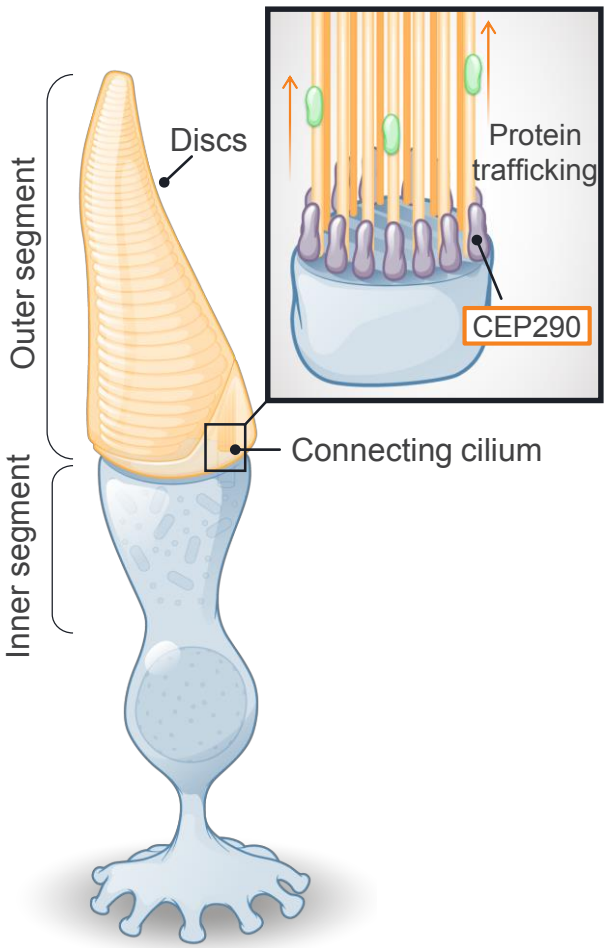
Therapeutic Correction of an LCA-Causing Splice Defect in the *CEP290* Gene by CRISPR/Cas-Mediated Genome Editing

