Screening *S. aureus* CRISPR-Cas9 Paired Guide RNAs for Efficient Targeted Deletion in Duchenne Muscular Dystrophy

Josh Tycko[1], Nick Huston[1], Jacqueline Robinson-Hamm[2], Chris Wilson[1], Charles A. Gersbach[2], Patrick D. Hsu[1], David Bumcrot[1]

1. Editas Medicine, 300 Third St., Cambridge, MA 02142 2. Department of Biomedical Engineering, Duke University, Durham, North Carolina 27708

Background

- Duchenne muscular dystrophy (DMD) is a X-linked recessive neuromuscular disorder that results in progressive muscle degeneration and premature death. Most patients have frameshifting mutations in the dystrophin gene that result in a nonfunctional protein.
- In contrast, Becker muscular dystrophy (BMD) patients generally have mutations in dystrophin that do not disrupt the reading frame, leading to a milder disease phenotype.
- Thus, CRISPR/Cas9 targeted deletions that restore the reading frame could convert DMD genotypes into BMD-like genotypes and potentially ameliorate the phenotype of this disease. The smaller Cas9 ortholog from *S. aureus* can be packaged with paired guide RNAs in an AAV vector for *in vivo* gene editing.

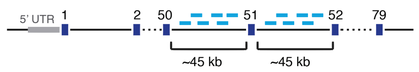
Experimental Overview

- Deleting Exon 51 would restore the dystrophin reading frame for the greatest number of patients of any single exon deletion.
- From the set of 10,553 21-nt *S.aureus* Cas9 (SaCas9) guide RNAs targeting human DMD introns 50 and 51, we selected pairs that passed design filters intended to improve guide RNA expression, improve SaCas9 cleavage efficiency, and minimize off-target editing concerns.

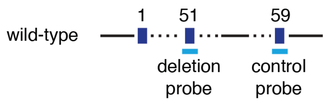
Conclusions

- Screening identified several candidate guide RNA pairs, including pairs with up to 25% deletion efficiency in DMD myoblasts.
- Lead guide RNAs mediate the precise desired deletion in immortalized DMD myoblasts.
- Deletion size plays a role in deletion efficiency, but is not highly predictive.

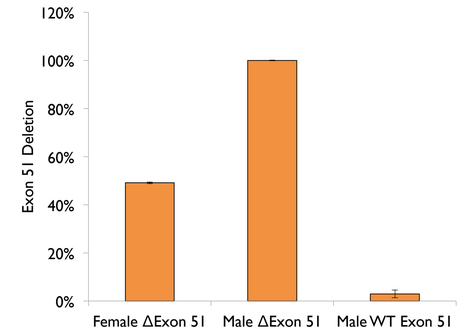
Absolute quantification of deletions with digital droplet PCR

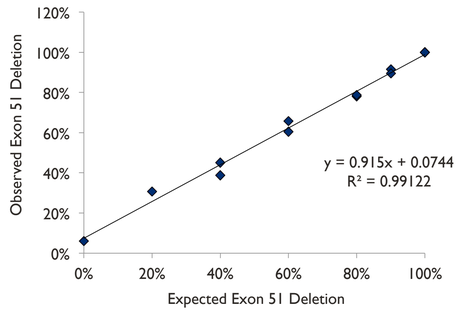


There is ~90 kb of intronic space wherein targeted paired guide RNAs (light blue) could mediate a deletion of Exon 51.

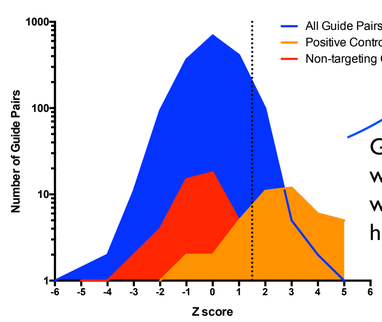


A multiplex Taqman ddPCR assay to quantify Exon 51 deletion.

