Development of High Quality CRISPR/Cas9 Agents

TIDES
May 7th, 2018

Terence Ta

Agenda

- Overview of CRISPR and Editas platform
- Development of NGS-based method for guide RNA QC
- Covalently-coupled dual guide RNA (cc dgRNA)
- Guide RNA contamination
- Summary and closing

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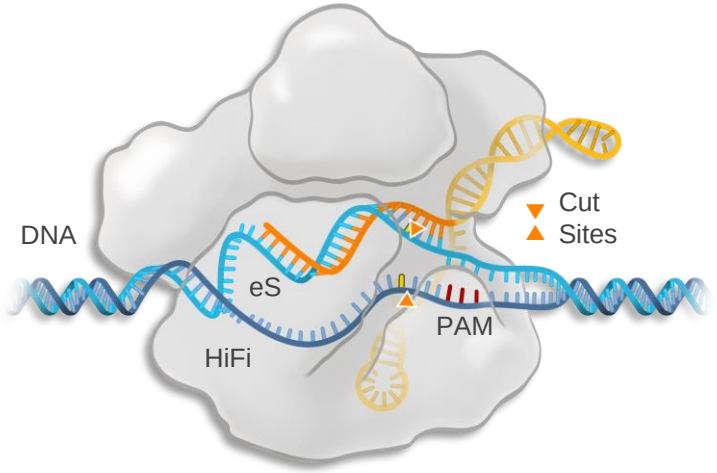
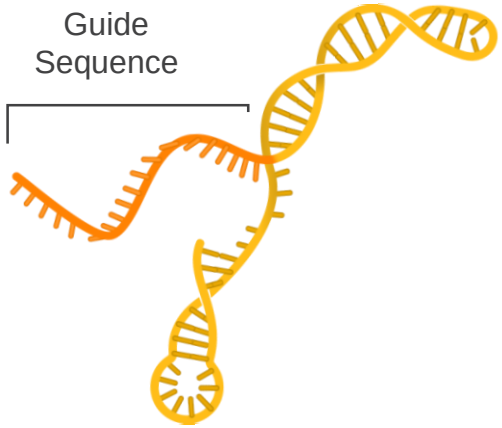
CRISPR Unlocks Genome Editing

Nuclease



Guide RNA



Complex of nuclease and guide RNA (RNP) precisely locates and cuts genomic sites

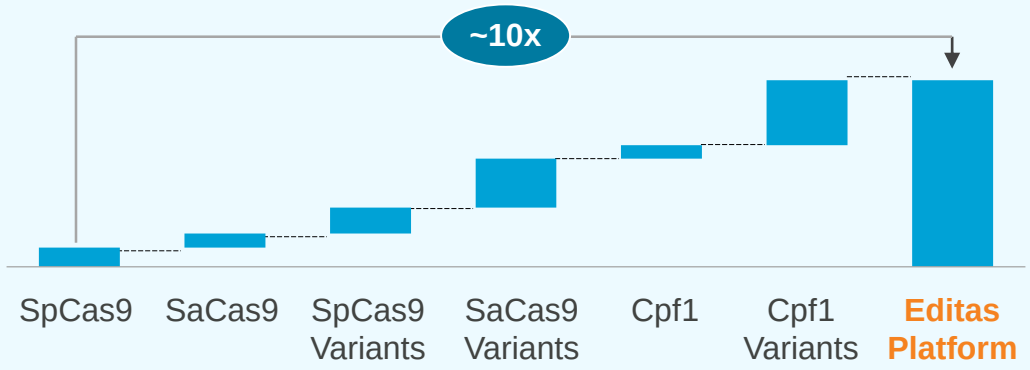
Ability to target many sites simultaneously using numerous guide RNAs

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

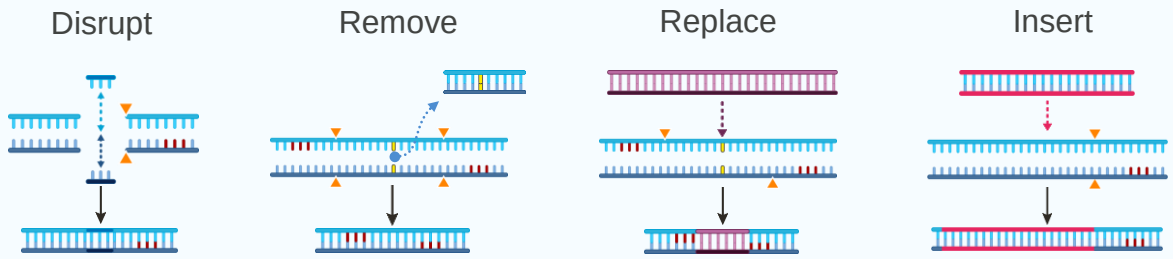
DNA: Deoxyribonucleic Acid; HiFi: High Fidelity; eS: Enhanced Specificity; PAM: Protospacer Adjacent Motif

Platform Enables Broad Product Pipeline

Broad Range of Sites



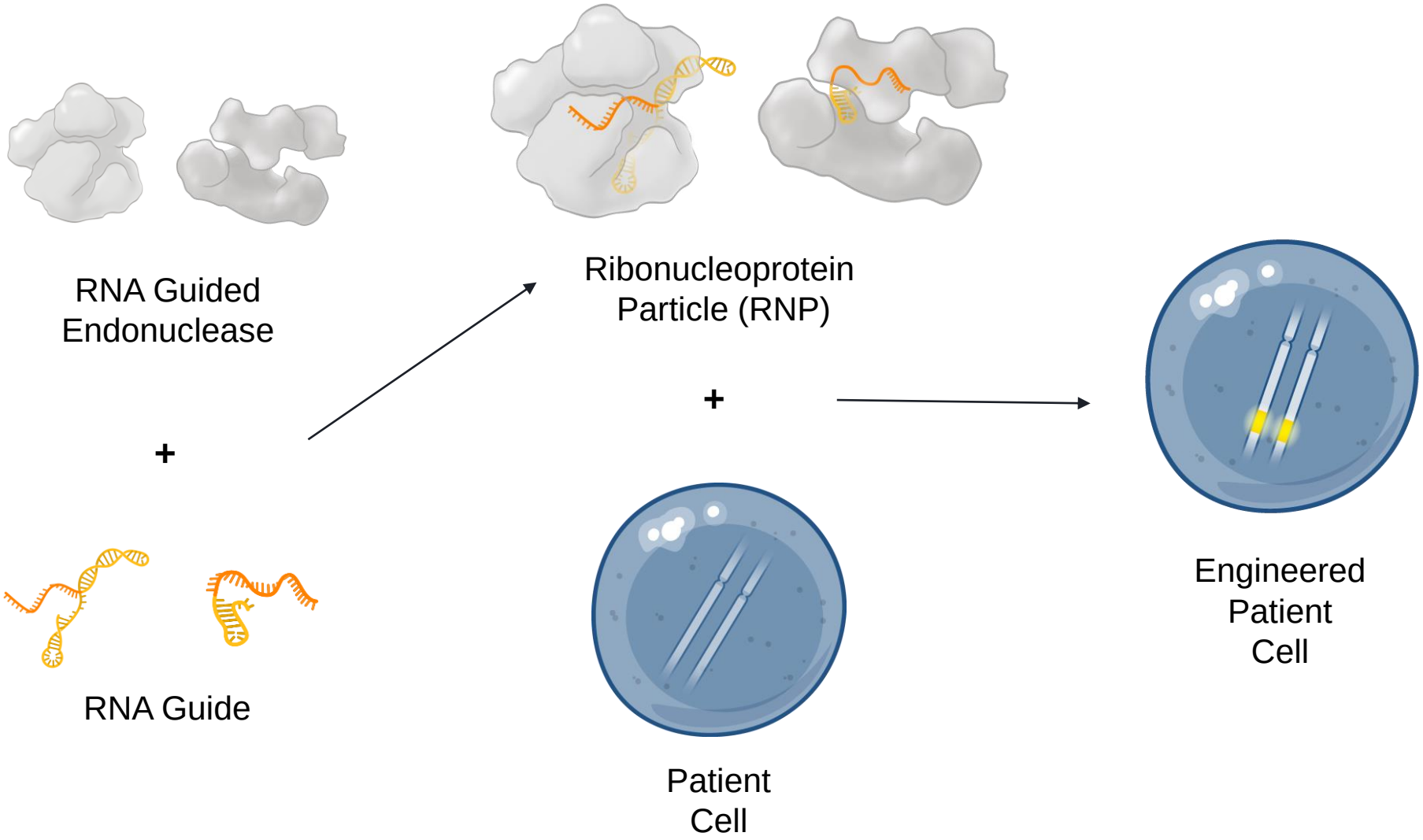
Diverse Spectrum of Edits



SpCas9: *Streptococcus pyogenes* Cas9; SaCas9: *Staphylococcus aureus* Cas9

Ex Vivo Drug Development

High Quality Ribonucleoprotein Particle Delivery



Challenges with chemical synthesis of gRNA

Single gRNA

- Direction of synthesis is 3’ to 5’, more critical 5’ end (Cas9) 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

