大阪大学共同研・ゲノム編集センター
使用説明会
～明日から始めるゲノム編集～

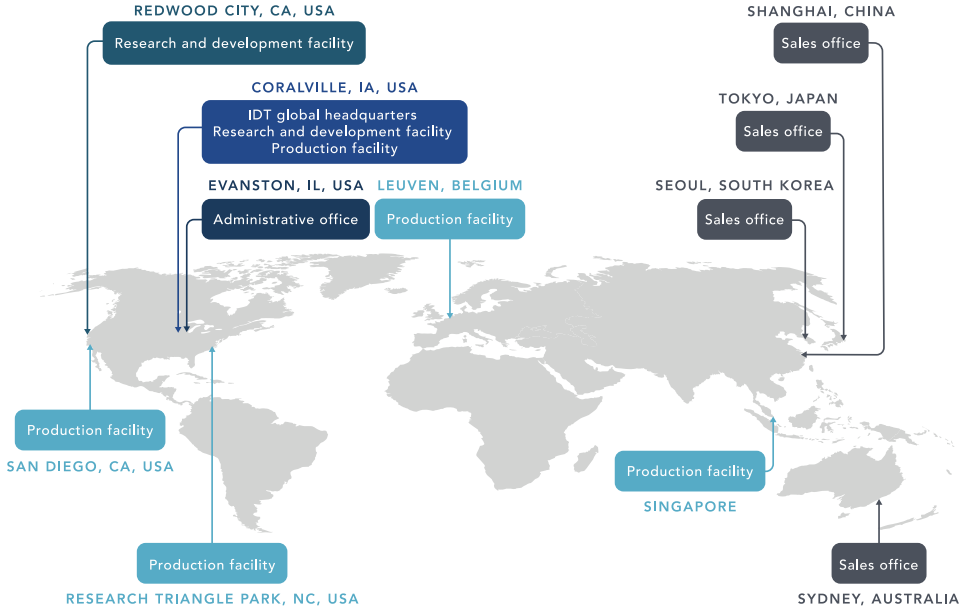
インテグレーテッドDNAテクノロジーズ株式会社

04-21-2026

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



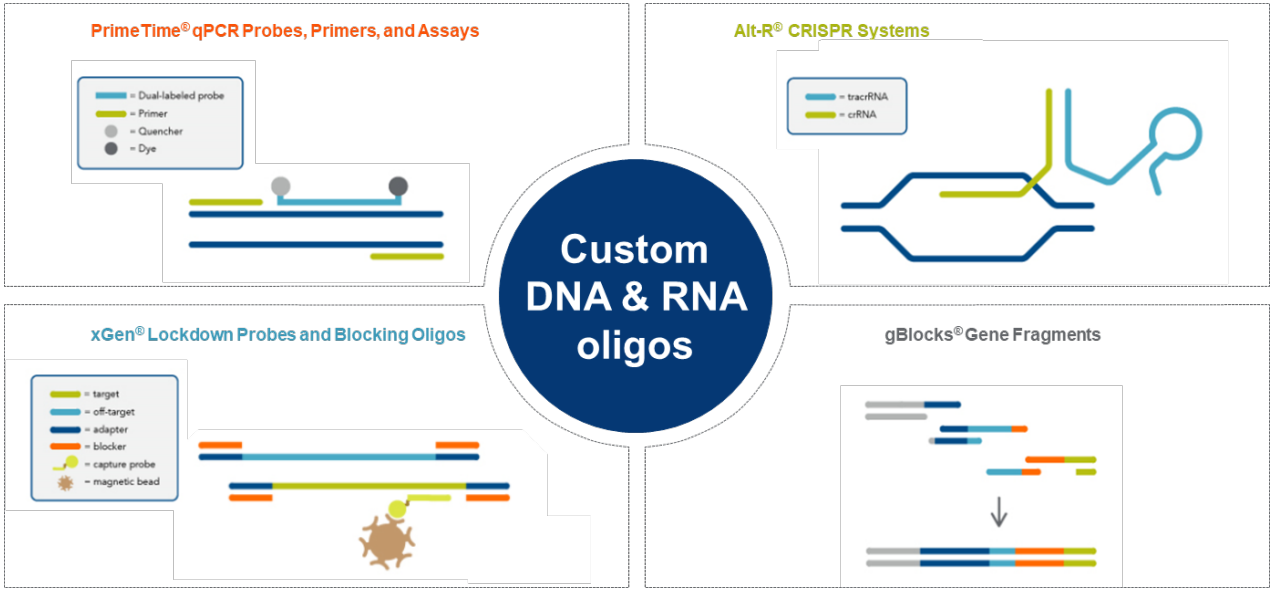
IDT INTEGRATED DNA TECHNOLOGIES

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



ゲノム編集技術について

これまでと比べて、画期的である理由

- 汎用性: 動物、植物、昆虫、微生物、、、各種の生物に適用可能
- 高効率: 改変効率が極めて高く、改変個体や細胞の同定、単離が容易
- 多用途: どのゲノム領域でもターゲットにできる、いろいろな改変可能
- 特異的: ターゲットのみを改変し、ほかに痕跡を残さない
- 簡便: 試薬・機器を比較的簡単に安価に入手し短期間で行える

既存の技術では、特定の生物でのみ、低い効率で、長い時間と大きな費用をかけてしか実現できなかった。

ゲノム編集がその制約を大きく乗り越えた。

（医療応用実用などには倫理面を含め、越えなければならないハードル未だ多し）

Alt-R™ CRISPR SYSTEM: A COMPLETE WORKFLOW

ターゲットのデザイン → 試薬の選定 →→→→→→→→→→ 解析

Design	CRISPR-Cas reagents	HDR reagents	Analyze

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

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

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

Alt-R HDR Donor Blocks
- Modified to reduce blunt integration
- Up to 3000 nt
- Sequence-verified via NGS

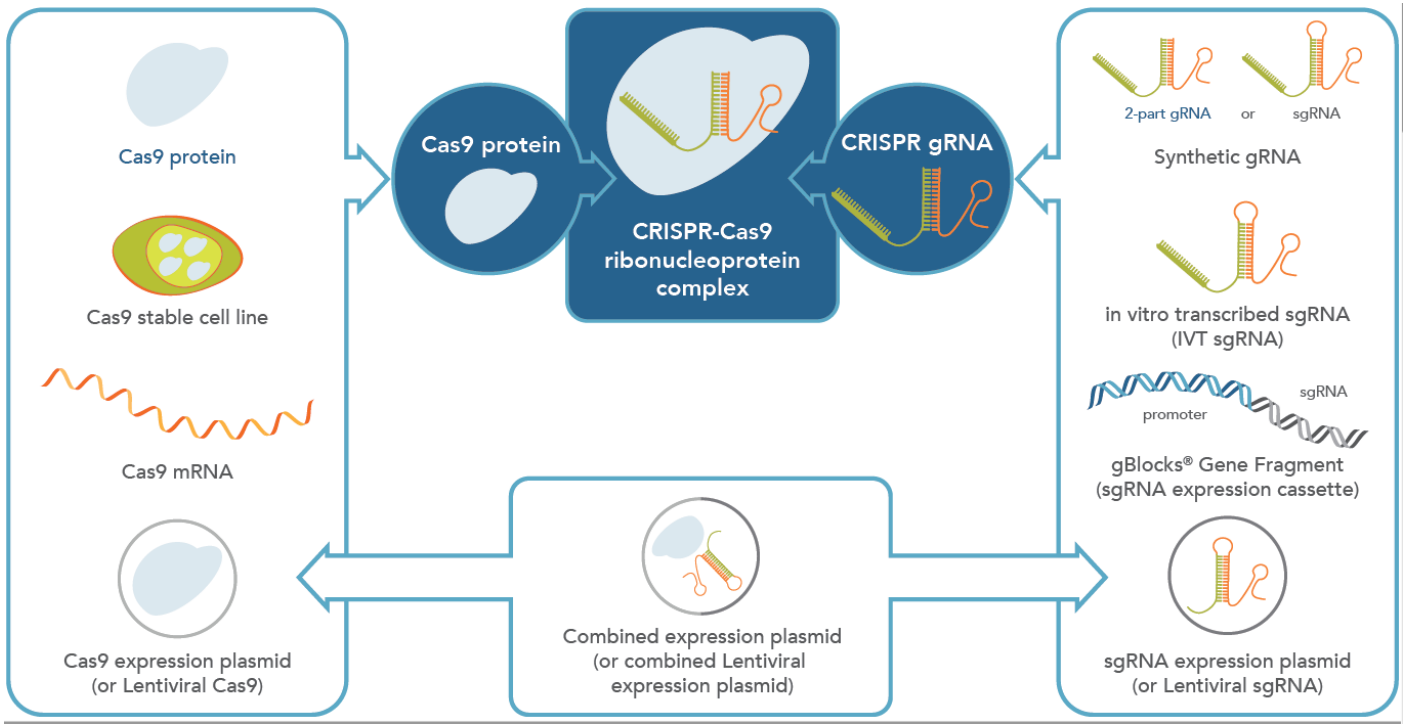
Alt-R HDR Enhancer
- V2

Alt-R HDR Enhancer
- Protein

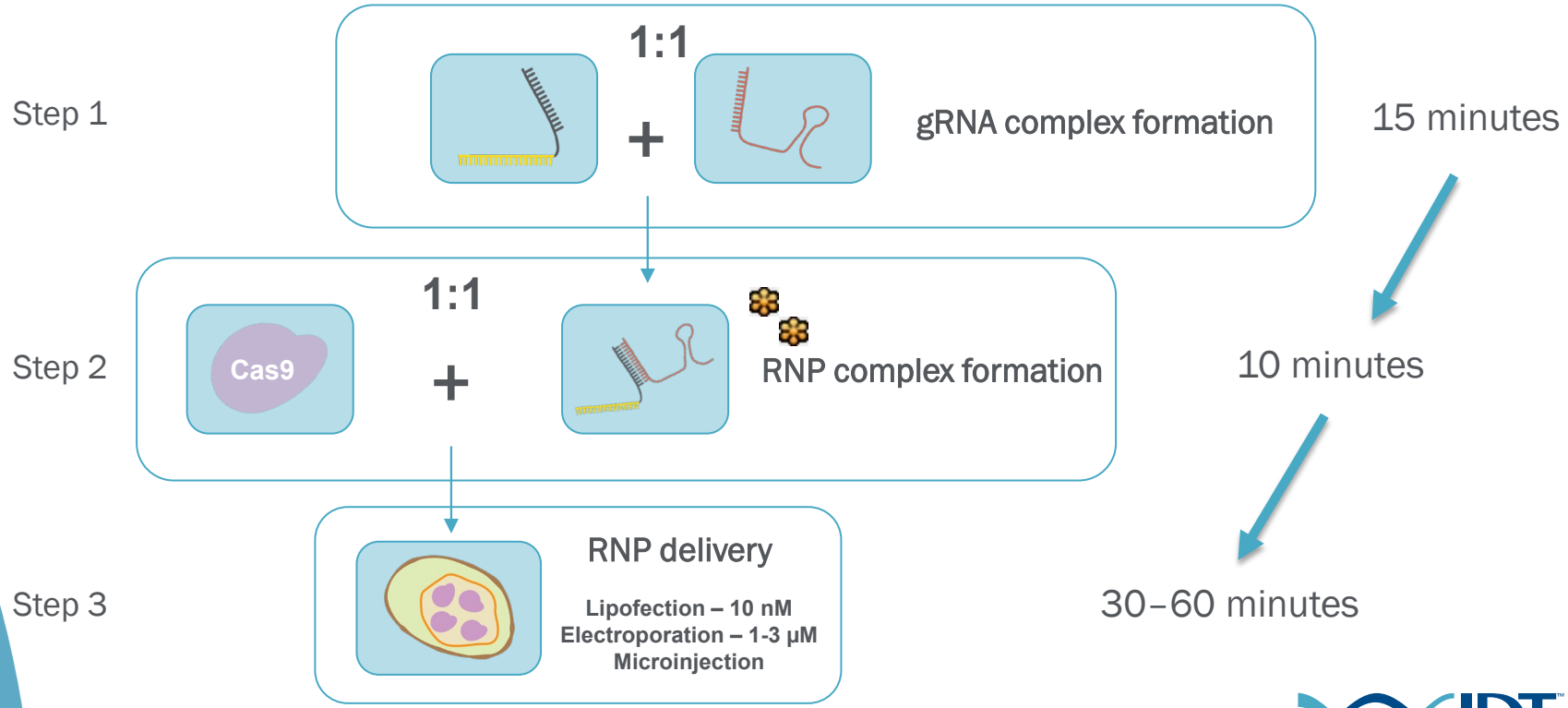
rhAmpSeq system for CRISPR
- Multiplexed amplicon sequencing

