Shieldin Genetics

SHLD1 CRISPR-Cas9 K/O

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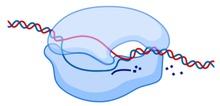
BACKGROUND:

DNA double strand breaks (DSBs) are a common type of DNA damage in both healthy and malignant/cancerous cells. Shieldin is a recently discovered four subunit complex involved in the DNA repair of DSBs, through a process called NHEJ. The gene interaction type between shieldin subunits was the initial proposed question, with the first step undertaken here; CRISPR-Cas9 editing of shieldin subunits.

METHODS

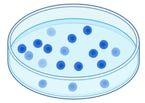
1. sgRNA oligomer design

2. Template DNA PCR and In-Vitro Transcription

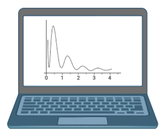


4. sgRNA Transfection

5. Single Colony Isolation



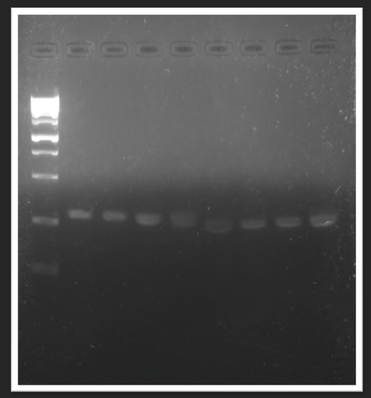
6. gDNA TIDE Analysis



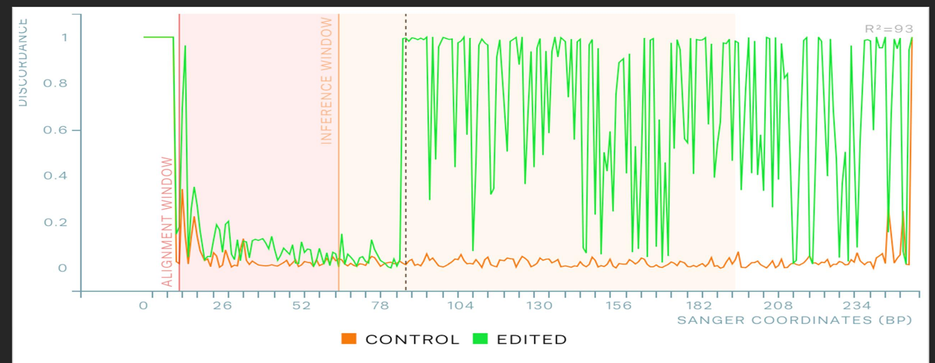
DISCUSSION

- CRISPR-Cas9 technology allowed the generation of cell lines with identical genomic background and SHLD1 knocked out. Future work could entail k/o of the second SHLD1 allele, SHLD2 alleles, and/or SHLD3 and REV7. These will be suitable for gene interaction analysis of the shieldin complex.

SHLD1 CRISPR K/O in Cas9+ Cells

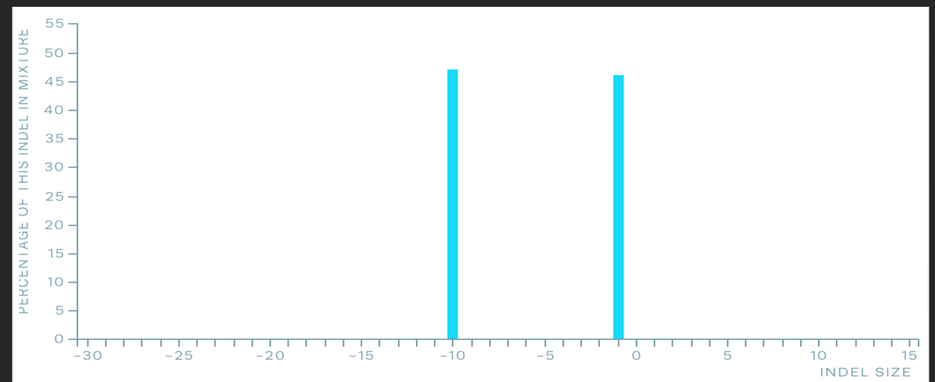


TIDE PCR product agarose gel electrophoresis

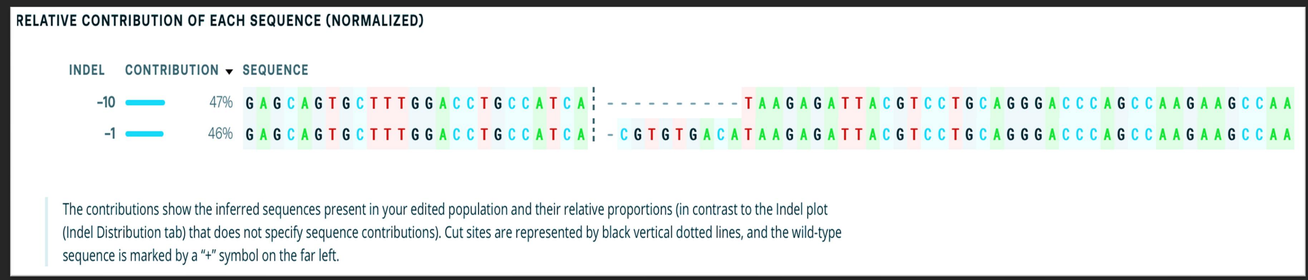


The discordance plot details the level of alignment per base between the wild type (control) and the edited sample in the inference window (the region around the cute site), i.e. it shows the average amount of signal that disagrees with the reference sequence derived from the control trace file. On the plot, the green line and orange line should be close together before the cut site, with a typical CRISPR edit resulting in a jump in the discordance near the cutsite and continuing to remain far apart after the cut site (representing a high level of sequence discordance).

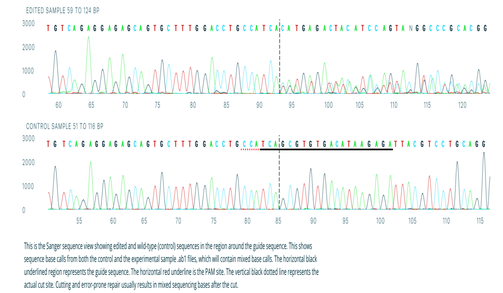
The figure to the right shows two bars at -1 and -10, indicating 1 and 10 base pair deletions. Both of these deletions are, hence, frameshift mutations. In this particular case, the k/o is homozygous, as suggested by the absence of a bar at indel size 0.



The Indel plot displays the inferred distribution of indels in the entire edited population of genomes. Hovering over each bar of the Indel plot shows the size of the insertion or deletion (+ or - 1 or more nucleotides), along with the percentage of genomes that contain it. Note: Each indel size represented in the Indel plot may not necessarily occur in the same sequence. The percentages of different indel sizes in the cell population are not the same as ICE/KO-Score scores.



The contributions show the inferred sequences present in your edited population and their relative proportions (in contrast to the Indel plot (Indel Distribution tab) that does not specify sequence contributions). Cut sites are represented by black vertical dotted lines, and the wild-type sequence is marked by a "+" symbol on the far left.



The black bar indicates location of sgRNA cut site. Following the cut site, there is variation between nucleotide sequence when control (top) and edited (bottom) are compared.

REFERENCES:

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