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Envisioning a Gene Editing Approach to Treat Inherited Blindness

Morgan Maeder

Why Gene Editing in the Eye?

Benefits of targeting inherited retinal diseases

Significant unmet medical need

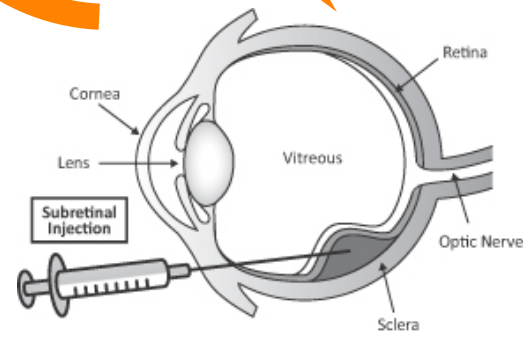
- Vision loss has huge negative impact on quality of life
- Most inherited retinal disease patients have no therapeutic options

Proven delivery

Gene therapy using viral vectors has demonstrated success in the clinic

Clear, non-invasive end points

- Visual acuity and other imaging measurements well characterized
- Built-in case-control: treated eye compared to untreated eye



Confined, immune-privileged location

- Limited immune response
- Any negative response easily detected and likely confined to the eye

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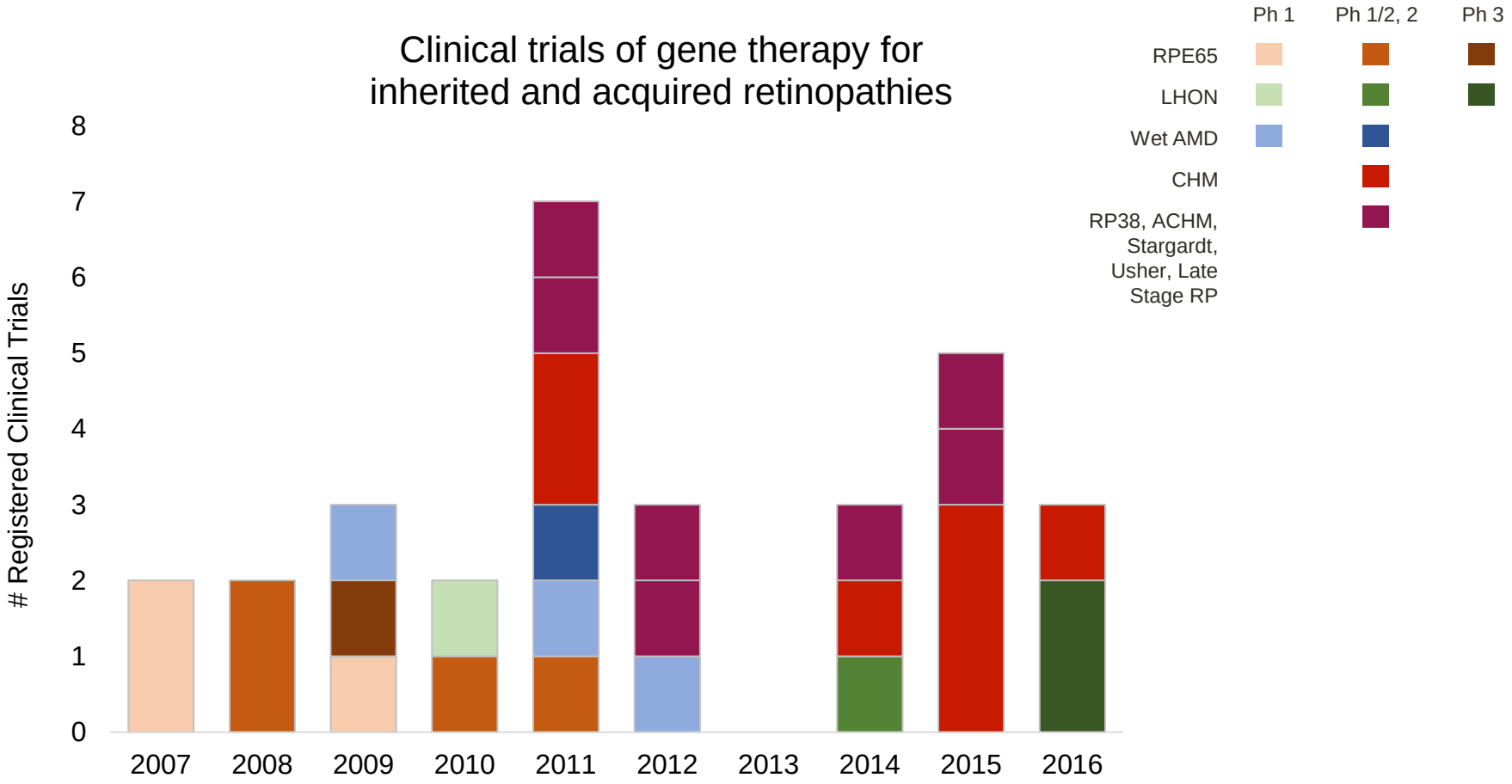
RPE65 Validates Retinal Gene Therapy

Clinical trials for a broad range of retinopathies

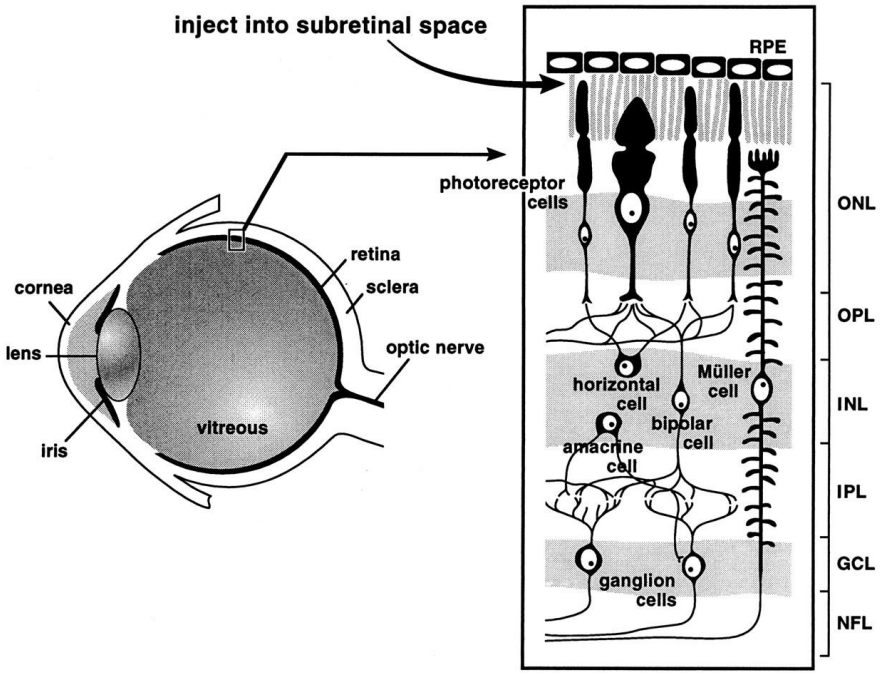


Data compiled from Petit, Khanna and Punzo, *Human Gene Therapy* 2016

AAV-Mediated Retinal Gene Therapy

On-going field of research to optimize current approach

Sub-retinal delivery of appropriate AAV serotype for target cell encoding cDNA for gene augmentation



Challenge	Potential solutions
Not all cell types are as easily transduced as RPE	Testing and optimization of non-AAV2 serotypes, modification and evolution of AAV
Sub-retinal injections are difficult and associated with risk	Evolution of AAV serotypes that can transduce the retina via intravitreal injection
Several disease-causing genes (ex. ABCA4, USH2A, CEP290) exceed the packaging limit of AAV	Lentivirus Trans-splicing AAV Gene Editing
Dominant diseases are difficult to address	Gene Editing

