Therapeutic Correction of an LCA-Causing Splice Defect in the *CEP290* Gene by CRISPR/Cas-Mediated Genome Editing

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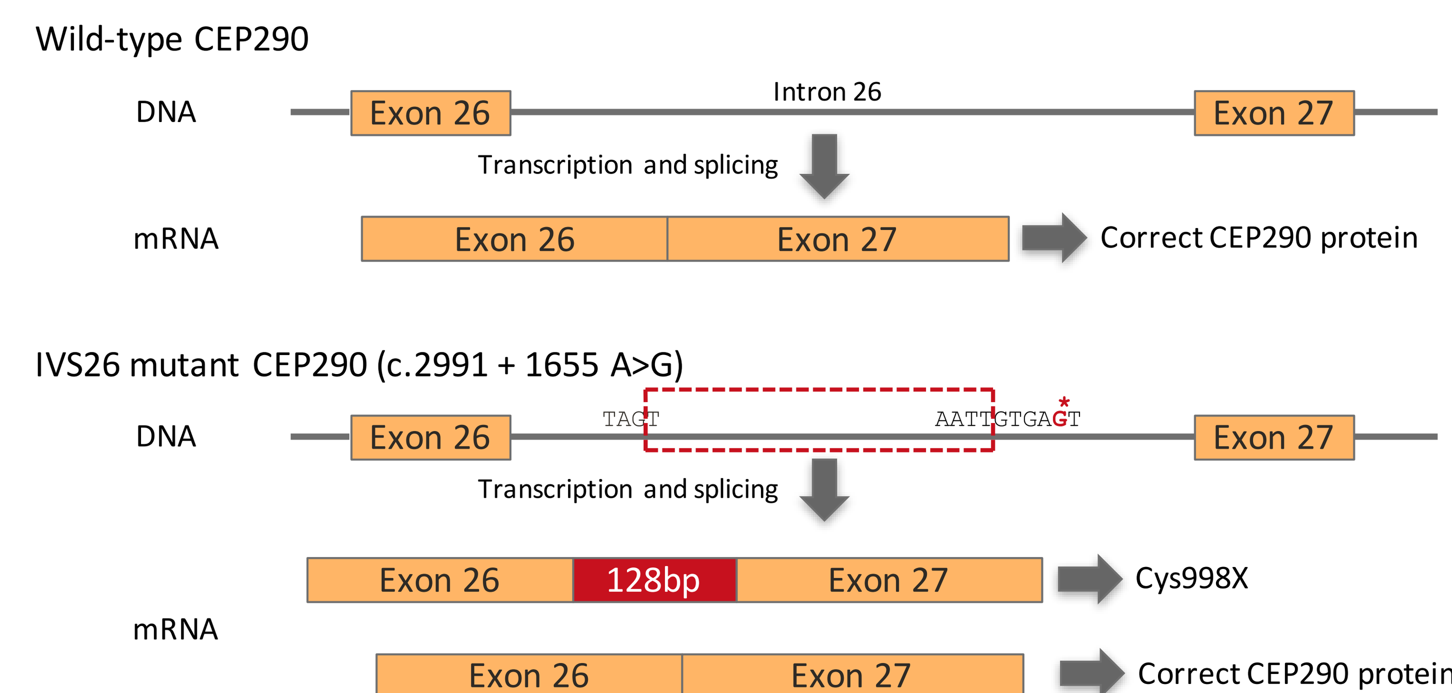
ABSTRACT

Leber congenital amaurosis (LCA) comprises a genetically heterogeneous group of early-onset retinal disorders characterized by severe loss of vision in the first years of life. Approximately 20% of LCA patients harbor mutations in the *CEP290* gene, which exceeds the packaging limit of AAV and is therefore not amenable to traditional gene therapy. Here, we report a gene editing approach in which the CRISPR/Cas9 system is used to modify the endogenous *CEP290* locus and restore normal function of the gene.

