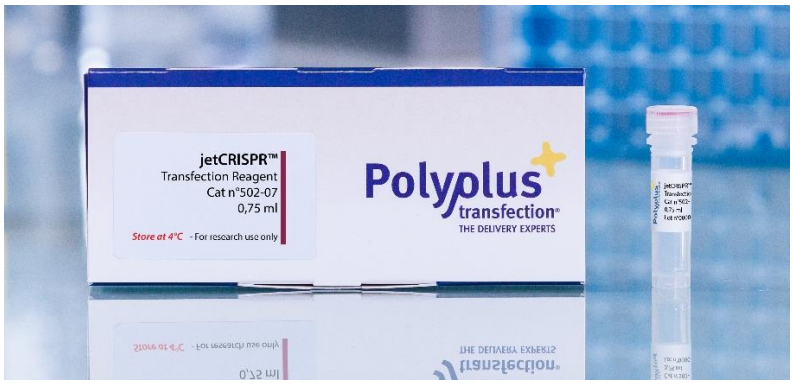
APPLICATION NOTE

Enhanced CRISPR/Cas9 genome editing using RNP delivery with jetCRISPR™

Alengo Nyamay'Antu[1], Fanny Prémartin[1], Thibaut Benchimol[1], Valérie Toussaint[1], Patrick Erbacher[1]



Introduction

The association of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) nucleases is an innovative technology to generate gene knock-outs, introduce defined sequences or deletions in the genome. The CRISPR/Cas9 engineered nuclease system is a two-component system with a guide RNA (gRNA) molecule that drives the Cas9 nuclease to create a double-strand break at a specific targeted sequence within the genome.

Successful targeted 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. Transfection is therefore a key and often limiting step to ensure successful genome editing. There are currently three main approaches to introduce gRNA and Cas9 in mammalian cells: DNA, RNA or RNP (gRNA + Cas9 protein) (**Fig. 1**).

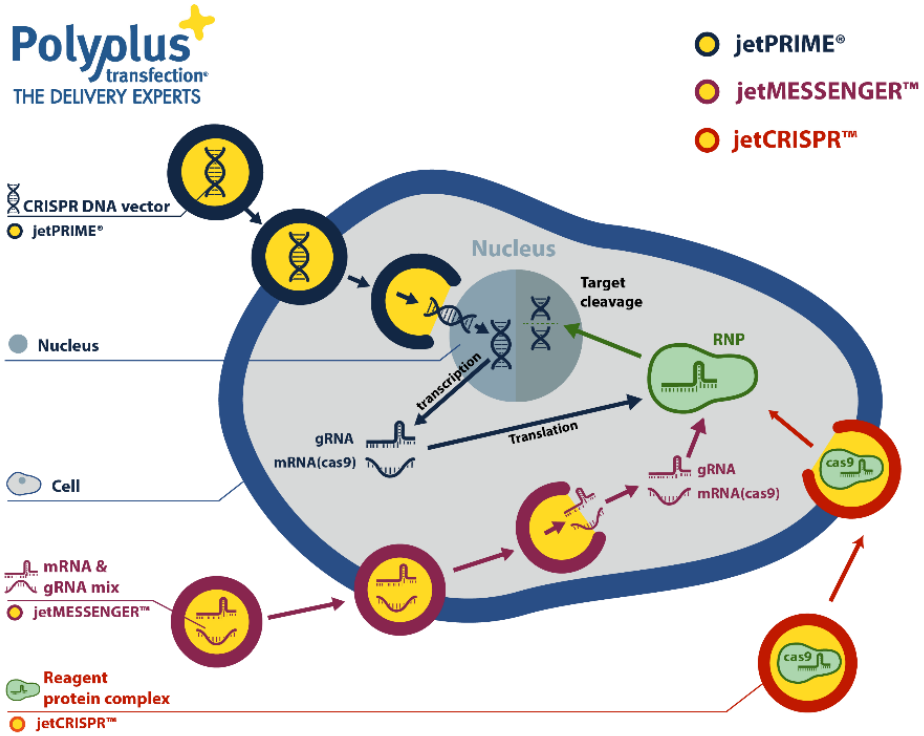


Fig. 1: *in vitro* transfection approaches for CRISPR experiments.