Hiroyuki Miyoshi et al. PNAS 1997

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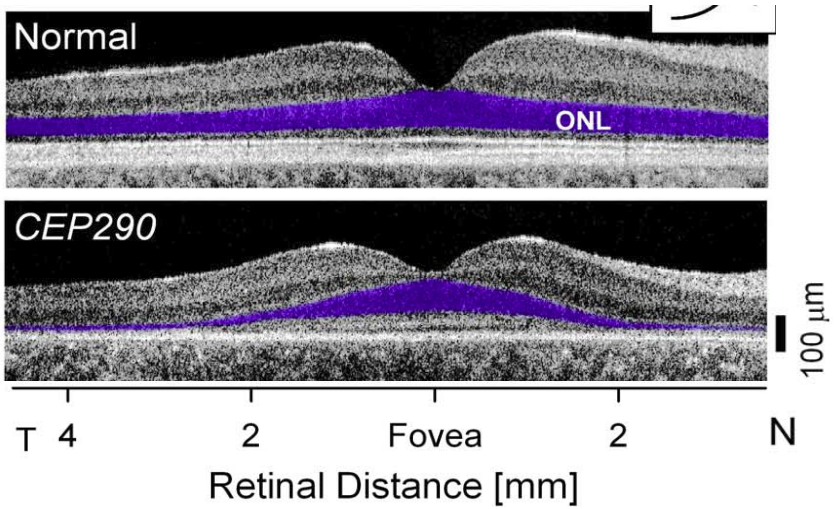
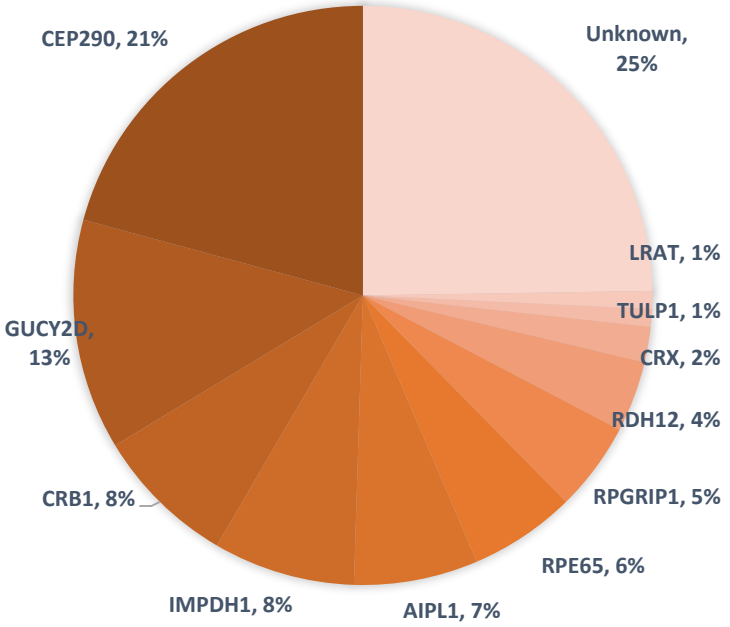
Leber Congenital Amaurosis

LCA10 caused by mutations in the CEP290 gene

- Group of heterogeneous inherited retinal dystrophies
- Early onset: infancy/early childhood
- CEP290 coding sequence = 7.5kb



Boye et al., PLOS ONE 2014



Sweeney MO, Mol Vis. 2007 Apr 5;13:588-93.

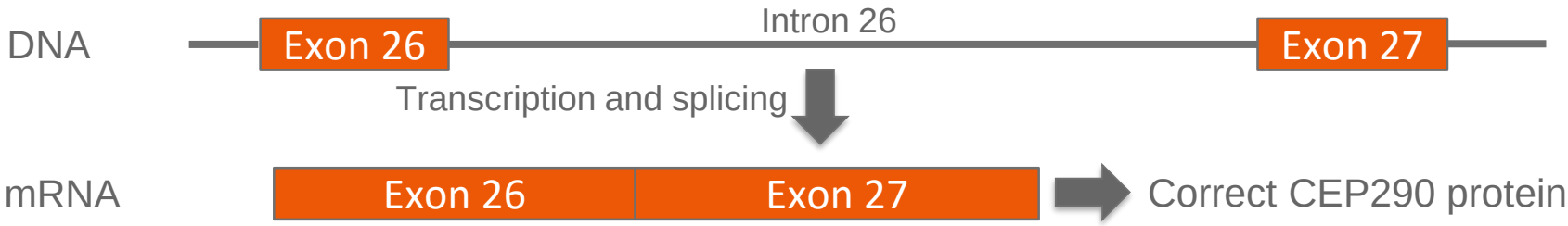
Target: Residual Photoreceptors

- Early loss of rod photoreceptors
- Survival of the central island of cone photoreceptors
- Normal intracranial visual pathways