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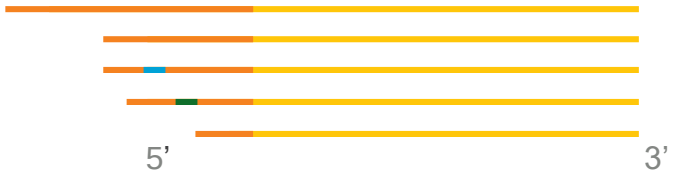
Heterogeneous product

Full-length, truncated, errors



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

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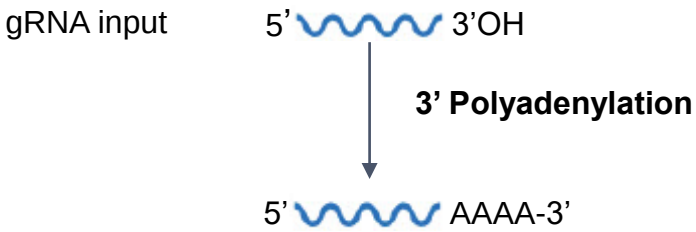
Development of NGS method for gRNA QC

- Reverse transcriptase w/template-switching activity generates cDNA libraries from gRNA templates which are then PCR-amplified
- NGS on PCR product, purity/fidelity evaluated using analysis pipeline developed in-house

Development of NGS method for gRNA QC



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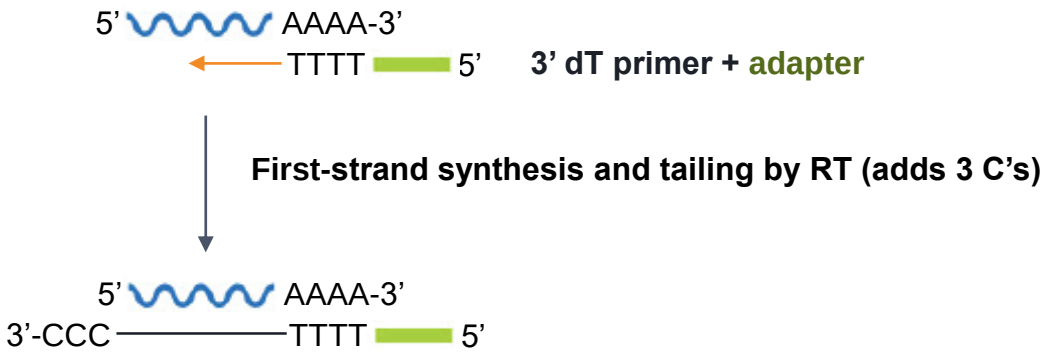

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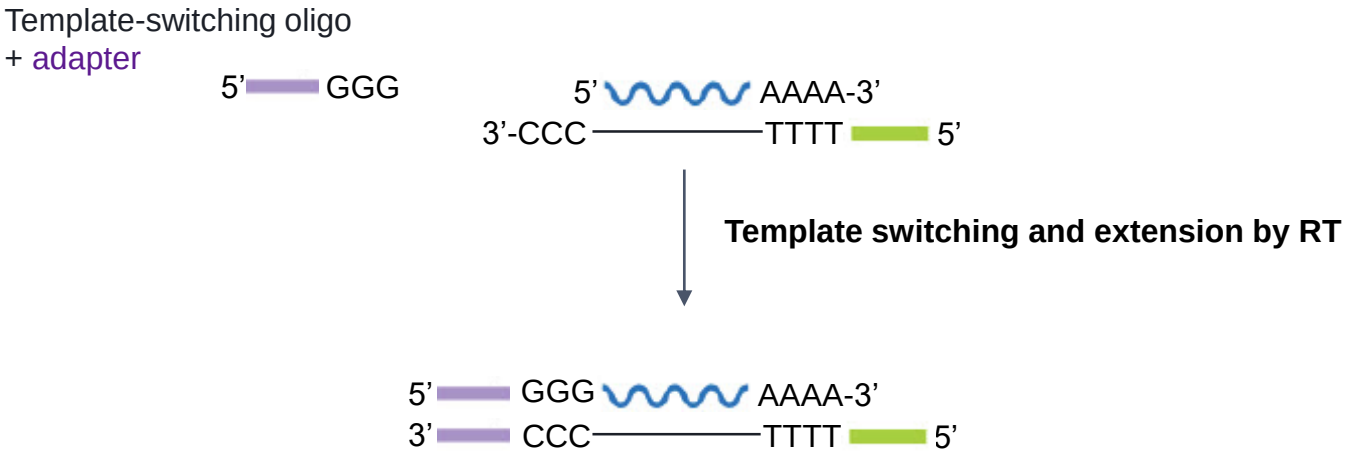

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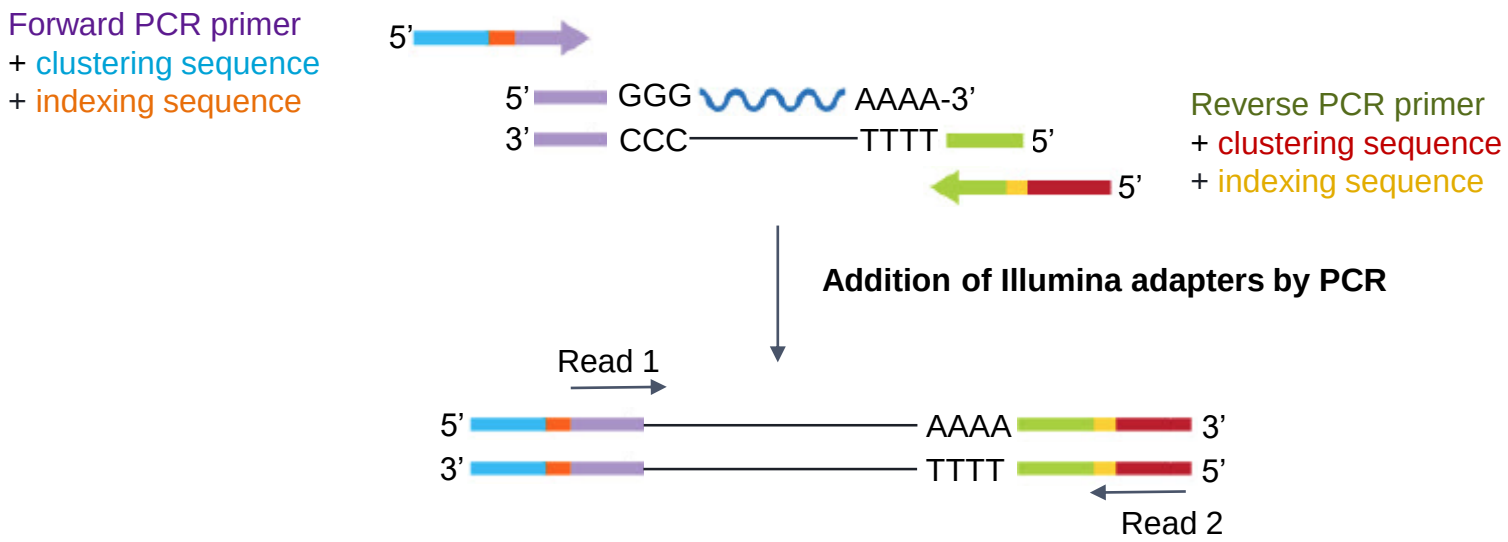

