Bill, living with transthyretin amyloidosis, and his wife, Maura

Avoiding Unintended Genome Editing for CRISPR/Cas9 Therapeutics

24th Annual Meeting of the American Society of Gene and Cell Therapy

Daniel J O'Connell, Ph.D. | May 10, 2021

Disclosure: Employee of Intellia Therapeutics, Inc.

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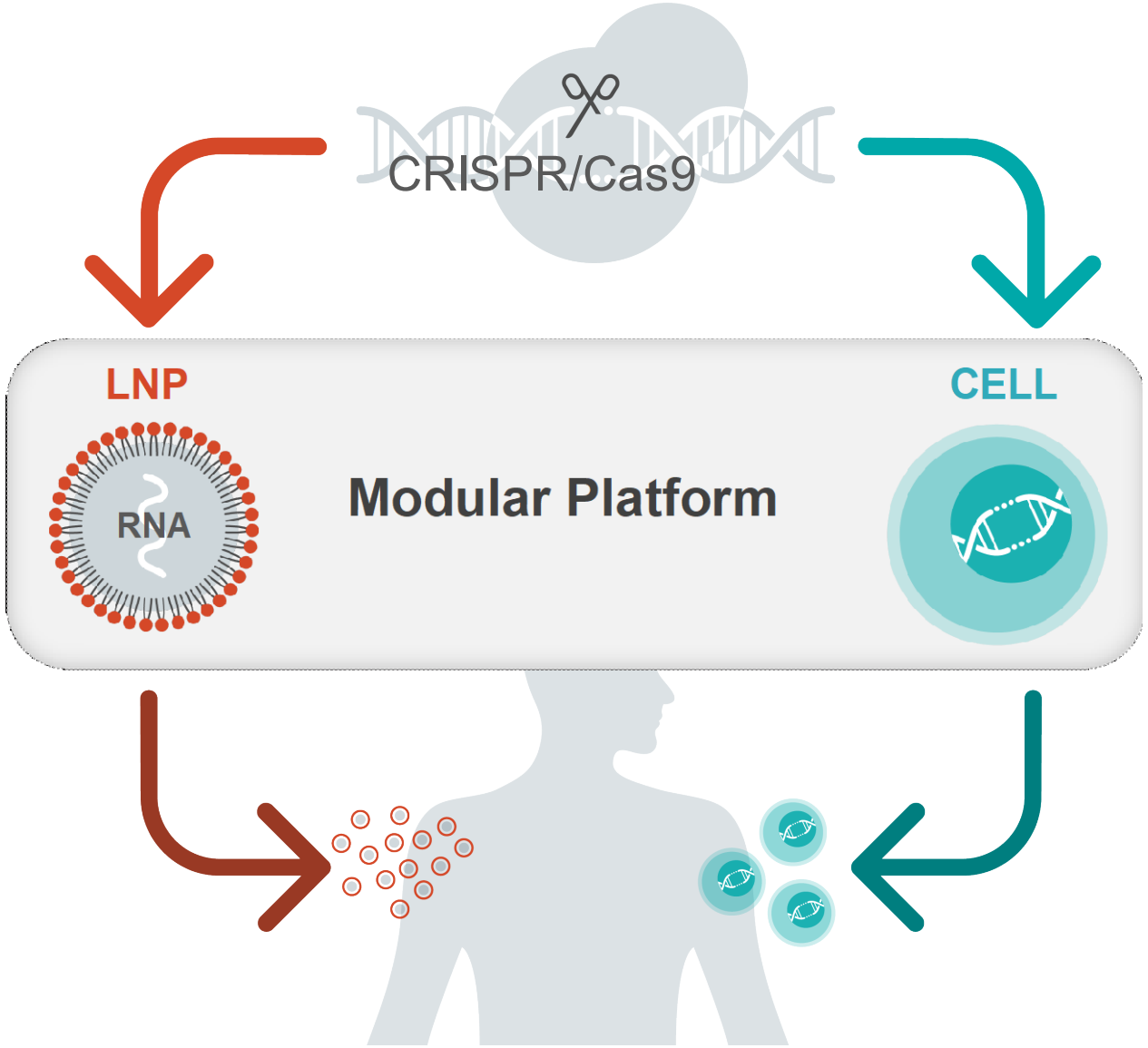
Developing CRISPR/Cas9 Genome Editing Therapies

In Vivo

CRISPR is the therapy

Non-viral delivery

Transient CRISPR expression and activity



Ex Vivo

CRISPR creates the therapy

LNP: Lipid Nanoparticle

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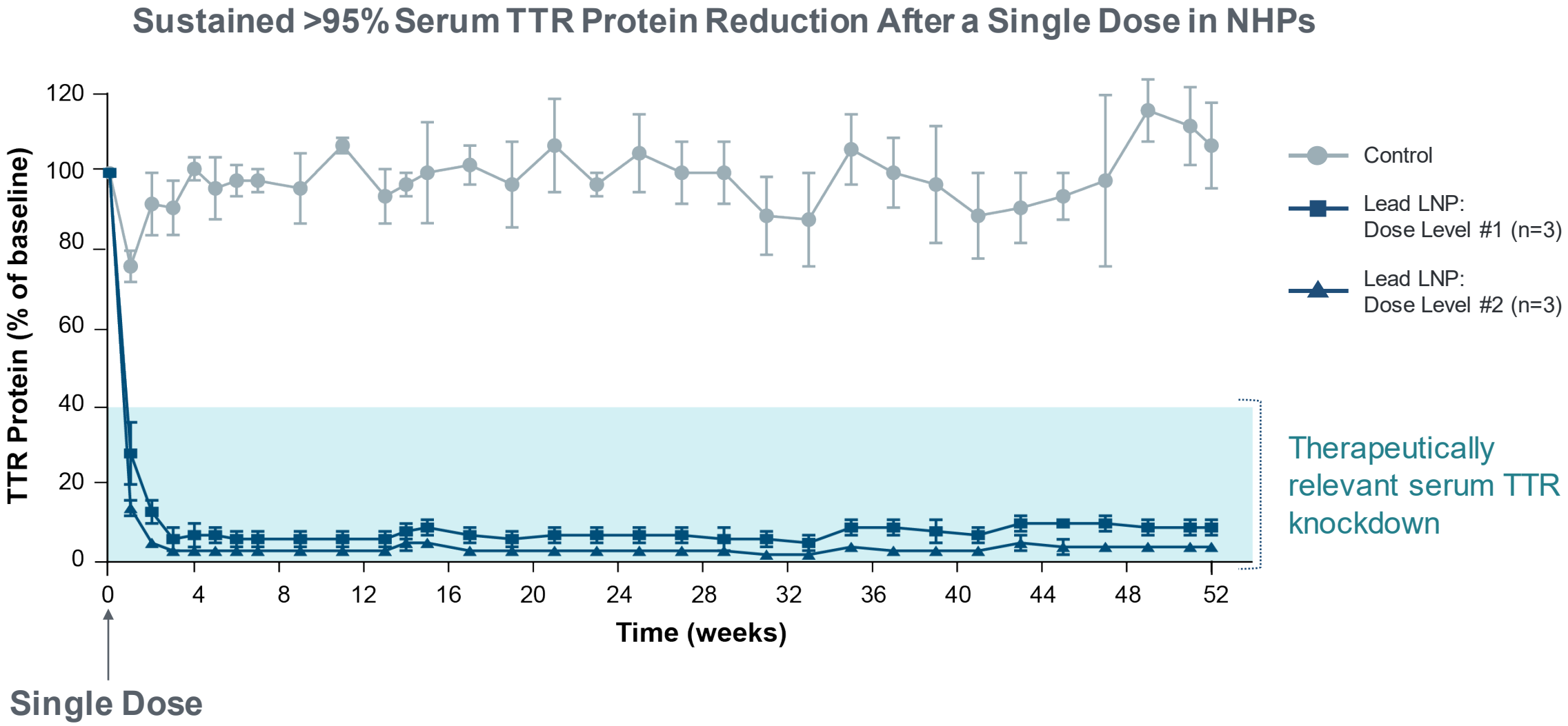
NTLA-2001 Phase 1 Follows Successful Preclinical Proof-of-Concept



