A ROBUST METHOD FOR TAGGING ENDOGENOUS GENES THROUGH PROMOTER TRAPPING AND SHORT HOMOLOGY ARMS

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ABSTRACT

Precise genome editing via homology-directed repair (HDR) pathway holds great promise for gene and stem cell therapy. However, the efficiency of integrating large DNA molecules into mammalian genome via HDR is intrinsically low. Recently, we showed that the use of short homology arms (≤30nt) was sufficient to introduce small changes in mammalian genome. Now, we take a step further and develop a novel method for tagging endogenous genes through promoter trapping and short homology arms, which synergize with a novel strategy, split targeted integration, that allows for the efficient creation of diverse targets. The HDR efficiency of tagging endogenous genes with a 1.4 kb promoterless GFP reporter ranged from 50% to 100% upon antibiotic selection with specifically occurring at the Cas9 RNP at the flanking protospacer adjacent motif (PAM) site. The method has broad applications in general genome engineering, DNA cloning, protein production and immune cell therapy.

INTRODUCTION

The recent advances in CRISPR/Cas9-mediated genome engineering enable researchers to efficiently introduce double-strand breaks (DSBs) in genomic DNA. The DSBs are then efficiently repaired by the non-homologous end joining (NHEJ) pathway or the homology-directed repair (HDR) pathway. While the NHEJ pathway is predominant and error-prone, which results in disruptive insertions or deletions (indels) at targeted loci among the efficiency of genome editing. The HDR pathway, which connects the DSBs via homologousDNA molecules into mammalian genome via HDR is inherently low. Recently, we showed that the use of short homology arms (≤30nt) was sufficient to introduce small changes in mammalian genome. Now, we take a step further and develop a novel method for tagging endogenous genes through promoter trapping and short homology arms.

MATERIALS AND METHODS

The gRNAs were designed using GeneArt™ CRISPR/gRNA Design Tool from Thermo Fisher Scientific and then synthesized using the GeneArt™ Precision gRNA Synthesis Kit. The concentration of gRNA was determined by QuantiBRITE® Pico Assay Kit. The HDR efficiency was determined by GeneArt® Genomic Cleavage Detection Kit. The GeneArt® Trapeze donor DNA design tool will soon be available from Thermo Fisher Scientific. The donor DNA was prepared by TrueTag™ Donor DNA Kit. The Truecut donor DNA design tool will soon be available from Thermo Fisher Scientific.

RESULTS

(A) Cleavage efficiency of CRISPR (N-ter)
(B) PMI location vs efficiency (N-ter)
(C) Cleavage efficiency of CRISPR (C-ter)
(D) PMI location vs efficiency (C-ter)

Figure 1. Donor Design for Gene Tagging

The G418-resistant colonies were transfected into 293FT cells along with various donor DNA. The percentages of Indel were determined by Sanger sequencing. The percentages of target integration were examined by fluorescence microscope (B). The HDR efficiency of ACTB was determined by flow cytometry staining with anti-GFP antibody. (A) HSC editing workflow. (B) Tagging efficiency of ACTB with EmGFP under different electroporation conditions. (C) The transfected wells were scored and co-transfected with CD90 and CD34 antibodies (D), and the GFP-positive cells were sorted into wells solid medium for 14 days and colony formation was visualized under fluorescence microscopy (C).

CONCLUSIONS

We developed a simple method for tagging endogenous genes efficiently without the need for preparation of donor plasmid. The tagging efficiency could reach nearly 100% upon antibiotic selection. The method has been validated with different targets in different cell lines.

REFERENCES


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TRADEMARKS/LICENSING

A series of gRNAs were designed and synthesized to target either N-ter ATG or C-ter stop codon of the ACTB locus. The gRNA sequences are shown in Table 1. Each gRNA sequence was transfected into 293FT cells along with donor DNA. The HDR efficiency of ACTB was determined by flow cytometry analysis to determine the percentage of GFP-positive cells using the Abcam anti-GFP antibody.

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