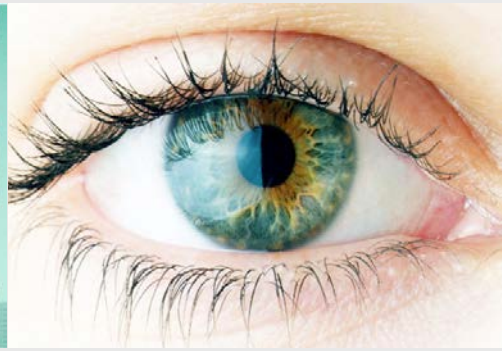


editas MEDICINE

Keeping an Eye on CRISPR

Gerry Cox, MD, PhD
Chief Medical Officer

Editas Medicine

Creating transformative genomic medicines for patients who have serious diseases with high unmet medical need

Leadership

- Katrine Bosley
  President & Chief Executive Officer
  Avila, Adnexus, Biogen
- Charles Albright, Ph.D.
  Chief Scientific Officer
  BMS, Incyte, DuPont
- Gerald Cox, M.D., Ph.D.
  Chief Medical Officer
  Sanofi Genzyme, Boston Children's Hospital
- Vic Myer, Ph.D.
  Chief Technology Officer
  Novartis, Akceli, Millennium
- Andrew Hack, M.D., Ph.D.
  Chief Financial Officer
  Millennium Management, HealthCor Management, Carlyle–Blue Wave
- Tim Hunt, J.D.
  Senior Vice President, Corporate Affairs
  Cubist, Biogen

Organization

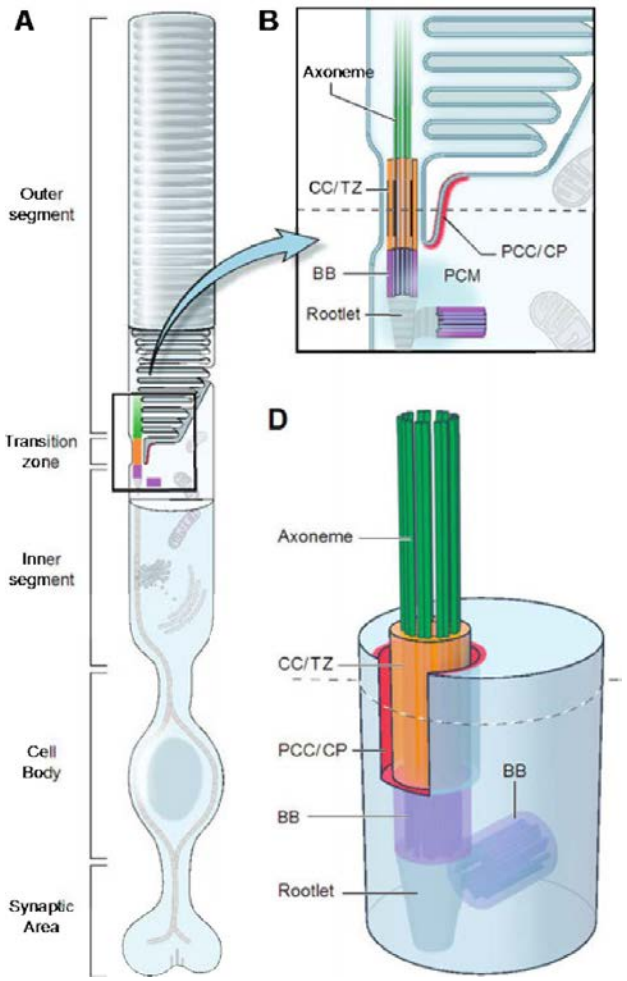
- Founded in 2013 by leading scientists and investors
- IPO in Feb. 2016 (EDIT)
- Over 100 FTEs today with approximately 75% in R&D
- Established capabilities include:
  Screening, disease biology, pharmacology, specificity, manufacturing, regulatory, clinical development
- Well capitalized with ~$325M current cash position
- Strong intellectual property position
  >40 issued patents in US/Europe and 500 pending applications
- 60,000 square foot facility in Cambridge, MA

Founders & Advisors

- Feng Zhang, Ph.D.
  Broad Institute; MIT
- George Church, Ph.D.
  Harvard Medical School; MIT; National Academy of Sciences
- David Liu, Ph.D.
  Howard Hughes Medical Institute; Harvard University; Broad Institue
- Keith Joung, M.D., Ph.D.
  Massachusetts General Hospital; Harvard Medical School

Leber Congenital Amaurosis Type 10

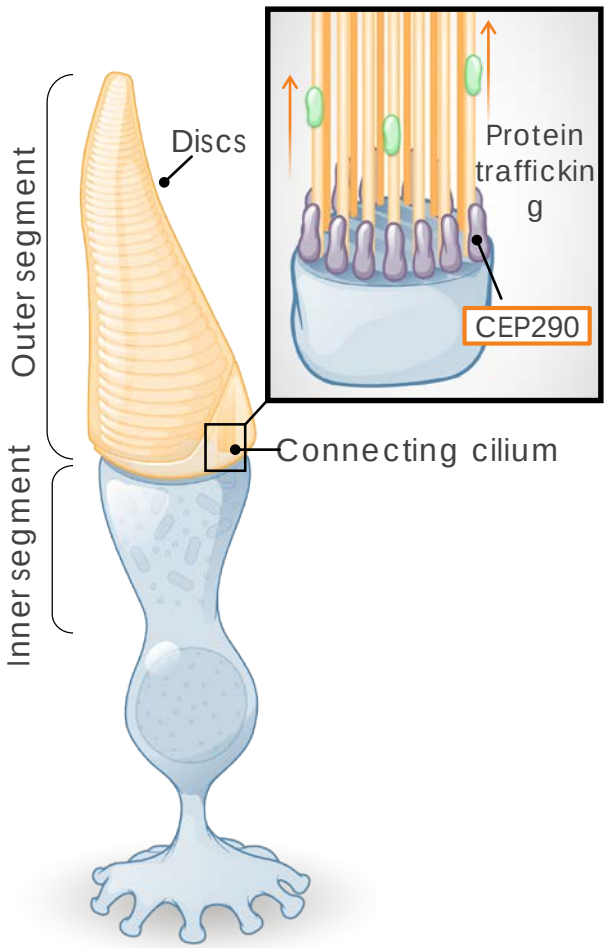
- Infantile-onset of poor vision, nystagmus, and a flat ERG[1]
- Visual acuity is typically counting fingers or worse, but can vary widely, even among siblings[1]
- LCA10 accounts for ~20-30% of all LCA patients[1]
- Caused by autosomal recessive mutations in the CEP290 gene at chromosome12q21.32[2]
- ~85% of LCA10 patients from north and west Europe have a common mutation in intron 26, c.2991+1655A>G, with geographic variation[1-7]
- CEP290 is present in the connecting cilium and is important for ciliogenesis, ciliary trafficking, and outer segment function and structure[8]



