

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.

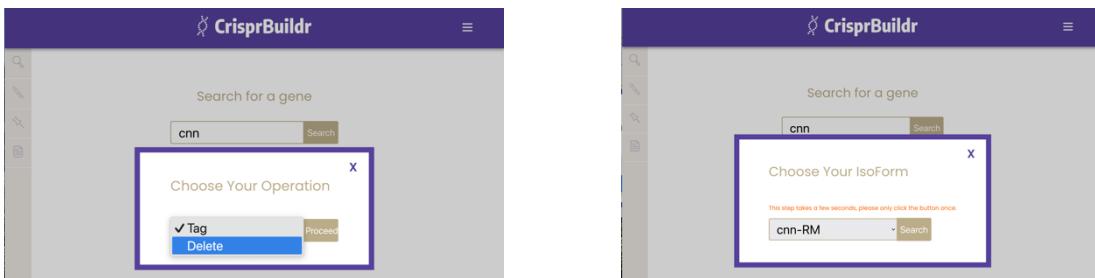
1. Gene deletions

1.1 Search for your gene of interest.

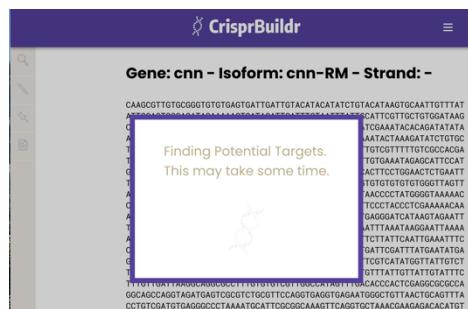
Type the name of your gene of interest into the search field. The application accepts Flybase gene symbols but not full names (e.g cnn instead of centrosomin). Instead of gene symbols, genes can also be searched by Flybase IDs (FBgn's).



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.

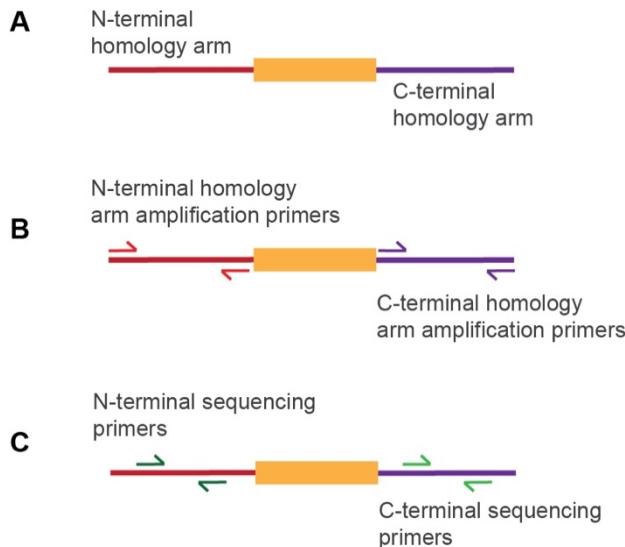
The selected site is highlighted in the sequence.

- 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 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.

(1) Displays all the information in a separate window.

(2) Downloads a locus map before targeting.

(3) Downloads a guide RNA vector.

(4) Downloads a locus map after targeting.

The screenshot shows the CrisprBuildr interface with the title 'cnn-RM'. On the left, there's a sidebar with 'Download Options' including 'View All Data', 'Genomic Template', 'Guide RNA Vector', and 'Plasmid Template'. Arrows point from numbered steps (1-4) to the corresponding sections in the sidebar. Step (3) points to the 'Guide RNA Vector' section, which is highlighted with a purple box and an arrow.

