Comparison of methods to study gene-to-gene variation of HDR promoting inhibitor effects

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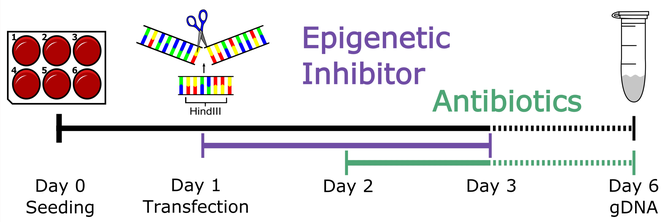
Introduction

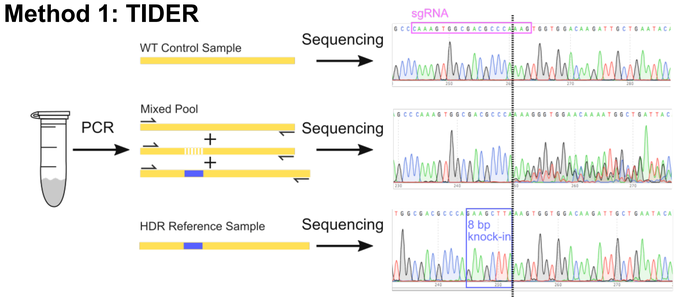
Despite the wide use of the CRISPR/Cas9 system, low efficiency of targeted genome editing still limits its applications. We have tested several small molecular weight compounds, inhibiting epigenetic modifiers, for their effect on double-strand break (DSB) repair pathway choice. Using an already published traffic light reporter system [1], expressed in HEK293 cells, identified an inhibitor with homology-directed repair (HDR) promoting effects. To further investigate the effects of our top hit compound for gene-to-gene variation reliable methods to evaluate DSB repair had to be established. Here we compare potential read-outs and confirm HDR promoting effects at several gene loci in HEK293T cells.

Experiment set-up



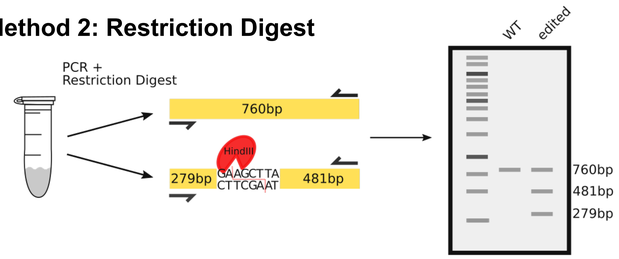
HEK293T cells were transfected with a targeting plasmid expressing sgRNA/Cas9/puromycin resistance and a single-stranded oligodeoxynucleotide repair template. HDR will result in the introduction of 8 additional nucleotides, including a HindIII restriction site. Cells are treated with inhibitor or DMSO for 48 hours. After antibiotic enrichment of positively transfected cells genomic DNA (gDNA) from the edited cell pool is isolated. Inhibitor effects on DSB repair are analysed.

Method 1: TIDER



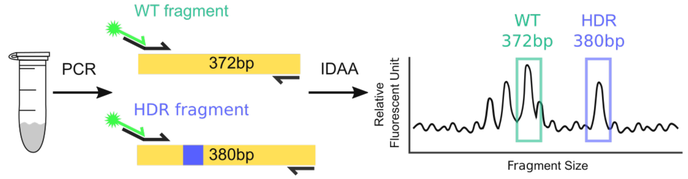
TIDER is a free-access web-tool that estimates HDR and NHEJ efficiency based on three Sanger sequencing files [2]. gDNA, isolated from the edited cell pool, was used for PCR amplification of the target site and sent for Sanger sequencing. Additionally, Sanger sequencing files representing the wild type sequence and the HDR reference sample were generated.

Method 2: Restriction Digest



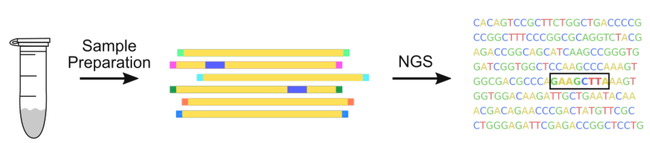
DSB repair via HDR inserts a HindIII restriction site. Isolated gDNA is PCR amplified and subsequently HindIII digested. Running fragments on a polyacrylamide gel allows quantification of HDR repair.