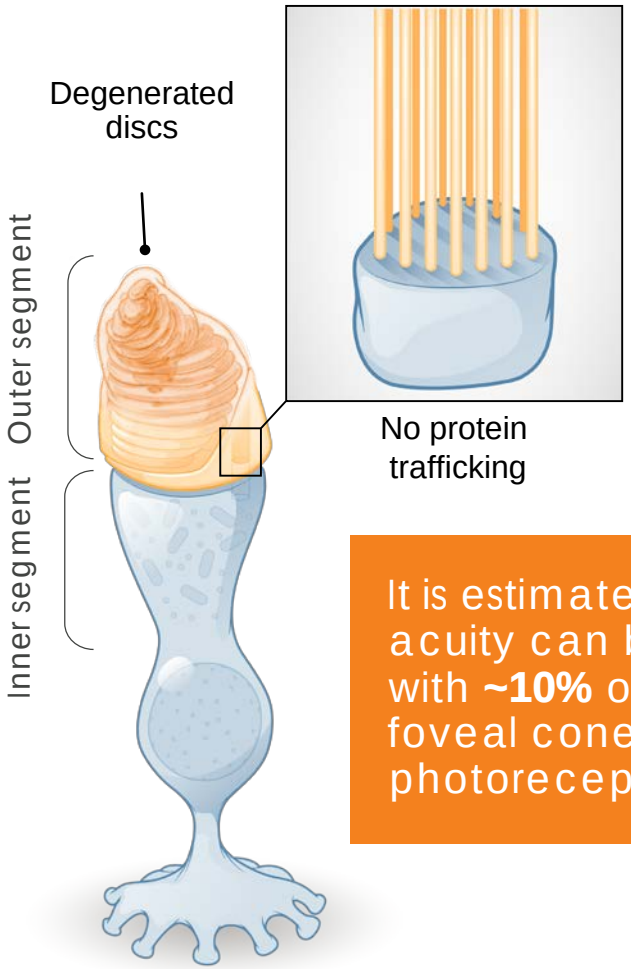
[1]Weleber RG, 2013 LCA Gene Reviews; [2]den Hollander AI, Koenekoop RK, Am J Hum Genet 2006;79:556-61; [3]Stone EM, Am J Ophthalmol 2007;144:791-811; [4]CEP290_databse 2017; [5]Perrault I, Hum Mutat 2007;28:416; [6]Vallespin E, IOVS 2007;48:5653-61; [7]Simonelli F, IOVS 2008;48:4284-90; [8]Rachel RA, Cilia 2012;1:22;

CEP290 in Ciliary Trafficking and Phototransduction

WT Photoreceptor



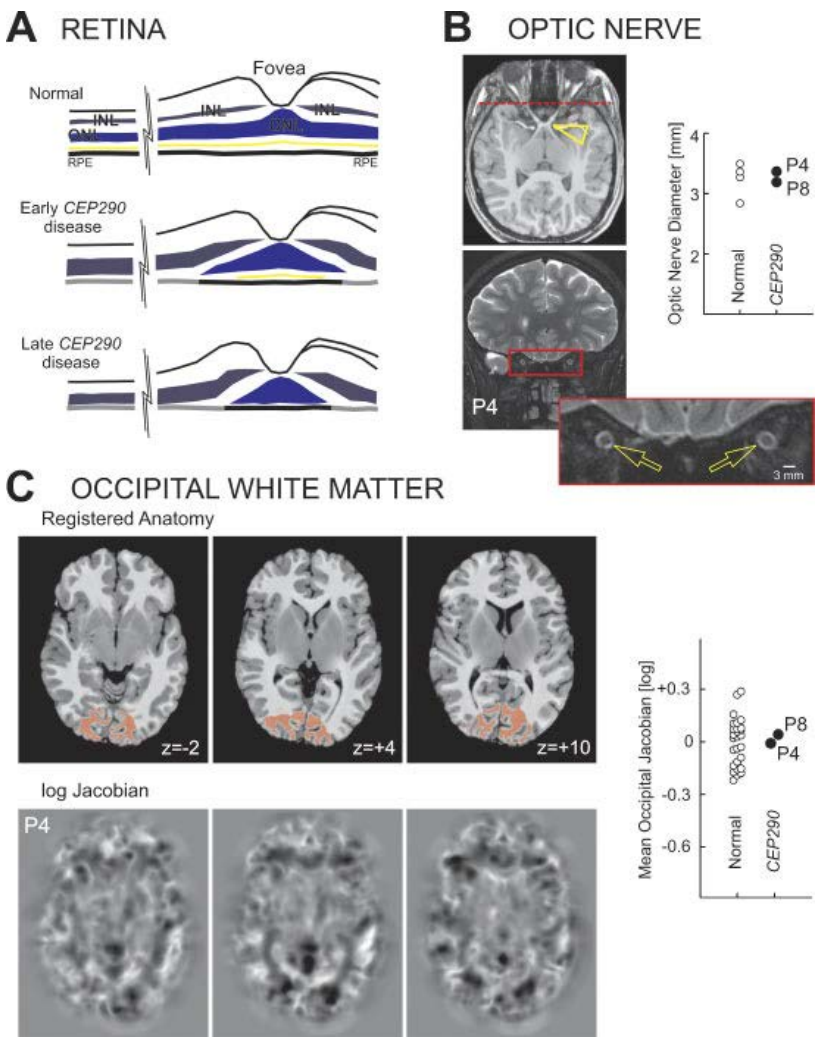
LCA10 Photoreceptor

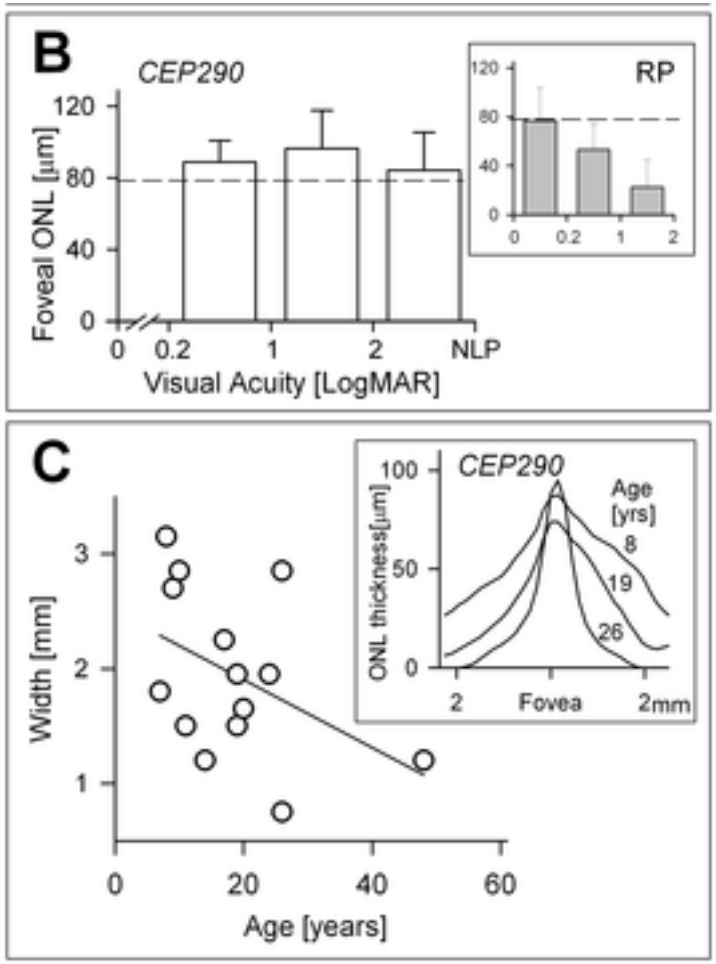


It is estimated that visual acuity can be achieved with **~10%** of functioning foveal cone photoreceptors[1,2]

[1]Geller AM, J Opt Soc AM 1992;9:472-77; [2]Geller AM, Vision Res 1993;33:1509-24.