Strand: -

```
TTGCATATTTATGAATGTTTGATTGATTTGAATATGAGTGACATTGACATCTGTTCAA
AAGCCTTCAAGATTCAAGTCAGTGCCTCTCGTATAGTTATTGCTTAAGATGCTTCTCGCATA
TCACTCGCTATAGTCTCGATCCACTCTGTGTTATTGTTATTGTTATTGTTATTGTTGATTAAGGCAG
GCGCCTTGTGTCGTTGCCATAGTTGACACCCACTCGAGGCGCGCAGGCAGCCAGGTAGA
TGAGTCGCTGCGTCCAGGTGAGGTGAGAAATGGGCTTAACTGCAGTTACCTGTCGATGT
GAGGGCCCTAAATGCACTCGCGCAAAGTTCAGGTGCTAAACGAAGAGACACATGTTCCGGCAC
GGTTCAAAATGACACCTGTTGCCCCAGTGACCAGGTGCGTCAGCTGTCGGCTAAACATTGA
TTTCATTACCATAGTAACCAGCGATAGCCAAATGCAAACAAAACAATGAGTGAATATG
TTGGCGAATTTCTTGTGCCCCTCAAAATTGCTGTTCTAATGCGGTGCGCCGTTGCTGTT
GCTCAATTCCAAAATAAACGACCCAGGGCGGTGCGTGGTAGTGTATGTTGTTAGTA
TCTTCACGATGCGTGGCCACACTTGTGAGACTCTTCTCCGCTACGGCTGAATGCTCTGATT
CGTCGTGCGCACGTCGATGCCAGCAGCCATGACTCATGGCTGCCGTCCTCGAAGACAGA
CATGGCGGATACGTAACGAAGTTGGCGTGGCCCGAAAGTATTAGGGATAAACGCAAGAAC
AACAAACAACACAGCAGCCGAGCAGCAGCCAGGAATGACGTGTCACCCGTGAAAGCAACT
AATTAAATCTAAATTAGAGTCGCTGCAATATCTACAGAAGAGAACTGAAGGAAAGTGAAGA
AATATAAAATAAACGCTAATAAGAGCGAAATGTTAAATGAAACATAGAAATGGGGTATCTT
CGATCTACCTCACTAAATCACTCGTGGATGACTGGCACCCATTCAAGAGAACTCTCTGAA
TTCAACAACACTCAACATTCTGGTAGGTTATGTTCTATAATAGTTGGCTCACTGTATGTAATC
TATAGCCCCCTCCCTGTTCCCGATTCAACGGCGGATTAAAGGGCGGAAGTGTAACTCATCCA
TTGCAGTTGTGGTTTTGGAAGTGTGATCATTCAAGTGTGACTTGAATGCTGAAATTAGTTCA
```

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.

(1) Guide RNA vector choices.

The screenshot shows the CrisprBuildr interface with the title 'cnn-RM'. On the left, there's a sidebar with 'Download Options' including 'View All Data', 'Genomic Template', 'Guide RNA Vector', and 'Plasmid Template'. The 'Guide RNA Vector' section has a dropdown menu open, showing options: 'Select a Vector...', 'pU6-gRNA', and 'Upload Your Own Guide RNA Vector'. The 'Select a Vector...' option is highlighted with a blue box and an arrow pointing from the text '(1) Guide RNA vector choices.'.

Strand: -

```
TTGCATATTTATGAATGTTTGATTGATTTGAATATGAGTGACATTGACATCTGTTCAA
AAGCCTTCAAGATTCAAGTCAGTGCCTCTCGTATAGTTATTGCTTAAGATGCTTCTCGCATA
TCACTCGCTATAGTCTCGATCCACTCTGTGTTATTGTTATTGTTATTGTTGATTAAGGCAG
GCGCCTTGTGTCGTTGCCATAGTTGACACCCACTCGAGGCGCGCAGGCAGCCAGGTAGA
TGAGTCGCTGCGTCCAGGTGAGGTGAGAAATGGGCTTAACTGCAGTTACCTGTCGATGT
GAGGGCCCTAAATGCACTCGCGCAAAGTTCAGGTGCTAAACGAAGAGACACATGTTCCGGCAC
GGTTCAAAATGCAACCTGTTGCCCCAGTGACCAGGTGCGTCAGCTGTCGGCTAAACATTGA
TTTCATTACCATAGTAACCAGCGATAGCCAAATGCAAACAAAACAATGAGTGAATATG
TTGGCAGAATTCTTGTCCGTTACAAATTGCTGTTCTAATGCGGTGCGGTTTGTGTT
GCTCAATTCCAAAATAAACGACAGGGCGGTGCTGGTAGTGTATGTTGTTAGTA
TCTTCACGATGCGTGGCCACATGTCGCTGTCACCCCGCTCTGGGGATGCTCC
CGTCGTGAGGAGGCCAGACTCTTGAAGACTCTTCTCCGCTACGGCTGAATGCTCTGATT
GCTGCTGCGCACGTCGATGCCAGCAGCCATGACTCATGGCTGCCGTCCTCGAAGACGA
CATGGCGGATACGTAACGAAGTTGGCGTGGCCGGAAAGTATTAGGGATAAACGCAAGAAC
AACAAACAACACAGCAGCCGAGCAGCAGCCAGGAATGACGTGTCACCCCGTAAAGCAACT
AATTAAATCTAAATTAGAGTCGCTGCAATATCTACAGAAGAGAACTGAAGGAAAGTGAAGA
AATATAAAATAAACGCTAATAAGAGCGAAATGTTAAATGAAACATAGAAATGGGGTATCTT
CGATCTACCTCACTAAATCACTCGTGGATGACTGGCACCCATTCAAGAGAACTCTCTGAA
TTCAACAACACTCAACATTCTGGTAGGTTATGTTCTATAATAGTTGGGCTCACTGTATGTAATC
TATAGCCCCCTCCCTGTTCCCGATTCAACGGCGGATTAAAGGGCGGAAGTGTAACTCATCCA
TTGCAGTTGTGGTTTTGGAAGTGTGATCATTCAAGTGTGACTTGAATGCTGAAATTAGTTCA
```