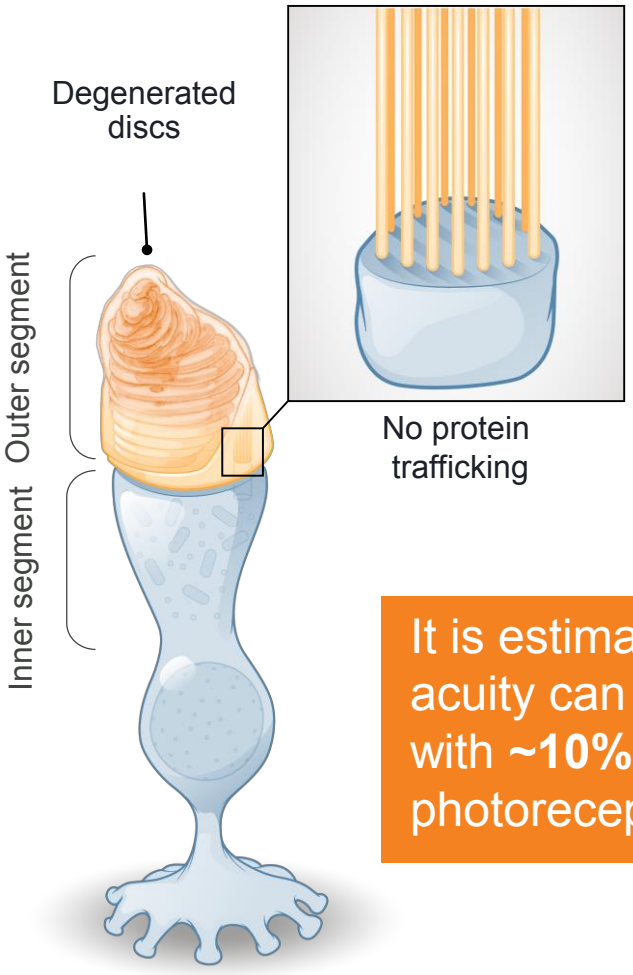
Maxwell N. Skor

CEP290 in Ciliary Trafficking and Phototransduction

WT Photoreceptor



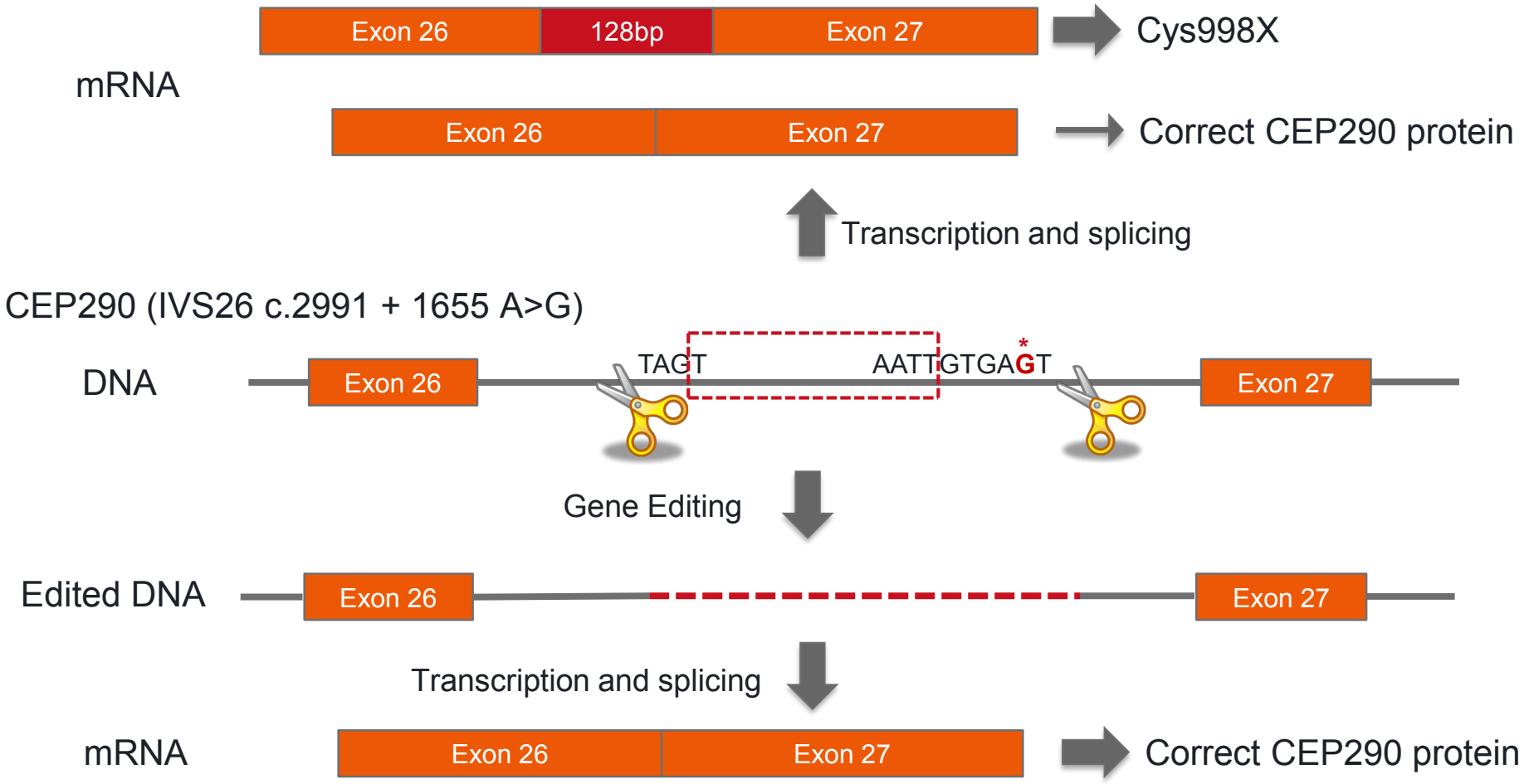
LCA10 Photoreceptor



It is estimated that visual acuity can be achieved with **~10%** of functioning photoreceptors[1,2]

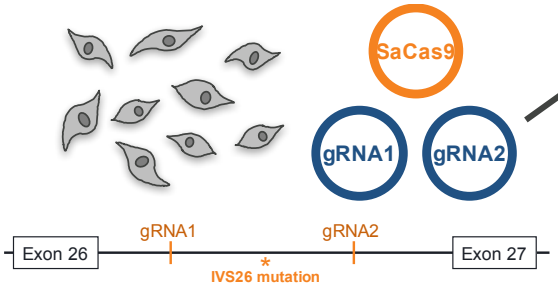
1.Geller, Sieving and Green, *J. Opt. Soc. Am.*, 1992
2.Geller and Sieving, *Vision Res.*, 1993

