Knockout of microRNAs using Cas9 nuclease and synthetic crRNA:tracrRNA

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Introduction

The CRISPR (clustered regularly interspaced short palindromic repeats) Cas9 system with synthetic crRNA and tracrRNA molecules is a revolutionary method that allows specific gene editing without the need for cloning. The system can be used to create deletions across clonal isolates of genes or across multiple genes without the requirement of any cloning steps. This synthetic approach to crRNA and tracrRNA enables fast assessment of multiple target sites per gene or across multiple genes without the requirement of any cloning steps. The use of a two-RNA system for programming of Cas9 nuclease enables rapid generation of site-selective genetic modifications.

Experimental design

Here we demonstrate a strategy for functional knockout of microRNAs using the CRISPR-Cas9 system with synthetic crRNA and tracrRNA molecules. We examine several parameters that affect the functional knockout efficiency, including the region of the microRNA sequence targeted and the number of crRNAs used. Through optimization of split-based transfection conditions, we utilized this platform to create microRNA knockouts in all alleles of a cell with high efficiencies. The methods presented should be broadly applicable as a strategy to knock out any microRNA.

Multiple crRNAs can be used to create large deletions

The CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated protein) system utilizes a Cas9 nuclease protein guided by two small RNA sequences, the tracrRNA and a targeting crRNA containing a 20 nucleotide guide sequence complementary to the genomic target of interest, to create double-strand breaks inside the genome. These breaks are repaired by non-homologous end joining (NHEJ), which can lead to gene disruptions including indels and small deletions, or homology-directed repair (HDR), which can be used to introduce new genetic sequences.

Gene editing with CRISPR-Cas9 generates populations of cells with variable deletions

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Conclusions

- CRISPR-Cas9 gene editing is an effective strategy for knocking out microRNA function to study the biological role of microRNA.
- The design space for targeting microRNAs is small, so it may not be possible to design a functional and specific crRNA targeting the sequence of the microRNA.
- It is possible to utilize crRNA design immediately upstream or downstream of a microRNA to knock out microRNA (Figures 6 & 8).
- crRNA pairs can be utilized to knock out larger sequences, leading to complete deletion of the microRNA sequence.

CrRNAs targeting different regions cause functional disruptions in the microRNA

To determine the effectiveness of the CRISPR-Cas9 system for microRNA knockout, the experiment was conducted using different crRNA sequences targeting different regions of the same microRNA sequence. The results showed that different crRNA pairs caused functional disruptions in the microRNA, with some causing complete knockout and others causing partial knockout. The figure above the sequence shows the different crRNA pairs used.

A single crRNA generates different deletions across clonal isolates

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