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SANTA CRUZ BIOTECHNOLOGY, INC.

SAMD1 HDR Plasmid (m): sc-437099-HDR

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: Samd1 (mouse) mapping to 8 C3.

PRODUCT

SAMD1 HDR Plasmid (m) consists of a pool of 2-3 plasmids, each containing a homology-directed DNA repair (HDR) template corresponding to the cut sites generated by the SAMD1 CRISPR/Cas9 KO Plasmid (m): sc-437099. Each HDR template contains two 800 bp homology arms designed to specifically bind to the genomic DNA surrounding the corresponding Cas9-induced double-strand DNA break site. Each vial contains 20 µg of lyophilized HDR Plasmid DNA. Suitable for up to 20 transfections.

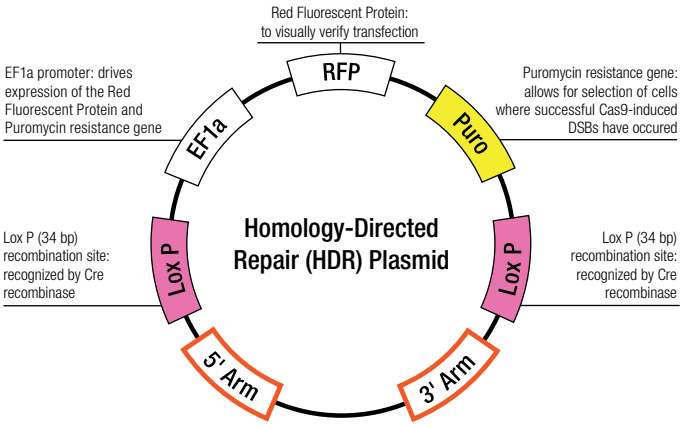
STORAGE AND RESUSPENSION

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

SAMD1 HDR Plasmid (m) is recommended for co-transfection with SAMD1 CRISPR/Cas9 KO Plasmid (m): sc-437099 and designed for repair of the site-specific Cas9-induced DNA cleavage within the Samd1 (mouse) gene. During repair, the SAMD1 HDR Plasmid (m) incorporates a puromycin resistance gene to enable selection of stable knockout (KO) cells and an RFP gene to visually confirm transfection.



SUPPORT REAGENTS

For optimal reaction efficiency with HDR Plasmids, Santa Cruz Biotechnology's UltraCruz® Transfection Reagent: sc-395739 (0.2 ml), Plasmid Transfection Medium: sc-108062 (20 ml) and L-755,507: sc-204045 (10 mg) are recommended. Puromycin dihydrochloride: sc-108071 (25 mg) is recommended for selection. Cre Vector: sc-418923 (20 µg in 20 µl) is also available for the optional removal of the puromycin resistance gene inserted during homology-directed repair.

GENE EXPRESSION MONITORING

SAMD1 (D-16): sc-244061 and SAMD1 (N-16): sc-244059 are recommended as control antibodies for monitoring of Samd1 (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 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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and