NANOMEDICINES INNOVATION NETWORK
RÉSEAU D'INNOVATION NANOMÉDECINES

Recent Advancements in CRISPR Reagents

HQP Capacity-Building Webinar | 12 January 2021 | 1:00 – 2:00 pm PST

IDT
INTEGRATED DNA TECHNOLOGIES

RECENT ADVANCEMENTS IN CRISPR REAGENTS

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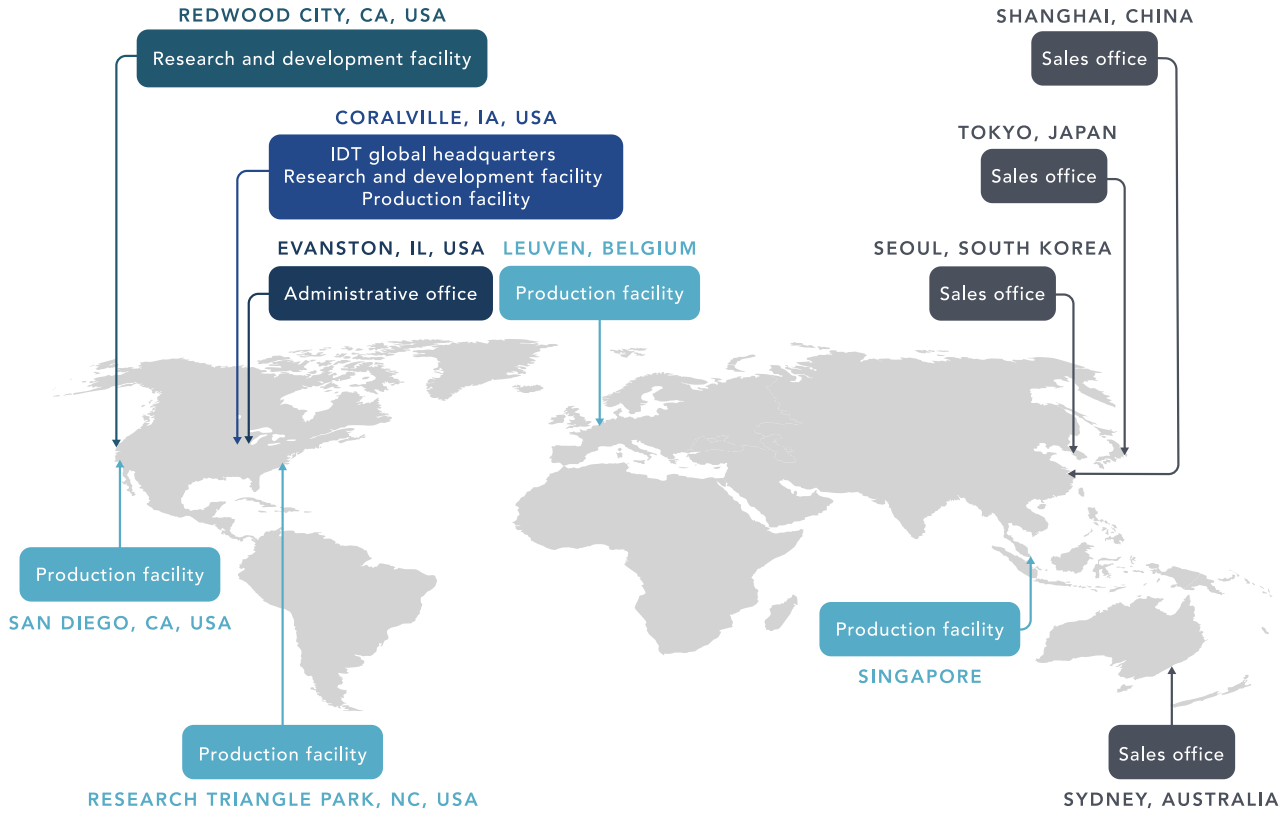
FIELD APPLICATION MANAGER – FUNCTIONAL GENOMICS

Jan. 12, 2021

INTEGRATED DNA TECHNOLOGIES

TODAY, IDT IS THE LEADING BRAND IN DNA AND RNA SYNTHESIS

- Founded in 1987 by Dr. Joseph Walder
- Largest custom oligonucleotide manufacturer worldwide
- >1500 employees in 9 locations
- >130,000 active customers
- >95% of ordered products are manufactured and shipped in less than 24 hours



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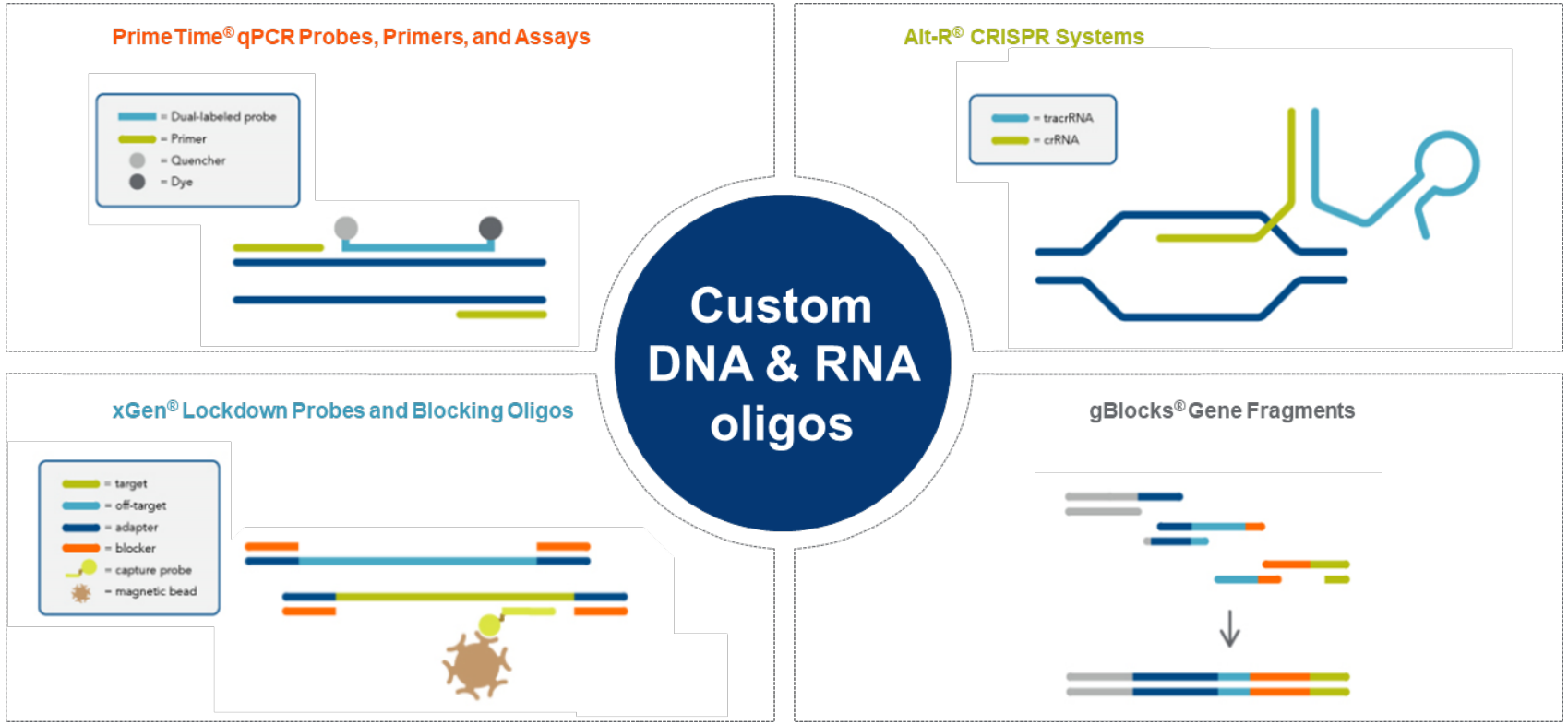
THE WORLD'S LARGEST SUPPLIER OF CUSTOM NUCLEIC ACIDS

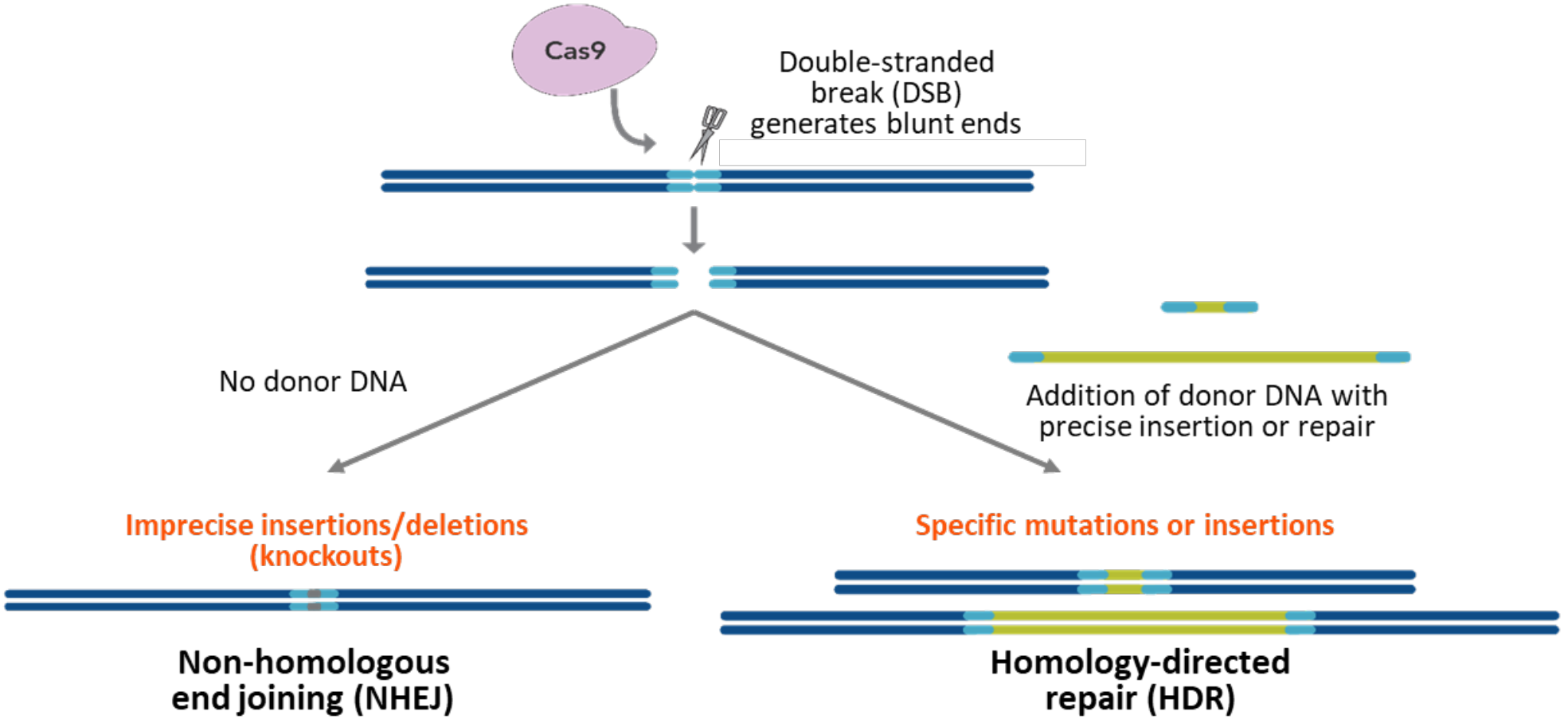
>64,000 oligos synthesized every day

- Major R&D teams (~80 employees)
- CRISPR
- NGS
- Genotyping
- qPCR
- Synthetic Biology
- RNAi
- Bioinformatics

