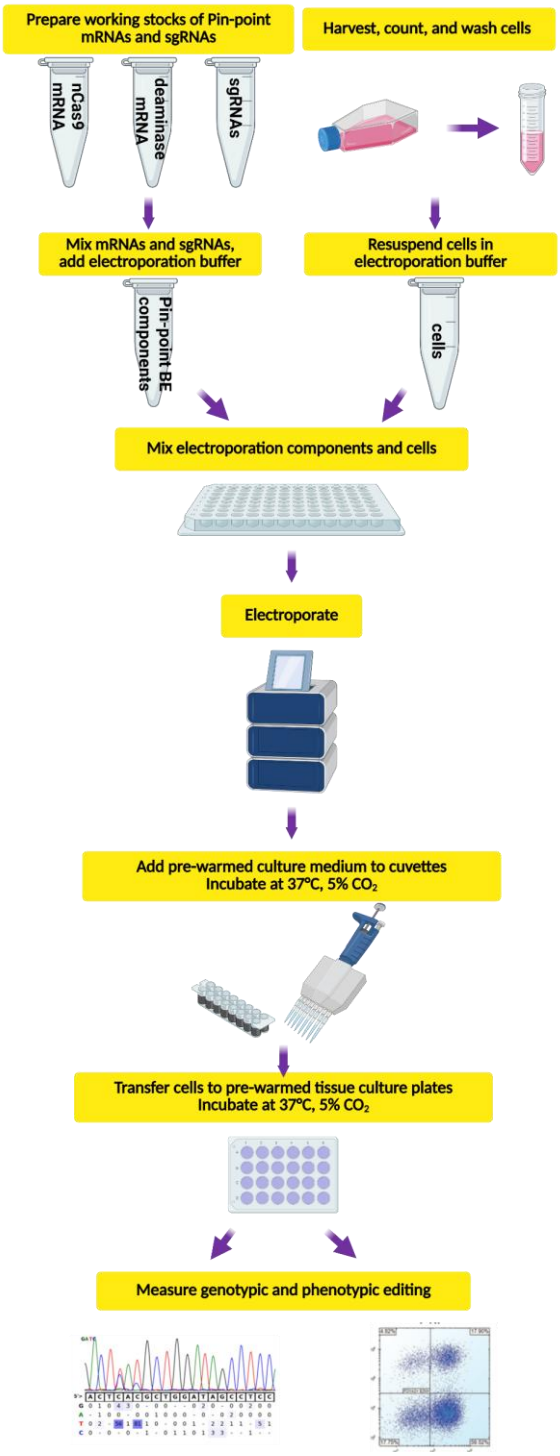
Pin-point™ cytidine base editing (CBE) platform

Short protocol for electroporation of HEK293T cells and activated human T cells using the Lonza 4D-Nucleofector® System

Experimental workflow:



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The following is a protocol for delivering unmodified Pin-point CBE nCas9 mRNA (Cat # PNP12744, PNP12746, PNP12748), Pin-point CBE rAPOBEC deaminase mRNA (Cat # PNP12745, PNP12747, PNP12749), and Pin-point CBE sgRNAs (validated controls or custom) to HEK293T or activated primary human T cells using the using the Lonza 4D-Nucleofector System with the SF or P3 Primary Cell 96-well Nucleofector® Kit. For more details, please refer to the Pin-point platform CBE technical manual. (EP = electroporation)

Day -2 — Plate cells

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

Day 0

Prepare post-EP plates

Add appropriate cell culture medium to plates
HEK293T cells: 96-well plates with 100 µL medium per well;
Activated T cells: 24-well plates with 500 µL 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 15 mL conical tube.
Wash with PBS, centrifuge at 200 x g for 10 minutes
Resuspend cell pellet in electroporation buffer.
HEK293T cells: 1 x 10^7 cells/mL;
Activated T cells: 5 x 10^7 cells/mL.

Mix EP components

Gently mix mRNAs + sgRNAs + cells:
HEK293T cells:

	Pin-point component	Working stock*	Final amount per 20 µL EP	Volume per 20 µL EP
mRNA and sgRNA mix	CBE nCas9 mRNA	2 µg/µL	1.75 µg	0.875 µL
	CBE rAPOBEC deaminase mRNA	0.2 µg/µL*	0.25 µg	1.25 µL
	synthetic sgRNA	200 µM	6µM	0.6 µL
	SF nucleofector solution	-	-	to 10 µL
Cells	Cells in SF nucleofector solution	-	50,000 cells	10 µL

Activated T cells:

	Pin-point component	Working stock*	Final amount per 20 µL EP	Volume per 20 µL EP
mRNA and sgRNA mix	CBE nCas9 mRNA	2 µg/µL	1.5 µg	0.75 µL
	CBE rAPOBEC deaminase mRNA	1 µg/µL*	0.6 µg	0.6 µL
	synthetic sgRNA	200 µM	2 - 6µM	0.2 µL of each sgRNA (0.6 µL total when using 3 sgRNAs)
	P3 nucleofector solution	-	-	to 10 µL
Cells	Cells in P3 nucleofector solution	-	250,000 cells	10 µL

* CBE nCas9 mRNA and CBE rAPOBEC deaminase mRNA shipped at 2 µg/µL

EP

Electroporate 20 µL on Lonza 4D-Nucleofector System with appropriate program:
HEK293T cells: CM-130; ***Activated T cells***: EO-115.
Add 80 µL of pre-warmed media without cytokines to the cuvette.
Incubate at 37°C, 5% CO_2 for 15 - 30 mins.
Gently transfer 100 µL of cells into prepared plates and disperse evenly by tilting/rocking. Incubate at 37°C, 5% CO_2.

Days 3-7 — Post-EP analysis

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

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