CRISPR Genome Editing: Considerations for Therapeutic Applications

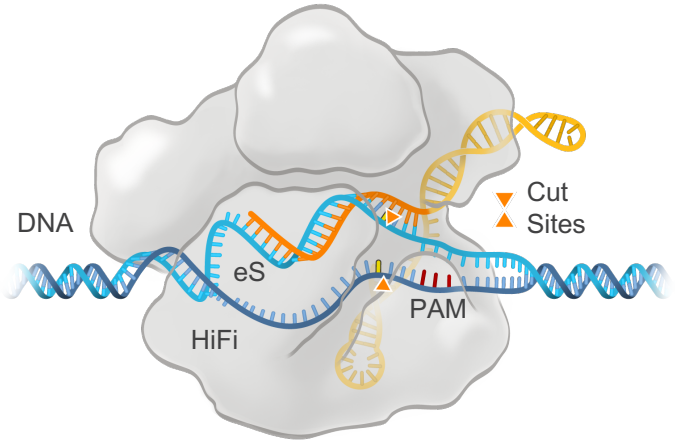
November 9, 2017

Cecilia Fernandez

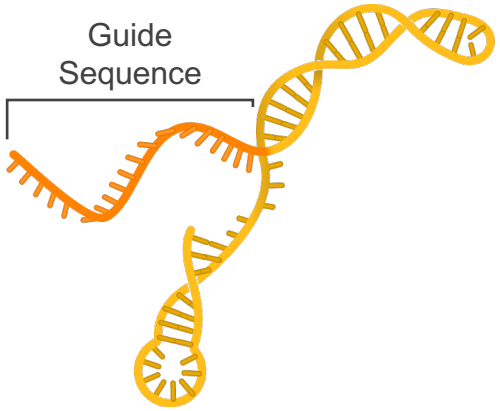
Medicines that Aim to Repair Any Broken Gene

Potential to create the next major category of transformative medicines

CRISPR Provides Versatile Genome Editing Systems



Nuclease

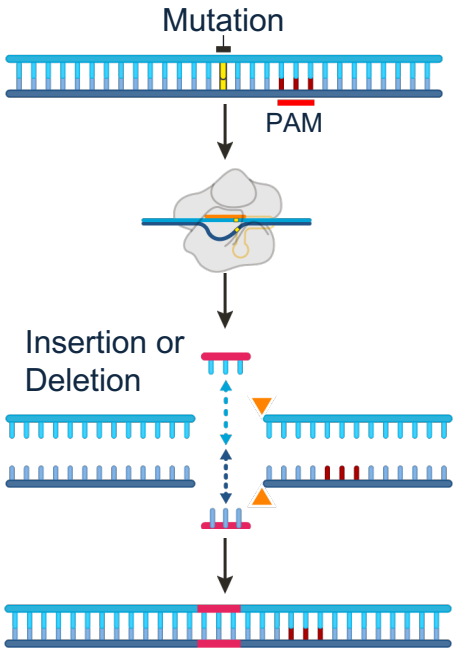


Guide RNA

- Complex of nuclease and guide RNA precisely locates and cuts genomic sites
- Ability to target several sites simultaneously using multiple guide RNAs
- Nuclease can be engineered to reach more sites and to modulate cutting

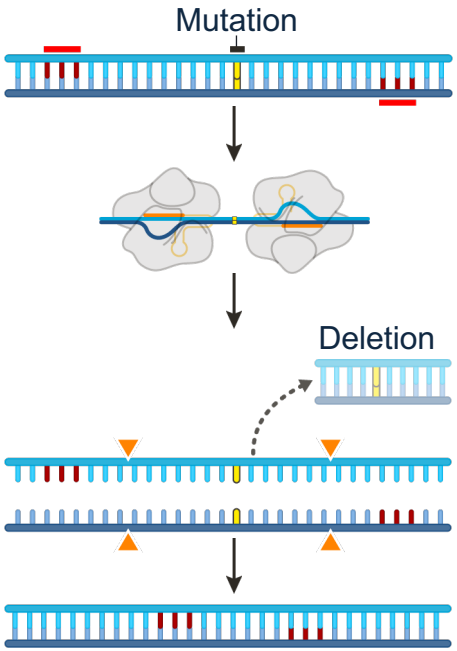
CRISPR Flexibility Addresses Diverse Mutations

Cut and **Disrupt**



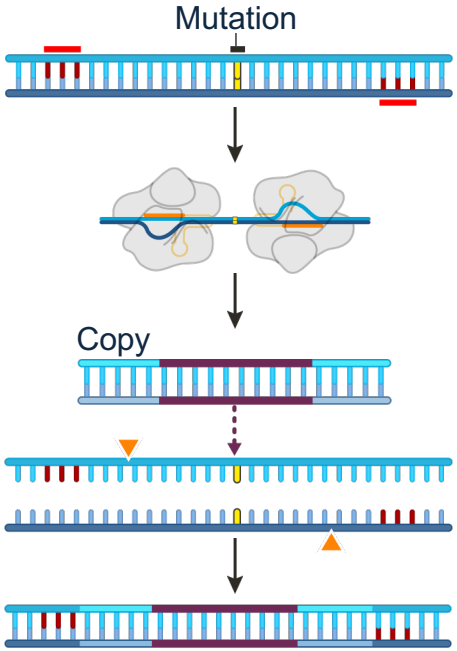
e.g., Engineered T cells

Cut and **Remove**



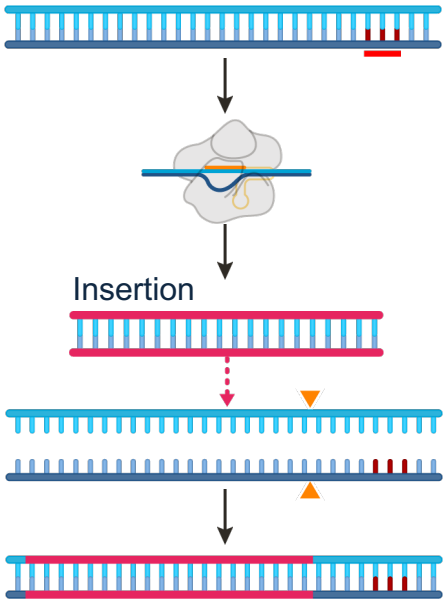
e.g., LCA10

Cut and **Replace**



e.g., Hemoglobin Beta

Cut and **Insert**



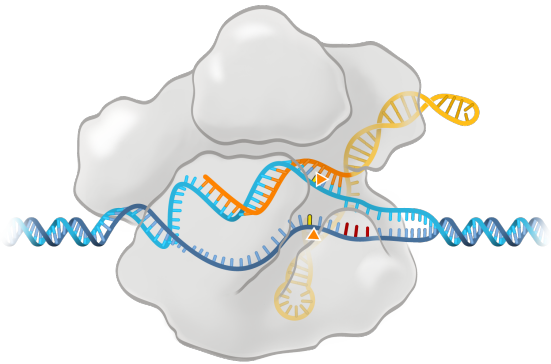
e.g., Safe harbor

Non-homologous end joining typically **disrupts a gene or eliminates a disease-causing mutation**

Homology-directed repair and targeted insertion aim to **promote expression of correct DNA sequences**

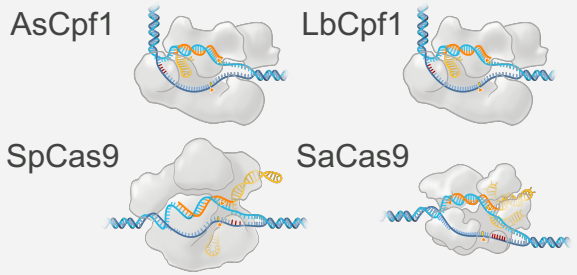
Broad Toolkit of CRISPR Nucleases

Cas9

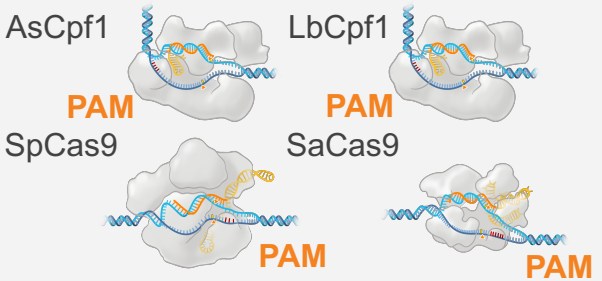


Cpf1

MULTIPLE EDITING SYSTEMS



ADVANCED FORMS FOR FLEXIBLE TARGETING

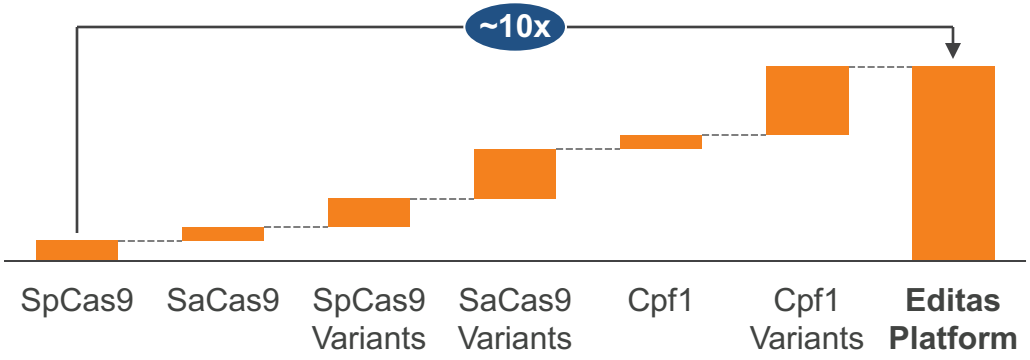


ADVANCED FORMS WITH INCREASED SPECIFICITY

AsCpf1: *Acidaminococcus* species Cpf1; LbCpf1: *Lachnospiraceae* bacterium Cpf1; PAM: Protospacer Adjacent Motif; HiFi: High Fidelity; eS: enhanced Specificity

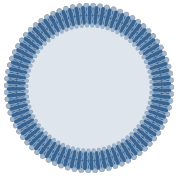
Platform Enables Broad Product Opportunities