Major Product Lines

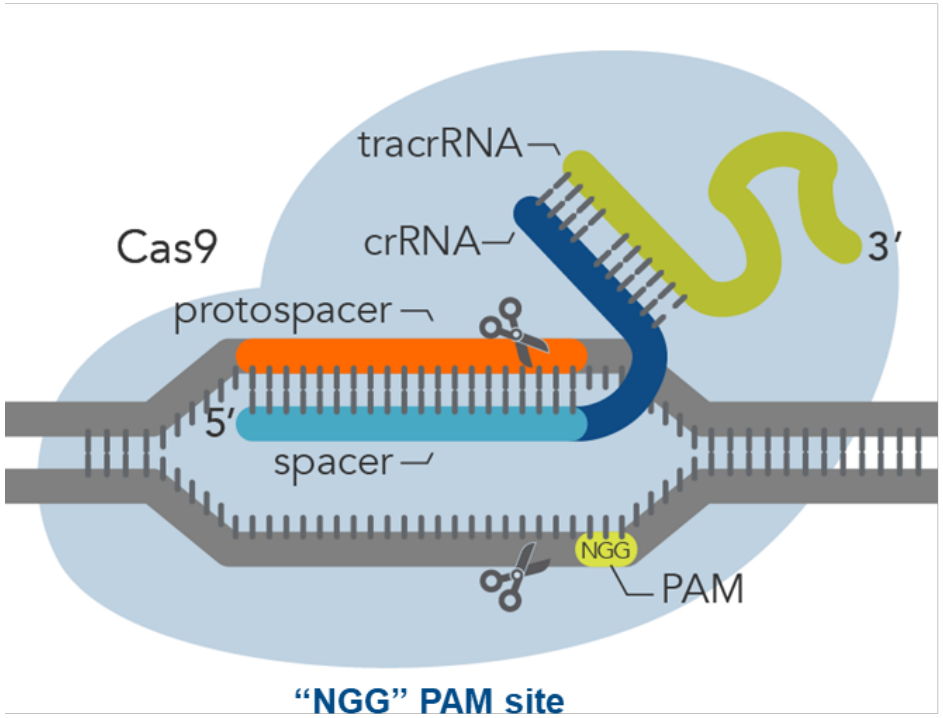


CUTTING AND EDITING



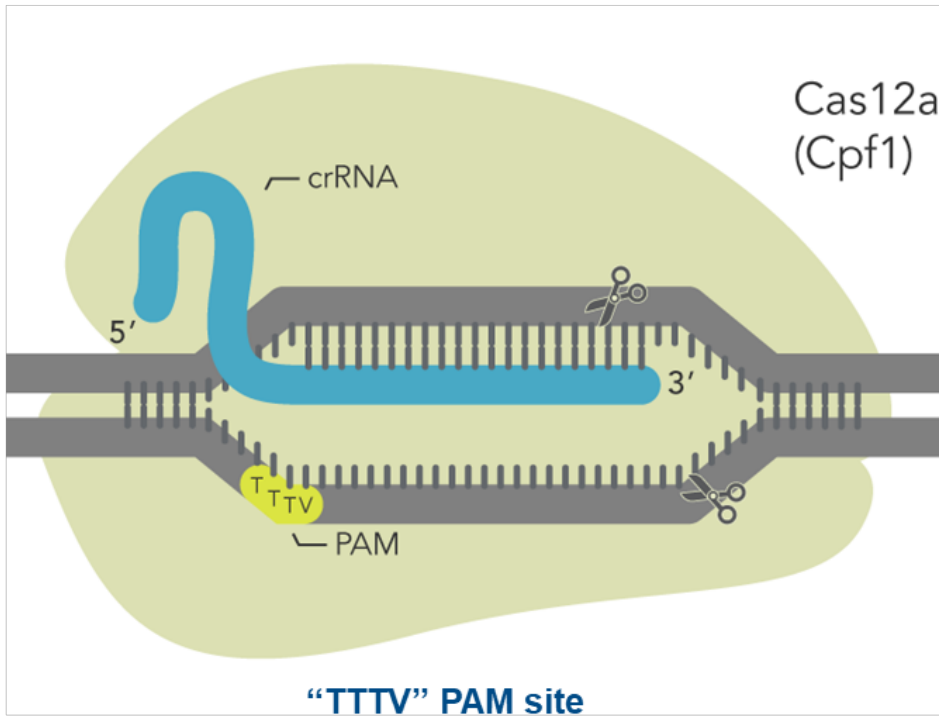
COMMON CRISPR ENZYMES

SpCas9



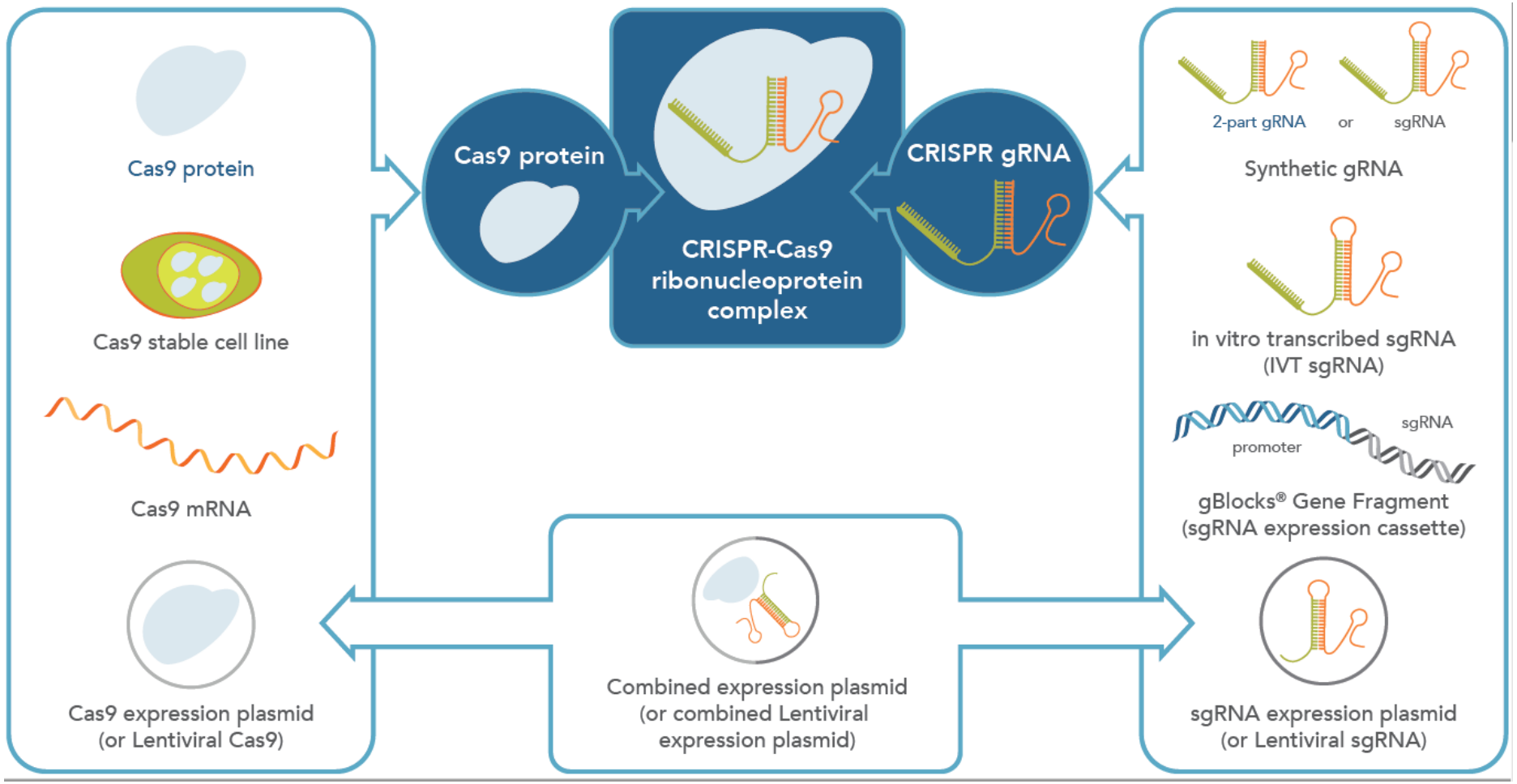
“NGG” PAM site
dsDNA cut with blunt ends
Separate crRNA + tracrRNA (annealed)
20 nt spacer

AsCas12a (Cpf1)



“TTTV” PAM site
5-base staggered cuts
Single, short crRNA
21–24 nt spacer

MANY WAYS TO DELIVER CRISPR INTO CELLS



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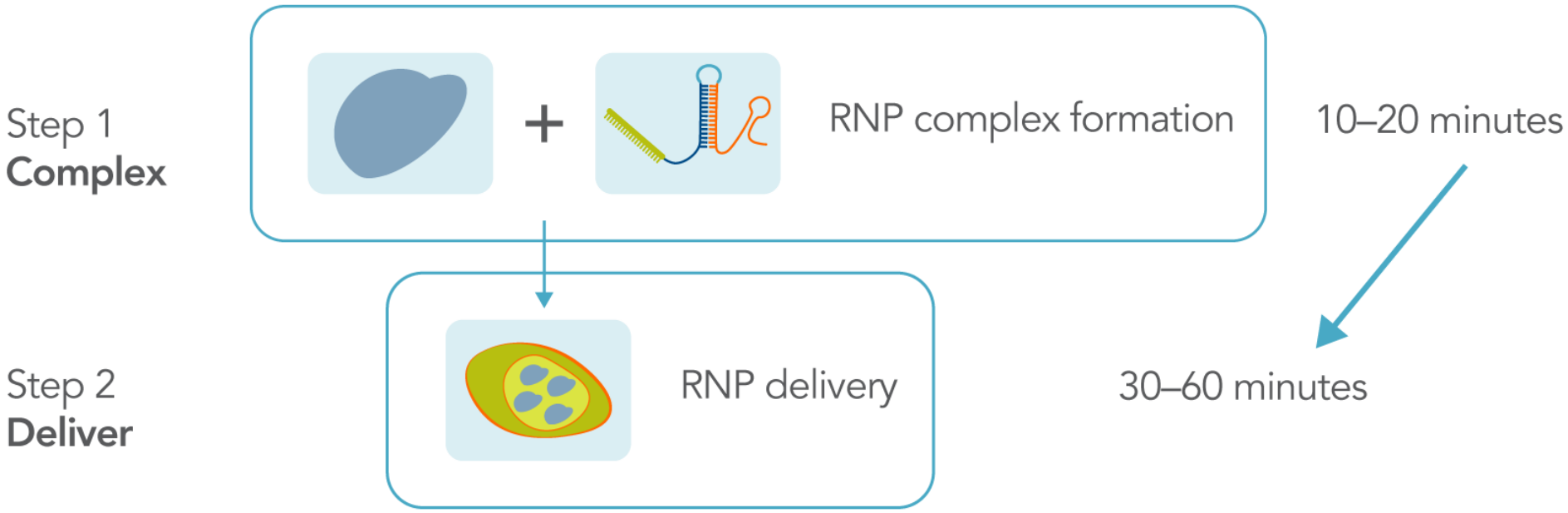
RNP DELIVERY