cnn-RM

Strand: -

```

TTGCATATTTATGAAATGTTGATTGATTGATTGAATATGAGTGACATTGACATCTGTTC
AAGCCTTCAAGATTCAAGTGGCTCCCTCGTCATATGGTTATTCCTTAAGATGCTTCCCTCGCAT
TCACTCGCTATAGTCTCGATCCTACTTGTATTGTATTGTATTCTTGTGATTAAGGCAG
GCGCCTTGTGTCGTTGCCATAGTTGACACCCACTCGAGGCCGCCAGGCCAGCAGTAGA
TGAGTCGCTGTCGTTCCAGGTGAGGTGAGAAATGGGCTTAACGTGACATTACCTGTGATGT
GAGGGCCTAAATGATTCCGCGCAAAGTTCAAGGTGCTAAACCAAGAGACACATGTTCCGGCAC
GGTCAAAATGACACCTGTGTTTCCCAGTGCACAGGTGCTCAGCTGTCGGCTTAACCCATTG
TTTCAATTACACCATAGTAACCGCGATAGCCGAATGCCAAACAAAATGAGTGAATG
GCAGAATTCTTGTCCCGTCCAAATTGCTGTTCAATGCCGTGCCGTTTGTGTT
CAATTCCAAAATAAAATAACGACCAAGAGGCCGTCGTTGGGATGCATCC
TCAACGATGCCGTGCCAACATGTCGCTGTCACGGCTGCTCAACCCCGTCTGTTGGGATGCATCC
CGICCGTGGAGGCCAGACTCTTGAAGACTCTTCCGCTACGGCTGAATGCTCTGATT
GCTGCTGCCAGCTCGCATGCCAGCGCCATGACTCATGGCTGCCGCTCCCTGAAAGACGA
CATGGCGGATACGTAACGAAAGTTGGCGTGCGCCGCGAAGATATTAGGAATAAGCCAAGAAC
AACACAACACAGCAGCCAGCAGCAGCAGCAGGAATGACGTGTCACCCCGTGTAAAGCAACT
AATTAATCTCAAATTAGTCTGCTCAAATATCAGCAAGAGAACTGAAGGAAAGTGAAGA
AATATAATAAACGCTAATAAGGCCGAAATGTTAAATGAAACATAGAATATGCGGTATCTT
CGATCTACTCAAAATCACTGTCGGATGTCCTGCCACCCATTCAAGAGATACTCTGAA
TTCCAACAACCAACATTCTGGTAGGTTATGTTCTATAATAGTTGGCTCACTGTATGTAATC
TATAGCCCCCTCCCTGTTCCCGATTCAAGCGGGATTAAAGCGCGGAAGTGTAAATCTCATCA
TTGCAGTGTGTTTGGAAAGTGTGATCATTCAAGTGTACTTAAAGTGTGAAATTAGTTCACA

