DNA Ends Matter: The Impact of Using CRISPR/Cas9 Variants on DNA Repair Pathway Choice and Editing Profiles at The HBB Locus

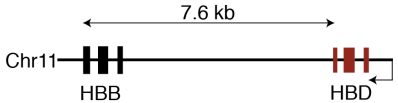
Tanushree Phadke, Anne Bothmer, Christina Lee, Luis Barrera, Hari Jayaram, Vic Myer, Cecilia Cotta-Ramusino

Editas Medicine, 300 Third Street, Cambridge, MA 02142

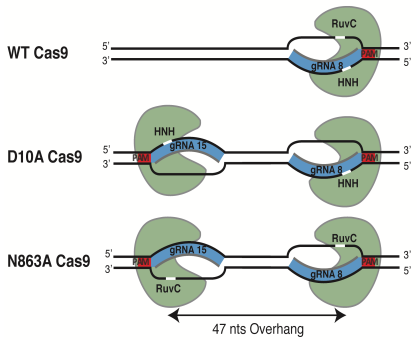
Introduction

- Cas9 and its variants can be used to introduce a variety of different DNA cuts including blunt double stranded break (DSB) or dual nicks leaving either a 3' or 5' overhang.
- The type of cut and donor used can play a role in triggering different repair pathways, thus resulting in various editing outcomes.
- Using a single strand oligonucleotide (ssODN), we characterize different DNA repair outcomes at the in the human beta globin gene including indel mutations resulting from Non Homologous End-Joining (NHEJ), Homology-Dependent Repair (HDR) using the donor as a template, and, finally, Gene Conversion (a kind of HDR event) using the closely related HBD gene as an endogenous template.

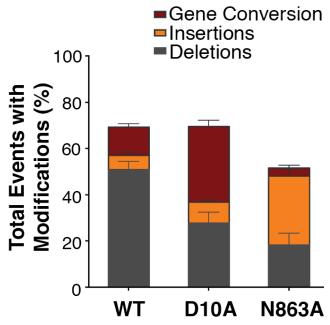
Illustration of the HBB and HBD loci



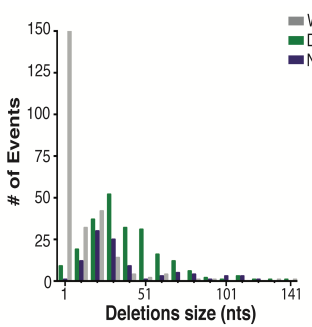
Schematic of Cas9 variants



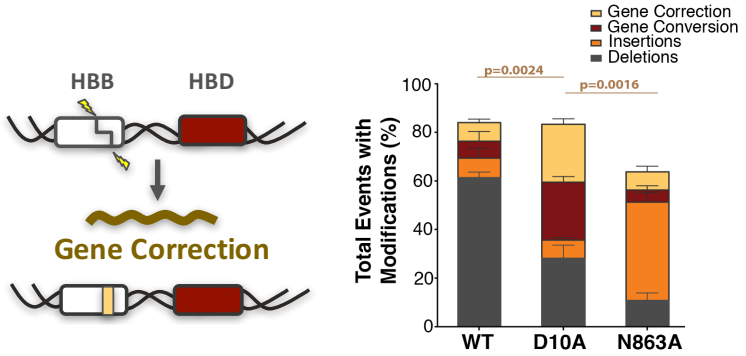
Overall modification frequency



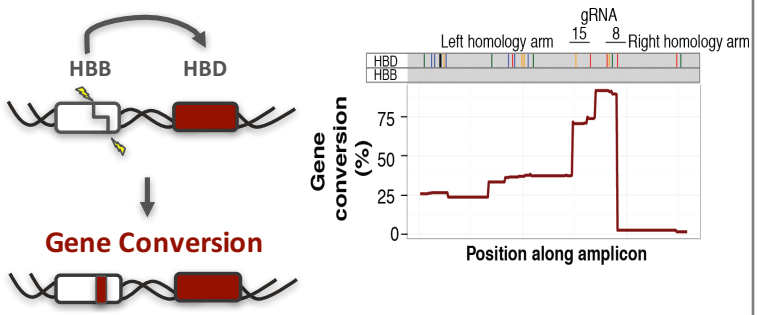
Deletions across Cas9 variants



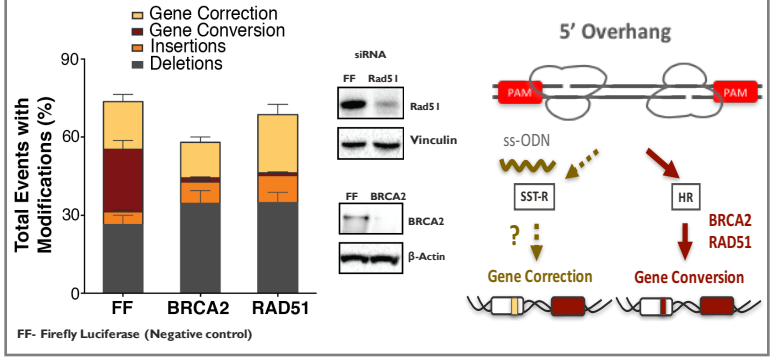
Modification frequency in the presence of ssODN Donor



Characterization of Gene Conversion with D10A Cas9

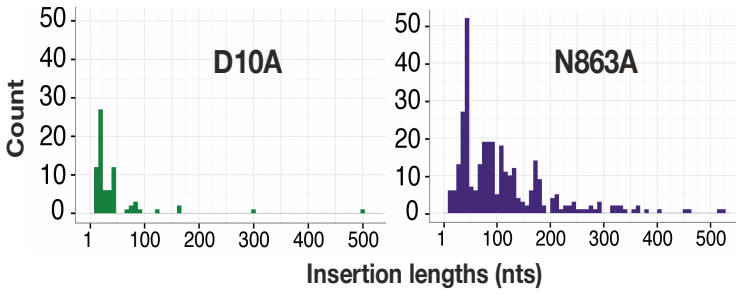


Characterization of genetic requirements for HDR

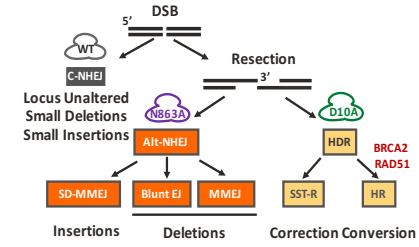


FF- Firefly Luciferase (Negative control)

Insertions across Cas9 variants



Model of possible repair outcomes



C-NHEJ: Canonical Non-Homologous End Joining
Alt-NHEJ: Alternative Non-Homologous End Joining
SD-MMEJ: Synthesis Dependent Microhomology Mediated End Joining
MMEJ: Microhomology Mediated End Joining
Blunt-EJ: Blunt End Joining
SST-R: Single Strand Template Repair
HR: Homologous Recombination

Conclusions

- Different ends generated by Cas9 variants yield different repair outcomes, with the 5' overhang yielding more Gene Correction and Gene Conversion.
- The length of insertions and deletions seen across Cas9 variants is substantially different suggesting the role of multiple repair pathways.
- Gene Conversion appears to be RAD51 and BRCA2 dependent, but Gene Correction does not.