Alt-R™ CRISPR SYSTEM: A COMPLETE WORKFLOW

Design

Alt-R Cas9 gRNA design tool
- Predesigned guides
- Custom designs
- Design checking

Alt-R Cas9 HDR design tool
- Friendly UI
- Empirically defined design rules
- Integration with Cas9 gRNA designs

rhAmpSeq design tool

CRISPR-Cas reagents

Alt-R gRNAs
- Cas9 crRNA:tracrRNA
- Fluorescently labeled tracrRNAs
- Cas9 sgRNA
- Cas12a crRNA
- Custom ordering for any gRNA (e.g. pegRNA, Cas13)

Alt-R CRISPR proteins
- WT Cas9
- HiFi Cas9
- Cas9 nickases
- A.s. Cas12a *Ultra*
- L.b. Cas12a *Ultra*
- Fluorescently labeled Cas9

Alt-R Electroporation Enhancers

HDR reagents

Alt-R HDR Donor Oligos
- Up to 200 nt
- Modified ssODNs

Megamer ssDNA Fragments
- Up to 2000 nt
- Sequence-verified via NGS

Linear dsDNA Fragments
- Modified to reduce blunt integration
- Up to 3000 nt
- Sequence-verified via NGS

Alt-R HDR Enhancer
- V2 coming soon!

Analyze

rhAmpSeq system for CRISPR
- Multiplexed amplicon sequencing

CRISPAltRations NGS analysis tool
- Cloud-hosted UI for analysis of CRISPR on- and off-target editing

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Alt-R GUIDE RNAs

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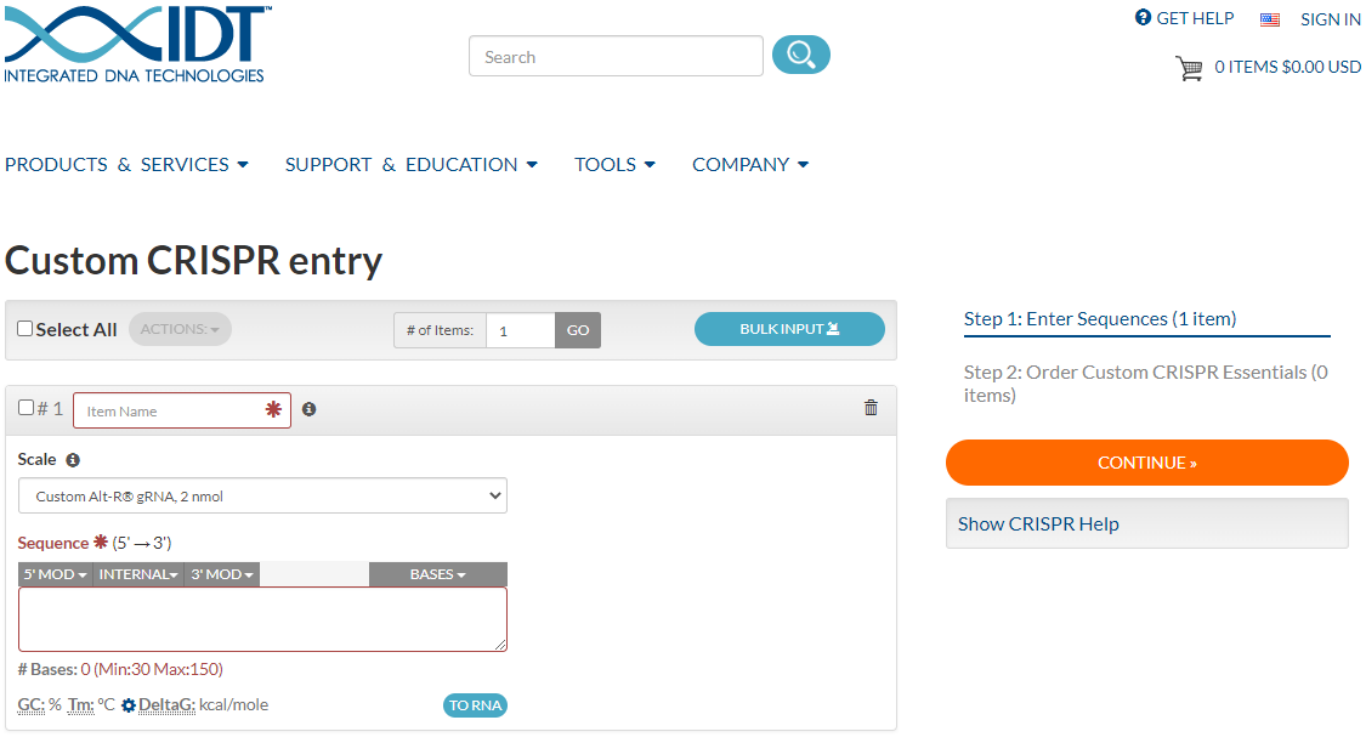
Alt-R gRNA Options for *S.p.* Cas9 & *A.s.* Cas12a

Guide RNAs	Cas9 guide RNAs			Cas12a guide RNAs
Structure	Alt-R 2-part 	Alt-R 2-part XT 	Alt-R sgRNA 	Alt-R Cas12a crRNA 
gRNA format	Alt-R CRISPR-Cas9 crRNA & tracrRNA	Alt-R CRISPR-Cas9 crRNA XT & tracrRNA	Alt-R CRISPR-Cas9 sgRNA	Alt-R CRISPR-Cas12a crRNA
Components	crRNA tracrRNA	crRNA XT tracrRNA	sgRNA	crRNA
Size (nt)	36 67	36 67	100	40–44 (41 nt recommended)
Annealing required	Yes	Yes	No	No
Stability	++	+++	++++	+++
Applications	• Cas9-expressing cells • RNP in most cell types	• Co-delivery with Cas9 plasmid/Cas9 mRNA • RNP under diffcult expermental condiitions (e.g., high nuclease environments)		• KO/KI, RNP in most cell types • Cas12a-expressing cells

IDT™ INTEGRATED DNA TECHNOLOGIES

NOW AVAILABLE!
CUSTOM ORDERING TOOL FOR ANY gRNAs



- Online ordering of any custom gRNA: including Cas13, pegRNA, gRNAs for novel nuclease systems
- Lengths: 30 to 150 nt
- Scales: 2, 10, 50, & 100 nmols
- Supports: RNA bases, Alt-R modifications, 2'OMe bases, and phosphorothioate linkages

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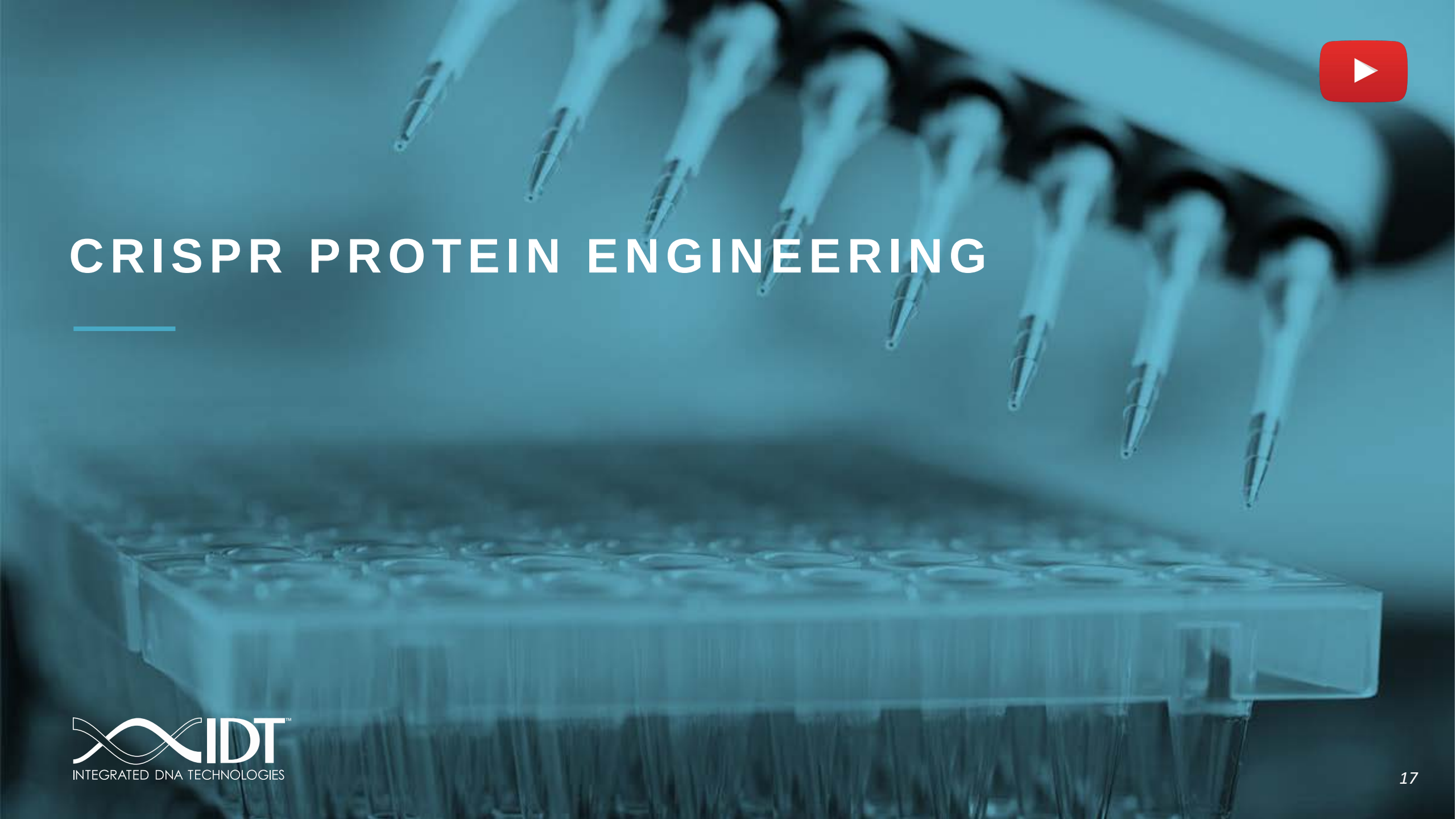

