Advancing CRISPR Technologies for Therapeutic Application

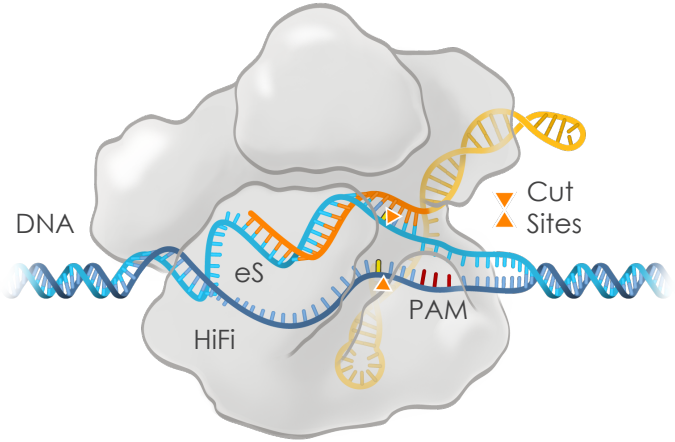
Christopher Wilson

TIDES: Oligonucleotide & Peptide Therapeutics

May 3, 2017

CRISPR Unlocks Genome Editing

Editing machinery can be engineered to target nearly any genomic location



Nuclease

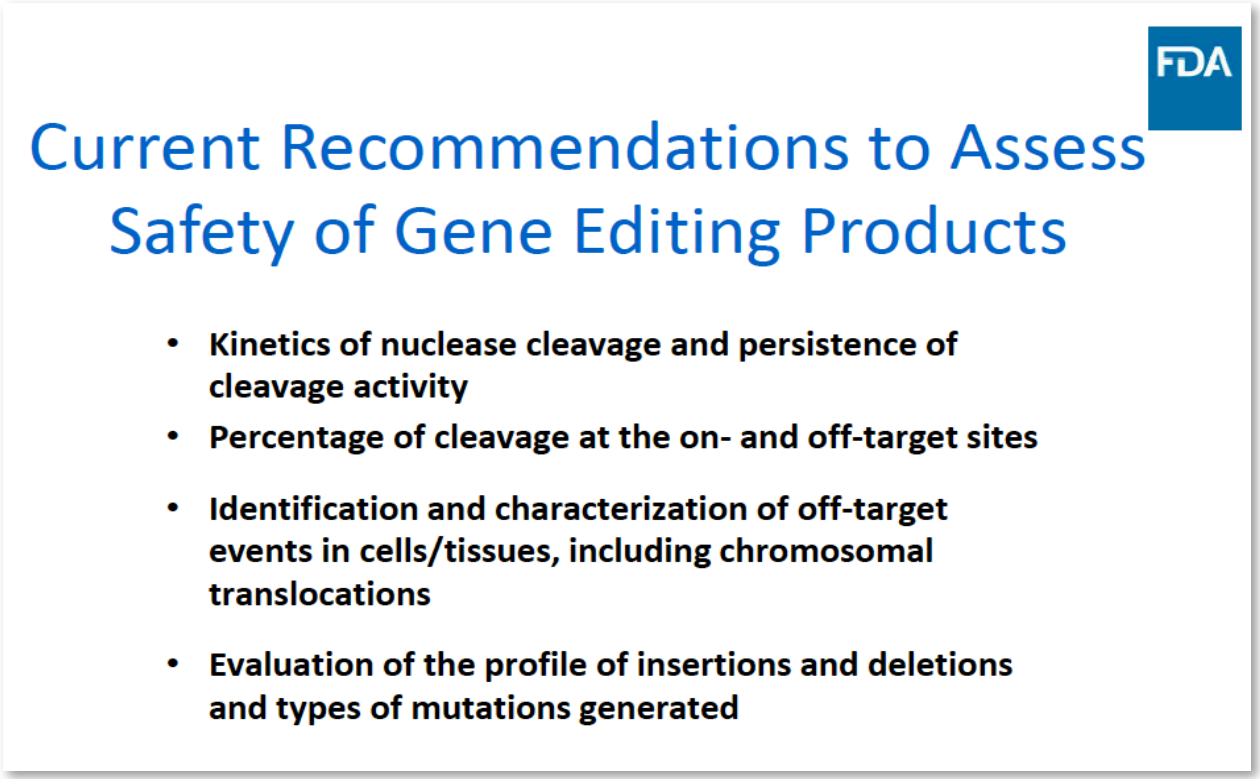
Guide RNA

- 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

Drug Development Fundamentals

- Reliably manufacture high quality drug substance
- Key understandings:
  - Pharmacokinetics: “what the body does to the drug”
  - Pharmacodynamics: “what the drug does to the body”



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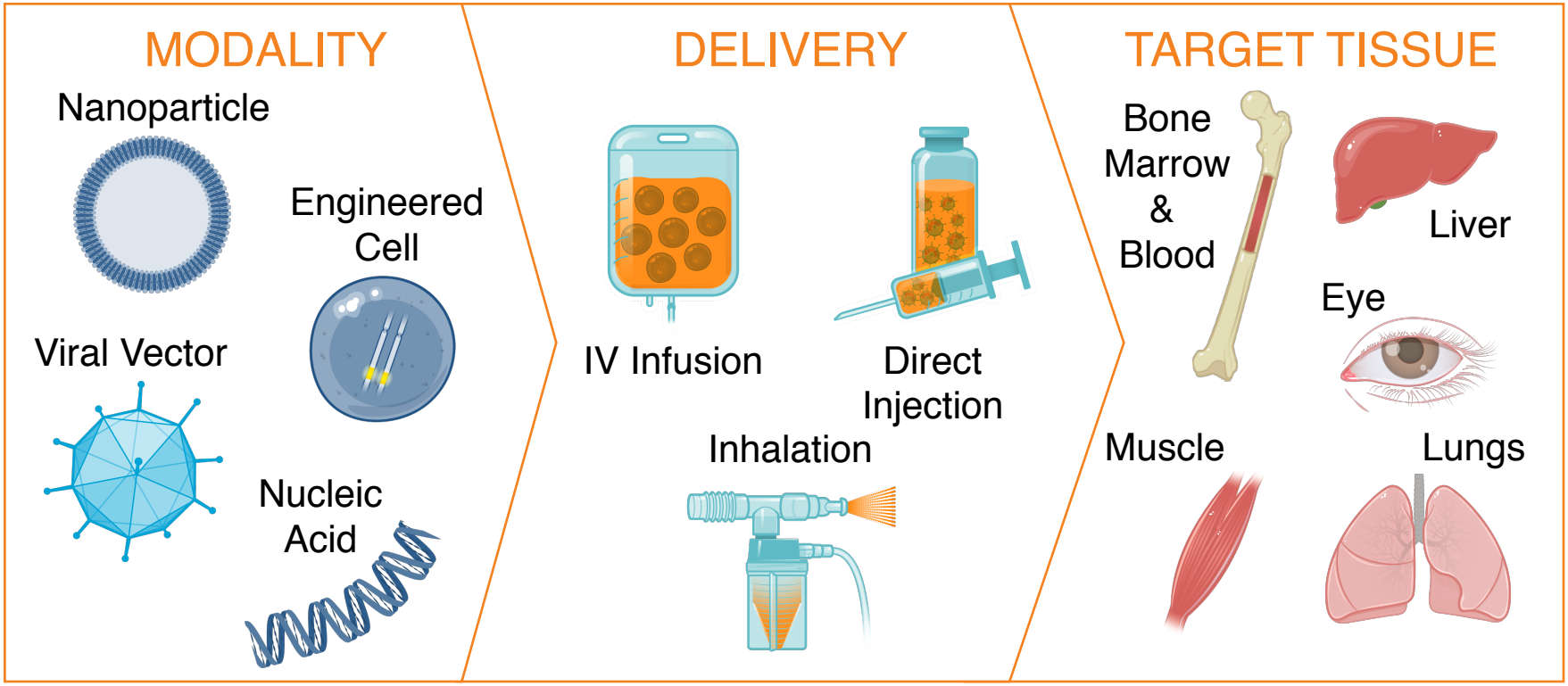
Strategy to Widely Enable Efficient Delivery

