



Technical Manual

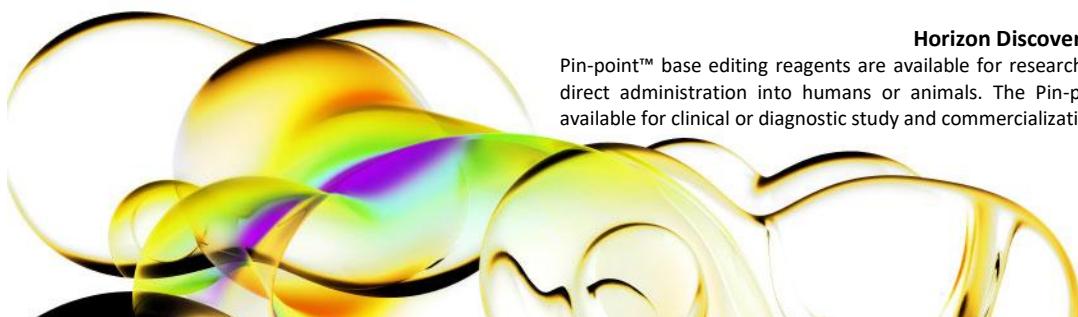
Pin-point™ base editing platform – adenine deaminase configuration

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Horizon Discovery Biosciences Ltd. (a Revvity Company)

Pin-point™ base editing reagents are available for research use only and are not for diagnostic use or direct administration into humans or animals. The Pin-point™ base editing platform technology is available for clinical or diagnostic study and commercialization under a commercial license from Revvity.



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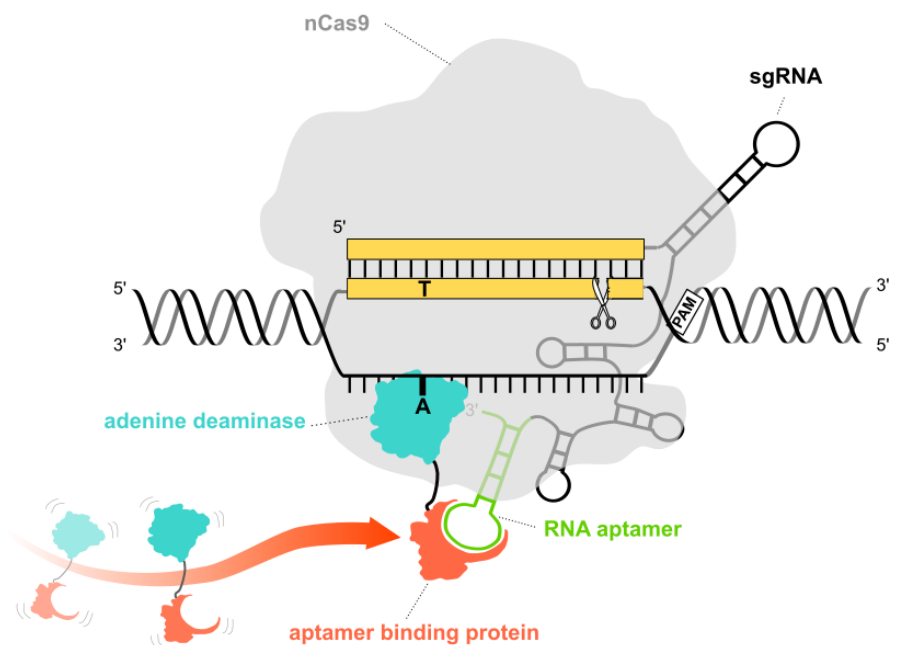
1. Introduction to the Pin-point base editing platform

CRISPR-based technologies provide powerful tools for gene editing and modulation (Mali, 2014; Sampson, 2014). Base Editing is a CRISPR-based technology that has been developed for precise and efficient genome editing without the induction of double-strand DNA cleavage. Adenine base editors (ABEs) enable recruitment of adenine deaminase enzymes to a DNA target site, specifically enabling conversion of A-T base pairs to G-C base pairs (Gaudelli, 2023), while Cytidine base editors (CBEs) convert C-G base pairs to T-A base pairs (Komor, 2016; Nishida, 2016). This ability to make specific nucleotide changes can be harnessed to then produce gene knockouts through introduction of premature stop codons (Billon, 2017) or disruption of splice donor and splice acceptor sites (Webber, 2019), while avoiding DNA double-strand breaks and imprecise insertion or deletion (indel) formation. In addition, base editors offer the opportunity for more advanced gene engineering such as epitope editing (Casirati 2023, Wellhausen 2023) and correction of pathogenic single nucleotide variants (Musunuru et al 2025).

The Pin-point base editing platform (**Figure 1**) was developed to efficiently recruit base-modifying enzymes using RNA aptamers (Collantes, 2021). The Pin-point base editing platform effectively induces target specific nucleotide changes without the formation of DNA double-strand breaks or indels. This system consists of three components: **[1]** a nuclease-defective “nickase” nCas9 that only cuts or “nicks” a single strand of DNA. **[2]** An adenine deaminase (ABE-flex or ABE-exact) fused to an aptamer binding protein. ABE-flex converts A-T base pairs to G-C base pairs if the base pairs are within positions 4 to 8 of the base editing window, while ABE-exact converts A-T base pairs to G-C base pairs if the base pairs are within positions 5 to 6 of the base editing window. **[3]** An aptameric single guide RNA (sgRNA) that recruits the nCas9 and the aptamer-deaminase fusion to a specific DNA target site.

Delivery of all three components into mammalian cells induces base editing at the target site. Pin-point ABE system can be used to make gene knockouts (through the disruption of splice donor and splice acceptor sites (Webber, 2019)), or to introduce point mutations. See our **Pin-point base editing sgRNA design tool** to design your own sgRNAs for gene knockout.

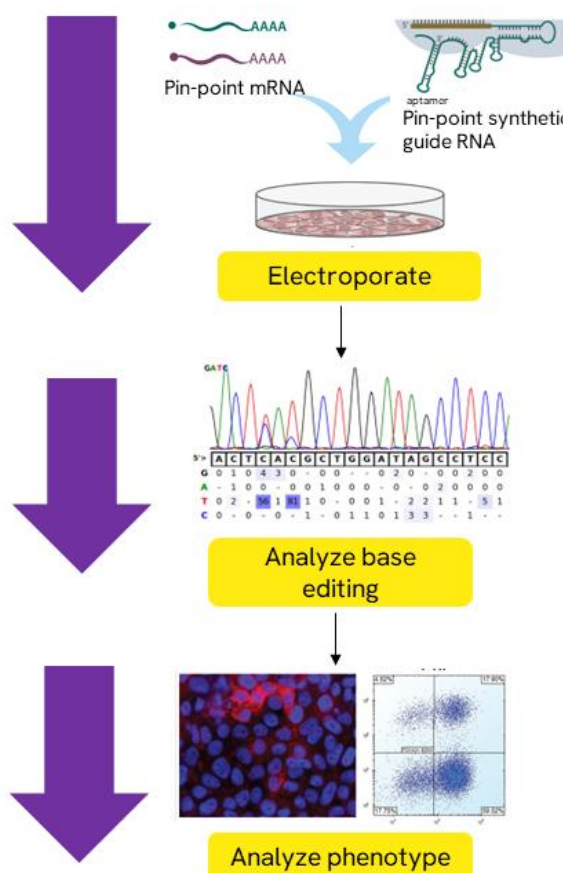
Figure 1. Illustration of Pin-point ABE system. The utilization of nCas9 (light gray) ensures that no DNA double-strand breaks occur and DNA damage response pathway is not triggered. Pin-point sgRNA contains an aptamer (green) that is used to recruit adenine deaminase (turquoise) via aptamer binding protein (orange) to perform base editing.



