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Chemical modifications of synthetic guide RNA for enhanced RNA stability and reduced cellular toxicity in CRISPR-Cas9 genome editing

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Abstract
The ability to modify the genome of organisms and mammalian cells has been established. In parallel to genome editing, the understanding of the mechanism of action of sequence-specific reagents for Cas9, Cas10, and the ability to enhance their activity, has led to the development of gene editing and genome editing. These reagents and reagents can be used to modify DNA and RNA, and can be used to enhance gene editing and genome editing. The ability to modify the genome of organisms and mammalian cells has been established. In parallel to genome editing, the understanding of the mechanism of action of sequence-specific reagents for Cas9, Cas10, and the ability to enhance their activity, has led to the development of gene editing and genome editing. These reagents and reagents can be used to modify DNA and RNA, and can be used to enhance gene editing and genome editing.

Sequential electroporation is required for delivery of unmodified crRNA:tracrRNA and Cas9 mRNA
For sequential Cas9 electroporation, Cas9 mRNA was electroporated and followed 4 hours later with either a crRNA:tracrRNA complex or a crRNA:tracrRNA:Cas9 mRNA complex. Electroporation parameters were optimized for each step. Electroporation of crRNA:tracrRNA and Cas9 mRNA was more efficient than electroporation of crRNA:tracrRNA alone. Unmodified crRNA:tracrRNA and Cas9 mRNA were more efficient than modified crRNA:tracrRNA and Cas9 mRNA.

Chemical modification of guide RNAs did not consistently improve gene editing in co-electroporation with Cas9 protein
Modified crRNA:tracrRNA complexes were transfected into a stably expressing Cas9-expressing cell line (HEK293T) and used to edit a reporter gene. Electroporation parameters were optimized for each step. Electroporation of crRNA:tracrRNA and Cas9 protein was more efficient than electroporation of crRNA:tracrRNA alone. Modified crRNA:tracrRNA complexes were not consistently more efficient than unmodified crRNA:tracrRNA complexes.

Stabilizing modifications of synthetic guide RNAs for resistance to degradation by nucleases
Synthetic guide RNAs were modified with one or more 2'-O methyl and backbone modifications. Electroporation parameters were optimized for each step. Electroporation of modified crRNA:tracrRNA complexes was more efficient than electroporation of unmodified crRNA:tracrRNA complexes.

RNA modifications do not affect gene editing activity when transfected into Cas9-expressing cells, but some patterns negatively affect cell viability
Unmodified and modified crRNA:tracrRNA complexes were transfected into a stably expressing Cas9-expressing cell line (HEK293T) and used to edit a reporter gene. Electroporation parameters were optimized for each step. Electroporation of crRNA:tracrRNA and Cas9 protein was more efficient than electroporation of crRNA:tracrRNA alone. RNA modifications did not affect gene editing activity, but some patterns negatively affected cell viability.

Synthetic guide RNAs show comparable editing to in vitro transcribed sgRNA, but elicit no immune response
Synthetic guide RNAs were used to edit a reporter gene in HEK293T cells. Electroporation parameters were optimized for each step. Electroporation of synthetic guide RNAs was more efficient than electroporation of in vitro transcribed sgRNA. Synthetic guide RNAs did not elicit an immune response.

Modification of both ends of synthetic sgRNAs stabilize RNAs for co-electroporation with Cas9 mRNA
Synthetic guide RNAs were modified with one or more 2'-O methyl and backbone modifications. Electroporation parameters were optimized for each step. Electroporation of modified crRNA:tracrRNA complexes was more efficient than electroporation of unmodified crRNA:tracrRNA complexes.

Modification of both ends of crRNA:tracrRNA stabilize the RNAs for co-electroporation with Cas9 mRNA
Unmodified and modified crRNA:tracrRNA complexes were transfected into a stably expressing Cas9-expressing cell line (HEK293T) and used to edit a reporter gene. Electroporation parameters were optimized for each step. Electroporation of crRNA:tracrRNA and Cas9 mRNA was more efficient than electroporation of crRNA:tracrRNA alone. Modified crRNA:tracrRNA complexes were not consistently more efficient than unmodified crRNA:tracrRNA complexes.

Modification of the single-stranded regions of crRNA:tracrRNA is sufficient for stabilization in co-electroporation with Cas9 mRNA
Unmodified and modified crRNA:tracrRNA complexes were transfected into a stably expressing Cas9-expressing cell line (HEK293T) and used to edit a reporter gene. Electroporation parameters were optimized for each step. Electroporation of crRNA:tracrRNA and Cas9 mRNA was more efficient than electroporation of crRNA:tracrRNA alone. Modified crRNA:tracrRNA complexes were not consistently more efficient than unmodified crRNA:tracrRNA complexes.

CRISPR-Cas9 genome editing for DNA-free CRISPR-Cas9 gene editing
Synthetic crRNA:tracrRNA for DNA-free CRISPR-Cas9 gene editing. Electroporation parameters were optimized for each step. Electroporation of synthetic crRNA:tracrRNA complexes was more efficient than electroporation of in vitro transcribed crRNA:tracrRNA complexes.

Benefits of Dharmacon® 2'-O methyl and backbone modifications for synthetic guide RNA
• Enhanced stability and resistance to degradation
• Increased efficiency of gene editing
• Reduced toxicity to cells

Electroporation workflow for synthetic guide RNA and Cas9 mRNA
1. Preparation of Cas9 mRNA and crRNA:tracrRNA complexes
2. Electroporation of Cas9 mRNA
3. Electroporation of crRNA:tracrRNA complexes
4. Co-electroporation of Cas9 mRNA and crRNA:tracrRNA complexes
5. Selection and expansion of edited cells

Conclusions
• Stabilizing modifications on synthetic guide RNAs (crRNA:tracrRNA or sgRNA) to resist nuclease degradation are required for co-electroporation with Cas9 protein.
• Some modifications improve gene editing efficiency of some gene targets in co-electroporation with Cas9 protein.
• RNA modifications do not affect gene editing activity when transfected into Cas9-expressing cells.
• Some modification patterns are toxic to cells with long tracrRNA.
• Stabilization of the single-stranded regions of the crRNA:tracrRNA molecule requires gene editing in lipid-coated vesicles with Cas9 mRNA.
• Modified crRNA:tracrRNA complexes are more efficient than unmodified crRNA:tracrRNA complexes in co-electroporation with Cas9 protein.

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