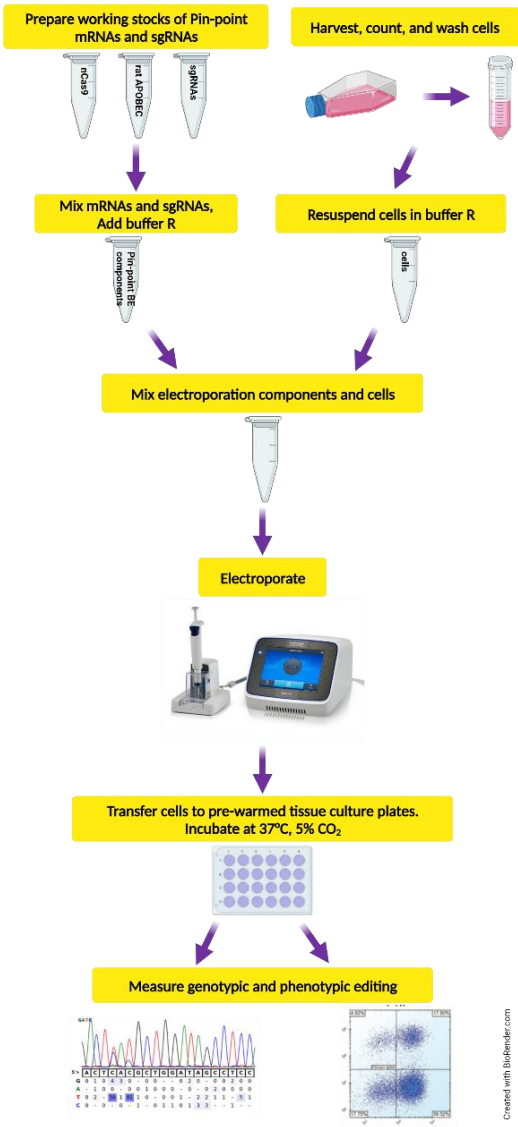
Pin-point™ base editing platform – short protocol

Version 2 January 2024



The following is a protocol for delivering Pin-point nCas9 mRNA (Cat # PNP12579, PNP12577, PNP12581), Pin-point rat APOBEC mRNA (Cat # PNP12580, PNP12578, PNP12582), and Pin-point sgRNAs (Cat # PNP-02000-01, PNP-02200-01, PNP-02300-01) to cultured mammalian cells using the Neon™ electroporation system. For more details, please see full protocol.

This protocol is written for electroporation of HEK293T cells and activated primary human T cells.

Day -2/-1

Plate cells

Seed cells at appropriate density.
HEK293T cells: 3 x 10^6 cells/10cm dish; ***Activated T cells***: 1 x 10^6 cells/mL

Day 0

Prepare post-electroporation plates

Add appropriate cell culture medium to plates
HEK293T cells: 96-well plates with 100µL medium per well; ***Activated T cells*** : 24-well plates with 500µL medium per well.
Incubate at 37°C and 5% CO_2

Prepare Pin-point base editing components

Prepare working stock solutions of mRNAs and sgRNAs according to the table below

Prepare the cells

Harvest and count the cells. Transfer the desired number of cells for electroporation into a centrifuge tube.
Wash with PBS (avoid centrifuge speeds > 400 x g)
Resuspend cell pellet in R buffer: ***HEK293T cells***: 1 x 10^7 cells/mL; ***Activated T cells*** : 5 x 10^7 cells/mL.

Mix electroporation components

Gently mix mRNAs + sgRNAs + cells

HEK293T cells:

Pin-point component	Working stock*	Final amount per reaction	Volume per reaction
nCas9 mRNA	2 µg/µL	1 µg	0.5 µL
Rat APOBEC mRNA	200 ng/µL	100 ng	0.5 µL
synthetic sgRNA	100 µM	6 µM	0.6 µL
Buffer R	-	-	3.4 µL
Cells		50,000 cells	5 µL

* nCas9 mRNA and Rat APOBEC mRNA shipped at 2µg/µl

Activated T cells:

Pin-point component	Working stock*	Final amount per reaction	Volume per reaction
nCas9 mRNA	2 µg/µL	1.56 µg	0.78 µL
Rat APOBEC mRNA	1 µg/µL	0.222 µg	0.222 µL
synthetic sgRNA	200 µM	2 - 6µM	0.1 µL of each sgRNA (0.3µL total when using 3 sgRNAs)
Buffer R	-	-	to 10 µL
Cells		250,000 cells	5 µL

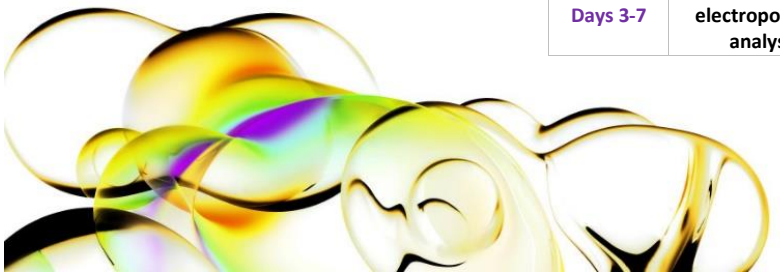
Electroporate

Electroporate on Neon transfection or electroporation system with the following conditions:
HEK293T cells: 1150V, 20ms, 2 pulses; ***Activated T cells***: 1600V, 10ms, 3 pulses.
Pipette cells into prepared plates.
Incubate at 37°C, 5% CO_2 for 48-72 hours.

Days 3-7

Post-electroporation analysis

Proceed with desired genotypic (Sanger sequencing) and phenotypic (flow cytometry) analyses of base editing levels.



If you have questions or comments, please reach out to Scientific Support.