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Dharmacon™ CRISPR Design Tool

The CRISPR Design Tool allows you to quickly and easily generate guide RNA sequences for ordering either synthetic single guide RNA (sgRNA), crRNA, or lentiviral sgRNA (Figure 1).

There are several options for designing guide RNAs:

- **Input a gene ID or gene symbol: design anywhere within gene.** Generate guide RNA designs to target a known gene of interest. The gene target must be from a species that is supported by the CRISPR RNA Design Tool (Table 1).
- **Input a gene ID or gene symbol: specific cleavage location for HDR.** Generate guide RNA designs in a specific region to facilitate HDR and knock-in experiments. Weighted functionality and specificity scoring is applied. The gene target must be from a species that is supported by the CRISPR Design Tool (Table 1).
- **Provide a DNA region for design.** Generate guide RNA designs to target a particular DNA sequence; often used for non-standard species or to target particular gene regions.
- **Input my own guide RNA sequence.** You can input your own nucleotide target sequence for custom synthesis of synthetic sgRNA(s), crRNA(s), or generation of lentiviral sgRNA(s).

The CRISPR Design Tool supports guide RNA design for targeting protein, microRNA or noncoding RNA gene loci (Table 2). In addition, the CRISPR Design Tool Advanced Settings allow for customization of criteria such as the protospacer-adjacent motif (PAM), transcripts targeted, and GC percentage, among others. By default, the CRISPR Design Tool will select guide RNA designs that target all transcripts of the selected gene.

Guide RNA designs are included for only NGG PAM-adjacent sites and for GC percentages between 20 and 80%. The Specificity Check performs a rigorous alignment that excludes from results any guide RNAs that have PAM-adjacent target sites (NGG/NAG) with two or fewer mismatches or gaps elsewhere in the selected genome.

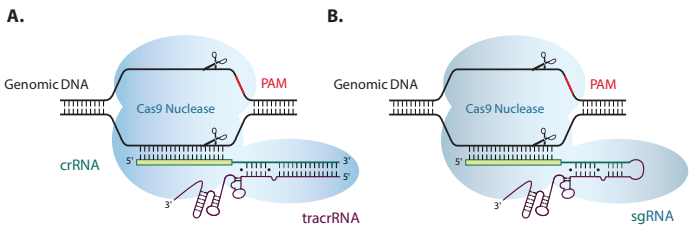

Figure 1. The Dharmacon™ Edit-R™ CRISPR-Cas9 platform includes two general approaches based on *Streptococcus pyogenes* CRISPR-Cas9: A. The native two-part guide RNA approach, requires a long, chemically synthesized trans-activating CRISPR RNA (tracrRNA, purple) and a chemically synthesized guide RNA (crRNA, green) in addition to Cas9 nuclease (as a plasmid, mRNA, lentivirus, or protein (light blue)). **B**. The sgRNA approach utilizes a long transcript that fuses elements of the tracrRNA and crRNA into a single molecule that is either chemically synthesized or expressed from a lentiviral vector. The synthetic sgRNA method is similar to the 2-part guide RNA approach where a Cas9 nuclease (plasmid, mRNA, lentivirus, or protein) is required in addition to the guide RNA.

Viewing results

Depending on the design option selected, results are presented as either a List or Graphical view. Results are sorted by earliest to latest position in the target gene or DNA region. Specificity rankings and the abovementioned filters are applied but there is no scoring from the Edit-R algorithm for functionality. It is recommended to test 3-5 different designs to find one that is most efficient. If you have an understanding of the functional domain(s) of your target gene, select designs across those exon(s). If not, choose targets in more than one exon, but always including an early exon for a better chance of disrupting translation.

Table 1. Species with integrated gene identifiers and genome-wide alignment capability in the CRISPR Design Tool. The following species may be selected in the "Organism" field when specifying the source species for a particular gene target, and for a genome-wide specificity check to ensure the resulting guide RNA(s) only has a perfect match to the intended target, and has two or more mismatches to other regions of the genome.

Common name	Scientific name
Human	*Homo sapiens*
Mouse	*Mus musculus*
Rat	*Rattus norvegicus*
Zebrafish	*Danio rerio*
Western clawed frog	*Xenopus tropicalis*
Fruit fly	*Drosophila melanogaster*
Chinese hamster	*Cricetulus griseus*
Pig	*Sus scrofa*
Cow	*Sus scrofa*
Marmoset	*Callithrix jacchus*
Dog	*Canis familiaris*
Roundworm	*Caenorhabditis elegans*
Sea Squirt	*Ciona intestinalis*
Horse	*Equus caballus*
Cat	*Felis catus*
Fugu	*Takifugu rubripes*
Chicken	*gallus gallus*
Stickleback	*Gasterosteus aculeatus*
Opossum	*Monodelphis domestica*
Ferret	*Mustela putorius furo*
Nile tilapia	*Oreochromis niloticus*
Platypus	*Ornithorhynchus anatinus*
Rabbit	*Oryctolagus cuniculus*
Medaka	*Oryzias latipes*
Sheep	*Ovis aries*
Bonobo	*Ovis aries*
Chimp	*Pan troglodytes*
Baboon	*Papio Anubis*
Orangutan	*Pongo pygmaeus abelii*
Rhesus	*Macaca mulatta*
Alligator	*Alligator mississippiensis*
Purple sea urchin	*Strongylocentrotus purpuratus*
Zebra finch	*Taeniopygia guttata*