Gene Editing to Repair *CEP290* Splicing Defect

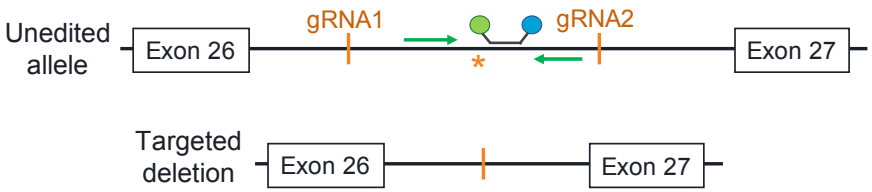


Targeted Deletion in Patient Fibroblasts

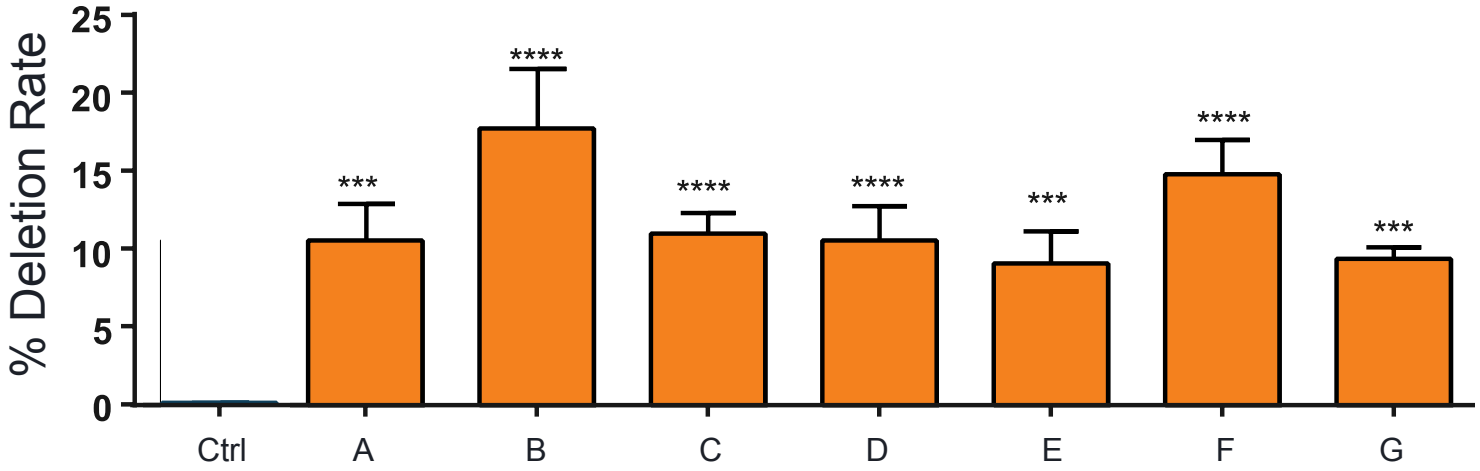
Targeted deletion of IVS26 mutation-containing region of intron 26