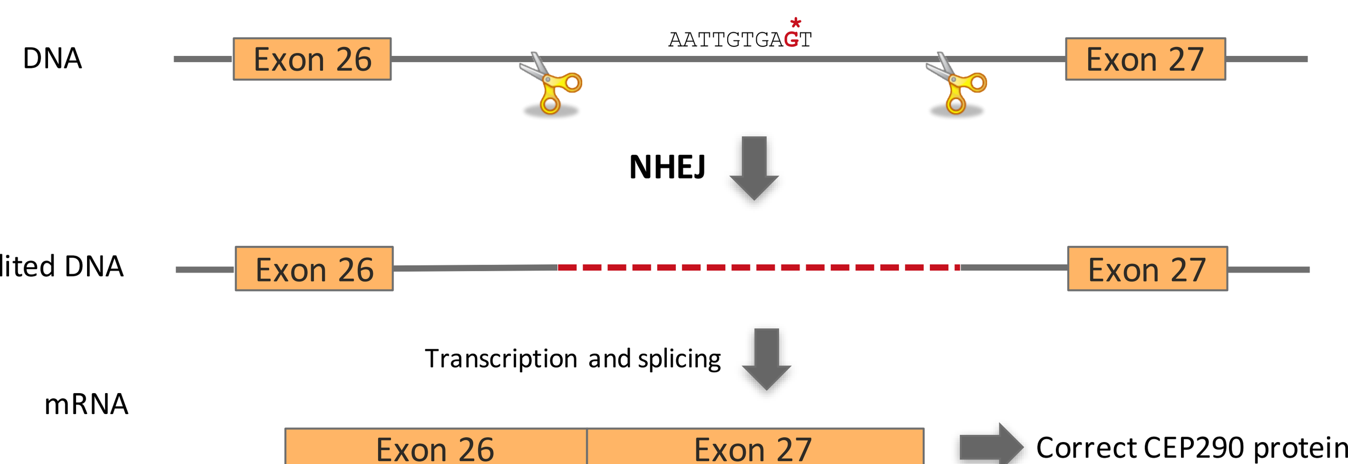
METHODS

Gene editing in LCA10 patient fibroblasts using *S. aureus* Cas9 and two guide RNAs.

Molecular basis of disease



Gene Editing Strategy



RESULTS

Figure 1: Targeted genomic deletion

Quantification of targeted genomic deletion in primary fibroblasts transfected with Cas9 and gRNAs by droplet digital PCR.

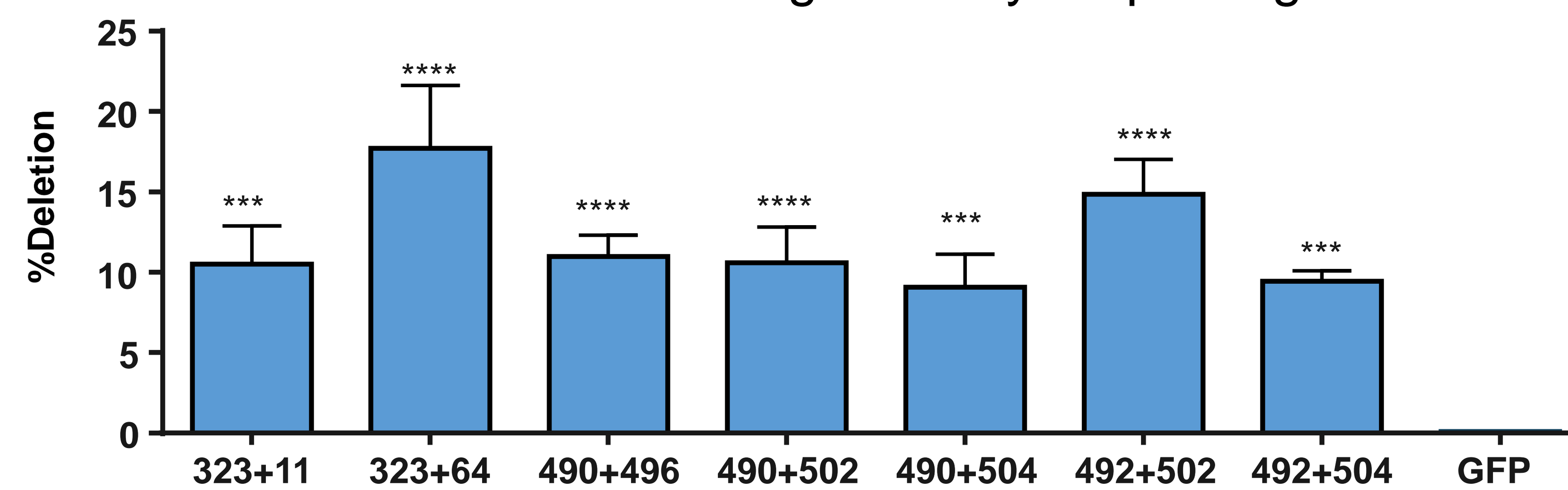


Figure 2: Targeted deletion corrects splicing

Increased expression of wildtype transcript and decreased expression of mutant transcript in primary patient fibroblasts transfected with Cas9 and gRNAs as measured by qRT-PCR.