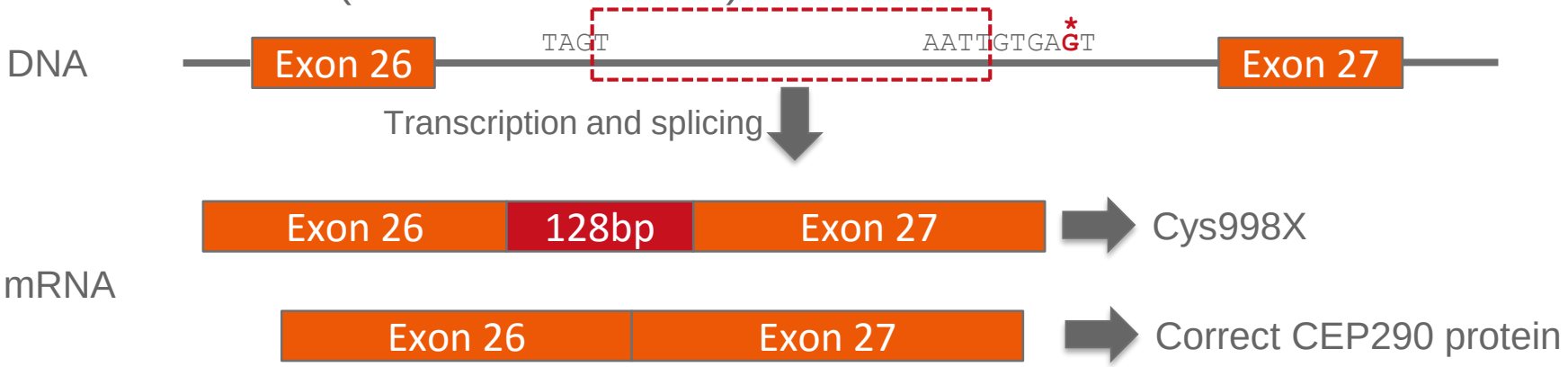
CEP290-Associated LCA10

Molecular mechanism of IVS26 mutation

Wild-type CEP290

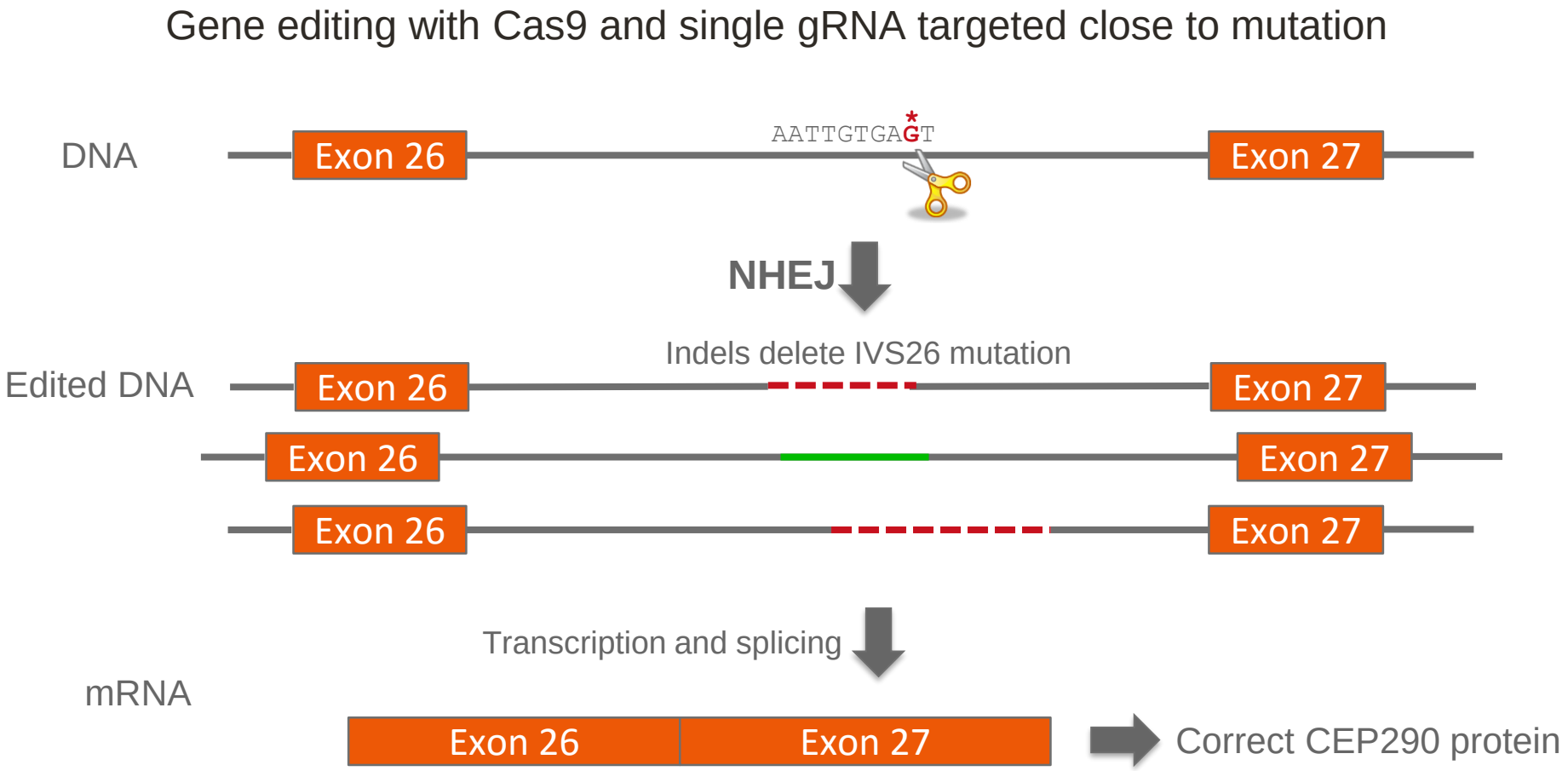


IVS26 mutant CEP290 (c.2991 + 1655 A>G)



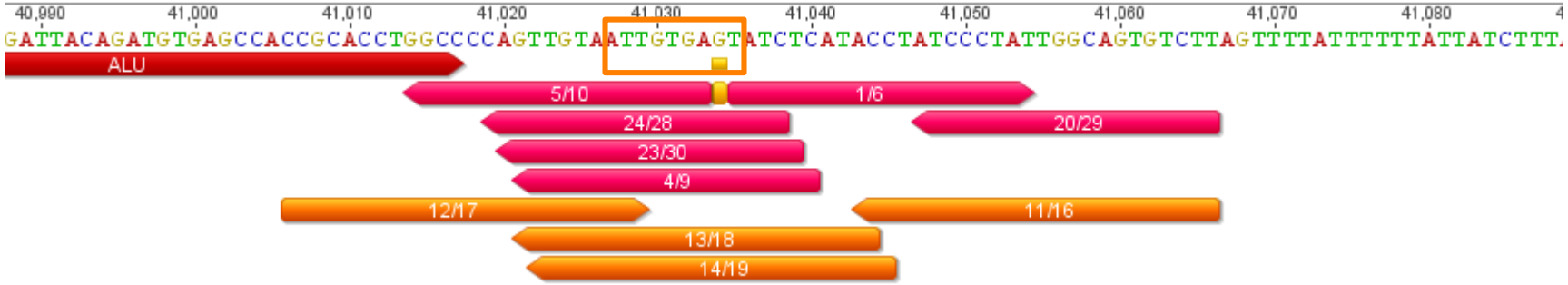
CEP290 Single gRNA Gene Editing Approach

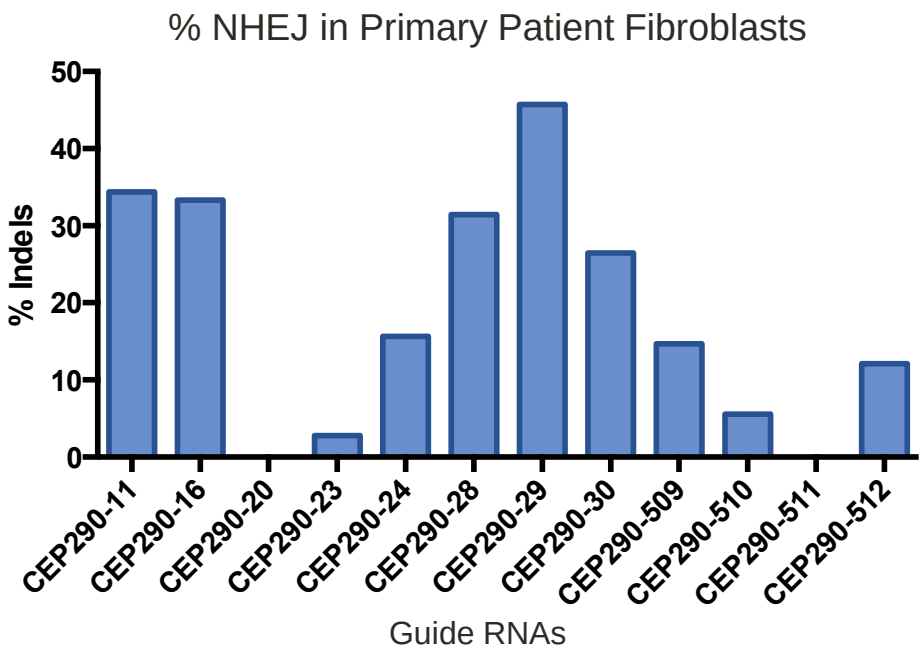
Single gRNA induces NHEJ to delete splice mutation



