Genome Editing: Considerations for Therapeutic Applications

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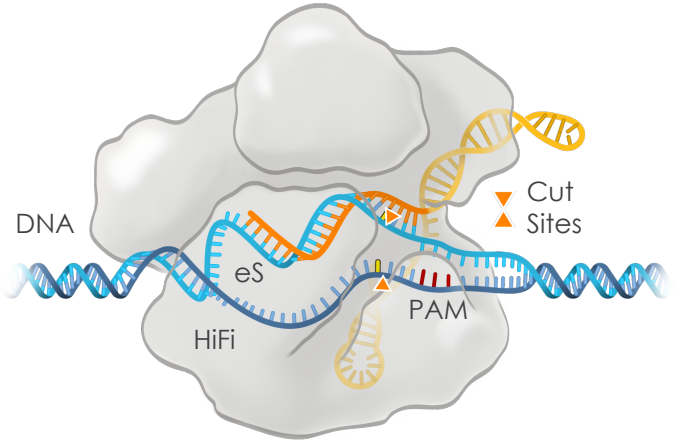
TIDES, 2017

The Potential to Repair Any Broken Gene

Transformative new category of medicines
for genetically-defined and genetically-treatable diseases

CRISPR Unlocks Genome Editing

Editing machinery can be engineered to target nearly any genomic location



Nuclease

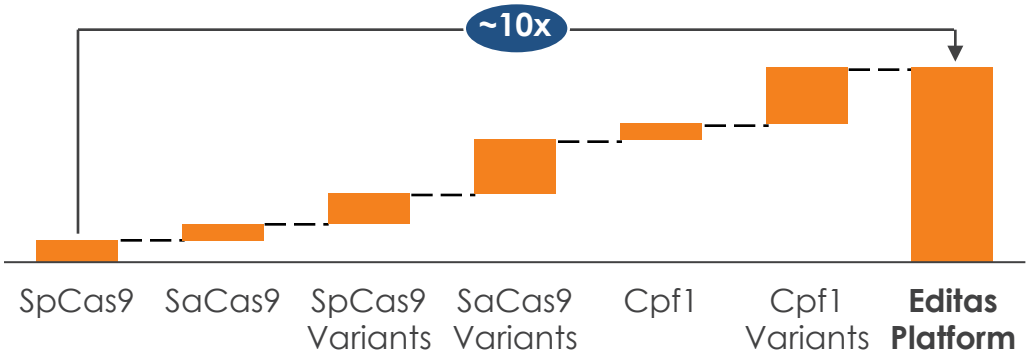
Guide RNA

- 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

PAM: Protospacer Adjacent Motif; HiFi: High Fidelity; eS: enhanced Specificity

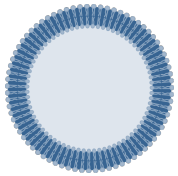
Unparalleled Platform for Gene Editing Medicines

