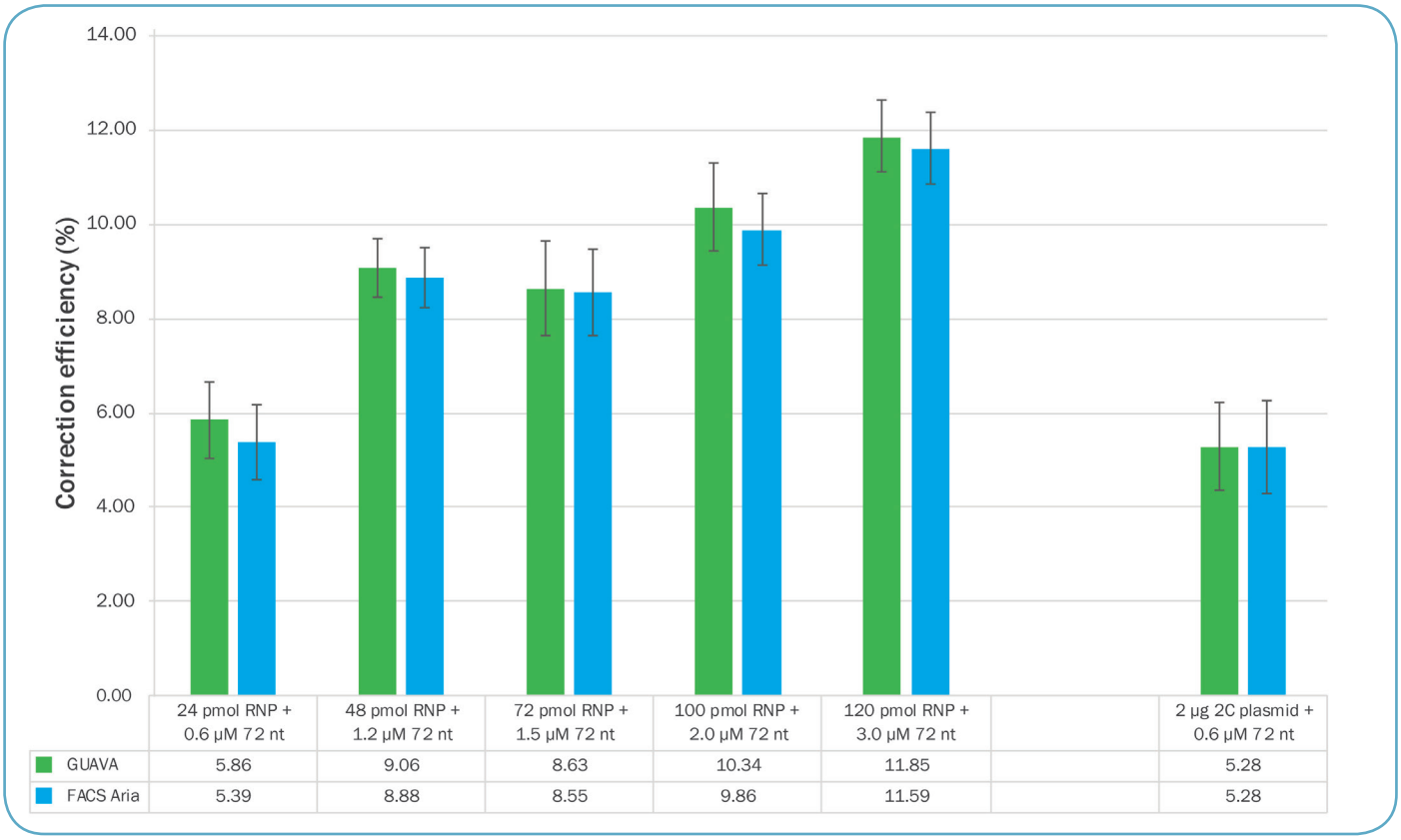
Improved homology-directed gene repair using the Alt-R™ CRISPR-Cas9 System

CRISPR-Cas9 genome editing reagents delivered as a ribonucleoprotein complex increase repair efficiency compared to plasmid-based delivery methods

Dr Eric Kmiec at the Gene Editing Institute, Helen F. Graham Cancer Center and Research Institute, Christiana Care Health System, compared the Alt-R CRISPR-Cas9 System ribonucleoprotein (RNP) to plasmid-based CRISPR-Cas9 tools for their ability to facilitate homology-directed repair of a mutated gene. His research group used a human colorectal carcinoma–derived cell line (HCT116), containing a single, stably incorporated eGFP with a nonsense, point mutation. In addition to the CRISPR-Cas9 components, a 72-nucleotide, single-stranded, donor oligo was used to correct the eGFP sequence to produce a functional eGFP protein for counting successful events by fluorescence-activated cell sorting (FACS). All components were delivered by electroporation. The researchers found that the RNP CRISPR-Cas9 approach doubled the correction efficiency in their experiments (Figure 1) and eliminated toxicity observed with the plasmid-based system.



	24 pmol RNP + 0.6 µM 72 nt	48 pmol RNP + 1.2 µM 72 nt	72 pmol RNP + 1.5 µM 72 nt	100 pmol RNP + 2.0 µM 72 nt	120 pmol RNP + 3.0 µM 72 nt		2 µg 2C plasmid + 0.6 µM 72 nt
GUAVA	5.86	9.06	8.63	10.34	11.85		5.28
FACS Aria	5.39	8.88	8.55	9.86	11.59		5.28

Figure 1. Improved homology-directed repair by ribonucleoprotein delivery of CRISPR-Cas9 genome editing reagents. Optimization of gene editing was performed in an HCT116 cell line with an integrated, single copy of an eGFP mutant gene containing a nonsense mutation at position +67 (TAC ⟶TAG). Alt-R™ CRISPR-Cas9 System components were assembled into an RNP to target the nonsense mutation site, as instructed (www.idtdna.com/CRISPR-Cas9). For comparison, a chimeric single-guide RNA was cloned into a pX330 backbone vector (Addgene) that also expresses a codon optimized SpCas9 (2C plasmid). A single-stranded 72 nt oligonucleotide was used as donor template for homology-directed repair of the nonsense mutation. CRISPR-Cas9 components and donor template were delivered into the cells using a Gene Pulser Xcell™ Electroporation System (Bio-Rad Laboratories). Cells were assayed for eGFP repair 72 hr after electroporation by using the Guava® easyCyte 5HT Benchtop Flow Cytometer (Millipore; green bars) and FACSAria™ II Flow Cytometer (BD Biosciences; blue bars) instruments. Error bars represent standard deviation.

"Having been in the field of gene editing for almost 20 years, the development and evolution of the CRISPR-Cas9 system has simply changed everything. However, the overexpression of the guide RNA and Cas9 components in transfected vectors creates a standard problem for most biomedical systems—an unhealthy and often deleterious environment inside the targeted cells. Fortunately, we were introduced to the Alt-R CRISPR-Cas9 System and RNP delivery method developed by IDT. Now, not only has the frequency of single-nucleotide repair improved significantly, but the cells maintain a high degree of viability and the capability to carry out metabolic processes, including cell division, in a normal fashion."

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IDT recommends using Alt-R S.p. Cas9 Nuclease 3NLS combined with Alt-R CRISPR-Cas9 crRNA and tracrRNA to generate a ribonucleoprotein editing complex for high editing efficiency across most target sites. View the *Alt-R CRISPR-Cas9 User Guide for ribonucleoprotein transfections* for delivery of the RNP into your cell lines (available at www.idtdna.com/CRISPR-Cas9).