jetCRISPR™ - a novel RNP transfection reagent for efficient genome editing

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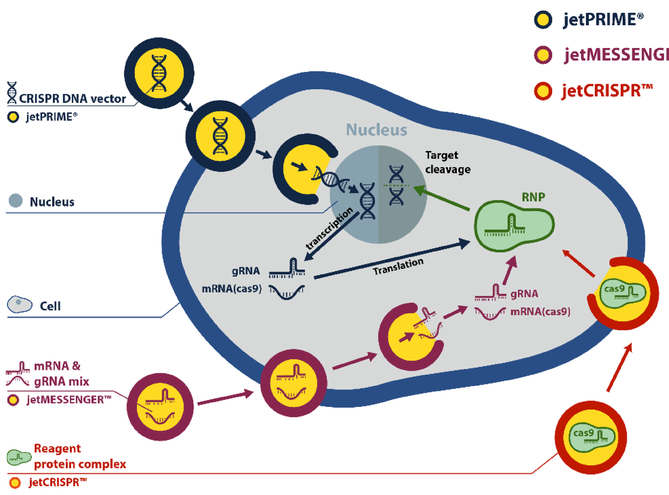
Introduction

The CRISPR/Cas9 engineered nuclease system is a powerful and highly specific genome editing tool. CRISPR/Cas9 is a two-component system with a guide RNA (gRNA) molecule that drives the Cas9 nuclease to a specific targeted sequence within the genome in order to introduce genetic modifications (mutations, insertions or deletions).

Successful delivery of the gRNA and the Cas9 into cells is indispensable to guarantee the high genome editing efficiency that is required to generate new cell or animal models. There are three main systems to transfect gRNA and to express Cas9 protein which are DNA, RNA or RNP (gRNA + Cas9 protein) approaches. While delivery of both gRNA and Cas9 as plasmid DNA is very efficient in easy to transfect cell lines, it is less adapted for difficult to transfect primary cells and cancer cell lines. DNA-free delivery systems in which the Cas9 protein is delivered as mRNA or as a protein have become attractive alternatives, as they overcome the main obstacle that DNA entry into the nucleus constitutes in harder to transfect cells.

CRISPR/Cas9 experiment using different approaches



RNP transfection approach

The most efficient CRISPR-CAS9 method is based on the direct delivery of pre-complexed gRNA and Cas9 protein, referred to Ribonucleoprotein (RNP) delivery. Delivering RNP complexes enables a better control of the Cas9 and gRNA concentration, as well as a more controlled temporal gene editing activity to attenuate off-target effects.

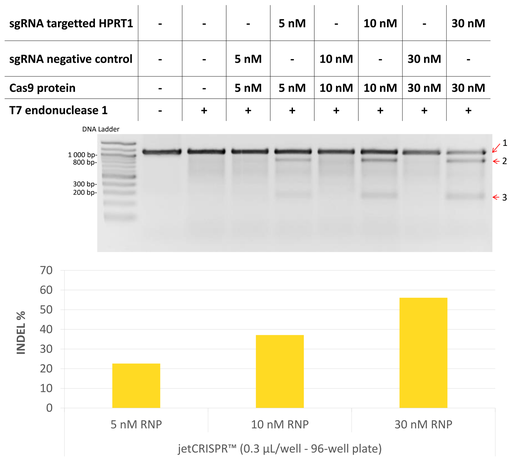
jetCRISPR™ is an innovative reagent especially designed to deliver RNP in a CRISPR/Cas9 experiment. jetCRISPR™ leads to high CRISPR genome editing efficiency and keep an excellent cell viability and morphology.