2. Pin-point mRNA and synthetic guide RNA for base editing

The Pin-point ABE reagents workflow includes all three of the critical Pin-point system components required for base editing in mammalian cells: (i) an mRNA for translation of a mammalian codon-optimized nCas9, (ii) an mRNA for translation of an adenine deaminase with aptamer binding protein fusion, and (iii) an aptameric Pin-point synthetic ABE sgRNA. All three components are electroporated into mammalian cells to enable the gene editing reaction. **Figure 2** summarizes the general experimental workflow.

Figure 2. Base editing workflow using Pin-point ABE nCas9 mRNA, Pin-point ABE deaminase mRNA, and Pin-point synthetic ABE sgRNA. All three components are electroporated into a specific cell type of interest. Base editing may then be observed using analysis primers to amplify and Sanger sequence the base editing region of interest. A phenotype may be analyzed to assess target protein knockout.



Pin-point ABE nCas9 mRNA (unmodified or 5moU-modified)

The Pin-point ABE nCas9 mRNA contains a human codon-optimized version of the *S. pyogenes* nickase Cas9 (D10A) with both, a 5' and 3' nuclear localization signal (NLS). The nCas9 mRNA is in vitro transcribed, capped using CleanCap® AG, and polyadenylated for translation and nuclear localization of the protein.

Pin-point ABE-exact deaminase mRNA (unmodified or 5moU-modified)

The Pin-point ABE-exact deaminase mRNA contains an adenine deaminase generated by Profluent using frontier AI models and is experimentally validated for very precise base editing at positions 5-6 of the protospacer (**Figure 3**). The ABE-exact deaminase mRNA is in vitro transcribed, capped using CleanCap® AG, and polyadenylated for translation and nuclear localization of the protein.

Pin-point ABE-flex deaminase mRNA (unmodified or 5moU-modified)

The Pin-point ABE-flex deaminase mRNA contains an adenine deaminase generated by Profluent using frontier AI models and is experimentally validated for broader base editing window (positions 4-7 of the protospacer, **Figure 3**). The ABE-flex deaminase mRNA is in vitro transcribed, capped using CleanCap® AG, and polyadenylated for translation and nuclear localization of the protein.

Pin-point ABE synthetic sgRNA

The Pin-point synthetic sgRNA is a synthetically produced 128-nucleotide chimeric oligo: fusing the crRNA, tracrRNA, and aptamer regions. It is chemically modified for nuclease resistance on both 5' and 3' ends of the molecule.

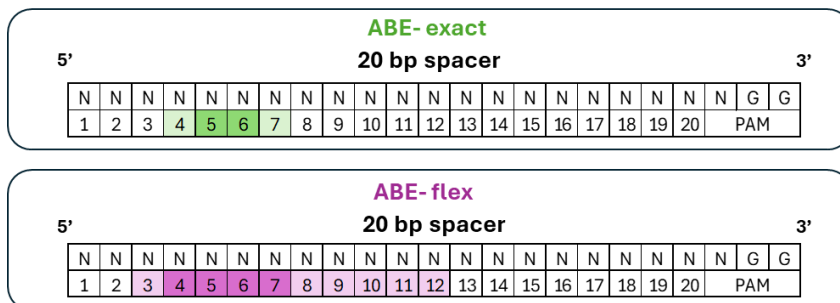


Figure 3. Editing Windows of ABE-exact and ABE-flex. The editing window represents the positions within a 20-nt spacer where a base editor efficiently converts target nucleotides. ABE-exact shows a narrow editing window centered on positions 5–6, with reduced editing at positions 4 and 7. ABE-flex primarily edits positions 4–7, with lower-efficiency editing extending to positions 3 and 8–12.

3. Guidelines for electroporation of Pin-point base editing components

Successful electroporation of Pin-point ABE nCas9 mRNA, Pin-point ABE deaminase mRNA, and Pin-point synthetic ABE sgRNA requires careful optimization of delivery conditions for each cell type of interest. The protocol below assumes experimental conditions have been optimized as recommended and is for electroporation of both mRNA components and a synthetic sgRNA in mammalian cells.

Materials required for base editing using Pin-point ABE nCas9 mRNA, Pin-point deaminase ABE mRNA (choose one of the options: ABE-exact or ABE-flex) and Pin-point synthetic sgRNA

- Pin-point ABE nCas9 mRNA, unmodified or 5moU-modified (2 µg/µL), (20 µg; Cat # PNP13767, PNP13770) (100 µg; Cat # PNP13768, PNP13771) (500 µg; Cat # PNP13769, PNP13772)
- Pin-point ABE-exact deaminase mRNA, unmodified or 5moU-modified (2 µg/µL), (10 µg; Cat # PNP13781, PNP13785) (20 µg; Cat # PNP13782, PNP13786) (100 µg; Cat # PNP13783, PNP13787)
- Pin-point ABE-flex deaminase mRNA, unmodified or 5moU-modified (2 µg/µL), (10 µg; Cat # PNP13773, PNP13777) (20 µg; Cat # PNP13774, PNP13778) (100 µg; Cat # PNP13775, PNP13779)
- Pin-point synthetic ABE sgRNA validated controls
 - CD52 (Cat # PNP-003200-01); Targeting sequence: CTCTTACCTGTACCATAACC
 - PDCD1 (Cat # PNP-003300-01); Targeting sequence: CACCTACCTAAGAACCATCC
 - TRAC (Cat # PNP-003000-01); Targeting sequence: CTTACCTGGGCTGGGGAAGA
 - CD58 (Cat # PNP-003500-01); Targeting sequence: CTCACCGCTGCTTGGGATAC
 - HBG1/2 (Cat # PNP-003600-01); Targeting sequence: CTTGACCAATAGCCTTGACA
 - BCL11A +58 (Cat # PNP-003700-01); Targeting sequence: TTTATCACAGGCTCCAGGAA
 - Site2 (Cat # PNP-003800-01); Targeting sequence: GAACACAAAGCATAGACTGC
- Pin-point Non-targeting Control synthetic sgRNA
 - Control #1 (Cat # PNP-01000-01)
 - Control #2 (Cat # PNP-01000-02)
- Pin-point custom synthetic sgRNA (Optional)

Additional materials required

Electroporation experiments require cell culture reagents and instruments appropriate for the maintenance of cells as described in the relevant sample protocols. The following additional materials may be required but not supplied:

- Electroporation option:
 - Neon™ Nxt Electroporation system (ThermoFisher Cat # NEON1S)
 - Electroporation reagents (ThermoFisher Cat # N10096)
- Nucleofection option:
 - Lonza 4D-Nucleofector® Core Unit (Cat # AAF-1003B)
 - Lonza 4D-Nucleofector® X Unit (Lonza Cat # AAF-1003X)
 - P3 Primary Cell 4D-Nucleofector® X Kit S (Cat # V4XP-3032)



Terms “electroporation” and “nucleofection” may be used interchangeably in this technical manual.

- Multi-well tissue culture plates or tissue culture dishes
- Antibiotic-free complete medium: Cell culture medium (including serum and/or supplements) recommended for maintenance and passaging of the cells of interest without antibiotic
- Phosphate-buffered saline (PBS)
- 10 mM Tris pH 7.4, nuclease free buffer (Tris buffer) solution (Dharmacon, Cat # B-006000-100) for synthetic guide resuspension

4. General protocol for base editing activated human T cells using the Pin-point ABE platform

The following is a general protocol using electroporation to deliver unmodified Pin-point ABE nCas9 mRNA, unmodified Pin-point ABE-exact or ABE-flex deaminase mRNA, and a Pin-point synthetic sgRNA for ABE into activated T cells (CD3+ Pan T Cells). Reagent amounts given here are recommended starting points for new users and untested targets to ensure high editing efficiency, but base editing activity has been observed at some sites with as low as 100-fold less mRNA, with reduced deaminase mRNA amounts usually resulting in reduced bystander/off-target editing while maintaining high on-target editing. Exact reagent concentrations and parameters for electroporation should therefore be empirically determined through careful optimization in the cells of interest in accordance with the electroporation instrument manufacturer’s recommendations and according to the user’s priorities. The protocol below describes delivery conditions in activated human T cells using the Lonza nucleofection system (Lonza) and is provided for illustrative purposes only. Reagent volumes can be calculated for including replicate samples as necessary.

