Off-Target Analysis Services and Prime Editor Innovation

Capabilities Overview

June 2026

Adam Chernick, PhD MBA

Sr. Commercial Product Manager

Agenda

- IDT Introduction
- Off-target services
  - Nomination (UNCOVERseq)
  - Confirmation (rhAmpSeq)
- Prime editor innovation
  - pegRNA chemical modifications
  - mRNA optimization

Industry-leading accomplishments

IDT
INTEGRATED DNA TECHNOLOGIES

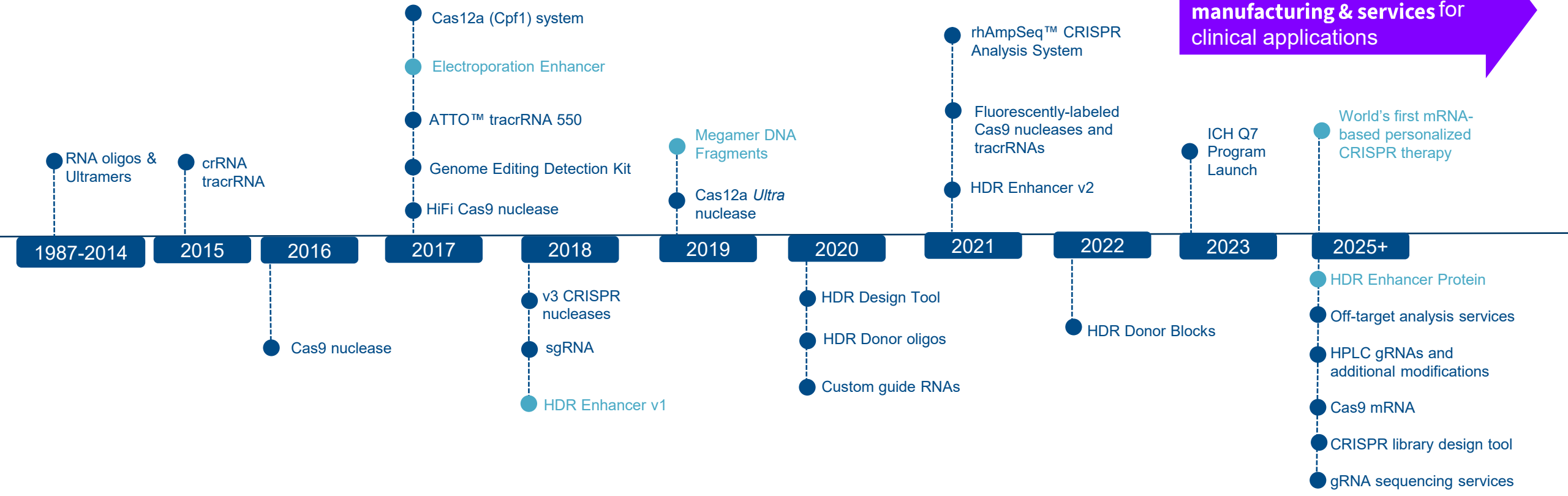
CRISPR Innovation Journey at IDT

Oligo Manufacturer: DNA and RNA Oligonucleotides

Oligo Manufacturer: Guide RNAs

Alt-CRISPR Systems & Workflow Developer: Knock-out (NHEJ), Knock-in (HDR), Edit Analysis Workflows

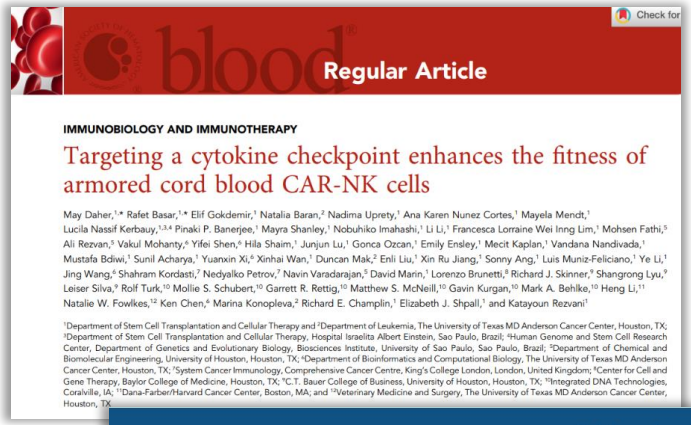
Therapeutic oligo manufacturing & services for clinical applications



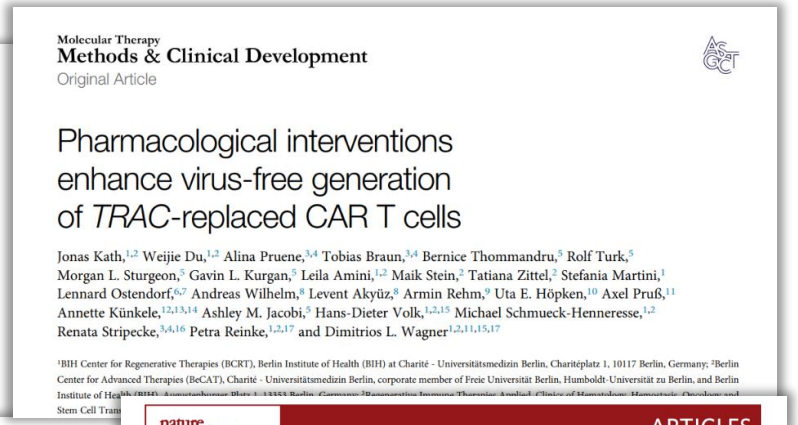
IDT INTEGRATED DNA TECHNOLOGIES

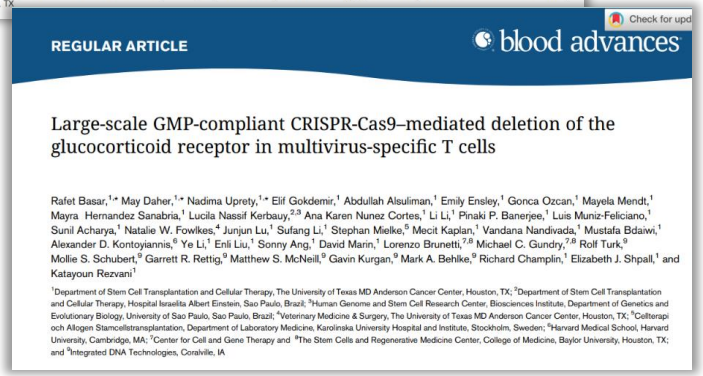
First to market

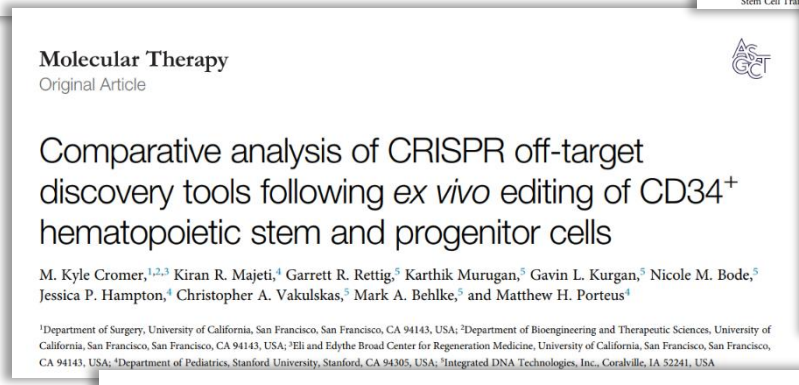
Off-target nomination → collaborations


blood
Regular Article
IMMUNOBIOLOGY AND IMMUNOTHERAPY
Targeting a cytokine checkpoint enhances the fitness of armored cord blood CAR-NK cells

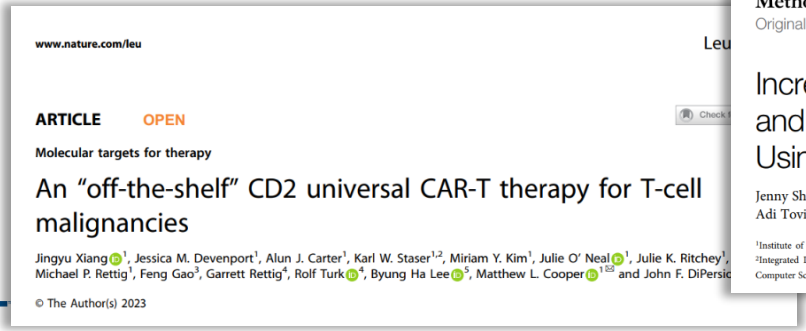

Balke-Want et al. Molecular Cancer (2023) 22:100
https://doi.org/10.1186/s12943-023-01799-7
Molecular Cancer
RESEARCH
Open Access
Homology-independent targeted insertion (HITI) enables guided CAR knock-in and efficient clinical scale CAR-T cell manufacturing

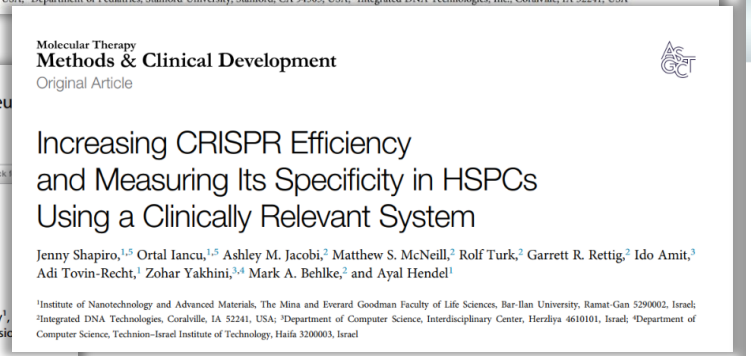

Molecular Therapy
Methods & Clinical Development
Original Article
Pharmacological interventions enhance virus-free generation of *TRAC*-replaced CAR T cells


REGULAR ARTICLE
blood advances
Large-scale GMP-compliant CRISPR-Cas9–mediated deletion of the glucocorticoid receptor in multivirus-specific T cells


Molecular Therapy
Original Article
Comparative analysis of CRISPR off-target discovery tools following *ex vivo* editing of CD34+ hematopoietic stem and progenitor cells


nature medicine
ARTICLES
https://doi.org/10.1038/s41591-021-01284-y
Gene replacement of α-globin with β-globin restores hemoglobin balance in β-thalassemia-derived hematopoietic stem and progenitor cells

