

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.

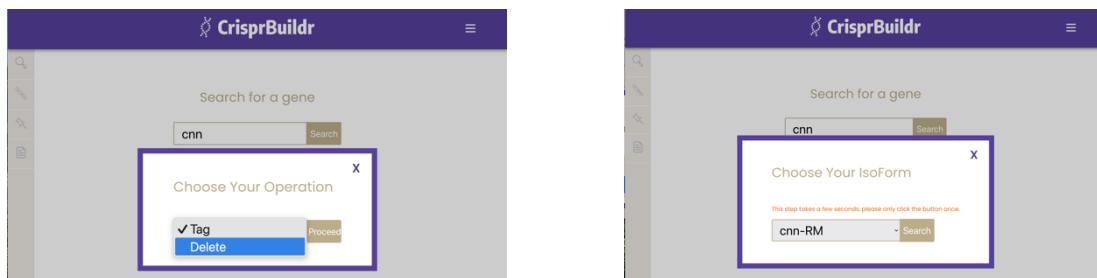
1. Gene deletions

1.1 Search for your gene of interest.

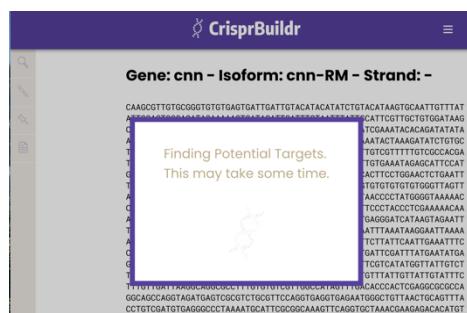
Type the name of your gene of interest into the search field. The application accepts Flybase gene symbols but not full names (e.g cnn instead of centrosomin). Instead of gene symbols, genes can also be searched by Flybase IDs (FBgn's).



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 is listing potential cutting sites. The selected cut site is highlighted in red 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.

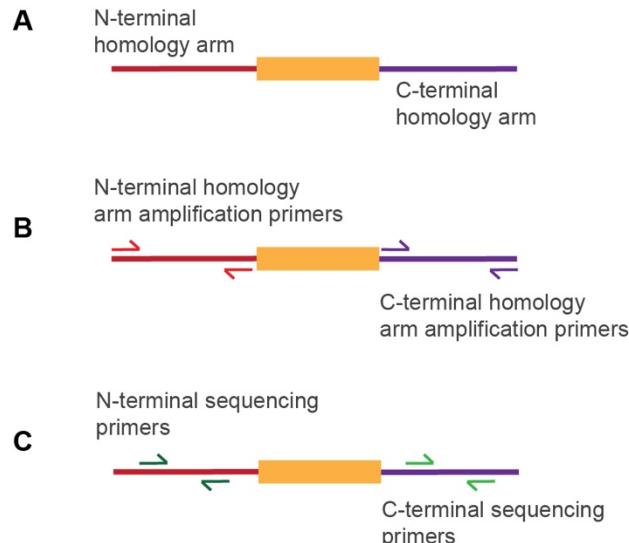
The selected site is highlighted in the sequence.

- 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.

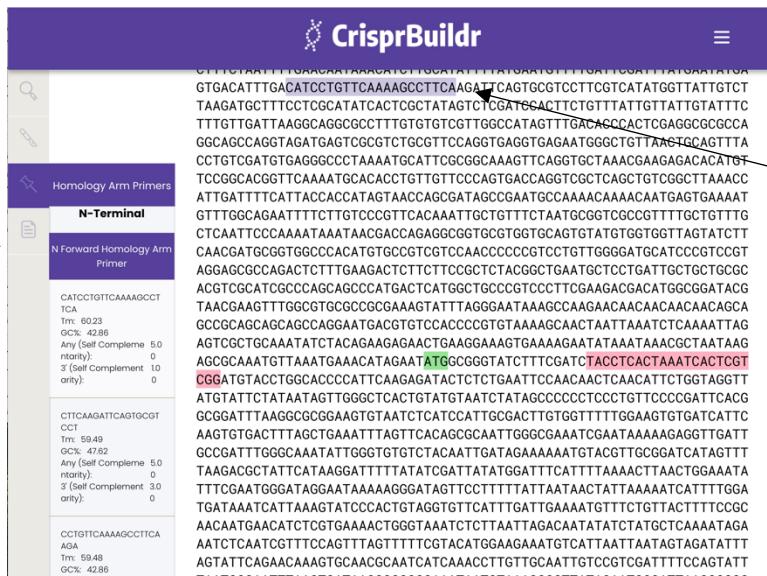
The selected site is highlighted in the sequence.