Visual Pathway is Anatomically Intact in LCA10





Cideciyan AV, Hum Mutat 2007;28:1074-83

Boye SE, PloS One 2014;9(3)e92928

Visual Acuity is not Related to Age in LCA10

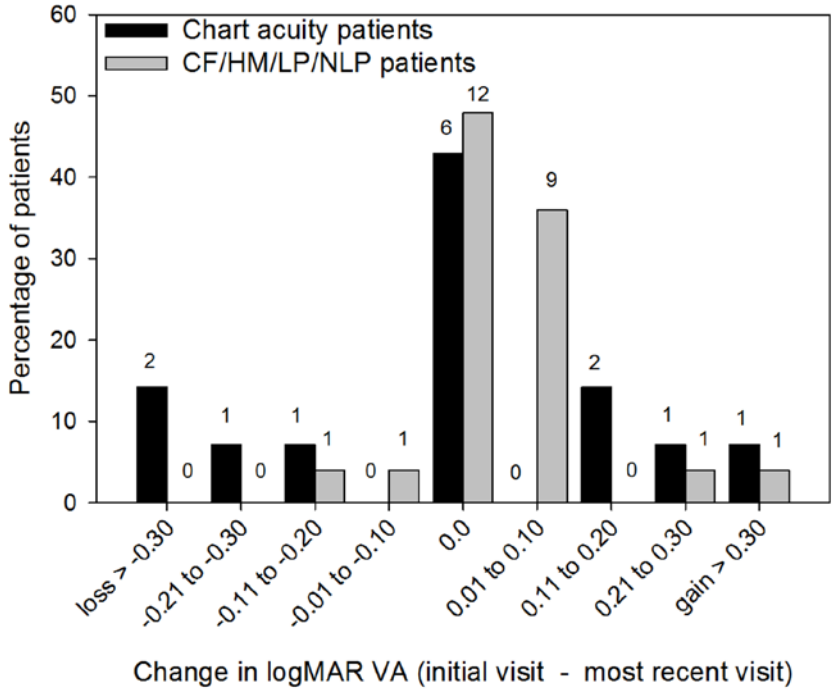
Mean logMAR (n)

Age (yrs)	CEP290
0–10	2.83 (10)
11–20	2.2 (13)
21–30	2.54 (5)
31–40	2.75 (4)
41–50	2.47 (4)
51–60	0.8 (1)
61–70	None
71–80	None

N=39
82% Counting Fingers or worse
Range 20/50 – No Light Perception

Walia S, Ophthalmology 2010;117:1190-1198

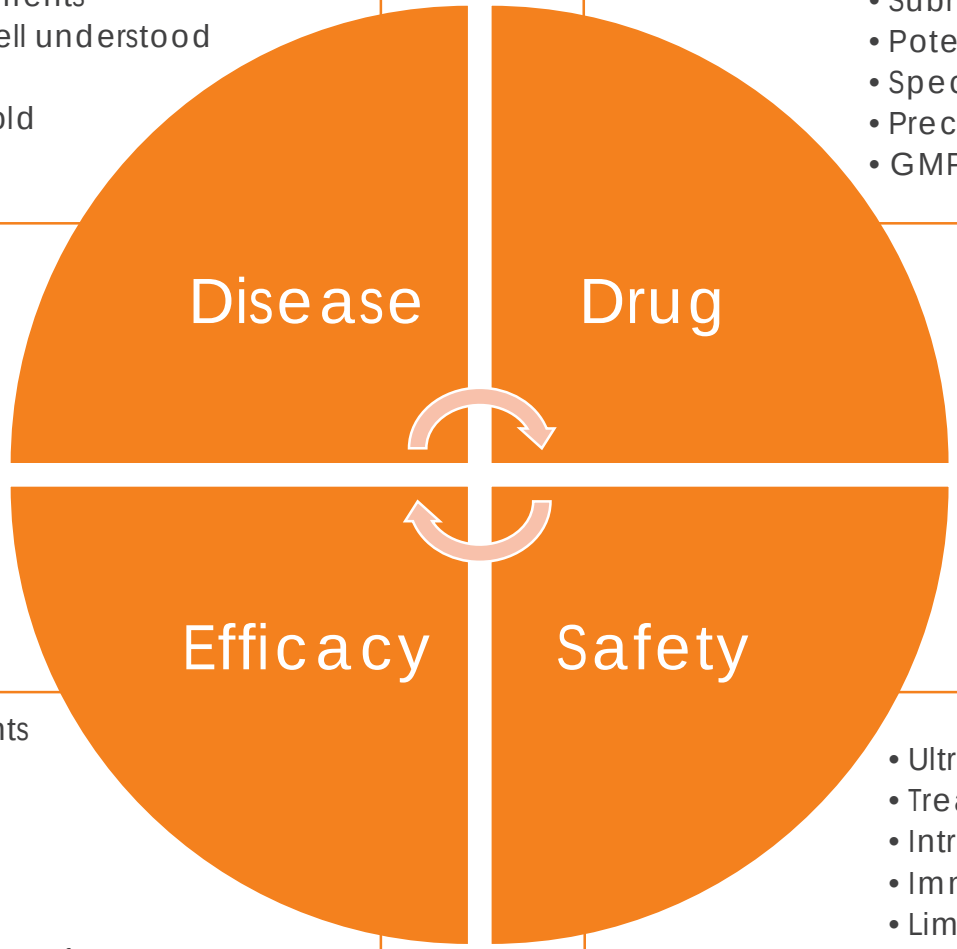
Mean follow-up 10.4 yr (range 2-47 yr)



N=43
64% Counting Fingers or worse
77% with IVS26 mutation
Mean age at initial visit 12.7 yr (0.2-57 yr)

McAnany JJ, JAMA Opthal 2013;131:178-182

Clinical Development Considerations for LCA10
Therapeutic goal is to improve vision



- High unmet need
- No approved treatments
- Pathophysiology well understood
- Treatment window
- Therapeutic threshold

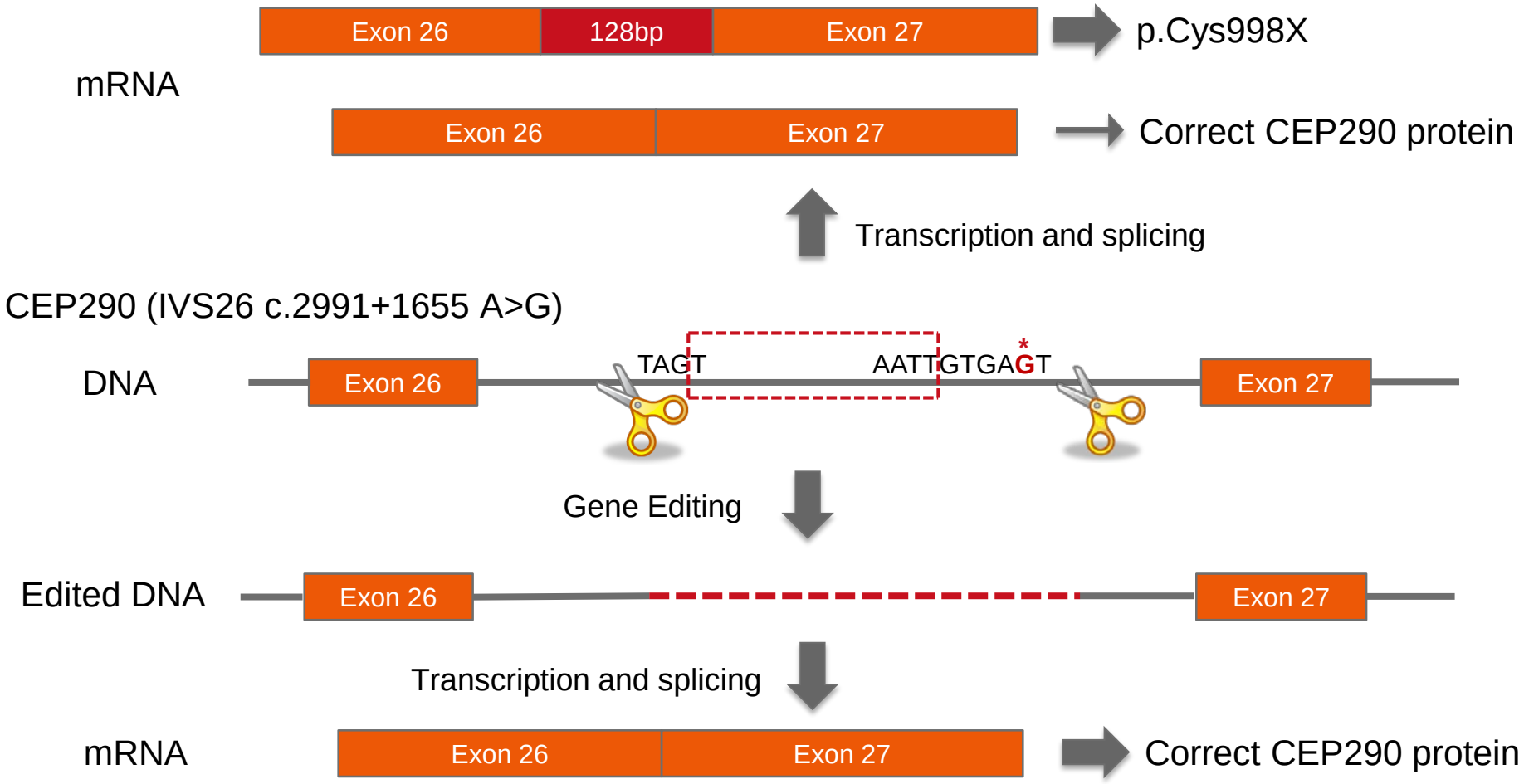
- AAV vector well tolerated
- Subretinal delivery established
- Potency (on-target editing)
- Specificity (off-target editing)
- Preclinical studies
- GMP grade material