CEP290 Single gRNA Gene Editing Approach

Single gRNAs induce targeted indels





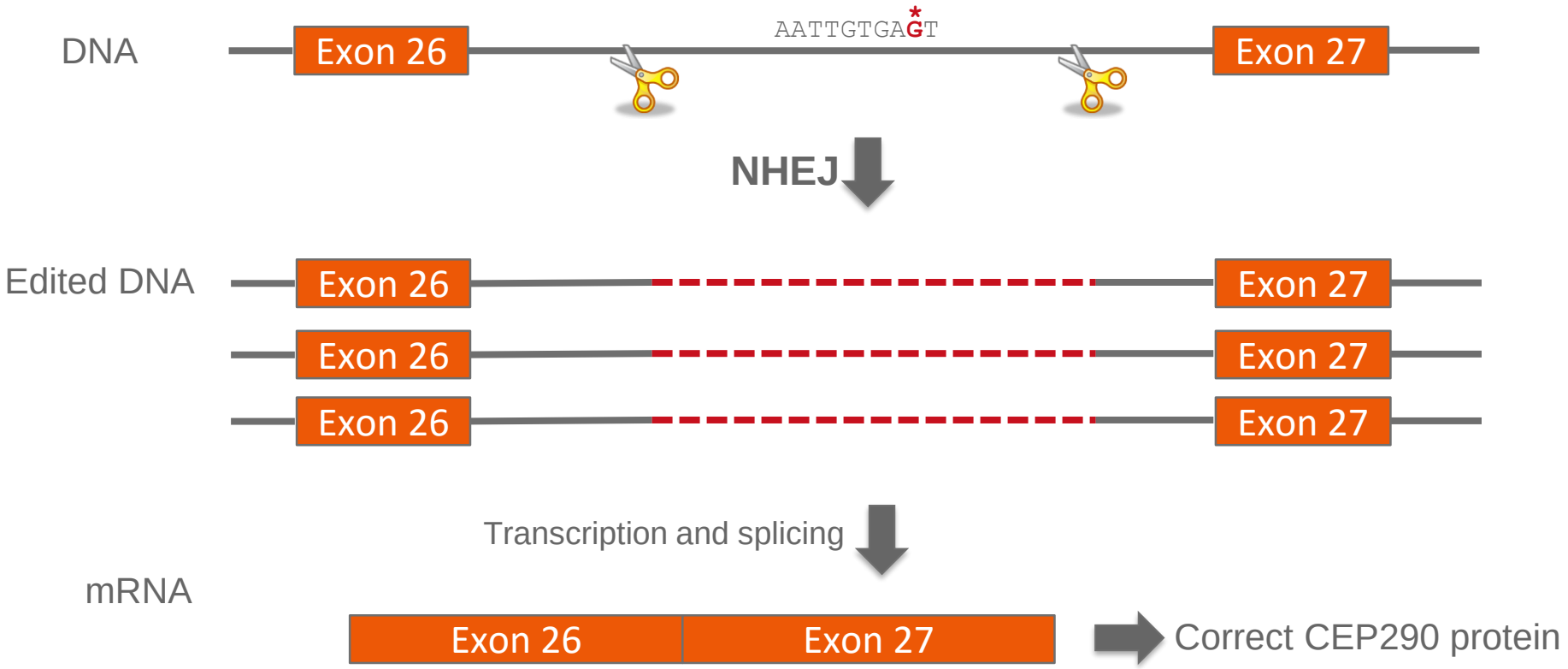
CEP290 Single gRNA Gene Editing Approach

Single gRNA-induced indels fail to efficiently delete IVS26 mutation

CEP290 Dual gRNA Gene Editing Approach

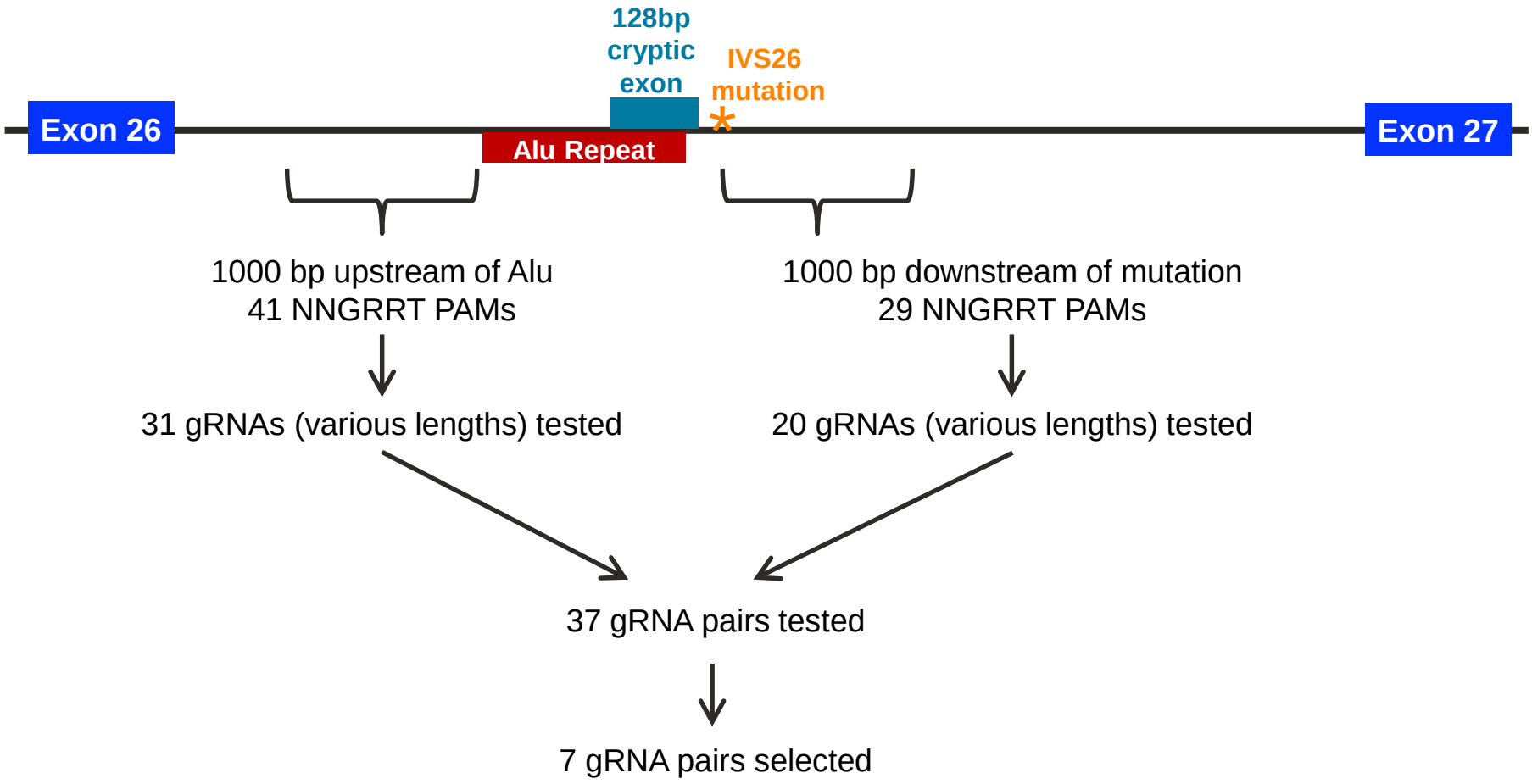
A targeted deletion approach to excise mutation

