# Clone Manager 11

## CRISPR Knockout Method

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) is a powerful genetic modification technique adapted from a simple bacteria immune-like system. The system uses a specially designed nucleotide guide sequence to direct action at a specific gene target. The guide sequence works with a Cas enzyme (CRISPR associated protein) to introduce a double strand cut in the target. The cut is then inefficiently repaired, using non homologous end joining, resulting in a deletion, or insertion, of base(s) at the target site. The net effect is the disruption of the translation coding frame resulting in a knockout of protein function.

There are three components required for the design of a CRISPR experiment. A guide sequence that is homologous to the specific target region of the molecule. A Protospacer Adjacent Motif (PAM) sequence adjacent to the guide sequence is required to enable a Cas nuclease to bind the complex and cleave the target.

### CRISPR design guide sequence

The CRISPR wizard is designed to assist in guide sequence selection. The guide needs to be in the region of interest and it needs to have a PAM sequence adjacent. In general, the guide location should be located nearer the amino terminal end of the gene to maximize knockout. This prevents the likelihood that a truncated protein will still be functional. Similarly, if the knockout occurs too early in the translation, there is a possibility that a second translation initiation codon will allow a functional gene product.

The wizard will identify potential guide sequences by searching for PAM sequences within the target region. The guides will then be evaluated for off-target homologies which represent alternate sites that could be acted upon and cause unintended mutations in other genes.

### CRISPR enzyme selection

There are an increasing number of enzymes that can be used for CRISPR experiments. Each enzyme has a corresponding PAM sequence and this will affect the selection of potential guides. If the wizard does not find good potential guides, you can select a different Cas enzyme.

Each Cas enzyme has an optimal guide sequence length. When you select a different Cas enzyme, the wizard will update the design parameters to match your preference for that enzyme. Your settings preferences will be remembered when you complete the wizard. You can also click the ‘Reset’ button to return the settings to default.

## CRISPR wizard conditions

Length of guide RNA: The default guide sequence length is set to your preference for the selected Cas enzyme (typically 20-24 bases). You can adjust this value if preferred.

PAM adjacent perfect match bases: This is used when evaluating possible off target sites. Bases adjacent to the PAM site are most important for binding of the Cas complex and mismatches within this close region will be most likely to prevent binding and reduce off target consequences. The default value requires 10 adjacent bases to be perfectly matched. Selecting a lower number will provide more sensitively to finding potential problems, but the search for off target sites will be slower.

Max mismatches permitted: Off target sites will be ignored if there are more that the specified number of mismatches.

## CRISPR wizard Off Target Search

This allows you to specify the genomic sequence that will be used to search for off target sites that may also be affected by Cas cleavage.

If you do not select an off-target sequence, the wizard will use the full molecule used for the guide design. This is most appropriate if you are designing a knockout experiment using a region of a full bacterial genome. For other organisms you can download genome sequences from NCBI (see references).

You can select a file using the ‘Browse’ button. Your most recently used selections are shown by clicking the ‘Recent’ button.

Sequence files may be either a single molecule file or a FASTA format multi molecule file. Single molecule files can be complete bacterial genome sequences (mega-base). FASTA format files can include complete eukaryotic genome sequence collections, such as the Human genome (GRCh38, file size 3 giga-bases).

The Clone Manager wizard has been optimized for rapid processing of very large sequences. Time to complete the analysis will typically be a few seconds. However, larger guide design target regions and large off-target sequences will increase the time.

## CRISPR wizard results

The results view displays a list of possible guide sequences. For each possible guide, its position and sequence are reported together with the result of the search for off target hits. The number of off target hits are reported together with the sequence of the worst homology. Mismatches are shown as upper case bases so you can evaluate the significance of the off target site.

## CRISPR wizard results toolbar

The results view toolbar enables you to change the displayed results. Hovering your mouse over a control will show a tooltip explaining the function.

Position: shows the base position of the PAM sequence which is usually close to the Cas nuclease cut position. The other option is to show the position of the guide sequence.

Sort: defaults to showing results in position sorted order. Optimal position of a guide will be earlier in the target gene but not too close to the start. The other option is to sort by specificity and will show guides at the top of the list that have no, or fewer, off target homologies. Higher specificity is indicative of fewer unintended off target mutations.

Binding: defaults to show all off target homologies found. For very large off target database files there will usually be many possible off target sites found. This combo box allows you to show only those sites that have strong, or moderate, binding.

Export guide sequence: clicking this icon will copy the sequence of the selected guide to the clipboard.

Redefine: icon is located at the right end of the toolbar and enables you to redefine the wizard design settings. You can change the guide design region, evaluation conditions and off target search.

## CRISPR wizard results output

The results view can be copied to the clipboard or a file using the menu View, Send view options. Results view can be printed using the menu File, Print.

## References:

A guide to the CRISPR process: https://www.addgene.org/guides/crispr/

Download Genome sequences for off target searches: https://www.ncbi.nlm.nih.gov/genome/