Precise CRISPR Gene Editing with Chemically Ligated Guide RNA (LgRNA) and LgRNA-Polymerase Editing

September 27, 2025

GENELANCET BIOSCIENCES

https://www.genelancetbio.com
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Executive Summary

GeneLancet is developing a novel RNA-guided gene editing and modulation platform — **STAR** (Seek-Tag-Amend-Release) Editor, leveraged with proprietary ligated guide RNAs (LgRNAs).

LgRNA technology enables scalable precise RNA-guided gene editing.

The **STAR** (LgRNA-polymerase) system enables targeted, durable corrections of disease drivers without permanent DNA cleavage.

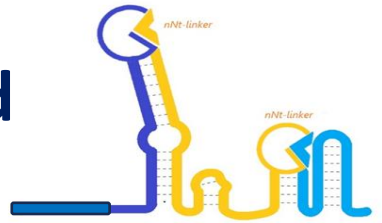
GeneLancet Tech Portfolio

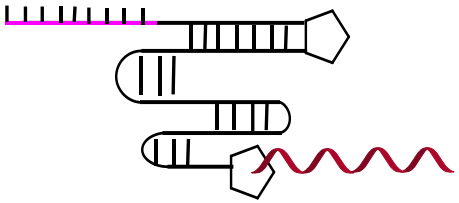
GL-Tech 1:
LgRNA – High purity, Precision and Scale Redefined

US10059940B2 (LgRNA + Antiviral)

US11667914B2 (Anti-Herpes virus)

Pending: US2021222165; US2023167441; US2025263693

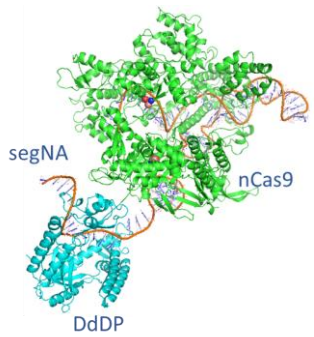


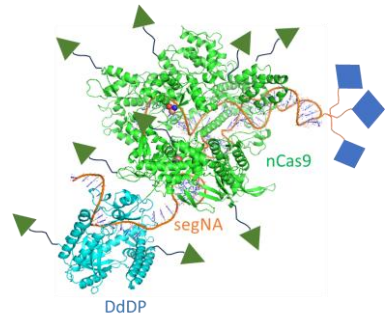


SAFE guides

GL-Tech 2:
STAR™ Editing – Precision and Efficacy

Pending: WO2021034373 (US2021054371, CN118922536); US2025027115





Problem: Chemical Manufacturing of Synthetic sgRNA

Formidable challenges with critical implications for patient safety and public health

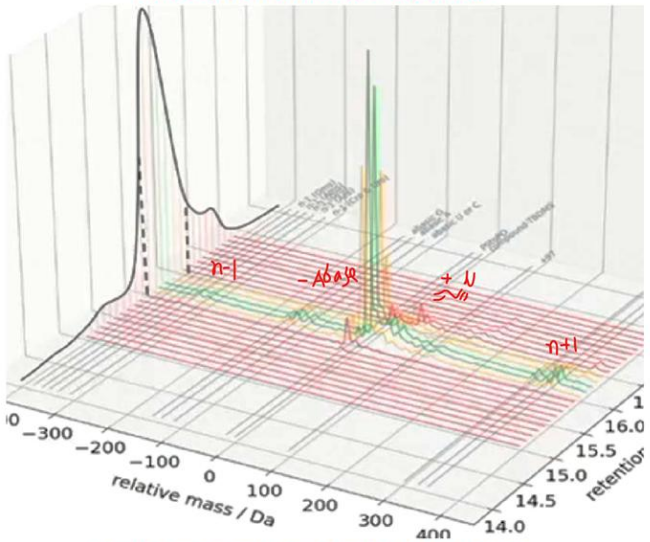
Current state-of-art commercial productions of sgRNA

CRO/CDMO	product/scale	HPLC purity*/yield
AX	1.0, 1.5g/mmol (90 nt, 1 mmol) (2'-OTBS)	74, 84%/3; 5%
Bio	15g/30 mmol (2'-OTBS) (2'-OMe)	~80%/1.6%
Ag	1.8g/mmol (7 mmol) (2'-OTBS)	~88%/0.8%

Source: TIDES-USA 2024

- LC-MS purity was not reported, and thus the content of truncated n-1 impurities in the product peak was undisclosed, the LC-MS purities are usually much lower.
- sgRNA s were mostly terminally modified only.

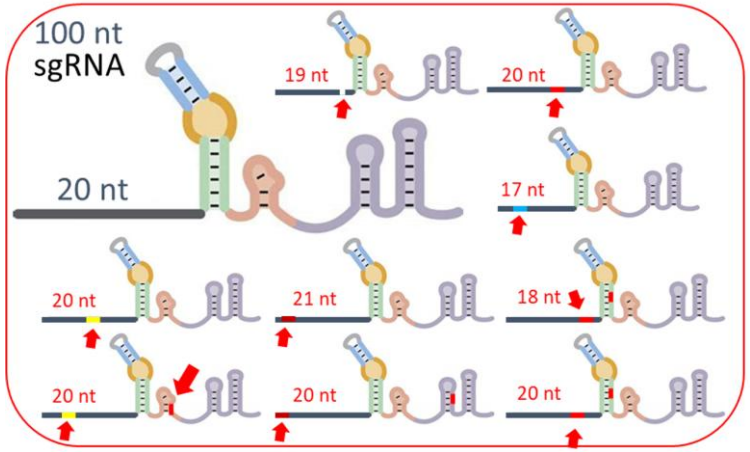
No separation of n-1 and n+1



Adapted from a vendor's report at 26th ASGCT meeting

Factors leading to challenging separations:

- ❖ Long RNA: complicated product mixtures
- ❖ RNA secondary structures
- ❖ Labile to RNase contaminations, etc.



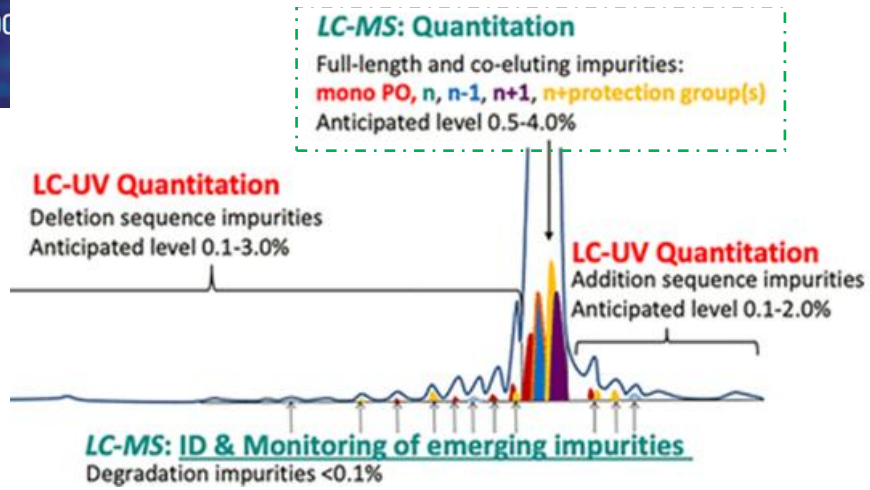
many truncated spacers
→ **catastrophic** off-targets

- Gene editing is at DNA level;
- CRISPR is an enzyme capable of many cuttings

FDA guidance for validation and purity control of sgRNA--

Current standard is HPLC purity, which significantly over-estimates product purity.

Analysis of HPLC product peak by LC-MS



Sutton JM and et al. Current State of Oligonucleotide Characterization Using Liquid Chromatography-Mass Spectrometry: Insight into Critical Issues. J Am Soc Mass Spectrom. **2020** Sep 2;31(9):1775-1782.

What are FDA's recommendations for guide RNA (gRNA) purity analysis?

DR. KWILAS: Thanks so much, Denise. This is a question that we get quite often, so I'm hoping that I can provide some additional feedback regarding gRNA purity analysis. So gRNA purity can be assessed in multiple different ways. For high-performance liquid chromatography (HPLC) and mass spectrometry purity analyses, as anyone who's submitted an application to FDA knows, we recommend the purity of gRNA full-length product to be greater than or equal to 80% and that you identify any impurities that are

- ≥ 80% FLP purity
- Identify impurities ≥ 1%

If < 80% purity

- Justify purity
- Conduct risk assessment for off-target

ge that the acceptance
sed to determine purity.

that can be used.
r equal to 80% due to
n for the proposed
data on the impurities
he safety of your product as it pertains to off-target editing.

