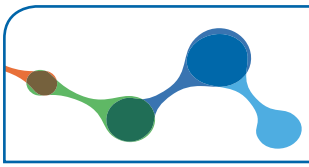


CRISPR/Cas9 editing: cotransfection of Cas9 protein and synthetic RNA oligos

This CRISPR/Cas9 protocol describes how to transfect cultured cells with the CRISPR machinery using conventional lipofection reagents. Transient or stable transfection of large construct(s) for Cas9 and guide RNA (gRNA) expression is not needed. Instead, you will deliver a gene-editing ribonuclear protein (RNP) complex that is comprised of the Cas9 protein and two synthetic RNA oligos: a CRISPR targeting RNA (crRNA) duplexed to a trans-activating crRNA (tracrRNA).

The gene editing results are specific to the potency of the guide RNA trigger, complexed at a 1:1 molar ratio with Cas9 protein, without variations introducted by the transfection efficiency of a Cas9 plasmid. In addition, this method avoids other common pitfalls associated with transfection of Cas9 plasmids, including complications of low transfection efficiency and the potential for plasmid integration into genomic DNA. High-quality synthetic RNA oligos also eliminate the time and expense needed for generating cloned plasmids or *in vitro* transcribed RNAs.

For additional information about and protocols for CRISPR/Cas9 editing, visit www.idtdna.com/CRISPR. For assistance with RNA designs or CRISPR/Cas9 transfection methods, email applicationsupport@idtdna.com.

The CRISPR/Cas9 system

CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats) are part of an adaptive defense mechanism in bacteria and archaea. Use of the CRISPR/Cas9 system for genome editing has been a major technological breakthrough, making genome modification in cells or organisms fast, more efficient, and much more robust than previous genome editing methods. Single guide RNAs (sgRNAs) or guide RNAs (gRNAs) direct and activate the Cas9 endonuclease at a specific genomic sequence. Cas9 then cleaves the target DNA, making it available for repair by the non-homologous end joining (NHEJ) system or for creating an insertion site for exogenous donor DNA by homologous recombination.

Types of CRISPR/Cas9 RNA species:

- crRNA (CRISPR-targeting RNA)—provides target specificity (20 bases) and an interaction domain with the tracrRNA
- tracrRNA (trans-activation crRNA)—acts as a scaffold between the crRNA and Cas9 endonuclease
- sgRNA (single guide RNA) or gRNA (guide RNA)—fusion of crRNA and tracrRNA sequences into a single RNA that includes sequences for DNA targeting specificity and Cas9 endonuclease binding

Discover more at www.idtdna.com

CRISPR/Cas9 editing: transfection of Cas9 protein and synthetic RNA oligos

Required materials

Kits and reagents	Ordering information*
RNA Oligos (crRNA, tracrRNA)	**applicationsupport@idtdna.com**
Primers (Forward and Reverse)	**www.idtdna.com (Custom DNA Oligos page)**
Nuclease-Free Duplex Buffer	**IDT (Cat #11-01-03-01)**
Nuclease-Free Water	**IDT (Cat #11-04-02-01)**
Cas9 Protein	PNA Bio (Cat #CP01)
DTT	General laboratory supplier
Glycerol	General laboratory supplier
HEPES	General laboratory supplier
KCl	General laboratory supplier
Opti-MEM® Media	Thermo Fisher (Cat #51985091)
PBS	General laboratory supplier
Lipofectamine® RNAiMAX Transfection Reagent	Thermo Fisher (Cat #13778100)

* These are suggested sources for reagents used by the IDT R&D team when this protocol was written. Individual components may be substituted with some optimization.

Go to www.idtdna.com for safety data sheets (SDSs) and certificates of analysis (COAs) for IDT products.

A

Assemble the RNP complex (duplex the RNA oligos and mix with Cas9 protein)

1. Resuspend each RNA oligo (crRNA and tracrRNA) in Nuclease-Free IDTE Buffer. For example, use a final concentration of 100 µM. For assistance, use the IDT Resuspension Calculator at www.idtdna.com/scitools.

2. Mix the two RNA oligos in equimolar concentrations in a sterile microcentrifuge tube. For example, create a final duplex concentration of 3 µM using the following table:

Component	Amount
100 µM crRNA	3 µL
100 µM tracrRNA	3 µL
Nuclease-Free Duplex Buffer	94 µL
Final volume	**100 µL**

3. Heat at 95° C for 5 min.
4. Remove from heat and allow to cool to room temperature (15–25°C) on your bench top.
5. If needed, dilute duplexed RNA to a working concentration (for example, 3 µM) in Nuclease-Free Duplex Buffer.
6. Dilute Cas9 protein to a working concentration (for example, 5 µM) in Cas9 Working Buffer (20 mM HEPES, 150 mM KCl, 5% Glycerol, 1mM DTT, pH 7.5).
7. For each transfection, combine 1.5 pmol of duplexed RNA oligos (Step A5) with 1.5 pmol of Cas9 protein (Step A6) in Opti-MEM Media to a final volume of 12.5 µL.
8. Incubate at room temperature for 5 min to assemble the RNP complexes.

B

Reverse transfect gRNA:Cas9 protein in a 96-well plate

1. Incubate the following at room temperature for 20 min to form transfection complexes:

Component	Amount
RNP (Step A8)	12.5 µL
Lipofectamine® RNAiMAX Transfection Reagent	1.2 µL
Opti-MEM® Media	11.3 µL
Total volume	**25.0 µL**

B

2. During incubation (Step B1), dilute cultured cells to 400,000 cells/mL using complete media without antibiotics.

3. When incubation is complete, add 25 µL of transfection complexes (from Step B1) to a 96-well tissue culture plate.

4. Add 125 µL of diluted cells (from Step B2) to the 96-well tissure culture plate (50,000 cells/well; final concentration of RNP will be 10 nM).

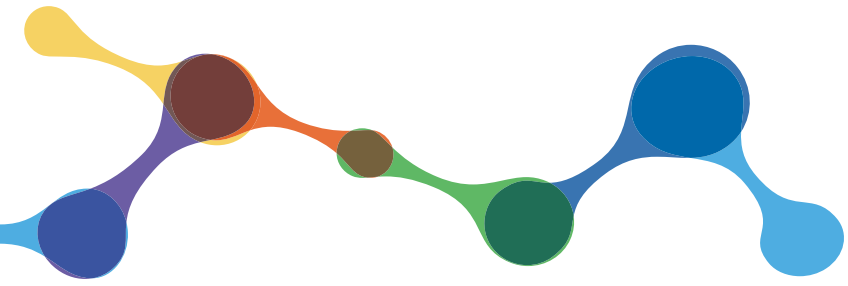
5. Incubate the plate containing the transfection complexes and cells in a tissure culture incubator (37°C, 5% CO_2) for 48 hr.

To detect on-target mutations, use protocol, *CRISPR/Cas9 editing: mutation detection* (available at www.idtdna.com/CRISPR).

For additional information or assistance, visit www.idtdna.com/CRISPR or email us at applicationsupport@idtdna.com.

Protocol revision history

Version	Description of changes
1	Original protocol.



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