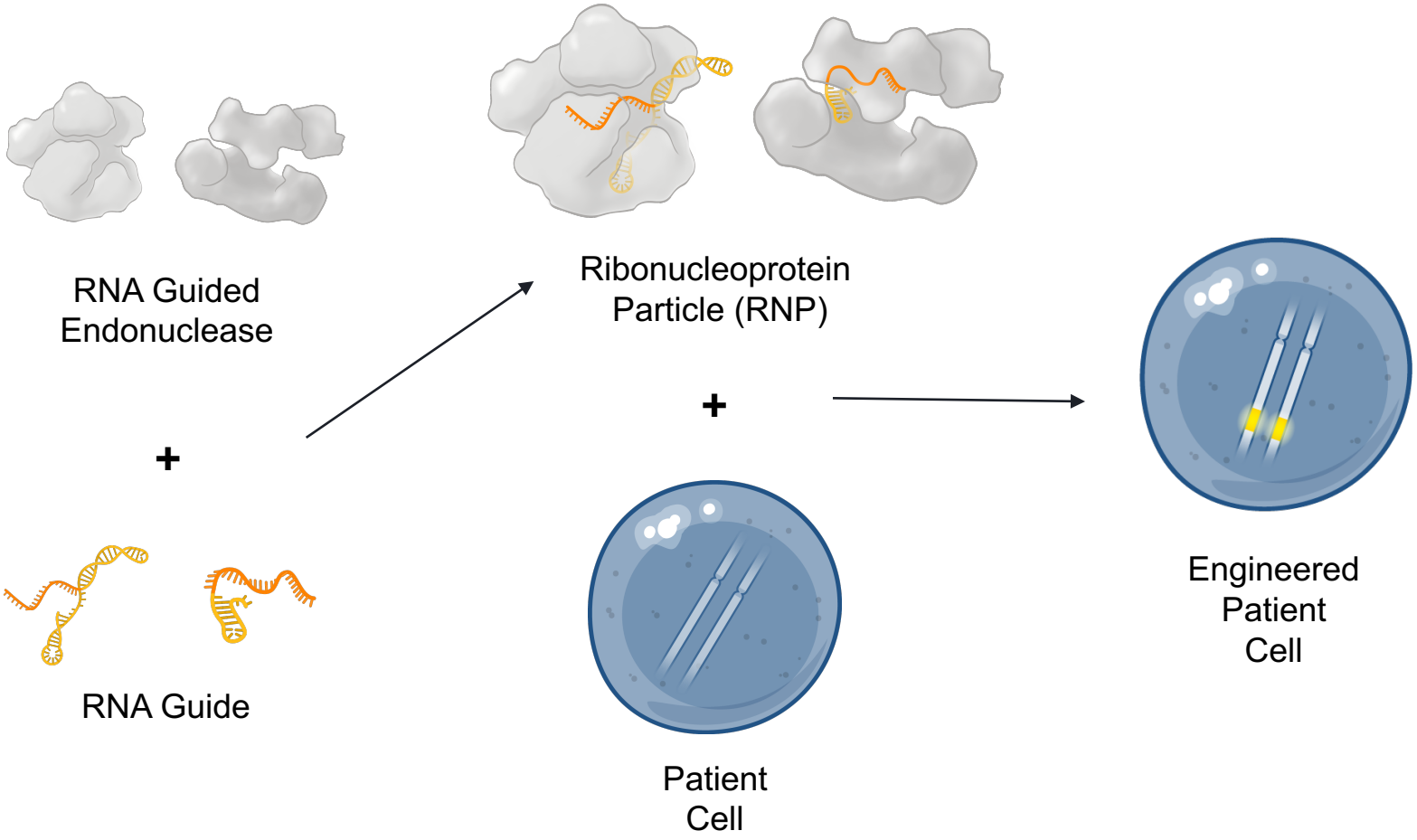
Success across a spectrum of delivery modalities in preclinical studies



- Tailor delivery approach for each product candidate to match specific disease
- Leverage existing technologies while investing in new approaches

High Quality Autologous Drug Development

Engineered autologous therapy requires multiple components

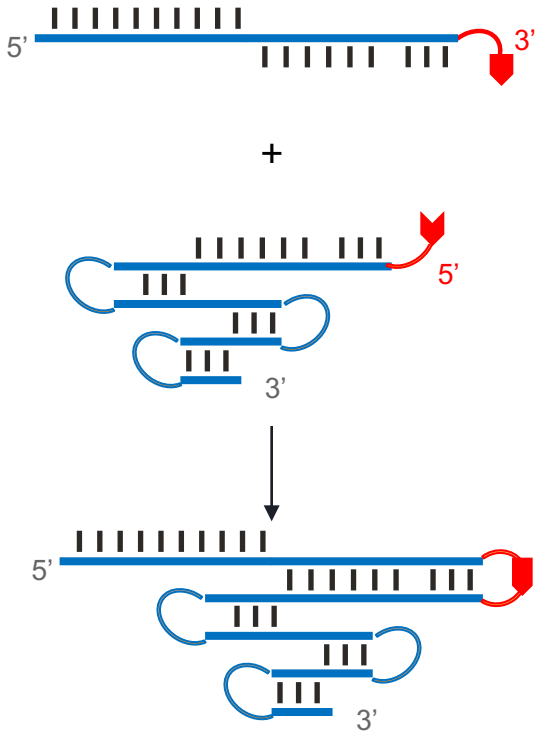


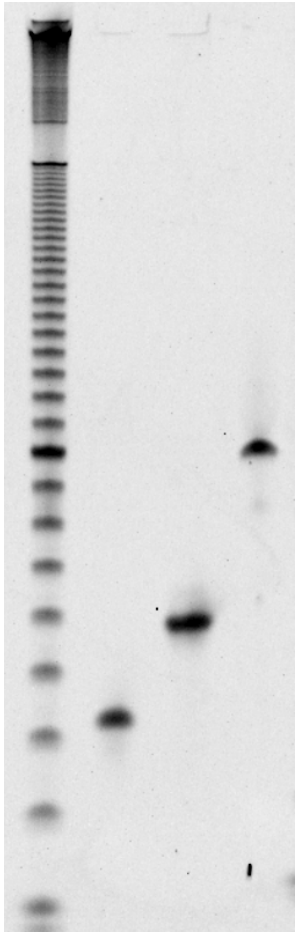
Generating Covalently-Coupled Dual gRNA

A completely non-enzymatic process for guide production

Why make a synthetic guide?

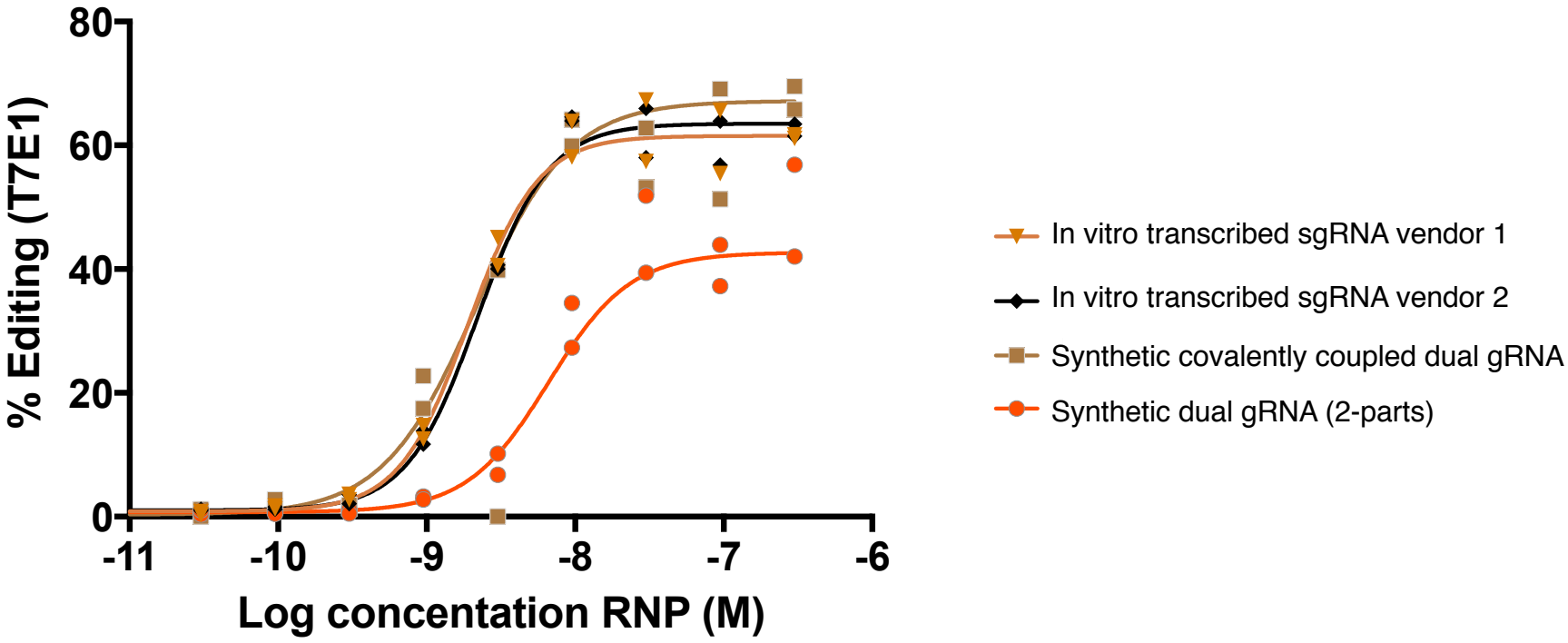
- Targeted chemistries anywhere in the molecule
- Unhindered ends and modifications
- Scale up and purity are more compatible with CMC requirements





Cellular Editing Activity

In vitro transcribed & synthetic gRNAs are equivalent in cells

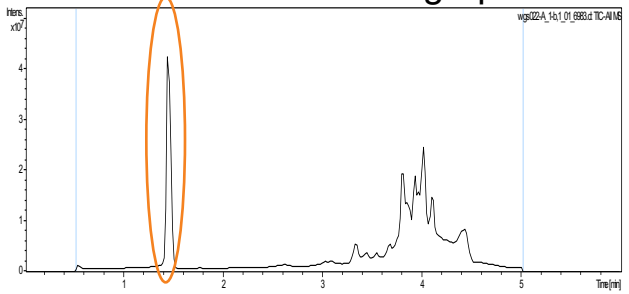


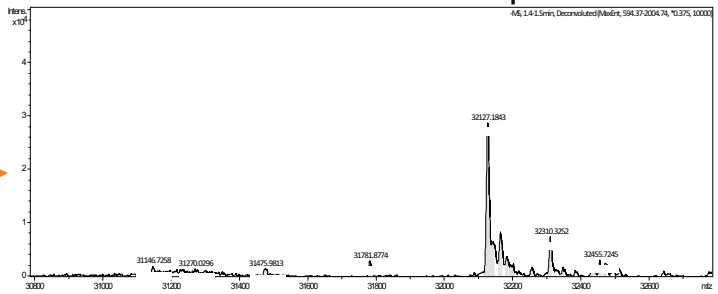
Analytics Demonstrates High Quality Material