Broad Range of Sites



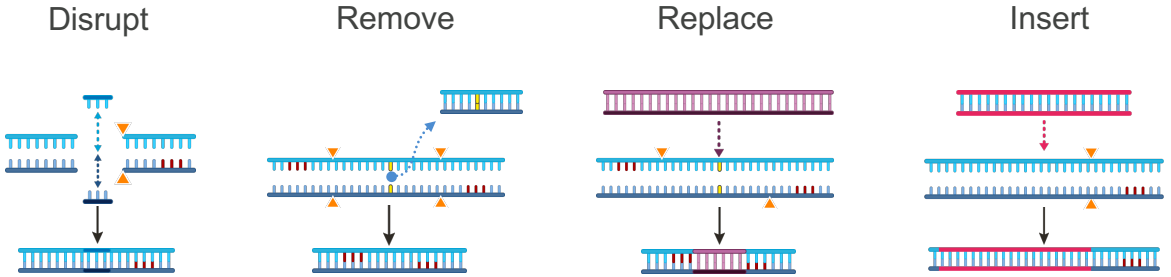
Wide Delivery Options


Viral Vector


Lipid Nanoparticle


Electroporation

Diverse Spectrum of Edits



SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9

Lead Finding for Nuclease/gRNA and Specificity

Identify, Measure, Minimize

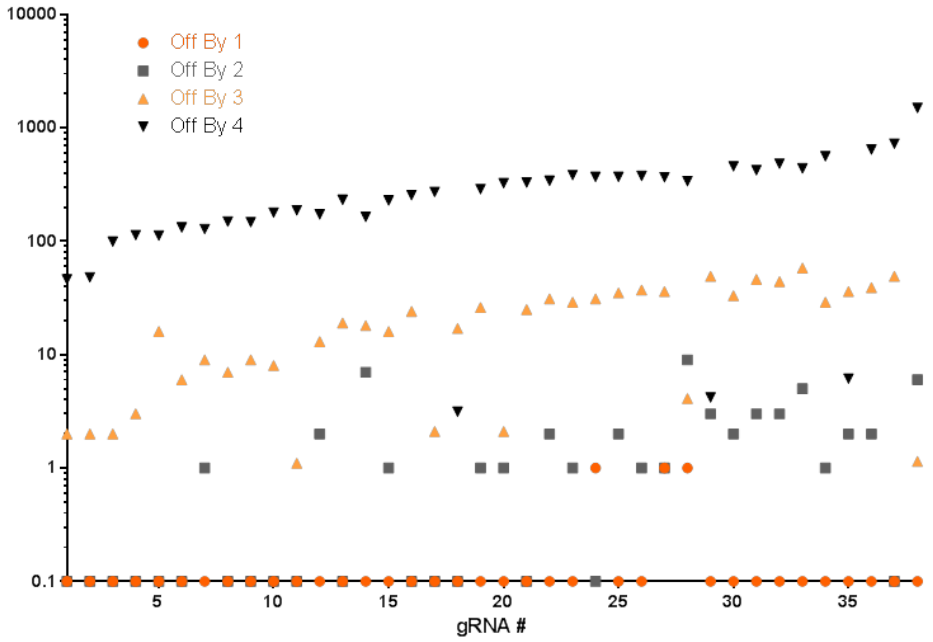
Proprietary *in silico* Prediction of Cutting Sites

Testing of On-Target Cutting (Cas9, Cpf1 WT, nickase)

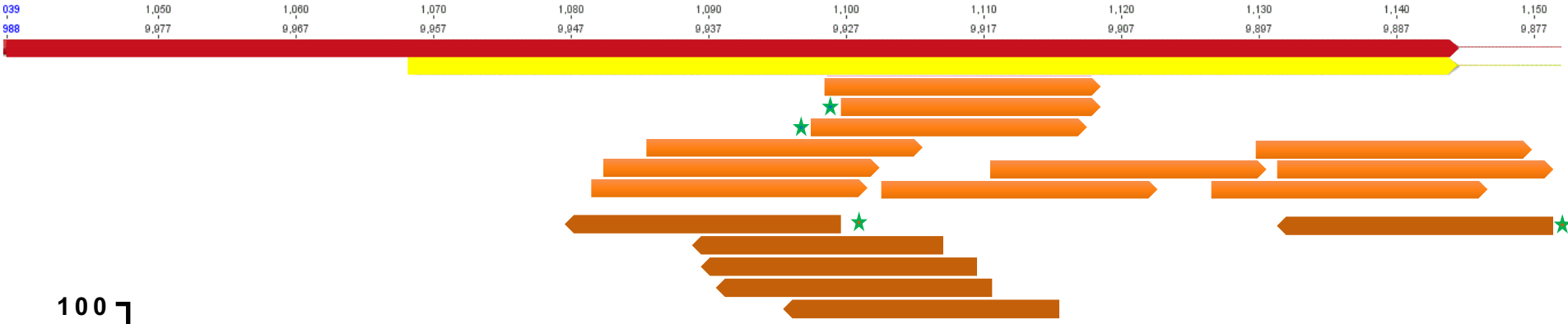
Unbiased Detection of Off-Target Cuts and Genomic Alterations (e.g., GUIDE-Seq, UDiTaS™)

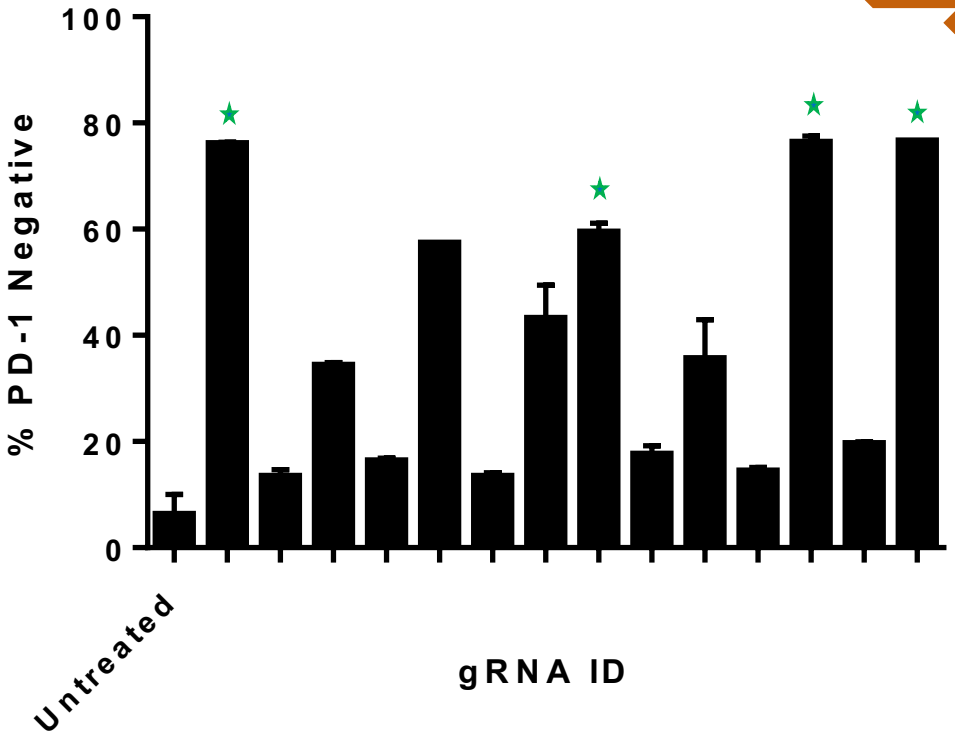
Targeted Panels for Detection of Sites from Biased & Unbiased Screens