- Established endpoints
  - -Anatomic
  - -Electrophysiologic
  - -Psychophysical
  - -Visual function
  - -Functional vision
- Expect improvements <1yr

- Ultra-orphan disease
- Treat one eye initially
- Intronic editing
- Immune privileged site
- Limited systemic exposure
- Photoreceptor expression
- Visible monitoring

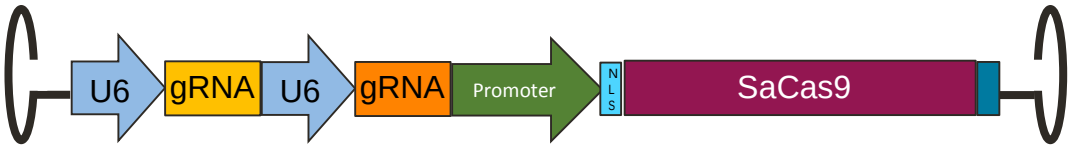
AAV, adeno-associated virus; GMP, good manufacturing practice

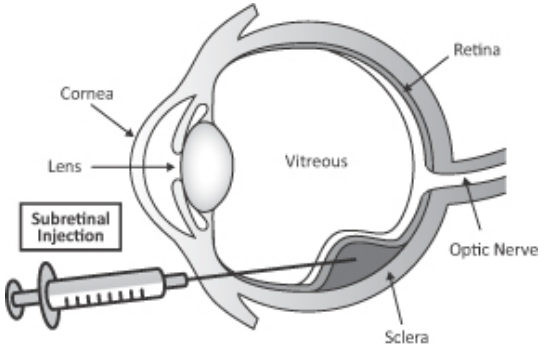
Gene Editing to Repair *CEP290* Splicing Defect



CRISPR-based Medicine for CEP290-LCA10

Subretinal injection of AAV5 vector encoding CRISPR/SaCas9 components intended to delete the IVS26 mutation and restore CEP290 splicing in photoreceptors





On-going in vivo experiments to understand:

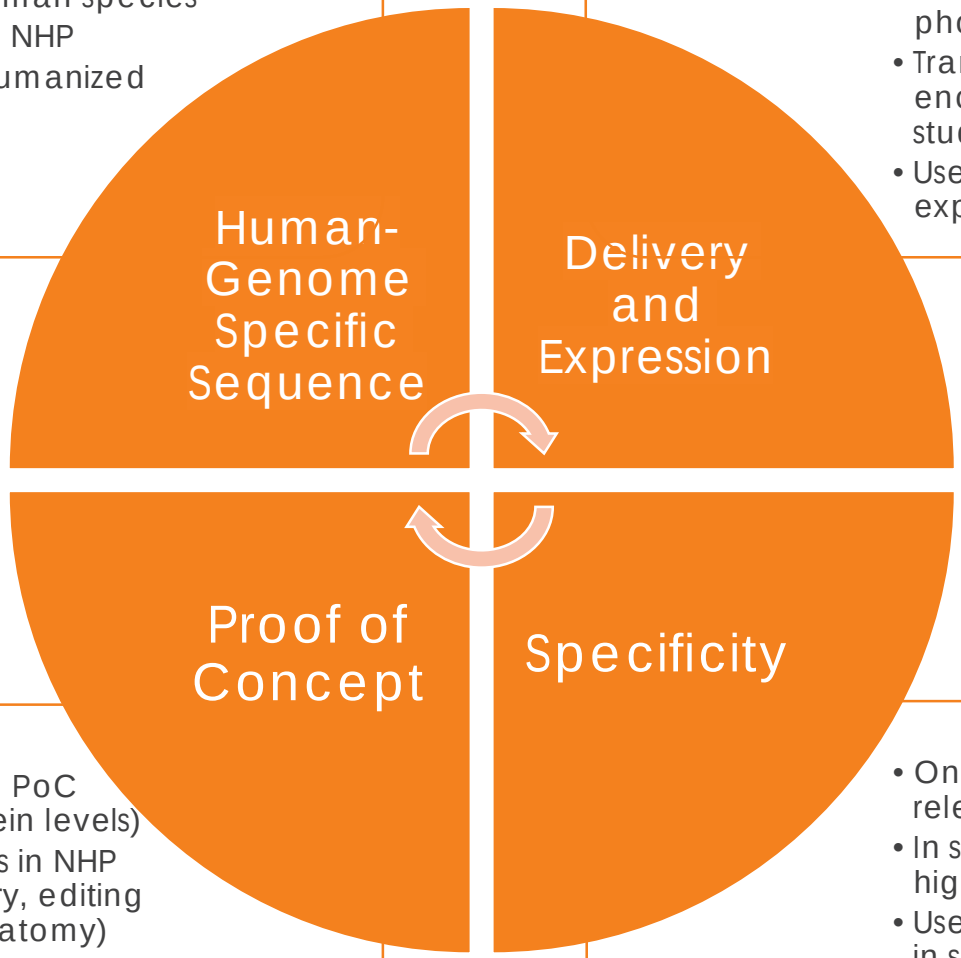
1. Level of gene editing in photoreceptors
2. Specificity
3. Tolerability

gRNA, guide RNA; NLS, nuclear localization signal
Sa Cas9, *Staphylococcus aureus* Cas9

CRISPR Development Challenges

- Genomic target and medicine are not recognized by non-human species
- Use surrogate gRNA in NHP
- Use human gRNA in humanized knock-in mice

- AAV5 and GRK1 promoter restrict Cas9 expression to photoreceptors
- Transfect gRNAs and plasmids encoding Cas9 for *in vitro cell* studies
- Use AAV5 in human retinal explant and in vivo