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Key Attributes for Identifying Therapeutic guide RNA (gRNA)

High Efficacy

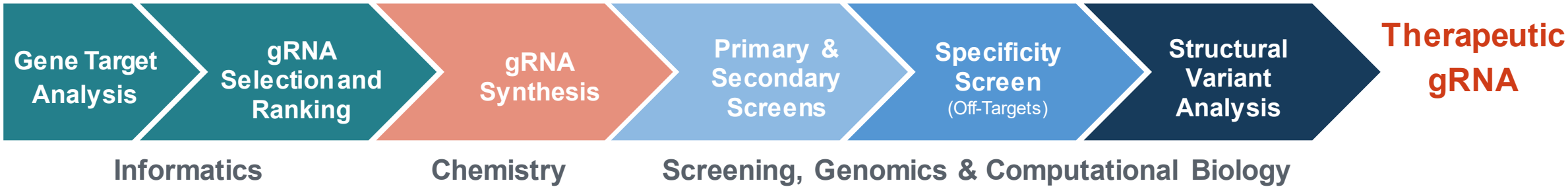
- Edit the genome at the intended target site
- Target site conserved across patient population
- High potency
- Edit results in desired pharmacological outcome

High Specificity

- Avoid validated unintended edits elsewhere in the genome
- Avoid DNA structural variants associated with toxicity and transformation
- Genotoxicity safety window vs. expected therapeutic exposure

Industrialized gRNA Selection and Qualification Platform

Intellia's workflow identifies the most active and specific gRNAs for therapeutic genome editing of a given target



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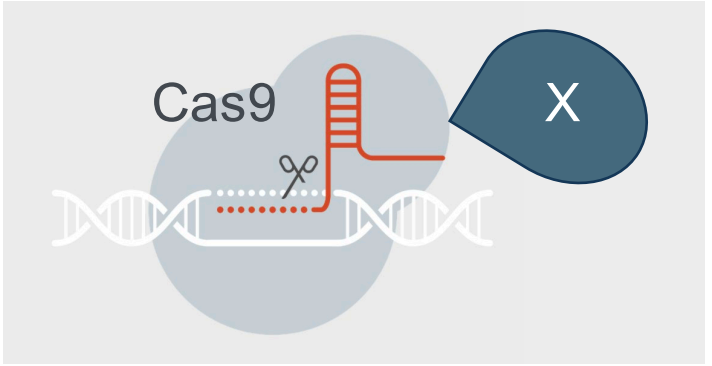
Potential Off-Target Editing with CRISPR/Cas9 is Exclusively RNA-Dependent

CRISPR Cleavase*

gRNA sequence-dependent off-target editing only

Other Modalities



gRNA sequence-dependent off-target editing

+

Stochastic, gRNA sequence-independent off-targets
Dependent on X functionality

*Cas9 adopts an auto-inhibited conformation until properly bound to target site

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Comprehensive gRNA Specificity Assessment: Off-Target Workflow

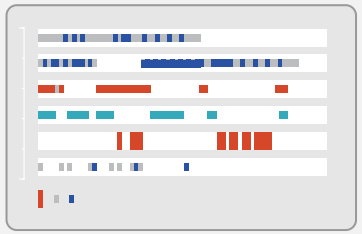
1: Discovery of Potential Off-Target Edits

- Computational prediction
- \+
- SITE-Seq Genomic DNA digest
- \+
- GUIDE-seq Cell-based DNA repair

Aggregate **ALL** potential off-target genomic loci

2: Validation of Off-Target Edits in Cells

- Multiplex panel for NGS
- Targeted Amp-Seq NGS follow-up



Ex Vivo

- Cell drug product

In Vivo

- Edit cells in vitro using drug product formulation
- Therapeutically relevant human primary cell type(s) (2 donors)
- Dose range to exceed projected therapeutic exposure (≥10X)

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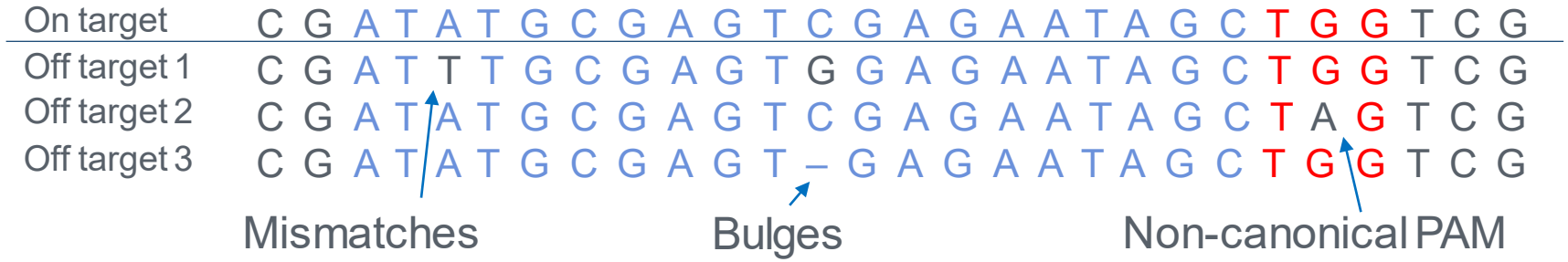
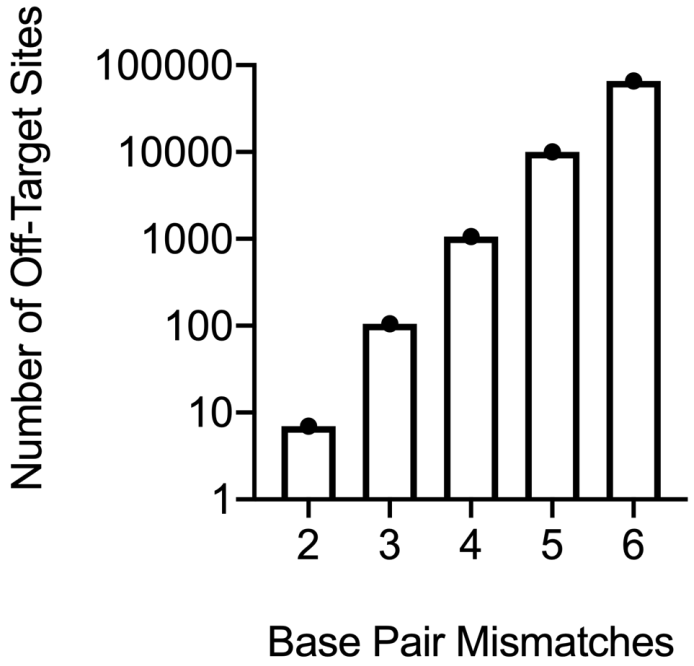
Computational Prediction of Off-Target Editing

Computational prediction





- gRNA target sequence defines on and off-target sequence space
- Number of predicted potential off-target loci has a log-linear relationship with the number of allowed mismatches
- Cas-OFFinder conditions <4 mismatches in non-coding DNA and up to <5 mismatches in exonic DNA

