bioNova científica, s.l.

mirus bio®

CRISPR/Cas GENOME EDITING

CRISPR/Cas9

Delivery Methods

CRISPR/Cas9 mediated genome engineering is a powerful tool enabling researchers to rapidly and efficiently modify genomic DNA. Mirus Bio offers a suite of transfection reagents capable of adapting to a variety of genome editing tools and techniques. These products are designed to enable high-efficiency delivery in many cell types, including hard-to-transfect cells, without the cost or complication of other systems.

"I was recently tasked with developing a CRISPR protocol for primary and bone-derived cell lines. *Trans*IT-X2® was simple to use, 2-3 times better for transfection and much gentler on my cells than other products! I feel I have hit the jackpot and have already passed this exciting information on to my colleagues."

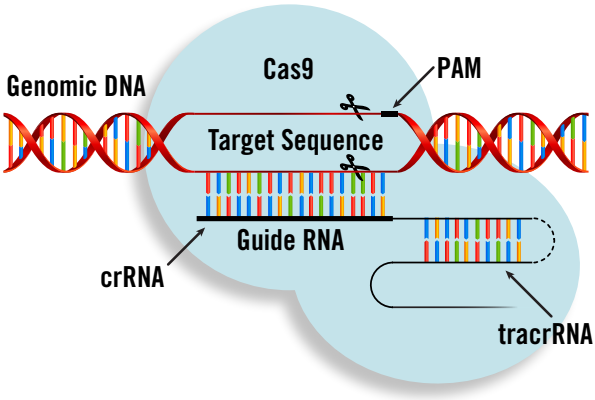
-Joshua Chou, Ph.D.
Harvard School of Dental Medicine

Editing Mammalian Genomes with CRISPR

- CRISPR Gene Editing Workflow
- Plasmid DNA and Guide RNA Transfection
- mRNA and Guide RNA Transfection
- Cas9/gRNA RNP Chemical Transfection
- Cas9/gRNA RNP Electroporation

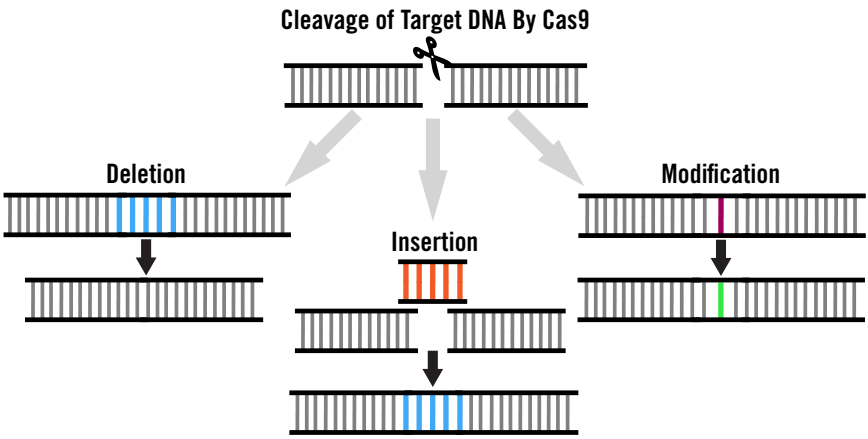
What is CRISPR/Cas9 Genome Editing?

The CRISPR/Cas9 system is a powerful tool for genome editing in mammalian cells that allows researchers to generate genetic variants at lower cost and with higher throughput than alternative methods like zinc finger nuclease (ZFN) or transcription activator-like effector nuclease (TALEN) genome editing.



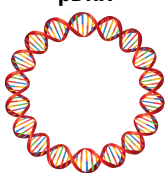
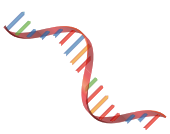
The CRISPR/Cas9 RNP Complex. The CRISPR associated protein 9 (Cas9) endonuclease (blue) is targeted to DNA by a guide RNA (gRNA), which can be supplied as a two-part system consisting of CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) or as a single guide RNA (sgRNA), where the crRNA and tracrRNA are connected by a linker (dotted line). Target recognition is facilitated by the protospacer-adjacent motif (PAM). A double strand break (DSB) occurs 3 bp upstream of the PAM.

