Methods for Gene Editing Measurement and Off-Target Discovery

NIST-FDA Genome Editing Workshop

April 23, 2018 Gaithersburg, MD

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Disclosures for Chris Wilson

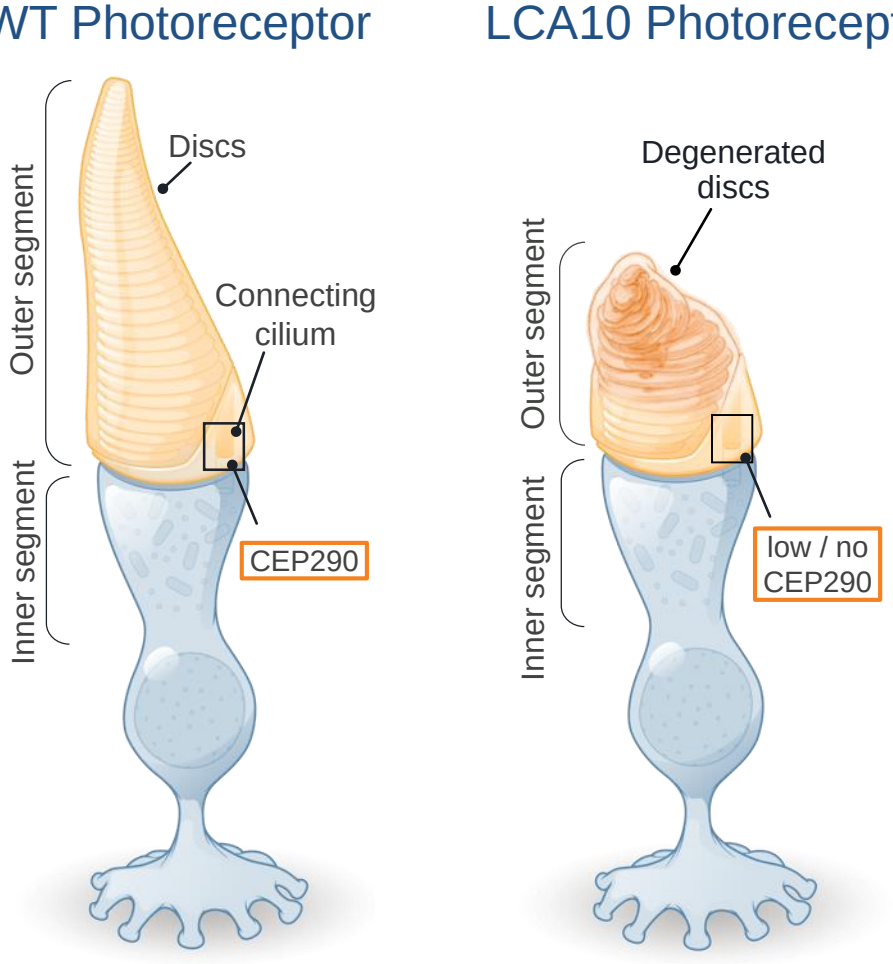
- Employee and stock option holder of Editas Medicine

Agenda

- LCA10 and CEP290 background
- UDiTaS
  - to measure large deletions and inversions at the CEP290 editing site
  - to measure translocations
  - development of accuracy standards for structural changes
- Specificity approaches
  - Digenome
  - Statistical framework for describing off-target verification

