Designing gRNA using CRISPRdirect

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

This protocol is designed to help guide the user through the use of CRISPRdirect to design gRNA for CRISPR/Cas9 applications in the model mouse organism Mus musculus. The target gene for these gRNA is the IGF1 gene, more specifically the C-domain located within Exon 3.

The planned edits to be made requiring the gRNA to be designed, are at positions 83-84 respectively within the C-domain. These edits are SS -> GN.

PROTOCOL CITATION

Gatesgibson 2020. Designing gRNA using CRISPRdirect. **protocols.io** https://protocols.io/view/designing-grna-using-crisprdirect-bpnnmmde

KEYWORDS

CRISPR, gRNA, CRISPRdirect, Benchling

IMAGE ATTRIBUTION

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DISCLAIMER:

This protocol is intended for the Schwartz lab

ABSTRACT

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Software used

1 In this protocol, the software used to generate both inputs and outputs are CRISPRdirect and Benchling

CRISPRdirect
by Naito Y, Hino K, Bono H, Ui-Tei K.

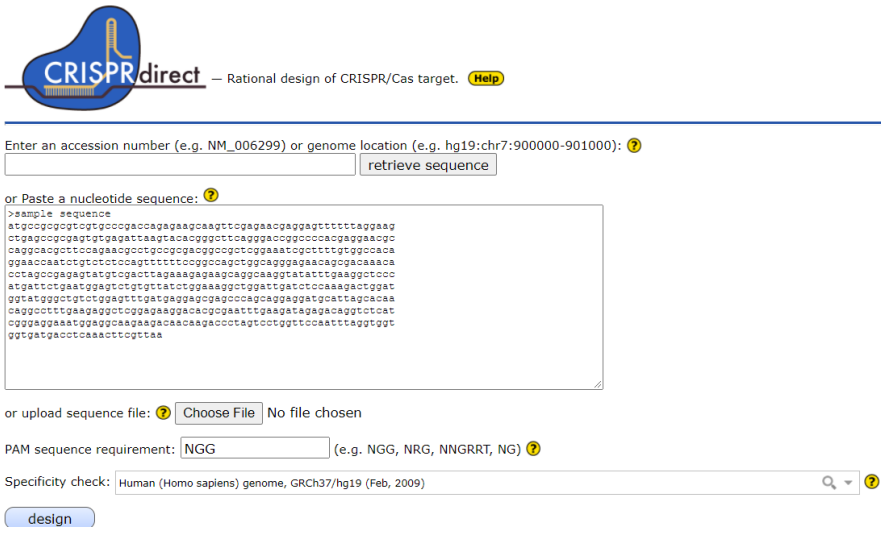
Benchling

2 Using Benchling, the user can easily access the genetic information needed to begin designing gRNA using the selected gRNA software.

In this example, the gene in question is the IGF1 gene in the model organism Mus musculus.. More specifically, the C-domain within Exon 3.

CRISPRdirect Input

3



In the above image, there exists:

- Help page (top right)
- Input boxes (accession numbers or raw sequences)
- sequence file upload
- PAM entry
- Host organism selection

If any of the above is not familiar or creates confusion, the "**help**" button provides a detailed description on how to use this software, including a tutorial video.

3.1 The gRNA design process should begin by inputting your selected (to be edited) sequence through one of the three entry methods. (In this example, the raw FASTA format was used)

FASTA format:
This should be preceeded by the following heading in the insert box

">[insert desired output name]"

entering down to the next line, the user will paste the DNA sequence. This is where knowledge about the aforementioned gene sequence in question is recommended before use of the gRNA design software. For this example, Benchling was used to identify the target area and the sequence pasted in the entry box was derived from there.

In this example, the following should look like

>Mus musculus IGF1 C-domain

GGCTATGGCTCCAGCATTCGGAGGGCACCTCAGACA

3.2 Next, it is imperative to select the proper PAM sequence for gRNA design (An arbitrary sequence using IUB codes (N, R, Y, ...) can be specified.)