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

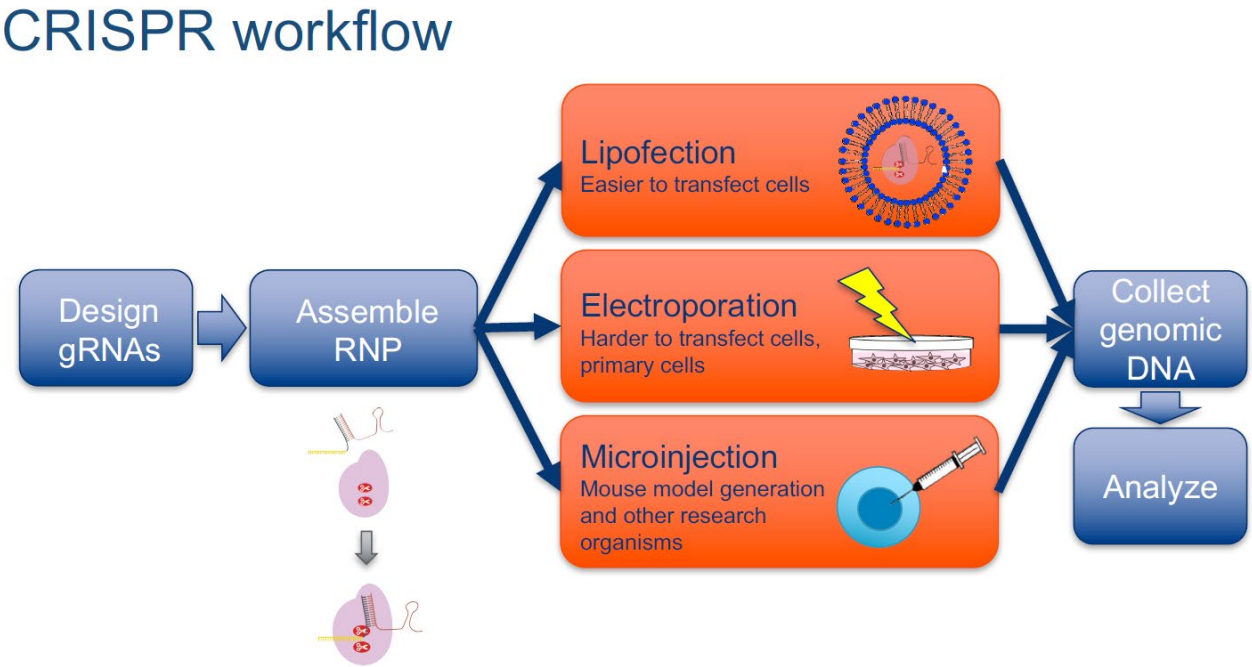
ゲノム編集に必要な 構成試薬（GRNAとCAS9タンパク質）



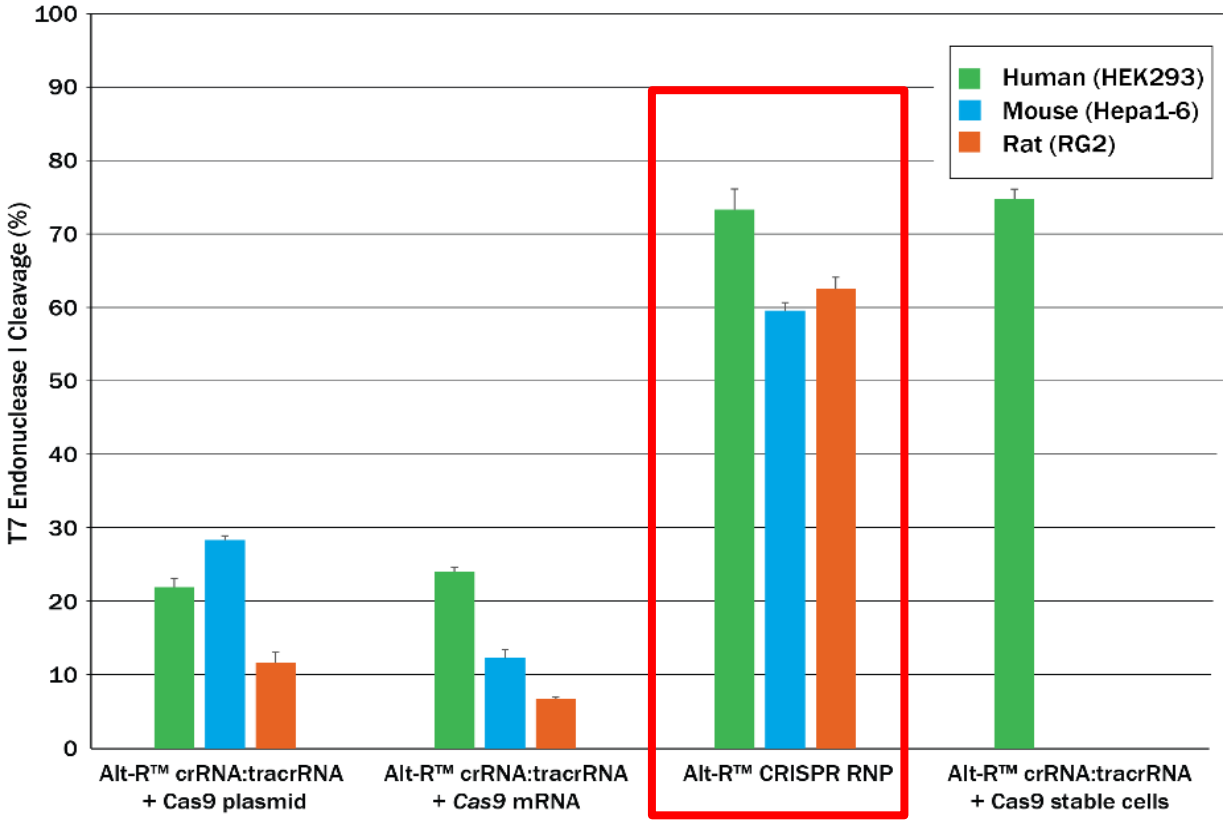
簡便なプロトコール


Step 1
1:1
+
gRNA complex formation
15 minutes
Step 2
1:1
Cas9
+
RNP complex formation
10 minutes
Step 3
RNP delivery
Lipofection – 10 nM
Electroporation – 1-3 µM
Microinjection
30–60 minutes

CAS9 RIBONUCLEOPROTEIN(RNP)導入法



リコンビナントCAS9タンパク質利用による編集効率UP



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 CRISPR-CAS9 GRNA DESIGN TOOL

- 3種類のツールがあります。

- 1) Search for Predesigned CRISPR： プレデザインを検索
- ヒト、マウス、ラット、ゼブラフィッシュ、線虫の遺伝子を対象

- 2) Design Custom crRNAs： カスタムで設計
- お客様が指定するターゲット配列情報から設計
- 全生物種の遺伝子を対象

- 3) CRISPR-Cas9 crRNA Checker： 既存のターゲット配列の検証
- 論文などに記載されているproto spacer 配列のデザインを検証する

Alt-R Cas9 CRISPR LIBRARIES

genome editing

Alt-R™ CUSTOM CRISPR gRNA LIBRARIES

Chemically modified guide RNA libraries for CRISPR screening research

Fast turnaround time, flexible formulation

Adaptable for different CRISPR systems

Complete CRISPR workflow solution from custom design to analysis

Alt-R Custom CRISPR gRNA libraries are available for all CRISPR nucleases, including Cas9, Cas12a, Cas13, prime editing enzymes, and others. These libraries were developed to address the need for better CRISPR screening solutions. They are chemically modified guide RNAs (gRNAs) synthesized on the IDT proprietary high-fidelity RNA manufacturing platform to provide high quality, reliable gRNA libraries with fast delivery.

BENEFITS

1. Innovative solution offered by a global leader in RNA synthesis and CRISPR innovation
2. Reliable, consistent, and fast delivery, with custom formulations available to suit a variety of project needs
3. Adaptable for alternative CRISPR systems such as Cas12a, Cas13, and prime editing
4. Enhanced nuclease resistance for maximal editing in Cas9-expressing cells or via ribonucleoproteins (RNP)
5. Optimized RNA synthesis processes to mitigate cross-contamination risk

PRODUCT SPECIFICATIONS

Features	Options
Design	Predesigned, custom, user-provided
CRISPR systems	Cas9, Cas12a, Cas13, prime editing, and other alternative systems
Guaranteed yield	0.5 nmol, 2 nmol, 5 nmol, and custom normalized deliverables
Cas9 gRNA formats	2-part crRNA:tracrRNA complex and sgRNA
Custom lengths supported	30–150 nt
Chemical modifications	2′-O-methyl RNA, PS linkages, end-blocking Alt-R modifications
Plate types	96- & 384-well PCR, Deep-well, V-bottom, ECHO, custom options available
Formulation options	Multi-guide per well; pooled by gene Arrayed (single gRNA/well) Custom formulations upon request
QC	Individual ESI/MS
Supporting reagents & functional analysis pipeline (optional)	WT Cas9, HiFi Cas9, Cas12a. and Cas12a *Ultra* Glycerol-free options available in tubes or plates (ideal for robotics) Electroporation Enhancers rhAmpSeq™ CRISPR Analysis System (NGS-based on-/off-target editing analysis)

> WWW.IDTDNA.COM

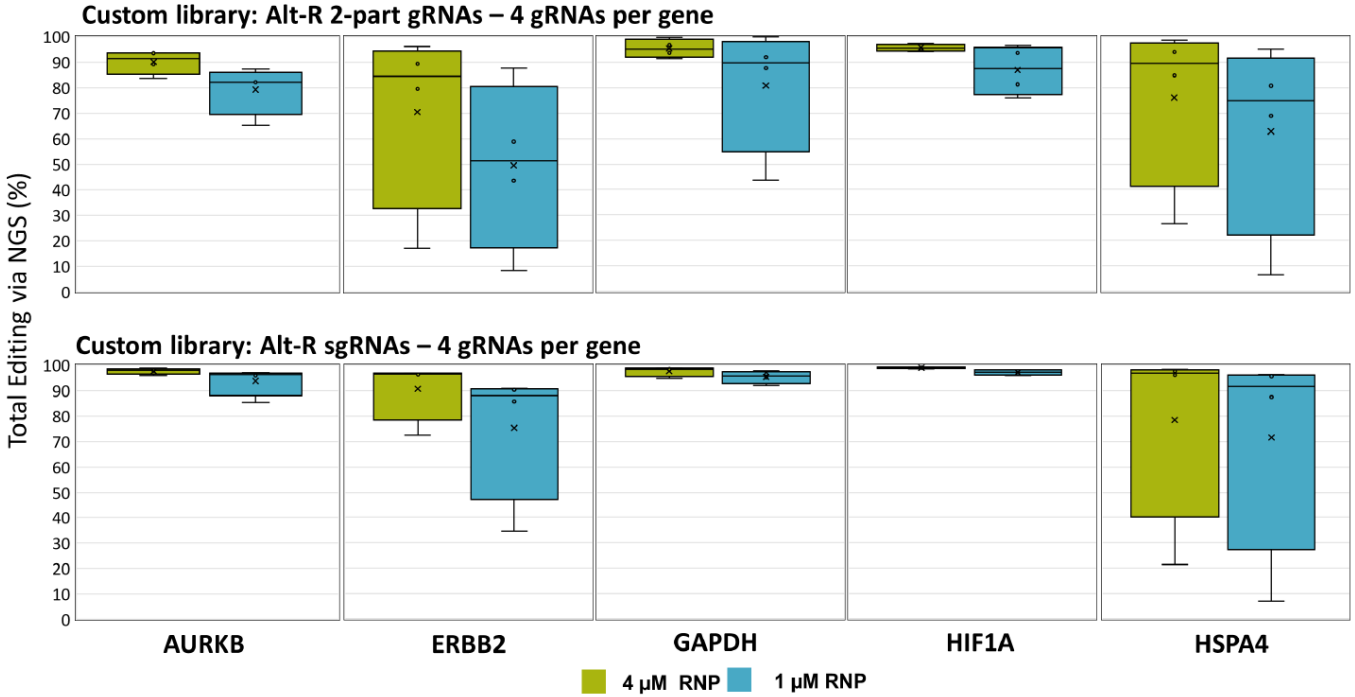
IDT INTEGRATED DNA TECHNOLOGIES

Custom gRNA libraries:

- 2 part (crRNA+tracrRNA) and sgRNA
- Standard offering provides desalted gRNAs, but purified versions, with custom mod patterns, large scale deliverables and likewise gRNAs for alternative applications built on Cas9 (i.e. Prime Editing) can easily be accommodated
- **Scale**s – 2nmol, 10nmol, 50nmol, 100nmol
- Available in tube or plates
- **Custom formats** – IDT can support any format to suit your needs
  - Arrayed – individual guides targeting different genes in different wells
  - Pooled – multi-guide pooled by gene
- **Custom Plates** – IDT has a variety for you to choose from including ECHO plates
- **Need help designing**? We can assist.

