Opportunities and Challenges in Development of CRISPR Medicines

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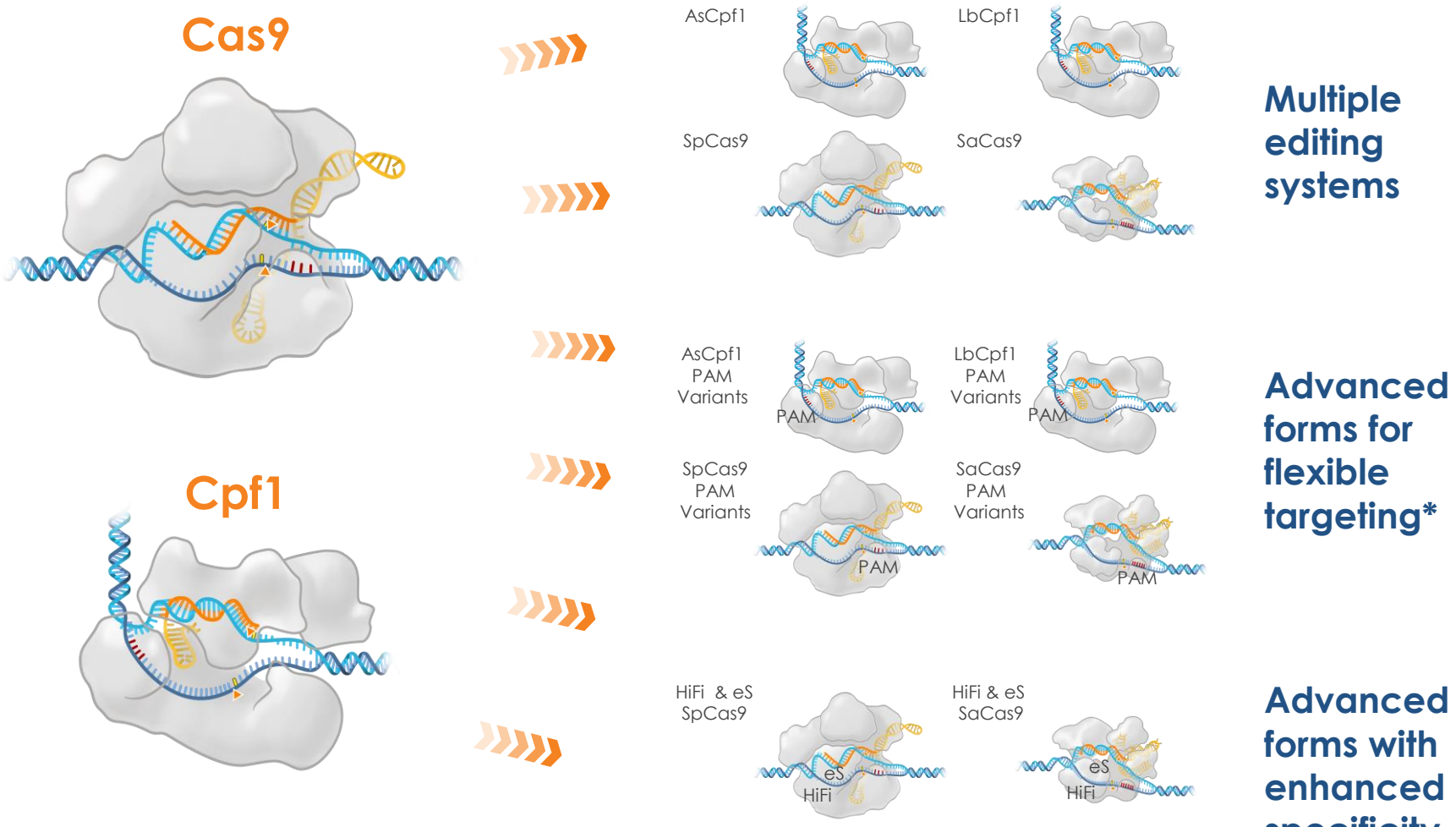
Genome Engineering 5.0
May 9, 2017

Unparalleled Platform for CRISPR Medicines

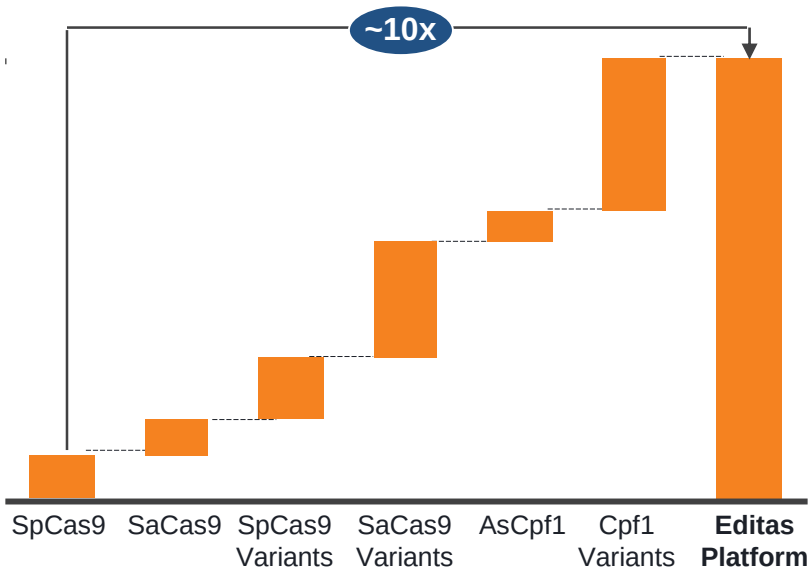


SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9; AsCpf1: *Acidaminococcus* species Cpf1; LbCpf1: *Lachnospiraceae* bacterium Cpf1; PAM: Protospacer Adjacent Motif; HiFi: High Fidelity; eS: enhanced Specificity

*Poster: Derek Cerchione et al, Directed evolution of targeted /Cas9 cleavage to the LCA10 splice donor mutation

Cas9 and Cpf1 Variants Expand Range of Targets

Platform investment to enable better medicines for more diseases



Variant	PAM	Expected Genome Frequency
SpCas9	NGG	1 per 16 bp
SaCas9	NNGRRT	1 per 64 bp
SaCas9 KKH	NNNRRT	1 per 16 bp
AsCpf1 WT	TTTV	1 per 85.3 bp
AsCpf1 RR	NYCV	1 per 10.7 bp
AsCpf1 RVR	TATV	1 per 85.3 bp

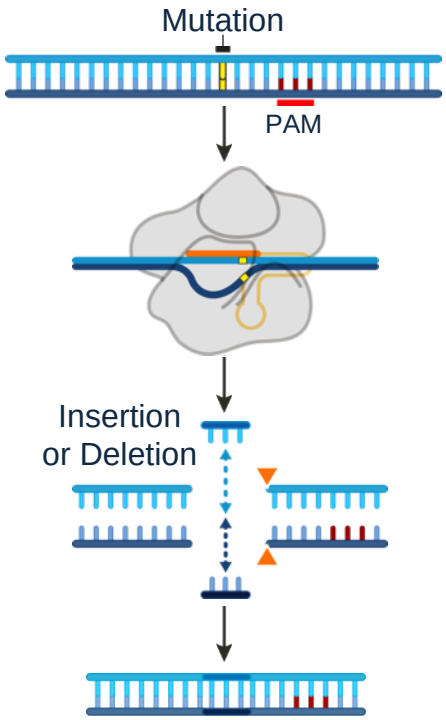
- Engineered Cas9 and Cpf1 variants substantially expand the range and location of targetable genomic sites