RNP transfection has many advantages compared to plasmid or mRNA approaches:

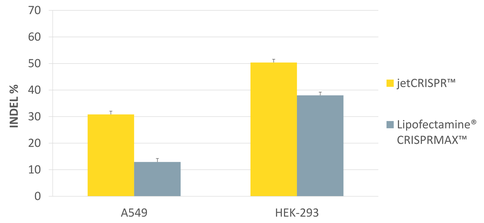
- ✓ Easier delivery in hard to transfect cells
- ✓ Fast gene editing activity
- ✓ Better Cas9 activity control
- ✓ Reduced off-target effects (faster Cas9 clearance)
- ✓ Easier gRNA screening
- ✓ Excellent cell viability

High Genome Editing efficiency

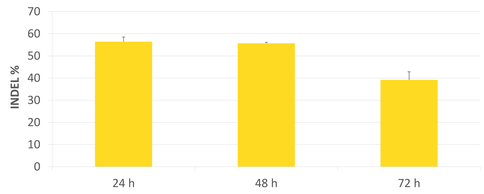
sgRNA targetted HPRT1	-	-	-	5 nM	-	10 nM	-	30 nM
sgRNA negative control	-	-	5 nM	-	10 nM	-	30 nM	-
Cas9 protein	-	-	5 nM	5 nM	10 nM	10 nM	30 nM	30 nM
T7 endonuclease 1	-	+	+	+	+	+	+	+



High genome editing efficiency using jetCRISPR™ in HeLa cells. RNP transfection was performed in HeLa cells using several RNP concentrations of HPRT1 sgRNA and Cas9 protein in combination with 0.3 µl of jetCRISPR™ reagent per well of a 96-well plate. At 48h post-transfection, T7 digestion products were run on a 2% agarose gel and stained with BET displayed by G:Box transilluminator (Syngene®). Acquisitions were carried out with the Genesnap software (Syngene®) and INDEL quantifications were performed with the Genetools software (Syngene®).
1: Uncleaved fragment of 1083 bp, 2: long-cleaved fragment of 827 bp, 3: short-cleaved fragment of 256 bp.



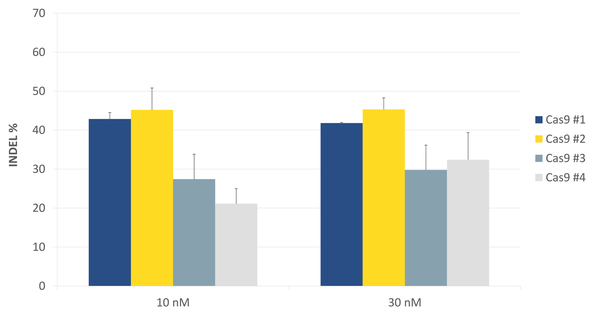
Superior genome editing efficiency obtained with jetCRISPR™ in comparison with Lipofectamine® CRISPRMAX™ in A549 and HEK-293 cells. RNP transfection was performed in A549 and HEK-293 cells using 30 nM RNP (Cas9 and HPRT1 sgRNA) with 0.3 µl of jetCRISPR™ reagent or 0.3 µl of Lipofectamine® CRISPRMAX™, per well of a 96-well plate. At 48h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was quantified using Genetools software (Syngene®).

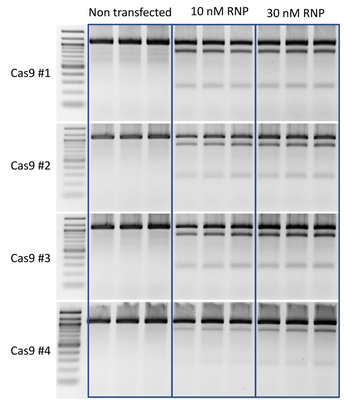


Fast and reliable gene editing efficiency obtained with jetCRISPR™ in HEK-293 cells. RNP transfection was performed in HEK-293 cells using 30 nM RNP (Cas9 and HPRT1 sgRNA) with 0.3 µl of jetCRISPR™ reagent or 0.3 µl of Lipofectamine® CRISPRMAX™, per well of a 96-well plate. At 48h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was quantified using Genetools software (Syngene®).