CRISPR PROTEIN ENGINEERING

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IDT CRISPR PROTEIN ENGINEERING

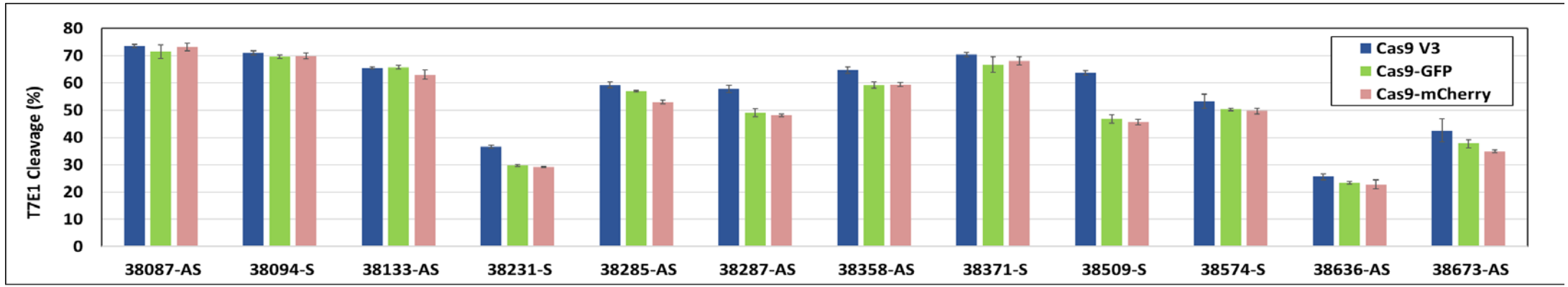
- Cas9 vs. Cas12a (Cpf1)
- Alt-R WT Cas9 vs. Alt-R HiFi Cas9
  - Problem with Cas9: off-target editing
  - Bacterial mutagenesis to evolve high-fidelity Cas9
- Alt-R WT Cas12a (Cpf1) vs. Alt-R Cas12a (Cpf1) *Ultra*
  - Problem with Cpf1: low on-target editing
  - Bacterial mutagenesis to evolve enhanced activity *A.s.* Cas12a
  - Actively working on developing mutants of *L.b.* Cas12a with increased activity

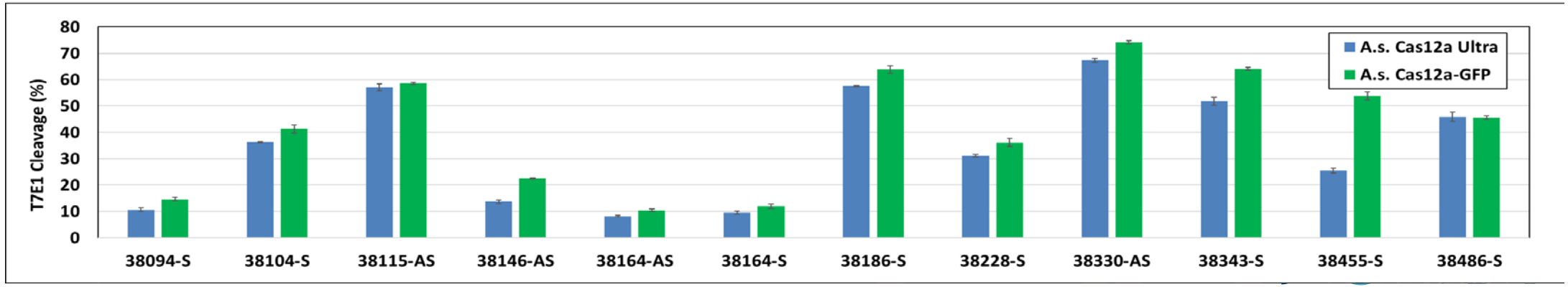
- Fluorescent CRISPR fusion proteins: coming soon
  - Alt-R Cas9-GFP
  - Alt-R Cas9-mCherry
  - Alt-R *A.s.* Cas12a *Ultra*-GFP

IDT-exclusive mutants

IDT FLUORESCENT CRISPR PROTEINS RETAIN HIGH ON-TARGET ACTIVITY

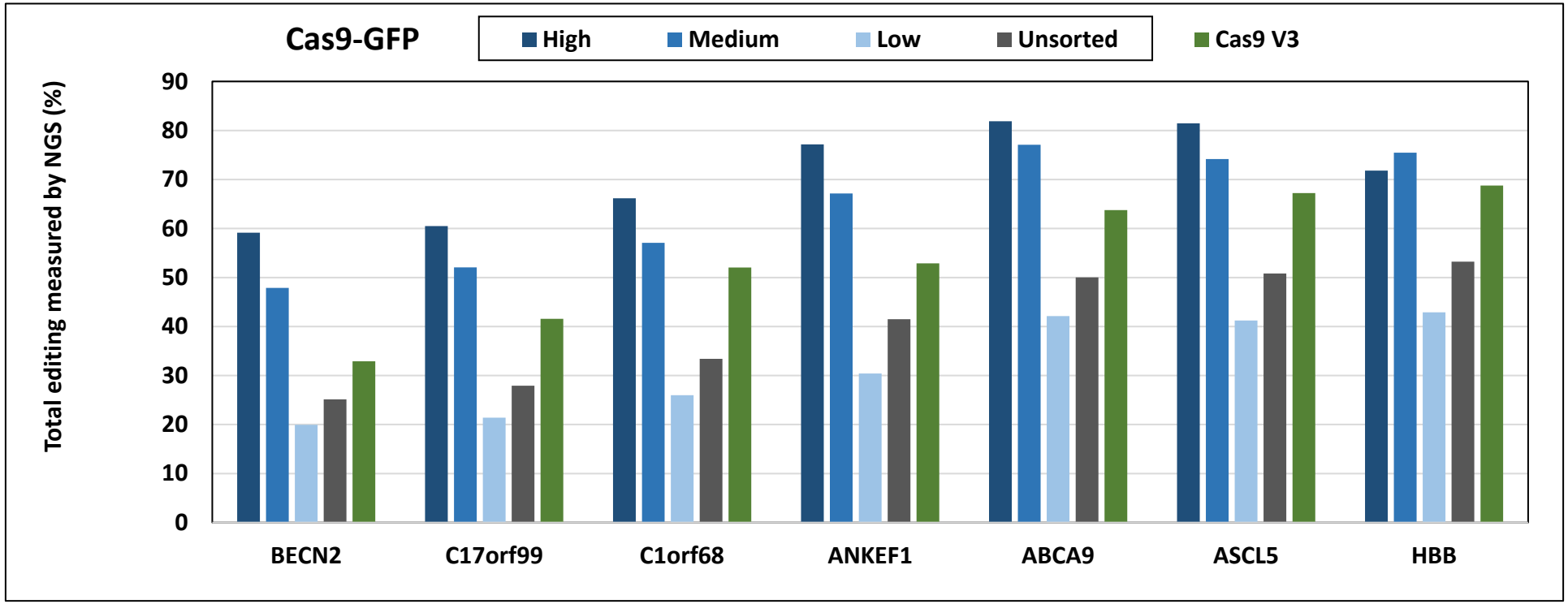
- *S.p.* Cas9 (Cas9 V3, Cas9-GFP, or Cas9-mCherry) was delivered at 2.0 µM RNP into HEK293 cells by Nucleofector™ Nucleofection (Lonza) targeting sites within the HPRT gene
- *A.s.* Cas12a RNP (Cas12a *Ultra* or Cas12a-GFP) was delivered at a suboptimal dose of 50 nM RNP into HEK293 cells by nucleofection to achieve a range of editing across the tested HPRT sites





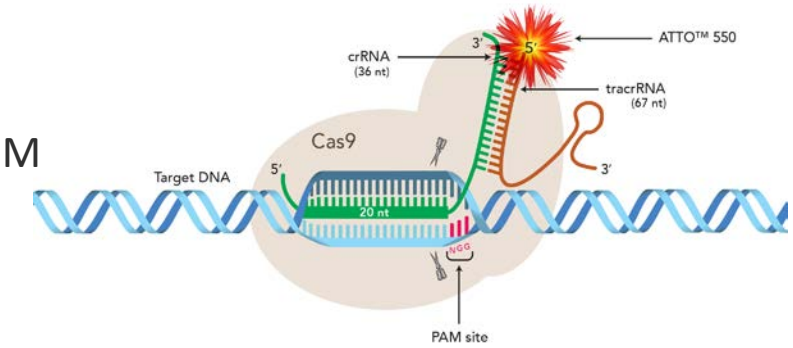
ENRICHMENT OF EDITED CELLS BY FACS USING Cas9-GFP

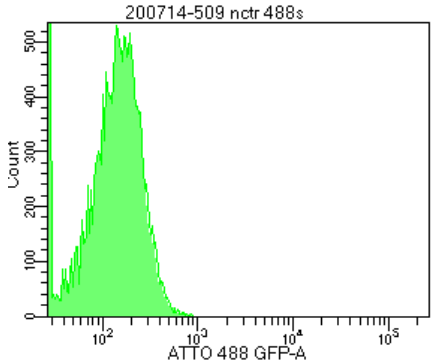
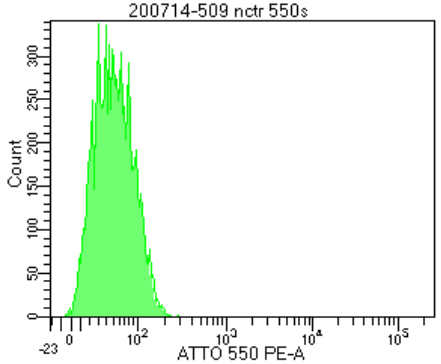
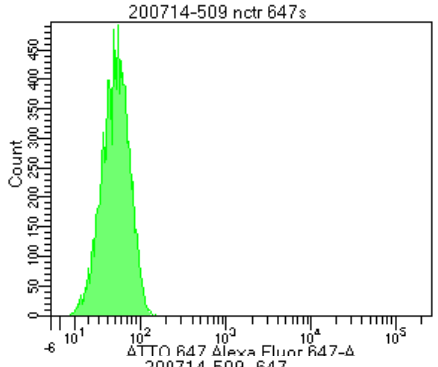
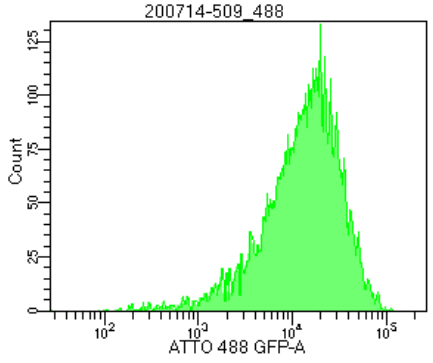
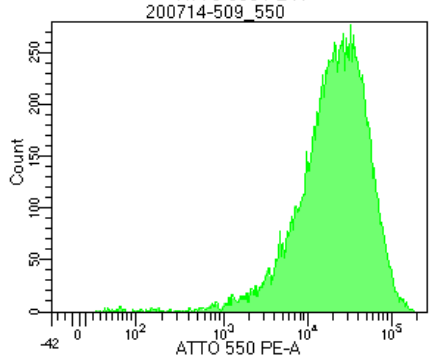
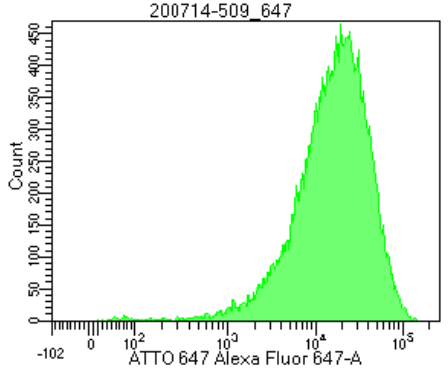
- Alt-R™ Cas9 V3 or Cas9-GFP was delivered by lipofection (10 nM RNP) into HEK293 cells
- After ~18 hours, cells were sorted into three populations. High: Top 20%, Medium 80–60%, Low: Bottom 60% of cells based on GFP signal



