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Advancing CRISPR Technologies for Therapeutic Application

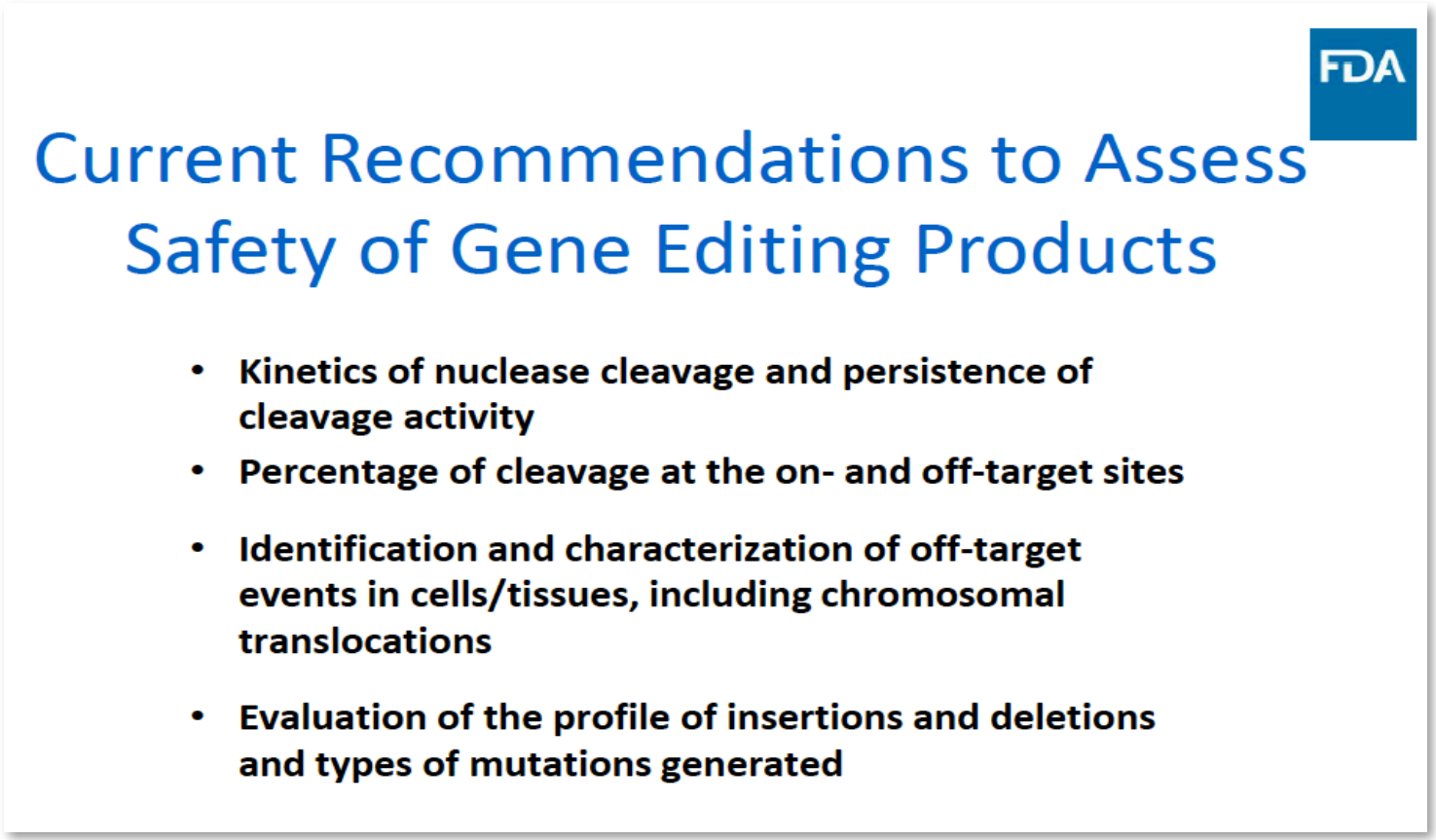
Vic Myer

Keystone Genome Editing Meeting

January, 2017

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”



Celia Witten,
OTAT→CBER→FDA
Cell and Gene Meeting on the Mesa
La Jolla, California,
October 6, 2016

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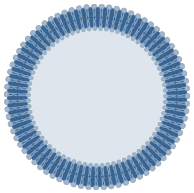
Strategy to Widely Enable Efficient Delivery

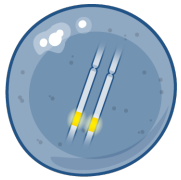
Success across a spectrum of delivery modalities in preclinical studies

MODALITY

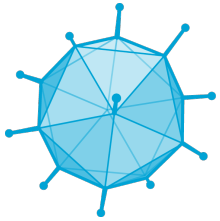
Nanoparticle



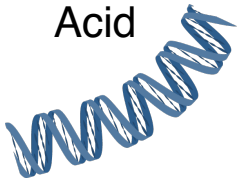
Engineered Cell



Viral Vector



Nucleic Acid

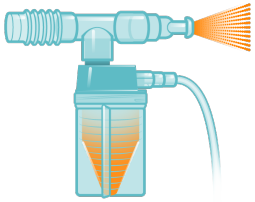


DELIVERY

IV Infusion

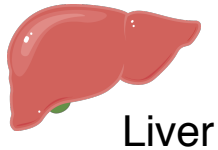
Direct Injection

Inhalation



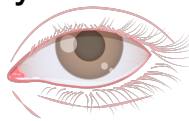
TARGET TISSUE

Bone Marrow & Blood

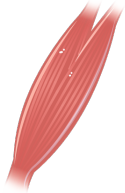


Liver

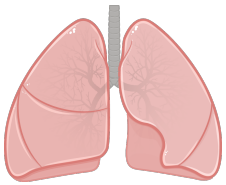
Eye



Muscle



Lungs



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

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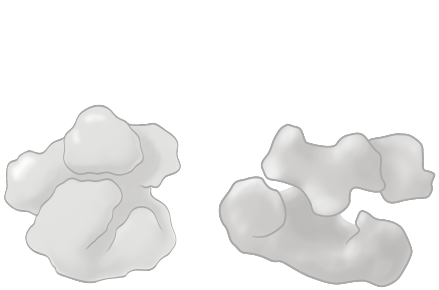
High Quality Autologous Drug Development

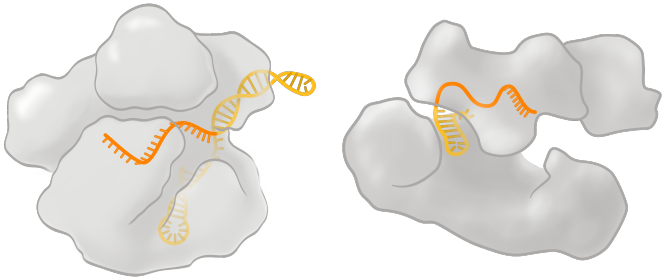
Engineered autologous therapy requires multiple components



RNA Guided Endonuclease

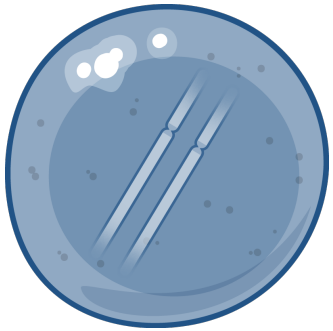
+

RNA Guide

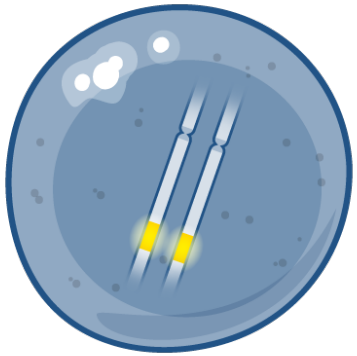


Ribonucleoprotein Particle (RNP)