Gene editing with S. aureus Cas9 and **two** gRNAs flanking the mutation



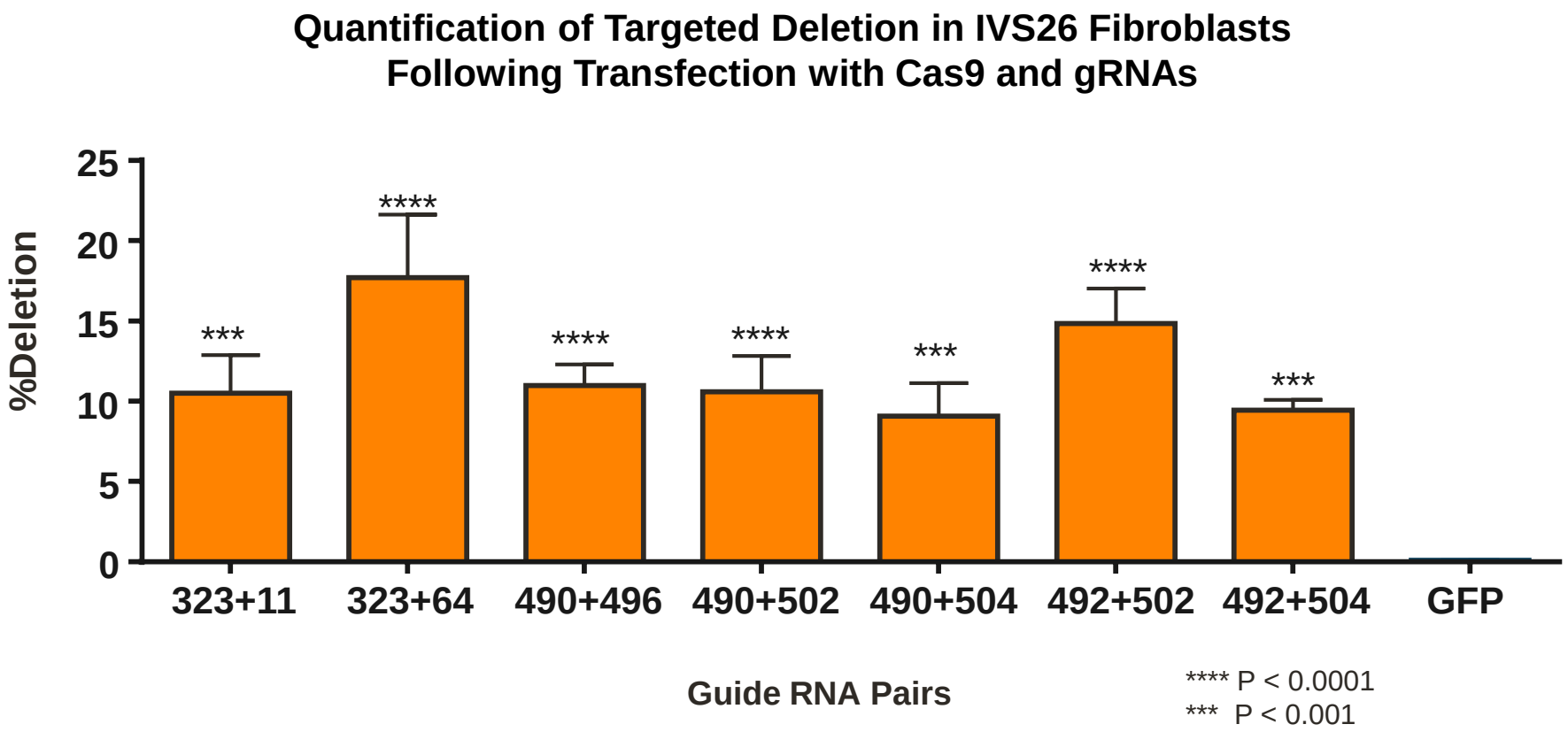
CEP290 Dual gRNA Gene Editing Approach

A targeted deletion approach to excise mutation



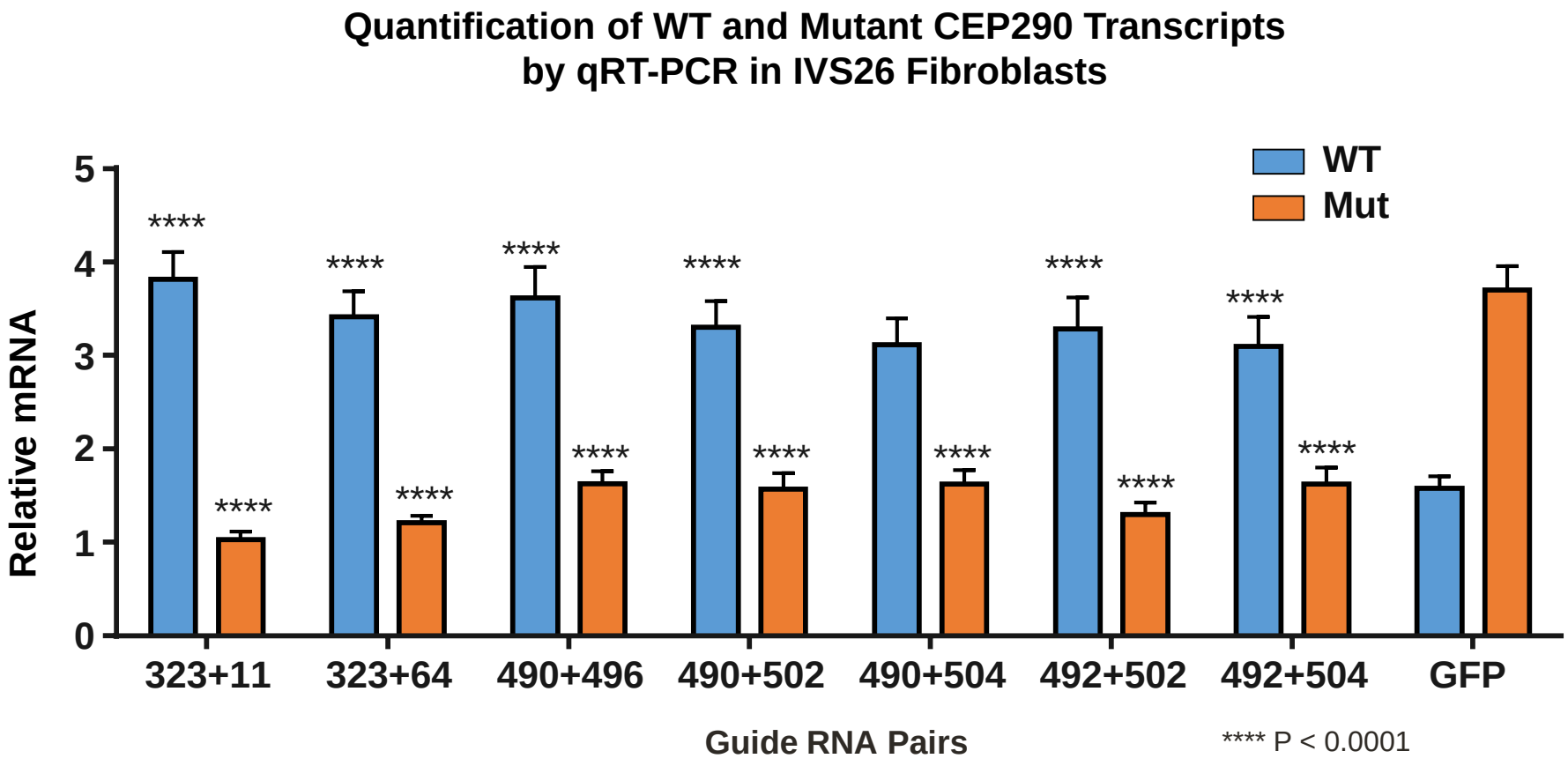
gRNA Pairs Induce Targeted Deletion

Targeted deletion quantification in primary patient fibroblasts by ddPCR



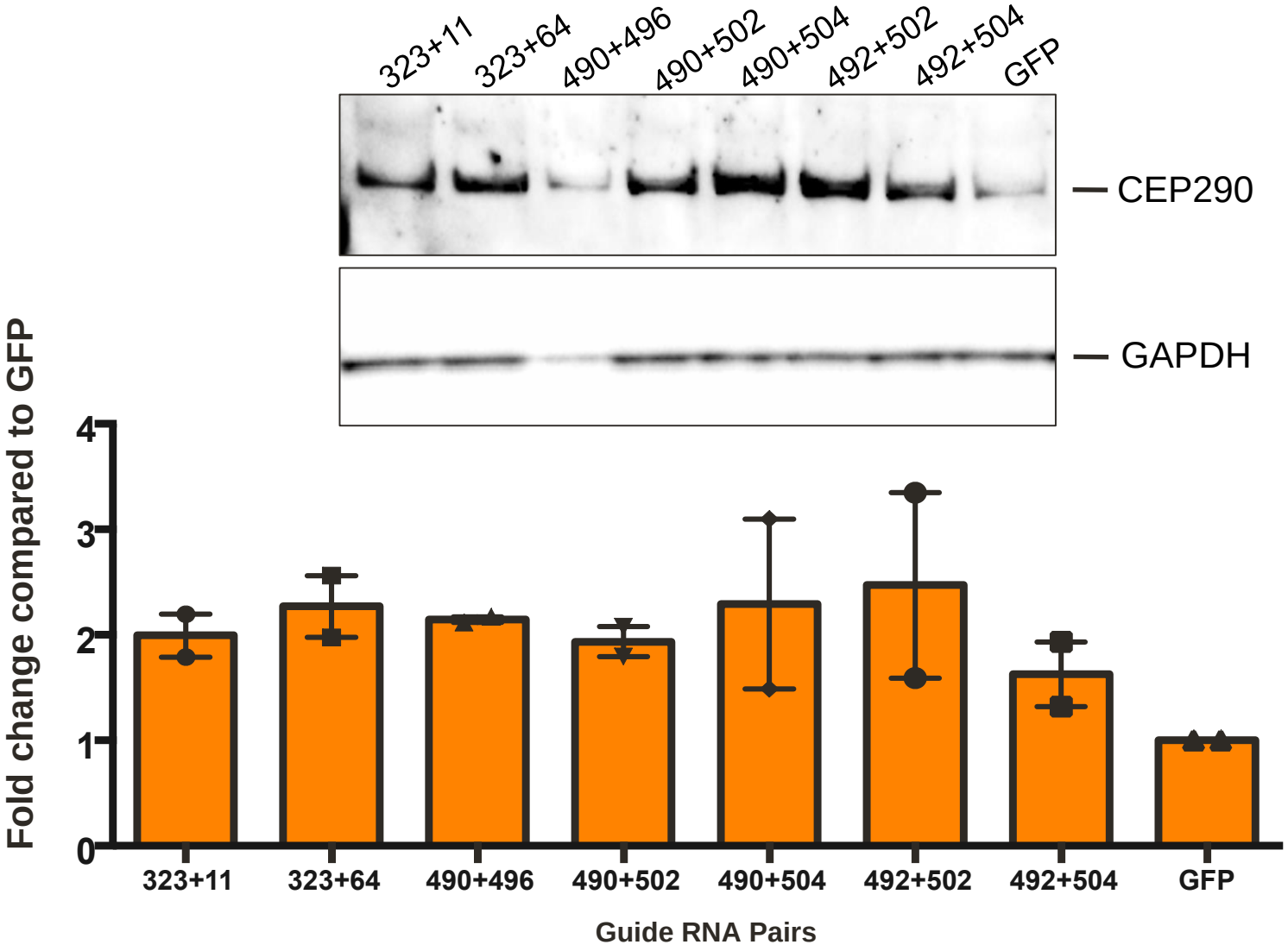
Targeted Deletion Corrects Splicing

Increased WT and decreased mut CEP290 mRNA measured by qRT-PCR



Targeted Deletion Corrects Splicing

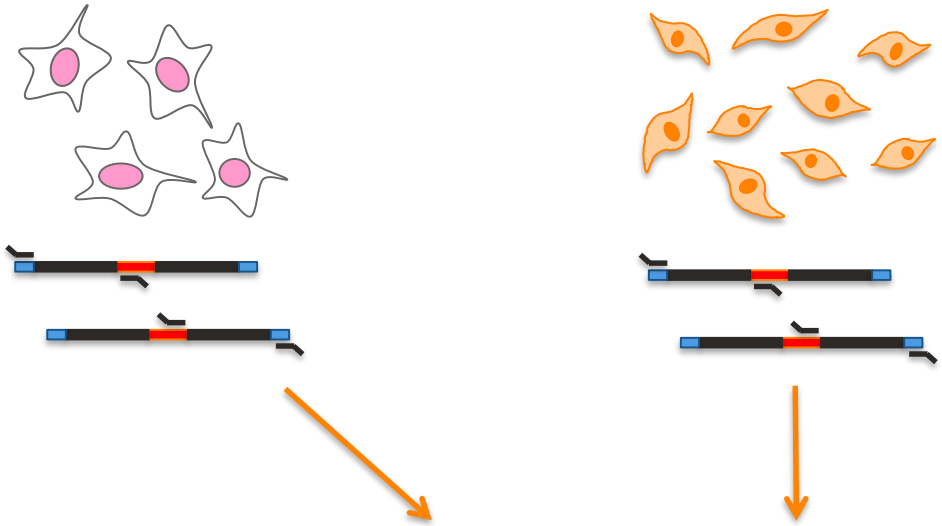
Increased WT CEP290 protein expression by Western



Specificity Analysis of Candidate gRNAs

Combining GUIDE-Seq and amplicon sequencing to assess specificity

GUIDE-Seq in multiple human cell lines



Computational identification of closely matched sites

TTGCACGTACGTAAACAGGATGG
TTGGACGTACGTAAACAGGATGG
TTGCACGAACGTAAGCAGGATGG
TTGCACGTACGTTAACAGGATGG
TAGCACGTACGTAAACAGGCTGG

Panel of sites analyzed by targeted NGS

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Specificity Analysis of Candidate gRNAs

Combining GUIDE-Seq and amplicon sequencing to assess specificity

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

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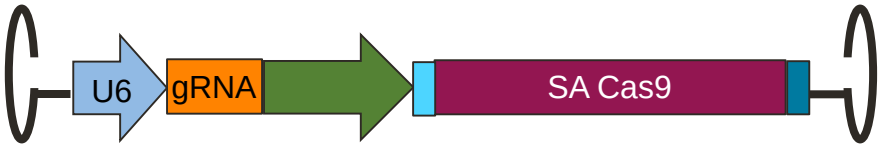