Nucleofect IVS26 fibroblasts with SaCas9 and gRNAs



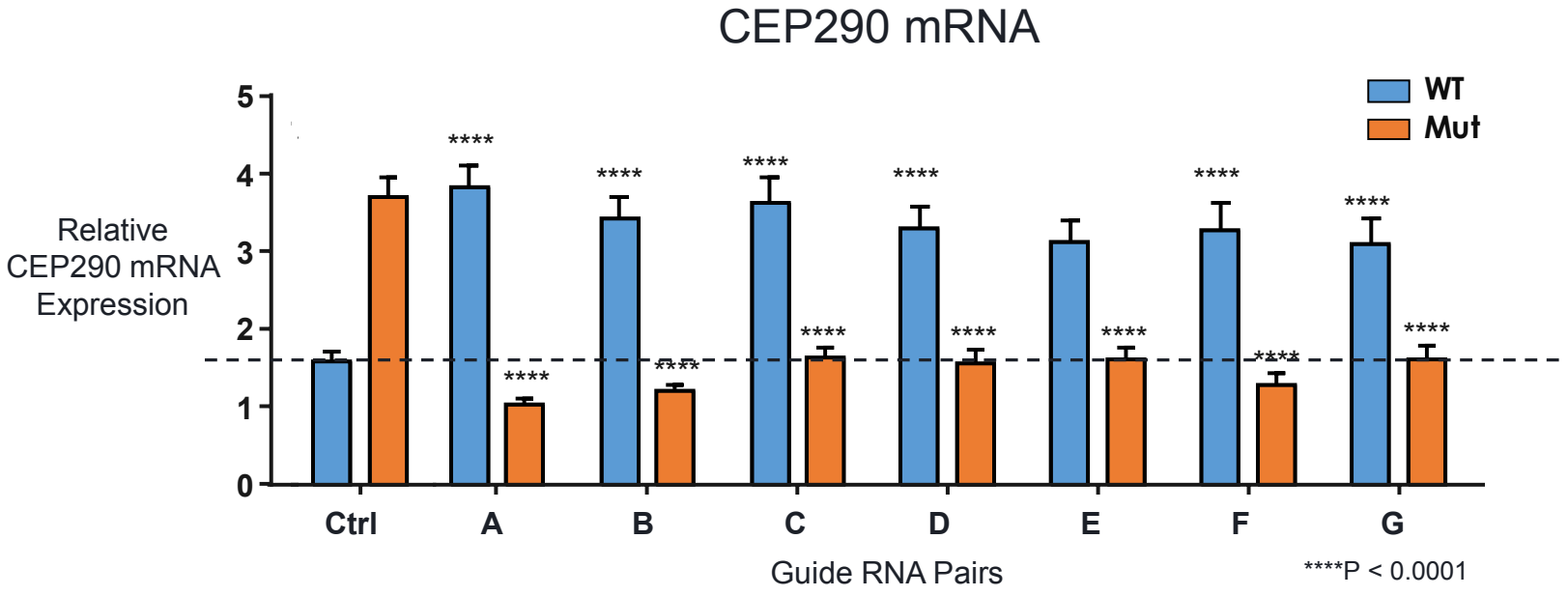
Quantify deletion rate by ddPCR

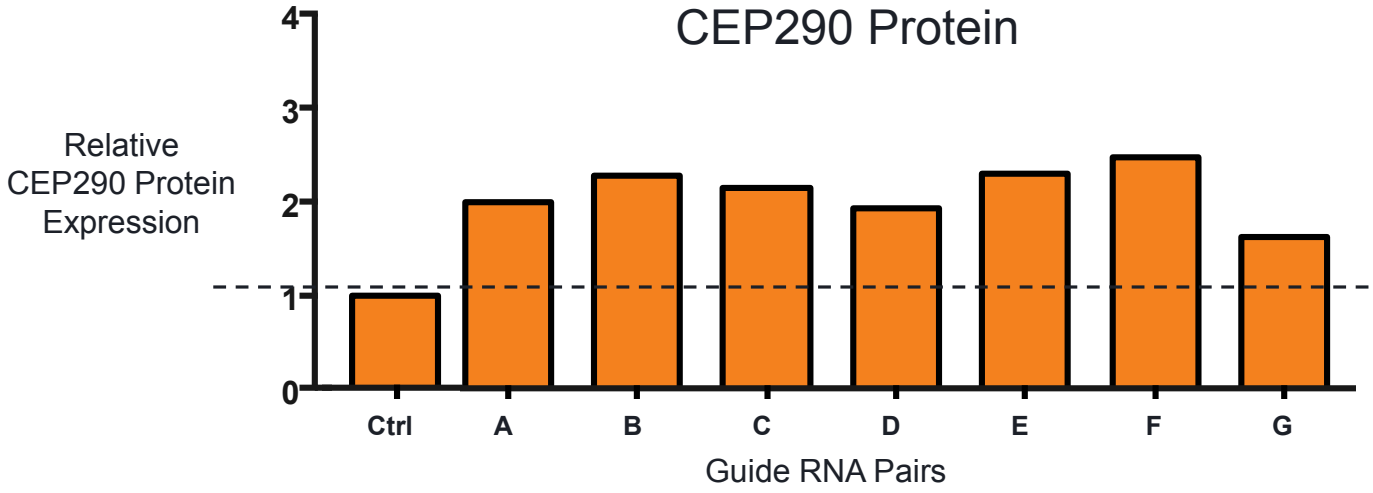


Guide RNA Pairs

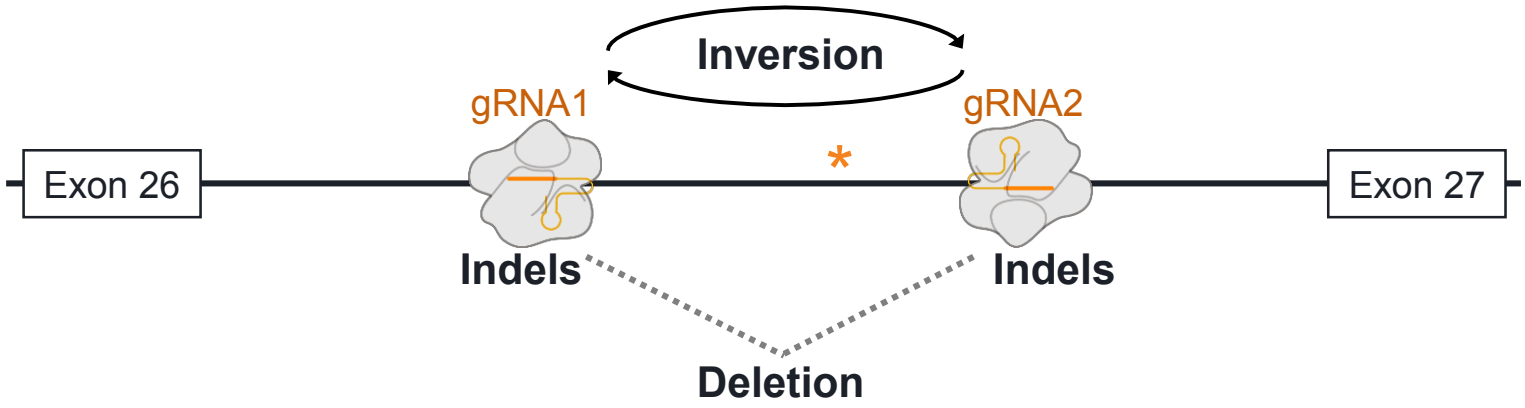
**** P < 0.0001
*** P < 0.001

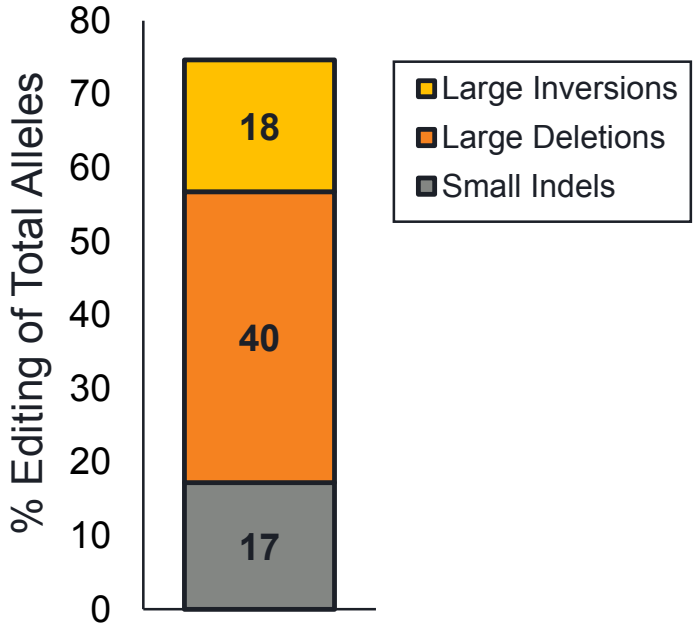
Editing Restores CEP290 Expression in Patient Fibroblasts