Broad Range of Sites



Wide Delivery Options

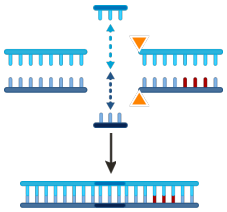

Viral Vector


Lipid Nanoparticle

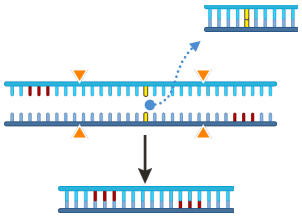

Electroporation

Diverse Spectrum of Edits

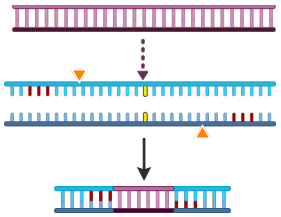
Disrupt



Delete

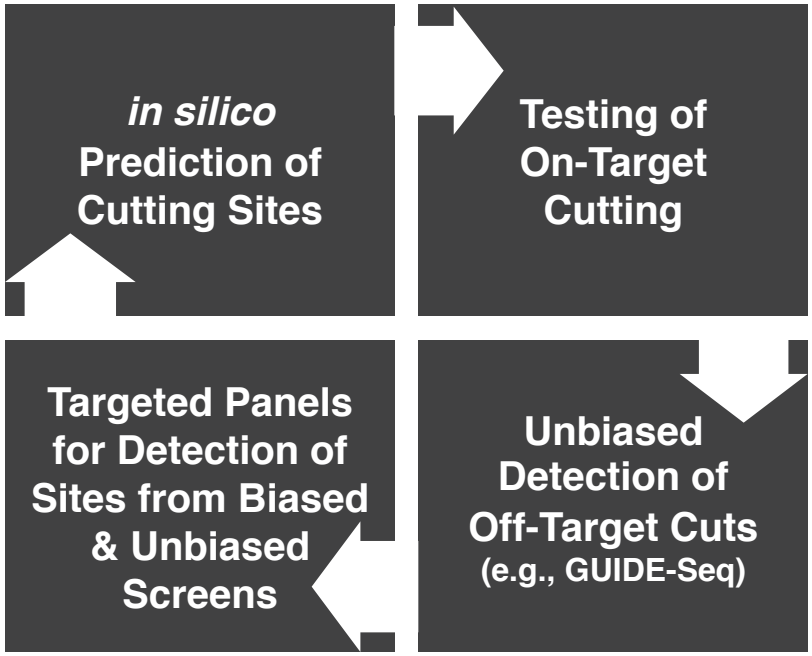


Correct

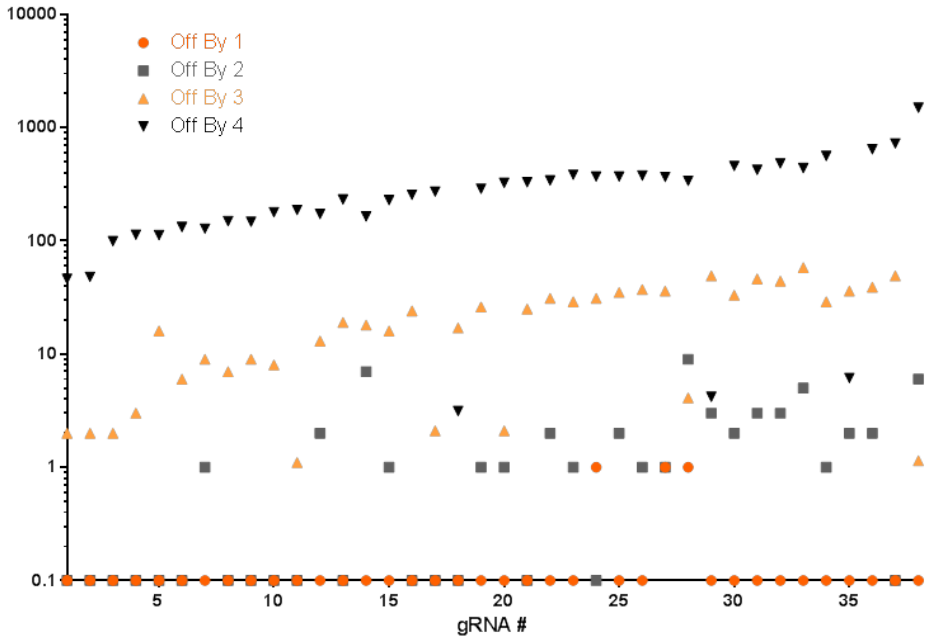


Lead Finding & Specificity to Select gRNA

Identify, Measure, Minimize

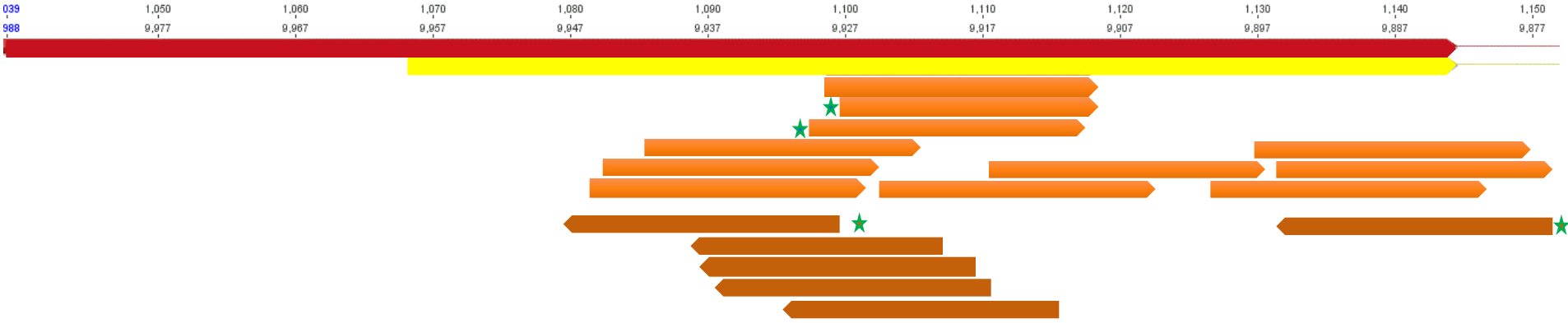


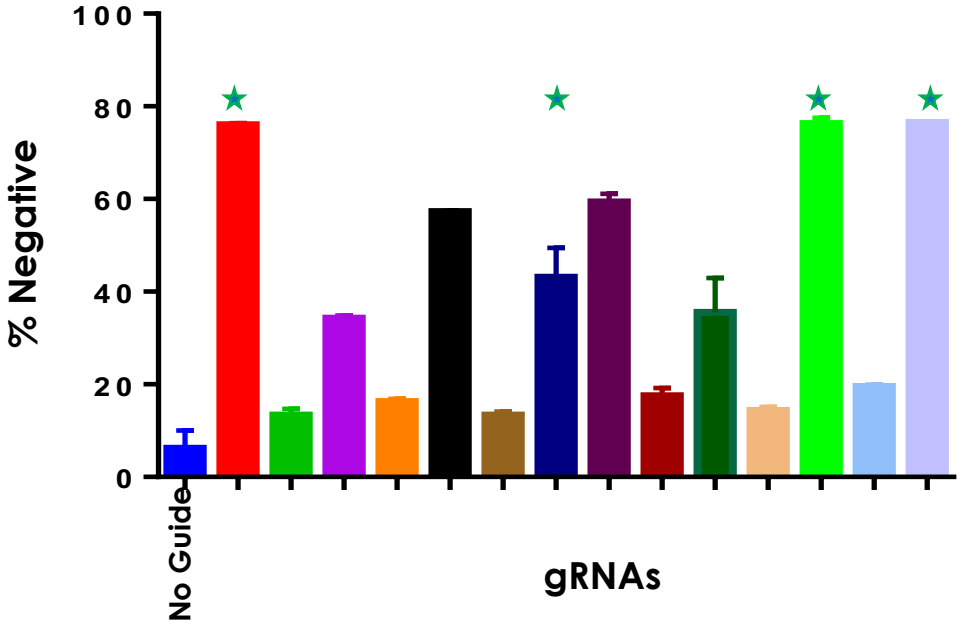
In silico Selection



