DELIVERING GENOME EDITING REAGENTS: RNP OR PLASMID?

One of the great features of CRISPR-Cas technology is its ease of use.

Now, molecular biologists can edit a genome in a matter of days using the CRISPR-Cas system. The process requires a Cas enzyme and guide RNA (gRNA) that directs Cas to the target DNA location.

When the technology was first discovered, it was necessary to construct plasmid vectors to express Cas enzyme and the gRNA, because the long synthetic RNAs were very expensive and not widely available. Transfected plasmids would use the cells' transcriptional and translational machinery to produce both the Cas protein and the gRNA. However, a large body of data now shows that this approach is inefficient and produces high off-target effects. Delivery of Cas protein and gRNA as a ribonucleoprotein (RNP) complex is an efficient and proven method that offers several advantages compared to plasmid expression vectors.

RNP complexes provide lower off-target editing because they are delivered directly to the cells as active complexes for high on-target editing, but do not persist in the cells to increase the risk of off-target editing.

A world leader in oligo production and CRISPR gene editing, IDT provides tools and reagents to make your CRISPR experiments a success. Explore our extensive portfolio of CRISPR editing products at **www.idtdna.com/CRISPR.**

Advantages of RNP over plasmid delivery of Cas enzymes and gRNAs:

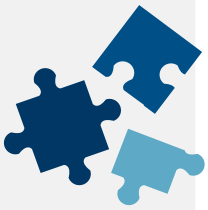
Genome editing with plasmid is laborious

You need to construct expression vectors, transform bacteria, select multiple clones, isolate DNA, perform restriction enzyme digests to identify positive clones, and sequence the positive clones to confirm their sequences.

Instead, simply order transfection-ready RNP components from IDT and jump-start your research.

Plasmid delivery is inherently inconsistent

Transfection efficiency differs between cell lines, and even varies from one experiment to the next. Plasmid delivery is particularly problematic for "hard-to-transfect" cell lines, resulting in inefficient editing.



The RNP complex can be delivered into cells with high efficiency by lipofection or electroporation, resulting in potent editing.

Plasmids leave behind molecular footprints

We and others have demonstrated that when gRNAs are introduced as expression plasmids, not only multiple bases but also large fragments of plasmid vectors are inserted into the genome.

RNP complex delivery produces fewer multiple-base insertions and avoids issues related to vector molecular footprint.

Long-lasting expression from plasmid vectors increases off-target editing

Plasmid-driven expression continues for a long time, which significantly increases off-target editing.

In contrast, RNP delivery produces a "fast on, fast off" reaction that increases targeting accuracy and decreases the chance of off-target editing.

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RUO23-1914_001 05/23