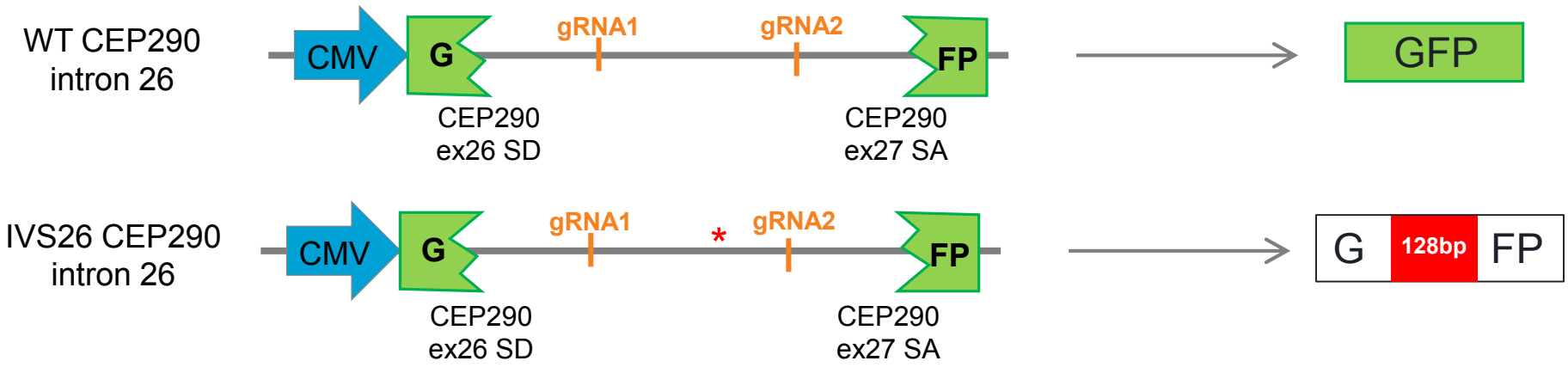
Total Editing Events Include Inversions

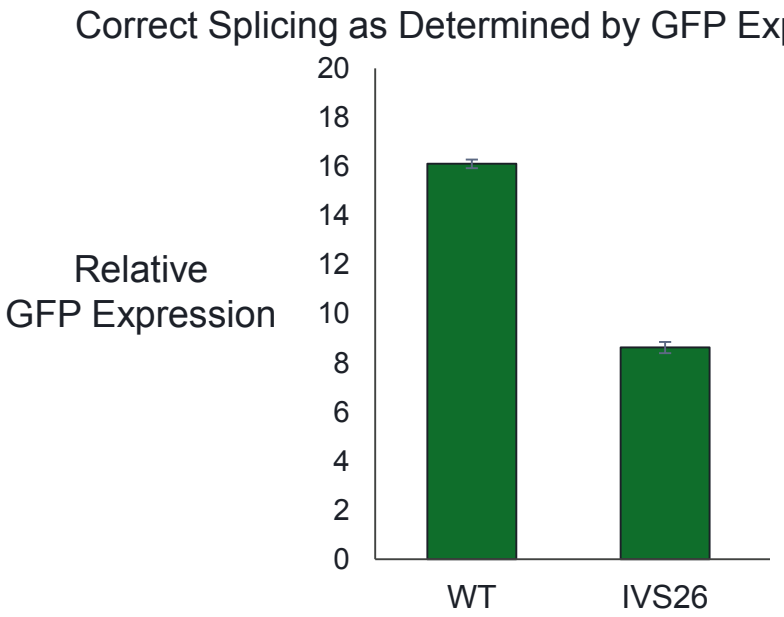




UDiTaS sequencing method was used to accurately quantify all gene editing events in U2OS cells (Please see Eugenio Marco's Poster for more details on UDiTaS)

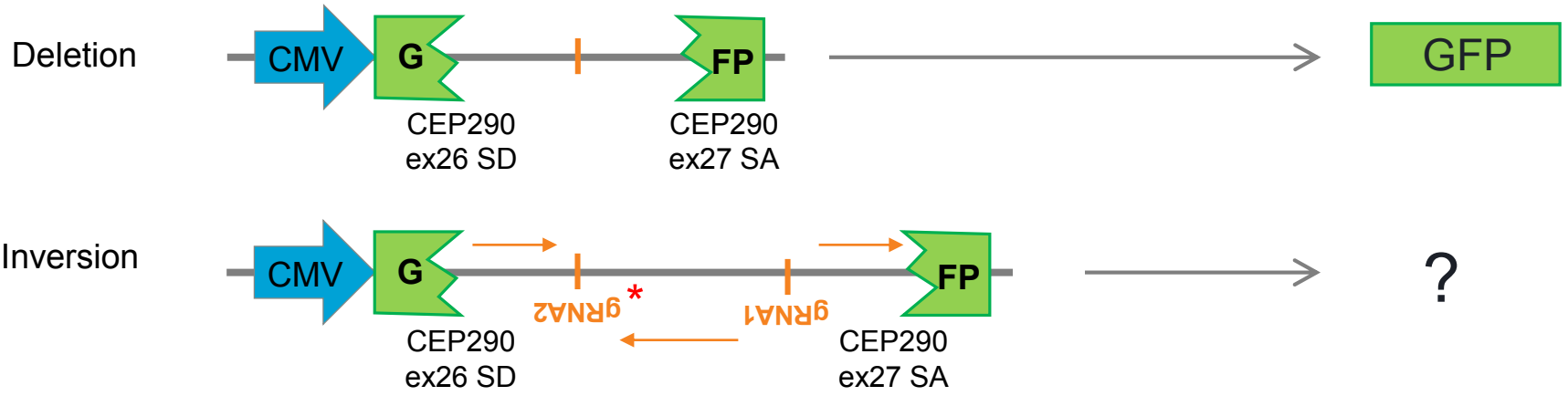
Construction of GFP Reporter Construct
Is the Inversion Event Functional?