Identification of Robust gRNAs

RNP (*S. pyogenes*) delivery to primary T cells

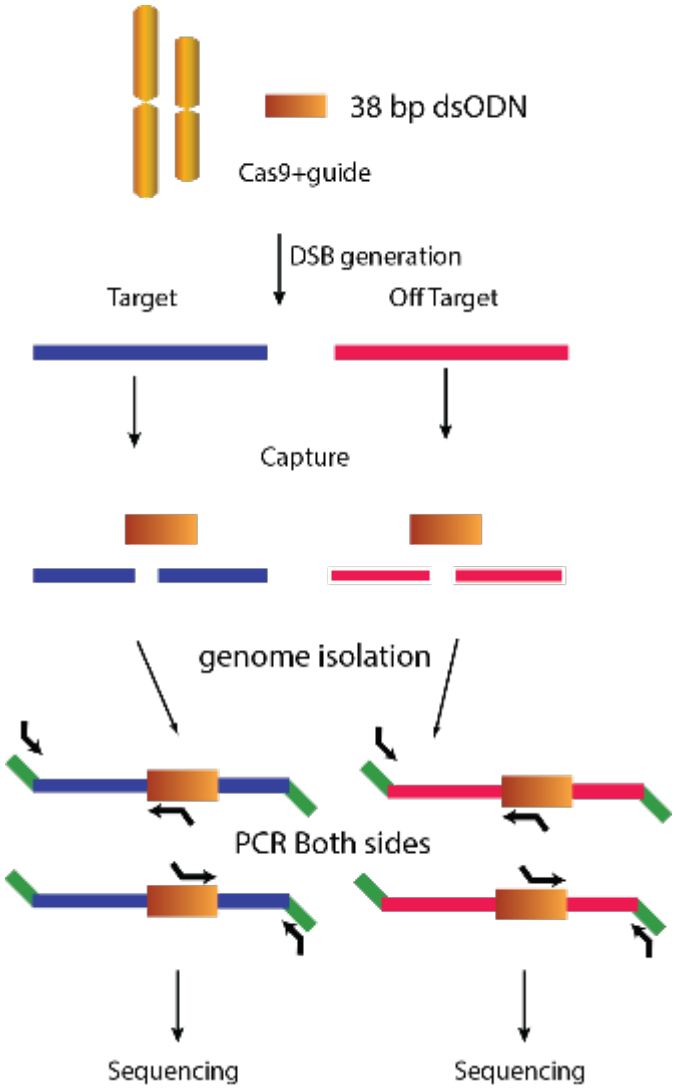


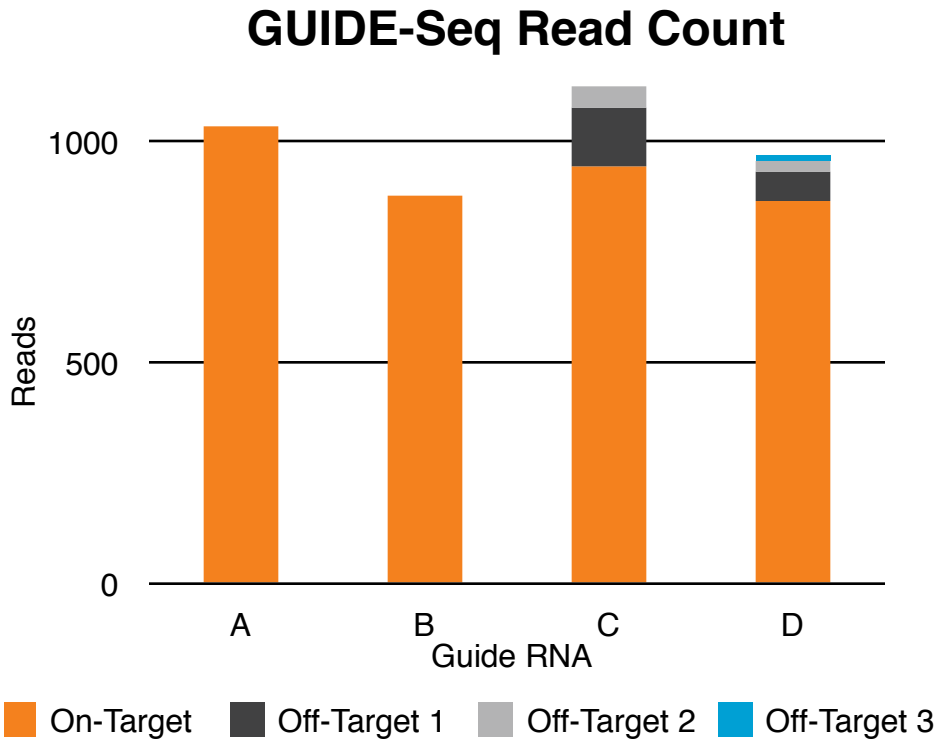


- Several gRNAs performed well when assessing target **expression** by FACS
- Sequencing of gDNA confirmed the presence of indels
- These gRNAs were analyzed by GUIDE-seq to identify off targets

Control and Specificity to Drive Precision

Guide-seq allows identification of highly selective candidates





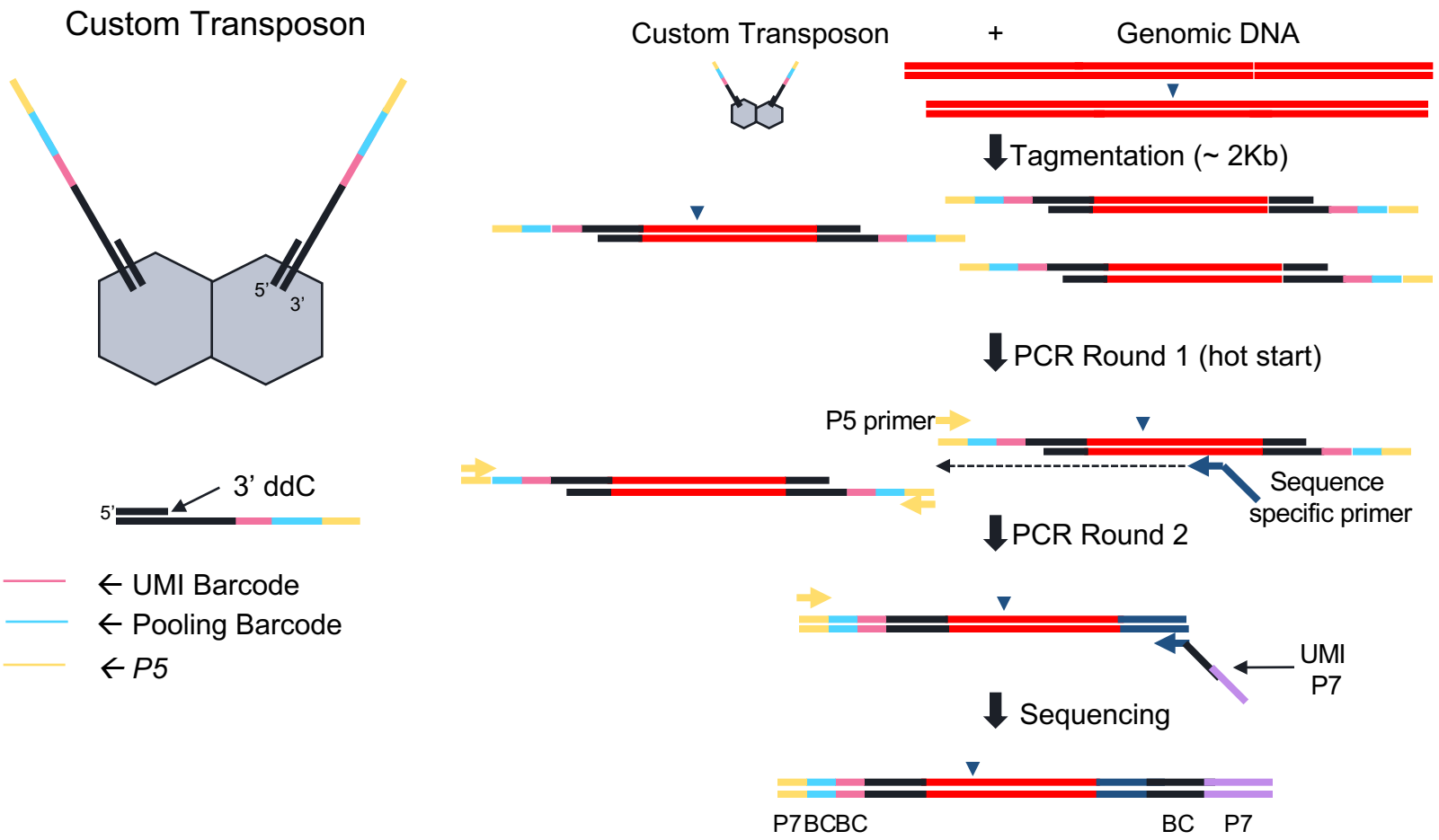
How Do You Best Measure Editing?