Different types of samples are recommended for a multiplex base editing experiment (Table 1). All steps of the protocol should be performed in a biosafety cabinet using sterile technique. A sample assay schedule is provided as a guide for a multiplex base editing workflow in T cells (Figure 4).

Table 1. Recommended samples for a multiplex base editing experiment using Pin-point platform.

Sample elements	Purpose
Pin-point ABE nCas9 mRNA, Pin-point ABE-exact or ABE-flex deaminase mRNA, and Multiple gene specific Pin-point synthetic sgRNAs	Multiplex base editing sample
Pin-point ABE nCas9 mRNA, Pin-point ABE-exact or ABE-flex deaminase mRNA, and Individual gene specific Pin-point synthetic sgRNA – validated control or custom sgRNA (one sample per each sgRNA involved in the multiplex experiment)	Single base editing samples, positive controls
Pin-point ABE nCas9 mRNA, Pin-point ABE-flex or ABE-exact deaminase mRNA, and Non-targeting control guide RNA	Negative control: provides a baseline to compare experimental conditions
Non-electroporated	No treatment control sample: confirmation of cell viability

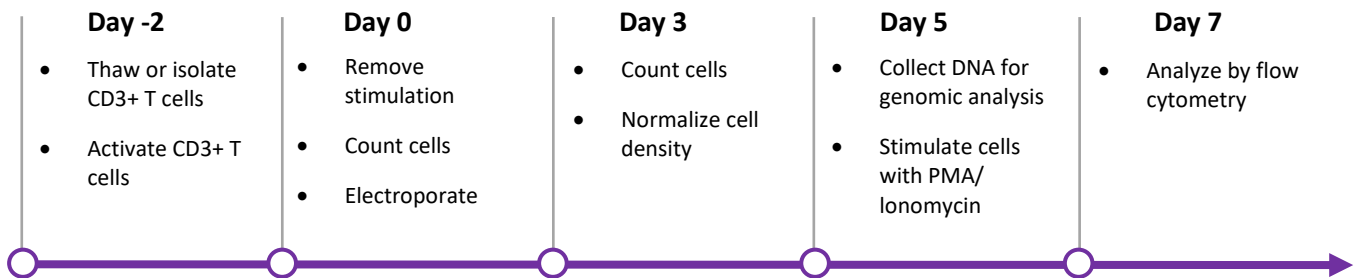


Figure 4. Example workflow for multiplex base editing in T cells that includes assessing base editing and protein knockout.

T cell isolation and activation prior to electroporation

1. CD3+ Pan T cells can be isolated from peripheral blood mononuclear cells using commercially available negative selection kits or can be acquired as cryopreserved cells. Quality control of the isolated cells by flow cytometry is recommended using the following markers: CD3, CD4, CD8, CD25.
2. Post isolation, activate CD3+ T cells for 48 hours with Human CD3/CD28 T Cell Activation Beads (BioLegend) in media supplemented with IL-2 according to the manufacturer's instructions.

T cell electroporation using Lonza 4D Nucleofector System



This protocol has been optimized for Lonza 4D nucleofector System in primary T cells. Using other electroporation platforms may require further optimization.

1. Cell preparation
 - a. Remove Human CD3/CD28 T Cell Activation Beads from the culture according to the manufacturer's instructions and count cells. Keep the cells at 37 °C and 5% CO₂ while preparing the electroporation reagents.
 - b. Prepare antibiotic-free complete T cell medium supplemented with the appropriate cytokines (IL-2, IL-7, and IL-15), allowing for 500 µL per electroporation reaction.
 - c. Prepare post-electroporation tissue culture plates by adding the appropriate volume of cell media to each recipient well. (Appropriate volume determined by size of tissue culture plates; e.g 500 µL/well in a 24-well plate for a standard 250,000 cell electroporation reaction)
 - d. Incubate the tissue culture plate(s) at 37°C and 5% CO₂ for a minimum of 30 minutes prior to electroporation to pre-warm the medium.

Table 2. Recommended working stocks and final concentration of Pin-point base editing components for one electroporation in T cells (250,000 cells in 20 µL reaction).

Pin-point component	Working stock*	Final amount per reaction
ABE nCas9 mRNA – unmodified	2 µg/µL	1.5 µg
ABE deaminase mRNA – unmodified		
ABE-exact mRNA	2 µg/µL	1 µg
ABE-flex mRNA	0.2 µg/µL	0.1 µg
sgRNA single-plex	200 µM	2 µM
sgRNA multi-plex	200 µM	2 µM per guide, 6 µM total

* ABE nCas9 mRNA and ABE deaminase mRNA shipped at 2µg/µl. See note about working stock preparation



We recommend preparing a 0.2 µg/µL working stock of the ABE-flex deaminase mRNA in nuclease-free water to reduce the risk of pipetting error from the higher concentration stock. Prepare only enough working stock for the current experiment as we do not recommend long-term storage at the lower concentration.

2. sgRNA preparation (see **Table 2** and **Table 3**)
 - a. Calculate the number of reactions required and include a minimum of 10% excess volume.
 - b. The concentration of each sgRNA in the electroporation mix is 2 µM sgRNA (0.2 µl of 200 µM stock sgRNA in a 20 µL electroporation mix).
 - c. Mix the calculated volumes of sgRNAs and maintain the compositions on ice until ready for electroporation.
3. mRNA preparation (see **Table 2** and **Table 3**)
 - a. Calculate the number of reactions required for the experiment and include a minimum of 10% excess volume. The mRNA mix is composed of 1.5 µg ABE nCas9 and 0.5 µg ABE deaminase mRNAs per reaction.
 - b. Transfer mRNA aliquots from the –80 °C freezer directly onto wet ice.

- c. Thaw mRNA on ice and mix the calculated volumes for the ABE nCas9 and ABE deaminase mRNAs.
- d. Keep mRNA mix on ice until ready for electroporation.

Table 3. Preparing electroporation samples for base editing experiment using Pin-point platform in T cells.

Sample	P3 nucleofactor solution	sgRNA (200 μM)	ABE nCas9 mRNA unmodified (2 μg/μL)	ABE-exact (2 μg/μL) or ABE-flex (0.2 μg/μL) deaminase mRNA unmodified	Cell suspension in P3 nucleofactor solution (250,000 cells)
Pin-point ABE nCas9 mRNA, Pin-point ABE deaminase mRNA, and multiple gene-specific Pin-point synthetic sgRNAs.	to 10 μL	0.2 μL of each sgRNA (0.6 μL total when using 3 sgRNAs)	0.75 μL	0.5 μL	10 μL
Pin-point ABE nCas9 mRNA, Pin-point ABE deaminase mRNA, and individual gene specific Pin-point synthetic sgRNA (one sample per each sgRNA involved in the multiplex experiment).	to 10 μL	0.2 μL	0.75 μL	0.5 μL	10 μL
Pin-point nCas9 mRNA, Pin-point ABE deaminase mRNA, and Non-targeting control guide RNA.	to 10 μL	0.2 μL as a control for the singleplex or 0.6 μL as control for the multiplex	0.75 μL	0.5 μL	10 μL
Non-electroporated	10 μL	0 μL	0 μL	0 μL	10 μL

Volumes are per single reaction. We recommend preparing sufficient sample volumes for the total number of replicates and to account for pipetting error. Exact reagent amounts for electroporation in other cell lines should be empirically determined through careful optimization prior to experimentation.