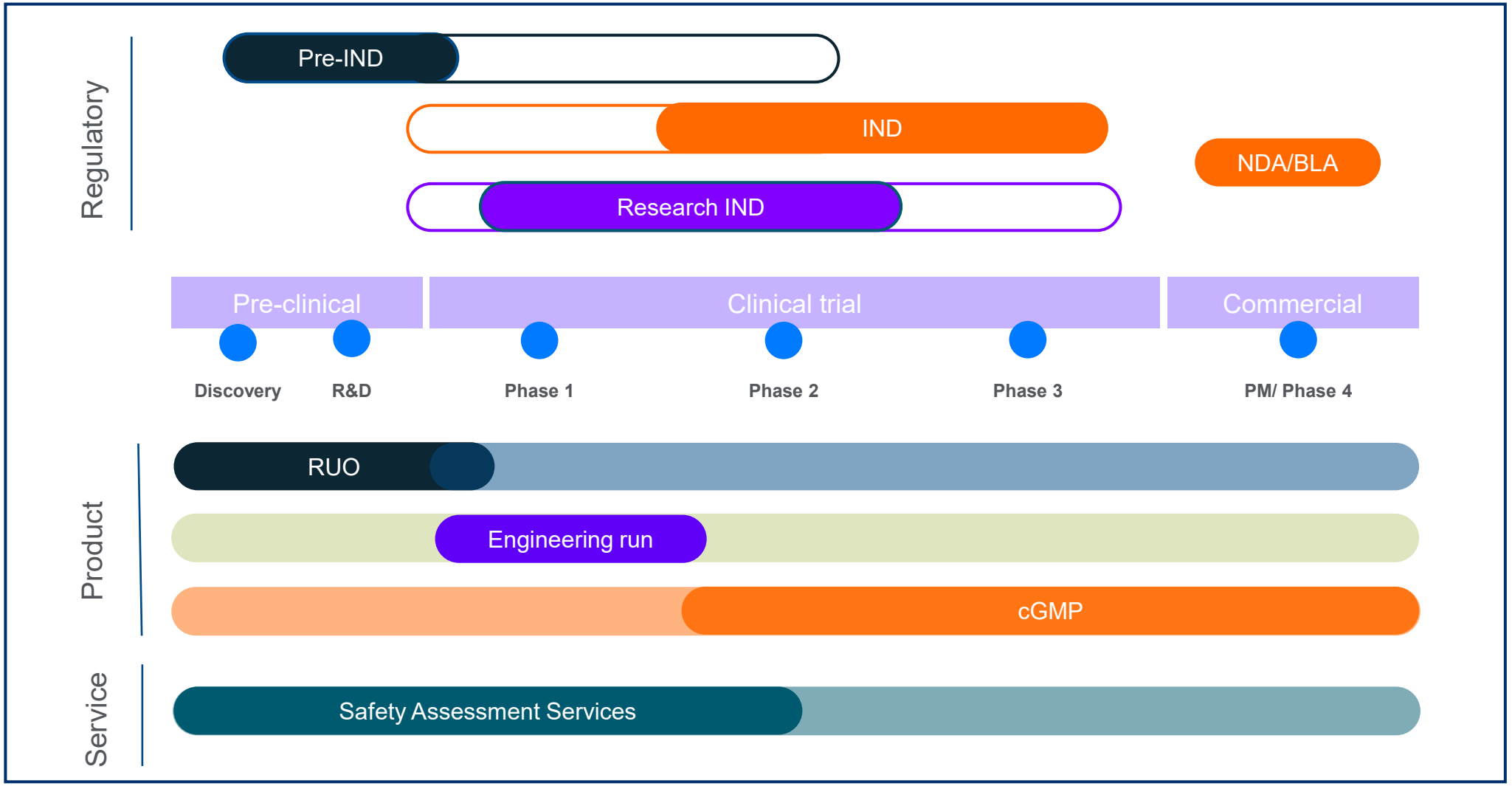

www.nature.com/leu
ARTICLE OPEN
Molecular targets for therapy
An "off-the-shelf" CD2 universal CAR-T therapy for T-cell malignancies
© The Author(s) 2023


Molecular Therapy
Methods & Clinical Development
Original Article
Increasing CRISPR Efficiency and Measuring Its Specificity in HSPCs Using a Clinically Relevant System


nature COMMUNICATIONS
ARTICLE
https://doi.org/10.1038/s41467-021-22417-4 OPEN
CRISPECTOR provides accurate estimation of genome editing translocation and off-target activity from comparative NGS data

IDT
INTEGRATED DNA TECHNOLOGIES

The right product to align with your development journey

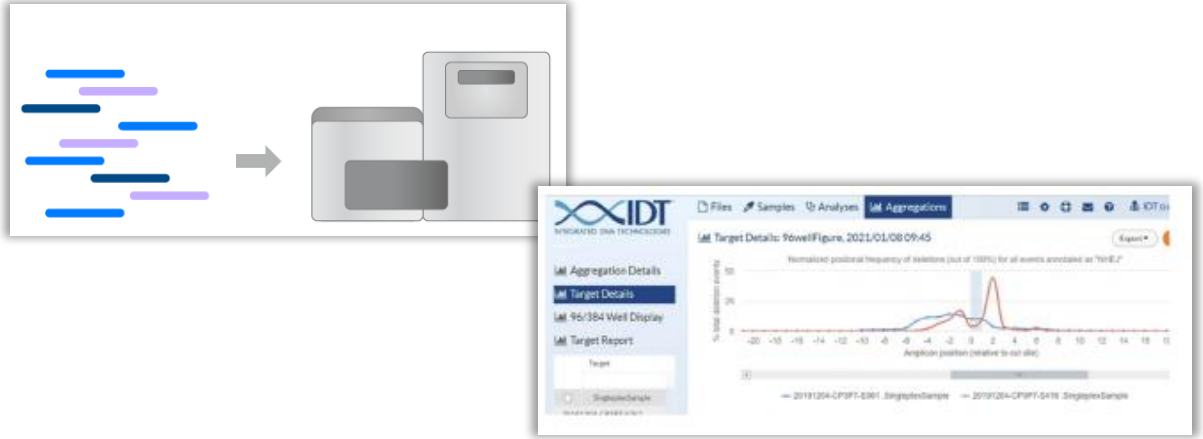


2-steps for off-target assessments

Step 1: Nomination (UNCOVERseq™)

GOAL: Identify genomic sites where cleavage is occurring
- Must be sensitive and genome-wide
- Sensitivity is critical at this stage, as these results are used to build the confirmation panel in step 2
- Various methods described in the literature:
  - Computational
  - Cell-based
  - Biochemical

Step 2: Confirmation (rhAmpSeq™)



GOAL: Confirm nominated cleavage sites in edited cells that have clinical associations
- Looks for more than just cut sites by assessing actual mutations at those locations
  - Indels, translocations, large deletions, etc.
- Some nominated sites may not occur in a clinically-relevant context, so confirmation helps determine those that are most critical to consider

Image above from Maeder and Gersbach, Mol Ther. 2016

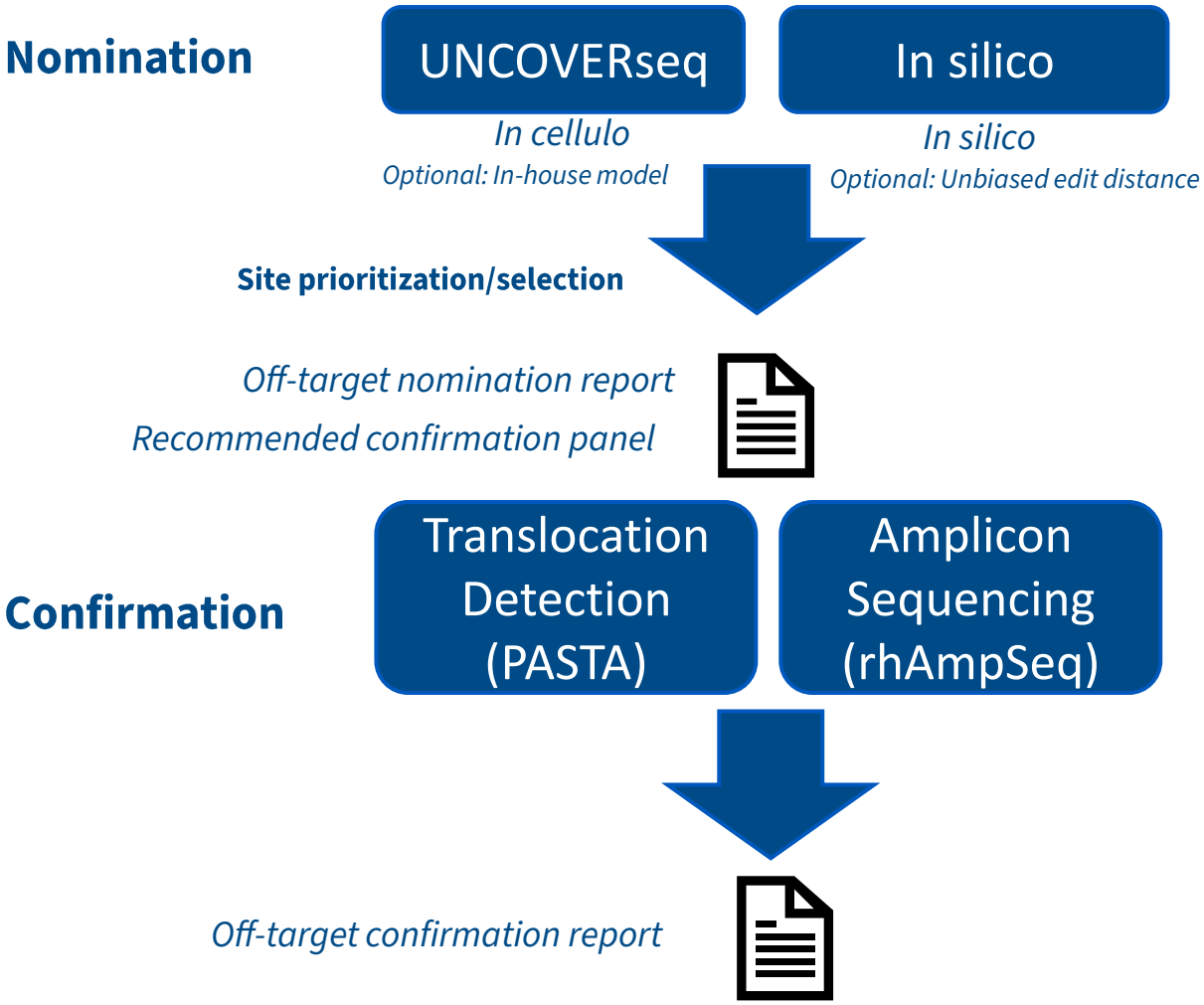
IDT's genotoxicity assessment covers current recommendations with orthogonality

Off-target nomination needs:

- Two orthogonal methods
  - UNCOVERseq and in silico nomination
    - *In silico* can be our method or client supplied
  - Annotation of potential risk of off-target regions for prioritization

Off-target confirmation needs

- Small indel detection
  - Defined analytical performance
- Chromosomal aberration detection
  - Defined analytical performance