IDT INTEGRATED DNA TECHNOLOGIES

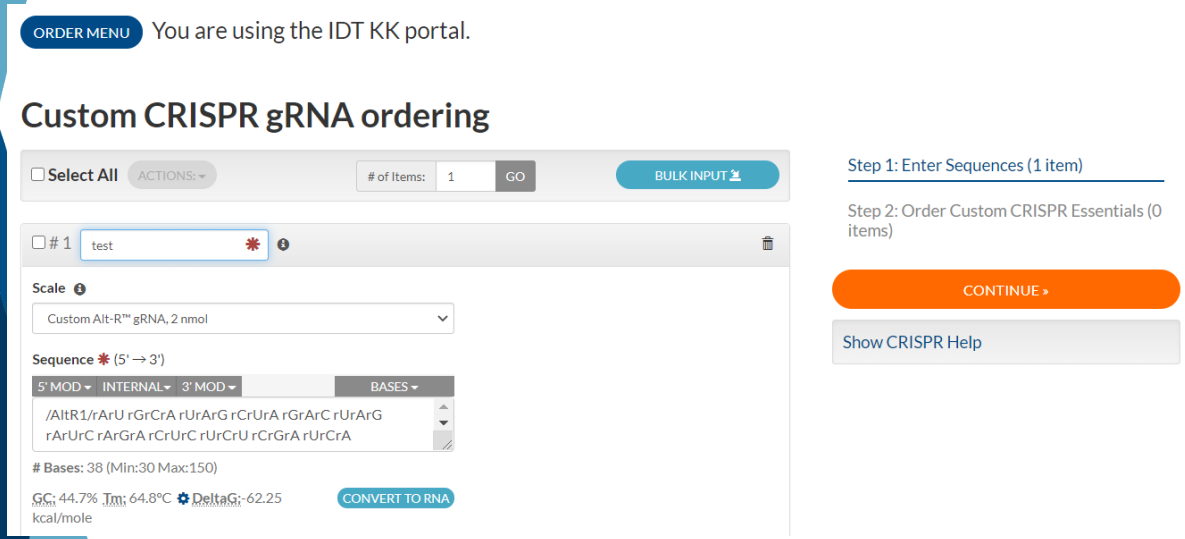
CRISPR SCREENS WITH CUSTOM Alt-R gRNA LIBRARIES



4 µM RNP Nucleofector™ Nucleofection (Lonza) into HAP1 cells, 4 gRNAs for 5 genes as 2-part or sgRNA gave >85% avg editing at all target loci

ALT-R™ CRISPR CUSTOM GUIDE RNAS

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

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:
  - Alt-R™ S.p. Cas9-GFP V3, 100 µg
  - Alt-R™ S.p. Cas9-RFP V3, 100 µg

IDT-exclusive mutants

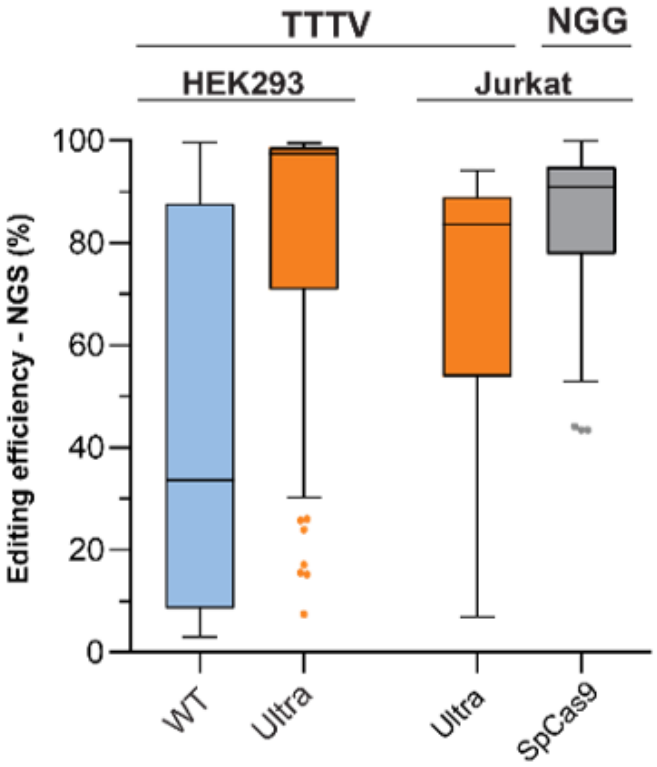
NUCLEASE

Product Name	Catalog #
Alt-R™ S.p. Cas9 Nuclease V3, 5 mg	10000735
Alt-R™ A.s. Cas12a (Cpf1) Ultra, 5 mg	10007804
Alt-R™ S.p. HiFi Cas9 Nuclease V3, 5 mg	10007803
Alt-R™ Cas9 Elec Enhancer, 100 nmol	10007805
Alt-R™ S.p.Cas9 V3, glycerol-free, 100µg	10007806
Alt-R™ S.p.Cas9 V3, glycerol-free, 500µg	10007807
Alt-R™ S.p.Cas9 V3, glycerol-free, 5 mg	10007808
Alt-R™ L.b. Cas12a (Cpf1) Ultra, 100 µg	10007922
Alt-R™ L.b. Cas12a (Cpf1) Ultra, 500 µg	10007923
Alt-R™ L.b. Cas12a (Cpf1) Ultra, 5 mg	10007924

Large Scale Cas9 Proteins:

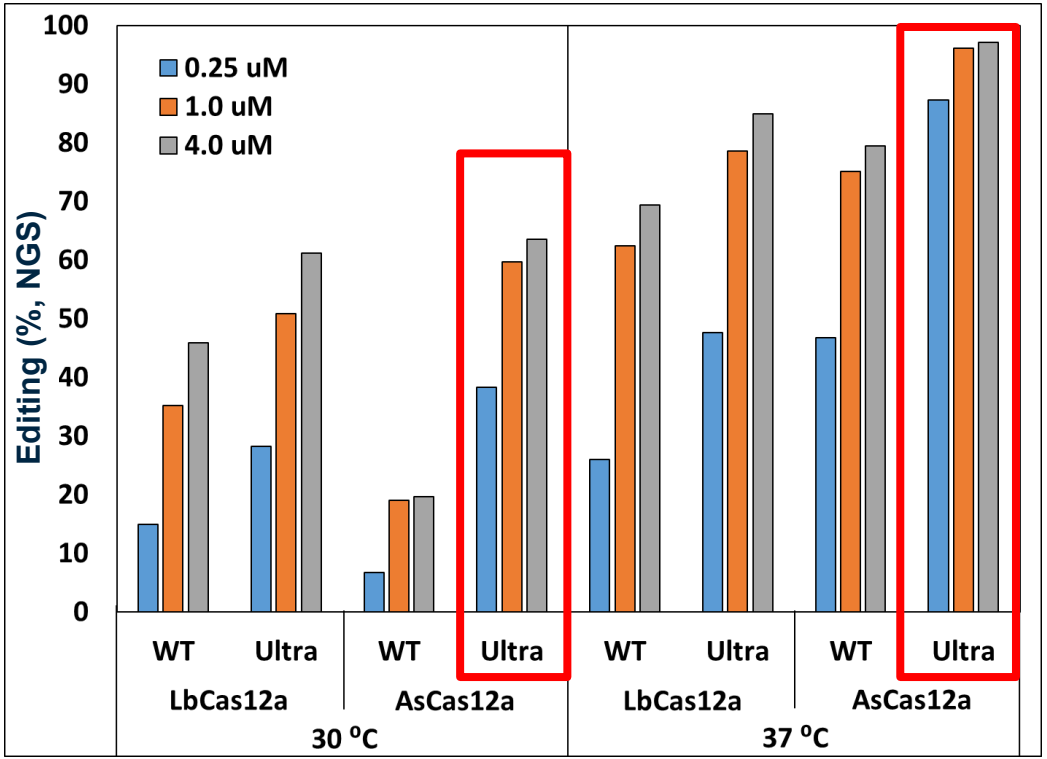
- Glycerol-free WTcas9v3 – ideal for sensitive cell lines
- As and Lbcas12a (Ultra) – useful for targeting AT-rich regions without available Cas9-specific PAM sequences. Much higher on-target potency than wild-type A.s. Cas12a (Cpf1). Can recognize many TTTT PAM sites in addition to TTTV motifs, increasing target range. Active at room temperature, making them flexible tools for applications requiring delivery at lower temperatures.

A.s. Cas12a *ULTRA* HAS EDITING ACTIVITY COMPARABLE TO *S.p.* Cas9



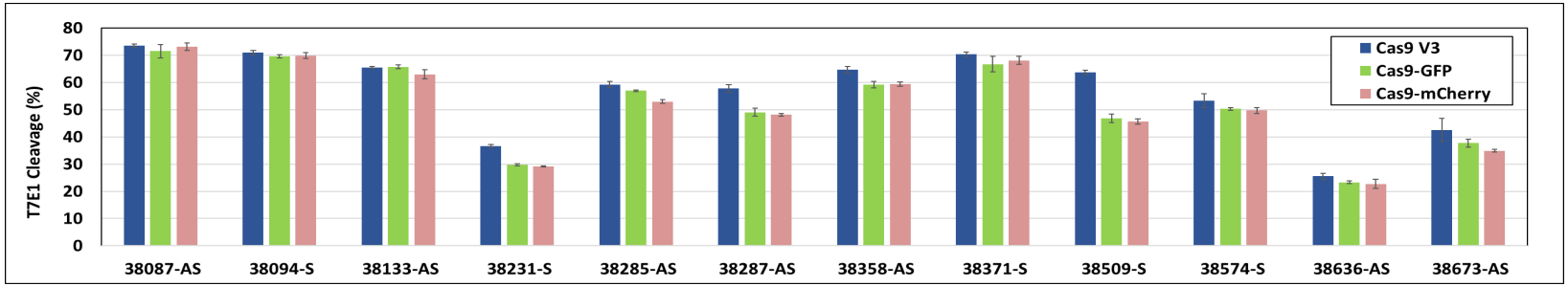
71 Cas9/Cas12a RNPs (TTTV PAMs) targeting multiple loci delivered by electroporation in HEK293 and Jurkat cells

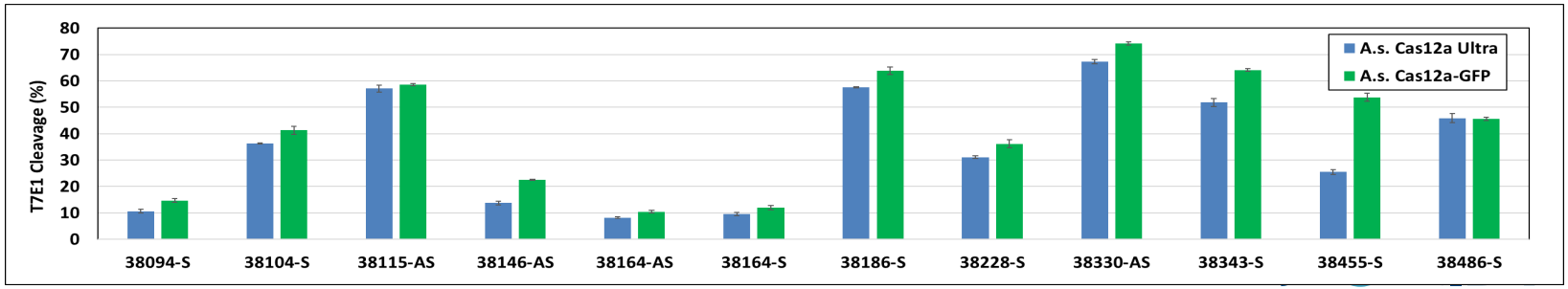
A.s. AND *L.b.* Cas12a *ULTRA* HAVE INCREASED TEMPERATURE TOLERANCE