- 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. 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.



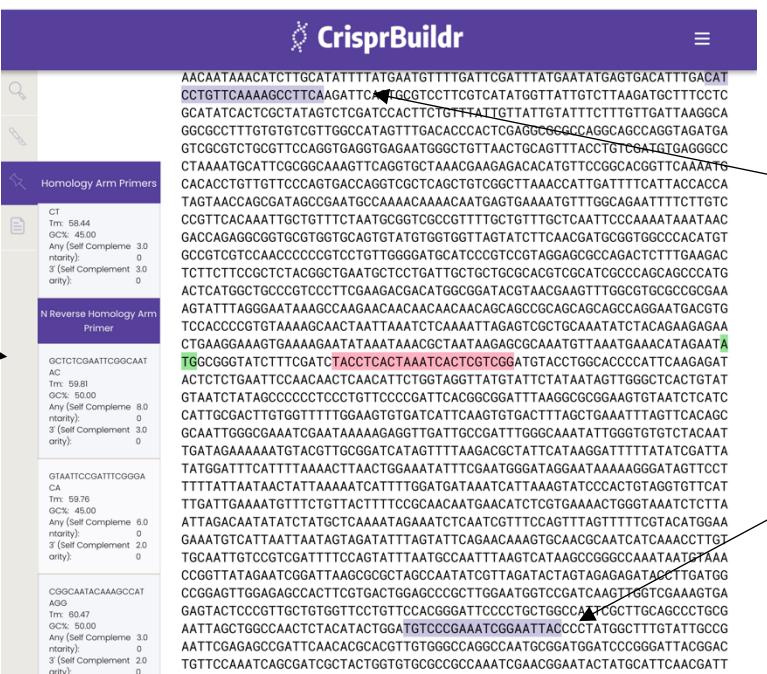
The images below only show the **primer selection for the N-terminus**. The process is the same for the C-Terminus

[Click here to select and lock-in forward amplification primers for the N-terminal homology arm.](#)



Selected forward amplification primer

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



Selected forward amplification primer

Selected reverse amplification primer

Click here to select and lock-in forward and reverse sequencing primers for the N-terminal and homology arms.

The screenshot shows the CrisprBuilder interface with the following details:

- Homology Arm Primers:**
 - Forward primer sequence: TTTTCTGTCCCGTTCACA
 - Tm: 59.14
 - GC%: 40.00
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 2.0
 - Arity: 0
- N Reverse Sequencing Primer:**
 - Forward primer sequence: GTTCAATTGTCGGAAAA
 - Tm: 59.71
 - GC%: 40.00
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 2.0
 - Arity: 0
- Guide Rna Vector:**
 - Forward primer sequence: GATGTCATTGTCGGAA
 - Tm: 59.52
 - GC%: 40.00
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 3.0
 - Arity: 0
- Plasmid Template:**
 - Forward primer sequence: ATGTCATTGTCGGAAA
 - Tm: 59.99
 - GC%: 38.10
 - Any (Self Complementarity): 0

On the right side of the interface, the selected forward amplification primer is highlighted in red, and the selected forward sequencing primer is highlighted in green.

Selected forward amplification primer

Selected forward sequencing primer

Selected reverse sequencing primer

Selected reverse amplification primer

- 1.7 Chose 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 at after a hypothetical successful targeting event.

The screenshot shows the CrisprBuilder interface with the following options for cloning maps:

- Download Data:**
 - View All Data:** A button with a purple background.
- Genomic Template:**
 - Download:** A button with a purple background.
- Guide Rna Vector:**
 - Download:** A button with a purple background.
- Plasmid Template:**
 - Choose A Template:** A dropdown menu with a purple border.
 - Download:** A button with a purple background.

Annotations on the left side of the interface indicate the following:

- (1) Displays all the information in a separate window.
- (2) Downloads a locus map before targeting.
- (3) Downloads a locus map after targeting.

On the right side of the interface, the selected forward amplification primer is highlighted in red, and the selected forward sequencing primer is highlighted in green.

