Technical Manual

Pin-point™ base editing platform – cytidine deaminase configuration

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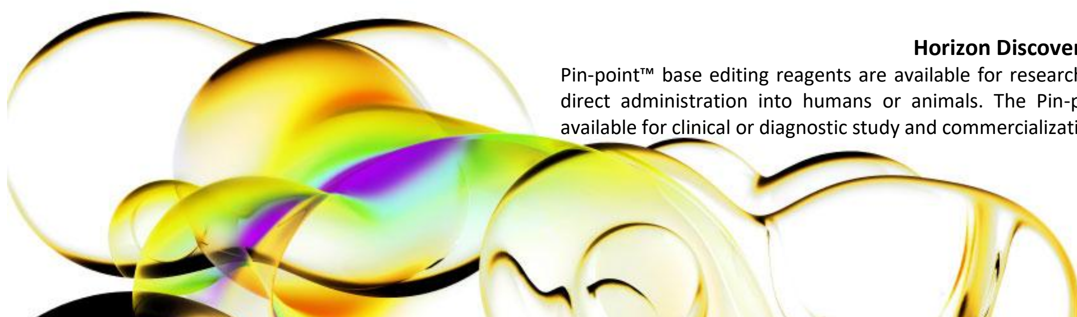

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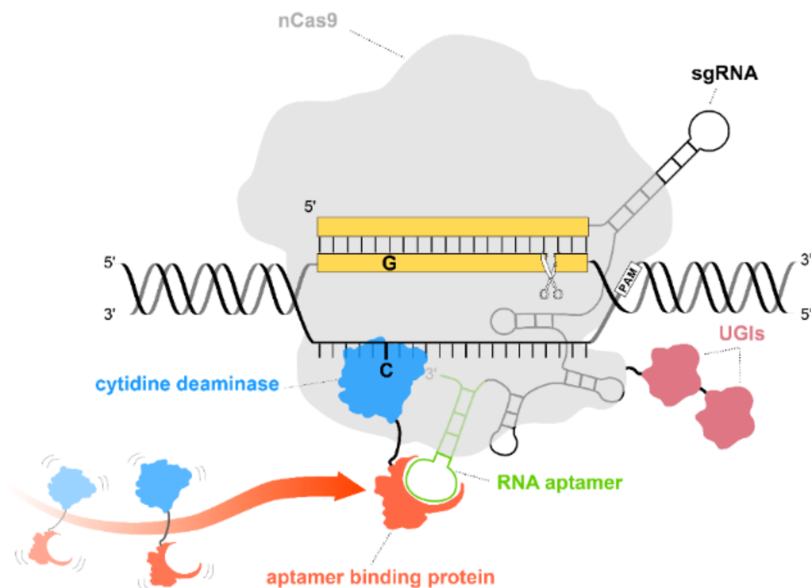
1. Introduction to the Pin-point base editing platform

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. Cytidine base editors (CBEs) enable recruitment of cytidine deaminase enzymes to a DNA target site, specifically enabling conversion of C-G base pairs to T-A base pairs (Komor, 2016; Nishida, 2016), while adenine base editors (ABEs) convert A-T to G-C pairs (Gaudelli, 2023). 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 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 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" CBE nCas9 that only cuts or "nicks" a single strand of DNA, fused to uracil glycosylase (UGI) inhibitor (Komor, 2016), [2] a cytidine deaminase base editor (CBE rAPOBEC) fused to an aptamer binding protein, and [3] an aptameric single guide RNA (sgRNA) that recruits both the CBE nCas9 and the aptamer-deaminase fusion to a specific DNA target site (**Figure 1**).

Delivery of these three components into a mammalian cell induces highly specific levels of C-G to T-A base conversion, suitable for cell and gene therapy applications involving either single amino-acid point mutations or for functional gene knockout. See our **Pin-point base editing sgRNA design tool** to design your own sgRNAs for gene knockout.

Figure 1. Illustration of Pin-point CBE system. The utilization of CBE 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 deaminase (blue) via aptamer binding protein (orange) to perform base editing.



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

The Pin-point immuno-oncology 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 a mammalian codon-optimized deaminase with aptamer binding protein fusion, (iii) and an aptameric Pin-point synthetic CBE sgRNA. All three components are electroporated into mammalian cells to enable the gene editing reaction. **Figure 2** summarizes the general experimental workflow.