```

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

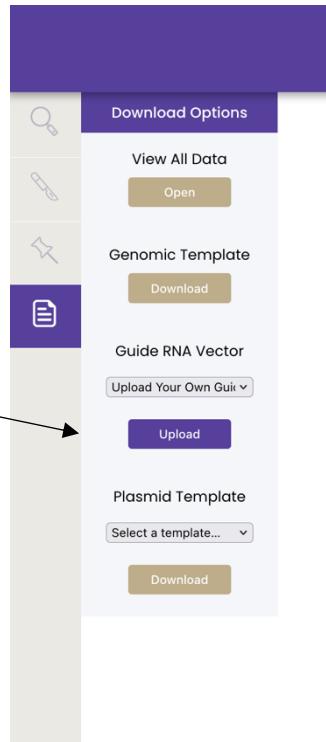
Strand: -

```

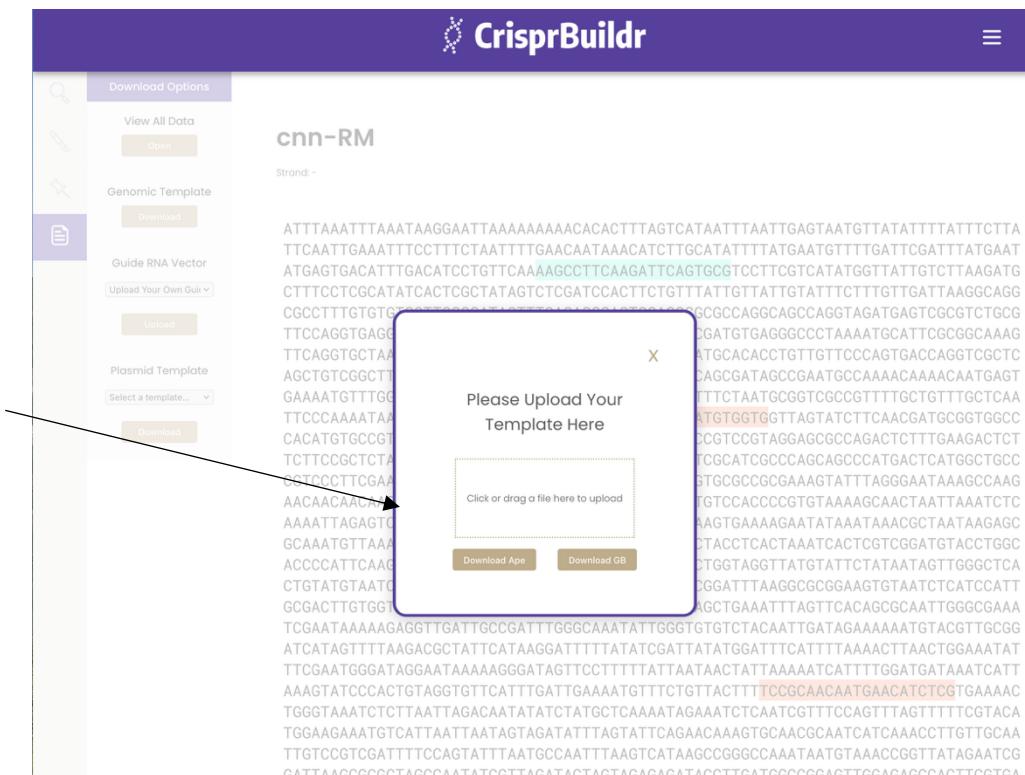
ATTAAATTAAAGGAATTAAAAAAACACTTTAGTCATAATTAAATTGAGTAATGTTATTTTCTTA
TTCAATTGAAATTCTCTTCAATTGAAACAAACATTGCACTTGCATATTTTAATGTTGATTGATTGATTGATTGAA
ATGAGTCACATTGACATCTGTCATGTTCAAAGCCTTCAAGATTCAGTCGCTCTTGTGATATGGTTATGCTTAAAGATG
TTTCTCGCATATCACTCGCTATAGTCGATCCTCTGTGTTATGTTGATTGTTCTTGTGATTAAGGCAGG
GCCCTTGTGCTGGCCATAGTTCATTACCCACATGTAACCCGCGATGCCGAAGCAGGTAGATGAGTCGGCTCTGGC
TCCAGGTGAGGTGAAAGTGGCTTAACTGCACTTACCTGTCGATGTCAGGGCCCTAAATGCAATTCCGGCAAAAG
TTCAGGTGCTAAACGAAGAGACATGTTCCGGCACGGTCAAATGCAACCTGTTCTCCAGTGCACGGCTC
AGCTGCGCTTAAACCATGTTTCAATTACCCACATGTAACCCGCGATGCCGAAGCAGGTAGATGAGTCGGCTCTGGC
GAAATGTTGGCAGAATTCTTGTCCCGTTCACAAATTGCTGTTCTAATGCGGTGCGTGTGTTGCTGTTGCTCAA
TTCCAAAATAAAATACGACCAAGGGCGTGGCTGTCAGTGTGTTGTTGAGTATCTTCAACGATGCCGTTG
CACATGTCGGCTCCACCCCGCTCTGTTGGGATGTCATCCGGCTCGTAGGAGGGCCAGACTCTTGAAGGACTCT
TCTCCCTCTACGGCTGAATCTCTGCTCCGATCCTGTCGCGATGCCAGGGCCATGACTCATGGCTCC
CGTCCCTCTGAAGACGACATGCCGATACGTAACGAAGTTGGCTGCCGGCGAAGATTTAGGAATAAGCCAAG
AAACAAACAAACACAGCAGCCGAGCAGCAGCAGCAGGAATGACGTGTCACCCCGTGTAAAGCAACTAATTAACCTC
AAAATTAGTCTGCTGAAATATCTCAAGAGAGAACTGAAGGAAGAATATAAAACGCTAATAAGAGC
GCAAAATGTTAAAGAACATAGAATATGCGGTATCTCTACTCAAAATCACTGTCGGATGACCTGCC
ACCCCATTAAGAGATACTCTGTAATTCAACAACTCAACATTCTGGTAGGTTATGTTCTATAATAGTTGGCTCA
CTGTTGTAATCTAATGCCCCCTCCCTGTCGGGATTCACGGGATTAAAGGCCGAAAGTGTACCTCATCCATT
GGGACTTGTGTTGGAAAGTGTGATCATTCAAGTGTGACTTACGGTAAATTAGTCAGCGCAATTGGCGAAA
TCGAATAAAAGAGGTTGATTGCCATTGGGAAATATTGGTGTCTACATTGATAGAAAATGACGTTGCGG
ATCATAGTTAAAGCCTATTCTATAAGGATTTTATATGCTTATTGATTTCATTGTTAAACTCTGAAATAT
TTGCAATGGGATAAGGAATAAAAGGGATAGTTCTTATTAAATAACTTAAACATTGTTGATGATAAATCATT
AAAGATCCCACCTGAGGTGTCATTGTTGAAATGTTCTGTTCTTCCGCAACATCTCGTCAAAC
TGGTAAATCTTAAATTAGACAAATATCTATGCTCAAATGAGAAATCTCACTGTTCCAGTTAGTTTCTGACA
