CRISPR Cheat Sheet

addgene

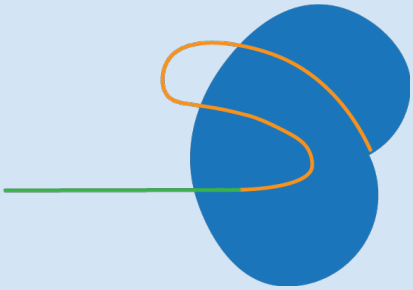
Clustered Regularly Interspaced Short Palindromic Repeats:

Sequences of DNA found in bacteria that allow the bacteria to target and destroy viruses that infect them. Commonly referred to as a bacterial immune system.

Genome Editing

Modifying the DNA sequence of an organism's genome usually for a research or applied goal.

Components of CRISPR



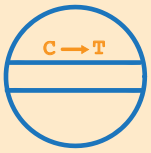
gRNAs: Sequences of RNA that direct the CRISPR system to cut other DNA or RNA sequences.

Nucleases: Proteins that bind to gRNAs and are directed by the gRNAs to cut particular DNA or RNA sequences. Cas9 is a very commonly used CRISPR nuclease.

Note: Most CRISPR plasmids from Addgene produce either a gRNA, a nuclease, or both.

Applications of CRISPR

Cut: CRISPR can be used to cut the DNA sequence. With a repair template, CRISPR can be used to introduce a functional change in the DNA sequence.



Base Editors: Modified versions of CRISPR nucleases that make single base changes in the DNA sequence without completely cutting the DNA.

RNA Editors: CRISPR nucleases that cut or modify RNA as opposed to DNA. Fusing the nuclease to an adenosine deaminase can convert adenosine to inosine.

Activate/Repress: Modified versions of CRISPR nucleases that can't cut DNA (dCas9, for example) that are used to increase or decrease expression of a gene.

Screen: A library of gRNAs with a CRISPR nuclease target multiple genes in a cell population. The resulting mutant cells are screened for phenotypes of interest.

Limitations of CRISPR

Precise edits are difficult. CRISPR is not 100% specific and can cut DNA sequences that researchers don't intend it to.

CRISPR cannot cut all sequences. However, prime editing and the identification of other Cas proteins continue to expand the targeting range.

CRISPR can be difficult to deliver. Not all cells efficiently take up plasmids used to produce CRISPR systems