Isolation of AAV-derived Inverted Repeats for Enhanced Gene Editing

Naveen Vridhachalam[1,2], Brian Golitz[3,4], Matthew L. Hirsch[1,2]

[1]Gene Therapy Center, University of North Carolina at Chapel Hill, [2]Department of Ophthalmology, University of North Carolina at Chapel Hill, [3]Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, [4]University of North Carolina School of Medicine, University of North Carolina at Chapel Hill

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

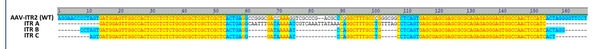
Abstract

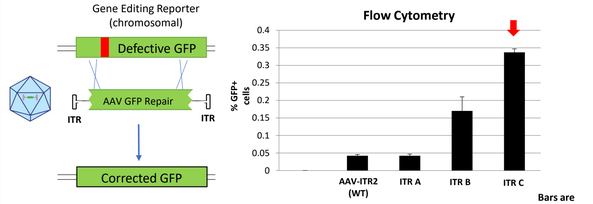
Previous studies have shown that a DNA double strand break (DSB) at or near a chromosomal target site enhances homologous recombination (HR) mediated genome editing. As such, designer endonucleases that recognize specific DNA sequences and generate a DSB at a site of interest have rose in popularity, however, concerns exist for in vivo therapeutic applications including the immune response to foreign epitopes and off-target mutagenic activity. A reported alternative to site-specific endonucleases are proximal inverted repeats (IRs), structured DNA elements processed to DSBs via endogenous DNA repair. To date, the influence of IRs engrafted on the DNA repair molecule have not been well characterized in a gene editing context. In this work, a truncated adeno-associated virus (AAV) IR enhanced for gene editing was randomly mutated in a manner that conserved sequences necessary for vector production thereby generating a mutant IR library of >10^6 diversity. Screening of individual mutant IRs in a gene editing selection identified AAV IR sequences decreased and enhanced for gene editing compared to the library parent and the wild-type AAV IR sequence. Currently, bioinformatic analyses correlating sequence/structure to chromosomal repair efficiencies and AAV vector production are in progress. Together, these results demonstrate that mutated viral IR sequences on the repair molecule effect gene editing frequency and offer mechanistic insights into the generation of AAV viral vectors that mediate high efficiency genetic engineering in the absence of a site-specific endonuclease.

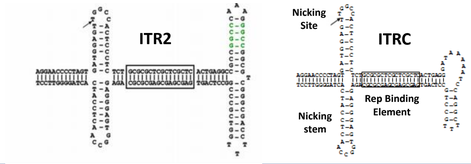
Objective: Gain insights into the generation of adeno-associated virus recombinant genomes enhanced for homology directed repair.
Hypothesis: Mutated AAV inverted repeat sequence will be enhanced and decreased for gene editing compared to the wild-type AAV inverted repeat.

Rationally Designed ITR Enhances AAV gene editing

Figure 1. A Rationally Designed ITR Enhances Viral Gene Editing.
A defective GFP reporter cell line was transduced with different AAV2-based ITRs flanking a GFP repair template. A rationally designed ITR (ITR C) enhanced viral gene editing.







Methods

Figure 2. Mutant Inverted Repeat Library Design
ITR C served as a parent for an AAV-based IR library. NGS shows successful library synthesis, with conserved and diverse mutated regions

