

CRISPR Design tool FAQ

1. What is the difference between “Knockout” and “Site-specific editing” applications?

The “Knockout” application should be used when knocking out the function of a protein via non-homologous end joining (NHEJ) is desired, and where the guide RNA targets the gene of interest is not of significant concern. Results generated with this tool prioritize the expected functionality and specificity of the guide RNA designs returned.

“Site-specific editing,” on the other hand, allows users the ability to easily search for guide RNAs specific to a unique location within the gene of interest. This is necessary for knock-in applications where the cut site location is critically important as it relates to the donor sequence and overall efficiency of the knock-in event. In this application, the functionality metric combines the general guide RNA functionality score and adds on a distance-weighted effect based on how many bases from the ideal cut site the guide will cut.

2. If I already know the guide RNA sequence required how can I order it?

Copy your targeting sequence(s) into [this tool](#).

3. I am attempting to find a guide that cuts near a specific location, but no guide options are showing up; what should I do?

A common reason for this result is a lack of a PAM with genomic uniqueness in the region of interest. You have two options:

- You can adjust where the desired cut site is and see if the tool can generate guide RNA options that target further upstream/downstream of your ideal edit location.
- If that is not an option, you can change the nuclease the design tool uses to base its design criteria on. The default nuclease is the standard, well-studied, and highly functional *S. pyogenes* Cas9. You can adjust this to *S. aureus* Cas9, or MAD7 nucleases. Note, going this route will require sourcing of non-SpCas9 nucleases to use along with the non-SpCas9 guides; therefore this should be attempted only after adjusting the cut site.

To change the nuclease and re-run the design tool click the link in the pop-up window:

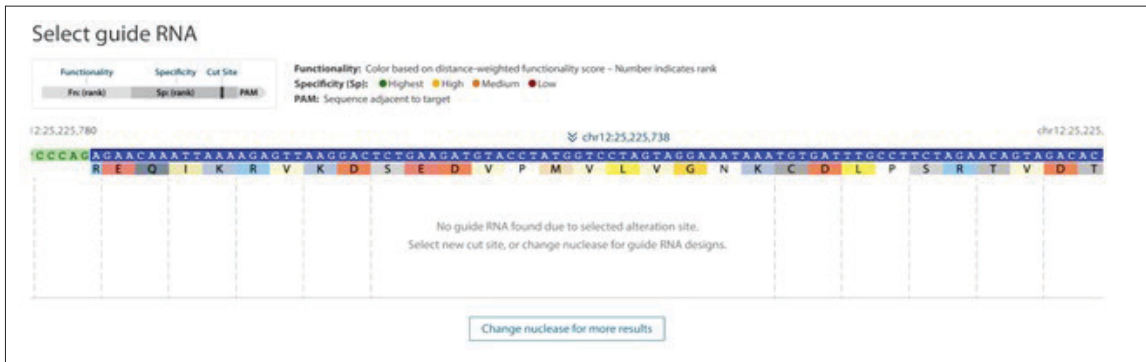
No Guide RNA Found

No CRISPR guide RNA options found within 50 bases of the alteration site. Please choose another alteration site.

For help in setting design parameters please [reach out to our Technical Support team](#).

[Try Advanced Options](#)