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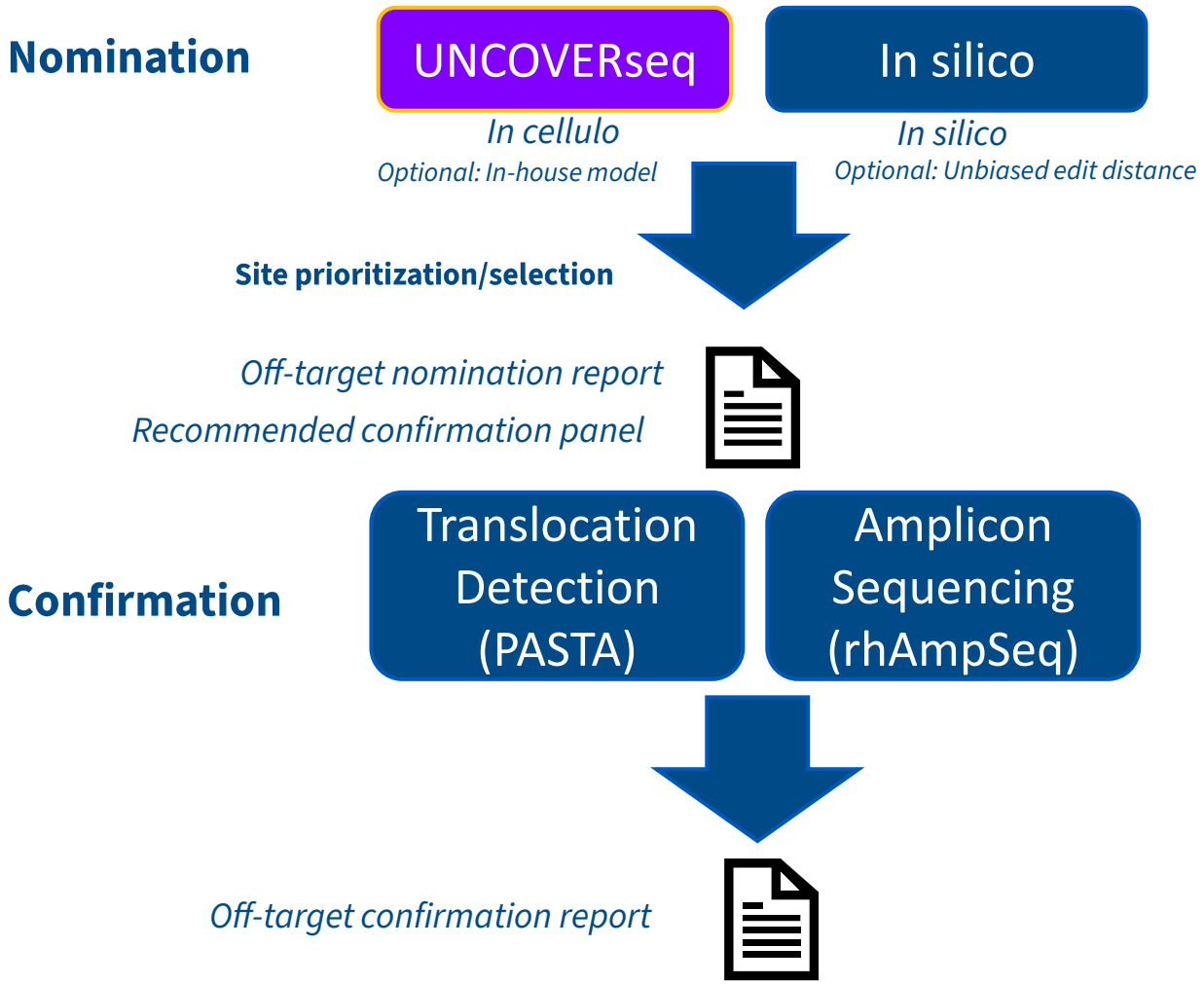
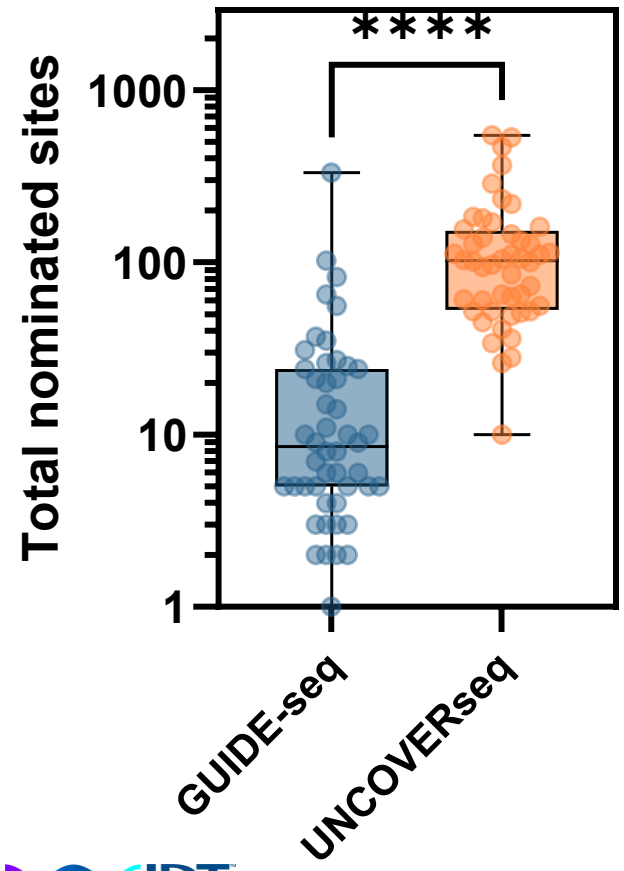


UNCOVERseq has an industry leading balance of sensitivity and precision

IDT uses a modified and improved version of the original GUIDE-seq methodology (UNCOVERseq™)

Accepted in Nature Communications

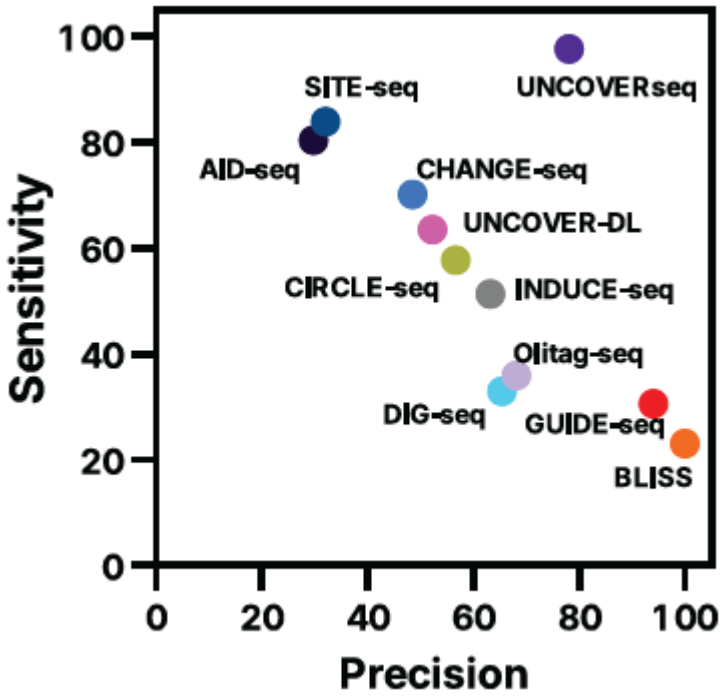
UNCOVERseq outperforms existing GUIDE-seq methodology across the same sites

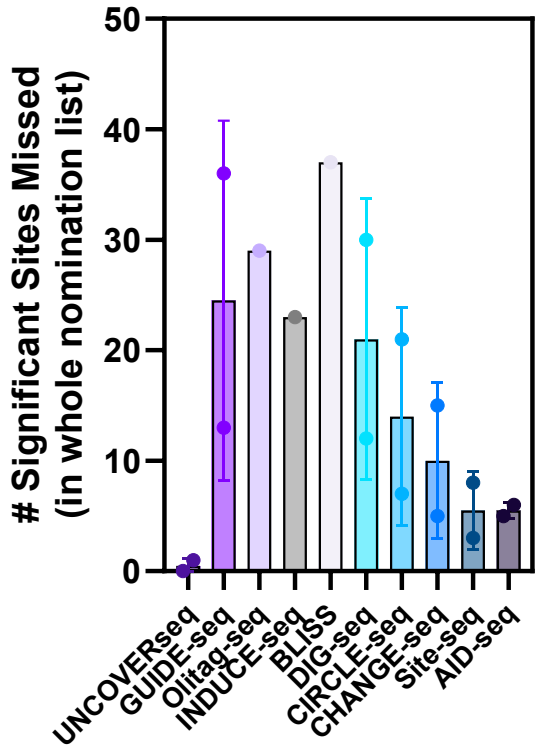


EMX1

FANCF

UNCOVERseq offers the best balance on sensitivity and precision for detecting true off-target sites compared to other methods



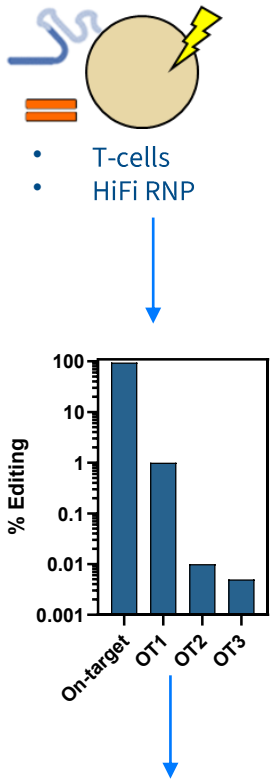


200+ sites sampled from up to 10 nomination empirical methods

Use of IDT's promiscuous nomination model serves as a sensitive proxy for clinical conditions

Clinical conditions

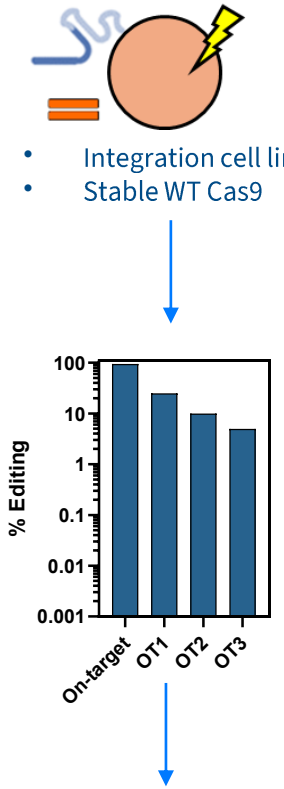
- T-cells
- HiFi RNP



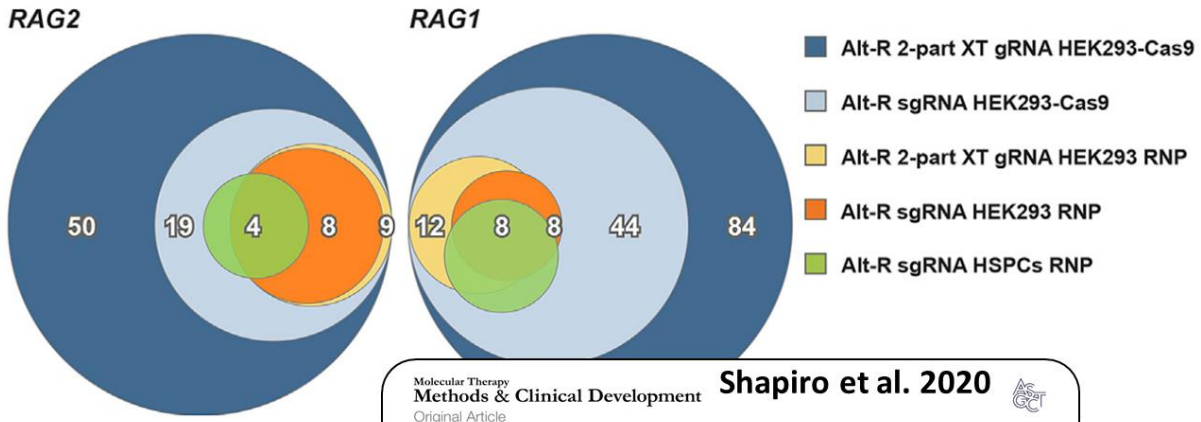
Harder to detect

Promiscuous conditions

- Integration cell line
- Stable WT Cas9



Easier to detect



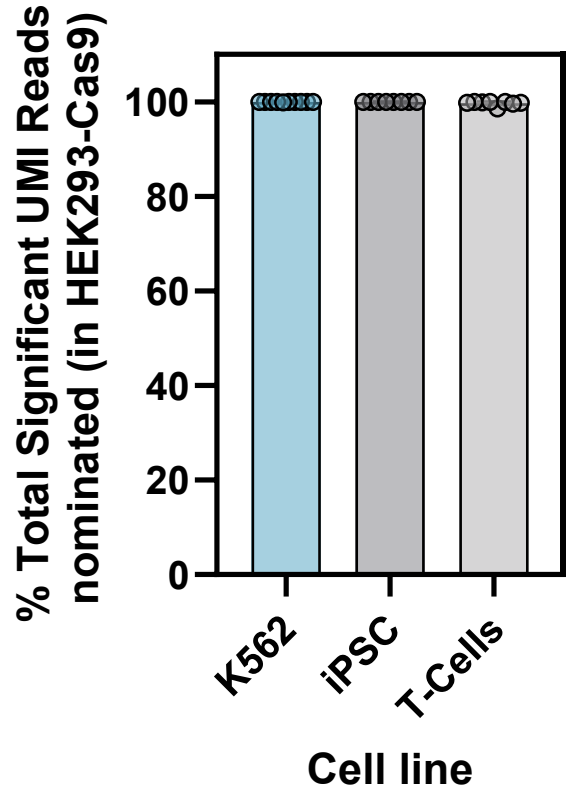
Molecular Therapy Methods & Clinical Development
Original Article

Shapiro et al. 2020

Increasing CRISPR Efficiency and Measuring Its Specificity in HSPCs Using a Clinically Relevant System

Literature precedent on cell lines and increasing levels of promiscuity being a suitable proxy

Low frequency off-target nomination



~99-100% of off-target events in clinically relevant cell lines/conditions are nominated in a model cell line that is overly promiscuous

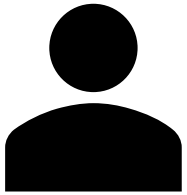
Process controls limit false-negatives and instill confidence in findings

Potential response from therapeutic developer:

"I saw there are no off-target effects for my gRNA in the conditions of interest!"

