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

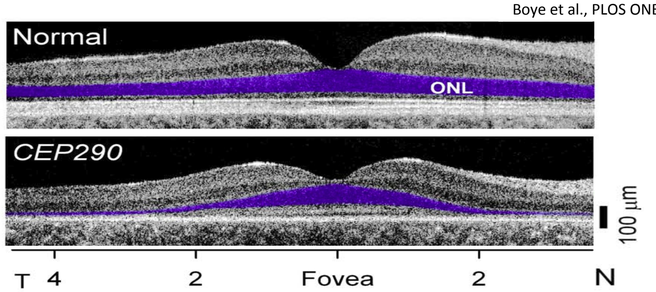
Morgan L. Maeder, Rina Mepani, Sebastian W. Gloskowski, Maxwell N. Skor, McKensie A. Collins, Gregory M. Gotta, Eugenio Marco, Luis A. Barrera, Hari Jayaram, David Bumcrot

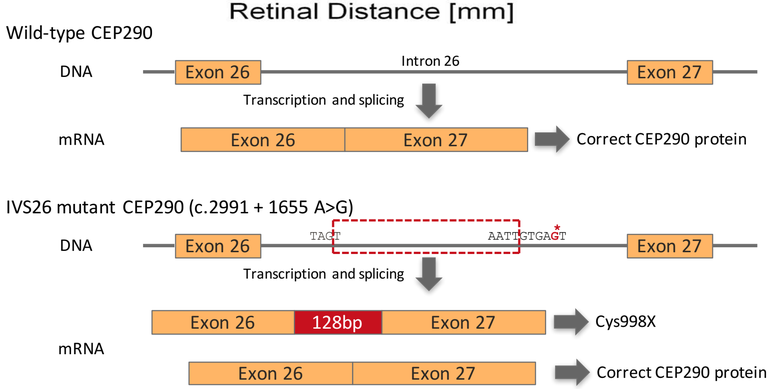
Editas Medicine, 300 3rd Street, Cambridge, MA 02142

Leber Congenital Amaurosis caused by mutations in the *CEP290* gene

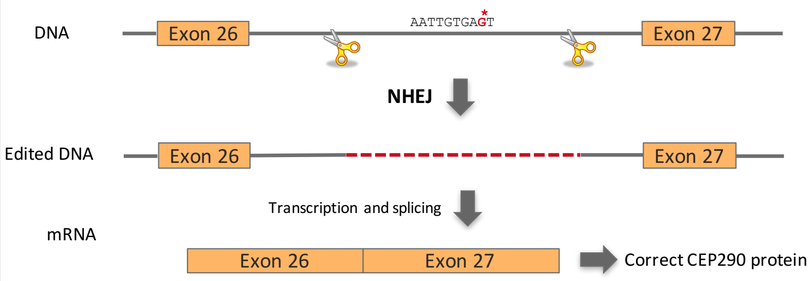
- Early onset retinal degeneration characterized by severe loss of vision in the first years of life.
- Approximately 20% of all LCA caused by mutations in the *CEP290* gene.
- Most common mutation in *CEP290* is the IVS26 c.2991+1655 A>G mutation in intron 26 which creates a strong splice donor.

Boye et al., PLOS ONE 2014



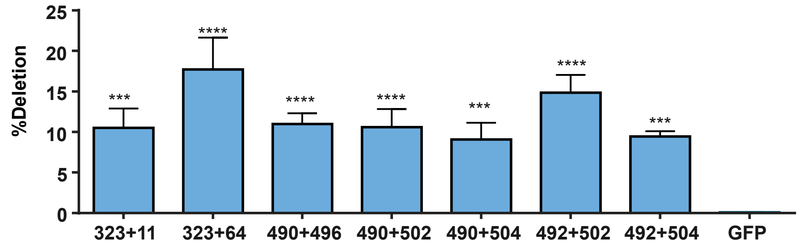


Gene Editing Strategy



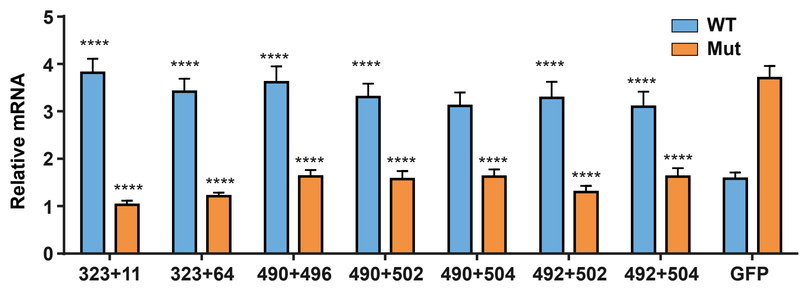
Targeted Genomic Deletion

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



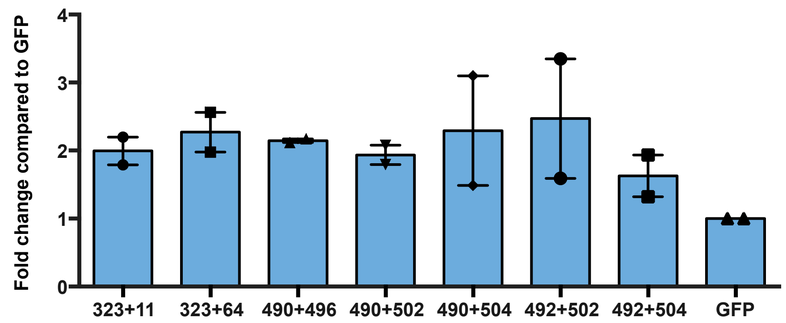
Targeted Deletion Corrects Splicing

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



Increased CEP290 Expression

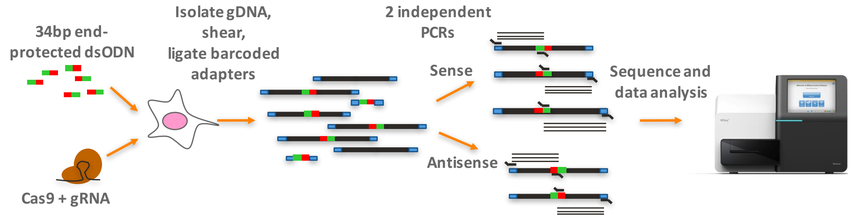
Increased expression of wildtype CEP290 protein as measured by Western blot in patient fibroblasts transfected with Cas9 and gRNAs



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

Specificity Profiling of Candidate gRNAs reveals few off-target sites

Specificity analysis performed by GUIDE-Seq and targeted amplicon sequencing in U2OS cells and primary fibroblasts.



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*	<0.01
		Chr3:71041347	Exon, *FOXP1*	0.27
502	93.34	Chr4:17268500	intergenic	8.81
		Chr10:46526823	intergenic	0.12
		Chr2:119441891	Intron, *SCTR*	<0.01
		Chr1:42755713	Intron, *LEPRE1*	<0.01
		Chr4:97307689	intergenic	0.05
		Chr1:247853709	intergenic	<0.01
		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.