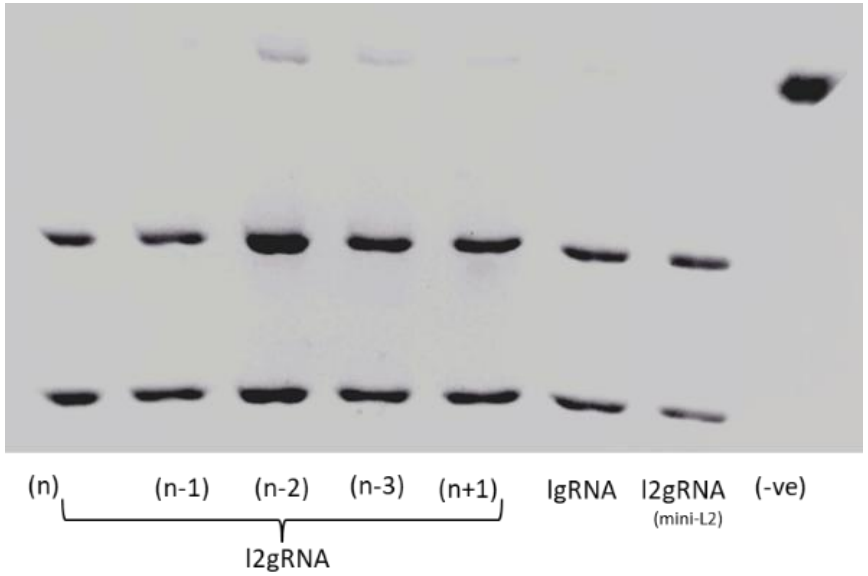
It's also important to note that we do not consider the direct sequencing assay as a strict identity assay. The sequencing assay should not only be used to confirm the correct full-length gRNA sequence, but it should also be used as an orthogonal purity assay to identify and quantify the presence of sequence variants.

Because sequence-related impurities can affect the safety and efficacy of the product, and to ensure that manufacturing yields product with a consistent impurity profile, characterizing the identities of these impurities in the gRNA is very crucial, which is why we recommend that the orthogonal assays are used. Therefore, we also recommend using a sensitive, high-throughput sequencing method, such as next-generation sequencing (NGS), which is more sensitive and has a better capacity for quantifying sequence variants as well.

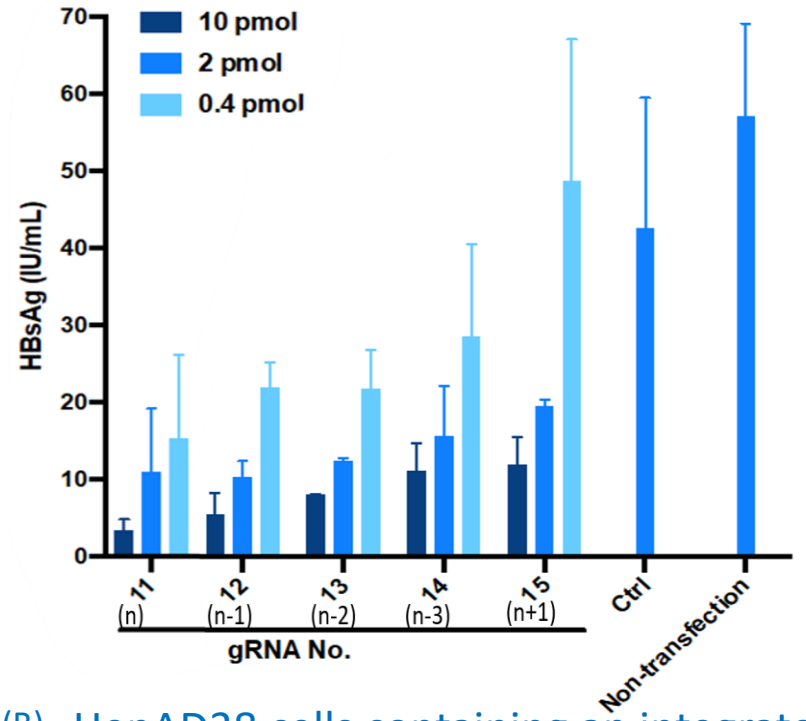
We also got a lot of questions about read depth. For this type of analysis, we recommend a read depth of 30X, which we believe would be sufficient in this application. Regarding impurity of gRNAs, I also would be remiss if I didn't mention that residual solvent and elemental impurities should also be assessed based on your guide manufacturing process. Thanks, Denise.

Problem: Truncated byproducts are fully functional

catastrophic unpredictable off-targets



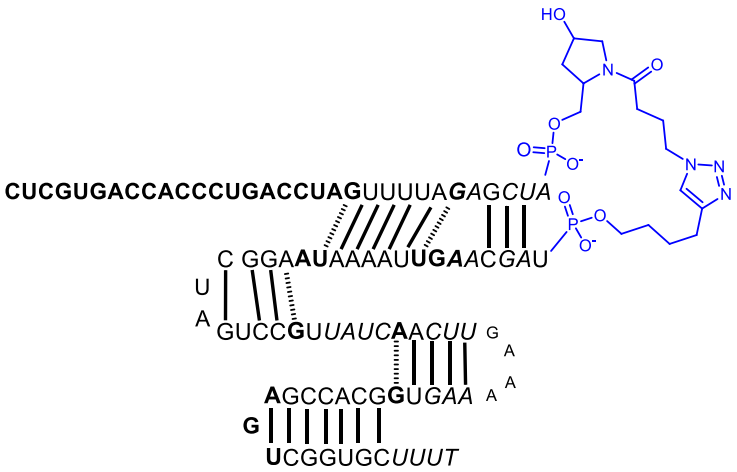
(A) In vitro cleavage assay

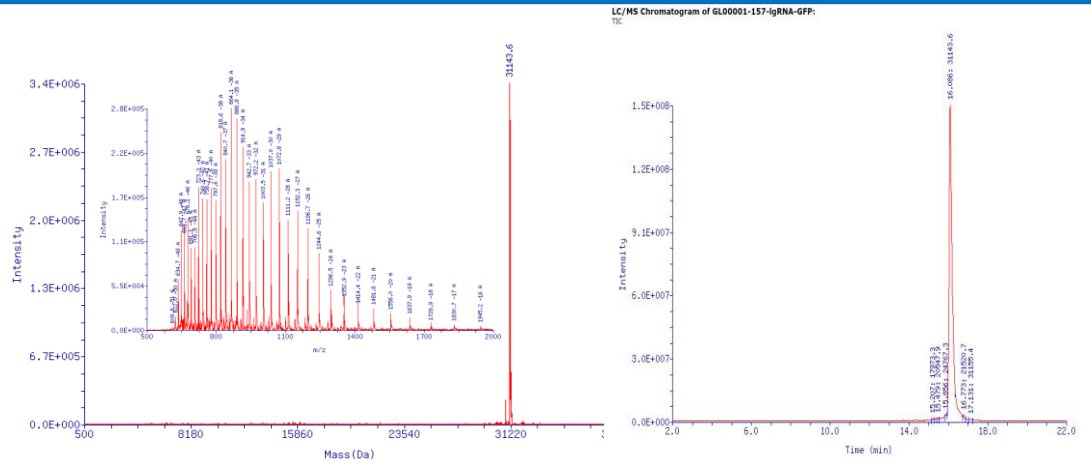


(B) HepAD38 cells containing an integrated full copy of HBV DNA

Fu Y and et al. Improving CRISPR-Cas nuclease specificity using truncated guide RNAs. Nat Biotechnol. 2014 Mar; 32(3):279-284. (This paper showed 5'-truncated sgRNAs up to n-3 are fully active.)