Potential reviewer response:

"Great, what were the analytical bounds of what you should have been able to detect during the protocol?"



Our process

"I expect analytical sensitivity down to at least 0.04% editing. This is confirmed with a parallel positive control. 100% of positive control sites were nominated (40) ranging from 51% to 0.04% editing with sufficient tag integration in my system of interest."

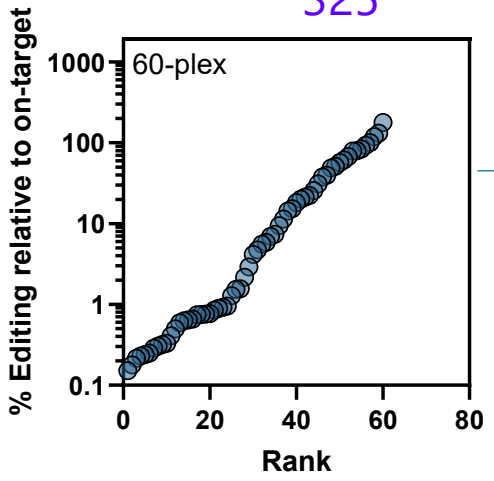
Experimental gRNA

+ Control gRNA

- Pre-nominated off-targets
- Pre-confirmed off-targets

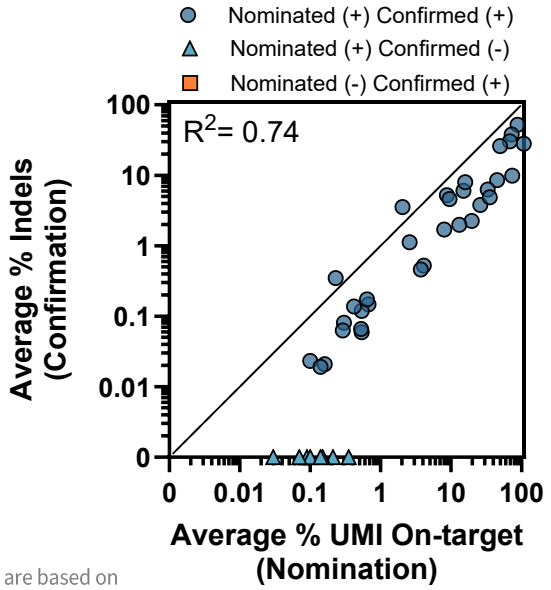
27% → Tag Integration QC → 34%

1 → Off-target nomination → 325



Off-target Confirmation

Metric	Value
# Sites meeting confirmation criteria	40
% Confirmed sites nominated	100%
Max % OTE editing nominated	51%
Min.% OTE editing nominated	0.04%
Max % OTE editing missed	N/A



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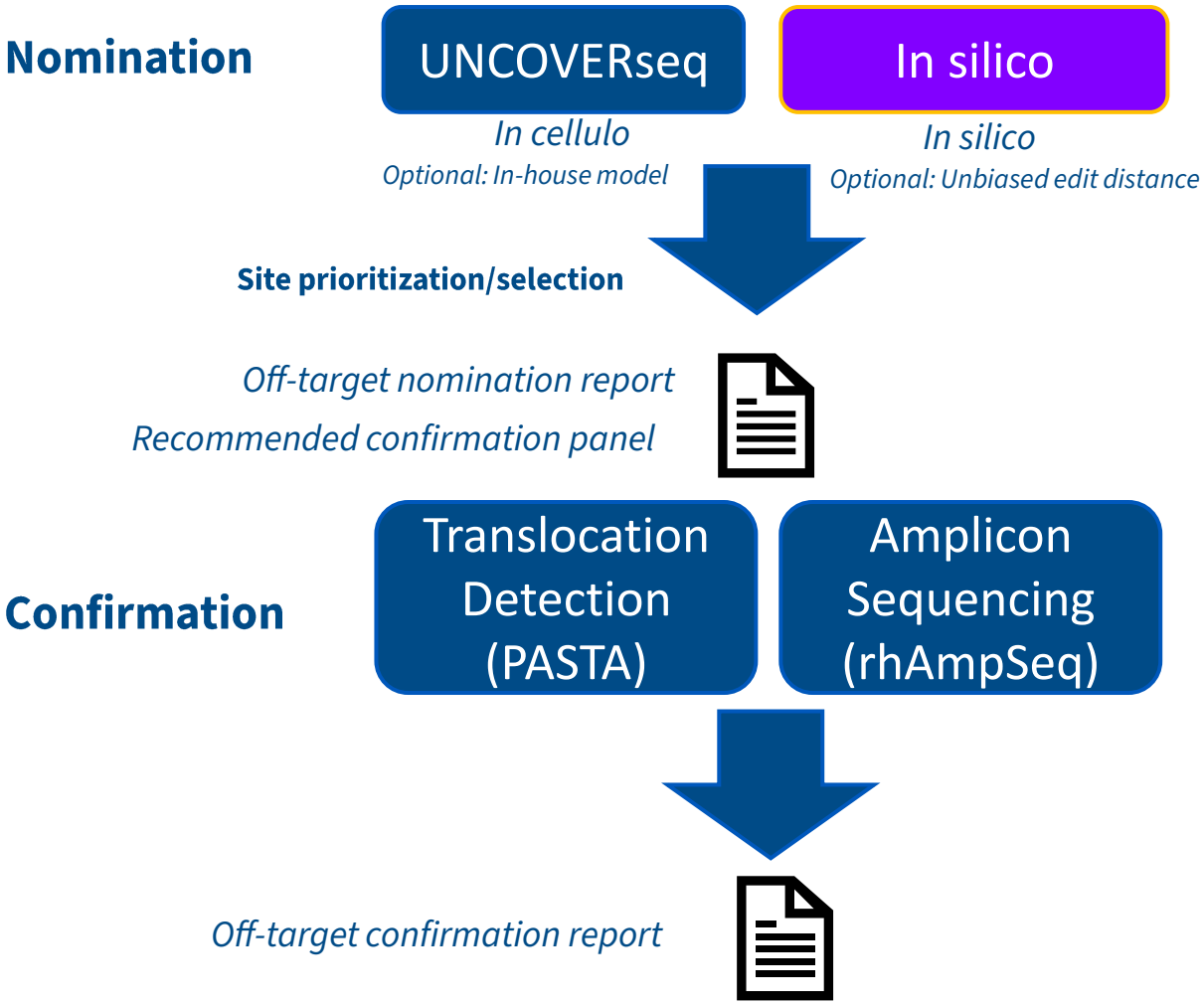
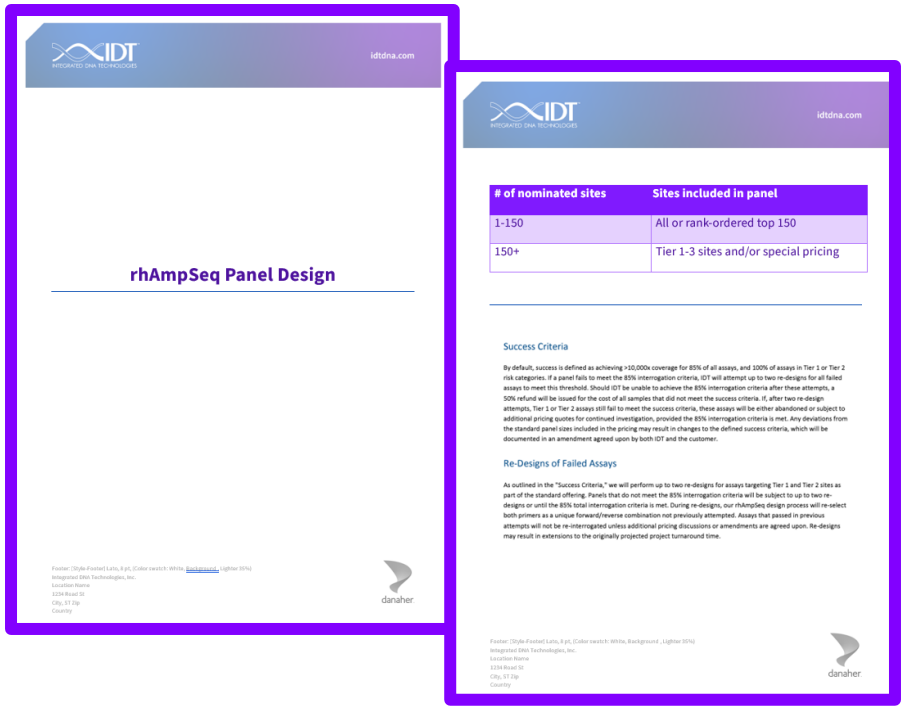


Review of nominated targets ensures proper customization & prioritization for programs

Off-target nomination → *Recommended sites* → Consultation + supplementation + sign-off → *Panel synthesis* → Off-target confirmation

- Example sign-off / acceptance criteria going to confirmation
  - 150 sites included; maximum of 24 pools
  - More sites / pools possible, but cost may change
- >85% of assays with >10,000x coverage
  - Two re-designs included to ensure we meet this #
- 100% of Tier 1 and 2 sites with >10,000x to be attempted
  - Two re-designs included to meet this (even if 85% met)
- Panel specifications
  - Which off-targets in which pool
  - Off-targets prioritized to attempt on-target/top off-targets get pooled together