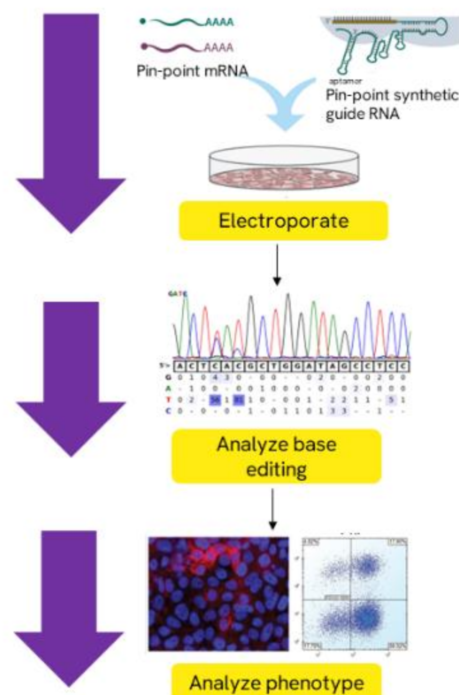


Figure 2. Base editing workflow using Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Pin-point synthetic CBE sgRNA. All three components are electroporated into a specific cell type of interest. Base editing may then be observed using analysis primers to amplify and sequence the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

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

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

Pin-point CBE rAPOBEC deaminase mRNA (unmodified or 5moU-modified)

The Pin-point CBE rAPOBEC deaminase mRNA contains a human codon-optimized version of an aptamer binding protein fused to a cytidine deaminase base editor enzyme with a 5′ NLS. The CBE rAPOBEC deaminase mRNA is in vitro transcribed, capped using CleanCap® AG, and polyadenylated for translation and nuclear localization of the protein.

Figure 3. Editing window of CBE rAPOBEC deaminase. The editing window represents the positions within a 20-nt spacer where a base editor efficiently converts target nucleotides. CBE rAPOBEC has high editing efficiency at positions 4-7 and lower editing at positions 2-3 and 8-9.

Pin-point CBE synthetic sgRNA

The Pin-point synthetic CBE 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.

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

Successful electroporation of Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Pin-point synthetic CBE 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 single sgRNA in mammalian cells.

Materials required for base editing using Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA and Pin-point synthetic CBE sgRNA validated controls

- Pin-point CBE nCas9 mRNA -Unmodified or -5moU modified (2 µg/µl), (20 µg; Cat # PNP12746, PNP12579) (100 µg; Cat # PNP12744, PNP12577) (500 µg; Cat # PNP12748, PNP12581)
- Pin-point CBE rAPOBEC deaminase mRNA -Unmodified or -5moU modified (2 µg/µl), (20 µg; Cat # PNP12747, PNP12580) (100 µg; PNP12745, PNP12578) (500 µg; PNP12749, PNP12582)
- Pin-point synthetic CBE sgRNA validated controls
  - TRAC (Cat # PNP-02000-01); Targeting sequence: TTCGTATCTGTAAAACCAAG
  - CD52 (Cat # PNP-02200-01); Targeting sequence: CTCTTACCTGTACCATAACC
  - PDCD1 (Cat # PNP-02300-01); Targeting sequence: CACCTACCTAAGAACCATCC
  - CIITA (Cat # PNP-002450-01); Targeting sequence: TGGTCAGGGCAAGAGCTATT
  - CD58 (Cat # PNP-002500-01); Targeting sequence: CTCACCGCTGCTTGGGATAC
  - HBG1/2 (Cat # PNP-002600-01); Targeting sequence: CTTGACCAATAGCCTTGACA
  - BCL11A+58 (Cat # PNP-002700-01); Targeting sequence: TTTATCACAGGCTCCAGGAA
  - Site 2 (Cat # PNP-002800-01); Targeting sequence: GAACACAAAGCATAGACTGC
  - PCSK9 (Cat # PNP-002900-01); Targeting sequence: CAGGTTCCACGGGATGCTCT
- 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 standard cell culture reagents and instruments appropriate for the maintenance of cells. 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 HEK293T cells using the Pin-point CBE platform

The following is a general protocol using electroporation to deliver unmodified Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Pin-point synthetic CBE sgRNA into HEK293T cells. Exact reagents, amounts and parameters for electroporation should be empirically determined through careful optimization in cells of interest, in accordance with electroporation instrument manufacturer's recommendations. The protocol below describes delivery conditions in HEK293T cells in a 96-well plate format using the Neon transfection or electroporation system and is given for illustrative purposes only. Reagent volumes can be calculated to include replicate samples as necessary and 10% excess.

Three different types of samples are recommended for a base editing experiment (see **Table 1)**. All steps of the protocol should be performed in a biosafety cabinet cell culture hood using sterile technique.

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

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

HEK293T Cell Plating

Optimal cell number for plating will vary with growth characteristics of specific cells and should be determined empirically.

1. Trypsinize and count cells.
2. Plate cells to achieve 70–80% confluence the next day. For example, plate 3 x 10^6 HEK293T cells in a 10 cm dish.
    a. Cell densities greater than 80% may reduce electroporation efficiency.
3. Incubate cells at 37°C in 5% CO_2 overnight.

Electroporation of HEK293T cells using Neon Nxt Electroporation System

This protocol has been optimized for Neon NxT Electroporation System in HEK293T cells. Using other electroporation platforms may require further optimization.

1. Cell preparation
    a. Prepare 96-well plates by transferring 100 µL of pre-warmed appropriate cell culture medium to the number of wells required for each sample in the experiment. Pre-incubate/equilibrate by placing at 37°C in 5% CO_2 while preparing samples.