Total Ion Chromatograph

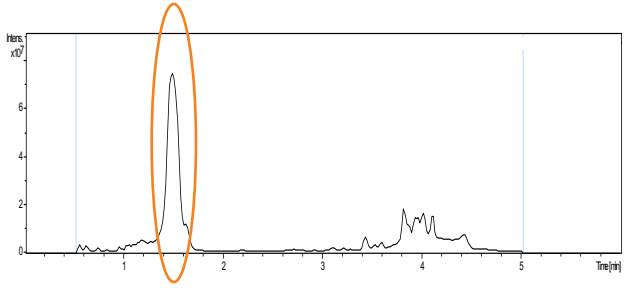
Deconvoluted Mass Spectrum

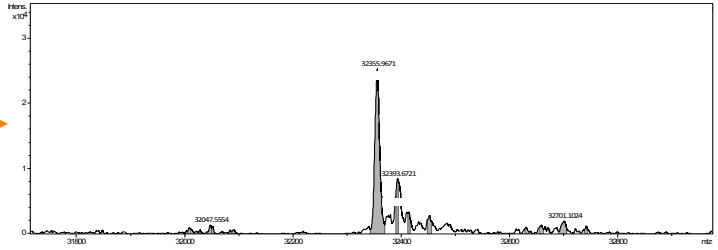
A



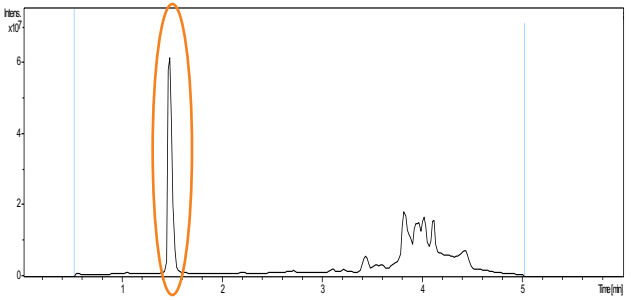


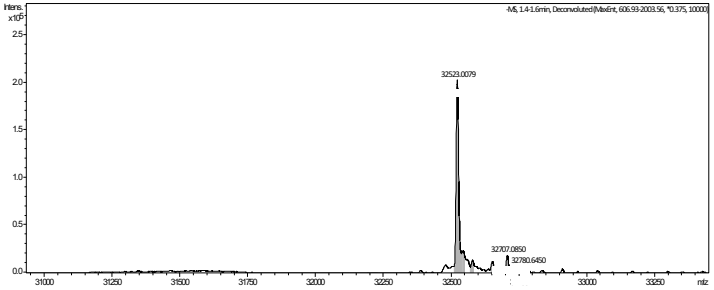
B





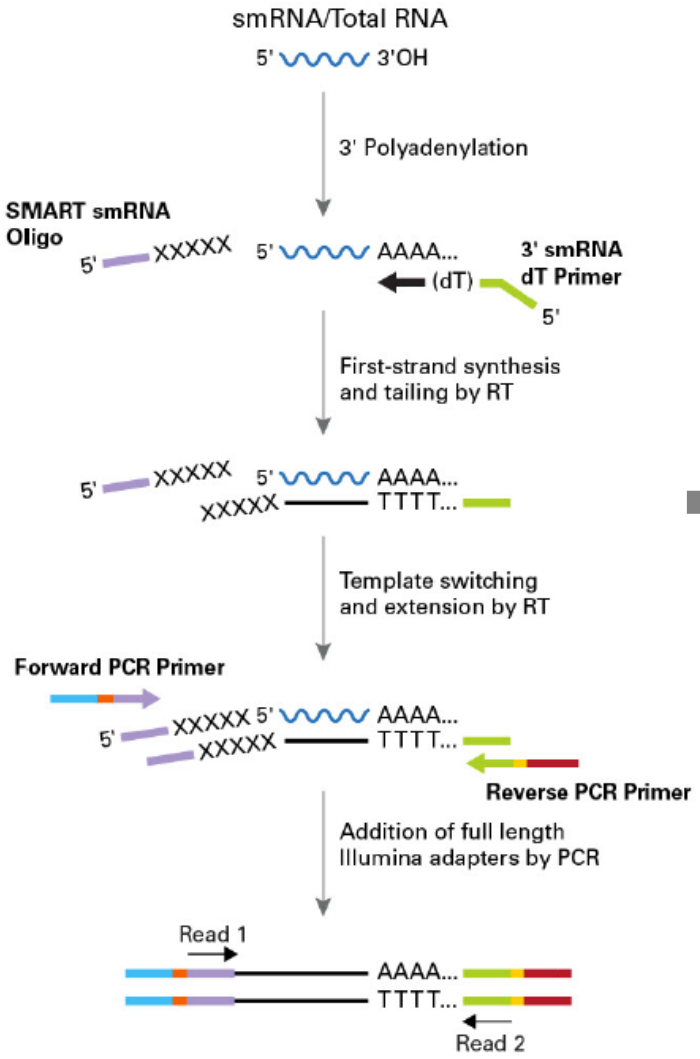
Covalently-coupled dual gRNA





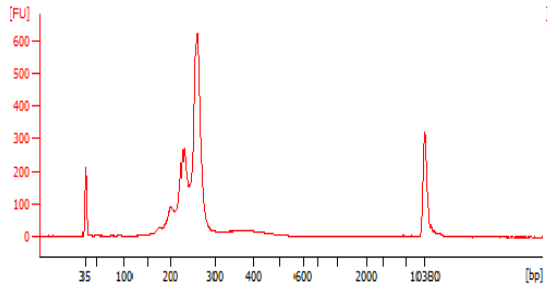
Assessing gRNA purity and sequence fidelity

Development of an RNA-Seq based method for gRNA QC

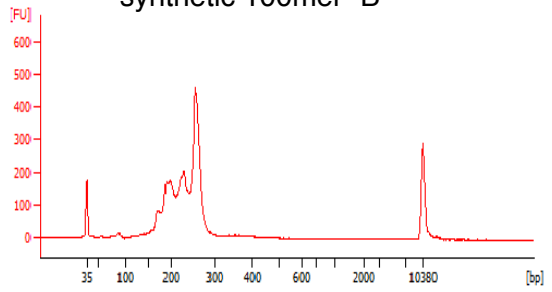


NGS library Prep

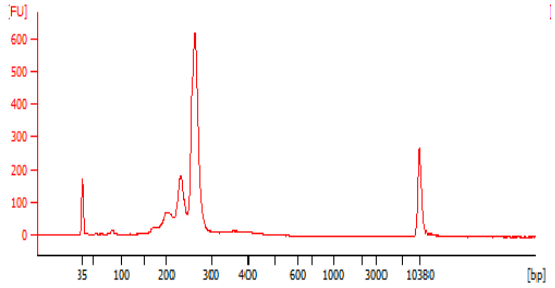
synthetic 100mer “A”



synthetic 100mer “B”



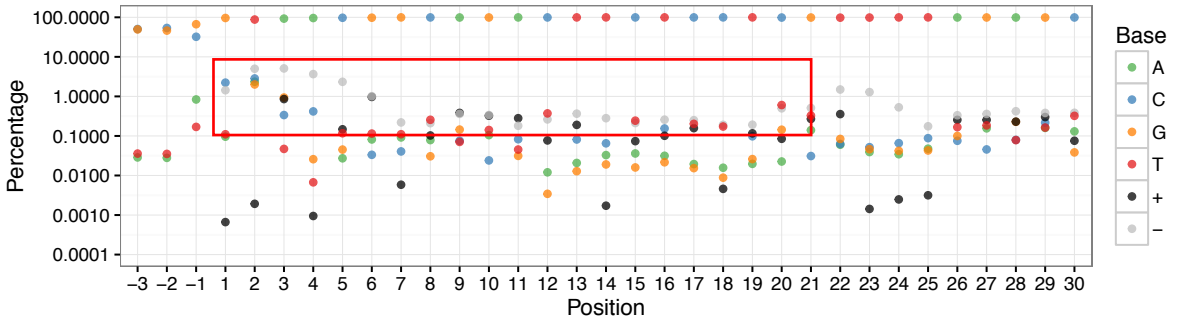
covalently-coupled dgRNA



gRNA purity and sequence fidelity

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

A



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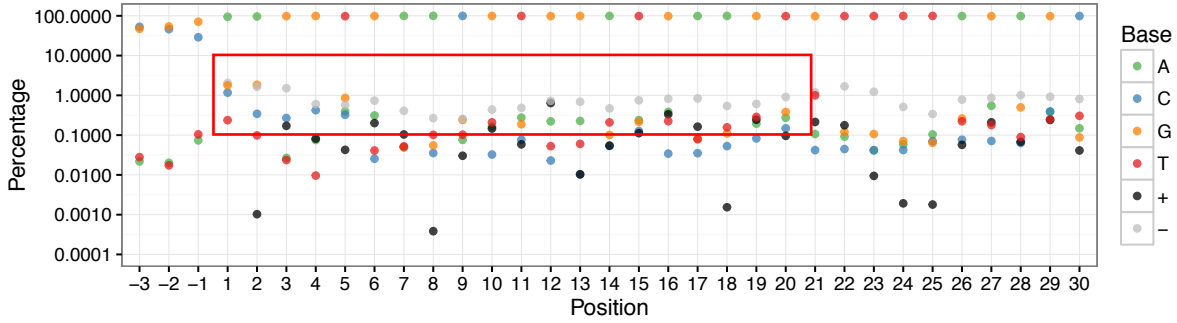
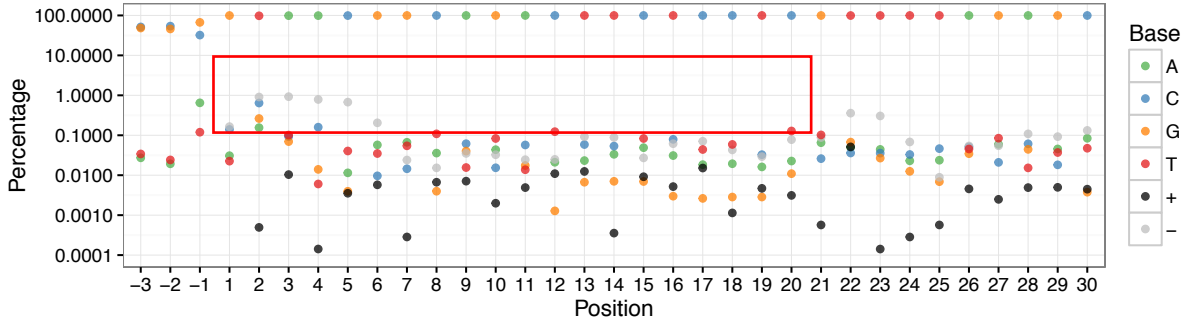