CRISPR Facilitates Multiple Types of Genome Modification

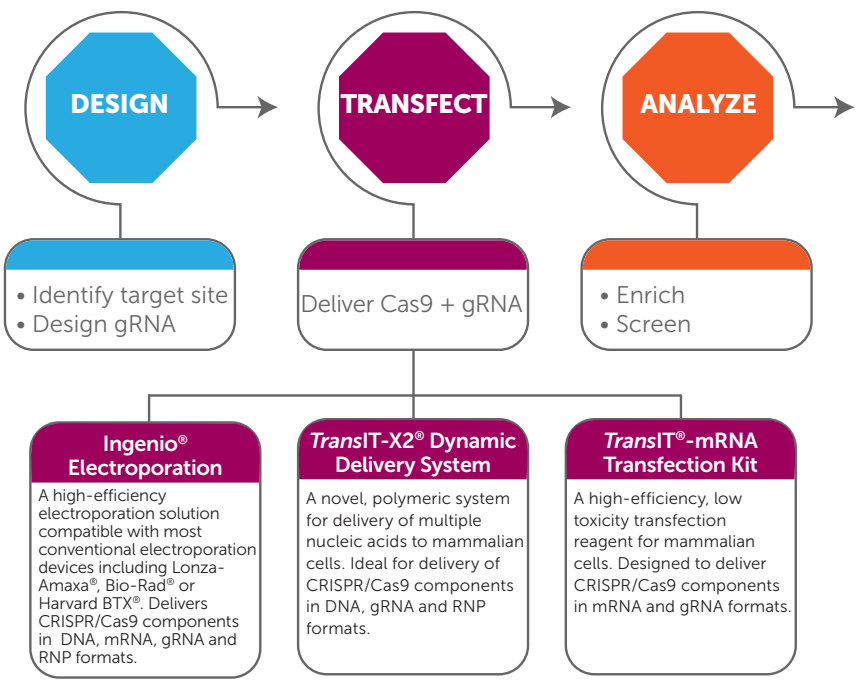


Multiple Genomic Alterations are Possible Following Cleavage of Target DNA by Cas9. Variable length insertions and/or deletions (indels) can result near the DNA break due to mistakes in DNA repair by the endogenous non-homologous end joining (NHEJ) pathway. These indels frequently result in disruption of gene function. Alternatively, by supplying a DNA repair template, researchers can leverage the homology-directed repair (HDR) pathway to create defined deletions, insertions or other modifications.

Comparison of Cas9 Formats: DNA, RNA and Protein

	Cas9 Delivery Methods		
	pDNA 	mRNA 	Protein 
High Efficiency	+++	++++	++++
Low Cost	++++	++++	+++
Specificity	+++	++++	++++

CRISPR Gene Editing Workflow



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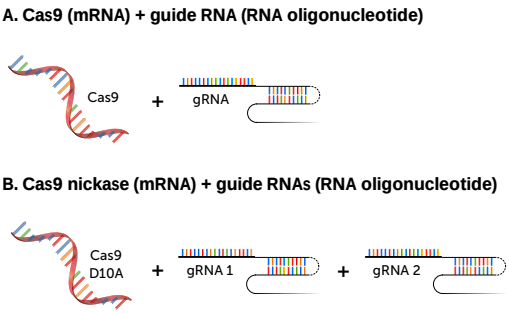
mRNA and Synthetic Guide RNA Transfection

In order to avoid off-target cleavage and unwanted genomic integration of plasmid DNA, Cas9-encoding mRNA can be co-transfected with guide RNA oligonucleotides. Benefits of RNA-based genome editing include:

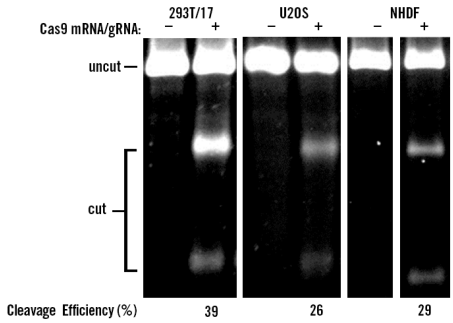
- High Specificity - Rapid gene expression generates a transient pulse of genome editing activity
- Ease-of-use - Deliver mRNA and guide RNA with a single reagent
- DNA Free - No risk of insertional mutagenesis



Cas9 mRNA + Guide RNA Oligonucleotides. Cas9 is supplied as messenger RNA, and guide RNAs are supplied as either synthetic or *in vitro* transcribed RNA oligonucleotides. (A) The wild-type Cas9 enzyme contains two endonuclease domains which cleave the target DNA on both strands when programmed with a guide RNA. (B) The D10A mutation converts Cas9 to a nickase that generates single-stranded breaks in the target DNA. For improved target specificity, Cas9 D10A can be used with paired guide RNAs targeting opposite strands to create staggered double-stranded breaks.

Cas9/gRNA Ribonucleoprotein (RNP) Electroporation

Efficient Genome Editing with Cas9 mRNA + Guide RNA Oligonucleotides. HEK293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding mRNA, 5meC (TriLink Biotechnologies) and 25 nM of PPIB targeting two-part gRNA (Dharmacon) using *Trans*IT®-mRNA Transfection Kit (0.5 µl/well of 24-well plate of both mRNA Reagent and Boost, Mirus Bio). A T7EI mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.



DNA-free Transfection Protocol Available Online

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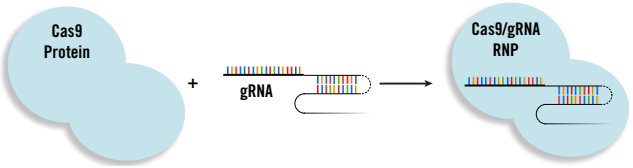
Cas9/gRNA Ribonucleoprotein (RNP) Transfection

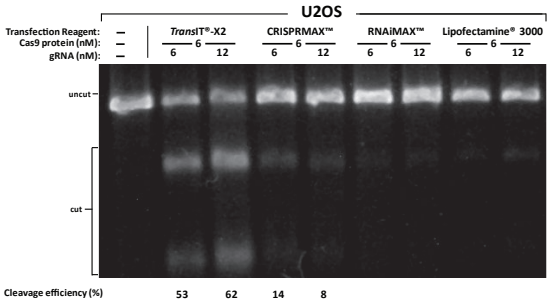
Purified Cas9 protein can be combined with guide RNA to form an RNP complex to be delivered to cells for rapid and highly efficient genome editing. Benefits of RNP-based genome editing include:

- High Efficiency Delivery - Deliver Cas9/gRNA complexes to multiple cell types, including hard-to-transfect cells such as immune and stem cells
- High Specificity - Pre-formed RNP complexes provide a rapid pulse of genome editing activity
- DNA Free - No risk of insertional mutagenesis



Cas9 RNP. Purified Cas9 protein and guide RNA oligonucleotides are combined to form a ribonucleoprotein (RNP) complex.

Cas9/gRNA Ribonucleoprotein (RNP) Chemical Transfection



*Trans*IT-X2® Outperforms Lipofectamine® for RNP Delivery. Ribonucleoprotein (RNP) complexes were delivered into U2OS cells using *Trans*IT-X2® Dynamic Delivery System (1 µl/well, Mirus Bio) or Lipofectamine CRISPRMAX™ (1.5 µl/well and 1 µl/well of Lipofectamine Cas9 Plus™ Reagent, ThermoFisher) or Lipofectamine RNAiMAX (1.5 µl/well, ThermoFisher) or Lipofectamine 3000 (1.5 µl/well and 1 µl/well of P3000™ Reagent, ThermoFisher) in a 24- well format according to the manufacturers' protocol. Varying levels of gRNA (6 nM or 12 nM) were tested with 6 nM Cas9 protein (PNA Bio). A T7EI mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.