Method 3: IDAA



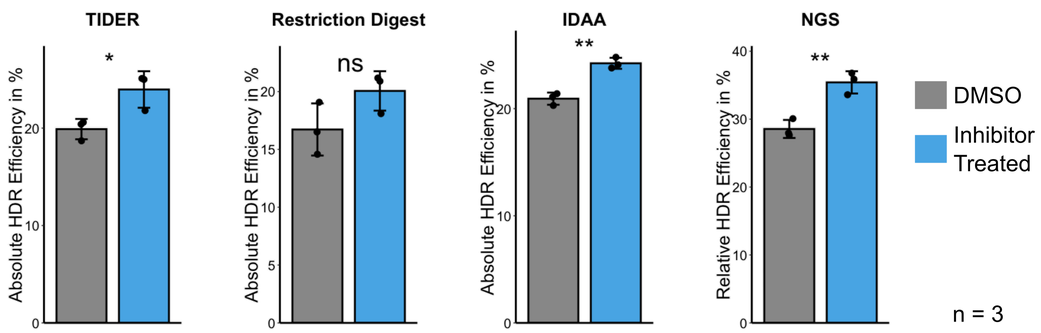
Insertion of 8 additional base pairs during HDR allows evaluation of DSB repair by fragment length analysis. Isolated gDNA is amplified in a tri-primer assay, resulting in FAM labelled fragments. A standard sequenator is used for fluorescent detection and quantification of amplicon length [3].

Method 4: Next-generation sequencing



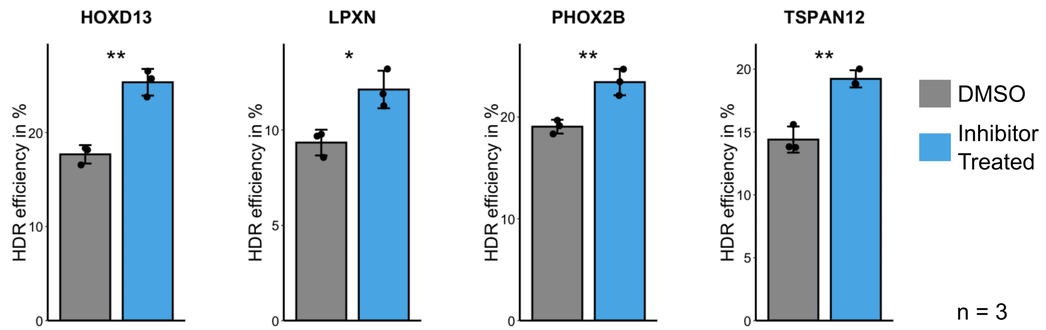
Next-generation sequencing (NGS) allows quantitative analysis of the exact nucleotide sequence. The amount of fragments containing the 8bp insert nucleotide string can thus be detected.

HDR promoting effects are confirmed for PAX2 editing with all methods



An increase for HDR efficiency was detected with all four methods, validating results. A significant effect was detected with TDER, IDAA and NGS. Unpaired t-test, shown are mean intensities with standard deviation, $*p \leq 0.05$; $**p \leq 0.01$.

Inhbitor treatment increases HDR efficiency in HEK293T cells independent of gene target



IDAA analysis in HEK293T cells showed significantly increased HDR efficiency for inhibitor treated cells independent of gene target. Unpaired t-test, shown are mean intensities with standard deviation, $*p \leq 0.05$; $**p \leq 0.01$.

Conclusion

All four methods can be used to study DSB repair, although sensitivity varies. NGS and IDAA show the smallest standard deviations, suggesting them to be highly precise precise and sensitive. Although sensitivity for Restriction Digest and TIDER might be limited, these cost-efficient methods can detect the same trend. IDAA combines cost-efficiency with high sensitivity but cannot detect nucleotide exchanges. The choice of method should thus depend on the application. Gene editing of the TLR locus in transgenic HEK293 cells as well as 5 loci in HEK293T cells have shown increased HDR efficiency for inhibitor treated cells.

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References

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