Covalently-Coupled dgRNA



Drug Development Fundamentals

- Reliably manufacture high quality drug substance
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  - Pharmacodynamics: “what the drug does to the body”

FDA

Current Recommendations to Assess Safety of Gene Editing Products

- **Kinetics of nuclease cleavage and persistence of cleavage activity**
- **Percentage of cleavage at the on- and off-target sites**
- **Identification and characterization of off-target events in cells/tissues, including chromosomal translocations**
- **Evaluation of the profile of insertions and deletions and types of mutations generated**

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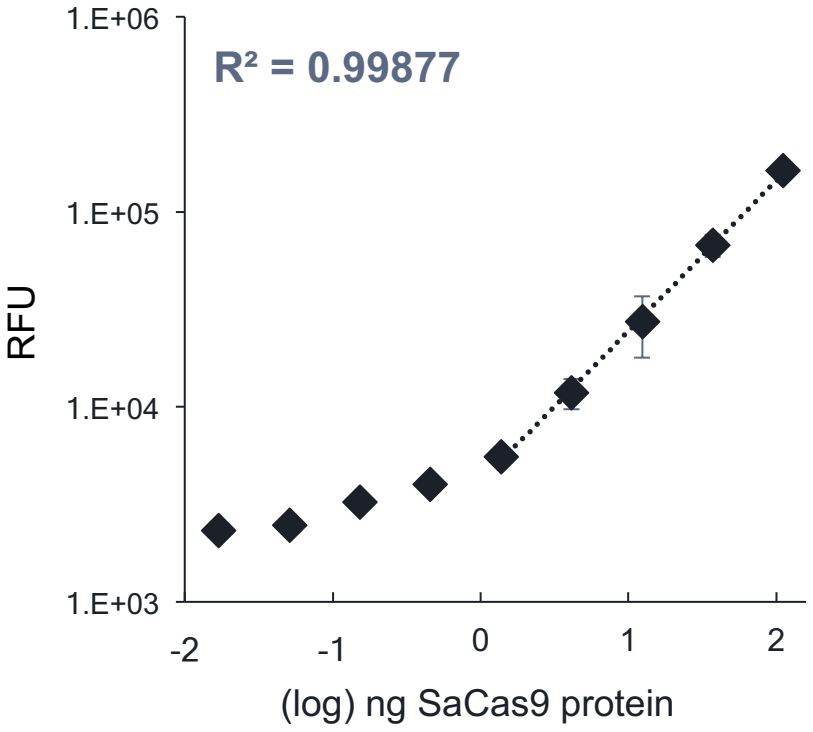
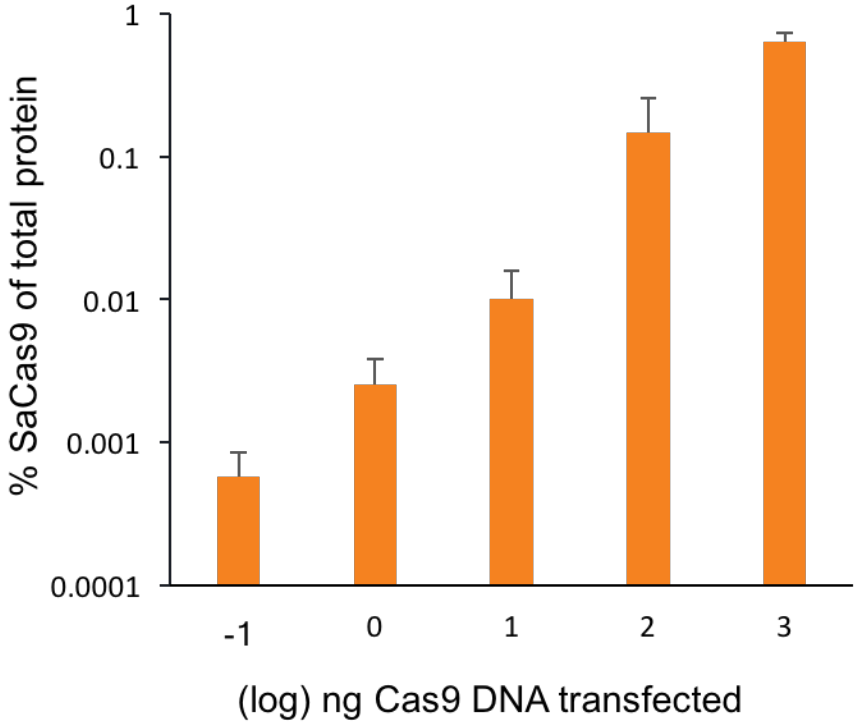
Sensitive Detection of SaCas9 Protein