Bioinformatics. 2014; 30(10):1473-1475

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Unbiased Empirical Genome-Wide Off-Target Discovery with NGS

SITE-Seq[1]	GUIDE-Seq[2]
Biochemical based, cell-free	Cell-based
Deproteinated DNA + Cas9-gRNA	Nuclear chromatin DNA + Cas9-gRNA + ODN
Most permissive cleavage	Cellular delivery, off-target access restrictions
Supra-physiologic concentrations of Cas9	Restricted by cellular delivery of Cas9
Cleavage, no repair	Cleavage plus cellular repair
Representative of all tissues	Limited to available primary cell types
Higher Sensitivity	Lower Sensitivity
Lower Specificity	Higher Specificity

False negatives in potential off-target editing discovery cannot be tolerated

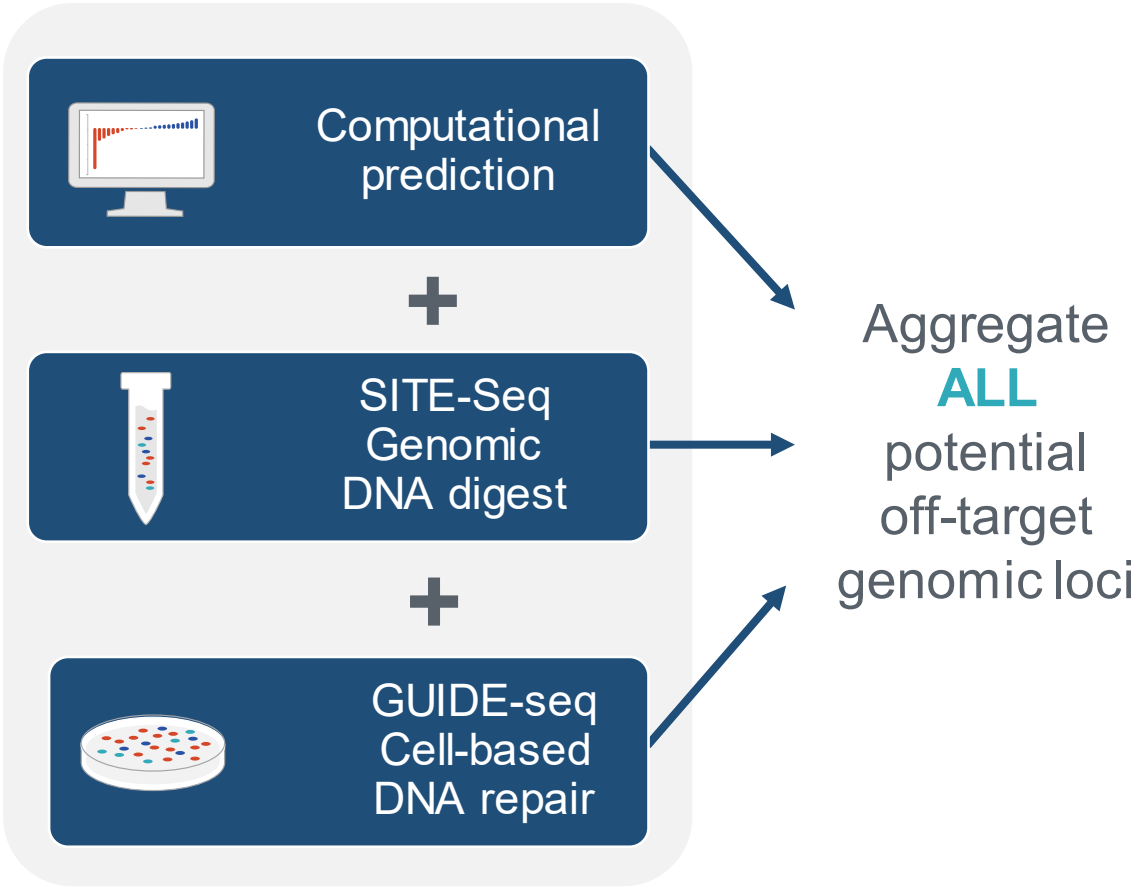
ODN = oligodeoxynucleotide

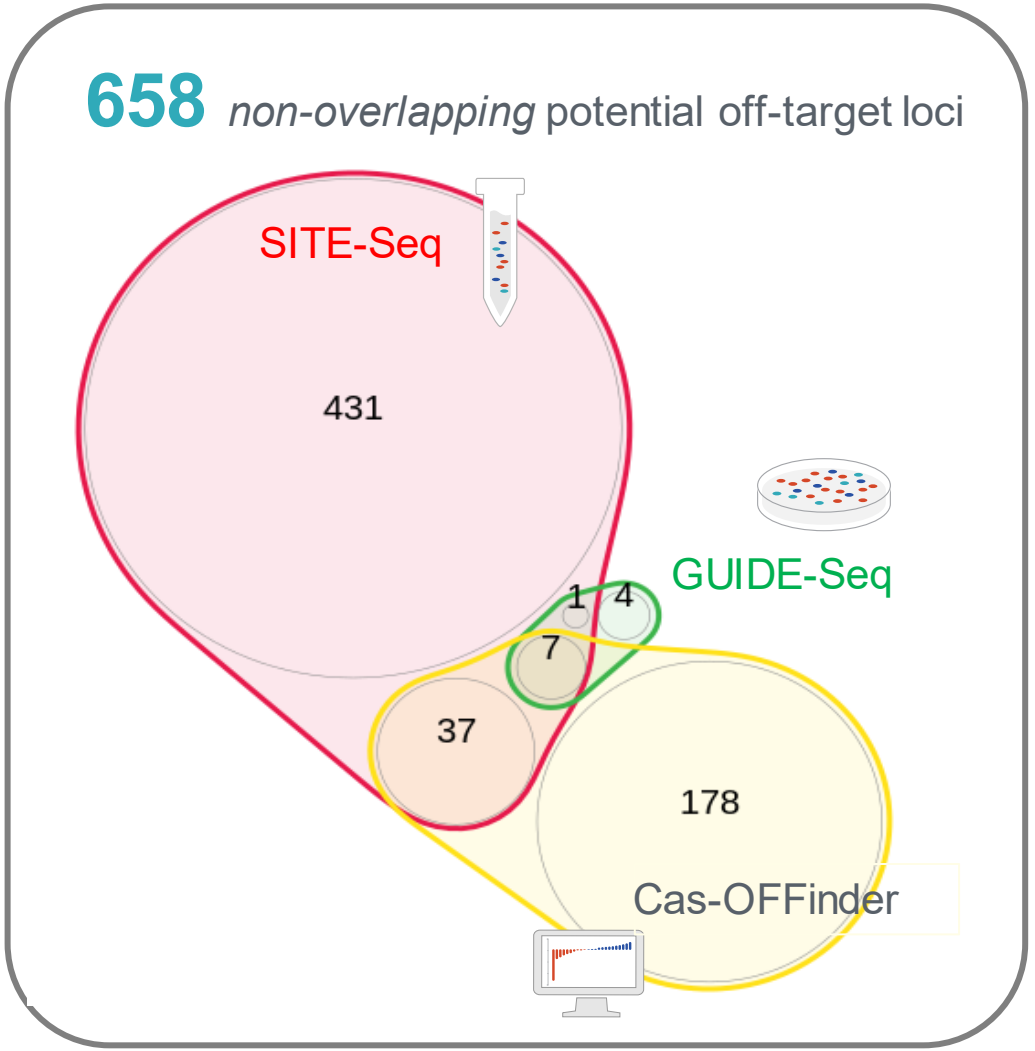
[1]*Nature Biotechnology* 2015; 33(2):187-197, [2]*Nature Methods*. 2017; 14(6):600-606