A simple question with a complex answer

- Sequence anchored detection approaches are limited to:
  - What is between the primers and
  - Amplicon size
- One cannot detect several events and has to build and "reassemble" answers from disparate technologies (e.g. ddPCR + targeted sequencing):
  - Large insertions
  - Large deletions
  - Inversions
  - Translocations
- Wanted a size insensitive, multiplex compatible, comparatively easy single tube method that can detect all of the above events

Uni-Directional Targeted Sequencing (UDiTaS™)

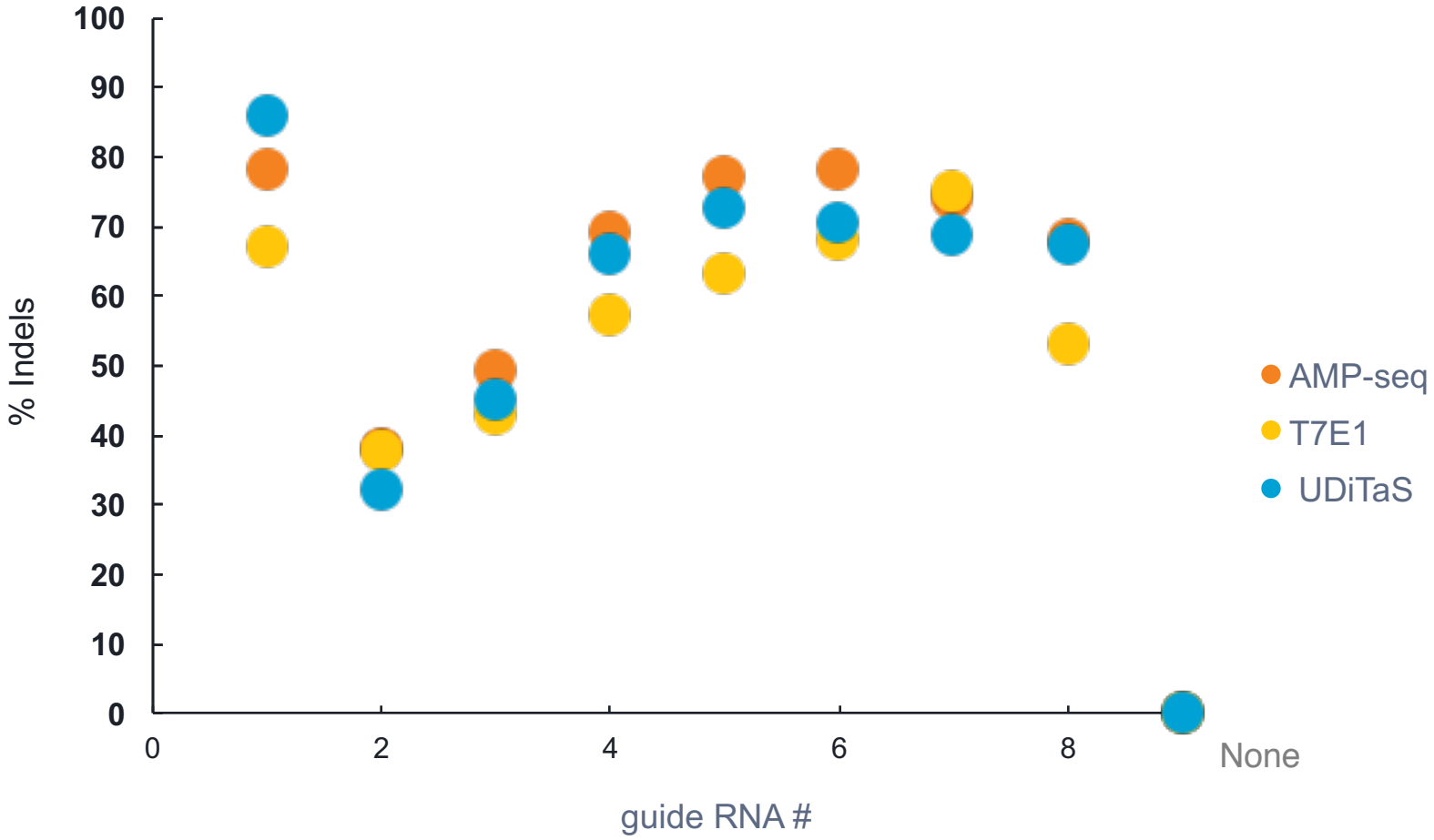
Blending tagmentation and AMP-seq



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

Comparison of Small Indel Measurements

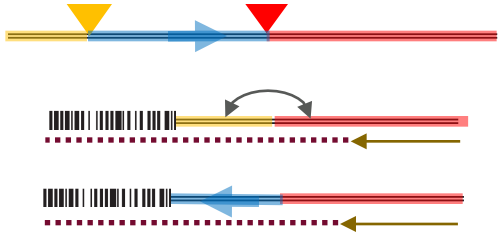
UDiTaS correlates well with targeted sequencing and T7E1 assays



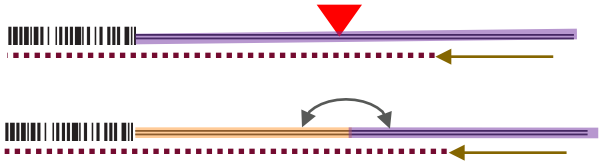
UDiTaS™ (Uni-Directional Targeted Sequencing)

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

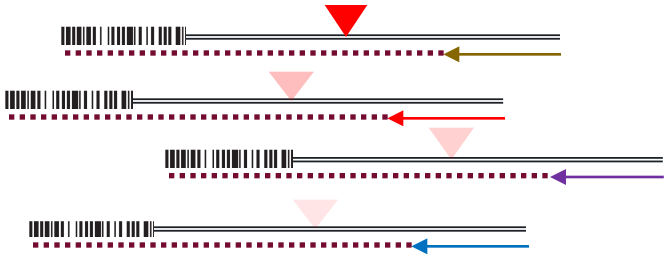
1. Quantitation of editing



2. Quantitation and discovery of large deletions and inversions



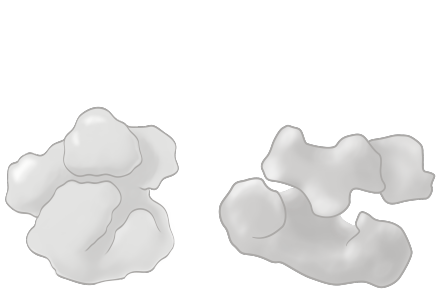
3. Translocation discovery and quantitation



4. Multiplexing

Ex Vivo Drug Development

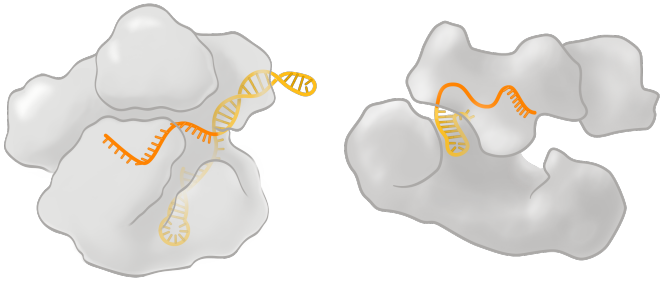
High Quality Ribonucleoprotein Particle Delivery