RNP Transfection Protocols Available Online

*Trans*IT-X2® Dynamic Delivery System for CRISPR/Cas9 Ribonucleoprotein (RNP) Delivery Protocol

*Trans*IT-X2® Dynamic Delivery System for CRISPR/Cas9 Ribonucleoprotein (RNP) + DNA Oligo (ssODN) Delivery Protocol

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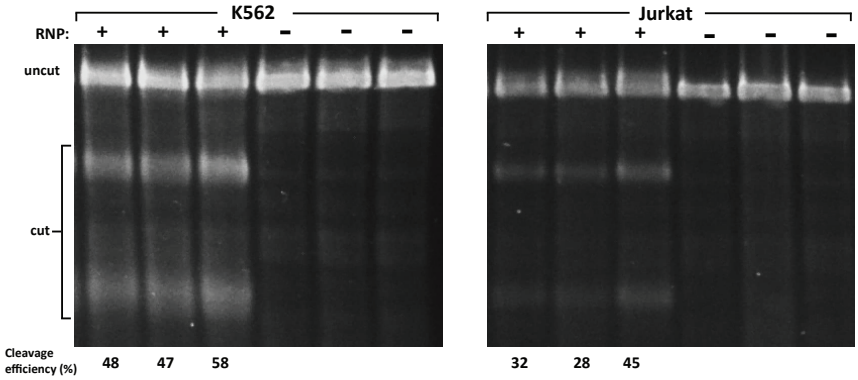
Cas9/gRNA Ribonucleoprotein (RNP) Electroporation



Efficient CRISPR RNP Delivery with Ingenio® Electroporation Solution. Ribonucleoprotein (RNP) complexes targeting WTAP were electroporated into K562 and Jurkat cells. The RNP complex, composed of 750 nM Cas9 protein (EnGen® Cas9 NLS, New England Biolabs) and 1,500 nM pre-complexed two-part gRNA (IDT), was electroporated using the Ingenio® Electroporation Solution (Mirus Bio) and a Gene Pulser Xcell™ Eukaryotic System (Bio-Rad® Laboratories). Exponential pulse conditions of 130V & 150V, 950 µF for K562 and 150V, 950 µF for Jurkat cells were applied to triplicate 0.2 cm cuvettes, 100 µl volume, 10 x 10^6 cells/ml +/- RNP complex. A T7EI mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection. Non-specific bands (NSP) were observed in the negative control of both cell lines. Cleavage efficiency was calculated based on the ratio of cleaved band intensities to the sum of cleaved and uncleaved band intensities minus the average signal of the non-specific band(s) in negative control lanes.

RNP Electroporation Protocol Available Online

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Ordering Information

DNA/RNP TRANSFECTION PRODUCTS	PRODUCT NO.	QUANTITY
*Trans*IT-X2® Dynamic Delivery System	MIR 6003	0.3 ml
	MIR 6004	0.75 ml
	MIR 6000	1.5 ml
	MIR 6005	5 x 1.5 ml
	MIR 6006	10 x 1.5 ml
mRNA TRANSFECTION PRODUCTS		
*Trans*IT®-mRNA Transfection Kit	MIR 2250	1 ml
	MIR 2225	0.4 ml
	MIR 2255	5 x 1 ml
	MIR 2256	10 x 1 ml
ELECTROPORATION PRODUCTS		
Ingenio® EZporator® Electroporation System	MIR 51000	EACH
Ingenio® Electroporation Kits, 0.2 cm Cuvettes	MIR 50112	25 RXN
	MIR 50115	50 RXN
	MIR 50118	100 RXN
Ingenio® Electroporation Kits. 0.4 cm Cuvettes	MIR 50113	25 RXN
	MIR 50116	50 RXN
	MIR 50119	100 RXN
Ingenio® Electroporation Solution (Stand-alone)	MIR 50111	25 RXN (6.25 ml)
	MIR 50114	50 RXN (12.5 ml)
	MIR 50117	100 RXN (25 ml)
Ingenio® Electroporation Accessories	MIR 50121	0.2 cm cuvettes (50PK)
	MIR 50123	0.4 cm cuvettes (50PK)
	MIR 50125	Cell Droppers (50PK)

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