In silico Selection



Identification of Robust gRNAs

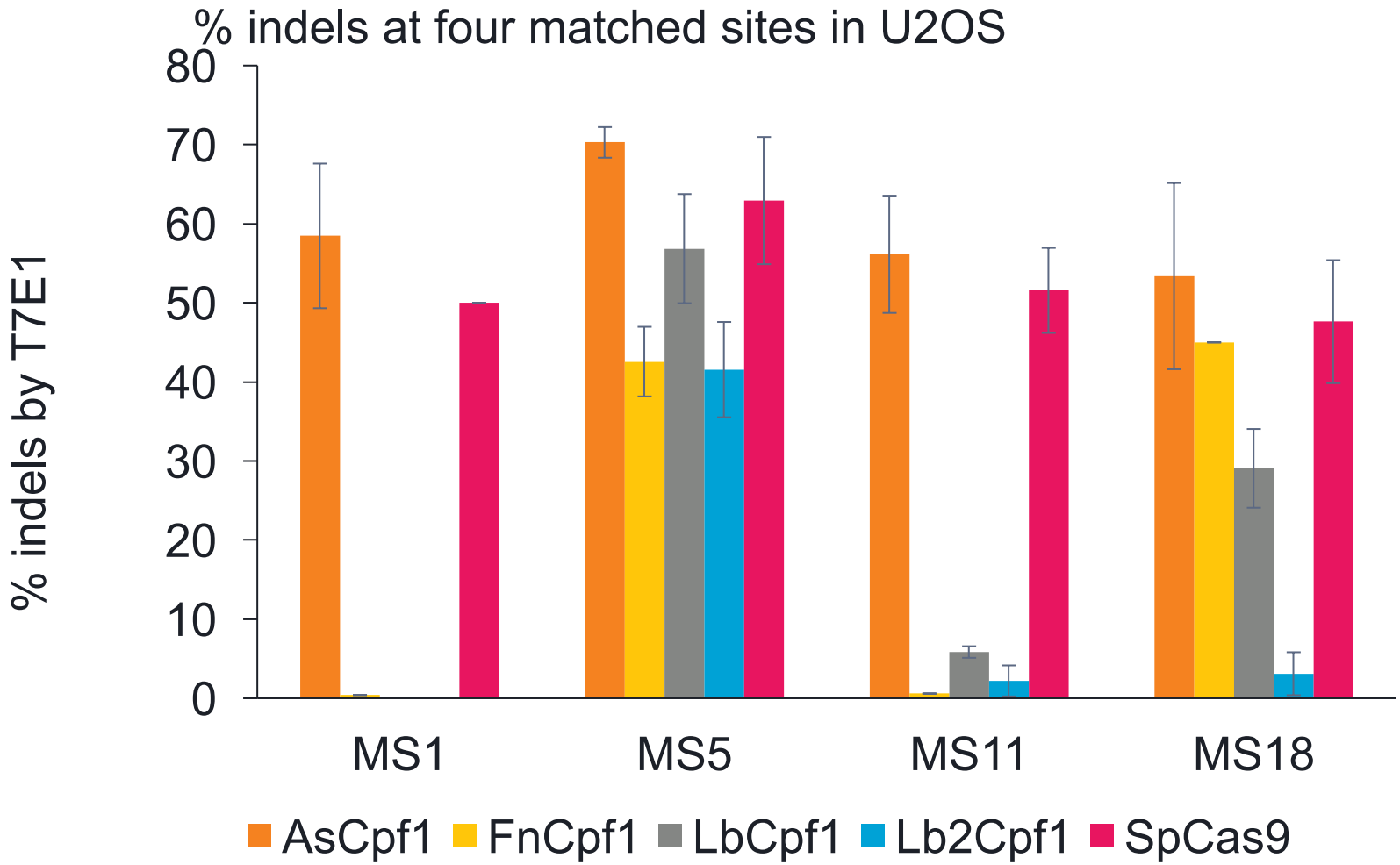




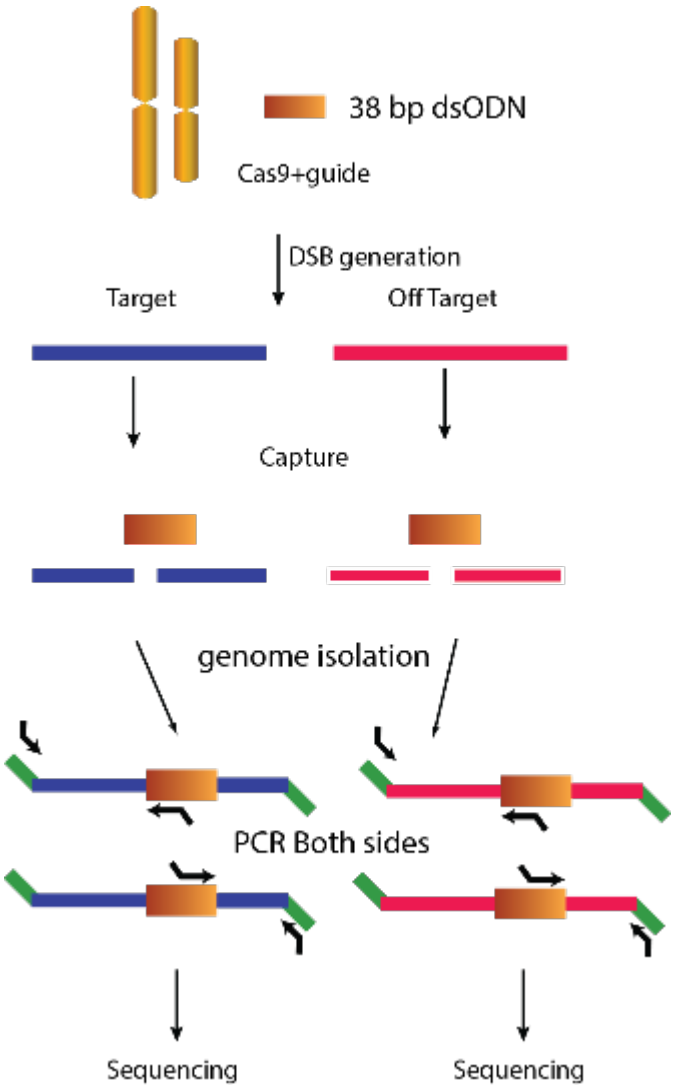
- S. pyogenes Cas9 RNPs in primary human T cells to knock-out PD-1
- Several gRNAs performed well as assessed by FACS
- Sequencing confirmed indels
- gRNAs analyzed by GUIDE-Seq to identify off targets

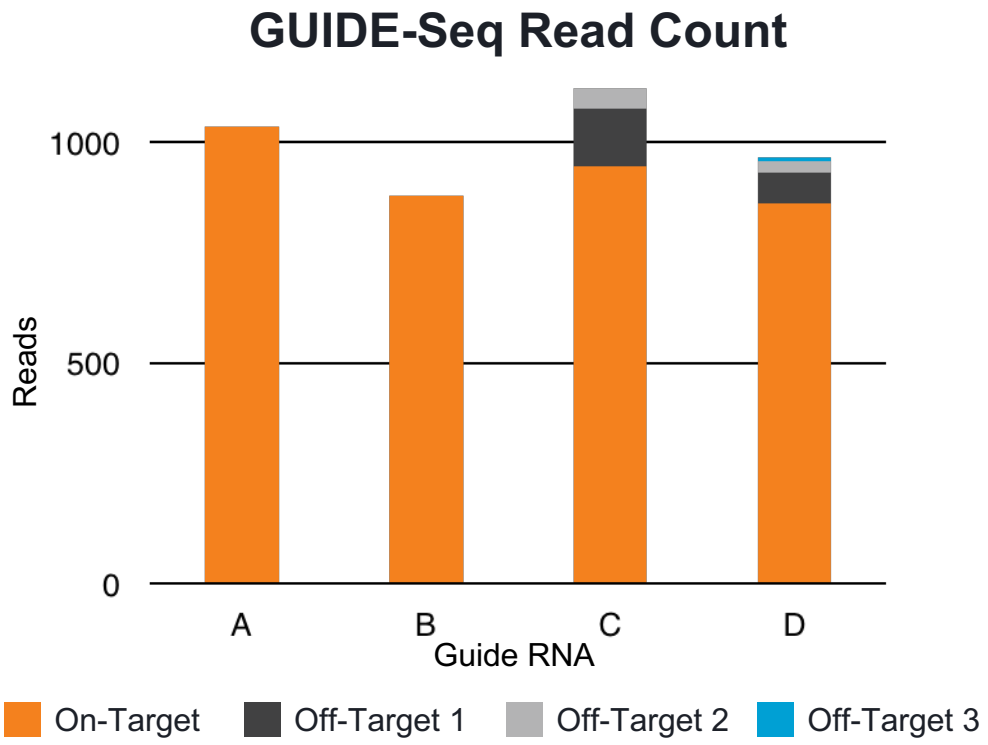
Screening of multiple Cpf-1 orthologs and variants

AsCpf1 emerging as the “go to” Cpf1 with Robust activity



Control and Specificity to Drive Precision



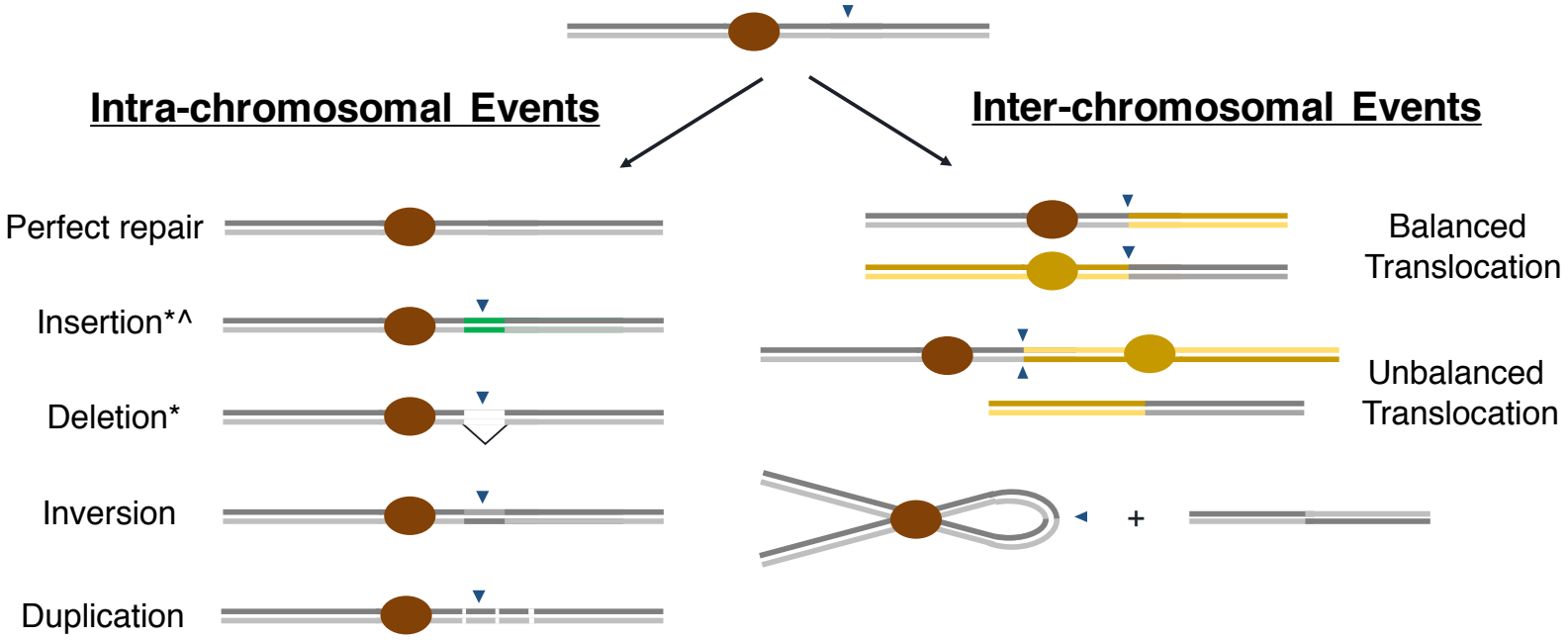


- GUIDE-Seq drives empirical demonstration of selectivity of product candidates
- Off-targets identified by GUIDE-Seq would not be accurately predicted by *in silico* methods alone

How Do You Best Measure Editing?