+



Patient Cell



Engineered Patient Cell

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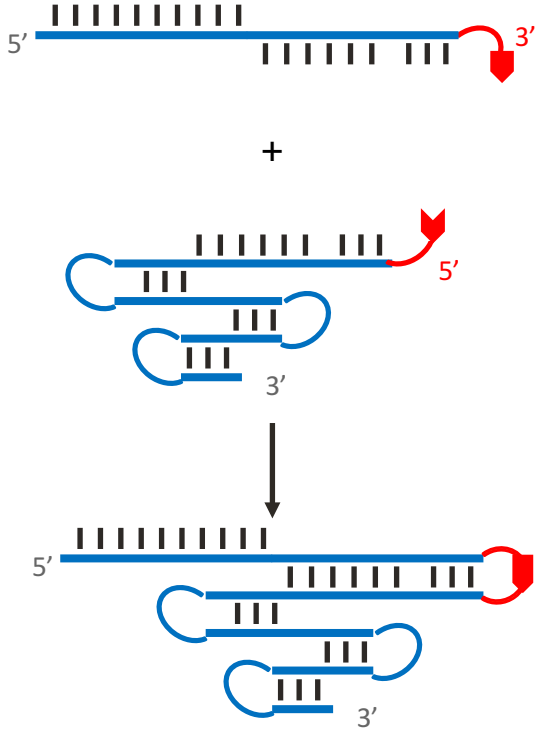
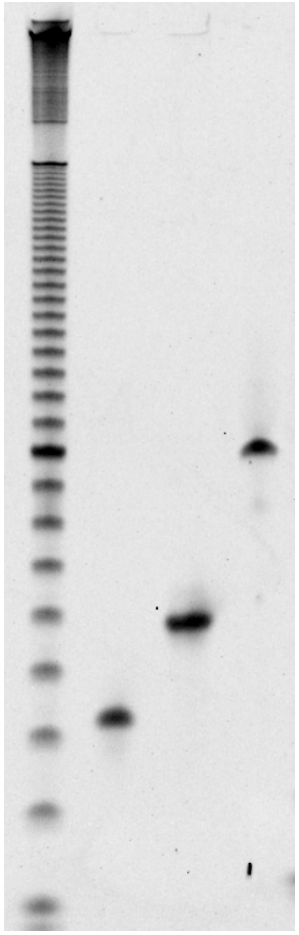
Generating Synthetic Single gRNA

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





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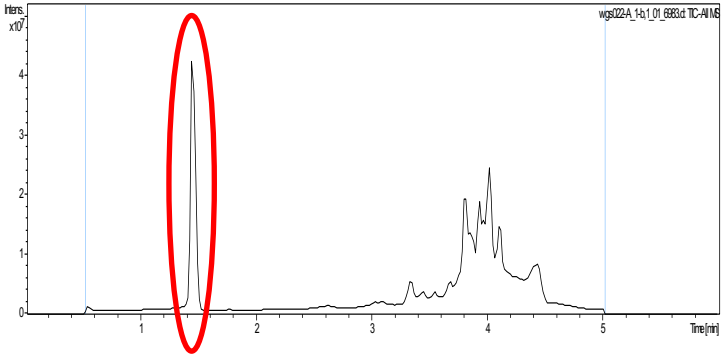
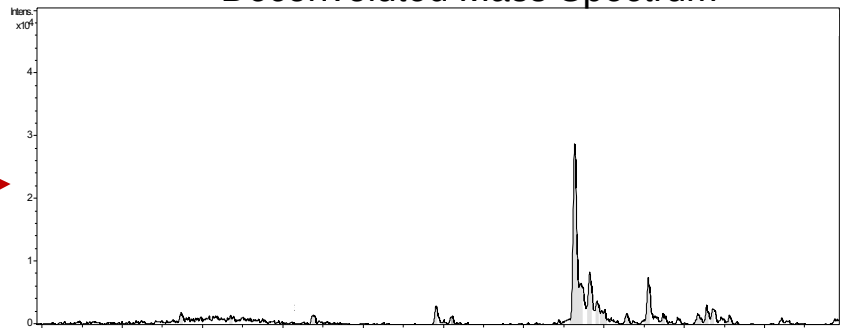
Analytics Demonstrates High Quality Material

Total Ion Chromatograph

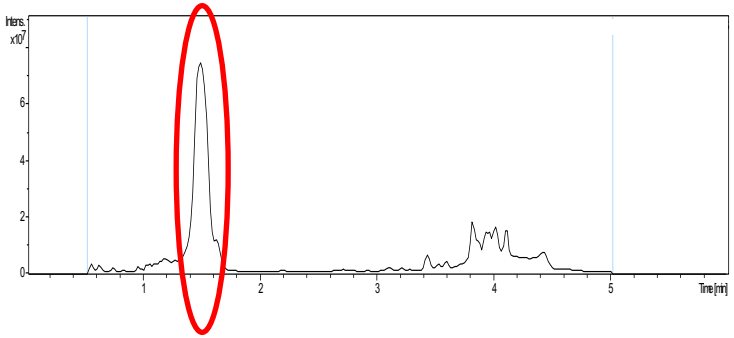
Deconvoluted Mass Spectrum

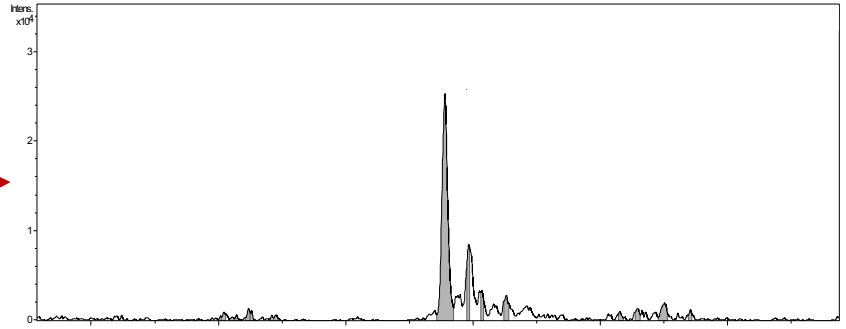
A



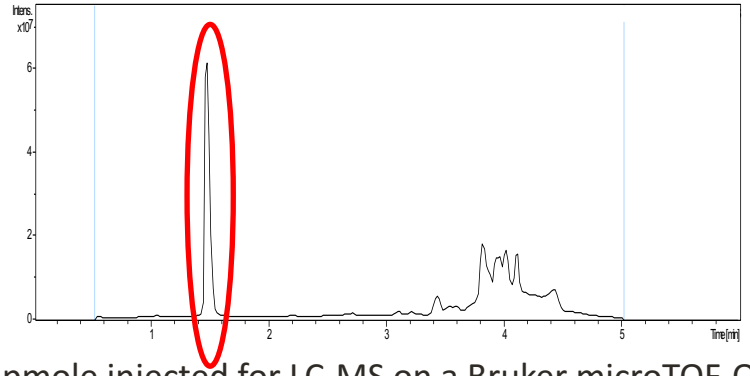


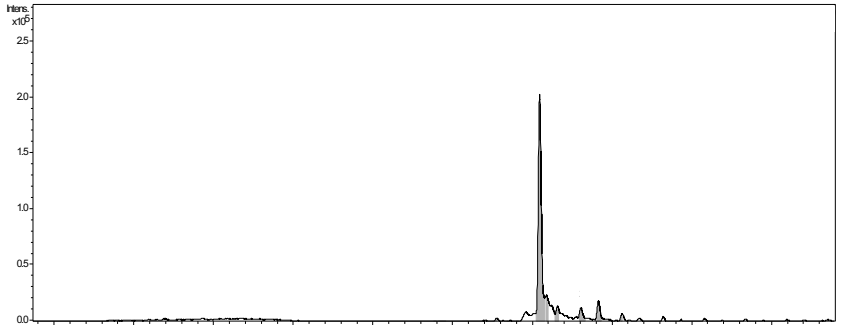
B





i2 Pharma





100 pmole injected for LC-MS on a Bruker microTOF-QII mass spec equipped with a Waters ACQUITY UPLC system (C18 column)

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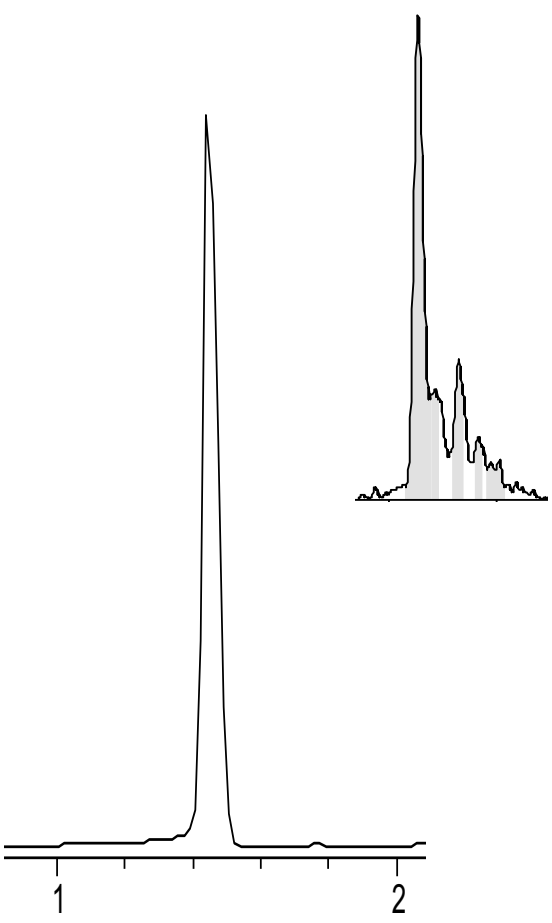
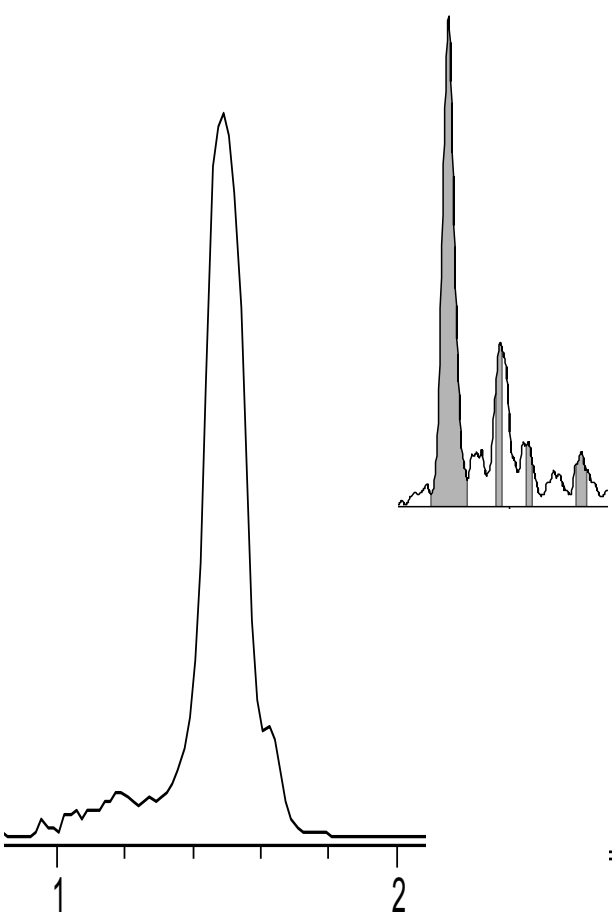
Comparative Mass Spec Data

