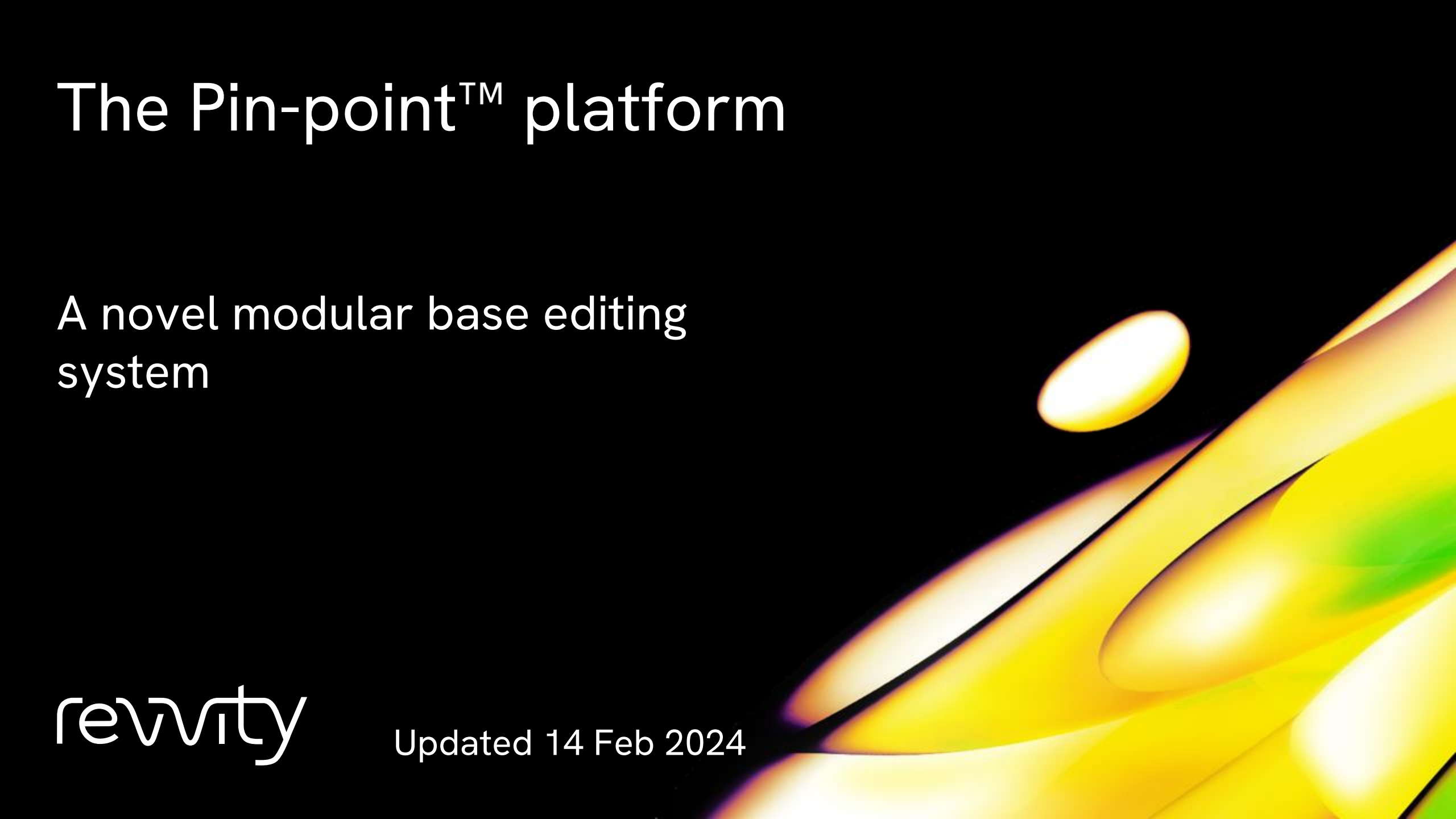
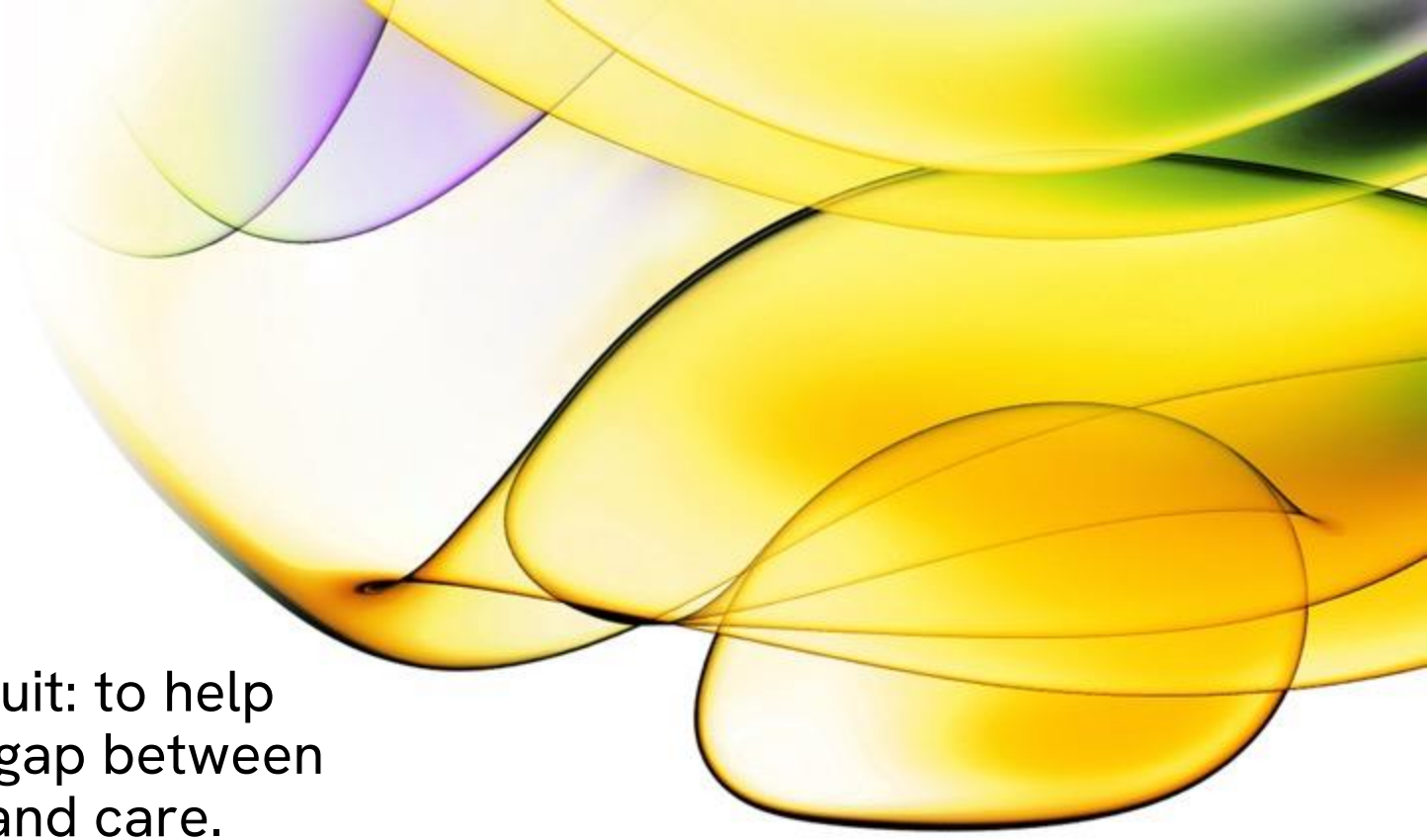
The Pin-point™ platform

A novel modular base editing system



Updated 14 Feb 2024

revvity

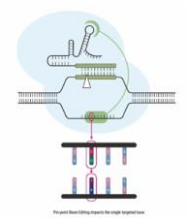


Revvity is born of a single-minded pursuit: to help improve human health by bridging the gap between science and people through precision and care.

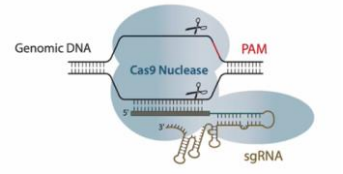
We innovate and collaborate to empower our partners to see science in unexpected ways that deliver breakthrough results.

Revvity's Cell & Gene Therapy Research Portfolio

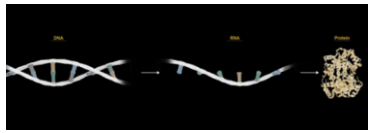
GENE EDITING & MODULATION



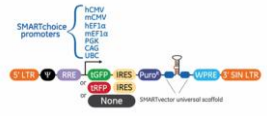
Base editing



CRISPR-Cas9

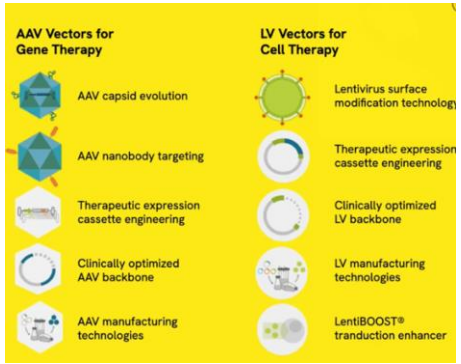


Dharmacon RNAi

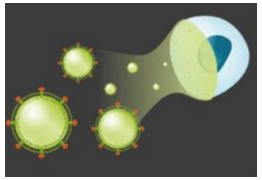


shRNA & siRNA

VIRAL VECTORS

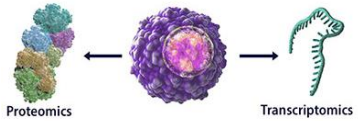


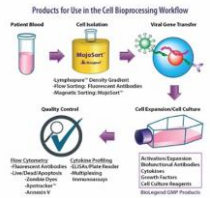
AAV and Lentiviral development and production



LentiBoost
Improved lentiviral transduction

CELL ANALYSIS





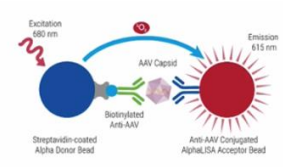
Total-Seq

Cell selection, culture and proteogenomics

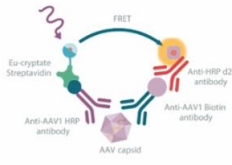
CELL COUNTING

Cell viability, potency and yield

DISCOVERY & QC



AlphaLISA General Assay Principle

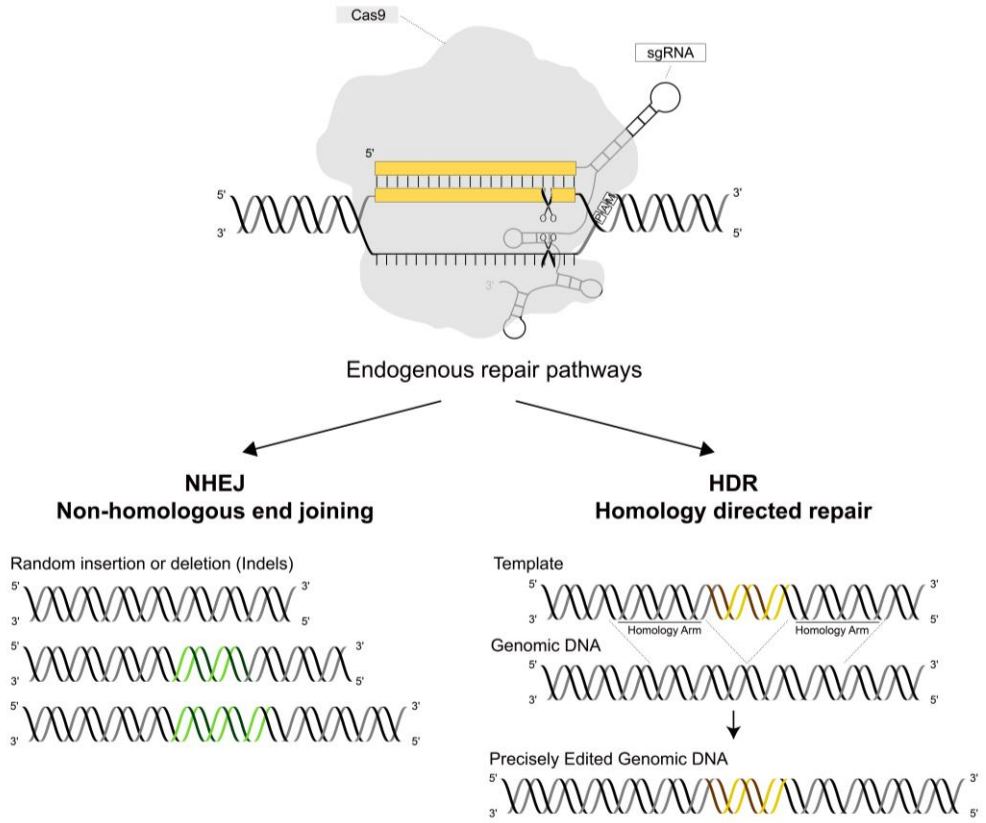


HTRF General Assay Principle

LabChip AAV empty/full

Cell and AAV characterization and QC

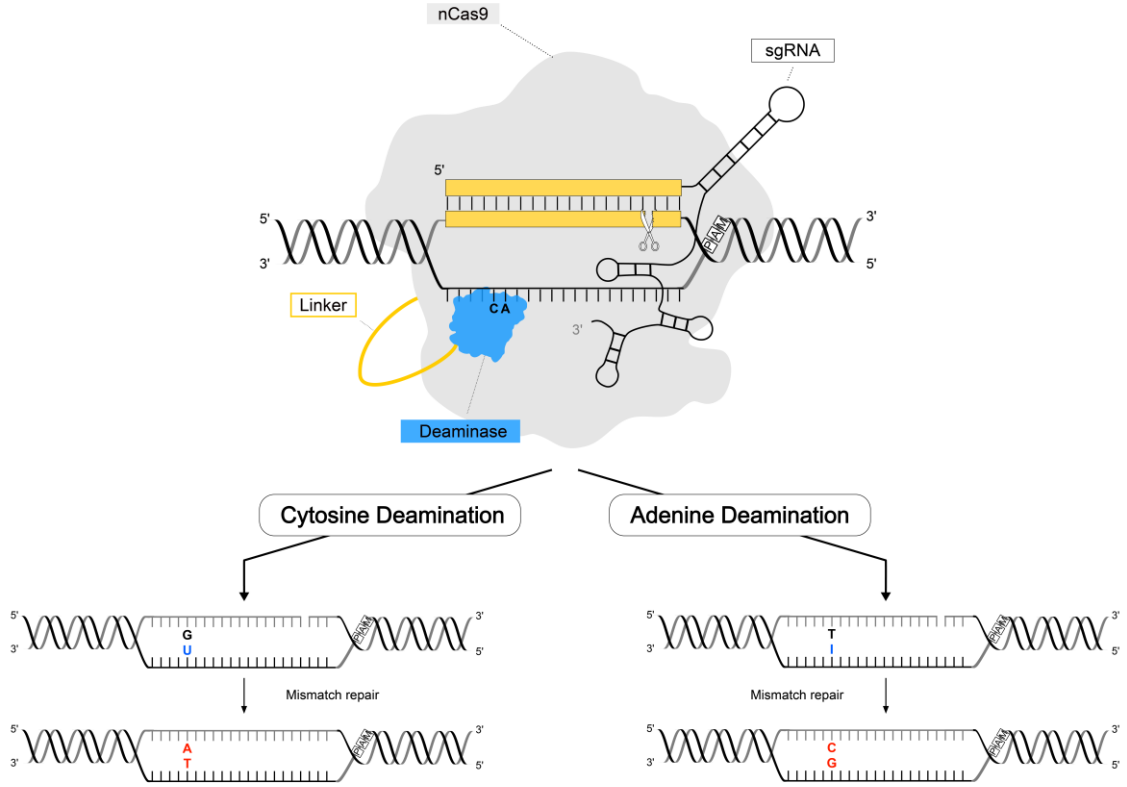
CRISPR gene editing



GENE DISRUPTION BY A DSDNA BREAK

- Indel formation to disrupt gene sequence
- complex population of indels

Base editing



GENE MODIFICATION BY POINT MUTATIONS

- Creation of stop codons or splice site disruption for knockout
- Introduction of single base conversion