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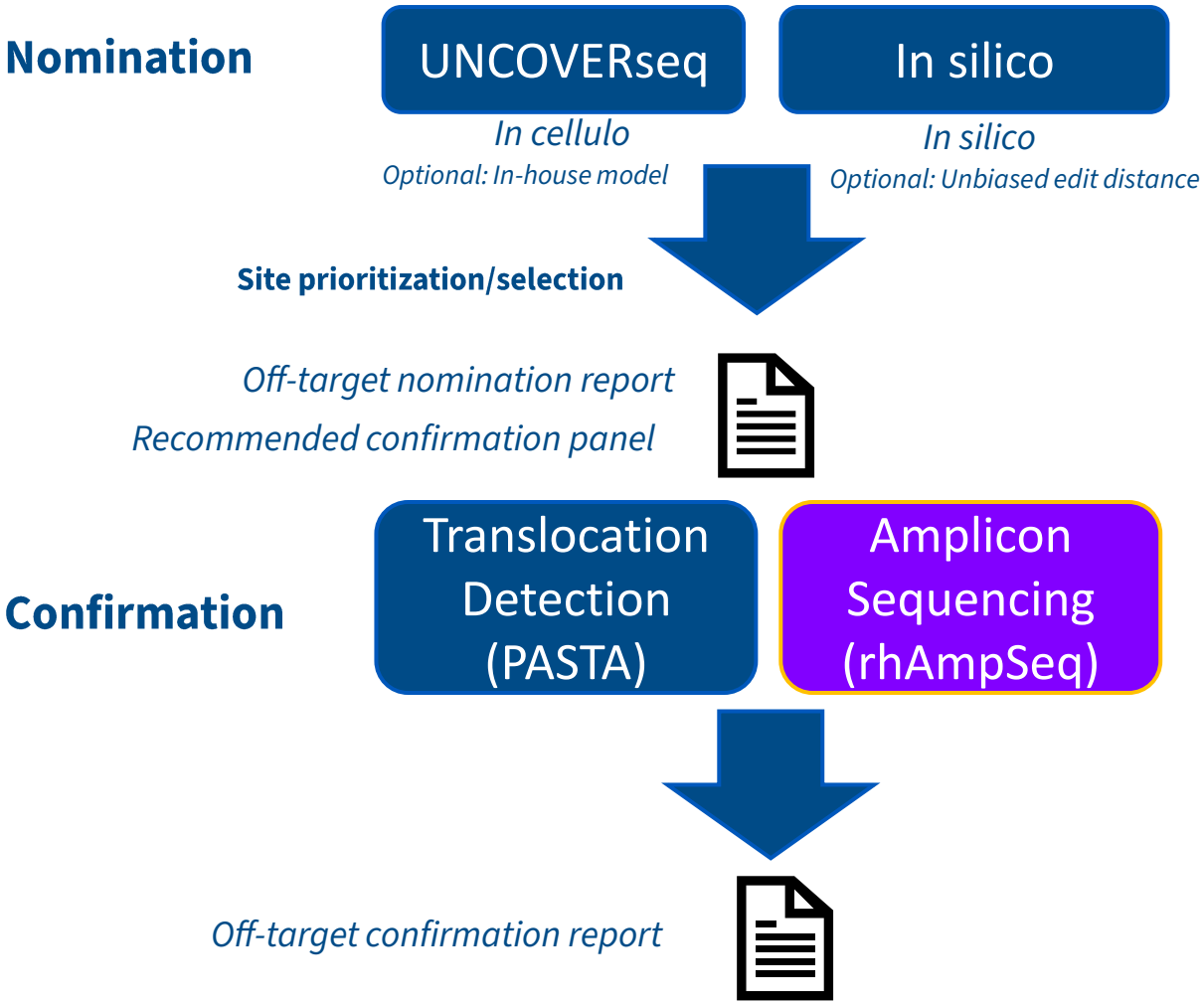
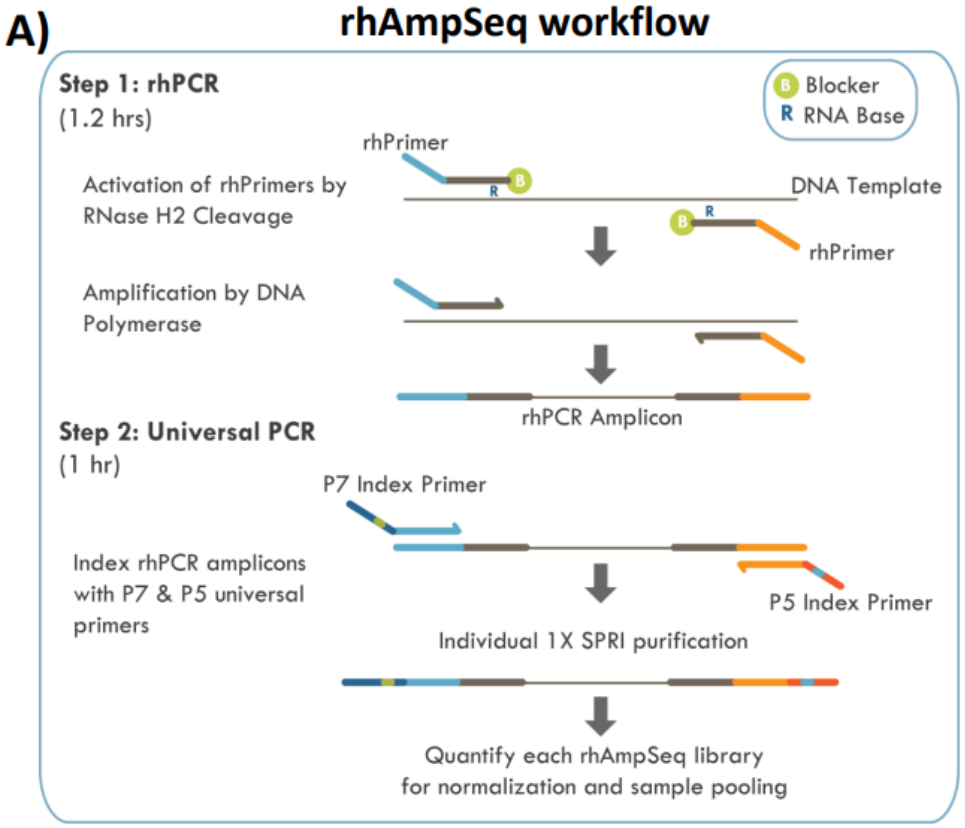
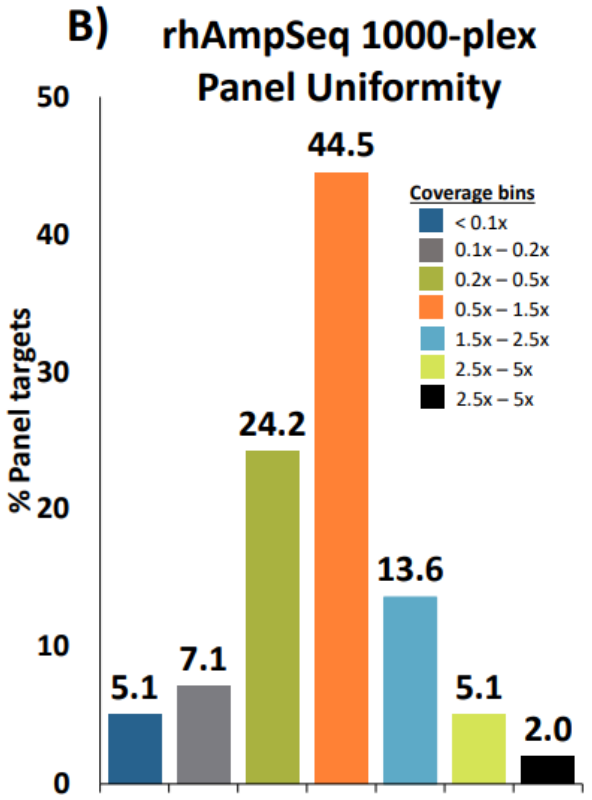


On-and-Off-Target editing quantification

rhAmpSeq™ CRISPR Analysis System



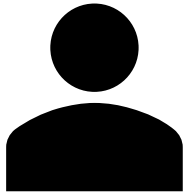


Benefits

- **Customizable:** rhAmpSeq panel built from user-provided or IDT-nominated loci
- **Technical support:** technical consultation for panel design
- **Results:** comprehensive results report
- **Performance:** high analytical specificity and sensitivity; detect translocations using rhAmpSeq data

Downstream confirmation requires rigorous analytical standards which IDT provides

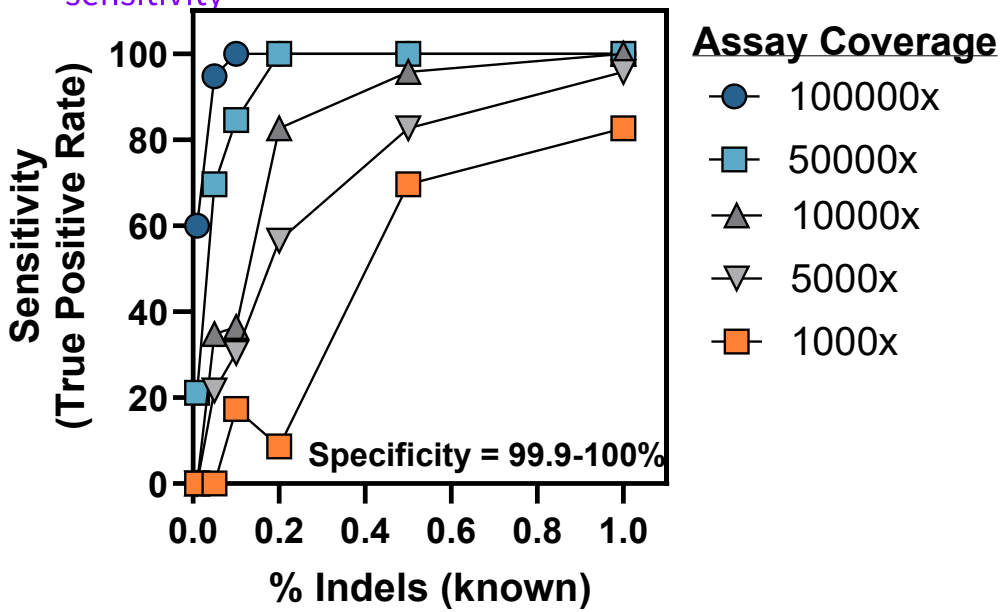
Potential response from therapeutic developer:



"My off-target confirmation showed no evidence of off-target editing"

"My method's efficacy varies based on read depth. 100% of my most at-risk sites (Tier 1 and 2) should be accurate to sub-0.2% with >95% sensitivity. 100% of sites have at least the ability to be annotated accurately to 1% editing frequencies with >95% analytical sensitivity"

Publication in preparation



Potential reviewer response:

"Great! What were the analytical bounds of what you should have been able to detect executing the protocol?"

Myth: 0.1% editing is detectable using NGS

Truth: Actual editing frequencies detectable is highly dependent on method used, DNA sequence motif variability and sequencing depth.