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

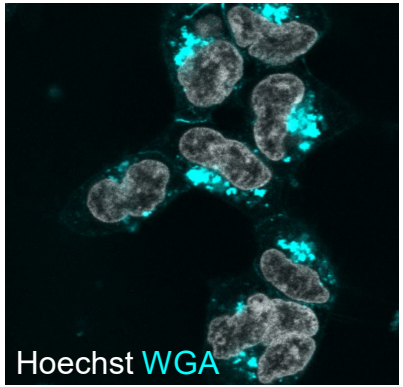


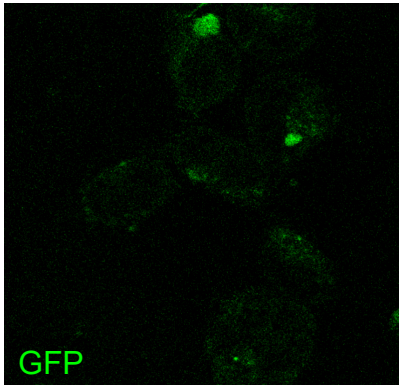


Cas9-GFP ALLOWS FOR VISUALIZATION OF DELIVERY

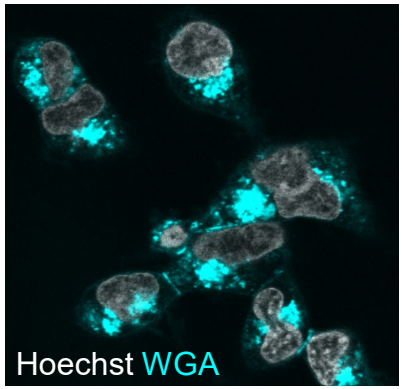
2 µM RNP delivery by Nucleofector™ Nucleofection (Lonza) into HEK293 cells, imaged ~18 hours after delivery using a confocal microscope

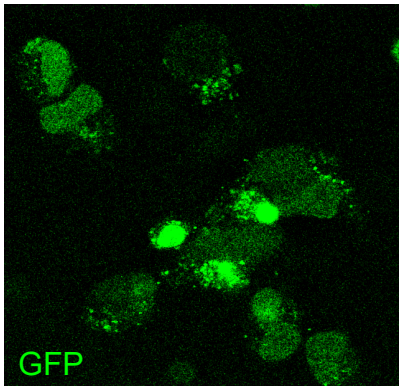
Cas9 V3





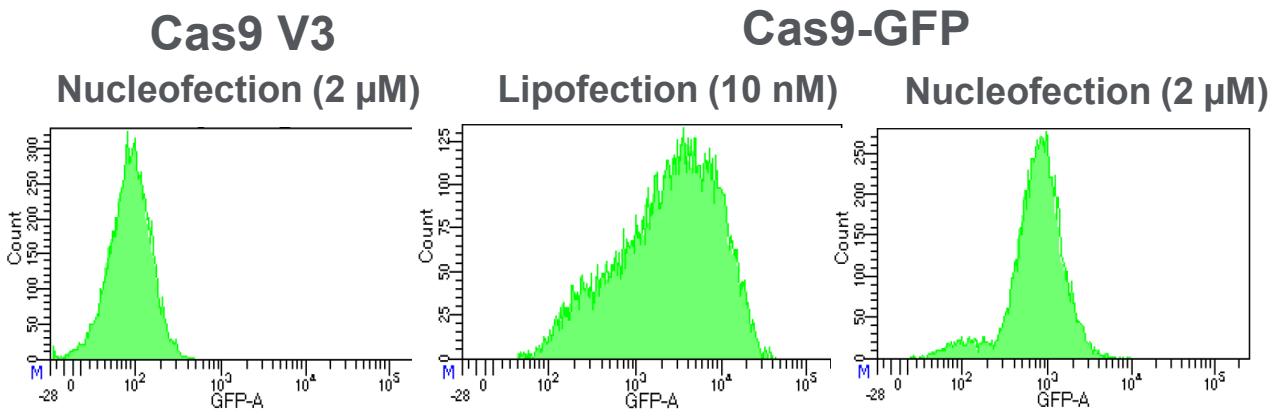
Cas9-GFP

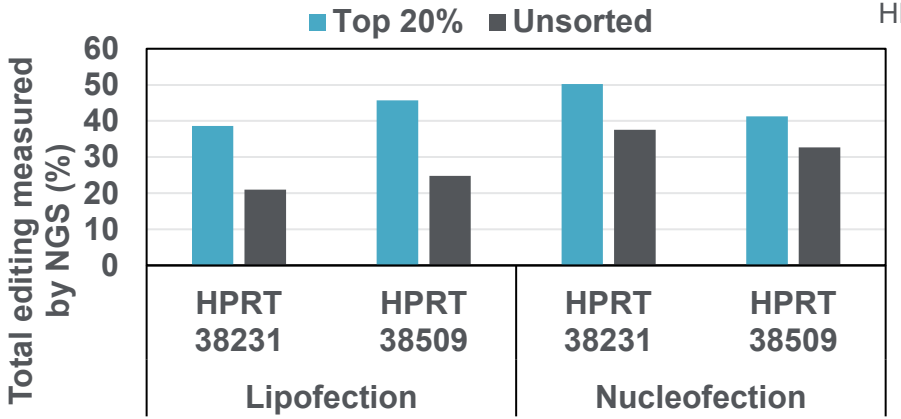




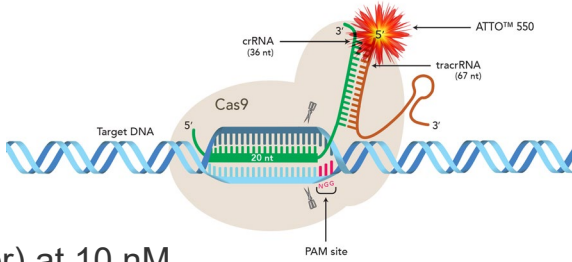
IDT INTEGRATED DNA TECHNOLOGIES

DELIVERY PROFILE IS REFLECTIVE OF EDITING

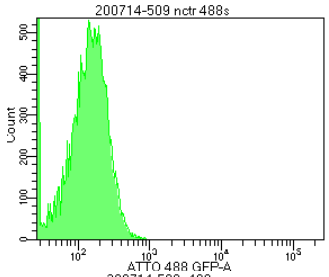
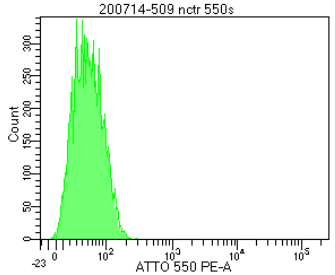
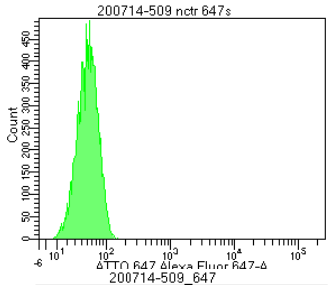
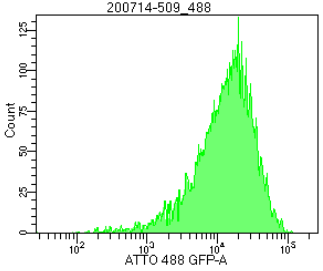
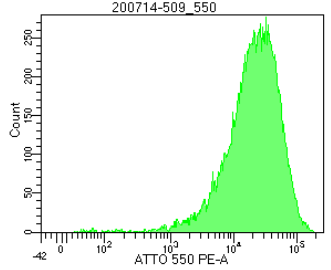
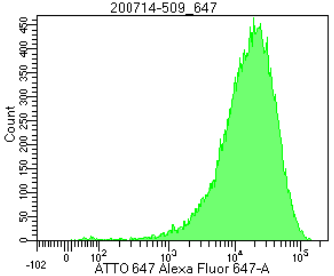




FLUORESCENTLY-LABELED tracrRNA



- 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			

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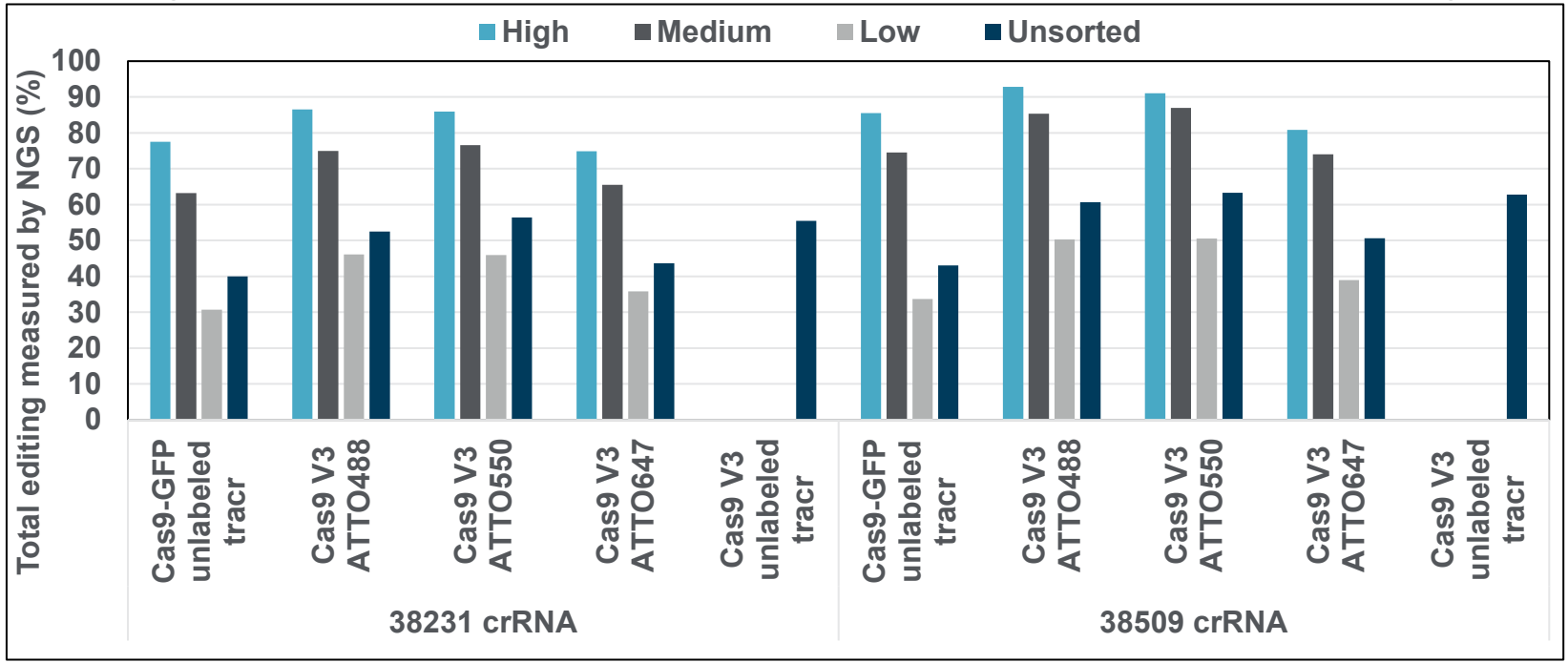
INTEGRATED DNA TECHNOLOGIES

ENRICHMENT OF EDITED CELLS USING LABELED tracrRNA BY FACS

High: Top 20%, Medium 80–60%, Low: Bottom 60% of cells based on fluorescent signal

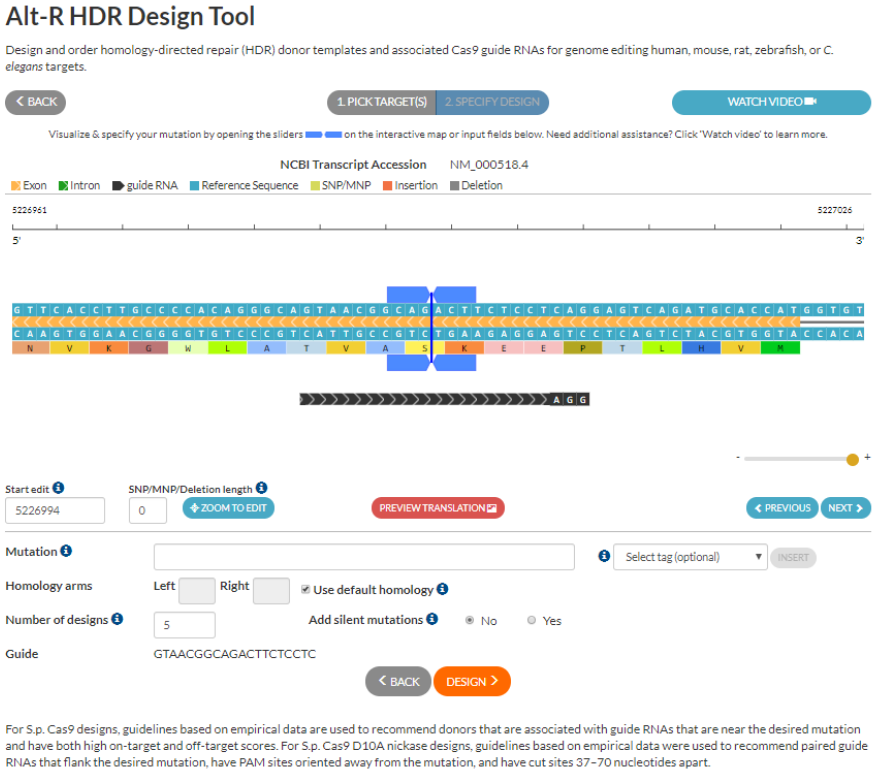


(10 nM RNP delivery by Lipofectamine™ RNAiMAX (Thermo Fisher) into HEK293 cells, sorted after ~18 hours)