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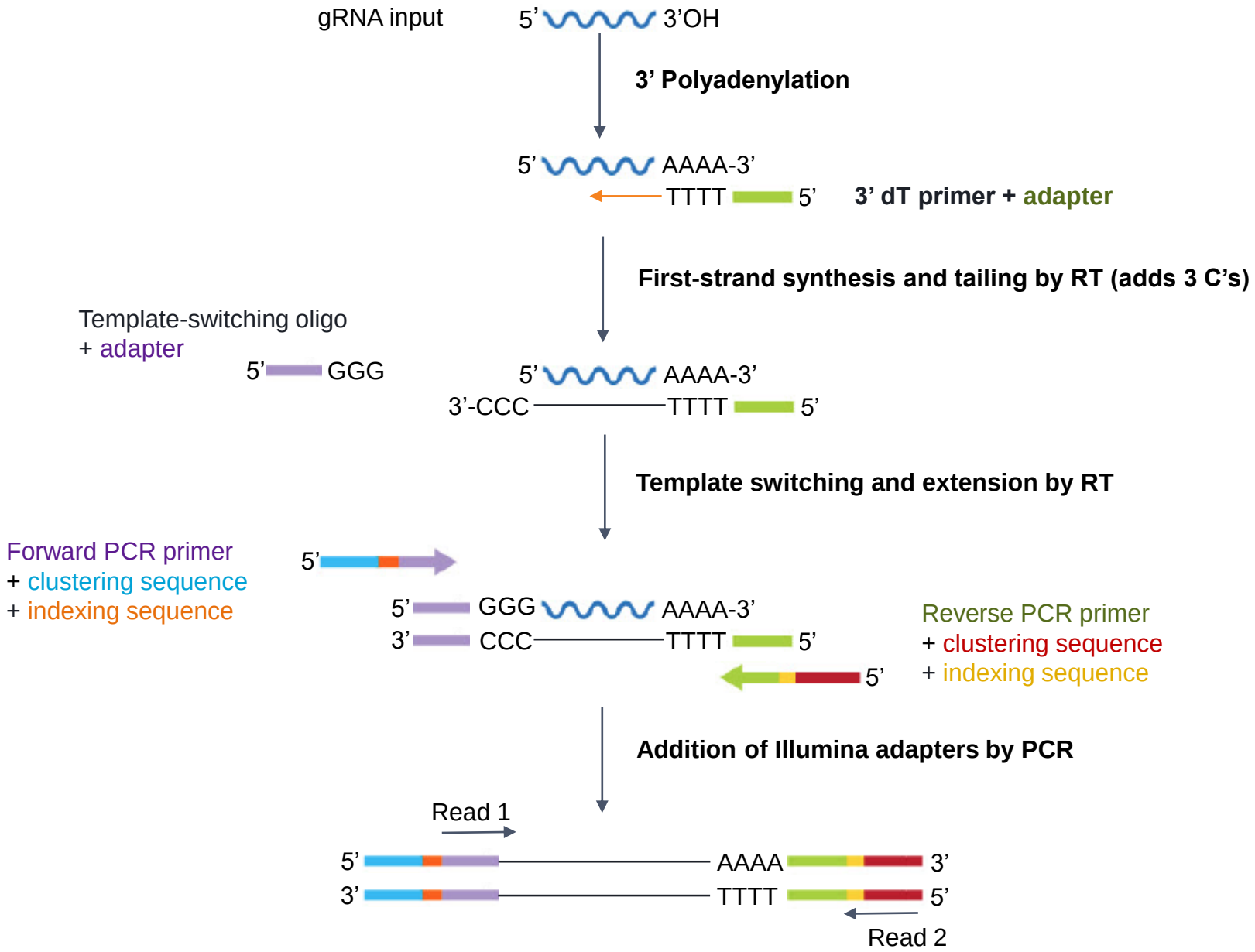

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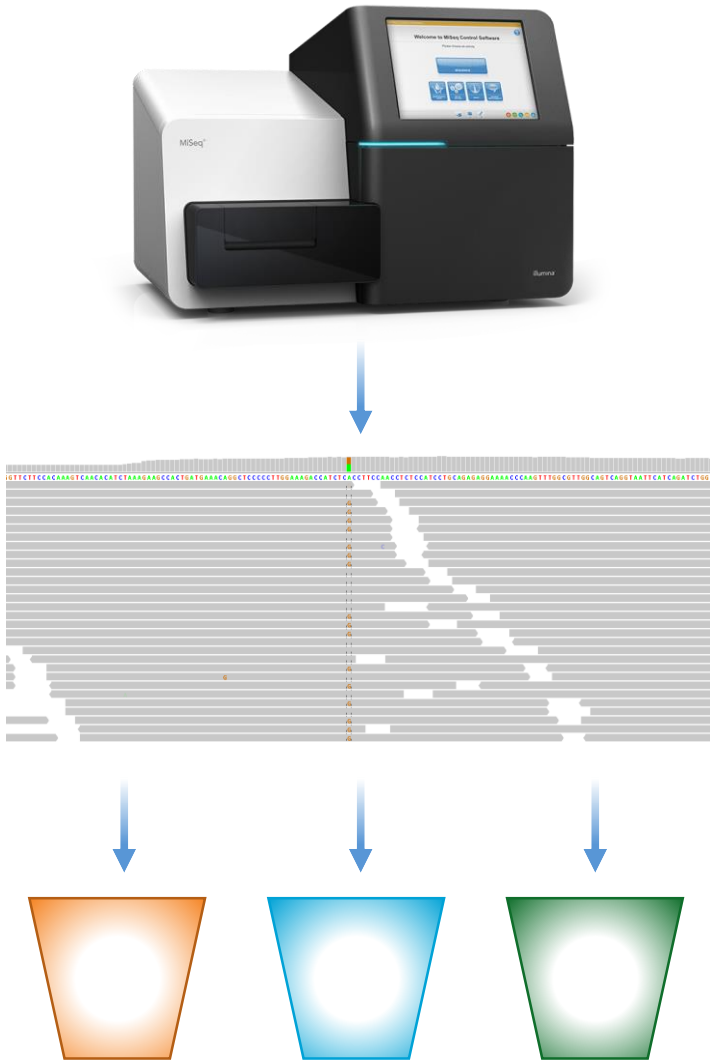


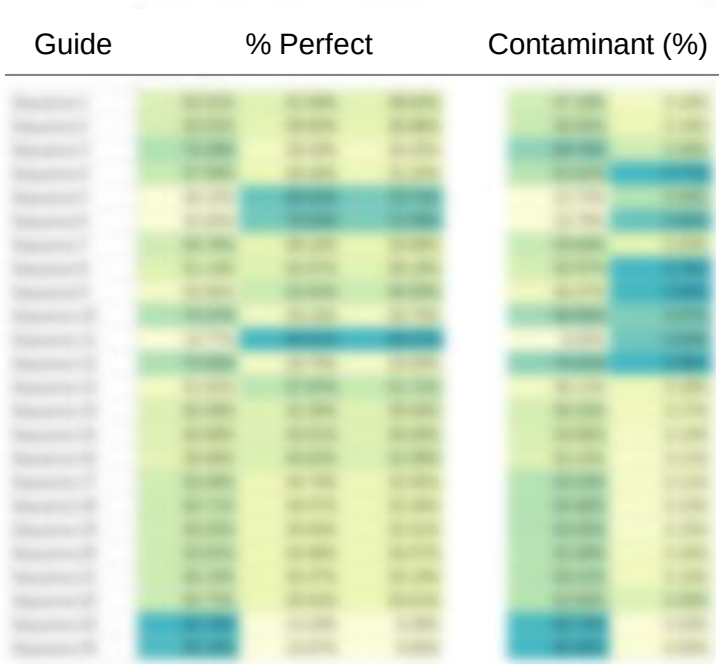
~400k reads sequenced for each oligo that are run through **in-house analysis pipeline**