Is There a Benefit to a Single gRNA Approach?

Single gRNA frees up additional space in the AAV

Continued technology optimization



Additional elements that could be added to free space in AAV:

- Alternative promoters driving Cas9 expression
- Longer intron sequences downstream of promoter to increase Cas9 expression
- Additional gRNA for Cas9 inactivation

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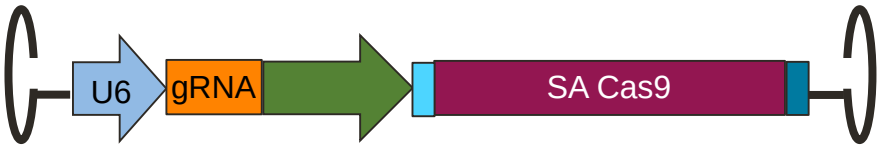
Cas9 Engineering to enable a single gRNA approach

Engineered Cas9s with altered PAM recognition broaden targeting range



LETTER

doi:10.1038/nature14592

Engineered CRISPR–Cas9 nucleases with altered PAM specificities

Benjamin P. Kleinstiver[1,2,3], Michelle S. Prew[1,2], Shengdar Q. Tsai[1,2,3], Ved V. Topkar[1,2], Nhu T. Nguyen[1,2], Zongli Zheng[1,3,4], Andrew P. W. Gonzales[5,6,7], Zhuyun Li[5], Randall T. Peterson[5,6,7], Jing-Ruey Joanna Yeh[5,8], Martin J. Aryee[1,3,9] & J. Keith Joung[1,2,3]

LETTERS

nature biotechnology

Broadening the targeting range of *Staphylococcus aureus* CRISPR-Cas9 by modifying PAM recognition

Benjamin P Kleinstiver[1–4], Michelle S Prew[1–3], Shengdar Q Tsai[1–4], Nhu T Nguyen[1–3], Ved V Topkar[1–3], Zongli Zheng[5] & J Keith Joung[1–4]