The gene editing evolution is now

1st generation Cas enzymes

Gene disruption by a dsDNA break

2nd generation base editing

Gene modification by point mutation

- *creation of stop codons or splice site disruption for knockout*
- *not reliant on dsDNA break*
- *introduction of single base conversion*

New generation Pin-point™ base editing system

- ☑ Predictable, precise and efficient single and multi-gene editing
- ☑ Simultaneous knock-in and knockout in a single reaction
- ☑ Nuclease and deaminase flexible
- ☑ Modular control over target and editing window to specifically reach your gene of interest

Why choose the Pin-point™ system?

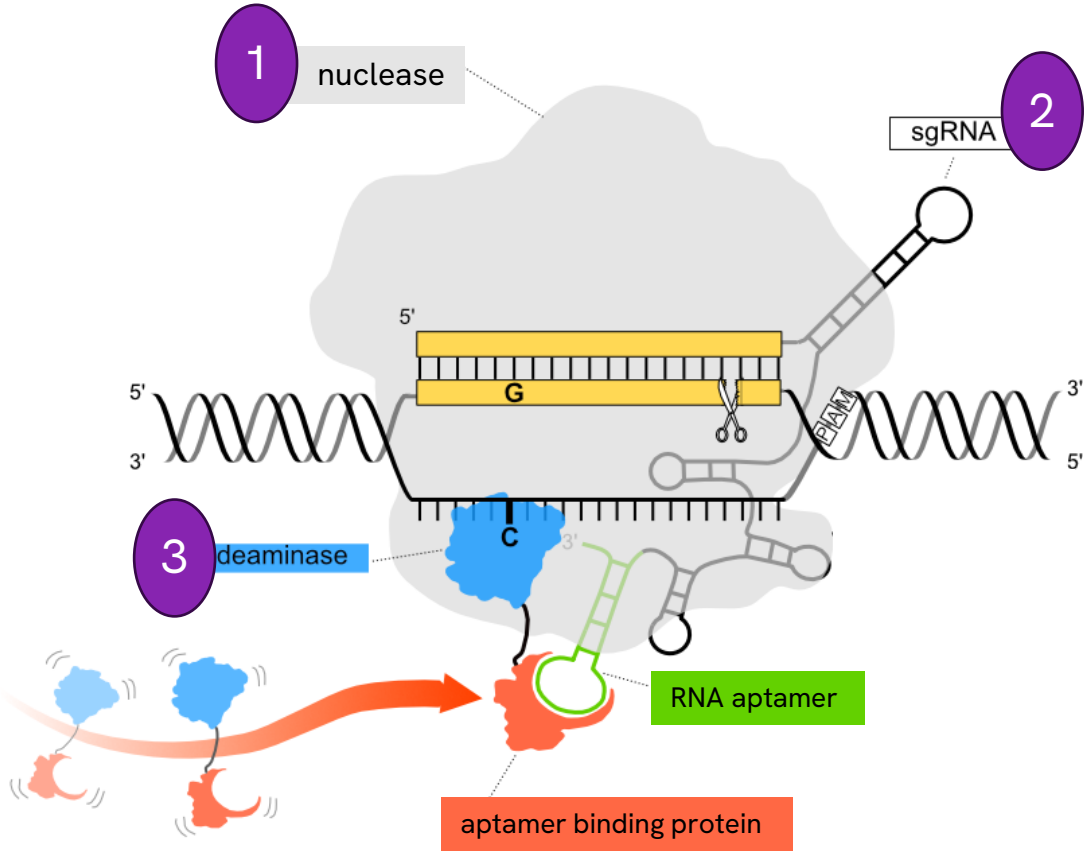
Novel, patented aptamer-recruited base editing platform that can be optimized for your research

Modular, tunable system helps you to reach your targets of interest

Exemplary safety profile with reduced unintended impact on cell viability or functionality

What is the Pin-point™ system?

Based on a patented aptamer-recruited base editing arrangement



*Schematic depicts nCas9 configuration

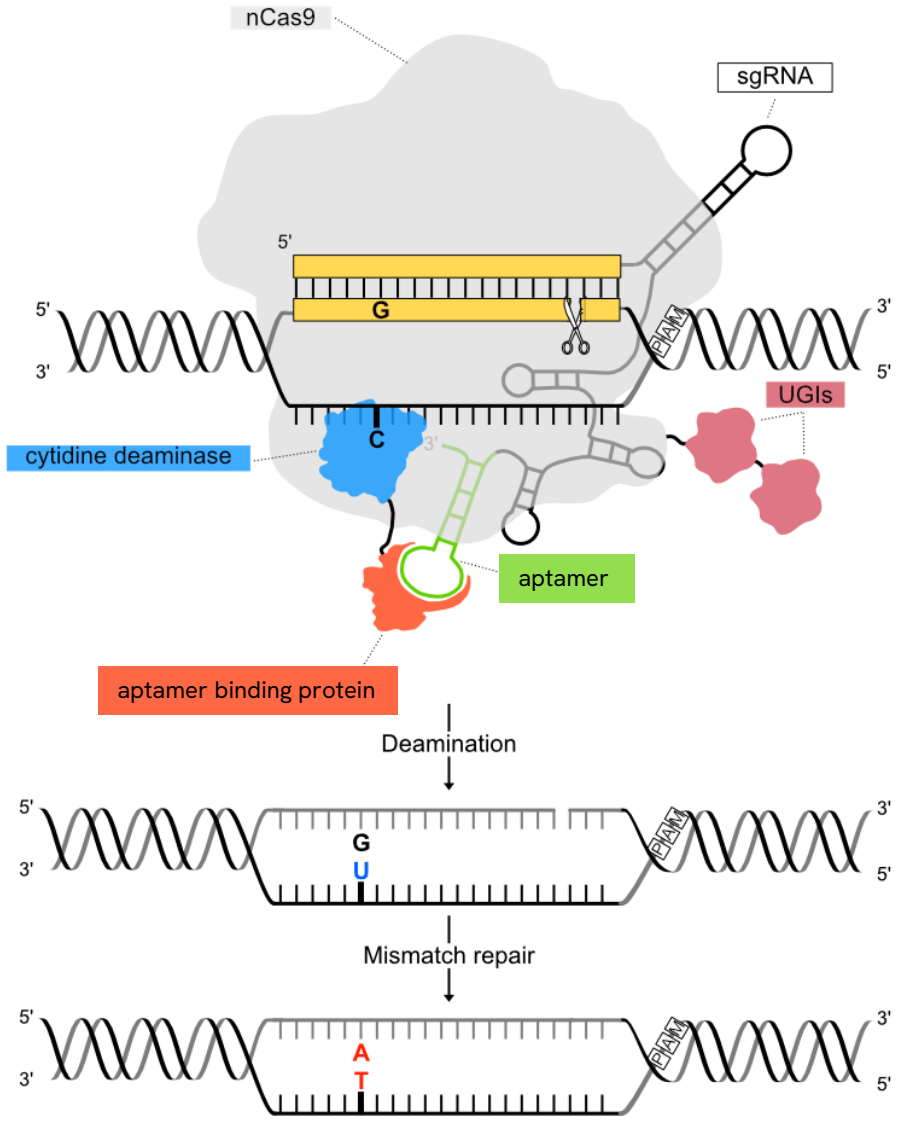
3 component system

1. RNA-guided enzyme
2. Deaminase and recruitment protein
3. Guide RNA with aptamer

Demonstrated advantages

- ☑ Multiplex gene editing including knock-in and knockout with high efficiency and safety
- ☑ Validated performance in T cells, iPSCs, and HSPCs
- ☑ Mix-and-match for target specificity and efficiency

Base editing terminology



"Base editing window"

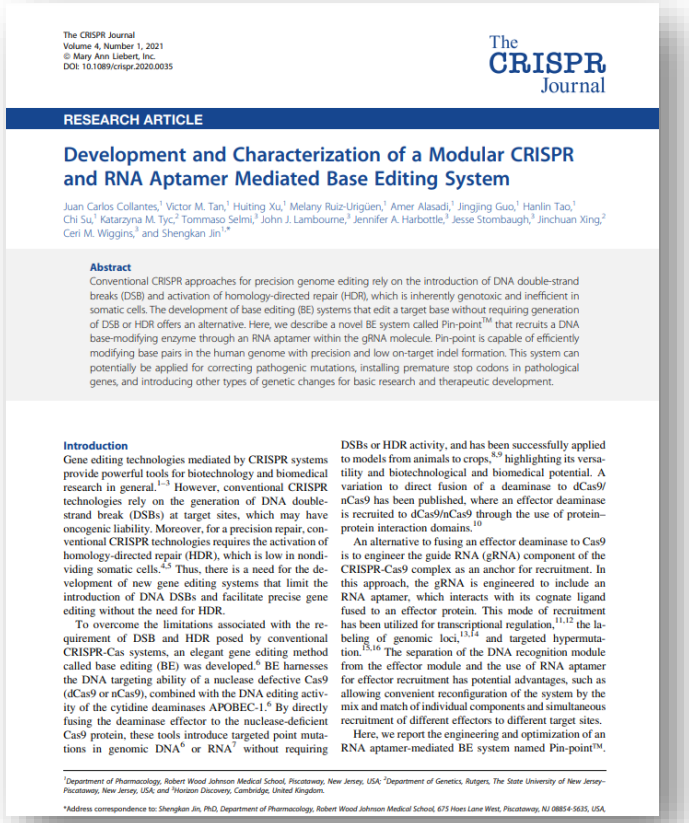
5′	20bp spacer																			3′
N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	G	G
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	PAM	

nCas9/rat APOBEC is most likely to edit C's in positions 4-7

"Bystander editing" is any editing other than the target base of interest

"Off-target editing" is any editing other than at the locus that is targeted

The Pin-point™ base editing technology is accelerating therapeutic development research



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The CRISPR Journal

RESEARCH ARTICLE

Development and Characterization of a Modular CRISPR and RNA Aptamer Mediated Base Editing System

Juan Carlos Collantes,[1] Victor M. Tan,[1] Huiting Xu,[1] Melany Ruiz-Urigüen,[1] Amer Alasadi,[1] Jingjing Guo,[1] Hanlin Tao,[1] Chi Su,[1] Katarzyna M. Tyc,[2] Tommaso Selmi,[3] John J. Lambourne,[3] Jennifer A. Harbottle,[3] Jesse Stombaugh,[3] Jinchuan Xing,[2] Ceri M. Wiggins,[3] and Shengkan Jin[1,*]

Abstract

Conventional CRISPR approaches for precision genome editing rely on the introduction of DNA double-strand breaks (DSB) and activation of homology-directed repair (HDR), which is inherently genotoxic and inefficient in somatic cells. The development of base editing (BE) systems that edit a target base without requiring generation of DSB or HDR offers an alternative. Here, we describe a novel BE system called Pin-point™ that recruits a DNA base-modifying enzyme through an RNA aptamer within the gRNA molecule. Pin-point is capable of efficiently modifying base pairs in the human genome with precision and low on-target indel formation. This system can potentially be applied for correcting pathogenic mutations, installing premature stop codons in pathological genes, and introducing other types of genetic changes for basic research and therapeutic development.

Introduction

Gene editing technologies mediated by CRISPR systems provide powerful tools for biotechnology and biomedical research in general.[1–3] However, conventional CRISPR technologies rely on the generation of DNA double-strand break (DSBs) at target sites, which may have oncogenic liability. Moreover, for a precision repair, conventional CRISPR technologies requires the activation of homology-directed repair (HDR), which is low in nondividing somatic cells.[4,5] Thus, there is a need for the development of new gene editing systems that limit the introduction of DNA DSBs and facilitate precise gene editing without the need for HDR.