Poster: Ramya Viswanathan et al, Comparison of RNP-mediated editing by Type V Cpf1 variants

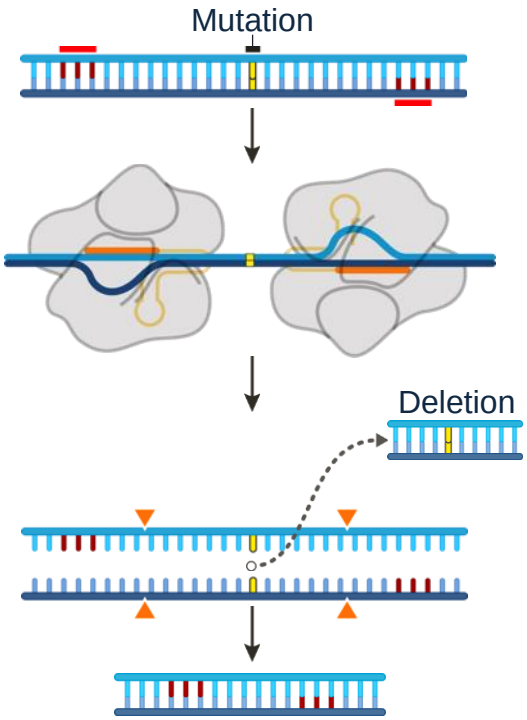
CRISPR Flexibility Addresses Diverse Mutations