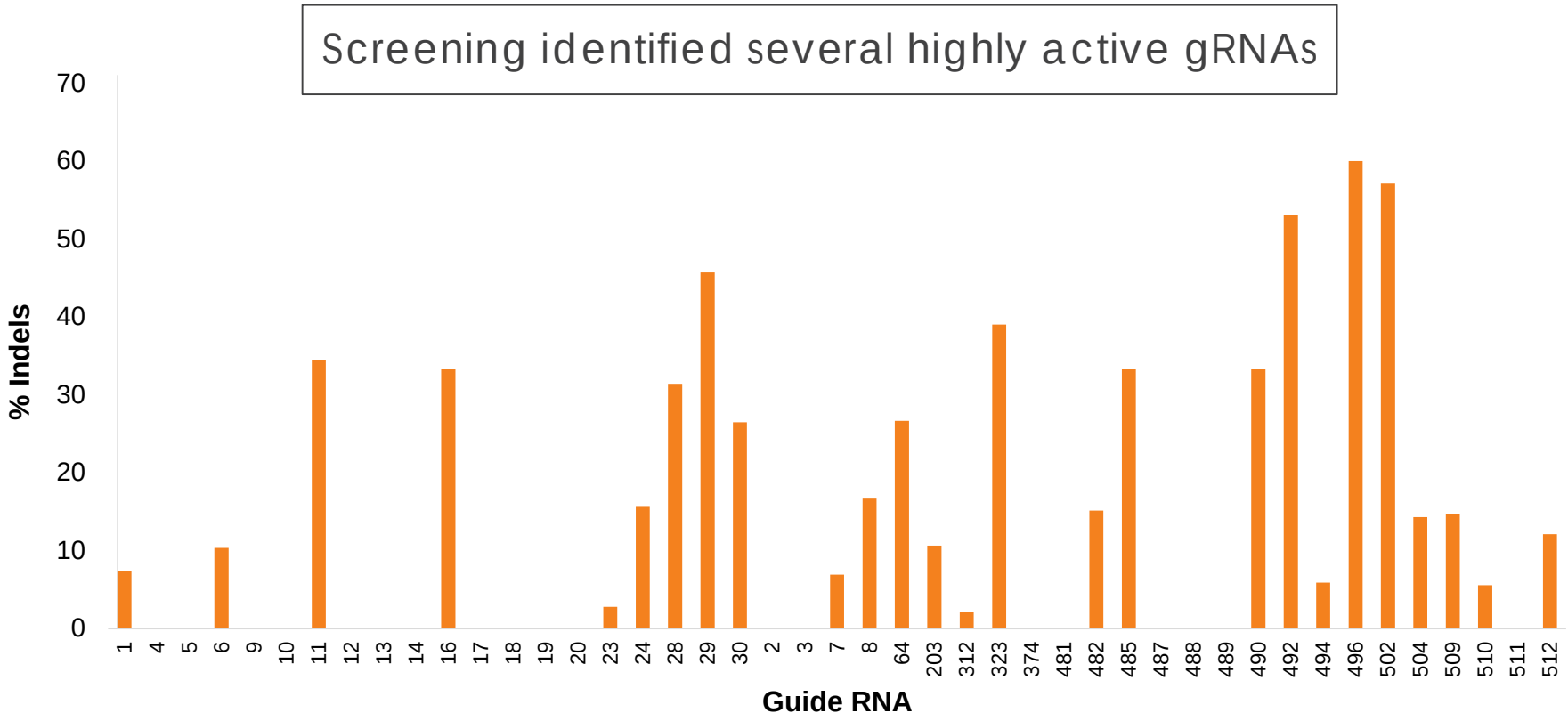


- No animal phenotype
- Human cells for in vitro PoC (editing, splicing, protein levels)
- AAV5 surrogate gRNAs in NHP for in vivo PoC (delivery, editing threshold, macular anatomy)
- AAV5 human gRNAs in humanized knock-in mouse for in vivo PoC (delivery, editing threshold) and retinal explant for in vitro PoC

- Only human cells are relevant
- In silico methods are not highly predictive
- Use orthogonal methods – in silico and cellular

AAV, adeno-associated virus; NHP, non-human primate; PoC, proof of concept

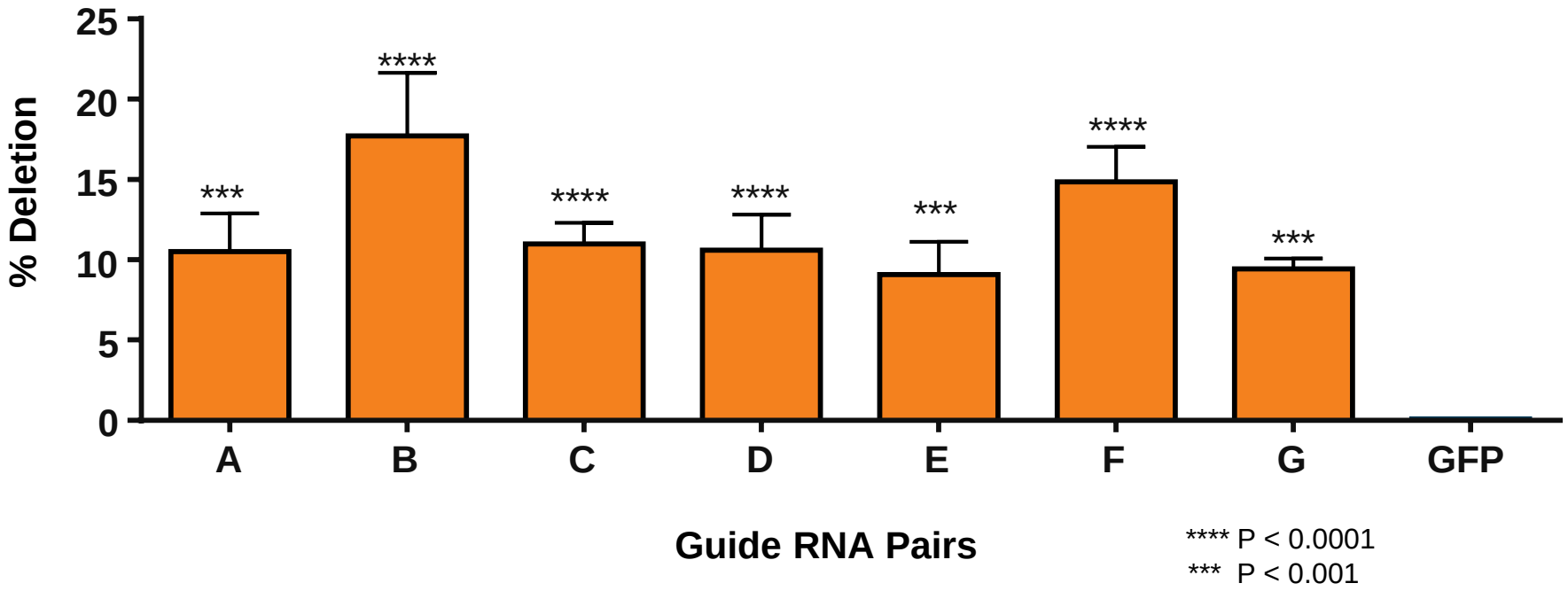
Single gRNA Screening for Editing Efficiency



- Single gRNAs were co-transfected with Cas9-encoding plasmid into either patient fibroblasts or HEK293 cells
- Gene editing rates were determined by quantifying indels at the target locus by Sanger sequencing

gRNA Pairs Induce Targeted Deletion

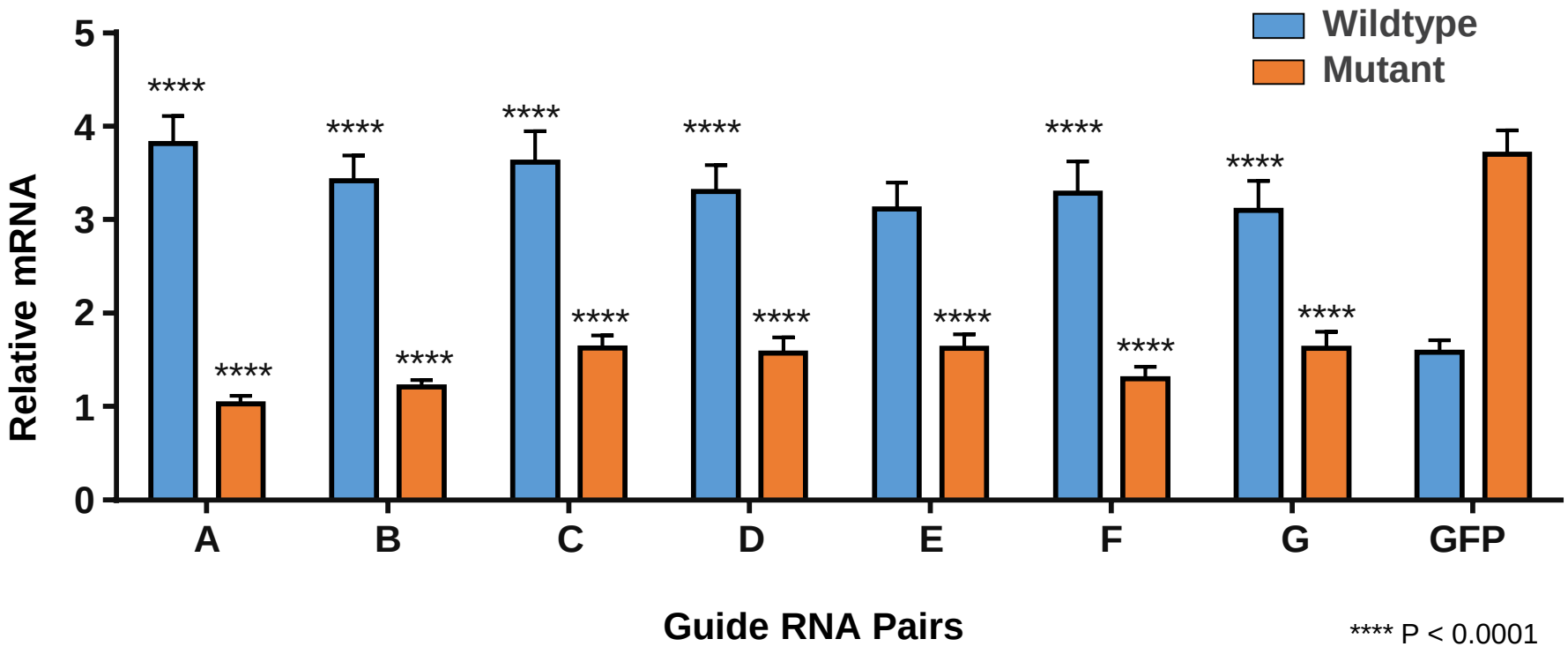
Quantification of targeted deletion by ddPCR following transfection of SaCas9 and gRNA pairs into LCA10 patient fibroblasts with a homozygous IVS26 mutation