2. sgRNA preparation (see **Table 2** and **Table 3**)
    a. Prepare a 100 µM synthetic sgRNA stock solution by adding the appropriate volume of Tris Buffer to the sgRNA. Verify the RNA concentration using UV spectrophotometry at 260 nm and adjust the volume necessary to obtain 100 µM.

3. mRNA preparation (see **Table 2** and **Table 3**)
    a. Prepare a 200 ng/µL stock of CBE rAPOBEC deaminase mRNA by diluting in nuclease-free water.

b. Prepare each sample to be electroporated in a 1.7 mL microcentrifuge tube by mixing sgRNA, CBE nCas9 mRNA, CBE rAPOBEC deaminase mRNA, and buffer R. Appropriate volumes (µL) are outlined in **Table 3**, corresponding to one well per sample.

We recommend preparing a working stock of the CBE rAPOBEC 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.

Table 2. Recommended working stocks and final amounts of Pin-point base editing components for one electroporation (50,000 cells in 10 µL reaction).

Pin-point component	Working stock*	Final amount per reaction
CBE nCas9 mRNA – unmodified	2 µg/µL	1 µg
CBE rAPOBEC deaminase mRNA – unmodified	200 ng/µL	100 ng
synthetic sgRNA	100 µM	6 µM

Table 3. Preparing electroporation samples for Pin-point base editing experiment in a 96-well plate format.

Sample	buffer R	sgRNA (100 µM)	CBE nCas9 mRNA unmodified (2 µg/µL)	CBE rAPOBEC deaminase mRNA unmodified (200 ng/µL)	Cell suspension in buffer R (50,000 cells)
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and gene specific Pin-point synthetic CBE sgRNA validated controls or custom sgRNA	3.4 µL	0.6 µL	0.5 µL	0.5 µL	5 µL
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Non-targeting control guide RNA	3.4 µL	0.6 µL	0.5 µL	0.5 µL	5 µL
Non-electroporated	5 µL	0 µL	0 µL	0 µL	5 µL

Volumes (µL) are for a single well of a 96-well plate for HEK293T cells to be electroporated. 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 types should be empirically determined through careful optimization prior to experimentation.

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

4. Cell harvest and centrifugation
    a. Harvest cells and collect 50,000 cells for each sample and centrifuge at 200 × g for 10 minutes at room temperature.
    b. Aspirate medium from the cell pellet, wash once with phosphate-buffered saline (PBS) by adding buffer to gently resuspend cells, and repeat centrifugation.

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

    c. Aspirate PBS from the cell pellet and resuspend the cell pellet with buffer R for a final concentration of 1×10^7 cells/mL.

5. Final mix of components and electroporation
    a. Gently mix components and using a Neon Pipette and 10 µL electroporation tips, pipette 10 µL of the sample, ensuring there are no air bubbles in the pipette.
    b. Electroporate sample following the manufacturer's protocol and using the following conditions: 1150 V, 20 ms, 2 pulses.

c. Pipette electroporation cell mixture into the pre-incubated medium in one well of a 96-well plate.

6. Post electroporation
    a. Incubate cells at 37°C in 5% CO_2 for a total of 48 to 72 hours post electroporation; proceed with base editing analysis.
    b. Collect DNA for genomic analysis following **Section 8: Guidelines for analyzing base editing with Pin-point analysis primers** (page **24**).

5. General protocol for multiplex base editing in activated human T cells using The Pin-point CBE platform

The following is a general protocol using electroporation to deliver unmodified Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and a multiplex of up to three independent Pin-point synthetic CBE sgRNA sequences into T cells (CD3+ Pan T Cells). Exact reagents, amount, and parameters for electroporation should be empirically determined through careful optimization in the cells of interest in accordance with the electroporation instrument manufacturer's recommendations. The protocol below describes delivery conditions in human T cells using the Neon transfection or electroporation system (Thermo Fisher Scientific) and is given 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 4**). 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 4 Recommended samples for a multiplex base editing experiment using Pin-point platform.

Sample elements	Purpose
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Multiple gene specific Pin-point synthetic CBE sgRNAs	Multiplex base editing sample
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Individual gene specific Pin-point synthetic CBE sgRNA (one sample per each sgRNA involved in the multiplex experiment)	Singleplex base editing samples, positive control
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Non-targeting control guide RNA	Negative control sample
Non-electroporated	No treatment control sample

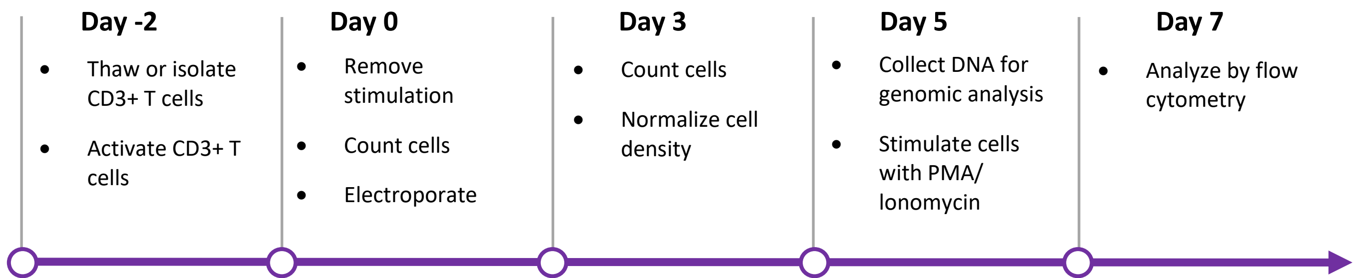

Figure 4. Example workflow for multiplex base editing in T cells that includes assessing base editing and protein knockout.