Edits are completed by the cell's natural DNA repair mechanisms

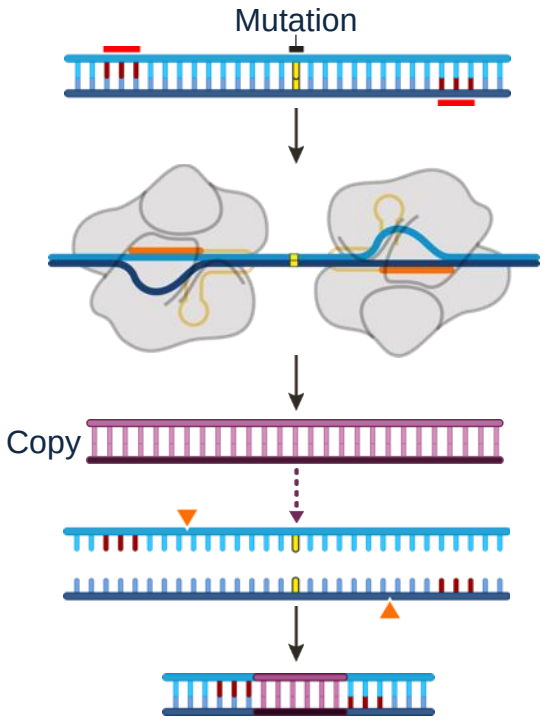
Cut and **Revise**



Cut and **Remove**



Cut and **Replace**

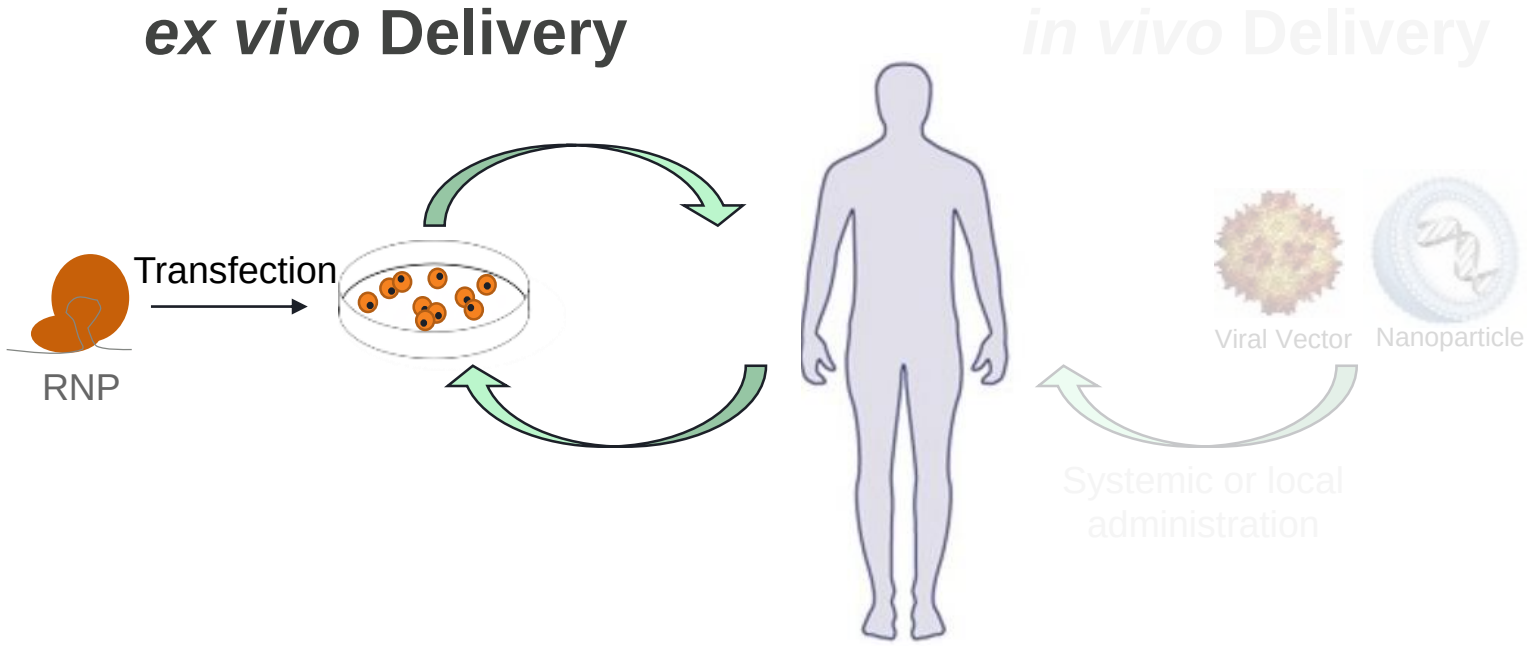


Typically disrupts a gene or eliminates a disease-causing mutation

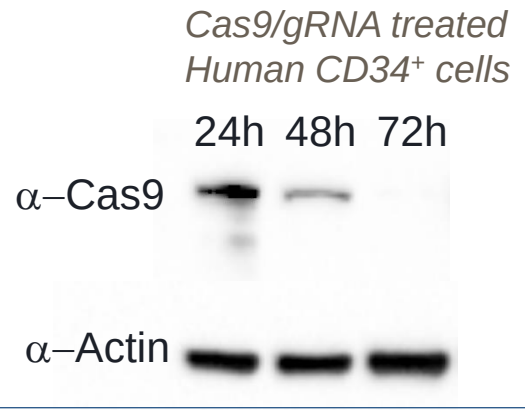
Aims to correct defective DNA sequences

PAM: Protospacer Adjacent Motif; LCA10: Leber Congenital Amaurosis 10

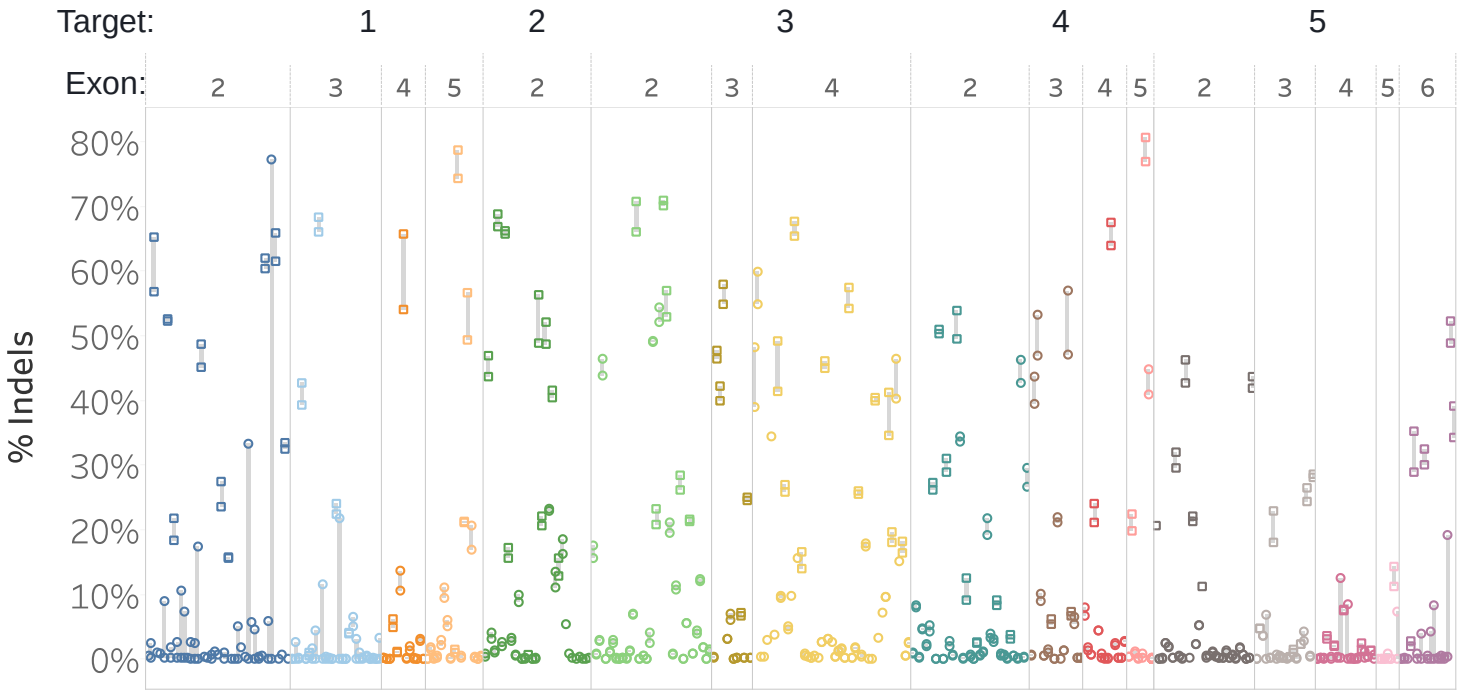
Target-specific Product Configurations



- **Electroporation of Cas9/gRNA ribonucleoprotein (RNP)**
  - Maximize gene editing efficiency
  - Minimize exposure to gene editing machinery



Scale Up Powers Lead Finding & Optimization

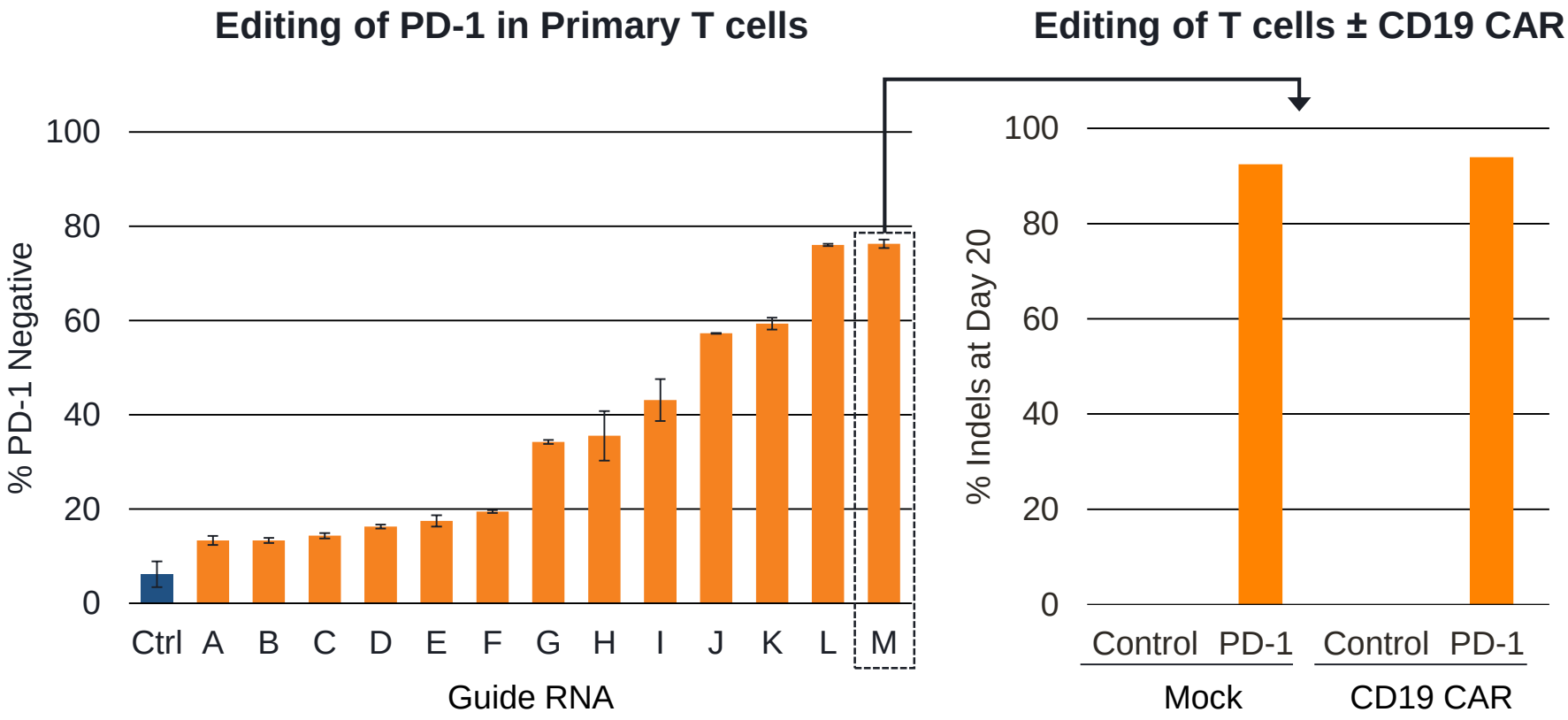


Primary screening of 5 targets with 2 enzymes in primary human T cells demonstrating high activity and reproducibility

- Fully tracked and automated process
- RNP and target agnostic (any variant or enzyme with a sequencing readout)
- Flexible format for single point screening, dose response or any combination

