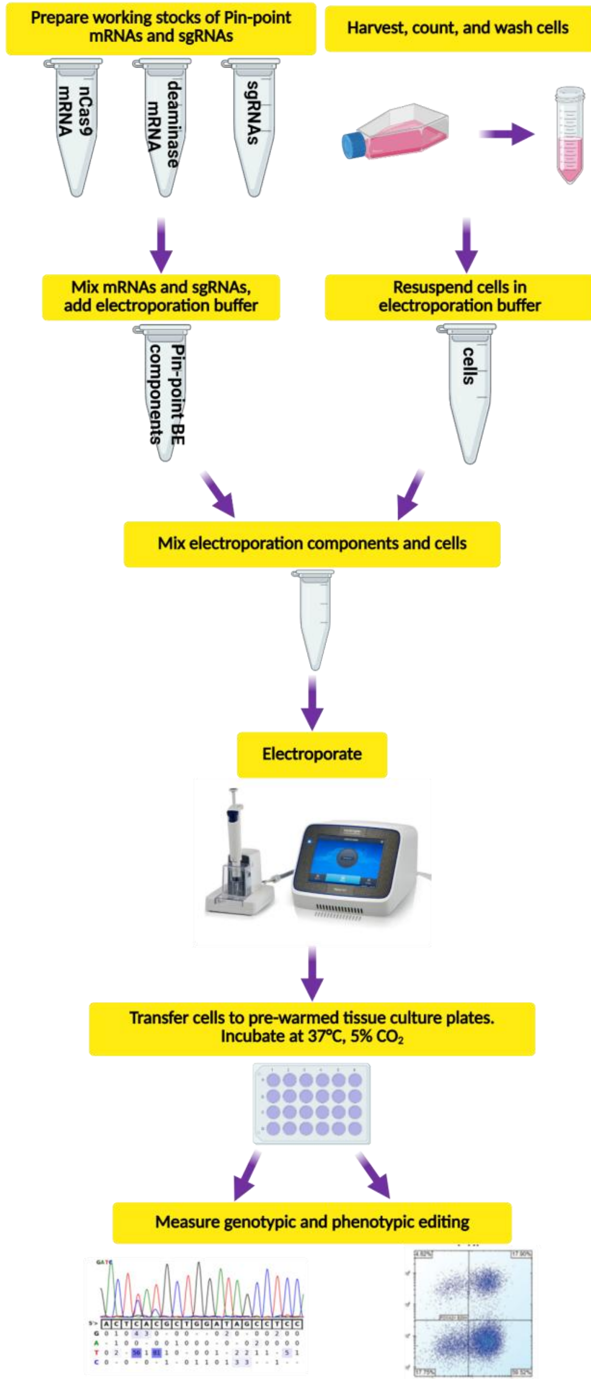
Pin-point™ cytidine base editing (CBE) platform

Short protocol for electroporation of HEK293T cells and activated human T cells using the Neon™ Nxt Electroporation 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 cells and activated primary human T cells using the Neon NxT Electroporation System. For more details, please refer to the Pin-point platform CBE technical manual. (EP = electroporation)

Day	Step	Procedure
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 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 on ice 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, centrifuge at 200 x g for 10 minutes Resuspend cell pellet in buffer R: ***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: (see tables below)
	EP	Electroporate 10 µL on Neon NxT Electroporation System using the following conditions: ***HEK293T cells***: 1150 V, 20 ms, 2 pulses; ***Activated T cells***: 1600 V, 10 ms, 3 pulses. Pipette cells into prepared plates. Incubate at 37°C, 5% CO_2 for 48-72 hours.
Days 3 - 7	**Post-EP analysis**	Proceed with desired genotypic (Sanger sequencing) and/or phenotypic (flow cytometry) analyses of base editing levels.

Mix EP components — Gently mix mRNAs + sgRNAs + cells:

HEK293T cells:

Pin-point component	Working stock*	Final amount per 10 µL EP	Volume per 10 µL EP
CBE nCas9 mRNA	2 µg/µL	1 µg	0.5 µL
CBE rAPOBEC deaminase mRNA	0.2 µg/µL*	0.1 µg	0.5 µL
synthetic sgRNA	200 µM	6µM	0.1 µL of each sgRNA (0.3 µL total when using 3 sgRNAs)
Buffer R	-	-	to 5 µL
Cells in buffer R		50,000 cells	5 µL

Activated T cells:

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

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

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

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