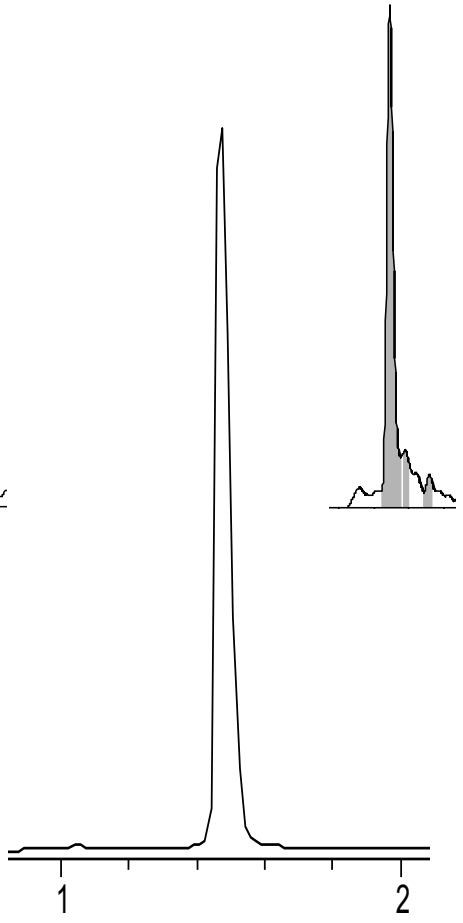
Our strategy yields high quality research grade molecules



Vendor A



Vendor B



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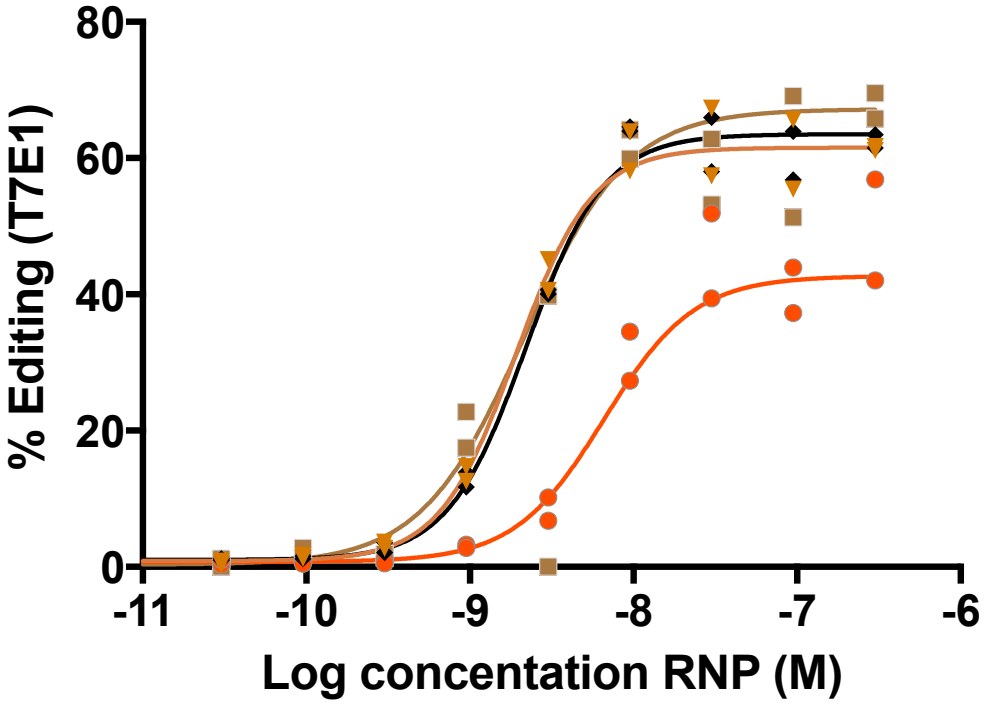
Cellular Editing Activity

In vitro transcribed & synthetic gRNAs are equivalent in cells



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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”

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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OTAT→CBER→FDA
Cell and Gene Meeting on the Mesa
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October 6, 2016

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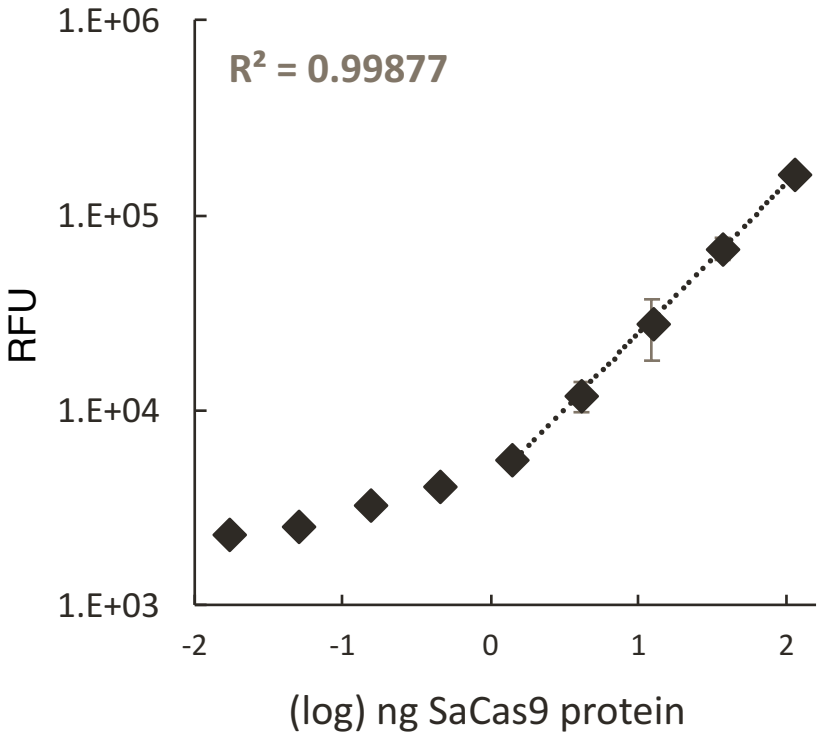
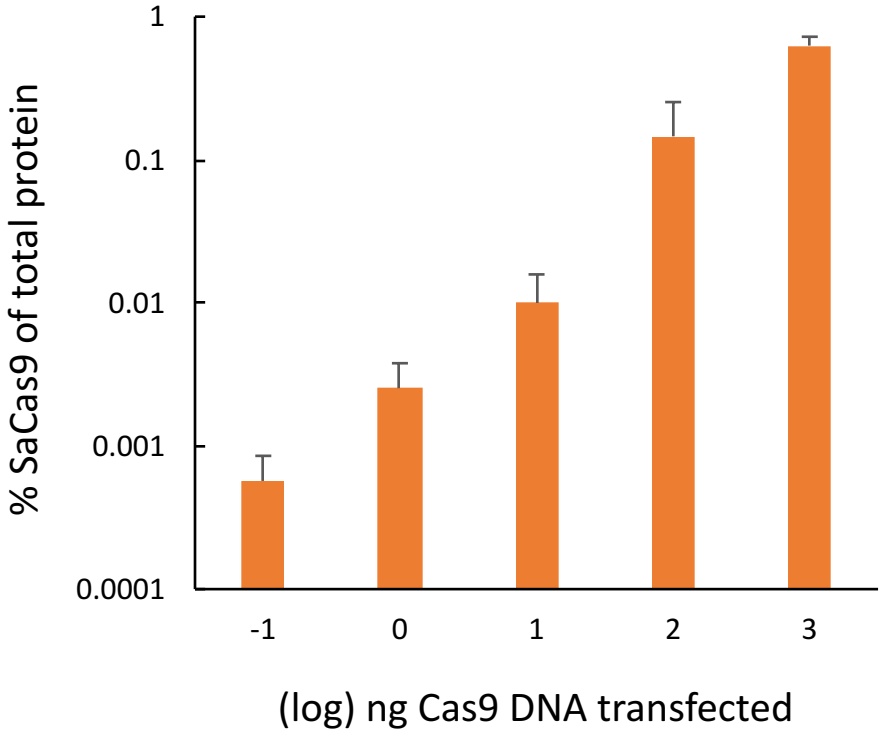
Sensitive Detection of SaCas9 Protein