To overcome the limitations associated with the requirement of DSB and HDR posed by conventional CRISPR-Cas systems, an elegant gene editing method called base editing (BE) was developed.[6] BE harnesses the DNA targeting ability of a nuclease defective Cas9 (dCas9 or nCas9), combined with the DNA editing activity of the cytidine deaminases APOBEC-1.[6] By directly fusing the deaminase effector to the nuclease-deficient Cas9 protein, these tools introduce targeted point mutations in genomic DNA[6] or RNA[7] without requiring DSBs or HDR activity, and has been successfully applied to models from animals to crops,[8,9] highlighting its versatility and biotechnological and biomedical potential. A variation to direct fusion of a deaminase to dCas9/nCas9 has been published, where an effector deaminase is recruited to dCas9/nCas9 through the use of protein–protein interaction domains.[10]

An alternative to fusing an effector deaminase to Cas9 is to engineer the guide RNA (gRNA) component of the CRISPR-Cas9 complex as an anchor for recruitment. In this approach, the gRNA is engineered to include an RNA aptamer, which interacts with its cognate ligand fused to an effector protein. This mode of recruitment has been utilized for transcriptional regulation,[11,12] the labeling of genomic loci,[13,14] and targeted hypermutation.[15,16] The separation of the DNA recognition module from the effector module and the use of RNA aptamer for effector recruitment has potential advantages, such as allowing convenient reconfiguration of the system by the mix and match of individual components and simultaneous recruitment of different effectors to different target sites.

Here, we report the engineering and optimization of an RNA aptamer-mediated BE system named Pin-point™.

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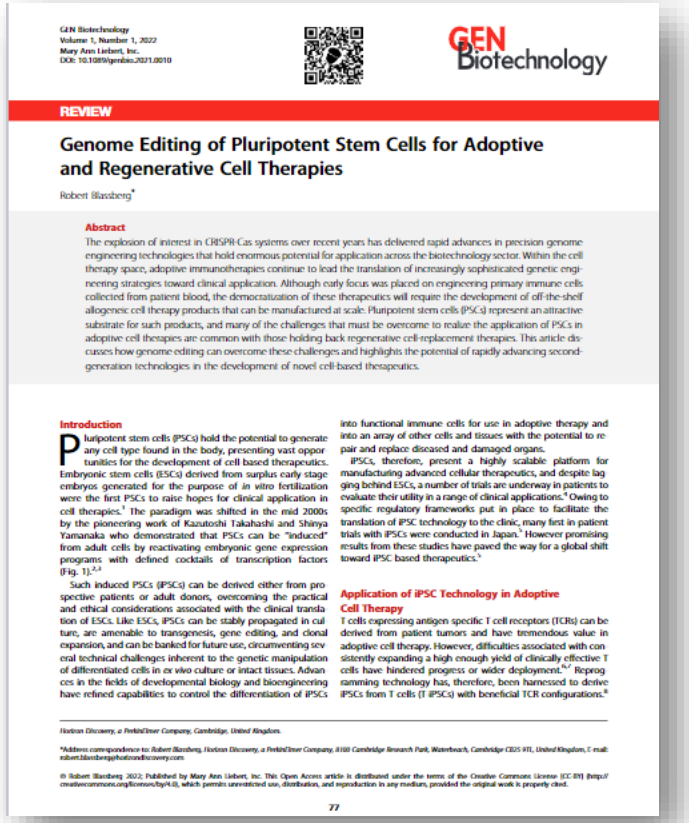
*Address correspondence to: Shengkan Jin, PhD, Department of Pharmacology, Robert Wood Johnson Medical School, 675 Hoes Lane West, Piscataway, NJ 08854-5635, USA



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Volume 1, Number 1, 2022
Mary Ann Liebert, Inc.
DOI: 10.1089/genbio.2021.0010

GEN Biotechnology

REVIEW

Genome Editing of Pluripotent Stem Cells for Adoptive and Regenerative Cell Therapies

Robert Blassberg*

Abstract

The explosion of interest in CRISPR-Cas systems over recent years has delivered rapid advances in precision genome engineering technologies that hold enormous potential for application across the biotechnology sector. Within the cell therapy space, adoptive immunotherapies continue to lead the translation of increasingly sophisticated genetic engineering strategies toward clinical application. Although early focus was placed on engineering primary immune cells collected from patient blood, the democratization of these therapeutics will require the development of off-the-shelf allogeneic cell therapy products that can be manufactured at scale. Pluripotent stem cells (PSCs) represent an attractive substrate for such products, and many of the challenges that must be overcome to realize the application of PSCs in adoptive cell therapies are common with those holding back regenerative cell-replacement therapies. This article discusses how genome editing can overcome these challenges and highlights the potential of rapidly advancing second-generation technologies in the development of novel cell-based therapeutics.

Introduction

Pluripotent stem cells (PSCs) hold the potential to generate any cell type found in the body, presenting vast opportunities for the development of cell based therapeutics. Embryonic stem cells (ESCs) derived from surplus early stage embryos generated for the purpose of *in vitro* fertilization were the first PSCs to raise hopes for clinical application in cell therapies.[1] The paradigm was shifted in the mid 2000s by the pioneering work of Kazutoshi Takahashi and Shinya Yamanaka who demonstrated that PSCs can be "induced" from adult cells by reactivating embryonic gene expression programs with defined cocktails of transcription factors (Fig. 1).[2,3]

Such induced PSCs (iPSCs) can be derived either from prospective patients or adult donors, overcoming the practical and ethical considerations associated with the clinical translation of ESCs. Like ESCs, iPSCs can be stably propagated in culture, are amenable to transgenesis, gene editing, and clonal expansion, and can be banked for future use, circumventing several technical challenges inherent to the genetic manipulation of differentiated cells in *ex vivo* culture or intact tissues. Advances in the fields of developmental biology and bioengineering have refined capabilities to control the differentiation of iPSCs into functional immune cells for use in adoptive therapy and into an array of other cells and tissues with the potential to repair and replace diseased and damaged organs.

iPSCs, therefore, present a highly scalable platform for manufacturing advanced cellular therapeutics, and despite lagging behind ESCs, a number of trials are underway in patients to evaluate their utility in a range of clinical applications.[4] Owing to specific regulatory frameworks put in place to facilitate the translation of iPSC technology to the clinic, many first in patient trials with iPSCs were conducted in Japan.[5] However promising results from these studies have paved the way for a global shift toward iPSC based therapeutics.[5]

Application of iPSC Technology in Adoptive Cell Therapy

T cells expressing antigen specific T cell receptors (TCRs) can be derived from patient tumors and have tremendous value in adoptive cell therapy. However, difficulties associated with consistently expanding a high enough yield of clinically effective T cells have hindered progress or wider deployment.[6,7] Reprogramming technology has, therefore, been harnessed to derive iPSCs from T cells (T iPSCs) with beneficial TCR configurations.[8]

Horizon Discovery, a PerkinElmer Company, Cambridge, United Kingdom.

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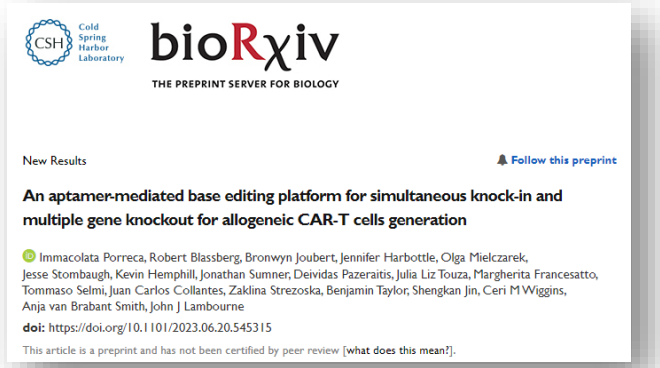

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An aptamer-mediated base editing platform for simultaneous knock-in and multiple gene knockout for allogeneic CAR-T cells generation

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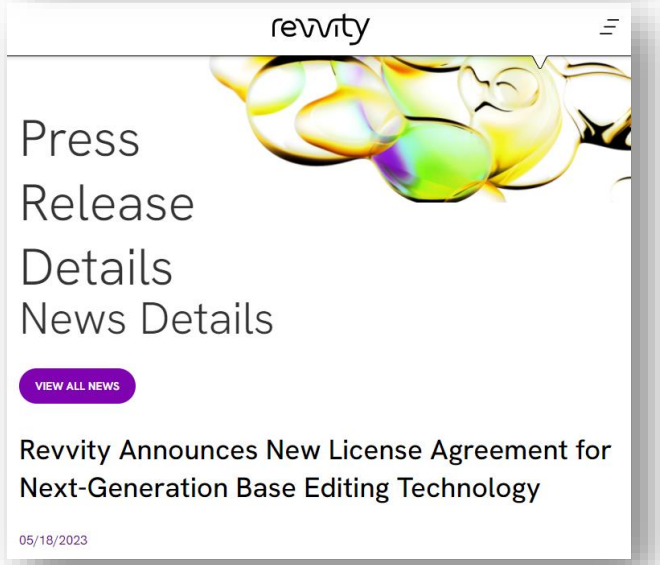
doi: https://doi.org/10.1101/2023.06.20.545315



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Press Release Details

News Details

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Revvity Announces New License Agreement for Next-Generation Base Editing Technology

05/18/2023

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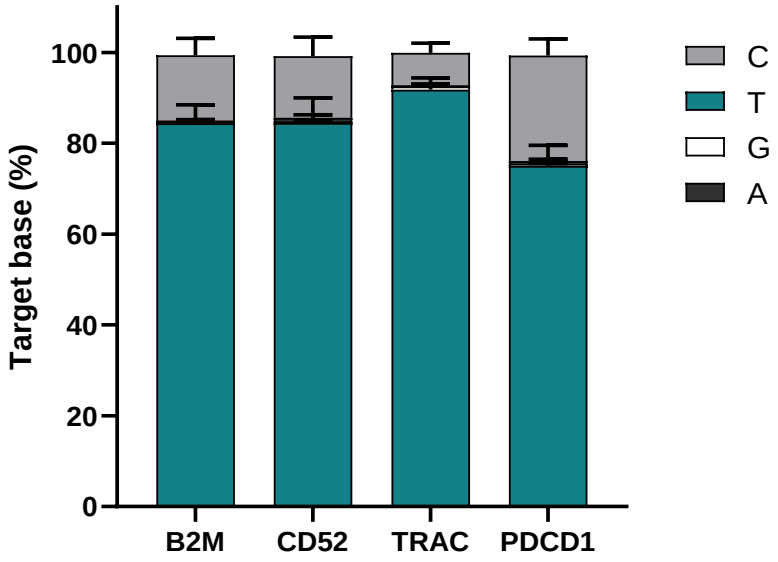
https://doi.org/10.1089/genbio.2021.0010
https://www.biorxiv.org/content/10.1101/2023.06.20.545315v1
Press release

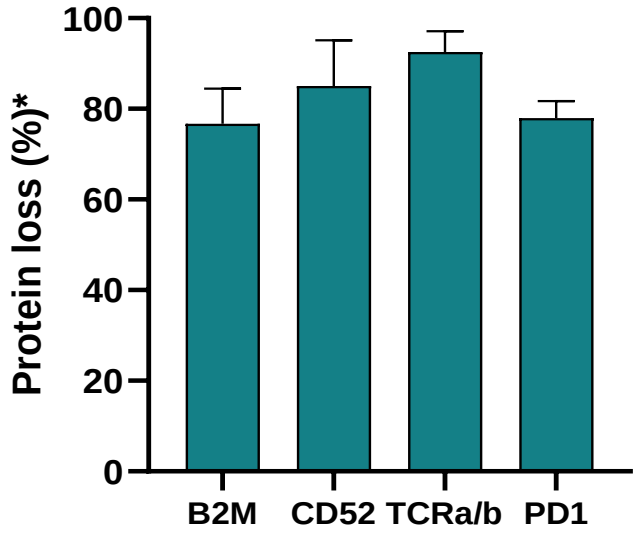
Validated performance in primary T cells

Highly efficient and precise multiplex T cell editing

>75% editing in each target base without enrichment

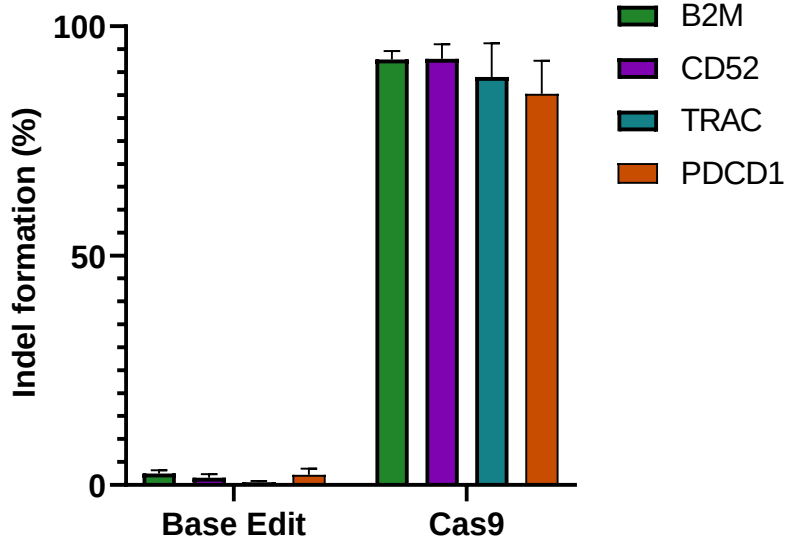


~50% of all 4 proteins knocked out (no enrichment)