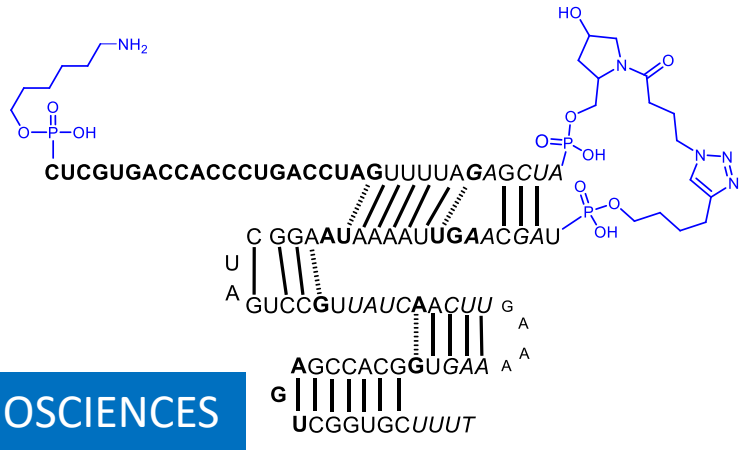
LgRNAs – Highest Quality Single-Molecule Guide RNAs

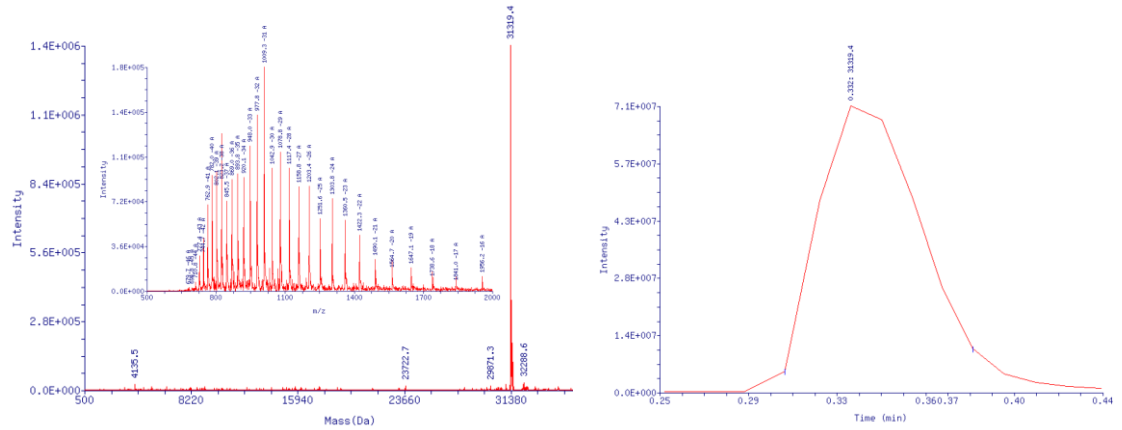




Chromatogram Summary

RT (min)	Base Peak Mass (Da)	Intensity	Spectral Quality	LC/MS Peak Area	LC/MS Area Percent
15.207	17973.3	1.70E+004	ok	2.28E+006	0.17
15.479	20547.9	5.35E+004	ok	2.43E+006	0.18
15.856	24767.3	4.64E+004	ok	1.79E+006	0.13
16.086	31143.6	3.35E+006	ok	1.35E+009	99.15
16.773	21520.7	1.67E+004	ok	3.74E+006	0.27
17.131	31155.4	1.30E+003	ok	1.40E+006	0.10





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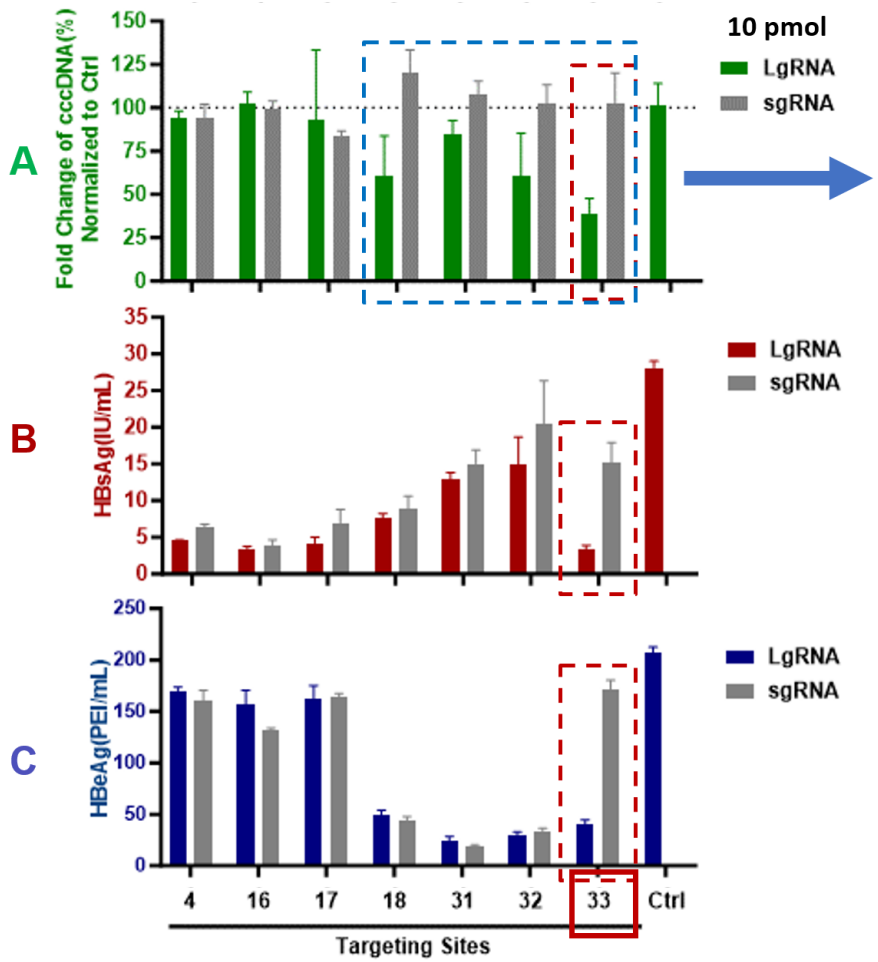
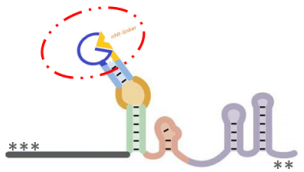
LgRNAs are Superior to sgRNAs in Editing HBV DNA in Human Hepatocytes

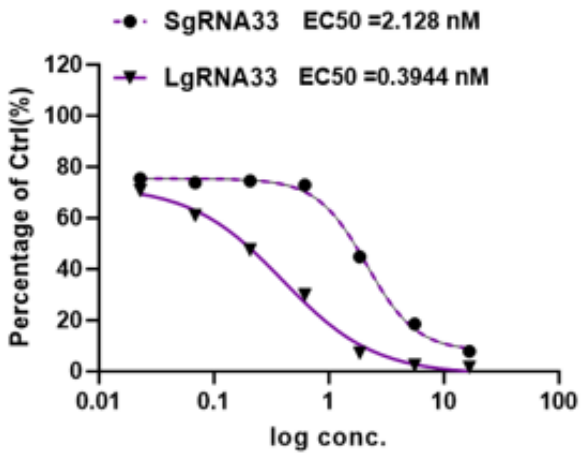




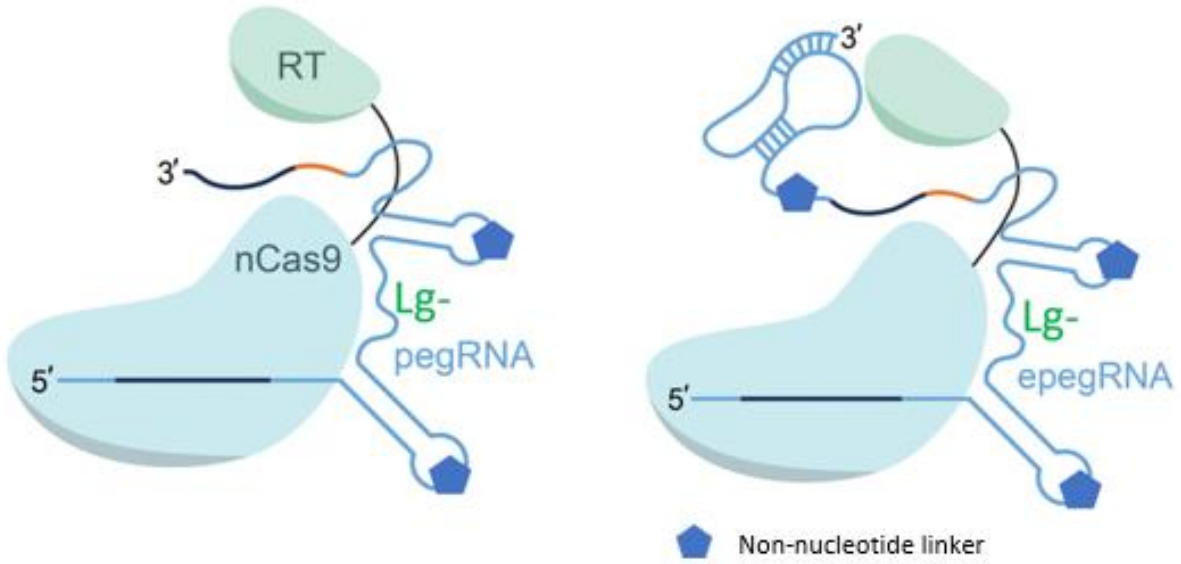
LgRNA degrades HBV cccDNA much better than sgRNA at multiple sites