FLUORESCENTLY-LABELED tracrRNA

- Labeled tracrRNA was duplexed with Alt-R™ crRNA XTs
- crRNA:tracrRNA duplex complexed with Alt-R™ Cas9 V3
- RNP was delivered into cells using Lipofectamine™ RNAiMAX (Thermo Fisher) at 10 nM
- HEK293 cells were incubated for ~18 h at 37°C.
- Cells were washed twice with PBS and run on a flow cytometer



	ATTO488	ATTO550	ATTO647
Cas9 V3 + Unlabeled tracrRNA			
Cas9 V3 + Labeled tracrRNA			

IDT SOLUTIONS TO IMPROVE HOMOLOGY-DIRECTED REPAIR

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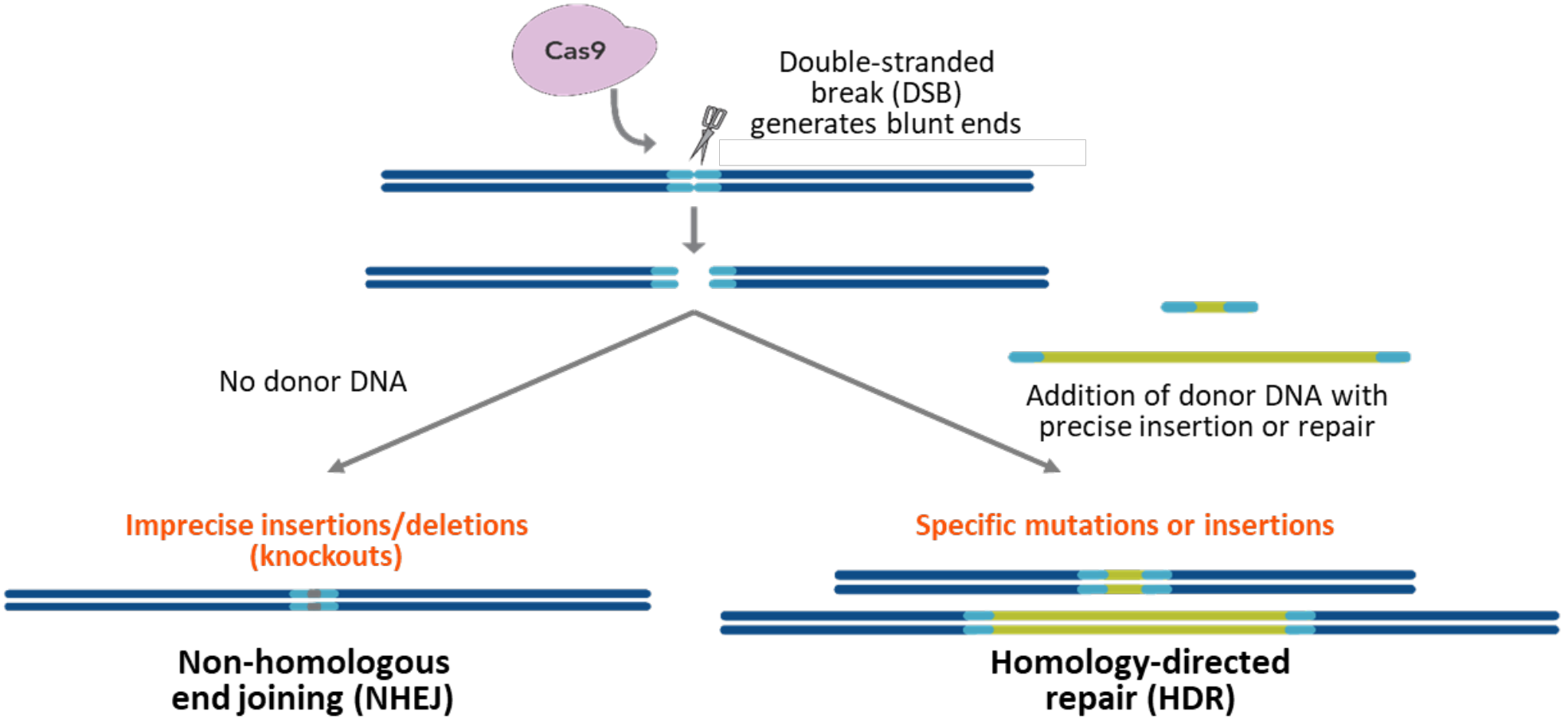
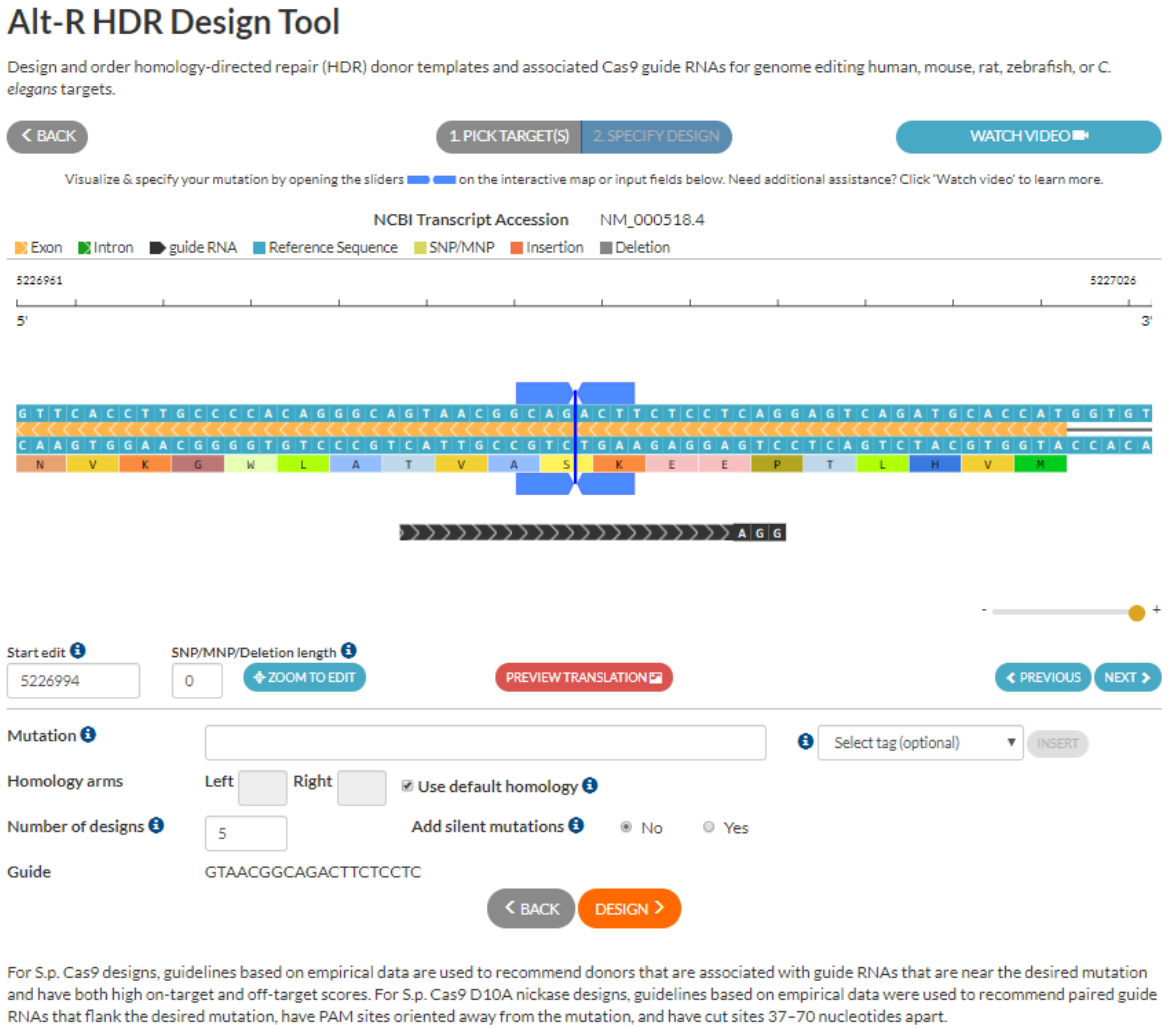
EDITING OUTCOMES



