gRNA Quality for CRISPR Medicines

TIDES

May 10, 2018

Vic Myer, Ph.D.
Chief Technology Officer

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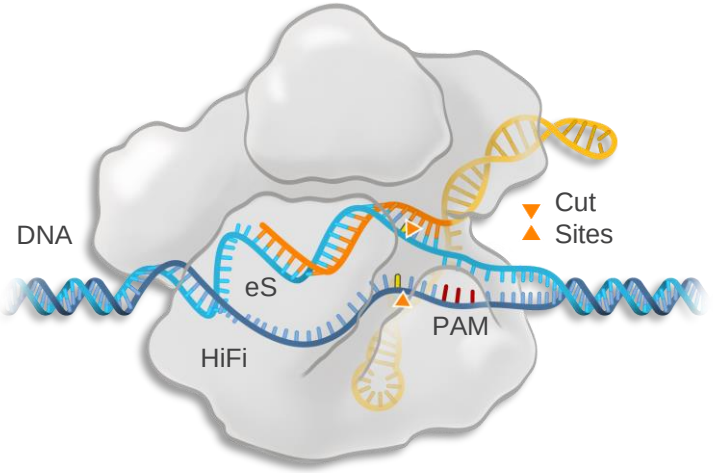
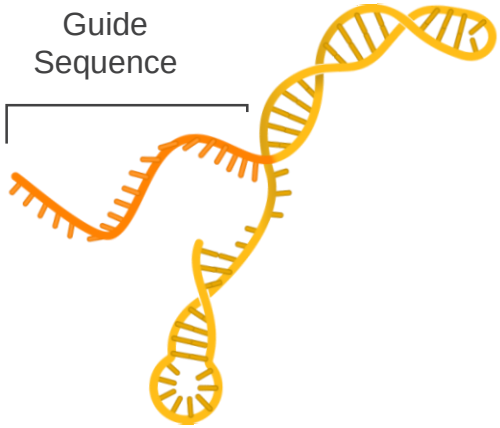
VM is an employee and shareholder of Editas Medicine

CRISPR Unlocks Genome Editing

Nuclease



Guide RNA



Complex of nuclease and guide RNA precisely locates and cuts genomic sites

Ability to target many sites simultaneously using numerous guide RNAs

Nuclease can be engineered to reach more sites and to modulate cutting

DNA: Deoxyribonucleic Acid; HiFi: High Fidelity; eS: Enhanced Specificity; PAM: Protospacer Adjacent Motif

Genome Editing Medicines

- Potential for transformative therapies
  - Genetically-defined and also genetically-treatable diseases
  - Achieves *durable* changes to edited cells
- Multiple translational science questions
  - Editing efficiency, cellular context, delivery & strategy
  - Biological and genetic context is critical
  - Specificity: demands focus and has progressed well
- Balance of potential therapeutic benefit with risk
- Regulatory science is key to realize these medicines

Developing Best-in-Class CRISPR Medicines

Ocular Medicines

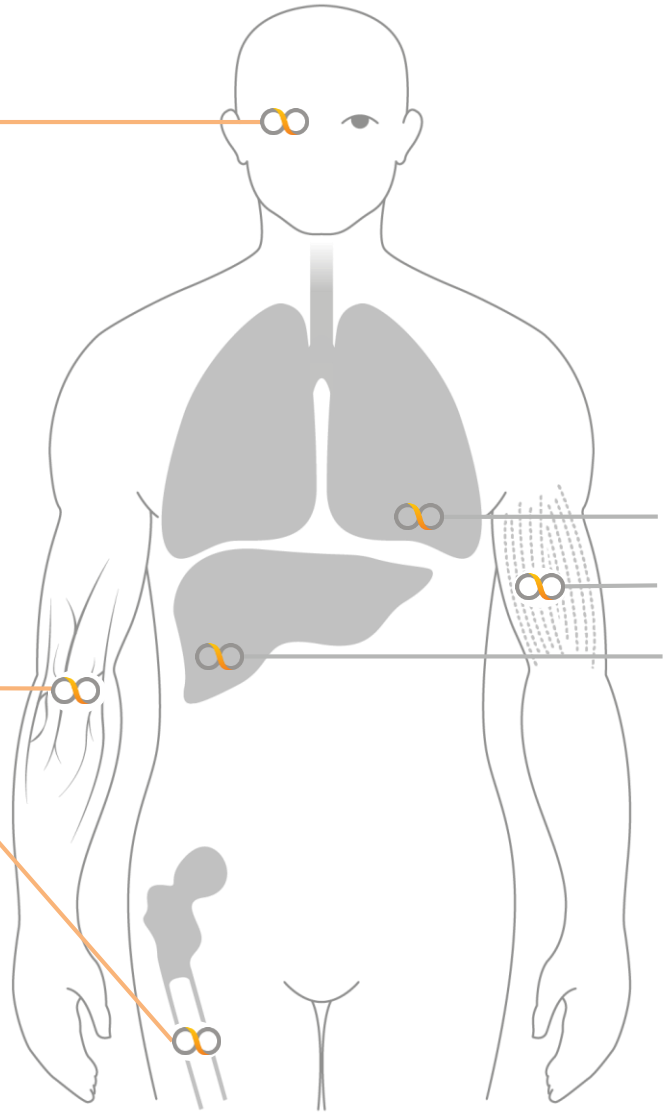
Inherited Retinal Diseases
- LCA10 (EDIT-101)
- USH2A
- Additional unnamed targets

Infectious Diseases
- Ocular HSV

Engineered Cell Medicines

Immune Cells
- T Cells – Cancer
- T Cells – Immunomodulation

Stem Cells
- HSCs – Sickle Cell Disease
- HSCs – Beta-thalassemia



Early Discovery
- Lung – CF
- Muscle – DMD
- Liver – AATD

in vitro proof-of-concept

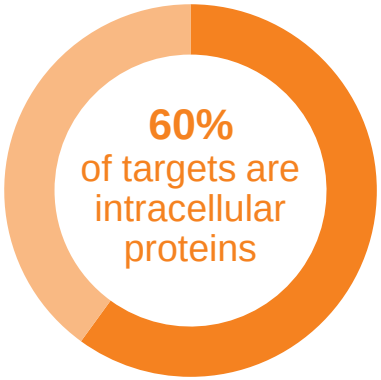
in vivo proof-of-concept

LCA10: Leber Congenital Amaurosis Type 10; USH2A: Usher Syndrome Type 2A; HSV: Herpes Simplex Virus; CF: Cystic Fibrosis; DMD: Duchenne Muscular Dystrophy; AATD: Alpha-1 Antitrypsin Deficiency; HSC: Hematopoietic Stem Cell

Next-Gen Engineered T Cells for Cancer

JUNO THERAPEUTICS A Celgene COMPANY

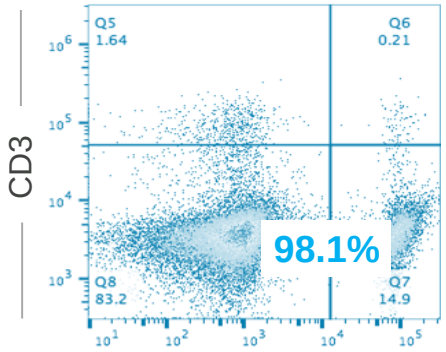
"Top 50" Cancer Antigen Targets[1]



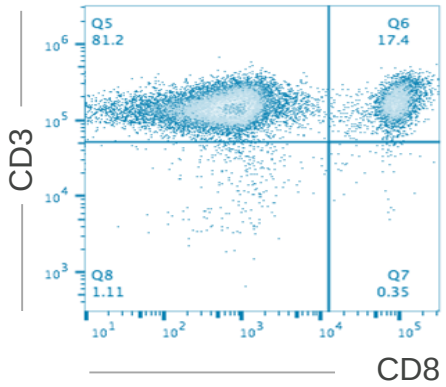
Rank	Antigen	Mechanism
1	WT1	Oncogenic
3	LMP2	Viral
4	HPV	Viral/Oncogenic
8	MAGE A3	Mixed
9	P53 WT	Oncogenic
10	NY-ESO-1	Prognosis
14	MelanA/ MART1	Differentiation
15	Ras Mutant	Oncogenic
16	gp100	Differentiation
17	p53 Mutant	Oncogenic

