Enhancing CRISPR/Cas9 HDR Efficiency through Covalent Tethering of Donor DNA Template

Eric J. Aird, Klaus N. Lovendahl, Wendy R. Gordon

Department of Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN 55455

HUH endonucleases are domains from viral replication proteins that covalently react with single-stranded DNA via a tyrosine residue in a metal-dependent fashion. This protein-DNA interaction is stable, even when heated to 95°C and exposed to SDS. HUH endonucleases react in a sequence-specific manner. The reaction occurs rapidly (in minutes) at 37°C and is essentially irreversible. A strength of HUH endonucleases is their small size and ease to fuse to other proteins. One such application is to fuse PCV (a type of HUH endonuclease) to Cas9. This allows us to tether a single-stranded oligo deoxynucleotide (ssODN) to Cas9 via PCV. By covalently linking the donor template to Cas9, we hypothesize the effective local concentration of the template will be increased, leading to increased HDR (homology directed repair). Here we show a consistent 2 to 3 fold increase in HDR efficiency when using Cas9 fused to PCV compared to Cas9 alone. This increase is seen at both the protein level using a split luciferase assay as well as at the DNA level utilizing qPCR. For the GAPDH target locus, the percent integration of a split luciferase is increased from 1.3% to 4% with the addition of PCV to Cas9. We believe this increased HDR efficiency can have wide ranging applications across many fields in biology and chemistry.