TGAGAAATGCTCAATTAAATAGTAGATATTAGTATTGAGAATCTGCAACAGGCAACATCAACCTGTTGCAA
TTGTCGCTGATTTCAGTATTGCAATTAAAGTCTGATAAGGAGATACCTGATGGCCGAGGTGAGAAGCCACTCTG
GATTAAGCCGCTGAGCCAATATCTGTAAGTACTAGAGAGATACCTGATGGCCGAGGTGAGAAGCCACTCTG
CTGGAGCCGCTGGGATGGTCGATCAAGTTGGCTGAAGGTGAGAGTACTCCGGTCTGTTGTTCTGTTCCAGGG
ATCCCCCTGCTGCCATTGGCTTCAGGCTGCAACTAGTCTGCAACTCTACATACTGATGTCGGAAATCGGAAT
TACCCCTATGGTTGATTGCGAATTGCGAAGAGCGGATTCAACCGCACGTTGAGGGCCAGGCAATGCGGATGGATC
CCGGGATTACGGACTGTCCTAACAGCGATCGCAGTGTGACTGTCGCGCCGAAATCGGAACCGAATATGCAAC

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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.



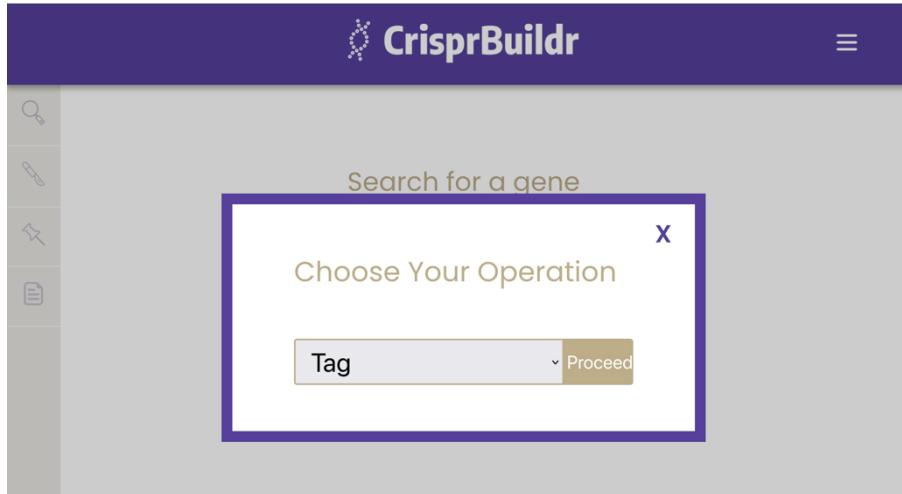
(4) Hit 'Download BG or 'Download Ape'. The Genbank (GB) file can be opened in most cloning applications.

The screenshot shows the CrisprBuilder interface with the following details:

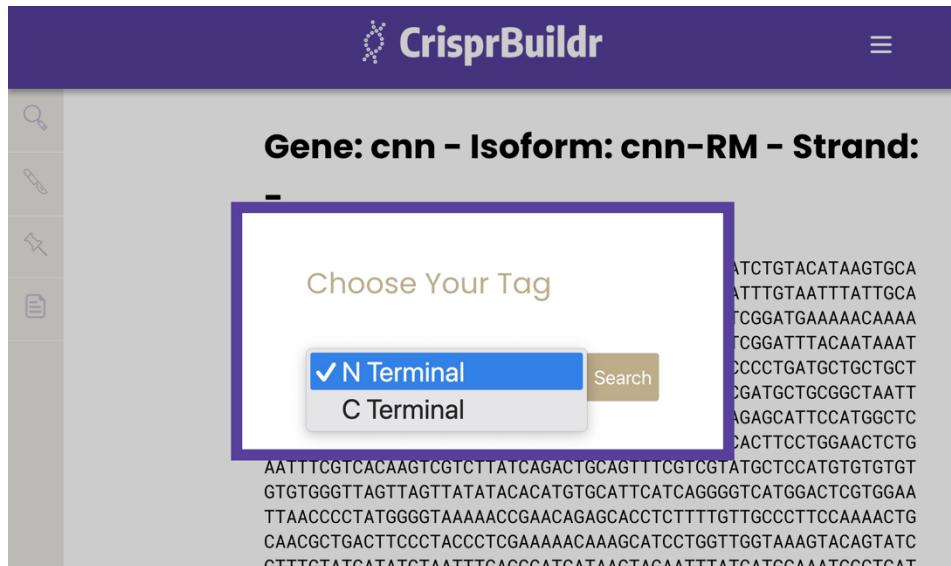
- Download Options** panel on the left:
 - View All Data**: Open
 - Genomic Template**: Download
 - Guide RNA Vector**: Upload Your Own Guide
 - Plasmid Template**: Select a template... Downloaded
- cnn-RM** project details:
 - Strand: -
 - Sequence: A large block of DNA sequence starting with ATTTAAATTAAATAAGGAATTAAAAAAAACACTTGTCAATAATTGAGTAATGTTATTTTCTTA...
- Please Upload Your Template Here** modal window:
 - File input field: pu6.txt
 - Buttons: Download Ape, Download GB

2. Gene tagging

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



- 2.2 Choose 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.

CrisprBuilder

▼ Select Cut Site

| | |
|-----------------------------|---------------------|
| TACCTCACTAAATCACTC GTCGG | Efficiency: 3.6613 |
| AAATGAAACATAGAATAT GCCGG | Efficiency: 4.22992 |
| GTTAAATGAAACATAGAAT ATGG | Efficiency: 5.43676 |
| ATCCGACGAGTGATTAG TGAGG | Off Targets: 0 |