Nearly Complete TCR Knockout

TCR KO

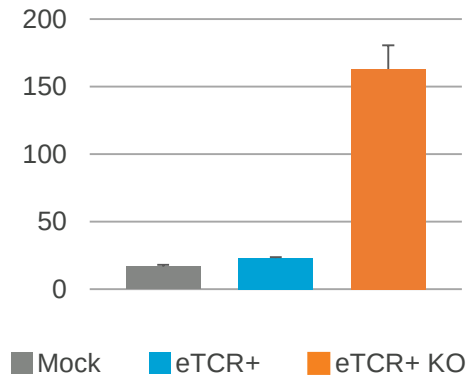


Control

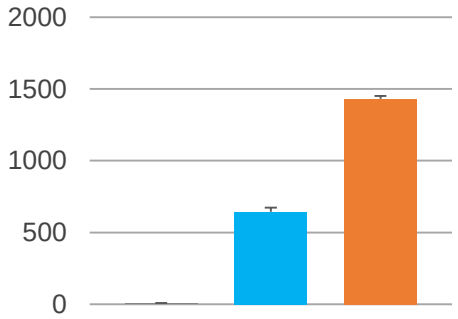


Increase in Functional Activity

CD4 IFNγ (pg/mL)



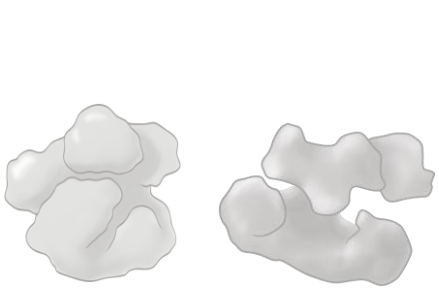
CD8 IFNγ (pg/mL)



[1]*Clin Cancer Res*. 2009 Sep 1, 15(17); KO: Knockout; eTCR: Engineered T Cell Receptor; IFNγ: Interferon Gamma

Ex Vivo Drug Development

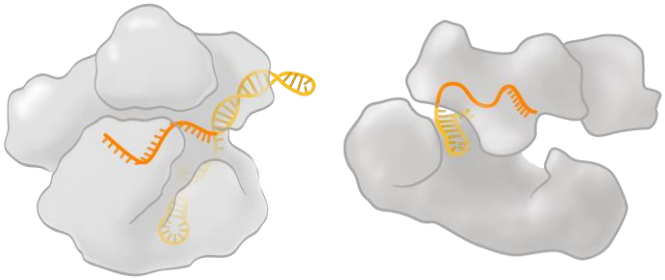
High Quality Ribonucleoprotein Particle Delivery



RNA Guided Endonuclease

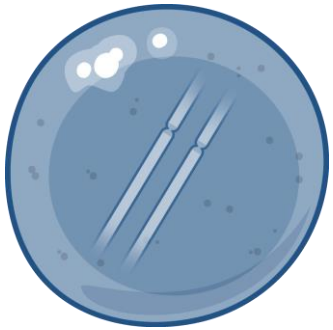
+

RNA Guide

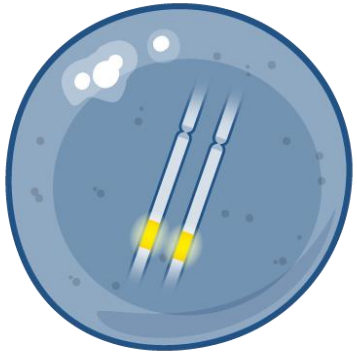


Ribonucleoprotein Particle (RNP)

+

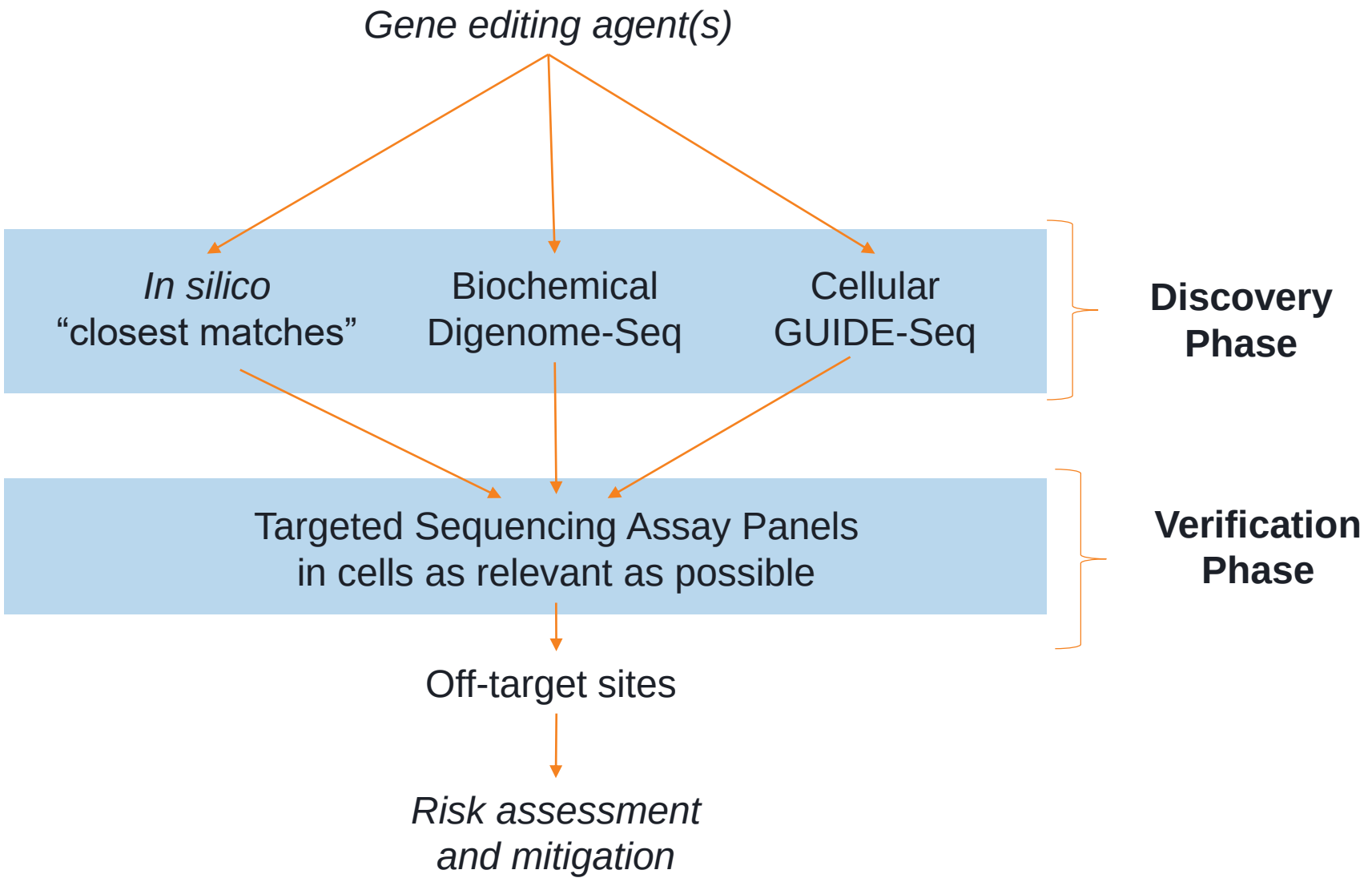


Patient Cell



Engineered Patient Cell

Editas Approach to Editing Specificity



In silico Prediction Yields Many Sites to Monitor

Using the enzyme specific sequences to generate a list of sites to monitor

- Computational methods compare gRNA sequence to human genome data base
- These methods allow for gRNA: genome mismatches (called “Off by”) and bulges in the gRNA and genomic DNA
- These analysis generally give ~ 100 sites to monitor

```
GUCUGGGCGGUGCUACAACU NGG
||||||.|||||||||||||  |||
GTCTGGATGGTGCTACAACT TGG
```