Reads aligned to expected oligo sequence

Reads classified as:
Match, Truncation, Contaminant

Development of NGS method for gRNA QC

High level metrics for fidelity and purity



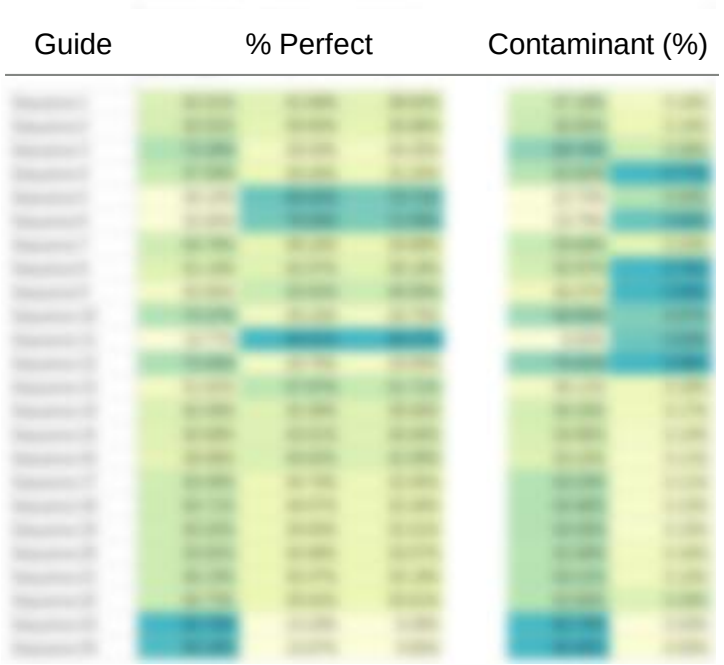
Contaminant profiles for individual guides

Contaminant sequence	Frequency (%)
Alternate Sequence 1	0.58
Alternate Sequence 2	0.14
Alternate Sequence 3	0.02
Alternate Sequence 4	0.02

- % Perfect: fraction of total reads that have a perfect guide sequence
- Identify, quantify contaminant sequences

Development of NGS method for gRNA QC

High level metrics for fidelity and purity



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Tool for measuring guide quality

Can we improve it?

How important are these attributes?

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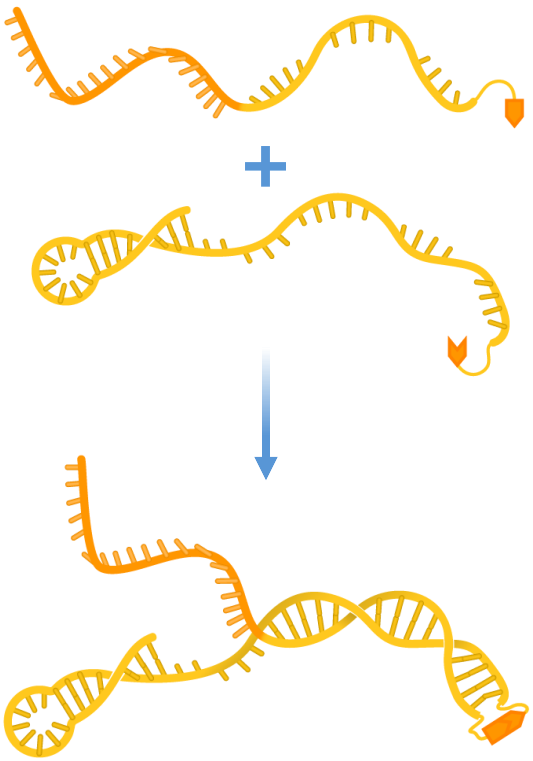
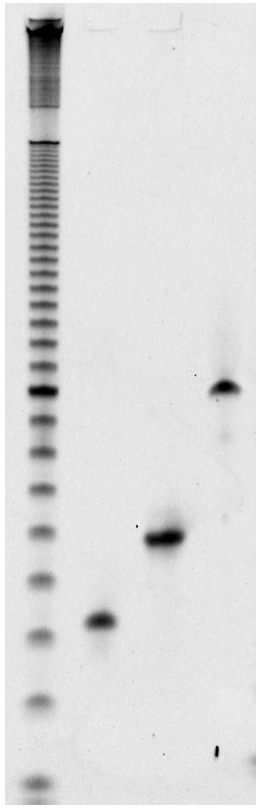
Synthetic Covalently-Coupled Dual gRNA

A completely non-enzymatic process for guide production





covalently-coupled dual gRNA (cc dgRNA)

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

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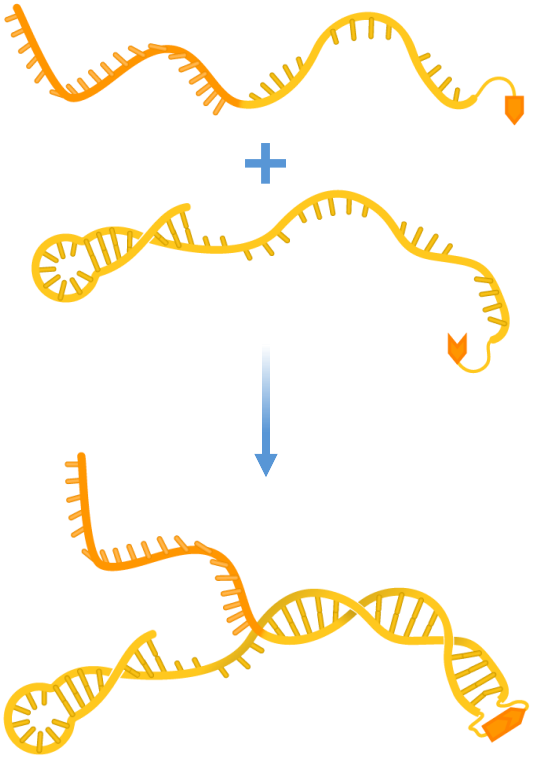

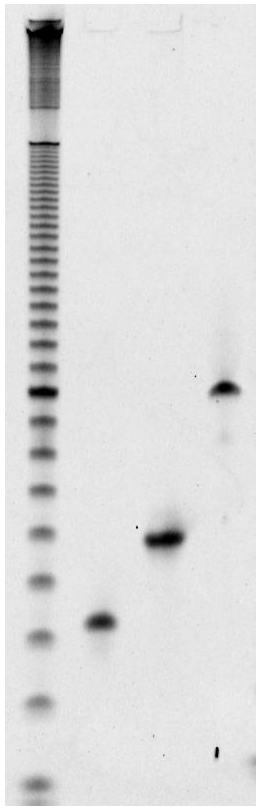

covalently-coupled dual gRNA (cc dgRNA)