Limited Overlap in Discovered Off-Target Loci by Three Leading Methods

Representative Example

1: Discovery of Potential Off-Target Edits





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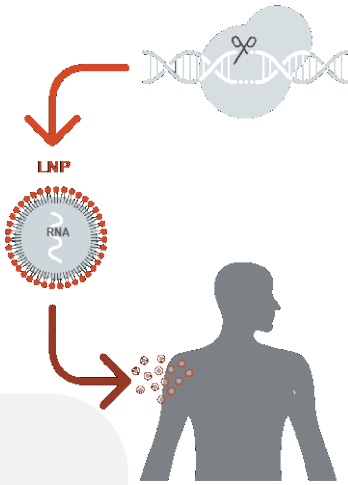
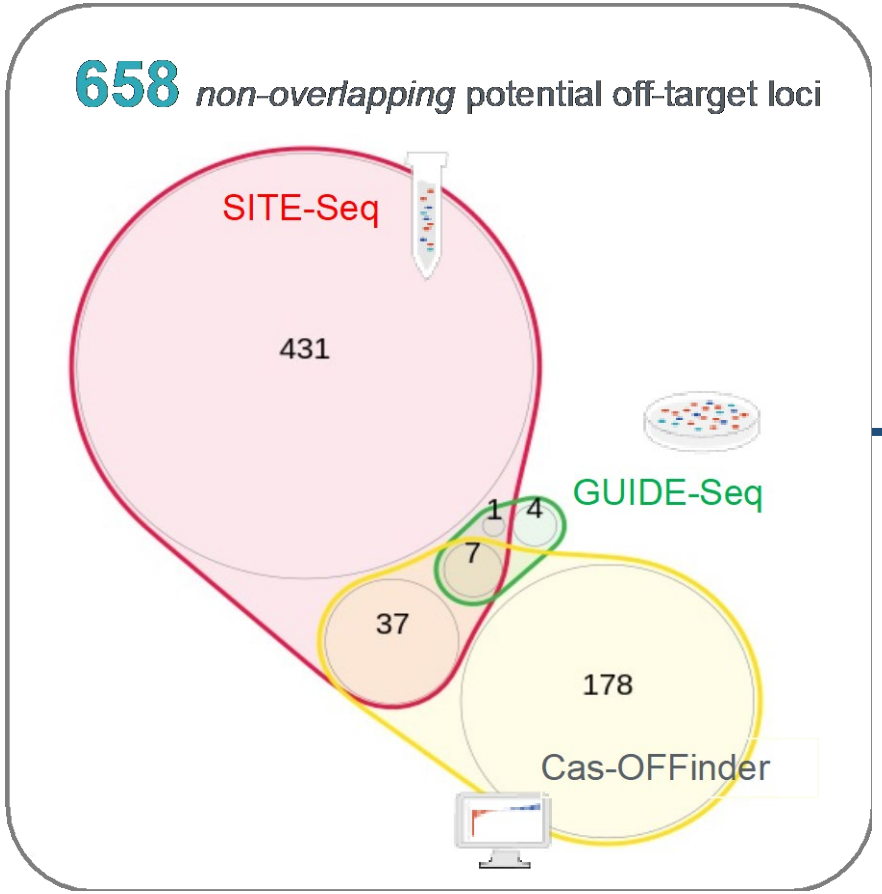
Off-Target Workflow In Practice: Representative Example



1: Discovery of Potential Off-Target Edits



2: Validation of Off-Target Edits in Cells

Multiplex panel for NGS

Targeted Amp-Seq NGS follow-up

In Vivo Programs

- Dose responses using drug product formulation
- Therapeutically relevant human primary cell type(s) (2 donors)
- Dose range to exceed projected therapeutic exposure (≥10X)
- Validation: off-target indels detected in edited cells

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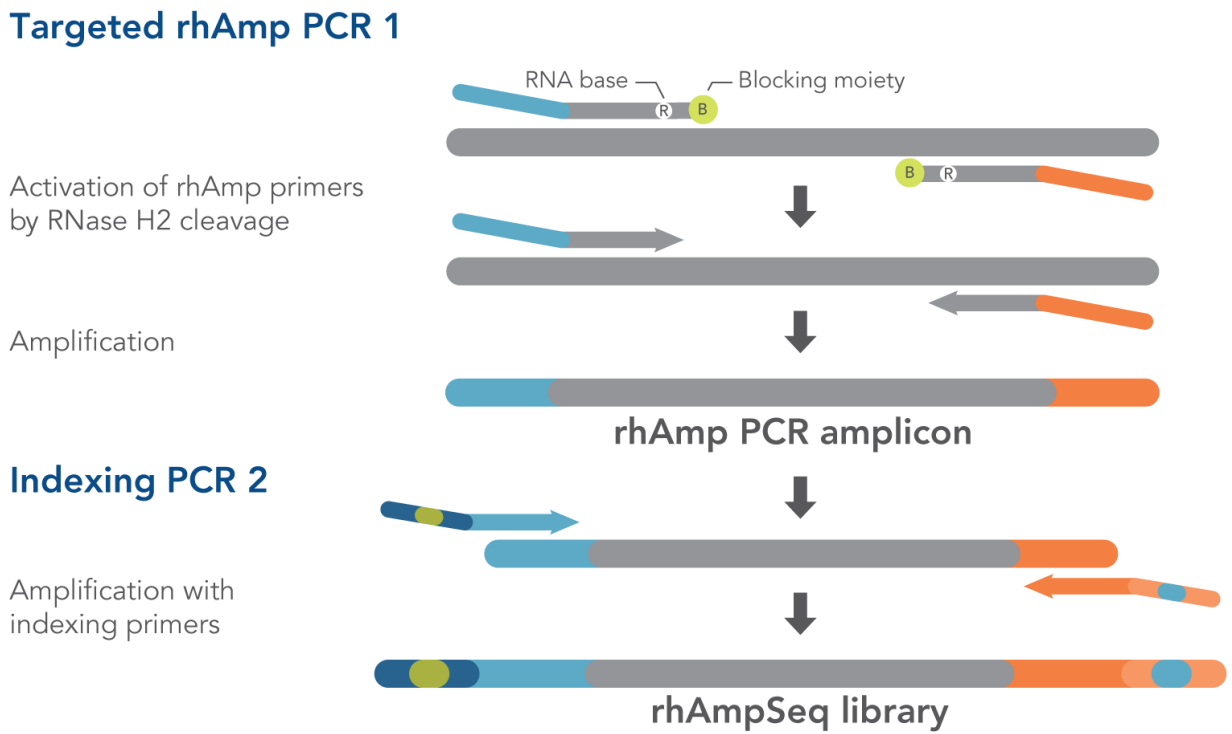
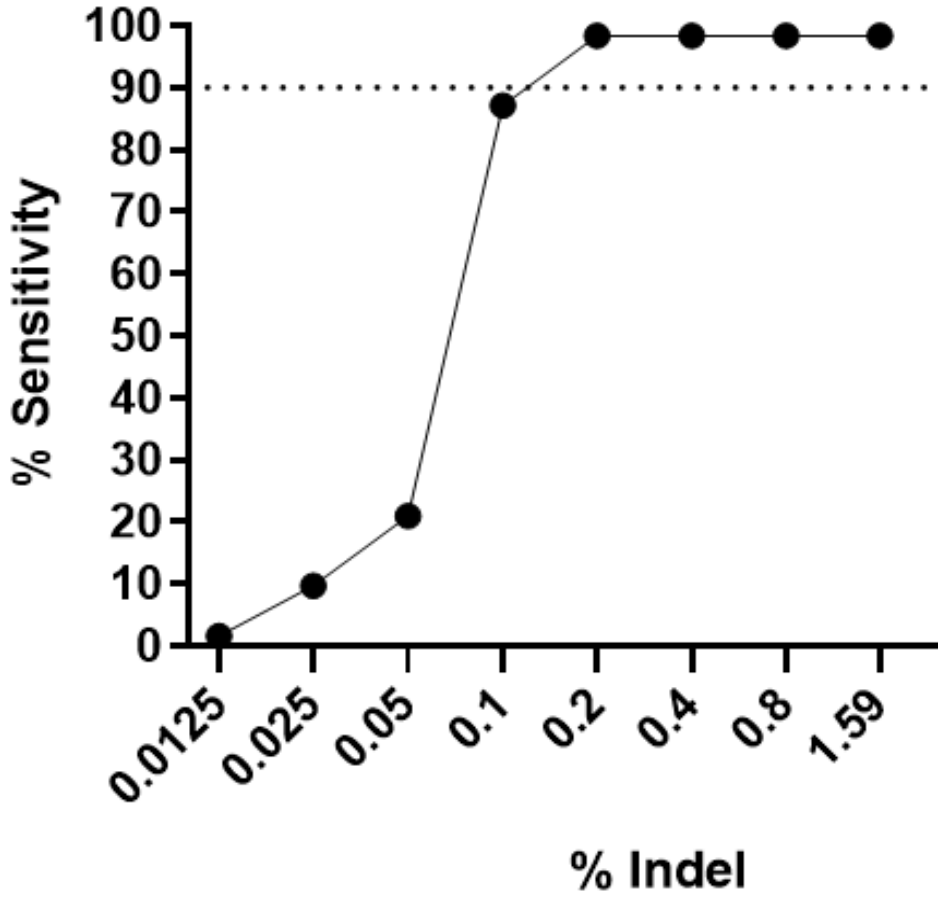
Validation of All Potential Off-Target Editing Loci is Performed by rhAmpSeq