In this example, CRISPRdirect will be using the default **PAM - NGG**

3.3 Lastly for the input section, the host organism should be selected from the dropdown menu. In this experiment, the host organism is **Mouse *(Mus musculus)* genome, GRCm39/mm39 (Jun, 2020)**

Hit the design button

CRISPRdirect Output

4 **Results:**

Sequence name: Mus musculus IGF1 C-domain
PAM sequence: NGG
Specificity check: Mouse (Mus musculus) genome, GRCm39/mm39 (Jun, 2020)
Time: 2020-11-13 05:39:36

- Highlighted target positions (e.g., **45 - 67**) indicate sequences that are highly specific and have fewer off-target hits.
- Target sequences with '0' in '20mer+PAM' (in number of target sites column) are shown in gray. Such sequences may possibly span over exon-exon junctions, so avoid using these.
- Target sequences with TTTTs are also shown in gray. Avoid TTTTs in gRNA vectors with pol III promoter.

☐ show **highly specific** target only

Show 20 entries Search:

position		target sequence	sequence information				number of target sites		
start - end	+ −	20mer+PAM (total 23mer)	GC% of 20mer	Tm of 20mer	TTTT in 20mer	restriction sites	20mer +PAM	12mer +PAM	8mer +PAM
2 - 24	+	GCTATGGCTCCAGCATTCGG AGG [gRNA]	60.00 %	77.32 °C	-		1 [detail]	8 [detail]	634 [detail]
3 - 25	+	CTATGGCTCCAGCATTCGGA GGG [gRNA]	55.00 %	75.20 °C	-		1 [detail]	35 [detail]	743 [detail]
11 - 33	-	CCA GCATTCGGAGGGCACCTCAG [gRNA]	65.00 %	79.05 °C	-	BmeI580I	1 [detail]	4 [detail]	1338 [detail]

Showing 1 to 3 of 3 entries First Previous 1 Next Last

Graphical View:

```
        10        20        30
GGCTATGGCTCCAGCATTCGGAGGGCACCTCAGACA
 >>                              <
```

Data Export:

- Tab-delimited text: Open in new window | Download
- JSON format: Open in new window | Download

Tab-delimited text can be copy-pasted into spreadsheet softwares (e.g. Excel) or text editors.

```
# [ CRISPRdirect | 2020-11-13 05:39:36 ]
# sequence_name:        Mus musculus IGF1 C-domain
# pam_sequence: NGG
# specificity_check:    Mouse (Mus musculus) genome, GRCm39/mm39 (Jun, 2020)
# start end     strand  sequence        GC      Tm      TTTT    RE_sites        hit_20mer
hit_12mer       hit_8mer
#
2       24      +       GCTATGGCTCCAGCATTCGGAGG 60.00   77.32   0               1       8       634
3       25      +       CTATGGCTCCAGCATTCGGAGGG 55.00   75.20   0               1       35      743
11      33      -       CCAGCATTCGGAGGGCACCTCAG 65.00   79.05   0       BmeI580I        1       4
1338
```

In the above image, there exists:

- A recap of the selected input
- position in the selected sequence
- target sequence for gRNA design
- GC%
- Tm
- Restriction sites if applicable

- Number of sites
- graphical view
- Data export

comparable to the input page, there exists a **"?"** button next to the **"results"** header that will explain the output in a detailed manner. Another tool for information is the **"?"** button next to the **"number of sites"** header that explains the relative importance of the numbers given in that sections.

In this example, CRISPRdirect generated 3 results for the search inquiry.

4.1 Based on the position of the edit you'd like to make, the results given may be more or less useful to your experimental protocol. In this example, the first two results show PAM sites on the left-most ends of the + strand. This placement may be too far downstream from the planned edit and therefore the third option was picked as it places the complex

4.2 Lastly, next to the target sequence is a [gRNA] button that loads the guide-RNA for the selected location. In this example, the third gRNA was chosen at is is closest to the selected site for editing. Likewise, this choice has the added bonus of a restriction enzyme site which may be used for future efficacy studies.