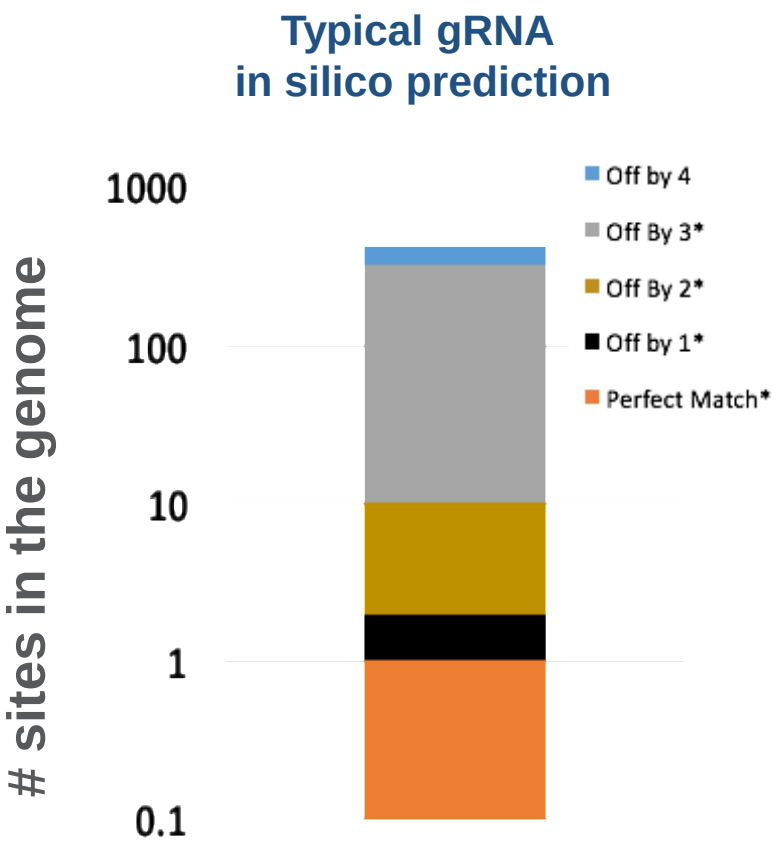
Off By 1

```
GUCUGGGCGGUGCUACAACU NGG
..|.|||||||.||||||||  |||
AACGGGGCGGTACTACAACT TGG
```

Off By 4

```
GUCUGGGCGGUGCUACAACU NGG
||||  ||.|||||||||||||  |||
GTCT-GGTGGTGCTACAACT TGG
```

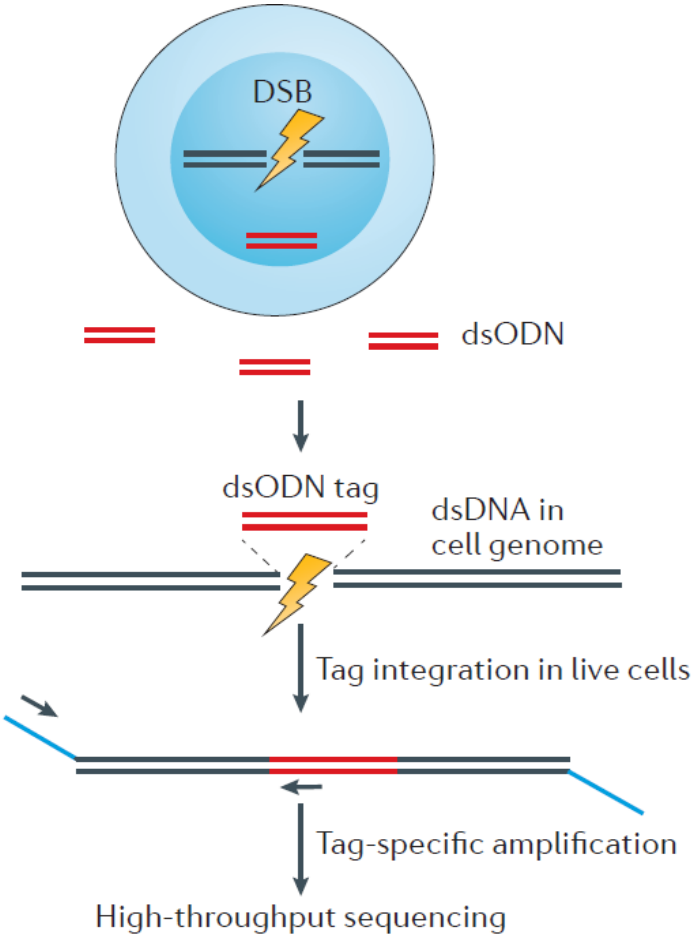
Off By 1 with a gRNA bulge



*These numbers allow for a single RNA or DNA bulge

Cellular Off-Target Discovery: GUIDE-Seq Assay

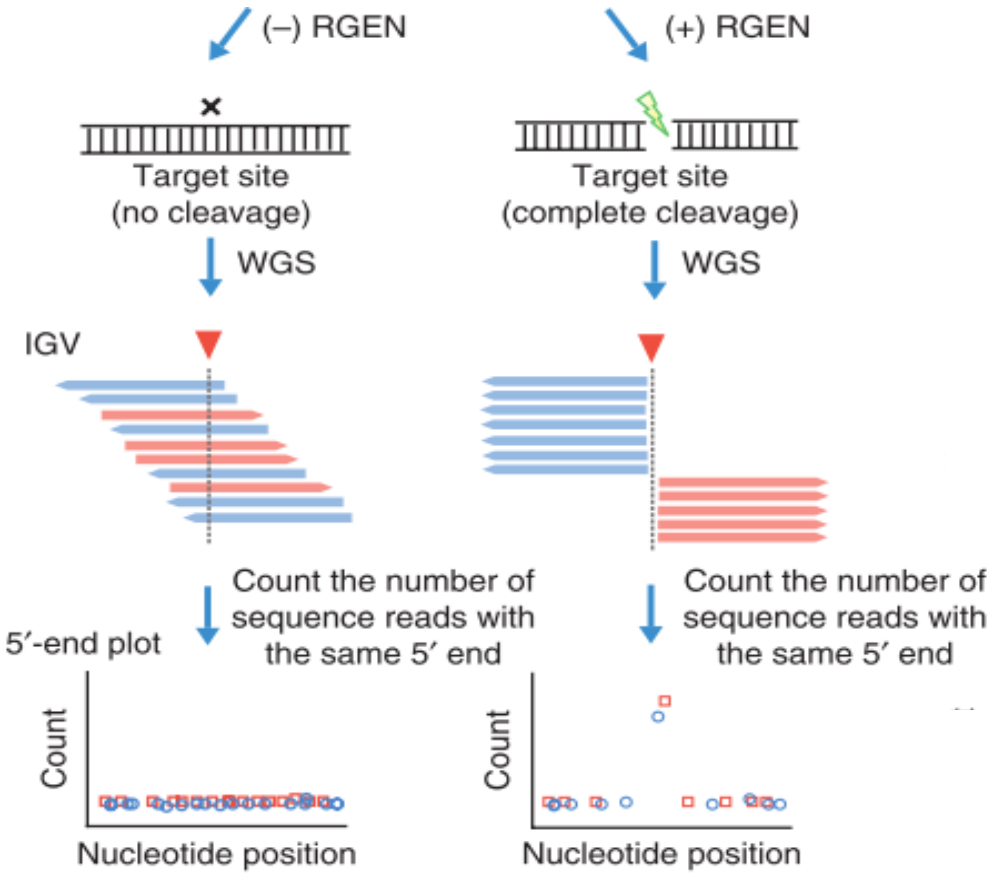
Introduction of a dsODN into cells along with Cas9 and gRNA tags sites of double-strand breaks. Tag is then used to identify sites of DNA double strand breaks via next gen sequencing



Tsai et al., NBT 2015

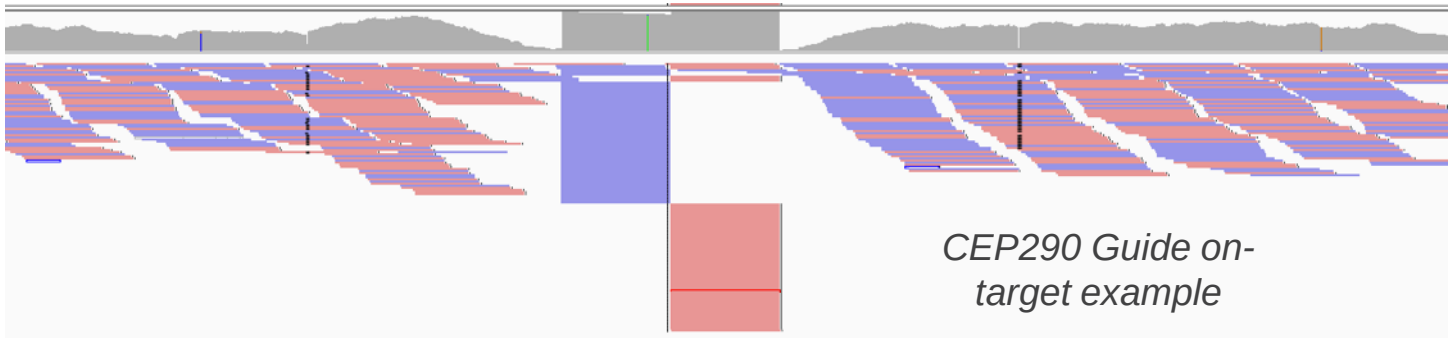
Biochemical Off-Target Discovery: Digenome Assay