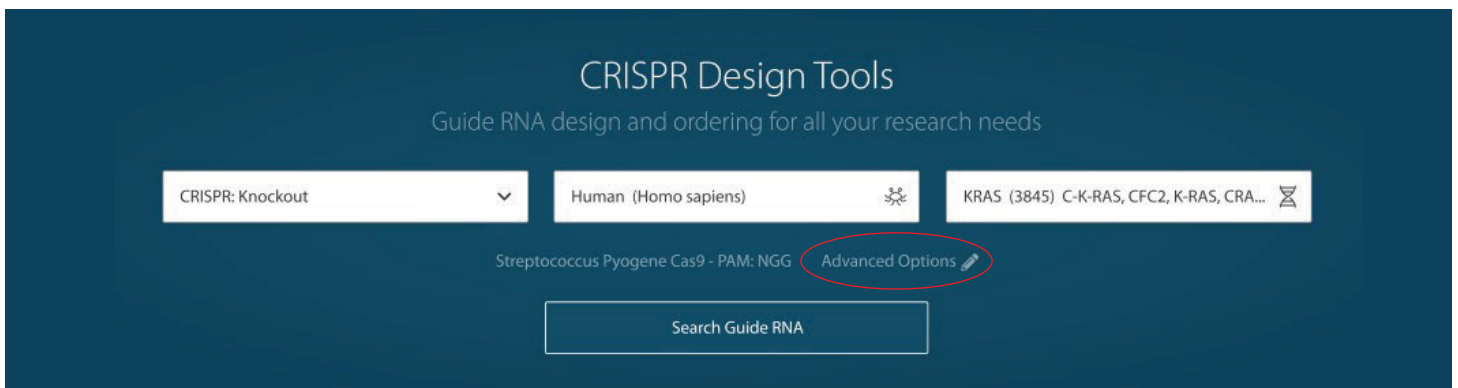
Or, if you closed the pop-up window, you can click the “Change nuclease for more results” button towards the bottom of the screen:



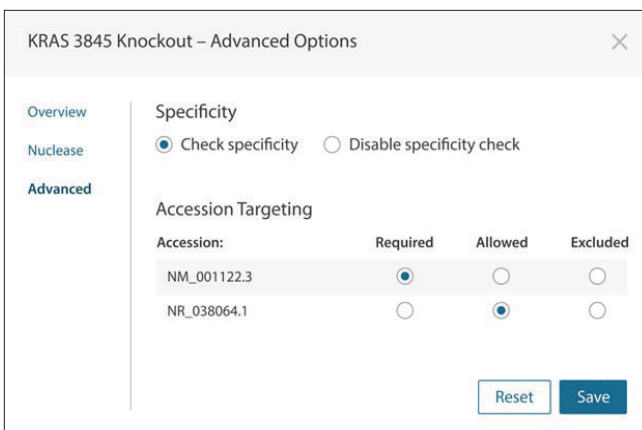
4. My gene has both coding and non-coding transcripts, and I want to design guides that only target the non-coding transcripts; how do I do that?

Our knockout algorithm prioritizes designs within the coding region and avoids non-coding transcripts.

If you want only to target non-coding transcripts, you can do so using the Advanced Options link on the CRISPR Design Tool homepage when conducting a new search.



Once the Advanced Options window is opened, you can specify which transcripts you want to require, allow, or wholly exclude for generating guide designs. In the example of only wanting to return non-coding transcripts, you would “Require” those while “Excluding” any coding transcripts.



5. If I want unmodified synthetic guides, how can I package my designs into that format?

You will need to know the 20nt targeting sequence for your guide RNA design(s). Once you have those sequences, you can paste them into [this tool](#) and select the unmodified version of synthetic crRNA or sgRNAs, as shown below.

Synthetic sgRNA crRNA All-in-one Lentiviral sgRNA Lentiviral sgRNA

Edit-R Modified Synthetic sgRNA

Custom0322 2 nmol - \$199.00

Add To Cart Generate Custom Plate

Edit-R Synthetic Custom sgRNA

Custom0286
Unit Size
2 nmol
Price
\$199.00
Add To Cart

6. I am using the 'Knockout' application, but no guides show up for my gene of interest; what should I do?

A common reason for this happening is specific to coding genes. If all coding transcripts are set to be “required” in the Advanced Options window, and not all of the transcripts overlap with each other, it's impossible to design a guide that targets all transcripts. In those cases, setting one or more coding transcripts to “allowed” enables designs to be generated.

To do this, return to the design tool homepage and open the Advanced Options window—click where the red box is below,

You can then specify which transcripts you want to require and those you will allow; this should loosen the design criteria, thus enabling the gene of interest to have designs available.

CRISPR Design Tools

Guide RNA design and ordering for all your research needs

CRISPR: Knockout Human (Homo sapiens) KRAS (3845) C-K-RAS, CFC2, K-RAS, CRA...