*Normalized to controls

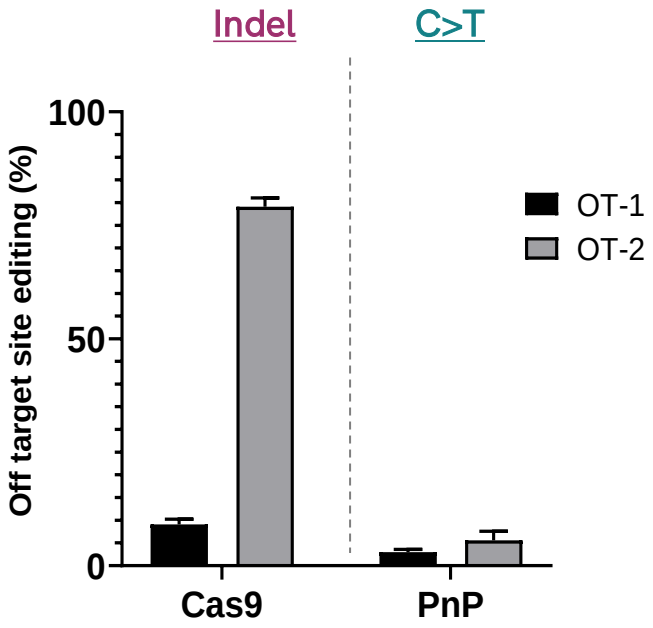
No indel formation



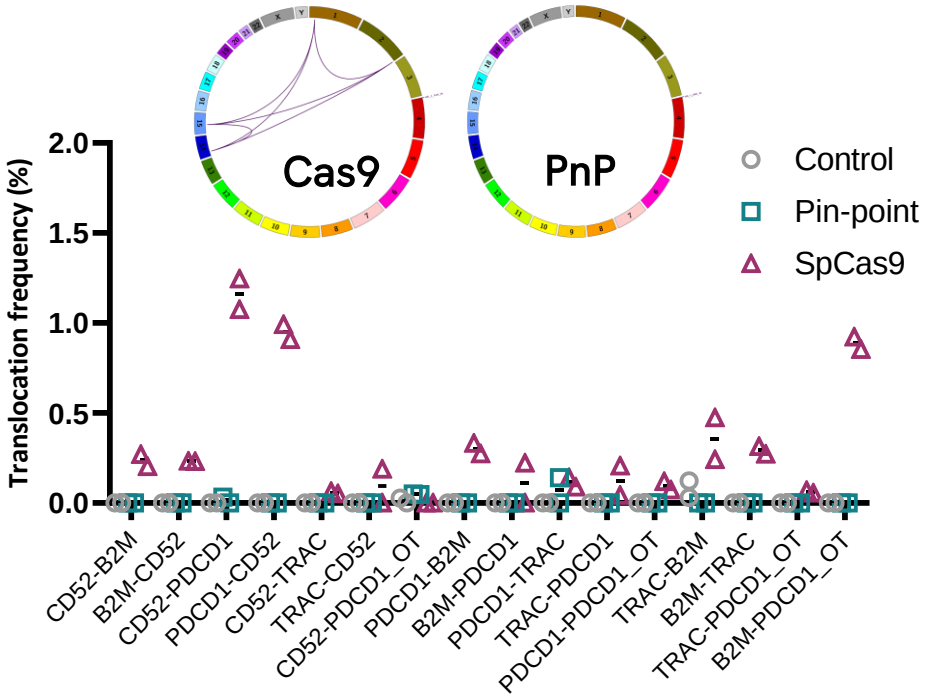
Pin-point™ base editing system is highly efficient and avoids potentially catastrophic DNA damage

Strong safety profile in T cells

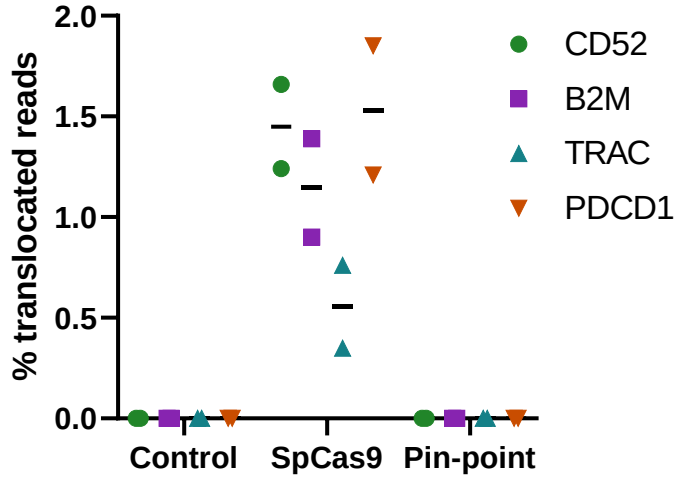
Negligible off-target edits



No detected translocations: targets and known off-target sites



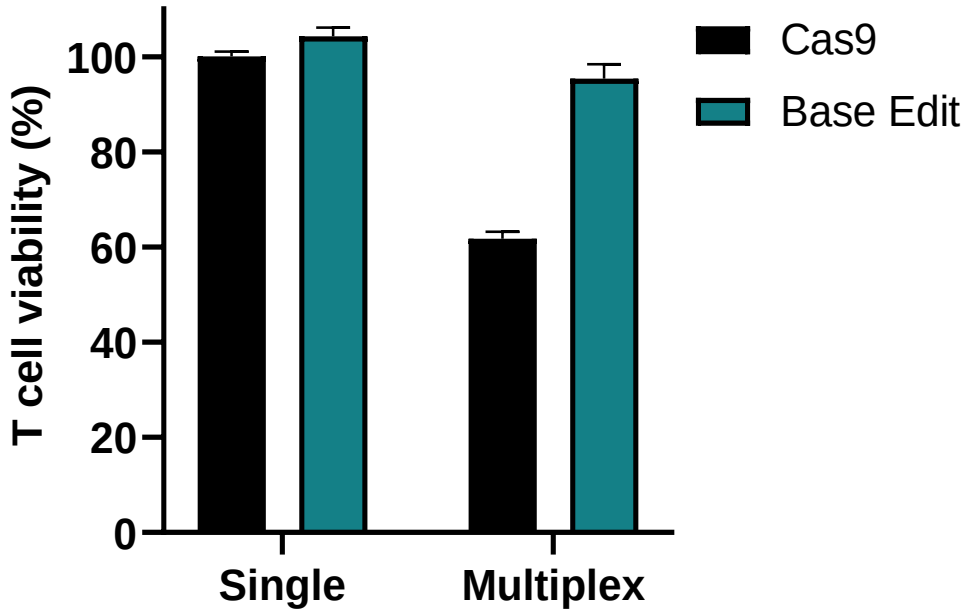
No detected translocations: fusions



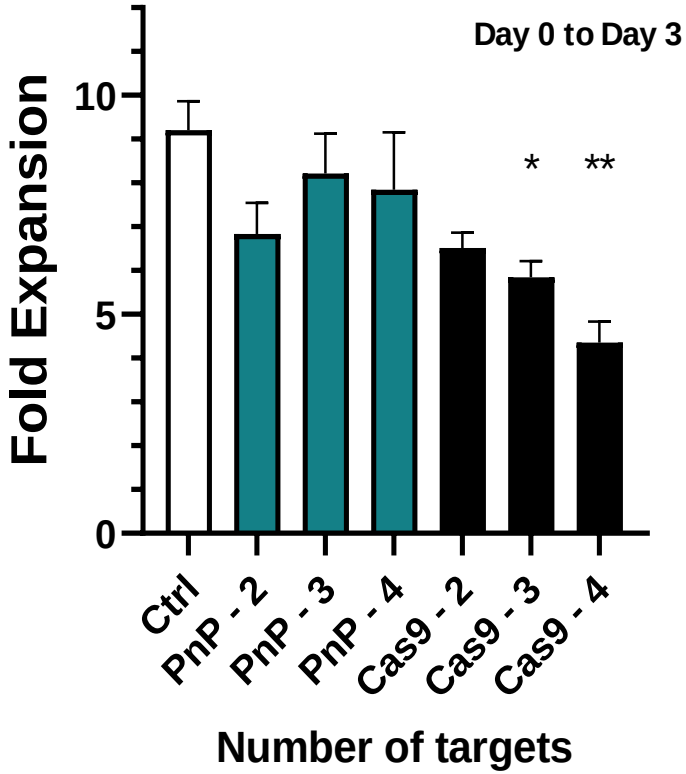
A cleaner and safer approach to multiplex gene editing in T cells

No impact on T cell health

Cell viability maintained



Rate of cell expansion unaffected



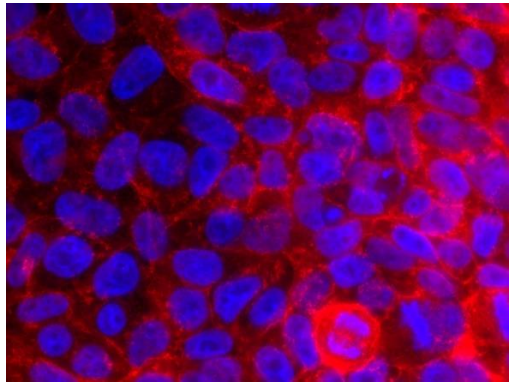
High multiplexing does not compromise cellular health or yield

Biological replicates = 3 | Data normalized to controls

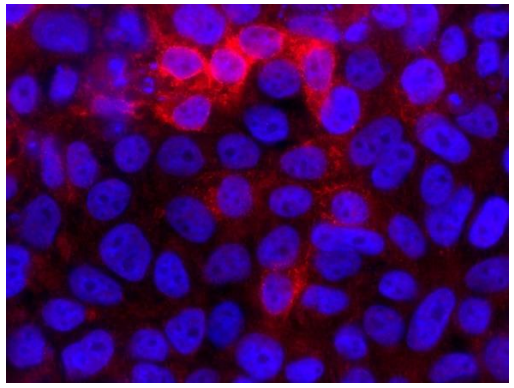
Validated performance in iPSCs

Base editing with a Pin-point™ system in iPSCs

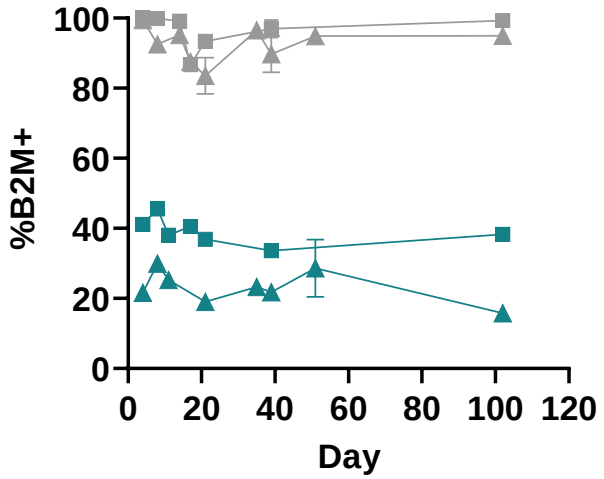
Mock

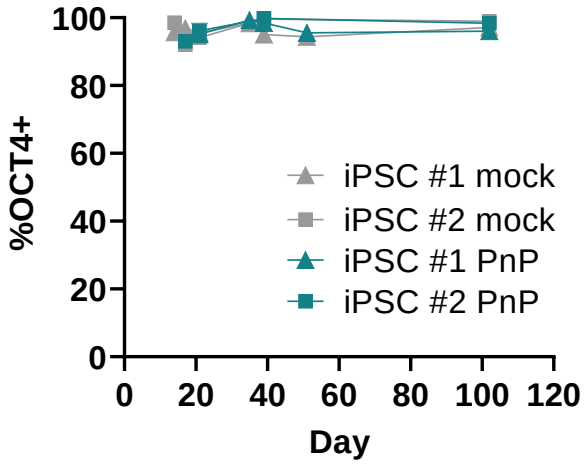


Pin-point BE

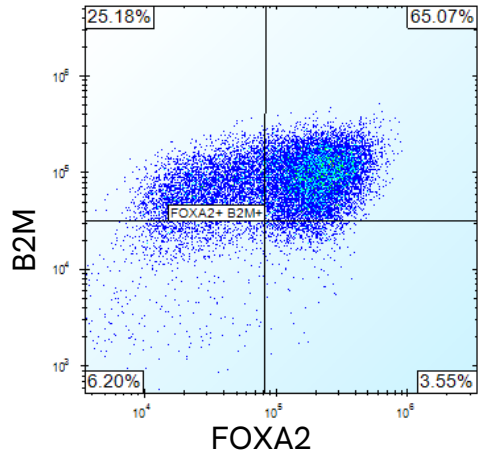


DAPI (nucleus) B2M

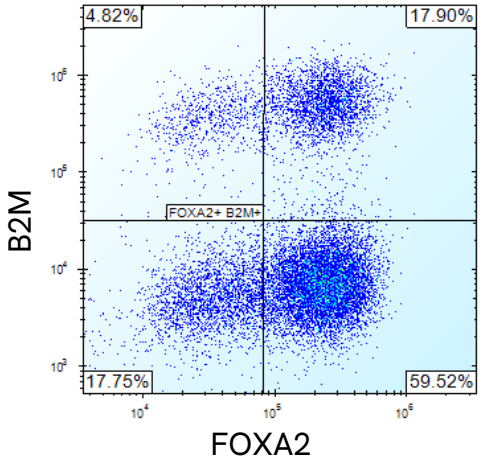




Mock



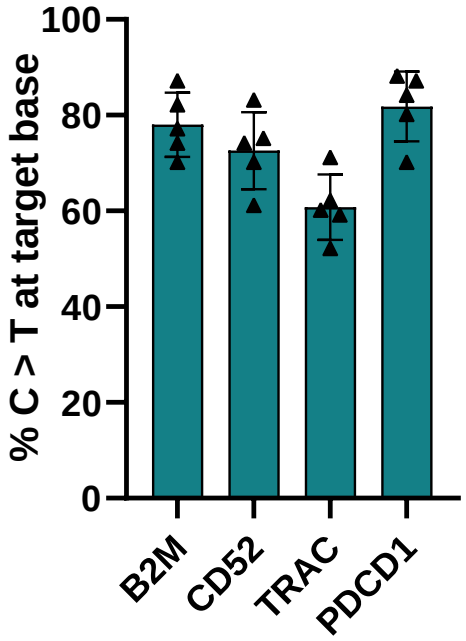
Pin-point BE



Edited iPSCs are stable with no growth defects when cultured up to 100 days and retain differentiation potential