LgRNA is Superior in inhibiting HBsAg production by integrated HBV DNA

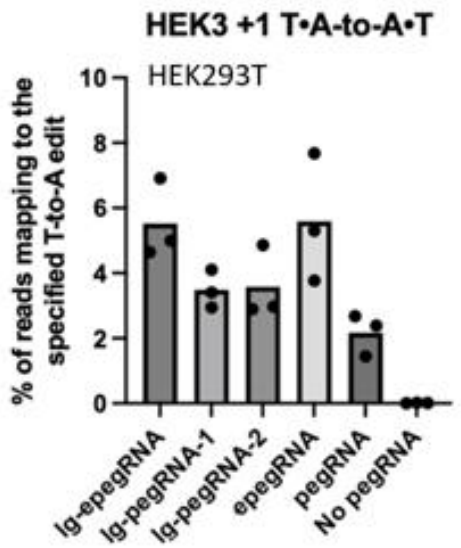
sgRNA33 → lgRNA33
(2.13 nM) (0.39 nM)



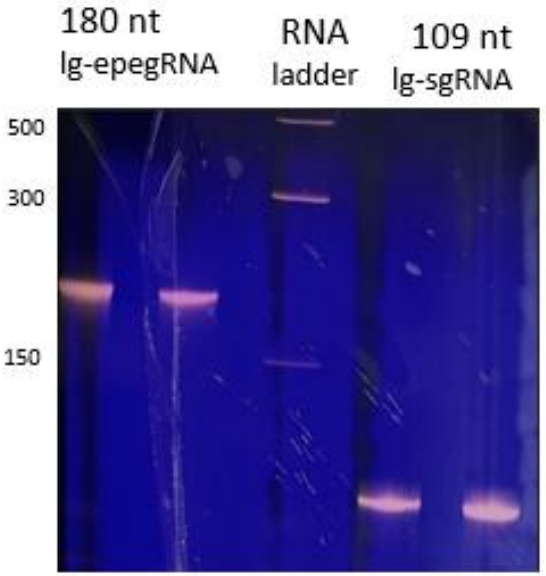
LgRNAs for SAFE Prime Editing, 180 nt -- High Quality Improves Precision



Adapted from Nelson, J.W. et al. Engineered pegRNAs improve prime editing efficiency. Nat Biotechnol 2022, 40, 402.



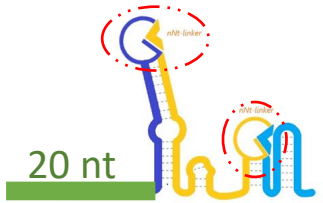
Comparison between sgRNAs and lgRNAs for prime editing



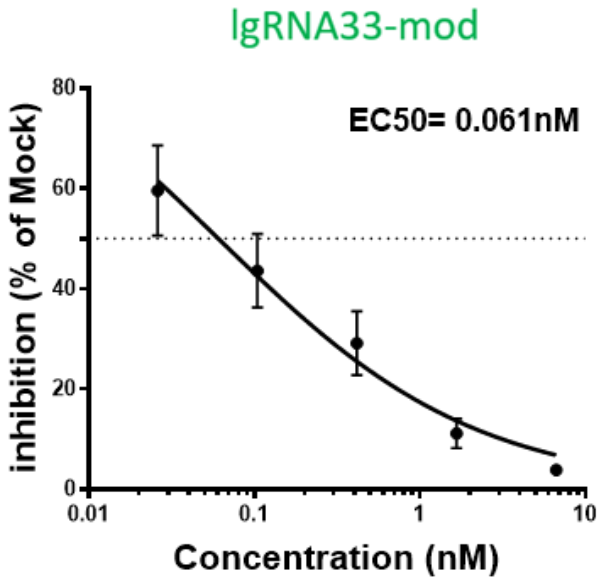
Quality-control of long guide RNAs at GeneLancet

- **ultra-purity;**
- **production scalability;**
- **Precision: preventing mis-incorporation of guide RNA scaffold by prime editors with epegRNAs.**

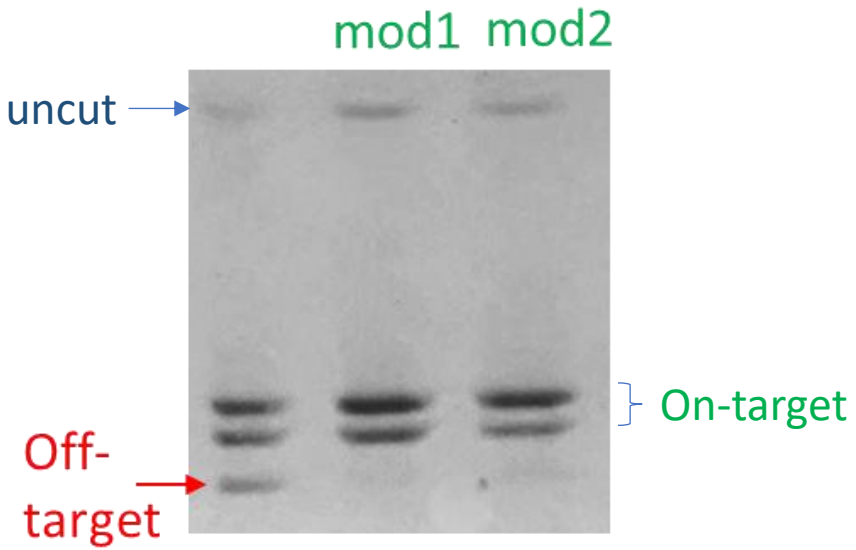
LgRNAs are Compatible to Versatile Chemical Modifications Critical for Efficacy and Safety