Streptococcus Pyogenes Cas9 - PAM: NGG Advanced Options

Search Guide RNA

KRAS 3845 Knockout - Advanced Options

Overview Nuclease Advanced

Specificity

Check specificity Disable specificity check

Accession Targeting

Accession:	Required	Allowed	Excluded
NM_001122.3	<input checked="" type="radio"/>	<input type="radio"/>	<input type="radio"/>
NR_038064.1	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>

Reset Save

7. How are specificity and functionality scores assigned?

Details on the Edit-R algorithm and how raw [functionality](#) & [specificity](#) scores are calculated can be found by following their respective links. From there, we bin the scores into Highest, High, Medium, and Low buckets using defined cutoff values.

Please note that the design rules only apply to SpCas9. If you design guides using an alternative nuclease, we will only return a specificity score as functionality is nuclease dependent.

8. What is a PAM, and is it included in my guide RNA design?

The PAM, or protospacer adjacent motif, is a short sequence of bases located in the genomic DNA, adjacent to the guide RNA targeting region. All gene editing nucleases have a specific sequence and location of the PAM. The PAM is required to recruit the nuclease & destabilize nearby bases allowing the targeting sequence of the guide RNA to bind.

The genomic DNA target sequence requires a PAM of the correct sequence, and location relative to the guide RNA's targeting sequence. When using SpCas9, the PAM is located immediately downstream of the 20 nucleotide target sequence and has a sequence of NGG (where N is any base). Also, the guide RNA includes a constant region that helps it bind to the nuclease. The sequence and length of this constant region depends on the specific nuclease and whether a single guide RNA or two-part system (crRNA & tracrRNA) is used.

9. Is it okay if I design guide RNA is complementary to the target DNA strand?

The CRISPR-Cas9 mechanism cuts both DNA strands. Therefore, it is possible to target a region using a guide RNA that targets either DNA strand. What is essential is ensuring the PAM is in the correct orientation relative to the target sequence used.

10. What is the difference between modified and unmodified guide RNA?

Modified synthetic guide RNA has been shown to increase overall editing efficiency by improving the guide's nuclease resistance. Because of this, we strongly recommend using modified guides.

- For our modified synthetic sgRNAs, - the first two 5' nucleotides and 3' nucleotides are 2'-O-methyl with phosphorothioate linkages in the backbone (2xMS).
- For our modified crRNAs, the first two 5' nucleotides are 2'-O-methyl with phosphorothioate linkages in the backbone (2xMS). For the associated, modified tracrRNA, the first two 3' nucleotides are also 2xMS, resulting in a similar modification pattern to our synthetic sgRNAs.

The minimal modification pattern used has not shown a detrimental effect on the efficiency of editing or cell viability in a large number of cell lines. However, if your cell line is unusually sensitive to these style of modifications, unmodified sgRNAs or crRNAs can also be purchased.

11. How much synthetic guide RNA do I need for my experiment?

This table provides the approximate number of experiments that can be carried out for lipid transfection methods at the recommended synthetic sgRNA working concentration (25 nM) in various plate/well formats. Calculations do not account for pipetting errors.

Synthetic sgRNA nmol	96-well plate 100 μ L reaction volume	24-well plate 500 μ L reaction volume	12-well plate 1000 μ L reaction volume	6-well plate 2500 μ L reaction volume
2	800	160	80	32
5	2000	400	200	80
10	4000	800	400	160

*Note, if using synthetic crRNA & tracrRNA instead of synthetic sgRNA, you will use equal molar amounts of crRNA & tracrRNA to generate the full crRNA:tracrRNA complex, and at that point, you can follow the estimates shown above.

12. Where do I find Cas9 nuclease?

We offer many formats of Cas9 nuclease; they can be found at the links below:

- [Cas9 protein](#)
- [Cas9 mRNA](#) (including fluorescent mRNA options)
- [Lentiviral Cas9](#) (includes fluorescent and inducible lentiviral options)
- [Cas9 stable cell lines](#)

For more information

To find the contact information in your country for your technology of interest, please visit us at horizondiscovery.com/contact-us

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