USE THE Alt-R HDR DESIGN TOOL FOR OPTIMAL DESIGN

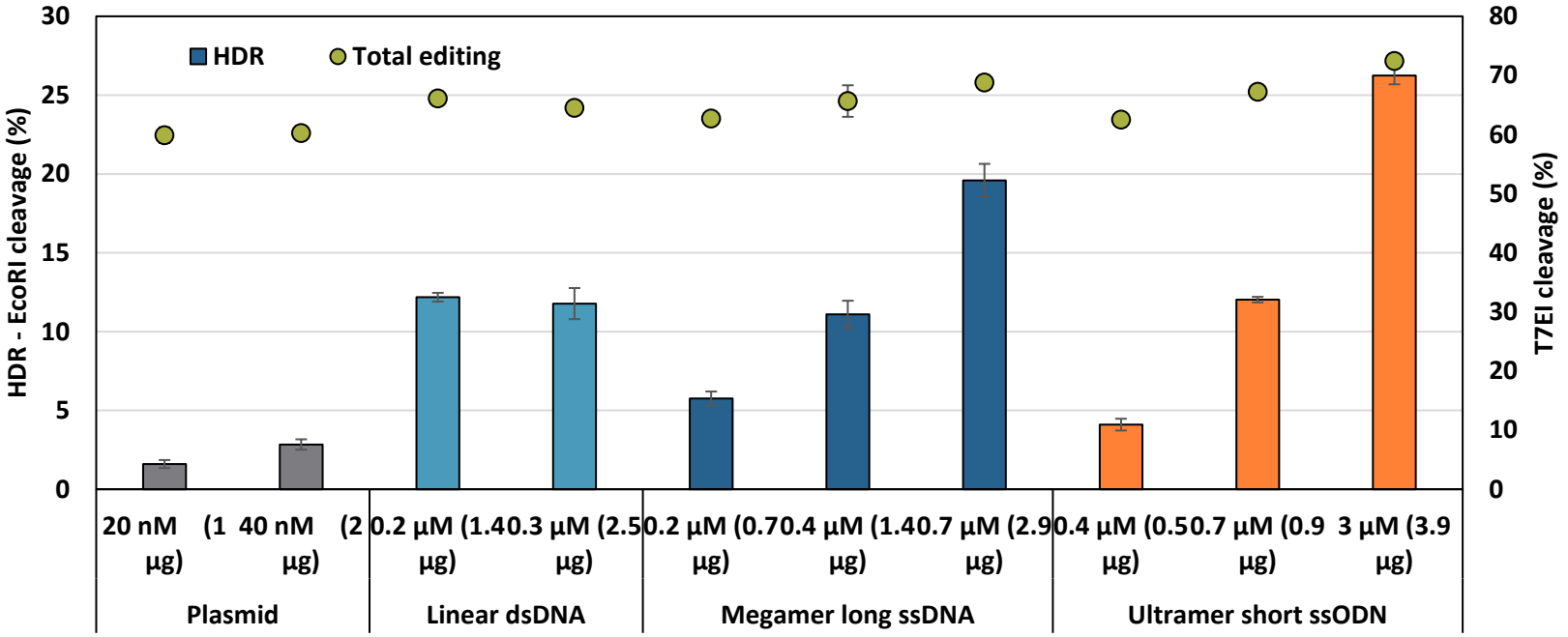


Alt-R HDR Design tool: a novel bioinformatics tool for ssDNA HDR template design

- Human, mouse, rat, zebrafish, nematode, or custom input
- gRNA selection using IDT Alt-R gRNA design tool
- Addition of silent mutations improves rates of HDR
- Supports WT and Cas9 Nickase (D10A) strategies
- Single or multiple (batch) design
- Alt-R modified donors available in output

www.idtdna.com/HDRdesigntool

DONOR TEMPLATE OPTIONS

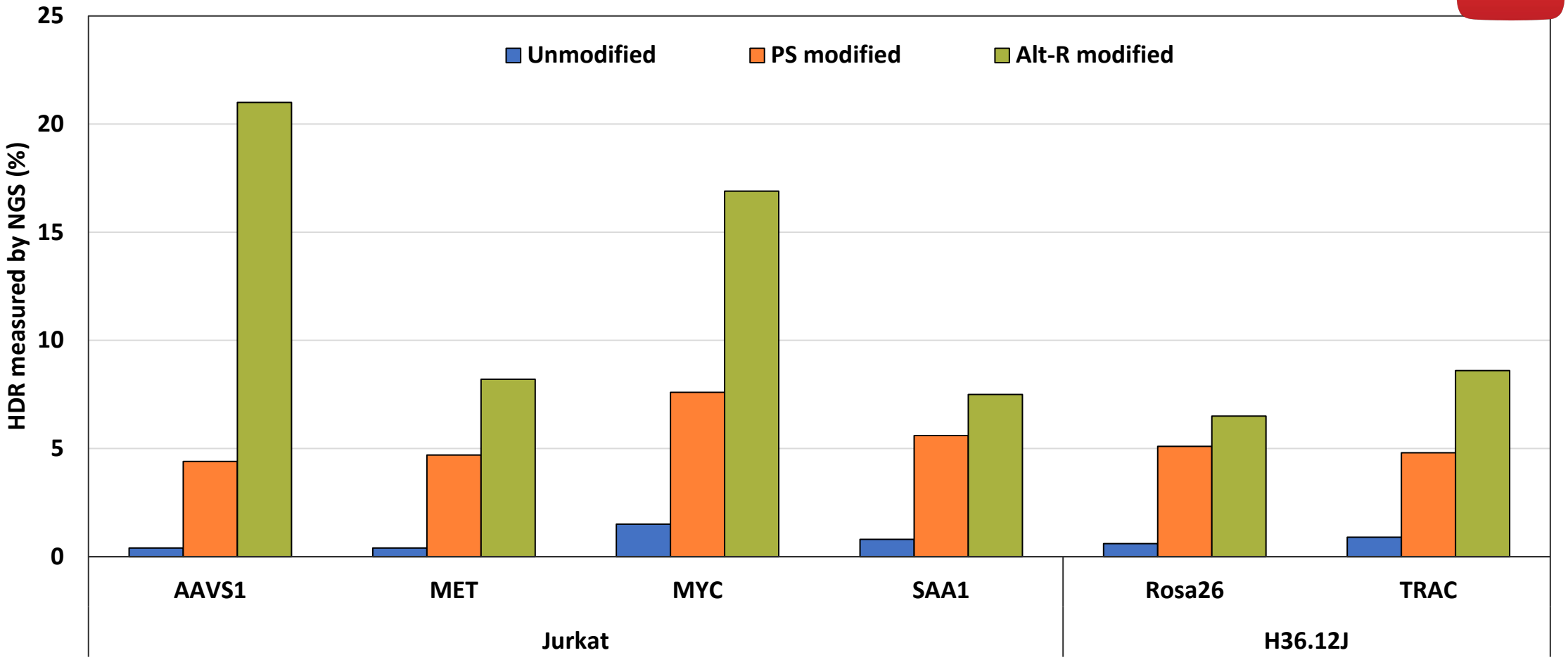


- Blunt insertion by NHEJ observed for the linear dsDNA donor, but not for ssDNA templates
- Doses titrated up until toxicity was observed.

Amaxa nucleofection
Jurkat cells – SERPINC1 locus – 2 µM Alt-R Cas9 RNP, 2 µM Alt-R Cas9 Electroporation Enhancer

Donor type for long HDR	Product	Size (nt or bp)	Toxicity	HDR efficiency
ssODN	Ultramer	45-200	Low	++++
ssDNA	Megamer	201-2000	Low-Med	+++
dsDNA (linear)	gBlock	125-3000	High	++
dsDNA (plasmid)	Gene	125-3000	Very high	+

ssDNA DONOR TEMPLATES



Unmodified	ATAATCAGGTTCTGTTCCATAAACTCTGGATTGCATTCCT**gaattc**ACATGGAAATGCCTCTGGAGTGTATTCTCACAGAAAAGAG
PS modified	A*T*AATCAGGTTCTGTTCCATAAACTCTGGATTGCATTCCT**gaattc**ACATGGAAATGCCTCTGGAGTGTATTCTCACAGAAAAG*A*G
Alt-R modified	/Alt-R/A*T*AATCAGGTTCTGTTCCATAAACTCTGGATTGCATTCCT**gaattc**ACATGGAAATGCCTCTGGAGTGTATTCTCACAGAAAAG*A*G/Alt-R/

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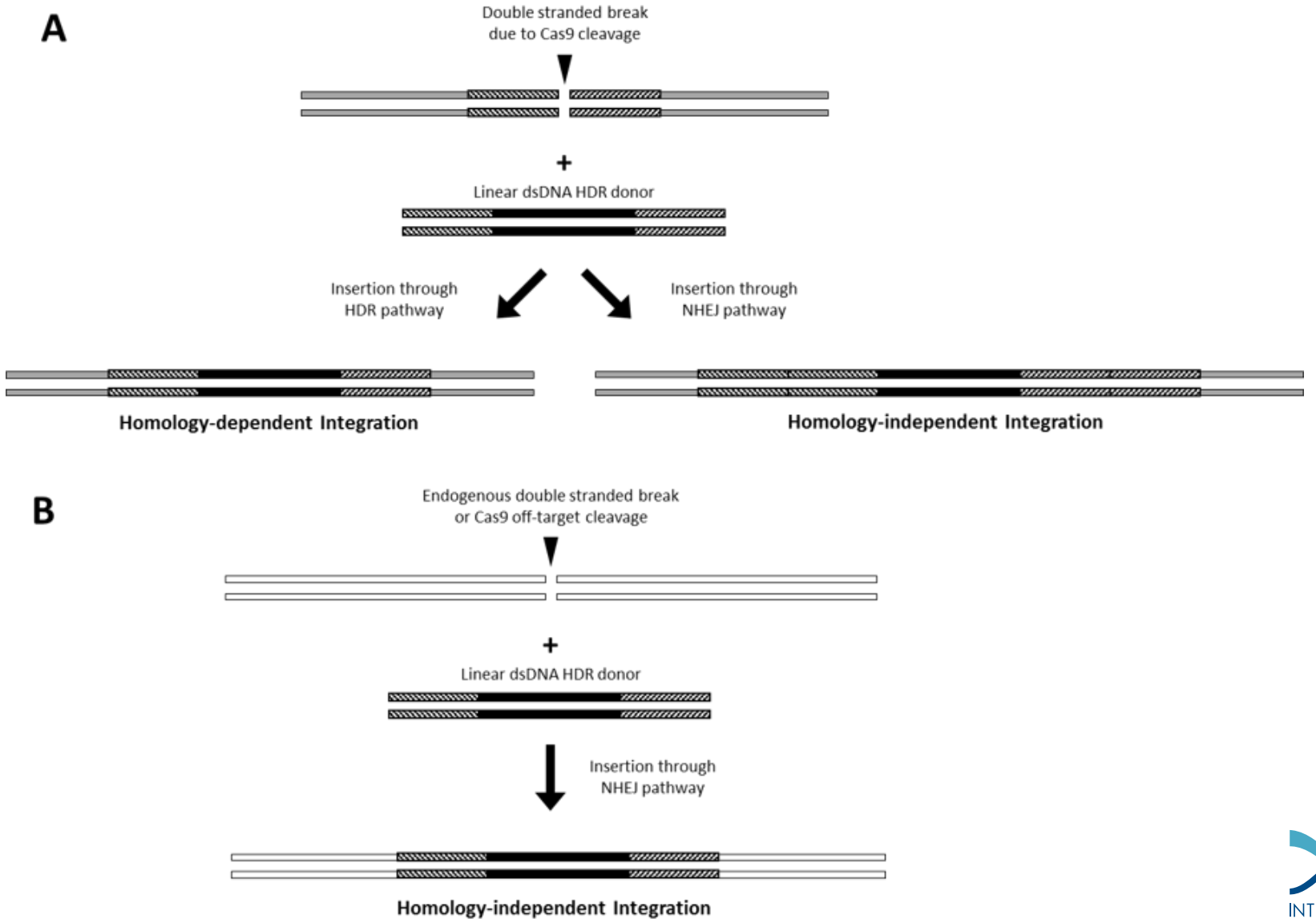
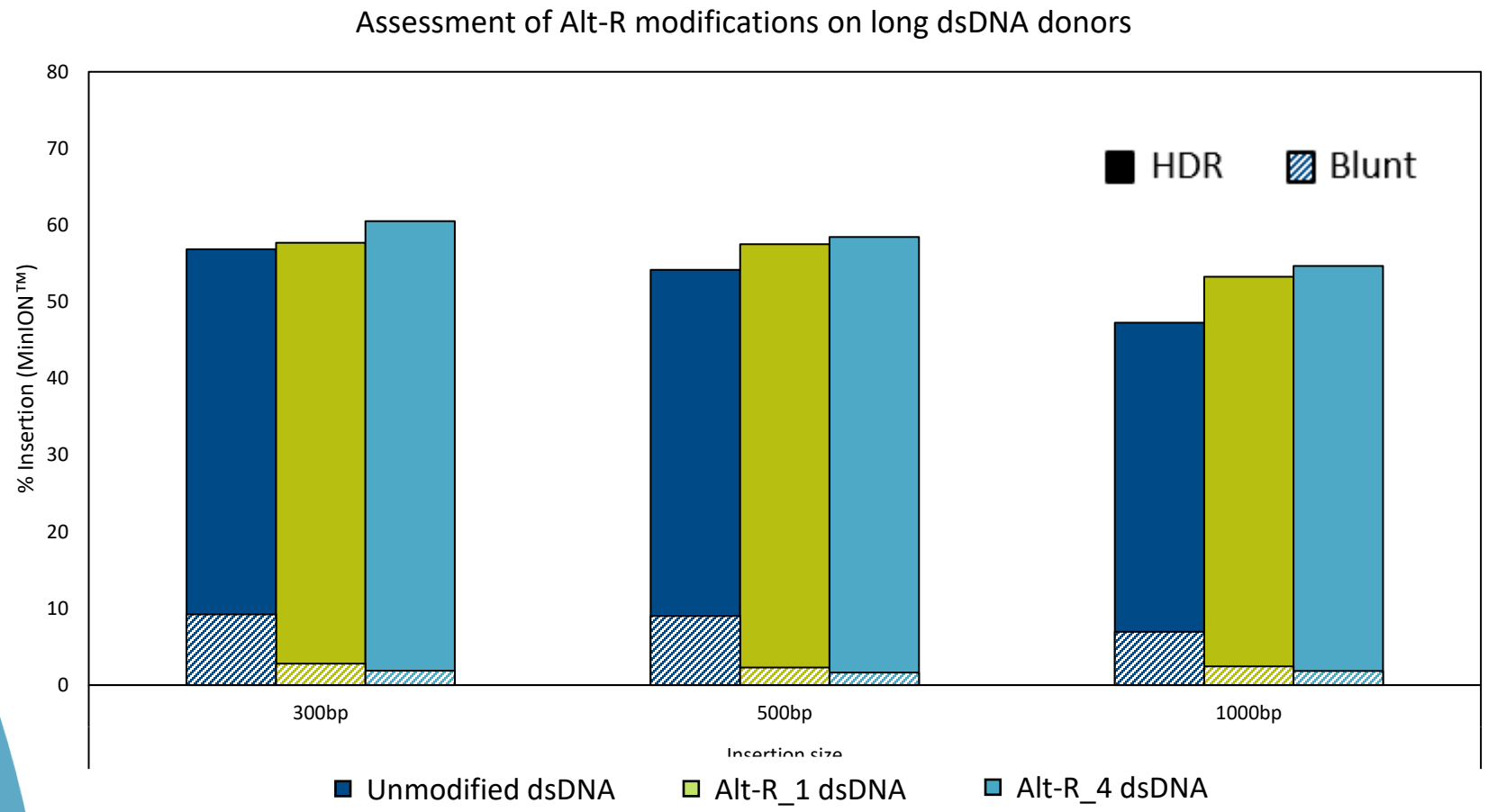
SOLUTIONS TO REDUCE BLUNT INTEGRATION WITH dsDNA HDR TEMPLATES