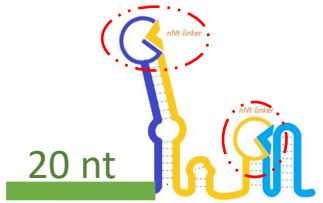
Unprecedent Potency

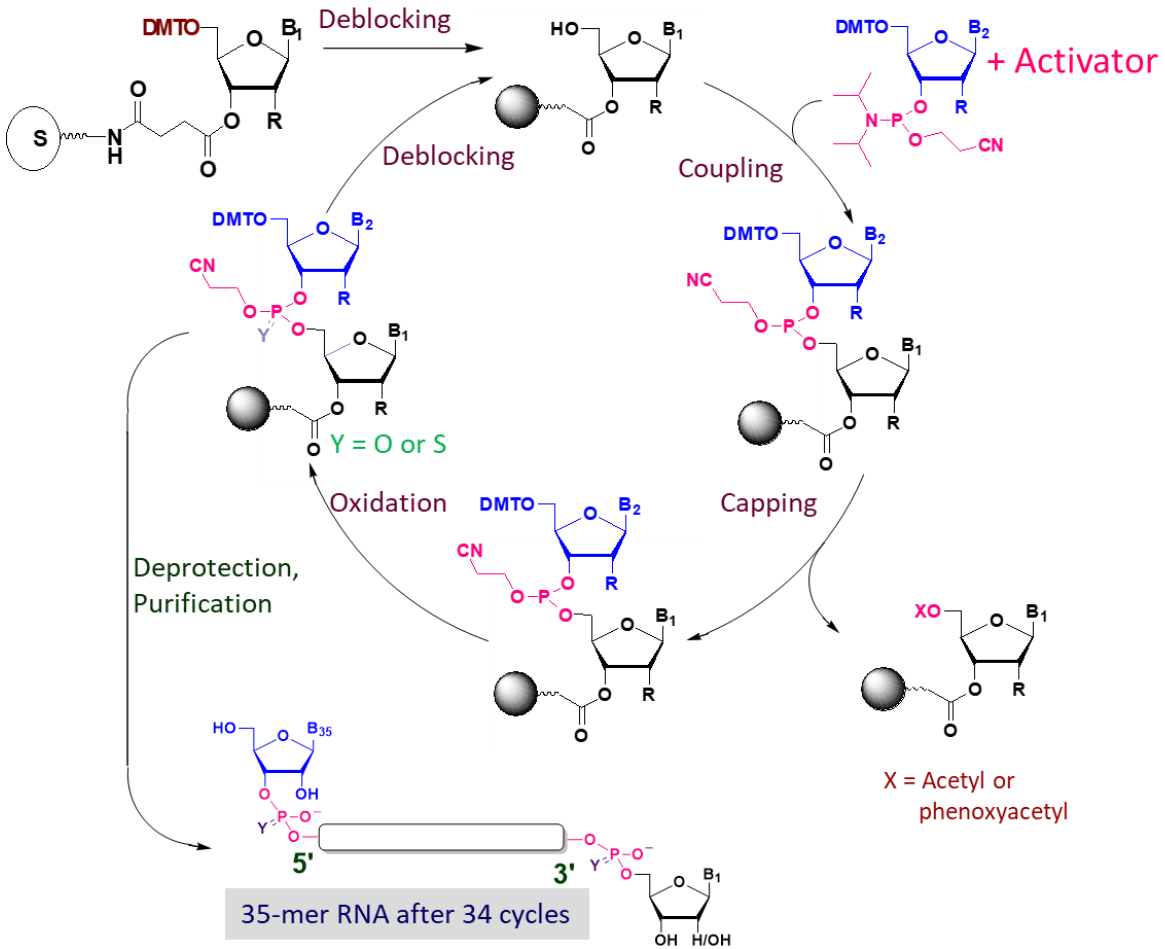


Enhanced Specificity

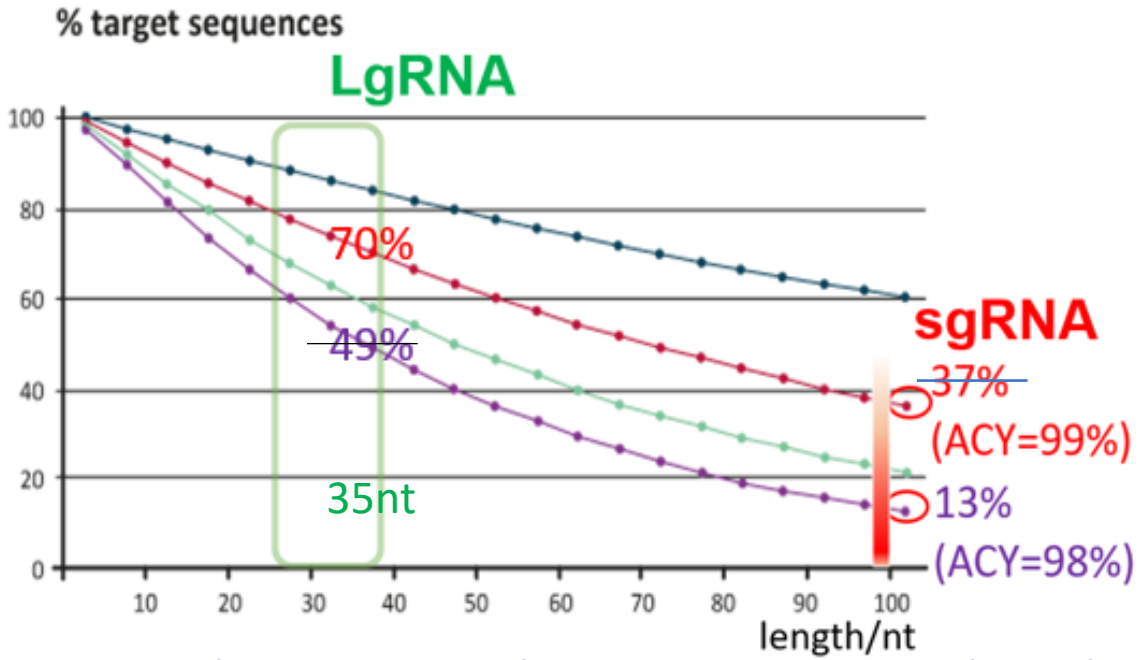


LgRNA Addresses Precision at Scale





Segmented Nucleic Acids: LgRNA



Much CLEANER products

complicated product mixtures

Problem:
Limitations of Present CRISPR Gene Editors

MegN Editing	ZFP Editing	TALEN

CRSIPR Editing	Base Editing (CRISPR)	Prime Editing (RT) (CRISPR)

DSBs

Off-targets

Immunogenicity

Delivery

Efficacy

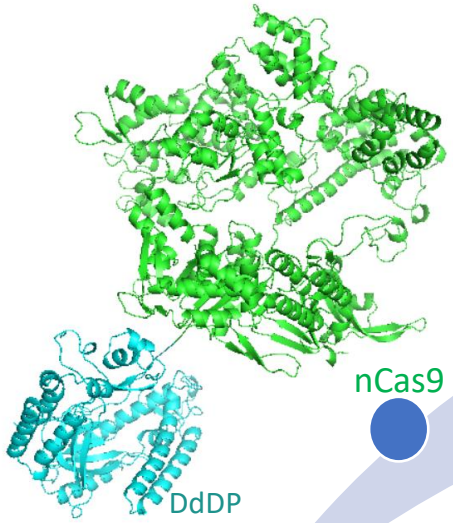
Epigenome Editing (CRISPR)

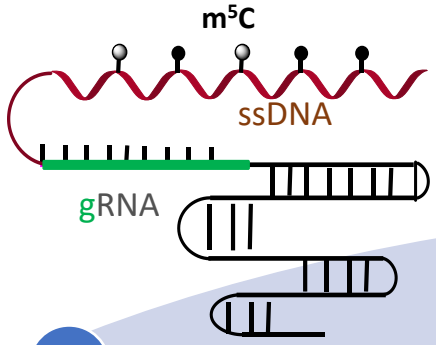
Polymerase editing (CRISPR)	