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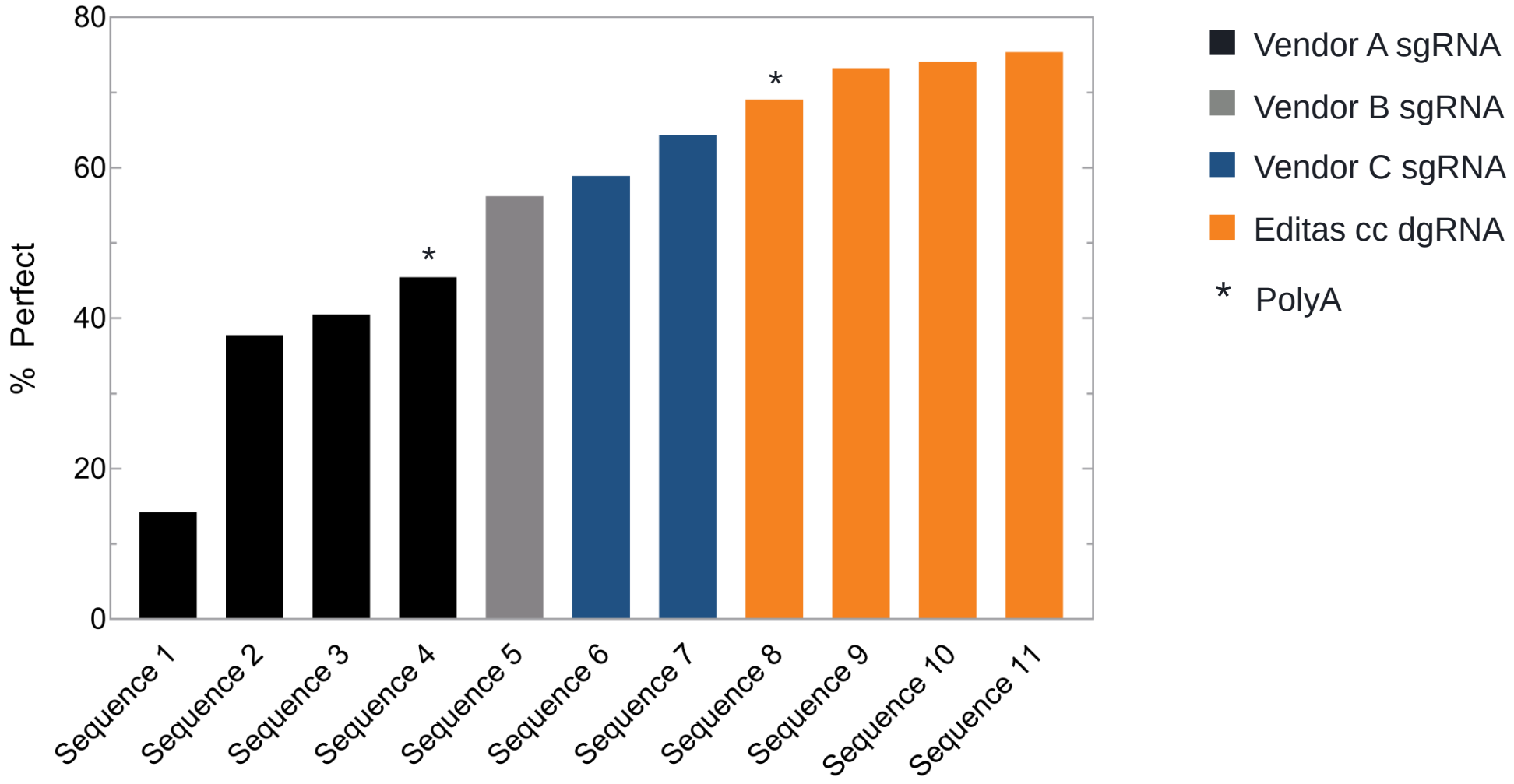
- Scale up and purity are more compatible with CMC requirements

Well-defined product

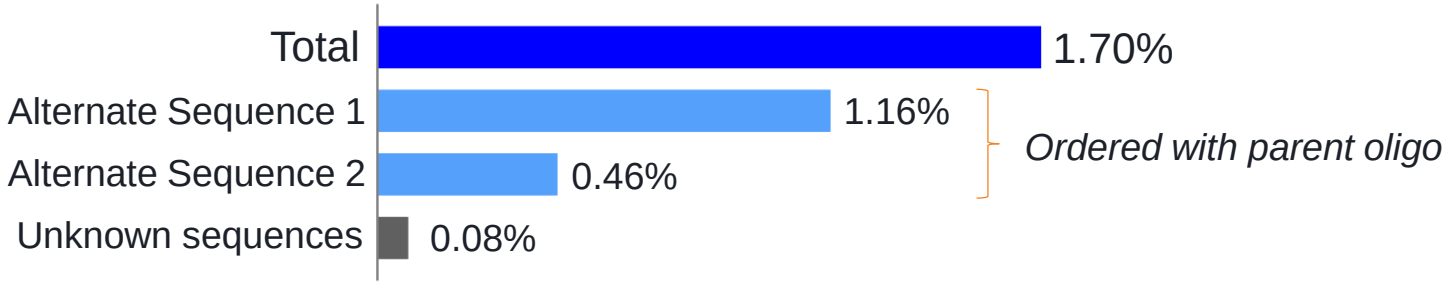
Full-length, less errors

cc dgRNAs demonstrate greater sequence fidelity as determined by NGS assay

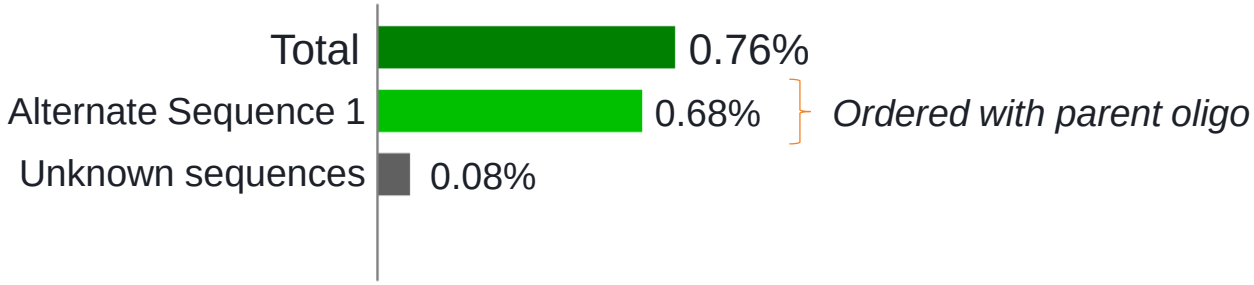


cc dgRNA process generates less impurities

Vendor A sgRNA

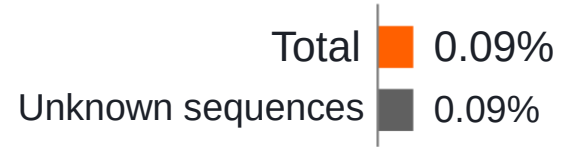


Vendor B sgRNA



Vendor C sgRNA

Editas cc dgRNA



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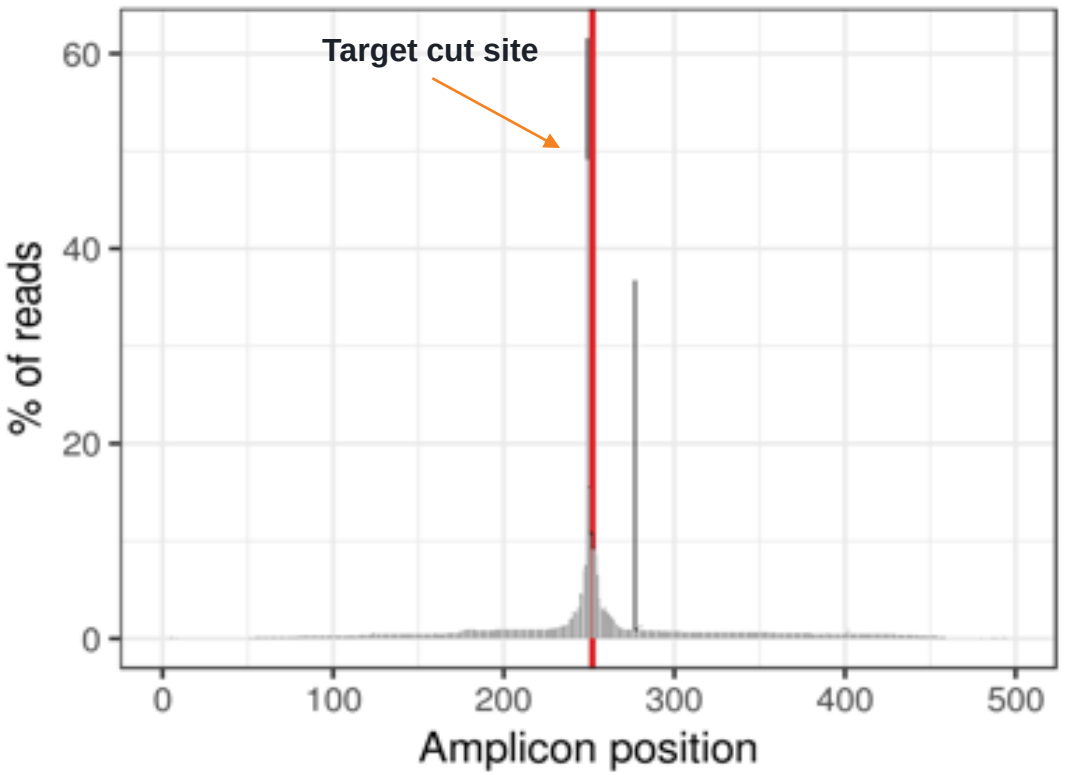
Measurement of cellular editing