IDT SOLUTIONS TO IMPROVE HOMOLOGY-DIRECTED REPAIR

INTEGRATED DNA TECHNOLOGIES

USE THE Alt-R HDR DESIGN TOOL FOR OPTIMAL DESIGN



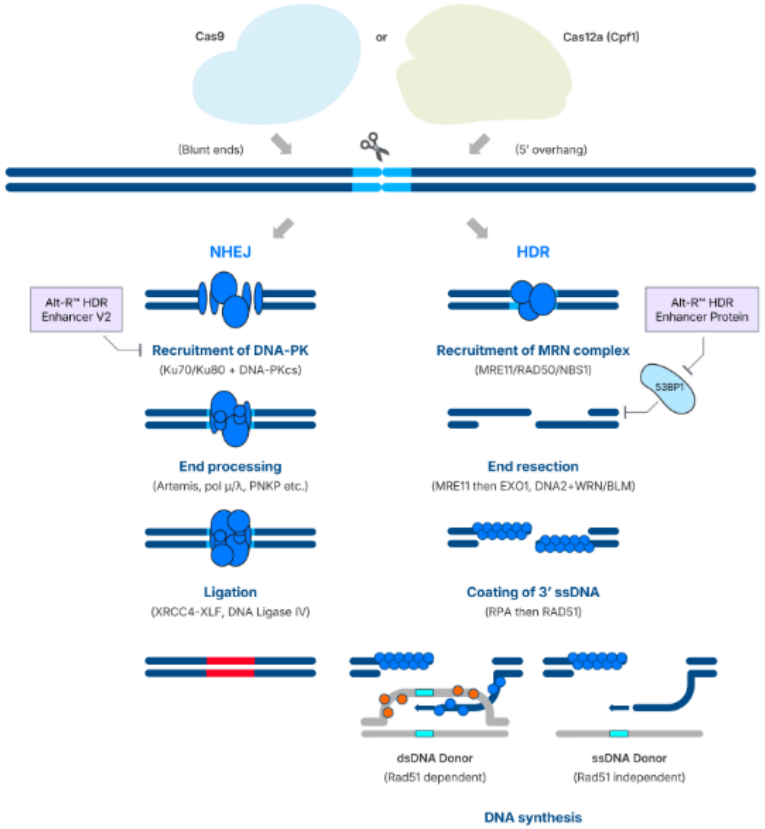
Alt-R HDR Design tool: a novel bioinformatics tool for ssDNA HDR template design

- 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

HDR ENHANCER PROTEIN

Alt-R HDR Enhancer V2



CRISPR-induced double-strand breaks are repaired via NHEJ or HDR. The Alt-R™ HDR Enhancer Protein promotes HDR by inhibiting 53BP1, making it specific to the HDR pathway, in contrast to DNA-PK inhibitors that enhance HDR by blocking the NHEJ pathway directly.

New

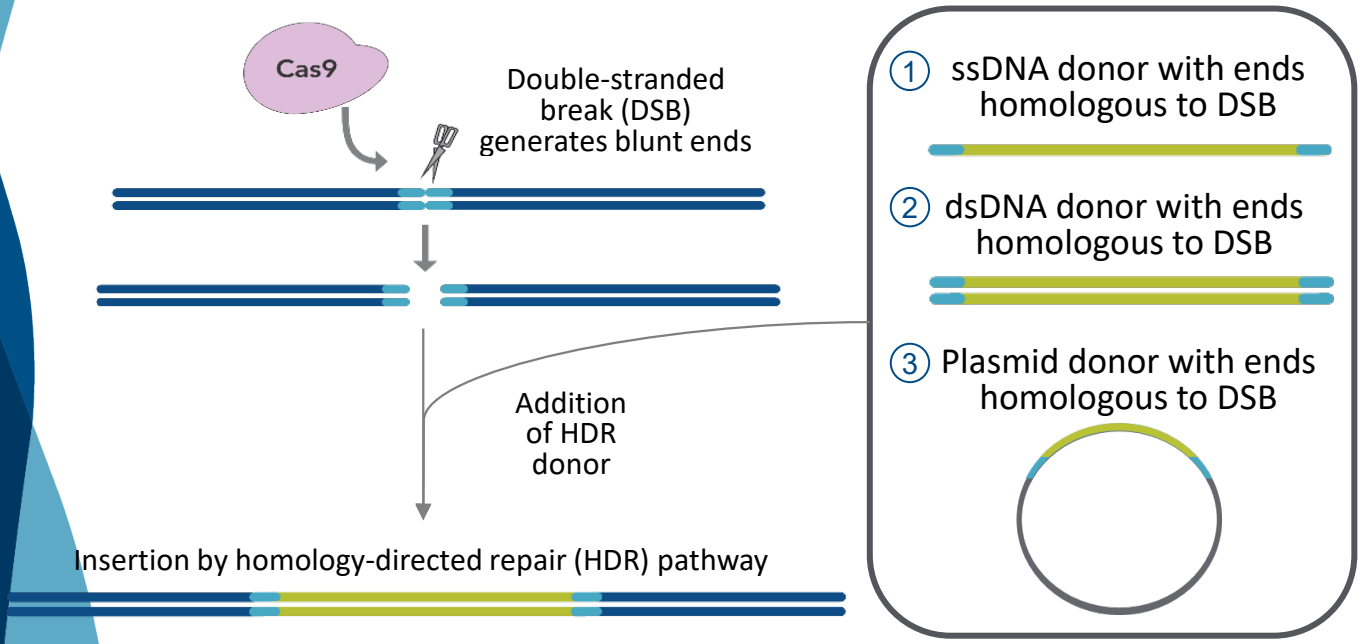
Alt-R™ HDR Enhancer Protein

IDT INTEGRATED DNA TECHNOLOGIES

HDR ENHANCER V2 OR PROTEIN

項目	HDR Enhancer V2	HDR Enhancer Protein
分類	小分子	タンパク質
作用の考え方	**NHEJ を弱める**	**HDR のブレーキ（53BP1）を外す**
作用点	経路レベル	分子レベル（53BP1）
性格	出力強め	精密・安全寄り
プロトコール上の位置	**核酸導入後（培養中）**	**RNPと同時導入**
「効く時間帯」	DSB修復が進行している間	DSB発生直後

ノックイン：大きなサイズのテンプレートの選択肢 (120塩基～



- **Ultramer® Oligonucleotide:ssODNドナーテンプレート(200mer)**
- **gBlocks® Gene Fragments (2本鎖DNAフラグメント 125～3000bp)**
- **人工遺伝子合成 (Genes 40円/bp)**

HDR ドナーテンプレート　まとめ

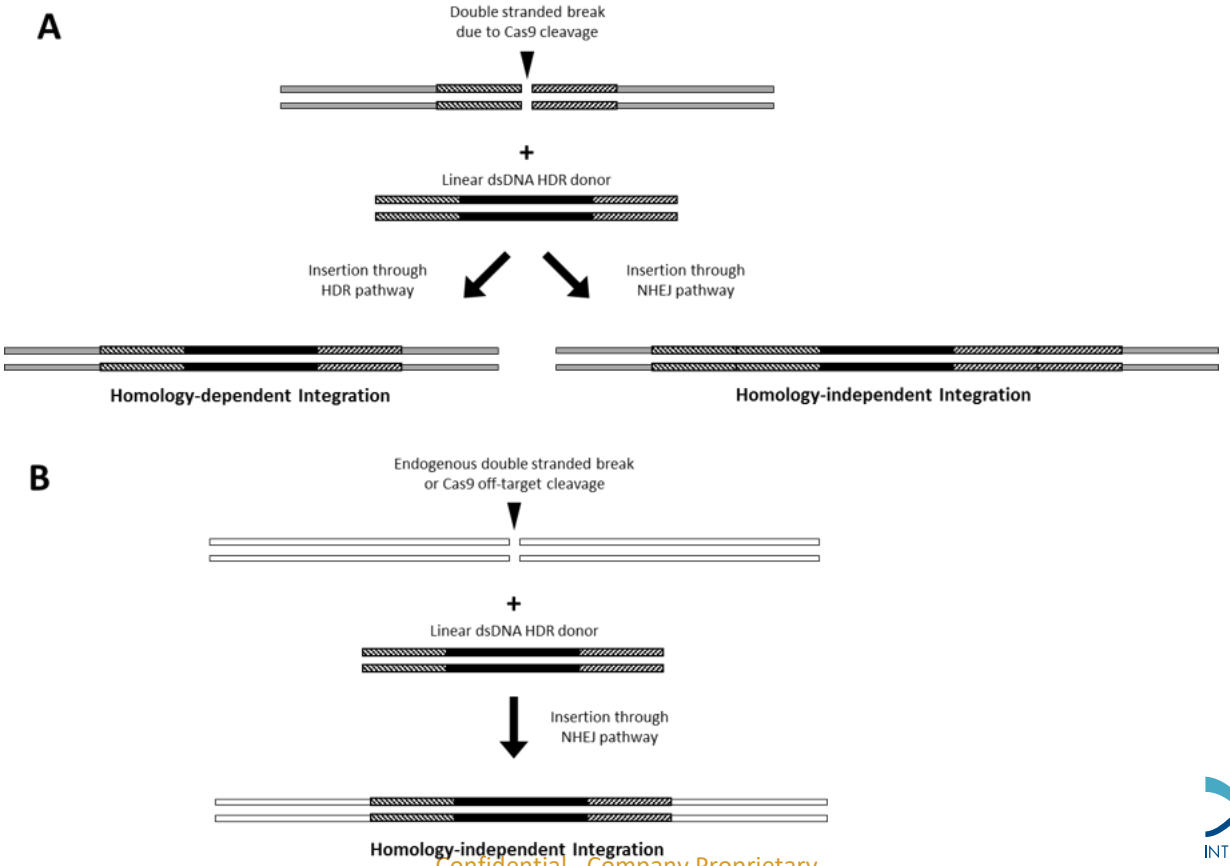
タイプ	製品	サイズ	毒性	HDR効率	コスト	納期	HDR Enhancer 併用時の効率＊
ssODN	**Alt-R HDR Donor Oligo/ PSmod**	**45-200mer**	**低い**	**＋＋＋＋**	**安価** **¥17,560~**	**5-10営業日**	**✓✓**
dsDNA (直鎖二本鎖)	**Alt-R™ HDR Donor Blocks**	**201-3,000bp**	**低い～中程度**	**＋＋＋**	比較的安価 **¥34,620 or ¥72/bp~**	**3週間～**	**✓✓**
dsDNA (環状)	Genes	25-3000bp、それ以上	より高い	＋	高価 ¥40/bp~	数週間	✓

大きいサイズのドナーテンプレートの特長

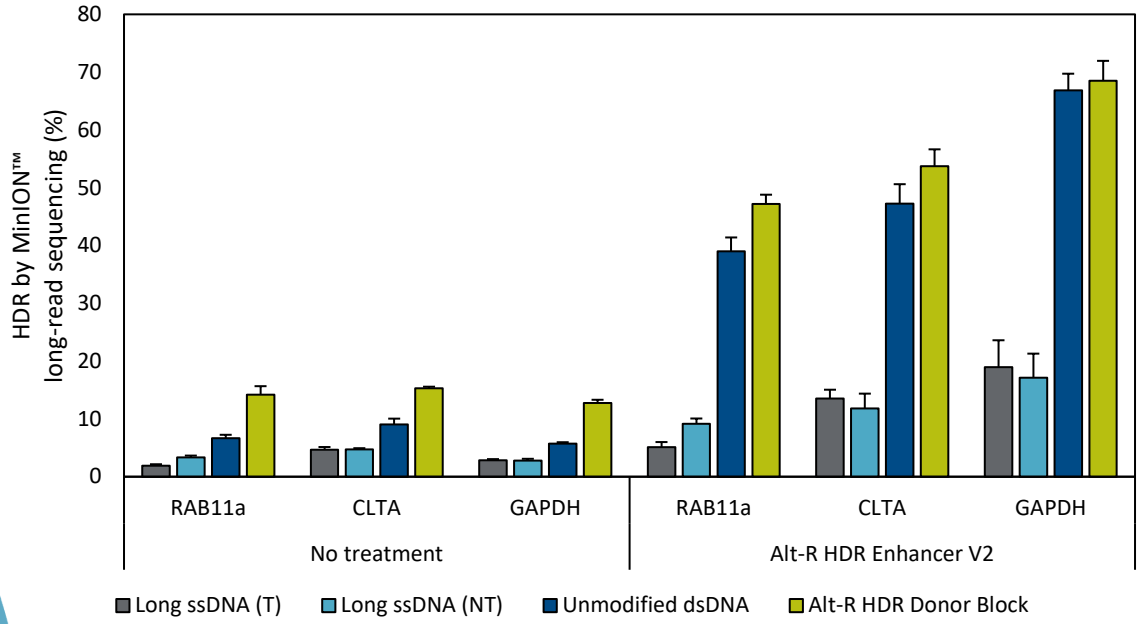
	ドナータイプ		
	Plasmid	Linear ssDNA	Linear dsDNA
HDR効率	低	高	高
細胞毒性	高	中	中
コストと納期	低	高	低～中
Off-target挿入リスク	中	低	中