ssDNA

Solution: GL-Tech 2
Proprietary STAR™ Editing – Precision and Scale Redefined

WO2021034373
WO2023101993

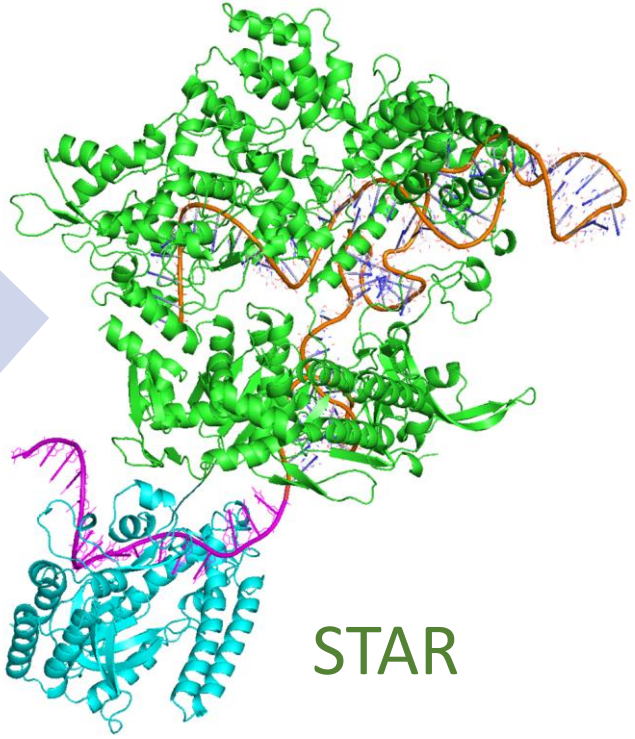




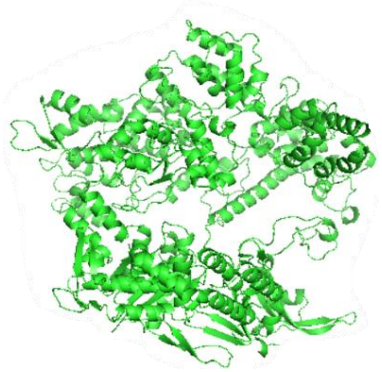
segNA

Uniquely accessible
by **LgRNA technology**

***STAR™* Editor**



STAR



Cas9

Polymerase editing

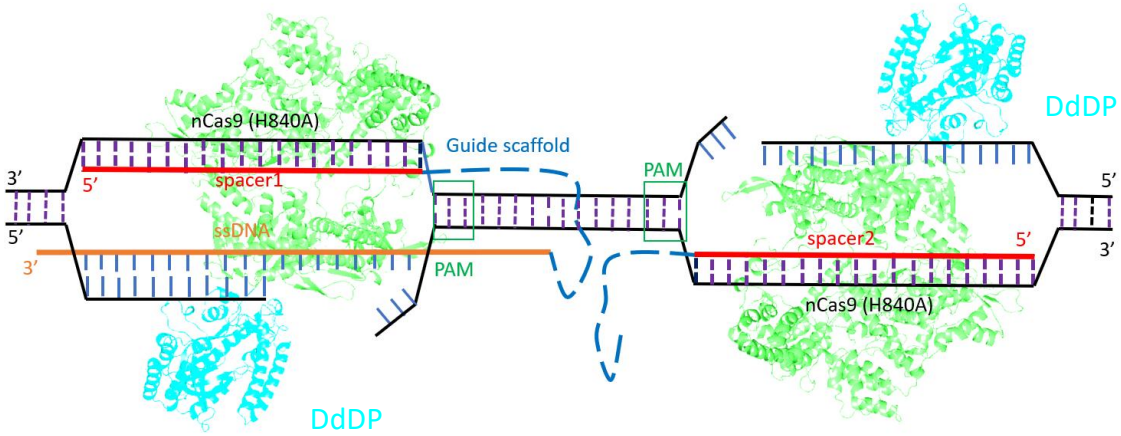
Proprietary platform

- High fidelity
- High processivity

DdDP: DNA-directed DNA polymerase

- ✓ Multiplexing **full-spectrum gene editing**
- ✓ **Precise Epigenome editing**
- ✓ Concurrent genome and epigenome editing: better efficacy
- ✓ No double-strand breaks
- ✓ High fidelity: DdDP + multiple proof-reading steps
- ✓ No viral vector template (dsDNA) is needed
- ✓ No dsDNA integrations

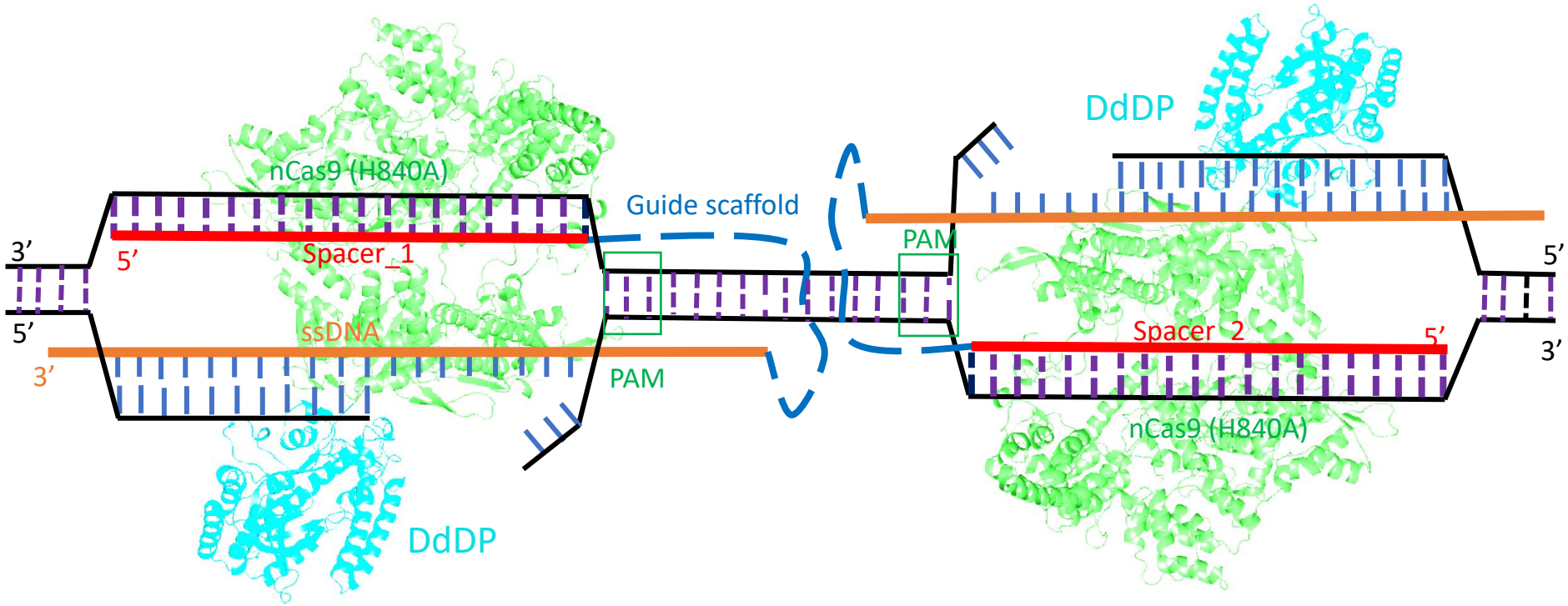
Principle of *Duo*STAR Editing



A multiple-turnover STAR (Seek-Tag-Amend-Release) genome editor for editing HBV genes with enhanced release of the acting ribonucleoprotein (RNP) complex. 1. Seek: a nCas9(H840A)/segNA complex binds HBV DNA that contains a sequence match to the first 17-20 nucleotides of the segNA and immediately before a protospacer adjacent motif (PAM) to form an R-loop. 2. Tag: cleavage and further 3'-end processing by RuvC leave a PAM distal strand of various lengths which is asymmetrically released from the nCas9:segNA:DNA complex. The released DNA strand acts as a primer (3'-end of the primer is further processed by DNA polymerase) and hybridizes with the 3'- homology arm of the conjugated ssDNA strand, which acts as a template for DNA repair. 3. Amend: the tagged DNA nick is repaired by ssDNA-templated synthesis and the 5'- flaps are removed and the gaps are ligated by cellular enzymes (mismatch repairing). The editing efficiency can be enhanced by a second nick (see the figure) at non-editing strand, the mismatches in non-editing strand can be efficiently corrected via edited-strand templated DNA synthesis, while indels can be minimal because of the high processivity of DNA polymerase. For deletion/insertion of DNA of interest, a second segNA can be used. 4. Release: Cas9:segNA complex is released from the repaired DNA, and is ready for next cycle.

DuoSTAR Editing

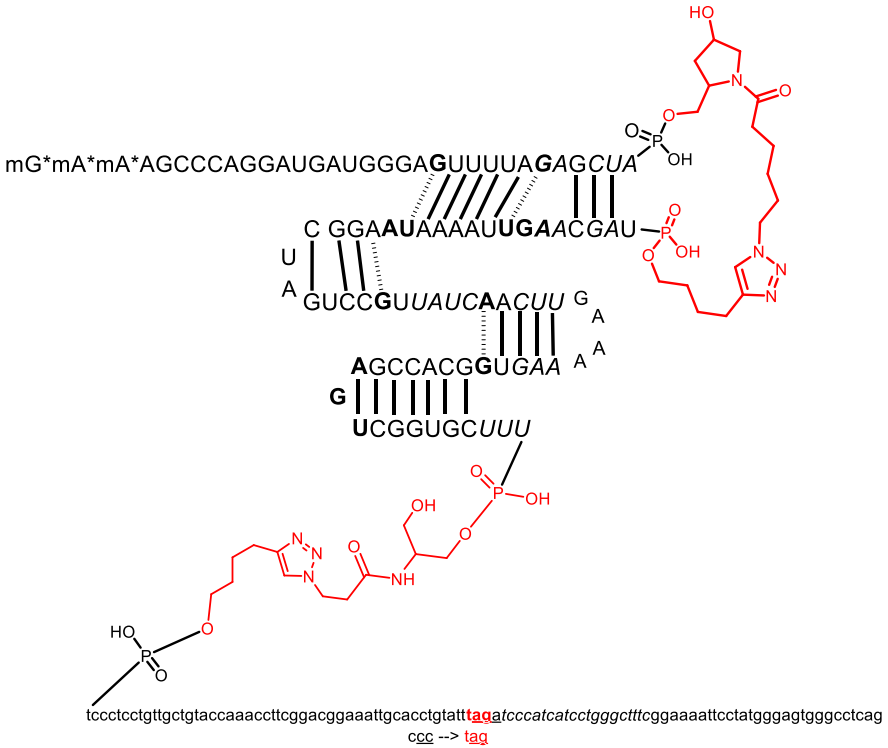
(segNA1/segNA2)



DuoSTAR (with a segNA pair) enables diverse editing including efficient precise single base editing, deletion of nucleotides and long repeats and insertions of longer DNAs.