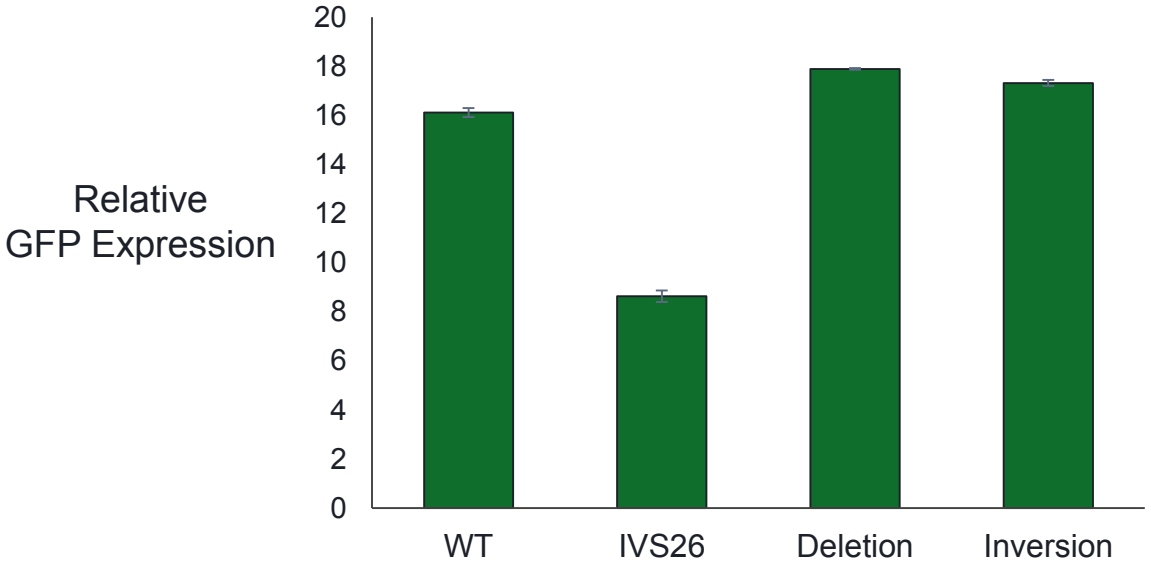


IVS26 mutation leads to aberrant splicing and a non-functional GFP-signal

Targeted Deletions and Inversions Correct Splicing



Correct Splicing as Determined by GFP Expression

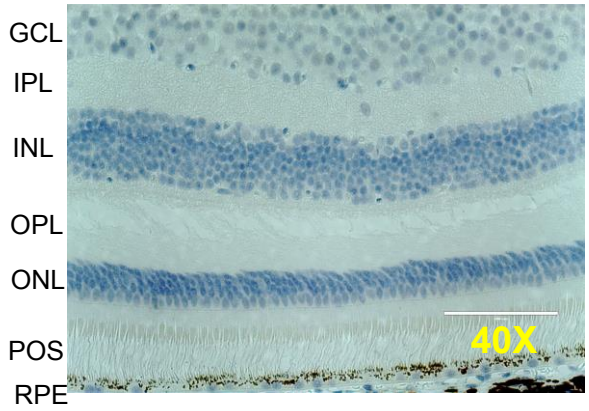


Inversions are <u>productive</u> editing events

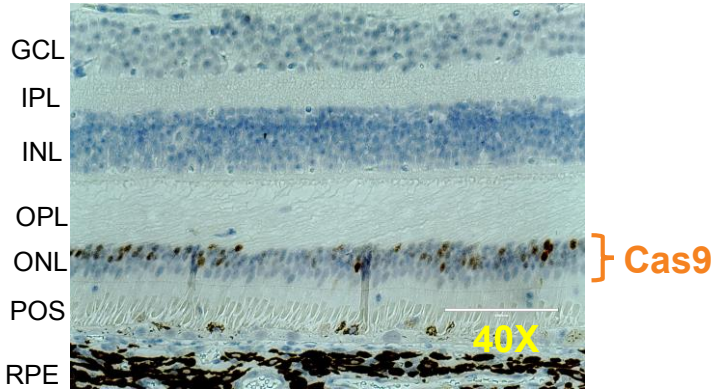
In vivo CEP290 Editing in Non-Human Primates