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 Neon Nxt Electroporation System

This protocol has been optimized for Neon NxT Electroporation 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% CO_2 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% CO_2 for a minimum of 30 minutes prior to electroporation to pre-warm the medium.

Table 5. Recommended working stocks and final concentration of Pin-point base editing components for one electroporation in T cells (250,000 cells in 10 µL reaction).

Pin-point component	Working stock*	Final amount per reaction
CBE nCas9 mRNA – unmodified	2 µg/µL	1.56 µg
CBE rAPOBEC deaminase mRNA – unmodified	1 µg/µL	0.222 µg
sgRNA singleplex	200 µM	2 µM
sgRNA multiplex (up to 3 sgRNAs)	200 µM	2 µM per guide, 6 µM total

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

We recommend preparing a 1 µg/µL working stock of the CBE rAPOBEC 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 5** and **Table 6**).
    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 (0.1 µl of 200 µM stock sgRNA in a 10 µL electroporation mix),
    c. If performing triplex editing (three targets), use 0.1 µL of each of the three sgRNA per 10 µL reaction.
    d. Mix the calculated volumes of sgRNAs and maintain the compositions on ice until ready for electroporation.

Table 6. Preparing electroporation samples for base editing experiment using Pin-point platform in T cells.

Sample	buffer R	sgRNA (200 µM)	CBE nCas9 mRNA unmodified (2 µg/µL)	CBE rAPOBEC deaminase mRNA unmodified (1 µg/µL)	Cell suspension in buffer R (250,000 cells)
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and multiple gene-specific Pin-point synthetic CBE sgRNA validated controls of custom sgRNAs	3.6 µL	0.1 µL of each sgRNA (0.3 µL total when using 3 sgRNA)	0.78 µL	0.22 µL	5 µL
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and individual gene specific Pin-point synthetic CBE sgRNA validated controls or custom sgRNAs (one sample per each sgRNA involved in the multiplex experiment).	3.9 µL	0.1 µL	0.78 µL	0.22 µL	5 µL
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Non-targeting control guide RNA.	3.6 – 3.9 µL	0.1 µL as a control for the singleplex or 0.3 µL as control for the multiplex	0.78 µL	0.22 µL	5 µL
Non-electroporated	5 µL	0 µL	0 µL	0 µL	5µL

Volumes are per single sgRNA to be electroporated. 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 5** and **Table 6**)
    a. Calculate the number of reactions required for the experiment and include a minimum of 10% excess volume; each 10 µL electroporation reaction will require a total of 1 µL mRNA mix. The mRNA mix is composed of 1.56 µg CBE nCas9 and 0.222 µg CBE rAPOBEC 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 CBE nCas9 and CBE rAPOBEC deaminase mRNAs.
    d. Keep mRNA mix on ice until ready for electroporation.

4. Cell harvest and centrifugation
    a. Calculate the total number of samples needed for the experiment and divide the total number of electroporations into subsets of 6-10 reactions, which will be processed together. This is to avoid prolonged exposure of cells to the buffer R which will lead to increased toxicity.
    b. Calculate the number of cells required for each subset, using 250,000 cells per electroporation, including 10% excess.
    c. Transfer the required number of cells to a centrifuge tube.
    d. Pellet the cells at 400 x g for 5 minutes at room temperature.
    e. Aspirate the supernatant to waste.
    f. Flick gently to dissociate the cell pellet.
    g. Dilute the cell solution with PBS to wash.
    h. 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.

i. After T cell centrifugation, carefully remove the supernatant from the pelleted cells.
j. Resuspend the cell pellet in buffer R at a final concentration of 5 x 10^7 cells/mL; each 5 µL electroporation reaction will contain 250,000 cells.

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

5. Final mix of components and electroporation
   a. As outlined in **Table 6**, prepare each sample to be electroporated in a 0.6 mL microcentrifuge tube by mixing buffer R, sgRNAs, CBE nCas9, and CBE rAPOBEC deaminase mRNA mix. Appropriate volumes (µL) correspond to one electroporation.
   b. Add 5 µL of the cell suspension in buffer R (250,000 cells) per reaction.
   c. Proceed immediately to electroporation following the manufacturer's protocol and using the following conditions: 1600 V, 10 ms, 3 pulses.
   d. Pipette electroporation cell mixture into the pre-incubated medium in one well of a 24-well plate.
   e. Once electroporations have been completed, incubate the electroporated 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-electroporation.

