTGCAATGGGATAGGAATAAAAGGGATA

- 2.4** Select homology arm amplification and sequencing primers. These primers are used to (1) amplify and (2) sequence the targeted region to verify, and possibly correct for, small genome aberrations. For tagging, only primers for the corresponding homology arm are selected. In the case of N-terminal tagging, a pair of homology arm primers and a pair of nested sequencing primers are selected.

CrisprBuildr

Homology Arm Primers

- TTTCTATTTGAAACAATAACATCTGCATATTTATGAATGTTTATTGATTAATATG
GTGACATTGACATCTGTCAAAAGCCTCAAGTGCAGTGCCTCTCGTCAATGGTTATTGCT
TAAGATGTTCTCGCATATCACTCGCTATGTCGATCCACTCTCTGTTATGGTTATTGTT
TTTGTGATTAAGGCAGGCCTTGTGTCGTTGGCCATAGTTGACACCCACTCGAGGCC
GGCACCGAGCTAGTGAATGCTCGCTCGGTTCCAGGTGAGGGTGAATGGCTGTTAAGCTT
CCTGCGATGTGAGGGCCCAAATGCTCCGGCAAAGTTCAGGTGCTAAACGAGAGACATGT
TCCGGCACGGTCAAACGACACTGTTGTCGTTCCAGTGCACCGTCGCTCAGTGCCTAAAC
ATTGATTTCATTACACCATAGTAACCGCGATAACCGAAATGCCAAACAAAACATGAA
GTTGGCAGAATTCTGTCCGTTCAAATTGCTTTCTAATGCGTGCCTGGTTGCTGTTG
CTCAATTCCAAAATAAAACGACCAGGGCGTCCGTGAGTGTGATCTCCGATCCGCGT
CAACGATGGCGTGGCCACATGTGCGTCGTCACCCCGCTCTGTTGGGATGATCCGCGT
AGGAGGCCAGACTCTTGAAGACTCTTCCGCTACGGCTGAATGCTCTGCTGCG
ACGTCGCATGCCAGCAGGCCATGACTCATGGCTGCCCTTCGAAGAGACATGGGATACG
TAACGAAGTTGGCGTGCGCCGAAAGTATTAGGAATAAGCCAAAGAACACAAACACAGCA
GCCGCGCAGCAGCCAGGAATGACGTGTCACCCCGTGTAAAGCAACTAATTAACTCAA
AGTCGCTGCAATATCAGAAGAGAACTGAAGGAAAGTGAAGAAATATAAAACGCTAA
AGGCCAAATGTTAAATGAAACATGAAATGCGGGTATCTTCCGATCACCTCAACTAC
CGGATGTCATTCGGCCACCCATTCAAGAGATACTCTGTAATCCAAACTCACATTCTGGT
ATGTTATTCTATAATGTTGGGCTACTGTATGTAATCTAGGCCCCCTCCCTGTTCCCGATT
CGGGATTTAAGGGCGGAAGTGTAACTCATCATTGCGACTTGTGGTTTGGAAAGTGTGAT
AAGTGTGACTTTAGCTGAATTAGTCAGCGCAATTGGGCAAATGAAATAAAAGAGGTTG
GCCGATTTGGCAAATATTGGGTGTCACAATTGATAGAAAAAATGACGTGCGGATCATGTT
TAAGACGCTATTCTATAAGGATTTTATATCGATTATGATTTCATTAAACTTAACTGGAATA
TTTCGAATGGGATAGGAATAAGGGATAGTCCTTGTGTTGATTTAAACTTAACTGGAATA
TGATAAAATCTTAAAGTACCCACTGTTGAGTGTGTTGATTTGAAAGTGTGTTCT
AACAAATGAAACATCTCGTAAACTGGTAAATCTTAAATTAGACAATATCTATGCT
AAATCTCAATGTTCCAGTTAGTTCTGATCGAATGAAAGAAATGCTTAAATTAACTG
AGTATTGAGACAAAGTGCAACGCAATCATCAACCTTGTGCAATTGCGTCAATTCT
TAATGCCAATTAAAGTCATAAGCCGGCCTAAATGTAACCGGTTAGAAATCGGATTAAG
TAGCCAATATCGTAGATACTAGTAGAGAGATACCTTGATGGCCGGAGTTGGAGAGCC
TGGAGCCCCCTTGGAAATGGTCCGATCAAGTTGCGAAAGTGAAGACTCCCGTGTGG
GTTCCACCGGATTCCCTGCTGGCATTGCGTCAAGTGGCAATTAGCTGGCAACTCT
TGGATGTCGGGAAATCGGAAATTACCCCTATGCTTGTATTGCGAATTGAGAGCG
CACGTTGTGGGCCAGGCCATCGGATGGATCCGGGATTACGGACTGTTCAAATCG
CTGTTGAGCGACGACATCACCATGGCGTCCAGTGCGCCCGCTATTGAGAACAGGG
GACATGGGACATGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG

Selected cut sites are highlighted in the sequence.