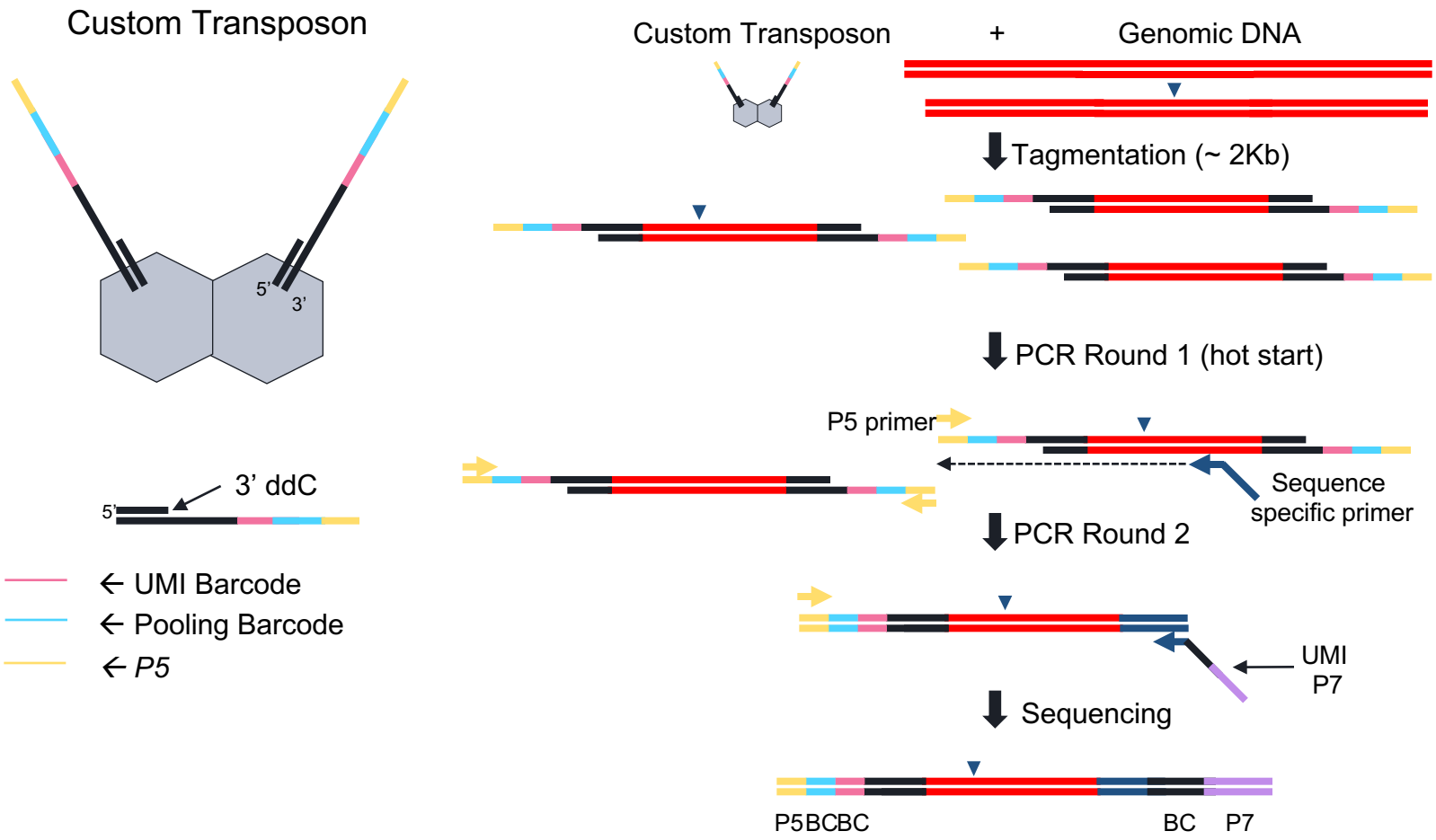
A simple question with a complex answer

- Sequence anchored detection approaches are limited to:
  - What is between the primers
  - Amplicon size



UDiTaS™ (Uni-Directional Targeted Sequencing)

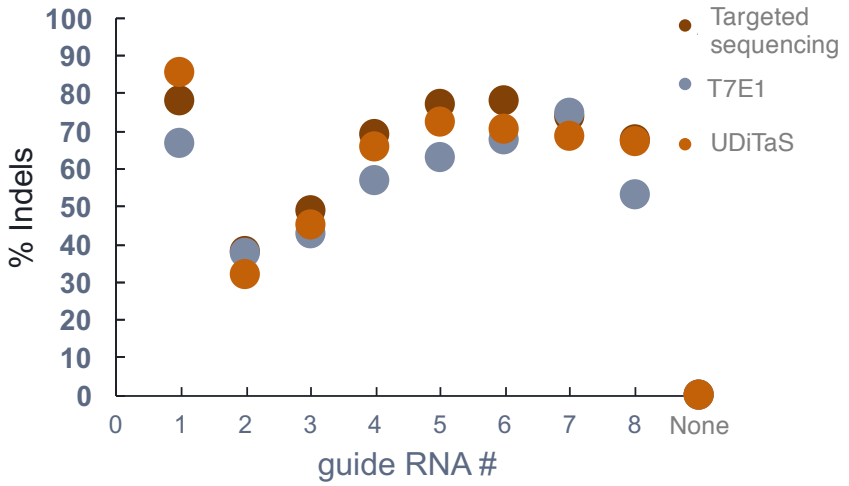
A simple, robust method for capturing complex editing events in a single reaction



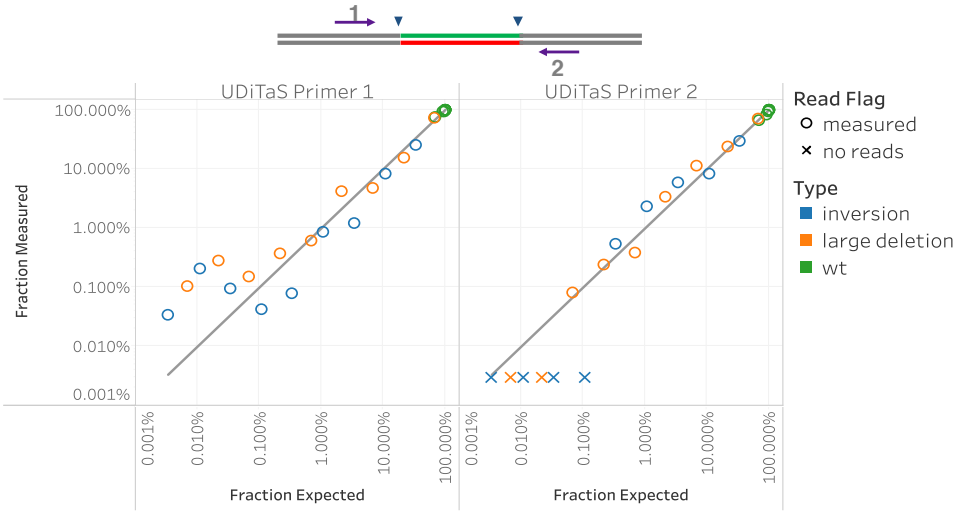
Picelli, S., et. al (2014). Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Research*, *24*(12), 2033–2040.

Measuring Structural Changes Using UDiTaS™

- Measurement of small Indels correlates well with targeted sequencing and T7E1 assays

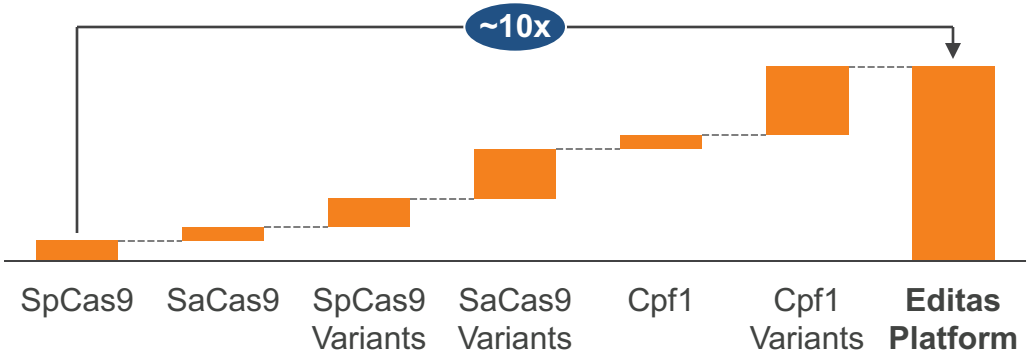


- Measurement of Inversions and Large Deletions



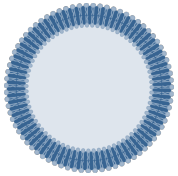
Platform Enables Broad Product Opportunities