6. Post electroporation
   a. We recommend checking cell viability 3 days following the electroporation and adjusting cell culture density to maintain approximately 100,000 – 300,000 cells/mL.
   b. At 5 days post electroporation, collect DNA for genomic analysis following **Section 8: Guidelines for analyzing base editing with Pin-point analysis primers** (page **24**).

T cell electroporation using the 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% CO_2 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% CO_2 for a minimum of 30 minutes prior to electroporation to pre-warm the medium.

Table 7. 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
CBE nCas9 mRNA – unmodified	2 µg/µL	1.5 µg
CBE rAPOBEC deaminase mRNA – unmodified	1 µg/µL*	0.6 µg
sgRNA singleplex	200 µM	2 µM
sgRNA multiplex	200 µM	2 µM per guide, 6 µM total

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

We recommend preparing a 1 µg/µL working stock of the CBE rAPOBEC 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 7** and **Table 8**).
    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 7** and **Table 8**).
    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 CBE nCas9 and 0.5 µg CBE rAPOBEC 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 CBE nCas9 and CBE rAPOBEC deaminase mRNA.
    d. Keep mRNA mix on ice until ready for electroporation.

Table 8. Preparing electroporation samples for base editing experiment using Pin-point platform in T cells.

Sample	P3 nucleofector solution	sgRNA (200 µM)	CBE nCas9 mRNA unmodified (2 µg/µL)	CBE rAPOBEC deaminase mRNA unmodified (1 µg/µL)	Cell suspension in P3 nucleofector solution (250,000 cells)
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and multiple gene-specific Pin-point synthetic CBE sgRNA validated controls or custom sgRNAs	to 10 µL	0.2 µL of each sgRNA (0.6 µL total when using 3 sgRNA)	0.78 µL	0.6 µL	10 µL
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and individual gene specific Pin-point synthetic CBE sgRNA validated controls or custom sgRNAs (one sample per each sgRNA involved in the multiplex experiment).	to 10 µL	0.2 µL	0.78 µL	0.6 µL	10 µL
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC 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	0.78 µL	0.6 µL	10 µL
Non-electroporated	10 µL	0 µL	0 µL	0 µL	10 µL

Volumes are per single sgRNA to be electroporated. 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 nucleofector 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 nucleofector solution to the mixture shortly before the electroporation.

5. Final mix of components and electroporation
    a. As outlined in **Table 8**, prepare each sample to be electroporated in a sterile V-bottom 96-well plate by mixing CBE nCas9 mRNA, CBE rAPOBEC deaminase mRNA, CBE sgRNA, and P3 nucleofector solution. Appropriate volumes (µL) correspond to one electroporation.
    b. Add 10 µL of the cell suspension in P3 nucleofector solution (250,000 cells) per reaction.
    c. Transfer 20ul of cells + reagents mix to a well of nucleofection cuvette. Avoid bubbles.
    d. Nucleofect following the manufacturer's protocol and using the following conditions: EO-115.

  e. 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.
  f. Pipette 100 µL of electroporation cell mixture into the pre-incubated medium in one well of a 24-well plate.
  g. 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 x 10^6 cells/mL.
   b. At 5 days post electroporation, collect DNA for genomic analysis following **Section 8: Guidelines for analyzing base editing with Pin-point analysis primers** (page **24**).

Considerations for flow cytometry read-out to assess functional gene knockout in edited T cells

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.

Table 9. 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

2. As a proxy for TRAC knockout, we recommend measuring the surface level of TCR a/b (T cell receptor a/b).
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% CO_2.

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

The following is a general protocol using electroporation to deliver unmodified Pin-point CBE nCas9 mRNA, unmodified Pin-point CBE rAPOBEC deaminase mRNA, and a Pin-point synthetic CBE sgRNA into human induced pluripotent cells (iPSCs). 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 given 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 10**. All steps of the protocol should be performed in a biosafety cabinet using sterile technique.

Table 10. Recommended samples for a base editing experiment using Pin-point platform.

Sample type	Sample elements
Base editing sample, positive control	Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Pin-point synthetic CBE sgRNAs validated controls or custom sgRNA
Negative control	Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Non-targeting control guide RNA
Mock nucleofected	No mRNA or sgRNA

iPSC electroporation using Lonza 4D Nucleofector 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% CO_2.
    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 11** and **Table 12**).
    a. Determine the number of 20 µL electroporation reactions using each sgRNA, and multiply by 0.2 µL of 200 µM stock per reaction. Include a minimum of 10% excess.
    b. If desired, sgRNAs may be pre-diluted 1:5 in Ultrapure water (to 40 µM) to instead require 1 µL per reaction, or they may be mixed with the mRNA components as a pool.
    c. Keep on ice until ready for electroporation.

Table 11. 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	Volume per reaction	Final amount per reaction
CBE nCas9 mRNA – unmodified	2 µg/µL	1.28 µL	2.56 µg
CBE rAPOBEC deaminase mRNA – unmodified	2 µg/µL	0.37 µL	0.74 µg
sgRNA single-plex	200 µM	0.2 µL	2 µM

* CBE nCas9 mRNA and CBE rAPOBEC deaminase mRNA are shipped at 2µg/µl.