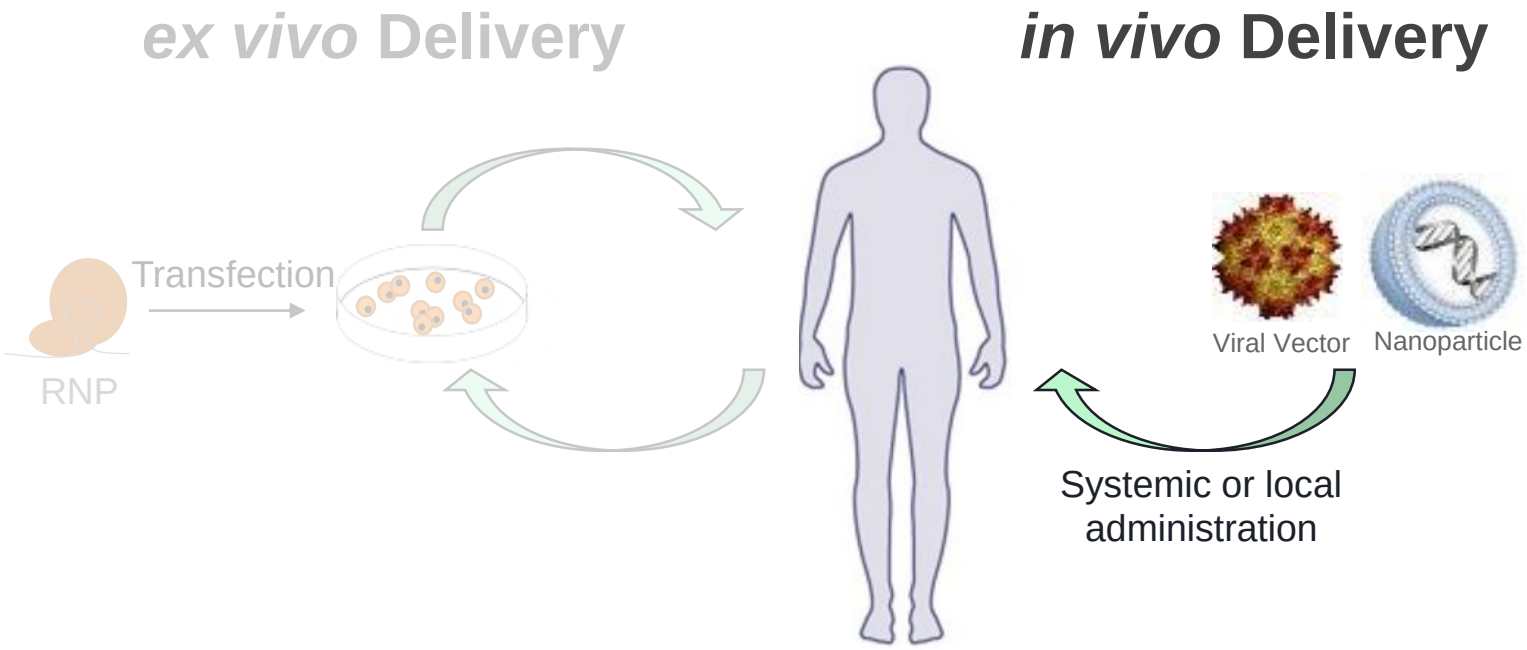
Poster: Eric Tillotson, Guillaume Harmangeet et al, Assessing Cas9-gRNA ribonucleoprotein complex formation for development of ex-vivo therapeutics

High Efficiency Editing in Primary Human T Cells



- Significant advances across multiple targets for improving T cell therapy
- Achieved over 90% PD-1 knockout in T cells also carrying CAR

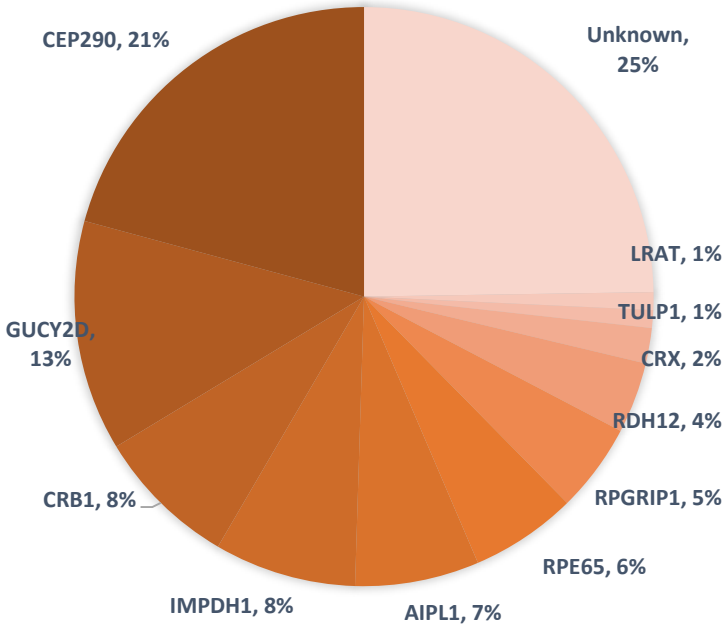
Target-specific Product Configurations



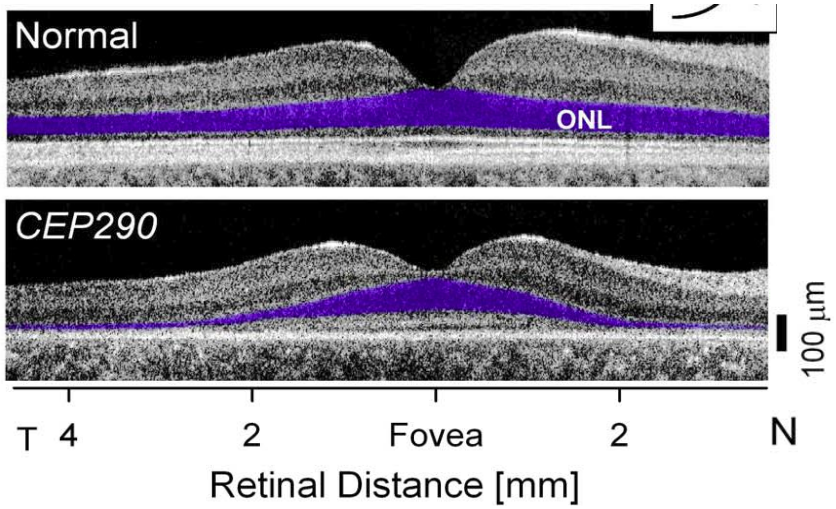
- AAV-mediated tissue-specific expression of Cas9 and gRNA
  - Leverage existing human experiences with AAV vectors via systemic or local delivery
- Nanoparticles delivering mRNA/gRNA or RNP
  - Enable repeated dosing
  - Transient exposure to Cas9 and gRNAs

Leber Congenital Amaurosis 10

- LCA is a group of heterogeneous and inherited retinal dystrophies, characterized by severe loss of vision in the first years of life
- LCA10 is caused by autosomal recessive mutations in the CEP290 gene at 12q21.32



