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miRNA CRISPR KITS - powered by NAWGEN

Our unique dual-gRNA system allows for the complete excision of miRNAs.

INCLUDED

1 µg of all-in-one plasmid containing:

- Dual CRISPRs, targeting both the 3' and 5' ends of your miRNA of interest
- Cas9 plasmid

www.canopybiosciences.com

TECH NOTE NAW003

miRNA CRISPR KNOCKOUT KITS
powered by NAWGEN

CRISPR-CAS9 SYSTEM

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. The type II system uses single effector enzymes such as Cas9 to cleave dsDNA. The CRISPR/Cas9 system is a genome editing tool that is both quick and simple compared to other genome editing tools. Cas9 is a nuclease guided by the crRNA and tracrRNA (or trans-activating crRNA) to cleave specific DNA sequences. A 20 nucleotide (nt) guide RNA (gRNA) can be designed to include a hairpin that mimics the tracrRNAcrRNA complex. This allows for highly specific genome editing with the help of the 20nt gRNA. Binding specificity is based on the gRNA and a three nucleotide NGG sequence called the protospacer adjacent motif (PAM) sequence.

WHAT ARE THE CHALLENGES OF USING CRISPR-CAS9 PLASMIDS TO TARGET MIRNA?

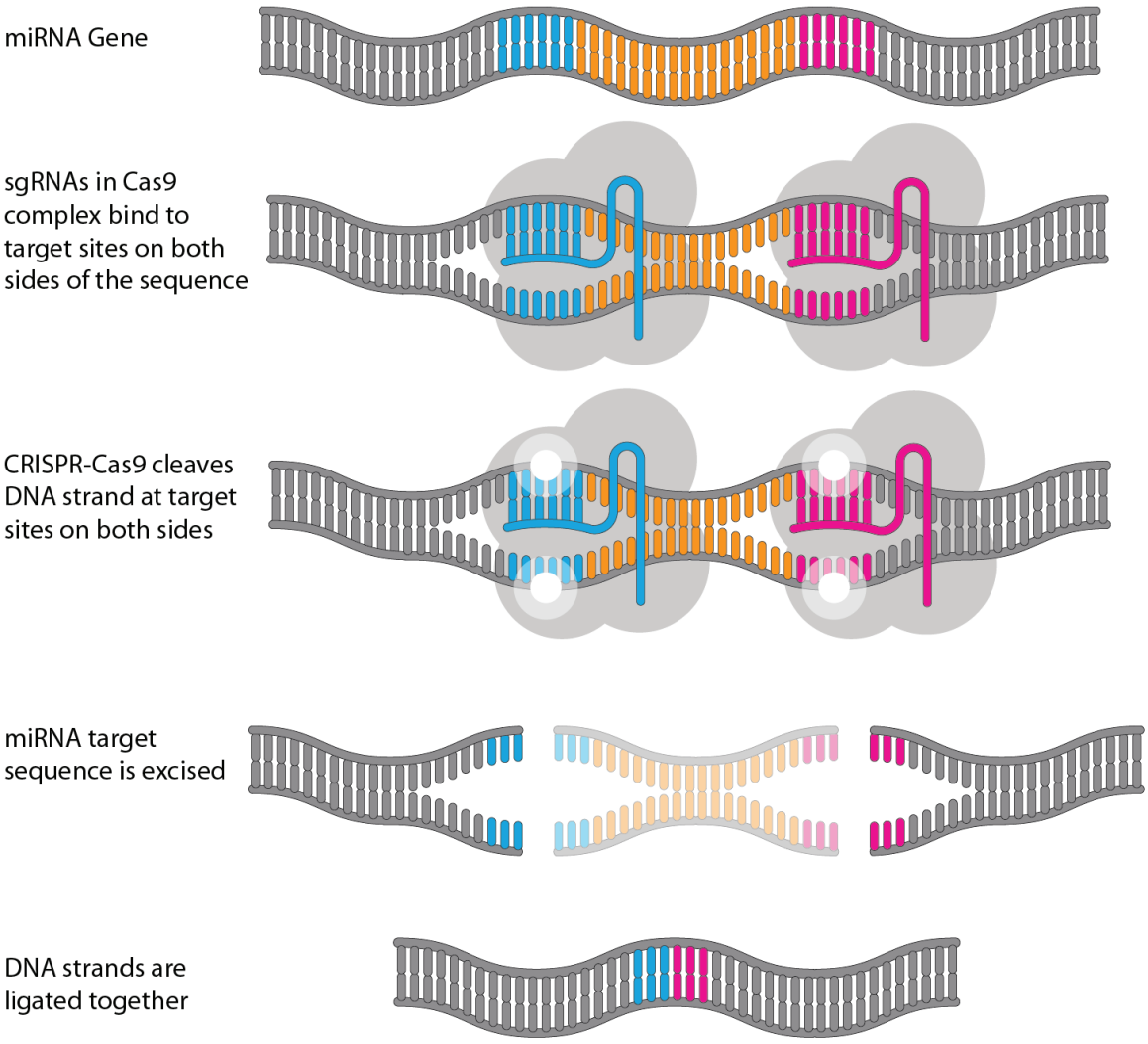
Knockout of protein-coding genes is relatively easy, because a small deletion or insertion can disrupt the open reading frame and result in a complete loss of function. However, this is not the case for miRNAs and other non-coding genes. A small deletion or insertion may result in no loss of function, or may even create an unintended miRNA product. Additionally, a mature miRNA is only ~22-nt long, which makes the use of a PAM sequence and 20nt guide RNA for targeting impractical.

Challenges with traditional CRISPR-Cas9 systems:

- Target area too small for PAM sequence and gRNA
- No loss of function from single base mutations on non-coding genes
- miRNA knockdown not possible or efficient with single-gRNA cutting

WHAT ARE THE BENEFITS OF USING OUR ALL-IN-ONE DUAL-GRNA CRISPR/CAS9 PLASMIDS?

We designed a dual-gRNA construct to target flanking regions of the pre-miRNA genome and completely excise the miRNA gene. This approach is advantageous for a variety of reasons; it allows for complete loss of function of the miRNA, provides a larger target area which allows for only the most ideal gRNAs to be used, and genome editing can be confirmed by genomic PCR instead of the more laborious T7 endonuclease assay.



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