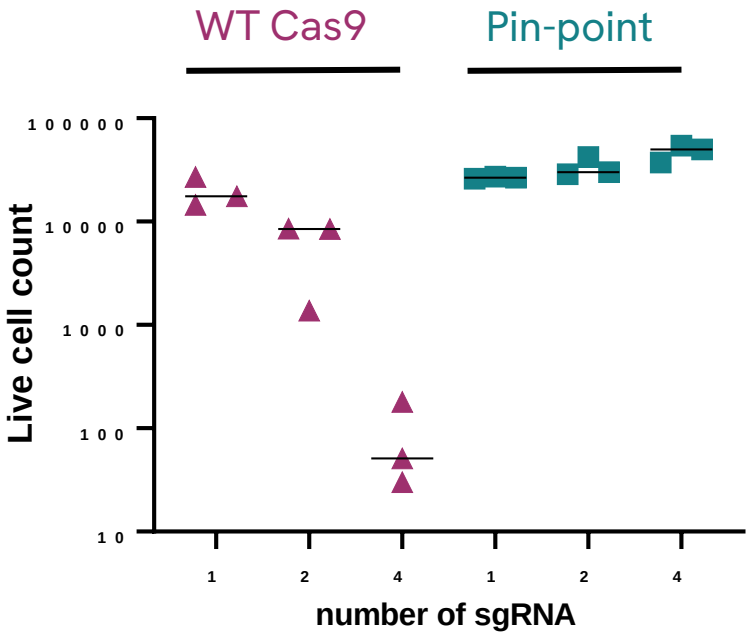
Multi-gene editing in iPSCs

Effective multiplex base editing



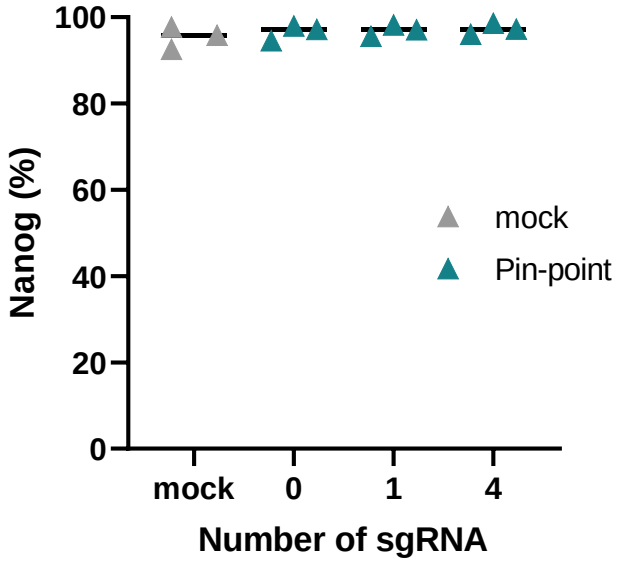
High base editing efficiency at target loci in a multiplex setting

Edited cells are viable



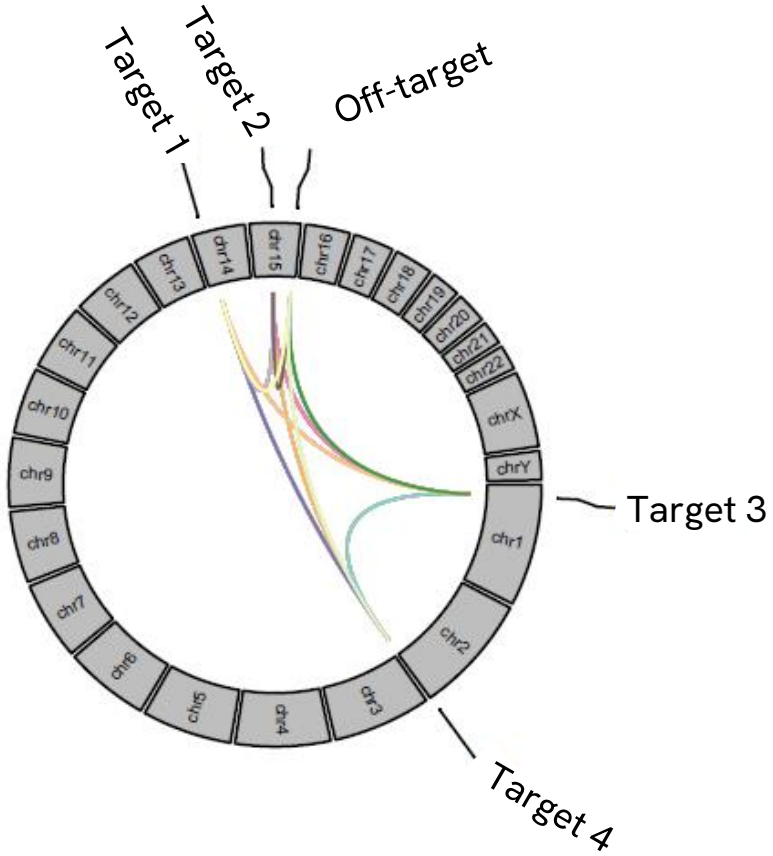
High survival of multi-edited iPSCs with a Pin-point system

Edited cells retain their pluripotency



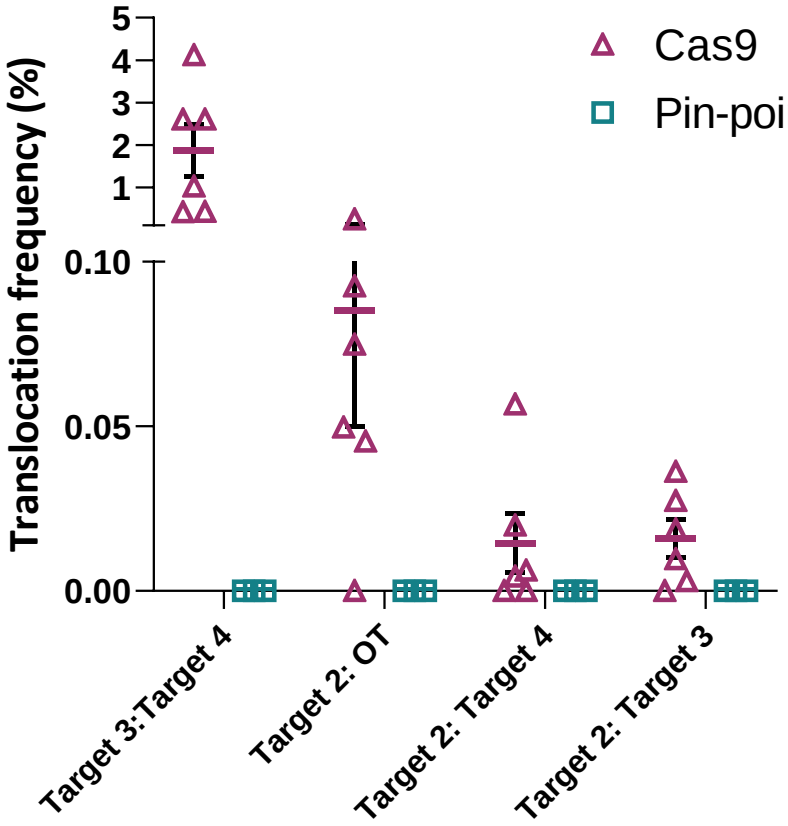
Pluripotency is retained in iPSCs edited with a Pin-point system

Strong safety profile in iPSCs



in-silico predicted translocations previously validated in T cells

Undetectable translocations after multiplex base editing with a Pin-point system



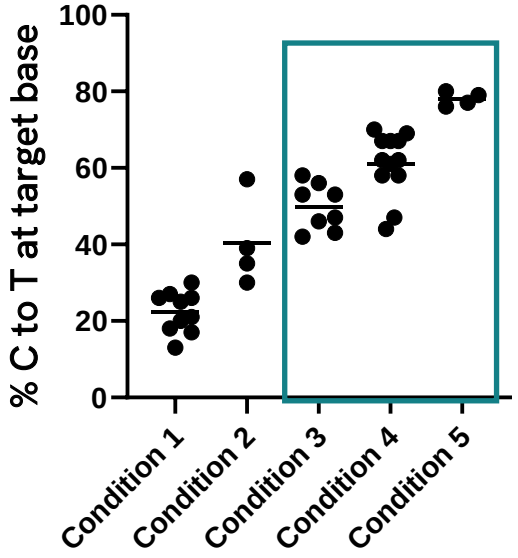
A cleaner and safer approach to multiplex gene editing in iPSCs

Validated performance in HSPCs

Highly efficient base editing in HSPCs

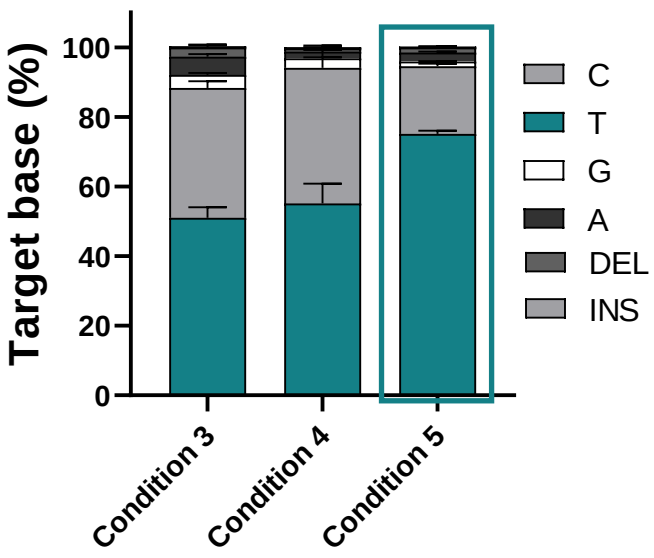
High level of editing achieved with optimised conditions

Sanger sequencing

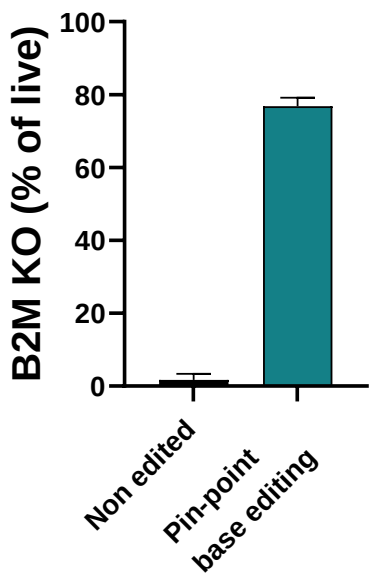


High level of editing and purity achieved at the target site

NGS



High level of B2M phenotypic knock-out



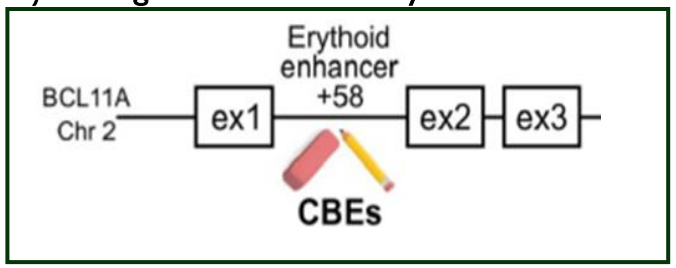
Pin-point base editing system achieves high level of editing in HSPCs with high purity of C to T conversion

Therapeutic editing of HSPCs with the Pin-point platform

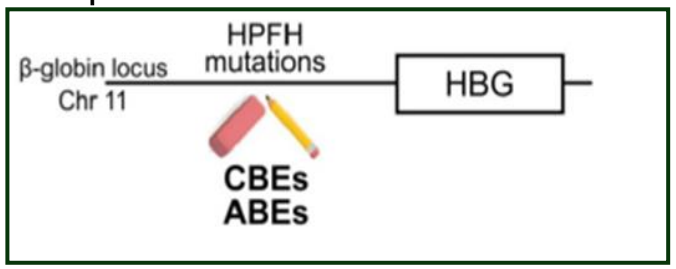
https://doi.org/10.3389/fgeed.2021.618406

Reactivating Fetal Haemoglobin

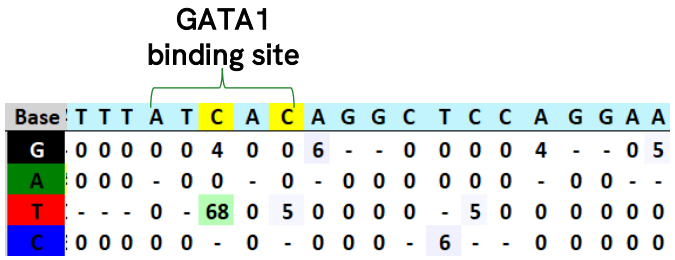
1) Editing of the BCL11A erythroid enhancer

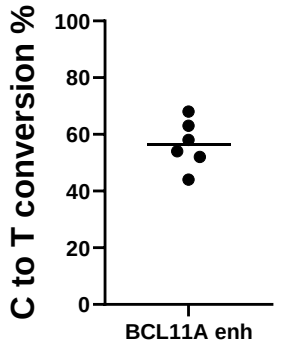


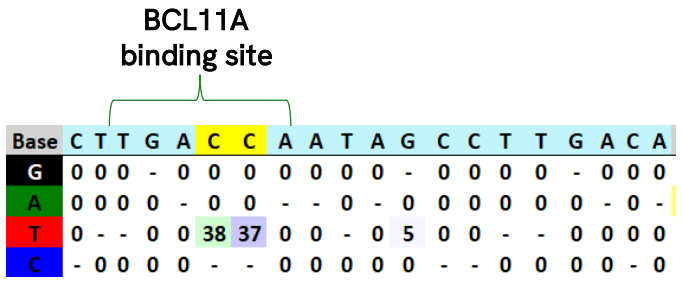
2) Editing of the BCL11A binding site in the HBG promoter

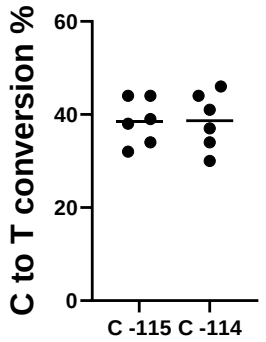


Editing Efficiency

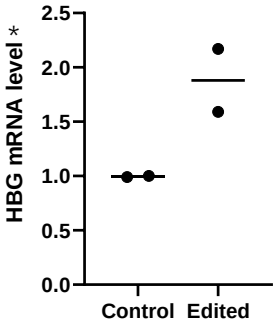


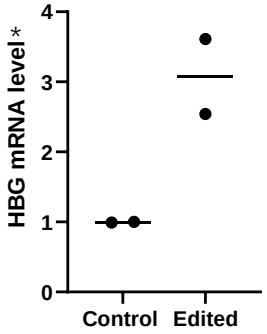






Induction of Fetal Haemoglobin





*qPCR data expressed as HBG/HBA and normalised on expression in control samples

Pin-point™ base editing system achieves therapeutic editing in HSPCs

Uniquely capable of complex engineering

A solution for complex engineering

One-step simultaneous knock-in and multiple knockout in T cells

One transfection

Pin-point deaminase

Pin-point aptamer gRNA (3)