Table 12. Preparing electroporation samples for base editing experiment using Pin-point platform in iPSC cells.

Sample	P3 nucleofector solution	sgRNA (200 µM)	CBE nCas9 mRNA unmodified (2 µg/µL)	CBE rAPOBEC deaminase mRNA unmodified (2 µg/µL)	Cells in P3 nucleofector solution (Pelleted & resuspended at 1×10^7 cells/mL)
Base editing sample, positive control	to 10 µL	0.2 µL	1.28 µL	0.37 µL	10 µL
Negative control	to 10 µL	0.2 µL	1.28 µL	0.37 µL	10 µL
Mock nucleofected	10 µL	0 µL	0 µL	0 µL	10 µL

Volumes are per single sgRNA to be electroporated. 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 11** and **Table 12**)
    a. Determine the number of reactions required, include a minimum of 10% excess.
    b. Transfer mRNA aliquots from the –80°C freezer directly onto wet ice to thaw.
    c. For each sample, prepare a reaction mix using the indicated volumes of the CBE nCas9 mRNA, CBE rAPOBEC deaminase mRNA, and sgRNA.
    d. Keep electroporation mix on ice.

4. Cell harvest and centrifugation
    a. Calculate the total number of cells required, using 200,000 cells per 20 µL electroporation reaction, including at least 10% excess.
    b. Prepare a single-cell suspension with Accutase.
    c. Count cell density and transfer the required volume to a 15 mL centrifuge tube.
    d. Pellet the cells at 200 x g for 10 minutes at room temperature.
    e. Aspirate the supernatant to waste.
    f. Resuspend the cell pellet in P3 nucleofector solution at a final concentration of 1 × 10^7 cells/mL

5. Final mix of components and electroporation
    a. Add 10 µL of the cell suspension in P3 nucleofector solution (200,000 cells) per reaction to each reaction mix.
    b. Transfer 20ul of cells + reaction mix to a well of nucleofection cuvette. Avoid bubbles.
    c. Nucleofect following the manufacturer's protocol. We recommend using the program CM-138.
    d. 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 µM RI (900 µL per well of a 12-well plate).
    f. Add 100 µL 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% CO_2, 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 8: Guidelines for analyzing base editing with Pin-point analysis primers** (page **24**).

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

The following is a general protocol using electroporation to deliver 5moU-modified Pin-point CBE nCas9 mRNA, 5moU-modified Pin-point CBE rAPOBEC deaminase mRNA, and a Pin-point synthetic CBE sgRNA into human hematopoietic stem and progenitor cells (HSPCs). Exact reagents, amount, and parameters for electroporation should be empirically determined through careful optimization in the cells of interest in accordance with the electroporation instrument manufacturer's recommendations. 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 13**. 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 13. Recommended samples for a multiplex base editing experiment using Pin-point platform.

Sample elements	Purpose
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Pin-point synthetic CBE sgRNAs validated controls or custom sgRNAs	Base editing sample, positive control
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC 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 µL Neon tips. Using other electroporation platforms or 100 µL 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 µM 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% CO_2.
    c. Prepare antibiotic-free complete StemSpan SFEM II medium supplemented with StemSpan CD34+ expansion supplement, 1 µM StemRegenin 1 (SR1) and 1000 nM UM729, allowing for either 200 or 500 µL per electroporation reaction depending on downstream application.
    d. Prepare post-electroporation tissue culture plates by adding either 200 µL/well in a 96-well plate or 500 µL/well in a 24-well plate. 200 µL/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 µL/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% CO_2 for a minimum of 30 minutes prior to electroporation to pre-warm the medium.

Table 14. Recommended working stocks and final concentration of Pin-point base editing components for one electroporation in HSPCs (50,000 cells in 10 µL reaction).

Pin-point component	Working stock*	Final amount per reaction
CBE nCas9 mRNA – 5moU-modified	2 µg/µL	2.8 µg
CBE rAPOBEC deaminase mRNA – 5moU-modified	2 µg/µL	2 µg
sgRNA single-plex	200 µM	6.25 µM

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

Table 15. Preparing electroporation samples for base editing experiment using Pin-point platform in HSPCs.

Sample	buffer R	sgRNA (200 µM)	CBE nCas9 mRNA 5moU-modified (2 µg/µL)	CBE rAPOBEC deaminase 5moU-modified mRNA (2 µg/µL)	Cell suspension in buffer R (50,000 cells)
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and gene-specific Pin-point synthetic CBE sgRNA.	to 5 µL	0.312 µL	1.4 µL	1 µL	5 µL
Pin-point CBE nCas9 mRNA, Pin-point CBE rAPOBEC deaminase mRNA, and Non-targeting control guide RNA.	to 5 µL	0.312 µL	1.4 µL	1 µL	5 µL
Non-electroporated	5 µL	0 µL	0 µL	0 µL	5 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.