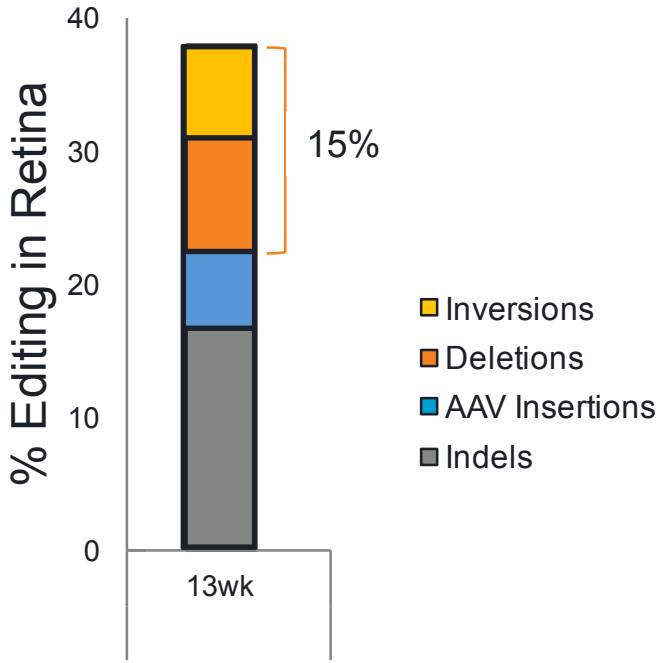
Cyno macaque injected sub-retinally with 4E11 vg of AAV5-NHPCEP290gRNAs-GRK1-SaCas9

Vehicle



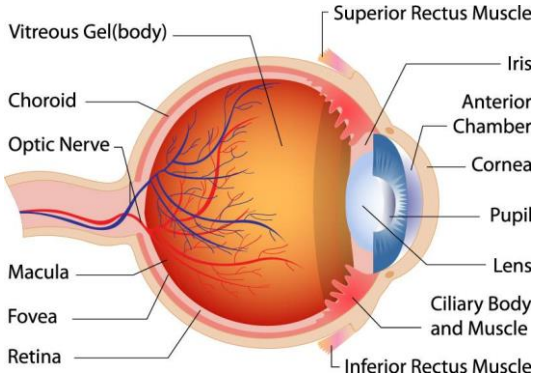
AAV5-NHPCEP290gRNAs-GRK1-Cas9





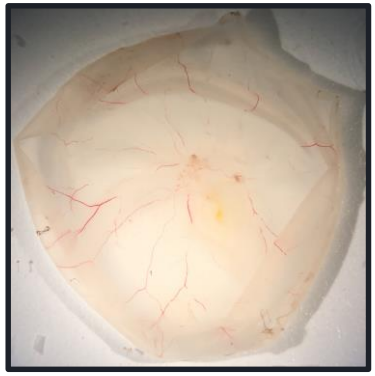
Proof of concept that we are able to reach >15% productive editing in NHP bulk retina and potentially as high as 50% productive editing in photoreceptors

Developing a Human Retinal Explant System



Obtain Human Eyes 3-5 hrs Post-Mortem

Remove Neural Retina



3 mm punches

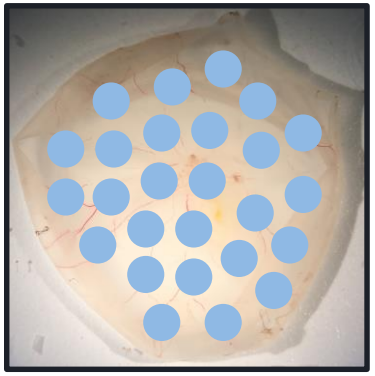
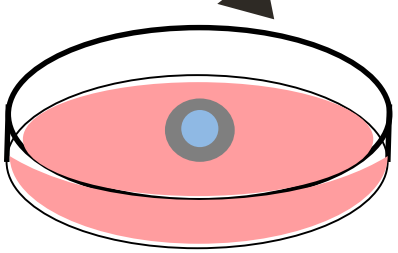
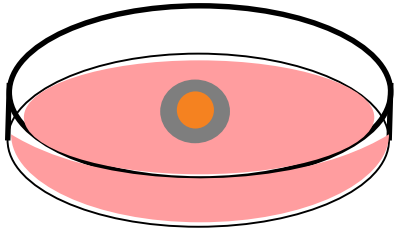