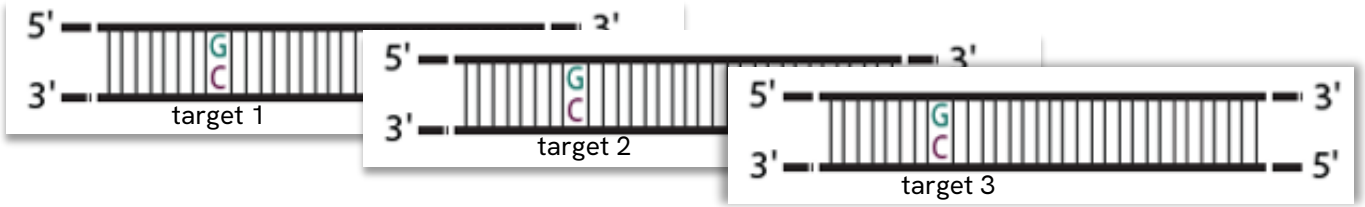
Nickase Cas

Nicking gRNAs (2)

Donor insert template

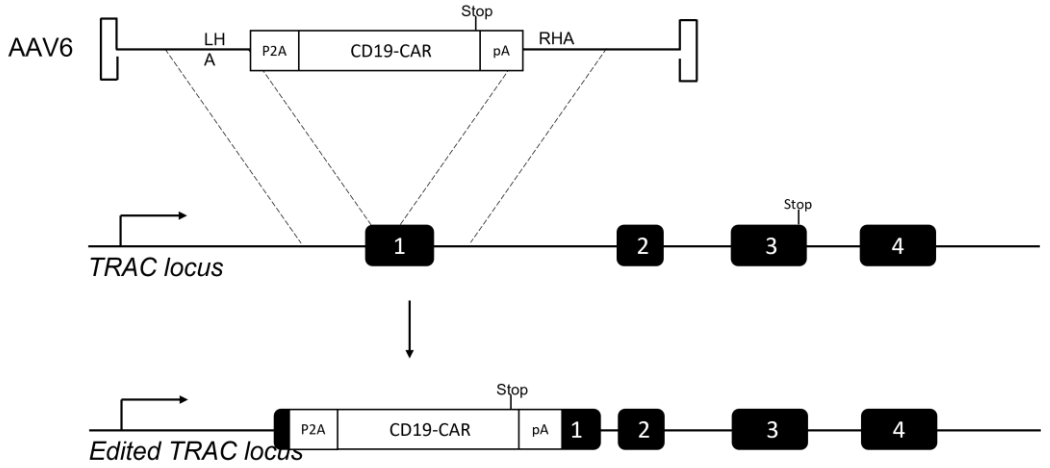
Base Editing with aptamer gRNAs

Knockout B2M, CD52, PDCD1



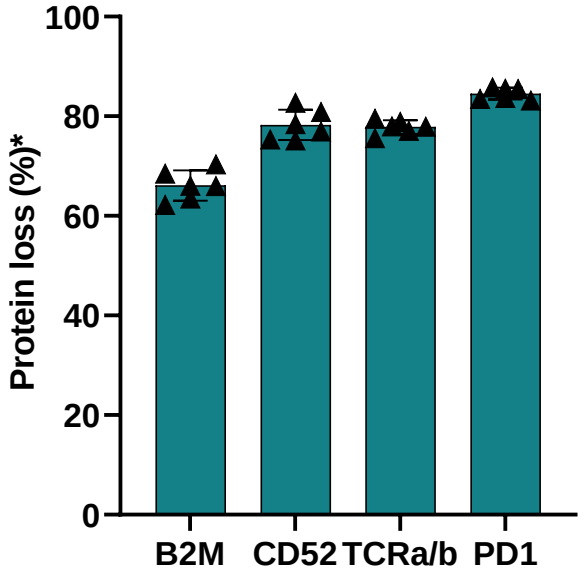
Insertion of a transgene by non-aptamer nicking gRNAs

CAR in TRAC

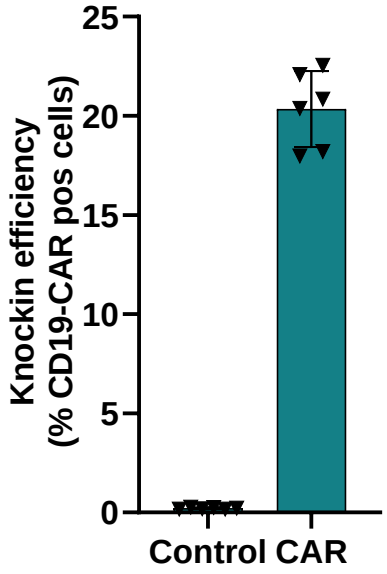


Streamlined creation of CAR-T cells is enabled with the Pin-point™ platform

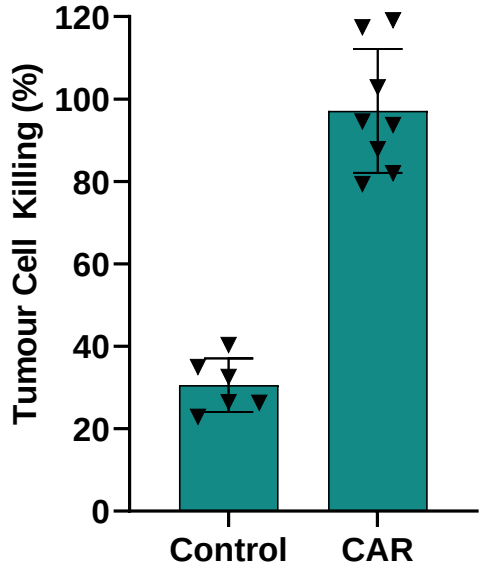
Multiple proteins are knocked out



... while enabling protein knock-in



... and weaponizing T cells against cancer cells



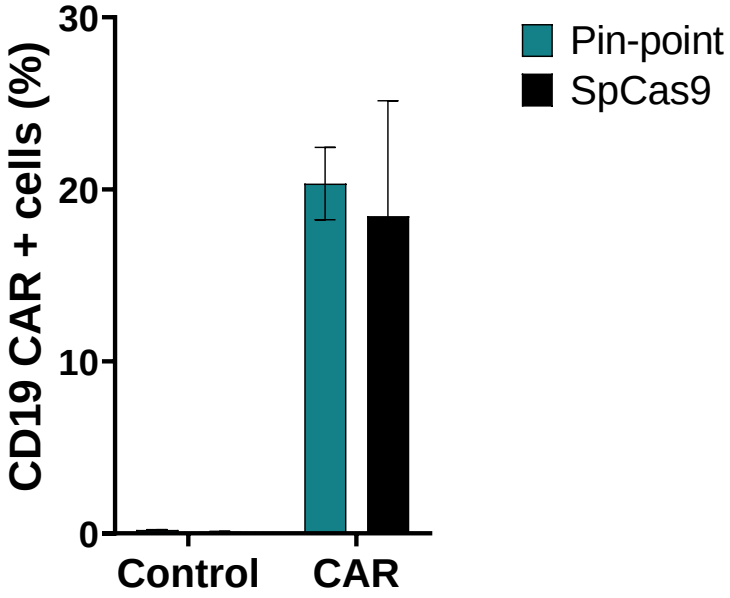
The Pin-point platform is efficient and accurate for concurrent transgene insertion and multiplex base editing

Biological replicates = 2

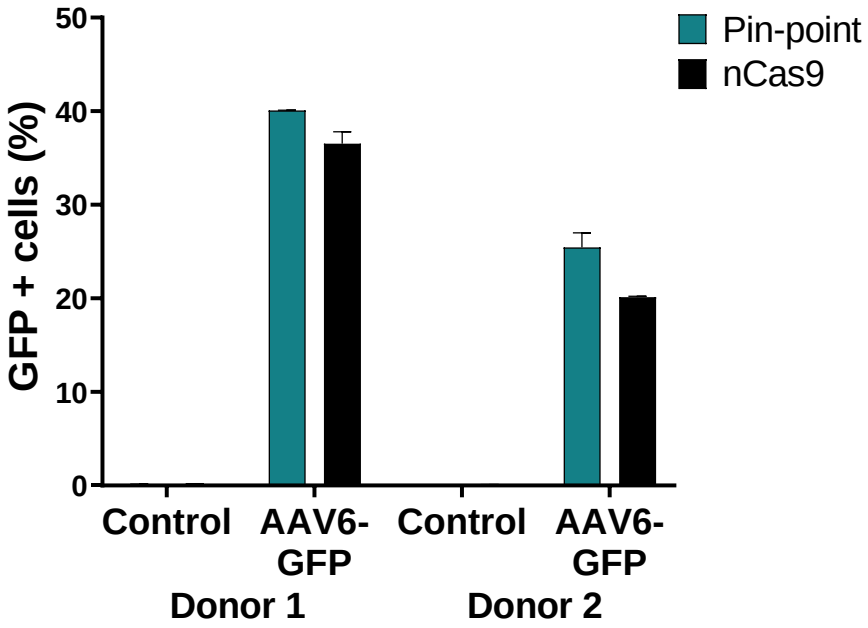
*Normalized to controls

No loss of efficiency in payload deliveries

Equivalent to dsDNA knock-in



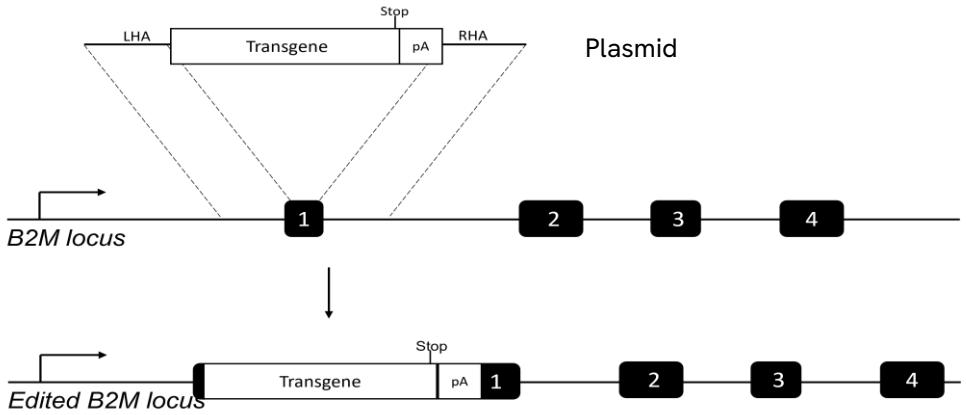
Presence of modular deaminase has no impact on knock-in



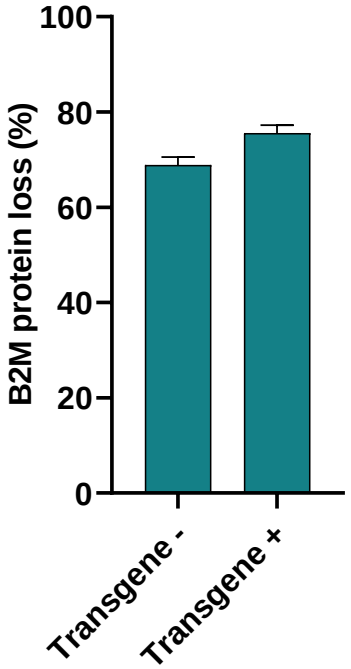
The Pin-point platform can deliver payloads equivalently to standard Cas9 or nCas9 knock-in strategies

Demonstrated simultaneous knock-in and multiple knockout in iPSCs

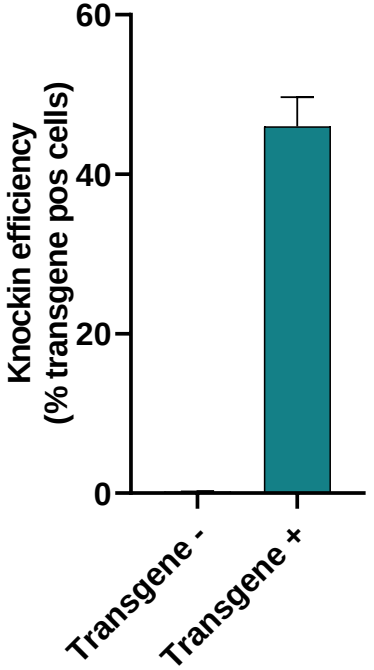
Base editing
Knockout CIITA
&
Insertion of a transgene
in B2M (promoter-less GFP)



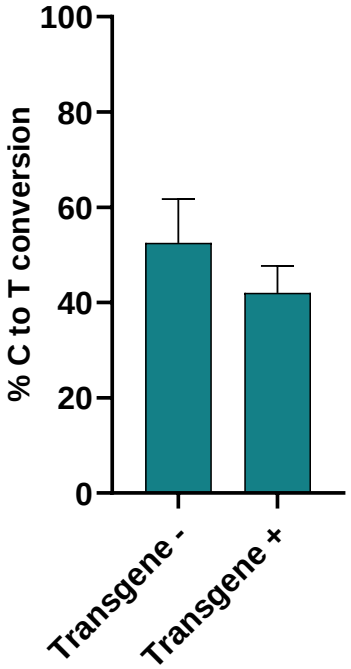
High level of B2M knockout



High level of transgene knock-in



… and base editing (CIITA KO)

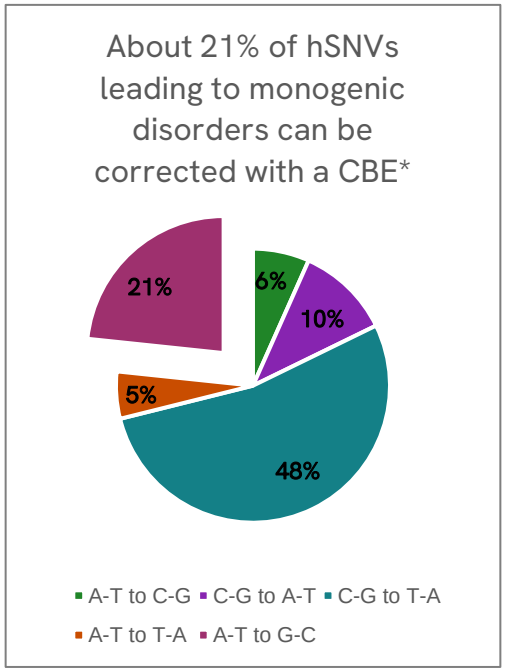


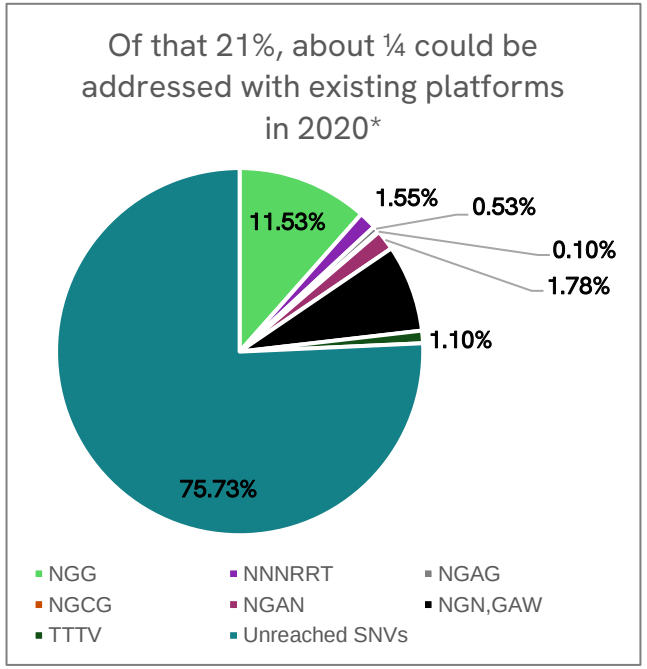
The Pin-point platform enables one-step simultaneous knock-in and multiple knockout in iPSCs