Alt-R MODIFIED dsDNA DONORS IMPROVE HDR RATES WHILE REDUCING BLUNT INTEGRATION EVENTS FOR LARGE INSERTIONS



Alt-R modified dsDNA donors mediating a 300 bp, 500 bp, or 1 kb insertion at the SerpinC1 locus (with symmetrical 100 bp homology arms).

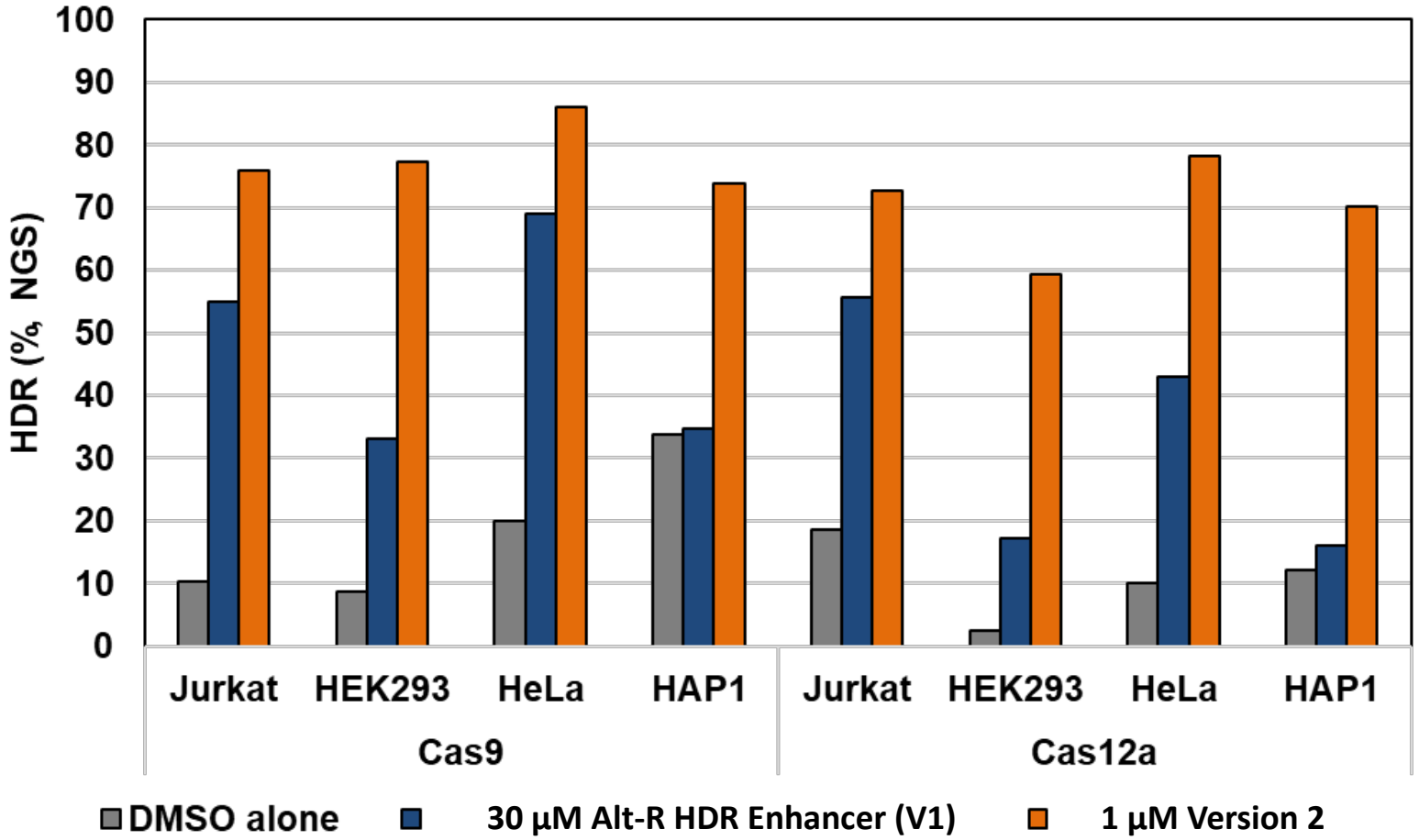
Donors delivered into HEK293 cells at 100 nM with 2 µM Cas9 RNP.

Cells were treated with 1 µM Alt-R HDR Enhancer V2 for 24 hrs.

Editing outcomes assessed by NGS: Targeted amplification long-read MinION™ sequencing (Oxford Nanopore Technologies).

Analyzed via an internal data analysis pipeline.

NEW: HDR ENHANCER V2 FURTHER IMPROVES HDR RATES



rhAmpSeq FOR EDITING QUANTIFICATION

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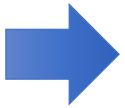
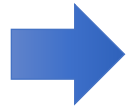
EXAMPLE CRISPR EXPERIMENT WORKFLOW

Off-target Identification (Nomination)
(stable Cas9, modified gRNAs)



rhAmpSeq design, amplification, and sequencing
(multiple conditions, high-throughput)



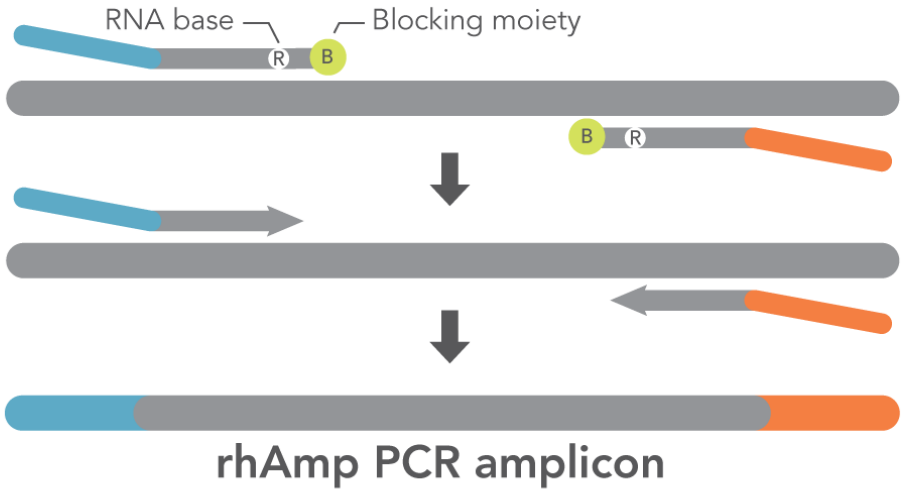
Data analysis pipeline
(CRISPAltRations)