RNA Guided Endonuclease

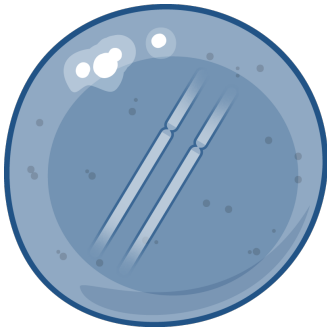
+

RNA Guide

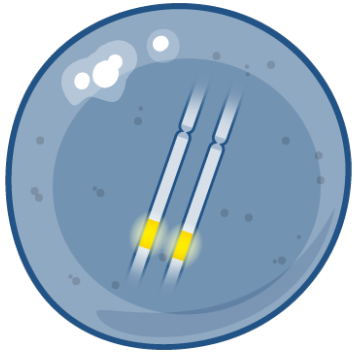


Ribonucleoprotein Particle (RNP)

+



Patient Cell



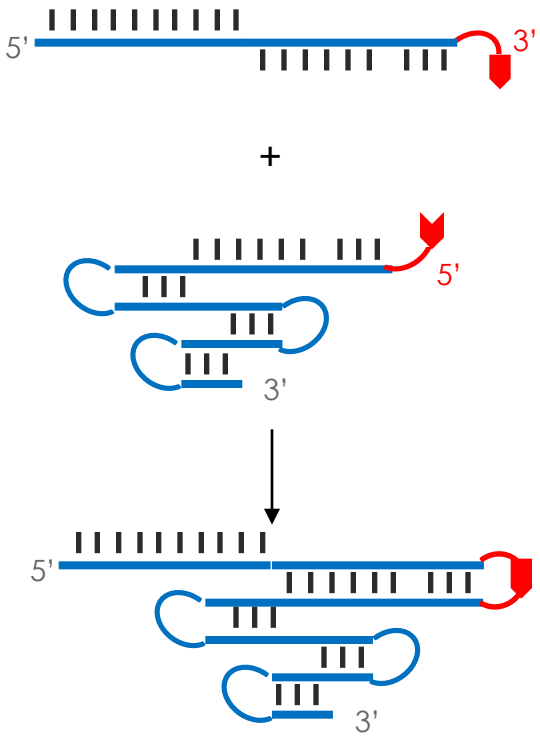
Engineered Patient Cell

Generating Synthetic Covalently-Coupled Dual gRNA

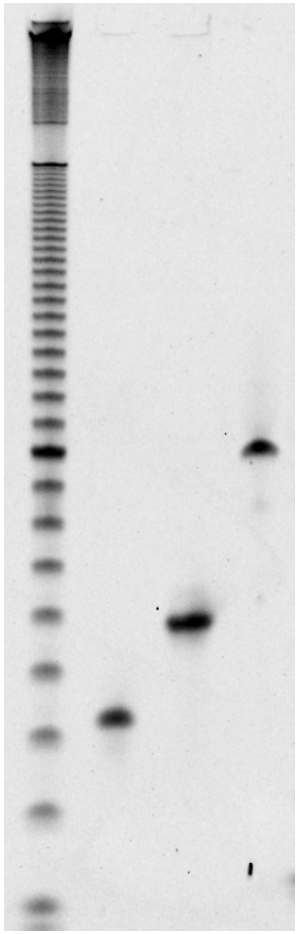
A completely non-enzymatic process for guide production

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

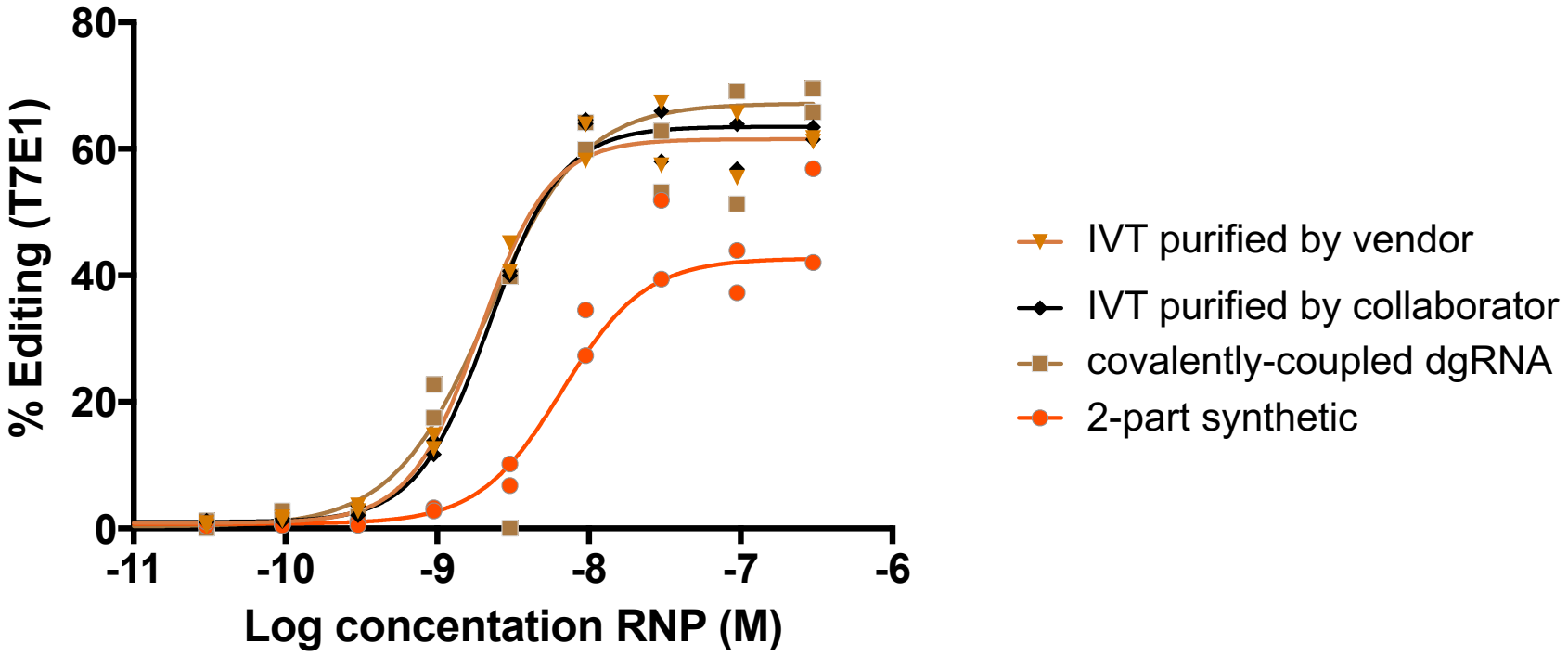


covalently-coupled dual gRNA (dgRNA)



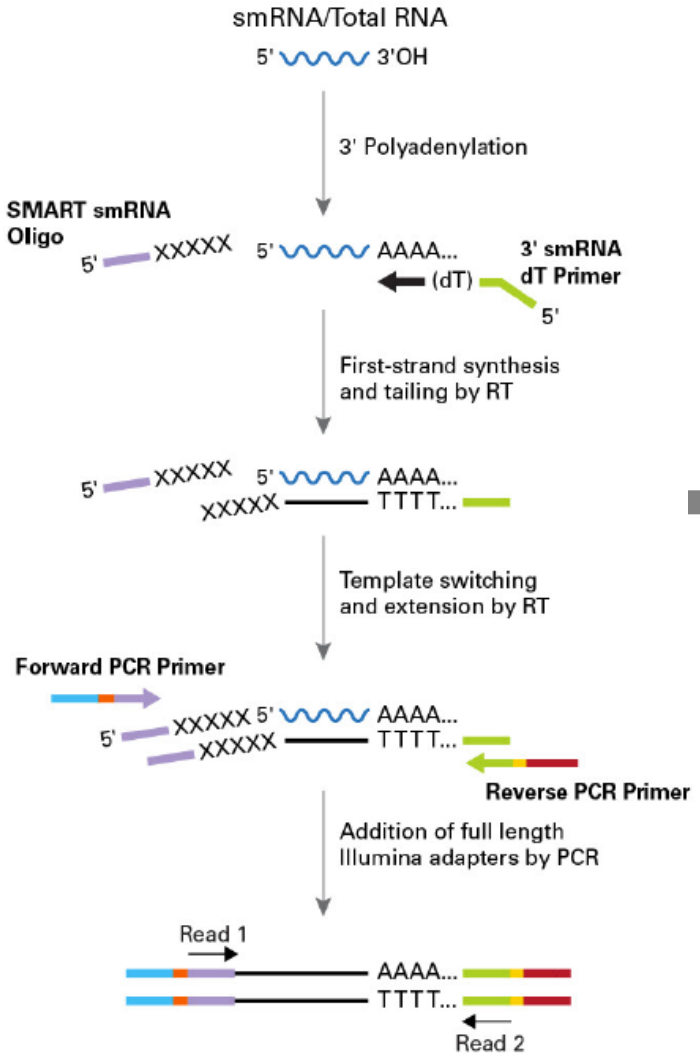
Cellular Editing Activity

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

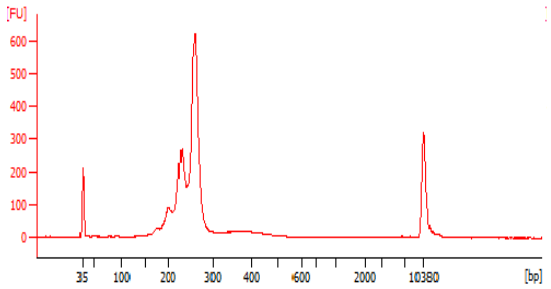


Assessing gRNA purity and sequence fidelity

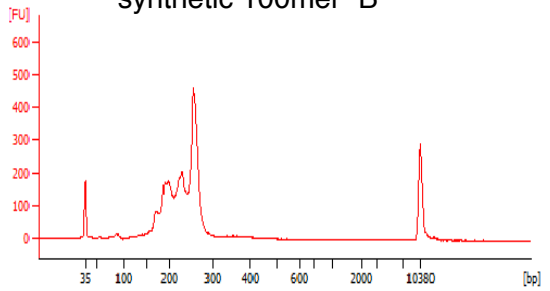
Development of an RNA-Seq based method for gRNA QC



