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Edit-R™ CRISPR Design Tool

The Edit-R™ CRISPR Design Tool allows you to quickly and easily generate guide RNA sequences for ordering either synthetic and lentiviral guides.

There are several options for designing guide RNAs:

- **Gene Knockout: Input a gene ID or gene symbol design anywhere within gene.** Generate guide RNA designs to target a known gene of interest. (Figure 1) The gene target must be from a species that is supported by the CRISPR RNA Design Tool (Table 1).
- **Site-specific editing: 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 Edit-R™ 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).

Edit-R™ CRISPR Design Tool Advanced Settings allow for customization of criteria such as nuclease, transcripts targeted, and specificity analysis. By default, the Edit-R™ CRISPR Design Tool will select guide RNA designs that target all transcripts of the selected gene.

The Specificity Check performs a rigorous alignment that excludes results from any guide RNAs that have PAM-adjacent target sites with two or fewer mismatches or gaps elsewhere in the selected genome.

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. Functionality (when using the default *S. pyogenes* Cas9 nuclease) and Specificity rankings, as well as location filters are available to aide in guide RNA design selection.

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.

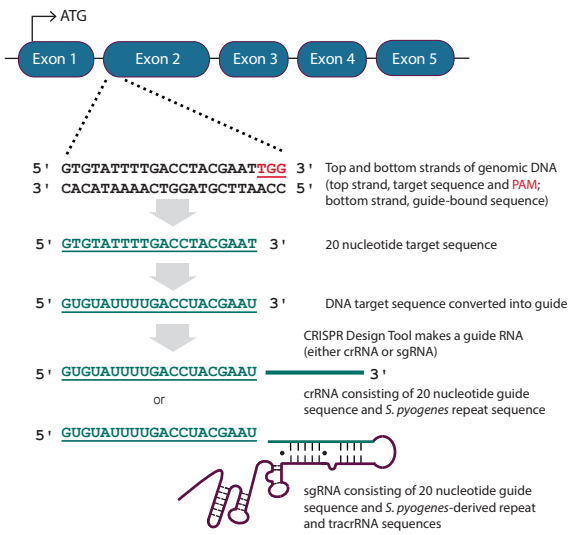

Figure 1. Example of how the Edit-R™ CRISPR Design Tool will select a 20 nucleotide sequence targeting the human gene PPIB. The target sequence can be located on either strand of the genomic DNA as long as it is in the 5' to 3' orientation and there is a NGG PAM (when using the default S. pyogenes Cas9) 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 Edit-R™ CRISPR Design Tool will return results from across the entire coding region so particular exons or protein domains can be targeted, if desired.

Table 1. Species with integrated gene identifiers and genome-wide alignment capability in the Edit-R™ 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	*Bos taurus*
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	*Pan paniscus*
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*
Macaque	*Macaca fascicularis*
Gorilla	*Gorilla gorilla*
Japanese Rice	*Oryza sativa subsp. Japonica*
Maize	*Zea mays*
Soybean	*Glycine max*
Apple	*Malus domestica*
Cotton	*Gossypium hirsutum*
Green Alga	*Chlamydomonas reinhardtii*

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

Type of locus	Targeting
Protein coding	Include all protein-coding transcripts and allow 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

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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