Selected forward amplification primer for the N-terminal homology arm

Selected forward sequencing primer

Selected reverse sequencing primer

Selected reverse amplification primer

- 2.5** Choose your cloning maps. In the last step, users can (1) view and print all the data, (2) download the genomic template without any alterations, or (3) pick from EGFP or mCherry-containing tagging vectors. You may also choose your own plasmid template vector. If so, see section 2.6 to modify your vector to make it compatible with CrisprBuildr. You can then download a locus map that shows the chosen deletion vector after a hypothetical successful targeting event.

Choose the tagging vector and download the plasmid map.

cnn-RM

Strand: -

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ATTTAAATTAAAGGAATTAAAAAAAACACACTTATGCATAATTAAATTGAGTAATGTTATTTTATTCTTA
TTCATTGAAATTCCCTTCAATTGGACAATAAACATCTGCATATTGGATGTTTGATTGATTGATTTGAAAT
ATGAGTGACATTGACATCCTGTTCAAAGGCTTCAGATTGCGCTCTCGTCAATGGTTATTGCTTAAGATG
CTTCCCTCGCATACTCGCTAGTCTGATCCACTCTGTATTGTTATTGTTATTCTTGTGATTAAGGCAGG
CGCTTGTGTCGTTGGCATAGTTGACACCCACTGAGGCGGCCAGGCAGGAGTAGATGAGTCGCGCTCGC
TTCAGGTGCTAACGAAGAGACACATGTTCCGGCACGGCTAAATGCAACCTGTTGTTCCAGTGACCAGG
TTCAAGGTGAGGTGAGAATGGGCTTAACTGCAGTTACCTGTGATGTTGAGGGCCCTAAATGCAATTG
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CAAATAAAATAACGACAGGAGCGGCCGAGCTGGCAGTGTATGGTGGTTGATATCTTCAACGATGGG
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CGTCTGGCGAGACGACATGGCGGATACGTAACGAAGTTGGCTGCGCCGCAAAGTATTAGGAATAAGCCAAG
AACAAACAAACAAACAGCAGGCCGAGCAGCAGGCCGAAATGCTGCTGCGCTGAAAGCAACTAATTAAATCTC
AAAATTAGAGTCGCTGCAAAATATCTACAGAAGAGAACTGAAGGAAGTGAAGGAAGAATATAAAACGCTAATAGAGC
GCAAAATGTTAAATGAAACATAGAATATGGGGTATCTTCGATCTCCTACTAAATCACTGTCGGATGACCTGGC
ACCCCATCAAGGATACTCTGAAATTCAACAACACTAACATTCTGTAGGTTATGTTCTATAATAGTTGGCTCA
CTGTATGTAATCTATAGGCCCCCTCTGGGGATTCAGGGGAGATTAAAGGCCGGAAGTGTAAATCTCATCCATT
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ATCATAGTTAAAGAGCTATTATAAGGATTTTATATGATTATATGGATTTCATTAAACTTAAGGAAATAT
TTGAATGGGATAGGAATAAAAGGATAGTTCTTTTATTAATAACTTTAAATCATTGGATGATAAAATCATT
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TGGGTAATCTCTTAAATTAGACAATATCTGTCAAAATAGAAATCTCAATCTGTTCTGAGTTTGTGACA
TGGAAAGAAATGTCATTAATTAGAGATATTGAGTATTGAGTATTCAGAACAAAGTGCACCGCAATCATCAA
TTGTCGCTGATTTCCAGTATTAAGCCAATTTCAGTCAAGCCGGCAAATAATGTAACCGGTTATAAGATCG
GATTAAGCCGCTAGCCAATATCTGTAGATAGTAAAGAGATACTTGTGAGCCGGACTTGGAGGCCACTCTG
CTGGAGCCGCTGGGATGGTCCGATCAAGTGGTCAAGAGTGAAGAGTACTCCGTTGCTGTTGCTGACCGG
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TACCCCTATGGTTGATTGGCGAATTGCGAGAGGCCATTACACCGCACGTTGTGGGCCAGGCCAATGCGGATGG
CCGGGATTACGGACTGTTCCAATCAGCGATCGCTACTGGTGTGGGCCAGGCCAATGCGGAAACTATG
CATTCAAC

```

2.6 Modify your guide RNA or Plasmid template vector

2.6.1 Modify your guideRNA vector

1. Save your vector as Genbank (.gb) or text file (.txt) file. These formats ensure that the header information, containing your own annotations in the vector are preserved.