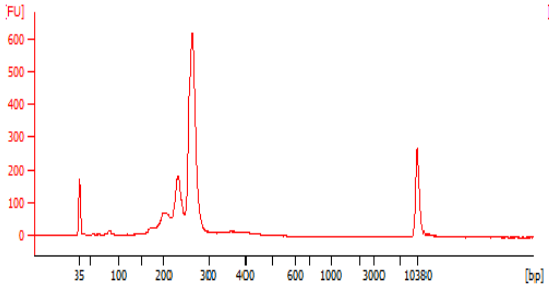
synthetic 100mer “A”



synthetic 100mer “B”



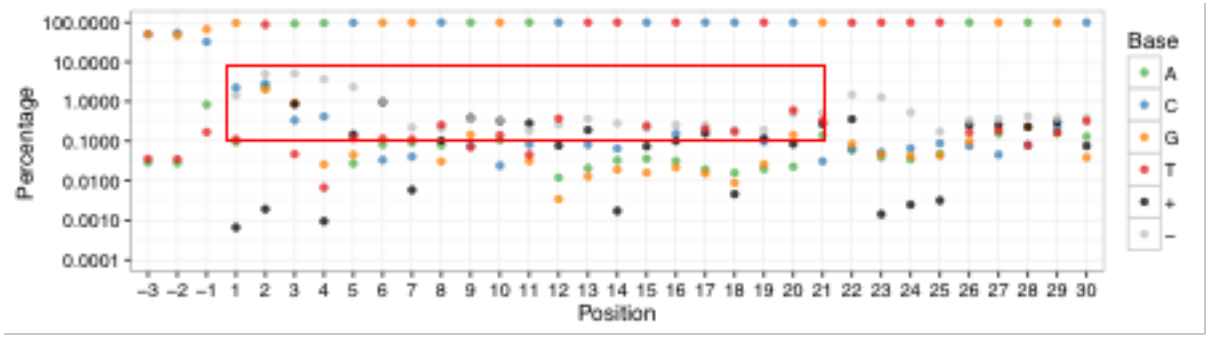
covalently-coupled dgRNA



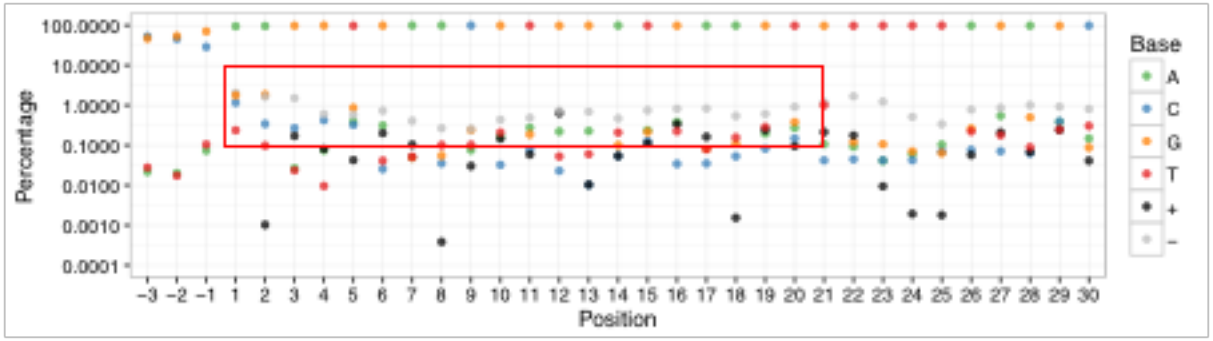
gRNA purity and sequence fidelity

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

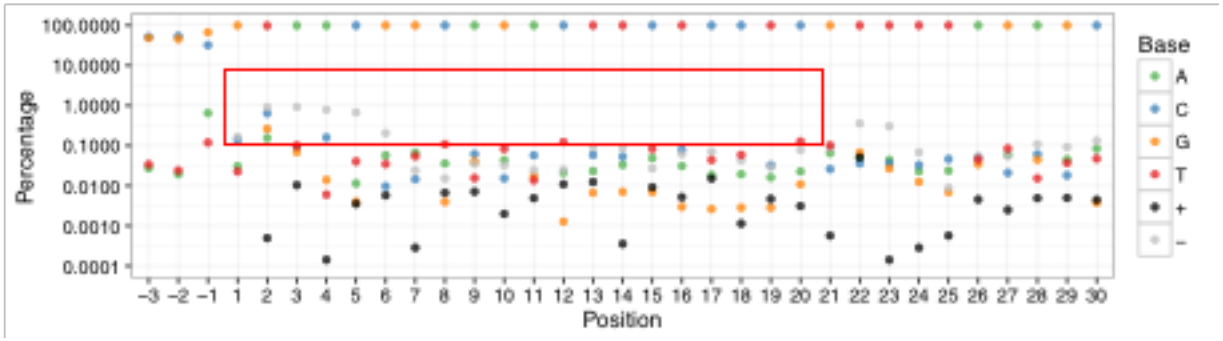
A



B



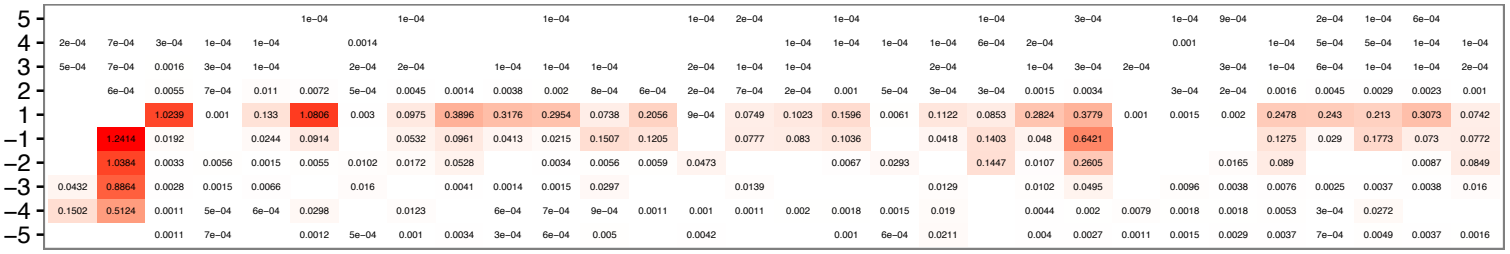
Covalently-Coupled dgRNA



gRNA Purity and Sequence Fidelity

Covalently-coupled dgRNA result in fewer truncated molecules

A



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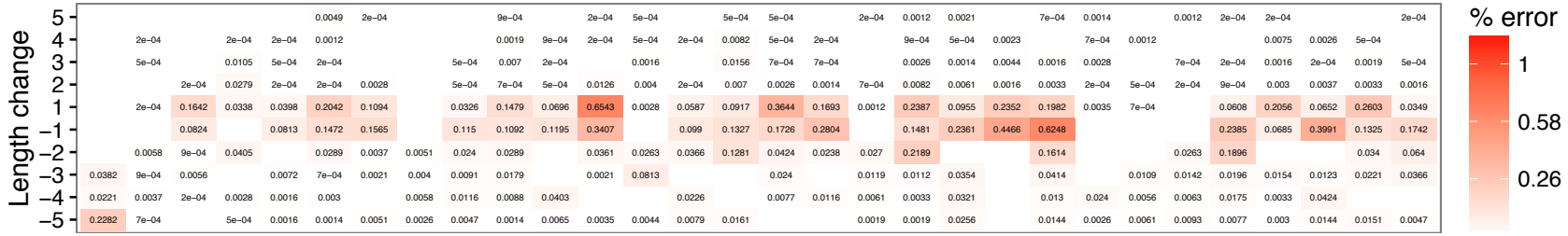
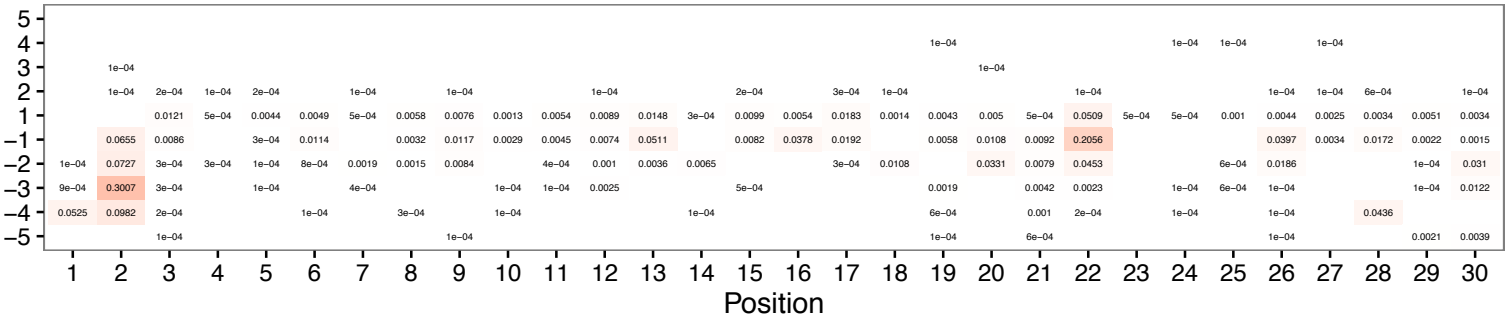


Covalently-Coupled dgRNA

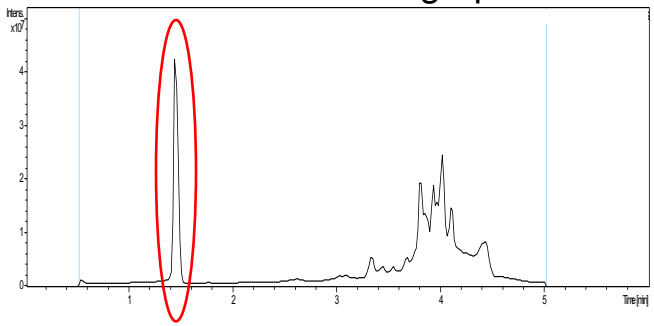


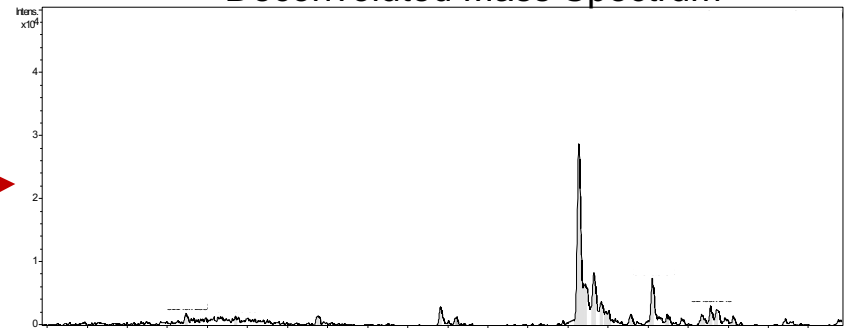
Orthogonal Analytics Demonstrates Superior Material

Total Ion Chromatograph

Deconvoluted Mass Spectrum

A





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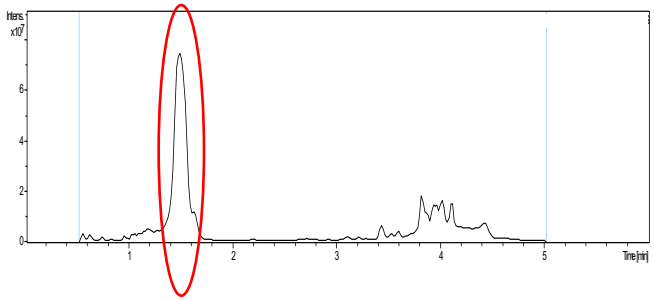
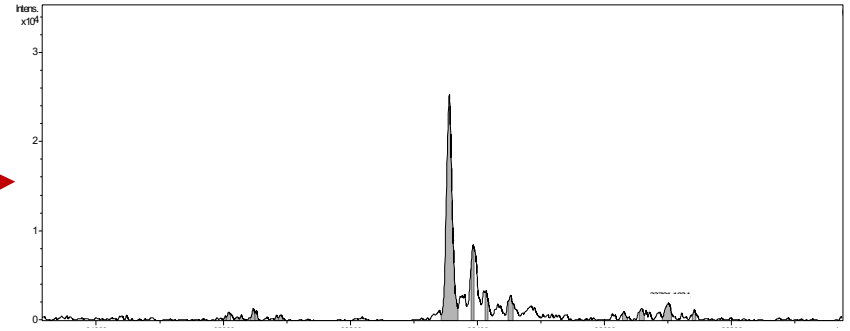
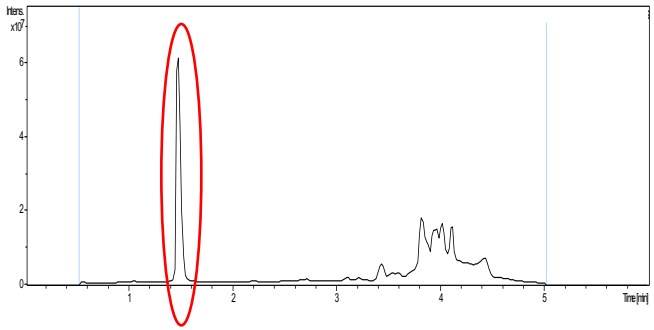
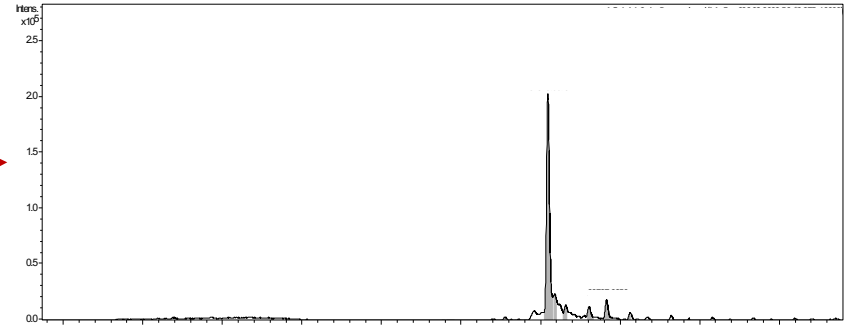




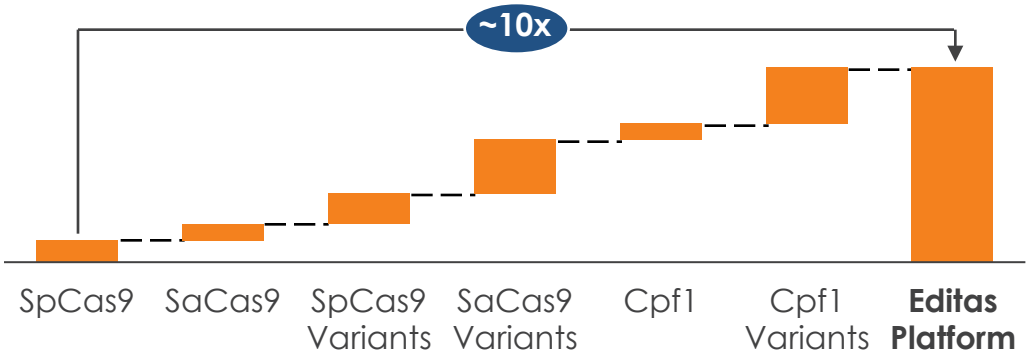
Covalently-coupled dgRNA





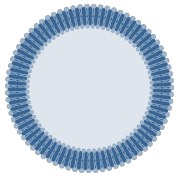
Unparalleled Platform for Gene Editing Medicines

