Multiplex gene editing with CRISPR Nuclease mRNA

GeneArt CRISPR Nuclease mRNA is a ready-to-transfect wild-type Cas9 mRNA for performing CRISPR/Cas9-mediated genome editing. When co-transfected with a guide RNA (gRNA) that targets your genomic locus of choice, the Cas9 protein is directed by the gRNA to cleave the gene at that locus.

The CRISPR Nuclease mRNA system allows multiplex genome editing, in which multiple target gene sequences can be edited simultaneously in a single transfection reaction by adding multiple gRNAs. The system is versatile and simple to use. Changing the target requires only a change in the gRNA design.

TIP: If you're new to CRISPR editing, we recommend using **TrueCut Cas9 Proteins** as your nuclease. The protein process does not require mRNA translation and allows you to deliver a ready-to-edit Cas9 ribonucleoprotein complex.

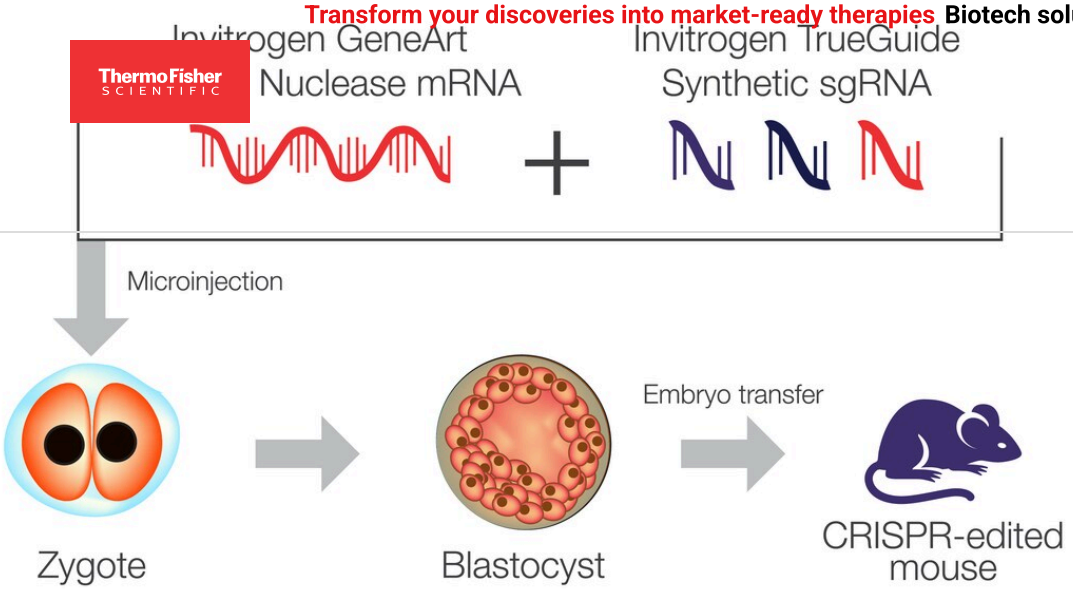
Order GeneArt CRISPR Nuclease mRNA
Design your edit and order reagents with our free TrueDesign Genome Editor

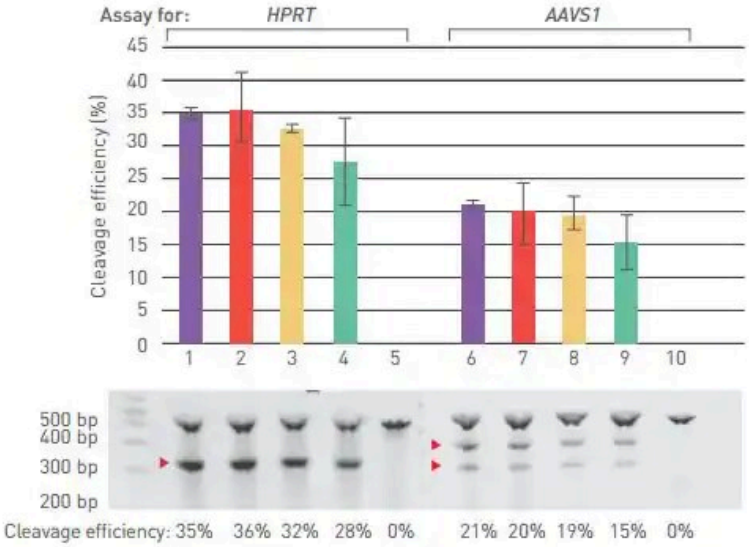
NOTE: CRISPR-Cas9 genome editing requires a guide RNA (gRNA) in order to cleave genomic DNA at a target sequence of interest. Consult **Guide RNAs for CRISPR-Cas9** and **CRISPR Cas9 Screening Libraries** for more information.

Cas9 mRNA workflow for multiplex gene editing and transgenic model system applications

With GeneArt CRISPR Nuclease mRNA, you can simultaneously transfect up to four different gRNAs in a single well and assess the cleavage efficiency of multiple genes simultaneously. You can use this approach to determine which gRNA sequence works best for a particular target, or to edit multiple genomic loci with one transfection.