4. Cell harvest and centrifugation

- a. Calculate the number of cells required for each subset, using 250,000 cells per electroporation, including 10% excess.
- b. Transfer the required number of cells to a centrifuge tube.
- c. Pellet the cells at 400 x g for 5 minutes at room temperature.
- d. Aspirate the supernatant to waste.
- e. Flick gently to dissociate the cell pellet.
- f. Dilute the cell solution with PBS to wash.
- g. Pellet the cells at 200 x g for 10 minutes at room temperature.



During this centrifugation wait step, finalize the preparation of mRNA and sgRNAs.

- h. After T cell centrifugation, carefully remove the supernatant from the pelleted cells.
- i. Resuspend the cell pellet in P3 nucleofactor® solution at a final concentration of 2.5×10^7 cells/mL; each 20 μL electroporation reaction will contain 250,000 cells.



We recommend preparing the mRNA and sgRNA mixtures in advance and keeping on ice. Add P3 nucleofactor solution to the mixture shortly before the electroporation.

5. Final mix of components and electroporation.

- a. As outlined in **Table 3**, prepare each sample to be electroporated in a sterile V-bottom 96-well plate by mixing ABE nCas9 mRNA, ABE deaminase mRNA, sgRNA, and P3 nucleofactor solution. Appropriate volumes (μL) correspond to one electroporation.
- b. Add 10 μL of the cell suspension in P3 nucleofactor solution (250,000 cells) per reaction.

- c. Transfer 20 μL of cells + reagents mix to a well of nucleofection cuvette. Avoid bubbles. Nucleofect following the manufacturer's protocol and using the following conditions: EO-115.
 - d. Immediately after nucleofection, add 80 μL of pre-warmed media without cytokines to each well, and rest the nucleofector cuvette in the incubator for 15-30 minutes.
 - e. Pipette 100 μL of electroporation cell mixture into the pre-incubated medium in one well of a 24-well plate.
 - f. Once nucleofections have been completed, incubate the nucleofected cells and controls at 37°C and 5% CO_2 for 72 hours, being careful not to agitate the plates during the hours immediately post-nucleofection.
6. Post nucleofection
- a. We recommend checking cell viability 3 days following the electroporation and adjusting cell culture density to maintain approximately 1×10^6 cells/mL.
 - b. At 5 days post electroporation, collect DNA for genomic analysis following **Section 7. Guidelines for analyzing base editing with Pin-point analysis primers (Page 18)**.

Considerations for flow cytometry read-out to assess functional gene knockout

1. Flow cytometry read-out is performed 7 days post electroporation.
 - a. We recommend optimizing the antibody panel for the detection of the three surface markers and finalizing compensation matrix for the selected fluorophores ahead of the experiment.
2. As a proxy for TRAC knockout, we recommend measuring the surface level of TCR a/b (T cell receptor a/b).

Table 4. Flow cytometry antibody panel.

Target	Description	Provider	Cat. No.
TCR a/b	TCRa/b-BV421 antibody	BioLegend	306721
CD52	CD52-PE antibody	BioLegend	318904
PD1 (CD279)	PD1-APC antibody	BioLegend	329908

3. While TCR a/b, CD52 is regularly expressed on the surface of T cells, PD1 is present only on the membrane of exhausted T cells. For this reason, to evaluate the levels of functional knockout for PD1 we recommend stimulating T cells before the flow cytometry readout. Stimulation is not required for functional surface marker readout for TCRa/b, and CD52.
 - a. To increase PD1 levels on the surface, T cells can be stimulated using Human CD3/CD28 T Cell Activation Beads or by phorbol myristate acetate (PMA)-Ionomycin treatment. PD1 surface expression has displayed donor-specific behavior in our experience, therefore unedited controls are required for normalization.
 - b. Stimulation with Human CD3/CD28 T Cell Activation Beads should only be performed in the absence of TRAC knockout. When performing simultaneous PD1 and TRAC knockout, PMA-Ionomycin stimulation is recommended.
 - c. If stimulation is desired, PMA-Ionomycin treatment can also be used for TRAC, and CD52, but some surface antigens are known to internalize or be affected by the treatment. Therefore, non-edited control cells are paramount for accurate interpretation of results. Additionally, PMA-Ionomycin adversely affects the way cells take up DAPI and should be considered when using this as a live-dead stain.
4. Additional tips and recommendations
 - a. In single gene knockout experiments, do not stimulate cells for flow cytometry readout for TCRa/b, and CD52, but stimulate them with Human CD3/CD28 T Cell Activation Beads for measurement of PD1.
 - b. In multiple gene knockout experiments that do not include PD1, do not stimulate cells for flow cytometry readout.
 - c. In multiple gene knockout experiments that include PD1, stimulation is required for functional surface marker readout. Stimulation can be via Human CD3/CD28 T Cell Activation Beads if the TCRa/b is intact or by PMA-Ionomycin treatment when TRAC is knocked out.
5. Protocol for PMA-Ionomycin Stimulation
 - a. T cells are stimulated with PMA-Ionomycin 48 hours prior to flow cytometry read-out. The later the read-out time point is, the more effective the stimulation will be.
 - b. Prepare PMA-Ionomycin stimulation medium by adding PMA and Ionomycin to the T cell medium at 50 ng/mL and 250 ng/mL, respectively.
 - c. 100,000 – 300,000 cells are resuspended in 100 µl of stimulation medium in a 96-well plate.
 - d. Incubate the stimulated and non-stimulated cultures for 48 hours at 37°C and 5% CO₂.

5. General protocol for base editing in stimulated human induced pluripotent stem cells (iPSCs) using The Pin-point ABE platform

The following is a general protocol using electroporation to deliver unmodified Pin-point ABE nCas9 mRNA, unmodified Pin-point ABE-exact or ABE-flex deaminase mRNA, and a Pin-point synthetic sgRNA for ABE into human induced pluripotent cells (iPSCs). Reagent amounts given here are recommended starting points for new users and untested targets to ensure high editing efficiency, but base editing activity has been observed at some sites with as low as 100-fold less mRNA, with reduced deaminase mRNA amounts usually resulting in reduced bystander/off-target editing while maintaining high on-target editing. Exact reagent concentrations and parameters for electroporation should therefore be empirically determined through careful optimization in the cells of interest in accordance with the electroporation instrument manufacturer's recommendations and according to the user's priorities. The protocol below describes delivery conditions in human iPSCs using the Lonza nucleofection system (Lonza) and is provided for illustrative purposes only. Reagent volumes can be calculated for including replicate samples as necessary.

Recommended samples for singleplex base editing in iPSC are shown in **Table 5**. All steps of the protocol should be performed in a biosafety cabinet using sterile technique.

Table 5. Recommended samples for a base editing experiment using Pin-point platform.

Sample type	Sample elements	Purpose
Base editing sample, positive control	Pin-point ABE nCas9 mRNA, Pin-point ABE-exact or ABE-flex deaminase mRNA, and Pin-point synthetic sgRNA validated control or custom sgRNA	Perform on-target editing; assessment of bystander & off-target editing
Negative control	Pin-point ABE nCas9 mRNA, Pin-point ABE-flex or ABE-exact deaminase mRNA, and Non-targeting control guide RNA	Impact of component expression; ensure editing is not due to spurious activity
Mock nucleofected	No mRNA or sgRNA	Confirmation of cell viability & pre-existing genotype

iPSC electroporation using Lonza 4D Nucleofection System



This protocol has been optimized for the Lonza 4D Nucleofector System using 20 µL 16-well cuvette strips in P3 nucleofector solution. Using other electroporation platforms may require further optimization.