Broad Range of Sites



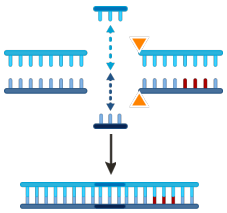
Wide Delivery Options

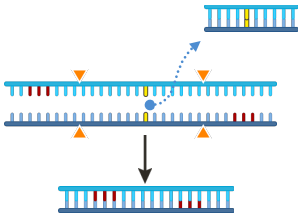

Viral Vector

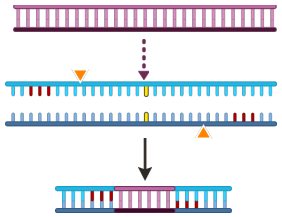

Lipid Nanoparticle


Electroporation

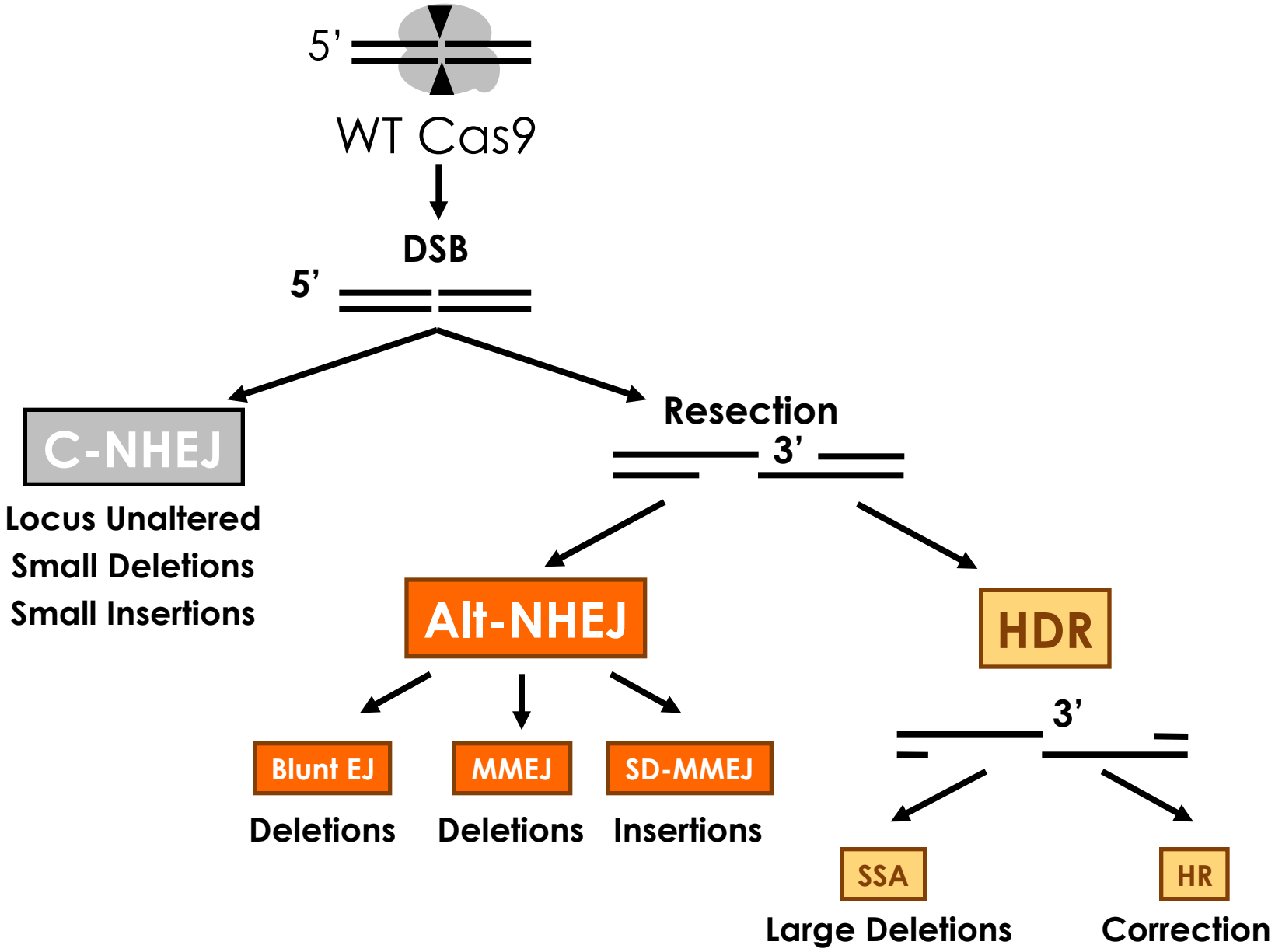
Diverse Spectrum of Edits



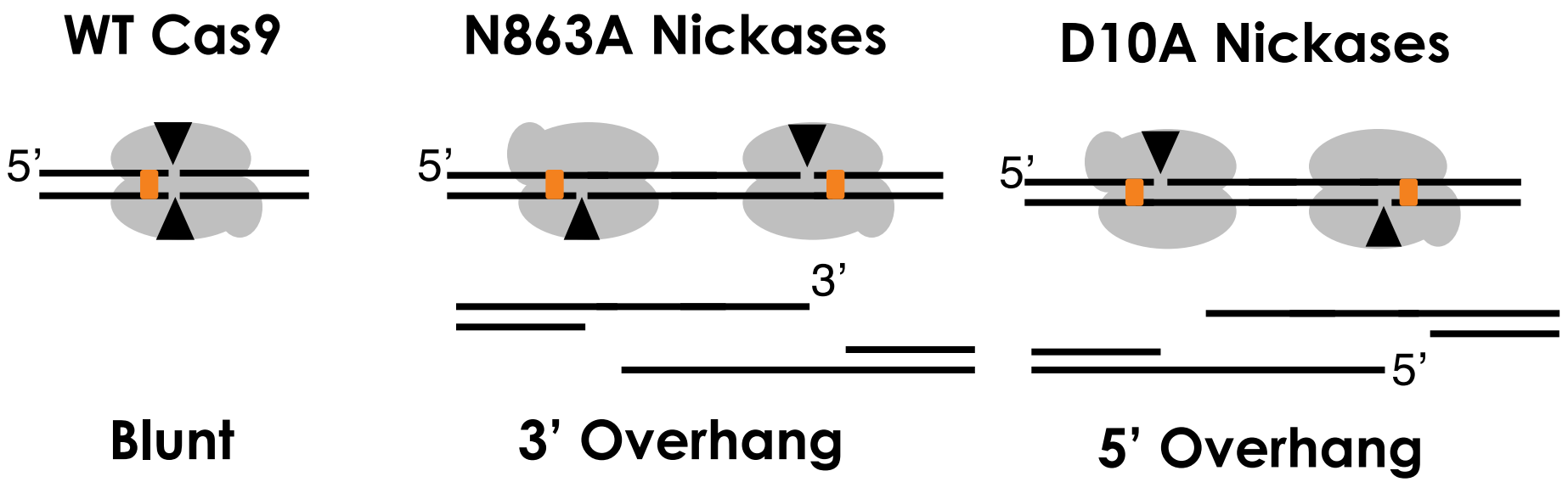




Cas9 Stimulates the Endogenous Repair Pathways

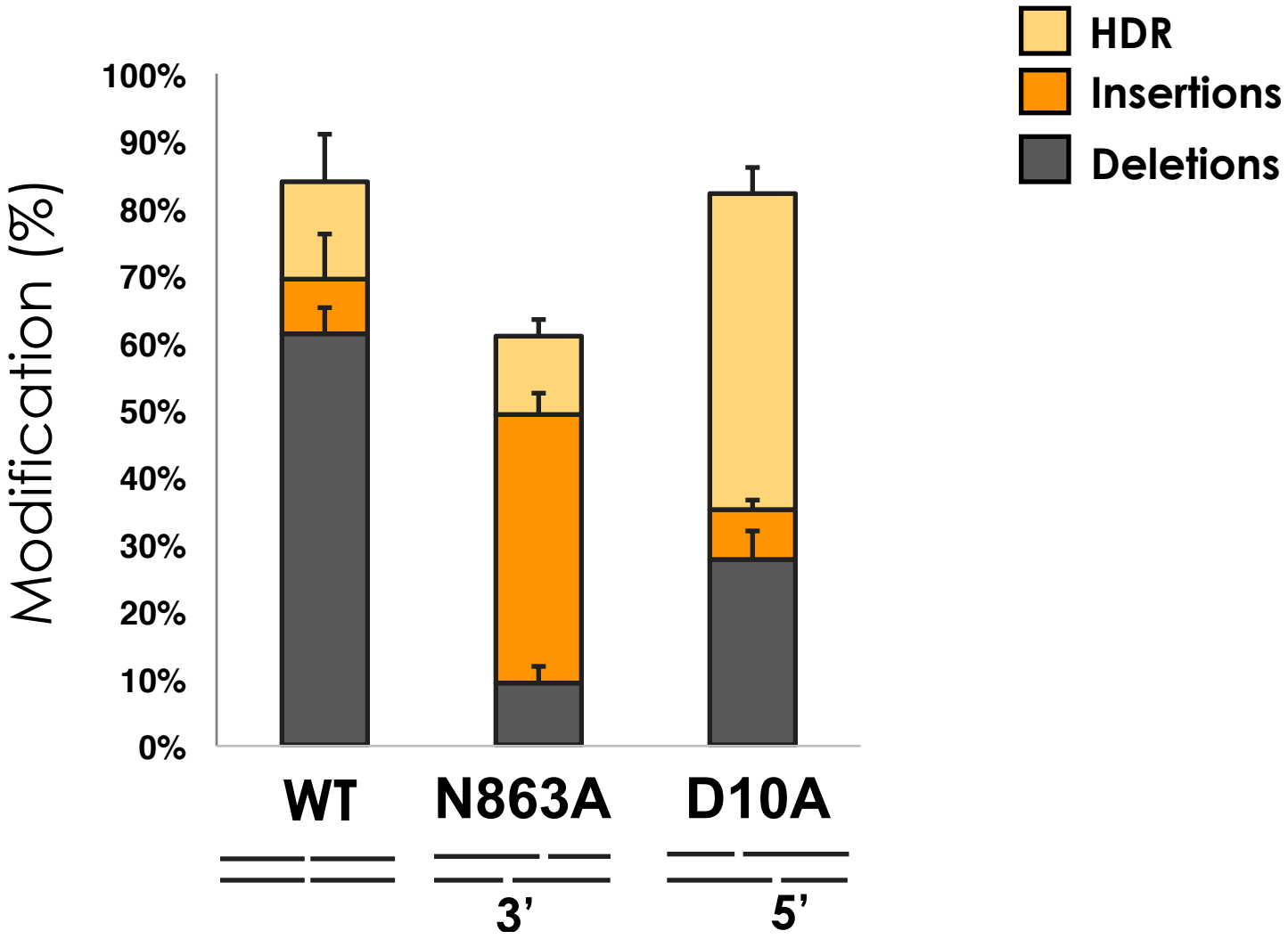


Cas9 is a Flexible Tool



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

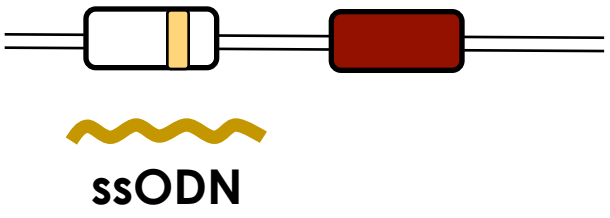
DSBs Generated by D10A are Predominantly Repaired by HDR



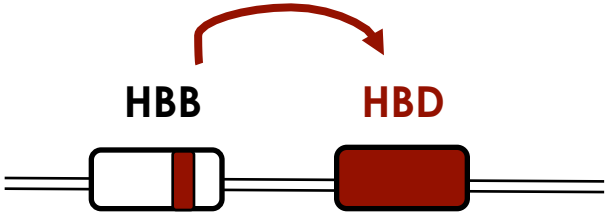
Bothmer et al., Nat Comm 2017

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

Gene Correction

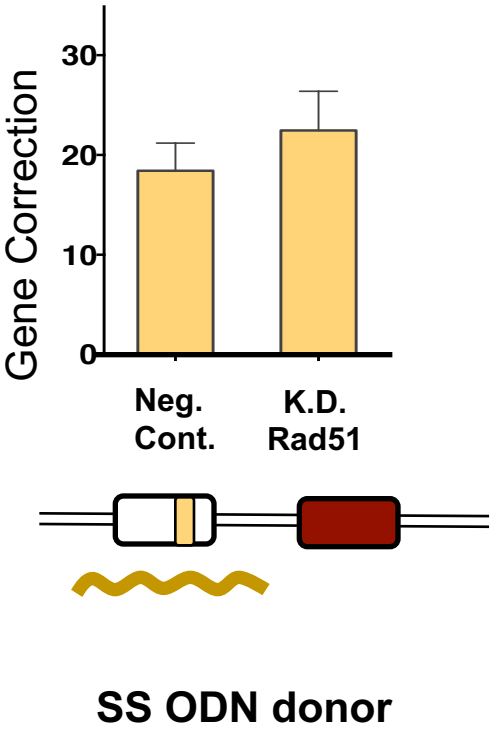


Gene Conversion