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



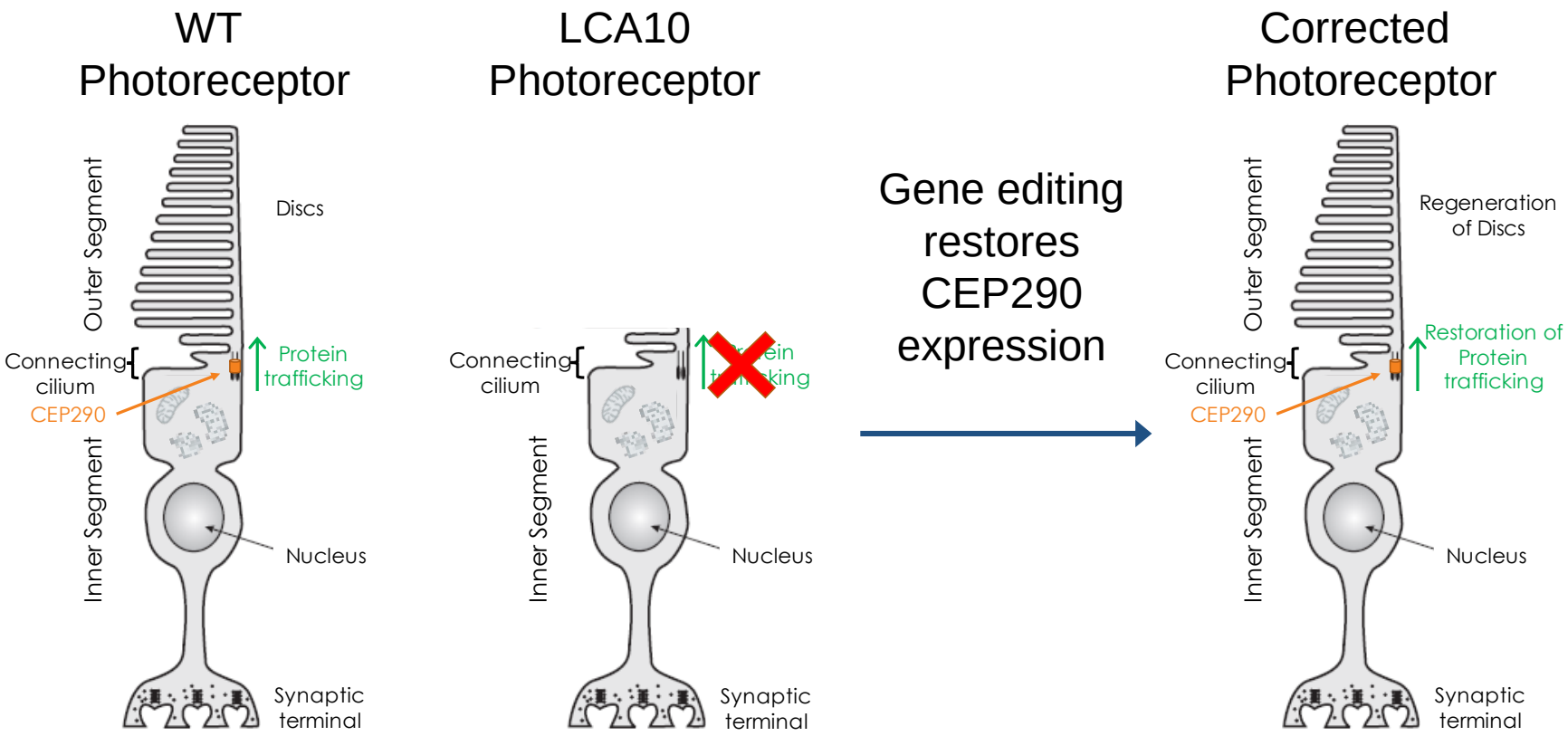
Boye et al., PLOS ONE 2014

Target: surviving foveal cones

- Despite severe loss of visual acuity, foveal cones remain and foveal thickness by OCT is similar to normal
- Normal intracranial visual pathways
- It is estimated that visual acuity can be achieved with ~10% of functioning photoreceptors

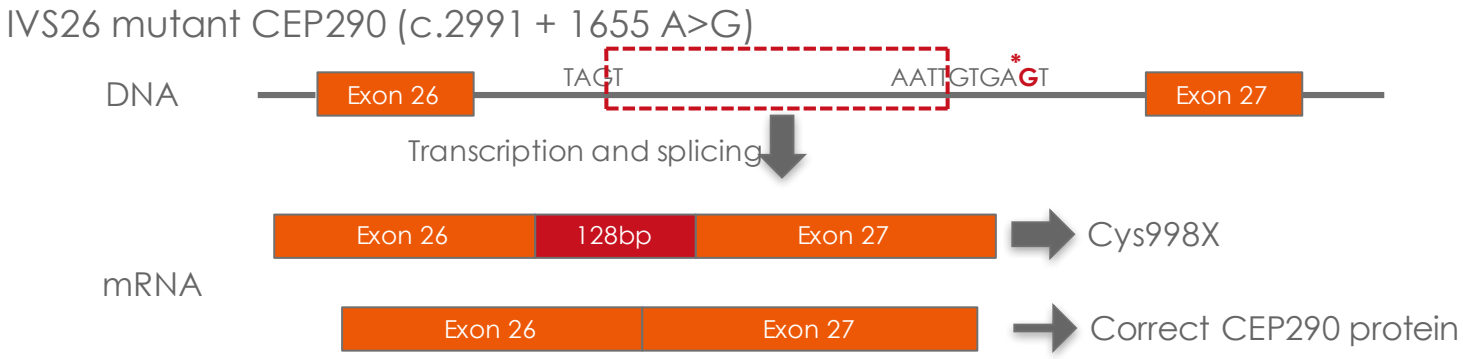
Leber Congenital Amaurosis 10

- ~30% of LCA10 patients have a common mutation in intron 26 of CEP290 gene (cDNA = 7.5kb)
- CEP290 localizes to the connecting cilium of photoreceptors and plays a role in ciliary structure and function