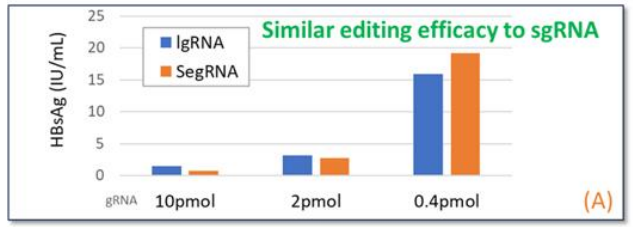
STAR Editing in development

segNA: our expertise

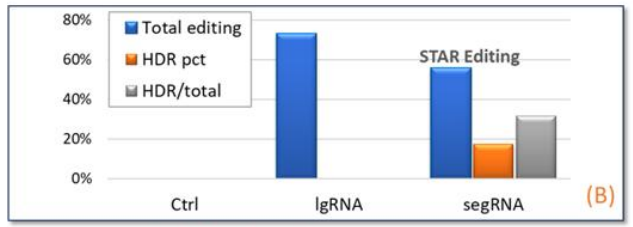


Proof-of-Concept: Validated in cells

Cas9 + segNA in AD38 cells



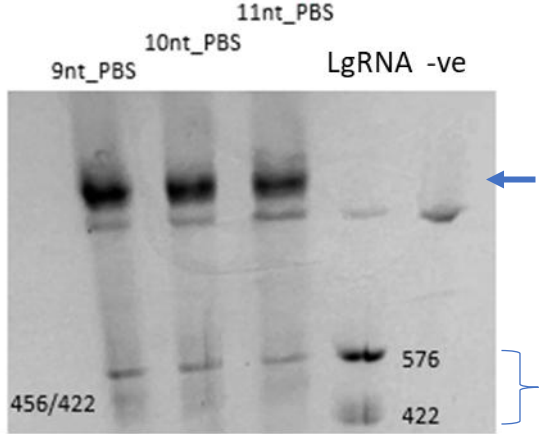
STAR editor dose-dependently decreased HBsAg levels in hepatocytes



STAR editor precisely deactivated HBV DNA by inserting a stop codon

Optimization: High efficiency

In vitro STAR Editing: Extension assay



fN*fN*fN*fNfNfN*fN*fN*fN*fN*fN
rNrNrNrNrNrGrGrUrU-gRNA-ssDNA

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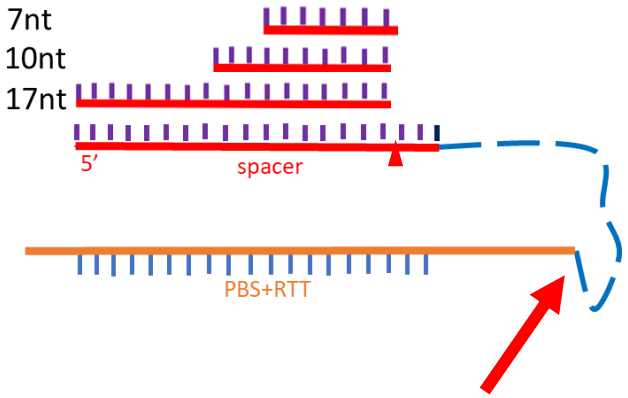
STAR vs. PEs:

1. segNA has no auto inhibition or over extension

pegRNA

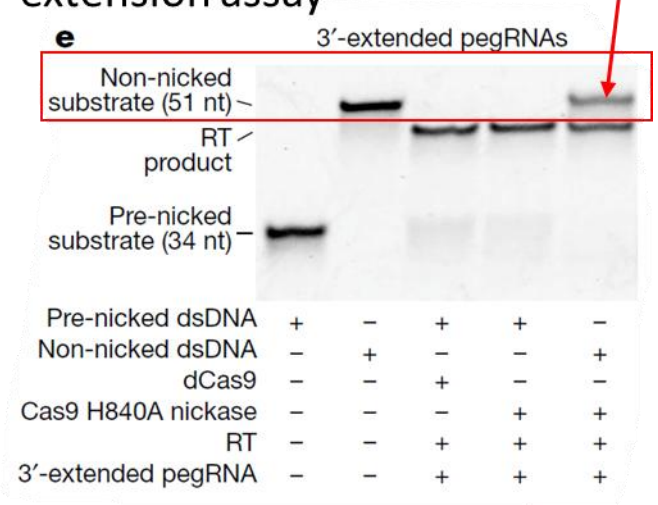
Auto-inhibitory



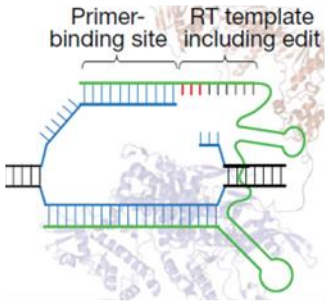
PE mis-incorporates guide scaffold.