rhAmpSeq WORKFLOW

Targeted rhAmp PCR 1

Activation of rhAmp primers by RNase H2 cleavage

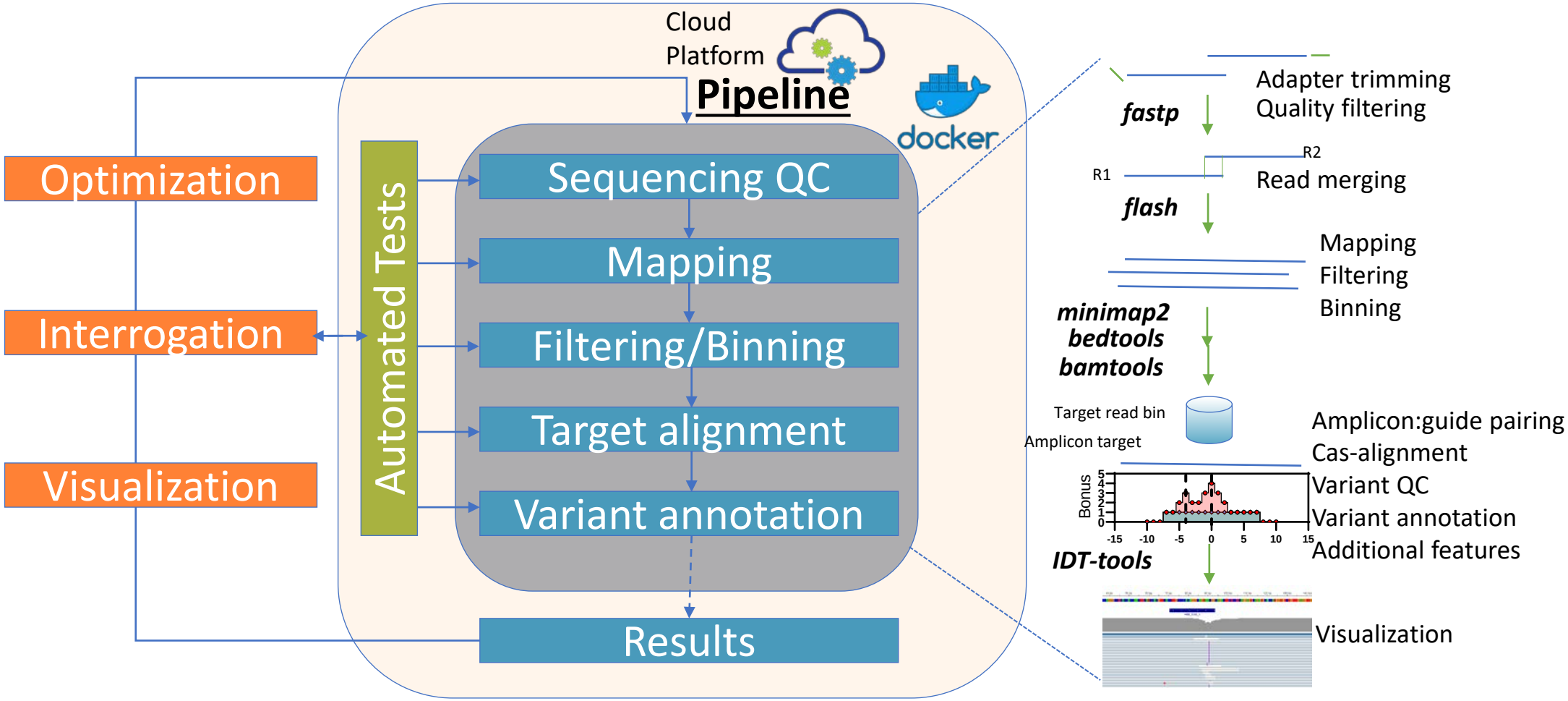
Amplification



Indexing PCR 2

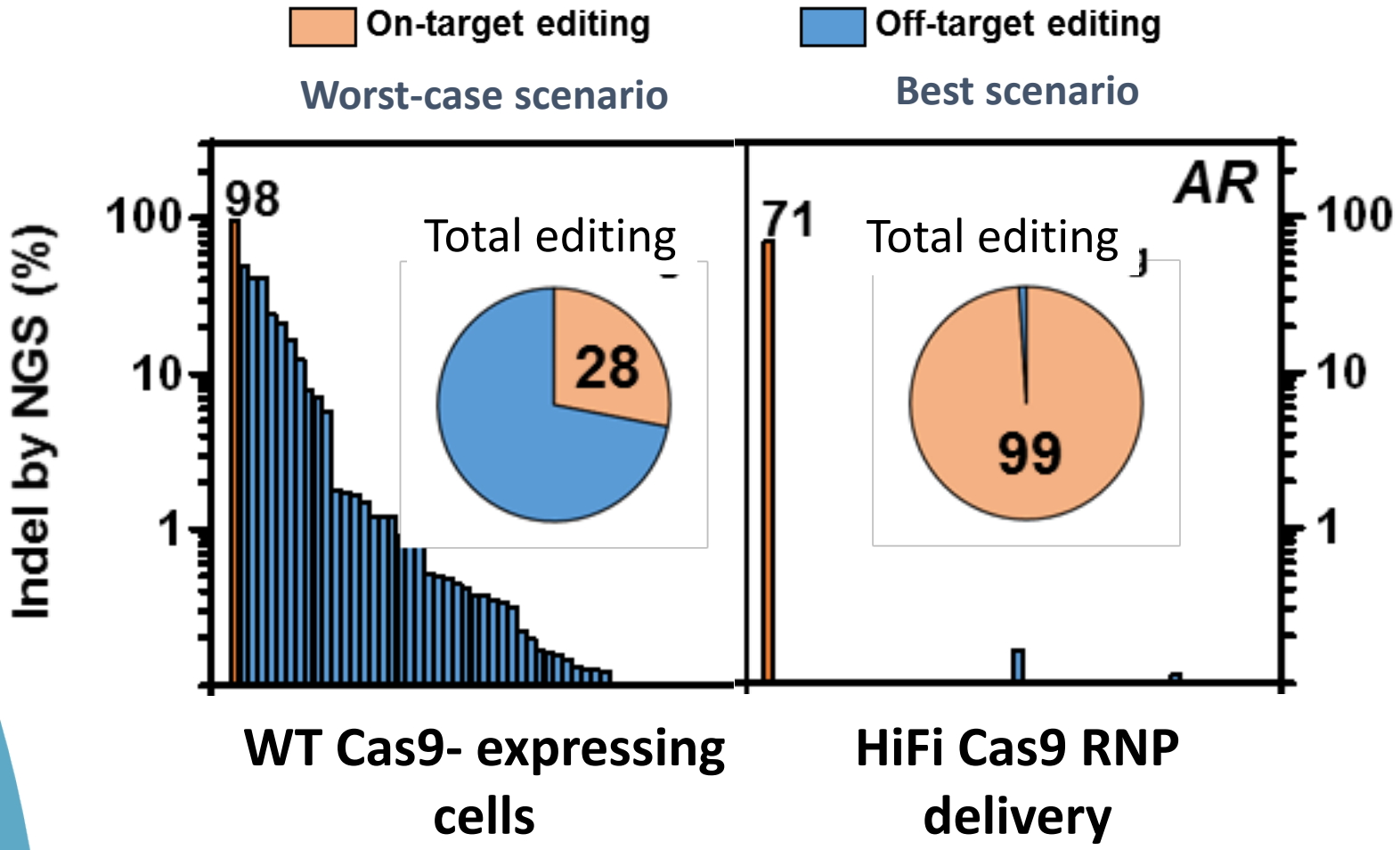
Amplification with indexing primers

CRISPAltRations FOR THE ANALYSIS OF rhAmpSeq DATA



rhAmpSeq TECHNOLOGY AND Alt-R HiFi Cas9

Verification and validation Alt-R *S.p.* HiFi Cas9 Nuclease V3



Vakulskas, *et al.* (2018) *Nat Med* 24:1216

rhAmpSeq system savings:

- 8 x 40 assay designs
- ~1500 individual PCRs reduced to <96
  - Master mix
  - gDNA
- Library quantification
- Full-time equivalent hours
  - Months to days

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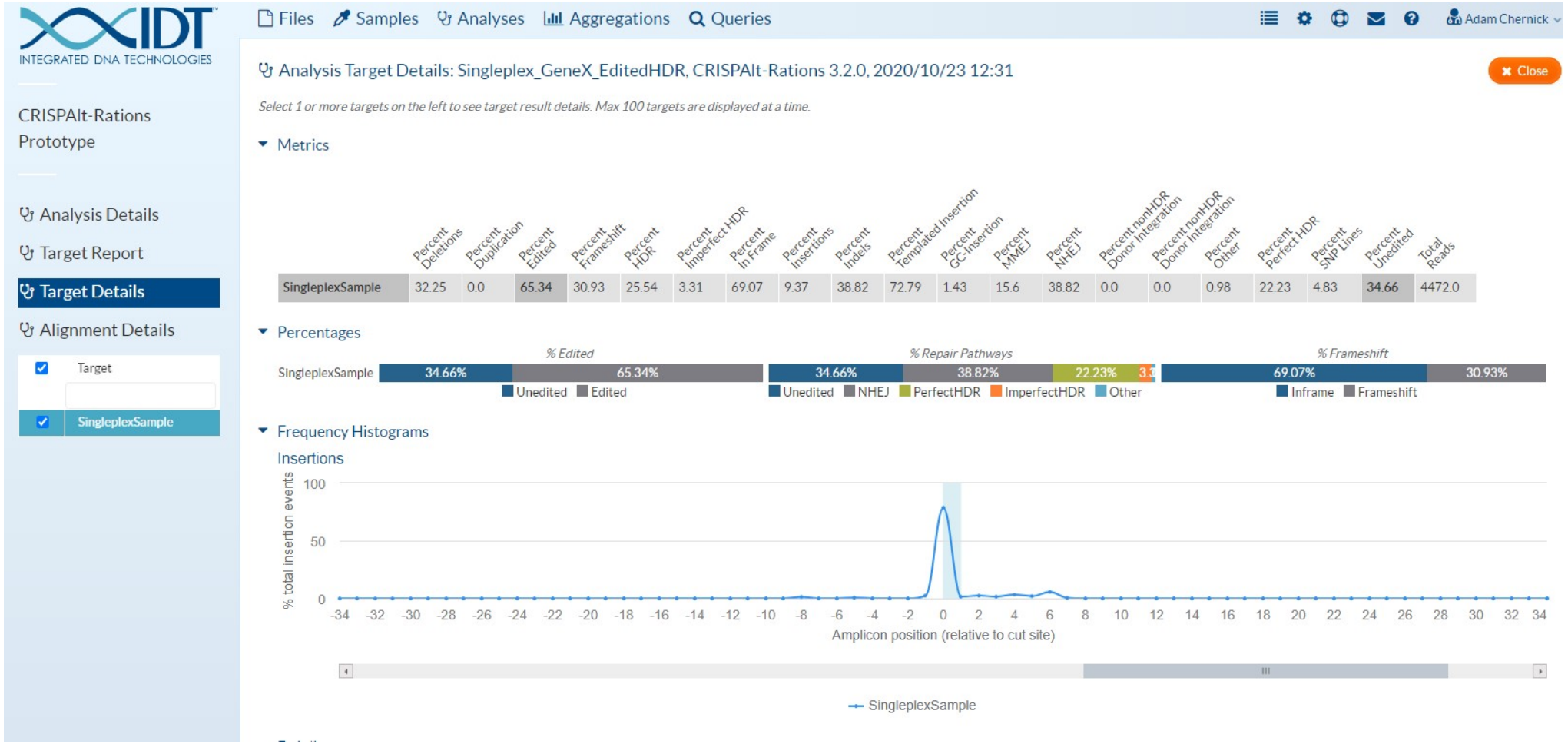
ANALYSIS TOOL OUTPUT



THANK YOU

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achernick@idtdna.com

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NMIN Capacity-Building Webinar

Q&A

Adam Chernick

Field Application Manager
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