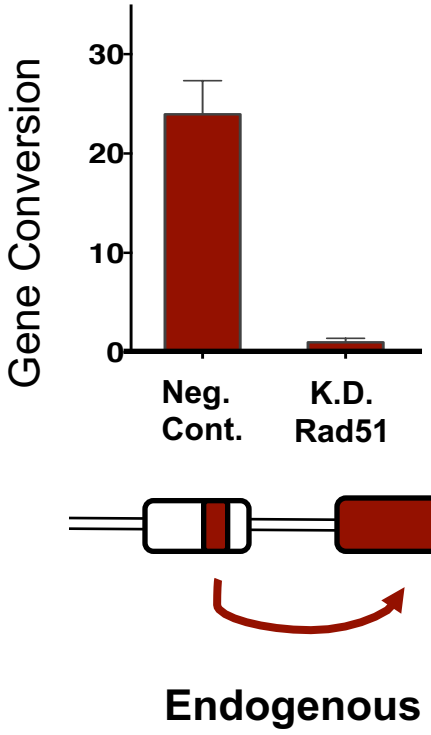


Do they both dependent on the HR pathway?

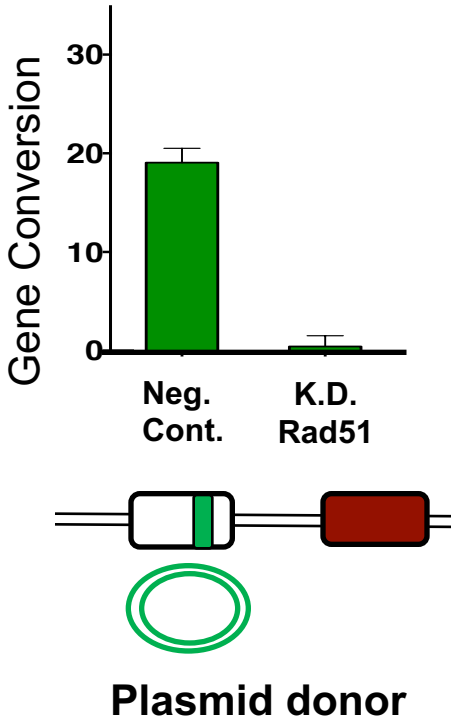
Gene Conversion and Gene Correction have Different Genetic Requirements



SS ODN donor



Endogenous HBD

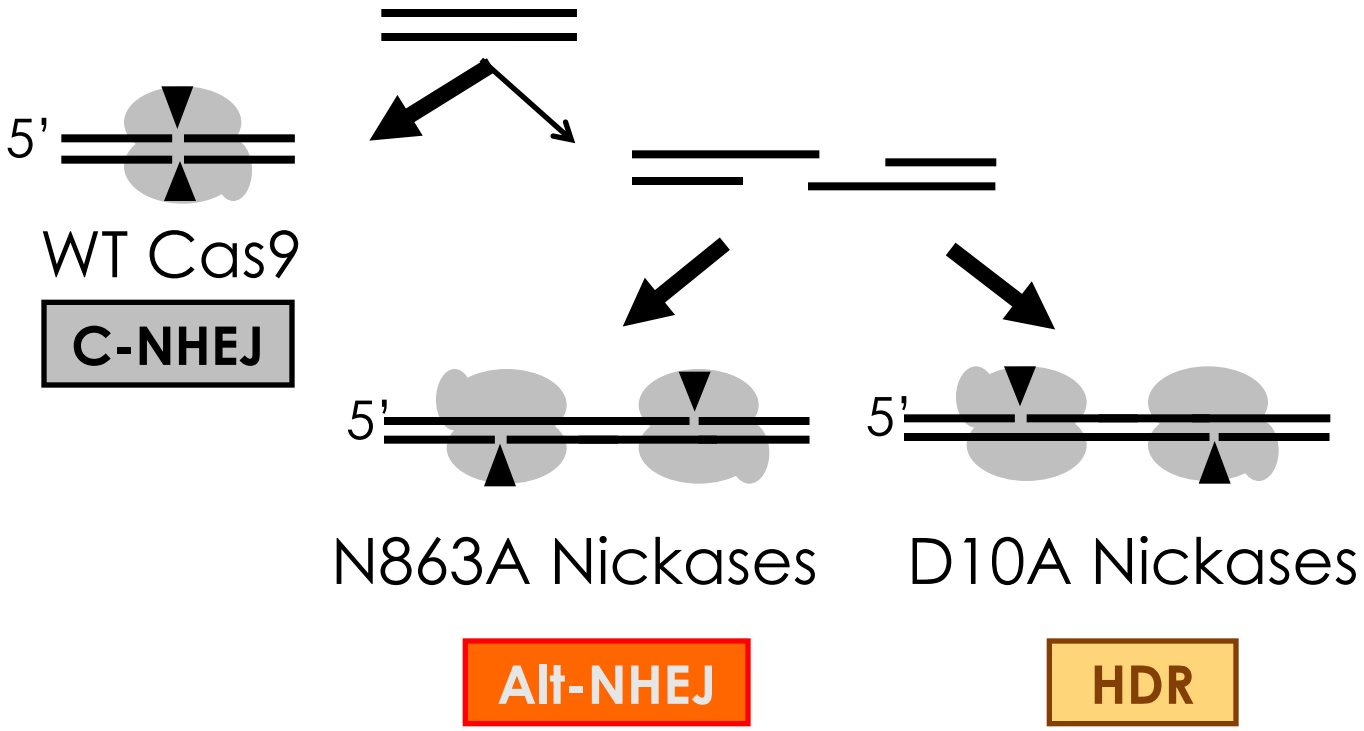


Plasmid donor

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

Summary

- We are developing precise and durable therapies by selecting and directing nuclease activity, delivery and the cellular repair response.
- We can effectively measure off-target and other genomic outcomes.
- We can make fully synthetic covalently-coupled dgRNAs of high quality.
- Our understanding of how different lesions are repaired and the connection with the nature of the donor allow us to engineer for the desired outcomes.

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