In vitro Prime editing: extension assay

Auto-inhibitory

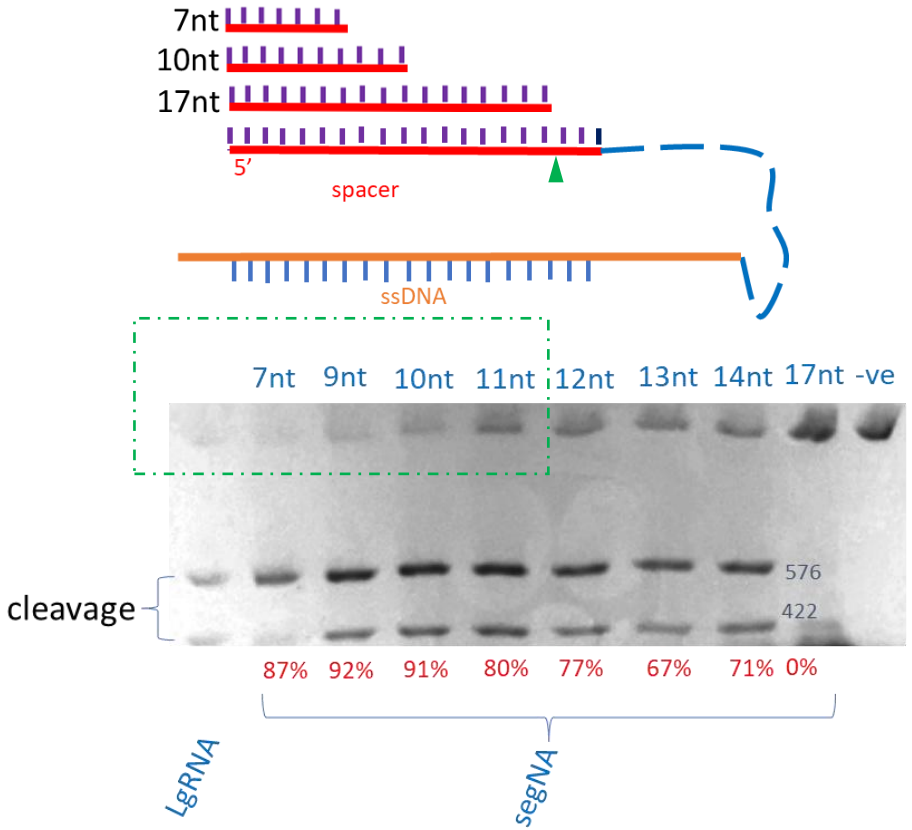


Anzalone et al. 2019



Limits: Inhibition of nickase activity in PE

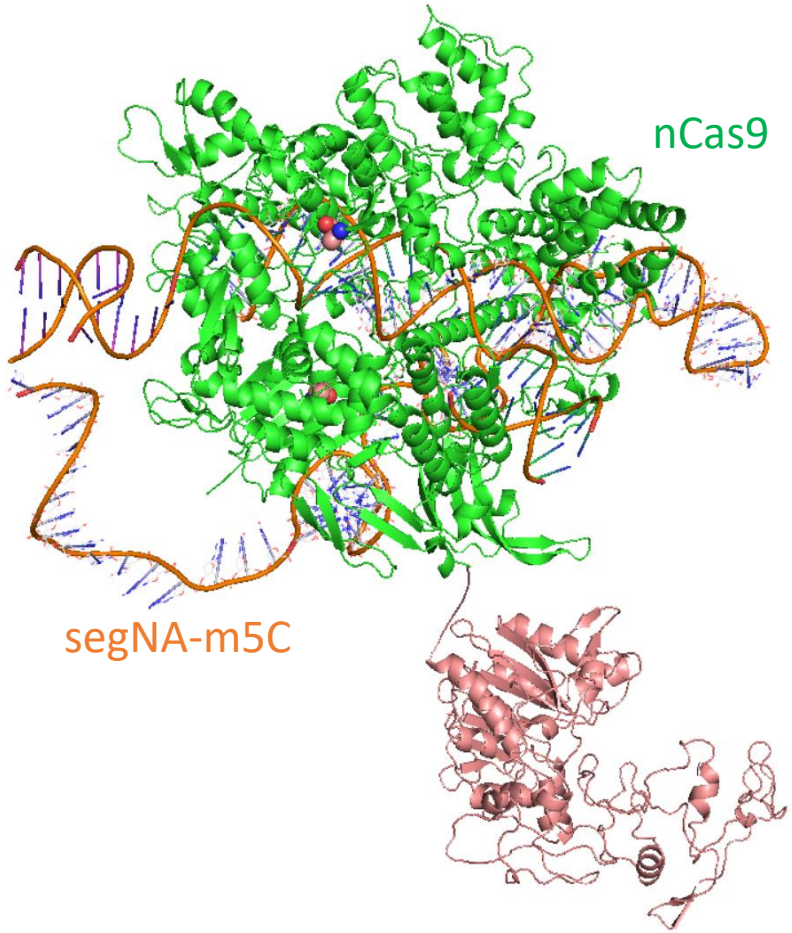
segNA design

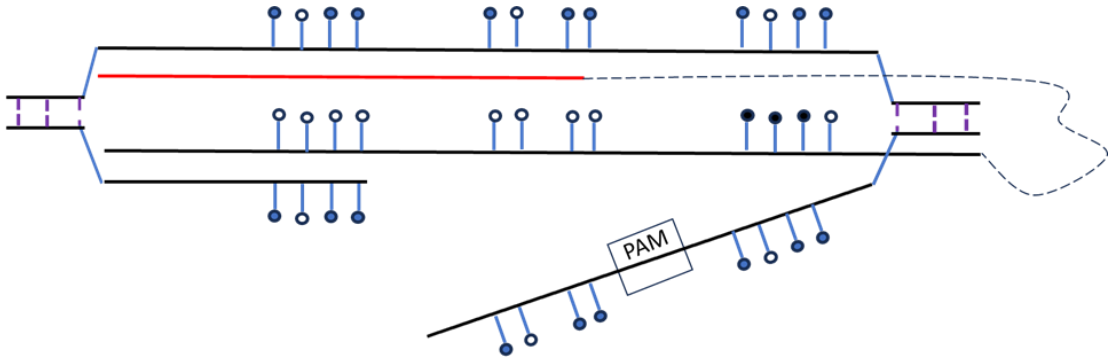


PE: prime editing, of which pegRNA is auto-inhibitory.

STAR vs. PEs:

2. segNA for epigenetic editing





segNA-m5C

ssDNA template of segNA can be specifically methylated, which allows DNA methylation at a single CpG resolution.

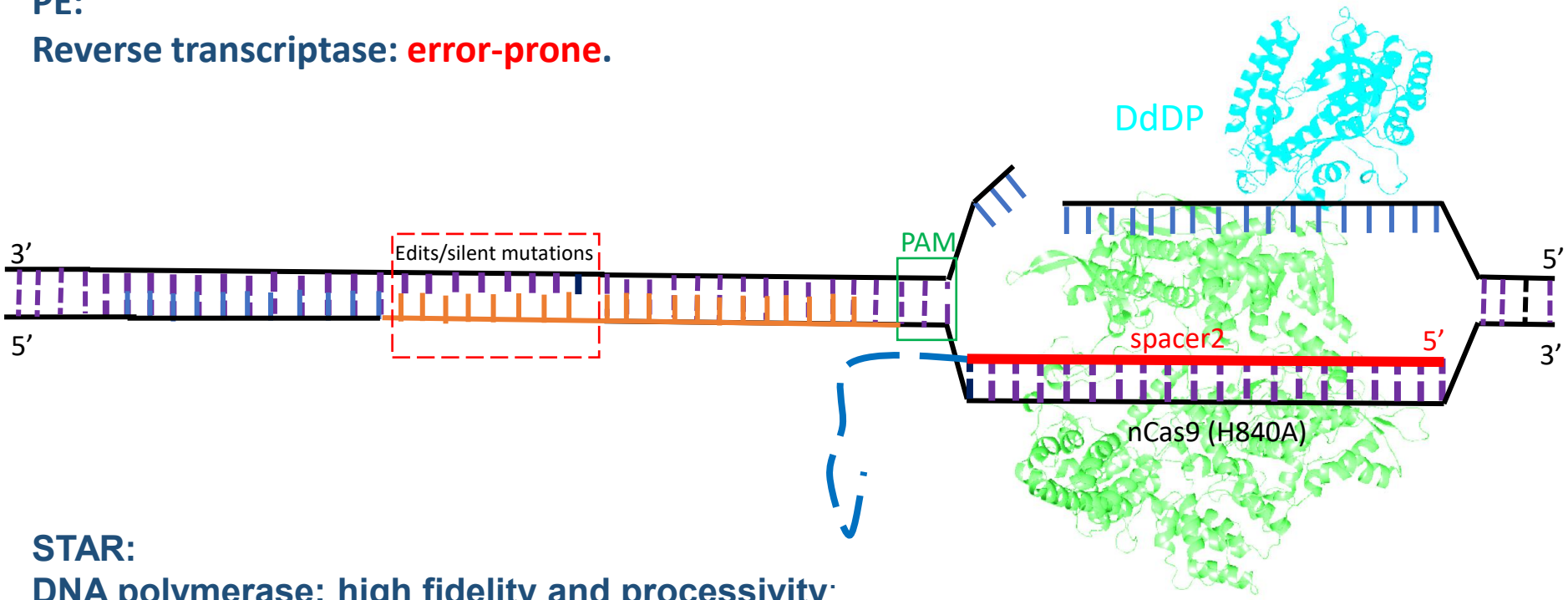
PE uses RNA template, and thus lacks this function.

DuoSTAR vs. PE3:

3. polymerase is superior to reverse transcriptase

PE:

Reverse transcriptase: error-prone.



STAR:

DNA polymerase: high fidelity and processivity;

Efficiency: 5’-flap removal

DuoSTAR uses edited DNA strand as a second template and a fused (or endogenous) polymerase for flap resolution.

PE3: prime editor with one additional sgRNA to nick unedited DNA strand. PE3 usually has increased efficiency but also significantly increased indels.

Precise Gene Editing: Competitive Landscape

Challenge	CRSIPR Editing	Base Editing	Prime Editing (RT)	STAR Editing (polymerase) (GeneLancet Bio)
Guide RNAs*	sgRNA/LgRNA	sgRNA/LgRNA	(pegRNA + sgRNA)/ (Lg-pegRNA + LgRNA)	segNA + LgRNA
Minimal DNA double strand breaks	-	+++	+++	+++++
Efficient/safe delivery	++	++	Dual AAVs ++	mRNA; RNP conjugates ++++ #
Minimal off-target effects	+	+	+++	++++
Minimal immune response$	+++	+++	+++	+++
Efficacy	++++	++++	+	++++

* LgRNA, Lg-pegRNA and segNA are our proprietary guide RNAs.
$ Multiple dosing LNP-mRNA-gRNA has been achieved.
# STAR does not require a fusion protein.