- NGS libraries are generated for all potential off-target loci
- Multiplex primers allow the enrichment of >1,000 loci in a single PCR reaction
- rhAmpSeq libraries are evaluated by Illumina NGS
- Loci that are not captured by rhAmpSeq are characterized by standard singleplex Amp Seq



rhAMPSeq Sensitivity Determined Using Well-Characterized Natural Indels

- NIST **Genome in a Bottle Consortium** has a catalog of the best characterized human genomes
- 62 naturally-occurring indels (1-20 bp) amongst a variety of DNA sequence contexts were curated from individuals NA12878 and NA24385
- Genomic DNA from NA12878 was titrated against NA24385, with 10,000 reads targeted per loci
- Down-sampling revealed a minimum of 1,000 sequencing reads from 100ng gDNA are necessary to achieve **>90% indel sensitivity down to 0.2% frequency**



National Institute of Standards and Technology: Genome in a Bottle Consortium
https://www.nist.gov/programs-projects/genome-bottle

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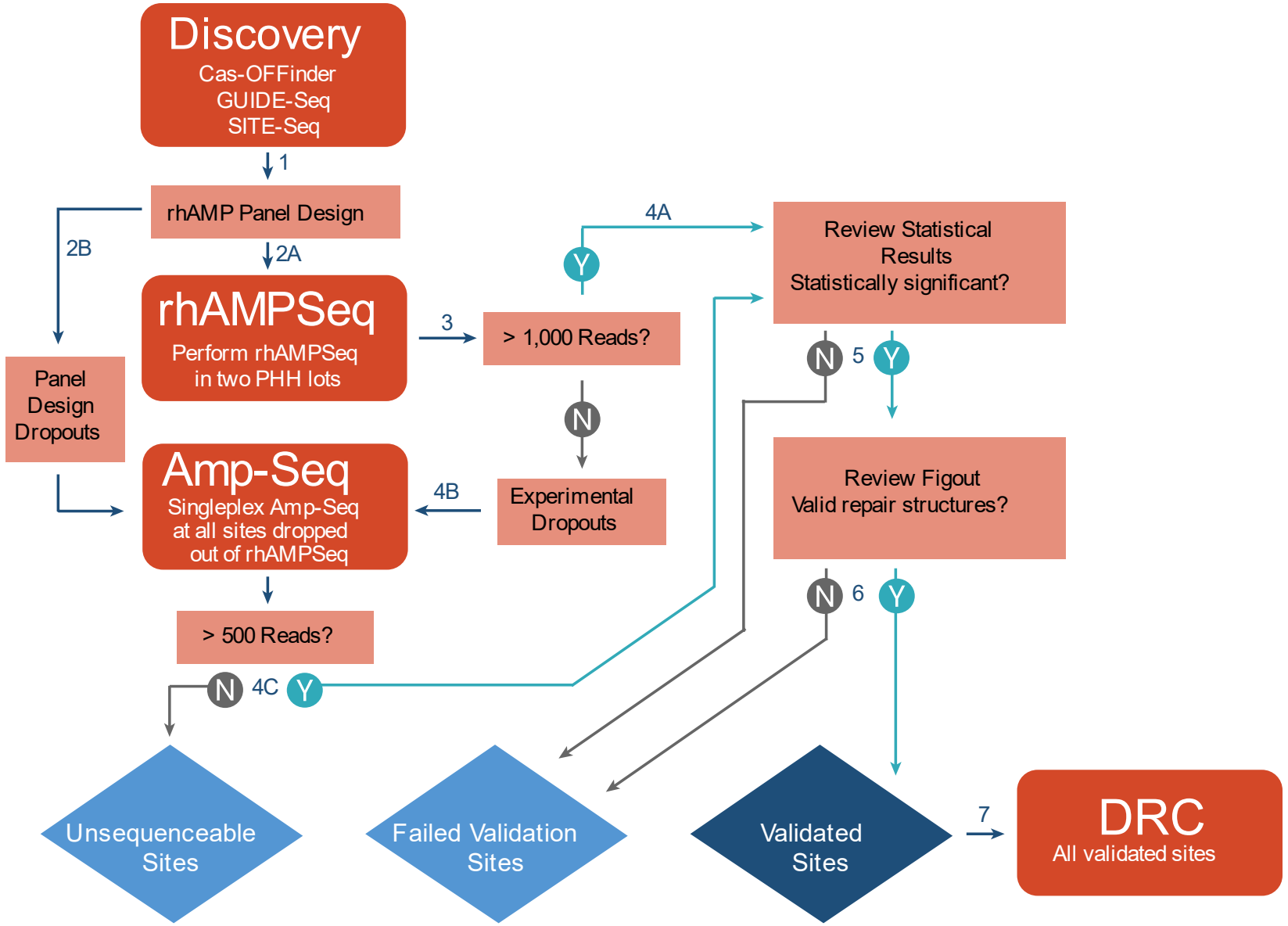
Potential Off-Target Editing Characterization Workflow