Cellular assay

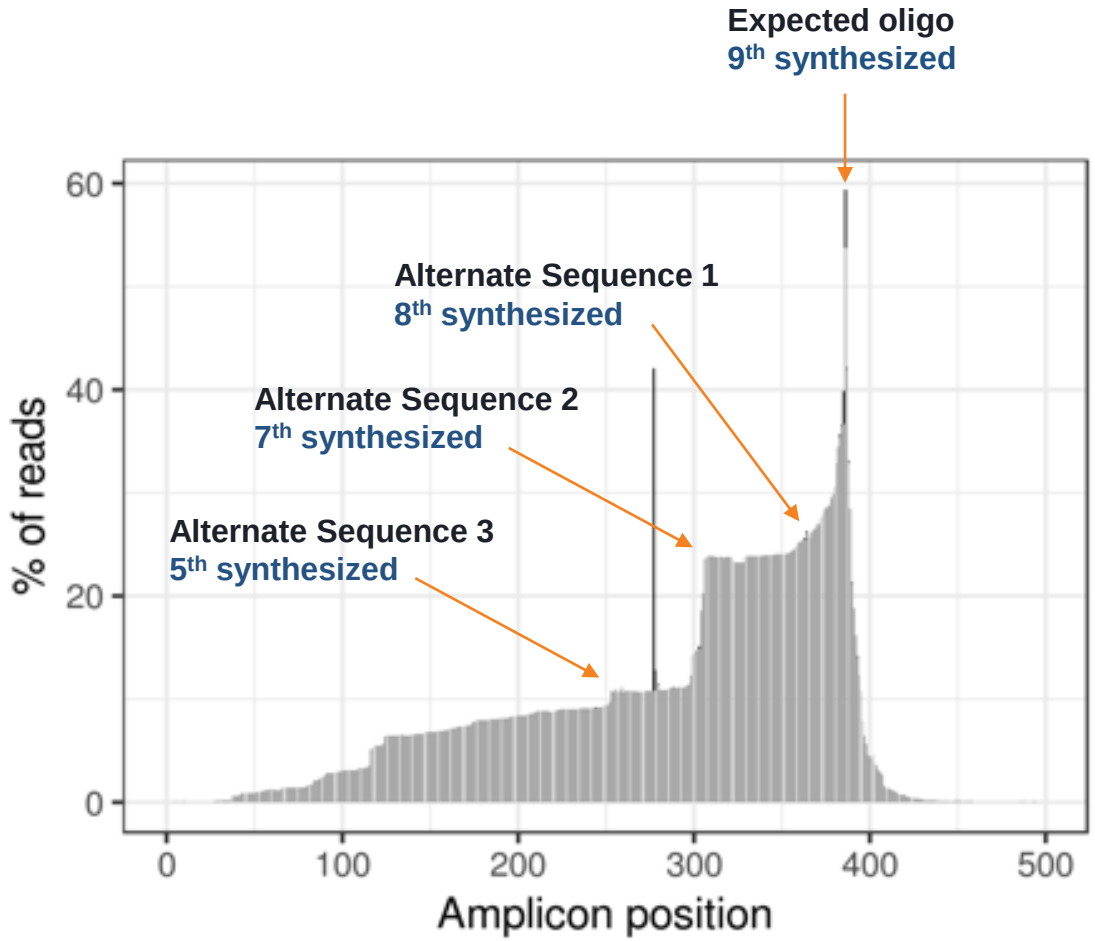
- Cells transfected with ribonucleoprotein-gRNA complex
- Amplicon assessed for editing (NGS)
- Editing events measured across amplicon
- Symmetric cutting profile with peak in editing at target site



Contaminating oligos can cut

Case where contamination caused 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



Sequencing reveals poor sequence fidelity and high contaminant levels

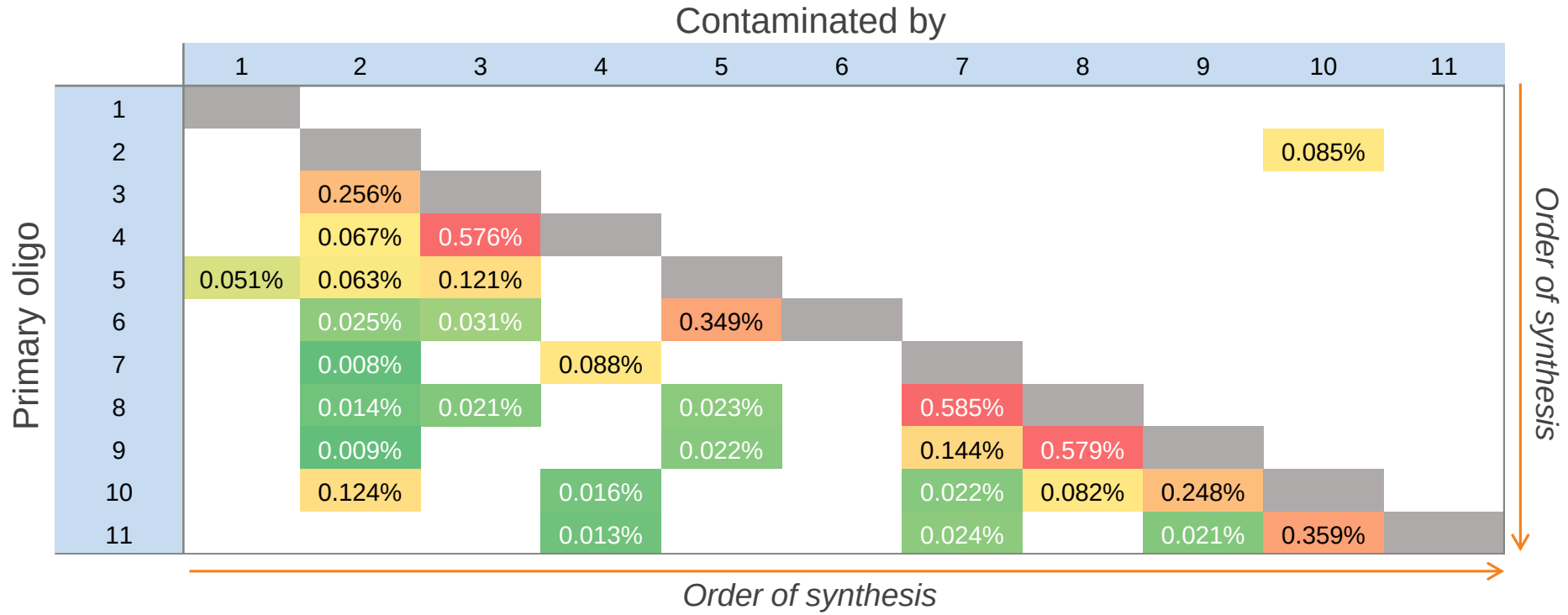
	Fraction Truncated 5′	% Perfect	Fraction Contaminant
Sequence 1	41.94%	57.18%	0.15%
Sequence 2	39.90%	58.95%	0.19%
Sequence 3	28.33%	69.74%	0.34%
Sequence 4	34.26%	62.62%	0.77%
Sequence 5	83.02%	22.72%	0.43%
Sequence 6	79.28%	23.79%	0.66%
Sequence 7	38.16%	59.69%	0.23%
Sequence 8	42.57%	55.97%	0.76%
Sequence 9	53.02%	46.37%	0.99%
Sequence 10	29.16%	66.95%	0.57%
Sequence 11	94.61%	8.32%	0.63%