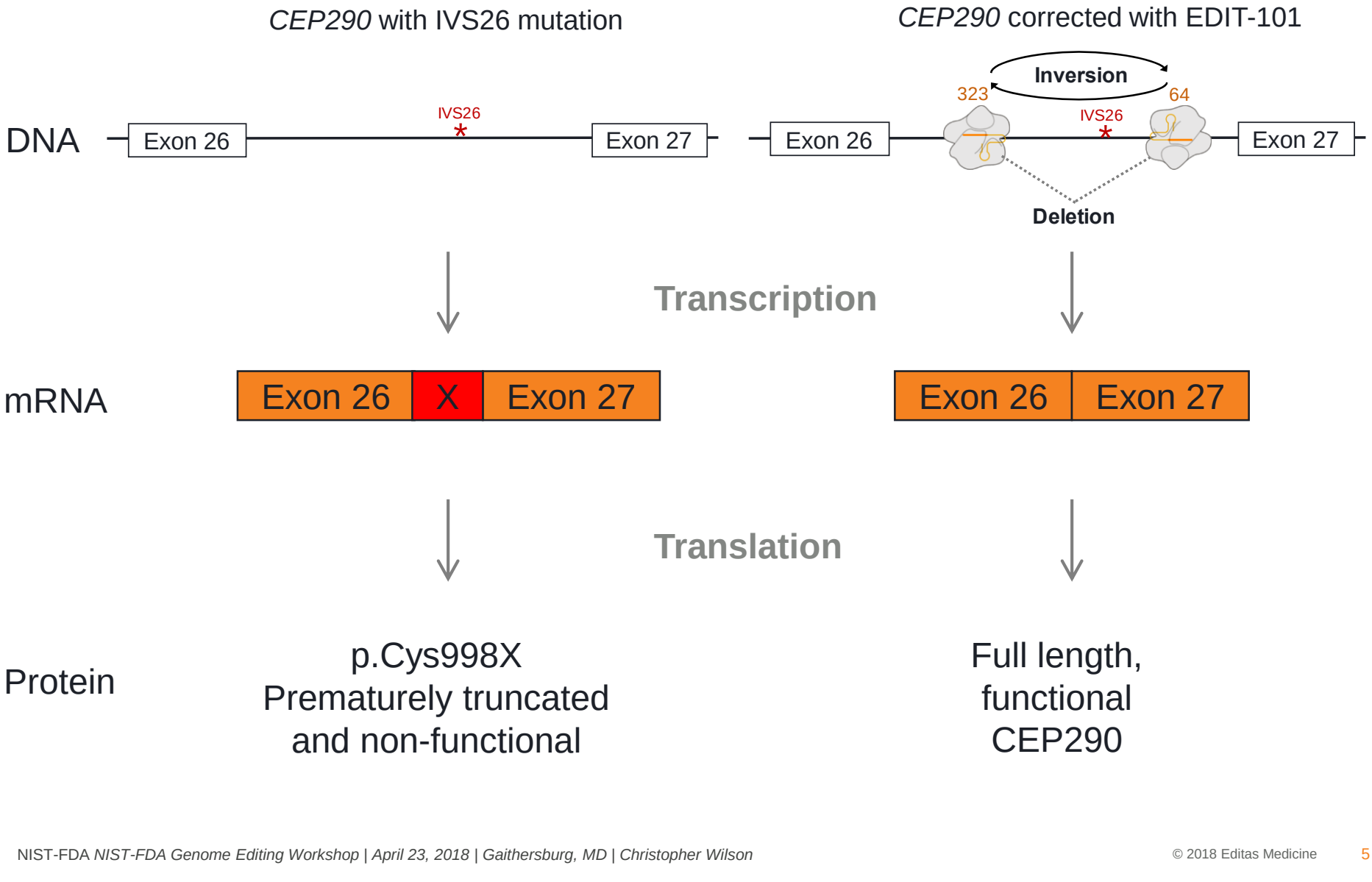
Leber Congenital Amaurosis Type 10

- Infantile-onset of poor vision, nystagmus, and a flat electroretinogram[1]
- Caused by autosomal recessive mutations in the **CEP290** gene at chromosome12q21.32[2]
- ~85% of LCA10 patients from northwest Europe have a “IVS26” mutation in intron 26, c.2991+1655A>G[1-7]
- CEP290 is present in the connecting cilium and is important for ciliogenesis, ciliary trafficking, and outer segment function and structure[8]