Broad Range of Sites



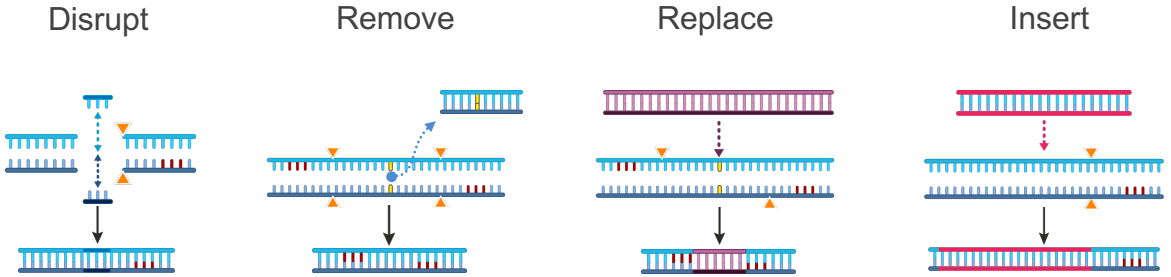
Wide Delivery Options


Viral Vector


Lipid Nanoparticle

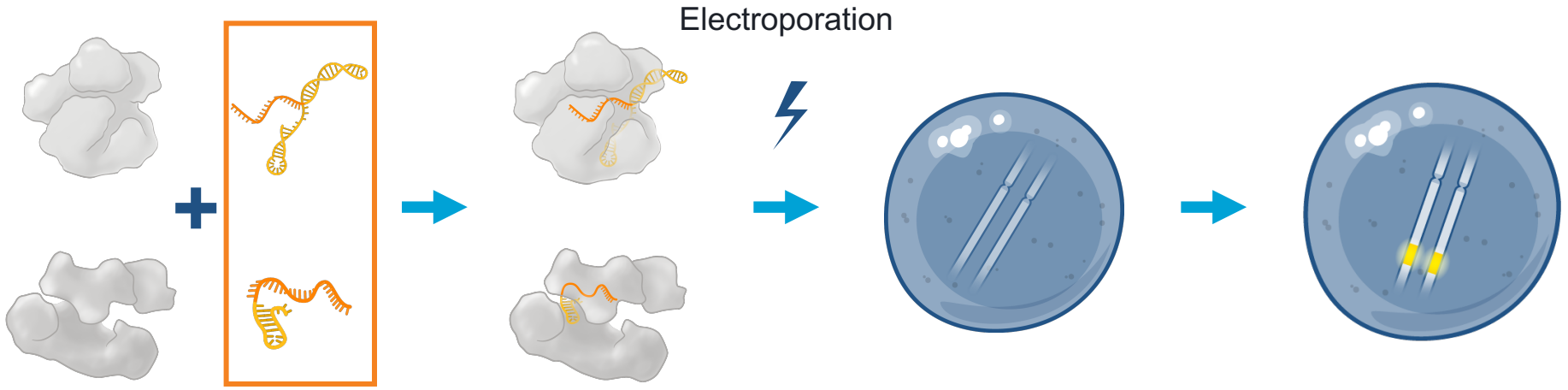

Electroporation

Diverse Spectrum of Edits



SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9

Scalable, Consistent Engineered Cell Therapies



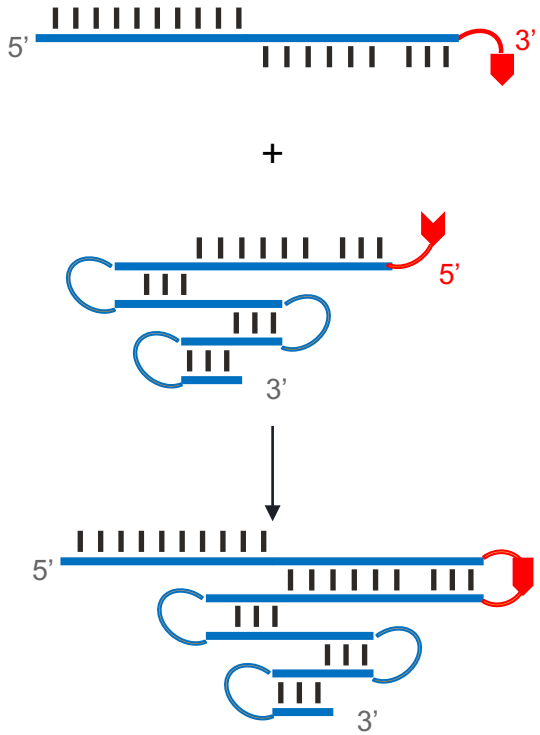
Optimized Delivery of RNP to Primary T cells Via Electroporation

Generating Synthetic Covalently-Coupled Dual gRNA

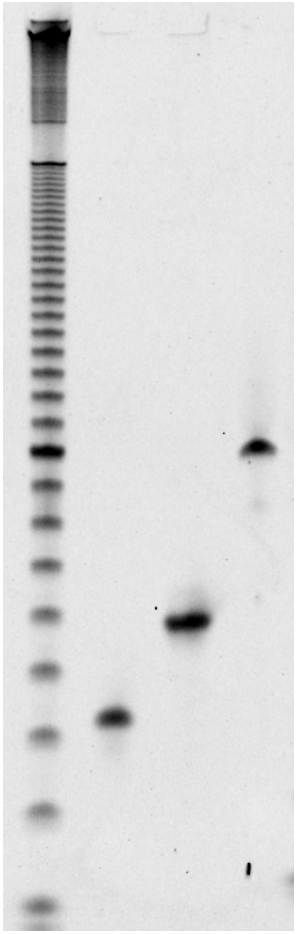
A completely non-enzymatic process for guide production

Why make a synthetic guide?

- Targeted chemistries anywhere in the molecule
- Unhindered ends and modifications
- Scale up and purity are more compatible with CMC requirements

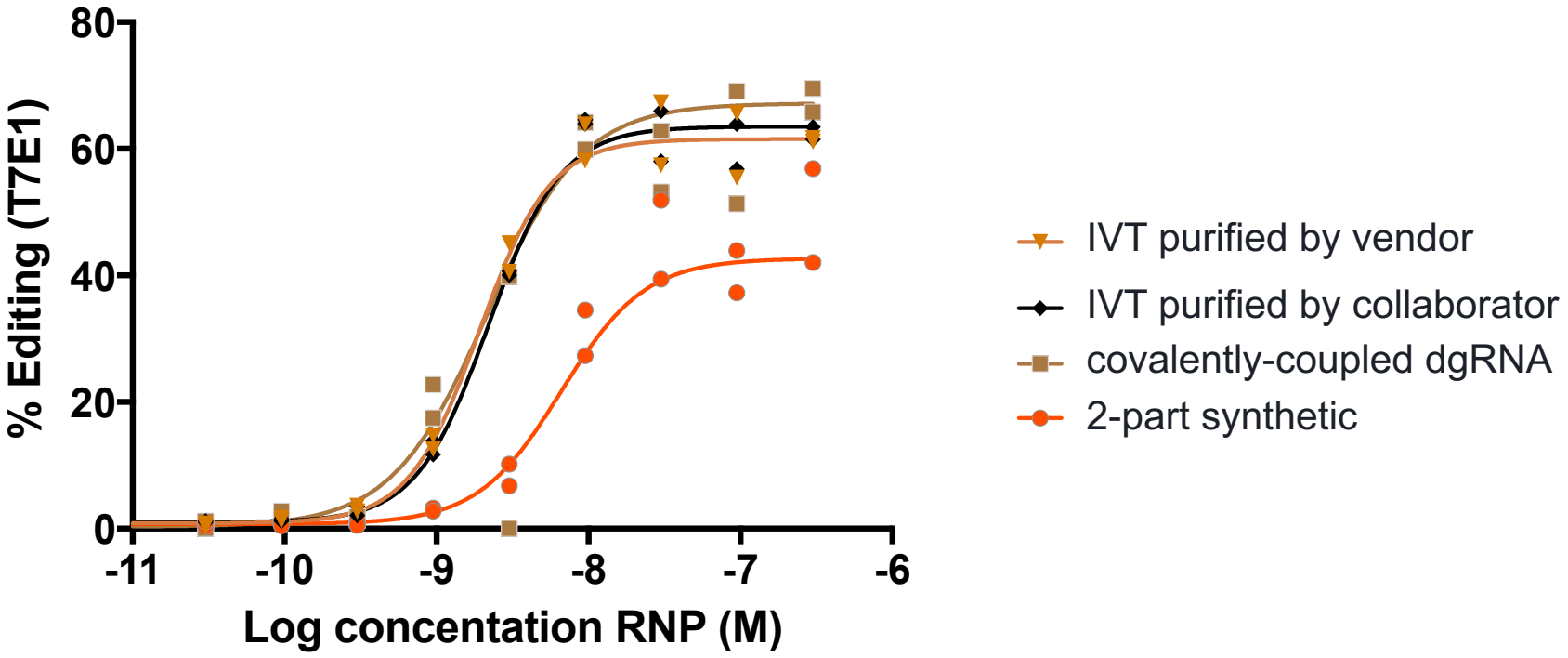


covalently-coupled dual gRNA (dgRNA)



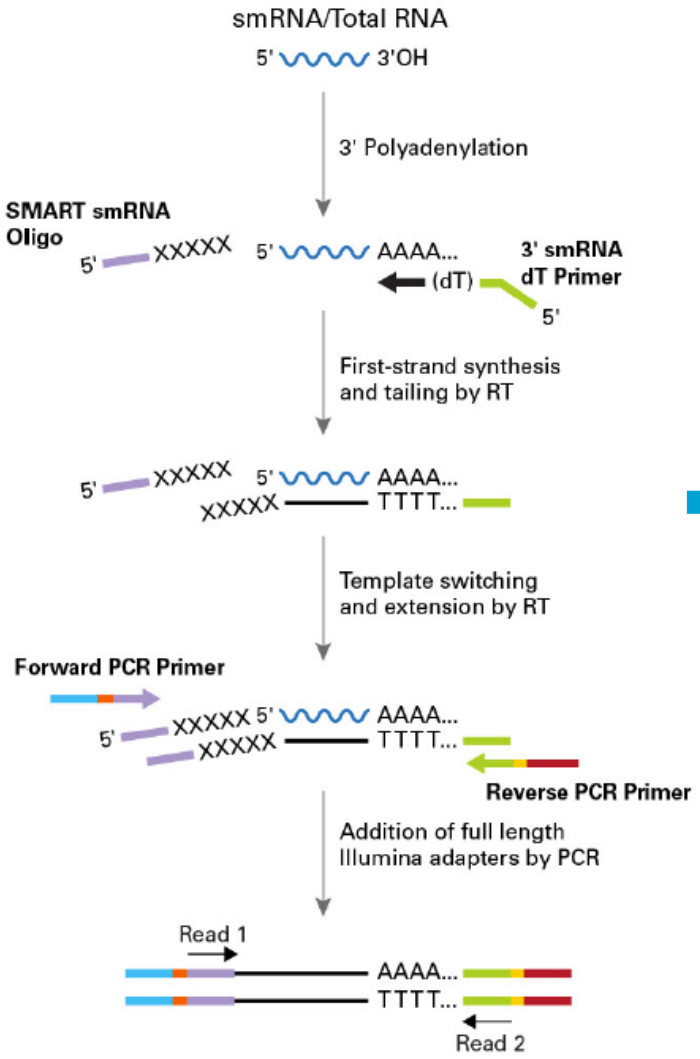
Cellular Editing Activity

In vitro transcribed and synthetic covalently-coupled dgRNA are equivalent in cells

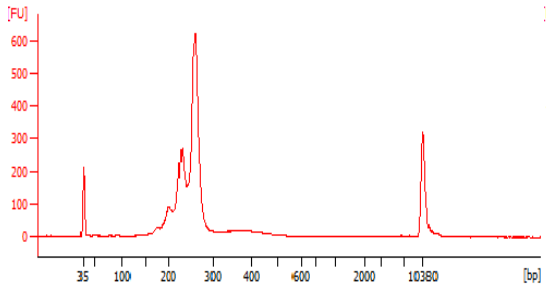


Assessing gRNA purity and sequence fidelity

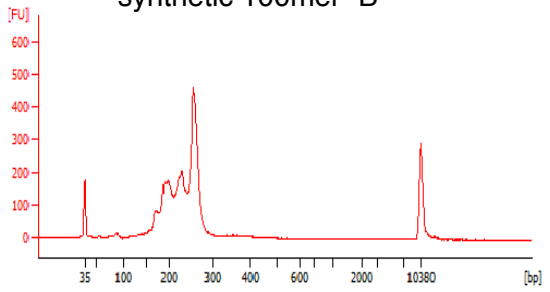
Development of an RNA-Seq based method for gRNA QC