CrisprBuilder



Download Data

[View All Data](#)

Genomic Template

[Download](#)

Guide Rna Vector

[Download](#)

Plasmid Template

Choose A Template

[pHD-DsRed-X](#)

[pHD-dsRed-attP-X](#)

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AGCAGCTAGACGAAGCACTCTGCAGGCCCTCAAGGGCGGTATGGAGCGCACAAAGGCCTACAACGGACA
AGCTGCAACTTGAGAACGGCTTCGAGGAATTGAGGTGCAACTGGAGGCCTCAAGGAAGAGCATCAA
AGCTGCTCGAGAGCGCTCAACAGCAGGACGTTCCAGTCCGGTTACACATCGAAGAGGTGCGG
TGCCCATGGGCCACCTCGGGTACAGCTACAACTGCAACACAGGCTGCTGCCAGTTGGCCAGA
GGGTGAACACATCATCTCCGATCTGGCATAGAAAGCGATGCCGCAGGATATCTAGCTGAGAAGTAT
CCAACGCCCACGCTGCGATGTTAACGACTGTAGAGATGAAAACCGGAGGATCACCGAGTCCAAAGGCAA
AGTCAGAGGGTAGTTGGATTAACCTGCCAGGAAGAGATCACCCAACACTAAAGTGTATTTCATA
CAGAACTCATCACCGACAGCAAGAGCACTGCAACTGTCAGGCCACAGTACACGACTGTGCCA
AGGTAGATCTGAAAACGCCAGTTGCCGCGCAAACTAATCCGCCAAAGCGCAGCTTGAAGACACCT
ACGAAAAGTTCGCTATGGCTAACAAAGCACAAGTGGAGAAAGAGCATCAAAATCAAATCTAA
AAACGCCAACATGTGCGGAAACGCTGCTAACATGGAGAATGTTATAAAGCTGCCGCCGACGTC
CAGTATATCAGCATTAACCCGAGGCCATTCCCCATGTCATTGCCATTCTGCCATTCTTATA
TTTGTATGCTATTGTTGTTCTGCTTCACTTGTATATTATCTGCCATTCTTATA
TTTTTTTTTTGTTAAAAGACCGTAAACACAGCAGCTATTAGTACGAAATACCCATGCTTTCTAA
AGACTGTTGAGGAATCGAACCCATTGTAATGAACTATCACACACACACACACACTGCTTTAAGC
ACAAAACGAAACTAACCTGAAACTTGTGAGTGTAAACCCGGCCTACATTCTAT
TGAATGACACATGTATTAGAATGTATATATATATCTCCCTAGTACATACTTTAAGCAATT
CTCTAGCATACTGCACCTGATTCTGCTCGCTAACCTGCTTAACCTTCAATTCTGCTGTAGTGC
TGCTGATTATGTATTCTCTGCTGCTTAACCTGCTTAACCTTCAATTCTGCTGTAGTGC
GATATTGAAATAATTGTTGACTTGCCTGGACAAAACAGTTAGCTACGTTGCTACATCCAAATACCCCTT
TGTGCGAACACATCCATTGTAACATGTAAAGTAGTGTGGCTTGTCTGGAGAACAGATCTAGAA
CTTAAGCTATTGCTACCTGCTTATTGAAATAATTGCTTAAAGTGTAGTGTGGCTTGTCTGGAGAACAGATCTAGAA
ATTAACTTGTGTTGGCTTGTCTGCTTAAAGTGTAGTGTGGCTTGTCTGGAGAACAGATCTAGAA
GGCGATCAAGACTAAAAATGCTATATATATATTTTACAATTATATTATCTTATAAGC
AAGTACTTTTAAACGGACTTTTGAAACGGCTCTGCTAGCGATATTGCTAGTGTGGCAACAGCTGTATG
AGGTAGCGCTGCCAGACTCTCAATTGCAAGTAAGGACAGACGCTGTAGTAAATTAAACCCAAAATGGCA
AAAAGCAAAATAACCGTCCGACTAATCAACTAACAGACAACACTCTTACGCCATTCTGGTTTACGCC
TTATCACTTATATTGATGTCAGCTGGTATACACATCGCATTATTACCCGGCTTAAGTGAAGA

```

2. Gene tagging

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



CrisprBuildr

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Search for a gene

X

Choose Your Operation

Tag

Proceed

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

The screenshot shows the CrisprBuildr web application. At the top, it displays the gene information: Gene: cnn - Isoform: cnn-RM - Strand: -. Below this, a modal window titled "Choose Your Tag" is open. Inside the modal, there are two options: "N Terminal" (which is checked) and "C Terminal". To the right of the modal, a portion of the DNA sequence is visible, starting with ATCTGTACATAAGTGCA. The "N Terminal" button is highlighted with a blue background and white text.

