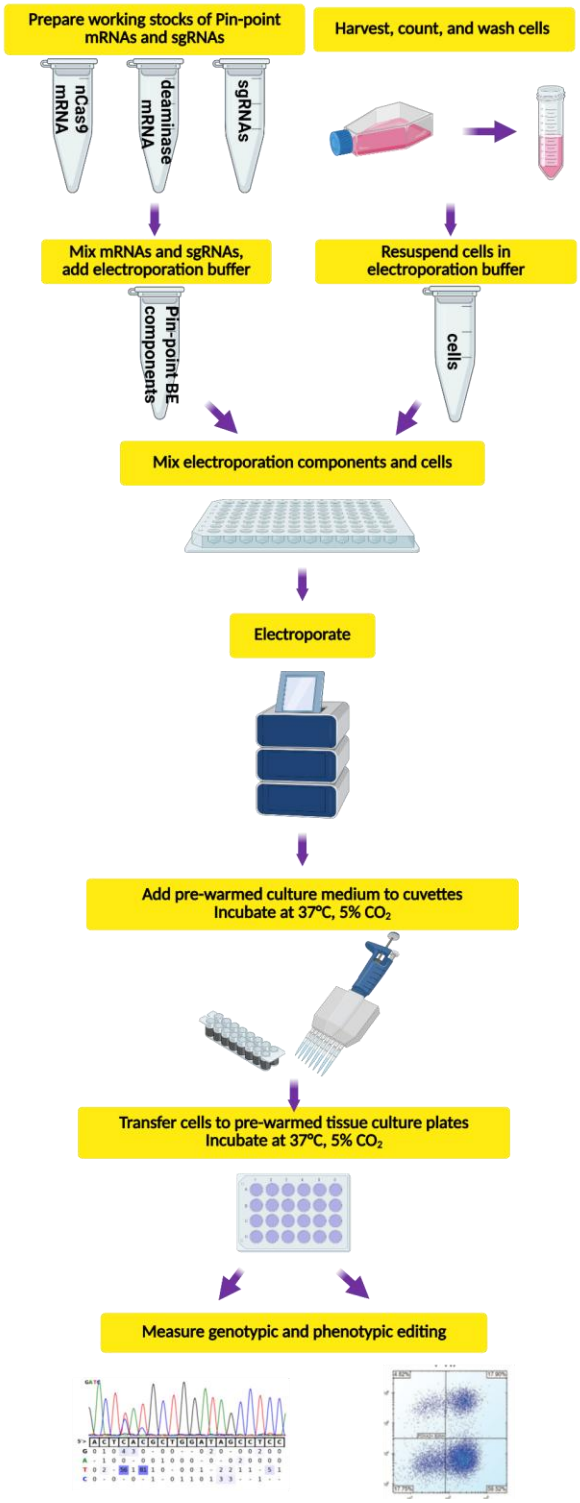
Pin-point™ adenine base editing (ABE) platform

Short protocol for electroporation of 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 ABE nCas9 mRNA (Cat # PNP13767, PNP13768, and PNP13769), Pin-point ABE-exact deaminase mRNA (Cat # PNP13781, PNP13782, and PNP13783) or Pin-point ABE-flex deaminase mRNA (Cat # PNP13773, PNP13774, and PNP13775), and Pin-point ABE sgRNAs (validated controls or custom) to activated human T cells using the Lonza 4D-Nucleofector System with the P3 Primary Cell 96-well Nucleofector® Kit. For more details, please refer to the Pin-point platform ABE technical manual. (EP = electroporation)

Day -2	**Plate cells**	Seed cells at 1 x 10^6 cells/mL and activate using CD3/28 beads in T cell culture medium containing IL-2. Incubate at 37°C and 5% CO_2.
Day 0	**Prepare post-EP plates**	Add 500 µL culture medium with cytokines (IL-2, IL-7, IL-15) to 24-well plates. 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 15 mL conical tube. Wash with PBS, centrifuge at 200 x g for 5 - 10 mins. Resuspend cell pellet in P3 nucleofector solution: 1 x 10^7 cells/mL (250,000 cells per 20 µL EP).
	Mix EP components	Gently mix mRNAs + sgRNAs + cells:
	EP	Electroporate 20 µL on Lonza 4D-Nucleofector System using program EO-115. Add 80 µL of pre-warmed culture medium without cytokines to the cuvette. Incubate at 37°C, 5% CO_2 for 15 - 30 mins. Gently transfer 100 µL of electroporated cells into prepared plates and disperse evenly by tilting/rocking. Incubate at 37°C, 5% CO_2.
Days 3 - 7	**Post-EP culture and analysis**	On day 3 post EP, adjust cell density to 1 x 10^6 cells/mL in culture medium with IL-2. Proceed with desired genotypic (Sanger sequencing) and/or phenotypic (flow cytometry) analyses of base editing levels.

	Pin-point component	Working stock*	Final amount per 20 µL EP	Volume per 20 µL EP
mRNA and sgRNA mix	ABE nCas9 mRNA	2 µg/µL	1.5 µg	0.75 µL
	ABE deaminase mRNA	2 ug/µL ABE-exact, 0.2 ug/µL ABE-flex*	1 µg ABE-exact, 0.1 µg ABE-flex	0.5 µL
	Synthetic sgRNA	200 µM	40 pmol	0.2 µL
	P3 nucleofector solution	-	-	to 10 µL
Cells	Cells in P3 nucleofector solution	-	2.5 x 10^5 cells	10 µL

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

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