Tier	Coverage Depth				
	>100k	>50k	>10k	>5k	>1k
1	54.2%	100.0%	100.0%	100.0%	100.0%
2	44.4%	100.0%	100.0%	100.0%	100.0%
3	91.7%	100.0%	100.0%	100.0%	100.0%
4	53.6%	84.1%	97.8%	100.0%	100.0%
5	53.6%	74.6%	100.0%	100.0%	100.0%
6	44.4%	70.1%	91.7%	100.0%	100.0%

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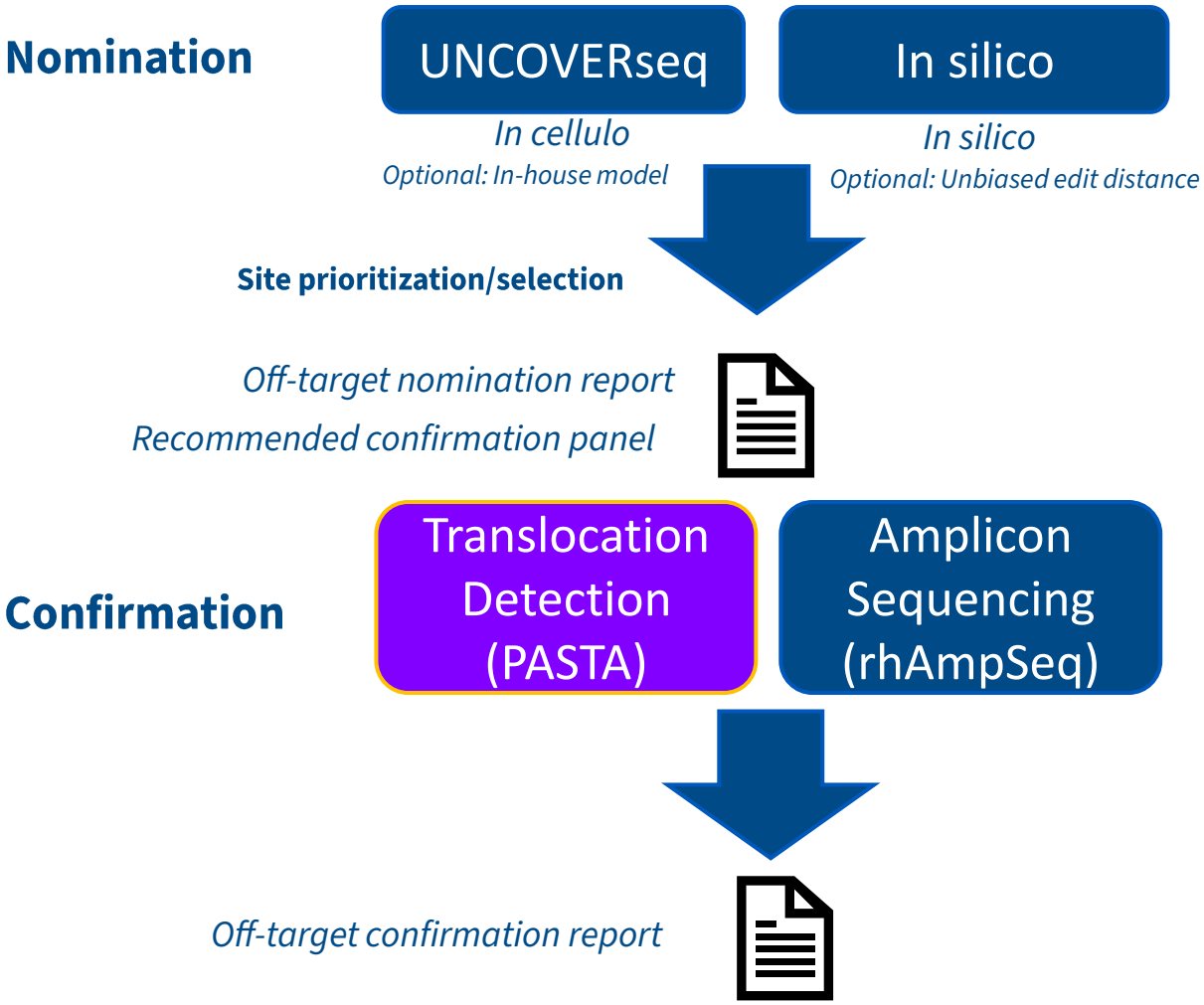
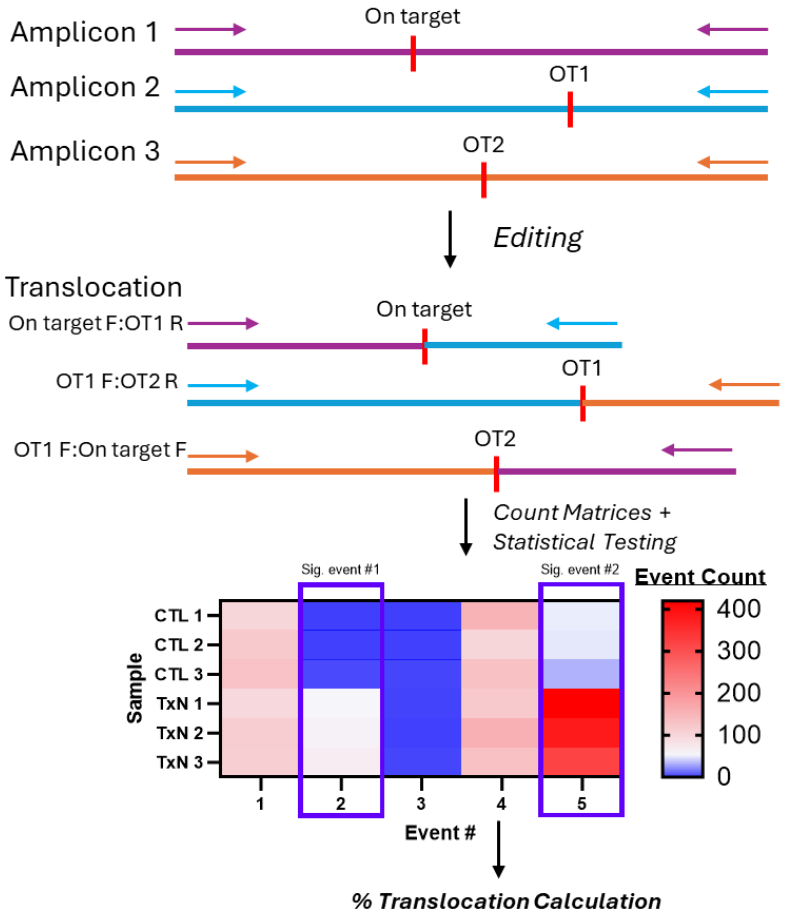


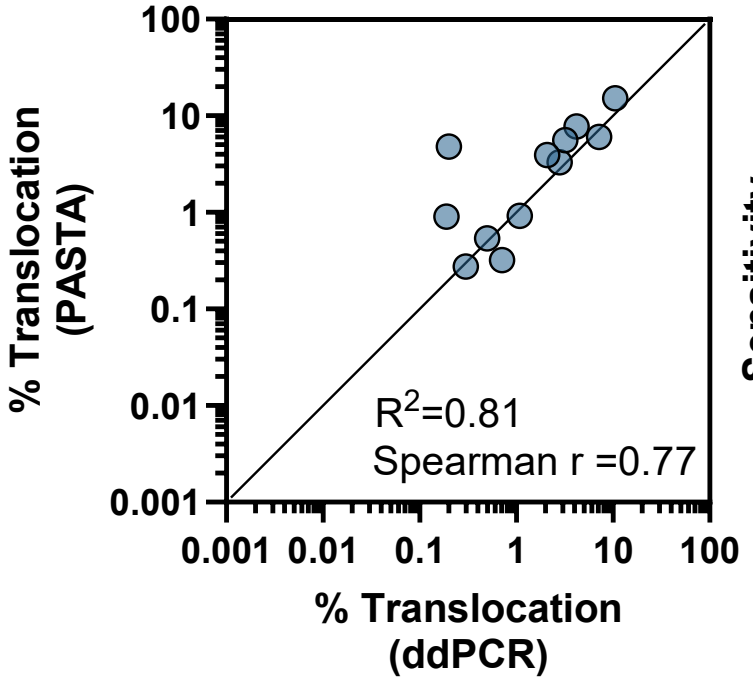
Amplicon sequencing allows sensitive detection of translocations

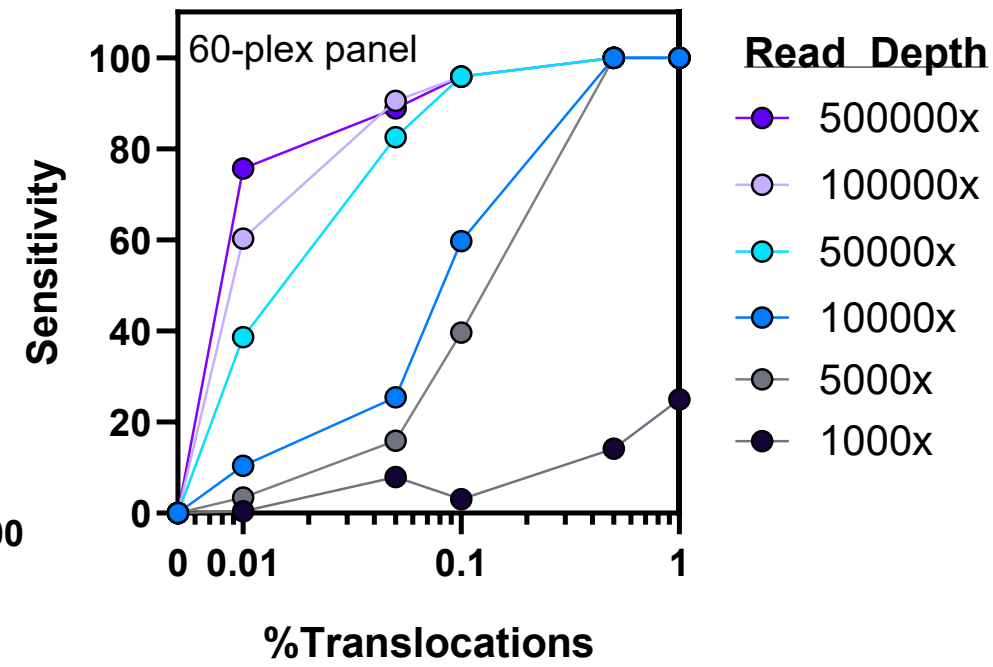
rhAmpSeq + bioinformatics pipeline (PASTA)



High-throughput translocation **detection down to 0.01% frequencies** with strong concordance to ddPCR compared to other methods