GCCCTAAAAATGCATTCGGCAAAGTTCAGGTGCTAAACGAAGAGACACATGTCGGC
ACGGTTCAAAATGCACACCTGTTGTCAGTGACCGAGTCGCTAGCTGTCGGCTAA
ACCATTGATTTCATTAACACCATAGTAACCAGCGATAGCCGAATGCCAAAACAAACA
ATGAGTGAAGAATGTTGGCAGAATTTCCTGTCAGCTGCTGGCTCACAAATTGCTGTTCTAATG
CGTCGCGCTTCTGCTCAATCCAAAATAAAACGACAGAGGGCGTGG
TGGTGCAGTGTATGTTGGTAGTATCTCAACGATGCGTGGCCACATGTCGGCTC
GTCAACCCCCCGTCTGTTGGATGTCATCCGCTAGGAGGCCAGACTCTTGA
AGACTCTTCTCGCTACGGCTGAATGCTCTGATTGCTGCGCACGTCGATCG
CCCAGCAGCCATGACTCATGCTGCCGTCCCTCGAAGACGACATGGCGATACGTA
ACGAAGTTGGCGTGGCCGCGAAAGATTAGGAAATAAGCAAGAACACAACAC
AACAGCAGCCGAGCAGCAGCAGCAGGAAATGACGTGTCACCCGTAAAAGCAACTAAT
TAAATCTCAAATTAGAGTCGCTGCAAATATCTACAGAAGAGAACTGAAGGAAGTGA
AAGAATATAAAACGCTAAAGAGCGCAAATGTTAAATGAAACATAGAAATGGCG
GGTATCTTCGATCTACCTCAAAATCACTCGTCTGATGTCAGTGCACCCATTCAA
GAGATACTCTGAATTCCAACAACCTAACATTCTGTTAGGTTATGATTCTATAATAG
TTGGGCTCACTGTATGTAATCTAGCCCCCTCCCTGTTCCCGATTCAACGGGATT
TAAGGCGCGGAAGTGTAAATCTCATCCATTGCGACTTGTGGTTTGGAAAGTGTGATCAT
TCAAGTGTACTTAACTGAAATTAGTTCACCGCAATTGGGAAATCGAATAAAA
AGAGGTTGATTGCCGATTGGCAAATATTGGGTGTTGCTACAATTGATAGAAAAAATG
TACGTTGCGGATCATAGTTAAAGACGCTATTCTAAAGGATTATATCGATTATG
GATTCATTTAAACTTAACGGAAATATTGCAATGGGATAGGAATAAAAGGGATA

- 2.4** Select homology arm amplification and sequencing primers. 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.

CrisprBuilder

Homology Arm Primers

| | |
|------------------------------|-------------------------------|
| TTTCCTTGTCCGTCACA A | Tm: 59.14 |
| GC%: 40.00 | Any (Self Complementarity): 0 |
| 3' (Self Complementarity): 0 | 0 |

N Reverse Sequencing Primer

| | |
|------------------------------|-------------------------------|
| TGTTCATTTGCGGGAAA G | Tm: 59.71 |
| GC%: 40.00 | Any (Self Complementarity): 0 |
| 3' (Self Complementarity): 0 | 0 |

GATGTTCATTTGCGGA
AA

| |
|-------------------------------|
| Tm: 59.52 |
| GC%: 40.00 |
| Any (Self Complementarity): 0 |