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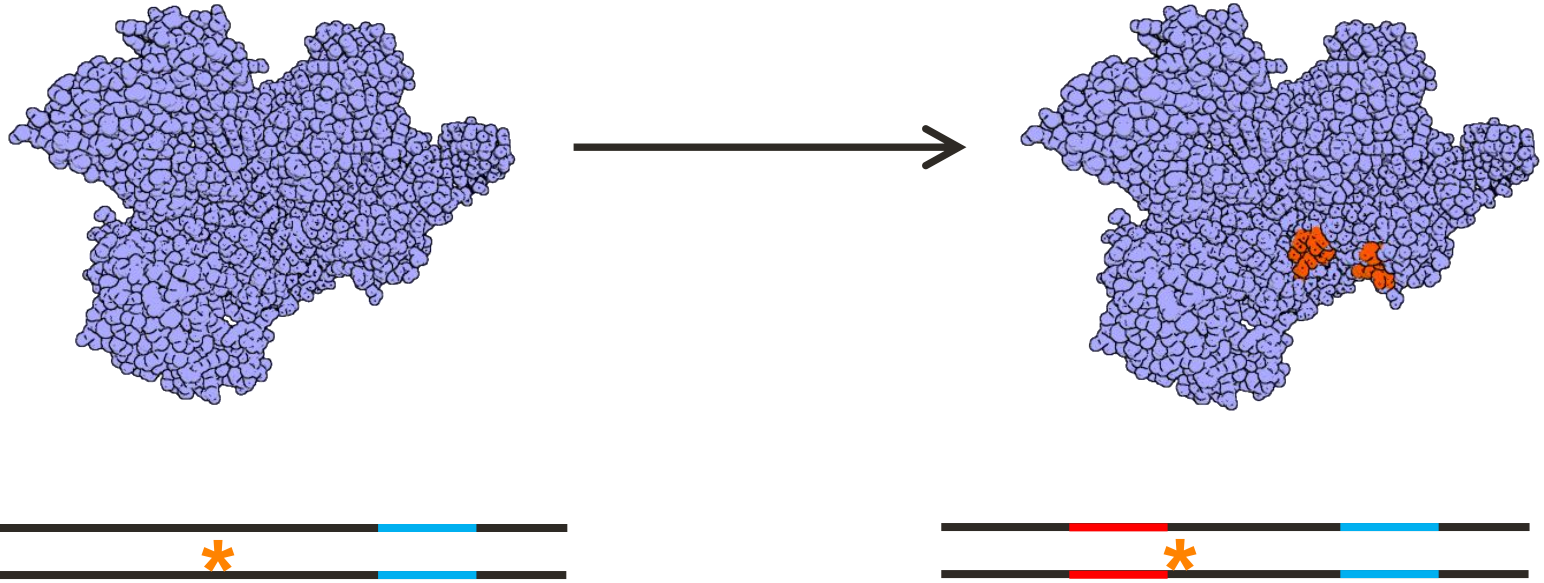
Engineering Alternate PAM Recognition

Cas9 Engineering could enable a single gRNA approach

WT Cas9

Engineered Cas9 Variant



No PAMs close enough to mutation

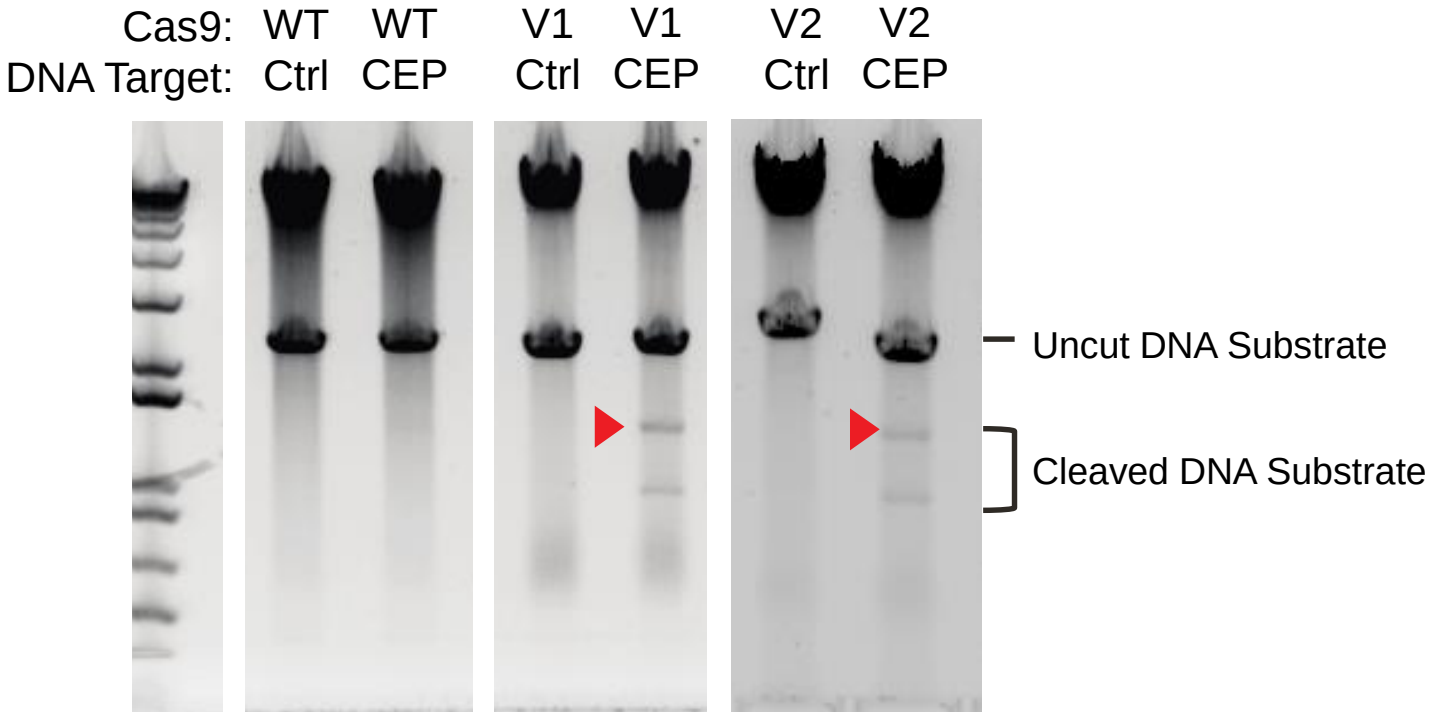
Novel PAM immediately adjacent to mutation