Plate 3 mm punches photoreceptor down



In a 24-well format



Transduced retina

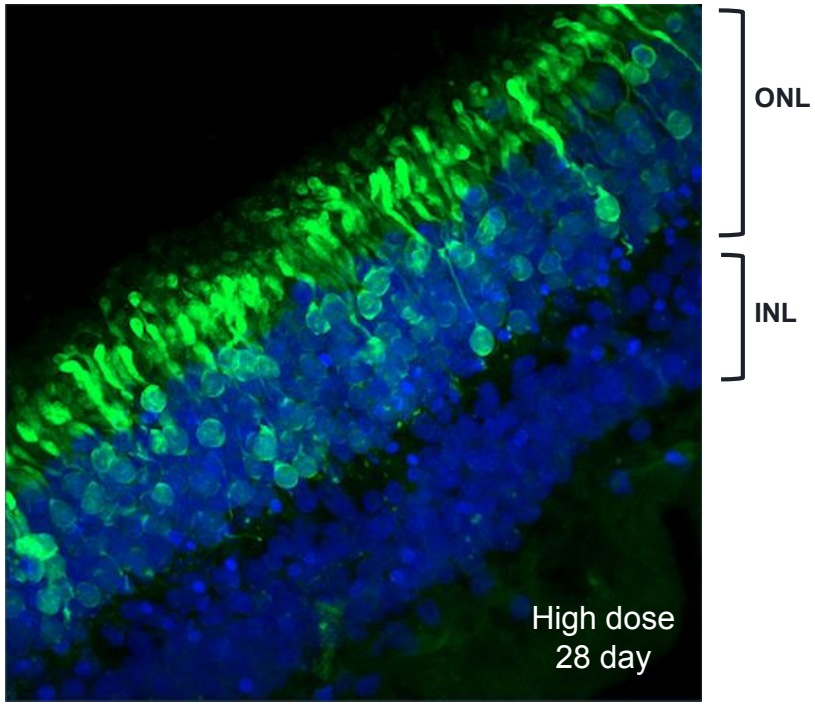
Retina were collected 28 days post-transduction

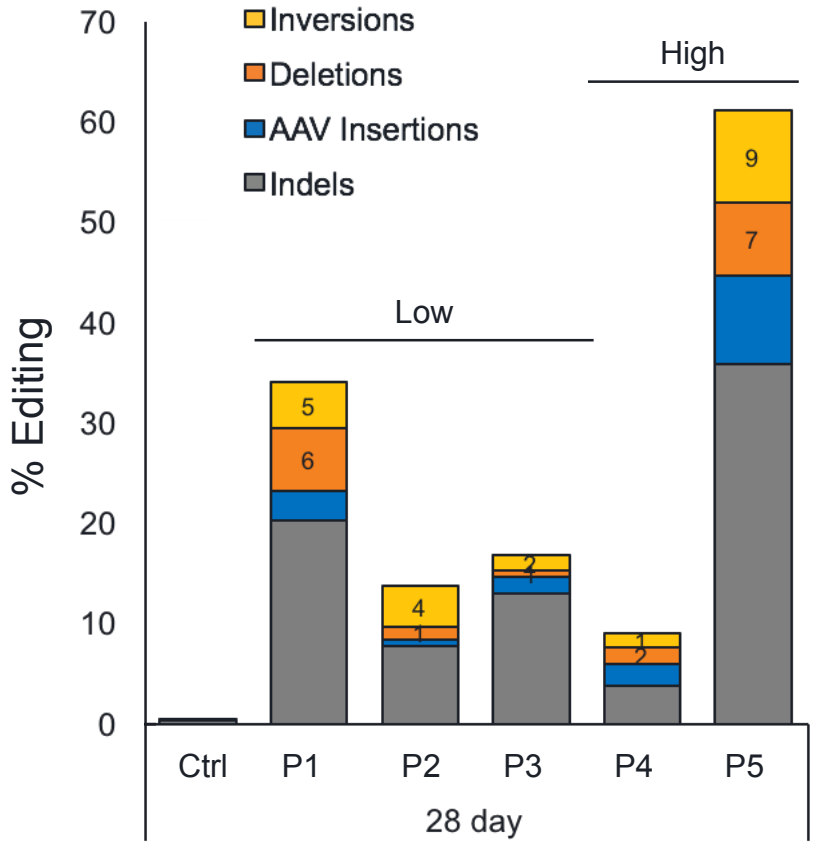
Histology

UDiTaS

Preliminary Data Demonstrating Editing of CEP290 in Human Retinal Explants

Human retina transduced with AAV5-GRK1-GFP



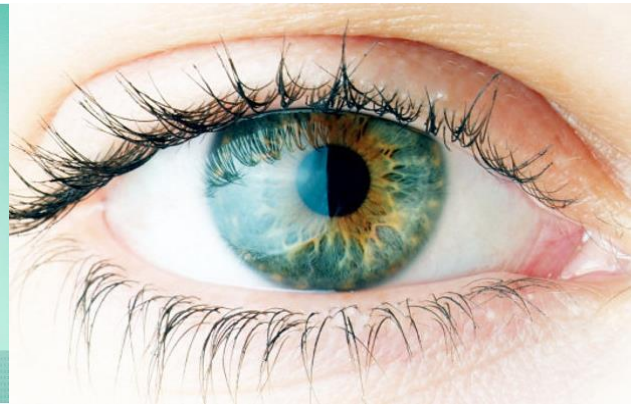


Low dose = 1e11 vg
High dose = 5e11 vg

Targeted CEP290 gene editing in mature human photoreceptors

Towards a Therapy for LCA10

- LCA10 patient fibroblast experiments demonstrate proof of concept for a CRISPR-based gene editing approach to treat LCA10 caused by the IVS26 splice mutation.
- *In vivo* experiments support pre-clinical development of a LCA10 therapeutic.
- Development of a human retinal explant assay demonstrates efficient editing in mature human photoreceptors, which enables ongoing specificity studies in the therapeutically relevant cell type



CEP290 gene editing therapeutic has the potential to have a major impact on vision in LCA10 patients

Acknowledgements

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Lions Eye Institute FOR TRANSPLANT & RESEARCH
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