Publication in preparation





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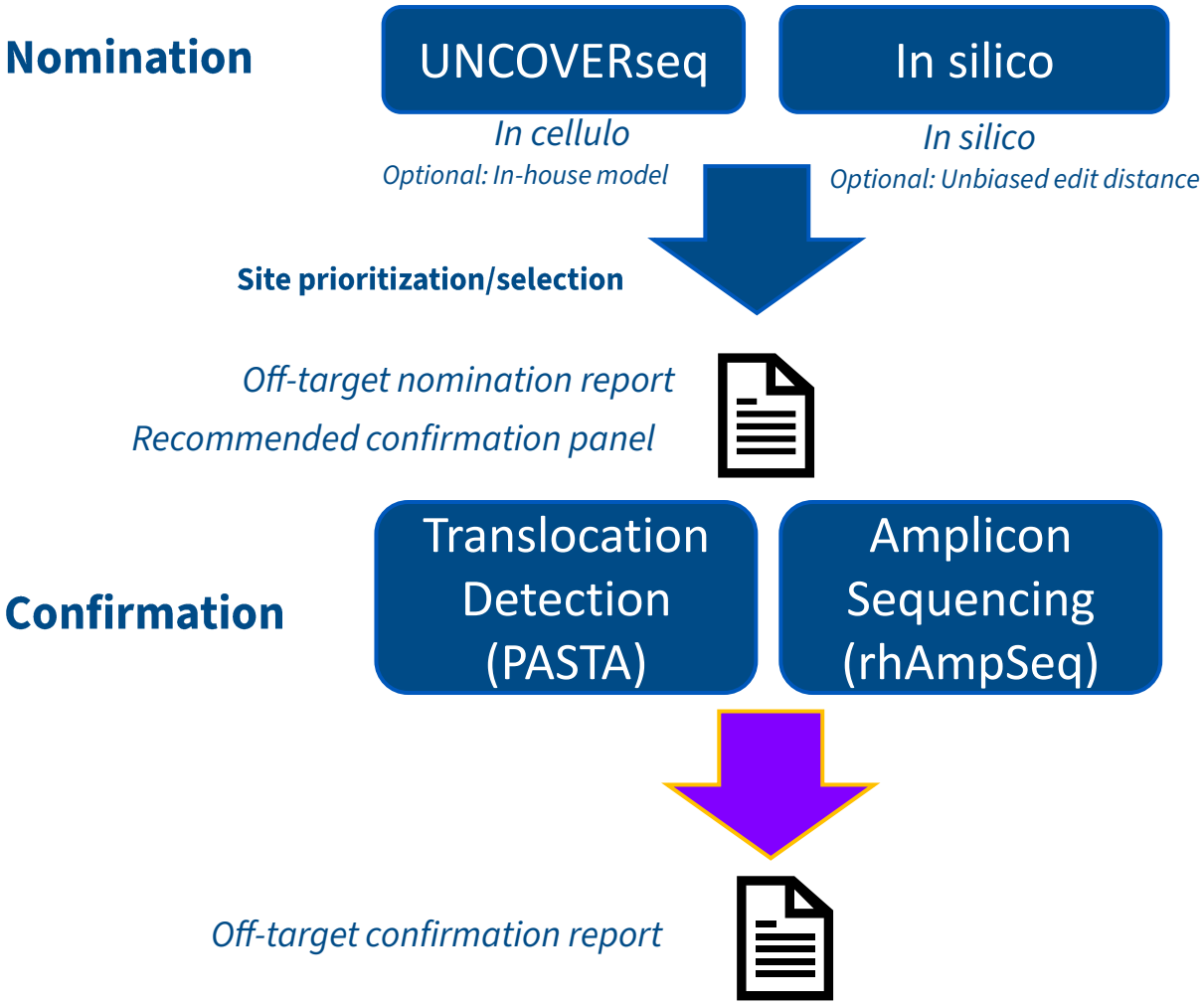
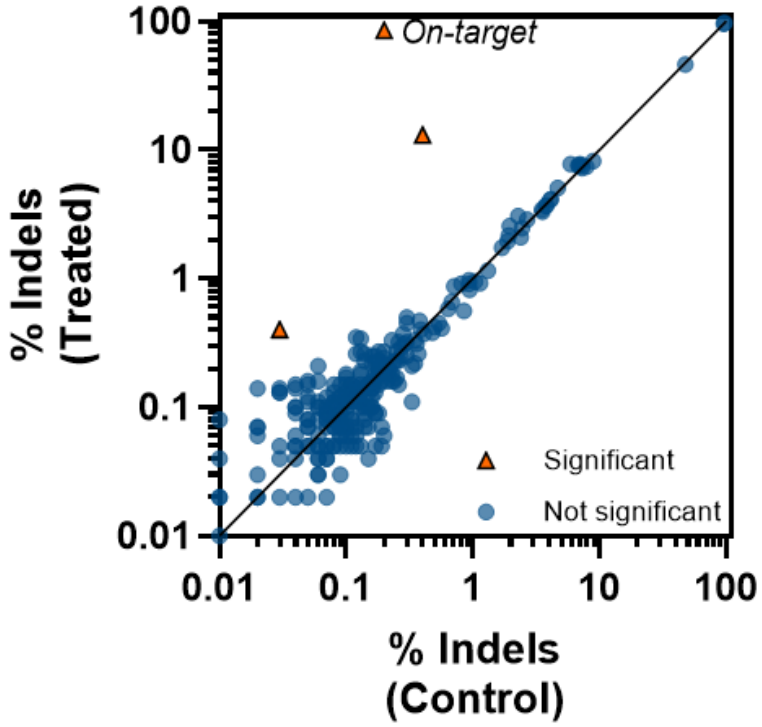


Off-target confirmation reports come with relevant information to determine editing and risk

- Confirmation and quantification of editing
  - On- and off-target
  - Donor-dependent and independent
- Gene feature annotation (shown previously)
- Variant(s) description and effect interpretation
- Cumulative off-target burden quantification
  - Small indels, SNPs (Base Editors), and SVs



Location	Avg Edit Frequency	P-value	Gene	Annotation	Tier	# Donors	Nomination Method
chr19:50490620-50490640(-)	13%	3.00E-06	EMC10	Exon	1	3	GUIDE-seq+ONE-seq
chr14:51095017-51095036(+)	0.70%	0.001	N/A	Intergenic	2a	1	GUIDE-seq+ONE-seq

Conclusions

- IDT currently offering end-to-end genotoxicity assessments for gene editing
  - Tailored for IND filings
  - Includes off-target nomination and confirmation
  - A la carte available for those that just need one or the other

- IDT services have high performance and well described analytical and process standards
  - High reproducibility and experimental set up recommendations guided by empirical data
  - Nomination
  - Described sensitivity and expected analytical range of nomination using process controls

- Confirmation
  - Sub-0.1% analytical sensitivity for confirmation with high specificity (>99%)
  - Translocation detection down to 0.1% frequencies

- Consultation end-to-end to ensure you have a strong partner going into IND filings
  - Success in IND filings using our process and personnel's expertise

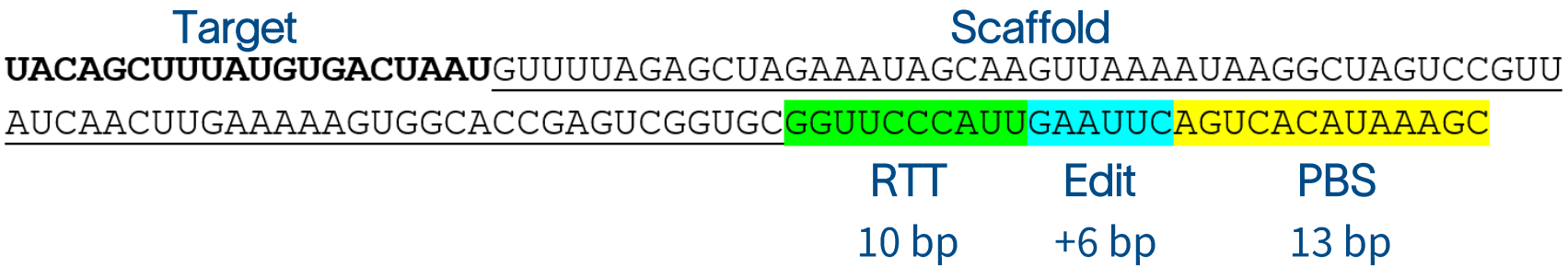
- Constantly improving methods and quality controls with FDA guidance
  - Save internal R&D FTEs for therapeutic development work, leave off-target analysis to IDT as a partner

IDT
INTEGRATED DNA TECHNOLOGIES

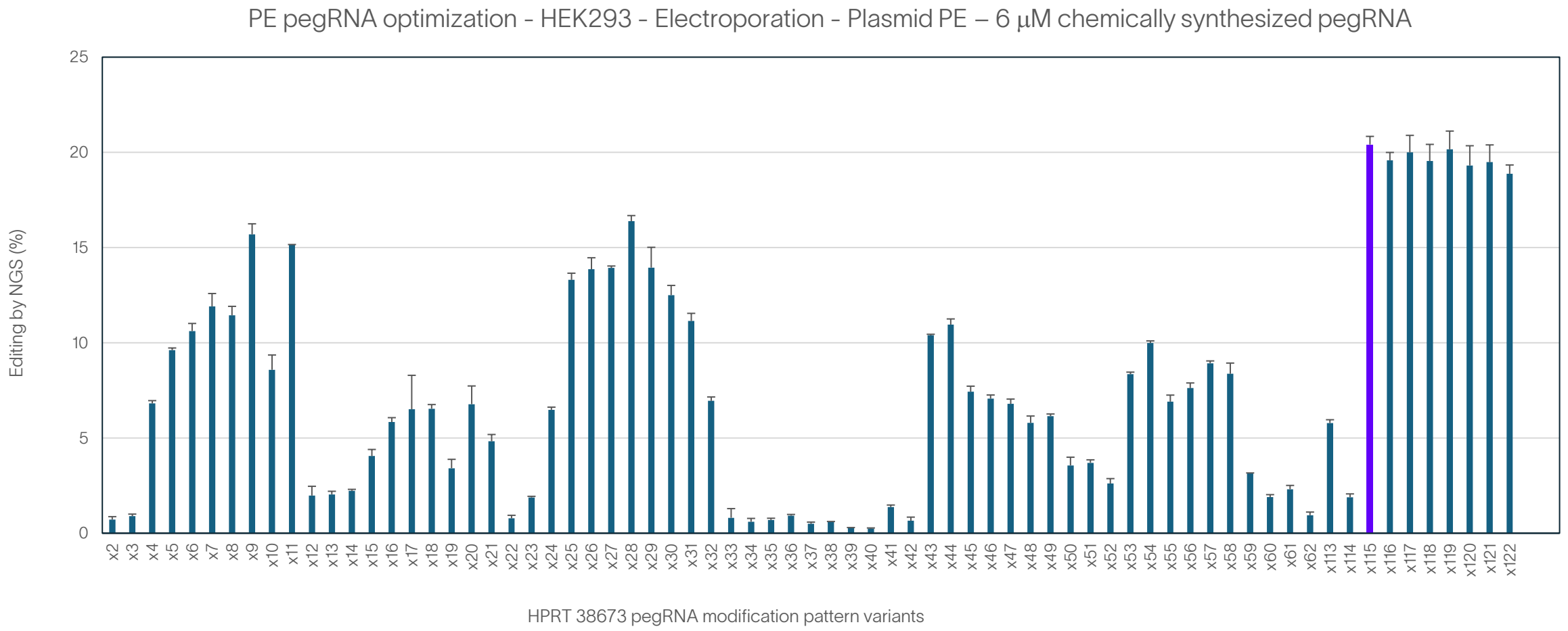
Prime Editor Innovation

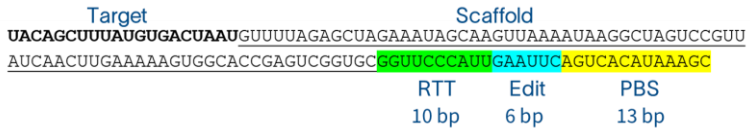
pegRNA optimization and innovations

- **Leveraging IDT's…**
  - Ability to do high-throughput synthesis long sgRNAs and pegRNAs
  - Extensive experience in chemical modifications that increase *in vivo* stability
  - Desire to test PE utility broadly and increase usability genome-wide
  - High-throughput analytical and testing capabilities for pegRNA variants
  - rhAmpSeq genome editing analysis package
- **IDT x115 pegRNA modification provides superior PE performance**
  - Tested with a systematic analysis of increased chemical modifications
  - Chemical substitution appears to universally increase editing
    - Cell lines, primary cells, etc.
    - Beneficial for all types of edits
  - Compatible with CGMP manufacturing