```
LOCUS      pU6_2_
DEFINITION .
ACCESSION
VERSION
SOURCE
ORGANISM
COMMENT
FEATURES
misc_feature    698..1093
/locus_tag="u6 promoter"
/label="u6 promoter"
/ApEinfo_label="u6 promoter"
/ApEinfo_fwdcolor="#1fe0d7"
/ApEinfo_revcolor="green"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
misc_feature    1195..1200
/locus_tag="U6 terminator"
/label="U6 terminator"
/ApEinfo_label="U6 terminator"
/ApEinfo_fwdcolor="#ff0000"
/ApEinfo_revcolor="green"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
misc_feature    625..646
/locus_tag="T7 primer"
/label="T7 primer"
/ApEinfo_label="T7 primer"
/ApEinfo_fwdcolor="#c0c0c0"
/ApEinfo_revcolor="green"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
misc_feature    1204..1209
/locus_tag="EcoRI"
/label="EcoRI"
/ApEinfo_label="EcoRI"
/ApEinfo_fwdcolor="#ff8080"
/ApEinfo_revcolor="green"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
```

Header information,
containing vector
annotations

2. Insert the string, containing 21 G's, below where you wish to insert gRNA spacer sequence:

injection_startGGGGGGGG GGGGGGGGGG GGGG**injection_end**

```

COMMENT      APOLINTO.METHOD.LATEX.1
FEATURES      Location/Qualifiers
promoter      7..406
/note="RNA polymerase III promoter for Drosophila U6-2
snRNA (Das et al., 1987)"
/locus_tag="dU6-2 promoter"
/label="dU6-2 promoter"
/ApEinfo_label="dU6-2 promoter"
/ApEinfo_fwdcolor="#346ee0"
/ApEinfo_revcolor="#346ee0"
/ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"