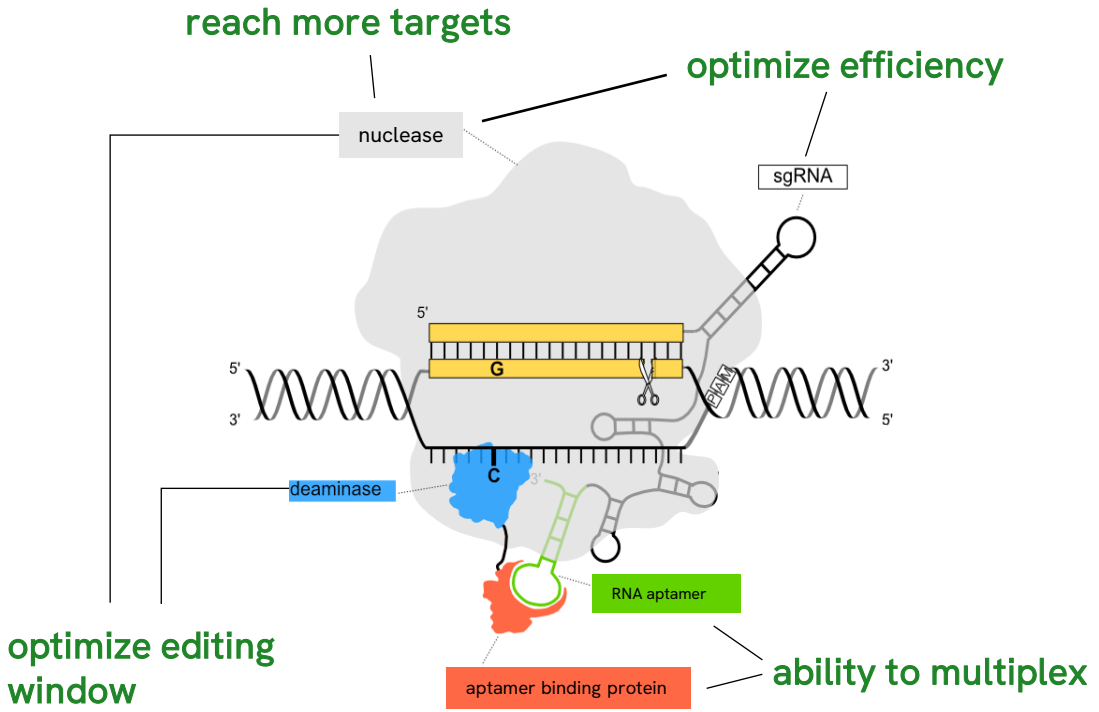
Flexibility for target optimization

Choose components for locus-specific optimization

Most pathogenic SNVs with potential CBE correction are not reachable with published systems*







Schematic depicts nCas9 configuration

The modular Pin-point platform can be customized to combine optimal components for a wide range of base editing applications

*Graphed from data published by Lavrov et al. *BMC Med Genomics* 2020

A benefit of modularity of the Pin-point™ platform

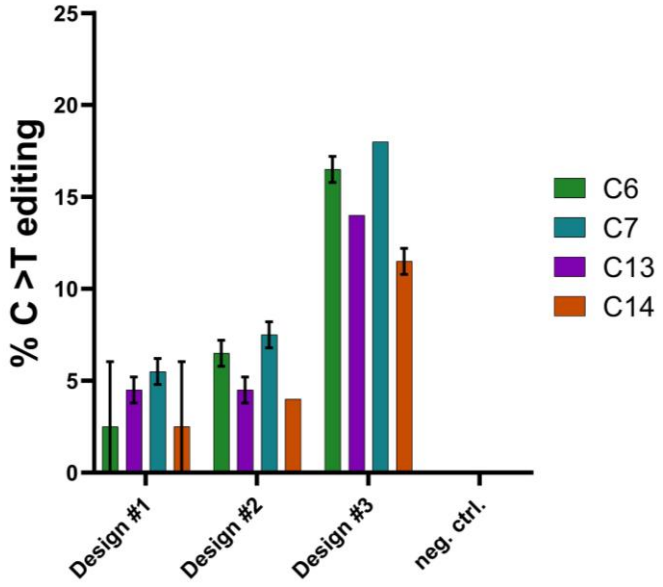
Demonstrated compatibility with numerous nucleases

	Type II			Type V						
	A	B	C	D	E	F	G	H	I	J
Enzyme activity	nickase	nickase	nickase	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated	deactivated
Demonstrated nuclease activity in mammalian cells	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Demonstrated with the Pin-point system	✓	✓	In progress	✓	In progress	✓	In progress	✓	✓	In progress
sgRNA optimized	✓	In progress		In progress		✓		✓	✓	
Enzyme optimized	✓					✓				
Confirmed at multiple targets (2+)	✓	In progress				✓		✓	✓	
Demonstrated in multiple cell types (2+)	✓	In progress				✓		✓		
Demonstrated with multiple deaminases (2+)	✓							✓	✓	

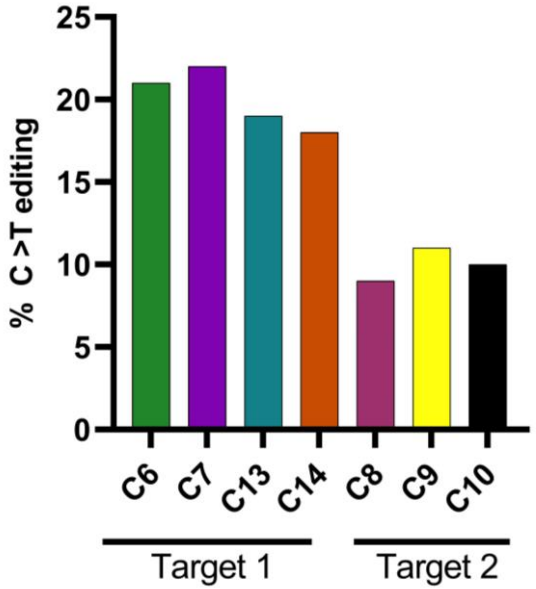
The Pin-point platform enables utilization of a variety of RNA-guided nucleases, which can be further optimized for editing efficiency

Preliminary evaluation of a deactivated Type V enzyme with the Pin-point base editing system

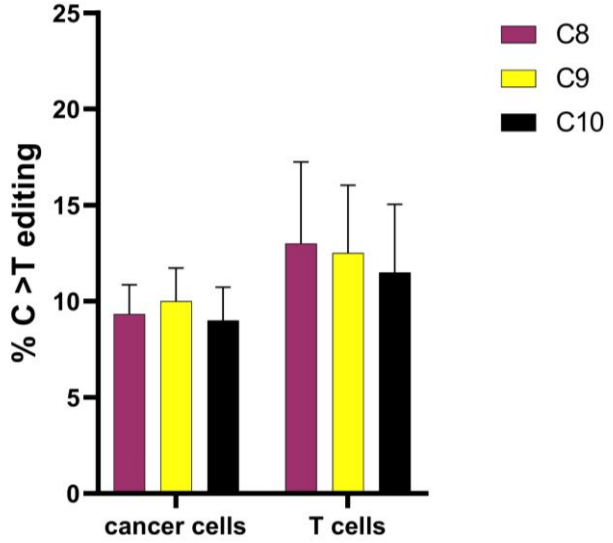
gRNA scaffold evaluation



Validated at two target sites



Validated in two cell types

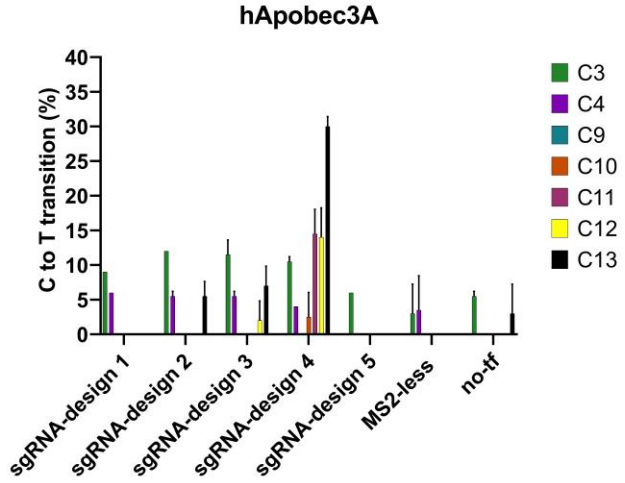


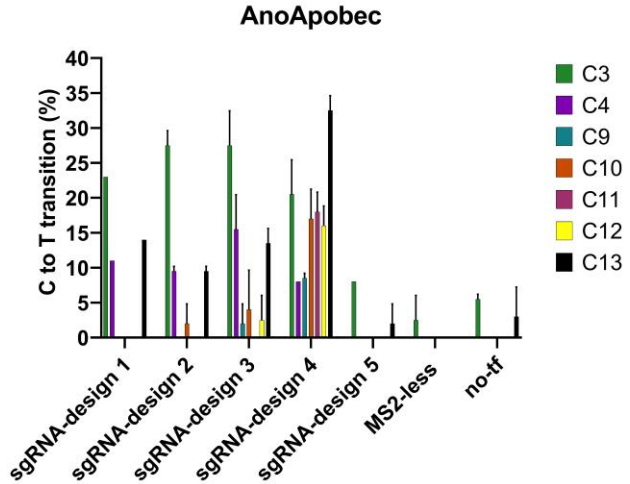
Increased activity achieved through design of the guide RNA scaffold persists over multiple targets and cell types

Read the full Application Note

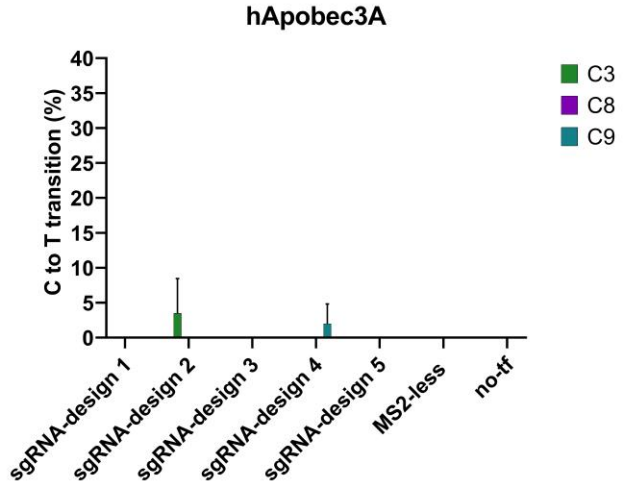
The Pin-point™ platform configured with the compact Type V effector protein dCasMINI

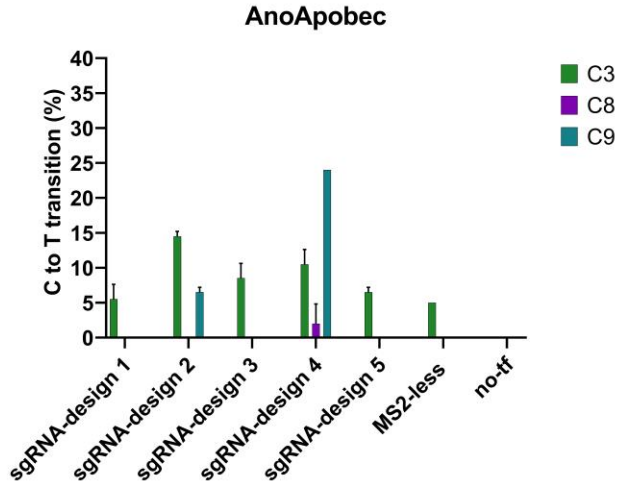
Target site 1





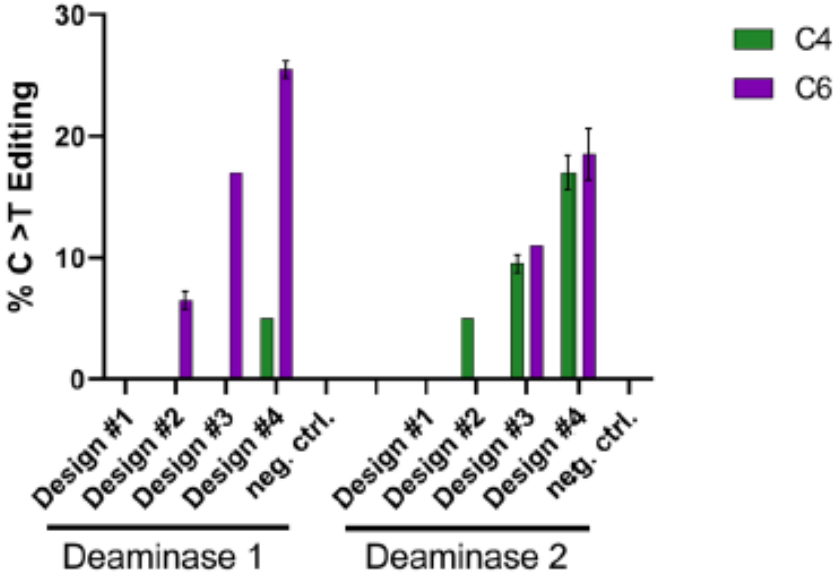
Target site 2

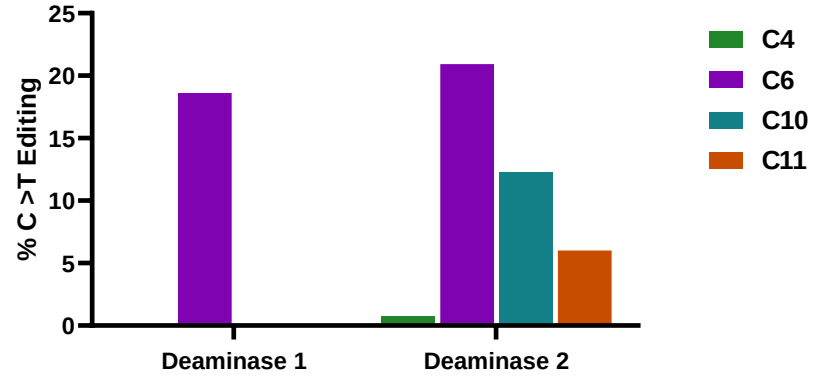


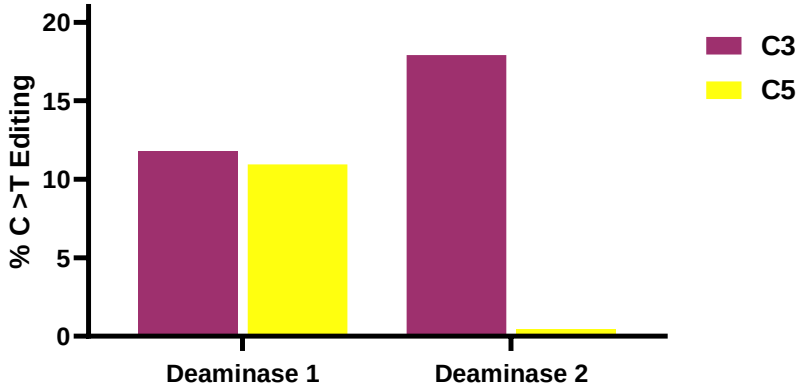


Read the full Application Note

Preliminary evaluation of a nickase Type II enzyme with the Pin-point base editing system