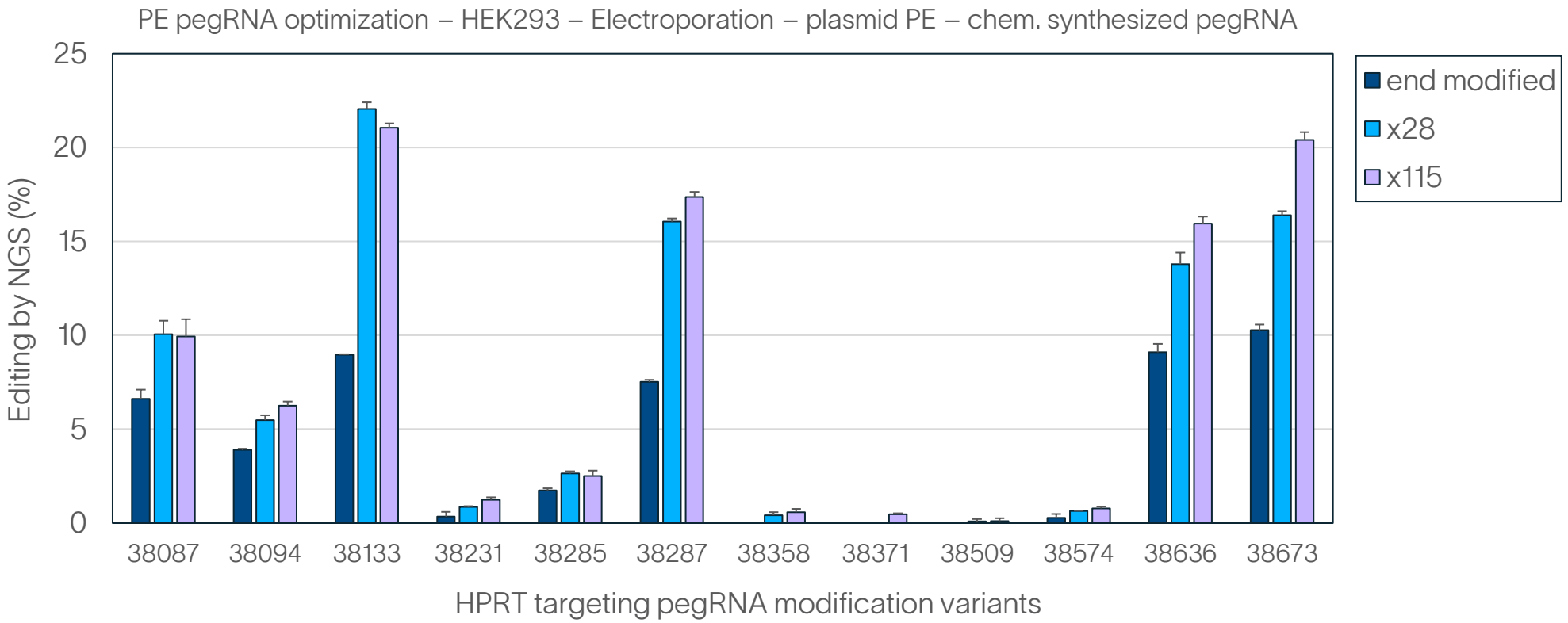


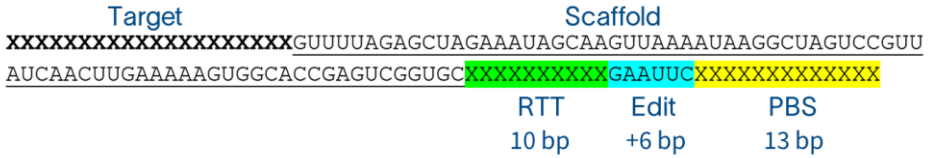
Chemically modified pegRNAs improve PE efficiency





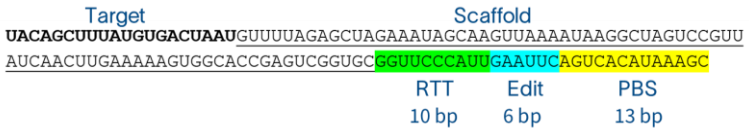
Chemically modified pegRNAs improve PE efficiency

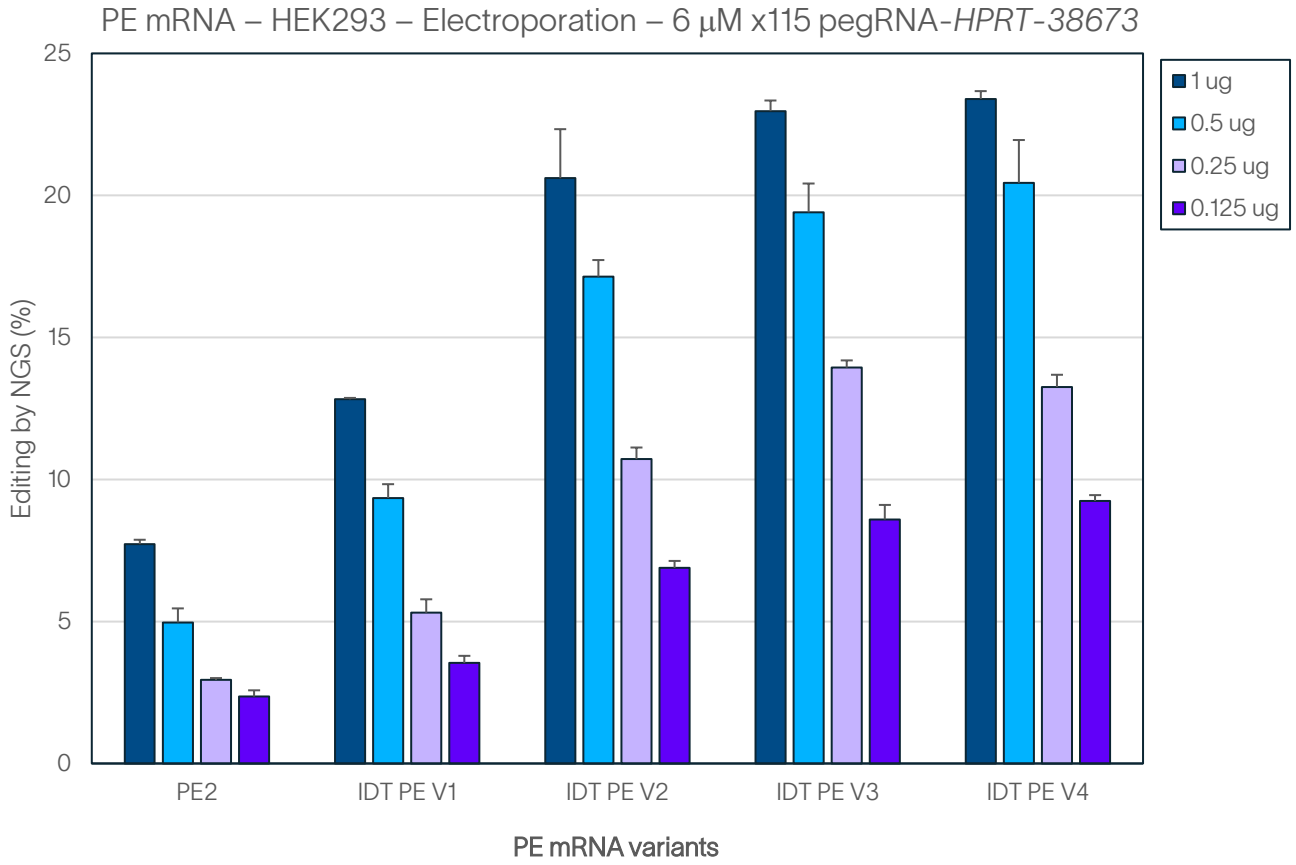




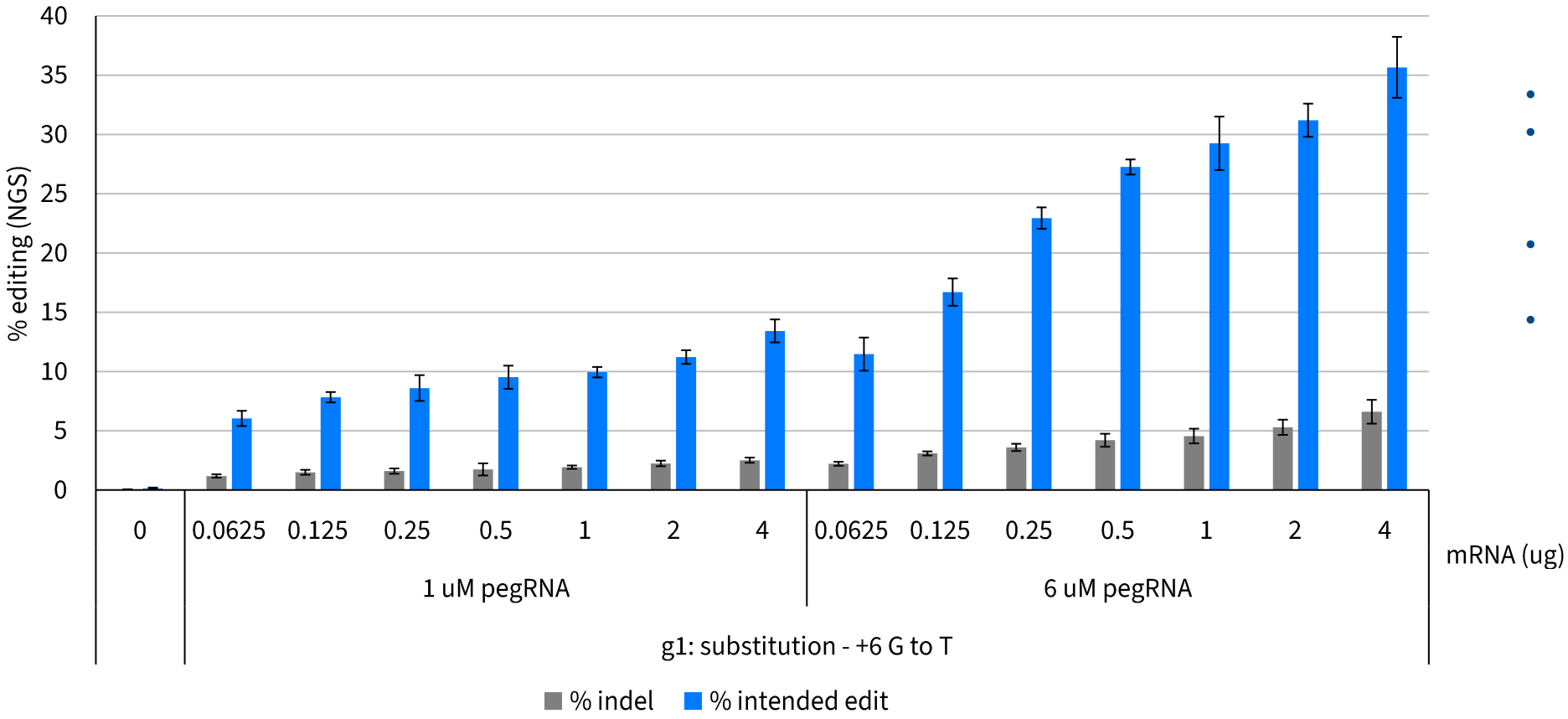
IDT Engineered PE mRNAs improve editing efficiency

- **Where we started to where we are today**
  - **PE2**
    - starting construct
  - **IDT PE V1**
    - IDT PE with IDT RTase
  - **IDT PE V2**
    - IDT PE with optimization and partial protein engineering
  - **IDT PE V3, V4, and V5**
    - IDT PE with optimization and complete protein engineering
  - **Future-state constructs**
    - mRNA expression optimization





ACAT2: Substitution +6 G to T (iPS cells)



- Dose dependent editing activity
- Boost Prime editing with higher pegRNA concentration
- Up to ~7% indel
- 0.5 ug mRNA dose data consistent with previous testing

n = 6 (3x nucleofection and replicate plating)

Thank you