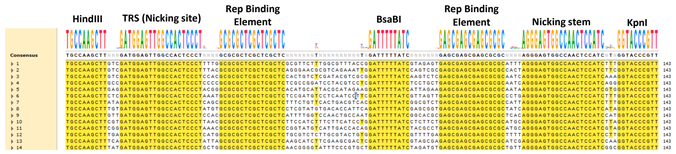


Figure 3. Isolation of Mutant Inverted Repeats from the IR Library

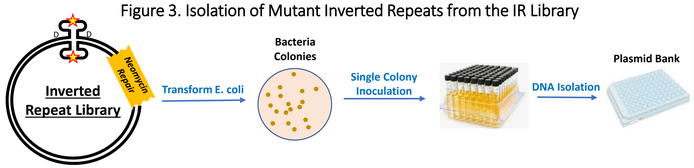


Figure 4. Gene Editing Assay

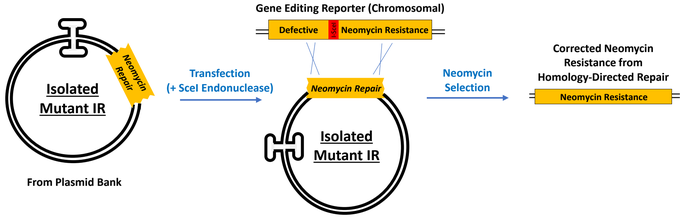
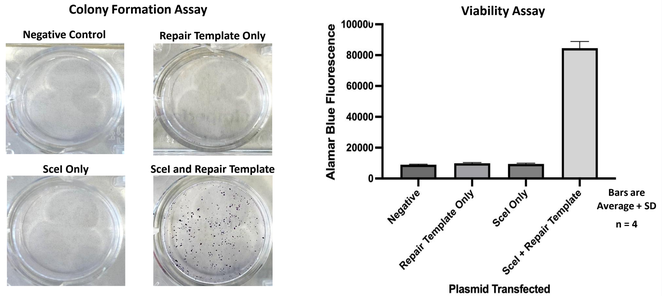
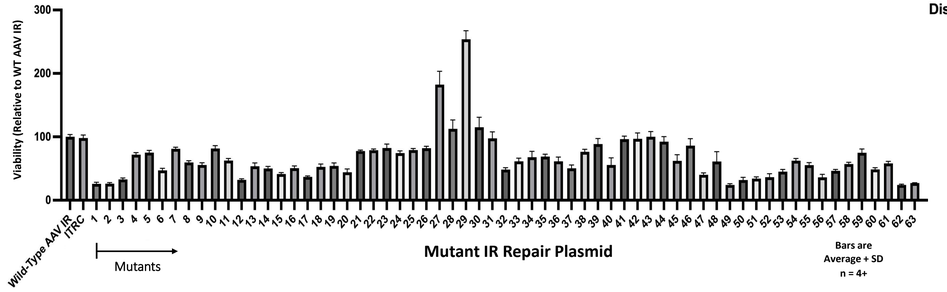


Figure 5. Colony Formation and Viability Assay Validation of Gene Editing
(a) A colony formation assay was used to fix colonies of corrected neomycin cells.
(b) Alamar Blue, a viability assay, was used to measure differences in the number of viable cells per well.



Results

Figure 6. Relative Viability of Cells Repaired by Different Mutant Inverted Repeats Compared to Wild-Type ITR2



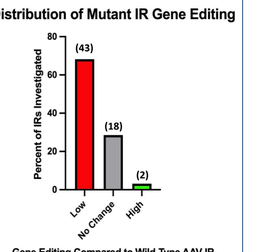


Figure 7. Sequence Alignment of Select Inverted Repeats Decreased or Enhanced For Gene Editing

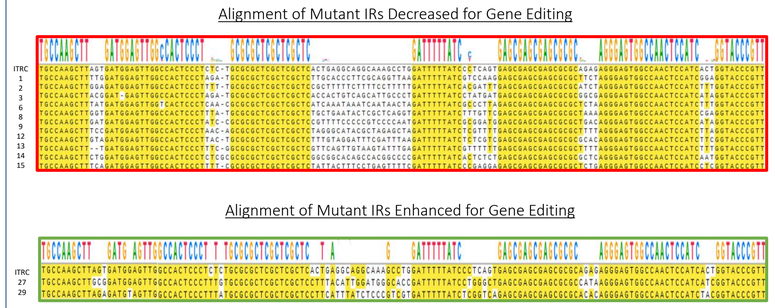
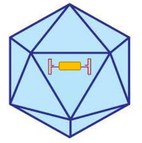
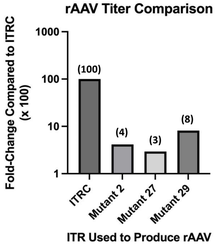


Figure 8. Mutant IRs rAAV Production
Small scale rAAV production was attempted with select mutant IRs and measured via qPCR. Mutant IRs produced virus, though significantly less than the library parent.





Conclusions and Future Directions

- Mutations of various AAV ITR regions changes gene editing frequency after transduction
- Different IR sequences proximal to a repair template effect gene editing frequency after transfection
- Most mutated sequences have significantly decreased gene editing compared to the wild-type AAV IR
- Mutated IRs produce rAAV, although at significantly low titer compared to the library parent
- Certain base pairs and mutation regions may be correlated to enhanced/decreased gene editing
- Future Directions include evaluating mutant IR viral production in depth, test gene editing in viral context, and investigate more IRs for gene editing to be able to do bioinformatic analyses of significantly different sequences

Acknowledgements

- UNC CRISPR Screening Core
- Dr. Jaquelyn Bower
- Funding: UNC Office of Technology Commercialization

- Disclaimer: NV and MLH are co-inventors of presented technologies with potential royalties