AlphaLISA protein assay with 2-3 logs linear sensitivity

AlphaLISA standard curve for SaCas9



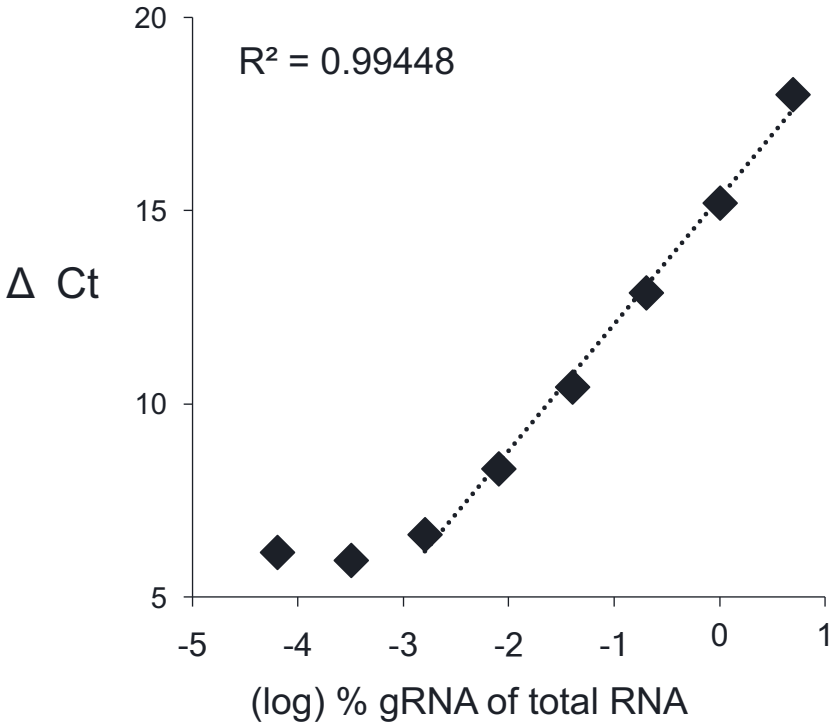
Dose response in transfected cells



Sensitive Detection of *S.aureus* gRNAs

Generic gRNA detection assay with 4 logs linear sensitivity

RT-qPCR for SaCas9 gRNA TRACR standard curve



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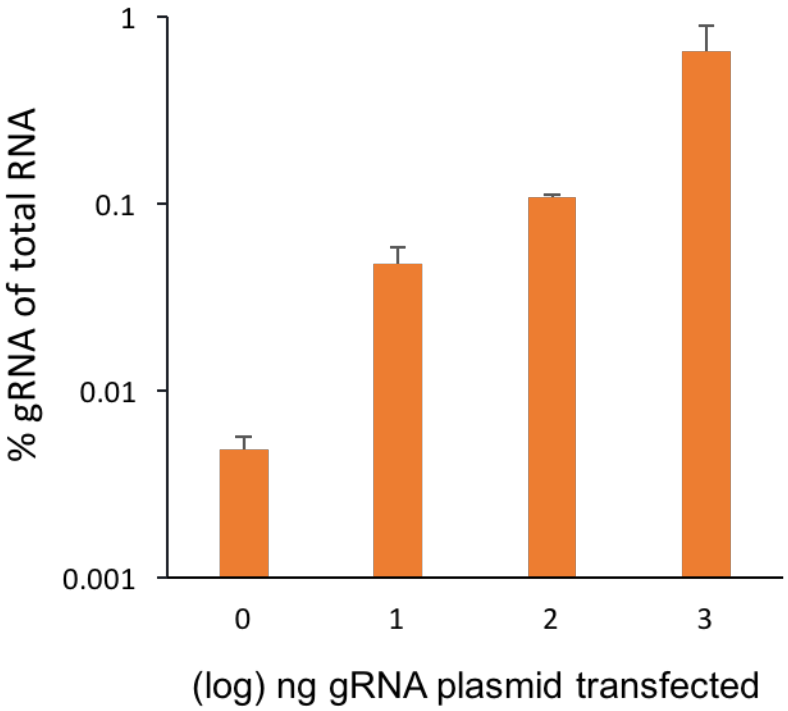
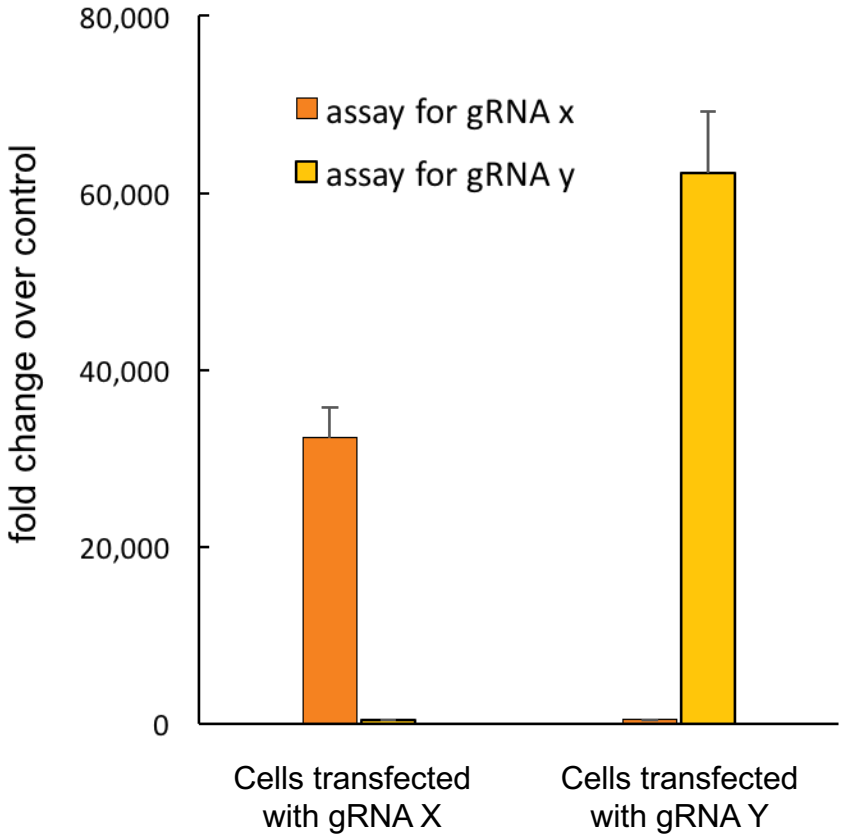


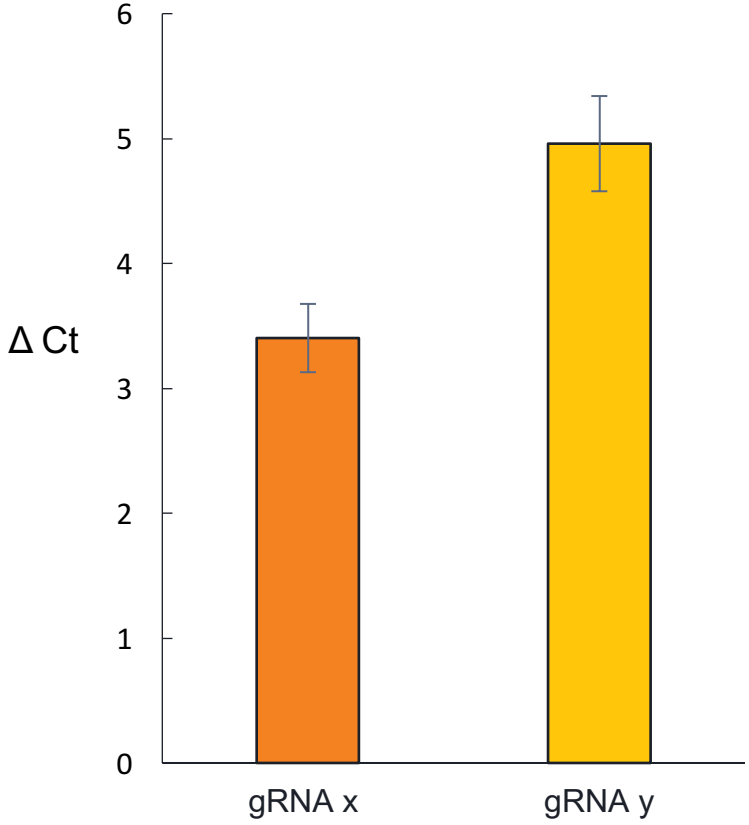
Sensitive and Specific Detection of gRNAs

Multi gRNA edits require specific assays

Taqman assays are specific for each gRNA



Expression differences *in vivo* with two gRNAs from a single AAV



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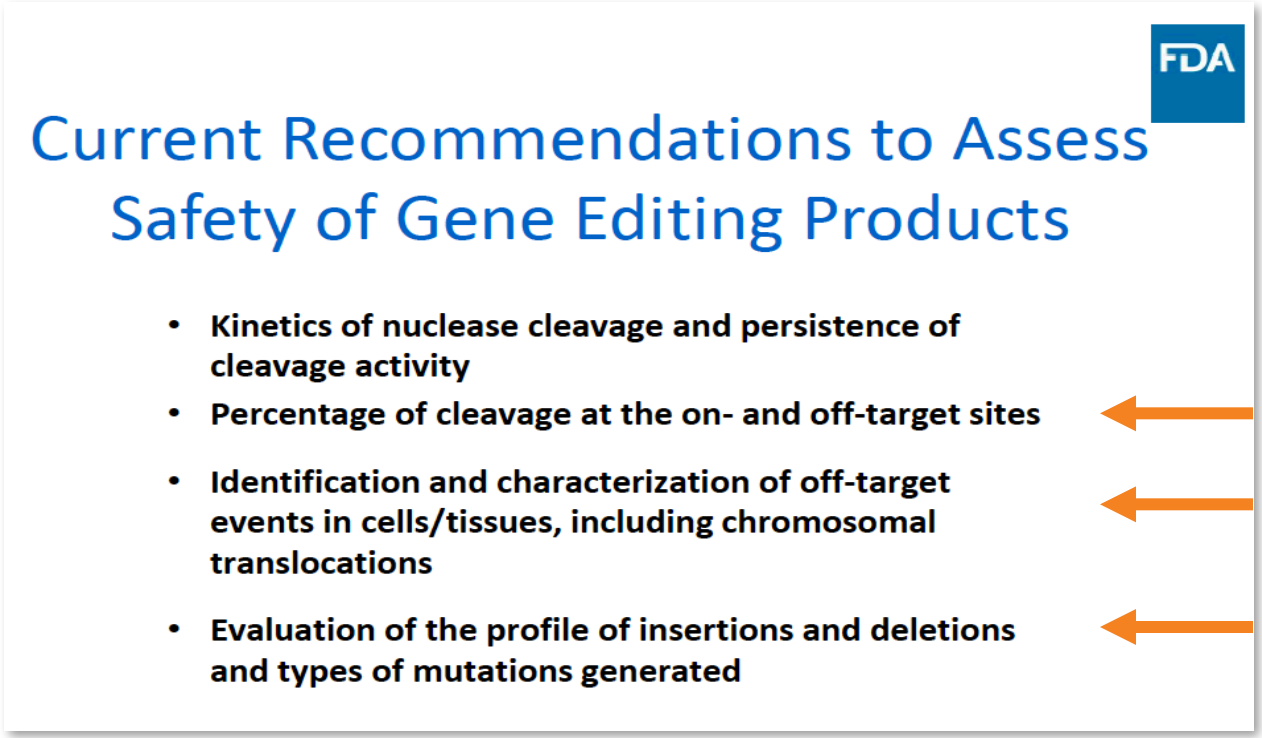

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How Do You Best Measure Editing?

A simple question with a complex answer

- PCR based 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

Single Primer Approaches Achieve Many Goals

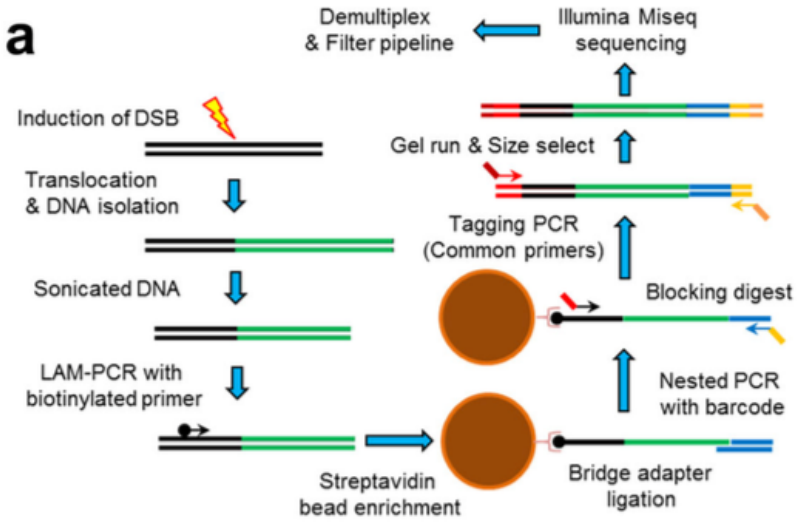
While effective, these approaches (HTGTS, AMP-Seq) can be cumbersome