1. Cell preparation
 - a. 3-4 days before nucleofection, perform an EDTA split (e.g., Versene). Cells should only be used for editing if they are growing rapidly, below 80% confluency in small-to-medium colonies, lack significant cell death/debris, and do not have visible differentiation.
 - b. On the day of nucleofection, pre-treat cells with media containing 10 µM Y-27632 ROCK inhibitor (RI) for at least two hours at 37°C and 5% CO₂.
 - c. Pre-coat tissue culture plates needed for later cell seeding with a basement membrane matrix of choice (e.g., Matrigel, Geltrex, Vitronectin, etc.). This protocol assumes one well of a 12-well plate per nucleofection sample, but volumes may be split between two wells of a 24-well plate or four wells of a 96-well plate.



We recommend preparing the following sgRNA and mRNA component dilutions and reaction mixtures in sterile 0.5 mL tubes or in a sterile v-bottom 96-well plate

2. sgRNA preparation (see **Table 6** and **Table 7**)
 - a. Determine the number of electroporation reactions required and include a minimum of 10% excess.
 - b. Keep on ice until ready for electroporation.

Table 6. Recommended working stocks, volumes, and final concentration of Pin-point base editing components for one electroporation in iPSCs (100,000 cells in 20 µL reaction).

Pin-point component	Working stock	Final amount per reaction
ABE nCas9 mRNA – unmodified	2 µg/µL	1 µg
ABE deaminase mRNA – unmodified		
ABE-exact mRNA	2 µg/µL	1 µg
ABE-flex mRNA*	0.2 µg/µL*	0.1 µg*
sgRNA single-plex	200 µM	2 µM

* ABE nCas9 mRNA and ABE mRNA are shipped at 2µg/µL.



We recommend preparing a 0.2 µg/µL working stock of the ABE-flex deaminase mRNA in sterile nuclease-free water to reduce the risk of pipetting error from the higher concentration stock. Prepare only enough working stock for the current experiment as we do not recommend long-term storage at the lower concentration.

Table 7. Preparing electroporation samples for base editing experiment using Pin-point platform in iPSCs.

Sample	P3 nucleofactor solution	sgRNA (200 µM)	ABE nCas9 mRNA unmodified (2 µg/µL)	ABE-exact (2 µg/µL) or ABE-flex (0.2 µg/µL) deaminase unmodified mRNA	Cell suspension in P3 nucleofactor solution (100,000 cells)
Base editing sample, positive control	8.8 µL	0.2 µL	0.5 µL	0.5 µL	10 µL
Negative control	8.8 µL	0.2 µL	0.5 µL	0.5 µL	10 µL
Mock nucleofected	10 µL	0 µL	0 µL	0 µL	10 µL

Volumes are per single reaction. We recommend preparing sufficient sample volumes for the total number of replicates and to account for pipetting error. Exact reagent amounts for electroporation in other cell lines should be empirically determined through careful optimization prior to experimentation.

3. mRNA preparation (see Table 6 and Table 7)

- Determine the number of reactions required and include a minimum of 10% excess volume.
- Transfer mRNA aliquots from the –80 °C freezer directly onto wet ice to thaw.
- If using ABE-flex, prepare a 1:10 dilution in Ultrapure water (to 0.2 µg/µL).
- For each sample, prepare a reaction mix using the indicated volumes of the ABE nCas9 mRNA, ABE deaminase mRNA, and sgRNA.
- Keep electroporation mix on ice.

4. Cell harvest and centrifugation

- Calculate the total number of cells required, using 100,000 cells per 20 µL electroporation reaction and including at least 10% excess.
- Prepare a single-cell suspension with Accutase.
- Count cell density and transfer the required volume to a 15 mL centrifuge tube.
- Pellet the cells at 200 x g for 5 minutes at room temperature.
- Aspirate the supernatant to waste.
- Resuspend the cell pellet in P3 nucleofactor solution at a final concentration of 5×10^6 cells/mL

5. Final mix of components and electroporation

- Add 20 µL of the cell suspension in P3 nucleofactor solution (100,000 cells) per reaction to each reaction mix.
- Transfer 20µl of cells + reaction mix to a well of nucleofection cuvette. Avoid bubbles.
- Nucleofect following the manufacturer’s protocol. We recommend using the program CM-138.
- Immediately after nucleofection, add 80 µL of pre-warmed media supplemented with 10 µM RI to each well of the nucleofection cuvette, and rest in the incubator for 5 minutes.

- e. Aspirate coating medium from recipient wells and add pre-warmed media supplemented with 10 μ M RI (900 μ L per well of a 12-well plate).
 - f. Add 100 μ L of nucleofected cells into each prepared recipient well.
 - g. Gently shake the plate to evenly distribute cells in the well, and incubate cells overnight at 37°C and 5% CO₂, being careful not to agitate the plates during the hours immediately post-nucleofection.
6. Post nucleofection
- a. One day after nucleofection, check on cell attachment and survival. Some cell death/debris is normal, but a PBS wash may be performed to remove excess debris if desired. Change media to remove RI.
 - b. 3 days after nucleofection, cells may be lysed and genomic DNA collected for analysis following **Section 7. Guidelines for analyzing base editing with Pin-point analysis primers (Page 18)**.

6. General protocol for base editing in stimulated human hematopoietic stem and progenitor cells (HSPCs) using The Pin-point ABE platform

The following is a general protocol using electroporation to deliver 5moU-modified Pin-point ABE nCas9 mRNA, 5moU-modified Pin-point ABE-exact or ABE-flex deaminase mRNA, and a Pin-point synthetic sgRNA for ABE into human hematopoietic stem and progenitor cells (HSPCs). Reagent amounts given here are recommended starting points for new users and untested targets to ensure high editing efficiency, but base editing activity has been observed at some sites with as low as 100-fold less mRNA, with reduced deaminase mRNA amounts usually resulting in reduced bystander/off-target editing while maintaining high on-target editing. Exact reagent concentrations and parameters for electroporation should therefore be empirically determined through careful optimization in the cells of interest in accordance with the electroporation instrument manufacturer’s recommendations and according to the user’s priorities. The protocol below describes delivery conditions in human HSPCs using the Neon Nxt Electroporation System with 10 µL Neon tips and is given for illustrative purposes only. Reagent volumes can be calculated for including replicate samples as necessary.

Recommended samples for singleplex base editing of HSPCs are shown in **Table 8**. All steps of the protocol should be performed in a biosafety cabinet using sterile technique. A sample assay schedule is provided as a guide for a base editing workflow in CD34+ HSPCs obtained from mobilized peripheral blood of healthy adults (**Figure 5**).

Table 8. Recommended samples for a base editing experiment using Pin-point platform.

Sample elements	Purpose
Pin-point ABE nCas9 mRNA, Pin-point ABE-exact or ABE-flex deaminase mRNA, and Pin-point synthetic sgRNA validated control or custom sgRNA	Base editing sample, positive control
Pin-point ABE nCas9 mRNA, Pin-point ABE-flex or ABE-exact deaminase mRNA, and Non-targeting control guide RNA	Negative control sample
Non-electroporated	No treatment control sample



Figure 5. Schematic of the timeline for base editing in CD34+ HSPCs.

HSPC electroporation using Neon Nxt Electroporation System



This protocol has been optimized for Neon Nxt Electroporation System in HSPCs using 10 μ L Neon tips. Using other electroporation platforms or 100 μ L tips may require further optimization.