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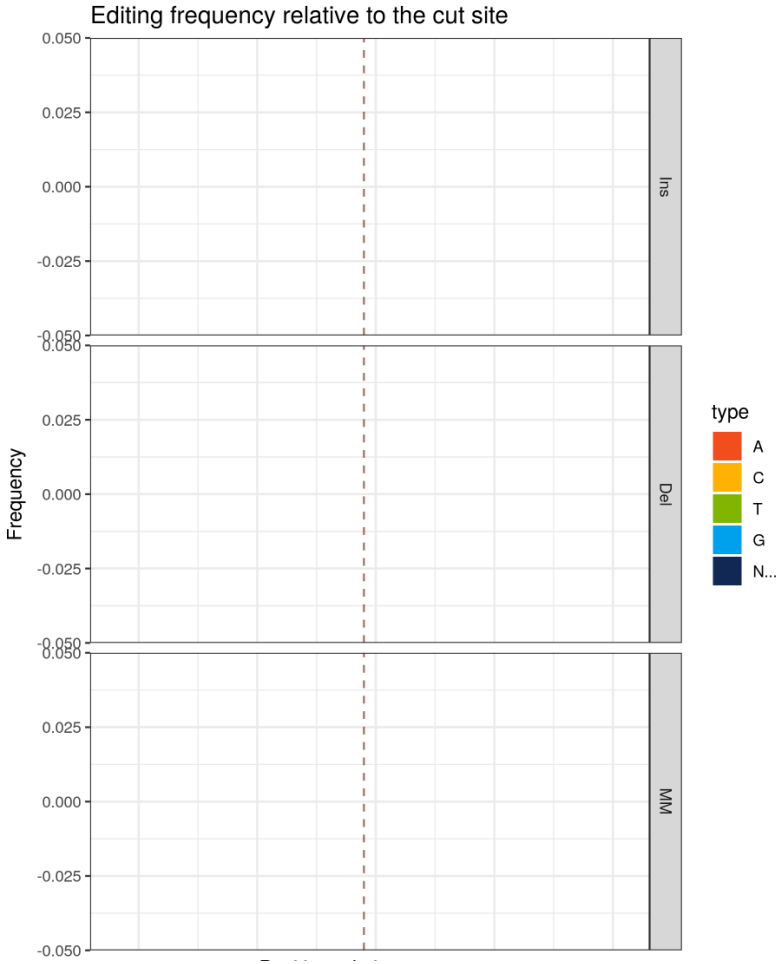
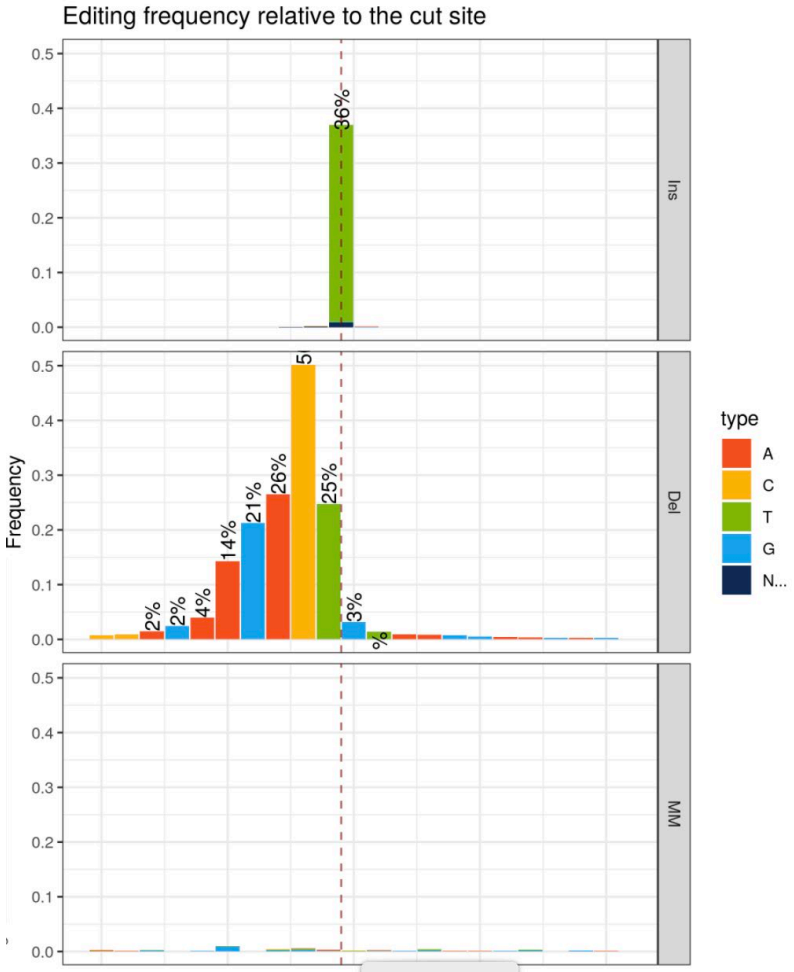
Manual Review of Off-Target Indel Editing Characterization