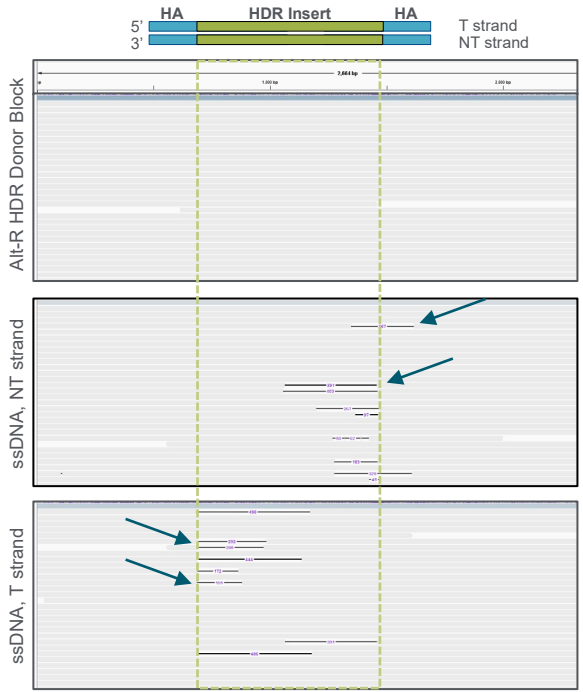
Can modifications reduce the risk of blunt integration with dsDNA?

SOLUTIONS TO REDUCE BLUNT INTEGRATION WITH dsDNA HDR TEMPLATES



Alt-R HDR Donor BlocksとAlt-R HDR Enhancer V2の併用がlarge HDR insertions効率を改善

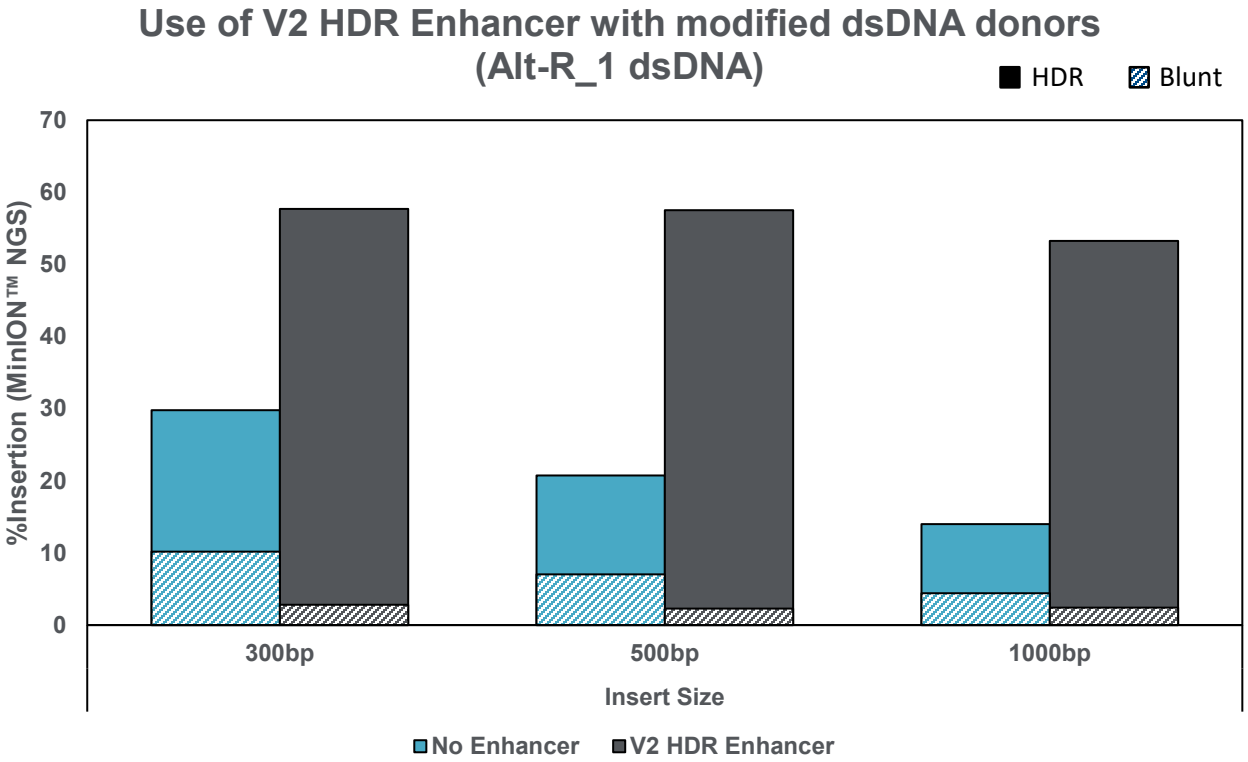




GFP insertion at CLTA

Lonza Nucleofector™ delivery system:
K562 or HEK-293 cells, 2 µM Cas9 RNP, 50 nM ss or dsDNA (GFP tag, 200bp HA), 0 or 1 µM Alt-R HDR Enhancer V2 (24 hrs) (n=4)

HDR ENHANCER V2 ALSO IMPROVES HDR RATES FOR LARGE INSERTIONS

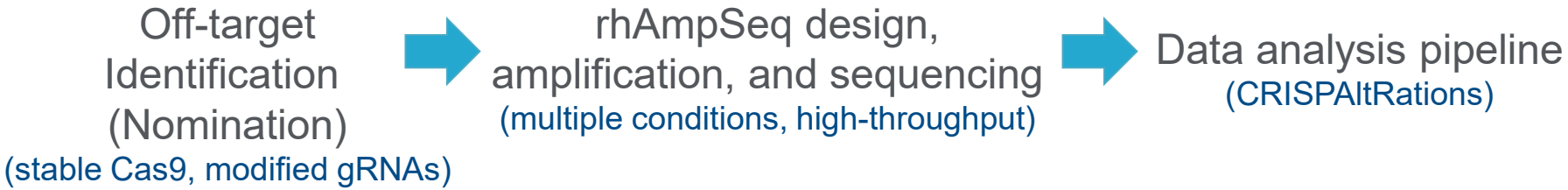


NGSでのオフターゲット解析
rhAmpSeq™ MULTIPLEXED AMPLICON SEQUENCING FOR CRISPR

CRISPR 編集・オフターゲットの検出

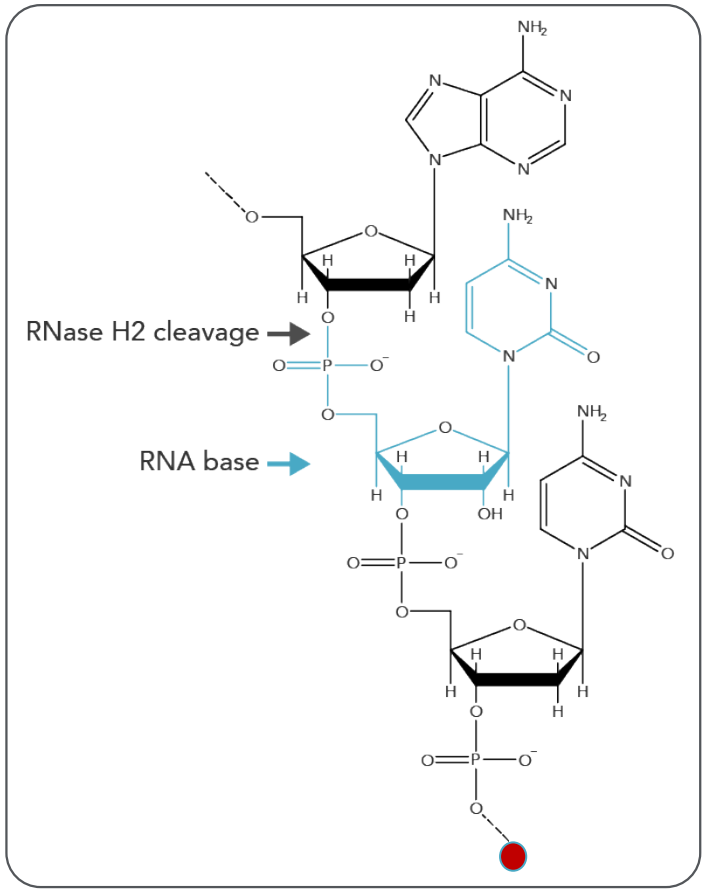
- 多くの検証・予測されたオフターゲットサイト-ガイドRNAの特異性
  - In silicoの予測ツール
    - 予測の困難性、重要なサイトの見落とし、予測過剰
  - 可能性のあるオフターゲット効果を定義するIn vitro アッセイ
    - SITE-seq, CIRCLE-seq　など.
    - 予測過剰傾向、シーケンスリード・スペースの無駄
  - GUIDEseqによる オフターゲット効果の識別
    - 目的の細胞株による、定量的
  - rhAmpSeq™ for CRISPR
    - マルチプレックス・アンプリコンベースのターゲットエンリッチメントNGS解析
    - オンターゲットサイトに対しての定量的評価、最大1000個までのオフターゲットサイトのマルチプレックス反応が可能

EXAMPLE CRISPR EXPERIMENT WORKFLOW



WHAT IS rhAmp™ PCR?

- A novel, proprietary technology developed at IDT
- More specific than standard PCR
- Unique characteristics of rhAmp PCR:
  - Primers are blocked at the 3′ end
  - Primers have a single RNA base near the 3′ end
  - *Taq* polymerase and RNase H2 are both required