INDEL efficiency optimization

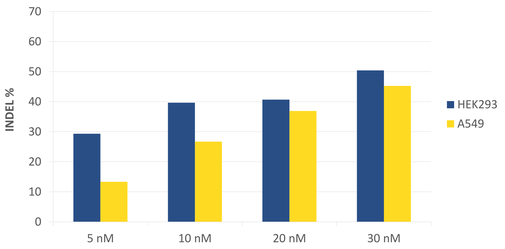
Select the best Cas9 protein

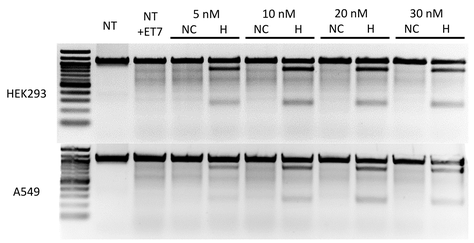




Different Cas9 proteins lead to different indel efficiency. RNP transfection was performed in HEK-293 cells using 10 or 30 nM RNP (Cas9 and HPRT1 sgRNA) with 0.3 µl of jetCRISPR™ reagent, per well of a 96-well plate. At 48h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was quantified using Genetools software (Syngene®).

Optimize the Cas9 protein concentration



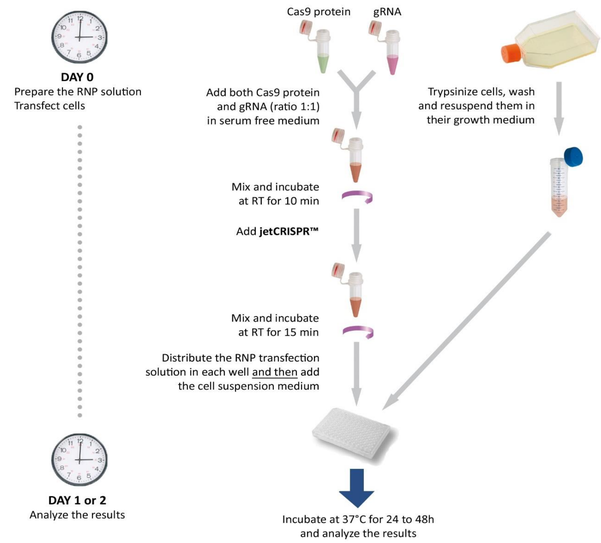


NT: Non tranfected, ET7: Endonuclease T7, NC: sgRNA negative control, H: sgRNA HPRT1

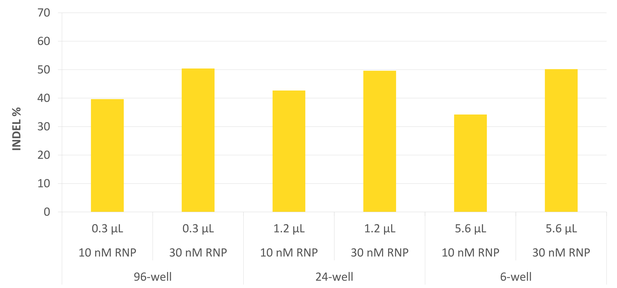
Optimal RNP concentration for efficient genome editing. RNP transfection was performed in HEK293 or A549 cells using different concentrations of RNP (Cas9 and HPRT1 sgRNA) with 0.3 µl of jetCRISPR™ reagent, per well of a 96-well plate. At 48h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was quantified using Genetools software (Syngene®).

jetCRISPR™ easy protocol

Reverse protocol



Easy to scale-up and down



Reproducible gene editing efficiency obtained with jetCRISPR™ in HEK-293 cells and different plate formats. RNP transfection was performed in HEK-293 cells using 10 and 30 nM RNP (Cas9 and HPRT1 sgRNA) with jetCRISPR™ reagent in different plate formats. At 48h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was quantified using Genetools software (Syngene®).

Conclusion

Advantages of jetCRISPR™

- ✦ Specifically designed for Cas9 protein and guide RNA delivery
- ✦ High genome editing efficiency
- ✦ Excellent cell viability and morphology
- ✦ Easy and ready to use reagent to fit your needs

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