AlphaLISA protein assay with 2-3 logs linear sensitivity

AlphaLISA standard curve for SaCas9



Dose response in transfected cells



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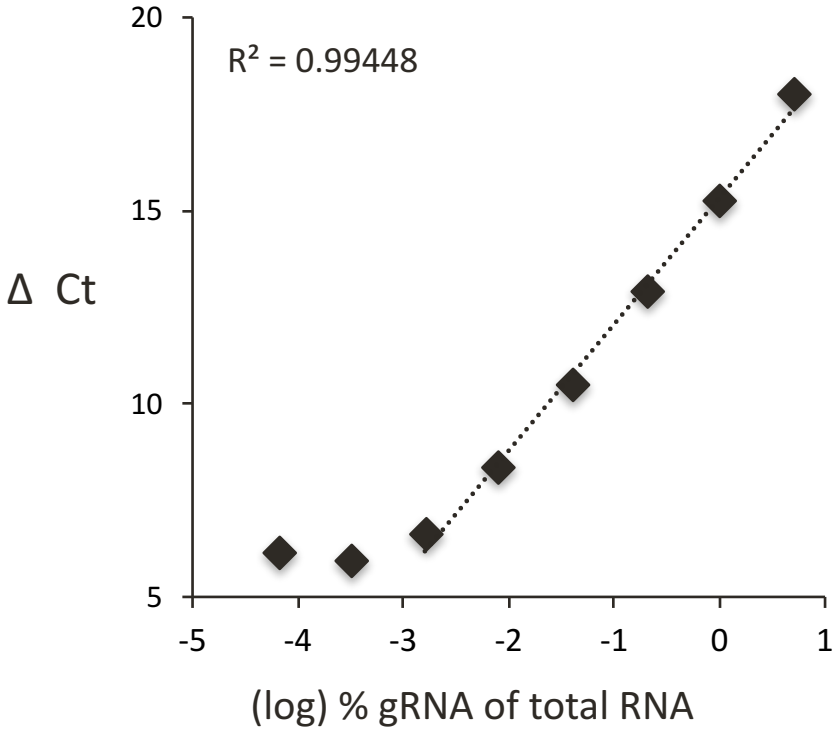
Sensitive Detection of *S.aureus* gRNAs

Generic gRNA detection assay with 4 logs linear sensitivity

RT-qPCR for SaCas9 gRNA TRACR standard curve



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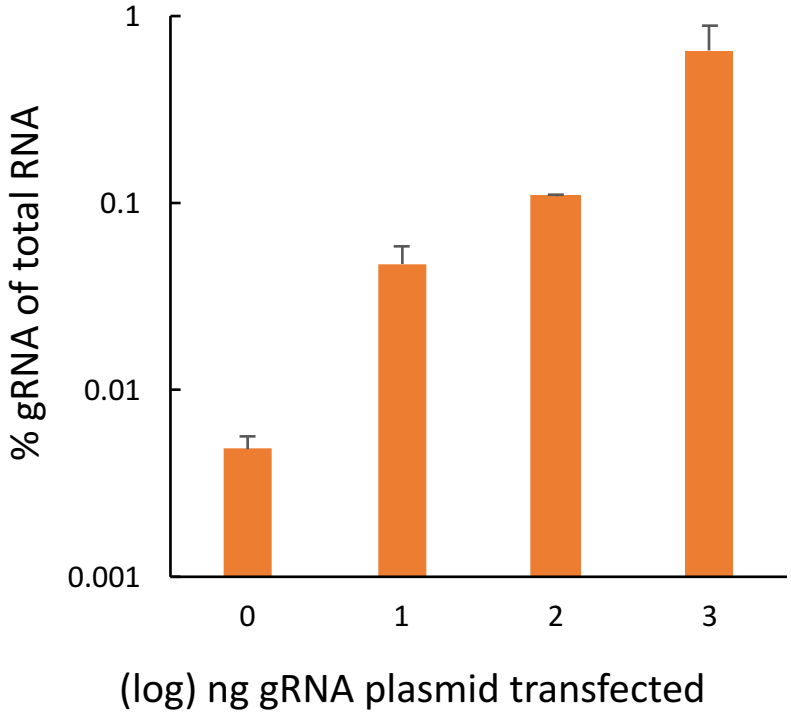

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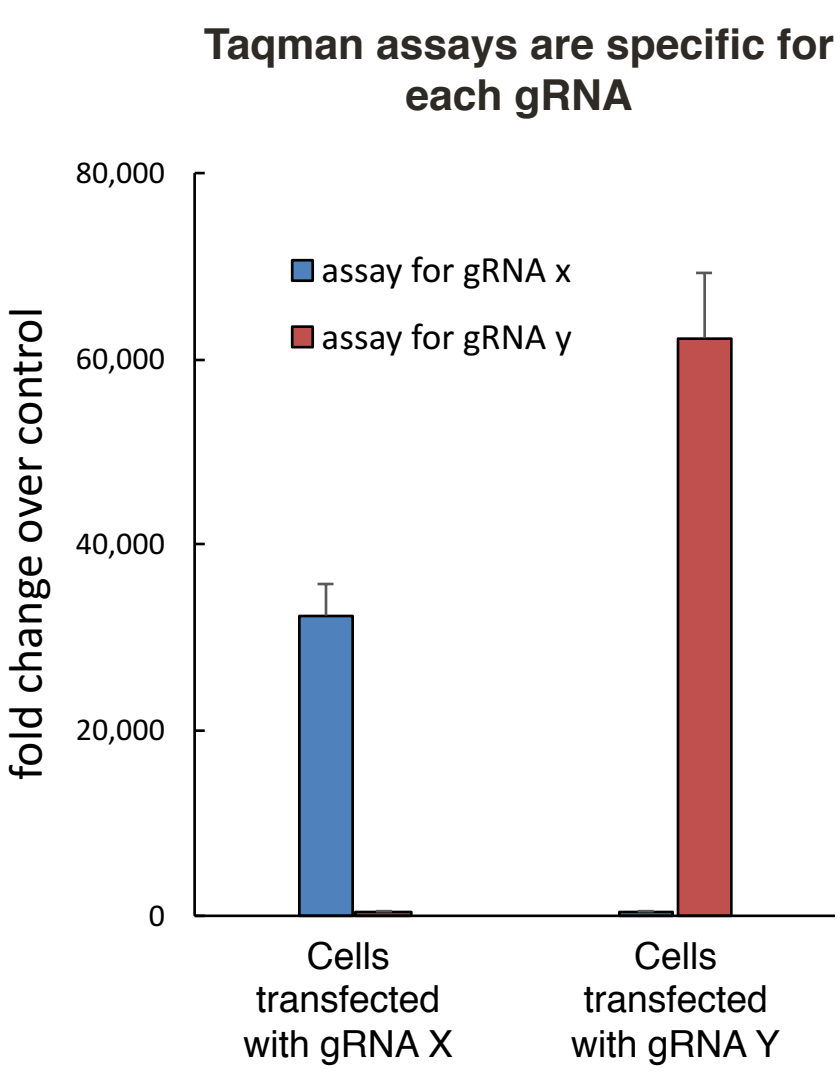
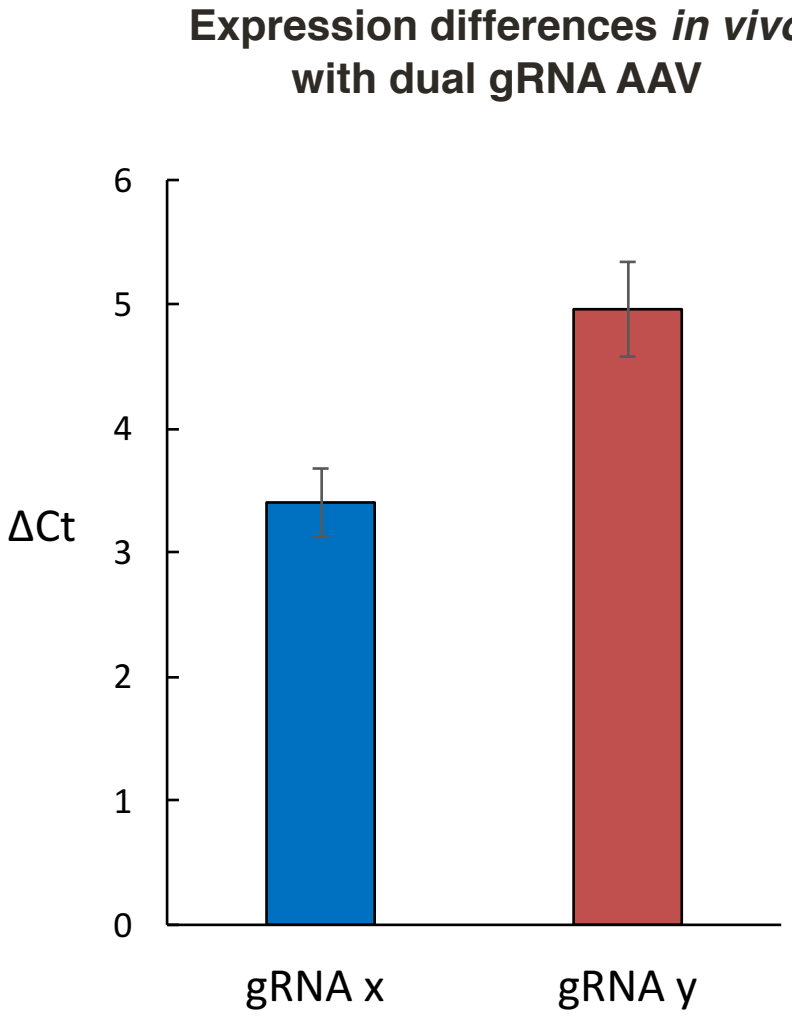
Sensitive and Specific Detection of gRNAs

