Towards predictable editing: influence of target sequence, cell type, and choice of type II nuclease on desired repair outcomes

Luis Barrera, Barrett Steinberg, Derek Cerchione, Christopher Wilson, Cecilia Cotta-Ramusino, Vic Myer, Hari Jayaram

Abstract

Targeted DNA cleavage by type II CRISPR nucleases has enabled a variety of genome editing applications.

However, the apparently stochastic repair of DNA breaks can create a broad range of edited sequences, providing a unique challenge should a specific edit be desired.

Here, we investigated the distributions of insertions and deletions following cleavage by Cas9 from *S. pyogenes (Sp) and S. aureus* (Sa) when paired with different guide RNAs in primary human T-cells and HEK 293T cell line.

We quantified the degree to which specific editing conditions create highly dominant alleles and observed significant variability. In addition, we observed shifts in the types and frequencies of repair outcomes, particularly when comparing editing in primary cells and cell lines.

The analysis of repair outcome distributions provides opportunities for improving models of non-homologous end joining and suggests mechanistic hypotheses for how factors inherent to the nuclease activity influence the repair process.

Question?

Given the wealth of CRISPR nucleases, how do we pick between them to engineer a therapeutic edit?

Conclusion

gRNA range from showing a dominant edit to a large spectrum of edits dependent on CRISPR variant and cell type. Understanding these relationships can allow streamlining of gRNA screening and engineering efforts.

References:*

1. In vivo genome editing using Staphylococcus aureus Cas9. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F. Nature. 2015 Apr 9;520(7546):186-91. doi: 10.1038/nature14299.

Editas RNP lead discovery

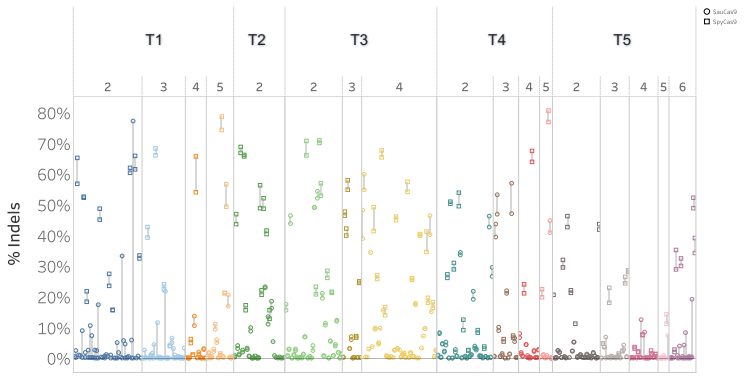

Figure 1.

RNP for Spy and Sau Cas9 variants is delivered to HEK293T and T-cells (T cell data shown) and efficacy determined by NGS sequencing in dose response mode.

Number of active guides across locus for both Spy Cas9 and Sa Cas9.

Variant, gRNA sequence and cell type influence edit

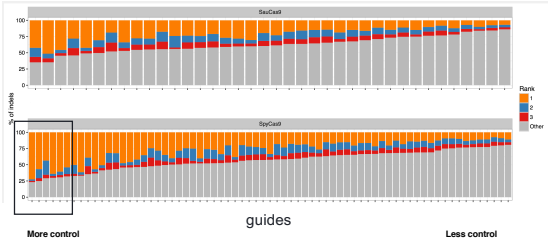

Figure 2. The indel spectrum of Cas9 edits across a series of gRNA in T-cells. gRNA range from those that result in a dominant indel to those that have a wider spectrum of indels.

Indel profiles depend on cell type and gRNA locus

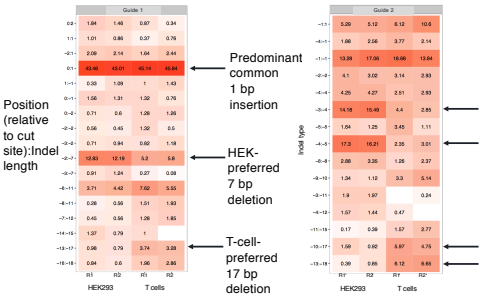

Figure 3. Comparison of guides with dominant indels between T-cells and HEK-293T cells. Key deletions indicated by arrows show similarities and differences between cell types.

T-cells prefer deletions and HEK-293T cells insertions

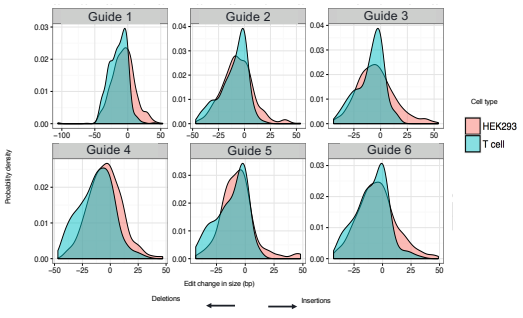

Figure 4. Probability density of edit sizes in T-cells vs HEK293T cells. The skewing of indel patterns under equivalent conditions indicates a cell type dependence.

Dominance of the +1 edit shown by Spy Cas9

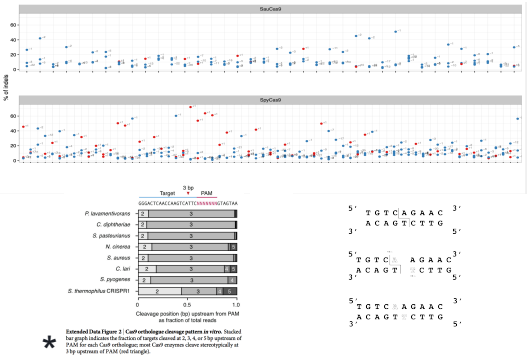

Figure 5. Nuclease dependent staggered cutting possibly leads to predominant +1 edit in Spy Cas9 over Sau Cas9.

11 Hurley Street | Cambridge, MA 02141