2. sgRNA preparation (see **Table 14** and **Table 15**)
    a. Calculate the number of reactions required for the experiment and include a minimum of 10% excess volume.
    b. Keep on ice until ready for electroporation.

3. mRNA preparation (see **Table 14** and **Table 15**)
    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 CBE nCas9 and CBE rAPOBEC 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.

4. Cell harvest and centrifugation
    a. 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.
    b. Transfer the required number of cells to a centrifuge tube.
    c. Pellet the cells at 200 x g for 10 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 and transfer cells to the required number of V-bottom microcentrifuge tubes for each subset. Pellet the cells at 200 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

Set the desired pulse conditions on the Neon transfection system:
- 1400V
- 10 ms
- 3 pulses

    g. After centrifugation, carefully remove the supernatant from the pelleted cells.
    h. Resuspend the cell pellet in buffer R at a final concentration of 1 x 10^7 cells/mL; each complete10 µL 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
    a. As outlined in **Table 15** prepare each sample to be electroporated in a sterile V-bottom microcentrifuge tubes by mixing CBE nCas9 mRNA, CBE rAPOBEC deaminase mRNA, sgRNA, and buffer R. Appropriate volumes (µL) correspond to one electroporation.
    b. Add 5 µL of the cell suspension in buffer R (50,000 cells) per reaction. Considering technical replicates, ensure a minimum of 2 µL excess volume in the microcentrifuge tube to ensure sufficient sample can be collected for the final 10 µL reaction.
    c. Pipette 10 µL of the cells mixed with mRNA/sgRNA into the Neon 10 µL tip
    d. Insert the Neon pipette with the sample vertically into the Neon tube placed in the Neon pipette station until you hear a click sound
    e. 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 µL 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% CO_2 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 8: Guidelines for analyzing base editing with Pin-point analysis primers** (page **24**).
   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.

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

The following protocol is an example of cell lysis and thermal cycling conditions for base editing analysis. For direct cell lysis, cells were treated for base editing 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 CBE Validated Control TRAC Forward Primer
  GGGGATATGCACAGAAGCTGC
- Pin-point Synthetic sgRNA CBE Validated Control TRAC Reverse Primer
  CTCAGAGCTTAGGATGCACCC
- Pin-point Synthetic sgRNA CBE Validated Control CD52 Forward Primer
  AAGCTGCTACCAAGACAGCC
- Pin-point Synthetic sgRNA CBE Validated Control CD52 Reverse Primer
  CAGGTTTCTCTCAGGGCAGC
- Pin-point Synthetic sgRNA CBE Validated Control PDCD1 Forward Primer
  GGCACCCTCCCTTCAACCT
- Pin-point Synthetic sgRNA CBE Validated Control PDCD1 Reverse Primer
  CTCCAGACCCCTCGCTCC
- Pin-point Synthetic sgRNA CBE Validated Control CIITA Forward Primer
  GGCATCCTAGTGGAGGTGGA
- Pin-point Synthetic sgRNA CBE Validated Control CIITA Reverse Primer
  CCCGGACGGTTCAAGCAATG
- Pin-point Synthetic sgRNA CBE Validated Control CD58 Forward Primer
  TGCTCAAGGAGTTTGTCTGCTCA
- Pin-point Synthetic sgRNA CBE Validated Control CD58 Reverse Primer
  AACCTTGTGTTAGTCACCACATCT
- Pin-point Synthetic sgRNA CBE Validated Control HBG1/2 Forward Primer
  AGCAGTATCCTCTTGGGGG
- Pin-point Synthetic sgRNA CBE Validated Control HBG1/2 Reverse Primer
  AACCTCAGACGTTCCAGAAGC
- Pin-point Synthetic sgRNA CBE Validated Control BCL11A+58 Forward Primer
  GGACAGCCCGACAGATGAAA
- Pin-point Synthetic sgRNA CBE Validated Control BCL11A+58 Reverse Primer
  AGTGAGATGGCTGAAAAGCGA
- Pin-point Synthetic sgRNA CBE Validated Control Site 2 Forward Primer
  AGGACGTCTGCCCAATATGT
- Pin-point Synthetic sgRNA CBE Validated Control Site 2 Reverse Primer
  CCAAGTGAGAAGCCAGTGGA
- Pin-point Synthetic sgRNA CBE Validated Control PCSK9 Forward Primer
  TAGCAGGGACAAGGTGGGAGGC
- Pin-point Synthetic sgRNA CBE Validated Control PCSK9 Reverse Primer
  CAACAGGAAGGCAGGCAGTGGG

  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 µL of 1X Phusion High-Fidelity buffer (Thermo Fisher Scientific) with additives (see **Table 16**).

Table 16. Preparing cell lysis reaction

Reagent	Volume per sample (µL)	Final Concentration
5X Phusion HF Buffer	20	1x
Proteinase K (~ 20mg/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 µL PCR for each primer set, for each sample to be analyzed (see **Table 17**).