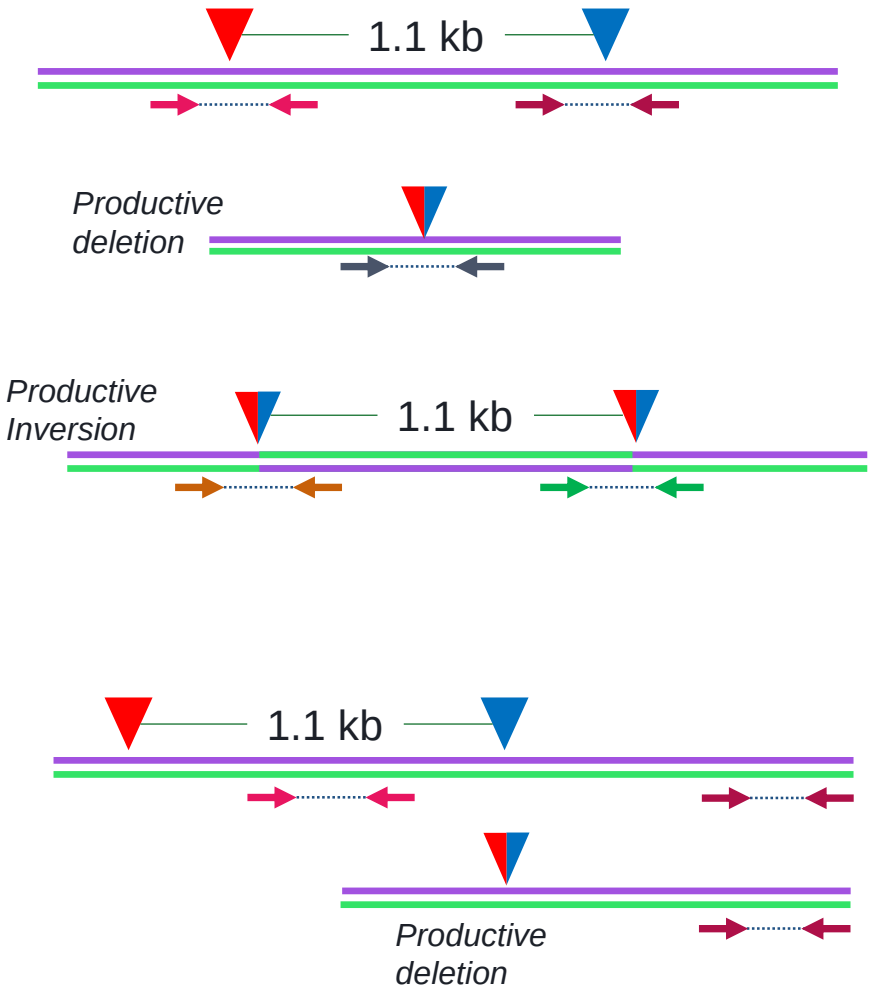


[1]Weleber RG, 2013 LCA Gene Reviews; [2]den Hollander AI, Koenekoop RK, Am J Hum Genet 2006;79:556; [3]Stone EM, Am J Ophthalmol 2007;144:791; [4]CEP290_database 2017; [5]Perrault I, Hum Mutat 2007;28:416; [6]Vallespin E, IOVS 2007;48:5653; [7]Simonelli F, IOVS 2008;48:4284; [8]Rachel RA, Cilia 2012;1:22

Gene Editing to Repair *CEP290* Splicing Defect



Challenges with PCR-NGS assays when making multiple edits

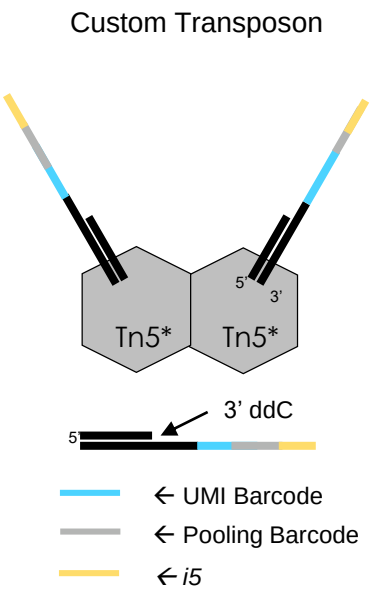


- 3 PCR assays needed to measure editing at CEP290 intron 26 locus
- Even with rigorous standards it is difficult to cross compare assays
- Another set need for inversions
- ddPCR sufficient to measure the deletion but unable to distinguish inversions from wild type locus

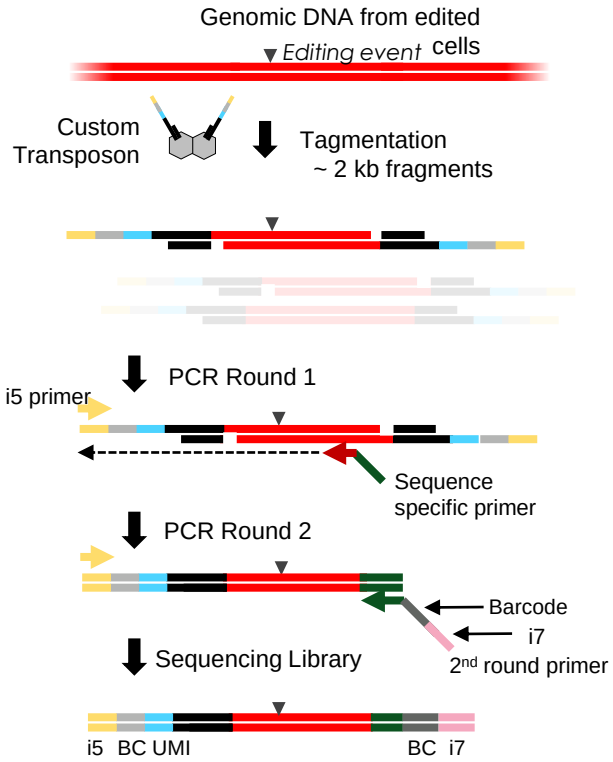
Uni-Directional Targeted Sequencing UDiTaS*

An NGS method for measuring junctions (and indels!)