1. Cell preparation
 - a. CD34+ HSPCs can be obtained as cryopreserved cells from many commercially available suppliers.
 - b. Thaw cryopreserved cells following the manufacturer's instructions. Count cells and resuspend at 200,000 cells/mL in StemSpan™ SFEM II medium (Stemcell technologies #09655) supplemented with StemSpan™ CD34+ expansion supplement (Stemcell Technologies #02691), 1 μ M StemRegenin 1 (SR1) (Cayman (Cambridge Biosciences #CAY10625), 1000 nM UM729 (Stemcell Technologies #72332) and Penicillin-Streptomycin (Gibco #15140). Culture overnight at 37 °C and 5% CO₂.
 - c. Prepare antibiotic-free complete StemSpan SFEM II medium supplemented with StemSpan CD34+ expansion supplement, 1 μ M StemRegenin 1 (SR1) and 1000 nM UM729, allowing for either 200 or 500 μ L per electroporation reaction depending on downstream application.
 - d. Prepare post-electroporation tissue culture plates by adding either 200 μ L/well in a 96-well plate or 500 μ L/well in a 24-well plate. 200 μ L/well in a 96-well plate is sufficient to sustain the cells for 4 days to measure gene editing, but a larger initial volume, i.e. 500 μ L/well in a 24-well plate is recommended if the cells are to be expanded for long term culture or differentiation.
 - e. Incubate the tissue culture plate(s) at 37°C and 5% CO₂ for a minimum of 30 minutes prior to electroporation to pre-warm the medium.

Table 9. Recommended working stocks and final concentration of Pin-point base editing components for one electroporation in HSPCs (50,000 cells in 10 μ L reaction).

Pin-point component	Working stock*	Final amount per reaction
ABE nCas9 mRNA – 5moU-modified	2 μ g/ μ L	1.5 μ g
ABE deaminase mRNA – 5moU-modified		
ABE-exact mRNA	2 μ g/ μ L	1 μ g
ABE-flex mRNA	0.8 μ g/ μ L	0.4 μ g
sgRNA single-plex	200 μ M	6.25 μ M

* ABE nCas9 mRNA and ABE deaminase mRNAs shipped at 2 μ g/ μ L. See note about working stock preparation



We recommend preparing an 800 ng/ μ L working stock of the ABE-flex deaminase mRNA in water to reduce the risk of pipetting error from the higher concentration stock. Prepare only enough working stock for the current experiment as we do not recommend long-term storage at the lower concentration.

2. sgRNA preparation (see **Table 9** and **Table 10**)
 - a. Determine the number of electroporation reactions required and include a minimum of 10% excess.
 - b. Keep on ice until ready for electroporation.
3. mRNA preparation (see **Table 9** and **Table 10**)
 - a. Calculate the number of reactions required for the experiment and include a minimum of 10% excess volume.
 - b. Transfer mRNA and sgRNA aliquots from the –80 °C freezer directly onto wet ice.
 - c. Thaw mRNA and sgRNA on ice.
 - d. Mix the calculated volumes for the ABE nCas9 and ABE deaminase mRNAs and dispense into microcentrifuge tubes
 - e. Add sgRNA/Buffer R to prepared microcentrifuge tubes as necessary
 - f. Keep mRNA/sgRNA mix on ice until ready for electroporation.

Table 10. Preparing electroporation samples for base editing experiment using Pin-point platform in HSPCs.

Sample	Buffer R	sgRNA (200 μ M)	nCas9 mRNA 5moU-modified (0.4 μ g/ μ L)	ABE-exact (2 μ g/ μ L) or ABE-flex (0.8 μ g/ μ L) deaminase 5moU-modified mRNA	Cell suspension in Buffer R (50,000 cells)
Pin-point ABE nCas9 mRNA, Pin-point ABE deaminase mRNA, and gene-specific Pin-point synthetic sgRNA validated controls or custom sgRNA	3.44 μ L	0.312 μ L	0.75 μ L	0.5 μ L	5 μ L
Pin-point ABE nCas9 mRNA, Pin-point ABE deaminase mRNA, and Non-targeting control guide RNA	3.44 μ L	0.312 μ L	0.75 μ L	0.5 μ L	5 μ L
Non-electroporated	5 μ L	0 μ L	0 μ L	0 μ L	5 μ L

Volumes are per single reaction. We recommend preparing sufficient sample volumes for the total number of replicates and to account for pipetting error. Exact reagent amounts for electroporation in other cell lines should be empirically determined through careful optimization prior to experimentation.

4. Cell harvest and centrifugation

- Calculate the total number of cells required, and the number of cells in each subset, using 50,000 cells per electroporation, including 10% excess. Prolonged time in Buffer R decreases editing efficiency and post thaw viability. We recommend resuspending enough cells for 6-8 samples in Buffer R at a time to minimize time spent in Buffer R.
- Transfer the required number of cells to a centrifuge tube.
- Pellet the cells at 300 x g for 10 minutes at room temperature.
- Aspirate the supernatant to waste.
- Flick gently to dissociate the cell pellet.
- Dilute the cell solution with PBS to wash and transfer cells to the required number of V-bottom microcentrifuge tubes for each subset. Pellet the cells at 300 x g for 10 minutes at room temperature.



During this centrifugation wait step, setup the Neon transfection system:

Dispense 2 mL Electrolytic Buffer E10 per Neon tube and place the Neon tube in the Neon pipette station. A fresh Neon tube with fresh buffer should be used for samples prepared using different HSPC donors or different mRNA/sgRNA mixes, or after every 6 samples

- After centrifugation, carefully remove the supernatant from the pelleted cells.
- Resuspend the cell pellet in Buffer R at a final concentration of 1×10^7 cells/mL; each complete 10 μ L electroporation reaction will contain 50,000 cells.



We recommend preparing the mRNA and sgRNA mixtures before preparing the cells and keeping on ice while the cells are prepared. Add the cells resuspended in Buffer R immediately before electroporation.

5. Final mix of components and electroporation.

- As outlined in **Table 10**, prepare each sample to be electroporated in a sterile V-bottom microcentrifuge tubes by mixing ABE nCas9 mRNA, ABE deaminase mRNA, sgRNA, and Buffer R. Appropriate volumes (μ L) correspond to one electroporation.
- Add 5 μ L of the cell suspension in Buffer R (50,000 cells) per reaction. Considering technical replicates, ensure a minimum of 2 μ L excess volume in the microcentrifuge tube to ensure sufficient sample can be collected for the final 10 μ L reaction.
- Pipette 10 μ L of the cells mixed with mRNA/sgRNA into the Neon 10 μ L tip
- Insert the Neon pipette with the sample vertically into the Neon tube placed in the Neon pipette station until you hear a click sound
- Press “electroporate” and wait a few seconds until the electroporation is complete

- f. Immediately transfer the electroporated cells into the culture dish with prewarmed culture medium
 - g. Repeat for technical replicates, then switch to a new Neon 10 μ L tip for each cell/mRNA/sgRNA mix
 - h. Once all electroporations have been completed, incubate the electroporated cells and controls at 37°C and 5% CO₂ for 4 days, being careful not to agitate the plates during the hours immediately post-electroporation.
6. Post electroporation
- a. We recommend collecting DNA for genomic analysis 4 days post electroporation following **Section 7. Guidelines for analyzing base editing with Pin-point analysis primers (Page 18)**.
 - b. If the cells are to be kept beyond 4 days post electroporation for downstream assays, we recommend assessing cell viability and adjusting cell culture density and replacing medium as required at this point.

7. Guidelines for analyzing base editing with Pin-point analysis primers

This protocol outlines example cell lysis and thermal cycling conditions used for base editing analysis. For direct lysis, cells were edited and processed in a 96-well plate format.