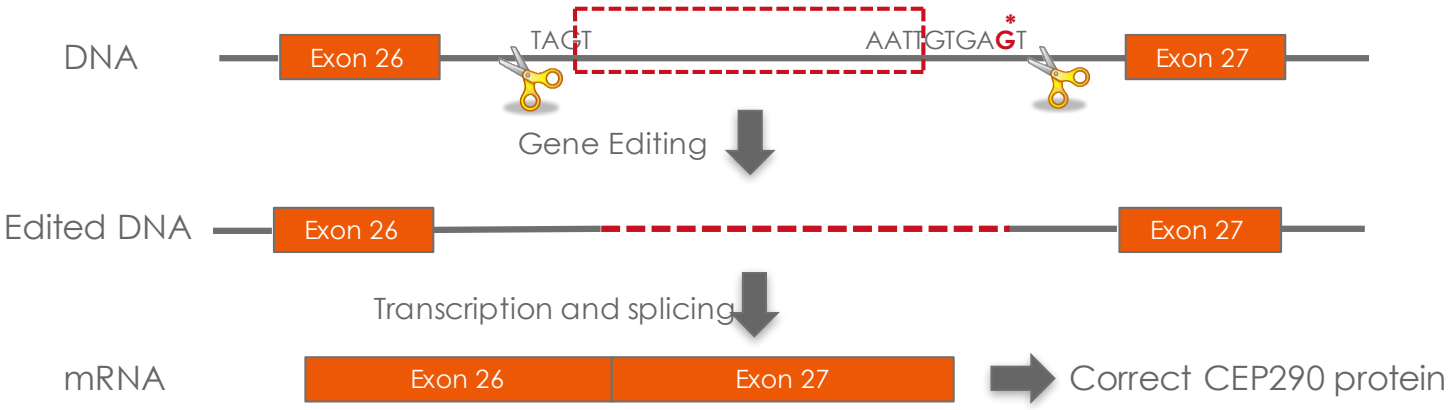


CEP290 Gene Editing to Treat LCA10

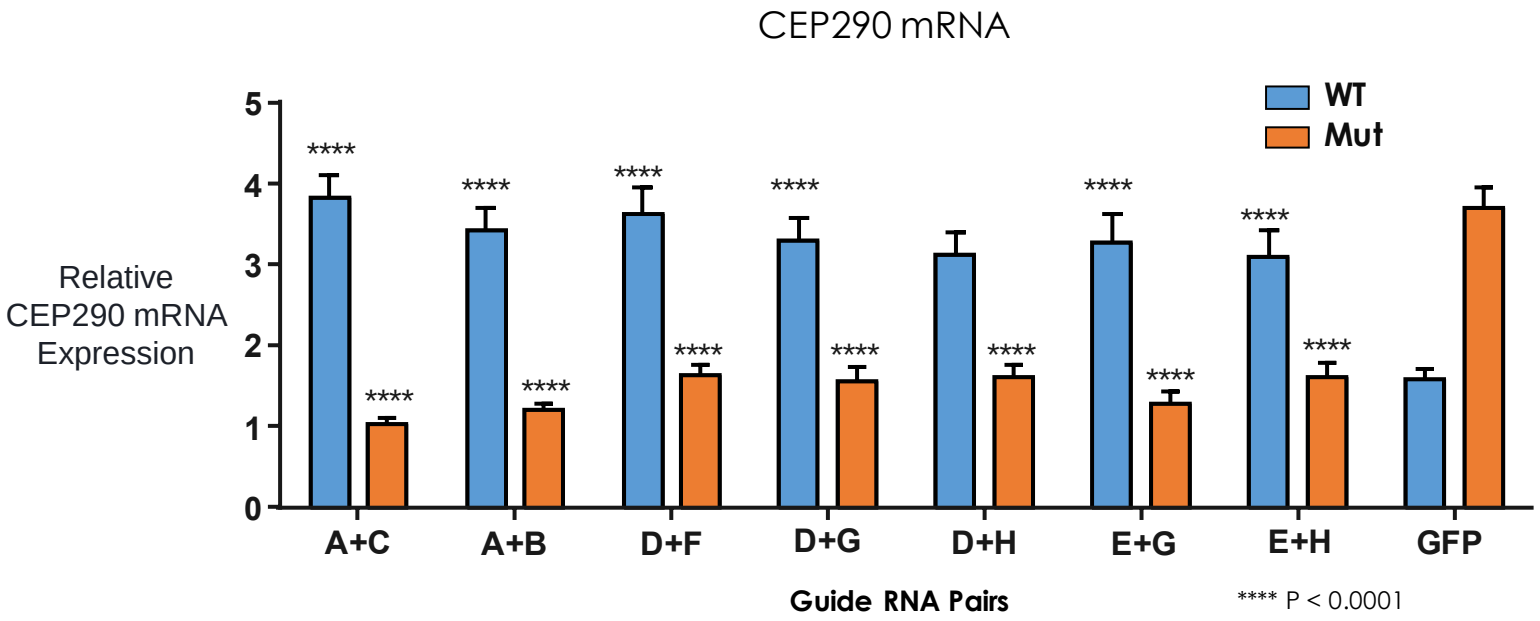
- Most common mutation is the IVS26 c.2991+1655A>G splice mutation

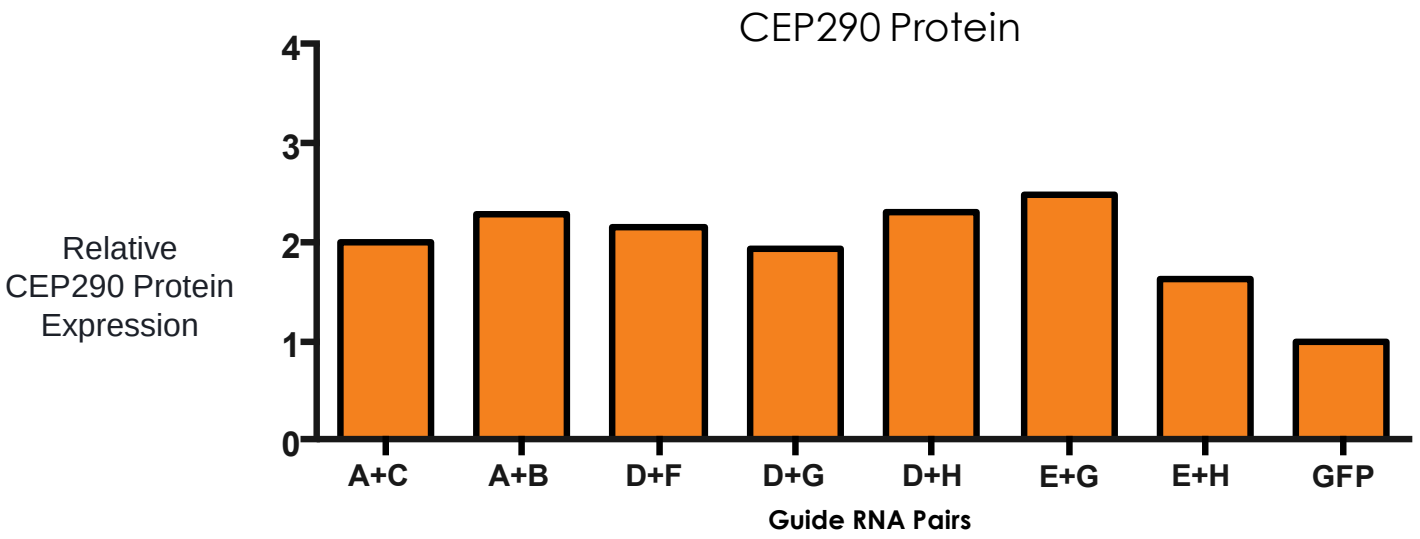


- Recessive disease – correction of 1 allele should restore cell to full function



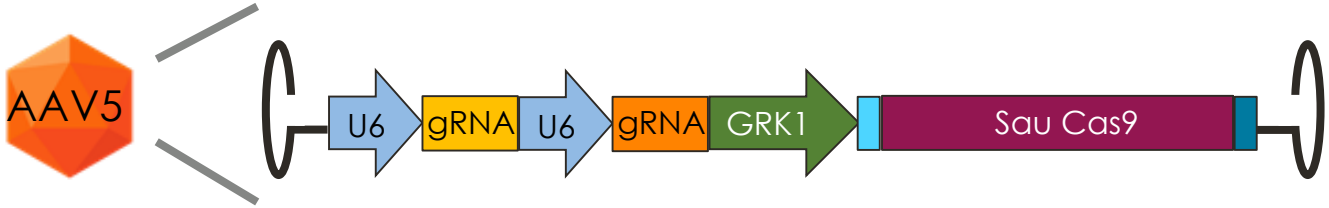
Editing Restores CEP290 Expression in Patient Fibroblasts

