CRISPR Cheat Sheet

addgene

The Basics

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.

Natural CRISPR Systems Have 2 Major Components

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

2. Repeat Regions: These are used to create gRNAs - sequences of RNA that direct the CRISPR system to cut other DNA sequences.

Note - Most CRISPR plasmids from Addgene are used to produce either a gRNA or a nuclease or both.

Genome Editing

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

Some Applications of CRISPR

Knockout : CRISPR can be used to cut the DNA sequence of a gene making it so that the gene no longer encodes a functional protein. Knockout experiments give researchers an idea of the role a gene plays in normal biology.

Activate/Repress : Modified versions of CRISPR nucleases that can no-longer cut DNA (dCas9 for example) can be used to increase or decrease the production of a protein encoded by a gene.

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

RNA Editors : CRISPR nucleases that cut or modify RNA as opposed to DNA.

Knockin : CRISPR can be used to facilitate a functional change in a DNA sequence that may give cells or organisms new or modified genes with new or modified functionality. Knockins place repair templates encoding the desired DNA change into the genome - the CRISPR system cuts the DNA, but the target cells use the repair template to fix the cut, thereby acquiring the function encoded by the repair template.

Limitations of CRISPR

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

CRISPR cannot cut all sequences - certain requirements of CRISPR systems prevent them from targeting all sequences.

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