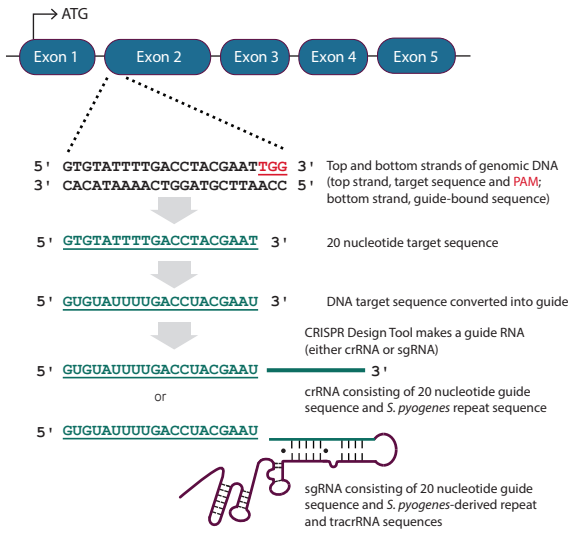

Figure 2. Example of how the CRISPR Design Tool will select a 20 nucleotide sequence targeting the human gene PPIB. The target sequence can be located on either strand of the genomic DNA as long as it is in the 5' to 3' orientation and there is a NGG PAM on the 3′ end of that strand. Cas9 nuclease will cut both strands of DNA at the position three nucleotides upstream of the NGG PAM. It is suggested to choose a target site located entirely within an early constitutive exon of the coding gene, but the CRISPR Design Tool will return results from across the entire coding region so particular exons or protein domains can be targeted, if desired.

Table 2. Default design parameters for Input a gene ID or gene symbol[1]

Type of locus	Targeting
Protein coding	Include all protein-coding transcripts and exclude all non-coding transcripts Require cleavage in the coding DNA sequence (CDS)
microRNA	Include all noncoding transcripts and exclude all protein-coding transcripts Cleavage allowed anywhere in the transcript
Long noncoding RNA	Include all noncoding transcripts and exclude all protein-coding transcripts Cleavage allowed anywhere in the transcript

In some cases, no guide RNA design results are returned using the default parameters for **Input a gene ID or gene symbol: design anywhere within gene**. Example genes where default settings returned no results are shown in Table 3 along with troubleshooting strategies for obtaining guide RNA designs. When using **Input a gene ID or gene symbol: precise cleavage for HDR**, if no designs are displayed you may need to scroll left or right. You will see an error message if there is not an NGG PAM 50 nt in either direction of the designated alteration site. It is not recommended to design guide RNAs outside of this range due to poor experimental efficiency.

Table 3. Troubleshooting for genes that return no designs with design option: Input a gene ID or gene symbol: design within gene default parameters

Reasons for no designs	Corrective action	Considerations	Examples
Gene consists of overlapping coding and noncoding transcript(s) and coding transcripts(s)	In Advanced Options, check the box to allow the non coding (NR_** RefSeq Accession) transcript	Noncoding and protein-coding transcripts will likely both be targeted in the gene editing experiment.	Gene locus: Protein-coding Species: Human Gene symbol: *BRCA1* 
Gene sequence is not annotated with untranslated and coding DNA regions	In Advanced Options, uncheck box "Require cuts to be in CDS"	Gene annotation may be incomplete in reference sequence databases. Manual sequence examination may be necessary to ensure targeting within the protein coding region.	Gene locus: Protein-coding Species: Medaka Gene symbol: *tac1* 
Gene sequence shares identity with other target(s) in genome of targeted organism	In Advanced Options, uncheck box for Specificity Check	Designs without Specificity Check are very likely to off-target closely related sequence.	Gene locus: Protein-coding Species: Horse Gene symbol: *SAA1* Gene locus: Protein-coding Species: Sheep Gene symbol: *BAMBI* 
The transcript occurs in two or more distinct genomic sites	Go to Advanced Options and select "Allowed" for all transcripts or design to each transcript individually (it may be necessary to remove specificity checking if transcripts are closely related or identical)	Designs returned may target any or all sites.	Gene locus: Protein-coding Species: Chicken Gene symbol: *FOXN4* Gene locus: Protein-coding Species: Pig Gene symbol: *ARSE* 
Selected target gene does not correspond to the selected parameters (for example, protein-coding locus selected, but noncoding gene ID entered)	Ensure that the selected design configuration is aligned with the gene target type selected (protein-coding, long noncoding, or microRNA) the gene target type selected (protein-coding, long noncoding, or microRNA)	Genes may consist of protein-coding (NM_** RefSeq Accession) and/or noncoding (NR_** RefSeq Accession) transcripts. Ensure that guide designs correspond to intended experiment.	Gene locus: Noncoding Species: Cow Gene symbol: *MEG3* Gene locus: Noncoding Species: Rhesus Gene symbol: *MIR155HG* 

If you have any questions, contact

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