Table 17. Preparing PCR for amplification of base editing region of interest

Reagent	Volume per sample (µL)	Final Concentration
5X Phusion HF Buffer	10	1x
Forward primer (50 µM)	0.5	500 nM
Reverse primer (50 µM)	0.5	500 nM
dNTPs (10 mM)	1	200 µM each
Phusion Hot Start II High-fidelity DNA polymerase (2 U/µL)	1	0.04 U/µL
Cell lysate	1 – 5	N/A
Water, nuclease free	up to 50	N/A

4. Gently mix, centrifuge plate, and run PCR samples (see **Table 18**).

Table 18. Thermal cycling conditions

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	98 °C	3 min	1
Denature	98 °C	10 s	10
Touchdown annealing	72 °C – 1 °C/cycle	15 s	
Extension	72 °C	30 s	
Denature	98 °C	10 s	25
Annealing	62 °C	15 s	
Extension	72 °C	30 s	
Final extension	72 °C	10 min	1

5. PCR product (2–5 µL) 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 and Reverse Primers used in the PCR product generation.

In some instances (different cell types, donors, etc.) manual design of 2-3 additional analysis primer sets may be required to succesfully 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 C to T 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 replacing 5X Phusion HF Buffer with 5X Phusion GC Buffer, or supplementing the reaction with 5% DMSO in order to optimize the PCR.
- 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.

9. Appendix

Stability and Storage

Pin-point CBE nCas9 and CBE rAPOBEC deaminase mRNA

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 CBE sgRNAs

Pin-point synthetic CBE 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.

10. 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 CBE deaminase?
The Pin-point base editing system uses the cytidine deaminase CBE rAPOBEC, which can convert C-G base pairs in the editing window to T-A base pairs. In published base editing systems, the canonical APOBEC1 activity window is positions 4–8 at the PAM-distal end of the protospacer (**Figure 3** on **page 4** and https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7898459). The base targeted by pre-designed sgRNAs is on the product page, written as the base and the position, counting from the 5′ end of the target sequence. For example, "C4" means the targeted base is the cytosine at position 4, if the PAM was positions 21–23.

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 CBE?
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 CBE have been validated in-house to produce a functional knockout at the protein level.

Can I design custom sgRNAs for the CBE 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 cytosine 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 CBE nCas9 mRNA and CBE 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 CBE rAPOBEC deaminase mRNA and Pin-point CBE 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.

11. References

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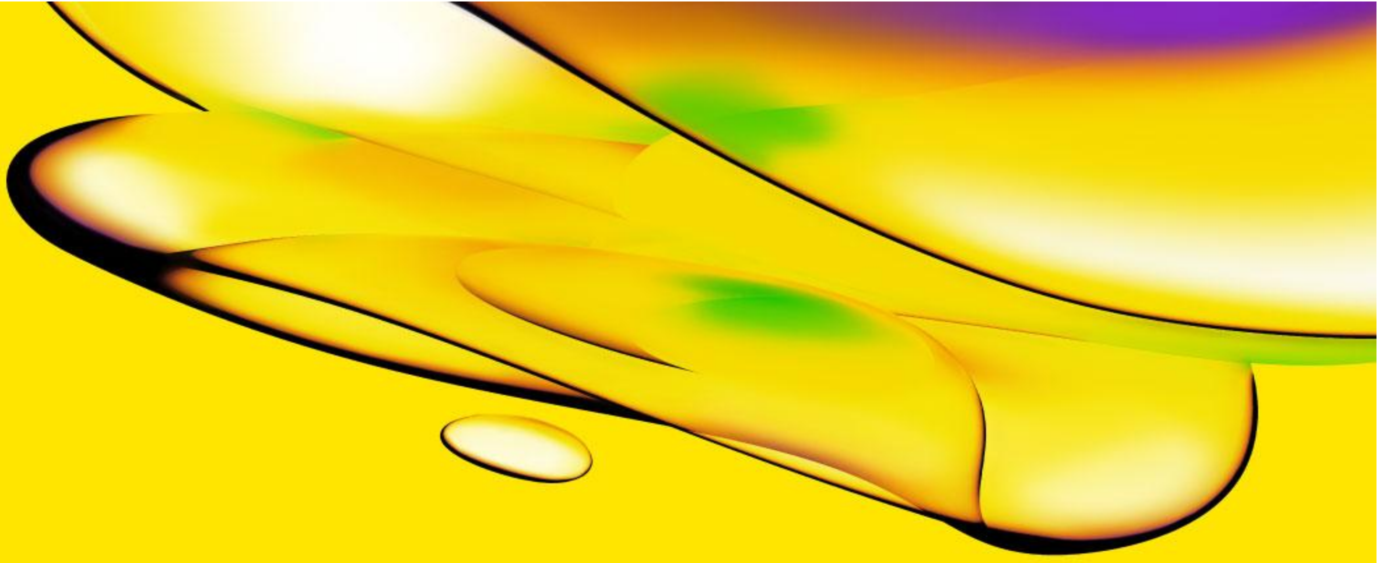

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