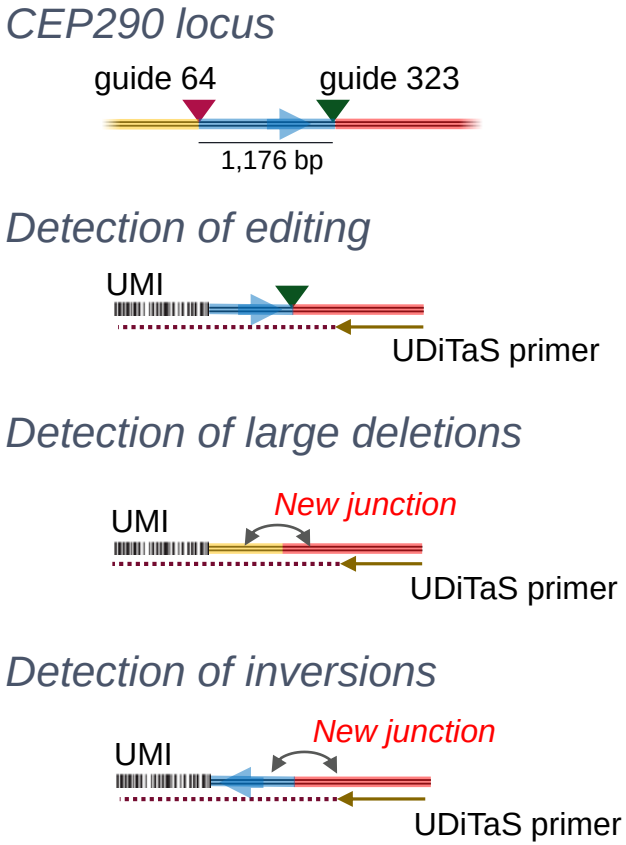
a



b



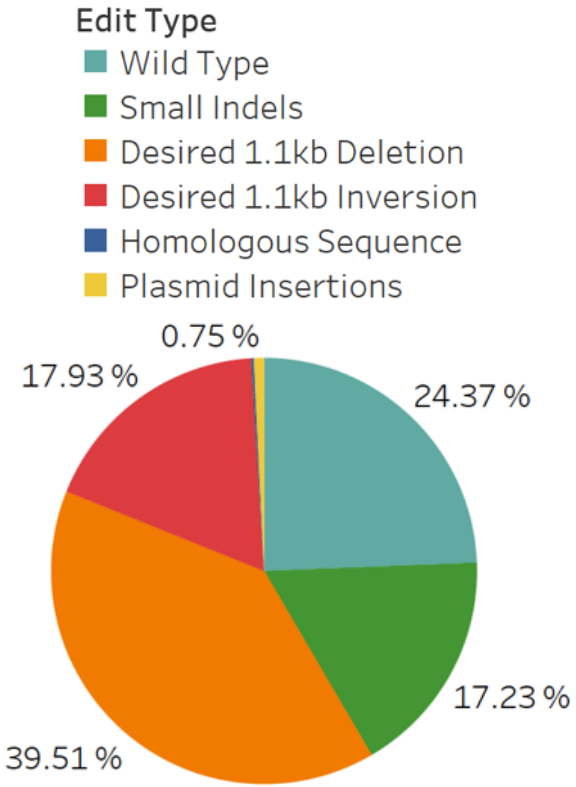
c



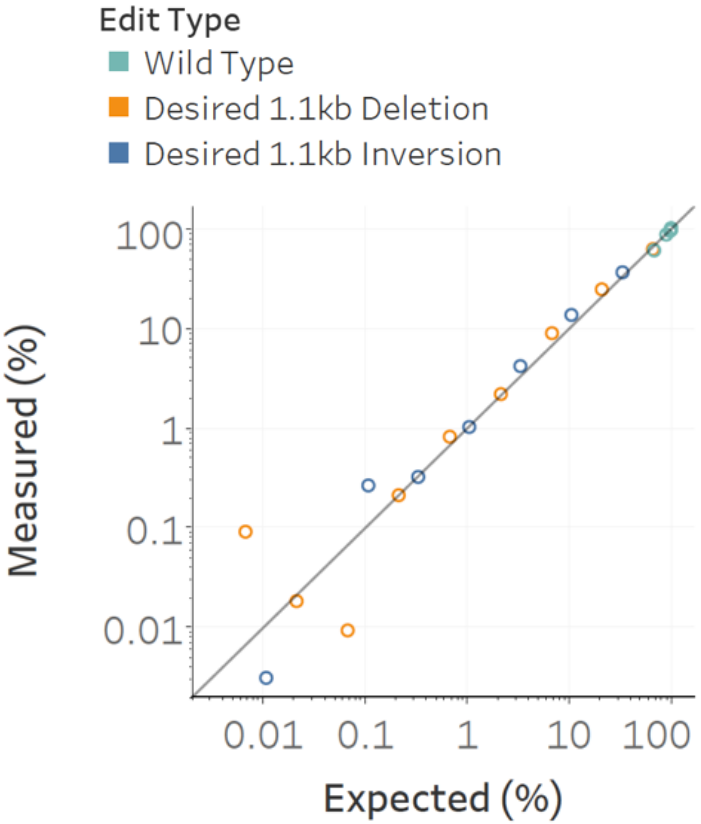
* Giannoukos, et al., "UDiTaS™, a genome editing detection method for indels and genome rearrangements", BMC Genomics, 2018 **19**:212