[1] Polyplus-transfection®, 850 Bld Sébastien Brant, 67400 Illkirch, France, support@polyplus-transfection.com

DNA-free delivery systems in which Cas9 is delivered as mRNA or as a protein have thus become attractive alternatives, as they overcome the main obstacle that DNA entry into the nucleus constitutes in harder to transfect cells. In contrast to a plasmid DNA approach, delivery of Cas9 as mRNA or even more so as a protein leads to a more transient and time-restricted presence of Cas9 within cells, before being rapidly cleared via the protein degradation pathways. Consequently, delivery of Cas9 as a protein is currently the best-suited approach to reduce potential off-target nuclease activity, when specificity is a main concern.

Here, we present jetCRISPR™ our latest innovative reagent designed to directly deliver Cas9 as a protein along with the guide RNA, also known as ribonucleoprotein (RNP) delivery. Both transcription and translation to obtain a functional Cas9 protein is bypassed, which makes RNP delivery using jetCRISPR™ the fastest and most precise gene editing approach currently available for a wide variety of cells.

High Genome Editing efficiency using RNP transfection approach

jetCRISPR™ transfection reagent is an innovative transfection reagent which efficiently delivers RNP complexes to reach highest Cas9-mediated genome editing. During RNP transfection optimization, we carefully optimized gRNA and Cas9 protein concentration and ratio as well as the Cas9 protein sequence. Genome editing efficiency measured as the percentage of INDEL (%), was assessed within a range of RNP concentrations to target one of the frequently used housekeeping gene HPRT1 **(Fig. 2)**. Several RNP concentrations were tested ranging from 5-30 nM, with a gRNA to Cas9 ratio of 1:1. While transfection of RNP at the lowest range of 5 nM generated 20% of INDEL in HeLa cells, we could demonstrate that by increasing RNP concentration to 10 nM, we could further increase by 2-fold editing of the targeted HPRT1 gene. Furthermore, with up to 30 nM of RNP, 50 % of INDEL was reached with minimal impact on cell viability and cell morphology

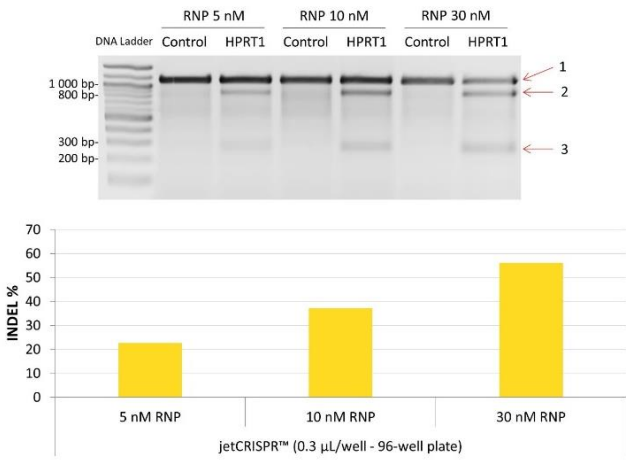


Fig. 2: High genome editing efficiency using jetCRISPR™ in HeLa cells. RNP transfections were performed in HeLa cells using several RNP concentrations of Cas9 protein and HPRT1 gRNA or negative control in combination with 0.3 µl of jetCRISPR™ reagent per well of a 96-well plate. At 48 h post-transfection, 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.

We could further show that the Cas9 protein sequence can also lead to significant variations in genome editing efficiency. RNP transfection optimization with commercially available recombinant Cas9 proteins was performed in HEK-293 cells to target one the housekeeping gene HPRT1. As shown in **Fig. 3**, The number of Nuclear Localization Sequence (NLS) and protein tags, as well as their positioning in N- or C-terminus could affect the genome editing efficiency. To this end, Polyplus-transfection also provides the Cas9 protein - SpCas9 nuclease - that led to the highest genome editing, irrespective of the concentration of RNP tested within the optimal range.