Materials required for PCR amplification and sequence verification of base editing with Pin-point platform

Pin-point analysis primers

- Pin-point Synthetic sgRNA ABE Validated Control TRAC Forward Primer
AAGTCTGTCTGCCTATTCACC
- Pin-point Synthetic sgRNA ABE Validated Control TRAC Reverse Primer
TTCCCGTGCATTCTCTGG
- Pin-point Synthetic sgRNA ABE Validated Control CD52 Forward Primer
AAGCTGCTACCAAGACAGCC
- Pin-point Synthetic sgRNA ABE Validated Control CD52 Reverse Primer
CAGGTTTCTCTCAGGGCAGC
- Pin-point Synthetic sgRNA ABE Validated Control PDCD1 Forward Primer
GGCACCTCCCTTCAACCT
- Pin-point Synthetic sgRNA ABE Validated Control PDCD1 Reverse Primer
CTCCAGACCCCTCGCTCC
- Pin-point Synthetic sgRNA ABE Validated Control CD58 Forward Primer
TGCTCAAGGAGTTTGTCTGCTCA
- Pin-point Synthetic sgRNA ABE Validated Control CD58 Reverse Primer
AACCTTGTGTTAGTCACCACATCT
- Pin-point Synthetic sgRNA ABE Validated Control HBG1/2 Forward Primer
AGCAGTATCCTCTTGGGGG
- Pin-point Synthetic sgRNA ABE Validated Control HBG1/2 Reverse Primer
AACCTCAGACGTTCCAGAAGC
- Pin-point Synthetic sgRNA ABE Validated Control BCL11A +58 Forward Primer
GGACAGCCCACAGATGAAA
- Pin-point Synthetic sgRNA ABE Validated Control BCL11A +58 Reverse Primer
AGTGAGATGGCTGAAAAGCGA
- Pin-point Synthetic sgRNA ABE Validated Control Site 2 Forward Primer
AGGACGTCTGCCCAATATGT
- Pin-point Synthetic sgRNA ABE Validated Control Site 2 Reverse Primer
CCAAGTGAGAAGCCAGTGGG

Additionally, custom primers can be ordered from our [website](#).

Cell Lysis and PCR

1. Aspirate cell culture media (for adherent cells), or spin cells down and aspirate media (for suspension cells). Lyse cells by adding 100 μ L of 1X Phusion High-Fidelity buffer (Thermo Fisher Scientific) with additives (see **Table 11**).

Table 11. Preparing cell lysis reaction.

Reagent	Volume per sample (μ L)	Final Concentration
5X Phusion HF Buffer	20	1x
Proteinase K (~ 20 mg/mL)	5	~ 1 mg/mL
RNase A (10 mg/mL)	5	0.5 mg/mL
Water, nuclease free	70	N/A

2. Seal the 96-well plate with a plate seal to minimize evaporation and cross contamination. Incubate for 15–30 minutes at 56°C, followed by deactivation for 5 minutes at 96°C. Briefly centrifuge to collect liquid at bottom of wells.
3. Set up a 50 μ L PCR for each primer set, for each sample to be analyzed (see **Table 12**).

Table 12. Preparing PCR for amplification of base editing region of interest.

Reagent	Volume per sample (μ L)	Final Concentration
5X GoTaq reaction buffer	5	1x
Forward primer (100 μ M)	0.125	500 nM
Reverse primer (100 μ M)	0.125	500 nM
dNTPs (10 mM)	0.5	200 μ M each
GoTaq	0.125	0.04 U/ μ L
Cell lysate	2.5	N/A
Water, nuclease free	up to 25	N/A

4. Gently mix, centrifuge plate, and run PCR samples (see **Table 13**).

Table 13. Thermal cycling conditions.

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	95°C	2 min	1
Denature	95°C	30 s	10
Touchdown annealing	67°C – 1°C/cycle	15 s	
Extension	72°C	30 s	
Denature	98°C	10 s	25
Annealing	57°C	15 s	
Extension	72°C	30 s	
Final extension	72°C	10 min	1

5. PCR product (2–5 μ L) can be run on a 2% agarose gel to confirm amplification.
6. Submit samples for sequencing with the provider of your choice. We recommend sending for Sanger sequencing using the same Forward or Reverse Primers used in the PCR product generation. Aim to have at least 50 bases between the sequencing primer and the sgRNA sequence to allow for cleaner Sanger sequencing results of the base editing region.



In some instances (different cell types, donors, etc.) manual design of 2-3 additional analysis primer sets may be required to successfully PCR amplify and sequence the base edited region of interest.

Data Analysis

Multiple programs are available to assist in the analysis of base editing to quantify levels of target A to G conversion. Two examples for analysis of base editing from Sanger Sequencing include:

- BEAT: <https://hanlab.cc/beat> (Xu, 2019)
- EditR: https://moriaritylab.shinyapps.io/editr_v10 (Kluesner, 2018)

Additional Tips

- If the PCR results in a faint band or if there is low final product quantity, consider increasing the PCR annealing time to 30 seconds and extension time to 60 seconds, and performing a temperature gradient for the PCR primers. Additionally, supplementing the reaction with 5% DMSO in order to optimize the PCR might be beneficial.
- The number of cells used/lysed should be considered for analysis purposes; a minimum of 1000 cells (2000 alleles) is needed to assess complex indel formation, however lower amounts can be used for base editing. In the event of low cell density, caution should be taken to avoid underrepresentation.
- Sanger sequencing should be used to quickly interrogate base editing efficiency.
- Next Gen Sequencing (NGS) can be used to get more reliable and in-depth data on the target locus and guide-dependent off-target sites; we recommend designing primers for paired-end short read sequencing (300PE or 250PE) for NGS analysis.

8. Appendix

Stability and Storage

Pin-point ABE nCas9 and ABE deaminase mRNAs

Pin-point mRNA reagents are shipped on dry ice for overnight domestic delivery or priority international for delivery outside of the U.S. Upon receipt, mRNA should be stored at -80°C . When stored properly, mRNA is stable for at least 12 months from shipment date. Each thaw can reduce functionality, so freeze-thaws should be avoided as much as possible. At first thaw, aliquot mRNA stock or working solution into RNase-free tubes at convenient volumes and store at -80°C to minimize the number freeze-thaws prior to each experiment.

Pin-point synthetic sgRNAs

Pin-point synthetic sgRNA reagents are shipped as dried pellets at room temperature (23°C). The reagents are stable for at least four weeks. Upon receipt, synthetic guide RNAs should be stored at -20°C to -80°C . Under these conditions, the reagents are stable for at least two years. Always resuspend RNA in nuclease-free solutions, such as Tris buffer. In solution and stored at -20°C , the aliquoted reagents are stable for at least one year.

Pin-point analysis primers

Pin-point analysis primer reagents are shipped as dried pellets at room temperature (23°C). Under these conditions, they are stable for at least four weeks. Upon receipt, primers should be stored at -20°C to -80°C . Under these conditions, the reagents are stable for at least two years. In solution and stored at -20°C , the aliquoted reagents are stable for at least one year.

9. Frequently Asked Questions

How do I choose between Pin-point CBE and ABE?

CBE converts C to T, while ABE converts A to G. Both enzymes can introduce protein knockout through mutation of splice donor and splice acceptor sites. Additionally, CBE can introduce a stop codon. Depending on your specific application, one enzyme might be preferred, or either enzyme might work equally well. If you need further help with enzyme or reagent choice, please contact our [Scientific Support team](#).

Which deaminases are available in the Pin-point system?

For CBE, we offer rAPOBEC deaminase. For ABE, we offer ABE-flex and ABE-exact deaminases.

What is the editing window for the Pin-point ABE deaminases?

The editing window represents the positions within a 20-nt spacer where a base editor efficiently converts target nucleotides. ABE-flex primarily edits positions 4–7, with lower-efficiency editing extending to positions 3 and 8–12. ABE-exact shows a narrower window centered on positions 5–6, with reduced editing at positions 4 and 7. Refer to Figures 3 and 4 on our [Pin-point sgRNA design guidelines page](#) for more information.