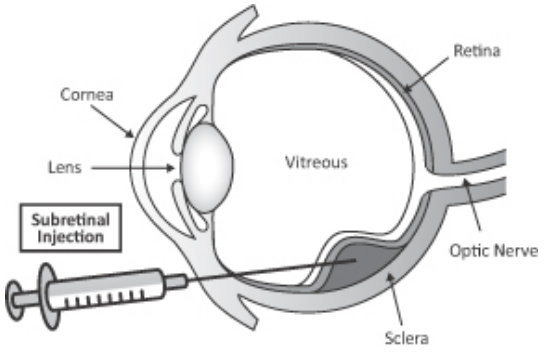




LCA10 Product Candidate Advancing Toward Clinic

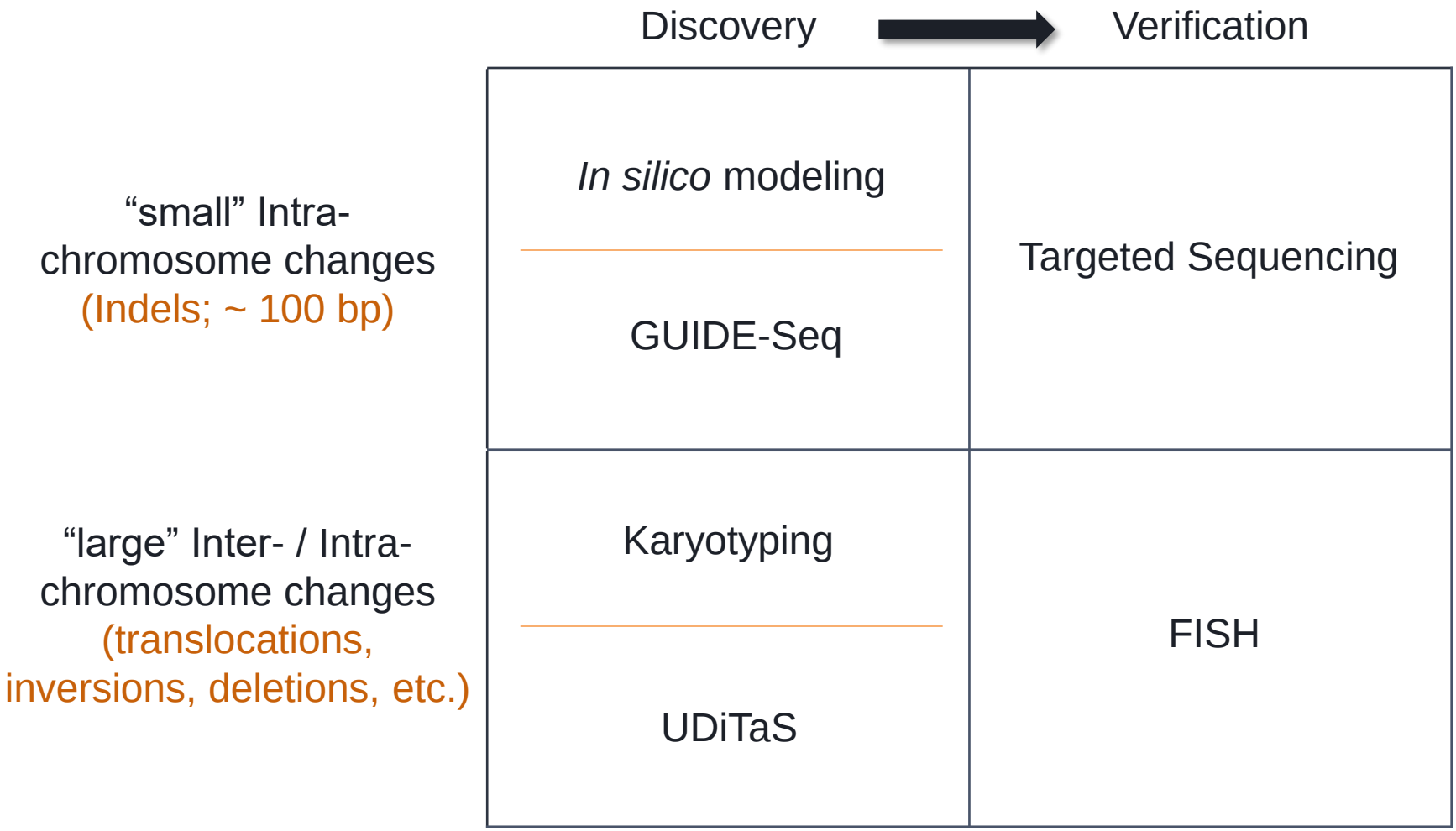
Program Goal		Status
Efficient editing *in vitro*	☑	**Achieved** – cells from LCA10 patients
High selectivity *in vitro*	☑	**No off-target editing detected**, using multiple methods
Efficient delivery	☑	All components delivered in a **single AAV**
Optimize product	☑	Promoter **specific** for photoreceptor cells
In vivo proof-of-editing	☑	**Achieved – retina of non-human primates***





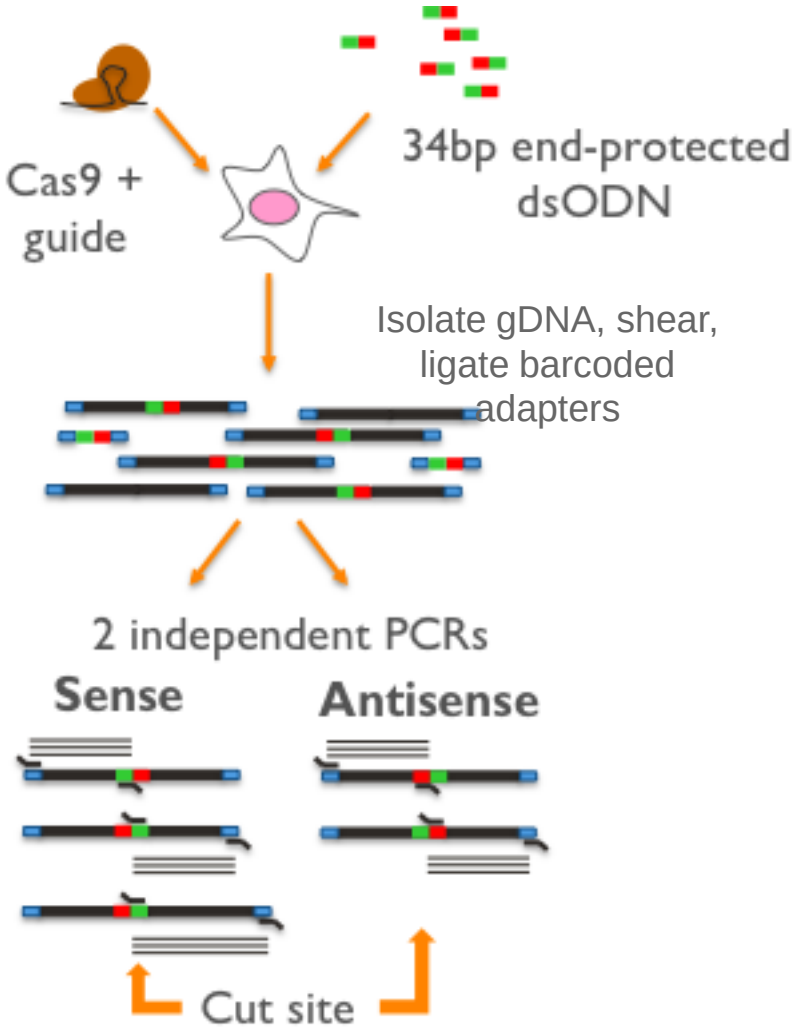
* Maeder, M et al, ASGCT, May 13, 2017

Approaches for Off-target Assessment



Unbiased Analysis for Off-target Discovery

GUIDE-Seq Allows Identification of Highly Selective Candidate gRNAs



gRNA	Cells Used for GUIDE-Seq				# off-target sites identified
	ARPE19	Fibroblasts	SH-SY5Y	U2OS	
A	✓	✓	✓	✓	4
B	✓	✓	✓	✓	0
C	✓	✓	✓	✓	0
D	✓		✓	✓	0
E	✓		✓	✓	1
F	✓		✓	✓	0
G	✓		✓	✓	8
H	✓		✓	✓	0
VEGFA site3, Spy**				✓	59