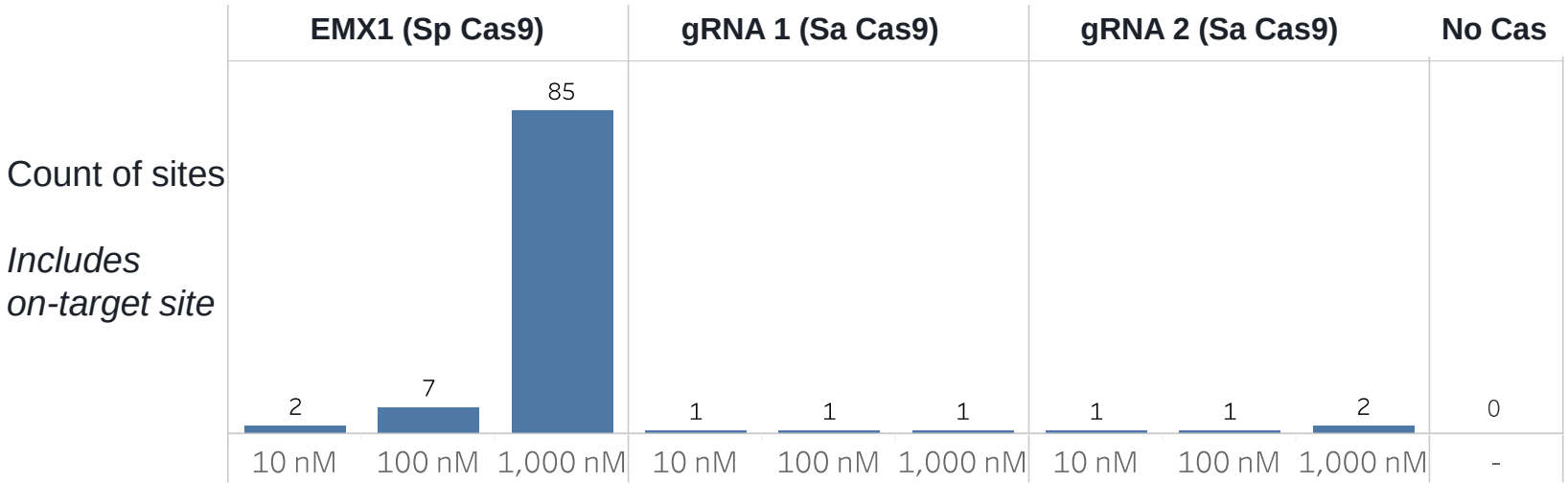
In vitro cleavage of genomic DNA followed by whole genome sequencing identifies potential off-target sites