ORIGIN
1 GAATTCGTT GACTTGCAGC CTGAAATACG GCACGAGTAG GAAAAGCCGA GTCAAATGCC
61 GAATGCAGAG TCTCATTACA GCACAAATCAA CTCAAGAAAA ACTCGACACT TTTTTACCAT
121 TTGCACTTAA ATCCTTTTT ATTCTGTTATG TATACTTTTT TTGGTCCCTA ACCAAAACAA
181 AACCCAAACTC TCTCTAGTGT GCCTCTATAT TTAAAACCTAT CAATTTATTA TAGTCAATAA
241 ATCGAACTGT GTTTCAACAA AACGAACAA AGGACACTTT GATTCTAAAG GAAATTGGAA
301 AAATCTTAAAG CAGAGGGTTC TTAAAGCATC TTGCAATTTC TTATAATTCT CAACTGCTCT
361 TTCTCTGATGT TGATCATTAA TATAAGGTATG TTTTCTCAA TA**injection_start**GGGGGGGG GGGGGGGGGGG GGGG**injection_end**GGT CTTCGAGAAG
421 ACCTGTTTA GAGCTAGAAA TAGCAAGTTA AAATAAGGCT AGTCGTTTAT CAACCTGAA
481 AAGTGGCACC GAGTCGGTGC TTTTTGCTT ACCTGGAGCC TGAGAGTTGT TCAATAAAAT
541 AAAATGTTT CGTTTTTG CTTTCGCCAG TATTCTTATTAT TTTTCATCAA TATGTATTCA
601 ATTTGGTATG TATTTAGTAA TTGTAATATA TAGACAATGG TTTTCCGTG ACGTACATAC
661 ATCTGACGTG TGTTTATTA GACATAATAG TTATGTTTC ACATCTTTTT AATGTTCGCT
721 TAATGGTAT GCATTCTAGA CAATTGTGCT CGGCAACAGT ATATTGTGG TGTGCCAAC
781 AACAAACCTGC AGGAGCTCA GCTTTGTT CTTTAGTGA GGGTTAATTG CGCGCTTGGC
841 GTAATCATGG TCATAGCTGT TTCTCTGTGT AAATTGTTAT CCCTCTACAA TTCCACACAA
901 CATACTGAGCC GGAAGCTAA AGTGTAAAGC CTGGGGTGC TAATGAGTGA GCTAACTCAC
961 ATTAATTGGC TTGGCTCAC TGCCCGCTT CCAGTCGGGA AACCTGCTGT GCCAGCTGCA
1021 TTAATGAATC GGCCAACGCG CGGGGAGAGG CGGTTTGCCT ATTGGGGCGT CTTCGGCTTC
1081 CTCGCTCACT GACTCGCTGC GCTCGGTGCT TCGGCTGCG CGAGCGGTAT CAGCTCACTC
1141 AAAGGCGGTA ATACGGTTAT CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC
1201 AAAAGGCCAG CAAAGGCCA GGAACCGTAA AAAGGCCGG TTGCTGGCGT TTTTCATAG
1261 GCTCCGGCCC CCTGACGAGC ATCACAAAAA TCGACGCTCA AGTCAGAGGT GGCAGAACCC
1321 GACAGGACTA TAAAGATACC AGGCCTTTCC CCTCTGGAAAGC TCCCTCGTGC GCTCTCCGT
1381 TCCGACCTG CGCCTTACCG GATACTGTC CGCCTTCTC CCTTCGGAA GCGTGGCGCT
1441 TTCTCATAGC TCACGCTGA GGTATCTAG TTCGGTGTAG GTCTGGCGT CCAAGCTGGG
1501 CTGTGTGCAC GAACCCCCCG TTCAGCCCGA CCAGCTGCC TTATCCGTA ACTATCGTCT
1561 TGAGTCAAC CCGTAAAGAC ACGACTTATC GCCACTGGCA GCAGCCACTG GTAACAGGAT

```

- Save the resulting file again as .txt or .gb. It can now be uploaded as a new template.

2.6.2 Modify your plasmid template vector

1. Save your vector as Genbank (.gb) or text file (.txt) file. These formats ensure that the header information, containing your own annotations in the vector are preserved.
 2. Insert the string of g's flanked by **arm_1_start** and **arm_1_end** as placeholders for the 5' homology arms and **arm_2_start**, **arm_2_end** as placeholder for the 3' homology arm.

```
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
misc_feature 1249..2248
    /locus_tag="Homology Arm 1"
    /label="Homology Arm 1"
    /ApEinfo_label="Homology Arm 1"
    /ApEinfo_fwdcolor="#b7ffb7"
    /ApEinfo_revcolor="green"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
misc_feature 4241..5240
    /locus_tag="Homology Arm 2"
    /label="Homology Arm 2"
    /ApEinfo_label="Homology Arm 2"
    /ApEinfo_fwdcolor="#b7ffb7"
    /ApEinfo_revcolor="green"
    /ApEinfo_graphicformat="arrow_data {{0 1 2 0 0 -1} {} 0}
width 5 offset 0"
```

Header information,
containing vector
annotations

```
insert **arm_1_start**
```

insert 1000 g's
This is where the 5'
homology arm will go

| insert **arm_1_end**

◀ insert **arm_2_start**

insert 1000 g's
This is where the 3'
homology arm will go

insert **arm 2 end**

3. Other features

The screenshot shows the CrisprBuildr website interface. On the left, there's a sidebar with various options like 'Download Options', 'Genomic Template', 'Guide RNA Vector', and 'Plasmid Template'. The main area displays a genomic sequence with a highlighted region. A modal window titled 'Report a Bug!' is overlaid on the page. It contains fields for 'Full Name' (with 'First Name' and 'Last Name' sub-fields), 'E-mail' (with an example 'ex: myname@example.com'), and a 'Type:' dropdown with three options: 'Bug' (selected), 'Feature Suggestion', and 'Comment'. At the bottom of the modal is a 'Jotform' logo and a 'Create your own Jotform' button.

Click here for additional features. The menu contains a link to the

- User Manual
- Bug report form
- Citations
- Manuscript