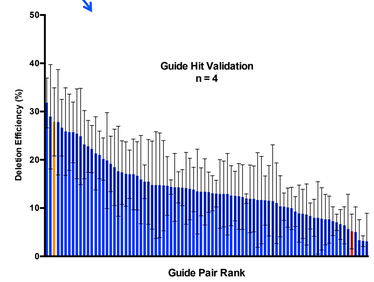




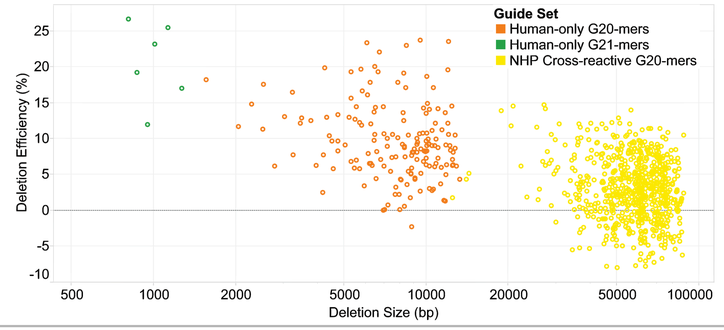
Screen analysis and validation of 78 hits



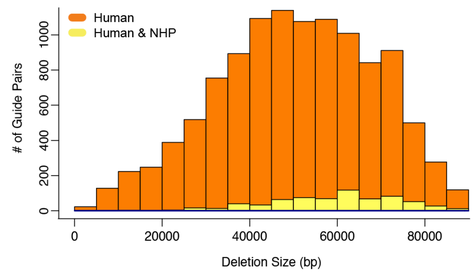
Guide RNA pairs with a Z score > 1.5 were selected as hits for validation



Deletion size is one factor affecting paired guide RNA deletion efficiency.

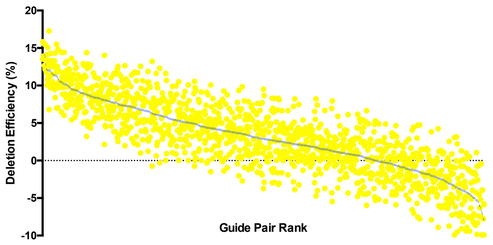


Deletion efficiency of 857 SaCas9 paired guide RNAs

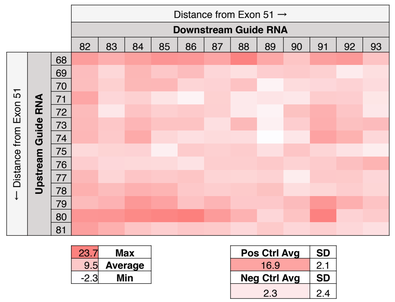


Guide RNAs were cloned into plasmids and transfected into HEK293T cells along with an SaCas9 plasmid. Exon 51 deletion efficiency was measured after 3 days by ddPCR.

Human-NHP cross reactive guide RNA pairs
Deletions >14kb



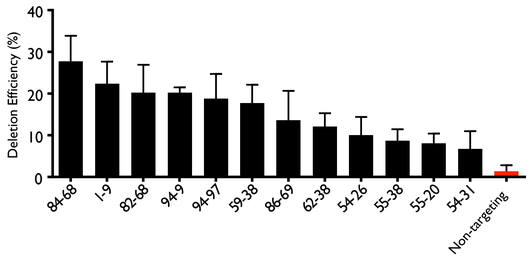
Human-only guide RNA pairs
Deletions <14kb



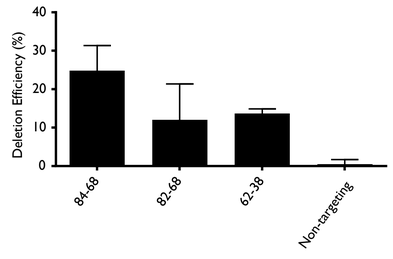
23.7	Max
9.5	Average
-2.3	Min

Pos Ctrl Avg	SD
16.9	2.1
Neg Ctrl Avg	SD
2.3	2.4

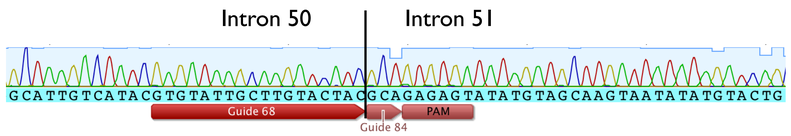
Activity of lead candidate guide RNA pairs confirmed



12 lead guide RNA pairs were validated by plasmid transfection in HEK293T cells.



A subset of leads were electroporated into immortalized DMD myoblasts.



Sequencing one of the immortalized DMD myoblast samples showed that a pair of guide RNAs can mediate the precise expected deletion.