S. Q. Tsai *et al.*,*Nat Biotechnol*, 2015

Target-Seq Verify the Selectivity of Lead gRNA

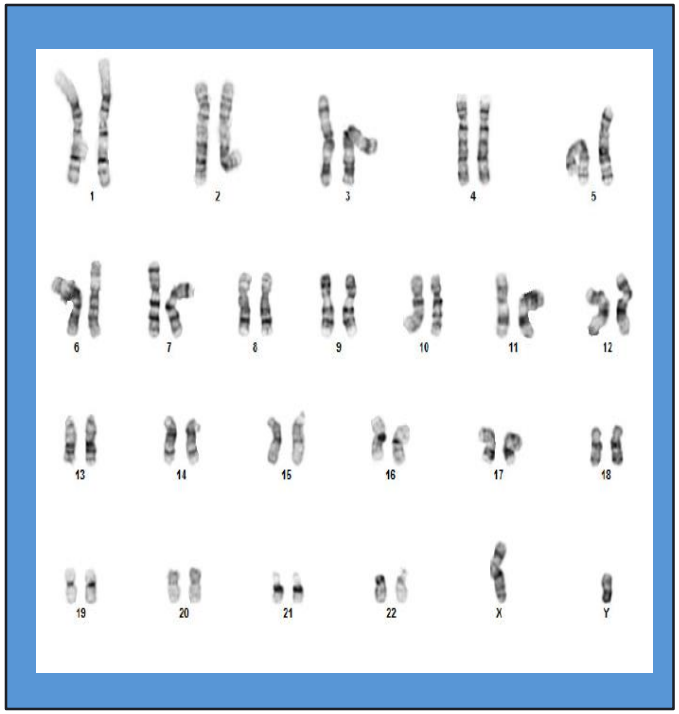
- Reference genome used for in silico identification of sites was Hg38 and variants described in the 1,000 Genomes Project with a minor allele frequency >1% were considered
- Prioritized sites in coding sequence or in non-coding regions of tumor suppressors, oncogenes or IRD disease-related genes (GEDi list*)
- Approximate sensitivity of detection = 0.1%

Guide	Cells	# of sites sequenced	# off- target sites identified
A	U2OS	6	1
	ARPE19	3	1
B	U2OS	81	0
	ARPE19	61	0
C	U2OS	90	0
	ARPE19	57	0
D	U2OS	74	0
	ARPE19	48	0
E	U2OS	95	1
	ARPE19	56	0
F	U2OS	72	0
	ARPE19	58	0
G	U2OS	12	6
	ARPE19	12	0
H	U2OS	71	0
	ARPE19	53	0

*courtesy of Eric Pierce, MEEI

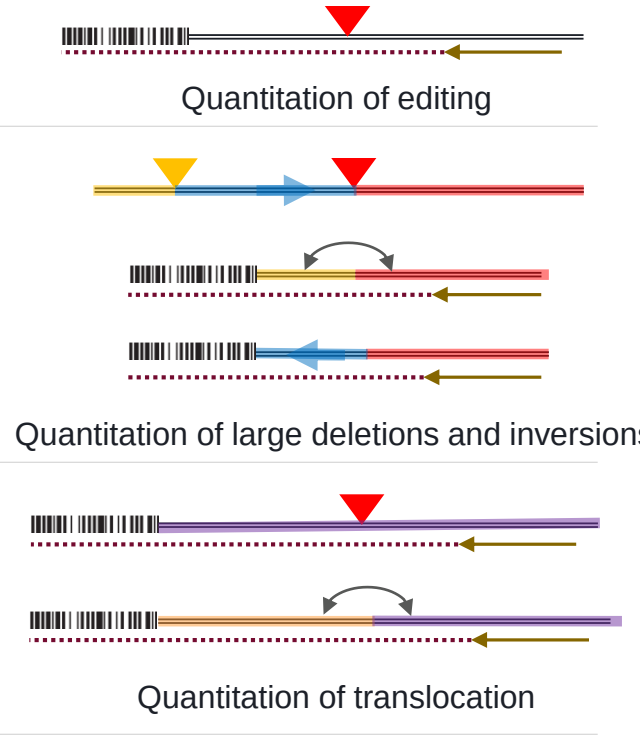
Approaches for Genotoxicity Assessment

Karyotyping



- Unbiased global assessment of genetic integrity (structural and/or numerical chromosome alterations)
- Protocol follows American College of Medical Genetics guidelines
- low resolution ~5-7 Mb,
- low sensitivity >5%

UDiTaS (Uni-Directional Targeted Sequencing)*



- Targeted assessment of on-target cleavage mediated structural alterations (large deletions, insertions or translocations)
- high resolution to single base,
- high sensitivity 0.1%

*Giannoukos, G et al, ASGCT, May 12, 2017

Safety Considerations for Locally Delivered AAV-CRISPR/Cas9 Product

In vivo Assessments Limited by gRNA Specificity for Human Genome

Aim	Approach
Assess risk of genotoxicity	Small and large chromosomal alterations GUIDE-Seq, Karyotype & UDiTaS Human surrogate cell types
Evaluate tumorigenicity potential	In vitro transformation assay in diploid fibroblasts
In vivo assessments • Kinetics of Cas9 expression and CEP290 gene editing • Target organ and systemic toxicity • Vector biodistribution • Immune response to AAV vector and/or constitutively expressed Cas9 • Effective and safe dose range	Pharmacology/toxicology studies using • Human clinical candidate AAV vector • Surrogate AAV vector encoding monkey gRNAs • Human CEP290 IVS26 knockin mouse • Non-human primate

Advancing CRISPR Medicines Towards Clinics

PRECLINICAL DEVELOPMENT	▪ Science-based and product-specific benefit-risk assessment
PHARMACOLOGY	▪ Unique target feature of Cas9/gRNA, and many unknowns in its pharmacological mechanism of action ▪ Understanding the pharmacology of CRISPR product is essential to design effective safety studies
OFF-TARGET EFFECTS	▪ Next gen sequencing has significantly improved the sensitivity in identifying potential off-targets ▪ Innovation is much needed in developing sensitive functional assays to assess genotoxicity and tumorigenicity
CLINICAL MONITORING	▪ Limited in vivo preclinical testing of human sequence-specific CRISPR product ▪ Developing clinical assays and safety biomarkers to enable vigilant clinical monitoring

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Developing
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Building the Leading
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