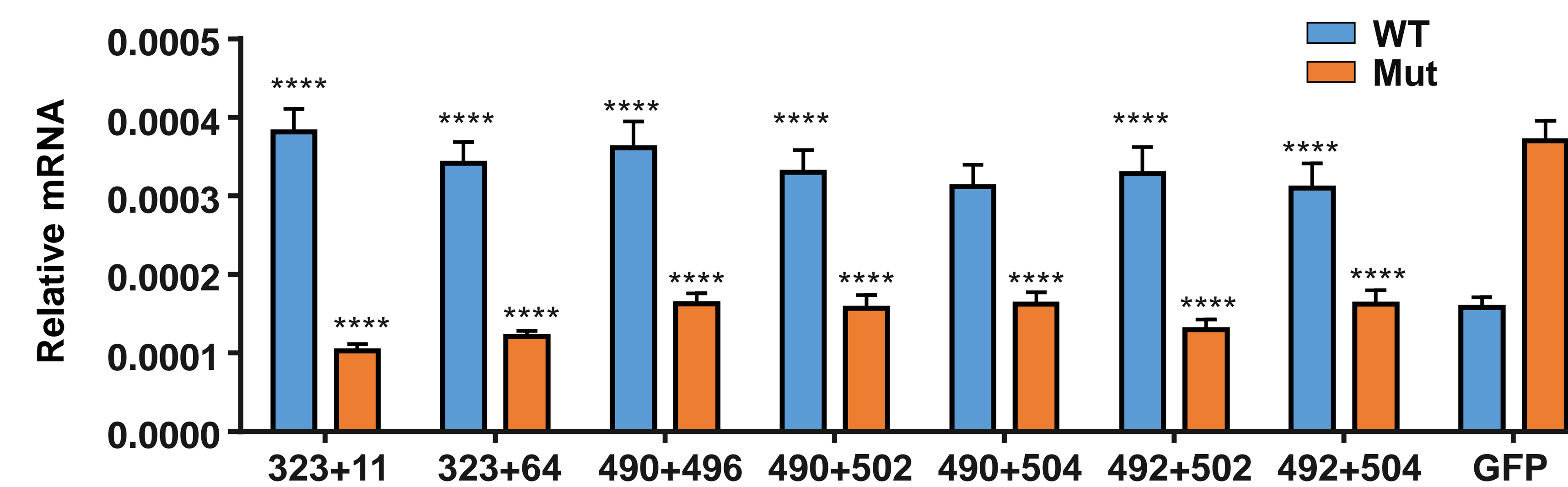
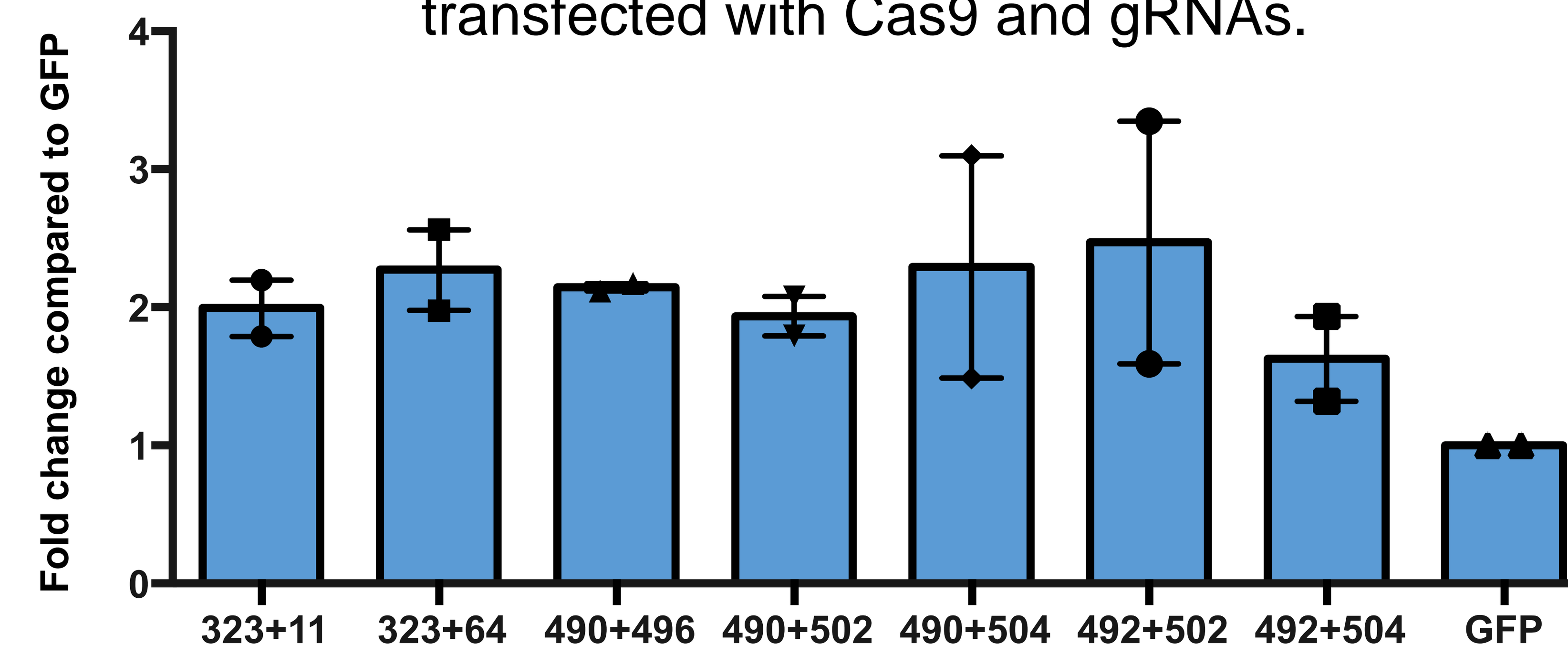