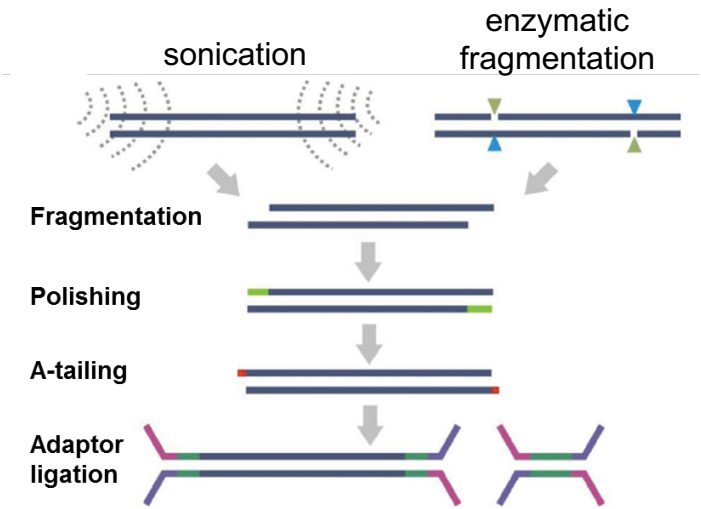
Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases

Richard L. Frock[1,2,3,4], Jiazhi Hu[1,2,3,4], Robin M. Meyers[1,2,3], Yu-Jui Ho[1,2,3], Erina Kii[1,2,3], and Frederick W. Alt[1,2,3,5]

Anchored multiplex PCR for targeted next-generation sequencing

Zongli Zheng[1,2], Matthew Liebers[1], Boryana Zhelyazkova[1], Yi Cao[1], Divya Panditi[1], Kerry D Lynch[1], Juxiang Chen[1,3], Hayley E Robinson[1], Hyo Sup Shim[1,4], Juliann Chmielecki[5], William Pao[5], Jeffrey A Engelman[6], A John Iafrate[1,6] & Long Phi Le[1,6]



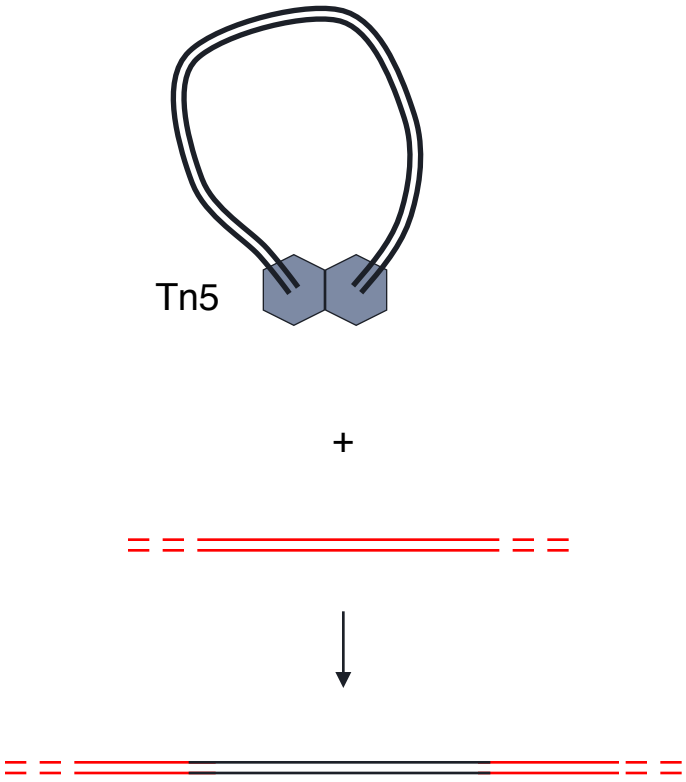


Typically a ½ day Multiple steps

Can induce oxidative DNA Damage (8-oxo-dG)

Transposase Engineering for Sequencing

Highly efficient and scalable



Adey, A., et al.(2010). Rapid, low-input, low-bias construction of shotgun fragment libraries by high-density in vitro transposition.*Genome Biology*, *11*(12), R119. http://doi.org/10.1186/gb-2010-11-12-r119

Transposase Engineering for Sequencing

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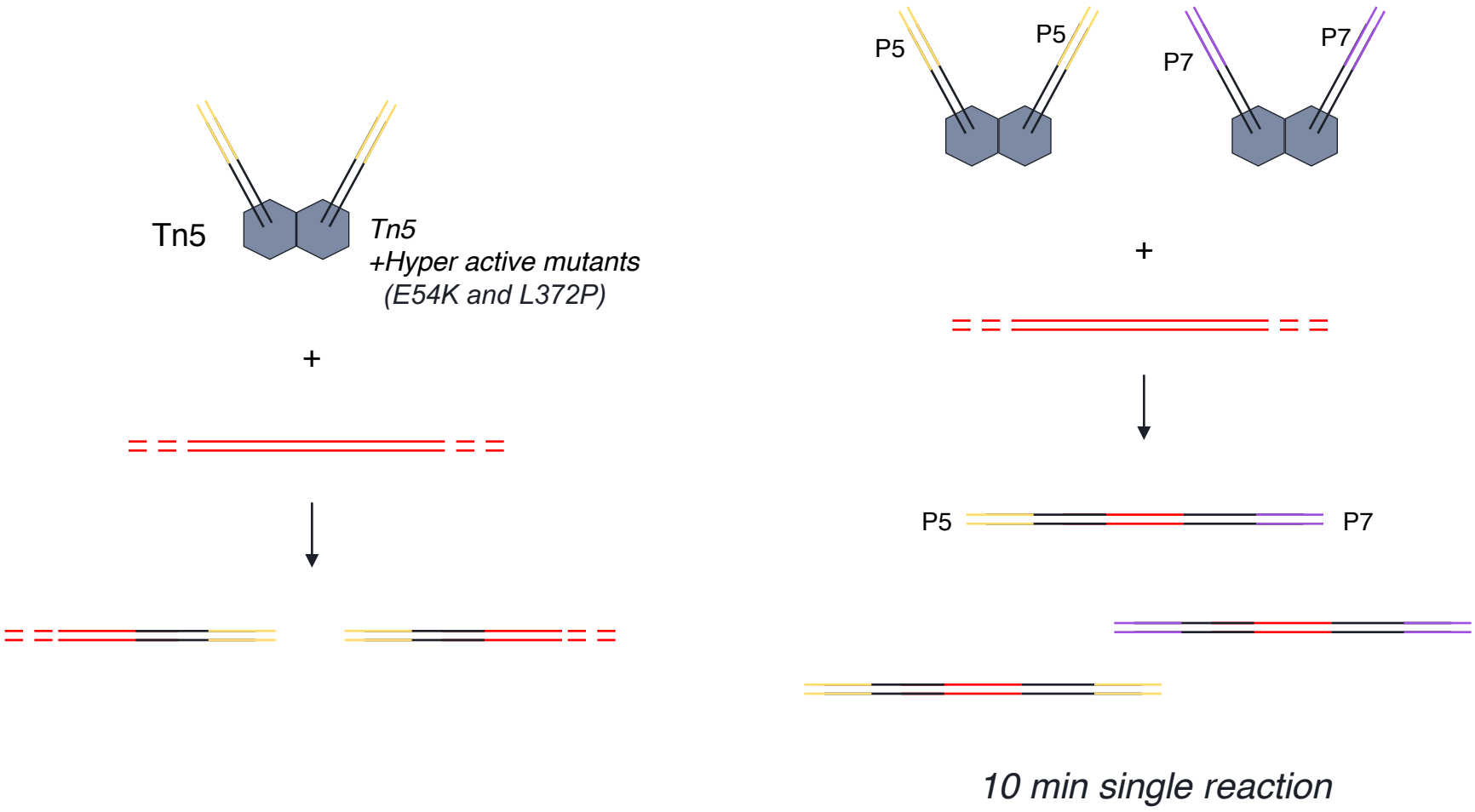
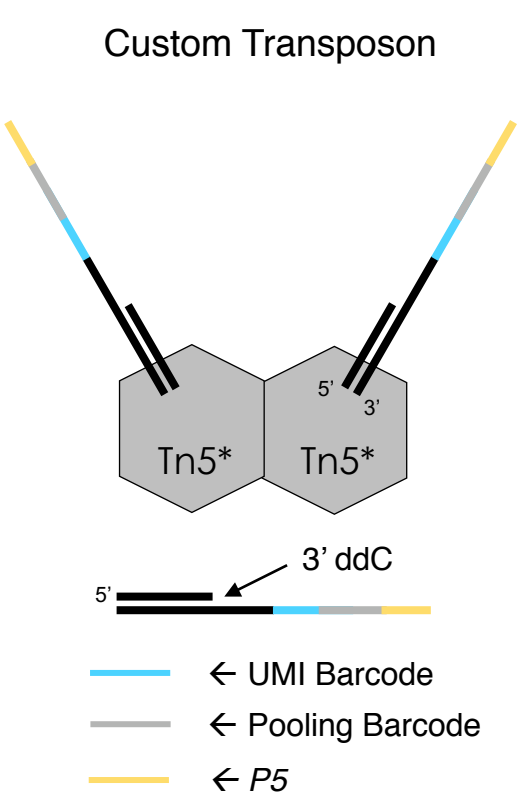