Multi gRNA edits require specific assays





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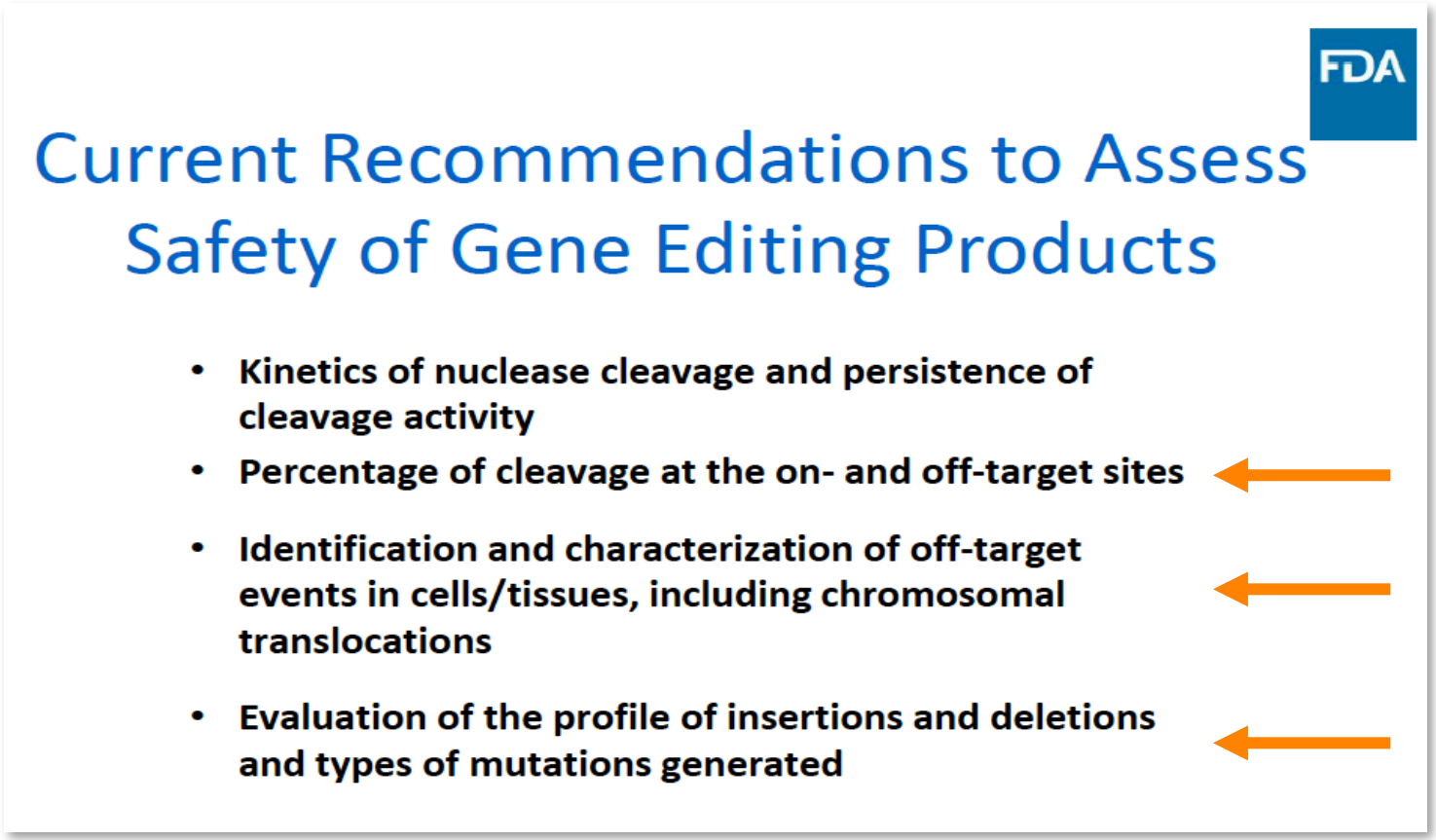

Celia Witten, OTAT→CBER→FDA Cell and Gene Meeting on the Mesa La Jolla, California, October 6, 2016

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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

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Single Primer Approaches Achieve Many Goals

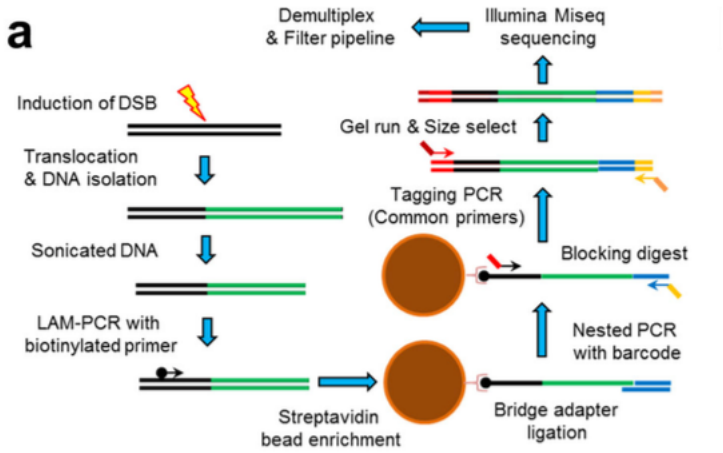
While effective, these approaches (HTGTS, AMP-Seq) can be cumbersome

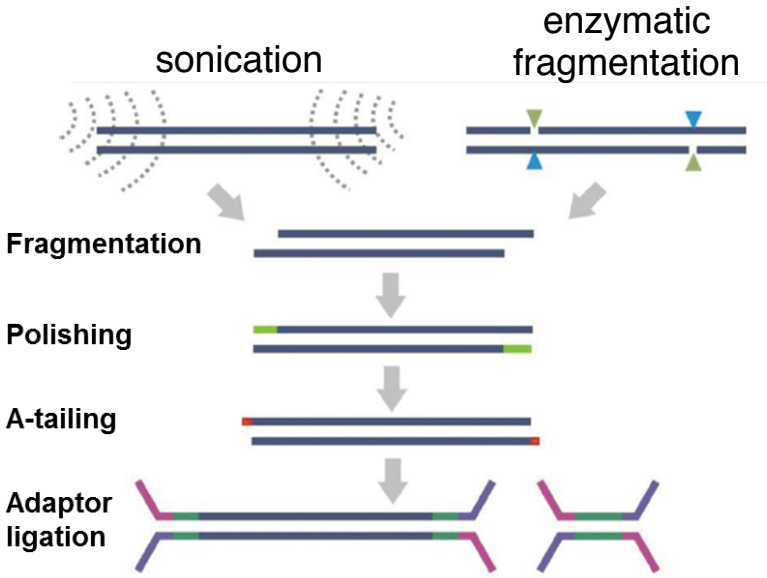
Genome-wide detection of DNA double-stranded breaks induced by engineered nucleases