UDiTaS finds novel structural changes and is robust

U-2 OS cells nucleofected with plasmids expressing SaCas9 + gRNA64 + gRNA323

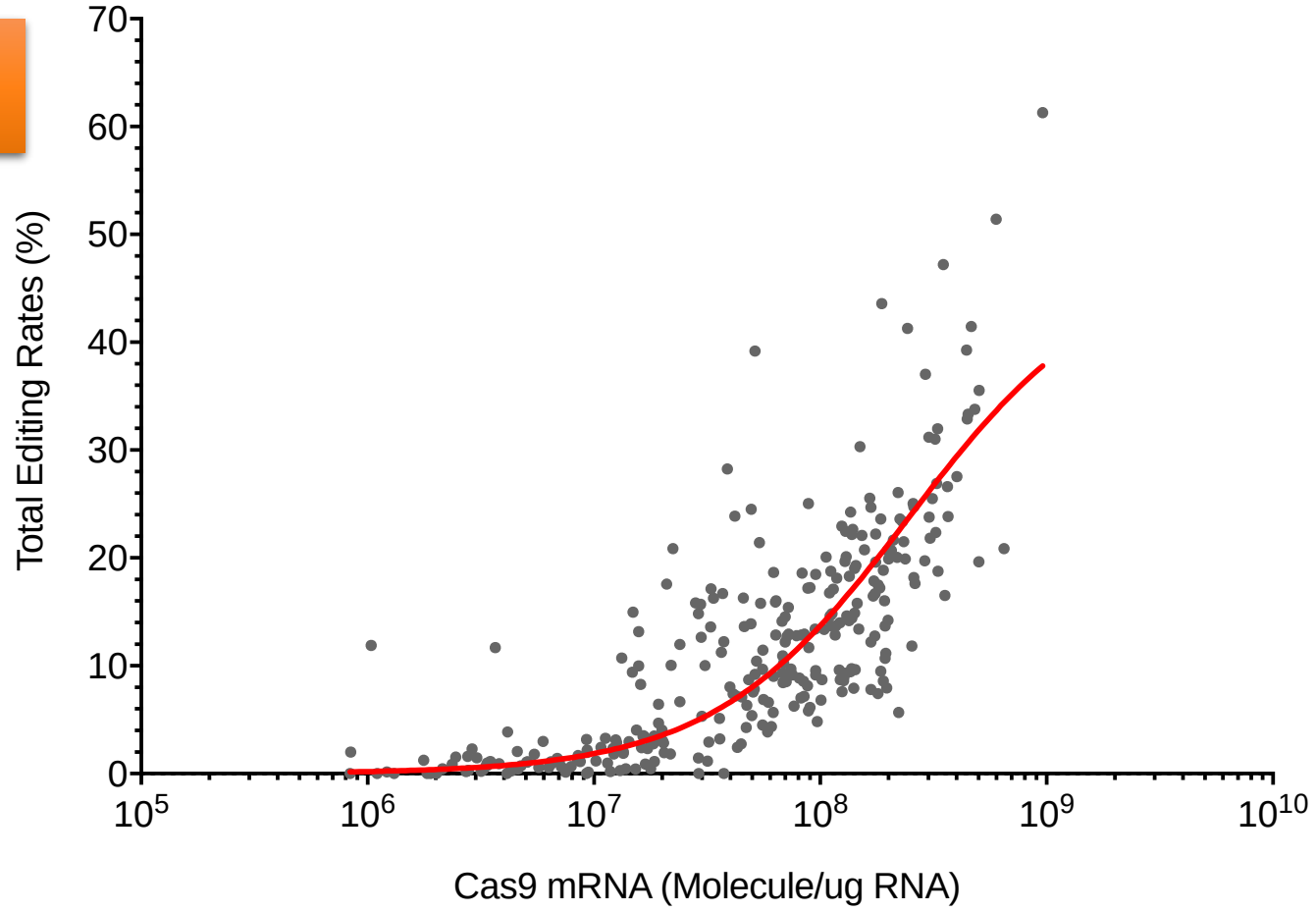


gDNA from stable HEK293 line with deletion and inversion mixed with HEK293 gDNA at various ratios



UDiTaS in action: hundreds of samples from mouse pharmacology experiments

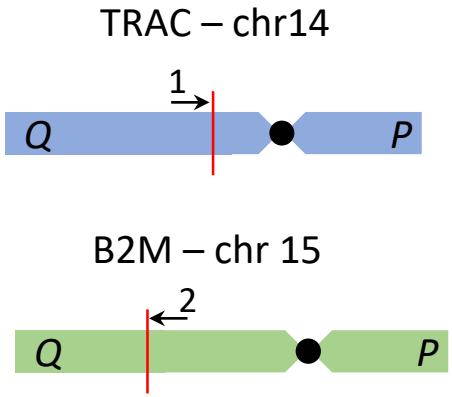
Measured by UDiTaS



PK/PD relationship: On-target Editing Correlates with Transgene Expression by EDIT-101 in HuCEP290 KI Mice

UDiTaS can measure translocations

CD4+ human primary T cell nucleofected with 2 RNPs:

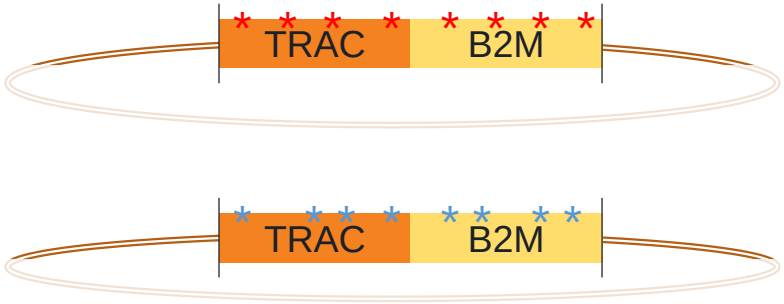


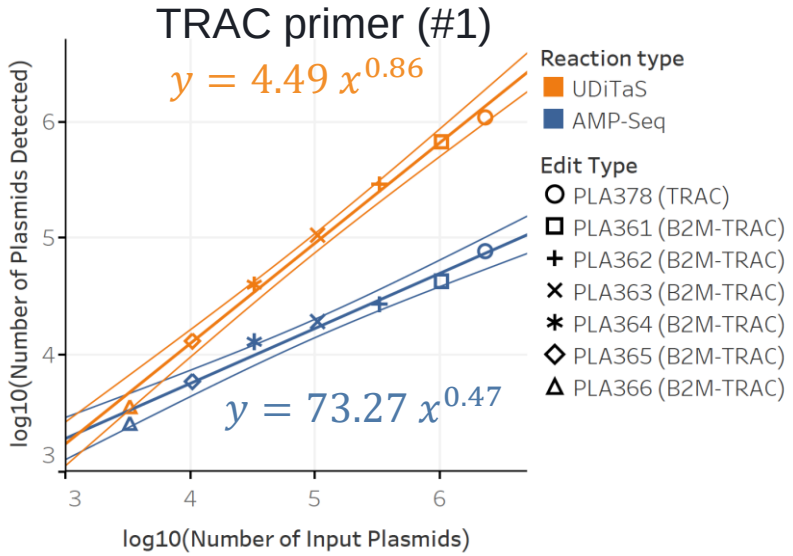
- 10 possible outcomes
- 7 measurable with primers 1 and 2
- All 7 events detected

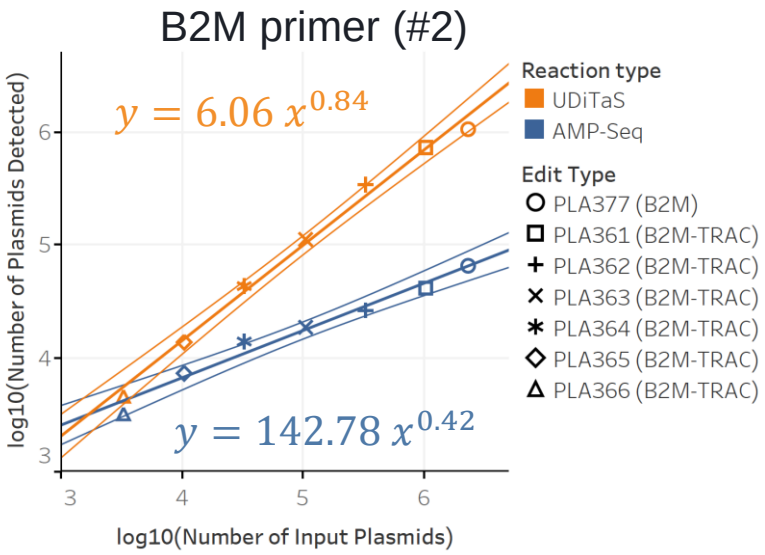
Schematic	Type	1 (%)	2 (%)
	No edits	**14.87**	n/a
	Indels	**82.35**	n/a
	Dicentric	n/a	n/a
	Acentric	**0.001**	n/a
	No edits	n/a	**3.89**
	Indels	n/a	**91.38**
	Dicentric	n/a	**0.42**
	Acentric	n/a	n/a
	Balanced	n/a	n/a
	Balanced	**2.04**	**2.63**
	Acentric	**0.66**	n/a
	Dicentric	n/a	**1.62**

Accuracy of translocation measurements

- Created series of 6 plasmid standards at the predicted TRAC-B2M translocation adding SNPs every ~10bp

