CRISPR KN2.0: A Universal Solution for Genome-Wide Gene Knockout/Knockin

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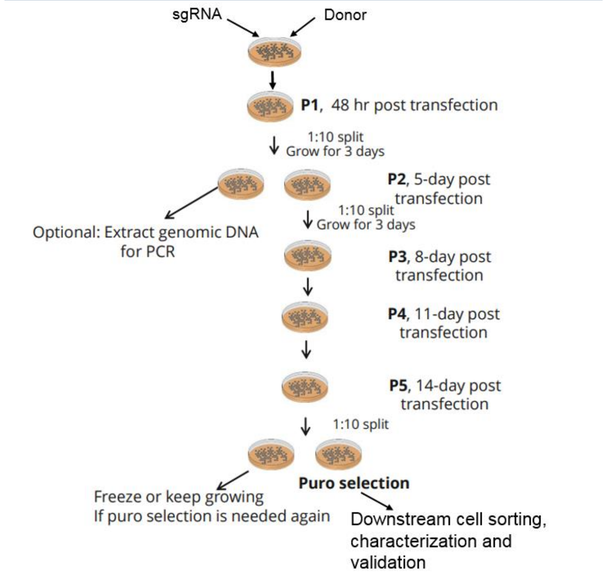
Abstract

Although homology directed repair (HDR)-mediated gene knockout / knockin is well established, it cannot necessarily be applied in some cell types and organisms with low HDR frequencies. Herein we describe an improved technology for genome-wide gene knockout / knockin using CRISPR-Cas9 targeted genome editing: CRISPR KN2.0. CRISPR KN2.0 technology is specifically designed to provide an easy-to-use and universal solution for all the researchers' knockout / knockin needs in every cell type and organism. This gene knockout / knockin technology is designed to knock out any DNA locus and knock in any functional cassettes including Green Fluorescence Protein (GFP) and puromycin resistant gene to facilitate screening process. Both of these cassettes are expressed under EF1A promoter after genomic integration. CRISPR KN 2.0 has been used to successfully knock out many genes / knocked in functional cassette in HeLa, HEK293T and MIA PaCa-2 (a human pancreatic carcinoma cell line) cells. Studies carried out in house and by some colleagues show that CRISPR KN 2.0 is highly efficient and render improved knockout/knockin rate (over 50% positively integrated colonies after puromycin selection).

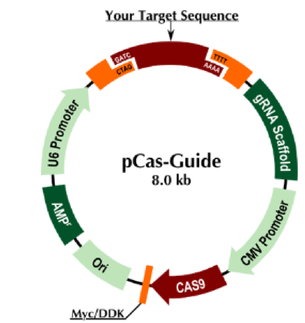
Overview



Procedures And Results

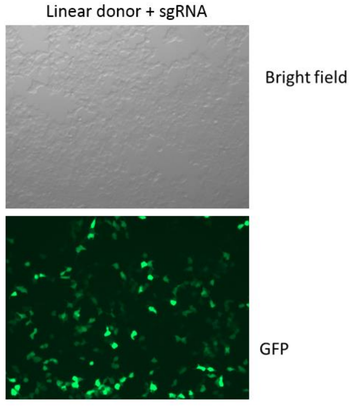
A. All-in-one CRISPR/Cas9 Vector.

gRNA sequence: AACTTGTTTCACGCTATATC

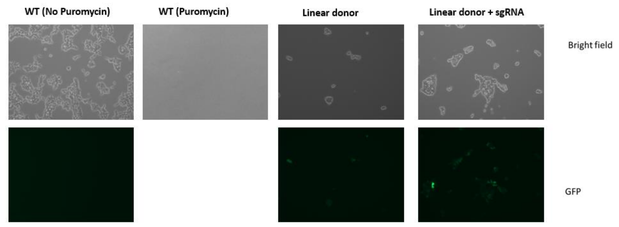


B. Linear Donor.

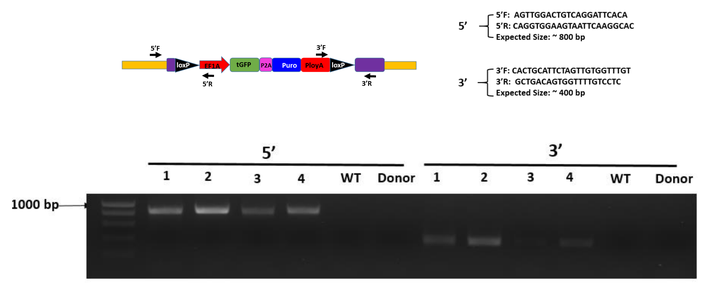
C. Validation: HEK293 Cells (24 h after Transfection)



D. Validation: P5 Cells (1 Week after Puro Selection)



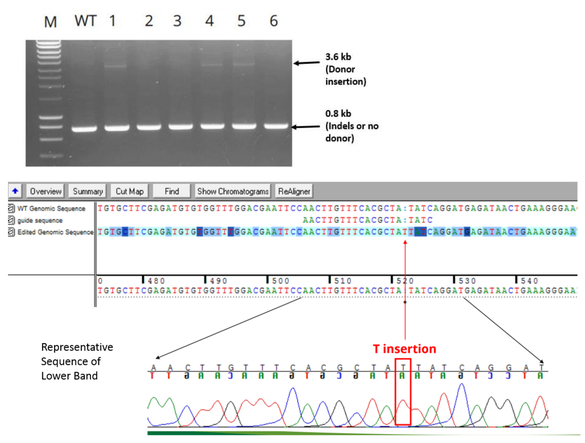
E. Validation: PCR on P2 Cells (Cell Pool)



(data not shown)

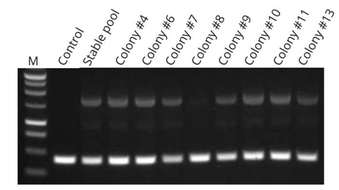
F. Validation: PCR on Single Colony Cells (1 Week after Puro Selection)

Forward Primer: AGTTGGACTGTCAGGATTCACA
Reverse Primer: TGTTGTCATGATGCGTTCTGG

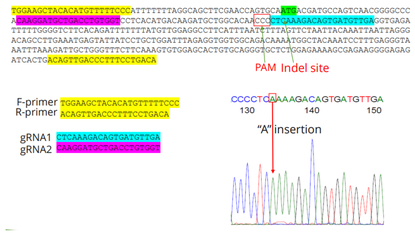


G. Human SHMT1 Knockout Using KN2.0 in MIA PaCa-2 Cells (Data from a customer)

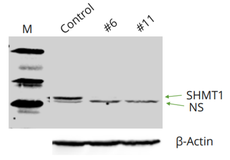
7 out of 8 Single Cell Clones (87%) Have Donor Insertion



Representative Sequence of Lower Band



SHMT1 Biallelic Knockout was Confirmed by WB



NS: Non-specific band

Conclusions

1. CRISPR KN2.0 technology is highly efficient in knockout/knockin gene or gene cassette (over 50% positively integrated colonies after puromycin selection).
2. CRISPR KN2.0 technology provides a fast (Less than 1 month) and versatile tool to meet your genome-wide gene knockout/knockin needs.

For more information, please contact our customer support:

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