In addition, CRISPR-Cas9 genome editing using Cas9 mRNA has been cited for use with microinjections and other *in vivo*-mediated delivery methods for transgenic model system generation in a wide variety of organisms, including mice [1], zebrafish [2], and *Drosophila*. For microinjection experiments and mouse model generation, we recommend testing at least three synthetic sgRNAs and validating them in the cell line of choice.





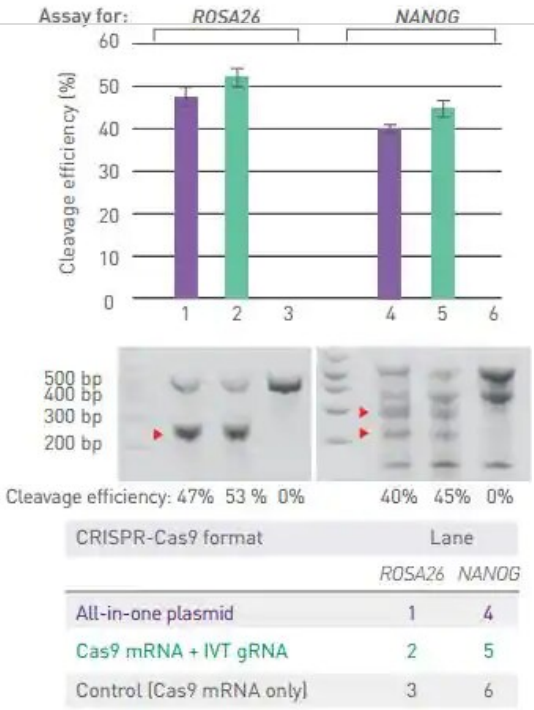
CRISPR-Cas9 format	Target	Lane	CRISPR-Cas9 format	Target	Lane
All-in-one plasmid	*HPRT*	1	All-in-one plasmid	*AAVS1*	6
Cas9 mRNA + IVT gRNA	*HPRT*	2	Cas9 mRNA + IVT gRNA	*AAVS1*	7
Cas9 mRNA + IVT gRNA	*HPRT* *AAVS1*	3	Cas9 mRNA + IVT gRNA	*HPRT* *AAVS1*	8
Cas9 mRNA + IVT gRNA	*HPRT* *AAVS1* *RELA*	4	Cas9 mRNA + IVT gRNA	*HPRT* *AAVS1* *RELA*	9
Control (IVT gRNA only)	*HPRT* *AAVS1* *RELA*	5	Control (IVT gRNA only)	*HPRT* *AAVS1* *RELA*	10

Figure 1. Efficient multiplexed genome editing using GeneArt CRISPR Nuclease mRNA and IVT gRNA. Genome editing efficiency is shown in HEK293 (human embryonic kidney) cells transfected with **GeneArt CRISPR Nuclease mRNA** and *in vitro* transcribed (IVT) gRNA at one target (red) or simultaneously targeted with either double (yellow) or triple (green) targets at the HPRT, AAVS1, and/or RelA genes. Control cells transfected with IVT gRNA only (gray) and cells transfected with GeneArt CRISPR Nuclease reporter plasmid (purple) are shown for comparison. Editing efficiency was consistent across conditions, regardless of the number of targets.

CRISPR mRNA applications across hosts and in transgenic model systems

Data: Ge… Nuclease mRNA achieves high editing efficiency in mouse neural cells

Th… np… rmat, delivered with our Lipofectamine transfection reagents, provides superior delivery combined with high genome editing efficiency in stem cells and difficult-to-transfect cell lines.



CRISPR-Cas9 format	Lane	
	ROSA26	NANOG
All-in-one plasmid	1	4
Cas9 mRNA + IVT gRNA	2	5
Control (Cas9 mRNA only)	3	6

Figure 2. The GeneArt CRISPR Nuclease mRNA system enables efficient genome editing across hosts. The system was used with IVT gRNA to edit Mouse Neuro-2A (mouse neuroblast) cells at the ROSA26 and NANOG loci. Cells were transfected in 24-well format using **Lipofectamine 2000 Reagent** and analyzed 72 hours post-transfection using the **GeneArt Genomic Cleavage Detection Kit**. For both genes, cleavage efficiency exceeded the all-in-one CRISPR plasmid.

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2. Shah AN, Davey CF, Whitebirch AC, Miller AC, Moens CB. Rapid reverse genetic screening using CRISPR in zebrafish. *Nat Methods*. 2015; 12: 535–540.
3. Hashimoto M, Takemoto T. Electroporation enables the efficient mRNA delivery into the mouse zygotes and facilitates CRISPR/Cas9-based genome editing. *Sci Rep.* 2015; 5: 11315.

Gene editing resources and support

Resources

- Genome Editing Learning Center
- Genome Editing Resource Library
- CRISPR Genome Editing Resource Guide
- CRISPR Genome Editing Brochure

Support

- Genome Editing Support Center
- Request Genome Editing Support
- Cell Line Engineering Services
- Contact Technical Support