ddPCR, digital droplet PCR; SaCas9, Staphylococcus aureas Cas9; gRNA, guide RNA; GFP, green fluorescent protein

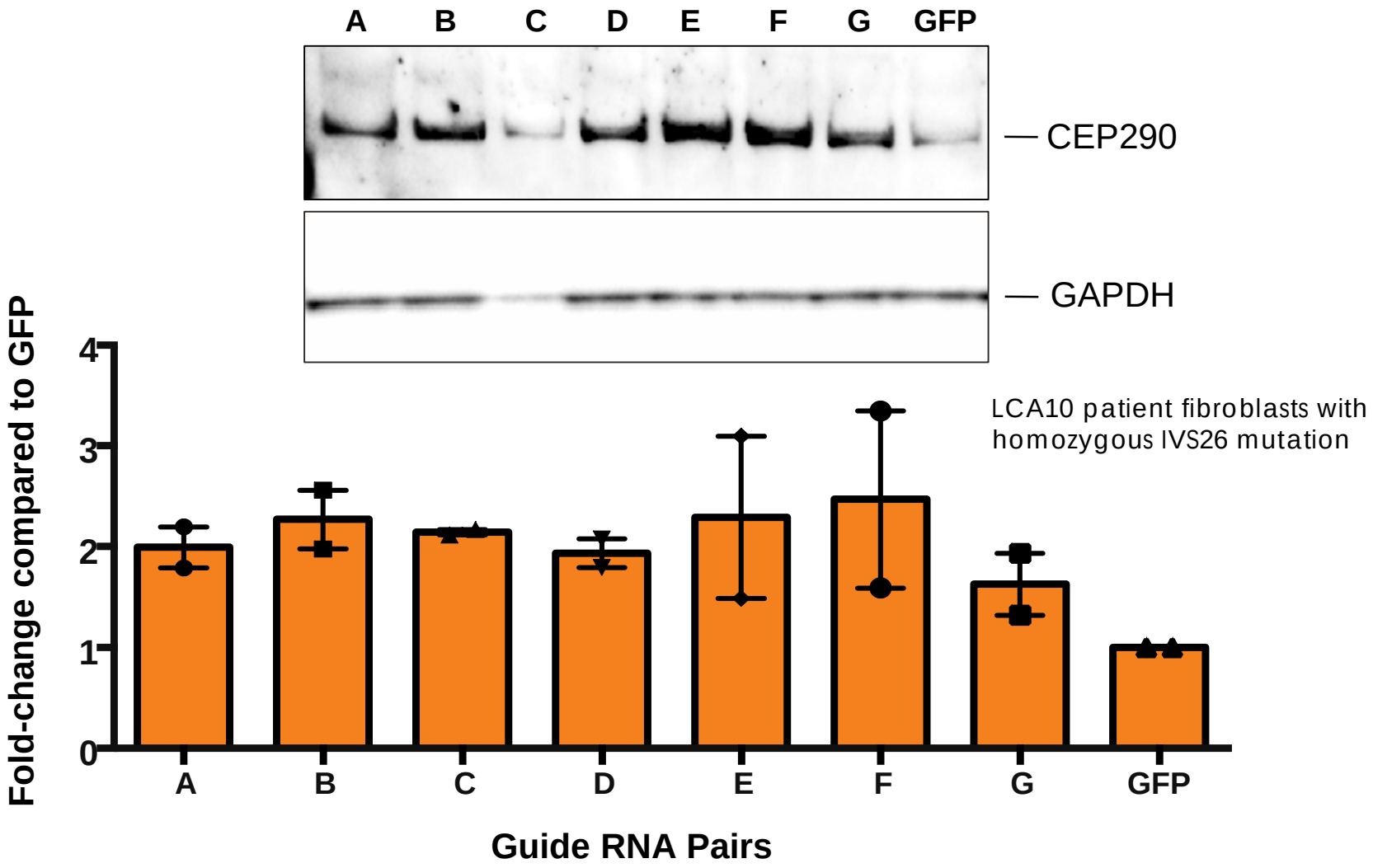
Targeted Deletion Corrects Splicing

Quantification of wildtype and mutant CEP290 mRNA by qRT-PCR in LCA10 patient fibroblasts



qRT-PCR, quantitative real time PCR
GFP, green fluorescent protein

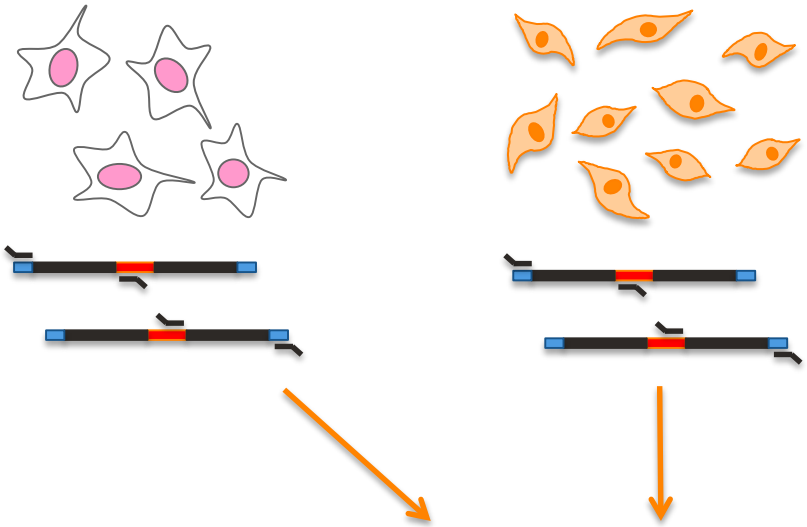
Targeted Deletion Increases CEP290 Level



GAPDH, glyceraldehyde 3-phosphate dehydrogenase
GFP, green fluorescent protein

Specificity Analysis of Candidate gRNAs

GUIDE-Seq in multiple human cell lines **(Unbiased Method)**



Computational identification of closely matched sites **(Biased Method)**

TTGCACGTACGTAAACAGGATGG
TTGGACGTACGTAAACAGGATGG
TTGCACGAACGTAAGCAGGATGG
TTGCACGTACGTTAACAGGATGG
TAGCACGTACGTAAACAGGCTGG

Panel of sites analyzed by targeted NGS

NGS, next-generation sequencing

GUIDE-Seq Summary for CEP290 gRNAs

5 of 8 gRNAs had no detectable off-targets in 3-4 cell lines

Guide RNA	Cells Used for GUIDE-Seq				# Off-target Sites Identified
	ARPE19	Fibroblasts	SH-SY5Y	U2OS	
1	✓	✓	✓	✓	4
2	✓	✓	✓	✓	0
3	✓	✓	✓	✓	0
4	✓		✓	✓	0
5	✓		✓	✓	1
6	✓		✓	✓	0
7	✓		✓	✓	8
8	✓		✓	✓	0
VEGFA site3, Sp**				✓	59

