SANTA CRUZ BIOTECHNOLOGY, INC.

UROS CRISPR/Cas9 KO Plasmid (m): sc-423627

BACKGROUND

DNA containing double-strand breaks (DSB) created by the CRISPR/Cas9 system can be repaired by either the non-homologous end-joining (NHEJ) or the homology-directed repair (HDR) pathway (1,2,3). The NHEJ repair pathway introduces non-specific insertions or deletions at the cleavage site, whereas the HDR pathway allows for precise gene editing at the DSB site (1,2,3). Target-specific HDR Plasmids provide a DNA repair template for a DSB and, when co-transfected with CRISPR/Cas9 KO Plasmids, enable the insertion of specific selection markers where Cas9-induced DNA cleavage has occurred (1,2). The HDR plasmid can incorporate a Red Fluorescent Protein (RFP) gene to visually confirm transfection and an antibiotic resistance gene (puromycin) for selection of cells containing a successful CRISPR/Cas9 double-strand break. The puromycin resistance and RFP encoding genes are flanked by two LoxP sites that are recognized by the Cre Vector, which can be used to later remove these selection markers from the genomic DNA (4,5).

REFERENCES

1. Mali, P., et al. 2013. RNA-guided human genome engineering via Cas9. Science 339: 823-826.
2. Ran, F.A., et al. 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8: 2281-2308.
3. Hsu, P., et al. 2014. Development and applications of CRISPR-Cas9 for genome editing. Cell 157: 1262-1278.
4. Ma, Y. 2014. Generation of eGFP and Cre knockin rats by CRISPR/Cas9. FEBS J. 281: 3779-3790.
5. Ma, Y., et al. 2014. Generating rats with conditional alleles using CRISPR/Cas9. Cell Res. 24: 122-125.

CHROMOSOMAL LOCATION

Genetic locus: Uros (mouse) mapping to 7 F3.

PRODUCT

UROS CRISPR/Cas9 KO Plasmid (m) is designed to disrupt gene expression by causing a double-strand break (DSB) in a 5' constitutive exon within the Uros (mouse) gene.

UROS CRISPR/Cas9 KO Plasmid (m) consists of a pool of 3 plasmids, each encoding the Cas9 nuclease and a target-specific 20 nt guide RNA (gRNA) designed for maximum knockout efficiency. Each vial contains 20 µg of lyophilized CRISPR/Cas9 Plasmid DNA. Suitable for up to 20 transfections. Also see UROS HDR Plasmid (m): sc-423627-HDR for selection of cells containing a DSB induced by UROS CRISPR/Cas9 KO Plasmid (m).

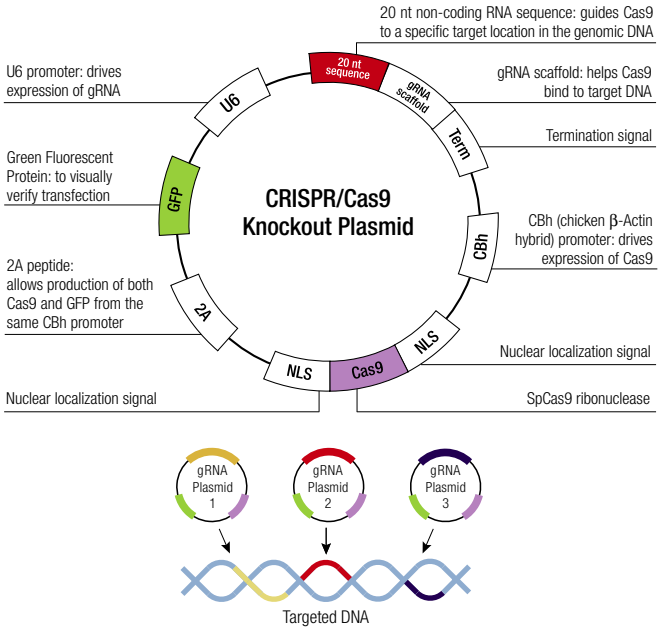
STORAGE AND RESUSPENSION

Store lyophilized plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -20° C for long-term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized plasmid DNA in 200 µl of the provided ultrapure, sterile, DNase-free water. Resuspension of the plasmid DNA makes a 0.1 µg/µl solution in a 10 mM TRIS EDTA, 1 mM EDTA buffered solution.

APPLICATIONS

UROS CRISPR/Cas9 KO Plasmid (m) is recommended for the disruption of gene expression in mouse cells.



SUPPORT REAGENTS

For optimal reaction efficiency with CRISPR/Cas9 KO Plasmids, Santa Cruz Biotechnology's UltraCruz® Transfection Reagent: sc-395739 (0.2 ml) and Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control CRISPR/Cas9 Plasmid: sc-418922 (20 µg) negative control is also available.

GENE EXPRESSION MONITORING

UROS (A-8): sc-365877 is recommended as a control antibody for monitoring of Uros (mouse) gene expression prior to and after knockout by Western blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

RESEARCH USE

The CRISPR/Cas9 KO Plasmids are considered "Licensed Products" and are to be used in accordance with the Limited License stated on www.scbt.com/limitedlicense.

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.