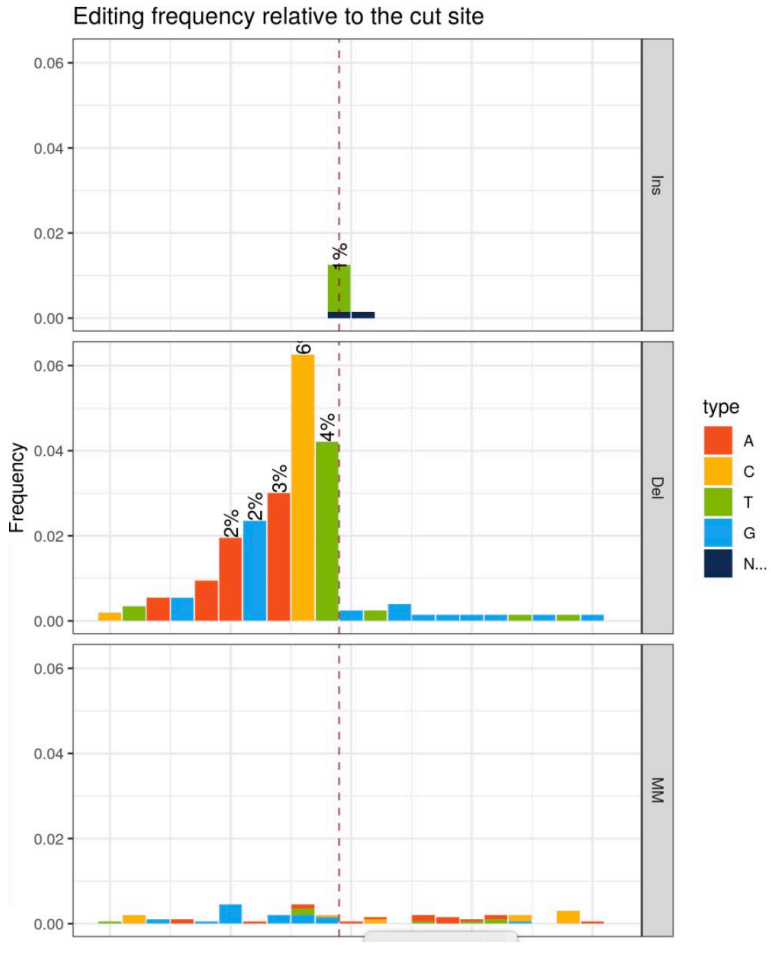
On-Target Control



On-Target Edit

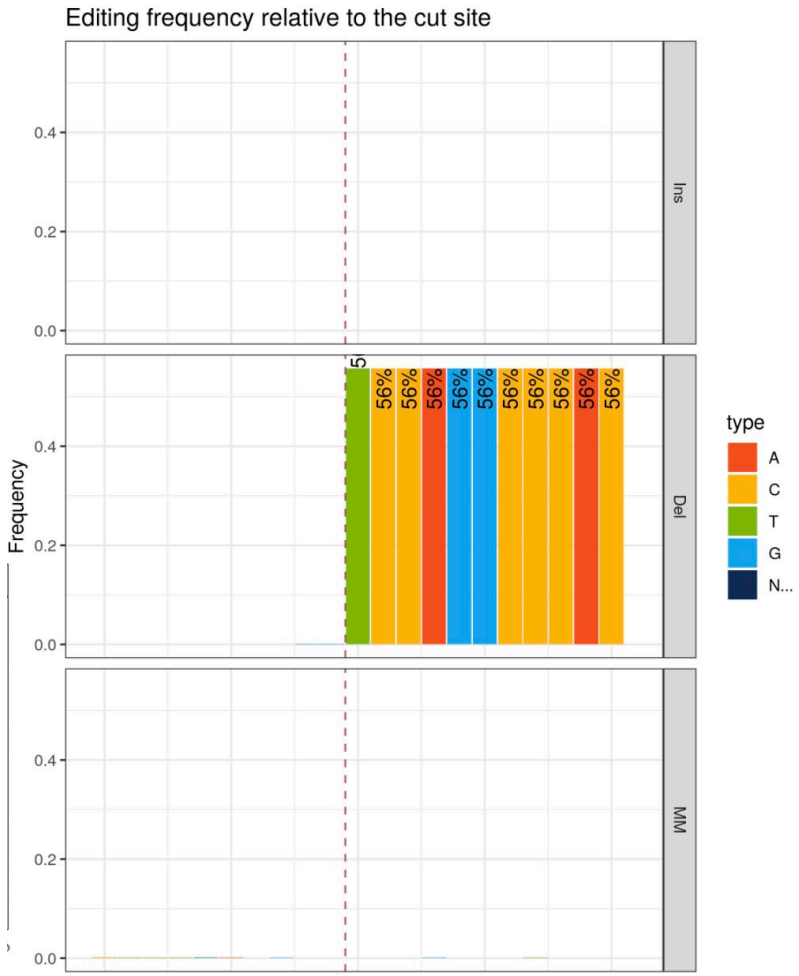


Validated Off-Target Edit



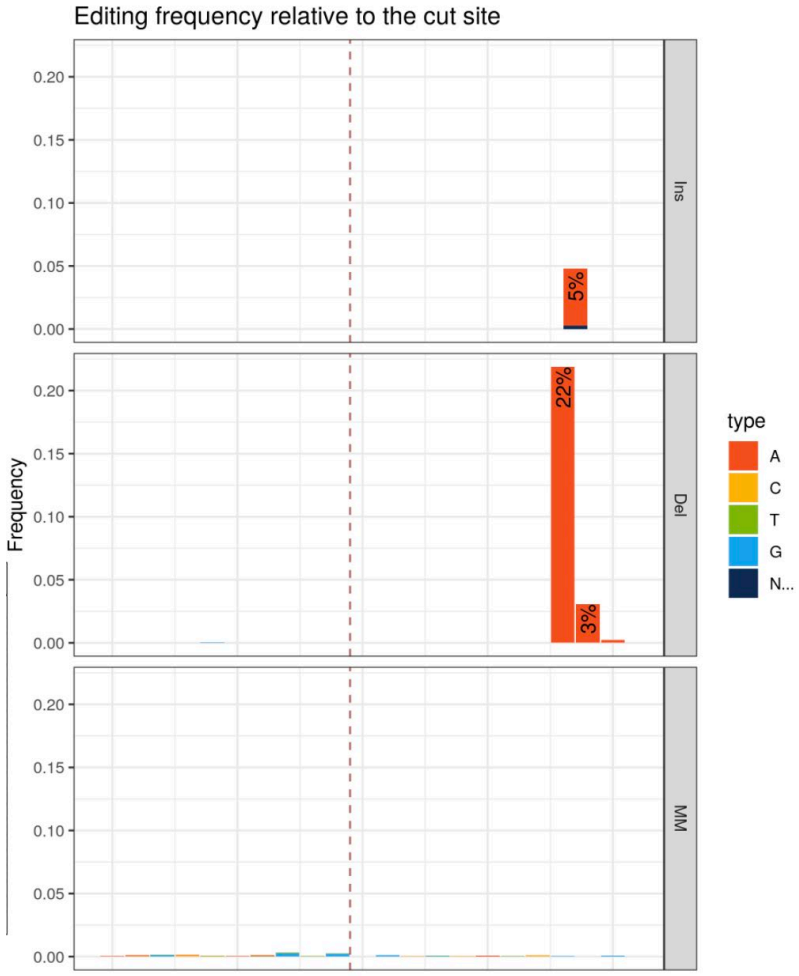
Common Errors in NGS Indel Detection

Natural Deletion



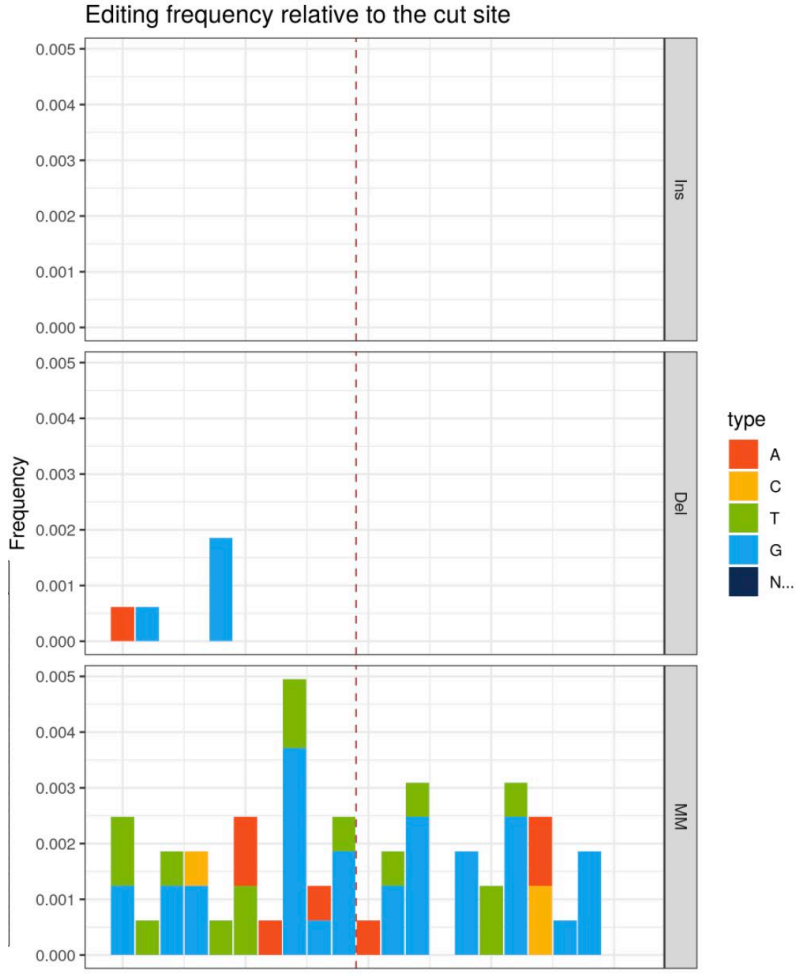
Position Relative to Cut

Slippage



Position Relative to Cut

Poor Alignment



Position Relative to Cut

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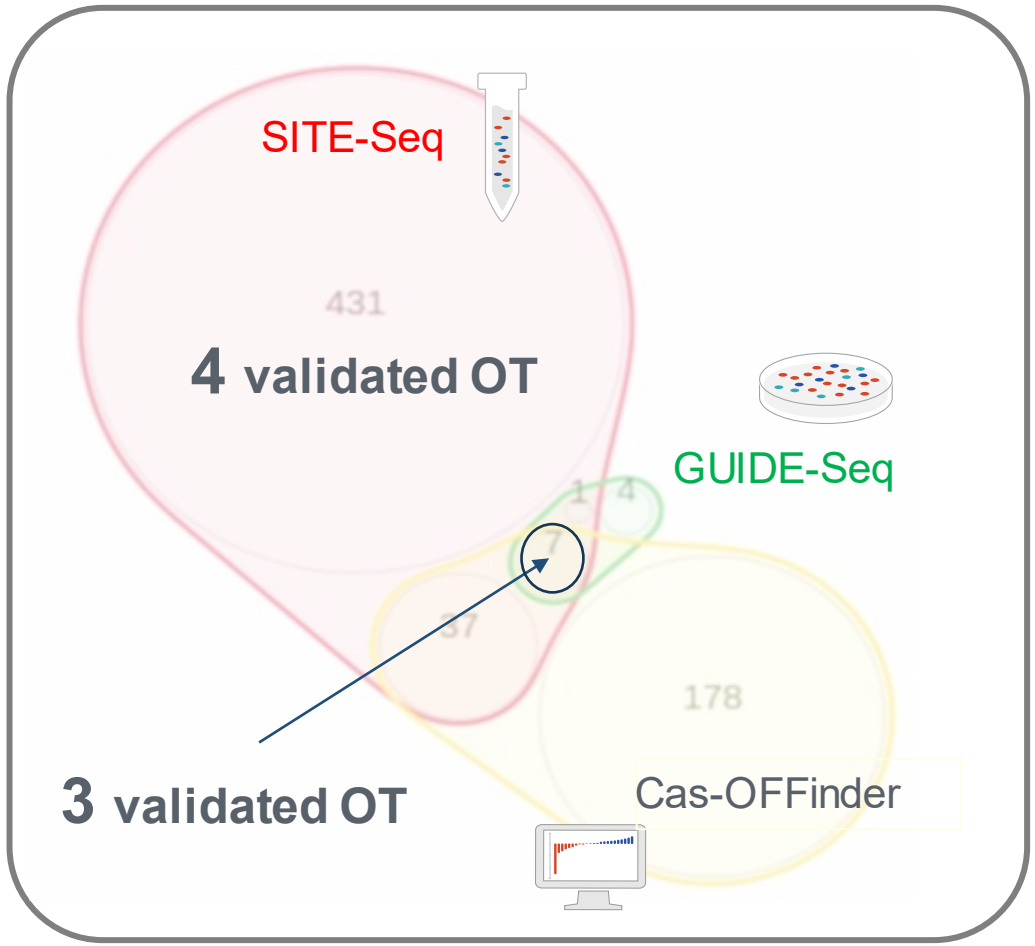
Validation of Off-Target Editing in Primary Human Hepatocytes at Supersaturating LNP CRISPR Concentrations to Maximize Sensitivity



658 potential off-target loci

7 validated off-target (OT) loci

2 in introns and 5 in intergenic regions

- SITE-Seq discovered **100%**
- GUIDE-Seq and Cas-OFFinder discovered the same 3 out of 7 validated off-target loci **43%**
- Eliminate gRNA with validated off-target indels in regions of the genome associated with cancer

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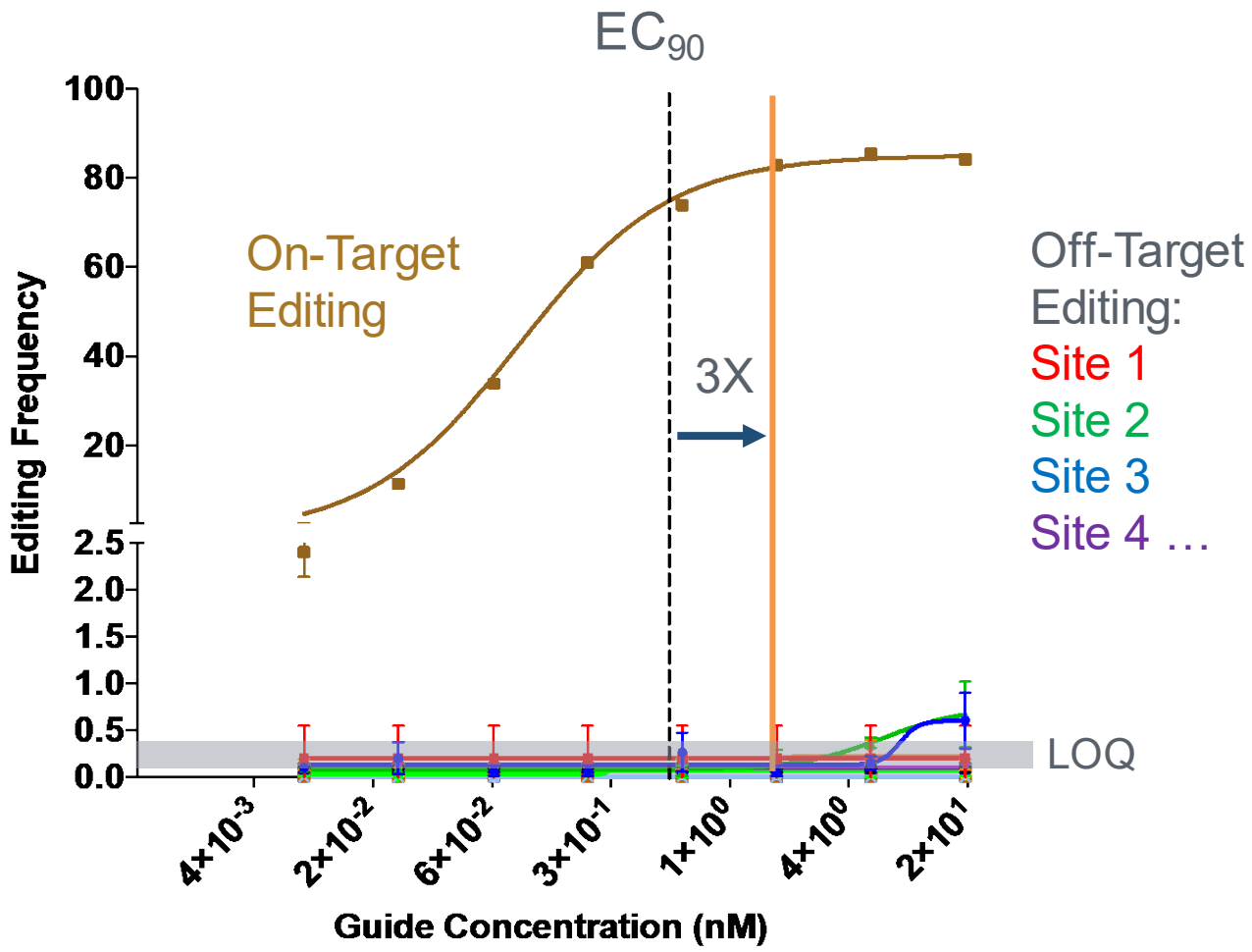
Zero Detectable Off-Target Editing Observed at LNP Concentrations Up To 3X Greater than Pharmacologic Dose



- Dose response in primary human hepatocytes (2 donors)
- Super-saturating concentrations of LNP CRISPR/Cas9 exceeding what is pharmacologically achievable *in vivo*
- Large genotoxicity safety window

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Key Takeaways

1. Selection of gRNAs for therapeutic gene editing with CRISPR/Cas9 requires in-depth analysis of off-target editing and unintended DNA structural variants
2. Comprehensive off-target characterization consists of discovery and validation phases
   - Off-target editing *discovery* using a biochemical approach has proven superior to the widely used cell-based experimental technology
   - Off-target editing *validation* of potential loci with targeted sequencing is done in primary cells representative of the intended target tissue
3. Therapeutic gRNA can be identified and qualified to have high activity and high specificity, with a large genotoxicity safety window

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