The Pin-point platform effectively supports optimization of the editing window by selecting the best guide RNA and deaminase pairs

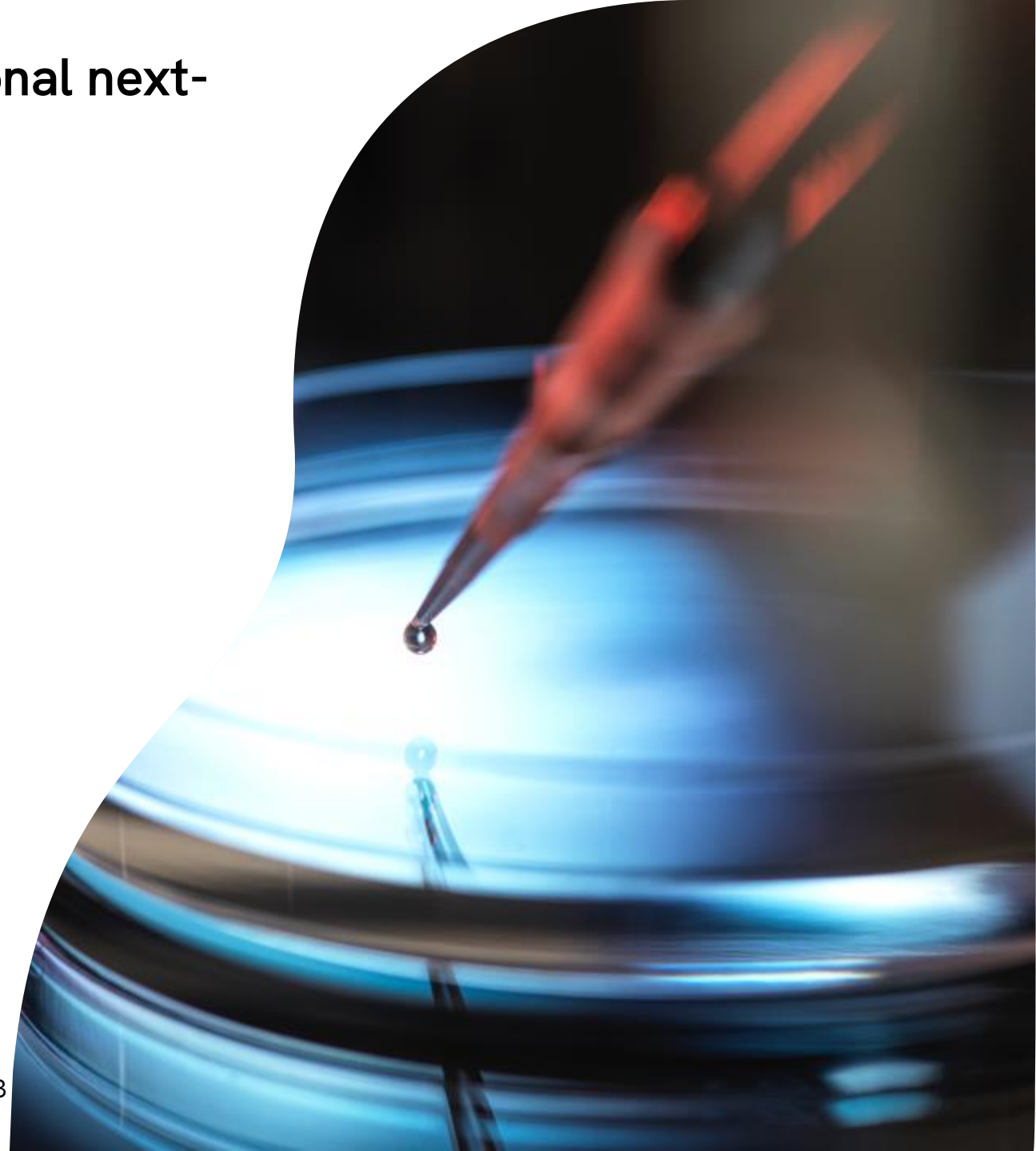
Read the full Application Note

The Pin-point™ system is a transformational next-generation gene editing technology

Highly effective editing platform, even for **complex edits**

Versatile technology modular and capable of generating locus-specific effects for novel therapies

Improved safety compared to standard CRISPR-Cas9 systems



Access Pin-point base editing

Licensing

Licenses for therapeutic development

Comprehensive support

Collaboration opportunities

Research Reagents

Synthetic off-the-shelf reagents

Validated controls

Custom guide RNAs

Services

Tiled pooled screening

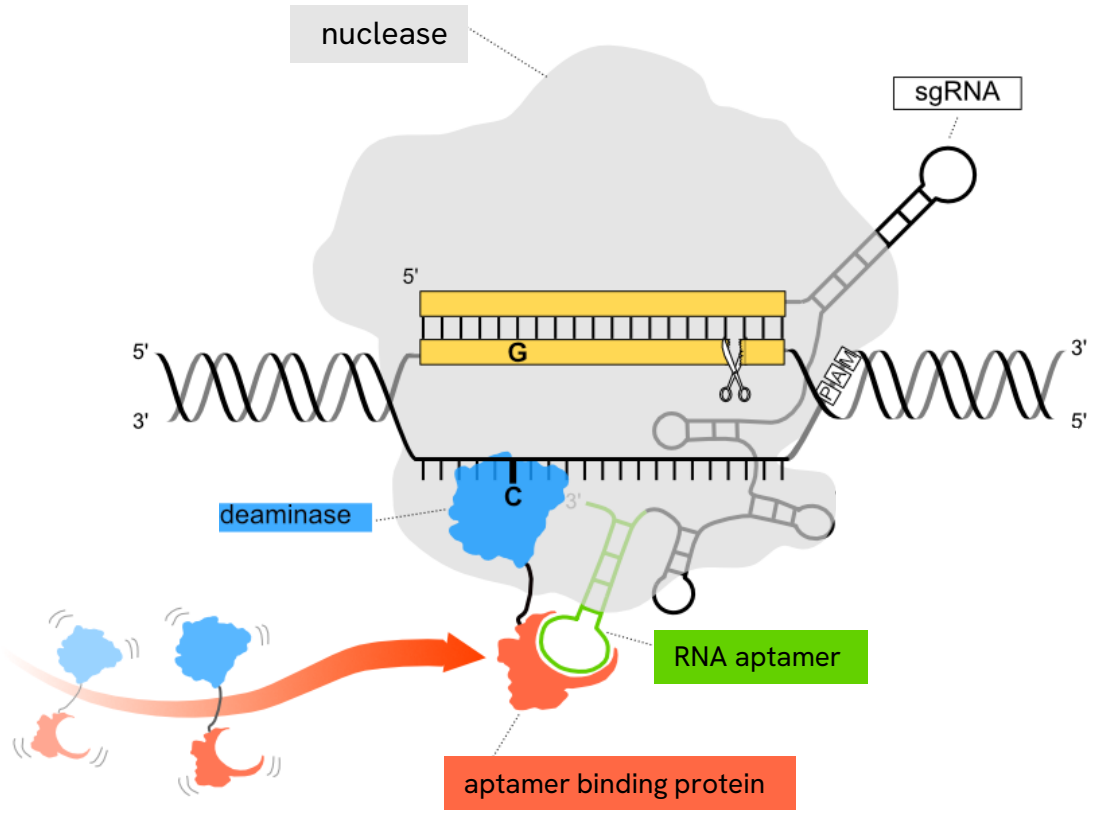
Functional genomics

Cell models

https://horizondiscovery.com/en/gene-editing/pin-point-base-editing-platform

BaseEditing@HorizonDiscovery.com

Pin-point base editing reagents – now available to all researchers



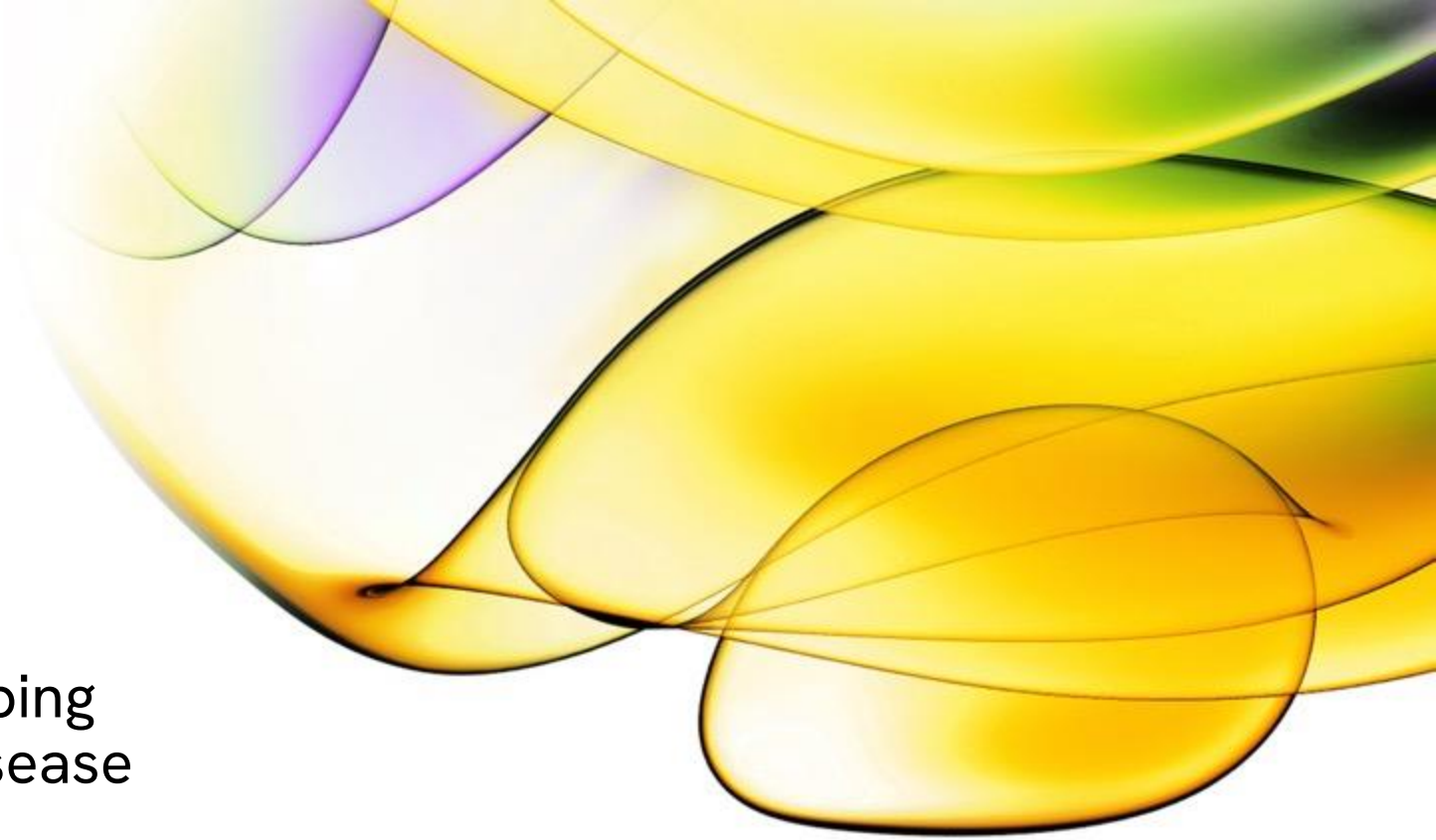
What's included?

nCas9 mRNA
APOBEC mRNA
Synthetic sgRNA

- Validated Control sgRNAs to knockout CD52, PDCD1, TRAC
- Non-targeting control sgRNA
- Custom sgRNA for your target

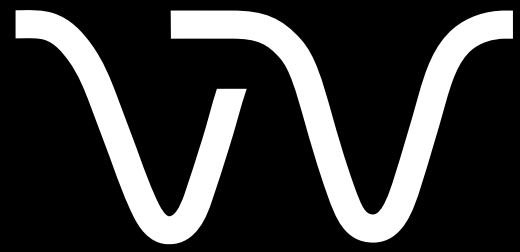
Protocols for T cells & cancer lines, technical manual
Comprehensive technical support

Reagents available at horizondiscovery.com



We are a visionary partner in developing technologies and solutions across disease research pathways.

Here for a healthier humankind.



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