| 3' (Self Complementarity): 0 |

ATGTTCATTTGCGGGAAA
AG

| |
|-------------------------------|
| Tm: 59.99 |
| GC%: 38.10 |
| Any (Self Complementarity): 0 |
| 3' (Self Complementarity): 0 |

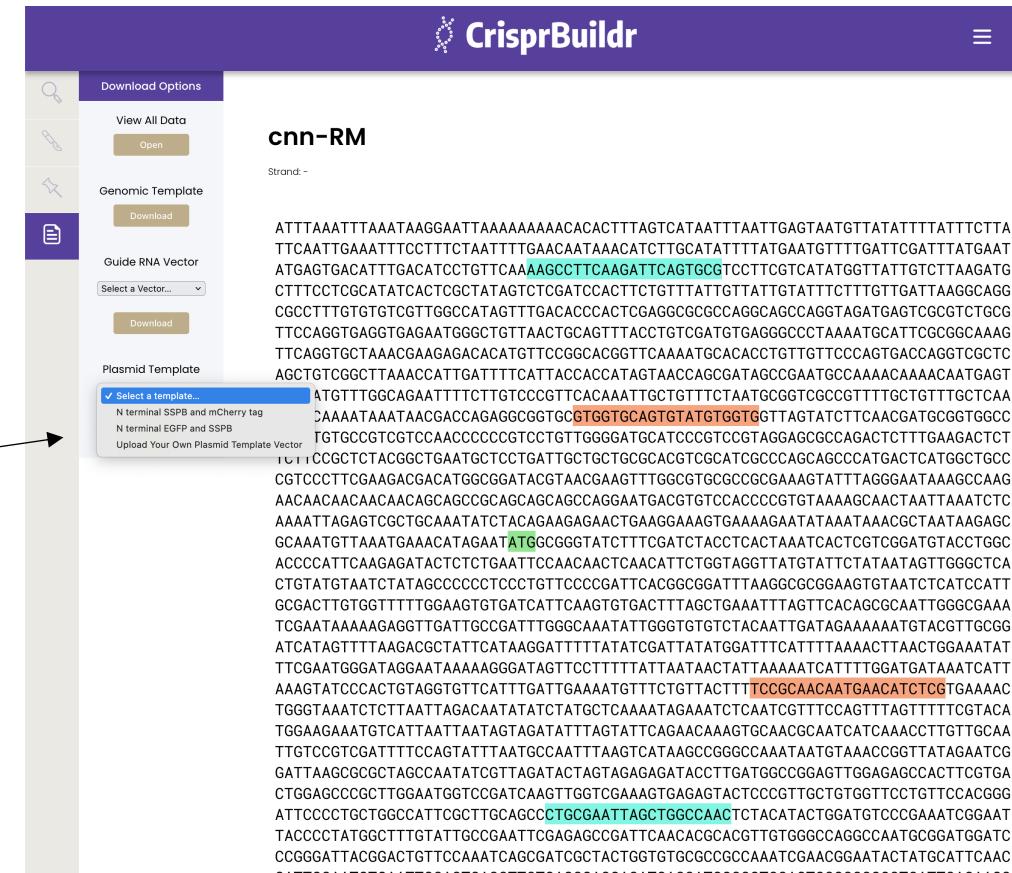
CTTCTAATTGAAACAATACCTGATATTATGAAATGTTGATTCGATTTGAAATATG
GTGACATTGACATCTGTCGAAAGCCTTCAGATTACGTCGCTCCTCGTATAGGTTATTGCT
TAAGATGTTCTTCGTCATACCTCGCTATAGTCGATCCACTCTCTGTTATTGTTATTGTT
TTGGTGTGTTAAGGCAGGCCCTTGTGTCGTTGGCCATAGTTGACACCCACTCGAGGCC
GGCAGCAGGAGTAGTGTGTCGCTCGTCCAGGTGAGGTGAGAAATGGGCTGTTACTGCA
CTCTGCGATGTTGAGGGCTTAAATGCACTCCGCGAAAGTTCAGGTGCTAAACGAGACACATGT
TCCGGCACGGTCAAATGCACACCTGTTGTCAGTGACCCGCTCACGTCGCCCTAAACC
ATTGATTTCTTACCAACCATAGTAACAGCGATAGCGGAATGCCAAACAAACAAATGAGTGA
GTTGGCAGAATTCTGTCGCCGTCACAAATTGCTTCTAATGCGTCCGCTTGTGTT
CTCAATTCCCAAATAAACGACGAGGGGGTGCCTGAGGTGAGTGTGTTGCTTCT
CAACGATGCGGGGCCACATGCGTCGTCACCCCGCTCTGTTGGGATGCACTCCGCG
AGGAGCGCAGACTCTTGAAGACTCTTCCGCTCTGCGTAAATGCTCTGATTGCTGCG
ACGTCGCATGCCAGCGCCCATGACTCATGCTGCCGCTCCCTCGAAGACGACATGGGGATACG
TAACGAAGTTGGCGTGCGCCGCGAAAGTATTAGGAAATAAGCAAGAACACAACACAGCA
GCCGCAGCAGCCAGGAATGACGTGTCACCCGTGTAAGAACACTAATTAAATCTCAAATTAG
AGTCGCTGAAATATCAGAAGAGAACTGAAGGAAAGTGAAGAACATATAAAACGCTAATAAG
AGCGCAATTTAAATGAAACATAGAATTGCGGGTATCTCGATCTACCTCACTCGTCTGTT
CGGATGTTGACTTAACTGAAATTAGTTCACAGCGCAATTGGCGAAATCGAATAAAAGAGGTTGATT
GCCGATTTGGCAAATATTGGGTGTCATAATTGATAGAAAGATGACCTTGTGCGGATCATGTT
TAAGACGCTATTCTAAAGGTTTATGCAATTATGATTCTATTAAACTACTGGAAATA
TTCTGCAATGGGATAGGAATAAAAGGGATAGTCTTCTTATTAAACTATTAAACATTGTT
TGATAAATCTTAAAGTATCCACTGTAAGTGTGTCATTGATTGAAAATGTTCTGTTACTTTCCGC
AACATGAACATCTCGTAAACACTGGGTAATCTTAAATTAGACAATATCTATGCTCAAATAGA
AATCTCAATCGTCTTCAGTTAGTTCTGTCATGAAAGAAATGCTTAAATTAGATGAGTATT
AGTATTGAGAACAAAGTGCACCGCAATCATCAACACCTTGTGCAATTGTCGCTGATTTCAGTATT
TAATGCAATATCGTTAGATACTAGTAGAGAGATACTCTGATGGCGGAGTTGGAGAGCCACTTCGTGAC