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

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

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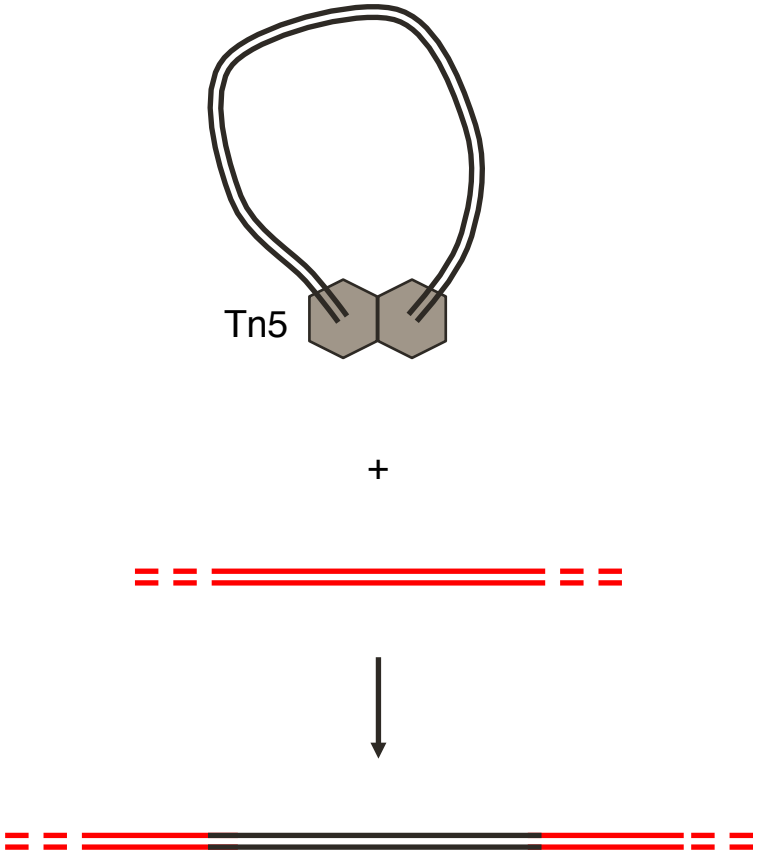
Transposase Engineering for Sequencing

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

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Transposase Engineering for Sequencing

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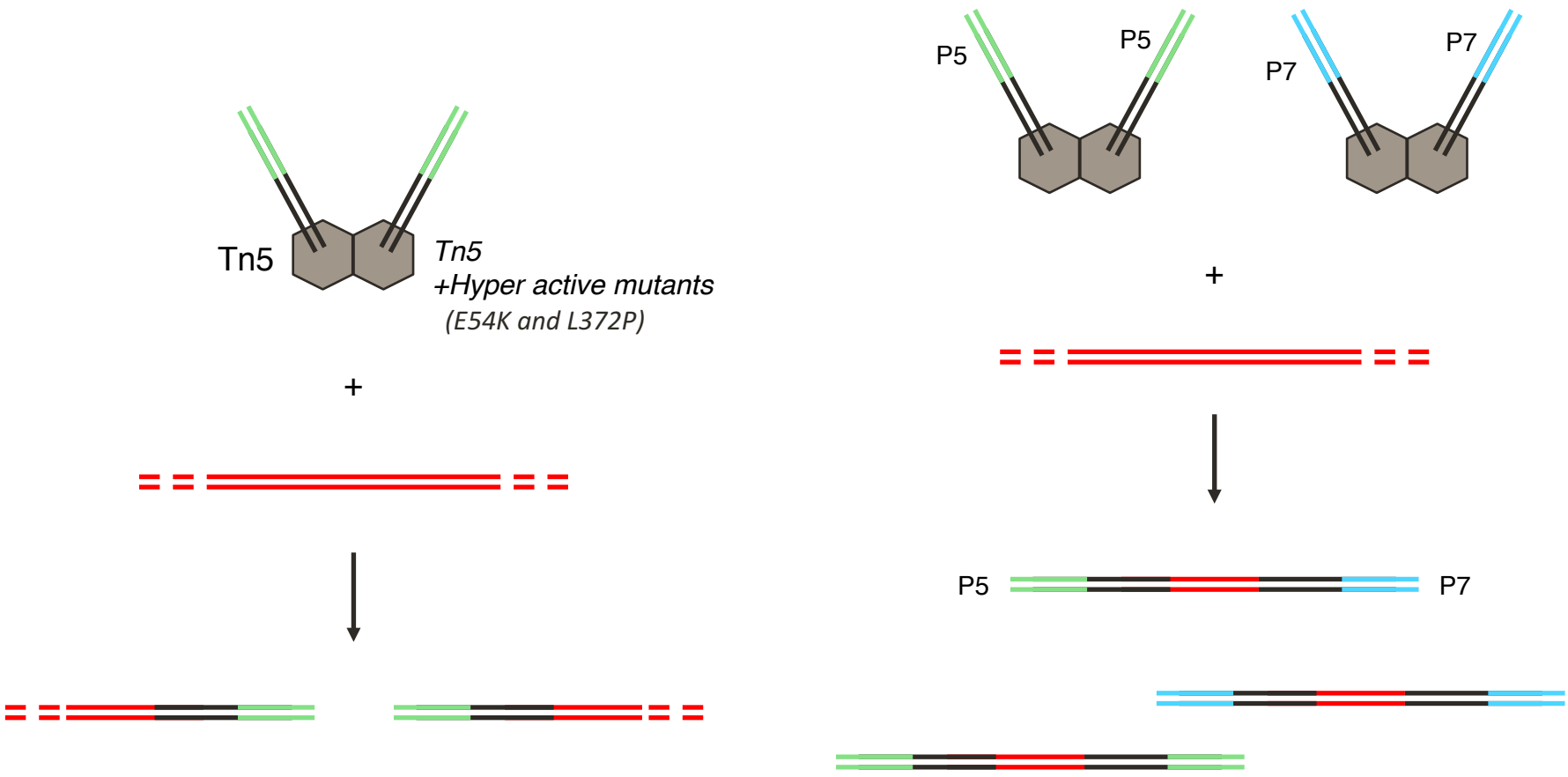

10 min single reaction

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

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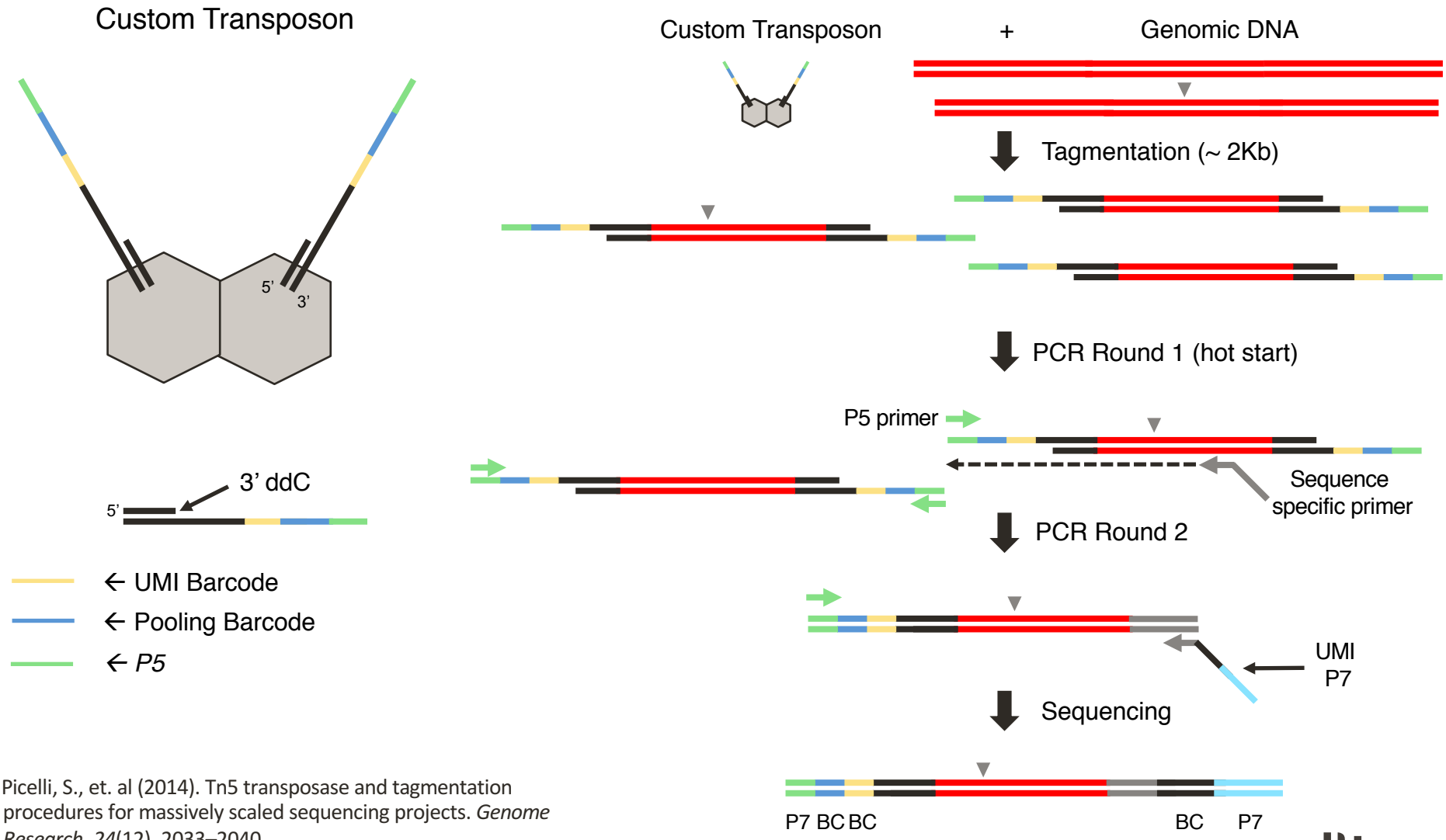
Uni-Directional Targeted Sequencing (UDiTaS)

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.