Contaminated set

	Fraction Truncated 5′	% Perfect	Fraction Contaminant
Sequence P1	38.23%	61.72%	0.06%
	39.16%	61.00%	0.05%
	40.74%	59.44%	0.09%
Sequence P2	30.34%	67.92%	0.07%
	29.63%	68.66%	0.06%
	29.67%	68.60%	0.06%
Sequence P3	27.65%	71.25%	0.14%
	27.68%	71.43%	0.13%
	27.90%	71.13%	0.12%

Standard panel

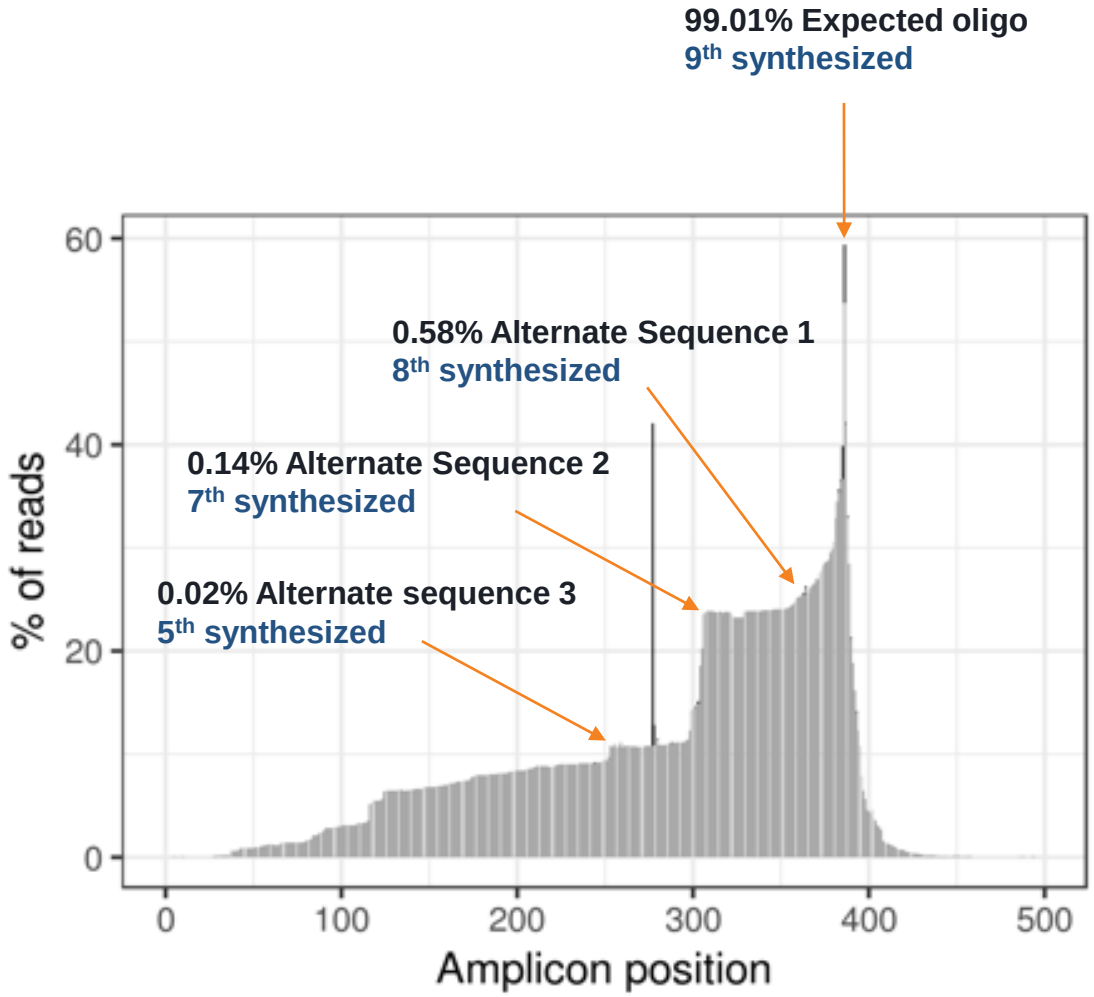
Sequencing detects directional contamination



Confirms directionality of contamination observed in cellular editing assay, at the oligo level:

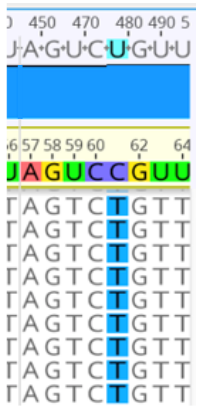
- For given oligo, the major contaminating sequence is almost always the oligo synthesized/purified immediately prior
- Many instances of multiple contaminating species where sequences closer in synthesis order to the primary oligo are present at greater levels

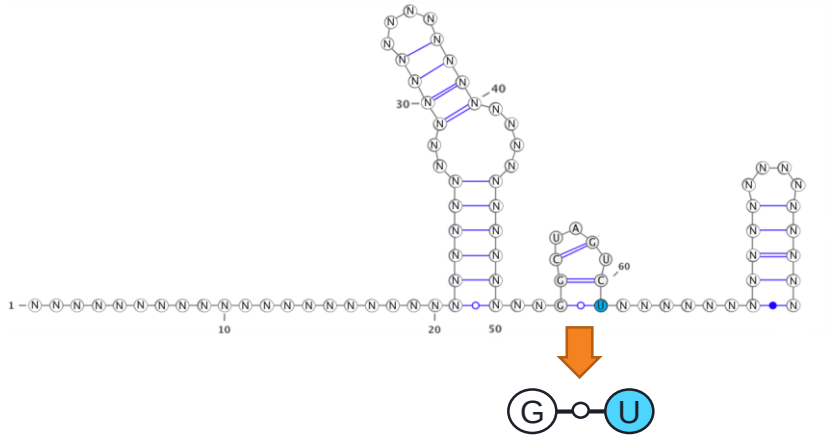
Quantifying contaminants and impact on editing



Sequencing assay reveals critical mutation in nexus region

C-001: C to U mutation (<1 Da difference) at base of **nexus** (position 61)





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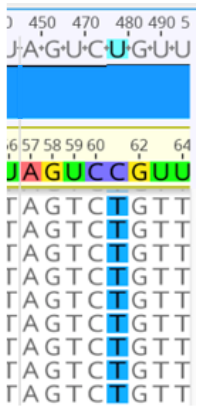

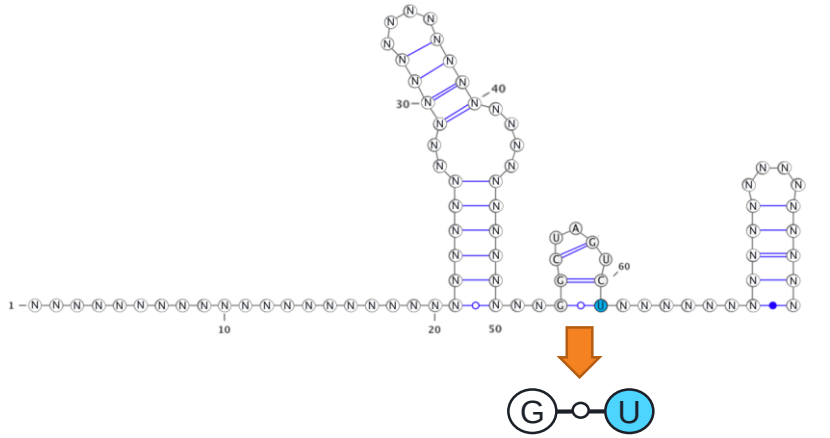

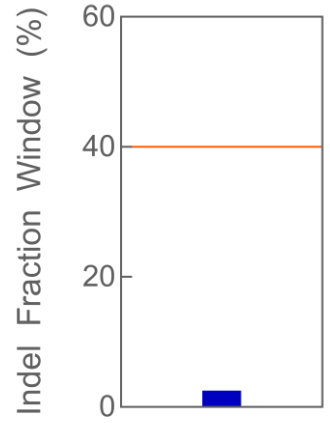

No activity in HSCs

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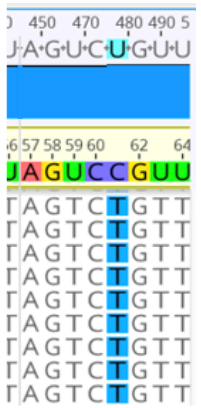