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 screenshot shows the CrisprBuildr interface with the "Select Cut Site" menu open on the left. The menu lists several potential cut sites with their sequences, efficiencies, strands, and off-target counts. An arrow points from the text "The menu on the left lists several cut sites." to the menu. On the right, a large portion of a DNA sequence is shown, with several red boxes highlighting specific segments of the sequence, indicating the locations of the selected cut sites.

The menu on the left lists several cut sites.

Sequence	Efficiency	Strand	Off Targets
TACCTCACTAAATCACTCGGG	3.66153	-	0
AAATGAAACATAGAATATGCCG	4.22992	-	0
GTTAAATGAAACATAGAATATGG	5.43676	-	0
ATCCGACGAGTGATTAGTGAGG			

Selected cut sites are highlighted in the sequence.

- 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.

The screenshot shows the CrisprBuilder software interface with the following details:

- Homology Arm Primers:**
 - Forward Amplification Primer:** TTTCTAATTTGAAACAATAAACATCTTCATATTATGAATGTTTGATTGATTCGATTTATGAATATGA
 - Forward Sequencing Primer:** GTGACATTGACATCCTGTTCAAAGCCTCAAGCTTCAGTGCCTCCTCGTCATATGGTTATTGTCT
 - Reverse Sequencing Primer:** TAAGATGCTTCTTCGATATCACTCGCTATAGTCTCGATCCACTCTCTGTTATTGTATTGTCT
 - Reverse Amplification Primer:** TTTGTTGATTAAGGCAGGCCCTTGTGTGCTGGCATAGTTGACACCCACTCGAGGCCGCC
- N Reverse Sequencing Primer:** CCTGTCGATGTGAGGGCCCTAAATGCATTCGGGAAAGTTCAGTGTAAACGAAGAGACACATGT
- Other Primers:**
 - GATTCATTGTTGCCGAAAGA
 - Ttt: 59.14
 - GC%: 40.00
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 0
 - 4' (Self Complementarity): 0
 - TGTCATTGCGCCGAAAGTAAAGGAAAGTGAAGGAAAGAATATAAAACGCTAAAG
 - Ttt: 59.71
 - GC%: 40.00
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 0
 - 4' (Self Complementarity): 0
 - GATTCATTGTTGCCGAAAGA
 - Ttt: 59.52
 - GC%: 40.00
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 0
 - 4' (Self Complementarity): 0
 - ATGTCATTGCGCCGAAATTTGGGTGTCTACATTGATAGAAGAAATGACGTGCGGATCATAGTT
 - Ttt: 59.99
 - GC%: 38.10
 - Any (Self Complementarity): 0
 - 3' (Self Complementarity): 0
 - 4' (Self Complementarity): 0

Annotations on the right side highlight specific primers:

- Selected forward amplification primer for the N-terminal homology arm:** The forward amplification primer (top) and its complementary strand (bottom).
- Selected forward sequencing primer:** The forward sequencing primer (middle).
- Selected reverse sequencing primer:** The reverse sequencing primer (bottom).
- Selected reverse amplification primer:** The reverse amplification primer (bottom).

- 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 and download a locus map that shows the chosen deletion vector at after a hypothetical successful targeting event.

 **CrisprBuildr**



Download Data

[View All Data](#)

Genomic Template

[Download](#)

Guide Rna Vector

[Download](#)

Plasmid Template

[Choose A Template](#)

N terminal SSPB and mCherry tag

N terminal EGFP and SSPB tag with Extended Linker

N terminal EGFP and SSPB tag

ACGTGCGATGCCAGGCCCATGACTCATGGCTGCCGCTCCCTGAAGAGCACATGGCGGATACG
TAACAAGATTGGCGGCCGAAAGTATTAGGAATAAAGCCAAGAACACAAACAGCA
GCCGCAGCAGCAGCCAGAACATGCTGCCACCGCTGAAAGCAACTAATTAAACTCAAATTAG
AGTCCTGAAATATCTACAGAAGAGAACTGAAGAAAAGTAAAGAATAATAAACGCTAATAAG