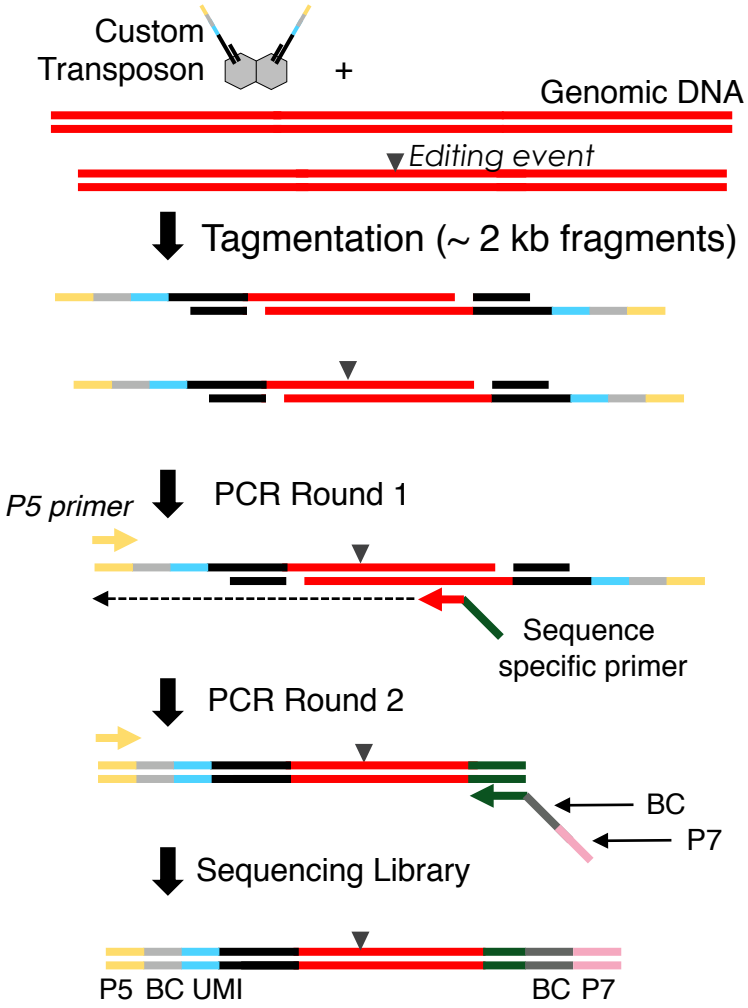
10 min single reaction

Adey, A., et al.(2010). Rapid, low-input, low-bias construction of shotgun fragment libraries by high-density in vitro transposition.*Genome Biology*, *11*(12), R119. http://doi.org/10.1186/gb-2010-11-12-r119

Uni-Directional Targeted Sequencing (UDiTaS)

Blending tagmentation and AMP-seq

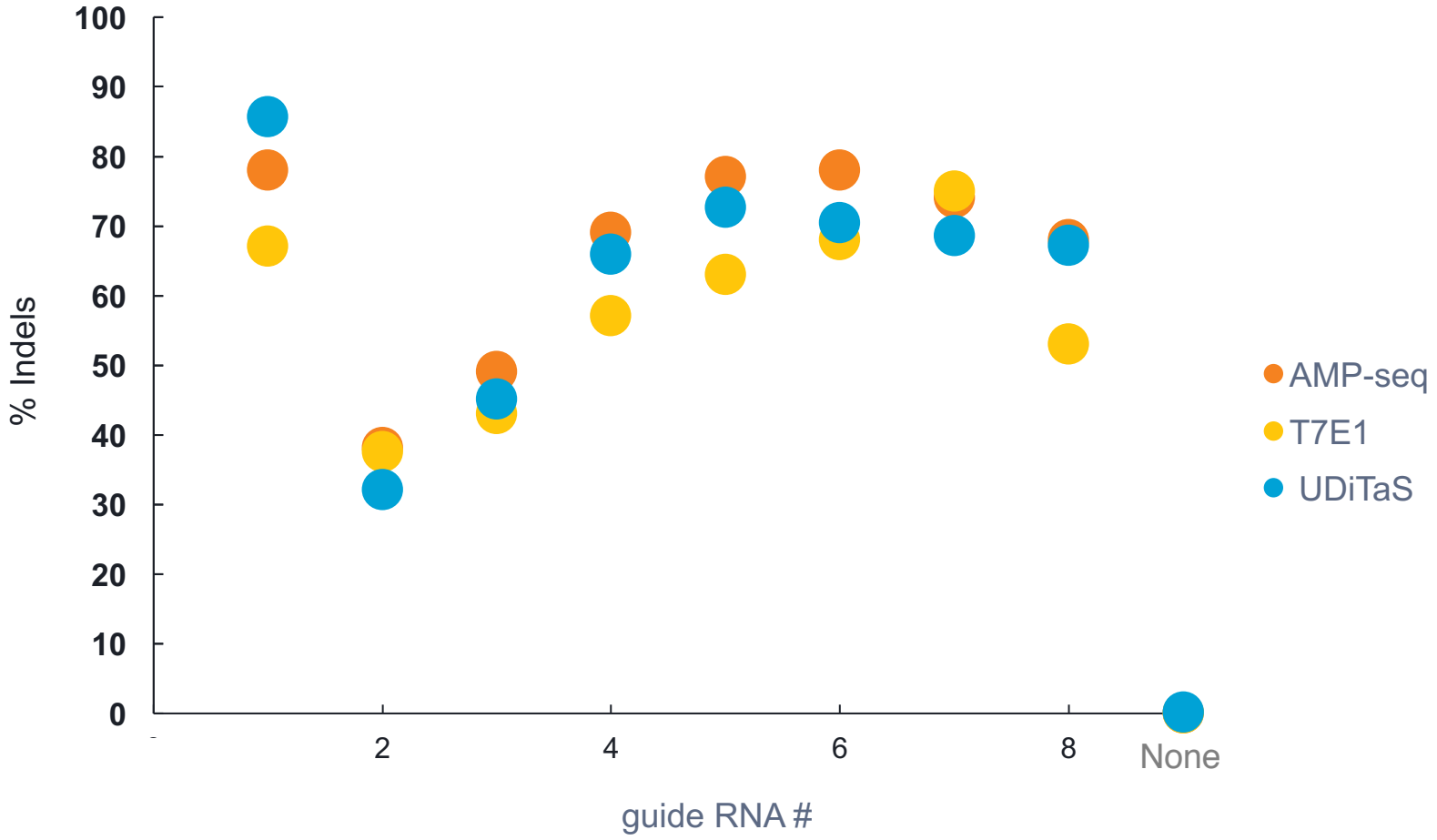




Picelli, S., et. al (2014). Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. *Genome Research*, *24*(12), 2033–2040.