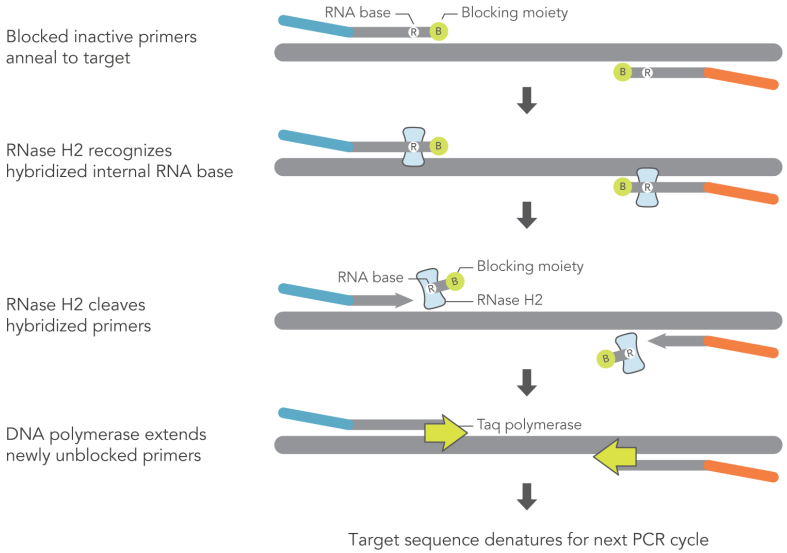
rhAmp PCR TECHNOLOGY INNOVATED AT IDT

BMC Biotechnology (2011) 11:80

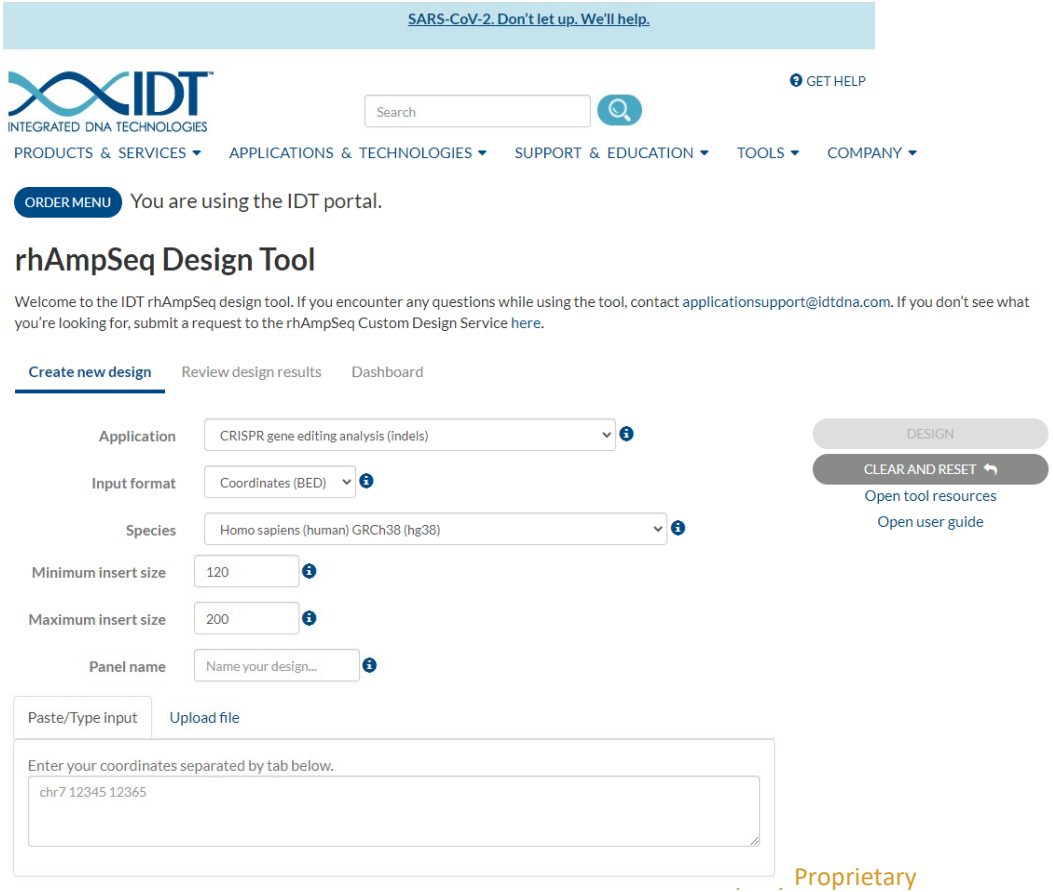
METHODOLOGY ARTICLE **Open Access**

RNase H-dependent PCR (rhPCR): improved specificity and single nucleotide polymorphism detection using blocked cleavable primers

Joseph R Dobosy, Scott D Rose, Kristin R Beltz, Susan M Rupp, Kristy M Powers, Mark A Behlke* and Joseph A Walder



rhAmpSeq DESIGN TOOL



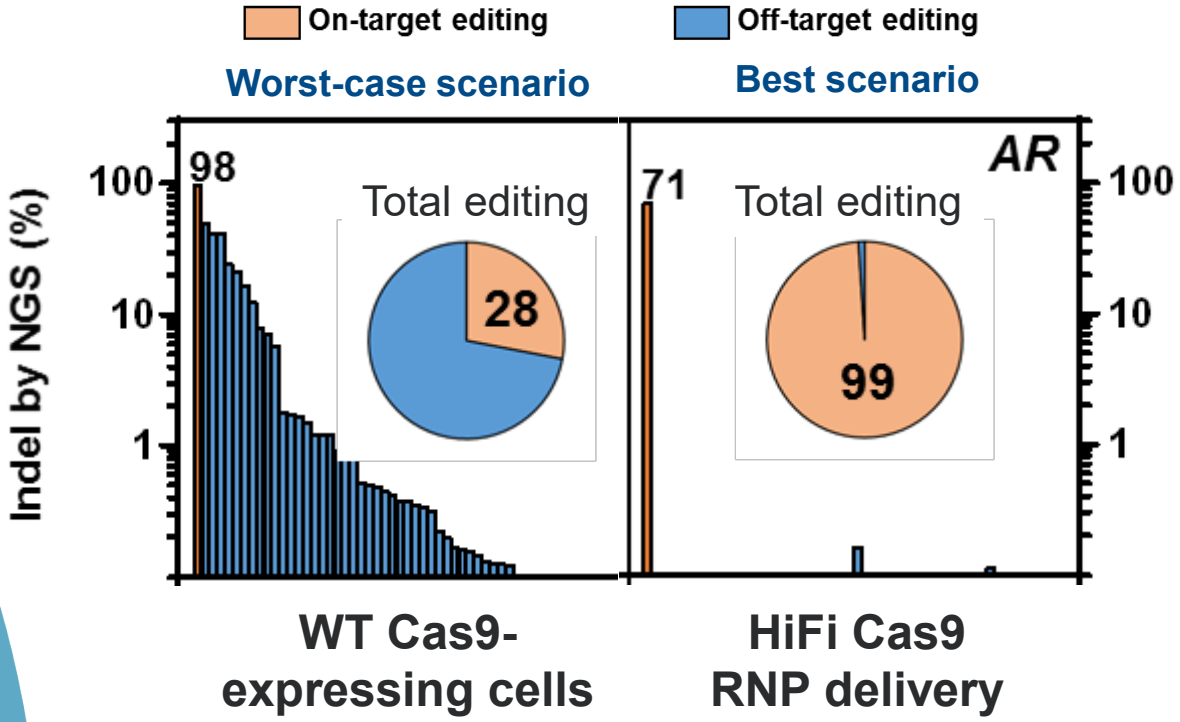
rhAmpSeq DESIGN TOOL

Supported Reference Genomes

Species	Reference genome
Homo sapiens (human)	GRCh38
Homo sapiens (human)	GRCh37
Mus musculus (mouse)	GRCm38
Rattus norvegicus (rat)	Rnor6.0
Bos taurus (cow)	ARS UCD1.2
Bos taurus (cow)	UMD3.1
Brassica napus (canola)	Bra napus v2.0
Caenorhabditis elegans (nematode)	WBcel235
Danio rerio (zebrafish)	GRCz11
Gallus gallus (chicken)	GRCg6a
Glycine max (soybean)	Glycine max v2.1
Gossypium hirsutum (cotton)	ASM98774v1
Oryza sativa (rice)	IRGSP 1.0
Solanum lycopersicum (tomato)	SL3.0
Solanum tuberosum (potato)	SolTub 3.0
Sus scrofa (pig)	Sscrofa11.1
Triticum aestivum (wheat)	IWGSC v1.0
Zea mays (maize)	B73 RefGen v4

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

【基礎研究から臨床応用まで利用可能 ： GMPグレード製品】

Aldevron CRISPRヌクレアーゼ

遺伝子編集を利用して遺伝子疾患や遺伝性疾患の調査・治療を行うには、このヌクレアーゼが臨床への近道です。なぜなら研究グレードと同等で、完全なcGMPで製造されており、規制当局への提出に必要な品質文書でサポートされているからです。

CRISPR with confidence. 詳しくはこちらから。

Aldevron SpCas9 Nuclease and SpyFi® Nuclease

ADD TO ORDER

Recombinant Streptococcus pyogenes Cas9 nuclease, purified from an E. coli strain expressing the SpCas9 nuclease. Contain N- and C-terminal SV40 nuclear localization sequences (sNLS) and no affinity purification tags. Provided in solution at 10 mg/mL. SpyFi® Cas9 nuclease has similar on-target potency to wild-type SpCas9, but with reduced off-target effects, allowing for more precise genome editing.

Quantity	Product	Catalog #	Price
0	sNLS-SpCas9-sNLS Nuclease, 0.25 MG	10017687	¥44,760 JPY
0	sNLS-SpCas9-sNLS Nuclease, 5 MG	10017688	¥744,600 JPY
0	SpyFi Cas9 Nuclease, 0.25 MG	10017689	¥69,700 JPY
0	SpyFi Cas9 Nuclease, 5 MG	10017690	¥946,500 JPY

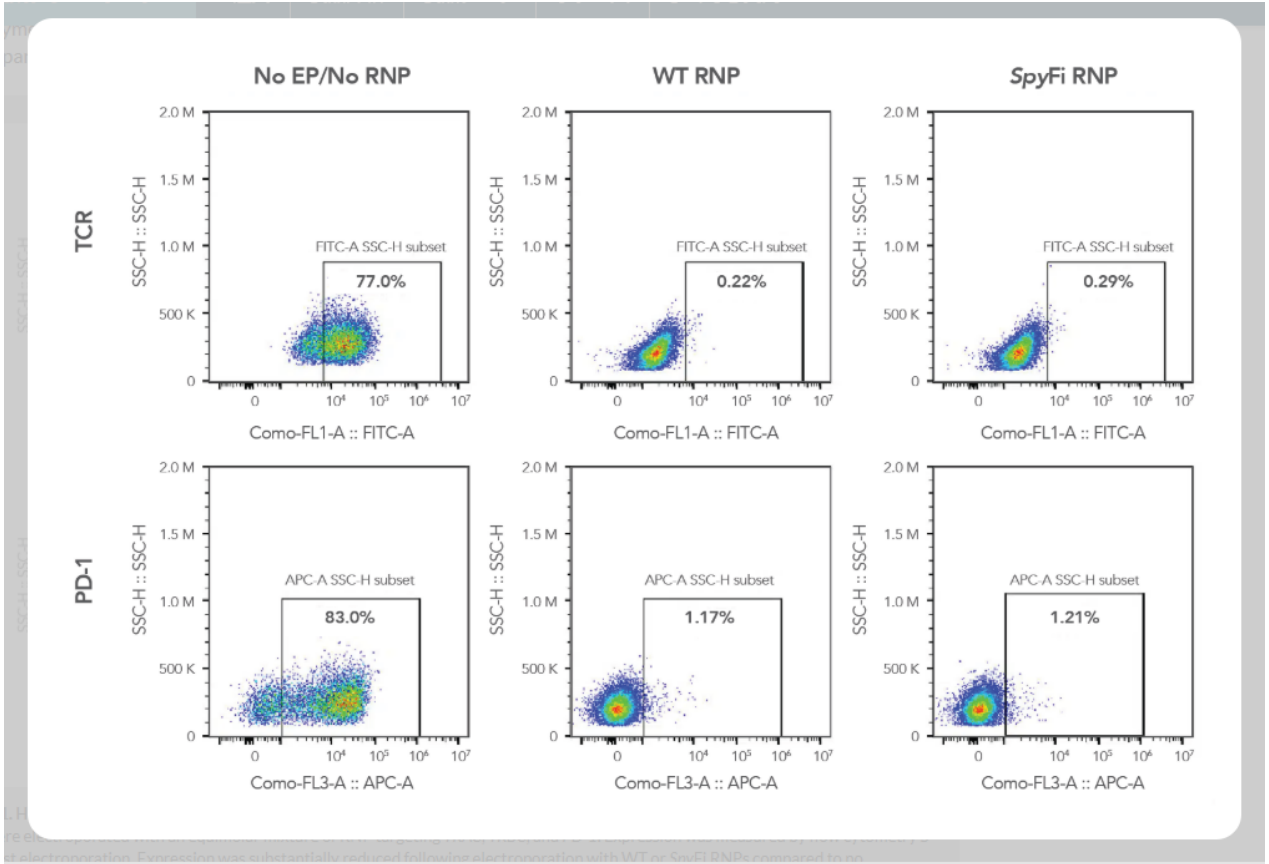
Aldevron Eureca-V™ Nuclease

ADD TO ORDER

Recombinant Class 2, Type V CRISPR-Cas nuclease, purified from an E. coli strain expressing the recombinant nuclease. Contains N- and C-terminal SV40 nuclear localization sequences (sNLS) and no affinity purification tags. Provided in solution at 10 mg/mL. Requires acknowledgment of Limited Use Label License (LULL).

Quantity	Product	Catalog #	Price
0	Eureca-V Nuclease, 1MG	10017800	¥211,500 JPY
0	Eureca-V Nuclease, 5MG	10017811	¥964,700 JPY

High-efficiency multiplexed T cell editing



High multiplexed knockout efficiency with wild type (WT) and *Spy*Fi RNP. To knockout both *TCR* and *PD-1*, activated human T cells were electroporated with an equimolar mixture of RNP targeting *TRAC*, *TRBC*, and *PD-1*. Expression was measured by flow cytometry 5 days post electroporation. Expression was substantially reduced following electroporation with WT or *Spy*Fi RNPs compared to no electroporated cells, $n = 1$.