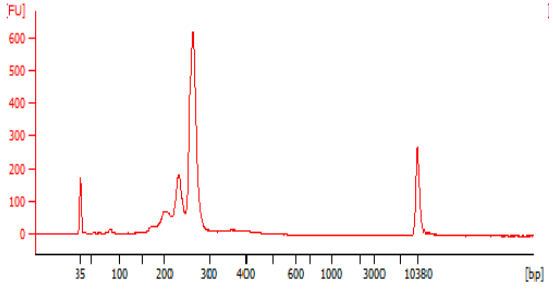
synthetic 100mer “A”



synthetic 100mer “B”



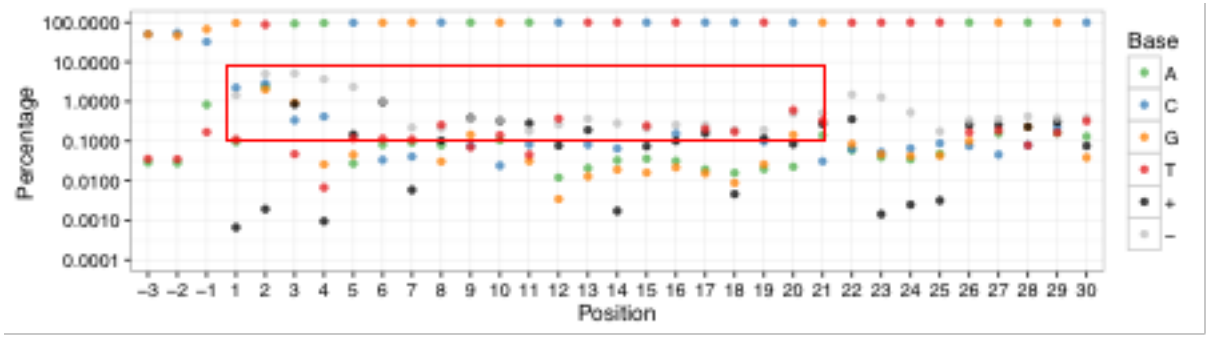
covalently-coupled dgRNA



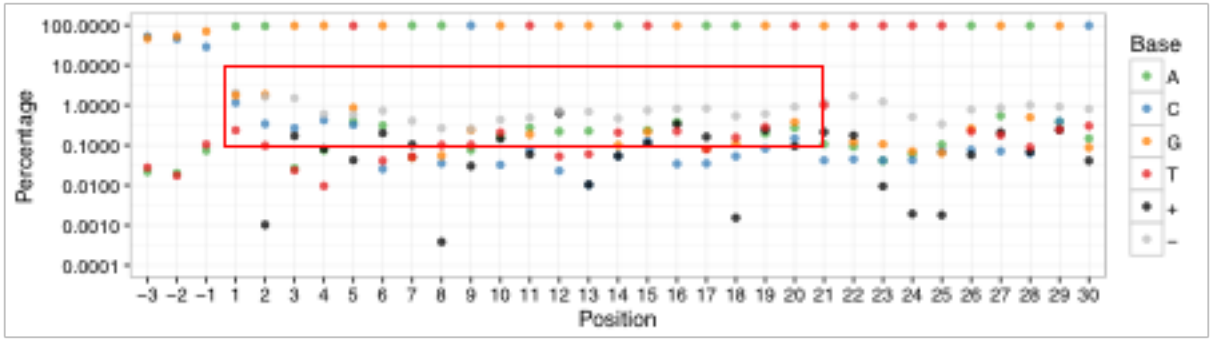
gRNA purity and sequence fidelity

Covalently-coupled dgRNA result in greater sequence fidelity in target region

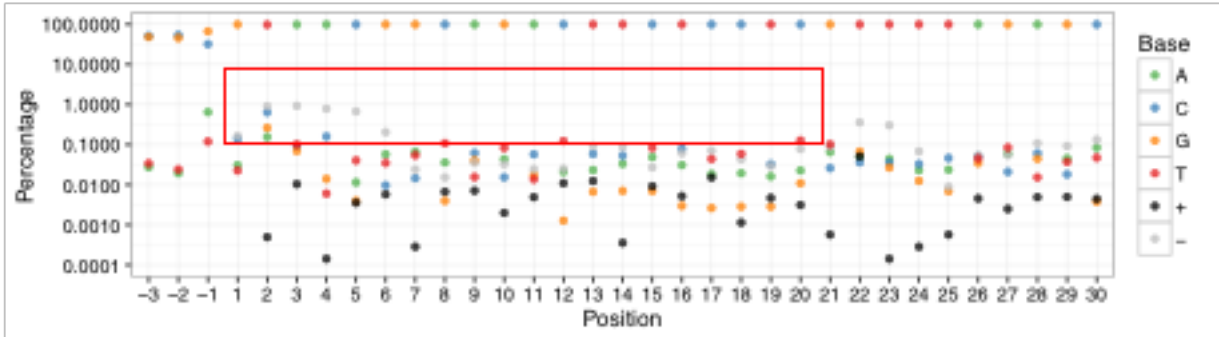
A



B



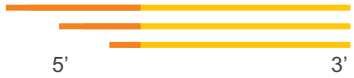
Covalently-Coupled dgRNA

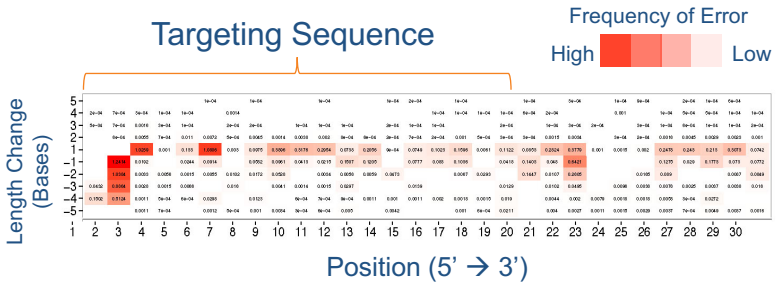


Improved and Proprietary Guide RNA Structures

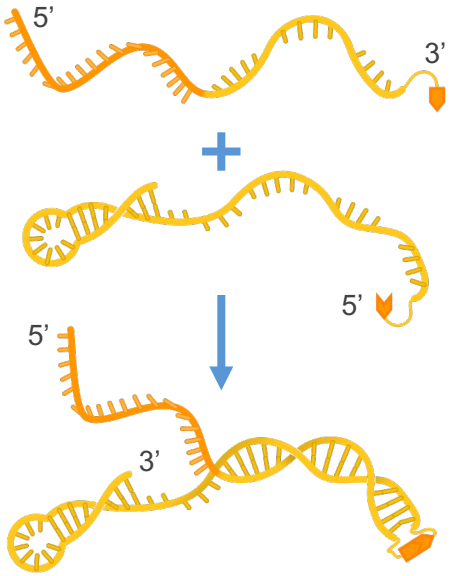
Single gRNA

Heterogeneous product
(full-length, truncated, errors)

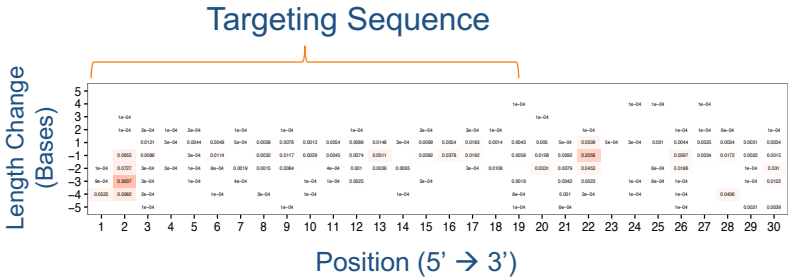




Covalently-Coupled Dual gRNA



Well-defined product
(full-length)



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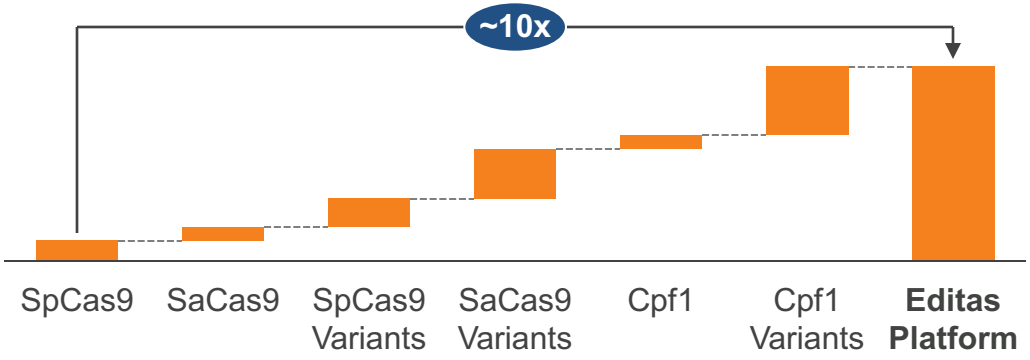