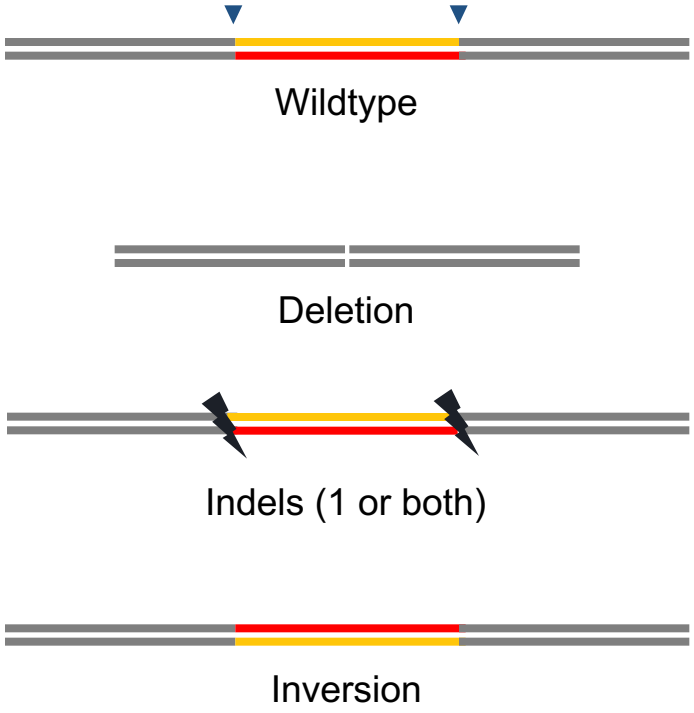
Comparison of Small Indel Measurements

UDiTaS correlates well with targeted sequencing and T7E1 assays



Intra-Chromosomal Rearrangement Detection

Dual guide edits have multiple possible outcomes

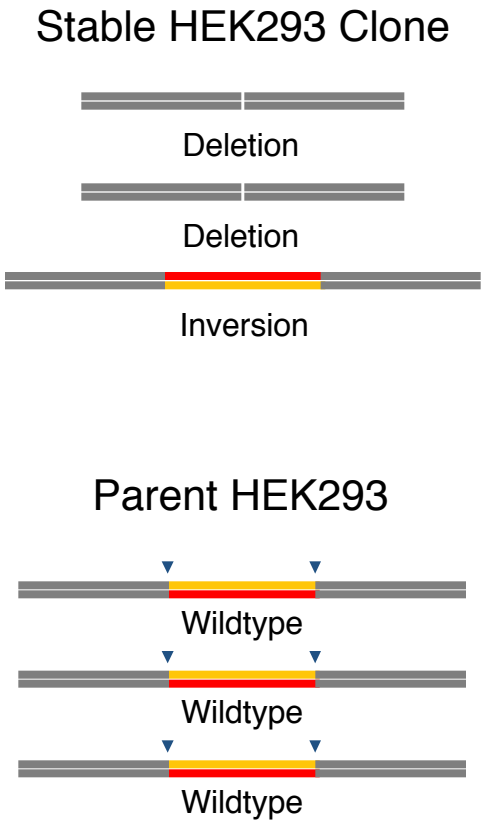


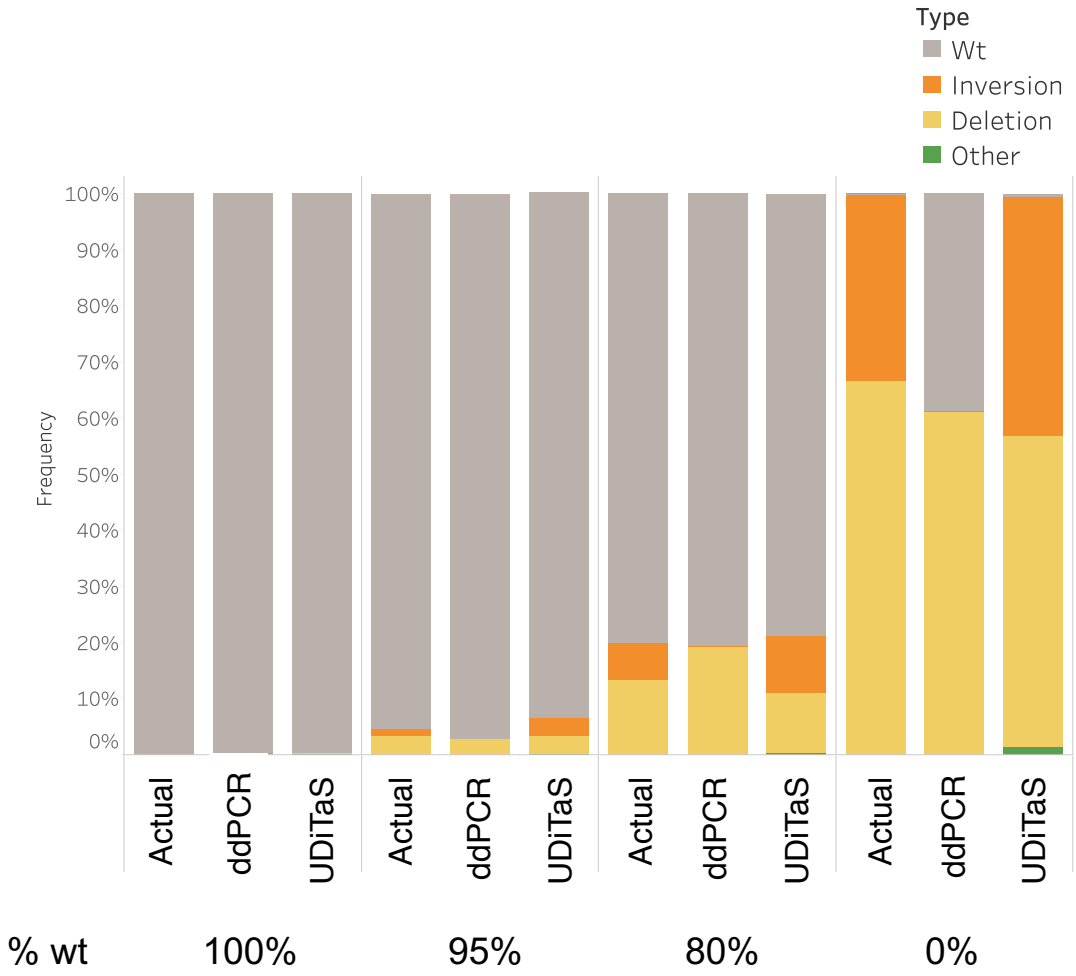
Digital Droplet PCR: measures the ratio of 2 qPCR assays on single molecules of genomic DNA

UDiTaS: counts the sequences post junction

Intra-Chromosomal Rearrangement Detection

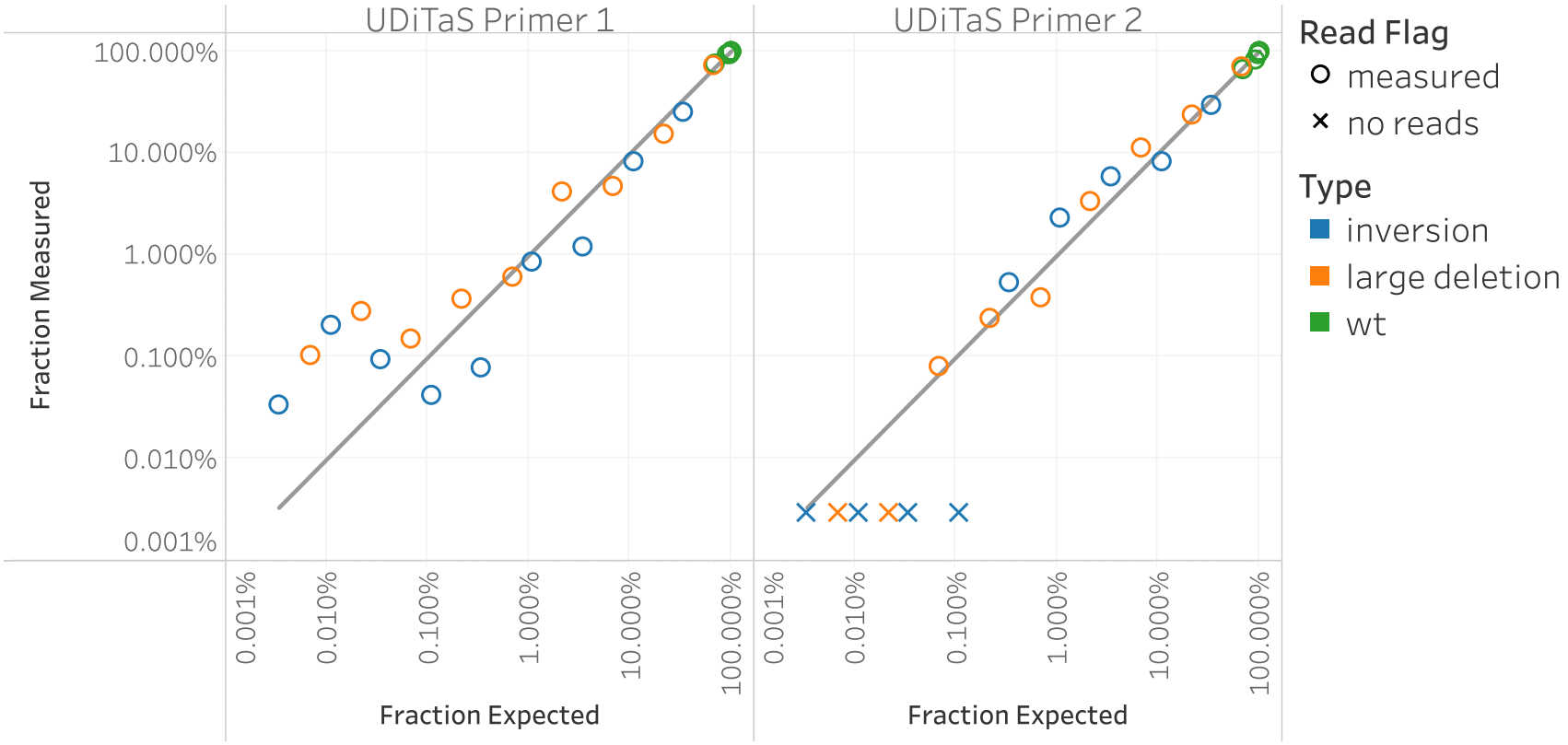
Validation using genomic DNA mixing of a stable HEK293T clone





Intra-Chromosomal Rearrangement Detection

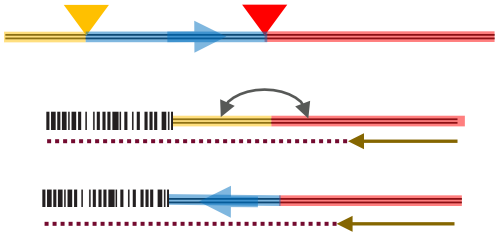
Follow up experiment defines LLOD (limited by input material and sequencing depth)



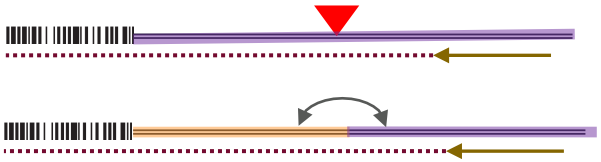
UDiTaS (Uni-Directional Targeted Sequencing)

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

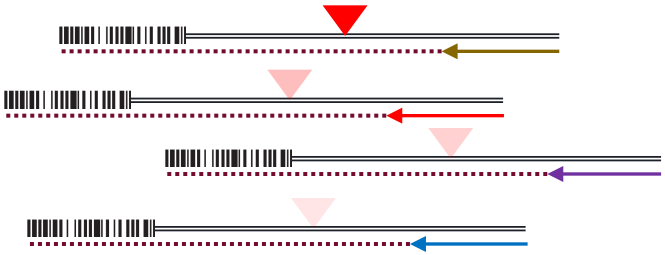
1. Quantitation of editing



2. Quantitation and discovery of large deletions and inversions



3. Translocation discovery and quantitation



4. Multiplexing

Summary

- We can make fully synthetic single gRNAs of high quality
- Pharmacokinetics: high sensitivity and specificity assays measure drug levels
- Pharmacodynamics: UDiTaS is a simple, robust method for capturing complex editing events in a single reaction

Reshica Baral
Luis Barrera
Dawn Ciulla
Cecilia Fernandez
Ari Friedland
Georgia Giannoukos
Sebastian Gloskowski
Kiran Gogi
Jenn Gori
Greg Gotta
Fred Harbinski

Jack Heath
Joy Horng
Hari Jayaram
Morgan Maeder
Eugenio Marco
Rina Mepani
Andrew Sadowski
Will Selleck
Max Skor
Terence Ta

Bruce Eaton &
The i2 Pharma team