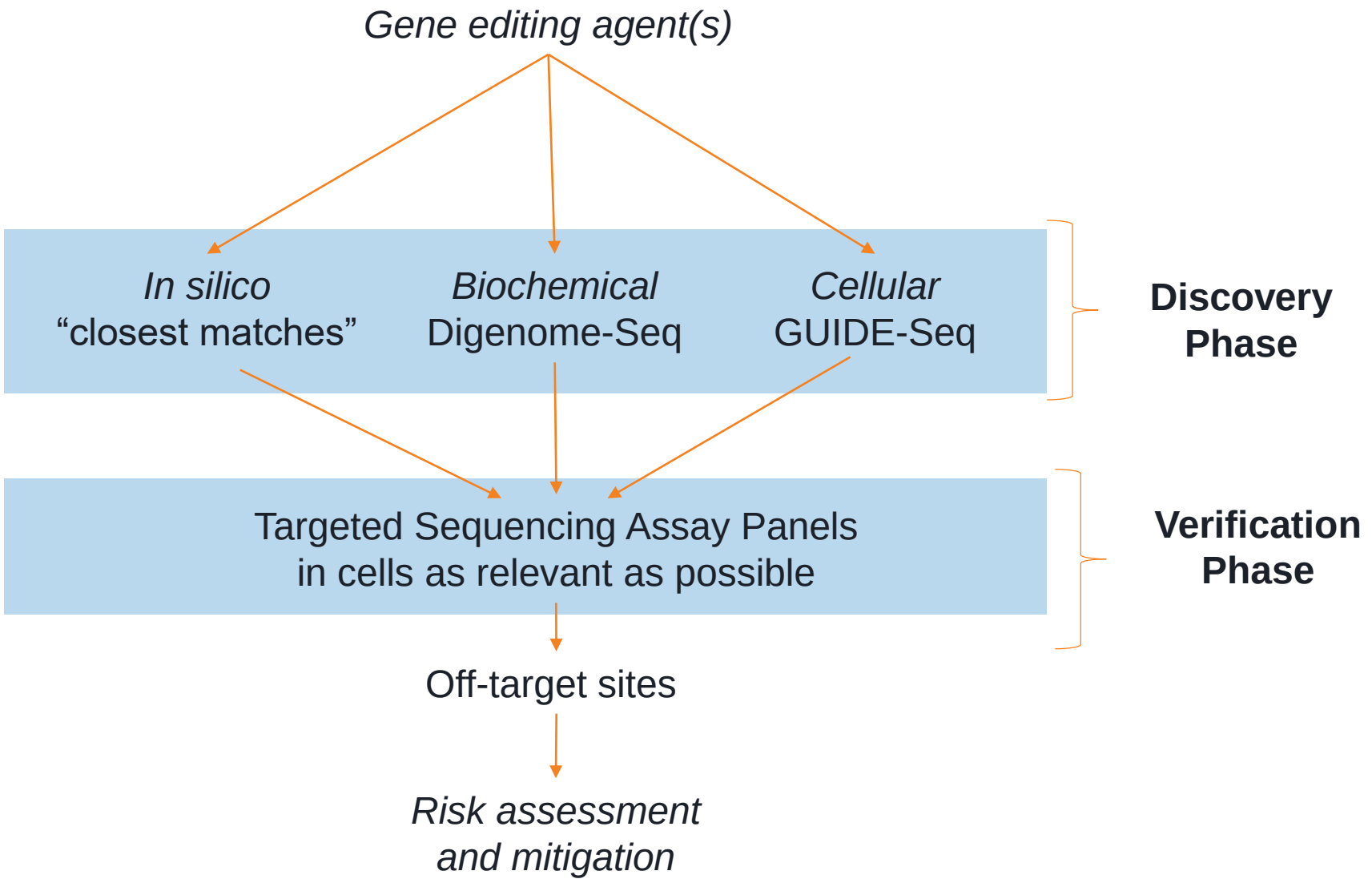




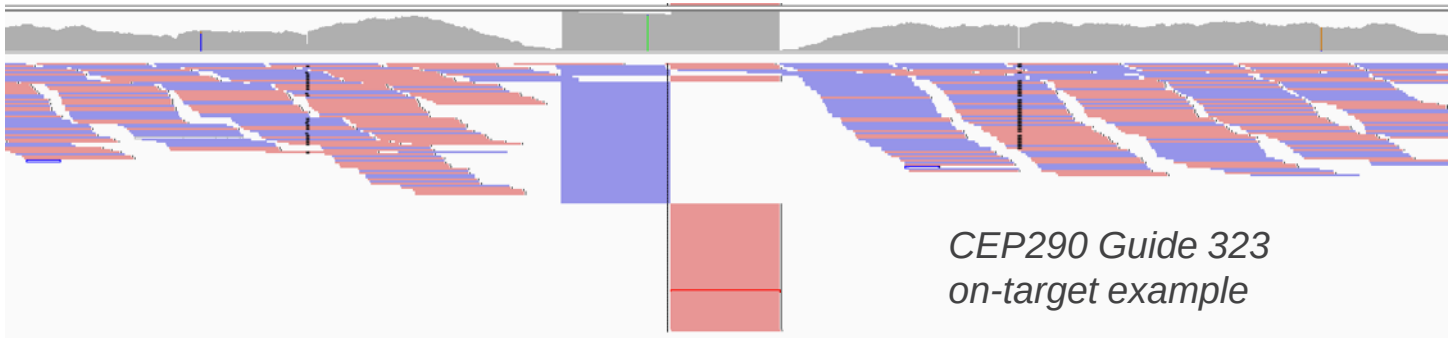


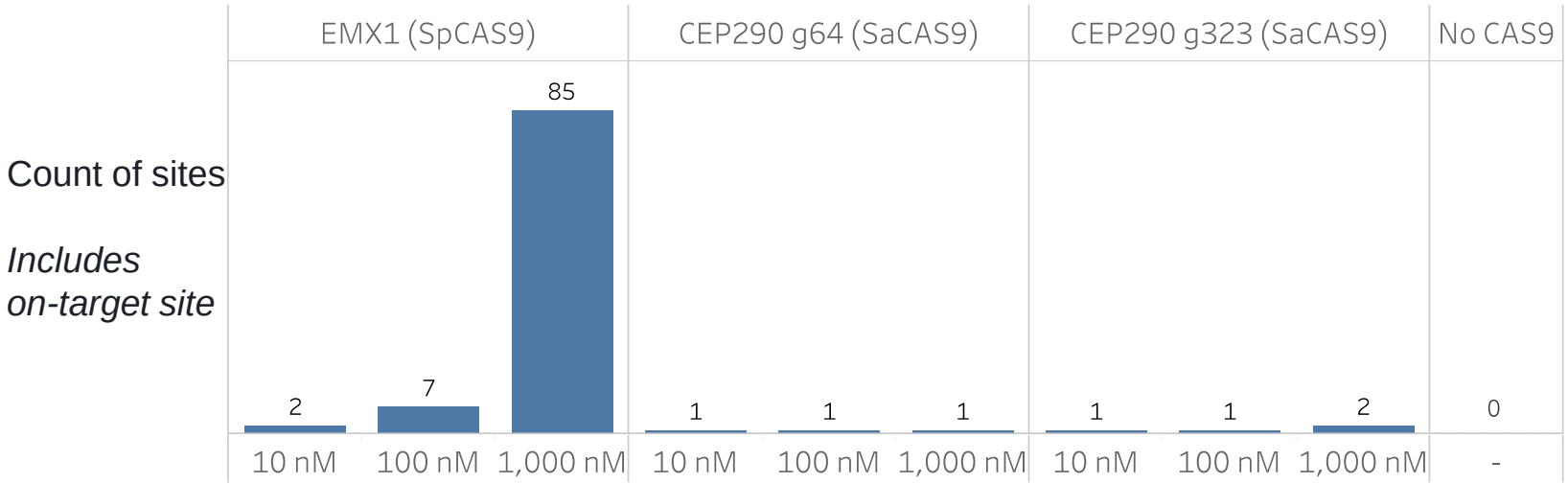
- Individually dilute each plasmid ~3,000 molecules to 3e6 molecules (3 logs)
- Spike into mouse genomic DNA
- Run UDiTaS and AMP-Seq
- Demonstrates high accuracy and linearity of UDiTaS