TAGCCAATATCGTTAGATACTAGTAGAGAGATACTCTGATGGCGGAGTTGGAGAGCCACTTCGTGAC
TGGATGTCGGCGAATCGAATTACCCCTATGGCTTGTATTGCGAATTGCGAGAGGACCTTCAC
CACGTTGTCGGCGAGGCAATGCGGATGGATCCGGGATTACGGACTGTTCAAACTCAGCGATCGCTA
CTGTTGTCGGCGGCAATCGAACGGAATACTATGCAATTGCAACGATTGCAATTGCAATTGCACTCACC
TTCTGAGCGACGACATCACCATGGCGTCCAGTGCGCCCGCTATTCAAGAACAGGGCTGGACG

Selected forward amplification primer for the N-terminal homology arm

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.



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
COMMENT     ApEinfo:methylated:1
FEATURES      Location/Qualifiers
  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 below where you wish to insert your gRNA:

injection_startGGGGGGGG GGGGGGGGGG GGGGGGGGG**injection_end**

3. 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"
```

3. Other features

The screenshot shows the CrisprBuilder interface. On the left, there's a sidebar with various download options like 'View All Data', 'Genomic Template', 'Guide RNA Vector', and 'Plasmid Template'. The main area displays a sequence 'cnn-RM' with a strand indicator. A central modal window titled 'Report a Bug!' is open, asking for details about a problem. It includes fields for 'Full Name' (First Name and Last Name), 'E-mail' (with placeholder 'ex: myname@example.com'), and a 'Type' dropdown with options: Bug (selected), Feature, Suggestion, and Comment. At the bottom of the modal are two buttons: 'Jotform' (in blue) and 'Create your own Jotform!' (in green). To the right of the modal is a vertical sidebar menu with links: Home, Font Size (with a dark mode option), Open Design, Save Design, User Manual, and Bug Report. An arrow points from the text 'Click here for additional features.' to the 'User Manual' link.

Click here for additional features.

The menu contains a link to the User Manual and a Bug report form.