GUIDE-Seq, Genome-wide Unbiased Identification of Double-stranded breaks Enabled by Sequencing

Sp**, *Streptococcus pyogenes* positive control

Targeted Amplicon Sequencing Summary for CEP290 gRNAs

5 of 8 gRNAs had no detectable off-targets in 2 cell lines

- Hg38 reference genome used for in silico identification of sites
- Variants in the 1,000 Genomes Project with a minor allele frequency >1% and a 3' *S. aureus* PAM (NNGRR) were identified using Cas-OFFinder[1]
- Sites prioritized in coding sequence or in non-coding regions of tumor suppressors, oncogenes or IRD genes (GEDi-R)
- Approximate sensitivity of detection = 0.1%

[1]Bae S, Bioinformatics 2014;30-1473-75

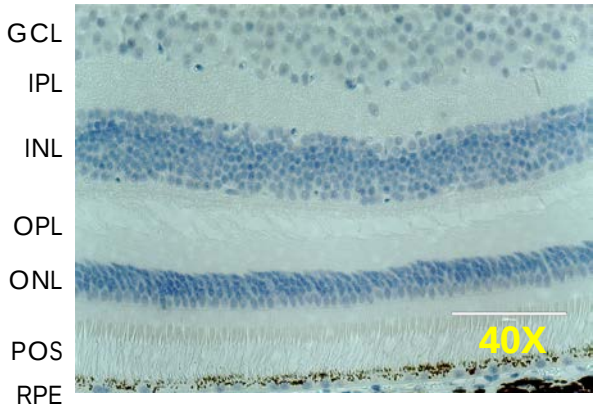
PAM, protospacer adjacent motif
GEDi-R, genetic eye disease panel for retinal genes
gRNA, guide RNA

Guide	Cells	# of Sites Sequenced	# Off- target Sites Identified
1	U2OS	6	1
	ARPE19	3	1
2	U2OS	81	0
	ARPE19	61	0
3	U2OS	90	0
	ARPE19	57	0
4	U2OS	74	0
	ARPE19	48	0
5	U2OS	95	1
	ARPE19	56	0
6	U2OS	72	0
	ARPE19	58	0
7	U2OS	12	6
	ARPE19	12	0
8	U2OS	71	0
	ARPE19	53	0

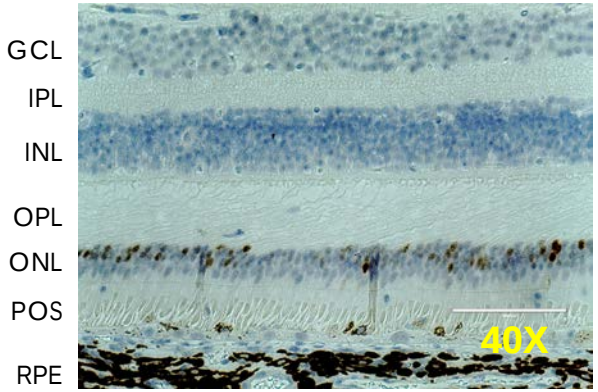
In vivo CEP290 Editing in Non-Human Primate Photoreceptors Using AAV5 and Surrogate gRNAs

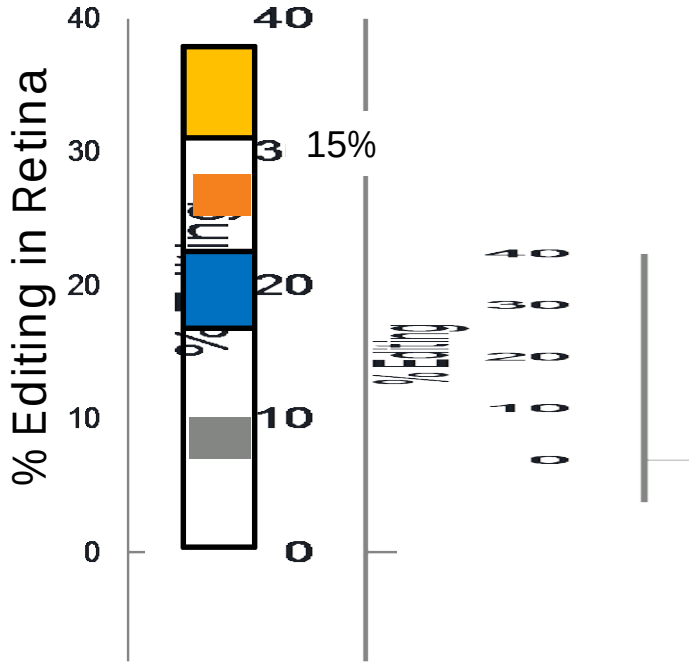
Cynomolgus macaque injected sub-retinally with 4e11 vg of AAV5-NHPCEP290gRNAs-GRK1-SaCas9

Vehicle



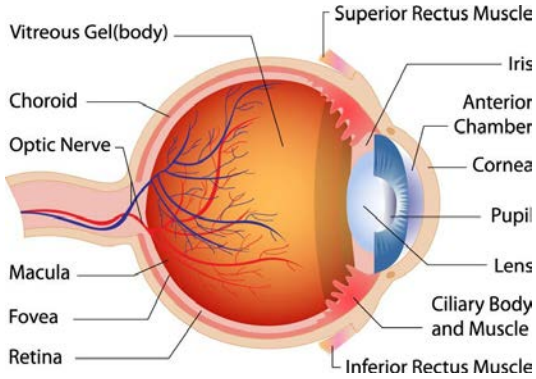
AAV5-NHPCEP290gRNAs-GRK1-Cas9





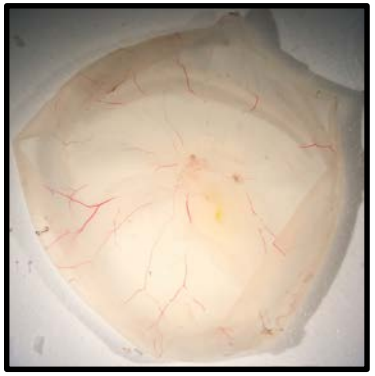
Proof of concept that CRISPR/SaCas9 can reach >15% productive editing in NHP bulk retina and potentially as high as 50% productive editing in photoreceptors

Developing a Human Retinal Explant System



Obtain Human Eyes 3-5 hrs Post-Mortem

Remove Neural Retina



3 mm punches

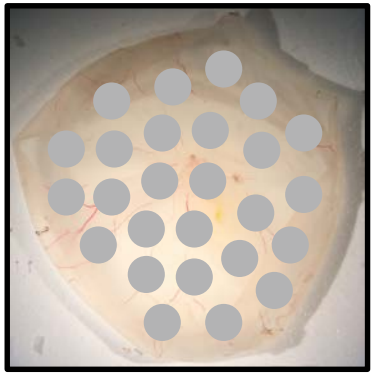
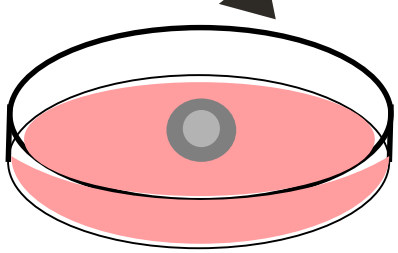
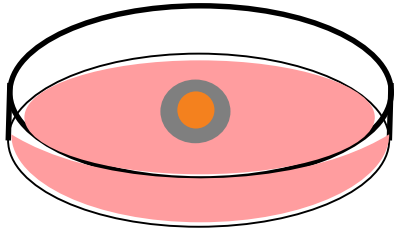