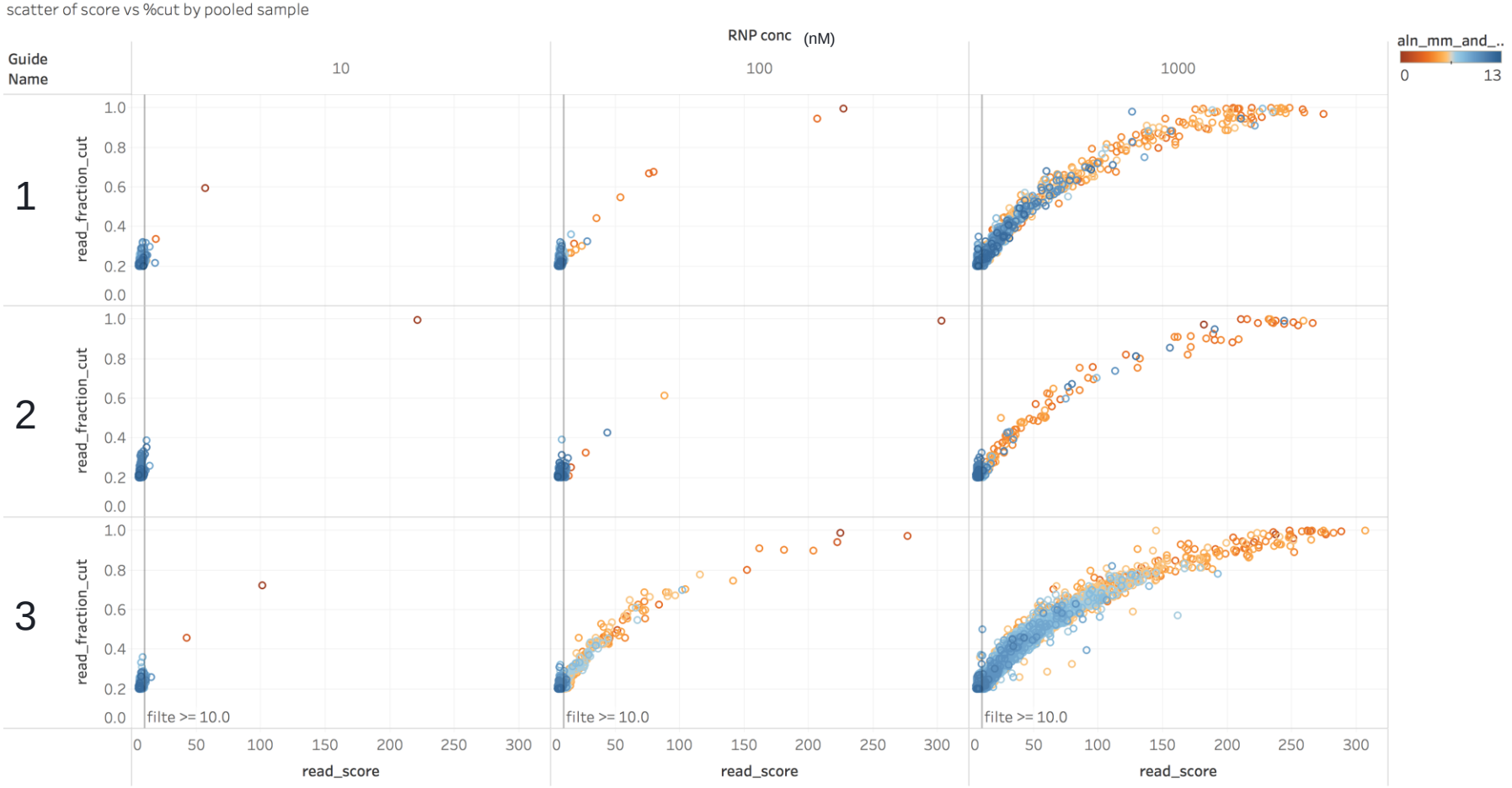
Kim et al., Nature Methods 2015

Digenome-Seq with Optimized Guides





Digenome-Seq Results for 3 Candidates



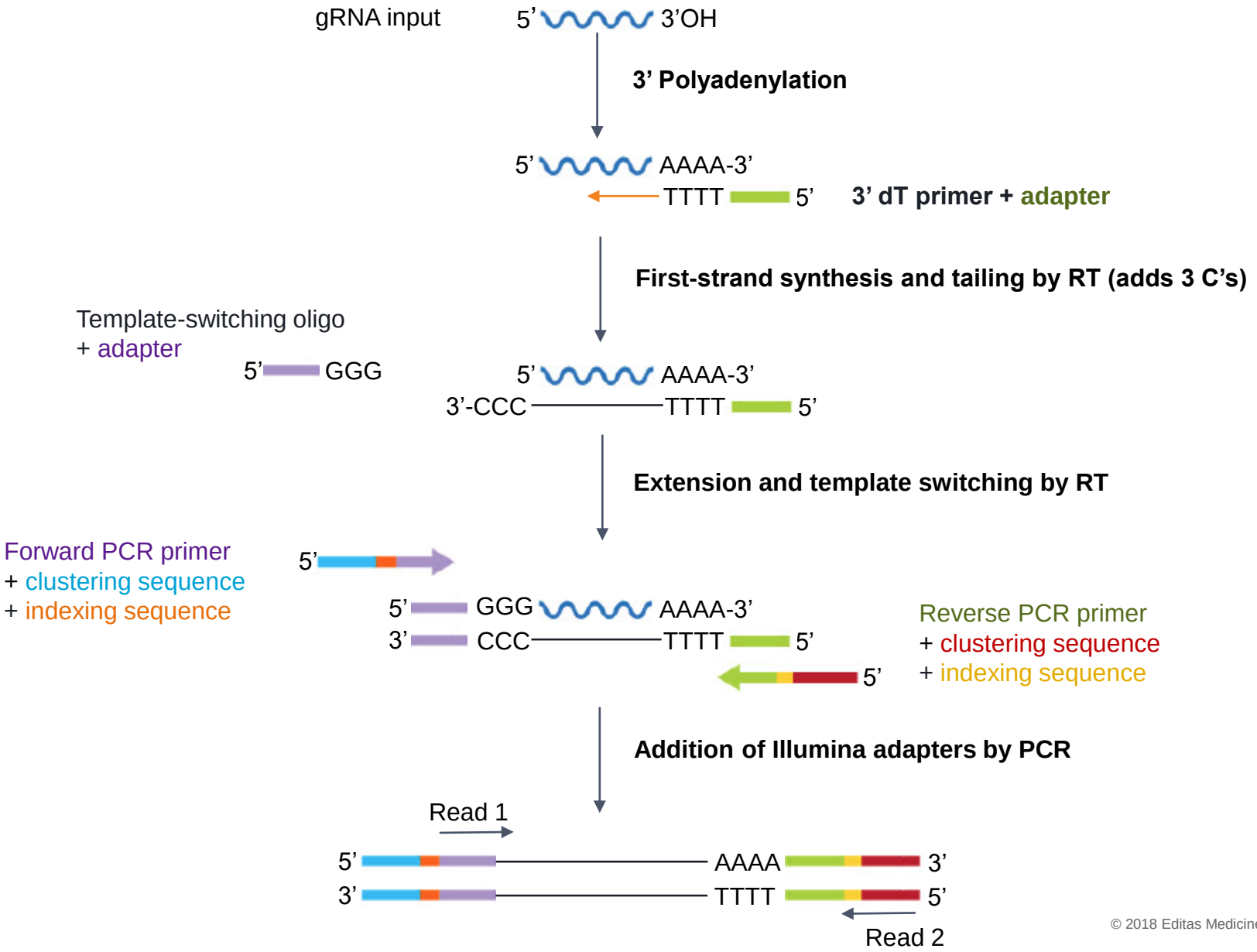
Read_score vs. read_fraction_cut broken down by RNP conc vs. Guide Name. Color shows aln_mm_and_gaps.

Digenome Results Show Unexpected Pattern

- Sites Identified by Digenome:
  - Distinct count of Alignment coordinates were broken down by guide and alignment (as mismatch gaps)
  - Interesting pattern emerged
  - Targeted sequencing panel showed no bone-fide off targets for all sites

Alignment mm+gaps	2
0	1
1	
2	1
3	25
4	52
5	23
6	1
7	5
8	5
9	7
10	16
11	20
12	4
13	
Grand Total	160

Development of NGS-based Method for gRNA QC



Sequencing Assay Results

Guide	Vendor	Fraction_perfect_guide (%)	Contaminant (% of reads)
2	A	68.52	0.884
2	B	81.94	0.029

Vendor A

Contaminant sequence	Frequency (%)
Alternate Sequence 1	0.753
Alternate Sequence 2	0.018
Alternate Sequence 3	0.011
Alternate Sequence 4	0.011
Similar to expected with 3NT deletion	0.009
Unknown sequences	0.082

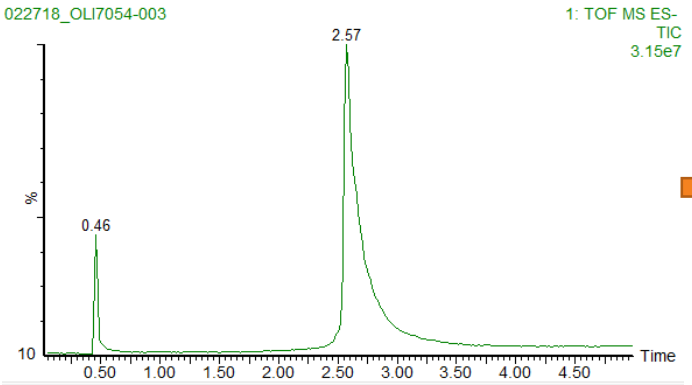
Vendor B

Contaminant sequence	Frequency (% of reads)
Similar to expected with 3NT deletion	0.019
Unknown sequences	0.010

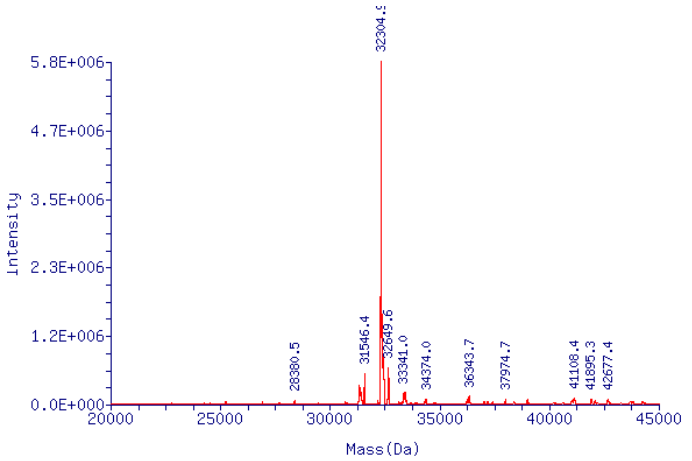
Contamination Confirmation by MS

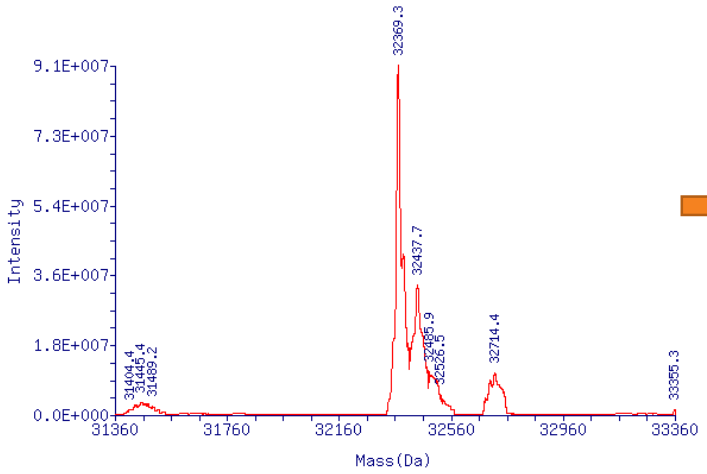
- Alternate analysis suggested presence of contaminating guides in vendor material
- Mass spec analysis and deconvolution of main peak reveal contaminating masses

