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MAGE-b4 HDR Plasmid (m): sc-436700-HDR

BACKGROUND

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas9) system is an adaptive immune response defense mechanism used by archaea and bacteria for the degradation of foreign genetic material (4,6). This mechanism can be repurposed for other functions, including genomic engineering for mammalian systems, such as gene knockout (KO) (1,2,3,5). CRISPR/Cas9 KO Plasmid products enable the identification and cleavage of specific genes by utilizing guide RNA (gRNA) sequences derived from the Genome-scale CRISPR Knock-Out (GeCKO) v2 library developed in the Zhang Laboratory at the Broad Institute (3,5).

REFERENCES

1. Mali, P., et al. 2013. RNA-guided human genome engineering via Cas9. *Science* 339: 823-826.
2. Cong, L. et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. *Science* 339: 819-823.
3. Ran, F.A., et al. 2013. Genome engineering using the CRISPR-Cas9 system. *Nat. Protoc.* 8: 2281-2308.
4. Van der Oost, J., et al. 2014. Unraveling the structural and mechanistic basis of CRISPR-Cas systems. *Nat. Rev. Microbiol.* 7: 479-492.
5. Shalem, O., et al. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science* 343: 84-87.
6. Hsu, P., et al. 2014. Development and applications of CRISPR-Cas9 for genome editing. *Cell* 157: 1262-1278.

CHROMOSOMAL LOCATION

Genetic locus: *Mageb4* (mouse) mapping to X C1.

PRODUCT

MAGE-b4 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 MAGE-b4 CRISPR/Cas9 KO Plasmid (m): sc-436700. 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.

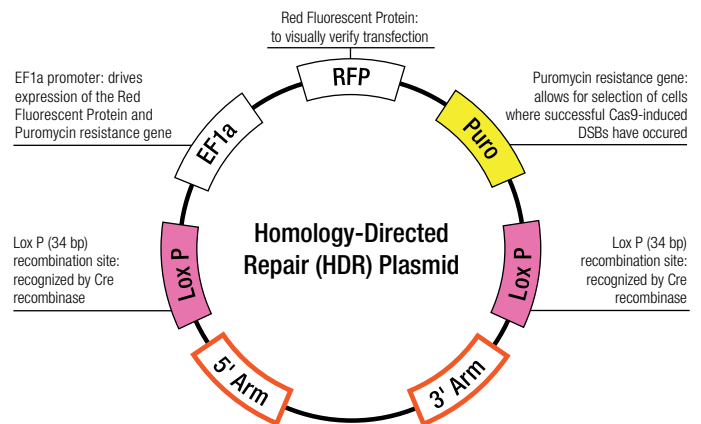
Resuspend lyophilized plasmid DNA in 200 µl of the provided ultrapure, sterile, DNase-free water. Resuspension of the plasmid DNA makes a 0.1 µg/µl solution in a 10 mM TRIS EDTA, 1 mM EDTA buffered solution.

PROTOCOLS

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

APPLICATIONS

MAGE-b4 HDR Plasmid (m) is recommended for co-transfection with MAGE-b4 CRISPR/Cas9 KO Plasmid (m): sc-436700 and designed for repair of the site-specific Cas9-induced DNA cleavage within the *Mageb4* (mouse) gene. During repair, the MAGE-b4 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. 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

MAGE-b4 (M-90): sc-367409 is recommended as a control antibody for monitoring of *Mageb4* (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.