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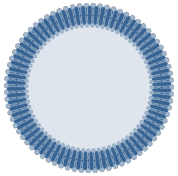
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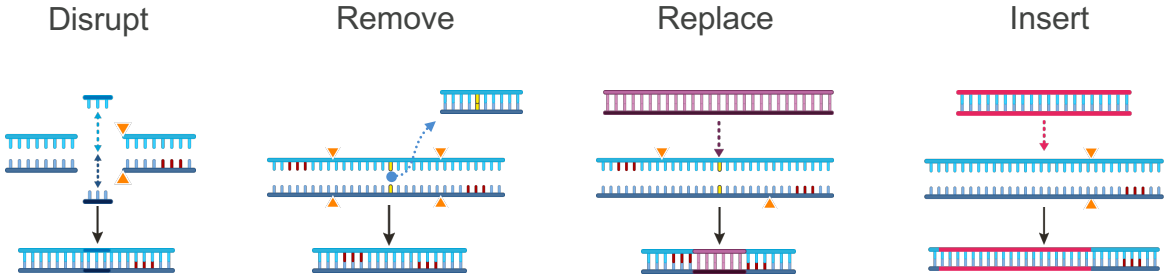
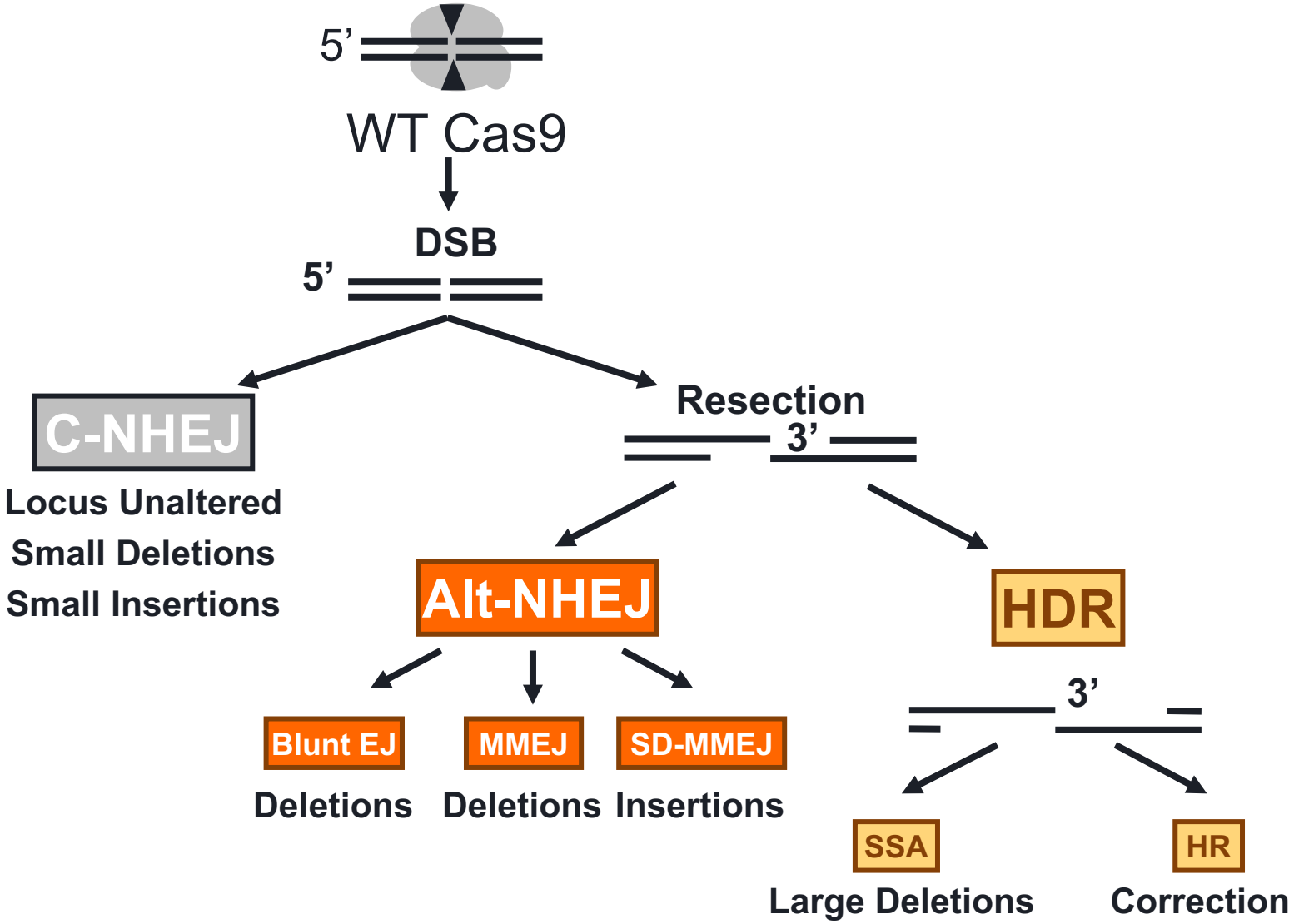
Diverse Spectrum of Edits

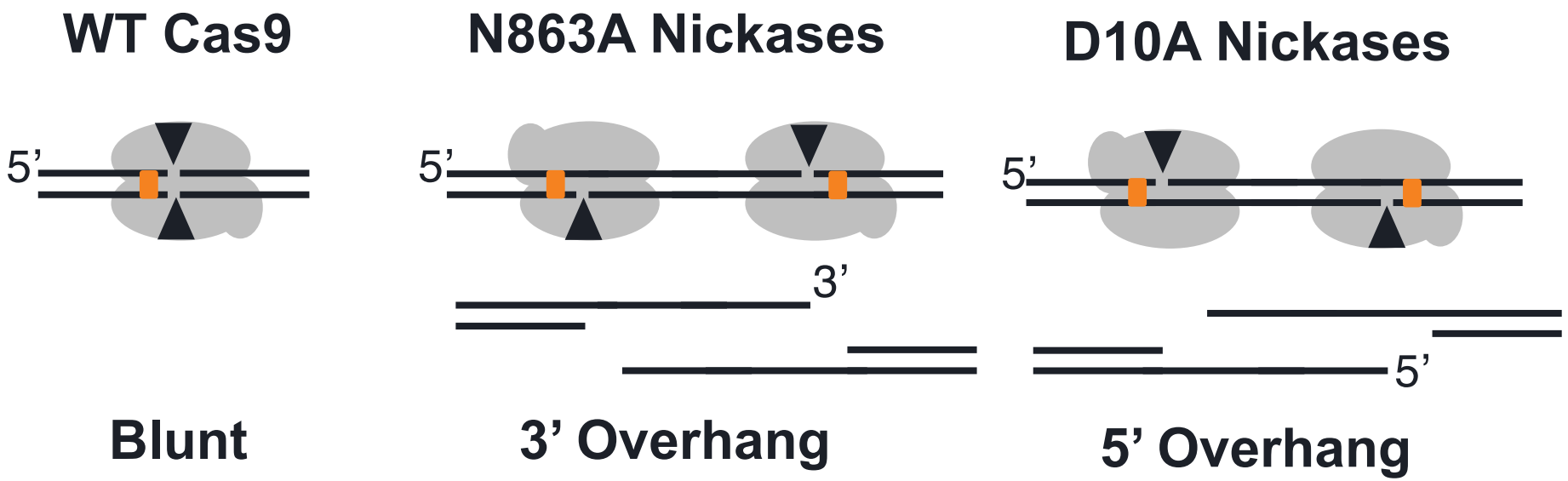


SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9

Cas9 Stimulates the Endogenous Repair Pathways

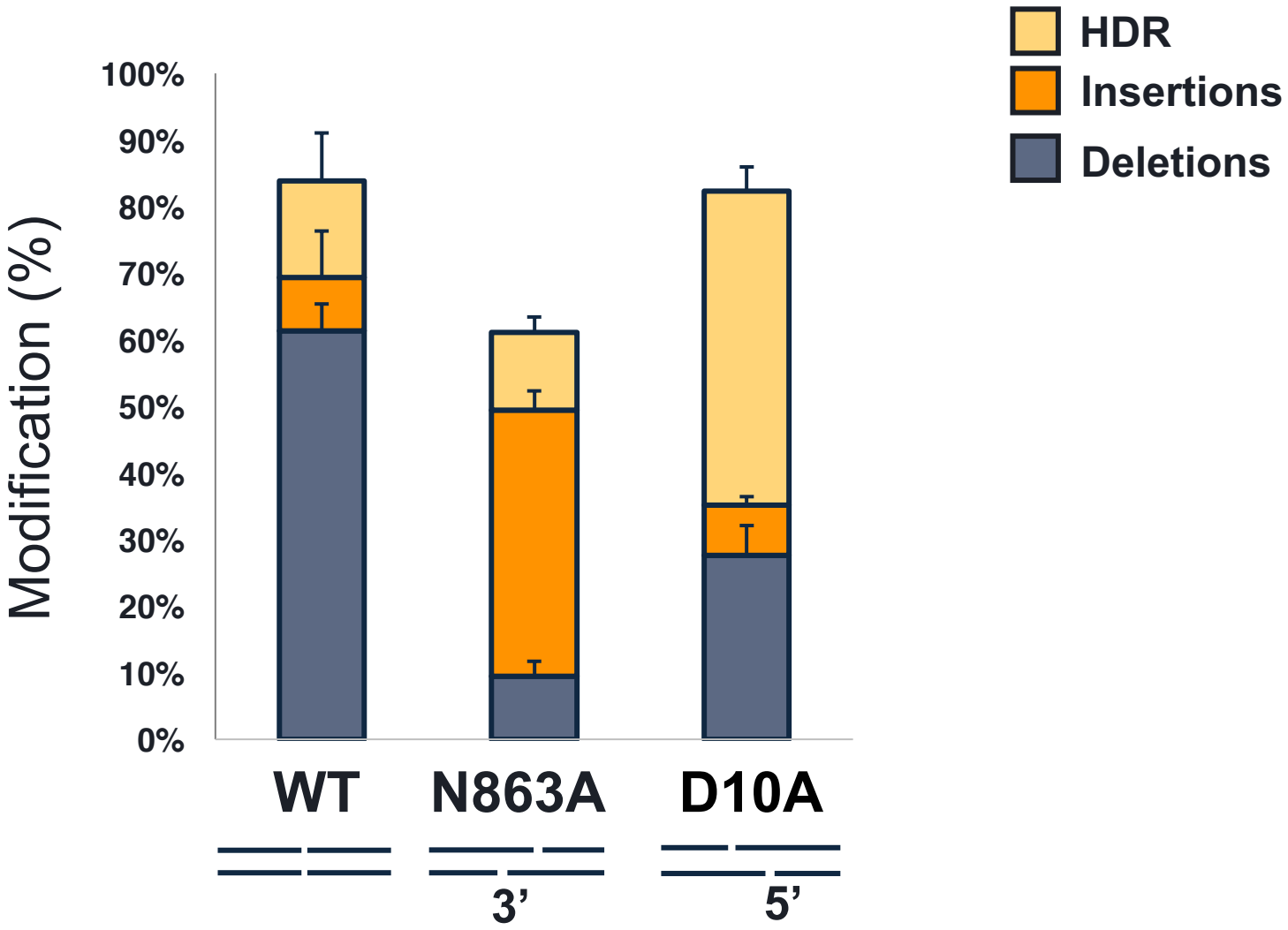


Cas9 is a Flexible Tool



- Could we engage different pathways by using these different variants?
- Could we selectively stimulate HDR?