Editas approach to editing specificity



Digenome-Seq with Lead Guides (64 and 323)





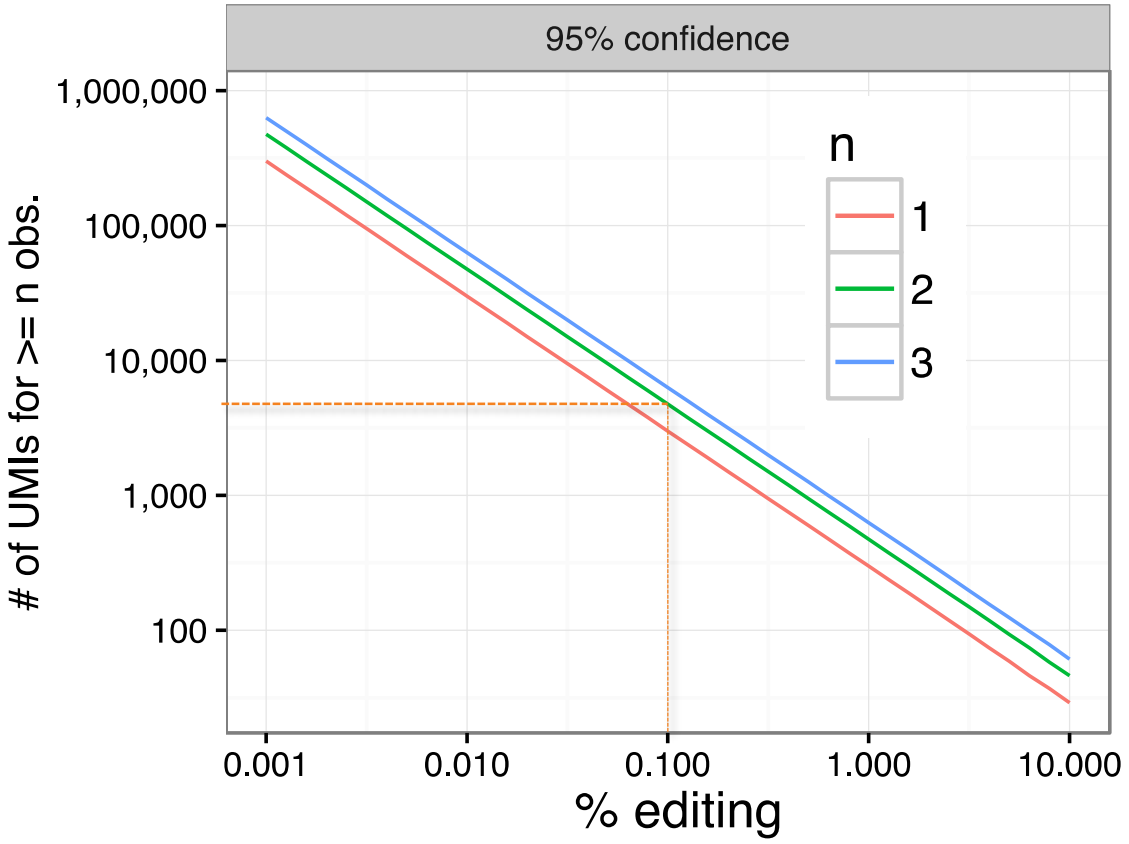
Statistical framework for sensitivity calculations

- Similar to small molecule safety profiling
- Off-target measurements needs to include the limit of detection
- Express no detected off-targets as <LLoD; eg:

  “Editing at chr1:124245 is <0.1%”

- Main determinants are:
  - Read count
  - Input DNA amount

Binomial sampling distribution in NGS as part of an LLoD determination



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  - to measure translocations
  - development of accuracy standards for structural changes
- Specificity approaches
  - Digenome
  - Statistical framework for describing off-target verification (and lack of off-target measurement)


Thank you.