AGCCAAATGTTAAAGAACATAGAATGGCGGATCTTCGTCATCCTGCATCCTGGTACCTCTGGTACCTG
CGGATGTACTTGCACCCATTCAAGAGACATCTCTGAATTCCAACTACACATTCTGGTAGTT
ATGATTCTATAATAGTGGGCTACTGTATGTAATCTATAGCCCCCTCCGTGCCCCGATTACG
GGCGATTAAAGGCCGGAAGTGAATCTACATCCATTGGGACTATGGTTTTGGAGTGTGATTAC
AAGTGTACTTGCTGAAATTGGCTACAGGCAATTGGGCAAGTCAAGAAAAGAGGTT
GGCATTGGGCAAAATTGGGTGTCTCACAAATTGATAGAAAAAATGTACGGTGGGATCATAGTT
TAAGGCCATTCTACAAAGTTTATGCTGTTATTGATGGTTTATTAAACTAAGTGGAAATA
TTTCGAATGGGATAGGAAATAAAAGGGATAGTCTCTTATTAACTAATTTAAACTATTGG
TGATAAATCTAAAGATCTTCACTGTAGGTGTCATTGATTGAAATGTTCTGTTACTTTCCGC
AACAAATGACATCTGGTAAACCTGGTAACCTTCAATTGACAAATACTATCTCTCAAATAAG
AACTCTAATGTTCTGGATTAGTCTTCTGATCAAGTGGAAAGTACATTAATTAGTAGATATT
AGTATTCAAGAACAAAGTCAAGCGCAATCATCAAACCTGTGCAATTGCGTGTGATTTCAGATT
TAATGCCATTAACTTGAAGCCGGGCAAAATACTGAAAGCCGTTTATAGAATGGGATTAAGCCGC
TACCGAAATCTGTTAGACTAGTAGAGAGATACTTGGCGGAGTGGCGGAGGACCTCTGG
TGGAGCCGCTGGATGGTCCGATCAAGTGGTGCAGAAGTGGAGACTCCGGTGTGTTCT
GTTGGGAGGATTCCTCCGTCGCTTCCCTGGGACCCCTGGAAATTGCGGAACTCTACAC
TGGATGTCGGAAATGGCAATTCCCTATGGCTTGTGTTGGGAGGACCTTACACAGC
CAGCTTCTGCCAGGCGGAAATGCCGATGGATCCGGGATTACGGACTGTTCAATCAGCGATCGCTA
CCGGCAAACTGAGCGGAATCTACTATCTTCAAGGATCTGGGATTCAGGACTGTTCAATCAGCGATCGCTA
CGACATCACCATGGCGCTGCGATGGCGGCCGCTTCAAGGACACAAAGCGGCTGGAGC
TTTATCCGGAGTTCTGCAATGGCACATTGGATGCCATGATGTGTTCCAGGCCAGG
AAAGCCGCAAGACATGCGGAGCTGGCGAGGCTCAGAGGAACTTGGCAAGGATCTGGCC
ATTAGGTCTCATCTGGCAAAATGAGAATTATGTTGCAAGGAAATTAAATCTTAAACTGGTAAA
GTGCACTGTTTATGGATGTTGTCGAGGTACACAGCGAGTGTGCTTTAGTGCAGCTTAAT
AACAGATTCT
CATGGAGAAGCTCATGTAAGTTCAGGATGATTCAGGAAATTCAGGCAAAATTCTGG
ACTCTGGTTAGAATTGAGAGATCTTGTGAAATTGCGCTGTTTCCACTGCTTCAAGCAGCTT
GAACATTATGTTTATGGAGGAAACCTGGCATGATATTAGGATTCTGGCTTGGCAACACA
CGAGCATTCAGCAATTCTGGCGCAGCTGGCTGTTTATAGTGTGTTCTCCAC
GGCTAGGAGAAGCTAAATCATGAATGAGTAACTGAGCTGGCTTCCGCACAACTGG
AAGATTCTTCTGAGTCAAGAAGAACCTGGTATTAGTCTGAGACTCTAAATCAATCC
TGTGTTCTTGGAGTCACTGGCTTCCAGGACCTCCGGCGGCCGACACTGGCTTGG
AAGGCTTCTGGAGTCACTGGCTTCCAGGACCTCCGGCGGCCGACACTGGCTTGG

Chose the tagging vector and download the plasmid map.

3. Other features

The screenshot shows the CrisprBuildr interface. At the top left is the logo and the title "CrisprBuildr". On the right is a navigation menu with a three-line icon, followed by a link "Click here for additional features." A dropdown menu is open from the three-line icon, listing "New Project", "Upload Project", "Save Project", "Switch to Dark Theme" (which is selected, indicated by a black dot), "Font Size", and "Bug Report".

The main area displays a DNA sequence editor. The sequence is shown as a long string of letters (A, T, C, G) with several regions highlighted in different colors: green, red, pink, and purple. A cursor is positioned over a green-highlighted segment.

To the right of the sequence editor is a "Report a Bug!" form. It includes fields for "Full Name" (with "First Name" and "Last Name" sub-fields), "E-mail" (with an example provided), and "Type:" (with options for "Bug" and "Feature Suggestion").

At the bottom right is a "Jotform" logo with the tagline "Create your own Jotform".