CRISPR-Cas9 genome editing utilizing chemically synthesized RNA

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Introduction
The CRISPR-Cas9 system permits researchers to rapidly create genome engineering in mammalian and plant genomes, among others, and consequently two biologically transformative biological research. The CRISPR-Cas9 system requires two key components: first, a guide RNA (gRNA) that is complementary to the part of the genome to be edited; and second, a Cas9 nuclease. The gRNA binds to the Cas9 nuclease to create a complex that cuts the DNA at the target site. The CRISPR-Cas9 gene editing process requires the delivery of the Cas9 nuclease and the gRNA into the cell, which can be achieved through transfection, infection, or transfection with viral particles. Once the Cas9 nuclease is delivered, it can be used to cut the DNA at the desired location. This allows researchers to introduce mutations, insertions, or deletions into the genome, which can be used to study the function of specific genes.

Synthetic crRNA:tracrRNA for CRISPR-Cas9 gene editing

Why dual guide? - Pairs the natural bacterial system - crRNA:tracrRNA - Synthetic RNA complex that is highly specific and fixed - Endogenous repair sequence - TracrRNA: long synthetic RNA that hybridizes with crRNA, a universal component that targets specific DNA sequences

Why synthetic? - Easier for research into cloning, sequencing, etc. - Meets high purity, stable, less toxic - Enables high-throughput applications like screening - Provides for the production of complex nucleic acids for heat and protein expression.

DNA-free gene editing efficiency using Cas9 mRNA and synthetic crRNA:tracrRNA

Using Cas9 mRNA with synthetic crRNA:tracrRNA allows for a completely DNA-free workflow and produces gene editing at levels comparable to Cas9 integrated lines. The DNA-free system reduces the concerns of unwanted integration as well as potential off-targets.

GFP knockout in vivo using Cas9 mRNA and synthetic crRNA:tracrRNA

Conclusions

- CRISPR-Cas9 gene editing using synthetic crRNA:tracrRNA is highly efficient and easy to use.
- Synthetic crRNA:tracrRNA is suitable for in vitro and in vivo applications, in particular, RNAi approach with Cas9 mRNA or Cas9 protein.
- Chemical synthesis of guide RNA allows accurate and rapid production of all required RNA oligomers for high-throughput, large-scaled genome engineering.

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