Project lead by Barrett Steinberg

Engineering Alternate PAM Recognition

Cas9 Engineering could enable a single gRNA approach

In vitro cleavage assay of novel Cas9 variants at IVS26 splice donor

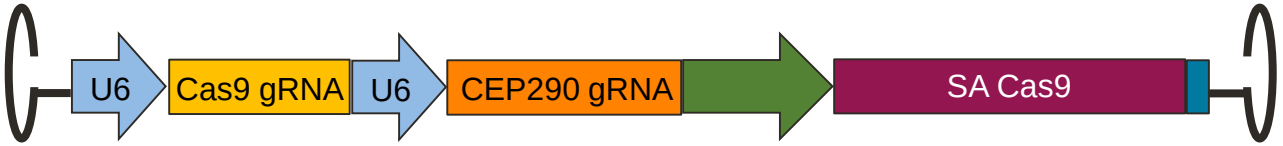


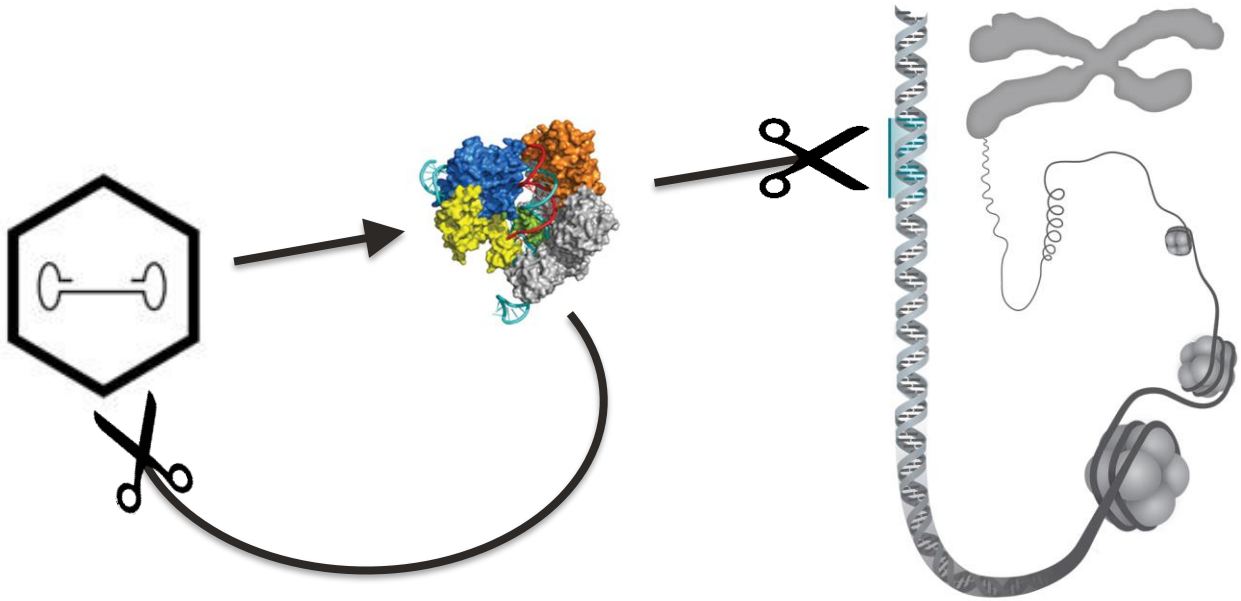
Project lead by Barrett Steinberg

What Can We Do with Extra Space in the AAV?

Single gRNA approach allows for inclusion of Cas9-inactivating gRNA

Long term transgene expression is ideal for standard gene therapy, but gene editing may benefit from a “hit and run” approach.





Project lead by Ari Friedland

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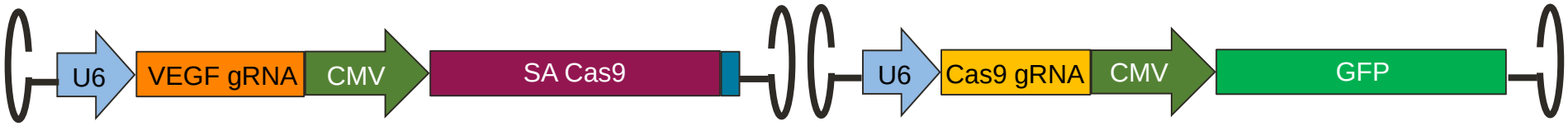
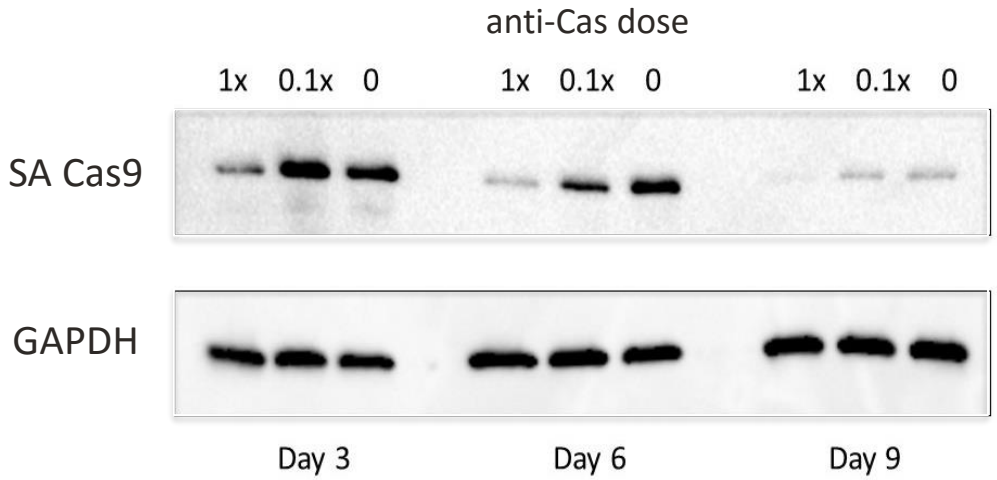
Self-Inactivating AAV-Cas9

Inclusion of Cas9-targeting gRNA decreases Cas9 expression

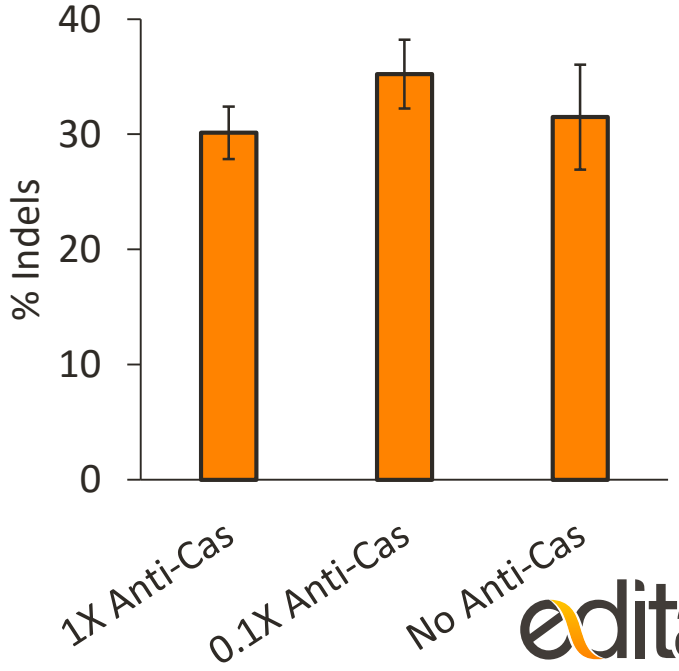
Initial experiment performed with 2 AAVs in HEK293 cells



Western blotting shows knockdown of Cas9 protein



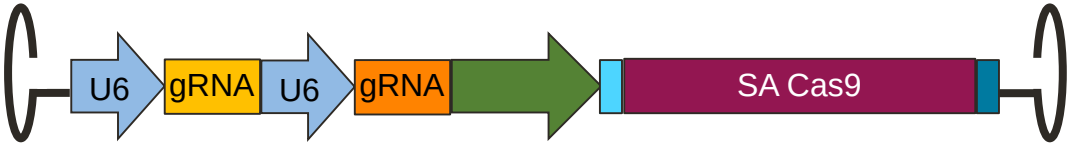
T7E1 shows no effect on on-target editing efficiency

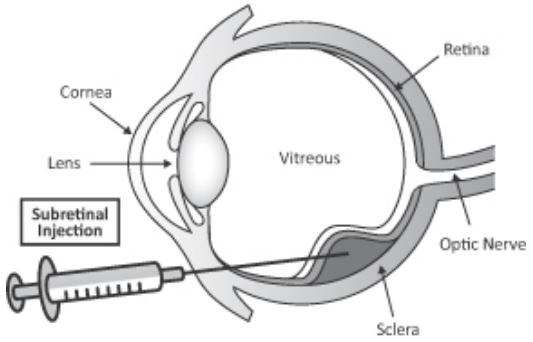


Project lead by Ari Friedland

Gene Editing Therapeutic for CEP290-LCA

Subretinal AAV delivers gene editing components for deletion of IVS26 mutation





On-going *In vivo* experiments to understand:

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

Acknowledgments

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Protein Engineering:
Barrett Steinberg
Derek Cerchione

Sequencing & Bioinformatics:
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Hari Jayaram
Eugenio Marco
Luis Barrera
Georgia Giannoukos
Dawn Ciulla
Tongyao Wang

And the entire Editas Team!

ddPCR Calibration