How do I choose between the two ABE deaminase mRNA offerings?

For a highly specific ABE and targets where the absence of bystander editing is critical, choose Pin-point ABE-exact. For a more permissive ABE with a broader editing window, choose Pin-point ABE-flex. If you need further help with enzyme or reagent choice, please contact our [Scientific Support team](#).

How do I choose between unmodified and 5moU mRNAs?

Unmodified mRNA is preferred for work in T cells and iPSCs, while 5moU-modified mRNAs provide better editing efficiency in HSPCs. For more information, please refer to the [App Note: Guidance for using unmodified versus 5-methoxyuridine \(5moU\) modified mRNAs with chemically synthesized sgRNAs](#) found under Resources tab.

Which formats of the Pin-point system are available?

For Cytidine base editing (CBE) we offer Pin-point CBE rAPOBEC mRNA, Pin-point CBE nCas9 mRNA, and synthetic Pin-point single guide RNA. For Adenine base editing (ABE) we offer Pin-point ABE-exact mRNA or Pin-point ABE-flex mRNA, Pin-point ABE nCas9 mRNA and Pin-point single guide RNA. The Pin-point mRNA offerings for both CBE and ABE are available as -Unmodified or -5moU modified formats for optimized usage in different cell types and experimental applications.

Can I multiplex Pin-point sgRNAs for ABE?

Yes – we have used up to 5 sgRNAs in-house to knock out 5 genes simultaneously.

Are there positive controls for the Pin-point system?

The [pre-designed gene targeting sgRNAs](#) for ABE have been validated in-house to produce a functional knockout at the protein level.

Can I design custom sgRNAs for the ABE system?

Yes, design and order custom Pin-point sgRNAs on our [website](#).

What is the Pin-point sgRNA sequence?

The targeting sequence can be found on the product page and on **page 5** of this manual. The sequence of the full sgRNA is confidential.

Are the Pin-point sgRNAs modified?

Pin-point sgRNAs have two 2'-O-methyl nucleotides and phosphorothioate linkages in the backbone (2xMS) on both the 5' and 3' ends of the oligonucleotide to increase nuclease resistance. Pin-point sgRNAs also include an aptamer to recruit the adenine deaminase base editor, which is fused to an aptamer binding protein.

Can I use Pin-point sgRNA with Edit-R Cas9?

No, Pin-point sgRNAs should not be used with Edit-R Cas9 for DNA cutting. For knockout, please consider using pre-designed Edit-R sgRNAs, or designing a custom sgRNA with the CRISPR design tool.

Can I use Edit-R sgRNAs with Pin-point ABE nCas9 mRNA and ABE deaminase mRNA?

No, the Pin-point guide RNA contains an aptamer sequence to recruit the deaminase. Therefore, to conduct a base editing experiment, you must use a Pin-point sgRNA.

Can I use Pin-point reagents for simultaneous knock-in and knockout?

For knockout alongside HDR-mediated knock-in applications, Pin-point sgRNAs can be multiplexed with a pair of custom-designed Edit-R sgRNAs. This allows for simultaneous targeted knock-in via introduction of a staggered dsDNA break by the nCas9 component of the Pin-point system when an appropriate donor template is provided. This feature enables complex multiplex knock-in and knockout genome editing outcomes.

How are the Pin-point base editing components shipped and stored?

sgRNAs are shipped as dried pellets at room temperature. Pin-point ABE deaminase mRNAs and Pin-point ABE nCas9 mRNA are re-suspended at 2 mg/mL in 1 mM Sodium Citrate buffer and shipped on dry ice. All components should be stored at -80°C upon receipt. Upon first thaw, please consider aliquoting the mRNA and resuspending sgRNA into smaller volumes to limit the number of freeze-thaw cycles.

What is the best way to confirm base editing has taken place?

Base editing can be confirmed at the DNA level by sequencing the target site. For pre-designed Pin-point sgRNAs, PCR and sequencing primers are available for purchase. Knockout at the protein level can be confirmed by functional assay.

Can I use the Pin-point system in non-mammalian cells?

We use the Pin-point system in human cell lines and primary cells in-house but use in other species has not been tested. The Pin-point mRNAs contain a mammalian codon-optimized nCas9, and a mammalian codon-optimized deaminase and aptamer binding protein fusion. Base editing with the Pin-point system requires that the guide RNA targets the desired edit site. Therefore, custom sgRNAs may be required for base editing in species other than human.

Can I use electroporation method other than the ones described here?

This protocol has been optimized on the Lonza nucleofection of primary T cells and iPSCs, and Neon NxT Electroporation System for human HSPCs. Using other electroporation platforms may require further optimization of reagent amounts, ratios of reagents, number of cells, and electroporation settings.

Can lipid transfection be used to deliver Pin-point base editing reagents?

We recommend delivering Pin-point base editing reagents by electroporation for optimal editing efficiency.

All Pin-point base editing reagents referenced in this manual are for Research Use Only (RUO).

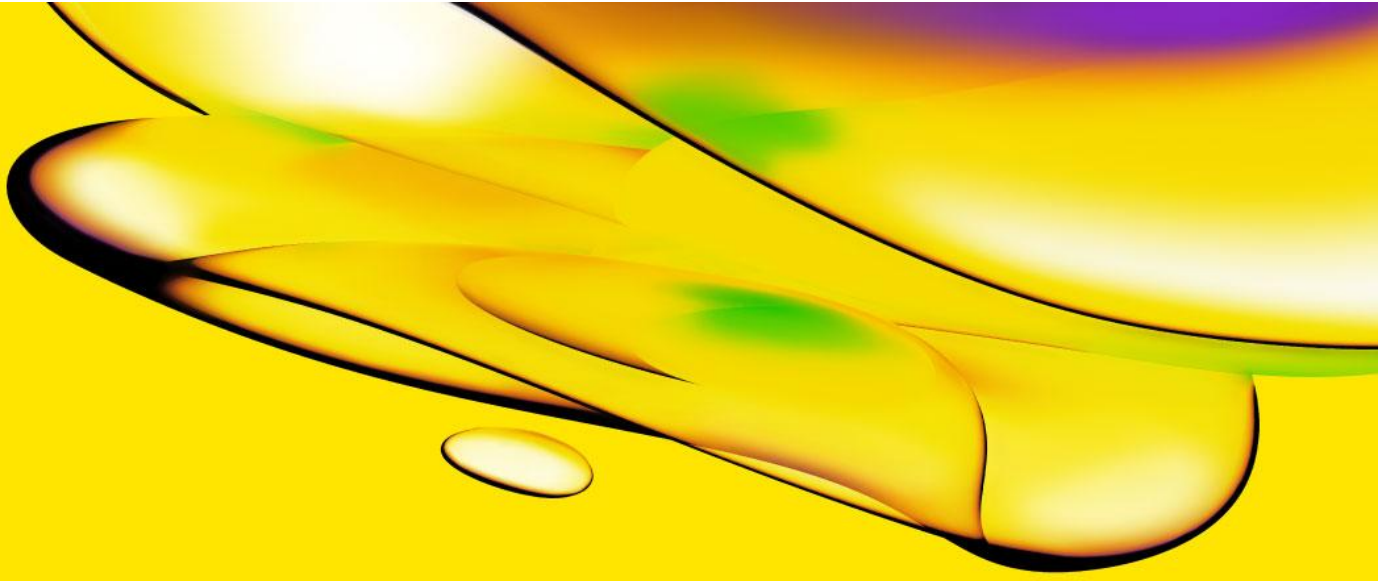
If you have questions or comments, please reach out to [Scientific Support](#).

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