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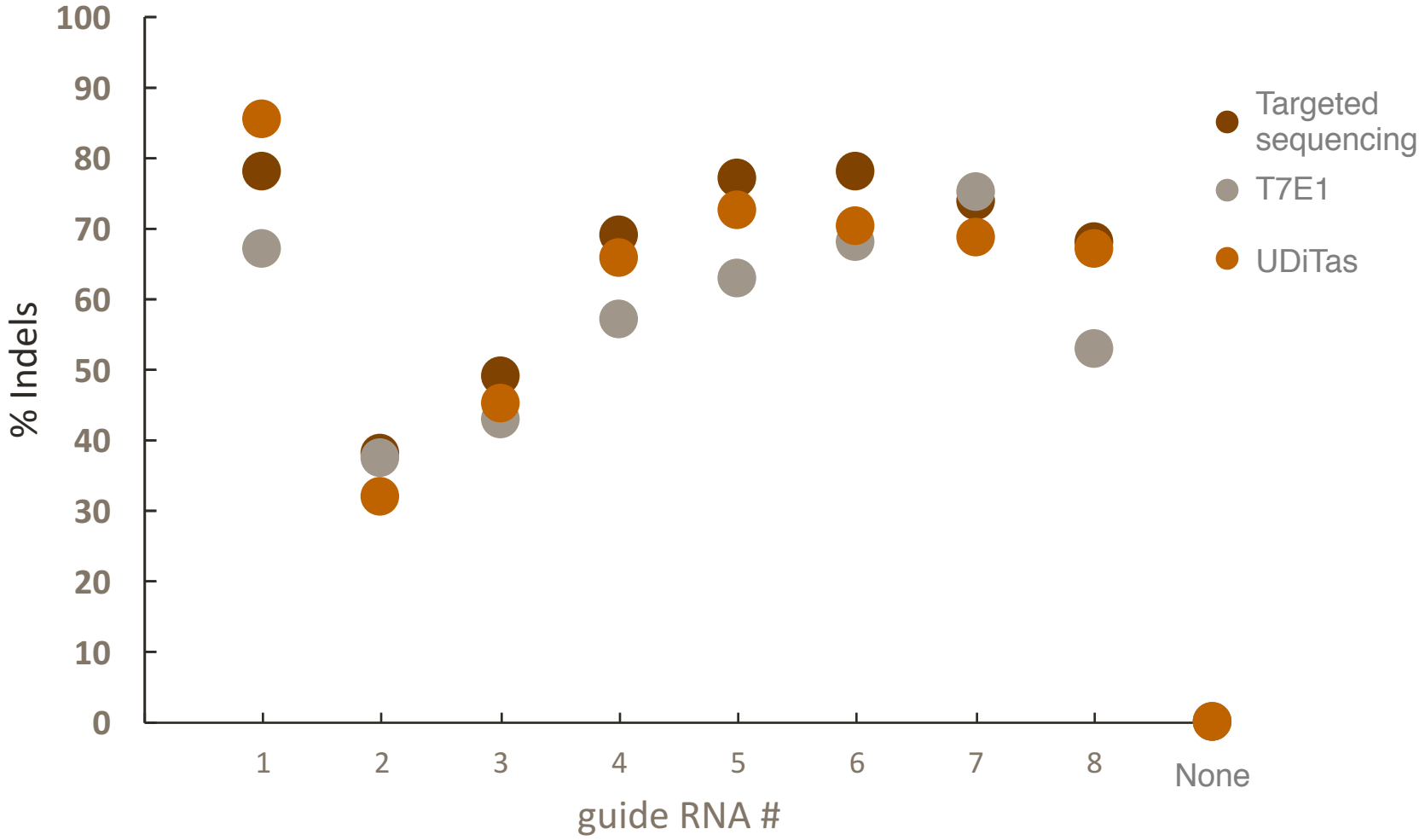
Comparison of Small Indel Measurements

UDiTaS correlates well with targeted sequencing and T7E1 assays



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Intra-Chromosomal Rearrangement Detection

Dual guide edits have multiple possible outcomes

Wildtype

Deletion

Indels (1 or both)

Inversion

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

Uditas: counts the sequences post junction

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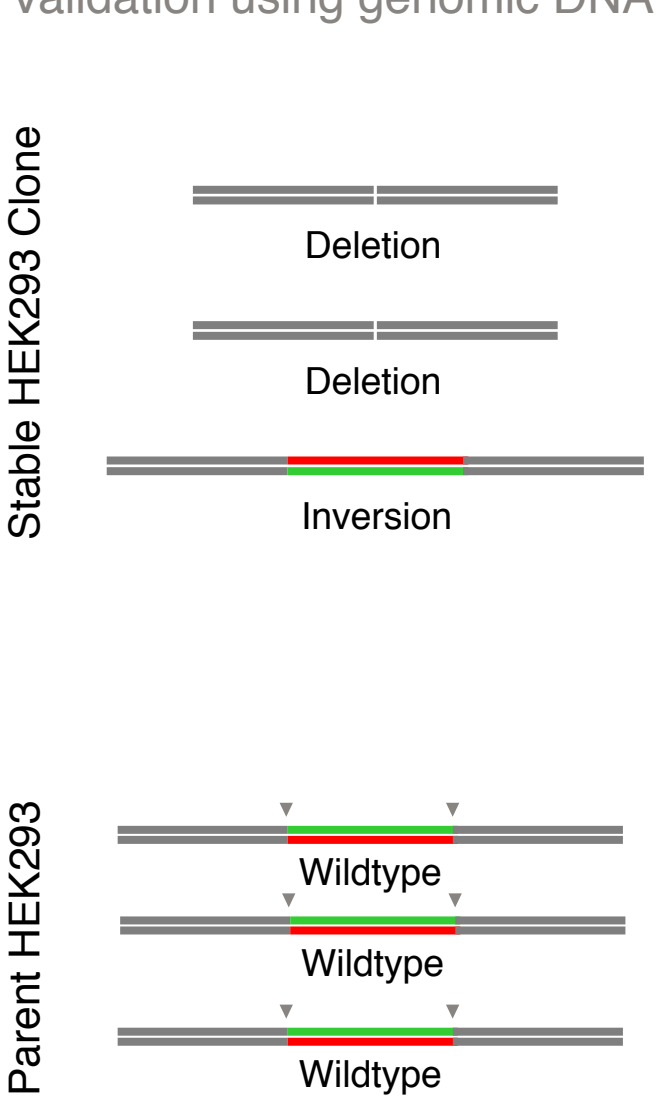
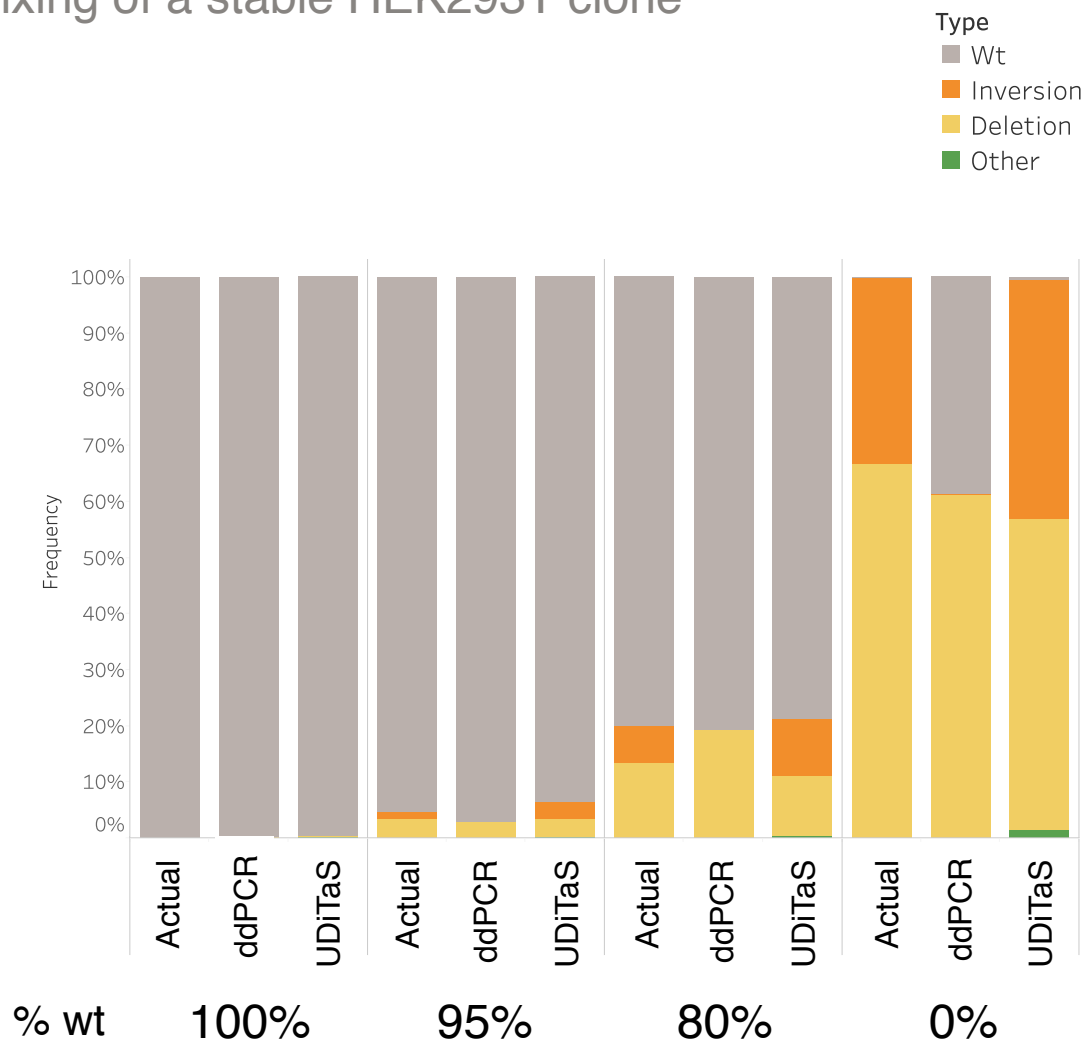
Intra-Chromosomal Rearrangement Detection