LC-MS



Deconvoluted MS





ProMass Sample Browser

Target Masses

32369.8 32438.9 32462.9 32479.9

Observed Masses

32369.3 32437.7 32462.7 32485.9

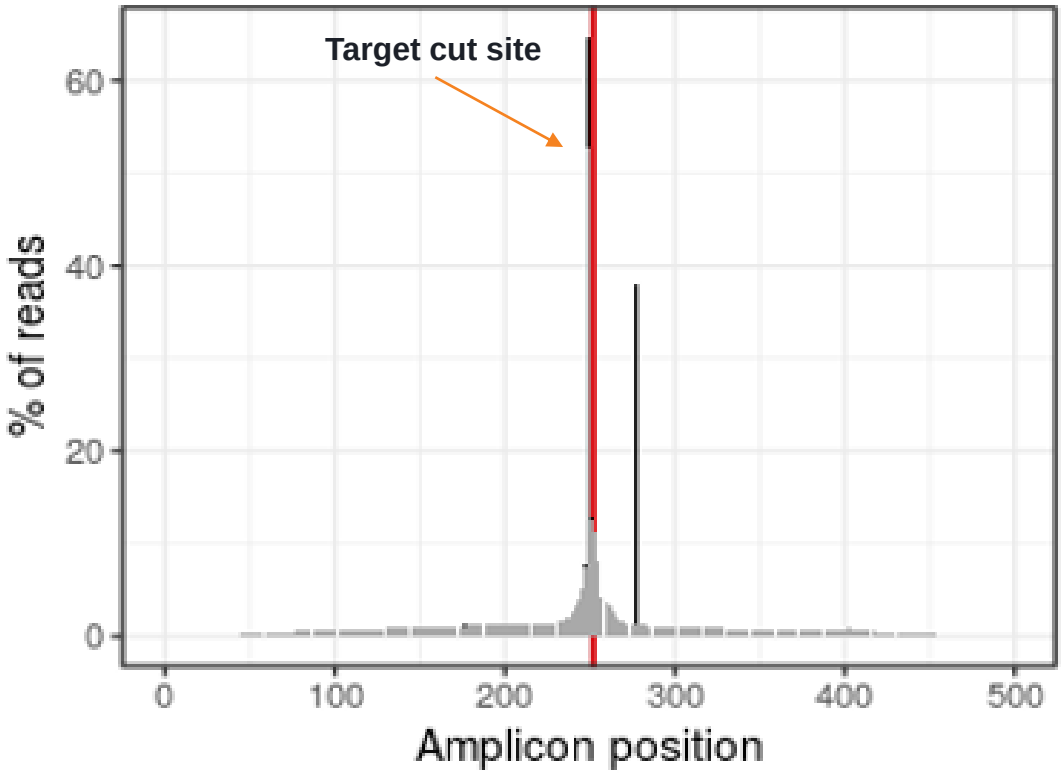
Purity

40.87 10.42 6.46

Measurement of Cellular Editing

Cellular assay

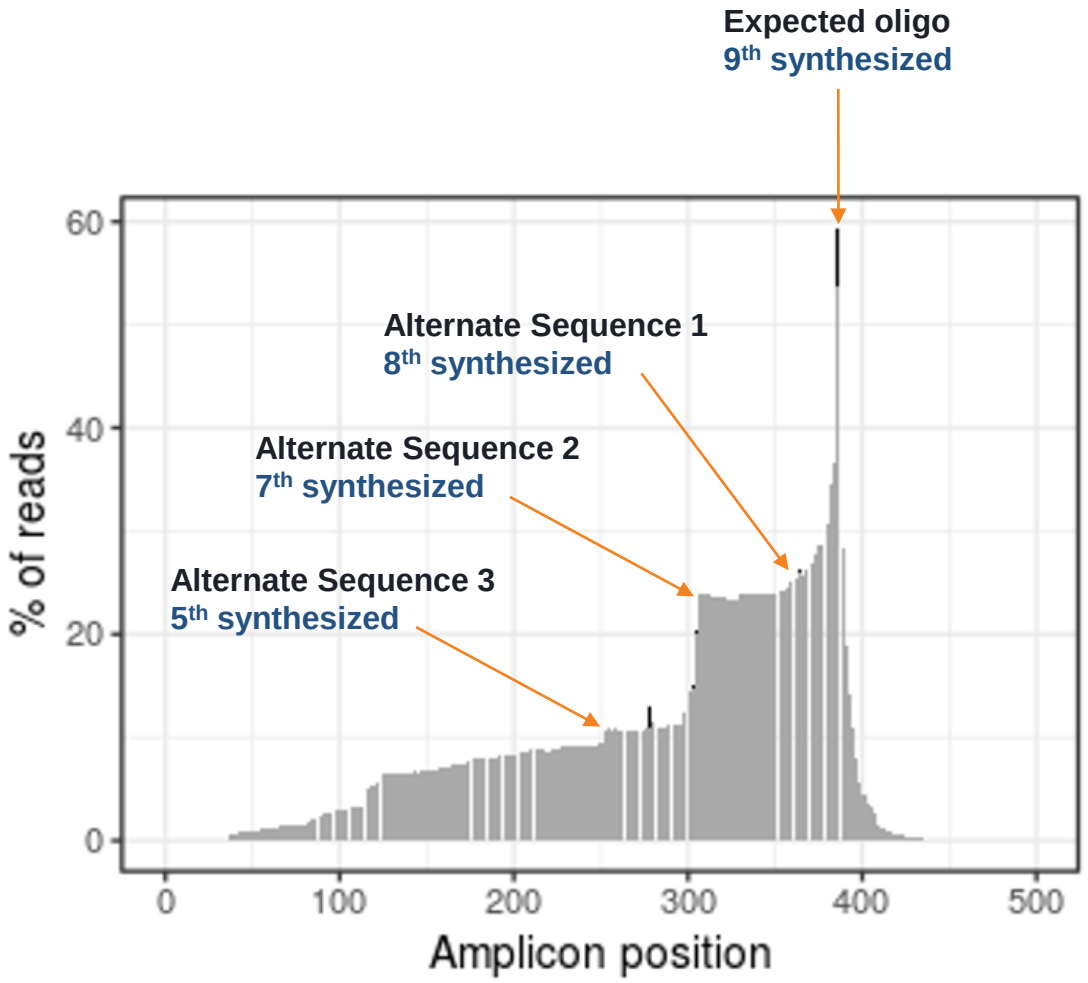
- Cells transfected with ribonucleoprotein-gRNA complex
- Amplicon assessed for editing
- Editing events measured across amplicon
- Symmetric cutting profile with peak in editing at target site



Contaminating Oligos Can Cut

Cellular assay – extension in editing region

- Observed in >20 oligos
- Contamination appears to be directional, in the order of synthesis
- Observed with multiple vendors
- Not picked up by mass spec



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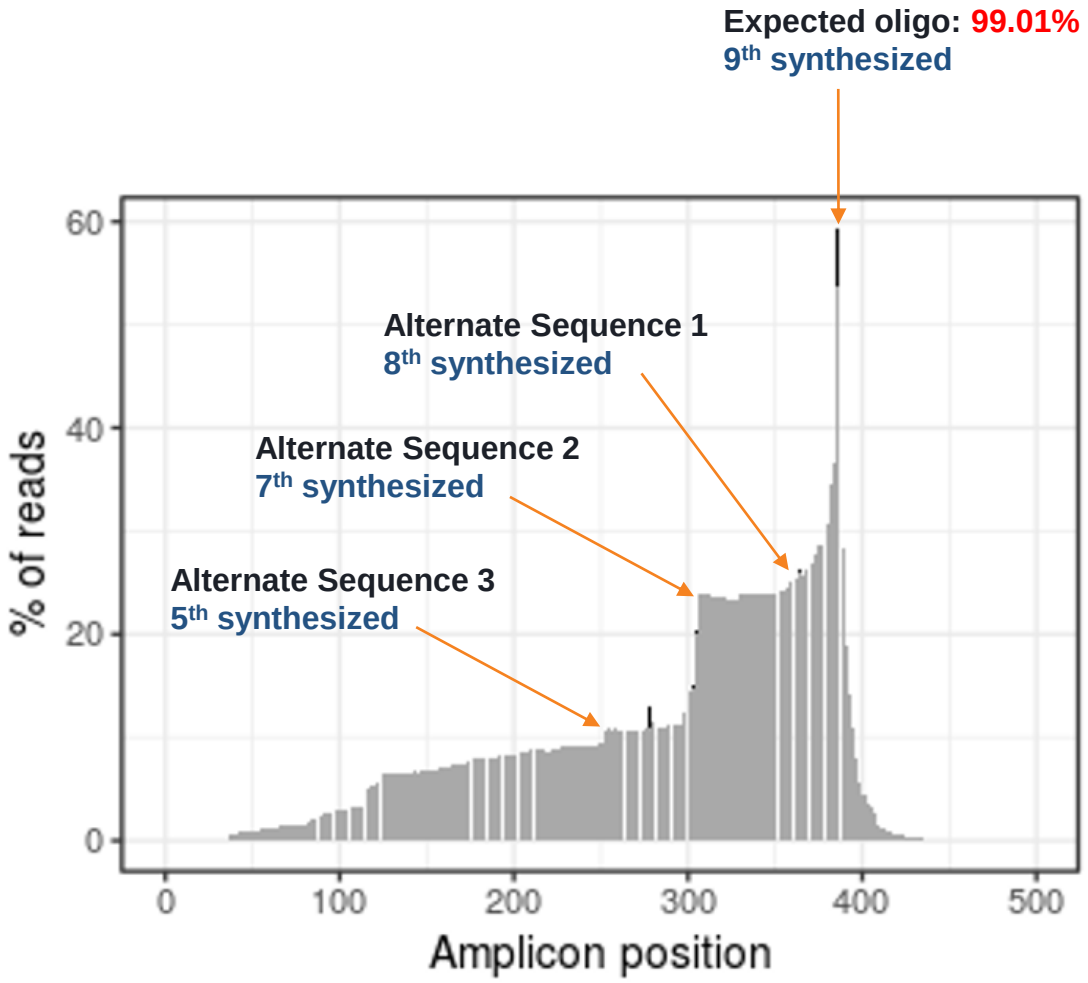