Reduced off-target effects

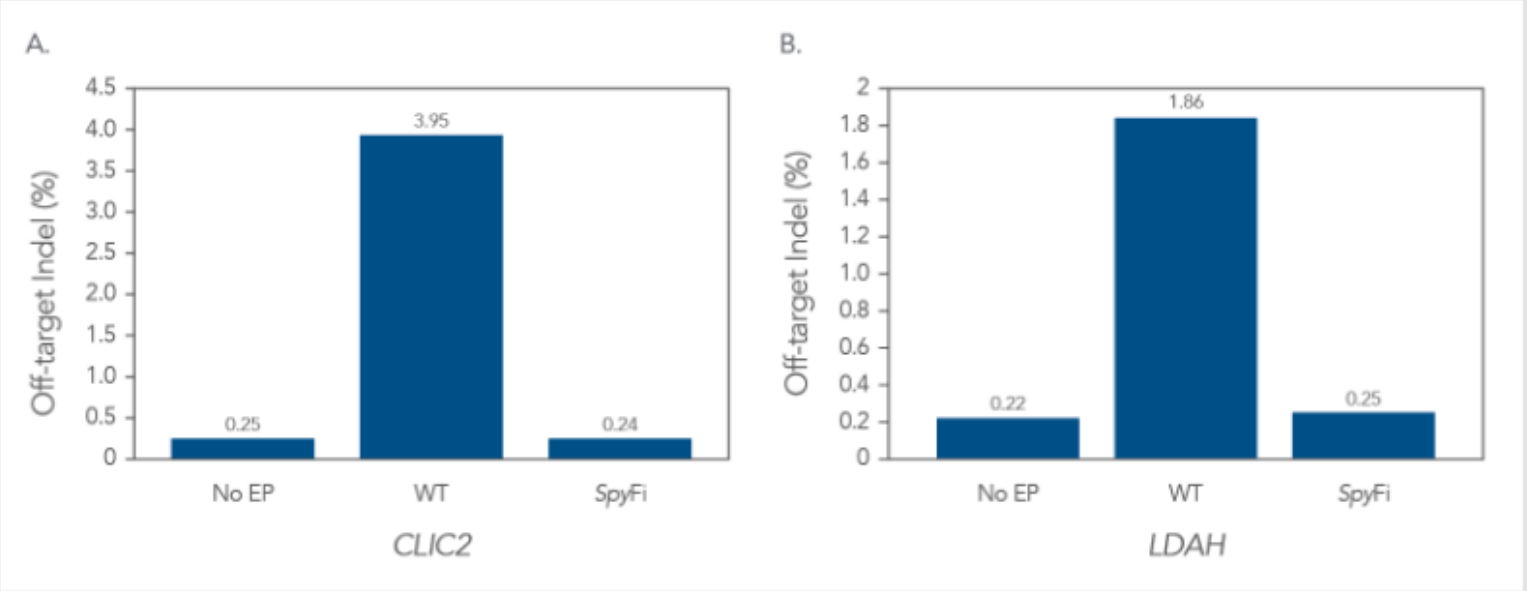


Figure 2. Fewer off-target events with *Spy*Fi vs WT RNP. SpCas9 or *Spy*Fi Cas9 RNP targeting the *TRAC* locus was transfected into activated human T cells using Maxcyte electroporation (EP) technology. The frequency of off-targeting editing for reach RNP was determined by measuring InDel percentages at four off-target binding sites previously identified by iGUIDE [1] . DNA flanking off-target binding sites in *CLIC2*, *LDAH*, *ANKS1B*, and *ADCY10* were amplified by PCR, gel-purified, and sequenced by next generation sequencing (NGS). (A) At the *CLIC2* locus *Spy*Fi RNP reduced off target editing by 94% compared to WT RNP. (B) Similarly, at the *LDAH* locus there was an 87% reduction in off-target editing. At both these loci *Spy*Fi had off-target effects comparable to no electroporation controls. At two other sites (*ANKS1B* and *ADCY10*), off-target editing was generally less frequent but was diminished by *Spy*Fi (33% less at *ANKS1B* locus, 15% less at *ADCY10*, data not shown), *n*=1.

CRISPR cGMP gRNA Manufacturing Overview

For customers looking to accelerate their CRISPR gene editing program from discovery to clinical trials, our Engineering Run and full cGMP Compliant gRNA Manufacturing Services offer a streamlined and regulated solution. Backed by comprehensive documentation, our guide RNA CRISPR services simplify regulatory filings, offering a straightforward path to clinical success.

Lab to life changing advances. 私たちがお手伝いします。

	Engineering Run	cGMP
Availability	Delivery times after placing the order	Delivery times after placing the order
Cleanroom	Certification not required	ISO 8 Clean Room – Certified
Changeover	In-place with cleaning validation	In-place with cleaning validation
Materials	QA release on raw materials	QA release on raw materials
Batch Records	Draft based on customer's specifications	Based on customer specification
Release Testing	Qualified methods	Validated methods

ゲノム編集でのHIBITの利用

1. HiBiT とは何か
   HiBiT は NanoLuc ルシフェラーゼを分割した 11 アミノ酸（33 Nucleotide seq）の超小型ペプチドタグ
   ゲノム編集で 内在性遺伝子の末端にノックインすると、細胞内で LgBiT と結合して強い発光シグナルを出す

2. ゲノム編集における最大のメリット
   **HDR ノックインが「成功したかどうか」を最短・最も確実に判断できる

『内在性タンパク質のノックイン成功を、迅速・高感度・定量的に評価できる点』

従来のノックイン確認方法の課題

方法	課題
PCR / シーケンス	時間がかかる、労力大、スループット低
Western blot	抗体依存、感度・特異性にばらつき
蛍光タグ（GFP等）	タグが大きく、HDR効率が下がる／機能影響

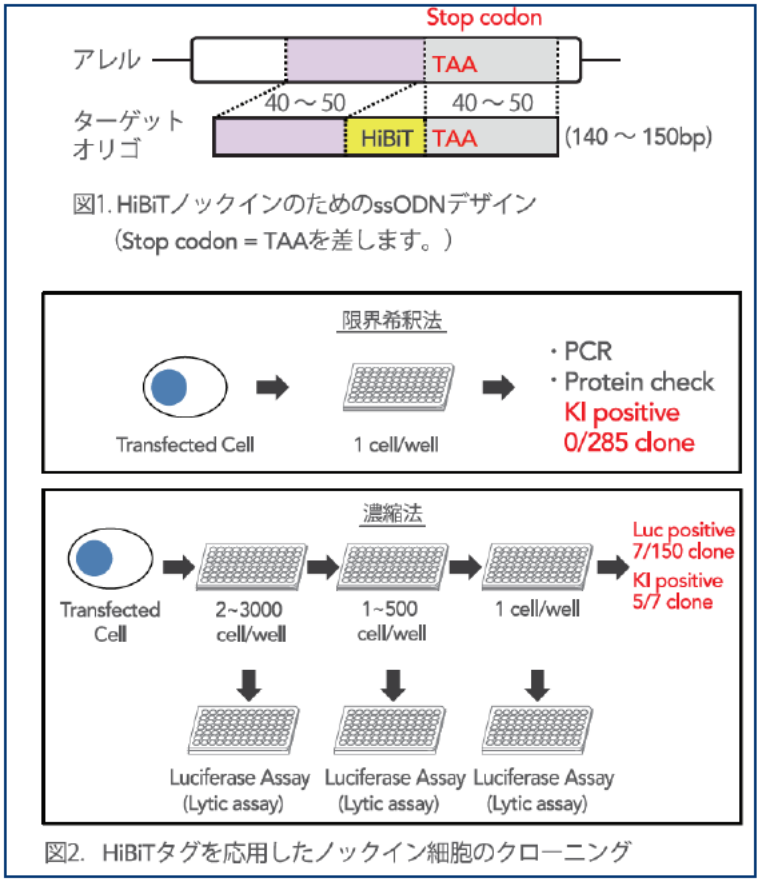
HiBiT を使うと何が変わるか

ポイント	HiBiT の強み
タグサイズ	11 aa（極小） → HDR成功率が高い
検出	**超高感度発光**
定量性	発光＝タンパク質量（半定量〜定量）
操作	細胞溶解→試薬添加のみ
スピード	**編集後 24–48 時間で評価可能**

「HDR がどれくらい起きたか」を“見える数字”で即判断できる
タンパク質の定量が可能

＊注意点：タグ挿入位置の設計（最重要）、フレーム設計（HiBiT はここに非常に厳しい）など慎重に確認する事が重要なポイントとなります。万が一塩基にズレが生じた場合、フレームシフトが起きて正しいタンパク発現が起きずに、発光での検出も出来なくなってしまいます。

例：細胞のクローニング作業を簡便化



まとめ

ALT-R™CRISPR CAS9 SYSTEM の初期費用の目安

gRNA:2パートシステム

例①:ヒト/マウス/ラット対象、ターゲット遺伝子用とコントロール用、及びCas9タンパク質を購入

- **Alt-R® CRISPR crRNA, 2 nmol（ターゲット遺伝子） 10,300円**
- **2 nmol Alt-R® CRISPR Control Kit 22,120円**
  - **tracrRNA、crRNA（HPRT）、crRNA（Negative)、プライマーセット、バッファー**
- **Alt-R® S.p. Cas9 Nuclease V3, 100 µg 24,860円**

初回費用定価合計
￥57,280～

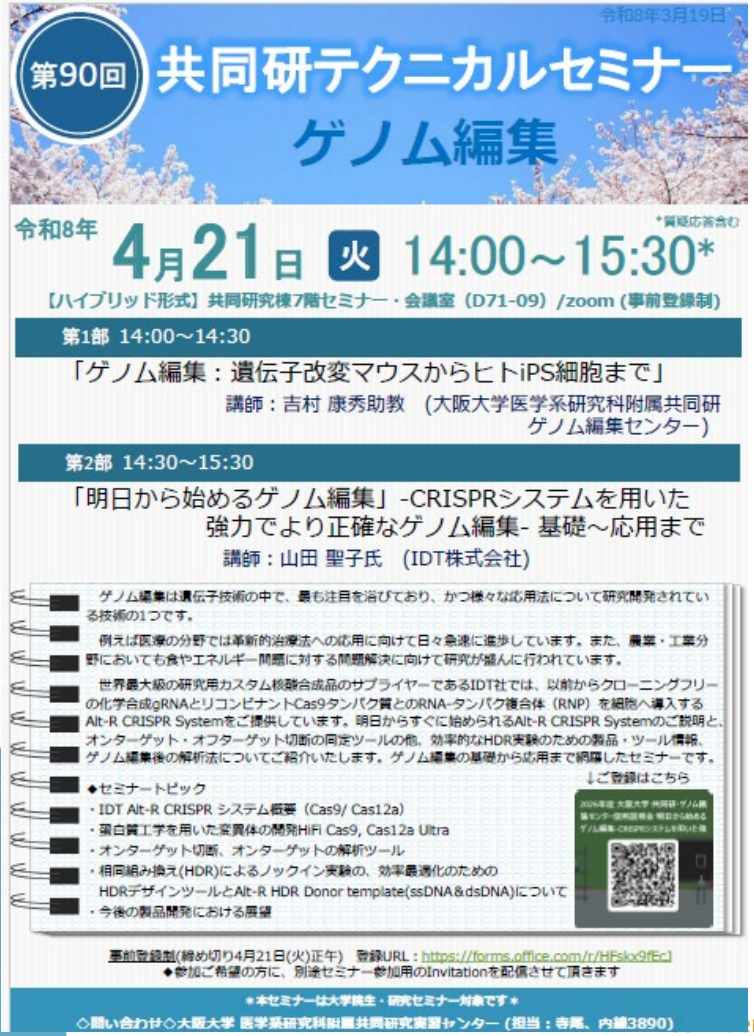
例②:ターゲット遺伝子用のみ購入、あるいはヒト/マウス/ラット以外の実験用に購入

- **Alt-R® CRISPR crRNA, 2 nmol 10,300円**
- **5 nmol Alt-R® CRISPR tracrRNA 12,160円**
- **Alt-R® S.p. Cas9 Nuclease V3, 100 µg 24,860円**

初回費用定価合計
￥47,320～

※ Alt-R® S.p. HiFi Cas9 Nuclease V3, 100 µg 30,200円
※ Alt-R® Genome Editing Detection Kit, 25 rxn 19,040円
※ **2パートシステムの場合、残ったtracrRNAは、新たに購入する別のcrRNA（10,300円）と一緒に使用できます。**

★初回ご注文時は、該当品目に限り30%オフでのトライアルキャンペーンを実施しております



◆大阪大学大学院医学系研究科附属共同研ゲノム編集センターゲノム編集サポート事業

◆ポイント

・IDTではゲノム編集関連製品をワークフローで揃える事が出来る

・設計サポート（有償）

・ゲノム編集分野では、高いシェアを誇り安心して使用できる

・製品開発に力を入れている

ご購入の際は、是非ゲノム編集サポート事業をご利用ください

◆共同研へのご質問・お問い合せ
order@ctrlab.med.osaka u.ac.jp

〒565-0871 大阪府吹田市山田丘 2-2
大阪大学大学院医学系研究科附属共同研
ゲノム編集センター

THANK YOU

記載内容については予告なく変更される可能性があります。最新情報は弊社HPでご確認いただくか、下記までお問合せください。

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03-4510-4061