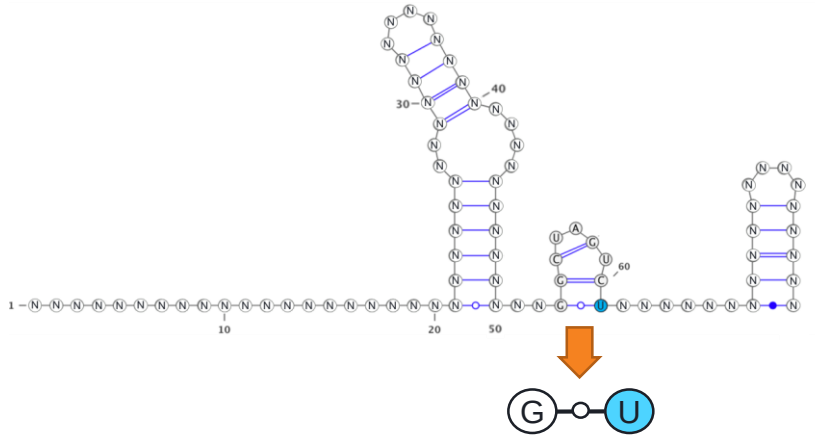

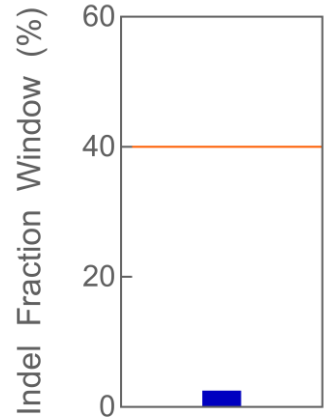

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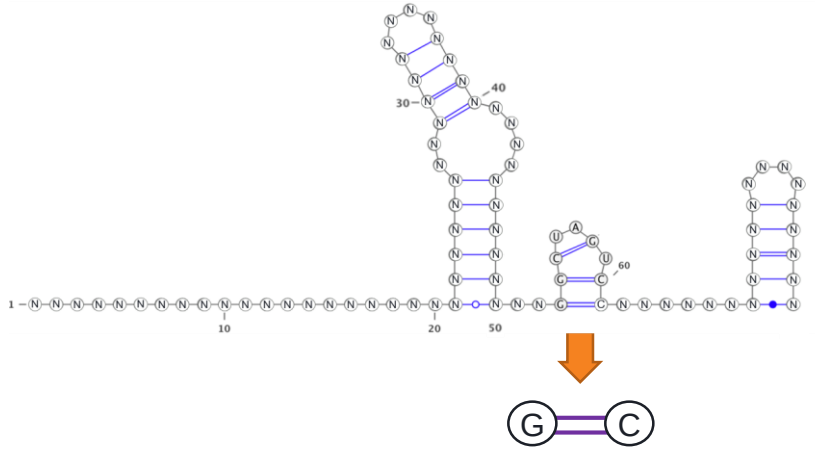
C-002: NGS assay confirms correction



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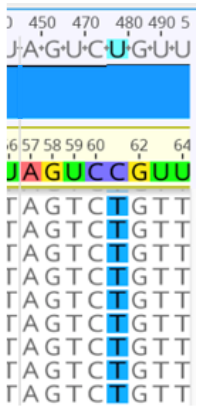

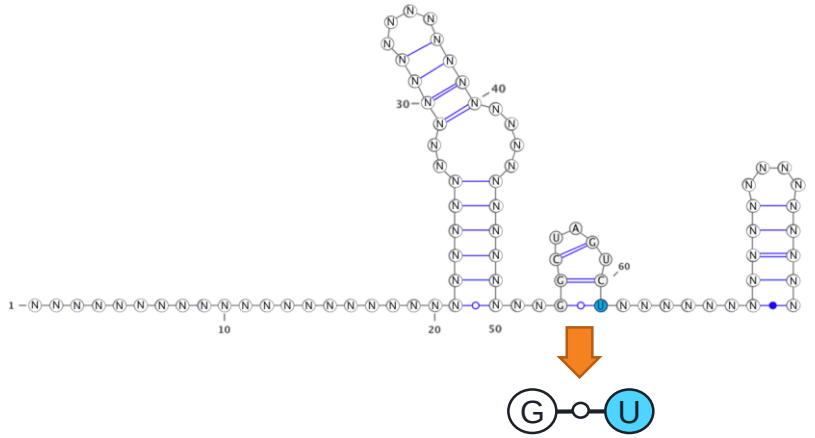

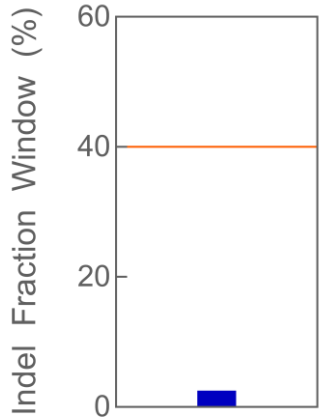

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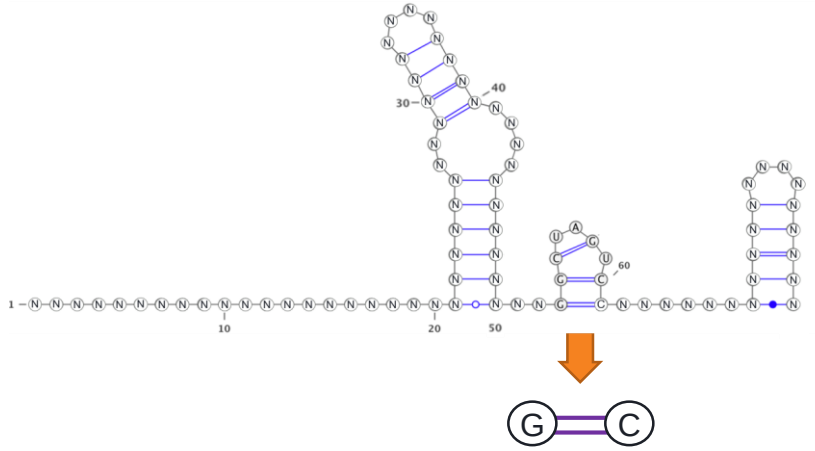

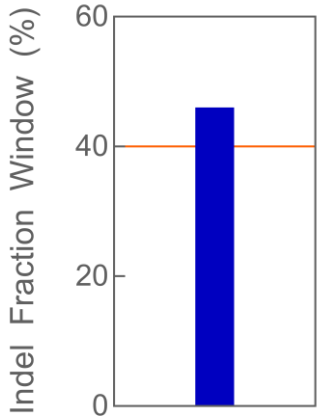

> 40% editing in HSCs

Summary

- gRNA quality is important as these are potent enzymes
- Sequence fidelity and purity are critical, we have developed methods to assess these
- Minor contaminants can have activity, we have developed methods to identify and quantify them
- Orthogonal mass spec assays also being developed
  - Sensitivity of MS: 1-5%
  - Sensitivity of sequencing assay: 0.1%
- Editas has developed state of the art synthesis and analytics for guide RNAs

Thank you!