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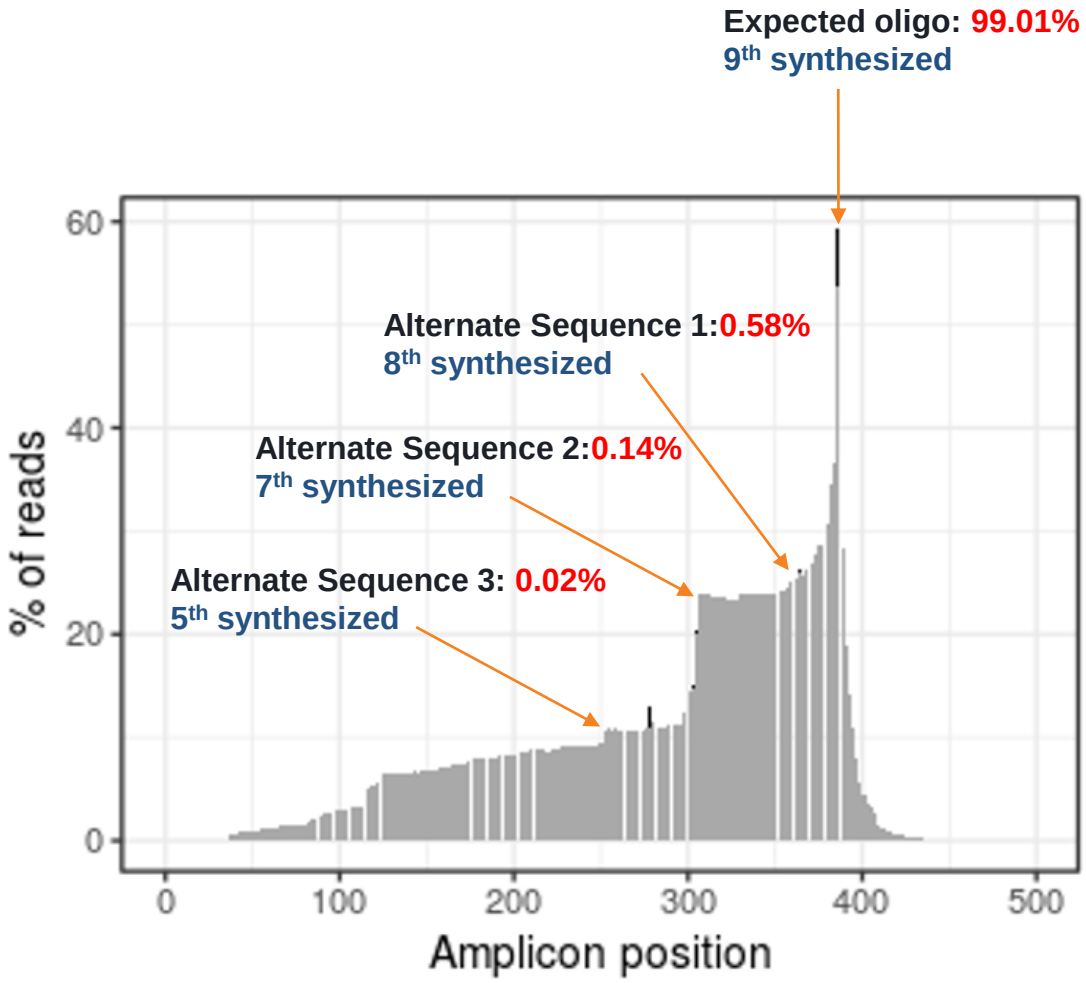


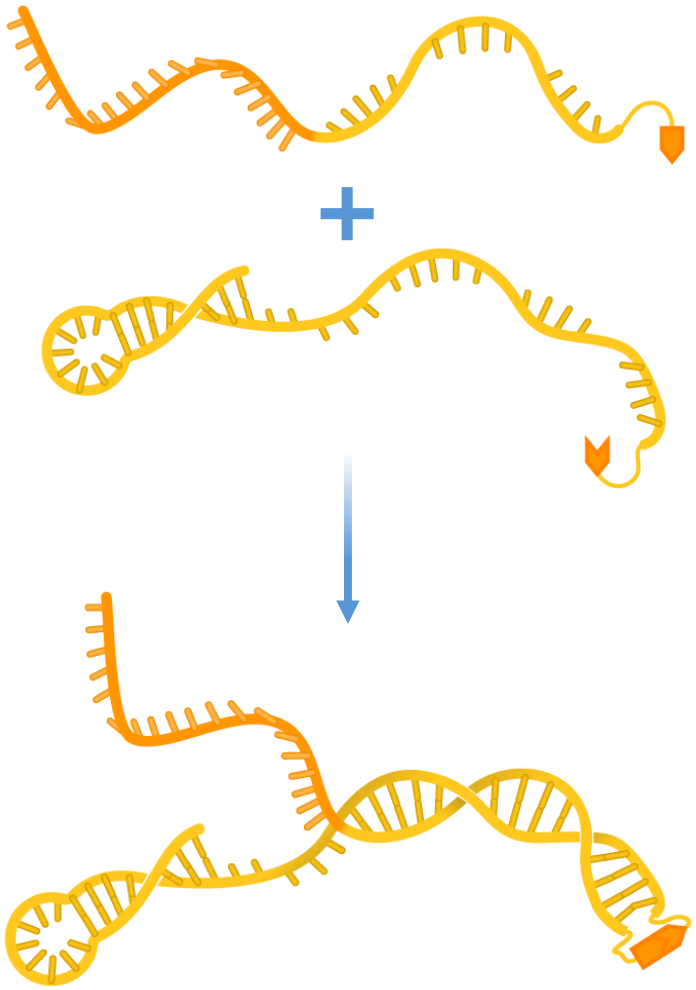
Contaminates are active at low levels

Editas Medicine: Unmatched gRNA Expertise

World class RNA chemistry expertise

Enables best-in-class CRISPR medicines

Proprietary classes of guide RNAs with distinct intellectual property



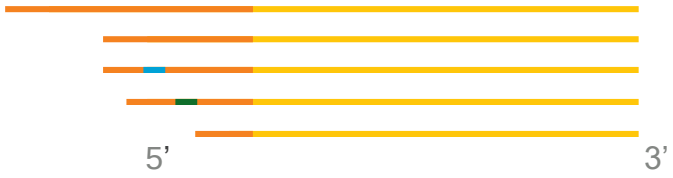
Challenges with Chemical Synthesis of gRNA

Single gRNA

- Direction of synthesis is 3’ to 5’, more critical 5’ end especially error prone
- Independent coupling reactions at 98.5% success rate: for 100mer, 20% full-length product
- Purification to enrich full-length product can introduce low level contamination