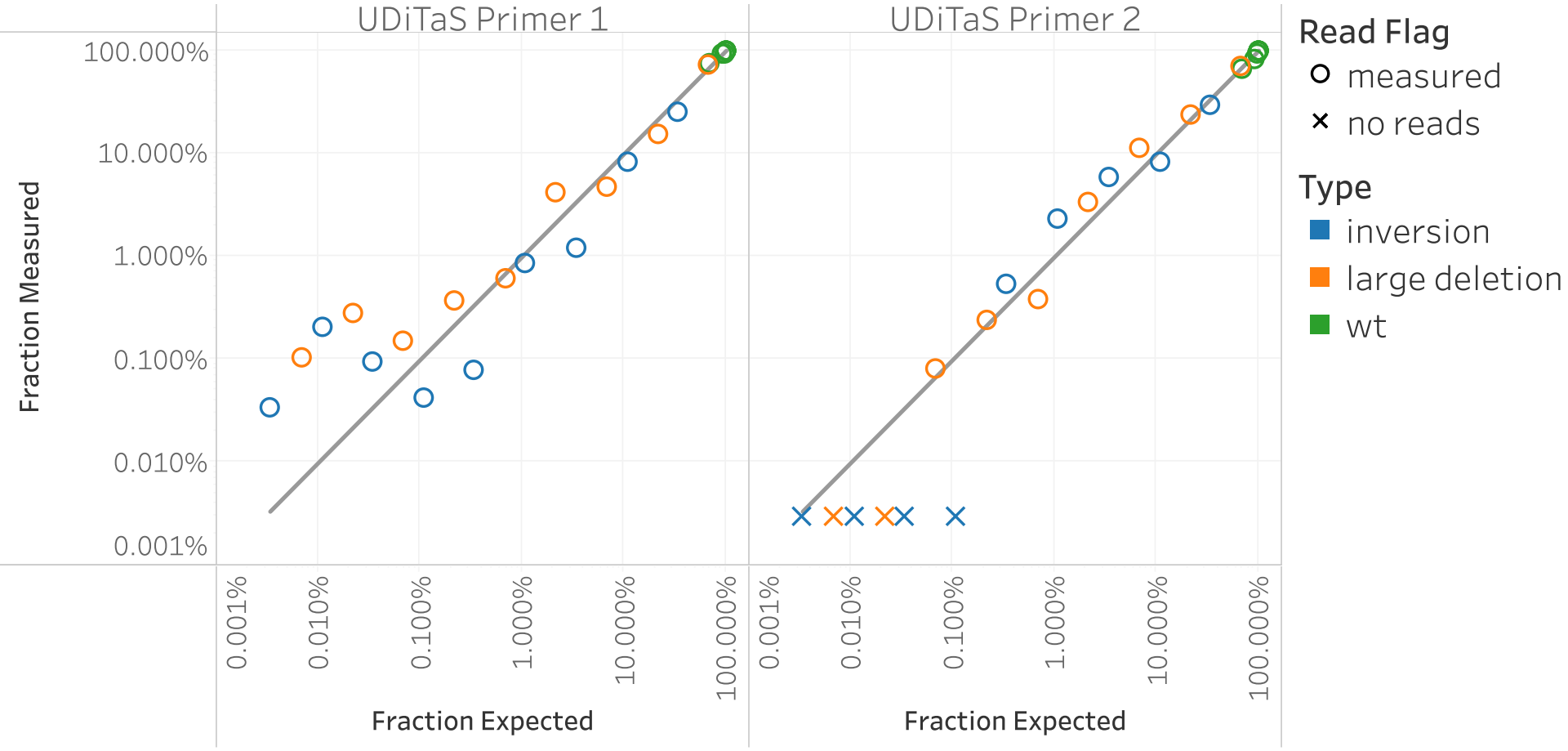
Validation using genomic DNA mixing of a stable HEK293T clone





Intra-Chromosomal Rearrangement Detection

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



3,500 input genomes

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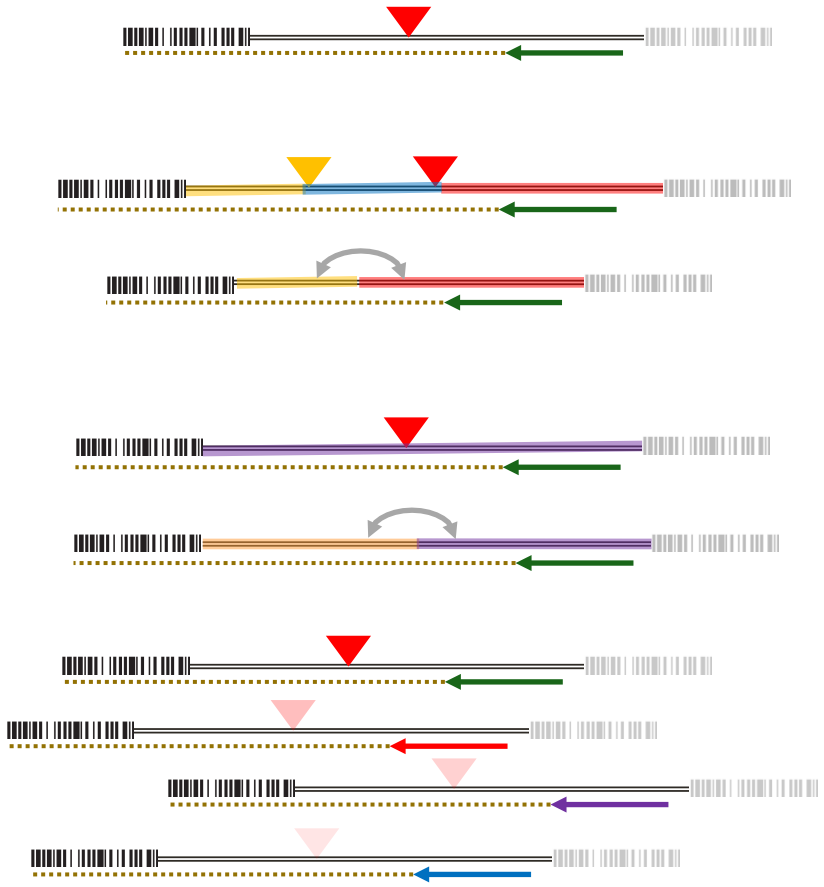
UDiTaS (Uni-Directional Targeted Sequencing)

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



1. Quantitation of editing
2. Quantitation and discovery of large deletions
3. Translocation discovery and quantitation
4. Multiplexing assays
5. Robust and shorter process

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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
Fred Harbinski
Jack Heath
Joy Horng
Hari Jayaram
Morgan Maeder
Eugenio Marco
Rina Mepani
Andrew Sadowski
Will Selleck
Terence Ta
Chris Wilson
Bruce Eaton &
The i2 Pharma team

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