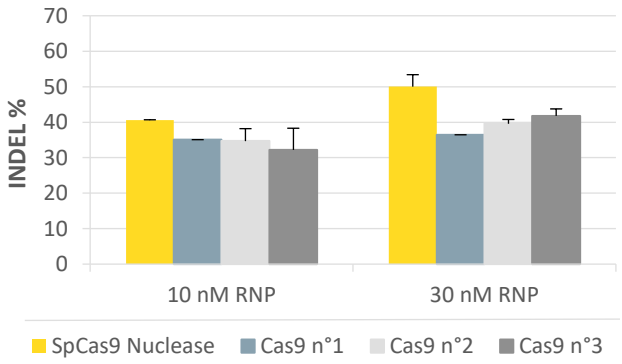


Fig. 3: Higher genome editing obtained with SpCas9 Nuclease compared to other Cas9 competitors using jetCRISPR™ transfection reagent. RNP transfections were performed in HEK-293 cells using 10 or 30 nM RNP (HPRT1 sgRNA + Cas9 protein) with 0.3 µl of jetCRISPR™ reagent per well of 96-well plate. At 48 h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was determined by using Genetools software (Syngene®).

Superior genome editing efficiency obtained with jetCRISPR™

RNP transfection using jetCRISPR™ is a straightforward 3-step process: assembling RNP complexes, mixing in of the transfection reagent and adding complexes to cells. Both reverse and forward transfections have been successfully optimized with jetCRISPR™, hence facilitating the implementation of a protocol for a wide variety of cell types. In addition to its compatibility with different cell culture systems, jetCRISPR™ transfection reagent systematically outperforms its main competitor by achieving higher genome editing efficiency, as illustrated in **Fig. 4.**

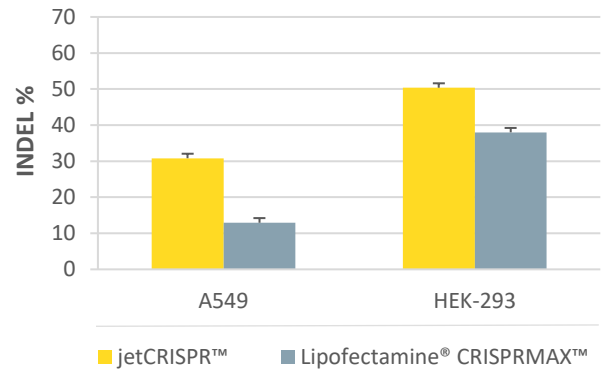


Fig.4: Superior genome editing efficiency obtained with jetCRISPR™ in comparison with Lipofectamine® CRISPRMAX™. RNP transfections were performed in A549 and HEK-293 cells using 30 nM RNP (Cas9 Protein and HPRT1 gRNA) with 0.3 µl of jetCRISPR™ reagent or 0.3 µl of Lipofectamine® CRISPRMAX™, per well of a 96-well plate. At 48 h post-transfection, genome editing was assessed by calculating the percentage (%) of INDEL using the T7 endonuclease method. The INDEL % was determined by using Genetools software (Syngene®).

Conclusion

Successful delivery of the gRNA and the Cas9 into cells is essential to guarantee the high genome editing efficiency that is required to generate new cell or animal models. The most efficient CRISPR-Cas9 method is based on the direct delivery of pre-complexed gRNA and Cas9 protein, referred to as Ribonucleoprotein (RNP) delivery. RNP transfection has many advantages compared to plasmid or mRNA approaches, of which a better Cas9 activity control and a reduced amount of off-target effects while maintaining excellent cell viability.

With our recently launched RNP transfection reagent jetCRISPR™, we now provide a full range of reagents for all CRISPR approaches **(Table 1).** Use the leading technology for CRISPR/Cas9 RNP delivery!

in vitro - in vivo	Delivered molecule	Cas9	Guide RNA	Our solutions
in vitro	Protein/RNA	Protein	RNA	**jetCRISPR™** RNP transfection reagent
in vitro	DNA	DNA	DNA	**jetPRIME®** DNA transfection reagent
in vitro	RNA	mRNA	RNA	**jetMESSENGER™** mRNA transfection reagent
in vivo	DNA/RNA	DNA/mRNA	DNA/RNA	***in vivo*-jetPEI®** *in vivo* delivery reagent

Table 1: Polyplus-transfection's range of transfection reagents for CRISPR experiments.

References

Product	Cat N°	Reagent size
jetCRISPR™	502-01	0.1 ml
	502-07	0.75 ml
	502-15	1.5 ml
SpCas9 Nuclease	722-100	100 µg

Resources

Phone: +33 (0)3 90 40 61 87

Email: support@polyplus-transfection.com

Web site: www.polyplus-transfection.com