Plate 3 mm punches photoreceptor down



In a 24-well format



Transduced retina

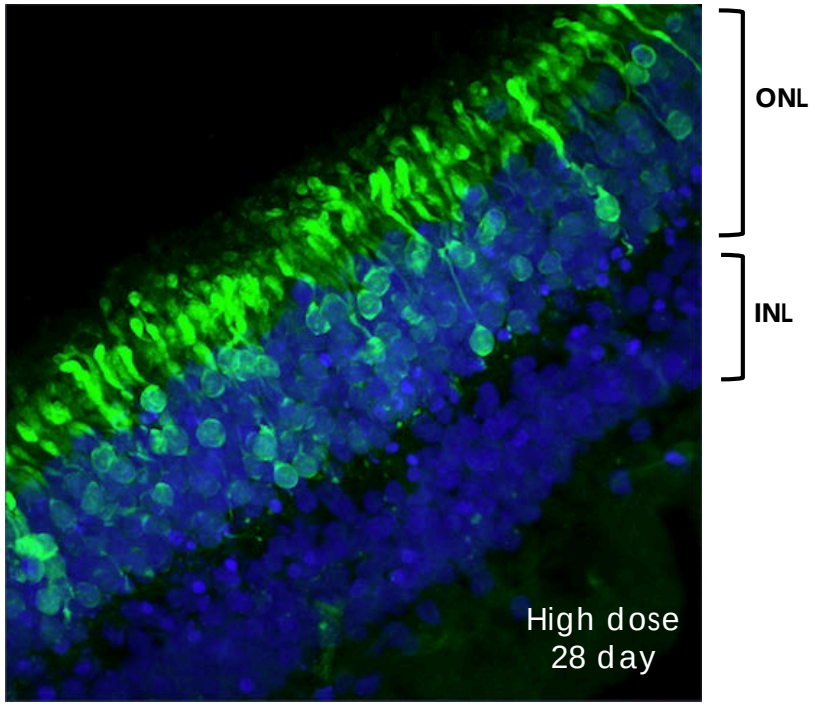
Retina were collected 28 days post-transduction

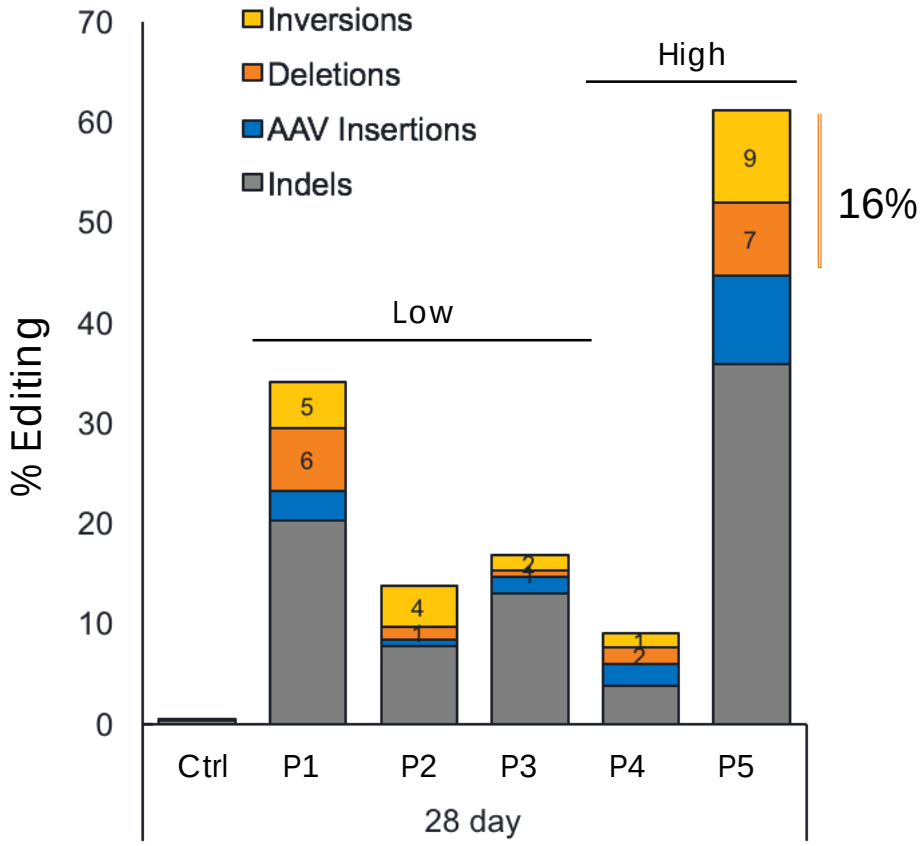
Histology

UDiTaS

Preliminary Data Demonstrating Editing of CEP290 in Human Retinal Explants using AAV5

Human retina transduced with AAV5-GRK1-GFP





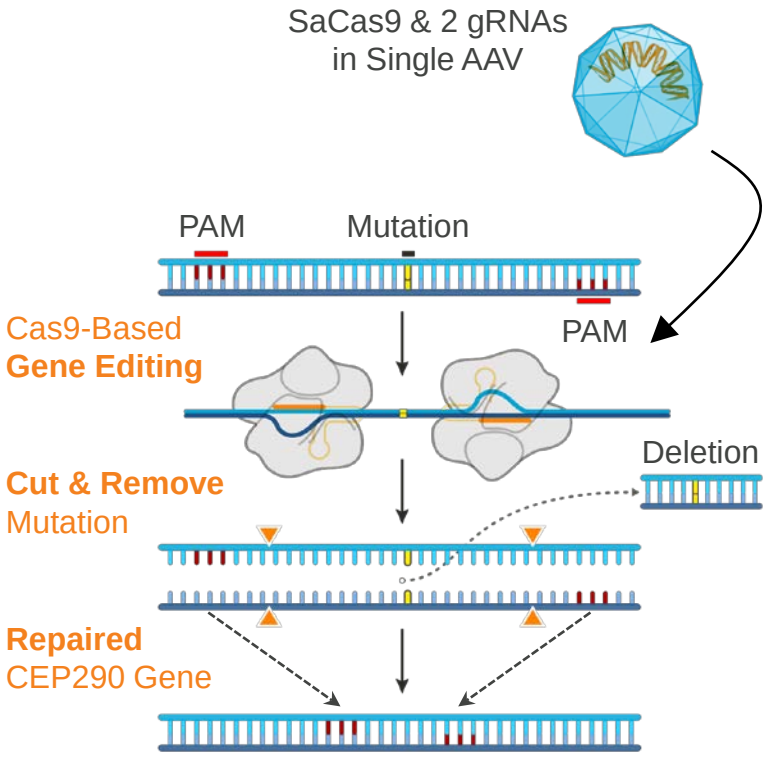
Low dose = 1e11 vg
High dose = 5e11 vg

Targeted CEP290 gene editing in normal mature human photoreceptors

LCA10 Product Candidate Advancing Toward Clinic

Attractive Target Product Profile Emerging

- ☑ Efficient editing in LCA10 patient-derived cells corrects mutation *in vitro*
- ☑ Highly selective with no off-target editing detected with multiple methods
- ☑ Efficient delivery of all components in a single, well-validated AAV5 vector
- ☑ Optimized configuration to include photoreceptor-specific promoter
- ☑ Demonstrated editing with drug product configuration in human eye explants
- ☑ Demonstrated therapeutically-relevant editing in non-human primates *in vivo*



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Maxwell Skor

Michael Stefanidakis
Steven Samuelsson
Pam Stetkiewicz
Haiyan Jiang
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Vic Myer
Hari Jayaram
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Eugenio Marco
Luis Barrera
Gregory Gotta
Georgia Giannoukos
Dawn Ciulla
Tongyao Wang
Vidya Dhanapal

Mitch McCartney
Nicholas Sprehe

Medicines which Aim to Repair Any Broken Gene

Potentially transformative new category of medicines
for genetically-defined and genetically-treatable diseases