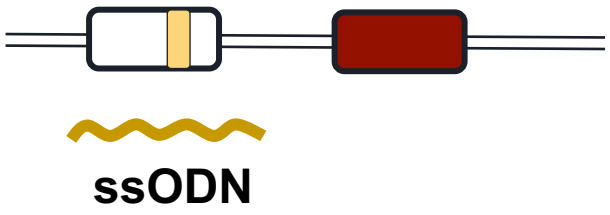
DSBs Generated by D10A are Predominantly Repaired by HDR



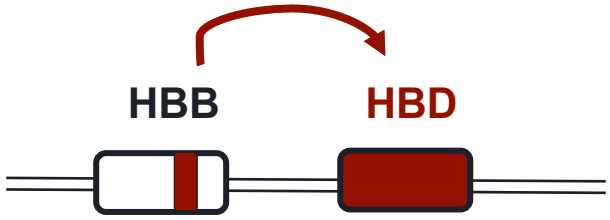
Bothmer et al., Nat Comm 2017

Do Gene Conversion and Gene Correction have the same Genetic Requirement?

Gene Correction

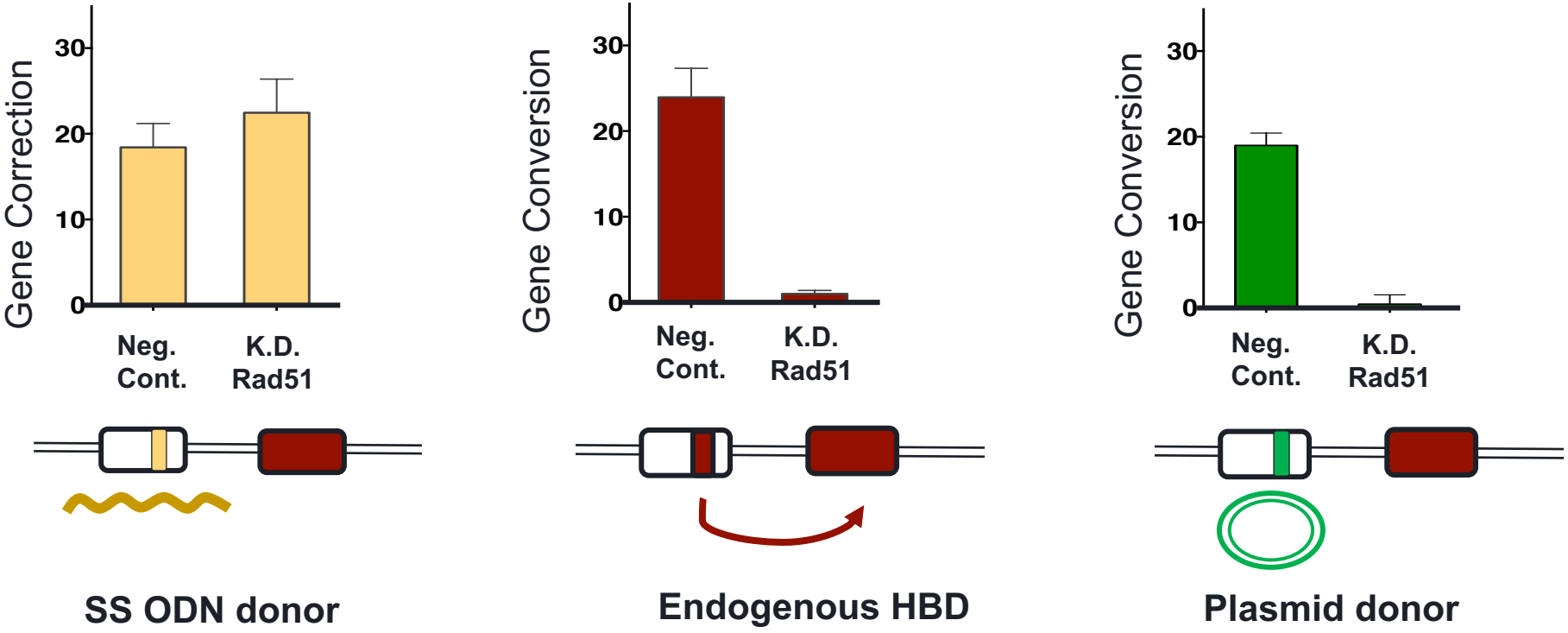


Gene Conversion



Do they both dependent on the HR pathway?

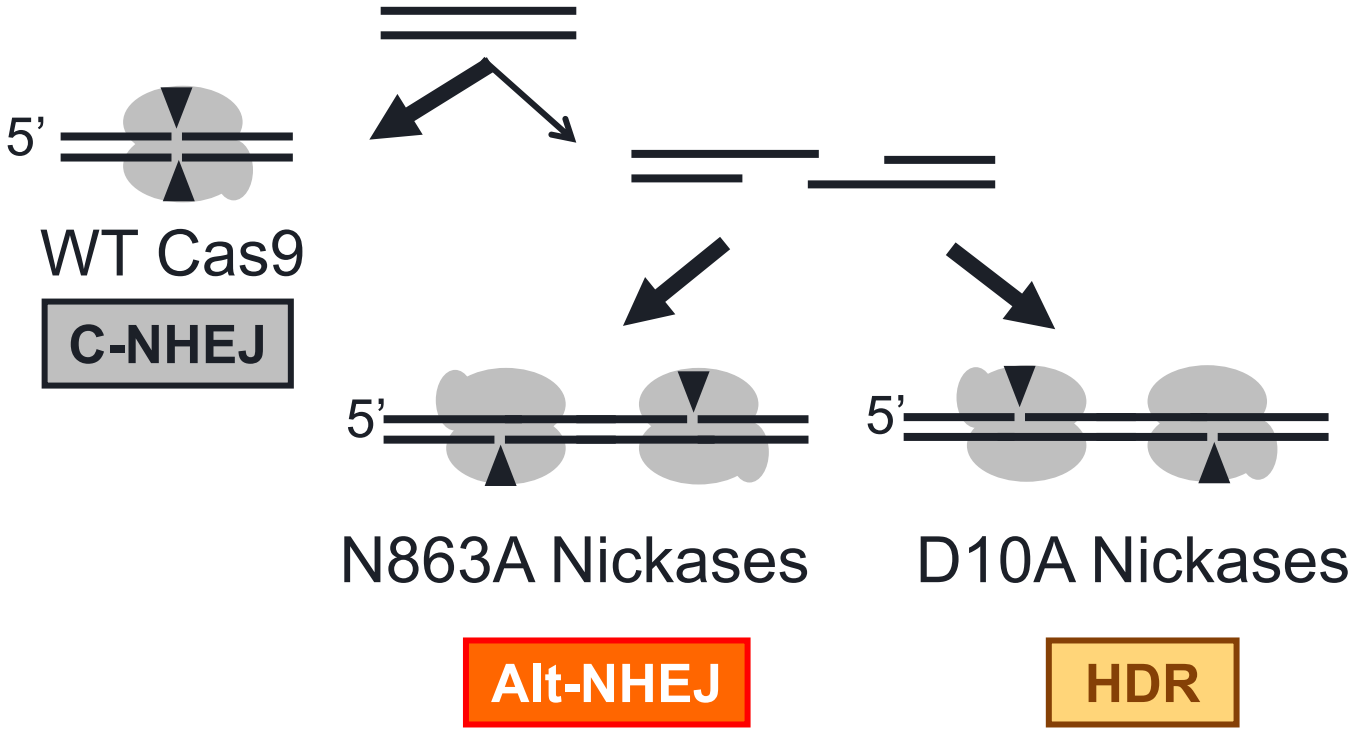
Gene Conversion and Gene Correction have Different Genetic Requirements



HR is required for repair from double stranded donors (endogenous homology tracks or plasmids) but not single stranded donors

Conclusions from the Dual Nick Analysis

- **Different ends activate different DNA repair pathways**



- **Different donors stimulate different pathways**

  Gene Correction mediated by ssODN is not HR dependent

Bothmer et al., Nat Comm 2017

Pipeline Strategy to Enable Successful Medicines

Prioritization Principles

Medical Need

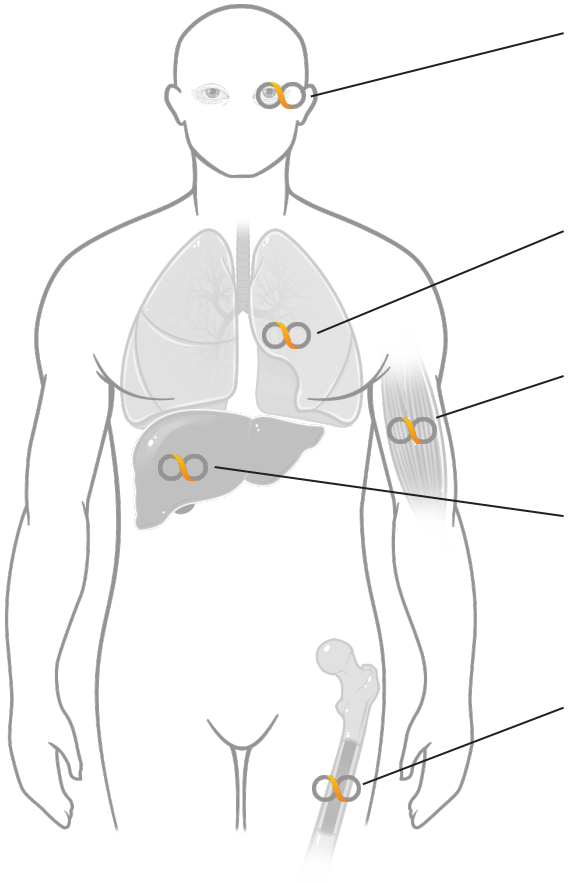
- Severe diseases where current treatments, if any, are poor
- Potential for durable therapies to provide unique benefit

Biology & Clinical

- Clear biological hypothesis for genomic intervention
- Favorable clinical and regulatory path

Technical

- Validated delivery approaches
- Mutation feasibly corrected



Product Pipeline

Eye

- LCA10 (EDIT-101)
- Ocular HSV
- Additional ocular indications

Lung

- Cystic Fibrosis

Muscle

- Duchenne Muscular Dystrophy

Liver

- Alpha-1 Antitrypsin Deficiency
- Infectious diseases of liver

Bone Marrow & Blood

- Hemoglobinopathies
- Engineered T cells for cancer
- Additional bone marrow and blood indications

LCA10: Leber Congenital Amaurosis type 10; HSV: Herpes Simplex Virus

Thank You

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