Figure 3: Increased CEP290 expression

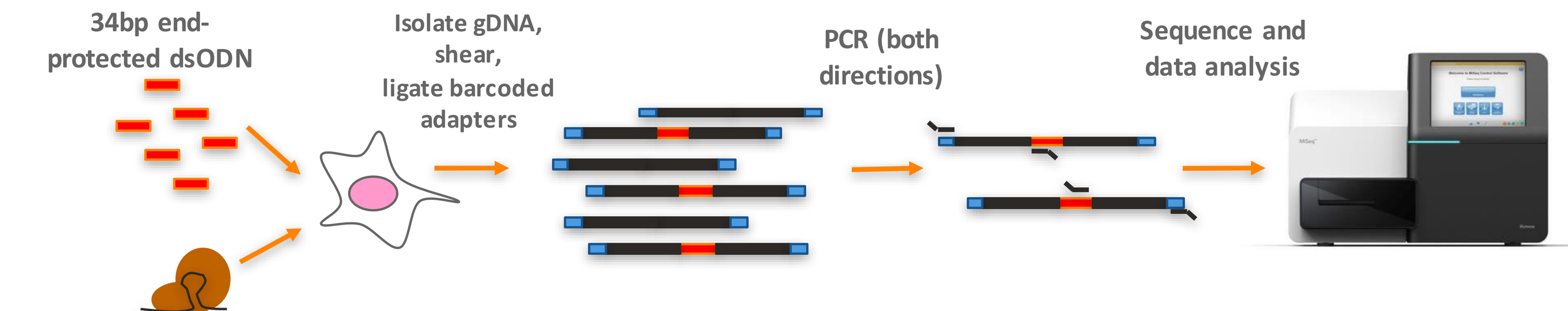
Increased expression of wildtype CEP290 protein as measured by Western blot in primary patient fibroblasts transfected with Cas9 and gRNAs.



The patient fibroblasts were generously provided by Budd Tucker at the University of Iowa.

Figure 4: Specificity profiling of candidate gRNAs reveals few off-target sites

GUIDE-Seq in U2OS cells and primary patient fibroblasts, followed by targeted amplicon sequencing reveals few off-target sites.



Target Site	On-target editing rate (% indels)	Off-Target Site	Off-Target Location	Off-target editing rate (% indels)
64	96.5	No off-targets identified		
323	94.8	No off-targets identified		
490	78.38	No off-targets identified		
492	93.76	No off-targets identified		
496	94.38	No off-targets identified		
504	72.09	No off-targets identified		
11	93.5	Chr17:55416466	Intron, *MMD*	0.03
		Chr2:10678496	Intron, *NOL10*	ND
		Chr3:71041347	Exon, *FOXP1*	0.27
502	93.34	Chr4:17268500	intergenic	8.81
		Chr10:46526823	intergenic	0.12
		Chr2:119441891	Intron, *SCTR*	ND
		Chr1:42755713	Intron, *LEPRE1*	ND
		Chr4:97307689	intergenic	0.05
		Chr1:247853709	intergenic	ND
		Chr2:2526357	intergenic	0.12

CONCLUSIONS

This work supports the development of a gene-editing approach for therapeutic treatment of *CEP290*-associated disease caused by the IVS26 c.2991+1655 A>G mutation. The use of the *S. aureus* CRISPR/Cas9 system enables efficient packaging of the Cas9 gene, as well as two gRNA genes, into a single AAV vector and provides a method for delivery into patient photoreceptors.

The authors declare competing financial interests. All authors are employees of Editas Medicine.