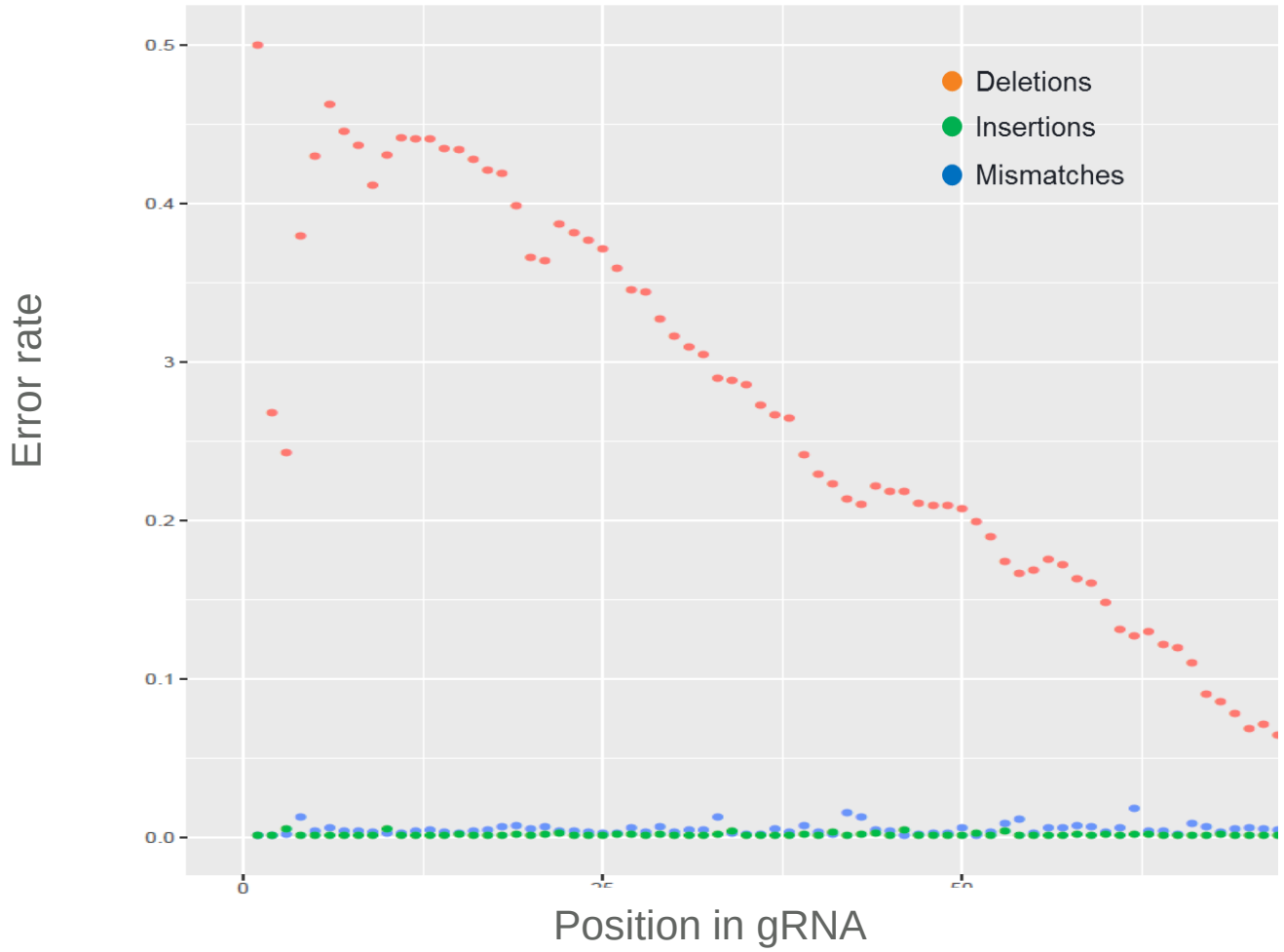
Heterogeneous product

Full-length, truncated, errors

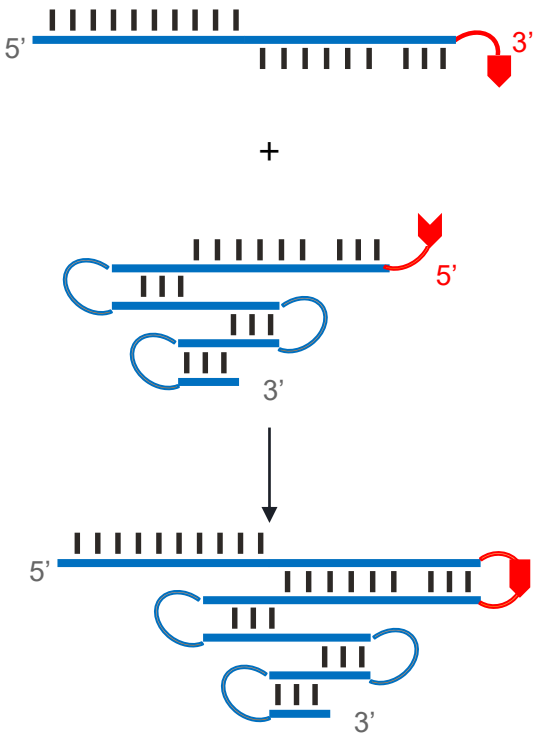


Need methods to measure guide **sequence fidelity** and **purity**

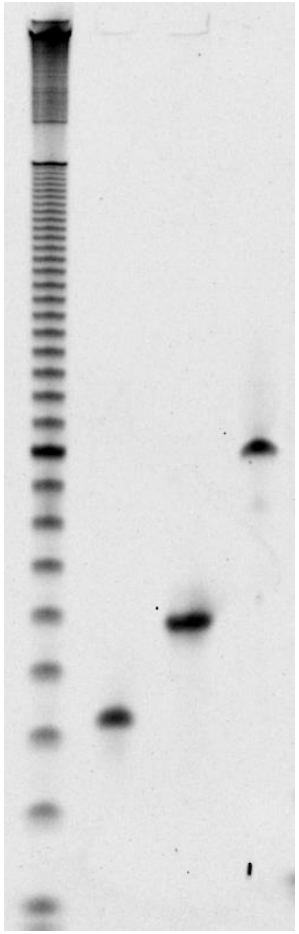
NGS-Based Method for 5’ End Evaluation



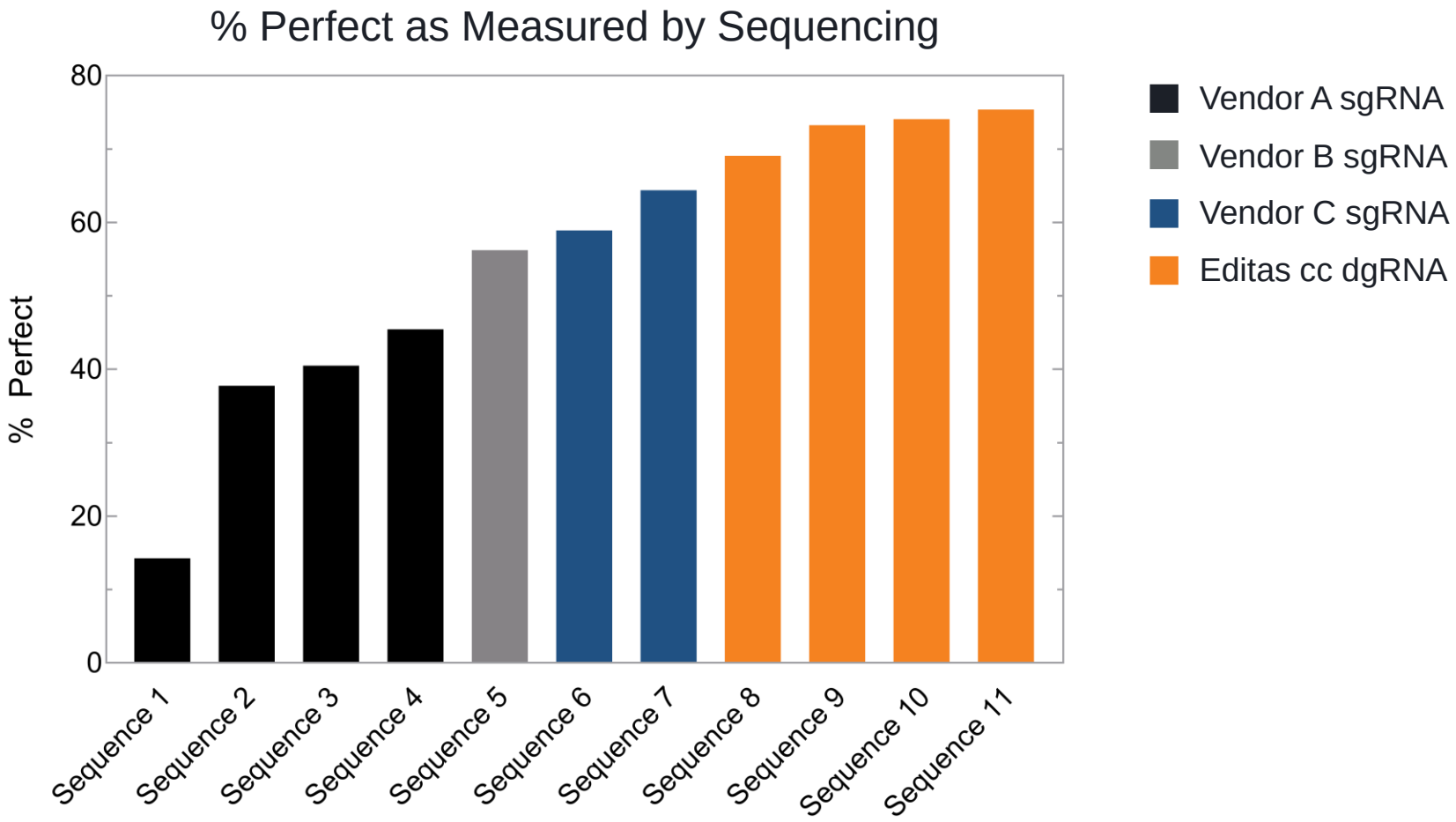
Generating Synthetic Covalently-Coupled Dual gRNA



covalently-coupled dual gRNA (dgRNA)



cc dgRNAs Demonstrate Greater Sequence Fidelity



Summary

- gRNA quality is important as these are potent enzymes
  - Sequence fidelity and purity should be understood
  - Minor contaminants can have activity
- Editas has developed state of the art synthesis and analytics for gRNAs


Thank you.