In vivo blunt-end cloning through CRISPR/Cas9-facilitated non-homologous end-joining.

Journal: Nucleic Acids Res
Publication Year: 2016
Authors: Jonathan M Geisinger, Soren Turan, Sophia Hernandez, Laura P Spector, Michele P Calos
PubMed link: 26762978
Funding Grants: Engineered iPSC for therapy of limb girdle muscular dystrophy type 2B

Public Summary:
CRISPR/Cas9 is an exciting new method for making precise changes in the DNA of chromosomes. In this study, we improved understanding of how the CRISPR/Cas9 system functions in mammalian cells, including stem cells. We showed that when CRISPR/Cas9 is used to cut DNA, the cut ends are often rejoined precisely. We used this information to develop a new method. In the method, we demonstrate that it is possible to fuse an introduced DNA fragment into the cut ends of DNA. The ends are then sealed up by the cell’s DNA repair machinery, resulting in precise addition of the introduced fragment of DNA. This method is a useful and efficient way to add specific fragments of DNA to chromosomes at precise locations. This ability is valuable for marking genes, manipulating gene expression, and many other uses. Therefore, this study adds new tools for creating useful modifications in mammalian cells, including stem cells.

Scientific Abstract:
The CRISPR/Cas9 system facilitates precise DNA modifications by generating RNA-guided blunt-ended double-strand breaks. We demonstrate that guide RNA pairs generate deletions that are repaired with a high level of precision by non-homologous end-joining in mammalian cells. We present a method called knock-in blunt ligation for exploiting these breaks to insert exogenous PCR-generated sequences in a homology-independent manner without loss of additional nucleotides. This method is useful for making precise additions to the genome such as insertions of marker gene cassettes or functional elements, without the need for homology arms. We successfully utilized this method in human and mouse cells to insert fluorescent protein cassettes into various loci, with efficiencies up to 36% in HEK293 cells without selection. We also created versions of Cas9 fused to the FKBP12-L106P destabilization domain in an effort to improve Cas9 performance. Our in vivo blunt-end cloning method and destabilization-domain-fused Cas9 variant increase the repertoire of precision genome engineering approaches.

Source URL: https://www.cirm.ca.gov/about-cirm/publications/vivo-blunt-end-cloning-through-crisprcas9-facilitated-non-homologous-end